Discovery of an Orally Active Non-Peptide Fibrinogen Receptor Antagonist Based on the Hydantoin Scaffold

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Antagonists of the platelet fibrinogen receptor (GP IIb/IIIa receptor) are expected to be a promising new class of antithrombotic agents. The binding of fibrinogen to the fibrinogen receptor depends on an Arg-Gly-Asp-Ser (RGDS) tetrapeptide recognition motif. Structural modifications of the RGDS lead have led to the discovery of a non-peptide RGD mimetic GP IIb/IIIa antagonist **44** (S 1197). Compound **44** inhibited, in a dose dependent and reversible manner, human and dog platelet aggregation as well as ^{125}I-fibrinogen binding to ADP-activated human gel filtered platelets and isolated GP IIb/IIIa with K_i values of 9 nM and 0.17 nM, respectively. A pharmacophore mapping procedure with QXP and a 3D-QSAR analysis applying the GRID/GOLPE methodology yielded a stable, rather predictive model and revealed structural features which are important for binding. Hydrophobic substitutions both at the hydantoin nucleus and at the C-terminus increase the affinity toward the fibrinogen receptor. The crystalline ethyl ester prodrug **48** (HMR 1794) is an orally active antithrombotic agent which is a promising drug candidate for the treatment of thrombotic diseases in humans.

Introduction

Vascular injury, thrombus formation, and their ischemic complications culminate in a variety of vasoocclusive disorders such as unstable angina, myocardial infarction, stroke, or peripheral arterial disease.[1−3] The acute vascular occlusion is caused by excessive platelet deposition and aggregation at the sites of atherosclerotic plaques or damaged arterial surfaces leading to platelet-rich thrombus formation.[4] Platelet aggregation may be stimulated by collagen, thrombin, thromboxane A2, serotonin, adenosine diphosphate (ADP), platelet activating factor (PAF), epinephrine, or most probably by a combination of these factors. The primary mechanism of platelet aggregation is initiated by activation of the platelet glycoprotein GP IIb/IIIa and linking of platelets by fibrinogen bound to the receptor GP IIb/IIIa.[5,6] Many of the present antiplatelet agents including aspirin, ticlopidine, and clopidogrel only interfere with one single agonistic pathway. However, the specific inhibition of a single agonistic pathway leaves alternative routes to platelet activation unaffected and is thus of limited effectiveness in preventing thrombus formation. Therefore blocking of GP IIb/IIIa offers a superior approach in effectively preventing arterial thrombosis.[7] This has been demonstrated with abciximab (ReoPro), the Fab fragment of the chimeric anti-GP IIb/IIIa monoclonal antibody. Abciximab has proven to be beneficial in several clinical studies of percutaneous transluminal coronary angioplasty (PTCA), unstable angina, and non-Q-wave myocardial infarction. Efficacy has been demonstrated after short- and long-term treatment.[9b]

The glycoprotein GP IIb/IIIa belongs to the integrin superfamily and is the most abundant platelet cell surface protein. A normal platelet contains approximately 50 000 receptor complexes which bind like several other integrins to an Arg-Gly-Asp-Ser (RGDS) tetrapeptide recognition sequence.[8] Despite the common motif, integrins are quite specific in their interaction with different adhesive proteins, such as fibrinogen, vitronectin, fibronectin, laminin, collagen, or von Willebrand factor (vWF).

Platelet GP IIb/IIIa antagonists described to date can be divided into different classes. Monoclonal antibodies,[9,10] polypeptides from snake venoms[11] and leeches,[12] linear and cyclic peptides containing either the arginine-glycine-aspartic acid (RGD) recognition sequence[13−17] or the carboxyl-terminal sequence from the fibrinogen γ-chain,[18] and non-peptide antagonists.[7,19−28] Several series of non-peptide antagonists which mimic the RGD sequence have been identified. Many of these compounds have been shown to inhibit platelet aggregation independently of the activating agonist, as well as to prevent thrombus formation in animal models or in humans.[7,15b,19,20a,22,23,29,30]

Derived from the RGDS recognition sequence, we have generated a novel series of potent, selective, and orally active heterocyclic non-peptide GP IIb/IIIa antagonists, incorporating a hydantoin substructure. In this publication, we describe the design and synthesis of the hydantoin series which finally resulted in the ethylester prodrug **48** (HMR 1794) as a promising candidate for further clinical profiling. A 3D-QSAR model applying the GRID/GOLPE [43,44] methodology was developed to rationalize the SAR data and to highlight structural features important for binding to the fibrinogen receptor.

Chemistry

The hydantoin ring represents a common feature of all GP IIb/IIIa inhibitors described in this paper.

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Scheme 1

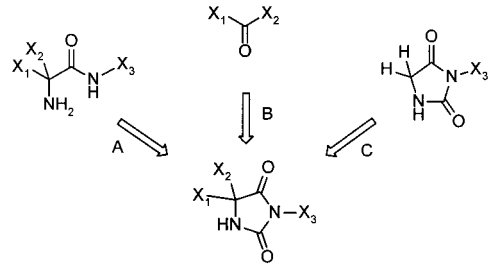
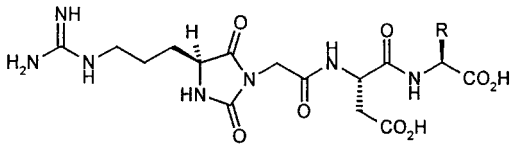


Table 1. Arginine Based GP IIb/IIIa Inhibitors



compd	R	IC_{50} (μM)[a]
RGDS		100 (n=2)
4	$HOCH_2$	10.0 (n=2)
5	$(CH_3)_2CHCH_2$	3.0 (n=4)
6	$(CH_3)_2CH$	1.0 (n=4)
7	C_6H_{11}	1.5 (n=4)
8	$C_6H_5CH_2$	10.0 (n=4)
9	C_6H_5	0.9 (n=4)

[a] Human platelets were isolated from platelet rich plasma (PRP) by gel filtration on Sepharose 2B. The resulting suspension of gel-filtered platelets was activated with 10 μM ADP in the presence of 1 mg/mL fibrinogen. Aggregation was measured as the maximal increase in light transmittance. Compounds were added to GFP 2 min before activation with ADP. Inhibition of aggregation was expressed as IC_{50} value, i.e., the mean concentration requiring 50% inhibition in GFP samples of two to six different donors.

However, a variety of different synthetic routes were used to build up the hydantoin scaffold, depending on the availability of the corresponding starting materials (Scheme 1). Amino acids (route A), ketones/aldehydes (route B), and unsubstituted hydantoins (route C) were used as precursors.

Compounds **4**−**9** in Table 1 were prepared as outlined in Scheme 2 (route A). Arginine methyl ester dihydrochloride **1** was reacted with ethoxycarbonylmethyl isocyanate in DMF in the presence of *N*-ethylmorpholine to give the urea derivative **2**, which was cyclized using 6 N HCl under reflux to obtain the hydantoin **3** in 99% yield.

The final steps in Scheme 2 were carried out by standard peptide chemistry. Briefly, hydantoin **3** and Boc protected dipeptide fragments were coupled using DCC and HOBt in DMF. The arginine based inhibitors **4**−**9** were obtained after deprotection and purification by chromatography. During all these different steps the guanidino group of arginine was always protonated and did not cause significant side reactions.Many of the compounds listed in Table 2 were obtained from hydantoin building blocks prepared as shown in Scheme 3 (route B), Scheme 4 (route A), and Scheme 5 (route C).

The hydantoin building blocks **28** were synthesized starting from the *p*-bromophenyl ketones **24** by treatment with copper cyanide in DMF to furnish the *p*-cyanophenyl ketones **25.**[31] Reaction of **25** with potassium cyanide and ammonium carbonate in water/ethanol yielded the hydantoin derivatives **26**.[32] Treatment of **26** with chloroacetic acid methylester in the presence of potassium iodide and sodium methylate using methanol as solvent provided intermediates **27**, which were converted to the hydantoin building blocks **28** using the Pinner reaction (Scheme 3).[33] This approach was used to prepare the target compounds **16**−**18** and **21**−**23** (Table 2). Compound **13** was synthesized analogously using 4-nitroacetophenone instead of the *p*-bromophenyl ketones for the hydantoin formation.

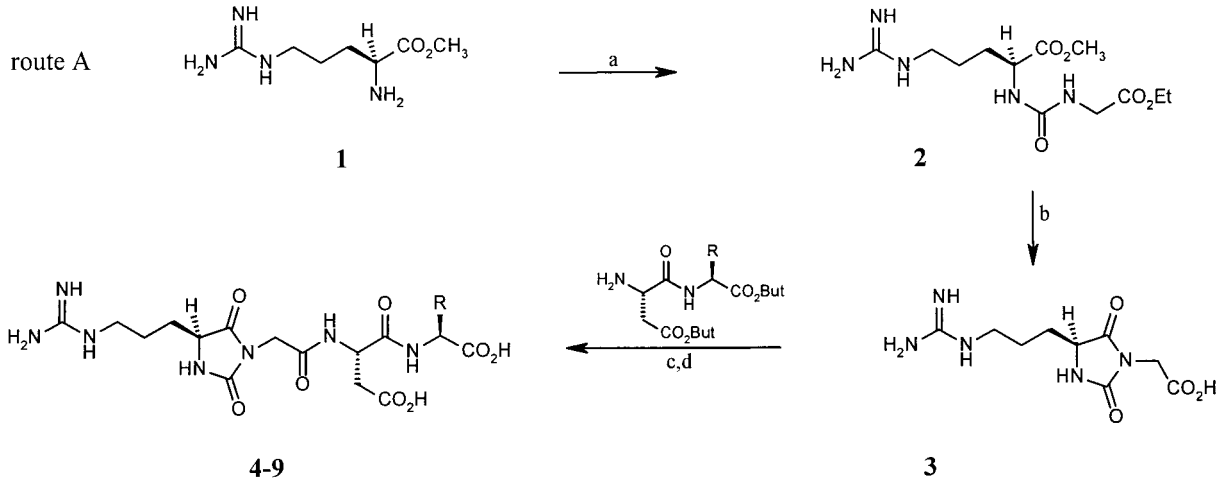
Scheme 4 shows the synthesis of the hydantoin building block **34** starting from methyl 2-(*p*-nitrophenyl)-acetic acid **29** which was treated with isoamylnitrite and sodium methylate in methanol/diethyl ether to yield oxime **30.** Subsequent hydrogenolysis with Pd/C as catalyst afforded the amino acid derivative **31**. Compound **31** was then reacted with ethoxycarbonylmethylisocyanate in DMF in the presence of *N*-ethylmorpholine to give the urea derivative **32** which was converted with benzyloxycarbonyl-*S*-methylisothiourea to the corresponding protected guanidino derivative **33**. Cyclization of **33** was conducted in HCl/water/acetic acid at 80 °C to yield hydantoin **34** and finally the target compound **12**. The hydantoin precursors of compounds **10** and **11** were prepared analogously starting from (*R,S*)-amino-(3-nitro-phenyl)-acetic acid methylester and 2-(*R,S*)-amino-3-(4-nitro-phenyl)-propionic acid methylester, respectively. Route A was also used to prepare **15** from (*S*)-2-amino-5-benzyloxycarbonylamino-pentanoic acid benzyl ester and 3-isocyanato-benzoic acid ethyl ester.

The synthesis of hydantoin building block **20c** is shown in Scheme 5 (route C). The initial step was the condensation of (2,5-dioxo-imidazolin-1-yl)acetic acid methyl ester with 4-cyanobenzaldehyde followed by the Pinner reaction and cleavage of the ester group to yield the acetic acid derivative **20c**.

The final step in the synthesis of compounds **10**−**23** and compounds **35**−**43** was carried out by coupling (DCC, HOBt) of H-Asp(OBu^t)-Phg-OBu^t hydrochloride (Table 2) with the corresponding hydantoin building blocks and by coupling of hydantoin **28a** with selected amino building blocks H-R_2 (Table 3). The coupling of building block **14c** to the GPIIb/IIIa inhibitor **14** serves as an illustrative example (Scheme 6). Precursor **14c** was prepared from **27a** in a three-step synthesis.

The four individual stereoisomers **44**, **45**, **46**, and **47** (Table 4) of the diastereomeric mixture **39** were pre-

Scheme 2[a]



[a] (a) Ethoxycarbonylmethyl isocyanate, *N*-ethylmorpholine, DMF; (b) HCl, reflux; (c) DCC, HOBt, DMF; (d) TFA.

pared by a convergent synthesis using (*S* or *R*)-3-amino-3-phenylpropionic acid ethyl ester hydrochloride (**49a** or **49b**) and (4-(*R* or *S*)-((4-amino-imino-methyl)-phenyl)-4-methyl-2,5-dioxoimidazolidin-1-yl)-acetic acid (**57a** or **57b**) as building blocks as has been reported previously.[29c,d] The synthesis of the enantiomerically pure hydantoin **57a** (*R*) was carried out by separation of the racemate **52** to the enantiomer **53a** (*R*, > 99% ee) as outlined in Scheme 7. The corresponding (*S*) enantiomers were prepared analogously.

Results and Discussion

To determine the potential of the compounds for the treatment of thrombotic diseases, they were evaluated in vitro for their ability to inhibit ADP-mediated platelet aggregation of human gel filtered platelets (IC_{50}, Table 1). The RGDS tetrapeptide, was used as an initial lead compound with only moderate activity (IC_{50} = 100 μM). Although our early compound **4** is closely related to the RGDS structure, we were surprised to find that the potency could be improved by a factor of 10 (IC_{50} = 10 μM). On the basis of that result we decided to conserve the hydantoin scaffold which is responsible for conformationally constraining the linear peptide.

Further variations at the carboxy terminus were made to optimize the potency. The serine residue in **4** was replaced by a variety of different amino acids. The most active compounds were obtained by incorporating amino acids having a lipophilic side chain (Table 1). Replacing the serine residue by a leucine in compound **5** improved the activity by a factor of 3 (IC_{50} = 3 μM). Even more potent molecules were obtained by replacing serine by valine in compound **6** (IC_{50} = 1 μM), with hexahydrophenylglycine in compound **7** (IC_{50} = 1.5 μM), or with phenylglycine in compound **9** (IC_{50} = 0.9 μM). In addition, **9** inhibited ^{125}I-fibrinogen binding to ADP-activated human GFP in a concentration dependent manner with a K_i value of 0.12 μM. Interestingly, the serine derivative **4** and the phenylalanine derivative **8** showed the same activity (IC_{50} = 10 μM) in inhibition of platelet aggregation. In conclusion, the activity of compounds can be improved by replacing the carboxy terminal serine with aliphatic or aromatic residues. This indicates that the GPIIb/IIIa receptor exposes a hydrophobic pocket close to the carboxylic acid binding site as has already been suggested by others.[7]

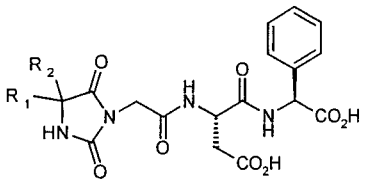
A variety of different arginine mimetics were evaluated in order to further constrain the molecules (Table 2). Replacement of the flexible arginine side chain by a meta substituted phenyl ring provided the diastereomeric mixture **10** which was less active than the parent compound **9**. Compounds **10** and **12** demonstrate the influence of the guanidino position. The guanidino group in the para position (**12**) enhances potency 30-fold in comparison to the meta position (**10**) and nearly 5-fold in comparison to **9**. These results correlate quite well with the data from the ^{125}I-fibrinogen binding assay[29b] (**12**, K_i = 0.03 μM, 4-fold more active than **9**). This dramatic increase is reversed on incorporation of a more flexible phenylmethylene linker (**11**).

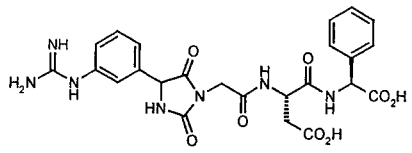
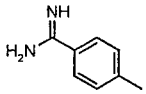
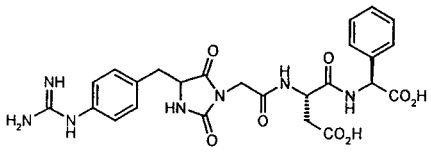
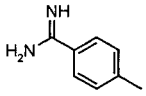
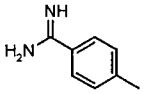
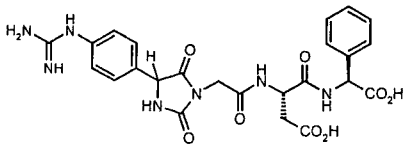
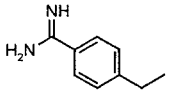
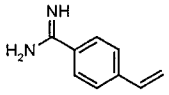
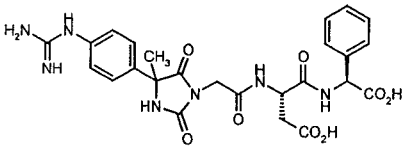
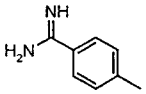
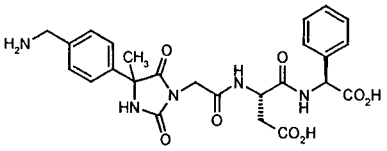
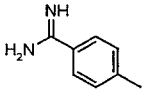
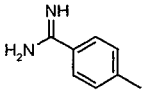
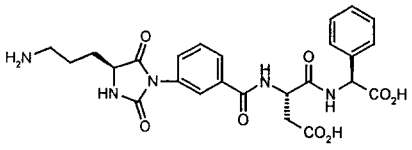
Substitution of the hydrogen atom at the 4-position of the hydantoin ring by a methyl group (**13**) enhances potency and makes this stereocenter stable against racemization, which is favorable for the isolation of the individual stereoisomers.

Another approach to improve the potency of the compounds involves replacement of the strong basic guanidino center by the less basic amino group. Surprisingly, compound **14**, the amino analogue of **13**, exhibited similar activity. Interestingly, the phenyl ring can be moved to the position of the glycine moiety as in compound **15** without a dramatic loss of activity compared to **14**. However, we could not further improve the activity within the amino series.

After intensive structure−activity relationship studies, the amidino derivative **16** turned out to be the most active molecule inhibiting human platelet aggregation with an IC_{50} of 40 nM. The diastereomeric mixture **16** was separated by chromatography, providing the two stereoisomers **17** and **18**. Stereoisomer **18** is the more active fibrinogen receptor antagonist (IC_{50} = 25 nM). Compound **18** is 36-fold more active than the parent arginine derivative **9**, suggesting that a benzamidine is an excellent substitute for the arginine as has been reported by others as well.[7a,b,15b] Less active compounds **19** and **20** were obtained by replacing the phenyl linker in **16** by a phenylmethylene (**19**) or a phenylmethine

Table 2. Arginine Replaced GP IIb/IIIa Inhibitors



compd	structure	IC_{50} (μM)[a]	compd	R_1	R_2	IC_{50} (μM)[a]
10		6.00 (n=4)	16		CH_3	0.04 (n=4)
11		5.50 (n=4)	17*		CH_3	0.80 (n=4)
			18*	CH_3		0.025 (n=4)
12		0.20 (n=4)	19		H	0.20 (n=4)
			20		-	0.15 (n=4)
13		0.15 (n=4)	21		CH_3CH_2	0.025 (n=4)
14		0.15 (n=4)	22			0.02 (n=4)
			23			0.05 (n=4)
15		0.60 (n=4)				

[a] Diastereomers separated.

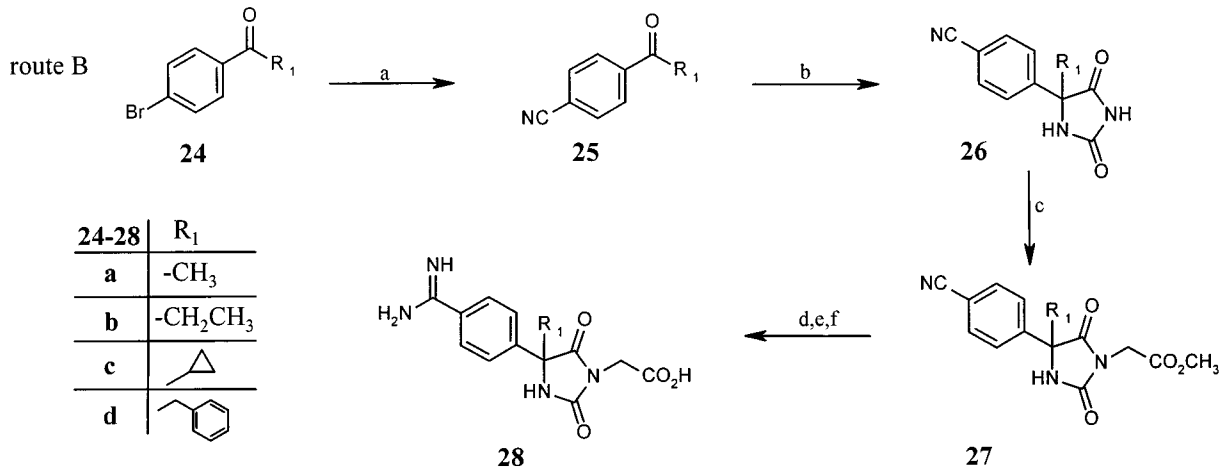
(**20**) linker, indicating that a specific three-dimensional separation of the amidino group and of the carboxylic acid group of the aspartic acid is particularly favorable for receptor binding.

Replacement of the methyl group of the hydantoin in **16** by selected groups such as ethyl, cyclopropyl, and benzyl should further elucidate the substituent effects at the 4-position of the hydantoin. Compounds **21** (IC_{50} = 25 nM) and **22** (IC_{50} = 20 nM) are slightly more active than **16**. Compound **23** (IC_{50} = 50 nM) with a larger side chain as in **16** is slightly less active.

The compounds shown in Table 2 are potent platelet aggregation inhibitors. However, the bioavailability and pharmacokinetic profile of these first generation inhibitors was not yet optimal. For example, the oral bioavailability of compound **12** was determined to be 3% (dog, 3 mg/kg p.o.; duration of action 1.1 h). This behavior called for further structural modification. One effort was aimed at reducing the peptidic nature of the compounds by replacement of the C-terminal amino acids. This might lead to metabolically more stable compounds with prolonged duration of action. Another effort was aimed at improving the oral bioavailability. Attempts to improve the oral activity of **16** by the synthesis of a variety of prodrugs in order to mask the charged ionic residues by suitable protective groups led to compounds with increased bioavailability whose property profile, however, still did not meet our desire.

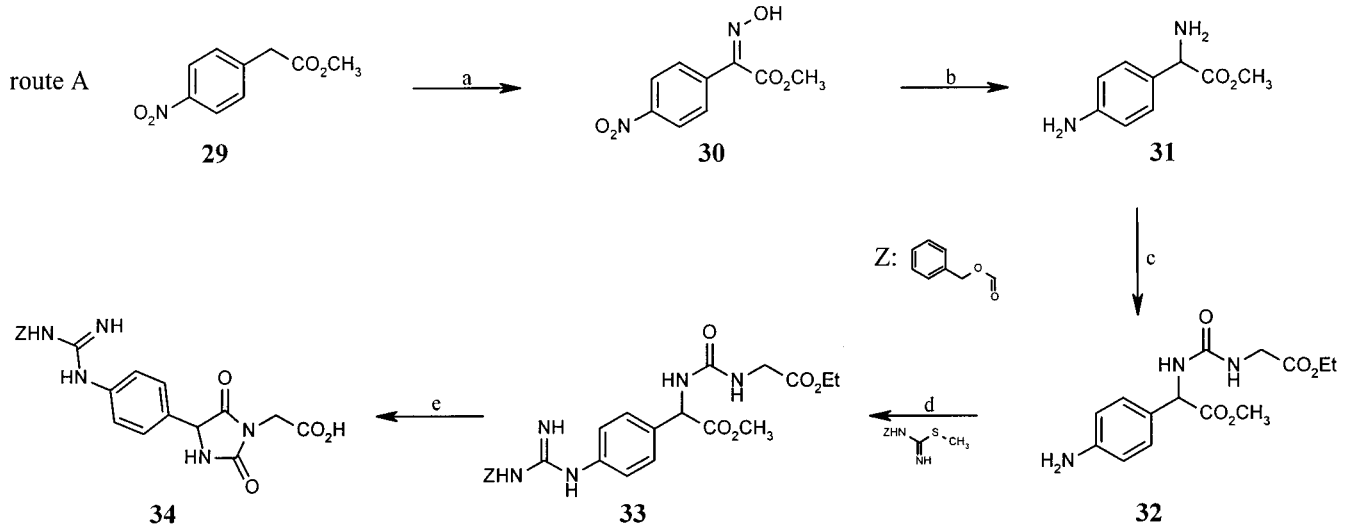
Variations of the C-terminal α-amino acids are given in Table 3.

Scheme 3[a]



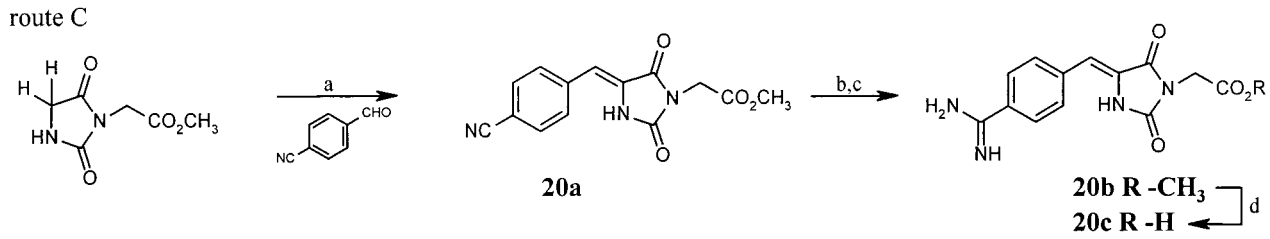
[a] (a) CuCN, DMF, reflux; (b) KCN, $(NH_4)_2CO_3$; (c) Cl-$CH_2CO_2CH_3$, KI, $NaOCH_3$; (d) HCl, ethanol; (e) NH_3, isopropanol; (f) HCl, reflux.

Scheme 4[a]



[a] (a) Isoamylnitrite, methanol, $NaOCH_3$; (b) HCl, methanol, DMF, 10% Pd/C, H_2; (c) ethoxycarbonylmethyl isocyanate, *N*-ethylmorpholine, DMF, −20 °C; (d) 1-benzyloxycarbonyl-2-methyl-isothiourea, CH_3CO_2H, methanol; (e) HCl, CH_3CO_2H, 80 °C.

Scheme 5[a]

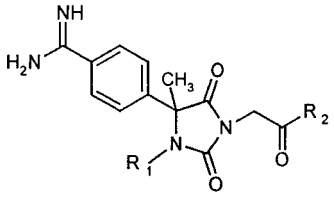


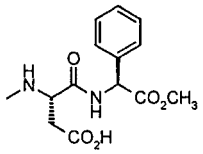
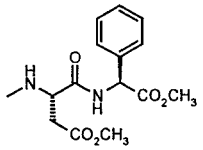
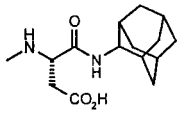
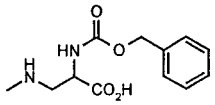
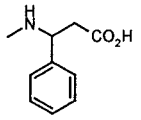
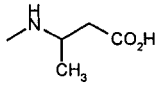
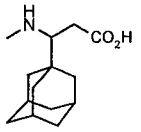
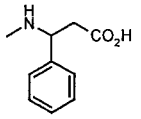
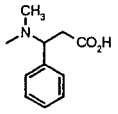
[a] (a) NaOAc, CH_3CO_2H, 135 °C, 3 h; (b) HCl, methanol; (c) NH_3, methanol; (d) 6 N HCl.

Again, it is worth noting that the presence of a free carboxylic acid group of the aspartic acid unit is favorable for high activity. Blockade of this carboxylic acid group by an ester as in compound **36** resulted in molecules that were less active in the in vitro platelet aggregation assay. In contrast, blockade of the second carboxylic acid group had a very limited influence on the activity as indicated by compound **35**. We therefore focused our attention on replacing the carboxyl terminal dipeptide moiety with structural elements having only one carboxylic acid group. Active compounds were discovered within three different structural classes including aspartic acid amides (**37**), diaminopropionic acid derivatives (**38**), and β-amino acid derivatives (**39**). A variety of different β-amino acids were synthesized to study this structural class in more detail. Incorporation of smaller alkyl substituents such as a methyl group or larger aliphatic residues such as an adamantyl residue into the side chain of the β-amino acids resulted in the less active compounds **40** and **41**. The phenyl residue proved to be a particularly favorable residue within the β-amino acids series.

Compound **39** is a potent inhibitor of human platelet aggregation with an IC_{50} value of 0.2 μM. In addition,

Table 3. C-Terminal Variation of GPIIb/IIIa Inhibitors



compd	R_1	R_2	IC_{50} (μM)[a]
35	H		0.07 (n=4)
36	H		70.0 (n=4)
37	H		0.025 (n=4)
38	H		0.30 (n=4)
39	H		0.20 (n=4)
40	H		2.00 (n=4)
41	H		30.0 (n=4)
42	CH_3CH_2		0.50 (n=4)
43	H		45.0 (n=4)

it was found that the compound is orally active in dogs.[29] Interestingly, orally active fibrinogen receptor antagonists where a β-amino acid derivative was incorporated at the carboxy terminus but based on quite different scaffolds have also been reported by other research groups.[20c,23a] Some limited structure/activity studies on compound **39** were carried out. In particular, the importance of the two NH groups for receptor binding was explored. Alkylation of the NH group by a methyl group at the carboxy-terminus resulted in the less active diastereomeric mixture **43**. Presumably, the respective NH group has a convenient influence on receptor binding or the N-alkylation results in a conformational change unfavorable for binding. Alkylation of the NH group of the hydantoin ring in derivative **42** had only a minor effect on receptor binding. Interestingly, this alkylation influences the pharmacokinetics of the molecule. Oral dosing of respective ester prodrugs resulted in higher drug plasma levels in the case of **39** than in the case of **42**. For this reason, further studies were concentrated on compound **39**.

Compound **39** is a mixture of the four possible stereoisomers. As has been reported elsewhere, the *SS* stereoisomers **44** proved to be the most potent fibrinogen receptor antagonist, inhibiting ADP-activated human platelet aggregation with an IC_{50} of 20 nM.[29c] The other three stereoisomers, **45**, **46**, and **47**, are less active (Table 4). Platelet aggregation activatation by other potent agonists like collagen (IC_{50} = 65 nM), PAF (IC_{50} = 30 nM), and thrombin (IC_{50} = 40 nM) was also effectively inhibited by **44**. The ^{125}I-binding to ADP-activated human GFP and isolated GP IIb/IIIa was inhibited in a concentration dependent manner with K_i values of 9 and 0.17 nM, respectively, suggesting that **44** is a reversible inhibitor of GP IIb/IIIa-mediated platelet aggregation. Selectivity of **44** for GP IIb/IIIa was indicated by testing the inhibition of ristocetin-induced von Willebrand factor binding to GP Ib-IX[40] (IC_{50} > 100 μM) or vitronectin binding to integrin $\alpha_v\beta_3$[39] (IC_{50} > 100 μM).

The drug **44** (S 1197) is a zwitterion containing a positively charged amidino group and a negatively charged carboxylic acid group. Probably due to its zwitterionic nature, the compound is not very soluble in water. We also assumed that the zwitterionic character of the molecule could be limiting for the oral bioavailability. In addition, this compound is amorphous, making its large-scale production less feasable. Several ester prodrugs were synthesized in order to protect the ionizable carboxylic-acid group of the drug. The crystalline maleic acid salt **48** (HMR 1794) of the ethylester prodrug was selected for further studies.

The oral antiplatelet activity of the ethylester prodrug **48** was measured by ex vivo platelet aggregometry. The prodrug was given orally at a dose of 1 mg/kg to a group of eight conscious dogs. Between 1 and 8 h after administration of **48**, ADP-induced aggregation was almost completely inhibited. Platelet function normalized within 24 h, indicating that the fibrinogen receptor blockade was fully reversible. A favorable terminal elimination half-life of 9.9 h for the active drug was observed. Primary hemostasis as determined by skin bleeding time was not significantly influenced at any time point during the experiment. These results confirm that **48** is a promising drug substance for the chronic treatment and prophylaxis of thrombotic diseases in humans.

Pharmacophore Modeling and 3D-QSAR Analysis

From the conformational analysis of cyclic peptides were derived various pharmacophoric models exhibiting turn-extended-turn, cupped, Gly-Asp β-turn, and Arg-Gly β-turn motifs for the RGD triad.[34] These models formed the basis for rational design and 3D database searches and eventually led to potent nonpeptide inhibitors with structurally diverse central scaffolds. The common motif between these different structural classes

Scheme 6[a]

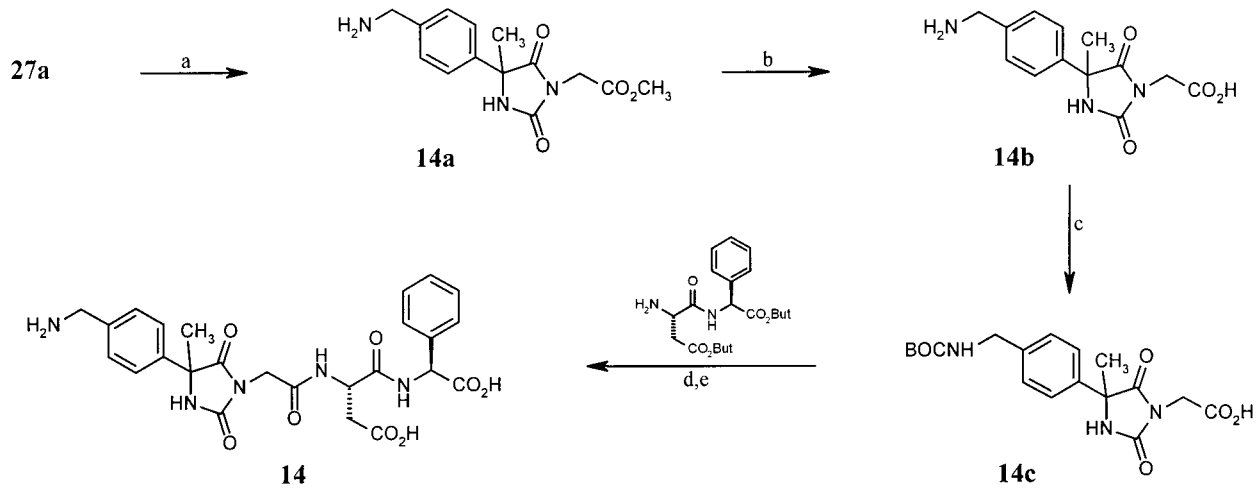
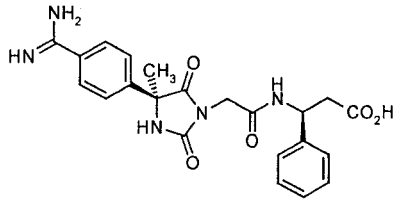
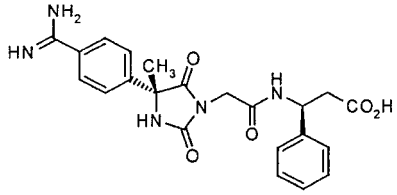
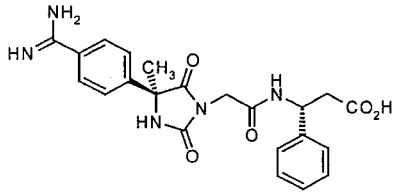
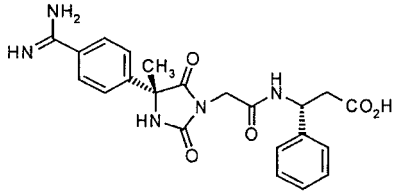
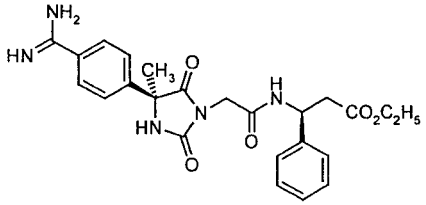


Table 4. Stereoisomers of **39**

compd	structure	IC_{50} [μM]
44		0.02 (n=4)
45		6.00 (n=4)
46		50.00 (n=4)
47		5.00 (n=4)
48	 maleic acid salt	prodrug

of fibrinogen receptor antagonists is a distance between 10 and 16 Å between the acidic and basic center depending on the central scaffold. This structural diversity strongly hints at an enlarged binding site which was originally proposed by Ku, Miller et al.[35] The validity and the predictivity of a 3D-QSAR analysis is critically dependent on the assumption that all the examined molecules have the same binding mode. Since the enlarged binding site hypothesis implies that a common binding mode can only be assumed within a given structural class of GP IIb/IIIa inhibitors, separate 3D-QSAR models have to be developed for each scaffold such as the hydantoins presented here.

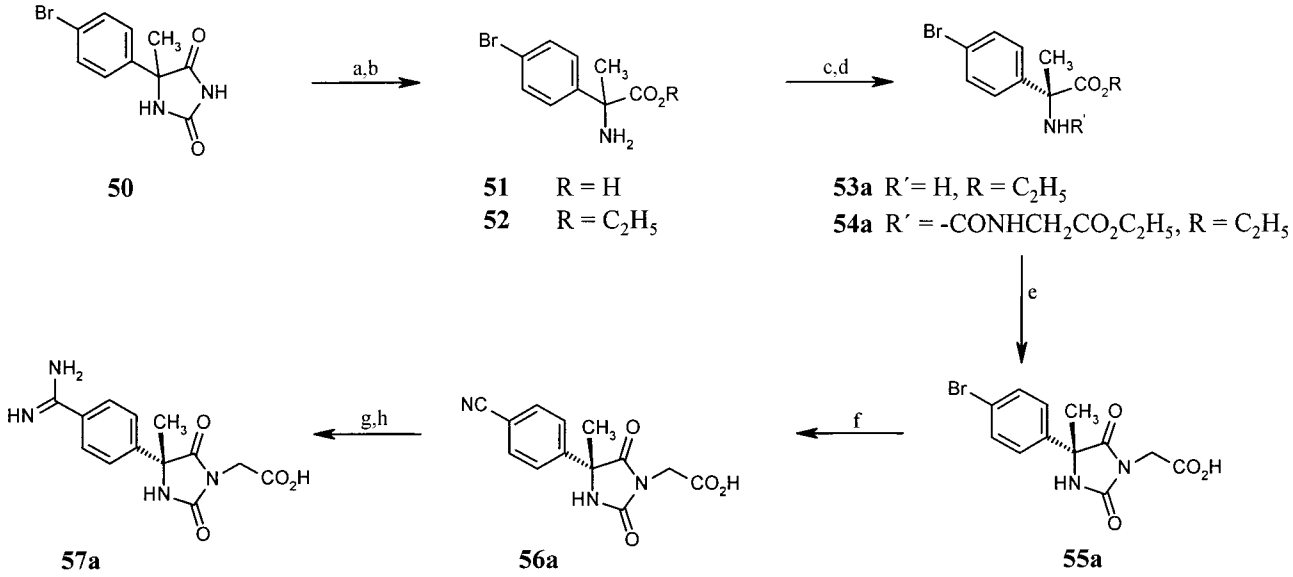
The development of a pharmacophore model involves the following three main aspects. First, key features for determining the biological activity have to be selected, i.e., the pharmacophore hypothesis has to be formulated. Second, a common conformation has to be defined which can be adopted by all the active compounds in the data set, assuming a single binding mode. Ideally this common conformation is identical to the bioactive conformation, but there may also be other common conformations yielding stable, predictive models. The last aspect in pharmacophore mapping deals with the superposition of a series of compounds onto a reference conformation which defines the so-called alignment rule.

In 3D-QSAR the aligned compounds are described by thousands of molecular field variables which are derived by calculating at each grid node the interaction energy between the respective compound and a probe which represents a chemical group. Each compound will have different interaction energies around the surface, and these differences in interaction energies between the examined molecules are correlated with the observed changes in biological activity by a partial least squares (PLS) model. Thus, 3D-QSAR methods can be used to establish statistical models for the quantitative correlation between 3D molecular structures and biological activities. Furthermore, 3D-QSAR models can also be interpreted in terms of molecular structures by highlighting regions in 3D space which either increase or diminish the activity.

Within this general context, the pharmacophore mapping procedure for the hydantoin-based fibrinogen receptor antagonists applying QXP and the resulting 3D-QSAR model generated with GRID/GOLPE will be described.[43,44]

The structures of compounds **22**, **35**, **37**, and **44** were selected for defining the 3D pharmacophore (Figure 1)

Scheme 7[a]



[a] (a) NaOH, 145 °C, 10 bar; (b) HCl, ethanol; (c) *R*-mandelic acid, isopropanol, diisopropyl ether; (d) ethoxycarbonylmethyl isocyanate, DMF, *N*-ethylmorpholine; (e) 6 N HCl; (f) CuCN, DMF; (g) HCl, ethanol; (h) NH_3, ethanol.

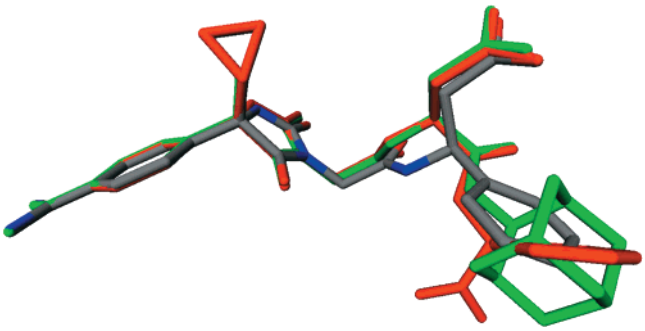


Figure 1. Pharmacophore derived by superposition of **44**, **22**, and **37** with QXP. For the sake of clarity, **35** has been omitted.

because they represent the most diverse subset of active compounds within the hydantoin series. Compound **22** is a mimic of the tetrapeptide template RGDF, **37** is an analogue of the tripeptide RGD C-terminally capped with an adamantyl moiety, and **44** is the most potent member with a C-terminal β-amino acid. Compound **35** was added to enforce the superposition of the carboxylic acid function following glycine, because this compound proved that the second C-terminal acid moiety of the tetrapeptide analogues was not essential for interacting with the fibrinogen receptor. To define a common pharmacophoric pattern in 3D space, the program QXP was applied.[36] QXP uses a superposition force field which automatically assigns short-range attractive forces to atoms with similar hydrogen bonding character or hydrophobicity in different molecules. QXP allows the molecules to adopt low-energy conformations which maximize the overlap of similar atoms. The interaction energies between the superimposed molecules and the phenolic OH probe were calculated by GRID with a grid spacing of 1 Å.[37] Thus, for each molecule 16 588 descriptors (interaction energies) were generated, the so-called **X**-matrix. The corresponding IC_{50} values were divided by the number of diastereomers (where applicable), and the negative log-transform was applied to linearize the range of activities. Since a lot of the *X*-variables do not contribute to the correlation with the inhibition of fibrinogen receptor binding, the recently developed variable selection methodology *smart region definition* (SRD) was defined.[43] This procedure discards noninformative interaction energies and is embedded in the program GOLPE for building, validating, and interpreting 3D-QSAR models.[44] Thus, the original 16 588 *X*-variables were finally reduced to 1623 *X*-variables.

The predictive ability of the models was evaluated by cross-validation using five randomly assigned groups of approximately the same size. Here, a two-component model with a $q^2 = 0.88$ and a standard error of prediction of 0.34 log units was obtained. The corresponding r^2 value is 0.97 for the model generated with the complete data set (Figure 2). This is an excellent result and proves both the validity of the proposed pharmacophore and the predictive quality of the 3D-QSAR model for the hydantoin series of fibrinogen receptor antagonists.

The 3D-QSAR model can also be readily interpreted by the use of PLS coefficient plots. For each grid point, the values of the coefficients are a measure for the correlation of the probe-ligand interaction energies with the biological activity. In this study the phenolic OH probe was used; therefore, negative coefficients indicate that favorable polar interactions increase the activity, whereas unfavorable steric interactions, i.e., bulky groups, decrease the activity. Conversely, positive coefficients indicate that polar interactions decrease the activity, whereas large, hydrophobic moieties increase the biological activity. As it can be seen in Figure 3, the contour maps of the positive PLS coefficients (yellow) at the level 0.002 encompass a much greater volume than the negative PLS coefficients (cyan) and, therefore, have a greater impact on correlating structural features with biological activity. The yellow regions indicate that bulky, hydrophobic substitutions at the hydantoin nucleus carrying the amidino moiety and at the C-terminus increase the affinity toward the fibrinogen receptor. This is in accordance with the qualitative SAR derived above.

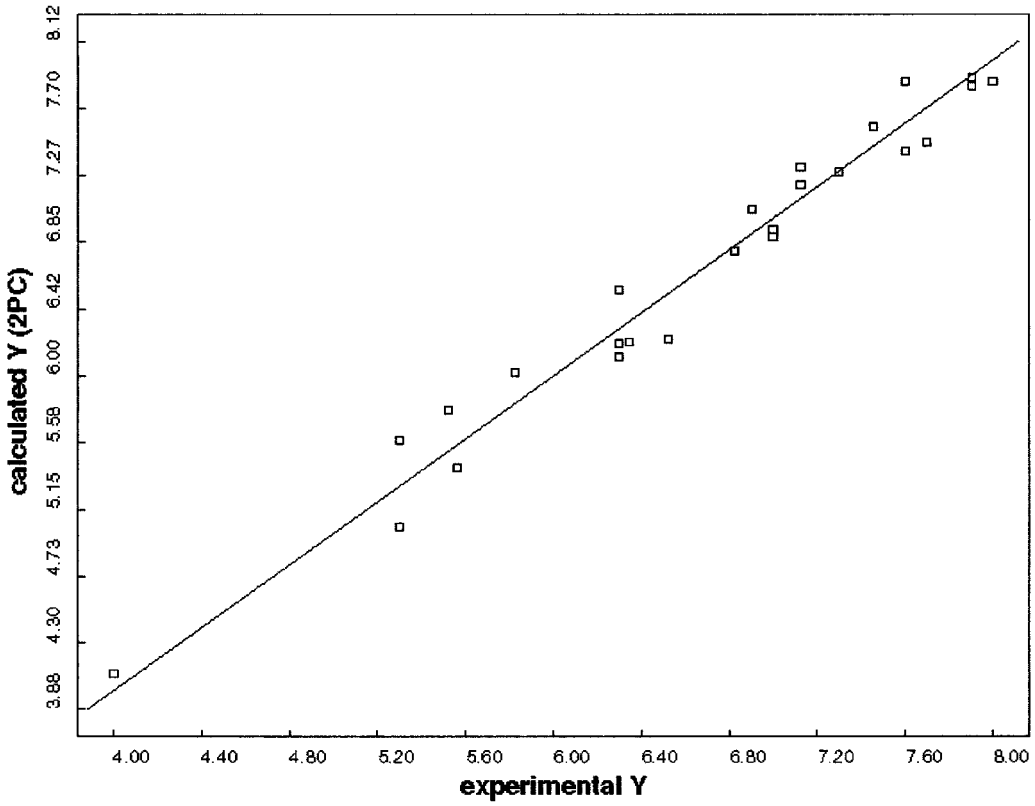


Figure 2. Experimental vs calculated activities (pIC_{50}). The highly active compounds are positioned in the upper right corner of the plot; the less active compounds are located in the lower left corner.

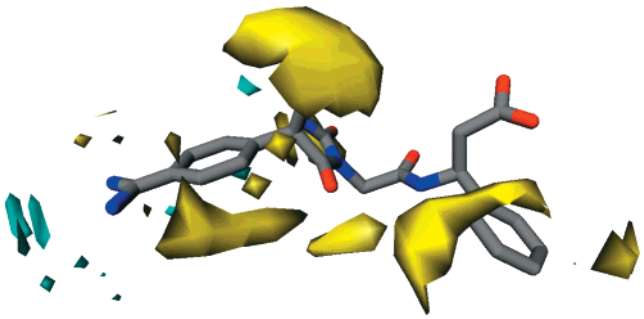


Figure 3. Contour maps of the negative (cyan) and positive (yellow) PLS regression coefficients contoured at 0.002 and −0.002, respectively. Steric interactions in the yellow regions will increase the activity. To aid the association of the contour maps with molecular features, **44** has been displayed for better orientation.

Conclusions

Structural modifications of an RGDS tetrapeptide lead structure have led to the discovery of a non-peptide fibrinogen receptor antagonist. The drug **44** is a highly potent, competitive, and selective fibrinogen receptor antagonist and a much more effective antithrombotic agent than aspirin, which is currently used to prevent arterial thrombosis.[29] The design of **44** is based on the hydantoin heterocycle onto which the important pharmacophoric groups are assembled. The conformational rigidity and well-defined stereochemistry at the two chiral centers of **44** most likely account for the very high affinity and specificity of **44** for the fibrinogen receptor. In addition, the structural rigidity might contribute to the enhanced metabolic stability and oral activity of **44** compared to the much more flexible tetrapeptide lead structure.

A pharmacophore mapping procedure and 3D-QSAR analysis resulted in a predictive and stable statistical model. The model can be readily interpreted in chemical terms, and the main determinants of affinity toward the fibrinogen receptor are hydrophobic substitutions at the hydantoin nucleus and the C-terminus. This is confirmed by the qualitative SAR derived from the comparison of the different optimization series.

The ethyl ester prodrug **48** (HMR 1794) is an orally active antithrombotic agent with a favorable terminal half-life of 9.9 h in dogs. Further studies with respect to the use of **48** for the chronic treatment and prophylaxis of thrombotic diseases in humans including unstable angina pectoris, myocardial infarction, stroke, or peripheral arterial disease are under investigation.

Experimental Section

All starting materials not described below were purchased from commercial sources. All reagents and solvents were used as received from commercial sources without additional purification.

Melting points were determined on a Büchi capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian Gemini 200 (200 MHz), a Bruker AM 400 (400 MHz), a Bruker Avance DRX 400 (400 MHz), or a Bruker ARX 500 (500 MHz) spectrometer; δ values in ppm relative to tetramethylsilane are given. Low-mass spectra were recorded with fast atom bombardment positive ionization (+FAB): VG ZAB SEQ; dissociation chemical ionization (DCI): Kratos MS 80 or VG Trio 2000; positive electrospray ionization (+ESI): VG BIO-Q. High-resolution spectra were recorded on a VG ZAB SEQ (+FAB). Optical rotations were determined on a Perkin-Elmer 241 polarimeter. The specific rotation has not been corrected. Normal phase silica gel (EM Science silica gel 60 (70−230 mesh) was used for chromatography. TLC plates coated with silica gel 60 F254 (Merck) were

used; detection by UV light (254 nm) or ninhydrin solution or iodine vapor was applied. The $KHSO_4/K_2SO_4$ solution was prepared by dissolving a mixture of $KHSO_4$ (50 g) and K_2SO_4 (100 g) in water (1000 mL). The following solvent mixtures were used: solvent A: *n*-butanol (60):water (24):pyridine (20): glacial acetic acid (6); solvent B: CH_2Cl_2 (9):methanol (1): glacial acetic acid (0.1):water (0.1); solvent C: *n*-butanol (17): glacial acetic acid (1):water (2).

2-(*S*)-(3-Ethoxycarbonylmethylureido)arginine Ethyl Ester (2). At 0 °C, *N*-ethylmorpholine (19.5 mL, 150 mmol) and ethoxycarbonylmethylisocyanate (18.45 mL, 150 mmol) were added to a solution of arginine methyl ester dihydrochloride **1** (39.18 g, 150 mmol) in DMF (450 mL). The mixture was stirred at 0 °C for 1 h and at 22 °C for 4 h. The reaction was monitored by TLC [solvent A]. After completion, the precipitate was filtered off and the filtrate was evaporated under reduced pressure to give 103.33 g of a DMF containing oil.

(4-(*S*)-(3-Guanidinopropyl-2,5-dioxo-imidazolidin-1-yl)acetic Acid (3). Crude material of **2** (103.33 g) was refluxed in HCl (6 N, 1000 mL) for 30 min. The solvent was removed under reduced pressure, and the remaining residue was adjusted to pH = 7 with saturated $NaHCO_3$ solution. The product crystallized from the aqueous solution. The crystals were sucked, washed with water, and dried under reduced pressure to give 31.75 g (99%) of **3**. ^{1}H NMR (DMSO-d_6) δ 1.40−1.80 (m, 4H), 2.13 (m, 2H), 3.05 (m, 2H), 3.43 (m, 2H), 4.00 (m, 1H), 7.60−7.90 (m, 5H, NH), 8.20 (m, 1H, NH) 9.40 (m, 1H, NH); MS *m*/*z* 272 $(M + H)^+$.

Similar methods were employed for compounds **4**−**9**.

{2-[4-(*S*)-(3-Guanidinopropyl)-2,5-dioxo-imidazolidin-1-yl]acetyl}-Asp(OBu^t)-Ser(But)-OBu^t (4a). At 0 °C, DCC (660 mg, 3 mmol) was added to a solution of **3** (770 mg, 3 mmol), H-Asp(OBu^t)-Ser(But)-OBu^t hydrochloride (1.27 g, 3 mmol), and HOBt (410 mg, 3 mmol) in DMF (20 mL). The mixture was stirred at 0 °C for 1 h and at 22 °C for 15 h. The precipitate (urea) was filtered off, and the filtrate was evaporated. After chromatography (silica gel, solvent B) the residue was freeze-dried to give 745 mg (40%) of **4a**. ^{1}H NMR (DMSO-d_6) δ 1.13 (s, 9H), 1.40 (s, 18H), 1.50−1.83 (m, 4H), 2.40 (dd, J = 15 Hz, J = 7.5 Hz, 1H), 2.68 (dd, J = 15 Hz, J = 7.5 Hz,-1H), 3.13 (m, 2H), 3.43 (dd, J = 10 Hz, J = 5 Hz, 1H), 3.61 (dd, J = 10 Hz, J = 5 Hz, 1H), 3.98 (s, 2H), 4.11 (m, 1H), 4.25 (m, 1H), 4.68 (m, 1H), 7.14 (br s, 4H, NH), 7.64 (br t, 1H, NH), 7.91 (br d, 1H, NH), 8.38 (s, 1H, NH), 8.50 (br d, 1H, NH); MS *m*/*z* (FAB) 628.6 $(M + H)^+$.

{2-[4-(*S*)-(3-Guanidinopropyl)-2,5-dioxo-imidazolidin-1-yl]acetyl}-Asp-Ser-OH Trifluoroacetic Acid Salt (4). Compound **4a** (700 mg, 1.12 mmol) was treated with TFA (7 mL, 90%) for 1 h at 22 °C. The solvent was removed, and the residue was distributed between water and diethyl ether. The aqueous solution was freeze-dried to give 579 mg (90%) of **4**. $[\alpha]^{21}_D$ = −19.1° (c = 1, water); ^{1}H NMR (DMSO-d_6) δ 1.48−1.83 (m, 4H), 2.45 (m, 1H), 2.69 (m, 1H), 3.10 (m, 2H), 3.65 (m, 2H), 4.00 (s, 2H), 4.13 (br s, 1H) 4.18 (m, 1H), 4.52 (m, 1H), 7.05−7.33 (m, 4H, NH), 7.69 (br s, 1H, NH), 7.93 (m, 1H, NH), 8.35 (m, 1H, NH), 8.50 (m, 1H, NH). Mass calcd for $C_{16}H_{26}N_7O_9$, 460.1792; found, 460.1786.

{2-[4-(*S*)-(3-Guanidinopropyl)-2,5-dioxo-imidazolidin-1-yl]acetyl}-Asp(OBu^t)-Leu-OBu^t (5a). mp 79−81 °C; $[\alpha]^{21}_D$ = −37.9° (c = 1, methanol); ^{1}H NMR (DMSO-d_6) δ 0.84 (d, J = 6 Hz, 3H), 0.90 (d, J = 6 Hz, 3H), 1.38 (s, 18 H), 1.40−1.80 (m, 7H), 2.40 (dd, J = 8 Hz, J = 16 Hz, 1H), 2.65 (dd, J = 5 Hz, J = 16 Hz, 1H), 3.15 (m, 2H), 3.99 (m, 2H), 4.03−4.16 (m, 2H), 4.65 (m, 1H), 7.16 (br s, 4H, NH), 7.66 (t, 1H, NH), 8.07 (d, 1H, NH), 8.38 (s, 1H, NH), 8.47 (d, 1H, NH); MS *m*/*z* (FAB) 597.9 $(M + H)^+$.

{2-[4-(*S*)-(3-Guanidinopropyl)-2,5-dioxo-imidazolidin-1-yl]acetyl}-Asp-Leu-OH Trifluoroacetic Acid Salt (5). $[\alpha]^{20}_D$ = −40.5° (c = 1, water); ^{1}H NMR (D_2O) δ 0.87 (d, J = 6 Hz, 3H), 0.94 (d, J = 6 Hz, 3H), 1.53−2.03 (m, 7H), 2.82 (dd, J = 8 Hz, J = 18 Hz, 1H), 2.96 (dd, J = 6 Hz, J = 18 Hz, 1H), 3.23 (m, 2H), 4.28 (m, 2H), 4.38 (m, 2H). Mass calcd for $C_{19}H_{32}N_7O_8$, 486.2312; found, 486.2308.

{2-[4-(*S*)-(3-Guanidinopropyl)-2,5-dioxo-imidazolidin-1-yl]acetyl}-Asp(OBu^t)-Val-OBu^t (6a). ^{1}H NMR (DMSO-d_6) δ 0.88 (d, J = 7.5 Hz, 6H), 1.38 (s, 18H), 1.55 (m, 2H), 2.00 (m, 1H), 2.40 (dd, J = 7.5, J = 15 Hz, 1H), 2.65 (dd, J = 5 Hz, J = 15 Hz, 1H), 3.10 (m, 2H), 3.95 (br s, 3H), 4.10 (br s, 1H), 4.70 (m, 1H), 7.65 (br s, NH), 7.90 (br s, NH), 8.60 (m, NH); MS *m*/*z* (FAB) 584.4 $(M + H)^+$.

{2-[4-(*S*)-(3-Guanidinopropyl)-2,5-dioxo-imidazolidin-1-yl]acetyl}-Asp-Val-OH Trifluoroacetic Acid Salt (6). $[\alpha]^{21}_D$ = −36.6° (c = 1, water); ^{1}H NMR (DMSO-d_6) δ 0.88 (d, J = 5 Hz, 6H), 1.55 (m, 2H), 2.05 (m, 1H), 2.45 (m, 1H), 2.70 (m, 1H), 3.10 (m, 2H), 3.95−4.10 (m, 4H), 4.63 (m, 1H), 7.15 (br s, 4H, NH), 7.75 (m, 2H, NH), 8.38 (s, 1H, NH), 8.50 (d, 1H, NH). Mass calcd for $C_{18}H_{30}N_7O_8$, 472.2156; found, 472.2159.

{2-[4-(*S*)-(3-Guanidinopropyl)-2,5-dioxo-1-yl]acetyl}-Asp(OBu^t)-(*S*)-hexahydrophenylglycine-OBu^t (7a). $[\alpha]^{20}_D$ = −26.9° (c = 1, methanol); ^{1}H NMR (DMSO-d_6) δ 0.95−1.16 (m, 7H), 1.38 (s, 18 H), 1.50−1.80 (m, 8H), 2.40 (m, 1H), 2.66 (m, 1H), 2.96 (m, 2H), 3.07 (m, 2H), 3.95 (m, 2H), 4.68 (m, 1H), 7.13 (br s, 4H, NH), 7.60 (br s, 1H, NH), 7.88 (m, 1H, NH), 8.35 (m, 1H, NH), 8.50 (m, 1H, NH); MS *m*/*z* (FAB) 624.3 $(M + H)^+$.

{2-[4-(*S*)-(3-Guanidinopropyl)-2,5-dioxo-imidazolidin-1-yl]acetyl}-Asp-(*S*)-Hexahydrophenylglycine-OH Trifluoroacetic Acid Salt (7). $[\alpha]^{20}_D$ = −30.6° (c = 1, water); ^{1}H NMR (DMSO-d_6) δ 0.9−1.16 (m, 7H), 1.26−1.80 (8H), 2.45 (m, 1H), 2.70 (m, 1H), 3.10 (m, 4H), 4.00 (m, 1H), 4.08 (m, 1H), 4.63 (m, 1H), 7.13 (br s, 4H, NH), 7.60 (t, 1H, NH), 7.75 (d, 1H, NH), 8.36 (s, 1H, NH), 8.50 (d, 1H, NH); Mass calcd for $C_{21}H_{34}N_7O_8$, 512.2469; found, 512.2469.

{2-[4-(*S*)-(3-Guanidinopropyl)-2,5-dioxo-imidazolidin1-yl]acetyl}-Asp(OBu^t)-Phe-OBu^t (8a). $[\alpha]^{22}_D$ = −25.1° (c = 1, methanol); ^{1}H NMR (DMSO-d_6) δ 1.40 (s, 18H), 1.48−1.93 (m, 4H), 2.50 (m, 1H), 3.08 (m, 1H), 4.13−4.58 (m, 7H), 7.39 (m, 5H); MS *m*/*z* (FAB) 632.6 $(M + H)^+$.

{2-[4-(*S*)-(3-Guanidinopropyl)-2,5-dioxo-imidazolidin-1-yl]acetyl}-Asp-Phe-OH Trifluoroacetic Acid Salt (8). $[\alpha]^{23}_D$ = −18.2° (c = 1, water); ^{1}H NMR (DMSO-d_6) δ 1.90 (m, 2H), 2.70 (m, 1H), 2.83 (m, 1H), 3.02 (m, 1H), 3.23 (m, 5H), 4.22 (m, 2H), 4.38 (m, 1H), 4.70 (m, 2H); Mass calcd for $C_{22}H_{30}N_7O_8$, 520.2156; found, 520.2156.

{2-[4-(*S*)-(3-Guanidinopropyl)-2,5-dioxo-imidazolidin-1-yl]acetyl}-Asp(OBu^t)-(*R*,*S*)-(*S*)-phenylglycine-OBu^t (9a). $[\alpha]^{19}_D$ = −0.5° (c = 1, methanol); ^{1}H NMR (DMSO-d_6) δ 1.33 (s, 9H), 1.40 (s, 9H), 1.50−1.80 (m, 4H), 2.45 (m, 1H), 2.70 (m, 1H), 3.13 (m, 2H), 3.98 (s, 2H), 4.10 (br s, 1H), 4.73 (m, 1H), 5.23 (d, J = 7.5 Hz, 1H), 7.13 (br s, 4H, NH), 7.35 (s, 5H), 7.65 (m, 1H, NH), 8.38 (s, 1H, NH), 8.50 (d, 1H, NH), 8.59 (d, 1H, NH); MS *m*/*z* (FAB) 618.2 $(M + H)^+$.

{2-[4-(*S*)-(3-Guanidinopropyl)-2,5-dioxo-imidazolidin-1-yl]acetyl}-Asp-(*S*)-phenylglycine-OH Trifluoroacetic Acid Salt (9). $[\alpha]^{19}_D$ = +20.7° (c = 1, water); ^{1}H NMR (DMSO-d_6) δ 1.60 (m, 4H), 2.43 (m, 1H), 2.75 (m, 1H), 3.10 (m, 2H), 4.00 (s, 2H), 4.15 (m, 1H), 4.65 (m, 1H), 5.13 (m, 1H), 7.18 (br s, 4H, NH), 7.34 (m, 5H), 8.29−8.50 (m, 2H, NH), 8.55 (m, 1H, NH). Mass calcd for $C_{21}H_{28}N_7O_8$, 506.1999; found, 506.2000.

Similar methods were employed for compounds **10** and **11**.

(*R*,*S*)-(3-Ethoxycarbonylmethylureido)-(3-nitro-phenyl)-acetic Acid Methyl Ester (10a). To a solution of (*R*,*S*)-amino-(3-nitro-phenyl)-acetic acid methyl ester hydrochloride (4.6 g, 19 mmol) and isocyanato-acetic acid ethyl ester (2.4 g, 19 mmol) in DMF (25 mL) was added *N*-ethylmorpholin (2.5 mL, 20 mmol). The solution was stirred at room temperature for 12 h. The solution was evaporated and treated with CH_2Cl_2 and aqueous hydrochloric acid. The organic layer was separated and evaporated to yield 4.55 g (70%) of **10a**.

[4-(*R*,*S*)-(3-Nitro-phenyl)-2,5-dioxo-imidazolidin-1-yl]-acetic Acid (10b). Compound **10a** (4.5 g, 13.2 mmol) was heated in a mixture of water (25 mL), concentrated HCl (25 mL), and acetic acid (15 mL) at 100 °C for 30 min. The mixture was cooled, and the product filtered to yield 3.1 g (84%) of **10b**.

[4-(*R*,*S*)-(3-Amino-phenyl)-2,5-dioxo-imidazolidin-1-yl]-acetic Acid (10c). Compound **10b** (300 mg, 1 mmol) was hydrogenated in methanol (50 mL) and DMF (10 mL) with

Pd/C (100 mg, 10%). After 8 h the catalyst was filtered, and the filtrate evaporated to yield 140 mg (56%) of **10c**.

[4-(*R,S*)-(3-Nitro-guanidino-phenyl)-2,5-dioxo-imidazolidin-1-yl]acetic Acid (10d). Compound **10c** (700 mg, 2.8 mmol) and 1-nitro-2-methyl-isothiourea (570 mg, 4.2 mmol) were stirred in NaOH (1 N, 3.5 mL) for 11 h at 80 °C. The solution was filtered, the aqueous layer was acidified to pH 3 with HCl, and the product was extracted with ethyl acetate. The compound was purified over Sephadex LH20 using solvent C and freeze-dried to yield 48 mg (5%) of **10d**.

{**2-[4-(*R,S*)-(3-Nitro-guanidino-phenyl)-2,5-dioxo-imidazolidin-1-yl]acetyl**}**-Asp(OBu^t)-(*S*)-phenylglycine-OBu^t (10e).** To a solution of **10d** (30 mg, 0.09 mmol) in DMF (5 mL) were added at 0 °C DCC (21 mg, 0.1 mmol), HOBt (12.2 mg, 0.09 mmol), Asp(OBu^t)-(*S*)-phenylglycine-OBu^t hydrochloride (38.6 mg, 0.09 mmol), and *N*-ethylmorpholin (10.4 mg, 0.09 mmol). The solution was stirred for 2 h at 0 °C and 12 h at room temperature. The solvent was removed in vacuo and the residue treated with ethyl acetate. The dicyclohexyl urea was filtered, and the filtrate was extracted with aqueous $NaHCO_3$ solution and $KHSO_4$ solution, washed with water, dried, and evaporated to give 69 mg of crude material of **10e** which was used in the next step without further purification.

{**2-[4-(*R,S*)-(3-Guanidino-phenyl)-2,5-dioxo-imidazolidin-1-yl]acetyl**}**-Asp-(*S*)-phenylglycine-OH Trifluoroacetic Acid Salt (10).** Compound **10e** (65 mg, 0.09 mmol) was stirred with TFA (5 mL, 90%) for 3 h and evaporated. The residue was dissolved in methanol (50 mL) and hydrogenated at room temperature with Pd/C (0.1 g, 10%) for 2 h. The catalyst was removed by filtration, and the filtrate was evaporated, purified over Sephadex LH20 using solvent C, and freeze-dried to yield 19.5 mg (39%) of **10**. mp 150 °C; ^{1}H NMR (DMSO-d_6) δ 2.4−2.5 (m, 1H), 2.6−2.8 (m, 1H), 4.05−4.15 (m, 2H), 4.6−4.7 (m, 1H), 5.1−5.25 (m, 1H), 5.33 (s, 1H), 7.1−7.7 (m, 10H), 8.5−8.7 (b, NH), 8.8 (s, NH). Mass calcd for $C_{24}H_{26}N_7O_8$, 540.1843; found, 540.1850.

{**2-[4-(*R,S*)-(4-Guanidino-benzyl)-2,5-dioxo-imidazolidin-1-yl]acetyl**}**-Asp-(*S*)-phenylglycine-OH Trifluoroacetic Acid Salt (11).** mp 195−200 °C; ^{1}H NMR (DMSO-d_6) δ 2.4−2.5 (m, 1H), 2.6−2.75 (m, 1H), 2.85−2.95 (m,1H), 3.0−3.15 (m,1H), 3.9 (s, 2H), 4.4−4.5 (m, 1H), 4.6−4.7 (m, 1H), 5.2−5.3 (m, 1H), 7.1−7.2 (m, 2H), 7.4−7.7 (m, 11H), 8.2 (s, NH), 8.4 (d, NH), 8.5 (d, NH), 9.8−10 (b, NH), 12.1−12.8 (b, OH). Mass calcd for $C_{25}H_{28}N_7O_8$, 554.1999; found, 554.1990.

Hydroxyimino-(4-nitro-phenyl)-acetic Acid Methyl Ester (30). To a solution of (4-nitro-phenyl)-acetic acid methyl ester **29** (245 g, 1.255 mol) in diethyl ether (2500 mL) were added a solution of isoamyl nitrite (323.5 g, 2.76 mol) in diethyl ether (1400 mL) and then sodium methoxide (108.3 g, 2.0 mol) in methanol (1600 mL). The mixture was stirred at room temperature for 12 h. The solid was filtered, and the filtrate was evaporated to 3000 mL and acidified to pH 2. The solid was dissolved in water and acidified with HCl to pH 2. The precipitations of both treatments were combined and recrystallized from methanol to give 175.9 g (62.5%) of **30**. mp 200−201 °C.

(*R,S*)-Amino-(4-amino-phenyl)acetic Acid Methyl Ester Hydrochloride (31). A solution of **30** (30 g, 133.8 mmol) in methanol (1000 mL) and DMF (250 mL) was hydrogenated over Pd/C (2 g, 10%). The pH value was adjusted between 3 and 4 by addition of HCl in methanol (3.3 mol/L). After 8 h the catalyst was filtered, the filtrate evaporated, and after addition of ethyl acetate the product was precipitated. Recrystallization from 2-propanol/ethyl acetate gave 24.5 g (84.5%)-of **31**. mp 155−158 °C.

(*R,S*)-(4-Amino-phenyl)-(3-ethoxycarbonylmethyl-ureido)acetic Acid Methyl Ester (32). To a solution of **31** (30 g, 138.5 mmol) and *N*-ethylmorpholin (41.5 g, 360 mmol) in DMF (200 mL) was slowly added isocyanato acetic acid ethyl ester (18 g, 139.4 mmol) at −20 to −30 °C. After warming to room temperature the mixture was evaporated, and water and ethyl acetate were added. The organic layer was separated, and the aqueous layer was adjusted to pH 8 and extracted with ethyl acetate. The combined organic layers were evaporated, and the compound crystallized (ethyl acetate) to yield **32** (29.75 g, 69.4%). mp 105−110 °C.

(*R,S*)-(3-Ethoxycarbonylmethyl-ureido)-(4-benzyloxycarbonyl-guanidino-phenyl)acetic Acid Methyl Ester (33). A solution of **32** (11.03 g, 35.7 mmol)**,** 1-benzyloxycarbonyl-2-methyl-isothiourea (8 g, 35.7 mmol) and acetic acid (7.5 g, 124.9 mmol) in methanol (72 mL) was stirred for 12 h. The solid was filtered and dried to yield **33** (10.68 g, 62%). mp 173−174 °C.

(*R,S*)-[4-(4-Benzyloxycarbonyl-guanidino-phenyl)-2,5-dioxo-imidazolidin-1-yl]acetic Acid Hydrochloride (34). A mixture of **33** (5.6 g, 11.5 mmol) in water (42 mL), concentrated HCl (28 mL), and acetic acid (112 mL) was heated at 80 °C for 90 min. The mixture was evaporated and stirred with water and methanol to give 4.25 g (80%) **34**. mp 214−216 °C.

{**2-[4-(*R,S*)-(4-Benzyloxycarbonyl-guanidino-phenyl)-2,5-dioxo-imidazolidin-1-yl]acetyl**}**-Asp(OBu^t)-(*S*)-phenylglycine-OBu^t (12a).** The method for compound **4a** was employed to give 3.46 g (63%) of **12a** starting from **34**.

{**2-[4-(*R,S*)-(4-Guanidino-phenyl)-2,5-dioxo-imidazolidin-1-yl]acetyl**}**-Asp-(*S*)-phenylglycine-OH Trifluoroacetic Acid Salt (12).** Compound **12a** (1.6 g, 2 mmol) was stirred with TFA (16 mL, 90%) for 1 h, evaporated, and lyophylized. The residue was dissolved in methanol (100 mL) and hydrogenated at room temperature with Pd/C (0.5 g, 10%) for 3 h. The catalyst was removed by filtration, and the filtrate was evaporated, purified over Sephadex LH20 using solvent C, and freeze-dried to give 0.72 g (65.5%) of **12**. ^{1}H NMR (DMSO-d_6) δ 2.4−2.55 (m, 1H), 2.65−2.75 (m, 1H), 3.95−4.05 (m, 2H), 4.6−4.75 (m, 1H), 5.2−5.3 (m, 1H), 5.32 (s, 1H), 7.20−7.70 (m, 11H), 8.4−8.5 (m, NH), 8.80 (s, NH), 9.90 (s, NH), 12.5 (b, OH). Mass calcd for $C_{24}H_{26}N_7O_8$, 540.1843; found, 540.1835.

(*R,S*)-5-Methyl-5-(4-nitro-phenyl)-imidazolidine-2,4-dione (13a). To potassium cyanide (20.8 g, 0.32 mol) and ammonium carbonate (96.1 g, 1 mol) in water (250 mL) was added carefully 4-nitroacetophenone (49.5 g, 0.3 mol) in ethanol (250 mL). The solution was stirred at 50 °C for 5 h. After cooling, the product was filtered and washed with diethyl ether to give 52.7 g (75%) of **13a**. mp 237−240 °C.

(*R,S*)-[4-Methyl-4-(4-nitro-phenyl)-2,5-dioxo-imidazolidin-1-yl]acetic Acid Methyl Ester (13b). Under inert atmosphere sodium (1.68 g, 73 mmol) was carefully dissolved in methanol (200 mL). After completion, **13a** (17.17 g, 73 mmol) was added and refluxed for 2 h. After addition of chloroacetic acid methylester (7.92 g, 73 mmol) and KI (12.12 g, 73 mmol), the mixture was refluxed for 10 h and cooled, and the precipitate was removed by filtration. Evaporation of the filtrate afford more crude material. Recrystallization (ethyl acetate) yielded 18.1 g (81%) of **13b**.

[**4-(*R,S*)-(4-Amino-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetic Acid Methyl Ester (13c).** To a solution of **13b** (22.2 g, 72.2 mmol) in ethanol (600 mL) and water (133 mL) were added calcium chloride (7.4 g) in water (11 mL), acetic acid (7.4 mL), and zinc powder (37 g). The mixture was heated under reflux for 2 h and filtered hot. The residue was extracted with ethyl acetate, and the filtrates were combined and evaporated to yield 10.2 g (51%) of **13c**.

[4-(*R,S*)-(4-Benzyloxycarbonylguanidino-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetic Acid Methyl Ester (13d). The method for compound **33** was employed to give 2.85 g (58%) of **13d** starting from **13c.**

[4-(*R,S*)-(4-Benzyloxycarbonylguanidino-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetic Acid (13e). The method for compound **34** was employed to give 850 mg (31%) of **13e**.

{**2-[4-(*R,S*)-(4-Benzyloxycarbonyl-guanidino-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetyl-amino**}**-Asp-(OBu^t)-(*S*)-phenylglycine-OBu^t (13f).** The method for compound **4a** was employed to give 370 mg (74%) of **13f** starting from of **13e**.

{**2-[4-(*R,S*)-(4-Guanidino-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetyl**}**-Asp-(*S*)-phenylglycine-OH (13).** The method for compound **10** was employed to give 120 mg

(47%) of **13** starting from **13f**. mp > 180 °C;^{1}H NMR (DMSO-d_6) δ 1.7 (s, 3H), 2.4−2.55 (m, 1), 2.6−2.75 (m, 1H), 3.95−4.05 (m, 2H), 4.6−4.7 (m, 1H), 5.15−5.25 (m, 1H), 7.2−7.67 (m, 14H), 8.45 (d, NH), 8.55 (d, NH), 9. 0 (m, NH), 9.9 (b, OH). Mass calcd for $C_{25}H_{28}N_7O_8$, 554.1999; found, 554.1996.

2-[4-(*R*,*S*)-(4-Aminomethyl-phenyl)-4-methyl-2,5-dioxoimidazolidin-1-yl]acetic Acid Methyl Ester (14a). To a solution of **27a** (1 g, 3.48 mmol) in ethanol (8 mL) and acetic acid (2 mL, 50%) was added Pd/C (200 mg, 10%). The mixture was hydrogenated at room temperature for 2 h at 3 bar. The catalyst was filtered off (Celite), and the filtrate was evaporated to give 800 mg (79%) after chromatography (silica gel, CH_2Cl_2/MeOH, 8:2); MS *m*/*z* (FAB) 292.1 $(M + H)^+$.

2-[4-(*R*,*S*)-(4-Aminomethyl-phenyl)-4-methyl-2,5-dioxoimidazolidin-1-yl]acetic Acid Hydrochloride (14b). A solution of **15a** (750 mg, 2.57 mmol) in concentrated HCl (15 mL) was heated for 6 h at 100 °C. The solution was evaporated and the residue freeze-dried to give 700 mg (87%). ^{1}H NMR (DMSO-d_6) δ 1.70 (s, 3H), 4.00 (q, $J = 5$ Hz, 2H), 4.08 (s, 2H), 7.53 (s, 4H), 8.48 (br s, 3H, NH), 9.10 (s, 1H, NH), MS *m*/*z* (FAB) 278 $(M + H)^+$.

2-[4-(*R*,*S*)-(4-*tert*-Butoxycarbonylaminomethyl-phenyl)-4-methyl-2,5-dioxoimidazolidin-1-yl]acetic Acid (14c). A mixture of **14b** (300 mg, 0.96 mmol) in dioxane (2 mL) and water (1 mL) was adjusted to pH 8.0 with NaOH (1 N, 1 mL) and subsequently cooled to 0 °C. Di-*tert*-butyl dicarbonate (230 mg, 1.05 mmol) was added while stirring. The reaction mixture was stirred at room temperature for 3 h. NaOH (1 N, 1.2 mL) was added continueously to maintain pH at 8.0. The reaction mixture was evaporated. The residue was adjusted to pH 2.0 ($KHSO_4$, 0 °C) and distributed between water and ethyl acetate. The organic extracts were washed with water and dried, and the solvent was evaporated. The residue was freeze-dried to give 340 mg (94%). ^{1}H NMR (DMSO-d_6) δ 1.40 (s, 9H), 1.70 (s, 3H), 4.00−4.19 (m, 4H), 7.25 (d, $J = 9$ Hz, 2H), 7.40 (m, 1H, NH), 7.48 (d, $J = 9$ Hz, 2H), 8.98 (s, 1H, NH), 13.00 (br s, 1H, COOH).

{**2-[4-(*R*,*S*)-(4-*tert*-Butoxycarbonylaminomethyl-phenyl)-4-methyl-2,5-dioxoimidazolidin-1-yl]acetyl**}-**Asp(OBu^t)-(*S*)-phenylglycine-OBu^t (14d).** The method for compound **4a** was employed to give 320 mg (54%) of **14d** starting from **14c**. ^{1}H NMR (DMSO-d_6) δ 1.33 (s, 9H), 1.40 (s, 18H), 1.70 (s, 3H), 2.43 (m, 1H), 2.68 (m, 1H), 4.00 (br s, 2H), 4.09 (d, $J = 5$ Hz, 2H), 4.70 (m, 1H), 5.20 (m, 1H), 7.24 (d, $J = 9$ Hz, 2H), 7.35 (m, 5H), 7.45 (d, $J = 9$ Hz, 2H), 7.95 (s, 1H, NH), 8.50 (m, 1H, NH), 8.60 (d, $J = 7.5$ Hz, 1H, NH), 8.86 (s, 1H, NH).

{**2-[4-(*R*,*S*)-(4-Aminomethyl-phenyl)-4-methyl-2,5-dioxoimidazolidin-1-yl]acetyl**}-**Asp-(*S*)-phenylglycine-OH Trifluoroacetic Acid Salt (14).** The method for compound **10** was employed to give 160 mg (59%) of **14** starting from **14d**. $[\alpha]^{23}_D = +1.7°$ ($c = 1$, methanol); ^{1}H NMR (DMSO-d_6) δ 1.70 (s, 3H), 2.45 (m, 1H), 2.70 (m, 1H), 4.00 (br s, 2H), 4.00 (m, 4H), 4.63 (m, 1H), 5.20 (m, 1H), 7.20−7.60 (m, 9H), 8.38 (m, 1H, NH), 8.53 (d, 1H, $J = 7.5$ Hz, NH), 8.95 (s, 1H, NH). Mass calcd for $C_{25}H_{28}N_5O_8$, 526.1938; found, 526.1938.

3-[3-(1-Benzyloxycarbonyl-4-benzyloxycarbonylamino-butyl)-ureido]benzoic Acid Ethyl Ester (15a). To a solution of (*S*)-2-amino-5-benzyloxycarbonylamino-pentanoic acid benzyl ester hydrochloride (4.1 g, 10.5 mmol) and 3-isocyanato-benzoic acid ethyl ester (2.0 g, 10.5 mmol) in DMF (20 mL) was added 1.7 g of *N*-ethylmorpholin (14.7 mmol), and the solution was stirred at room temperature for 12 h. The solution was evaporated and treated with ethyl acetate and $KHSO_4$ solution. The organic layer was separated and evaporated to yield 4.1 g (72%) of **15a**.

3-[4-(*S*)-(3-Amino-propyl)-2,5-dioxo-imidazolidin-1-yl]-benzoic Acid Hydrochloride (15b). The method for compound **12e** was employed to give 230 mg (10%) of **15b** starting from **15a**. mp 174 °C.

3-[4-(*S*)-(3-*tert*-Butoxycarbonylamino-propyl)-2,5-dioxo-imidazolidin-1-yl]benzoic Acid (15c). A solution of **15b** (230 mg, 0.73 mmol), di-*tert*-butyl dicarbonate (270 mg, 1.2 mmol), and *N*-ethylmorpholin (200 mg, 1.7 mmol) in DMF (20 mL) was stirred for 2 h. The solution was evaporated and treated with CH_2Cl_2 and potassium hydrogen sulfate solution. The organic layer was separated and evaporated to yield 187 mg (68%) of **15c**. ^{1}H NMR (DMSO-d_6) δ 1.50 (s, 9H), 1.55−1.85 (m, 4H), 2.95 (m, 2H), 4.25 (m, 1H), 6.85 (m, 1H, NH), 7.60 (m, 2H), 7.90 (m, 2H), 8.60 (m, 1H, NH).

{**3-[4-(*S*)-(3-*tert*-Butoxycarbonylamino-propyl)-2,5-dioxo-imidazolidin-1-yl]benzoyl**}-**Asp(OBu^t)-(*S*)-phenylglycine-OBu^t (15d).** The method for compound **4a** was employed to give 500 mg of crude material **15d** starting from **15c** which was used for the next step without further purification.

{**3-[4-(*S*)-(3-Amino-propyl)-2,5-dioxo-imidazolidin-1-yl]-benzoyl**}-**Asp-(*S*)-phenylglycine-OH (15).** The method for compound **10** was employed to give 244 mg (93%, based on **15c**) of **15** starting from **15d**. mp 201 °C; ^{1}H NMR (DMSO-d_6) δ 1.5−1.95 (m, 4H), 2.65−2.95 (m, 4H), 4.00 (br s, 2H), 4.3 (t, 1H), 4.9 (m, 1H), 5.3 (d, 1H), 7.25−8.0 (m, 12H), 8.6 (d, NH), 8.65 (d, NH), 8.8 (d, NH), 12−13.2 (b, OH). Mass calcd for $C_{25}H_{28}N_5O_8$, 526.1938; found, 526.1949.

{**2-[4-(*R*,*S*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetyl**}-**Asp-(*S*)-phenylglycine-OH Trifluoroacetic Acid Salt (16).** The method for compound **4a** was employed starting from **28a** followed by treatment with TFA to give 390 mg. $[\alpha]^{25}_D = +1.3$ ° ($c = 1$, in methanol); ^{1}H NMR (DMSO-d_6) δ 1.74 (s, 3H), 2.48 (m, 1H), 3.70 (m, 1H), 4.05 (s, 2H), 4.65 (m, 1H), 5.24 (m, 1H), 7.35 (m, 5H), 7.79 (m, 4H), 8.54 (m, 1H, NH), 8.55 (m, 1H, NH), 9.08 (s, 1H, NH), 9.30 (br s, 4H, NH). Mass calcd for $C_{25}H_{27}N_6O_8$, 539.1890; found, 539.1892.

{**2-[4-(*S* or *R*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetyl**}-**Asp-(*S*)-phenylglycine Trifluoroacetic Acid Salt (17).** The diastereomeric mixture **16** was resolved by chromatography on a LiChroprep-RP-18 reversed-phase column (10 μm) using a water/acetonitrile mixture (880 mL of water; 120 mL of acetonitrile; 1 mL of TFA). Fractions containing the first peak were concentrated and freeze-dried. $[\alpha]^{30}_D = -14$ ° ($c = 1$, in water); ^{1}H NMR (DMSO-d_6) δ 1.75 (s, 3H), 2.45 (m, 1H), 2.70 (m, 1H), 4.03 (s, 2H), 4.63 (m, 1H), 5.30 (d, $J = 7.5$ Hz, 1H), 7.38 (m, 5H), 7.70−7.90 (AA′BB′, 4H), 8.53 (m, 2H, NH), 9.05 (s, 1H,NH), 9.15 (br s, 2H, NH, 9.30 (br s, 2H, NH), 12.70 (br s, 2H, COOH); MS *m*/*z* (FAB) 539 $(M + H)^+$.

{**2-[4-(*R* or *S*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetyl**}-**Asp-(*S*)-phenylglycine-OH Trifluoroacetic Acid Salt(18).** Compound **18** was isolated from the diastereomeric mixture **16** by chromatography on a LiChroprep-RP-18 reversed-phase column (10 μm). Fractions containing the second peak were concentrated and freeze-dried; $[\alpha]^{30}_D = +20°$ ($c = 1$, in water); ^{1}H NMR (DMSO-d_6) δ 1.75 (s, 3H), 2.45 (m, 1H), 2.70 (m, 1H), 4.03 (s, 2H), 4.63 (m, 1H), 5.30 (d, $J = 7.5$ Hz, 1H), 7.38 (m, 5H), 7.70−7.90 (AA′BB′, 4H), 8.53 (m, 2H, NH), 9.05 (s, 1H, NH), 9.15 (br s, 2H, NH), 9.30 (br s, 2H, NH), 12.70 (br s, 2H, COOH; MS *m*/*z* (FAB) 539 $(M + H)^+$.

{**2-[4-(*R*,*S*)-(4-Aminoiminomethyl-benzyl)-2,5-dioxo-imidazolidin-1-yl]acetyl**}-**Asp-(*S*)-phenylglycine-OH Trifluoroacetic Acid Salt (19).** The method for compound **4** was employed; $[\alpha]^{28}_D = +9.7$ ° ($c = 1$, in water); ^{1}H NMR (DMSO-d_6) δ 1.74 (s, 3H), 2.65−2.75 (m, 2H), 3.05 (m, 2H), 3.93 (m, 2H), 4.53 (m, 1H), 4.68 (m, 1H), 5.20 (m, 1H), 7.28−7.40 (m, 5H), 7.48 (m, 2H), 7.78 (m, 2H), 8.35−8.53 (m, 2H, NH), 8.90 (m, 3H, NH), 9.40 (m, 2H, NH). Mass calcd for $C_{25}H_{27}N_6O_8$, 539.1890; found, 539.1882.

[4-(4-Cyano-benzylidene)-2,5-dioxo-imidazolidin-1-yl]-acetic Acid Methyl Ester (20a). A mixture of (2,5-dioxo-imidazolidin-1-yl)acetic acid methyl ester (30 g, 174 mmol), 4-cyanobenzaldehyde (28.8 g, 220 mmol), and anhydrous sodium acetate (36 g, 432 mmol) was heated to 135 °C. Acetic acid (60 mL) was added, and the mixture was stirred for 3 h. After cooling the mixture was added to 600 mL of water and 70 mL of methanol. The precipitation was filtered and dried in a vacuum to yield 37.39 g (75%) of **20a**. mp 265 °C.

[4-(4-Aminoiminomethyl-benzylidene)-2,5-dioxo-imidazolidin-1-yl]acetic Acid Methyl Ester Hydrochloride (20b). Dry HCl was bubbled through a solution of **20a** (10 g,

35.1 mmol) in CH_2Cl_2 (600 mL) and methanol (100 mL) at 0 °C for 7 h. The mixture was stirred overnight at room temperature and the precipitate filtered. The precipitate was dissoved in methanol (1400 mL), and NH_3 in methanol (17 mL, 2.935 mol/L) was added. After the mixture was stirred overnight, diethyl ether was added and the precipitate filtered and dried to yield 9.3 g (78%) of **20b**. mp 290 °C.

[4-(4-Aminoiminomethyl-benzylidene)-2,5-dioxo-imidazolidin-1-yl]acetic Acid Hydrochloride (20c). Compound **21b** (1.61 g, 4.75 mmol) was heated in HCl (6 N, 32 mL) for 2 h under reflux. After cooling, the solid was filtered and dried to give 1.42 g (92%) of **20c**. mp > 300 °C; ^{1}H NMR (DMSO-d_6) δ 4.20 (s, 2H), 6.66 (s, 1H), 2.70 (m, 1H), 7.75−7.95 (m, 4H), 9.15−9.50 (m, 4H), 11.23 (s, 1H, NH).

{2-[4-(4-Aminoiminomethyl-benzylidene)-2,5-dioxo-imidazolidin-1-yl]acetyl-Asp-(*S*)-phenylglycine-OH Trifluoroacetic Acid Salt (20). The method for compound **4a** was employed starting from **20c** followed by treatment with TFA to give 0.21 g (13%) of **20**. mp 255 °C; ^{1}H NMR (DMSO-d_6) δ 2.4−2.55 (m, 1H), 2.65−2.8 (m, 1H), 4.15 (s, 2H), 4.65−4.75 (m, 1H), 5.05−5.2 (m, 1H), 6.65 (s, 1H), 7.20−7.45 (m, 8H), 7.7−7.95 (m, 7H), 8.2−8.4 (m, NH), 8.6−8.75 (m, NH), 9.05−10.1 (b, NH), 10.5−11.0 (b,OH). Mass calcd for $C_{25}H_{25}N_6O_8$, 537.1734; found, 537.1740.

{2-[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-4-ethyl-2,5-dioxo-imidazolidin-1-yl]acetyl}-Asp(OBu^t)-(*S*)-phenylglycine-OBu^t Hydrochloride (21a). The method for compound **16a** was employed starting from **28b.** ^{1}H NMR (DMSO-d_6) δ 1.85 (m, 3H), 1.30−1.40 (s, 18H), 1.90−2.20 (m, 2H), 2.43 (dd, J = 9 Hz, J = 15 Hz, 1H), 2.70 (dd, J = 5 Hz, J = 15 Hz, 1H), 4.00 (s, 2H), 4.70 (m, 1H), 5.20 (m, 1H), 7.38 (m, 5H), 7.70−7.90 (AA′BB′, 4H); MS *m/z* (FAB) 665.4 (M + H)$^+$.

{2-[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-4-ethyl-2,5-dioxo-imidazolidin-1-yl]acetyl}-Asp-(*S*)-phenylglycine Trifluoroacetic Acid Salt (21). The method for compound **16** was employed starting from **21a**. ^{1}H NMR (D_2O) δ 0.90 (m, 3H), 2.10−2.40 (m, 2H), 2.70−3.00 (m, 2H), 4.25 (m, 2H), 5.35 (d, J = 10 Hz, 1H), 7.25−7.40 (m, 5H), 7.70−7.85 (m, 4H). Mass calcd for $C_{26}H_{29}N_6O_8$, 553.2047; found, 553.2048.

{2-[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-4-cyclopropyl-2,5-dioxo-imidazolidin-1-yl]acetyl}-Asp(OBu^t)-(*S*)-phenylglycine-OBu^t Hydrochloride (22a). The method for compound **16a** was employed starting from **28c**. Compound **22a** was used without further purification.

{2-[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-4-cyclopropyl-2,5-dioxo-imidazolidin-1-yl]acetyl}-Asp-(*S*)-phenylglycine-OH Trifluoroacetic Acid Salt (22). The method for compound **16** was employed starting from **22a**. ^{1}H NMR (DMSO-d_6) δ 0.30−0.75 (m, 4H), 1.70 (m, 1H), 2.40 (m, 1H), 2.75 (m, 1H), 4.08 (m, 2H), 4.65 (m, 1H), 4.88 (m, 1H), 7.10−7.33 (m, 5H), 7.73−8.00 (m, 5H), 8.70−9.10 (m, 4H, NH), 11.20 br s, 1H, NH), 11.40 (br s, 1H, NH). Mass calcd for $C_{27}H_{29}N_6O_8$, 565.2047; found, 565.2038.

{2-[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-4-benzyl-2,5-dioxo-imidazolidin-1-yl]acetyl}-Asp(OBu^t)-(*S*)-phenylglycine-OBu^t Hydrochloride (23a). The method for compound **16a** was employed starting from **28d**. Compound **23a** was used without further purification.

{2-[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-4-benzyl-2,5-dioxo-imidazolidin-1-yl]acetyl}-Asp-(*S*)-phenylglycine-OH Trifluoroacetic Acid Salt (23). The method for compound **16** was employed starting from **23a**. ^{1}H NMR (DMSO-d_6) δ 2.38 (m, 1H), 2.70 (m, 1H), 3.50−3.80 (m, 4H), 4.55 (m, 1H), 4.88 (m, 1H), 7.25 (m, 1OH), 7.78−7.98 (AA′BB′, 4H); MS *m/z* (FAB) 615 (M + H)$^+$.

Similar methods were employed for compounds **25b**, **25c**, and **25d**.

4-Cyanphenylethylmethanone (25b) from 4-bromphenylethylmethanone. ^{1}H NMR (DMSO-d_6) δ 1.10 (t, J 6.3 Hz, 3H), 3.10 (q, J = 6.3 Hz, 2H), 7.95−8.15 (AA′BB′, 4H); MS *m/z* (FAB) 160 (M + H)$^+$.

4-Cyanphenylcyclopropylmethanone (25c). 4-Bromophenylcyclopropylmethanone (22.5 g, 100 mmol) and CuCN (10.3 g, 100 mmol) were dissolved in DMF (15 mL) and stirred for 4 h under reflux. The suspension was allowed to cool to 70 °C, and poured into a solution of of iron(III) chloride (40 g), concentrated HCl (10 mL), and water (60 mL). The mixture was stirred at 70 °C for 20 min and extracted three times with toluene (90 mL). The organic extracts were washed with HCl (2 N, 250 mL) and with NaOH (2 N, 250 mL) and evaporated. The solid residue was triturated with ligroine to give 14.57 g (85%). ^{1}H NMR (DMSO-d_6) δ 1.10 (m, 4H), 2.93 (m, 1H), 8.00−8.24 (AA′BB′, 4H); MS *m/z* (FAB) 172 (M + H)$^+$.

4-Cyanphenylbenzylmethanone (25d) starting from 4-bromphenylbenzylmethanone. ^{1}H NMR (DMSO-d_6) δ 4.45 (s, 2H), 7.30 (m, 5H), 8.00−8.25 (AA′BB′, 4H); MS *m/z* (FAB) 222 (M + H)$^+$.

Similar methods were employed for compounds **26a**, **26b**, **26c**, and **26d**.

4-(*R,S*)-(4-Cyanophenyl)-4-methyl-2,5-dioxoimidazolidine (26a). 4-Acetyl benzonitrile (20 g, 138 mmol), ammonium carbonate (115.6 g, 1.21 mol), and potassium cyanide (11.6 g, 178 mmol) were dissolved in ethanol/water (600 mL, 1:1). The mixture was stirred for 5 h at 55 °C and for 12 h at room temperature. The solution was adjusted to pH = 6.3 with 6 N HCl and subsequently stirred at room temperature for 2 h. The precipitate was filtered off, washed with water, and dried over phosphorus pentoxide under high vacuum to give 22.33 g (75%). ^{1}H NMR (DMSO-d_6) δ 1.66 (s, 3H), 7.68 (d, J = 7.5 Hz, 2H), 7.90 (d, J = 7.5 Hz, 2H), 8.74 (s, 1H, NH), 10.9 (s, 1H, NH); MS *m/z* (FAB) 216 (M + H)$^+$.

(*R,S*)-(4-Cyanophenyl)-4-ethyl-2,5-dioxoimidazolidine (26b). ^{1}H NMR (DMSO-d_6) δ 0.80 (t, J = 7.5 Hz, 3H), 1.80−2.10 (m, 2H), 7.68−7.93 (AA′BB′, 4H), 8.75 (s, 1H, NH), 10.90 (s, 1H, NH); MS *m/z* (FAB) 230 (M + H)$^+$.

(*R,S*)-(4-Cyanophenyl)-4-cyclopropyl-2,5-dioxoimidazolidine (26c). ^{1}H NMR (DMSO-d_6) δ 0.40 (m, 2H), 0.50 (m, 2H), 1.65 (m, 1H), 7.70−7.95 (AA′BB′, 4H), 8.50 (s, 1H, NH), 10.95 (s, 1H, NH); MS *m/z* (FAB) 242 (M + H)$^+$.

(*R,S*)-(4-Cyanophenyl)-4-benzyl-2,5-dioxoimidazolidine (26d). ^{1}H NMR (DMSO-d_6) δ 3.00 (d, J = 15 Hz, 1H), 3.50 (d, J = 15 Hz, 1H), 7.25 (m, 5H), 7.50 (m, 1H, NH), 7.83−7.95 (AA′BB′, 4H), 8.78 (s, 1H, NH); MS *m/z* (FAB) 292 (M + H)$^+$.

Similar methods were employed for compounds **27a**, **27b**, **27c**, and **27d**.

[(*R,S*)-4-(4-Cyanophenyl)-4-methyl-2,5-dioxoimidazolidin-1-yl]acetic Acid Methyl Ester (27a). Sodium (1.068 g, 46.47 mmol) was dissolved under nitrogen in dry methanol (110 mL). Compound **26a** (10 g, 46.47 mmol) was added to the clear solution, and the mixture was boiled under reflux for 2 h. Potassium iodide (7.75 g, 46.48 mmol) was added, and a solution of methyl chloroacetate (4.53 mL, 51.3 mmol) in methanol (5 mL) was added dropwise within 1 h. The mixture was heated to reflux for 6 h, left to stand at room temperature overnight, and then concentrated. The oily residue was chromatographed on silica gel using CH_2Cl_2/ethyl acetate (9:1) to give 8.81 g (66%). ^{1}H NMR (DMSO-d_6) δ 1.75 (s, 3H), 3.65 (s, 3H), 4.25 (s, 2H), 7.73 (d, J = 9 Hz, 2H), 7.9 5 (d, J = 9 Hz, 2H), 9.2 0 (s, 1H, NH); MS *m/z* (FAB) 288.1 (M + H)$^+$.

[(4-(*R,S*)-(4-Cyanophenyl)-4-ethyl-2,5-dioxoimidazolidin-1-yl]acetic Acid Methyl Ester (27b). ^{1}H NMR (DMSO-d_6) δ 0.88 (t, J = 7.5 Hz, 3H), 1.90−2.30 (m, 2H), 3.65 (s, 3H), 4.24 (s, 2H), 7.70−7.98 (AA′BB′, 4H), 9.25 (s, 1H, NH); MS *m/z* (FAB) 302 (M + H)$^+$.

[(4-(*R,S*)-(4-Cyanophenyl)-4-cyclopropyl-2,5-dioxoimidazolidin-1-yl]acetic Acid Methyl Ester (27c). ^{1}H NMR (DMSO-d_6) δ 0.45−0.65 (m, 4H), 1.66 (M, 1H), 3.70 (s, 3H, OCH_3), 4.24 (s, 2H), 7.75−7.98 (AA′BB′, 4H), 9.00 (s, 1H, NH); MS *m/z* (FAB) 314 (M + H)$^+$.

[(4-(*R,S*)-(4-Cyanophenyl)-4-benzyl-2,5-dioxoimidazolidin-1-yl]acetic Acid Methyl Ester (27d). MS *m/z* (FAB) 364 (M + H)$^+$.

Similar methods were employed for compounds **28a**, **28b**, **28c**, and **28d**.

[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetic Acid Hydrochloride (28a). A cooled (0 °C) solution of **27a** (28.8 g, 100 mmol) in ethanol

(400 mL) was purged with dry HCl while maintaining the temperature below 10 °C until the characteristic absorption of the nitrile group had disappeared (IR). The solvent was evaporated to give 38.4 g (99%) of (4-(*R,S*)-((4-ethoxyimino-methyl)-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl) acetic acid ethyl ester hydrochloride: MS *m/z* (FAB) 348 $(M + H)^+$. This intermediate was immediately suspended in 2-propanol (380 mL) and treated with a solution of NH_3 in 2-propanol (2 N, 115 mL). The reaction mixture was stirred for 2 h at 50 °C and allowed to cool to room temperature. The product was precipitated by addition of diethyl ether (2000 mL). The material was filtered and dried in high vacuum to give 24.8 g (71%) of (4-(*S*)-((4-amino-imino-methyl)- phenyl)-4-methyl-2,5-dioxoimidazolidin-1-yl)-acetic acid ethylester hydrochloride: MS *m/z* (FAB) 319 $(M + H)^+$. The ester (24.7 g/mmol) was dissolved in concentrated HCl (375 mL) and refluxed for 6 h. The solvent was removed under reduced pressure. The residue was freeze-dried to give 21.65 g (95%) of **28a**. ^{1}H NMR (DMSO-d_6) δ 1.74 (s, 3H), 4.09 (s, 2H), 7.7−7.9 (AA′BB′, 4H), 9.2 (s, 1H), 9.28 (s, 2H), 9.45 (s, 2H); MS *m/z* (FAB) 291.1 $(M + H)^+$.

[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-4-ethyl-2,5-dioxo-imidazolidin-1-yl]acetic Acid Hydrochloride (28b). ^{1}H NMR (DMSO-d_6) δ 0.88 (s, 3H), 2.10 (m, 2H), 4.10 (s, 2H), 7.70−7.90 (AA′BB′, 4H), 9.25 (m, 3H, NH), 9.45 (s, 2H, NH); MS *m/z* 305 (FAB) $(M + H)^+$.

[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-4-cyclopropyl-2,5-dioxo-imidazolidin-1-yl]acetic Acid Hydrochloride (28c). ^{1}H NMR (DMSO-d_6) δ 0.55 (m, 4H), 1.68 (m, 1H), 4.10 (s, 2H), 7.80−7.93 (AA′BB′, 4H), 9.00 (s, 1H, NH), 9.25 (br s, 2H, NH), 9.44 (br s, 2H, NH); MS *m/z* (FAB) 317 $(M + H)^+$.

[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-4-benzyl-2,5-dioxo-imidazolidin-1-yl]acetic Acid Hydrochloride (28d). ^{1}H NMR (DMSO-d_6) δ 3.20 (d, J = 15 Hz, 1H), 3.55 (d, J = 15 Hz, 1H), 3.78 (d, J = 5 Hz, 2H), 7.25 (m, 5H), 7.90 (s, 4H), 9.20−9.50 (m, 5H, NH); MS *m/z* (FAB) 367.2 $(M + H)^+$.

{2-[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetyl}-Asp-(*S*)-phenylglycine-OCH_3 Hydrochloride (35). The method for compound **4a** was employed. ^{1}H NMR (DMSO-d_6) δ 1.88 (s, 3H), 2.80 (dd, J = 7.5 Hz, J = 17.5 Hz, 1H), 3.96 (dd, J = 5 Hz, J = 17.5 Hz, 1H), 3.65−3.74 (s, 3H), 4.30 (br s, 3H), 5.46 (m, 1H), 7.25−7.40 (m, 5H), 7.70−7.90 (AA′BB′, 4H). Mass calcd for $C_{26}H_{29}N_6O_8$, 553.2047; found, 553.2048.

{2-[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetyl}-Asp-OCH_3-(*S*)-phenylglycine-OCH_3 Hydrochloride (36). The method for compound **4a** was employed starting from **28a** to give 437 mg (16%). ^{1}H NMR (DMSO-d_6) δ 1.75 (s, 3H), 2.45 (m, 1H), 2.65 (m, 1H), 3.60 (OCH_3, 6H), 4.03 (br s, 2H), 4.70 (m, 1H), 5.40 (m, 1H), 7.35 (m, 5H), 7.75 (d, J = 7.5 Hz, 2H), 7.85 (d, J = 7.5 Hz, 2H), 8.55 (m, 1H, NH), 8.80 (m, 1H, NH), 9.10 (br s, 3H), 9.40 (br s, 2H). Mass calcd for $C_{27}H_{31}N_6O_8$, 567.2203; found, 567.2194.

{2-[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetyl}-Asp-2-Adamantyl-amide Trifluoroacetic Acid Salt (37). The method for compound **4a** was employed starting from **28a** followed by treatment with TFA to give 615.8 mg. ^{1}H NMR (DMSO-d_6) δ 1.35−1.90 (m, 17H), 2.35−2.65 (m, 2H), 3.78 (m, 1H), 4.58 (m, 1H), 7.70−7.90 (AA′BB′, 4H), 7.80 (1H, NH), 8.50 (m, 1H, NH), 9.08 (br s, 1H, NH). Mass calcd for $C_{27}H_{35}N_6O_6$, 539.2618; found, 539.2621.

3-Amino-2-S-benzyloxycarbonyl-aminopropionic Acid *tert*-Butyl Ester (38a). A cooled suspension of 3-amino-2-L-benzyloxycarbonylaminopropionic acid (5 g, 21 mmol) in dioxane (50 mL) was treated with concentrated H_2SO_4 (5 mL). The slightly yellowish solution was cooled with dry ice, and condensed isobutylene (50 mL) was added. The mixture was shaken at room temperature for 3 d under nitrogen at 20 atm. Subsequently, excess isobutylene was removed by nitrogen. The solution was adjusted to a pH of 10 with $NaCO_3$ (2 M, 70 mL) and extracted three times with diethyl ether (200 mL). The organic phase was washed with water, dried, and evaporated to give 4.31 g of oil (70%). ^{1}H NMR (DMSO-d_6) δ 1.38 (s, 9H), 2.80 (m, 2H), 3.90 (m, 1H), 5.05 (s, 2H), 7.35 (br s, 5H), 7.50 (m, 1H, NH); MS *m/z* (FAB) 295.1 $(M + H)^+$.

3-{2-[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetylamino}-2-S-benzyloxy-carbonylamino-propionic Acid Hydrochloride (38). The method for compound **4a** was employed starting from **28a** to give crude material (6.32 g) which was treated with TFA (3.6 mL), water (0.4 mL), and ethanedithiole (0.4 mL) to give 350 mg. ^{1}H NMR (DMSO-d_6) δ 1.75 (s, 3H), 3.50 (m, 2H), 4.00 (br s, 3H), 5.03 6 (s, 2H), 7.33 (br s, 6H), 7.80 (AA′BB′, 4H), 8.25 (m, 1H, NH), 9.05 (s, 1H, NH), 9.25 (br s, 2H, NH), 9.50 (br s, 2H, NH). Mass calcd for $C_{24}H_{27}N_6O_7$, 511.1941; found, 511.1940.

3-{2-[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetylamino}-3-(*R,S*)-phenyl-propionic Acid Ethyl Ester Hydrochloride (39a). The method for compound **4a** was employed starting from **28a** to give 597 mg (61%). ^{1}H NMR (DMSO-d_6) δ 1.10 (q, 3H), 1.70 (s, 3H), 1. 80 (s, 3H), 2.75 (m, 2H), 3.90−4.10 (m, 4H), 5.15 (m, 1 H), 7.20−7.40 (m, 5H), 7.68−7.85 (AA′BB′), 8.80 (d, 1H, NH), 9.60 (brs, 4H, NH); MS *m/z* 466 $(M + H)^+$.

3-{2-[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetylamino}-3-(*R,S*)-phenyl-propionic Acid Hydrochloride (39). Compound **39a** (580 mg, 1.19 mmol) was dissolved in concentrated HCl (55 mL) and left at room temperature for 5.5 h. The solution was evaporated, and the residue (540 mg) was chromatographed on Sephadex LH20 (solvent C). The residue was freeze-dried to give 477 mg (85%). ^{1}H NMR (DMSO-d_6) δ 1.75 (s, 3H), 2.60 (d, J = 7.5 Hz, 2H), 4.03 (s, 2H), 5.10 (dd, J = 7.5 Hz, J = 15 Hz, 1H), 7.30 (m, 5H), 7.70−7.90 (AA′BB′, 4H), 8.75 (d, J = 7.5 Hz, 1H, NH), 9.07 (br s, 1H, NH), 9.60 (br s, 4H). Mass calcd for $C_{22}H_{24}N_5O_5$, 438.1777; found, 438.1761.

3-{2-[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetylamino}-3-(*R,S*)-methyl-propionic Acid Methyl Ester Hydrochloride (40a). The method for compound **4a** was employed starting from **28a.** ^{1}H NMR (DMSO-d_6) δ 1.18 (t, J 7.5 Hz, 3H), 1.63 (m, 1H), 1.73 (s, 3H), 2.30 (m, 2H), 3.08 (m, 3H), 3.84 (m, 2H), 4.04 (q, J = 7.5 Hz, 2H), 7.70−7.88 (AA′BB′), 8.18 (m, 1H, NH); MS *m/z* 404 $(M + H)^+$.

3-{2-[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetylamino}-3-(*R,S*)-methyl-propionic Acid Hydrochloride (40). The method for compound **39** was employed to give 477 mg (85%). ^{1}H NMR (DMSO-d_6) δ 1.60 (m, 1H), 1.73 (s, 3H), 2.21 (m, 2H), 3.05 (m, 3H), 3.53 (m, 2H), 3.98 (s, 2H), 7.73−7.88 (AA′BB′), 8.15 (m, 1H, NH), 8.95 (m, 1H, NH), 9.03 (br s, 2H, NH), 9.33 (br s, 2H, NH), 12.08 (br s, 1H). Mass calcd for $C_{17}H_{22}N_5O_5$, 376.1621; found, 376.1617.

1-Adamantyl carbaldehyde (41a). A solution of 1-(hydroxymethyl)adamantane (19.98 g, 120 mmol) in CH_2Cl_2 (180 mL) was added to a suspension of pyridinium chlorochromate (36.9 g, 171 mmol) in CH_2Cl_2 (240 mL) at 12−18 °C during 15 min. After the mixture was stirred at 12−18 °C for 90 min, heptane (900 mL) was added. The mixture was filtered, and the filtrate was evaporated under reduced pressure. The residue was dissolved in pentane, and the pentane phase was washed with NaOH (0.2 N, 3 × 120 mL) and water (2 × 120 mL). The solvent was dried and evaporated to give 14.58 g of crude material which was used for the next step without further purification

(*R,S*)-3-Amino-3-(1-adamantyl)propionic Acid (41b). Malonic acid (5.64 g 54.2 mmol), ammonium acetate (8.67 g, 112.5 mmol), and **41a** (8.9 g, 54.2 mmol) in absolute ethanol (14 mL) were stirred under reflux for 4 h. The precipitate was filtered and washed with ethanol. The solvent was removed, and the residue distributed between CH_2Cl_2 and water. The water phase was freeze-dried and the residue treated with a small amount of water. lt was filtered and dried in vacuo over phosphorus pentoxide to give 1.75 g (15%) of **41b** as a white solid. ^{1}H NMR (CD_3OD) δ 1.5−2.05 (m, 15H), 2.1 (dd, J = 5.5 Hz, J = 10.0 Hz, 1H), 2.5 (dd, J = 1.5 Hz, J = 10.0 Hz, 1H), 2.95 (dd, J = 5.5 Hz, J = 1.5 Hz, 1H).

(*R,S*)-3-Amino-3-(1-adamantyl)propionic Acid Methyl Ester (41c). A solution of **41b** (1.75 g, 7.87 mmol) in methanolic HCl (2 N) was stirred under reflux for 4 h. The solvent was removed in vacuo and the residue distributed between diethyl ether (50 mL) and water (50 mL). The phases were separated, and the water phase was extracted with diethyl ether (50 mL). The water phase was adjusted to pH 9.3 with NaOH (2 N) and extracted with CH_2Cl_2 (2 × 50 mL). After drying over anhydrous Na_2SO_4, the solvent was evaporated to give 1.45 g (78%), pale yellow oil. ^{1}H NMR (DMSO-d_6) δ 1.22−1.72 (m, 15H), 1.85−2.05 (m, 4H), 2.43−2.55 (m, 1H), 3.58 (s, 3H).

3-{2-[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetylamino}-3-(*R,S*)-(1-adamantyl)propionic Acid Methyl Ester Hydrochloride (41d). The method for compound **4a** was employed starting from **28a** to give 800 mg (45%). ^{1}H NMR (DMSO-d_6) δ 1.3−2.0 (m, 18H), 2.1−2.3 (m,1H), 2.4−2.6 (m,1H), 3.5, 3.55 (2s, 3H), 3.73−3.87 (m, 1H), 3.85−4.05 (AB, 2H), 7.68−7.85 (m, 4H), 7.92 (d, J = 5.0 Hz, 1H), 9.0−9.9 (bs, 5H); MS *m/z* (ESI) 510 (M + H)$^+$.

3-{2-[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetylamino}-3-(*R,S*)-(1-adamantyl)propionic Acid Hydrochloride (41). The method for compound **39** was employed to give 260 mg (89%). ^{1}H NMR (DMSO-d_6) δ 1.3−2.1 (m, 19H), 2.3−2.45 (m, 1H), 2.4−2.6 (m, 1H), 3.73−3.9 (m, 1H), 3.87−4.03 (AB, 2H), 7.7−8.0 (m, 5H) 8.8−11.0 (2bs, 5H). Mass calcd for $C_{26}H_{34}N_5O_5$, 496.2660; found, 496.2582.

2-[4-(*R,S*)-(4-Cyanophenyl)-3-ethyl-4-methyl-2,5-dioxoimidazolidin-1-yl]acetic Acid Methyl Ester (42a). A solution of 2-[4-(*S,R*)-(4-cyanophenyl)-4-methyl-2,5-dioxoimidazolidin-1-yl]acetic acid methyl ester (4.5 g, 15.7 mmol) in dried DMF (25 mL) was treated (Argon) with NaH (753.6 mg, 17.3 mmol, 50% oil dispersion). After the mixture was stirred for 15 min, ethyl iodide (1.39 mL, 17.3 mmol) was added, and stirring was continued for 4 h at room temperature and left overnight. The solution was concentrated, and the residue was chromatographed on silica CH_2Cl_2/ethyl acetate (9.5:0.5) to give 2.55 g of oil (51%). ^{1}H NMR (DMSO-d_6) δ 0.98 (t, J = 6.3 Hz, 3H), 1.85 (s, 3H), 3.10 (m, 2H), 3.70 (s, 3H), 4.30 (s, 2H), 7.63 (d, J = 9 Hz, 2H), 7.96 (d, J = 9 Hz, 2H); MS *m/z* (FAB) 316 (M + H)$^+$.

2-[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-3-ethyl-4-methyl-2,5-dioxoimidazolidin-1-yl]acetic Acid (42b). The method for compound **28a** was employed starting from **42a**. ^{1}H NMR (DMSO-d_6) δ 1.00 (t, 3H), 1.88 (s, 3H), 3.05 (m, 2H), 4.18 (s, 2H), 7.65 (d, J = 7.5 Hz, 2H), 7.90 (d, J = 7.5 Hz, 2H), 9.30−9.50 (m, 4H, NH); MS *m/z* (FAB) 319.2 (M + H)$^+$.

3-{2-[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-3-ethyl-4-methyl-2,5-dioxoimidazolidin-1-yl]acetylamino}-3-(*R,S*)-phenylpropionic Acid Ethyl Ester Hydrochloride (42c). The method for compound **4a** was employed starting from **42b**. ^{1}H NMR (DMSO-d_6) δ 0.95 (t, J = 6.3 Hz, 3H), 1.10 (m, 3H), 1.73 (s, 3H, CH_3COOH), 1.84 (s, 3H), 2.75 (m, 2H), 2.90−3.40 (m, 2H), 4.00 (q, J = 6.3 Hz, 2H), 4.10 (br s, 2H), 5.10 (m, 1H), 7.30 (m, 5H), 7.65−7.90 (AA′BB′, 4H), 8.80 (d, 1H, NH); MS *m/z* (FAB) 494 (M + H)$^+$.

3-{2-[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-3-ethyl-4-methyl-2,5-dioxoimidazolidin-1-yl]acetylamino}-3-(*R,S*)-phenylpropionic Acid Hydrochloride (42). The method for compound **39** was employed.

(*R,S*)-3-*N*-Methylamino-3-phenylpropionic acid (43a). Methylammonium chloride (20.26 g, 300 mmol) and sodium acetate (24.61 g, 300 mmol) in absolute ethanol (40 mL) were stirred for 30 min. Malonic acid (10.4 g, 100 mmol) and benzaldehyde (10.61 g, 100 mmol) were added, and the mixture was stirred under reflux for 3.5 h. The precipitate was filtered and washed with ethanol. The solvent was removed and the residue distributed between ethyl acetate and water. The water phase was concentrated, and the residue was purified by flash chromatography on silica gel using methanol as eluent to give 26.23 g of **43a** (contains some amounts of methylammonium acetate). ^{1}H NMR (DMSO-d_6) δ 2.3 (s, 3H), 2.2−2.4 (m, 2H), 3.85 (m, 1H), 7.2−7.4 (m, 5H); MS *m/z* (CI) 180 (M + H)$^+$.

(*R,S*)-3-*N*-Methylamino-3-phenylpropionic Acid Methyl Ester Hydrochloride (43b). Thionyl chloride (10.7 mL, 145 mmol) was added dropwise to absolute methanol (100 mL) at − 15 °C. Compound **43a** (13 g, 73.4 mmol) was added, and the solution was allowed to stand overnight at room temperature. The solvent was removed, and the residue was purified by flash chromatography on silica gel (CH_2Cl_2/methanol 9:1) to give 7.1 g (50%). ^{1}H NMR (DMSO-d_6) δ 2.32 (s, 3H), 3.1 (m, 2H), 3.52 (s, 3H), 4.48 (dd, J = 4.5 Hz, J = 2.5 Hz, 1H), 7.4−7.6 (m, 5H), 8.6−9.8 (bs, 2H, NH_2^+); MS *m/z* (ESI) 194 (M + H)$^+$.

3-{2-[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetyl-*N*-methylamino}-3-(*R,S*)-phenylpropionic Acid Methyl Ester Hydrochloride (43c). TOTU (*O*-(cyano(ethoxycarbonyl)-methyleneamino)-1,1,3,3-tetramethyluronium-tetrafluoroborate (328 mg, 1 mmol) was added to a solution of **28a** (327 mg, 1 mmol), **43b** (230 mg, 1 mmol), and *N*-ethylmorpholine (115 mg, 1 mmol) in DMF (15 mL) at 0 °C. The solution was allowed to stir at room temperature for 24 h. The solvent was evaporated and the residue was chromatographed on Sephadex LH20 (solvent C) and freeze-dried to give 160 mg (32%). ^{1}H NMR (DMSO-d_6) δ 1.78 (s, 3H), 2.78, 3.23 (2s, 3H), 2.8−3.0 (m, 1H), 3.05−3.25 (m, 1H), 3.52, 3.58, 3.68, 3.7 (4s, 3H), 4.18−4.38, 4.55 (2AB, 2H), 5.47−5.55, 5.9−6.03 (2m, 1H), 7.2−7.5 (m, 5H), 7.73−7.95 (m, 4H), 8.85−9.4 (bs, 5H); MS *m/z* (ESI) 466 (M + H)$^+$.

3-{2-[4-(*R,S*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetyl-*N*-methylamino}-3-(*R,S*)-phenylpropionic Acid Hydrochloride (43). Compound **43c** (270 mg, 0.54 mmol) was dissolved in HCl (6 N, 15 mL) and stirred at room temperature for 7 h. The solution is diluted with water and freeze-dried to give 240 mg (91%). ^{1}H NMR (DMSO-d_6) δ 1.78 (s, 3H), 2.52,2.75 (2s, 3H), 2.63−2.83,3.0−3.2 (2m, 2H), 4.18−4.35,4.48−4.7 (2,AB, 2H), 5.45−5.55,5.95−6.0 (2m, 1H), 7.13−7.65 (2s, 1H), 7.2−7.45 (m, 5H), 7.73−7.95 (m, 4H), 9.15 (s, 1H), 9.35−9.65 (2bs, 4H), 12.35−12.65 (bs, 1H); MS *m/z* (ESI) 452 (M + H)$^+$.

(*S*)-3-Amino-3-phenylpropionic Acid Ethyl Ester Hydrochloride (49a). To a solution of (*R,S*)-3-amino-3-phenylpropionic acid ethyl ester (46.27 g, 239.4 mmol) in absolute ethanol (492 mL) was slowly added Z-L-Phe-OH (71.67 g, 239.4 mmol) under stirring. The solution was kept at 4 °C for 12 h. The precipitate was filtered, washed with a small proportion of cold absolute ethanol, and dried in vacuo over phosphorus pentoxide to give 53.19 g of the *S* isomer as Z-L-Phe-OH salt containing 3.2% of the *R* isomer as determined by GC analysis. It was recrystallized from absolute ethanol to give 47.49 g which was confirmed by GC analysis to be optically pure (>99% ee). The optically pure Z-L-Phe-OH salt (47.49 g, 96.4 mmol) was suspended in a mixture of ethyl acetate (2400 mL) and of water (2400 mL). Solid $NaHCO_3$ was added until a pH of 7.5 was reached. The organic phase was separated, and the aqueous phase was washed twice with ethyl acetate (1000 mL). The combined organic phases were washed with water (100 mL) and dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure, and the residue was taken up in absolute ethanol (1000 mL). A saturated solution of HCl (10 mL) in ethanol was added. The mixture was stirred, filtered, and the solvent was removed under reduced pressure. The remaining residue was treated with diethyl ether. It was filtered, washed with diethyl ether, and dried in vacuo over phosphorus pentoxide to give 20 g (30%) of **49a**. $[\alpha]^{22}_D$ = +5.8° (c = 1; methanol); ^{1}H NMR (DMSO-d_6) δ 1.1 (t, J = 5 Hz, 3 H), 1.96 (br s, 2H), 2.6 (m, 2H), 4.0 (q, J = 5 Hz, 2H), 4.2 (t, J = 5 Hz, 3H), 7.17−7.4 (m, 5H); MS *m/z* (FAB) 194 (M + H)$^+$.

(*R*)-3-Amino-3-phenylpropionic Acid Ethyl Ester Hydrochloride (49b). The method for compound **49a** was employed starting from (*R,S*)-3-amino-3-phenyl-propionic acid ethyl ester hydrochloride by using Z-D-Phe-OH to resolve the racemate. $[\alpha]^{22}_D$ = −5.8° (c = 1; methanol); ^{1}H NMR (DMSO-d_6) δ 1.1 (t, J = 5 Hz, 3H), 1.96 (br s, 2H), 2.6 (m, 2H), 4.0 (q,

J = 5 Hz, 2H), 4.2 (t, J = 5 Hz, 3H), 7.17−7.4 (m, 5H); MS m/z (FAB) 194 $(M + H)^+$.

(*R*,*S*)-4-(4-Bromophenyl)-4-methyl-2,5-dioxoimidazolidine (50). The method for compound **26a** was employed starting from 4-bromoacetophenone to give 65.3 g (97%). ^{1}H NMR (DMSO-d_6) δ 1.65 (s, 3 H), 7.4−7.65 (AA′BB′, 4 H), 8.63 (s, 1 H), 10.85 (br s, 1 H); MS m/z (FAB) 269 $(M + H)^+$.

(*R*,*S*)-2-Amino-2-(4-bromophenyl)-propionic Acid (51). A solution of NaOH (3 N, 50 mL) was added to **50** (5.3 g, 20 mmol). The resulting suspension was heated in an autoclave under nitrogen (10 bar) for 1 h at 145 °C. The reaction mixture was allowed to cool to room temperature. It was diluted with water (150 mL), and the pH was adjusted to 4 by addition of acetic acid using an ice bath for cooling. The reaction mixture was stirred for 2 h at 0 °C. The precipitate was isolated, washed with water, and dried in vacuo over phosphorus pentoxide to give 3.65 g (75%)of **51** as a white solid. ^{1}H NMR (DMSO-d_6) δ 1.6 (s, 3H), 7.4−7.6 (AA′BB′, 4H); MS m/z (FAB) 244 $(M + H)^+$.

(*R*,*S*)-2-Amino-2-(4-bromophenyl)-propionic Acid Ethyl Ester (52). A solution of HCl (9.8 N, 150 mL) in ethanol was added to **51** (27.3 g, 112.3 mmol). The suspension was refluxed for 18 h. The solvent was removed, and the remaining residue was distributed between ethyl acetate and an saturated aqueous solution of $NaHCO_3$. The organic layer was washed with water and dried over anhydrous magnesium sulfate. The solvent was removed and the crude product (23.22 g) was distilled in vacuo to give 20.7 g (68%) of **52** as a colorless oil, bp 129−130 °C/2 mm. ^{1}H NMR (DMSO-d_6) δ 1.14 (t, J = 8 Hz, 3H), 1.5 (s, 3H), 2.38 (br s, 2H), 4.08 (q, J = 8 Hz, 2H), 7.38−7.58 (AA′BB′, 4H); MS m/z (FAB) 272 $(M + H)^+$.

(*S*)-2-Amino-2-(4-bromophenyl)-propionic Acid Ethyl Ester (53a). To a solution of **52** (44.3 g, 163 mmol) and *R*-mandelic acid (24.8 g, 163 mmol) in 2-propanol (138 mL) was added diisopropyl ether (414 mL) at room temperature. The suspension was kept at 0 °C for 12 h. The precipitated salt was isolated and recrystallized twice in the same way to give 20 g of enantiopure **53a** as mandelic acid salt, $[\alpha]^{22}_D$ = −14° (c = 1, 2.15 N HCl in ethanol). The precipitate was distributed between ethyl acetate and an aqueous solution of $NaHCO_3$. The organic layer was washed with water and dried over anhydrous magnesium sulfate. The solvent was removed to give 12.5 g (28%) of **53a** as a colorless oil which was confirmed by means of the *R*(−)-α-methoxy-α-trifluor-methylphenyl acetic acid derivative (Mosher) to be optically pure (>99% ee). $[\alpha]^{22}_D$ = +52.7° (c = 1; 2.15 N HCl in ethanol); ^{1}H NMR (DMSO-d_6) δ 1.14 (t, J = 8 Hz, 3H), 1.5 (s, 3H), 2.38 (br s, 2H), 4.08 (q, J = 8 Hz, 2H), 7.38−7.58 (AA′BB′, 4H); MS m/z (FAB) 272 $(M + H)^+$.

(R)-2-Amino-2-(4-bromophenyl)-propionic Acid Ethyl Ester (53b). The method for compound **53a** was employed by using *S*-mandelic acid to resolve the racemate. $[\alpha]^{22}_D$ = −52.7° (c = 1; 2.15 N HCl in ethanol). ^{1}H NMR (DMSO-d_6) δ 1.14 (t, J = 8 Hz, 3H), 1.5 (s, 3H), 2.38 (br s, 2H), 4.08 (q, J = 8 Hz, 2H), 7.38−7.58 (AA′BB′, 4H); MS m/z (FAB) 272 $(M + H)^+$.

2-(*S*)-(4-Bromophenyl)-2-(3-ethoxycarbonyl-methyl-ureido)-propionic Acid Ethyl Ester (54a). The method for compound **2** was employed starting from **53a** to give 18.1 g (99%) of **54a** as a white solid. $[\alpha]^{22}_D$ = +10.7° (c = 1; 2.15 N HCl in ethanol); ^{1}H NMR (DMSO-d_6) δ 1.17 (t, J = 7.5 Hz, 3H), 1.25 (t, J = 7.5 Hz, 3H), 2.02 (s, 3H), 3.91 (d, J = 6 Hz, 2H), 4.05−4.25 (m, 4H), 5.02 (t, J = 6 Hz, 1H), 6.08 (s, 1H), 7.29−7.51 (AA′BB′, 4H); MS m/z (FAB) 401 $(M + H)^+$.

[4-(*S*)-(4-Bromophenyl)-4-methyl-2,5-dioxoimidazolidin-1-yl]acetic Acid (55a). The method for compound **3** was employed starting from **54a** to give 11.4 g (78%) of **55a**. $[\alpha]^{22}_D$ = +32.8° (c = 1; 2.15 N HCl in ethanol); ^{1}H NMR (DMSO-d_6) δ 1.69 (s, 3H), 4.06 (s, 2H), 7.4−7.65 (AA′BB′, 4H), 9.05 (s, 1H), 13.1 (br s, 1H); MS m/z (FAB) 327 $(M + H)^+$.

[4-(*S*)-(4-Cyanophenyl)-4-methyl-2,5-dioxoimidazolidin-1-yl]acetic Acid (56a). The method for compound **22c** was employed starting from **55a** to give 9.3 g (95%). $[\alpha]^{22}_D$ = +33.4° (c = 1; 2.15 N HCl in ethanol); ^{1}H NMR (DMSO-d_6) d 1.73 (s, 3H), 4.07 (s, 2 H), 7.68−7.95 (AA′BB′, 4H), 9.15 (s, 1H), 13.15 (br s, 1H); MS m/z (FAB) 274 $(M + H)^+$.

[4-(*S*)-((4-Aminoiminomethyl)-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetic Acid Hydrochloride (57a). The method for compound **25a** was employed starting from **56a** to give 21.65 g (95%). $[\alpha]^{22}_D$ = +42.7° (c = 1; 1 N HCl); ^{1}H NMR (DMSO-d_6) δ 1.74 (s, 3H), 4.09 (s, 2H), 7.7−7.9 (AA′BB′, 4H), 9.2 (s, 1H), 9.28 (s, 2H), 9.45 (s, 2H); MS m/z (FAB) 291 $(M + H)^+$.

[4-(*R*)-((4-Aminoiminomethyl)-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetic Acid Hydrochloride (57b). The method for compound **57a** was employed starting from **53b**. $[\alpha]^{22}_D$ = - 42.7° (c = 1; 1 N HCl); ^{1}H NMR (DMSO-d_6) δ 1.74 (s, 3 H), 4.09 (s, 2 H), 7.7−7.9 (AA′BB′, 4 H), 9.2 (s, 1 H), 9.28 (s, 2 H), 9.45 (s, 2 H); MS m/z (FAB) 291 $(M + H)^+$.

3-{2-[4-(*S*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetylamino}-3-(*S*)-phenylpropionic Acid Ethyl Ester Acetate (44a). The method for compound **4a** was employed starting from **57a** and **49a** to give 35 g (83%). $[\alpha]^{22}_D$ = −56.7° (c = 1; water); ^{1}H NMR (DMSO-d_6) δ 1.12 (t, J = 6 Hz, 3H), 1.73 (s, 6H), 2.75 (d, J = 6 Hz, 2H), 3.95−4.1 (m, 4H), 5.18 (q, J = 6 Hz, 1H), 7.2−7.38 (m, 5H), 7.64−7.85 (AA′BB′, 4H), 8.78 (d, J = 8 Hz, 1H), 9.6 (br s, 5 H). Mass calcd for $C_{24}H_{28}N_5O_5$, 466.2090; found, 466.2093.

3-{2-[4-(*S*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetylamino}-3-(*S*)-phenylpropionic Acid Hydrochloride (44). The method for compound **12e** was employed starting from **44a** to give 10.55 g (94%). $[\alpha]^{22}_D$ = −54.0° (c = 1; water); ^{1}H NMR (DMSO-d_6) δ 1.73 (s, 3H), 2.7 (d, J = 7 Hz, 2H), 4.03 (s, 2H), 5.13 (q, J = 7 Hz, 1H), 7.2−7.38 (m, 5H), 7.7−7.9 (AA′BB′, 4H), 8.78 (d, J = 8 Hz, 1H), 9.08 (s, 1H), 9.19 (s, 2H), 9.39 (s, 2H), 12.3 (s, 1H). Mass calcd for $C_{22}H_{24}N_5O_5$, 438.1777; found, 438.1782.

3-{2-[4-(*R*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetylamino}-3-(*S*)-phenylpropionic Acid Ethyl Ester Acetate (45a). The method for compound **4a** was employed starting from **57b** and **49a.** ^{1}H NMR (DMSO-d_6) δ 1.10 (t, J = 6 Hz, 3H), 1.75 (s, 6H), 2.78 (d, J = 6 Hz, 2H), 3.95−4.1 (m, 4H), 5.18 (q, J = 6 Hz, 1H), 7.2−7.38 (m, 5H), 7.64−7.85 (AA′BB′, 4H), 8.83 (d, J = 8 Hz, 1H), 9.09 (s, 1H), 9.24 (br s, 2H), 9.45 (br s, 2H); MS m/z (FAB) 466 $(M + H)^+$.

3-{2-[4-(*R*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetylamino}-3-(*S*)-phenylpropionic Acid Hydrochloride (45). The method for compound **12e** was employed starting from **45a.** $[\alpha]^{22}_D$ = −110.0° (c = 1; water); ^{1}H NMR (DMSO-d_6) δ 1.75 (s, 3H), 2.78 (d, J = 7 Hz, 2H), 4.00 (s, 2H), 5.15 (q, J = 7 Hz, 1H), 7.20−7.40 (m, 5H), 7.7−7.9 (AA′BB′, 4H), 8.73 (d, J = 8 Hz, 1H), 9.09 (s, 1H), 9.24 (s, 2H), 9.45 (s, 2H), 12.3 (s, 1H). Mass calcd for $C_{22}H_{24}N_5O_5$, 438.1777; found, 438.1765.

3-{2-[4-(*S*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetylamino}-3-(*R*)-phenylpropionic Acid Ethyl Ester Acetate (46a). The method for compound **4a** was employed starting from **57a** and **49b.** ^{1}H NMR (DMSO-d_6) δ 1.12 (t, J = 6 Hz, 3H), 1.73 (s, 6H), 2.75 (d, J = 6 Hz, 2H), 3.95−4.1 (m, 4H), 5.18 (q, J = 6 Hz, 1H), 7.2−7.38 (m, 5H), 7.64−7.85 (AA′BB′, 4H), 8.78 (d, J = 8 Hz, 1H), 9.6 (br s, 5H); MS m/z (FAB) 466 $(M + H)^+$.

3-{2-[4-(*S*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetylamino}-3-(*R*)-phenylpropionic Acid Hydrochloride (46). The method for compound **12e** was employed starting from **46a**. ^{1}H NMR (DMSO-d_6) δ 1.75 (s, 3H), 2.38 (m, 2H), 4.03 (m, 2H), 5.18 (m, 1H), 7.10 (m, 1H), 7.25−7.33 (m, 5H), 7.7−7.9 (AA′BB′, 4H), 8.70 (m, 1H), 9.20−9.80 (br s, 4H), 12.3 (s, 1H); MS m/z (FAB) 438 $(M + H)^+$.

3-{2-[4-(*R*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetylamino}-3-(*R*)-phenylpropionic Acid Ethyl Ester Acetate (47a). The method for compound **4a** was employed starting from **57b** and **49b**. $[\alpha]^{22}_D$ = +56.7° (c = 1; water); ^{1}H NMR (DMSO-d_6) δ 1.12 (t, J = 6 Hz, 3H), 1.73 (s, 6H), 2.75 (d, J = 6 Hz, 2H), 3.95−4.1 (m, 4H), 5.18 (q, J = 6 Hz, 1H), 7.2−7.38 (m, 5H), 7.64−7.85

(AA′BB′, 4H), 8.78 (d, J = 8 Hz, 1H), 9.6 (br s, 5H); MS m/z (FAB) 466 $(M + H)^+$.

3-{2-[4-(*R*)-(4-Aminoiminomethyl-phenyl)-4-methyl-2,5-dioxo-imidazolidin-1-yl]acetylamino}-3-(*R*)-phenylpropionic Acid Hydrochloride (47). The method for compound **12e** was employed starting from **47a.** $[\alpha]^{22}_D = -60.7°$ (c = 1; water); ^{1}H NMR (DMSO-d_6) δ 1.75 (s, 3H), 2.70 (d, J = 7 Hz, 2H), 4.03 (s, 2H), 5.13 (q, J = 7 Hz, 1H), 7.23 (m, 1H), 7.26−7.35 (m, 5H), 7.73−7.85 (AA′BB′, 4H), 8.78 (d, J = 8 Hz, 1H), 9.06 (s, 1H), 9.15 (s, 2H), 9.36 (s, 2H), 12.3 (s, 1H); MS m/z (FAB) 438 $(M + H)^+$.

QXP Pharmacophore Modeling. The routine *TFIT* (as implemented within the QXP program, version flo99.6S) with 2000 iterations was applied. As an additional constraint, the superposition of the carbon atoms of the essential carboxylic acid functions was enforced by adding zero-order bonds.[36] The lowest energy pharmacophore was chosen, since the remaining 49 solutions hardly differed. In contrast to the procedure suggested in the manual, explicit hydrogen atoms were also attached to carbon atoms. The final pharmacophore consisted of an ensemble of the above-mentioned four molecules, and the remaining molecules in the dataset with the exception of compounds **16** (diasteromeric mixture of compound **17**), **18** (wrong stereoisomer), **36** (double ester), and **39** (diastereomeric mixture of **44**) were separately superimposed by template fitting with *TFIT*. Each superposition was visually inspected, and with few exceptions the highest scoring solution was selected. In the case of RGDS, **4** and **19** the second and both the second and fifth solutions, respectively, was chosen. For **20** both the first and fourth superposition and for **11** and **40** both the first and second solutions were chosen, and during the later QSAR analysis the conformers with the greatest deviations in predicted activity were discarded.

GRID Calculations. The interaction energies between the superimposed molecules and the phenolic OH probe were calculated by GRID (Molecular Discovery Ltd., version 17) with a grid spacing of 1 Å.[37] Thus, for each molecule, 16 588 descriptors (interaction energies) were generated.

GOLPE Analysis. For building, validating, and interpreting the 3D-QSAR model, the program GOLPE (version 4.5.1) with the new methodology *smart region definition* (SRD) was applied.[43,44] After eliminating columns in the **X**-matrix with a total sum of squares < 10^{-7}, interaction energies with a range between −0.02 and 0.02 were zeroed and columns with a standard deviation < 0.21 kcal/mol were removed. To avoid a skewed distribution in the **X**-matrix unique ($N - 1$) variables were also eliminated. This variable pretreatment reduced the original number of 16 588 to 2556 variables. A principal components analysis projects the high-dimensional information into a few latent variables. The score plot of the first two dimensions, $t1$ and $t2$ (data not shown), shows a clear separation between highly active and low active compounds. The only exceptions are **41** and **43** which are close to **44** and which were also detected as outliers in the PLS analysis correlating the grid variables with the IC_{50}s. Since both compounds exhibit a high internal strain energy after superposition onto the pharmacophore, they were omitted from the further analysis.

To improve the interpretability and the predictivity of the 3D-QSAR model, the novel SRD algorithm (critical distance = 1.0 Å, collapsing distance = 2.0 Å) implemented in GOLPE was applied. Regions around the molecules carrying the same statistical information are comprised into groups, and those regions which do not contribute to the predictivity of the model are eliminated. As a consequence, the risk of chance correlations is minimized and the grouping of variables in 3D space facilitates the interpretation of the loading contours. To evaluate the effect of the grouped X-variables on the predictivity, a fractional factorial design (FFD) scheme for variable selection (two dimensions, the remaining parameters were at their default settings) was applied to check the effect of temporarily removed groups on the predictivity of the model. Those regions which did not increase the predictive power of the model were eliminated. Thus, the original 16 588 X-variables were finally reduced to 1623 X-variables.

The predictive ability of the models was evaluated by cross-validation using five groups of approximately the same size to which the objects were assigned randomly. Each of these groups was omitted from the data set at a time, and a reduced PLS model was built with the remaining compounds. The whole procedure was repeated 20 times. This cross-validation scheme yields a more realistic estimate of the predictivity of the statistical model than the usual leave-one-out (LOO).

Blood Sampling and Platelet Preparation. Blood was collected from healthy adult volunteers who denied taking any medication for the previous two weeks. A total of 8.4 mL of blood was drawn without tourniquet from the antecubital vein into 1.4 mL of acid citrate-dectrose (ACD, 0.8% citric acid, 2.2% sodium citrate, 2.45% glucose). Platelet rich plasma (PRP) was separated by centrifugation of blood for 15 min at 160g at room temperature. Gel-filtered platelets (GFP) were prepared as described. Briefly, PRP was acidified to pH 6.5 with ACD (approximately 1 mL of ACD + 10 mL of PRP) and centrifuged for 20 min at 400g. Platelets were resuspended with Tyrode's solution containing hirudin (1 IU/mL), albumin (0.35%), and apyrase (500 μg/mL). The platelet suspension was applied to a Sepharose CL 2B acrylic glass column, and platelets were eluted at 2 mL/min using Tyrode's solution without additives. The resulting GFP was set to a platelet count of 4×10^8 cells/mL.

Platelet Aggregation Assay. A suspension of gel filtered platelets (GFP) containing 3×10^8 platelets/mL was activated with 10 μM ADP in the presence of 1 mg/mL fibrinogen and stirred at 1000 rpm at 37 °C in an aggregometer (PAP 4, Biodata, Hatboro, PA). Aggregation was measured as the maximal increase in light transmittance. Compounds were added to GFP at 37 °C 2 min before the activation with ADP. Inhibition of aggregation was expressed as IC_{50} value, i.e., mean concentration requiring 50% inhibition in GFP samples of two to six different donors.

Fibrinogen Binding to GFP. ^{125}I-Fibrinogen binding to GFP was determined according to Marguerie et al.[38] with slight modifications from Kornecki et al.[41] and Bennett and Vilaire.[42] In displacement experiments, the inhibition of 40 nM ^{125}I-fibrinogen by increasing concentrations of the competitor was characterized in the presence of the platelet agonist ADP (10 μM). The incubation was started by the addition of 250 μL of GFP (4×10^8 cells/mL), yielding a total sample volume of 500 μL. After 30 min incubation at room temperature, 100 μL aliquots were layered onto a 20% sucrose solution, centrifuged for 2 min at 10700g, and the radioactivity of the resulting platelet pellet was measured in a γ counter.

Fibrinogen Binding Assay. GP IIb/IIIa isolation and immobilization, fibrinogen binding assay, and data analysis were carried out as described.[29d]

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Supporting Information Available: Analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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