Non-Peptide Fibrinogen Receptor Antagonists. 7. Design and Synthesis of a Potent, Orally Active Fibrinogen Receptor Antagonist†

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The design, synthesis, and pharmacological evaluation of L-734,217, a potent, low-molecular weight, orally active fibrinogen receptor antagonist, is reported. A strategy for producing low-molecular weight inhibitors from the peptide c-[(Ac)CRGDC] A, previously reported from these laboratories, is outlined. This strategy combines a retrodesign analysis of the conformationally defined cyclic peptide A with stereochemical information present in the arginine-glycine-aspartic acid (RGD) tripeptide sequence, culminating with the discovery of L-734,217. L-734,217 inhibited the aggregation of human, dog, and chimpanzee platelets at concentrations below 100 nM and was found to be >15000-fold less effective at inhibiting the attachment of human umbilical vein endothelial cells to fibrinogen, fibronectin, and vitronectin than it was at inhibiting the aggregation of platelets. L-734,217 showed significant ex vivo antiplatelet activity following oral administration in dogs and chimpanzees at doses of 1.0 and 2.0 mg/kg, respectively, and has been selected as a clinical candidate for development as an antithrombotic agent.

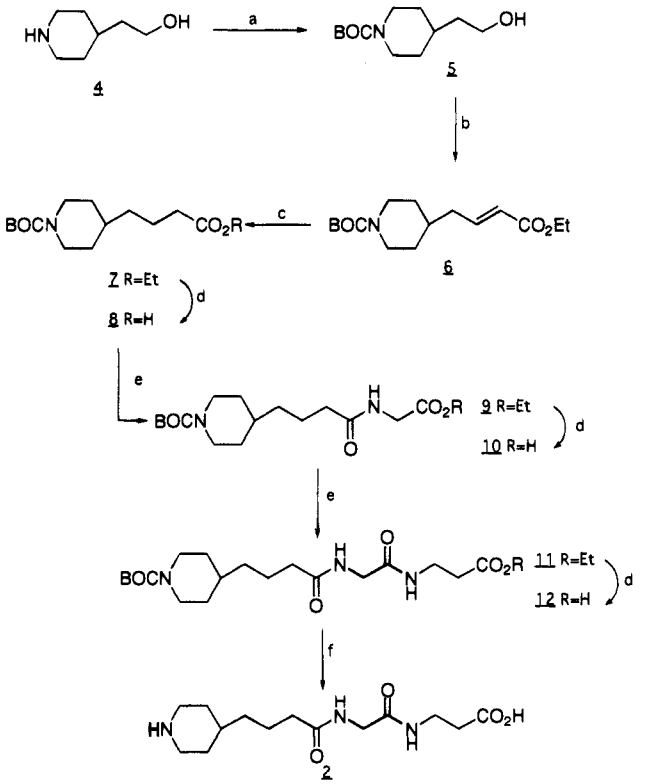
Introduction

The fibrinogen receptor GP IIb/IIIa, a heterodimeric glycoprotein present on the surface of platelets, plays a crucial role in platelet adherence and aggregation.[1] Upon vascular injury, mediators of platelet aggregation, such as ADP, thrombin, and collagen, are released, initiating platelet activation, with subsequent exposure of GP IIb/IIIa, resulting in aggregation through its binding to the homodimeric plasma protein fibrinogen.[2-4] In atherosclerotic arteries, the disruption of plaque eventually leads to platelet activation and hence aggregation, leading to thrombotic and thromboembolic occlusions resulting in heart attacks, strokes, and angina.[5-8] Inhibition of platelet aggregation at the activation stage has proven difficult due to the plethora of activation pathways available to platelets. However, a common end point of all activation pathways is the binding of fibrinogen to GP IIb/IIIa, defining intervention at this stage of aggregation as an attractive strategy for controlling thrombus formation.[9,10] In fact, GP IIb/IIIa-specific antibodies that inhibit the binding of fibrinogen to its receptor have been shown to be potent inhibitors of platelet aggregation in vitro and effective antithrombotic agents in vivo, offering early clinical relevance for this approach.[11,12]

GP IIb/IIIa as well as other members of the integrin superfamily of cell adhesion receptors, such as fibronectin and vitronectin, recognizes ligands containing the three-amino acid sequence arginine-glycine-aspartic acid (RGD).[13] The RGD sequence occurs twice in the Aα chain of fibrinogen and is believed to mediate, at least in part, the binding of fibrinogen to its platelet receptor.[14,15]

Several structurally diverse RGD-based fibrinogen receptor antagonists that inhibit platelet aggregation at low nanomolar concentrations have been disclosed.

Scheme 1[a]



[a] (a) Boc_2O, DMF; (b) Swern oxidation then (carbethoxymethylene)triphenylphosphorane; (c) 10% Pd/C, H_2, EtOAc; (d) 1 N NaOH, ethanol; (e) EDC, HOBT, NEt_3, DMF; (f) TFA/CH_2Cl_2.

† Abbreviations: Boc, *tert*-butyloxycarbonyl; EDC, 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride; HOBT, 1-hydroxybenzotriazole·H_2O; NMM, 4-methylmorpholine; NaHMDS, sodium hexamethyldisilylazide; DMF, dimethylformamide.

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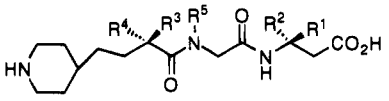
Table 1. Physical Properties and in vitro IC_{50} Values



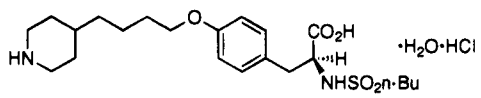
compound	R^1	R^2	R^3	R^5	R^4	mp (°C)	plaggin[a] IC_{50} (μM)	formula[b]	FAB-MS (*m/e*) (M + H)
A	–	–	–	–	–	–	0.68[c]	–	593
1	H	Me	H	$(CH_2)_3$	$(CH_2)_3$	240 (dec)	0.032	$C_{18}H_{31}N_3O_4$	354
1a	Me	H	H	$(CH_2)_3$	$(CH_2)_3$	–	7.8	$C_{18}H_{31}N_3O_4$	354
2	H	H	H	H	H	–	8.4	$C_{14}H_{25}N_3O_4 \cdot HCl \cdot 0.68H_2O$	300
3	H	H	H	$(CH_2)_3$	$(CH_2)_3$	–	0.083	$C_{17}H_{29}N_3O_4 \cdot 1.8H_2O$	340
3a	H	H	$(CH_2)_3$	$(CH_2)_3$	H	–	7.7	$C_{17}H_{29}N_3O_4$[d]	340.2232

[a] Platelet aggregation with human gel-filtered platelets (GFP) was measured by the light transmittance method at 37 °C with 2×10^8 platelets/mL, 0.1 mg/mL of human fibrinogen, and 1 mM $CaCl_2$. Aggregation was initiated by adding 10 μM ADP after all other components were added. The rate of aggregation in the absence of inhibitor served as the control, and values reported are the concentration necessary to inhibit the rate of aggregation by 50%. At least two determinations are made for each compound. [b] Analysis for C, H, and N within ±0.4% except where indicated. [c] IC_{50} value obtained from ref 29. [d] Characterized by high-resolution FAB-MS.

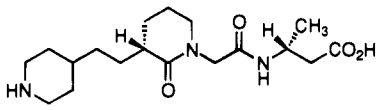
These include the viper venom peptides termed disintegrins, RGD-based linear and cyclic peptides, and nonpeptidal inhibitors.[16-28] Common to all of the potent inhibitors reported to date is a basic moiety, mimicking the arginine guanidine, and a carboxylic acid, representing the side chain carboxylic acid of aspartic acid. These basic and acidic functional groups share spatial requirements congruent with those found in the tripeptide. In addition, significant specificity for GP IIb/IIIa over other integrins has been reported for classes of inhibitors containing either an amine or a benzamidine moiety in place of guanidine.[20,23,26]

Efforts in these laboratories have focused on preparing GP IIb/IIIa selective, orally active fibrinogen receptor antagonists that inhibit platelet aggregation at concentrations below 100 nM. Previously, our group disclosed the structures of the cyclic pentapeptide c-[(Ac)CRGDC] A and the nonpeptidal exosite inhibitor MK-383,[23,24] a potent, selective ligand for GP IIb/IIIa that is currently being evaluated in human clinical trials via iv administration as an acute antithrombotic agent.[30]

In this report, we disclose our approach toward the design of potent, low-molecular weight fibrinogen receptor antagonists that culminated with the discovery of the orally active nonpeptide **1** (L-734,217). This approach utilizes configurational as well as conformational information derived from the cyclic peptide A.



MK-383



1 (L-734,217)

Chemistry

Preparation of the compounds contained in Table 1 is illustrated in Schemes 1–4. 4-Piperidineethanol (**4**) served as the progenitor to the key intermediate **8** shown in Scheme 1. Protection of **4** with Boc_2O in DMF followed by a one-pot oxidation/Wittig sequence furnished the olefin **6** in 30% yield. Hydrogenation of **6** and then saponification afforded **8** in 88% yield. EDC coupling of glycine ethyl ester to **8** followed by saponification produced the acid **10** in 83% yield. This process was repeated for the conversion of **10** to **12** in 80% yield using β-alanine ethyl ester in place of glycine ethyl ester. Removal of the Boc group was accomplished with TFA in methylene chloride to give **2** in 53% yield.

The chemistry developed to generate the chiral synthons **18** and **21** is displayed in Schemes 2 and 3. Using technology developed in the Evans group,[31] the key intermediates **18** and **18a** were obtained in high optical purity. The acid **8** was activated and then treated with lithium (*S*)-4-benzyl-2-oxazolidinone to give **13** in 90% yield. Alkylation of the titanium enolate of **13** with acrylonitrile furnished **14** as a single diastereomer in 66% yield. Nitrile reduction with PtO_2/H_2 followed by stirring of the resulting amine·HCl **15** in acetonitrile and $NaHCO_3$ gave the lactam **16** in 91% yield. Lactam alkylation with ethyl bromoacetate followed by saponification afforded **18** in 78% yield with 98% ee, as determined by chiral HPLC. The corresponding enantiomer **18a** was prepared as described for **18** using the chiral auxilary (*R*)-4-benzyl-2-oxazolidinone.

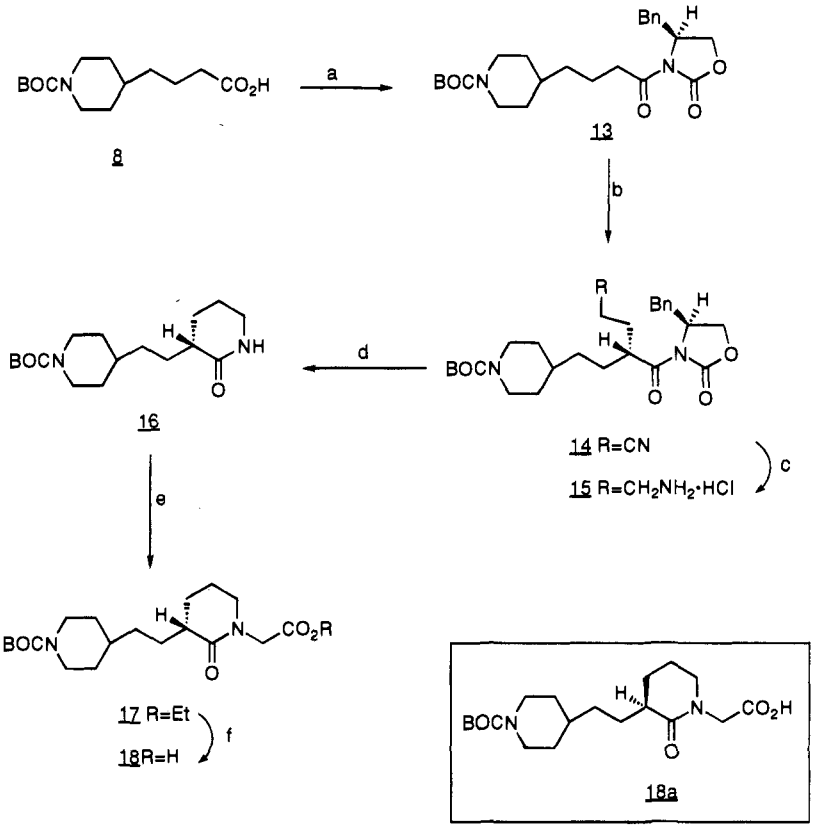
As shown in Scheme 3, the optically pure β-alanine esters **21** and **21a** were prepared in two steps from the commercially available Boc-protected D- and L-alanine, respectively. Boc-D-alanine **19** was treated with isobutyl chloroformate to form the mixed anhydride. Addition of diazomethane to the mixed anhydride furnished the intermediate diazo ketone which underwent silver benzoate-catalyzed Wolff rearrangement to form **20** in 50% yield. Deprotection of the resulting carbamate with HCl gave the amine·HCl **21** in 92% yield. The enantiomer **21a** was prepared in an analogous manner starting from Boc-L-alanine. The enantiomers **21** and **21a** were determined to be >99% optically pure by HPLC analysis.

Scheme 4 depicts the chemistry developed for preparing compounds **1**, **1a**, **3**, and **3a**. Standard EDC coupling conditions were used to condense the acid **18** with either **21** or **21a** to furnish **22** and **22a**, respectively, in high yield. Saponification and TFA deprotection of **22** and **22a** afforded **1** and **1a** in 47 and 38% yield, respectively. Alternatively, **18** was coupled to β-alanine *tert*-butyl ester to furnish **24** in 64% yield. TFA deprotection provided **3** in 65% yield. The corresponding enantiomer **3a** was prepared in a similar fashion from **18a**.

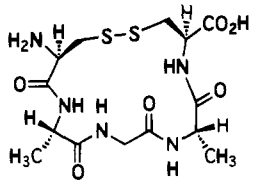
Molecular Modeling

The NMR-based structure A1, c-[CAGAC], was previously used to describe the cyclic peptide A, c-[(Ac)-CRGDC].[29] The Advanced Modeling Facility[32] was used

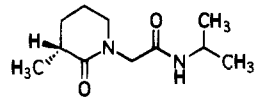
Scheme 2[a]



[a] (a) Pivaloyl chloride, THF, NEt_3, (*S*)-4-benzyl-2-oxazolidinone; (b) $Ti(O\text{-}i\text{-}Pr)Cl_3$, $i\text{-}Pr_2EtN$, CH_2Cl_2, acrylonitrile; (c) PtO_2, H_2, $CH_3OH/CHCl_3$; (d) $NaHCO_3$, CH_3CN; (e) NaHMDS, THF, ethyl bromoacetate; (f) 1 N NaOH, CH_3OH.



A1



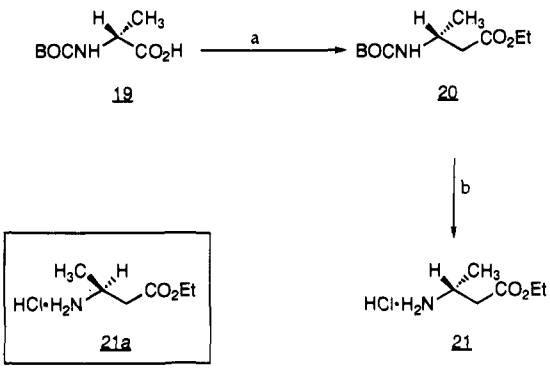
X

to produce a starting structure for the lactam core unit, X. Conformers of structure A1, D094 and F179 (see Figure 2), were both used or comparison to the lactam core unit X.

Pairs of structures where X was superposed onto the peptide conformers D094 and F179 were produced with the distance geometry program JIGGLE.[33] For these comparisons, X was completely flexible while the cyclic peptide conformers D094 and F179 were held rigid. In each instance, 50 pairs of structures were requested. Stereochemistry at all chiral centers was maintained, and a match of X onto the peptide was defined by superposing, within 0.2 Å, the positions given below: cyclic peptide A1, X; Arg carbonyl O, lactam O; Gly amide N, lactam N; Gly carbonyl O, secondary amide O; and Asp amide N, secondary amide N.

The aligned pairs of molecules were energy minimized using the OPTIMOL methodology with the MMFF-93 force field.[34] A distance-dependent dielectric model, with $\epsilon = 4.0$, was employed. Initial minimizations imposed distance constraints between the matched atoms. The resulting structures were subsequently minimized without constraints. Visualization of superposed ligands was accomplished using C_View.[35] A set of 150 random conformations of X was produced using

Scheme 3[a]



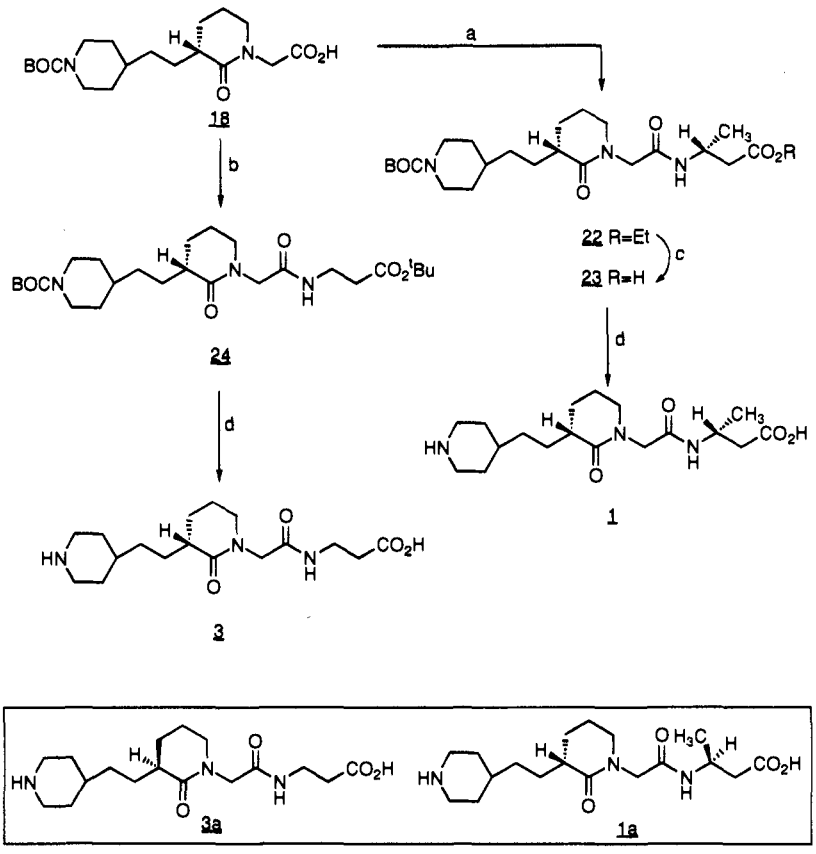
[a] (a) Isobutyl chloroformate, EtOAc, NMM, diazomethane then silver benzoate, NEt_3, MeOH; (b) EtOAc, HCl.

JIGGLE. These structures were energy minimized via OPTIMOL using the MMFF force field,[34] as described above.

Results and Discussion

Figure 1 illustrates our approach to designing potent, low-molecular weight, nonpeptidal fibrinogen receptor antagonists from the cyclic RGD-containing pentapeptide c-[(Ac)CRGDC] A. This template was an attractive starting point since A exhibited significant antiaggregatory potency in vitro, IC_{50} = 0.68 uM, and offered sufficient conformational bias to allow a pair of low-energy conformers to be determined by computational analysis of ^1H NMR data. Key bond disconnections were made at the arginyl α-amino and aspartyl α-carbonyl bonds of the cyclic peptide to generate compound B. These disconnections retain the arginyl guanidine and the aspartyl side chain carboxylic acid while

Scheme 4[a]



[a] (a) EDC, HOBT, NEt_3, DMF, **21** or **21a**; (b) EDC, HOBT, NEt_3, DMF, β-alanine *tert*-butyl ester; (c) 1 N NaOH, CH_3OH; (d) TFA/CH_2Cl_2.

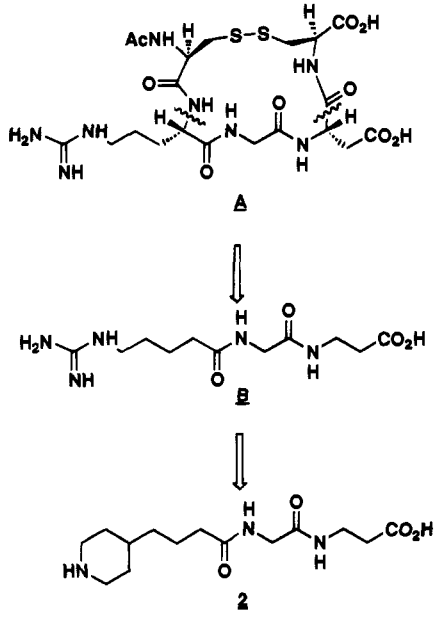


Figure 1.

significantly reducing molecular weight. The guanidine could then be replaced by an amino group, in this case a piperidine, to provide compound **2**.[21,23]

Compounds described in this report were evaluated for their ability to inhibit the aggregation of ADP-induced, gel-filtered platelets (Table 1). Although compound **2** was 12-fold less effective than A at inhibiting the aggregation of human platelets in vitro, **2** provided a low-molecular weight lead structure for further modifications.

Since the difference between the in vitro potencies of the conformationally restricted cyclic peptide A and the flexible amino acid **2** may, in part, be entropically driven, a conformational constraint was incorporated into structure **2**. A three-methylene tether was introduced from the glycine nitrogen to the carbon α to piperidinebutyryl carbonyl, to form a six-membered lactam. This strategy reintroduced the arginyl bond, present in the cyclic peptide A, without a large increase in molecular weight. Of the enantiomeric lactams prepared, only the *R*-isomer **3** afforded an increase in potency compared to **2**. Lactam **3** retained the stereogenicity of arginine present in both the cyclic peptide A and the RGD units of fibrinogen.

The antiaggregatory potency of **3** was further enhanced through the introduction of a conformational bias into the β-alanine moiety of **3**. To present a bioactive conformation similar to that anticipated for the cyclic peptide, the bond broken at the aspartic acid residue in our retrodesign analysis, Figure 1, was reestablished as a methyl group, to minimize molecular weight increase. Compound **1**, $IC_{50} = 32$ nM, possessing a methyl group with *R*-configuration at the 3-position of the β-alanine unit, was 2–3-fold more potent than **3** at inhibiting platelet aggregation in vitro. In contrast to **1**, diastereomer **1a** was 10-fold less potent than **3** in vitro. Diastereomer **1** retains the relative stereochemistry observed in the cyclic peptide A at the 3-position of the β-alanine. Thus, the stereochemical information revealed in the arginine and aspartic acid residues of the cyclic peptide and fibrinogen was utilized to furnish a potent, low-molecular weight ligand for GP IIb/IIIa.

To define the conformational similiarity between **1** and the cyclic peptide A, the lactam core unit X was superposed onto the NMR-based conformers, D094 and F179, of the cyclic portion of A. Selected superpositions

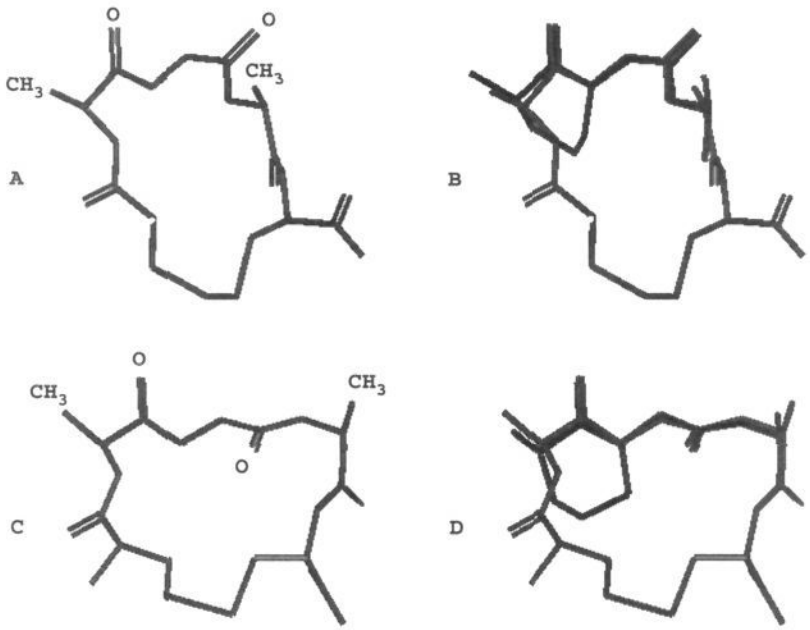


Figure 2. Comparison of core unit X with NMR-based cyclic peptide structures. Parts A and C show the D094 and F179 conformations of the peptide, respectively. For clarity, the Arg and Gly carbonyl oxygens and the truncated side chains of Arg and Asp, represented as methyl groups, are shown. Parts B and D illustrate the core unit (black) aligned to the D094 and F179 peptide conformers (gray), respectively.

Table 2. Energies for X Given in Kilocalories per Mole

study	highest energy	lowest energy
random conformations	22.197	15.445
Compared to D094 Peptide		
minimized with constraints	29.509	19.103
minimized free of constraints	21.014	15.944
Compared to F179 Peptide		
minimized with constraints	25.542	16.944
minimized free of constraints	20.593	15.445

of X are illustrated in Figure 2. Comparisons of X to conformers D094 and F179 suggest that this lactam-containing moiety can mimic the geometrical constraints imposed by the peptide backbone. The elements of the Arg-Gly and Gly-Asp amide linkages align nicely with the lactam and amide moieties, respectively, of X. In addition, the alignment of the Arg and Asp side chain vectors of the cyclic peptide with that of X is in good agreement.

Energy minimization of the superpositioned lactam core unit X was performed with and without the imposed constraints, and the results are summarized in Table 2. These studies indicate that the energy cost for X to adopt conformations that superpose well with the peptide is reasonable. For the F179 form of the peptide, the energy of the constraint-imposed structure was approximately 1.5 kcal/mol higher than that of the free form of X. In addition, the energy calculated for the lowest energy free form of X, derived from F179, was comparable to that of its lowest energy random conformer. For the D094 form of the peptide, the energy of the constraint-imposed structure was calculated to be approximately 3.6 kcal higher than that of the free form of X. These data indicate that the core unit X can mimic the F179 conformer of the cyclic peptide more closely than the D094 conformer.

Table 3 summarizes the inhibitory concentrations required for compound **1** to inhibit the aggregation of human, dog, and chimp platelets in vitro. The aggregation of human platelets in platelet rich plasma was inhibited by compound **1** in the presence of ADP, epinephrine, collagen, and thrombin with IC_{50} values of 23, 36, 67, and 27 nM, respectively. Compound **1** demonstrated comparable inhibitory potencies of 42 and 40 nM toward dog platelets in the presence of ADP and epinephrine or collagen and 42 and 40 nM toward chimp platelets in the presence of ADP and collagen, respectively.

Table 3. Inhibition of Platelet Aggregation by **1**[a]

	reaction conditions	IC_{50} (nM) (platelet rich plasma)
A.	human	
	10 μM ADP	23 ± 3
	10 μM epinephrine	36 ± 3
	10 μg/mL of collagen	67 ± 10
	0.5 U/mL of thrombin and 1 mM GPRP	27 ± 5
B.	dog	
	10 μM ADP and 1 μM epinephrine	41 ± 8
	10 μg/mL of collagen and 1 μM epinephrine	40 ± 13
C.	chimp	
	20 μM ADP	42 ± 8
	10 μg/mL of collagen	40 ± 13

[a] X ± SE (n = 3–7). Platelet rich plasma (PRP) was obtained by centrifuging the blood sample at 150g for 5 min. Platelet counts were adjusted to 200 000 cells/mm^3 with platelet poor plasma (PPP). The adjusted PRP (300 μL) was incubated at 37 °C in a siliconized cuvette for 3 min before the agonist was added. Aggregation was induced by the addition of agonist. Platelet aggregation was measured as a percent change in light transmission with a BioData platelet aggregometer. The aggregometer was standardized with PPP representing 100% light transmittance. The effect of L-734,217 treatment on the extent of aggregation was expressed as percent inhibition of aggregation, using the base line pretreatment aggregation response as 100%.

Since the fibrinogen receptor on platelets is a member of the integrin superfamily of receptors, specificity for GP IIb/IIIa over other RGD-recognizing integrins is thought to be an important requirement for generating a safe, effective antiplatelet agent. Specificity was

Table 4. Effect of 1 on Cell Attachment to Adhesive Proteins[a]

	IC_{50} (nM) (HUVEC)		
IC_{50} (nM) (platelet)	Fg	Vn	Fn
32	$>5 \times 10^5$	$>5 \times 10^5$	$>5 \times 10^5$

[a] Human umbilical vein endothelial cells (HUVEC) were used between passages 2 and 7. Following removal from the monolayer, cells were allowed to attach to microtiter plates containing human fibrinogen (Fg), human vitronectin (Vn), or human fibronectin (Fn). After 75 min at 37 °C, unattached cells were removed by gentle washing and adherent cells were quantitated by measuring glucosaminidase activity. Binding of HUVEC to fibrinogen was blocked by specific monoclonal antibodies directed against α_v (AMAC) or $\alpha_v\beta_3$ (23C6) but not α_5 or α_2, binding to vitronectin by antibodies directed against $\alpha_v\beta_3$ (AMAC) but not α_5 or α_2, and binding to fibronectin by antibodies directed against α_5 (Telios) but not α_v or $\alpha_v\beta_3$. Thus, HUVEC attachment to these three substrates monitors $\alpha_v\beta_3$ and $\alpha_5\beta_1$ activity.

Table 5. Effect of 1, 1.0 mg/kg po by Capsule, on ex Vivo Platelet Aggregation Responses and Platelet Count in Conscious Dogs[a]

	ex vivo platelet aggregation (% inhibition)		platelet counts	
time (min)	ADP (10 μM + 1 μM Epi)[b]	collagen (10 μM + 1 μM Epi)[b]	($10^3/\mu$L)	(Δ% from base line)
0	0	0	339 ± 22	0
20	50 ± 29	48 ± 28	310 ± 39	−8.7 ± 8.6
40	86 ± 8	79 ± 10	344 ± 34	+1.2 + 1.3
70	93 ± 6	79 ± 12	353 ± 25	+4.2 ± 2.3
90	96 ± 4	75 ± 5	353 ± 25	+4.2 ± 2.3
150	96 ± 3	94 ± 4	338 ± 30	−0.5 ± 3.5
200	94 ± 4	78 ± 10	331 ± 25	−2.7 ± 1.3
250	90 ± 6	72 ± 10	332 ± 13	−1.4 ± 3.1
300	91 ± 4	67 ± 10	322 ± 20	−4.8 ± 1.5
350	81 ± 9	54 ± 17	339 ± 17	+0.3 ± 1.7
480	63 ± 23	31 ± 21	309 ± 21	−8.9 ± 2.1
1440	2 ± 2	3 ± 2	313 ± 37	−8.2 ± 7.3

[a] Data are mean ± SEM (n = 4). [b] Epi: epinephrine.

evaluated by determining the IC_{50} for inhibition of human umbilical vein endothelial cell (HUVAC) attachment to fibrinogen (Fg), vitronectin (Vn), and fibronectin (Fn).[36] The results summarized in Table 4 demonstrate that compound **1** is >15000-fold less effective at inhibiting cell attachment than inhibiting the aggregation of platelets. This selectivity may, in part, be attributed to the presence of an amine in place of the arginine guanidine as reported previously.[21,23]

The antiplatelet activity of compound **1** was assessed following oral administration of a solid crystalline compound in gelatin capsules at a dose of 1 mg/kg to conscious dogs (n = 4). After administration, blood samples were drawn at specified time points, and the derived platelet rich plasma was assayed for the extent of aggregation in the presence of collagen or ADP. Whole blood platelet counts were determined separately. The effects of oral administration of compound **1** on ex vivo platelet aggregation responses to ADP and collagen are summarized in Table 5 and are depicted graphically in Figures 3 and 4. Oral administration of compound **1** in a capsule resulted in an inhibition of the platelet aggregation response to ADP that was equal to or greater than 90% from 70−300 min after administration. Aggregation responses to collagen were inhibited 67−94% during the same time frame. Platelet aggregation responses to ADP and collagen remained inhibited 63 and 31%, respectively, 8 h after the oral administration of **1**. Platelet responsiveness to ADP and collagen returned to base line by 24 h. Platelet count was not altered by the oral administration of compound **1** at this dose.

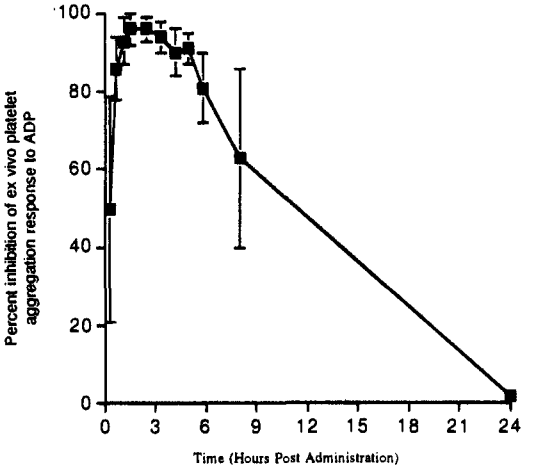


Figure 3. Effect of 1, 1.0 mg/kg po by capsule, on ex vivo platelet aggregation responses to ADP (10 μM + 1 μM epinephrine) expressed as percent inhibition over time (h) in conscious dogs.

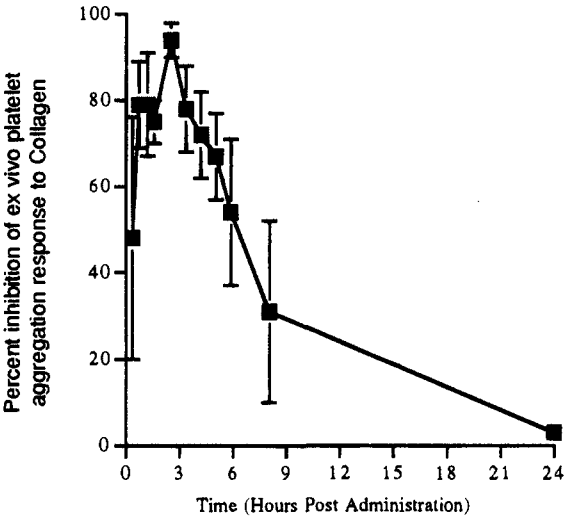


Figure 4. Effect of 1, 1.0 mg/kg po by capsule, on ex vivo platelet aggregation responses to collagen (10 μM + 1 μM epinephrine) expressed as percent inhibition over time (h) in conscious dogs.

Compound **1** was also evaluated in a primate model for its antiplatelet activity following oral administration. In this study, four male chimps were made to fast overnight before receiving, under ketamine sedation, a 2 mg/kg dose (aqueous solution) of **1** by gavage. The chimpanzees were repeatedly sedated with ketamine for blood sampling, and the platelet rich plasma was evaluated for the extent of aggregation in the presence of ADP. These data are summarized graphically in Figure 5. Following oral administration of **1**, ex vivo platelet aggregation was inhibited at a level of >50% from 2 to 16 h after the dose with platelet activity returning to base line over 36 h. In addition, no significant changes in platelet counts were observed during the 36 h period in all four chimps (data not shown).

In conclusion, we have described our approach for the design of potent, low-molecular weight inhibitors of platelet aggregation. This strategy begins with the bioactive RGD-containing cyclic peptide c[(AcCRGDC] A. Retrodesign analysis of this conformationally biased template combined with the stereochemical information present in the Arg-Gly-Asp tripeptide sequence led to the discovery of L-734,217, a potent, integrin-selective inhibitor of GP IIb/IIIa. This point is illustrated in

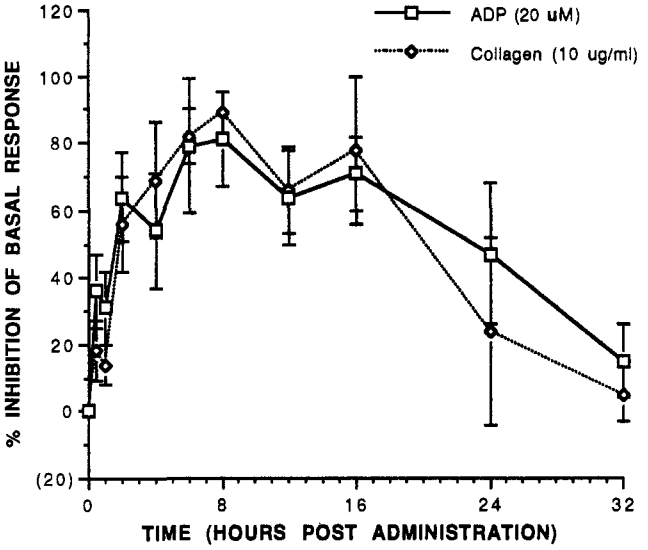
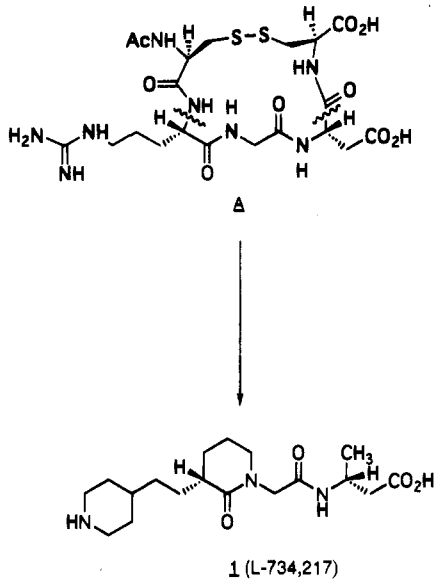


Figure 5. Effect of L-734,217 (2.0 mg/kg oral) on ex vivo aggregation of platelets in chimpanzees ($n = 4$).



A

1 (L-734,217)

Figure 6. Structural comparison of L-734,217 and peptide A.

Figure 6. The bonds broken in the peptide A were ultimately restored to maintain the stereogenicity present in the arginyl and aspartyl units of the cyclic peptide A. These features provided the requisite elements of conformational bias and constraint. Molecular-modeling studies, comparing the core lactam unit of L-734,217 with NMR-based conformers of A, suggest that low-energy conformations of L-734,217 overlap well with that of the peptide conformers D094 and F179. L-734,217 exhibited significant oral activity in dogs and chimpanzees at doses of 1.0 and 2.0 mg/kg, respectively, and has been chosen for further evaluation in clinical trials. Furthermore, the approach disclosed herein may offer advantages in other areas of ligand design where a di-, tri-, or tetra-peptide sequence, representing the shortest sequence necessary for binding, can be incorporated into cyclic peptide structures.

Experimental Section

Unless otherwise noted, starting materials were obtained from commercial suppliers and used without further purification. Silica gel (E. Merck, 230–400 mesh) was used for column chromatography, and silica gel (Analtech) plates were used for analytical thin-layer chromatography. All nuclear magnetic resonance spectra were recorded on a Varian XL-300 spectrometer except where noted (Varian VXR-400S). Chemical shifts are reported in parts per million relative to TMS as the internal standard. Melting points were determined on a Thomas-Hoover melting point apparatus and are corrected. Mass spectra were obtained on an LKB-9000S mass spectrometer at 70 eV. Optical rotations were obtained on a Perkin-Elmer polarimeter. Spectrophysics SP8800 HPLC pump and SP100 detector were used to determine optical purities.

***N*-Boc-4-piperidineethanol (5).** A stirred solution of 4-piperidineethanol (4) (18.7 g, 140 mmol) and DMF at 0 °C was treated with *N-tert*-butoxycarbonyl anhydride (31 g, 140 mmol). After 1 h, the cooling bath was removed and the reaction mixture stirred for an additional 20 h. The reaction mixture was then diluted with ether, washed with H_2O (2×) and brine, dried ($MgSO_4$), and concentrated to furnish **5** (26 g, 82%) as a colorless oil: TLC R_f 0.25 (40% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$) δ 4.09 (bs, 2H), 3.72 (t, $J = 7$ Hz, 2H), 2.70 (m, 2H), 1.75–1.10 (m, 7H), 1.46 (s, 9H).

Ethyl 4-(*N*-Boc-piperidin-4-yl)*trans*-crotonate (6). To a stirred solution of oxalyl chloride (0.43 mL, 5.0 mmol) in CH_2Cl_2 (29 mL) at −78 °C was added DMSO dropwise. After gas evolution subsided (5 min), the alcohol **5** (0.8 g, 3.5 mmol) in CH_2Cl_2 (20 mL) was added in a stream. After 20 min, (carbethoxymethylene)triphenylphosphorane (1.4 g, 4.0 mmol) was added. After 2 h, the reaction mixture was diluted with petroleum ether, washed sequentially with H_2O, 5% $KHSO_4$, and brine, dried ($MgSO_4$), and concentrated. Flash chromatography (15% EtOAc/hexanes) gave the ester **6** (0.57 g, 38%) as a colorless oil: TLC R_f 0.79 (50% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$) δ 6.91 (dt, $J = 16$ and 7 Hz, 1H), 5.81 (bd, $J = 17$ Hz, 1H), 4.18 (q, $J = 7$ Hz, 2H), 4.08 (m, 2H), 2.67 (m, 2H), 2.14 (t, $J = 7$ Hz, 2H), 1.70–1.05 (m, 5H), 1.44 (s, 9H), 1.28 (t, $J = 7$ Hz, 3H).

Ethyl 4-(*N*-Boc-piperdin-4-yl)butyrate (7). The olefin **6** (26 g, 87 mmol) in EtOAc (500 mL) at ambient temperature was stirred under a hydrogen atmosphere (1 atm) in the presence of 10% Pd/C (5.0 g) overnight. The reaction mixture was then purged with argon, followed by filtration through a Celite pad. Concentration of the filtrate followed by flash chromatography (10% EtOAc/hexanes) gave the ester **7** (24 g, 92%) as a crystalline solid: TLC R_f 0.42 (20% EtOAc/hexanes); ^{1}H NMR ($CDCl_3$) δ 4.16 (q, $J = 7$ Hz, 2H), 4.10 (m, 2H), 2.69 (m, 2H), 2.31 (t, $J = 7$ Hz, 2H), 1.68 (s, 9H), 1.40 (m, 1H), 1.11 (m, 2H).

(*N*-Boc-piperidin-4-yl)butanoic Acid (8). A solution of the ester **7** (19 g, 63 mmol), ethanol (300 mL), and 1 N NaOH (100 mL) was stirred at ambient temperature for 3 h, followed by concentration. The residue was diluted with 5% $KHSO_4$ and EtOAc and then transferred to a separatory funnel. The phases were shaken, and separated, and the organic portion was washed with brine, dried ($MgSO_4$), and concentrated to give **8** (18 g, 96%) as a colorless oil that crystallized on standing: mp 80–81 °C; TLC R_f 0.68 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 4.10 (m, 2H), 2.71 (m, 2H), 2.38 (t, $J = 7$ Hz, 2H), 1.70 (m, 4H), 1.60–1.30 (m, 3H), 1.48 (s, 3H), 1.12 (m, 2H).

(*N*-Boc-piperidin-4-yl)butyrylglycine Ethyl Ester (9). To a stirred solution of **8** (4.0 g, 14.7 mmol), 4-methylmorpholine (1.6 mL, 14.7 mmol), and EtOAc (200 mL) at −15 °C was added isobutyl chloroformate (1.9 mL, 14.7 mmol). After 15 min, glycine ethyl ester·HCl (4.1 g, 29 mmol) and 4-methylmorpholine (4.8 mL, 45 mmol) were added. After an additional 45 min, the reaction mixture was washed with H_2O, saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and concentrated. Flash chromatography (60% EtOAc/hexanes) gave the dipeptide **9** (5.0 g, 96%) as a colorless oil: TLC R_f 0.60 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 5.98 (m, 1H), 4.22 (q, $J = 7$ Hz, 2H), 4.08 (m, 2H), 4.05 (d, $J = 5$ Hz, 2H), 2.68 (m, 2H), 2.24 (t, $J = 7$ Hz, 2H), 1.70 (m, 4H), 1.47 (s, 9H), 1.30 (t, $J = 7$ Hz, 3H), 1.10 (m, 2H).

(*N*-Boc-piperidin-4-yl)butyrylglycine (10). A mixture of the ester **9** (5.0 g, 14 mmol), 1 N NaOH (21 mL), and CH_3OH (100 mL) was stirred at ambient temperature for 3 h. The reaction mixture was then concentrated to dryness, dissolved in H_2O, and acidified to pH 3 with 5% $KHSO_4$. Extraction with EtOAc followed by washing the EtOAc portion with brine,

drying ($MgSO_4$), and concentration gave the acid **10** (4.2 g, 86%) as a colorless oil: TLC R_f 0.50 (9:0.5:0.5 CH_2Cl_2/CH_3OH/AcOH).

(*N*-Boc-piperidin-4-yl)butyrylglycine-β-alanine Ethyl Ester (11). To a stirred solution of **10** (158 mg, 0.48 mmol), 4-methylmorpholine (53 μL, 0.48 mmol), and EtOAc (5 mL) at −15 °C was added isobutyl chloroformate (63 μL, 0.48 mmol). After 15 min, β-alanine ethyl ester·HCl (220 mg, 1.45 mmol) and 4-methylmorpholine (160 μL, 1.45 mmol) were added. After an additional 45 min, the reaction mixture was washed with H_2O, saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and concentrated. Flash chromatography (7.5% 2-propanol/$CHCl_3$) furnished **11** (164 mg, 80%) as a colorless oil: TLC R_f 0.58 (10% 2-propanol/$CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 6.39 (m, 1H), 6.12 (m, 1H), 4.17 (q, J = 7 Hz, 2H), 4.07 (m, 2H), 3.90 (d, J = 6 Hz, 2H), 3.55 (m, 2H), 2.66 (m, 2H), 2.53 (t, J = 6 Hz, 2H), 2.22 (t, J = 7 Hz, 2H), 1.70−1.00 (m, 9H), 1.45 (s, 9H), 1.26 (t, J = 7 Hz, 3H).

(*N*-Boc-piperidin-4-yl)butyrylglycine-β-alanine (12). A mixture of the ester **11** (160 mg, 0.37 mmol), 2 N NaOH (0.37 mL), and CH_3OH (5 mL) was stirred at ambient temperature for 16 h. The reaction mixture was then concentrated to dryness, dissolved in H_2O, and acidified to pH 2 with 5% $KHSO_4$. Extraction with $CHCl_3$ followed by washing the organic portion with brine, drying ($NaSO_4$), and concentration gave the acid **12** (164 mg, 100%) as a colorless oil: TLC R_f 0.56 (9:1:1 CH_2Cl_2/CH_3OH/AcOH); ^1H NMR δ (400 MHz, $CDCl_3$) δ 6.90 (m, 1H), 6.43 (m, 1H), 4.08 (m, 2H), 3.99 (d, J = 6 Hz, 2H), 3.55 (m, 2H), 2.68 (m, 2H), 2.58 (m, 2H), 2.23 (m, 2H), 1.70−1.00 (m, 9H).

(Piperidin-4-yl)butyrylglycine-β-alanine (2). HCl gas was passed through a stirred suspension of **12** (114 mg, 0.29 mmol) in CH_2Cl_2 (5 mL) at −10 °C for 20 min. After 20 min, the cooling bath was removed and the reaction mixture stirred for an additional 5 min. The reaction mixture was then purged with argon to remove excess HCl. The resulting precipitate was collected by filtration to give **2** (51 mg, 53%) as a white solid: TLC R_f 0.21 (10:1:1 ethanol/NH_4OH/H_2O); ^1H NMR (400 MHz, DMSO) δ 8.62 (m, 1H), 8.30 (m, 1H), 8.00 (m, 1H), 7.88 (m, 1H), 3.61 (d, J = 6 Hz, 2H), 3.50 (m, 2H), 3.23 (m, 4H), 2.70 (m, 2H), 2.38 (m, 2H), 2.13 (t, J = 7 Hz, 2H), 1.79 (m, 2H), 1.50 (m, 3H), 1.30−1.15 (m, 4H); FAB-MS *m/e* 300 $(M + H)^+$. Anal. ($C_{14}H_{25}N_3O_4$·HCl·0.68 H_2O) C, H, N.

4(*S*)-Benzyl-3-[4-(*N*-Boc-piperidin-4-yl)butyroyl]oxazolidinone (13). To a solution of **8** (15.3 g, 56 mmol), NEt_3 (9.4 mL, 67 mmol), and dry THF (240 mL) at −78 °C was added trimethylacetyl chloride (7.6 mL, 61 mmol) in a stream. After 10 min, the cooling bath was removed and replaced with an ice bath. After 1 h, the heterogeneous mixture was recooled to −78 °C, followed by cannula addition of lithium (*S*)-(−)-4-benzyl-2-oxazolidinone (62 mmol) in dry THF (150 mL), prepared by treating (*S*)-(−)-4-benzyl-2-oxazolodinone in dry THF (150 mL) at −78 °C with *n*-BuLi (38.8 mL, 62 mmol, 1.6 M/hexanes). After addition was complete, the reaction mixture was warmed to 0 °C for 1 h, diluted with EtOAc, washed with H_2O, saturated $NaHCO_3$, 5% $KHSO_4$, and brine, dried, MgSO_4), and concentrated. Flash chromatography (30% EtOAc/hexanes) gave **13** (21.8 g, 90%) as a colorless oil: TLC R_f 0.45 (30% EtOAc/hexanes); ^1H NMR ($CDCl_3$) δ 7.36−7.20 (m, 5H), 4.67 (m, 1H), 4.18 (m, 2H), 3.30 (dd, J = 13 and 3 Hz, 1H), 2.93 (m, 2H), 2.77 (dd, J = 13 and 10 Hz, 1H), 2.69 (m, 2H), 1.70 (m, 4H), 1.50−1.30 (m, 3H), 1.45 (s, 9H), 1.11 (m, 2H).

4(*S*)-Benzyl-3-[4-(*N*-Boc-piperidin-4-yl)-2(*R*)-(2-cyanoethyl)butyroyl]-2-oxazolidinone (14). To a stirred solution of $TiCl_4$ (42 mL, 42 mmol, 1 M/CH_2Cl_2) and CH_2Cl_2 (250 mL) at 0 °C was added titanium(IV) isopropoxide (4.2 mL, 14 mmol). After 15 min, diisopropylethylamine (11.0 mL, 63 mmol) was added dropwise to form a dark brown solution. After 10 min, **13** (21.8 g, 51 mmol) in CH_2Cl_2 (75 mL) was added, followed by continued stirring at 0 °C for 1 h. Acrylonitrile (33.4 mL, 0.50 mol) was added dropwise at 0 °C to the deep red solution. After 4 h, the reaction was quenched with saturated NH_4Cl (150 mL) at 0 °C and then the mixture extracted with CH_2Cl_2 (3 × 250 mL). The combined organic extracts were washed with saturated $NaHCO_3$ and brine, dried ($MgSO_4$), and concentrated. Flash chromatography (25% EtOAc/hexanes) gave crude **14** (19.9 g) as a yellow oil. Crude **14** was rechromatographed (2.5% acetone/CH_2Cl_2) to yield **14** (16.6 g, 66%) as an oil: TLC R_f 0.35 (2.5% acetone/CH_2Cl_2); ^1H NMR ($CDCl_3$) δ 7.50−7.30 (m, 5H), 4.68 (m, 1H), 4.21 (m, 2H), 4.07 (m, 2H), 3.80 (m, 1H), 3.33 (dd, J = 13 and 4 Hz, 1H), 2.77 (dd, J = 13 and 10 Hz, 1H), 2.65 (m, 2H), 2.38 (m, 2H), 2.13 (m, 1H), 1.89 (m, 1H), 1.63 (m, 2H), 1.50 (m, 2H), 1.45 (s, 9H), 1.35 (m, 1H), 1.25 (m, 2H), 1.08 (m, 2H).

4(*S*)-(Cyclohexylmethyl)-3-[4-(*N*-Boc-piperidin-4-yl)-2(*R*)-(2-aminoethyl)butyroyl]-2-oxazolidinone·HCl (15). A mixture of **14** (19.2 g, 40 mmol), PtO_2 (2.0 g), CH_3OH (70 mL), and $CHCl_3$ (7.0 mL) was shaken on the Parr apparatus under a hydrogen atmosphere (60 psi) at ambient temperature for 3 h. The reaction mixture was filtered through a Celite pad and then concentrated to furnish the crude amine·HCl **15** (20.5 g, 97%) as a white solid: TLC R_f 0.50 (10:1:1 CH_2Cl_2/CH_3OH/HOAc); ^1H NMR ($CDCl_3$) δ 8.28 (bs, 2H), 4.50 (m, 1H), 4.36 (m, 1H), 4.14 (m, 1H), 4.03 (m, 2H), 3.63 (m, 1H), 3.02 (m, 2H), 2.63 (m, 2H), 2.00−1.00 (m, 24H), 1.45 (s, 9H).

3(*R*)-[2-(*N*-Boc-piperidin-4-yl)ethyl]-2-piperidone (16). The crude amine·HCl **15** (16.6 g, 31 mmol), acetonitrile (750 mL), and $NaHCO_3$ (10.0 g) was stirred at ambient temperature for 20 h. The heterogeneous mixture was then treated with di-*tert*-butyldicarbonate (3.0 g), followed by continued stirring for 1 h to reprotect minor amounts of free piperidine that formed in the previous reaction. The $NaHCO_3$ was removed by filtration and the filtrate concentrated. Flash chromatography (5% CH_3OH/EtOAc) gave the lactam **16** (8.8 g, 91%) as a colorless crystalline solid: mp 110−111 °C; TLC R_f 0.65 (20% CH_3OH/EtOAc); ^1H NMR ($CDCl_3$) δ 6.31 (bs, 1H), 4.06 (m, 2H), 3.31 (m, 2H), 2.67 (m, 3H), 2.28 (m, 1H), 2.00−1.20 (m, 11H), 1.45 (s, 9H), 1.10 (m, 2H).

Ethyl 3(*R*)-[2-(*N*-Boc-piperidin-4-yl)ethyl]-2-oxopiperidineacetate (17). To a stirred solution of **16** (6.7 g, 22 mmol) and dry THF (150 mL) at −78 °C was added NaN$(TMS)_2$ (24.5 mL, 24.5 mmol, 1 M/hexanes) dropwise. After 15 min, ethyl bromoacetate (5.2 mL, 45 mmol) was added and then the reaction mixture warmed to 0 °C for 1 h. The reaction was quenched with AcOH (1.0 mL) and then the mixture diluted with EtOAc, washed with H_2O and brine, dried ($MgSO_4$), and concentrated. Flash chromatography (40% EtOAc/hexanes) gave the ester **17** (6.7 g, 78%) as a yellow oil: TLC R_f 0.26 (40% EtOAc/hexanes); ^1H NMR ($CDCl_3$) δ 4.20 (q, J = 7 Hz, 2H), 4.17 (d, J = 18 Hz, 1H), 4.08 (m, 2H), 3.98 (d, J = 18 Hz, 1H), 3.37 (m, 2H), 2.68 (m, 2H), 2.32 (m, 1H), 2.00−1.25 (m, 11H), 1.45 (s, 9H), 1.30 (t, J = 7 Hz, 3H), 1.11 (m, 2H).

3(*R*)-[2-(*N*-Boc-piperidin-4-yl)ethyl]-2-oxopiperidineacetic Acid (18). A solution of **17** (6.0 g, 15 mmol), 1 N NaOH (50 mL, 50 mmol), and CH_3OH (75 mL) was stirred at ambient temperature for 1 h. The reaction mixture was then acidified with 5% $KHSO_4$ and then extracted with EtOAc. The organic portion was washed with brine, dried ($MgSO_4$), and concentrated to give the carboxylic acid **18** (5.6 g, 100%) as a yellow oil: TLC R_f 0.31 (9:0.5:0.5 CH_2Cl_2/CH_3OH/AcOH); ^1H NMR (400 MHz, $CDCl_3$) δ 4.13 (d, J = 17 Hz, 1H), 4.07 (m, 2H), 4.06 (d, J = 17 Hz, 1H), 3.39 (m, 2H), 2.66 (m, 1H), 1.95 (m, 3H), 1.81 (m, 1H), 1.70−1.25 (m, 7H), 1.45 (s, 9H), 1.08 (m, 2H).

3(*S*)-[2-(*N*-Boc-piperidin-4-yl)ethyl]-2-oxopiperidineacetic Acid (18a). This enantiomer **18a** was prepared through the same reaction sequence as was compound **18**. The optical purities of **18** and **18a** were determined by HPLC analysis to be 98% ee using a chiral AGP-I (10 × 4.6 mm) column, eluting with 92:8 solution of pH 4.7 buffer (aqueous sodium monobasic phosphate/acetonitrile) at 0.7 mL/min. Retention times for **18** and **18a** were 11.2 and 16.3 min, respectively, at 215 nM.

Ethyl 3(*R*)-Methyl-*N*-Boc-β-alanine (20). To a stirred solution of **19** (3.8 g, 20 mmol), 4-methylmorpholine (2.2 mL, 20 mmol), and EtOAc (200 mL) at −15 °C was added isobutyl chloroformate (2.6 mL, 20 mmol). After 1 h, the reaction mixture was washed with H_2O and brine, dried ($MgSO_4$), and gravity filtered into a round bottom flask. After cooling to 0 °C, the mixed anhydride was treated portionwise with an ethereal solution of diazomethane (80 mL, 40 mmol, 0.5 M solution). After 2 h, the cooling bath was removed and the excess diazomethane removed by purging the solution with argon for 30 min. Concentration gave the intermediate diazo

ketone (4.3 g) which was used directly for the next step: TLC R_f 0.37 (30% EtOAc/hexanes).

The diazo ketone was dissolved in ethanol (250 mL) and treated sequentially with NEt_3 (3.4 mL, 24 mmol) and silver benzoate (1.4 g, 6.0 mmol) to effect vigorous gas evolution and afford a black precipitate. After 1 h, the reaction mixture was concentrated and the residue purified by flash chroamtography (10% EtOAc/hexanes) to give the ethyl ester **20** (2.3 g, 50%) as a colorless oil: TLC R_f 0.42 (30% EtOAc/hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.96 (m, 1H), 4.19 (q, J = 7 Hz, 2H), 4.04 (m, 1H), 2.52 (dd, J = 15 and 6 Hz, 1H), 2.46 (dd, J = 15 and 6 Hz, 1H), 1.44 (s, 9H), 1.26 (t, J = 7 Hz, 3H), 1.21 (d, J = 7 Hz, 3H).

Ethyl 3(*R*)-Methyl-β-alanine·HCl (21). Through a mechanically stirred solution of **20** (2.2 g, 9.7 mmol) in EtOAc (180 mL) at −15 °C was vigorously bubbled HCl gas for 30 min. The cooling bath (ethanol/ice) was removed and the solution purged with argon for 1 h to remove the excess HCl. Concentration furnished the amine·HCl **21** (1.5 g, 92%) as a yellow glass: $[\alpha]^{23}_D$ −19.0 (*c* 0.6, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 4.23 (q, J = 7 Hz, 2H), 3.78 (m, 1H), 2.79 (m, 2H), 1.38 (d, J = 7 Hz, 3H), 1.29 (t, J = 7 Hz, 3H).

Ethyl 3(*R*)-Methyl-β-alanine·HCl (21a). With *N*-Boc-L-alanine as starting material, **21a** was prepared through the same reaction sequence as **21**. The optical purity values of >99% ee for **20** and **20a** were determined by the following method. Each compound was treated with Marfy reagent in an aqueous solution of $NaHCO_3$ and then heated at 50 °C for 1 h. The cooled solutions were then neutralized with 2 M HCl and then ejected onto a Beckman ODS (5 μm, 25 × 4.6 mm) column, eluting with a linear gradient of 20 to 50% acetonitrile/aqueous pH 3 buffer (triethylamine/H_3PO_4) for 60 min at a flow rate of 1.5 mL/min. The retention times for the derivatized products of **21** and **21a** detected at 340 nM were 34.9 and 35.5 min, respectively.

[3(*R*)-[2-(*N*-Boc-piperidin-4-yl)ethyl]-2-oxopiperidinyl]-acetyl-3(*R*)-methyl-β-alanine Ethyl Ester (22). To a stirred solution of **18** (1.0 g, 2.7 mmol), **21** (0.48 g, 2.8 mmol), HOBT (0.39 g, 2.8 mmol), N(*i*-Pr)$_2$Et(1.5 mL, 8.5 mmol), and dry DMF (27 mL) at −15 °C was added EDC (0.95 g, 2.8 mmol), followed by removal of the cooling bath. After 20 h, the reaction mixture was diluted with EtOAc and then washed with H_2O, saturated $NaHCO_3$, 5% $KHSO_4$, and brine, dried ($MgSO_4$), and concentrated. Flash chromatography (EtOAc) gave **22** (1.3 g, 97%) as a colorless oil: TLC R_f 0.35 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 6.82 (bd, 1H), 4.32 (m, 1H), 4.13 (q, J = 7 Hz, 2H), 4.10 (d, J = 15 Hz, 1H), 4.08 (m, 2H), 3.82 (d, J = 15 Hz, 1H), 3.36 (m, 2H), 2.67 (m, 2H), 2.48 (dd, J = 5 and 1 Hz, 2H), 2.33 (m, 1H), 2.00−1.20 (m, 11H), 1.45 (s, 9H), 1.27 (t, J = 7 Hz, 3H), 1.21 (d, J = 7 Hz, 3H), 1.10 (m, 2H).

[3(*R*)-[2-(*N*-Boc-piperidin-4-yl)ethyl]-2-oxopiperidinyl]-acetyl-3(*R*)-methyl-β-alanine (23). A solution of **22** (1.2 g, 2.5 mmol), 1 N NaOH (10 mL, 10 mmol), and CH_3OH (18 mL) was stirred at ambient temperature for 1 h. The reaction mixture was acidified with 5% $KHSO_4$ and then extracted with EtOAc. The organic portion was then washed with brine, dried ($MgSO_4$), and concentrated. Flash chromatography (10:0.5:0.5 CH_2Cl_2/CH_3OH/AcOH) gave the carboxylic acid **23** (0.75 g, 66%) as a colorless oil after azeotropic removal of the residual AcOH with toluene: TLC R_f 0.40 (10:0.5:0.5 CH_2Cl_2/CH_3OH/AcOH); ^{1}H NMR ($CDCl_3$) δ 7.02 (bd, 1H), 4.35 (m, 1H), 4.12 (d, J = 16 Hz, 1H), 4.08 (m, 2H), 3.87 (d, J = 16 Hz, 1H), 3.32 (m, 2H), 2.69 (m, 2H), 2.56 (m, 2H), 2.00−1.25 (m, 11H), 1.45 (s, 9H), 1.03 (m, 2H).

[3(*R*)-[2-(Piperidin-4-yl)ethyl]-2-oxopiperidinyl]acetyl-3(*R*)-methyl-β-alanine (1). A solution of **23** (0.74 g, 1.6 mmol), trifluoroacetic acid (10 mL), and CH_2Cl_2 (10 mL) was stirred at ambient temperature for 1 h. The reaction mixture was then concentrated and the residual trifluoroacetic acid removed azeotropically with toluene. Flash chroamtography (10:1:1 CH_3OH/NH_4OH/H_2O) afforded 1 (0.41 g, 71%) as an amorphous solid. Crystallization of 1 from hot ethanol furnished fiberous crystals after filteration at ambient temperature: mp 240 °C dec; $[\alpha]^{23}_D$ −23.4 (*c* 1.1, H_2O); TLC R_f 0.48 (10:1:1 CH_3OH/NH_4OH/H_2O); ^{1}H NMR (400 MHz, D_2O) δ 4.18 (m, 1H), 4.06 (d, J = 16 Hz, 1H), 3.95 (d, J = 16 Hz, 1H), 3.41 (m, 4H), 2.99 (m, 2H), 2.45 (m, 1H), 2.42 (dd, J = 18 and 6 Hz, 1H), 2.33 (dd, J = 18 and 7 Hz, 1H), 1.97 (m, 4H), 1.90−1.55 (m, 5H), 1.38 (m, 4H), 1.19 (d, J = 7 Hz, 3H).

[3(*R*)-[2-(*N*-Boc-piperidin-4-yl)ethyl]-2-oxopiperidinyl]-acetyl-3(*S*)-methyl-β-alanine Ethyl Ester (22a). Utilizing the same procedure for coupling **21** to **18**, **21a** (152 mg, 0.9 mmol) was condensed with **18** (123 mg, 0.33 mmol) to give **22a** (140 mg, 88%) as an oil after flash chromatography (EtOAc): TLC R_f 0.20 (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.77 (bd, 1H), 4.32 (m, 1H), 4.13 (q, J = 7 Hz, 2H), 4.05 (m, 2H), 4.00 (d, J = 16 Hz, 1H), 3.89 (d, J = 16 Hz, 1H), 3.36 (m, 2H), 2.67 (m, 2H), 2.48 (d, J = 6 Hz, 2H), 2.32 (m, 1H), 2.00−1.00 (m, 13H), 1.45 (s, 9H), 1.27 (t, J = 7 Hz, 3H), 1.21 (d, J = 3 Hz, 3 H).

[3(*R*)-[2-(*N*-Boc-piperidin-4-yl)ethyl]-2-oxopiperidinyl]-acetyl-3(*S*)-methyl-β-alanine (23a). Utilizing the same procedure for converting **22** to **23**, **22a** (140 mg, 0.29 mmol) furnished **23a** (137 mg, 100%) after flash chroamtography (20:1:1 CH_2Cl_2/CH_3OH/HOAc) and azeotropic removal of residual HOAc with a toluene azeotrope: TLC R_f 0.60 (20:1:1 CH_2Cl_2/CH_3OH/HOAc); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.90 (m, 1H), 4.30 (m, 1H), 4.10−3.85 (m, 4H), 3.37 (m, 2H), 2.67 (m, 2H), 2.52 (m, 2H), 2.32 (m, 1H), 2.00−1.00 (m, 13H), 1.45 (s, 9H), 1.23 (d, J = 6 Hz, 3H).

[3(*R*)-[2-(Piperidin-4-yl)ethyl]-2-oxopiperidinyl]acetyl-3(*S*)-methyl-β-alanine (1a). Utilizing the same procedure for converting **23** to 1, **23a** (137 mg, 0.30 mmol) afforded impure **1a** (83 mg) after flash chromatography (10:1:1 ethanol/H_2O/NH_4OH). Final purification was accomplished by preparative HPLC (delta pak C-18 column), eluting with a linear gradient of CH_3CN:H_2O w/0.1% TFA, 0 to 20%, over 20 min. Lyophilization gave pure **1a** (60 mg, 38%) as a glass: TLC R_f 0.25 (10:1:1 ethanol/H_2O/NH_4OH); ^{1}H NMR (400 MHz, CD_3OD) δ 4.25 (m, 1H), 4.18 (d, J = 16 Hz, 1H), 3.77 (d, J = 16 Hz, 1H), 3.48 (m, 1H), 3.30 (m, 3H), 2.95 (m, 2H), 2.40 (m, 1H), 2.33 (m, 2H), 2.00−1.30 (m, 13H), 1.20 (d, J = 6 Hz, 3H); FAB-MS *m/e* 354 (M + H)$^+$. Anal. ($C_{18}H_{31}N_3O_4$·1.4CF_3CO_2H·0.5H_2O) C, H, N.

[3(*R*)-[2-[2-(Piperidin-4-yl)ethyl]-2-oxopiperidinyl]-acetyl-β-alanine *tert*-Butyl Ester (24). To a stirred solution of **18** (0.40 g, 1.1 mmol), *tert*-butyl β-alanine·HCl (0.24 g, 1.3 mmol), HOBT (175 mg, 1.3 mmol), DMF (10 mL), and N(*i*-Pr)$_2$Et (0.68 mL, 3.9 mmol) at ambient temperature was added EDC (250 mg, 1.3 mmol). After 18 h, the reaction mixture was diluted with EtOAc, washed sequentially with H_2O, 10% $KHSO_4$, saturated $NaHCO_3$, and brine, dried ($MgSO_4$), and concentrated. Flash chromatography (EtOAc) gave **24** (350 mg, 64%) as a colorless oil: TLC R_f 0.26 (EtOAc); ^{1}H NMR ($CDCl_3$) δ 6.78 (m, 1H), 4.10 (m, 2H), 4.08 (d, J = 18 Hz, 1H), 3.93 (d, J = 18 Hz, 1H), 3.50 (m, 2H), 3.40 (m, 2H), 2.70 (m, 2H), 2.47 (t, J = 7 Hz, 2H), 2.33 (m, 1H), 2.05−1.00 (m, 13H), 1.46 (s, 18H).

[3(*R*)-[2-(Piperidin-4-yl)ethyl]-2-oxopiperidinyl]acetyl-β-alanine (3). A solution of **24** (0.35 g, 0.7 mmol), trifluoroacetic acid (5 mL), and CH_2Cl_2 (5 mL) was stirred at ambient temperature for 2 h. The reaction mixture was then concentrated, and the residual trifluoroacetic acid was removed azeotropically with toluene. Flash chroamtography (10:1:1 CH_3OH/NH_4OH/H_2O) gave **3** (0.16 g, 65%) as a white solid: $[\alpha]^{23}_D$ −29.8 (*c* 0.5, H_2O); TLC R_f 0.45 (10:1:1 CH_3OH/NH_4OH/H_2O); ^{1}H NMR (400 MHz, D_2O) δ 4.02 (m, 2H), 3.40 (m, 6H), 2.99 (m, 2H), 2.42 (3H), 2.05−1.30 (m, 13H); FAB-MS *m/e* 340 (M + H)$^+$. Anal. ($C_{17}H_{29}N_3O_4$·1.8H_2O) C, H, N.

[3(*S*)-[2-(Piperidin-4-yl)ethyl]-2-oxopiperidinyl]acetyl-β-alanine (3a). Compound **3a** was prepared from **18a** through the same sequence of reactions described for the preparation of **3**: $[\alpha]^{23}_D$ +29.7 (*c* 0.4, H_2O); FAB-MS *m/e* 340 (M + H)$^+$.

Single Dose Oral Administration of Crystalline L-734,217 to Conscious Dogs by Capsule: Inhibition of ex Vivo Response. Four conscious, purpose-bred mongrel dogs were administered L-734,217 orally with 1.0 mg/kg crystalline compound in gelatin capsules. During these studies, dogs rested comfortably in nylon slings. At specified time points, blood samples were drawn from either saphenous or cephalic veins into a plastic syringe containing 0.5 mL of 3.8% sodium citrate; 5 mL of blood was withdrawn for ex vivo platelet aggregation studies (described in text), with an additional 1 mL of blood drawn for the measurement of whole platelet

counts. The whole blood platelet count was determined with an automated hematology analyzer (Serono-Baker Diagnostics). For all four dogs administered L-734,217 po by capsule, blood samples were obtained before compound administration (base line) and at 20, 40, 70, 90, 150, 200, 250, 300, 350, and 1440 min after drug administration.

Single Oral Administration of L-734,217 to Sedated Chimpanzees. L-734,217 was dissolved in 5 mL of 0.9% phosphate-buffered water and filtered through 0.2 μm acrodisks prior to administration. Chimps were made to fast overnight and sedated with ketamine (10 mg/kg im), intubated, and administered 2.0 mg/kg of L-734,217 po by gavage in solution. Ketamine sedation was repeated for blood sampling. Blood samples were taken via a syringe containing 3.8% sodium citrate before dosing (5–15 min prior to dosing) and 15, 30, 45, 60, 90, 120, 180, 240, 300, 360, 420, 480, 540, and 600 min after dosing and evaluated for the extent of ex vivo platelet aggregation.

References

(1) Kieffer, N.; Phillips, D. R. Platelet Membrane Glycoproteins: Functions in Cellular Adhesion and Aggregation. *Annu. Rev. Cell Biol.* **1990**, *6*, 329–357.

(2) Bennett, J. S.; Vilaire, G. Exposure of Platelet Fibrinogen Receptors by ADP and Epinephrine. *J. Clin. Invest.* **1979**, *64*, 1393–1401.

(3) Margurie, G. A.; Plow, E. F.; Edgington, T. S. Human Platelets Possess an Inducible Saturable Receptor Specific for Fibrinogen. *J. Biol. Chem.* **1979**, *254*, 5357–5363.

(4) Sims, P. J.; Ginsberg, M. H.; Plow, E. F.; Shattil, S. J. Effect of Platelet Activation on the Conformation of the Plasma Membrane Glycoprotein IIb–IIIa Complex. *J. Biol. Chem.* **1991**, *266*, 7345–7350.

(5) Kessler, C. M. Anticoaggulation and Thrombolytic Therapy. *Chest* **1989**, *95*, 245S–255S.

(6) Fitzgerald, D. J.; Roy, L.; Catella, F.; Fitzgerald, G. A. Platelet Activation in Unstable Coronary Disease. *N. Engl. J. Med.* **1986**, *315*, 983–989.

(7) Splawinska, B.; Kuzniar, J.; Splawinski, J. Platelet Aggregation and Deaggregation in Unstable Angina and Myocardial Infarction. *Med. Actual.* **1991**, *27*, 83–106.

(8) Colman, R. W.; Marder, V. J.; Salzman, E. W.; Hirsch, J. *Hemostasis and Thrombosis: Basic Principles and Clinical Practices*; Lippincott Publishers: Philadelphia, PA.

(9) Shebuski, R. J. Interuption of Thrombosis and Hemostasis by Anti-Platelet Agents. *Tox. Pathol.* **1993**, *21*, 180–189.

(10) Ruggeri, Z. M. Receptor-Specific Antiplatelet Therapy. *Circulation* **1989**, *80*, 1920–1922.

(11) Yasuda, T.; Gold, H. K.; Fallon, J. T.; Leinbach, R. C.; Guerrero, J. L.; Scudder, L. E.; Kanke, M.; Shealy, D.; Ross, M. J.; Collen, D.; Coller, B. S. Monoclonal Antibody Against the Platelet Glycoprotein (GP) IIb/IIIa Receptor Prevents Coronary Artery Reocclusion after Reperfusion with Recombinant Tissue-Type Plasminogen Activator in Dogs. *J. Clin. Invest.* **1988**, *81*, 1284–1291.

(12) Gold, H. K.; Gimple, L.; Yasuda, T.; Leinbach, R. S.; Jordan, R.; Iuliucci, J.; Coller, B. S. Phase I Human Clinical Trials of the Potent Antiplatelet Agent 7E3 (ab')2, a Monoclonal Antibody to the GP IIb/IIIa Receptor. *Circulation* **1989**, *80* (Supplement II), 267.

(13) Pytela, R.; Pierschbacher, M. D.; Ginsberg, M. H.; Plow, E. F.; Ruoslahti, E. Platelet Membrane Glycoprotein IIb/IIIa: Member of a Family of Arg-Gly-Asp-Specific Adhesion Receptors. *Science* **1986**, *231*, 1559.

(14) Hawiger, J.; Kloczewiak, M.; Bednarek, M. A.; Timmons, S. Platelet Receptor Recognition Domains on the a Chain of Human Fibrinogen: Structure-Function Analysis. *Biochemistry* **1989**, *28*, 2909–2914.

(15) Plow, E. F.; Pierschbacher, M. D.; Ruoslathi, E.; Margurie, G. A.; Ginsberg, M. H. The Effect of Arg-Gly-Asp-containing Peptides on Fibrinogen and von Willebrand Factor Binding to Platelets. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 8057–8061.

(16) Gan, Z. R.; Gould, R. J.; Jacobs, J. W.; Friedman, P. A.; Polokoff, M. A. Echistatin. A Potent Platelet Aggregation Inhibitor From the Venom of the Viper, Echis. Carinatus. *J. Biol. Chem.* **1988**, *263*, 19827–19832.

(17) Huang, T.-F.; Holt, J. C.; Niewiarowski, S. Disintegrins: A Family of Integrin Inhibitory Proteins From Viper Venoms. *Proc. Soc. Exp. Biol. Med.* **1990**, *195*, 168–171.

(18) Scarborough, R. M.; Rose, J. W.; Naughton, M. A.; Phillips, D. R.; Nannizzi, L.; Arfsten, A.; Campbell, A. M.; Charo, I. F. Characterization of the Integrin Specificities of Disintegrins Isolated from American Pit Viper Venoms. *J. Biol. Chem.* **1993**, *268*, 1058–1065.

(19) Pierschbacher, M. D.; Ruoslahti, E. Influence of Stereochemistry of the Sequence Arg-Gly-Asp-Xaa on Binding Specificity in Cell Adhesion. *J. Biol. Chem.* **1987**, *262*, 17294–17298.

(20) Ku, T. W.; et al. Direct Design of a Potent Non-Peptide Fibrinogen Receptor Antagonist Based on the Structure and Conformation of a Highly Constrained Cyclic RGD Peptide. *J. Am. Chem. Soc.* **1993**, *115*, 8861–8862.

(21) Nutt, R. F.; Brady, S. F.; Sisko, J. T.; Ciccarone, T. M.; Colton, C. D.; Levy, M. R.; Gould, R. J.; Zhang, G.; Friedman, P. A.; Veber, D. F. Structure- and conformation-activity studies leading to potent fibrinogen receptor antagonists containing Arg-Gly-Asp. In *Peptides 1990 (proceeding of the 21st European Peptide Symposium)*; Giralt, E., Andreu, D., Eds.; ESCOM: Leiden, 1991; pp 784–786.

(22) Cheng, S.; Craig, W. S.; Mullen, D.; Tschopp, J. F.; Dixon, D.; Piersbascher, M. D. Design and Synthesis of Novel Cyclic RGD-containing Peptides as Highly Potent and Selective Integrin αIIbβ3 Antagonists. *J. Med. Chem.* **1994**, *37*, 1–9.

(23) Hartman, G. D.; Egbertson, M. S.; Halczenko, W.; Laswell, W. L.; Duggan, M. E.; Smith, R. L.; Naylor, A. M.; Manno, P. D.; Lynch, R. J.; Zhang, G.; Chang, C. T.-C.; Gould, R. J. Non-Peptide Fibrinogen Receptor Antagonists 1. Discovery and Design of Exosite Inhibitors. *J. Med. Chem.* **1992**, *35*, 4640–4642.

(24) Egbertson, M. S.; Chang, C. T.-C.; Duggan, M. E.; Gould, R. J.; Halczenko, W.; Hartman, G. D.; Laswell, W. L.; Lynch, J. J.; Lynch, R. J.; Manno, P. D.; Naylor, A. M.; Prugh, J. D.; Ramjit, D. R.; Sitko, G. R.; Smith, R. S.; Turchi, L. M.; Zhang, G. Non-Peptide Fibrinogen Receptor Antagonists. 2. Optimization of a Tyrosine Template as a Mimic for Arg-Gly-Asp. *J. Med. Chem.*, in press.

(25) Zablocki, J. A.; Miyano, M.; Garland, R. B.; Pireh, D.; Schretzman, L.; Rao, S. N.; Linmark, R. J.; Panzer-Knodle, S. G.; Nicholson, N. S.; Taite, B. B.; Salyers, A. K.; King, L. W.; Campion, J. G.; Feigen, L. P. Potent in Vitro and in Vivo Inhibitors of Platelet Aggragtion Based upon the Arg-Gly-Asp-Phe Sequence of Fibrinogen. A Proposal on the Nature of the Binding Interaction between the Arg-guanidine of RGDX Mimetics and the Platelet GP IIb–IIIa Receptor. *J. Med. Chem.* **1993**, *36*, 1811–1819.

(26) Alig, L.; Edenhofer, A.; Hadvary, P.; Hurzeler, M.; Knopp, D.; Muller, M.; Steiner, B.; Trzeciak, A.; Weller, T. Low Molecular Weight, Non-Peptide Fibrinogen Receptor Antagonists. *J. Med. Chem.* **1992**, *35*, 4393–4407.

(27) Callahan, J. F.; Bean, J. W.; Burgess, J. L.; Eggleston, D. S.; Hwang, S. M.; Kopple, K. D.; Koster, P. F.; Nichols, A.; Peishoff, C. E.; Samanen, J. M.; Vasko, J. A.; Wong, A.; Huffman, W. F. Design and Synthesis of a C_7 Mimetic for the Predicted γ-Turn Conformation Found in Several Constrained RGD Antagonists. *J. Med. Chem.* **1992**, *35*, 3970–3972.

(28) Barker, P. L.; Bullens, S.; Bunting, S.; Burdick, D. J.; Chan, K. S.; Deisher, T.; Eigenbrot, C.; Gadek, T. R.; Gantzos, R.; Lipari, M. T.; Muir, C. D.; Pitti, R. M.; Padua, A.; Quan, C.; Stanley, M.; Struble, M.; Tom, J. Y. K.; Burnier, J. P. Cyclic RGD Peptide Analogues as Antiplatelet Antithrombotics. *J. Med. Chem.* **1992**, *35*, 2040–2048.

(29) Bogusky, M. J.; Naylor, A. M.; Pitzenberger, S. M.; Nutt, R. F.; Brady, S. F.; Colton, C. D.; Sisko, J. T.; Anderson, P. S.; Veber, D. F. NMR and molecular modeling characterization of RGD containing peptides. *Int. J. Protein Res.* **1992**, *39*, 63–76.

(30) Peerlik, K.; De Lepeleire, I.; Goldberg, M.; Farrell, D.; Barrett, J.; Hand, E.; Panebianco, D.; Deckmyn, H.; Vermylen, J.; Arnout, J. MK-383 (L-700,462), a Selective Nonpeptide Platelet Glycoprotein IIb/IIIa Antagonist, Is Active in Man. *Circulation* **1993**, *88*, 1512–1517.

(31) Evans, D. A.; Urpi, F.; Somers, T. C.; Clark, J. S.; Bilodeau, M. T. New Procedure for the Direct Generation of Titanium Enolates. Diastereoselective Bond Constructions with Representative Electrophiles. *J. Am. Chem. Soc.* **1990**, *112*, 8215–8216.

(32) *AMF·The Advanced Modeling Facility*, Version 2.0; Molecular Systems Department, Merck Research Laboratories, a division of Merck & Co., Inc.: October 1992.

(33) *JIGGLE·A Distance Geometry Algorithm*; Molecular Systems Department, Merck Research Laboratories, a division of Merck & Co., Inc.: April 1993.

(34) The MMFF force field used in this work is implemented in $CHARM_m$, available from Molecular Simulation, Inc., Burlington, MA.

(35) *C_View-Molecular Visualization*; Molecular Systems Department, Merck Research Laboratories, a division of Merck & Co., Inc.: April 1993.

(36) Albeida, S. M.; Daise, M.; Levine, E. M.; Buck, C. A. Identification and Characterization of cell·stratum adhesion receptors on cultured human endothelial cells. *J. Clin. Invest.* **1989**, *83*, 1992–2002.

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