Potent *in Vitro* and *in Vivo* Inhibitors of Platelet Aggregation 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

Jeffery A. Zablocki,*,† Masateru Miyano,† Robert B. Garland,† Daisy Pireh,† Lori Schretzman,† Shashidhar N. Rao,‡ Richard J. Lindmark,§ Susan G. Panzer-Knodle,‡ Nancy S. Nicholson,‡ Beatrice B. Taite,‡ Anita K. Salyers,‡ Lucy W. King,‡ James G. Campion,‡ and Larry P. Feigen‡

Departments of Medicinal Chemistry and Pharmacology, Searle Research & Development, 4901 Searle Parkway, Skokie, Illinois 60077, and Department of Chemistry, Monsanto Central Research, 700 Chesterfield Village Parkway, St. Louis, Missouri 63198

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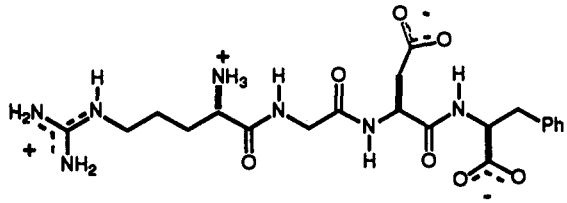
Peptide mimetics of the RGDF sequence in which Arg-Gly has been replaced with 5-(4-amidinophenyl)pentanoyl mimetic has led to a 1000-fold increase in inhibitory potency over the natural RGDF ligand. The guanidine residue of the arginine may be involved in a reinforced ionic interaction with a carboxylate of the receptor which could explain the dramatic increase in potency upon replacement with benzamidine. This hypothesis is supported by the observation of low inhibitory potency of the corresponding benzylamine (18) and no activity with the corresponding imidazoline derivative (19); plus, *ab initio* calculations on the respective complexes suggest that the benzamidine–carboxylate is more favorable than the guanidine–carboxylate interaction. The ED_{50} for the inhibition of *ex vivo* collagen induced platelet aggregation in the dog for SC-52012 (1) was 0.32 μg/kg/min by iv infusion with a pharmacodynamic half-life for recovery of approximately 40 min.

Introduction

The tripeptide sequence Arg-Gly-Asp (RGD) found in the α chain of fibrinogen (RGDF 95–98, RGDS 572–575) is a key recognition domain for the platelet membrane protein, glycoprotein IIb-IIIa (GP IIb-IIIa).[1-3] It is now well documented that tetrapeptides containing the RGD sequence are capable of effectively inhibiting the binding of fibrinogen to the GP IIb-IIIa receptor.[4-7] The competitive inhibition of fibrinogen binding prevents platelet aggregation and subsequent white thrombus formation which can lead to arterial occlusion. The RGDX antiplatelet agents show promise for improving the immediate treatment of a myocardial infarct (MI) when used in combination therapy with a fibrinolytic agent (e.g. r-tissue type plasminogen activator [rt-TPA] or streptokinase [SK]).[8a]



RGDF
IC_{50} = 90 μM

7E3 is an antibody directed against the platelet fibrinogen receptor which inhibits platelet aggregation and is currently in phase III clinical trials for the treatment of MI.[8b] The encouraging preliminary findings with this antibody have accelerated efforts to obtain a low molecular weight peptide mimetic based inhibitor. Through the process of maximizing receptor–RGDX pharmacophore interactions, we arrived at SC-52012 (1) which is our leading candidate as an iv antiplatelet agent. Similarily, scientists at Merck[8c] and Hoffmann-LaRoche[8d] have recently disclosed their efforts in the antiplatelet area which have also resulted in linear potent inhibitors of platelet aggregation affording inhibitors exemplified by 2 and 3 (Figure 1).

Previously, we had shown that the inherent inhibitory potency of Arg-Gly-Asp-Phe [RGDF, 4][6] for disrupting the fibrinogen–GP IIb-IIIa interaction can be enhanced 15-fold by removing the Arg-NH_2 and the Arg-Gly amide bond to obtain 8-guanidinooctanoyl-Asp-Phe [GOA-Asp-Phe, 5].[9] Furthermore, we have demonstrated that the Phe-carboxylate can be replaced with tetrazole, compound 6, without a loss in inhibitory potency; however, replacing the Asp-carboxylate with tetrazole, compound 7, completely abolishes activity.[10] Herein, we describe our work probing the nature of the remaining key pharmacophore of compound 5, the guanidine–receptor interaction which ultimately led to the selection of 1 as an iv antiplatelet agent suitable for development.

Guanidine can interact with the receptor in three common modes: hydrogen bonding, simple ionic, and reinforced ionic (Figure 2).[11a,11b] The derivative 8 in which the guanidine of 5 is replaced with urea was prepared to test the hydrogen bonding mode, and it was found to be completely inactive. Based upon Markwardt and coworkers' success in utilizing *p*-amidinophenylalanine as an effective arginine mimic in the preparation of thrombin inhibitors,[12a] we decided to incorporate benzamidine into our RGDF antiplatelet agents.[12b,c] Intuitively benzamidine was an ideal choice to test the ionic and reinforced ionic mode of guanidine–receptor interaction for the following reasons: the benzamidine has the charge localized on two nitrogens, as opposed to three, allowing for

† Department of Medicinal Chemistry, Searle Research & Development.
‡ Department of Pharmacology, Searle Research & Development.
§ Department of Chemistry, Monsanto Central Research.

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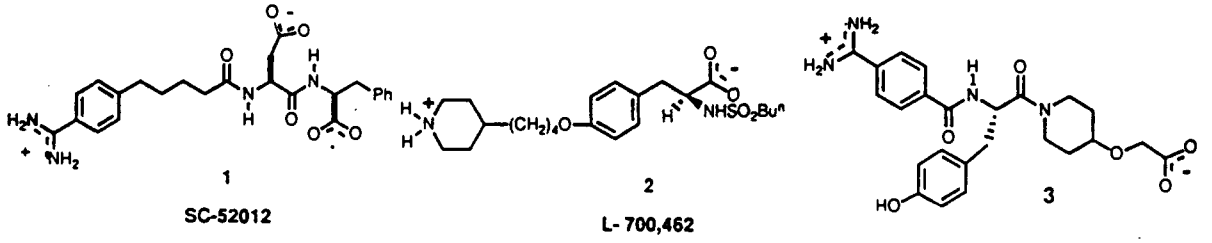


1 SC-52012 2 L-700,462 3

Figure 1. Linear RGD-based antiplatelet agents of Searle (1), Merck (2), and Hoffmann LaRoche (3).

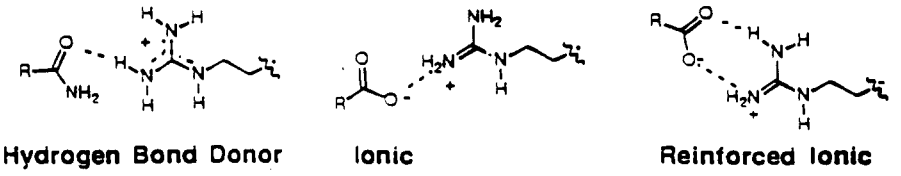
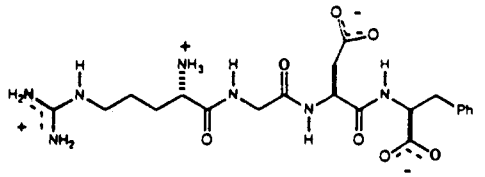


Figure 2. The three common modes of guanidine–receptor interactions and their associated binding energy.[13]



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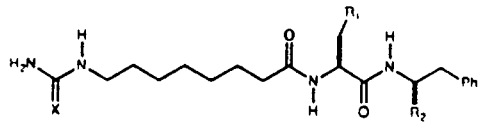


5 R_1 R_2 = CO_2^- X = NH

6 R_1 = CO_2^- R_2 = Tetrazole X = NH

7 R_1 = Tetrazole R_2 = CO_2^- X = NH

8 R_1 = CO_2^- R_2 = CO_2^- X = O

more favorable electrostatic interactions with a negatively charged receptor site (Figure 3); the reinforced ionic interaction provides a favorable alignment of dipole moments, as illustrated in Figure 3, which is likely to be more favorable with benzamidine (7.1 Debye) as opposed to guanidine (3.4 Debye).[13] Therefore, a series of benzamidine derivatives was prepared in order to optimize the presentation of the amidine moiety to the receptor.

Chemistry

The novel platelet aggregation inhibitors can be prepared following the general synthetic sequence as outlined in Scheme I. The amide bonds were prepared using standard coupling reagents, e.g. 1,3-dicyclohexylcarbodiimide (DCC). The benzonitrile was converted to the amidine via the thioimidate in nearly quantitative yield. The thioimidate was formed by first treating the benzonitrile with hydrogen sulfide (H_2S) followed by alkylation with methyl iodide. Next, treatment of the thioimidate with ammonium acetate affords the benzamidine as the salt (HI). The final compounds for biological testing were obtained by purification by reverse-phase high-pressure liquid chromatography.[14]

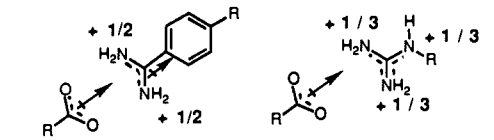
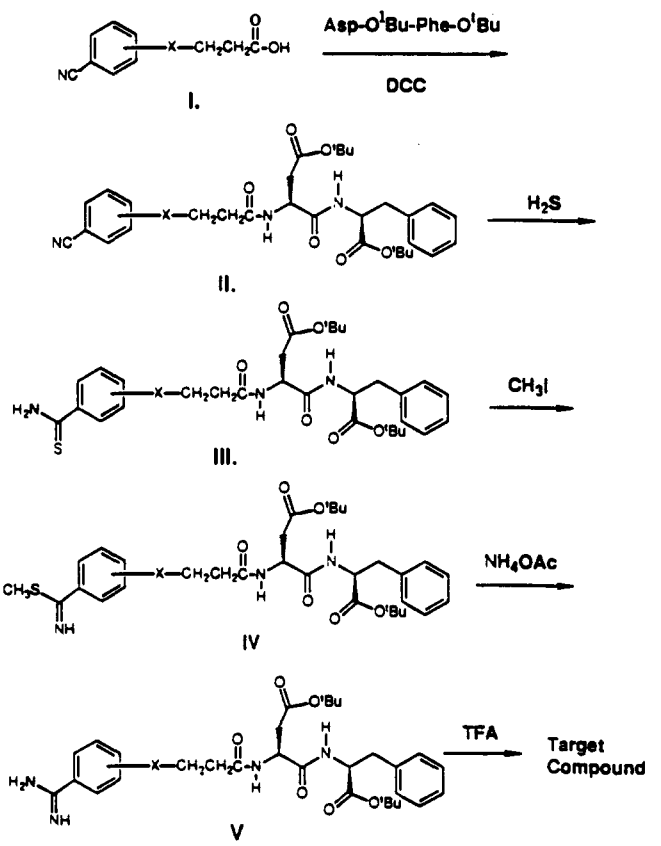


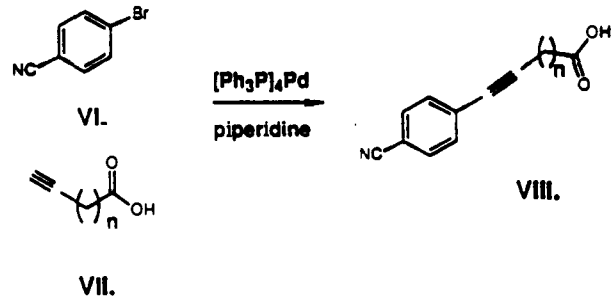
Figure 3. Charge and dipole moment. The reinforced ionic interaction has a favorable alignment of dipole moments whose interaction is enhanced with the benzamidine vs the guanidine.

Scheme I

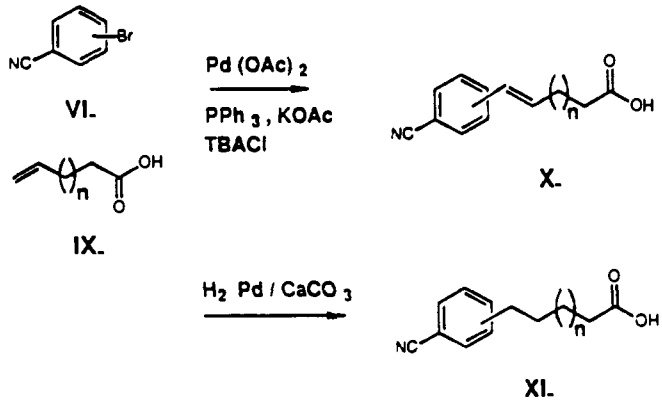


The benzonitrile acid of Scheme I where X = alkenyl, alkynyl, alkyl, alkyl carbonyl, or alkyl hydroxy having 2–4 carbon atoms can be prepared in the following manner: The halobenzonitrile is coupled to an ω alkenoic or alkynoic acid using a palladium(0)-based coupling reaction.[15] The conditions for the palladium coupling reaction differed for the alkynoic acid and the alkenoic acid coupling

Scheme II



Scheme III



components. For the alkynoic acid the conditions for the palladium coupling reaction utilized tetrakis(triphenylphosphine)palladium(0) as catalyst and piperidine as the solvent (Scheme II).[16] The conditions for the alkenoic acid coupling component utilized the phase-transfer conditions of Jeffery and Larock (Scheme III).[17] These conditions [phase-transfer agent, tetrabutylammonium salt; catalyst, palladium(II) acetate; base, potassium acetate; solvent, dimethylformamide] are extremely mild conditions which afforded a good yield of coupled olefin. Compounds where X = alkyl were obtained through a selective reduction of the double bond by catalytic reduction over palladium on calcium carbonate. Interestingly, when the phase-transfer conditions of Jeffery and Larock were used with the alkynoic acid coupling component, an enol–lactone was isolated in good yield (Scheme IV).[18] The enol–lactone can be directly coupled to the dipeptide or dipeptide mimetic by refluxing in acetonitrile to afford X = alkyl carbonyl and alkyl hydroxy derivatives (after reduction, Scheme IV).

Results and Discussion

***In Vitro* Pharmacology.** The inhibition of ADP-induced platelet aggregation by the benzamidine-based RGDX peptide mimetics was measured in canine platelet-rich plasma (PRP),[4,6] and the results are summarized in Tables I and II. In the alkyl series (**9**, **10**, **1**), the pentanoyl spacer was optimum, affording an extremely potent inhibitor of platelet aggregation, compound **1** (Table I). In fact, **1** is more potent than the snake venom echistatin which is a protein (49 amino acids) containing an RGD sequence and multiple disulfide bonds that has served as a landmark in terms of potency for inhibitors of platelet aggregation.[19] The ketomethylene (**11**) and hydroxyethylene (**12**) isosteres of **1** which correspond to the natural Arg-Gly amide bond were found to be inferior to the ethylene isostere, **1**; however, the 4-pentenyl (**13**) and 4-pentynyl (**14**) derivatives provided good inhibition of

Scheme IV

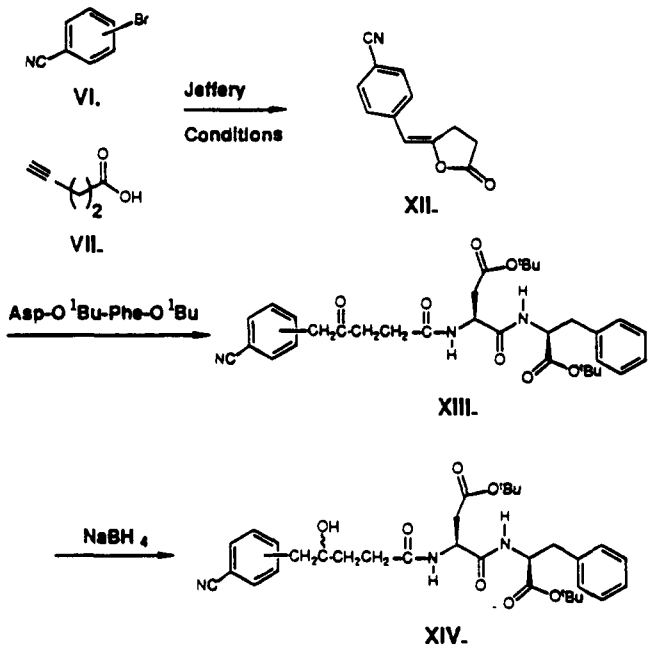
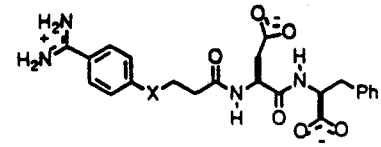
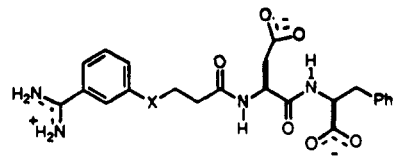


Table I. *In Vitro* Biological Evaluation of *p*-Amidinophenyl-Based RGDX Mimetics



compound	X	dog PRP IC_{50} (nM)	human PRP IC_{50} (nM)
9	CH_2	inactive	
1	CH_2CH_2	53	42
10	$CH_2CH_2CH_2$	1100	
11	CH_2CO	180	
12	CH_2CHOH	50000	
13	t-CH=CH	82	90
14	C≡C	90	120
echistatin			80

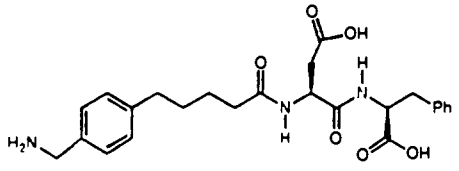
Table II. *In Vitro* Biological Evaluation of *m*-Amidinophenyl-Based RGDX Mimetics



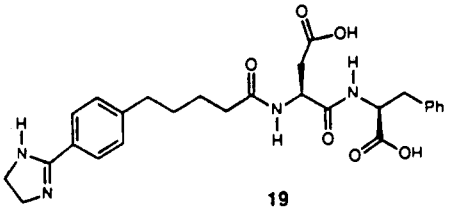
compound	X	dog PRP IC_{50} (nM)	human PRP IC_{50} (nM)
15	CH_2CH_2	50000	
16	$CH_2CH_2CH_2$	5800	
17	c-CH=$CHCH_2$	4300	3200

platelet aggregation. The alkyl (**15–16**) and *cis*-alkenyl (**17**) *m*-benzamidine derivatives were found to be dramatically less potent than the *p*-amidino series (Table II).

After optimization of the inhibition of platelet aggregation in the benzamidine series, we turned to answer the original question of probing the nature of the guanidine–receptor interaction. Specifically, two compounds were prepared to determine whether the simple ionic or reinforced ionic mode of interaction was operative for this particular RGDX series.[20] The benzylamine derivative **18** (Figure 4) was used as a test of the simple ionic mode,



18



19

Figure 4. Support for reinforced ionic mode of binding: 18 has low activity and 19 is inactive.

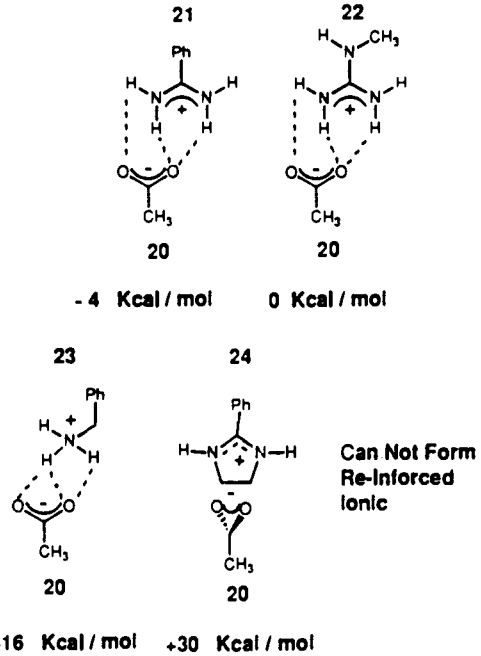


Figure 5. Relative energies of complexes based on *ab initio* calculations.

and it was found to have extremely low inhibitory potency (IC_{50} = 2000 nM). The imidazoline derivative **19** (Figure 4) was used as a negative test for the reinforced ionic interaction, since the required planar array of dual hydrogen bonds (Figure 2) is disrupted by the intervening ethylene bridge. The imidazoline derivative **19** was completely inactive. Therefore, the dramatic increase in potency of **1** relative to the guanidine derivative **5** coupled with these results supports a reinforced ionic interaction with the receptor for this particular series.

To lend further support for this hypothesis, the energies of the benzamidine–acetate complex, the guanidine–acetate, the benzylammonium–acetate, and the benzimidazoline–acetate complexes were calculated using the truncated model systems shown in Figure 5 using semiempirical gas-phase methods for all of the complexes as described in the experimental section; plus, a more rigorous molecular dynamics method with the inclusion of solvent was applied specifically to the benzamidine–acetate and guanidine–acetate complexes. In the gas-phase calculations, the benzamidine–acetate complex was lower in energy than the methylguanidine–acetate complex by 4 kcal/mol. As expected, the benzylammonium–acetate complex is intermediate in energy, and the imidazoline–acetate complex is energetically much less favorable. Inclusion of solvent using molecular dynamic methods for the benzamidine–acetate and the guanidine–acetate complexes resulted in a similar energy difference of 3.5 ± 1.2 kcal/mol (see the Experimental Section).

The lower energy of the benzamidine–acetate complex versus the guanidine–acetate complex is consistent with the increase in platelet inhibition observed by benzamidine **1** versus alkylguanidine **5** (42 versus 1600 nM, respectively, human PRP). A more accurate measure of each of these specific compounds affinity for the receptor was obtained by determining their inhibition of [^{125}I]fibrinogen binding (K_D = 120 nM) to platelets. Benzamidine **1** was 100 times more potent than alkylguanidine **5** with IC_{50}'s of 8 and 800 nM, respectively. Applying the Gibbs–Helmholtz equation for binding of biologically active peptides as previously described by Farmer,[11a,22] one obtains a difference in the free energy of binding between **1** and **5** of 3.0 ± 0.1 kcal/mol as illustrated below.

$$\Delta G^\circ = -RT \ln K_i$$

$$(5)\Delta G^\circ = -(1.987)(310\ \text{K}) \ln (6.8 \times 10^{-8}) = 10.2\ \text{kcal/mol}$$

$$(1)\Delta G^\circ = -(1.987)(310\ \text{K}) \ln (8.3 \times 10^{-6}) = 7.2\ \text{kcal/mol}$$

Therefore: $(5)\Delta G^\circ - (1)\Delta G^\circ = 3.0 \pm 0.1$ kcal/mol

Thus, the calculated free energy of binding difference between the benzamidine–acetate complex versus the guanidine–acetate complex is in good agreement with the observed difference in biological affinities of inhibitors **1** and **5**. Williams has recently commented on the importance of rigidification and the effect of removing degrees of freedom on improving the apparent affinity of ligands for their receptors by decreasing the entropy term of $\Delta G^\circ = \Delta H - T\Delta S$.[11b] Kouns and co-workers of LaRoche have noted that the benzamidine removes degrees of freedom relative to the alkylguanidine of arginine which may *contribute* to the increase in observed affinity.[23] Our calculations suggest that enthalpy changes are likely to be the major contributor to the observed energy differences which are the result of more favorable electrostatic interactions with the receptor.

In a very elegant study, D'Souza and co-workers found that the region containing the second calcium binding site of GP IIb, residues 296–306 (TDVNGDGRHDL), is implicated in the ligand binding function of GP IIb-IIIa.[24] It is noteworthy that through analysis of similar EF hand calcium binding sites, such as parvalbumin,[25] it is likely that one of the carboxylates of the second calcium binding site of GP IIb is free for ligand interaction with a guanidine or benzamidine.

***In Vivo* Pharmacology of 1.** The dramatic impovement in the *in vitro* potency of the benzamidinylpentanoyl derivative **1** versus the 8-guanidinyloctanoic acid derivative **5** was also observed *in vivo* in the dog where the inhibition of collagen-induced *ex vivo* platelet aggregation was measured. Both compounds were infused to achieve an approximate steady state (**1**, 4 h; **5**, 2 h), and the ED_{50} of *ex vivo* collagen-induced platelet aggregation of **1** was 0.32 μg/kg/min versus 30 μg/kg/min for **5**. A short half-lived compound may be desirable for an iv antiplatelet agent, since platelet recovery may be required in the event of a surgical emergency. The pharmocodynamic half-life for

recovery of compound 1 was estimated to be 40 min while that of compound 5 was 29 min. The dramatic improvement in potency of compound 1 offset the slightly longer half-life; thus, compound 1 was chosen for development.

Conclusion

Benzamidine was found to be an excellent guanidine surrogate for the RGDX antiplatelet agents. In particular, compound 1, 5-(*p*-amidinophenyl)pentanoyl-Asp-Phe, was found to be an extremely potent inhibitor of platelet aggregation *in vitro*. The nature of the dramatic increase in inhibitory potency is postulated to involve a reinforced ionic interaction with a carboxylate of the receptor which has been demonstrated to be energetically more favorable than a guanidine–carboxylate interaction based on both gas-phase semiempirical and solvent inclusion molecular dynamic simulations of the energy differences between the respective complexes. Compound 1 was determined to be extremely potent against collagen-induced *ex vivo* platelet aggregation when administered iv in dogs. Further promising *in vivo* pharmacology including the results of evaluation of 1 in a canine model of a myocardial infarct will be published elsewhere.

Experimental Section

Pharmacology. *In Vitro* Inhibition of Collagen-Induced Dog Platelet Aggregation. The *in vitro* platelet aggregation assay using canine platelet-rich plasma was done in the following manner:[6] 30 mL of whole blood was collected using a butterfly needle and a 30-cm³ plastic syringe with 3 mL of 0.129 M buffered sodium citrate (3.8%). The syringe was rotated carefully as blood was drawn to mix the citrate. Platelet-rich plasma (PRP) was prepared by centrifugation at 975*g* for 3.17 min at room temperature. The PRP was removed from the blood with a plastic pipette and placed in a plastic-capped, 50-mL Corning conical sterile centrifuge tube which was held at room temperature. Platelet-poor plasma (PPP) was prepared by centrifuging at 2000*g* for 15 min at room temperature, allowing the centrifuge to coast to a stop without braking. The PRP was adjusted with PPP to a count of (2–3) × 10^8 platelets/mL. Four hundred microliters of the PRP preparation and 50 μL of the compound to be tested, or saline, were preincubated for 2 min at 37 °C in an aggregometer (PAP-4C, Biodata, Hatboro, PA); 50 μL of collagen (50 μL of a 100 mg/mL, final concentration 10 μg/mL) was added, and the aggregation was monitored for 4 min. All of the compounds were tested in duplicate. Results were calculated as follows: [observed percent aggregation (inhibitor)] divided by [maximum percent aggregation (control)] equals the percent of control. The percent inhibition = 100 − (percent of control).

Fibrinogen Binding Assay. Fibrinogen binding was performed essentially as described by Plow.[4] Briefly, blood from humans who had not been given any antiplatelet drugs in the previous 2 weeks was collected into $^1/_{10}$ volume of CCD buffer (100 mM sodium citrate, 136 mM glucose, pH 6.5). The blood was centrifuged for 3 min at 1000*g*, and platelet-rich plasma was transferred to a 50-mL centrifugation tube and placed on ice. After 15 min, $^1/_2$ volume of ice-cold CCD buffer was added, and the sample was centrifuged at 900*g* for 10 min at 2 °C. The supernatant was decanted, and the platelet pellet was gently resuspended in half the original volume of ice-cold modified Tyrode's buffer (137 mM NaCl, 2.6 mM KCl, 12 mM $NaHCO_3$, 5.5 mM glucose, 15 mM HEPES, 0.5% bovine serum albumin, pH 7.4). After incubation for 30 min at 37 °C, the platelet count was adjusted to 4 × 10^8 platelets/mL with modified Tyrode's buffer. To test samples (final concentration = 1 × 10^8 platelets/mL) were added in sequence to achieve the final concentration noted in a volume of 200 μL: ADP (10 μM), $CaCl_2$ (2 mM), test compound, and [^{125}I]fibrinogen (0.3 μM). The samples were incubated for 40 min at 22 °C, and 50-μL aliquots were centrifuged at 8000*g* through a 20% sucrose solution (300 μL). The tubes were quick frozen, and the tips containing the platelet pellet were cut and assayed for bound [^{125}I]fibrinogen by γ scintillation counting. Specific binding was determined in each test by subtracting from the total binding the amount of [^{125}I]fibrinogen bound in the presence of a 60-fold excess of unlabeled fibrinogen. The potency of test compounds (IC_{50}) was determined as the concentration of compound to inhibit 50% of [^{125}I]fibrinogen binding.

The K_i's were determined using a competitive binding assay as described above, but substitituting ^{3}H-1 for [^{125}I]fibrinogen which afforded the IC_{50} of 1 and 5. The K_i's were determined using the Cheng/Prusoff equation $K_i = IC_{50}/([^3H\text{-}1]/K_D + 1)$ where K_D of 1 equals 31 ± 5 nM and [^{3}H-1] equals 100 nM. The K_D of 1 was determined by Scatchard analysis.

Inhibition of *ex Vivo* Collagen-Induced Canine Platelet Aggregation. Dogs (Beagle, obtained from White Eagle, Doylestown, PA; 7–12 kg, $n = 3$) were anesthetized with sodium pentobarbital [65 mg/mL]. Animals were intubated and allowed to breathe room air unassisted. Dogs were maintained on a heating pad (37 °C). A lateral saphenous vein was cannulated with a 18 g × 2-in. catheter for compound infusion. A small cut was made over the left jugular vein, and the vein was cannulated with a polyethylene catheter (PE-240) fitted with the appropriate size needle adapter and a three way plastic stopcock. Blood samples were withdrawn through this catheter using the two syringe technique. The blood was centrifuged (TechnoSpin R centrifuge, Sorvall Instruments, Wilmington, DE) at 266*g* for 6 min to prepare platelet-rich plasma (PRP). After the PRP was removed, the remaining blood was centrifuged (Eppendorf centrifuge, Westbury, NY) for 2 min at 12000*g* to prepare platelet-poor plasma (PPP) for calibration of the aggregometer (PAP 4, BioData, Hatboro, PA.). PRP (450 μL) was tested for aggregatory response to collagen (50 μL of a 100 mg/mL, final concentration 10 μg/mL) (aggregation monitored for 4 min). A minimum of four control samples were tested to establish a baseline control aggregation.

A concentrated stock solution of compound 5 (50 mg/mL) was prepared in distilled water and adjusted to pH = 9.0 with 0.5 M Na_2CO_3. Working solutions were prepared by diluting an aliquot from the stock solution with saline to achieve the desired concentration for each dose. Compound 5 solutions (15–80 μg/kg/min) were infused iv at a rate of 0.15 mL/min [Harvard infusion pump, Model 22] for 2 h. Blood samples were taken at 15-min intervals during the infusion, and PRP was prepared. The PRP was tested for aggregation. Inhibition of aggregation was calculated by comparing the aggregation response during the infusion with the average response during the control period. Steady state was visually determined from a graph of the responses over time and determined to be achieved after 45 min of infusion. Platelet responses from 45 to 120 min were averaged and plotted in a dose response curve. The ED_{50} was calculated by linear regression of the dose response data. After 2 h the infusion was terminated, and the aggregation was monitored periodically until the platelet aggregatory response returned to control levels.

Compound 1 was infused intravenously into dogs for 4 h in doses ranging from 0.15 μg/kg/min to 0.5 μg/kg/min. Blood samples were collected at selected time intervals during and after infusion, and platelet-rich plasma (PRP) was prepared and tested for *ex vivo* inhibition of collagen-induced aggregation as described above.

Theoretical Studies. The energies of the benzamidine–acetate, guanidine–acetate, benzylammonium–acetate, and benzimidazoline–acetate complexes were calculated using the truncated model systems shown in Figure 5. Models of acetate, benzamidine, methylguanidine, benzylammonium, and benzimidazoline were built and optimized using MacroModel MM2 executing on a Personal Iris 4D/35. Their energies were evaluated using the *ab initio* program GAUSSIAN92 with a 6-311G**/RHF basis set. The above complexes were also model built and optimized using MacroModel MM2, and the refined structures were then subjected to *ab initio* calculations with a 6-311G**/RHF basis set. During the *ab initio* calculations the geometries were kept fixed at those obtained by molecular mechanics. The energies of interaction between the anionic acetate on one hand and a cation on the other was calculated by subtracting the sum of individual energies from the total energy. Since the goal of the computational studies is to determine *relative* rather than

absolute energies of binding of acetate to any two cations, the following sets of equations were employed:

$$E_{Ace} + E_{Cat1} + E_{Ace.Cat1} = E_{Compl} \quad (1)$$

$$E_{Ace} + E_{Cat2} + E_{Ace.Cat2} = E_{Comp2} \quad (2)$$

where E_{Ace}, $E_{Cat1/2}$, $E_{Ace.Cat1/2}$, and $E_{Compl/2}$ are the energies of acetate, cation, their interactions, and the total complex, respectively. Subtracting eq 2 from eq 1 gives

$$E_{Ace.Cat1} - E_{Ace.Cat2} = E_{Compl} - E_{Comp2} - E_{cat2} + E_{cat1} \quad (3)$$

If ($E_{Ace.Cat1} - E_{Ace.Cat2}$) is negative, then the interactions between Cat1 and acetate are favored compared to those between Cat2 and acetate.

In addition to the gas-phase energy calculations, simulations were also carried out on the acetate–benzamidine and acetate–methylguanidine complexes with explicit solvent environment using molecular dynamics and free energy perturbation methods in AMBER (v. 4.0[26]). The goal of these calculations was to determine if the presence of explicit solvent environment alters the energetic preferences observed in the gas-phase calculations.

Parameters were developed for the benzamidine, acetate, and methylguanidine moieties consistent with the protocol prescribed in the AMBER force field. The electrostatic charges were obtained by fitting with the electrostatic potentials obtained using the *ab initio* program at the 6-31+G* basis set, following the Merz–Kollman–Singh (POP=MK) method and are available upon request. The bond length, angle, and torsional parameters for the amidine, guanidine, and acetate moieties were taken from the analogous systems in amino acids (e.g. arginine, aspartic acid). The MacroModel built complexes (described above) were surrounded by a shell of about 450 TIP3P water molecules,[27] and the solvated complexes were energy optimized until a root mean square gradient of 0.1 or less was achieved. This was followed by molecular dynamics equilibration to 300 K over a period of 10 ps with a nonbonded cutoff of 12 Å.

In order to estimate the relative binding interactions of acetate with benzamidine and methylguanidine, the acetate molecule was "disappeared" from the two solvated complexes using the free energy perturbation simulations. This was achieved over a simulation time of 80 ps using the window method. The disappearance was carried out over 20 windows with 4000 steps of equilibration and 4000 steps data collection coupled to a step size of 0.0005 ps. The free energy contributions from changes in bond lengths, bond angles, dihedral angles, and intramolecular 1–4 nonbonded interactions were included. Simulations were done in both the forward and backward directions employing electrostatic decoupling, and the variations about their averages were found to be around 1.2 kcal/mol.

Chemistry. High-field ^{1}H NMR and ^{13}C NMR were recorded on a GE QE-300 spectrometer at 300 and 75 MHz, respectively. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Microanalyses were performed at the Searle Physical Methodology Department. Final compounds purified by reverse-phase HPLC using a Waters LC-3000 and a Waters C-18 column (5 × 30 cm) using a linear gradient (5% CH_3CN/0.05% TFA in H_2O to 40% CH_3CN/0.05% TFA in H_2O) over 30 min.

5-(*p*-Amidinophenyl)pentanoyl-(*S*)-aspartyl-(*S*)-phenylalanine (1). General Procedure. Section A. 5-(*p*-Cyanophenyl)-4-pentenoic Acid (25). Tetrabutylammonium chloride (hydrate, 17.8 g) was dried by azeotroping with benzene (250-mL round-bottom flask equipped with a Dean–Stark apparatus). The benzene was removed *in vacuo* affording anhydrous tetrabutylammonium chloride (17.0 g, 61.2 mmol). To this flask under argon were added triphenylphosphine (820 mg, 3.13 mmol), palladium acetate (703 mg, 3.13 mmol), 4-bromobenzonitrile (16.9 g, 92.8 mmol), potassium acetate (36.8 g, 375 mmol), and 100 mL of degassed anhydrous dimethylformamide (degassed by bubbling argon through for 10 min, dried over molecular sieves). A solution of 4-pentenoic acid (6.27 g, 62.6 mmol) and degassed anhydrous DMF (35 mL) was then added to the rapidly stirring reaction mixture at 23 °C. After 21 h at 23 °C, the reaction mixture was poured slowly into a sodium carbonate solution (3%, 400 mL) and extracted with ethyl acetate (500 mL). The aqueous layer was treated with decolorizing carbon and filtered. Then, the aqueous layer was acidified to a pH of 2 with 10% HCl which afforded a white solid (6.82 g, 54%): mp 150–167 °C.

The above procedure affords **25** in sufficient purity to move on to the next step without complications. An analytical sample was obtained by submitting the sample to further puification by flash chromatography (ethyl acetate–methylene chloride–acetic acid, 1:4:0.05) and recrystallization from ethyl acetate (2 times): mp 154–156 °C; ^{1}H NMR (CD_3OD) δ 2.20–2.35 (m, 4H), 6.23–6.35 (m, 2H), 7.34 (d, J = 7 Hz, PhH), 7.54 (d, J = 7 Hz); ^{13}C NMR (CD_3OD) δ 28.0, 33.1, 109.3, 119.0, 128.9, 129.5, 132.6, 133.9, 141.9, 173.8. Anal. ($C_{12}H_{11}NO_2$) C, H, N.

Section B. 5-(*p*-Cyanophenyl)pentanoic Acid (26). A solution of 1.47 g (7.32 mmol) of **18** in 90 mL of methanol was hydrogenated over 200 mg of 5% Pd/$CaCO_3$ at 5 psi of hydrogen over a 1.2-h period. After removing the catalyst by filtration and evaporation of the solvent *in vacuo*, the residue was triturated with ether followed by hexane which afforded **26** as a white solid: mp 101–102 °C; ^{1}H NMR (CD_3OD) δ 1.52–1.69 (m, 4H), 2.28 (t, J = 7 Hz, CH_2), 2.69 (t, J = 7 Hz, CH_2), 7.42 (d, J = 7 Hz, PhH), 7.75 (d, J = 7 Hz); ^{13}C NMR (CD_3OD) δ 24.9, 30.7, 34.3, 35.7, 109.5, 119.9, 130.2, 133.0, 149.0, 175.2. Anal. ($C_{12}H_{13}NO_2$) C, H, N.

Section C. 5-(*p*-Cyanophenyl)pentanoyl-(*S*)-Aspartyl(*O*-*tert*-butyl)-(*S*)-phenylalanine(*O*-*tert*-butyl) (27). To a solution of 650 mg (3.20 mmol) of **26** in 30 mL of methylene chloride at 23 °C was added 727 mg (3.52 mmol) of *N,N*-dicyclohexylcarbodiimide, followed immmediately by 1.26 g (3.20 mmol) of (*S*)-aspartyl(*O*-*tert*-butyl)-(*S*)-phenylalanine(*O*-*tert*-butyl) (**28**). The mixture was stirred for 20 h under an argon atmosphere. After dilution with ether (100 mL), the solids were removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was dissolved in ethyl acetate (300 mL), washed with $KHSO_4$ (1 N, 1 × 80 mL), saturated $KHCO_3$ (1 × 80 mL), and brine (1 × 80 mL), and dried (Na_2SO_4). Purification by flash chromatography (gradient 1 L of ethyl acetate–hexane (3:7) followed by 1.5 L of ethyl acetate–hexane (1:1)) afforded 1.48 g (80%) of **27** as an oil: ^{1}H NMR (CD_3OD) δ 1.30 and 1.42 (2 s, $C(CH_3)_3$), 1.55–1.63 (m, 4H), 2.28 (t, J = 7 Hz, CH_2), 2.51–2.82 (m, CH_2), 2.93–3.08 (m, 2-CH_2), 4.57 (m, CH), 4.73 (m, CH), 7.10–7.22 (m, PhH), 7.43 (d, J = 7 Hz, PhH); ^{13}C NMR (CD_3OD) δ 24.6, 27.5, 27.6, 30.0, 35.4, 35.6, 36.7, 37.5, 48.9, 53.6, 81.2, 81.8, 109.2, 118.7, 126.5, 127.9, 128.9, 129.2, 131.8, 135.8, 147.5, 169.5, 169.8, 170.7, 172.2.

Section D. 5-(*p*-Amidinophenyl)pentanoyl-(*S*)-aspartyl-(*S*)-phenylalanine (1). Hydrogen sulfide was bubbled through a solution of 740 mg (1.28 mmol) of **27** in pyridine–triethylamine (12 mL:1.2 mL) for 3 min at 23 °C (caution: hydrogen sulfide is highly toxic, and the reaction should be done in a hood). After 24 h at 23 °C in an enclosed flask, the reaction mixture was concentrated under a steady stream of nitrogen in the hood (caution: hydrogen sulfide is highly toxic). The residue was diluted with ethyl acetate (200 mL), washed with $KHSO_4$ (2 N, 2 × 50 mL) and brine (1 × 50 mL), and dried (Na_2SO_4). Concentration *in vacuo* afforded a quantitative yield of thioamide **29**: ^{1}H NMR ($CDCl_3$) δ 1.30 and 1.42 (2 s, $C(CH_3)_3$), 1.55–1.63 (m, 4H), 2.28 (t, J = 7 Hz, CH_2), 2.51–2.82 (m, CH_2), 2.93–3.08 (m, 2-CH_2), 4.57 (m, CH), 4.73 (m, CH), 7.10–7.22 (m, PhH), 7.72 (d, J = 7 Hz, PhH), 8.21 and 8.52 (2 s, NH_2); ^{13}C NMR ($CDCl_3$) δ 25.0, 27.5, 27.8, 30.4, 35.3, 35.9, 37.1, 37.8, 49.4, 54.1, 81.7, 82.2, 126.8, 127.4, 128.1, 129.5, 136.0, 136.6, 146.4, 170.0, 170.5, 170.7, 173.2, 201.6.

Thioamide **29** (690 mg, 1.13 mmol) was dissolved in a solution of acetone–iodomethane (14 mL:1 mL). The reaction mixture was warmed to achieve reflux for 25 min. Concentration *in vacuo* afforded a quantitative yield of thioimidate **30** as the HI salt.

A solution of **30** (705 mg, 1.13 mmol) and ammonium acetate (130 mg, 1.69 mmol) in methanol (10 mL) was warmed to achieve reflux for 3.5 h. After cooling to 23 °C, the reaction mixture was concentrated under a steady stream of nitrogen in the hood which afforded a quantitative yield of **31**: ^{1}H NMR (CD_3OD) δ 1.38 and 1.42 (2 s, $C(CH_3)_3$), 1.55–1.63 (m, 4H), 2.28 (t, J = 7 Hz, CH_2), 2.51–2.82 (m, CH_2), 2.72 (t, J = 7 Hz, CH_2), 2.93–3.08 (m, CH_2), 4.48 (m, CH), 4.73 (m, CH), 7.12–7.39 (m, PhH), 7.43 (d, J = 7 Hz, PhH), 7.73 (d, J = 7 Hz, PhH); ^{13}C NMR (CD_3OD) δ 22.8,

24.9, 26.9, 27.0, 30.1, 35.0, 35.2, 36.8, 37.2, 49.8, 54.4, 81.0, 81.8, 125.7, 126.5, 127.5, 128.1, 129.1, 136.5, 149.2, 166.8, 169.8, 170.3, 171.2, 174.4.

A mixture of 31 (390 mg, 0.656 mmol), trifluoroacetic acid (9 mL), and water (1 mL) was stirred at 23 °C for 1 h and then evaporated under a slow nitrogen stream overnight. The product was purified on a Waters reverse-phase C-18 microbondapak column (1.9 cm × 15 cm) using a linear gradient of 10% methanol/water 0.5% acetic acid to 100% methanol (40 min) with a flow rate of 3 mL/min to afford 1. The product purity was verified by ^{1}H NMR (CD_3CO_2D) δ 1.55–1.73 (m, 4H), 2.35 (m, CH_2), 2.63–2.92 (m, 2-CH_2), 3.15 (m, CH_2), 4.82 (m, CH), 4.93 (m, CH), 7.12–7.39 (m, PhH), 7.43 (d, J = 7 Hz, PhH), 7.82 (d, J = 7 Hz, PhH); ^{13}C NMR (CD_3CO_2D) δ 24.9, 29.9, 35.0, 35.1, 35.6, 36.9, 49.6, 54.0, 125.2, 126.8, 127.8, 128.3, 129.3, 136.3,149.7 166.4, 171.6, 174.6, 174.6, 175.3; FAB MS (MH^+ = 483). Anal. ($C_{25}H_{30}N_4O_6$ plus 1.0 H_2O and 0.8 acetic acid) C, H, N.

6-(*p*-Amidinophenyl)butanoyl-(*S*)-aspartyl-(*S*)-phenylalanine (9). Section A. 4-(*p*-Cyanophenyl)butanoic Acid (32). The 4-(*p*-cyanophenyl)butanoic acid (32) was prepared in the following manner: A mixture of 3-buten-1-ol (3.03 g, 42.0 mmol), 4-bromobenzonitrile (7.27 g, 39.9 mmol), triethylamine (6.05 g, 59.9 mmol), tri-*o*-tolylphosphine (0.841 g, 2.77 mmol), palladium acetate (0.224 g, 1 mmol), and acetonitrile (40 mL) was heated in a Teflon sealed vial at 80 °C for 20 h. After cooling to 23 °C, the reaction mixture was concentrated in vacuo, diluted with Na_2CO_3 (5%, 300 mL), extracted with ethyl acetate (2 × 300 mL), washed with brine (1 × 100 mL), and dried (Na_2SO_4). After concentration *in vacuo*, purification of the resultant residue by flash chromatography (ethyl acetate–hexane, 1:1) afforded 4.06 g (58.7%) of 4-(*p*-cyanophenyl)-3-buten-1-ol (33). The product was converted to 4-(*p*-cyanophenyl)butan-1-ol (34) by reduction of the double bond using the conditions of section B of compound 1. The 4-(*p*-cyanophenyl)butan-1-ol (34) (1.49 g, 8.51 mmol) was oxidized to 4-(*p*-cyanophenyl)butanoic acid (32) by treatment with 8 N Jones reagent (4 mL) in acetone (30 mL) at 10 °C for 10 min. The reaction was quenched with 2-propanol (5 mL), concentrated *in vacuo*, diluted with H_2O (80 mL), extracted with ethyl acetate (2 × 200 mL), and washed with $KHCO_3$ (2 × 250 mL). The aqueous layer was acidified with HCl (1 N), extracted with ether (2 × 400 mL), and dried (Na_2SO_4). Concentration *in vacuo* afforded 1.07 g (78%, based on starting material consumed) of 4-(*p*-cyanophenyl)butanoic acid.

Section B. *N*-[*N*-[4-(*p*-Amidinophenyl)-1-oxobutyl]-(*S*)-aspartyl]-(*S*)-phenylalanine (9). To a solution of 4-(*p*-cyanophenyl)butanoic acid (32) (1.07 g, 5.27 mmol), dimethylformamide (10 mL), and pyridine (2 mL) were added *N,N'*-disuccinimidylcarbonate (1.35 g, 5.26 mmol) and 4-(dimethylamino)pyridine (64.4 mg, 0.527 mmol) under an argon atmosphere at 23 °C. After 4 h, Asp(*O-tert*-butyl)-Phe(*O-tert*-butyl) (28) (2.06 g, 5.27 mmol) was added followed immediately by *N,N'*-diisopropylethylamine (0.680 g, 5.26 mmol). After 20 h at 23 °C, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in ethyl acetate (250 mL), washed with $KHSO_4$ (1 N, 100 mL), washed with brine (1 × 100 mL), and dried (Na_2SO_4). After concentration *in vacuo*, the residue was purified by flash chromatography (ethyl acetate–hexane, 2:3) to afford 1.70 g (56%) of the coupled product. The title compound was prepared by conversion of the benzonitrile to the benzamidine via the conditions of compound 1 followed by deprotection as in compound 1: ^{1}H NMR (CD_3OD) δ 1.82–1.93 (m, 2H), 2.23 (t, J = 7 Hz, CH_2), 2.53–2.82 (m, CH_2), 2.68 (t, J = 7 Hz, CH_2), 2.93–3.22 (m, CH_2), 4.62 (m, CH), 4.74 (m, CH), 7.12–7.29 (m, PhH), 7.43 (d, J = 7 Hz, PhH), 7.73 (d, J = 7 Hz, PhH); ^{13}C NMR (CD_3OD) δ 26.8, 34.7, 34.8, 35.4, 37.0, 50.0, 53.8, 125.8, 126.6, 127.8, 128.2, 129.2, 129.4, 136.8, 149.2, 166.6, 171.5, 172.6, 173.0, 174.3.

6-(*p*-Amidinophenyl)hexanoyl-(*S*)-aspartyl-(*S*)-phenylalanine (10). Compound 10 was prepared in the manner of compound 1 with the following modifications: the 6-(*p*-cyanophenyl)-5-hexenoic acid (35) was prepared using standard Wittig chemistry from commercially available starting materials using the following procedure. Potassium bis(trimethylsilyl)-amide (231 mL of a 0.66 M solution in toluene, 152.5 mmol) was added dropwise to a suspension of 4-(carboxybutyl)triphenylphosphonium bromide (72.75 mmol) in 500 mL of dry THF at 23 °C under a nitrogen atmosphere. After 1 h at 23 °C, the reaction was cooled to −70 °C, and 4-cyanobenzaldehyde (10.0 g, 76.3 mmol) in 50 mL of dry THF was added over 20 min. The reaction mixture was allowed to warm to 23 °C and stir for 20 h. After concentration of the reaction mixture, the residue was dissolved in ether (500 mL) and washed with water (300 mL) and aqueous sodium carbonate (300 mL, 5%). The combined aqueous layers were acidified to a pH of 1, extracted with ether (2 × 300 mL), and dried (Na_2SO_4). After concentration *in vacuo*, the crude product was esterified by treatment with iodomethane (2 equiv) in dimethylformamide using potassium carbonate (2.5 equiv) as base. After concentration, the residue was dissolved in ethyl acetate (300 mL), washed with water (2 × 100 mL) and brine (100 mL), and dried (Na_2SO_4). After concentration, the residue was purified by flash chromatography (ethyl acetate–hexane, 1:1). A small portion (2.2 mmol) of the purified material was reduced using the conditions of compound 1 (section B) which afforded methyl 6-(*p*-cyanophenyl)hexanoate (36). The methyl ester (2.6 mmol) was cleaved using aqueous sodium hydroxide (1 N, 1.1 equiv) in methanol at 23 °C for 20 h. After concentration, the residue was dissolved in water (50 mL), acidified with HCl (1 N) to a pH of 2, extracted with ether (2 × 200 mL), washed with water (1 × 100 mL), washed with brine (1 × 100 mL), and dried (Na_2SO_4). Concentration *in vacuo* afforded 6-(*p*-cyanophenyl)hexanoic acid (35) as a white solid: mp 61–62 °C. This starting material was used in place of the product of section B, compound 1, in the procedure of section C, compound 1, to afford compound 10 after completion of the sequence of reactions in the manner of compound 1 with the above substitution. ^{1}H NMR (CD_3OD) δ 1.23–1.73 (m, 6H), 2.18 (m, CH_2), 2.24–2.45 (m, CH_2), 2.83 (m, CH_2), 2.63–2.92 (m, CH_2), 4.45 (m, CH), 4.63 (m, CH), 7.05–7.28 (m, PhH), 7.42 (d, J = 7 Hz, PhH), 7.72 (d, J = 7 Hz, PhH); ^{13}C NMR (CD_3CO_2D) δ 26.2, 29.1, 29.2, 31.3, 36.1, 36.3, 36.4, 49.2, 49.3, 126.2, 127.7, 128.9, 129.4, 130.1, 130.4, 138.5, 151.0, 167.3, 176.7, 177.1, 178, 178. Anal. ($C_{26}H_{32}N_4O_6$ 0.3 H_2O) C, H, N.

***N*-[5-(*p*-Amidinophenyl)-1,4-dioxopentyl]-(*S*)-aspartyl-(*S*)-phenylalanine (11). Section A.** To a solution of tetrakis(triphenylphosphine)palladium(0) (100 mg, 0.09 mmol) and triethylamine (1.45 g, 14.3 mmol) in 50 mL of acetonitrile were added 4-bromobenzonitrile (1.82 g, 10 mmol) and 4-pentynoic acid (1.0 g, 10.2 mmol). The reaction mixture was warmed to 82 °C for 4 h followed by cooling to 23 °C. After concentration in vacuo, the residue was purified by flash chromatography (gradient, 1 L of hexane–ethyl acetate (4:1) followed by hexane–ethyl acetate (1:1)) which afforded the enol–lactone 37 (1.48 g, 74%). Anal. ($C_{12}H_9NO_2$) C, H, N.

Section B. A mixture of the enol–lactone (287 mg, 1.43 mmol), Asp(*O-tert*-butyl)-Phe(*O-tert*-butyl) (28) (565 mg, 1.43 mmol) and acetonitrile (15 mL) was warmed to 82 °C for 40 h followed by cooling to 23 °C. After concentration *in vacuo*, the residue was purified by flash chromatography (hexane–ethyl acetate (1:1)) which afforded the amide 38 (748 mg, 88.4%).

Section C. The title compound was prepared by conversion of the benzonitrile to the benzamidine following the conditions of compound 1 followed by deprotection as in compound 1. The final product was verified by ^{1}H NMR (DMSO-d_6) δ 2.23–2.47 (m, 2-CH_2), 2.58–2.82 (m, CH_2), 2.87–3.07 (m, CH_2), 3.89 (s, CH_2), 4.14 (m, CH), 4.52 (m, CH), 7.10–7.22 (m, PhH), 7.41 (d, J = 7 Hz, PhH), 7.72 (d, J = 7 Hz, PhH), 8.28 (d, J = 6 Hz), 8.98 (br s, NH_2); ^{13}C NMR (DMSO-d_6) δ 28.9, 36.6, 37.1, 48.3, 49.8, 54.6, 125.8, 127.0, 127.6, 127.7, 129.4, 130.1, 138.1, 140.7, 165.9, 169.9, 171.0, 172.6, 174.1, 206.2. Anal. ($C_{25}H_{28}N_4O_7$ plus H_2O) C, H, N.

***N*-[5-(*p*-Amidinophenyl)-4-hydroxy-1-oxopentyl]-(*S*)-aspartyl-(*S*)-phenylalanine (12).** The ketone 38 of step B of compound 11 (622 mg, 105 mmol) was reduced to the alcohol 39 by treatment with $NaBH_4$ (65.0 mg, 1.72 mmol) in 2-propanol (5 mL) at 23 °C for 2 h. The reaction was quenched through the addition of 5% HCl (5 mL) and subsequent stirring for 1 h at 23 °C. The reaction mixture was diluted with ethyl acetate (150 mL), washed with water (50 mL) and brine (50 mL), and dried (Na_2SO_4). After concentration *in vacuo*, the residue was purified by flash chromatography (gradient, ethyl acetate–hexane (3:2) to ethyl acetate, 100%) to afford the alcohol (152 mg). The title compound 12 was prepared by conversion of the benzonitrile to

the benzamidine following the conditions for compound 1 followed by deprotection as in compound 1. The final product was verified by ^{1}H NMR (diastereomeric mixture) (CD_3OD) δ 1.97–2.04 (m, CH_2), 2.30–2.61 (m, 2-CH_2), 2.75–3.07 (m, CH_2), 3.11 (m, CH_2), 3.22–3.33 (m, CH), 4.14 (m, CH), 4.64–4.87 (m, CH), 7.19–7.32 (m, PhH), 7.53 (d, J = 7 Hz, PhH), 7.77 (d, J = 7 Hz, PhH); ^{13}C NMR (CD_3OD) δ 27.2, 29.3, 35.9, 37.9, 38.7, 42.9, 50.7, 54.1, 55.4, 82.2, 127.8, 127.9, 128.9, 129.4, 130.1, 130.2, 131.4, 138.7, 145.3, 168.4, 170.1, 173.2, 174.8, 179.6.

5-(*p*-Amidinophenyl)-4(*E*)-pentenoyl-(*S*)-aspartyl-(*S*)-phenylalanine (13). Compound 13 was prepared in the manner of compound 1 with the following modification: The recrystallized 25 was used and step B was omitted: mp 215–218 °C; ^{1}H NMR (CD_3OD) δ 2.68–2.74 (m, 4H), 2.96–3.16 (m, CH_2), 3.24–3.49 (m, CH_2), 5.05 (m, CH), 5.23 (m, CH), 7.42–7.57 (m, PhH), 7.80 (d, J = 7 Hz, PhH), 8.04 (d, J = 7 Hz, PhH); ^{13}C NMR (CD_3CO_2D) δ 27.9, 33.8, 35.0, 36.0, 48.7, 53.2, 124.8, 125.7, 125.8, 127.2, 127.4, 128.3, 128.4, 132.0, 135.4, 142.5, 165.5, 170.6, 173.6, 173.9, 174.1. Anal. ($C_{25}H_{28}N_4O_6$) C, H, N.

5-(*p*-Amidinophenyl)-4-pentynoyl-(*S*)-aspartyl-(*S*)-phenylalanine (14). Compound 14 was prepared in the manner of compound 1 with the following modification: substituting 5-(*p*-cyanophenyl)-4-pentynoic acid (40) for 5-(*p*-cyanophenyl)pentanoic acid in section C. 5-(*p*-Cyanophenyl)-4-pentynoic acid (40) was prepared using the following procedure: A solution of 4-pentynoic acid (2.15 g, 22 mmol), 4-bromobenzonitrile (3.64 g, 20 mmol), and piperidine (40 mL) was degassed by bubbling nitrogen through the solution for 5 min prior to the addition of tetrakis(triphenylphosphine)palladium(0) (240 mg, 0.2 mmol). The reaction vial was sealed and warmed to 80 °C for 1.5 h. After being cooled to 23 °C, the reaction mixture was diluted with ethyl acetate (200 mL), filtered, and concentrated *in vacuo*. The residue was diluted with ethyl acetate (300 mL), washed with 5% HCl (2 × 100 mL), washed with water (1 × 100 mL), and extracted with 3% sodium carbonate (2 × 200 mL). The basic aqueous layer was treated with decolorizing carbon, filtered, and acidified to pH = 2. The resultant solid was filtered, washed with water, dried, and purified by flash chromatography (gradient ethyl acetate–methylene chloride–acetic acid, 1:9:0.005) and fractional recrystallization (methylene chloride–ether) to afford 5-(*p*-cyanophenyl)-4-pentynoic acid (40) as a white solid: mp 149–152 °C. Anal. ($C_{12}H_9NO_2$) C, H, N.

The final target compound (14) was prepared following the procedure of compound 1: ^{1}H NMR (DMSO-d_6) δ 2.24–2.52 (m, 4H), 2.58–2.72 (m, CH_2), 2.87–3.15 (m, CH_2), 4.19 (m, CH), 4.59 (m, CH), 7.05–7.28 (m, PhH), 7.43 (d, J = 6 Hz, NH), 7.58 (d, J = 7 Hz, PhH), 7.74 (d, J = 7 Hz, PhH), 8.38 (d, J = 6 Hz, NH), 9.00 (br s, NH_2); ^{13}C NMR (DMSO-d_6) δ 34.8, 37.9, 52.1, 56.5, 80.5, 94.5, 126.8, 128.6, 128.7, 128.8, 128.9, 130.3, 132.4, 139.0, 166.2, 171.0, 171.1, 174.1, 176.2.

5-(*m*-Amidinophenyl)pentanoyl-(*S*)-aspartyl-(*S*)-phenylalanine (15). Compound 15 was prepared in the manner of compound 1 with the following changes in section A: 3-bromobenzonitrile was substituted for 4-bromobenzonitrile, and the reaction was conducted at 50 °C. The final product was verified by ^{1}H NMR (CD_3CO_2D) δ 1.55–1.73 (m, 4H), 2.35 (m, CH_2), 2.63–2.92 (m, 2-CH_2), 3.15 (m, CH_2), 4.82 (m, CH), 5.00 (m, CH), 7.12–7.39 (m, PhH), 7.43–7.72 (m, PhH); ^{13}C NMR (CD_3CO_2D) δ 24.8, 29.9, 34.7, 35.1, 35.6, 36.8, 49.5, 53.8, 125.2, 126.8, 127.6, 128.4, 129.2, 134.3, 136.2, 143.9, 166.7, 171.6, 174.6, 174.8, 175.4; FAB MS (MH^+ = 483).

6-(*m*-Amidinophenyl)hexanoyl-(*S*)-aspartyl-(*S*)-phenylalanine (16). Section A. 6-(*m*-Cyanophenyl)hexanoic Acid (41). A solution of 1.22 g (5.70 mmol) of 6-(*m*-cyanophenyl)-5(*Z*)-hexenoic acid (42) in 30 mL of methanol was hydrogenated over 200 mg of 5% Pd/$CaCO_3$ at 2 psi of hydrogen over a 1.2-h period. After removing the catalyst by filtration, evaporation of the solvent *in vacuo* afforded 1.24 g of 6-(*m*-cyanophenyl)hexanoic acid (41). The target compound was prepared by substituting 6-(*m*-cyanophenyl)hexanoic acid (41) for 5-(*p*-cyanophenyl)pentanoic acid in section C of compound 1: ^{1}H NMR (CD_3CO_2D) δ 1.27–1.73 (m, 6H), 2.28 (t, J = 7 Hz, CH_2), 2.68 (t, J = 7 Hz, CH_2), 2.63–2.92 (m, CH_2), 3.00–3.27 (m, CH_2), 4.77 (m, CH), 4.93 (m, CH), 7.15–7.28 (m, PhH), 7.42–7.71 (m, PhH); ^{13}C NMR (CD_3CO_2D) δ 26.2, 29.2, 31.6, 36.1, 36.5, 37.0, 38.1, 50.8, 55.3, 126.4, 127.9, 128.9, 129.1, 129.5, 130.5, 135.4, 137.5, 145.4, 167.8, 172.8, 176.0, 176.1, 176.7. Anal. ($C_{26}H_{32}N_4O_6$ plus 0.25 H_2O) C, H, N.

***N*-[6-(*m*-Amidinophenyl)-1-oxo-5(*Z*)-hexenyl]-(*S*)-aspartyl-(*S*)-phenylalanine (17).** The title compound was prepared in the manner of compound 1 with the following modifications: substituting 6-(*m*-cyanophenyl)-5 (*Z*)-hexenoic acid (42) for 5-(*p*-cyanophenyl)pentanoic acid in section C. The 6-(*m*-cyanophenyl)-5 (*Z*)-hexenoic acid (42) was prepared using standard Wittig chemistry following the procedure for compound 10 with the following substitutions: sodium bis(trimethylsilyl)amide for potassium bis(trimethylsilyl)amide and 3-cyanobenzaldehyde for 4-cyanobenzaldehyde. The 6-(*m*-cyanophenyl)-5(*Z*)-hexenoic acid was obtained after purification by flash chromatography (hexane–ethyl acetate–acetic acid, 8:2:0.005) and fractional crystallization (ether–hexane) (note: following this procedure one can separate the *E* and *Z* isomers on a preparative scale). The reduction step of section C of compound 1 was omitted. The final product was verified by ^{1}H NMR (DMSO-d_6) δ 1.58–1.72 (m, 2H), 2.15 (t, J = 7 Hz, CH_2), 2.24–2.68 (m, 2-CH_2), 2.84–3.11 (m, CH_2), 4.20 (m, CH), 4.55 (m, CH), 5.78 (m, CH=), 6.45 (d, J = 8 Hz, CH=), 7.07–7.23 (m, PhH), 7.52–7.72 (m, PhH), 8.22 (d, J = 7 Hz, NH), 9.05 (br s, NH_2); ^{13}C NMR (DMSO-d_6) δ 25.1, 27.6, 34.5, 37.0, 37.1, 49.9, 54.7, 125.8, 125.9, 127.4, 127.5, 127.7, 128.1, 128.7, 128.9, 129.2, 129.4, 133.2, 134.1, 137.7, 138.2, 166.1, 170.2, 171.9, 172.8, 174.6. Anal. ($C_{26}H_{30}N_4O_6$) C, H, N.

5-[*p*-(Aminomethyl)phenyl]pentanoyl-(*S*)-aspartyl-(*S*)-phenylalanine (18). 5-(*p*-Cyanophenyl)-4-pentynoyl-(*S*)-aspartyl-(*S*)-phenylalanine (40) (750 mg, 1.29 mmol) was reduced by hydrogenation over palladium on carbon (10%) at 60 psi (23 °C) for 4 h in 20 mL of methanol containing 1 equiv of HCl (1 N). After removing the catalyst by filtration, evaporation of the solvent *in vacuo* afforded 750 mg of 5-[*p*-(aminomethyl)phenyl]pentanoyl-(*S*)-*O*-*tert*-butylaspartyl-(*S*)-*O*-*tert*-butylphenylalanine which was deprotected using TFA via the conditions of compound 1 to afford the title compound 18. The product was verified by ^{1}H NMR (CD_3OD) δ 1.52–1.68 (m, 4H), 2.19 (m, CH_2), 2.54–2.75 (m, 2-CH_2), 3.08 (m, CH_2), 4.05 (br s, NH_2), 4.62 (m, CH), 4.74 (m, CH), 7.15–7.28 (m, PhH), 7.38 (d, J = 7 Hz, PhH); ^{13}C NMR (CD_3OD) δ 25.0, 30.6, 34.9, 35.3, 35.4, 37.1, 43.0, 49.9, 53.8, 126.7, 128.3, 128.9, 129.1, 129.3, 130.5, 136.8, 143.6, 171.5, 172.6, 172.9, 174.8; CI MS ($M^+ - 17$ = 452). Anal. ($C_{25}H_{31}N_3O_6$ plus 1 HCl and 1.6 H_2O) C, H, N.

5-[*p*-(2-Imidazolinyl)phenyl]pentanoyl-(*S*)-aspartyl-(*S*)-phenylalanine (19). Compound 1 (600 mg, 1.25 mmol) and ethylenediamine (899 mg, 14.95 mmol) in methanol (20 mL) were warmed to achieve reflux for 20 h. After concentration in vacuo, purification by reverse-phase HPLC afforded the desired product 19 after concentration *in vacuo* and freeze-drying: ^{1}H NMR (CD_3CO_2D) δ 1.61–1.68 (m, 4H), 2.32 (m, CH_2), 2.72 (m, CH_2), 2.56–2.93 (m, CH_2), 3.15 (m, CH_2), 4.11(br s, 2-CH_2), 4.82 (m, CH), 4.94 (m, CH), 7.15–7.28 (m, PhH), 7.43 (d, J = 7 Hz, PhH), 7.83 (d, J = 7 Hz, PhH); ^{13}C NMR (CD_3OD) δ 24.7, 29.6, 34.9, 35.2, 36.5, 44.4, 49.3, 53.5, 119.1, 126.6, 128.1, 128.2, 129.0, 129.2, 135.9, 150.3, 165.9, 171.4, 174.1, 174.4, 175.3; CI MS ($M^+ - 17$ = 491); exact mass (CI) calcd for $C_{27}H_{31}N_4O_5$ 491.2332, found 491.2313.

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