Fibrin-Stabilizing Factor Inhibitors. 12. 5-Dibenzylaminopentylamine and Related Compounds, a New Type of FSF Inhibitors

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A series of ω-dibenzylaminoalkylamines and related compounds have been prepared and tested as inhibitors of fibrin cross-linking. This structural type was chosen in an attempt to develop noncompetitive inhibitors of fibrinoligase. By the combination of the dibenzylamino moiety at one end and the primary amino group at the other end of a polymethylene chain, the same compound could function both as a pseudo donor substrate and as a noncompetitive alkylating inhibitor. Some of the compounds, notably **74–79**, are among the most active fibrinoligase inhibitors described. However, the data indicate that the compounds probably function only as pseudo donor inhibitors.

The final step in the formation of a fibrin clot is the stabilization of the protein, *i.e.*, the cross-linking of the fibrin molecules. This reaction is a transamidation, catalyzed by the thrombin and Ca^{2+} activated fibrin-stabilizing factor (FSF; F XIIIa) or fibrinoligase. The inhibition of this reaction may lead to development of anticoagulants. We have therefore considered it worthwhile to explore the possibilities for therapeutic use of such inhibitors in various coagulation diseases.

A large number of compounds have already been tested *in vitro* as potential inhibitors of fibrin cross-linking.[1,2] These inhibitors may be considered as pseudo substrates for the enzyme, functioning either as "donors" or "acceptors" in the transamidation reaction.[3] Monodansylcadaverine[1] is the most studied inhibitor of the donor type while recently reported thiol esters[4,5] function as acceptor substrates.

We have previously reported[6] that an inhibitor of the donor type should have the general structure $ArX(CH_2)_5NH_2$, where the X group is an electron-withdrawing moiety, *e.g.*, a sulfonyl group imparting a relative low electron density to the aromatic nucleus. It has been proposed[2] that a pseudo donor of this type is aligned in the proximity of the active center of the enzyme *via* a charge-transfer type complex between the aromatic part of the substrate and the tryptophan residue adjacent to the active site cysteine of the enzyme. The side chain carrying the nucleophilic amino group must then have a specific length (preferably five methylene groups) to permit the amino group to attack the thiol ester of the acyl–enzyme intermediate and form an amide bond between the fibrin and the inhibitor, thus blocking the cross-linking of fibrin.

We now wish to report on a new structural type of fibrinoligase inhibitors. We have found that primary *n*-alkylamines, particularly *n*-pentylamines carrying an arylmethylamino group at the other end of the polymethylene chain, are highly active as inhibitors of fibrin cross-linking. Similar to the sulfonamides, the specificity of these compounds is based on the structure of the aromatic moiety of the molecule. The inhibitors and their activities are presented in Table II and intermediates used in the synthesis are listed in Table I.

Chemistry. The compounds presented in Table II were prepared using mainly four different methods designated A–D in Scheme I. Most of the compounds tested were prepared by alkylation of the appropriate secondary amine, in most cases a dibenzylamine. These amines (compounds 1–13) were synthesized *via* two different routes as shown in Scheme I.

Scheme I

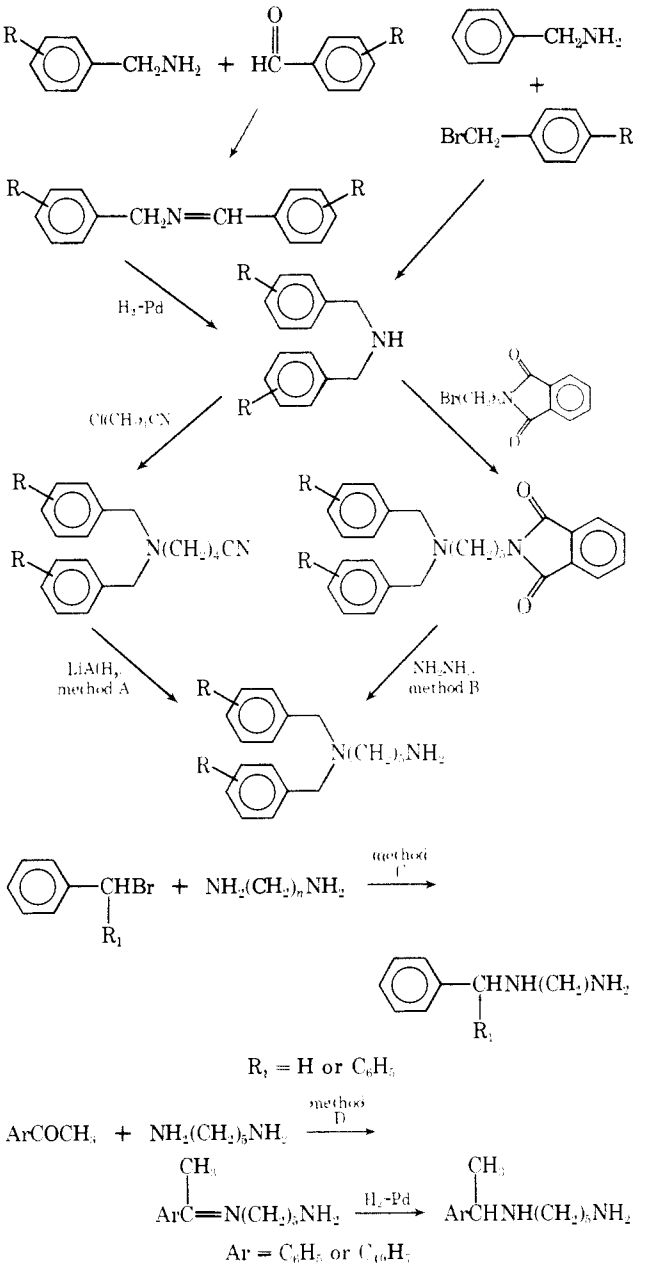

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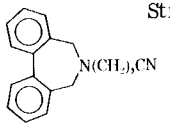
Table I. Physical Data for Nitriles and Phthalimido Derivatives

Compd no.	$R_1R_2N(CH_2)_nR_3$ R_1	R_2	n	R_3	Yield, %	Mp and/or bp (mm), °C	Formula	Recrystn solvent
14	β-$C_{10}H_7CH_2$–	CH_3–	4	–CN	80	*a*	$C_{17}H_{20}N_2$	
15	C_6H_5–	C_2H_5–	4	–CN	30	145 (0.7)	$C_{13}H_{18}N_2$	
16	$C_6H_5CH_2$–	$C_6H_5CH_2$–	2	–CN	64	152–155 (0.01), 45–46	$C_{17}H_{18}N_2$	
17	$C_6H_5CH_2$–	$C_6H_5CH_2$–	3	–CN	64	167–169 (0.01), 45–49	$C_{18}H_{20}N_2$	
18	$C_6H_5CH_2$–	$C_6H_5CH_2$–	4	–CN	77	170–174 (0.5)	$C_{19}H_{22}N_2$	
19	$C_6H_5CH_2$–	$C_6H_5CH_2$–	5	–CN	65	180–182 (0.01), 40–41	$C_{20}H_{24}N_2$	
20	$C_6H_5CH_2$–	$C_6H_5CH_2$–	6	–CN	70	185–187 (0.01)	$C_{21}H_{26}N_2$	
21	CH_2=$CHCH_2$–	CH_2=$CHCH_2$–	4	–CN	70	135–136 (15)	$C_{11}H_{18}N_2$	
22	C_3H_7–	C_3H_7–	4	–CN	88	72–75 (1)	$C_{11}H_{22}N_2$	
23	$C_6H_5CH_2$–	$HOCH_2CH_2$–	4	–CN	80	153–155 (0.05)	$C_{14}H_{20}N_2O$	
24	4-$ClC_6H_5CH_2$–	$C_6H_5CH_2$–	4	–CN	38	120–122	$C_{19}H_{21}ClN_2 \cdot C_2H_2O_4$	2-Propanol
25	4-$(CH_3)_2N \cdot C_6H_5CH_2$–	$C_6H_5CH_2$–	4	–CN	51	153–155	$C_{21}H_{27}N_3 \cdot 2HCl$	Absolute ethanol–ether
26	4-$CH_3C_6H_5CH_2$–	$C_6H_5CH_2$–	4	–CN	45	126–128	$C_{20}H_{24}N_2 \cdot C_2H_2O_4$	2-Propanol
27	4-$CH_3OC_6H_5CH_2$–	$C_6H_5CH_2$–	5	Phthalimido	55	232–233	$C_{28}H_{30}N_2O_3 \cdot C_2H_2O_4$	Water
28	$C_6H_5CH_2CH_2$–	$C_6H_5CH_2CH_2$–	5	Phthalimido	63	142–145 dec	$C_{29}H_{32}N_2O_2 \cdot C_2H_4O_4$	Acetone
29	3,4-$Cl_2C_6H_4CH_2$–	$C_6H_5CH_2$–	4	–CN	49	238–239 dec	$C_{19}H_{20}Cl_2N_2 \cdot C_2H_4O_4$	Absolute ethanol–ether
30	4-$CH_3CONHC_6H_5CH_2$–	$C_6H_5CH_2$–	5	Phthalimido	68	212–214	$C_{29}H_{31}N_3O_3 \cdot C_2H_4O_4$	Absolute ethanol
31	4-$CNC_6H_5CH_2$–	$C_6H_5CH_2$–	5	Phthalimido	45	68–69	$C_{23}H_{27}N_3O_2$	Toluene
32	4-$NO_2C_6H_5CH_2$–	$C_6H_5CH_2$–	5	Phthalimido	40	76–78	$C_{27}H_{27}N_3O_4$	Toluene
33	4-$FC_6H_5CH_2$–	$C_6H_5CH_2$–	4	–CN	58	*a*	$C_{19}H_{21}FN_2$	
34	α-$C_{10}H_7CH_2$–	$C_6H_5CH_2$–	4	–CN	69	*a*	$C_{23}H_{24}N_2$	
35	4-$BrC_6H_5CH_2$–	$C_6H_5CH_2$–	5	Phthalimido	55	54–56	$C_{27}H_{27}BrN_2O_2$	Petroleum ether
36	4-$ClC_6H_5CH_2$–	4-$ClC_6H_5CH_2$–	4	–CN	59	132–133	$C_{19}H_{20}ClN_2 \cdot C_2H_4O_4$	Ethanol–water
37	Structure 				40	230–233	$C_{19}H_{20}N_2 \cdot HCl$	Absolute ethanol
38	HC≡CCH_2CH_2-phthalimido				88	134–135	$C_{12}H_9NO_2$	Glacial acetic acid
39	$(C_6H_5CH_2)_2NCH_2C$≡CCH_2CH_2-phthalimido				50	85–86	$C_{27}H_{24}N_2O_2$	70% ethanol
40	$(C_6H_5CH_2)_2NCH_2CH_2OCH_2CN$				58	154–155	$C_{18}H_{20}N_2O \cdot HCl$	Ethanol–ether
41	$(C_6H_5CH_2)_2NCO(CH_2)_4$-phthalimido				80	84–86	$C_{27}H_{26}N_2O_3$	Ethyl acetate

[a] Oil which could not be dlstilled; hydrochloride and oxalate not crystalline. [b] M. V. Gittos and N. Wilson, *J. Chem. Soc.*, 2371 (1955).

Table II. Physical Data and Inhibitory Activities of the Compounds Tested[a]

Compd no.	$R_1R_2NXNH_2$ R_1	R_2	X	Method	Yield, %	Mp and/or bp (mm), °C	Formula	Derivative	Activity[b]
Monodansylcadaverine				*d*					100
42	C_2H_5–	C_2H_5–	–$(CH_2)_5$–	*d*	68	90–93 (10)	$C_9H_{22}N_2$	Base	1
43	$C_6H_5CH_2$–	H–	–$(CH_2)_4$–	C	64	125–127 (5)	$C_{11}H_{18}N_2$	Base	5
44	$C_6H_5CH_2$–	H–	–$(CH_2)_5$–	C	66	110–112 (0.4)	$C_{12}H_{20}N_2$	Base	6
45	$C_6H_5CH_2$–	H–	–$(CH_2)_6$–	C	69	136–140 (1)	$C_{13}H_{22}N_2$	Base	10
46	$C_6H_5CH_2$–	CH_3–	–$(CH_2)_5$–	*d*		114–115 (1.5)	$C_{13}H_{22}N_2$	Base	11
47	β-$C_{10}H_7CH_2$–	CH_3–	–$(CH_2)_5$–	A	67	160–164 (0.8)	$C_{17}H_{24}N_2$	Base	19
48	C_6H_5–	C_2H_5–	–$(CH_2)_5$–	A	65	134 (1)	$C_{13}H_{22}N_2$	Base	1
49	$C_6H_5CH(CH_3)$–	H–	–$(CH_2)_5$–	D	33	110–112 (0.7)	$C_{13}H_{22}N_2$	Base	7
50	α-$C_{10}H_7CH(CH_3)$–	H–	–$(CH_2)_5$–	D	52	136–140 (0.3)	$C_{17}H_{24}N_2$	Base	5
51	$(C_6H_5)_2CH$–	H–	–$(CH_2)_5$–	C	65	256–257	$C_{18}H_{24}N_2$	2HCl	40
52	$C_6H_5CH_2$–	$C_6H_5CH_2$–	–$(CH_2)_3$–	A	79	137–139 (0.01), 104–105	$C_{17}H_{22}N_2$	2HCl	2
53	$C_6H_5CH_2$–	$C_6H_5CH_2$–	–$(CH_2)_4$–	A	76	145–146 (0.01)	$C_{18}H_{24}N_2$	Base	5
54	$C_6H_5CH_2$–	$C_6H_5CH_2$–	–$(CH_2)_5$–	A	63	200–202	$C_{19}H_{26}N_2$	2HCl	110
55	$C_6H_5CH_2$–	$C_6H_5CH_2$–	–$(CH_2)_6$–	A	78	158–160 (0.01), 198–199	$C_{20}H_{28}N_2$	HCl	58
56	$C_6H_5CH_2$–	$C_6H_5CH_2$–	–$CH_2CH_2OCH_2CH_2$–	A	80	146–148	$C_{18}H_{24}N_2O$	HCl	3
57	$C_6H_5CH_2$–	$C_6H_5CH_2$–	–$CH_2CH_2SCH_2CH_2$–	*c*	85	140–142	$C_{18}H_2N_2S$	0.5 Fumarate	2
58	$C_6H_5CH_2$–	$C_6H_5CH_2$–	–$CH_2C{\equiv}CCH_2CH_2$–	B	67	156–158	$C_{19}H_{22}N_2$	Fumarate	7
59	$C_6H_5CH_2$–	$C_6H_5CH_2$–	–$COCH_2SCH_2CH_2$–	*c*	91	139–140	$C_{18}H_{22}N_2OS$	Fumarate	2
60	$C_6H_5CH_2$–	$C_6H_5CH_2$–	–$COCH_2CH_2CH_2CH_2$–	*c*	86	Oil	$C_{19}H_{20}N_2O$	Base	5
61	$CH_2{=}CHCH_2$–	$CH_2{=}CHCH_2$–	–$(CH_2)_5$–	A	55	120–123 (20)	$C_{11}H_{22}N_2$	Base	4
62	*n*-C_3H_7–	*n*-C_3H_7–	–$(CH_2)_5$–	A	70	64–65 (0.5)	$C_{11}H_{26}N_2$	Base	1
63	$C_6H_5CH_2CH_2$–	$C_6H_5CH_2CH_2$–	–$(CH_2)_5$–	B	87	138–140	$C_{21}H_{30}N_2$	Fumarate	40
64	$C_6H_5CH_2$–	–CH_2CH_2OH	–$(CH_2)_5$–	A	76	133–135 (0.1)	$C_{14}H_{24}N_2O$	Base	6
65	4-$NO_2C_6H_4CH_2$–	$C_6H_5CH_2$–	–$(CH_2)_5$–	B	75	152–153	$C_{19}H_{25}N_3O_2$	Fumarate	60
66	4-$CNC_6H_4CH_2$–	$C_6H_5CH_2$–	–$(CH_2)_5$–	B	51	146–147	$C_{20}H_{25}N_3$	2HCl	51
67	4-$HOCOC_6H_4CH_2$–	C_6H_5CH–	–$(CH_2)_5$–	*c*	37	202–205	$C_{20}H_{26}N_2O_2$	Base	26
68	4-$CH_3CONHC_6H_4CH_2$–	$C_6H_5CH_2$–	–$(CH_2)_5$–	B	86	Oil	$C_{21}H_{29}N_3O$	Base	43
69	4-$C_2H_5NHC_6H_4CH_2$–	$C_6H_5CH_2$–	–$(CH_2)_5$–	*c*	51	Oil	$C_{21}H_{31}N_3$	Base	30
70	4-$CH_3OC_6H_4CH_2$–	$C_6H_5CH_2$–	–$(CH_2)_5$–	B	63	149–152	$C_{20}H_{22}N_2I$	2HCl	55
71	4-$(CH_3)_2NC_6H_4CH_2$–	$C_6H_5CH_2$–	–$(CH_2)_5$–	A	85	Oil	$C_{21}H_{31}N_3$	Base	56
72	4-$NH_2CH_2C_6H_4CH_2$–	$C_6H_5CH_2$–	–$(CH_2)_5$–	*c*	92	149–150	$C_{20}H_{29}N_3$	Fumarate	40
73	4-$CH_3C_6H_4CH_2$–	$C_6H_5CH_2$–	–$(CH_2)_5$–	A	77	175–177	$C_{20}H_{28}N_2$	2HCl	92
74	4-$FC_6H_4CH_2$–	$C_6H_5CH_2$–	–$(CH_2)_5$–	A	82	128–130	$C_{19}H_{25}FN_2$	2HCl	105
75	4-$ClC_6H_4CH_2$–	$C_6H_5CH_2$–	–$(CH_2)_5$–	A	83	177–179	$C_{19}H_{25}ClN_2$	2HCl	150

76	4-Br-$C_6H_4CH_2$–	$C_6H_5CH_2$–	–$(CH_2)_5$–	B	87	184–186	$C_{19}H_{25}BrN_2$	2HCl	105
77	3,4-$Cl_2C_6H_3CH_2$–	$C_6H_5CH_2$–	–$(CH_2)_5$–	A	69	148–150	$C_{19}H_{24}Cl_2N_2$	Fumarate	100
78	4-$ClC_6H_4CH_2$–	4-$ClC_6H_4CH_2$–	–$(CH_2)_5$–	A	73	160–161	$C_{19}H_{24}Cl_2N_2$	Fumarate	200
79	α-$C_{10}H_7CH_2$–	$C_6H_5CH_2$–	–$(CH_2)_5$–	A	86	170–171	$C_{23}H_{28}N_2$	Fumarate	158
80	Structure			A	60	175–176	$C_{13}H_{24}N_2$	2HCl	18
81		$(C_6H_5CH_2)_2N(CH_2)_5N(CH_3)_2$		c	88	163–165 (0.05), 214–216	$C_{21}H_{30}N_2 \cdot 2HCl$	Base	0
82		$(C_6H_5CH_2)_2N^+(CH_2)_5NH_2$ Br^-		c	41	Oil	$C_{20}H_{29}BrN_2$		0

[a] The compounds are presently subjected to extended pharmacological studies. [b] Activity expressed in per cent of that of monodansylcadaverine. [c] Special synthetic method. [d] See ref 8.

Method A. The appropriate secondary amine was alkylated using ω-chloroalkylnitriles, usually 5-chlorovaleronitrile. The nitriles thus formed (compounds **14–26, 29, 33, 34, 36, 37,** and **40,** Table I) were then reduced to the corresponding primary amine using $LiAlH_4$. This yielded the inhibitors **47, 48, 52–56, 61, 62, 64, 71, 73–75** and **77–80.**

Method B. The appropriate dibenzylamine was alkylated using *N*-(5-bromopentyl)phthalimide. The derivatives thus obtained (compounds **27, 28, 30–32, 35,** and **39,** Table I) were treated with hydrazine hydrate and the primary amines were isolated in 50–80% yield (compounds **58, 63, 65, 66, 68, 70,** and **76**).

Method C. To prepare compounds **43–45** and **51,** benzyl bromide or benzhydryl bromide was treated with an excess of the appropriate α,ω-diaminoalkane, whereupon the monoalkylated amine could be isolated in 60–70% yield.

Method D. Compounds **49** and **50** were prepared by condensing cadaverine with acetophenone or 1-acetonaphthalene, respectively, whereupon the Schiff base formed was hydrogenated over platinum at atmospheric pressure.

Compounds **57, 59, 60, 67, 69, 72, 81,** and **82** were prepared using special methods as described in the Experimental Section.

Bioassays. The biological assays were carried out *in vitro* as previously described.[7] The inhibitory activity of each compound is expressed in per cent of that of monodansylcadaverine (activity of monodansylcadaverine = 100). The screening results listed in Table II are means of duplicate determinations, whereas compound **54** was tested in four different experiments in order to study the accuracy of the assay method. This gave a mean activity ± S.E. of 110 ± 10.

Results and Discussion

The inhibitors and their activities are presented in Table II. The activities are expressed in per cent of monodansylcadaverine which was used as a reference compound.

A majority of the compounds in Table II are ω-dibenzylaminoalkylamines. This structural type was chosen in an attempt to develop noncompetitive inhibitors of fibrinoligase. The idea was based on the observation that benzylamines or benzylammonium compounds can be displaced by nucleophiles, thus alkylating the nucleophilic functional group.[8] As inhibitors of fibrinoligase, the benzylammonium salts could either S-benzylate the cysteine SH group at the active site of the enzyme or N-benzylate the ε-amino group of lysine at the cross-linking site of fibrin. By a combination of the dibenzylamino moiety and the primary amino group at the other end of the polymethylene chain, the same compound could function both as a pseudo donor substrate and as a noncompetitive alkylating inhibitor.

The first compounds to be investigated were the monobenzyl type derivatives **43–47** and **49–51.** The activities of these compounds are 5–40 times higher than that of 5-diethylaminopentylamine (**42**) or 5-ethylphenylaminopentylamine (**48**). These results supported our hypothesis and indicated that the *N*-benzyl group had a specific function in the molecule. It also showed that a ω-dialkylamino group (**42**) or a weakly basic ω-alkylarylamino group (**48**) does not give the alkylamines any specificity as FSF inhibitors. Instead, compounds **42** and **48** have about the same activity as pentylamine itself.

Dibenzylaminopentylamines were found to be very potent FSF inhibitors, 10–15 times more active than the corresponding monobenzyl derivatives (*cf.* compounds **44** or **45** with **54**). The dibenzyl compounds show very different activity depending on the length of the polymethylene

chain (*cf.* compounds **52–55**). A similar specificity for the chain length was observed for the dansyl derivatives previously studied.[9] In the monobenzyl compounds **43–45** the length of the side chain is not very critical for the effect.

To study further the possibility that the benzyl compounds might function as alkylating inhibitors, as discussed above, compounds **61–63, 81,** and **82** were synthesized. A diallylamine (**61**) may function in the same manner as the dibenzylamine[8] and alkylate the attacking nucleophilic species, whereas the closely related dipropylamine derivative **62** or the diphenethylamine derivative **63** would not have these properties. Of these compounds, **61** and **62** have only low activity while **63** has an activity of 40 as compared to 110 for 5-dibenzylaminopentylamine (**54**). When the primary amino group of **54** is methylated to a tertiary amine as in **81** or when the dibenzylamino nitrogen is quaternized as in **82,** all the activity is lost. The results thus indicate that the compounds probably are pseudo donors and not alkylating inhibitors as assumed in our working hypothesis. This is particularly well illustrated by compound **63** which should be inactive as an alkylating inhibitor and by **81** and **82** which both theoretically should be potent inhibitors.

The data also show that potent FSF inhibitors of the dibenzyl type should have a primary amino group, a pentamethylene chain, and a basic tertiary nitrogen substituted with preferentially two benzyl groups. The importance of the basic tertiary nitrogen is also illustrated by compound **60** which is an amide. This substance has only low activity.

In a previous study we demonstrated[10] that monodansyl derivatives of 3-oxacadaverine and of 3-thiacadaverine were considerably more active inhibitors of fibrin cross-linking than monodansylcadaverine itself. This is probably due to the low basic strength of the primary amine in these compounds. The low basicity would increase the amount of the primary amine present in the base form at physiological pH and as a consequence also increase the activity of the compound when it functions as a nucleophile in the reaction with the acyl–enzyme intermediate. When we tested the same idea for the dibenzyl compounds **56–59** these inhibitors had only low activity. This may indicate that the specificity of the dansyl compounds is accomplished in a different way than that for the dibenzyl derivatives. The latter compounds could be bound to the receptor structure of the enzyme in a different way than the dansyl compounds.[2] An electron-rich structure, an oxygen (**56**), a sulfur (**57**), or a triple bound (**58**) in the chain of the dibenzyl derivatives may bind to some macromolecular functional group at the receptor and divert the nucleophilic H_2N group from reacting with the thiol ester of the acyl–enzyme intermediate. It is also possible that these side-chain modifications might affect the protonation of the tertiary amine, reducing the enzyme–inhibitor binding.

To study the structural requirements of the dibenzylamino moiety of the inhibitors, compounds **64–80** were synthesized and tested. First we found that compound **80** where the two phenyl rings are bound together, forming a comparatively flat tricyclic structure, had only 20% of the activity of the parent compound **54,** showing that higher activity is obtained when the benzyl groups are free.

In compounds **64–79** we have introduced a variety of substituents in one or both of the aromatic rings. Generally speaking, the results of these experiments show that the introduction of a hydrophilic substituent with hydrogen bonding properties decreases the activity compared to that of **54.** Lipophilic substituents, on the other hand, increase the activity of the inhibitor. Some of the compounds with lipophilic substituents, notably **74–79,** are among the most active fibrinoligase inhibitors described.

Experimental Section

General Comments. Melting points were determined using calibrated Anschütz thermometers in an electrically heated metal block. Ir spectra were run on a Perkin-Elmer 237 spectrophotometer and nmr spectra were recorded with a Varian A-60 instrument using $CDCl_3$ solutions and with TMS as internal standard. New compounds, which were analyzed for C, H, and N, gave values within ±0.4% of the theoretical ones.

4-Chlorodibenzylamine[11] (**1**), 4-dimethylaminodibenzylamine[11] (**2**), 4-nitrodibenzylamine[11] (**3**), 4-methoxydibenzylamine[11] (**4**), 4-methyldibenzylamine[11] (**5**), 3,4-dichlorodibenzylamine[12] (**6**), 4,4′-dichlorodibenzylamine[13] (**7**), and benzylnaphthylmethylamine[14] (**8**) were prepared as described in the literature.

4-Acetamidodibenzylamine (9). A mixture of 4-acetamidobenzaldehyde (3.7 g, 0.02 mol) and benzylamine (2.2 g, 0.02 mol) was refluxed for 2 hr in 250 ml of ethanol. The solution was cooled and hydrogenated over platinum. The solvent was evaporated and the product (4 g, 80%) crystallized upon standing: mp 74–75° (from ethanol–ether). *Anal.* ($C_{16}H_{18}N_2O$) C, H, N.

4-Cyanodibenzylamine (10). A mixture of 4-cyanobenzylbromide (20 g, 0.1 mol) and benzylamine (21.8 g, 0.2 mol) in 300 ml of absolute ethanol was refluxed for 1 hr. Water (400 ml) was then added and the product was extracted with chloroform (3 × 300 ml). The extract was dried (Na_2SO_4), the solvent evaporated, and the residual yellow oil distilled. This yielded 14.5 g (65%) of the title compound: bp 154° (0.01 mm); the hydrochloride had mp 253–254° (from ethanol). *Anal.* ($C_{15}H_{14}N_2 \cdot HCl$) C, H, N.

4-Fluorodibenzylamine (**11**) was prepared as described for **10** in 53% yield: bp 105–108° (0.1 mm); mp (hydrochloride) 270–271° (from ethanol). *Anal.* ($C_{14}H_{14}FN \cdot HCl$) C, H, N.

4-Bromodibenzylamine (**12**) was prepared as described for **10** in 55% yield: bp 147–150° (0.1 mm); mp (hydrochloride) 260–261° (from ethanol). *Anal.* ($C_{14}H_{14}BrN \cdot HCl$) C, H, N.

6,7-Dihydro-5*H*-dibenz[*c,e*]azepine (13). This compound was obtained by $LiAlH_4$ reduction of diphenimide.[15] It had the same physical constants as previously reported for the compound,[16] obtained *via* a different route.

Synthesis of the Inhibitors. Method A. This procedure is illustrated by the synthesis of 5-(4-methyldibenzylamino)pentylamine (**73**).

5-(4-Methyldibenzylamino)valeronitrile (26). 4-Methyldibenzylamine (5 21.1 g, 0.1 mol) and 5-chlorovaleronitrile (5.8 g, 0.05 mol) were dissolved in dry xylene (300 ml), 0.1 g of NaI was added, and the mixture was stirred and refluxed overnight. The amine hydrochloride formed was filtered off, and the filtrate was washed with saturated $NaHCO_3$ solution and extracted with 2 *N* HCl (500 ml). The acidic aqueous solution was made alkaline and the base extracted with ether (3 × 100 ml). The extract was dried (Na_2SO_4) and the solvent evaporated *in vacuo*. The residual oil was purified by column chromatography on silica gel eluted with ether–petroleum ether mixtures of increasing polarity. The title compound was obtained as an oil (6.6 g, 45%) and precipitated as oxalate: mp 126–128° (from 2-propanol). *Anal.* ($C_{20}H_{24}N_2 \cdot C_2H_2O_4$) C, H, N.

5-(4-Methyldibenzylamino)pentylamine (73). To a suspension of $LiAlH_4$ (1.2 g, 32 mmol) in anhydrous ether (100 ml) was slowly added a solution of **26** (in the basic form) (6 g, 0.02 mmol) in anhydrous ether, whereupon the mixture was stirred at room temperature for 3 hr. After addition of water the amine was extracted with 2 *N* HCl (200 ml), the aqueous phase realkalized, and the amine extracted into ether. The extract was dried (Na_2SO_4) and the solvent evaporated yielding 4.7 g (77%) of a yellow oil which was converted to its hydrochloride: mp 175–177° (from 2-propanol–ether). *Anal.* ($C_{20}H_{28}H_2 \cdot 2HCl$) C, H, N.

Method B is illustrated by the synthesis of 5-(4-bromodibenzylamino)pentylamine (**76**).

1-(4-Bromodibenzylamino)-5-phthalimidopentane (35). A solution of 4-bromodibenzylamine (**12**, 7.8 g, 28.2 mmol), *N*-(5-bromopentyl)phthalimide (8.3 g, 28.2 mmol), and triethylamine (2.85 g, 28.2 mmol) in dry DMF (200 ml) was heated to 150° for 3 hr. The solution was cooled and diluted with 500 ml of water and the product extracted with chloroform (3 × 100 ml). The extract was dried (Na_2SO_4) and the solvent evaporated *in vacuo*. The brown oil was purified by column chromatography on silica gel eluted with ether–petroleum ether mixtures of increasing polarity. This yielded the title compound as an oil which solidified upon standing: yield 7.5 g (55%); mp 54–55° (from petroleum ether). *Anal.* ($C_{27}H_{27}BrN_2O_2$) C, H, N.

5-(4-Bromodibenzylamino)pentylamine (76). Compound **35**

(6.3 g, 13 mmol) and hydrazine hydrate (0.64 g, 13 mmol) in absolute ethanol (100 ml) were refluxed for 3 hr. The reaction mixture was then cooled to 5°, 10 ml of concentrated HCl was added, and the suspension was stirred at 5° for 1 hr. The precipitate was filtered and washed with 100 ml of absolute ethanol. The filtrate and washings were pooled and concentrated *in vacuo* and alkalized and the product was extracted with ether. The ether extract was dried (Na_2SO_4) and evaporated yielding 4 g (87%) of an oil which was converted to its hydrochloride: mp 184–186° (from ether–ethanol). *Anal.* ($C_{19}H_{25}BrN_2 \cdot 2HCl$) C, H, N.

Method C is illustrated by the synthesis of **44**.

5-Benzylaminopentylamine (44). A mixture of benzyl bromide (3.4 g, 0.02 mol) and cadaverine (6.1 g, 0.06 mol) in chloroform (100 ml) was stirred at room temperature overnight. Aqueous NaOH (50 ml, 1 *N*) was added, the mixture was stirred for 5 min, and the layers were separated. The chloroform layer was dried (Na_2SO_4) and evaporated and the residue distilled. This yielded 2.5 g (66%) of the title compound, bp 110–112° (0.4 mm). *Anal.* ($C_{12}H_{20}N_2$) C, H, N.

Method D is illustrated by the synthesis of **49**.

5-(1-Phenylethylamino)pentylamine (49). A mixture of acetophenone (2.4 g, 0.02 mol) and cadaverine (3 g, 0.03 mol) in ethanol (50 ml) was heated to reflux for 1 hr. Platinum oxide was then added and the cool solution was hydrogenated at atmospheric pressure and room temperature until the theoretical amount of hydrogen had been consumed. After filtration, the solvent was removed *in vacuo* and the residue distilled: bp 110–112° (0.7 mm); yield 1.3 g (33%). *Anal.* ($C_{13}H_{22}N_2$) C, H, N.

***N*-But-3-ynylphthalimide (38).** Phthalic acid anhydride (11.8 g, 0.08 mol) was suspended in glacial acetic acid (80 ml) whereupon 4-amino-1-butyne[17] (5.5 g, 0.08 mol) was added dropwise. The mixture was stirred and refluxed until a clear solution was obtained and then for another 45 min. Upon cooling, the product separated as white needles: mp 134–135° (from glacial acetic acid); yield 14 g (88%); ir (KBr) 3250 (≡CH), 1760 and 1710 cm^{-1} (imide); nmr δ 1.9 (t, 1 H, J = 2.6 Hz, ≡CH), 2.55 (triplet of doublets, 2 H, J = 7.0 and 2.6 Hz, respectively, $NCH_2CH_2C{\equiv}CH$), 4.75 (t, 2 H, J = 7.0 Hz, $NCH_2CH_2C{\equiv}CH$), and 7.6 ppm (m, 4 H, ArH). *Anal.* ($C_{12}H_9NO_2$) C, H, N.

1-Dibenzylamino-5-phthalimido-2-pentyne (39). Compound **38** (10 g, 0.05 mol), paraformaldehyde (2.0 g, 0.067 mol), and cuprous chloride (0.1 g, catalyst) were suspended in a mixture of glacial acetic acid (7 ml) and dry peroxide-free dioxane (20 ml). The suspension was stirred for 15 min whereupon dibenzylamine (11.0 g, 0.06 mol) was added dropwise. The mixture was then heated at 80° for 45 min. To the cool reaction mixture was then added water (100 ml) and sulfuric acid (1 *M*, 30 ml). The acidic solution was washed with two 100-ml portions of ether and the washings were discarded. The aqueous phase was then alkalized using K_2CO_3 and 25% ammonia (10 ml) was added to dissolve precipitated copper salts. The alkaline aqueous mixture was then extracted with chloroform (3 × 100 ml) and the extracts were dried (Na_2SO_4). After evaporation of the solvent, 10.2 g (50%) of the title compound was obtained: mp 85–86° (from 70% ethanol); ir (KBr) 1770 and 1720 cm^{-1} (imide); nmr δ 2.63 (triplet of triplets, 2 H, J = 7.0 and 2.0 Hz, respectively, $NCH_2CH_2C{\equiv}CCH_2N$), 3.10 (t, 2 H, J = 2.0 Hz, $NCH_2CH_2C{\equiv}CCH_2N$), 3.45 (s, 4 H, $ArCH_2$–), 3.82 (t, 2 H, J = 7.0 Hz, NCH_2CH_2–), 7.10 (s, 10 H, ArH in benzylic groups), and 7.59 ppm (m, 4 H, ArH in phthalimido group). *Anal.* ($C_{27}H_{24}N_2O_2$) C, H, N.

5-Phthalimidopentanoic Acid. Phthalic acid anhydride (14.8 g, 0.1 mol) and 5-aminopentanoic acid (11.7 g, 0.1 mol) were refluxed in 100 ml of glacial acetic acid for 1 hr. The solvent was evaporated leaving the crude product as a dark mass, which was macerated with 600 ml of ether. A small amount of tarry material remained undissolved and was discarded. The ether was evaporated and the product recrystallized three times from ethyl acetate: yield 12.0 g (48%); mp 118.5–119.5°. *Anal.* ($C_{13}H_{13}NO_4$) C, H, N.

***N*-(4-Dibenzylcarbamoylbutyl)phthalimide (41).** 5-Phthalimidopentanoic acid (6.0 g, 0.024 mol) was suspended in dry benzene. Triethylamine (2.4 g, 0.024 mol) was added. The acid dissolved rapidly. To the clear solution was added ethyl chloroformate (2.6 g, 0.024 mol). Almost immediately a finely crystalline precipitate started to form. The mixture was stirred for 1 hr. Dibenzylamine (4.7 g, 0.024 mol) was added and the mixture was stirred for another hour. The precipitate was filtered off and the solvent was evaporated leaving 9 g of crude product as a yellowish, soft solid. Recrystallization from 70% EtOH yielded 7.0 g (70%) of white crystals, mp 85–86°. *Anal.* ($C_{27}H_{26}N_2O_3$) C, H, N.

2-Amino-2′-dibenzylaminodiethyl Sulfide (57). Sodium (2.6 g, 0.113 g-atom) was dissolved in absolute ethanol (150 ml) and 2-mecaptoethylamine (4.3 g, 0.056 mol) was slowly added under nitrogen atmosphere. 2-Chloroethyldibenzylamine hydrochloride (15 g, 0.051 mol) was then added and the mixture was refluxed under nitrogen for 4 hr. After filtration, the solvent was removed *in vacuo*, and the residual oil was dissolved in ether, washed twice with saturated $NaHCO_3$ solution (200 ml) and with water (200 ml), and dried (Na_2SO_4). The product was precipitated as its fumarate: mp 140–142° (from ethanol–ether); yield 18.3 g (85%); nmr δ 1.20 (s, 2 H, $-NH_2$), 2.10–2.80 (m, 8 H, $-CH_2$–), 3.49 (s, 4 H, $ArCH_2$–), and 7.18 ppm (m, 10 H, ArH). *Anal.* ($C_{18}H_{24}N_2S \cdot 0.5C_4H_4O_4$) C, H, N.

***N,N*-Dibenzyl-5-amino-3-thiapentanoic Acid Amide (59).** Sodium (0.7 g, 0.03 g-atom) was dissolved in absolute ethanol (80 ml) and 2-mercaptoethylamine (2.5 g, 0.03 mol) was added under nitrogen atmosphere, followed by *N,N*-dibenzylchloroacetamide[18] (8.2 g, 0.03 mol). A precipitate was formed and the mixture was stirred and refluxed under nitrogen for 2.5 hr. After filtration, the solution was neutralized using acetic acid and the solvent was evaporated *in vacuo*. The residue was dissolved in chloroform (100 ml), washed twice with saturated $NaHCO_3$ solution (100 ml) and once with water (100 ml), and dried (Na_2SO_4). The product was then precipitated as the fumarate: mp 139–140° (from ethanol–ether); yield 11.6 g (90%); ir (film) of the base, 3480 and 3410 (NH_2), 1630 cm^{-1} (CO); nmr δ 1.40 (s, 2 H, $-NH_2$), 2.6–3.0 (m, 4 H, $-SCH_2CH_2N$), 4.45 (s, 4 H, $ArCH_2$–), and 7.10 ppm (m, 10 H, ArH). *Anal.* ($C_{18}H_{22}N_2OS \cdot C_4H_4O_4$) C, H, N.

4-(Dibenzylcarbamoyl)butylamine (60). *N*-(4-Dibenzylcarbamoylbutyl)phthalimide (5.0 g, 0.012 mol) was treated with hydrazine hydrate in refluxing ethanol according to the method described by Ing and Manske.[19] The crude product was placed on an alumina column and eluted with ether. The purified product was isolated as a viscous, colorless oil, which gave only one spot, R_f 0.7, on tlc (silica gel, eluent 75% Et_2O + 20% MeOH + 5% NH_4OH): mass spectrum parent ion *m/e* 296 (5%); mol wt ($C_{19}H_{24}N_2O$) 296.42.

5-(4-Carboxydibenzylamino)pentylamine (67). Compound **66** (1 g, 3.2 mmol) was dissolved in 50 ml of 80% ethanol containing 10% KOH. The solution was refluxed overnight, cooled, and neutralized with 2 *M* HCl. Inorganic salts were precipitated by addition of ether (200 ml) and removed by filtration. The filtrate was evaporated *in vacuo* and the remaining water was removed by azeotropic distillation with benzene. This yielded 0.4 g (37%) of **67** as white crystals, mp 202–205° (from ethanol–water). *Anal.* ($C_{20}H_{26}N_2O_2$) C, H, N.

5-(4-Ethylaminodibenzylamino)pentylamine (69). Compound **68** (1 g, 2.9 mmol) was stirred with $LiAlH_4$ (0.3 g, 8 mmol) in dry THF at 70° for 24 hr. After addition of water, the amine was extracted with 50 ml of 2 *M* HCl, the aqueous layer alkalized, and the product extracted with ether (2 × 50 ml). The extract was dried (Na_2SO_4), the solvent evaporated, and the title compound was obtained as an oil (0.5 g, 51%). The compound could not be obtained in crystalline form as the base or as salts. A small amount was purified by preparative tlc for elementary analysis: ir (film) 3450–3200 (NH_2), 1600 and 1500 cm^{-1} (phenyl). *Anal.* ($C_{21}H_{31}N_3$) C, H, N.

5-(4-Aminomethyldibenzylamino)pentylamine (72). This compound was prepared from **66** by reduction with $LiAlH_4$ as described for **69**: yield 92%; mp 149–150° (fumarate, from ethanol). *Anal.* ($C_{20}H_{20}N_3 \cdot C_4H_4O_4$) C, H, N.

***N,N*-Dibenzyl-*N′,N′*-dimethyl-1,5-diaminopentane (81).** *N,N*-Dibenzyl-1,5-diaminopentane (28.2 g, 0.1 mol) and 98% formic acid (42.0 g, 0.9 mol) were mixed and cooled in an ice bath. Ice-cold 37% formaldehyde solution (18.0 g, 0.22 mol) was added and the mixture was heated on a steam bath. A vivid evolution of carbon dioxide indicated that the reaction had started. The heating was continued for 45 min whereupon 100 ml of ice–water was added to the reaction mixture, which was then made alkaline by the addition of solid K_2CO_3. The product separated as an oil and was taken up in 200 ml of ether. The ether extract was dried (K_2CO_3) and the solvent was evaporated leaving the crude product as a faintly yellow oil. Distillation yielded 27.3 g (88%) of pure product, bp 163–165° (0.05 mm). The ir spectrum of the base showed no NH band. The dihydrochloride had mp 214–216°. *Anal.* ($C_{21}H_{30}N_2 \cdot 2HCl$) C, N, H.

5-(Dibenzylmethylammonio)pentylamine Bromide (82). Compound **54** (0.9 g) was dissolved in dry acetone (50 ml) and the solution was left at room temperature overnight. Methyl bromide (1 g) was then added and the solution left at room temperature for

another 3 days. An oil separated, which could not be crystallized: ir (film) a band at 1690 cm^{-1} indicated the presence of a $-N{=}C(CH_3)_2-$ structure. The oil was dissolved in water (25 ml) and the solution left at room temperature overnight. Evaporation yielded 0.5 g of an oil which could not be crystallized: ir (film) 3200–3300 cm^{-1} (NH_2), no band at 1690 cm^{-1}. A small amount was purified for analysis by preparative tlc developed in ethanol. *Anal.* ($C_{20}H_{29}BrN_2$) C, H, N.

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References

(1) J. L. G. Nilsson, P. Stenberg, Ch. Ljunggren, K.-J. Hoffmann, R. Lundén, O. Eriksson, and L. Lorand, *Ann. N. Y. Acad. Sci.*, **202**, 286 (1972).
(2) L. Lorand and J. L. G. Nilsson, "Drug Design," Vol. 3, Academic Press, New York, N.Y., 1972, p 415.
(3) L. Lorand, *Thromb. Diath. Haemorrh., Suppl.*, **39**, 75 (1970).
(4) L. Lorand, C. H. Chou, and I. Simpson, *Proc. Nat. Acad. Sci. U. S.*, **69**, 2645 (1972).
(5) Ch. Ljunggren, K.-J. Hoffmann, P. Stenberg, and J. L. G. Nilsson, *J. Med. Chem.*, **16**, 1186 (1973).
(6) P. Stenberg, Ch. Ljunggren, J. L. G. Nilsson, R. Lundén, and O. Eriksson, *J. Med. Chem.*, **15**, 674 (1972).
(7) J. L. G. Nilsson, P. Stenberg, O. Eriksson, and R. Lundén, *Acta Pharm. Suecica*, **7**, 441 (1970).
(8) J. H. Brewster and E. L. Eliel, *Org. React.*, 7, 99 (1953).
(9) J. L. G. Nilsson, P. Stenberg, Ch. Ljunggren, O. Eriksson, and R. Lundén, *Acta Pharm. Suecica*, **8**, 497 (1971).
(10) Ch. Ljunggren, K.-J. Hoffman, P. Stenberg, U. Svensson, J. L. G. Nilsson, A. Hartkoorn, and R. Lundén, *J. Med. Chem.*, **17**, 649 (1974).
(11) R. L. Hinmann and K. L. Hamm, *J. Org. Chem.*, **23**, 529 (1958).
(12) W. J. Houlihan, German Patent 1231690; *Chem. Abstr.*, **66**, 55248e (1967).
(13) C. Winans, *J. Amer. Chem. Soc.*, **61**, 3564 (1939).
(14) H. Dahn, U. Solms, and P. Zoller, *Helv. Chim. Acta*, **35**, 2117 (1952).
(15) H. W. Underwood, Jr., and L. A. Clough, *J. Amer. Chem. Soc.*, **51**, 583 (1929).
(16) J. O. Hawthorne, E. L. Mihelic, M. S. Morgan, and M. H. Witt, *J. Org. Chem.*, **28**, 2831 (1963).
(17) J. L. Dumont, J. Tohier, and P. Cadiot, *C. R. Acad. Sci.*, **256**, 3146 (1963).
(18) A. J. Speziale and P. C. Hamm, *J. Amer. Chem. Soc.*, **78**, 2556 (1956).
(19) H. R. Ing and R. F. Manske, *J. Chem. Soc.*, 2348 (1926).

[1-β-Mercapto-β,β-pentamethylenepropionic acid]oxytocin, a Potent Inhibitor of Oxytocin†

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[1-β-Mercapto-β,β-pentamethylenepropionic acid]oxytocin was prepared from β-Mpa(β-$(CH_2)_5$)(Bzl)-Tyr(Bzl)-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 by removal of the Bzl-protecting groups with Na–NH_3 followed by cyclization of the resulting disulfhydryl compound with $K_3Fe(CN)_6$. The analog was purified by desalting on Sephadex G-15 in 50% HOAc and gel filtration on Sephadex G-25 and LH-20. The protected intermediate above was synthesized from Z-Cys(Bzl)-Pro-Leu-Gly-NH_2 by the stepwise *p*-nitrophenyl ester method using N^{α}-Boc protection at the penta-, hexa-, and octapeptide stages. The analog was found to be a potent inhibitor of the oxytocic and avian vasodepressor effects of oxytocin (pA_2 values of 7.43 and 8.30, respectively) but was only a weak inhibitor of the rat pressor effect of 8-lysine-vasopressin. The rat antipressor potency of [1-deaminopenicillamine]oxytocin was also determined in this study: pA_2 = 6.27. Of the alkyl-substituted 1-position analogs of oxytocin studied so far, [1-β-mercapto-β,β-pentamethylenepropionic acid]oxytocin is the most potent antioxytocic agent.

When [1-L-penicillamine]oxytocin, [1-D-penicillamine]-oxytocin, and [1-deaminopenicillamine]oxytocin ([1-β-mercapto-β,β-dimethylpropionic acid]oxytocin) were found to be potent inhibitors[2] of the oxytocic and avian vasodepressor (AVD) activities of oxytocin (Figure 1), studies were undertaken of related modifications[3] in the 1 position of the highly potent deaminooxytocin.[4] [1-β-Mercapto-β,β-diethylpropionic acid]oxytocin[3] ([1-β-Mpa(β-Et_2)]oxytocin) was found to be the most potent inhibitor of the compounds that had been prepared, having pA_2 values of 7.24 in the antioxytocic and 8.11 in the anti-AVD assays in comparison with corresponding pA_2 values for [1-deaminopenicillamine]oxytocin of 6.94 and 7.88, respectively.

As a further variation, the incorporation of a β-mercapto-β,β-pentamethylenepropionic acid residue [β-Mpa(β-$(CH_2)_5$)] into the 1 position of oxytocin is reported here. This residue is structurally similar to β-Mpa(β-Et_2) and can be looked upon as having the β-geminal ethyl groups connected by a methylene bridge. The possible conformations of the β-alkyl substituents are thus limited and the overall lipophilicity of the residue is increased.

[1-β-Mpa(β-$(CH_2)_5$)]oxytocin was prepared from the protected polypeptide β-Mpa(β-$(CH_2)_5$)(Bzl)-Tyr(Bzl)-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2 by removal of the benzyl-protecting groups with Na–NH_3[5] followed by cyclization with potassium ferricyanide[6] solution. The hormone analog was purified by desalting in 50% HOAc on Sephadex G-15 by the method of Manning[7] followed by gel filtration in 0.2 *N* HOAc on Sephadex G-15 and in DMF on Sephadex LH-20.

N^{α}-*tert*-Butyloxycarbonyl (Boc) protection was used on the penta-, hexa-, and octapeptide intermediates. Boc removal was accomplished by treatment with trifluoroacetic acid (TFA) at room temperature. In the case of the Boc-Tyr(Bzl)-terminal octapeptide it is important that the deprotection period be no longer than 15 min. Longer periods lead to removal of the benzyl ether protection. *p*-Nitrophenyl esters[8] were used for coupling reactions throughout. β-(*S*-Benzylmercapto)-β,β-pentamethylenepropionic acid was prepared from ethyl cyclohexylidineacetate[9] by means

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