New Aromatic Diamidines with Central α-Oxyalkane or α,ω-Dioxyalkane Chains. Structure–Activity Relationships for the Inhibition of Trypsin, Pancreatic Kallikrein,† and Thrombin and for the Inhibition of the Overall Coagulation Process‡

J. D. Geratz,* M. C.-F. Cheng, and R. R. Tidwell

Department of Pathology, University of North Carolina, School of Medicine, Chapel Hill, North Carolina 27514.
Received November 11, 1974

A series of ω-amidinophenylalkyl amidinophenyl ethers was synthesized and examined for inhibitory activity against trypsin, pancreatic kallikrein, and thrombin. Modifications of the compounds included lengthening of the alkane chain, variation in the position of the amidino groups, and substitution of halogen on the benzene rings. The compounds act as competitive reversible inhibitors, and many of them possess considerable potency. An outstanding trypsin inhibitor was found in 4-amidinophenylethyl 4-amidino-2-bromophenyl ether (compound **7**) with a K_i value of 7.3×10^{-8} *M* (pH 8.1, 37°). A number of aromatic diamidines with a central dioxyalkane chain were similarly studied. Here, 1-(4-amidino-2-iodophenoxy)-5-(3-amidinophenoxy)pentane (compound **32**) was a highly effective inhibitor of bovine thrombin ($K_i = 1.1 \times 10^{-6}$ *M*), of human thrombin, and of the overall clotting process of human plasma.

Unchecked proteolytic activity is thought to be of crucial importance in the development of a number of disease states in man. These include pancreatitis and other inflammations, thrombotic and fibrinolytic processes, complement dependent immune reactions, and certain types of shock. Some of the proteases involved in the pathogenetic pathways have been well identified and characterized, and this has stimulated the search for inhibitors which might be able to block the undesirable effects. Such an endeavor has been highly successful with regard to arginine- and lysine-specific esteroproteases where it led to the discovery of aromatic diamidines as powerful inhibitory agents against enterokinase,[1] trypsin,[1,2] thrombin,[3-5] plasmin and SK-plasmin(ogen) activator,[3,4,6] pancreatic kallikrein,[7] and Cls and Clr.[8] In a recent publication we have shown how the potency of diamidines with a central dioxyalkane chain is modified by variation in the length of the alkane chain, by the substitution of halogen on the benzene moieties, and by altering the position of the amidino groups.[9] We now wish to report on the structure–activity relationships for diamidines possessing only a single ether bond on the central hydrocarbon moiety. The compounds were examined for their effect on bovine trypsin, porcine pancreatic kallikrein, and bovine thrombin, and they include the most active competitive reversible inhibitor of trypsin known so far. In addition, we will present data on a number of novel bis ether compounds, the development of which was an outgrowth of our earlier studies and led to a considerable increase in antitrypsin and anticoagulant properties.

Results

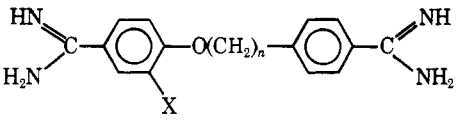
ω-Amidinophenylalkyl Amidinophenyl Ethers. All compounds were tested in amidase assays for their inhibitory effect against the three enzymes. In each instance the kinetic behavior conformed to that of a reversible competitive inhibitory pattern, and thus the enzyme–inhibitor dissociation constants could be used to compare the potency of the various diamidines.

The data in Table I show that lengthening of the monooxyalkane chain of bis(*p*-amidinophenyl) derivatives from 1 to 3 carbons led to a progressive increase in inhibitory strength against kallikrein and thrombin, while for trypsin the optimal length was already reached with the 2-carbon chain. Halogen substitution on the amidinophenoxy moiety increased the inhibitory strength of a given compound and, as a rule, iodine was a more effective substituent than bromine, and bromine again was more effective than chlorine. With trypsin as well as with thrombin, however, the bromine-substituted ethoxy compound **7** was superior to its iodine-substituted counterpart, and, in fact, compound **7** was the strongest trypsin inhibitor in the series reported here.

The influence of single or double halogen substitutions on the potency of 4- or 3-amidinobenzyl-4-amidinophenyl ethers is demonstrated in Table II. For compounds possessing both amidino groups in the para position, double substitutions on the amidinophenoxy moiety were always less effective than single substitutions, except in the case of kallikrein. When the amidino group on the amidinobenzyl moiety was placed in the meta position, however, all compounds with two substitutions were more powerful than those with a single substitution. Compounds **20** and **21** with their two bromine and two iodine substitutions, respectively, were noteworthy for their marked antithrombin acivity.

The importance of the position of the two cationic

Table I. Effect of Variation in the Length of the Hydrocarbon Chain and of Halogen Substitution on the Inhibitory Strength of 4-Amidino-ω-phenalkyl 4-Amidinophenyl Ethers



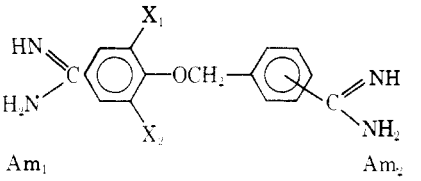
Compd no.	X	n	K_i, μM[a] Trypsin	Kallikrein	Thrombin
1	H	1	4.5 ± 0.1	56 ± 9.6	44 ± 8.4
2	H	2	1.4 ± 0.2	55 ± 10.6	18 ± 3.3
3	H	3	2.6 ± 0.1	13 ± 1.4	12 ± 0.3
4	Cl	1	1.5 ± 0.3	11 ± 0.4	25 ± 3.0
5	Cl	3	2.3 ± 0.3	7.0 ± 0.6	11 ± 2.2
6	Br	1	1.1 ± 0.01	13 ± 1.0	25 ± 1.4
7	Br	2	0.073 ± 0.007[b]	12 ± 1.2	6.0 ± 0.8
8	Br	3	2.1 ± 0.3	3.8 ± 0.5	10 ± 0.4
9	I	1	0.9 ± 0.3	12 ± 2.3	24 ± 1.3
10	I	2	0.11 ± 0.02[b]	10 ± 1.2	6.8 ± 0.7
11	I	3	1.3 ± 0.2	2.2 ± 0.2	7.1 ± 1.0

[a] K_i, dissociation constant of the enzyme–inhibitor complex. Values are means ± SD; $n = 3$. [b] BPVANA used as substrate in amidase assay.

† Kallikrein is a registered trademark assigned to Farbenfabriken Bayer AG, Leverkusen, Federal Republic of Germany.

‡ Presented in part at the 168th National Meeting of the American Chemical Society, Atlantic City, N.J., Sept 1974.

Table II. Effect of Halogen Substitution on the Inhibitory Strength of Amidinobenzyl 4-Amidinophenyl Ethers



Compd no.	Am_2	X_1	X_2	K_i, μM[a] Trypsin	Kallikrein	Thrombin
1	4	H	H	4.5 ± 0.1	56 ± 9.6	44 ± 8.4
4	4	Cl	H	1.5 ± 0.3	11 ± 0.4	25 ± 3.0
6	4	Br	H	1.1 ± 0.01	13 ± 1.0	25 ± 1.4
9	4	I	H	0.9 ± 0.3	12 ± 2.3	24 ± 1.3
12	4	Cl	Cl	4.3 ± 0.4	10 ± 1.2	32 ± 3.8
13	4	Br	Br	3.7 ± 0.1	7.8 ± 1.4	33 ± 2.3
14	4	I	I	4.8 ± 0.6	3.4 ± 0.6	26 ± 2.8
15	3	H	H	5.7 ± 0.1	81 ± 2.5	14 ± 3.6
16	3	Cl	H	5.9 ± 0.01	25 ± 6.1	9.6 ± 0.07
17	3	Br	H	5.2 ± 0.6	22 ± 0.6	9.1 ± 0.2
18	3	I	H	2.6 ± 0.4	12 ± 1.3	7.8 ± 0.9
19	3	Cl	Cl	3.2 ± 0.2	11 ± 1.2	7.8 ± 0.7
20	3	Br	Br	1.8 ± 0.2	7.0 ± 0.7	2.1 ± 0.4
21	3	I	I	2.4 ± 0.4	7.5 ± 1.1	2.1 ± 0.4

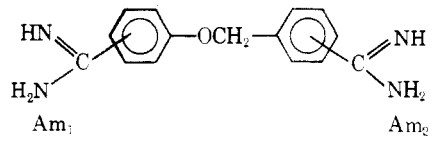
[a] K_i, dissociation constant of the enzyme-inhibitor complex. Values are means ± SD; $n = 3$.

groups for maximal potency was confirmed by our next study where either one or both amidino groups of amidinobenzyl amidinophenyl ethers were shifted from the para to the meta location (Table III). With trypsin the di-para position was most beneficial while with thrombin all three compounds having one or both amidino groups in the meta position surpassed the potency of the di-para compound 1 by a margin of 3–4. A comparison of the activity of compounds 15 and 22, respectively, against trypsin and kallikrein also reveals that it mattered which of the two amidino groups had been moved into the meta position, the group on the amidinophenoxy moiety or the one on the amidinobenzyl moiety.

Diamidines with a Central Dioxyalkane Chain. Amidase Assay. Previously we had shown that single or double bromine substitutions on each of the two benzene moieties of 1,5-bis(4-amidinophenoxy)pentane lead to highly effective inhibitors of kallikrein as well as of trypsin.[9] For the former enzyme the tetrabromine derivative was more active than the dihalo compound while the converse was observed with trypsin. To determine the effect of variation in the chain length on the potency of the tetrabromine compounds we have now prepared compounds **24** and **26** (Table IV), the 3- and 8-carbon homologs of the original 5-carbon compound **25**. The data in Table IV reveal that the propane derivative was considerably superior to the pentane derivative as an inhibitor of trypsin and, in fact, it is the most effective antitrypsin agent among all bis ether compounds examined so far. Against kallikrein, however, compound **24** was only half as potent as compound **25**. With thrombin, finally, the effectiveness of the three inhibitors was not significantly affected by the variation in chain length.

As the shift of one of the amidino groups of 1,5-bis(4-amidinophenoxy)pentane from the para into the meta position (**28**) markedly increased the thrombin inhibitory strength,[9] we have now also investigated the 3-, 6-, and 8-carbon chain homologs of compound **28**. The tests with the amide substrate revealed for each enzyme a remarkably small variation in potency from the 3- through the 8-carbon

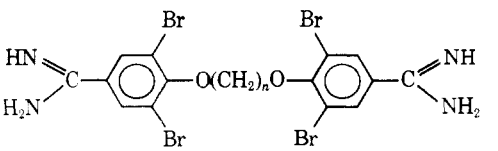
Table III. Effect of Variation in the Position of the Amidino Groups on the Inhibitory Strength of Amidinobenzyl Amidinophenyl Ethers



Compd no.	Am_1	Am_2	K_i, μM[a] Trypsin	Kallikrein	Thrombin
1	4	4	4.5 ± 0.1	56 ± 9.6	44 ± 8.4
15	4	3	5.7 ± 0.1	81 ± 2.5	14 ± 3.6
22	3	4	9.5 ± 0.9	32 ± 3.3	10 ± 1.2
23	3	3	9.0 ± 0.6	32 ± 4.0	14 ± 0.5

[a] K_i, dissociation constant of the enzyme-inhibitor complex. Values are means ± SD; $n = 3$.

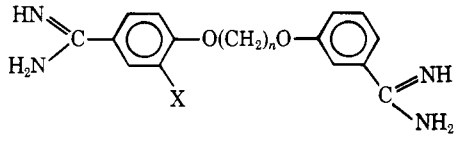
Table IV. Effect of Variation in the Length of the Hydrocarbon Chain on the Inhibitory Strength of α,ω-Bis(4-amidino-2,6-dibromophenoxy)alkanes



Compd no.	n	K_i, μM[a] Trypsin	Kallikrein	Thrombin
24	3	0.17 ± 0.02	1.5 ± 0.1	7.0 ± 0.7
25	5	0.78 ± 0.06	0.81 ± 0.1	9.6 ± 1.0
26	8	0.73 ± 0.4		7.2 ± 1.1

[a] K_i, dissociation constant of the enzyme-inhibitor complex. Values are means ± SD; $n = 3$.

Table V. Effect of Variation in the Length of the Hydrocarbon Chain and of Halogen Substitution on the Inhibitory Strength of α-(4-Amidinophenoxy)-ω-(3-amidinophenoxy)alkanes



Compd no.	n	X	K_i, μM[a] Trypsin	Kallikrein	Thrombin
27	3	H	3.2 ± 0.2	11 ± 1.6	3.8 ± 0.08
28	5	H	3.5 ± 0.1	8.3 ± 1.5	4.3 ± 0.6
29	6	H	3.8 ± 0.08	10 ± 1.5	4.5 ± 1.2
30	8	H	3.2 ± 0.6	7.4 ± 1.2	3.7 ± 0.8
31	5	Br	1.3 ± 0.1	2.6 ± 0.3	2.7 ± 0.4
32	5	I	1.0 ± 0.4	1.1 ± 0.2	1.1 ± 0.1

[a] K_i, dissociation constant of the enzyme-inhibitor complex. Values are means ± SD; $n = 3$.

chain compound (Table V). Halogen substitution fortified considerably the inhibitory properties, and the iodine-bearing compound **32** proved to be as powerful an antithrombin agent as our previously most active inhibitor, 1,8-bis(4-amidino-2-iodophenoxy)octane.[9]

Diamidines with a Central Dioxyalkane Chain. Clotting Tests. As one of our chief goals is the production of powerful anticoagulants for use in man, we also determined the inhibitory influence of α-(4-amidinophenoxy)-ω-(3-amidinophenoxy)alkanes on clotting tests involving the human coagulation components. To this end, we selected the thrombin clotting test employing stabilized human thrombin and human fibrinogen and also the partial thromboplastin time test (PTT). The former assay is sensitive only to inhibition of thrombin, while the latter is susceptible also to inhibition of earlier steps in the coagulation process. In Table VI we have listed side by side the effect of α-(4-amidinophenoxy)-ω-(3-amidinophenoxy)alkanes on the two tests, and we have also included 1,5-bis(4-amidinophenoxy)pentane (**33**) and 1,5-bis(3-amidinophenoxy)pentane (**34**) among the compounds examined. Turning first to the thrombin clotting test, we can note that compounds **27–30** with their 3-, 5-, 6-, and 8-carbon chains, respectively, were of equal potency and were about twice as active as compounds **33** and **34**. The most effective inhibitor was compound **32** which had also been the leading inhibitor of bovine thrombin in the amidase assay. In the PTT, it is interesting that the pentane derivative **28** was clearly superior to the homologs with shorter or longer central chains. Iodine substitution on the 5-carbon chain compound improved inhibitory performance also in the PTT and made compound **32** again surpass all other diamidines in inhibitory strength. However, it should be noted that the results in the PTT did not always parallel those in the thrombin clotting test. Compounds **30** and **31**, for example, were equally effective in the latter test, but compound **31** was considerably more potent than **30** in the PTT.

Conclusions

The most notable difference between the monooxyalkane compounds described above and the dioxyalkane derivatives reported earlier[9] was the observation that the 2-carbon chain was optimal for trypsin in the former series while in the latter series maximal strength had been reached only with the 12-carbon chain. It was also remarkable that the single bromine substitution on the ethoxy derivative increased potency by roughly 20 times and made the compound (**7**) the most effective low-molecular-weight reversible inhibitor of trypsin reported to date. The inhibitor is endowed with considerable specificity in that its K_i value for trypsin is 164 times lower than for kallikrein and 82 times lower than for thrombin. These data lend further support to the notion that significant differences exist in the topography of the binding sites of the three enzymes.

The experience with our inhibitors makes it clear that neither the K_i values obtained for bovine thrombin nor the performance in the human thrombin clotting test are reliable indicators of the overall anticoagulant activity of the compounds in human plasma. Such information can only be gathered from tests as the PTT which will reflect interference at more than one of the multiple steps in the clotting cascade. Diamidines can be expected to inhibit coagulation and thus prolong the PTT, not only by blocking the final thrombin–fibrinogen reaction but also by suppressing the activity of factor X_a, another arginine-specific esteroprotease.[10] In addition, phospholipid micelles play an important part in coagulation by furnishing binding sites and points of interaction for several clotting factors. The positively charged diamidines can be envisaged as occupying

Table VI. Inhibitory Effect of Diamidines with a Central Dioxyalkane Chain on the Thrombin Clotting Test and on the Partial Thromboplastin Time

Compd no.	Formula[a]	Thrombin clotting test,[b] concn of inhibitor producing 50% inhibn, μM	Partial thromboplastin time,[c] sec (control 56.8 ± 4.1 sec), at inhibitor concn (M) 10^{-4}	10^{-5}	5×10^{-6}
33	Am-RO(CH$_2$)$_5$OR-Am	22	248 ± 13.8	80 ± 1.2	
34	Am(3)-RO(CH$_2$)$_5$OR-Am(3)	25	328 ± 5.8	106 ± 2.3	
27	Am-RO(CH$_2$)$_3$OR-Am(3)	9	475 ± 14.4	115 ± 2.9	86 ± 1.0
28	Am-RO(CH$_2$)$_5$OR-Am(3)	10	>600	141 ± 5.8	107 ± 3.2
29	Am-RO(CH$_2$)$_6$OR-Am(3)	9.5	387 ± 3.9	113 ± 4.2	87 ± 1.0
30	Am-RO(CH$_2$)$_8$OR-Am(3)	15	272 ± 4.0	69 ± 1.9	60 ± 0.2
31	Am-RBr(2)O(CH$_2$)$_5$OR-Am(3)	15	721 ± 5.2	175 ± 1.3	131 ± 1.7
32	Am-RI(2)O(CH$_2$)$_5$OR-Am(3)	2.8	945 ± 8.0	191 ± 2.8	156 ± 1.4

[a] Am, amidino group; R, benzene ring. The numbers placed in parentheses after certain amidino (Am) groups and after the halogens indicate the location on the respective benzene rings. Amidino groups without numbers are present in the para position with respect to the central linkages. [b] Values are the means of two determinations. [c] Values are means ± SD; $n = 30$ for the control and $n = 3$ for the inhibitor assays.

Table VII. Diamidino Derivatives

Compd no.	Mp, °C	Yield, %	Formula	Analyses
1	293–295 dec	28	$C_{15}H_{16}N_4O \cdot 2HCl$	C, H
2	290	23	$C_{16}H_{18}N_4O \cdot 2HCl$	C, H
3	298	44	$C_{17}H_{20}N_4O \cdot 2HCl$	C, H, N
4	296–298 dec	42	$C_{15}H_{15}ClN_4O \cdot 2HCl$	C, H
5	305	55	$C_{17}H_{19}ClN_4O \cdot 2HCl \cdot 1.5H_2O$	C, H
6	290–293 dec	39	$C_{15}H_{15}BrN_4O \cdot 2HCl$	C, H
7	160–163	41	$C_{16}H_{17}BrN_4O \cdot 2HCl$	C, H
8	297–298	72	$C_{17}H_{19}BrN_4O \cdot 2HCl$	C, H, N
9	>300	34	$C_{15}H_{15}IN_4O \cdot 2HCl$	C, H, N
10	320	88	$C_{16}H_{17}IN_4O \cdot 2HCl$	C, H, N
11	277–279 dec	71	$C_{17}H_{19}IN_4O \cdot 2HCl$	C, H, N
12	240–243 dec	43	$C_{15}H_{14}Cl_2N_4O \cdot 2HCl \cdot 0.5H_2O$	C, H, N
13	254–256	24	$C_{15}H_{14}Br_2N_4O \cdot 2HCl$	C, H, N
14	248–251 dec	26	$C_{15}H_{14}I_2N_4O \cdot 2HCl$	C, H, N
15	165–168	25	$C_{15}H_{16}N_4O \cdot 2HCl$	C, H, N
16	170–174	80	$C_{15}H_{15}ClN_4O \cdot 2HCl \cdot 2H_2O$	C, H
17	177–180	13	$C_{15}H_{15}BrN_4O \cdot 2HCl \cdot H_2O$	C, H, N
18	156–158	88	$C_{15}H_{15}IN_4O \cdot 2HCl \cdot H_2O$	C, H, N
19	167–172	59	$C_{15}H_{14}Cl_2N_4O \cdot 2HCl$	C, H, N
20	280	53	$C_{15}H_{14}Br_2N_4O \cdot 2HCl \cdot 2H_2O$	C, H, N
21	193–198 dec	91	$C_{15}H_{14}I_2N_4O \cdot 2HCl$	C, H, N
22	165	25	$C_{15}H_{16}N_4O \cdot 2HCl$	C, H
23	130–135	42	$C_{15}H_{16}N_4O \cdot 2HCl$	C, H
24	292–294	61	$C_{17}H_{16}Br_4N_4O_2 \cdot 2HCl$	C, H
25	278 dec	11	$C_{19}H_{20}Br_4N_4O_2 \cdot 2HCl$	C, H
26	184–186	41	$C_{22}H_{26}Br_4N_4O_2 \cdot 2HCl \cdot 2H_2O$	C, H, N
27	148–150	58	$C_{17}H_{20}N_4O_2 \cdot 2HCl$	C, H
28	133–135	64	$C_{19}H_{24}N_4O_2 \cdot 2HCl$	C, H, N
29	128–131	77	$C_{20}H_{26}N_4O_2 \cdot 2HCl$	C, H, N
30	220	76	$C_{22}H_{30}N_4O_2 \cdot 2HCl \cdot 1.5H_2O$	C, H, N
31	185–190	78	$C_{19}H_{23}BrN_4O_2 \cdot 2HCl$	C, H
32	165–167	60	$C_{19}H_{23}IN_4O_2 \cdot 2HCl$	C, H, N

some of these sites on the anionic phospholipid layers and thus further retarding the coagulation process. As to the question whether diamidines are already potent enough to affect coagulation in man at reasonable doses, we may recall that for our most potent anticoagulant, compound **32**, a concentration of only 5×10^{-6} *M* was necessary to prolong the PTT to 2.5 times the control value, i.e., to achieve a clinical useful level of inhibition. In vivo studies of compounds **28** and **32** are in progress and will evaluate anticoagulant activity as well as possible hypotensive side effects.

Experimental Section

Amidase Assays. The dissociation constants (K_i) of diamidines with trypsin, pancreatic kallikrein, and thrombin were determined in amidase assays employing either N^{α}-benzoyl-DL-arginine-*p*-nitroanilide hydrochloride[11] (BANA, Bachem, Inc.) or *N*-benzoyl-L-phenylalanyl-L-valyl-L-arginine-*p*-nitroanilide hydrochloride[12] (BPVANA, AB Bofors, Nobel Div.) as substrate. The latter material was used with trypsin when the K_i value fell to around 10^{-7} *M* or less. For a given diamidine, identical dissociation constants were obtained with either substrate, but because of the lower K_m value with BPVANA and the faster reaction rate lesser amounts of enzymes were needed in the incubation mixtures. The K_i values were obtained graphically according to Dixon.[13]

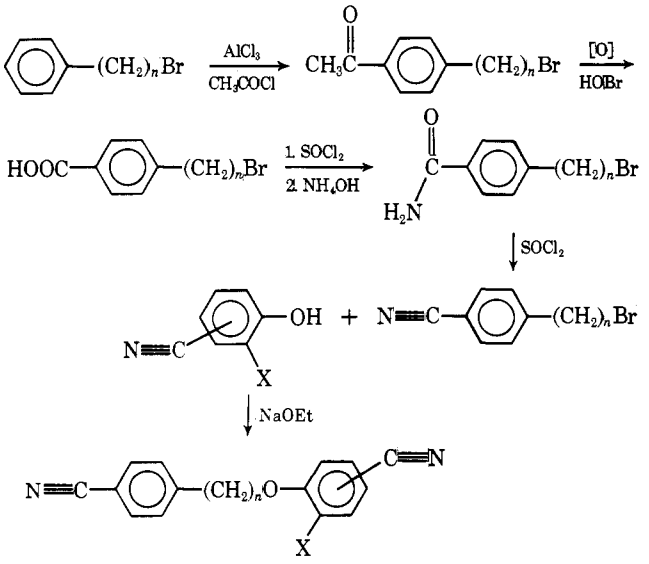
The reaction mixtures with each enzyme amounted to 1.6 ml and included 0.09 *M* Tris-HCl (pH 8.1), 10% by volume dimethyl sulfoxide, and BANA (3×10^{-3} or 1×10^{-3} *M*) or BPVANA (5×10^{-4} or 2.5×10^{-4} *M*). In the case of trypsin and thrombin they were fortified with 0.02 *M* $CaCl_2$. The following amounts of enzymes were used per assay: trypsin, 3.3 μg in the tests with BANA and 0.04 μg in the tests with BPVANA; pancreatic kallikrein, 17.7–71 Frey units; thrombin, 50 NIH units. All tests were carried out at 37°, and the length of the incubation period varied from 15 to 30 min for trypsin, to 40 min with thrombin, and to 40–160 min with kallikrein.

Trypsin (bovine, twice crystallized, salt-free) was obtained from Schwartz/Mann. Active-site titration revealed the preparation to contain 56.6% active trypsin by weight. The material was not further purified. Pancreatic kallikrein was kindly supplied by Farbenfabriken Bayer AG, Wuppertal-Elberfeld, Germany. The preparation contained 500 Frey units per milligram. Thrombin (bovine, topical) was a product of Parke, Davis and Co.

Thrombin Clotting Test. This test was performed in a similar manner as previously described.[4,5] A solution of stabilized human thrombin was prepared by clotting plasma in the presence of 20% by volume of ethanol, thus avoiding inactivation of the thrombin formed during the coagulation process.[14] After separation from the fibrin coagulum, the solution could be stored for months in the refrigerator without losing any of its thrombin activity. For the clotting test, a dilution of the solution was prepared, usually 1:4, to give a clotting time of about 50 sec at 37° when 0.2 ml was added to 0.1 ml of 0.154 *M* NaCl solution and 0.2 ml of human fibrinogen solution (400 mg of fibrinogen/100 ml of 0.146 *M* NaCl–0.0055 *M* sodium citrate solution). The diluent employed for the thrombin solution was composed of 20% by volume ethanol and 80% by volume 0.05 *M* Tris-HCl buffer (pH 7.4), the latter including 0.05 *M* NaCl and 0.005 *M* $CaCl_2$. To judge the potency of a diamidine, the amount of thrombin in the assay containing the inhibitor was doubled over that in the controls, and then that concentration of inhibitor was determined empirically which would retard clotting to a time matching the control value. At this point there would be 50% inhibition of the thrombin in the inhibitor-containing assay mixtures.

Partial Thromboplastin Time Test (PTT). This assay was derived from the method of Nye et al.[15] Citrated human plasma (0.1 ml) was mixed with 0.1 ml of thromboplastin solution (Thrombofax, Ortho Diagnostics) and 0.1 ml of 0.154 *M* NaCl solution or 0.154 *M* NaCl–inhibitor solution. After 30 sec of incubation at 37°

Scheme I



n = 2 or 3; X = H, I, Br, or Cl

0.1 ml of 0.02 M $CaCl_2$ solution was added. The time from the addition of the $CaCl_2$ solution to the formation of a firm clot represents the partial thromboplastin time.

Organic Synthesis. Melting points were determined on a Thomas-Hoover capillary melting point apparatus. Compound **33**, 1,5-bis(4-amidinophenoxy)pentane diisethionate, and compound **34**, 1,5-bis(3-amidinophenoxy)pentane dihydrochloride dihydrate, were obtained from May & Baker, Ltd., Dagenham, England. The following chemicals were prepared by previously described methods: 4-cyano-2-iodophenol,[16] 2,6-dibromo-4-cyanophenol,[17] 2-chloro-4-cyanophenol,[9,16] and 2-bromo-4-cyanophenol.[9,16] The target amidino compounds were prepared from the corresponding dicyano derivatives by slight modifications of a procedure developed by Ashley et al.[18] and by Berg and Newberry.[16] Analyses for all amidino derivatives were within ±0.3% of the theoretical values and were determined by either Atlantic Microlab, Inc., Atlanta, Ga., or Galbraith Laboratories, Inc., Knoxville, Tenn. Yields for the conversion of the dicyano intermediates to the diamidino products as well as the melting points and analyses for the diamidino compounds are given in Table VII. All symmetrical cyano derivatives (diethers) were prepared according to methods described in an earlier paper.[9] The monoether dicyano intermediates with two and three carbon chains were prepared according to a method described by Forman and McElvain[19] and by Blicke and Lilienfeld.[20] The stepwise procedure is outlined in Scheme I. The corresponding single carbon monoethers were prepared in one step from commercially available starting materials.

Acknowledgments. The authors are grateful to Mrs. Lynda B. Fox and to Mr. Robert E. Lee for their skillful technical help. Our thanks are also due to Farbenfabriken Bayer AG, Verfahrensentwicklung Biochemie, Wuppertal-Elberfeld, Germany, for the gift of pancreatic kallikrein. This study was supported by U.S. Public Health Service Grants HL 14228 (Thrombosis Center) and AM 10746.

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