Articles

Isoxazolines as Potent Antagonists of the Integrin $\alpha_v\beta_3$

William J. Pitts,[†] John Wityak,*[,†] Joanne M. Smallheer, A. Ewa Tobin, James W. Jetter, Jennifer S. Buynitsky, Patricia P. Harlow, Kimberly A. Solomon, Martha H. Corjay, Shaker A. Mousa, Ruth R. Wexler, and Prabhakar K. Jadhav

DuPont Pharmaceuticals Company, P.O. Box 80500, Wilmington, Delaware 19880-0500

Received January 21, 1999

Starting with lead compound **2**, we sought to increase the selectivity for $\alpha_v\beta_3$-mediated cell adhesion by examining the effects of structural changes in both the guanidine mimetic and the substituent α to the carboxylate. To prepare some of the desired aminoimidazoles, a novel reductive amination utilizing a trityl-protected aminoimidazole was developed. It was found that guanidine mimetics with a wide range of pK_a's were potent antagonists of $\alpha_v\beta_3$. In general, it appeared that an acylated 2-aminoimidazole guanidine mimetic imparted excellent selectivity for $\alpha_v\beta_3$-mediated adhesion versus $\alpha_{IIb}\beta_3$-mediated platelet aggregation, with selectivity of approximately 3 orders of magnitude observed for compounds **3g** and **3h**. It was also found in this series that the α-substituent was required for potent activity and that 2,6-disubstituted arylsulfonamides were optimal. In addition, the selective $\alpha_v\beta_3$ antagonist **3h** was found to be a potent inhibitor of $\alpha_v\beta_3$-mediated cell migration.

Introduction

Integrins are heterodimeric transmembrane receptors that mediate cell adhesion, migration, and cellular signaling. Pharmacological modulation of integrin-mediated processes is of current interest. The integrin $\alpha_v\beta_3$ is distributed on numerous cell types, such as platelets, endothelial cells, melanoma, smooth muscle cells (SMCs), and osteoclasts. On most cell types, it is expressed at relatively low levels; however, up-regulation of the receptor occurs under pathophysiological conditions.[1] Like GPIIb/IIIa ($\alpha_{IIb}\beta_3$), $\alpha_v\beta_3$ binds a variety of RGD-containing adhesive proteins such as vitronectin, fibronectin, von Willebrand factor (vWF), fibrinogen, osteopontin, bone sialo protein II, and thrombospondin. Recently, small molecule antagonists to the $\alpha_v\beta_3$ receptor have been reported to be effective when tested in animal models of osteoclast-mediated bone resorption.[2] The discovery of selective antagonists to the $\alpha_v\beta_3$ receptor could, in principle, offer new therapeutic strategies for the treatment of osteoporosis, restenosis, angiogenic ocular disorders, and cancer.

Since its introduction in 1978,[3] percutaneous transluminal coronary angioplasty (PTCA) has been transformed into a routine procedure that benefits thousands of coronary artery disease patients annually. Despite the clinical benefit and advances in techniques, restenosis occurs typically 3−6 months after the procedure at an incidence of 30−50%.[4] The pathogenesis of restenosis is a multicomponent process dependent upon one or more of the following factors: inadequate initial luminal cross section due to elastic recoil; neointimal hyperplasia; extracellular matrix deposition; or unfavorable vascular remodeling.[5] The relative importance of these factors is believed to be dependent on the individual patient, lesion, and revascularization procedure. The $\alpha_v\beta_3$ integrin is expressed in migrating SMCs during restenosis in the process of neointimal hyperplasia.[6] Several studies in animal models have shown that selective inhibition of $\alpha_v\beta_3$ leads to the inhibition of neointimal hyperplasia and stenosis.[7]

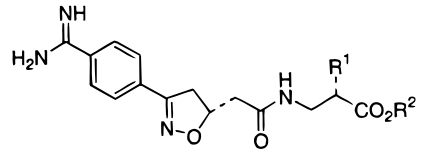
Earlier, we disclosed a series of isoxazolines represented by XR299 (**1a**) and DMP 754 (**1b**) that are potent and selective GPIIb/IIIa antagonists (Figure 1).[8] As shown in Table 1, XR299 shows a greater than 800-fold selectivity for the inhibition of platelet aggregation in the PRP assay, an $\alpha_{IIb}\beta_3$-driven process, versus the adhesion of 293 cells engineered to express the $\alpha_v\beta_3$ receptor to immobilized fibrinogen,[9] a process mediated by this integrin. Using a strategy that relied upon solid-phase synthesis of a small library of 3,5-disubstituted isoxazolines and in vitro screening via ELISA format, optimization of tether lengths and isoxazoline orientation led to the identification of **2** (Figure 1) as a potent inhibitor of the binding of vitronectin to purified $\alpha_v\beta_3$ receptor.[10] However, selectivity for the $\alpha_v\beta_3$ integrin relative to $\alpha_{IIb}\beta_3$ was still an issue. In this work, we disclose our efforts to reverse the integrin selectivity observed with agents such as XR299 and DMP 754, with the goal of discovering a new series of potent, selective $\alpha_v\beta_3$ antagonists.

Chemistry

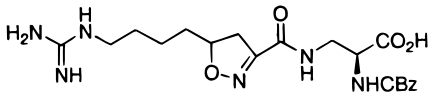
The majority of the isoxazolines **3** were synthesized from intermediate **4** using a variety of synthetic strategies. Isoxazoline **4** was prepared in 70−90% yield from the 1,3-dipolar cycloaddition of 1-hexen-6-ol (**5**) with

* To whom correspondence should be addressed. Tel: 609-252-4832. Fax: 609-252-6804. E-mail: john.wityak@bms.com.
[†] Present address: Bristol-Myers Squibb Pharmaceutical Research Institute, P.O. Box 4000, Princeton, NJ 08543-4000.

10.1021/jm9900321 CCC: $19.00
Published on Web 12/10/1999



1a: $R^1 = R^2 = H$
1b: $R^1 = NHCO_2n\text{-}Bu$, $R^2 = Me$



2

Figure 1. Early leads toward a selective $\alpha_v\beta_3$ antagonist.

ethyl chlorooximidoacetate (**6**). The simple guanidine and guanidine mimetics **3a, 3b, 3i,** and **3j** were prepared from **4** using the route depicted in Scheme 1. Tosylation of **4**, followed by displacement using azide and saponification gave the acid **7**. Coupling of **7** to diaminopropionate **8a** followed by Staudinger reduction afforded amine **9a**. Reaction of **9a** with reagents such as **10**,[11] trimethylsilyl isocyanate, or chlorosulfonyl isocyanate followed by protecting group cleavage afforded the desired analogues.

The 2-aminoimidazol-4-yl **3c** was prepared as shown in Scheme 2. The alcohol **4a** was subjected to Jones oxidation to provide acid **11**. This acid was converted to the acyl chloride, reacted with diazomethane, and treated with HBr gas to provide bromoketone **12**. Reaction of **12** with acetylguanidine, in a manner analogous to that published by Little and Webber,[12] followed by careful saponification yielded carboxylic acid **13**. Coupling of **13** to diaminopropionate **8a** followed by protecting group cleavage afforded the desired aminoimidazole.

The imidazol-2-ylaminoacyl bearing isoxazoline **3g** was prepared by coupling acid **11** with 2-aminoimidazole sulfate **14** at elevated temperature, followed by hydrolysis with lithium hydroxide to form acid **15** (Scheme 3). Coupling of **15** to diaminopropionate **8a** followed by protecting group cleavage afforded the desired acylaminoimidazole.

The pyridin-2-ylamino derivatives **3e**, **3r**, **3s**, **3u**, **3x**, **3y**, and **3aa** were prepared according to the protocol outlined in Scheme 4. Oxidation of alcohol **4** to aldehyde **16** followed by reductive amination using 2-aminopyridine under Maryanoff conditions, Boc protection, and ester saponification gave the carboxylate **17**. Coupling of **17** to diaminopropionate **8b** provided the bis(carbamate) ester **18** which was converted to **3aa**. Alternatively, intermediate **18** underwent chemoselective hydrogenolysis of the Cbz group to afford amine **19** using conditions adapted from Nikam.[13] Refunctionalization of this amine using a series of sulfonyl chlorides, followed by protecting group cleavage, afforded the desired aminopyridines. The isoquinolines **3k** and **3l** were prepared from aldehyde **16** in a similar manner.

Synthesis of the 6-aminopyridin-2-yl **3f** is depicted in Scheme 5. The synthesis began with the protection of 2-amino-6-methylpyridine **(20)** as the Boc derivative. Lithiation and alkylation of the dianion using 4-bromo-1-butene followed by dipolar cycloaddition with **6** and saponification gave the isoxazoline acid **21**. Coupling of **21** to diaminopropionate **8a** followed by protecting group cleavage afforded the desired aminopyridine.

Synthesis of (Imidazol-2-ylamino)alkyl Derivatives. Two potential strategies for the introduction of the imidazol-2-ylamino moiety were known in the literature. The first methodology relies upon variations on the de novo synthesis of the imidazole ring.[14] A second method demonstrates the utility of the Mitsunobu reaction of 2-(allyloxycarbonylamino)imidazole with a carbapenam-derived alcohol.[15] This latter method was viewed as a potentially efficient entry into the series; however, its application met with incomplete reaction and an arduous chromatographic separation unsuitable for scale-up. As an alternative, reductive amination of aldehyde **16** with 1-trityl-2-aminoimidazole **(22)** was investigated (Scheme 6). Imidazole **22** was prepared from 2-aminoimidazole (**14**) by protection of the 2-amino functionality as the phthalimide, followed by tritylation and hydrazinolysis. Under Maryanoff conditions,[16] reaction of **22** with **16** resulted not in the desired amine but instead reduction of the aldehyde to afford alcohol **4a**. It was apparent that imine formation was the rate-determining step; under the best conditions (toluene at reflux), the half-life for this reaction was 1.5 h.[17] After 7 half-lives, the mixture was cooled to room temperature and $NaBH(OAc)_3$ added, resulting in the desired aminoimidazole adduct. Saponification then gave the desired acid **23** in 66% overall yield from **16**. Coupling to **8**, followed by protecting group cleavage, afforded the desired aminoimidazoles **3d**, **3m**−**q**, **3t**, **3v**, **3w**, **3z**, and **3bb**.

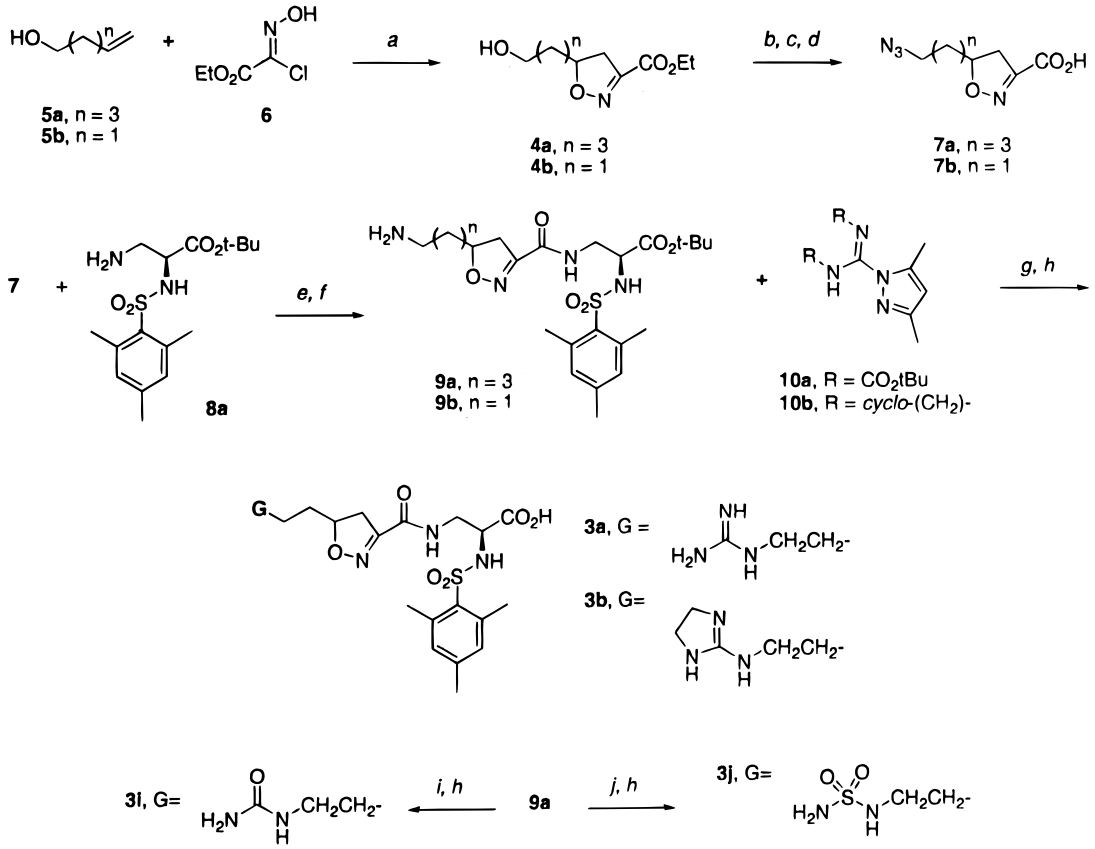
The urea **3h** was prepared from **9b** via reaction with phosgene followed by the addition of **22** to the intermediate isocyanate. Protecting group cleavage then afforded the desired target.

Results and Discussion

The screening strategy used to discover potent and selective $\alpha_v\beta_3$ antagonists involved measuring the relative activity and selectivity of compounds using whole-cell assays. Human 293 cells were engineered to overexpress the $\alpha_v\beta_3$ receptor and were used to assess the potency of antagonists as measured by the inhibition of their binding to immobilized fibrinogen. Compounds were also assayed for relative selectivity of antagonism of the $\alpha_v\beta_3$ versus $\alpha_{IIb}\beta_3$ receptor by comparing this result with the IC_{50} values obtained for the inhibition of platelet aggregation in human platelet-rich plasma (PRP)[18] and/or in human gel-purified platelets containing 1 mg/mL fibrinogen (GPP + Fg).[19] Since the protein concentration differs greatly between these two platelet aggregation assays, the potencies obtained could provide a rough estimate of the protein binding of the test compounds. These data are summarized in Tables 1 and 2. In addition, a selective $\alpha_v\beta_3$ antagonist was further assayed using a microporous filter assay for the ability to inhibit the migration of transfected 293 cells in response to vitronectin.

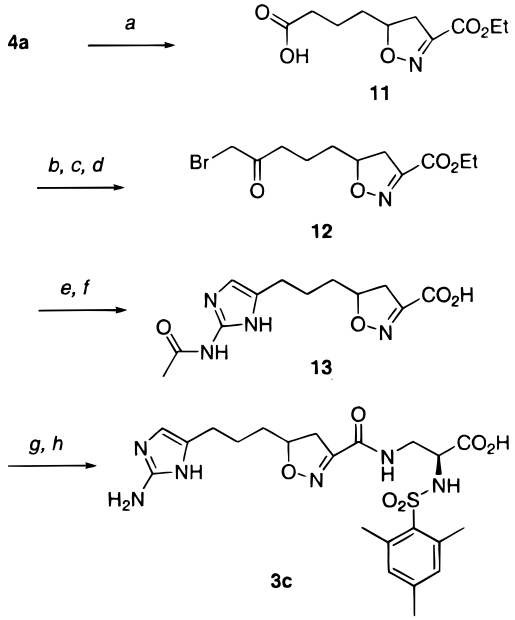
Guanidine Mimetic SAR. A working hypothesis regarding selectivity for $\alpha_v\beta_3$- versus $\alpha_{IIb}\beta_3$-mediated effects was that selectivity could be optimized through careful choice of the pK_a, geometry, and hydrogen-bonding characteristics of the guanidine mimetic (Table 1). As a starting point, guanidine **3a**, which has an

Scheme 1. Synthesis of the Guanidine Mimetics **3a**, **3b**, **3i**, and **3j**[a]



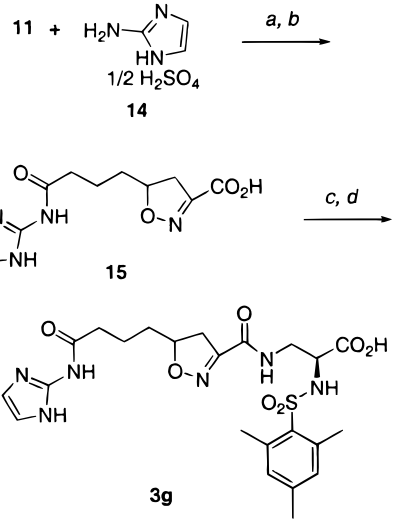
[a] Reagents: (a) $NaHCO_3$(aq), THF, 0 °C−rt; (b) *p*-TsCl, pyridine; (c) NaN_3, DMF; (d) NaOH, H^+; (e) BOP, **8a**, Hunig's base; (f) Ph_3P, dioxane, NH_4OH(aq); (g) **10**, 80 °C, dioxane; (h) TFA; (i) TMSNCO; (j) $ClSO_2NCO$, *t*-BuOH.

Scheme 2. Synthesis of Imidazole **3c**[a]



[a] Reagents: (a) Jones reagent; (b) oxalyl chloride, cat. DMF; (c) diazomethane; (d) HBr(g); (e) acetylguanidine; (f) NaOH, HCl; (g) BOP, **8a**, *N*-methylmorpholine; (h) H_2SO_4, 60 °C.

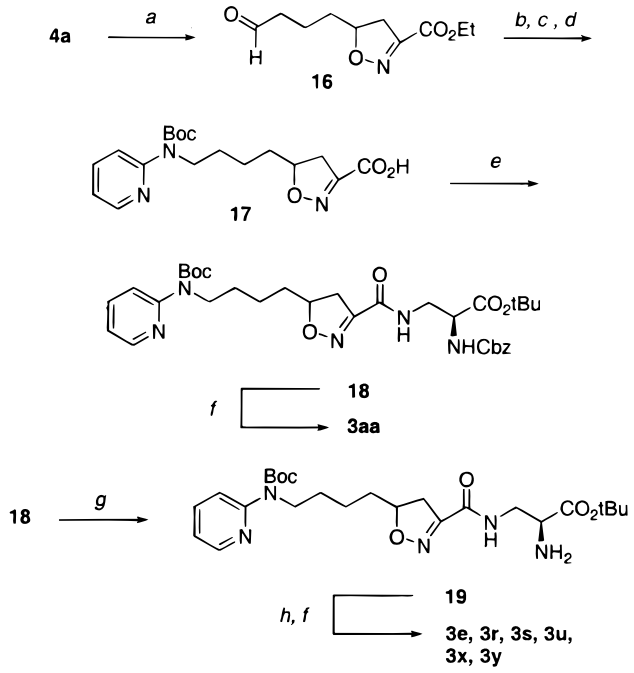
Scheme 3. Synthesis of the Acylaminoimidazole **3g**[a]



[a] Reagents: (a) **14**, BOP, Hunig's base, 70 °C; (b) LiOH, dioxane(aq), H^+; (c) BOP, **8a**, *N*-methylmorpholine; (d) TFA.

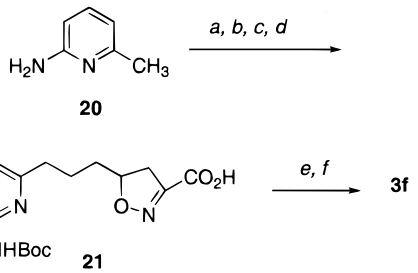
estimated pK_a of 12,[20] had modest potency against the $\alpha_v\beta_3$-mediated adhesion of 293 cells to fibrinogen and was an equipotent inhibitor of platelet aggregation in the PRP assay. Embedding the guanidine in an imidazoline ring as in **3b** to hinder possible binding modes involving participation of its imino(aminomethyl) structural motif had a modest positive effect on both potency and selectivity when compared to **3a**. The aminoimidazoles **3c** and **3d** further probed geometry and pK_a. Aminoimidazole **3d** has an estimated pK_a of 8.6 and was envisioned as a less basic alternative to **3b**. It has excellent potency in the 293 cell adhesion assay and moderate selectivity versus $\alpha_{IIb}\beta_3$ receptor-driven platelet aggregation. In contrast, the isomeric aminoimidazole **3c** was approximately 2−3-fold less potent and appeared to be less selective. The decreased selectivity

Scheme 4. Synthesis of 2-Aminopyridines **3aa**, **3e**, **3r**, **3s**, **3u**, **3x**, and **3y**[a]



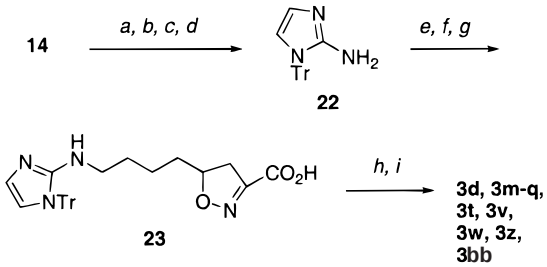
[a] Reagents: (a) oxalyl chloride, DMSO, Et_3N, −70 °C; (b) sodium triacetoxyborohydride, 2-aminopyridine; (c) Boc_2O, pyridine, cat. DMAP; (d) LiOH, dil H^+; (e) BOP, **8b**, *N*-methylmorpholine; (f) TFA; (g) H_2, 40 psi, $Pd/BaSO_4$; (h) arylsulfonyl chloride, pyridine.

Scheme 5. Synthesis of the 2-Aminopyridin-6-yl **3f**[a]



[a] Reagents: (a) Boc_2O, 40 °C; (b) LDA, −78 °C, then 4-bromobutene; (c) **6**, $NaHCO_3$, THF(aq), 0 °C−rt; (d) NaOH, dil H^+; (e) BOP, **8a**, *N*-methylmorpholine; (f) TFA.

Scheme 6. Synthesis of the 2-Aminoimidazoles **3d**, **3m**−**q**, **3t**, **3v**, **3w**, **3z**, and **3bb**[a]



[a] Reagents: (a) $NaOCH_3$, −78 °C−rt; (b) phthalic anhydride, melt; (c) triphenylmethyl chloride, pyridine; (d) N_2H_4, EtOH, reflux; (e) **16**, toluene, reflux; (f) sodium triacetoxyborohydride; (g) LiOH, H^+; (h) TBTU, **8**, *N*-methylmorpholine; (i) TFA, reflux.

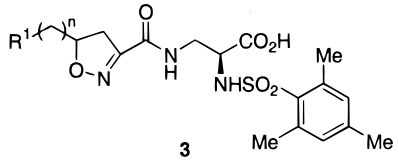
observed with aminoimidazole **3c** hints at the possibility that the nitrogen at position 2 of the imidazole ring may mimic an interaction normally made by the ω' nitrogen of the arginine guanidine moiety (Figure 2). Assuming that this interaction is a more important component toward the binding of ligands to $\alpha_{IIb}\beta_3$ than to $\alpha_v\beta_3$, it might be reasonably expected that such interaction could be more readily accommodated by aminoimidazole **3c** than aminoimidazole **3d**. The aminopyridines were selected as examples of guanidine mimetics having a pK_a of approximately 7. Both aminopyridine **3e** and its isomer **3f** were approximately equipotent to **3d** in the 293 cell adhesion assay and appeared to have enhanced selectivity versus $\alpha_{IIb}\beta_3$ receptor-driven platelet aggregation. The acylated aminoimidazoles **3g** and **3h** ($pK_a < 4$) are analogues having lower basicity than the aminopyridines. These were also potent $\alpha_v\beta_3$ antagonists and had the best selectivity yet observed in the series. Two other nonbasic guanidine mimetics, urea **3i** and sulfamide **3j**, were inactive when tested at a concentration of 1 μmol. The isomeric isoquinolines **3k** and **3l** were also inactive, suggesting that additional lipophilicity or steric bulk adjacent to the guanidine mimetic is not tolerated.

The empirical results suggest that a positively charged guanidine mimetic is not a prerequisite for potency against $\alpha_v\beta_3$ mediated adhesion. The separation of $\alpha_v\beta_3$ mediated adhesion and antiplatelet effects was readily achieved by the incorporation of a less basic guanidine mimetic such as an acylated aminoimidazole. As observed with aminoimidazoles **3c** and **3d**, selectivity could also be gained through an orientation of the guanidine mimetic that discouraged certain bidentate binding arrangements involving both a δ-like nitrogen (arginine numbering) and an amino(iminomethyl) ω'-nitrogen.

Effect of the α-Substituent. Much of the SAR with respect to the substituent α to the carboxylate was elucidated via analogues having the aminoimidazole and aminopyridine guanidine mimetics (Table 2). Aminoimidazoles which possessed a 2,6-disubstituted arylsulfonamide (**3d**, **3m**−**p**) had approximately 5-fold increased potency relative to phenylsulfonamide **3q**. However, this finding was not observed with the aminopyridines (**3e**, **3r** versus **3s**). The 1-naphthylsulfonamides **3t** and **3u** and the 3,5-dimethylisoxazol-4-yl analogue **3v** were approximately equipotent with their respective phenylsulfonamide analogues. Likewise, the presence of a phenyl or isopropyl substituent at the 4-position of the arylsulfonamide in **3w**−**y** appeared to confer neither increased potency nor selectivity for $\alpha_v\beta_3$-mediated adhesion when compared to their respective phenylsulfonamide analogues. However, as shown with **3q**, the potency of **3w** could be increased through the addition of substituents at the 2- and 6-positions (**3o, 3p**). A prerequisite for potent activity in this series of isoxazolines appears to be the derivatization of the α-amino moiety as a sulfonamide. As shown with analogues **3z** and **3aa**, compounds lacking this structural feature were inactive against $\alpha_v\beta_3$-mediated adhesion.

Effect of Overall Length and Stereochemistry. In an earlier study, we had observed that activity in an $\alpha_v\beta_3$ ELISA was optimal when the guanidine and isoxazoline moieties were separated by a tether length of either 2 or 4 methylene units.[10] This curious finding was repeated by the observation of submicromolar activity for both **3d** and **3bb** in our cell-based assay. It was initially hypothesized that compounds having a shorter overall distance between the guanidinium mi-

Table 1. In Vitro Activity of Isoxazolines **3** with Respect to Guanidine Mimetic

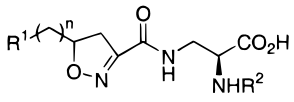


3

			IC$_{50}$, nM			
compd	R^1	*n*	293β_3z-Fg adhesion[a]	PRP[b]	GPP+Fg[b]	GPP+Fg/293β_3z-Fg
1a			>100000	240	140	<1
3a	$H_2NC{=}NH(NH)$	4	169 ± 87	160		
3b	imidazolin-2-ylNH	4	62 ± 43	690	600	10
3c	2-aminoimidazol-4-yl	3	46 ± 1.0	280	180	4
3d	imidazol-2-ylNH	4	15 ± 4.3	850		
3e	pyridin-2-ylNH	4	54 ± 38	16000	6500	120
3f	2-aminopyridin-6-yl	3	26 ± 24	78000		
3g	imidazol-2-ylNHCO	3	31 ± 16	61000	>100000	>3200
3h	imidazol-2-ylNHCONH	2	34 ± 12	120000	32000	940
3i	$H_2NC{=}O(NH)$	4	>1000		6300	
3j	$H_2NSO_2(NH)$	4	>1000		>100000	
3k	isoquinolin-1-ylNH	4	>1000	78000	6500	
3l	isoquinolin-3-ylNH	4	>1000	11000	7700	

[a] Number of measurements = 3. [b] Number of measurements = 1. The error in the GPP and PRP measurements is ±10%.

Table 2. In Vitro Activity of Isoxazolines **3** with Respect to α-Substituent, Stereochemistry, and Overall Length



3

				IC$_{50}$, nM			
compd	R^1	R^2	*n*	293β_3z-Fg adhesion[a]	PRP[b]	GPP+Fg[b]	GPP+Fg/293β_3z-Fg
3d	imidazol-2-ylNH	2,4,6-$(CH_3)_3C_6H_2SO_2$	4	15 ± 4.3	850		
3e	pyridin-2-ylNH	2,4,6-$(CH_3)_3C_6H_2SO_2$	4	54 ± 38	16000	6500	120
3m	imidazol-2-ylNH	2,6-$(Cl)_2C_6H_3SO_2$	4	18 ± 14	350	190	10
3n	imidazol-2-ylNH	2-Cl-6-$(CH_3)C_6H_3SO_2$	4	7.1 ± 3.9	390		
3o	imidazol-2-ylNH	2,6-$(Cl)_2$-4-$(Ph)C_6H_2SO_2$	4	4.6 ± 4.0	1600		
3p	imidazol-2-ylNH	2,6-$(CH_3)_2$-4-$(Ph)C_6H_2SO_2$	4	8.8 ± 3.2	3100	690	80
3q	imidazol-2-ylNH	$C_6H_5SO_2$	4	66 ± 7.3	1000	930	10
3r	pyridin-2-ylNH	2,6-$(CH_3)_2$-4-$(Ph)C_6H_2SO_2$	4	44 ± 19	>100000		
3s	pyridin-2-ylNH	$C_6H_5SO_2$	4	81 ± 6.4	30000	18000	220
3t	imidazol-2-ylNH	1-$C_{10}H_7SO_2$	4	51 ± 10	7800	570	10
3u	pyridin-2-ylNH	1-$C_{10}H_7SO_2$	4	160 ± 59	>100000		
3v	imidazol-2-ylNH	3,5-$(CH_3)_2$isoxazol-4-ylSO$_2$	4	73 ± 42	1500		
3w	imidazol-2-ylNH	4-$(Ph)C_6H_4SO_2$	4	240 ± 140	43000	5000	20
3x	pyridin-2-ylNH	4-$(Ph)C_6H_4SO_2$	4	130 ± 68	70000		
3y	pyridin-2-ylNH	4-(*i*-Pr)$C_6H_4SO_2$	4	170 ± 70	>100000		
3z	imidazol-2-ylNH	H	4	>1000	84000	47000	
3aa	pyridin-2-ylNH	$(C_6H_5CH_2O)CO$	4	ND[c]	>100000	85000	
3bb	imidazol-2-ylNH	2,4,6-$(CH_3)_3C_6H_2SO_2$	2	106 ± 39	4700	3000	30
3cc	imidazol-2-ylNH	**3s** isomer 1	4	10.6 ± 1.1	980	890	80
3dd	imidazol-2-ylNH	**3s** isomer 2	4	4.5 ± 3.6	1100	690	150
3ee	imidazol-2-ylNH	(*R*)-2,4,6-$(CH_3)_3C_6H_2SO_2$	4	2800 ± 580	68000	28000	10

[a] Number of measurements = 3. [b] Number of measurements = 1. The error in the GPP and PRP measurements is ±10%. [c] Not determined. The IC$_{50}$ against an $\alpha_v\beta_3$ ELISA is 23 nM. For comparison, the IC$_{50}$ for **3d** in this ELISA is <1 nM.

metic and the carboxylate moiety might have enhanced inherent selectivity for inhibiting $\alpha_v\beta_3$-mediated adhesion relative to $\alpha_{IIb}\beta_3$-mediated antiplatelet activity; however, the data for compounds **3d** and **3bb** did not support this hypothesis, and shorter tether length compounds such as **3bb** were not studied further. The inherent conformational flexibility of the compounds precluded a more detailed analysis.

The majority of compounds prepared in this study were diastereomeric mixtures having the (*S*)-absolute configuration about the diaminopropionate stereocenter. Holding the diaminopropionate α-substituent of **3d** in the (*S*)-configuration, two isoxazoline epimers **3cc** and **3dd** were separated using chiral supercritical fluid chromatography. As with a related series of $\alpha_{IIb}\beta_3$ antagonists,[8] the absolute configuration of this stereocenter did not have a significant effect on either potency or selectivity. Conversely, inversion of the diaminopropionate α-substituent as in **3ee** resulted in a large decrease in potency for both $\alpha_v\beta_3$- and $\alpha_{IIb}\beta_3$-mediated effects. Apart from the $\alpha_v\beta_3$ activity, it is interesting to note that in the related series targeted to $\alpha_{IIb}\beta_3$, the absolute configuration of this stereocenter had very little effect on antiplatelet potency,[8a] while in the present series, it appears to be critical to both $\alpha_v\beta_3$- and $\alpha_{IIb}\beta_3$-mediated activities.

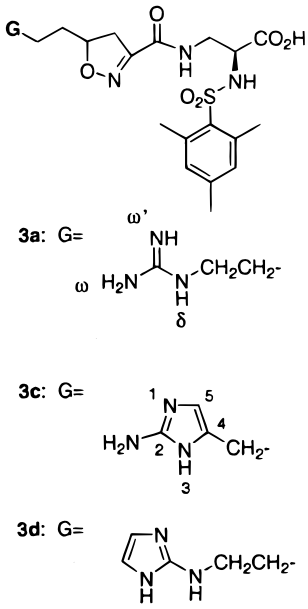


Figure 2. Orientation of the guanidine mimetic may relate to integrin selectivity.

$\alpha_v\beta_3$-Mediated Migration. During the process of neointimal hyperplasia, $\alpha_v\beta_3$ integrin expression is up-regulated in migrating SMCs. In a chemotaxis assay, the selective $\alpha_v\beta_3$ inhibitor **3h** inhibited the migration of $\alpha_v\beta_3$-transfected 293 cell migration in response to vitronectin with an IC_{50} value of 140 ± 60 nM (n = 4). It has been suggested that different activation states of $\alpha_v\beta_3$, each possessing its own unique conformation of the integrin, are important in relation to functional activity.[21] We have demonstrated that a selective example within the current series of isoxazolines is a potent antagonist of both $\alpha_v\beta_3$-mediated adhesion and migration. Migration is thought to require $\alpha_v\beta_3$-mediated outside-in signaling. It is believed that both adhesion and migration play important roles in the processes of vascular remodeling and restenosis.

Conclusions

In the present work, the effect of structural changes in both the guanidine mimetic and the substituent α to the carboxylate of guanidine **2** led to the observation of increased selectivity and potency for the adhesion of 293 cells expressing $\alpha_v\beta_3$ to fibrinogen. To prepare some of the desired aminoimidazoles, a novel reductive amination utilizing a trityl-protected aminoimidazole was developed. It was found that guanidine mimetics with a wide range of pK_a's were potent antagonists of $\alpha_v\beta_3$-mediated adhesion of transfected 293 cells to fibrinogen. In general, it appeared that the less basic guanidine mimetics imparted greater selectivity for $\alpha_v\beta_3$-mediated adhesion versus $\alpha_{IIb}\beta_3$-mediated platelet aggregation. In particular, compounds **3g** and **3h** had excellent potency and selectivity of approximately 3 orders of magnitude. It was also found that the α-substituent was required for potent activity in this series and that 2,6-disubstituted arylsulfonamides appeared optimal in this regard. The absolute stereochemistry of the diaminopropionate-derived stereocenter was also determined to be a critical factor affecting potency. In addition, aminoimidazolylurea **3h** was found to be a potent inhibitor of $\alpha_v\beta_3$-mediated chemotaxis in response to vitronectin in a migration assay.

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 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. ^{1}H 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 were isolated yields.

***tert*-Butyl 3-Amino-*N*-(2,4,6-trimethylphenyl)sulfonyl-L-alanine (8a).** L-Asparagine (20.0 g, 0.15 mol) was suspended in a mixture of THF (130 mL) and water (250 mL). Triethylamine (49 g, 0.48 mol) was added followed by mesitylenesulfonyl chloride (49.7 g, 0.227 mol). The reaction mixture became slightly exothermic and the solids dissolved over a period of 0.5 h to yield a yellow solution. The mixture was stirred for 3 h at room temperature and washed with Et_2O and CH_2Cl_2. The aqueous layer was separated and acidified to a pH of 1.5 using concentrated HCl, during which time a heavy precipitate formed. After 0.5 h, the product was filtered, washed with water, and dried ($MgSO_4$) to yield a white solid (34 g, 72%): mp 193.5−195 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.58 (bs, 1H), 7.82 (d, J = 9.2 Hz, 1H), 7.32 (bs, 1H), 6.99 (s, 2H), 6.88 (bs, 1H), 3.98 (m, 1H), 2.55 (s, 6H), 2.45 (apparent dd, J = 15.4, 7.0 Hz, 1H; partially coincident with solvent), 2.28 (dd, J = 15.8, 6.2 Hz, 1H), 2.24 (s, 3H); MS (ESI) m/z 315.2 [(M + H)$^+$ 100]. Anal. Calcd for $C_{13}H_{18}N_2O_5S$: C, 49.67; H, 5.77; N, 8.91. Found: C, 49.79; H, 5.90; N, 8.84.

Sodium hydroxide (32 g, 0.80 mol) was dissolved in water (200 mL) and cooled in an ice bath. Bromine (19.2 g, 0.12 mol) was added dropwise over 5 min and the mixture allowed to stir for 15 min. *N*-(2,4,6-Trimethylphenyl)sulfonyl-L-asparagine (31.44 g, 0.10 mol) was added in several portions over a period of 10 min, during which time the yellow color faded. The reaction mixture was gently heated on a steam bath during which time the internal temperature rose to ca. 85 °C. After 1 h, the reaction mixture was allowed to cool to room temperature; then it was cooled in an ice bath. After cautiously acidifying to pH 6 with concentrated HCl, a solid formed and gas was evolved. The solid was filtered, washed with cold water, and dried overnight to give the product as a white solid (23.9 g, 83%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.06 (s, 2H), 3.07 (dd, J = 11.4, 4.4 Hz, 1H), 2.94 (dd, J = 9.5, 4.4 Hz, 1H), 2.80 (m, 1H), 2.59 (s, 6H), 2.26 (s, 3H); MS (ESI) m/z 287.2 [(M + H)$^+$ 100].

Into a Parr bottle was placed a suspension of 3-amino-*N*-(2,4,6-trimethylphenyl)sulfonylamino-L-alanine (11.45 g, 0.04 mol) in dioxane (170 mL). Into the cooled reaction mixture (dry ice−acetone bath) was condensed isobutylene (ca. 185 mL) and concentrated sulfuric acid (11 mL) was added. The bottle was sealed and agitated for 114 h. The bottle was depressurized and purged with nitrogen for a brief time. The reaction mixture was poured into a rapidly stirred mixture of water (225 mL) containing NaOH (17 g) and Et_2O (600 mL) which had been precooled in an ice bath. The layers were separated and the aqueous layer was extracted with additional Et_2O. The pH of the aqueous layer was carefully adjusted to 11 with concentrated HCl and the mixture extracted four times with Et_2O. The organic layers from the pH 11 adjusted extraction were combined, dried (Na_2SO_4), filtered, and evaporated to yield the product as a viscous oil which solidified upon standing (8.64 g, 63%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.95 (s, 2H), 3.69 (m,

1H; partially coincident with residual dioxane), 2.98 (dd, J = 13.6, 4.4 Hz, 2H), 2.88 (dd, J = 13.6, 5.5 Hz, 1H), 2.67 (s, 6H), 2.28 (s, 3H), 1.28 (s, 9H); MS (ESI) m/z 343 [$(M + H)^+$ 100]. Anal. Calcd for $C_{16}H_{26}N_2O_4S$: C, 56.12; H, 7.65; N, 8.18. Found: C, 56.13; H, 7.45; N, 7.98.

3-Ethoxycarbonyl-5-(4-hydroxybutyl)isoxazoline (4a). To a solution of 5-hexen-1-ol (**5**; 5.0 g, 0.05 mol) in THF (30 mL) was added a solution of $NaHCO_3$ (29.4 g, 0.35 mol) in water (20 mL) and the reaction mixture was cooled in an ice bath. Ethyl chlorooximidoacetate (**6**; 11.4 g, 0.075 mol) was added over 15 min in several portions. The reaction mixture was stirred while still immersed in an ice bath for 6 h. An additional amount of **6** (7.75 g, 0.05 mol) was added and the reaction mixture was allowed to warm to room temperature overnight. The mixture was partitioned between EtOAc and water; the organic layer was separated, dried ($MgSO_4$), filtered, and evaporated. The crude product was purified using flash chromatography (3:1 = hexane:EtOAc) to yield the desired isoxazoline as a colorless oil (9.22 g, 86%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.90−4.78 (m, 1H), 4.34 (q, J = 7 Hz, 2H), 3.66 (bs, 2H), 3.54−3.20 (m, 1H), 2.94−2.80 (m, 1H), 1.90−1.40 (m, 6H), 1.3 (t, J = 7 Hz, 3H); MS (NH_3-CI) m/z 233 [$(M + NH_4)^+$, 100], 216 $(M + H)^+$. Anal. Calcd for $C_{10}H_{17}NO_4$: C, 55.80; H, 7.96; N, 6.51. Found: C, 55.50; H, 7.72; N, 6.37.

3-Ethoxycarbonyl-5-(2-hydroxyethyl)isoxazoline (4b). The title compound was synthesized from 3-buten-1-ol following the general procedure for **4a:** ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.90−4.78 (m, 1H), 4.34 (q, J = 7 Hz, 2H), 3.66 (bs, 2H), 3.54−3.20 (m, 1H), 2.94−2.80 (m, 1H), 1.90−1.85 (m, 1H), 1.80−1.70 (m, 1H), 1.3 (t, J = 7 Hz, 3H); MS (NH_3-CI) m/z 205 [$(M + NH_4)^+$, 100], 188 $(M + H)^+$.

2-[(*S*)-2,4,6-Trimethylphenylsulfonylamino]-3-[5-(4-aminobutyl)isoxazolin-3-ylcarbonyl]aminopropionic Acid *tert*-Butyl Ester (9a). To an ice-cold solution of **4a** (6.45 g, 30 mmol) in CH_2Cl_2 (anhyd, 200 mL) was added pyridine (2.57 g, 33 mmol) followed by *p*-toluenesulfonyl chloride (6.27 g, 33 mmol). After 0.5 h, the ice bath was removed and the reaction mixture stirred at room temperature for 18 h. The reaction mixture was washed with water (2 × 200 mL), dried over $MgSO_4$, filtered, and evaporated to yield the crude product as a colorless oil. Purification using flash chromatography (2% MeOH−CH_2Cl_2) yielded 10.0 g (90%) of the desired tosylate: ^{1}H NMR (300 MHz, CD_3OD) δ 7.80 (d, J = 12 Hz, 2H), 7.36 (d, J = 12 Hz, 2H), 4.80−4.68 (m, 1H), 4.36 (q, J = 7 Hz, 2H), 4.02 (t, J = 6 Hz, 2H), 3.30−3.20, (m, 1H), 2.84−2.72 (m, 1H), 2.46 (s, 3H), 1.88−1.40 (m, 6H), 1.3 (t, J = 7 Hz, 3H).

The tosylate (10.0 g, 27 mmol) was dissolved in DMF (15 mL) and cooled in an ice bath. Sodium azide (4.5 g, 69 mmol) was added, and the reaction mixture stirred for 18 h, during which time the reaction mixture warmed to room temperature. After dilution with water (150 mL), the mixture was extracted with EtOAc. The organic layer was dried over Na_2SO_4, filtered, and evaporated to yield the azido ester as a colorless oil (5.96 g, 92%): ^{1}H NMR (300 MHz, CD_3OD) δ 4.86−4.76 (m, 1H), 4.36 (q, J = 7 Hz, 2H), 3.34−3.22, (m, 3H), 2.90−2.80 (m, 1H), 1.82−1.40 (m, 6H), 1.3 (t, J = 7 Hz, 3H). Anal. Calcd for $C_{10}H_{16}N_4O_3$: C, 49.99; H, 6.71; N, 25.32. Found: C, 49.78; H, 6.61; N, 25.02.

The azido ester (5.96 g, 25 mmol) was dissolved in 70% EtOH (20 mL) and 1 N NaOH (4 mL) was added. After stirring for 2 h at room temperature, the solvent was evaporated under reduced pressure (bath temperature < 25 °C), the residue acidified with a 20% solution of citric acid, and the product extracted with EtOAc. The organic layer was dried over $MgSO_4$, filtered, and evaporated to yield the azido acid **7a** as a white solid (4.52 g, 86%): ^{1}H NMR (300 MHz, CD_3OD) δ 4.82−4.72 (m, 1H), 3.34−3.20, (m, 3H), 2.90−2.74 (m, 1H), 1.82−1.38 (m, 6H); MS (ESI) m/z 211.3 [$(M - H)^-$, 25].

The azido acid **7a** (1.03 g, 4.9 mmol), **8a** (1.82 g, 5.3 mmol), *N*-methylmorpholine (1.08 g, 10.6 mmol), and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP; 3.22 g, 7.2 mmol) were dissolved in dioxane (4 mL) and stirred at room temperature for 18 h. The solvent was evaporated, the residue dissolved in EtOAc, and the resulting mixture washed with 1 M citric acid. The organic layer was dried over $MgSO_4$ and evaporated and the residue purified using flash chromatography to afford the desired amide (2.42 g, 94%): ^{1}H NMR (300 MHz, CD_3OD) δ 6.96 (s, 2H), 4.80−4.68 (m, 1H), 4.00−3.92 (m, 1H), 3.58−3.36 (m, 2H), 3.34−3.20, (m, 3H), 2.82−2.70 (m, 1H), 2.60 (s, 6H), 2.24 (s, 3H), 1.78−1.38 (m, 6H). 1.20 (d, J = 3 Hz, 9H); MS (ESI) m/z 537.4 [$(M - H)^-$, 81].

The above amide (2.42 g, 4.5 mmol) was dissolved in dioxane (anhyd, 3 mL), triphenylphosphine (1.99 g, 7.5 mmol) added, and the mixture stirred at room temperature for 18 h. 1 N Ammonium hydroxide (5 mL) was added and the reaction mixture stirred for an additional 6 h. The solvents were evaporated and the residue purified using flash chromatography (89:10:1 = CH_2Cl_2:MeOH:1 M NH_4OH) to give the desired amine **9a** (1.92 g, 83%) as a white solid: ^{1}H NMR (300 MHz, CD_3OD) δ 6.96 (s, 2H), 4.80−4.68 (m, 1H), 4.00−3.92 (m, 1H), 3.58−3.30 (m, 2H), 3.20−3.08, (m, 3H), 2.80−2.68 (m, 1H), 2.58 (s, 6H), 2.24 (s, 3H), 1.78−1.38 (m, 6H), 1.20 (d, J = 3 Hz, 9H); MS (ESI) m/z 511.3 [$(M + H)^+$, 100]. Anal. Calcd for $C_{24}H_{38}N_4O_6S\cdot1.20CF_3CO_2H$: C, 48.97; H, 6.10; N, 8.65; F, 10.56. Found: C, 49.22; H, 6.19; N, 9.01; F, 10.84.

2-[(*S*)-2,4,6-Trimethylphenylsulfonylamino]-3-[5-(2-aminoethyl)isoxazolin-3-ylcarbonyl]aminopropionic Acid *tert*-Butyl Ester (9b). This material was prepared in an analogous manner to **9a** and was isolated as a white solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.94 (s, 2H), 6.91−6.83 (b, 1H), 4.93−4.83 (m, 1H), 3.89−3.86 (m, 1H), 3.72−3.54 (m, 2H), 3.28 (dd, J = 10.8, 2.4 Hz, 1H), 2.92−2.81 (m, 3H), 2.64 (s, 6H), 2.28 (s, 3H), 1.95−1.84 (m, 1H), 1.79−1.70 (m, 1H), 1.32 (s, 9H); MS (ESI) m/z 483.3 [$(M + H)^+$, 100].

2-[(*S*)-2,4,6-Trimethylphenylsulfonylamino]-3-[5-(4-(aminoiminomethyl)aminobutyl)isoxazolin-3-ylcarbonyl]aminopropionic Acid Trifluoroacetate (3a). To a solution of **9a** (160 mg, 0.31 mmol) in dioxane (anhyd, 5 mL) was added 2-*(N,N′*-di-*tert*-butyloxycarbonylguanyl)-3,5-dimethylpyrazole (**10a**; 127 mg, 0.37 mmol), and the reaction mixture heated at 80 °C for 16 h. The solvent was evaporated and the residue dissolved TFA (4 mL). The reaction mixture was stirred for 1 h, the solvent evaporated, and the residue purified using reverse-phase HPLC. Concentration of the appropriate fractions in vacuo, trituration with Et_2O, and drying under vacuum gave the desired guanidine **3a** (50 mg, 26% overall from **9a**): ^{1}H NMR (300 MHz, CD_3OD) δ 6.96 (s, 2H), 4.80−4.68 (m, 1H), 4.08−4.00 (m, 1H), 3.64−3.56 (m, 1H), 3.42−3.30, (m, 1H), 3.22−3.02 (m, 3H), 2.78−2.68 (m, 1H), 2.58 (s, 6H), 2.24 (s, 3H), 1.78−1.38 (m, 6H); MS (ESI) m/z 497.3 [$(M + H)^+$, 100]. Anal. ($C_{21}H_{32}N_6O_6S\cdot1.4CF_3CO_2H$) C, H, N, F.

2-[(*S*)-2,4,6-Trimethylphenylsulfonylamino]-3-[5-(4-(imidazolin-2-yl)aminobutyl)isoxazolin-3-ylcarbonyl]aminopropionic Acid Trifluoroacetate (3b). To a solution of **9a** (180 mg, 0.35 mmol) and 2-(3,5-dimethylpyrazolyl)-4,5-dihydroimidazole hydrobromide (**10b**; 95 mg, 0.39 mmol) was added 4-(dimethylamino)pyridine (5 mg, 0.04 mmol), and the resulting mixture heated at 80 °C for 16 h. The solvent was evaporated and the residue dissolved in TFA (4 mL). The reaction mixture was stirred for 1 h, the solvent evaporated, and the residue purified using reverse-phase HPLC. Concentration of the appropriate fractions in vacuo, trituration with Et_2O, and drying under vacuum afforded 50 mg (20% overall from **9a**) of the desired guanidine: ^{1}H NMR (300 MHz, CD_3OD) δ 6.94 (s, 2H), 4.80−4.68 (m, 1H), 4.08−4.00 (m, 1H), 3.64 (s, 4H), 3.62−3.56 (m, 1H), 3.42−3.30, (m, 1H), 3.22−3.04 (m, 3H), 2.80−2.70 (m, 1H), 2.58 (s, 6H), 2.24 (s, 3H), 1.78−1.38 (m, 6H); MS (ESI) m/z 523.3 [$(M + H)^+$, 100]. Anal. ($C_{23}H_{34}N_6O_6S\cdot1.4CF_3CO_2H$) C, H, N, F.

2-[(*S*)-2,4,6-Trimethylphenylsulfonylamino]-3-[5-(4-(aminocarbonylamino)butyl)isoxazolin-3-ylcarbonyl]aminopropionic Acid (3i). To a solution of **9a** (100 mg, 0.20 mmol) in CH_2Cl_2 (0.5 mL) was added trimethylsilyl isocyanate (50 mg, 0.44 mmol), and the reaction mixture was allowed to stir at room temperature for 18 h. The mixture was diluted with CH_2Cl_2 (5 mL) and washed with 0.1 N HCl (5 mL). The organic layer was dried over $MgSO_4$, filtered, evaporated, and purified

using flash chromatography (2% MeOH−CH_2Cl_2) to yield the *tert*-butyl ester (67 mg, 62%): ^{1}H NMR (300 MHz, CD_3OD) δ 6.96 (s, 2H), 4.80−4.64 (m, 1H), 4.00−3.92 (m, 1H), 3.60−3.30 (m, 2H), 3.20−3.04, (m, 3H), 2.80−2.68 (m, 1H), 2.58 (s, 6H), 2.24 (s, 3H), 1.78−1.38 (m, 6H). 1.20 (d, J = 1.5 Hz, 9H).

The above *tert*-butyl ester (67 mg, 0.12 mmol) was dissolved in TFA (2 mL) and stirred for 1 h. The solvent was evaporated and the residue purified using reverse-phase HPLC to yield **3i** as a white solid (51 mg, 81%): ^{1}H NMR (300 MHz, CD_3OD) δ 6.96 (s, 2H), 4.80−4.68 (m, 1H), 4.08−3.98 (m, 1H), 3.64−3.54 (m, 1H), 3.42−3.30, (m, 1H), 3.22−3.02 (m, 3H), 2.78−2.68 (m, 1H), 2.58 (s, 6H), 2.24 (s, 3H), 1.78−1.38 (m, 6H); MS (ESI) m/z 496.2 [$(M - H)^-$, 100]. Anal. ($C_{23}H_{34}N_6O_6S\cdot 1.5CF_3CO_2H$) C, H, N, F.

2-[(*S*)-2,4,6-Trimethylphenylsulfonylamino]-3-[5-(4-(aminosulfonylamino)butyl)isoxazolin-3-ylcarbonyl]aminopropionic Acid (3j). Chlorosulfonyl isocyanate (55 mg, 0.39 mmol) was dissolved in CH_2Cl_2 (anhyd, 2 mL) and cooled in an ice bath, and *tert*-butyl alcohol (29 mg, 0.39 mmol) was added. After 1 h, **9a** (200 mg, 0.39 mmol) was added, the cooling bath was removed, and the reaction stirred for 4 h. The mixture was washed with water and the organic layer was dried ($MgSO_4$), filtered, and evaporated. The residue was purified using reverse-phase HPLC to yield the *tert*-butyl ester (50 mg, 20%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.0 (m, 1H), 6.92, (s, 2H), 5.74 (m, 1H), 5.30 (bs, 1H), 4.78−4.70 (m, 1H), 3.92−3.84 (m, 1H), 3.68−3.54 (m, 2H), 3.30−3.04, (m, 2H), 2.90−2.78 (m, 1H), 2.60 (s, 6H), 2.24 (s, 3H), 1.78−1.42 (m, 6H), 1.45 (s, 9H), 1.24, (s, 9H).

The above *tert*-butyl ester Boc derivative was dissolved in TFA (0.5 mL) and stirred for 1 h. The solvent was evaporated and the residue purified using reverse-phase HPLC. Concentration of the appropriate fractions in vacuo, trituration with Et_2O, and drying under vacuum gave the desired sulfamide **3j** (32 mg, 76%): ^{1}H NMR (300 MHz, CD_3OD) δ 6.96 (s, 2H), 4.80−4.68 (m, 1H), 4.08−3.98 (m, 1H), 3.64−3.54 (m, 1H), 3.42−3.30, (m, 1H), 3.22−3.02 (m, 3H), 2.78−2.68 (m, 1H), 2.58 (s, 6H), 2.24 (s, 3H), 1.78−1.38 (m, 6H); MS (ESI) m/z 534.4 [$(M + H)^+$, 100]; HRMS (FAB) m/z 534.1682 [$(M + H)^+$ calcd for $C_{20}H_{32}N_5O_8S_2$ 534.1692].

4-(3-Ethoxycarbonylisoxazolin-5-yl)butyric Acid (11). To a solution of **4a** (5.0 g, 22 mmol) in acetone (100 mL) was added Jones reagent until an orange color persisted for 10 min. 2-Propanol (2 mL) was added and the reaction mixture was poured into water (200 mL) and extracted with Et_2O (3 × 100 mL). The organic layer was dried (Na_2SO_4) and evaporated, and the residue was purified using flash chromatography (10% MeOH−CH_2Cl_2) to yield the title compound (3.1 g, 62%): ^{1}H NMR (300 MHz $CDCl_3$) δ 4.90−4.78 (m, 1H), 4.34 (q, J = 7 Hz, 2H), 3.34−3.20, (m, 1H), 2.94−2.82 (m, 1H), 2.20 (t, br, 2H), 1.90−1.40 (m, 4H), 1.30 (t, J = 7 Hz, 3H).

3-Ethoxycarbonyl-5-(5-bromo-4-oxopentyl)isoxazoline (12). To a solution of **11** (5.0 g, 21.8 mmol) in CH_2Cl_2 (15 mL) was added DMF (2 drops) followed by the dropwise addition of oxalyl chloride (2.03 g, 15.9 mmol). The reaction mixture was stirred at room temperature for 16 h, and the solvent was removed in vacuo to yield the crude acid chloride: ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.88−4.68 (m, 1H), 4.34 (q, J = 8 Hz, 2H), 3.38−3.24, (m, 1H), 3.02−2.80 (m, 3H), 1.90−1.60 (m, 4H), 1.38 (t, J = 8 Hz, 3H).

The crude acid chloride was dissolved in Et_2O (100 mL) and cooled in an ice bath. An ether solution of diazomethane (300 mL) was added, and the ice bath was removed after 1 h. The reaction mixture was allowed to stand at room temperature for 2 h. The solvent was degassed by passing a stream of nitrogen through the solution. The resulting solvent evaporation and cooling caused the diazoketone to precipitate. When the volume had been reduced to approximately 100 mL, the product was filtered to afford the crude diazoketone (4.0 g) as a solid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.26 (bs, 1H), 4.84−4.68 (m, 1H), 4.34 (q, J = 8 Hz, 2H), 3.38−3.24, (m, 1H), 2.90−2.80 (m, 1H), 2.38 (m, 2H), 1.84−1.64 (m, 4H), 1.38 (t, J = 8 Hz, 3H).

To a suspension of the crude diazoketone in Et_2O (100 mL) was bubbled gaseous HBr until the starting material was no longer present (approximately 5 min). The mixture was cautiously added to an ice-cold solution of saturated $NaHCO_3$. After the gas evolution had subsided, the mixture was extracted with Et_2O (2 × 100 mL). The combined organic layers were dried over Na_2SO_4, filtered, and evaporated to yield the title product as a white solid (4.0 g, 37% overall from **11**): ^{1}H NMR (300 MHz $CDCl_3$) δ 4.80−4.70 (m, 1H), 4.28 (q, J = 8 Hz, 2H), 3.82 (s, 2H), 3.28−3.18, (m, 1H), 2.84−2.74 (m, 1H), 2.70 (m, 2H), 1.78−1.58 (m, 4H), 1.30 (t, J = 8 Hz, 3H). Anal. Calcd for $C_{11}H_{16}BrNO_4$: C, 43.15; H, 5.27; N, 4.58. Found: C, 43.44; H, 5.07; N, 4.52.

5-[3-(2-Acetylaminoimidazol-4-yl)propyl]isoxazoline-3-carboxylic Acid Hydrochloride (13). To a solution of **12** (2.00 g, 6.5 mmol) in DMF (20 mL) was added acetylguanidine (1.97 g, 19.5 mmol). The reaction mixture was stirred at room temperature for 4 days and poured into water, and the resulting precipitate was collected and dried under vacuum to yield the desired acetylaminoimidazole (700 mg, 35%): ^{1}H NMR (300 MHz, CD_3OD) δ 6.50 (s, 1H), 4.84−4.74 (m, 1H), 4.28 (q, J = 8 Hz, 2H), 3.30−3.20, (m, 1H), 2.90−2.78 (m, 1H), 2.54 (m, 2H), 1.78−1.58 (m, 4H), 1.28 (t, J = 8 Hz, 3H); MS (ESI) m/z 309.3 [$(M + H)^+$, 100].

To the acetylaminoimidazole (700 mg, 2.2 mmol) in 95% EtOH (20 mL) was added 20% NaOH (1 mL). After stirring for 5−10 min, a heavy precipitate formed. Water (1 mL) was added and the reaction mixture filtered to remove undissolved material. The solvent was evaporated and the residue redissolved in water (20 mL). The pH was adjusted to 6 using 1 N HCl, causing a copious precipitate to form. This was filtered and washed with a small amount of water. The solid was resuspended in MeOH (20 mL) and 1 N HCl (7 mL) was added. After stirring for 1 h, the mixture was filtered and evaporated. The residue was dissolved in a small amount of water and placed on a column of C18 reverse-phase silica, washed with water, and eluted with acetonitrile. Evaporation of the appropriate fractions provided the carboxylic acid (652 mg, 91%): ^{1}H NMR (300 MHz, CD_3OD) δ 6.90 (s, 1H), 4.88−4.78 (m, 1H), 3.36−3.22, (m, 1H), 2.90−2.80 (m, 1H), 2.64 (m, 2H), 1.78−1.62 (m, 4H).

2-[(*S*)-2,4,6-Trimethylphenylsulfonylamino]-3-[5-[3-(2-aminoimidazol-4-yl)propyl]isoxazolin-3-ylcarbonyl]aminopropionic Acid Trifluoroacetate (3c). To a solution of **13** (474 mg, 1.5 mmol), **8a** (512 mg, 1.5 mmol), and BOP (729 mg, 1.6 mmol) in DMF (1 mL) was added *N*-methylmorpholine (605 mg, 6.0 mmol), and the resulting mixture stirred at room temperature for 16 h. The solvent was evaporated in vacuo and the residue purified using flash chromatography (3% MeOH−CH_2Cl_2) to give the sulfonamide (700 mg, 79%): ^{1}H NMR (300 MHz, CD_3OD) δ 6.94 (s, 2H), 6.62 (s, 1H), 4.80−4.70 (m, 1H), 4.00−3.92 (m, 1H), 3.60−3.50 (m, 1H), 3.42−3.30 (m, 1H), 3.24−3.10, (m, 1H), 2.84−2.74 (m, 1H), 2.65−2.52 (m, 14H), 2.24 (s, 3H), 1.84−1.60 (m, 4H), 1.22 (d, J = 0.5 Hz, 9H).

To a suspension of the above sulfonamide (350 mg, 0.60 mmol) in water (7 mL) was added concentrated sulfuric acid (15 drops), and the reaction mixture was heated at 60 °C for 16 h. The solvent was evaporated in vacuo, and the residue was dissolved in TFA (2 mL), evaporated in vacuo, and purified using reverse-phase column chromatography. Concentration of the appropriate fractions in vacuo and drying under vacuum afforded **3c** as a white powder (314 mg, 86%): ^{1}H NMR (300 MHz, CD_3OD) δ 6.94 (s, 2H), 6.52 (s, 1H), 4.84−4.76 (m, 1H), 4.06−3.98 (m, 1H), 3.66−3.58 (m, 1H), 3.40−3.28 (m, 1H), 3.24−3.08, (m, 1H), 2.82−2.70 (m, 1H), 2.62−2.50 (m, 8H), 2.24 (s, 3H), 1.74−1.60 (m, 4H); MS (ESI) m/z 507.4 [$(M + H)^+$, 100]; HRMS (FAB) m/z 507.2026 [$(M + H)^+$ calcd for $C_{22}H_{30}N_6O_6S$ 507.2038].

5-[4-(Imidazol-2-ylamino)-4-oxobutyl]isoxazoline-3-carboxylic Acid (15). To a solution of acid **11** (2.29 g, 10 mmol), 2-aminoimidazole sulfate (**14**,; 3.96 g, 30 mmol), and *N*,*N*-diisopropylethylamine (6.35 g, 50 mmol) in DMF (10 mL) was added BOP (6.63 g, 15 mmol). The resulting mixture was stirred at 70 °C for 16 h. The reaction mixture was cooled to

room temperature, poured into water (200 mL), and allowed to stand for 1 h, resulting in the formation of a precipitate. The product was filtered and dried under vacuum to yield the acylaminoimidazole (1.20 g, 41%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.64 (s, 2H), 4.80−4.70 (m, 1H), 4.20 (q, J = 8 Hz, 2H), 3.30−3.20 (m, 1H), 2.84−2.74 (m, 1H), 2.32 (m, 2H), 1.90−1.40 (m, 4H), 1.22 (t, J = 7 Hz, 3H); MS (ESI) m/z 295.4 [(M + H) $^+$, 100].

To a solution of the above ester (1.00 g, 34 mmol) in 50% aqueous dioxane (10 mL) was added LiOH (214 mg, 50 mmol), and the reaction mixture stirred at room temperature for 0.5 h. The mixture was passed through a short column of C18 reverse-phase silica, and the product eluted using acetonitrile. The solvent was evaporated in vacuo and the residue dissolved in water (3 mL) and acidified with N HCl. The solid was filtered and dried under vacuum to afford **15** (0.84 g, 92%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.64 (s, 2H), 4.80−4.70 (m, 1H), 3.30−3.20 (m, 1H), 2.84−2.74 (m, 1H), 2.32 (m, 2H), 1.90−1.40 (m, 4H); MS (ESI) m/z 267.3 [(M + H)$^+$, 100].

2-[(*S*)-2,4,6-Trimethylphenylsulfonylamino]-3-[5-[4-(imidazol-2-ylamino)-4-oxobutyl]isoxazolin-3-ylcarbonyl]-aminopropionic Acid Trifluoroacetate (3g). To a solution of **15** (266 mg, 1.0 mmol), **8a** (342 mg, 1.0 mmol), and BOP (486 mg, 1.1 mmol) in DMF (1 mL) was added *N*-methylmorpholine (404 mg, 4.0 mmol). After 16 h at room temperature, the mixture was diluted with water and the crude product filtered. The product was purified using prep TLC to yield the desired amide (170 mg, 29%): ^{1}H NMR (300 MHz, CD_3OD) δ 6.94 (s, 2H), 6.74 (bs, 2H), 4.80−4.70 (m, 1H), 4.00−3.92 (m, 1H), 3.58−3.50 (m, 1H), 3.42−3.30 (m, 1H), 3.24−3.10 (m, 1H), 2.84−2.74 (m, 1H), 2.58 (s, 6H), 2.42 (bs, 2H), 2.24 (s, 3H), 1.84−1.60 (m, 4H), 1.20 (d, J = 0.5 Hz, 9H); MS (ESI) m/z 591.4 [(M + H)$^+$, 12].

The above amide (150 mg, 0.26 mmol) was dissolved in TFA (2 mL) and stirred at room temperature for 1 h. The solvent was evaporated in vacuo and the residue purified using reverse-phase HPLC. Concentration of the appropriate fractions in vacuo, trituration with Et_2O, and drying under vacuum gave **3g** (140 mg, 84%): ^{1}H NMR (300 MHz, CD_3OD) δ 7.10 (d, J = 3 Hz, 2H), 6.94 (s, 2H), 4.82−4.72 (m, 1H), 4.06−3.98 (m, 1H), 3.64−3.58 (m, 1H), 3.42−3.30 (m, 1H), 3.28−3.10 (m, 1H), 2.84−2.72 (m, 1H), 2.58 (s, 6H), 2.48 (bs, 2H), 2.24 (s, 3H), 1.84−1.60 (m, 4H); MS (ESI) m/z 535.3 [(M + H)$^+$, 100]. Anal. ($C_{23}H_{30}N_6O_7S\cdot 0.3CF_3CO_2H$) C, H, N, F.

Ethyl 5-(4-Oxobutyl)isoxazoline-3-carboxylate (16). Oxalyl chloride (7.30 g, 0.0575 mol) was dissolved in CH_2Cl_2 (anhyd, 100 mL) and cooled to −60 °C in a dry ice/$CHCl_3$ bath. To this solution was added dropwise over 30 min a solution of DMSO (9.38 g, 0.12 mol) in CH_2Cl_2 (anhyd, 50 mL). After an additional 30 min, a solution of alcohol **4a** (10.75 g, 0.05 mol) in CH_2Cl_2 (anhyd, 30 mL) was added dropwise to the reaction mixture over 45 min and allowed to stir for an additional 30 min. To this mixture was added Et_3N (25.25 g, 0.25 mol) dropwise over 15 min. The cooling bath was removed and the reaction mixture allowed to warm to room temperature. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and washed with water, 1 N HCl, water, and brine. The organic layer was separated, dried ($MgSO_4$), and evaporated to yield aldehyde **16** as a colorless oil (9.54 g, 90%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.58 (s, 1H), 4.88−4.74 (m, 1H), 4.36 (q, J = 7 Hz, 2H), 3.36−3.22 (m, 1H), 2.92−2.80 (m, 1H), 2.52 (t, J = 6 Hz, 2H), 1.92 (m, 6H), 1.37 (t, J = 7 Hz, 3H).

5-[4-(*N*-(Pyridin-2-yl)-*N*-(*tert*-butyloxycarbonyl)amino)-butyl]isoxazoline-3-carboxylic Acid (17). A solution of **16** (9.40 g, 0.044 mol) in CH_2Cl_2 (100 mL) was cooled in an ice bath and 2-aminopyridine (4.57 g, 0.048 mol) added followed by the addition of sodium triacetoxyborohydride (14.0 g, 0.066 mol). The ice bath was removed and the reaction mixture allowed to stir at room temperature for 4 h. The reaction mixture was cautiously poured into a saturated solution of $NaHCO_3$ (200 mL) and extracted with EtOAc. The organic layer was dried over anhydrous Na_2CO_3, filtered, and evaporated to yield a semisolid. The crude product was triturated with a mixture of Et_2O and hexane and the resulting powder collected by filtration (8.93 g, 70%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (d, J = 4 Hz, 1H) 7.42 (t, J = 6 Hz, 1H), 6.48 (t, J = 6 Hz, 1H), 6.30 (d, J = 9 Hz, 1H), 4.90−4.70 (m, 2H), 4.34 (q, J = 7 Hz, 2H), 3.34−3.20 (m, 2H), 2.80−2.92 (m, 1H), 1.90−1.40 (m, 6H), 1.30 (t, J = 7 Hz, 3H).

The above aminopyridine (8.93 g, 0.031 mol) was dissolved in CH_2Cl_2 (100 mL) and DMAP (374 mg, 0.003 mol) and di-*tert*-butyl dicarbonate (14.73 g, 0.067 mol) were added. The reaction mixture was allowed to stir at room temperature overnight. The mixture was diluted with water and the organic layer separated, dried ($MgSO_4$), filtered, and evaporated. The crude product was purified using flash chromatography (3:1 = hexane:EtOAc) to afford the ester (9.7 g, 81%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.36 (d, J = 4 Hz, 1H), 7.86−7.54 (m, 2H), 7.01 (t, J = 6 Hz, 1H), 4.82−4.70 (m, 1H), 4.32 (q, J = 7 Hz, 2H), 3.93 (t, J = 7 Hz, 2H), 3.30−3.18 (m, 1H), 2.86−2.76 (m, 1H), 1.90−1.40 (m, 15H), 1.38 (t, J = 7 Hz, 3H).

A solution of the above ester (10.7 g, 0.027 mol) in a mixture of THF (20 mL) and water (20 mL) was cooled in an ice bath. To this was added a solution of LiOH (1.73 g, 0.41 mol) in water (5 mL). The reaction mixture was stirred for 45 min. A 1 M solution of citric acid (40 mL) was added and the mixture extracted several times using EtOAc (until TLC of the organic layer showed no product). The combined organic layers were dried ($MgSO_4$), filtered, evaporated, and dried under high vacuum to give **17** as a light yellow semisolid (9.9 g, 99%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.42 (t, J = 4 Hz, 1H), 7.70 (t, J = 6 Hz, 1H), 7.52 (d, J = 8 Hz, 1H), 7.10 (t, J = 6 Hz, 1H), 4.90−4.78 (m, 1H), 3.90 (t, J = 7 Hz, 2H), 3.32−3.20 (m, 1H), 2.78−2.69 (m, 1H), 1.82−1.24 (m, 12H); MS (ESI) m/z 364.3 [(M + H)$^+$, 100].

***tert*-Butyl 2(*S*)-Benzyloxycarbonylamino-3-[5-(4-(*N*-(pyridin-2-yl)-*N*-(*tert*-butyloxycarbonyl)amino)butyl)-isoxazolin-3-ylcarbonyl]aminopropionate (18).** *tert*-Butyl N^2-benzyloxycarbonyl-2(*S*)-2,3-diaminopropionate[22] (**8b**; 2.59 g, 8.81 mmol) and **17** (3.20 g, 8.81 mmol) were dissolved in DMF (20 mL). *N*-Methylmorpholine (2.72 g, 26.9 mmol) and BOP (4.09 g, 9.25 mmol) were added and the reaction mixture stirred for 48 h at room temperature. Concentration in vacuo gave an orange oil which was purified using flash chromatography (2:1 = hexane:EtOAc) to yield the title compound as a pale yellow syrup (4.56 g, 83%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.39−8.35 (dd, J = 4.7, 1.8 Hz, 1H), 7.67−7.58 (td, J = 7.0, 1.8 Hz, 1H), 7.59−7.51 (td, J = 7.0, 1.8 Hz, 1H), 7.37−7.28 (m, 5H), 7.06−6.98 (dd, J = 4.8, 1.8 Hz, 1H), 6.97−6.94 (t, J = 5.4 Hz, 1H), 5.69−5.66 (d, J = 7.0 Hz, 1H), 5.11 (s, 2H), 4.79−4.67 (m, 1H), 4.41−4.35 (m, 1H), 3.95−3.90 (t, J = 7.0 Hz, 2H), 3.79−3.68 (m, 2H), 3.24−3.14 (ddd, J = 17.6, 8.0, 1.1 Hz, 1H), 2.83−2.74 (dd, J = 17.6, 8.0 Hz, 1H), 1.50 (s, 9H), 1.48 (s, 9H), 1.47−1.35 (m, 6H); MS (ESI) m/z 640.4 [(M + H)$^+$, 60].

2(*S*)-Benzyloxycarbonylamino-3-[5-(4-(pyridin-2-ylamino)butyl)isoxazolin-3-ylcarbonyl]aminopropionic Acid Trifluoroacetate (3aa). The ester carbamate **18** (240 mg, 0.37 mmol) was dissolved in TFA (2 mL) and stirred at room temperature for 1 h. The solvent was evaporated in vacuo and the residue purified using reverse-phase HPLC. Concentration of the appropriate fractions in vacuo, trituration with Et_2O, and drying under vacuum afforded the desired compound as an amorphous solid (158 mg, 87%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (t, J = 5 Hz, 1H), 7.78 (d, J = 4 Hz, 1H), 7.28 (s, 5H), 7.02 (d, 6 Hz, 1H), 6.83 (m, 1H), 5.02 (t, J = 8 Hz, 2H), 4.55 (bs, 1H), 4.40 (bs, 1H), 3.80−3.55 (m, 3H), 3.40−3.20 (m, 3H), 2.83−2.74 (m, 2H), 1.77−1.35 (m, 6H); MS (ESI) m/z 484.3 [(M + H)$^+$, 100]. Anal. ($C_{24}H_{29}N_5O_6\cdot 1.25CF_3CO_2H$) C, H, N.

***tert*-Butyl 2(*S*)-Amino-3-[5-(4-(*N*-(*tert*-butyloxycarbonyl)pyridin-2-ylamino)butyl)isoxazolin-3-ylcarbonyl]aminopropionate (19).** To **18** (1.5 g, 2.408 mmol) in MeOH (50 mL) was added Pd/$BaSO_4$ unreduced (0.300 g, 0.482 mmol). The reaction mixture was placed under an atmosphere of H_2 (41 psi) at room temperature for 15 h. The mixture was filtered through a Celite pad and concentrated in vacuo to afford the amine as a light yellow syrup (1.22 g, quantitative): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.38−8.36 (dd, J = 4.8, 1.8 Hz, 1H), 7.64−

7.58 (td, J = 7.0, 1.8 Hz, 1H), 7.56−7.51 (td, J = 7.0, 1.8 Hz, 1H), 7.14−7.12 (t, J = 5.4 Hz, 1H), 7.02−6.98 (dd, J = 4.8, 1.8 Hz, 1H), 4.78−4.69 (m, 1H), 3.91−3.86 (t, J = 7.0 Hz, 2H), 3.66−3.59 (m, 2H), 3.78−3.81 (m, 1H), 3.28−3.19 (ddd, J = 17.6, 8.0, 1.1 Hz, 1H), 2.87−2.78 (dd, J = 17.6, 8.0 Hz, 1H), 2.48−2.29 (bs, 2H), 1.76−1.34 (m, 6H), 1.48 (s, 9H), 1.46 (s, 9H); MS (ESI) m/z 506.4 [$(M + H)^+$, 40].

2(*S*)-Benzenesulfonylamino-3-[5-(4-((pyridin-2-yl)amino)butyl)isoxazolin-3-ylcarbonyl]aminopropionic Acid Trifluoroacetate (3s). To a solution of **19** (250 mg, 0.50 mmol) and pyridine (202 mg, 2.58 mmol) in CH_2Cl_2 (5 mL) was added benzenesulfonyl chloride (87 mg, 0.50 mmol) and the mixture stirred at room temperature under nitrogen for 8 h. Saturated $NaHCO_3$ (15 mL) was added and the organic layer was separated, dried over anhydrous Na_2CO_3, and concentrated in vacuo to a syrup. The product was dissolved in TFA (5 mL), stirred at room temperature for 1 h, and then concentrated in vacuo to an oil. Toluene (10 mL) was added and the mixture again concentrated in vacuo. The resulting viscous oil was purified using reverse-phase HPLC. The fractions containing product were concentrated in vacuo and placed on a lyophilization apparatus overnight, to afford **3s** as a white powder (108 mg, 45% over two steps): ^{1}H NMR (300 MHz, CD_3OD) δ 7.87−7.76 (d, J = 6.96, 4H), 7.59−7.57 (t, J = 7.33 Hz, 1H), 7.51−7.46 (t, J = 7.7 Hz, 2H), 7.03−7.00 (dd, J = 9.2, 2.6 Hz, 1H), 6.85−6.79 (d, J = 4.8 Hz, 1H), 5.86−5.80 (dd, J = 7.7, 3.4 Hz, 1H), 4.75−4.70 (m, 1H), 4.12−4.06 (m, 1H), 3.69−3.62 (m, 2H), 3.36−3.30 (t, J = 7.3 Hz, 2H), 3.25−2.88 (ddd, J = 17.6, 10.6, 3.7 Hz, 1H), 2.96−2.88 (d, J = 22.3 Hz, 1H), 2.83−2.71 (ddd, J = 17.6, 8.4, 2.2 Hz, 1H), 2.68−2.59 (dd, J = 22.3, 9.5 Hz, 1H), 1.77−1.34 (m, 6H); MS (ESI) m/z 490.2 [$(M + H)^+$, 100]. Anal. ($C_{22}H_{27}N_5O_6S \cdot 1.1CF_3CO_2H \cdot 0.5H_2O$) C, H, N, F.

Also isolated as white powders using the general procedure reported for **3s** were **3e**, **3k**, **3l**, **3r**, **3u**, **3x**, and **3y**.

2-[(*S*)-2,4,6-Trimethylbenzenesulfonylamino]-3-[5-(4-((pyridin-2-yl)amino)butyl)isoxazolin-3-ylcarbonyl]aminopropionic Acid Trifluoroacetate (3e): ^{1}H NMR (300 MHz, CD_3OD) δ 7.86−7.81 (t, J = 4.4 Hz, 1H), 7.78−7.75 (t, J = 5.9 Hz, 1H), 7.03−6.98 (dd, J = 4.4, 0.05 Hz, 1H), 6.94 (s, 2H), 6.85−6.78 (dd, J = 5.9, 0.7 Hz, 1H), 4.82−4.74 (m, 1H), 4.06−4.00 (m, 1H), 3.65−3.31 (dd, J = 13.6, 5.1 Hz, 2H), 3.36−3.31 (t, J = 7.0 Hz, 2H), 3.16−3.07 (ddd, J = 17.6, 8.0, 1.1 Hz, 1H), 2.82−2.74 (dd, J = 17.6, 8.0 Hz, 1H), 2.58 (s, 9H), 2.25 (s, 3H), 1.79−1.49 (m, 6H); MS (ESI) m/z 532.2 [$(M + H)^+$, 100]; HRMS (FAB) m/z 532.2230 [$(M + H)^+$ calcd for $C_{25}H_{34}N_5O_6S$ 532.2243].

2-[(*S*)-2,4,6-Trimethylbenzenesulfonylamino]-3-[5-(4-((isoquinolin-1-yl)amino)butyl)isoxazolin-3-ylcarbonyl]-aminopropionic Acid Trifluoroacetate (3k). The title compound was synthesized from **16** following the general procedure for reductive amination and ester cleavage reported for **3aa**: ^{1}H NMR (300 MHz, CD_3OD) δ 8.42 (d, J = 6.0 Hz, 1H), 8.00−7.70 (m, 3H), 7.50 (t, J = 5.0 Hz, 1H), 7.18 (t, J = 6.0 Hz, 1H), 6.90, (s, 2H), 4.82−4.74 (m, 1H), 4.06−4.00 (m, 1H), 3.65−3.31 (m, 2H), 3.30−3.10 (m, 3H), 2.84−2.74 (m, 1H), 2.58 (s, 9H), 2.25 (s, 3H), 1.79−1.49 (m, 6H); MS (ESI) m/z 582.2 [$(M + H)^+$, 100]. Anal. ($C_{29}H_{35}N_5O_6S \cdot 1.2CF_3CO_2H$) C, H, N, F.

2-[(*S*)-2,4,6-Trimethylbenzenesulfonylamino]-3-[5-(4-((isoquinolin-3-yl)amino)butyl)isoxazolin-3-ylcarbonyl]-aminopropionic Acid Trifluoroacetate (3l). The title compound was synthesized from **16** following the general procedure for reductive amination and ester cleavage reported for **3aa**: ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.94 (s, 1H), 8.30−8.18 (m, 1H), 8.00−7.82 (m, 2H), 7.66−7.52 (m, 2H), 7.20 (t, J = 5.0 Hz, 1H), 6.90, (s, 2H), 6.84 (s, 1H), 4.78−4.70 (m, 1H), 3.92−3.82 (m, 1H), 3.42−3.00 (m, 5H), 2.78−2.60 (m, 1H), 2.48 (s, 9H), 2.20 (s, 3H), 1.79−1.49 (m, 6H); MS (ESI) m/z 582.2 [$(M + H)^+$, 100]. Anal. ($C_{29}H_{35}N_5O_6S \cdot 1.2CF_3CO_2H$) C, H, N, F.

2-[(*S*)-2,6-Dimethyl-4-phenylbenzenesulfonylamino]-3-[5-(4-((pyridin-2-yl)amino)butyl)isoxazolin-3-ylcarbonyl]-aminopropionic Acid Trifluoroacetate (3r): ^{1}H NMR (300 MHz, CD_3OD) δ 7.90−7.75 (m, 2H), 7.70−7.62 (m, 2H), 7.50−7.30 (m, 5H), 7.00 (d, J = 4.4, 1H), 6.85−6.78 (m, 1H), 4.82−4.74 (m, 1H), 4.06−4.00 (m, 1H), 3.70−3.58 (m, 1H), 3.42−3.18 (m, 4H), 3.20−2.90 (m, 2H), 2.70 (s, 6H), 1.70−1.30 (m, 6H); MS (ESI) m/z 594.3 [$(M + H)^+$, 100]; HRMS (FAB) m/z 594.2386 [$(M + H)^+$ calcd for $C_{30}H_{36}N_5O_6S$ 594.2386].

2-[(*S*)-1-Naphthylenesulfonylamino]-3-[5-(4-((pyridin-2-yl)amino)butyl)isoxazolin-3-ylcarbonyl]aminopropionic Acid Trifluoroacetate (3u): ^{1}H NMR (300 MHz, CD_3OD) δ 8.62 (d, J = 10.0 Hz, 1H), 8.18 (d, J = 8.0 Hz, 1H), 8.10 (d, J = 10.0 Hz, 1H), 7.96 (d, J = 8.0 Hz, 1H), 7.85−7.50 (m, 5H), 7.03−6.98 (m, 1H), 6.84−6.76 (m, 1H), 4.78−4.65 (m, 1H), 4.14−4.06 (m, 1H), 3.70−3.58 (m, 1H), 3.42−3.24 (m, 4H), 3.10−2.96 (m, 2H), 2.76−2.60 (m, 1H), 1.80−1.40 (m, 7H); MS (ESI) m/z 540.1 [$(M + H)^+$, 100]; HRMS (FAB) m/z 540.1922 [$(M + H)^+$ calcd for $C_{26}H_{30}N_5O_6S$ 540.1917].

2-[(*S*)-4-Phenylbenzenesulfonylamino]-3-[5-(4-((pyridin-2-yl)amino)butyl)isoxazolin-3-ylcarbonyl]aminopropionic Acid Trifluoroacetate (3x): ^{1}H NMR (300 MHz, CD_3OD) δ 7.90−7.64 (m, 7H), 7.50−7.34 (m, 4H), 7.00 (d, J = 4.8 Hz, 1H), 6.85−6.80 (m, 1H), 4.82−4.74 (m, 1H), 4.20−4.10 (m, 1H), 3.70−3.58 (m, 1H), 3.42−3.18 (m, 4H), 3.00−2.78 (m, 2H), 1.75−1.40 (m, 6H); MS (ESI) m/z 566.1 [$(M + H)^+$, 100]; HRMS (FAB) m/z 566.2074 [$(M + H)^+$ calcd for $C_{28}H_{32}N_5O_6S$ 566.2073].

2-[(*S*)-4-Isopropylbenzenesulfonylamino]-3-[5-(4-((pyridin-2-yl)amino)butyl)isoxazolin-3-ylcarbonyl]aminopropionic Acid Trifluoroacetate (3y): ^{1}H NMR (300 MHz, CD_3OD) δ 7.90−7.70 (m, 4H), 7.36 (d, J = 6.0 Hz, 2H), 7.03−6.98 (dd, J = 4.4, 0.05 Hz, 1H), 6.85−6.78 (dd, J = 5.8, 0.7 Hz, 1H), 4.82−4.74 (m, 1H), 4.06−4.00 (m, 1H), 3.70−3.58 (m, 1H), 3.42−3.18 (m, 4H), 3.00−2.78 (m, 2H), 1.79−1.49 (m, 7H), 1.14 (d, J = 10.0 Hz, 6H); MS (ESI) m/z 532.4 [$(M + H)^+$, 100]; HRMS (FAB) m/z 532.2235 [$(M + H)^+$ calcd for $C_{25}H_{34}N_5O_6S$ 532.2230].

5-[3-(*N*-(2-Aminopyridin-6-yl)-*N*-(*tert*-butyloxycarbonyl)-amino)propyl]isoxazoline-3-carboxylic Acid (21). To a solution of 2-amino-6-picoline (**20**; 15 g, 0.14 mol) in CH_2Cl_2 (450 mL) was added di-*tert*-butyl dicarbonate (33.3 g, 0.152 mol), and the reaction mixture was heated at reflux overnight (18 h) with stirring under N_2. The solvent was removed in vacuo and the residue purified using filtration chromatography through silica gel (CH_2Cl_2 as eluent) to provide the carbamate as a white waxy solid (23.7 g, 82%): ^{1}H NMR (300 MHz, CD_3-Cl) δ 7.70 (d, J = 8.4 Hz, 1H), 7.54 (app t, J = 8.1, 7.7 Hz, 1H), 7.16 (bs, 1H), 6.81 (d, J = 7.7 Hz, 1H), 2.42 (s, 3H), 1.51 (s, 9H). Anal. Calcd for $C_{11}H_{16}N_2O_2$: C, 63.44; H, 7.74; N, 13.45. Found: C, 63.51; H, 7.62; N, 13.40.

A solution of the above carbamate (4 g, 19.2 mmol) in THF (40 mL) was cooled to −23 °C under N_2 and treated with a 2 M solution of LDA in THF (24 mL, 48 mmol). After stirring for 30 min, the resulting red solution was cooled to −78 °C and 4-bromobutene (2.9 mL, 28.8 mmol) was added dropwise. The resulting mixture was stirred for 15 min, quenched by the addition of saturated NH_4Cl solution, and allowed to assume room temperature. Extraction with EtOAc and separation of product from unreacted starting material using flash chromatography (Biotage 40S cartridge, 9:1 = hexane:Et_2O) provided the alkene as a light yellow oil (0.29 g, 5.8%): ^{1}H NMR (300 MHz, CD_3Cl) δ 7.71 (d, J = 8.4 Hz, 1H), 7.55 (app t, J = 8.1, 7.7 Hz, 1H), 7.14 (bs, 1H), 6.80 (d, J = 7.7 Hz, 1H), 5.77−5.90, (m, 1H), 4.94−5.07 (m, 2H), 2.65 (t, J = 7.7 Hz, 2H), 2.05−2.13 (m, 2H), 1.72−1.84 (m, 2H), 1.51 (s, 9H).

To a solution of the above alkene (0.29 g, 1.1 mmol) in THF (3 mL) were added water (2 mL) and $NaHCO_3$ (0.65 g, 7.7 mmol) followed by **6** (0.25 g, 1.66 mmol). The reaction mixture was stirred at 0 °C under N_2 for 5 h, then warmed to room temperature overnight (18 h). The mixture was again cooled to 0 °C and a second aliquot of **6** added followed by stirring for 4 h at 0−25 °C. The reaction mixture was diluted with water and extracted with EtOAc (3×). The combined extracts were washed with brine, dried (Na_2SO_4), filtered, and evaporated. Purification using flash chromatography (Biotage Flash 40S cartridge, 4:1 then 3:1 = hexane:EtOAc) afforded the

isoxazoline as a yellow oil (0.3 g, 72%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.73 (d, J = 8.4 Hz, 1H), 7.56 (app t, J = 8., 7.7 Hz, 1H), 7.14 (bs, 1H), 6.79 (d, J = 6.5 Hz, 1H), 4.88−4.75 (m, 1H), 4.34 (q, J = 7.3 Hz, 2H), 3.25 (dd, J = 17.6, 11 Hz, 1H), 2.85 (dd, J = 17.3, 8.5 Hz, 1H) 2.69 (t, J = 7 Hz, 2H), 1.60−1.90 (m, 4H), 1.52 (s, 9H), 1.36 (t, J = 7.3 Hz, 3H); MS (ESI) m/z 400.2 $(M + Na)^+$, 378.2 $(M + H)^+$, 322.2 $[(M + H - t\text{-}Bu)^+$, 100].

A solution of the above isoxazoline (0.3 g, 0.8 mmol) in THF (3.6 mL) was treated with 1 M LiOH solution (1.2 mL, 1.2 mmol). MeOH (0.5 mL) was added to achieve a homogeneous solution, which was stirred for 2 h at room temperature. The reaction mixture was diluted with water, adjusted to pH 4.0 with 5% aqueous citric acid solution, and then extracted with EtOAc (3×). The combined extracts were washed with brine, dried (Na_2SO_4), filtered, and evaporated to yield acid **21** as a white foam (0.25 g, 89%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 (d, J = 8.4 Hz, 1H), 7.75 (dd, J = 8.6, 7 Hz, 1H), 6.88 (d, J = 7 Hz, 1H), 4.93−4.80 (m, 1H), 3.33 (dd, J = 17.4, 11 Hz, 1H), 2.85 (dd, J = 17.7, 8.1 Hz, 1H) 2.83−2.70 (m, 2H), 1.65−1.94 (m, 4H), 1.54 (s, 9H).

2-[(*S*)-2,4,6-Trimethylbenzenesulfonylamino]-3-[5-(3-((2-aminopyridin-6-yl)amino)propyl)isoxazolin-3-ylcarbonyl]aminopropionic Acid Trifluoroacetate (3f). A mixture of acid **21** (0.23 g, 0.66 mmol), **8a** (0.25 g, 0.73 mmol), *N*-methylmorpholine (0.16 mL, 1.4 mmol), and BOP (0.44 g, 0.99 mmol) in DMF (5 mL) was stirred under N_2 at room temperature for 3 days. The reaction mixture was poured into water (50 mL) and extracted with EtOAc (3×). The combined extracts were washed with saturated $NaHCO_3$ and brine, dried (Na_2SO_4), filtered, and evaporated. Purification of the residue using flash chromatography (Biotage Flash 40S cartridge, 1:1 = EtOAc:hexane) gave the amide (380 mg, 85%): ^{1}H NMR (300 MHz, CD_3Cl) δ 7.73 (d, J = 8 Hz, 1H), 7.56 (t, J = 8, 7.7 Hz, 1H), 7.15 (bs, 1H) 6.94 (s, 2H) 6.85 (t, J = 7.5 Hz, 1H) 6.80 (d, J = 7 Hz, 1H), 5.58 (d, J = 7.3 Hz, 1H), 4.83−4.71 (m, 1H), 3.92−3.83 (m, 1H), 3.77−3.50 (m, 2H), 3.81 (dd, J = 18, 11 Hz, 1H), 2.81 (dd, J = 18, 8.4 Hz, 1H), 2.69 (t, J = 7.5 Hz, 2H), 2.64 (s, 6H), 2.28 (s, 3H), 1.63−1.88 (m, 4H), 1.52 (s, 9H), 1.30 (s, 9H); MS (ESI) m/z 672.3 $[(M + H)^+$, 100].

A solution of the above amide (380 mg, 0.56 mmol) in CH_2Cl_2 (6 mL) was treated with TFA (1.5 mL), and the resulting solution stirred at room temperature under N_2 overnight (18 h). Evaporation in vacuo and trituration with Et_2O gave **3f** as a white solid (344 mg, 100%): ^{1}H NMR (300 MHz, CD_3OD) δ 7.84−6.97 (m, 1H), 6.95 (s, 2H), 6.79 (dd, J = 8, 5.6 Hz, 1H), 6.75 (d, J = 7.5 Hz, 1H), 4.05−4.00 (m, 1H), 3.63 (dt, J = 15, 3.5 Hz, 1H), 3.10−3.37 (m, 3H), 2.74−2.85 (m, 3H), 2.58 (s, 6H), 2.26 (s, 3H), 1.91−1.65 (m, 4H); MS (ESI) m/z 540.1 $(M + Na)^+$, 518.2 $[(M + H)^+$, 100]. Anal. ($C_{24}H_{31}N_5O_6S\cdot 1.25CF_3CO_2H$) C, H, N, S, F.

2-Amino-1-(triphenylmethyl)imidazole (22). 2-Aminoimidazole sulfate (**14**; 2.64 g, 20 mmol) was dissolved in MeOH (anhyd, 200 mL) and cooled to −78 °C. A 25% solution of sodium methoxide in methanol (4.57 mL, 20 mmol) was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for an additional 3 h. The solution was filtered and concentrated in vacuo to afford a brown oil (1.6 g, 96.4%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.32, (s, 2H), 5.0 (bs, 2H).

Phthalic anhydride (freshly recrystallized, 4.14 g, 29.2 mmol) and 2-aminoimidazole (2.32 g, 29.2 mmol) were quickly blended and placed in a heating bath at a temperature of 170 °C for 15 min. After cooling to room temperature, the crude reaction mixture was purified using flash chromatography (95:5 = $CHCl_3$:MeOH, 80:20 = $CHCl_3$:MeOH) to yield 4.66 g (75%) of a brown solid: ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.35 (bs, 1H), 8.06−7.94 (m, 4H), 7.16 (bs, 2H); MS (ESI) m/z 214.2 $(M + H)^+$.

To a solution of the above (4.66 g, 21.9 mmol) in pyridine (anhyd, 100 mL) was added triphenylmethyl chloride (9.15 g, 32.82 mmol). The reaction mixture was heated at reflux for 24 h. Pyridine was removed and the residue purified using flash chromatography (5−10% $CHCl_3$/MeOH) to yield the desired product (2.74 g, 27.5% yield): mp 212.6−215.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (d, 6H, 7.64, s 4H), 7.17 (t, J = 7.7 Hz, 7H), 7.06 (t, J = 7.3 Hz, 3H), 6.80 (d, J = 1.1 Hz, 1H); MS (NH_3-DCI) m/z 456 $(M + H)^+$.

To a solution of the above phthalimide (2.60 g, 5.7 mmol) in EtOH (anhyd, 250 mL) was added freshly distilled hydrazine (1.83 g, 57 mmol) and the resulting mixture was heated at reflux for 1 h. The reaction mixture was cooled and the solvent removed in vacuo. The solid residue was purified using flash chromatography (10:1 = $CHCl_3$:MeOH) to yield 1.8 g (97%) of **22** as a yellow solid: mp 207−208 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.44−7.33 (m, 9H), 7.13 (d, J = 7.0 Hz, 6H), 6.51 (d, J = 1.8 Hz, 1H), 6.26 (d, J = 1.8 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 149.13, 141.74, 130.02, 129.29, 128.08, 127.94, 127.30, 123.03, 117.27, 74.08; MS (NH_3-DCI) m/z 326 $(M + H)^+$. The analytical sample was crystallized from EtOH/EtOAc. Anal. Calcd for $C_{22}H_{19}N_3\cdot 0.33H_2O$: C, 79.75; H, 5.98; N, 12.68. Found: C, 79.80; H, 5.97; N, 12.56.

5-[4-(1-Triphenylmethylimidazol-2-ylamino)butyl]isoxazoline-3-carboxylic Acid (23). A solution of **22** (3.00 g, 9.23 mmol) and aldehyde **16** (2.16 g, 10.2 mmol) was stirred in toluene (250 mL) at reflux for 27 h. After cooling to room temperature, sodium triacetoxyborohydride (3.74 g, 17.65 mmol) was added and the mixture stirred for an additional 20 h. Water (100 mL) was added, the organic layer was separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were concentrated and purified using flash chromatography ($CHCl_3$−5% MeOH/$CHCl_3$) to yield the product as a brown oil (5.56 g, >100%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.50 (d, J = 1.5 Hz, 1H), 7.44−7.33 (m, 6H), 7.09 (m, 6H), 6.26 (d, J = 1.5 Hz, 1H), 4.6 (m, 1H), 4.25 (q, J = 7.3 Hz, 2H), 3.07 (bs, 1H), 3.20 (dd, J = 11, 17.6 Hz, 1H), 2.90 (q, J = 2.3 Hz, 2H), 2.70 (dd, J = 8.4, 17.6 Hz, 1H), 1.48−1.31 (m, 2H), 1.26 (t, J = 7.3 Hz, 3H), 1.0 (m, 2H), 0.92−0.84 (m, 2H); MS (NH_3-DCI) m/z 523 $(M + H)^+$.

To a solution of the crude ester (5.56 g, 9.23 mmol) in THF (50 mL) was added a 0.5 M solution of LiOH (37 mL, 18.5 mmol). The reaction mixture was stirred for 1 h and adjusted to pH 2 using 1 N HCl, and the solvent was evaporated. The crude product was purified using flash chromatography (10:1 = $CHCl_3$:MeOH) to afford **23** as a white solid (3.00 g, 66%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.44−7.34 (m, 9H), 7.10 (appt d, 6H), 6.52 (d, J = 1.6 Hz, 1H), 6.26 (d, J = 1.8 Hz, 1H), 4.36 (m, 1H), 4.15−4.0 (bs, 1H), 3.07 (dd, J = 17.6, 10.6 Hz, 1 H), 2.89 (q, J = 6.6 Hz, 1H), 2.57 (dd, J = 17.6, 8.4 Hz, 1H), 1.31−0.84 (m, 6H); MS (NH_3-DCI) m/z 451 $(M + H - CO_2H)^+$.

2-[(*S*)-2,4,6-Trimethylbenzenesulfonylamino]-3-[5-(4-((imidazol-2-yl)amino)butyl)isoxazolin-3-ylcarbonyl]aminopropionic Acid Trifluoroacetate (3d). To a solution of acid **23** (98 mg, 0.198 mmol) and amine **8a** (68 mg, 0.198 mmol) in DMF (2 mL) were added 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU; 76 mg, 0.24 mmol) and Et_3N (69 mL, 0.495 mmol). After stirring at room temperature for 1.5 h, the solvent was evaporated in vacuo and the residue purified using flash chromatography (10:1 = $CHCl_3$:MeOH) to yield the amide as a white solid (135 mg, 83%): MS (ESI) m/z 819.4 $[(M + H)^+$ 100].

To the above amide (130 mg, 0.16 mmol) was added a 1:1 mixture of TFA and CH_2Cl_2 (2 mL) and the resulting solution stirred at room temperature for 5 h. The solvent was evaporated in vacuo and the crude product purified using reverse-phase HPLC to provide the desired trityl-protected imidazole (75 mg, 73%): ^{1}H NMR (300 MHz, acetone-d_6) δ 8.03 (s, 1H), 7.70−7.66 (m, 1H), 7.48 (m, 9H), 7.33 (m, 6H), 7.14 (d, J = 2.6 Hz, 1H), 6.98 (s, 2H), 6.85 (d, 1H), 6.70 (d, J = 2.6 Hz, 1H), 5.25 (bs, 1H), 4.60 (m, 1H), 4.14 (m, 1H), 3.74−3.52 (m, 1H), 3.31 (q, J = 6.6 Hz), 3.21−3.04 (m, 1H), 2.67 (m, 1H), 2.62 (s, 6H), 2.27 (s, 3H), 1.48−0.99 (m, 6H); MS (NH_3-DCI) m/z 763.3; HRMS (NH_3-CI) m/z 763.3278 $[(M + H)^+$ calcd for $C_{42}H_{46}N_6O_6S$ 763.3291].

The above trityl-protected imidazole (20 mg, 27 μmol) was dissolved in TFA (2 mL). Water (0.01 mL) was added and the reaction mixture was heated at reflux for 1 h. The solvent was evaporated in vacuo and the crude product purified using

reverse-phase HPLC. Concentration of the appropriate fractions in vacuo and lyophilization of the remaining aqueous solution afforded **3d** as a white powder (10 mg, 58%): ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.8−12.5 (bs, 1H), 11.9 (s, 2H), 8.23 (m, 1H), 7.94 (m, 2H), 6.97 (s, 2H), 6.95 (s, 2H), 6.50 (bs, 1H), 4.70 (m, 1H), 3.91 (q, J = Hz, 1H), 3.48−3.30 (m, 2H), 3.20 (m, 2H), 3.14−3.06 (m, 1H), 2.76−2.67 (m, 1H), 2.53 (s, 6H), 2.24 (s, 3H), 1.66−1.34 (m, 6H); IR (KBr) 3294, 2944, 1680, 1204, 1156, 1136, cm^{-1}; MS (ESI) m/z 521.4 [(M + H)$^+$ 100]; HRMS (NH_3-CI) m/z 521.2182 [(M + H)$^+$ calcd for $C_{23}H_{32}N_6O_6S$ 521.2190]. Anal. ($C_{23}H_{32}N_6O_6S\cdot1.3CF_3CO_2H$) C, H, N.

Also prepared using the general precedures described for **3d** were **3m**−**q**, **3t**, **3v**, **3w**, **3z**, **3bb**, and **3cc.**

2-[(*S*)-2,6-Dichlorobenzenesulfonylamino]-3-[5-(4-((imidazol-2-yl)amino)butyl)isoxazolin-3-ylcarbonyl]aminopropionic Acid Trifluoroacetate (3m): ^{1}H NMR (300 MHz, DMSO-d_6) δ 13.2−12.5 (bs, 1H), 11.98 (s, 2H), 8.85 (d, J = 8.8 Hz, 1H), 8.30−8.22 (m, 1H), 7.96 (m, 1H), 7.60−7.51 (m, 3H), 6.96 (s, 2H), 4.75−4.63 (m, 1H), 4.18−4.10 (m, 1H), 3.57−3.32 (m, 2H), 3.23−3.08 (m, 3H), 2.78−2.69 (m, 1H), 1.69−1.50 (m, 4H), 1.47−1.28 (m, 2H); MS (NH_3-DCI) m/z 326 [(M + H)$^+$ 100]. Anal. ($C_{20}H_{24}Cl_2N_6O_6S\cdot1.8CF_3CO_2H$) C, H, N, S.

2-[(*S*)-2-Chloro-6-methylbenzenesulfonylamino]-3-[5-(4-((imidazol-2-yl)amino)butyl)isoxazolin-3-ylcarbonyl]-aminopropionic Acid Trifluoroacetate (3n): ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.05−11.95 (bs, 2H), 8.29−8.22 (m, 1H), 8.15 (d, J = 8.8 Hz, 1H), 7.96 (t, J = 5.6 Hz, 1H), 7.44−7.39 (m, 2H), 7.34−7.30 (m, 1H), 6.95 (s, 2H), 4.71−4.67 (m, 1H), 4.11−4.05 (m, 1H), 3.52−3.31 (m, 2H), 3.23−3.09 (m, 3H), 2.77−2.69 (m, 1H), 2.60 (s, 3H), 1.69−1.53 (m, 4H), 1.41−1.34 (m, 2H); MS (ESI) m/z 527.3 [(M + H)$^+$ 100], 529 (40). Anal. ($C_{21}H_{27}ClN_6O_6S\cdot2CF_3CO_2H\cdot H_2O$) C, H, N; S: calcd, 4.15; found, 5.16.

2-[(*S*)-2,6-Dichloro-4-phenylbenzenesulfonylamino]-3-[5-(4-((imidazol-2-yl)amino)butyl)isoxazolin-3-ylcarbonyl]-aminopropionic Acid Trifluoroacetate (3o): ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.1−12.0 (bs, 2H), 8.56 (dd, J = 8.8, 3.3 Hz, 1H), 8.33−8.27 (m, 1H), 8.00 (t, J = 5.1 Hz, 1H), 7.98 (s, 2H), 7.89−7.81 (m, 2H), 7.52−7.47 (m, 3H), 6.96 (s, 2H), 4.59−4.48 (m, 1H), 4.38−4.18 (m, 1H), 3.21−3.04 (m, 4H), 2.76−2.67 (m, 1H), 1.6−1.2 (m, 6H); MS (ESI) m/z 623.2 [(M + H)$^+$ 100]. Anal. ($C_{26}H_{28}Cl_2N_6O_6S\cdot1.3CF_3CO_2H$) C, H, N.

2-[(*S*)-2,6-Dimethyl-4-phenylbenzenesulfonylamino]-3-[5-(4-((imidazol-2-yl)amino)butyl)isoxazolin-3-ylcarbonyl]-aminopropionic Acid Trifluoroacetate (3p): ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.9−12.6 (bs, 1H), 12.0−11.9 (bs, 2H), 8.27−8.21 (m, 1H), 8.08 (d, J = 9.5 Hz, 1H), 7.96−7.90 (b, 1H), 7.75−7.72 (m, 2H), 7.43−7.39 (m, 1H), 6.96 (s, 2H), 4.63−4.45 (m, 1H), 4.02−3.96 (m, 1H), 3.50−3.43 (m, 1H), 3.20−2.98 (m, 3H), 2.68−2.60 (m, 1H), 2.66 (s, 6H), 1.57−1.46 (m, 4H), 1.35−1.27 (m, 2H); MS (ESI) m/z 583.5 [(M + H)$^+$ 100]; HRMS (FAB) m/z 583.2339 [(M + H)$^+$ calcd for $C_{28}H_{34}N_6O_6S$ 583.2326]. Anal. ($C_{28}H_{34}N_6O_6S\cdot1.7CF_3CO_2H$) C, H, N.

2-[(*S*)-Benzenesulfonylamino]-3-[5-(2-((imidazol-2-yl)-amino)butyl)isoxazolin-3-ylcarbonyl]aminopropionic Acid Trifluoroacetate (3q): ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.2−12.05 (bs, 2H), 8.34−8.26 (m, 1H), 8.20 (t, J = 8.4 Hz, 1H), 8.05 (t, J = 5.5 Hz, 1H), 7.77−7.74 (m, 2H), 7.64−7.50 (m, 3H), 6.96 (s, 2H), 4.74−4.64 (m, 1H), 4.02−3.92 (m, 1H), 3.46−3.31 (m, partially coincident with H_2O), 3.29−3.06 (m, 4H), 2.77−2.66 (m, 1H), 1.72−1.45 (m, 4H), 1.45−1.25 (m, 2H); MS (ESI) m/z 479.3 [(M + H)$^+$ 100]. Anal. ($C_{20}H_{26}N_6O_6S\cdot1.4CF_3CO_2H$) C, H, N.

2-[(*S*)-1-Naphthalenesulfonylamino]-3-[5-(4-((imidazol-2-yl)amino)butyl)isoxazolin-3-ylcarbonyl]aminopropionic Acid Trifluoroacetate (3t): ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.85−12.48 (bs, 1H), 12.0−11.9 (bs, 2H), 8.63 (d, J = 8.6 Hz, 1H), 8.49 (t, J = 10.0 Hz, 1H), 8.22−8.18 (m, 1H), 8.16−8.05 (m, 2H), 7.93 (t, J = 5.4 Hz, 1H), 7.71−7.59 (m, 3H), 6.95 (s, 2H), 6.6−6.4 (bs, 2H), 4.71−4.60 (m, 1H), 4.02−3.97 (m, 1H), 3.42−3.17 (m, coincident with water), 3.09−2.96 (m, 1H), 2.64 (dd, J = 17.6, 8.6 Hz, 1H), 1.68−1.5s (m, 4H), 1.46−1.27 (m, 2H); MS (ESI) m/z 529.3 [(M + H)$^+$ 100].

2-[(*S*)-3,5-Dimethylisoxazolesulfonylamino]-3-[5-(4-((imidazol-2-yl)amino)butyl)isoxazolin-3-ylcarbonyl]aminopropionic Acid Trifluoroacetate (3v): ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.2−12.8 (bs, 1H), 12.04−11.93 (bs, 2H), 8.53 (dd, J = 9.3, 1.7 Hz, 1H), 8.31 (t, J = 5.6 Hz, 1H), 7.94 (t, J = 5.9 Hz, 1H), 6.95 (s, 2H), 4.76−4.66 (m, 1H), 4.03−3.98 (m, 1H), 3.49−3.42 (m, 2H), 3.33−3.28 (m, 1H), 3.23−3.14 (m, 3H), 2.79−2.71 (m, 1H), 2.53 (s, 3H), 2.30 (s, 3H), 1.69−1.53 (m, 4H), 1.44−1.35 (m, 2H); MS (ESI) m/z 498.3 [(M + H)$^+$ 100]. Anal. ($C_{19}H_{27}N_7O_7S\cdot2.2CF_3CO_2H$) C, H, N, S.

2-[(*S*)-4-Phenylbenzenesulfonylamino]-3-[5-(4-((imidazol-2-yl)amino)butyl)isoxazolin-3-ylcarbonyl]aminopropionic Acid Trifluoroacetate (3w): ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.0−11.95 (bs, 2H), 8.27 (dt, J = 18.6, 5.9 Hz, 1H), 8.19 (dd, J = 12.9, 8.6 Hz, 1H), 7.92 (t, J = 5.6 Hz, 1H), 7.87−7.82 (m, 4H), 7.76−7.73 (m, 2H), 7.53−7.49 (m, 2H), 7.45−7.41 (m, 1H), 6.96 (s, 2H), 4.65−4.46 (m, 1H), 4.06−3.98 (m, 1H), 3.34−3.00 (m, 4H), 2.74−2.62 (m, 1H), 1.58−1.43 (m, 4H), 1.41−1.28 (m, 2H); MS (ESI) m/z 555.3 [(M + H)$^+$ 100]; HRMS (FAB) m/z 555.2026 [(M + H)$^+$ calcd for $C_{26}H_{30}N_6O_6S$ 555.2026]. Anal. ($C_{26}H_{30}N_6O_6S\cdot1.2CF_3CO_2H\cdot H_2O$) C, H, N, F.

2(*S*)-Amino-3-[5-(4-((imidazol-2-yl)amino)butyl)isoxazolin-3-ylcarbonyl]aminopropionic Acid Trifluoroacetate (3z): ^{1}H NMR (400 MHz, CD_3OD) δ 6.82 (s, 2H), 4.2−4.1 (bs, 1H), 3.87−3.81 (m, 2H), 2.87 (dd, J = 17.1, 8.3 Hz, 1H), 1.72−1.64 (m, 4H), 1.57−1.48 (m, 2H); MS (ESI) m/z 339.3 [(M + H)$^+$ 75], 170.2 (100). Anal. ($C_{14}H_{22}N_6O_4\cdot2.6CF_3CO_2H\cdot 2H_2O$) C, H, N.

2-[(*S*)-2,4,6-Trimethylbenzenesulfonylamino]-3-[5-(2-((imidazol-2-yl)amino)ethyl)isoxazolin-3-ylcarbonyl]aminopropionic Acid Trifluoroacetate (3bb): ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.2−12.1 (bs, 2H), 8.34−8.27 (m, 1H), 8.08−7.99 (m, 2H), 6.98 (d, J = 3.3 Hz, 2H), 4.77−4.74 (m, 1H), 3.95−3.88 (m, 1H), 3.34−3.08 (m, partially coincident with H_2O), 2.53 (s, 6H), 2.23 (s, 3H), 1.92−1.79 (m, 2H); MS (ESI) m/z 493.4 [(M + H)$^+$ 100]. Anal. ($C_{21}H_{28}N_6O_6S\cdot1.3CF_3CO_2H$) C, H, N.

2-[(*R*)-2,4,6-Trimethylbenzenesulfonylamino]-3-[5-(4-((imidazol-2-yl)amino)butyl)isoxazolin-3-ylcarbonyl]aminopropionic Acid Trifluoroacetate (3cc): ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.0−12.1 (bs, 1H), 11.98 (s, 2H), 8.22 (t, J = 5.9 Hz, 1H), 7.96 (m, 1H), 7.96 (t, J = 5.7 Hz, 1H), 7.92 (d, J = 9.0 Hz, 1H), 6.97 (s, 2H), 6.94 (s, 2H), 4.71−4.66 (m, 1H), 3.94−3.88 (m, 1H), 3.7−3.4 (bs, 2H), 3.45−3.30 (m, 2H), 3.23−3.14 (m, 3H), 2.70 (dd, J = 17.6, 8.5 Hz, 1H), 2.54 (s, 6H), 2.24 (s, 3H), 1.65−1.53 (m, 4H), 1.42−1.34 (m, 2H); MS (ESI) m/z 543 [(M+Na)$^+$ 80], 521 [(M+H)$^+$ 100]. Anal. ($C_{23}H_{32}N_6O_6S\cdot1.4CF_3CO_2H$) C, H, N.

2-[(*S*)-2,4,6-Trimethylbenzenesulfonylamino]-3-[5-(4-(imidazol-2-ylamino)-4-oxo-3-azabutyl)isoxazolin-3-yl]-aminopropionic Acid Trifluoroacetate (3h). To a solution of **9b** (82 mg, 0.17 mmol) in CH_2Cl_2 (anhyd, 10 mL) was added triethylamine (0.05 mL, 0.36 mmol) followed by a 20% solution of phosgene in toluene (0.097 mL, 0.19 mmol). After stirring for 3 h at room temperature, **22** (60 mg, 0.17 mmol) was added, and the mixture stirred an additional 18 h. Following by partitioning between water and EtOAc, the combined organic fraction was washed with water and saturated NaCl, dried ($MgSO_4$), filtered, and evaporated in vacuo. Using a combination of reverse-phase HPLC and mass spectral analysis, two components were identified: the first component (t_R = 14.2 min) was determined to be the detritylated product: MS (ESI) m/z 592 (M + H)$^+$. The second component (t_R = 18.8 min) was the desired product: MS (ESI) m/z 834 (M + H)$^+$. The desired material was placed in 20% HOAc/MeOH overnight, combined with the detritylated material, and purified using reverse-phase HPLC. Combination of the appropriate fractions, concentration in vacuo, and lyophilization resulted in the detritylated *tert*-butyl ester (60 mg, 43%). Cleavage of the ester in TFA as described for **3s** gave **3h**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 13.1−11.8 (bs, 2H), 11.5−10.0 (bs, 1H), 8.27−8.21 (m, 1H), 7.93 (bd, J = 9.0 Hz, 1H), 7.42−7.33 (bs, 1H), 7.05 (s, 2H), 6.97 (s, 2H), 6.60−6.40 (bs, 2H), 4.80−4.68 (m, 1H), 3.95−3.86 (m, 1H), 2.83−2.73 (m, 1H), 2.54 (s, 3H), 2.53 (s, 3H), 2.24

(s, 3H), 1.88−1.72 (m, 2H); MS (ESI) *m/z* 558.1 [$(M + Na)^+$ 100], 536.1 [$(M + H)^+$ 60]. Anal. ($C_{20}H_{29}N_7O_7S \cdot 1.9CF_3CO_2H$) C, H, N.

In Vitro Pharmacology. 1. Human PRP−Light Transmittance Aggregometry Assay. Venous blood was obtained from healthy human donors who were aspirin-free for at least 2 weeks prior to blood collection or from other species as previously described.[18b] Blood was collected into citrate Vacutainer tubes. The blood was centrifuged for 10 min at 150*g* in a Sorvall RT6000 tabletop centrifuge with H-1000 B rotor at room temperature, and PRP was removed. The remaining blood was centrifuged for 10 min at 2500 rpm at room temperature, and platelet-poor plasma (PPP) was removed. Samples were assayed on a PAP-4 platelet profiler, using PPP as the blank (100% transmittance). PRP (200 μL, 2×10^8 platelets/mL) was added to each micro test tube, and transmittance was set to 0%. The platelet agonist ADP (20 μL, 100 μM final concentration) was added to each tube, and the aggregation profiles were plotted (% transmittance versus time). Maximal aggregation was obtained with ADP at 10 μM final concentration. The test agent (20 μL) was added at different concentrations 8 min prior to the addition of ADP (10 μM). The IC_{50} values (μM) for the different test agents were then calculated.

2. GPP + Fibrinogen Aggregation. Human PRP (h-PRP) or PRP obtained from healthy volunteers was applied to a sepharose column to prepare GPP as previously described (Mousa et al., 1994). Aliquots of GPP (2×10^8 platelets/mL) along with 1 mM calcium chloride and 1 mg/mL fibrinogen with or without the test agent at different concentrations were then added to generate an inhibition curve from which IC_{50} values were calculated.

3. Generation of 293β_3z Cells. The 293 human embryonic kidney cells were obtained from ATCC and cultured in Dulbecco minimal essential media (Gibco) containing 10% fetal bovine serum and pen/strep at 37 °C, 5% CO_2. The cDNA for the human β_3 integrin subunit was obtained by PCR amplification of RNA from HEL cells and cloned into the expression vector pcDNAneo (Invitrogen). After characterization of the cloned DNA, 293 cells were transfected with the β_3 expression construct using calcium phosphate. Transfected cells were first selected in media containing 600 μg/mL active G418 (Gibco) and then sorted via FACS (Becton-Dickinson) using the $\alpha_v\beta_3$ complex-specific antibody, LM609 (Chemicon). A population of cells expressing the highest levels of $\alpha_v\beta_3$ were plated at low density and a single clone was selected. These 293β_3 cells were transfected by electroporation with the SVzeo/lacZ construct from Invitrogen and selected in media containing 50 μg/mL zeocin (Invitrogen). Individual clones were picked and assayed for β-galactosidase specific activity. Clones expressing the highest β-galactosidase activity were characterized by FACS analysis for integrin receptor number, $\alpha_v\beta_3$, α_v, β_3, $\alpha_v\beta_5$, $\alpha_4\beta_1$, and $\alpha_5\beta_1$ using a Quantum Simply Cellular microbead kit (Sigma). The parent 293β_3 and the clone (293β_3z) selected for use in the adhesion and migration assays expressed 1.1×10^5 $\alpha_v\beta_3$, $<2.5 \times 10^3$ $\alpha_v\beta_5$, 1.2×10^5 α_v, 1.0×10^5 β_3, 2.6×10^4 $\alpha_5\beta_1$, and 2.0×10^5 β_1 receptors per cell. In contrast, the untransfected 293 cells expressed $<2.5 \times 10^3$ $\alpha_v\beta_3$, 1.2×10^4 $\alpha_v\beta_5$, 1.6×10^4 α_v, $<2.5 \times 10^3$ β_3, 2.6×10^4 $\alpha_5\beta_1$, and 1.9×10^5 β_1 receptors per cell. Although the 293β_3 cells were always cultured in the presence of 300 μg/mL G418 and 50 μg/mL zeocin, continuous monitoring of cells indicated decreases in both $\alpha_v\beta_3$ receptor and β-galactosidase expression with time in culture. New batches of cells were then started when expression decreased by 50%.

4. 293β_3z-Fg Adhesion Assay. Human fibrinogen (Enzyme Research Laboratories Inc.) was further purified by precipitation with glycine.[23] Polystyrene EIA plates (Costar 3590) were coated with 25 μg of fibrinogen overnight at 4 °C. The plates were washed two times with phosphate-buffered saline without calcium and magnesium (PBS), blocked with 5% bovine serum albumin in PBS for 2 h, and washed two times prior to the assay. Using polypropylene plates (Costar 9482), compounds were diluted in MCDB131 media[24] adjusted to pH 7.0 with 20 mM HEPES buffer such that the DMSO concentration was constant (0.2%). Dilutions of a control compound and negative DMSO control were run on each plate. Log phase 293β_3z cells were harvested and adjusted to 10^6 viable cells/mL of MCDB131 media/20 mM HEPES. The 293β_3z cells were added to the compound dilutions in the polypropylene plates and preincubated for 15 min at 37 °C in a 5% CO_2 incubator. The compound/cell mixture was then added to the fibrinogen-coated plate (10^5 cells/well) and incubated for 1 h at 37 °C in a 5% CO_2 incubator. After incubation, the nonadhered cells were removed and the wells washed once with PBS using an automated multichannel pipet. The adherent cells (10% of input in absence of inhibitors) were lysed and an aliquot was taken for determination of β-galactosidase activity using a Galactolight Plus assay (Tropix) and a Dynatech Microlite 1 luminometer. Nonspecific binding was negligible and determined using 1 μM of a compound having an IC_{50} below 20 nM. The specificity of the assay was indicated by >95% inhibition of 293β_3z adhesion by the $\alpha_v\beta_3$ neutralizing antibody LM609 (Chemicon) and the negligible adhesion of untransfected 293 cells to purified fibrinogen under the conditions used in the assay. The mass of β-galactosidase per well was determined by comparison to a standard concentration curve of purified β-galactosidase (Sigma) assayed simultaneously with the samples.

5. 293β_3z-Vn Migration Assay. Vitronectin was purified from human plasma by heparin affinity chromatography.[25] Migration media (Dulbecco minimal essential media (Gibco) containing 0.1% bovine serum albumin was added beneath the microporous membrane inserts of Costar Transwell plates (6.5 mm, 8-μm pore). Compounds were diluted in migration media such that the concentration of DMSO was constant (0.5%). DMSO controls in the absence and presence of vitronectin were run in each assay along with dilutions of a control compound. Log phase 293β_3z cells were harvested and adjusted to 10^6 viable cells/mL of migration media. The 293β_3z cells (10^5 cells/well) were added to the compound dilutions in the polypropylene plates and preincubated for 15 min at room temperature. The compound/cell mixture was then added into the inserts of the prepared plates. Vitronectin was added to the media below the insert and the plates were incubated for 17−19 h at 37 °C in a 5% CO_2 incubator. The amount of vitronectin used to induce migration in the assay was that concentration that induced half of the maximal migration observed in titration with that batch of vitronectin (typically 1 μg/mL vitronectin final concentration for the best preparations). After incubation, the nonmigrated cells still within the insert were removed with a cotton swab. The cells that had migrated to the opposite side of the filter were fixed and stained using a Diff-Quick kit (Baxter). Excess stain was removed by several rinses with water. The inserts were inverted and allowed to dry overnight. The membrane was removed from the insert and mounted on a microscope slide. The number of migrated cells per membrane was quantified using an Optomax Domino image analyzer (Hollis, NH). Generally, 7.5% of the input 293β_3z cells migrated in the absence of inhibitors to the opposite side of the filter in the presence of vitronectin, while less than 0.3% migrated in the absence of vitronectin. Specificity of the assay was indicated by >95% inhibition of migration by LM609 and minimal inhibition (<30%) by antibodies to $\alpha_v\beta_5$ (P1F6, Chemicon) and $\alpha_5\beta_5$ (JSB5, Chemicon) in comparison to isotype controls. Additionally, 293 cells that had not been transfected with the β_3 contruct showed negligible (0.3%) migration toward vitronectin.

Acknowledgment. We express our thanks to F. Wayne Rankin for technical assistance; Alfred J. Mical and Andrew M. Blum for performing preparative chiral HPLC separations; Gregory A. Nemeth and Ernest M. Schubert for assistance in acquiring NMR data; and Carl Schwarz and Michael J. Haas for providing low- and high-resolution mass spectral data. We also thank Frank A. Barbera, Jeffrey M. Bozarth, Sandra K. Flint, Mark S. Forsythe, Lori L. Foster, Maureen A. Kearney,

William F. Lorelli, Karen L. Schlingmann, and Susan M. Spitz for technical assistance in support of in vitro studies.

References

(1) (a) Brooks, P. C.; Clark, R. A. F.; Cheresh, D. A. Requirement of Integrin $\alpha_v\beta_3$ for Angiogenesis. *Science* **1994**, *264*, 569−571. (b) Brooks, P. C.; Montgomery, A. M. P.; Rosenfeld, M.; Reisfeld, R. A.; Hu, T.; Klier, G.; Cheresh, D. A. Integrin $\alpha_v\beta_3$ Antagonists Promote Tumor Regression by Inducing Apoptosis of Angiogenic Blood Vessels. *Cell* **1994**, *79*, 1157−1164.

(2) (a) Engleman, V. W.; Nickols, G. A.; Ross, F. P.; Horton, M. A.; Griggs, D. W.; Settle, S. L.; Ruminski, P. G.; Teitelbaum, S. L. A Peptidomimetic Antagonist of the $\alpha_v\beta_3$ Integrin Inhibits Bone Resorption In Vitro and Prevents Osteoporosis In Vivo. *J. Clin. Invest.* **1997**, *99*, 2284−2292. (b) Keenan, R. M.; Miller, W. H.; Kwon, C.; Ali, F. A.; Callahan, J. F.; Calvo, R. R.; Hwang, S.-M.; Kopple, K. D.; Peishoff, C. E.; Samanen, J. M.; Wong, A. S.; Yuan, C.-K.; Huffman, W. F. Discovery of Potent Nonpeptide Vitronectin Receptor ($\alpha_v\beta_3$) Antagonists. *J. Med. Chem.* **1997**, *40*, 2289−2292.

(3) Gruentzig, A. R.; Senning, A.; Siegenthaler, W. F. Nonoperative Dilatation of Coronary-Artery Stenosis. *N. Engl. J. Med.* **1979**, *301*, 61−68.

(4) Popma, J. J.; Califf, R. M.; Topol, E. J. Clinical Trials of Restenosis After Coronary Angioplasty. *Circulation* **1991**, *84*, 1426−1436.

(5) Kovach, J. A.; Mintz, G. S.; Pichard, A. D.; Kent, K. M.; Popma, J. J.; Satler, L. F.; Leon, M. B. Sequential Intravascular Ultrasound Characterization of the Mechanisms of Rotational Atherectomy and Adjunct Balloon Angioplasty. *J. Am. Coll. Cardiol.* **1993**, *22*, 1024−1032. (b) Mintz, G. S.; Douek, P. C.; Bonner, R. F.; Kent, K. M.; Picherd, A. D.; Satler, L. F.; Leon, M. B. Intravascular Ultrasound Comparison of De Novo and Restenotic Coronary Artery Lesions. *J. Am. Coll. Cardiol.* **1993**, *21*, 118A (Abstract).

(6) Charo, I. F.; Bekeart, L. S.; Phillips, D. R. Platelet Glycoprotein IIb-IIIa-Like Proteins Mediate Endothelial Cell Attachment to Adhesive Proteins and the Extracellular Matrix. *J. Biol. Chem.* **1987**, *262*, 9935−9938. (b) Yue, T.-L.; Ohlstein, E.; Louden, C.; Gu, J.-L.; Vickery, L. M.; Johanson, K.; Stadel, J. M. SK&F107260, a Cyclic RGD Peptide, Inhibits Integrin $\alpha_v\beta_3$-Mediated Vascular Smooth Muscle Cell Migration, In Vitro, and Reduces Neointima Formation Following Balloon Injury to the Rat Carotid Artery, In Vivo. *Pharmacol. Rev. Commun.* **1998**, *10*, 9−18.

(7) Srivatsa, S. S.; Fitzpatrick, L. A.; Tsao, P. W.; Reilly, T. M.; Holmes Jr., D. R.; Schwartz, R. S.; Mousa, S. A. Selective $\alpha_v\beta_3$ Integrin Blockade Potently Limits Neointimal Hyperplasia and Lumen Stenosis Following Deep Coronary Arterial Stent Injury: Evidence for the Functional Importance of $\alpha_v\beta_3$ and Osteopontin Expression During Neointimal Formation. *Cardiovasc. Res.* **1998**, *36*, 408−428. (b) Choi, E. T.; Engel, L.; Callow, A. D.; Sun, S.; Trachtenberg, J.; Santoro, S.; Ryan, U. S. Inhibition of Neointimal Hyperplasia by Blocking $\alpha_v\beta_3$ Integrin with a Small Peptide Antagonist G*penGRGDSPC*A. *J. Vasc. Surg.* **1994**, *19*, 125−134. (c) Matsuno, H.; Stassen, J. M.; Vermeylen, J.; Deckmyn, H. Inhibition of Integrin Function by a Cyclic RGD-Containing Peptide Prevents Neointima Formation. *Circulation* **1994**, *90*, 2203−2206.

(8) Xue, C.-B.; Wityak, J.; Sielecki, T. M.; Pinto, D. J.; Batt, D. G.; Cain, G. A.; Sworin, M.; Rockwell, A. L.; Roderick, J. J.; Wang, S.; Orwat, M. J.; Frietze, W. E.; Bostrom, L. L.; Liu, J.; Higley, C. A.; Rankin, F. W.; Tobin, A. E.; Emmett, G.; Lalka, G. K.; Sze, J. Y.; Di Meo, S. V.; Mousa, S. A.; Thoolen, M. J.; Racanelli, A. L.; Hausner, E. A.; Reilly, T. M.; DeGrado, W. F.; Wexler, R. R.; Olson, R. E. Discovery of an Orally Active Series of Isoxazoline GPIIb/IIIa Antagonists. *J. Med. Chem.* **1997**, *40*, 2064−2084. (b) Wityak, J.; Sielecki, T. M.; Pinto, D. J.; Sze, J. Y.; Liu, J.; Tobin, A. E.; Wang, S.; Jiang, B.; Emmett, G.; Ma, P.; Mousa, S. A.; Ma, P.; Wexler, R. R.; Olson, R. E. Discovery of Potent Isoxazoline Glycoprotein IIb/IIIa Receptor Antagonists. *J. Med. Chem.* **1997**, *40*, 50−60.

(9) Bodary, S. C.; Napier, M. A.; McLean, J. W. Expression of Recombin ant Platelet Glycoprotein IIbIIIa Results in a Functional Fibrinogen-binding Complex. *J. Biol. Chem.* **1989**, *264*, 18859−18862.

(10) Rockwell, A. L.; Rafalski, M.; Pitts, W. J.; Batt, D. G.; Petraitis, J. J.; DeGrado, W. F.; Mousa, S. A.; Jadhav, P. K. Rapid Synthesis of RGD Mimetics with Isoxazoline Scaffold on Solid Phase: Identification of $\alpha_v\beta_3$ Antagonists Lead Compounds. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 937−942.

(11) Bernatowicz, M. S.; Wu, Y.; Matsueda, G. R. Urethane Protected Derivatives of 1-Guanylpyrazole for the Mild and Efficient Preparation of Guanidines. *Tetrahedron Lett.* **1993**, *34*, 3389−3392.

(12) Little, T. L.; Webber, S. E. A Simple and Practical Synthesis of 2-Aminoimidazoles. *J. Org. Chem.* **1994**, *59*, 7299−7305.

(13) Nikam, S. S.; Kornberg, B. E.; Johnson, D. R.; Doherty, A. M. Synthesis of Hydroxamic Acids: $Pd/BaSO_4$ as a New Catalyst for the Deprotection of O-Benzyl Hydroxamates. *Tetrahedron Lett.* **1995,** *36*, 197−200.

(14) Czarnik, A. W.; Leonard, N. J. Unequivocal Assignment of the Skeletal Structure of the Guanine-Glyoxal Adduct. *J. Org. Chem.* **1980**, *45*, 3514−3517. (b) Lawson, A. The Reaction of Cyanamide with α-Amino-acetals and α-Amino-aldehydes. *J. Chem. Soc.* **1956**, 307−310.

(15) Arnould, J. C.; Landier, F.; Pasquet, M. J. New Applications of the Mitsunobu Reaction in the Synthesis of C-2 *N*-Methyl Carbapenems. *Tetrahedron Lett.* **1992**, *33*, 7133−7136.

(16) Abdel-Magid, A. F.; Maryanoff, C. A. Reductive Amination of Aldehydes and Ketones with Weakly Basic Anilines Using Sodium Triacetoxyborohydride. *Synlett* **1990**, 537−539.

(17) Determined using ^{1}H NMR through integration of the aldehyde proton over time.

(18) Mousa, S. A.; Bozarth, J. M.; Forsythe, M. S.; Lorelli, W.; Ramachandran, N.; Jackson, S.; De Grado, W. F.; Reilly, T. M. Antiplatelet Efficacy and Specificity of DMP 728, A Novel Platelet GPIIb/IIIa Receptor Antagonist. *Cardiology* **1993**, *83*, 374−382. (b) Mousa, S. A.; Bozarth, J. M.; Forsythe, M. S.; Jackson, S. M.; Leamy, A.; Diemer, M. M.; Kapil, R. P.; Knabb, R. M.; Mayo, M. C.; Pierce, S. K.; De Grado, W. F.; Thoolen, M. J.; Reilly, T. M. Antiplatelet and Antithrombotic Efficacy of DMP 728, a Novel Platelet GPIIb/IIIa Receptor Antagonist. *Circulation* **1994**, *89*, 3−12.

(19) Wityak, J.; Tobin, E. A.; Mousa, S. A.; Wexler, R. R.; Olson, R. E. Isoxazoline GPIIb/IIIa Antagonists Bearing a Phosphoramidate. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 123−126.

(20) All pK_a values cited in this manuscript are estimates based upon literature precedent. For reference, see: (a) Albert, A. Ionization Constants. In *Physical Methods in Heterocyclic Chemistry*; Katritzky, A. R., Ed.; Academic Press: New York, 1963; Vol. 1, pp 2−103. (b) Perrin, D. D. *pK_a Prediction for Organic Acids and Bases*; Chapman and Hall: New York, 1981.

(21) Hu, D. D.; Barbas III, C. F.; Smith, J. W. An Allosteric Ca^{2+} Binding Site on the β_3-Integrins That Regulates the Dissociation Rate for RGD Ligands. *J. Biol. Chem.* **1996**, *271*, 21745−21751. (b) Pelletier, A. J.; Kunicki, T.; Quaranta, V. Activation of the Integrin $\alpha_v\beta_3$ Involves a Discreet Cation-Binding Site That Regulates Conformation. *J. Biol. Chem.* **1996**, *271*, 1364−1370.

(22) Mokotoff, M.; Logue, L. W. Potential Inhibitors of L-Asparagine Biosynthesis. 5. Electrophilic Amide Analogues of (*S*)-Diaminopropionic Acid. *J. Med. Chem.* **1981**, *24*, 554−559.

(23) Jilg, W.; Priess, H.; Hörman, H. Isolation of residual fibrinogen from a supernatant of plasma precipitated with 2.1 M glycine. *Thromb. Res.* **1976**, *9*, 479−489.

(24) Knedler, A.; Ham, R. G. Optimized Medium for Clonal Growth of Human Microvascular Endothelial Cells with Minimum Serum. *In Vitro Cell. Dev. Biol.* **1987**, *23*, 481−491.

(25) Yatohgo, T.; Izumi, M.; Kashiwagi, H.; Hayashi, M. Novel purification of vitronectin from human plasma by heparin affinity chromatography. *Cell Struct. Funct.* **1988**, 281−292.

JM9900321