Tetrahydro-isoquinoline-Based Factor Xa Inhibitors

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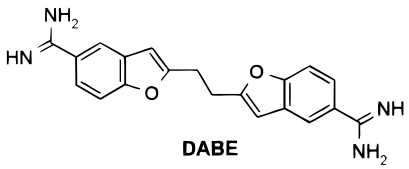
Derivatives of (2-amidino-1,2,3,4-tetrahydro-isoquinolin-7-yloxy)phenylacetic acid (TIPAC) were developed as inhibitors of factor Xa (fXa). The compounds are prepared using 15 synthetic steps on average. The most potent compounds **(14**, **17**, **22**−**26)** display inhibition constants of $K_i = 21-55$ nM but do not inhibit thrombin ($K_i = 5->100$ μM) and only weakly inhibit trypsin ($K_i = 0.08-5$ μM). They bear a second basic moiety, e.g., substituted 1-(iminomethyl)piperidines, which is linked to C-4 of the phenyl group of TIPAC via an oxygen atom. The inhibition constants of these compounds are almost independent of the size of the (iminomethyl)piperidine substituent. Due to the fact that fXa displays two cation binding sites, namely, the S1 and S4 sites, in principle two binding modes are conceivable for the novel dibasic fXa inhibitors. Molecular modeling experiments based on the X-ray structures of uninhibited fXa and the DX-9065a/fXa complex were carried out. The results taken together with the inhibition constants clearly favor one binding mode: the tetrahydro-isoquinoline fills the S1 pocket even better than the naphthalene moiety of DX-9065a, and the (iminomethyl)piperidine residues occupy the S4 site.

Introduction

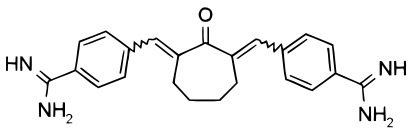
Factor Xa (fXa), a trypsin-like serine protease, plays a pivotal role in the coagulation process. It is produced from its zymogen factor X[1] by cleavage of the Arg194−Ile195 peptide bond upon activation of either the intrinsic or extrinsic pathway of the coagulation cascade.[2] Released fXa participates in the prothrombinase complex, which includes cofactor Va and calcium ions assembled on a phospholipid membrane. This complex generates thrombin from prothrombin, which ultimately activates platelets and causes fibrin deposition.

The two branches of the coagulation cascade converge on fXa which has therefore become a major target in the field of anticoagulant drug research.[3] A variety of fXa inhibitors have been isolated from natural sources: e.g., tick anticoagulant peptide from extracts of *Ornithodorus mubata*,[4] antistasin from the salivary glands of the Mexican leech *Haementeria officinalis*,[5] the anclystoma caninum anticoagulant peptide from soluble protein extracts of canine hookworms,[6] and ecotin from the periplasm of *Escherichia coli*.[7]

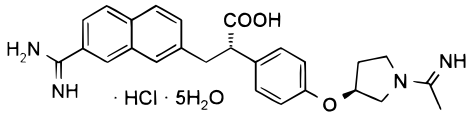
Considerable efforts are being focused on synthetic, low-molecular-weight fXa inhibitors. Benzamidine moderately inhibits diverse serine proteases including fXa by occupation of the enzymes' S1 subsites ($K_{i_{fXa}} = 410$ μM, $K_{i_{thrombin}} = 220$ μM, $K_{i_{trypsin}} = 35$ μM, $K_{i_{plasmin}} = 350$ μM).[8] The lack of selectivity is due to the similarity of the S1 sites of the enzymes. The selectivity is improved by the introduction of a second basic moiety. Various arylbisamidine derivatives such as DABE[9a] or BABCH[9b] have therefore been prepared in order to improve anti-fXa activity as well as selectivity.[10] Daiichi's naphthamidine-derived dibasic DX-9065a[11] combines reasonable anti-fXa activity with a satisfactory selectivity profile ($K_{i_{fXa}} = 0.041$ μM, $K_{i_{thrombin}} > 2000$ μM, $K_{i_{trypsin}} = 0.62$ μM, $K_{i_{plasmin}} = 23$ μM).[11c] However, the favorable profile of



DABE



BABCH



DX-9065a

this compound has to be paid for with a toilsome synthesis. DX-9065a has been prepared in a sequence comprising more than 20 reaction steps,[11b] of which the main effort is attributed to the poor accessibility of the 2,7-di-C,C-substituted naphthamidine precursor.[12] Hence, our idea was to design novel P1 building blocks suitable for fXa inhibition. Initially, modeling studies based on the X-ray structure of fXa[13] and on the structure of fXa complexed with DX-9065a[14] were carried out in order to scan the enzymes' S1 site. This approach resulted in the assumption that a bioisosteric substitution, of the 2,7-di-C,C-substituted naphthami-

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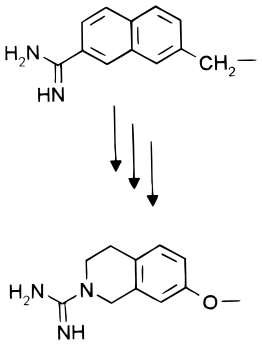


Figure 1. Bioisosteric transformation of the amidino-naphthylmethyl residue.

dine moiety with the 2-carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy template (Figure 1) would provide a novel P1 residue suitable for fXa inhibition. Herein we report on the synthesis and activity of (1,2,3,4-tetrahydro-isoquinolin-7-yloxy)phenylacetic acid derivatives ("TIPAC derivatives"), a novel class of low-molecular-weight inhibitors of factor Xa.

Chemistry

The synthesis of the central building block **6**, which provides the common scaffold for the tetrahydro-isoquinoline-based compounds, is depicted in Scheme 1. 3-(Benzyloxy)benzaldehyde is reacted with aminoacetaldehyde diethyl acetal to give the corresponding Schiff base **1**. Pomeranz−Fritsch type cyclization mediated by boron trifluoride and trifluoroacetic anhydride[15] and simultaneous cleavage of the *O*-benzyl group afford **2**, which is subsequently hydrogenated to give 1,2,3,4-tetrahydro-isoquinolin-7-ol (**3**). This four-step synthesis is performed in a convenient one-pot procedure without purification of the intermediates in an overall yield of more than 60%. Boc protection of the crude product followed by alkylation of the phenolic hydroxy group with the chloromandelate derivative **9** and subsequent O-debenzylation provide the key intermediate **6** in good yields.

Scheme 2 pictures the preparation of **9** via its cyanohydrin precursor **7**, which is obtained from 4-(benzyloxy)benzaldehyde and potassium cyanide in the presence of ammonium chloride. Pinner reaction followed by acid-catalyzed alcoholysis releases the mandelic acid derivative **8**, which is subsequently chlorinated by phosphorus pentachloride.

As outlined in Schemes 3 and 4, the key intermediate **6** is condensed with **10a**−**f** under Mitsunobu conditions[16] to give **11a**−**f**. Acid-catalyzed removal of the Boc groups yields the tetrahydro-isoquinolines **12a**−**f**, which afford the corresponding guanidines **13**−**16**, **27**, and **28** after treatment with 1*H*-pyrazole-1-carboxamidine hydrochloride.[17] Palladium-catalyzed cleavage of the allyl carbamate **13** in the presence of dimedone[18] releases the piperidine derivative **20**.

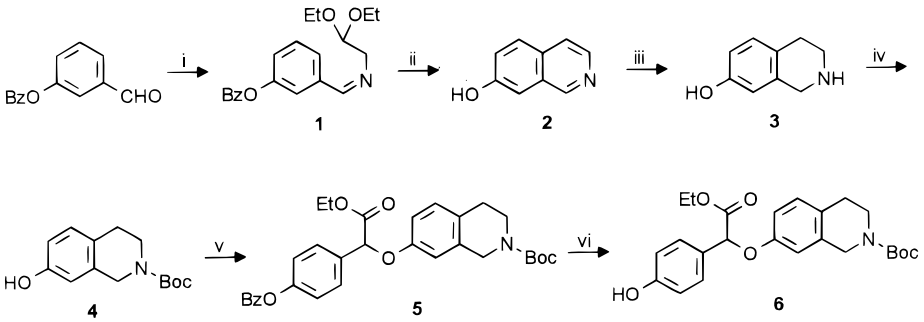
Attempts to deprotect the pyrrolidine moiety of **27** in the same manner did not afford the desired amine **30** but yielded a mixture of the enamine **35** and the allyl-substituted pyrrolidine **34** (Scheme 5). The latter is being formed due to retro allyl migration after carbon dioxide elimination. Hence, the intermediate pyrrolidine **30** is a more effective allyl scavenger than dimedone[19] and a more effective one than the corresponding piperidine derivative **20** as well. The enamine **35** results from condensation of released **30** with excess dimedone.[20] Modification of the deprotection conditions by replacing dimedone with tributyltin hydride[21] finally afforded **30** in good yields. **20** and **30** were reacted with various ethyl imidates to give **22**−**24** and **32**. The carboxylic acid derivatives **17**−**19**, **21**, **25**, **26**, **29**, **31**, **33**, and **36** were obtained upon conventional alkaline hydrolysis of the corresponding ethyl esters **14**−**16**, **20**, **22**, **23**, **28**, **30**, **32**, and **35**.

Results and Discussion

The inhibition constants of **13**−**36** toward fXa and the structurally related serine proteases thrombin, trypsin, and plasmin are summarized in Table 1. The dibasic compounds **14**, **17**, and **22**−**26** are the most potent derivatives with K_i values toward fXa in the lower nanomolar range. A closer inspection of the inhibition constants led to a better understanding of the binding mode of the new inhibitors. It has been suggested earlier that the S1[22] and S4 pockets of fXa are very similar.[14] Both consist of hydrophobic walls and a negatively charged bottom. Hence, two binding modes are possible for the dibasic inhibitors. In the first one, the amidino-tetrahydro-isoquinoline is the P1 residue and the second basic group, e.g., the amidino-piperidine of **14** or **17**, occupies the S4 site. This binding mode resembles that of DX-9065a in fXa.[14] In the second binding mode, the positions of the basic groups are inverted; i.e., the amidino-piperidine is the P1 residue. This mode is also conceivable, since amidino-piperidines are P1 residues of potent inhibitors of thrombin,[23] which has an almost identical S1 pocket. To solve this dichotomy, we took advantage of the small structural differences of the S1 and S4 pockets of fXa: The S1 pocket is accessible only from the top, while one wall of the S4 site is open to the solvent, and thus it can potentially accommodate bulkier groups which will not be able to enter the S1 site. Hence, we prepared the compounds **22**−**26** which bear bulkier substituents at the piperidine moiety. The inhibition constants turned out to be independent of the size of the substituents and very similar to those of the amidino-piperidines (Table 1). Therefore, the piperidines are most likely the P4 residues (first binding mode).

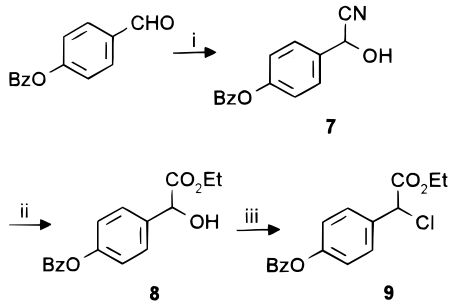
To further corroborate this interpretation, we undertook a simple molecular dynamics simulation of the S1 pocket residues and inhibitor docking experiments.[24] This was thought to be important to exclude the possibility of bulky groups entering the S1 site of fXa, since it had been observed previously that the S1 site is flexible: its rim moved by almost 2 Å toward the naphthalene moiety upon binding of DX-9065a.[14] Moreover, the temperature factors of the corresponding atoms in the fXa/DX-9065a structure are remarkably high compared to those of several thrombin/inhibitor structures. The molecular dynamics simulation of the S1 pocket residues revealed a high degree of flexibility also on the bottom of the S1 site which is most pronounced in the orientations of the side chain of Asp189 and the carbonyl group of Ala190. These residues are involved in the binding of the amidino group of DX-9065a in the fXa/DX-9065a[14] and of the guanidino group of the C-terminal Arg138 side chain

Scheme 1[a]



[a] Reagents and conditions: (i) $H_2N\text{-}CH_2\text{-}CH\text{-}(OCH_3)_2$, toluene, reflux; (ii) $(CF_3CO)_2O$, $BF_3{-}OEt_2$, toluene, rt; (iii) PtO_2, H_2, glacial acetic acid, rt; (iv) Boc_2O, NEt_3, CH_2Cl_2, 5 °C; (v) K_2CO_3, CH_3CN, reflux; (vi) Pd/C, H_2, ethyl acetate, rt.

Scheme 2[a]



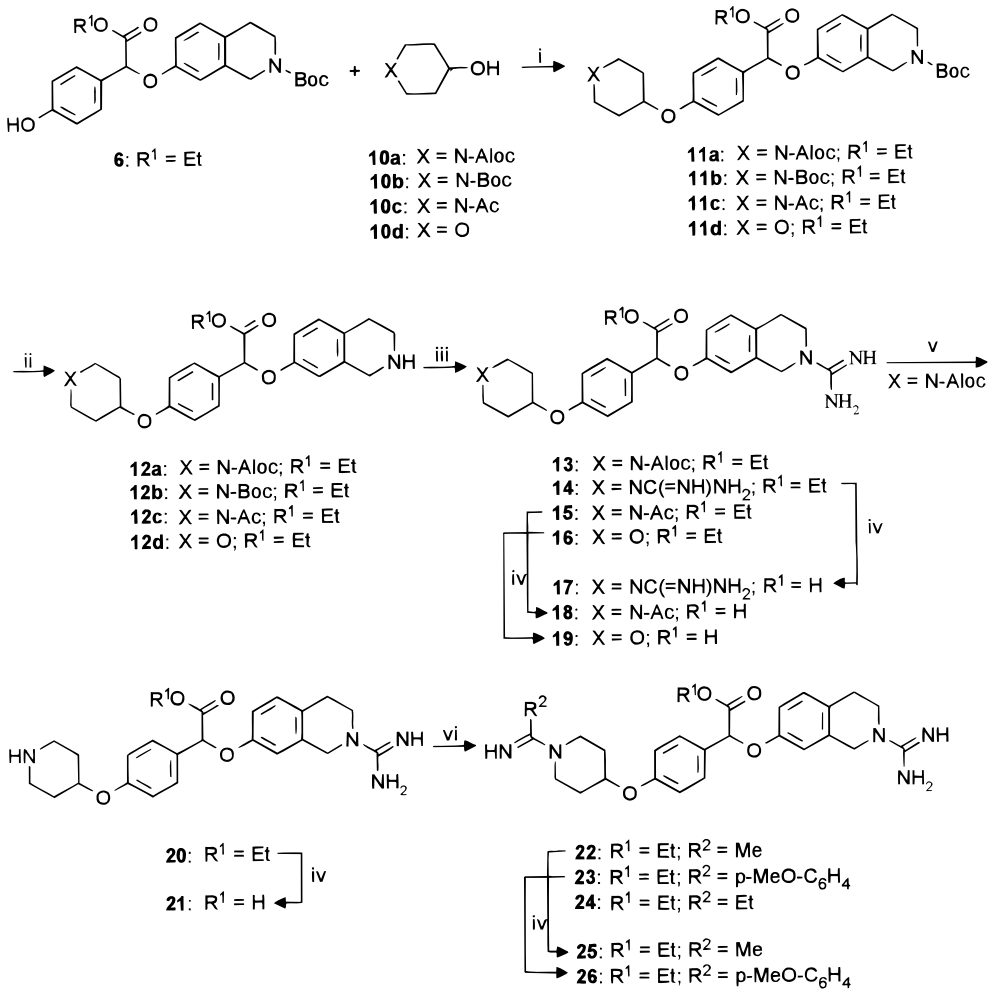
[a] Reagents and conditions: (i) NaCN, NH_4Cl, H_2O, 5 °C; (ii) HCl, EtOH, 5 °C; (iii) PCl_5, CH_2Cl_2, rt.

of the EGF2 domain of a neighboring symmetry equivalent fXa molecule in the inhibitor-free X-ray structure of fXa.[13] In the docking experiments it became evident that the amidino-tetrahydro-isoquinoline residue fills the S1 site even slightly better than the naphthamidine of DX-9065a. The bulky substituted piperidine moiety of **23** and **26** cannot enter this site, despite its flexibility, but it fits into the S4 pocket with the methoxyphenyl group exposed to the bulk solvent. In the S1 site, the tetrahydro-isoquinoline moiety can arrange in such a way that one C-1 hydrogen atom occupies the same position as the C-1 hydrogen atom of the naphthalene ring of DX-9065a, while the second C-1 hydrogen atom occupies a spatial gap which appears in the fXa/DX-9065a structure at the interface between enzyme and inhibitor (Figure 2).

As in the fXa/DX-9065a complex the second main interaction site is presented by the fXa-specific aryl-binding site (S4). The pyrrolidine ring of DX-9065a is completely buried by the aromatic moieties of Phe174 and Tyr99. The aromatic residues align almost parallel to the ring (stacking interaction), while the aliphatic part of the Glu97 side chain and Trp215 arrange in a perpendicular orientation. In our series the iminoalkyl- and amidino-substituted piperidine moieties of **14**, **17**, and **22**−**26** bind in a more effective way (K_i = 0.021−0.055 μM) compared with the corresponding pyrrolidine residues of **28**, **29**, **32**, and **33** (K_i = 0.08−0.2 μM) due to a more favorable alignment of the positively charged group in the aryl-binding site (S4). A similar trend is observed when comparing the pyrrolidine-based DX-9065a with its piperidine analogue.[25] As confirmed by modeling, one of the amidino nitrogen atoms of the amidino-piperidine moiety of compound **14**—and the appropriate one of **17**—forms a hydrogen bond to Glu97. This ionic interaction contributes considerably to the overall binding energy. Substitution of the second nitrogen atom by a hydrophobic methyl group (**22**, **25**) illuminates specificity features between the different enzymes. While this modification is unfavorable for thrombin and trypsin inhibition, there is almost no effect on the activity versus fXa. However, the equivalent transformation in the corresponding pyrrolidine series **(28**, **29)** results in a slight increase of potency on a generally lower level (**32**, **33**). An enlargement of the methyl group does not effect considerable changes in potency toward fXa (compare **22**/**23**/**24** and **25**/**26**). A conspicuous difference is observed only toward trypsin, where a 10-fold increase in potency occurs when the methyl group of compound **25** is replaced with the *p*-methoxyphenyl substituent (**26**). Obviously this hydrophobic substitution does not cause an essential contribution to the overall binding energy on fXa. Two slightly different binding modes of the piperidine moieties in the S4 site can be considered, depending on the nature of the terminal residue. Compounds substituted with a small terminal group on the piperidine nitrogen atom, such as the amidino-piperidine derivative **17**, arrange in a DX-9065a-like manner, whereas the bulky substituted piperidine nitrogen atom of **26** shifts slightly to the line formed by Glu97 and Phe174 due to the linear alignment of the methoxyphenyl substituent between Glu97, Thr98, and Phe174. Replacing the basic hydrogen donor substituent on the P4 residue with a neutral hydrogen acceptor fragment, for instance, iminoethyl (**22**) versus acetyl (**15**), results in a dramatic loss of potency toward fXa by a factor of 100. This effect is caused by the lack of the ionic interaction in the S4 site and demonstrates the importance of the correct pharmacophore in this position. Finally the elimination of substituent on the P4 heterocycle (Table 1, X = NH) also results in less potent compounds. Although the P4 residue of **20**, for instance, still provides the piperidine NH as hydrogen donor functionality, the observed activity is poor. Moreover, the replacement of the hydrogen donor group $>NH-$ with the hydrogen acceptor group $-O-$ does not considerably influence the affinity. Apparently in this position a hydrogen bond does not play an important role. This is in agreement with modeling data and the structure of DX-9065a in fXa.[14] The appropriate position is too far away from any hydrogen donor or hydrogen acceptor site.

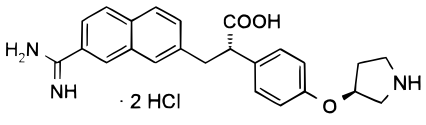
The nature of the carboxylate moieties of the TIPAC derivatives does not influence their inhibitory activity toward fXa. In any case the K_i value of a particular

Scheme 3[a]



[a] Reagents and conditions: (i) DEAD, PPh_3, THF, rt; (ii) HCl, Et_2O, 5 °C; (iii) 1*H*-pyrazole-1-carboxamidine hydrochloride, DIPEA, DMF, 5 °C; (iv) EtOH, NaOH aq, 5 °C; (v) $Pd(PPh_3)_4$, dimedone, THF, rt; (vi) R(=NH)OEt, NEt_3, EtOH, rt.

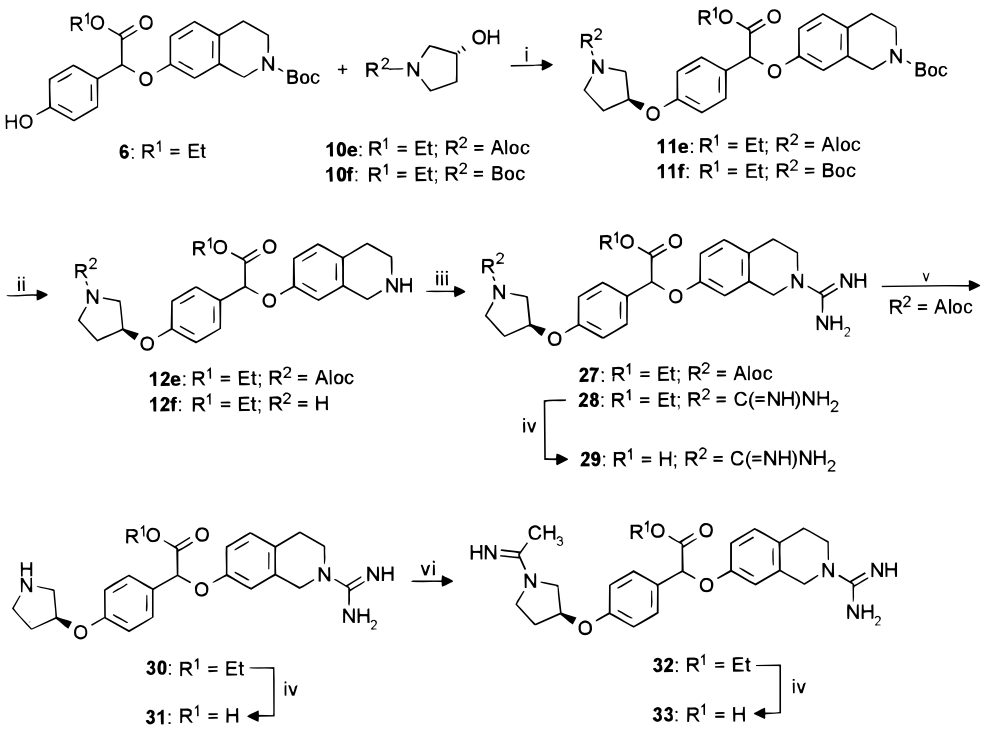
acid derivative is similar to that of the corresponding ethyl ester (e.g., the couples **14**/**17**, **22**/**25**, and **23**/**26**). This demonstrates that the carboxylate moieties are involved neither in direct ionic interaction nor in donor−acceptor interactions with fXa. They are exposed to the solvent as suggested by modeling studies and in analogy to the binding of DX-9065a in fXa. However, regarding the selectivity a distinct difference between acid and ester derivatives emerges. The ester derivatives, for instance, **14**, **22**, and **23**, show moderate inhibitory activity toward thrombin (K_i = 8−15 μM), trypsin (K_i = 0.2−0.4 μM), and plasmin ($K_i \approx$ 25 μM) similar to the observed selectivity profile of the ethyl ester of DX-9065a.[26] The corresponding acids (**17**, **25**, **26**) also moderately inhibit trypsin and plasmin, but in analogy to DX-9065a, they do not inhibit thrombin. The structural reasons for the observed selectivity profiles become clear upon contemplation of the X-ray structure of BM 12.1700, a precursor of DX-9065a in thrombin (PDB code: luvu).[27] In this structure the binding of the



BM 12.1700

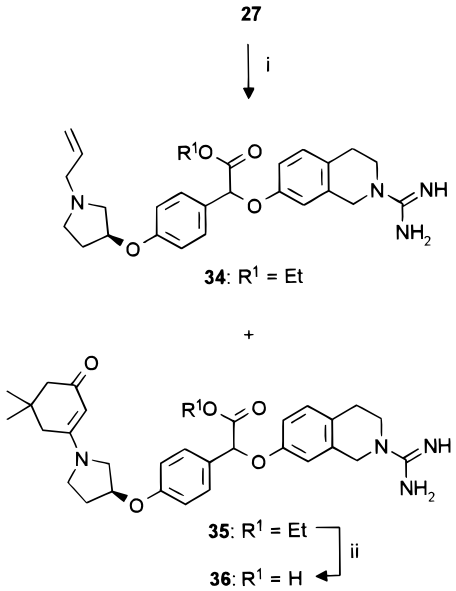
naphthamidine moiety (P1) is accompanied by a main chain transformation which results in a constriction of the S1 site. The energy required to alter the thrombin structure is (over)compensated by the interaction of the ester group of the inhibitor with the hydrophobic S2 site. An analogous binding mode is assumed for the ester derivatives of this work: interaction of the amidino-tetrahydro-isoquinoline group with a constricted S1 site along with the extension of the ester groups into the hydrophobic S2 site results in moderate thrombin inhibition. Removal of the ester groups eliminates the essential S2 site interaction and thus withdraws the basis for a reasonable occupation of the S1 site. In other words: thrombin is not inhibited by the acid derivatives. In trypsin the situation is different. The S1 site is less pronounced as in thrombin and does not require a movement of its rim toward the amidino-tetrahydro-isoquinoline residue, as previously shown in the X-ray structures of naphthamidine-bound trypsin.[25] A particular S2 pocket is not present in trypsin, and further interactions are only of minor importance. This results in a moderate inhibition of both ester- and acid-derived compounds of this series. Plasmin inhibition is assumed to follow similar principles, although the inhibition constants are significantly lower (by about 2 orders of magnitude compared to those found for trypsin).

Scheme 4[a]



[a] Reagents and conditions: (i) DEAD, PPh_3, THF, rt; (ii) HCl, Et_2O, 5 °C; (iii) 1*H*-pyrazole-1-carboxamidine hydrochloride, DIPEA, DMF, 5 °C; (iv) EtOH, NaOH aq, 5 °C; (v) $Pd(PPh_3)_4$, Bu_3SnH, CH_2Cl_2, 5 °C; (vi) R(=NH)OEt, NEt_3, EtOH, rt.

Scheme 5[a]



[a] Reagents and conditions: (i) $Pd(PPh_3)_4$, dimedone, THF, rt; (ii) EtOH, NaOH aq, 5 °C.

The anticoagulant activity and the selectivity profile of the compounds **14**, **17**, and **22**−**26** are also demonstrated by the results of the plasma coagulation assays. The influence of the inhibitors on the activated partial thromboplastin time (aPTT) mirrors both the fXa and thrombin inhibition, while the thrombin time (TT) is sensitive to thrombin inhibition, but not for fXa inhibition. The inhibitors prolongate the aPTT considerably. This is expressed by the values of the aPTT doubling time concentration (ED_{200}(aPTT): 3−11 μM). The selectivity profile is confirmed by the results of the TT assay. The ED_{200} values of the aPPT and TT assays are listed in Table 1.

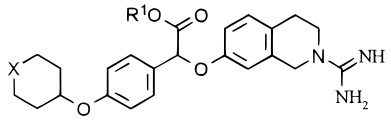
Conclusion

We have introduced the amidino-tetrahydro-isoquinolin-7-yloxy template as a novel P1 residue for the inhibition of the blood coagulation factor Xa. On the basis of this moiety, we-have developed (2-amidino-1,2,3,4-tetrahydro-isoquinolin-7-yloxy)phenylacetic acid derivatives (TIPAC derivatives) as a novel class of low-molecular-weight fXa inhibitors. They were prepared in 15 reaction steps on average. Substituted iminoalkyl- or amidino-piperidine moieties proved to be the most effective P4 residues in this series. The best compounds (**17**, **25**, and **26**) inhibit fXa with K_i values in the lower nanomolar range and display a reasonable selectivity profile.

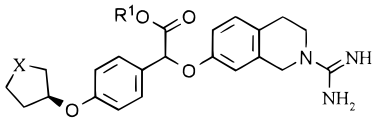
Experimental Section

General. Starting materials and reagents were purchased from commercial sources (Aldrich or Lancaster) and used without further purification, unless otherwise noted. Dry solvents: acetone and dichloromethane distilled from P_2O_5 distilled using a mirrored Vigreux column; DMF distilled from calcium hydride; methanol distilled from sodium methanolate (10 g of sodium/L of methanol); tetrahydrofuran distilled from $LiAlH_4$; toluene distilled from sodium hydride; distilled solvents stored over molecular sieves (3 Å; Merck Darmstadt); diethyl ether kept over Al_2O_3 (alumina N, activity grade I; ICN Biomedicals). Air- and moisture-sensitive reactions were carried out under inert gas atmosphere (argon or nitrogen) using oven-dried (110 °C) glassware. ^{1}H NMR: DMSO-d_6 (unless otherwise stated), Bruker AC 250 spectrometer, TMS as internal standard, chemical shifts in δ ppm. ^{13}C NMR: DMSO-d_6 (unless otherwise stated), Bruker AC 250 spectrometer at 63 MHz, TMS as internal standard, chemical shifts in δ ppm. Mass spectra: Finnigan MAT 312, Micro PDP-11 computer, Finnigan program SS300. Melting points: Büchi

Table 1. Inhibition Constants and Anticoagulant Activity of Compounds **13**−**36**



A



B

				K_i[a]				ED_{200}[a]	
compd	formula	X	R^1	fXa	thrombin	trypsin	plasmin	aPTT	TT
13	A	N-Aloc	Et	3	4	nd[b]	14	260	>500
14	A	N-C(=NH)NH_2	Et	0.028	8	0.2	25	3	54
15	A	N-acetyl	Et	3	>100	10	10	100	200
16	A	O	Et	2	8	3	26	250	500
17	A	N-C(=NH)NH_2	H	0.044	>100	0.2	44	9	>500
18	A	N-acetyl	H	0.4	>100	5	nd[b]	150	>500
19	A	O	H	3	>100	12	25	>500	>500
20	A	N-H	Et	2	16	3	25	26	71
21	A	N-H	H	2	>100	6	25	190	>500
22	A	N-C(=NH)Me	Et	0.030	15	0.4	25	3	53
23	A	N-C(=NH)Ar[c]	Et	0.021	5	0.2	nd[b]	6	32
24	A	N-C(=NH)Et	Et	0.030	20	0.08	nd[b]	10	70
25	A	N-C(=NH)Me	H	0.055	>100	2	52	11	>500
26	A	N-C(=NH)Ar[c]	H	0.026	>100	0.2	nd[b]	7	>500
27	B	N-Aloc	Et	0.2	>100	9	10	200	500
28	B	N-C(=NH)NH_2	Et	0.2	13	2	45	10	300
29	B	N-C(=NH)NH_2	H	0.2	>100	1	nd[b]	40	>500
30	B	N-H	Et	0.4	>100	6	40	26	140
31	B	N-H	H	1	>100	10	10	50	>500
32	B	N-C(=NH)Me	Et	0.1	>100	2	nd[b]	10	100
33	B	N-C(=NH)Me	H	0.080	>100	10	nd[b]	30	>500
34	B	N-allyl	Et	1	>100	10	50	27	240
35	B	N-dmoche[d]	Et	0.2	>100	10	50	24	90
36	B	N-dmoche[d]	H	0.2	>100	10	50	200	>500
DX-9065a				0.040[e]	>100[e]	2[e]	11[e]		

[a] In μM. [b] nd, not determined. [c] Ar = 4-MeO-C_6H_4−. [d] dmoche = 5,5-dimethyl-3-oxocyclohex-l-enyl. [e] As determined according to the procedures described in the Experimental Section (for original data, see ref 11c).

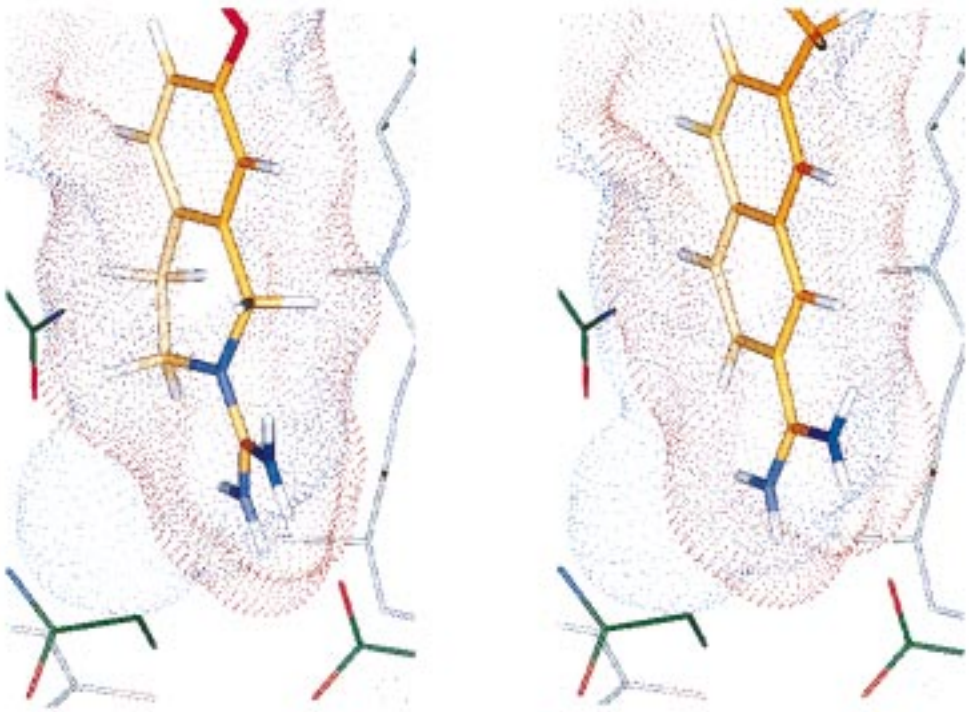


Figure 2. Proposed binding of the isoquinoline moiety of TIPAC derivative **25** to the S1 site of factor Xa (left) in comparison with the binding of the naphthamidine moiety of DX-9065a (right) as determined in the X-ray structure.[14]

model 530 apparatus, uncorrected. TLC: TLC plates 0.25-mm silica gel 60 F_{254} (Merck Darmstadt); detection achieved under UV light (254 nm) or by spraying with ninhydrin or phosphomolybdic acid or Awe's reagent (sodium azide/iodine). Column chromatography: Merck silica gel 60 (70−230 mesh). Analytical HPLC: reverse-phase LiChrospher 60 RP-Select-B column, 5 μm, size 4 × 125 mm (Merck Darmstadt); eluents acetonitrile/water (10/90−30/70) or methanol/water (10/90−

30/70). Preparative HPLC: reverse-phase LiChroprep RP-18 column, 15−25 μm, size 4 × 25 cm (Merck Darmstadt). Microanalytical results indicated by the symbols of the elements were within ±0.4% of the theoretical values; if only mass spectral data are given, the purity of these compounds has been assessed to be >98% by TLC or HPLC. Compounds were named following IUPAC rules as applied by AUTONOM, Beilstein Informationssysteme GmbH, Frankfurt/Main, Germany. Modeling studies have been carried out with the Insight II/Discover program package (MSI, San Diego, CA). DX-9065a was sythesized according to published procedures.[11b,12]

Isoquinolin-7-ol (2). Aminoacetaldehyde dimethyl acetal (79 g, 0.750 mol) was added to 3-(benzyloxy)benzaldehyde (0.500 mol, 106 g) in 1100 mL of toluene and the mixture refluxed for 6 h using a Dean−Stark trap. After cooling to 5 °C trifluoracetic acid anhydride (212 mL, 1.500 mol) and boron trifluoride etherate (185 mL, 1.500 mol) were added under nitrogen in succession at such a rate that the internal temperature was kept below 10 °C. After stirring for 5 days at room temperature the precipitated material was separated by filtration, washed with diethyl ether several times, and dissolved in 750 mL of water. The pH value was adjusted to pH 9 by adding concentrated aqueous ammonia and the precipitated product separated by filtration, followed by washing with diethyl ether and drying in vacuo: yield 53.2 g (73%); light yellow solid; mp 228−230 °C. EI-MS: 145 (M^+). ^{1}H NMR: δ = 7.35 (s, 1H), 7.37 (m, 1H), 7.72 (d, 1H), 7.83 (d, 1H), 8.30 (d, 1H), 9.12 (s, 1H), 10.25 (s, 1H). ^{13}C NMR: δ = 107.8, 120.0, 123.2, 128.1, 129.7, 129.9, 139.9, 150.4, 156.3. Anal. Calcd (C_9H_7NO) C, H, N.

1,2,3,4-Tetrahydro-isoquinolin-7-ol Hydroacetate (3). **2** (53.2 g, 0.365 mol) was dissolved in 1000 mL of glacial acetic acid and hydrogenated for 48 h at room temperature using prehydrogenated platinum dioxide (3.5 g) as catalyst. Filtration followed by concentration and addition of 50 mL of acetone gave a clear solution. Addition of diethyl ether resulted in precipitation of pure 1,2,3,4-tetrahydro-isoquinolin-7-ol hydroacetate: yield 44.9 g (83%); white solid; mp 179−182 °C. EI-MS: 149 (M^+). ^{13}C NMR: δ = 20.8, 26.2, 42.4, 46.0, 112.3, 113.8, 123.6, 129.5, 134.1, 155.3, 171.9. Anal. Calcd ($C_{11}H_{15}NO_3$) C, H, N.

7-Hydroxy-3,4-dihydro-1*H*-isoquinoline-2-carboxylic Acid *tert*-Butyl Ester (4). A mixture of 17.1 g (0.079 mol) of di-*tert*-butyl dicarbonate in 170 mL of dichloromethane was added dropwise to a stirred suspension of 16.4 g (0.079 mol) of **3** and 32.6 mL (0.235 mol) of triethylamine in 164 mL of dichloromethane at 5 °C. Stirring was continued at 5 °C for 1 h, followed by evaporation of the volatiles. The residue was redissolved in ethyl acetate and this solution washed in succession with aqueous acetic acid, saturated sodium hydrogen carbonate solution, and brine and dried (Na_2SO_4). Removal of the solvent in vacuo gave pure **4** as a white solid: yield 17.5 g (89%); mp 140−142.5 °C; EI-MS: 249 (M^+). ^{1}H NMR: δ = 1.44 (s, 9H), 2.64 (t, 2H), 3.50 (t, 2H), 4.37 (s, 2H), 6.55 (m, 2H), 6.92 (d, 1H), 9.17 (s, 1H). ^{13}C NMR: δ = 27.3, 28.0, 41.5, 45.1, 78.1, 112.2, 113.7, 124.3, 129.3, 134.2, 153.9, 155.5. Anal. Calcd ($C_{14}H_{19}NO_3$) C, H, N.

7-[(4-(Benzyloxy)phenyl)(ethoxycarbonyl)methoxy]-3,4-dihydro-1*H*-isoquinoline-2-carboxylic Acid *tert*-Butyl Ester (5). A mixture of 7.5 g (0.030 mol) of **4**, 11.4 g (0.037 mol) of **9**, and 8.4 g (0.060 mol) of K_2CO_3 in 150 mL of acetonitrile was refluxed for 24 h. Filtration followed by concentration in vacuo and column chromatography (isohexane/ethyl acetate, 9:1, 8:2, 7:3) yielded 15.2 g (98%) of the title compound as a white solid: mp 85−87 °C. (+)-FAB-MS: 518 (MH^+). ^{13}C NMR: δ = 13.8, 27.3, 28.0, 41.3, 45.0, 60.8, 69.2, 76.7, 78.8, 112.5, 113.6, 114.7, 127.2, 127.5, 127.6, 127.7, 127.8, 128.3, 128.7, 129.5, 136.8, 153.8, 155.1, 158.7, 169.5. Anal. Calcd ($C_{31}H_{35}NO_6$) C, H, N.

7-[(Ethoxycarbonyl)(4-hydroxyphenyl)methoxy]-3,4-dihydro-1*H*-isoquinoline-2-carboxylic Acid *tert*-Butyl Ester (6). **5** (10.8 g, 0.020 mol) was dissolved in 200 mL of ethyl acetate and hydrogenated for 3 h at room temperature using 2.0 g of palladium on charcoal (10%) as catalyst. Filtration followed by concentration in vacuo afforded 7.8 g (91%) of the title compound as a white solid: mp 152−155 °C. (+)-FAB-MS: 428 (MH^+). ^{1}H NMR: δ = 1.11 (t, 3H), 1.42 (s, 9H), 2.68 (t, 2H), 3.50 (t, 2H), 4.11 (m, 2H), 4.43 (s, 2H), 5.75 (s, 1H), 6.76 (m, 4H), 7.02 (d, 1H), 7.31 (d, 2H), 9.60 (s, 1H). ^{13}C NMR: δ = 14.0, 27.4, 28.1, 41.4, 44.8, 60.9, 77.1, 78.9, 112.6, 113.7, 115.4, 125.8, 127.2, 128.9, 129.6, 134.7, 153.9, 155.4, 158.0, 169.8. Anal. Calcd ($C_{24}H_{29}NO_6$) C, H, N.

(4-Benzyloxy)phenyl)hydroxyacetonitrile (7). 4-(Benzyloxy)benzaldehyde (42.6 g, 0.200 mol) was dissolved in 500 mL of ether, and 32.0 g (0.600 mol) of ammonium chloride in 90 mL of water and 29.4 g (0.600 mol) of sodium cyanide in 80 mL of water were added in succession at 5 °C. The reaction mixture was allowed to stir for 24 h at 5 °C. Organic and aqueous layers were separated, the latter was extracted with ethyl acetate, the combined organic extracts were washed with water and brine and dried, and the solvent was removed in vacuo. The remaining white solid [47.6 g, 99%; EI-MS 240 (M^+)] was used in the next step without further purification.

(4-(Benzyloxy)phenyl)hydroxyacetic Acid Ethyl Ester (8). **7** (47.6 g, 0.198 mol) was dissolved in 200 mL of diethyl ether; 12.8 mL (0.260 mol) of ethanol and 8.0 g (0.220 mol) of dry hydrogen chloride were added at 5 °C. The reaction mixture was allowed to stand for 24 h at 5 °C. The precipitated material was collected by filtration and suspended in a mixture of 300 mL of ethanol and 600 mL of water. After stirring for 72 h at ambient temperature the precipitated white solid was collected, washed with water, and dried: yield 50.0 g (88%); mp 89−92 °C. (+)-FAB-MS: 287 (MH^+). ^{1}H NMR: δ = 1.15 (t, 3H), 4.07 (m, 2H), 5.00 (d, 1H), 5.05 (s, 2H), 5.88 (d, 1H), 6.95 (d, 2H), 7.27−7.47 (m, 7H). Anal. Calcd ($C_{17}H_{18}O_4$) C, H.

(4-(Benzyloxy)phenyl)chloroacetic Acid Ethyl Ester (9). PCl_5 (10.4 g, 0.050 mol) was added to a solution of **8** (14.3 g, 0.050 mol) in 250 mL of dichloromethane at 5 °C and the reaction mixture stirred for 24 h at room temperature. Concentration in vacuo followed by column chromatography (isohexane/ethyl acetate, 9:1) yielded 13.0 g (85%) of the title compound as a yellow, crystalline solid: mp 45−47 °C. EI-MS: 304 (M^+). ^{1}H NMR: δ = 1.18 (t, 3H), 4.16 (m, 2H), 5.11 (s, 2H), 5.81 (d, 1H), 7.04 (d, 2H), 7.30−7.46 (m, 7H). ^{13}C NMR: δ = 13.8, 58.4, 62.0, 69.4, 115.0, 127.7, 127.9, 128.3, 128.5, 129.5, 136.8, 159.0, 168.1. Anal. Calcd ($C_{17}H_{17}ClO_3$) C, H, Cl.

4-Hydroxypiperidine-1-carboxylic Acid Allyl Ester (10a). A solution of 11.7 mL of allyl chloroformate (0.11 mol) in 20 mL dichloromethane was added dropwise to a mixture of 10.1 g (0.10 mol) of 4-hydroxypiperidine and 18.0 mL (0.12 mol) of DBU in 200 mL of dichloromethane at 5 °C. The organic layer was extracted with aqueous acetic acid and saturated sodium hydrogen carbonate solution and dried (Na_2SO_4). Removal of the solvent in vacuo gave pure **10a** as a colorless oil: yield 18.4 g (99%). EI-MS: 185 (M^+). ^{1}H NMR: δ = 1.18−1.36 (m, 2H), 1.60−1.78 (m, 2H), 3.05, (m, 2H), 3.57−3.78 (m, 3H), 4.48 (d, 2H), 4.72 (d, 1H), 5.25 (dd, 2H), 5.91 (m, 1H). ^{13}C NMR: δ = 33.9, 41.1, 65.1, 65.3, 116.7, 133.6, 154.2.

4-Hydroxypiperidine-l-carboxylic Acid *tert*-Butyl Ester (10b). To a solution of 20.2 g (0.20 mol) of 4-hydroxypiperidine and 56.0 mL (0.40 mol) of triethylamine in 200 mL of dichloromethane was added 50.0 g (0.22 mol) of di-*tert*-butyl dicarbonate in portions at 5 °C. After stirring at room temperature for 16 h the mixture was worked up following the procedure used for **10a**: yield 37.8 g (94%); white crystals; mp 68 °C. EI-MS: 201 (M^+). ^{1}H NMR: δ = 1.13−1.31 (m, 2H), 1.39 (s, 9H), 1.61−1.72 (m, 2H), 2.94, (t, 2H), 3.61−3.70 (m, 3H), 4.66 (s, 1H). ^{13}C NMR: δ = 28.0, 34.0, 43.8, 65.6, 78.3, 153.8. Anal. Calcd ($C_{10}H_{19}NO_3$) C, H, N.

1-(4-Hydroxypiperidin-1-yl)ethanone (10c). To a solution of 5.60 g (0.050 mol) of 4-hydroxypiperidine in 100 mL of methanol was added 18.8 mL (0.200 mol) of acetic acid anhydride dropwise at 5 °C. After stirring for 72 h at room temperature the volatiles were removed in vacuo and the residue was recrystallized from diethyl ether to give 4.10 g (0.029 mol; 58.0%) of pure **10c** as a white solid: mp 70−73

°C. El-MS: 143 (M^+). ^{1}H NMR: δ = 1.13−1.42 (m, 2H), 1.71 (m, 2H), 2.00 (s, 3H), 2.97 (t, 1H), 3.12 (t, 1H), 3.67 (m, 2H), 3.88 (m, 1H), 4.72 (d, 1H). ^{13}C NMR: δ = 21.2, 33.9, 34.6, 38.4, 43.3, 65.6, 167.9. Anal. Calcd ($C_7H_{13}NO_2$) C, H, N.

3(*R*)-Hydroxypyrrolidine-1-carboxylic Acid Allyl Ester (10e). Prepared according to the procedure used for **10a**: yield 88%; colorless oil. EI-MS: 171 (M^+). ^{1}H NMR: δ = 1.82 (m, 2H), 3.13−3.41 (m, 4H), 4.23 (m, 1H), 4.51 (d, 2H), 4.90 (s, 1H), 5.21 (dd, 2H), 5.92 (m, 1H). ^{13}C NMR: δ = 32.8, 33.6, 43.5, 44.0, 53.7, 54.3, 64.6, 68.7, 69.2, 116.6, 133.6, 153.9. Anal. Calcd ($C_8H_{13}NO_3$) C, H, N.

3(*R*)-Hydroxypyrrolidine-1-carboxylic Acid *tert*-Butyl Ester (10f). Prepared according to the procedure used for **10b**: yield 99%; colorless oil. El-MS: 187 (M^+). ^{1}H NMR: δ = 1.40 (s, 9H), 1.78 (m, 2H), 3.20 (m, 4H), 4.20 (m, 1H), 4.84 (s, 1H). ^{13}C NMR: δ = 28.2, 32.9, 33.6, 43.5, 43.7, 53.8, 54.0, 68.4, 69.2, 78.0, 153.6. Anal. Calcd ($C_9H_{17}NO_3$) C, H, N.

General Procedure A. Mitsunobu Reaction Affording Compounds 11a−f from 10a−f and 6. Under nitrogen a solution of 0.025 mol of diethyl azodicarboxylate in 20 mL of tetrahydrofuran was added at 5 °C to a mixture of 0.020 mol of phenol **6**, 0.025 mol of alcohol **10**, and 0.025 mol of triphenylphosphine in 150 mL of tetrahydrofuran. After stirring for 72 h at room temperature the volatiles were removed in vacuo, and the residue was chromatographed on silica gel.

7-{[4-(1-(Allyloxycarbonyl)piperidin-4-yloxy)phenyl](ethoxycarbonyl)methoxy}-3,4-dihydro-1*H*-isoquinoline-2-carboxylic Acid *tert*-Butyl Ester (11a). Colorless oil; yield 90%. (+)-FAB-MS: 595 (MH^+). ^{1}H NMR: δ = 1.14 (t, 3H), 1.40 (s, 9H), 1.55 (m, 2H), 1.92 (m, 2H), 2.68 (t, 2H), 3.25 (m, 2H), 3.50 (t, 2H), 3.70 (m, 2H), 4.11 (m, 2H), 4.41 (s, 2H), 4.52 (d, 2H), 4.61 (m, 1H), 5.23 (dd, 2H), 5.83 (s, 1H), 5.90 (m, 1H), 6.75 (m, 2H), 7.01 (m, 3H), 7.43 (m, 2H). ^{13}C NMR: δ = 13.9, 27.9, 28.0, 30.1, 40.5, 41.4, 44.8, 60.8, 65.1, 71.5, 76.6, 78.8, 112.4, 113.6, 115.6, 116.8, 127.2, 127.5, 128.8, 129.6, 133.4, 134.7, 153.9, 154.1; 155.1, 157.2, 169.5. Anal. Calcd ($C_{33}H_{42}N_2O_8$) C, H, N.

7-{[4-(1-(*tert*-Butoxycarbonyl)piperidin-4-yloxy)phenyl](ethoxycarbonyl)methoxy}-3,4-dihydro-1*H*-isoquinoline-2-carboxylic Acid *tert*-Butyl Ester (11b). Light yellow oil; yield 75%. EI-MS: 610 (M^+).

7-{[4-(1-Acetylpiperidin-4-yloxy)phenyl](ethoxycarbonyl)methoxy}-3,4-dihydro-1*H*-isoquinoline-2-carboxylic Acid *tert*-Butyl Ester (11c). White solid; yield 91%. (+)-FAB-MS: 553 (MH^+). ^{1}H NMR: δ = 1.13 (t, 3H), 1.41 (s, 9H), 1.42−1.69 (m, 2H), 1.89 (m, 2H), 2.02 (s, 3H), 2.68 (t, 2H), 3.21 (m, 1H), 3.31 (m, 1H), 3.50 (t, 2H), 3.62 (m, 1H), 3.80 (m, 1H), 4.11 (m, 2H), 4.42 (s, 2H), 4.62 (m, 1H), 5.84 (s, 1H), 6.77 (m, 2H), 7.01 (m, 3H), 7.42 (d, 2H). ^{13}C NMR: δ = 13.9, 21.2, 27.3, 28.0, 30.1, 30.8, 37.9, 42.0, 42.8, 45.5, 60.9, 71.7, 76.7, 78.8, 112.5, 114.5, 115.7, 127.2, 127.5, 128.8, 129.5, 133.2, 153.8, 155.1; 157.3, 167.9, 169.5. Anal. Calcd ($C_{31}H_{40}N_2O_7$) C, H, N.

7-{(Ethoxycarbonyl)[4-(tetrahydro-pyran-4-yloxy)phenyl]methoxy}-3,4-dihydro-1*H*-isoquinoline-2-carboxylic Acid *tert*-Butyl Ester (11d). Colorless oil; yield 58%. EI-MS: 511 (M^+). ^{1}H NMR: δ = 1.12 (t, 3H), 1.41 (s, 9H), 1.59 (m, 2H), 1.99 (m, 2H), 2.66 (t, 2H), 3.49 (m, 4H), 3.84 (m, 2H), 4.11 (m, 2H), 4.43 (s, 2H), 4.59 (m, 1H), 5.82 (s, 1H), 6.75 (m, 2H), 7.01 (m, 3H), 7.42 (d, 2H). ^{13}C NMR: δ = 14.0, 28.0, 28.1, 31.7, 40.6, 43.6, 61.0, 64.5, 71.3, 76.8, 79.0, 112.8, 113.8, 115.8, 127.4, 127.6, 128.9, 129.6, 129.7, 153.9, 155.3; 157.4, 169.7. Anal. Calcd ($C_{29}H_{37}NO_7$) C, H, N.

7-{[4-(1-(Allyloxycarbonyl)pyrrolidin-3(*S*)-yloxy)phenyl](ethoxycarbonyl)methoxy}-3,4-dihydro-1*H*-isoquinoline-2-carboxylic Acid *tert*-Butyl Ester (11e). Colorless oil; yield 91%. (+)-LSIMS: 579.9 (MH^+). ^{1}H NMR: δ = 1.12 (m, 3H), 1.42 (s, 9H), 2.12 (m, 2H), 2.68 (t, 2H), 3.50 (m, 6H), 4.12 (m, 2H), 4.43 (s, 2H), 4.52 (d, 2H), 5.02 (m, 1H), 5.22 (dd, 2H), 5.85 (s, 1H), 5.92 (m, 1H), 6.76 (m, 2H), 7.02 (m, 3H), 7.45 (d, 2H). ^{13}C NMR: δ = 13.9, 14.6, 27.3, 28.0, 29.8, 30.8, 41.4, 43.5, 44.1, 44.8, 50.8, 51.4, 60.3, 60.9, 64.8, 75.1, 76.0, 77.0, 78.8, 112.6, 113.7, 115.4, 116.8, 127.3, 127.9, 128.8, 129.5, 133.4, 134.7, 153.8, 155.1, 156.5, 157.1, 169.5. Anal. Calcd ($C_{32}H_{40}N_2O_8$) C, H, N.

7-([4-(1-(*tert*-Butoxycarbonyl)pyrrolidin-3(*S*)-yloxy)-phenyl](ethoxycarbonyl)methoxy}-3,4-dihydro-1*H*-isoquinoline-2-carboxylic Acid *tert*-Butyl Ester (11f). Colorless oil; yield 41%. EI-MS: 596 (M^+).

General Procedure B. N-Boc Deprotection Affording Compounds 12a−f from 11a−f. At 5 °C 50 mL of a saturated solution of hydrogen chloride in diethyl ether was added dropwise to a mixture of 0.010 mol of N-Boc-protected compound **11** in 50 mL of diethyl ether and the reaction mixture stirred for 5 h at 5 °C. The precipitated product (hydrochloride) was separated by filtration, washed with diethyl ether several times, and dried in vacuo.

4-{4-[(Ethoxycarbonyl)(1,2,3,4-tetrahydro-isoquinolin-7-yloxy)methyl]phenoxy}piperidine-1-carboxylic Acid Allyl Ester Hydrochloride (12a). White solid; yield 70%; mp 132 °C. (+)-FAB-MS: 495 (MH^+). ^{1}H NMR: δ = 1.15 (t, 3H), 1.60 (m, 2H), 1.95 (m, 2H), 2.98 (t, 2H), 3.33 (m, 4H), 3.72 (m, 2H), 4.16 (m, 4H), 4.55 (m, 3H), 5.23 (dd, 2H), 5.82 (s, 1H), 5.90 (m, 1H), 6.85 (m, 2H), 7.02 (m, 3H), 7.39 (m, 2H), 9.70 (s, 2H). ^{13}C NMR: δ = 13.9, 23.9, 30.3, 40.7, 43.4, 43.5, 61.0, 65.2, 71.6, 76.7, 113.2, 114.7, 115.8, 116.9, 124.9, 127.5, 128.9, 129.9, 130.1, 133.5, 154.2; 155.4, 157.4, 169.5. Anal. Calcd ($C_{28}H_{35}ClN_2O_6$) C, H, Cl, N.

[4-(Piperidin-4-yloxy)phenyl](1,2,3,4-tetrahydro-isoquinolin-7-yloxy)acetic Acid Ethyl Ester Dihydrochloride (12b). White solid; yield 72%; mp 215−222 °C. EI-MS: 410 (M^+). ^{1}H NMR: δ = 1.13 (t, 3H), 1.89 (m, 2H), 2.11 (m, 2H), 2.92 (t, 2H), 3.06 (m, 2H), 3.18 (m, 2H), 3.30 (t, 2H), 4.11 (m, 4H), 4.68 (m, 1H), 5.88 (s, 1H), 6.86 (m, 2H), 7.02 (d, 2H), 7.10 (d, 1H), 7.42 (d, 2H), 9.40 (s, 2H), 9.80 (s, 2H). ^{13}C NMR: δ = 13.8, 23.7, 26.9, 39.5, 40.2, 43.3, 61.0, 69.0, 76.6, 113.0, 114.5, 115.7, 124.8, 127.7, 128.8, 129.7, 129.9, 155.2; 157.0, 169.3. Anal. Calcd ($C_{24}H_{32}Cl_2N_2O_4$) C, H, Cl, N.

[4-(1-Acetylpiperidin-4-yloxy)phenyl](1,2,3,4-tetrahydro-isoquinolin-7-yloxy)acetic Acid Ethyl Ester Hydrochloride (12c). Yellow oil; yield 97%. (+)-FAB-MS: 453 (MH^+).

(1,2,3,4-Tetrahydro-isoquinolin-7-yloxy)[4-(tetrahydropyran-4-yloxy)phenyl]acetic Acid Ethyl Ester Hydrochloride (12d). Yellow solid; yield 99%. (+)-FAB-MS: 412 (MH^+). ^{1}H NMR: δ = 1.16 (t, 3H), 1.59 (m, 2H), 1.98 (m, 2H), 2.91 (t, 2H), 3.38 (m, 4H), 3.85 (m, 2H), 4.11 (m, 4H), 4.58 (m, 1H), 5.85 (s, 1H), 6.82 (m, 2H), 6.99 (d, 2H), 7.09 (d, 1H), 7.42 (d, 2H), 9.61 (s, 2H). ^{13}C NMR: δ = 14.0, 24.0, 31.7, 40.6, 43.5, 61.1, 64.6, 71.3, 76.8, 113.2, 114.7, 115.8, 124.9, 127.4, 129.0, 129.9, 130.1, 155.4; 157.4, 169.5. Anal. Calcd ($C_{24}H_{30}ClNO_5$) C, H, Cl, N.

3(*S*)-{4-[(Ethoxycarbonyl)(1,2,3,4-tetrahydro-isoquinolin-7-yloxy)methyl]phenoxy}pyrrolidine-1-carboxylic Acid Allyl Ester Hydrochloride (12e). Colorless oil; yield 99%. EI-MS: 480 (M^+). ^{1}H NMR: δ = 1.15 (m, 3H), 2.10 (m, 2H), 2.90 (t, 2H), 3.20−3.70 (m, 6H), 4.05 (m, 2H), 4.15 (d, 2H), 4.50 (s, 2H), 5.05 (m, 1H), 5.20 (dd, 2H), 5.83 (s, 1H), 5.91 (m, 1H), 6.82 (m, 2H), 6.98 (d, 2H), 7.11 (d, 1H), 7.44 (d, 2H), 9.68 (s, 2H). ^{13}C NMR: δ = 13.9, 14.5, 23.8, 29.9, 30.7, 43.4, 43.5, 44.1, 46.0, 51.0, 51.6, 60.3, 60.9, 64.8, 75.1, 76.0, 76.6, 113.1, 114.6, 115.4, 116.8, 124.8, 127.7, 128.9, 129.8, 130.0, 133.4, 153.7, 155.3, 157.2, 169.3.

[4-(Pyrrolidin-3(*S*)-yloxy)phenyl](1,2,3,4-tetrahydro-isoquinolin-7-yloxy)acetic Acid Ethyl Ester Dihydrochloride (12f). Light brownish solid; yield 46%; mp 50 °C. (+)-FAB-MS: 397 (MH^+). ^{1}H NMR: δ = 1.12 (t, 3H), 2.18 (m, 2H), 2.92 (t, 2H), 3.30−3.60 (m, 6H), 4.11 (m, 4H), 5.12 (m, 1H), 5.90 (s, 1H), 6.88 (m, 2H), 7.01 (d, 2H), 7.12 (d, 1H), 7.47 (d, 2H), 9.71 (s, 2H), 9.82 (s, 2H). ^{13}C NMR: δ 13.8, 23.8, 30.6, 40.7, 43.1, 43.3, 49.3, 61.0, 75.4, 76.5, 113.1, 114.9, 115.6, 124.7, 128.1, 128.9, 129.7, 129.9, 155.2; 156.7, 169.3.

General Procedure C. Guanylation of Tetrahydroisoquinolines. To a stirred mixture of the appropriate tetrahydro-isoquinoline hydrochloride (0.005 mol) and 1*H*-pyrazole-1-carboxamidine hydrochloride (0.010 mol) in 5 mL of *N*,*N*-dimethylformamide was added 5.2 mL (0.030 mol) of

diisopropylethylamnine under nitrogen at 5 °C. Stirring was continued at room temperature for 24 h. Diethyl ether was added to afford precipitation of the crude product which was collected, washed with ether, and dissolved in water and the aqueous solution adjusted to pH 3. Preparative HPLC chromatography (H_2O, pH 3; H_2O/CH_3OH, 7:3, pH 3) afforded pure guanidine hydrochloride.

4-{4-[(2-Carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy)(ethoxycarbonyl)methyl]phenoxy}piperidine-1-carboxylic Acid Allyl Ester Hydrochloride (13). Colorless oil; yield 94%. (+)-FAB-MS: 537 (MH^+). ^{1}H NMR: δ = 1.17 (t, 3H), 1.60 (m, 2H), 1.92 (m, 2H), 2.82 (t, 2H), 3.30 (m, 2H), 3.58 (t, 2H), 3.69 (m, 2H), 4.15 (m, 2H), 4.58 (m, 5H), 5.20 (dd, 2H), 5.85 (s, 1H), 5.92 (m, 1H), 6.75 (s, 1H), 6.85 (d, 2H), 7.00 (d, 2H), 7.13 (d, 1H), 7.47 (d, 2H), 7.70 (4, 2H). ^{13}C NMR: δ = 13.9, 26.9, 30.3, 40.7, 43.2, 46.6, 61.1, 65.2, 71.6, 76.9, 112.8, 114.2, 115.8, 116.9, 127.2, 127.6, 129.0, 129.4, 133.2, 133.5, 154.2; 155.5, 156.4, 157.4, 169.6. Anal. Calcd ($C_{29}H_{37}ClN_4O_6$) C, H, Cl, N.

[4-(1-Carbamimidoyl-piperidin-4-yloxy)phenyl](2-carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy)acetic Acid Ethyl Ester Dihydrochloride (14). White solid; yield 78%; mp 120 °C. (+)-FAB-MS: 495 (MH^+). ^{1}H NMR: δ = 1.15 (t, 3H), 1.66 (m, 2H), 2.03 (m, 2H), 2.86 (t, 2H), 3.41 (m, 2H), 3.63 (t, 2H), 3.70 (m, 2H), 4.15 (m, 2H), 4.59 (s, 2H), 4.70 (m, 1H), 5.83 (s, 1H), 6.75 (s, 1H), 6.85 (d, 1H), 7.06 (d, 2H), 7.17 (d, 1H), 7.49 (d, 2H), 7.74 (s, 4H), 7.79 (s, 4H). ^{13}C NMR: δ = 13.9, 26.7, 29.8, 42.3, 43.0, 46.5, 61.0, 70.6, 76.8, 112.6, 114.0; 115.7, 127.0, 127.6, 128.8, 129.3, 133.0, 155.3; 156.1, 156.3, 157.2, 169.5. Anal. Calcd ($C_{26}H_{36}Cl_2N_6O_4$) C, H, Cl, N.

[4-(1-Acetylpiperidin-4-yloxy)phenyl](2-carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy)acetic Acid Ethyl Ester Hydrochloride (15). Yellow solid; yield 43%; mp 103 °C. (+)-FAB-MS: 495 (MH^+). ^{1}H NMR: δ = 1.13 (t, 3H), 1.50 (m, 1H), 1.61 (m, 1H), 1.86 (m, 1H), 1.92 (m, 1H), 2.02 (s, 3H), 2.82 (t, 2H), 3.23 (m, 1H), 3.48 (m, 1H), 3.61 (t, 2H), 3.69 (m, 1H), 3.82 (m, 1H), 4.12 (m, 2H), 4.56 (s, 2H), 4.61 (m, 1H), 5.83 (s, 1H), 6.72 (s, 1H), 6.82 (d, 1H), 7.00 (d, 2H), 7.11 (d, 1H), 7.45 (d, 2H), 7.72 (s, 4H). ^{13}C NMR: δ = 14.0, 21.3, 26.9, 30.2, 30.9, 38.1, 43.0, 43.2, 46.6, 61.1, 71.8, 76.9, 112.7, 114.2, 115.9, 127.2, 127.5, 129.0, 129.4, 133.2, 155.4, 156.4; 157.4, 168.1, 169.6. Anal. Calcd ($C_{27}H_{35}ClN_4O_5$) C, H, N.

(2-Carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy)[4-(tetrahydro-pyran-4-yloxy)phenyl]acetic Acid Ethyl Ester Hydrochloride (16). Light yellow solid; yield 42%; mp 95 °C. (+)-FAB-MS: 454 (MH^+); ^{1}H NMR: δ = 1.12 (t, 3H), 1.58 (m, 2H), 1.97 (m, 2H), 2.82 (t, 2H), 3.48 (m, 2H), 3.59 (t, 2H), 3.85 (m, 2H), 4.11 (m, 2H), 4.52 (s, 2H), 4.59 (m, 1H), 5.85 (s, 1H), 6.74 (s, 1H), 6.85 (d, 1H), 7.01 (d, 2H), 7.14 (d, 1H), 7.42 (d, 2H), 7.73 (s, 4H). ^{13}C NMR: δ = 14.0, 26.8, 31.7, 43.1, 46.6, 61.1, 64.6, 71.3, 76.9, 112.7, 114.2, 115.8, 127.2, 127.5, 129.0, 129.4, 133.2, 155.5; 156.4, 157.4, 169.6. Anal. Calcd ($C_{25}H_{32}ClN_3O_5$) C, H, Cl, N.

[4-(1-Carbamimidoyl-piperidin-4-yloxy)phenyl](2-carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy]acetic Acid Dihydrochloride (17). 14 (567 mg, 1.0 mmol) was dissolved in a mixture of 20 mL of water and 20 mL of ethanol and 1 N aqueous sodium hydroxide solution (4 mL) added at 5 °C while stirring. After stirring for another hour at room temperature the solution was adjusted to pH 3 by adding 2 N aqueous hydrochloric acid and concentrated. The crude product was purified using reverse-phase HPLC chromatography (H_2O, pH 3; H_2O/CH_3CN, 7:3, pH 3) to give 400 mg (74%) of the title acid as a white solid: mp 95 °C. (+)-FAB-MS: 467 (MH^+). ^{1}H NMR: δ = 1.60 (m, 2H), 1.95 (m, 2H), 2.78 (t, 2H), 3.40 (m, 2H), 3.61 (t, 2H), 3.67 (m, 2H), 4.54 (s, 2H), 4.63 (m, 1H), 5.40 (s, 1H), 6.70 (s, 1H), 6.79 (d, 1H), 6.96 (d, 2H), 7.08 (d, 1H), 7.44 (d, 2H), 7.89 (s, 8H). ^{13}C NMR: δ = 26.9, 30.0, 42.4, 43.1, 46.6, 70.7, 78.5, 112.4, 113.8, 115.5, 126.0, 128.7, 128.9, 130.4, 132.8, 155.5; 156.3, 156.5, 157.6, 172.6. Anal. Calcd ($C_{24}H_{32}Cl_2N_6O_4$) C, H, Cl, N.

[4-(1-Acetylpiperidin-4-yloxy)phenyl](2-carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy)acetic Acid Hydrochloride (18). Prepared from **15** using the procedure described for **17**: white solid; yield 53%; mp 102 °C. (+)-FAB-MS: 467 (MH^+). ^{1}H NMR: δ = 1.55 (m, 2H), 1.89 (m, 2H), 2.02 (s, 3H), 2.81 (t, 2H), 3.28 (m, 2H), 3.58 (t, 2H), 3.64 (m, 1H), 3.79 (m, 1H), 4.52 (s, 2H), 4.60 (m, 1H), 5.71 (s, 1H), 6.70 (s, 1H), 6.81 (d, 1H), 6.99 (d, 2H), 7.11 (d, 1H), 7.45 (d, 2H), 7.72 (s, 4H). ^{13}C NMR: δ = 21.2, 26.7, 30.1, 30.8, 37.9, 42.8, 43.0, 46.5, 71.7, 76.8, 112.4, 114.0, 115.7, 126.8, 128.1, 128.8, 129.2, 133.0, 155.6, 156.3; 157.2, 168.0, 170.8. Anal. Calcd ($C_{25}H_{31}ClN_4O_5$) C, H, Cl, N.

(2-Carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy)[4-(tetrahydro-pyran-4-yloxy)phenyl]acetic Acid Hydrochloride (19). Prepared from **16** using the procedure described for **17**: pale yellow solid; yield 25%; mp 112 °C. (+)-FAB-MS: 426 (MH^+). ^{1}H NMR: δ = 1.59 (m, 2H), 1.97 (m, 2H), 2.82 (t, 2H), 3.48 (m, 2H), 3.58 (t, 2H), 3.84 (m, 2H), 4.52 (s, 2H), 4.59 (m, 1H), 5.70 (s, 1H), 6.72 (s, 1H), 6.84 (d, 1H), 6.99 (d, 2H), 7.13 (d, 1H), 7.42 (d, 2H), 7.71 (s, 4H). ^{13}C NMR: δ = 26.9, 31.7, 43.2, 46.6, 64.5, 71.3, 77.0, 112.5, 114.1, 115.7, 126.9, 128.1, 128.9, 129.4, 133.0, 155.7; 156.4, 157.2, 171.1. Anal. Calcd ($C_{23}H_{28}ClN_3O_5$) C, H, Cl, N.

(2-Carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy)[4-(piperidin-4-yloxy)phenyl]acetic Acid Ethyl Ester Dihydrochloride (20). Under nitrogen 720 mg of tetrakis(triphenylphosphine)palladium was added at 5 °C to a solution of 1.90 g (3.32 mmol) of **13** and 4.20 g (33.20 mmol) of dimedone in 160 mL of tetrahydrofuran, and the mixture was stirred for 48 h at room temperature. The solvent was pumped off, and 75 mL of 0.1 N aqueous hydrogen chloride solution was added followed by filtration in order to remove the precipitated material. The aqueous solution was washed with ethyl acetate and diethyl ether, concentrated, and adjusted to pH 3. Preparative HPLC chromatography (H_2O, pH 3; H_2O/CH_3CN, 75:25, pH 3) afforded 1.70 g (97%) of the title compound as a yellow solid: mp 130 °C. (+)-FAB-MS: 453 (MH^+). ^{1}H NMR: δ = 1.11 (t, 3H), 1.90 (m, 2H), 2.11 (m, 2H), 2.81 (t, 2H), 3.07 (m, 2H), 3.19 (m, 2H), 3.57 (t, 2H), 4.12 (m, 2H), 4.52 (s, 2H), 4.70 (m, 1H), 5.85 (s, 1H), 6.72 (s, 1H), 6.83 (d, 1H), 7.04 (d, 2H), 7.12 (d, 1H), 7.46 (d, 2H), 7.71 (s, 4H), 9.33 (s, 2H). ^{13}C NMR: δ = 14.0, 27.0, 30.7, 40.6, 43.2, 46.6, 61.1, 69.0, 76.9, 112.7, 114.2, 115.8, 127.2, 127.8, 129.0, 129.4, 133.2, 155.4, 156.4, 157.1, 169.6. Anal. Calcd ($C_{25}H_{34}Cl_2N_4O_4$) C, H, Cl, N.

(2-Carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy)[4-(piperidin-4-yloxy)phenyl]acetic Acid Dihydrochloride (21). Prepared from **20** using the procedure described for **17**: light yellow solid; yield 89%; mp 157 °C. (+)-FAB-MS: 425 (MH^+). ^{1}H NMR: δ = 1.81 (m, 2H), 2.08 (m, 2H), 2.80 (t, 2H), 3.03 (m, 2H), 3.13 (m, 2H), 3.57 (t, 2H), 4.49 (s, 2H), 4.61 (m, 1H), 5.46 (s, 1H), 6.69 (s, 1H), 6.80 (d, 1H), 7.00 (d, 2H), 7.09 (d, 1H), 7.43 (d, 2H), 7.87 (s, 4H), 9.50 (s, 2H). ^{13}C NMR: δ = 26.9, 30.6, 43.1, 43.8, 46.6, 69.0, 78.9, 112.8, 114.2, 115.5, 126.2, 128.7, 129.1, 130.0, 132.8, 156.0, 156.1, 156.3, 172.0. Anal. Calcd ($C_{23}H_{30}Cl_2N_4O_4$) C, H, Cl, N.

(2-Carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy){4-[1-(1-iminoethyl)piperidin-4-yloxy]phenyl}acetic Acid Ethyl Ester Dihydrochloride (22). Under nitrogen triethylamine (2.2 mL, 0.016 mol) was added at 5 °C to a stirred mixture of 1.05 g (0.002 mol) of **20** and 0.50 g (0.004 mol) of acetimidic acid ethyl ester hydrochloride in 50 mL of ethanol. Stirring was continued for 48 h at room temperature followed by evaporation of the solvent. The residue was dissolved in water (20 mL) and the solution adjusted to pH 3 and chromatographed (HPLC: H_2O, pH 3; H_2O/CH_3CN, 8:2, pH 3) to give 0.60 g (53%) of the title compound as a colorless oil. (+)-FAB-MS: 494 (MH^+). ^{1}H NMR: δ = 1.17 (t, 3H), 1.75 (m, 2H), 2.08 (m, 2H), 2.36 (s, 3H), 2.85 (t, 2H), 3.46−3.96 (m, 6H), 4.13 (m, 2H), 4.58 (s, 2H), 4.77 (m, 1H), 5.85 (s, 1H), 6.75 (s, 1H), 6.84 (d, 1H), 7.06 (d, 2H), 7.15 (d, 1H), 7.48 (d, 2H), 7.79 (s, 4H), 9.00 (s, 1H), 9.56 (s, 1H). ^{13}C NMR: δ = 13.9, 18.3, 26.7, 29.2, 30.0, 42.5, 43.0, 44.7, 46.5, 61.0, 70.0, 76.7, 112.6, 114.0, 115.7, 127.0, 127.6, 128.9, 129.3, 133.1, 155.3; 156.3, 157.1, 162.8, 169.4. Anal. Calcd ($C_{27}H_{37}Cl_2N_5O_4$) C, H, Cl, N.

(2-Carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy)(4-{1-[imino(4-methoxyphenyl)methyl]piperidin-4-yloxy}phenyl)acetic Acid Ethyl Ester Dihydrochloride (23). Prepared from 4-methoxybenzimidic acid ethyl ester hydrochloride (obtained from 4-methoxybenzonitrile, ethanol, and gaseous HCl) and **20** according to the procedure described for compound **22**: white solid; mp 165 °C; yield 58%. (+)-FAB-MS: 586 (MH^+). ^{1}H NMR: δ = 1.11 (t, 3H), 1.71 (m, 1H), 1.88 (m, 1H), 2.00 (m, 1H), 2.20 (m, 1H), 2.82 (t, 2H), 3.40 (m, 1H), 3.53 (m, 1H), 3.58 (t, 2H), 3.78 (m, 1H), 3.87 (s, 3H), 3.99 (m, 1H), 4.10 (m, 2H), 4.57 (s, 2H), 4.78 (m, 1H), 5.82 (s, 1H), 6.72 (s, 1H), 6.82 (d, 1H), 7.03 (d, 2H), 7.12 (m, 3H), 7.45 (d, 2H), 7.57 (d, 2H), 7.78 (s, 4H), 9.43 (s, 1H), 9.58 (s, 1H). ^{13}C NMR: δ = 13.6, 26.7, 29.3, 30.2, 43.0, 43.8, 46.4, 46.9, 55.5, 61.0, 70.2, 76.7, 112.6, 114.0, 114.3, 115.8, 121.0, 127.0, 127.7, 128.9, 129.3, 130.4, 133.1, 155.3; 156.3, 157.1, 162.0, 163.6, 169.5. Anal. Calcd ($C_{33}H_{41}Cl_2N_5O_5$) C, H, Cl, N.

(2-Carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy){4-[1-(1-iminopropyl)piperidin-4-yloxy]phenyl}-acetic Acid Ethyl Ester Dihydrochloride (24). Prepared from propionimidic acid ethyl ester hydrochloride (obtained from propionitrile, ethanol, and gaseous HCl) and **20** according to the procedure described for compound **22**: white solid; mp 64−66 °C; yield 53%. (+)-FAB-MS: 508 (MH^+). ^{1}H NMR: δ = 1.14 (t, 3H), 1.17 (t, 3H), 1.76 (m, 2H), 2.07 (m, 2H), 2.67 (q, 2H), 2.84 (t, 2H), 3.50−3.99 (m, 6H), 4.12 (m, 2H), 4.60 (s, 2H), 4.78 (m, 1H), 5.88 (s, 1H), 6.78 (s, 1H), 6.87 (d, 1H), 7.04 (d, 2H), 7.13 (d, 1H), 7.49 (d, 2H), 7.87 (s, 4H), 9.09 (s, 1H), 9.51 (s, 1H). ^{13}C NMR: δ = 11.1, 13.9, 24.5, 26.7, 29.3, 30.5, 42.8, 43.0, 44.4, 46.5, 61.0, 70.0, 76.7, 112.6, 114.0, 115.8, 127.0, 127.7, 128.9, 129.3, 133.1, 155.3; 156.3, 157.1, 166.7, 169.5. Anal. Calcd ($C_{28}H_{39}Cl_2N_5O_4$) C, H, Cl, N.

(2-Carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy){4-[1-(1-iminoethyl)piperidin-4-yloxy]phenyl}-acetic Acid Dihydrochloride (25). Prepared from **22** using the procedure described for **17**: white solid; yield 74%; mp 162 °C. (+)-FAB-MS: 466 (MH^+). ^{1}H NMR: δ = 1.73 (m, 2H), 2.02 (m, 2H), 2.31 (s, 3H), 2.78 (t, 2H), 3.58 (t, 2H), 3.67 (m, 2H), 3.81 (m, 2H), 4.53 (s, 2H), 4.68 (m, 1H), 5.48 (s, 1H), 6.70 (s, 1H), 6.80 (d, 1H), 6.95 (d, 2H), 7.06 (d, 1H), 7.46 (d, 2H), 7.89 (s, 4H), 9.00 (s, 1H), 9.50 (s, 1H). ^{13}C NMR: δ = 18.4, 26.9, 29.3, 30.1, 42.6, 43.2, 44.8, 46.9, 70.1, 78.5, 112.5, 113.9, 115.6, 126.1, 128.8, 128.9, 129.3, 132.9, 156.3; 156.5, 156.6, 162.9, 172.1. Anal. Calcd ($C_{25}H_{33}Cl_2N_5O_4$) C, H, Cl, N.

(2-Carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy)(4-{1-[imino(4-methoxyphenyl)methyl]piperidin-4-yloxy}phenyl)acetic Acid Dihydrochloride (26). Prepared from **23** using the procedure described for **17**: white solid; yield 72%; mp 223 °C. (+)-LSIMS: 558.2 (MH^+). ^{1}H NMR: δ = 1.72 (m, 1H), 1.89 (m, 1H), 2.00 (m, 1H), 2.18 (m, 1H), 2.80 (t, 2H), 3.40 (m, 1H), 3.55 (m, 1H), 3.60 (t, 2H), 3.79 (m, 1H), 3.83 (s, 3H), 3.99 (m, 1H), 4.55 (s, 2H), 4.78 (m, 1H), 5.82 (s, 1H), 6.71 (s, 1H), 6.82 (d, 1H), 7.03 (d, 2H), 7.12 (m, 3H), 7.48 (d, 2H), 7.59 (d, 2H), 7.82 (s, 4H), 9.45 (s, 1H), 9.60 (s, 1H). ^{13}C NMR: δ = 26.7, 29.3, 30.2, 43.0, 43.8, 46.5, 46.9, 55.5, 70.1, 76.7, 112.4, 114.0,.114.3, 115.7, 121.0, 126.7, 128.3, 128.8, 129.3, 130.4, 133.0, 155.5; 156.3, 156.9, 162.0, 163.6, 170.7. Anal. Calcd ($C_{31}H_{37}Cl_2N_5O_5$) C, H, Cl, N.

3(*S*)-{4-[(2-Carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy)(ethoxycarbonyl)methyl]phenoxy}-pyrrolidine-1-carboxylic Acid Allyl Ester Hydrochloride (27). Prepared from **12e** according to general procedure C: white solid; yield 74%; mp 80−83 °C. (+)-FAB-MS: 523 (MH^+). ^{1}H NMR: δ = 1.15 (m, 3H), 2.10 (m, 2H), 2.84 (t, 2H), 3.30−3.70 (m, 6H), 4.13 (m, 2H), 4.54 (m, 4H), 5.06 (m, 1H), 5.22 (dd, 2H), 5.84 (s, 1H), 5.92 (m, 1H), 6.72 (s, 1H), 6.84 (d, 1H), 6.99 (d, 2H), 7.12 (d, 1H), 7.47 (d, 2H), 7.71 (s, 4H). ^{13}C NMR: δ = 14.0, 26.9, 29.9, 30.7, 43.1, 43.7, 44.2, 46.6, 51.0, 51.6, 61.1, 64.9, 75.2, 76.1, 76.9, 112.8, 114.2, 115.5, 116.9, 127.2, 127.8, 129.0, 129.4, 133.2, 133.6, 154.0, 155.4, 156.4, 157.3, 169.6. Anal. Calcd ($C_{28}H_{35}ClN_4O_6$) C, H, Cl, N.

[4-(1-Carbamimidoyl-pyrrolidin-3(*S*)-yloxy)phenyl](2-carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy)-acetic Acid Ethyl Ester Dihydrochloride (28). Prepared from **12f** according to general procedure C: pale yellow solid; yield 53%; mp 90 °C. (+)-FAB-MS: 481 (MH^+). ^{1}H NMR: δ = 1.14 (t, 3H), 2.23 (m, 2H), 2.85 (t, 2H), 3.60 (m, 6H), 4.16 (m, 2H), 4.51 (s, 2H), 5.20 (m, 1H), 5.88 (s, 1H), 6.75 (s, 1H), 6.85 (d, 1H), 7.02 (d, 2H), 7.15 (d, 1H), 7.49 (d, 2H), 7.59 (s, 4H), 7.82 (s, 4H). ^{13}C NMR: δ = 13.9, 26.7, 30.0, 43.0, 45.2, 46.5, 52.4, 61.0, 75.3, 76.7, 112.6, 114.0, 115.5, 127.1, 128.0, 128.9, 129.3, 133.1, 154.9, 155.2; 156.3, 156.9, 169.4. Anal. Calcd ($C_{25}H_{34}Cl_2N_6O_4$) C, H, Cl, N.

[4-(1-Carbamimidoyl-pyrrolidin-3(*S*)-yloxy)phenyl](2-carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy)-acetic Acid Dihydrochloride (29). Prepared from **28** using the procedure described for **17**: light brownish solid; yield 69%; mp >155 °C dec. (+)-FAB-MS: 453 (MH^+). ^{1}H NMR: δ = 2.27 (m, 2H), 2.85 (t, 2H), 3.59 (m, 6H), 4.58 (s, 2H), 5.20 (m, 1H), 5.78 (s, 1H), 6.73 (s, 1H), 6.85 (d, 1H), 7.02 (d, 2H), 7.14 (d, 1H), 7.49 (d, 2H), 7.51 (s, 4H), 7.74 (s, 4H). ^{13}C NMR: δ = 26.7, 30.0, 43.0, 45.1, 46.5, 52.3, 75.2, 76.7, 112.4, 114.0, 115.4, 126.8, 128.6, 128.8, 129.2, 133.0, 154.8, 155.5; 156.3, 156.7, 170.7. Anal. Calcd ($C_{23}H_{30}Cl_2N_6O_4$) C, H, Cl, N.

(2-Carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy)[4-(pyrrolidin-3(*S*)-yloxy)phenyl]acetic Acid Ethyl Ester Dihydrochloride (30). Tetrakis(triphenylphosphine)-palladium (333 mg, 0.0003 mol) was added to a stirred mixture of **27** (1.50 g, 0.0029 mol) and tributyltin hydride (1.56 mL, 0.0058 mol) in 300 mL of dichloromethane at 5 °C. Stirring was continued at 5 °C for 3 h followed by extraction with aqueous hydrochloric acid (0.1 N), separation of precipitated material by filtration, and concentration of the combined aqueous extracts affording 1.37 g (0.0027 mol, 93%) of **30** as a white solid: mp >170 °C dec. (+)-FAB-MS: 439 (MH^+). ^{1}H NMR: δ = 1.15 (t, 3H), 2.15 (m, 2H), 2.83 (t, 2H), 3.32 (m, 4H), 3.60 (t, 2H), 4.15 (m, 2H), 4.56 (s, 2H), 5.13 (m, 1H), 5.87 (s, 1H), 6.76 (s, 1H), 6.87 (d, 1H), 7.02 (d, 2H), 7.14 (d, 1H), 7.49 (d, 2H), 7.74 (s, 4H), 9.70 (s, 1H), 10.00 (s, 1H). ^{13}C NMR: δ = 14.0, 26.8, 30.6, 43.2, 43.3, 46.6, 49.5, 61.1, 75.6, 76.8, 112.8, 114.2, 115.8, 127.2, 128.3, 129.0, 129.4, 133.2, 155.4, 156.4, 156.9, 169.6. Anal. Calcd ($C_{24}H_{32}Cl_2N_4O_4$) C, H, Cl, N.

(2-Carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy)[4-(pyrrolidin-3(*S*)-yloxy)phenyl]acetic Acid Dihydrochloride (31). Prepared from **30** using the procedure described for **17**: pale yellow solid; yield 31%; mp 100−105 °C. (+)-FAB-MS: 411 (MH^+). ^{1}H NMR: δ = 2.16 (m, 2H), 2.83 (t, 2H), 3.38 (m, 4H), 3.60 (t, 2H), 4.53 (s, 2H), 5.16 (m, 1H), 5.73 (s, 1H), 6.71 (s, 1H), 6.82 (d, 1H), 7.00 (d, 2H), 7.13 (d, 1H), 7.49 (d, 2H), 7.66 (s, 4H), 9.56 (s, 1H), 9.81 (s, 1H). ^{13}C NMR: δ = 26.7, 30.4, 43.0, 43.2, 46.5, 49.5, 75.4, 76.7, 112.4, 114.0, 115.6, 126.8, 128.7, 128.8, 129.2, 132.9, 155.5, 156.2, 156.5, 170.8. Anal. Calcd ($C_{22}H_{28}Cl_2N_4O_4$) C, H, Cl, N.

(2-Carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy){4-[1-(1-iminoethyl)pyrrolidin-3(*S*)-yloxy]phenyl}-acetic Acid Ethyl Ester Dihydrochloride (32). Prepared from **30** using the procedure described for **22**: yellowish solid; yield 77%; mp >130 °C dec. (+)-FAB-MS: 480 (MH^+). ^{1}H NMR: δ = 1.16 (t, 3H), 2.23 (m, 2H), 2.30 (d, 3H), 2.82 (t, 2H), 3.60 (m, 6H), 4.14 (m, 2H), 4.56 (s, 2H), 5.21 (m, 1H), 5.88 (s, 1H), 6.72 (s, 1H), 6.84 (d, 1H), 7.01 (d, 2H), 7.13 (d, 1H), 7.48 (d, 2H), 7.79 (s, 4H), 8.67 (d, 1H), 9.52 (d, 1H). ^{13}C NMR: δ = 14.0, 18.5, 26.8, 30.0, 43.2, 43.3, 46.6, 47.3, 61.1, 75.4, 76.8, 112.8, 114.2, 115.7, 127.2, 128.2, 129.1, 129.4, 133.2, 155.4, 156.5, 156.9, 162.9, 169.6. Anal. Calcd ($C_{26}H_{35}Cl_2N_5O_4$) C, H, Cl, N.

(2-Carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy)-{4-[1-(1-iminoethyl)pyrrolidin-3(*S*)-yloxy]phenyl}-acetic Acid Dihydrochloride (33). Prepared from **32** using the procedure described for **17**: pale yellow solid; yield 96%; mp >180 °C dec. (+)-FAB-MS: 452 (MH^+). ^{1}H NMR: δ = 2.12 (m, 2H), 2.25 (d, 3H), 2.79 (t, 2H), 3.62 (m, 6H), 4.51 (s, 2H), 5.11 (m, 1H), 5.43 (s, 1H), 6.69 (s, 1H), 6.78 (d, 1H), 6.90 (d, 2H), 7.05 (d, 1H), 7.48 (d, 2H), 7.94 (s, 4H), 8.74 (d, 1H), 9.58 (d, 1H). ^{13}C NMR: δ = 18.5, 26.9, 30.0, 43.2, 43.3, 46.7, 47.1, 75.3, 78.9, 112.4, 114.1, 115.3, 126.1, 128.8, 129.2, 130.9,

132.9, 156.1, 156.3, 156.5, 162.9, 172.3. Anal. Calcd ($C_{24}H_{31}Cl_2N_5O_4$) C, H, Cl, N.

[4-(1-Allylpyrrolidin-3(*S*)-yloxy)phenyl](2-carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy)acetic Acid Ethyl Ester Hydrochloride (34) and (2-Carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy)(4-[1-(5,5-dimethyl-3-oxocyclohex-1-enyl)pyrrolidin-3(*S*)-yloxy]phenyl}-acetic Acid Ethyl Ester Hydrochloride (35). Under nitrogen 240 mg (0.0002 mol) of tetrakis(triphenylphosphine)-palladium was added at 5 °C to 0.56 g (0.001 mmol) of **27** and 1.40 g (0.010 mmol) of dimedone in 50 mL of tetrahydrofuran and the mixture stirred for 48 h at room temperature. The solvent was pumped off, and 50 mL of 0.1 N aqueous hydrogen chloride solution was added followed by filtration in order to remove the precipitated material. The aqueous solution washed with ethyl acetate and diethyl ether, concentrated, adjusted to pH 3, and chromatographed (HPLC: H_2O, pH 3; H_2O/CH_3CN, 8:2, pH 3; H_2O/CH_3CN, 7:3, pH 3) yielding 98 mg (20%) of **34** as a yellow oil. EI-MS: 478 (M^+). ^{1}H NMR: δ = 1.14 (t, 3H), 2.12 (m, 2H), 2.82 (t, 2H), 3.26 (m, 4H), 3.60 (t, 2H), 3.82 (d, 2H), 4.12 (m, 2H), 4.54 (s, 2H), 5.17 (m, 1H), 5.48 (dd, 2H), 5.88 (s, 1H), 5.99 (m, 1H), 6.73 (s, 1H), 6.83 (d, 1H), 7.01 (d, 2H), 7.14 (d, 1H), 7.49 (d, 2H), 7.63 (s, 4H). ^{13}C NMR: δ = 13.8, 26.7, 30.6, 43.2, 43.8, 46.2, 50.9, 51.0, 61.0, 74.8, 76.7, 112.7, 114.0, 115.5, 115.6, 123.8, 127.1, 128.2, 128.9, 129.3, 133.0, 155.3, 156.2, 156.7, 169.4. Anal. Calcd ($C_{27}H_{35}ClN_4O_4$) C, H, Cl, N. Also yielded was 230 mg (40%) of **35** as a white solid: mp >160 °C dec. (+)-FAB-MS: 561 (MH^+). ^{1}H NMR: δ = 1.10 (m, 9H), 2.20−2.72 (m, 6H), 2.81 (t, 2H), 3.59 (t, 2H), 3.61−4.02 (m, 4H), 4.09 (m, 2H), 4.55 (s, 2H), 5.24 (m, 1H), 5.86 (m, 2H), 6.73 (s, 1H), 6.86 (d, 1H), 7.03 (d, 2H), 7.15 (d, 1H), 7.50 (d, 2H), 7.72 (s, 4H). ^{13}C NMR: δ = 13.9, 26.7, 27.2, 27.9, 29.7, 32.2, 40.4, 42.8, 43.0, 43.7, 46.5, 55.9, 61.0, 74.9, 76.7, 95.4, 112.6, 114.0, 115.5, 127.0, 128.1, 128.9, 129.3, 133.1, 155.3, 156.3, 156.8, 169.4, 171.4, 186.1. Anal. Calcd ($C_{32}H_{41}ClN_4O_5$) C, H, Cl, N.

(2-Carbamimidoyl-1,2,3,4-tetrahydro-isoquinolin-7-yloxy){4-[1-(5,5-dimethyl-3-oxocyclohex-1-enyl)pyrrolidin-3(*S*)-yloxy]phenyl}acetic Acid Hydrochloride (36). Prepared from **35** using the procedure described for **17**: pale yellow solid; yield 42%; mp 175−178 °C. (+)-FAB-MS: 533 (MH^+). ^{1}H NMR: δ = 1.07 (s, 6H), 2.26 (m, 2H), 2.30−2.75 (m, 4H), 2.82 (t, 2H), 3.60 (t, 2H), 3.65−4.25 (m, 4H), 4.57 (s, 2H), 5.22 (m, 1H), 5.74 (m, 2H), 6.74 (s, 1H), 6.86 (d, 1H), 7.02 (d, 2H), 7.15 (d, 1H), 7.51 (d, 2H), 7.67 (s, 4H). ^{13}C NMR: δ = 26.7, 27.2, 27.9, 29.7, 32.2, 40.4, 43.0, 43.1, 46.4, 48.8, 56.0, 74.8, 76.6, 95.5, 112.4, 114.0, 115.4, 126.8, 128.7, 128.9, 129.2, 133.0, 155.5; 156.2, 156.6, 170.6, 170.8, 186.3. Anal. Calcd ($C_{30}H_{37}ClN_4O_5$) C, H, Cl, N.

Enzyme Kinetics. Inhibition constants were, determined by means of chromogenic assays as previously described.[8] For results, see Table 1.

Coagulation Assays. Citrated plasma: blood from healthy human donors (9 parts) was mixed with aqueous sodium citrate (0.11 mol/L, 1 part) and centrifuged at 3000 rpm for 10 min at room temperature. The plasma was stored at room temperature for up to 8 h.

Activated partial thromboplastin time (aPTT): citrated plasma (100 μL), aPTT reagent (100 μL STA aPTT; Diagnostica Stago/Boehringer Mannheim GmbH, Germany), and dimethyl sulfoxide (10 μL, control sample) or the test compound in dimethyl sulfoxide (10 μL) were incubated in a KC10 coagulometer (Amelung, Lemgo, Germany) for 3 min at 37 °C. Coagulation was started by addition of calcium chloride solution (100 μL STA Calcium Chloride; Diagnostica Stago/Boehringer Mannheim GmbH, Germany). The addition of this reagent started a stop watch, which stopped when the plasma had coagulated. Quadruplicate determinations were performed for each individual assay. The aPTT was 28−35 s in the control experiments; samples containing a test compound exhibited prolonged clotting times. The aPTT increase (i.e., the difference to the control experiment) in seconds was determined at concentrations of the test compound in the citrated plasma of 1000, 200, 100, 10, and 1 μM. ED_{200} values (aPTT doubling concentrations) were determined using the equation $y = a + b \times x^c$, where y is the experimental coagulation time (s), x is the concentration of the inhibitor (μM), a is the coagulation time in the absence of the inhibitor, and b and c are parameters which determine the shape and the location on the x-axis of the coagulation time/concentration curve. These parameters were determined from the experimental coagulation times using a nonlinear regression with the starting values $b = 10$ and $c = 0.65$. The resulting ED_{200} values (from quadruplicate determinations) were within ±20% of the values given in Table 1.

Thrombin clotting time (TT): citrated plasma (190 μL) and dimethyl sulfoxide (10 μL, control sample) or the test compound in dimethyl sulfoxide (10 μL) were incubated in a KC10 coagulometer (Amelung, Lemgo, Germany) for 2 min at 37 °C. Coagulation was started by addition of thrombin reagent (200 μL STA thrombin, contains 3 U/mL equine thrombin and 0.0125 mol Ca^{2+}/L; Diagnostica Stago/Boehringer Mannheim GmbH, Germany). The addition of this reagent started a stop watch, which stopped when the plasma had coagulated. Quadruplicate determinations were performed for each individual assay. The TT was 24 ± 2 s in the control experiments; samples containing a test compound exhibited prolonged clotting times. The TT increase (i.e., the difference to the control experiment) in seconds was determined at concentrations of the test compound in the citrated plasma of 500, 100, 50, 5, and 0.5 μM. ED_{200} values (TT doubling concentrations) were determined using the equation $y = a + b \times x^c$, where y is the experimental coagulation time (s), x is the concentration of the inhibitor (μM), a is the coagulation time in the absence of the inhibitor, and b and c are parameters which determine the shape and the location on the x-axis of the coagulation time/concentration curve. These parameters were determined from the experimental coagulation times using a nonlinear regression with the starting values $b = 10$ and $c = 0.65$. The resulting ED_{200} values (from quadruplicate determinations) were within ±20% of the values given in Table 1.

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References

(1) Hertzberg, M. Biochemistry of Factor X. *Blood Rev.* **1994**, *8*, 56−62.

(2) Davie, E. W.; Fujikawa, K.; Kisiel, W. The Coagulation Cascade: Initiation, Maintenance, and Regulation. *Biochemistry* **1991**, *30*, 10363−10370.

(3) (a) Bode, W.; Brandstetter, H.; Mather, T.; Stubbs, M. T. Comparative Analysis of Haemostatic Proteinases: Structural Aspects of Thrombin, Factor Xa, Factor IXa and Protein C. *Thromb. Haemostasis* **1997**, *78*, 501−511. (b) Prasa, D.; Svendsen, L.; Stürzebecher, J. Inhibition of Thrombin Generation in Plasma by Inhibitors of Factor Xa. *Thromb. Haemostasis* **1997**, *78*, 1215−1220. (c) Quan, M. L.; Pruitt, J. R.; Ellis, C. D.; Liauw, A. Y.; Galemmo, R. A., Jr.; Stouten, P. F. W.; Wityak, J.; Knabb, R. M.; Thoolen, M. J.; Wong, P. C.; Wexler, R. R. Bisbenzamidine Isoxazoline Derivatives as Factor Xa Inhibitors. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2813−2818. (d) Edmunds, J. J.; Rapundalo, S. T. Thrombin and Factor Xa Inhibition. *Annu. Rep. Med. Chem.* **1996**, *31*, 51−60. (e) Stubbs, M. T. Structural Aspects of Factor Xa Inhibition. *Curr. Pharm. Des.* **1996**, *2*, 543−552. (f) Wong, P. C.; Crain, E. J., Jr.; Nguan, O.; Watson C. A.; Racanelli, A. Antithrombotic Actions of Selective Inhibitors of Blood Coagulation Factor Xa in Rat Models of Thrombosis. *Thromb. Res.* **1996**, *83*, 117−126. (g) Yamazaki, M. Factor Xa Inhibitors. *Drugs Future* **1995**, *20*, 911−918. (h) Mao, S. Factor Xa Inhibitors. *Perspec. Drug Discovery Des.* **1994**, *1*, 423−430.

(4) Waxman, L.; Smith, D. E.; Arcuri, K. E.; Vlasuk, G. P. Tick Anticoagulant Peptide (TAP) Is a Novel Inhibitor of Blood Coagulation Factor Xa. *Science* **1990**, *248*, 593−596.

(5) (a) Nutt, E.; Gasic, T.; Rodkey, J.; Gasic, G. J.; Jacobs, J. W.; Friedman, P. A.; Simpson, E. The Amino Acid Sequence of Antistasin: A Potent Inhibitor of Factor Xa Reveals a Repeated Internal Structure. *J. Biol. Chem.* **1988**, *263*, 10162−10167. (b) Lapatto, R.; Krengel, U.; Schreuder, H. A.; Arkema, A.; de Boer, B.; Kalk, K. H.; Hol, W. G. J.; Grootenhuis, P. D. J.; Mulders, J. W. M.; Dijkema, R.; Theunissen, H. J. M.; Dijkstra, B. W. X-ray Structure of Antistasin at 1.9 Å Resolution and its Modelled Complex with Blood Coagulation Factor Xa. *EMBO J.* **1997**, *16*, 5151−5161.

(6) Cappello, M.; Vlasuk, G. P.; Bergum, P. W.; Huang, S.; Hotez, P. J. Anclystoma Caninum Anticoagulant Peptide: A Hookworm derived Inhibitor of Human Coagulation Factor Xa. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 6152−6156.

(7) Seymour, J. L.; Lindquist, R. N.; Dennis, M. S.; Moffat, B.; Yansura, D.; Reilly, D.; Wessinger, M. E.; Lazarus, R. A. Ecotin Is a Potent Anticoagulant and Reversible Tight-Binding Inhibitor of Factor Xa. *Biochemistry* **1994**, *33*, 3949−3958.

(8) von der Saal, W.; Engh, R. A.; Eichinger, A.; Gabriel, B.; Kucznierz, R.; Sauer, J. Syntheses and Selective Inhibitory Activities of Terphenyl-Bisamidines for Serine Proteases. *Arch. Pharmacol. Pharm. Med. Chem.* **1996**, *329*, 73−82.

(9) (a) Tidwell, R. R.; Webster, W. P.; Shaver, S. R.; Geratz, J. D. Strategies for Anticoagulation with Synthetic Protease Inhibitors: Xa Inhibitors Versus Thrombin Inhibitors. *Thromb. Res.* **1980**, *19*, 339−349. (b) Stürzebecher, J.; Stürzebecher, U.; Vieweg, H.; Wagner, G.; Hauptmann, J.; Markwardt, F. Synthetic Inhibitors of Bovine Factor Xa and Thrombin: Comparison of Their Anticoagulant Efficiency. *Thromb. Res.* **1989**, *54*, 245−252.

(10) Very recently a novel series of bis-phenylamidine carboxylate-derived inhibitors of factor Xa has been published: Maduskuie, T. P., Jr.; McNamara, K. J.; Ru, Y.; Knabb, R. M.; Stouten, P. F. W. Rational Design and Synthesis of Novel, Potent Bisphenylamidine Carboxylate Factor Xa Inhibitors. *J. Med Chem.* **1998**, *41*, 53−62.

(11) (a) Katakura, S.; Nagahara, T.; Hara, T.; Iwamoto, M. A Novel Factor Xa Inhibitor: Structure-Activity Relationships and Selectivity Between Factor Xa and Thrombin. *Biochem. Biophys. Res. Commun.* **1993**, *197*, 965−972. (b) Nagahara, T.; Yokoyama, Y.; Inamura, K.; Katakura, S.; Komoriya, S.; Yamaguchi, H.; Hara, T.; Iwamoto, M. Dibasic (Amidinoaryl)propanoic Acid Derivatives as Novel Blood Coagulation Factor Xa Inhibitors. *J. Med. Chem.* **1994**, *37*, 1200−1207. (c) Hara, T.; Yokoyama, A.; Ishihara, H.; Yokoyama, Y.; Nagahara, T.; Iwamoto, M. DX-9065a, a New Synthetic, Potent Anticoagulant and Selective Inhibitor for Factor Xa. *Thromb. Haemostasis* **1994**, *71*, 314−319. (d) Hara, T.; Yokoyama, A.; Tanabe, K.; Ishihara, H.; Iwamoto, M. DX-9065a, an Orally Active, Specific Inhibitor of Factor Xa, Inhibits Thrombosis without Affecting Bleeding Time in Rats. *Thromb. Haemostasis* **1995**, *74*, 635−639. (e) Hara, T.; Yokoyama, A.; Tanabe, K.; Ishihara, H.; Iwamoto, M. Molecular Model of an Interaction Between Factor Xa and DX-9065a, a Novel Factor Xa Inhibitor: Contribution of The Acetimidoylpyrrolidine moiety of The Inhibitor To Potency and Selectivity for Serine Proteases. *Eur. J. Med. Chem.* **1995**, *30*, 387−394.

(12) For an improved synthesis of DX-9065a, see: Kehr, C.; Neidlein, R.; Engh, R. A.; Brandstetter, H.; Kucznierz, R.; Leinert, H.; Marzenell, K.; Strein, K.; von der Saal, W. A Short Synthesis of the Factor Xa Inhibitor DX-9065a Using Palladium-Catalyzed Key Steps. *Helv. Chim. Acta* **1997**, *80*, 892−896.

(13) Padmanabhan, K.; Padmanabhan, K. P.; Tulinsky, A.; Park, C. H.; Bode, W.; Huber, R.; Blankenship, D. T.; Cardin, A. D.; Kisiel, W. Structure of Human Des(1-45) Factor Xa at 2.2 Å Resolution. *J. Mol. Biol.* **1993**, *232*, 947−966.

(14) Brandstetter, H.; Kühne, A.; Bode, W.; Huber, R.; von der Saal, W.; Wirthensohn, K.; Engh, R. A. X-ray Structure of Active Site-inhibited Clotting Factor Xa: Implications for Drug Design and Substrate Recognition. *J Biol. Chem.* **1996**, *271*, 29988−29992.

(15) Bevis, M. J.; Forbest, E. J.; Naik, N. N.; Uff, B. C. The Synthesis of Isoquinolines, Indoles and Benzthiophen by an Improved Pomeranz-Fritsch Reaction Using Boron Trifluoride in Trifluoroacetic Anhydride. *Tetrahedron* **1971**, *27*, 1253−1259.

(16) For reviews on Mitsunobu reactions, see: (a) Hughes, D. L. The Mitsunobu Reaction. *Org. React.* **1992**, *42*, 335−656. (b) Mitsunobu, O. The Use of Diethyl Azodicarboxylate and Triphenylphosphine in Synthesis and Transformation of Natural Products. *Synthesis* **1981**, 1−28.

(17) Bernatowicz, M. S.; Wu, Y.; Matsueda, G. R. 1*H*-Pyrazole-l-carboxamidine Hydrochloride: An Attractive Reagent for Guanylation of Amines and Its Application to Peptide Synthesis. *J. Org. Chem.* **1992**, *57*, 2497−2502.

(18) Kunz, H.; Unverzagt, C. Der Allyloxycarbonyl(Aloc)-Rest - die Verwandlung einer untauglichen in eine wertvolle Aminoschutzgruppe für die Peptidsynthese. *Angew. Chem.* **1984**, *96*, 426−427.

(19) The application of pyrrolidine as an allyl scavenger in the palladium-catalyzed cleavage of allyl carbamates has been reported recently: (a) Howard, P. W.; Thurston, D. E. The Use of Carbamate Protecting Groups in the Synthesis of Monomeric and Dimeric Pyrrolo[2,1-c]benzodiazepines. *J. Pharm. Pharmacol.* **1995**, 1078. (b) See also: Roos, E. C.; Bernabé, P.; Hiemstra, H.; Speckamp, W. N. Palladium-Catalyzed Transprotection of Allyloxycarbonyl-Protected Amines: Efficient One-Pot Formation of Amides and Dipeptides. *J. Org. Chem.* **1995**, *60*, 1733−1740.

(20) The enamine derivative of **20** has not been detected under these conditions, not even in traces. This is attributed to the generally enhanced tendency of pyrrolidines to form enamines.

(21) Dangles, O.; Guibé, F.; Balavoine, G.; Lavielle, S.; Marquet, A. Selective Cleavage of the Allyl and Allyloxycarbonyl Groups through Palladium-Catalyzed Hydrostannolysis with Tributyltin Hydride: Application to the Selective Protection-Deprotection of Amino Acid Derivatives and in Peptide Synthesis. *J. Org. Chem.* **1987**, *52*, 4984−4993.

(22) Concerning the nomenclature of the subsites of fXa, see ref 14.

(23) Hilpert, K.; Ackermann, J.; Banner, D. W.; Gast, A.; Gubernator, K.; Hadváry, P.; Labler, L.; Müller, K.; Schmid, G.; Tschopp, T. B.; van de Waterbeemd, H. Design and Synthesis of Potent and Highly Selective Thrombin Inhibitors. *J. Med. Chem.* **1994**, *37*, 3889−3901.

(24) For the MD all residues of the S1 pocket were allowed to move freely. All other residues were kept fixed. Solvents have not been used. Trajectories were not written out, but the movement of the residues was followed on the screen. For docking experiments the inhibitors were manually adjusted and minimized to convergence using the CVFF force field of the Insight II/Discover (MSI, San Diego, CA) program package. The protein atoms were fixed for this purpose, and a constraint was given from the exocyclic amino nitrogen atoms to Asp189 in the S1 pocket.

(25) Stubbs, M. T.; Huber, R.; Bode, W. Crystal Structures of Factor Xa Specific Inhibitors in Complex with Trypsin: Structural Grounds for Inhibition of Factor Xa and Selectivity Against Thrombin. *FEBS Lett.* **1995**, *375*, 103−107.

(26) Inhibition constants of the ethyl ester of DX-9065a (unpublished results): $K_{i_{fXa}} = 0.040\ \mu M$, $K_{i_{thrombin}} = 16\ \mu M$, $K_{i_{trypsin}} = 0.28\ \mu M$, $K_{i_{plasmin}} = 13\ \mu M$.

(27) Engh, R. A.; Brandstetter, H.; Sucher, G.; Eichinger, A.; Baumann, U.; Bode, W.; Huber, R.; Poll, T.; Rudolph, R.; von der Saal, W. Enzyme Flexibility, Solvent and Weak Interactions Characterize Thrombin-Ligand Interactions: Implications for Drug Design. *Structure* **1996**, *4*, 1353−1362.

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