Low Molecular Weight, Non-Peptide Fibrinogen Receptor Antagonists

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The tetrapeptide H-Arg-Gly-Asp-Ser-OH (1) (RGDS), representing a recognition sequence of fibrinogen for its platelet receptor GP IIb-IIIa (integrin $\alpha_{IIb}\beta_3$), served as lead compound for the development of highly potent and selective fibrinogen receptor antagonists. Replacement of the N-terminal arginine by *p*-amidinophenylalanine or the Gly moiety by *m*-aminobenzoic acid led to compounds which are superior to the lead peptide with regard to activity and selectivity for GP IIb-IIIa vs the closely related vitronectin receptor $\alpha_v\beta_3$. By random screening [(*p*-amidinobenzenesulfonamido)ethyl]-*p*-phenoxyacetic acid derivatives have been identified as fibrinogen receptor antagonists. Further structure–activity relationship studies culminated in the preparation of *N*-[*N*-[*N*-(*p*-amidinobenzoyl)-β-alanyl]-L-α-aspartyl]-3-phenyl-L-alanine (**29h**, Ro 43-5054) and [[1-[*N*-(*p*-amidinobenzoyl)-L-tyrosyl]-4-piperidinyl]oxy]acetic acid (**37f**, Ro 44-9883), which exhibit very high activity as platelet aggregation inhibitors (IC_{50}s 0.06 and 0.03 μM, respectively, human PRP/ADP) as well as marked selectivity for GP IIb-IIIa vs $\alpha_v\beta_3$. Since the activity of **37f** in dogs declines according to a two-compartment model with an initial phase having a $t_{1/2}$ of 8 min and a second phase with a $t_{1/2}$ of 110 min, this compound is a suitable candidate for the development as iv platelet inhibitor.

Introduction

Adhesion of platelets to the subendothelium of damaged vessel walls as well as platelet aggregation are critical events in hemostasis and arterial thrombosis.[1] The uncontrolled deposition of platelets on thrombogenic surfaces such as ruptured atherosclerotic plaques followed by formation of larger aggregates may provoke acute vascular occlusion resulting in myocardial infarction, unstable angina, transient ischemic attacks, or stroke.[2] While the interaction of tissue-bound von Willebrand factor with its platelet receptor, glycoprotein Ib-IX (GP Ib-IX), initiates the adherence of platelets to surfaces lacking an intact endothelial lining,[3] the binding of fibrinogen to its platelet receptor, glycoprotein IIb-IIIa complex (GP IIb-IIIa or $\alpha_{IIb}\beta_3$), was shown to be essential for thrombus growth.[4] A plethora of agonists including adenosine diphosphate (ADP), collagen, epinephrine, thromboxane A_2, platelet-activating factor (PAF), serotonin, or thrombin might be involved in the physiological process of platelet stimulation leading finally to the functionalization of GP IIb-IIIa and to platelet aggregation.[5,6] Antiplatelet agents inhibiting only one single agonistic pathway exhibit clear limitations.[7] Therefore, the blockade of GP IIb-IIIa receptors might constitute a superior approach in effectively preventing arterial thrombus formation.[7]

Like other members of the integrin family, GP IIb-IIIa embodies a recognition site for the peptide sequence Arg-Gly-Asp (RGD).[8-10] Besides several monoclonal antibodies,[11] polypeptides having an RGD sequence isolated from either snake venoms[12] or leeches[13] are able to block the platelet fibrinogen receptor. In addition, small linear and cyclic peptides containing either an RGD sequence[14-20] or

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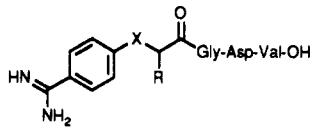
Table I. *p*-Amidinophenylalanine Derivatives and Related Compounds

no.	compound	platelet aggregation in h-PRP:[a] IC_{50} (μM) ADP	Coll	Thr	solid-phase assay GP IIb-IIIa–FG:[b] IC_{50comp}/IC_{50RGDS}	solid-phase assay $\alpha_v\beta_3$–FG:[b] IC_{50comp}/IC_{50RGDS}
1	H-Arg-Gly-Asp-Ser-OH	88	142	135	1[c]	1[d]
2	H-Arg-Gly-Asp-Phe-OH	19	38	21	0.3	3.5
3	H-Arg-Gly-Asp-Val-OH	127	151	98	0.63	0.6
						
7a	X = CH_2, R = (*R*,*S*)-NH_2	2.2	2.8	2.5	0.01	650
7b	X = CH_2, R = H	2.6	2.3	2.4	0.001	530
7c	X = O, R = H	0.7	0.6	0.5	0.02	700
7d	X = –, R = H	0.3	0.5	0.3	0.001	560

[a] Inhibition of platelet aggregation was determined in platelet rich plasma of at least four donors. In the examples with more than 10 donors tested (*n* up to 40), the SD was always lower than ±40% of the mean. Therefore it is expected that the IC_{50}s given in the tables have a SEM of lower than ±20%. [b] IC_{50}s were calculated with a log linear regression analysis. The mean value of at least two independent experiments is shown. In examples with more than five independent experiments the SD was always lower than ±75% of the mean. [c] The reference compound 1 (RGDS) showed an IC_{50} value of 3.6 ± 0.4 μM (*n* = 165, mean ± SEM). [d] Compound 1 showed an IC_{50} value of 9.4 ± 1.8 nM (*n* = 9, mean ± SEM).

the carboxy-terminal sequence of the fibrinogen gamma chain[21,22] have been shown to inhibit fibrinogen binding to GP IIb-IIIa and thereby to prevent platelet aggregation. So far only little information about peptide mimetics interfering with fibrinogen binding to GP IIb-IIIa has been published.[23]

Our goal was to find molecules which (i) are as potent as the snake venom polypeptides but significantly smaller in size, (ii) have shorter plasma half-lives than monoclonal antibodies,[11] and (iii) display selectivity for the fibrinogen receptor GP IIb-IIIa versus the vitronectin receptor $\alpha_v\beta_3$ in the same order as the snake venom polypeptide barbourin.[24]

Initial structure–activity relationship studies with tetrapeptides of the type RGDX (X = Ser (1), Phe (2), Val (3)) led to similar conclusions as already disclosed by others.[14–20] In this paper we report on the synthesis and the biological properties of related compounds in which (i) the N-terminal arginine was replaced by *p*-amidinophenylalanine and (ii) the backbone was modified by incorporation of a *m*-aminobenzoic acid building block. In addition, *p*-amidinobenzoyl derivatives of *p*-(2-aminoethyl)phenoxyacetic acids have been discovered by random screening as a new class of potent, non-peptide antagonists of the fibrinogen receptor. Furthermore, the combination of beneficial structural features of the different classes of compounds resulted in the identification of potent, small hybrid molecules with high GP IIb-IIIa specificity like the (piperidinyloxy)acetic acid derivatives.

Chemistry

All target compounds mentioned in Tables I–V were prepared according to Schemes I–V.

Coupling of the protected tripeptide intermediate **4**[25] (Scheme I) with either racemic *N*-Boc-*p*-cyanophenylalanine (**5a**),[26] *p*-cyanohydrocinnamic acid (**5b**),[27] 4-cyanophenoxyacetic acid (**5c**),[28] or 4-cyanophenylacetic acid (**5d**)[29] led to the corresponding intermediates **6a–d**. A known[26] three-step protocol was used for the conversion of the cyano group to the amidino group. Deprotection finally led to the target compounds **7a–d**.

The preparation of the *p*-(2-aminoethyl)phenoxyacetic acid derivatives is outlined in Scheme II. The amino esters **8a–c** were sulfonated or acylated to the corresponding nitriles **9a–e**. Acylation of **12** with either 4-cyanobenzoyl

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(28) Hatano, K.; Matsui, M. Synthesis of 2,4-Dicyanophenoxyacetic Acid and its Analogues. *Agric. Biol. Chem.* **1973**, *37*, 2917–2919.

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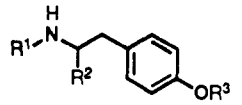
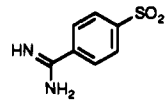
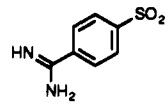
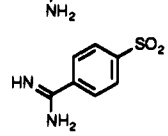
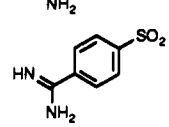
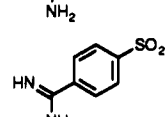
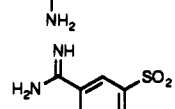
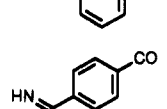
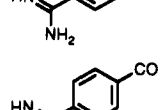
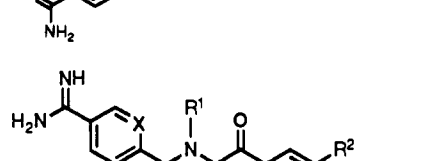
Table II. (2-Aminoethyl)phenoxyacetic Acid Derivatives



no.	R^1	R^2	R^3	platelet aggregation in h-PRP:[a] IC_{50} (μM) ADP	Coll	Thr	solid-phase assay GP IIb-IIIa–FG:[b] IC_{50comp}/IC_{50RGDS}	solid-phase assay $\alpha_v\beta_3$–FG:[b] IC_{50comp}/IC_{50RGDS}
10a		(*S*)-CH_3	CH_2COOCH_3	9.8	11	9.6	2.8	nt[c]
11a		(*S*)-CH_3	CH_2COOH	7.4	11	9.3	0.34	1700
11b		(*R*)-CH_3	CH_2COOH	115	102	103	10.5	>30000
11c		H	CH_2COOH	15	12	12	0.2	nt[c]
18		H	$C(CH_3)_2COOH$	>200	>200	>200	>100	nt[c]
11d		H	CH_2COOH	>200	>200	>200	24	nt[c]
10e		H	CH_2COOCH_3	0.9	0.3	0.5	0.45	2300
11e		H	CH_2COOH	0.8	0.3	0.4	0.028	1300



	X	R^1	R^2					
15a	CH	H	H	0.09	0.13	0.09	0.004	8400
15b	N	H	H	0.03	0.02	0.04	0.001	>20000
21	CH	CH_3	OCH_2COOH	0.07	0.07	0.06	0.0001	37000

[a,b] See Table I. [c] nt = not tested.

chloride or 5-cyano-2-pyridinecarboxylic acid, respectively, followed by oxidation with manganese dioxide provided the ketones **13a,b**. The standard sequence described above was used to produce the amidino esters **10a–e** and **14a,b** which could be hydrolyzed to the amidino acids **11a–e** and **15a,b** mentioned in Table II. A slightly modified sequence, sulfonation or acylation followed by etherification, was applied for the synthesis of **17**, **18** and **20**, **21** respectively.

Mixed anhydride coupling of the protected *m*-aminobenzoic acid derivative **22a** (Scheme III) with H-Asp(*O*-Bzl)-Val-*O*-Bzl[25] followed by standard deprotection/acylation gave the arginine derivative **23c**. Activation of **22b** with CDMT[30] followed by reaction with β-alanine benzyl ester yielded **25a**, which was deprotected to the desired *p*-(aminomethyl)benzamide **25b**. The β-alanine derivative **24b** served as starting material in the coupling reactions leading to the intermediates **25c** and **27a**. These in turn were subjected to catalytic hydrogenation giving the corresponding target compounds **25d** and **27d**, respectively. From the coupling reaction of **24b** with *N*-Z-*p*-cyanophenylalanine[26] the corresponding nitrile **26a** was obtained. Transformation of the cyano group to the amidino group was carried out in the usual way to give, after deprotection, the pseudopeptide **26c**.

As illustrated in Scheme IV, standard acylation of the peptide derivatives **28b** and **28d** with *p*-cyanobenzoic acid led to the intermediates **29a** and **29d**, which were converted to the corresponding amidinobenzoyl pseudopeptides **29c** and **29f**, respectively. The synthesis of the *p*-amidinobenzamides **29h**, **29k**, and **29m** starting with **28e**, **28g**, and **28h** has been accomplished by use of *p*-amidinobenzoyl

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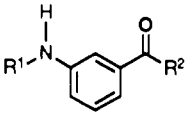
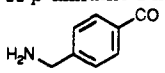
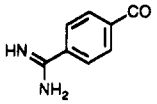
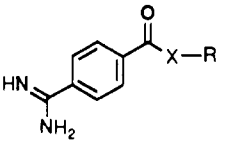
Table III. *m*-Aminobenzoic Acid Derivatives

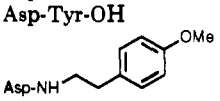


	R¹	R²	platelet aggregation in h-PRP:[a] IC_{50} (μM) ADP	Coll	Thr	solid-phase assay GP IIb-IIIa–FG:[b] IC_{50comp}/IC_{50RGDS}	solid-phase assay $\alpha_v\beta_3$–FG:[b] IC_{50comp}/IC_{50RGDS}
23c	Boc-Arg	Asp-Val-OH	4.9	7.0	4.4	0.06	250
25d	H-Arg	β-Ala-OH	51	50	68	1.3	900
26c	H-*p*-amidino-Phe (*R*/*S*)	β-Ala-OH	95	153	95	10	2500
25b		β-Ala-OH	8.7	9.6	6.8	0.1	26000
27b		β-Ala-OH	0.2	0.4	0.5	0.0005	8000

[a,b] See Table I.

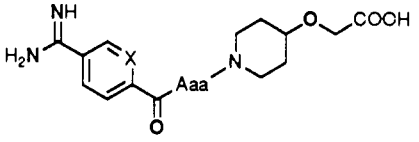
Table IV. *p*-Amidinoaroyl Hybrid Compounds



	X	R	platelet aggregation in h-PRP:[a] IC_{50} (μM) ADP	Coll	Thr	solid-phase assay GP IIb-IIIa–FG:[b] IC_{50comp}/IC_{50RGDS}	solid-phase assay $\alpha_v\beta_3$–FG:[b] IC_{50comp}/IC_{50RGDS}
29c	$NHCH_2CO$	Asp-Phe-OH	67	76	78	1	570
29h (Ro 43-5054)	$NH(CH_2)_2CO$	Asp-Phe-OH	0.06	0.06	0.07	0.0003[c]	4500[d]
29k	$NH(CH_2)_2CO$	Asp-Tyr-OH	0.05	0.04	0.02	0.00006	nt[e]
31d	$NH(CH_2)_2CO$		0.12	0.10	0.13	0.002	8000
31b	$NH(CH_2)_2CO$	β-Ala-OH	1.1	0.9	0.9	0.007	nt[e]
29f	$NH(CH_2)_3CO$	Asp-Val-OH	0.7	0.7	0.7	0.002	1700
29m	$NH(CH_2)_4CO$	Asp-Phe-OH	0.2	0.4	0.1	0.004	280

[a,b] See Table I. [c] Compound **29h** showed an IC_{50} value of 0.6 ± 0.1 nM (n = 12, mean ± SEM). [d] Compound **29h** showed an IC_{50} value of 36 ± 3.8 μM (n = 3, mean ± SEM). [e] nt = not tested.

Table V. (Piperidinyloxy)acetic Acid Derivatives



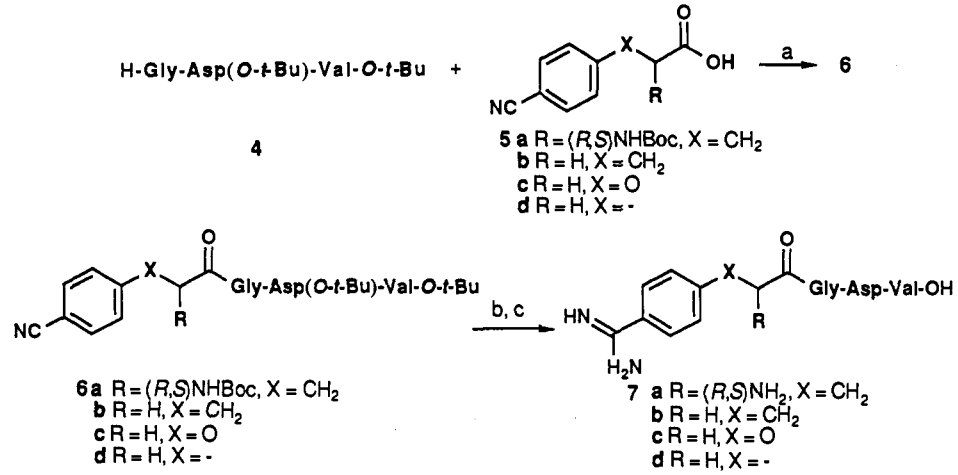
	X	Aaa	platelet aggregation in h-PRP:[a] IC_{50} (μM) ADP	Coll	Thr	solid-phase assay GP IIb-IIIa–FG:[b] IC_{50comp}/IC_{50RGDS}	solid-phase assay $\alpha_v\beta_3$–FG:[b] IC_{50comp}/IC_{50RGDS}
37a	CH	Gly	0.11	0.10	0.09	0.008	>8000
37b	CH	Ala	0.05	0.07	0.05	0.0007	>27000
37c	CH	D-Ala	0.9	1.1	1.1	0.007	>9000
37d	CH	Orn	0.1	0.07	0.07	0.009	>30000
37e	CH	Asp	0.35	0.34	0.38	0.02	>30000
37f (Ro 44-9883)	CH	Tyr	0.03	0.02	0.03	0.0002	17500
37g	CH	Pro	0.06	0.11	0.05	0.002	nt[c]
37h	CH	(*S*)-α-Me-Pro	13	15	49	0.37	12000
37i	N	Ala	0.02	0.03	0.03	0.0001	>30000

[a,b] See Table I. [c] nt = not tested.

chloride in the acylation step. In the case of the intermediate product **29l** carbamoylation of the amidino group greatly facilitated the isolation and purification. The same is true for the preparation of the C-terminally truncated analog **31b** from **30a** via **31a**. The *O*-methyltyramine analog **31d** was obtained in a highly convergent way upon coupling of the Boc-protected benzamidine derivative **30c** with the corresponding aspartate building block followed by removal of the protective groups by catalytic hydrogenation in acidic medium.

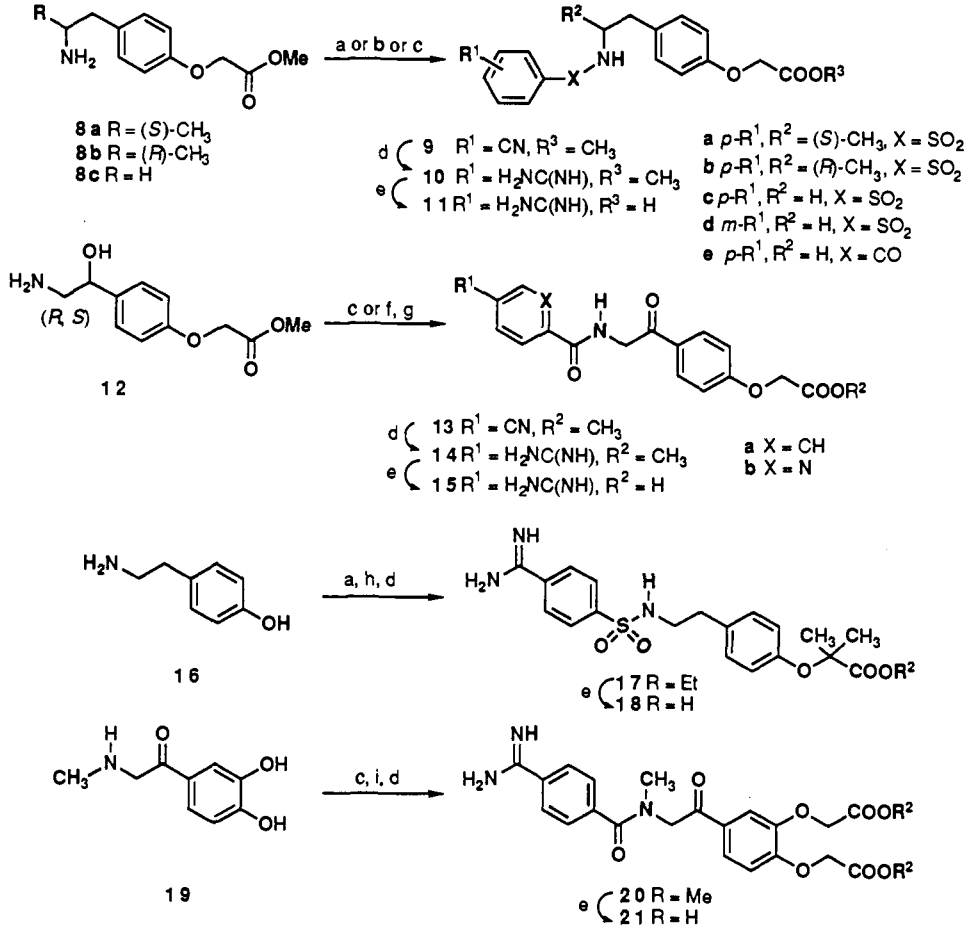
Scheme V shows the syntheses of the (piperidinyloxy)-acetic acid derivatives **37a–i**. An α-*N*-Cbz-protected α-amino acid was coupled with the piperidine building block **33** followed by hydrogenolytic removal of the Cbz

Scheme I. [a] *p*-Amidinophenylalanine Derivatives and Related Compounds



[a] (a) HBTU, *N*-methylmorpholine, THF or DMF; (b) 1. H_2S, Et_3N, pyridine, 2. CH_3I, acetone, 3. NH_4OAc, MeOH; (c) TFA, CH_2Cl_2.

Scheme II. [a] Preparation of (2-Aminoethyl)phenoxyacetic Acid Derivatives



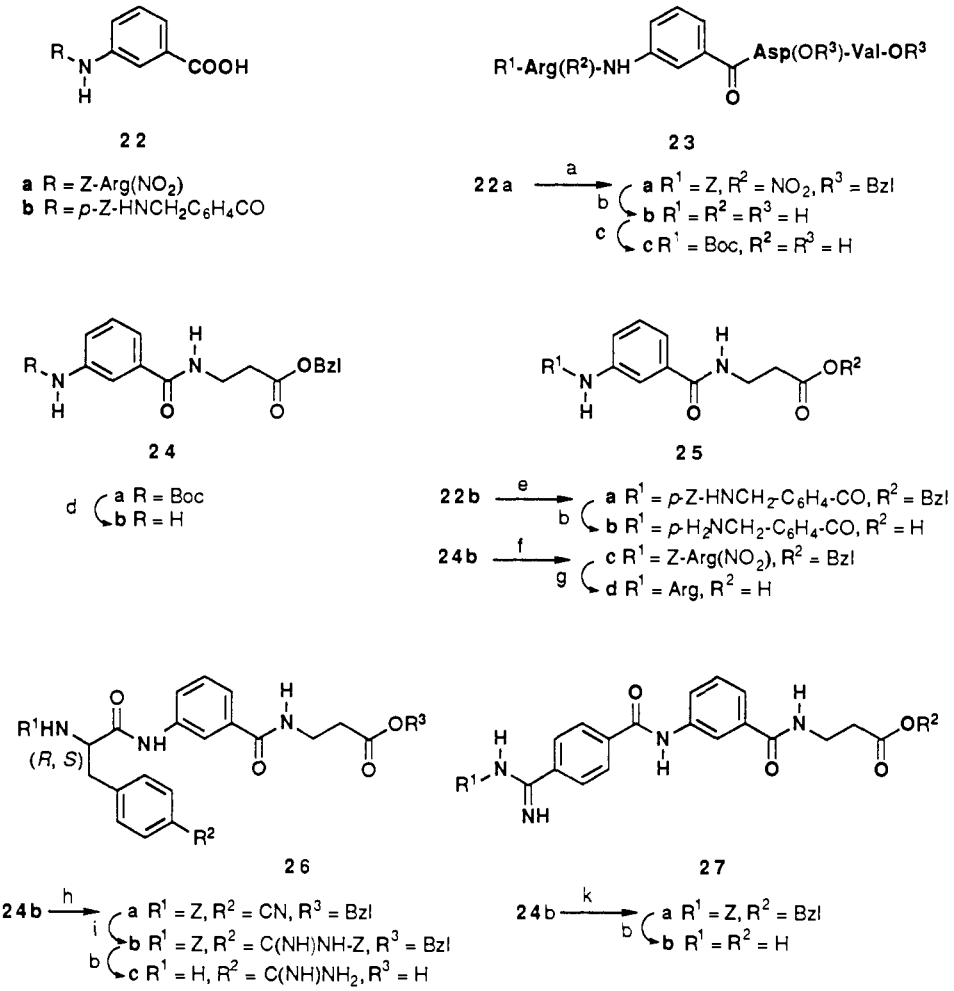
[a] (a) 4-$NCC_6H_4SO_2Cl$, pyridine; (b) 3-$NCC_6H_4SO_2Cl$, pyridine, (c) 4-NCC_6H_4COCl, Et_3N, or 4-NCC_6H_4COOH, CDMT, NMM; (d) 1. H_2S, Et_3N, pyridine, 2. CH_3I, acetone, 3. NH_4OAc, MeOH; (e) hydrolysis under basic or acidic conditions; (f) 5-cyano-2-pyridinecarboxylic acid, ethyl chloroformate, 4-ethylmorpholine, THF; (g) MnO_2, $CHCl_3$; (h) $Br(CH_3)_2CCOOC_2H_5$, KOH, acetone; (i) $BrCH_2COOCH_3$, K_2CO_3, acetone or DMF.

protecting group to give the corresponding amines **34a–f**. Subsequent acylation with either *p*-amidinobenzoyl chloride or 5-cyano-2-pyridinecarboxylic acid and conversion of the cyano group to the amidino group led after cleavage of the *tert*-butyl ester function to the desired products **37a–f** and **37i**. In case of the proline derivatives **35a,b** acylation with *p*-cyanobenzoyl chloride preceded coupling with **33** to **36a,b**. The final steps to **37g,h** remained the same as mentioned above.

Results and Discussion

Assay Systems. In order to assess the biological activity of the compounds discussed in this study, three assay systems were used: (i) Platelet aggregation in human platelet rich plasma (h-PRP) induced by either adenosine 5′-diphosphate (ADP), collagen, or thrombin. Independent of the agonist used for platelet activation the IC_{50} values for a given compound were in the same range, as

Scheme III. [a] *m*-Aminobenzoic Acid Derivatives



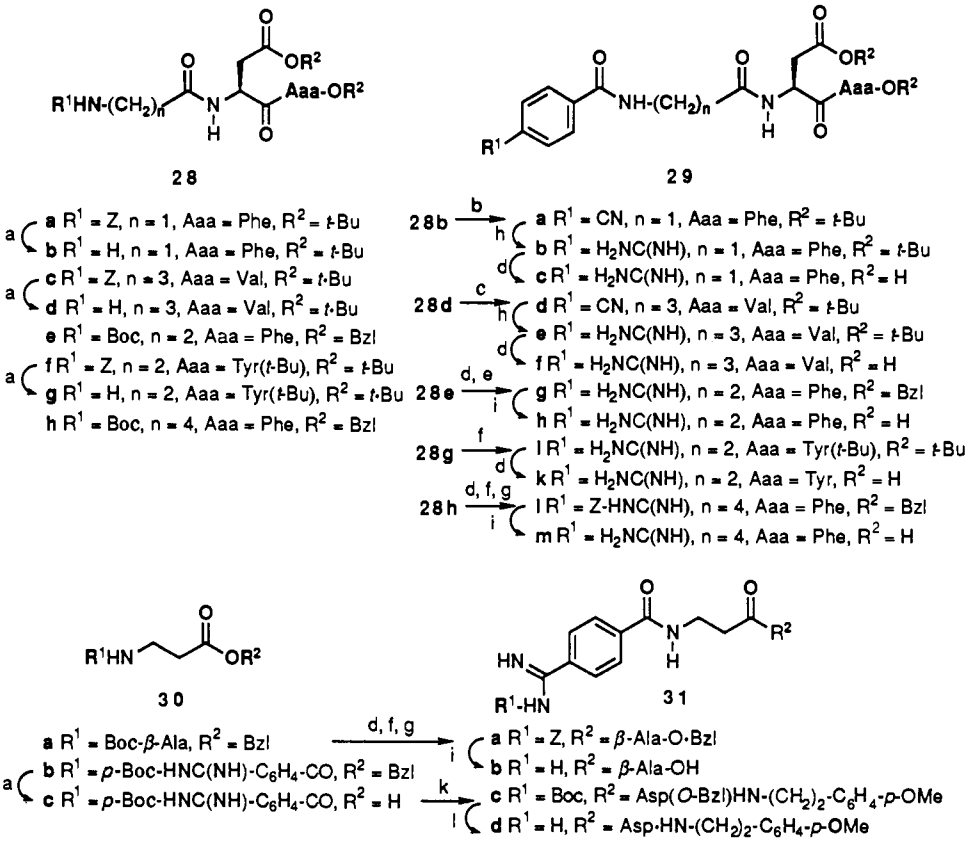
[a] (a) $(CH_3)_2CHCH_2OCOCl$, H-Asp(*O*-Bzl)-Val-*O*-Bzl, NMM, DMF; (b) H_2, Pd/C, AcOH; (c) $(Boc)_2O$, $NaHCO_3$, dioxane–H_2O; (d) TFA, CH_2Cl_2; (e) CDMT, NMM, THF, H-β-Ala-*O*-Bzl; (f) Z-Arg(NO_2)-OH, $(CH_3)_2CHCH_2OCOCl$, TEA, THF; (g) H_2, Pd/C, HCOOH; (h) Z-*p*-NC-Phe-OH, CDMT, NMM, THF; (i) 1. H_2S, TEA, pyridine, 2. CH_3I, acetone, 3. NH_4OAc, MeOH, 4. ZCl, TEA, DMF; (k) 1. *p*-$H_2NC(NH)C_6H_4COCl$, CH_2Cl_2, H_2O, $NaHCO_3$, 2. ZCl, Na_2CO_3.

indicated in Tables I–V. This clearly demonstrates that a step common to all these agonistic pathways is inhibited. (ii) Fibrinogen binding to purified immobilized GP IIb-IIIa (integrin $\alpha_{IIb}\beta_3$). This assay proves that a given compound exhibits fibrinogen receptor antagonistic activity. Since the sensitivity of the assay might vary with the receptor and ligand preparations as well as the coated plates used, the ratios between the IC_{50} value for a given compound and the IC_{50} value for RGDS (1) determined in every experiment are given in Tables I–V. The large difference of the IC_{50} values of the inhibitors observed for inhibition of platelet aggregation and for inhibition of fibrinogen binding to GP IIb-IIIa in the solid-phase assay (for RGDS (1), Table I, ~100 μM vs 3.6 ± 0.4 μM) is largely due to the different fibrinogen concentrations present (about 10 μM in human plasma vs 1.5 nM in the solid-phase assay). In general, a good correlation between the order of potency in the solid-phase GP IIb-IIIa assay and the platelet aggregation assay was observed. However, since the receptor concentration in plasma is high (approximately 20 nM) compared to that in the receptor assay (approximately 1 nM), the theoretical limit of the IC_{50} value in the aggregation assay is about 10 nM, while one could expect a substantially lower limit in the receptor assay. As can be seen in Tables I–V the few discrepancies observed occurred with the most potent fibrinogen receptor antagonists. (iii) Fibrinogen binding to the purified immobilized vitronectin receptor $\alpha_v\beta_3$ in order to examine the integrin selectivity of the compounds. The vitronectin receptor was chosen for these studies because it is closely related to GP IIb-IIIa in that it is composed of the same β_3 subunit and a related α subunit. Furthermore, both receptors can bind fibrinogen, vitronectin, and von Willebrand factor in an RGD-dependent manner.[8] For the same reasons as mentioned above, the ratios between the IC_{50} value for a given compound and the IC_{50} value for RGDS (1), which was determined in every experiment, are given in Tables I–V. The larger the ratio in this assay and the smaller the ratio in the GP IIb-IIIa assay described above, the more pronounced is the selectivity of a given compound for the platelet fibrinogen receptor GP IIb-IIIa.

***p*-Amidinophenylalanine Derivatives and Related Compounds.** From studies in the field of inhibitors of serine proteases like trypsin and thrombin it has been known for quite some time that the *p*-amidinophenyl moiety can mimic the arginine side chain.[31] Since no reports about the replacement of arginine by *p*-amidinophenylalanine in RGD-containing peptides are available, it was decided to explore the effects of such a structural

(31) Wagner, G.; Horn, H.; Richter, P.; Vieweg, H.; Lischke, I.; Kazmirowski, H.-G. Synthesis of Antiproteolytically Active Nα-Arylsulfonylated Amidinophenylalaninamides. *Pharmazie* 1981, *36*, 597–603.

Scheme IV. [a] *p*-Amidinoaroyl Hybrid Compounds



[a] (a) H_2, Pd/C, EtOH; (b) *p*-CN-C_6H_4COOH, HBTU, NMM, DMF; (c) *p*-CN-C_6H_4COOH, CDMT, NMM, DMF; (d) CH_2Cl_2, TFA; (e) *p*-$H_2NC(NH)C_6H_4COCl$, pyridine; (f) *p*-$H_2NC(NH)C_6H_4COCl$, CH_2Cl_2, H_2O, $NaHCO_3$; (g) ZCl; (h) 1. H_2S, Et_3N, pyridine, 2. CH_3I, acetone, 3. NH_4OAc, MeOH; (i) H_2, Pd/C, AcOH; (k) H-Asp(*O*-Bzl)HN-$(CH_2)_2$-C_6H_4-*p*-OMe, CDMT, NMM, THF; (l) H_2, Pd/C, HCOOH.

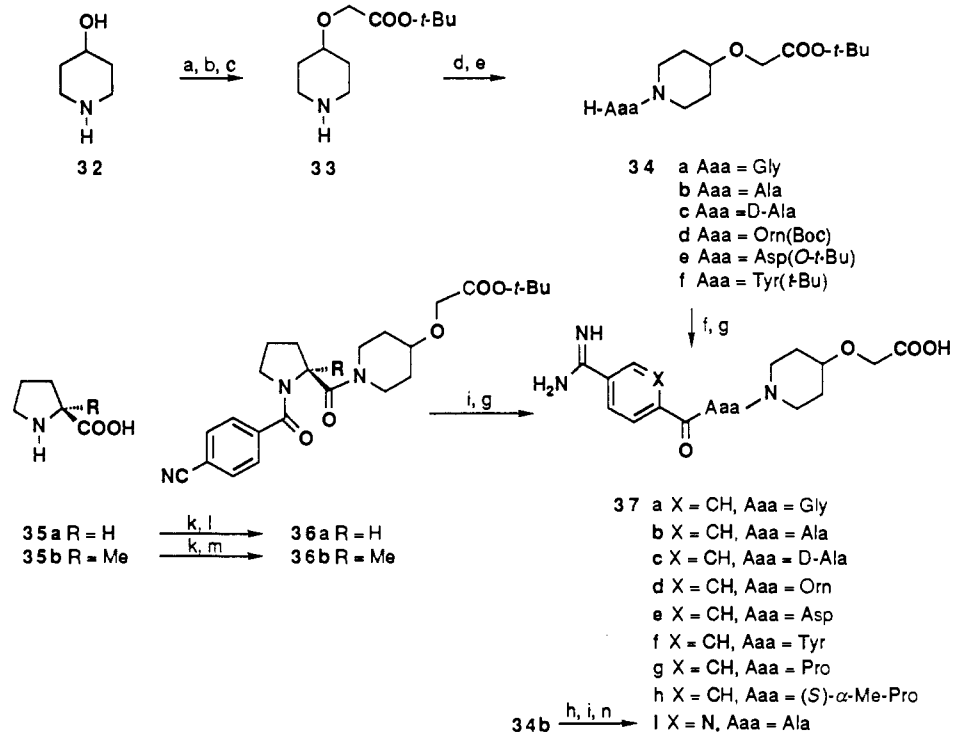
modification. As indicated in Table I, compound **7a** in which the N-terminal arginine of the lead peptides **1–3** has been replaced by racemic *p*-amidinophenylalanine exhibited significantly greater affinity for GP IIb-IIIa and, as expected, higher antiaggregatory activity than **1–3**. Omission of the N-terminal amino group in **7b** results in retention of activity. This finding is in accordance with results from RGD-containing peptides published by others.[14-20] Replacement of the benzylic methylene group by an oxygen atom as demonstrated in **7c** or the deletion of one methylene group as shown in **7d** was even accompanied by a slight increase in activity. In addition, a high selectivity for inhibition of fibrinogen binding to GP IIb-IIIa versus $\alpha_v\beta_3$ was observed for the compounds **7a–d**. The reasons for such a behavior are not clear at the moment. However, compared to the arginine side chain the *p*-amidinophenyl group is considerably less flexible. This might lead to a preference of a conformation which is more suitable for the interaction with GP IIb-IIIa.

***p*-(2-Aminoethyl)phenoxyacetic Acid Derivatives.** In parallel to the studies discussed above, the *p*-amidino sulfonamide **10a** (Table II), originally prepared as a thromboxane A_2 receptor antagonist,[32] was found to inhibit platelet aggregation induced by all three agonists used. This unusual behavior called for further clarification since a typical thromboxane A_2 receptor antagonist potently inhibits collagen induced platelet aggregation only. In the solid-phase receptor assay, **10a** turned out to be a weak GP IIb-IIIa antagonist. Hence, a non-peptide lead compound blocking the platelet fibrinogen receptor has been identified by routine screening. While being a platelet aggregation inhibitor of comparable activity, the corresponding acid **11a** exhibited higher affinity for GP IIb-IIIa than does **10a**. It is also interesting to note that the intermediate **9a**[33] with a cyano group at the same position as the amidino group of **10a/11a** behaves like a thromboxane A_2 receptor antagonist but is devoid of any activity in the GP IIb-IIIa assay (results not shown). These observations clearly indicate that not only the presence of a basic amidino group but also of a free carboxyl group is important for the interaction with GP IIb-IIIa. Furthermore, inspection of molecular models shows that upon superimposing the side chain carboxylate function of aspartic acid in **1** (RGDS) with the carboxylate group of **11a**, the guanidino group of the former can be perfectly matched to the amidino group of the latter if both molecules are allowed to adopt an extended conformation. Since with the ester/acid pair **10e/11e** (Table II) similar trends were observed as with **10a/11a**, it has been hypothesized that the ester function is rapidly cleaved in h-PRP. Indeed, after incubating an aqueous solution of **10e** with h-PRP for 30 min, the free carboxylic acid **11e** could be detected by TLC. A structure–activity relationship (SAR) study of this novel class of fibrinogen receptor antagonists revealed that the (*S*)-configuration in **11a** is critical since the (*R*)-enantiomer **11b** was considerably less

(32) Stegmeier, K.; Pill, J.; Müller-Beckmann, B.; Schmidt, F. H.; Witte, E.-C.; Wolff, H.-P.; Patscheke, H. The Pharmacological Profile of the Thromboxane A_2 Antagonist BM 13.177. A New Anti-platelet and Anti-thrombotic Drug. *Thromb. Res.* **1984**, *35*, 379–395.

(33) While the nitrile **9a** does inhibit the U-46619 induced contraction of isolated rat aortic rings the amidines **11c** and **15a** are completely inactive in this test system. Therefore it might be concluded that all fibrinogen receptor antagonists in this series are devoid of TXA_2 antagonistic activity.

Scheme V. [a] (Piperidinyloxy)acetic Acid Derivatives



[a] (a) ZCl, Et_3N; (b) $BrCH_2COOt$-Bu, $(n\text{-Bu})_4NHSO_4$, 50% NaOH, toluene 1:1; (c) H_2, Pd/C MeOH; (d) α-*N*-Z-protected α-amino acid, CDMT or HBTU, NMM, DMF or CH_2Cl_2; (e) H_2, Pd/C, EtOH, (f) *p*-$H_2NC(NH)C_6H_4COCl$, CH_2Cl_2, H_2O, $NaHCO_3$; (g) TFA, CH_2Cl_2; (h) 5-cyano-2-pyridinecarboxylic acid, CDMT, NMM, CH_2Cl_2; (i) 1. H_2S, Et_3N, pyridine, 2. CH_3I, acetone, 3. NH_4OAc, MeOH; (k) *p*-cyanobenzoyl chloride, base; (l) **33**, HBTU, NMM, DMF; (m) $SOCl_2$, THF followed by **33**; (n) AcOH saturated with HCl.

active. The same trend was found for the compounds of Table V (see below). However, removal of the methyl group responsible for chirality as realized in **11c**[33] restored the activity. Neither substitution in α-position to the acid function (**18**) nor shifting the amidino group from the para to the meta position (**11d**) were tolerated. Two structural variations proved to be beneficial to further improvements: (i) replacement of the sulfonamide function by a carboxamide as demonstrated in **10e/11e** yielded compounds with IC_{50} values in the submicromolar range in the platelet aggregation assay and (ii) introduction of a carbonyl group in the benzyl position as seen in **15a**.[33] Model considerations suggest that this ketone function could play the role of the amide carbonyl group of arginine in **1** (RGDS). Attachment of a second oxyacetic acid side chain in a 1,2-relationship as realized in **21** led to a further improvement in activity and selectivity. In this case, inspection of molecular models points to the possibility that the additional carboxyl group in **21** can be matched to the C-terminal carboxylate of **1** (RGDS), and therefore might contribute to a more favorable interaction with the platelet fibrinogen receptor. Finally, replacement of the *p*-amidinobenzoyl residue by a 5-amidino-2-pyridoyl group as shown in **15b** produced the most potent non-peptide GP IIb-IIIa antagonist in this series. The high selectivity of **15a,b** and **21** for GP IIb-IIIa vs $\alpha_v\beta_3$ is remarkable.

***m*-Aminobenzoic Acid Derivatives.** The idea to prepare the substances listed in Table III emerged from earlier work with template fixed cyclic peptides of type A (Figure 1; results to be published elsewhere). From the data discussed above it is evident that the distance between the basic guanidino or amidino group and the side-chain carboxylate of aspartic acid is a critical prerequisite for achieving good inhibitory activities. It was therefore reasoned that linear compounds of type **B** (Figure 1) where

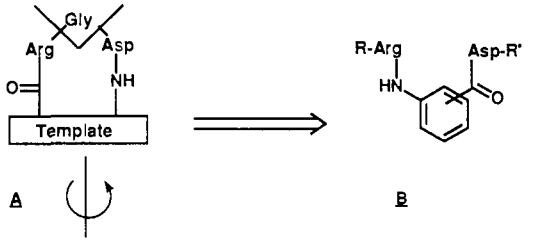


Figure 1.

the glycine portion in A has been excised and the N- and C-terminal ends exchanged could become interesting target molecules with the two important functional groups kept within the distance needed, provided that the template used is relatively small. This would then be equivalent to a modification of the peptide backbone at the glycine portion in **1–3**. As indicated in Table III, the replacement of glycine by the rigid *m*-aminobenzoic acid building block in combination with an N-terminal arginine as in **23c** was indeed accompanied by an increase of activity compared to the lead peptides **1–3**, while the selectivity warrants further improving. This result is remarkable because it has been reported that the glycine residue cannot be replaced by other amino acids without loss of activity.[19b] Truncation of the C-terminal end led to the β-alanine derivative **25d**, which was still equipotent to the lead peptides **1–3** although some activity was lost compared to **23c**. At this point it was obvious to replace the arginine by *p*-amidinophenylalanine as realized in **26c**. As indicated in Table III this modification was accompanied by a loss of activity as compared to **23c**. From model considerations it might be concluded that the guanidino group of **23c** can easily be matched to the guanidino group of **3** due to the flexibility of the arginine side chain present in both molecules. Such a match is hardly possible with

26c because here the amidino group is attached to a rigid aromatic ring. Therefore, the distance to the carboxylate of β-alanine lies outside the optimal range for high affinity binding to GP IIb-IIIa. The situation looks quite different when using the shorter *p*-amidinobenzoyl group, as demonstrated with the derivative **27b** for which not only high activity but also high selectivity for GP IIb-IIIa was observed. The aminomethyl analogue **25b** was found to be considerably less active than **27b**. It has to be stressed that neither the *p*- nor the *o*-aminobenzoic acid analogues of **25d** or **27b** reach the activities of the corresponding *m*-aminobenzoates (results not shown).

***p*-Amidinoaroyl Hybrid Compounds.** The results given in Table IV again demonstrate the importance of the distance between the amidino group and the carboxylic acid function of the aspartate side chain. The insertion of a β-alanine portion produced compounds (**29h**, **29k**) whose activities were far superior to that of the corresponding analogues incorporating either glycine (**29c**), 4-aminobutyric acid (**29f**), or 5-aminopentanoic acid (**29m**), respectively, at the same position. Removal of the C-terminal carboxyl group as in **31d** only slightly affected the activity. A related observation has recently been disclosed by others.[34] Upon replacement of the Asp-X-OH moiety (X = Phe, Tyr) by β-alanine (**31b**), i.e. a C-terminal truncation as already described for the compounds given in Table III, some activity was lost. With the exception of **29c** and **29m**, the selectivity of this type of derivatives for the fibrinogen receptor was excellent.

(Piperidinyloxy)acetic Acid Derivatives. In order to arrive at a more flexible scheme to introduce different substituents at the α-position to the ketone carbonyl group in the structures shown in Table II (e.g. **15a/b** and **21**), the possibility of using any α-amino acid from the chiral pool was assessed. One of the easiest ways to make use of α-amino acids in organic synthesis is the preparation of amides. As a consequence, a prototype compound structurally closely related to **15a/b** would be the glycine derivative **37a**, where the aromatic carbocyclic phenoxyacetic acid portion has been replaced by a saturated heterocyclic (piperidinyloxy)acetic acid building block. Indeed, inspection of molecular models demonstrates that the two important functional groups in **15a/b** and **37a**, i.e. the amidino group and the carboxylate, can be nicely matched if the piperidine ring of **37a** adopts a chair conformation with the acetic acid side chain in an equatorial position. As indicated in Table V, **37a** already exhibited promising activities as well as excellent selectivity. Once again, it has to be stressed that the glycine in **37a** does not play the same role as the glycine in the lead peptides **1–3**. With the two alanine derivatives **37b** and **37c** it became evident that the (*S*)-enantiomer is considerably more potent than the (*R*)-enantiomer. Thus, the same trend was observed as in the case of **11a/b** (Table II). While the use of (*S*)-proline (**37g**) resulted in retention of activity, the incorporation of (*S*)-α-methylproline (**37h**) was highly detrimental. An additional basic amino function as exemplified by **37d** was much better tolerated than a second carboxylate (**37e**) in the same molecule. With the tyrosine derivative **37f** and the amidino pyridoyl analog **37i**, the most potent examples which also exhibited remarkable selectivities for GP IIb-IIIa vs $\alpha_v\beta_3$, were identified.

Half-Life of Selected Compounds. In the course of an extended pharmacological characterization of the compounds **21**, **29h**, **37b**, **37f** as well as **37i**, the half-lives in dogs were determined. The compounds were infused into dogs for 4 h at a dose of 30–50 nmol/kg per min. The plasma concentration of active substance was measured in a bioassay during and for 90 min beyond the infusion. After stopping the infusion, the decline of activity was best fitted to a two-compartment model, with an initial phase having a $t_{1/2}$ of 8 min and a second phase with a $t_{1/2}$ of 110 and 87 min for **37f** and **37i**, respectively. The volume of distribution was in the range of 0.45 L/kg for both compounds, indicating a distribution into tissues. For **21**, **29h**, and **37b**, the limited number of samples did not allow a satisfactory fitting to a two-compartment model; therefore, a one compartment model was used which yielded a $t_{1/2}$ of 45, 50, and 40 min and a volume of distribution of 0.2, 0.2, and 0.5 L/kg, respectively.

Conclusions

Starting with the lead tetrapeptide **1** (RGDS), we have identified non-peptide fibrinogen receptor antagonists by rational modifications of either the side chains or the backbone as well as by random screening. While being much smaller in size, some of the compounds described were equipotent to monoclonal antibodies but exhibited a significantly shorter plasma half-life, making them suitable candidates for iv platelet inhibitors. In addition, they were highly selective for the fibrinogen receptor GP IIb-IIIa as compared to the vitronectin receptor $\alpha_v\beta_3$. The SAR information gained from this study might also facilitate the search for GP IIb-IIIa antagonists with improved bioavailibity after oral application.

Experimental Section

The abbreviations are used: CDMT, 2-chloro-4,6-dimethoxy-1,3,5-triazine; FG, fibrinogen; HBTU, *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; h-PRP, human platelet rich plasma; NMM, *N*-methylmorpholine; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

Chemistry. Reagent grade solvents were used without further purification. Evaporation means removal of solvent by use of a Büchi rotary evaporator at 40–50 °C in vacuo. All organic extracts were dried over Na_2SO_4 or $MgSO_4$. Normal phase silica gel used for flash chromatography (FC) was Kieselgel-60 (230–400 mesh); reverse-phase silica gel used was Lichroprep RP 18 (40–63 μm); both were supplied by E. Merck, A.G., Darmstadt, Germany. Sometimes MCI gel CHP20P from Mitsubishi Chemical Industries was used for liquid chromatography. TLC plates coated with silica gel 60 F_{254} (Merck) were used; detection by UV (254 nm), by I_2 vapor or by treatment with Cl_2/*o*-tolidine followed by heating was applied. Melting points were determined with a Büchi 510 apparatus and are uncorrected. Proton NMR spectra (NMR) were recorded on a Bruker AC 250 spectrometer; δ values in ppm relative to tetramethylsilane are given. IR spectra (IR) were recorded with the compound (neat) on a sodium chloride disk or as KBr pellets using a Nicolet 7199-FT-IR spectrometer. Optical rotations $[\alpha]^{20}_D$ were determined with a Perkin-Elmer 241 polarimeter, c in g/100 mL. Mass spectra (MS) were recorded with a MS 901 AEI apparatus. Results of elemental analysis were within 0.4% of the theoretical values unless otherwise noted.

H-D,L-*p*-Amidino-Phe-Gly-Asp-Val-OH (7a). (a) Preparation of Boc-D,L-*p*-cyano-Phe-Gly-Asp(*O*-*t*-Bu)-Val-*O*-*t*-Bu (6a) (Standard Procedure A). To a solution of H-Gly-Asp(*O*-*t*-Bu)-Val-*O*-*t*-Bu (**4**)[25] (4.02 g, 10 mmol) and Boc-D,L-*p*-cyano-

(34) Nicholson, N. S.; Panzer-Knodle, S. G.; Salyers, A. K.; Taite, B. B.; King, L. W.; Miyano, M.; Gorczynski, R. J.; Williams, M. H.; Zupec, M. E.; Tjeong, F. S.; Adams, S. P.; Feigen, L. P. In Vitro and In Vivo Effects of a Peptide Mimetic (SC-47643) of RGD as an Antiplatelet and Antithrombotic Agent. *Thromb. Res.* **1991**, *62*, 567–578.

Phe-OH (**5a**)[26] (2.9 g, 10 mmol) in THF (50 mL) were added at 0 °C HBTU (3.79 g, 10 mmol) and NMM (1.1 mL, 11 mmol). The pH of the solution was adjusted to 8.5 by further addition of NMM. The mixture was stirred at room temperature for 6 h. After removal of the solvents, the residue was partitioned between EtOAc/$NaHCO_3$ (5%). The combined organic extracts (3 × 50 mL) were washed with brine, dried, filtered, and evaporated. Purification by FC (silica gel, EtOAc) and recrystallization (diisopropyl ether/EtOAc) gave **6a** (4.82 g, 66%): mp 132–133 °C; IR (KBr) 2228, 1729, 1642, 1161 cm^{-1}; NMR (DMSO-d_6) δ 0.84 (d, J = 6 Hz, 3 H), 0.87 (d, J = 6 Hz, 3 H), 1.27 (s, 9 H), 1.37 (s, 9 H), 1.39 (s, 9 H), 2.05 (m, 1 H), 2.45 (m, 1 H), 2.68 (m, 1 H), 2.83 (m, 1 H), 3.08 (m, 1 H), 3.74 (m, 2 H), 4.01 (m, 1 H), 4.24 (m, 1 H), 4.70 (m, 1 H), 7.05 (d, J = 8 Hz, 1 H), 7.46 (part of AA'BB', 2 H), 7.75 (part of AA'BB', 2 H), 7.87 (d, J = 8 Hz, 1 H), 8.20 (d, J = 8 Hz, 1 H), 8.28 (t, J = 6 Hz, 1 H); MS m/z (FAB) 674 $(M + H)^+$. Anal. ($C_{34}H_{51}N_5O_9 \cdot 0.4C_6H_{14}O$) C, H, N.

(b) Preparation of Boc-D,L-*p*-amidino-Phe-Gly-Asp(*O*-*t*-Bu)-Val-*O*-*t*-Bu (Standard Procedure B). A solution of the nitrile **6a** (5.0 g, 7.4 mmol) in pyridine/TEA 5:1 (180 mL) was saturated with H_2S and allowed to stand at room temperature until no starting material could be detected by TLC. Excess H_2S was extruded by a gentle stream of argon, and the solvents were removed to give the corresponding thioamide (5.06 g, 96%) as a yellow foam; MS m/z (FAB) 708 $(M + H)^+$. A solution of the thioamide (5 g, 7 mmol) and iodomethane (5 mL) in acetone (150 mL) was kept at 50 °C until no starting material could be detected by TLC. Complete removal of the solvents yielded the corresponding thioiminomethyl ester as an amorphous hydriodide salt (4.9 g, 82%); MS m/z (FAB) 722 $(M + H)^+$. This material (4.8 g, 5.6 mmol) and ammonium acetate (0.65 g, 8.4 mmol) in methanol (100 mL) was kept at 70 °C until no starting material could be detected by TLC. After extrusion of the methyl mercaptan by a stream of argon, the reaction mixture was concentrated and the intermediate Boc-D,L-*p*-amidino-Phe-Gly-Asp(*O*-*t*-Bu)-Val-*O*-*t*-Bu precipitated as hydriodide salt by addition of Et_2O. The solid was collected by filtration, washed (EtOAc), and dried (4.1 g, 89%): mp 140 °C; NMR (DMSO-d_6) δ 0.84 (d, J = 6 Hz, 3 H), 0.88 (d, J = 6 Hz, 3 H), 1.28 (s, 9 H), 1.38 (s, 9 H), 1.40 (s, 9 H), 2.05 (m, 1 H), 2.45 (m, 1 H), 2.67 (m, 1 H), 2.85 (m, 1 H), 3.10 (m, 1 H), 3.74 (m, 2 H), 4.02 (m, 1 H), 4.22 (m, 1 H), 4.68 (m, 1 H), 7.06 (d, J = 8 Hz, 1 H), 7.50 (part of AA'BB', 2 H), 7.74 (part of AA'BB', 2 H), 7.95 (d, J = 8 Hz, 1 H), 8.10–8.30 (m, 3 H), 8.59 (br t, J = 8 Hz, 1 H), 9.0 (d, J = 5 Hz, 1 H); MS m/z (FAB) 691 $(M + H)^+$.

(c) Preparation of H-D,L-*p*-Amidino-Phe-Gly-Asp-Val-OH (7a) (Standard Procedure C). The hydriodide salt of Boc-D,L-*p*-amidino-Phe-Gly-Asp(*O*-*t*-Bu)-Val-*O*-*t*-Bu (1.5 g, 1.8 mmol) was dissolved in CH_2Cl_2/TFA 7:2 (90 mL). After 2 h at room temperature, the solvents were removed and the residue was recrystallized from MeOH/EtOAc to give the TFA salt of **7a** as a white powder (0.96 g, 75%): mp 174 °C; NMR (DMSO-d_6) δ 0.86 (d, J = 7 Hz, 6 H), 2.06 (m, 1 H), 2.48 (m, 1 H), 2.68 (dd, J = 5, 17 Hz, 1 H), 3.04 (dd, J = 7, 15 Hz, 1 H), 3.22 (dd, J = 4, 15 Hz, 1 H), 3.84 (m, 2 H), 4.13 (m, 2 H), 4.70 (m, 2 H), 7.54 (part of AA'BB', 2 H), 7.80 (part of AA'BB', 2 H), 7.96 (d, J = 8 Hz, 1 H), 8.40 (d, J = 8 Hz, 1 H), 8.78 (br t, J = 5 Hz, 1 H), 9.30 (br s, 4 H); MS m/z (FAB) 479 $(M + H)^+$. Anal. ($C_{21}H_{30}N_6O_7 \cdot 2.3C_2HF_3O_2 \cdot 2.5H_2O$) C, H, N.

Compounds **6b–d** were synthesized from **5b–d**, respectively, by coupling to **4** according to standard procedure A. Compounds **7b–d** were prepared from the intermediates **6b–d**, respectively, according to standard procedures B and C.

Methyl [*p*-[(*S*)-2-(*p*-Cyanobenzenesulfonamido)propyl]-phenoxy]acetate (9a). To a solution of methyl [*p*-[(*S*)-2-aminopropyl]phenoxy]acetate (**8a**)[35] (520 mg, 2 mmol) in pyridine (10 mL) was added 4-cyanobenzenesulfonyl chloride[36] (442 mg, 2.2 mmol). After 2 h at 50 °C, the solvents were evaporated, and the residue dissolved in CH_2Cl_2 and washed with water. After chromatography (silica gel, $CHCl_3$/1-propanol/NH_4OH 1000:2:0.2) and recrystallization from acetone/hexane **9a** (470 mg, 60%) was obtained: mp 118–119 °C; $[\alpha]^{20}_D$ = +25.8° (c = 0.8, MeOH); IR (KBr) 2233, 1746, 1212 cm^{-1}; NMR ($CDCl_3$) δ 1.18 (d, J = 7 Hz, 3 H), 2.52 (dd, J = 8, 14 Hz, 1 H), 2.79 (dd, J = 6, 14 Hz, 1 H), 3.47 (m, 1 H), 3.83 (s, 3 H), 4.63 (s, 2 H), 4.68 (d, J = 8 Hz, 1 H), 6.70 (m, 2 H), 6.89 (m, 2 H), 7.71 (br s, 4 H); MS m/z (EI) 388 (0.7, M^+), 209 (100). Anal. ($C_{19}H_{20}N_2O_5S$) C, H, N, S.

Compounds **9b** and **9c** were synthesized in the same way from **8b**[37] and **8c**,[38] respectively, and 4-cyanobenzenesulfonyl chloride;[36] compound **9d** was prepared in an analogous manner from **8c**[38] and 3-cyanobenzenesulfonyl chloride.[39]

Methyl [*p*-[2-(*p*-Cyanobenzamido)ethyl]phenoxy]acetate (9e) (Standard Procedure D). A solution of *p*-cyanobenzoic acid (1.47 g, 10 mmol), CDMT[30] (1.79 g, 10.2 mmol), and NMM (1.03 g, 10.2 mmol) in CH_2Cl_2 (40 mL) was stirred at 0 °C for 3 h. When no CDMT could be detected anymore by TLC, a solution of the hydrochloride salt of methyl [*p*-(2-aminoethyl)phenoxy]-acetate (**8c**)[38] (2.45 g, 10 mmol) and NMM (1.03 g, 10.2 mmol) in CH_2Cl_2 (40 mL) was added. After 12 h at room temperature, the solvents were evaporated. The residue was dissolved in EtOAc and successively washed with 0.2 N HCl at 0 °C, H_2O, 5% $NaHCO_3$, H_2O, and brine, respectively. The crude material obtained after evaporation of the solvents was triturated with Et_2O and dried to give **9e** (2.83 g, 84%): mp 149–151 °C; IR (KBr) 3431, 2228, 1751, 1676, 1223 cm^{-1}; NMR (DMSO-d_6) δ 2.79 (m, 2 H), 3.44 (m, 2 H), 3.69 (s, 3 H), 4.75 (s, 2 H), 6.85 (m, 2 H), 7.16 (m, 2 H), 7.96 (br s, 4 H), 8.86 (br t, J = 5 Hz, 1 H); MS m/z (EI) 338 (0.5, M^+), 192 (100).

Methyl [*p*-[(*S*)-2-(*p*-Amidinobenzenesulfonamido)propyl]phenoxy]acetate (10a). The nitrile **9a** (1.16 g, 3 mmol) was converted to the acetate salt of the corresponding amidine **10a** (470 mg, 34% overall after chromatography, MCI gel CHP20P, H_2O/MeOH) according to standard procedure B: mp 208–210 °C; $[\alpha]^{20}_D$ = +24.0° (c = 0.9, MeOH); IR (KBr) 3284, 1759, 1224 cm^{-1}; NMR (DMSO-d_6) δ 0.90 (d, J = 7 Hz, 3 H), 1.74 (s, 3 H), 2.54 (m, 2 H), 3.34 (m, 1 H), 3.70 (s, 3 H), 4.73 (s, 2 H), 6.75 (m, 2 H), 6.99 (m, 2 H), 7.84 (m, 4 H); MS m/z (EI) 346 (2, $M - COOCH_3^+$), 209 (100). Anal. ($C_{19}H_{23}N_3O_5S \cdot 1.0C_2H_4O_2$) C, H, N, S.

The amidines **10b–e** were produced in a similar manner from the corresponding nitriles **9b–e**.

[*p*-[(*S*)-2-(*p*-Amidinobenzenesulfonamido)propyl]phenoxy]acetic Acid (11a). A solution of **10a** (40 mg, 0.085 mmol) in EtOH/1 N NaOH 2:1 (4.5 mL) was stirred at room temperature for 5 h. The pH was then adjusted to 7 by addition of 1 N HCl, and the organic solvents were evaporated. The residue was passed through a column of MCI gel after acidification to pH 2.5 by addition of 0.1 N HCl. Removal of the solvents yielded the hydrochloride salt of **11a** (32 mg, 88%) as a colorless resin: IR (KBr) 3342, 1680, 1510 cm^{-1}; NMR (DMSO-d_6) δ 0.90 (d, J = 7 Hz, 3 H), 2.53 (m, 2 H), 3.34 (m, 1 H), 4.60 (s, 2 H), 6.73 (m, 2 H), 6.99 (m, 2 H), 7.89 (m, 4 H), 8.02 (d, J = 7.5 Hz, 1 H), 9.34 (br s, 2 H), 9.52 (br s, 2 H), 12.94 (br s, 1 H); MS m/z (FAB) 392 $(M + H)^+$. Anal. ($C_{18}H_{21}N_3O_5S \cdot 1.0HCl \cdot 0.5H_2O$) C, H, N: calcd, 9.62; found, 9.14; S: calcd, 7.34; found, 6.80.

The acids **11b–d** were prepared in a similar way from the corresponding methyl esters **10b–e**.

Methyl [*p*-[(*p*-Cyanobenzamido)acetyl]phenoxy]acetate (13a). The reaction of the hydrochloride salt of (*R*,*S*)-methyl [*p*-(2-amino-1-hydroxyethyl)phenoxy]acetate (**12**)[40] (1.30 g, 5 mmol) with *p*-cyanobenzoyl chloride (Aldrich, 830 mg, 5 mmol) in CH_2Cl_2 (120 mL) in the presence of triethylamine (1.49 mL, 10.7 mmol), as described for **9b**, afforded after recrystallization from acetone/hexane (*R*,*S*)-methyl *p*-[2-(*p*-cyanobenzamido)-1-hydroxyethyl]phenoxyacetate (1.3 g, 73%): mp 140–142 °C; MS m/z (EI) 337 (1.5, $M - OH^+$), 195 (100). To a solution of the intermediate alcohol (600 mg, 1.69 mmol) in $CHCl_3$ (100 mL) was added MnO_2 (3.0 g and again 1 g after 24 h). After 44 h at

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room temperature, the reaction mixture was filtered through a pad of Celite, and the solvents were evaporated. Chromatography (silica gel, $CHCl_3$/1-propanol/NH_4OH 1000:10:1) yielded **13a** (200 mg, 34%): mp 200–203 °C; NMR (DMSO-d_6) δ 3.72 (s, 3 H), 4.78 (br d, J = 6 Hz, 2 H), 4.96 (s, 2 H), 7.09 (m, 2 H), 8.03 (m, 6 H), 9.12 (br t, J = 6 Hz, 1 H); MS m/z (EI) 293 (1.0, M – $C_2H_3O_2^+$), 193 (100).

Methyl [*p*-[(5-Cyano-2-pyridinecarboxamido)acetyl]phenoxy]acetate (13b). To a solution of 5-cyano-2-pyridinecarboxylic acid[41] (1.48 g, 10 mmol), 4-ethylmorpholine (3.12 mL, 25 mmol), and ethyl chloroformate (1.2 mL, 10.2 mmol) in THF (120 mL) was added a solution of the hydrochloride salt of (*R,S*)-methyl [*p*-(2-amino-1-hydroxyethyl)phenoxy]acetate (**12**)[40] (2.67 g, 10 mmol) at –5 °C. After 1 h the reaction mixture was allowed to warm to room temperature. Usual workup and chromatography as described for **9a** yielded (*R,S*)-methyl [*p*-[2-(5-cyano-2-pyridinecarboxamido)-1-hydroxyethyl]phenoxy]acetate (2.1 g, 59%): mp 126–132 °C (from acetone/*n*-hexane); IR (KBr) 3514, 2238, 1742, 1672 cm^{-1}. This material (2.0 g, 5.6 mmol) was oxidized to methyl [*p*-[(5-cyano-2-pyridinecarboxamido)acetyl]phenoxy]-acetate (**13b**) (770 mg, 35%) in the same way as described for **13a**: mp 190–192 °C (from acetone/*n*-hexane); IR (KBr) 3408, 2233, 1764, 1689 cm^{-1}; NMR (DMSO-d_6) δ 3.72 (s, 3 H), 4.82 (d, J = 6 Hz, 2 H), 4.96 (s, 2 H), 7.08 (m, 2 H), 8.02 (m, 2 H), 8.20 (dd, J = 8, 0.6 Hz, 1 H), 8.55 (dd, J = 8, 1.5 Hz, 1 H), 9.18 (m, 2 H); MS m/z (EI) 294 (1.0, M – $C_2H_3O_2^+$), 193 (100). Anal. ($C_{18}H_{15}N_3O_5$) C, H, N.

The amidines **14a,b** were obtained in the same way as described for **10a** from the corresponding nitriles **13a,b**.

***p*-[(*p*-Amidinobenzamido)acetyl]phenoxyacetic Acid (15a).** From **14a** the hydrochloride salt of the corresponding acid **15a** was prepared as described for **11a**: mp >280 °C (from 2 N HCl); IR (KBr) 3413, 1737, 1677, 1633 cm^{-1}; NMR (DMSO-d_6) δ 4.78 (br d, J = 6 Hz, 2 H), 4.83 (s, 2 H), 7.06 (m, 2 H), 7.94 (m, 2 H), 8.02 (m, 2 H), 8.11 (m, 2 H), 9.14 (br t, J = 6 Hz, 1 H), 9.30 (br s, 2 H), 9.50 (br s, 2 H), 13.16 (br s, 1 H); MS m/z (CI) 356 (M + H)$^+$. Anal. ($C_{16}H_{17}N_3O_5$·1.0HCl) C, H, Cl, N.

***p*-[(5-Amidino-2-pyridinecarboxamido)acetyl]phenoxyacetic Acid (15b).** A solution of **14b** (110 mg, 0.27 mmol) in aq 1 N HCl was heated to reflux for 30 min. The reaction mixture was then cooled to 5 °C, and the precipitates formed were collected by filtration to give the hydrochloride salt of the corresponding acid **15b**: mp >250 °C; IR (KBr) 3351, 1683, 1660 cm^{-1}; NMR (DMSO-d_6) δ 4.83 (m, 4 H), 7.06 (m, 2 H), 8.02 (m, 2 H), 8.25 (br d, J = 8 Hz, 1 H), 8.43 (dd, J = 8, 1.5 Hz, 1 H), 9.08 (d, J = 1.5 Hz, 1 H), 9.20 (br t, J = 6 Hz, 1 H), 9.42 (br s, 2 H), 9.70 (br s, 2 H), 13.18 (br s, 1 H); MS m/z (FAB) 357 (M + H)$^+$. Anal. ($C_{17}H_{16}N_4O_5$·1.0HCl·0.3H_2O) C, H, Cl, N.

Dimethyl [[4-[(*p*-Amidino-*N*-methylbenzamido)acetyl]-*o*-phenylene]dioxy]diacetate (20). To a solution of adrenalon hydrochloride (**19**) (Fluka, 25.5 g, 0.12 mol) in DMF (250 mL) was added *p*-cyanobenzoyl chloride (Aldrich, 19.4 g, 0.12 mol) in portions. To this mixture was added pyridine (18.9 mL) in such a way that the reaction temperature did not exceed 35 °C. After 2 h at room temperature the reaction mixture was poured into ice water (1.5 L) and the resulting precipitate collected by filtration. A solution of the crude product in 1 N NaOH (600 mL) was filtered, cooled to 0 °C, and acidified with concentrated aqueous HCl to give the intermediate α-(*p*-cyano-*N*-methylbenzamido)-3,4-dihydroxyacetophenone (32 g, 88%): mp 227–229 °C dec; MS m/z (EI) 310 (4, M$^+$), 137 (100). A solution of this material (16 g, 51.5 mmol), methyl bromoacetate (19.7 g, 129 mmol) and K_2CO_3 (20 g, 144 mmol) in DMF (150 mL) was kept at 50–60 °C. After 2 h the reaction mixture was partitioned between EtOAc and H_2O. After drying and evaporation of the combined organic extracts, the residue was triturated with Et_2O to give dimethyl [[4-[(*p*-cyano-*N*-methylbenzamido)acetyl]-o-phenylene]dioxy]diacetate as an amorphous solid (14 g, 60%): MS m/z (EI) 454 (0.5, M$^+$), 281 (100). This compound (3 g, 6.6 mmol) was converted to the acetate salt of the corresponding amidine **20** (1.3 g, 37% overall) according to standard procedure B: mp 177–178 °C (H_2O/MeOH, dec); NMR (DMSO-d_6, rotamers) δ 1.72 (s, 3 H), 2.94 and 2.99 (2 s, 3 H), 3.67–3.72 (4 s, 6 H), 4.86–5.00 (4 br s, 6 H), 6.98–7.12 (m, 1 H), 7.40–7.92 (m, 6 H), 10.30 (br s, 3 H); MS m/z (FAB) 472 (M + H)$^+$. Anal. ($C_{23}H_{25}N_3O_8$·1.0$C_2H_4O_2$) C, H, N.

[[4-[(*p*-Amidino-*N*-methylbenzamido)acetyl]-*o*-phenylene]dioxy]diacetic Acid (21). A solution of **20** (1.25 g, 2.35 mmol) in H_2O/AcOH 4:1 (25 mL) was heated to reflux. After 12 h, the solvents were evaporated, and the residue was taken up in H_2O of 70 °C. The precipitate formed upon addition of EtOH was collected by filtration, washed with EtOH, and dried to give **21** (0.91 g, 87%): mp 230 °C dec; NMR (D_2O/CF_3COOD, rotamers) δ 2.94 and 3.02 (2 s, 3 H), 4.72–5.00 (m, 6 H), 6.93–7.08 (m, 1 H), 7.44–7.94 (m, 6 H); MS m/z (FAB) 444 (M + H)$^+$. Anal. ($C_{21}H_{21}N_3O_8$·0.2$C_2H_4O_2$) C, H, N.

Starting from tyramine and 4-cyanobenzenesulfonyl chloride[36] compound **18** was prepared in a similar manner via the ethyl ester **17**.

***m*-[[N^2-[(Benzyloxy)carbonyl]-N^5-(N^1-nitroamidino)-L-ornithyl]amino]benzoic Acid (22a).** To a solution of Z-Arg-(NO_2)-OH (7.07 g, 20 mmol) in DMF (50 mL) were added NMM (2.2 mL, 20 mmol) and isobutyl chloroformate (2.61 mL, 20 mmol) at –5 °C. After stirring for 2 min, a precooled (–5 °C) solution of 3-aminobenzoic acid (2.74 g, 20 mmol) and NMM (2.2 mL, 20 mmol) in DMF (40 mL) was added. Stirring was continued at –5 °C for 30 min and at 20 °C for 2 h. The reaction mixture was diluted with EtOAc, washed with aqueous 5% $KHSO_4$/10% K_2SO_4 solution and brine. Upon concentration of the dried and filtered organic extracts, **22a** (3.0 g, 31%) crystallized and was isolated by suction filtration: mp 232 °C; $[\alpha]^{20}_D$ = +5.9° (*c* = 1, DMF); NMR (DMSO-d_6) δ 1.47–1.84 (m, 4 H), 3.18 (m, 2 H), 4.16 (m, 1 H), 5.04 (s, 2 H), 7.13–7.41 (m, 5 H), 7.44 (t, J = 8 Hz, 1 H), 7.66 (m, 2 H), 7.84 (br d, J = 8 Hz, 1 H), 8.25 (br s, 1 H), 7.5–8.3 (br s, 2 H), 8.52 (br s, 1 H), 10.26 (br s, 1 H), 13.01 (br s, 1 H); MS m/z (ISP) 473 (M + H)$^+$.

Compound **22b** was produced from α-[1-(benzyloxy)formamido]-*p*-toluic acid[42] and *m*-aminobenzoic acid according to standard procedure D; compound **23a** was prepared from **22a** and H-Asp(*O*-Bzl)-Val-*O*-Bzl[25] as described for **22a**.

[*m*-[H-Arg-NH]benzoyl]-Asp-Val-OH (23b). The protected intermediate **23a** (750 mg, 0.86 mmol) was hydrogenated (1 atm) over 10% Pd/C in AcOH/H_2O 9:1 (50 mL) at 20 °C. After removal of the catalyst by filtration, the solution was freeze-dried to give **23b** (440 mg, 100%): NMR (DMSO-d_6) δ 0.80 (m, 6 H), 1.42–1.86 (m, 4 H), 2.00 (m, 1 H), 2.61 (m, 2 H), 3.08 (m, 2 H), 3.75 (m, 1 H), 3.88 (m, 1 H), 4.63 (m, 1 H), 7.35–8.00 (m, 6 H), 8.05 (m, 1 H), 8.96 (m, 1 H), 9.18 (m, 1 H); MS m/z (FAB) 508 (M + H)$^+$; amino acid anal. Arg, Asp, Val.

[*m*-[Boc-Arg-NH]benzoyl]-Asp-Val-OH (23c). To a solution of **23b** (142 mg, 0.16 mmol) in dioxane (1 mL) and H_2O (1 mL) were added pyridine hydrobromide (40 mg, 0.25 mmol), $NaHCO_3$ (105 mg, 1.25 mmol), and di-*tert*-butyl dicarbonate (76 mg, 0.35 mmol). The mixture was shaken for 3 h at room temperature and then acidified to pH = 4 with AcOH. Purification by (i) chromatography over Sephadex G-25S with 0.2 N AcOH, (ii) freeze-drying of the main fraction thereof, (iii) HPLC over Lichrosorb RP18 with a gradient solvent 0.05 M NH_4OAc–EtOH, and (iv) repeated lyophilization with H_2O gave the acetate salt of **23c** (6:5, 54 mg, 30%): NMR (DMSO-d_6) δ 0.78 (2 d, 6 H), 1.39 (s, 9 H), 1.44–1.74 (m, 4 H), 1.90 (s, 2.5 H), 1.98 (m, 1 H), 2.57 (m, 2 H), 3.03 (m, 2 H), 3.82 (m, 1 H), 4.13 (m, 1 H), 4.51 (m, 1 H), 7.10 (d, J = 6 Hz, 1 H), 7.33–7.95 (m, 7 H), 9.12 (m, 1 H), 9.44 (m, 1 H), 10.22 (m, 1 H); MS m/z (FAB) 608 (M + H)$^+$; amino acid anal. Arg, Asp, Val.

Compound **24b** was synthesized via **24a** from *m*-(1-*tert*-butoxyformamido)benzoic acid[43] and β-alanine benzyl ester *p*-toluenesulfonate according to standard procedure D followed by standard procedure C; compound **25a** was prepared from **22b** and β-alanine benzyl ester *p*-toluenesulfonate in THF according to standard procedure D.

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***N*-[*m*-(α-Amino-*p*-toluamido)benzoyl]-β-alanine (25b).** A suspension of **25a** (0.99 g, 1.76 mmol) and 5% Pd/C (0.33 g) in AcOH (19.9 mL) was stirred in a hydrogen atmosphere (1 atm) for 4 h. After filtration and removal of the solvents, the residue was evaporated several times with H_2O until a crystalline crude product was obtained. This was taken up in MeOH (7 mL) and stirred for 20 min to provide **25b** (497 mg, 83%): mp 232 °C dec; IR (KBr) 3291, 3038, 1649, 1587, 1531, 1434, 1398, 1316, 756, 688 cm^{-1}; NMR (D_2O) δ 2.70 (t, J = 6.6 Hz, 2 H), 3.61 (t, J = 6.6 Hz, 2 H), 4.24 (s, 2 H), 7.42–7.88 (m, 8 H); MS m/z (FAB) 342 (M + H)$^+$. Anal. ($C_{18}H_{19}N_3O_4 \cdot 0.4H_2O$) C, H, N.

***N*-[*m*-[*N*^{2}-[(Benzyloxy)carbonyl]-*N*^{5}-(*N*-nitroamidino)-L-ornithyl]benzoyl]-β-alanine Benzyl Ester (25c).** To a mixture of [*N*^{2}-(benzyloxy)carbonyl]-*N*^{5}-(*N*-nitroamidino)-L-ornithine (1.54 g, 4.1 mmol) and TEA (0.58 mL, 4.1 mmol) in THF (20 mL) was added isobutyl chloroformate (0.56 mL, 4.3 mmol) at −10 °C. After stirring for 5 min, **24b** trifluoroacetate (1.69 g, 4.1 mmol) in THF (5 mL) and TEA (0.58 mL, 4.1 mmol) were added. After 10 min the mixture was allowed to warm up to room temperature and stirring was continued for 3.5 h. After dilution with EtOAc, the reaction mixture was successively washed with 2 N HCl, H_2O, 2 N Na_2CO_3, H_2O, and brine. The organic layer was dried and evaporated in vacuo to give a red-colored resin which was purified by chromatography (silica gel, AcOEt/EtOH 49:1) to give **25c** (1.08 g, 42%): mp 157–160 °C (EtOAc); $[\alpha]^{20}_D$ = −4.6° (c = 0.5, AcOH); IR (KBr) 3312, 1721, 1635, 1590, 1533, 1434, 1267, 749, 698 cm^{-1}; NMR (DMSO-d_6) δ 1.44–1.81 (m, 4 H), 2.65 (t, J = 7 Hz, 2 H), 3.17 (m, 2 H), 3.53 (q, J = 7 Hz, 2 H), 4.15 (m, 2 H), 5.04 (s, 2 H), 5.10 (s, 2 H), 7.29–7.52 (m, 12 H), 7.64 (d, J = 8 Hz, 1 H), 7.78 (d, J = 8 Hz, 1 H), 8.04 (s, 1 H), 8.57 (t, J = 6 Hz, 1 H), 10.21 (s, 1 H); MS m/z (FAB) 342 (M + H)$^+$. Anal. ($C_{31}H_{35}N_7O_8 \cdot 0.5H_2O$) C, H, N.

***N*-[*m*-(L-Arginylamino)benzoyl]-β-alanine (25d).** The benzyl ester **25c** (0.9 g, 1.42 mmol), 5% Pd/C (0.3 g), and formic acid (20 mL) were stirred in a hydrogen atmosphere (1 atm) overnight. The catalyst was removed by filtration, and the solvents were evaporated in vacuo. The residue was evaporated twice with H_2O, giving **25d** formate as a white foam (545 mg, 88%); $[\alpha]^{20}_D$ = +39° (c = 0.5, H_2O); IR (KBr) 3344, 3259, 3168, 3081, 2957, 1631, 1564, 1400, 1346, 754 cm^{-1}; NMR (D_2O) δ 1.77 (m, 2 H), 2.09 (m, 2 H), 2.57 (t, J = 6.6 Hz, 2 H), 3.28 (t, J = 6.6 Hz, 2 H), 3.61 (t, J = 6.6 Hz, 2 H), 4.24 (t, J = 6.6 Hz, 2 H), 7.50–7.69 (m, 3 H), 7.83 (t, J = 1.5 Hz, 1 H), 8.46 (s, 1 H); MS m/z (FAB) 365 (M + H)$^+$. Anal. ($C_{16}H_{24}N_6O_4 \cdot 1.5CH_2O_2 \cdot 0.5H_2O$) C, H, N. The formate salt of **25d** in H_2O was loaded on an ion exchange resin (Amberlite IR 120 H^+). After washing to neutrality with H_2O, 2% aqueous ammonia eluated **25d**: mp 93–95 °C (H_2O); NMR (D_2O) δ 1.55–1.86 (m, 4 H), 2.53 (t, J = 6.6 Hz, 2 H), 3.22 (m, 2 H), 3.60 (t, J = 6.6 Hz, 3 H), 7.44–7.68 (m, 3 H), 7.77 (s, 1 H); MS m/z (FAB) 365 (M + H)$^+$.

Compound **26b** was prepared via **26a** from *N*-[(benzyloxy)carbonyl]-3-(*p*-cyanophenyl)-D,L-alanine[44] and **24b** according to standard procedure D followed by standard procedure B.

***N*-[*m*-[3-(*p*-Amidinophenyl)-D,L-alanyl]benzoyl]-β-alanine (26c) acetate (1:1)** was prepared from **26b** in AcOH as described above for **25d** and was isolated as a white foam in 90% yield: IR (KBr) 3273, 3056, 2961, 1690, 1638, 1559, 1483, 1398, 1301 cm^{-1}; NMR (D_2O) δ 1.96 (s, 3 H), 2.53 (t, J = 7 Hz, 2 H), 3.27–3.57 (m, 2 H), 3.60 (t, J = 7 Hz, 2 H), 4.41 (d, J = 6, 9 Hz, 1 H), 7.38–7.59 (m, 6 H), 7.77 (d, J = 8.5 Hz, 2 H); MS m/z (FAB) 398 (M + H)$^+$. Anal. ($C_{20}H_{23}N_5O_4 \cdot 1.25C_2H_4O_2 \cdot 0.5H_2O$) C, H, N.

***N*-[*m*-[*p*-[*N*-[(Benzyloxy)carbonyl]amidino]benzamido]benzoyl]-β-alanine Benzyl Ester (27a). (a) *p*-Amidinobenzoyl Chloride Hydrochloride.** A suspension of *p*-amidinobenzoic acid (820 mg, 5 mmol) in THF (5 mL), thionyl chloride (5 mL), and DMF (0.25 mL) was stirred overnight at 20 °C. The solvents were evaporated in vacuo, and the resultant *p*-amidinobenzoyl chloride hydrochloride was dried to constant weight. **(b) Preparation of 27a.** To a vigorously stirred mixture of the TFA salt of **24b** (2.06 g, 5 mmol), CH_2Cl_2 (75 mL), saturated $NaHCO_3$ (25 mL), and H_2O (10 mL) was added *p*-amidinobenzoyl chloride hydrochloride (1.09 g, 5 mmol) in portions. After stirring for 1 h, saturated Na_2CO_3 (10 mL) and benzyl chloroformate (0.8 mL, 5 mmol) were added, and the mixture was stirred for 2 h. After dilution of the reaction mixture with CH_2Cl_2/MeOH 9:1, the organic layer was separated, washed with H_2O, and evaporated in vacuo. Stirring the crude product in acetone gave **27a** (868 mg, 30%). An analytical sample was obtained by chromatography (silica gel, $CHCl_3$/EtOH 24:1) and stirring the purified product in acetone: mp 191–193 °C; IR (KBr) 3420, 3309, 1734, 1644, 1609, 1532, 1494, 1300, 1256, 820, 749, 696, 600 cm^{-1}; NMR (DMSO-d_6) δ 2.67 (t, J = 6.5 Hz, 2 H), 3.55 (q, J = 6.5 Hz, 2 H), 5.11 (s, 2 H), 5.13 (s, 2 H), 7.26–7.59 (m, 12 H), 7.96 (br d, J = 7.5 Hz, 1 H), 8.06 (d, J = 7.5 Hz, 2 H), 8.10 (d, J = 7.5 Hz, 2 H), 8.24 (br s, 1 H), 8.60 (t, J = 6 Hz, 1 H), 9.24 (br s, 2 H), 10.55 (s, 1 H); MS m/z (FAB) 579 (M + H)$^+$. Anal. ($C_{33}H_{30}N_4O_6$) C, H, N.

***N*-[*m*-(*p*-Amidinobenzamido)benzoyl]-β-alanine (27b).** A mixture of **27a** (980 mg, 1.7 mmol), 5% Pd/C (330 mg), AcOH (20 mL), and H_2O (10 mL) was stirred under H_2 gas (1 atm) for 4 h. The catalyst was removed by filtration, and the solvents were evaporated in vacuo. The residue was dissolved in H_2O and evaporated again to give a crystalline mass which, after suspension in H_2O (10 mL), was adjusted to pH 8 with ammonia. The solid was collected by filtration, washed with H_2O, and dried to give **27b** (574 mg, 89%): mp >280 °C; IR (KBr) 3495, 3409, 3279, 3060, 2951, 1700, 1656, 1552, 1481, 1404, 1310, 861, 749, 704, 589 cm^{-1}; NMR (1 N NaOD, D_2O) δ 2.53 (t, J = 7 Hz, 2 H), 3.59 (t, J = 7 Hz, 2 H), 7.26–7.51 (m, 4 H), 7.75 (part of AA'BB', 2 H), 7.94 (part of AA'BB', 2 H); MS m/z (thermospray) 355 (M + H)$^+$. Anal. ($C_{18}H_{18}N_4O_4 \cdot 1.25H_2O$) C, H, N.

Compound **28d** was prepared via **28c** from 4-[1-(benzyloxy)formamido]butyric acid and H-Asp(*O*-*t*-Bu)-Val-*O*-*t*-Bu[25] according to standard procedure A followed by catalytic hydrogenation (10% Pd/C, EtOH, 1 atm).

***N*-[3-(Benzyloxycarbonyl)-*N*-[*N*-(*tert*-butoxycarbonyl)-β-alanyl]-L-alanyl]-3-phenyl-L-alanine Benzyl Ester (28e).** From H-Asp(*O*-Bzl)-Phe-*O*-Bzl·TFA (12 g, 21 mmol, prepared as described for **28h**) and Boc-β-alanine (4.35 g, 23 mmol) according to standard procedure D after FC (hexane/EtOAc 1:1) **28e** (10.6 g, 80%) was obtained: mp 124–125 °C; $[\alpha]^{20}_D$ = −29° (c = 0.5, MeOH); IR (KBr) 3301, 1732, 1689, 1645, 1537, 1173, 688 cm^{-1}; NMR (DMSO-d_6) δ 1.37 (s, 9 H), 2.24 (t, J = 6 Hz, 2 H), 2.50 (m, 1 H), 2.72 (dd, J = 4, 12 Hz, 1 H), 3.05 (m, 4 H), 4.50 (m, 1 H), 4.70 (m, 1 H), 5.06 (m, 4 H), 6.74 (m, 1 H), 7.24 (m, 15 H), 8.20 (d, J = 7 Hz, 1 H), 8.36 (d, J = 6 Hz, 1 H); MS m/z (FAB) 532 (M + H − CO_2 − C_4H_8)$^+$.

Compound **28g** was produced via **28f** from Z-β-Ala-OH and H-Asp(*O*-*t*-Bu)-Tyr(*t*-Bu)-O-*t*-Bu[45] according to standard procedure A followed by catalytic hydrogenation (10% Pd/C, EtOH, 1 atm).

***N*-[3-[(Benzyloxy)carbonyl]-*N*-[5-(1-*tert*-butoxyformamido)valeryl]-L-alanyl]-3-phenyl-L-alanine Benzyl Ester (28h).** (a) Boc-Asp(*O*-Bzl)-OH was coupled with H-Phe-*O*-Bzl according to standard procedure D to give Boc-Asp(*O*-Bzl)-Phe-*O*-Bzl (64%): mp 93–94 °C (diisopropyl ether); $[\alpha]^{20}_D$ = −20.7° (c = 1.0, MeOH); IR (KBr) 3361, 1738, 1669, 1530, 1309, 1178, 1002, 750, 728, 698 cm^{-1}; NMR (DMSO-d_6, mixture of rotamers ca. 6:1) δ 1.22 and 1.36 (2 s, 9 H), 2.50–2.71 (m, 2 H), 3.01 (m, 2 H), 4.38 (m, 1 H), 4.52 (q, J = 7 Hz, 1 H), 5.00–5.15 (m, 4 H), 7.12 (d, J = 9 Hz, 1 H), 7.16–7.40 (m, 15 H), 8.28 (d, J = 7 Hz, 1 H); MS m/z (FAB) 561 (M + H)$^+$. (b) This material was deprotected to the TFA salt of H-Asp(*O*-Bzl)-Phe-*O*-Bzl using the conditions of standard procedure C. Coupling of the intermediate amine with 5-(1-*tert*-butoxyformamido)valeric acid (standard procedure D) provided **28h**: mp 119.5–120.5 °C (EtOH); $[\alpha]^{20}_D$ = −25.6° (c = 0.5, MeOH); IR (KBr) 3361, 3312, 1732, 1682, 1646, 1600, 1529, 1281, 1172, 872, 749, 698 cm^{-1}; NMR (DMSO-d_6) δ 1.37 (s, 9 H, overlapped by m, 4 H), 2.03 (t, J = 7 Hz, 2 H), 2.45–3.10 (m, 6 H), 4.50 (q, J = 7 Hz, 1 H), 4.70 (m, 1 H), 5.06 (m, 4 H), 6.76 (t, J = 5 Hz, 1 H), 7.14–7.42 (m, 15 H), 8.09 (d, J = 8 Hz, 1 H), 8.33 (d, J = 7 Hz, 1 H); MS m/z (FAB) 660 (M + H)$^+$. Anal. ($C_{37}H_{45}N_3O_8$) C, H, N.

(44) Voigt, B.; Wagner, G. Synthesis of Nα-Benzyloxycarbonyl-4-amidinophenylalanine Amides as Thrombin Inhibitors. *Pharmazie* 1985, *40*, 305–306.

(45) Hamada, Y.; Rishi, S.; Shioiri, T.; Yamada, S. Amino Acids and Peptides. XXVI. Phosphorus in Organic Synthesis XV. Application of Diphenyl Phosphorazidate (DPPA) and Diethyl Phosphorocyanidate (DEPC) to the Synthesis of the N-Terminal Decapeptide of Gastric Inhibitory Polypeptide. *Chem. Pharm. Bull.* 1977, *25*, 224–230.

N-[3-(tert-Butoxycarbonyl)-N-[N-(p-cyanobenzoyl)glycyl]-L-alanyl]-3-phenyl-L-alanine tert-Butyl Ester (29a). From H-Gly-Asp(O-t-Bu)-Phe-O-t-Bu **28b**[25] (674 mg, 1.5 mmol, prepared from **28a** by hydrogenolytic removal of the benzyloxycarbonyl group) and p-cyanobenzoic acid (243 mg, 1.65 mmol) according to standard procedure A and crystallization (EtOAc) **29a** (477 mg, 55%) was obtained: mp 177–178 °C; NMR (DMSO-d_6) δ 1.39 (s, 9 H), 1.43 (s, 9 H), 2.59 (dd, J = 7, 14 Hz, 1 H), 2.89 (dd, J = 4, 14 Hz, 1 H), 3.08 (m, 2 H), 4.09 (m, 2 H), 4.67 (m, 1 H), 4.82 (m, 1 H), 7.23 (m, 8 H), 7.71 (part of AA'BB', 2 H), 7.90 (part of AA'BB', 2 H); MS m/z (EI) 579 (M + H)$^+$.

Compound **29c** was synthesized via **29b** from **29a** according to standard procedure B followed by standard procedure C. Compound **29d** was prepared from p-cyanobenzoic acid and **28d** according to standard procedure D. Transformation of **29d** to **29e** and further to **29f** was carried out according to standard procedure B and C, respectively.

N-[N-[N-(p-Amidinobenzoyl)-β-alanyl]-3-(benzyloxycarbonyl)-L-alanyl]-3-phenyl-L-alanine Benzyl Ester (29g). The deprotection of **28e** (10.5 g, 16.6 mmol) was carried out according to standard procedure C. After removal of the solvents, the residue was crystallized in Et_2O to give the TFA salt of H-β-Ala-Asp(O-Bzl)-Phe-O-Bzl in quantitative yield. The TFA salt of H-β-Ala-Asp(O-Bzl)-Phe-O-Bzl (6.45 g, 10 mmol) was added to a suspension of p-amidinobenzoyl chloride hydrochloride (2.63 g, 12 mmol) in pyridine (80 mL) at room temperature. After 16 h the solvents were evaporated. FC of the residue (silica gel, CH_2Cl_2/MeOH 0–50%) gave the HCl salt of **29g** (2.53 g, 33%): mp 124 °C dec; IR (KBr) 3291, 1737, 1648, 1541, 698 cm^{-1}; NMR (DMSO-d_6) δ 2.41 (m, 2 H), 2.50 (m, 1 H), 2.73 (m, 1 H), 3.00 (m, 2 H), 3.48 (m, 2 H), 4.50 (q, J = 7 Hz, 1 H), 4.75 (m, 1 H), 5.07 (m, 4 H), 7.29 (m, 15 H), 7.88 (part of AA'BB', 2 H), 8.02 (part of AA'BB', 2 H), 8.33 (d, J = 8 Hz, 1 H), 8.43 (d, J = 8 Hz, 1 H), 8.77 (t, J = 5 Hz, 1 H), 9.28 (br s, 4 H); MS m/z (ISP) 678 (M + H)$^+$.

N-[N-[N-(p-Amidinobenzoyl)-β-alanyl]-L-α-aspartyl]-3-phenyl-L-alanine (29h). A solution of **29g** (3.7 g, 5.2 mmol) in AcOH (40 mL) was hydrogenated at 50 °C and 10 bar over 10% Pd/C. After 6 h the catalyst was filtered off, the solvents were evaporated, and the residue was purified by chromatography (RP18, 0.04% TFA/THF 0–30%) to yield the TFA salt of **29h** (1.1 g, 35%): mp 205 °C dec; IR (KBr) 3289, 1651, 1542, 1195, 1163, 700 cm^{-1}; NMR (DMSO-d_6) δ 2.43 (m, 3 H), 2.66 (dd, J = 5, 16 Hz, 1 H), 2.91 (dd, J = 8, 14 Hz, 1 H), 3.06 (dd, J = 5, 14 Hz, 1 H), 3.50 (m, 2 H), 4.40 (m, 1 H), 4.63 (m, 1 H), 7.25 (m, 5 H), 7.87 (part of AA'BB', 2 H), 8.02 (m, 3 H), 8.23 (d, J = 10 Hz, 1 H), 8.73 (t, J = 6 Hz, 1 H), 9.19 (s, 1 H), 9.40 (s, 1 H), 12.60 (br s, 2 H); MS m/z (ISP) 498 (M + H)$^+$. Anal. ($C_{24}H_{27}N_5O_7 \cdot 1.0C_2HF_3O_2$) C, H, F, N.

Compound **29k** was produced from **28g** via **29i** followed by deprotection according to standard procedure C.

N-[3-(Benzyloxycarbonyl)-N-[5-[p-[N-(benzyloxycarbonyl)amidino]benzamido]valeryl]-L-alanyl]-3-phenyl-L-alanine Benzyl Ester (29l). The Boc derivative **28h** was deprotected in TFA/CH_2Cl_2 1:1 (standard procedure C), coupled with p-amidinobenzoyl chloride, and treated with benzyl chloroformate in the presence of $NaHCO_3$ as described above for **27a**. After chromatography (silica gel, CH_2Cl_2/2-propanol) and crystallization from MeOH **29l** was obtained in 21% overall yield: mp 169–171 °C; NMR (DMSO-d_6) δ 1.50 (m, 4 H), 2.10 (m, 2 H), 2.45–2.58 (dd, overlapped by DMSO, 1 H), 2.71 (dd, J = 5, 15, Hz, 1 H), 3.00 (m, 2 H), 3.26 (m, 2 H), 4.50 (q, J = 7 Hz, 1 H), 4.72 (m, 1 H), 5.05 (s, 4 H), 5.12 (s, 2 H), 7.14–7.46 (m, 20 H), 7.93 (part of AA'BB', 2 H), 8.05 (part of AA'BB', 2 H), 8.14 (d, J = 9 Hz, 1 H), 8.33 (d, J = 8 Hz, 1 H), 8.60 (t, J = 6 Hz, 1 H), 9.20 (br s, 2 H); MS m/z (FAB) 840 (M + H)$^+$.

N-[N-[5-(p-Amidinobenzamido)valeryl]-L-α-aspartyl]-3-phenyl-L-alanine (29m) was prepared from **29l** as described for **25b**. Stirring the crude crystalline mass in H_2O provided after filtration **29m**: yield 88%; mp 241 °C; $[\alpha]^{20}_D$ = −16.2° (c = 0.5, DMSO); IR (KBr) 3700–2200, 1642, 1546, 1398, 1311, 1227, 864, 700 cm^{-1}; NMR (DMSO-d_6) δ 1.52 (m, 4 H), 2.12 (m, 2 H), 2.38 (dd, J = 9, 16 Hz, 1 H), 2.65 (dd, J = 5, 16 Hz, 1 H), 2.90–3.14 (m, 2 H), 3.25 (m, 2 H), 4.13 (q, J = 6 Hz, 1 H), 4.53 (q, J = 7 Hz, 1 H), 7.08–7.25 (m, 5 H), 7.42 (d, J = 6 Hz, 1 H), 7.84 (part of AA'BB', 2 H), 8.06 (part of AA'BB', 2 H), 8.25 (d, J = 8 Hz, 1 H), 8.82 (br t, J = 6 Hz, 1 H), 9.12 (br s, 2 H), 11.19 (br s, 2 H); MS m/z (FAB) 526 (M + H)$^+$. Anal. ($C_{26}H_{31}N_5O_7 \cdot 2H_2O$) C, H, N.

Compound **30a** was prepared from Boc-β-alanine and β-alanine benzyl ester according to standard procedure D.

N-[p-[N-(tert-Butoxycarbonyl)amidino]benzoyl]-β-alanine Benzyl Ester (30b). β-Alanine benzyl ester p-toluenesulfonate (3.51 g, 10 mmol) was added to a well stirred mixture of CH_2Cl_2 (60 mL) and saturated aqueous $NaHCO_3$ (50 mL). When evolution of CO_2 had ceased, p-amidinobenzoyl chloride·HCl (2.2 g, 10 mmol) was added to the vigorously stirred mixture. After 3.5 h saturated aqueous Na_2CO_3 (40 mL) and di-tert-butyl dicarbonate (2.62 g, 12 mmol) was added and stirring continued overnight. The reaction mixture was then diluted with $CHCl_3$ and a small amount of MeOH and washed successively with ice cold 0.2 N HCl, diluted $NaHCO_3$, and H_2O. The organic layer was filtered, and the solvents were removed in vacuo. Crystallization of the crude product from MeCN gave **30b** (2.85 g, 67%): mp 127–128 °C; IR (KBr) 3346, 3245, 1737, 1634, 1532, 1277, 1169, 1139, 740, 696 cm^{-1}; NMR (DMSO-d_6) δ 1.45 (s, 9 H), 2.68 (t, J = 7 Hz, 2 H), 3.55 (q, J = 6 Hz, 2 H), 5.11 (s, 2 H), 7.36 (m, 5 H), 7.89 (part of AA'BB', 2 H), 8.01 (part of AA'BB', 2 H), 8.73 (t, J = 6 Hz, 1 H), 9.07 (br s, 1 H); MS m/z (FAB) 426 (M + H)$^+$. Anal. ($C_{23}H_{27}N_3O_5$) C, H, N.

N-[p-[N-(tert-Butoxycarbonyl)amidino]benzoyl]-β-alanine (30c) was obtained as a white foam in quantitative yield by catalytic hydrogenation of **30b** over Pd/C in EtOH: IR (KBr) 3600–2300, 1716, 1623, 1546, 1279, 1145, 863 cm^{-1}; NMR (DMSO-d_6) δ 1.45 (s, 9 H), 2.50 (m overlapped by DMSO, 2 H), 3.46 (q, J = 6.5 Hz, 2 H), 7.92 (part of AA'BB', 2 H), 8.01 (part of AA'BB', 2 H), 8.69 (t, J = 6 Hz, 1 H), 9.06 (br s, 2 H); MS m/z (FAB) 336 (M + H)$^+$.

Compound **31b** was obtained from **30a** via **31a** in the same way as described for **29m**.

Benzyl (S)-3-[[N-[p-[N-(tert-butoxycarbonyl)amidino]-benzoyl]-β-alanyl]amino]-3-[(p-methoxyphenethyl)carbamoyl]propionate (31c) was prepared by activation of **30c** with CDMT (standard procedure D) and coupling with H-Asp(O-Bzl) 2-(4-methoxyphenyl)ethyl amide.[46] Pure **31c** was obtained by chromatography (silica gel, CH_2Cl_2/2-propanol 19:1 to 12:1) in 14% yield: mp 150 °C dec; IR (KBr) 3382, 3312, 1734, 1654, 1616, 1513, 1280, 1250, 1171 cm^{-1}; NMR (DMSO-d_6) δ 1.45 (s, 9 H), 2.43 (m, 2 H), 2.59 (m, 3 H), 2.76 (dd, J = 6, 16 Hz, 1 H), 3.19 (m, 2 H), 3.50 (q, J = 6 Hz, 2 H), 3.70 (s, 3 H), 4.66 (q, J = 7 Hz, 1 H), 5.06 (s, 2 H), 6.85 (part of AA'BB', 2 H), 7.08 (part of AA'BB', 2 H), 7.35 (m, 5 H), 7.92 (m, 3 H), 8.02 (part of AA'BB', 2 H), 8.29 (d, J = 9 Hz, 1 H), 8.65 (t, J = 6 Hz, 1 H), 9.07 (br s, 2 H); MS m/z (FAB) 674 (M + H)$^+$.

(S)-3-[[N-(p-Amidinobenzoyl)-β-alanyl]amino]-3-[(p-methoxyphenethyl)carbamoyl]propionic Acid (31d). The benzyl ester **31c** (0.30 g, 0.45 mmol) and 5% Pd/C (75 mg) in formic acid (6 mL) was stirred under H_2 (1 atm) for 4 h and allowed to stand under argon overnight. The catalyst was removed by filtration, and the solvents were evaporated in vacuo. Residual formic acid was removed by repeated evaporation with H_2O. The crude solid was taken up in H_2O and the suspension adjusted to pH = 8 with ammonia. The precipitates were collected by filtration to give **31d** (151 mg, 64%): mp 217 °C; IR (KBr) 3650–2300, 1646, 1549, 1511, 1393, 1245 cm^{-1}; NMR (D_2O) δ 2.53–2.83 (m, 6 H), 3.29 (t, J = 7 Hz, 2 H), 3.65 (m, 2 H), 3.76 (s, 3 H), 4.61 (t, J = 6.6 Hz, 1 H), 6.89 (part of AA'BB', 2 H), 7.11 (part of AA'BB', 2 H), 7.83 (part of AA'BB', 2 H), 7.89 (part of AA'BB', 2 H); MS m/z (FAB) 484 (M + H)$^+$. Anal. ($C_{24}H_{29}N_5O_6 \cdot 0.2CH_2O_2 \cdot 1.8H_2O$) C, H, N.

tert-Butyl (4-Piperidinyloxy)acetate (33). To a solution of 4-hydroxypiperidine (**32**) (Fluka, 50 g, 0.496 mol) in CH_2Cl_2 (500 mL) were added successively triethylamine (69 mL, 0.496 mol) and benzyl chloroformate (70.2 mL, 0.496 mol) at 0 °C. The reaction mixture was left at room temperature for 16 h. The resulting suspension was filtered, the solvents were evaporated, and the residue was taken up in EtOAc (500 mL). After washing with H_2O (2 × 200 mL) and 1 N HCl (200 mL), the organic extracts were dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo to give benzyl 4-hydroxypiperidinecarboxylate

(46) Tjoeng, F. S.; Adams, S. P. US Patent 4,879,313, November 7, 1989.

(73.6 g, 63%) as a pale yellow oil: R_f = 0.56 (EtOAc/MeOH 1:1); NMR ($CDCl_3$) δ 1.51 (m, 2 H), 1.64 (br s, 1 H), 1.85 (m, 2 H), 3.14 (ddd, J = 4, 7, 13 Hz, 2 H), 3.87 (m, 3 H), 5.12 (br s, 2 H), 7.34 (m, 5 H); MS m/z (EI) 235 (1, M^+), 91 (100). To a solution of benzyl 4-hydroxypiperidinecarboxylate (30.1 g, 128 mmol) was added successively *tert*-butyl bromoacetate (28 mL, 192 mmol) and tetra-*n*-butylammonium hydrogen sulfate (1.4 g, 4.1 mmol) dissolved in H_2O (10 mL). Dropwise addition of a solution of sodium hydroxide (125 g) in H_2O (125 mL) was followed by vigorous stirring for 16 h. The organic layer was then separated, dried over anhydrous sodium sulfate, filtered, and evaporated in vacuo to give benzyl 4-[(*tert*-butyloxycarbonyl)methoxy]-1-piperidinecarboxylate (34.1 g, 76%) as a white crystalline solid: R_f = 0.76 (EtOAc); mp 66–67 °C (hexane); NMR ($CDCl_3$) δ 1.48 (s, 9 H), 1.60 (m, 2 H), 1.86 (m, 2 H), 3.23 (ddd, J = 4, 7, 13 Hz, 2 H), 3.58 (m, 1 H), 3.84 (m, 2 H), 4.00 (s, 2 H), 5.12 (br s, 2 H), 7.34 (m, 5 H); MS m/z (EI) 293 (0.4, M – $C_4H_8^+$), 91 (100). A mixture of 10% Pd/C (1.5 g) and benzyl 4-[(*tert*-butyloxycarbonyl)methoxy]-1-piperidinecarboxylate (30 g, 86 mmol) in EtOH (500 mL) was shaken under a hydrogen atmosphere (1 atm) until the hydrogen consumption stopped (3 h). The solution was degassed and filtered, and the solvents were evaporated in vacuo to give *tert*-butyl (4-piperidinyloxy)acetate (**33**) (17.4 g, 94%) as a pale yellow oil: R_f = 0.14 (EtOAc/MeOH 1:1); NMR ($CDCl_3$) δ 1.48 (s, 9 H), 1.54 (m, 2 H), 1.95 (m, 2 H), 2.43 (br s, 1 H), 2.64 (ddd, J = 3, 10, 14 Hz, 2 H), 3.12 (m, 2 H), 3.47 (m, 1 H), 4.00 (s, 2 H); MS m/z (EI) 215 (0.6 M^+), 84 (100).

***tert*-Butyl [(1-Glycyl-4-piperidinyl)oxy]acetate (34a).** The reaction of Z-Gly-OH (Fluka, 5.8 g, 27.9 mmol) and **33** (6.0 g, 27.9 mmol) according to standard procedure D as described for **9e** afforded benzyl [[4-[(*tert*-butoxycarbonyl)methoxy]piperidinyl]-carbonyl]methyl]carbamate (10 g, 88%) as a pale yellow oil: MS m/z (EI) 406 (0.8, M^+), 91 (100). A solution of this material (10 g, 24.6 mmol) in EtOH (200 mL) and AcOH (1.4 mL) was hydrogenated over 10% Pd/C (0.7 g) at atmospheric pressure. After 3 h the solution was degassed and filtered, and the solvents were evaporated in vacuo. The residue was purified by chromatography (silica gel, EtOAc/MeOH 1:1) to give **34a** (4.1 g, 61%) as colorless oil; IR (film) 3367, 1746, 1648 cm^{-1}; NMR ($CDCl_3$) δ 1.48 (s, 9 H), 1.68 (m, 2 H), 1.84 (m, 2 H), 2.80 (br s, 2 H), 3.25 (m, 1 H), 3.41 (br s, 1 H), 3.50 (br s, 1 H), 3.40–3.70 (m, 2 H), 3.85 (m, 1 H), 4.01 (s, 2 H), 4.10 (m, 1 H); MS m/z (EI) 273 (1, M + H^+), 30 (100).

Compounds **34b–f** were prepared in a similar manner from **33** and Z-Ala-OH, Z-D-Ala-OH, Z-Orn(Boc)-OH, Z-Asp-(*O*-*t*-Bu)-OH, and Z-Tyr(*t*-Bu)-OH, respectively.

Compounds **36a,b** were obtained by acylation of L-proline **35a** and (*S*)-2-methylproline hydrobromide (**35b**),[47] respectively, with *p*-cyanobenzoyl chloride followed by coupling with **33**.

[[1-[*N*-(*p*-Amidinobenzoyl)glycyl]-4-piperidinyl]oxy]acetic Acid (37a). To a mixture of the acetate salt of **34a** (4.1 g, 12.3 mmol) and tetra-*n*-butylammonium hydrogen sulfate (30 mg) in CH_2Cl_2 (120 mL) and saturated aqueous $NaHCO_3$ was added in portions *p*-amidinobenzoyl chloride (2.95 g, 13.5 mmol, prepared as described for **27a**) at room temperature. After vigorous stirring for 16 h, the reaction mixture was diluted by addition of CH_2Cl_2/H_2O 1:1 (100 mL). The organic layer was separated and dried over Na_2SO_4. Evaporation of the solvents gave *tert*-butyl [[1-*N*-(*p*-amidinobenzoyl)glycyl]-4-piperidinyl]-oxy]acetate (2.43 g, 47%) as a pale yellow foam: MS m/z (FAB) 419 (M + H^+). This material was subjected to standard procedure C as described for **7a** to give after chromatography (RP 18, H_2O/MeOH 0–15%) the TFA salt of **37a** (1.56 g, 56%): mp 233–236 °C; IR (KBr) 3322, 1667, 1631 cm^{-1}; NMR (D_2O) δ 1.62 (m, 2 H), 2.08 (m, 2 H), 3.17 (m, 1 H), 3.35 (m, 1 H), 3.81 (m, 2 H), 4.01 (s, 2 H), 4.06 (m, 1 H), 4.39 (s, 2 H), 7.94 (AA'BB', 4 H); MS m/z (EI) 363 (M + H^+). Anal. ($C_{17}H_{22}N_4O_5 \cdot 0.35C_2HF_3O_2$) C, N, H: calcd, 5.60; found, 6.09.

[[1-[*N*-(*p*-Amidinobenzoyl)-L-tyrosyl]-4-piperidinyl]oxy]-acetic Acid (37f). Starting from **34f** the usual two-step procedure described for **37a** yielded the TFA salt of **37f** (26% overall yield). To a solution of the crude TFA salt of **37f** (123 g) in H_2O (800 mL) was added charcoal. The mixture was filtered and the pH of the filtrate adjusted to 7.2 by careful addition of concentrated NaOH (28% in H_2O). The solution was concentrated to a volume of 300 mL and passed through silica gel RP-18 (600 g, H_2O/AcOH 0–10%). The fractions containing **37f** were evaporated, and the resulting residue was crystallized from H_2O to give the zwitterionic **37f** (39.1 g): mp over 200 °C dec; $[\alpha]^{20}_D$ = +29.8° (c = 0.86, 1 N HCl); IR (KBr) 3407, 1617, 1540, 1105 cm^{-1}; NMR (DMSO-d_6, rotamers) δ 1.05–1.45 (m, 2 H), 1.55–1.95 (m, 2 H), 2.75–3.15 (m, 3 H), 3.20–3.54 (m, 3 H), 3.64 and 3.70 (2 br s, 2 H), 3.80 and 4.10 (2 m, 1 H), 5.06 (m, 1 H), 6.62 (part of AA'BB', 2 H), 7.05 (part of AA'BB', 2 H), 7.83 (part of AA'BB', 2 H), 8.04 (part of AA'BB', 2 H), 9.00 and 9.12 (2 d, J = 8 Hz, 1 H), 9.86 (br s, 5 H); MS m/z (ISP) 469.2 (M + H^+). Anal. ($C_{24}H_{28}N_4O_6$) C, H, N.

Compounds **37b–e** were synthesized in a similar manner from the corresponding amines **34b–e**.

Compounds **37g,h** were prepared from the corresponding nitriles **36a,b**, respectively, according to standard procedures B and C.

1-[[[*N*-[(5-Amidino-2-pyridyl)carbonyl]-L-alanyl]-4-piperidinyl]oxy]acetic Acid (37i). Coupling of **34b** (12.0 g, 34.7 mmol) with 5-cyano-2-pyridinecarboxylic acid[41] (5.0 g, 33.8 mmol) as described in standard procedure D for **9e** provided *tert*-butyl [[1-[*N*-[(5-cyano-2-pyridyl)carbonyl]-L-alanyl]-4-piperidinyl]-oxy]acetate (11.9 g, 81%) as a pale yellow oil: IR (film) 3376, 2234, 1747, 1680, 1643 cm^{-1}; MS m/z (FAB) 417 (M + H^+). This material (11.9 g, 28.6 mmol) was converted to the acetate salt of the corresponding amidine (6.6 g, 47% overall yield after chromatography RP-18, H_2O/MeOH 0–15%) as outlined in standard procedure B for **7a**: mp 142–145 °C; IR (KBr) 3138, 1745, 1676, 1636, 1400 cm^{-1}; NMR (DMSO-d_6, rotamers) δ 1.34 (br d, J = 7 Hz, 3 H), 1.42 (s, 9 H), 1.35–1.95 (m, 4 H), 1.75 (s, 3 H), 3.02–3.44 (m, 2 H), 3.50–4.00 (m, 3 H), 4.03 and 4.05 (2 s, 2 H), 4.97 (m, 1 H), 8.20 (m, 1 H), 8.40 (m, 1 H), 8.95 (m, 1 H), 9.03 (m, 1 H); MS m/z (FAB) 434 (M + H^+). To a solution of this material (1.6 g, 3.25 mmol) in AcOH (5 mL) was added at room temperature AcOH saturated with HCl (5 mL). After 45 min, the solvents were evaporated, and the residue was purified by chromatography (RP-18, H_2O/MeOH 0–15%). The product obtained was dissolved in THF and reprecipitated with EtOAc to give **37i** (0.76 g, 62%); mp >200 °C; $[\alpha]^{20}_D$ (hydrochloride salt) = +34° (c = 1.0, H_2O); IR (KBr) 3385, 1677, 1640, 1517, 1107 cm^{-1}; NMR (D_2O, rotamers) δ 1.49 (2 d, J = 7 Hz, 3 H), 1.45–1.76 (m, 2 H), 2.05 (m, 2 H), 3.06–3.58 (m, 2 H), 3.77 (m, 1 H), 3.88–4.22 (m, 2 H), 3.99 (2 s, 2 H), 5.10 (2 q, J = 7 Hz, 1 H), 8.23 (m, 1 H), 8.40 (m, 1 H), 9.01 (m, 1 H); MS m/z (FAB) 378 (M + H^+). Anal. ($C_{17}H_{23}N_5O_5 \cdot 0.25C_4H_8O_2$) C, H, N.

Biochemistry/Pharmacology. Purification of Glycoprotein IIb-IIIa (GP IIb-IIIa). Outdated, washed human platelets were lysed with 1% Triton X-100, 150 mmol/L NaCl, 20 mmol/L Tris-HCl, 1 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, 0.02% NaN_3, 10 μmol/L leupeptin, 0.5 mmol/L phenylmethanesulfonyl fluoride, and 2 mmol/L *N*-ethylmaleimide, pH 7.3, at 4 °C for 15 h. The glycoproteins were isolated using a concanavalin A-Sepharose 4B and an aminoethylglycine (Aeg)-RGDS affinity column as described previously.[10] The GP IIb-IIIa retained on the Aeg-RGDS column (active GP IIb-IIIa) was specifically eluted with buffer A (0.1% Triton X-100, 150 mmol/L NaCl, 20 mmol/L Tris-HCl, 1 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, 0.05% NaN_3, pH 7.0) containing 3 mmol/L RGDV. The protein concentrations were determined by the method of Bradford,[48] and the purity of the eluted GP IIb-IIIa was determined to be >95% by electrophoretic evaluation. Aliquots of the purified GP IIb-IIIa were stored at −80 °C.

Purification of the Vitronectin Receptor ($\alpha_v\beta_3$). Human $\alpha_v\beta_3$ was purified from human placenta. The placentas were stored at −80 °C immediately after delivery. To extract the receptor, each placenta was thawed slightly and sliced into small pieces with a scalpel. The pieces were washed twice with a buffer containing 150 mmol/L NaCl, 1 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$,

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and 20 mmol/L Tris-HCl, pH 7.4. The proteins were extracted by incubating them at room temperature for 1 h in a lysis buffer containing 1% Triton X-100, 150 mmol/L NaCl, 1 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, 20 mmol/L Tris-HCl, 0.02% NaN_3, 0.5 mmol/L phenylmethanesulfonyl fluoride, 1 mmol/L leupeptin, and 2 mmol/L *N*-ethylmaleimide, pH 7.4, and filtered through sterile gauze. The filtrate was centrifuged for 30 min at 30000*g* at 4 °C and the vitronectin receptor was purified according to the procedure described above for platelet GP IIb-IIIa. Briefly, the glycoproteins were first isolated on a concanavalin A-Sepharose 4B column, and the bound and eluted proteins were then applied on an Aeg-RGDS affinity column. Following extensive washing, the bound vitronectin receptors were eluted by including 3 mmol/L RGDS in buffer A.

Fibrinogen Binding to Immobilized GP IIb-IIIa and $\alpha_v\beta_3$ (Solid-Phase Assays). The wells of plastic microtiter plates (Nunc-Immunoplate MaxiSorp) were coated overnight at 4 °C with purified active GP IIb-IIIa or $\alpha_v\beta_3$ at 0.5 μg/mL (100 μL/well) in a buffer containing 150 mmol/L NaCl, 1 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, 0.0005% Triton X-100, and 20 mmol/L Tris-HCl, pH 7.4. Blocking of nonspecific binding sites was achieved by incubating the wells with 3.5% bovine serum albumin (Fluka) for at least 1 h at 20 °C. Prior to initiation of the binding assay, the plates were washed once with 150 mmol/L NaCl, 1 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, and 20 mmol/L Tris-HCl, pH 7.4 (buffer B). The coated plates can be stored in the presence of 0.05% NaN_3 (in buffer B) at 4 °C in a humid chamber for at least 2 months without any loss in binding activity. Fibrinogen (IMCO, fibronectin free) was diluted in buffer B containing 1% bovine serum albumin (BSA) to 0.5 μg/mL for the binding to GP IIb-IIIa and to 1.5 μg/mL for the binding to $\alpha_v\beta_3$. The receptor-coated wells were incubated with fibrinogen (100 μL/well) overnight at room temperature in the absence or presence of increasing concentrations of RGDS or the compounds of interest. Nonbound fibrinogen was removed by three washes with buffer B and bound fibrinogen was detected by ELISA. Rabbit anti-human fibrinogen antibodies (Dakopatts, Denmark) diluted in buffer B containing 0.1% BSA were incubated for 1 h at room temperature, followed by incubation with biotinylated anti-rabbit immunoglobulin antibodies (Amersham) for 30 min. The nonbound antibodies were removed by three washes with buffer B. The preformed streptavidin-biotinylated peroxidase complex (Amersham) was then added for an additional 30 min. The wells were washed three times with buffer B, and after the addition of the peroxidase substrate ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid), Boehringer Mannheim), the enzyme activity in the wells was measured by a multichannel photometer (UVmax, Molecular Devices). The difference between total (in the absence of the inhibitor) and nonspecific (in the presence of 100 μM RGDS) binding was designated as specific binding. The concentration of inhibitor required to inhibit 50% of specific binding was defined as IC_{50}. In the Tables I–V the ratios between the IC_{50} of a given compound and the IC_{50} of the reference peptide RGDS are indicated.

Inhibition of Platelet Aggregation in Human Plasma. Human platelet rich plasma (h-PRP) was prepared from blood anticoagulated with $^1/_{10}$ volume 90 mM trisodium citrate and aggregation of platelets was measured by recording the velocity of light transmission change with an aggregometer (Elvi, Milano, type 840) essentially as described.[49] Serial dilutions of the inhibitors were added, and aggregation was induced by collagen (Horm), ADP, or thrombin at a final concentration in h-PRP of 2.5 μg/mL, 10 μM and 0.2 unit/mL, respectively. Essentially the same IC_{50} values were found for all three agonists.

Determination of the Half-Life in Dogs. Anesthetized German shepherd dogs with implanted venous catheters (jugularis and carotis) were continuously infused with the inhibitors for 4 h. Blood samples were taken during and after the infusion, and plasma was prepared. Serial dilutions of this plasma were added to 3 volumes of h-PRP, and the inhibition of ADP induced platelet aggregation was determined as described above. The inhibitory activity in the dogs plasma ex vivo was calculated using a standard curve with the inhibitor added in vitro and the half-life for the disappearance of inhibitory activity determined using the computer program ELSFIT.

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Supplementary Material Available: Experimental details and characterization data (NMR spectra, mass spectra, IR spectra, melting points, and microanalytical) for all compounds contained in Schemes I–V as well as in Tables I–V which do not appear in the Experimental Section (12 pages). Ordering information is given on any current masthead page.

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