Discovery of an Orally Active Series of Isoxazoline Glycoprotein IIb/IIIa Antagonists

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Using isoxazoline XR299 (**1a**) as a starting point for the design of highly potent, long-duration GPIIb/IIIa antagonists, the effect of placing lipophilic substituents at positions α and β to the carboxylate moiety was evaluated. Of the compounds studied, it was found that the *n*-butyl carbamate **24u**[1,2] exhibited superior potency and duration of *ex vivo* antiplatelet effects in dogs. Replacement of the benzamidin-4-yl moiety with alternative basic groups, elimination of the isoxazoline stereocenter, and reversal of the orientation of the isoxazoline ring resulted in lowered potency and/or duration of action.

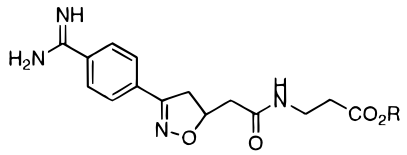
Introduction

Platelet adhesion and aggregation are important events in hemostasis and in the pathophysiology of thrombosis.[3] Uncontrolled deposition of platelets on thrombogenic surfaces may lead to the occlusion of vessels,[4−6] a condition associated with numerous pathophysiological conditions, such as acute myocardial infarction and unstable angina, or the ischemic complications of coronary intervention and stroke.

Platelet glycoprotein IIb/IIIa (GPIIb/IIIa, $\alpha_{IIb}\beta_3$) is a member of the integrin family of adhesive proteins. Platelets are activated by a wide variety of agonists, including adenosine diphosphate (ADP), serotonin, arachidonic acid, thrombin, and collagen. Agonist activation causes a morphological change in platelets, placing the GPIIb/IIIa receptors in a conformation having a high affinity for the binding of fibrinogen. The binding of fibrinogen to the activated form of GPIIb/IIIa is both a necessary and sufficient event that mediates the process of platelet aggregation.[7,8] Currently prescribed antiplatelet drugs, such as aspirin and ticlopidine, inhibit only one agonistic pathway and are therefore of limited efficacy. To address this issue, a number of parenterally active GPIIb/IIIa antagonists, such as c7E3-Fab[9] and Integrelin,[10] among others,[11,12] are undergoing development for use in acute settings. Antagonism of GPIIb/IIIa with an orally acting agent represents an attractive therapy for chronic treatment of arterial thrombosis.

Currently, the nature of the interaction of fibrinogen with GPIIb/IIIa is a point of some debate. It has been proposed that the binding of human fibrinogen to GPIIb/IIIa is mediated through two RGD motifs located on the fibrinogen α chain.[13,14] This belief is supported by the fact that many small RGD-containing linear and cyclic peptides antagonize the binding of fibrinogen to the receptor. A second proposal states that this interaction is mediated through the fibrinogen γ chain. Supporting this proposal is the essential role of residues 401−411 of the γ chain (denoted a platelet receptor recognition domain) in mediating aggregation[15,16] and the lack of a functional significance of the two RGD sequences in the α chain.[17]

We recently described the discovery of XR299 (**1a**),[18] a novel and selective isoxazoline-containing GPIIb/IIIa receptor antagonist. Upon chiral resolution of XR299, the (*R*)-enantiomer was found to be a potent antagonist of GPIIb/IIIa (PRP $IC_{50} = 0.06\ \mu M$).[18] In addition, the ethyl ester prodrug of XR299, XR300 (**1b**), provided substantial inhibition of *ex vivo* platelet aggregation for several hours when administered orally to dogs at a dose of 1 mg/kg. In this report, we describe efforts to further increase the potency and duration of action of XR299/XR300. These studies describe improved methods for the synthesis of analogs, the effect of replacing the benzamidin-4-yl moiety with alternative basic groups, and the pharmacological effects of incorporating substituents at a position α[19,20] or β[21] to the acidic group.



1a: R = H
1b: R = Et

In this regard the variety of hydrophobic groups appended to the RGD-mimetic backbone of known fibrinogen receptor antagonists was noted. DMP 728,[22] with a hydrophobic linker group, and the phenethyl-substituted SB 208651 (**2**)[23] feature centrally located hydrophobic regions. The tyrosine-based GPIIb/IIIa antagonist MK-383 (**3**) demonstrated the ability of GPIIb/IIIa to interact with hydrophobic groups near the carboxy terminus of nonpeptide antagonists through a proposed receptor "exosite".[19] Several GPIIb/IIIa antagonists reported in the literature, such as SC 54684 (**4**) and related compounds,[24] the hydantoin S1752 (**5**),[25]

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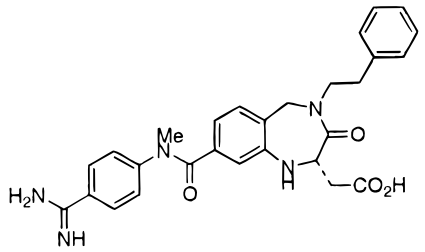
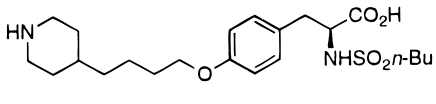
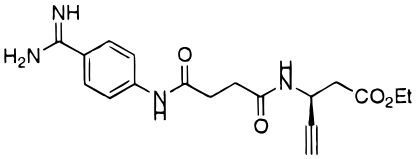
and the linear peptide RGDV,[26] feature hydrophobic β-substituents.



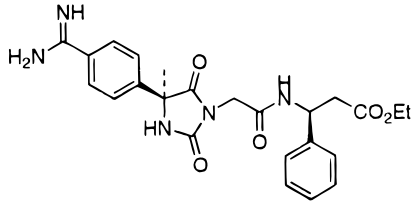
SB 208651, **2**



MK-383, **3**



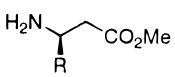
SC-54684, **4**



S 1762, **5**

Chemistry

Synthesis of Substituted β-Alanines. Syntheses of the β-formamido-β-alanines **6** were accomplished from (*tert*-butyloxycarbonyl)-L-aspartic acid β-methyl ester (**7**) by coupling to an amine followed by Boc deprotection (Scheme 1). Syntheses of scalemic β-aryl- and β-alkyl-β-alanines **8** were accomplished using the method of Davies.[27]



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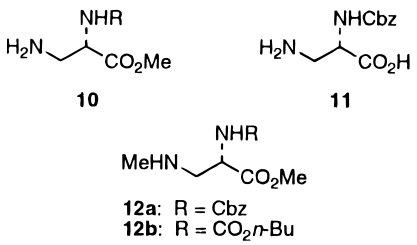
The preparation of the scalemic β-aminomethyl-β-alanines **9** was accomplished from **7** according to Scheme 2. Amide formation using a secondary amine, followed by a borane-mediated reduction and subsequent Boc cleavage, gave the desired β-amino esters.

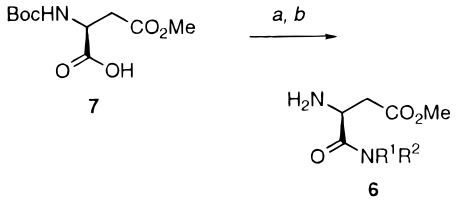
The diaminopropionates **10** were prepared as described from N^2-(benzyloxycarbonyl)-L-2,3-diaminopropionic acid (**11**).[28] N^3-Methylated versions of the N^2-Cbz and N^2-*n*-butyloxycarbonyl diaminopropionates (**12a** and **12b**, respectively) were prepared using the



10 **11**

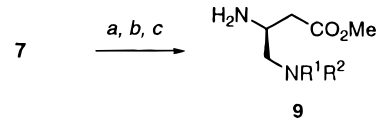
12a: R = Cbz
12b: R = CO_2*n*-Bu

Scheme 1. Preparation of Aspartic Acid β-Amides[a]



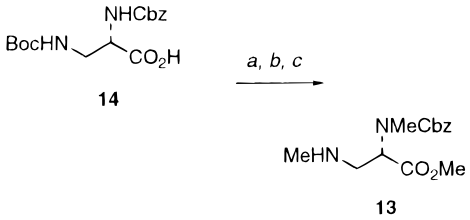
[a] Reagents: (a) NHR^1R^2, Et_3N, TBTU, EtOAc; (b) 4 M HCl−dioxane.

Scheme 2. Synthesis of β-(Aminomethyl)-β-alanines[a]



[a] Reagents: (a) NHR^1R^2, Et_3N, TBTU, EtOAc; (b) BH_3−THF; (c) 4 M HCl−dioxane.

Scheme 3. *N*-Methylation of Diaminopropionate **14**[a]

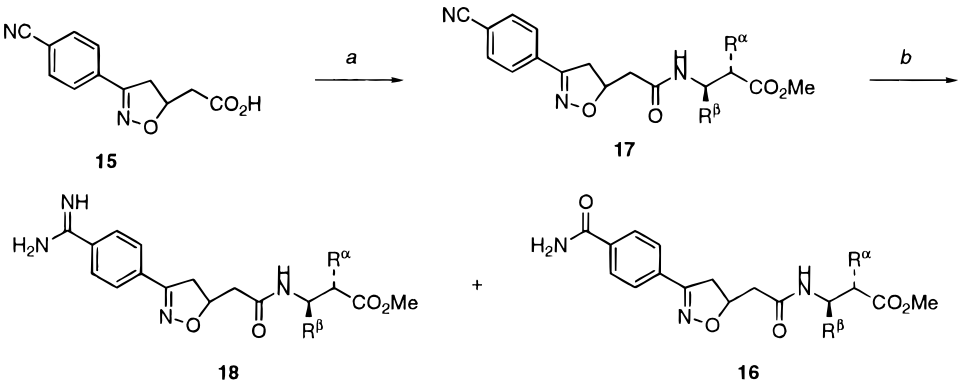


[a] Reagents: (a) NaH, MeI, THF; (b) *p*-TsOH, MeOH; (c) TFA, CH_2Cl_2.

method of Ohfune.[29] The N^2,N^3-dimethylated N^2-(benzyloxycarbonyl)diaminopropionate **13** was prepared from N^2-(benzyloxycarbonyl)-N^3-(*tert*-butyloxycarbonyl)-L-2,3-diaminopropionic acid (**14**) *via* permethylation using sodium hydride/methyl iodide in DMF followed by esterification and cleavage of the Boc protecting group (Scheme 3).

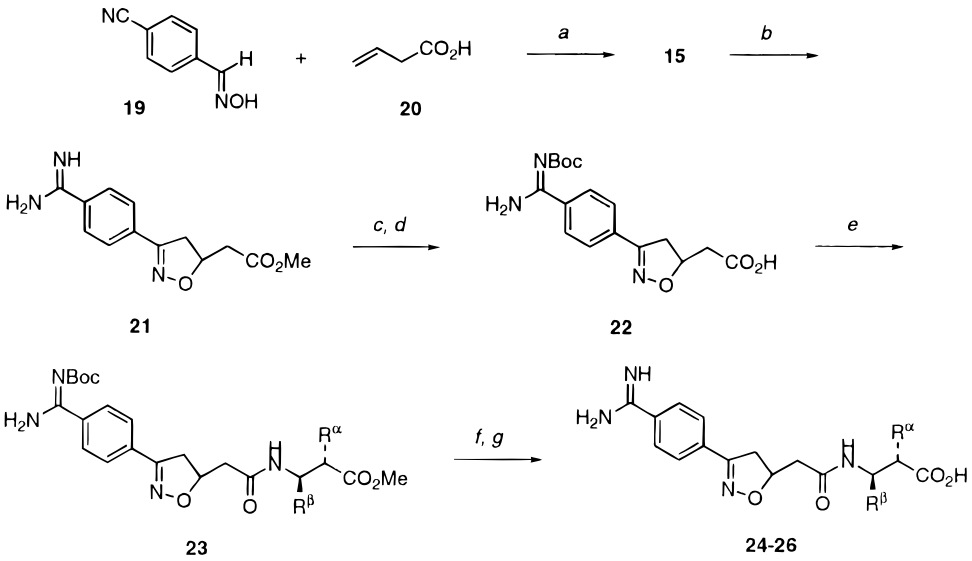
Synthesis of XR299 Analogs. Initially, the synthesis of XR299 analogs involved coupling of nitrile acid **15**[18] with amines **6**, **8**, **9**, or **10** (Scheme 4). However, due to the formation of variable amounts of amide **16** that was observed in the conversion of nitrile **17** to amidine **18**, an improved method of synthesis was developed (Scheme 5). Nitrile acid **15** was prepared from the cycloaddition of oxime **19** with vinylacetic acid (**20**) in the presence of sodium hypochlorite solution (Clorox). As compared to our prior synthesis of **15**, this method of preparation held the advantages of being a single step, scaleable procedure that afforded a crystalline product, greatly easing purification. Conversion of the nitrile to the imidate, followed by reaction with ammonia in methanol, smoothly afforded amidine ester **21** with very little amide formation. Protection of **21** as the Boc derivative, followed by careful saponification of the ester, gave protected amidine acid **22**. This material proved to be a key intermediate and was typically coupled to an optically active β-amino ester to give the amide **23** as a mixture of diastereomers. These diastereomeric mixtures were then carried through to the targets *via* deprotection of the amidine followed by hydrolysis of the ester to afford the α-substituted compounds **24** or the β-substituted compounds **25** and **26**. Due to the diverse functionality present, one of three methods of ester hydrolysis was used: saponifica-

Scheme 4. Early Method for Synthesis of Isoxazolinylacetamides[a]



[a] Reagents: (a) **6**, **8**, **9**, or **10**, TBTU, DMF, Et_3N; (b) HCl(anhyd), MeOH, 0 °C, then NH_3, MeOH, 0 °C.

Scheme 5. Convergent Method for Preparation of Isoxazolinylacetamides[a]



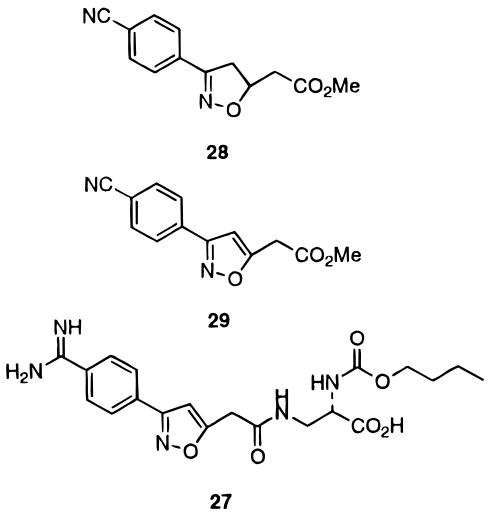
[a] Reagents: (a) Clorox, THF; (b) HCl(anhyd), MeOH, 0 °C, then NH_3, MeOH, 0 °C; (c) Boc_2O, Et_3N, DMF; (d) LiOH, MeOH−H_2O; (e) **6**, **8**, **9**, or **10**, TBTU, Et_3N, DMF; (f) TFA, CH_2Cl_2; (g) ester hydrolysis (see text).

tion using lithium hydroxide in aqueous methanol, acidic hydrolysis using aqueous 6 M HCl in dioxane or 40% concentrated HCl/formic acid, or esterase hydrolysis using rabbit liver esterase.

The isoxazole **27** was prepared by bromination of nitrile ester **28**[18] using NBS/AIBN followed by elimination (KOAc/HOAc) to afford **29**. Further processing of **29** in a fashion similar to **15** *via* Scheme 5 then gave the desired isoxazole.

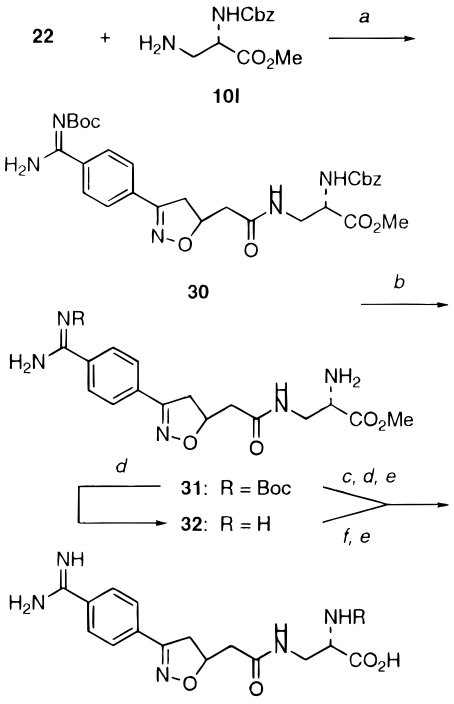
When a diaminopropionate was used as the carboxy-bearing terminus, the chemistry could be further optimized for the rapid synthesis of analogs as is illustrated in Scheme 6. Coupling of methyl N^2-(benzyloxycarbonyl)-L-2,3-diaminopropionate (**10l**) to **22** gave amide **30**. Hydrogenolysis of the Cbz group was accomplished in the presence of the isoxazoline in good yield using catalytic transfer hydrogenation to give amine **31**. Following derivatization of the α-amino moiety, deprotection of the amidine and ester hydrolysis afforded the target compounds **24**. Alternatively, hydrogenolysis and Boc cleavage of **30** gave **32**. Selective derivatization of the α-amino group and saponification then afforded **24**.

It was anticipated that the synthesis of single diastereomers could be accomplished starting with (*R*)-**15** using the route illustrated in Scheme 5. It was found that (*R*)-**15** could be conveniently prepared through a



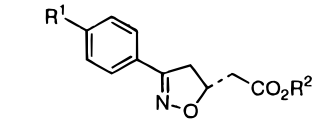
lipase-mediated hydrolysis of the racemic *n*-butyl ester (*R*,*S*)-**33**. Unreacted (*S*)-**33** was recycled through racemization using potassium *tert*-butoxide. Early attempts at the conversion of (*R*)-**15** to (*R*)-**22** under basic conditions indicated that the protection of amidine ester (*R*)-**34** would be problematic due to racemization. Additionally, protection of amidine acid (*R*)-**35** was hampered by poor solubility. Single diastereomers were successfully prepared from (*R*)-**15** using the route illustrated

Scheme 6. Selective Functionalization of the α-Amino Group[a]

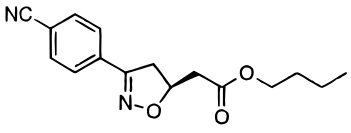


[a] Reagents: (a) TBTU, Et_3N, DMF; (b) 1,4-cyclohexadiene, 10% Pd/C, MeOH; (c) RSO_2Cl, RCOCl, etc., Et_3N, CH_2Cl_2; (d) TFA, CH_2Cl_2; (e) LiOH, THF(aq); (f) RSO_2Cl, RCOCl, etc., $NaHCO_3$, MeCN(aq).

in Scheme 4. Compounds prepared using this protocol were shown to be ≥99% of a single isomer.[30] This chemistry was also successfully combined with the improvements noted in Scheme 6 to provide an efficient entry to the synthesis of single diastereomers.



(*R*)-**15**: R^1 = CN, R^2 = H
(*R*)-**22**: R^1 = C(NBoc)NH_2, R^2 = H
(*R*)-**33**: R^1 = CN, R^2 = *n*-Bu
(*R*)-**34**: R^1 = C(NH)NH_2, R^2 = Me
(*R*)-**35**: R^1 = C(NH)NH_2, R^2 = H

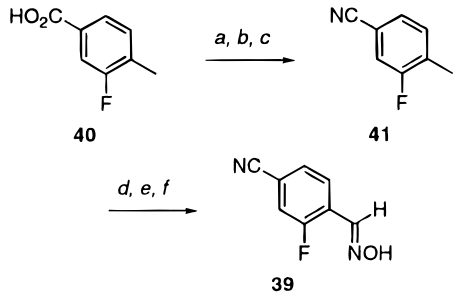


(*S*)-**33**

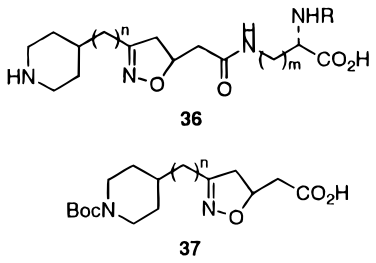
Synthesis of Targets Bearing Alternative Basic Moieties. A number of analogs containing a (piperidin-4-yl)alkyl moiety (**36**) were prepared from acids represented by **37**[18] using a route analogous to that depicted in Scheme 5.

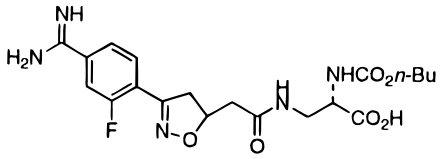
Synthesis of the fluoro-substituted benzamidine **38** was accomplished from oxime **39** using a route analogous to that depicted in Scheme 5. Oxime **39** was prepared from 3-fluoro-4-methylbenzoic acid **40** as shown in Scheme 7. Conversion of **40** to nitrile **41** was accomplished by reaction with thionyl chloride followed by ammonia and subsequent dehydration of the resulting amide. Bromination of **41**, followed by oxidation using trimethylamine *N*-oxide−DMSO[31] and oxime formation, completed the preparation of **39**.

Scheme 7. Preparation of Oxime **39**[a]



[a] Reagents: (a) $SOCl_2$, Δ; (b) NH_3(aq); (c) $ClCOCCl_3$, Et_3N, CH_2Cl_2; (d) NBS, CCl_4; (e) $Me_3NO{\cdot}2H_2O$, DMSO, CH_2Cl_2; (f) $NH_2OH{\cdot}HCl$, K_2CO_3, MeOH(aq), Δ.



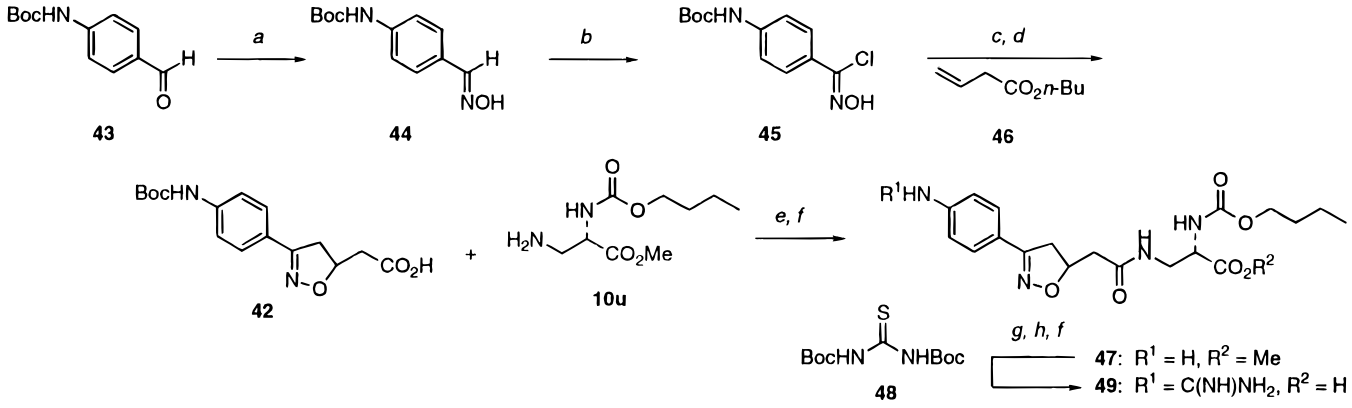


Replacement of the benzamidin-4-yl moiety with the *N*-(aminoiminomethyl)anilin-4-yl group required the preparation of Boc-protected aniline **42** as a key intermediate, as illustrated in Scheme 8. 4-[(*tert*-Butoxycarbonyl)amino]benzaldehyde (**43**) was sequentially converted to oxime **44** and oximinoyl chloride **45**.[32] Formation of the nitrile oxide under basic conditions in the presence of butyl vinylacetate (**46**) then afforded the isoxazoline in moderate yield. Saponification of the butyl ester followed by careful acidification with acetic acid afforded the Boc-anilin-4-ylisoxazolineacetic acid **42**. Coupling of **42** with amino ester **10u** and removal of the Boc protecting group afforded the aniline **47** as its TFA salt. Reaction of this intermediate with *N*,*N*′-bis(*tert*-butyloxycarbonyl)thiourea (**48**),[33] saponification of the ester, and removal of the Boc groups using TFA afforded the desired guanidine **49**.

The synthesis of the alkylguanidine **50** followed the protocol outlined below (Scheme 9). Starting with oximinoyl chloride **51**,[34] generation of the nitrile oxide *in situ* in the presence of butyl vinylacetate (**46**) and saponification of the ester gave acid **52**. Coupling with amino ester **10u**, Boc cleavage, and reaction of the amine with 1*H*-pyrazole-1-carboxamidine hydrochloride (**53**)[35] afforded the bis(Boc)-protected guanidine **54**. Saponification and Boc cleavage afforded the desired carboxylic acid **50**.

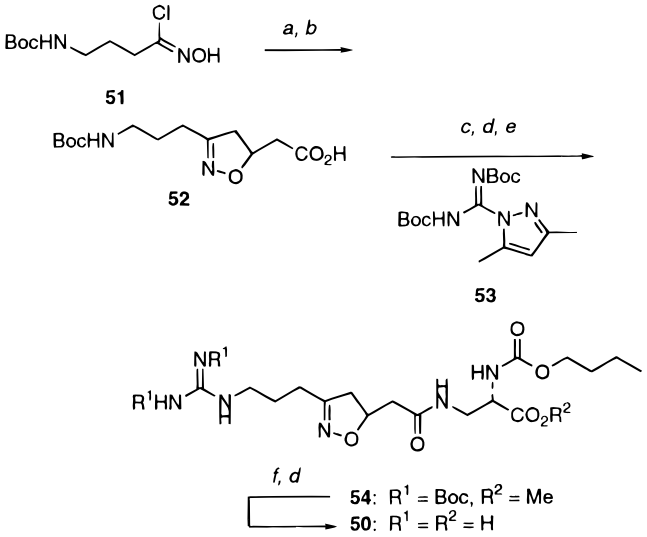
Synthesis of a Reversed Orientation Isoxazoline. Synthesis of the reversed orientation isoxazoline **55** is illustrated in Scheme 10. The nitrile oxide

Scheme 8. Synthesis of *N*-Formamidinoaniline **49**[a]



[a] Reagents: (a) $NH_2OH{\cdot}HCl$, Na_2CO_3, EtOH; (b) NCS, DMF; (c) Na_2CO_3, **46**, THF(aq); (d) LiOH, THF(aq), then HOAc; (e) **10u**, TBTU, Et_3N, EtOAc; (f) TFA, CH_2Cl_2; (g) **48**, Et_3N, $HgCl_2$, DMF; (h) LiOH, THF(aq).

Scheme 9. Preparation of Alkylguanidine **50**[a]



[a] Reagents: (a) Na_2CO_3, **46**, THF(aq); (b) LiOH, THF(aq); (c) **10u**, TBTU, Et_3N, EtOAc; (d) TFA, CH_2Cl_2; (e) **53**, Et_3N, CH_2Cl_2; (f) LiOH, THF(aq).

cycloaddition of 4-cyanostyrene (**56**) with the oximinoyl chloride derived from *tert*-butyl formylacetate oxime (**57**)[36] gave the *tert*-butyl isoxazolinylacetate **58**. Conversion of nitrile **58** to the amidine was accompanied by ester exchange. Protection as the Boc derivative and saponification of the methyl ester afforded carboxylic acid **59**. Coupling with **10u** and Boc cleavage afforded the desired ester.

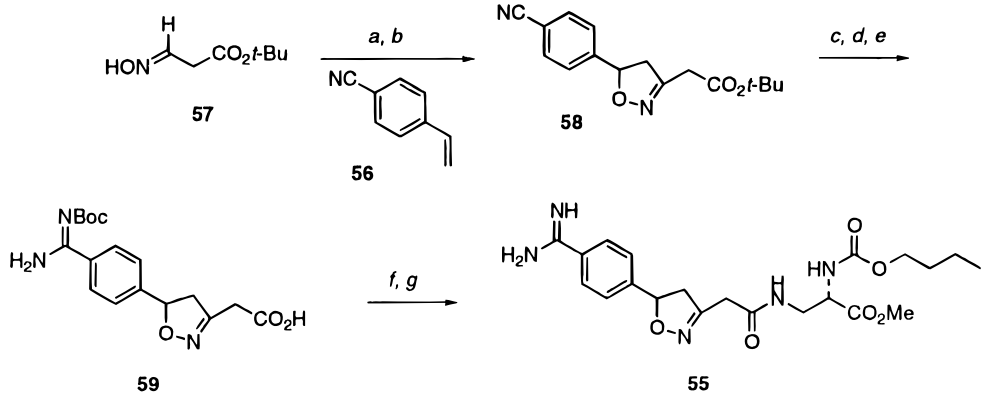
Results and Discussion

***β*-Substituted Analogs.** The addition of a variety of substituents placed β to the carboxylate moiety was found to provide improved potency in an *in vitro* aggregation assay[37] relative to XR299 (Tables 1 and 2). Considerable steric bulk was tolerated, as shown by the aspartate-derived amides **25a** and **25c** and the β-benzyl- and β-isobutyl-substituted analogs **26e** and **26j**. However, β,β-disubstitution, such as in **26a**, resulted in an almost complete loss of antiplatelet activity. This result was consistent with literature precedent[38] and may reflect unfavorable steric interactions of **26a** with the receptor or a stabilization of low-affinity conformations. Among a series of β-2-, -3-, and -4-pyridylethyl-β-alanine derivatives (**26b**, **26c**, and **26d**), the β-2-pyridylethyl analog was found to be the most potent.

Most of the β-substituted analogs were prepared as epimeric mixtures at the isoxazolin-5-yl position, using the method outlined in Scheme 5. A stereochemical preference for (3*R*)-substitution ((*S*)-aspartate configuration) was indicated by comparison of the (3*R*)- and (3*S*)-benzyl-substituted derivatives **26e** and **26f**. Acidic substituents such as carboxymethyl (**26h**) were well tolerated; however, highly basic substituents such as pyrrolidin-1-ylmethyl (**26k**) were not favored.

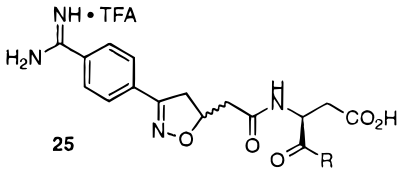
The *in vivo* activity of the β-substituted analogs is represented by the data shown for **25a** in Figure 1. When administered orally or intravenously (bolus) at a dose of 0.4 mg/kg in a canine model,[39] **25a** demonstrated a short-lived *ex vivo* antiplatelet effect.

Scheme 10. Synthesis of Reverse-Orientation Isoxazoline **55**[a]

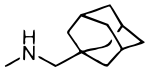
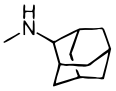
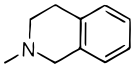


[a] Reagents: (a) Cl_2, CH_2Cl_2, −40 °C; (b) **56**, Na_2CO_3, THF(aq); (c) HCl(anhyd), MeOH, 0 °C, then NH_3, MeOH, 0 °C; (d) Boc_2O, Et_3N, dioxane; (e) LiOH, THF−H_2O; (f) **10u**, TBTU, Et_3N, EtOAc; (g) TFA, CH_2Cl_2.

Table 1. *In Vitro* Potencies of Aspartic Acid β-Amides **25**

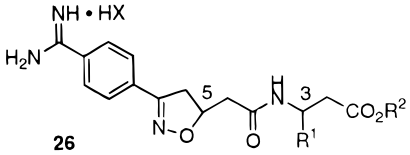


25

compd	R	hPRP $IC_{50} \pm$ SEM, μM^a
XR299	---	0.24 ± 0.063
a	-NH(CH$_2$)$_2$Ph	0.068 ± 0.023
b		0.21 ± 0.030
c		0.095 ± 0.051
d		0.14 ± 0.022
e		0.36 ± 0.035
f		0.17 ± 0.020
g	-N(Bn)*n*-Bu	0.18 ± 0.064

[a] Inhibition of ADP-induced platelet aggregation was determined in three donors. See reference 37 for assay protocol.

Table 2. *In Vitro* Potencies of β-Substituted β-Alanines **26**



26

compd	R^1	R^2	stereochemistry 5	stereochemistry 3	HX	hPRP $IC_{50} \pm$ SEM, μM^a
XR299	H	H	(*R*,*S*)		TFA	0.24 ± 0.063
a	*gem*-dimethyl	H	(*R*,*S*)		TFA	39 ± 3.2
b	CH$_2$CH$_2$-2-Py	H	(*R*,*S*)	(*R*)	TFA	0.18 ± 0.036
c	CH$_2$CH$_2$-3-Py	H	(*R*,*S*)	(*R*)	TFA	0.36 ± 0.058
d	CH$_2$CH$_2$-4-Py	H	(*R*,*S*)	(*R*)	TFA	0.50 ± 0.083
e	CH$_2$Ph	H	(*R*,*S*)	(*R*)	TFA	0.078 ± 0.0075
f	CH$_2$Ph	H	(*R*,*S*)	(*S*)	HCl	1.4 ± 0.12
g	3-Py	H	(*R*)	(*R*)	TFA	0.17 ± 0.026
h	CH$_2$CO$_2$H	H	(*R*,*S*)		TFA	0.21 ± 0.11
i	Et	H	(*R*)	(*R*)	TFA	0.054 ± 0.0099
j[b,c]	CH$_2$CH(CH$_3$)$_2$	CH$_3$	(*R*) or (*S*)	(*R*)	TFA	0.050 ± 0.014
k[b]	CH$_2$N(CH$_2$)$_4$	CH$_3$	(*R*,*S*)	(*S*)	TFA	3.3 ± 0.41
l[b,c]	CH$_2$N(CH$_3$)$_2$	CH$_3$	(*R*) or (*S*)	(*S*)	TFA	7.0 ± 0.89
m[b,c]	CH$_2$N(CH$_3$)$_2$	CH$_3$	(*S*) or (*R*)	(*S*)	TFA	2.7 ± 0.47

[a] See corresponding footnote in Table 1. [b] Converted to the free acid form prior to assay using porcine liver esterase. [c] Stereochemistry at the 5-position indicates a single, but unassigned, stereoisomer.

Diaminopropionate Derivatives. As with the β-substituted analogs, most of the α-substituted analogs were prepared as epimeric mixtures at the isoxazolin-5-yl position. The addition of an amino substituent at the α(*S*)-position of the β-alanine chain resulted in **24a**, which was somewhat more potent than the unsubstituted parent, XR299 (Table 3). Acylation of the α-nitrogen gave improved *in vitro* potency relative to XR299, as evidenced by **24b**−**e**. The phenyl- and benzylurea derivatives **24f** and **24g** were also slightly more potent *in vitro* than the free amine. The *n*-butyl sulfonamide **24h** was approximately equipotent to the best amide and urea analogs. A series of carbamates (**24i**−**u**) was prepared and generally found to afford somewhat higher *in vitro* potency than other α-amine derivatives. In general, these α-amine derivatives also had higher *in vivo* potency than the β-substituted analogs. In particular, the *n*-butyl carbamate **24u** was notable for its relatively high *in vitro* potency and enhanced duration of action when administered intravenously in a canine model.

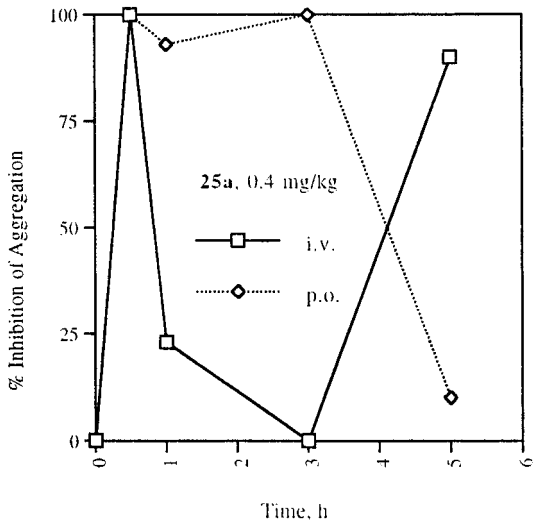


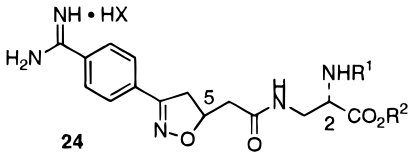
Figure 1. Inhibition of ADP (100 μM)-mediated *ex vivo* platelet aggregation in the canine dosed orally or intravenously (bolus) with 0.4 mg/kg of **25a** ($n = 1$).

It is known that the *N*-methylation of amides is a useful method for the stabilization of amide bonds to hydrolysis. In addition, *N*-methylation restricts the available conformational space of an amino acid residue.[40,41] *In vitro* testing of the *N*-methylamides **60a** and **60b** and the *N*-methylamide *N*-methyl-*N*-benzylcarbamate, **60c** (Table 4), indicated that *N*-methylation of the amide (**60a**, **60b**) resulted in a 4-fold loss in potency when compared to the secondary amides **24l** and **24u**. The addition of the carbamate *N*-methyl group (**60c**) resulted in a further 3−4-fold loss in potency.

Alternatives to the Benzamidin-4-yl Moiety. From the study of serine protease inhibitors, the benzamidine moiety was a known mimetic of the arginine side chain.[42] Several alternative basic moieties were examined to determine the effect that this group had on the pharmacological profile. A series of carbamates (**36**) were studied having a (piperidin-4-yl)alkyl group[19] in which the overall distance between the acidic and basic termini and the relative position of the isoxazolinylacetamide core were altered (Table 5). These compounds were found to be of lower potency *in vitro* than the corresponding benzamidines.

One example of a simple alkylguanidine, *n*-butyl carbamate **50**, was studied. It had an IC_{50} of 2.1 ± 0.48 μM ($n = 3$), nearly 2 orders of magnitude less potent *in vitro* than the corresponding benzamidine **24u**. This result was again consistent with those reported in the literature[24,43,44] and possibly reflected the loss of a

Table 3. *In Vitro* Potencies of Diaminopropionates **24**

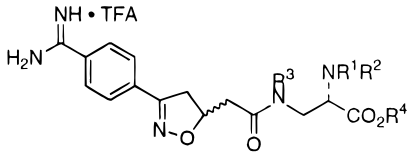


24

compd	stereochemistry R¹	R²	5	2	HX	hPRP IC_{50} ± SEM, μM[a]
XR299	–	H	(*R*,*S*)	–	TFA	0.24 ± 0.063
a	H	H	(*R*,*S*)	(*S*)	TFA	0.091 ± 0.0095
b	$CO(CH_2)_2Ph$	H	(*R*,*S*)	(*S*)	TFA	0.031 ± 0.0077
c	CO-2-naphthyl	H	(*R*,*S*)	(*S*)	TFA	0.061 ± 0.022
d	CO-C_6H_4-4-Et	H	(*R*,*S*)	(*R*)	TFA	0.049 ± 0.0062
e	CO-C_6H_4-4-Ph	H	(*R*,*S*)	(*S*)	TFA	0.067 ± 0.017
f[b]	CONHPh	CH_3	(*R*,*S*)	(*S*)	TFA	0.069 ± 0.023
g	$CONHCH_2Ph$	H	(*R*,*S*)	(*S*)	TFA	0.067 ± 0.0096
h	$SO_2(CH_2)_3CH_3$	H	(*R*,*S*)	(*S*)	TFA	0.031 ± 0.0082
i	CO_2CH_3	H	(*R*,*S*)	(*S*)	TFA	0.065 ± 0.0089
j	$CO_2CH(CH_3)_2$	H	(*R*,*S*)	(*S*)	TFA	0.13 ± 0.021
k	$CO_2(CH_2)_5CH_3$	H	(*R*,*S*)	(*S*)	TFA	0.041 ± 0.013
l	CO_2CH_2Ph	H	(*R*,*S*)	(*S*)	TFA	0.047 ± 0.0085
m	$CO_2(CH_2)_2Ph$	H	(*R*,*S*)	(*S*)	TFA	0.055 ± 0.0092
n	$CO_2CH_2CH(CH_3)_2$	H	(*R*,*S*)	(*S*)	TFA	0.047 ± 0.014
o	$CO_2(CH_2)_2CH{=}CH_2$	H	(*R*)	(*S*)	TFA	0.044 ± 0.016
p	$CO_2(CH_2)_2$c-C_5H_9	H	(*R*,*S*)	(*S*)	TFA	0.032 ±0.013
q	$CO_2(CH_2)_2$c-C_3H_5	H	(*R*,*S*)	(*S*)	TFA	0.036 ± 0.011
r	$CO_2(CH_2)_2CF_3$	H	(*R*,*S*)	(*S*)	TFA	0.031 ± 0.012
s	CO_2CH_2-C_6H_4-4-Br	H	(*R*,*S*)	(*S*)	TFA	0.044 ± 0.020
t	CO_2CH_2-C_6H_4-2-Cl	H	(*R*,*S*)	(*S*)	TFA	0.032 ± 0.014
u	$CO_2(CH_2)_3CH_3$	H	(*R*,*S*)	(*S*)	TFA	0.042 ± 0.0093
v[b]	$CO_2(CH_2)_3CH_3$	CH_3	(*R*)	(*S*)	HCl	0.030 ± 0.0068
w	$CO_2(CH_2)_3CH_3$	H	(*R*)	(*S*)	TFA	0.050 ± 0.003
x	$CO_2(CH_2)_3CH_3$	H	(*S*)	(*R*)	HCl	0.10 ± 0.017
y	$CO_2(CH_2)_3CH_3$	H	(*S*)	(*S*)	HCl	0.032 ± 0.0070
z	$CO_2(CH_2)_3CH_3$	H	(*R*)	(*R*)	HCl	0.027 ± 0.0084

[a] See corresponding footnote in Table 1. [b] See corresponding footnote in Table 2.

Table 4. *In Vitro* Potencies of *N*-Methylated Diaminopropionates **60**

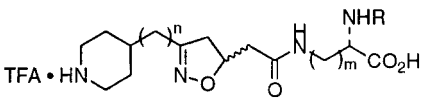


60

compd	R^1	R^2	R^3	R^3	hPRP IC_{50} ± SEM, μM[a]
a[b]	CO_2CH_2Ph	H	CH_3	CH_3	0.16 ± 0.017
b	$CO_2(CH_2)_3CH_3$	H	CH_3	H	0.20 ± 0.010
c	CO_2CH_2Ph	CH_3	CH_3	H	0.57 ± 0.084

[a] See corresponding footnote in Table 1. [b] See corresponding footnote in Table 2.

Table 5. *In Vitro* Potencies of Piperidines **36**



36

compd	R	*n*	*m*	hPRP IC_{50} ± SEM, μM[a]
a	$CO_2(CH_2)_3CH_3$	0	1	6.3 ± 0.33
b	$CO_2(CH_2)_3CH_3$	1	1	10 ± 0.37
c	$CO_2(CH_2)_3CH_3$	1	2	35 ± 4.0
d	$CO_2(CH_2)_3CH_3$	2	1	0.18 ± 0.0067
e	$SO_2(CH_2)_3CH_3$	2	1	0.23 ± 0.047
f	CO_2CH_2Ph	2	1	0.21 ± 0.0088
g	$CO_2(CH_2)_3CH_3$	3	1	0.18 ± 0.012

[a] See corresponding footnote in Table 1.

beneficial "hydrophobic shielding" effect of the aryl moiety on the amidino group or a loss of conformational control in the "arginine region". Interestingly, much of the loss in potency suffered in **50** was regained in the phenyl derivative **49**, which had an IC_{50} of 0.088 ± 0.013 μM ($n = 3$), lending support to the hydrophobic shielding hypothesis. Although longer in overall length than the benzamidines by one atom and offering an alternative presentation of the basic group, **49** nonetheless retained much of the *in vitro* potency of **24u**. While potent *in vitro*, its duration of action was shorter than **24u** when dosed intravenously in a canine model (data not shown).

One modification of the benzamidin-4-yl moiety resulted in the fluoro-substituted benzamidine **38**. It was predicted that the electron-withdrawing *m*-fluoro substituent would lower the pK_a of the amidine by approximately 0.5 pK_a unit[45] and increase lipophilicity, possibly resulting in a modified pharmacological profile. When tested *in vitro* **38** had an IC_{50} of 0.073 ± 0.0078 μM ($n = 3$), less potent than **24u** by nearly a factor of 2. In addition, it showed no improvement over **24u** when administered intravenously in the dog (data not shown).

Isoxazoline Stereochemistry. Since it was recognized that elimination of the isoxazolin-5-yl stereocenter would greatly simplify further development of the series, the need for this asymmetric center was investigated through the study of isoxazole **27**. Upon testing *in vitro*, **27** had an IC_{50} of 0.075 ± 0.0089 μM ($n = 3$) and was somewhat less potent than **24u**. It appeared as though the isoxazoline stereocenter was necessary to obtain antagonists having the highest potency. Reversing the orientation of the isoxazoline as in **55** resulted in an

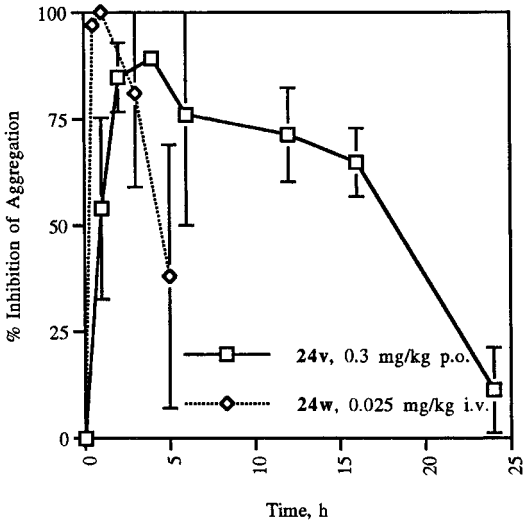


Figure 2. Inhibition of ADP (100 μM)-mediated *ex vivo* platelet aggregation in the canine dosed with **24v** and **24w** (n = 4).

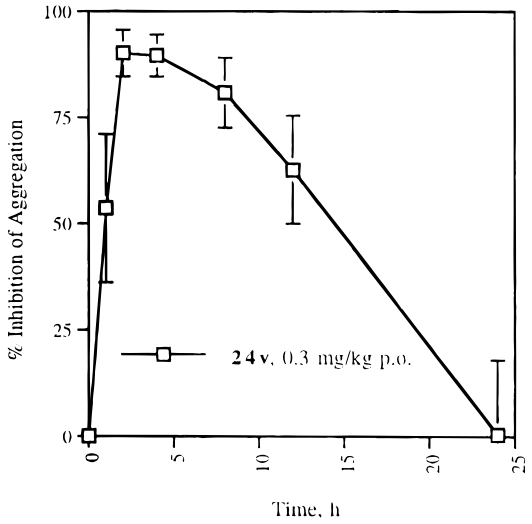


Figure 3. Inhibition of ADP (100 μM)-mediated *ex vivo* platelet aggregation in rhesus monkeys dosed orally with **24v** (n = 8).

almost complete loss of antiplatelet activity (IC_{50} = 34 ± 17 μM, ester cleaved *via* pretreatment with porcine liver esterase (n = 3)).

Preparation of all 4 isomers of **24u** afforded **24w** (5*R*,2*S*), **24x** (5*S*,2*R*), **24y** (5*S*,2*S*), and **24z** (5*R*,2*R*). Surprisingly, upon testing their *in vitro* antiplatelet potencies, all isomers of **24u** were sub-micromolar inhibitors of platelet aggregation, with potencies within a factor of 3 of each other (Table 3). This was in sharp contrast to the results observed from the study of MK-383 (**3**) and its enantiomer, (*R*)-MK-383, in which a 90-fold difference in potency was noted,[46] and in the study of (*R*)- and (*S*)-XR299, in which a 40-fold difference in potency was observed.[18]

***In Vitro* and *in Vivo* Antiplatelet Activity of the *n*-Butyl Carbamates 24v and 24w.** Additional studies of the (5*R*,2*S*)-isomer **24w** with regard to specificity, duration, and potency revealed an attractive pharmacological profile. A high degree of specificity for the inhibition of fibrinogen binding to the GPIIb/IIIa receptor was observed with **24w**.[47,48] Against a battery of agonists, **24w** was a potent, agonist independent inhibitor of platelet aggregation. It was shown to selectively inhibit the binding of [^{125}I]fibrinogen to activated human platelets (IC_{50} = 11 ± 3 nM) and to purified platelet GPIIb/IIIa receptor (IC_{50} = 0.25 ± 0.05 nM).[48]

In assessing the relative affinity of **24w** for activated versus unactivated platelets, it was found that **24w** bound equally well to both unactivated and activated platelets.[47] The presence of a substituent α to the carboxylate appeared to play a key role in the affinity for binding to unactivated platelets, as XR299 demonstrated only a weak affinity for unactivated platelets.[49]

The administration of an iv bolus dose of 0.025 mg/kg of **24w** to dogs resulted in a 90−100% inhibition of *ex vivo* platelet aggregation which declined to approximately 40% over 5 h. After oral administration of the methyl ester prodrug of **24w**, **24v**, to dogs at 0.1−0.4 mg/kg, *ex vivo* ADP (100 μM)-induced platelet aggregation was inhibited in a dose-dependent manner (Figure 2). Importantly, significant (>60%) inhibition of platelet aggregation was maintained for 12 h after oral doses of 0.3−0.4 mg/kg of **24v**. It was determined that the prodrug form **24v** had little antiaggregatory effect when tested *in vitro* and that **24v** afforded **24w** when incubated in human or canine liver homogenates or plasma.[48]

When **24v** was administered at an oral dose of 0.3 mg/kg to rhesus monkeys,[50] a profile similar to that in dogs was observed; a maximal inhibition of *ex vivo* aggregation of 90% was achieved within 2 h, which declined to approximately 60% over 12 h (Figure 3).

The oral administration of **24v** to anesthetized baboons produced a dose dependent inhibition of ADP-mediated *ex vivo* aggregation (Figure 4).[50] In this species a slower onset of action was observed, possibly attributable to slower absorption due to the anesthetization procedure. When administered at a dose of 0.3 mg/kg, **24v** had a duration of action of approximately 24 h, considerably longer than the 12 h duration of action noted in the dog or rhesus monkey.

Conclusions

Using XR299 as a starting point for the design of highly potent, long-duration GPIIb/IIIa antagonists, the effect of placing lipophilic substituents at the positions α and β to the carboxylate moiety was evaluated. Of the compounds studied, it was found that those bearing a carbamate substituent α to the carboxylate moiety exhibited superior potency and enhanced duration of action. Replacement of the benzamidin-4-yl moiety of **24u** with alternative basic groups, elimination of the isoxazoline stereocenter, and reversal of the orientation of the isoxazoline ring were associated with reduced potency and/or duration of action. The *n*-butyl carbamate **24u** was notable for its relatively high potency and long duration of action when studied in dog, rhesus monkey, and baboon animal models.

Experimental Section

Chemistry. Melting points were determined on an Electrothermal melting point apparatus and are uncorrected. Unless otherwise stated, preparative HPLC separations were accomplished on a Vydac C18 column operated at room temperature and eluted at a 10 mL/min flow rate, using a

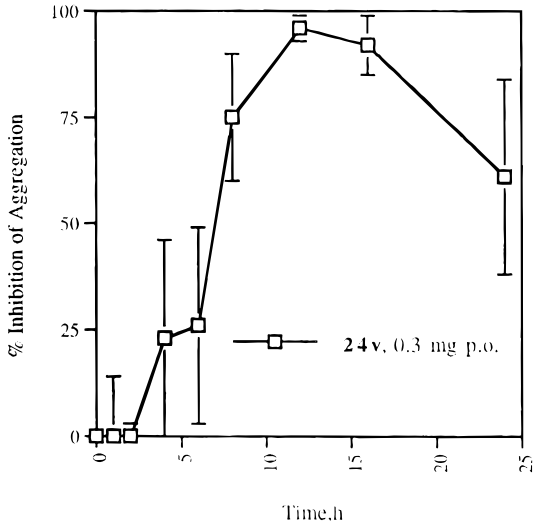


Figure 4. Inhibition of ADP (100 μM)-mediated *ex vivo* platelet aggregation in ketamine anesthetized baboons dosed orally with **24v** ($n = 3$).

linear gradient of 100% water containing 0.05% TFA−20% water/acetonitrile containing 0.05% TFA over 50 min, with UV detection at 254 nm. Proton and ^{13}C NMR data were obtained using Varian Unity 300, Unity 400, or VXR400 spectrometers and were referenced to TMS, $CDCl_3$, or residual HOD. Mass spectral data were obtained on either VG 70-VSE (FAB, high res FAB, high res DCI) or Finnigan MAT 8230 (DCI) mass spectrometers. Combustion analyses were performed by Quantitative Technologies, Inc., Bound Brook, NJ. Solvents and reagents were used as purchased from Aldrich Chemical Co. unless otherwise stated. The yields quoted in this paper are isolated yields.

(*R,S*)-3-(4-Cyanophenyl)-4,5-dihydro-5-isoxazoleacetic Acid (15). To a solution of 4-cyanobenzaldoxime (**19**) (312 g, 2.13 mol) in THF (3000 mL) at room temperature was added vinylacetic acid (**20**) (552 g, 6.41 mol). The yellow solution was cooled in an ice bath and sodium hypochlorite solution (Clorox, 5200 mL) was added dropwise over 2 h. After being stirred overnight at room temperature the reaction was quenched with a 5% citric acid solution and diluted with Et_2O (200 mL). The layers were separated, and the aqueous layer was acidified to pH 4 using citric acid. The acid layer was washed with Et_2O (2 × 200 mL), and the Et_2O layers were combined and washed with a saturated $NaHCO_3$ solution. After the aqueous layer was acidified to pH 4 with citric acid, the product was extracted into Et_2O (400 mL). The organic phase was washed with water (3 × 150 mL) and saturated NaCl, dried ($MgSO_4$), filtered, and concentrated *in vacuo*, yielding 220 g of the title compound as a colorless solid. Recrystallization from 25% water/EtOH yielded 165 g (34%) of analytically pure material: ^1H NMR (300 MHz, $CDCl_3$) δ 7.77−7.76 (d, $J = 1.8$ Hz, 2H), 7.72−7.71 (d, $J = 1.8$ Hz, 2H), 5.22−5.14 (m, 1H), 3.63−3.54 (dd, $J = 10.6$, 16.8 Hz, 1H), 3.19−3.11 (dd, $J = 7.3$, 16.8 Hz, 1H), 3.00−2.93 (dd, $J = 6.2$, 16.5 Hz, 1H), 2.79−2.72 (dd, $J = 7.3$, 16.5 Hz, 1H); IR (KBr pellet, cm^{-1}) 3202, 2244, 1736, 1610, 1432, 1416, 1194, 1152, 928, 840, 562. Anal. ($C_{12}H_{10}N_2O_3$) C, H, N.

Methyl (*R,S*)-3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazoleacetate Hydrochloride (21). To an ice-cold suspension of **15** (23.1 g, 100 mmol) in MeOH (anhyd, 200 mL) was bubbled HCl (anhyd) until a clear solution was obtained (3 h). The reaction flask was sealed and allowed to warm to room temperature, with stirring, for 24 h. The solution was poured into Et_2O (anhydrous, 600 mL) precipitating the product, and the resulting slurry was chilled to −25 °C for 2.5 h. The slurry was further diluted with chilled Et_2O (anhydrous, 100 mL). The precipitate was filtered, washed with chilled Et_2O (anhydrous, 2 × 100 mL), and suction-dried under nitrogen to afford 23.3 g (73%) of methyl (*R,S*)-3-[4-(methoxyiminomethyl)phenyl]-4,5-dihydro-5-isoxazoleacetate hydrochloride: ^1H NMR (300 MHz, $CDCl_3$) δ 12.9 (bs, 1H), 12.2 (bs, 1H), 8.46 (d, $J = 8.8$ Hz, 2H), 7.86 (d, $J = 8.8$ Hz, 2H), 5.20 (bm, 1H), 4.59 (s, 3H), 3.74 (s, 3H), 3.53 (dd, $J = 16.8$, 10.6 Hz, 1H), 3.15 (dd, $J = 16.8$, 7.7 Hz, 1H), 2.90 (dd, $J = 16.1$, 6.2 Hz, 1H), 2.70 (dd, $J = 16.1$, 7.3 Hz, 1H), 1.77 (bs, 1H); CIMS (NH_3) *m/z* 277 [$(M + H)^+$, 100].

A solution of methyl (*R,S*)-3-[4-(methoxyiminomethyl)phenyl]-4,5-dihydro-5-isoxazoleacetate hydrochloride (22.9 g, 73.0 mmol) in 1 M ammonia in MeOH (anhydrous, 500 mL) was stirred at room temperature for 14 h, during which time all solids dissolved. The solution was concentrated *in vacuo*, yielding 22.1 g (100%) of crude hydrochloride salt **21** as a tan solid: ^1H NMR (300 MHz, $CDCl_3$) δ 9.6−9.2 (m, 3H), 7.91 (d, $J = 8.8$ Hz, 2H), 7.87 (d, $J = 8.8$ Hz, 2H), 5.08 (bm, 1H), 3.64 (s, 3H), 3.3−3.1 (m, 2H), 2.8 (m, 2H); MS (ESI) *m/z* 264 [$(M + H)^+$, 100].

(*R,S*)-3-[4-[[*N*-(Dimethylethoxycarbonyl)imino]-aminomethyl]phenyl]-4,5-dihydro-5-isoxazoleacetic Acid (22). To a solution of **21** (21.6 g, 72.5 mmol) in DMF (350 mL) cooled in an ice bath were added Et_3N (20.2 mL, 145 mmol) and *tert*-butyl dicarbonate (17.4 g, 79.8 mmol). After the reaction mixture was stirred for 16 h at 22 °C, it was poured into water (1500 mL) with stirring. A colorless precipitate formed and was filtered and dried on the filter under nitrogen to give methyl (*R,S*)-3-[4-[[*N*-(dimethylethoxycarbonyl)imino]-aminomethyl]phenyl]-4,5-dihydro-5-isoxazoleacetate (19.6 g, 75%) as a colorless solid: ^1H NMR (300 MHz, DMSO-d_6) δ 7.90 (d, $J = 8.4$ Hz, 2H), 7.70 (d, $J = 8.4$ Hz, 2H), 5.14 (m, 1H), 3.74 (s, 3H), 3.56 (dd, $J = 16.8$, 16.8 Hz, 1H), 3.14 (dd, $J = 16.8$, 16.8 Hz, 1H), 2.90 (dd, $J = 16.1$, 16.1 Hz, 1H), 2.68 (dd, $J = 16.1$, 16.1 Hz, 1H), 1.56 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.93, 165.76, 164.04, 156.86, 136.24, 132.79, 128.51, 126.91, 78.35, 77.89, 51.98, 39.58, 39.31, 28.46; MS (ESI) *m/z* 362 [$(M + H)^+$, 100].

To a solution of methyl (*R,S*)-3-[4-[[*N*-(dimethylethoxycarbonyl)imino]aminomethyl]phenyl]-4,5-dihydro-5-isoxazoleacetate (18.95 g, 52.4 mmol) in MeOH (500 mL) at 22 °C was added $LiOH \cdot H_2O$ (2.42 g, 57.7 mmol) in water (75 mL). The mixture was stirred for 16 h and filtered, and the filtrate was concentrated *in vacuo* to remove MeOH. The residual aqueous phase was cooled with an ice bath and acidified with 6 N and 1 N HCl to pH 4, causing a colorless solid to precipitate. After being left to stand at −4 °C overnight, the solid was filtered and dried on the filter under nitrogen to give **22** (17.74 g, 97%) as an off-white powder: ^1H NMR (300 MHz, DMSO-d_6) δ 7.94 (d, $J = 8.4$ Hz, 2H), 7.78 (d, $J = 8.4$ Hz, 2H), 5.04 (m, 1H), 3.62 (dd, $J = 16.8$, 17.2 Hz, 1H), 3.22 (dd, $J = 17.2$, 17.2 Hz, 1H), 2.68 (m, 2H), 1.50 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 171.91, 165.58, 158.61, 156.76, 133.87, 132.78, 129.43, 126.87, 81.55, 78.39, 40.44, 39.30, 28.27; MS (ESI) *m/z* 348 [$(M + H)^+$, 100]; HRMS (NH_3-CI) *m/z* 348.1556 [$(M + H)^+$ calcd for $C_{17}H_{21}N_3O_5$ 348.1559].

Butyl (*R,S*)-3-(4-Cyanophenyl)-4,5-dihydro-5-isoxazoleacetate ((*R,S*)-33). This compound was synthesized following the reported procedure:[18] ^1H NMR (300 MHz, $CDCl_3$) δ 7.78 (d, $J = 8.4$ Hz, 2H), 7.69 (d, $J = 8.4$ Hz, 2H), 5.18 (m, 1H), 4.13 (t, $J = 6.6$ Hz, 2H), 3.55 (dd, $J = 17.1$, 10.6 Hz, 1H), 3.14 (dd, $J = 16.8$, 7.7 Hz, 1H), 2.90 (dd, $J = 16.1$, 5.8 Hz, 1H), 2.68 (dd, $J = 16.1$, 7.7 Hz, 1H), 1.61 (m, 2H), 1.38 (m, 2H), 0.94 (t, $J = 7.3$ Hz, 3H). Anal. ($C_{16}H_{18}N_2O_3 \cdot 0.5H_2O$) C, H, N.

(*R*)-3-(4-Cyanophenyl)-4,5-dihydro-5-isoxazoleacetic Acid ((*R*)-15). To a solution of (*R,S*)-**33** (415 g, 1.45 mol) in acetonitrile (2 L) was added $NaHCO_3$ (150 g) in water (5.0 L) followed by a suspension of Amano P30 lipase (15 g) in water (100 mL). After 18 h, the reaction mixture was filtered. The *n*-butyl ester (*S*)-**33** was recovered as the solid filter cake while the (*R*)-acid remained in the aqueous phase. The filtrate was then extracted with CH_2Cl_2 (2 × 1 L), resulting in an emulsion which separated upon standing overnight. The aqueous layer was transferred to a 12 L flask and the pH lowered to 5.02 using concentrated HCl (165 mL). The solid was filtered, dried on a fritted funnel overnight, and placed in a vacuum oven, affording 128.3 g of desired material. A second crop was collected and dried (10.6 g), giving a combined yield of (*R*)-**15** of 138.9 g (83%). The material was identical to that previously reported.[18] Anal. ($C_{12}H_{10}N_2O_3$) C, H, N.

(*S*)-3-(4-Cyanophenyl)-4,5-dihydro-5-isoxazoleacetic Acid ((*S*)-15). Butyl (*S*)-3-(4-cyanophenyl)-4,5-dihydro-5-isoxazoleacetate ((*S*)-**33**, 30 g, 0.10 mol) was dissolved in 4 N HCl/dioxane (125 mL), and water (25 mL) was added. The reaction mixture was stirred for 18 h when an equal volume of water was added. The mixture was made basic using saturated $NaHCO_3$ and extracted with EtOAc (2×), and the aqueous layer was acidified using 1 N HCl. A colorless solid precipitated, was filtered, and was washed with water. Recrystallization from acetonitrile gave the desired material (22.8 g, 99%) as a colorless solid, identical to that previously reported.[18] Anal. ($C_{12}H_{10}N_2O_3$) C, H, N.

Methyl (*R*)-3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-L-alanine Bis(trifluoroacetate) ((*R*)-32). To a solution of (*R*)-**15** (5.0 g, 22 mmol) and **10l** (6.27 g, 21.7 mmol) in DMF (50 mL) was added *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU, 6.97 g, 21.7 mmol). After the mixture was stirred for 15 min, diisopropylethylamine was added (7.57 mL, 43.4 mmol) and the resulting mixture stirred at room temperature for 16 h. The reaction mixture was partitioned between EtOAc (100 mL) and water (100 mL), the aqueous layer was washed with EtOAc (100 mL), and the organic extracts were combined and washed successively with water (100 mL), 0.25 M potassium phthalate solution (100 mL), 5% aqueous $NaHCO_3$ (100 mL), and saturated NaCl (50 mL). The organic extract was dried (Na_2SO_4), filtered, and evaporated *in vacuo* to yield a solid residue which was purified using silica gel column chromatography (1:1 to 95:5 EtOAc/hexane gradient) to give the amide as a colorless solid (9.0 g, 89% yield) having >99% de (HPLC): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, J = 8.8 Hz, 2H), 7.67 (d, J = 8.4 Hz, 2H), 7.35 (m, 5H), 6.30 (m, 1H), 5.81 (m, 1H), 5.10 (m, 3H overlap), 4.45 (m, 1H), 3.80−3.57 (m, 5H), 3.52−3.40 (m, 1H), 3.20 (m, 1H), 2.65 (m, 1H), 2.52 (m, 1H); HRMS (NH_3-CI) m/z 465.1772 [$(M + H)^+$ calcd for $C_{24}H_{25}N_4O_6$ 465.1774].

Anhydrous HCl gas was bubbled into a suspension of the amide (4.8 g, 10.3 mmol) in MeOH (100 mL) at −10 °C for 2 h. The reaction vessel was sealed, allowed to warm to room temperature, and stirred for 5 h. The volatiles were evaporated *in vacuo*, the residue was taken up in MeOH (100 mL), and ammonium carbonate (4.97 g, 51.67 mmol) was added. The reaction mixture was sealed and stirred at room temperature for 16 h. Concentration *in vacuo* gave a residue which was purified using column chromatography on silica gel (5:95 to 35:65 MeOH/$CHCl_3$ gradient) to yield the amidine·HCl salt as a colorless solid (2.4 g, 45%) of suitable purity for the next reaction: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.40 (s, 2H), 9.30 (s, 2H), 8.21 (t, J = 8.3 Hz, 1H), 7.87 (m, 4H), 7.69 (d, J = 8.1 Hz, 1H), 7.32 (m, 5H), 5.03 (m, 3H), 4.21 (m, 1H), 3.64 (s, 3H), 3.55 (m, 2H), 3.22 (m, 2H), 2.51 (m, 2H); MS (ESI) m/z 482.2 [$(M + H)^+$, 100].

The amidine·HCl salt (25 mg) was dissolved in 1:1 water/acetonitrile, three drops of TFA were added, and the mixture was purified using reverse phase HPLC. After pooling and evaporation of the acetonitrile from the appropriate fractions, lyophilization of the remaining aqueous solution afforded the amidine·TFA salt (25 mg) as a colorless solid: ^{1}H NMR identical to that of the amidine·HCl salt; MS (ESI) m/z 482.4 [$(M + H)^+$, 100]; HRMS (NH_3-CI) m/z 482.2057 [$(M + H)^+$ calcd for $C_{24}H_{28}N_5O_6$ 482.2040].

The amidine·TFA salt (690 mg) was stirred in neat TFA for 16 h at room temperature. The mixture was concentrated *in vacuo* at room temperature, and the crude residue was purified using reverse phase HPLC to yield (*R*)-**32** as a colorless solid (470 mg, 85%, >99% de (HPLC)): ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.40 (s, 2H), 9.28 (s, 2H), 8.42 (bs, 3H), 8.32 (t, J = 5.5 Hz, 1H), 7.87 (m, 4H), 5.06 (m, 1H), 4.13 (m, 1H), 3.75 (s, 3H), 3.68−3.20 (m, 4H), 2.65−2.45 (m, 2H); MS (ESI) m/z 348.3 [$(M + H)^+$, 100]; HRMS (NH_3-CI) m/z 348.1672 [$(M + H)^+$ calcd for $C_{16}H_{21}N_5O_4$ 348.1671].

Methyl 3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-L-alanine Bis(trifluoroacetate) (32). To a solution of **22** (5.0 g, 14 mmol) and **10l** (4.16 g, 14.4 mmol) in DMF (50 mL) were added TBTU (4.85 g, 15.1 mmol) and Et_3N (8.52 mL, 61.2 mmol). After being stirred at room temperature for 16 h, the reaction mixture was concentrated and partitioned between EtOAc (100 mL) and water (100 mL). The aqueous layer was washed with EtOAc (2 × 100 mL), and the organic extracts were combined and washed successively with water (2 × 100 mL), 0.25 M potassium phthalate solution (100 mL), 5% aqueous $NaHCO_3$ (100 mL), and saturated NaCl (50 mL). The organic extract was dried (Na_2SO_4), filtered, and concentrated *in vacuo* to yield a residue which was purified on silica gel (75:25 to 95:5 EtOAc/hexane gradient) to yield amide **30** as a colorless foam (7.4 g, 89%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.83 (m, 2H), 7.65 (m, 2H), 7.35 (m, 5H), 6.43 (m, 1H), 5.85 (m, 1H), 5.70 (m, 3H), 4.44 (m, 1H), 3.77 (s, 3H), 3.68 (m, 2H), 3.45 (m, 1H), 3.13 (m,1H), 2.6 (m, 2H), 1.56 (s, 9H); HRMS (FAB) m/z 582.2536 [$(M + H)^+$ calcd for $C_{29}H_{36}N_5O_8$ 582.2564].

To a 1 L round bottom flask fitted with a condenser was added MeOH (200 mL), **30** (12.4 g, 21.3 mmol), and 1,4-cyclohexadiene (100 mL). The solution was degassed, and Pearlman's catalyst (12.4 g) was introduced. After being stirred at room temperature for 30 min, the reaction mixture began to exotherm. The reaction flask was cooled in an ice bath, and after the solution was stirred for another 30 min it was filtered through Celite and the filter cake was washed with MeOH. The solvents were evaporated *in vacuo*, and the residue was purified by column chromatography on silica gel (5:95 to 20:80 MeOH/$CHCl_3$ gradient) to yield methyl 3-[[[3-[4-[[[(1,1-dimethylethoxy)carbonyl]amino]iminomethyl]phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(phenylmethoxy)-carbonyl]-L-alanine (**31**) as a pale yellow glass (5.93 g, 62% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (m, 2H), 7.68 (m, 2H), 6.44 (m, 1H), 5.15 (m, 1H), 3.75 (s, 3H), 3.7−3.1 (m, 5H), 2.64 (m, 2H), 1.57 (s, 9H); HRMS (NH_3-CI) m/z 448.2215 [$(M + H)^+$ calcd for $C_{21}H_{30}N_5O_6$ 448.2196].

A solution of **31** (50 mg, 112 μmol) in CH_2Cl_2 (2 mL) was treated with TFA (1 mL) and stirred at room temperature for 3 h. The solution was diluted with Et_2O, and the resulting precipitate was collected by filtration, washed with Et_2O, and concentrated *in vacuo* to provide **32** (60 mg, 94%) as a pale yellow powder: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.41 (bs, 2H), 9.26 (bs, 2H), 8.44 (bs, 3H), 8.33 (m, 1H), 7.88 (m, 4H), 5.06 (m, 1H), 4.13 (q, J = 7.0 Hz, 1H), 3.75 (s, 3H), 3.7−3.3 (m, 4H), 3.24 (dd, J = 16.0, 8.0 Hz, 1H), 2.60 (dd, J = 16.0, 8.0 Hz, 1H); MS (ESI) m/z 348 [$(M + H)^+$, 100]. Anal. ($C_{16}H_{21}N_5O_4 \cdot 1.8CF_3CO_2H \cdot 0.25H_2O$) C, H, N, F.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(butoxycarbonyl)-L-alanine Trifluoroacetate (24u). To an ice-cold solution of **32** (200 mg, 0.35 mmol) in water (1 mL) and acetonitrile (1 mL) was added $NaHCO_3$ (112 mg, 1.4 mmol) followed by *n*-butyl chloroformate (48 mg, 0.35 mmol). After being stirred for 1 h, the solution was acidified using TFA and purified using reverse phase HPLC to yield the desired carbamate as a colorless solid (120 mg, 61%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.42 (s, 2H), 9.18 (s, 2H), 8.19 (m, 1H), 7.88 (m, 4H), 7.48 (m, 1H), 5.02 (m, 1H), 4.15 (m, 1H), 3.95 (m, 2H), 3.62 (s, 3H), 3.50 (m, 2H), 3.26 (m, 2H), 2.50 (m, 2H), 1.52 (m, 2H), 1.32 (m, 2H), 0.86 (m, 3H); MS (ESI) m/z 448.3 [$(M + H)^+$, 100].

The carbamate (60 mg, 0.107 mmol) was dissolved in THF (1 mL), and 1 N LiOH (1 mL, 1 mmol) was added. After being stirred at room temperature for 1 h, the solution was acidified with TFA to pH 4. Purification using preparative reverse phase HPLC, pooling of the appropriate fractions, evaporation of the acetonitrile *in vacuo*, and lyophilization of the aqueous gave the title compound as a colorless powder (45 mg, 77%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.40 (s, 2H), 9.17 (s, 2H), 8.16 (m, 1H), 7.86 (m, 4H), 7.35 (m, 1H), 5.02 (m, 1H), 4.12 (m, 1H), 3.94 (m, 2H), 3.50 (m, 2H), 3.26 (m, 2H), 2.50 (m, 2H), 1.52 (m, 2H), 1.32 (m, 2H), 0.88 (m, 3H); MS (ESI) m/z 434.3 [$(M + H)^+$, 100]. Anal. ($C_{20}H_{27}N_5O_6 \cdot 1.3CF_3CO_2H$) C, H, N.

***β*-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*γ*-oxo-3-azabicyclo[3.2.2]nonane-3-butanoic Acid Trifluoroacetate (25b).** To a solution of *N*-(*tert*-butoxycarbonyl)aspartic acid β-methyl ester (**7**) (989 mg, 4.00 mmol), 3-azabicyclo[3.2.2]nonane (500 mg, 4.0 mmol), and TBTU (1.29 g, 4.02 mmol) in EtOAc (25 mL) was added Et_3N (1.7 mL, 12 mmol). The mixture was stirred at room

temperature overnight (18 h) and was washed with 0.1 M HCl, saturated $NaHCO_3$, and saturated NaCl. The solution was dried ($MgSO_4$), filtered, concentrated *in vacuo*, and placed under high vacuum until constant weight was achieved, affording 1.39 g (98%) of the desired amide as an amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.50 (bd, J = 9.5 Hz, 1H), 5.09 (m, 1H), 3.90 (dd, J = 13.6, 4.8 Hz, 1H), 3.80 (dd, J = 13.9, 4.8 Hz, 1H), 3.69 (s, 3H), 3.53 (m, 2H), 2.81 (dd, J = 15.4, 6.6 Hz, 1H), 2.59 (dd, J = 15.4, 5.9 Hz, 1H), 2.08 (m, 2H), 1.65 (m, 8H), 1.43 (s, 9H); MS (ESI) m/z 355 [$(M + H)^+$, 100]. Anal. Calcd for $C_{18}H_{30}N_2O_5 \cdot 0.33H_2O$: C, 59.98; H, 8.58; N, 7.77. Found: C, 60.12; H, 8.49; N, 7.69.

To the neat Boc-protected aspartic acid β-amide (1.23 g, 3.47 mmol) was added 4 M HCl in dioxane (5 mL, 20 mmol). The mixture was stirred at room temperature for 1 h, concentrated *in vacuo*, and placed under high vacuum until constant weight was achieved, affording the desired amine hydrochloride **6b** (785 mg, 78%) as an amorphous solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.71 (bs, 3H), 5.04 (bs, 1H), 3.95 (bd, J = 10.2 Hz, 1H), 3.75 (s, 3H, coincident with m, 1H), 3.52 (bd, J = 12.0 Hz, 2H), 3.14 (bs, 2H), 2.11 (bs, 2H), 1.64 (m, 8H); MS (ESI) m/z 255 [$(M + H)^+$, 100]. Anal. ($C_{13}H_{23}N_2O_3 \cdot HCl \cdot H_2O$) C, H, N.

To a solution of **22** (225 mg, 0.653 mmol) and **6b** (188 mg, 0.646 mmol) in DMF (5 mL) was added Et_3N (0.36 mL, 2.6 mmol) followed by TBTU (210 mg, 0.654 mmol). The resulting mixture was stirred at room temperature for 2 h, after which time it was diluted with EtOAc, washed with water, saturated $NaHCO_3$, saturated NaCl, and dried over $MgSO_4$. Following filtration and EtOAc washing of the solid, the combined filtrate was concentrated *in vacuo* and placed under vacuum until constant weight was achieved, affording 346 mg (92%) of amide **23b** as an amorphous solid (80% purity by ^{1}H NMR, contained tetramethylurea): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (d, J = 8.8 Hz, 2H), 7.69 (dd, J = 5.5, 3.7 Hz, 2H), 6.88 (dd, J = 8.4, 2.6 Hz, 1H), 5.39 (m, 1H), 5.14 (m, 1H), 3.81 (dd, J = 13.9, 4.4 Hz, 1H), 3.74−3.46 (m, 4H, coincident with tetramethylurea), 3.19 (dt, J = 17.2, 7.0 Hz, 1H), 2.88−2.53 (m, 4H), 2.08 (bs, 2H), 1.77−1.47 (m, 8H), 1.56 (s, 9H); MS (ESI) m/z 584 [$(M + H)^+$, 100].

To the neat Boc derivative **23b** (290 mg, 0.497 mmol) was added 4 M HCl in dioxane (5 mL, 20 mmol). After 10 h at room temperature, the mixture was concentrated *in vacuo* to give an oily residue. Trituration with Et_2O afforded an off-white solid, which was dried under vacuum until constant weight was achieved, affording 113 mg (44%) of the crude methyl ester hydrochloride. To the neat ester (100 mg, 0.172 mmol) was added 40% concentrated HCl in formic acid (1.25 mL). The resulting solution was stirred at room temperature overnight. After concentration *in vacuo*, the residue was purified using preparative reverse phase HPLC. Fractions containing the desired product **25b** were pooled and concentrated *in vacuo* to remove acetonitrile, and the aqueous solution was lyophilized to yield 52 mg (52%) of the desired TFA salt as a colorless fluffy powder: ^{1}H NMR (300 MHz, CD_3OD) δ 7.87 (AB quartet, J = 8.8 Hz, Δ = 19.4 Hz, 4H), 5.34 (dd, J = 8.0, 6.1 Hz, 0.5H), 5.14 (m, 1H), 4.69 (t, J = 6.6 Hz, 0.5 H), 3.72−3.50 (m, 3H), 2.91−2.46 (m, 4H), 2.15 (bs, 1H), 2.05 (bs, 1H), 1.84−1.62 (m, 8H); MS (ESI) m/z 470 [$(M + H)^+$, 100]. Anal. ($C_{24}H_{31}N_5O_5 \cdot 1.8CF_3CO_2H$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(1-oxo-3-phenylpropyl)-L-alanine Trifluoroacetate (24b). This compound was synthesized in a manner similar to that used for **24u**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.40 (s, 2H), 9.24 (s, 2H), 8.24 (m, 1H), 8.18 (m, 1H), 7.86 (m, 4H), 7.20 (m, 5H), 5.04 (m, 1H), 4.39 (m, 1H), 3.50 (m, 2H), 3.24 (m, 2H), 2.80 (m, 2H), 2.50 (m, 4H); MS (ESI) m/z 466.3 [$(M + H)^+$, 100]. Anal. ($C_{24}H_{27}N_5O_5 \cdot 1.3CF_3CO_2H$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(2-naphthalenylcarbonyl)-L-alanine Mono(trifluoroacetate) (24c). This compound was synthesized in a manner similar to that used for **24u**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.38 (s, 2H), 9.20 (s, 2H), 8.82 (m, 1H), 8.50 (s, 1H), 8.32 (m, 1H), 8.00 (m, 4H), 7.80 (m, 4H), 7.60 (m, 2H), 5.06 (m, 1H), 4.60 (m, 1H), 3.70 (m, 2H), 3.20 (m, 2H), 2.55 (m, 2H); MS (ESI) m/z 488.2 [$(M + H)^+$, 100]. Anal. ($C_{26}H_{25}N_5O_5 \cdot CF_3CO_2H \cdot H_2O$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(4-ethylbenzoyl)-L-alanine Mono(trifluoroacetate) (24d). This compound was synthesized in a manner similar to that used for **24u**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.40 (s, 2H), 9.24 (s, 2H), 8.60 (m, 1H), 8.30 (m, 1H), 7.80 (m, 6H), 7.30 (m, 2H), 5.04 (m, 1H), 4.50 (m, 1H), 3.60 (m, 2H), 3.20 (m, 2H), 2.62 (m, 2H), 2.50 (m, 2H), 1.18 (m, 3H); MS (ESI) m/z 466.3 [$(M + H)^+$, 100]. Anal. ($C_{24}H_{27}N_5O_5 \cdot CF_3CO_2H \cdot 0.7H_2O$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-([1,1′-biphenyl]-4-ylcarbonyl)-L-alanine Trifluoroacetate (24e). This compound was synthesized in a manner similar to that used for **24u**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.40 (s, 2H), 9.22 (s, 2H), 8.75 (m, 1H), 8.35 (m, 1H), 8.00 (m, 2H), 7.80 (m, 8H), 7.42 (m, 3H), 5.04 (m, 1H), 4.58 (m, 1H), 3.60 (m, 2H), 3.20 (m, 2H), 2.50 (m, 4H); MS (ESI) m/z 466.3 [$(M + H)^+$, 100]. Anal. ($C_{28}H_{27}N_5O_5 \cdot 1.3CF_3CO_2H$) C, H, N.

Methyl 3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(phenylamino)carbonyl]-L-alanine Trifluoroacetate (24f). To a solution of methyl 3-(*tert*-butoxycarbonylamino)-L-alanine[27] (502 mg, 2.3 mmol) in CH_2Cl_2 (10 mL) was added phenyl isocyanate (275 μL, 2.5 mmol). The resulting mixture was stirred under N_2 for 7 h, diluted with EtOAc, and extracted with water and saturated NaCl. The organic layer was removed, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude product was purified using column chromatography on silica gel (20−40% EtOAc/hexane) to obtain the desired urea (618 mg, 80%). Cleavage of the Boc group as described for the synthesis of **25b** then afforded **10f**. The coupling of **22** with **10f** and further elaboration as described previously for **25b** afforded **24f**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.37 (s, 2H), 9.03 (s, 2H), 8.84 (s, 5H), 8.28 (bs, 1H), 7.85 (s, 4H), 7.38 (d, J = 8.4 Hz, 2H), 7.25−7.18 (m, 2H), 6.90 (t, J = 7.4 Hz, 1H), 6.55 (m, 5H), 5.08−5.02 (m, 1H), 4.38 (m, 1H), 3.66 (s, 3H), 3.61−3.35 (m, 3H), 3.27−3.19 (m, 1H), 2.58 (m, 1H), 2.45 (m, 1H); HRMS (NH_3-CI) m/z 467.2037 [$(M + H)^+$ calcd for $C_{16}H_{24}N_3O_5$ 467.2043].

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[[(phenylmethyl)amino]carbonyl]-L-alanine Trifluoroacetate (24g). This compound was synthesized following the procedure used for **25b**: ^{1}H NMR (300 MHz, CD_3OD) δ 7.89 (m, 4H), 7.26 (m, 5H), 7.17 (m, 1H), 5.08 (m, 1H), 4.46 (m, 1H), 4.29 (m, 2H), 3.74−3.36 (m, 3H), 3.22 (dd, J = 17.2, 9.0 Hz, 1H), 2.68 (dd, J = 14.3, 6.4 Hz, 1H), 2.49 (m, 1H); MS (ESI) m/z 467 [$(M + H)^+$, 100]. Anal. ($C_{23}H_{26}N_6O_5 \cdot 1.5CF_3CO_2H$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(butylsulfonyl)-L-alanine Trifluoroacetate (24h). This compound was synthesized in an analogous manner to that used for **25b**. The ester was cleaved using the LiOH protocol described in the preparation of **22**: ^{1}H NMR (300 MHz, CD_3OD) δ 7.88 (AB quartet, J = 8.4 Hz, Δ = 33.0 Hz, 4H), 5.14 (m, 1H), 4.18 (m, 1H), 3.79 (dd, J = 13.6, 4.4 Hz, 0.5H), 3.59 (m, 2H), 3.44 (dd, J = 13.9, 8.8 Hz, 0.5H), 3.35 (t, J = 3.7 Hz, 0.5H), 3.23 (dd, J = 13.6, 8.8 Hz, partially coincident with CHD_2OD, 0.5H), 3.06 (t, J = 8.1 Hz, 2H), 2.73 (m, 1H), 2.54 (m, 1H), 1.78 (m, 2H), 1.44 (m, 2H), 0.94 (t, J = 7.3 Hz, 3H); MS (ESI) m/z 454 [$(M + H)^+$, 100]. Anal. ($C_{19}H_{27}N_5O_6S \cdot 1.6CF_3CO_2H \cdot 1.8H_2O$) C, H, N, S.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(methoxycarbonyl)-L-alanine Trifluoroacetate (24i). To a solution of **31** (200 mg, 0.447 mmol) in CH_2Cl_2 (15 mL) was added methyl chloroformate (0.035 mL, 0.447 mmol) and Et_3N (0.062 mL, 0.447 mmol). The reaction mixture was stirred for 16 h at room temperature and was then directly added to a silica gel column for purification (1:1 to 85:15 EtOAc/hexane) to yield the methyl carbamate as a colorless foam (205 mg, 91%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (m, 2H), 7.69 (m, 2H), 6.44 (m, 1H), 5.70 (m, 1H), 5.11 (m, 1H), 4.42 (m, 1H), 3.77 (s, 3H), 3.67 (m, 5H), 3.51 (m, 1H), 3.17 (m, 1H), 2.62 (m, 2H), 1.57 (s, 9H); HRMS (FAB) m/z 506.2249 [$(M + H)^+$ calcd for $C_{23}H_{32}N_5O_8$ 506.2251].

The methyl carbamate (170 mg, 0.337 mmol) was dissolved in a solution of 1:1 TFA/CH_2Cl_2 (7 mL) and stirred at room temperature for 2 h. The solution was evaporated *in vacuo*, and the residue was purified using reverse phase HPLC to yield the amidine as a colorless solid (103 mg, 59% yield): ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.38 (s, 2H), 9.12 (s, 2H), 8.17 (m, 1H), 7.87 (s, 4H), 7.55 (m, 1H), 5.03 (m, 1H), 4.17 (m, 1H), 3.62 (s, 3H), 3.52 (s, 3H), 3.64−3.15 (m, 4H), 2.50 (m, 2H); HRMS (FAB) *m/z* 406.1743 [(M + H)$^+$ calcd for $C_{18}H_{24}N_5O_6$ 406.1726].

To a solution of the amidine (72 mg, 0.178 mmol) in MeOH (2 mL) and water (1 mL) was added a 1 M LiOH solution (0.26 mL, 0.26 mmol). The reaction mixture was stirred at room temperature for 5 h. Three drops of TFA were then added to the mixture, and it was evaporated *in vacuo*. The residue was purified by reverse phase HPLC to yield **24i** as a colorless solid (31 mg, 34% yield): ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.39 (s, 2H), 9.12 (s, 2H), 8.16 (m, 1H), 7.87 (s, 4H), 7.38 (m, 1H), 5.03 (m, 1H), 4.17 (m, 1H), 3.53 (s, 3H) 3.60−3.15 (m, 4H), 2.63−2.41 (m, 2H); HRMS (FAB) *m/z* 392.1582 [(M + H)$^+$ calcd for $C_{17}H_{21}N_5O_6$ 392.1570].

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(1-methylethoxy)carbonyl]-L-alanine Trifluoroacetate (24j). This compound was synthesized in a manner analogous to that used for the synthesis of **24i**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.43 (s, 2H), 9.35 (s, 2H), 8.15 (m, 1H), 7.87 (m, 4H), 7.19 (m, 1H), 5.15 (m, 1H), 4.74 (m, 1H), 4.15 (m, 1H), 3.60−3.15 (m, 4H), 2.50 (m, 2H), 1.17 (d, J = 6.6 Hz, 3H), 1.15 (d, J = 6.6 Hz, 3H); HRMS (FAB) *m/z* 420.1876 [(M + H)$^+$ calcd for $C_{19}H_{25}N_5O_6$ 420.1883].

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(2-hexyloxy)carbonyl]-L-alanine Trifluoroacetate (24k). This compound was synthesized in a manner similar to that used for **24u**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.42 (s, 2H), 9.21 (s, 2H), 8.16 (m, 1H), 7.86 (m, 4H), 7.45 (m, 1H), 5.02 (m, 1H), 4.12 (m, 1H), 3.90 (m, 2H), 3.50 (m, 2H), 3.26 (m, 2H), 2.50 (m, 2H), 1.55 (m, 2H), 1.26 (m, 4H), 0.88 (m, 3H); MS (ESI) *m/z* 462.3 [(M + H)$^+$, 100]. Anal. ($C_{22}H_{31}N_5O_6 \cdot 1.3CF_3CO_2H$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(phenylmethoxy)carbonyl]-L-alanine Trifluoroacetate (24l). This compound was synthesized in a manner similar to that used for **24u**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.40 (s, 2H), 9.22 (s, 2H), 8.18 (m, 1H), 7.86 (m, 4H), 7.65 (m, 1H), 7.35 (m, 5H), 5.04 (s, 2H), 5.00 (m, 1H), 4.20 (m, 1H), 3.50 (m, 2H), 3.26 (m, 2H), 2.50 (m, 2H); MS (ESI) *m/z* 468.2 [(M + H)$^+$, 100]. Anal. ($C_{23}H_{25}N_5O_6 \cdot CF_3CO_2H \cdot 0.5H_2O$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(2-phenylethoxy)carbonyl]-L-alanine Mono(trifluoroacetate) (24m). This compound was synthesized in a manner similar to that used for **24u**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.44 (s, 2H), 9.22 (s, 2H), 8.18 (m, 1H), 7.86 (m, 4H), 7.52 (m, 1H), 7.25 (m, 5H), 5.02 (m, 1H), 4.16 (m, 2H), 4.05 (m, 1H), 3.50 (m, 2H), 3.26 (m, 2H), 2.86 (m, 2H), 2.50 (m, 2H); MS (ESI) *m/z* 482.5 [(M + H)$^+$, 100]. Anal. ($C_{24}H_{27}N_5O_6 \cdot CF_3CO_2H \cdot 0.7H_2O$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(2-methylpropoxy)carbonyl]-L-alanine Trifluoroacetate (24n). This compound was synthesized using a procedure analogous to that used for **24u**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.40 (s, 2H), 9.21 (s, 2H), 8.16 (m, 1H), 7.86 (s, 4H), 7.45 (m, 1H), 5.02 (m, 1H), 4.12 (m, 1H), 3.90 (m, 2H), 3.50 (m, 2H), 3.26 (m, 2H), 2.50 (m, 2H), 1.82 (m, 1H), 0.88 (m, 6H); MS (ESI) *m/z* 434.3 [(M + H)$^+$, 100]. Anal. ($C_{20}H_{27}N_5O_6 \cdot 1.3CF_3CO_2H$) C, H, N.

(*R*)-3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(3-butenyloxy)carbonyl]-L-alanine Trifluoroacetate (24o). To a solution of (*R*)-**32** (440 mg, 0.765 mmol) in 2:1 water/acetonitrile were added $NaHCO_3$ (160.7 mg, 1.91 mmol) and 3-butenyl chloroformate (0.095 mL, 0.765 mmol). The reaction mixture was stirred at room temperature for 1.5 h. The mixture was then acidified to pH 3−4 by the addition of TFA and concentrated *in vacuo*. The residue was dissolved in CH_3OH (5 mL), and a solution of LiOH (1.1 equiv) in 1.5 mL of water was added. The resulting reaction mixture was stirred at room temperature for 6.5 h. After concentration *in vacuo*, the residue was taken up in 1:1 TFA/CH_2Cl_2 (4 mL) and concentrated to an amber residue which after purification using reverse phase HPLC and concentration *in vacuo* yielded **24o** as a colorless solid (240 mg, 73% yield, >99% de (chiral SFC[30]): ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.40 (s, 2H), 9.17 (s, 2H), 8.17 (t, J = 5.5 Hz, 1H), 7.87 (s, 4H), 7.39 (d, J = 8.4 Hz, 1H), 5.79 (m, 1H), 5.05 (m, 3H), 4.09 (m, 1H), 4.00 (m, 2H), 3.65−3.54 (m, 2H), 3.2 (m, 2H), 2.61−2.41 (m, 2H), 2.31 (dd, J = 7.0, 6.6 Hz, 2H); MS (ESI) *m/z* 432.3 [(M + H)$^+$, 100]; HRMS (FAB) *m/z* 432.1891 [(M + H)$^+$ calcd for $C_{20}H_{26}N_5O_6$ 432.1883]. Anal. ($C_{20}H_{25}N_5O_6 \cdot 1.5CF_3CO_2H$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(2-cyclopentylethoxy)carbonyl]-L-alanine Trifluoroacetate (24p). This compound was synthesized in a manner similar to that used for **24u**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.40 (s, 2H), 9.18 (s, 2H), 8.18 (m, 1H), 7.86 (m, 4H), 7.35 (m, 1H), 5.02 (m, 1H), 4.08 (m, 1H), 3.98 (m, 2H), 3.50 (m, 2H), 3.22 (m, 2H), 2.50 (m, 2H), 1.75 (m, 3H), 1.55 (m, 6H), 1.05 (m, 2H); MS (ESI) *m/z* 474.2 [(M + H)$^+$, 100]. Anal. ($C_{23}H_{31}N_5O_6 \cdot 1.4CF_3CO_2H$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(2-cyclopropylethoxy)carbonyl]-L-alanine Trifluoroacetate (24q). This compound was synthesized in a manner similar to that used for **24u**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.38 (s, 2H), 9.32 (s, 2H), 8.14 (m, 1H), 7.82 (m, 4H), 7.45 (m, 1H), 5.00 (m, 1H), 4.12 (m, 1H), 3.96 (m, 2H), 3.50 (m, 2H), 3.26 (m, 2H), 2.50 (m, 2H), 1.40 (m, 2H), 0.70 (m, 1H), 0.40 (m, 2H), 0.10 (m, 2H); MS (ESI) *m/z* 446.3 [(M + H)$^+$, 100]. Anal. ($C_{21}H_{27}N_5O_6 \cdot 1.4CF_3CO_2H$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[(4,4,4-trifluorobutoxy)carbonyl]-L-alanine Mono(trifluoroacetate) (24r). This compound was synthesized in a manner similar to that used for **24u**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.42 (s, 2H), 9.28 (s, 2H), 8.18 (m, 1H), 7.86 (m, 4H), 7.45 (m, 1H), 5.02 (m, 1H), 4.18 (m, 1H), 4.02 (m, 2H), 3.50 (m, 2H), 3.26 (m, 2H), 2.50 (m, 2H), 2.30 (m, 2H), 1.76 (m, 2H); MS (ESI) *m/z* 488.1 [(M + H)$^+$, 100]. Anal. ($C_{20}H_{24}F_3N_5O_6 \cdot CF_3CO_2H \cdot 0.3H_2O$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[[(4-bromophenyl)methoxy]carbonyl]-L-alanine Mono(trifluoroacetate) (24s). This compound was synthesized in a manner similar to that used for **24u**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.42 (s, 2H), 9.28 (s, 2H), 8.20 (m, 1H), 7.86 (m, 4H), 7.82 (m, 1H), 7.62 (m, 1H), 7.44 (m, 2H), 7.30 (m, 1H), 5.12 (s, 2H), 5.02 (m, 1H), 4.22 (m, 1H), 3.50 (m, 2H), 3.26 (m, 2H), 2.52 (m, 2H); MS (ESI) *m/z* 548.3 [(M + H)$^+$, 100]. Anal. ($C_{23}H_{24}BrN_5O_6 \cdot CF_3CO_2H \cdot 0.5H_2O$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-[[(2-chlorophenyl)methoxy]carbonyl]-L-alanine Mono(trifluoroacetate) (24t). This compound was synthesized in a manner similar to that for **24u**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.40 (s, 2H), 9.16 (s, 2H), 8.20 (m, 1H), 7.86 (m, 4H), 7.66 (m, 1H), 7.48 (m, 2H), 7.38 (m, 2H), 5.12 (s, 2H), 5.02 (m, 1H), 4.18 (m, 1H), 3.50 (m, 2H), 3.26 (m, 2H), 2.50 (m, 2H); MS (ESI) *m/z* 502.4 [(M + H)$^+$, 100]. Anal. ($C_{23}H_{24}ClN_5O_6 \cdot CF_3CO_2H \cdot 0.5H_2O$) C, H, N.

Methyl (*R*)-3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(butoxycarbonyl)-L-alanine Hydrochloride (24v). Following the general procedure described for the preparation of **25b**, (*R*)-**15** (1.0 g, 4.3 mmol) was coupled to **10u** (1.27 g, 5.00 mmol) to yield the nitrile as a colorless solid (1.75 g, 95%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.12 (t, J = 7.0 Hz, 1H), 7.94 (d, J = 7.4 Hz, 2H), 7.83 (d, J = 7.4 Hz, 2H), 7.46 (d, J = 7.6 Hz, 1H), 5.04 (m, 1H), 4.16 (m, 1H), 3.96 (t, J = 6.4 Hz, 2H), 3.64 (s, 3H), 3.58 (dd, J = 16.0, 8.0 Hz, 1H), 3.40 (m, 2H), 3.20 (dd, J = 16.0, 8.0 Hz, 1H), 2.56 (dd, J = 15.0, 7.5 Hz, 1H), 2.43 (dd, J = 15.0, 7.5 Hz, 1H), 1.52 (m, 2H), 1.32 (m, 2H), 0.88 (t, J = 7.3 Hz, 3H).

Into a solution of the nitrile (1.7 g, 4.0 mmol) in MeOH (50 mL) at 0 °C was bubbled HCl gas for 1 h. The solution was stirred at room temperature for 5 h, and after concentration

in vacuo the residue was taken up in MeOH (20 mL) and $(NH_4)_2CO_3$ (1.1 g, 11 mmol) was added. The mixture was stirred at room temperature overnight and then concentrated *in vacuo*. The solid residue was purified using column chromatography ($CHCl_3$:MeOH = 90:10) to give **24v** (1.0 g, 45%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.40 (m, 4H), 8.22 (t, J = 7.0 Hz, 1H), 7.86 (m, 4H), 7.47 (d, J = 7.6 Hz, 1H), 5.00 (m, 1H), 4.16 (m, 1H), 3.91 (t, J = 6.4 Hz, 2H), 3.60 (s, 3H), 3.50 (m, 2H), 3.26 (m, 2H), 2.50 (m, 2H), 1.52 (m, 2H), 1.32 (m, 2H), 0.88 (t, J = 7.3 Hz, 3H); MS (ESI) m/z 448.3 [(M + H)$^+$, 100]. Anal. ($C_{21}H_{29}N_5O_6$·1.3HCl) C, H, N.

(*R*)-3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(butoxycarbonyl)-L-alanine Trifluoroacetate (24w). A solution of **24v** (600 mg, 1.24 mmol) in MeOH (24 mL) and water (24 mL) was cooled in an ice bath. LiOH (1 N, 1.3 mL, 1.3 mmol) was added, and the solution was stirred at room temperature for 5 h. The solvents were removed *in vacuo* at room temperature, and the crude product was purified using reverse phase HPLC to afford **24w** (610 mg, 90%) as a colorless powder: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.42 (s, 2H), 9.21 (s, 2H), 8.16 (t, J = 7.0 Hz, 1H), 7.86 (m, 4H), 7.45 (d, J = 7.6 Hz, 1H), 5.02 (m, 1H), 4.12 (m, 1H), 3.90 (t, J = 6.4 Hz, 2H), 3.50 (m, 2H), 3.26 (m, 2H), 2.50 (m, 2H), 1.52 (m, 2H), 1.32 (m, 2H), 0.88 (t, J = 7.3 Hz, 3H); MS (ESI) m/z 434.3 [(M + H)$^+$, 100]. Anal. ($C_{20}H_{27}N_5O_6$·CF_3CO_2H·0.5H_2O) C, H; N: calcd, 12.58; found, 11.26.

(*S*)-3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(butoxycarbonyl)-D-alanine Trifluoroacetate (24x). The title compound was synthesized following the procedure used for **24w**:[51] ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.99 (m, 1H), 7.71 (d, J = 8.4 Hz, 2H), 7.67 (d, J = 8.8 Hz, 2H), 6.47 (d, J = 7.3 Hz, 1H), 4.92 (m, 1H), 3.95 (t, J = 6.6 Hz, 2H), 3.84 (m, 1H), 3.63 (m, 1H), 3.33 (s, 7H), 3.27 (m, 1H), 2.69 (dd, J = 12.6, 4.8 Hz, 1H), 2.33 (m, 1H), 1.54 (d, J = 6.6 Hz, 2H), 1.34 (t, J = 7.3 Hz, 2H), 0.90 (t, J = 7.3 Hz, 3H); HRMS (FAB) m/z 434.2040 [(M + H)$^+$ calcd for $C_{20}H_{27}N_5O_6$ 434.2029].

(*S*)-3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(butoxycarbonyl)-L-alanine Hydrochloride (24y). The title compound was synthesized following the procedure used for **24w**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.95 (m, 1H), 7.79 (s, 4H), 6.52 (d, J = 7.7 Hz, 1H), 4.97 (m, 1H), 3.94 (t, J = 6.6 Hz, 2H), 3.86 (m, 1H), 3.6−3.4 (m, 2H), 3.20 (m, 1H), 2.60 (dd, J = 17.0, 8.1, Hz, 1H), 2.4 (dd, J = 13.6, 9.5 Hz, 1H), 1.53 (m, 2H), 1.33 (m, 2H), 0.89 (t, J = 7.3 Hz, 3H); MS (NH_3-CI) m/z 434 [(M + H)$^+$, 100]. Anal. ($C_{20}H_{27}N_5O_6$·0.3HCl) C, H, N.

(*R*)-3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(butoxycarbonyl)-D-alanine Monohydrochloride (24z). The title compound was synthesized following the procedure used for **24w**:[51] ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.94 (m, 1H), 7.77 (s, 4H), 6.5 (d, J = 7.7 Hz, 1H), 3.94 (t, J = 6.6 Hz, 3H), 3.85 (m, 1H), 3.63−3.43 (m, 3H), 3.28−3.18 (m, 2H), 2.61 (dd, J = 13.4, 5.1 Hz, 1H), 2.41 (dd, J = 13.5, 9.2 Hz, 1H), 1.53 (m, 2H), 1.33 (m, 2H), 0.89 (t, J = 7.3 Hz, 3H); MS (NH_3-CI) m/z 434 [(M + H)$^+$, 100]. Anal. ($C_{20}H_{27}N_5O_6$·HCl·H_2O) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-4-[(phenylethyl)amino]-4-oxobutanoic Acid Mono(trifluoroacetate) (25a). This compound was synthesized following the synthesis reported for **25b**. Analytical reverse phase HPLC showed two equal peaks, which were ascribed to the diastereomers: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.40 (s, 2H), 9.26 (s, 2H), 8.33 (dd, J = 8.42, 8.79 Hz, 1H), 7.88 (s, 4H), 7.98−7.85 (m, 1H), 7.31−7.17 (m, 5H), 5.05 (m, 1H), 4.56 (m, 1H), 3.61−3.51 (m, 1H), 3.26−3.18 (m, 3H), 2.73−2.59 (m, 4H), 2.53−2.40 (m, 2H); MS (ESI) m/z 466 [(M + H)$^+$, 100]. Anal. ($C_{24}H_{27}N_5O_5$·CF_3CO_2H·H_2O) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-4-oxo-4-[(tricyclo[3.3.1.1^{3,7}]dec-1-ylmethyl)amino]butanoic Acid Trifluoroacetate (25c). This compound was synthesized following the general procedure used for **25b**: ^{1}H NMR (300 MHz, CD_3OD) δ 7.87 (AB quartet, J = 8.6 Hz, Δ = 18.3 Hz, 4H), 7.75 (m, 1H), 5.13 (m, 1H), 4.71 (m, 1H), 3.56 (dd, J = 17.2, 10.6 Hz, 1H), 3.31 (m, 1H, partially coincident with solvent), 3.31−2.58 (m, 5H), 1.92 (bs, 3H), 1.68 (AB quartet (broad), J = 11.9 Hz, Δ = 22.5 Hz, 5H), 1.50 (d, J = 2.2 Hz, 5H); MS (ESI) m/z 510 [(M + H)$^+$, 100]. Anal. ($C_{27}H_{35}N_5O_5$·1.6CF_3CO_2H) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-4-oxo-4-(tricyclo[3.3.1.1^{3,7}]dec-2-ylamino)butanoic Acid Trifluoroacetate (25d). This compound was synthesized following the general sequence described for **25b**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.40 (s, 2H), 9.22 (s, 2H), 8.41 (dd, J = 14.6, 7.9 Hz, 1H), 7.88 (m, 4H), 7.67 (t, J = 7.0 Hz, 1H), 5.06 (m, 1H), 4.67 (m, 1H), 3.79 (d, J = 6.6 Hz, 1H), 3.57 (dd, J = 17.2, 10.6 Hz, 1H), 3.23 (m, 1H), 2.69−2.44 (m, 5H), 1.94−1.68 (m, 10H), 1.49 (bd, J = 12.5 Hz, 2H); MS (ESI) m/z 496 [(M + H)$^+$, 100]. Anal. ($C_{26}H_{33}N_5O_5$·1.7CF_3CO_2H) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-4-(4-thiopyridyl)butanoic Acid Bis(trifluoroacetate) (25e). The title compound was synthesized following the general procedure reported for **25b**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.3 (bs, 1H), 9.37 (bs, 2H), 9.16 (bs, 2H), 8.58 (d, J = 9 Hz, 1H), 7.88 (m, 4H), 6.51 (s, 1H), 5.04 (m, 2H), 3.91 (m, 1H), 3.74 (m, 2H), 3.57 (m, 2H), 3.23 (dd, J = 17.1, 7.3 Hz, 1H), 2.78 (dd, J = 16.4, 9.2 Hz, 1H), 2.69−2.45 (m, 4H, coincident with DMSO-d_5), 2.38 (dd, J = 16.4, 5.1 Hz, 1H); MS (ESI) m/z 448 [(M + H)$^+$, 100]. Anal. ($C_{20}H_{25}N_5O_5S$·2CF_3CO_2H) C, H, N.

β-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-3,4-dihydro-γ-oxo-2(1*H*)-isoquinolinebutanoic Acid Trifluoroacetate (25f). The title compound was synthesized following the general procedure reported for **25b**: ^{1}H NMR (300 MHz, CD_3OD) δ 7.84 (m, 5H), 7.14 (m, 4H), 5.31 (m, 1H), 5.11 (m, 1H), 4.75−4.55 (m, 2H), 3.84 (m, 2H), 3.66−3.34 (m, 2H), 3.14 (dd, J = 17.2, 7.3 Hz, 1H), 2.90 (m, 3H), 2.66−2.46 (m, 3H); MS (ESI) m/z 478 [(M + H)$^+$, 100]. Anal. ($C_{25}H_{27}N_5O_5$·1.8CF_3CO_2H) H, N; C: calcd, 50.31; found, 49.89.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-4-[butyl(phenylmethyl)amino]-4-oxobutanoic Acid Trifluoroacetate (25g). This compound was synthesized following the method reported for **25b**. Analytical HPLC showed two equal peaks, which were ascribed to the diastereomers: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.40 (s, 2H), 9.33 (s, 2H), 8.63 (t, J = 7.2 Hz, 1H), 7.87 (m, 4H), 7.31 (m, 3H), 7.22 (m, 2H), 5.17−4.84 (m, 2H), 4.55 (m, 2H), 3.61−3.42 (m, 1H), 3.26−3.06 (m, 2H), 2.82 (m, 1H), 2.68−2.23 (m, 4H), 1.53 (m, 1H), 1.37 (m, 1H), 1.21 (m, 2H), 0.84 (m, 3H); MS (ESI) m/z 508 [(M + H)$^+$, 100]; HRMS (FAB) m/z 508.2572 [(M + H)$^+$ calcd for $C_{27}H_{34}N_5O_5$ 508.255995]. Anal. ($C_{27}H_{33}N_5O_5$·1.5CF_3CO_2H) C, H, N.

β-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]benzenebutanoic Acid Trifluoroacetate (26e). A solution of phenylacetaldehyde (12.02 g, 0.1 mol) and methyl(triphenylphosphoranylidene)acetate (33.44 g, 0.1 mol) in THF was stirred at reflux for 5 h. The reaction mixture was concentrated *in vacuo* and the residue purified using column chromatography (hexane:EtOAc = 8:2). The desired alkene was obtained as a clear liquid (12.64 g, 72%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40−7.10 (m, 5H), 6.55−6.25 (m, 1H), 5.90−5.80 (m, 1H), 3.75 (s, 3H), 3.55 (dd, J = 6.6, 2.8 Hz, 1H), 2.25 (dd, J = 6.9, 2.7 Hz, 1H).

A mixture of the alkene (3.52 g, 0.02 mol) and (*R*)-methylbenzylamine (8.46 g, 0.04 mol) was heated at 110 °C over 94 h. The cooled reaction mixture was purified using flash chromatography (hexane:EtOAc = 9:1) to afford the secondary amine (4.81 g, 31%) as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40−7.10 (m, 17H), 3.95−3.85 (m, 1H), 3.75−3.60 (m, 4H), 3.45 (s, 2H), 2.95−2.85 (m, 1H), 2.65−2.55 (m, 1H), 2.15 (d, J = 6.9 Hz, 1H), 1.20 (d, J = 6.9 Hz, 2H).

A mixture of the secondary amine (4.0 g, 0.0103 mol), 20% $Pd(OH)_2$/C (2.0 g), cyclohexene (0.36 mol, 36.52 mL), glacial HOAc (0.61 mL, 0.0103 mol), and MeOH (70 mL) was heated at reflux under N_2 for 20 h. After cooling, the catalyst was removed by filtration through a Celite plug and washed with MeOH, and the filtrate was concentrated *in vacuo*. The residue was triturated with hexane to afford amine **8e** as a colorless solid (1.22 g, 47%): mp 94−96 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.35−7.15 (m, 5H), 6.00 (bs, 2H), 3.70 (s, 3H),

3.60−3.50 (m, 1H), 2.85−2.70 (m, 2H), 2.60−2.40 (m, 2H), 2.05 (s, 3H); $[\alpha]^{25}_D$ −2.15° (c = 0.186, MeOH).

The amine **8e** was used in the general coupling procedure previously described in the synthesis of **25b** to afford amide **23e** as a colorless solid (0.204 g, 63%) and a 1:1 mixture of diastereomers: mp 174−176 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.35 (bs, 4H), 8.10−8.05 (m, 1H), 7.90−7.80 (m, 4H), 7.30−7.10 (m, 5H), 5.10−4.90 (m, 1H), 4.40−4.10 (m, 1H), 3.60 (s, 3H), 3.50−3.45 (m, 1H), 3.20−2.95 (m, 2H), 2.80−2.70 (m, 2H), 2.50−2.30 (m, 2H).

Using the procedure described for the synthesis of **25b**, amide **23e** afforded **26e** as a colorless solid (20 mg, 27%, 1:1 mixture of diastereomers): mp 219−222 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.30 (bs, 1H), 9.40 (s, 1H), 9.10 (s, 1H), 8.10−8.00 (m, 1H), 7.90−7.80 (m, 6H), 7.50−6.00 (m, 4H), 5.45−5.15 (m, 2H), 5.00−4.90 (m, 1H), 4.10−4.00 (m, 1H), 3.00−2.70 (m, 2H), 2.45−2.40 (m, 2H), 1.90−1.70 (m, 2H). Anal. ($C_{22}H_{24}N_4O_4 \cdot 1.17CF_3CO_2H \cdot 0.2H_2O$) C, H; F: calcd, 10.27; found, 9.79.

(*R,S*)-3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-3-methylbutanoate Trifluoroacetate (26a). The compound was prepared following the coupling procedure presented for **26e** using *gem*-dimethyl-β-alanine[52,53] as the β-alanine moiety: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.36 (bs, 2H), 8.99 (bs, 2H), 7.87 (s, 4H), 7.67 (s, 1H), 5.00 (m, 1H), 3.56−3.50 (dd, J = 10.5, 17.0 Hz, 1H), 3.31 (s, 2H), 3.24−3.18 (dd, J = 7.6, 17.0 Hz, 1H), 2.55 (m, 1H), 2.40−2.35 (dd, J = 7.6, 14.2 Hz, 1H), 1.31 (s, 3H), 1.30 (s, 3H); HRMS (FAB) m/z 347.1719 [(M + H)$^+$ calcd for $C_{17}H_{23}N_4O_4$ 347.1709]. Anal. ($C_{17}H_{22}N_4O_4 \cdot 1.3CF_3CO_2H$) C, H, N.

β-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-2-pyridinepentanoic Acid Trifluoroacetate (26b). This material was prepared as previously described in the synthesis of **26e**. The crude material was triturated with cold Et_2O to afford an amorphous solid (182 mg, 66%) as a 1:1 mixture of diastereomers: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.60 (s, 2H), 9.40 (s, 2H), 8.80−8.70 (m, 1H), 8.35−8.25 (m, 1H), 8.20−7.80 (m, 8H), 5.10−5.00 (m, 1H), 4.10−4.00 (m, 1H), 3.40−3.20 (m, 1H), 3.20−2.90 (m, 2H), 2.60−2.40 (m, 4H), 2.10−1.80 (m, 2H). Anal. ($C_{22}H_{25}N_5O_4 \cdot 2.5CF_3CO_2H$) C, H, N.

β-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-3-pyridinepentanoic Acid Trifluoroacetate (26c). This material was prepared as previously described in the procedure for **26e**. The crude product was triturated with cold Et_2O to yield an amorphous solid (911 mg, 87%) as a 1:1 mixture of diastereomers: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.70 (s, 2H), 9.50 (s, 2H), 8.80−8.70 (m, 2H), 8.35 (s, 2H), 8.00−7.80 (m, 5H), 5.10−5.00 (m, 1H), 4.10−4.00 (m, 2H), 3.30−3.20 (m, 2H), 2.70−2.60 (m, 1H), 2.50−2.40 (m, 4H), 1.90−1.70 (m, 2H). Anal. ($C_{22}H_{25}N_5O_4 \cdot 2.2CF_3CO_2H$) C, H, N.

β-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-4-pyridinepentanoic Acid Trifluoroacetate (26d). This material was prepared as previously described in the procedure for **26e**. The crude product was triturated with cold Et_2O to yield an amorphous solid (120 mg, 42%) as a 1:1 mixture of diastereomers: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.70 (s, 2H), 9.50 (s, 2H), 8.80−8.70 (m, 2H), 8.35−8.25 (m, 1H), 8.00−7.80 (m, 8H), 5.10−5.00 (m, 1H), 4.10−4.00 (m, 2H), 3.00−2.70 (m, 3H), 2.45−2.40 (m, 2H), 1.90−1.70 (m, 2H). Anal. ($C_{22}H_{25}N_5O_4 \cdot 2.2CF_3CO_2H$) C, H, N.

β-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]benzenebutanoic Acid Hydrochloride (26f). This material was prepared as previously described in the procedure for **26e** to afford a colorless solid (34 mg, 27%) as a 1:1 mixture of diastereomers: mp 85−90 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.28 (bs, 1H), 9.45 (s, 1H), 9.10 (s, 1H), 8.15−8.00 (m, 1H), 7.90−7.80 (m, 6H), 7.50−6.00 (m, 4H), 5.50−5.10 (m, 2H), 5.00−4.90 (m, 1H), 4.10−4.00 (m, 1H), 3.00−2.70 (m, 2H), 2.45−2.40 (m, 2H), 1.90−1.70 (m, 2H). Anal. ($C_{22}H_{24}N_4O_4 \cdot 1.4HCl$) C, H, N, Cl.

(*R,R)-β-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl*]amino]-3-pyridinepropanoic Acid Trifluoroacetate (26g).** The title compound was synthesized following the procedure used for **24w** to yield a colorless foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.48 (bs, 1.5H), 9.40 (bs, 1.5H), 8.82 (d, J = 7.8 Hz, 1H), 8.65 (d, J = 8.0 Hz, 2H), 8.30 (m, 1H), 7.80 (m, 4H), 6.16 (s, 1H), 5.24 (m, 1H), 5.00 (m, 1H), 3.48 (m, 1H), 3.18 (dd, J = 8.0, 16.0 Hz, 1H), 2.80 (m, 2H), 2.54 (m, 2H); MS (ESI) m/z 396 [(M + H)$^+$, 20]; HRMS (FAB) m/z 396.1679 [(M + H)$^+$ calcd for $C_{20}H_{22}N_5O_4$ 396.1672].

(*R,S*)-3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]pentanedioic Acid Trifluoroacetate (26h). This material was synthesized following the procedure reported for **26e**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 12.30 (s, 1H), 9.38 (bs, 1.5H), 9.14 (bs, 1.5H), 8.08 (d, J = 7.7, 1H), 7.87 (s, 3H), 5.01 (m, 1H), 4.31 (m, 1H), 3.54 (dd, J = 17.3, 10.7, 1H), 3.20 (dd, J = 17.2, 7.3, 1H), 2.46 (m, 6H). Anal. ($C_{17}H_{20}N_4O_6 \cdot 1.4CF_3CO_2H$) C, H, F; N: calcd, 10.79; found, 9.44.

(*R,R)-3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl*]amino]pentanoic Acid Trifluoroacetate (26i).** The title compound was synthesized following the procedure used for **24w**: ^{1}H NMR (300 MHz, CD_3OD) δ 7.89 (AB quartet, J = 8.4 Hz, Δ = 18.3 Hz, 4H), 5.14 (m, 1H), 4.13 (m, 1H), 3.60 (dd, J = 17.2, 10.6 Hz, 1H), 3.3 (m, coincident with solvent), 2.69 (dd, J = 14.3, 6.2 Hz, 1H), 2.57 (dd, J = 14.3, 6.6 Hz, 1H), 2.49 (d, J = 6.6 Hz, 2H), 1.62, (m, 1H), 1.50 (m, 1H), 0.94 (t, J = 7.3 Hz, 3H); MS (ESI) m/z 347 (M + H$^+$, 100). Anal. ($C_{17}H_{22}N_4O_4 \cdot CF_3CO_2H \cdot 0.5H_2O$) C, H, N.

Methyl 3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-5-methylhexanoate Hydrochloride (26j). This material was synthesized following the procedure reported for **26e**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.22 (bs, 2H), 7.88 (m, 5H), 5.03 (m, 1H), 4.13 (m, 1H), 3.57 (m, 1H), 3.58 (s, 3H), 3.23 (dd, J = 17.2, 7.3 Hz, 1H), 2.50 (m, 2H), 2.40 (d, J = 6.5 Hz, 2H), 1.58 (m, 1H), 1.36 (m, 1H), 1.21 (m, 1H), 0.84 (m, 6H); IR (KBr pellet, cm^{-1}) 3204−2956, 1734, 1676, 1646; $[\alpha]^{25}_D$ = −72.55° (c = 0.102, MeOH). Anal. ($C_{20}H_{28}N_4O_4 \cdot 1.3HCl \cdot 0.9H_2O$) C, H, N, Cl.

Methyl 3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-4-(dimethylamino)butanoate Bis(trifluoroacetate) (26l) and Methyl 3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-4-(dimethylamino)butanoate Bis(trifluoroacetate) (26m). To a solution of **7** (10.0 g, 40 mmol) in EtOAc (200 mL) was added Et_3N (18.4 mL, 132 mmol), dimethylamine hydrochloride (3.30 g, 40 mmol), and TBTU (13.0 g, 41 mmol), and the mixture was stirred overnight at room temperature under a N_2 atmosphere. The resulting solution was extracted with 5% aqueous citric acid (2×), saturated $NaHCO_3$, and saturated NaCl. The organic layer was separated, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The crude product was purified using column chromatography on silica gel (50−100% EtOAc/hexane) to obtain the amide as an orange oil (7.2 g, 65%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.38 (d, J = 9.2 Hz, 1H), 5.00 (m, 1H), 3.69 (s, 3H), 3.15 (s, 3H), 2.97 (s, 3H), 2.77 (dd, J = 15.8, 7.6 Hz, 1H), 2.59 (dd, J = 15.7, 5.9 Hz, 1H), 1.43 (s, 9H); IR (KBr pellet, cm^{-1}) 3294, 1730, 1666, 1632; $[\alpha]^{25}_D$ = −3.30° (c = 0.364, CH_2Cl_2). Anal. Calcd for $C_{12}H_{22}N_2O_5$: C, 52.53; H, 8.10; N, 10.21. Found: C, 52.74; H, 8.11; N, 10.02.

The amide (3.0 g, 11 mmol) was dissolved in THF (anhydrous, 25 mL) and cooled to 0 °C under a N_2 atmosphere. A 1.0 M solution of BH_3 in THF (22.0 mL, 22.0 mmol) was added dropwise over 30 min. The solution was stirred an additional 10 min at 0 °C before being warmed gradually to reflux. After being heated at reflux overnight, the solution was cooled to room temperature and MeOH (25 mL) was added dropwise over 75 min. The resulting mixture was heated at reflux for an additional 2 h and then concentrated *in vacuo*. The clear oil was evaporated from MeOH (2×) and purified using column chromatography on silica gel (2−10% MeOH/$CHCl_3$) to give the desired amine (948 mg, 33%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.09 (bs, 1H), 3.97 (m, 1H), 3.68 (s, 3H), 2.71 (dd, J = 15.4, 4.8 Hz, 1H), 2.53 (dd, J = 15.7, 5.8 Hz, 1H), 2.35 (d, J = 6.9 Hz, 2H), 2.23 (s, 6H), 1.44 (s, 9H); IR (KBr pellet, cm^{-1}) 3368, 1740, 1716; $[\alpha]^{25}_D$ = +0.96° (c = 0.520, CH_2Cl_2). Anal. Calcd for $C_{12}H_{24}N_2O_4$: C, 55.35; H, 9.31; N, 10.76. Found: C, 55.36; H, 9.45; N, 10.89.

The amine (860 mg, 3.3 mmol) was dissolved in 4 M HCl/dioxane (5 mL) and stirred under a N_2 atmosphere for 2 h. The resulting solution was triturated with Et_2O and filtered to yield the diamine **9l** as a colorless solid (693 mg, 90%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.71 (bs, 2H), 3.99 (m, 1H), 3.67 (s, 3H), 3.45 (dd, J = 14.3, 7.7 Hz, 1H), 3.35 (dd, J = 13.9, 2.9 Hz, 1H), 2.92 (d, J = 6.6 Hz, 2H), 2.86 (s, 6H); IR (KBr pellet, cm^{-1}) 2946, 1722; $[\alpha]^{25}_D$ = +0.32° (c = 0.628, MeOH); HRMS (NH_3-CI) m/z 161.1289 [(M + H)$^+$ calcd for $C_7H_{17}N_2O_2$ 161.1290].

Synthesized from **9l** following the procedure reported for **26e** was a diastereomeric mixture containing **26l** and **26m**. These materials were separated using preparative reverse phase HPLC. **26l**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.39 (bs, 1H), 9.09 (bs, 1H), 8.21 (d, J = 8.4 Hz, 1H), 7.88 (s, 4H), 5.05 (m, 1H), 4.46 (m, 1H), 3.63 (s, 3H), 3.43 (m, 3H), 3.21 (m, 3H), 2.83 (s, 6H), 2.59 (m, 2H). Anal. ($C_{19}H_{27}N_5O_4 \cdot 2CF_3CO_2H \cdot H_2O$) C, H, N, F. **26m**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.40 (bs, 1H), 9.18 (bs, 1H), 8.23 (d, J = 8.8 Hz, 1H), 7.88 (s, 4H), 5.05 (m, 1H), 4.46 (m, 1H), 3.63 (s, 3H), 3.48 (m, 3H), 3.23 (m, 3H), 2.58 (m, 2H), 2.82 (d, J = 4.4 Hz, 6H). Anal. ($C_{19}H_{27}N_5O_4 \cdot 2CF_3CO_2H \cdot H_2O$) C, H, N, F.

Methyl *β*-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-1-pyrrolidinebutanoate Bis(trifluoroacetate) (26k). Synthesized following the procedure reported for **26l**. The product was isolated as a 1:1 mixture of diastereomers: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.40 (bs, 2H), 9.15 (bs, 1H), 8.25 (m, 1H), 7.88 (m, 4H), 5.06 (m, 1H), 4.44 (m, 1H), 3.64 (s, 1.5 H), 3.62 (s, 1.5 H), 3.49 (m, 3H), 3.25 (m, 3H), 3.07 (m, 2H), 2.54 (m, 4H), 2.01 (m, 2H), 1.87 (m, 2H); IR (KBr pellet, cm^{-1}) 3314, 1736, 1668. Anal. ($C_{21}H_{29}N_5O_4 \cdot 2CF_3CO_2H$) C, H, N, F.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-5-isoxazolyl]-acetyl]amino]-*N*-(butoxycarbonyl)-L-alanine Trifluoroacetate (27). To a suspension of **28** (5.28 g, 21.62 mmol) in $CHCl_3$ (150 mL) was added NBS (4.23 g, 23.78 mmol) and AIBN (100 mg), and the mixture was warmed to reflux.[54] A small amount of AIBN (100−200 mg) was added at 1 h intervals until TLC indicated a complete reaction. Potassium acetate (17.3 g) and HOAc (6.5 mL) were added, and the reaction mixture was heated at reflux for 1 h, cooled, and then poured into 1 N NaOH (325 mL). The organic layer was separated, the aqueous layer was extracted with EtOAc (3 × 100 mL), and the organic layers were combined and washed with saturated NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified using column chromatography on silica gel (15% to 35% EtOAc in hexane) to yield ester **29** as an off-white solid (2.2 g, 42%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (m, 2H), 7.76 (m, 2H), 6.67 (s, 1H), 3.92 (s, 2H), 3.8 (s, 3H).

A suspension of **29** (2.19 g, 9.04 mmol) in MeOH (anhyd, 100 mL) was chilled in an ice bath, and dry HCl gas was bubbled over 2 h through the reaction mixture until a solution was obtained. The reaction flask was sealed and the reaction mixture allowed to warm to room temperature, with stirring, over a period of 24 h. The methanolic solution was poured into Et_2O (anhyd, 500 mL), precipitating the product, and the resulting slurry was chilled to −25 °C for 3 h. The precipitate was filtered, washed with chilled Et_2O (anhyd, 2 × 100 mL), and suction-dried under nitrogen to afford the imidate (2.3 g, 82%): ^{1}H NMR (300 MHz, suspension in $CDCl_3$) δ 8.52 (d, J = 8.06 Hz, 2H), 8.03 (d, J = 8.4 Hz, 2H), 6.67 (s, 1H), 4.6 (s, 3H), 3.93 (s, 2H), 3.8 (s, 3H).

A solution of the imidate (2.3 g, 7.4 mmol) in MeOH (anhyd, 50 mL) was chilled in an ice bath, and 2 M ammonia in MeOH (18.5 mL, 37 mmol) was added. The reaction flask was sealed, and the reaction mixture was allowed to warm to room temperature, with stirring, over a period of 24 h. The amber solution was then concentrated *in vacuo* to give the amidine as a yellow foam (2.2 g, quantitative yield): ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.12 (d, J = 8.4 Hz, 2H), 7.97 (d, J = 8.4 Hz, 2H), 7.14 (s, 1H), 4.15 (s, 2H), 3.70 (s, 3H).

To an ice-cold solution of the amidine (2.2 g, 7.4 mmol) in DMF (30 mL) were added Et_3N (2.06 mL, 14.8 mmol) and di-*tert*-butyl dicarbonate (1.78 g, 8.14 mmol). The reaction mixture was warmed to room temperature and stirred for 64 h. The reaction mixture was then partitioned between EtOAc and water, the aqueous layer was washed with EtOAc, and the organic layers were combined, washed with water and saturated NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo*. The residue was purified using column chromatography on silica gel (15% to 25% EtOAc in hexane) to afford the Boc-protected amidine (1.45 g, 54%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (d, J = 8.4 Hz, 2H), 7.87 (d, J = 8.4 Hz, 2H), 6.65 (s, 1H), 3.91 (s, 2H), 3.80 (s, 3H), 1.56 (s, 9H).

To a solution of the Boc-protected amidine (1.45 g, 4.03 mmol) in MeOH (30 mL) was added a solution of $LiOH \cdot H_2O$ (0.195 g, 4.64 mmol) in water (5 mL). The mixture was stirred at room temperature for 16 h and concentrated *in vacuo*, the residue was diluted with water, and the resulting mixture was cooled using an ice bath. HCl (1 N) was slowly added until the solution had a pH of 3−4, and the resulting acidic aqueous mixture was extracted repeatedly with EtOAc. The organic layers were combined, washed with saturated NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to yield the acid as an off-white powdery solid (0.97 g, 70%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.07 (d, J = 8.8 Hz, 2H), 7.97 (d, J = 8.4 Hz, 2H), 7.03 (s, 1H), 3.99 (s, 2H), 1.45 (s, 9H).

To a solution of the acid (0.262 g, 0.76 mmol), **10u** (0.193 g, 0.76 mmol), and TBTU (0.256 g, 0.8 mmol) in DMF (15 mL) was added Et_3N (0.45 mL, 3.23 mmol), and the resulting reaction mixture was allowed to stir at room temperature for 16 h. The reaction mixture was partitioned between EtOAc and water, the water layer was washed with EtOAc (2×), and the organic layers were combined, washed with water, pH 4 buffer, 5% $NaHCO_3$, and saturated NaCl, dried (Na_2SO_4), filtered, and evaporated *in vacuo*. The residue was purified using column chromatography on silica gel (100% EtOAc) to yield the amide as an amber foam (0.315 g, 76%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (d, J = 8.4 Hz, 2H), 7.83 (d, J = 8.4 Hz, 2H), 6.60 (s, 1H), 6.57 (m, 1H), 5.66 (m, 1H), 4.45 (m, 1H), 4.05 (m, 2H), 3.77 (s, 5H), 3.70 (m, 2H), 1.57 (s, 9H), 1.56 (m, 2H), 1.35 (m, 2H), 0.90 (t, J = 7.3 Hz, 3H).

A solution of the amide (0.215 g, 0.39 mmol) in 1:1 CH_2Cl_2/TFA (20 mL) was stirred at room temperature for 16 h. The reaction mixture was then concentrated *in vacuo* and the residue purified using column chromatography on silica gel (10% to 30% MeOH in $CHCl_3$) to yield the amidine as a colorless solid (0.11 g, 50%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.40 (bs, 2H), 9.15 (bs, 2H), 8.45 (m, 1H), 8.11 (d, J = 8.42 Hz, 2H), 7.94 (d, J = 8.42 Hz, 2H), 7.53 (d, J = 8.1 Hz, 1H), 7.01 (s, 1H), 4.21 (m, 1H), 3.95 (m, 2H), 3.81 (s, 2H), 3.62 (s, 3H), 3.55 (m, 1H), 3.34 (m, 1H), 1.50 (m, 2H), 1.30 (m, 2H), 0.87 (t, J = 7.3 Hz, 3H); MS (ESI) m/z 446.3 [(M + H)$^+$, 100].

To a solution of the amidine (50 mg, 0.09 mmol) in MeOH (2 mL) was added a 1 M aqueous solution of LiOH (0.18 mL, 0.18 mmol) and water (1 mL). The mixture was stirred at room temperature for 16 h and then evaporated *in vacuo*, and the residue was dissolved in 1:1 TFA/CH_2Cl_2 (3 mL) and again evaporated *in vacuo*. The residue was purified using reverse phase HPLC to yield **27** as a colorless solid (31 mg, 63%) of >98% purity by analytical HPLC: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.40 (bs, 2H), 9.14 (bs, 2H), 8.42 (m, 1H), 8.10 (d, J = 8.4 Hz, 2H), 7.94 (d, J = 8.4 Hz, 2H), 7.36 (d, J = 8.4, 1H), 7.00 (s, 1H), 4.11 (m, 1H), 3.94 (t, J = 6.6 Hz, 2H), 3.81 (s, 2H), 3.56 (m, 1H), 3.30 (m, 1H), 1.50 (m, 2H), 1.31 (m, 2H), 0.87 (t, J = 7.3 Hz, 3H). MS (ESI) m/z 432.4 (M + H$^+$, 100); HRMS (FAB) m/z 432.1888 [(M + H)$^+$ calcd for $C_{20}H_{25}N_5O_6$ 432.1883]. Anal. ($C_{20}H_{25}N_5O_6 \cdot 1.3CF_3CO_2H$) C, H, N.

***N*-(Butoxycarbonyl)-3-[[[4,5-dihydro-3-(4-piperidinyl)-5-isoxazolyl]acetyl]amino]-L-alanine Trifluoroacetate (36a).** A solution of 4-*N*-(*tert*-butoxycarbonyl)piperidinecarboxaldehyde[55] (7.3 g, 34 mmol) and hydroxylamine hydrochloride (2.86 g, 41 mmol) in MeOH (50 mL) at 0 °C was treated with 3 M NaOH (13.7 mL, 41 mmol), and the resulting mixture was allowed to warm to room temperature overnight. The MeOH was removed *in vacuo*, and the aqueous layer was extracted with EtOAc (2 × 100 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield the oxime as a colorless solid (6.4 g, 82%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (s, 1H), 7.36 (d, J = 5.5 Hz, 1 H), 4.16 (m, 2H), 2.84 (t, J = 12.0 Hz,

2H), 2.43 (m, 1H), 1.79 (d, J = 12.0 Hz, 2H), 1.49 (m, 2H), 1.46 (s, 9H); MS m/z 229.2 [(M + H)$^+$, 100]; IR (KBr pellet, cm^{-1}) 3390,1676, 1438, 1240, 1160.

To a solution of the oxime (3.4 g, 14.9 mmol) in $CHCl_3$ (20 mL) was added NCS (1.99 g, 14.9 mmol) followed by 3 drops of pyridine. The reaction was allowed to stir for 4 h at room temperature, and the solvent was removed *in vacuo*. To the crude chloro oxime in 1:1 THF/water were added *n*-butyl vinylacetate (2.70 g, 19 mmol) and $NaHCO_3$ (3.8 g, 44.7 mmol). The reaction was stirred for 48 h and concentrated *in vacuo*. The residue was taken up in CH_2Cl_2, washed with water and saturated NaCl, filtered, and dried ($MgSO_4$). The crude product was purified using column chromatography on silica gel (hexanes/EtOAc (4:1)) and then recrystallized from CH_2Cl_2/hexanes to afford the ester as colorless crystals (2.5 g, 45%): mp 53−54 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.96 (m, 1H), 4.12 (m, 4H), 3.16 (dd, J = 10.2, 17.2 Hz, 1H), 2.84 (m, 4H), 2.56 (m, 1H), 2.53 (dd, J = 7.7, 15.7 Hz, 1H), 1.84 (d, J = 12.8 Hz, 2H), 1.62 (m, 4H), 1.46 (s, 9H), 1.42 (m, 2H), 0.94 (t, J = 7.3 Hz, 3H); MS (NH_3-CI) m/z 369.3 [(M + H)$^+$, 100] 386.3 (M + NH_4^+); IR (KBr pellet, cm^{-1}) 2958, 1732, 1694, 1414, 1238, 1176, 1120. Anal. Calcd for $C_{19}H_{32}N_2O_5$: C, 61.93; H, 8.75; N, 7.60. Found: C, 62.03; H, 8.85; N, 7.51.

To the ester (1.0 g, 2.7 mmol) in THF (5 mL) at 0 °C was added 0.5 M LiOH (7 mL, 3.5 mmol) and the reaction stirred overnight at room temperature. The solvents were removed *in vacuo*, and the residue was taken up in water, acidified with HOAc, extracted with EtOAc, dried ($MgSO_4$), and filtered. Concentration of the filtrate *in vacuo* afforded carboxylic acid **37a** as a viscous clear oil (0.95 g): ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.94 (m, 1H), 4.18 (m, 2H), 3.18 (dd, J = 10.2, 17.2 Hz, 1H), 2.90 (m, 4H), 1.46 (s, 9H); MS (DCI) m/z 330 [(M + NH_4)$^+$, 100]; IR (KBr pellet, cm^{-1}) 3100, 1734, 1690, 1648, 1430, 1276, 1168, 758.

To a solution of **37a** (0.35 g, 1.12 mmol) and **10u** (0.34 g, 1.3 mmol) in CH_2Cl_2 (5 mL) was added *N*,*N*-diisopropylethylamine (0.61 mL, 3.50 mmol) followed by 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (0.28 g, 1.45 mmol). The resulting mixture was stirred at room temperature overnight. The reaction mixture was diluted with CH_2Cl_2, washed successively with 5% citric acid, saturated $NaHCO_3$, and saturated NaCl, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The crude product was purified using flash chromatography on silica gel with 1% MeOH/CH_2Cl_2 as eluent to afford the amide as a colorless foam (0.43 g, 75%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.70 (m, 1H), 5.85 (m, 1H), 4.90 (m, 1H), 4.41 (m, 1H), 4.15 (m, 1H), 4.09 (t, J = 6.6 Hz, 2H), 3.77 (s, 3H), 3.67 (t, J = 5.5 Hz, 2H), 3.10 (dd, J = 10.2, 17.2 Hz, 1H), 2.81 (m, 2H), 2.59 (m, 2H), 2.03 (s, 2H), 1.85 (d, J = 10.9 Hz, 2H), 1.62 (m, 4H), 1.46 (s, 9H), 1.41 (m, 3H), 0.95 (t, J = 7.3 Hz, 3H); MS (ESI) m/z 513.5 [(M + H)$^+$, 100].

To the amide (0.27 g, 0.53 mmol) was added a mixture of formic acid (2.25 mL) and concentrated HCl (1.5 mL), and the reaction was stirred overnight at room temperature. The solvents were removed *in vacuo*, and the crude material was purified *via* preparative reverse phase HPLC to afford **36a** as a colorless solid (0.171 g, 63%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.62 (m, 1H), 8.38 (m, 1H), 8.10 (m, 1H), 7.33 (dd, J = 2.9, 8.4 Hz, 1H), 4.74 (m, 1H), 4.09 (q, J = 8.1 Hz, 1H), 3.96 (t, J = 6.6 Hz, 2H), 3.52 (m, 1H), 3.26 (m, 3H), 3.11 (dd, J = 10.2, 17.6 Hz, 1H), 2.97 (q, J = 10.6 Hz, 2H), 2.76 (dd, J = 7.0, 17.2 Hz, 1H), 2.66 (m, 1H), 2.47 (dd, J = 14.6, 6.2 Hz, 1H), 2.32 (dd, J = 7.0, 14.3 Hz, 1H), 1.98 (d, J = 13.6 Hz, 2 H), 1.70 (m, 2H), 1.55 (q, J = 7.0 Hz, 2H), 1.39 (m, 2H), 0.91 (t, J = 7.3 Hz, 3H); MS (ESI) m/z 399.4 [(M + H)$^+$, 100]. Anal. ($C_{18}H_{30}N_4O_6$·1.2CF_3CO_2H·0.5H_2O) C, H, N.

***N*-(Butoxycarbonyl)-3-[[[4,5-dihydro-3-(4-piperidinylmethyl)-5-isoxazolyl]acetyl]amino]-L-alanine Trifluoroacetate (36b).** Following the general procedure for **36a**, starting with 4-*N*-(*tert*-butoxycarbonyl)piperidinylmethanecarboxaldehyde,[56] acid **37b** was obtained as a colorless foam (0.38 g, 67%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.95 (m,1H), 4.10 (m, 2H), 3.15 (dd, J = 10.2, 17.2 Hz, 1H), 2.85 (m, 4H), 2.30 (d, J = 7.0 Hz, 1H), 2.05 (m, 2H), 1. 80 (m, 1H), 1.75 (d, J = 14.6 Hz, 2H), 1.46 (s, 9H), 1.22 (m, 2H); MS (ESI) m/z 327.3 [(M + H)$^+$, 100].

From the coupling of **37b** (1.0 g, 3.1 mmol) with **10u** (0.9 g, 3.6 mmol), followed by subsequent processing as described in the preparation of **36a**, was obtained **36b** as a colorless solid (0.23 g): ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.57 (m, 1H), 8.29 (m, 1H), 8.10 (m, 1H), 7.33 (dd, J = 4.4, 8.1 Hz, 1H), 4.74 (m, 1H), 4.09 (q, J = 7.7 Hz, 1H), 3.96 (t, J = 6.6 Hz, 2H), 3.52 (m, 1H), 3.28 (d, J = 13.2 Hz, 2H), 3.21 (m, 1H), 3.09 (dd, J = 10.2, 17.2 Hz, 1H), 2.97 (q, J = 11.7 Hz, 2H), 2.73 (dd, J = 7.3, 17.6 Hz, 1H), 2.47 (dd, J = 14.6, 8.4 Hz, 1H), 2.32 (d, J = 7.3 Hz, 1H), 2.25 (d, J = 6.6 Hz, 2H), 1.86 (m, 1H), 1.81 (d, J = 14.3 Hz, 2H), 1.55 (m, 2H), 1.36 (m, 4H), 0.91 (t, J = 7.0 Hz, 3H); MS (ESI) m/z 413.4 [(M + H)$^+$, 100]. Anal. ($C_{19}H_{32}N_4O_6$·1.4CF_3CO_2H·0.4H_2O) C, H, N.

2-[[(1,1-Dimethylethoxy)carbonyl]amino]-4-[[[4,5-dihydro-3-(4-piperidinylmethyl)-5-isoxazolyl]acetyl]amino]-butanoic Acid Trifluoroacetate (36c). *N*-(*tert*-Butoxycarbonyl)-L-asparagine (5.0 g, 22 mmol) was dissolved in pyridine (40 mL), and Ac_2O (3.1 g, 30.2 mmol) was added. The reaction was stirred at room temperature overnight, the solvent was removed *in vacuo*, and the residue was taken up in EtOAc, washed with 10% citric acid (100 mL) and saturated NaCl (50 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield the nitrile as a colorless oil (4.9 g): MS (DCI) m/z 232 [(M + NH_4)$^+$, 100].

To a solution of the nitrile (2.0 g, 9.3 mmol) in CH_2Cl_2 at 0 °C was added the reagent made from *t*-BuOH and 1,3-diisopropylcarbodiimide[57] (9.4 g, 46.7 mmol). The reaction was stirred at room temperature overnight, filtered through a layer of Celite, washed with citric acid (3 × 50 mL), saturated $NaHCO_3$ (100 mL), and saturated NaCl (50 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give the *tert*-butyl ester as an oil (1.1 g): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.59 (m, 1H), 4.44 (q, J = 5.9 Hz, 1H), 4.09 (t, J = 6.6 Hz, 2H), 2.96 (m, 2H), 1.62 (m, 2H), 1.53 (s, 9H), 1.37 (m, 2H), 0.94 (t, J = 7.3 Hz, 3H); MS (DCI) m/z 288.2 [(M + NH_4)$^+$, 100].

The *tert*-butyl ester was hydrogenated at 50 psi in MeOH (10 mL) and 4 M HCl (15 mL) for 2 h. Filtration through a layer of Celite and concentration *in vacuo* afforded the amine as a colorless solid (1.2 g): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.27 (m, 2H), 5.78 (m, 1H), 4.28 (m, 1H), 4.06 (m, 2H), 3.2 (m, 2H), 1.63 (m, 2H), 1.48 (s, 9H), 1.38 (m, 4H), 0.93 (t, J = 6.9 Hz, 3H); MS (ESI) m/z 275 [(M + H)$^+$, 100].

The coupling of the amine with **37b** using the procedure reported for the synthesis of **25b** afforded the amide: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.7 (m, 1H), 5.4 (m, 1H), 4.9 (m, 1H), 4.2 (m, 1H), 4.05 (t, J = 6.6 Hz, 4H), 3.1 (m, 1H), 3.0 (m, 1H), 2.8−2.4 (m, 5H), 2.25 (d, J = 7.0 Hz, 2H), 1.72−1.58 (m, 6H), 1.47 (s, 9H), 1.45 (s, 9H), 1.43 (m, 3H), 1.2 (m, 3H), 0.96 (t, J = 7.3 Hz, 3H); MS (ESI) m/z 583 [(M + H)$^+$, 100].

The preceding amide (360 mg, 0.06 mmol) was stirred in CH_2Cl_2 (3 mL) and TFA (1 mL) overnight. The volatiles were removed *in vacuo*, and the crude material was purified *via* preparative reverse phase HPLC to afford **36c** as a colorless solid (226 mg): ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.5 (m, 1H), 8.2 (m, 1H), 8.0 (m, 1H), 7.46 (d, J = 8.4 Hz, 1H), 4.74 (m, 1H), 3.94 (m, 3H), 3.60 (m, 1H), 3.28 (m, 2H), 3.10 (m, 3H), 2.88 (m, 2H), 2.71 (m, 1H), 2.44 (m, 2H), 2.26 (m, 3H), 1.78 (m, 2H), 1.70 (m, 1H), 1.55 (m, 2H), 1.34 (m, 4H), 0.89 (t, J = 7.3 Hz, 3H); MS (ESI) m/z 427 [(M + H)$^+$, 100]. Anal. ($C_{20}H_{34}N_4O_6$·1.4CF_3CO_2H) C, H, N.

***N*-[(1,1-Dimethylethoxy)carbonyl]-3-[[[4,5-dihydro-3-[2-(4-piperidinyl)ethyl]-5-isoxazolyl]acetyl]amino]-L-alanine Trifluoroacetate (36d).** The compound was synthesized using the general procedure reported for **25b**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.85 (m, 1H), 4.28 (m, 1H), 4.02 (t, J = 6.6 Hz, 2H), 3.62 (m, 1H), 3.38 (m, 3H), 3.12 (dd, J = 17.7, 10.1 Hz, 1H), 2.95 (dt, J = 12.8, 2.6 Hz, 2H), 2.80 (dd, J = 17.2, 7.2 Hz, 1H), 2.55 (ddd, J = 16.8, 4.0, 2.6 Hz, 1H), 2.40 (m, 3H), 1.96 (bd, J = 14.3 Hz, 2H), 1.57 (m, 5H), 1.37 (m, 4H), 0.92 (t, J = 7.3 Hz, 3H); MS (ESI) m/z 427 [(M + H)$^+$, 100]. Anal. ($C_{20}H_{34}N_4O_6$·1.5CF_3CO_2H) C, H, N.

***N*-(Butoxysulfonyl)-3-[[[4,5-dihydro-3-[2-(4-piperidinyl)-ethyl]-5-isoxazolyl]acetyl]amino]-L-alanine Trifluoroacetate (36e).** The compound was synthesized using the general procedure reported for **25b**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.5 (m, 1H), 8.12 (m, 2H), 7.55 (m, 1H), 4.71 (m, 1H), 3.96 (m,

1H), 3.44 (m, 1H), 3.28 (m, 3H), 3.09 (m, 1H), 2.98 (m, 2H), 2.9−2.6 (m, 4H), 2.45 (d, J = 6.2 Hz, 1H), 2.30 (m, 3H), 1.84 (m, 2H), 1.7−1.6 (m, 2H), 1.48−1.2 (m, 6H), 0.88 (t, J = 7.3 Hz, 3H); MS (ESI) m/z 447 [(M + H)$^+$, 100]. Anal. ($C_{19}H_{34}N_4O_6S\cdot1.2CF_3CO_2H$) C, H, N.

3-[[[4,5-Dihydro-3-[2-(4-piperidinyl)ethyl]-5-isoxazolyl]-acetyl]amino]-*N*-[(phenylmethoxy)carbonyl]-L-alanine Trifluoroacetate (36f). This material was prepared using the general procedure reported for **25b**: ^{1}H NMR (300 MHz, CD_3OD) δ 7.32 (m, 5H), 5.08 (AB quartet, J = 12.6 Hz, Δ = 18.0 Hz, 2H), 4.81 (m, 1H), 4.34 (m, 1H), 3.69 (m, 1H), 3.47−3.28 (m, 3H, coincident with CHD_2OD), 3.07 (m, 1H), 2.93 (bt, J = 13.2 Hz, 2H), 2.78 (dd, J = 17.2, 7.3 Hz, 1H), 2.52 (ddd, J = 14.3, 6.2, 2.2 Hz, 1H), 2.36 (m, 3H), 1.94 (bd, J = 14.3 Hz, 2H), 1.55 (m, 3H), 1.33 (m, 2H); MS (ESI) m/z 461 [(M + H)$^+$, 100]. Anal. ($C_{23}H_{32}N_4O_6\cdot1.6CF_3CO_2H$) C, H, N.

***N*-(Butoxycarbonyl)-3-[[[4,5-dihydro-3-[3-(4-piperidinyl)propyl]-5-isoxazolyl]acetyl]amino]-L-alanine Trifluoroacetate (36g).** The material was synthesized using the general procedure reported for **25b**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.5 (m, 1H), 8.2−8.07 (m, 2H), 7.70 (m, 1H), 7.32 (m, 1H), 4.05 (m, 1H), 3.94 (t, J = 7.0 Hz, 2H), 3.47 (m, 1H), 3.22 (m, 3H), 3.03 (m, 1H), 2.85 (m, 2H), 2.71 (m, 1H), 2.43 (m, 1H), 2.26 (t, J = 7.3 Hz, 3H), 1.81 (m, 2H), 1.6−1.4 (m, 6H), 1.38−1.17 (m, 7H), 0.89 (t, J = 7.3 Hz, 3H); MS (ESI) m/z 441 [(M + H)$^+$, 100]. Anal. ($C_{21}H_{36}N_4O_6\cdot1.7CF_3CO_2H$) C, H, N.

3-[[[3-[4-(Aminoiminomethyl)-2-fluorophenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(butoxycarbonyl)-L-alanine (38). Thionyl chloride (300 mL) was added to 3-fluoro-4-methylbenzoic acid (**40**, 28.9 g, 187 mmol) and heated at reflux for 100 min. The excess thionyl chloride was removed by distillation, the resulting solution was cooled to 0 °C and diluted with CH_2Cl_2 (300 mL), and ammonia (28−30%, 60 mL) was added over 40 min. The solids were removed by filtration and washed with EtOAc, and the filtrate was extracted with saturated Na_2CO_3 (2×) and saturated NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to afford a yellow solid (5.3 g). The filtered solids were partitioned between EtOAc and water, extracted with saturated Na_2CO_3 (2×) and saturated NaCl, dried (Na_2SO_4), filtered, and concentrated *in vacuo* to yield an additional 15.6 g. A third batch of white solid (1.2 g) was obtained by extracting the combined aqueous with CH_2Cl_2 (4×) and EtOAc (2×), giving the amide in a combined yield of 77%: ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.99 (bs, 1H), 7.62 (m, 2H), 7.44 (bs, 1H), 7.37 (t, J = 8.0 Hz, 1H).

The above amide (21.9 g, 143 mmol) was dissolved in CH_2Cl_2 (200 mL), and Et_3N (40 mL, 287 mmol) was added. After the mixture was cooled to 0 °C, trichloroacetyl chloride (17.6 mL, 158 mmol) was added dropwise over 50 min and the reaction mixture stirred at 0 °C for 1 h. The reaction mixture was diluted with Et_2O, extracted with 1 M HCl, saturated $NaHCO_3$, water, and saturated NaCl, dried (Na_2SO_4), filtered, and evaporated without heat to yield nitrile **41** as a tan solid (22.5 g, >100% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 (m, 3H), 2.35 (d, J = 2.2 Hz, 3H).

A solution of NBS (25.4 g, 143 mmol) and **41** (22.4 g, 143 mmol based on theoretical yield) in CCl_4 was heated at reflux in the presence of high-intensity visible light for 4 h. The resulting mixture was cooled and filtered through Celite. The filtrate was evaporated and recrystallized twice from hot cyclohexane to yield the bromide as tan needles (8.1 g, 27%). The mother liquors were combined and purified using column chromatography (0−15% EtOAc/hexane) to yield an additional 10.7 g (35%) of product: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (t, J = 7.3 Hz, 1H), 7.46 (d, J = 8.4 Hz, 1H), 7.38 (dd, J = 9.1, 1.4 Hz, 1H), 4.49 (s, 2H); MS (CH_4-CI/GC) m/z 214, 216 [(M + H)$^+$, 100]. Anal. Calcd for C_8H_5NBrF: C, 44.89; H, 2.36; N, 6.54; F, 8.88. Found: C, 44.74; H, 2.25; N, 6.41; F, 8.62.

To a solution of the bromide (18.5 g, 86 mmol) in DMSO (150 mL) and CH_2Cl_2 (75 mL) cooled to 0 °C was added trimethylamine *N*-oxide dihydrate (39.0 g, 350 mmol). The resulting solution was warmed to room temperature over 2 h. The reaction was stirred overnight at room temperature, water was added, and the solution was extracted with Et_2O (2×). Saturated NaCl was added to the combined aqueous, and it was extracted again with Et_2O. The combined organic was dried (Na_2SO_4 and $MgSO_4$), filtered, concentrated *in vacuo*, and purified using column chromatography (CH_2Cl_2) to yield the aldehyde as a yellow solid (7.1 g, 55%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.41 (s, 1H), 8.00 (t, J = 7.7 Hz, 1H), 7.59 (d, J = 8.1 Hz, 1H), 7.53 (d, J = 9.5 Hz, 1H).

To a solution of the aldehyde (5.76 g, 39 mmol) in 90% MeOH/water were added K_2CO_3 (5.34 g, 39 mmol) and hydroxylamine hydrochloride (5.37 g, 77 mmol), and the mixture was heated at reflux for 160 min. The reaction was diluted with water and filtered to yield oxime **39** as a white solid (5.76 g, 91%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.07 (s, 1H), 8.26 (s, 1H), 7.93 (m, 2H), 7.72 (d, J = 8.4 Hz, 1H).

Oxime **39** was elaborated to lithium (*R*,*S*)-3-[4-[[*N*-(dimethylethoxy)imino]aminomethyl]-2-fluorophenyl]-4,5-dihydro-5-isoxazolyl acetate following the procedure outlined for **22**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.21 (bs, 1H), 7.83 (m, 3H), 4.90 (m, 1H), 3.50 (dd, J = 17.5, 11.3 Hz, 1H), 3.14 (dd, J = 17.2, 8.4 Hz, 1H), 2.38 (m, 1H), 2.13 (m, 1H), 1.45 (s, 9H); HRMS (FAB) m/z 366.1479 [(M + H$^+$ calcd for $C_{17}H_{20}N_3O_5$ 366.1465]. Anal. Calcd for $C_{17}H_{19}N_3O_5Li$: C, 54.94; H, 5.16; N, 11.31; Li, 1.87. Found: C, 51.67; H, 5.45; N, 10.49; Li, 2.12.

Compound **38** was prepared from the preceding isoxazoline and **10u** in a synthesis analogous to **25b**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.74 (bs, 1H), 9.51 (bs, 1.5H), 9.27 (bs, 1.5H), 8.16 (bs, 1H), 7.95 (t, J = 8.0 Hz, 1H), 7.85 (d, J = 11.7 Hz, 1H), 7.73 (d, J = 8.0 Hz, 1H), 7.31 (m, 1H), 5.05 (m, 1H), 4.07 (m, 1H), 3.94 (m, 2H), 3.49 (m, 2H), 3.28 (m, 2H), 2.52 (m, 2H), 1.55 (m, 2H), 1.32 (m, 2H), 0.88 (m, 3H); HRMS (FAB) m/z 452.1941 [(M + H)$^+$ calcd for $C_{20}H_{27}FN_5O_6$ 452.1945].

3-[[[3-[4-[(Aminoiminomethyl)amino]phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(butoxycarbonyl)-L-alanine Trifluoroacetate (49). To a solution of 4-[(*tert*-butoxycarbonyl)amino]benzaldehyde **43**[32] (5.21 g, 23.6 mmol) in EtOH (50 mL) were added hydroxylamine hydrochloride (1.63 g, 23.6 mmol) and Na_2CO_3 (4.98 g, 47 mmol). The reaction mixture was stirred at room temperature overnight and poured into water. The mixture was washed with EtOAc (2 × 100 mL) and the combined organic washed with saturated NaCl, dried ($MgSO_4$), and filtered. Concentration *in vacuo* afforded oxime **44** (5.23 g, 93%) as colorless crystals: ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.0 (s, 1H), 10.1 (bs, 1H), 8.03 (s, 1H), 7.59 (d, J = 8 Hz, 1H), 7.48 (d, J = 8 Hz, 2H), 1.59 (s, 9H).

Oxime **44** (3.28 g, 14.1 mmol) was dissolved in DMF (50 mL) followed by the addition of NCS (1.89 g, 14.1 mmol). The reaction mixture was stirred at room temperature for 3 h and then quenched with water (200 mL). The crude chloro oxime **45** was extracted with EtOAc (2 × 100 mL), dried ($MgSO_4$), filtered, and evaporated to a pale yellow oil which was redissolved in THF/water (4:1, 50 mL) and subjected to the cycloaddition reaction protocol used for the preparation of **36a**, affording the desired isoxazolinyl acetate (1.9 g, 49%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.58 (d, J = 8.3 Hz, 2H), 7.38 (d, J = 8.4 Hz, 2H), 6.60 (bs, 1H), 5.03 (m, 1H), 4.10 (m, 2H), 3.48 (dd, J = 10.3, 16.5 Hz, 1H), 3.06 (dd, J = 7.4, 16.9 Hz, 1H), 2.84 (dd, J = 5.9, 16 Hz, 1H), 2.60 (dd, J = 7.7, 16.5 Hz, 1H), 1.61 (m, 2H), 1.50 (s, 9H), 1.35 (m, 2H), 0.99 (m, 3H); IR (KBr pellet, cm^{-1}) 2966, 1734, 1740, 1610, 1578, 1528, 1508, 1458, 1442, 1412, 1392, 1368 1234, 1160, 1058, 916, 878, 828, 772, 612; HRMS (FAB) m/z 377.2073 [(M + H)$^+$ calcd for $C_{20}H_{28}N_2O_5$ 377.2076].

Saponification of the above isoxazolinyl acetate using the conditions described in the preparation of **22** gave the acid **42** as colorless crystals (88%): mp 178−180 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.03 (m, 1H), 3.48 (dd, J = 10.3, 16.5 Hz, 1H), 3.06 (dd, J = 9.5, 16.9 Hz, 1H), 2.89 (dd, J = 8.3, 16.0 Hz, 1H), 2.67 (dd, J = 7.8, 16.0 Hz, 1H), 1.52 (s, 9H).

Acid **42** was condensed with **10u** following the protocol described in the synthesis of **25b** to give the amide: mp 80−82 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.6 (s, 1H), 10.4 (s, 1H), 7.84 (d, J = 7.8 Hz, 2H), 7.58 (d, J = 7.8 Hz, 2H), 6.77 (m, 1H), 5.88 (dd, J = 5.0, 20.0 Hz, 1H), 5.00 (m, 1H), 4.38 (m, 1H), 4.00 (m, 2H), 3.74 (s, 3H), 3.63 (m, 2H), 3.40 (dd, J = 12.0, 18.5 Hz, 1H), 3.09 (dd, J = 2.5, 17.0 Hz, 1H), 2.61 (dd, J = 6.4, 18.0 Hz, 1H), 2.53 (m, 1H), 1.88 (t, J = 8.0 Hz, 3H),

1.47 (m, 20H), 1.30 (m, 2H); IR (KBr pellet, cm^{-1}) 3286, 2964, 1722, 1646, 1546, 1414, 1368, 1340, 1312, 1294, 1240, 1156, 1122, 1100, 1058, 1030, 844, 776; MS (NH_3-CI) *m/z* 663 [(M + H)$^+$, 20], 463 (100).

The above amide (350 mg, 0.67 mmol) was treated with TFA in CH_2Cl_2 as described in the preparation of **36c** to afford the corresponding aniline (344 mg, 95%) as an oily TFA salt. To a solution of this material in DMF (20 mL) was added thiourea **48** (0.19 g, 0.71 mmol), $HgCl_2$ (0.18 g, 0.65 mmol), and pyridine (0.13 mL, 1.6 mmol). The reaction mixture was stirred overnight at room temperature and then was diluted with water (100 mL). The aqueous was washed with EtOAc (2 × 50 mL), and the combined organic was washed with saturated NaCl, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield an oily residue. Purification using column chromatography on silica gel (CH_2Cl_2:MeOH, 9:1) afforded the bis(Boc)-protected guanidine (0.25 g, 59%) as a colorless foam. Cleavage of the Boc protecting groups using the procedure described for the preparation of **36c** gave the guanidine ester (90% yield): ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.84 (bs, 1H), 7.68 (d, J = 8.0 Hz, 2H), 7.64 (bs, 3H), 7.28 (d, J = 8.1 Hz, 2H), 6.80 (m, 1H), 5.02 (m, 1H), 4.31 (m, 1H), 4.00 (t, J = 8.0 Hz, 2H), 3.70 (s, 3H), 3.65 (m, 1H), 3.57−3.40 (m, 2H), 3.25−3.17 (m, 1H), 2.64 (m, 1H), 2.58 (t, J = 6.2 Hz, 2H), 2.44 (dd, J = 7.0, 17.1 Hz, 1H), 1.89 (t, J = 7.8 Hz, 3H), 1.57 (m, 2H), 1.34 (m, 2H); MS (ESI) *m/z* 463 [(M + H)$^+$, 100]; HRMS (FAB) *m/z* 463.2307 [(M + H)$^+$ calcd for $C_{21}H_{31}N_6O_6$ 463.2305].

Saponification of the above guanidine ester using the procedure described for the synthesis of **22** afforded acid **49**: ^{1}H NMR (300 MHz, CD_3OD) δ 7.67 (d, J = 7.9 Hz, 2H), 7.25 (d, J = 8.1 Hz, 2H), 4.80 (m, 2H), 4.18 (m, 1H), 3.95 (t, J = 7.5 Hz, 2H), 3.65−3.30 (m, 4H), 3.10 (m, 2H), 2.68−2.40 (m, 2H), 1.18 (m, 2H), 1.10 (t, J = 8.1 Hz, 2H), 0.84 (t, J = 7.9 Hz, 3H); IR (KBr pellet, cm^{-1}) 3328, 2962, 1676, 1604, 1560, 1520, 1432, 1406, 1356, 1256, 1202, 1074, 834, 722, 548; HRMS (FAB) *m/z* 449.2162 [(M + H)$^+$ calcd for $C_{20}H_{28}N_6O_6$ 449.2149].

3-[[[3-[3-[(Aminoiminomethyl)amino]propyl]-4,5-dihydro-5-isoxazolyl]acetyl]amino]-*N*-(butoxycarbonyl)-L-alanine Trifluoroacetate (50). The isoxazolinylacetic acid **52** was prepared as an oil from chloro oxime **51**[34] and **46** *via* the general method previously outlined for **25b**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.90 (m, 1H), 4.70 (bs, 1H), 3.08 (m, 3H), 2.68 (m, 2H), 2.57 (dd, J = 7.8, 5.0 Hz, 2H), 2.34 (m, 2H), 1.75 (m, 2H), 1.41 (s, 9H); MS (ESI) *m/z* 287 [(M + H)$^+$, 100].

Following the procedure outlined for **25b**, isoxazoline **52** was coupled with **10u** to afford the amide in 93% yield as a colorless foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.01 (bs, 1H), 6.04 (m, 1H), 5.98 (m, 1H), 4.91 (m, 1H), 4.40 (m, 1H), 4.05 (m, 2H), 3.77 (s, 3H), 3.22−3.04 (m, 4H), 2.85−2.70 (m, 1H), 2.61−2.33 (m, 6H), 1.82 (m, 3H), 1.66 (m, 2H), 1.44 (s, 9H), 1.40 (m, 2H), 0.91 (m, 3H); HRMS (FAB) *m/z* 487.2781 [(M + H)$^+$ calcd for $C_{22}H_{39}N_4O_8$ 487.2770].

To a solution of the amide in CH_2Cl_2 (10 mL) was added TFA (0.5 mL). The reaction mixture was stirred at room temperature for 3 h and then concentrated to a colorless foam. The residue was redissolved in CH_2Cl_2 (25 mL) followed by the addition of (aminoiminomethyl)pyrazole **53**[35] (0.31 g, 0.91 mmol) and Et_3N (3 mL, 21 mmol). The reaction mixture was stirred at room temperature overnight and concentrated *in vacuo*. The residue was dissolved in water and washed with EtOAc (2 × 50 mL), and the combined organic was dried ($MgSO_4$), filtered, and concentrated *in vacuo* to afford the crude bis(Boc)guanidine. Purification using column chromatography (silica gel, CH_2Cl_2/MeOH) afforded **54** (0.39 g, 70%) as tan crystals: ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.40 (bs, 1H), 6.84 (m, 1H), 5.84 (bs, 1H), 4.86 (m, 1H), 4.40 (m, 1H), 3.78 (s, 3H), 3.50−3.31 (m, 4H), 3.13 (m, 1H), 2.73 (m, 1H), 2.58−2.36 (m, 4H), 1.86 (m, 4H), 1.51 (s, 18H), 1.40 (m, 2H), 0.93 (m, 3H). MS (ESI) *m/z* 617 [(M + H)$^+$, 48], 517 [(M + H − Boc)$^+$, 100].

Saponification as described for the preparation of **22** and Boc cleavage as described for the synthesis of **36c** afforded **50** in 40% overall yield as a colorless foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.93 (m, 1H), 4.2 (m, 1H), 4.02 (m, 2H), 3.65 (m, 1H), 3.32 (m, 1H), 3.21 (m, 2H), 3.09 (dd, J = 10.2, 17.6 Hz, 1H), 2.79 (dd, J = 7.3, 17.1 Hz, 1H), 2.52 (dd, J = 8.0, 17.0 Hz, 1H), 2.39 (m, 3H), 1.84 (m, 2H), 1.58 (m, 2H), 1.36 (m, 2H), 0.90 (m, 3H); MS (ESI) *m/z* 529 [(M + H)$^+$, 100]; HRMS (FAB) *m/z* 415.4612 [(M + H)$^+$ calcd for $C_{17}H_{30}N_6O_6$ 415.4638].

Methyl 3-[[[5-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-3-isoxazolyl]acetyl]amino]-*N*-(butoxycarbonyl)-L-alanine Trifluoroacetate (55). Through a solution of oxime **57**[36] (5.58 g, 34.9 mmol) in $CHCl_3$ (100 mL) at −40 °C was gently bubbled chlorine gas for 1 h. The reaction mixture was warmed to room temperature, concentrated *in vacuo* to a yellow oil, and redissolved in 4:1 THF/water (50 mL). To this solution was added a solution of **56** (11.2 g, 87.3 mmol) in THF (10 mL) and excess solid Na_2CO_3 (18.4 g, 174 mmol) in small portions over 1 h. The reaction mixture was stirred at room temperature overnight, filtered, and concentrated *in vacuo* to a reddish brown oil. Purification using column chromatography on silica gel (hexane:EtOAc, 7:3) afforded the desired isoxazoline **58** (7.20 g, 73%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (d, J = 8.0 Hz, 2H), 7.47 (d, J = 8.0 Hz, 2H), 5.60 (dd, J = 9.0, 4.5 Hz, 1H), 3.48 (m, 1H), 3.35 (AB quartet, Δ = 18.0 J = 8.3 Hz, 2H), 3.00 (dd, J = 8.3, 17.0 Hz, 1H), 1.40 (s, 9H); IR (KBr pellet, cm^{-1}) 2235, 1718, 1610; MS (NH_3-CI) *m/z* 287 [(M + H)$^+$, 100].

Isoxazoline **58** (7.0 g, 25 mmol) was subjected to the Pinner synthesis/ammonia sequence described for compound **21** to afford the crude amidine. To a solution of this material in dioxane (50 mL) was added Et_3N (10.5 mL, 74.7 mmol) and di-*tert*-butyl dicarbonate (13.1 g, 60.0 mmol) and the reaction mixture stirred at room temperature overnight. Water (100 mL) was added, followed by extraction with EtOAc (2 × 100 mL), washing with saturated NaCl (50 mL), drying ($MgSO_4$), filtration, and evaporation *in vacuo* to yield a yellow oil. Purification using column chromatography on silica gel (CH_2Cl_2:MeOH, 9.5:0.5) afforded the desired isoxazolinyl acetate (2.51 g, 28%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (d, J = 8.2 Hz, 2H), 7.38 (d, J = 8.2 Hz, 2H), 5.63 (dd, J = 8.0, 11.4 Hz, 1H), 3.71 (s, 3H), 3.53 (m, 1H), 3.49 (s, 2H), 2.98 (dd, J = 8.0, 17.0 Hz, 1H), 1.54 (s, 9H); MS (ESI) *m/z* 262 [(M + H − Boc)$^+$, 100].

The above isoxazolinyl acetate (1.51 g, 4.18 mmol) was saponified using the conditions described for the synthesis of **22** to afford acid **59** (0.06 g, 5%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (d, J = 8.2 Hz, 2H), 7.38 (d, J = 8.2 Hz, 2H), 5.63 (dd, J = 8.0, 11.4 Hz, 1H), 3.53 (m, 1H), 3.51 (s, 2H), 3.00 (dd, J = 8.0, 17.0 Hz, 1H), 1.54 (s, 9H); MS (ESI) *m/z* 248 [(M + H − Boc)$^+$, 100].

Acid **59** and amine **10u** were coupled following the procedure described for **25b** to give the amide in 80% yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (d, J = 8.2 Hz, 2H), 7.33 (d, J = 8.2 Hz, 2H), 6.70 (bs, 1H), 5.70 (bs, 1H), 5.60 (dd, J = 8.0, 11.4 Hz, 1H), 4.31 (m, 1H), 4.00 (m, 2H), 3.73 (s, 3H), 3.60 (m, 2H), 3.46 (m, 1H), 3.33 (s, 2H), 2.95 (dd, J = 8.0, 17.0 Hz, 1H), 1.53 (s, 9H), 1.32 (m, 2H), 1.17 (m, 2H), 0.89 (t, J = 8.0 Hz, 3H); MS (ESI) *m/z* 534 (M + H$^+$, 30), 434 [(M + H − Boc)$^+$, 100].

Deprotection by treatment of the above Boc-amidine with excess TFA in CH_2Cl_2 provided **55** as the TFA salt: ^{1}H NMR (300 MHz, $CDCl_3$/DMSO-d_6) δ 9.05 (bs, 2H), 8.03 (bs, 1H), 7.80 (d, J = 8.2 Hz, 2H), 7.50 (d, J = 8.0 Hz, 2H), 6.80 (m, 1H), 5.60 (dd, J = 8.0, 11.0 Hz, 1H), 4.29 (m, 1H), 3.98 (m, 2H), 3.63 (m, 3H), 3.40−3.63 (m, 3H), 3.32 (s, 2H), 3.00 (dd, J = 8.0, 17.0 Hz, 1H), 1.88 (t, J = 7.5 Hz, 3H), 1.53 (m, 2H), 1.30 (m, 2H); IR (KBr pellet, cm^{-1}) 3388, 1718, 1664, 1620, 1528, 1456, 1436, 1384, 1366, 1280, 1254, 1168, 1144, 1074, 980, 882, 778; MS (ESI) *m/z* 448 [(M + H)$^+$, 100]; HRMS (NH_3-CI) 448.2183 [(M + H)$^+$ calcd for $C_{21}H_{29}N_5O_6$ 448.2196].

Methyl 3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]methylamino]-*N*-[(phenylmethoxy)carbonyl]-L-alanine Mono(trifluoroacetate) (60a). The coupling of **22** with **12a**[29] as described for the synthesis of **25b** gave the amide: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (m, 2H), 7.69 (m, 2H), 7.33 (m, 5H), 5.79 (bd, J = 9.0 Hz, 1H), 5.09 (m, 3H), 4.58 (m, 1H), 3.86 (m, 1H), 3.77 (2s, 3H), 3.63 (m, 2H), 3.14 (m, 1H), 3.01 (2s, 3H), 2.90 (m, 1H), 2.53 (m, 1H), 1.66 (bs, 2H), 1.56 (s, 9H); MS (ESI) *m/z* 596 [(M + H)$^+$, 100]; HRMS (FAB) *m/z* 596.2699 [(M + H)$^+$ calcd for $C_{30}H_{38}N_5O_8$ 596.2720].

The title compound was synthesized from the amide following the procedure used for **25b**: ^{1}H NMR (300 MHz, DMSO-

d_6) δ 9.38 (bs, 2H), 9.19 (bs, 2H), 7.87 (m, 4H), 7.32 (m, 5H), 5.03 (m, 3H), 4.40 (m, 2H), 3.89 (m, 1H), 3.65 (3s, 3H), 3.60−3.20 (m, 3H), 3.14 (dd, J = 16.0, 7.5 Hz, 1H), 2.95, 2.93, 2.82 (3s, 3H), 2.75 (m, 1H); MS (ESI) m/z 496 [(M + H)$^+$, 100]; HRMS (FAB) m/z 496.2195 [(M + H)$^+$ calcd for $C_{25}H_{30}N_5O_6$ 496.2196]; HPLC t_R 12.3 min (95%). Anal. ($C_{25}H_{29}N_5O_6 \cdot CF_3CO_2H$): C, H, N, F.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]methylamino]-*N*-(butoxycarbonyl)-L-alanine Mono(trifluoroacetate) (60b). A mixture of **60a** (900 mg, 1.5 mmol) and 10% Pd on charcoal (90 mg) in EtOH (40 mL) was stirred at room temperature under hydrogen (1 atm) for 19 h. The mixture was filtered through Celite, and the solids were washed with additional EtOH. The filtrate was concentrated *in vacuo*, and the resulting colorless glass was purified using column chromatography (CH_2Cl_2/CH_3OH, step gradient from 97:3 to 90:10) to provide methyl 3-[[[3-[4-[[[(1,1-dimethylethoxy)carbonyl]amino]iminomethyl]phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]methylamino]-L-alanine (408 mg, 59%) as a pale yellowish glassy foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (d, J = 8 Hz, 2H), 7.72 (d, J = 8 Hz, 2H), 5.18 (m, 1H), 3.75 (2s, 3H), 3.7−3.5 (m, 4H), 3.49, 3.10 (2s, 3H), 3.15 (m, 1H), 2.96 (m, 1H), 2.70 (m, 1H), 1.55 (s, 9H), 1.60 (bs, 4H); MS (ESI) m/z 462 [(M + H)$^+$, 17], 362 [(M + H − Boc)$^+$, 42], 195 (100); HRMS (FAB) m/z 462.2337 [(M + H)$^+$ calcd for $C_{22}H_{32}N_5O_6$ 462.2353]; HPLC t_R 9.9 min (>98%).

A solution of methyl 3-[[[3-[4-[[[(1,1-dimethylethoxy)carbonyl]amino]iminomethyl]phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]-methylamino]-L-alanine (175 mg, 380 μmol) in CH_2Cl_2 (4 mL) was stirred at room temperature and treated sequentially with n-butyl chloroformate (58 μL, 450 μmol) and Et_3N (66 μL, 475 μmol). After 26 h, the mixture was concentrated *in vacuo*. Purification of the residue using column chromatography (EtOAc) provided methyl *N*-(butoxycarbonyl)-3-[[[3-[4-[[[(1,1-dimethylethoxy)carbonyl]amino]iminomethyl]phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]methylamino]-L-alanine (194 mg, 91%) as a colorless glassy foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (d, J = 8.0 Hz, 2H), 7.72 (d, J = 8.0 Hz, 2H), 5.66 (m, 1H), 5.17 (m, 1H), 4.53 (m, 1H), 4.05 (m, 2H), 3.84 (m, 1H), 3.77 (2s, 3H), 3.65 (m, 2H), 3.15 (m, 1H), 3.07 (2s, 3H), 3.00 (m, 1H), 2.63 (m, 1H), 1.55 (s + m, 11H), 1.50 (m, 2H), 1.34 (m, 2H), 0.91 (m, 3H); MS (ESI) m/z 562 [(M + H)$^+$, 100]; HPLC t_R 14.6 min (>98%).

A solution of methyl *N*-(butoxycarbonyl)-3-[[[3-[4-[[[(1,1-dimethylethoxy)carbonyl]amino]iminomethyl]phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]methylamino]-L-alanine (70 mg, 125 μmol) in MeOH (1 mL) was treated with aqueous 0.69 M LiOH (0.2 mL, 138 μmol) and stirred at room temperature for 72 h. The volatiles were removed *in vacuo*, and the residue was dissolved in water (0.5 mL) and acidified with 2 drops of aqueous 1 M HCl. The resulting gummy precipitate was extracted into EtOAc. The organic phase was dried ($MgSO_4$), filtered, and concentrated *in vacuo* to provide 3-[[[3-[4-[[[(1,1-dimethylethoxy)carbonyl]amino]iminomethyl]phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]methylamino]-*N*-(butoxycarbonyl)-L-alanine (56 mg, 82%) as a colorless solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.01 (d, J = 8.0 Hz, 2H), 7.75 (d, J = 8.0 Hz, 2H), 7.55 (m, 1H), 5.02 (m, 1H), 4.27 (m, 1H), 3.92 (m, 2H), 3.60 (m, 2H), 3.15 (m, 2H), 2.97 + 2.80 (2s, 3H), 2.90 (m, 1H), 2.63 (m, 1H), 1.50 (m, 2H), 1.47 (s, 9H), 1.30 (m, 2H), 0.86 (m, 3H); MS (ESI) m/z 548 [(M + H)$^+$, 100]; HPLC t_R 12.4 min (>98%).

A solution of 3-[[[3-[4-[[[(1,1-dimethylethoxy)carbonyl]amino]iminomethyl]phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]-methylamino]-*N*-(butoxycarbonyl)-L-alanine (43 mg, 78 μmol) in CH_2Cl_2 (2 mL) was treated with TFA (1 mL) and stirred at room temperature for 2.5 h. The solution was concentrated *in vacuo*, and the residue was triturated with Et_2O. The resulting precipitate was collected by filtration, washed with Et_2O, and dried *in vacuo* to provide **60b** (31 mg, 72%) as a colorless powder, which was a mixture of isomers by ^{1}H NMR: ^{1}H NMR (300 MHz, DMSO-d_6) δ 9.38 (bs, 2H), 9.13 (bs, 2H), 7.88 (s, 4H), 5.03 (m, 1H), 4.26 (m, 1H), 3.92 (m, 3H), 3.62 (m, 2H), 3.18 (m, 1H), 2.99, 2.97 + 2.80 (3s, 3H), 2.90 (m, 1H), 2.80 (m, 1H), 1.50 (m, 2H), 1.29 (m, 2H), 0.86 (m, 3H); MS (ESI) m/z 448 [(M + H)$^+$, 100]; HRMS (FAB) m/z 448.2216 [(M + H)$^+$ calcd for $C_{21}H_{30}N_5O_6$ 448.2196]; HPLC t_R 10.0 min (95%). Anal. ($C_{21}H_{29}N_5O_6 \cdot 0.95CF_3CO_2H$) C, H, N, F.

3-[[[3-[4-(Aminoiminomethyl)phenyl]-4,5-dihydro-5-isoxazolyl]acetyl]methylamino]-*N*-methyl-*N*-(benzyloxycarbonyl)-L-alanine Trifluoroacetate (60c). To a solution of **11** (3.53g, 14.8mmol) in dioxane (50 mL) was added NaOH (1.30 g, 32.43 mmol) followed by di-*tert*-butyl dicarbonate (3.23 g, 14.8 mmol). The reaction mixture was stirred at room temperature overnight, quenched with water (150 mL), and extracted with EtOAc (2 × 50 mL). The aqueous layer was acidified using 1 N HCl, and the product was extracted with EtOAc (2 × 50 mL), washed with brine (50 mL), dried ($MgSO_4$), and evaporated to give **14** as a colorless foam (3.36 g, 70% yield). The foam was dissolved in THF (anhyd, 25 mL) and cooled to 0 °C. To this solution was added NaH (60% in oil, 1.36 g, 34.2 mmol). The reaction mixture was stirred at 0 °C for 0.5 h, iodomethane (4.5 g, 31.7 mmol) was added, the mixture was stirred at room temperature for 24 h and quenched with saturated NH_4Cl (25 mL). After the pH was adjusted to 4 with 1 N HCl, the mixture was extracted with EtOAc (2 × 50 mL). The combined organic was washed with saturated NaCl (50 mL), dried ($MgSO_4$), and filtered. Evaporation *in vacuo* afforded a colorless oil which was purified using column chromatography (silica gel, hexane:EtOAc, 1:1) to afford the desired dimethylated diaminopropionic acid (2.82 g, 74%) as a colorless foam: ^{1}H NMR ($CDCl_3$) δ 7.40−7.32 (m, 5H), 5.17 (s, 2H), 4.84−4.75 (bt, J = 7.5 Hz, 2H), 3.85 (bs, 1H), 3.70−3.58 (bs, 2H), 2.99 (s, 3H), 2.84 (s, 3H), 1.43 (s, 9H); MS (ESI) m/z 367 [(M + H)$^+$, 100].

To a solution of the above acid (2.50 g, 6.8 mmol) in methanol (anhyd, 25 mL) was added *p*-TsOH (spatula tip). The reaction mixture was gently heated for 24 h. Solvent was removed *in vacuo*, and the residue was quenched with saturated $NaHCO_3$ (25 mL). The mixture was extracted with EtOAc (2 × 50 mL), and the combined organic was dried ($MgSO_4$) and evaporated to a colorless oil. Cleavage of the Boc protecting group as described for the preparation of **60b** afforded **13** (2.01 g, 97% yield): ^{1}H NMR ($CDCl_3$/DMSO-d_6) δ 7.40−7.32 (m, 5H), 5.15 (s, 2H), 4.80−4.65 (bt, J = 7.8 Hz, 2H), 3.85 (s, 1H), 3.70 (bs, 2H), 2.99 (s, 3H), 2.82 (s, 3H); MS (ESI) m/z 269 [(M + H)$^+$, 100].

The title compound was prepared from **22** and **13** following the procedure reported for **25b** to yield a colorless foam (20%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.45 (bs, 1.5 H), 9.40 (bs, 1.5H), 7.85 (m, 2H), 7.69 (m, 2H), 7.27 (m, 5H), 5.24−5.00 (m, 3H), 4.90 (m, 1H), 4.78 (m, 1H), 4.03−3.80 (m, 3H), 3.68−3.50 (m, 2H), 3.08 (dd, J = 9.0, 17.0 Hz, 1H), 2.96 (s, 3H), 2.93 (s, 3H), 2.80 (m, 1H), 2.48−2.40 (m, 1H); HRMS (FAB) m/z 496.2209 [(M + H)$^+$ calcd for $C_{25}H_{29}N_5O_6$ 496.2196].

***In Vivo* Pharmacology. Studies of Antiplatelet Efficacy in Rhesus Monkeys.** Sixteen rhesus monkeys of either sex, 8−15 years of age and weighing 6−10 kg, were administered **24v** as a solution in 0.9% saline (total dosing volume 0.5 mL/kg) at oral doses of 0.1, 0.3, and 1.0 mg/kg. Each dose was given to at least two animals/sex. Oral dosing was achieved by passing a 16 Fr feeding tube through the oral cavity into the stomach. Administration of **24v** was followed by a 10−20 mL water flush. Blood sampling at various time points was accomplished by accessing the appropriate sample site (femoral or saphenous vein). The total volume of blood sampled during the study did not exceed 1% of body weight. Blood samples were withdrawn in citrate containing Vacutainer tubes for an assessment of the *ex vivo* inhibition of ADP (100 μM)-mediated platelet aggregation.

Studies of Antiplatelet Efficacy in Baboons. For these studies, eight baboons (three per group, each made up of two females and one male) weighing 15.4−31.9 kg were fasted overnight, administered atropine (0.04 mg/kg) followed by ketamine (10 mg/kg) and xylazine (2 mg/kg), and restrained on a treatment table. Either the femoral or saphenous vein was cannulated for blood sampling at various time points. Using a nasogastric tube, **24v** was administered as a 0.2 or 0.6 mg/mL solution in 5% EtOH−95% saline (0.9%) at oral doses of 0.1, 0.3, 1.0, and 3.0 mg/kg. Blood samples were withdrawn in citrate containing Vacutainer tubes for an

assessment of the *ex vivo* inhibition of ADP (100 μM)-mediated platelet aggregation.

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Supporting Information Available: 300 MHz ^{1}H NMR spectra of **22**, **24a**, **24f**, **24i**, **24j**, **24x**, **26g**, **38**, **49**, **50**, **56**, and **60c**, reverse phase HPLC chromatograms of **24f**, **24i**, **24j**, **24x**, **26g**, **38**, **49**, **56**, and **60c**, and chiral phase supercritical fluid chromatograms of **24u** and various salt forms of **24y** (22 pages). Ordering information is given on any current masthead page.

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