Synthesis of N-Phosphorylated Derivatives of Nitrogen Mustards with Latent Cytotoxicity[1]

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The phosphoramide nitrogen mustards reported include series of phosphoro-triamides, diamidic esters, amidic diesters, diamidic acids, ester amidic acids and *sym*-pyrophosphorodiamidic diesters that were synthesized because of their possible selective action against tumors. Some of these compounds already tested in experimental animals have shown significant antitumor activity.

N-Phosphorylated derivatives of nitrogen mustards of the type reported here have been of interest in cancer chemotherapy since compounds of this type were initially synthesized[2] because of their possible selectivity of action against malignant tumors. These compounds are non-toxic precursors from which cytotoxic mustards would be liberated by enzymatic hydrolysis.[3,4] By the action of phosphamidases that are abundant in neoplastic cells, for example, a greater concentration of a nitrogen mustard given in this inactive phosphorylated form could be localized in the cells of a malignant tumor than would reach them by the administration of a tolerated dose of the parent mustard.

Recent work in this laboratory has provided evidence for the existence of phosphomonoamidase[5] and pyrophosphamidase[6] as discrete enzymes; and it is probable from the results of Gomori and others[7a-k] that mammalian tissues also contain an enzyme, phosphodiamidase, specific for phosphorodiamidic acids. A study of the former two enzymes in rat and mouse tissues has been made and some preliminary information is available on the enzyme content of normal and malignant human tissues.[6] There have also been reports of high concentrations of the phosphodiamidase in human tumors.[7c,d,e,f,i,j,k] These enzymes are of a type that could possibly "activate" phosphoramide mustards with the appropriate structural requirements.

Certain of the compounds, the syntheses of which are reported here, that have been studied in biological systems have shown various degrees of cytotoxicity against tumor and bacterial cells grown in culture; and of those studied to date in experimental animals some have produced significant inhibition of the growth of tumors.[8] A preliminary report of the results obtained at the Children's Cancer Research Foundation, Inc., and the CCNSC Screening Program is given in a later section. A structurally related cyclic phosphoramide ester of the same type, cyclophosphamide, prepared by Arnold *et al.*,[9] has proven to be an extraordinarily active inhibitor of many experimental tumors in animals; and although much less dramatic in its effects against human cancer it is a useful drug that has been studied very widely in the clinic. Phosphamidase has been presumed to be involved in the activation of this compound although the exact mechanism of action still remains to be elucidated. In a recent study[10] evidence was found that indicated that cyclophosphamide undergoes activation in the liver of rats since extracts of liver of animals pretreated with the drug are significantly cytotoxic to tumor cells growing in culture whereas the parent drug prior to biological activation shows no cytotoxicity to the same cells *in vitro*. This metabolic intermediate which is presumably the active form of the drug has not been identified as yet. It may possibly be either the phosphoramidic acid ester or the phosphorodiamidic acid XXXVII if the metabolism of this drug can be represented as a simple one-step hydrolytic process. The synthesis of the latter, cytoxyl alcohol, is included among the compounds reported here.

In the present paper we report the syntheses of a number of new phosphoramide mustards, some of which are derived from bis-(β-chloroethyl)-amine-(norHN2) and others from the more potent secondary β-chloroethylamines[11a,b] that had been developed previously in relation to this program in cancer chemotherapy. These derivatives include both aliphatic, aromatic and cyclic phosphorodiamidates, phosphorotriamidates, phosphorodiamidic monoesters, phosphoramidic diesters as well as various phosphorodiamidic acids and phosphoramidic acid monoesters. These compounds are listed in Tables I-VII.

The cyclic phosphoramide mustard derivatives II, III, IV, VI and VIII listed in Table I were prepared by the same general method that had been used earlier.[2] The known dichlorophosphoramides were condensed with the appropriate propylenediamine, propylenediol

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(2) O. M. Friedman and A. M. Seligman, *J. Am. Chem. Soc.*, **76**, 655 (1954).

(3) A. M. Seligman, M. M. Nachlas, L. H. Manheimer, O. M. Friedman and G. Wolf, *Ann. Surg.*, **130**, 333 (1949).

(4) A. M. Rutenburg, L. Persky, O. M. Friedman and A. M. Seligman, *J. Pharmacol. Exptl. Therap.*, **3**, 483 (1954).

(5) To be published: see O. M. Friedman and E. Boger, Abstr. 139th A.C.S. Meeting, St. Louis, Mo., March 1961, pp. 26-C.

(6) See O. M. Friedman, S. Schichor and E. Boger, *Proc. Am. Soc. Cancer Res.*, **3**, 320 (1962).

(7) (a) E. Waldschmidt-Leitz, *Biochem. Ztschr.*, **257-258**, 360 (1933); (b) M. Ichihara, *J. Biochem., Japan*, **18**, 87 (1933); (c) G. Gomori, *Proc. Soc. Exp. Biol. & Med.*, **69**, 407 (1948); (d) J. Meyer and J. P. Weinman, *J. Histochem. & Cytochem.*, **5**, 354 (1957); (e) J. Meyer and J. P. Weinman, *J. Histochem. & Cytochem.*, **1**, 305 (1953); and *J. Dent. Res.*, **32**, 669 (1953); (f) P. Sepp, *Strahlentherapie*, **99**, 393 (1956); (g) H. Holter and Si-Oh Li, *Compt. rend. trav. lab. Carlsberg Ser. Chim.*, 293 (1951); (h) G. E. Perlman, "Phosphorus Metabolism," Vol. II, ed. W. D. McElroy and B. Glass, The Johns Hopkins Press, 1952, p. 167; (i) H. Bredereck and E. Ceyer, *Z. physiol. Chem.*, **254**, 223 (1938); (j) E. H. Strecker, *Deut. Med. Wochschr.*, **74**, 1268 (1951); (k) L. A. Tseitlin, *Biokhimiya*, **17**, 208 (1952).

(8) These studies have been done and are being continued by Dr. George E. Foley, Dr. Charlotte L. Maddock and Dr. Alfred H. Handler and their associates at the Children's Cancer Research Foundation, Inc., Boston, Mass. The biological data will be published in detail elsewhere.

(9) H. Arnold, F. Bourseaux and N. Brock, *Nature*, **181**, 131 (1958).

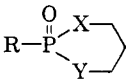
(10) G. E. Foley, B. P. Drolet and O. M. Friedman, *Cancer Res.*, **21**, 57 (1961).

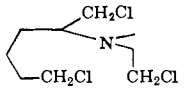
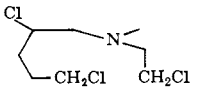
(11) (a) O. M. Friedman and A. M. Seligman, *J. Am. Chem. Soc.*, **76**, 658 (1954); (b) O. M. Friedman and E. Boger, *ibid.*, **78**, 4659 (1956).

or propanolamine in the presence of two moles of base. The base generally used was triethylamine although in the case of the triamide II, the use of excess of propylenediamine as the base gave much better results. This series includes cyclophosphamide (IV) the preparation of which had been reported earlier,[9] although the details of synthesis have not been given. This compound was isolated as the monohydrate which was also the form in which the two nitrogen mustard analogs VI and VIII were obtained.

TABLE I

CYCLIC PHOSPHORAMIDE MUSTARDS

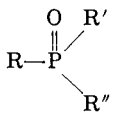


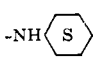
No.	R	X	Y
II	$(ClCH_2CH_2)_2N-$	—NH—	—NH—
III	$(ClCH_2CH_2)_2N-$	—O—	—O—
IV	$(ClCH_2CH_2)_2N-$	—NH—	—O—
VI		—NH—	—O—
VIII		—NH—	—O—

The phosphorotriamidate mustard derivatives XVIII, XIX and XX listed in Table II were also prepared from the dichlorophosphoramide of norHN2 by the stepwise replacement of chlorine through condensation with the appropriate amines in sequence, a procedure again analogous to that reported earlier.[2] Two of the compounds, XVIII and XIX, were prepared by way of the chloroaziridinyl intermediate XVII.

TABLE II

PHOSPHOROTRIAMIDATE MUSTARDS



No.	R	R′	R″
XVIII	$(ClCH_2CH_2)_2N-$	$-NH_2$	
XIX	$(ClCH_2CH_2)_2N-$		
XX	$(ClCH_2CH_2)_2N-$		

The phosphorodiamidates XXI through XXXIV, XLVII, L and LI listed in Table III were prepared by sequence of reactions analogous to those above by way of the intermediate chlorophosphoramide esters formed by condensation of the particular dichlorophosphoramide with the appropriate carbinol or phenol. The very useful although unstable intermediate chlorobenzylphosphoramide from which compounds XXI through XXVI, L and LI were derived was obtained by condensation of the dichlorophosphoramide of norHN2 with one mole of sodium benzyloxide. The indicated products were formed by condensation of these chloroesters with the various amines used in excess or with one mole of the particular amine in the presence of triethylamine. The phosphoramidic mustard diesters XXXV and XXXVI listed in Table IV were prepared by sequential replacement of chlorine atoms by the groups indicated.

The phosphorodiamidic acid mustards XXXVII through XLIII, LII and LIII were prepared by hydrogenolysis of either the phenyl or benzyl ester precursors. The phosphoramidic acid monoester XLV was prepared by an alternate method in which the chlorophosphoramide phenyl ester was condensed with triethylammonium acetate to give the acetylphosphoramide from which the product was obtained on treatment with cyclohexylamine. Compound XLV had been prepared previously by selective hydrogenolysis of the benzylphenyl diester with palladium.[2]

The symmetrical diphenyl XLVIII and dibenzyl XLIX, pyrophosphodiamide derivatives of norHN2 illustrated in Table VII were prepared by treatment of the intermediate chloroesters with small amounts of water in the presence of a base such as lutidine or triethylamine.

Biological Data.—The results of biological testing of some of these compounds at the Cancer Chemotherapy National Service Centre, NIH, are summarized in Table VIII. The seven compounds listed are structurally related in the sense that they are either phosphorodiamides containing the propanolamino grouping or they are isologs of cyclophosphamide. Although the tumor system used here, L1210 leukemia in BDF_1 mice, is generally responsive to the more "active" alkylating agents, it is possibly significant that only cyclophosphamide (IV) and cytoxyl alcohol (XXXVII) (a primary hydrolytic product that would derive from cyclophosphamide), showed substantial activity, whereas the other compounds which are structural variants of these two were completely inactive.

A summary of some preliminary data from the primary screening program at the Children's Cancer Research Foundation, Boston, on ten of these compounds shown in Table IX essentially corroborates the CCNSC results with regard to the L1210 leukemia system. These results indicate, in addition, that four of the compounds including cyclophosphamide (IV) the phosphorotriamidate mustard XVIII and the two phosphorodiamidic acid mustards XXXVII and XXXVIII are very active inhibitors of the other three tumor systems generally. An assessment of structure-activity relationships in this series may be possible when the results of testing of several of the other compounds now in progress become available.

Cyclophosphamide, IV, already has undergone very extensive clinical study. The other three, XVIII, XXXVII and XXXVIII, are in the process of preclinical pharmacologic evaluation at the Children's Cancer Research Foundation, Inc.

Experimental[12]

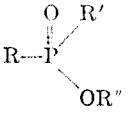
The reagents and solvents used in these preparations including dioxane, benzene, triethylamine and propanolamine were dried carefully and distilled just prior to use.

1,3,2-Diazaphosphorine, 2-[Bis(2-chloroethyl)amino]pentahydro-, 2-Oxide (II).—A solution of 3 g. of the dichlorophosphoramide (I) in 30 ml. of dioxane was added dropwise to a stirred

(12) Microanalyses by Dr. Carol Fitz, Needham, Mass.; all melting points are uncorrected.

TABLE III

PHOSPHORODIAMIDATE MUSTARDS



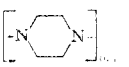
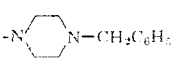
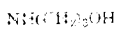
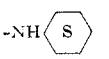
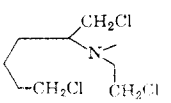
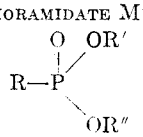
No.	R	R′	R″
XXI	$(ClCH_2CH_2)_2N$—	—NH_2	—$CH_2C_6H_5$
XXII	$(ClCH_2CH_2)_2N$—		—$CH_2C_6H_5$
XXIII	$(ClCH_2CH_2)_2N$—		—$CH_2C_6H_5$
XXIV	$(ClCH_2CH_2)_2N$—		—$CH_2C_6H_5$
XXV	$(ClCH_2CH_2)_2N$—		—$CH_2C_6H_5$
XXVI	$(ClCH_2CH_2)_2N$—		—$CH_2C_6H_5$
L	$(ClCH_2CH_2)_2N$—		—$CH_2C_6H_5$
LI	$(ClCH_2CH_2)_2N$—		—$CH_2C_6H_5$
XXVII	$(ClCH_2CH_2)_2N$—		—C_6H_5
XXVIII	$(ClCH_2CH_2)_2N$—		—C_6H_5
XXIX	$(ClCH_2CH_2)_2N$—		—CH_3
XXX	$(ClCH_2CH_2)_2N$—		—C_2H_5
XXXI	$(ClCH_2CH_2)_2N$—		—CH_3
XXXII	$(ClCH_2CH_2)_2N$—		—C_2H_5
XXXIII	$(ClCH_2CH_2)_2N$—	—NH_2	—CH_2CH_2Cl
XXXIV	$(ClCH_2CH_2)_2N$—		—CH_2CH_2Cl
XLVII		—$NH(CH_2)_3OH$	—C_6H_5

TABLE IV

PHOSPHORAMIDATE MUSTARDS



No.	R	R′	R″
XXXV	$(ClCH_2CH_2)_2N$—	—$(CH_2)_3NHCO_2CH_2C_6H_5$	—$CH_2C_6H_5$
XXXVI	$(ClCH_2CH_2)_2N$—	—CH_2CH_2Cl	—C_6H_5

solution of 0.87 ml. of 1,3-diaminopropane and 4.5 ml. of triethylamine in 15 ml. of dioxane. Stirring was continued for an additional hr. and the mixture was stored at 4° overnight. After removal of the precipitated triethylamine hydrochloride, the dioxane was evaporated, leaving an oil which was taken up in acetone-ether (1:2). Filtration and evaporation of the solvent gave 1.6 g. (53%) of white crystalline product, m.p. 106–107°. Recrystallization from acetone-petroleum ether raised the m.p. to 109–110°.

Anal. Calcd. for $C_7H_{16}PCl_2N_3O$: C, 32.31; H, 6.15; N, 16.15; P, 11.92; Cl, 27.31. Found: C, 32.65; H, 6.17; N, 16.22; P, 11.86; Cl, 27.60.

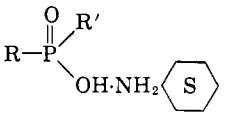
2H-1,3,2-Dioxaphosphorine, 2-[Bis(2-chloroethyl)amino]trihydro-, 2-Oxide (III).—A solution of 14 g. of the dichlorophosphoramide I in 50 ml. of dioxane was added dropwise over a period of 1.5 hr. to a solution of 4.5 ml. of 1,3-dihydroxypropane and 20.5 ml. of triethylamine in 100 ml. of dioxane. Reflux was maintained during and for 2 hr. after addition. After standing overnight the precipitated triethylamine hydrochloride was removed by filtration. The dioxane was evaporated leaving an oil which was taken up in benzene, washed 4 times with water, dried and evaporated. The residual oil on refrigeration yielded 7.8 g. (55%) of product as a thick semi-crystalline syrup.

Anal. Calcd. for $C_7H_{14}Cl_2NO_3$: C, 32.06; H, 5.34; N, 5.34; P, 11.83; Cl, 27.10. Found: C, 31.73; H, 5.65; N, 5.43; P, 11.75; Cl, 27.0.

2H-1,3,2-Oxazaphosphorine, 2-[Bis(2-chloroethyl)amino]tetrahydro-, 2-Oxide (IV).—A solution of 0.3 ml. of propanolamine and 1.5 ml. of triethylamine in 4 ml. of dioxane was added dropwise to a stirred solution of 1 g. of the dichlorophosphoramide (I) in 10 ml. of dioxane. After standing overnight the precipitated triethylamine hydrochloride was removed by filtration. The dioxane was evaporated leaving an oily residue which on trituration with petroleum ether–benzene (3:1) solution with which a crystal of ice was added yielded 0.55 g. (55.5%) of white crystalline product, m.p. 42–45°. This material was identical with

Table V

Phosphorodiamidic Acid Mustards



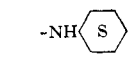
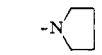
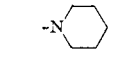
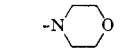
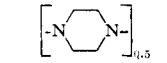
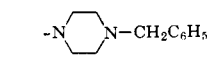
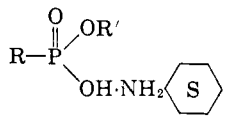
No.	R	R′
XXXVII	$(ClCH_2CH_2)_2N$—	—$NH(CH_2)_3OH$
XXXVIII	$(ClCH_2CH_2)_2N$—	—NH_2
XXXIX	$(ClCH_2CH_2)_2N$—	
XL	$(ClCH_2CH_2)_2N$—	
XLI	$(ClCH_2CH_2)_2N$—	
XLII	$(ClCH_2CH_2)_2N$—	
XLIII	$(ClCH_2CH_2)_2N$—	
LII	$(ClCH_2CH_2)_2N$—	
LIII	$(ClCH_2CH_2)_2N$—	

Table VI

Phosphoramidic Acid Ester Mustards



No.	R	R′
XLV	$(ClCH_2CH_2)_2N$—	—C_6H_5

respect to m.p., mixture m.p., and infrared spectrum with a sample of the compound kindly supplied by the CCNSC, National Institutes of Health, USPHS.

2H-1,3,2-Oxazaphosphorine, 2-[N-2-Chloroethyl-N-5-chloro-(1-chloromethyl)pentylamino]tetrahydro-, 2-Oxide (VI).—A solution of 4 ml. of propanolamine and 12 ml. of triethylamine in 20 ml. of dry benzene and 10 ml. of chloroform was added dropwise to a stirred solution of 12.6 g. of the dichlorophosphoramide (V) in 50 ml. of dry benzene. Stirring was continued for an additional 1.5 hr. After standing overnight at room temperature the precipitated triethylamine hydrochloride was filtered off. The filtrate after treatment with charcoal was washed 4 times with a total of 75 ml. of water and dried over sodium sulfate. Evaporation of the solvent gave 9 g. (72%) of oily product.

Anal. Calcd. for $C_{11}H_{22}Cl_3N_2O_2P$: N, 7.95; Cl, 30.3. Found: N, 7.7; Cl, 29.6.

2H-1,3,2-Oxazaphosphorine, 2-(N-2-Chloroethyl-N-3,5-dichloropentylamino)tetrahydro-, 2-Oxide (VIII).—A solution of 6.5 ml. of propanolamine and 19.5 ml. of triethylamine in 30 ml. of dry benzene and 10 ml. of chloroform was added dropwise to a stirred solution of 18.5 g. of the dichlorophosphoramide (VII) in 200 ml. of dry benzene. Reflux was maintained during and for 3 hr. after addition. After standing overnight the precipitated triethylamine hydrochloride was removed by filtration. The filtrate was evaporated, and the residual oil treated with charcoal in a benzene solution. Evaporation gave 12.5 g. (67%) of oily product.

Anal. Calcd. for $C_{10}H_{20}Cl_3O_3N_2P \cdot H_2O$: N, 7.88; Cl, 29.96. Found: N, 7.8; Cl, 30.6.

Benzyl-di-(2-chloroethyl)-phosphoramidic Chloride (X).—Sodium hydride suspension (53.5%, 4.48 g.) was washed 3 times with petroleum ether to remove the mineral oil. A solution of 10.29 ml. of benzyl alcohol in 25 ml. of dry benzene was added dropwise to a stirred suspension of the pure sodium hydride in 70 ml. of dry benzene, ice-cooled and under anhydrous conditions. The reaction mixture was allowed to stir at 5° overnight. The sodium benzylate suspension thus obtained was added dropwise and anhydrously over a period of 1 hr. to a solution of 25.9 g. of the dichlorophosphoramide (I) in 100 ml. of dry benzene with stirring and cooling in ice. After an additional 2 hr. of stirring, the sodium chloride was filtered off in a large Büchner funnel. This process was accelerated by the addition of 200 ml. of ether to the mixture in the Büchner. The filtrate was evaporated and the residual oil was taken up in ether and treated with 10 g. of a 50% Nuchar–Norit mixture. Evaporation of the filtrate afforded 24 g. (72%) of oil which was stored in solid carbon dioxide, $n^{25}D$ 1.5300.

Anal. Calcd. for $C_{11}H_{15}Cl_3NO_2$: N, 4.24; Cl, 32.2. Found: N, 4.2; Cl, 32.4.

Benzyl-N,N′-di-(2-chloroethyl)-N′,N′-ethylene Phosphorodiamidate (XXIII).—To a solution of 0.99 g. of the benzyl phosphoramidic chloride (X) in 15 ml. of dry benzene was added quickly 0.63 ml. of triethylamine, and 0.155 ml. of ethylenimine was added dropwise to the mixture with stirring and ice cooling. The reaction was allowed to proceed to completion by stirring for an additional hr. and then stored at 5° overnight. The precipitated triethylamine hydrochloride was filtered off and washed with benzene. After treatment with Norit the filtrate was evaporated at reduced pressure, leaving 0.8 g. (78%) of product as a light oil, $n^{25}D$ 1.5305.

Anal. Calcd. for $C_{13}H_{19}Cl_2O_2N_2P$: C, 46.29; H, 5.64; N, 8.3; Cl, 21.07. Found: C, 46.45; H, 5.76; N, 8.2; Cl, 20.90.

Benzyl-N,N-di-(2-chloroethyl)-N′,N′-tetramethylene Phosphorodiamidate (XXIV).—This compound was prepared in essentially the same manner as (XXIII). Reaction of 3.3 g. of the benzyl chlorophosphoramide (X), 1.4 ml. of triethylamine and 0.835 ml. of pyrrolidine yielded 2.27 g. (62%) of oily product.

Anal. Calcd. for $C_{15}H_{23}Cl_2N_2O_2P$: N, 7.67; Cl, 19.45. Found: N, 7.60; Cl, 19.6.

Benzyl-N,N-di-(2-chloroethyl)-N′,N′-pentamethylene Phosphorodiamidate (XXV).—A solution of 1.98 ml. of piperidine in 30 ml. of dry benzene was added dropwise to a stirred solution of 3.3 g. of the benzyl phosphoramidic chloride (X) in 30 ml. of dry benzene. The reaction was allowed to proceed to completion by stirring for an additional 30 min. and then was stored at 5° overnight. The precipitated piperidine hydrochloride was filtered off and washed with benzene. The combined filtrate was evaporated at reduced pressure and the residual oil dissolved in ethyl ether and treated with 2 g. of a 50% Nuchar–Norit mixture. Evaporation of the filtrate afforded 2.7 g. (65%) of product as an oil.

Anal. Calcd. for $C_{16}H_{25}Cl_2N_2O_2P$: N, 7.38; Cl, 18.73. Found: N, 7.4; Cl, 18.9.

Benzyl-N,N-di-(2-chloroethyl)-N′,N′-(2,2-diethyleneoxido) Phosphorodiamidate (XXVI).—This compound was prepared in essentially the same manner as (XXV). Reaction of 1.74 ml. of morpholine and 3.3 g. of (X) yielded 2.85 g. (74%) of oily product.

Anal. Calcd. for $C_{15}H_{23}Cl_2N_2O_3P$: N, 7.35; Cl, 18.63. Found: N, 7.3; Cl, 18.80.

1,4-Bis-[benzyl-N,N-di-(2-chloroethyl)phosphorodiamidate]-piperazine (L).—This compound was also prepared in essentially the same manner as (XXV). Reaction of 0.77 g. of piperazine and 2.97 g. of the benzyl chlorophosphoramide (X) yielded 2.18 g. (36%) of an oil which was purified by treatment with Norit in acetone.

Anal. Calcd. for $C_{26}H_{38}Cl_4N_4O_4P_2$: N, 8.31; Cl, 21.07. Found: N, 8.00; Cl, 21.0.

Benzyl-N,N-di-(2-chloroethyl)-N′,N′-diethyleneaminobenzyl Phosphorodiamidate (LI).—This compound was prepared in essentially the same manner as (XXV). Monobenzylpiperazine (2.11 g.) and 1.98 g. of (X) yielded 2.10 g. (74%) of product as an oil.

Anal. Calcd. for $C_{22}H_{30}Cl_2N_3O_2P$: N, 8.94; Cl, 15.10. Found: N, 8.90; Cl, 15.0.

Benzyl-N,N-di-(2-chloroethyl)-N′-cyclohexyl Phosphorodiamidate (XXII).—This compound was prepared in essentially the same manner as (XXV). Reaction of 0.96 ml. of cyclohexylamine and 1.32 g. of the benzyl chlorophosphoramide (X) yielded an oily product which after dilution to cloudiness with petroleum ether gave on refrigeration 0.77 g. (49%) of white crystalline product, m.p. 85–86°.

Anal. Calcd. for $C_{17}H_{27}Cl_2N_2O_2P$: C, 51.91; H, 6.87; N, 7.12; Cl, 18.09. Found: C, 51.37; H, 7.0; N, 7.30; Cl, 18.00.

Benzyl-N,N-di-(2-chloroethyl) Phosphorodiamidate (XXI).—Ammonia gas was bubbled through a solution of 1.5 g. of the benzyl phosphoramidic chloride (X) in 50 ml. of dry benzene

cooled in ice for 2 hr. until the precipitation of ammonium chloride was complete. The ammonium chloride was separated by filtration. The residual oil obtained after evaporation of the filtrate was taken up in ether and treated with Norit. The resulting clear filtrate on evaporation afforded 1.07 g. (76%) of light-colored oil, $n^{24}D$ 1.5352.

Anal. Calcd. for $C_{11}H_{17}Cl_2N_2O_2P$: C, 42.44; H, 5.46; N, 9.0; Cl, 22.83. Found: C, 42.77; H, 5.59; N, 9.0; Cl, 22.80.

Cyclohexylammonium Hydrogen-N,N-di-(2-chloroethyl)-N′,N′-tetramethylene Phosphorodiamidate (XLI).—Hydrogenation of 1.10 g. of the benzyl phosphorodiamidate (XXIV) in 25 ml. of dioxane in the presence of 0.4 ml. of cyclohexylamine and 0.25 g. of palladium–charcoal at ice-bath temperature was complete within 30 min. with the absorption of 67 ml. of hydrogen. After filtration, the palladium and precipitated white solid were extracted with ethyl alcohol. Evaporation of the alcohol filtrate gave a solid product which was suspended in acetone and filtered yielding 0.38 g. (34%) of white crystalline product, m.p. 115–116°.

Anal. Calcd. for $C_{14}H_{30}Cl_2N_3O_2P$: N, 11.23; Cl, 18.98. Found: N, 11.23; Cl, 18.70.

Cyclohexylammonium Hydrogen-N,N-di-(2-chloroethyl)-N′,N′-pentamethylene Phosphorodiamidate (XLII).—This compound was prepared in a manner essentially similar to (XLI). The benzylpiperidine intermediate (XXV, 1.4 g.) took up 70 ml. of hydrogen in 25 min. The alcoholic filtrate yielded on evaporation 0.54 g. (33%) of white crystalline product, m.p. 120–122°.

Anal. Calcd. for $C_{15}H_{32}Cl_2N_3O_2P$: N, 10.8; Cl, 18.3. Found: N, 10.6; Cl, 18.1.

Cyclohexylammonium Hydrogen-N,N-di-(2-chloroethyl)-N′,N′-(2,2′-diethyleneoxido) Phosphorodiamidate (XLIII).—This compound was prepared in a manner similar to XLI. The benzylmorpholine intermediate (XXVI, 1.4 g.) absorbed 65 ml. of hydrogen in 15 min. The alcoholic filtrate gave on evaporation at reduced pressure a white solid, which was suspended in acetone and filtered; 0.31 g. (26%) of white crystalline product, m.p. 118–119°, was obtained.

Anal. Calcd. for $C_{14}H_{30}Cl_2N_3O_3P$: N, 10.77; Cl, 18.2. Found: N, 10.80; Cl, 18.0.

1,4-Bis[cyclohexylammonium Hydrogen-N,N-di-(2-chloroethyl) Phosphorodiamidate]-piperazine (LII).—Hydrogenation of 2 g. of the benzyl phosphoramide (XLIV) in the presence of 0.4 ml. of cyclohexylamine over 0.25 g. of palladium-charcoal in alcohol in the cold resulted in an uptake of 52 ml. of hydrogen in 20 min. The white solid that precipitated during the course of the reaction was leached from the catalyst by repeated washing with alcohol which on evaporation yielded 0.1 g. (5%) of white crystalline product, m.p. 138–140°.

Anal. Calcd. for $C_{24}H_{52}Cl_4N_6O_4P_2$: N, 12.14; Cl, 20.58. Found: N, 11.9; Cl, 20.5.

Cyclohexylammonium Hydrogen-N,N-di-(2-chloroethyl)-N′-cyclohexyl Phosphorodiamidate (XXXIX).—Hydrogenation of 0.5 g. of the benzyl phosphorodiamidate XXII in 25 ml. of absolute ethanol over 0.15 g. of palladium–charcoal in the cold was complete in 14 min. with the absorption of 32 ml. (100%) of hydrogen. The palladium was filtered off and 0.20 ml. of cyclohexylamine added to the filtrate. Evaporation afforded 0.24 g. (47%) of crystalline product, m.p. 110–112°. Purification of the crude material with charcoal in a chloroform solution raised the m.p. to 117–118°.

Anal. Calcd. for $C_{16}H_{34}Cl_2N_3O_2P$: C, 47.76; H, 8.46; N, 10.45; Cl, 17.66. Found: C, 47.7; H, 8.39; N, 10.50; Cl, 17.30.

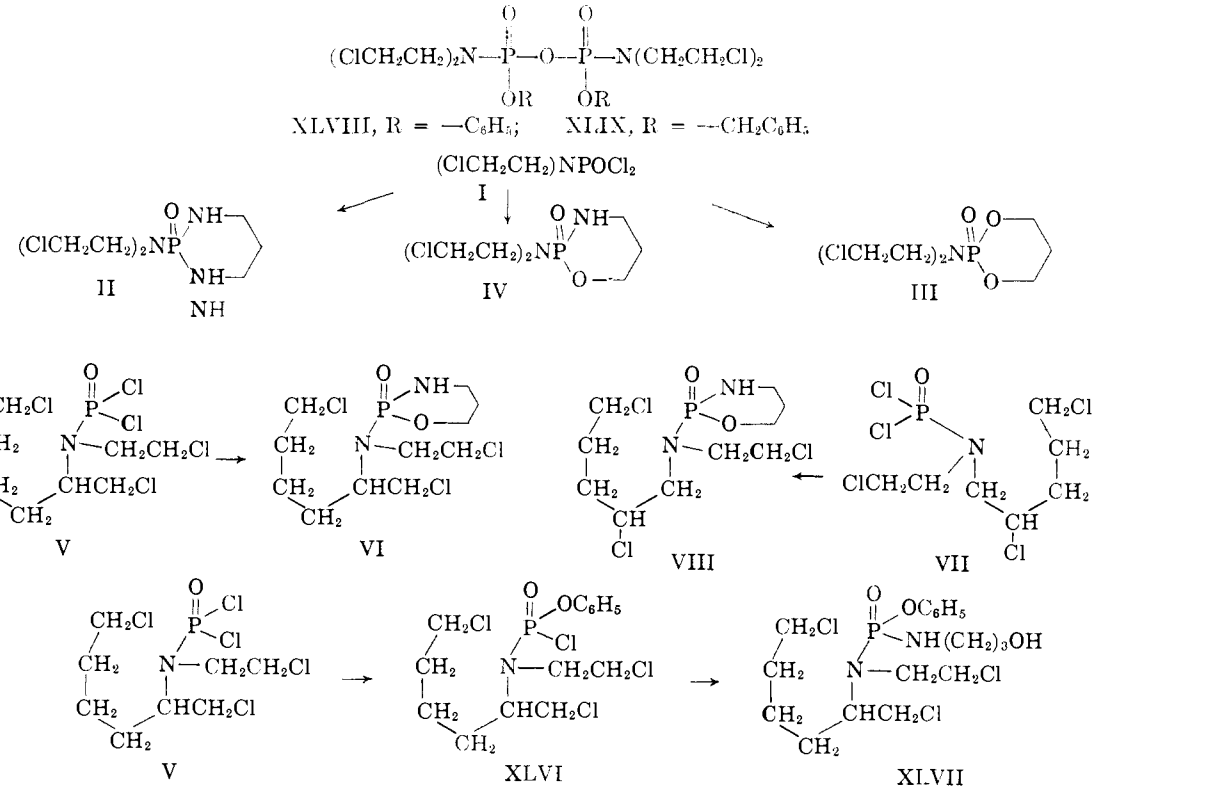
Cyclohexylammonium Hydrogen-N,N-di-(2-chloroethyl)-N′,N′-diethylenebenzylamino Phosphorodiamidate (LIII).—Hydrogenation of 2.35 g. of the monobenzylpiperazine intermediate (XXXIX) in absolute alcohol over 0.7 g. of palladium–charcoal in the cold was complete in 100 min. with the absorption of 93 ml. of hydrogen (86% of the amount required to replace one benzyl group). The palladium–charcoal was filtered off and 0.7 ml. of cyclohexylamine added to the filtrate. Evaporation at reduced pressure gave a yellowish solid residue which when stirred in acetone gave a white crystalline material, 0.32 g. (13%), m.p. 127–128°.

Anal. Calcd. for $C_{21}H_{43}Cl_2N_4O_2P$: N, 11.69; Cl, 14.8. Found: N, 11.50; Cl, 15.0.

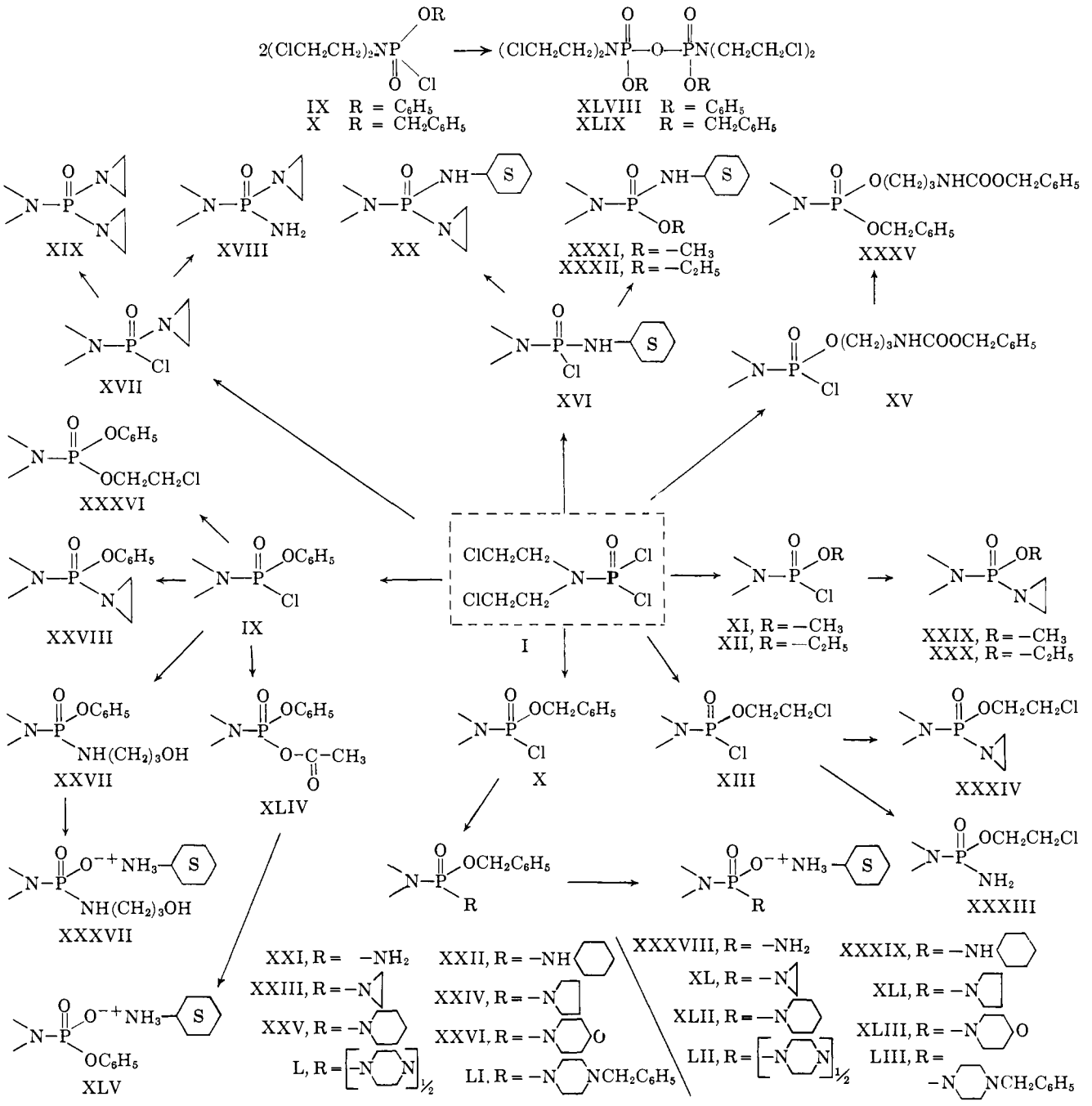
Cyclohexylammonium Hydrogen-N,N-di-(2-chloroethyl) Phosphorodiamidate (XXXVIII).—This compound was prepared by a procedure similar to that for XXXIX. The benzyl intermediate

TABLE VII

PYROPHOSPHORODIAMIDATE MUSTARDS



(continued)



(XXI, 2.6 g.) took up essentially the theoretical amount of hydrogen. After filtration the palladium and precipitated white solid were stirred in a solution of 1.1 ml. of cyclohexylamine in 50 ml. of ethyl alcohol for 30 min. The mixture was again filtered and the filtrate evaporated to give a solid product which was suspended in ether. On filtration 1.9 g. (51.2%) of white crystalline product, m.p. 107–108°, was obtained in pure form.

Anal. Calcd. for $C_{10}H_{24}Cl_2N_3O_2P$: C, 37.5; H, 7.50; N, 13.12; Cl, 22.18. Found: C, 37.06; H, 7.08; N, 13.10; Cl, 22.2.

Cyclohexylammonium Hydrogen-N,N-di-(2-chloroethyl)-N′,N′-ethylene Phosphorodiamidate (XL).—Hydrogenation of 0.8 g. of XXIII in 25 ml. of ethyl alcohol over 0.2 g. of palladium–charcoal in the cold was complete with the absorption of 106 ml. of hydrogen in 25 min. The palladium was filtered off and 0.75 ml. of cyclohexylamine added to the filtrate. Evaporation of the filtrate gave an oily residue which was taken up in ether and stored at 5° overnight. A precipitate, 0.26 g. (31.3%), of crystalline product, m.p. 103–105°, separated. The crude material, purified by treatment with Norit in methanol, melted at 110–112°.

Anal. Calcd. for $C_{12}H_{26}Cl_2N_3O_2$: N, 12.14; Cl, 20.52. Found: N, 11.9; Cl, 20.30.

N,N-Di-(2-Chloroethyl)-N′,N′-ethylene Phosphorotriamidate (XVIII).—Ethyleneimine (0.26 ml.) was added dropwise to a mixture of 1.3 g. of the dichlorophosphoramide I and 0.7 ml. of triethylamine in 20 ml. of dry benzene with stirring and ice cooling. After refrigeration for 1 hr. the precipitated triethylamine hydrochloride was filtered off. Ammonia gas was bubbled through the cooled filtrate for 2 hr. when the precipitated ammonium chloride was separated by filtration and petroleum ether added to the oily residue obtained after evaporation. The petroleum ether was removed by evaporation leaving a solid residue which when stirred with ether gave 0.22 g. (17.6%) of crystalline material, m.p. 83–85°.

Anal. Calcd. for $C_6H_{14}Cl_2N_3O$: C, 29.27; H, 5.69; N, 17.08; Cl, 28.86. Found: C, 29.24; H, 5.58; N, 16.9; Cl, 28.7.

Methyl-N,N-di-(2-chloroethyl)-N′,N′-ethylene Phosphorodiamidate (XXIX).—The intermediate methyl phosphoramidic chloride XI was prepared in the same manner as the benzyl phosphoramidic chloride X. Reaction of 4.5 g. of a sodium hydride suspension (53.5%), 4.0 ml. of methyl alcohol and 25.9 g. of the dichlorophosphoramide (I) gave 14.8 g. (62%) of **methyl-di-(2-chloroethyl)-phosphoramidic choride (XI)** as an oil. To a stirred solution of 1.27 g. of crude XI and 0.7 ml. of tri-

TABLE VIII

CCNSC SCREENING DATA SUMMARY[a] ON ANTITUMOR ACTIVITY OF SOME PHOSPHORAMIDE NITROGEN MUSTARDS AGAINST L1210 LEUKEMIA IN BDF_1 MICE[b]

Compound	Daily dose, mg./kg.	Survivors	Response: Test/Control (T/C) Animal wt. change, g.	Survival, days	Per cent
IV	100	7/7	−3.3/−0.2	11.1/9.2	121
	50	7/7	−1.6/−0.2	16.2/9.2	176
	25	7/7	−0.6/−0.2	12.0/9.2	130
	12	7/7	0.0/−0.2	13.0/9.2	141
II	100	6/6	−1.7/0.5	10.0/9.5	105
	50	6/6	−0.4/0.5	9.7/9.5	102
	75	6/6	0.7/0.5	9.0/9.5	94
III	100	6/6	1.9/0.5	10.4/9.5	109
	50	5/6	0.9/0.5	8.7/9.5	91
	25	6/6	0.6/0.5	8.7/9.5	71
VI	50	6/6	0.3/0.9	10.5/9.5	110
	25	6/6	1.0/0.9	10.6/9.5	111
	12.5	6/6	0.8/0.9	10.0/9.5	105
VIII	200	6/6	−0.3/0.9	11.2/9.5	117
	100	6/6	0.8/0.9	9.8/9.5	103
	50	6/6	1.2/0.9	9.7/9.5	102
XXXVII	100	6/6	−2.5/0.7	13.8/8.9	155
	50	6/6	−1.2/0.7	11.3/8.9	126
	25	6/6	−0.1/0.7	11.3/8.9	126
	12.5	6/6	−0.3/0.7	10.5/8.9	117
XXVII	1000	6/6	−0.9/−0.5	9.0/8.9	101
	500	6/6	0.6/0.8	9.8/9.6	102
	250	6/6	0.4/0.8	9.8/9.6	102

[a] Kindly supplied by Dr. Joseph E. Leiter, CCNSC, National Institutes of Health, Bethesda, Md. [b] Administered intraperitoneally in saline. Assays for activity were performed according to specifications established by the CCNSC (*Cancer Chemotherapy Reports*, **1**, 42 (1959), Cancer Chemotherapy National Service Centre, National Cancer Institute, Bethesda, Md.).

ethylamine in 25 ml. of dry benzene, 0.26 ml. of ethyleneimine in 15 ml. of dry benzene was added dropwise. After standing overnight at 5° the triethylamine hydrochloride was removed by filtration. The benzene was evaporated and purification of the residue with charcoal in an ether solution gave 0.7 g. (50%) of product as an oil.

Anal. Calcd. for $C_{10}H_{15}Cl_2N_2O_2$: N, 10.73; Cl, 27.2. Found: N, 10.6; Cl, 27.0.

Ethyl-N,N-di-(2-chloroethyl)-N′N′-ethylene Phosphorodiamidate (XXX).—The intermediate ethyl phosphoramidic chloride (XII) was prepared in the same manner as the benzyl phosphoramidic chloride (X). Reaction of 4.5 g. of a sodium hydride suspension (53.5%), 5.8 ml. of ethyl alcohol and 25.9 g. of the dichlorophosphoramide (I) gave 15.1 g. (60%) of **ethyl-di-(2-chloroethyl)-phosphoramidic chloride (XII)** as an oil. To a stirred solution of 1.34 g. of the crude product (XII) and 0.7 ml. of triethylamine in 25 ml. of dry benzene, 0.26 ml. of ethyleneimine was added dropwise. After standing overnight at 5° the triethylamine hydrochloride was removed by filtration. The benzene was evaporated and purification of the residue with charcoal in an ether solution gave 0.75 g. (54%) of oily product, $n^{25}D$ 1.4850.

Anal. Calcd. for $C_{11}H_{17}Cl_2N_2O_2P$: N, 10.16; Cl, 25.8. Found: N, 10.0; Cl, 25.5.

N-Carbobenzoxypropanolamine (XIV).—A cooled solution of 10 g. of carbobenzoxy chloride in 20 ml. of chloroform was added dropwise to a solution of 11 ml. of propanolamine in 50 ml. of chloroform with stirring and cooling in ice. After standing overnight the mixture was washed with water, dilute hydrochloric acid, dilute potassium carbonate and again with water. The chloroform layer was dried over sodium sulfate and evaporated. The oily residue obtained on dilution with petroleum ether gave a crystalline product, m.p. 48–51°. Recrystallization from chloroform–petroleum ether (1:4) yielded 5.5 g. (45%), m.p. 53–55°.

Anal. Calcd. for $C_{11}H_{15}NO_3$: C, 63.16; H, 7.17; N, 6.69. Found: C, 63.1; H, 7.1; N, 6.7.

Benzyl-N-carbobenzoxyaminopropyl-di-(2-chloroethyl)-phosphoramidate (XXXV).—A solution of 2.09 g. of carbobenzoxypropanolamine in 40 ml. of benzene was added to 0.44 g. of sodium hydride suspension (53.5%). After stirring for 2 hr., 2.59 g. of the dichlorophosphoramide (I) was added to the mixture and the stirring was continued for an additional 70 min. The precipitated sodium chloride was removed by filtration and the filtrate treated with charcoal. The benzene was removed by evaporation, leaving an oily residue which was washed three times with petroleum ether to remove the mineral oil. The residue was treated with charcoal in a benzene solution and evaporation gave 2.95 g. (68.4%) of **N-carbobenzoxyaminopropyl-di-(2-chloroethyl)-phosphoramidic chloride (XV)** as an oil. A solution of 22 g. of crude XV in 100 ml. of dry benzene was added dropwise to a suspension of 6.6 g. of sodium benzyloxide (preparation previously recorded in X) in 100 ml. of dry benzene with stirring and cooling in ice. The stirring was continued for an additional 2 hr. and the mixture was stored at 5° overnight. The benzene was evaporated leaving an oily residue which was taken up in ether. After separation of the sodium chloride precipitate by centrifugation and filtration, the ether was evaporated leaving 14.3 g. (55%) of oily product.

Anal. Calcd. for $C_{22}H_{29}Cl_2N_2O_5P$: N, 5.56; Cl, 14.11. Found: N, 5.5; Cl, 14.0.

2-Chloroethyl-N,N-di-(2-chloroethyl) Phosphorodiamidate (XXXIII).—A solution of 3.32 ml. of chloroethanol in 15 ml. of dry benzene was added dropwise to a stirred solution of 12.95 g. of the dichlorophosphoramide (I) and 6.99 ml. of triethylamine in

TABLE IX[a]

CCRF RESULTS IN PRIMARY TUMOR SCREEN WITH SOME PHOSPHORAMIDE MUSTARDS[b]

Compound	L1210 Survival increase %		P1534 Survival increase %		P1534 Tumor inhibition %		C1498 Survival increase %		C1498 Tumor inhibition %		DBRB Survival increase %		DBRB Tumor inhibition %	
IV	+51[c]	(20)[d]	+41	(5)	+100	(5)	+96	(20)	+100	(20)	+71	(5)	+100	(5)
VIII	+24	(80)									+23	(80)	+22	(80)
VI	+28	(40)	−15	(10)	+51	(10)					Toxic			
XXXVII	+47	(80)	−6	(80)	+100	(80)	−1	(20)	+30	(20)	−17	(20)	+72	(20)
XXVII	−20	(640)									+22	(160)	+10	(160)
III	+36	(320)									±0	(160)	+43	(160)
II	+4	(20)	−8	(20)	+14	(20)					+54	(5)	−4	(5)
XXXIX	+7	(20)	−16	(20)	+69	(20)	+8	(20)	+8	(20)	+44	(20)	+35	(20)
XVIII	+48	(20)	−9	(80)	+100	(80)	+2	(20)	+94	(20)	+67	(5)	+90	(20)
XXXVIII	+112	(80)	+46	(20)	+100	(20)	+13	(20)	+72	(20)	+69	(20)	+100	(20)

[a] We are indebted to Dr. Sidney Farber, Director of the Children's Cancer Research Foundation, Inc., Boston, for kind permission to publish these preliminary results, and to Dr. Charlotte L. Maddock of the Foundation for these data and for the preparation of this summary. [b] Tumors utilized by CCRF are L1210, an ascitic lymphatic leukemia in BDF1 or AKD2F1 mice; P 1534, a lymphatic leukemia in DBA/2 mice; C1498, a myelogenous leukemia in C57B1/6 mice, and DBRB, a mammary gland adenocarcinoma in DBA/1 mice. [c] Survival increases above 20% and tumor inhibitions above 50% denote significant anti-tumor activity. [d] Numbers in parenthese refer to the daily dose in mg./kg. given intraperitoneally.

25 ml. of benzene. After standing overnight at 5° the triethylamine hydrochloride was removed by filtration. The residue obtained after evaporation of the benzene was purified by charcoal in an ether solution. Evaporation of the solvent gave 8.9 g. (56%) of **2-chloroethyl-di-(2-chloroethyl)-phosphoramidic chloride (XIII)** as an oil, $n^{25}D$ 1.5020. Ammonia gas was bubbled through a solution of 1.52 g. of crude XIII in 100 ml. of dry benzene for 2 hr. until the precipitation of ammonium chloride was complete. The ammonium chloride was removed by filtration. The oily residue obtained after evaporation of the benzene was treated with charcoal in an ether solution. Evaporation of the solvent gave 0.9 g. (63%) of oily product, $n^{25}D$ 1.5010.

Anal. Calcd. for $C_6H_{14}Cl_3N_2O_2P$: N, 9.88; Cl, 37.56. Found: N, 9.70; Cl, 36.70.

Methyl-N,N-di-(2-chloroethyl)-N′-cyclohexyl Phosphorodiamidate (XXXI).—A solution of 5.4 ml. of triethylamine in 10 ml. of dry benzene was added dropwise to a stirred and cooled solution of 10 g. of the dichlorophosphoramide (I) in 60 ml. of dry benzene, followed by the dropwise addition of a solution of 4.64 ml. of cyclohexylamine in 10 ml. of dry benzene. The reaction was allowed to proceed to completion by stirring for an additional hr. and the mixture was then stored at 5° overnight. After removal of the precipitated triethylamine hydrochloride the filtrate was treated with a 50% Nuchar–Norit mixture. The benzene was removed by evaporation leaving 9.6 g. (78%) of **N,N-di-(2-chloroethyl)-N′-cyclohexyl phosphorodiamidate chloride (XVI)** as a light oil which was stored in Dry Ice, $n^{25}D$ 1.5165. To a solution of 1.28 g. of crude XVI in 16 ml. of methanol 0.54 ml. of triethylamine was added and the mixture was stored at 5° for 40 hr. After evaporation and addition of ether to the residue, precipitated triethylamine hydrochloride was removed by filtration. The filtrate was freed from ether at reduced pressure, the oily residue was taken up in 25 ml. of fresh methanol and 0.3 ml. of triethylamine and refluxed for 2 hr. to complete the reaction. Evaporation at reduced pressure and addition of ether to the oily residue gave an additional precipitation of triethylamine hydrochloride. The filtrate, after treatment with charcoal, gave on evaporation 0.6 g. (48%) of oily product, $n^{25}D$ 1.4940.

Anal. Calcd. for $C_{11}H_{23}Cl_2N_2O_2P$: N, 8.83; Cl, 22.4. Found: N, 8.70; Cl, 22.0.

Ethyl-N,N-di-(2-chloroethyl)-N′-cyclohexyl Phosphorodiamidate (XXXII).—This compound was prepared in essentially the same manner as (XXXI). Reaction of 1.6 g. of the phosphoramidic chloride (XVI) in ethanol yielded 1.33 g. (80%) of oily product, $n^{25}D$ 1.5130.

Anal. Calcd. for $C_{12}H_{25}Cl_2N_2O_2P$: N, 8.45; Cl, 21.44. Found: N, 8.40; Cl, 21.70.

N,N-Di-(2-chloroethyl)-N′,N″-diethylene Phosphorotriamidate (XIX).—A solution of 0.52 ml. of ethylenimine in 15 ml. of dry benzene was added dropwise to a stirred and cooled solution of 1.3 g. of the dichlorophosphoramide (I) and 1.5 ml. of triethylamine in 25 ml. of benzene. After standing overnight at 5°, the triethylamine hydrochloride was removed by filtration. The residue obtained after evaporation of the benzene was purified with charcoal in an ether–acetone solution. Evaporation of the solvent gave 0.94 g. (70%) of product as an oil.

Anal. Calcd. for $C_8H_{16}Cl_2N_3OP$: C, 35.99; H, 5.88; N, 15.4; Cl, 26.1. Found: C, 35.28; H, 6.03; N, 14.8; Cl, 26.0.

Phenyl-N,N-di-(2-chloroethyl)-N′,N′-ethylene Phosphorodiamidate (XXVIII).—This compound was prepared in the same manner as XIX. Reaction of 0.26 ml. of ethylenimine, 0.75 ml. of triethylamine and 1.58 g. of the phenyl phosphoramidic chloride (IX) yielded 1.35 g. (83%) of oily product after purification of the benzene filtrate with charcoal, $n^{25}D$ 1.5330.

Anal. Calcd. for $C_{12}H_{17}Cl_2N_2O_2P$: C, 44.58; H, 5.26; N, 8.67; Cl, 21.98. Found: C, 44.47; H, 5.32; N, 8.50; Cl, 22.0.

2-Chloroethyl-N,N-di-(2-chloroethyl)-N′,N′-ethylene Phosphorodiamidate (XXXIV).—This compound was also prepared in the same manner as XIX. Reaction of 0.26 ml. of ethylenimine, 0.75 ml. of triethylamine with 1.515 g. of the chlorophosphoramide ester XIII—prepared according to the procedure given for XXXIII—yielded 0.6 g. (39%) of oily product after purification with charcoal in an ether solution.

Anal. Calcd. for $C_{11}H_{16}Cl_2N_2O_2P$: N, 9.05; Cl, 34.41. Found: N, 9.12; Cl, 34.0.

N,N-Di-(2-chloroethyl)-N′,N′-ethylene-N″-cyclohexyl Phosphorotriamidate (XX).—This compound was prepared in the same manner as XIX except that the addition was carried out at room temperature. Reaction of 0.26 ml. of ethylenimine, 0.7 ml. of triethylamine and 1.6 g. of the chlorophosphorodiamidate (XVI)—prepared according to the procedure for XXXI—yielded 1.27 g. (77%) of oily product, $n^{25}D$ 1.5055.

Anal. Calcd. for $C_{12}H_{24}Cl_2N_3OP$: N, 12.8; Cl, 21.65. Found: N, 12.3; Cl, 21.40.

2-Chloroethyl-phenyl-di-(2-chloroethyl) Phosphoramidate (XXXVI).—This compound was prepared by a procedure similar to that for XX in that reaction was carried out at room temperature. Reaction of 10 ml. of 2-chloroethanol, 5.67 g. of the phenyl phosphoramidic chloride (IX) and 2.63 ml. of triethylamine yielded 4.4 g. (65%) of oily product, after purification with charcoal in ether.

Anal. Calcd. for $C_{12}H_{17}Cl_3NO_3P$: N, 3.88; Cl, 29.54. Found: N, 4.0; Cl, 29.40.

Cyclohexylammonium Hydrogen-N,N-di-(2-chloroethyl)-N′-3-hydroxypropyl Phosphorodiamidate (XXXVII). A. From the Phenyl Phosphoramidic Chloride, IX.—The phenyl phosphoramidic chloride (IX, 34 g.) and 68 ml. of propanolamine were mixed and ice cooled. After standing at room temperature for 5 hr., the mixture was diluted with benzene, washed with water and dried over sodium sulfate. The benzene was removed by evaporation, leaving an oily residue which gave on refrigeration crude **phenyl-N,N-di-(2-chloroethyl)-N′-3-hydroxypropyl phosphorodiamidate (XXVII)** as a crystalline product, m.p. 55–58°. Hydrogenation of 2.0 g. of XXVII in 20 ml. of anhydrous ethanol containing 0.16 ml. (5 drops) of concd. hydrochloric acid over 0.8 g. of platinum oxide at 0° was complete in 2 hr. and 11 min. with uptake of 681 ml. of hydrogen at 10 cm. overpressure. The grey precipitate containing platinum oxide and the product was filtered, washed repeatedly with ether and stirred for 15 min. at 0° in 500 ml. of ethanol and filtered. Cyclohexylamine (3 ml.) was added to the filtrate. Evaporation at reduced pressure yielded a white crystalline material, which was suspended in ether, filtered and washed with ether repeatedly, 1.46 g. (70%), m.p. 116–116.5°.

Anal. Calcd. for $C_{13}H_{30}Cl_2N_3O_3P$: C, 41.27; H, 7.93; N, 11.11; Cl, 18.78; P, 8.20. Found: C, 41.33; H, 8.35; N, 11.0; Cl, 18.0; P, 8.70.

B. From the Benzyl Phosphoramidic Chloride X.—The chlorophosphoramide (X, 6 g.) was treated with a total of 15 ml. of freshly distilled 3-aminopropanol in small portions with stirring and ice cooling. The very viscous syrupy mixture was stirred for 2 more hr. and refrigerated overnight. The reaction mixture was shaken with 250 ml. of benzene and 200 ml. of water and the benzene layer extracted twice with a total of 300 ml. of water. After drying over sodium sulfate and purification with Norit, evaporation at reduced pressure yielded the intermediate **monobenzyldi(2-chloroethyl)-3-hydroxopropyl phosphorodiamidate** as a straw-colored oil, 3.3 g. (49.3%), $n^{24}D$ 1.5212. Hydrogenation of 3.3 g. of this intermediate in 15 ml. of absolute alcohol over 0.6 g. of palladium–charcoal in the cold at a slight overpressure of hydrogen was completed in 50 min. with absorption of 213 ml. of hydrogen. After filtration, the palladium–charcoal and the precipitated white solid were extracted with 200 ml. of alcohol, containing 0.8 ml. of cyclohexylamine, by stirring for 30 min. Evaporation of the alcoholic filtrate gave a white solid, which was suspended in ether and filtered, 1.81 g. (48.7%), m.p. 112–113.5°.

Anal. Calcd. for $C_{13}H_{30}Cl_2N_3O_3P$: N, 11.11; Cl, 18.78. Found: N, 10.9; Cl, 18.1.

Phenyl-N-2-chloroethyl-N-5-chloro(1-chloromethyl)pentyl-N-3-hydroxypropyl Phosphorodiamidate (XLVII).—This compound was prepared by a procedure similar to that described for XXVII. Reaction of 2.7 g. of the phenyl phosphoramidic chloride XLVI with 5.5 ml. of propanolamine yielded 1.9 g. (65%) of product as an oil.

Anal. Calcd. for $C_{17}H_{28}Cl_3N_2O_3P$: N, 6.29; Cl, 23.9. Found: N, 6.40; Cl, 23.6.

Cyclohexylammonium Phenyl Hydrogen-di-(2-chloroethyl) Phosphoramidate (XLV).—Glacial acetic acid (0.115 ml.) was added to a mixture of 0.63 g. of the phenyl phosphoramidic chloride IX and 0.28 ml. of triethylamine in 20 ml. of benzene. Reflux was maintained by heating for 3.5 hr. After removal of the triethylamine hydrochloride by filtration 0.55 ml. of cyclohexylamine was added to the filtrate and reflux was maintained for a further 2 hr. Crude product, m.p. 162–165° (0.38 g., 49%), was obtained by filtration of the hot reaction mixture. Trituration with acetone and recrystallization from methanol–ethyl acetate gave a white crystalline product, m.p. 172–174°, which showed no depression of melting point when mixed with the compound previously prepared.[11a]

sym-**Dibenzyl-N,N,N′,N′-tetrakis-(2-chloroethyl) Pyrophosphorodiamidate (XLIX).**—A mixture of 0.99 g. of the benzyl phosphoramidic chloride X, 0.34 ml. of 2,6-lutidine and 0.03 ml. of water was agitated until solid separated from the mixture. After refrigeration overnight and dilution with ether the oily solid precipitate that formed was removed by filtration. The filtrate was evaporated leaving an oily residue which after washing several times with petroleum ether was taken up in ether. The ether extracts treated with Norit after evaporation gave 0.62 g. of oily product, $n^{25}D$ 1.5320.

Anal. Calcd. for $C_{22}H_{30}Cl_4N_2O_5P_2$: N, 4.62; Cl, 23.43. Found: N, 4.7; Cl, 23.70.

sym-**Diphenyl-N,N,N′,N′-tetrakis-(2-chloroethyl) Pyrophosphorodiamidate (XLVIII).**—This compound was prepared in the same manner as XLIX. Reaction of 1 g. of the phenyl phosphoramidic chloride (IX), 0.36 ml. of 2,6-lutidine and 0.057 ml. of water yielded 0.86 g. (48%) of oily product.

Anal. Calcd. for $C_{20}H_{26}Cl_4N_2O_5P_2$: C, 41.52; H, 4.5; N, 4.84; P, 10.73; Cl, 24.57. Found: C, 41.98; H, 4.62; N, 4.74; P, 10.42; Cl, 23.84.

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2-Amino-5-pyrimidinesulfonamides

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2-Amino-5-pyrimidinesulfonamides have been prepared from 2-(5-chlorosulfonylpyrimidyl)-phosphoramidic dichloride, readily obtainable from 2-aminopyrimidine in two steps.

When 2-chloro-5-pyrimidinesulfonyl chloride, recently described by Caldwell and Jaffe,[1] was treated with ammonia or amines, both halogens were replaced; hence this intermediate is not suited to the preparation of compounds of the type 2-aminopyrimidine-5-(N-phenyl)sulfonamide, an isomer of sulfadiazine. The object of the work described here was to obtain an intermediate from which a variety of 2-amino-5-pyrimidinesulfonamides could be prepared readily.

Like pyridine, pyrimidine is resistant to electrophilic attack and, as yet, a simple mononitropyrimidinesulfonic acid has not been described. Substituted nitropyrimidines, pyrimidinesulfonic acids or sulfonyl chlorides are known[2-7] but, unless prepared from pyrimidines containing at least two such substituents as hydroxyl or amino groups, have not been obtained by either direct nitration or sulfonation. The compounds described by Caldwell and Jaffe are the first to have been made by direct electrophilic action upon a monoaminopyrimidine.

Preparation of 1-aminobenzene-2,4,6-trisulfonyl chloride by Lustig and Katscher[8] and the report of the formation of sulfanilyl chloride directly from aniline[9] gave support to the thought that 2-amino-5-pyrimidinesulfonyl chloride might be obtained since it also had been shown previously[10-12] that a strongly electron-withdrawing group in position 5 of the pyrimidine nucleus deactivates an amino group in position 2 toward electrophilic attack. The normal incompatibility to be expected in the simultaneous existence of free primary amino and sulfonyl chloride groups might not prevail in this particular case.

Attempts to acetylate 2-amino-5-pyrimidinesulfonic acid or its sodium salt by methods analogous to those by which 2-acetamido-5-nitropyrimidine was prepared[12] from 2-amino-5-nitropyrimidine failed in our hands; furthermore, we obtained only 2-amino-5-pyrimidinesulfonic acid upon treating 2-acetamidopyrimidine with chlorosulfonic acid.

We therefore turned to a reaction between the sodium salt of 2-amino-5-pyrimidinesulfonic acid and phosphorus pentachloride from which we derived, not the expected 2-aminopyrimidinesulfonyl chloride but, instead, 2-(5-chlorosulfonylpyrimidyl)phosphoramidic dichloride (I) by a sequence of reactions presumably like those given by Bieber and Kane[13] for an analogous reaction between sodium sulfanilate and phosphorus pentachloride. Its preparation and conversion to the desired compounds are shown.

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Experimental

2-Amino-5-pyrimidinesulfonic Acid.—This compound was prepared by a modification of the method of Caldwell and Jaffe.[1] To 100 ml. (1.5 moles) of chlorosulfonic acid was added, with stirring, 16.0 g. (0.168 mole) of 2-aminopyrimidine and the

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