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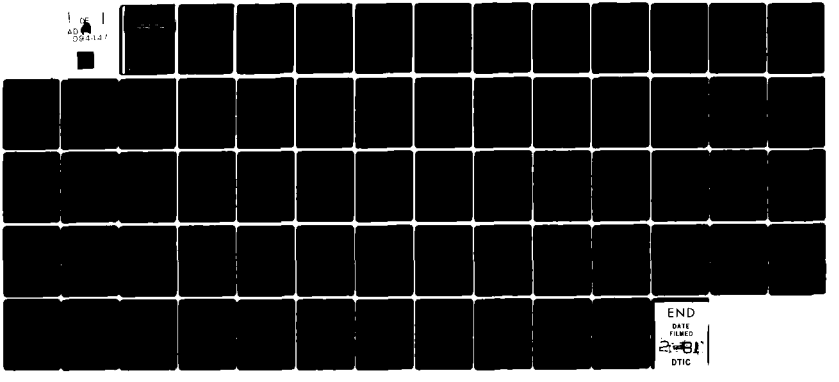

RESEARCH AND DEVELOPMENT BRANCH
DEPARTMENT OF NATIONAL DEFENCE
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PART XVIII

SYNTHESIS AND PROTECTIVE ACTIVITY OF A SERIES OF S-ω[ω-(ω- AMINOALKYLAMINO) ALKYLAMINO] ALKYLPHOSPHOROTHIOIC ACIDS AND DISULPHIDES

by

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OTTAWA

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ABSTRACT

The syntheses of the polyamines phosphorothioic acids $NH_2(CH_2)_n"NH(CH_2)_n'NH(CH_2)_nSPO_3H$ were achieved where n= 2 and 3, n' = 2, 3, 4 and 5; n" = 2, 3, 4. The toxicities and radiation dose reduction factors (DRF) were determined in mice. The compounds were given intraperitoneally before irradiation. The mice were irradiated with ^{134}Cs gamma radiation source at dose rate of approximately 90 r/min.

The best DRF (1.8) was obtained for the compounds with n = n' = n" = 2 and when n = n" = 2 and n' = 3. The toxicity increased and protective activity decreased when the number of carbon atoms in the alkyl chains was increased. The preparation of pure thiol derivatives was not totally successful. The one impure thiol compound when n = n' = n" = 2 obtained had a DRF of 1.41. The disulfide derivatives were prepared but were more toxic and less protective than the parent compounds. The compounds were not protective when given orally or after irradiation. The relationship between chemical structure and radiation protective activity is discussed.

RÉSUMÉ

On a effectué les synthèses d'acides phosphorothioiques polyaminés $NH_2(CH_2)_n"NH(CH_2)_n'NH(CH_2)_nSPO_3H$ pour n = 2 et 3, n' = 2, 3, 4 et 5, et n" = 2, 3, 4. Les toxicités et les facteurs de réduction de dose (FRD) ont été mesurés chez les souris. On a administré les composés par injection intrapéritonéale avant irradiation. Les souris ont été irradiées à partir d'une source de rayonnement gamma au Cs^{134}, à une dose d'environ 90 R/min.

Les meilleurs FRD (1.8) se retrouvaient chez les composés avec n = n' = n" = 2, ou n = n" = 2 et n' = 3. La toxicité augmentait et la capacité protectrice diminuait avec l'accroissement du nombre d'atomes de carbone dans les chaînes alkyl. La préparation de thiols purs n'a pas réussi complètement. Le seul thiol non pur obtenu, avec n = n' = n" = 2, avait un FRD de 1.41. Des disulfures ont pu être préparés, mais ils étaient plus toxiques et moins protecteurs que les substances apparentées. Les composés n'avaient pas de capacité protectrice après administration orale ou irradiation. La relation entre la configuration chimique et la capacité protectrice contre les radiations est examinée.

SYNTHESIS AND PROTECTIVE ACTIVITY OF A SERIES S-ω[ω-(ω-aminoalkylamino)alkylamino] alkylphosphorothioic Acids and Disulphides

G.A. Grant, W. Sowa*, B. Licht*, and K. Leach

INTRODUCTION

Derivatives of 2-aminoethanethiol (cysteamine) in which the amino substituent is an alkyl group, with and without latentiating S-substitution groups, did not provide more effective radiation protection agents than the parent compound (1-8). Much work has been focused on the amino-substituted compounds and it has been shown that di-substitution and mono-substitution usually increase toxicity and decrease protective activity (8), (9), (10), (11). The relationship between structure and protective activity has been previously reviewed (12), (13), (14).

The most important advance to achieving more effective protective agents was made by Piper, Stringfellow, Elliott and Johnston (15). Substitution of an ω-aminoalkyl group in 2-aminoethanephosphorothioate produced a series of compound superior to cysteamine. Substitution of the ω-aminoalkyl group decreased toxicity and this is the only functional group that decreases toxicity and increases protective activity. The protective activity of one of the compounds in this series, S-2-(3-aminopropylamino)ethyl dihydrogen phosphorothioate (WR2721), has been extensively investigated (15, 16, 17, 18, 19, 20, 21, 22, 23) and has a dose reduction factor over two.

At the present time it is not well known why the amino substitution with the ω-aminoalkyl group and S-substitution with a phosphate group promote protective activity and decrease the toxicity of the compounds in mice. The purpose of the S-substitution or latentiating groups is to overcome the pharmacological effects of thiols and disulphides (24, 25). Usually, substitution with acid groups such as phosphate or sulfate introduces more charged groups into the molecule and increases the difficulty of the compound to penetrate membrane barriers. However, it is possible that in case of the ω-(ω-aminoalkylamino)alkylphosphorothioic acids the acid function can be neutralized internally by two amino groups, providing that the number of methylene groups between the amino groups is optimum to permit folding of the molecule to allow internal neutralization. If this were the case, the compound would be able to penetrate vascular tissue walls and cellular membranes much more easily than the zwitter ion

$$\overset{+}{N}H_3(CH_2)_n\ \overset{+}{N}H_2(CH_2)_nSPO_3^{2-}.$$

Since substitution with the ω-aminoalkyl groups in cysteamine phosphate appears to be the most profitable approach to improving the protective activity of radioprotective compounds it was of interest to explore this lead. Therefore, it was decided to synthesize a series of homologous ω-[ω-(ω-aminoalkylamino)alkylamino]alkylphosphorothioic acids,

$$NH_2(CH_2)_n''NH(CH_2)_n'NH(CH_2)_nSPO_3H_2 \ .$$

This series of compounds should also be of interest as the acid group can neutralize two of the three amino-groups and the compound is left with a positive charge, if one amino group is protonated extramolecularly and the other two amino groups are protonated intramolecularly.

$$\overset{+}{N}H_3(CH_2)_n''\overset{+}{N}H_2(CH_2)_n'\overset{+}{N}H_2(CH_2)_nSPO_3^{2-}$$

Since the extent of the internal neutralization is unknown the phosphorothiol compounds synthesized will be written in the nonzwitterionic form.

This research is an extension of the investigation of Piper, Johnston, Stringfellow, Elliott and Johnston (15) and Piper and Johnston (26) who prepared 2-[3-(4-aminobutylamino)-propylamino]ethanethiol triphosphate from S-2-[3-(4-aminobutylamino)propylamino]ethylphosphorothioic acid. The intermediates were not isolated.

The investigation of Piper et al, (15) provided routes (Scheme 1) which were successful in preparing the homologous series:

$$NH_2(CH_2)_n'NH(CH_2)_nSPO_3H_2$$

SCHEME 1

$$H_2N(CH_2)_nNH_2 + CH_2\text{—}CH_2 \rightarrow H_2N(CH_2)_nNHCH_2CH_2OH\cdot HBr$$

$$2HBr\cdot H_2N(CH_2)_nNHCH_2CH_2Br \xrightarrow{Li_3SPO_3} H_2N(CH_2)_nNHCH_2CH_2SPO_3H_2$$

A similar reaction sequence was envisaged for symmetrical triamines of the type $H_2N(CH_2)_n''NH(CH_2)_n'NH(CH_2)_nNH_2$ where n' = n''. The final products would have n = 2 derived from ethylene oxide. Alternative routes are necessary for n' = n'' and for n = 3. When n = n' = 2 and n'' = 3 a method based on the reaction of a diamine with oxazolidinone was found suitable (26, 27). For preparation of compounds in which n = n'' = 2 and n' = 3 to 5, the reactions of 3-(3-chloroalkyl)-2-oxazolidinone (27) with 1,2-diamine provided the necessary intermediates. For simiplicity they are called the 2/2/2 and 2/3/3 compounds, respectively, with the carbon groups numbered from the end of the molecule having the S-group or potential S-group (e.g., alcohol

halide).

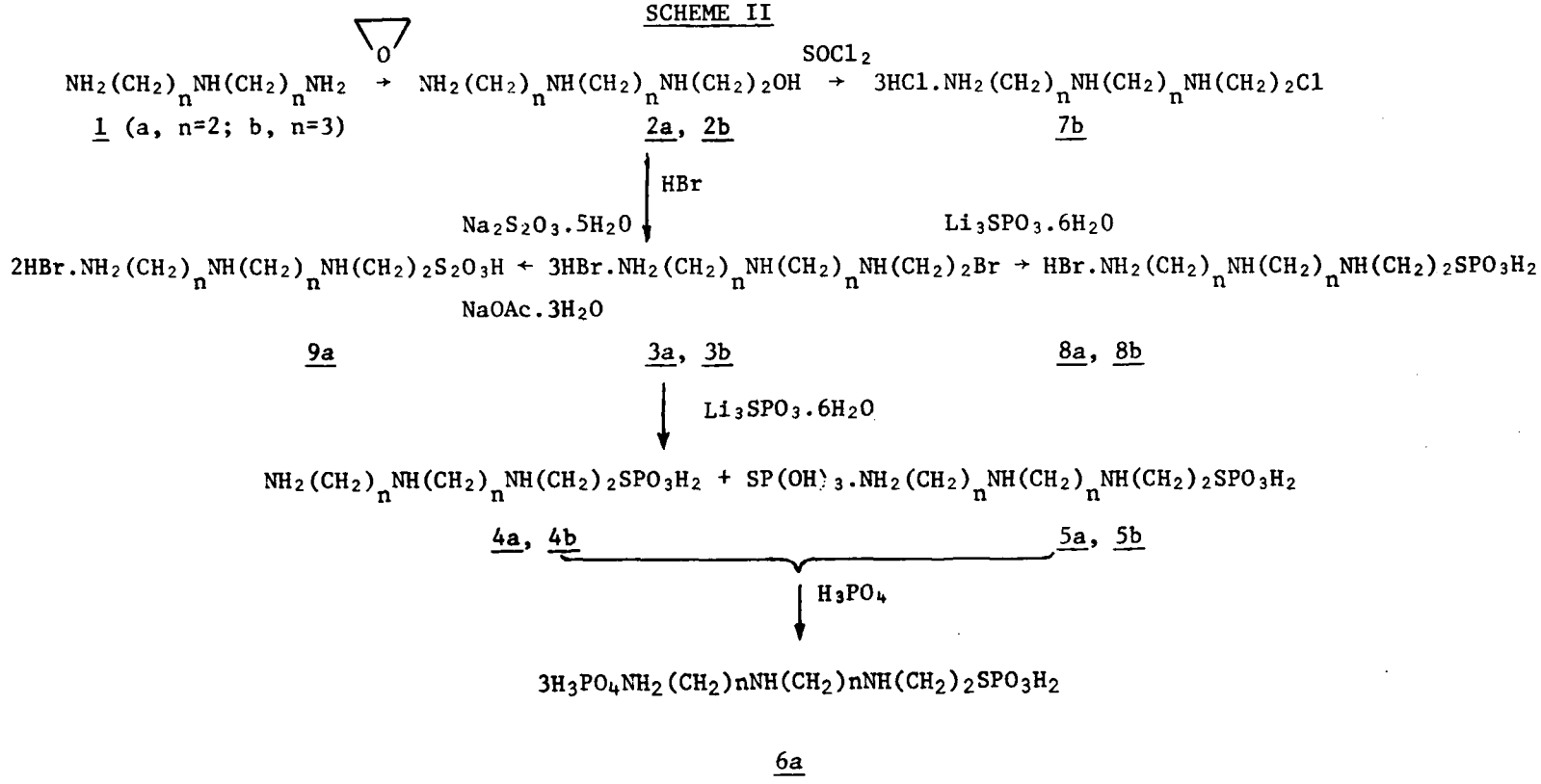
The polyamine derivatives are similar to 2/3/4 compounds related to spermine and spermidine which have been reported by Piper and Johnston (26).

RESULTS AND DISCUSSION

Preparation of Compounds

The reaction sequences used to prepare compounds when n = n' = n" = 2 and n = 2 and n' = n" = 3 are shown in Scheme II. Diethylenetriamine (1a) and 3,3'-diaminodipropylamine (1b) were treated with ethylene oxide (15) to give the respective hydroxyethyl derivatives 2a and 2b. The use of about 5-10 per cent molar excess of polyamine gave the best yields. Alcohol 2a and 2b were isolated in about 30 and 45 per cent yield, respectively, with the aid of a spinning band distillation column. The separation was followed by thin-layer chromatography (tlc). Compound 2a is an extremely viscous, hygroscopic material. The extra methylene between amine groups in 2b renders it less hygroscopic. The two hydroxyethylpolyamines were readily converted to bromoethylpolyamines by successively refluxing and distilling with 48-percent hydrobromic acid (26), (28). The bromide trihydrobromide derivatives 3a and 3b are easily crystallized, stable substances. Attempts were made to prepare the corresponding chloride derivative 7b from the 2/3/3 alcohol via the trihydrochloride salt, but the salt was too insoluble in either dichloroethane or dimethylformamide (DMF) for reaction with thionyl chloride to proceed (29). Initial efforts to prepare the 2/2/2 phosphorothioate from alkyl bromide 3a by Akerfeldt's procedure using lithium phosphorothioate with DMF as catalyst (30) were unsuccessful. Experiments were performed with and without the addition of lithium hydroxide to neutralize HBr. The isolated products were gummy mixtures (tlc) with an S-like odour. Piper and Johnston (26) have discussed the problems of stoichiometry associated with the conversion of polyfunctional N-(2-bromoethyl)amine hydrobromides into Bunte salts and S-phophorothioates by reactions with divalent thiosulfate and trivalent phosphorothioate anions. The hydrobromides are acidic while the reagents, sodium thiosulfate and trilithium (or trisodium) phosphorothioate, are acid labile. If the ratio of covalent bromine to ionic bromine (RBr:Br) is too low, the excess HBr decomposes the inorganic reagent. Any organic phosphorothioate which forms also may be decomposed. Buffering or partial neutralization was recommended by Piper and Johnston (26) for reactions with low RBr:Br- ratios. The ideal ratio is 1:1 in the preparation of Bunte salts or monosodium organic phosphorothioates. An ideal ratio of 1:2 for RBr:Br^- in preparing phosphorothioates leads to internal salts between amine and acid groups, without any alkali metal.

SCHEME II

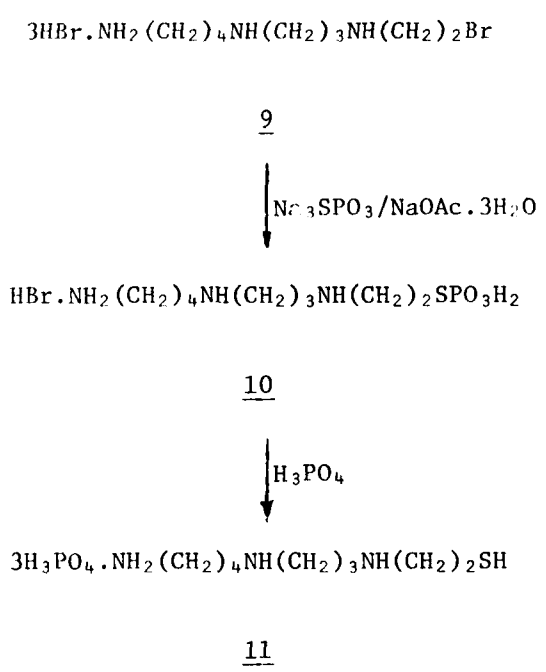


Piper and Johnston (26) prepared the 2/3/4 phosphorothioate (10 in Scheme III) by treatment of alkyl bromide 9 with sodium phosphorothioate and sodium acetate in aqueous DMF for 1 h at 25-30°. The product, a white gum, was presumed to be the hydrobromide 10 as shown. It was not examined further but hydrolysed directly to the thiol 11.

Syntheses by Akerfeldt (30) were done at room temperature while Piper and Johnston (26) used an even higher temperature of 25-30°. In view of the instability of reagents and products in the present work, it was decided to use lower temperatures and conduct reactions under nitrogen. At the same time, buffering or neutralization was omitted so that products would not be contaminated by inorganic salts which are difficult to remove and inevitably lead to decomposition and loss of product in subsequent work-up procedures. Thus, by treatment of alkyl bromides 3a and 3b under N_2 with lithium phosphorothioate in aqueous DMF at 10°, it was possible to isolate the organic phosphorothiates. They are white, crystalline materials, initially odour-free. The products were not the expected hydrobromide salts 8a and 8b, analogous to Piper and Johnston's (26) intermediate 10, but were the free amines 4a and 4b. The analysis also indicated a higher than expected proportion of S and P, in equivalent amounts. With the aid of paper chromatography, each product was found to contain a second, slower moving component in minor quantity. Although the second substance had almost the same mobility as lithium phosphorothioate, it was unlikely to be unreacted by phosphorothioate since the end of the reaction had been indicated by a negative Akerfeldt test (nitric acid-silver nitrate) for inorganic phosphorothioate (30). Atomic absorption spectroscopy of the products 4a and 4b proved that lithium was absent. The pmr spectra of the two products in D_2O corresponded with the expected structures and confirmed the absence of any impurities of similar structure. The ir spectra were consistent with the expected structures but in addition showed some absorption at 850 cm^{-1}. This is characteristic of P-S in a number of organic derivatives, (45). The analytical and ir evidence suggested that the slower-moving compounds accompanying 4a and 4b were their phosphorothioic acid salts. Lower mobility is consistent with more salt-like character. When the products were hydrolysed and examined chromatographically, a single spot for thiol was observed in each case. This again is consistent with the presence of a free amine mixed with its phosphorothioic acid salt, since phosphorothioic acid is known to be acid labile (26), (30), (31). Pmr in D_2O would not detect the phosphorothioic acid salt of the amine, but only the protons on carbon atoms. Additional evidence for the existence of an inorganic phosphorothioic acid was obtained by spraying the chromatograms first with dilute nitric acid and then silver nitrate, in an adaptation of Akerfeldt's test (30). The major products, 4a and 4b, did not react, while the minor components rapidly appeared as brown spots. The test on paper is more sensitive than in a test tube, however, the latter was quite adequate for following the consumption of lithium phosphorothioate during the course of its reaction with alkyl bromide.

The organic phosphorothioates 4a and 4b isolated in the present work appear to be free amines mixed with their phosphorothioic acid salts 5a and 5b. Amine salts of phosphorothioic acid have not been reported previously. At this time there is no information on whether the trapping of the

SCHEME III


3HBr.NH2(CH2)4NH(CH2)3NH(CH2)2Br
9
Na3SPO3/NaOAc.3H2O
HBr.NH2(CH2)4NH(CH2)3NH(CH2)2SPO3H2
10
H3PO4
3H3PO4.NH2(CH2)4NH(CH2)3NH(CH2)2SH
11

phosphorothioic acid is a result of the polyamine structure or the cold reaction condition or both. There is a possibility that the ratio of free amine to salt is variable. It is also probable that the products are hydrated, but it is not known to what extent the hydration is associated with the amines (4a, 4b) or their salts (5a, 5b).

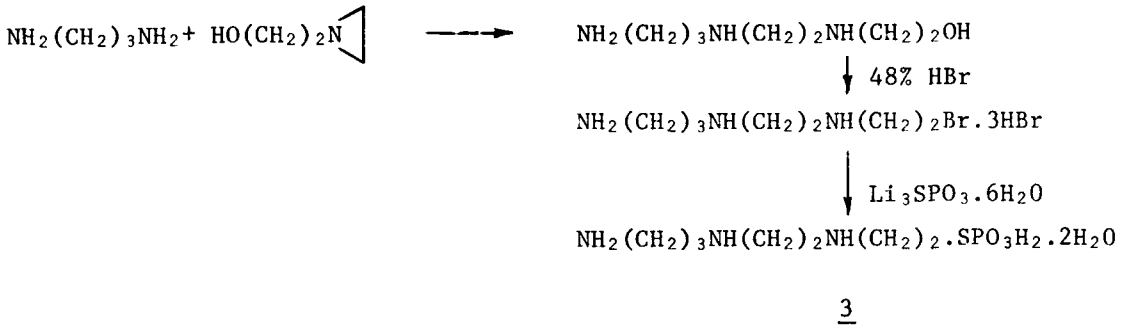
The 2/2/2 thiol triphosphate (6a) was prepared by hydrolysis of the phosphorothioate with phosphoric acid (26). The product was gummy and did not have a satisfactory microanalysis, although it was chromatographically pure ninhydrin.

Preparation of the 2/2/2 thiosulfate (9a) by treatment of alkyl brom - 3a with thallous thiosulfate was unsuccessful; practically no reaction was observed. There appeared to be some reaction with sodium thiosulfate in the presence of sodium acetate (26), but the Bunte salt could not be separated from inorganic material.

Two routes to the 2/2/3 phosphorothioate were developed. The objective was to extend diaminopropane from one end by attachment of an entity containing C-C-N-C-C. One pathway, using aziridinethanol (32), is shown in Scheme IV, and the other, based on the oxazolidinone method, of Piper et al (26), (27), is in Scheme V. The aziridinethanol route is based on a known reaction of ethylenimine with monoamino compounds. With aziridinethanol and diaminopropane, an extended reaction time and a very large excess (10:1) of amine were necessary. Results were highly inconsistent due to polymerization of the aziridinethanol. The best yield of hydroxyethyl compound in five trials was 30%, based on aziridinethanol. The lack of reproducibility was a major drawback of the method and it was abandoned in favour of the oxazolidinone procedure for preparation of N-2(2-bromoethyl)amines (27).

The sequence in Scheme V was used by Piper et al (26) with 1,4-butanediamine. While one amine group is protected as an aliphatic amide (N-acetyl), the other is converted to an aromatic amide (N-toluenesulfonyl) having a reactive hydrogen on the nitrogen atom. On treatment with a 3-haloalkyl-2-oxazolidinone, followed by cleavage with hydrogen bromide in acetic acid, chain extension by an alkylaminoethylbromide substitutent occurs. Piper et al (26) attempted to prepare S-2-[3-(4-aminobutylamino)propylamino]-ethylphosphorothioic acid starting with 1,4-butanediamine and 3-(3-chloropropyl) -2-oxazolidinone. They obtained a gum which was believed to be the hydrobromide salt of the product. It was not identified but hydrolyzed immediately with phosphoric acid to 2-[3-(4-aminobutylamino)propylamino] ethanethiol triphosphate. In the present work with polyamines, isolation of the phosphorothioates has been achieved by modifying the final step. The alkyl bromide in DMF is treated with $Li_3SPO_3.6H_2O$ at 5-10° under nitrogen rather than with Na_3SPO_3 and $NaOAC.3H_2O$ at 25-30°. The final product is not a hydrogen bromide salt of the polyaminoalkylphosphorothioate but a mixture of the phosphorothioate with variable amounts of the phosphorothioic acid salt of the polyaminoalkylphosphorothioate. The overall yield of 2/2/2 phosphorothioate dihydrate from diaminopropane is about 7%. Most of the variability in yields occurs at the first and fourth steps of the synthesis. The first step is the selective acetylation of a single amine group and the fourth step is the cleavage of the oxazilidinone ring with hydrogen bromide in acetic acid.

SCHEME IV



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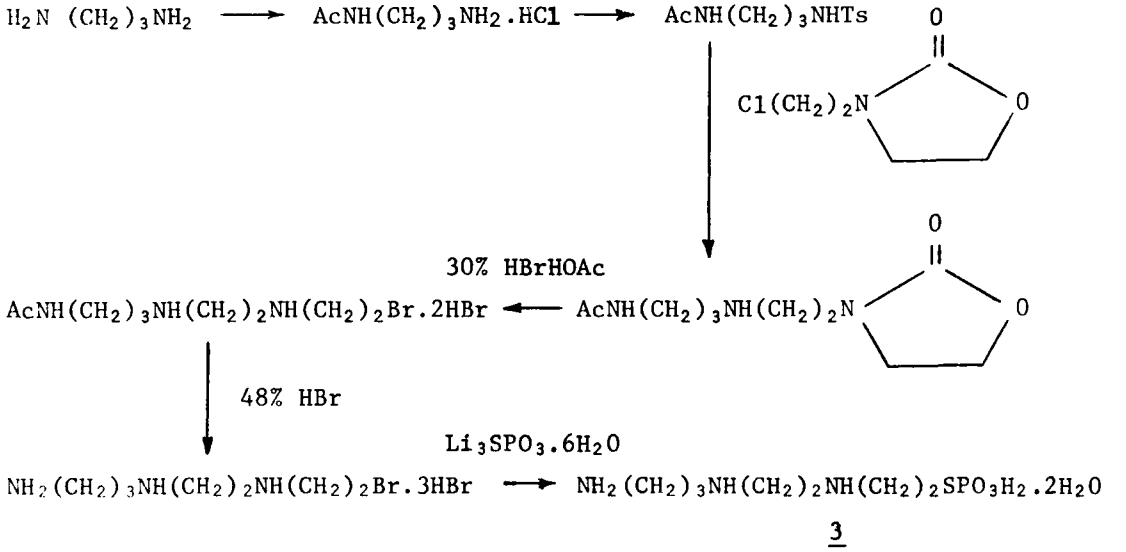
SCHEME V



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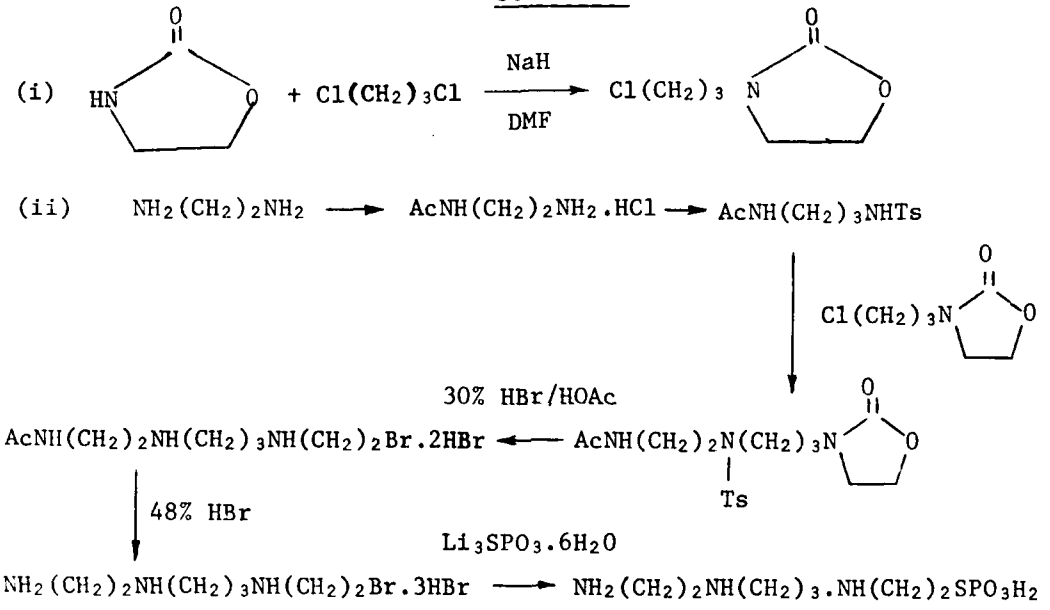
Two routes were attempted for the 2/3/2 phosphorothioate. As in the alternative procedure for the 2/2/3 compound discussed previously, the most direct route by way of a polyaminoethanol was tried first. Hydroxyethyldiaminopropane, prepared from diaminopropane and ethylene oxide, was treated with ethylenimine in the presence of 3 mole percent of the amine hydrochloride as catalyst. The reaction mixture was distilled at 1 mm to yield mainly starting aminoalcohol and a residue which could not be fractionated. Further work on this route was not continued and efforts were concentrated on the oxazolidinone method (26). The procedures in Scheme VI, based on the generalized route were used for the preparation of 2/3/2 phosphorothioate. The yields at each stage vary from batch to batch with the overall yield of phosphorothioate dihydrate from ethylenediamine generally amounting to 5%.

The identity and purity of the 2/2/3 and 2/3/2 phosphorothioates were confirmed by paper chromatography and by infrared and nuclear magnetic resonance spectroscopy. Microanalyses have been highly inconsistent because of the hygroscopic and sensitive nature of the compounds and because of the presence of variable amounts of the phosphorothioic acid salts. Reasonably good analyses were obtained with samples of the 2/3/3 and 2/2/3 phosphorothioates after purification by preparative paper chromatography. Better analyses (C and H only) were obtained with one batch of the 2/3/2 phosphorothioate by not attempting chromatographic purification. Apparently the 2/3/2 compound is not a dihydrate.

SCHEME VI



4

The disulfide hexaphosphates and disulfide hexachlorides were prepared by hydrolysing the corresponding phosphorothioates with phosphoric acid or hydrochloric acid, respectively. Hydrolyses were carried out at 100° with air bubbling through the reaction mixtures. The 2/2/2 and 2/3/3 disulfide hexahydrochlorides had unexplainable low microanalyses. All disulfides are solids except the 2/2/3 disulfide hexaphosphate.

Biological Testing of Compounds

The effects of structural changes on toxicity and protective activity in the series of polyaminoalkylphosphorothioates and disulfides are given in Tables I and II and Appendix A, Tables A-1 to A-27.

From Table I it is evident that increasing the number of carbon atoms in n' beyond 3 increased toxicity and decreased protective activity.

Dose reduction factors of approximately 1.8 were obtained with the compound when n = n" = 2 and n' = 3. The toxicity increased and protective activity decreased for the compounds when n = n' = 2 and n' is increased beyond 3. In general, except for compounds when n = n" = 2 and n' = 3 an increase in the number of carbon atoms in the alkyl chain beyond two increased toxicity and decreased protective activity.

The toxicity and dose reduction factors for the s-[ω-ω-(ω-aminoalkylamino) alkylamino]alkyl disulphides are given in Table II. The hydrochloride salts were more toxic than the hexaphosphate salts. A comparison of Table I and Table II shows the disulphides are more toxic and less protective than the corresponding phosphorothioic acids. Dose reduction factors of up to 1.41 were obtained when compounds were given intraperitoneally. The compounds were not protective when given orally.

The Effect of Administered Dose

The effects of administered dose on protective activity of the compounds are summarized in Table III. Further data obtained are given in Appendix B, Tables B-1 to B-7.

All the compounds showed an increase in protection with an increased dose. The minimum dose which does not provide protection varied with the compounds. The two compounds when n = n' = 2 and n' = 2 or 3 which provide a DRF of approximately 1.8 had different levels of minimum dose. The compound when n = n' = n" = 2 had a lower minimum dose requirement than the compounds with the greater toxicity and lower potency such as compound 2/2/3, had the lowest minimum dose and gave full protection at lower dose.

Administration Time

As with the majority of other known protectors the compounds had to be administered prior to irradiation to provide protection. The effect of pre-irradiation time of administration was studied for a number of the compounds. The results are given in Table IV and Appendix C, Tables C-1 to C-15 for the series of phosphorothioic acids. When the compounds are given by the I.P. route the maximum activity is obtained when the compounds are given 30 to 50 min prior to irradiation. The optimum pre-irradiation time is not related to the number of methylene groups in the alkyl chains. The duration of action was only approximately estimated as it was necessary to vary the radiation and compound doses to obtain data. It is evident that the protective activity decreases with an increase in the time between administration and irradiation. As estimated by the time when 100% mortality was obtained the compounds when n" = 2 and n' = 2 to 5 in general showed protective activity up to 120 min when n' = 2 and n" = 3 or 4, the time of duration of action was decreased. The other compounds in Table IV, although having a low DRF, did show duration of action of 90 to 180 min.

The investigation of the compounds in the series s-[ω-ω'-(ω-amino-alkylamino)alkylamino]alkylphosphorothioic acids and their disulphides gave interesting results with regard to the relationship between radioprotective activity and chemical structure. The substitution of the amino group in the compound 2-aminoethanethiol or 2-aminoethylphosphorothioic acid for a large number of previously prepared derivatives produced compounds with increased toxicity and decreased radiation protection. However, the substitution by an aminoalkyl group is an exception to the general rule. It was reported by Fatome at the meeting of NATO AC/225 Panel VII GEC in London in 1977 (47) that substitution of an unsaturated alkyl chain did not cause a great reduction in radiation protection. The preparation of the series of s-ω-(ω-aminoalkylamino) alkylphosphorothioates by Piper et al (15) produced compounds with greater dose reduction factors than 2-aminoethylphosphorothioic acid or 2-aminoethane thiol (16), (17). Also the compounds had longer duration times of action than 2-aminoethanethiol or 2-aminoethylphosphorothioic acid (17): MEA reaches its optimum protective activity approximately 10 min after injection and has only a slight residue of activity after 30 min (33). The fact that the aminoethyl group can be substituted in the present series of compounds on the ω-amino group of WR2721 and result in only a slight decrease in protection and toxicity indicates the uniqueness of this chemical group. The reasons for the ability of this (substituted) group to provide improved protection and toxicity have not been elucidated. Also, it is of interest to note that substitution of the aminoalkyl group contributed to increasing the duration of action of compounds over the duration times of 2-aminoethanethiol or 2-aminoethylphosphorothioic acid but did not contribute to increasing the effectiveness of the compounds when they are administered orally.

It is pertinent to discuss some of the factors which may contribute to the improvement of the radiation protection agent. Firstly, it is well established that administration of thiols by the intraperitoneal route produces undesirable pharmacological effects such as a drop in blood pressure and cardiac effects which increase the toxicity of the compounds in animals (34), (35) and man (36), (37). Substitution of a long alkyl chain increases the probability of toxicity (38) due to increasing lipid solubility and acceptability to the beta adrenergic receptors. To alleviate some of the toxicity problems associated with the thiol group, a phosphate group is added to this group temporarily until the compound reaches the sites in the tissue containing phosphatases which then remove the phosphate group (16). Thus the thiol is released more slowly and at dose levels which can provide protection, but is not as active pharmacologically. This is evident from the comparison of the pharmacology (39) and of the protection and toxic doses of aminoethanethiol and 2-aminoethanethiolphosphorothioates. The LD_{50} toxic doses of 2-aminoethanethiol and (lithium) 2-aminoethanephosphorothioate are approximately 385 and 1,028 mg/kg, respectively, while the doses required to produce a DRF of 1.5 are 200 mg/kg and 400 mg/kg for 2-aminoethanethiol and 2-aminoethylphosphorothioic acid, respectively. Tissue-culture studies showed that 2-aminoethanephosphorothioate is not toxic or protective until the phosphate group is removed and it is not hydrolyzed by all cells (22). The kidney cells do not hydrolyze the phosphorothioic acid at a sufficient rate to provide protection. The compounds are hydrolyzed by red cells to liberate the thiol (22). The hydrolysis of WR2721 to produce the thiol is more difficult than for 2-aminoethylphosphorothioic acid (23). The incubation period with red cells takes a long time to produce sufficient thiol to provide

protection (22). Harris and Phillips (23) have studied the dephosphorylation reaction and it is intracellular and the duration of action is longer for WR2721 than for compound WR638 in tissue culture. The thiol of WR2721 penetrates cells slowly and provide protection (40). It would be interesting to determine if the 2/2/2 and 2/3/2 compounds follow the same general trend. The 2/2/2 compound is not very protective in isolated kidney cell system (41), and probably has to be dephophorylated prior to providing full protection. Preliminary studies with one thiol derivative (2/2/2) supported this view as it was protective. The disulphides were protective in animals but not in tissue culture, therefore, they must be reduced by tissue reductase enzymes to provide protection.

The substitution of WR2721 with an aminoethyl group on the ω-amino group produced a compound which had slightly less protective action. A prophylactic dose of 500 mg/kg of the 2/3/2 compound gave a DRF of 1.8. In comparison, a dose of WR2721 of approximately 350 mg/kg gave a DRF of 2.0 (42). In the series of diamines prepared by Piper et al (15), the 3-2 and 2-3 (WR2721) had long duration of action (16). In the triamine series the 2/3/2 and 2/2/2 compounds had long duration of action, although slightly less than that of WR2721. Thus it would appear that in this family of compounds, WR2721 may have the best chemical structures for providing protective activity and duration of action. However, the thiols, disulphides and phosphorothioic acids of this series when given orally are not effective protective agents. The intrinsic protective activity on a molecular basis is difficult to evaluate as the multiple amine function of the compounds made them difficult to characterize. The efficiency of the protector is expressed as the molecular weight of the free base. An approximate estimation of the molecular efficiency was obtained by employing the molecular weight of the free base, WR2721 is taken as 214 and the 2/3/2 compounds as 257. Therefore, 1.64 m moles/kg of WR2721 gave a DRF of 2.02 for an efficiency of 1.23 and 1.95 m moles/kg of compound 2/3/2 gave a DRF 1.79 for an efficiency of 1.04. Therefore on a molar basis the efficiency of WR2721 and compound 2/3/2 is approximately equal.

It has been suggested that the efficiency of WR2721 may be due to its internal charge neutralization which allows it to penetrate some cellular membranes more easily than a charged species. The addition of the aminoethyl group to the ω-amino group of WR2721 adds an additional positive charge, due to the protonation of the amino-groups, which cannot be neutralized. Therefore, it is likely to have a residual positive charge. Protection by cysteamine phosphate at a dose of 600 mg/kg (4.76 m mole/kg) provides a DRF of 1.5, i.e. an efficiency of 0.11, which is far less than either WR2721 or the 2/3/2 compound. The difference may be due to lack of penetration of the cells and suggests that a molecule does not enter the cells as readily when it has a negative charge.

TABLE I

The Effect on Toxicity and Radiation Protective Activity of the Number of Carbon Atoms in the Alkyl Chains between the Amino-Nitrogens in the Compounds Aminoalklaminoalkylaminoalkylphosphorothioic Acids

$NH_2(CH_2)_{n''}NH(CH_2)_{n'}NH(CH_2)_nSPO_3H_2$

Compounds n	n'	n''	Toxicity* LD_{50} mg/kg	Dose*** mg/kg	$LD_{50}/_{30}$ rads	DRF
2	2	2	1600	600	1388 1349 - 1430**	1.79 1.73 - 1.84
2	3	2	900	500	1327 1295 - 1357	1.79 1.74 - 1.83
2	4	2	547 483 - 588	400	1166 1046 - 1288	1.47 1.32 - 1.63
2	5	2	225	150	892 861 - 920	1.12 1.08 - 1.15
2	2	3	892 821 - 940	450	1170 1049 - 1335	1.58 1.41 - 1.80
2	2	4	721 646 - 779	300	925 890 - 948	1.20 1.16 - 1.24
2	3	3	409 348 - 462	200	1143 1088 - 1191	1.43 1.40 - 1.53
2	3	4	483 452 - 511	325	1069 1042 - 1095	1.42
3	2	2	641 624 -659	500	950 932 - 985	1.19

* mice injected intraperitoneally
** 95% fiducial limits
*** Dose given 30 min. prior to irradiation

TABLE II

The Effect on Toxicity and Radiation Protection Activity of the Number of of Carbon Atoms in Alkyl Chain between the Amino-Nitrogen in the Compounds Aminoalkylaminoalkylaminoalkyl Disulphides

$[NH_2(CH_2)_{n''}NH(CH_2)_{n'}NH(CH_2)_nS]_2$

Compounds n	n'	n''	Toxicity LD_{50} mg/kg	Dose*** mg/kg	$LD_{50}/_{30}$ rads	DRF
2	2	2^2	500* 66 (64-73)**IV	300	1092 (1055 - 1133)**	1.41 (1.36 - 1.46)**
2	3	2^1	400*	275	830 (812 - 847)	1.05 (1.03 - 1.08)
2	3	2^2	550*	500	1044 (1008 - 1092)	1.27 (1.23 - 1.33)
2	3	$.3^2$	130* 30 IV 71000 oral	80	801 (765 - 830)	1.08 (1.03 - 1.12)
2	2	3^1	125*	100	841 (820 - 861)	1.07 (1.04 - 110)
2	2	3^2	300*	105	801 (782 - 819)	1.01 (0.99 - 1.03)

1 hexahydrochloride salt
2 hexaphosphate salt
IV intravenously
* mice injected intraperitoneally
** 95% fiducial limits
*** Dose given 30 min. prior to irradiation

TABLE III

Effect of Administered Dose on Protective Activity for
S-ω-[ω-(ω aminoalkylamino)alkylamino]alkylphosphorothioic Acids

$NH_2(CH_2)_{n''}NH(CH_2)_{n'}$, $NH(CH_2)_n$ SPO_3H_2

Compounds n	n'	n''	Dose rad	Mortality mg/kg	%	max mg/kg	Mortality %
2	2	2	1100	100	(100%)	600 -	(24)
2	3	2	1200	400	(100%)	600 -	(70)
2	5	2	950	150	(55)	200 -	(30)
2	2	3	1000	50	(100)	450	(0)
2	2	4	950	300	(75)	400	(45)
2	3	3	1000	50	(100)	200	(5)
2	3	3	1000	400*	(100)		

*Administered Orally.

TABLE IV

Effect of Length of Alkyl Chains in the Series of Compounds aminoalkylaminoalkylaminoalkylphosphorothioic Acids and on Optimum Pre-Irradiation and Duration-of-Action Times

$NH_2(CH_2)_{n''}NH(CH_2)_{n'}NH(CH_2)_nSPO_3H_2$

Compounds n	n'	n''	Dose rad	Dose mg/kg	Optimum time min	Duration time min.
2	2	2	1100	600	30	60 to 180
2	3	2	1200	500	60	120
2	4	2	1000	400	30	180
2	5	2	925	150	60	120

TABLE IV (continued)

Compounds n	n'	n''	Dose rad	Dose mg/kg	Optimum time min.	Duration time min.
2	2	3	1200	450	15 - 30	60
2	2	4	950	400	30	60
2	3	3	1000	200	30	100
2	3	4	900	325	60	120
3	2	2	900	500	15 to 60	90

EXPERIMENTAL

Biological Methods

The compounds were dissolved in phosphate buffer pH 7.2 and 0.1 ml was injected intraperitoneally (ip) or administered orally by use of a ball-point needle. The mice were Charles River strain COB's female and weighed 25 to 28 gm. The mice were irradiated in a special cesium - 134 irradiator (43) at a dose-rate of approximately 90 rads/min. For protective studies 20 mice per radiation dose were used. The mice were held 5 per cage up to 30 days. The $LD_{50/30}$ values were calculated from the response curves. The dose reduction factors were calculated:

$$\text{Dose Reduction Factor (DRF)} = \frac{\text{treated } LD_{50}}{\text{untreated } LD_{50}}$$

Relative-potency analyses were done to determine whether the slopes of the response curves were parallel. With some of the compounds the slopes of the response curves were significantly different, therefore the dose reduction factors were calculated separately and reported as approximate values.

SYNTHETIC METHODS

2[2-(2-Aminoethylamino)ethylamino]ethanol (2a)

Diethylenetriamine 1a (Fisher, Technical) was purified by distillation through a spinning band column (Nester Faust). The pressure was 13-16 mm with head temperature 102-103°. This method was more convenient than crystallization as the sulfate followed by regeneration with NaOH. Purity was confirmed by tlc on silica gell G using aqueous ammonia (25%)-ethanol (2:1, v/v) as solvent and ninhydrin or iodine for detection. The refractive index of the pure amine was 1.4820 at 25°.

To prepare the n = n' = n'' = 2 alcohol, diethylenetriamine (30.15 g, 0.292 mole) was dissolved in 90% methanol (225 ml) and treated with ethylene oxide (13.5 ml, 0.272 mole). The ethylene oxide was added dropwise over 1 hr to the well-stirred amine solution. During the addition the reaction mixture, condenser and jacketed dropping funnel for the ethylene oxide were all maintained at -5°. Stirring was continued for 1 h at -5° and then for 1 hr at room temperature. Finally the reaction mixture was refluxed for 1 hr. Methanol and water were removed by concentration of the mixture on a rotary evaporator (water-pump vacuum, bath temperature 50°). Removal of the water is essential for subsequent fractionation to be efficient on the spinning band column. The alcohol (2a) (13.4 g, 31%) was obtained with the spinning band column at 0.1 mm pressure, head temperature ca 113°. Distillation was slow and difficult because the alcohol is extremely viscous. In some runs the formation of a small amount of white solid caused the spinning band to seize. Formation of the solid was traced to absorption of CO_2 from the air by the diethylenetriamine in the mixture. The hygroscopic, syrupy, viscous nature of the product caused microanalytical results to be erratic. The following results were obtained by two analytical laboratories for the same sample: Calc. for $C_6H_{17}N_3O$: C, 48.95; H, 11.64; N, 28.55. Found: (Gygli) C, 48.92; H, 11.65; N, 31.84. Found: (Daessel) C, 46.14; H, 11.75, N,26.22. Found: (Gygli) C, 47.28; H, 12.59; N, 27.80.

Preparation of sulfate or oxalate derivatives was not successful. A crystalline sulfate was not obtainable by the procedure used for diethylenetriamine (from aqueous acetone).

A number of solvents and reagents were evaluated for tlc of the 2/2/2 alcohol. For example, tlc on cellulose was done in (a) n-propanol-triethylamine-H_2O (85:3:15 v/v), (b) n-propanol-NH_4OH-H_2O (6:3:1 v/v), (c) n-propanol-NH_4OH-H_2O (11:2:7 v/v) and (d) n-propanol-1% aqueous NH_4OH (2:1 v/v). The first solvent was the best of the group; all required 7-9 hr. Most often tlc was done on silica gel G in 25% aqueous NH_4OH-ethanol (2:1 v/v). Detection reagents were ninhydrin and iodine. The presence of the hydroxyl group in the molecule considerably decreased the response with ninhydrin compared with the starting amine. Another reagent for differentiation was cobalt thiocyanate (ammonium thiocyanate and 1 g $CoCl_2$ in 20 ml water) for use with cellulose plates. The colour with the starting amine is much stronger than with the 2/2/2 alcohol. The alcohol moves faster

than diethylenetriamine on both silica gel G and on cellulose. An attempt was made to separate diethylenetriamine and the alcohol as Dansyl derivatives. Such derivatives of 5-dimethylamino-1-naphthalenesulfonyl chloride are commonly used for amines and amino acids (4a). There was no separation by tlc on silica gel G in benzene-triethylamine (83:17 v/v).

N-[2-(2-Bromoethylamino)ethyl]-1,2-ethanediamine trihydrobromide (3a)

The alkyl bromide 3a was prepared by successive refluxing and distillation with hydrobromic acid according to the procedure of Piper et al (15) and Cortese (28). The n = n' = n" = 2 alcohol (34 g, 0.23 mole) was dissolved in 48% HBr (220 ml) and the solution was refluxed for 2 hrs. It was than partially distilled (39 ml) through a vigreux column (30 cm) and refluxed overnight. It was distilled 5 more times (25-30 ml each time) with refluxing in between, generally for 90 min but once overnight. The remaining HBr was removed with a rotary evaporator to obtain a syrup which solidified. The solid was washed several times with either and then with ether-acetone. It was recrystallized three times from hot methanol with charcoal treatment. The final product (48.0 g, 46%) had m.p. 194-195°. An analytical sample had m.p. 196-197°.

Calc. for $C_6H_{19}N_3Br_4$:C, 15.91; H, 4.21; N, 9.28; Br, 70.58.

Found: C, 16.04; H, 4.27; N, 9.29; Br, 70.48

S-2-[2-(2-Aminoethylamino)ethylamino]ethylphosphorothioic acid (4a)

A solution of $Li_3SPO_3.6H_2O$ (5) (4.80 g, 0.20 mole) and 3a (9.5 g, 0.021 mole) in water (70 ml) was stirred under N_2 in an ice-water bath and treated with DMF (30 ml). Stirring was continued under N_2 for 2 hr at ca 10° until the Akerfeldt silver nitrate test for inorganic phosphorothioate was negative (36). The reaction mixture was added dropwise under N_2 to stirred ethanol (800 ml). The supernatant liquid was decanted from the deliquescent residue which was then dissolved in water (50 ml). The solution was filtered and added slowly to stirred ethanol (500 ml). This precipitation was repeated and the product was washed with ethanol-ether. All operations were under N_2. The white, odourless product melted around 150° with decomposition. The yield was 5.40 g.

Tlc of 4a on cellulose in ethanol-water-NH OH (6:3:1 v/v) with silver nitrate (1 ml saturated $AgNO_3$ in 100 ml acetone) and ninhydrin detection reagents indicated the presence of a small amount of a second compound slower than the product. A more effective separation was obtained by paper chromatography on Whatman No. 4 paper run in the same solvent. The compound had R_f 0.49, comparable to a similar reference compound S-2-(5-aminopentylamino)ethylphosphorothioic acid monohydrate which has R_f 0.53 on the same chromatogram. The starting alkyl bromide had R_f 0.86. Lithium phosphorothioate ($Li_3SPO_3.6H_2O$) has R_f 0.28, similar to the unidentified

compound, and appears purple with ninhydrin. However, on standing the inorganic spot fades to yellow while the unknown and the product (4a) remain purple. Violuric acid, a spray for lithium, was not very useful since it was strongly positive with lithium phosphorothioate and slightly positive with all organic compounds (including the alkyl bromide). All spots became more strongly positive with violuric acid on standing. Quantitative analysis for Li by atomic absorption spectroscopy, demonstrated that only a small amount of Li was present in product 4, while reference compound (diaminoalkylphosphorothioate) had no lithium, contrary to the violuric acid spray test. To determine whether the unknown with R_f 0.25 was the thiol, the product (4a) containing it was treated with hydrochloric acid at room temperature for 24 hr (ca. 25 mg in 0.5 ml NHCl) and chromatographed. The reference gave a single ninhydrin-positive spot with R_f 0.88, Product 4a also gave one ninhydrin-positive spot which had R_f 0.81. Lithium phosphorothioate appeared little changed by the treatment. A trace of thiol (R_f 0.81) was detected in the 4a product. Compound 4a in solution is unstable and a S-like odour is detectable in a few minutes when 4 is dissolved in water.

The ir spectrum of the product in KBr showed the following absorption: ca. 3400 cm^{-1}(NH_3^+,NH), ca. 3200-2300 cm^{-1}(NH,P=O(OH), 1610 cm^{-1}(NH), 1450 cm^{-1} (CH_2), 1200-1000 cm^{-1} (P=O, C-N), 950 cm^{-1} (P-O), 650-550 cm^{-1} (P-S). There was also some absorption in the 850 cm^{-1} region suggestive of P=S(45). It is possible that absorption in the 3400 cm^{-1} is also due to H_2O as hydrate.

The pnr spectrum at 200 MHz was consistent with the assigned structure (see Appendix "D"). For pmr the sample was dissolved in D_2O with TMS as internal standard. Methylene hydrogen multiplets were assigned values as follows:

6.60 $NH_2(CH_2)$ NH; 6.70 $NH(CH_2)_2NH$ and $NHCH_2CS$; 6.97 CH S

2-[2-(2-Aminoethylamino)ethylamino]ethanethiol Triphosphate

Piper and Johnston's Method B (26) was followed in which the alkyl bromide is treated with $Li_3SPO_3.6H_2O$ and the resulting reaction mixture is hydrolyzed directly with H_3PO_4 by refluxing. All operations were under N_2. The thiol was precipitated from boiling ethanol as semi-solid. It was again isolated in a semi-solid state when reprecipitated from ethanol-water (N_2). Freeze-drying was not effective. On drying in vacuo over P_2O_5 at successive temperatures of 22°, 70° and 110°, it became hard and clear (glassy). The yield from 3 (9.52g, 0.021 mole) was 72% (6.5 g, 0.0152 mole). A satisfactory microanalysis was not obtained. The product gives a single spot (R_f 0.81, ninhydrin) on paper chromatography in ethanol-NH_4OH-water. The ir spectrum showed broad bands around 2700-2500 and 1140-900 cm^{-1} consistent with the presence of phosphate.

Attempted Preparation of S-2[2-(2-Aminoethylamino)ethylamino]ethylthiosulfuric Acid Dihydrobromide

Aminoalkylbromide 3a (9.0g) in water (20 ml) was treated with thallous thiosulfate (19.0g) at 50° for 24 h, followed by more thallous thiosulfate (9.5g) for an additional 24 h at 50° (46). The reaction mixture was examined by tlc on cellulose (butanol-ethanol-water, and ninhydrin spray). The major spot was starting material, with a small second spot.

A solution of $Na_2S_2O_3.5H_2O$ (2.48g. 10 mmoles), NaOAc. $3H_2O$ (2.72g, 20 mmoles) and 3a (4.53g, 10 mmoles) in water (12.5 ml) was heated at 90-95° for 2 h (2). The reaction mixture was evaporated to dryness to give a syrup in about 30 min. and refluxing was continued for 2 h. The crude product was removed by filtration (under N_2), dried in vacuo (over P_2O_5) and then stirred in boiling methanol (ca. 250 ml). Only a small amount dissolved. The methanol solution was filtered and evaporated to dryness. The syrupy residue was stirred with boiling methanol, the mixture was filtered, and ethanol was added to the filtrate. There was only slight precipitation after several days.

3-(3-Chloropropyl)-2-oxazolidinone (Scheme IV)

2-Oxazolidinone (34.37 g, 0.354 mole) and 1,3-dichloropropane (208.0 g, 1.84 mole) in DMF (177 ml) were added over 3 hr to a stirred suspension of sodium hydride (oil free, 8.5 g, 0.354 mole) in DMF (17.7 ml) at 25-30° (cooling). The mixture was filtered and the filtrate was evaporated in vacuo. The residue was dissolved in ethanol (200 ml) and the solution was treated with charcoal. The mixture was filtered and evaporated to dryness to isolate the product (62 g).

N-(2-Aminoethyl)acetamide hydrochloride

The 2-aminoethyl compound was prepared by acetylation of ethylenediamine according to the procedure for the 2-aminopropyl compound described previously. The product had mp 113-115° and yields varied from 21 to 52%.

N-2-(Acetamidoethyl)-p-toluenesulfonamide

The toluenesulfonamide was prepared in the same manner as the corresponding N-3-(acetamidopropyl)-p-toluenesulfonamide. The product has mp 108-109° and yields varied from 37 to 60%, generally closer to the latter.

N-(2-Acetamidoethyl)-N- 3-(2-oxo-3-oxazolidinyl)propyl-p-toluenesulfonamide

The oxazolidinyl compound was prepared from N-(-2-acetamidoethyl)-p-toluenesulfonamide and 3-(3-chloropropyl)-2-oxazolidinone in DMF containing K_2CO_3 in 99% yield. The ir spectrum was satisfactory and the crude product was cleaved directly with HBr-acetic acid.

N-[3-(2-Bromoethylamino)propyl]-,2-ethanediamine trihydrobromide

The oxazolidinyl derivative was cleaved by treatment with 30% HBr-acetic acid for 7 days. The resulting N-2[-3-(2-bromoethylamino)propylamino] ethylacetamide dihydrobromide, mp 163-164°, was obtained in 45% yield. It was treated with 48% HB in the usual manner to hydrolyse the N-acetyl group. The final alkyl bromide had mp 262-265° (dec.) and was obtained in 61% yield from the N-acetyl compound.

S-2-[3-(2-Aminoethylamino)propylamino]ethylphosphorothioic acid

The 2/3/2 phosphorothioate was obtained in the usual manner by treatment of the alkyl bromide with $Li_3SPO_3.6H_2O$ in DMF under N_2 in the cold. The yield of product varied from 34 to 88%. It had a tendency to form a gum and to become yellow. Paper chromatography indicated that it contained some phosphorothioic acid salt which was also detectable by ir in some batches (P=S at 850 cm^{-1}). The nmr spectrum was consistent with the expected structure. A sample for microanalysis was separated by paper chromatography with very poor results. A sample of the same batch was analysed (C and H) without chromatography with much better results.

Calc. for $C_7H_{20}N_3O_3PS$: C, 32.68; H, 7.84. Found: (Gygli) C, 32.88; H, 8.31.

The result suggests that the 2/3/2 compound is not a hydrate.

Attempted Preparation of 2-[3-(-2-Aminoethylamino)propylamino]ethanol

A solution of 1,3-diaminopropane (37 g) in 90% methanol (330 ml) was stirred at -5° and ethylene oxide (24 ml) was added slowly. The reaction mixture was stirred at -5° for 1 hr, at room temperature for 1 hr and then it was refluxed for 1 hr. The methanol was removed by evaporation under reduced pressure and the 2-(3-aminopropylamino)ethanol (16.0 g) was isolated by distillation, $b.p._{15\ mm}$ 153-154°.

A mixture of ethylenimine (2.92 g), 2-(3-aminopropylamino)ethanol (16.00 g), and 2-(3-aminopropylamino)ethanol hydrochloride (0.39 g) in ethanol (22.4 ml) was refluxed for 55 hr. The ethanol was removed under

reduced pressure and the residue was distilled at 15 mm and at 0.7 mm. Only the starting 2-(3-aminopropylamino)ethanol was distillable (nmr).

S-2-[4-(2-Aminoethylamino)butylamino]ethylphosphorothioic acid

N-(2-Acetamidoethyl)-p-toluenesulfonamide, m.p. 112-113°, was prepared in 52% yield by treatment of a mixture of N-(2-aminoethyl)acetamide hydrochloride and sodium carbonate with p-toluenesulfonylchloride. A mixture of the sulfonamide (81.7 g) and K_2CO_3 (46 g) in DMF (253 ml) was heated with stirring to 120 . A solution of 3-(4-chlorobutyl)-2-oxazolidinone (59.3 g) in DMF (127 ml) was added over 20 min. and the reaction mixture was stirred at 115-120° for 4 hrs. The solvent was removed by distillation in vacuo, the residue was suspended in water (330 ml), and the mixture was extracted with $CHCl_3$ (466 ml). The $CHCl_3$ solution was washed with water, dried and evaporated to dryness. The oily residue of N-(2-acetamindoethyl)-N-[4-(2-oxazolidinyl)butyl]-p-toluenesulfonamide (105 g) had an ir spectrum with absorption frequencies similar to those reported by Piper and Johnston (21) for N-(4-acetamidobutyl)-N-[3-(2-oxo-3-oxazolidinyl)propyl]-p-toluenesulfonamide.

N-(2-Acetamidoethyl)-N-[4-(2-oxo-3-oxazolidinyl]butyl]-p-toluenesulfonamide (105 g) was stirred in a freshly-prepared solution of hydrogen bromide in acetic acid (ca 35%) (600 ml) for 1 week at room temperature. The mixture was poured with stirring into ether and dried in vacuo over P_2O_5. The product was resuspended in acetone-ether, filtered and washed until the washings remained colour-free. The N-2{[4-(2-bromoethylamino)butylamino] ethyl}-acetamide dihydrobromide (77.6 g) had m.p. 168-178° and its ir spectrum showed the absence of oxazolidinyl carbonyl. It was hydrolyzed with 48% HBr (250 ml) by refluxing for 90 min. The solution was cooled, treated with charcoal, filtered and poured into ethanol. The mixture was stored overnight and the solid was collected by filtration. After it had been washed with ethanol and ether and dried, it had 220-221° (yield, 29.9 g). The ir spectrum showed that hydrolysis was complete.

Calcd. for $C_8H_{23}N_3Br_4$: C, 19.98; H, 4.82; N, 8.74; Br, 66.46.
Found: Gygli C, 19.79; H, 4.85; N, 8.76; Br, 66.30.

N-2-{[4-(2-bromoethylamino)butylamino]ethyl}amine trihydrobromine (24.05 g) and $Li_3SPO_3.6H_2O$(11.55 g) in water (200 ml) were stirred at 5-10° under nitrogen. DMF (80 ml) was added and stirring was continued for an additional 4 h. The filtered reaction mixture was poured into ethanol (2 l) with stirring under nitrogen. The supernatant was decanted and the residue was redissolved in water and treated with charcoal. The solution was filtered and freeze dried. The product, slightly coloured, was redissolved and treated with charcoal. Yield, 12.1 g.

Calcd. for $C_8H_{22}N_3SPO_3$: C, 35.41; H, 8.17; N, 15.49; P, 11.42; S, 11.82. Found: Galbraith C, 22.70; H, 7.21; N, 10.29; P, 11.84; S, 12.12, Gygli C, 22.41; H, 6.68.

S-2-[5-(2-Aminoethylamino)pentylamino]ethylphosphorothioic acid

The reaction sequence for the 2/5/2 phosphorothioate was analogous to the 2/4/2 compound described above. N-2(2-Acetamidoethyl)-p-toluenesulfonamide (m.p. 112-113°, 57.55 g) was treated with 3-(5-chloropentyl)-2-oxazolidinone (45.2 g) in DMF in the presence of K_2CO_3 (32.43 g) at 115-120° for 4 hrs. The reaction mixture was worked up to yield 95 g of crude oxazolidine derivative having an ir spectrum with expected absorption.

The N-(2-acetamidoethyl)-N-[5-(2-oxazolidinyl)pentyl]-p-toluenesulfonamide (95 g) was treated with hydrogen bromide in acetic acid (30%, 450 ml) for 7 days and worked up as with the butyl compound to yield N-2[5-(2-bromoethylamino)pentylamino]ethyl acetamide (53.6 g) with m.p. 173-175° after recrystallization from ethanol. The ir spectrum showed the oxazolidinone ring had been cleaved. Further hydrolysis with 48% HBr under reflux for 90 min. yielded N-2-[5-(2-bromoethylamino)pentylamino]ethylamine trihydrobromide (46.2 g) with m.p. 229-230°C.

Calcd. for $C_9H_{24}N_3Br_4$: C, 21.80; H, 4.99; N, 8.50; Br, 64.71;
Found: Gygli C, 20.81; H, 5.18; N, 8.63; Br, 63.27.
Duplicate, Gygli C., 20.65; H, 4.81; N, 8.48; Br, 65.79.

The N-2- 5-(2-bromoethylamino)ethylamine trihydrobromide (21.6 g, 0.094 mole) was treated with $Li_3SPO_36H_20$ (0.09 mole) in the presence of DMF for 4 hrs. in the cold until the silver nitrate test was negative in the usual manner. The product was precipitated in ethanol and purified by redissolving in water, charcoal treatment and freeze drying four times. The yield of S-2-[5-(2-aminoethylamino)pentylamino]ethylphosphorothioic acid was 24.2 g of which 14.0 g was submitted for screening.

The ir spectrum was similar to other polyamine phosphorothioates. There was broad absorption from 3400 cm^{-1} to 2300 cm^{-1} for NH_3+, NH, P=O(OH) and possibly hydrate; at 1610 cm^{-1} for NH; 1450 cm^{-1} (CH_2); 1200-100 cm^{-1} (P=O, C-N); 950 cm^{-1} (P-O), 600 cm^{-1} (P-S). There was no absorption at 850 cm^{-1} for P=S suggesting that phosphorothioic acid as a salt of the amine was not present.

Calcd. for $C_9H_{22}N_3SPO_3H_2$: C, 27.88; H, 8.48; N, 14.73; P, 10.85; S, 11.24. Found: Galbraith C, 27.72; H, 7.52; N, 11.02; P, 9.49; S, 9.67 Gygli C, 28.25; H, 7.80.

2-[2-(3-Aminopropylamino)ethylamino]ethanol

Mixtures of aziridinethanol and excess 1,3-diaminopropane in various ratios were refluxed in ethanol in the presence of 3 mole of 1,3-diaminopropane dihydrochloride. The hydroxyethylpolyamine was isolated by distillation under reduced pressure (0.1 mm). The conditions finally adopted consisted of refluxing aziridinethanol (8.7 g, 0.1 mole) and 1,3-diaminopropane (74 g, 1.0 mole) with 1,3-diaminopropane dihydrochloride (2.2 g) in ethanol (37 ml)

for 65 hr. The excess diamine was removed under reduced pressure and the remainder was distilled at 0.1 mm to yield the product, b.p.$_{0.1}$ 126° (4.9 g, 30% yield based on aziridinethanol). The reaction was not consistently reproducible. A single spot with mobility between that of 1,3-diaminopropane and aziridinethanol was observed on tlc of the product on silica gel G in ammonium hydroxide-ethanol (2:1). Its colour reaction with ninhydrin is less intense than that of the starting compounds. The ir and nmr spectra were satisfactory.

Calc. for $C_7H_{19}N_2O$: C, 62.15; H, 11.88; N, 26.06. Found: (Gygli) C, 62.57; H, 11.82; N, 24.72. (Daesslé) C, 52.72; H, 12.64; N. 26.32.

N-[2-(2-Bromoethylamino)ethyl]-1,3-propanediamine trihydrobromide

2-[2-(3-Aminopropylamino)ethylamino]ethanol (4.0 g, 0.0248 mole) was refluxed in 48% HBr (30 ml) for 18 hr. The reaction mixture was partially distilled (5 ml) and then refluxed for an additional 1.5 hr. The mixture was then distilled again (3 ml), refluxed (1.5 hr), distilled (3 ml), and finally refluxed for 18 hr. The product partially crystallized from the reaction mixture which was then cooled. The product was precipitated with ether, removed by filtration, and recrystallized from methanol. The yield of alkyl bromide (8.5 g), m.p. 220-221°, was 74%.

Calc. for $C_{11}H_{30}N_4Br_2 \cdot 3HBr$; C, 18.01; H, 4.53; N, 9.00; Br, 68.46. Found: (Gygli) C, 18.07; H, 4.43; N, 9.10; Br, 67.76.

S-2-[2-(3-Aminopropylamino)ethylamino]ethylphosphoroethioic acid

A solution of 2-[2-(2-bromoethylamino)ethyl]1,3-propanediamine trihydrobromide (4.90 g, 0.0105 mole) and $Li_3SPO_3.6H_2O$ (2.40 g, 0.010 mole) in water (35 ml) was stirred under N_2 and dimethylformamide (DMF) (15 ml) was added. Stirring was continued for 4 h and the reaction mixture was filtered. The solution was stirred into ethanol (ca 300 ml) under N and the resulting fine precipitate was collected by filtration. The product was redissolved in a minimum amount of water and freeze dried. Yield of dihydrate, 2.35 g, 76%. The ir spectrum (KBr) showed broad absorption from 3400 cm^{-1} to 2300 cm^{-1} for hydrate, NH_3^+, NH and P=O(OH), at 1610 cm^{-1}(NH), 1450 cm^{-1}(CH_2), 1200-1000 cm^{-1}(P=O, C-N), 950 cm^{-1}(P-O) and 650-550 cm^{-1}(P=S). There was no acid salt in the product detectable with the HNO_3-$AgNO_3$ reagent after paper chromatography in ethanol-water-ammonia (6:3:1 v/v). The small spot was negative with violuric acid and positive with ninhydrin. It was therefore not $Li_3SPO_3.6H_2O$.

S-2-[2-(4-Aminobutylamino)ethylamino]ethylphosphorothioic acid

N-(4-Aminobutyl)acetamide hydrochloride (76.8 g), m.p. 141°-142°, was prepared from 1, 4-diaminobutane (194 g) by the standard procedure for monoacetylation of diaminoalkanes. The N-toluenesulfonyl derivative was prepared by adding p-toluenesulfonyl chloride (38.2 g) in small portions to a mixture of the N-acetyl compound (30.0 g) and sodium carbonate (42.4 g) in water (450 ml) stirred at 60°. The reaction mixture was stirred at 60 for 3 hr. and at room temperature overnight. The precipitate was removed by filtration, washed, dried and recrystallized from ethyl acetate to obtain N-(4-acetamidobutyl)-p-toluenesulfonamide (36.5 g), m.p. 129-130°. A second batch (45.6 g) from 45 g of starting N-acetyl compound also had m.p. 129-130°.

The oxazolidine derivative of the tosyl compound was prepared using 3-(2-chloroethyl)-2-oxazolidinone by the method described above for the 2/2/4 phosphorothioate. The yield of N-(4-acetamidobutyl)-N-2-(2-oxo-3-oxazolidinyl)ethyl-p-toluenesulfonamide was 50 g from 36.5 g of N-(4-acetamidobutyl)-p-toluenesulfonamide. The ir spectrum was satisfactory. A second batch (60 g) with an identical spectrum was obtained from 45.6 g of starting material.

N-(4-acetamidobutyl)-N-2-(2-oxo-3-oxazolidinyl)ethyl-p-toluenesulfonamide (110 g) was treated with a freshly-prepared solution of hydrogen bromide in acetic acid (ca. 35%) (600 ml) at room temperature, with stirring. The mixture solidified after several hours and was left unstirred for 7 days. The solid was added to stirred ether (3 l) and stirring was continued overnight. The product was collected by filtration and washed with ether until the washings were colourless. It was then dried in vacuo to constant weight (100.1 g). Its m.p. was 175-179° and the ir spectrum showed that oxazolidinone was absent. The N-acetyl group was removed by treating the N-4{[2-(2-bromoethylamino)ethylamino]butyl}acetamide dihydrobromide (100 g) with 48% HBr (300 ml) under reflux for 90 min. The reaction mixture was cooled treated with charcoal, filtered and stirred into ethanol (3.5l). The precipitate was removed by filtration, washed with ethanol, and dried. The products (48.9 g), m.p. 215-218° was recrystallized from methanol-water to yield purified material (24 g) with m.p. 222-224°. The ir spectrum was satisfactory.

Calcd. for $C_8H_{23}N_3Br_4$: C, 19.98; H, 4.82; N, 8.74; Br, 66.46.
Found: Gygli C, 19.81; H, 5.01; N, 8.63; Br, 66.44.

N-4-[2-(2-bromethylamino)ethylamino]butylamine trihydrobromide (24 g) was stirred with $Li_3SPO_3.6H_2O$ (11.55 g) in water (200 ml) under nitrogen with cooling 5°-10°). DMF (80 ml) was added and stirring was continued for 4 hrs. The reaction mixture was filtered and the filtrate was added dropwise to ethanol (2 l) with stirring under nitrogen. The product precipitated as a semisolid residue which was isolated by decantation of the supernatant liquid. The residue was redissolved in water, treated with carbon and freeze dried several times to yield 13.8 g. Paper chromatography of the product in ethanol-water-ammonia (6:3:1 v/v) using ninhydrin and $AGNO_3$ sprays for detection showed one main spot accompanied by a small amount of material

positive with both sprays, indicative of phosphorothioic acid salt of the amine. The ir absorption spectrum was typical of polyamine phosphorothioates.

Calcd. for $C_8H_{22}N_3SPO$: C, 35.41; H, 8.17; N, 15.49; P, 11.42; S, 11.82. Found: Galbraith C, 22.72; H, 7.04; N, 10.29; P, 11.90; S, 12.21. Cyglic C, 22.61; H, 6.51.

2-[3-(3-Aminopropylamino)propylamino]ethanol (2b)

3,3'-Diaminodipropylamine (1b) from Eastman was found to be pure by tlc. The triamine (34.1 g, 0.26 mole) in 90% methanol (200 ml) was treated with ethylene oxide (12.0 ml, 0.24 mole) as described above for 2a. The hydroxyethyl derivative 2b was isolated in 45% yield by distillation on a spinning band column. The product was not as hygroscopic as the 2/2/2 alcohol 2a.

N-[3-(3-Bromopropylamino)propyl]-1,2-ethanediamine trihydrobromide 3b

The hydroxyethyl compound 2b (10 g) was treated with 48% hydrobromic acid (60 ml) by successive reflux and distillation as for 3a. Refluxing was stopped when the product (3b) began to crystallize from the boiling solution. On cooling, the reaction mixture solidified. The solid was washed with ether to obtain the crude product, m.p. 265°. On recrystallization from methanol-water, the purified product (20.6 g, 75% yield) had m.p. 266-267°. The ir spectrum was satisfactory.

Calc. for $C_8H_{20}N_3Br.3HB$: C, 19.98; H, 4.82; N, 8.74; Br, 66.46. Found: C, 20.04; H, 5.03; N, 9.15; Br, 66.10.

S-2-[3-(3-Aminoethylamino)propylamino]ethylphosphorothioic acid (4b)

The alkyl bromide (3b) (5.04 g, 0.0105 mole) was treated with $Li_3SPO_3.6H_2O$ (2.40 g, 0.010 mole) in aqueous solution (35 ml) in the presence of DMF (15 ml) under N_2 and in the cold (10°) as in the preparation of 4a. The resulting phosphorothioate 4b (1.8 g) was isolated in a similar manner. The product was a white crystalline material with an indefinite m.p. around 140° (decomp.). The ir spectrum in KBr showed the same absorption as 4a; ca. 3400 cm^{-1} (NH_3^+, NH, possibly H_2O), ca. 3200-2300 (NH, P=O(OH), 1610 cm^{-1} (NH), 1460 cm^{-1} (CH_2), 1200-1000 cm^{-1} (P=O, C-N), 950 cm^{-1} (P-O), 860 cm^{-1} (P=S), 650-550 cm^{-1} (P-S). In addition, there was also absorption at 750 cm^{-1} for the CH_2 rocking mode of polymethylene compounds.

The pmr spectrum (220 MHz) was obtained for a sample dissolved in D_2O with TMS as internal standard and the temperature at 18 . Methylene hydrogen multiplets were assigned values as follows: 6.61 [NH_2CH_2]: 6.82 [CH_2NHCH_2]: 7.01 [CH_2S]: 7.84 [$C-CH_2-C$].

S-2-[3-(3-Aminopropylamino)propylamino]ethylphosporothioic acid (2)

The ir and proton nmr spectra were satisfactory. A minor amount of phosphorothioic acid salt was detected by paper chromatography. A sample purified by preparative paper chromatography was submitted for microanalysis (Galbraith). The results indicated that the 2/3/3 compound is a dihydrate.

Calc. for $C_8H_{22}N_2SPO_3 \cdot 2H_2O$: 31.26; H, 8.53; N, 13.67; P, 10.08; S, 10.40. Found: C, 30.76; H, 7.88; N, 12.16; P, 9.93; S, 10.54. Same sample to Gygli, N, 12.61.

Calc. for $C_8H_{22}N_3O_3PS.HBr$: C, 27.29; H, 6.58; N, 11.94; Br, 22.69; P, 8.80; S, 9.11. Found: C, 26.20; H, 7.61; N, 11.39; Br, 1.08; P, 12.58; S, 12.93.

Atomic absorption analysis indicated that Li was absent.

Paper chromatography in ethanol-NH_4OH-water (6:1:3 v/v) and detection with silver nitrate or ninhydrin showed one major compound, R_f 0.50, and one minor compound with R_f 0.25. When the chromatogram was sprayed first with dilute HNO_3 (1% v/v), followed by silver nitrate in acetone, the major compound did not react while the minor compound gave a brown spot.

Attempted Preparation of N-[3-(2-Chloroethylamino)propyl]-1,3-propanediamine trihydrochloride

Hydroxyethylpolyamine 2b (3.05 g, 0.02 mole) was converted to the hydrochloride salt by treatment with hydrochloric acid (6 g, 0.06 mole). The salt was isolated by precipitation with ethanol and filtration. After drying to constant weight, it had m.p. 242-243°. It was then suspended in dry dichloroethane (25 ml) and thionyl chloride (3.45 g, 0.029 mole) was added slowly to the stirred and cooled (-5°) suspension. The reaction mixture was held for successive 1-h intervals at -5°, room temperature, 50-60°, and under reflux. The mixture was left overnight at 4° and then filtered. It had m.p. of 244-245° and an ir spectrum identical to the starting material. Absorption at ca 720 cm^{-1} expected for alkyl chloride was absent. Identification was confirmed by microanalysis. The reaction with thionyl chloride was repeated several times with variation of temperature and time, but in all cases starting material was recovered.

In one experiment, 3b trihydrochloride (2.85 g, 0.01 mole) was suspended in dimethylformamide (20 ml) and treated with thionyl chloride (0.78 ml, 0.011 mole) (4). The mixture was stirred at 100-120° for 1 hr. The solid isolated, m.p. 244-246°, was starting material (ir).

3-[2-(2-Aminoethylamino)ethylamino]propanol

3-Bromopropanol (35 g, 0.25 mole) was added to freshly distilled diethylenetriamine (103 g, 1.0 mole). The temperature of the reaction mixture was controlled at 70-75°C and stirring was continued for an addition 1½ hr, The crude product was separated by fractional distillation and purified further by two distillations on a spinning band column. Fractionation and purification were followed by GC (column: 3% OV-101, temp-program: 100°-180°C). The product has a $b.p._{0.01}$ of 92°-97°C and was ca. 95% pure by nmr.

2-[2-(3-Bromopropylamino)ethylamino]ethylamine trihydrobromide

3-[2-(2-Aminoethylamino)ethylamino]propanol (33 g) was refluxed in 48% HBr (400 ml) for 18 hr. The reaction mixture was partially distilled (75 ml), then refluxed for an additional 18 hr, distilled (75 ml) and refluxed for 18 hr. The remaining HBr was removed by distillation under reduced pressure. The residue was suspended in ether, filtered, washed and dried. The product after recrystallization from 90% methanol had m.p. 217°-219°C. Yield, 47.0 g.

Calc. for $C_7H_{21}N_3Br_4$: C, 18.01; H, 4.53; N, 9.00; Br, 68.46. Found: C, 18.08; H, 4.81; N, 8.51; Br, 67.82.

S-3-[2-(2-Aminoethylamino)ethylamino]propylphosphorothioic acid

A solution of 2-[2-(3-bromopropylamino)ethylamino]-ethylamine trihydrobromide (46.7 g, 0.1 mole) and $Li_3SPO_3.5.5H_2O$ (23.1 g, 0.1 mole) in water (300 ml) was stirred under N_2. Dimethylformamide (127.5 ml) was added and stirring was continued for 4 h. The reaction mixture was filtered, the filtrate was stirred into ethanol (1200 ml), and the resulting fine precipitate was collected by filtration. The product was redissolved in water (200 ml), treated with charcoal, precipitated with ethanol, and dried. This procedure was repeated until the final product was white (3 times). Yield, 20.5 g. The ir spectrum (KBr) was satisfactory. Only a very small amount of phosphorothioic acid salt was detectable by paper chromatography in ethanol-water-ammonia (6:3:1 v/v). The structure was also confirmed by nmr which indicated that the product was ca. 95% pure.

Preparation of S-2-[3-(4-Aminobutylamino)propylamino]ethylphosphorothioic acid

N-(4-Aminobutyl)acetamide hydrochloride (I)

Butanediamine (193.6 g, 2.2 mole) was added slowly with stirring to acetic acid (1200 ml). On completion of the addition the temperature of the mixture was brought to 55°-60°C and acetic anhydride (180 ml, 1.9 mole) was

added. The reaction mixture was then evaporated to dryness and the residue obtained was dissolved in hot water. Hydrochloric acid (6N, 410 ml) was added to the solution and the mixture was evaporated to dryness. The residue was extracted with hot 2-propanol and the solution was filtered and concentrated. Upon refrigeration of the solution, the product was obtained as a precipitate which was removed by filtration and recrystallized from 2-propanol.

Yield: 96.3 g m.p.: 140° - 142°C

N-(4-Acetamidobutyl)-p-toluenesulfonamide (II)

I (180 g, 1.28 mole) and sodium carbonate (254.4 g, 2.4 mole) were dissolved in water (2700 ml). The solution was stirred at 60°C and p-toluenesulfonyl chloride (229.2 g, 1.2 mole) was added in portions. Stirring was continued for 3 hrs at 60°C and then overnight at room temperature. The precipitate was removed by filtration, washed, dried and recrystallized from ethylacetate.

Yield: 123.5 g m.p.: 129° - 130°C

N-(4-Acetamidobutyl)-N-[3-(2-oxo-3-oxazolidinyl)propyl]p-toluenesulfonamide (III)

A mixture of II (121.5 g, 0.412 mole), anhydrous potassium carbonate (59.7 g, 0.433 mole) and dimethylformamide (333 ml) was heated over a 1-h period until the temperature reached 150° - 120°C. 3-(3-(chloropropyl)oxazolidinone (71 g, 0.435 mole) in dimethylformamide (166.5 ml) was added dropwise. The mixture was stirred for an additional 4 h at 115° - 120°C and evaporated. The residue was suspended in water (400 ml) and the mixture was extracted with chloroform (600 ml). The washed and dried chloroform extract was evaporated to dryness. The residue was redissolved in petroleum ether and the solution was treated with activated charcoal and celite, and evaporated to dryness.

Yield: 154.3 g

N-4{[3-(2-Bromoethylamino)propylamino]butyl}acetamide dihydrobromine (IV)

III (154.3 g) was dissolved in 35% hydrobromic acid acetic acid and stirred for 7 days at room temperature. The reaction mixture was poured into ether. The ether was decanted and the residue was treated with fresh ether until the residue solidified.

Yield: 82.7 g

N-4-[3-(2-Bromoethylamino)propylamino]butylamine trihydrobromide (V)

Hydrobromic acid (0.48%, 300 ml) was added to IV (82.7 g) and refluxed for 2 hrs. The reaction mixture was cooled, treated with activated charcoal, filtered and poured into 2 ml of stirred ethanol. The precipitate which formed was removed by filtration and recrystallized from methanol.

Yield: 46.6 g m.p.: 254° - 255°C
(Microanalysis: Calc. C, 21.84; H, 5.09; N, 8.49; Br, 64.58
Found C, 21.63; H, 5.01; N, 8.43; Br, 64.59)

S-2-[3-(4-Aminobutylamino)propylamino]ethylphosphorothioic acid

Trilithiumphosphorothioate (19.2 g, 80 mmole) and V (41.6 g, 84 mmole) were dissolved in water (280 ml) and dimethylformamide (120 ml) was added at 10°C under N_2. The mixture was stirred for 4 h under N_2, refrigerated overnight and then poured into a mixture of ethanol-methanol (2500 ml). The precipitate which formed was removed by filtration and redissolved in water. The solution was treated with activated charcoal and the product was isolated by precipitation in ethanol-methanol. It was examined by paper chromatography in ethanol (ammonia) water (6:1:3) using ninhydrin and silver nitrate spray reagents. The final product contained a small amount of the phosphorothioic acid salt of the amine.

Yield: 13.1 g

2-[2-(2-Aminoethylamino)ethylamino]ethanethiol Triphosphate

Piper and Johnston's method B (2b) was followed in which the alkyl bromide is treated with $Li_3SPO_3.6H_2O$ and the resulting reaction mixture is hydrolyzed directly with H_3PO_4 by refluxing. All operations were under N_2. The thiol was precipitated from boiling ethanol as semi-solid. It was again isolated in a semi-solid state when reprecipitated from ethanol-water (N_2). Freeze drying was not effective. On drying in vacuo over P_2O_5 at successive temperatures of 22°, 70° and 110°, it became hard and clear (glassy). The yield from 3a (9.52 g, 0.021 mole) was 72% (6.5 g, 0.0152 mole). A satisfactory microanalysis was not obtained. The product gives a single spot (R_f 0.81, ninhydrin) on paper chromatography in ethanol-NH_4OH-water (6:1:3 v/v). The ir spectrum showed broad bands around 2700-2500 and 1140-900 cm^{-1} consistent with the presence of phosphate.

2-[2-(2-Aminoethylamino)ethylamino]ethyl disulfide hexaphosphate

The 2/2/2 phosphoroethioate (10.5 g) was heated in 17% H_3PO_4 (130 ml) for 3 hr at 100° with air bubbling through the mixture. The reaction mixture

was then cooled and the solution was stirred into ethanol (1.91). The precipitate was redissolved in water and reprecipitated twice more. The product (12.9 g) had m.p. 92-95° and had a satisfactory ir spectrum. Paper chromatography in ethanol-water-ammonia (6:3:1 v/v) and detection with ninhydrin and specific thiol and disulfide sprays indicated only a single spot for disulfide. For thiol (red) the spray is 5 g sodium nitroprusside in 5 ml 2N HCl + 95 ml CH_3OH + 10 ml 25% NH (filtered). For disulfide (red), the previous spray is followed by 2 g sodium cyanide in 5 ml water + 100 ml CH_3OH.

Calc. for $C_{12}H_{32}N_6S_2.6H_3PO_4$: C, 15.79; H, 5.52; N, 9.21; P, 20.37; S, 7.03. Found: (Galbraith) C, 15.62; H, 5.65; N, 9.14; P, 20.54: S, 6.93.

2-[3-(3-Aminopropylamino)propylamino]ethyl disulfide hexaphosphate

The 2/3/3 disulfide was prepared in the same manner as the 2/2/2 derivative, and had m.p. 210-215°.

Calc. for $C_{16}H_{40}N_6S.6H_3PO_4$: C, 19.84; H, 6.04; N, 8.67; P, 19.20; S, 6.62. Found: (Galbraith) C, 19.63; H, 6.36; N, 8.60; P, 19.32; S, 6.57.

2-[2-(3-Aminopropylamino)ethylamino]ethyl disulfide hexaphosphate

The 2/2/3 disulfide hexaphosphate was prepared by hydrolysis with 17% phosphoric acid as with previous disulfides. The product was a syrup.

Calc. for $C_{14}H_{36}N_6S_2.6H_3PO_4$: C, 17.88; H, 5.79; N, 8.94; P, 19.76; S, 6.82. Found for sample dried over P_2O_5 in vacuo at room temperature: C, 15.33; H, 5.96; N, 8.33; P, 20.23; S, 6.09. Found for sample heated at 130-150° in vacuo for 8 hr: C, 16.95; H, 6.04; N, 7.92; P, 20.49, S, 6.21 (Galbraith).

2-[3-(2-Aminoethylamino)propylamino]ethyl disulfide hexaphosphate

The 2/3/2 disulfide hexaphosphate was prepared from the 2/3/2 phosphoroethioate by hydrolysis with phosphoric acid in the presence of air bubbling). The product had m.p. 134-35°.

Calc. for $C_{14}H_{36}N_6S_2.6H_3PO_4$: C, 17.88; H, 5.79; N, 8.94; P, 19.76; S, 6.82. Found: (Galbraith) C, 17.59; H, 6.36; N, 8.55; P, 20.49; S, 6.19.

2-[2-(2-Aminoethylamino)ethylamino]ethyl disulfide hexahydrochloride

In a typical preparation a solution of phosphorothioate (10 g) in 6 N HCl (80 ml) was heated at 100° for 3 hr with bubbling air. The reaction mixture was cooled, poured with stirring into ethanol (1500 ml), and the precipitate was removed by filtration, washed with ethanol and ether, and dried to constant weight over P_2O_5 in vacuo.

The 2/2/2 disulfide hexahydrochloride had m.p. 195-200°.

Calc. for $C_{12}H_{32}N_6S_2.6HCl$: C, 26.53; H, 7.05; N, 15.47; Cl, 39.15; S, 11.70. Found: (Galbraith) C, 25.97; H, 7.52; N, 15.12; Cl, 37.80; S, 4.95 Daesslé: S, 5.40.

2-[3-(3-Aminopropylamino)propylamino]ethyl disulfide hexhydrochloride

The 2/3/3 disulfide hexachloride (12.7 g) prepared from the phosphorothioate (13.6 g) had m.p. 268-269° (decomp).

Calc. for $C_{16}H_{40}N_6S_2.6HCl$: C, 32.06; H, 7.73; N, 14.021; Cl, 35.49; S, 10.70. Found: (Galbraith) C, 29.61; H, 7.43; N, 13.10; Cl, 32.12; S, 9.94 Daesslé S, 9.50.

2-[2-(3-Aminopropylamino)ethylamino]ethyl disulfide hexahydrochloride

From 10.3 g 2/2/3 phosphorothioate there was obtained 6.5 g of 2/2/3 disulfide hexahydrochloride having m.p. 251-252° (decomp.).

Calc. for $C_{14}H_{42}N_6S_2.6HCl$: C, 29.43; H, 7.41; N, 14.71; Cl, 37.23; S, 11.22. Found: (Gygli) C, 30.10; H, 7.57; N, 14.63; Cl, 36.58; S, 10.74. (Galbraith) C, 29.45; H, 7.8; N, 14.95; Cl, 36.45; S, 10.89; Daesslé S, 10.83.

2-[3-(2-Aminoethylamino)propylamino]ethyl disulfides hexahydrochloride

From 11.5 g 2/3/2 phosphorothioate there was obtained 9.2 g of 2/3/2 disulfide hexahydrochloride with m.p. 201-202° (decomp.).

Calc. for $C_{14}H_{36}N_6S_2.6HCl$: C, 29.43; H, 7.41; N, 14.71; Cl, 37.23; S, 11.22. Found: (Daesslé) S, 10.65.

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APPENDIX "A"

TABLE A-1

Toxicity in Mice by Intraperitoneal Route of Some Aminoalkylaminoalkylaminoalkylphosphorothioic Acids $NH_2(CH_2)_n$" $NH(CH_2)_n$'$NH(CH_2)_nSPO_3H_2$

Dose mg/kg	Percent Mortality							
	2/2/2	2/3/2	2/4/2	2/5/2	2/2/3	2/2/4	2/3/3	2/3/4
150				0				
200				0			0	
250				80				
300				100			20	
450								0
400	0		0					
450						0	40	10
500		0	20					20 70
550			70					90
600	0		70		0	20	90	90
650			70					
700			100		10	40		
750					0			
800	0	0			70	73	100	
850		10				70		
900					60			
1000					90			
1600	50							
LD 50/2 (Limits 95%)	-	-	547 483- 588	892 821- 940	721 646- 779	409 348- 462	482 452- 511	

TABLE A-2

Toxicity in Mice by Intraperitoneal Route of
S-2-[2-(2-aminoethylamino)ethylamino]ethyl disulfide hexaphosphate

Dose mg/kg	No. mice	No. deaths	Percent mortality
400	10	0	0
500	10	6	60
700	10	10	100

TABLE A-3

Toxicity in Mice by Intravenous Route of
S-2-[2-(2-aminoethylamino)ethylamino]ethyl disulfide hexaphosphate

Dose mg/kg	No. mice	No. deaths	Percent mortality
50	10	0	0
62.5	10	0	0
63.5	10	4	40
70	10	8	80
75	10	10	100

Calculated $LD_{50/30}$ 66 (64-73) mg/kg (95% Fiducial Limits)
Slope Function 19.33 ± 6.90

TABLE A-4

Toxicity in Mice by Intraperitoneal Route of
S-2-[3-(2-aminoethylamino)propylamino]ethyl disulfide hexahydrochloride

Dose mg/kg	No. mice	No. deaths	Percent mortality
200	10	0	0
400	5	2	40
500	10	10	100
600	10	10	100

TABLE A-5

Toxicity in Mice by Intraperitoneal Route of
S-2-[3-(2-aminoethylamino)propylamino]ethyl disulfide hexaphosphate

Dose mg/kg	No. mice	No. deaths	Percent mortality
300	10	0	0
400	10	0	0
500	10	0	0
600	10	8	80
650	10	10	100

TABLE A-6

Toxicity in Mice by Intraperitoneal Route of
S-2[2-(3-aminopropylamino)ethylamino]ethyl disulfide hexahydrochloride

Dose mg/kg	No. mice	No. deaths	Percent mortality
100	10	0	0
150	10	8	80
200	10	10	100
300	10	10	100

TABLE A-7

Toxicity in Mice by Intraperitoneal Route of
S-2[2-(3-aminopropylamino)ethylamino]ethyl disulfide hexaphosphate

Dose mg/kg	No. mice	No. deaths	Percent mortality
150	10	0	0
200	10	1	10
300	10	2	20
350	10	9	90
400	10	8	80

TABLE A-8

Toxicity of Mice by Intraperitoneal Route of
S-2-[3-(3-aminopropylamino)propylamino]ethyl disulfide hexaphosphate

Dose mg/kg	No. mice	No. deaths	Percent mortality
110	10	0	0
130	10	5	50
150	10	7	70
225	10	8	80
250	10	10	100

Calculated $LD_{50/30}$ 145 (125-167) mg/kg (95% Fiducial Limits)
Slope Function 3.30 ± 0.82

TABLE A-9

Toxicity in Mice by Intravenous Route of
S-2-[3-(3-aminopropylamino)propylamino]ethyl disulfide hexaphosphate

Dose mg/kg	No. mice	No. deaths	Percent mortality
25	10	0	0
30	10	7	70
37.5	10	10	100
50	10	10	100

TABLE A-10

Toxicity in Mice by Oral Route of
S-2-[3-(3-aminopropylamino)propylamino]ethyl disulfide hexaphosphate

Dose mg/kg	No. mice	No. deaths	Percent mortality
600	9	0	0
800	10	1	10
1000	10	3	30

TABLE A-11

Toxicity in Mice by Intraperitoneal Route of
2-[2-(2-aminoethylamino)ethylamino]ethanethiol triphosphate

Dose mg/kg	No. mice	No. deaths	Percent mortality
200	10	0	0
400	10	0	0
600	10	0	0
800	10	0	0

TABLE A-12

Thirty-day Survival of Mice given 600 mg/kg of
S-2-[2-(2-aminoethylamino)ethylamino]ethylphosphorothioic
Acid Intraperitoneally 30 min Prior to Irradiation

Dose (rads)	No. mice	No. deaths	Percent mortality
1100	20	0	0
1150	20	1	5
1200	20	2	10
1300	20	4	20
1400	20	13	65
1500	20	16	80
1600	20	16	80
1700	20	20	100

Calculated $LD_{50}/_{30}$ 1388 (1349-1430) rads (95% Fiducial Limits)

Slope Function 9.23 ± 1.13

DRF $\frac{1388}{776} = 1.79$ (1.73-1.84)

TABLE A-13

Thirty-day Survival of Mice given 500 mg/kg of
S-2-[3-(2-aminoethylamino)propylamino]ethylphosphorothioic acid
Intraperitoneally 30 min Prior to Irradiation

Dose (rads)	No. mice	No. deaths	Percent mortality
1175	18	1	6
1250	20	5	25
1325	20	9	45
1400	20	15	75
1475	20	18	90
1550	20	20	100

Calculated $LD_{50/30}$ 1327 (1295-1357) rads (95% Fiducial Limits)
Slope Function 12.82 ± 1.94
DRF $\frac{1327}{741}$ = 1.79 (1.74-1.83)

TABLE A-14

Thirty-day Survival of Mice given 400 mg/kg of
S-2-[4-(2-aminoethylamino)butylamino]ethylphosphorothioic acid
Intraperitoneally 30 min Prior to Irradiation

Dose (rads)	No. mice	No. deaths	Percent mortality
1050	18	1	6
1125	20	5	25
1200	20	17	85
1325	20	17	85
1350	19	19	100

Calculated $LD_{50/30}$ 1166 (1046-1288) rads (95% Fiducial Limits)
Slope Function 12.94 ± 3.82
DRF $\frac{1166}{790}$ = 1.47 (1.32-1.63)

TABLE A-15

Thirty-day Survival of Mice given 150 mg/kg of
S-2-[5-(2-aminoethylamino)pentylamino]ethylphosphorothioic acid
Intraperitoneally 30 min Prior to Irradiation

Dose (rads)	No. mice	No. deaths	Percent mortality
800	19	5	26
850	18	4	22
900	20	8	40
925	20	14	60
975	20	17	85
1050	20	18	90
1125	20	19	95
1200	20	20	100

Calculated $LD_{50/30}$ 893 (861-920) rads (95% Fiducial Limits)
Slope Function 8.17 ± 1.28
DRF $\frac{893}{796} = 1.12$ (1.08-1.15)

TABLE A-16

Thirty-day Survival of Mice given 450 mg/kg of
S-2-[2-(3-aminopropylamino)ethylamino]ethylphosphorothioic acid
Intraperitoneally 30 min Prior to Irradiation

Dose (rads)	No. mice	No. deaths	Percent mortality
1025	20	4	20
1050	19	5	26
1075	17	7	41
1100	20	5	25
1270	20	10	50
1370	20	20	100

Calculated $LD_{50/30}$ 1170 (1049-1335) rads (95% Fiducial Limits)
Slope Function 6.71 ± 1.97
DRF $\frac{1170}{741} = 1.58$ (1.41-1.80)

TABLE A-17

Thirty-day Survival of Mice given 300 mg/kg of S-2-[2-(4-aminobutylamino)ethylamino]ethylphosphorothioic acid Intraperitoneally 30 min Prior to Irradiation

Dose (rads)	No. mice	No. deaths	Percent mortality
900	20	6	30
950	20	15	75
1000	20	16	80
1050	20	19	95
1100	20	20	100

Calculated $LD_{50/30}$ 925 (890-948) rads (95% Fiducial Limits)
Slope Function 13.58 ± 2.85
DRF = $\frac{925}{765}$ = 1.20 (1.16-1.24)

TABLE A-18

Thirty-day Survival of Mice given 200 mg/kg of S-2-[3-(3-aminopropylamino)propylamino]ethylphosphoroethioic acid Intraperitoneally 30 min Prior to Irradiation

Dose (rads)	No. mice	No. deaths	Percent mortality
900	20	2	10
1000	20	2	10
1200	20	13	65
1300	20	17	85
1350	20	17	85
1400	20	18	90

Calculated $LD_{50/30}$ 1143 (1088-1191) rads (95% Fiducial Limits)
Slope Function 6.65 ± 0.98
DRF $\frac{1143}{776}$ = 1.47 (1.40-1.53)

TABLE A-19

Thirty-day Survival of Mice given 300 mg/kg of
S-2-[2-(2-aminoethylamino)ethylamino]ethyl disulfide hexaphosphate
Intraperitoneally 30 min Prior to Irradiation

Dose (rads)	No. mice	No. deaths	Percent mortality
800	18	2	11
850	20	0	0
950	20	3	15
1050	20	6	30
1150	20	13	65
1200	20	17	85

Calculated $LD_{50}/_{30}$ 1092 (1055-1133) rads (95% Fiducial Limits)
Slope Function 9.23 ± 1.67
DRF $\frac{1092}{771}$ = 1.41 (1.36-1.46)

TABLE A-20

Thirty-day Survival of Mice given 275 mg/kg of
S-2-[3-(2-aminoethylamino)propylamino]ethyl disulfide hexahydrochloride
Intraperitoneally 90 min Prior to Irradiation

Dose (rads)	No. mice	No. deaths	Percent mortality
750	20	1	5
800	20	7	35
850	20	10	50
900	20	19	95
950	20	18	90

Calculated $LD_{50}/_{30}$ 830 (812-847) rads (95% Fiducial Limits)
Slope Function 16.45 ± 2.70
DRF $\frac{830}{785}$ = 1.05 (1.03-1.08)

TABLE A-21

Thirty-day Survival of Mice given 500 mg/kg of
S-2-[3-(2-aminoethylamino)propylamino]ethyl disulfide hexaphosphate
Intraperitoneally 30 min Prior to Irradiation

Dose (rads)	No. mice	No. deaths	Percent mortality
750	19	0	0
850	20	1	5
900	16	2	13
950	18	4	22
1000	16	5	31
1100	14	9	64
1150	17	14	82

Calculated $LD_{50/30}$ 1044 (1008-1092) rads (95% Fiducial Limits
Slope Function 8.33 ± 1.44
DRF $\frac{1044}{817}$ = 1.27 (1.23-1.33)

TABLE A-22

Thirty-day Survival of Mice given 80 mg/kg of
2-[3-(3-aminopropylamino)propylamino]ethyl disulfide hexaphosphate
Intraperitoneally 30 min Prior to Irradiation

Dose (rads)	No. mice	No. deaths	Percent mortality
700	21	5	24
800	19	6	32
850	20	12	60
900	20	17	85
950	20	20	100

Calculated $LD_{50/30}$ 801 (765-830) rads (95% Fiducial Limits)
Slope Function 8.02 ± 1.49
DRF $\frac{801}{738}$ = 1.08 (1.03-1.12)

TABLE A-23

Thirty-day Survival of Mice given 100 mg/kg of S-2-[2-(3-aminopropylamino)ethylamino]ethyl disulfide hexahydrochloride Intraperitoneally 30 min Prior to Irradiation

Dose (rads)	No. mice	No. deaths	Percent mortality
750	20	0	0
800	20	8	40
850	20	11	55
900	20	15	75
950	20	19	95

Calculated $LD_{50/30}$ 841 (820-861) rads (95% Fiducial Limits)
Slope Function 12.79 ± 2.12
DRF $\frac{841}{785} = 1.07$ (1.04-1.10)

TABLE A-24

Thirty-day Survival of Mice given 150 mg/kg of S-2-[2-(3-aminopropylamino)ethylamino]ethyl disulfide hexaphosphate Intraperitoneally 30 min Prior to Irradiation

Dose (rads)	No. mice	No. deaths	Percent mortality
700	20	0	0
750	16	4	25
800	20	8	40
850	20	16	80
900	20	20	100

Calculated $LD_{50/30}$ 801 (783-819) (95% Fiducial Limits)
Slope Function 16.26 ± 2.73
DRF $\frac{801}{790} = 1.01$ (0.99-1.03)

TABLE A-25

Thirty-day Survival of Mice given 600 mg/kg of
S-2[2-(2-aminoethylamino)ethylamino]ethane thiol triphosphate
Intraperitoneally 30 min Prior to Irradiation

Dose (rads)	No. mice	No. deaths	Percent mortality
800	10	1	10
850	10	3	30
900	10	7	70
950	10	8	80
1000	10	8	80

Calculated $LD_{50/30}$ 887 (845-925) rads (95% Fiducial Limits)
Slope Function 10.20 + 2.76
DRF $\frac{887}{776}$ = 1.15 (1.08-1.19)

TABLE A-26

Thirty-day Survival of Mice given 325 mg/kg of
S-2-[3-(4-aminobutylamino)propylamino]ethylphosphorothioic acid
Intraperitoneally 30 min Prior to Irradiation

Dose (rads)	No. mice	No. deaths	Percent mortality
850	19	1	5
950	20	1	5
1000	18	3	17
1050	19	9	47
1100	18	11	61
1150	19	15	79
1200	20	17	85
1250	17	17	100

Calculated $LD_{50/30}$ 1069 (1042-1095) rads (95% Fiducial Limits)
Slope Function 10.46 + 1.48
DRF $\frac{1069}{750}$ = 1.42 (1.38-1.46)

TABLE A-27

Thirty-day Survival of Mice given 500 mg/kg of S-3-[2-(2-aminoethylamino)ethylamino]propylphosphorothioic acid Intraperitoneally 30 min Prior to Irradiation

Dose (rads)	No. mice	No. deaths	Percent mortality
800	19	3	16
900	20	3	15
950	20	10	50
1000	19	13	68
1050	20	17	85
1100	20	18	90
1150	18	17	94

Calculated $LD_{50}/_{30}$	960 (932-985) rads	(95% Fiducial Limits)
Slope Function	9.80 ± 1.47	
DRF	$\frac{960}{801}$ = 1.19 (1.16 - 1.22)	

APPENDIX "B"

TABLE B-1

Effect on Thirty-day Survival of Mice Treated with Various Doses of S-2-[2-(2-aminoethylamino)ethylamino]ethylphosphorothioic acid hydrobromide given Intraperitoneally 30 min Prior to Irradiation with 1100 rads

Dose mg/kg	No. mice	No. deaths	Percent mortality
50	25	25	100
100	25	25	100
200	25	20	80
350	25	14	56
600	25	6	24

TABLE B-2

The Effect on Thirty-day Survival of Mice Treated with Various Doses of S-2-[3-(2 aminoethylamino)propylamino]ethylphosphorothioic acid given Intraperitoneally 30 min Prior to Irradiation with 1200 rads

Dose mg/kg	No. mice	No. of deaths	Percent mortality
300	40	40	100
400	40	40	100
500	39	35	90
600	40	28	70

TABLE B-3

Effect on Thirty-day Survival of Mice Treated with Various Doses of S-2-[5-(2-aminoethylamino)pentylamino]ethylphosphorothioic acid given Intraperitoneally 30 min Prior to Irradiation with 950 rads

Dose mg/kg	No. mice	No. deaths	Percent mortality
150	20	11	55
200	20	6*	30

*early deaths occurred on Day 1

TABLE B-4

Effect on Thirty-day Survival of Mice treated with Various Doses of S-2-[2-(3 aminopropylamino)ethylamino]ethylphosphorothioic acid given 30 min Prior to Irradiation with 1000 rads

Dose mg/kg	No. mice	No. of deaths	Percent mortality
50	20	20	100
100	20	16	80
200	20	1	5
450	20	0	0

TABLE B-5

Effect on Thirty-day Survival of Mice Treated with Various Doses S-2-[2-(4-aminobutylamino)ethylamino]ethylphosphorothioic acid given Intraperitoneally 30 min Prior to Irradiation with 950 rads

Dose mg/kg	No. mice	No. deaths	Percent mortality
300	20	15	75
400	20	9	45

TABLE B-6

Effect on Thirty-day Survival of Mice Treated with Various Doses of S-2-[3-(3-aminopropylamino)propylamino]ethylphosphorothioic acid given Intraperitoneally 30 min Prior to Irradiation with 1000 rads

Dose mg/kg	No. mice	No. deaths	Percent mortality
200	20	1	5
100	20	16	80
50	20	20	100
*400	20	20	100
Radiation control	20	20	100

*administered orally

TABLE B-7

The Effect on Thirty-day Survival of Mice Treated with Various Doses of S-2[2-(2 aminoethylamino)ethylamino]ethyl disulfide hexaphosphate given 30 min Prior to Irradiation with 1100 rads

Dose mg/kg	No. mice	No. of deaths	Percent mortality
125	20	20	100
225	20	18	90
325	20	8	40

APPENDIX "C"

TABLE C-1

Effect of Administration Time on Protection Afforded by 600 mg/kg of S-2-[2-(2-aminoethylamino)ethylamino]ethylphosphorothioic acid hydrobromide given Intraperitoneally to Mice Irradiated with 1100 rads

Pre-Irradiation Time (min)	No. mice	No. deaths	Percent mortality
15	25	11	44
30	20	0	0
60	25	8	32
180	25	25	100
240	25	24	96
360	25	24	96

TABLE C-2

Effect of Administration Time on Protection Afforded by 500 mg/kg of S-2-[3-(2-aminoethylamino)propylamino]ethylphosphorothioic acid given Intraperitoneally to Mice Irradiated with 1200 rads

Pre-Irradiation Time (min)	No. mice	No. deaths	Percent mortality
15	20	17	85
30	20	18	90
60	20	4	20
90	20	18	90
120	20	19	95
150	20	20	100

TABLE C-3

Effect of Administration Time on Protection Afforded by 400 mg/kg of S-2-[4-(2-aminoethylamino)butylamino]ethylphosphorothioic acid given Intraperitoneally to Mice Irradiated with 1000 rads

Pre-Irradiation Time (min)	No. mice	No. deaths	Percent mortality
15	20	7	35
30	20	1	5
60	20	4	20
90	20	5	25
120	20	12	60
150	20	15	75
180	18	14	78

TABLE C-4

Effect of Administration Time on Protection Afforded by 150 mg/kg of S-2-[5-(2-aminoethylamino)pentylamino]ethylphosphorothioic acid given Intraperitoneally to Mice Irradiated with 925 rads

Pre-Irradiation Time (min)	No. mice	No. deaths	Percent mortality
15	19	10	52.6
30	20	16	80
60	20	6	30
90	19	11	57.9
120	20	17	85

TABLE C-5

Effect of Administration Time on Protection Afforded by 450 mg/kg of S-2[2-(3-aminopropylamino)ethylamino]ethylphosphorothioic acid given Intraperitoneally to Mice Irradiated with 1200 rads

Pre-Irradiation Time (min)	No. mice	No. deaths	Percent mortality
15	16	10	62
30	20	14	70
60	14	13	93
90	20	20	100
120	19	19	100
150	20	20	100

TABLE C-6

Effect of Administration Time on Protection Afforded by 400 mg/kg of S-2-[2-(4-aminobutylamino)ethylamino]ethylphosphorothioic acid given Intraperitoneally to Mice Irradiated with 950 rads

Pre-Irradiation Time (min)	No. mice	No. deaths	Percent mortality
30	20	9	45
60	20	18	90
90	20	16	80

TABLE C-7

Effect of Administration Time on Protection Afforded by 200 mg/kg of S-2-[3-(3-aminopropylamino)propylamino]ethylphosphorothioic acid given Intraperitoneally to Mice Irradiated with 1000 rads

Pre-Irradiation Time (min)	No. mice	No. deaths	Percent mortality
30	20	1	5
60	20	2	10
180	20	17	85
360	20	20	100

TABLE C-8

Effect of Administration Time on Protection Afforded by 425 mg/kg of S-2-[3-(4-aminobutylamino)propylamino]ethylphosphorothioic acid given Intraperitoneally to Mice Irradiated with 900 rads

Pre-irradiation Time (min)	No. mice	No. deaths	Percent mortality
15	19	2	11
30	19	4	21
60	20	1	5
90	20	10	50
120	20	15	75

TABLE C-9

Effect of Administration Time on Protection Afforded by 500 mg/kg of S-3-[2-(2-aminoethylamino)ethylamino]propylphosphorothioic acid given Intraperitoneally to Mice Irradiated with 900 rads

Pre-irradiation Time (min)	No. mice	No. deaths	Percent mortality
15	20	3	15
30	20	3	15
60	19	3	16
90	18	7	39

TABLE C-10

Effect of Administration Time on Protection Afforded by 325 mg/kg of 2-[2-(2-aminoethylamino)ethylamino]ethyl disulfide hexaphosphate given Intraperitoneally to Mice Irradiated with 1100 rads

Pre-irradiation Time (min)	No. mice	No. deaths	Percent mortality
15	20	6	30
30	20	9	45
60	20	13	65
90	19	18	95
120	20	18	90

TABLE C-11

Effect of Administration Time on Protection Afforded by 275 mg/kg of S-2-[3-(2-aminoethylamino)propylamino]ethyl disulfide hexahydrochloride given Intraperitoneally to Mice Irradiated with 850 rads

Pre-irradiation Time (min)	No. mice	No. deaths	Percent mortality
15	20	14	70
30	20	13	65
60	19	12	63
90	20	10	50
	Orally 500 mg/kg 900 rads		
30	19	19	100

TABLE C-12

Effect of Administration Time on Protection Afforded by 500 mg/kg of S-2-[3-(2-aminoethylamino)propylamino]ethyl disulfide hexaphosphate given Intraperitoneally to Mice Irradiated with 850 rads

Pre-irradiation Time (min)	No. mice	No. deaths	Percent mortality
15	20	5	25
30	20	1	5
60	20	6	30
90	20	2	10
	Orally 900 rads		
30	20	17	85

TABLE C-13

Effect of Administration Time on Protection Afforded by 550 mg/kg of S-2-[3-(3-aminopropylamino)propylamino]ethyl disulfide hexaphosphate given Orally to Mice Irradiated with 800 rads

Pre-irradiation Time (min)	No. mice	No. deaths	Percent mortality
30	20	17	85
45	17	17	100
60	20	19	90

TABLE C-14

Effect of Administration Time on Protection Afforded by 100 mg/kg of S-2-[2-(3-aminopropylamino)ethylamino]ethyl disulfide hexahydrochloride given Intraperitoneally to Mice Irradiated with 900 rads

Pre-irradiation Time (min)	No. mice	No. deaths	Percent mortality
15	20	17	85
30	20	15	75
60	20	20	100
90	19	19	100

TABLE C-15

Effect of Administration Time on Protection Afforded by 150 mg/kg of S-2-[2-(3-aminopropylamino)ethylamino]ethyl disulfide hexaphosphate given Intraperitoneally to Mice Irradiated with 850 rads

Pre-irradiation Time (min)	No. mice	No. deaths	Percent mortality
15	20	11	55
30	20	16	80
60	20	11	55
90	20	16	80

APPENDIX "D"

PMR of Polyamine Phosphorothioates and Disulfides
s = Singlet, t = Triplet

X	Y	Right to Left	H_2NCH_2	$(CH_2)_X$	CH_2NH	CH_2	$(CH_2)_Y$	CH_2NH	CH_2	$CH_2SPO_3H_2$
0	0	2,2,2	3.4(s)	-	.4(s)	3.3	-	3.3	~3.2	3.04
0	1	2,3,2	3.4(s)	-	3.4(s)	3.2 or 3.4	2.18	3.4 or 3.2	~3.2	3.0
0	2	2,4,2	3.2	-	3.3	3.37 or 3.1	1.83	3.1 or 3.7	~3.1 ~3.1	~ 2.9 2.9
0	3	2,5,2	3.43(s)	-	3.43 (s)	3.12	1.79, 1.5, 1.78	3.12 or 3.36	~3.1	3.0
1	0	2,2,3	3.40,	2.15	3.2 or 3.44	3.48 (s)	-	3.48 (s)	~3.1	2.9
1	1	2,3,3	3.18 or 3.39	2.16	3.39 or 3.18	3.18	2.16	3.18	~3.2	3.0
3	2	2,0,5	3.35 or 3.05	1.75 1.0, 1.75	3.05 or 3.35	X	-	X	3.3	2.96

X	Y	Left to Right	H_2NCH_2	$(CH_2)_X$	CH_2NH	CH_2	$(CH_2)_Y$	CH_2NH	CH_2	CH_2S-R
0	0									2.88
0	1									
0	2	2,4,2	3.44	-	3.44	3.21	2.20	3.27 (t)	3.29 (s)	2.87(t)
0	3	2,5,2	3.42(s)		3.42(s)	3.11?	2.19, 1.51, 2.19	3.11?	3.26 (t)	2.86(t)
0	1									
2	0	2,2,3	3.40(t)	1.03	~3.1	3.48 (s)	-	3.48 (s)	~3.0	$(CH_2)_2CH_2SR$
0	0	3,2,2	3.2(I_2)	-	3.2(t)	3.2 (t)	-	3.26 (t)	3.3 (t)	1.7 2.88

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13. ABSTRACT

The synthesis of the polyamines phosphorothioic acids $NH_2(CH_2)_n''NH(CH_2)_n'NH(CH_2)_nSPO_3H$ was achieved where n=2 and 3, n' = 2, 3, 4 and 5; n'' = 2, 3 and 4. The toxicity and radiation dose reduction factor (DRF) were determined in mice. The compounds were given intraperitoneally before irradiation. The mice were irradiated with ^{134}Cs gamma radiation source at dose-rate of approximately 90 r/min.

The best DRF (1.8) was obtained for the compounds with n = n' = n'' = 2 and when n = n'' = 2 and n' = 3. The toxicity increased and protective activity decreased when the number of carbon atoms in the alkyl chains was increased. The preparation of pure thiol derivatives was not totally successful. The one impure thiol compound when n = n' = n'' = 2 obtained had a DRF of 1.41. The disulfide derivatives were prepared but were more toxic and less protective than the parent compounds. The compounds were not protective when given orally or after irradiation. The relationship between chemical structure and radiation protective activity is discussed.

DSIS

KEY WORDS

Synthesis

Toxicity

Radiation Protection

Mice

Aminoalkylaminoalkylaminoalkylphosphorothioic

Aminoalkylaminoalkylaminoalkylphosphorothioates

Dose-reduction Factor

Gamma

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