Potent *in Vitro* and *in Vivo* Inhibitors of Platelet Aggregation Based Upon the Arg-Gly-Asp Sequence of Fibrinogen. (Aminobenzamidino)succinyl (ABAS) Series of Orally Active Fibrinogen Receptor Antagonists

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Our initial orally active fibrinogen receptor antagonist benzamidinopentanoyl (BAP) series which was discovered through truncation of our iv antiplatelet agent (SC-52012) demonstrated modest oral activity in canine studies (ethyl [5-(4-amidinophenyl)pentanoyl]-3-amino-3-(3-pyridyl)propionate, **1e**). Introduction of an amide bond adjacent to the benzamidine led to a novel series with an (aminobenzamidino)succinyl (ABAS) Arg-Gly surrogate that had improved *in vitro* potency (5−17 times) relative to the BAP series. Four ester prodrug/acid active metabolite pairs (**2a/2e, 60a/60e, 62a/62e, 63a/63e**) from the ABAS series which varied in their 3-substituent on the β-amino ester "aspartate mimetic" were prepared in enantiomerically enriched form (>95:5), and they were evaluated in canine studies for their ability to block collagen-induced aggregation in platelet-rich plasma, the elimination profile ($t_{1/2}$ β-phase), repeated oral dosing studies, and oral systemic availability. Of the four ester prodrug/acid active metabolite pairs, **2e/2a** (SC-54684A/SC-54701A) had the most favorable properties in the above studies with an $IC_{50} = 67 \pm 5$ nM (dog platelet-rich plasma, collagen), $t_{1/2}$ $\beta = 1.6$ h (ester) and 6.5 h (acid), no adverse effects upon repeated dosing, and a drug oral systemic availability of 62% (area under curve (AUC) of acid **2a** (drug) following ig administration of ester **2e** (prodrug, 2.5 mg/kg) divided by AUC of acid **2a** (drug) following iv administration of ester **2e** (prodrug, 2.5 mg/kg) as determined by HPLRC). In further pharmacokinetic studies using nonlabeled **2e/2a**, the oral systemic availability (ester **2e** ig/ester **2e** iv) of **2e** was measured to be in the range of 44.7−53.0%. The more biologically relevant oral systemic availability (ester **2e** ig/acid **2a** iv) of **2e** was found to be in the range of 22.0−26.4%. A pharmacophore model based on inhibitors from several different benzamidine classes including **2a** (ABAS class) was developed using a combination of molecular modeling (MM2) and pharmacophore identification (APOLLO) methods.

Introduction

Many new therapeutic approaches for the treatment or prevention of a myocardial infarct (MI), stroke, and unstable angina are currently under clinical investigation.[1] Fibrinogen receptor (glycoprotein, GP α_{IIb}-β_{IIIa}) antagonists disrupt the obligatory platelet−fibrinogen interaction for white thrombus formation.[2] We[3] and others[4] have designed peptide mimetics based on the RGD sequence of fibrinogen that effectively disrupt platelet aggregation and possess short half-lives ideally suited for critical intervention in combination with a fibrinolytic agent. Chronic administration of an orally active fibrinogen receptor antagonist has the potential

to prevent the above vascular complications which has led to a continued interest in the RGD mimetic area.

Previously, we described our initial orally active RGD series, the benzamidinopentanoyl (BAP) series (compound **1e**, Figure 1) which had modest oral activity.[5] We sought to improve upon the oral activity with a new RGD series, since low oral activity can lead to larger variations in plasma levels from patient to patient. The therapeutic index is unknown for this class of compounds, but certainly a narrow window of safety would be complicated by low oral activity. Herein, we describe the design and the synthesis of our lead series, (aminobenzamidino)succinyl (ABAS), and the structure−activity relationships (SAR) which led to choosing **2e** (SC-54684A; Figure 1) for clinical development. Recently, several structurally unique fibrinogen receptor antagonists have been disclosed that have oral activity in animals (Figure 1, compounds **3**,[6] **4**,[7] and **5**[8]). The structural variance between the linkers of guanidine surrogate and Asp-carboxylate surrogate for the different classes of fibrinogen receptor antagonists is note-

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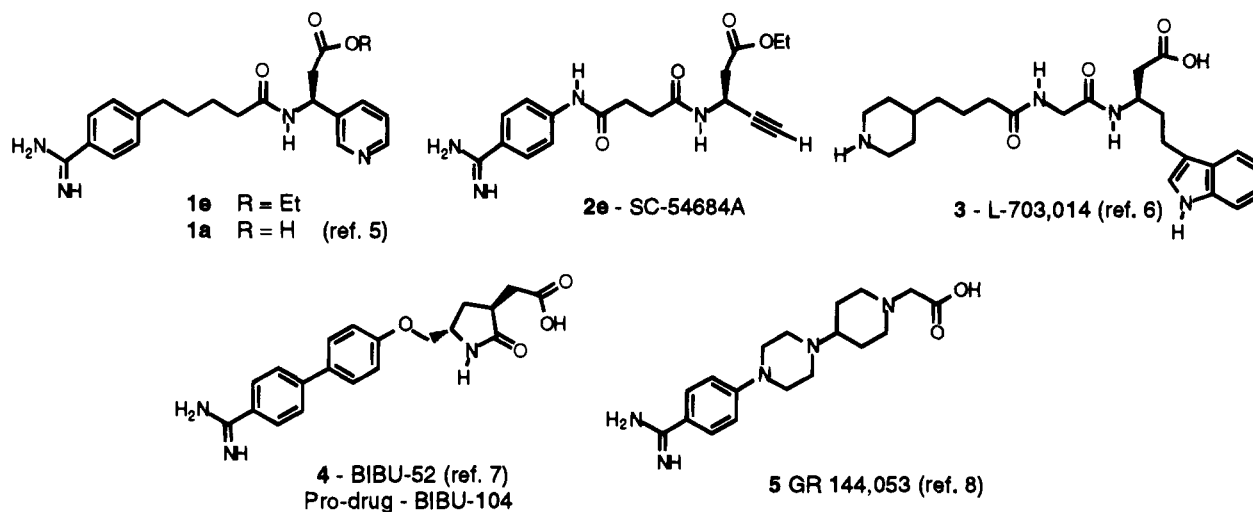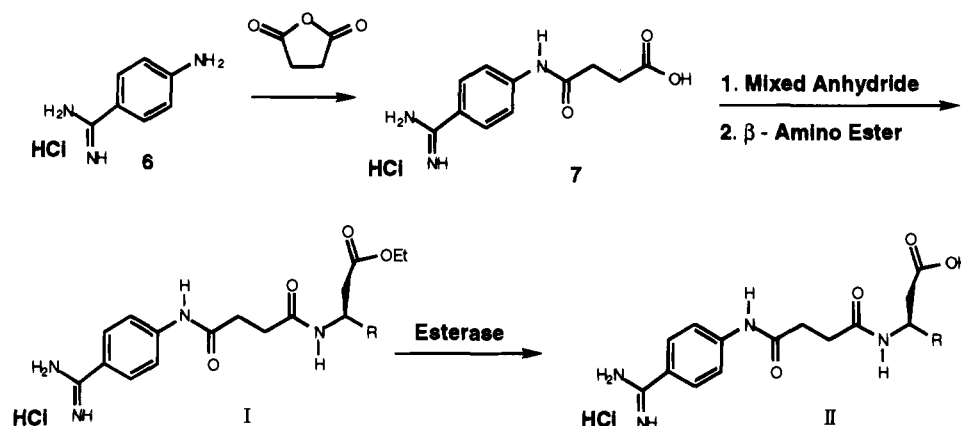[⊛] Abstract published in *Advance ACS Abstracts*, June 1, 1995.

**Figure 1.** Orally active fibrinogen receptor antagonists.

Scheme 1

worthy. Previous studies utilizing cyclic RGD-containing peptides[9,10] have resulted in constrained peptide mimetics exhibiting a γ-turn[9b] motif and a cup-shaped topology of the RGD pharmacophore.[10b] We have developed a unified pharmacophore model utilizing several different classes of fibrinogen receptor antagonists, and the features of and the methods used to generate the model will be discussed.

Chemistry

The novel platelet aggregation inhibitors can be prepared following the general synthetic sequence outlined in Scheme 1. Commercially available 4-aminobenzamidine hydrochloride (**6**) was reacted with succinic anhydride in warm DMF utilizing 4-(dimethylamino)-pyridine (DMAP) catalysis which afforded the zwitterion product of **7** upon cooling. After conversion of the zwitterion to the HCl salt, the ABAS acid **7** was coupled directly to a β-amino ester "aspartate mimetic" via the mixed anhydride method. Careful addition of 1 equiv of *N*-methylmorpholine followed immediately by 1 equiv of isobutyl chloroformate minimized acylation of the basic benzamidine which was protected as the hydrochloride salt. Purification by reverse phase high-pressure liquid chromatography (RPHPLC) afforded the prodrug form **I** of the platelet aggregation inhibitor. For *in vitro* testing, a portion of the prodrug ester **I** was cleaved to the active β-amino acid **II** by treatment with esterase or base.

The requisite β-amino esters were prepared as outlined in Schemes 2–4 or as previously described (β-aryl derivatives).[5,11] The ethynyl β-amino ester derivatives were prepared through the addition of 2 equiv of the corresponding lithioacetylide anion to 4-(benzoyloxy)-azetidinone (**8**) at low temperature which presumably ensues through the acylimine intermediate.[12] The resultant β-lactam is opened to the required β-amino ester by treatment with anhydrous HCl in ethanol. An illustrative addition-ring-opening process for 1-lithio-2-(trimethylsilyl)acetylene (**9**) is depicted in Scheme 2. Enantiomerically enriched 3-ethynyl β-amino ester **17** was prepared through a modification of Hacksell's procedure[13] for the resolution of propargylamines in which the diastereomic amides obtained from *O*-methylmandelic acid were chromatographically resolved on multigram scales. Both diastereomeric amides **13** and **14** were separately activated by treatment with BOC anhydride and then subsequently cleaved by treatment with tetramethylguanidine in methanol which concommitantly cleaved the TMS-protected acetylene (Scheme 2). After protecting group manipulation, chiral HPLC analysis (crown-pak-(+))[14] of the β-amino ester **17** indicated an enantiomeric ratio of greater than 98:2. The 3-allyl β-amino ester **21** was prepared through Lewis acid-mediated addition of allylsilane to 4-(benzoyloxy)azetidinone (**8**) followed by treatment with anhydrous HCl in ethanol (Scheme 3).[15]
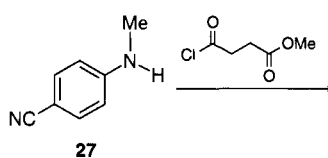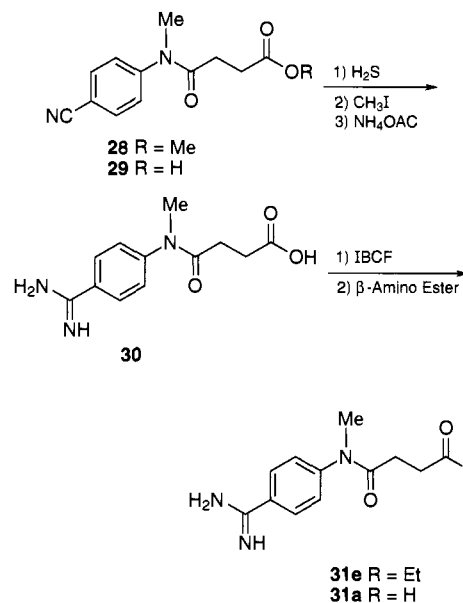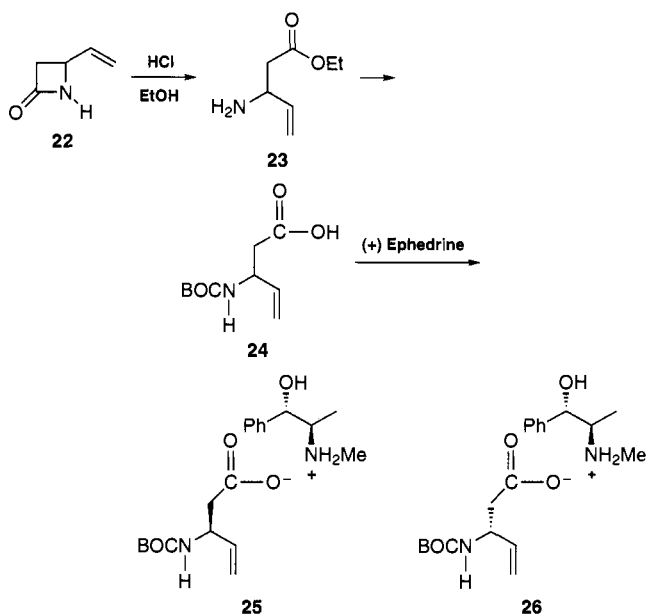
Scheme 2
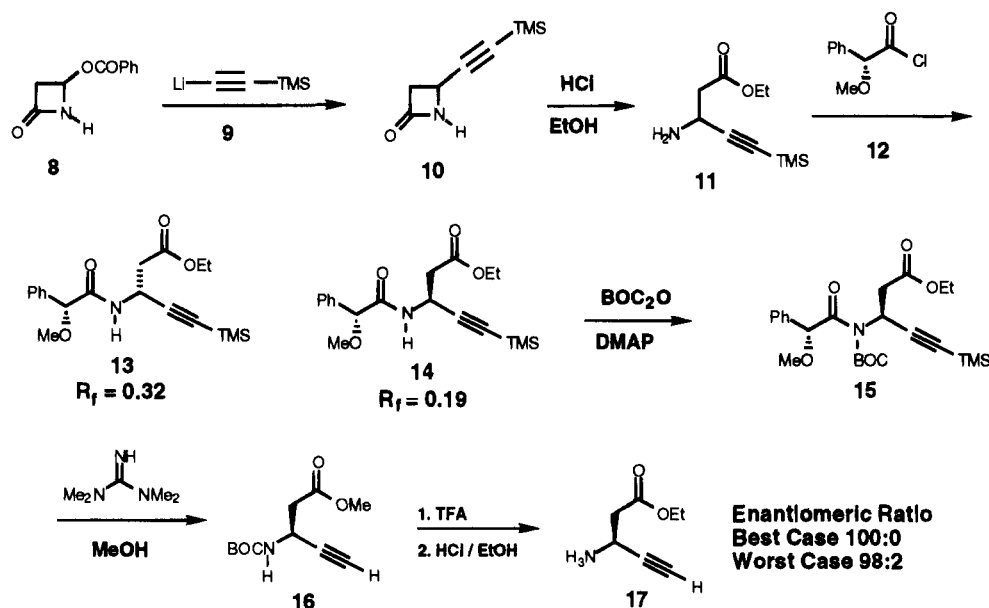
**Scheme 3**
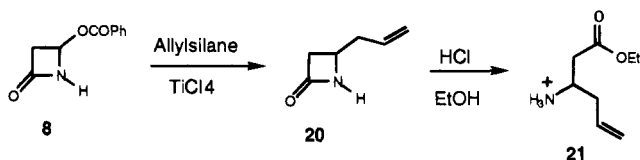
Scheme 4

Scheme 5

The vinyl β-amino ester was prepared by reaction of chlorosulfonyl isocyanate (CSI) with 1,3-butadiene as previously described,[16] which afforded the vinyl β-lactam **22** in good yield followed by opening to the β-amino ester as above (Scheme 4). The racemic vinyl β-amino ester was resolved classically through complexation with (+)-ephedrine and repeated fractional crystallization of the diastereomeric salts (Scheme 4). The (phenylmethylene)dioxy β-amino ester of derivative **49** was prepared racemically via a modified Knoevenagel process[17] which was subsequently resolved chromatographically by forming the diastereomeric amides of phenylalaninol.[11] The enantiomerically enriched 3-pyridyl β-amino ester was

prepared through a modification of Davies β-amino ester synthesis.[11,18]

Several derivatives were prepared in which the amide bond adjacent to the benzamidine was modified or replaced. The *N*-methyl ABAS derivatives **31e,a** were prepared in several steps as outlined in Scheme 5. Briefly, 4-(*N*-methylamino)benzonitrile (**27**) was acylated with 3-carbomethoxypropionyl chloride followed by conversion to the benzamidine **30** by sequential reaction with hydrogen sulfide, methyl iodide, and ammonium acetate. The *N*-methyl ABAS derivative **30** was coupled to a β-amino ester as described above (Scheme 5).

The aniline derivatives **36e,a** were prepared in several steps as outlined in Scheme 6. Initially, the

Scheme 6
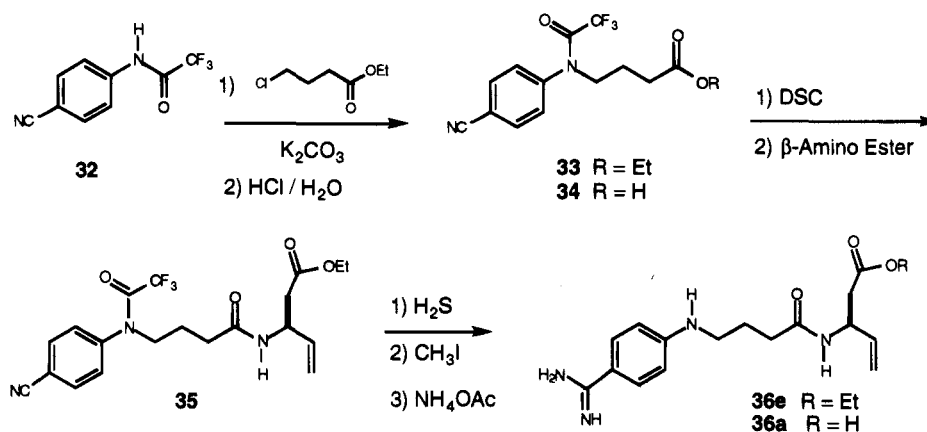
N-trifluoroacetamide derivative **32** was alkylated by reaction with ethyl 4-chlorobutyrate to give **33** which was subsequently subjected to acid-mediated ester hydrolysis affording derivative **34**. The resultant acid **34** was coupled to the vinyl β-amino ester **72**, and the benzonitrile **35** was converted to the benzamidine **36** via the thioamide in high yield as illustrated in Scheme 6 (note: the trifluoroacetamide was cleaved in the conversion process of **35** to **36**).

Results and Discussion

Our progression from the natural ligand Arg-Gly-Asp-Phe (RGDF, IC_{50} = 29 ± 8.0 μM, dog platelet-rich plasma, PRP[19]) found on the α-chain of fibrinogen to our lead orally active ABAS series is shown in Figure 2. In brief, the Arg-Gly dipeptide was replaced by an 8-guanidinooctanoyl fragment which resulted in a 10-fold enhancement in activity (**37**, SC-49992, IC_{50} = 3.0 ± 0.1 μM, dog PRP). On the basis of Markwardt's work in the thrombin inhibitor area,[20] we replaced the guanidine with a benzamidine which resulted in a dramatic enhancement in the inhibitory potency (42-fold) which

Figure 2. Progression from the natural RGDF ligand to orally active **2e**.

afforded our iv antiplatelet agent **38** (SC-52012, IC_{50} = 72 ± 11 nM, dog PRP). Our first orally active series was obtained through removal of the metabolically labile Asp-Phe amide bond which was accomplished by truncation to a β-amino ester "aspartate mimetic" (BAP series,[5] Figure 2). The β-amino ester also served the dual purpose of masking the zwitterionic nature of the inhibitor which has been shown to be detrimental for the passive absorption of similar drugs in the intestine.[21] The initial compounds in the BAP series had diminished inhibitory potency (2−5 μM) relative to the iv antiplatelet agent **38** (100 times less active); however, by varying the 3-substituent on the β-amino acid, the potency was enhanced considerably as exemplified by the 3-pyridyl derivative **1a** (Figure 1; IC_{50} = 152 ± 15 nM, dog PRP). The BAP derivative **1a** possessed good *in vitro* potency, but efforts were continued to increase the duration of action at low oral doses. The ABAS series was designed primarily to enhance potency through acquiring additional hydrogen-bonding interactions with the receptor which may be present in the natural RGDF ligand (Figure 2).

A comparison of the *in vitro* potency of the BAP versus ABAS series in which the 3-substituent of the β-amino acid is varied in four analogs demonstrates an increase in the *in vitro* potency resulting from the introduction of the retro-amide bond of the ABAS series (Table 1). In the simplest case, the unsubstituted β-alanine ABAS derivative **40a** was approximately 5 times more active than the corresponding BAP derivative **39a** (dog PRP). The 3-phenyl-β-alanine ABAS derivative **44a** was approximately 16 times more active than the corresponding substituted BAP derivative **43a**. An enhancement in the duration of oral activity for the 3-methyl derivative **42e** and the 3-benzyl derivative **46e** of the ABAS series over the corresponding BAP derivatives **41e** and **45e** further intensified our efforts in the ABAS series.

A series of derivatives related to 3-phenyl-β-alanine derivative **44a** were prepared with the intent of enhancing inherent *in vitro* potency (Table 2). The 3-(3,4-difluorophenyl)-β-alanine derivative **47a** was comparable to the 3-phenyl-β-alanine derivative **44a** in inhibitory potency; however, the 3-(pentafluorophenyl)-β-alanine derivative **48a** was approximately 17 times less active than **44a**. The 3-[5-(benzo-1,3-dioxole)]-β-alanine derivative **49a** showed good inhibitory potency *in vitro*, and the corresponding ethyl ester demonstrated a long duration of activity upon oral administration in
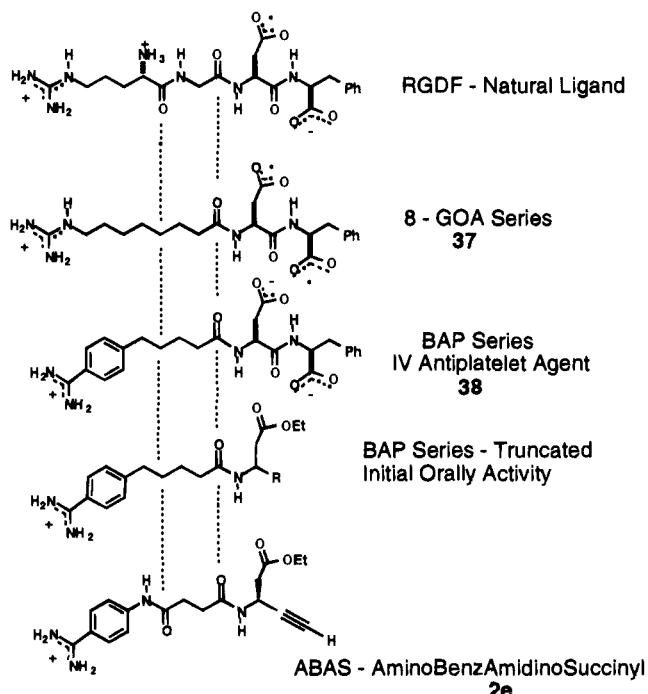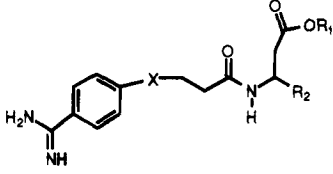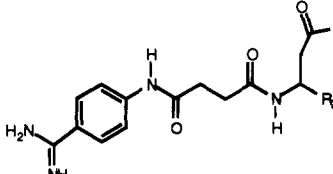
Table 1. Comparison of BAP and ABAS Series: *In Vitro* Inhibition of Platelet Aggregation and Duration of Action Post-ig Dosing in Dogs


compd	R$_1$	R$_2$	X	IC$_{50}$ (nM)^a	oral dose (mg/kg)	duration^b time (h)
39a	H	H	CH$_2$CH$_2$	5880 ± 390		
40a	H	H	HNCO	1090 ± 80		
40e	Et	H				
41a	H	CH$_3$	CH$_2$CH$_2$	2870 ± 380		
41e	Et	CH$_3$	CH$_2$CH$_2$		20	>6, <24
42a	H	CH$_3$	HNCO	170		
42e	Et	CH$_3$	HNCO		20	>30
43a	H	Ph	CH$_2$CH$_2$	4880 ± 420		
43e	Et	Ph	CH$_2$CH$_2$		10	>6, <24
44a	H	Ph	HNCO	294 ± 34		
44e	Et	Ph	HNCO		20	>6, <24
45a	H	CH$_2$Ph	CH$_2$CH$_2$	4720 ± 290		
45e	Et	CH$_2$h	CH$_2$CH$_2$		20	>3, <6
46a	H	CH$_2$Ph	HNCO	321 ± 20		
46e	Et	CH$_2$Ph	HNCO		20	>6, <24

^{a} Dog platelet-rich plasma collagen, $n = 3$, pooled blood, ±standard error. ^{b} Based on a >20% inhibition of *ex vivo* collagen-induced platelet aggregation. See the Experimental Section for details.

dogs. The addition of an o-nitro group to the benzo-1,3-dioxole ring, derivative **50a**, led to a considerable decrease (>10 times) in the observed *in vitro* potency. Replacement of the 3-phenyl of **44a** with 3-pyridyl led to a slight enhancement in the *in vitro* potency for **51a**, but a marked enhancement of duration of activity upon oral dosing was observed with **51e** relative to **44e** (Table 2). Attempts to further enhance the potency of the 3-(3-pyridyl)-β-alanine derivative **51a** through the introduction of a hydrogen bond acceptor, notably a 4-ethoxy substituent on the pyridyl ring of derivative **52a**, were not met with success. Furthermore, extending the π-cloud of the pyridyl ring by expanding to a 3-(3-quinolinyl)-β-alanine derivative (**53a**) led to comparable *in vitro* activity but diminished duration of activity after oral dosing. On the basis of the *in vitro* potency and the duration of oral activity, the acid/ester pairs of 3-[5-(benzo-1,3-dioxole)]-β-alanine derivatives **49a/49e** and 3-(3-pyridyl)-β-alanine derivatives **51a/51e** were chosen for further study.

We prepared several unsaturated 3-alkyl-substituted β-alanine derivatives which are shown in Table 3. The 3-vinyl-β-alanine derivative **54a** was more potent than the 3-allyl-β-alanine derivative **55a** but comparable to the 3-ethynyl-β-alanine derivative **56a**. The sterically demanding 3-[1-(trimethylsilyl)ethynyl]-β-alanine (**58a**) and 3-(1-*tert*-butylethynyl)-β-alanine (**57a**) derivatives did not lead to a significant decrease in activity relative to the 3-ethynyl-β-alanine derivative **56a** which suggests that steric bulk is tolerated in this position. Efforts to enhance the inhibitory potency of the 3-ethynyl-β-alanine derivative **56a** through the incorporation of a hydrogen bond-accepting group (CH$_3$OCH$_2$) in derivative **59a** were not met with success; therefore, the simple 3-vinyl-β-alanine derivative **54a** and the 3-ethynyl-β-alanine derivative **56a** were chosen for further study.

Table 2. ABAS β-Phenylalanine Derivatives: *In Vitro* Inhibition of Platelet Aggregation and Duration of Action Post-ig Dosing in Dogs
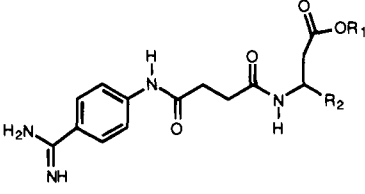

compd	R$_1$	R$_2$	IC$_{50}$ (nM)^a	oral dose (mg/kg)	duration^b time (h)
44a	H		294 ± 34		
44e	Et	phenyl		20	>6, <24
47a	H		158 ± 3		
47e	Et	3,4-difluorophenyl			NT
48a	H	pentafluorophenyl	4940 ± 750		NT
49a	H		104 ± 12		
49e	Et	benzodioxole		5	>24
50a	H	nitrobenzodioxole	1170 ± 10		NT
51a	H		122 ± 8		
51e	Et	pyridyl		10 / 5	>24 / >6
52a	H		108 ± 5		
52e	Et	ethoxypyridyl		5	<6
53a	H		101 ± 8		
53e	Et	quinolinyl		5	<6

^{a} Dog platelet-rich plasma, collagen, $n = 3$, pooled blood, ±standard error. ^{b} Based on a >20% inhibition of *ex vivo* collagen-induced platelet aggregation. NT = not tested.

The SAR of the amide adjacent to the benzamidine on *in vitro* potency was briefly investigated for a series of 3-vinyl-β-alanine derivatives (Table 4). Replacement of the trans amide of derivative **60a** with a trans carbon–carbon double bond in derivative **61a** resulted in a 7-fold loss in inhibitory potency. The N-methyl-amide derivative **31a** which has a mixture of cis and trans amides is 10 times less active than the trans amide derivative **60a**. The carbonyl of the amide bond is critical for activity, since the corresponding aniline derivative **36a** was devoid of activity. These results suggest that a trans orientation is favorable for activity and there may be a hydrogen-bonding component to the receptor interaction in this region.

Four of the more promising ABAS acid/ester pairs (**49**, **51**, **54**, and **56**) based on *in vitro* potency were prepared in enantiomerically enriched form (>95:5 ratio of enantiomers),[22,23] and each pair was subjected to the following tests: *in vitro* platelet aggregation and long term oral dosing to test for side effects; finally, each pair was radiolabeled to determine the oral systemic availability (ig ester AUC/iv ester AUC) and $t_{1/2}$ β-phase. All four

Table 3. ABAS Unsaturated Alkyl Derivatives: *In Vivo* Inhibition of Platelet Aggregation and Duration of Action Post-ig Dosing in Dogs
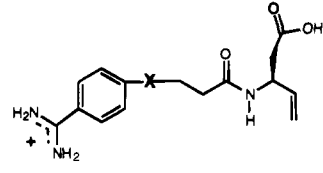

compd	R_1	R_2	IC_{50} $(nM)^a$	oral dose (mg/kg)	duration^b time (h)
54a	H		115 ± 8		
54e	Et			20	>24
55a	H		491 ± 32		NT
56a	H		256 ± 12		
56e	Et			10	>24
57a	H	Bu^t	302 ± 56		NT
58a	H	TMS	580 ± 60		NT
59a	H	OMe	224 ± 9		NT

^{a} Dog platelet-rich plasma, collagen, $n = 3$, pooled blood, $\pm$ standard error. ^{b} Based on a >20% inhibition of *ex vivo* collagen-induced platelet aggregation. NT = not tested.

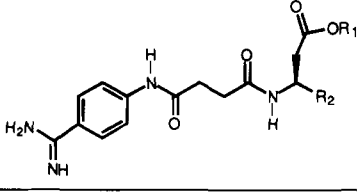**Table 4.** SAR of Retro-Amide of ABAS: *In Vitro* Inhibition of Platelet Aggregation

compd	X	IC_{50} $(nM)^a$
60a	HNCO	133 ± 8
61a	t-C=C	904 ± 152
31a	MeNCO	1240 ± 60
36a	$HNCH_2$	>10000

^{a} Dog platelet-rich plasma, collagen, $n = 3$, pooled blood, $\pm$ standard error.

acids were very potent in their ability to inhibit the *in vitro* collagen-induced platelet aggregation with IC_{50}'s ranging from 50 to 133 ± 8 nM (Table 5).[22b] Preliminary dose ranging of the ester prodrugs was used to select two BID doses that would lead to trough level (minimal level) inhibition greater than 50% and less than 50%.[27b] The doses used for each prodrug and the trough level inhibition over an extended period are plotted in Figure 3. The 3-pyridyl-β-alanine ethyl ester prodrug **63e** led to a 35% reduction in platelet count after 9 days, and the study was truncated to one high dose (3.0 mg/kg). From these studies it is evident that the lead candidates from the ABAS class of fibrinogen antagonists are potent inhibitors of platelet aggregation upon oral administration. The thrombocytopenia associated with **63e** has been noted with other fibrinogen receptor antagonists from different classes of compounds,[24] and the mechanism for induction of platelet loss is not known.[25] The observation that the platelet count initially remained steady for a few days upon oral administration of **63e** illustrates the importance of extended studies with this class of agents.

In a preliminary screening study, each ethyl ester prodrug was prepared in the ^{14}C-radiolabeled form and administered at 2.5 mg/kg ester ig/2.5 mg/kg ester iv to

Table 5. *In Vitro* Inhibition of Platelet Aggregation and Pharmacokinetic Data of Radiolabeled ABAS Derivatives in Dogs

compd	R_1	R_2	IC_{50} $(nM)^a$	$t_{1/2}$ $(h)^b$	$OSAV^c$ (%)
2a	H		67 ± 5	6.5	62
2e	Et			1.6	
60a	H		133 ± 8	5.4	30
60e	Et			1.3	
62a	H		52 ± 4.7	5.4	3
62e	Et			1.4	
63a	H		50^d	5.6	11
63e	Et			0.5	

^{a} Dog platelet-rich plasma, collagen, $n = 3$, pooled blood, $\pm$standard error, see ref 22b for the activity of the corresponding antipode of each compound which is generally 100 times less active. ^{b} β-Phase half-life after iv administration at a dose of 2.5 mg/kg. ^{c} OSAV = oral systemic availability = AUC of acid **2a** following ig administration of ester **2e** (2.5 mg/kg)/AUC of acid **2a** following iv administration of ester **2e** (2.5 mg/kg) as determined by HPLRC. ^{d} Average of $n = 2$ determinations.

dogs. After iv administration, all of the ethyl ester prodrugs were rapidly converted to the acid as evidenced by high-performance liquid radiochromatography (HPLRC) analysis of plasma levels. All four active constituents had similar $t_{1/2}$ (β-phase), and the oral systemic availability (OSAV) was the discerning feature (Table 5). The highest OSAV (62%) of drug was obtained for the 3-ethynyl-β-alanine derivative **2a** when administered as the ethyl ester prodrug (area under curve (AUC) of acid **2a** following ig administration of ester **2e** (2.5 mg/kg)/AUC of acid **2a** following iv administration of ester **2e** (2.5 mg/kg) as determined by HPLRC) followed by the 3-vinyl-β-alanine derivative **60a** at 30% (administered as prodrug; Table 5). The amount of active constituent **2a** in the plasma after ig and iv administration is plotted versus time in Figure 4. The C_{max} of **2a** upon oral administration of **2e** was 172 ng/mL with a value of approximately 72 ng/mL corresponding to the IC_{50} for *ex vivo* collagen-induced platelet aggregation.

On the basis of these preliminary studies, the 3-ethynyl-β-alanine derivative **2e** was further studied. Nonlabeled **2e,a** were administered iv at a dose of 2.27 mg/kg to female dogs ($n = 3$), and the mean AUC of **2a** following iv administration is shown in Table 6. Compound **2e** was administered at 5.45 and 54.5 mg/kg ig to female dogs ($n = 3$) either in solution or in capsule form, and the AUC of **2a** following ig administration of **2e** is shown in Table 6. Thus, the oral systemic availabilities AUC of acid **2a** (drug) following ig administration of ester **2e** (prodrug, given dose)/AUC of acid **2a** (drug) following iv administration of ester **2e** (prodrug, 2.27 mg/kg) as determined by HPLC; AUC of acid **2a** (drug) following ig administration of ester **2e** (prodrug, given dose)/AUC of acid **2a** (drug) following iv administration of acid **2a** (drug, 2.27 mg/kg) as deter-
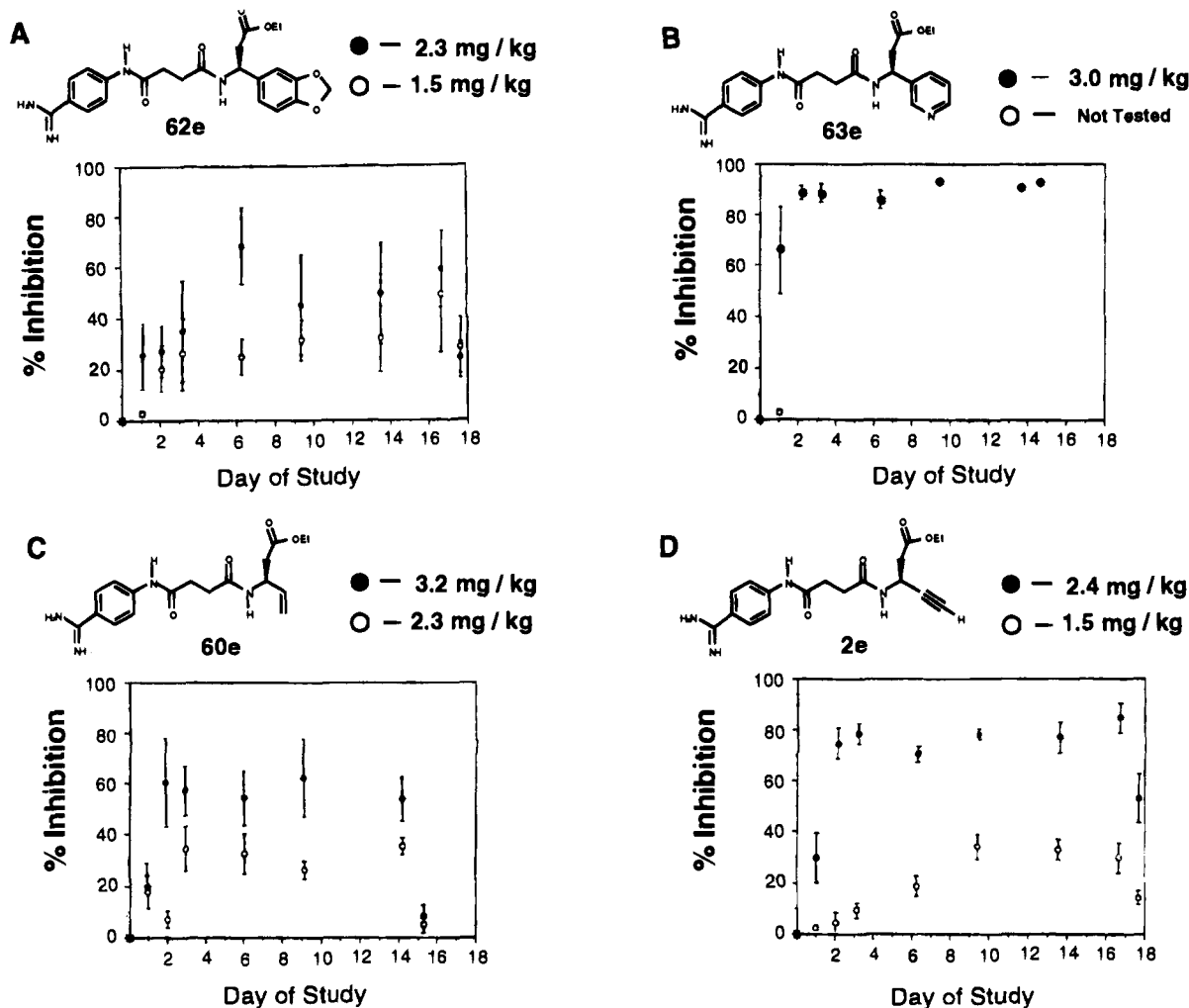
Figure 3. Long term study—minimum daily level inhibition upon BID oral dosing in dogs ($n = 4$).
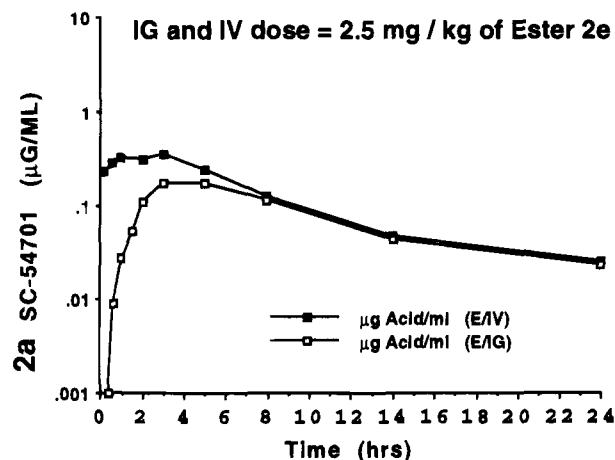
Figure 4. Plasma levels of **2a** upon ig and iv administration of ester **2e** versus time as determined by radio-HPLC.

mined by HPLC) were calculated at the low and high dose for both solution and capsule form, and the data are shown in Table 6. The data indicate that the oral systemic availability is independent of dose and whether the compound is administered by solution or capsule. Furthermore, the oral systemic availability measurement ester (ester **2e** ig/ester **2e** iv) of 44.7—53% is in good agreement with the earlier radiolabeled studies for **2e** (62%). Arguably the more biologically relevant comparison of ester **2e** ig/acid **2a** iv is still a respectable

Table 6. Pharmacokinetic Data of Nonlabeled **2a/2e**

2a R = H
2e R = Et

compd—dose method	mean AUC[a] (mg h/L)	$t_{1/2}$ (β)[b] (h)	OSAV (%) 2e ig/ 2e iv[c]	OSAV (%) 2e ig/ 2a iv[d]
2e—2.27 mg/kg, iv, bolus	1.38 ± 0.85	1.36 ± 0.81		
2a—2.27 mg/kg, iv, bolus	2.49[e]	4.43[e]		
2e—5.45 mg/kg, ig, gavage	2.73 ± 0.98		45.6	22.7
2e—5.45 mg/kg, ig, capsule	2.66 ± 1.12		44.7	22.0
2e—54.5 mg/kg, ig, gavage	31.7 ± 1.12		53.0	26.4
2e—54.5 mg/kg, ig, capsule	31.2 ± 9.95		52.2	22.0

[a] Mean AUC of acid **2a** as determined by HPLC, ±standard deviation. [b] β-Phase half-life in hours. [c] OSAV = oral systemic availability = AUC of acid **2a** following ig administration of ester **2e** (given dose)/AUC of acid **2a** following iv administration of ester **2e** (2.27 mg/kg) as determined by HPLC. [d] OSAV = oral systemic availability AUC of acid **2a** following ig administration of ester **2e** (given dose)/AUC of acid **2a** following IV administration of acid **2a** (2.27 mg/kg) as determined by HPLC. [e] Average of two values.

value for a peptide mimetic at 22.0—26.4% (Table 6). The difference in measurements reflects the conversion of prodrug **2e** to acid **2a** upon iv administration versus the clearance of **2e**.

Figure 5. Different benzamidine classes used in the pharmacophore model.

Selective inhibition of GP α_{IIb}-β_{IIIa} over other RGD dependent integrin receptors is desirable particularly for a chronically administered agent to avoid potential side effects. The vitronectin receptor, GP $\al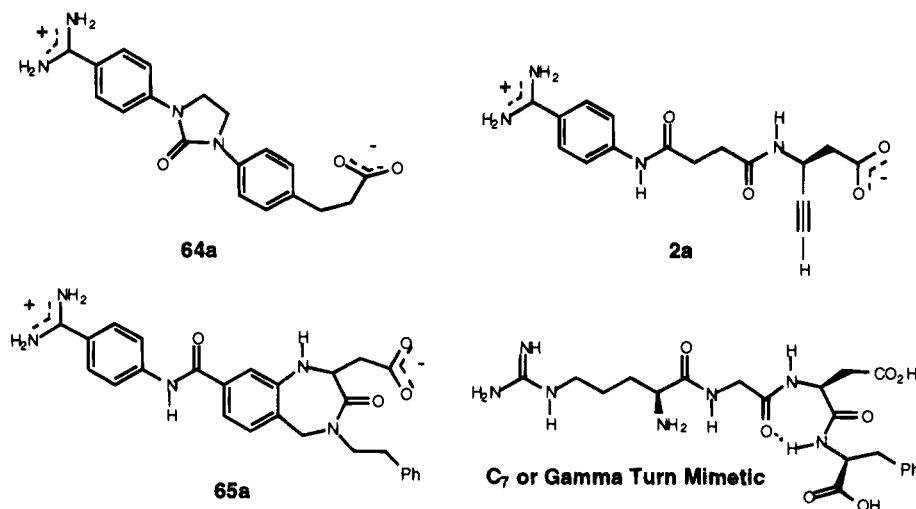pha_v\beta_{IIIa}$, has the same β-subunit as GP α_{IIb}-β_{IIIa}, but it is more widely distributed residing on membranes of platelets, endothelial cells, osteoclasts, etc.[26] Competitive binding experiments using 3-ethynyl-β-alanine derivative **2a** displayed potent high inhibition of vitronectin binding to isolated GP α_{IIb}-β_{IIIa} receptors in a solid phase assay with an IC$_{50}$ of (4.7 ± 0.7) × 10^{-10} as expected; however, the 3-ethynyl-β-alanine **2a** was much less effective in inhibiting vitronectin binding to isolated GP $\alpha_v\beta_{IIIa}$ with an IC$_{50}$ of (1.6 ± 0.1) × 10^{-5}. Thus, the 3-ethynyl-β-alanine derivative **2a** demonstrated a 34 000-fold selectivity for GP α_{IIb}-β_{IIIa} over GP $\alpha_v\beta_{IIIa}$ based on solid phase assays.[27]

Pharmacophore Model Development. Due largely to the potential of fibrinogen receptor antagonists as a therapeutic class, there is a high interest in the area with a vast number of classes of inhibitors now disclosed in the literature.[28] We chose three compounds from the benzamidine class of inhibitors with varying degrees of conformational constraint to build a first-generation pharmacophore model (Figure 5). Initially, we chose the rigid benzamidine derivative **64a** which has a 3-[4-(2-carboxyethyl)phenyl]imidazolidin-2-one spacer with five rotatable bonds to the right of the benzamidine moiety.[29] Energetically preferred conformations of **64a** were obtained by generating conformations at 30° intervals about the single rotatable bonds which were further refined using a combination of molecular mechanics optimizations within MacroModel (MM2).[30] Thus, a subset of conformations for **64a** were generated in which all of the energetically favorable conformations adopted an extended conformation in which an interatomic distance of greater than 10 Å between the central carbon atoms of the carboxylate and amidine moieties was observed. Furthermore, the global minimum conformation had the central carbon atoms of the key pharmacophores separated by 14 Å. Thus, in generating conformations for **65a** and **2a**, the carboxylate and amidine central carbons were constrained to a minimum interatomic distance of 11 Å (note: no constraints on maximum interatomic distance were imposed). A unique subset of conformations for each molecule was generated

and then subsequently pooled together for the generation of a 3-D pharmacophore using the *a*utomated *p*harmacophore *l*ocation through *l*igand overlap (APOLLO) program.[31] A root mean square distance constraint between the amidine of each molecule and the carboxylate of each molecule of less than 0.5 Å was applied, and conformations which were destabilized by more than 3 kcal/mol relative to their respective global minima were eliminated. The 3-D pharmacophore is illustrated in the panels of Figure 6. The flexible ABAS derivative **2a** is shown in panel B in an extended conformation. It is noteworthy that the addition of a retro-amide bond adjacent to the benzamidine in the ABAS series enhances the inhibitory potency over the corresponding alkyl BAP series as demonstrated by analogs in Table 1. The retro-amide bond reduces the degree of conformational freedom relative to the alkyl series and more importantly favors the extended conformation of **2a** which is suggested as a biologically relevant conformation based on the pharmacophore model.[32] Furthermore, in related studies, we have prepared additionally constrained molecules which are further locked in an extended conformation by replacing the ethylene moiety of ABAS with a trans cyclopropyl group resulting in potent inhibitors of platelet aggregation *in vitro*, and they are the subject of another publication.[11] Not unexpectedly, there is excellent overlap between the pharmacophores (amidine and carboxylate) of the flexible **2a** and **64a** (Figure 6, panel C). As noted with our iv antiplatelet class containing the benzamidinylpentanoyl linker, wherein the pentanoyl spacer (five atoms) was found optimum, the five-atom spacer corresponding to the ABAS led to greater activity over the six-atom glutaryl spacer.[11] The carboxylate of **65a** is within the 0.5 Å of **2a** and **64a** providing reasonable overlap between the three classes of fibrinogen receptor antagonists (panel D). The RGD conformation found in cyclic peptides was consistent with a "turn−extended−turn" motif which led to the design of **65a**.[33] Although elements of turn mimetics in **2a** are lacking, the extended conformation of **2a** is consistent with the developed pharmacophore model. The unified pharmacophore model will guide further introduction of conformational constraints into subsequent classes of fibrinogen receptor antagonists.
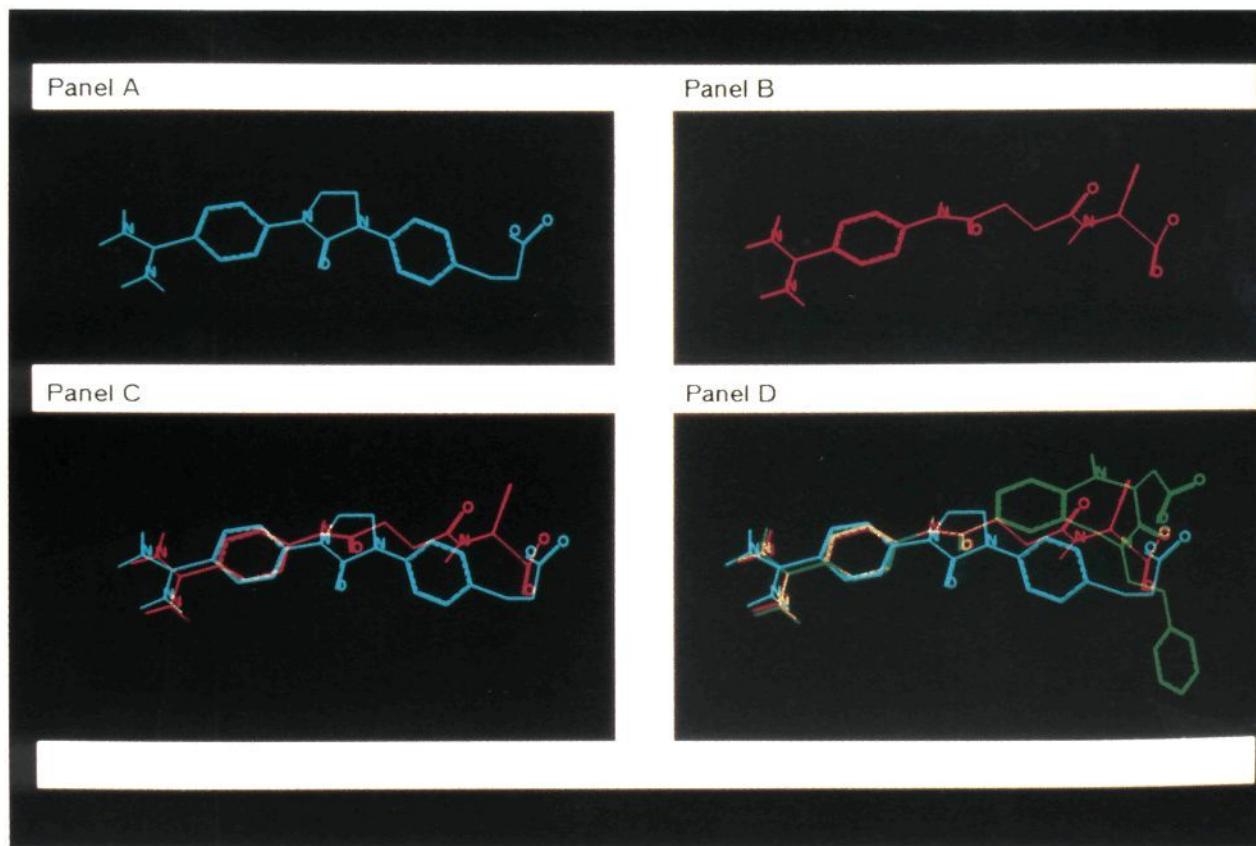
Figure 6. Pharmacophore model based on **64a, 2a**, and **65a**.

Conclusion

We have discovered a novel class of orally active fibrinogen receptor antagonists through structural modification of our initial orally active BAP series. The introduction of a retro-amide bond adjacent to the benzamidine resulted in the ABAS series which displayed enhanced inhibitory potency and oral activity relative to the BAP series. The enhanced inhibitory potency imparted by the amide bond is attributed to favorable conformational effects in which an extended conformation is favored which is consistent with our pharmacophore model and potential hydrogen-bonding interactions with the receptor imparted by the amide. A series of ABAS analogs in which the 3-substituent on the β-amino ester/acid was evaluated on the basis of the *in vitro* potency of the acid and the duration of action of isolated esters upon oral administration led to the selection of four compounds for extensive study. The ABAS acid/ester pairs (**49, 51, 54**, and **56**) were prepared in enantiomerically enriched form (>95:5 ratio of enantiomers), and each pair was subjected to the following tests: *in vitro* PRP of acid and repeated oral dosing to test for side effects; finally, each pair was radiolabeled to determine the oral systemic availabilities and $t_{1/2}$ (β-phase). On the basis of these studies, **2e** emerged as a favorable candidate for development as an orally active fibrinogen receptor antagonist with the following properties: $IC_{50} = 67 \pm 5$ nM (PRP, collagen, dog[34]), no apparent side effects upon repeated oral dosing for extended periods, favorable oral systemic availabilities (22.0–26.4% ester **2e** ig/acid **2a** iv and 44.7–60% ester **2e** ig/ester **2e** iv), and favorable half-life (β-phase), **2e** 1.4–1.6 h/**2a** 4.4–6.5 h, dog). Further

in vivo preclinical pharmacology studies on **2e/2a** will be reported elsewhere.[36]

Experimental Section

Pharmacology. *In Vitro* **Inhibition of Collagen-Induced Canine Platelet Aggregation.**[19] Thirty milliliters of whole blood (pooled from ≥2 dogs) was collected into 0.129 M buffered sodium citrate (3.8%, 1:10). PRP was prepared by centrifugation at 975g for 3.17 min at room temperature. Platelet-poor plasma (PPP) was prepared by centrifuging at 6000g for 8 min at room temperature. The PRP was adjusted with PPP to a count of $(2–3) \times 10^8$ platelets/mL. PRP (400 μL) was preincubated with 50 μL of the compound to be tested, or saline, for 2 min at 37 °C in an aggregometer (PAP-4C; Biodata, Hatboro, PA). Collagen (equine tendon, 50 μL, final concentration 33 μg/mL; Chronolog, Havertown, PA) was added, and the aggregation was monitored for 3 min. All the compounds were tested in duplicate at concentrations of 0.05–100 μM. Results were calculated as follows: [observed percent aggregation (inhibitor)] ÷ [maximum percent aggregation (control)] = percent of control. The percent inhibition = 100 − (percent of control). The IC_{50} was calculated by logit analysis of the dose–response curve. The percent inhibition values were used to choose a minimum of four concentrations which were run in duplicate in three separate experiments ($n = 3$). The IC_{50} (mean ± SE) was calculated by linear regression of individual plots of percent inhibition (logit) versus concentration (log).

Ex Vivo **Studies.** The ester prodrugs (Tables 1–3) were administered orally (by capsule) to conscious beagle dogs ($n = 2$).[35] Blood samples (2 mL) were withdrawn via venipuncture of the cephalic vein into citrated vacutainers at predetermined times before and after dosing for a period of 24 h or until activity had returned to base line. PRP was prepared by centrifuging blood samples at 260g for 6 min. Aggregation was measured and percent inhibition for individual dogs calculated as described above.

Pharmacokinetic Studies. The preliminary radiolabeled studies were done as described.[19,37] The cold pharmacokinetic studies on **2e/2a** were done in the following manner. Six female dogs (White Eagle, Doylestown, PA; 7–12 kg) were used in the study. Three dogs were used in each study group. The dogs were fasted 18 h before each administration. The week prior to the first administration, a catheter was placed in a femoral vein for blood sampling. Oral administrations were done either by intragastric administration of an aqueous solution (2 mL/kg of body weight) or by means of a gelatin capsule. Intravenous administration of an aqueous solution of the drug in saline were performed in a radial vein (0.5 mL/kg of body weight) over 2–3 min. Blood was withdrawn (3.5 mL) from the femoral vein following iv administration at the following time points: 0.25, 0.5, 1, 2, 3, 5, 8, 13, and 24 h. Blood was withdrawn (3.5 mL) from the femoral vein following ig administration at the following time points: 0.5, 1, 1.5, 2, 3, 5, 8, 13, 24, and 48 h. Blood samples were immediately placed on ice and centrifuged, and the plasma were placed on dry ice and then at −20 °C until HPLC analysis. Pharmacokinetic parameters were calculated using the Siphar program on a IBM PS/2 computer. AUC's were calculated using the trapezoidal method and extrapolated to infinity and back to zero. Statistical data are expressed as the mean ± the standard deviation.

Theoretical Studies. Molecular modeling studies were carried out on **2a, 64a,** and **65a** with a view to develop a 3-D pharmacophore model consistent with their *in vitro* biological activity (*vide infra*). Preliminary models of these compounds were built using the computer graphics package MacroModel.[30] The amidine and carboxylate moieties were modeled in their cationic and anionic forms, respectively. The partial atomic charges were obtained by fitting to the electrostatic potential surface obtained through GAUSSIAN 92[38] *ab initio* calculations performed with the 6-31+G* level basis set. The preliminary models were refined using a combination of steepest descent, block-diagonalized Newton–Raphson, and full matrix Newton–Raphson minimization techniques, with a dielectric of 10.0. Throughout the study, the force field used for molecular mechanics optimizations was the MacroModel version of MM2.

Initially, the most rigid of the GP IIb/IIIa antagonists (**64a**) was analyzed for its energetically preferred structures. This was done by generating conformations about its five rotatable single bonds on the right-hand side of the phenyl amidine moiety (Figure 5) at 30° intervals. Conformations with severe interatomic short contacts were eliminated from the resultant collection, which was minimized in two stages using the BatchMin module of MacroModel.[30] In the first stage, the torsions defining the conformations were constrained to their starting value with a harmonic force constant of 1000 kcal/mol/deg, while allowing all the other degrees of freedom to optimize. Conformations with energies of more than 10 kcal/mol above the "global minimum" were eliminated from further consideration. The resultant list was subjected to a full refinement in which the torsion angle constraints were removed. Distinct conformations with energies of more than 10 kcal/mol above the global minimum were again eliminated from further consideration. Two structures were considered distinct from one another only if the atoms determining the varied torsion angles (in the conformation generation) overlapped by more than 0.25 Å.

The global minimum of **64a** adopted an extended conformation in which the central carbon atoms on the amidine and carboxylate groups were separated by 14 Å. In all the energetically favored conformations of **64a**, this distance was at least 10 Å. In this light, conformations of **2a** and **65a** were generated with a minimum constraint of 11 Å on the corresponding distance (note: no upper limit), as a function of the flexible torsion angles. The amides were assumed to be trans during all the simulations. The collections of conformations of **2a** and **65a** were optimized in two stages just as in the case of **64a**.

Unique conformations resulting from the two stages of optimization and elimination were pooled together for each of the three molecules and employed in the generation of a 3-D

pharmacophore using the program APOLLO.[31] This was done by overlapping the non-hydrogen atoms of benzamidine and the carboxylate in all the three molecules. The following criteria were employed in determining an overlap to be acceptable: (1) only conformations which were destabilized by less than 3 kcal/mol relative to their respective global minima were considered; (2) the root mean square distance between each pair of the overlapping atoms was less than 0.5 Å; and (3) the benzamidine and carboxylate groups from each of the molecules occupied similar volumes in space. Any overlap that did not satisfy these three conditions simultaneously was eliminated from further consideration. The resultant best overlap between **64a, 2a,** and **65a** was deemed to constitute a 3-D pharmacophore for *in vitro* antithrombotic potency and was visualized using the computer graphics package SYBYL version 6.04 for further evaluations.

Chemistry. High-field ¹H and ¹³C NMR were recorded on a GE QE-300 spectrometer at 300 and 75 MHz, respectively. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Microanalyses were performed at the Searle Physical Methodology Department. Final compounds were purified by reverse phase HPLC using a Waters LC-3000 instrument and a Waters C-18 column (5 × 30 cm) using a linear gradient (5% CH₃CN/0.05% TFA in H₂O to 40% CH₃CN/0.05% TFA in H₂O) over 30 min. The experimental details for compounds **39a, 41a/41e, 43a/43e,** and **45a/45e** are found in ref 5. The experimental details for compounds **40e/40a, 42e/42a, 51e/51a, 62a/62e,** and **63a/63e** are found in ref 11a.

General Procedure A. 3-[[4-[[4-(Aminoiminomethyl)-phenyl]amino]-1,4-dioxobutyl]amino]-3-phenylpropionic acid (44a). Section A. 4-[[4-(Aminoiminomethyl)-phenyl]amino]-4-oxobutanoic Acid (7). 4-Aminobenzamidine dihydrochloride (**6**) (25.0 g, 120 mmol) was added to dry DMF (100 mL). To this solution were added dry pyridine (100 mL) and succinic anhydride (12.0 g, 120 mmol) followed by (dimethylamino)pyridine (DMAP; 1.50 g, 12.0 mmol). The product precipitated after heating for 0.5 h at 100 °C. The product was filtered and washed with water (2 × 100 mL), acetonitrile (2 × 100 mL), and ether (2 × 100 mL). The product was suspended in dioxane, and 4 N HCl in dioxane (100 mL) was added. After 1 h at 23 °C, the product was filtered and dried in a desiccator to give 28.0 g (88%) of 4-[[4-(aminoiminomethyl)phenyl]amino]-4-oxobutanoic acid (**7**) as a pale yellow solid which decomposes between 270 and 290 °C.

Section B. D,L-3-[[4-[[4-(Aminoiminomethyl)phenyl]-amino]-1,4-dioxobutyl]amino]-3-phenylpropionic Acid (44a). 4-[[4-(Aminoiminomethyl)phenyl]amino]-4-oxobutanoic acid hydrochloride (**7**) prepared in section A (1.0 g, 3.7 mmol) was added to dry DMF (35 mL) followed by N-methylmorpholine (0.39 g, 1 equiv) and isobutyl chloroformate (0.53 g, 3.9 mmol) at 25 °C. The mixture was stirred for 5 min. D,L-3-Amino-3-phenylpropionic acid (**66**) (0.67 g, 4.05 mmol) was added followed by diisopropylethylamine (0.68 mL, 3.9 mmol) and a catalytic amount of DMAP (10 mg). After 1 h, the solvent was removed *in vacuo*. The residue was dissolved in acetonitrile/water and purified on a Waters Deltapak C-18 HPLC column (30 cm × 5 cm) with a flow rate of 80 mL/min. A linear gradient (30 min) of 5–40% acetontrile/water/0.05% TFA followed by an increase to 60% acetonitrile in 10 min was used. The product was freeze-dried to give 340 mg of **44a** as a white solid: ¹H NMR (DMSO-*d₆*) δ 2.45 (m, 2H), 2.6 (m, 2H), 2.7 (d, 2H, J = 7 Hz), 4.2 (dd, 1H, J = 7, 8 Hz), 7.3 (m, 4H), 7.8 (s, 4H), 8.45 (d, 1H, J = 8 Hz), 9.0 (bs, 2H), 9.2 (bs, 2H), 10.4 (s, 1H); FAB MS (MH⁺ = 383). Anal. (C₂₀H₂₂N₄O₄ plus F₃C₂O₂H and H₂O) C, H, N.

Section C. Ethyl D,L-3-[[4-[[4-(Aminoiminomethyl)-phenyl]amino]-1,4-dioxobutyl]amino]-3-phenylpropanoate (44e). To a solution of **44a** (1.30 g, 2.61 mmol) and 200 mL of absolute ethanol was added 10 mL of anhydrous HCl in dioxane (4 N) solution. After 16 h at 23 °C, the reaction mixture was concentrated *in vacuo* and the residue was purified by reverse phase HPLC as in the procedure for **44a** to provide **44e** as a white solid (0.65 g, 47%): ¹H NMR (DMSO-*d₆*) δ 1.10 (t, J = 7 Hz, CH₃), 2.45 (m, 2H), 2.6 (m, 2H), 2.75 (d, 2H, J = 7 Hz), 4.0 (q, J = 7 Hz, CH₂), 4.2 (dd, 1H, J = 7,

8 Hz), 7.3 (m, 4H), 7.8 (s, 4H), 8.45 (d, 1H, $J = 8$ Hz), 9.05 (bs, 2H), 9.2 (bs, 2H), 10.4 (s, 1H); FAB MS (MH$^+$ = 411). Anal. (C$_{22}$H$_{26}$N$_4$O$_4$ plus F$_3$C$_2$O$_2$H and H$_2$O) C, H, N.

Ethyl 3-(S)-[[4-[[4-(Aminoiminomethyl)phenyl]methylamino]-1,4-dioxobutyl]amino]-4-pentenoate (31a). 4-(Methylamino)benzonitrile (27). To a cold solution (−30 °C) of 4-aminobenzonitrile (10.25 g, 86.76 mmol) and pyridine (50 mL) was added trifluoroacetic anhydride (20.8 g, 99 mmol) over 15 min which resulted in an exotherm (note: reaction mixture was not allowed to warm above 10 °C). After addition was complete, the reaction mixture was allowed to warm to 23 °C. After 1 h, the reaction mixture was concentrated under a steady stream of nitrogen. The residue was diluted with ethyl acetate (400 mL) and washed with water (1 × 100 mL), 5% HCl (2 × 100 mL), water (1 × 100 mL), and brine (1 × 100 mL). The organic layer was dried (Na$_2$SO$_4$) and concentrated *in vacuo*. The residue was crystallized from ethyl acetate–ether to afford a first crop (8.66 g, mp 169−170 °C), and then the mother liquor was concentrated and digested with ether to afford a second crop (6.43 g, mp 169−170 °C) followed by further dilution of the mother liquor with hexane which afforded a third crop (2.72 g, mp 169−170 °C). All of the crops were combined to afford *p*-(trifluoroacetamido)benzonitrile (**32**). Anal. (C$_9$H$_5$N$_2$OF$_3$) C, H, N.

To a solution of *p*-(trifluoroacetamido)benzonitrile (**32**) (4.35 g, 20.3 mmol) and acetone (50 mL) was added iodomethane (11.4 g, 80.3 mmol) followed by potassium carbonate (80.3 mmol). The reaction mixture was warmed to achieve reflux for 4 h followed by cooling to 23 °C and then filtration. The filtrate was concentraetd *in vacuo*, diluted with ethyl acetate (300 mL), washed with water (2 × 100 mL) and brine (1 × 100 mL), and dried (Na$_2$SO$_4$). After concentration *in vacuo*, the residue was recrystallized from ethyl acetate−ether (4.23 g, mp 121−122 °C). The solid obtained was diluted with methanol and treated with 20 mL of 1 N NaOH (slow addition dropwise over 10 min). The reaction mixture was diluted with water (10 mL), and a resultant precipitate was filtered, washed with water, and dried under vacuum to afford **27** as a white solid (2.55 g, 95%): ^{1}H NMR (CDCl$_3$) δ 2.84 (d, $J = 4$ Hz, CH$_3$), 4.47 (bs, NH), 6.56 (d, $J = 6$ Hz, ArH), 7.39 (d, $J = 6$ Hz, ArH); ^{13}C NMR (CDCl$_3$) δ 29.4, 97.5, 111.3, 120.2, 133.1, 152.7. Anal. (C$_8$H$_8$N$_2$) C, H, N.

4-[(4-Cyanophenyl)-N-methylamino]-4-oxobutanoic Acid (29). To a cold solution (0 °C) of 4-(methylamino)-benzonitrile (**27**) (2.43 g, 18.3 mmol) and methylene chloride (50 mL) was added diisopropylethylamine (2.82 g, 21.8 mmol) followed by 3-carbomethoxypropionyl chloride (3.06 g, 20.3 mmol). The reaction mixture was allowed to warm to 23 °C after addition was complete. After 1 h, the reaction mixture was concentrated *in vacuo*, diluted with ethyl acetate (300 mL), and washed with water (1 × 100 mL), 5% HCl (2 × 100 mL), water (1 × 100 mL), and brine (1 × 100 mL). The organic layer was dried (Na$_2$SO$_4$) and concentrated *in vacuo*. The residue was crystallized from ethyl acetate−ether to afford a first crop of **28** (2.74 g, mp 88−89 °C). The mother liquor was purified by flash chromatography (1:5 ethyl acetate:hexane− 1:1 ethyl acetate:hexane) to afford an additional 1.14 g of **28** (combined 86% yield): ^{1}H NMR (CDCl$_3$) δ 2.41−2.49 (m, 2H), 2.65 (t, $J = 6$ Hz, CH$_2$), 3.32 (s, CH$_3$), 3.68 (s, OCH$_3$), 7.42 (d, $J = 6$ Hz, ArH), 7.75 (d, $J = 6$ Hz, ArH); ^{13}C NMR (CDCl$_3$) δ 30.0, 38.2, 52.7, 119.0, 128.9, 134.6, 148.7, 171.8, 174.2. Anal. (C$_{13}$H$_{14}$N$_2$O$_3$) C, H, N.

To a methanol (20 mL) suspension of methyl 4-[(4-cyanophenyl)-N-methylamino]-4-oxobutanoate (**28**) (3.84 g, 15.6 mmol) at 0 °C was added 1 N NaOH (17 mL). After 1 h, the reaction mixture was diluted with water (10 mL). The reaction mixture was concentrated *in vacuo*, extracted with ether (1 × 100 mL), acidified with 1 N HCl, extracted with ethyl acetate (3 × 100 mL), washed with water (1 × 100 mL) and brine (1 × 100 mL), and dried (Na$_2$SO$_4$). After concentration *in vacuo*, the residue was crystallized from methylene chloride:ether to afford **29** as a white solid (2.90 g, 80.0%, mp 128−129 °C): ^{1}H NMR (CDCl$_3$) δ 2.39−2.49 (m, 2H), 2.68 (t, $J = 6$ Hz, CH$_2$), 3.32 (s, CH$_3$), 7.42 (d, $J = 6$ Hz, ArH), 7.75 (d, $J = 6$ Hz, ArH); ^{13}C NMR (CDCl$_3$) δ 29.7, 38.1, 118.6, 128.5, 134.4, 148.1, 171.8, 178.2. Anal. (C$_{12}$H$_{12}$N$_2$O$_3$) C, H, N.

Hydrogen sulfide was bubbled through a solution of benzonitrile **29** (2.84 g, 12.2 mmol), pyridine (20 mL), and triethylamine for 5 min (caution: hydrogen sulfide is highly toxic). After 28 h at 23 °C in a closed system, the reaction mixture was concentrated with a nitrogen stream (bleach trap). The residue was diluted with ethyl acetate (350 mL), washed with 1 N KHSO$_4$ (2 × 100 mL), water (1 × 100 mL), and brine (1 × 100 mL), and dried (Na$_2$SO$_4$). The reaction mixture was concentrated *in vacuo*, and the residue was taken directly on to the next step without further purification. The thioamide residue (3.50 g, 13.14 mmol) was diluted with acetone (50 mL) and reacted with iodomethane (6.84 g, 48.0 mmol) at 56 °C for 30 min under a nitrogen atmosphere. After cooling to 23 °C, the reaction mixture was diluted with ether (50 mL), cooled to 0 °C, and then subsequently filtered to afford a quantitative yield of the thioimidate salt. The solid thioimidate salt was taken on to the next step without further purification. To a solution of the thioimidate (5.36 g, 13.14 mmol) and methanol ((20 mL) was added solid anhydrous ammonium acetate (2.01 g, 26.2 mmol); the resultant solution was warmed to 65 °C for 3 h. After cooling to 23 °C, the reaction mixture was filtered and washed with methanol (10 mL) and acetone (20 mL) to afford benzamidine **30** as the HI salt (2.218 g). The salt was exchanged by repeated (three times) treatment with 1 N HCl (1.5 equiv) followed by triturating with acetone to afford (2.04 g) of benamidine **30** as the HCl salt: ^{1}H NMR (CDCl$_3$) δ 2.36−2.51 (m, 4H), 3.25 (s, CH$_3$), 7.62 (d, $J = 6$ Hz, ArH), 7.97 (d, $J = 6$ Hz, ArH), 9.41 (bs, NH$_2$), 9.58 (bs, NH$_2$); ^{13}C NMR (CDCl$_3$) δ 28.8, 28.9, 36.6, 126.0, 127.1, 129.3, 148.3, 164.9, 170.5, 173.6. Anal. (C$_{12}$H$_{16}$-N$_3$O$_3$Cl) C, H, N, Cl.

4-[(4-Amidinophenyl)-N-methylamino]-4-oxobutanoic acid (**30**) was coupled to (S)-ethyl 3-amino-4-pentenoate (**72**) in a manner similar to that of compound **44a**. Purification by reverse phase HPLC afforded **31e**: ^{1}H NMR (CD$_3$OD) δ 1.22 (t, $J = 6$ Hz, CH$_3$), 2.43−2.59 (m, 6H), 3.32 (s, CH$_3$), 4.10 (q, $J = 6$ Hz, CH$_2$), 4.72−4.83 (m, NH), 5.03−5.24 (m, =CH$_2$), 5.78− 5.91 (m =CH), 7.59 (d, $J = 6$ Hz, ArH), 7.92 (d, $J = 6$ Hz, ArH), 9.18 (bs, NH$_2$), 9.38 (bs, NH$_2$); ^{13}C NMR (CD$_3$OD) δ 14.1, 30.1, 31.3, 37.3, 39.8, 49.2, 61.2, 128.0, 128.6, 130.0, 137.6, 149.6, 167.2, 171.9, 173.2, 173.3.

31e was converted to **31a** by treatment with esterase in a manner similar to that of **46a**: ^{1}H NMR (CD$_3$OD) δ 2.48−2.59 (m, 6H), 3.32 (s, CH$_3$), 4.73−4.80 (m, NH), 5.09−5.32 (m, =CH$_2$), 5.79−5.92 (m, =CH), 7.59 (d, $J = 6$ Hz, ArH), 7.92 (d, $J = 6$ Hz, ArH), 9.08 (bs, NH$_2$), 9.35 (bs, NH$_2$); ^{13}C NMR (CD$_3$-OD) δ 29.3, 30.5, 36.6, 38.7, 48.4, 114.4, 127.7, 127.9, 129.3, 136.9, 136.9, 148.8, 166.7, 172.5, 172.6, 173.0. Anal. (C$_{17}$H$_{22}$-N$_4$O$_4$ plus 1.05HCl and 1H$_2$O) C, H, N, Cl.

3-(S)-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-4-oxobutyl]amino]-4-pentenoic Acid (36a). To a solution of *p*-(trifluoroacetamido)benzonitrile (**32**) (30.0 g, 0.140 mol), KI (25.9 g, 0.156 mol), ethyl 4-bromobutyrate (109 g, 0.56 mol), and acetone (700 mL) was added potassium carbonate (78.0 g, 0.560 mol). The reaction mixture was warmed to 56 °C for 4 h and then cooled to 23 °C. The reaction mixture was filtered and concentrated *in vacuo*. The residue was diluted with ether (400 mL), washed with water (2 × 100 mL) and brine (2 × 100 mL), and dried (Na$_2$SO$_4$). After concentration *in vacuo*, the residue was purified by flash chromatography (step gradient 5% ethyl acetate:hexane; 10% ethyl acetate:hexane; 20% ethyl acetate:hexane; 40% ethyl acetate:hexane) which afforded **33** (8.25 g) as a white solid (73−74 °C). A portion of the ester **33** (4.30 g, 13.1 mmol) was treated with 6 N HCl (50 mL) and dioxane (30 mL) for 20 h. After removal of the dioxane *in vacuo*, the reaction mixture was extracted with ethyl acetate (1 × 400 mL), washed with water (1 × 100 mL) and brine (1 × 100 mL), and dried (Na$_2$SO$_4$). After concentration *in vacuo*, the residue was purified by flash chromatography (ethyl acetate:hexane:acetic acid, 2:3:0.0001) to afford 4-[(4-cyanophenyl)-N-(trifluoroacetyl)amino]-4-oxobutanoic acid (**34**) as a white solid (2.85 g, 72.5%, mp 90−92 °C). Anal. (C$_{13}$H$_{11}$N$_2$O$_3$F$_3$) C, H, N.

To a solution of **34** (1.022 g, 3.40 mmol), acetonitrile (10 mL), and pyridine (2.5 mL) was added solid DSC (874 mg, 3.41 mmol) followed by DMAP (30 mg). After 30 min at 23 °C under

nitrogen, solid (S)-ethyl 3-amino-4-pentenoate hydrochloride (72) (613 mg, 3.41 mmol) was added followed by 4-methylpiperidine (310 mg, 3.2 mmol). After 3 h at 23 °C, the reaction mixture was concentrated *in vacuo*. The residue was diluted with ether (300 mL), washed with 5% HCl (2 × 100 mL) and brine (2 × 100 mL), and dried (Na$_2$SO$_4$). After concentration *in vacuo*, the residue was purified by flash chromatography (ethyl acetate:hexane, 2:3 then 3:2) which afforded ethyl 3-(S)-[[4-[(4-cyanophenyl)-N-(trifluoroacetyl)amino]-4-oxobutyl]amino]-4-pentenoate (35) as an oil (1.076 g): ^{1}H NMR (CDCl$_3$) δ 1.25 (t, J = 6 Hz, CH$_3$), 1.86−2.00 (m, 2H), 2.31 (t, J = 6 Hz, CH$_2$), 2.57−2.63 (m, CH$_2$), 3.78−3.92 (m, 2H), 4.14 (q, J = 6 Hz, CH$_2$), 4.78−4.89 (m, CHN), 5.11−5.23 (m, =CH$_2$), 5.78−5.92 (m, =CH), 6.69 (d, J = 6 Hz, NH), 7.47 (d, J = 6 Hz, 2H, ArH), 7.81 (d, J = 6 Hz, 2H, ArH); ^{13}C NMR (CDCl$_3$) δ 14.2, 22.9, 33.1, 38.7, 48.0, 51.3, 60.7, 115.7, 116.1 (q, J = 321 Hz, CF$_3$), 117.7, 129.4, 133.6, 136.7, 143.0, 156.2 (q, J = 11 Hz), 170.8, 171.2.

The benzonitrile 35 was converted to the benzamidine in a manner similar to that of 30 to afford 1.418 g of crude 36e which was purified by reverse phase HPLC: ^{1}H NMR (CD$_3$-OD) δ 1.22 (t, J = 6 Hz, CH$_3$), 1.89−2.01 (m, 2H), 2.39 (t, J = 6 Hz, CH$_2$), 2.53−2.69 (m, CH$_2$), 3.21 (t, J = 6 Hz, CH$_2$), 4.12 (q, J = 6 Hz, CH$_2$), 4.78−4.89 (m, CHN), 5.02 (bs, NH), 5.11−5.27 (m, =CH$_2$), 5.82−5.95 (m, =CH), 6.78 (d, J = 6 Hz, 2H, ArH), 7.68 (d, J = 6 Hz, 2H, ArH); ^{13}C NMR (CD$_3$OD) δ 14.2, 22.7, 25.6, 34.0, 39.8, 42.7, 49.2, 61.5, 112.3, 113.2, 115.7, 120.6, 128.8, 130.2, 137.5, 154.7, 166.1, 172.0, 174.3.

36e was treated with 6 N HCl (10 mL) and dioxane (5 mL) for 20 h at 23 °C. After concentration *in vacuo*, the residue was purified by reverse phase HPLC in a manner similar to that of 44a substituting acetic acid for TFA to afford 36a as a white solid: ^{1}H NMR (CD$_3$OD) δ 1.94−2.01 (m, 2H), 2.32 (t, J = 6 Hz, CH$_2$), 2.51−2.66 (m, CH$_2$), 3.19 (t, J = 6 Hz, CH$_2$), 4.78−4.85 (m, CHN), 5.07 (bs, NH), 5.08−5.23 (m, =CH$_2$), 5.78−5.92 (m, =CH), 6.71 (d, J = 6 Hz, 2H, ArH), 7.62 (d, J = 6 Hz, 2H, ArH); ^{13}C NMR (CD$_3$OD) δ 24.5, 32.7, 38.3, 41.6, 48.0, 111.1, 112.5, 114.2, 129.0, 136.5, 153.8, 165.4, 171.9, 172.9. Anal. (C$_{16}$H$_{22}$N$_4$O$_3$ plus 1.8acetic acid) C, H, N.

Ethyl β-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]benzenebutanoate (46e). 46e was prepared in a manner similar to that of 44a substituting D,L-ethyl 3-amino-4-phenylbutanoate for D,L-3-amino-3-phenylpropionic acid. The product was purified by reverse phase HPLC and freeze-dried to afford 46e as a white solid: ^{1}H NMR (CD$_3$OD) δ 1.23 (t, J = 6 Hz, CH$_3$), 2.42−2.55 (m, 4H), 2.65 (t, J = 7 Hz, CH$_2$), 2.83 (t, J = 7 Hz, CH$_2$), 4.08 (q, J = 6 Hz, CH$_2$), 4.42−4.53 (m, CHN), 7.13−7.28 (m, ArH), 7.75−7.85 (m, ArH); ^{13}C NMR (CD$_3$OD) δ 13.6, 30.8, 32.2, 38.8, 40.4, 48.7, 60.8, 119.7, 122.7, 126.6, 128.5, 129.0, 129.5, 138.4, 144.8, 166.1, 171.2, 172.0, 172.5; FAB MS (MH$^+$ = 425). Anal. (C$_{23}$H$_{28}$N$_4$O$_4$·1.0CF$_3$CO$_2$H·0.5H$_2$O) C, H, N.

β-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]benzenebutanoic Acid (46a). Porcine liver esterase (200 mL, 11 mg/mL in 3.2 M (NH$_4$)$_2$SO$_4$ at pH 8; Sigma) was added to 46e in 20 mL of 0.1 M phosphate buffer (pH 7.4). After 20 h at 23 °C, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in 1 N HCl (3 mL) and subsequently diluted with acetonitrile (5 mL) followed by immediate purification by reverse phase HPLC using the conditions of the procedure for 44a. The major peak was freeze-dried to afford 46a as a white powder: ^{1}H NMR (CD$_3$OD) δ 2.42−2.54 (m, 4H), 2.65 (t, J = 7 Hz, CH$_2$), 2.75−2.90 (m, CH$_2$), 4.42−4.53 (m, CHN), 7.15−7.31 (m, 5-ArH), 7.78−7.88 (m, 4-ArH), 8.7 and 9.1 (2 bs, 2-NH$_2$); ^{13}C NMR (CD$_3$-OD) δ 30.2, 31.7, 37.8, 39.6, 47.1, 119.2, 122.1, 126.1, 128.6, 129.0, 138.0, 144.3, 166.2, 172.0, 172.5, 173.2; FAB MS (MH$^+$ = 397). Anal. (C$_{21}$H$_{24}$N$_4$O$_4$·1.4CF$_3$CO$_2$H), C, H, N.

Ethyl 3-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-3-(3,4-difluorophenyl)propanoate (47e). 47e was prepared in a manner similar to that of 44a substituting ethyl 3-amino-3-(3,4-difluorophenyl)propanoate for D,L-3-amino-3-phenylpropionic acid (66). The product was purified by reverse phase HPLC and freeze-dried to afford 47e as a white solid: ^{1}H NMR (DMSO-d_6) δ 2.11 (t, 2H, J = 7.1 Hz), 2.62 (t, 2H, J = 7.3 Hz), 3.47 (t, 2H, J = 7.0 Hz), 3.5 (s,

6H), 3.51 (m, 1H), 7.20−7.40 (m, 3H), 7.79 (s, 4H), 8.1 (t, 1H, J = 7.1 Hz), 8.7 (bs, 2H), 9.09 (bs, 2H), 10.42 (s, 1H); FAB MS (MH$^+$ = 379). Anal. (C$_{17}$H$_{22}$N$_4$O$_6$·F$_3$C$_2$O$_2$H·H$_2$O) C, H, N.

β-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-3-(3,4-difluorophenyl)propanoic Acid (47a). 47e (700 mg) was added to water/acetonitrile (20 mL) followed by lithium hydroxide (100 mg) at 25 °C. The mixture was stirred for 30 min. The course of the reaction was monitored by RPHPLC. After satisfactory acid was formed, the reaction mixture was neutralized with TFA, purified by reverse phase chromatography, and freeze-dried to afford 620 mg of 47a as a white solid: ^{1}H NMR (DMSO-d_6) δ 2.38 (t, 2H, J = 7.3 Hz), 2.44 (d, 2H, J = 6.4 Hz), 2.61 (t, 2H, J = 7.3 Hz), 4.32 (m, 1H), 7.30 (m, 3H), 7.78 (s, 4H), 7.90 (d, 1H, J = 8.1 Hz), 8.92 (bs, 2H), 9.16 (bs, 2H), 10.39 (s, 1H); FAB MS (MH$^+$ = 365). Anal. (C$_{16}$H$_{20}$N$_4$O$_6$·F$_3$C$_2$O$_2$H·H$_2$O) C, H, N.

Ethyl β-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-3-(pentafluorophenyl)propanoate (48e). 48e was prepared in a manner similar to that of 44a substituting ethyl 3-amino-3-(pentafluorophenyl)propanoate for D,L-3-amino-3-phenylpropionic acid. The product was purified by reverse phase HPLC and freeze-dried to afford 48e as a white solid: ^{1}H NMR (DMSO-d_6) δ 2.57 (t, 2H, J = 7.3 Hz), 2.07 (t, 2H, J = 7.1 Hz), 3.47 (t, 2H, J = 7.0 Hz), 3.5 (s, 6H), 3.51 (m, 1H), 7.79 (s, 4H), 8.1 (t, 1H, J = 7.1 Hz), 8.7 (bs, 2H), 9.09 (bs, 2H), 10.32 (s, 1H); FAB MS (MH$^+$ = 379). Anal. (C$_{17}$H$_{22}$N$_4$O$_4$F$_5$F$_3$C$_2$O$_2$H·H$_2$O) C, H, N.

β-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-3-(pentafluorophenyl)propanoic Acid (48a). 48e (600 mg) was added to water/acetonitrile (20 mL) followed by lithium hydroxide (100 mg) at 25 °C. The mixture was stirred for 30 min. The course of the reaction was monitored by RPHPLC. After satisfactory acid was formed, the reaction mixture was neutralized with TFA, purified by reverse phase chromatography, and freeze-dried to afford 620 mg of 48a as a white solid: ^{1}H NMR (DMSO-d_6) δ 2.40 (d, 2H, J = 6.4 Hz), 2.42 (t, 2H, J = 7.3 Hz), 2.56 (t, 2H, J = 7.3 Hz), 4.32 (m, 1H), 7.78 (s, 4H), 7.99 (d, 1H, J = 8.1 Hz), 8.92 (bs, 2H), 9.16 (bs, 2H), 10.39 (s, 1H); FAB MS (MH$^+$ = 365). Anal. (C$_{15}$H$_{18}$N$_4$O$_4$F$_5$·CF$_3$CO$_2$H·H$_2$O) C, H, N.

Ethyl β-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-1,3-benzodioxole-5-propanoate (49e). An ethanol solution (350 mL) of 3,4-(methylenedioxy)benzaldehyde (6.0 g, 40 mmol), malonic acid (5.2 g, 50 mmol), and ammonium acetate (4.0 g, 52 mmol) was heated at reflux for 20 h. The reaction mixture was allowed to cool down to room temperature, and the solid precipitate was collected by filtration and washed with ethanol/water (1:1, 2 × 100 mL). The air-dried free acid β-amino-1,3-benzodioxole-5-propanoic acid (3.0 g, [FAB MS: MH$^+$ = 210) was suspended in absolute ethanol (200 mL). The solution was cooled in an ice bath, and dry HCl gas was bubbled through for 1 h. The reaction mixture was stirred at room temperature overnight followed by solvent removal *in vacuo*. The residue was dried in a vacuum dessicator to give 3.2 g of ethyl β-amino-1,3-benzodioxole-5-propanoate hydrochloride (67) ester (FAB MS: MH$^+$ = 238). This material was used without any further purification.

4-[[4-(Aminoiminomethyl)phenyl]amino]-4-oxobutanoic acid hydrochloride (7) (2.75 g, 10 mmol) was dissolved in DMF (50 mL). Isobutyl chloroformate (1.5 g, 11 mmol) was added dropwise with stirring followed by N-methylmorpholine (1.0 g, 10 mmol). In a separate flask, ethyl β-amino-1,3-benzoxazole-5-propanoate hydrochloride (3.0 g, 12.5 mmol) and N,N-diisopropyl-N-ethylamine (1.3 g, 10 mmol) were dissolved in DMF (20 mL). Both solutions were combined and stirred at room temperature for 2 h. Saturated sodium bicarbonate solution (30 mL) was added with stirring, and the mixture was filtered. The filtrate was taken down to dryness on a rotavapor. The remaining residue was purified by RPHPLC and freeze-dried to afford 49e: ^{1}H NMR (DMSO) δ 1.11 (t, J = 7 Hz, 3H), 2.45 and 2.57 (t, 4H), 2.69 (m, 2H), 4.0 (q, J = 7 Hz, 2H), 5.13 (m, 1H), 5.97 (s, 2H, OCH$_2$O), 6.80−6.91 (m, 3H, Ar), 7.77 (s, 4H, Ar), 8.48 (m, 1H, CONH), 8.87 and 9.15 (2s, 4H, H$_2$NCNH$_2$); FAB MS (M$^+$ = 454). Anal. (C$_{23}$H$_{23}$N$_4$O$_6$·CF$_3$COOH) H, N, C: calcd, 52.81; found, 52.10.

β-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-1,3-benzodioxole-5-propanoic Acid (49a). 49e (100 mg) was stirred in 2 N LiOH (5 mL) and methanol (5 mL) at room temperature for 20 min. The mixture was neutralized with 4 N HCl and diluted with water (20 mL). This material was then purified by RPHPLC and freeze-dried to afford (49a): ¹H NMR (DMSO) δ 2.45 (m, 2H), 2.57 (m, 2H), 2.60 (m, 2H), 5.08 (m, 1H), 5.97 (s, 2H, OCH₂O), 6.80–6.90 (m, 3H, ArH), 7.77 (s, 4H, Ar), 8.44 (m, 1H, CONH), 8.93 and 9.12 (2s, 4H, H₂NCNH₂); FAB MS (MH⁺ = 426). Anal. (C₂₁H₂₂N₄O₆·CF₃COOH) H, N; C: calcd, 51.11; found, 50.30.

Ethyl β-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-6-nitro-1,3-benzodioxole-5-propanoate (50e). 50e was prepared and purified according to the procedure of 49e substituting ethyl β-amino-6-nitro-1,3-benzodioxazole-5-propanoate for ethyl β-amino-1,3-benzodioxole-5-propanoate hydrochloride: ¹H NMR (DMSO-d₆) δ 1.16 (t, J = 7 Hz, 3H), 2.43 (m, 2H), 2.54 (m, 2H), 2.73 (m, 2H), 4.06 (m, 2H), 5.63 (m, 1H, NHCH), 6.20 (s, 2H, OCH₂O), 7.2 and 7.5 (2s, 2H, Ar), 7.74 (s, 4H, Ar), 8.62 (m, 1H, CONH), 8.74 and 9.12 (2s, 4H, H₂NCNH₂). Anal. (C₂₃H₂₅N₅O₈·CF₃COOH) H; C: calcd, 48.94; found, 48.17. N: calcd, 11.42; found, 10.88.

β-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-6-nitro-1,3-benzoxazole-5-propanoic Acid (50a). 50a was prepared and purified according to the procedure described for 49a starting with ester 50e: ¹H NMR (DMSO-d₆) δ 2.45 (m, 2H), 2.54 (m, 2H), 2.65 (m, 2H), 5.57 (m, 1H, NHCH), 6.20 (s, 2H, OCH₂O), 7.17 (s, 1H, ArH), 7.51 (s, 1H, ArH), 7.74 (s, 4H, Ar), 8.60 (m, 1H, CONH), 8.83 and 9.14 (2s, 4H, H₂NCNH₂); FAB MS (MH⁺ = 472). Anal. (C₂₁H₂₁N₅O₈·CF₃COOH·H₂O) C, H, N.

Ethyl 3-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-3-(6-ethoxypyrid-3-yl)propanoate (52a). 52e was prepared in a manner similar to that of 44a substituting tert-butyl 3-amino-3-[3-(6-ethoxypyridyl)]propanoate for D,L-3-amino-3-phenylpropionic acid. The product 52e was treated with trifluoroacetic acid:water (9:1) which afforded the acid 52a as a white solid after purification by reverse phase HPLC and freeze-drying: ¹H NMR (DMSO-d₆) δ 1.43 (t, 3H, J = 7 Hz), 2.54–2.97 (m, 3-CH₂), 4.37 (q, J = 7 Hz, CH₂), 5.28–5.34 (m, CHN), 7.08 (d, J = 6 Hz, 1H), 7.73–7.83 (m, 4H), 8.05–8.24 (m, ArH); ¹³C NMR (CD₃OD) δ 13.9, 30.6, 32.0, 39.7, 47.7, 64.9, 110.0, 119.8, 122.3, 127.5, 129.2, 142.2, 142.3, 144.5, 146.9, 166.7, 172.6, 173.3. Anal. (C₂₁H₂₅N₅O₅·1.5 F₃C₂O₂H·1H₂O) C, N; H: calcd, 4.66; found, 4.16.

D,L-β-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-3-(3-quinolinyl)propanoic Acid (53a). 53e was prepared in a manner similar to that of 44a substituting ethyl 3-amino-3-(3-quinolinyl)propioniate for D,L-3-amino-3-phenylpropionic acid (66). The product was purified by reverse phase HPLC and freeze-dried to afford 53e as a white solid. The ester 53e was converted to the acid 53a using lithium hydroxide in a manner similar to that of 47a which afforded 250 mg of 53a as a white solid: ¹H NMR (DMSO-d₆) 2.50 (m, 2H), 2.61 (m, 2H), 3.22 (m, 2H), 5.75 (m, 1H), 7.98–8.80 (m, 12H), 8.89 (bs, 2H), 8.72 (bs, 1H), 9.14 (bs, 2H), 10.72 (s, 1H); FAB MS (MH⁺ = 434). Anal. (C₁₆H₂₂N₄O₅·1F₃C₂O₂H·O·1H₂O) C, H, N.

Ethyl 3-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-pentenoate (54e). 54e was prepared in a manner similar to that of 44a substituting ethyl 3-amino-4-pentenoate (23) for D,L-3-amino-3-phenylpropionic acid (66). The product was purified by reverse phase HPLC and freeze-dried to afford 54e as a white solid: ¹H NMR (CD₃OD) δ 1.24 (t, J = 6 Hz, CH₃), 2.56–2.77 (m, 6H), 4.12 (q, J = 6 Hz, CH₂), 4.74–4.85 (m, CHN), 5.06–5.24 (m, CH₂), 5.78–5.92 (m, CH), 7.75–7.85 (m, ArH); ¹³C NMR (CD₃OD) δ 12.8, 29.5, 31.0, 47.5, 59.5, 69.3, 113.8, 118.2, 121.1, 128.2, 136.5, 143.7, 165.9, 169.9, 170.9, 171.0; FAB MS (MH⁺ = 361). Anal. (C₁₈H₂₄N₄O₄·1.0CF₃CO₂H·0.5H₂O) C, H, N.

3-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-pentenoic Acid (54a). 54e was prepared by treating 54e with porcine liver esterase in a manner similar to that of 46a. The product was purified by reverse phase HPLC and freeze-dried to afford 54a as a white solid: ¹H NMR (CD₃OD) δ 2.53–2.77 (m, 6H), 4.12 (q, J = 6 Hz, CH₂), 4.74–

4.85 (m, CHN), 5.06–5.26 (m, CH₂), 5.80–5.92 (m, CH), 7.73–7.84 (m, ArH); ¹³C NMR (CD₃OD) δ 30.0, 31.3, 38.2, 47.9, 114.0, 118.9, 119.0, 128.1, 136.3, 165.9, 171.8, 172.1, 173.2; FAB MS (MH⁺ = 333). Anal. (C₁₆H₂₀N₄O₄·1.0CF₃CO₂H·1.45H₂O) C, H; N: calcd, 11.86; found, 12.29.

Ethyl 3-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-5-hexenoate (55e). 55e was prepared following general procedure A (44a) substituting ethyl 3-amino-5-hexenoate (21) for D,L-3-amino-3-phenylpropionic acid (66). The product was purified by reverse phase HPLC and freeze-dried to afford 55e as a white solid: ¹H NMR (CD₃OD) δ 1.08 (t, J = 6 Hz, CH₃), 2.05–2.41 (m, 4H), 2.45 (t, J = 6 Hz, CH₂), 2.61 (t, J = 6 Hz, CH₂), 3.96 (q, J = 6 Hz, CH₂), 4.13–4.24 (m, 1 H), 4.85–5.01 (m, CH₂), 5.54–5.72 (m, CH), 7.63–7.72 (m, 4-ArH), 8.71 and 8.95 (2bs, 2-NH₂), 10.15 (bs, NH); ¹³C NMR (CD₃OD) δ 12.6, 29.8, 31.3, 37.9, 45.9, 59.9, 116.5, 118.7, 121.5, 128.1, 133.4, 143.7, 165.6, 171.1, 171.6, 172.2; FAB MS (MH⁺ = 375).

3-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-5-hexenoic Acid (55a). 55a was prepared by treating 55e with porcine liver esterase in a manner similar to that of 46a. The product was purified by reverse phase HPLC and freeze-dried to afford 55a as a white solid: ¹H NMR (CD₃OD) β 2.21–2.52 (m, 4H), 2.51–2.74 (m, 4H), 4.21–4.32 (m, 1H), 5.01–5.13 (m, CH₂), 5.72–5.84 (m, CH), 7.74–7.84 (m, 4-ArH), 8.73 and 9.12 (2bs, 2-NH₂), 10.27 (bs, NH); ¹³C NMR (CD₃OD) δ 31.3, 32.8, 39.0, 39.2, 47.0, 117.8, 120.2, 123.1, 129.5, 135.1, 145.2, 165.6, 173.0, 173.6, 174.4; FAB MS (MH⁺ = 347). Anal. (C₁₇H₂₂N₄O₄·1.0 CF₃CO₂H·0.6H₂O) C, H, N.

Ethyl 3-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-pentynoate (56e). 56e was prepared in a manner similar to that of 44a substituting ethyl 3-amino-4-pentynoate for D,L-3-amino-3-phenylpropionic acid (66). The product was purified by reverse phase HPLC and freeze-dried to afford 56e as a white solid: ¹H NMR (CD₃OD) δ 1.24 (t, J = 6 Hz, CH₃), 2.54–2.75 (m, 7H), 4.12 (q, J = 6 Hz, CH₂), 5.01–5.09 (m, CHN), 7.75–7.85 (m, ArH); ¹³C NMR (CD₃OD) δ 13.6, 30.3, 31.9, 38.1, 40.4, 61.0, 71.9, 82.0, 119.6, 122.5, 129.1, 144.8, 166.5, 170.3, 172.1, 172.2; FAB MS (MH⁺ = 359). Anal. (C₁₆H₁₈N₄O₄ plus 1.5CF₃CO₂H plus 0.65H₂O) H; C: calcd, 44.48; found, 44.05. N: calcd, 10.92; found, 11.38.

3-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-pentynoic Acid (56a). 56a was prepared by treating 56e with porcine liver esterase in a manner similar to that of 46a. The product was purified by reverse phase HPLC and freeze-dried to afford 56a as a white solid: ¹H NMR (CD₃OD) δ 2.56–2.78 (m, 6H), 4.95–5.05 (m, CHN), 7.73–7.83 (m, ArH), 8.72 and 9.11 (2bs, 2-NH₂); ¹³C NMR (CD₃OD) δ 29.9, 31.4, 37.7, 39.5, 71.1, 81.5, 119.2, 122.1, 128.3, 144.2, 166.2, 171.8, 172.0, 172.1; FAB MS (MH⁺ = 331). Anal. (C₁₆H₁₈N₄O₄·1.5CF₃CO₂H·0.65H₂O) H; C: calcd, 44.48; found, 44.05. N: calcd, 10.92; found, 11.38.

Ethyl 3-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-6,6-dimethyl-4-heptynoate (57e). 57e was prepared in a manner similar to that of 44a substituting ethyl 3-amino-6,6-dimethyl-4-heptynoate (69) for D,L-3-amino-3-phenylpropionic acid (66). The product was purified by reverse phase HPLC and freeze-dried to afford 57e as a white solid: ¹H NMR (CD₃OD) δ 1.18 (s, 9H), 1.24 (t, J = 6 Hz, CH₃), 2.54–2.77 (m, 6H), 4.12 (q, J = 6 Hz, CH₂), 4.95–5.09 (m, CHN), 7.75–7.85 (m, ArH), 8.85 and 9.10 (2bs, 2-NH₂); ¹³C NMR (CD₃OD) δ 13.7, 30.4, 32.0, 38.6, 41.2, 61.0, 76.7, 92.7, 119.7, 122.8, 129.0, 144.7, 166.7, 170.7, 172.5; FAB MS (MH⁻ = 413).

3-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-6,6-dimethyl-4-heptynoic Acid (57a). 57a was prepared by treating 57e with porcine liver esterase in a manner similar to that of 46a. The product was purified by reverse phase HPLC and freeze-dried to afford 57a as a white solid: ¹H NMR (CD₃OD) δ 1.18 (s, 9H), 2.53–2.75 (m, 6H), 4.96–5.05 (m, CHN), 7.74–7.84 (m, ArH), 8.75 and 9.12 (2bs, 2-NH₂); ¹³C NMR (CD₃OD) δ 30.4, 30.5, 32.0, 38.6, 41.0, 76.9, 93.1, 119.8, 122.4, 129.1, 144.8, 166.7, 172.6, 172.6. Anal. (C₂₀H₂₆N₄O₄ plus 1.6CF₃CO₂H and 1.0H₂O) C, N; H: calcd, 5.08; found, 4.57.

Ethyl 3-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-5-(trimethylsilyl)-4-pentynoate (58e).
β**-Lactam 10.** To a solution of (trimethylsilyl)acetylene (7.77 mL, 54.97 mmol) and THF (100 mL) at −78 °C was added *n*-butyllithium (34.37 mL of a 1.6 M solution in hexane) over 5 min followed by warming to 0 °C for 1 h. After cooling to −78 °C, 4-(benzoyloxy)-2-azetidinone (5.25 g, 27.48 mmol) was added as a THF solution (50 mL) over 3 min. After 10 min at −78 °C, the reaction mixture was warmed to 0 °C for 0.75 h followed by quenching the reaction with 1 N KHSO₄ to a pH of 4. The reaction mixture was extracted with ether (2 × 200 mL), washed with KHCO₃ (1 × 100 mL), and dried (Na₂SO₄). After concentration *in vacuo*, the reaction mixture was purified by flash chromatography (ethyl acetate:hexane, 2:3) to afford 10 as an oil (3.40 g, 73%): ¹H NMR (CDCl₃) δ −0.03 (s, 9H, TMS), 2.83−3.27 (m, 2H), 4.03−4.14 (m, 1H), 6.48 (bs, NH); ¹³C NMR (CD₃OD) δ 0.0, 37.8, 47.0, 90.5, 103.4, 167.0.

To a solution of β-lactam 10 (5.30 g, 31.7 mmol) and ethanol (150 mL) at 0 °C was added anhydrous HCl in ethanol (saturated, 30 mL). After 1.5 h at 0 °C, the reaction mixture was concentrated *in vacuo* to afford 11 as an oil (7.90 g, 100%): ¹H NMR (CDCl₃) δ −0.03 (s, 9H, TMS), 1.11 (t, J = 7 Hz, CH₃), 2.67−2.86 (m, 2H), 4.04 (q, J = 7 Hz, CH₂), 4.03−4.14 (m, 1H), 4.28−4.36 (m, 1H); ¹³C NMR (CD₃OD) δ −2.3, 12.7, 37.0, 39.4, 60.7, 93.5, 98.2, 168.9.

58e was prepared in a manner similar to that of 44a substituting ethyl 5-(trimethylsilyl)-4-pentynoate for D,L-3-amino-3-phenylpropionic acid (66). The product was purified by reverse phase HPLC and freeze-dried to afford 58e as a white solid: ¹H NMR (CD₃OD) δ −0.03 (s, 9H, TMS), 1.03 (t, J = 7 Hz, CH₃), 2.43−2.67 (m, 6H), 4.01 (q, J = 7 Hz, CH₂), 4.89−4.98 (m, CHN), 7.61−7.72 (m, ArH), 8.72 and 8.98 (2bs, 2-NH₂); ¹³C NMR (CD₃OD) δ −1.5, 13.2, 30.0, 31.4, 38.6, 40.1, 60.6, 87.0, 103.3, 119.2, 122.0, 128.6, 144.2, 166.1, 170.0, 172.0, 172.1; FAB MS (MH⁺ = 431). Anal. (C₂₁H₃₀N₄O₄Si·1.3CF₃·CO₂H) C, H, N.

3-[[4-[[4-(Aminoinomethyl)phenyl]amino]-1,4-dioxobutyl]aminol]-5-(trimethylsilyl)-4-pentynoic Acid (58a). 58a was prepared by treating 58e with porcine liver esterase in a manner similar to that of 46a. The product was purified by reverse phase HPLC and freeze-dried to afford 58a as a white solid: ¹H NMR (CD₃OD) δ −0.03 (s, 9H, TMS), 2.43−2.67 (m, 6H), 4.89−4.98 (m, CHN), 7.61−7.72 (m, ArH), 8.65 and 8.99 (2bs, 2-NH₂); ¹³C NMR (CD₃OD) δ −0.6, 30.8, 32.3, 39.4, 40.7, 87.6, 104.5, 120.1, 123.0, 129.5, 145.2, 166.2, 172.8, 172.9, 173.0; FAB MS (MH⁺ = 403). Anal. (C₁₉H₂₆N₄O₄Si·1.4CF₃·CO₂H) C, H, N.

3-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-6-methoxy-5-hexynoic Acid (59a). 59e was prepared in a manner similar to that of 44a substituting ethyl 3-amino-6-methoxy-4-hexynoate (70) for D,L-3-amino-3-phenylpropionic acid (66). The product was purified by reverse phase HPLC and freeze-dried to afford 59 as a white solid: ¹H NMR (CD₃OD) δ 1.25 (t, J = 6.5 Hz, CH₃), 2.54−2.78 (m, 6H), 3.32 (s, OCH₃), 4.08 (d, J = 2.5 Hz, CH₂), 4.14 (q, J = 6.5 Hz, CH₂), 5.05−5.14 (m, CHN), 7.73−7.84 (m, ArH), 8.82 and 9.13 (2bs, 2-NH₂).

59a was prepared by treating 59e with porcine liver esterase in a manner similar to that of 46a. The product was purified by reverse phase HPLC and freeze-dried to afford 59a as a white solid: ¹H NMR (CD₃OD) δ 2.54−2.81 (m, 6H), 3.32 (s, OCH₃), 4.08 (d, J = 2.5 Hz, CH₂), 5.05−5.14 (m, CHN), 7.73−7.84 (m, ArH), 8.78 and 9.08 (2bs, 2-NH₂); ¹³C NMR (CD₃OD) δ 29.1, 30.6, 37.2, 38.7, 55.5, 58.2, 77.4, 83.5, 118.4, 121.3, 127.8, 143.4, 165.6, 171.1, 171.2, 171.4. Anal. (C₁₈H₂₂N₄O₅ plus 1.1CF₃CO₂H and 0.65H₂O) C, H, N.

Ethyl 3-(S)-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-pentenoate (60a). Section A. (±)-Ethyl 3-Amino-4-pentenoate Hydrochloride (23). A solution of 69.79 g (0.719 mol) of 4-vinyl-2-azetidinone[16] (22) (bp 76−78 °C/0.6 mmHg) in 500 mL of absolute ethanol was chilled in an ice−salt bath, and hydrogen chloride was bubbled in, keeping the temperature below 20 °C. After 2 h at room temperature, the solution was concentrated, and the residue was triturated with ether to yield 23 as a white solid (123.0 g, 95%): mp 84−85 °C; ¹H NMR (CDCl₃) δ 1.26 (3H, t, J = 7

Hz), 2.90 (1H, dd, J = 17, 7 Hz), 3.07 (1H, dd, J = 17, 6 Hz), 4.19 (2H, q, J = 7 Hz), 4.30 (1H, td, J = 7, 6 Hz), 5.48 (1H, dd, J = 11, 1 Hz), 5.56 (1H, dd, J = 17.5, 1 Hz), 6.05 (1H, ddd, J = 17.5, 11, 7 Hz), 8.65 (3H, bs); ¹³C NMR (CDCl₃) δ 14.0, 36.9, 50.4, 61.2, 121.0, 132.2, 169.8. Anal. (C₇H₁₄NO₂Cl) C, H, N, Cl.

Section B. (±)-3-[(*tert*-Butoxycarbonyl)amino]-4-pentenoic Acid (24). A solution of 50.64 g (0.282 mol) of (±)-ethyl 3-amino-4-pentenoate (23) in 200 mL of 6 N HCl was stirred at 24 °C for 4 h and then concentrated *in vacuo*. After redissolving in 200 mL of water, the mixture was again concentrated *in vacuo*. The residue was dissolved in 50 mL of water, and the solution was chilled to 10 °C and brought to pH 10 with 1 N NaOH. After the addition of 100 mL of *tert*-butyl alcohol, 70 mL (66.5 g, 0.30 mol) of di-*tert*-butyl dicarbonate was added in portions while maintaining the pH at 9−9.5 with 1 N NaOH. The cooling bath was removed, and the mixture was stirred for 2 h at 24 °C. After being extracted with ether (2 × 200 mL), the aqueous layer was acidified with 2 N KHSO₄, extracted with ethyl acetate (3 × 200 mL), washed with brine (2 × 200 mL), and dried (Na₂SO₄). After concentration *in vacuo*, the residue was crystallized from ether−hexane to provide 55.15 g (91%) of 24: mp 87−88 °C; ¹H NMR (CDCl₃) δ 1.45 (9H, bs), 2.64 (2H, bs), 4.51 (1H, bs), 5.14 (1H, dt, J = 11, 1 Hz), 5.22 (1H, dt, J = 17.5, 1 Hz), 5.38 (1H, bs), 6.86 (1H, ddd, J = 17.5, 11, 6 Hz); ¹³C NMR (CDCl₃) δ 28.2, 39.0, 49.0, 79.7, 115.5, 136.8, 155.2, 176.1.

Section C. (S)-3-[(*tert*-Butoxycarbonyl)amino]-4-pentenoic Acid (+)-Ephedrine Salt (25). To a chilled (10 °C) solution of 80 g of potassium carbonate in 400 mL of water were added 51 g (0.253 mol) of (1S,2R)-ephedrine hydrochloride and 600 mL of ethyl acetate. After shaking until dissolution was complete, the organic layer was separated and the aqueous layer extracted with ethyl acetate (400 mL). The combined organics were washed with brine (2 × 100 mL) and dried (Na₂SO₄). The solution of (1S,2R)-ephedrine was added to 68.37 g (0.317 mol) of (±)-3-[(*tert*-butyoxycarbonyl)amino]-4-pentenoic acid (25). After distilling part of the solvent to assure dryness, the mixture was diluted to a total volume of 1400 mL of ethyl acetate and then subsequently diluted further with hexane (600 mL). After 48 h at 23 °C, a precipitate had formed. The mixture was chilled at 5 °C for an additional 24 h, and then the precipitate was filtered, washed with ether (2 × 200 mL), and collected (45.6 g, mp 112−125 °C). The solid was recrystallized from 2 L of EtOAc to give 38.7 g (mp 126−129 °C). A second recrystallization afforded 25 (36.89 g, 42.7% yield based on recovered racemate) as a white powder: mp 128−130 °C, [α]₀ +36.0° (c 0.943, MeOH). Anal. (C₂₀H₃₂N₂O₅) C, H, N.

(S)-3-[(*tert*-Butoxycarbonyl)amino]-4-pentenoic Acid (71). To a suspension of 36.89 g of the above (+)-ephedrine salt in 400 mL of ethyl acetate was added 150 mL of cold 1 N HCl. After mixing until solution was complete, the organic layer was separated and washed with water (1 × 100 mL) and brine (1 × 100 mL). After drying (Na₂SO₄), the solvent was evaporated, and the residue was crystallized from ether−hexane to afford 71 as a white solid (20.48 g, 98%): mp 69−70 °C, [α]₀ +27.2° (c 1.023, CH₂Cl₂). Anal. (C₁₀H₁₇NO₄) C, H, N.

Ethyl (S)-3-Amino-4-pentenoate Hydrochloride (72). To 200 mL of absolute ethanol at −30 °C was added 11 mL (17.9 g, 0.15 mol) of thionyl chloride at a rate to keep the temperature below 0 °C. Then 20.48 g (95 mmol) of the above BOC acid 71 was added. The mixture was allowed to warm to room temperature, during which time solution occurred. The mixture was warmed to achieve reflux for 1 h and then concentrated. The residue was crystallized from ethyl acetate to afford 72 as a white solid (16.24 g, 95%): mp 106−107 °C, [α]₀ −7.1° (c 0.992, MeOH). Analysis of the β-amino ester by chiral HPLC using a crownpak ether column (CR-(+)) cooled to 5 °C using methanol:water (10:90) at pH at 1 (HClO₄) and a flow rate of 0.5 mL/min showed an enantiomeric ratio of 100:0: ¹H NMR (CDCl₃) δ 1.28 (t, J = 7 Hz, CH₃), 2.74−2.93 (m, CH₂), 4.13−4.25 (q, J = 7 Hz, CH₂), 5.37−5.54 (m, CH₂), 5.83−6.05 (m, CH); ¹³C NMR (CDCl₃) δ 14.9, 37.7, 51.4, 62.2, 121.9, 133.1, 171.0. Anal. (C₇H₁₄NO₂Cl) C, H, N, Cl.

Ethyl 3-(S)-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-pentenoate (60e). 60e was prepared in a manner similar to that of 44a substituting ethyl (S)-3-amino-4-pentenoate hydrochloride (72) for D,L-3-amino-3-phenylpropionic acid (66). The product was purified by reverse phase HPLC and freeze-dried to afford 60e as a white solid, and it was identical to the racemic 54e on the basis of ^{1}H and ^{13}C NMR. TFA salt of 60e: $[\alpha]_D$ +4.6° (c 1.03, MeOH). Anal. ($C_{18}H_{24}N_4O_4$ plus $1.1CF_3CO_2H$ and $0.65H_2O$) C, H, N.

3-(S)-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-pentenoic Acid (60a). 60a was prepared by treating 60e with porcine liver esterase in a manner similar to that of 46a. The product was purified by reverse phase HPLC and freeze-dried to afford 60a. 60a had identical ^{1}H and ^{13}C NMR to the racemic material 54a. Anal. ($C_{16}H_{20}N_4O_5$ plus 1.1HCl and $1.2H_2O$) C, N, Cl; H: calcd, 5.99; found, 5.52.

Ethyl 3-(S)-[[5-[4-(Aminoiminomethyl)phenyl]-4-(E)-pentenoyl]amino]-4-pentenoate (61e). 61e was prepared in a manner similar to that of 44a substituting ethyl (S)-3-amino-4-pentenoate hydrochloride (72) for D,L-3-amino-3-phenylpropionic acid (66) and 5-[4-(aminoiminomethyl)phenyl]-4-(E)-pentenoic acid for 4-[[4-(aminoiminomethyl)phenyl]amino]-4-oxobutanoic acid (7). The 5-[4-(aminoiminomethyl)phenyl]-4-(E)-pentenoic acid was prepared from the previously described benzonitrile, 5-(4-cyanophenyl)-4-(E)-pentenoic acid,[3a] in a manner similar to that of compound 30. The product was purified by reverse phase HPLC and freeze-dried to afford 61e: ^{1}H NMR (CDCl$_3$) δ 1.15 (t, J = 7 Hz, CH$_3$), 2.25–2.58 (m, 3-CH$_2$), 4.05 (q, J = 7 Hz, CH$_2$), 4.63–4.74 (m, CHN), 5.03–5.14 (m, =CH$_2$), 5.73–5.87 (m, =CH), 6.46–6.63 (m, =CH), 7.61 (d, J = 6 Hz, ArH), 7.81 (d, J = 6 Hz, ArH), 8.07 (d, J = 6 Hz, NH), 9.28 (bs, NH$_2$), 9.44 (bs, NH$_2$); ^{13}C NMR (CDCl$_3$) δ 14.0, 28.6, 34.6, 47.6, 59.9, 114.6, 125.9, 128.4, 128.6, 133.7, 137.7, 142.6, 165.2, 170.2, 170.5. Anal. ($C_{19}H_{25}N_3O_3$ plus $1.0CF_3CO_2H$) C, H, N.

61a was prepared by treating 61e with porcine liver esterase in a manner similar to that of 46a. The product was purified by reverse phase HPLC and freeze-dried to afford 61a: ^{1}H NMR (CDCl$_3$) δ 2.27–2.58 (m, 3-CH$_2$), 4.60–4.74 (m, CHN), 4.97–5.14 (m, =CH$_2$), 5.75–5.87 (m, =CH), 6.52–6.63 (m, =CH), 7.55–7.65 (m, ArH), 7.82 (d, J = 6 Hz, ArH), 8.09 (d, J = 6 Hz, NH), 9.35 (bs, NH$_2$), 9.59 (bs, NH$_2$); ^{13}C NMR (CDCl$_3$) δ 27.9, 33.0, 47.6, 114.3, 126.1, 128.4, 128.6, 133.4, 133.8, 138.0, 142.5, 142.6, 165.3, 170.5, 173.7. Anal. ($C_{17}H_{21}N_3O_3$ plus 1.25HCl and $0.5H_2O$) C, H, N, Cl.

Ethyl 3-(R)-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-pentenoate (73e). Ethyl 3-(R)-amino-4-pentenoate hydrochloride (74) was obtained in a manner similar to that of ethyl 3-(S)-amino-4-pentenoate hydrochloride (72) using (1R,2S)-ephedrine in place of (1S,2R)-ephedrine hydrochloride in section C (60e): mp 106–107 °C; $[\alpha]_D$ +8.1° (c 1.129, MeOH). 73e was prepared in a manner similar to that of 44a substituting ethyl 3-(R)-amino-4-pentenoate hydrochloride (74) for D,L-3-amino-3-phenylpropionic acid (66). The product was purified by reverse phase HPLC and freeze-dried to afford 73e as a white solid which had identical ^{1}H and ^{13}C NMR to the racemic. 73e (TFA salt): mp 206–208 °C; $[\alpha]_D$ –4.4° (c 0.999, MeOH). Anal. ($C_{18}H_{24}N_4O_4$·1.0 CF_3CO_2H) C, H, N.

3-(R)-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-pentenoic Acid (73a). 73a was prepared by treating 73e with porcine liver esterase in a manner similar to that of 46a. The product was purified by reverse phase HPLC and freeze-dried to afford 73a. 73a had identical ^{1}H and ^{13}C NMR to the racemic material 54a. Anal. ($C_{16}H_{20}N_4O_4$ plus HCl and $0.5H_2O$) C, H, N; Cl: calcd, 9.38; found, 8.91.

(3S)-Ethyl 3-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-pentynoate (2e). **(S)-Ethyl 3-Amino-4-pentynoate (17).** To a methylene chloride (25 mL) solution of acid chloride 12 (7.83 mmol, prepared from 1.30 g of O-methylmandelic acid by treatment with 35 mL of SOCl$_2$ at 23 °C for 20 h followed by concentration *in vacuo* at 23 °C) was added the β-amino ester hydrochloride 11 (1.50 g, 6.02 mmol) in 10 mL of water followed immediately by 1 N

NaOH (10 mL). After 24 h at 23 °C, the reaction mixture was diluted with methylene chloride (125 mL), washed with 1 N HCl (3 × 75 mL), saturated potassium bicarbonate (3 × 75 mL), and brine (1 × 75 mL), and dried (MgSO$_4$). After concentration *in vacuo*, the residue was separated by MPLC (1:1 ether:hexane) to afford 751 mg (53.2%) of 13 as an oil and 835 mg (59.2%) of 14 as an oil. 13: ^{1}H NMR (CDCl$_3$) δ 0.20 (s, 9H, TMS), 1.25 (t, J = 7 Hz, CH$_3$), 2.57–2.78 (m, CH$_2$), 3.42 (s, CH$_3$O), 4.09–4.25 (m, CH$_2$O), 5.10–5.31 (m, CHN), 7.33–7.53 (m, 5H, Ph); $[\alpha]_D$ –38.9° (c 0.914, CHCl$_3$).

14: ^{1}H NMR (CDCl$_3$) δ 0.20 (s, 9H, TMS), 1.30 (t, J = 7 Hz, CH$_3$), 2.71–2.90 (m, CH$_2$), 3.42 (s, CH$_3$O), 4.20–4.35 (m, CH$_2$O), 5.10–5.31 (m, CHN), 7.33–7.63 (m, 5H, Ph); $[\alpha]_D$ –153.8° (c 0.969, CHCl$_3$).

To a solution of 14 (835 mg, 2.31 mmol) and acetonitrile (18 mL) was added di-*tert*-butyl dicarbonate (5.05 g, 23.1 mmol) followed by 4-(dimethylamino)pyridine (342 mg, 2.80 mmol) at 23 °C under argon. After 24 h, an additional 2.0 g of di-*tert*-butyl dicarbonate and 200 mg of 4-(dimethylamino)-pyridine were added. After 48 h, the reaction mixture was concentrated *in vacuo*, diluted with ether (350 mL), washed with 1 N KHSO$_4$ (5 × 75 mL), saturated bicarbonate (5 × 75 mL), and brine (3 × 75 mL), dried (MgSO$_4$), and concentrated *in vacuo*. The residue was dissolved in methanol (10 mL) and treated with tetramethylguanidine (320 mg, 2.78 mmol) for 1.5 h at 23 °C. After concentration *in vacuo*, the residue was diluted with ether (350 mL), washed with 1 N KHSO$_4$ (5 × 75 mL), saturated bicarbonate (5 × 75 mL), and brine (3 × 75 mL), dried (MgSO$_4$), and concentrated *in vacuo*. The residue was dissolved in methylene chloride (15 mL), and cooled to 0 °C followed by dropwise addition of TFA (7.5 mL) over 10 min. The reaction mixture was allowed to warm to 23 °C for 1 h. The reaction mixture was diluted with 1 N HCl (75 mL), and the organic layer was separated from the aqueous layer. The aqueous layer was extracted with ether (2 × 75 mL), and then the aqueous layer was concentrated *in vacuo* to afford the methyl ester 75 (230 mg, 41.3%) which was quantitatively exchanged to the ethyl ester by treatment with anhydrous HCl in ethanol. Concentration *in vacuo* afforded 17 as the hydrochloride salt: ^{1}H NMR (CDCl$_3$) δ 1.31 (t, J = 7 Hz, CH$_3$), 2.96–3.18 (m, CH$_2$), 3.18 (d, J = 1.5 Hz, CH), 4.23 (q, J = 7 Hz, CH$_2$), 4.48–4.58 (m, CHN), 4.91 (bs, NH$_3$); ^{13}C NMR (CDCl$_3$) δ 13.5, 37.5, 39.3, 57.5, 61.5, 77.0, 169.2; $[\alpha]_D$ –3.4° (c 0.705, CH$_3$OH).

2e was prepared in a manner similar to that of 44a substituting (S)-ethyl 3-amino-4-pentynoate (17) for D,L-3-amino-3-phenylpropionic acid (66). The product was purified by reverse phase HPLC to afford the TFA salt and freeze-dried with 3 equiv of 1 N HCl to afford 2e as the HCl salt. The product had the same NMR as 56e. The ratio of enantiomers was determined to be 98:2 by chiral HPLC using an AGP protein column with a flow rate of 1.0 mL/min and a mobile phase of 0.01 M KH$_2$PO$_4$ (pH 7.0) and 1 mM N,N-dimethyloctylamine. Anal. ($C_{18}H_{22}N_4O_4$ plus $0.2CF_3CO_2H$, 0.8HCl, and $1.0H_2O$) C, H; H: calcd, 5.88; found, 5.45.

(3S)-3-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino-4-pentynoic Acid (2a). 2a was prepared by treating 2e with porcine liver esterase in a manner similar to that of 46a. The product was purified by reverse phase HPLC to afford the TFA salt and freeze-dried with 3 equiv of 1 N HCl to afford 2a. The product had the same NMR as 56a. HCl salt: $[\alpha]_D$ –33.7 (c 1.45, CH$_3$OH). Anal. ($C_{16}H_{18}N_4O_4$ plus 1.85HCl and $0.95H_2O$) C, H, N; Cl: calcd, 16.20; found, 15.68.

(3R)-Ethyl 3-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-pentynoate (77e). (R)-Ethyl 3-amino-4-pentynoate (76) was prepared in a manner similar to that of (S)-ethyl 3-amino-4-pentynoate by subjecting 13 to the same reaction sequence (61.9% yield from 13). (R)-Ethyl 3-amino-4-pentynoate hydrochloride salt (76): $[\alpha]_D$ +3.2° (c 0.124, CH$_3$OH).

77e was prepared in a manner similar to that of 44a substituting (R)-ethyl 3-amino-4-pentynoate (76) for D,L-3-amino-3-phenylpropionic acid (66). The product was purified by reverse phase HPLC to afford the TFA salt and freeze-dried with 3 equiv of 1 N HCl to afford 77e as the HCl salt. The product had the same NMR as 52e. The ratio of enantiomers

was determined to be 98:2 by chiral HPLC using an AGP protein column with a flow rate of 1.0 mL/min and a mobile phase of 0.01 M KH$_2$PO$_4$ (pH 7.0) and 1 mM *N,N*-dimethyloctylamine.

(3*R*)-3-[[4-[[4-(Aminoiminomethyl)phenyl]amino]-1,4-dioxobutyl]amino]-4-pentynoic Acid (77a). 77a was prepared by treating 77e with porcine liver esterase in a manner similar to that of 46a. The product was purified by reverse phase HPLC to afford the TFA salt and freeze dried with 3 equiv of 1 N HCl to afford 77a. The product had the same ^{1}H and ^{13}C NMR as 56a.

Supplementary Material Available: Radiochemical synthesis of 2e/2a and chromatograms (14 pages). Ordering information is given on any current masthead page.

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(22) (a) The absolute configuration of **2e/2a** has been confirmed to be *S* by an independent synthesis. (b) The opposite antipodes of **2a, 60a, 62a,** and **63a** were in general found to be 100 times less active than the *S*-antipode which is consistant with the configuration corresponding to the natural configuration of aspartic acid providing the predominant activity. The antipodes had the following activity in the PRP assay: ethynyl derivative **77a** had an $IC_{50} = 4510 \pm 270$ nM (contained 1% *S*-enantiomer by chiral HPLC), the vinyl derivative **73a** had an $IC_{50} > 10\,000$ nM, the antipode of **62a** (ref 11) had an $IC_{50} = 6800$ nM, and the antipode of **63a** had an $IC_{50} = 6410 \pm 920$ nM. Therefore, we expected an approximate doubling in activity upon comparing the enantiomerically enriched *S*-derivative with the racemic compound which was observed for **49a/62a** and **51a/63a**; however, the vinyl racemic/enantiomerically enriched derivatives **54a/60a** have similar potencies, and the enantiomerically enriched ethynyl **2a** is 3.8 times more active than the racemic **56a**. Although difficult to explain, there are several factors which may contribute to these anomalies including the following: slow on rates of the inhibitors, variable structure dependent protein binding, differential ligand-induced activation of the receptor, or experimental error. All attempts to safeguard against the latter have been made.

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