Articles

Conformational Preferences in a Benzodiazepine Series of Potent Nonpeptide Fibrinogen Receptor Antagonists

Richard M. Keenan,*,† James F. Callahan,*,† James M. Samanen,*,† William E. Bondinell,† Raul R. Calvo,† Lichong Chen,† Charles DeBrosse,▽ Drake S. Eggleston,§ R. Curtis Haltiwanger,§ Shing Mei Hwang,‖ Dalia R. Jakas,† Thomas W. Ku,† William H. Miller,† Kenneth A. Newlander,† Andrew Nichols,‡ Michael F. Parker,† Linda S. Southhall,† Irene Uzinskas,† Janice A. Vasko-Moser,‡ Joseph W. Venslavsky,† Angela S. Wong,‖ and William F. Huffman†

Research and Development Division, SmithKline Beecham Pharmaceuticals, P.O. Box 5089, Collegeville, Pennsylvania 19426-0989

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Previously, we reported the direct design of highly potent nonpeptide 3-oxo-1,4-benzodiazepine fibrinogen receptor antagonists from a constrained, RGD-containing cyclic semipeptide. The critical features incorporated into the design of these nonpeptides were the exocyclic amide at the 8-position which overlaid the Arg carbonyl, the phenyl ring which maintained an extended Gly conformation, and the diazepine ring which mimicked the γ-turn at Asp. In this paper, we investigate conformational preferences of the 8-substituted benzodiazepine analogues by examining structural modifications to both the exocyclic amide and the seven-membered diazepine ring and by studying the conformation of the benzodiazepine ring using molecular modeling, X-ray crystallography, and NMR. We found that the directionality of the amide at the 8-position had little effect on activity and the (*E*)-olefin analogue retained significant potency, indicating that the trans orientation of the amide, and not the carbonyl or NH groups, made the largest contribution to the observed activity. For the diazepine ring, with the exception of the closely analogous 3-oxo-2-benzazepine ring system described previously, all of the modifications led to a significant reduction in activity compared to the potent 3-oxo-1,4-benzodiazepine parent ring system, implicating this particular type of ring system as a desirable structural feature for high potency. Energy minimizations of a number of the modified analogues revealed that none could adopt the same low-energy conformation as the one shared by the active (*S*)-isomer of the 3-oxo-1,4-benzodiazepines and 3-oxo-2-benzazepines. The overall data suggest that the features contributing to the observed high potency in this series are the orientation of the 3−4 amide and the conformational constraint imposed by the seven-membered ring, both of which position the key acidic and basic groups in the proper spatial relationship.

Introduction

The aggregation of platelets is a critical event in hemostasis and arterial thrombosis. However, the uncontrolled deposition of platelets and the formation of large aggregates can lead to vascular occlusion resulting in serious ischemic disorders such as acute myocardial infarction (AMI), unstable angina, or thrombotic stroke. Platelets aggregate in response to a wide variety of agonists including adenosine diphosphate (ADP), thromboxane A_2, platelet activating factor (PAF), epinephrine, serotonin, thrombin, vasopressin, and collagen.[1] Independent of the agonist, the final step in platelet aggregation and subsequent thrombus formation is the cross-linking of the dimeric plasma protein fibrinogen to its receptor, the membrane-bound glycoprotein complex GPIIb/IIIa, on adjacent activated platelets.[2] Therefore, the development of fibrinogen receptor antagonists for the treatment of thrombogenic disorders has been the focus of considerable research in recent years.[3]

The minimum binding epitope of fibrinogen to its receptor, a member of the integrin superfamily of adhesion receptors, is defined by the tripeptide sequence: Arg-Gly-Asp (RGD). Extensive early work on modified peptides led to the identification of short linear peptides containing the RGD sequence which effectively block platelet aggregation. These studies pointed to the basic group of the arginine and the acidic group of the aspartic acid as the key elements for antagonist activity.[4,5] Subsequent studies showed that incorporating the RGD motif into constrained, cyclic peptides was a successful approach to the discovery of high-affinity fibrinogen receptor antagonists.[6−13] Structural and conformational analyses of such constrained peptides led to several related proposals for a biologically important conformation about the RGD backbone, including a "turn-extended-turn" conformation,[14−17] a "cupped" presentation,[18] and a Gly-Asp β-turn,[19,20] which were used

† Department of Medicinal Chemistry.
‖ Department of Cellular Biochemistry.
‡ Department of Cardiovascular Pharmacology.
▽ Department of Analytical Chemistry.
§ Department of Physical and Structural Chemistry.

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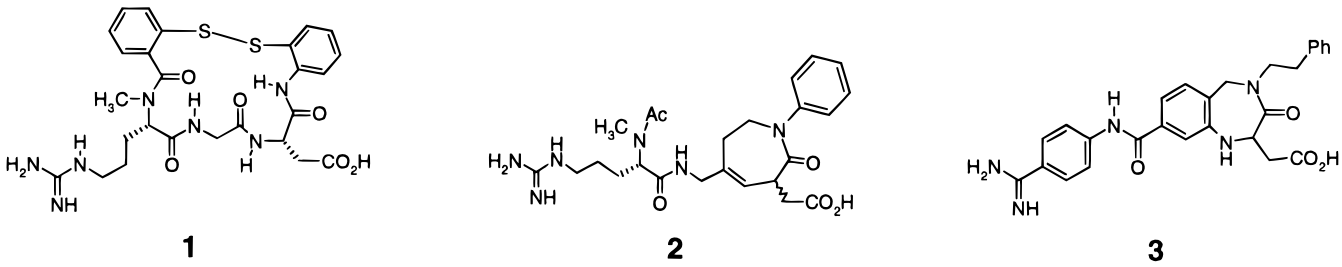
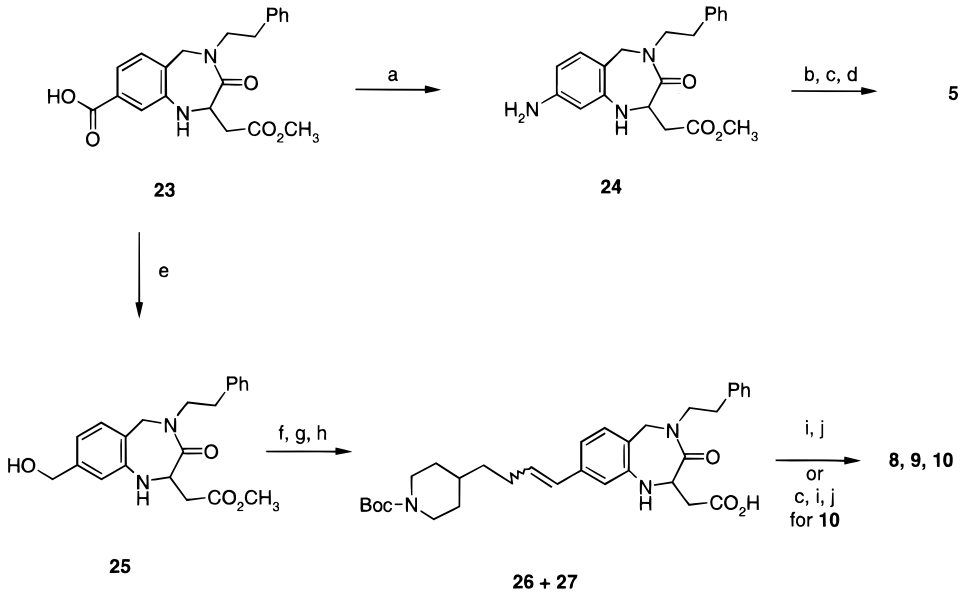


1 **2** **3**

Figure 1. Comparison of the structures of fibrinogen receptor antagonists cyclic peptide **1**, peptidomimetic **2**, and nonpeptide **3**.

Scheme 1[a]



23 **24** **5** **25** **26 + 27** **8, 9, 10**

[a] (a) $SOCl_2$, NaN_3, toluene, 80 °C; (b) 4-(*N*-Cbz-amidino)benzoyl chloride; (c) 10% Pd/C; (d) 3 N HCl; (e) $SOCl_2$, $NaBH_4$; (f) CBr_4, Ph_3P; (g) Ph_3P; (h) 3-(*N*-Boc-piperidin-4-yl)propionaldehyde, NaH; (i) 1 N NaOH; (j) TFA.

as templates for the successful design of potent nonpeptide RGD mimetics. The development of RGD mimetics as potent nonpeptide fibrinogen receptor antagonists therefore not only offers a promising new treatment for thrombotic disorders but also represents a considerable achievement in peptidomimetic drug design.[21]

In our previous work, we used NMR, X-ray crystallographic, and conformational data to identify a low-energy turn-extended-turn conformation for the RGD backbone of the highly potent cyclic peptide **1** (Figure 1).[14,15] The conformational mimetic **2** containing the seven-membered ring γ-turn mimetic about Asp retained a good deal of the activity of the cyclic peptide **1**, supporting the biological importance of the proposed model.[22] Finally, the 3-oxo-1,4-benzodiazepine nonpeptide fibrinogen receptor antagonist **3** had equal potency to the constrained cyclic peptide **1** used as the template in its design.[23] The critical features incorporated into the structure of nonpeptide **3** were the diazepine ring to mimic a γ-turn at Asp, the phenyl ring to maintain an extended Gly conformation, and the exocyclic amide at the 8-position which overlaid the Arg carbonyl. These structural features closely mimicked the turn-extended-turn Arg-Gly-Asp backbone found in **1** and were believed to contribute to the potent activity observed for **3**.

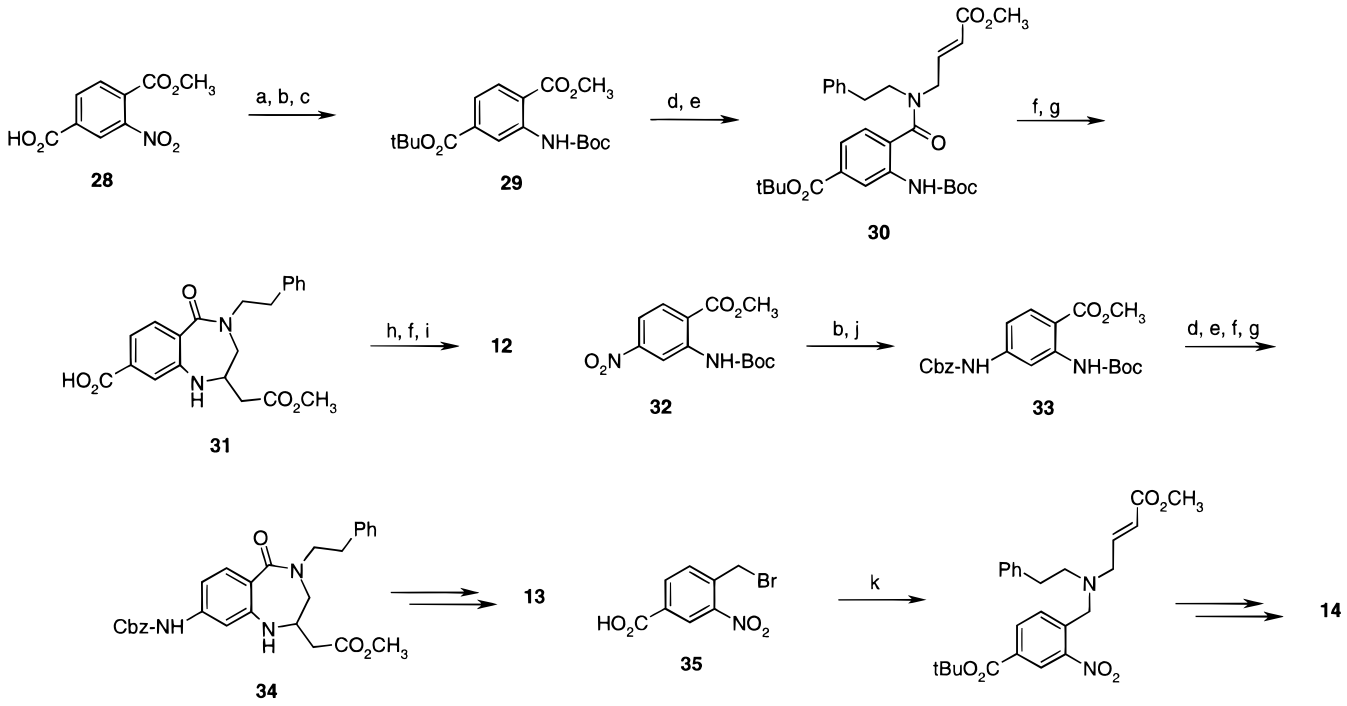
To date, we have described modifications to the Arg mimetic region of the nonpeptide which have led to surprising improvements in oral bioavailability and duration of action in vivo,[24] including a potent series of 7-substituted 3-oxo-1,4-benzodiazepines.[25] Also, we have reported that replacement of N-1 of the benzodiazepine nucleus with CH_2 afforded a series of 3-oxo-2-benzazepines with comparable activity, indicating that neither the nitrogen lone pair nor the N-H group at this position is required for high affinity.[26] In this paper, we report on the synthesis and activity of 8-substituted benzodiazepine analogues containing modifications to the exocyclic amide and to the seven-membered diazepine ring and also discuss the implications of the data on our original design hypothesis.

Chemistry

The syntheses of the analogues in Table 1 are depicted in Scheme 1. The carboxylic acid **23**[24a] was subjected to Curtius rearrangement via conversion to the acyl azide to furnish the 8-amino derivative **24** in modest yield. Coupling with the acid chloride derived from 4-(*N*-Cbz-amidino)benzoic acid[27] and global deprotection afforded the retro-amide analogue **5**. Reduction of carboxylic acid **23** gave the alcohol **25**, which was converted to a mixture of olefin isomers **26** and **27** via a Wittig reaction. The isomers were separated and deprotected to give the (*E*)- and (*Z*)-olefin analogues **8** and **9**. Hydrogenation of the olefin mixture and subsequent deprotection provided the saturated compound **10**.

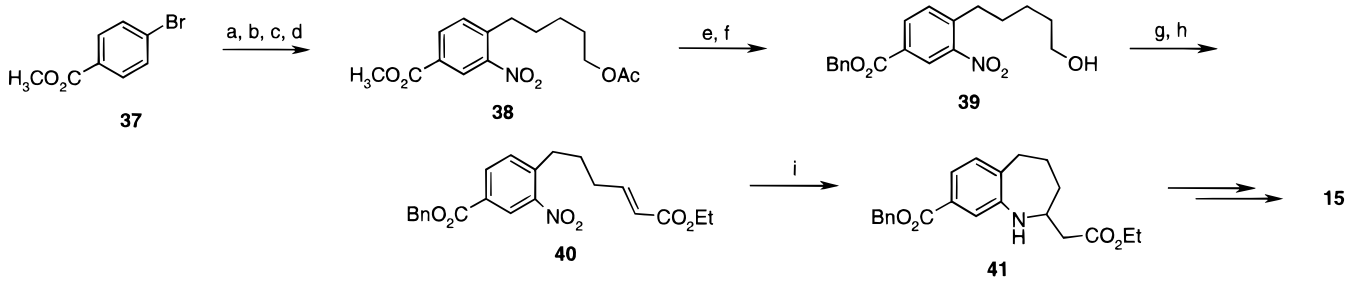
The synthesis of the other compounds described in this paper involved construction of the corresponding 6−7 ring system followed by attachment of the amidi-

Scheme 2[a]



[a] (a) ClCOCOCl, tBuOH; (b) H_2, Pd/C, MeOH; (c) $(Boc)_2O$, DMAP; (d) 1 N NaOH; (e) methyl 4-phenethylaminocrotonate, Et_3N, HOBt, BOP reagent; (f) TFA, CH_2Cl_2; (g) MeOH, reflux; (h) $SOCl_2$, 4-(Boc-amidino)aniline; (i) 20% HOAc, reflux; (j) Cbz-Cl; (k) methyl 4-phenethylaminocrotonate, Et_3N.

Scheme 3[a]

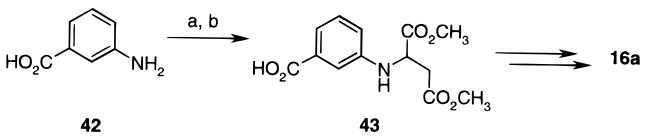


[a] (a) Mg, $ZnBr_2$, 5-bromo-1-pentene; (b) O_3, $MeOH/CH_2Cl_2$, $NaBH_4$; (c) Ac_2O, pyridine; (d) HNO_3/H_2SO_4; (e) 1 N NaOH; (f) $CsHCO_3$, benzyl bromide, DMF; (g) PCC, CH_2Cl_2; (h) $Ph_3P{=}CHCO_2Et$; (i) $SnCl_2$, EtOH.

nophenyl group to the aromatic ring via an amide linker using standard coupling conditions as previously described.[27] Global deprotection then afforded the desired product. The various ring systems were constructed utilizing a variety of synthetic pathways outlined below.

The modified seven-membered ring compounds **12**–**15** in Table 2 were made via an intramolecular conjugate addition of the appropriate aniline to an unsaturated ester. Scheme 2 shows the syntheses of analogues **12** and **13**, in which the amide carbonyl has been moved to the 5-position on the benzodiazepine ring. For compound **12**, partial hydrolysis of dimethyl nitroterephthalate afforded **28**, which was converted to **29** by reesterification as the *tert*-butyl ester and subsequent reduction of the nitro group and protection as its Boc derivative. Selective ester hydrolysis and coupling with methyl 4-(phenethylamino)crotonate[27] gave the cyclization precursor **30** which, after acid deprotection, underwent an intramolecular conjugate addition to furnish **31**. For the synthesis of compound **13**, esterification and protection of 5-nitroanthranilic acid yielded **32**. Nitro group reduction and protection gave the methyl ester

Scheme 4[a]

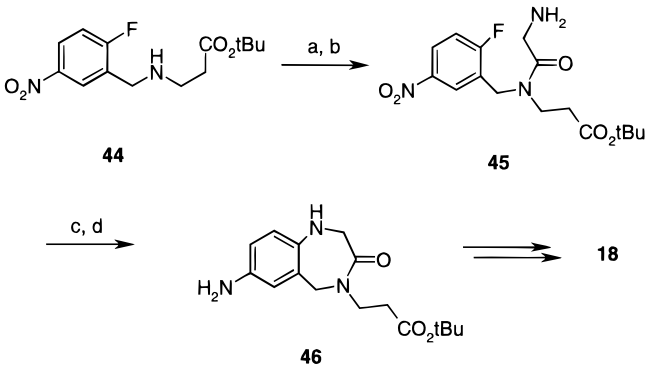


[a] (a) DMAD, MeOH; (b) H_2, Pd/C.

33, which was converted as before to **13** via the ring-closed intermediate **34**. Alkylation of the aminocrotonate with the benzyl bromide **35**[27] furnished **36**. Reduction of the nitro group and cyclization as before provided the bicyclic intermediate for the synthesis of the related reduced amide analogue **14**.

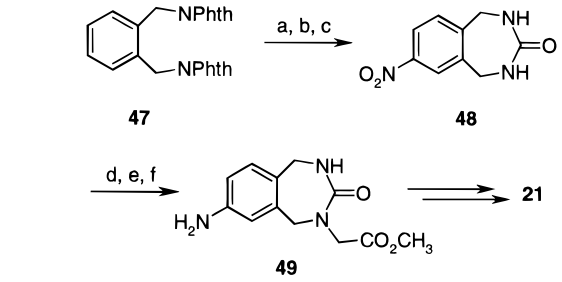
The precursor for the azepine analogue **15** was synthesized as depicted in Scheme 3. Palladium-catalyzed coupling of the bromide **37** with 4-pentenylzinc iodide, ozonolysis of the olefin followed by reductive workup, protection of the primary alcohol, and ring nitration afforded **38**. Treatment with base liberated the alcohol, and the benzoic acid was reesterified to yield the benzyl ester **39**. Oxidation and chain elongation

Scheme 5[a]



[a] (a) Boc-glycine, BOP reagent; (b) 4 M HCl, dioxane; (c) Et_3N, DMSO; (d) H_2, Pd/C.

Scheme 6[a]



[a] (a) KNO_3, H_2SO_4; (b) H_2NNH_2; (c) 1,1′-carbonyldiimidazole; (d) Lawesson's reagent; (e) CH_3I, K_2CO_3, $BrCH_2CO_2CH_3$; (f) H_2, Pd/C.

afforded the unsaturated ester **40**. Ring closure to **41** occurred in situ during reduction of the nitro group with stannous chloride. For the synthesis of the ring-opened analogue **16**, intermediate **43** was made from reaction of 3-aminobenzoic acid (**42**) with DMAD followed by catalytic reduction (Scheme 4).

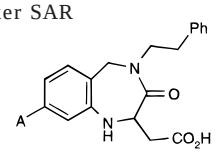
The regioisomeric benzodiazepine analogues **17** and **18** in Table 2 were made from an intramolecular nucleophilic aromatic substitution reaction. For **18**, reaction of 2-fluoro-5-nitrobenzaldehyde with *tert*-butyl 3-aminopropionate under reducing conditions afforded the amine **44**, which was acylated with Boc-glycine and deprotected to give **45** (Scheme 5). Ring closure to **46** was effected by treatment with triethylamine overnight in DMSO. Compound **17** was made in similar fashion, but substituting *tert*-butylglycine in the first step.

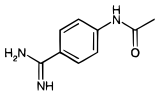
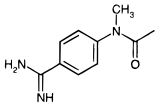
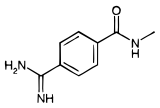
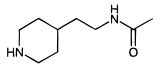
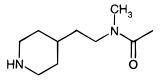
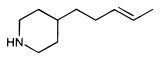
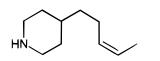
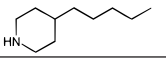
The 2-azepine analogues **19** and **20** were made using chemistry analogous to that developed for the synthesis of the benzazepine series of GPIIb/IIIa antagonists.[28] Here, methyl acrylate was employed in the palladium-catalyzed addition in place of dimethyl itaconate, and either glycine methyl ester or *β*-alanine methyl ester replaced phenethylamine as the amine component. The urea analogue **21** was synthesized via nonselective alkylation of the thiourea derived from urea **48** which was made in four steps from α,α′-dibromo-*o*-xylene (Scheme 6).

Results and Discussion

For the compounds in this study, fibrinogen receptor binding affinity was determined by assaying for inhibition of [^{3}H]-**1** binding to purified GPIIb/IIIa isolated from human platelets and reconstituted in liposomes. Also, the compounds were evaluated for their ability to inhibit ADP-induced platelet aggregation in human platelet-rich plasma.[29]

Table 1. Exocyclic Linker SAR



no.	A	GPIIb/IIIa binding K_i (nM)	platelet aggregation IC_{50} (nM)
3[a]		2.8 ± 0.1	150 ± 40
4[a]		1.6 ± 0.2	65 ± 3
5		2.0 ± 0.2	130 ± 40
6[b]		4.7 ± 0.2	510 ± 20
7[b]		1.9 ± 0.1	15 ± 3
8		19 ± 1	175 ± 40
9		750 ± 50	7400 ± 1500
10		80 ± 4	930 ± 60

[a] Reference 24a. [b] Reference 24b.

A series of analogues was prepared to investigate the role of the exocyclic amide at the 8-position of the benzodiazepine nucleus (Table 1). We have previously reported that N-methylation of the amide to yield **4** led to a slight increase in in vitro activity, most notably in the platelet aggregation assay.[24a] Interestingly, the retro-amide analogue **5** showed comparable activity to **3** in both binding and inhibition of platelet aggregation indicating the directionality of the exocyclic amide bond was not important for activity in this series. We also investigated olefin replacements, in this case in the piperidinoethyl series,[24b] where the *N*-methylamide **7** exhibited similar binding affinity but had much improved platelet aggregation inhibitory activity compared to the secondary amide **6**.[30] The (*E*)-olefin **8** had 40-fold better activity in both assays compared to the (*Z*)-olefin **9**, and only 4-fold diminished binding affinity relative to **6**. The data from this set of analogues revealed that the trans orientation of the amide was likely the bioactive conformation and suggested the carbonyl and NH had minimal contributions to the observed activity.

The incorporation of a seven-membered diazepine ring to mimic the *γ*-turn observed around Gly-Asp in the peptide model was another of the critical elements in our original design hypothesis.[23] A number of modifications to the seven-membered ring amide region were investigated to probe the structural requirements for activity (Table 2). Compounds **12** and **13**, containing the

Table 2. Seven-Membered Ring SAR

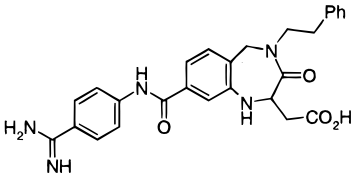
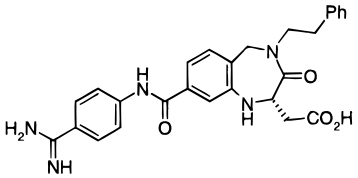
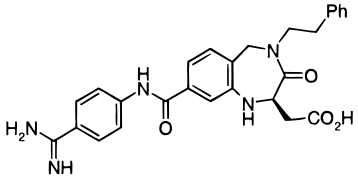
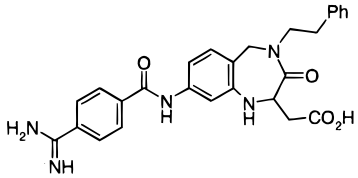
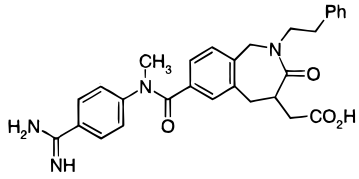
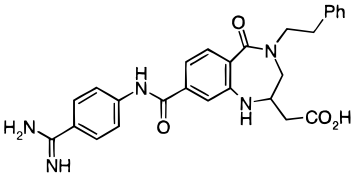
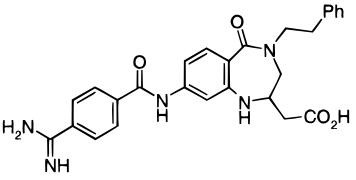
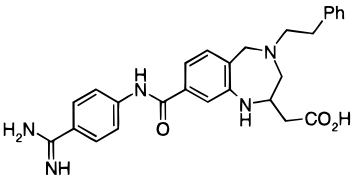
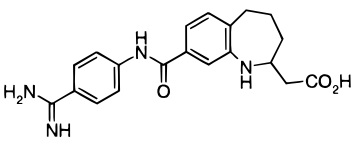
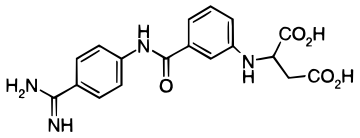
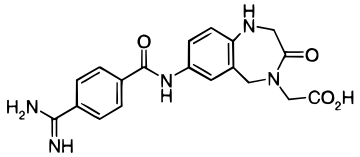
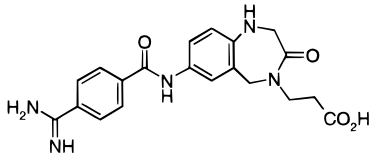
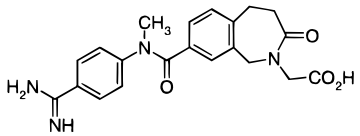
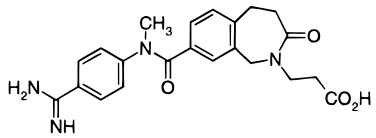
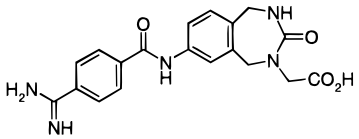
no.	structure	GPIIb/IIIa binding K_i (nM)	platelet aggregation IC_{50} (nM)	relevant model
3[a]		2.8 ± 0.1	150 ± 40	AM1
3s[a]		2.0 ± 0.1	34 ± 10	AM1
3r[a]		500 ± 40	2200 ± 300	R-AM1
5		2.0 ± 0.2	130 ± 40	AM1
11[b]		1.5 ± 0.1	12 ± 4	AM1'
12		830 ± 20	8300 ± 1100	AM2
13		14900 ± 100	>200,000	AM2
14		540 ± 10	3000 ± 100	-
15		1500 ± 200	3900 ± 700	AM3

Table 2 (Continued)

no.	structure	GPIIb/IIIa binding K_i (nM)	platelet aggregation IC_{50} (nM)	relevant model
16		560 ± 3	4700 ± 500	-
17		1200 ± 10	3100 ± 500	AM4
18		290 ± 3	2100 ± 300	AM4
19		>50000	>200000	AM4
20		22500 ± 500	>200000	AM4
21		1750 ± 70	23000 ± 3000	AM5

[a] Reference 24a. [b] Reference 26.

regioisomeric 5-oxo-1,4-benzodiazepine ring system, were much less potent than the parent 3-oxo compounds. In addition, reduction of the amide carbonyl (**14**) or removal of the amide altogether (**15**) also led to a significant reduction in activity compared to **3**. Finally, opening the seven-membered ring to the meta-disubstituted benzene compound **16** caused a similar reduction of activity, which is notable in light of the numerous reports of highly potent fibrinogen receptor antagonists which lack the constraints of a bicyclic ring system.[31] Thus, an intact seven-membered ring containing a 3−4 amide contributes significantly to the observed fibrinogen receptor antagonist activity of the potent 3-oxo-1,4-benzodiazepines such as **3**. The contribution to activity by the ring amide could be the result of a binding interaction between the receptor and the amide carbonyl group and/or the stabilization by the ring amide of a highly potent conformation of the seven-membered ring.

Previously, we have shown that replacement of N-1 of the benzodiazepine nucleus with CH_2 affords a 3-oxo-2-benzazepine series with comparable activity (i.e., **11**, Table 2), indicating that neither the nitrogen lone pair nor the N-H group at this position is required for high affinity.[26] We have also examined a number of related analogues in which the chiral carbon atom in the seven-membered ring has been replaced by a nitrogen, but the key carbonyl at position 3 has been retained (Table 2). All of the analogues **17**−**21** containing such modified seven-membered rings were much weaker in activity than the benzazepine **11**. Although **17**−**21** lack a phenethyl side chain on the seven-membered ring, this substitution has been shown not to be important for good activity in either the 3-oxo-2-benzazepine[26] or 3-oxo-1,4-benzodiazepine[24b] systems. Thus, the chiral center on the seven-membered ring appears to be crucial for good activity and possibly is instrumental in placing the carboxylate in the proper position for binding. Still, these less active analogues all contain the carbonyl group at position 3 found in the potent benzodiazepine and benzazepine analogues, which would suggest either that this carbonyl group is not a receptor-binding element or that the conformation of the seven-mem-

bered ring in these less active analogues positions the carbonyl group in an unfavorable orientation. To shed further light on this issue, we have performed molecular modeling studies to examine the families of conformations available to each of the 6−7 ring systems described in this paper.

Modeling and X-ray Crystallography. Each ring system was subjected to a Monte Carlo[32] simulation (1000 iterations) using an Amber[33] force field in a water matrix. All conformers within 50 kJ/mol of the global minimum were saved and ranked by relative energy. The substituent at N-4 was truncated to a methyl group, and the arginine mimetic was replaced by an *N*-methylamide for simplicity. The (*S*)-absolute stereochemistry of the 3-substituent was fixed in AM1, and all corresponding analogues maintained the same absolute stereochemistry.[34]

The modeling of the parent 3-oxo-1,4-benzodiazepine ring system (AM1) showed two low-energy families of conformers, AM1-A and AM1-B, whose lowest energy members differed by 10.6 kJ/mol. These consisted of the two flip forms of the seven-membered ring (Table 3). Replacement of N-1 with a methylene group to yield a 3-oxo-2-benzazepine ring system had no effect on the observed ring conformers C-AM1 or the relative energy of the two flip forms of the seven-membered ring, consistent with the similar biological activity observed for compounds containing these two closely related ring systems.

We previously reported an X-ray crystal structure of the racemic benzodiazepine **22a** (Table 4).[24a] As seen in Figure 2, the benzodiazepine ring of the (*S*)-enantiomer of **22a** is superimposable upon AM1-A. We now have obtained several X-ray crystal structures of various 3-oxo-1,4-benzodiazepine and 3-oxo-2-benzazepine compounds (**22b**−**f**, Table 4), all of which display the same AM1-A conformation about the seven-membered ring. Since the crystal packing should be different due to the different substituents in these analogues, the X-ray data strongly implies that AM1-A must be an inherently low-energy conformation for the 3-oxo-1,4-benzodiazepine ring system.

The proton NMR spectrum of **22c** in hexadeuterioacetone at 25 °C displays one set of multiplets for each proton in the spectrum and does not suggest the presence of multiple conformers. Lowering the temperature to −60 °C induces a shift in the proton at N-1 but has no effect on any of the other resonances, supporting the lack of dynamic behavior in the benzodiazepine ring. As with all other 3-oxo-1,4-benzodiazepine analogues, the methylene protons at position 5 of **22c** display a characteristic doublet at ∼5.5 ppm that arises from the different magnetic environments experienced by each methylene proton. In the conformation AM1-A, one proton (H-5a, Figure 3) resides above the plane of the adjacent aromatic ring, while the other (H-5b, Figure 3) sits within the plane. In the proton spectrum, the NOE enhancements between H-5a and H-2 and between H-5b and H-6 (Figure 3) are consistent with the lowest energy conformation of AM1 (AM1-A), wherein H-5a is separated by only 2 Å from H-2.

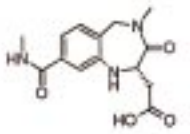
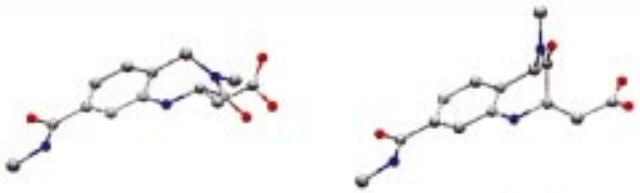
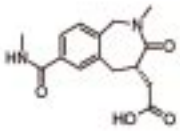
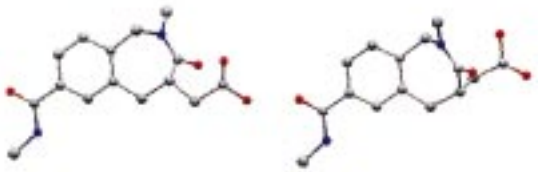
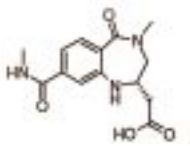
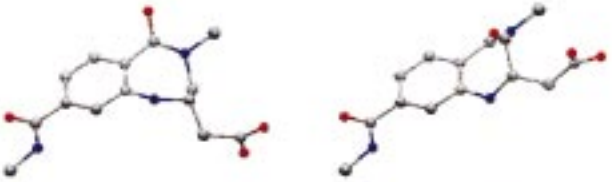
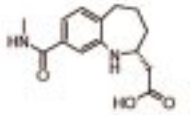
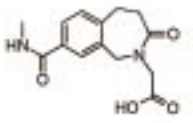
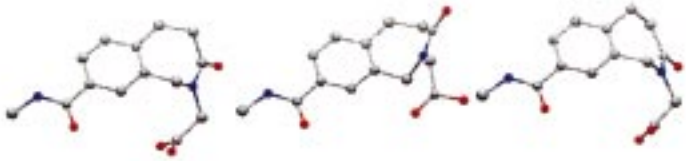
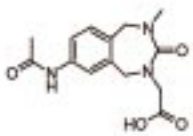
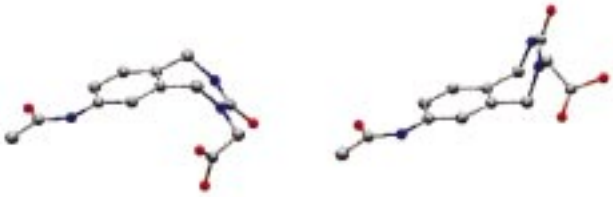
These molecular modeling studies of the 3-oxo-1,4-benzodiazepine ring system have identified a low-energy conformation AM1-A which is also the only conformation observed by both proton NMR spectroscopy and X-ray crystallography. Thus AM1-A may be the conformation responsible for the pharmacological activity of these 3-oxo-1,4-benzodiazepine fibrinogen receptor antagonists.

In Table 3, we have compared the conformations obtained from molecular modeling analysis of the other 6−7 ring systems to AM1-A, to probe for differences which may explain the reduction in biological activity. The analogous structure AM2, which contains a 4−5 amide in place of a 3−4 amide, also displays two families of conformations which represent two flip forms of the seven-membered ring. Both the vector of the acetic acid substituent and the ring pucker of the seven-membered ring in the lower energy conformers AM2-A are significantly different when compared to those in AM1-A. The analogue **12**, which contains an AM-2 type 6,7 template, displays considerably lower activity than the parent **3**, which contains an AM1 type template. These results suggest that the lower energy conformers AM2-A place the acetic acid group in an unfavorable orientation, while the higher energy conformers AM2-B place the ring amide in an unfavorable orientation.

The conformers of AM3, which are related to analogue **14**, display a variety of low-energy conformations due to the absence of the constraining 3−4 amide bond. Interestingly, the conformer AM3-A positions the acetic acid group in an equatorial orientation in the plane of the aromatic ring similar to that in AM1-A, with minor variations in the seven-membered ring conformation at C-3 and C-4 and the obvious loss of the carbonyl oxygen. The higher energy conformers AM3-B and AM3-C vary either the position of the acetic acid side chain or the conformation of the seven-membered ring. The lower affinity of **14** which lacks the ring amide suggests that the 3−4 amide may contribute a positive binding interaction in addition to its stabilizing conformational role. The potential importance of the amide's interaction is also seen in the strong preference for the (*S*)-absolute stereochemistry at C-2 in potent (*S*)-3-oxo-1,4-benzodiazepine GPIIb/IIIa antagonists (3*S* vs 3*R*, Table 2).[24a,25] The less potent (*R*)-isomers maintain an equatorial acetic acid side chain but have a different relative orientation of the seven-membered ring amide in interacting with the receptor (Figure 4). The reduction in activity for the acyclic compound **16**, where the appropriate pseudoequatorial conformation of the carboxylate-containing side chain should be accessible, also points to a positive binding interaction for the ring amide. However, in each of these examples, it is not possible to rule out a negative interaction at the receptor caused by the introduction of new functionality as the reason for the dropoff in activity.

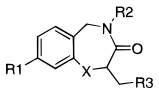
The conformers of AM4, which are related to analogue **19**, contain a 2−3 amide instead of a 3−4 amide. The vector of the acetic acid side chain in all of the conformers AM4-A, AM4-B, and AM4-C position the acetic acid side chain below the plane of the aromatic ring with a vector significantly different than seen in AM1-A. This is due to the fact that the side chain originates from an amide nitrogen and not a methylene group, providing a rationale for the low activity of **19**. Similar results were observed using the diazepine structure related to **17**, another indication that interchange of carbon and

Table 3. Conformations of the Different Seven-Membered Ring Systems

no.	structure(s)	analogs	conformations
AM1		3, 5	 AM1-A AM1-B Rel. Ener. 0.0 +10.6
C-AM1		11	 AM1-A' AM1-B' Rel. Ener. 0.0 +13.7
AM2		12, 13	 AM2-A AM2-B Rel. Ener. 0.0 +2.6
AM3		15	 AM3-A AM3-B AM3-C Rel. Ener. 0.0 +1.9 +3.8
AM4		17, 18, 19, 20	 AM4-A AM4-B AM4-C Rel. Ener. 0.0 +5.77 +8.49
AM5		21	 AM5-A AM5-B Rel. Ener. 0.0 +1.9

[a] Relative energy in kJ/mol.

Table 4. Solved Benzodiazepine and Benzazepine X-ray Structures



	R_1	R_2	R_3	X
(R)-22a	(4-BrPh)NHCO	$(CH_2)_2Ph$	CH_3OOC	NH
(R,S)-22b	PhNHCO	$(CH_2)_2Ph$	CH_3OOC	NH
(R,S)-22c	HOOC	$(CH_2)_2Ph$	CH_3OOC	NH
(R,S)-22d	HOOC	$(CH_2)_2Ph$	CH_3OOC	CH_2
(R,S)-22e	HOOC	iPr	CH_3OOC	CH_2
(R,S)-22f	4-[C(=N)NH_2)]C_6H_4-N(CH_3)CO	$(CH_2)_2Ph$	HOOC	CH_2

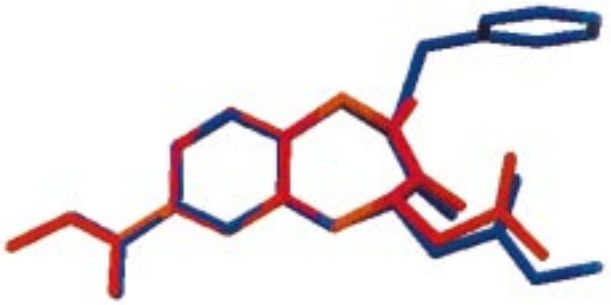


Figure 2. Overlay of AM1-A, the low-energy conformation calculated for the 3-oxo-1,4-benzodiazepine ring system, with the conformation derived from the X-ray structure of **22a**.

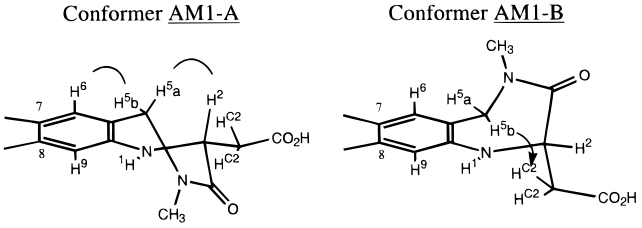


Figure 3. Depiction of conformations AM1-A and AM1-B of the 3-oxo-1,4-benzodiazepine ring system showing the observed NOE relationships consistent with conformation AM1-A.

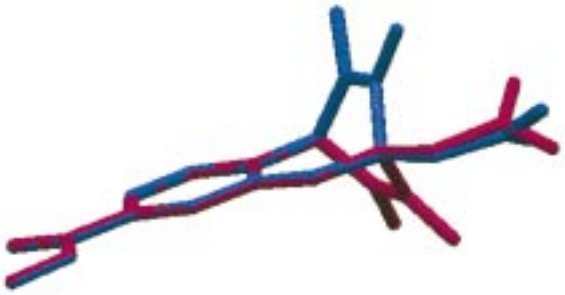


Figure 4. Overlay of the (*R*)- and (*S*)-enantiomers of the lowest energy conformation AM1-A.

nitrogen at position 1 of the benzodiazepine ring has little to no conformational impact in these ring systems. The fact that extension of the acetic acid side chains as in analogues **18** and **20** gives modest increases in affinity suggests that greater flexibility of the propionate side chain allows for a slightly better fit with the receptor by overcoming the original vector from the template.

Figure 5. Comparison of lowest energy conformations of AM1 (blue), AM1′ (green), AM2 (yellow), AM3 (red), AM4 (purple), and AM5 (light blue).

The low-energy conformations of the cyclic urea-containing analogue **21** are represented by AM5-A and AM5-B. Neither of these conformers is capable of positioning the acetic acid side chain within the plane of the aromatic ring, and therefore a favorable interaction with the receptor cannot be achieved.

In summary, the data collected in the Monte Carlo simulations allows for the rationalization of the biological data for this series of related compounds. Analogues containing the seven-membered ring conformation found in AM1-A showed the optimal biological profile of the ring systems described. The related 6−7 ring analogues with diminished activity adopt conformations in which either the acetic acid or the ring amide groups are positioned in regions of space other than those of AM1-A (Figure 5). Although not conclusive, these calculations suggest the ring amide of the 3-oxo-1,4-benzodiazepine and 3-oxo-2-benzazepine analogues may contribute a positive binding interaction in addition to its stabilizing conformational role.

Conclusion

The initial benzodiazepine analogues designed from the peptide model contain a number of structural features which map directly onto the peptide backbone of Arg-Gly-Asp in a proposed bioactive conformation.[23] Our subsequent studies have shown that the NH at position 1 of the benzodiazepine ring which aligns with the Gly-Asp amide bond NH is not necessary for good fibrinogen receptor-binding affinity.[26] In this paper, we have shown that the carbonyl at the 8-position which corresponds to the Arg-Gly amide bond carbonyl is also not important for good activity. In addition, we have demonstrated that the 3-oxo-1,4-benzodiazepine or 3-oxo-2-benzazepine displays substantially better antiaggregatory activity than several closely related ring systems. Computational analysis reveals that these two ring systems are able to adopt a specific conformation of the seven-membered ring which may be associated with enhanced activity. The overall data suggest that the high potency in this series of compounds arises from the orientation of the ring amide bond and the conformational constraint it imposes on the seven-membered ring[35] to position the key acidic and basic groups in the preferred relative orientation.

Experimental Section

General. Melting points were measured with a Thomas-Hoover melting point apparatus and are uncorrected. Proton NMR spectra were obtained using Bruker AM-250 or AM-400

spectrometers and are reported as ppm downfield from TMS with multiplicity, number of protons, and coupling constant(s) in hertz (Hz) indicated parenthetically. Elemental analyses were obtained using a Perkin-Elmer 240C elemental analyzer. Chromatography refers to flash chromatography using Kieselgel 60, 230−400 mesh silica gel.

NMR Spectroscopy of 22c. ^{1}H NMR spectra were measured at 400.13 MHz using a Bruker Instruments AMX 400 for 10 mg/mL solutions of **22c** in acetone-d_6. Nuclear Overhauser enhancements (NOE) were determined by difference spectroscopy, requiring collection of a series of spectra in which the resonance frequencies of interest, including an off-resonance control, were selectively irradiated with low (60-db) RF power for 4 s prior to acquisition. For each spectrum eight scans were averaged; then the process was repeated for four cycles. Following Fourier transformation, difference spectra were generating by subtracting the control spectrum from each irradiated spectrum.

Variable-temperature spectra were measured in both solvents with the AMX 400 instrument using a Eurotherm temperature controller, calibrated against an external methanol standard. Spectra were measured over a range of ca. 100 K with the lowest temperature (ca. −70 °C) limited by the characteristics of the solvent.

Methyl (*R,S*)-8-Amino-1,2,4,5-tetrahydro-3-oxo-4-(2-phenylethyl)-3*H*-1,4-benzodiazepine-2-acetate (24). A mixture of carboxylic acid **23**[24a] (0.6 g, 1.3 mmol) and thionyl chloride (3 mL) was heated to reflux under argon for 15 min. The mixture was concentrated in vacuo, treated with methylene chloride (3 × 20 mL), and concentrated in vacuo to give the acid chloride as a yellow solid. The acid chloride was dissolved in dry acetone (6 mL) and added dropwise to a solution of sodium azide (120 mg, 1.8 mmol) in water (3 mL) stirred in an ice bath. The mixture was stirred for 1 h, diluted with water (15 mL), and extracted with ethyl acetate. The organic phase was dried with magnesium sulfate and concentrated in vacuo. The residue was dissolved in dry toluene (12 mL) and heated to 80 °C in an argon atmosphere for 2 h. The mixture was concentrated in vacuo, and the residue was stirred in 3 N HCl (6 mL) and tetrahydrofuran (10 mL) for 1 h. The mixture was concentrated in vacuo, and solid sodium bicarbonate was added to pH 8. The mixture was extracted with ethyl acetate, and the organic phase was dried with magnesium sulfate and concentrated in vacuo to give **24** (0.3 g, 67%).

(*R,S*)-8-[(4-Amidinobenzoyl)amino]-1,2,4,5-tetrahydro-3-oxo-4-(2-phenylethyl)-3*H*-1,4-benzodiazepine-2-acetic Acid (5). A mixture of 4-[*N*-(benzyloxycarbonyl)amidino]-benzoic acid (0.23 g, 0.001 mol) and thionyl chloride (3 mL) in methylene chloride (3 mL) was heated to reflux for 10 min, concentrated in vacuo, treated with toluene, and concentrated in vacuo several times to give 4-[*N*-(benzyloxycarbonyl)-amidino]benzoyl chloride. The acid chloride was dissolved in methylene chloride (3 mL) and added dropwise to a solution of **24** (0.3 g, 0.9 mmol) and diisopropylethylamine (130 mg, 1 mmol) in dry methylene chloride (5 mL). The mixture was stirred for at room temperature under argon for 5 h, diluted with methylene chloride (20 mL), and extracted with water, 3 N HCl, 5% sodium bicarbonate, and brine. The organic phase was dried with magnesium sulfate and concentrated in vacuo. The residue was chromatographed on silica gel eluted with 2:98 methanol:methylene chloride to give the coupled product (0.17 g, 32%). A solution of the coupled product (0.1 g, 0.15 mmol) in methanol (40 mL) containing 3 N HCl (8 drops) and 10% Pd/C (20 mg) was shaken in a hydrogen atmosphere (45 psi) for 30 min. The mixture was filtered and the filtrate concentrated in vacuo. The crude amidine was dissolved in methanol (15 mL), water (2 mL), and 1 N NaOH (1 mL) and was stirred at room temperature under argon overnight. The mixture was treated with 3 N HCl (1 mL) and concentrated in vacuo. The residue was dissolved in 33:67 acetonitrile:water and purified by HPLC to give **5** (26 mg, 33%): MS (EI) *m/e* 486 $(M + H)^+$.

Methyl (*R,S*)-2,3,4,5-Tetrahydro-8-(hydroxymethyl)-3-oxo-4-(2-phenylethyl)-1*H*-1,4-benzodiazepine-2-acetate (25). Carboxylic acid **23** (3.8 g, 10 mmol) was treated with thionyl chloride (50 mL), and the resulting suspension was heated at reflux under argon with stirring for 15 min, cooled to room temperature, and concentrated. The residual oil was redissolved in toluene (25 mL) and concentrated (2×), and the residue was taken up in dry tetrahydrofuran (50 mL). The solution was stirred at room temperature, and sodium borohydride (2.0 g, 53 mmol) as added in one portion. After stirring for 16 h, the reaction was carefully quenched at 0 °C with 1 N hydrochloric acid, basified with 1 N sodium carbonate, extracted with ethyl acetate (150 mL), washed with brine, dried (magnesium sulfate), and concentrated. Purification by flash chromatography (1% methanol:chloroform) gave **25** (1.71 g, 47%) as a solid foam: ^{1}H NMR ($CDCl_3$) δ 2.62 (dd, 1H), 2.77 (m, 2H), 2.97 (dd, 1H), 3.27 (br s, 2H), 3.68 (m, 3H), 3.72 (s, 3H), 4.51 (s, 2H), 4.94 (t, 1H), 5.24 (d, J = 16.5 Hz, 1H), 6.56 (s, 1H), 6.62 (d, J = 7.4, 1H), 6.80 (d, J = 7.7 Hz, 1H), 7.20 (m, 5H).

Methyl (*R,S*)-8-[4-[*N*-(*tert*-Butoxycarbonyl)piperidin-4-yl]-1(*E*)-butenyl]-2,3,4,5-tetrahydro-3-oxo-4-(2-phenylethyl)-1*H*-1,4-benzodiazepine-2-acetate (26) and Methyl (*R,S*)-8-[4-[*N*-(*tert*-Butoxycarbonyl)piperidin-4-yl]-1(*Z*)-butenyl]-2,3,4,5-tetrahydro-3-oxo-4-(2-phenylethyl)-1*H*-1,4-benzodiazepine-2-acetate (27). (a) To a solution of alcohol **25** (1.20 g, 3.3 mmol) and carbon tetrabromide (1.4 g, 4.2 mmol) in dry tetrahydrofuran (40 mL) stirred at 0 °C under argon was added triphenylphosphine (1.1 g, 4.2 mmol) in one portion. After 10 min, the reaction was allowed to warm to room temperature and stirred for 3 h. The mixture was concentrated, and the residue was purified by flash chromatography (50% ethyl acetate:hexane) to yield the bromomethyl intermediate (1.17 g, 83%). To this compound (1.15 g, 2.67 mmol) in dry tetrahydrofuran (30 mL) was added triphenylphosphine (0.71 g, 2.7 mmol). The reaction was refluxed for 4 h, cooled to room temperature, concentrated, and triturated with ether, filtered, and dried under vacuum to give the triphenylphosphonium bromide Wittig reagent (1.89 g, 100%): ^{1}H NMR ($CDCl_3$) δ 2.01 (1H, s), 2.73 (2H, m), 2.90 (1H, dd), 3.08 (1H, dd), 3.54 (1H, m), 3.65 (1H, m), 3.70 (3H, s), 3.74 (1H, d, J = 16.7 Hz), 4.49 (2H, dt), 5.08 (1H, d, J = 15.5 Hz), 5.17 (1H, t), 6.40 (1H, d), 6.58 (1H, d, J = 11.4 Hz), 6.87 (1H, s), 7.18 (5H, m), 7.58−7.80 (15H, m).

(b) To a stirred solution of oxalyl chloride (2 mL, 22 mmol) in dry dichloromethane (50 mL) at −78 °C under argon was added dropwise a solution of dimethyl sulfoxide (3.4 mL, 44 mmol) in dichloromethane (10 mL). After 2 min, a solution of 3-[*N*-(*tert*-butoxycarbonyl)piperidin-4-yl]propanol (4.86 g, 20 mmol) in dichloromethane (10 mL) was added dropwise over 5 min. After stirring an additional 15 min at −78 °C, triethylamine (14 mL) was added dropwise. After 5 min, the reaction became a thick white slurry and was allowed to warm to room temperature and stirred for 16 h. The reaction mixture was washed with cold 1 N hydrochloric acid and with brine, dried (magnesium sulfate), and concentrated. The residue was purified by flash chromatography (30% ethyl acetate:hexane) to yield 3-[*N*-(*tert*-butoxycarbonyl)piperidin-4-yl]propionaldehyde (4.18 g, 87%) as a clear oil which solidified in the freezer: ^{1}H NMR ($CDCl_3$) δ 0.95−1.80 (7H, m), 1.48 (9H, s), 2.40−2.90 (4H, m), 4.14 (2H, br dt), 9.88 (1H, t).

(c) To a solution of the Wittig reagent (1.0 g, 1.44 mmol) and 3-[*N*-(*tert*-butoxycarbonyl)piperidin-4-yl]propionaldehyde (0.42 g, 1.75 mmol) in dry tetrahydrofuran (30 mL) was added, with stirring under argon, a 60% dispersion of sodium hydride in mineral oil (58 mg, 1.44 mmol) in one portion. After stirring for 16 h, the reaction was concentrated. The residue was purified by flash chromatography (5% ethyl acetate:chloroform) to give a mixture of the title compounds (0.717 g, 86%). Pure olefin isomers in the ratio of approximately 1:2 *Z*:*E* were obtained by HPLC chromatography [silica gel (Apex, 8 × 250 mm), 40% ethyl acetate:hexane]. (*E*)-Isomer **26**: ^{1}H NMR ($CDCl_3$) δ 1.11 (2H, m), 1.43 (4H, m), 1.45 (9H, s), 1.68 (2H, d, J = 12.5 Hz), 2.20 (2H, dd), 2.67 (2H, dt), 2.72 (1H, dd), 2.81 (1H, dd), 3.72 (3H, m), 3.74 (3H, s), 4.08 (2H, d, J = 13.7 Hz),

4.90 (1H, t), 5.18 (1H, d, J = 16.5 Hz), 6.14 (1H, dt, J_{trans} = 15.8 Hz), 6.24 (1H, d, J_{trans} = 15.8 Hz), 6.67 (1H, s), 6.72 (1H, dd, J = 7.8 Hz), 6.80 (1H, d, J = 7.8 Hz), 7.20 (5H, m); MS (ES) *m/e* 576.2 $(M + H)^+$. (*Z*)-Isomer **27**: ^{1}H NMR ($CDCl_3$) δ 1.08 (2H, m), 1.41 (4H, m), 1.45 (9H, s), 1.62 (2H, d, J = 12.9 Hz), 2.31 (2H, dd), 2.64 (2H, dt), 2.72 (1H, dd), 2.82 (2H, dt), 3.03 (1H, dd), 3.73 (3H, m), 3.75 (3H, s), 4.06 (2H, d, J = 13.2 Hz), 4.93 (1H, t), 5.22 (1H, d, J = 16.5 Hz), 5.59 (1H, dt, J_{cis} = 11.6 Hz), 6.27 (1H, d, J_{cis} = 11.7 Hz), 6.55 (1H, s), 6.63 (1H, d, J = 7.7 Hz), 6.82 (1H, d, J = 7.7 Hz), 7.20 (5H, m); MS (ES) *m/e* 576.2 $(M + H)^+$.

(*R*,*S*)-2,3,4,5-Tetrahydro-3-oxo-4-(2-phenylethyl)-8-[4-(piperidin-4-yl)-1(*E*)-butenyl]-1*H*-1,4-benzodiazepine-2-acetic Acid (8). Compound **26** (273 mg, 0.47 mmol) was dissolved in dioxane (50 mL), and 1.0 N NaOH (0.9 mL, 0.9 mmol) was added. The solution was stirred at room temperature overnight, acidified with 1.0 N HCl (0.9 mL, 0.9 mmol), diluted with $CHCl_3$ (50 mL), washed with brine, dried ($MgSO_4$), and evaporated. The residue which remained was treated with neat TFA (20 mL), stirred at room temperature for 30 min, evaporated to dryness, reevaporated from toluene several times, triturated with petroleum ether/ether, and then dried under vacuum. The solid which remained was purified by reversed-phase flash chromatography (Hamilton PRP-1 (10 × 250 mm) eluted with 40% CH_3CN, H_2O, 0.1% TFA) to give the title compound: ^{1}H NMR (CD_3OD) δ 1.38 (2H, m), 1.45 (2H, m), 1.61 (1H, br s), 1.95 (2H, d, J = 13.6 Hz), 2.22 (2H, dd), 2.61 (1H, m), 2.77 (2H, m), 2.91 (3H, m), 3.35 (2H, m), 3.67 (2H, m), 3.79 (1H, d, J = 16.8 Hz), 5.02 (1H, dd), 5.37 (1H, d, J = 16.5 Hz), 6.15 (1H, dt), 6.26 (1H, d, J = 15.9 Hz), 6.58 (1H, s), 6.60 (1H, d, J = 7.8 Hz), 6.81 (1H, d, J = 7.8 Hz), 7.17 (5H, m); MS (ES) *m/e* 462.2 $(M + H)^+$. Anal. ($C_{28}H_{35}N_3O_3 \cdot 1.5CF_3CO_2H$) Calcd: C, 58.86; H, 5.82; N, 6.64. Found: C, 58.68; H, 5.84; N, 6.32.

(*R*,*S*)-2,3,4,5-Tetrahydro-3-oxo-4-(2-phenylethyl)-8-[4-(piperidin-4-yl)-1(*Z*)-butenyl]-1*H*-1,4-benzodiazepine-2-acetic Acid (9). Compound **27** (185 mg, 0.32 mmol) was hydrolyzed and deprotected as in the preparation of compound **8** to give the title compound: ^{1}H NMR (CD_3OD) δ 1.30 (2H, m), 1.41 (2H, dd), 1.58 (1H, br s), 1.82 (2H, d, J = 13.1 Hz), 2.34 (2H, m), 2.60 (1H, m), 2.76 (2H, m), 2.88 (3H, m), 3.29 (3H, m), 3.69 (2H, m), 3.81 (1H, d, J = 16.9 Hz), 5.04 (1H, m), 5.40 (1H, d, J = 16.6 Hz), 5.58 (1H, dt), 6.31 (1H, d, J = 11.6 Hz), 6.48 (1H, d, J = 7.9 Hz), 6.50 (1H, s), 6.85 (1H, d, J = 7.6 Hz), 7.18 (5H, m); MS (ES) *m/e* 462.2 $(M + H)^+$. Anal. ($C_{28}H_{35}N_3O_3 \cdot 1.5CF_3CO_2H \cdot 0.5H_2O$) Calcd: C, 57.83; H, 5.73; N, 6.27. Found: C, 58.03; H, 5.89; N,6.55.

(*R*,*S*)-2,3,4,5-Tetrahydro-3-oxo-4-(2-phenylethyl)-8-[4-(piperidin-4-yl)butyl]-1*H*-1,4-benzodiazepine-2-acetic Acid (10). A mixture of the (*E*/*Z*)-isomers **26** and **27** and 10% palladium on carbon in acetic acid was shaken under hydrogen (50 psi) for 4 h to give the saturated compound, which was deprotected and hydrolyzed as in the preparation of compound **8** to give the title compound: ^{1}H NMR (CD_3OD) δ 1.32 (6H, m), 1.57 (3H, m), 1.87 (2H, d, J = 14.0 Hz), 2.49 (2H, t), 2.61 (1H, dd), 2.75 (2H, m), 2.90 (3H, m), 3.30 (2H, m), 3.68 (2H, m), 3.80 (1H, d, J = 16.7 Hz), 5.02 (1H, dd), 5.37 (1H, d, J = 16.5 Hz), 6.41 (1H, d, J = 8.0 Hz), 6.42 (1H, s), 6.80 (1H, d, J = 8.0 Hz), 7.17 (5H, m); MS (ES) *m/e* 464.2 $(M + H)^+$. Anal. ($C_{28}H_{37}N_3O_3 \cdot CF_3CO_2H \cdot 1.25H_2O$) Calcd: C, 59.91; H, 6.51; N, 6.73. Found: C, 60.04; H, 6.80; N, 7.00.

5-Carboxy-2-(methoxycarbonyl)nitrobenzene (28). To a stirred solution of dimethyl nitroterephthalate (12 g, 50 mmol) in dioxane (100 mL) was slowly added at room temperature aqueous 1 N NaOH (50 mL) dropwise over 30 min. After stirring overnight at room temperature the reaction was diluted with water, washed with ether, acidified with aqueous 1 N HCl (50 mL), and then extracted several times with ethyl acetate. After the ethyl acetate phase was dried over $MgSO_4$ and evaporated, the resulting residue was purified by flash chromatography eluting with 98:2:0.1 $CHCl_3$, MeOH, HOAc to give product **28** (8.07 g, 72%): ^{1}H NMR ($CDCl_3$) δ 3.96 (3H, s), 7.80 (1H, d), 8.36 (1H, dd), 8.57 (1H, d).

5,*N*-Bis(*tert*-butoxycarbonyl)-2-(methoxycarbonyl)aniline (29). To a stirred suspension of the acid **28** (7.0 g, 31 mmol) in toluene (25 mL) was added oxalyl chloride (5 mL, 57 mmol) followed by 1 drop of dry DMF. After stirring overnight (the solution became clear after ~30 min), the reaction was evaporated to dryness and reevaporated from fresh toluene two times. To the resulting acid chloride were added $CHCl_3$ (10 mL) and *t*-BuOH (1 mL) followed by pyridine (0.6 mL). After stirring for 6 h the reaction was evaporated, taken up in ethyl acetate, washed with 1 N $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated. Purification by flash chromatography eluting with 10% ethyl acetate in hexane gave the intermediate ester as an oil (7.28 g, 83%): ^{1}H NMR ($CDCl_3$) δ 1.6 (9H, s), 3.95 (3H, s), 7.83 (1H, d, J = 8 Hz), 8.3 (1H, dd), 8.5 (1H, d, J = 2 Hz). A solution of the ester (7.28 g, 26 mmol) in MeOH (100 mL) was hydrogenated over 5% Pd/C (0.5 g) at 50 psi H_2 in a Parr shaker for 4 h. After filtration of the catalyst through a pad of Celite and evaporation of the filtrate, the remaining residue was taken up in CH_2Cl_2 (100 mL) to which was added di-*tert*-butyl dicarbonate (6.2 g, 28.4 mmol) followed by DMAP (0.6 g, 5 mmol) with stirring. The reaction was refluxed for 16 h, then evaporated to dryness, taken up in ethyl acetate, washed with 1 N HCl and brine, dried over $MgSO_4$, and evaporated. Purification by flash chromatography on silica gel eluted with 10% ethyl acetate in *n*-hexane gave product **29** (3.68 g, 40%): ^{1}H NMR ($CDCl_3$) δ 1.55 (9H, s), 1.6 (9H, s), 3.93 (3H, s), 7.6 (1H, dd), 8.05 (1H, d, J = 8 Hz), 9.1 (1H, d, J = 2 Hz), 10.28 (1H, s).

(*Z*)-Methyl 4-[*N*-[4,*N*-Bis(*tert*-butoxycarbonyl)-2-aminobenzoyl]-*N*-(2-phenylethyl)amino]-2-butenoate (30). To a stirred solution of ester **29** (3.68 g, 10.5 mmol) in dioxane (50 mL) was added aqueous 1 N NaOH (12 mL). The reaction was stirred for 3 h, acidified with aqueous 1 N HCl (12 mL), extracted with ethyl acetate, washed with brine, dried over $MgSO_4$, and evaporated. To the remaining solid were added DMF (75 mL), (*Z*)-methyl 4-(phenethylamino)-2-butenoate[27] (3.7 g, 16.9 mmol), Et_3N (7.3 mL, 52 mmol), and HOBt (2.8 g, 20.7 mmol) followed by BOP reagent (5.6 g, 12.7 mmol). After stirring overnight the reaction was evaporated to dryness. Purification by flash chromatography eluting with 99:1 $CHCl_3$, MeOH gave product **30** as a solid foam (3.59 g, 62%).

Methyl (*R*,*S*)-8-Carboxy-1,2,4,5-tetrahydro-5-oxo-4-(2-phenylethyl)-5*H*-1,4-benzodiazepine-2-acetate (31). To **30** (3.59 g) was added 90% TFA in CH_2Cl_2 (50 mL). After stirring at room temperature for 45 min the reaction was evaporated to dryness and then reevaporated from anhydrous MeOH two times. The residue which remained was taken up in MeOH (100 mL) and refluxed for 2 days under Ar. Evaporation to dryness gave **31** as a slightly yellow solid (2.5 g, 100%): ^{1}H NMR ($CDCl_3$) δ 2.57 (2H, m), 2.88 (2H, m), 3.41 (1H, dd), 3.50 (2H, m), 3.66 (3H, s), 3.92 (2H, m), 6.18 (1H, s), 6.97 (1H, d), 7.10−7.38 (5H, m), 7.44 (1H, s), 7.64 (1H, d); MS $(M + H)^+$ = 383.3.

(*R*,*S*)-8-[[(4-Amidinophenyl)amino]carbonyl]-1,2,4,5-tetrahydro-5-oxo-4-(2-phenylethyl)-5*H*-1,4-benzodiazepine-2-acetic Acid (12). To a stirred solution of 4-amidinoaniline dihydrochloride (2.1 g, 10 mmol) in aqueous 1 N NaOH (20 mL), H_2O (10 mL), and THF (10 mL) was added di-*tert*-butoxycarbonyl dicarbonate (2.4 g, 11 mmol). After stirring for 5 h the reaction was extracted with ethyl acetate three times, dried over Na_2SO_4, and evaporated. Purification by flash chromatography on silica gel eluted with 96:4 $CHCl_3$, MeOH gave 4-(Boc-amidino)aniline as a white solid (1.31 g, 56%): ^{1}H NMR ($CDCl_3$) δ 1.44 (9H, s), 5.74 (2H, s), 6.52 (2H, d), 7.70 (2H, d); TLC R_f 0.38 (5% MeOH, $CHCl_3$). To the acid **31** (0.52 g, 1.4 mmol) was added thionyl chloride (5 mL, 69 mmol). After refluxing for 15 min under Ar the reaction was evaporated and then reevaporated from fresh toluene two times. To the resulting acid chloride in CH_2Cl_2 (30 mL) cooled to 0 °C, with stirring under Ar, was added pyridine (0.33 mL, 4 mmol) followed by 4-(Boc-amidino)aniline (0.48 g, 2 mmol). The reaction was stirred for 4 h at room temperature, diluted with chloroform, washed with aqueous 1 N $NaHCO_3$, dried over Na_2SO_4, and evaporated. Purification by flash chromatography eluting with 98:2 $CHCl_3$, MeOH gave the coupled

product as a solid (585 mg, 71%): ^{1}H NMR ($CDCl_3$) δ 1.52 (9H, s), 2.37 (1H, dd), 2.52 (1H, dd), 2.93 (2H, t), 3.17 (1H, dd), 3.30 (1H, d), 3.48 (1H, dt), 3.67 (3H, s), 3.90 (1H, br s), 4.03 (1H, dt), 4.67 (1H, s), 6.90−7.85 (12H, m).

To the above (585 mg, 1.0 mmol) was added 90% TFA in CH_2Cl_2 (20 mL). After stirring for 45 min the reation was evaporated to dryness. The remaining residue was then taken up in 20% HOAc in water and refluxed under Ar for 24 h. Evaporation left a residue which was purified by prep-HPLC on a PRP-1 column eluted with 26% CH_3CN, 0.1% TFA/0.1% TFA, H_2O to give product **12** as a white solid: ^{1}H NMR (DMSO-d_6, 2%TFA) δ 2.52 (4H, m), 2.89 (2H, m), 3.4−3.57 (3H, m), 3.92 (2H, m), 7.27 (6H, m), 7.37 (1H, s), 7.72 (1H, d), 7.83 (2H, d), 8.0 (2H, d), 8.97 (2H, s), 9.22 (2H, s); MS $(M + H)^+$ = 486.2. Anal. ($C_{27}H_{27}N_5O_4 \cdot 2CF_3CO_2H \cdot 0.5CH_3CO_2H \cdot 2.5H_2O$).

***N*-(*tert*-Butoxycarbonyl)-4-nitroanthranilic Acid Methyl Ester (32).** 5-Nitroanthranilic acid (10 g, 55 mmol) was treated with diazomethane from Diazald (34.5 g, 110 mmol) and then protected as its Boc derivative as in the preparation of **29** to give product **32** (6.61 g, 43%): ^{1}H NMR ($CDCl_3$) δ 1.58 (9H, s), 4.0 (3H, s), 7.8 (1H, dd), 8.2 (1H, d, J = 8 Hz), 9.38 (1H, d, J = 2 Hz), 10.4 (1H, br s).

***N*-(*tert*-Butoxycarbonyl)-4-(carbobenzyloxyamino)-2-aminobenzoic Acid Methyl Ester (33).** The nitro compound **32** (6.6 g, 23.4 mmol) was hydrogenated as in the preparation of **29** and then treated with Cbz-Cl (4.0 mL, 28 mmol) to give product **33** (8.24 g, 88%): ^{1}H NMR ($CDCl_3$) δ 1.5 (9H, s), 3.87 (3H, s), 5.2 (2H, s), 7.35 (5H, s), 7.44 (1H, dd), 7.57 (1H, s), 7.97 (1H, d, J = 8 Hz), 8.42 (1H, d, J = 2 Hz), 10.46 (1H, s).

Methyl (*R,S*)-8-(Carbobenzyloxyamino)-1,2,4,5-tetrahydro-5-oxo-4-(2-phenylethyl)-5*H*-1,4-benzodiazepine-2-acetate (34). The methyl ester **33** (7.91 g, 19.8 mmol) was saponified and coupled with 4-phenethylaminocrotonate as in the preparation of **30**, then deprotected with TFA, and cyclized as in the preparation of **31** to give 3.73 g (76%) of product **34**: ^{1}H NMR ($CDCl_3$) δ 2.40 (1H, dd), 2.53 (1H, dd), 2.94 (2H, t), 3.13 (1H, dd), 3.35 (1H, dd), 3.38 (1H, dt), 3.70 (3H, s), 3.90 (1H, m), 4.10 (1H, dt), 5.16 (2H, s), 6.67 (1H, dd), 7.00 (1H, s), 7.10−7.40 (10H, m), 7.71 (1H, d, J = 8.5); MS $(M + H)^+$ = 488.3.

(*R,S*)-8-[[(4-Amidinophenyl)carbonyl]amino]-1,2,4,5-tetrahydro-5-oxo-4-(2-phenylethyl)-5*H*-1,4-benzodiazepine-2-acetic Acid (13). The Cbz-aniline derivative **34** (0.5 g, 1 mmol) was hydrogenolyzed, coupled to 4-Cbz-amidinobenzoyl chloride, and deprotected as in the preparation of **5**. Ester hydrolysis as in the preparation of **10** gave product **13** (218 mg, 48%): ^{1}H NMR (DMSO-d_6, 2%TFA) δ 2.53 (4H, m), 2.89 (2H, m), 3.53−3.36 (3H, m), 3.9 (2H, m), 7.08 (1H, dd), 7.26 (5H, m), 7.44 (1H, d), 7.6 (1H, d), 7.95 (2H, d), 8.14 (2H, d), 9.2 (2H, s), 9.43 (2H, s); MS $(M + H)^+$ = 486.2. Anal. ($C_{27}H_{27}N_5O_4 \cdot 3CF_3CO_2H \cdot 2CH_3CO_2H \cdot 3H_2O$).

(*E*)-Methyl *N*-[(2-Nitro-4-(*tert*-butoxycarbonyl)phenyl)methyl]-4-[(2-phenylethyl)amino]-2-butenoate (36). To a solution of 2-nitro-4-(*tert*-butoxycarbonyl)benzyl bromide (**35**)[27] (4.06 g, 12.8 mmol) in THF (50 mL) was added with stirring at room temperature (*E*)-methyl 4-(phenethylamino)-2-butenoate (3.4 g, 15.5 mmol) followed by triethylamine (2.2 mL, 15.7 mmol). After stirring for 16 h the reaction was diluted with ethyl acetate (50 mL), washed with 1 N Na_2CO_3 (100 mL) and brine (100 mL), dried (Na_2SO_4), and evaporated to dryness. Purification by flash chromatography on silica gel eluted with 15% ethyl acetate in *n*-hexane gave **36** as an oil (5.16 g, 88%): ^{1}H NMR ($CDCl_3$) δ 1.60 (9H, s), 2.74 (4H, br s), 3.30 (2H, dd), 3.74 (3H, s), 4.00 (2H, s), 6.00 (1H, d, J = 15 Hz), 7.02 (1H, dt), 7.25 (5H, m), 7.67 (1H, d, J = 8 Hz), 8.15 (1H, dd), 8.45 (1H, d, J = 2 Hz).

(*R,S*)-8-[[(4-Amidinophenyl)amino]carbonyl]-1,2,4,5-tetrahydro-4-(2-phenylethyl)-1,4-benzodiazepine-2-acetic Acid (14). To a solution of the nitro compound **36** (1.03 g, 2.26 mmol) in 1:1 acetic acid, ethanol (50 mL) was added with stirring at room temperature iron powder (0.8 g, 14.3 mmol). After stirring for 16 h the reaction was evaporated at reduced pressure taken up in ethyl acetate, washed with 1 N Na_2CO_3 and brine, dried (Na_2SO_4), and evaporated to give the amine (0.96 g) as an oil which was used in the next reaction. The amine was dissolved in anhydrous methanol (50 mL) and refluxed under Ar for 72 h. After stripping off the solvent the crude material was purified by flash chromatography eluted with 60% ethyl acetate in *n*-hexane to give the cyclized product (0.87 g, 91%): ^{1}H NMR ($CDCl_3$) δ 1.59 (9H, s), 2.39 (1H, dd), 2.51 (1H, dd), 2.68−3.50 (6H, m), 3.56 (1H, br s), 3.73 (3H, s), 3.95 (2H, br s), 4.47 (1H, br s), 7.15−7.27 (6H, m), 7.42 (1H, s), 7.51 (1H, d, J = 7.4 Hz); MS (ES) *m/e* 425.0 $(M + H)^+$.

To the cyclized product was added 90% TFA in CH_2Cl_2 (30 mL). After stirring at room temperature for 45 min the reaction was evaporated to dryness and then reevaporated from anhydrous toluene two times. The free acid was converted to its acid chloride and coupled with (4-Boc-amidino)aniline, and the product deprotected and hydrolzed as in the preparation of **12**. Evaporation left a residue which was purified by prep-HPLC on a PRP-1 column eluted with 25% CH_3CN, 0.1% TFA/0.1% TFA, H_2O to give product **14** as a white solid: MS $(M + H)^+$ = 472.2. Anal. ($C_{27}H_{29}N_5O_3 \cdot 2.5CF_3CO_2H \cdot 1.0H_2O$).

Methyl 3-Nitro-4-(4-acetoxybutyl)benzoate (38). A mixture of dry Mg (900 mg, 37 mmol) and $ZnBr_2$ (5.63 g, 25 mmol) in THF (25 mL) was treated with 5-bromopent-1-ene (Aldrich; 4.45 mL, 37.6 mmol), and the subsequent suspension was heated at 56 °C (oil bath) for 18 h. The mixture was cooled to room temperature, methyl 4-bromobenzoate (**37**) (Eastman; 7.1 g, 33 mmol) was added along with tetrakis(triphenylphosphine)palladium(0) (1.0 g, 0.865 mmol), and the mixture was stirred at room temperature for 24 h. The mixture was quenched with 3 N HCl (aqueous) and extracted with ethyl acetate. The combined organic extracts were dried over anhydrous $MgSO_4$ and evaporated at reduced pressure. The residue was purified by flash chromatography (5% ethyl acetate in hexane) to give partially purified methyl 4-pent-4-enylbenzoate which was carried on to the next step without further purification.

The partially purified methyl 4-pent-4-enylbenzoate was dissolved in a mixture of MeOH/CH_2Cl_2, cooled to −78 °C, and treated with excess O_3. After the excess O_3 was purged, solid $NaBH_4$ (2.50 g, 66 mmol) was added and the solution slowly brought to room temperature overnight. The reaction mixture was evaporated at reduced pressure and the residue taken into ethyl acetate. It was then washed with 1 N HCl (aqueous), dried over anhydrous $MgSO_4$, and evaporated at reduced pressure. The residue was purified by flash chromatography (30−40% ethyl acetate in hexane) to give 2.13 g of a partially purified methyl 4-(4-hydroxybutyl)benzoate which was carried on to the next step without further purification.

The partially purified methyl 4-(4-hydroxybutyl)benzoate was dissolved in dry pyridine (25 mL) and treated with acetic anhydride (1.93 mL, 20.4 mmol) at room temperature for 24 h. The solvent was evaporated at reduced pressure, and the residue dissolved in ethyl acetate. This solution was washed with 1 N HCl (aqueous), dried over anhydrous $MgSO_4$, and evaporated at reduced pressure. The residue was purified by flash chromatography (15% ethyl acetate in hexane) to give partially purified methyl 4-(4-acetoxybutyl)benzoate which was carried on to the next step without further purification.

The partially purified methyl 4-(4-acetoxybutyl)benzoate was dissolved in acetic anhydride (5 mL) and the solution cooled to −12 °C. A mixture of fuming HNO_3 (4.1 mL) and concentrated H_2SO_4 (3.4 mL) was added dropwise over 20 min and the reaction stirred an additional 40 min at −12 °C. The reaction mixture was then poured onto ice and then extracted with Et_2O. The Et_2O extracts were washed carefully with 5% Na_2CO_3 (aqueous), dried over anhydrous $MgSO_4$, and evaporated at reduced pressure. The residue was first purified by flash chromatography (15−25% ethyl acetate in hexane) to give 1.12 g (11.5% overall) of **38**: ^{1}H NMR ($CDCl_3$) δ 1.57−1.90 (m, 4H), 2.03 (s, 3H), 2.77−3.10 (m, 2H), 3.93 (s, 3H), 3.97−4.20 (m, 2H), 7.43 (d, 1H, J = 7.5 Hz), 8.13 (d of d, 1H, J = 7.5, 1.5 Hz), 8.47 (d, 1H, J = 1.5 Hz); MS (ES) *m/e* 296 $(M + H)^+$.

Benzyl 3-Nitro-4-(4-hydroxybutyl)benzoate (39). The ester **38** (1.04 g, 3.51 mmol) in dioxane (36 mL) was treated

with 18 mL of 1 M NaOH (aqueous) at room temperature for 3 days. The reaction mixture was acidified with 1 N HCl (aqueous) and extracted with ethyl acetate. The combined organic extracts were dried over anhydrous $MgSO_4$ and evaporated at reduced pressure. The partially hydrolyzed material was resubmitted to the above conditions at room temperature for 3 days to give crude 3-nitro-4-(4-hydroxybutyl)benzoic acid which was used after workup without further purification.

The crude 3-nitro-4-(4-hydroxybutyl)benzoic acid was dissolved in aqueous MeOH and neutralized to pH = 7.6 with solid $CsHCO_3$. The solution was then evaporated under vacuum to dryness and evaporated from MeOH/toluene to remove traces of H_2O. The salt was then dissolved in anhydrous DMF (30 mL) and treated with benzyl bromide (1 mL, 8.41 mmol). The subsequent suspension was heated at 57 °C (oil bath) for 18 h. The reaction mixture was evaporated under vacuum and the residue dissolved in ethyl acetate. The solution was washed with 1 N HCl (aqueous), dried over anhydrous $MgSO_4$, and evaporated at reduced pressure. The residue was purified by flash chromatography (40% ethyl acetate in hexane) to give 624 mg (54%) of **39**: ^{1}H NMR ($CDCl_3$) δ 1.43−1.95 (m, 5H), 2.93 (t, 2H, J = 7.5 Hz), 3.67 (t, 2H, J = 6 Hz), 5.37 (s, 2H), 7.37 (s, 5H), 7.40 (d, 1H, J = 7.5 Hz), 8.13 (d of d, 1H, J = 7.5, 1.5 Hz), 8.74 (d, 1H, J = 1.5 Hz).

(*E*)-Benzyl 3-Nitro-4-(5-carbethoxypent-4-enyl)benzoate (40). The alcohol **39** (271 mg, 0.823 mmol) in CH_2Cl_2 (10 mL) was treated with pyridinium chlorochromate (355 mg, 1.65 mmol) at room temperature for 4 h. The reaction mixture was diluted with Et_2O, filtered through a pad of Florisil, and evaporated at reduced pressure. The residue was dissolved in ethyl acetate and refiltered through a pad of Florisil. The crude aldehyde in CH_2Cl_2 (10 mL) was treated with (carbethoxymethylene)triphenylphosphorane (344 mg, 0.988 mmol) and stirred at room temperature for 3 days. The reaction mixture was evaporated at reduced pressure, and the residue was purified by flash chromatography (20% ethyl acetate in hexane) to give 195 mg (60%) of **40**: ^{1}H NMR ($CDCl_3$) δ 1.27 (t, 3H, J = 7.5 Hz), 1.63−2.03 (m, 2H), 2.13−2.47 (m, 2H), 2.80−3.10 (m, 2H), 4.17 (q, 2H, J = 7.5 Hz), 5.37 (s, 2H), 5.83 (br d, 1H, J = 16.5 Hz), 6.93 (d of t, 1H, J = 16.5, 7.5 Hz), 7.40 (s, 5H), 7.40 (d, 1H, J = 9 Hz), 8.15 (d of d, 1H, J = 9, 1.5 Hz), 8.50 (d, 1H, J = 1.5 Hz).

2-(Carbethoxymethyl)-8-(carbobenzyloxy)-tetrahydro-1-benzazepine (41). The nitro compound **40** (304 mg, 0.765 mmol) in dry EtOH (10 mL) was treated with $SnCl_2$ (725 mg, 3.83 mmol), and the mixture was heated at reflux for 18 h. The reaction mixture was evaporated and the residue taken into ethyl acetate and 5% Na_2CO_3 (aqueous). The resulting precipitate was filtered off and discarded and the organic layer separated, dried over anhydrous $MgSO_4$, and evaporated at reduced pressure. The residue was purified by flash chromatography (15−50% ethyl acetate in hexane) to give 128 mg of **41** contaminated with about 20% of the corresponding diethyl ester. An additional amount (67 mg) of the uncyclized aniline was also isolated. Compound **41**: ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 3H, J = 6 Hz), 1.43−2.07 (m, 4H), 2.33−2.57 (m, 2H), 2.67−2.93 (m, 2H), 3.17−3.50 (m, 1H), 4.13 (q, 2H, J = 6 Hz), 4.30 (br s, 1H), 5.32 (s, 2H), 7.00−7.67 (m, 8H).

(*R,S*)-2-(Carboxymethyl)-8-[[(4-amidinophenyl)amino]-carboxy]-tetrahydro-1-benzazepine (15). A suspension of benzazepine **41** (128 mg, 0.348 mmol) contaminated with about 20% of the diethyl ester and 5% Pd/C (20 mg) in MeOH was treated with H_2 at 50 psi (Parr apparatus) at room temperature for 3 h. The reaction mixture was filtered through a plug of Celite and evaporated at reduced pressure to give the free acid. The acid was converted to its acid chloride and reacted with 4-(*N*-Cbz-amidino)aniline (141 mg, 0.522 mmol) as in the preparation of **12** to give the coupled product: ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 3H, J = 7.5 Hz), 1.30−2.03 (m, 4H), 2.30−2.50 (m, 2H), 2.60−2.90 (m, 2H), 3.10−3.50 (m, 1H), 4.10 (q, 2H, J = 7.5 Hz), 4.30 (br s, 1H), 5.17 (s, 2H), 6.93−7.90 (m, 14H), 8.40 (s, 1H); MS (ES) *m/e* 529 $(M + H)^+$. The coupled product was deprotected and hydrolyzed as in the preparation of **5** to give **15**: MS (ES) *m/e* 367 $(M + H)^+$.

(*R,S*)-Dimethyl *N*-(3-Carboxyphenyl)aspartate (43). To a solution of 3-aminobenzoic acid (**42**) (1.04 g, 7.5 mmol) in methanol (40 mL) was added dimethyl acetylenedicarboxylate (1.25 g, 8.75 mmol). The solution was heated at reflux for 1 h, cooled to room temperature, and concentrated. The resulting oil was chromatographed (5:95 methanol:dichloromethane−0.5% acetic acid) to give a yellow solid (2.0 g, 95%). The solid was dissolved in methanol (100 mL) containing 10% palladium on carbon (0.40 g), and the solution was shaken in a hydrogen atmosphere (35 psi) for 3 h. The mixture was filtered, and the filtrate was concentrated to give **43** as a pale-yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.55 (d, 1H), 7.45 (s, 1H), 7.3 (t, 1H), 4.55 (t, 1H), 3.8 (s, 3H), 3.7 (s, 3H), 2.95 (d, 2H).

(*R,S*)-*N*-[3-[[(4-Amidinophenyl)amino]carbonyl]phenyl]-aspartic Acid Dihydrochloride (16). The acid **43** (0.5 g, 1.78 mmol) was refluxed for 5 min in thionyl chloride. The solution was concentrated to an oil which was treated with dichloromethane (25 mL) and concentrated three times. The resulting oil was dissolved in dichloromethane (5 mL) and the solution added dropwise to a stirred solution of 4-(*N*-Cbz-amidino)aniline (0.5 g, 1.85 mmol) and diisopropylethylamine (0.24 g, 1.9 mmol) in dichloromethane (50 mL). After 16 h, the solution was treated with diisopropylethylamine (0.24 g, 1.9 mmol), extracted with water (2 × 25 mL), and dried (sodium sulfate). The organic phase was concentrated, and the resulting brown solid was purified by preparative TLC. The resultant pale-yellow solid was deprotected and the ester hydrolyzed as in the preparation of **5**. Purification by preparative HPLC gave a white solid which was dissolved in water (20 mL) and 6 N hydrochloric acid (1.0 mL). Lyophilization gave the title compound (0.04 g, 31%) as a colorless solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.3 (s, 2H), 8.9 (s, 2H), 8.0 (d, 2H), 7.7 (d, 2H), 7.3−7.1 (m, 3H), 6.85 (d, 1H), 4.4 (t, 1H), 2.8 (dd, 1H), 2.7 (dd, 1H); MS (ES) *m/e* 371.2 $(M + H)^+$.

***tert*-Butyl *N*-[(2-Fluoro-5-nitrophenyl)methyl]-3-aminopropionate (44).** 3-Aminopropionic acid *tert*-butyl ester (9.7 g, 53.3 mmol) and 2-fluoro-5-nitrobenzaldehyde (9.0 g, 53.3 mmol) were added to a suspension of sodium acetate (6.5 g, 78.3 mmol) in methanol (150 mL), followed by portionwise addition of sodium cyanoborohydride (6.5 g, 0.1 mol). After 2 h at room temperature, the reaction mixture was quenched with ice and diluted with sodium bicarbonate solution. The mixture was extracted with EtOAc, and the combined organic extracts were dried over $MgSO_4$. Filtration and evaporation of the solvent in vacuo yielded the title compound as a yellow oil (15.7 g, 99%): MS *m/e* 299 $(M + H)^+$.

***tert*-Butyl *N*-[(2-Fluoro-5-nitrophenyl)methyl]-*N*-(2-aminoacetyl)-3-aminopropionate (45).** A solution of compound **44** (17.3 g, 58.1 mmol) in CH_2Cl_2 (250 mL) was stirred at room temperature under an argon atmosphere. Triethylamine (18 mL, 128 mmol) and BOP reagent (28.2 g, 63.9 mmol) were added, followed by Boc-glycine (11.2 g, 63.9 mmol). The reaction mixture was stirred overnight at room temperature and poured into ice water (300 mL). The mixture was extracted with ethyl acetate. The combined organic extracts were washed with 1 M $KHSO_4$, water, 5% $NaHCO_3$, and brine and dried over $MgSO_4$. Filtration of the mixture and evaporation of the filtrate in vacuo yielded the intermediate Boc-protected amine which was stirred in CH_2Cl_2 (250 mL) and 4 M HCl/dioxane (25 mL) at 0 °C for 3 h. Evaporation of the solvents in vacuo yielded the free amine **45** (20.0 g, 100%): MS *m/e* 356 $(M + H)^+$.

***tert*-Butyl 7-Amino-3-oxo-1,2,3,5-tetrahydro-4*H*-1,4-benzodiazepine-2-propanoate (46).** To a solution of the amine **45** (12.0 g, 30.7 mmol) in DMSO (400 mL) were added triethylamine (15 mL) and water (5 mL), and the reaction was stirred overnight. The reaction mixture was poured into water (500 mL) and extracted with ethyl acetate. The combined organic phases were washed with aqueous brine and dried over sodium sulfate. Filtration and concentration of the organic extracts in vacuo yielded the cyclized nitro compound (1.8 g, 18%).

A solution of the nitro compound (2.2 g, 6.6 mmol) and platinum oxide catalyst (0.6 g) in ethyl acetate (100 mL) was shaken on a Parr shaker under hydrogen (40 psi) for 1 h. The catalyst was filtered from the solution, and the solvent was evaporated in vacuo to yield **46** (2.0 g, 98%): MS *m/e* 306 (M + H)$^+$.

7-[[(4-Amidinophenyl)carbonyl]amino]-3-oxo-1,2,3,5-tetrahydro-4*H*-1,4-benzodiazepine-4-propanoic Acid (18). A mixture of compound **46** (2.0 g, 6.6 mmol), 4-[*N*-(benzyloxycarbonyl)amidino]benzoic acid (2 g, 6.6 mmol), BOP reagent (2.9 g, 6.6 mmol), and triethylamine (1.6 mL, 13.2 mmol) in DMF (20 mL) was stirred overnight under an argon atmosphere. The solution was poured into a mixture of ice water (150 mL) and extracted with EtOAc. The combined extracts were washed with 5% $NaHCO_3$ solution, dried over sodium sulfate, filtered, and concentrated in vacuo to a yellow solid. This solid was chromatographed (silica gel, 3% methanol/CH_2Cl_2) to yield the coupled compound (150 mg, 4%); 10% palladium on carbon (150 mg, activated) was added to a solution of the compound (150 mg, 0.26 mmol) in glacial acetic acid: ethyl acetate (1:1, 30 mL) and concentrated hydrochloric acid (0.5 mL). The mixture was hydrogenated in a Parr shaker (45 psi) for 24 h. The reaction mixture was filtered, the filtrate was evaporated, and a portion (0.36 g) of the resulting residue was purified on HPLC (YMC ODS-AQ, 50 × 250 mm, 85 mL/min, 15% CH_3CN/H_2O−0.1% TFA, UV detection at 220 nm) to yield the title compound (15 mg): MS *m/e* 362 (M + H)$^+$. Anal. ($C_{20}H_{21}N_5O_4$·1.1TFA·0.25H_2O) Calcd: C, 47.46; H, 4.23; N, 12.47. Found: C, 47.76; H, 4.05; N, 12.17.

Diphthalimidoyl-*o*-xylene (47). A mixture of α,α′-dibromo-*o*-xylene (26 g, 98 mmol) and potassium phthalimide (54.7 g, 294 mmol) in dimethylformamide (500 mL) was refluxed for 18 h. The solution was cooled to room temperature, poured into water, and filtered and the filter cake was dried at reduced pressure to give the title compound (35 g, 90%).

2,3,4,5-Tetrahydro-7-nitro-3-oxo-1*H*-2,4-benzodiazepine (48). To a cold solution of potassium nitrate (6.2 g, 61.2 mmol) in concentrated sulfuric acid (350 mL) was added portionwise compound **47** (22 g, 55.6 mmol). The resulting solution was warmed to room temperature and stirred for 18 h. The mixture was carefully poured into ice water, and the mixture was filtered. The filter cake was washed with water and dried at reduced pressure to give the nitrated compound. This compound was added portionwise to a solution of hydrazine (26 mL, 535 mmol) in ethanol (1000 mL), and the solution was refluxed for 1 h. Additional hydrazine (26 mL, 535 mmol) was added followed by ethanol (500 mL). The solution was stirred at room temperature for 18 h and filtered, and the filtrate was concentrated. The residue was triturated with chloroform (600 mL) for 1 h. After filtration and concentration, the residue was dissolved in tetrahydrofuran (500 mL), and 1,1′-carbonyldiimidazole (9.2 g, 56.8 mmol) dissolved in tetrahydrofuran (250 mL) was added dropwise. The resulting mixture was stirred at room temperature for 6 days, filtered, and concentrated to yield the title compound (7.7 g, 71% yield).

Methyl 8-Amino-2,3,4,5-tetrahydro-3-oxo-1*H*-2,4-benzodiazepine-2-acetate (49). (a) A heterogeneous solution consisting of compound **48** (5.5 g, 26.6 mmol) and Lawesson's reagent (7.0 g, 17.3 mmol) in toluene (100 mL) was heated to 80 °C under an argon atmosphere for 1.5 h. The solution was cooled and filtered. The solid was triturated with 7:3 dichloromethane:methanol for 1 h, filtered, and concentrated to give the thiourea (4.3 g, 73%).

(b) To a solution of the thiourea (2.2 g, 9.9 mmol) in dimethylformamide (50 mL) was added dropwise iodomethane (0.614 mL, 9.9 mmol) dissolved in dimethylformamide (5 mL). The solution was stirred for 1 h, and potassium carbonate (3.0 g, 21.8 mmol) was added followed by methyl bromoacetate (0.934 mL, 9.9 mmol). The solution was stirred for 18 h, filtered, and concentrated to give a residue which was partitioned between ethyl acetate and water. The organic layer was concentrated, and the residue was taken up in water/dioxane (1:1) (50 mL) and refluxed for 18 h. The solution was concentrated, and the residue was triturated with water (30 mL), filtered, and dried at reduced pressure to give a mixture of the 7- and 8-nitro compounds (1.38 g, 68%).

(c) The mixture of the above compounds (470 mg, 1.7 mmol) was taken up in 1:1 methanol:dimethylformamide (20 mL). Argon was bubbled through the system, 10% palladium on carbon (80 mg) was added, and the mixture was shaken under hydrogen (40 psi) for 1.5 h. The solution was filtered through Celite and concentrated. The residue was chromatographed (silica gel, 4% methanol:dichloromethane) to separate the 7-amino regioisomer from the 8-isomer **49** (110 mg, 42%): ^{1}H NMR (360 MHz, DMSO-d_6) δ 3.6−3.65 (s, 3H), 4.0−4.1 (m, 4H), 4.25−4.3 (s, 2H), 5.7 (s, 2H), 6.35−6.45 (dd, 1H), 6.45−6.5 (d, 1H), 6.6−6.65 (t, 1H), 6.85−6.9 (d, 1H); MS (ES) *m/e* 250.2 (M + H)$^+$.

8-[(4-Amidinobenzoyl)amino]-2,3,4,5-tetrahydro-3-oxo-1*H*-2,4-benzodiazepine-2-acetic Acid (21). The amine **49** was coupled with 4-[(benzyloxycarbonyl)amidino]benzoic acid and deprotected as in the preparation of **5** to furnish the title compound: MS (ES) *m/e* 382.0 (M + H)$^+$; ^{1}H NMR (DMSO-d_6, 360 MHz) δ 2.5 (s, 3H), 3.9−4.0 (d, 2H), 4.2−4.3 (d, 2H), 4.4−4.5 (d, 2H), 6.5−6.6 (m, 1H), 7.2−7.3 (d, 1H), 7.6−8.1 (m, 10H). Anal. ($C_{21}H_{26}N_5O_{7.5}$) Calcd: C, 53.84; H, 5.59; N, 14.95. Found: C, 54.04; H, 5.92; N, 14.81.

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Supporting Information Available: X-ray crystallography data for compounds **22b**−**f** (Table 4). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Coller, B. S.; Anderson, K.; Weisman, H. F. New antiplatelet agents: platelet GPIIb/IIIa antagonists. *Thromb. Haemost.* **1995,** *74*, 302−308.
(2) Kieffer, N.; Phillips, D. R. Platelet Membrane Glycoproteins: Functions in Cellular Adhesion and Aggregation. *Annu. Rev. Cell. Biol.* **1990**, *6*, 329−357.
(3) See: (a) Lefkovits, J.; Plow, E. F.; Topol, E. J. Platelet Glycoprotein IIb/IIIa Receptors in Cardiovascular Medicine. *N. Engl. J. Med.* **1995**, *332*, 1553−1559. (b) Samanen, J. *Annu. Rep. Med. Chem.* **1996**, *31*, 91−100.
(4) D'Souza, S. E.; Ginsberg, M. H.; Plow, E. F. Arginyl-glycyl-aspartic acid (RGD): A Cell Adhesion Motif. *Trends Biochem. Sci.* **1991**, *16*, 246−250.
(5) Nichols, A. J.; Ruffolo, R. R., Jr.; Huffman, W. H.; Poste, G.; Samanen, J. *Trends Pharmacol. Sci.* **1992**, *13*, 413.
(6) Samanen, J.; Ali, F.; Romoff, T.; Calvo, R.; Sorenson, E.; Vasko, J.; Storer, B.; Berry, D.; Bennett, D.; Stroksacker, M.; Powers, D.; Stadel, J.; Nichols, A. Development of a Small RGD Peptide Fibrinogen Receptor Antagonist with Potent Antiaggregatory Activity in Vitro. *J. Med. Chem.* **1991**, *34*, 3114−3125.
(7) Nutt, R. F.; Brady, S. F.; Colton, C. D.; Sisko, J. T.; Ciccarone, T.; Levey, M. R.; Duggan, M. E.; Imagire, I. S.; Gould, R. J.; Anderson, P. S.; Veber, D. F. Development of Novel, Highly Selective Fibrinogen Receptor Antagonists as Potentially Useful Antithrombotic Agents. In *Peptides: Chemistry and Biology. Proceedings of the Twelfth American Peptide Symposium*; Smith, J. A., River, J. E., Eds.; ESCOM: Leiden, The Netherlands, 1992; pp 914−916.
(8) 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.; Napier, M. A.; Pitti, R. M.; Padua, A.; Quan, C.; Stanley, M.; Struble, M.; Tom, J. Y. K.; Burnier, J. P. Cyclic RGD Analogues as Antiplatelet Antithrombotics. *J. Med. Chem.* **1992**, *35*, 2040−2048.
(9) Scarborough, R. M.; Naughton, M. A.; Teng, W.; Rose, J. W.; Phillips, D. R.; Nannizzi, L.; Arfsten, A.; Campbell, A. M.; Charo, I. F. Design of Potent and Specific Integrin Antagonists. *J. Biol. Chem.* **1993**, *268*, 1066−1073.
(10) Ali, F. E.; Bennett, D. B.; Raul, C. R.; Elliott, J. D.; Hwand, S. M.; Ku, T. W.; Lago, M. A.; Nichols, A. J.; Romoff, T. T.; Shah, D. H.; Vasko, J. A.; Wong, A. S.; Yellin, T. O.; Yuan, C. K.; Samanen, J. M. Conformationally Constrained Peptides and Semipeptides Derived from RGD as Potent Inhibitors of the Platelet Fibrinogen Receptor and Platelet Aggregation. *J. Med. Chem.* **1994**, *37*, 769−780.

(11) Cheng, S.; Craig, W. S.; Mullen, D.; Tschopp, J. F.; Dixon, D.; Pierschbacher, M. D. Design and Synthesis of Novel Cyclic RGD-Containing Peptides as Highly Potent and Selective Integrin $\alpha_{IIb}\beta_{III}$ Antagonists. *J. Med. Chem.* **1994**, *37*, 1−8.
(12) Jackson, S.; DeGrado, W.; Dwivedi, A.; Parthasarathy, A.; Higley, A.; Krywko, J.; Rockwell, A.; Markwalder, J.; Wells, G.; Wexler, R.; Mousa, S.; Harlow, R. Template-Constrained Cyclic Peptides: Design of High-Affinity Ligands for GPIIb/IIIa. *J. Am. Chem. Soc.* **1994**, *116*, 3220−3230.
(13) Muller, G.; Gurrath, M.; Kessler, H. Pharmacophore refinement of gpIIb/IIIa antagonists based on comparative studies of anti-adhesive cyclic and acyclic RGD peptides. *J. Comput. Aided. Mol. Des.* **1994,** *8*, 709−730.
(14) Kopple, K. D.; Baures, P. W.; Bean, J. W.; D'Ambrosio, C. A.; Hughes, J. L.; Peishoff, C. E.; Eggleston, D. S. Conformations of Arg-Gly-Asp Containing Heterodetic Cyclic Peptides: Solution and Crystal Studies. *J. Am. Chem. Soc.* **1992**, *114*, 9615−9623.
(15) Peishoff, C. E.; Ali, F. E.; Bean, J. W.; Calvo, R.; D'Ambrosio, C. A.; Eggleston, D. S.; Hwang, S. M.; Kline, T. P.; Koster, P. F.; Nichols, A.; Powers, D.; Romoff, T.; Samanen, J. M.; Stadel, J.; Vasko, J. A.; Kopple, K. D. Investigation of Conformational Specificity at GPIIb/IIIa: Evaluation of Conformationally Constrained RGD Peptides. *J. Med. Chem.* **1992**, *35*, 3962−3969.
(16) (a) Bach, A. C., II; Eyermann, C. J.; Gross, J. D.; Bower, M. J.; Harlow, R. L.; Weber, P. C.; DeGrado, W. F. Structural Studies of Family of High Affinity Ligands for GP IIB-IIIa. *J. Am. Chem. Soc.* **1994**, *116*, 3207−3219. (b) Wityak, J.; Sielecki, T. M.; Pinto, D. J.; Emmet, G.; Sze, J. Y.; Liu, J.; Tobin, E.; Wang, S.; Jiang, B.; Ma, P.; Mousa, S. A.; Wexler, R. R.; Olson, R. Discovery of Potent Isoxazoline Glycoprotein IIb/IIIa Receptor Antagonists. *J. Med. Chem.* **1997**, *40*, 50−60.
(17) Eldred, C. P.; Evans, B.; Hindley, S.; Judkins, B. D.; Kelly, H. A.; Kitchin, J.; Lumley, B. D.; Porter, B.; Ross, B. C.; Smith, K. J.; Taylor, N. R.; Wheatcroft, J. R. Orally Active Non-Peptide Fibrinogen Receptor (GPIIb/IIIa) Antagonists: Identification of 4-[4-[-4(Aminoimino-methyl)Phenyl]-1-Piperazinyl-1-Piperidineacetic acid as a Long-Acting, Broad-Spectrum Antithrombotic Agent. *J. Med. Chem.* **1994**, *37*, 3882−3885.
(18) (a) McDowell, R. S.; Gadek, T. R.; Barker, P. L.; Burdick, D. J.; Chan, K. S.; Quan, C. L.; Skelton, N.; Struble, M.; Thorsett, E. D.; Tischler, M.; Tom, J. Y. K.; Webb, T. R.; Burnler, J. P. From Peptide to Non-Peptide. 1. The Elucidation of a Bioactive Conformation of the Arginine-Glycine-Aspartic Acid Recognition Sequence. *J. Am. Chem. Soc.* **1994**, *116*, 5069−5077. (b) McDowell, R. S.; Blackburn, B. K.; Gadek, T. R.; McGee, L. R.; Rawson, T.; Reynolds, M. E.; Robarge, K. D.; Somers, T. C.; Thorsett, E. D.; Tischler, M.; Webb, R. R.; Venuti, M. C. From Peptide to Non-Peptide. 2. The de Novo Design of Potent, Non-Peptidal Inhibitors of Platelet Aggregation Based on a Benzodiazepinedione Scaffold. *J. Am. Chem. Soc.* **1994**, *116*, 5077−5083.
(19) Fisher, M. J.; Gunn, B.; Harms, C. S.; Kline, A. D.; Mullaney, J. T.; Nunes, A.; Scarborough, R. M.; Arfsen, A. E.; Skelton, M. A.; Um, S. L.; Utterback, B. G.; Jacubowski, J. A. Non-Peptide RGD Surrogates Which Mimic a Gly-Asp β-turn: Potent Antagonists of Platelet Glycoprotein IIb-IIIa. *J. Med. Chem.* **1997**, *40*, 2085−2101.
(20) Hirschmann, R.; Sprengeler, P. A.; Kawasaki, T.; Leahy, J. W.; Shakespeare, W. C.; Smith, A. B., III. The First Design and Synthesis of a Steroidal Peptidomimetic. The Potential Value of Peptidomimetics in Elucidating the Bioactive Conformation of Peptide Ligands. *J. Am. Chem. Soc.* **1992**, *114*, 9699−9701.
(21) For recent reviews of the progress in the development of nonpeptide IIb/IIIa antagonists, see ref 3b and: Ojima, I.; Chakravarty, S.; Dong, Q. Antithrombotic agents: from RGD to peptide mimetics. *Bioorg. Med. Chem.* **1995,** *3*, 337−360.
(22) Callahan, J. F.; Bean, J. W.; Burgess, J. L.; Eggleston, D. E.; 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 C7 Mimetic for the Predicted γ-turn Conformation Found in Several Constrained RGD Antagonists. *J. Med. Chem.*, **1992**, *35*, 3970−3972.
(23) Ku, T. W.; Ali, F. E.; Barton, L. S.; Bean, J. W.; Bondinell, W. E.; Burgess, J. L.; Callahan, J. F.; Calvo, R. R.; Chen, L.; Eggleston, D. S.; Gleason, J. G.; Huffman, W. F.; Hwang, S.-M.; Jakas, D. R.; Karash, C. B.; Keenan, R. M.; Kopple, K. D.; Miller, W. H.; Newlander, K. A.; Nichols, A.; Parker, M. F.; Peishoff, C. E.; Samanen, J. M.; Uzinskas, I.; Venslavsky, J. W. 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.
(24) (a) Bondinell, W. E.; Keenan, R. M.; Miller, W. H.; Ali, F. E.; Allen, A. C.; DeBrosse, C. W.; Eggleston, D. S.; Erhard, K. F.; Haltiwanger, R. C.; Lee, C. P.; Nichols, A. J.; Ross, S. T.; Samanen, J. M.; Valocik, R. E.; Vasko-Moser, J. A.; Venslavsky, J. W.; Wong, A. S.; Yuan, C. K. Design of a Potent and Orally Active Nonpeptide Platelet Fibrinogen Receptor (GPIIB/IIIA) Antagonist. *Bioorg. Med. Chem.* **1994**, *2*, 897−908. (b) Bondinell, W. E.; et al. Manuscript in preparation.
(25) Samanen, J. M.; Ali, F. E.; Barton, L. S.; Bondinell, W. E.; Burgess, J. L.; Callahan, J. F.; Calvo, R. R.; Chen, W.; Chen, L.; Erhard, K.; Feuerstein, G.; Heys, R.; Hwang, S.-M.; Jakas, D. R.; Keenan, R. M.; Koster, P. F.; Ku, T. W.; Kwon, C.; Lee, C.-P.; Miller, W. H.; Newlander, K. A.; Nichols, A.; Parker, M.; Peishoff, C. E.; Rhodes, G.; Ross, S.; Shu, A.; Simpson, R.; Takata, D.; Vasko-Moser, J. A.; Valocik, R. E.; Yellin, T. O.; Uzinskas, I.; Venslavsky, J. W.; Wong, A.; Yuan, C.-K.; Huffman, W. F. Potent, Selective, Orally Active 3-Oxo-1,4-benzodiazepine GPIIb/IIIa Integrin Antagonists. *J. Med. Chem.* **1996**, *39*, 4867−4870.
(26) Miller, W. H.; Bondinell, W. E.; Callahan, J. F.; Eggleston, D. S.; Huffman, W. F.; Hwang, S. M.; Jakas, D. R.; Keenan, R. M.; Koster, P. F.; Ku, T. W.; Kwon, C. K.; Nichols, A. J.; Samanen, J. M.; Takata, D. T.; Uzinskas, I. N.; Valocik, R. E.; Vasko-Moser, J. A.; Wong, A. S. Structure−Activity Relationships in 3-Oxo-1,4-benzodiazepine-2-acetic Acid GPIIb/IIIa Antagonists. The 2-Benzazepine Series. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2481−2486.
(27) Bondinell, W. E.; Callahan, J. F.; Huffman, W. F.; Keenan, R. M.; Ku, T. W.; Newlander, K. A.; Samanen, J. M.; Uzinskas, I. N. WO 9414776, July 7, 1994.
(28) Miller, W. H.; Newlander, K. A.; Eggleston, D. S.; Haltiwanger, R. C.; Synthesis of a 2-Benzazepine Analogue of a Potent, Nonpeptide GPIIb/IIIa Antagonist. *Tetrahedron Lett.* **1995**, *36*, 373−376.
(29) For a full description of the in vitro assays, see ref 24a.
(30) The intestinal permeability of these compounds was the subject of a recent review; See: Samanen, J. M.; Lee, C.-P.; Smith, P. L.; Bondinell, W.; Calvo, R. R.; Jakas, D. R.; Newlander, K.; Parker, M.; Uzinskas, I.; Yellin, T. O.; Nichols, A. J. The Use of Rabbit Intestinal Permeability as an in vitro Assay in the Search for Orally Active GPIIb/IIIa Antagonists. *Adv. Drug Deliv. Rev.* **1996**, *23*, 133−142.
(31) For prototypical acyclic nonpeptide fibrinogen receptor antagonists, see: (a) 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.; et al. Non-peptide fibrinogen receptor antagonists. 1. Discovery and design of exosite inhibitors. *J. Med. Chem.* **1992**, *35*, 4640−4642. (b) Zablocki, J. A.; Rico, J. G.; Garland, R. B.; Rogers, T. E.; Williams, K.; Schretzman, L. A.; Rao, S. A.; Bovy, P. R.; Tjoeng, F. S.; Lindmark, R. J.; Toth, M. V.; Zupec, M. E.; McMackins, D. E.; Adams, S. P.; Miyano, M.; Markos, C. S.; Milton, M. N.; Paulson, S.; Herin, M.; et al. Potent in vitro and in vivo inhibitors of platelet aggregation based upon the Arg-Gly-Asp sequence of fibrinogen. (Aminobenzamidino)succinyl (ABAS) series of orally active fibrinogen receptor antagonists. *J. Med. Chem.* **1995**, *38*, 2378−2394.
(32) Monte Carlo simulations were done using MacroModel version 6.0 (Department of Chemistry, Columbia University, New York).
(33) Minimizations within BatchMin V6.0 (Department of Chemistry, Columbia University, New York) were performed using AMBER*. All force-field equations are identical with those of authentic AMBER from P. Kollman.
(34) The biological activity for the benzodiazepine series has been shown to reside in the (*S*)-configuration at the acetic acid stereocenter, as in L-aspartic acid; see refs 24a and 25.
(35) Blackburn, B. K.; Lee, A.; Baier, M.; Kohl, B.; Olivero, A. G.; Matamoros, R.; Robarge, K. D.; McDowell, R. S. From peptide to non-peptide. 3. Atropisomeric GPIIbIIIa antagonists containing the 3,4-dihydro-1H-1,4-benzodiazepine-2,5-dione nucleus. *J. Med. Chem.* **1997**, *40*, 717−729.

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