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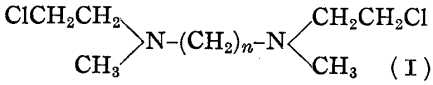
27. Morizo Ishidate, Yoshio Sakurai, and Kozo Maruyama : Studies on Carcinostatic Substances. XVIII.* Anticancer Action of N,N′-Bis(2-chloroethyl)-N,N′-dimethylpolymethylenediamines.

(*Iatrochemical Institute of Pharmacological Research Foundation***)

Variation of anticancer activity of N,N′-bis(2-chloroethyl)polymethylendiamine (I) with an increase in the length of the central methylene chain has not been investigated.



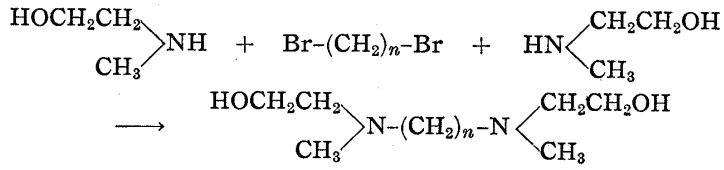
There has been the work of English authors[1] in the past literature with compounds having two (N-aryl-N-2-chloroethyl)amino groups apart in one molecule and they concluded that the activity on tumors was potent enough when n was 2 or 3, but decreased with further increase in the number of n.

The chemical attitude of nitrogen mustards derived from aromatic 2-chloroethylamine is believed to be not completely similar to those of aliphatic derivatives. In addition to it, it was deemed interesting to know whether there was any optimum length of methylene chain in order to obtain the highest selectivity of action of the compound among the tumor and host.

This paper deals with the preparation of the compound (I) ($n=2, 3, 4, 6$, and 9) and discussion on a correlation between their chemical property and biological behavior.

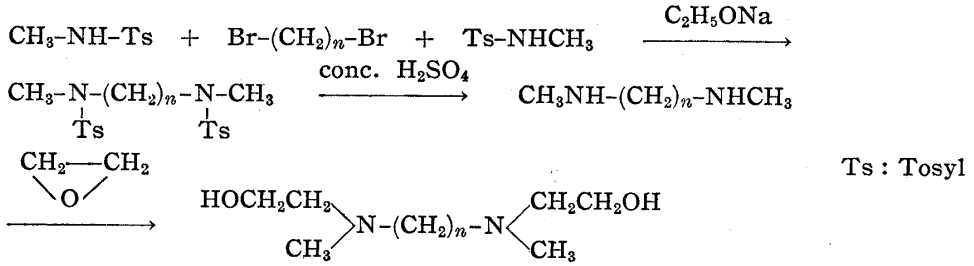
Various processes are available to prepare the dihydroxy-amines of this series.

(A)



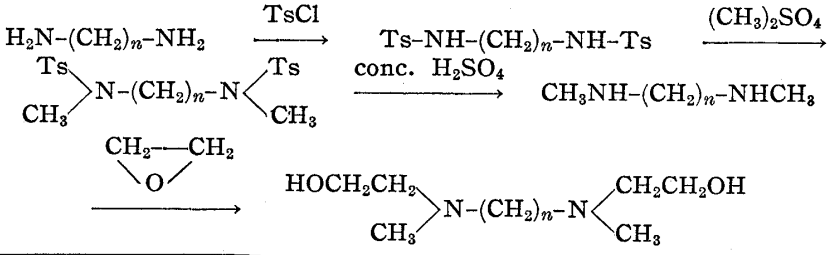
This process (A) was applicable to the preparation of compounds with $n=2, 3$, and 9, and also bis[(N-2-hydroxyethyl-N-methyl)aminoethyl] ether. However, the yield of

(B)



Ts : Tosyl

(C)



* Part XVII : This Bulletin, **5**, 435(1957).

** 26 Nishigahara 1-chome, Kita-ku, Tokyo (石舘守三, 桜井欽夫, 丸山幸三).

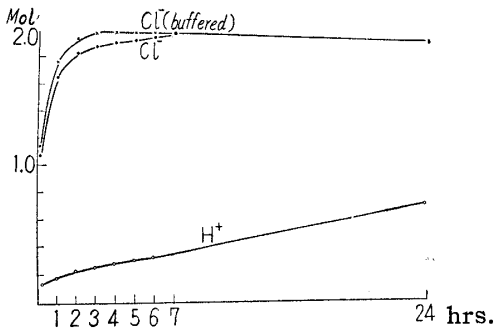
1) G. A. R. Kon, J. J. Robert : J. Chem. Soc., **1950**, 978.

the preparation of N-methyl-2-hydroxyethylamine itself by this process could not be raised over 30% of the calculated and also no small amount of the quaternary amine was often mixed in the end product of the reaction.

The processes (B) and (C) were found more useful than (A) and the compounds ($n=4$ and 6) were synthesized by these procedures in a good yield. The total yield depended chiefly on the yield of hydrolysis of tosyl groups.

There was generally no particular difficulty in chlorinating the hydroxy-amines with thionyl chloride, but in the case of bis[(N-2-chloroethyl-N-methyl)aminoethyl] ether alone, it should be kept at room temperature all through the chlorinating reaction, because the ether bond of the central chain was easily cleaved by the reagent at a higher temperature, yielding N-methyl-bis(2-chloroethyl)amine as the chief product. The hydrochloride of most of these bases were well crystallizing and not so unstable or hygroscopic.

The reaction of the compounds in an approximately neutral aqueous solution was traced by titrating Cl^- and H^+ liberation and the presumption that two 2-chloroethyl groups transformed into oxazetidinium rings at the same time and the same velocity was supported by the result of this experiment. A few titration curves are shown in Fig. 1.



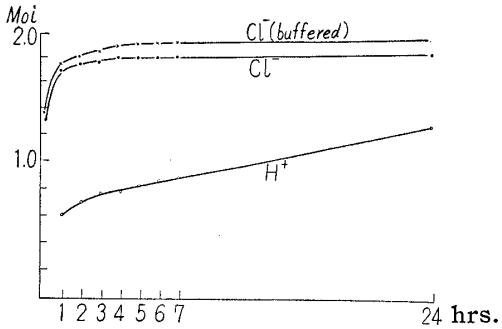
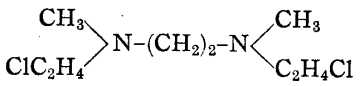
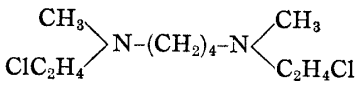


Fig. 1.





A delay of H^+- over Cl^--liberation in the figure meant the formation of an intermediate quaternary ring base at that instant and an inclination of the ascending curve of Cl^--liberation did not show a sudden decrease at the site corresponding to one molar equivalent liberation. On the contrary, it is well known[2] that out of the two functional groups of N-methyl-bis(2-chloroethyl)amine, the one reacted more than ten times faster than the other.

This one-step reaction of two functional groups of these compounds was also observed in the reaction with thiosulfate and, therefore, the compounds having one functional group on each of the two nitrogen atoms were expected to have a different biological reaction from the ordinary bifunctional nitrogen mustards.

2) M. Bergmann, *et al.*: J. Org. Chem., **11**, 518(1946).

Thiosulfate comsumptions of the series of compounds during 10 and 120 mins. of incubation tended to increase as the polymethylene chain became longer, but the apparent slow rate of the lower derivatives in alkylating reaction should be understood as a partial inactivation by hydrolysis before the main reaction ended, because the thiosulfate consumption of ethylenediamine derivative proceeded no longer after 10 mins.' incubation. The data are summarized in Table I.

Table I.

	Thiosulfate consumption		
	10 mins.	2 hrs.	24 hrs.
H_3C, ClH_4C_2 > N-$(CH_2)_n$-N < CH_3, C_2H_4Cl			
n: 2	0.32	0.34	0.34
3	0.62	1.22	
4	0.87	1.53	
6	0.96	1.71	
9	0.98	1.76	
$CH_3N(C_2H_4Cl)_2$	0.92	1.88	

The effect of a series of these compounds to lower the viscosity of polymethacrylate solution was also determined after the procedure described by Maruyama[3] and, from the results shown in Table II, a conclusion could be drawn that all these compounds exhibited the esterification rate as small as monofunctional agents such as N-2-chloroethyl-diethylamine, but their rate of viscosity depression was fully comparable to those of the typical bifunctional nitrogen mustards. It is worthy of note that, even in the case of highest homolog ($n=9$), influence on the viscosity was proved to be of the same level as that of N-methyl-bis(2-chloroethyl)amine. Judging from the molecular dimension of the compound, the sites of esterification on a molecule of polymethacrylate by the two functional groups of the compound could not be the adjacent two carboxyl groups, and this fact was not coincident with the opinion of Alexander[4] who stated that the viscosity depressing activity depended on the distance between two functional groups.

Table II.

Compounds	Rate of viscosity depression at 10 cm. (pressure) at 28°	Esterified-COOH in mol. equiv.
$(C_2H_5)_2NC_2H_4Cl$	7	0.2
H_3C, ClH_4C_2 > N-$(CH_2)_n$-N < CH_3, C_2H_4Cl	—	—
n: 2	34	0.25
3	40	0.3
4	40	0.25
6	40	0.25
9	35	0.5
$CH_3N(C_2H_4Cl)_2$	40	0.5

Relative viscosity of the control, 0.054% solution of sodium polymethacrylate, η_c 1.76.

The compounds described above were tested for their anticancer activity on the Yoshida sarcoma and the result is shown in Table III, while their bacteriostatic activities on *E. coli* are listed in Table IV.

The biological experiments are yet in progress but some of them were published elsewhere.[5]

The fact however should be noted that the respective activities of the compounds

3) K. Maruyama : This Bulletin, **2**, 220(1954).
4) P. Alexander : Nature, **169**, 226, 572(1952).
5) M. Ishidate, *et al.* : Gann, **46**, 469, 475(1955).

Table III.

H_3C, ClH_4C_2 > N-$(CH_2)_n$-N < CH_3, C_2H_4Cl	MTD (mg./kg.)	MED (mg./kg.)	LD_{50} (mg./kg.)	Effective period* (mins.)	$\frac{LD_{50}}{MED}$
n: 2	2.0	0.05	1.75	5	35
3	0.5	0.25	0.75	10	3
4	1.0	0.05	3.0	60	60
6	5.0	0.1	7.5	120	75
9	50.0	1.0	75.0		75
$CH_3N(C_2H_4Cl)_2$	1.0	0.1	1.6	45	16
$CH_3N(C_2H_4Cl)_2 \rightarrow O$	40.0	1.0	80.0	90	80

* Period during which the compounds remain effective in the peritoneal cavity of a rat after intraperitoneal injection.

Table IV.

CH_3, ClC_2H_4 > N-$(CH_2)_n$-N < CH_3, C_2H_4Cl	Min. concn. of inhibition* ($\times 10^{-3}$ mol.)	
	24 hrs.	49 hrs.
n: 2	0.8	1.7
4	1.5	3.1
6	1.4	1.4
9	0.2	0.6
$CH_3N(C_2H_4Cl)_2$	1.3	1.3

* A usual synthetic medium was used in the estimation.

on host and on tumor gradually fell together as the polymethylene chain of the compound became longer. The decrease in anticancer activity with lengthening of the polymethylene chain was found to be far less than that in toxicity, and it was anticipated that chemotherapeutic index, viz. LD_{50}/MED,* of the higher homologs might be rather improved, when it was compared with those of the lower homologs.

So far as the Yoshida sarcoma was concerned, the derivatives of hexamethylenediamine and nonamethylenediamine exhibited excellent efficacy, having the indices nearly equal to that of N-methyl-bis(2-chloroethyl)amine N-oxide.

How long the compound remained in the ascites of a rat at least in its minimum effective concentration when it was given intraperitoneally was examined and a brief data of this experiment are also given in Fig. 4. It was interesting that the compound having the highest chemotherapeutic index on the Yoshida sarcoma remained unchanged in the ascites of a rat for the longest period.

The authors are indebted to Prof. T. Yoshida of the University of Tokyo and Dr. H. Satoh of Iatrochemical Institute of Pharmacological Research Foundation, Tokyo, for the biological experiments, to Miss H. Komai for the bacteriostatic data and to Mr. D. Ohata for microanalytical data. This work was supported by a Grant in Aid of Scientific Research from the Ministry of Education for which the authors are greatly indebted.

Experimental

N,N′-Bis(2-hydroxyethyl)-N,N′-dimethylethylenediamine (I)—A mixture of 4 moles of N-methylethanolamine, 1 mole of ethylene dibromide, and 1.2 moles of anhyd. K_2CO_3 was dissolved in dehyd. EtOH and refluxed for 60~70 hrs. The insoluble inorganic salts were removed when cooled and the filtrate was evaporated to dryness. The residue was then extracted with Et_2O, Et_2O was removed, and the residue was evaporated *in vacuo* in order to distil the unreacted reagents. The final residue consisted of a nearly pure tertiary amine which could be used without purification for the next procedure. Yield, 50%. b.p_{15} 174°. It is identified with b.p. in the reference.[6] It was preferable to use an excess of N-methylethanolamine and to keep the reaction mixture at a low temperature to

* MED: Minimum effective dose given intraperitoneally on the Yoshida sarcoma rat to induce the morphologically abnormal findings on the tumor cells.

6) Chem. Zentr., **107** II, 4255(1936).

avoid the formation of quaternary amines as by-products.

N,N′-Bis(2-hydroxyethyl)-N,N′-dimethyltrimethylenediamine—Obtained by the same procedure as (I). Picrylsulfonate: m.p. 225°(from EtOH). *Anal.* Calcd. for $C_{21}H_{28}O_{20}N_8S_2$: C, 32.47; H, 3.60; N, 14.43. Found: C, 32.76; H, 3.54; N, 14.45.

N,N′-Bis(2-hydroxyethyl)-N,N′-dimethylnonamethylenediamine—Prepared by the same procedure as (I) starting from 1,9-dibromononane, prepared by brominating (PBr_3) 1,9-nonanediol, which was obtained from dimethyl azelanate by the Bouveault-Blanc reduction. Picrylsulfonate (from EtOH): m.p. 158°. *Anal.* Calcd. for $C_{27}H_{40}O_{20}N_8S_2$: C, 37.67; H, 4.65; N, 13.02. Found: C, 37.68; H, 4.58; N, 12.80.

Bis[(N-2-hydroxyethyl-N-methyl)aminoethyl] Ether—In this case, refluxing of bis(2-bromoethyl) ether with N-methylethanolamine should not be continued over 2.5 hrs. to avoid the formation of a quaternary by-product. $b.p_2$ 160~180°. Picrylsulfonate: Plates (from EtOH), m.p. 172°. *Anal.* Calcd. for $C_{22}H_{30}O_{21}N_8S_2$: C, 32.75; H, 3.72; N, 13.89. Found: C, 32.75; H, 3.57; N, 13.68.

N,N′-Dimethyl-N,N′-di(*p*-tosyl)tetramethylenediamine—N-Methyl-*p*-toluenesulfonamide (100 g.) was dissolved in EtOH (350 cc.) which was preliminarily added with Na (13.5 g.). The solution was then added with tetramethylene dibromide (65 g.) and refluxed for 13 hrs. The separated NaBr was removed while hot and the fiitrate was chilled. The precipitate was recrystallized from Me_2CO-EtOH. Colorless needles, m.p. 132~133°. Yield, 75 g. *Anal.* Calcd. for $C_{20}H_{28}O_4N_2S_2$: C, 56.57; H, 6.65; N, 6.60. Found: C, 56.40; H, 6.65; N, 6.70.

N,N′-Dimethyltetramethylenediamine (II)—A mixture of N,N′-dimethyl-N,N′-di(*p*-tosyl)tetramethylenediamine (0.1 mole) and 93% H_2SO_4 (5.0 g.) was heated at 120~130° in an oil bath for 17 hrs. When cool, the solution was cautiously neutralized with conc. NaOH under chilling and after removing the separated salts, the secondary amine was extracted automatically with Et_2O for 24 hrs. b.p. 168~170°. Yield, 8 g. Picrate: Yellow needles (from Me_2CO-EtOH), m.p. 198~200°. *Anal.* Calcd. for $C_{18}H_{22}O_{14}N_8$: C, 37.63; H, 3.85; N, 19.51. Found: C, 38.06; H, 3.90; N, 19.86.

N,N′-Bis(2-hydroxyethyl)-N,N′-dimethyltetramethylenediamine (III)—Ethylene oxide (0.2 mole) was passed into an aq. 50% solution of N,N′-dimethyltetramethylenediamine (0.1 mole) at room temperature. After evaporation, the residue was distilled *in vacuo*. $b.p_6$ 125°. Yield, 14 g. Picrylsulfonate: m.p. 197°(decomp.) (from H_2O). *Anal.* Calcd. for $C_{22}H_{30}O_{20}N_8S_2$: C, 33.41; H, 3.79; N, 14.17. Found: C, 33.24; H, 3.79; N, 14.44.

N,N′-Di(*p*-tosyl)hexamethylenediamine—A mixture of hexamethylenediamine (81 g.), H_2O (80 cc.), and *p*-toluensulfonyl chloride (267 g.) was added portionwise with 10% NaOH (560 g.) under stirring on a water bath during 2 hrs. After cool, the precipitate was washed with Et_2O to remove *p*-toluenesulfonyl chloride and recrystallized from EtOH, m.p. 154~155°. Yield, 300 g. *Anal.* Calcd. for $C_{20}H_{28}O_4N_2S_2$: C, 56.65; H, 6.66; N, 6.61. Found: C, 56.58; H, 6.72; N, 6.59.

N,N′-Dimethyl-N,N′-di(*p*-tosyl)hexamethylenediamine—N,N′-Di(*p*-tosyl)hexamethylenediamine (300 g.) was dissolved in 15% ethanolic KOH (550 g.). To the solution was added Me_2SO_4 (90 g.) under stirring, in which a part of the reaction product appeared instantly as colorless crystals. The rest of the product separated when diluted with H_2O. It was recrystallized from EtOH, m.p. 152~153°. Yield, 300 g. *Anal.* Calcd. for $C_{22}H_{32}O_4N_2S_2$: C, 58.40; H, 7.08; N, 6.20. Fonud: C, 58.21; H, 7.04; N, 6.27.

N,N′-Dimethylhexamethylenediamine—Obtained by the same procedure as for (II). $b.p_2$ 65~70°. Yield, 6.5 g. Picrate: Yellow needles (from Me_2CO-EtOH), m.p. 137°.

N,N′-Bis(2-hydroxyethyl)-N,N′-dimethylhexamethylenediamine—It was obtained by the same procedure as (III). $b.p_2$ 138~140°. Yield, 12 g. Picrylsulfonate (from H_2O): m.p. 199°(decomp.). *Anal.* Calcd. for $C_{24}H_{34}O_{20}N_8S_2$: C, 35.20; H, 4.15; N, 13.77. Found: C, 35.15; H, 4.12; N, 13.77.

Chlorination of N,N′-Bis(2-hydroxyethyl)-N,N′-dimethylpolymethylenediamine—N,N′-Bis(2-hydroxyethyl)-N,N′-dimethylpolymethylenediamine (0.05 mole) was dissolved in dehyd. $CHCl_3$ (50cc.) and $SOCl_2$ (15 g.) added dropwise under 50° with mechanical stirring. Refluxing for 2 hrs. at 60~70°, both the solvent and excess of $SOCl_2$ were evaporated. The dark colored residue was decolorized with activated carbon and recrystallized from dehyd. Me_2CO or dehyd. EtOH.

N,N′-Bis(2-chloroethyl)-N,N′-dimethylethylenediamine dihydrochloride: Colorless and hygroscopic needles, m.p. 182°. Yield, 81%. *Anal.* Calcd. for $C_8H_{20}N_2Cl_4$: C, 33.56; H, 6.99; N, 9.79. Found: C, 33.47; H, 7.02; N, 9.51.

N,N′-Bis(2-chloroethyl)-N,N′-dimethyltrimethylenediamine dihydrochloride: Colorless and hygroscopic needles, m.p. 243°. Yield, 42%. *Anal.* Calcd. for $C_9H_{22}N_2Cl_4$: C, 36.00; N, 7.33; N, 9.33. Found: C, 36.31; H, 7.08; N, 9.72. Picrate: m.p. 213°. *Anal.* Calcd. for $C_{21}H_{26}O_{14}N_8Cl_2$: C, 36.78; H, 3.79; N, 16.35. Found: C, 36.76; H, 3.51; N, 16.40.

N,N′-Bis(2-chloroethyl)-N,N′-dimethyltetramethylenediamine dihydrochloride: Colorless and hygroscopic needles, m.p. 218~220°. Yield, 85%. *Anal.* Calcd. for $C_{10}H_{24}N_2Cl_4$: C, 38.23; H, 7.70; N, 8.92. Found: C, 38.51; H, 8.12; N, 8.86.

N,N′-Bis(2-chloroethyl)-N,N′-dimethylhexamethylenediamine dipicrate (from Me_2CO-EtOH): m.p. 148~150°. Yield, 30%. *Anal.* Calcd. for $C_{24}H_{32}O_{14}N_8Cl_2$: C, 39.65; H, 4.44; N, 15.44. Found: C,

40.04; H, 4.37; N, 15.20. The hydrochloride became finally crystalline when it was prepared from its purified picrate, as colorless and hygroscopic needles, m.p. 182~183°. *Anal.* Calcd. for $C_{12}H_{28}N_2Cl_4$: C, 42.12; H, 8.25; N, 8.19. Found: C, 41.95; H, 8.31; N, 8.35.

N,N′-Bis(2-chloroethyl)-N,N′-dimethylnonamethylenediamine dipicrate (from EtOH): m.p. 147~148°. Yield, 70%. *Anal.* Calcd. for $C_{27}H_{38}O_{14}N_8Cl_2$: C, 42.13; H, 4.94; N, 14.56. Found: C, 42.42; H, 5.02; N, 14.57. The hydrochloride was prepared from the purified picrate as colorless and hygroscopic needles, m.p. 161° (from Me_2CO-EtOH). *Anal.* Calcd. for $C_{15}H_{34}N_2Cl_4$: C, 46.87; H, 8.85; N, 7.29. Found: C, 46.83; H, 8.77; N, 7.11.

Bis[(N-2-chloroethyl-N-methyl)aminoethyl] ether: In this case, the hydroxy compound should be kept with $SOCl_2$ at room temperature without any solvent for 1 day, otherwise the ether bond is cleaved and N-methyl-bis(2-chloroethyl)amine alone is formed. The picrate of the product could be purified by recrystallization from Me_2CO of the crude picrate obtained from the reaction mixture. m.p. 175°. Yield, a few %. Hydrochloride could not be obtained in crystalline state.

Determination of Cl^- and H^+ Liberation—An aq. solution of hydrochloride of the base (0.02 m. mole/cc.) was neutralized with 2 mol. equiv. of NaOH and incubated at 37°. An aliquot of the solution was successively taken out at every 1-hr. intervals. Cl^- and H^+ were titrated with 0.01*N* $AgNO_3$(indicator: Na-salt of tetrabromophenolphthalein ethyl ester) and 0.01*N* NaOH(indicator, thymolphthalein) respectively.

Determination of Cl^- in $NaHCO_3$-buffered Solution—A sample solution of the same concentration as above, but containing 0.08 m.mole/cc. of $NaHCO_3$, was titrated with 0.01*N* $AgNO_3$ by the same procedure as described above.

Determination of Thiosulfate Consumption in $NaHCO_3$-buffered Solution—The sample (0.5 m. mole) was added with 10% aq. $NaHCO_3$ (2 cc.) and 0.1*N* $Na_2S_2O_3$ (10 cc.), and immediately diluted to 50 cc. with H_2O. It was incubated at 37° and 10-cc. portion was taken out after 10 mins., 2 hrs., and 24 hrs., if necessary from the beginning of incubation, and titrated with 0.02*N* I_2.

Determination of Rate of Viscosity Depression of Polymetacrylate Solution—Experiment was carried out after the description published by one of the authors.[3]

Summary

Preparation, chemical properties, and carcinostatic activity on the Yoshida sarcoma of N,N′-bis(2-chloroethyl)-N,N′-dimethylpolymethylenediamines were discussed. It was concluded that the derivatives of hexamethylenediamine and nonamethylenediamine were more promising than the corresponding lower homologs with respect to chemotherapeutic indices.

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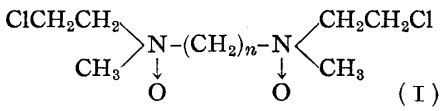
28. Morizo Ishidate, Yoshio Sakurai, and Kozo Maruyama: Studies on Carcinostatic Substances. XIX.* The Preparation and Properties of N,N′-Bis(2-chloroethyl)-N,N′-dimethylpolymethylenediamine N,N′-Dioxides.

(*Iatrochemical Institute of Pharmacological Research Foundation***)

In the preceding paper, the authors reported on some derivatives of N,N′-bis(2-chloroethyl)-N,N′-dimethylpolymethylenediamine. The present report deals with the preparation of the N,N′-dioxides (I) of those compounds.



(I) n=2, 4, 6, 9

The ordinary oxidation process using peracids was successful in most of the cases.

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