Design of Benzamidine-Type Inhibitors of Factor Xa

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Received April 13, 1998

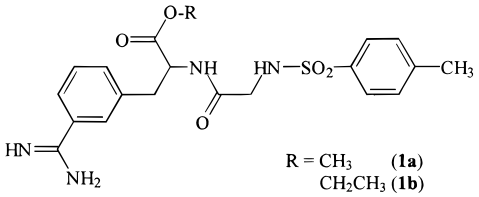
A series of derivatives of *rac*-benzenesulfonyl-glycyl-phenylalanine or its ethyl ester with a combination of thioamido/amidino or amidino/amidino substituents in the benzene rings was synthesized as potential inhibitors of factor Xa (fXa). Among these, the racemic 4′-amidinobenzenesulfonyl-glycyl-4-amidinophenylalanine ethyl ester was found to exhibit the highest affinity for fXa despite the unfavored location of the amidino substituent in the para position. X-ray structural analysis of the trypsin complex with this bis-benzamidine compound revealed a retro-binding mode if compared to those of similar compounds, so far analyzed in complexes with trypsin or fXa. This noncanonical binding mode as well as its slow plasma clearance rates in rats, if compared to those of other benzamidine derivatives, suggests this compound as an interesting new lead structure for the design of fXa inhibitors.

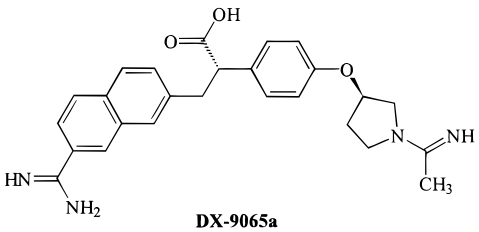
Introduction

Extrinsic and intrinsic pathways of the blood coagulation cascade intersect at factor Xa (fXa) making this enzyme directly responsible for thrombin activation while allowing the thrombin-catalyzed activation of the anticoagulant APC.[1] Because of this key position in the enzymatic cascade, fXa represents an attractive target for anticoagulant drug development. It is a trypsin-like enzyme whose architecture[2] is very similar to that of other procoagulant proteinases such as thrombin,[3,4] factor IXa,[5] and factor VIIa[6] as well as of the anticoagulant APC[7] despite major differences exhibited by these enzymes in natural substrate specificities and related biological functions.

Among the synthetic inhibitors of fXa reported so far, the most potent ones consist of two basic moieties linked by spacers of appropriate length like in the Daiichi compound DX-9065a[8] or the related YM-60828[9] as well as in the bis-benzamidine compounds 2,7-bis(4-amidinobenzylidene)cycloheptan-1-one or the 3-amidine isomer.[10] Initial efforts to identify the structural determinants of these low-mass synthetic compounds for specific inhibition of fXa were hampered by occlusion of the active site cleft of des(1−45)human fXa by the crystal packing.[2] Recently, however, it was reported that the binding mode of the synthetic inhibitor DX-9065a to des-Gla-fXa[11] is identical to that previously determined by X-ray structural analysis of the DX-9065a/trypsin complex,[12] thus suggesting the indirect approach based on crystallographic analyses of trypsin/inhibitor complexes as promising as was previously found to be in the case of thrombin inhibitors.[13−17]

Recently, we have applied this indirect approach with a series of 10 different mono- and bis-benzamidine compounds of different inhibitory potencies and specificities toward fXa and the plasminogen activators uPA and sc-tPA.[18] The binding mode of the inhibitors to the active-site cleft of trypsin, as determined by X-ray analysis, was then used for analogy modeling of the related complexes with des-Gla-fXa. By correlating the binding affinities and particularly the inhibitory specificities shown by this set of inhibitors[10] with the information gained in the structural analyses and modeling experiments, the N^{α}-tosyl-glycyl-D,L-3-amidinophenylalanine alkyl esters **1a,b** appeared to be interesting lead structures (Figure 1).





DX-9065a

	*K*i [*μM*]			
no.	hfXa	bfXa	thrombin	trypsin
1a	1.8	0.84	3.9	5.6
1b	1.3	0.46	46	17
DX-9065a	0.007	0.041	>2000	0.62

Figure 1. Structures and inhibitory potencies of compounds **1a,b**[10] and DX-9065a.[8]

The synthetic inhibitor DX-9065a binds in an extended conformation to the unprimed sites of fXa.[11]

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10.1021/jm980227t CCC: $15.00
Published on Web 09/24/1998

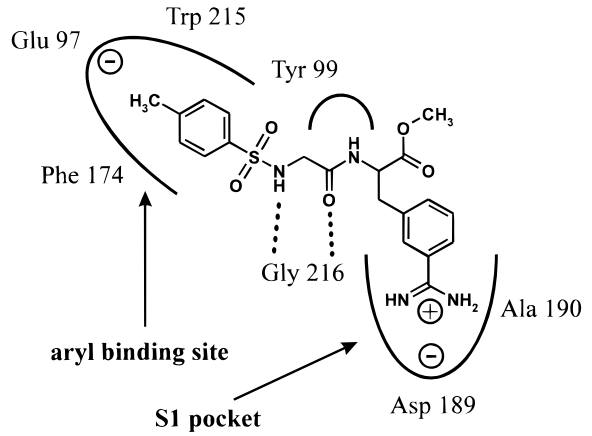


Figure 2. Schematic representation of the putative binding mode of compound **1a** to fXa as derived from the X-ray structure of the trypsin/N^{α}-tosyl-glycyl-D,L-3-amidinophenylalanine methyl ester complex[18] and the des-Gla-fXa/DX-9065a complex.[11]

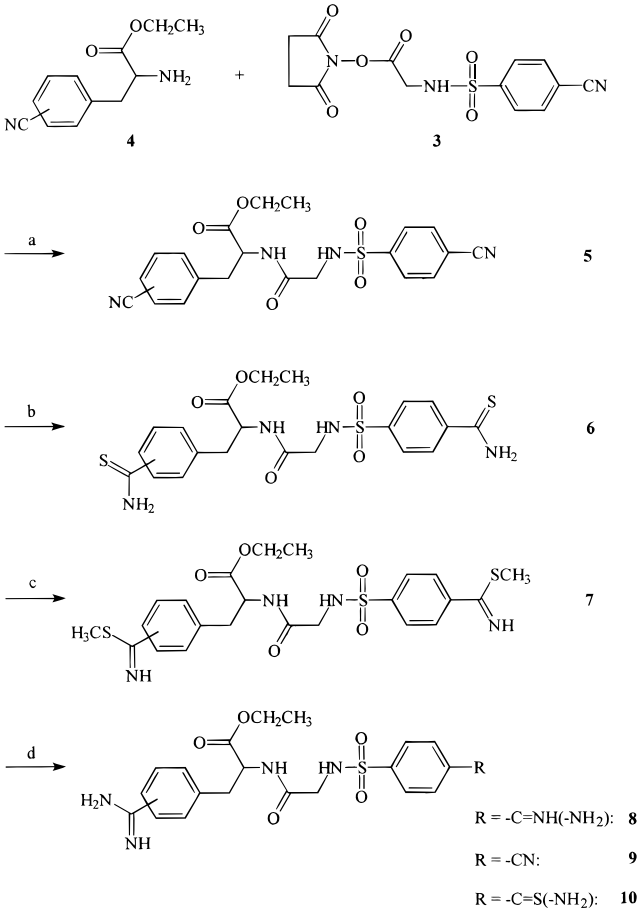
Thereby the bulky naphthylamidine moiety is inserted into the hydrophobic S1 subsite with its amidine group salt-bridged to Asp-189 on the bottom of the pocket. The aromatic benzyl spacer is oriented parallel to Trp-215 and the acetimidoyl-pyrrolidinyl moiety interacts with the hydrophobic box formed by the side chains of residues Tyr-99, Phe-174, and Trp-215, whereas the acetimido group maps to an electronegative cavity built up by the carbonyl oxygens of Lys-96, Thr-98, and Glu-97 and by the carboxylate group of the latter residue.

Modeling of the X-ray structure of compound **1a**, when bound to crystalline trypsin, onto the active-site cleft of des-Gla-fXa revealed a very similar interaction pattern whereby the restriction imparted by the side chain of Tyr-99 for the P2 residues in substrates and inhibitors of fXa is fulfilled with the glycine residue of compound **1a** (Figure 2). Conversely, the nitrogen cation hole identified in the DX-9065a/des-Gla-fXa complex[11] is not exploited with this inhibitor. In the present study we have, therefore, focused our attention primarily on the effect of hydrogen-bonding partners or positively charged groups in position 4 of the benzenesulfonyl group of the lead structure on the inhibitory potency and selectivity toward a series of trypsin-like proteinases.

Results

Chemistry. N^{α}-Tosyl-glycyl-D,L-3-amidinophenylalanine ethyl ester (**1b**) is slightly more potent than the related methyl ester **1a** (Figure 1). Correspondingly, the bis-benzamidine derivatives of **1b** with the amidino groups located in position 4′ of the benzenesulfonyl group and in position 3 or 4 of the phenylalanine moiety, respectively, were synthesized following the general route outlined in Scheme 1. For this purpose glycine was acylated with 4-cyanobenzenesulfonyl chloride, and the resulting N^{α}-(4-cyanobenzenesulfonyl)glycine was then converted to the *N*-hydroxysuccinimide ester which in turn was used to acylate D,L-4- and D,L-3-cyanophenylalanine as well as the related ethyl esters. The two cyanophenylalanine derivatives were prepared following essentially known procedures,[19] whereas D,L-4- and D,L-3-cyanophenylalanine ethyl esters were obtained by catalytic phase-transfer alkylation of the dibenzophenone imine of glycine ethyl ester with 3- and 4-cyanobenzyl bromide according to the method described by O′Donnell and Bennett,[20] followed by mild acid hydrolysis of the Schiff base.

Scheme 1. Syntheses of Bis-Substituted Derivatives of Racemic N^{α}-Benzenesulfonyl-glycyl-phenylalanine Ethyl Esters[a]

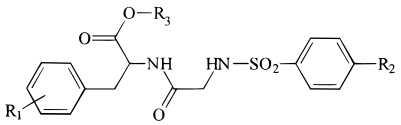


[a] (a) TEA, DMF; (b) H_2S, TEA, pyridine; (c) CH_3I, acetone; (d) NH_4OAc, MeOH, Δ; HPLC.

Subsequent conversion of the bis-cyano to the bis-amidino compounds was carried out by standard procedures based on addition of hydrogen sulfide, methylation of the resulting thioamido intermediates, and aminolysis of the thioimidates to the corresponding amidino derivatives. This conversion occurred almost quantitatively for the cyano group of the phenylalanine moiety, but in a significantly less efficient manner in the case of the 4′-cyanobenzenesulfonyl moiety. Indeed, aminolysis of the related 4′-thioimidate derivative, besides producing the desired 4′-amidino compound, led to its reconversion to the 4′-thioamido and 4′-cyano compounds with a product to side-product distribution of about 60:20:20. The side products were recovered from the reaction mixture, thus allowing for concomitant isolation of the 4′-cyano- and 4′-thioamidobenzenesulfonyl derivatives as interesting mono-amidino compounds.

Inhibition of fXa. The bis-benzamidine compounds as ethyl esters **8a,b** or free carboxylic acids **14a,b**, the mixed cyano/amidino compounds **9a,b**, and thioamido/amidino compounds **10a**,**b** were assayed for their inhibitory potencies against a larger series of trypsin-like proteinases, and the resulting K_i values are reported

Table 1. Inhibition of Various Trypsin-like Enzymes by Bis-Substituted Derivatives of Racemic N^{α}-Benzenesulfonyl-glycyl-phenylalanine



				K_i (μM)[a]									
no.	R_1	R_2	R_3	hfXa	bfXa	thrombin	APC	PK	plasmin	uPA	sc-tPA	trypsin	tryptase[b]
8a	4-amidine	4′-amidine	CH_2CH_3	0.50 (0.04)	0.53 (0.10)	41 (5)	43 (6)	8.6 (2.2)	27 (2)	>1000	70 (7)	4.2 (1.2)	29 (3)
8b	3-amidine	4′-amidine	CH_2CH_3	0.82 (0.10)	1.6 (0.2)	25 (2)	29 (3)	7.6 (0.7)	19 (4)	15 (4)	110 (12)	3.6 (1.1)	12 (1)
9a	4-amidine	4′-cyano	CH_2CH_3	8.7 (1.7)	4.8 (0.5)	11 (1)	>1000	18 (2)	>1000	>1000	>1000	20 (4)	>1000
9b	3-amidine	4′-cyano	CH_2CH_3	5.2 (1.6)	1.8 (0.5)	15 (1)	80 (2)	14 (1)	57 (9)	>1000	>1000	14 (3)	33 (8)
10a	4-amidine	4′-thioamide	CH_2CH_3	7.1 (0.9)	4.2 (0.2)	0.45 (0.09)	>1000	29 (3)	160 (45)	>1000	>1000	19 (4)	>1000
10b	3-amidine	4′-thioamide	CH_2CH_3	0.64 (0.09)	0.58 (0.05)	15 (1)	48 (8)	12 (1)	52 (4)	>1000	>1000	13 (2)	21 (4)
14a	4-amidine	4′-amidine	H	3.6 (0.7)	2.6 (0.7)	>1000	180 (28)	19 (3)	57 (2)	>1000	>1000	18 (4)	65 (7)
14b	3-amidine	4′-amidine	H	18 (2)	29 (3)	>1000	180 (12)	36 (5)	>1000	>1000	180 (24)	20 (1)	100 (27)

[a] K_i values were calculated according to Dixon[32] using a linear regression program; mean values (±SD, $n = 3-5$). [b] IC_{50} values.

in Table 1. The bis-benzamidine compound **8b** containing the 3-amidinophenylalanine moiety was found to exhibit the lowest degree of specificity, since it was capable of inhibiting in the micromolar range all the enzymes analyzed. A replacement of the 4′-amidino with the thioamido substituent in the benzenesulfonyl moiety (**10b**) did not affect the binding affinity, thus strongly suggesting the absence of any significant interaction of both these substituents with the nitrogen cation hole. With the related cyano derivative **9b**, only a moderate reduction in inhibitory potency toward bovine fXa is observed, if compared to the 4′-amidino compound, although inhibition of human fXa is sensibly affected.

By comparing the series of compounds with a 3-amidinophenylalanine as constituent (**8b**, **9b**, **10b**) with those containing the 4-amidinophenylalanine (**8a**, **9a**, **10a**), the most potent inhibitor of fXa was found to be the 4,4′-bis-amidino derivative **8a** with a K_i value of 0.5 μM for human fXa and 0.53 μM for bovine fXa. This observation contrasts previous findings that a 3-amidino group as substituent of aromatic moieties in the P1 position favors binding to fXa significantly more than the related 4-amidino isomer, whereas in the case of thrombin an opposite effect is generally observed.[21] Most surprising was the finding that for this 4,4′-series a replacement of the 4′-amidino substituent with the cyano (**9a**) or thioamido (**10a**) group leads to strongly reduced binding affinities. Both these observations suggest a different binding mode of the 3,4′- and 4,4′-series of compounds to fXa.

As expected from the X-ray structure of DX-9065a complexed to des-Gla-fXa where the carboxylate group is directed toward the bulk solvent, the compounds **14a**,**b** with the free C-terminal carboxyl group exhibit a high degree of selectivity for inhibition of fXa (Table 1). Again the 4,4′-bis-benzamidine compound **14a** is more active than the 3,4′-bis-benzamidine isomer **14b**, supporting the hypothesis of a putative different binding mode.

Inhibition of Other Trypsin-like Proteases. For an in vivo use of fXa inhibitors as antithrombotic drugs, the inhibitors are required to act in a highly selective manner. Particularly important is a minimal inhibition of enzymes of the fibrinolytic system and of APC which are counterbalancing prothrombotic stimuli.[22-24] In view of these crucial requirements we have examined the inhibitory activity of our set of compounds toward different serine proteinases.

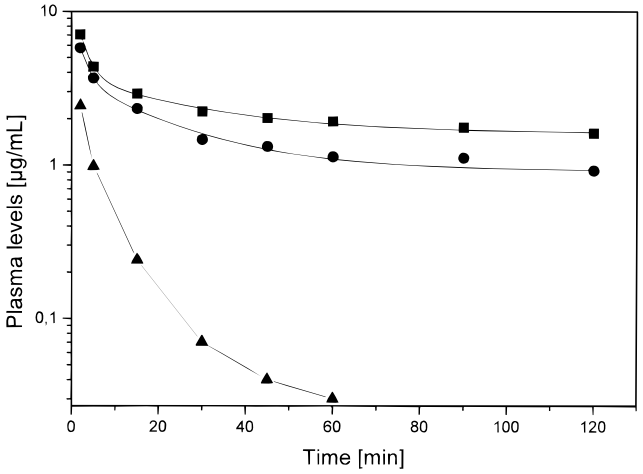


Figure 3. Plasma levels of the fXa inhibitors **14a** (■) and **14b** (●) administered iv at 1 mg/kg doses in anesthetized rats in comparison with NAPAP (▲).

Compared to fXa, the inhibitory activity of the most potent inhibitor of this series, i.e., of compound **8a**, toward thrombin is lower by nearly 2 orders of magnitude (Table 1). A similarly poor inhibition was observed in the case of the anticoagulantly effective APC as well as of PK, trypsin, and tryptase. For most of the compounds listed in Table 1 the K_i values were found to be 1 or 2 orders of magnitude higher than those related to inhibition of fXa. The same holds for inhibition of fibrinolytic enzymes such as plasmin and the plasminogen activators uPA and sc-tPA. Only compound **10a** showed a thrombin inhibition with a submicromolar K_i value possibly indicating a NAPAP-like binding mode.[14]

Pharmacokinetic Studies. Esters of 3-amidinophenylalanine are known to be hydrolyzed in blood or plasma of experimental animals.[25] We have therefore limited the pharmacokinetic studies to **14a**,**b** with their free C-terminal carboxyl function. The plasma levels of these two inhibitors were monitored after iv administration at a dose of 1 mg/kg of body weight to anesthetized rats in comparison to NAPAP. Figure 3 shows the plasma levels of the inhibitors over a period of 2 h. For NAPAP, as reported previously,[26,27] the

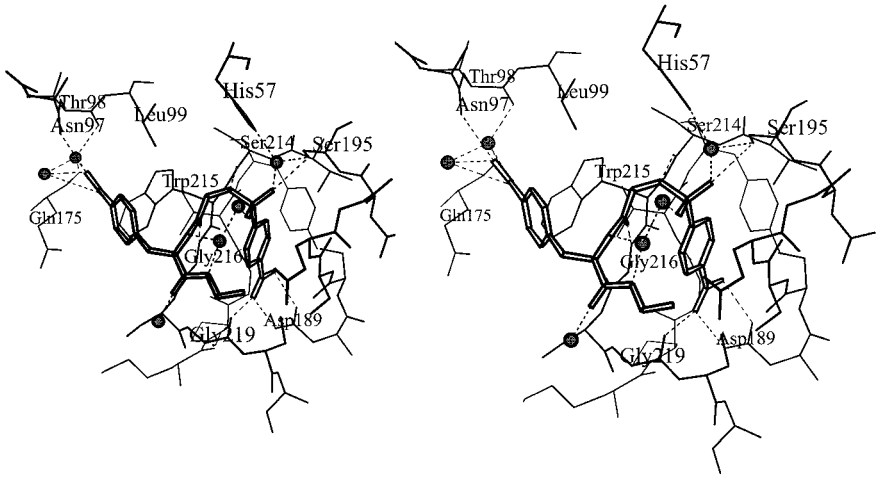


Figure 4. X-ray structure of the trypsin/**8a** complex.

plasma level declines rapidly reaching the detection limit at about 60 min after administration; thereby almost no distinction is observable between the initial distribution and subsequent elimination phase. Conversely, for the inhibitors **14a**,**b** significantly higher values were obtained in the initial distribution phase which is followed by a slow decline of the plasma level. For comparison, **14a** shows after 30 min a 32-fold and **14b** a 21-fold higher plasma level than NAPAP; after 120 min the concentrations of **14a**,**b** in plasma were still about 1 μg/mL, respectively, whereas the concentration of NAPAP dropped below the detection limit already after 60 min.

X-ray Structures of the 8a,b/Trypsin and 10b/Trypsin Complexes. To answer the question of whether the different substitution patterns affect the binding mode of the inhibitors to fXa, bovine β-trypsin crystals were soaked with the 4,4′- and 3,4′-bis-benzamidine derivatives **8a**,**b** as well as the mixed 3-amidino/4′-thioamido derivative **10b**. In the case of the trypsin complexes with **8b** and **10b**, the electron densities of the inhibitors were too weak to allow for refinement of the structures, a fact that has to be correlated with the relatively weak inhibition of trypsin by the two compounds.

For the **8b**/trypsin complex the electron density was very weak except for the region around the S1 pocket which allowed to identify unambiguously the 3-amidinophenylalanine side chain inserted into the S1 subsite; for the rest of the molecule possible interaction sites could not be assigned. Similarly, in the **10b**/trypsin complex the 3-amidinophenylalanine is inserted into the S1 pocket in full agreement with the previous findings obtained with compound **1a**.[18] Again assignment of the rest of the molecule was prevented by the weak difference electron density map.

The difference electron density map of the **8a**/trypsin complex is well-defined, thus allowing unambiguous identification of the whole inhibitor molecule as well as the assignment of the chirality *R* of the 4-amidinophenylalanine moiety when bound to the enzyme (Figure 4). Most surprising was the finding that the 4,4′-bis-benzamidine compound **8a** binds to the active site cleft of trypsin in a clearly different mode than the 3,4′-bis-benzamidine derivative **8b** as hypothetically postulated on the basis of the inhibition constants. At the entrance of the S1 pocket the unusual bulky electron density matches well with the sulfonyl group and thus with insertion of the 4′-amidinobenzene moiety into the S1 pocket. The typical salt bridge between the amidino function and the carboxylate oxygens of Asp-189 is formed, and an additional hydrogen bond is established with the carbonyl oxygen of Gly-219, as shown in Figure 4. One oxygen of the sulfonyl group is hydrogen-bonded to the hydroxyl group of the catalytic Ser-195 and to one water molecule that bridges to Ser-195O_γ and His-57$N_{\epsilon 2}$. As expected from the inverted binding mode of the inhibitor **8a**, the hydrogen-bonding pattern of the glycyl residue to the enzyme differs from the pattern observed so far for similar inhibitor/trypsin complexes.[18] In fact, the glycine NH binds to the Ser-214 carbonyl oxygen, whereas its carbonyl oxygen is hydrogen-bonded to the backbone NH of Gly-216. The well-defined ethyl ester group points toward bulk solvent with a water molecule bound to its carbonyl group. Two more water molecules are hydrogen-bonded to the second oxygen of the sulfonyl group, to the oxycarbonyl oxygen of the ester moiety and to the amide proton of the (*R*)-4-amidinophenylalanine residue. The 4-amidino substituent of the C-terminal phenylalanine is anchored via a hydrogen-bonding network, mediated by two water molecules, to the backbone oxygens of Asn-97, Thr-98, and Glu-175.

Discussion

Modeling of the newly synthesized bis-benzamidine compounds **8a**,**b** to fXa on the basis of the coordinates of the trypsin/compound **1a** complex suggested a better fitting of the 3- than the 4-amidinophenylalanine moiety into the S1 pocket for salt bridging to Asp-189. In both cases an additional interaction of the second 4′-amidino moiety with the electronegative cavity generated by the carbonyl oxygens of Lys-96, Thr-98, and Glu-97 and the side-chain carboxylate function of Glu-97 was highly probable. Surprising was, therefore, the finding that the 4,4′-bis-benzamidine compound **8a** showed an inhibition of fXa higher than that of compound **8b** despite the highly unfavored location of the amidino group in the para position as recently further confirmed with a series of differently substituted bis-benzamidine compounds.[28] Somehow expected was therefore the retro-binding mode of compound **8a** to trypsin in crystals, i.e.,

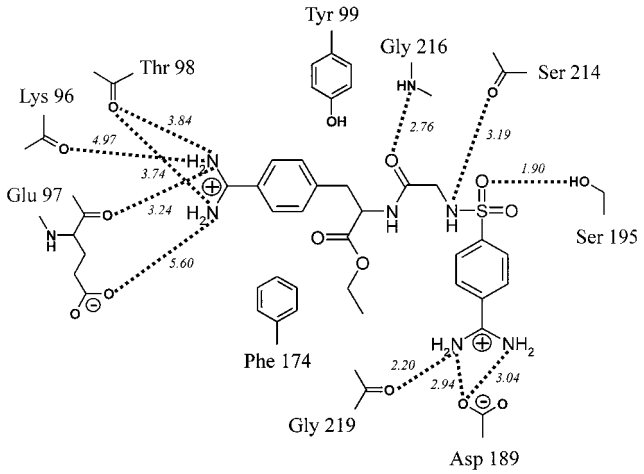


Figure 5. Schematic representation of the retro-binding mode of **8a** to human fXa and the potential interaction sites as resulting from modeling the **8a**/fXa complex on the basis of the X-ray structure of des-Gla-fXa[11] and the **8a**/trypsin complex.

with the 4′-amidinobenzenesulfonyl group inserted into the S1 pocket.

Using the X-ray structure of human des-Gla-fXa[11] and the coordinates of **8a** when bound to trypsin, a model of the fXa/**8a** complex was generated. As schematically outlined in Figure 5, the overall hydrogen-bonding pattern of this inhibitor is essentially retained even in fXa. The sulfonyl oxygen is hydrogen-bonded to the side-chain hydroxyl function of Ser-195, and this interaction leads to a different orientation of the benzamidine moiety in the S1 pocket that allows for a salt-bridge formation between the 4′-amidino group and Asp-189 with an additional hydrogen bond to the carbonyl group of Gly-219. The glycine carbonyl is hydrogen-bonded to the NH of Gly-216 thus orienting the aromatic ring of the C-terminal 4-benzamidine moiety into the aryl binding site (S4) of fXa, which is flanked by the side chains of Phe-174 and Tyr-99 not present in trypsin. The 4-amidino moiety is located behind the aryl binding site, points toward the carbonyl oxygens of Glu-97, Thr-98, and Lys-96, and is located within interaction distance to the side-chain carboxylate of Glu-97. This positioning of the amidino group within the nitrogen cation hole detected with the DX-9065a inhibitor cannot rationally explain the higher affinity of compound **8a** than **8b** for fXa, since modeling experiments with the latter compound, even when bound in the conservative mode, indicated a similar interaction in the electronegative cavity. This electrostatic interaction apparently does not suffice for increasing binding affinities, and in trypsin crystals, where the electronegative cavity is absent, immobilization of this portion of the molecule was not achieved as well-evidenced by the poor difference electron density map.

Recently, with a series of bis-benzamidine compounds of shorter spacers than the glycyl spacer used in the present study, Maduskuie et al.[28] succeeded to obtain nanomolar inhibition constants. Thereby the 4′,3-bis-benzamidine pattern proved to be essential for interactions of the 3-benzamidine group with the S1 pocket. Correspondingly, a retro-binding mode was postulated for the inverted 3′,4-substitution pattern on the basis of the experimental binding affinity data and modeling experiments. Our present findings fully confirm this hypothesis.

A retro-binding mode of inhibitors has previously been reported for thrombin when complexed, e.g., with hirudin,[29−31] ornithodorin,[32] the natural product Nazumamide A,[33] or hirudin-derived synthetic inhibitors.[34−36] The X-ray analysis of the trypsin/**8a** complex yielded an additional example of such retro-binding of synthetic inhibitors, and both the inhibitory data and modeling experiments are strongly supportive for this type of binding of **8a** even to fXa. Thus a new template molecule was disclosed which could serve for the development of low-mass fXa inhibitors with possibly selective interactions at the specificity sites of the enzyme.

For a synthetic inhibitor as an anticoagulant drug, the oral administration would certainly be the preferred one. However, most of the known synthetic inhibitors of thrombin and fXa show poor absorption after oral/intraduodenal administration and a marked hepatic first-pass effect causing low oral bioavailability. Additionally, the half-life of the compounds is relatively short, thus requiring iv infusion. In this context, we have examined the structure−pharmacokinetic relationships of various benzamidine-derived inhibitors in order to improve their oral bioavailability. In comparison with NAPAP, the two bis-benzamidine compounds **14a**,**b** that contain a free C-terminal carboxyl group were found to exhibit, upon iv administration, significantly reduced plasma clearance rates in rats that differ also from those of several other benzamidine-derived inhibitors.[26,27,37] The prolonged half-life measured for the two compounds represents the first example of a decisive improvement of pharmacokinetic properties in benzamidine-based inhibitors. This property taken together with the new binding mode may represent an interesting indication for further synthetic studies using compounds **8a** and **14a** as new lead structures.

Experimental Section

Abbreviations: fXa, factor X activated; APC, activated protein C; DX-9065a, (+)-2-[4-[((3*S*)-1-acetimidoyl-3-pyrrolidinyl)oxy]phenyl]-3-(7-amidino-2-naphthyl)propionic acid; YM-60828, [*N*-[4-[(1-acetimidoyl-4-piperidyl)oxy]phenyl]-*N*-[(7-amidino-2-naphthyl)methyl]sulfamoyl]acetic acid dihydrochloride; PK, plasma kallikrein; uPA, urokinase-type plasminogen activator; sc-tPA, single-chain tissue-type plasminogen activator; NAPAP, N^{α}-(2-naphthylsulfonyl-glycyl)-D,L-*p*-amidinophenylalanyl-piperidine; HOSu, *N*-hydroxysuccinimide; DCCI, *N*,*N*′-dicyclohexylcarbodiimide; DMF, *N*,*N*′-dimethylformamide; TEA, triethylamine; pNA, 4-nitroanilide; TFA, trifluoroacetic acid; Cbz, benzyloxycarbonyl.

Materials and Methods. All solvents and reagents used in the syntheses were of the highest quality commercially available and when required were further purified and dried by standard methods. Melting points were determined on a Büchi apparatus and are uncorrected. TLC silica gel 60 plates were from Merck AG (Darmstadt, Germany), and compounds were visualized with the chlorine/tolidine or permanganate reagent. Analytical HPLC was performed on either Nucleosil 300/C8 or C18 columns (Macherey-Nagel, Düren, Germany) using linear gradients of CH_3CN/2% H_3PO_4 from 5:95 (A) to 80:20 (B). Preparative reversed-phase chromatography was carried out on Lichroprep RP 18 (25−40 μm; Merck AG, Darmstadt, Germany) and preparative HPLC on Nucleosil RP 8 (Macherey-Nagel, Düren, Germany). FAB-MS spectra were recorded on a Finnigan MAT 900 spectrometer and EI-MS on a Finnigan MAT 312 spectrometer. IR spectra were recorded on a Perkin-Elmer FT-IR spectrometer (model 1760 X), ^{1}H

NMR spectra in DMSO-d_6 on a Bruker AM 400 or AMX 500 spectrometer, and ^{13}C NMR spectra on the AM 400 spectrometer. Chemical shifts are reported in ppm (δ) using tetramethylsilane as reference.

***N*$^{\alpha}$-(4-Cyanobenzenesulfonyl)glycine (2).** A suspension of 4-cyanobenzenesulfonyl chloride (10.0 g, 49.7 mmol) in 120 mL ether was added dropwise to a solution of glycine (3.39 g, 45.2 mmol) in 45 mL 1 M NaOH. The reaction mixture was stirred vigorously at room temperature for 20 h at pH 9. The organic layer was washed with water, and the combined aqueous extracts were acidified with HCl to pH 1. The precipitate was filtered off, washed with water, and dried at 60 °C over KOH pellets: yield 10.4 g (96%) of white powder; mp 181−182 °C; EI-MS *m*/*z* = 240.0 [M$^+$]; M_r = 240.02 calcd for $C_9H_8N_2O_4S$; IR (KBr) ν 3249 (s), 3196 (s, NH); 3097 (w), 3071 (w), 3048 (w), 3007 (w, =CH); 2936 (w, CH_2); 2516 (w, OH); 2248 (s, C≡N); 1752 (s), 1730 (s, C=O); 1493 (w, C=C); 1441 (s), 1427 (m, $\delta$$CH_2$); 1341 (s), 1165 (s, $ArSO_2N<$); ^{1}H NMR δ 3.67 (s, 2H, NH−CH_2−COOH), 7.95 (m, 2H, Ar-$H_{3/5}$, ^3J = 8.49 Hz, BB′), 8.06 (d, 2H, Ar-$H_{2/6}$, ^3J = 8.53 Hz, AA′), 8.35 (br s, 1H, CH_2−NH−SO_2); ^{13}C NMR δ 43.66 (NH−$C$$H_2$−COOH), 114.66 (Ar-$C_4$), 117.69 ($C$≡N), 127.19 (Ar-$C_{2/6}$), 133.11 (Ar-$C_{3/5}$), 144.97 (Ar-$C_1$), 169.98 ($CH_2$-$C$OOH).

***N*$^{\alpha}$-(4-Cyanobenzenesulfonyl)glycine *N*-Hydroxysuccinimidate (3).** To an ice-cold solution of **2** (1.2 g, 5.0 mmol) and HOSu (0.58 g, 5.05 mmol) in 35 mL dioxane was added DCCI (1.03 g, 5.0 mmol), and the reaction mixture was stirred overnight at 4 °C. The urea was filtered off, the solution evaporated, and the residue crystallized from 2-propanol: yield 1.53 g (91%) of white powder; mp 184−185 °C; FAB-MS *m*/*z* 338.2 [M + H$^+$]; M_r = 337.04 calcd for $C_{13}H_{11}N_3O_6S$; IR (KBr) ν 3385 (m, NH); 3070 (w), 3044 (w, =CH); 2980 (w), 2939 (w, CH_2); 2234 (m, C≡N); 1827 (s), 1784 (m), 1748 (s), 1712 (sh, C=O); 1630 (s, $\delta$$NH_2$); 1491 (w, C=C); 1434 (m), 1416 (s, $\delta$$CH_2$); 1341 (s Ar−$SO_2$N<); 1215 (s, C−O); 1175 (s, $ArSO_2N<$); 1079 (s, C−O); ^{1}H NMR δ 2.60 (s, 4H, OC−CH_2−CH_2−CO), 4.85 (s, 2H, =N−CH_2−COO), 8.16 (d, 2H, Ar-$H_{3/5}$, ^3J = 7.96 Hz, BB′), 8.21 (d, 2H, Ar-$H_{2/6}$, ^3J = 7.96 Hz, AA′), 10.50 (s, 1H, S−OH); ^{13}C NMR δ 25.13 (OC−$C$$H_2$−$C$$H_2$−CO), 41.41 (NH−$C$$H_2$−COOH), 114.94 (Ar-$C_4$), 117.63 ($C$≡N), 127.09 (Ar-$C_{2/6}$), 133.15 (Ar-$C_{3/5}$), 144.71 (Ar-$C_1$), 165.71 ($CH_2$−$C$O−N=), 172.59 (=N−$C$O−$CH_2$).

D,L-4-Cyanophenylalanine Ethyl Ester (4a). To a solution of 4-cyanobenzyl bromide (8.8 g, 44.9 mmol), *N*-diphenylmethylene-glycine ethyl ester[38] (10.0 g, 37.4 mmol), and tetrabutylammonium bromide (1.21 g, 3.74 mmol) in CH_3CN was added powdered K_2CO_3 (12.9 g, 93.5 mmol). After 48 h under vigorous stirring the K_2CO_3 was filtered off and the solution evaporated. The residue was dissolved in ether, and the organic layer was washed with brine, dried over Mg_2SO_4, and then evaporated to dryness: yield 14.2 g (99%) of yellowish powder; mp 69−72 °C; the IR (KBr) and ^{1}H and ^{13}C NMR spectra were consistent with the assigned structure.

The solution of the intermediate *N*-diphenylmethylene-D,L-4-cyanophenylalanine ethyl ester (5.58 g, 14.6 mmol) in 50 mL ether was stirred with 21.9 mL (21.9 mmol) 1 M HCl for 22 h. The reaction mixture was washed with 1 M HCl and water, and the combined aqueous extracts were neutralized with Na_2CO_3 (pH 9.5) and reextracted with AcOEt. The organic layer was dried over Na_2SO_4 and evaporated to dryness: yield 6.55 g (80%) of yellowish oil; FAB-MS *m*/*z*: 219.0 [M + H$^+$]; M_r = 218.11 calcd for $C_{12}H_{14}N_2O_2$; ^{1}H NMR δ 1.11 (t, 3H, OCH_2CH_3, ^3J = 7.15 Hz), 2.85 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.38 Hz, ^3J = 7.61 Hz), 2.95 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.35 Hz, ^3J = 6.22 Hz), 3.59 (dd, 1H, C$_\alpha$$H$, ^3J = 7.77 Hz, ^3J = 6.24 Hz), 4.03 (q, 2H, OCH_2CH$_3$, ^3J = 7.08 Hz), 7.41 (d, 2H, Ar-$H_{3/5}$, ^3J = 8.15 Hz, BB′), 7.73 (d, 2H, Ar-$H_{2/6}$, ^3J = 8.19 Hz, AA′); ^{13}C NMR δ 13.92 (OCH_2CH_3), 40.48 (Ar−CH_2−C$_\alpha$H), 55.21 (C$_\alpha$H), 59.97 (OCH_2CH$_3$), 109.02 (Ar-C_4), 118.87 (C≡N), 130.32 (Ar-$C_{2/6}$), 131.79 (Ar-$C_{3/5}$), 144.24 (Ar-C_1), 174.54 (C$_\alpha$H-COOCH$_2$CH$_3$).

D,L-3-Cyanophenylalanine Ethyl Ester (4b). The title compound was prepared as described for **4a** via the intermediate *N*-diphenylmethylene-D,L-3-cyanophenylalanine ethyl ester (yield 99% of yellow oil) and subsequent hydrolysis with 1 M HCl: yield 78% of yellowish oil (lit.[23] 56%); FAB-MS *m*/*z*: 219.1 [M + H$^+$]; M_r = 218.11 calcd for $C_{12}H_{14}N_2O_2$; ^{1}H NMR δ 1.12 (t, 3H, OCH_2CH_3, ^3J = 7.18 Hz), 2.83 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.50 Hz, ^3J = 7.67 Hz), 2.92 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.48 Hz, ^3J = 6.07 Hz), 3.58 (m, 1H, C$_\alpha$$H$, ^3J = 7.53 Hz, ^3J = 6.13 Hz), 4.03 (m, 2H, OCH_2CH$_3$, ^3J = 7.10 Hz), 7.48 (t, 1H, Ar-H_5, ^3J = 7.97 Hz), 7.56 (dvt, 1H, Ar-H_4, ^3J = 7.82 Hz, ^4J = 1.19 Hz), 7.66 (m, 1H, Ar-H_6), 7.67 (t, 1H, Ar-H_2, ^4J = 1.22 Hz); ^{13}C NMR δ 13.91 (OCH_2CH_3), 40.46 (Ar−$C$$H_2$−C$_\alpha$H), 55.20 (C$_\alpha$H), 59.96 (O$CH_2CH_3$), 110.91 (Ar-$C_3$), 118.80 ($C$≡N), 129.11 (Ar-$C_4$), 129.95 (Ar-$C_5$), 132.82 (Ar-$C_2$), 134.27 (Ar-$C_6$), 139.83 (Ar-$C_1$), 174.58 (C$_\alpha$H-$C$OOCH$_2CH_3$).

***N*$^{\alpha}$-(4′-Cyanobenzenesulfonyl)glycyl-D,L-4-cyanophenylalanine Ethyl Ester (5a).** Compound **4a** (1.52 g, 6.96 mmol) was reacted in DMF with the active ester **3** (1.85 g, 5.48 mmol) and 0.76 mL (5.48 mmol) TEA for 3 days. The bulk of the solvent was evaporated and the residue distributed between AcOEt and 5% $KHSO_4$. The organic layer was washed with water, dried over Na_2SO_4, and evaporated to dryness. The residue was recrystallized from $CHCl_3$/*n*-hexane: yield 2.38 g (99%) of orange foam; FAB-MS *m*/*z*: 441.3 [M + H$^+$]; M_r = 440.12 calcd for $C_{21}H_{20}N_4O_5S$; IR (KBr) ν 3355 (m, NH); 3095 (w, =CH); 2984 (w), 2936 (w, $CH_{2/3}$); 2232 (m, C≡N); 1738 (s, C=O); 1671 (s, N−C=O); 1610 (w, δNH); 1536 (m, C=C); 1447 (w, $\delta$$CH_2$); 1377 (w, $\delta$$CH_3$); 1342 (m, $ArSO_2N<$); 1217 (m, C−O); 1165 (s, $ArSO_2N<$); ^{1}H NMR δ 1.11 (t, 3H, OCH_2CH_3, ^3J = 7.0 Hz), 2.95 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 14.0 Hz, ^3J = 9.0 Hz), 3.08 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 14.0 Hz, ^3J = 6.0 Hz), 3.53 (m, 2H, CO−CH_2−NH, ^2J = 17.0 Hz, ^3J = 8.0 Hz), 4.05 (q, 2H, OCH_2CH$_3$, ^3J = 7.0 Hz), 4.43 (dvt, 1H, C$_\alpha$$H$, ^3J = 8.0 Hz, ^3J = 6.0 Hz), 7.38 (d, 2H, Ar-$H_{3/5}$, ^3J = 8.2 Hz, BB′), 7.73 (d, 2H, Ar-$H_{2/6}$, ^3J = 8.2 Hz, AA′), 7.91 (d, 2H, Ar-$H_{3'/5'}$, ^3J = 8.5 Hz, BB′), 8.03 (d, 2H, Ar-$H_{2'/6'}$, ^3J = 8.5 Hz, AA′), 8.27 (br s, 1H, CH_2−NH−SO_2), 8.38 (d, 1H, C$_\alpha$H−NH−CO, ^3J = 8.0 Hz); ^{13}C NMR δ 13.82 (OCH_2CH_3), 36.60 (Ar−$C$$H_2$−C$_\alpha$H), 44.53 (CO−$C$$H_2$−NH), 52.81 (C$_\alpha$H), 60.68 (O$CH_2$-$CH_3$), 109.44 (Ar-$C_4$), 114.69 (Ar-$C_{4'}$), 117.66 ($SO_2$−Ar−$C$≡N), 118.74 ($C$≡N), 127.23 (Ar-$C_{2'/6'}$), 130.19 (Ar-$C_{2/6}$), 131.99 (Ar-$C_{3/5}$), 133.06 (Ar-$C_{3'/5'}$), 142.90 (Ar-$C_1$), 144.65 (Ar-$C_{1'}$), 167.30 (NH−$C$O−$CH_2$), 170.59 (C$_\alpha$H-$C$OOCH$_2CH_3$).

***N*$^{\alpha}$-(4′-Cyanobenzenesulfonyl)glycyl-D,L-3-cyanophenylalanine Ethyl Ester (5b).** The title compound was prepared by reacting **4b** with **3** as described for **5a**: yield 96% of yellowish foam; FAB-MS *m*/*z*: 441.2 [M + H$^+$]; M_r = 440.12 calcd for $C_{21}H_{20}N_4O_5S$; IR (KBr) ν 3370 (m, NH); 3098 (w, =CH); 2984 (w), 2936 (w, $CH_{2/3}$); 2233 (m, C≡N); 1738 (s, C=O); 1667 (s, N−C=O); 1535 (m), 1485 (w, C=C); 1446 (w, $\delta$$CH_2$); 1377 (w, $\delta$$CH_3$); 1342 (m, $ArSO_2N<$); 1213 (m, C−O); 1164 (s, $ArSO_2N<$); ^{1}H NMR δ 1.11 (t, 3H, OCH_2CH_3, ^3J = 7.0 Hz), 2.92 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 14.0 Hz, ^3J = 9.0 Hz), 3.05 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 14.0 Hz, ^3J = 6.0 Hz), 3.53 (m, 2H, CO−CH_2−NH, ^2J = 16.6 Hz, ^3J = 6.0 Hz), 4.04 (q, 2H, OCH_2CH$_3$, ^3J = 7.0 Hz), 4.44 (dvt, 1H, C$_\alpha$$H$, ^3J = 8.0 Hz, ^3J = 6.0 Hz), 7.48 (t, 1H, Ar-H_5, ^3J = 7.6 Hz), 7.53 (dvt, 1H, Ar-H_4, ^3J = 7.6 Hz, ^4J = 1.6 Hz), 7.65 (s, 1H, Ar-H_2), 7.69 (dvt, 1H, Ar-H_6, ^3J = 7.6 Hz, ^4J = 1.6 Hz), 7.91 (d, 2H, Ar-$H_{3'/5'}$, ^3J = 8.5 Hz, BB′), 8.03 (d, 2H, Ar-$H_{2'/6'}$, ^3J = 8.5 Hz, AA′), 8.26 (t, 1H, CH_2−NH−SO_2, ^3J = 6.0 Hz), 8.37 (d, 1H, C$_\alpha$H−NH−CO, ^3J = 8.0 Hz); ^{13}C NMR δ 13.82 (OCH_2CH_3), 36.01 (Ar−$C$$H_2$−C$_\alpha$H), 44.52 (CO−$C$$H_2$−NH), 52.91 (C$_\alpha$H), 60.64 (O$CH_2CH_3$), 111.14 (Ar-$C_4$), 114.69 (Ar-$C_{4'}$), 117.65 ($SO_2$−Ar−$C$≡N), 118.67 ($C$≡N), 127.22 (Ar-$C_{2'/6'}$), 129.31 (Ar-$C_5$), 130.39 (Ar-$C_4$), 132.71 (Ar-$C_2$), 133.05 (Ar-$C_{3'/5'}$), 134.12 (Ar-$C_6$), 138.55 (Ar-$C_1$), 144.65 (Ar-$C_{1'}$), 167.32 (NH−$C$O−$CH_2$), 170.61 (C$_\alpha$H-$C$OOCH$_2CH_3$).

***N*$^{\alpha}$-(4′-Thioamidobenzenesulfonyl)glycyl-D,L-4-thioamidophenylalanine Ethyl Ester (6a).** Hydrogen sulfide was bubbled through the solution of **5a** (2.14 g, 4.86 mmol) and 4.03 mL (28.1 mmol) TEA in 30 mL of dry pyridine for 15 min. The solution was stirred for 5 days and then evaporated. The residue was washed in AcOEt with 1 M HCl and water, dried over Na_2SO_4, and evaporated to dryness: yield 2.3 g (93%) of yellowish foam; HPLC (isocratic elution with A for 5

min; linear gradient from A to B in 25 min): t_R = 20.07 min; FAB-MS *m/z*: 509.2 [M + H$^+$]; M_r = 508.09 calcd for $C_{21}H_{24}N_4O_5S_3$; IR (KBr) ν 3315 (s), 3194 (s, NH_2); 3089 (sh, =CH); 2975 (w), 2915 (w, $CH_{2/3}$); 1728 (s, C=O); 1659 (s, N−C=O); 1630 (s, δNH_2); 1565 (w), 1531 (m, C=C); 1423 (m, δCH_2); 1377 (w, δCH_3); 1330 (m, $ArSO_2N<$); 1275 (m, C−O); 1164 (s, $ArSO_2N<$); ^{1}H NMR δ 1.12 (t, 3H, OCH_2CH_3, ^3J = 7.03 Hz), 2.93 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.76 Hz, ^3J = 8.40 Hz), 3.02 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.80 Hz, ^3J = 5.92 Hz), 3.48 (m, 2H, CO−CH_2−NH, ^2J = 16.57 Hz, ^3J = 6.21 Hz), 4.04 (q, 2H, OCH_2CH_3, ^3J = 7.04 Hz), 4.44 (dvt, 1H, C$_\alpha H$, ^3J = 8.05 Hz, ^3J = 6.10 Hz), 7.22 (d, 2H, Ar-$H_{3/5}$, ^3J = 8.32 Hz, BB′), 7.79 (d, 2H, Ar-$H_{3'/5'}$, ^3J = 8.43 Hz, BB′), 7.84 (d, 2H, Ar-$H_{2/6}$, ^3J = 8.34 Hz, AA′), 7.97 (d, 2H, Ar-$H_{2'/6'}$, ^3J = 8.37 Hz, AA′), 8.20 (t, 1H, CH_2−NH−SO_2, ^3J = 6.23 Hz), 8.32 (d, 1H, C$_\alpha$H−NH−CO, ^3J = 7.72 Hz), 9.40 (s, 1H, C=S(−NH_2)), 9.65 (s, 1H, C=S(−NH_2)), 9.76 (s, 1H, C=S(−NH_2)), 10.06 (s, 1H, C=S(−NH_2)); ^{13}C NMR δ 13.85 (OCH_2CH_3), 36.37 (Ar−CH_2−C$_\alpha$H), 44.71 (CO-CH_2−NH), 53.14 (C$_\alpha$H), 60.63 (OCH_2CH_3), 126.13 (Ar-$C_{2'/6'}$), 127.24 (Ar-$C_{2/6}$), 127.68 (Ar-$C_{3'/5'}$), 128.54 (Ar-$C_{3/5}$), 137.54 (Ar-C_4), 140.30 (Ar-$C_{4'}$), 142.12 (Ar-C_1), 142.79 (Ar-$C_{1'}$), 167.43 (NH−CO−CH_2), 170.81 (C$_\alpha$H-COOCH$_2$CH$_3$), 198.84 (C=S(−NH_2)), 199.55 (C=S(−NH_2)).

***N*$^\alpha$-(4′-Thioamidobenzenesulfonyl)glycyl-D,L-3-thioamidophenylalanine Ethyl Ester (6b).** The title compound was prepared from **5b** as described for **6a**: yield 91% of yellow foam; HPLC (conditions as for **6a**): t_R = 20.20 min; FAB-MS *m/z*: 509.2 [M + H$^+$]; M_r = 508.09 calcd for $C_{21}H_{24}N_4O_5S_3$; IR (KBr) ν 3315 (s), 3194 (s, NH_2); 3090 (sh, =CH); 2978 (w, $CH_{2/3}$); 1729 (s, C=O); 1667 (s, N−C=O); 1630 (s, δNH_2); 1534 (m, C=C); 1412 (m, δCH_2); 1377 (w, δCH_3); 1332 (m, $ArSO_2N<$); 1277 (m, C−O); 1164 (s, $ArSO_2N<$); ^{1}H NMR δ 1.11 (t, 3H, OCH_2CH_3, ^3J = 7.06 Hz), 2.93 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.74 Hz, ^3J = 8.16 Hz), 3.02 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.80 Hz, ^3J = 6.12 Hz), 3.50 (m, 2H, CO−CH_2−NH, ^2J = 16.80 Hz, ^3J = 6.64 Hz), 4.04 (q, 2H, OCH_2CH_3, ^3J = 7.01 Hz), 4.44 (dvt, 1H, C$_\alpha H$, ^3J = 7.80 Hz, ^3J = 6.30 Hz), 7.30−7.32 (m, 2H, Ar-H_4 and Ar-H_5), 7.72−7.74 (m, 2H, Ar-H_2 and Ar-H_6), 7.79 (d, 2H, Ar-$H_{3'/5'}$, ^3J = 8.35 Hz, BB′), 7.97 (d, 2H, Ar-$H_{2'/6'}$, ^3J = 8.41 Hz, AA′), 8.04 (t, 1H, CH_2−NH−SO_2, ^3J = 6.14 Hz), 8.32 (d, 1H, C$_\alpha$H−NH−CO, ^3J = 7.70 Hz), 9.40 (s, 1H, C=S(−NH_2)), 9.65 (s, 1H, C=S(−NH_2)), 9.80 (s, 1H, C=S(−NH_2)), 10.06 (s, 1H, C=S(−NH_2)); ^{13}C NMR δ 13.83 (OCH_2CH_3), 36.62 (Ar−CH_2−C$_\alpha$H), 44.71 (CO-CH_2−NH), 53.35 (C$_\alpha$H), 60.62 (OCH_2-CH_3), 125.41 (Ar-C_4), 126.14 (Ar-$C_{3'/5'}$), 127.69 (Ar-$C_{2'/6'}$ and Ar-C_2), 128.20 (Ar-C_5), 131.83 (Ar-C_6), 136.50 (Ar-C_1), 139.32 (Ar-C_3), 142.12 (Ar-$C_{4'}$), 142.79 (Ar-$C_{1'}$), 167.40 (NH−CO−CH_2), 170.87 (C$_\alpha$H-COOCH$_2$CH$_3$), 198.84 (C=S(−NH_2)), 199.95 (C=S(−NH_2)).

***N*$^\alpha$-(4′-Methylthioimido-benzenesulfonyl)glycyl-D,L-4-methylthioimido-phenylalanine Ethyl Ester Bis(hydroiodide) (7a).** To **6a** (0.79 mg, 1.55 mmol) in 25 mL dry acetone was added methyl iodide (0.58 mL, 9.31 mmol) under nitrogen in the dark. After 2 days the reaction mixture was evaporated to dryness: yield 0.87 g (97%) of yellowish foam; HPLC (conditions as for **6a**): t_R = 14.69 min; FAB-MS *m/z*: 537.2 [M + H$^+$]; M_r = 536.12 calcd for $C_{23}H_{28}N_4O_5S_3$; IR (KBr) ν 3400 (sh), 3192 (sh, NH_2); 3048 (w, =CH); 2973 (w, $CH_{2/3}$); 1730 (m, C=O); 1659 (s, N−C=O); 1608 (m, δNH_2); 1566 (w), 1531 (m, C=C); 1400 (m, δCH_2); 1377 (w, δCH_3); 1335 (m, $ArSO_2N<$); 1277 (w), 1207 (sh, C−O); 1165 (s, $ArSO_2N<$); ^{1}H NMR δ 1.11 (t, 3H, OCH_2CH_3, ^3J = 7.15 Hz), 2.84 (s, 3H, −SCH_3), 2.85 (s, 3H, −SCH_3), 3.02 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.80 Hz, ^3J = 8.81 Hz), 3.13 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.81 Hz, ^3J = 5.89 Hz), 3.54 (d, 2H, CO−CH_2−NH, ^3J = 5.89 Hz), 4.05 (q, 2H, OCH_2CH_3, ^3J = 7.05 Hz), 4.48 (dvt, 1H, C$_\alpha H$, ^3J = 8.23 Hz, ^3J = 6.24 Hz, ^3J = 5.86 Hz), 7.49 (d, 2H, Ar-$H_{3/5}$, ^3J = 8.25 Hz, BB′), 7.80 (d, 2H, Ar-$H_{3'/5'}$, ^3J = 8.22 Hz, BB′), 7.97 (d, 2H, Ar-$H_{2/6}$, ^3J = 8.93 Hz, AA′), 8.01 (d, 2H, Ar-$H_{2'/6'}$, ^3J = 8.89 Hz, AA′), 8.28 (t, 1H, CH_2−NH−SO_2, ^3J = 6.10 Hz), 8.44 (d, 1H, C$_\alpha$H−NH−CO, ^3J = 7.80 Hz); ^{13}C NMR δ 13.88 (OCH_2CH_3), 15.68 (−SCH_3), 36.48 (Ar−CH_2−C$_\alpha$H), 44.66 (CO−CH_2−NH), 52.92 (C$_\alpha$H), 60.75 (OCH_2CH_3), 126.42 (Ar-$C_{1'}$), 127.22 (Ar-$C_{2'/6'}$), 127.96 (Ar-$C_{3/5}$), 128.79 (Ar-$C_{3'/5'}$), 129.23 (Ar-C_4), 130.22 (Ar-$C_{2/6}$), 134.61 (Ar-$C_{4'}$), 145.18 (Ar-C_1), 167.46 (NH−CO−CH_2), 170.59 (C$_\alpha$H-COOCH$_2$CH$_3$), 188.61 (C−SCH$_3$(=NH$_2^+$)).

***N*$^\alpha$-(4′-Methylthioimido-benzenesulfonyl)glycyl-D,L-3-methylthioimido-phenylalanine Ethyl Ester Bis(hydroiodide) (7b).** The compound was obtained from **6b** as described for **7a**: yield 88% of yellow foam; HPLC (conditions as for **6a**): t_R = 14.57 min; FAB-MS *m/z*: 537.2 [M + H$^+$]; M_r = 536.12 calcd for $C_{23}H_{28}N_4O_5S_3$; IR (KBr) ν 3406 (s), 3192 (sh, NH_2); 3061 (w, =CH); 2996 (w), 2935 (sh, $CH_{2/3}$); 1730 (m, C=O); 1666 (s, N−C=O); 1629 (sh, δNH_2); 1565 (w), 1535 (m, C=C); 1401 (m, δCH_2); 1377 (w, δCH_3); 1337 (m, $ArSO_2N<$); 1275 (w), 1210 (w, C−O); 1166 (s, $ArSO_2N<$); ^{1}H NMR δ 1.11 (t, 3H, OCH_2CH_3, ^3J = 7.12 Hz), 2.83 (s, 3H, −SCH_3), 2.85 (s, 3H, −SCH_3), 3.01 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.82 Hz, ^3J = 8.61 Hz), 3.12 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.77 Hz, ^3J = 6.04 Hz), 3.54 (d, 2H, CO−CH_2−NH, ^3J = 6.09 Hz), 4.05 (q, 2H, OCH_2CH_3, ^3J = 7.05 Hz), 4.48 (dvt, 1H, C$_\alpha H$, ^3J = 8.23 Hz, ^3J = 6.05 Hz), 7.58 (t, 1H, Ar-H_5, ^3J = 7.66 Hz), 7.64 (dvt, 1H, Ar-H_4, ^3J = 7.82 Hz), 7.72 (br s, 1H, Ar-H_2), 7.73 (dvt, 1H, Ar-H_6, ^3J = 7.68 Hz, ^4J = 1.06 Hz), 7.96 (d, 2H, Ar-$H_{3'/5'}$, ^3J = 8.94 Hz, BB′), 8.00 (d, 2H, Ar-$H_{2'/6'}$, ^3J = 8.65 Hz, AA′), 8.28 (t, 1H, CH_2−NH−SO_2, ^3J = 6.10 Hz), 8.42 (d, 1H, C$_\alpha$H−NH−CO, ^3J = 7.83 Hz); ^{13}C NMR δ 13.88 (OCH_2CH_3), 15.68 (−SCH_3), 36.20 (Ar−CH_2−C$_\alpha$H), 44.67 (CO−CH_2−NH), 53.14 (C$_\alpha$H), 60.73 (OCH_2CH_3), 126.34 (Ar-C_4), 127.19 (Ar-$C_{2'/6'}$), 128.07 (Ar-C_2), 128.61 (Ar-C_5), 128.70 (Ar-$C_{3'/5'}$), 129.41 (Ar-C_3), 131.02 (Ar-$C_{4'}$), 135.93 (Ar-C_6), 138.58 (Ar-C_1), 145.45 (Ar-$C_{1'}$), 167.47 (NH−CO−CH_2), 170.66 (C$_\alpha$H-COOCH$_2$CH$_3$), 188.93 (C−SCH$_3$(=NH$_2^+$)).

***N*$^\alpha$-(4′-Amidinobenzenesulfonyl)glycyl-D,L-4-amidinophenylalanine Ethyl Ester Bis(trifluoroacetate) (8a).** A solution of **7a** (0.8 g, 1.01 mmol) and ammonium acetate (195 mg, 2.53 mmol) in dry MeOH (25 mL) was refluxed for 4 h and then evaporated. The oily residue was chromatographed on a Lichroprep RP-18 column (2 × 200 cm) by elution with a linear gradient from 1% TFA/CH_3CN (95:5) to 1% TFA/CH_3-CN (55:45) in 10 h at a flow rate of 380 mL/h. The elution pattern exhibited three peaks which were separately pooled, evaporated, and dried over KOH pellets.

Peak 1 corresponds to the title compound: yield 0.26 g (37%) of white powder; HPLC (linear gradient from 100% A to 60% B in 18 min): t_R = 7.62 min; FAB-MS *m/z*: 475.2 [M + H$^+$]; M_r = 474.17 calcd for $C_{21}H_{26}N_6O_5S$; IR (KBr) ν 3333 (m, NH_2); 3104 (m, =CH); 2945 (sh), 2867 (sh, $CH_{2/3}$); 1738 (sh, C=O); 1670 (s, N−C=O); 1618 (w, δNH_2); 1537 (m), 1492 (m, C=C); 1442 (m, δCH_2); 1377 (w, δCH_3); 1340 (m, $ArSO_2N<$); 1206 (s, C−O); 1136 (s, $ArSO_2N<$); ^{1}H NMR δ 1.13 (t, 3H, OCH_2CH_3, ^3J = 7.20 Hz), 3.01 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.85 Hz, ^3J = 8.71 Hz), 3.13 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.84 Hz, ^3J = 5.87 Hz), 3.52 (dd, 2H, CO−CH_2−NH, ^2J = 16.58 Hz), 4.06 (q, 2H, OCH_2CH_3, ^3J = 7.09 Hz), 4.50 (dvt, 1H, C$_\alpha H$, ^3J = 8.36 Hz, ^3J = 5.99 Hz, ^3J = 5.67 Hz), 7.45 (d, 2H, Ar-$H_{3/5}$, ^3J = 8.40 Hz, BB′), 7.75 (d, 2H, Ar-$H_{2/6}$, ^3J = 8.38 Hz, AA′), 7.96 (d, 2H, Ar-$H_{3'/5'}$, ^3J = 9.19 Hz, BB′), 7.98 (d, 2H, Ar-$H_{2'/6'}$, ^3J = 9.15 Hz, AA′), 8.46 (d, 1H, C$_\alpha$H−NH−CO, ^3J = 7.82 Hz), 9.29 (br s, 7H, C−NH_2(=NH_2^+) and CH_2−NH−SO_2); ^{13}C NMR δ 13.86 (OCH_2CH_3), 36.40 (Ar−CH_2−C$_\alpha$H), 44.69 (CO-CH_2−NH), 53.07 (C$_\alpha$H), 60.72 (OCH_2CH_3), 126.25 (Ar-C_4), 126.76 (Ar-$C_{2'/6'}$), 127.92 (Ar-$C_{3/5}$), 129.00 (Ar-$C_{3'/5'}$), 129.66 (Ar-$C_{2/6}$), 131.79 (Ar-$C_{4'}$), 143.47 (Ar-C_1), 144.88 (Ar-$C_{1'}$), 164.77 (C−NH$_2$(=NH$_2^+$)), 165.21 (C−NH$_2$(=NH$_2^+$)), 167.50 (NH−CO−CH_2), 170.71 (C$_\alpha$H-COOCH$_2$CH$_3$). Anal. ($C_{21}H_{26}N_6O_5S(CF_3CO_2H)_2$) H, N; C: calcd, 42.73; found, 42.08.

Peak 2 corresponds to *N*$^\alpha$-(4′-cyanobenzenesulfonyl)glycyl-D,L-4-amidinophenylalanine ethyl ester mono(trifluoroacetate) (**9a**): yield 58 mg (10%) of white powder; HPLC (conditions as for **8a**): t_R = 12.32 min; FAB-MS *m/z*: 458.2 [M + H$^+$]; M_r = 457.14 calcd for $C_{21}H_{23}N_5O_5S$; IR (KBr) ν 3333 (m, NH_2); 3099 (m, =CH); 2973 (sh), 2940 (sh, $CH_{2/3}$); 2235 (m, C≡N); 1736 (sh, C=O); 1671 (s, N−C=O); 1617 (w, δNH_2); 1539 (m), 1492 (m, C=C); 1442 (m, δCH_2); 1378 (w, δCH_3); 1340 (m, $ArSO_2N<$); 1208 (s, C−O); 1162 (s, $ArSO_2N<$); ^{1}H NMR δ 1.13 (t, 3H, OCH_2CH_3, ^3J = 7.06 Hz), 2.96 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.81 Hz, ^3J = 8.90 Hz), 3.10 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J

= 13.85 Hz, ^3J = 5.79 Hz), 3.53 (dd, 2H, CO−CH_2−NH, ^2J = 16.73 Hz, ^3J = 8.82 Hz), 4.06 (q, 2H, OCH_2CH$_3$, ^3J = 7.09 Hz), 4.44 (dvt, 1H, C$_\alpha H$, ^3J = 8.57 Hz, ^3J = 5.80 Hz, ^3J = 5.32 Hz), 7.44 (d, 2H, Ar-$H_{3/5}$, ^3J = 8.32 Hz, BB′), 7.74 (d, 2H, Ar-$H_{2/6}$, ^3J = 8.35 Hz, AA′), 7.91 (d, 2H, Ar-$H_{3'/5'}$, ^3J = 8.44 Hz, BB′), 8.03 (d, 2H, Ar-$H_{2'/6'}$, ^3J = 8.63 Hz, AA′), 8.42 (d, 1H, C$_\alpha$H−NH−CO, ^3J = 7.85 Hz), 9.05 (br s, 4H, C−NH_2(=NH_2^+) and CH$_2$−NH−SO$_2$); ^{13}C NMR δ 13.87 (OCH$_2$CH_3), 36.40 (Ar−CH_2−C$_\alpha$H), 44.58 (CO−CH_2−NH), 53.01 (C$_\alpha$H), 60.69 (OCH_2-CH$_3$), 116.36 (Ar-$C_{4'}$), 117.57 (C≡N), 126.21 (Ar-C_4), 127.23 (Ar-$C_{2'/6'}$), 127.93 (Ar-$C_{3/5}$), 128.95 (Ar-$C_{1'}$), 129.64 (Ar-$C_{2/6}$), 133.09 (Ar-$C_{3'/5'}$), 143.47 (Ar-C_1), 165.10 (C−NH$_2$(=NH$_2^+$)), 167.87 (NH−CO−CH$_2$), 170.90 (C$_\alpha$H-COOCH$_2$CH$_3$). Anal. (C$_{21}$H$_{23}$N$_5$O$_5$S(CF$_3$CO$_2$H)$_1$) H; C: calcd, 48.32; found, 46.62. N: calcd, 12.26; found, 11.67.

Peak 3 corresponds to N^α-(4′-thioamidobenzenesulfonyl)-glycyl-D,L-4-amidinophenylalanine ethyl ester mono(trifluoroacetate) (**10a**): yield 40 mg (6%) of white powder; HPLC (conditions as for **8a**): t_R = 12.00 min; FAB-MS *m*/*z*: 492.2 [M + H$^+$]; M_r = 491.13 calcd for C$_{21}$H$_{25}$N$_5$O$_6$S$_2$; IR (KBr) ν 3329 (m, NH$_2$); 3119 (sh, =CH); 2936 (sh), 2855 (sh, CH$_{2/3}$); 1734 (w, C=O); 1671 (s, N−C=O); 1539 (m), 1492 (m, C=C); 1418 (m, δCH$_2$); 1377 (w, δCH$_3$); 1333 (m, ArSO$_2$N<); 1207 (s), 1187 (s, C−O); 1136 (s, ArSO$_2$N<); ^{1}H NMR δ 1.13 (t, 3H, OCH$_2$CH_3, ^3J = 7.14 Hz), 2.99 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.80 Hz, ^3J = 8.79 Hz), 3.12 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.83 Hz, ^3J = 5.77 Hz), 3.47 (t, 2H, CO−CH_2−NH, ^3J = 5.79 Hz), 4.06 (q, 2H, OCH_2CH$_3$, ^3J = 7.15 Hz), 4.49 (m, 1H, C$_\alpha H$, ^3J = 8.02 Hz, ^3J = 5.66 Hz), 7.45 (d, 2H, Ar-$H_{3/5}$, ^3J = 8.33 Hz, BB′), 7.74 (d, 2H, Ar-$H_{2/6}$, ^3J = 8.28 Hz, AA′), 7.79 (d, 2H, Ar-$H_{3'/5'}$, ^3J = 8.51 Hz, BB′), 7.97 (d, 2H, Ar-$H_{2'/6'}$, ^3J = 8.44 Hz, AA′), 8.08 (t, 1H, CH$_2$−NH−SO$_2$, ^3J = 6.10 Hz), 8.40 (d, 1H, C$_\alpha$H−NH−CO, ^3J = 7.85 Hz), 9.03 (s, 1H, C−NH_2(=NH_2^+)), 9.24 (s, 1H, C−NH_2-(=NH_2^+)), 9.67 (s, 1H, C=S(−NH_2)), 10.07 (s, 1H, C=S(−NH_2)); ^{13}C NMR δ 13.88 (OCH$_2$CH_3), 36.42 (Ar−CH_2−C$_\alpha$H), 44.71 (CO−CH_2−NH), 53.02 (C$_\alpha$H), 60.73 (OCH_2CH$_3$), 126.12 (Ar-C_4 and Ar-$C_{2'/6'}$), 127.70 (Ar-$C_{3'/5'}$), 127.93 (Ar-$C_{3/5}$), 129.66 (Ar-$C_{2/6}$), 142.06 (Ar-C_1), 142.82 (Ar-$C_{4'}$), 143.50 (Ar-$C_{1'}$), 165.21 (C−NH$_2$(=NH$_2^+$)), 167.49 (NH−CO−CH$_2$), 170.70 (C$_\alpha$H-COOCH$_2$CH$_3$), 198.81 (C=S(−NH$_2$)). Anal. (C$_{21}$H$_{25}$N$_5$O$_5$S$_2$(CF$_3$-CO$_2$H)$_1$) H; C: calcd, 45.61; found, 44.73. N: calcd, 11.57; found: 11.08.

***N*$^\alpha$-(4′-Amidinobenzenesulfonyl)glycyl-D,L-3-amidinophenylalanine Ethyl Ester Bis(trifluoroacetate) (8b).** After aminolysis of **7b** (0.80 g, 1.01 mmol) reversed-phase chromatography on Lichroprep RP-18 as described for **8a** led again to three peaks.

Peak 1 corresponds to the title compound: 225 mg (32%) of white powder; HPLC (conditions as for **8a**): t_R = 7.82 min; FAB-MS *m*/*z*: 475.5 [M + H$^+$]; M_r = 474.17 calcd for C$_{21}$H$_{26}$N$_6$O$_5$S; IR (KBr) ν 3320 (m), 3100 (m, NH$_2$); 3050 (sh, =CH); 2902 (sh), 2876 (sh, CH$_{2/3}$); 1730 (sh, C=O); 1670 (s, N−C=O); 1612 (w, (NH); 1527 (m), 1483 (w, C=C); 1444 (w, δCH$_2$); 1378 (w, δCH$_3$); 1340 (m, ArSO$_2$N<); 1206 (s, C−O); 1143 (s, ArSO$_2$N<); ^{1}H NMR δ 1.12 (t, 3H, OCH$_2$CH_3, ^3J = 7.09 Hz), 3.00 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.85 Hz, ^3J = 8.66 Hz), 3.10 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.79 Hz, ^3J = 5.79 Hz), 3.52 (d, 2H, CO−CH_2−NH, ^3J = 5.87 Hz), 4.06 (q, 2H, OCH_2CH$_3$, ^3J = 7.07 Hz), 4.52 (m, 1H, C$_\alpha H$, ^3J = 8.18 Hz, ^3J = 7.61 Hz, ^3J = 5.86 Hz), 7.52 (t, 1H, Ar-H_5, ^3J = 7.49 Hz), 7.57 (dvt, 1H, Ar-H_4, ^3J = 7.67 Hz), 7.68−7.70 (m, 2H, Ar-H_2 and Ar-H_6), 7.97 (s, 4H, Ar-$H_{2'/3'/5'/6'}$), 8.28 (t, 1H, CH$_2$−NH−SO$_2$, ^3J = 6.05 Hz), 8.43 (d, 1H, C$_\alpha$H−NH−CO, ^3J = 7.76 Hz), 9.25 (s, 3H, C−NH_2(=NH_2^+)), 9.47 (s, 3H, C−NH_2(=NH_2^+)); ^{13}C NMR δ 13.85 (OCH$_2$CH_3), 36.33 (Ar−CH$_2$−C$_\alpha$H), 44.66 (CO−CH_2−NH), 53.23 (C$_\alpha$H), 60.68 (OCH_2CH$_3$), 126.30 (Ar-C_4), 126.75 (Ar-$C_{2'/6'}$), 128.06 (Ar-C_2), 128.70 (Ar-C_5), 128.84 (Ar-C_3), 129.00 (Ar-$C_{3'/5'}$), 131.77 (Ar-$C_{4'}$), 134.52 (Ar-C_6), 138.00 (Ar-C_1), 144.92 (Ar-$C_{1'}$), 164.84 (C−NH$_2$(=NH$_2^+$)), 165.48 (C−NH$_2$(=NH$_2^+$)), 167.53 (NH−CO−CH$_2$), 170.80 (C$_\alpha$H-COOCH$_2$-CH$_3$). Anal. (C$_{21}$H$_{26}$N$_6$O$_5$S$_1$(CF$_3$CO$_2$H)$_2$) C, H, N.

Peak 2 corresponds to N^α-(4′-cyanobenzenesulfonyl)glycyl-D,L-3-amidinophenylalanine ethyl ester mono(trifluoroacetate) (**9b**): yield 66 mg (11%) of white powder; HPLC (conditions as for **8a**): t_R = 12.32 min; FAB-MS *m*/*z*: 458.4 [M + H$^+$]; M_r = 457.14 calcd for C$_{21}$H$_{23}$N$_5$O$_5$S; IR (KBr) ν 3488 (sh), 3335 (w, NH$_2$); 3087 (w, =CH); 2983 (sh), 2936 (sh), 2867 (sh, CH$_{2/3}$); 2236 (w, C≡N); 1741 (sh, C=O); 1673 (s, N−C=O); 1529 (m), 1481 (w, C=C); 1432 (w, δCH$_2$); 1383 (w, δCH$_3$); 1338 (m, ArSO$_2$N<); 1205 (s, C−O); 1164 (s, ArSO$_2$N<); ^{1}H NMR δ 1.11 (t, 3H, OCH$_2$CH_3, ^3J = 7.09 Hz), 2.95 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.79 Hz, ^3J = 8.68 Hz), 3.07 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.81 Hz, ^3J = 5.76 Hz), 3.54 (m, 2H, CO−CH_2−NH, ^3J = 6.02 Hz), 4.06 (q, 2H, OCH_2CH$_3$, ^3J = 7.10 Hz), 4.47 (m, 1H, C$_\alpha H$, ^3J = 8.36 Hz, ^3J = 8.00 Hz, ^3J = 5.84 Hz), 7.52 (t, 1H, Ar-H_5, ^3J = 7.42 Hz), 7.55 (m, 1H, Ar-H_4), 7.68 (m, 2H, Ar-H_2 and Ar-H_6), 7.90 (m, 2H, Ar-$H_{3'/5'}$, ^3J = 8.41 Hz, BB′), 8.02 (m, 2H, Ar-$H_{2'/6'}$, ^3J = 8.48 Hz, AA′), 8.29 (t, 1H, CH$_2$−NH−SO$_2$, ^3J = 6.10 Hz), 8.39 (d, 1H, C$_\alpha$H−NH−CO, ^3J = 7.82 Hz), 9.12 (s, 2H, C−NH_2(=NH_2^+)), 9.25 (s, 2H, C−NH_2(=NH_2^+)); ^{13}C NMR δ 13.85 (OCH$_2$CH_3), 36.34 (Ar−CH_2−C$_\alpha$H), 44.55 (CO−CH_2−NH), 53.16 (C$_\alpha$H), 60.66 (OCH_2CH$_3$), 114.69 (Ar-$C_{4'}$), 117.66 (C≡N), 126.31 (Ar-C_4), 127.22 (Ar-$C_{2'/6'}$), 128.02 (Ar-C_2), 128.68 (Ar-C_5), 128.83 (Ar-C_3), 133.08 (Ar-$C_{3'/5'}$), 134.52 (Ar-C_6), 137.97 (Ar-C_1), 144.62 (Ar-$C_{1'}$), 165.41 (C−NH$_2$(=NH$_2^+$)), 167.39 (NH−CO−CH$_2$), 170.74 (C$_\alpha$H-COOCH$_2$CH$_3$). Anal. (C$_{21}$H$_{23}$N$_5$O$_5$S(CF$_3$CO$_2$H)$_1$) H; C: calcd, 48.32; found, 45.87. N: calcd, 12.26; found, 11.56.

Peak 3 corresponds to N^α-(4′-thioamidobenzenesulfonyl)-glycyl-D,L-3-amidinophenylalanine ethyl ester mono(trifluoroacetate) (**10b**): 61 mg (10%) of white powder; HPLC (conditions as for **8a**): t_R = 12.02 min; FAB-MS *m*/*z*: 492.4 [M + H$^+$]; M_r = 491.13 calcd for C$_{21}$H$_{25}$N$_5$O$_6$S$_2$; IR (KBr) ν 3329 (w), 3202 (w, NH$_2$); 3098 (sh, =CH); 2983 (sh), 2936 (sh, CH$_{2/3}$); 1736 (sh, C=O); 1675 (s, N−C=O); 1531 (m), 1480 (w, C=C); 1425 (m, δCH$_2$); 1378 (w, δCH$_3$); 1334 (m, ArSO$_2$N<); 1204 (s, C−O); 1166 (s, ArSO$_2$N<); ^{1}H NMR δ 1.12 (t, 3H, OCH$_2$CH_3, ^3J = 7.03 Hz), 2.98 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.81 Hz, ^3J = 8.64 Hz), 3.09 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.83 Hz, ^3J = 5.72 Hz), 3.47 (m, 2H, CO−CH_2−NH, ^3J = 6.16 Hz), 4.06 (q, 2H, OCH_2CH$_3$, ^3J = 7.08 Hz), 4.52 (m, 1H, C$_\alpha H$, ^3J = 8.21 Hz, ^3J = 8.08 Hz, ^3J = 5.91 Hz), 7.52 (t, 1H, Ar-H_5, ^3J = 7.45 Hz), 7.55 (dvt, 1H, Ar-H_4, ^3J = 7.53 Hz, ^4J = 1.25 Hz), 7.67 (dvt, 1H, Ar-H_6, ^3J = 7.64 Hz, ^4J = 1.38 Hz), 7.69 (br s, 1H, Ar-H_2), 7.78 (d, 2H, Ar-$H_{3'/5'}$, ^3J = 8.46 Hz, BB′), 7.97 (d, 1H, Ar-$H_{2'/6'}$, ^3J = 8.45 Hz, AA′), 8.07 (t, 1H, CH$_2$−NH−SO$_2$, ^3J = 6.19 Hz), 8.36 (d, 1H, C$_\alpha$H−NH−CO, ^3J = 7.84 Hz), 9.07 (s, 2H, C−NH_2-(=NH_2^+)), 9.24 (s, 2H, C−NH_2(=NH_2^+)), 9.66 (s, 1H, C=S(−NH_2)), 10.07 (s, 1H, C=S(−NH_2)); ^{13}C NMR δ 13.86 (OCH$_2$CH_3), 36.36 (Ar−CH_2−C$_\alpha$H), 44.69 (CO−CH_2−NH), 53.16 (C$_\alpha$H), 60.69 (OCH_2CH$_3$), 126.11 (Ar-C_4), 126.31 (Ar-$C_{2'/6'}$), 127.69 (Ar-$C_{3'/5'}$), 128.03 (Ar-C_2), 128.71 (Ar-C_5), 128.82 (Ar-C_3), 134.55 (Ar-C_6), 137.98 (Ar-C_1), 142.08 (Ar-$C_{4'}$), 142.81 (Ar-$C_{1'}$), 165.42 (C−NH$_2$(=NH$_2^+$)), 167.53 (NH−CO−CH$_2$), 170.77 (C$_\alpha$H-COOCH$_2$CH$_3$), 198.81 (C=S(−NH$_2$)). Anal. (C$_{21}$H$_{25}$N$_5$O$_5$S$_2$(CF$_3$-CO$_2$H)$_1$) H; C: calcd, 45.61; found: 44.02. N: calcd, 11.57; found, 10.93.

***N*$^\alpha$-(4′-Cyanobenzenesulfonyl)glycyl-D,L-4-cyanophenylalanine (11a).** D,L-4-Cyanophenylalanine[19] (1.2 g, 6.31 mmol) was reacted with the active ester **3** (2.13 g, 6.31 mmol) in DMF with TEA (0.875 mL, 6.31 mmol) as auxiliary base. After 4 days the reaction mixture was evaporated, and the residue was distributed between AcOEt and 5% KHSO$_4$. The organic layer was dried over Na$_2$SO$_4$ and evaporated, and the residue was recrystallized from 2-propanol: yield 1.95 g (75%) of white powder; mp 202−203 °C; FAB-MS *m*/*z*: 413.1 [M + H$^+$]; M_r = 412.08 calcd for C$_{19}$H$_{16}$N$_4$O$_5$S; IR (KBr) ν 3386 (m), 3310 (m, NH); 3095 (w), 3046 (w, =CH); 2936 (w, CH$_2$); 2518 (w, OH); 2236 (m, C≡N); 1729 (s, C=O); 1619 (s, (NH); 1539 (s, C=C); 1407 (m, δCH$_2$); 1343 (m), 1166 (s, ArSO$_2$N<); ^{1}H NMR δ 2.92 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.78 Hz, ^3J = 8.96 Hz), 3.11 (dd, 1H, Ar−CH_2−C$_\alpha$H, ^2J = 13.82 Hz, ^3J = 4.98 Hz), 3.51 (dd, 2H, CO−CH_2−NH, ^2J = 16.70 Hz, ^3J = 5.45 Hz), 4.40 (dvt, 1H, C$_\alpha H$, ^3J = 8.51 Hz, ^3J = 5.01 Hz), 7.38 (d, 2H, Ar-$H_{3/5}$, ^3J = 8.15 Hz, BB′), 7.73 (d, 2H, Ar-$H_{2/6}$, ^3J = 8.16 Hz, AA′), 7.90 (d, 2H, Ar-$H_{3'/5'}$, ^3J = 8.39 Hz, BB′), 8.02 (d, 2H, Ar-$H_{2'/6'}$, ^3J = 8.40 Hz, AA′), 8.24 (d, 1H, C$_\alpha$H−NH−CO, ^3J = 8.11 Hz), 8.26 (t, 1H, CH$_2$−NH−SO$_2$, ^3J = 5.80 Hz), 12.91(s,

1H, $C_\alpha H-COOH$); ^{13}C NMR δ 36.66 (Ar-$CH_2-C_\alpha H$), 44.60 ($CO-CH_2-NH$), 52.71 ($C_\alpha H$), 109.32 (Ar-C_4), 114.68 (Ar-$C_{4'}$), 117.66 ($SO_2-Ar-C\equiv N$), 118.77 ($C\equiv N$), 127.21 (Ar-$C_{2'/6'}$), 130.17 (Ar-$C_{2/6}$), 131.93 (Ar-$C_{3/5}$), 133.05 (Ar-$C_{3'/5'}$), 143.32 (Ar-C_1), 144.64 (Ar-$C_{1'}$), 167.12 ($NH-CO-CH_2$), 171.99 ($C_\alpha H$-COOH).

***N*$^\alpha$-(4′-Cyanobenzenesulfonyl)glycyl-D,L-3-cyanophenylalanine (11b).** The title compound was prepared from D,L-3-cyanophenylalanine[19] as described for **11a**: yield 69% of white powder; mp 181−183 °C; FAB-MS *m/z*: 413.1 [M + H$^+$]; M_r = 412.08 calcd for $C_{19}H_{16}N_4O_5S$; IR (KBr) ν 3373 (s), 3198 (s, NH); 3094 (w, =CH); 2971 (sh), 2927 (sh, CH_2); 2234 (m, C≡N); 1718 (s), 1679 (s, C=O); 1551 (m), 1487 (w, C=C); 1450 (w), 1417 (m, δCH_2); 1363 (m), 1169 (s, $ArSO_2N<$); ^1H NMR δ 2.90 (dd, 1H, Ar−$CH_2-C_\alpha H$, ^2J = 13.96 Hz, ^3J = 8.82 Hz), 3.09 (dd, 1H, Ar−$CH_2-C_\alpha H$, ^2J = 13.91 Hz, ^3J = 5.06 Hz), 3.52 (dd, 2H, CO−CH_2−NH, ^2J = 16.74 Hz, ^3J = 6.11 Hz), 4.41 (dvt, 1H, $C_\alpha H$, ^3J = 8.46 Hz, ^3J = 4.84 Hz), 7.48 (t, 1H, Ar-H_5, ^3J = 7.58 Hz), 7.53 (dvt, 1H, Ar-H_4, ^3J = 7.85 Hz, ^4J = 1.35 Hz), 7.63 (s, 1H, Ar-H_2), 7.68 (dvt, 1H, Ar-H_6, ^3J = 7.62 Hz, ^4J = 1.52 Hz), 7.90 (d, 2H, Ar-$H_{3'/5'}$, ^3J = 8.39 Hz, BB′), 8.02 (d, 2H, Ar-$H_{2'/6'}$, ^3J = 8.35 Hz, AA′), 8.23 (d, 1H, $C_\alpha H-NH-CO$, ^3J = 8.21 Hz), 8.26 (t, 1H, $CH_2-NH-SO_2$, ^3J = 6.11 Hz), 12.86 (s, 1H, $C_\alpha H-COOH$); ^{13}C NMR δ 36.07 (Ar−$CH_2-C_\alpha H$), 44.59 ($CO-CH_2-NH$), 52.78 ($C_\alpha H$), 111.09 (Ar-C_4), 114.69 (Ar-$C_{4'}$), 117.67 ($SO_2-Ar-C\equiv N$), 118.73 ($C\equiv N$), 127.22 (Ar-$C_{2'/6'}$), 129.28 (Ar-C_5), 130.30 (Ar-C_4), 132.65 (Ar-C_2), 133.05 (Ar-$C_{3'/5'}$), 134.13 (Ar-C_6), 138.91 (Ar-C_1), 144.65 (Ar-$C_{1'}$), 167.16 ($NH-CO-CH_2$), 172.02 ($C_\alpha H$-COOH).

***N*$^\alpha$-(4′-Thioamidobenzenesulfonyl)glycyl-D,L-4-thioamidophenylalanine (12a).** Compound **11a** was reacted with hydrogen sulfide and worked up as described for **6a**: yield 2.1 g (98%) of yellow foam; HPLC (isocratic elution with A for 5 min; linear gradient from A to B in 40 min): t_R = 23.52 min; FAB-MS *m/z*: 481.3 [M + H$^+$]; M_r = 480.06 calcd for $C_{19}H_{20}N_4O_5S_3$; IR (KBr) ν 3315 (s), 3188 (s, NH_2); 3064 (sh, =CH); 2938 (sh, $CH_{2/3}$); 1726 (w, C=O); 1659 (w, N−C=O); 1630 (s, δNH_2); 1547 (w), 1531 (m, C=C); 1423 (m, δCH_2); 1328 (m), 1163 (s, $ArSO_2N<$); ^1H NMR δ 2.91 (dd, 1H, Ar−$CH_2-C_\alpha H$, ^2J = 13.80 Hz, ^3J = 8.50 Hz), 3.06 (dd, 1H, Ar−$CH_2-C_\alpha H$, ^2J = 13.83 Hz, ^3J = 5.20 Hz), 3.57 (dd, 2H, CO−CH_2−NH, ^2J = 16.56 Hz, ^3J = 6.23 Hz), 4.43 (dvt, 1H, $C_\alpha H$, ^3J = 8.10 Hz, ^3J = 5.43 Hz), 7.22 (d, 2H, Ar-$H_{3/5}$, ^3J = 8.30 Hz, BB′), 7.79 (d, 2H, Ar-$H_{3'/5'}$, ^3J = 8.46 Hz, BB′), 7.82 (d, 2H, Ar-$H_{2/6}$, ^3J = 8.30 Hz, AA′), 7.96 (d, 2H, Ar-$H_{2'/6'}$, ^3J = 8.45 Hz, AA′), 8.06 (t, 1H, $CH_2-NH-SO_2$, ^3J = 6.16 Hz), 8.17 (d, 1H, $C_\alpha H-NH-CO$, ^3J = 7.94 Hz), 9.40 (s, 1H, C=S(−NH_2)), 9.65 (s, 1H, C=S(−NH_2)), 9.75 (s, 1H, C=S(−NH_2)), 10.06 (s, 1H, C=S(−NH_2)), 12.82 (s, 1H, $C_\alpha H-COOH$); ^{13}C NMR δ 36.39 (Ar−$CH_2-C_\alpha H$), 44.79 ($CO-CH_2-NH$), 52.98 ($C_\alpha H$), 126.14 (Ar-$C_{2'/6'}$), 127.21 (Ar-$C_{2/6}$), 127.69 (Ar-$C_{3'/5'}$), 128.55 (Ar-$C_{3/5}$), 137.45 (Ar-C_4), 140.70 (Ar-$C_{4'}$), 142.09 (Ar-C_1), 142.89 (Ar-$C_{1'}$), 167.29 ($NH-CO-CH_2$), 172.21 ($C_\alpha H$-COOH), 198.90 (C=S(−NH_2)), 199.59 (C=S(−NH_2)).

***N*$^\alpha$-(4′-Thioamidobenzenesulfonyl)glycyl-D,L-3-thioamidophenylalanine (12b).** The title compound was prepared from **11b** as described for **6a**: yield 98% of yellow foam; HPLC (conditions as for **12a**): t_R = 23.82 min; FAB-MS *m/z*: 481.3 [M + H$^+$]; M_r = 480.06 calcd for $C_{19}H_{20}N_4O_5S_3$; IR (KBr) ν 3314 (s), 3192 (s, NH_2); 3051 (sh, =CH); 2938 (sh, $CH_{2/3}$); 1725 (m, C=O); 1665 (sh, N−C=O); 1630 (s, δNH_2); 1535 (m), 1486 (w, C=C); 1414 (m, δCH_2); 1329 (s), 1163 (s, $ArSO_2N<$); ^1H NMR δ 2.90 (dd, 1H, Ar−$CH_2-C_\alpha H$, ^2J = 13.88 Hz, ^3J = 8.51 Hz), 3.06 (dd, 1H, Ar−$CH_2-C_\alpha H$, ^2J = 13.83 Hz, ^3J = 5.21 Hz), 3.48 (dd, 2H, CO−CH_2−NH, ^2J = 16.45 Hz, ^3J = 6.17 Hz), 4.43 (dvt, 1H, $C_\alpha H$, ^3J = 8.10 Hz, ^3J = 5.21 Hz), 7.29 (s, 1H, Ar-H_5, ^3J = 7.65 Hz), 7.30−7.32 (m, 1H, Ar-H_4), 7.70−7.73 (m, 1H, Ar-H_6), 7.74 (s, 1H, Ar-H_2), 7.79 (d, 2H, Ar-$H_{3'/5'}$, ^3J = 8.37 Hz, BB′), 7.96 (d, 2H, Ar-$H_{2'/6'}$, ^3J = 8.42 Hz, AA′), 8.02 (t, 1H, $CH_2-NH-SO_2$, ^3J = 6.17 Hz), 8.18 (d, 1H, $C_\alpha H-NH-CO$, ^3J = 7.96 Hz), 9.39 (s, 1H, C=S(−NH_2)), 9.64 (s, 1H, C=S(−NH_2)), 9.79 (s, 1H, C=S(−NH_2)), 10.06 (s, 1H, C=S(−NH_2)), 12.80 (s, 1H, $C_\alpha H-COOH$); ^{13}C NMR δ 36.58 (Ar−$CH_2-C_\alpha H$), 44.78 ($CO-CH_2-NH$), 53.21 ($C_\alpha H$), 125.44 (Ar-C_4), 126.14 (Ar-$C_{3'/5'}$), 127.67 (Ar-$C_{2'/6'}$ and Ar-C_2), 128.09 (Ar-C_5), 131.80 (Ar-C_6), 136.93 (Ar-C_1), 139.40 (Ar-C_3), 142.09 (Ar-$C_{4'}$), 142.87 (Ar-$C_{1'}$), 167.26 ($NH-CO-CH_2$), 172.25 ($C_\alpha H$-COOH), 198.91 (C=S(−NH_2)), 200.09 (C=S(−NH_2)).

***N*$^\alpha$-(4′-Methylthioimido-benzenesulfonyl)glycyl-D,L-4-methylthioimido-phenylalanine Bis(hydroiodide) (13a).** The compound was prepared from **12a** as described for **7a**: yield 92% of yellow foam; HPLC (conditions as for **12a**): t_R = 21.07 min; FAB-MS *m/z*: 509.2 [M + H$^+$]; M_r = 508.09 calcd for $C_{21}H_{24}N_4O_5S_3$; ^1H NMR δ 2.81 (s, 3H, −SCH_3), 2.82 (s, 3H, −SCH_3), 2.98 (dd, 1H, Ar−$CH_2-C_\alpha H$, ^2J = 13.81 Hz, ^3J = 9.10 Hz), 3.16 (dd, 1H, Ar−$CH_2-C_\alpha H$, ^2J = 13.90 Hz, ^3J = 5.10 Hz), 3.51 (dd, 2H, CO−CH_2−NH, ^3J = 6.11 Hz), 4.46 (dvt, 1H, $C_\alpha H$, ^3J = 8.57 Hz, ^3J = 8.27 Hz, ^3J = 5.35 Hz), 7.47 (d, 2H, Ar-$H_{3/5}$, ^3J = 8.38 Hz, BB′), 7.79 (d, 2H, Ar-$H_{2/6}$, ^3J = 8.33 Hz, AA′), 7.92 (d, 2H, Ar-$H_{3'/5'}$, ^3J = 8.22 Hz, BB′), 7.97 (d, 2H, Ar-$H_{2'/6'}$, ^3J = 8.64 Hz, AA′), 8.20 (t, 1H, $CH_2-NH-SO_2$, ^3J = 6.16 Hz), 8.29 (d, 1H, $C_\alpha H-NH-CO$, ^3J = 8.04 Hz).

***N*$^\alpha$-(4′-Methylthioimido-benzenesulfonyl)glycyl-D,L-3-methylthioimido-phenylalanine Bis(hydroiodide) (13b).** Prepared as described for **13a**: yield 94% of yellow foam; HPLC (conditions as for **12a**): t_R = 21.22 min; FAB-MS *m/z*: 509.2 [M + H$^+$]; M_r = 508.09 calcd for $C_{21}H_{24}N_4O_5S_3$; ^1H NMR δ 2.75 (s, 3H, −SCH_3), 2.83 (s, 3H, −SCH_3), 2.97 (dd, 1H, Ar−$CH_2-C_\alpha H$, ^2J = 13.78 Hz, ^3J = 8.98 Hz), 3.15 (dd, 1H, Ar−$CH_2-C_\alpha H$, ^2J = 13.80 Hz, ^3J = 4.91 Hz), 3.52 (dd, 2H, CO−CH_2−NH, ^3J = 6.08 Hz), 4.45 (dvt, 1H, $C_\alpha H$, ^3J = 8.50 Hz, ^3J = 8.42 Hz, ^3J = 5.07 Hz), 7.56 (t, 1H, Ar-H_5, ^3J = 7.66 Hz), 7.63 (dvt, 1H, Ar-H_4, ^3J = 7.68 Hz), 7.71 (s, 1H, Ar-H_2), 7.72 (dvt, 1H, Ar-H_6, ^3J = 7.46 Hz), 7.93 (d, 2H, Ar-$H_{3'/5'}$, ^3J = 8.49 Hz, BB′), 7.98 (d, 2H, Ar-$H_{2'/6'}$, ^3J = 8.68 Hz, AA′), 8.23 (t, 1H, $CH_2-NH-SO_2$, ^3J = 6.12 Hz), 8.27 (d, 1H, $C_\alpha H-NH-CO$, ^3J = 8.09 Hz).

***N*$^\alpha$-(4′-Amidinobenzenesulfonyl)glycyl-D,L-4-amidinophenylalanine Bis(trifluoroacetate) (14a).** Aminolysis of **13a** and subsequent reversed-phase chromatography on Lichroprep RP-18 were carried out as reported for **8a**. Fractions of the main peak were collected, evaporated, and dried over KOH pellets: yield 35 mg (40%) of white powder; HPLC (conditions as for **8a**): t_R = 5.40 min; FAB-MS *m/z*: 447.1 [M + H$^+$]; M_r = 446.14 calcd for $C_{19}H_{22}N_6O_5S$; ^1H NMR δ 2.98 (dd, 1H, Ar−$CH_2-C_\alpha H$, ^2J = 13.95 Hz, ^3J = 8.92 Hz), 3.16 (dd, 1H, Ar−$CH_2-C_\alpha H$, ^2J = 13.88 Hz, ^3J = 5.12 Hz), 3.51 (dd, 2H, CO−CH_2−NH, ^3J = 5.77 Hz), 4.45 (dvt, 1H, $C_\alpha H$, ^3J = 8.34 Hz, ^3J = 8.20 Hz, ^3J = 5.32 Hz), 7.45 (d, 2H, Ar-$H_{3/5}$, ^3J = 8.25 Hz, BB′), 7.74 (d, 2H, Ar-$H_{2/6}$, ^3J = 8.42 Hz, AA′), 7.95 (d, 2H, Ar-$H_{3'/5'}$, ^3J = 8.84 Hz, BB′), 7.98 (d, 2H, Ar-$H_{2'/6'}$, ^3J = 8.79 Hz, AA′), 8.26 (t, 1H, $CH_2-NH-SO_2$, ^3J = 5.98 Hz), 8.30 (d, 1H, $C_\alpha H-NH-CO$, ^3J = 8.13 Hz), 9.08 (s, 2H, C−NH_2(=NH_2^+)), 9.24 (s, 2H, C−NH_2(=NH_2^+)), 9.36 (s, 2H, C−NH_2(=NH_2^+)), 9.45 (s, 2H, C−NH_2(=NH_2^+)), 12.91 (br s, 1H, $C_\alpha H-COOH$); ^{13}C NMR δ 36.56 (Ar−$CH_2-C_\alpha H$), 44.84 ($CO-CH_2-NH$), 53.06 ($C_\alpha H$), 126.19 (Ar-C_4), 126.88 (Ar-$C_{2'/6'}$), 127.96 (Ar-$C_{3/5}$), 129.11 (Ar-$C_{3'/5'}$), 129.74 (Ar-$C_{2/6}$), 131.88 (Ar-$C_{4'}$), 144.01 (Ar-C_1), 144.91 (Ar-$C_{1'}$), 164.91 ($C-NH_2$(=NH_2^+)), 165.33 ($C-NH_2$(=NH_2^+)), 167.42 ($NH-CO-CH_2$), 172.23 ($C_\alpha H$-COOH). Anal. ($C_{19}H_{22}N_6O_5S(CF_3CO_2H)_2$) H, N; C: calcd, 40.94; found, 40.04.

***N*$^\alpha$-(4′-Amidinobenzenesulfonyl)glycyl-D,L-3-amidinophenylalanine Bis(trifluoroacetate) (14b).** The title compound was prepared and purified as described for **14a**: yield 25% of white powder; HPLC (conditions as for **8a**): t_R = 6.80 min; FAB-MS *m/z*: 447.2 [M + H$^+$]; M_r = 446.14 calcd for $C_{19}H_{22}N_6O_5S$; ^1H NMR δ 2.98 (dd, 1H, Ar−$CH_2-C_\alpha H$, ^2J = 13.68 Hz, ^3J = 8.33 Hz), 3.13 (dd, 1H, Ar−$CH_2-C_\alpha H$, ^2J = 13.73 Hz, ^3J = 5.13 Hz), 3.51 (m, 2H, CO−CH_2−NH), 4.44 (dvt, 1H, $C_\alpha H$, ^3J = 7.57 Hz, ^3J = 5.13 Hz), 7.51 (t, 1H, Ar-H_5, ^3J = 7.48 Hz), 7.53 (dvt, 1H, Ar-H_4, ^3J = 7.37 Hz), 7.66 (dvt, 1H, Ar-H_6, ^3J = 7.78 Hz), 7.68 (br s, 1H, Ar-H_2), 7.96 (s, 4H, Ar-$H_{2'/3'/5'/6'}$), 8.17 (br s, 1H, $CH_2-NH-SO_2$), 8.27 (br s, 1H, $C_\alpha H-NH-CO$), 9.21 (s, 4H, C−NH_2(=NH_2^+)), 9.43 (s, 4H, C−NH_2(=NH_2^+)), 12.86 (br s, 1H, $C_\alpha H-COOH$); ^{13}C NMR δ 36.25 (Ar−$CH_2-C_\alpha H$), 44.70 ($CO-CH_2-NH$), 51.93 (OCH_3), 53.16 ($C_\alpha H$), 126.32 (Ar-C_4), 127.24 (Ar-$C_{2'/6'}$), 127.74 (Ar-C_2), 128.69 (Ar-C_5), 128.84 (Ar-C_3), 129.10 (Ar-$C_{3'/5'}$), 131.96 (Ar-$C_{4'}$), 134.46

(Ar-C_6), 138.12 (Ar-C_1), 142.77 (Ar-$C_{1'}$), 164.69 ($C-NH_2(=NH_2^+)$), 165.61 ($C-NH_2(=NH_2^+)$), 167.85 ($NH-CO-CH_2$), 171.40 (C_αH-COOH). Anal. ($C_{19}H_{22}N_6O_5S(CF_3CO_2H)_2$) H, N; C: calcd, 40.94; found: 40.09.

Determination of Inhibition Constants. The measurements were carried out on a microplate reader (MR 5000, Dynatech, Denkendorf, Germany) at 25 °C. The test medium consisted of 200 μL Tris buffer (0.05 M; 0.154 M NaCl, 5% ethanol, pH 8.0), 25 μL aqueous substrate solution, and 50 μL enzyme solution. Two concentrations of the substrate and five concentrations of the inhibitor were used; 3 min after the addition of the enzyme 25 μL acetic acid (50%) was added to quench the reaction and the optical density was measured at 405 nm. The K_i values were calculated according to Dixon[39] using a linear regression program. In case of tryptase determination of inhibition constants was not possible because of nonlinear Dixon kinetics as described previously.[40] Therefore, inhibitory potency was evaluated by determination of the inhibitor concentration required to reduce the enzyme activity by 50% (IC_{50}). The K_i and IC_{50} values reported are means from at least three determinations.

Enzymes and Substrates for K_i Determination. The following enzymes and the respective substrates were used at the final concentrations indicated: bovine thrombin prepared according to Walsmann[41] (2262 U/mg, final concentration 0.45 U/mL), substrate $MeSO_2$-D-hexahydrotyrosyl-Gly-Arg-pNA (final concentrations 0.18 and 0.09 mM); bovine fXa (5 U/vial, 0.11 U/mL; Diagnostic Reagents Ltd., Thame, U.K.), human fXa (4 mg/vial, 0.18 μg/mL; Kordia Lab. Supplies, Leiden, The Netherlands), substrate $MeSO_2$-D-Nle-Gly-Arg-pNA (0.36 and 0.18 mM); human APC (1 mg/vial, 1.13 μg/mL; Kordia Lab. Supplies, Leiden, The Netherlands), substrate H-D-Lys(Cbz)-Pro-Arg-pNA (0.36 and 0.18 mM); human PK (1 mg/vial, 4 μg/mL; Kordia Lab. Supplies, Leiden, The Netherlands), substrate Bz-Pro-Phe-Arg-pNA (0.36 and 0.18 mM); human plasmin (0.67 CTA-U/mg, 0.06 CTA-U/mL; Behringwerke AG, Marburg, Germany), substrate Tos-Gly-Pro-Lys-pNA (0.18 and 0.09 mM); human uPA (500 000 U/vial, final concentration 150 U/mL; Ribosepharm GmbH, Haan, Germany), substrate Bz-βAla-Gly-Arg-pNA (0.18 and 0.09 mM); sc-tPA purified from CHO cells[42] (4.1 mg/mL, 0.0031 μg/mL), substrate $MeSO_2$-D-hexahydrotyrosyl-Gly-Arg-pNA (0.54, 0.27, and 0.145 mM); bovine pancreatic trypsin (42 U/mg, 0.0038 U/mL; Serva, Heidelberg, Germany), substrate $MeSO_2$-D-hexahydrotyrosyl-Gly-Arg-pNA (0.18 and 0.06 mM), human lung tryptase prepared according to ref 43 (8 μM, 2.4 nM), substrate Tos-Gly-Pro-Arg-pNA (0.18 mM). The substrates were supplied by Pentapharm Ltd., Basel, Switzerland.

In Vivo Elimination Study. NAPAP and compounds **14a**,**b** were administered iv to anesthetized, bile-duct-cannulated (urethane, 1.5 g/kg ip) rats in aqueous solution at doses of 1 mg/kg. Citrated blood was drawn at various time intervals after administration from the cannulated carotid artery; bile was collected fractionally. Blood plasma samples were applied on Chromabond C18 solid-phase exctraction columns (Macherey-Nagel, Düren, Germany). The concentrations of the inhibitors in the plasma samples were measured by HPLC using Nucleosil 7 C18 columns (Macherey-Nagel, Düren, Germany) using CH_3CN/water/1 M perchloric acid (15:70:0.04; flow rate, 1 mL/min) as eluent; NAPAP was detected by fluorescence (λ_{ex} = 232 nm, λ_{em} = 343 nm); the compounds **14a**,**b** were detected by UV (238 nm) measurements.

X-ray Structure Analysis. Crystals of bovine β-trypsin were grown from small seeds of the "open" crystal form in 1.7−1.8 M ammonium sulfate, pH 6, and were soaked for 2 days in 2 mM inhibitor (**8a**,**b** and **10b**) solution, 2.5 M ammonium sulfate, pH 8.0, as described.[12] The crystals had *P*212121 symmetry, with cell dimensions *a*,*b*,*c* = 63.55, 69.19, and 63.84 Å for the trypsin/**8a**, *a*,*b*,*c* = 63.40, 69.08, and 63.77 Å for the trypsin/**8b**, and *a*,*b*,*c* = 63.40, 69.40, and 63.90 Å for the trypsin/**10b**. Data to 1.9 Å were collected on a Centronix area detector (Siemens) mounted on a Rigaku rotating anode X-ray generator and processed using XDS.[44] A total of 21 787 unique reflections (95.9% completeness) for trypsin/**8a**, 21 347 (completeness 94.0%) for trypsin/**8b**, and 18 573 (81.3% completeness) for trypsin/**10b** were collected. The corresponding *R* merges were 3.6% (11.5% for the 2.00−1.90 Å resolution shell), 4.2% (17.2%), and 5.5% (24.3%), respectively.

Conventional crystallographic rigid body, positional, and temperature factor refinements were carried out with XPLOR[45] using the β-trypsin structure (PDB, Brookhaven; accession code 1MTS) as starting model. Model building was carried out with TURBO-FRODO.[46] The structure was refined to a *R* factor of 18.7% (free *R* factor 23.6%) with rms deviations from target values of 0.009 Å for bonds and 1.952° for angles. The rms deviation of *B* factors of bonded atoms was 2.23 Å^2.

Modeling experiments on fXa were performed using the coordinates of des-Gla-fXa[11] (PDB entry 1FAX) and Insight II (version 95.0; MSI Technologies, San Diego, CA).

Acknowledgment. The authors thank Inge Buhrow for the mass spectra; Christa Böttner for the enzyme kinetics; Stella Fiori, Dr. Sabine Rudolph-Böhner, Dr. Michaela Schenk, and Dr. Isolde Sonnenbichler for the NMR measurements; Regina Scharf and Petra Schwille for technical assistance; and Günter Wünsche for elemental analyses. This study was supported by Grants MO 377/8-1, BO 1279/2-1, and STU 161/1-1 of the Deutsche Forschungsgemeinschaft.

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JM980227T