Synthesis of Potentially Cytoactive Amino Acid Amide Mustards[1a]

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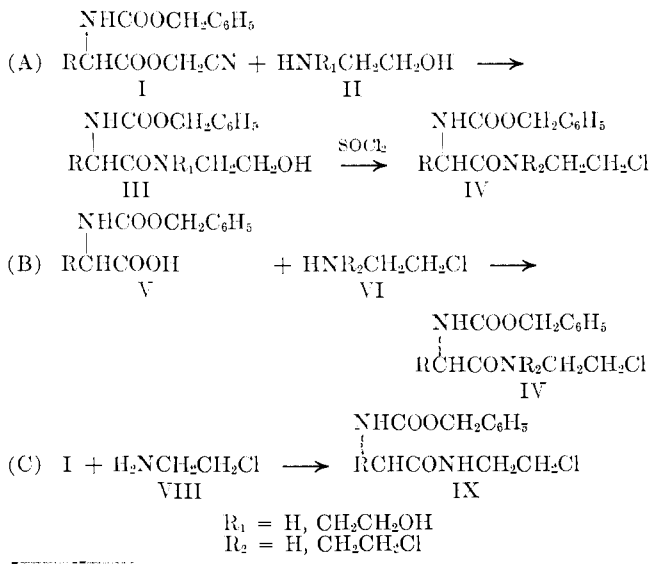
Methods have been developed for the synthesis of amino acid mono- and bis(2-chloroethyl)amides. These have been applied to the synthesis of the bis(2-chloroethyl)amides of DL-alanine, DL-phenylalanine, and DL-leucine, and to the 2-chloroethylamides of glycine, L-alanine, DL-alanine, DL-phenylalanine, DL-leucine, DL-asparagine, and α- and γ-L-glutamic acids. In most cases the products to which compounds of this type readily rearrange in water, the corresponding aminoethylcarboxylic esters, have been isolated and characterized. When tested against cells in tissue culture, the "one-armed" mustard derivatives were uniformly cytotoxic, whereas the corresponding "two-armed" derivatives were either inactive or showed borderline cytotoxicity.

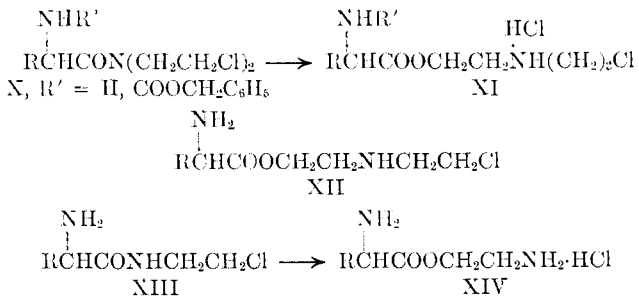
Nitrogen mustard amides and peptides[2] are of interest as antitumor agents because of their possible selectivity of action.[3] These compounds are, in a sense, latent derivatives of the alkylating agents that would be activated by hydrolysis of the amide bond. To the extent that such hydrolysis occurred selectively in the tumor, cytotoxic action would tend to localize at that site. Moreover, if amino acids from which these compounds are derived act as biological "carriers," they may serve to introduce these derivatives into cellular metabolism.

This paper deals with the synthesis of nitrogen mustard amides of a series of amino acids. These compounds have been submitted for cytotoxicity studies in tissue culture and animal testing for antitumor activity in the screening program at the Children's Cancer Research Foundation Inc., Boston. The results of these biological investigations will be published in detail elsewhere. Some preliminary results of the cytotoxicity studies are reported here.

In an earlier communication[4] we reported the synthesis of glycine bis(2-chloroethyl)amide by two different routes involving A, condensation of the activated cyanomethyl N-carbobenzoxyglycinate with diethanolamine and subsequent chlorination of the resulting diol; and B, direct condensation of N-carbobenzoxyglycine with bis(2-chloroethyl)amine (nor-HN2) in the presence of N,N′-dicyclohexylcarbodiimide (DCC) to give the same N-carbobenzoxyglycine bis(2-chloroethyl)amide intermediate; the desired product was produced by catalytic hydrogenolysis of the protecting carbobenzoxy group. Both routes afforded pure product, essentially free of the ester to which amides of this type readily rearrange.[5,6] We have now developed these synthetic procedures toward the preparation of the corresponding "two-armed" bis(2-chloroethyl) mustard amides of DL-alanine, DL-phenylalanine, and DL-leucine, as well as "one-armed" 2-chloroethyl mustard amides derived from glycine, L-alanine, DL-alanine, DL-phenylalanine, DL-leucine, α- and γ-L-glutamic acids, and DL-asparagine.



The acid-catalyzed rearrangement of N,N-di(2-chloroethyl)carboxamides to the corresponding 2-acyloxyethyl-2′-chloroethylammonium chloride is known.[6] We, however, effected this intramolecular acyl migration (N→O) in the derivatives X of bis(2-chloroethyl)amides of N-carbobenzoxyglycine, DL-alanine, and DL-phenylalanine by treatment with water. The resulting 2-acyloxyethyl-2′-chloroethylammonium salts XI in each case were isolated and identified. The corresponding decarbobenzoxylated derivatives XII of amino acids were obtained by catalytic (10% palladium–charcoal) hydrogenolysis. One-armed mustard (2-chloroethyl)amides (XIII) of glycine, L-alanine, and DL-phenylalanine also underwent similar rearrangement to the corresponding 2-acyloxyethylammonium salts (XIV) when treated with water, although reflux in water–ethanol (1:1) for 24 hr. was required to bring this change, in contrast to the two-armed



(1) (a) Supported by a research grant, (CA 02130) from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service. (b) To whom inquiries should be addressed.

(2) J. K. Chakrabarti and O. M. Friedman, *Chem. Ind.* (London), 898 (1965).

(3) O. M. Friedman and R. Chatterji, *J. Am. Chem. Soc.*, **81**, 3750 (1959).

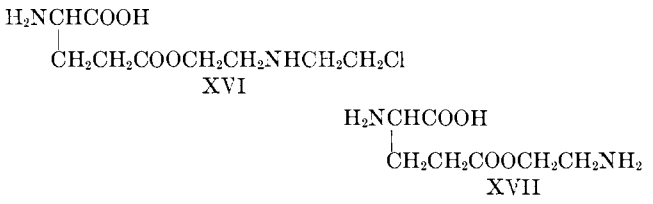
(4) S. Bien and O. M. Friedman, *Chem. Ind.* (London), 2062 (1962).

(5) T. S. Safonova and S. I. Sergievskaya, *J. Gen. Chem. USSR*, **31**, 1104 (1961); *Zh. Obshch. Khim.*, **32**, 1351 (1962).

(6) W. C. J. Ross and J. G. Wilson, *J. Chem. Soc.*, 3616 (1959).

compounds, where, under the same conditions, rearrangement was essentially complete within 2 hr. The nature of this rearrangement could be readily recognized from the characteristic shift of the amide carbonyl (1660 cm.$^{-1}$) to the ester carbonyl (1750 cm.$^{-1}$) in the infrared absorption during reaction. These rearranged products are listed in Table I.

A number of unsuccessful attempts were made to prepare the two-armed γ-glutamoyl mustard amide. Repeated attempts to prepare this compound by the DCC condensation of N-carbobenzoxy-α-benzylglutamate with bis(2-chloroethyl)amine (nor-HN2) and subsequent hydrogenolysis gave mixtures from which only the rearranged ester XVI could be isolated despite strict precautions to exclude moisture. Interestingly, an early attempt by an entirely different approach involving oxidation of the N-carbobenzoxy-α-benzyl-γ-glutamoyl hydrazide with iodine in the presence of nor-HN2 by a modification of the original Carpino procedure,[7] gave only the rearranged ester XVI (Table IIf) and no trace of the desired amide. However, the preparation of the corresponding one-armed mustard amides of γ-glutamic acid presented no such difficulty, when made through the route A. The rearrangement of this amide to the corresponding ester XVII was effected as usual by refluxing in 50% ethanol. Apparently, bis(2-chloroethyl)amide of γ-glutamic acid is extremely unstable and undergoes rapid rearrangement to the ester under the reaction conditions. The relevant γ-glutamoyl derivatives that were prepared are listed in Table II.



XVI

XVII

The desired reactive cyanomethyl esters (Table III) were prepared by the interaction of the N-carbobenzoxy amino acids with 0.5–1.0 molar excess of chloroacetonitrile and triethylamine preferably in the absence of solvent. These activated esters, when treated with 1 molar equiv. of N,N-diethanolamine or 2-hydroxyethanolamine underwent aminolysis to produce the desired amides (Table IV). The reactions, catalyzed by a few drops of acetic acid, were carried out in tetrahydrofuran or ethyl acetate at room temperature, although elevated temperatures were occasionally required. The resulting hydroxyamides were converted to the corresponding chloramides by treatment with thionyl chloride in chloroform (Table V). This aminolysis was also conveniently effected in certain cases when the cyanomethyl esters of N-carbobenzoxyamino acids were treated with 1.5–2 molar excess of freshly liberated base, 2-chloroethylamine, to yield the desired amides in one step (method C). This procedure has been particularly useful in the preparation of 2-chloroethylamides of N-carbobenzoxy-L-glutamine, and N-carbobenzoxy-DL-asparagine, where the DCC coupling produced very poor yields. Bis(2-chloroethyl)amides and 2-chloroethylamides of N-carbobenzoxyamino acids were also conveniently obtained (in better yields in some cases) through the action of 1 equiv. of N,N′-dicyclohexylcarbodiimide on 1 equiv. of N-carbobenzoxyamino acid and 2–3 equiv. of the appropriate amine, bis(2-chloroethyl)amine or 2-chloroethylamine, freshly prepared from their respective hydrochlorides (Table V). The desired final products, "two-armed" or "one-armed" mustard amides, were procured by hydrogenolysis of the N-protecting carbobenzoxy group in absolute ethanol with 10% palladium–charcoal catalyst in the presence of 1 molar equiv. of either hydrochloric acid or oxalic acid to give appropriate salts (Table VI).

Biological Results.—Initial results of testing of a series of these compounds against the KB cell line in tissue culture in the screening program[8] have given reproducible values for ID_{50} in the range of 5–50 μg./ml. for the mono-2-chloroethylamides of L-leucine, glycine, DL-alanine, DL-phenylalanine, and α- and γ-L-glutamic acid, and values in the range of 100 to >1000 μg./ml. for the corresponding bis(2-chloroethyl)amides of L-leucine, glycine, DL-alanine, and DL-phenylalanine. These results indicate a rather striking difference in behavior between the "one-armed" mustard derivatives which are uniformly cytotoxic and the "two-armed" derivatives which show only borderline cytotoxicity or are inactive in this system. The reason for this difference is not too clearly understood and may simply reflect the difference in the rates of rearrangement to the corresponding esters that the two classes of compounds undergo; or it may be related to the fact that the "two-armed" mustards, lacking an amide hydrogen, are less susceptible to enzymatic hydrolysis that would be required for "activation." Of particular interest, however, is the fact that the "one-armed" compounds are substantially more cytotoxic than the parent mustard, 2-chloroethylamine, from which they are derived. Possibly used as an amino acid amide, this simple alkylating agent reaches some more critical, intracellular site where it is released, than it would administered in its native form.

Experimental Section

The compounds just described were prepared generally by procedures closely analogous to those in the specific cases reported in detail below.

N-Carbobenzoxy-DL-phenylalanine Cyanomethyl Ester.—N-carbobenzoxy-DL-phenylalanine (1.5 g.) was treated with 1.05 ml. of triethylamine, and 0.95 ml. of chloroacetonitrile was added to the viscous mix, with a rise of temperature. The reaction mixture was left overnight at room temperature, and the excess chloroacetonitrile was removed under reduced pressure. The residue was taken up in ethyl acetate, and the solution was washed with dilute HCl, then with saturated $NaHCO_3$, dried (Na_2SO_4), and evaporated. The white residue (2.0 g.) was crystallized from CCl_4; yield 1.5 g., m.p. 96–97.5°.

N-Carbobenzoxy-DL-phenylalanine 2-Hydroxyethylamide (Method A).—To a solution of 1.0 g. of N-carbobenzoxy-DL-phenylalanine cyanomethyl ester in 7 ml. of ethyl acetate 0.17 ml. of 2-hydroxyethylamine was added, and the mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure and the residue was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed with a small volume of cold, saturated NaCl containing a few drops of dilute HCl, then with a small volume of cold, saturated $NaHCO_3$, dried (Na_2SO_4), filtered, and evaporated. The residue (1.1 g.) was crystallized from benzene; m.p. 116–117.5° (sintering).

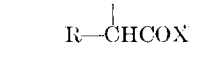
(7) L. A. Carpino, *J. Am. Chem. Soc.*, **79**, 96 (1957).

(8) These studies have been carried out under the direction of Dr. George E. Foley. We are grateful to Dr. Sidney Farber, Director, Children's Cancer Research Foundation Inc., Boston, for permission to refer to these preliminary results.

TABLE I

REARRANGED PRODUCTS OF AMINO ACID DERIVATIVES

2-ACYLOXYETHYLAMMONIUM SALTS

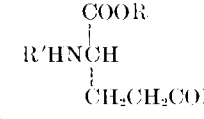


R (amino acid)	R'	X	M.p., °C.	ν_{max}, cm.$^{-1}$	Formula	Calcd., % C	H	Cl	N	Found, % C	H	Cl	N
H (Gly)	H	$OCH_2CH_2NH_2$	157–160	1740	$C_4H_{10}N_2O_2 \cdot 2HCl$	25.14	6.33	37.11	14.67	25.2	6.4	37.2	14.6
CH_3 (L-Ala)	H	$OCH_2CH_2NH_2$	182–184	1745	$C_5H_{12}N_2O_2 \cdot 2HCl$[a]	29.28	6.88	34.57	13.66	29.6	6.8	34.6	13.6
$CH_2C_6H_5$ (DL-Phe)	H	$OCH_2CH_2NH_2$	165–167	1750	$C_{11}H_{16}N_2O_2 \cdot 2HCl$	46.99	6.45	25.22	9.96	47.0	6.4	25.2	9.9
H (Gly)	$COOCH_2C_6H_5$	$O(CH_2)_2NH(CH_2)_2Cl$	106–107	1750	$C_{14}H_{19}ClN_2O_4 \cdot HCl$	47.86	5.70	20.25	7.98	47.7	5.8	20.0	8.3
H (Gly)	$COOCH_2C_6H_5$	$O(CH_2)_2NH(CH_2)_2Cl$	164–165	1750	$C_{14}H_{19}ClN_2O_4 \cdot C_2H_2O_4$	47.47	5.19	8.77	6.92	47.2	5.2	8.4	6.9
H (Gly)	H	$O(CH_2)_2NH(CH_2)_2Cl$	120–122	1754	$C_6H_{13}ClN_2O_2 \cdot 2HCl$	28.42	5.96	41.92	11.07	28.3	6.0	41.8	11.0
CH_3 (DL Ala)	$COOCH_2C_6H_5$	$O(CH_2)_2NH(CH_2)_2Cl$	138–140	1750	$C_{15}H_{21}ClN_2O_4 \cdot HCl$	49.32	6.07	19.41	7.67	49.3	5.9	19.2	7.7
CH_3 (DL-Ala)	$COOCH_2C_6H_5$	$O(CH_2)_2NH(CH_2)_2Cl$	165–167	1755	$C_{15}H_{21}ClN_2O_4 \cdot C_2H_2O_4$	48.75	5.50	8.48	6.69	48.8	5.6	9.0	6.6
CH_3 (DL-Ala)	H	$O(CH_2)_2NH(CH_2)_2Cl$	148–150	1755	$[C_7H_{15}ClN_2O_2]_2 \cdot (C_2H_2O_4)_3$	36.42	5.45	10.77	8.50	36.8	5.7	10.5	8.5
$CH_2C_6H_5$ (DL-Phe)	$COOCH_2C_6H_5$	$O(CH_2)_2NH(CH_2)_2Cl$	136–137	1755	$C_{21}H_{25}ClN_2O_4 \cdot HCl$	57.14	5.90	16.10	6.35	57.2	6.0	15.8	6.1
$CH_2C_6H_5$ (DL-Phe)	$COOCH_2C_6H_5$	$O(CH_2)_2NH(CH_2)_2Cl$	134–135	1750	$C_{21}H_{25}ClN_2O_4 \cdot C_2H_2O_4$	55.81	5.46	7.18	5.66	56.3	5.7	6.9	5.5

[a] $[\alpha]^{20}D$ +10.1° (c 0.89, water).

TABLE II

DERIVATIVES OF γ-L-GLUTAMIC ACID



	R	R'	X	Yield, %	M.p., °C.	$[\alpha]^{25}D$, deg. (concn.)	ν_{max}, cm.$^{-1}$	Formula	Calcd., % C	H	Cl	N	Found, % C	H	Cl	N
a	$CH_2C_6H_5$	$COOCH_2C_6H_5$	OCH_2CN	70	69–71	−17.8 (2.87, acetone)		$C_{22}H_{22}N_2O_6$	64.38	5.40	...	6.83	64.3	5.4	...	6.7
b	$CH_2C_6H_5$	$COOCH_2C_6H_5$	$NHCH_2CH_2OH$	84	100–102	−12.1 (3.70, ethanol)		$C_{22}H_{26}N_2O_5$	63.75	6.32	...	6.76	63.9	6.3	...	6.8
c	$CH_2C_6H_5$	$COOCH_2C_6H_5$	$NHCH_2CH_2Cl$	59	132–134	−2.1 (2.30, $CHCl_3$)		$C_{22}H_{25}ClN_2O_5$	61.02	5.82	8.19	6.47	61.2	5.8	8.5	6.7
d	H	H	$NHCH_2CH_2Cl$	82	141–143	+3.1 (2.56, water)	1660	$C_7H_{13}ClN_2O_3$	40.28	6.28	16.98	13.43	40.3	6.2	17.1	13.4
e	H	H	$OCH_2CH_2NH_2$		75–80	−11.6 (3.12, water)	1755	$C_7H_{14}N_2O_4 \cdot 2HCl$	31.96	6.13	26.95	10.64	31.7	6.4	26.8 (26.7 ionic)	10.6
f	H	H	$O(CH_2)_2NH(CH_2)_2Cl$	18	152–153	−16.1 (3.05, water)	1735 1635	$C_9H_{17}ClN_2O_4 \cdot 2HCl$	37.38	6.27	24.52	9.69	37.6	6.4	24.5 (12.2 ionic)	9.6
g	H	H	$O(CH_2)_2NH(CH_2)_2Cl$	12	152–153	...	Identical with f	$C_9H_{17}ClN_2O_4 \cdot 2HCl$	37.38	6.27	24.52	9.69	37.5	6.3	24.4	9.8

TABLE III

CYANOMETHYL ESTERS OF N-CARBOBENZOXYAMINO ACIDS

$$\begin{array}{l} \mathrm{NHCOOCH_2C_6H_5} \\ | \\ \mathrm{R{-}CHCOOCH_2CN} \end{array}$$

R (amino acid)	Yield, %	M.p., °C.	$[\alpha]^{20}D$, deg. (concn. in acetone)	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
CH_3 (DL-Ala)	74	50–51	...	$C_{13}H_{14}N_2O_4$	59.51	5.38	10.69	59.4	5.4	10.9
CH_3 (L-Ala)	84	40–42	−35.6 (2.98)	$C_{13}H_{14}N_2O_4$	59.51	5.38	10.69	59.6	5.4	10.8
$CH_2C_6H_5$ (DL-Phe)	96	96–97	...	$C_{19}H_{18}N_2O_4$	67.44	5.36	8.28	67.4	5.3	8.3
$CH_2CH(CH_3)_2$ (DL-Leu)	70	87–89	...	$C_{16}H_{20}N_2O_4$	63.14	6.62	9.21	63.31	6.68	9.39
$CH_2CH_2CO_2CH_2C_6H_5$ (L-γ-Bz-Glu)	68	88–89	−20.5 (3.52)	$C_{22}H_{22}N_2O_6$	64.38	5.40	6.83	64.2	5.4	6.8
$CH_2CH_2CONH_2$ (L-Glu-NH_2)	63	133–134	...	$C_{15}H_{17}N_3O_5$	56.42	5.37	13.16	56.51	5.46	12.96
CH_2CONH_2 (DL-Asp-NH_2)	30	108–110	...	$C_{14}H_{15}N_3O_5$	55.08	4.95	13.77	54.91	5.10	13.66
$CH_2CH_2CH_2{-}N{=}C(NHCOOCH_2C_6H_5)_2$ (L-Arg)	70	99–101	...	$C_{32}H_{33}N_5O_8$	62.43	5.40	11.38	62.33	5.51	11.27

TABLE IV

2-HYDROXYETHYL- AND BIS(2-HYDROXYETHYL)AMIDES OF N-CARBOBENZOXYAMINO ACIDS

$$\begin{array}{l} \mathrm{NHCOOCH_2C_6H_5} \\ | \\ \mathrm{R{-}CHCOX} \end{array}$$

R (amino acid)	X	Yield, %	M.p., °C.	$[\alpha]D$, deg. (concn. in MeOH)	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
H (Gly)	$N(CH_2CH_2OH)_2$	58	82–84	...	$C_{14}H_{20}N_2O_5$	56.7	6.8	9.5	56.6	6.8	9.6
H (Gly)	$NHCH_2CH_2OH$	82	114–115[a]	...							
CH_3 (DL-Ala)	$NHCH_2CH_2OH$	64	113–115	...	$C_{13}H_{18}N_2O_4$	58.63	6.81	10.52	58.5	6.8	10.6
CH_3 (L-Ala)	$NHCH_2CH_2OH$	80	113–114	−13.5 (2.29)	$C_{13}H_{18}N_2O_4$	58.63	6.81	10.52	58.4	6.6	10.7
$CH_2C_6H_5$ (DL-Phe)	$NHCH_2CH_2OH$	80	116–117	...	$C_{19}H_{22}N_2O_4$	66.65	6.48	8.18	67.0	6.6	8.0
$CH_2CH_2CO_2CH_2C_6H_5$ (γ-Bz-L-Glu)	$NHCH_2CH_2OH$	82	120–122	−4.5 (2.88)	$C_{22}H_{26}N_2O_6$	63.75	6.32	6.76	63.9	6.3	6.8

[a] D. Schäufele, B. Prijs, and H. Erlenmeyer [*Helv. Chim. Acta*, **38**, 1345 (1955)] report m.p. 115–116°.

TABLE V

2-CHLOROETHYL- AND BIS(2-CHLOROETHYL)AMIDES OF N-CARBOBENZOXYAMINO ACIDS

$$\begin{array}{l} \mathrm{NHCOOCH_2C_6H_5} \\ | \\ \mathrm{R{-}CHCOX} \end{array}$$

No.	R (amino acid)	X	Method	Yield, %	M.p., °C.	Formula	Calcd., % C	Calcd., % H	Calcd., % Cl	Calcd., % N	Found, % C	Found, % H	Found, % Cl	Found, % N
1	H (Gly)	$NHCH_2CH_2Cl$	A	55	115–117	$C_{12}H_{15}ClN_2O_3$	53.23	5.59	13.10	10.35	53.0	5.6	13.1	10.3
2	H (Gly)	$NHCH_2CH_2Cl$	C	17	115–117	$C_{12}H_{15}ClN_2O_3$	53.23	5.59	13.10	10.35	53.3	5.5	13.0	10.3
3	CH_3 (DL-Ala)	$NHCH_2CH_2Cl$	A	56	102–104	$C_{13}H_{17}ClN_2O_3$	54.83	6.02	12.46	9.84	54.9	6.2	12.5	9.9
4	CH_3 (DL-Ala)	$NHCH_2CH_2Cl$	B	65	102–104[a]	$C_{13}H_{17}ClN_2O_3$								
5	CH_3 (L-Ala)	$NHCH_2CH_2Cl$	A	84	124–126	$C_{13}H_{17}ClN_2O_3$[b]	54.83	6.02	12.46	9.84	55.1	6.1	12.3	10.1
6	$CH_2C_6H_5$ (DL-Phe)	$NHCH_2CH_2Cl$	A	56	125–127	$C_{19}H_{21}ClN_2O_3$	63.24	5.87	9.83	7.77	62.4	5.3	10.0	7.9
7	$CH_2C_6H_5$ (DL-Phe)	$NHCH_2CH_2Cl$	B	45	125–127	$C_{19}H_{21}ClN_2O_3$	63.24	5.87	9.83	7.77	63.5	6.0	9.6	7.8
8	$CH_2CH(CH_3)_2$ (DL-Leu)	$NHCH_2CH_2Cl$	B	65	119–121	$C_{16}H_{23}ClN_2O_3$	58.80	7.09	10.85	8.57	59.0	7.2	10.9	8.6
9	$CH_2CH_2CO_2CH_2C_6H_5$ (L-γ-Bz-Glu)	$NHCH_2CH_2Cl$	A	57	130–131	$C_{22}H_{25}ClN_2O_5$[c]	61.02	5.82	8.19	6.47	61.2	6.0	8.1	6.7
10	$CH_2CH_2CONH_2$ (L-Glu-NH_2)	$NHCH_2CH_2Cl$	B	29	180–182	$C_{15}H_{20}ClN_3O_4$	52.71	5.90	10.37	12.29	52.9	6.1	10.4	12.2
11	$CH_2CH_2CONH_2$ (L-Glu-NH_2)	$NHCH_2CH_2Cl$	C	58	182–184[d]	$C_{15}H_{20}ClN_3O_4$								
12	CH_2CONH_2 (DL-Asp-NH_2)	$NHCH_2CH_2Cl$	C	50	192–194	$C_{14}H_{18}ClN_3O_4$	51.30	5.54	10.81	12.82	51.3	5.5	10.7	12.7
13	CH_2CONH_2 (DL-Asp-NH_2)	$NHCH_2CH_2Cl$	B	18	192–193[e]	$C_{14}H_{18}ClN_3O_4$								
14	$CH_2CH_2CH_2{-}N{=}C(NHCO_2CH_2C_6H_5)_2$ (L-Arg)	$NHCH_2CH_2Cl$	B	68	165–166	$C_{32}H_{35}ClN_6O_7$	60.23	5.69	5.56	10.97	60.2	5.8	5.5	11.0
15	H (Gly)	$N(CH_2CH_2Cl)_2$	B	71	87–89	$C_{14}H_{18}Cl_2N_2O_3$	50.47	5.45	21.28	8.41	50.4	5.4	21.1	8.4
16	H (Gly)	$N(CH_2CH_2Cl)_2$	A		87–89[f]	$C_{14}H_{18}Cl_2N_2O_3$								
17	CH_3 (DL-Ala)	$N(CH_2CH_2Cl)_2$	B	68	102–104	$C_{15}H_{20}Cl_2N_2O_3$	51.87	5.76	20.46	8.07	51.9	5.9	20.5	7.9
18	$CH_2C_6H_5$ (DL-Phe)	$N(CH_2CH_2Cl)_2$	B	75	127–129	$C_{21}H_{24}Cl_2N_2O_3$	59.58	5.72	16.75	6.62	60.1	5.9	16.0	6.5
19	$CH_2C_6H_5$ (DL-Phe)	$N(CH_2CH_2Cl)_2$	A		126–128[g]	$C_{21}H_{24}Cl_2N_2O_3$								
20	$CH_2CH(CH_3)_2$ (DL-Leu)	$N(CH_2CH_2Cl)_2$	B	68	101–103	$C_{18}H_{26}Cl_2N_2O_3$	55.55	6.73	18.22	7.20	55.6	6.8	18.3	7.3
21	$CH_2CH_2SCH_3$ (DL-Met)	$N(CH_2CH_2Cl)_2$	B	54	99–101	$C_{17}H_{24}Cl_2N_2O_3S$	50.12	5.94	17.39	6.88 (S, 7.87)	50.3	5.8	17.3	7.1 (S, 7.7)

[a] Undepressed when admixed with **3**. [b] $[\alpha]^{20}D$ −35.6° (*c* 2.98, acetone). [c] $[\alpha]^{20}D$ −17.8° (*c* 1.52, methanol). [d] Undepressed when admixed with **10**. [e] Undepressed when admixed with **12**. [f] Undepressed when admixed with **15**. [g] Undepressed when admixed with **18**.

TABLE VI
2-CHLOROETHYL- AND BIS(2-CHLOROETHYL)AMIDES OF AMINO ACIDS

$$R-CHCON\begin{matrix}X\\ NH_2\end{matrix}$$

R (amino acid)	X	Yield, %	M.p., °C.	ν_{max}, cm.$^{-1}$	Formula	Calcd., % C	H	N	Cl	Found, % C	H	N	Cl
H (Gly)	$NHCH_2CH_2Cl$	77	165–166	1665	$C_4H_9ClN_2O\cdot HCl$	27.51	5.82	16.19	40.98	27.9	5.8	15.9	40.9
CH_3 (DL-Ala)	$NHCH_2CH_2Cl$	65	141–143	1660	$C_5H_{11}ClN_2O\cdot HCl$	32.01	6.47	14.97	37.90	32.2	6.4	14.9	38.1
CH_3 (L-Ala)	$NHCH_2CH_2Cl$	92	179–180	1660	$C_5H_{11}ClN_2O\cdot HCl$[a]	32.10	6.47	14.97	37.90	31.9	6.6	15.0	37.9
$CH_2C_6H_5$ (DL-Phe)	$NHCH_2CH_2Cl$	70	153–154	1665	$C_{11}H_{15}ClN_2O\cdot HCl$	50.20	6.13	10.65	26.95	50.1	6.2	10.6	26.9
$CH_2CH(CH_3)_2$ (DL-Leu)	$NHCH_2CH_2Cl$	81	136–138	...	$C_8H_{17}ClN_2O\cdot HCl$	41.95	7.92	12.23	30.95	42.2	7.9	12.2	31.2
$CH_2CH_2CO_2H$ (L-γ-Glu)	$NHCH_2CH_2Cl$	55	104–105	1570	$C_7H_{13}ClN_2O_3$[b]	40.28	6.28	13.43	16.98	40.3	6.4	13.4	17.6
CH_2CONH_2 (DL-Asp-NH_2)	$NHCH_2CH_2Cl$	30	154–156	1676, 1657	$C_6H_{12}ClN_3O_2\cdot C_2H_2O_4$	33.87	4.98	14.81	12.50	34.0	5.0	14.6	12.3
CH_2CONH_2 (DL-Asp-NH_2)	$NHCH_2CH_2Cl$	..	155–157	...	$C_6H_{12}ClN_3O_2\cdot C_6H_5N_3O_7$	34.09	3.58	19.88	8.38	34.1	3.7	19.8	8.2
H (Gly)	$N(CH_2CH_2Cl)_2$	86	163–165	1660	$C_6H_{12}Cl_2N_2O\cdot HCl$	30.59	5.56	11.90	45.14	30.5	5.5	10.7	45.0
H (Gly)	$N(CH_2CH_2Cl)_2$	46	114–115	1660	$C_8H_{12}Cl_2N_2O\cdot C_2H_2O_4\cdot H_2O$	31.90	5.25	9.12	23.09	31.5	4.9	9.1	23.0
CH_3 (DL-Ala)	$N(CH_2CH_2Cl)_2$	72	116–118	1665	$C_7H_{14}Cl_2N_2O\cdot C_2H_2O_4$	35.64	5.28	9.24	23.43	35.6	5.3	9.1	23.4
$CH_2C_6H_5$ (DL-Phe)	$N(CH_2CH_2Cl)_2$	85	158–161	1660	$C_{13}H_{18}Cl_2N_2O\cdot HCl$	45.41	6.17	8.15	30.91	45.4	6.2	8.1	31.0
$CH_2C_6H_5$ (DL-Phe)	$N(CH_2CH_2Cl)_2$	53	114–116	1660	$C_{13}H_{18}Cl_2N_2O\cdot C_2H_2O_4$	47.19	5.28	7.38	18.73	47.5	5.4	7.3	19.1
$CHCH_2(CH_3)_2$ (DL-Leu)	$N(CH_2CH_2Cl)_2$	80	180–182	1660	$C_{10}H_{20}Cl_2N_2O\cdot HCl$	41.18	7.26	9.61	35.47	41.2	7.2	9.8	36.7

[a] $[\alpha]^{20}D$ +5.2° (*c* 3.49, water). [b] $[\alpha]^{20}D$ +22.8° (*c* 1.25, water).

N-Carbobenzoxy-DL-phenylalanine 2-Chloroethylamide (Method A).—To 0.6 g. of N-carbobenzoxy-DL-phenylalanine 2-hydroxyethylamide in $CHCl_3$ (10 ml.) was added dropwise, 0.6 ml. of thionyl chloride at ice-bath temperature, while stirring magnetically. The ice bath was then removed, a drop of pyridine was added, and stirring was continued at room temperature for 1 hr. and at 40–45° for 2 hr. The solvent and excess thionyl chloride were removed *in vacuo* and the residual solid (0.55 g.) was crystallized from benzene–methylcyclohexane: m.p. 125–127°.

N-Carbobenzoxy-DL-asparagine 2-Chloroethylamide (Method C).—N-Carbobenzoxy-DL-asparagine cyanomethyl ester (0.77 g., 0.0025 mole) was dissolved in 30 ml. of ethyl acetate. To this solution cooled in ice, the freshly liberated base 2-chloroethylamine was added. (The latter reactant was prepared by neutralizing 2-chloroethylamine hydrochloride (0.45 g., 0.004 mole) to pH around 9 with 2 *N* NaOH in an ice bath, subsequent extraction into ethyl acetate, and drying (Na_2SO_4) while in an ice bath.) The reaction mixture was stirred at the ice-bath temperature for 2 hr. and at room temperature overnight. The white solid which separated was collected on a filter, washed with a little cold ethyl acetate, and crystallized from a mixture of ethyl acetate–ethanol; yield 0.4 g. (50%), m.p. 192–194°.

N-Carbobenzoxyamino Acid Bis(2-chloroethyl)- and 2-Chloroethylamides (Method B).—To a solution of 1 mole of N-carbobenzoxyamino acid in $CHCl_3$ or methylene chloride and 2–3 moles of bis(2-chloroethyl)amine or 2-chloroethylamine (freshly prepared from the equivalent amount of their respective hydrochlorides as described in the previous experiment excepting that the base was extracted into an ice-cold $CHCl_3$ solution instead of ethyl acetate) was added 1.0 mole of N,N′-dicyclohexylcarbodiimide. The mixture was stirred at ice-bath temperature for 1–2 hr. and left around 10° overnight. The precipitated dicyclohexylurea was filtered. The filtrate was washed successively with 1 *N* HCl, water, saturated $NaHCO_3$, and water then dried (Na_2SO_4). The residue obtained after evaporation of the solvent under reduced pressure at room temperature was taken up in ethyl acetate and the insoluble material was filtered. The ethyl acetate solution, on evaporation under reduced pressure at ordinary temperature, left a residue which was crystallized to produce analytically pure product.

Sulfonanilides. I. Monoalkyl- and Arylsulfonamidophenethanolamines[1,2]

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Incorporation of the alkyl- or arylsulfonamido moiety into the benzene ring of phenethanolamines leads to a series of compounds, members of which have significant biological actions. The chemical rationale for the use of this substituent, on the basis of its acidity and spacial geometry, is discussed. Prominent pharmacologic properties of this series of monosulfonamidophenethanolamines are β-adrenergic stimulation and blockade and α-adrenergic stimulation. Highly potent and specific β-adrenergic blocking agents were obtained with no demonstrable intrinsic β-mimetic action. The synthesis of the sulfonamidophenethanolamines is reported and the configuration of some *erythro* and *threo* racemates was confirmed by examination of their n.m.r. spectra.

Elucidation of the structure of the adrenal medullary hormones, epinephrine and norepinephrine,[3] provided the impetus for extensive molecular modifications of these catecholamines.[4] Although the benzenoid hydroxyl group of the catecholamine has been replaced with a variety of substituents including chlorine,[5a] fluorine,[5b] iodine,[5c] alkyl,[5d] nitro,[5e] amino,[5f] and alkoxy

(1) For preliminary reports of this work see A. A. Larsen and P. M. Lish, *Nature*, **203**, 1283 (1964).

(2) Presented in part at the Division of Medicinal Chemistry, 147th National Meeting of the American Chemical Society, Philadelphia, Pa., April 1964, Abstracts of Papers, p. 3M.

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