Non-Peptide Fibrinogen Receptor Antagonists. 2. Optimization of a Tyrosine Template as a Mimic for Arg-Gly-Asp

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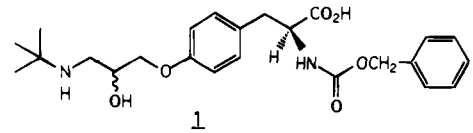
Inhibitors of platelet–fibrinogen binding offer an opportunity to interrupt the final, common pathway for platelet aggregation. Small molecule inhibitors of the platelet fibrinogen receptor GPIIb/IIIa were prepared and evaluated for their ability to prevent platelet aggregation. Compound **23m** (L-700,462/MK-383) inhibited *in vitro* platelet aggregation with an IC_{50} of 9 nM and demonstrated a selectivity of >24 000-fold between platelet and human umbilical vein endothelial cell fibrinogen receptors. Dose-dependent inhibition of *ex vivo* platelet aggregation induced by ADP was achieved with iv infusions of 0.1–10 μg/kg/min of **23m** in anesthetized dogs, with 10 μg/kg/min completely inhibiting platelet aggregation during the entire 6 h infusion protocol. Platelet aggregatability returned rapidly after the termination of the **23m** infusions. These features suggest that **23m** may be useful in the treatment of arterial occlusive disorders.

Platelet-rich thrombus formation is a key factor in arterial vasoocclusive disorders such as unstable angina, myocardial infarction, and reocclusion after angioplasty.[1–5] In the final stages of thrombus formation, activated platelets aggregate by binding to the multivalent protein fibrinogen via a membrane glycoprotein, the integrin GPIIb/IIIa.[6–14] Interruption of this process through inhibition of platelet–fibrinogen binding presents an attractive therapeutic target.[15–19]

Fibrinogen binding to GPIIb/IIIa is believed to be mediated by a dodecapeptide sequence (HHLGGAKQAGDV) present on the C-terminus of the fibrinogen γ chain and by two Arg-Gly-Asp (RGD) tripeptide sequences found at residues 95–97 and 572–574 of the fibrinogen α chain.[13,20–29] Antibodies raised to the fibrinogen receptor[30–32] and compounds possessing the RGD sequence have been found to inhibit platelet aggregation. Examples include the venom peptides echistatin and bitistatin[33–35] and lower molecular weight synthetic peptides.[36–40] As discussed previously,[41] our goal is to prepare small molecule, non-peptide inhibitors of GPIIb/IIIa-mediated fibrinogen binding that would have use as antithrombotic agents.[42] An intravenous antiaggregatory agent that combines selective *in vivo* activity with rapid diminution of antiplatelet activity after the cessation of infusion would allow for acute intervention with minimal complications and may minimize undesired bleeding.[43]

Previous peptide structure–activity studies have identified structural features that contribute to the activity of RGD-containing molecules. Both the guanidino of arginine and the carboxylate of aspartic acid are important for good antiaggregatory activity.[38,39,42] A constraint on steric bulk at the central glycine residue is indicated by the decrease in potency that accompanies α-carbon substitution at this site.[44] A search of the Merck sample collection for structures containing a basic and acidic moiety separated by a distance of 10–20 Å (based on a consideration of the distance separating the side chains of the RGD sequence in echistatin)[45,46] resulted in the identification of tyrosine derivative **1**. We now wish to report on the modification of this lead structure to give fibrinogen receptor antagonists of nanomolar potency.



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Chemistry

Compound **4a** was prepared using the method outlined in Scheme 1. *N*-Cbz-tyrosine (**2**) was treated with sodium hydride in DMF and alkylated with bromochloropropane. Treatment of the resulting terminal halide **3** with a 1:1 mixture of *tert*-butylamine/acetonitrile at reflux yielded compound **4a**. Compounds **4b–i** were prepared in a similar fashion (Table 1).

Compounds **6a** and **7a** (Table 2) were prepared by alkylation of *N*-Cbz-tyrosine (Scheme 2), utilizing the linear alkylamine **8a** and the constrained piperidine butyl halide **9** (Table 3) as alkylating agents. The t-BOC protecting group was removed using anhydrous HCl in ethyl acetate. Compounds **6b–d** and **7b** were prepared in a similar manner. Alternatively, the α-amino group of **5b** could be alkylated with NaH/MeI. Deprotection with HCl/ethyl acetate gave compound **7c** (Table 4).

Synthesis of compound **15a** (Table 4) began with formation of the phenolic anion of α-methyl-substituted ethyl ester **13a**[47] by treatment with NaH/DMF (Scheme 3). Alkylation with iodide **9** followed by ester cleavage with NaOH and BOC removal gave **15a**. A similar sequence beginning with **13b** and utilizing alkylating agent **10** yielded compound **15b** (Table 2). Alternatively, intermediate **14b** was hydrolyzed and then treated with isobutyl chloroformate followed by diazomethane/silver benzoate to yield C-terminal homologated product **16**.

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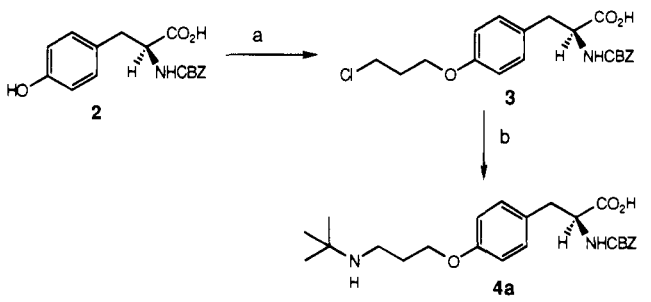
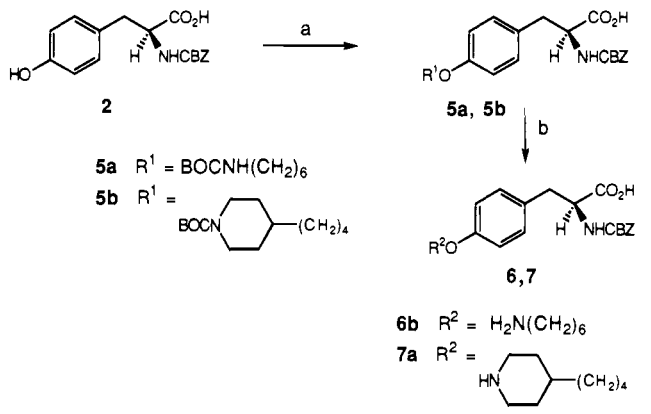
Scheme 1[a]



[a] (a) NaH/DMF, $Cl(CH_2)_3Br$; (b) CH_3CN, t-$BuNH_2$, reflux.

Scheme 2[a]



[a] (a) NaH/DMF, RX; (b) HCl/EtOAc.

Basic hydrolysis of **16** followed by BOC removal with trifluoroacetic acid provided compound **17**.

The α-unsubstituted 3-phenylpropanoate derivatives **19a,b** were prepared via the ester **18** (Scheme 4). Formation of the phenolic cesium salt of **18** with Cs_2CO_3 in methanol followed by alkylation and basic hydrolysis of the resulting ester gave **19**.

Two methods outlined in Scheme 5 were used to prepare α-amino derivatives **22b–j** and **23a–n** (Tables 4 and 5). The method used depended on the particular side chain required. For example, method A was used to prepare compound **22b**. Removal of the α-amino Cbz protecting group of compound **5a** with H_2 (Pd/C) in EtOH gave the amino acid **20a**. Acylation with 3-phenylpropanoyl chloride in NaOH/dioxane solution followed by BOC removal with HCl/ethyl acetate gave **22b**. In general, more lipophilic or higher molecular weight acid chlorides or sulfonyl chlorides, such as 3-phenylpropanoyl chloride, performed well under these conditions, but lower molecular weight agents, such as acetyl chloride or methanesulfonyl chloride, gave compounds **22e,h** in poor yield. Method B proved effective for preparing compounds with low molecular weight side chains. Esterification of **5a** with Cs_2CO_3/MeI followed by Cbz removal with H_2 (Pd/C) yielded the amino ester **21a**. Acylation by acetyl chloride in ethyl acetate with solid sodium bicarbonate or pyridine as the base was followed by ester hydrolysis with LiOH in 1:1:1 THF/MeOH/H_2O and BOC removal with HCl/ethyl acetate to produce **22e** in good yield. A similar sequence (Scheme 3) allowed the preparation of **24a,b** from **16**.

The enantiomeric purity of material prepared by methods A and B was compared.[48] Samples of compound **23m** prepared using methods A and B possessed enantiomeric excesses of 99.6% and 85.7%, respectively.[49]

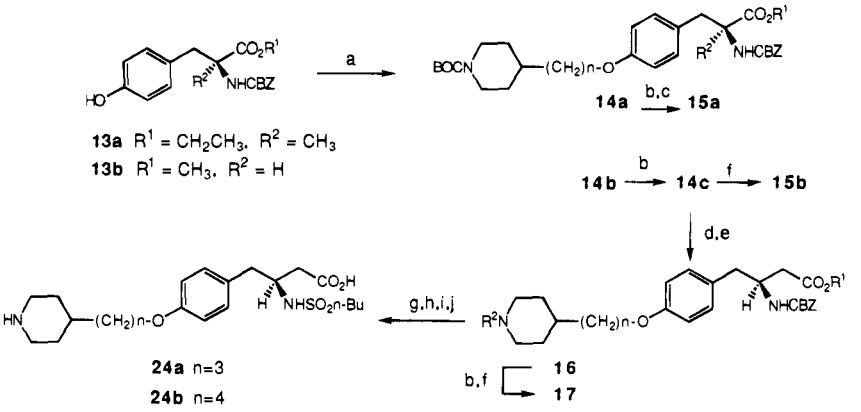
Palladium-catalyzed coupling of *N*-hexanoyl-4-iodophenylalanine methyl ester (**26**) and enyne **12** and reduction of both the olefinic and acetylenic bonds with H_2 (Pd/C) followed by deprotection (Scheme 6) yielded **27**, in which the phenolic oxygen of the tyrosine lead was replaced by methylene.

Results and Discussion

Compounds were evaluated *in vitro* for their ability to inhibit ADP-induced aggregation of human gel-filtered platelets (IC_{50}, Table 1).[50] Lead compound **1** displayed IC_{50} = 27 μM for inhibition of platelet aggregation. This potency is similar to the activity of simple synthetic tetrapeptides such as RGDS (IC_{50} = 26 μM).[29] Our first goal was to simplify and optimize **1**, as we characterized its mimicry of the RGD sequence. The hydroxyl group present in the side chain of compound **1** was removed, to give compound **4a**, which possessed comparable activity to the parent (Table 1). Since substitution of BOC for Cbz on the α-amino group resulted in a 3-fold drop in potency (**4b**), the Cbz group was utilized in subsequent compounds.

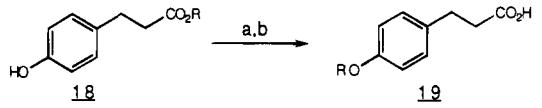
The N-terminus of compound **1** was examined further (Table 1). Replacement of the bulky *tert*-butylamine by phenyl resulted in compound **4c**, which did not demonstrate significant inhibitory activity. Replacement by the bulkier *tert*-octyl side chain also lowered potency dramatically (**4d**), although use of a diaminoalkyl chain (**4e**) restored potency. The tertiary pyrrolidine **4f** was

Scheme 3[a]



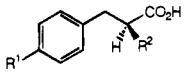
[a] (a) DMF, **9** or **10**; (b) NaOH; (c) HCl/EtOAc; (d) isobutyl chloroformate; (e) CH_2N_2, silver benzoate; (f) TFA/anisole; (g) H_2 (Pd/C); (h) $ClSO_2n$-Bu, $NaHCO_3$/EtOAc; (i) NaOH; (j) TFA/anisole.

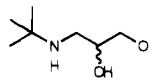
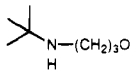
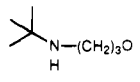
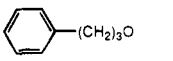
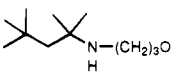
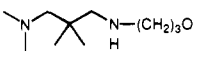
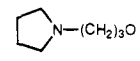
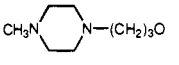
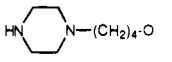
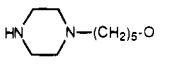
Scheme 4[a]



[a] (a) Cs_2CO_3/DMF, RX; (b) NaOH.

Table 1. N-Terminal Modifications of Lead Compound 1



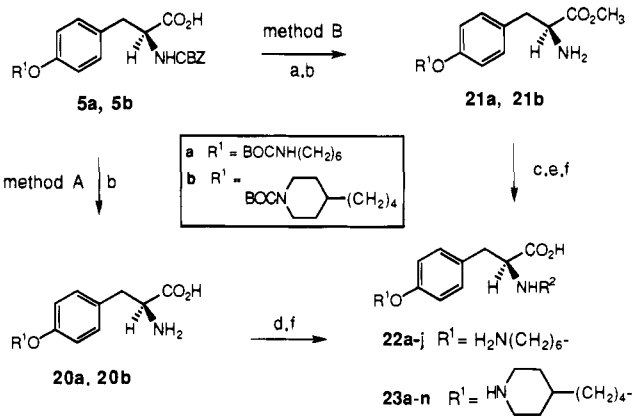
compound	R^1	R^2	IC_{50} (μM)[a,50]
1		NHCBZ	27
4a		NHCBZ	28
4b		NHBOC	95
4c		NHCBZ	>1000
4d		NHCBZ	>100
4e		NHCBZ	19
4f		NHCBZ	23
4g		NHCBZ	5.2
4h		NHCBZ	0.5
4i		NHCBZ	1.1

[a] Platelet aggregation was measured in a functional assay monitoring the increase in light transmittance that occurs when platelets aggregate. Gel-filtered platelets[33] were adjusted to a concentration of 2×10^8/mL and mixed with 0.1 mg/mL human fibrinogen, 1 mM $CaCl_2$, and the compound of interest. Aggregation was then initiated by addition of the agonist (10 μM adenosine diphosphate (ADP)). Inhibition of platelet aggregation was determined by comparison of light transmittance values for the control and subject samples. The IC_{50} was determined as the concentration necessary to inhibit the change in light transmittance by 50%. At least two determinations were made for each compound, and the IC_{50} was calculated by fitting to a four-parameter equation. The average standard error of the IC_{50} determinations was ±20%.

of comparable potency to **1**, while the *N*-methylpiperazine **4g** was 4-fold more potent. Removal of the methyl group and use of a butyl linkage gave the piperazine compound **4h**, which displayed an IC_{50} of 0.5 μM. Introduction of an additional methylene to the linker provided **4i**, which was 2-fold less potent than **4h**.

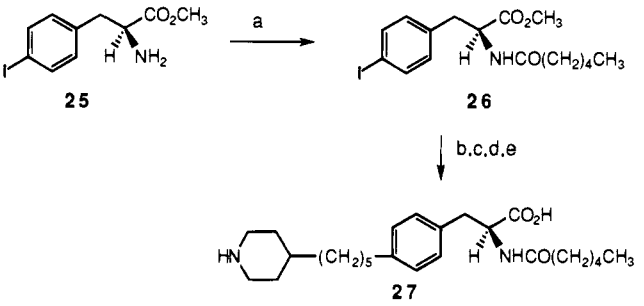
To explore the effect on potency of altering the number of methylene units between the amine terminus and the phenolic oxygen, a series of primary alkylamine compounds was prepared (**6a–d**, Table 2). The spacing requirement for optimal activity was quite specific: an array of seven methylenes (**6c**, $IC_{50} = 2.6$ μM) increased potency 10-fold over compound **1** and other analogs (**6b,d**). However, the potency of **6c** was less than that

Scheme 5[a]



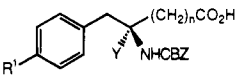
[a] (a) Cs_2CO_3, CH_3I; (b) H_2 (Pd/C); (c) R^2Cl, $NaHCO_3$, EtOAc; (d) R^2Cl/NaOH; (e) LiOH, THF, MeOH, H_2O; (f) HCl/EtOAc.

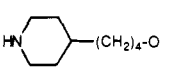
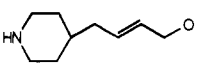
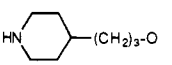
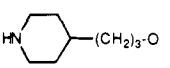
Scheme 6[a]



[a] (a) $ClCO(CH_2)_4CH_3$; (b) $Pd(PPh_3)_2Cl_2$, CuI, Et_2N, **12**; (c) H_2 (Pd/C); (d) LiOH; (e) HCl/EtOAc.

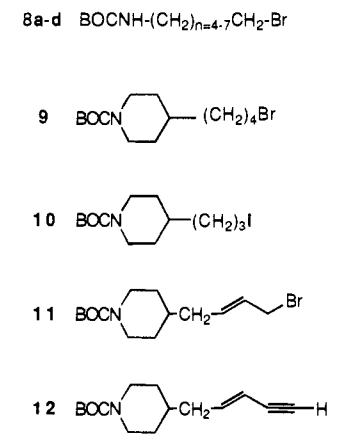
Table 2. Primary and Secondary Amine Derivatives of Lead Compound 1



compound	R^1	Y	n	IC_{50} (μM)
6a	H_2N-$(CH_2)_5$-O	H	0	36
6b	H_2N-$(CH_2)_6$-O	H	0	26
6c	H_2N-$(CH_2)_7$-O	H	0	2.6
6d	H_2N-$(CH_2)_8$-O	H	0	38
7a		H	0	0.26
7b		H	0	0.24
15b		H	0	6.2
17		H	1	0.88

observed in the compounds containing a terminal constrained secondary amine (**4h,i**). Constraint of the terminus of **6c** via a piperidine ring produced compound **7a** and led to an additional 10-fold increase in potency

Table 3. Alkylating Agents



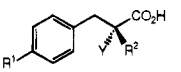
(IC_{50} = 0.26 μM). Incorporation of a trans olefin into the methylene linker had little effect on potency (**7b**, IC_{50} = 0.24 μM). Removal of a methylene from the N-terminal side chain was detrimental (**15b**, IC_{50} = 6.2 μM), while simultaneous extension of the C-terminal acid (**17**, IC_{50} = 0.88 μM) did not provide full recovery of activity. Overall, optimization at the N-terminus of compound **1** provided **7a**, which exhibited a 100-fold increase in potency along with elimination of a chiral center.

Further attempts were made to optimize potency at the carboxy terminus (Table 4). Addition of a methyl substituent, either to the α-amino group to give **7c** or to the α-carbon to give the quaternary derivative **15a**, was accompanied by a ~100-fold loss in potency. Although removal of the Cbz group from compound **6b** (IC_{50} = 26 μM) was not detrimental to potency (**22a**, IC_{50} = 35 μM), elimination of the α substituent had a profound negative effect (**19a**, 12% inhibition at 300 μM). Next, the Cbz group was modified by replacement of the Cbz benzyl oxygen with methylene to give the 3-phenylpropanoyl derivative **22b**, which demonstrated a 3-fold increase in potency (**22b**, IC_{50} = 8 μM). In addition, the shorter-chain 2-phenylacetyl and benzoyl derivatives **22c,d** and the *N*-acetyl derivative **22e** were slightly less potent than was **22b**. However, longer alkyl chain derivatives such as the *N*-hexanoyl derivative **22g** showed an additional 3-fold increase in potency (IC_{50} = 5.0 μM). Thus, the effect of the α substituent is both profound and complex, possessing at least two components, one arising from electrostatic interaction at the acidic N-H center and the other influenced by lipophilic substituents that conform to requirements of size and shape.

Systematic variation of the amine substituent showed that sulfonamide derivatives possessing suitable lipophilic side chains displayed an additional increase in *in vitro* activity (Table 4). While the methanesulfonamide derivative **22h** was comparable in potency to the *N*-acetyl derivative **22e**, the phenylmethanesulfonamide derivative **22i** and the butanesulfonamide **22j** demonstrated 7-fold increases in potency over their *N*-acyl counterparts and a 30-fold increase over the original lead **6b**.

Combination of optimized N- and C-termini into the same molecule provided the anticipated additive potency enhancements (Table 5). For example, the *N*-hexanoyl derivative **23d** (IC_{50} = 35 nM) demonstrated a 140-fold

Table 4. C-Terminal Derivatives



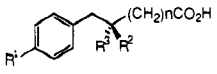
compound	R^1	R^2	Y	IC_{50} (μM)
7a	HN-(CH2)4-O	NHCBZ	H	0.26
7c	HN-(CH2)4-O	N(CH3)CBZ	H	22
15a	HN-(CH2)4-O	NHCBZ	CH_3	65
6b	H_2N-$(CH_2)_6$-O	NHCBZ	H	26
22a	H_2N-$(CH_2)_6$-O	NH_2	H	35
19a	H_2N-$(CH_2)_6$-O	H	H	12% inhibition @300 uM
22b	H_2N-$(CH_2)_6$-O	$NHCO(CH_2)_2Ph$	H	8
22c	H_2N-$(CH_2)_6$-O	$NHCOCH_2Ph$	H	14
22d	H_2N-$(CH_2)_6$-O	NHCOPh	H	10
22e	H_2N-$(CH_2)_6$-O	$NHCOCH_3$	H	18
22f	H_2N-$(CH_2)_6$-O	$NHCO(CH_2)_2CH_3$	H	5.7
22g	H_2N-$(CH_2)_6$-O	$NHCO(CH_2)_4CH_3$	H	5.0
22h	H_2N-$(CH_2)_6$-O	$NHSO_2CH_3$	H	10
22i	H_2N-$(CH_2)_6$-O	$NHSO_2CH_2Ph$	H	1.9
22j	H_2N-$(CH_2)_6$-O	$NHSO_2(CH_2)_3CH_3$	H	0.78

increase in potency over the aminohexyl analog **22g** (Table 4). This result was consistent with the conclusion derived from **6c** and **7a** (Table 2) that proper adjustment of chain length and constraint of the N-terminus are required for optimal potency. Compound **23d** also showed a 7-fold increase in potency over the derivative **7a**, an improvement consistent with the IC_{50} difference observed between **6b** (Table 2) and **22g** (Table 4). In a series of *N*-acyl analogs of varying chain length, the *N*-pentanoyl and *N*-hexanoyl compounds **23c,d**, respectively, were optimum.

The importance of the phenolic oxygen to the overall potency of the tyrosine analogs was evaluated by the preparation of compound **27** (Table 5) in which this oxygen was replaced by a methylene group. Comparison of **23d** and **27** shows that a 43-fold loss in potency accompanies this structural change.

Potency improvements of 5–8-fold accompanied replacement of the *N*-acyl group by *N*-sulfonyl, as shown by comparison of the *N*-acetyl and *N*-methylsulfonyl derivatives **23a,k** or comparison of the *N*-phenylpropanoyl and *N*-(phenylethyl)sulfonyl derivatives **23e,g**. The arenesulfonamides **23f–j** demonstrated potencies in the 15–43 nM range, with the more potent containing the sulfonyl directly attached to an aryl ring. The potency of alkanesulfonamides was sensitive to chain length, with the methane- or propanesulfonamide 7- and 2-fold less potent, respectively, than the *n*-butane-

Table 5. Combined N- and C-Terminal Variations



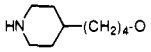
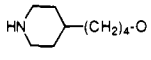
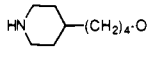
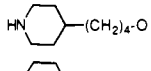
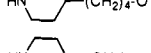
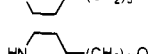
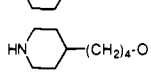
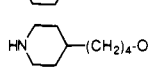
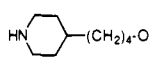
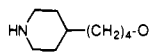
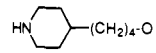
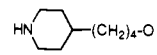
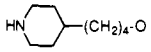
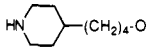
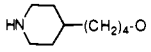
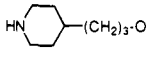
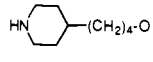
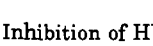
compound	R^1	R^2	R^3	n	IC_{50} (μM)
19b		H	H	0	24
23a		$NHCOCH_3$	H	0	0.5
23b		$NHCOCH_2CH_3$	H	0	0.105
23c		$NHCO(CH_2)_3CH_3$	H	0	0.047
23d		$NHCO(CH_2)_4CH_3$	H	0	0.035
27		$NHCO(CH_2)_4CH_3$	H	0	1.5
23e		$NHCO(CH_2)_2Ph$	H	0	0.10
23f			H	0	0.043
23g		$NHSO_2(CH_2)_2Ph$	H	0	0.029
23h		$NHSO_2CH_2Ph$	H	0	0.018
23i		$NHSO_2Ph$	H	0	0.018
23j			H	0	0.015
23k		$NHSO_2CH_3$	H	0	0.063
23l		$NHSO_2(CH_2)_2CH_3$	H	0	0.023
23m		$NHSO_2(CH_2)_3CH_3$	H	0	0.009
23n		H	$NHSO_2(CH_2)_3CH_3$	0	0.800
24a		$NHSO_2(CH_2)_3CH_3$	H	1	0.6
24b		$NHSO_2(CH_2)_3CH_3$	H	1	0.14

Table 6. Inhibition of HUVEC[a] Attachment to Fibrinogen (F_G), Vitronectin (V_N), and Fibronectin (F_N)

compound	IC_{50} (μM) (platelet)	IC_{50} (μM) (HUVEC) F_G	V_N	F_N
RGDW	1.7	34	14	27
echistatin	0.033	0.0005	0.0007	0.0007
1	35	>300	>300	>300
6c	2.6	315	147	>450
22j	0.78	330	150	>500
7a	0.26	158	56	300
23m	0.009	260	62	>300

[a] HUVEC—human umbilical vein endothelial cells. For details, see the *In Vitro* Pharmacology section of the experimental.

sulfonamide **23m** (IC_{50} = 9 nM). The effect of stereochemistry on potency was profound. The (*R*)-isomer of **23m** (**23n**, IC_{50} = 800 nM)[51] was ~100-fold less potent than **23m**.

Analogs **24a,b** were prepared to determine the optimal chain length in this inhibitor series. Homologation of the C-terminus by a single methylene diminished potency (**24b**, IC_{50} = 0.14 μM), while activity was further worsened by simultaneous shortening of the N-terminal side chain (**24a**, IC_{50} = 0.6 μM).

Molecular modeling comparison of **23m** to the cyclic peptide Ac-CRGDC-OH[37,52] (IC_{50} = 0.68 μM) offers insights as to how this ligand may mimic the RGD region of peptide inhibitors and how functional elements of **23m** may lead to enhanced potency. While the very flexible nature of the compounds involved precludes definitive conclusions, the exercise is useful in exploring the theory of RGD mimicry. Figures 1 and 2 demonstrate that the piperidinyl and carboxylic acid moieties of **23m** can substitute for the ionic groups of the Arg and Asp side chains, respectively. In addition, the ether oxygen of **23m** may interact with the receptor as a hydrogen bond acceptor. Modeling indicates that this interaction may be similar to that made by the Arg-Gly amide carbonyl oxygen of peptide inhibitors. Absence of this favorable interaction may be responsible for the drop in potency observed when the phenolic oxygen is replaced by methylene (**27**, 43-fold loss in potency). Additionally, orbital overlap of the oxygen π orbitals with the π cloud of the phenyl ring might result in a preference for a conformation that positions the $-OCH_2-$ unit in the same plane as the aromatic ring, as shown in Figure 2. This preference may confer an element of favorable conformational restriction on the oxygen-containing backbone of **23m** that is absent in the less potent methylene analog **27**.[53]

The aromatic ring of **23m** may be a direct surrogate for the Gly residue of the RGD tripeptide fragment, thus occupying the same site on the GPIIb/IIIa protein that the Gly residue fills (see Figure 1). Alternatively, the aromatic ring could serve as a scaffold to correctly space and position the necessary cationic and anionic sites of the ligand without directly replacing the Gly residue (see Figure 2).

The role of the *n*-butanesulfonamide α substituent is also of interest. The oxygen atoms of the sulfonamide group may interact with the receptor site occupied by the cysteine carboxylic acid of cyclic Ac-CRGDC-OH (see Figure 1), or, interestingly, the α substituent, or portions of it, may be interacting with a region of GpIIb/IIIa that is not exploited by the cyclic peptides (Figure 2). This region we refer to as the "exosite".[54] The ~100-fold difference in activity observed for **23m** and **23n** indicates a distinct receptor preference for the *S* configuration at the α position. Further modeling efforts are currently underway in an effort to understand this preference.

Since the sequence RGD is utilized by several integrin receptors to modulate cellular adhesion and migration, etc., the determination of specificity of **23m** for interaction at GPIIb/IIIa vs other receptors was of importance. The specificity of these inhibitors was determined by comparison of the inhibition of fibrinogen binding to platelet receptors with the inhibition of fibrinogen, vitronectin, and fibronectin binding to human umbilical vein endothelial cells (HUVEC) (Table 6).[56] The tetrapeptide RGDW inhibits fibrinogen binding to platelets at concentrations 15-fold less than those required to inhibit binding to the HUVEC cells. The snake venom peptide echistatin, while a more potent inhibitor, showed a greater affinity for HUVEC receptors than for fibrinogen receptors. Interestingly, for the series of compounds shown in Table 6, the affinity of the tyrosine derivatives for the *non-platelet* cell receptors did not change greatly

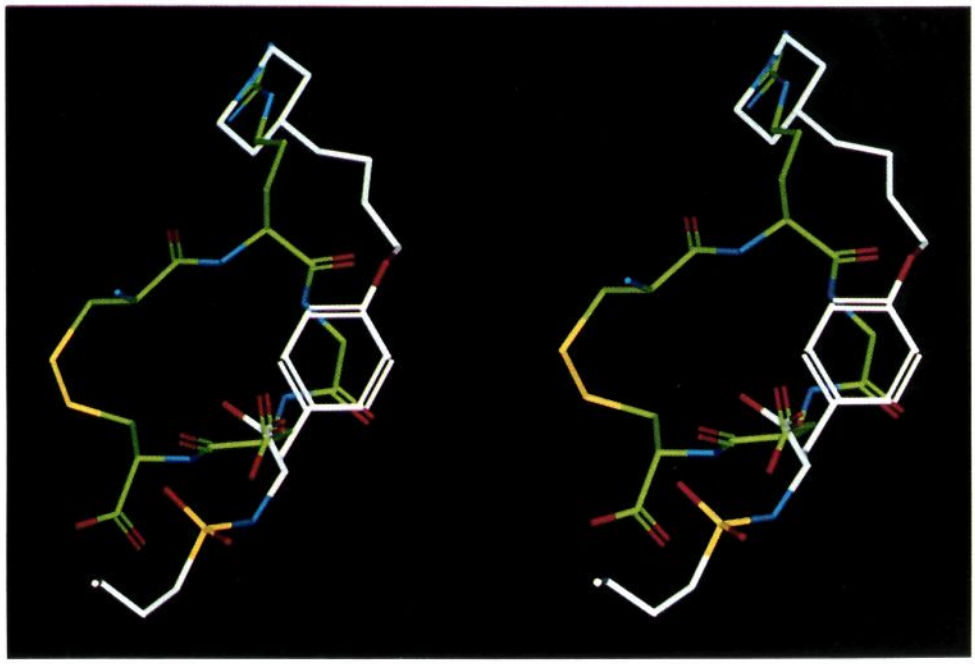


Figure 1. Stereoplot of one alignment of **23m** (backbone white, oxygen atoms red, nitrogen atoms light blue) with the NMR-based structure of cyclic Ac-CRGDC-OH (backbone green, oxygen atoms red, nitrogen atoms light blue, sulfur atoms yellow). In this view, the piperidine–Arg moieties are located at 12 o'clock and the acid–Asp groups are at 4 o'clock. In this comparison, the phenyl ring mimics the positional role of the Gly residue (3 o'clock position) while the sulfonamide oxygens may substitute for the C-terminal carboxylic acid of the peptide (6 o'clock position).

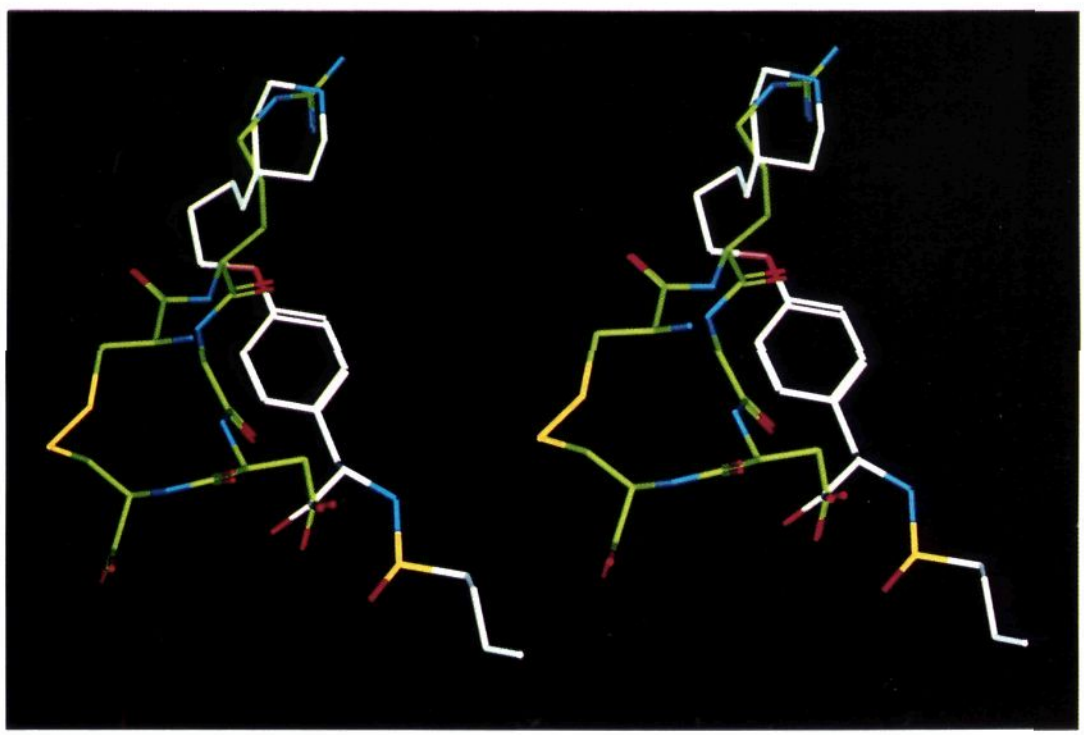


Figure 2. Stereoplot of one alignment of **23m** (backbone white, oxygen atoms red, nitrogen atoms light blue) with the NMR-based structure of cyclic Ac-CRGDC-OH (backbone green, oxygen atoms red, nitrogen atoms light blue, sulfur atoms yellow). In this view, the piperidine–Arg moieties are located at 12 o'clock and the acid–Asp groups are at 4 o'clock. In this comparison, the phenyl ring (3 o'clock position) acts as a scaffold to correctly position the piperidine and acid units while the ether oxygen atom substitutes for the Arg-Gly amide carbonyl and the ether–alkyl bond adopts a conformation that is coplanar with the aromatic ring (2 o'clock position).

while the affinity for the *platelet* receptor was improved significantly. Thus, compound **23m** demonstrated a selectivity of >24 000-fold between the platelet and HUVEC fibrinogen receptors.

The *in vivo* antiplatelet activity of **23m** was assessed by determining the extent of inhibition of *ex vivo* aggregation during 6 h of continuous iv infusions in pentobarbital-anesthetized dogs, using an experimental

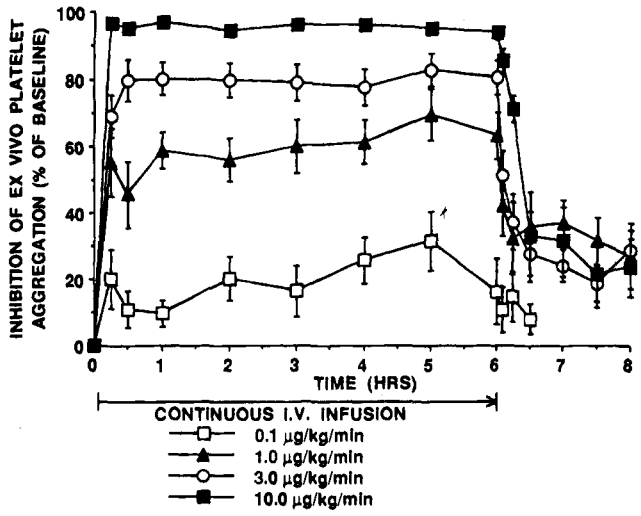


Figure 3. Inhibition of *ex vivo* platelet aggregation induced by ADP (10 μM) during and after 6 h of continuous iv infusions of 0.1, 1.0, 3.0, and 10.0 μg/kg/min of **23m** to anesthetized dogs (n = 4–5/group).

protocol and methods described previously.[57] Dose-dependent inhibition of *ex vivo* platelet aggregation induced by ADP was achieved with infusions of 0.1–10.0 μg/kg/min of **23m**, with the 10.0 μg/kg/min rate completely inhibiting platelet aggregation during the entire 6 h infusion protocol (Figure 3). Platelet aggregatability returned rapidly after the termination of the **23m** infusions (Figure 3). Platelet counts were unaffected by administration of **23m**.

Conclusions

Optimization of the lead compound **1** has led to the potent, selective fibrinogen receptor antagonist **23m**. Comparison of **23m** to the cyclic peptide Ac-CRGDC-OH suggests that elements of the non-peptide ligand may structurally mimic RGD-containing peptides while the crucial α-sulfonamido substituent may interact at a novel exosite on GpIIb/IIIa. The *in vivo* profile of this compound combines a rapid onset, dose-dependent, antithrombotic activity with an abrupt diminution of physiological activity after cessation of infusion. This profile may prove valuable for minimization of undesired bleeding should acute intervention become necessary. The preclinical and clinical evaluation of **23m** as an intravenous antiplatelet agent will be reported in due course.[58–60]

Experimental Section

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Varian XL-300 spectrometer unless otherwise noted. Chemical shifts are reported in parts per million relative to tetramethylsilane as the internal standard. Elemental analysis for carbon, hydrogen, and nitrogen were determined with Leeman Labs CEC 240XA and CE440 elemental analyzers and are within ±0.4% of the theoretical value unless noted otherwise. Low-mass spectra were recorded with a Vg Micromass MM7035 analytical spectrometer. High-resolution mass spectra were recorded with a Fisons VG AutoSpec Q high-resolution mass spectrometer. Normal phase silica gel (EM Science Silica Gel 60 (230–400 mesh)) was used for chromatography. HPLC separations were done using a semipreparative Vydac protein and peptide C_{18} column (cat. #218TP1022). TLC's were developed with ninhydrin or iodine stain. All starting materials and solvents were commercially available and used as received. Yields are unoptimized.

2-(*S*)-[[(Benzyloxy)carbonyl]amino]-3-[4-[(3-chloropropyl)oxy]phenyl]propionic Acid (3). *N*-Cbz-tyrosine (3.0 g, 9.9 mmol) was dissolved in dimethylformamide (DMF) (20 mL) and treated with NaH (50% dispersion in oil, 0.95 g, 19.8 mmol) for 1 h; then 1,3-bromochloropropane (1 mL, 9.9 mmol) was added and the reaction stirred for 16 h. The DMF was removed *in vacuo* and the residue dissolved in water, acidified to pH 3, and extracted with EtOAc. The EtOAc layer was dried with $MgSO_4$, filtered, and evaporated. Column chromatography (SiO_2, 97:3:1 $CHCl_3$/CH_3OH/HOAc) yielded 2.42 g (68%) of **3** as a yellow oil. R_f (97:3:1 $CHCl_3$/CH_3OH/HOAc): 0.3. NMR ($CDCl_3$): δ 7.3 (b s, 5H), 7.03 (d, J = 8.3 Hz, 2H), 6.8 (d, J = 8.3 Hz, 2H), 5.2 (d, J = 8 Hz, 1H), 5.05 (b s, 2H), 4.65 (m, 1H), 4.05 (t, J = 5.7 Hz, 2H), 3.73 (t, J = 6.3 Hz, 2H), 3.1 (m, 2H), 2.2 (m, 2H).

General Procedure for the Preparation of Compounds 4a–f. A solution of chloride **3** (0.4 g, 1.1 mmol) in acetonitrile (20 mL) was treated with the pertinent amine (20 mL) and refluxed for 72 h. The mixture was concentrated and the residue dissolved in water and washed with ether. The water layer was acidified to pH 2–3, extracted with EtOAc, dried with $MgSO_4$, filtered, and evaporated to give ~35% yield of the crude product as an amorphous solid. Further purification is described for each compound.

2-(*S*)-[[(Benzyloxy)carbonyl]amino]-3-[4-[[3-(*tert*-butylamino)propyl]oxy]phenyl]propionic Acid (4a). The amorphous crude product obtained as described above was precipitated from water at pH 7 to give pure **4a** (5%). R_f (9:1 EtOH/H_2O): 0.8. NMR (D_2O + NaOD): δ 7.4 (b s, 2H), 7.2 (b s, 4H), 6.85 (d, J = 8.55 Hz, 2H), 5.2 (d, J = 12.8 Hz, 1H), 5.0 (d, J = 12.8 Hz, 1H), 4.3 (dd, J = 4.0, 9.6 Hz, 1H), 4.0 (b s, 2H), 3.2 (dd, J = 4.0, 13.6 Hz, 1H), 2.8 (dd, J = 9.6, 13.6 Hz, 1H), 2.65 (t, J = 7.3 Hz, 2H), 1.8 (m, 2H), 1.09 (s, 9H). Exact mass (FAB, m + 1): calcd, 429.2389; found, 429.2378. Anal. ($C_{24}H_{32}N_2O_5$·1.6H_2O) C,H,N.

2-(*S*)-[[(*tert*-Butyloxy)carbonyl]amino]-3-[4-[[3-(*tert*-butylamino)propyl]oxy]phenyl]propionic Acid (4b). The general procedure was used, substituting *N*-BOC-tyrosine for **2**. Column chromatography (SiO_2, 9:2 EtOH/H_2O) gave **4b** (5%) as an amorphous solid. R_f (9:1 EtOH/H_2O): 0.8. NMR (CD_3OD): δ 7.17 (d, J = 8.5 Hz, 2H), 6.85 (d, J = 8.5 Hz, 2H), 4.28 (dd, J = 4.8, 9.1 Hz, 1H), 4.1 (t, J = 5.9 Hz, 2H), 3.2 (t, J = 7.7 Hz, 2H), 3.1 (dd, J = 4.8, 13.3 Hz, 1H), 2.8 (dd, J = 9.1, 13.3 Hz, 1H), 2.15 (m, 2H), 1.38 (s, 18H). Exact mass (FAB, m + 1): calcd, 395.2545; found, 395.2554. Anal. ($C_{12}H_{34}N_2O_5$) C,H,N.

2-(*S*)-[[(Benzyloxy)carbonyl]amino]-3-[4-[(3-phenylpropyl)oxy]phenyl]propionic Acid (4c). The general procedure was followed, using 1-bromo-3-phenylpropane as the alkylating agent. Column chromatography (SiO_2, 97:3:1 $CHCl_3$/CH_3OH/HOAc) followed by crystallization from EtOAc/hexanes gave **4c** (27%) as a crystalline solid (mp 88–89 °C). R_f (97:3:1 $CHCl_3$/CH_3OH/HOAc): 0.29. NMR (CD_3OD): δ 7.2 (m, 12H), 6.8 (d, J = 9 Hz, 2H), 5.1 (d, J = 13 Hz, 1H), 4.95 (d, J = 13 Hz, 1H), 4.35 (m, 1H), 3.9 (t, J = 6.5 Hz, 2H), 3.21 (dd, J = 1, 13.5 Hz, 1H), 2.8 (m, 1H), 2.77 (t, J = 13.5 Hz, 2H), 2.05 (m, 2H). Exact mass (FAB, m + 1): calcd, 434.1967; found, 434.1974. Anal. ($C_{26}H_{27}NO_5$) H,N; C: calcd, 72.04; observed, 72.48.

2-(*S*)-[[(Benzyloxy)carbonyl]amino]-3-[4-[[3-[(1,1,3,3-tetramethylbutyl)amino]propyl]oxy]phenyl]propionic Acid (4d). The general procedure was followed, using 1,1,3,3-tetramethylbutylamine as the amine component. Preparative reverse phase HPLC allowed the isolation of **4d** (10%) as the TFA salt. NMR (CD_3OD): δ 7.35 (m, 5H), 7.18 (d, J = 8.6 Hz, 1H), 6.85 (d, J = 8.6 Hz, 1H), 5.00 (s, 2H), 4.35 (dd, J = 4.8, 9.1 Hz, 1H), 4.10 (t, J = 6 Hz, 2H), 3.1 (m, 2H), 3.15 (dd, J = 4.7, 9.3 Hz, 1H), 2.50 (dd, J = 5, 9.3 Hz, 1H), 2.1 (m, 2H), 1.70 (s, 2H), 1.5 (s, 6H), 1.10 (s, 9H). Exact mass (FAB, m + 1): calcd, 485.3015; found, 485.3012. Anal. ($C_{28}H_{40}N_2O_5$·0.9TFA) C,H,N.

2-(*S*)-[[(Benzyloxy)carbonyl]amino]-3-[4-[[3-(*N*,*N*,2,2-tetramethyl-1,3-diaminopropyl)propyl]oxy]phenyl]-propionic Acid (4e). The general procedure was followed, using *N*,*N*,2,2-tetramethyl-1,3-diaminopropane as the amine component. Preparative reverse phase HPLC allowed the

isolation of **43** (10%) as the TFA salt. R_f (9:1:1 EtOH/H_2O/NH_4OH): 0.37. NMR (D_2O): δ 7.5 (b s, 3H), 7.4 (b s, 2H), 7.33 (d, J = 8.3 Hz, 2H), 7.0 (d, J = 8.3 Hz, 2H), 5.20 (d, J = 10 Hz, 1H), 5.10 (d, J = 10 Hz, 1H), 4.25 (m, 1H), 4.25 (t, J = 5.6 Hz, 2H), 3.4 (t, J = 7.8 Hz, 2H), 3.4 (s, 2H), 3.25–2.95 (m, 2H), 3.22 (s, 2H), 3.1 (s, 6H), 2.35 (m, 2H), 1.38 (s, 6H). Exact mass (FAB, m + 1): calcd, 486.2967; found, 486.2985. Anal. ($C_{27}H_{39}N_3O_5$·2.4TFA) C,H,N.

2-(*S*)-[[(Benzyloxy)carbonyl]amino]-3-[4-[(3-*N*-pyrrolidinylpropyl)oxy]phenyl]propionic Acid (4f). The general procedure was followed, using pyrrolidine as the amine component. Column chromatography (SiO_2, 9:1:1 EtOH/H_2O/NH_4OH) gave **4f** (37%) as an amorphous solid. R_f (9:1:1 EtOH/H_2O/NH_4OH): 0.81. NMR ($CDCl_3$): δ 7.3 (b s, 5H), 7.0 (d, J = 8.1 Hz, 2H), 6.7 (d, J = 8.1 Hz, 2H), 5.5 (d, J = 7.4 Hz, 1H), 5.0 (b s, 2H), 4.5 (m, 1H), 3.8 (m, 2H), 3.75 (b s, 1H), 3.4 (m, 2H), 3.18 (t, J = 8.5 Hz, 2H), 3.1 (b s, 2H), 2.8 (b s, 1H), 2.2–1.8 (m, 6H). Exact mass (FAB, m + 1): calcd, 427.2232; found, 427.2230. Anal. ($C_{24}H_{30}N_2O_5$·0.25CH_2Cl_2) C,H,N.

2-(*S*)-[[(Benzyloxy)carbonyl]amino]-3-[4-[[3-(*N*-methylpiperazyl)propyl]oxy]phenyl]propionic Acid (4g). The general procedure was followed, using *N*-methylpiperazine as the amine component. Preparative reverse phase HPLC allowed the isolation of **4g** (10%) as the TFA salt. R_f (9:1:1 EtOH/H_2O/NH_4OH): 0.46. NMR (D_2O): δ 7.5 (m, 3H), 7.4 (s, 2H), 7.3 (d, J = 8.5 Hz, 2H), 7.0 (d, J = 8.5 Hz, 2H), 5.18 (d, J = 13 Hz, 1H), 5.05 (d, J = 13 Hz, 1H), 4.5 (m, 1H), 4.2 (t, J = 6 Hz, 2H), 3.8 (s, 8H), 3.6 (t, J = 8 Hz, 2H), 3.3 (m, 1H), 3.1 (s, 3H), 3.0 (m, 1H), 2.4 (m, 2H). Exact mass (FAB, m + 1): calcd, 456.2498; found, 456.2488. Anal. ($C_{25}H_{38}N_3O_5$·2.3TFA) C,H,N.

2-(*S*)-[[(Benzyloxy)carbonyl]amino]-3-[4-[(4-piperazylbutyl)oxy]phenyl]propionic Acid (4h). The method was the same as that for **3** and **4**, except using 1,4-dibromobutane and piperazine, respectively. The product precipitated from the reaction mixture and was purified by preparative HPLC to give **4h** (5%) as the TFA salt. NMR (CD_3OD): δ 7.3 (m, 5H), 7.13 (d, J = 7 Hz, 2H), 6.83 (d, J = 7 Hz, 2H), 5.0 (b s, 2H), 4.35 (dd, J = 4, 8 Hz, 1H), 4.0 (t, J = 4.5 Hz, 2H), 3.6 (b s, 8H), 3.1 (dd, J = 4, 11.5 Hz, 1H), 2.85 (dd, J = 8, 11.5 Hz, 1H), 2.0–1.8 (m, 4H). Anal. ($C_{25}H_{33}N_3O_5$·2.95TFA) C,H,N.

2-(*S*)-[[(Benzyloxy)carbonyl]amino]-3-[4-[(5-piperazylpentyl)oxy]phenyl]propionic Acid (4i). The method was the same as that for **4h**, except using 1,5-dibromopentane. The crude reaction mixture was concentrated and chromatographed (SiO_2, 18:1:1 EtOH/H_2O/NH_4OH) to give **4i** (5%) as an amorphous white solid. R_f (18:1:1 EtOH/H_2O/NH_4OH): 0.35. NMR (CD_3OD): δ 7.2 (m, 5H), 7.09 (d, J = 7Hz, 2H), 6.7 (d, J = 7 Hz, 2H), 5.08 (d, J = 12 Hz, 1H), 4.95 (d, J = 12 Hz, 1H), 4.21 (dd, J = 4.5, 6 Hz, 1H), 3.94 (t, J = 5 Hz, 2H), 3.01–3.12 (m, 5H), 2.8 (dd, J = 4.5, 11 Hz, 1H), 2.6 (m, 4H), 2.4 (t, J = 6 Hz, 2H), 1.77 (m, 2H), 1.52 (m, 4H). Anal. ($C_{26}H_{35}N_3O_5$·1.2H_2O) C,H,N.

2-(*S*)-[[(Benzyloxy)carbonyl]amino]-3-[4-[[6-[*N*-[(*tert*-butyloxy)carbonyl]amino]hexyl]oxy]phenyl]propionic Acid (5a). *N*-Cbz-L-tyrosine (12.6 g, 40 mmol) was dissolved in 175 mL of DMF and cooled to 0–10 °C. Sodium hydride (50% dispersion, 4.2 g, 88 mmol) was added in portions, and the mixture was stirred for 1 h. Bromide **8b** (11.2 g, 40 mmol) was dissolved in DMF (25 mL) and added dropwise, and the resulting solution was stirred for 24 h. After solvent removal (*in vacuo*), the residue was taken up in EtOAc, and this was made acidic with 10% $KHSO_4$ solution. The organic phase was separated, washed with brine, dried (Na_2SO_4), and concentrated. Column chromatography (SiO_2, 98:2:1 $CHCl_3$/CH_3OH/HOAc) gave 14.06 g of **5a** (68%) as an oil. R_f (95:5:1 $CHCl_3$/CH_3OH/HOAc): 0.71. NMR (CD_3OD): δ 7.3 (m, 5H), 7.1 (d, J = 7.5 Hz, 2H), 6.8 (d, J = 7.5 Hz, 2H), 5.08 (d, J = 11 Hz, 1H), 5.00 (d, J = 11 Hz, 1H), 4.38 (m, 1H), 3.83 (t, J = 5 Hz, 2H), 3.12 (dd, J = 4, 12.5 Hz, 1H), 3.03 (t, J = 5.5 Hz, 2H), 2.86 (dd, J = 7.5, 12.5 Hz, 1H), 1.75 (m, 2H), 1.6–1.4 (m, 6H), 0.43 (s, 9H).

2-(*S*)-[[(Benzyloxy)carbonyl]amino]-3-[4-[(6-*N*-aminohexyl)oxy]phenyl]propionic Acid (6b). BOC-amine **5a** (51.4 mg, 0.1 mmol) was dissolved in 20 mL of EtOAc and cooled to −20 °C under nitrogen. HCl gas was bubbled into this solution for 10 min, and then the solution was placed in an ice bath for 1 h. The solvent was removed *in vacuo*, and the residue was chromatographed (SiO_2, 9:1:1 EtOH/NH_4OH/H_2O) to give **6b** (14.4 mg, 35%) as an amorphous white solid. R_f (SiO_2, 9:1:1 EtOH/NH_4OH/H_2O): 0.4. NMR (CD_3OD): δ 7.3 (m, 5H), 7.1 (d, J = 7 Hz, 2H), 6.77 (d, J = 7 Hz, 2H), 5.0 (m, 2H), 4.3 (m, 1H), 3.95 (t, J = 5 Hz, 2H), 3.13 (m, 1H), 2.90 (m, 3H), 1.73 (m, 4H), 1.45 (m, 6H). Anal. ($C_{23}H_{30}N_2O_5$·0.05EtOH·1.05H_2O) C,H,N.

2-(*S*)-[[(Benzyloxy)carbonyl]amino]-3-[4-[(5-*N*-aminopentyl)oxy]phenyl]propionic Acid (6a). The method was the same as that for **6b**, except using **8a**. After removal of EtOAc, the solid was triturated with 5% MeOH/EtOAc to give **6a** as an amorphous white solid. NMR (CD_3OD): δ 7.3 (m, 5H), 7.13 (d, J = 7 Hz, 2H), 6.82 (d, J = 7 Hz, 2H), 5.08 (d, J = 7.5 Hz, 1H), 5.0 (d, J = 7.5 Hz, 1H), 4.36 (dd, J = 4, 8.5 Hz, 1H), 3.95 (t, J = 5.5 Hz, 2H), 3.12 (dd, J = 5.5, 12 Hz, 1H), 2.95 (t, J = 6 Hz, 2H), 2.84 (dd, J = 8.5, 13 Hz, 1H), 1.82 (m, 2H), 1.73 (m, 2H), 1.6 (m, 2H). Anal. ($C_{22}H_{29}N_2O_5$·0.25H_2O) C,H,N.

2-(*S*)-[[(Benzyloxy)carbonyl]amino]-3-[4-[(7-*N*-aminoheptyl)oxy]phenyl]propionic Acid (6c). The method was the same as that for **6b**, except using **8c** gave **6c** (20%) as an amorphous white solid. NMR (CD_3OD): δ 7.33 (m, 5H), 7.13 (d, J = 6.5 Hz, 2H), 6.83 (d, J = 6.5 Hz, 2H), 5.06 (d, J = 11.5 Hz, 1H), 5.0 (d, J = 11.5 Hz, 1H), 2.6 (dd, J = 4, 8.5 Hz, 1H), 3.95 (t, J = 5.5 Hz, 2H), 3.2 (dd, J = 4, 12 Hz, 1H), 2.95 (t, J = 6.5 Hz, 2H), 2.85 (dd, J = 8.5, 12 Hz, 1H), 1.8 (m, 4H), 1.6 (m, 6H). Anal. ($C_{24}H_{32}N_2O_5$·0.2EtOH·0.75H_2O) C,H,N.

2-(*S*)-[[(Benzyloxy)carbonyl]amino]-3-[4-[(8-*N*-aminooctyl)oxy]phenyl]propionic Acid (6d). The method was the same as that for **6b**, except using **8d**. After removal of EtOAc, the solid was triturated with EtOAc to give **6d** (87%) as an amorphous white solid. NMR (CD_3OD): δ 7.3 (m, 5H), 7.2 (d, J = 6.5 Hz, 2H), 6.8 (d, J = 6.5 Hz, 2H), 5.05 (d, J = 11.5 Hz, 1H), 5.0 (d, J = 11.5 Hz, 1H), 4.35 (dd, J = 4, 8 Hz, 2H), 3.95 (t, J = 5 Hz, 2H), 3.1 (dd, J = 4, 12 Hz, 1H), 2.9 (t, J = 7 Hz, 2H), 2.85 (dd, J = 8, 12 Hz, 1H), 1.75 (m, 2H), 1.65 (m, 2H), 1.5 (m, 2H), 1.4 (b s, 6H). Anal. ($C_{25}H_{35}N_2O_5$·HCl·0.75H_2O) C,H,N.

2-(*S*)-[[(Benzyloxy)carbonyl]amino]-3-[4-[[(piperidin-4-yl)butyl]oxy]phenyl]propionic Acid (7a). Sodium hydride (60% dispersion, 2.88 g, 0.12 mol) was placed under nitrogen and washed with 2 × 50 mL portions of hexane. DMF (35 mL) was added, and the mixture was cooled to 0 °C. A solution of *N*-Cbz-L-tyrosine (17.58 g, 0.055 mol) in 50 mL of DMF was added dropwise; the mixture was stirred for 1 h and then treated with a solution of **9** (17.7 g, 0.055 mol) in 50 mL of DMF and allowed to stir for 18 h. The solvent was removed *in vacuo*, and the residue was partitioned between EtOAc (500 mL)/10% KHSO4 (100 mL). The acidic aqueous phase was re-extracted with EtOAc (300 mL), and the combined organic extracts were washed with brine, dried (Na_2SO_4), and concentrated. The residue was chromatographed (SiO_2, 98:2:0.5 $CHCl_3$/MeOH/HOAc) to provide **5b** (23.7 g, 77%) as an oil. R_f (98:2:0.5 $CHCl_3$/MeOH/HOac): 0.30. NMR ($CDCl_3$): δ 7.33 (m, 5H), 7.05 (d, J = 6.5 Hz, 2H), 6.8 (d, J = 6.5 Hz, 2H), 5.2 (b d, 1H), 5.1 (b s, 2H), 4.63 (m, 1H), 4.01 (b d, 2H), 3.92 (t, J = 6 Hz, 2H), 3.7 (m, 2H), 2.65 (b t, 7H), 1.75–1.4 (m, 7H), 1.45 (s, 9H), 1.3 (m, 2H), 1.1 (m, 2H). A solution of **5b** (160 mg, 0.297 mmol) in EtOAc (15 mL) was cooled to −40 °C, and HCl gas was bubbled through the solution until it was saturated. The reaction mixture was warmed to 0 °C for 1 h and then evaporated, first at room temperature and then at 40 °C, to give **7a** as an oil. Column chromatography (18:1:1 EtOH/H_2O/NH_4OH) gave **7a** (67 mg, 50%) as a white solid. R_f (9:1:1 EtOH/H_2O/NH_4OH): 0.59. NMR (400 MHz, DMSO): δ 7.3 (m, 5H), 7.0 (d, J = 8 Hz, 2H), 6.72 (d, J = 8 Hz, 2H), 6.52 (b s, 1H), 5.0 (d, J = 13 Hz, 1H), 4.96 (d, J = 13 Hz, 1H), 3.92 (t, J = 5 Hz, 2H), 3.87 (m, 1H), 3.2 (b s, 4H), 2.95 (dd, J = 5, 13

Hz, 1H), 2.83 (dd, J = 5, 13 Hz, 1H), 2.65 (t, J = 12 Hz, 4H), 1.65 (m, 3H), 1.37 (m, 2H), 1.15 (m, 2H). Mass spectroscopy (FAB, m + 1): 455. Anal. ($C_{26}H_{34}N_2O_5 \cdot 0.8H_2O$) C,H,N.

2-(*S*)-[[(Benzyloxy)carbonyl]amino]-3-[4-[[(piperidin-4-yl)but-2-enyl]oxy]phenyl]propionic Acid (7b). The method was the same as that for **7a**, except using alkylating agent 11 gave **7b** (65% for two steps) as an amorphous white solid. A small sample was purified on reverse phase HPLC to give the product as the TFA salt. R_f (9:1:1 EtOH/H_2O/NH_4OH): 0.45. NMR (D_2O): δ 7.2 (m, 3H), 7.1 (m, 4H), 6.7 (d, J = 8.5 Hz, 2H), 5.5 (m, 2H), 5.1 (d, J = 13 Hz, 1H), 4.8 (d, J = 13 Hz, 1H), 4.22 (b s, 3H), 3.2 (b d, J = 6 Hz, 2H), 3.0 (m, 3H), 2.25 (b t, J = 11.5 Hz, 2H), 1.8 (m, 2H), 1.4 (m, 3H), 1.2 (m, 1H), 0.9 (m, 2H). Exact mass (FAB, m + 1): calcd, 453.2384; found, 453.2373. Anal. ($C_{26}H_{32}N_2O_5 \cdot 1.2TFA$) C,H,N.

2-(*S*)-[*N*-[(Benzyloxy)carbonyl]-*N*-methylamino]-3-[4-[[(piperidin-4-yl)butyl]oxy]phenyl]propionic Acid (7c). A solution of **5b** (0.785 g, 1.4 mmol) in THF (10 mL) was cooled to 0 °C, treated consecutively with methyl iodide (1.61 g, 11.3 mmol) and sodium hydride (60% dispersion in oil, 0.17 g, 4.2 mmol), and allowed to stir and warm gradually over 18 h. The solution was diluted with 100 mL of EtOAc and 30 mL of H_2O and then acidified with 10% $KHSO_4$ to pH 3 and extracted. The organic layers were separated, dried with brine and Na_2SO_4, filtered, and concentrated, and the residue was chromatographed (SiO_2, 97:3:1 $CHCl_3$/MeOH/HOAc) to give the N-methylated product in 69% yield. R_f (97:3:1 $CHCl_3$/MeOH/HOAc): 0.3. NMR (CD_3OD): δ 7.3 (m, 3H), 7.2 (b s, 2H), 7.08 (d, J = 7.5 Hz, 1H), 7.04 (d, J = 7.5 Hz, 1H), 6.78 (d, J = 6 Hz, 1H), 6.76 (d, J = 6 Hz, 1H), 5.1 (d, J = 10.5 Hz, 1H), 5.0 (d, J = 10.5 Hz, 1H), 4.8 (m, 1H), 4.05 (b d, J = 11 Hz, 2H), 3.94 (b t, J = 5 Hz, 2H), 3.22 (m, 1H), 3.0 (m, 1H), 2.8 (s, 3H), 2.7 (m, 2H), 1.7 (m, 4H), 1.5 (m, 2H), 1.43 (s, 9H), 1.3 (m, 2H), 1.05 (m, 2H). This intermediate (0.2 g, 0.35 mmol) was dissolved in EtOAc (30 mL), cooled to −20 °C, and treated with HCl gas. The solution was warmed to 0 °C for 1 h and then evaporated to give **7c** (0.113 g, 88%) as a hygroscopic solid. R_f (9:1:1 EtOH/H_2O/NH_4OH): 0.5. NMR (CD_3OD): δ 7.3 (m, 3H), 7.2 (m, 2H), 7.1 (d, J = 6.5 Hz, 1H), 7.05 (d, J = 6.5 Hz, 1H), 6.78 (d, J = 5 Hz, 1H), 6.76 (d, J = 5 Hz, 1H), 5.1 (d, J = 11.5 Hz, 1H), 5.03 (d, J = 11.5 Hz, 1H), 4.8 (m, 1H), 3.95 (b t, J = 5 Hz, 2H), 3.35 (b d, J = 10 Hz, 2H), 3.23 (m, 1H), 2.9 (b t, J = 11.5 Hz, 4H), 2.8 (s, 3H), 1.95 (b d, J = 12 Hz, 2H), 1.8 (m, 2H), 1.5 (m, 4H), 1.4 (m, 5H). Anal. ($C_{27}H_{36}N_2O_5 \cdot HCl \cdot 0.75H_2O$) C,H,N.

General Procedure for Preparation of Compounds 8a–d. A 0.05 M THF solution of the pertinent *N*-[(*tert*-butyloxy)carbonyl]amino acid was cooled to 0 °C under argon. A 1 M THF solution of BH_3 (3 equiv) was added dropwise, and the solution was stirred for 1 h. A 1 N solution of NaOH (3.2 equiv) was added, keeping the temperature of the reaction mixture between 5 and 10 °C. The solution was then allowed to warm to room temperature. The THF was removed by evaporation, and the remaining aqueous solution was extracted with 3 × 250 mL of Et_2O. The organic layer was dried with brine and $MgSO_4$, filtered, and evaporated to give the desired *N*-[(*tert*-butyloxy)carbonyl]amino alcohol in >90% yield. A 0.2 M THF solution of the alcohol was treated sequentially with triphenylphosphine (1.3 equiv) and an acetonitrile solution of carbon tetrabromide (1.3 equiv) and allowed to stir for 24 h. The solution was concentrated and the residue chromatographed (SiO_2, 10% EtOAc/hexanes) to give the product (70%) as a white solid.

5-[*N*-[(*tert*-Butyloxy)carbonyl]amino]pentyl bromide (8a): R_f (1:4 EtOAc/hexanes) 0.57; NMR (CD_3OD) δ 6.6 (b s, 1H), 3.42 (t, 2H), 3.02 (dd, 2H), 1.85 (m, 2H), 1.5–1.4 (m, 13H).

6-[*N*-[(*tert*-Butyloxy)carbonyl]amino]hexyl bromide (8b): R_f (1:4 EtOAc/hexanes) 0.58; NMR ($CDCl_3$) δ 4.55 (b s, 1H), 3.4 (t, 2H), 3.1 (m, 2H), 1.85 (m, 2H), 1.6–1.2 (m, 15H).

7-[*N*-[(*tert*-Butyloxy)carbonyl]amino]heptyl bromide (8c): R_f (10% EtOAc/hexanes) 0.32; NMR (400 MHz, $CDCl_3$) δ 4.5 (b s, 1H), 3.4 (t, J = 7 Hz, 2H), 3.1 (m, 2H), 1.83 (m, 2H), 1.45 (s, 13H), 1.3 (m, 4H).

8-[*N*-[(*tert*-Butyloxy)carbonyl]amino]octyl bromide (8d): R_f (1:4 EtOAc/hexanes) 0.54; NMR (CD_3OD) δ 4.55 (b s, 1H), 3.4 (t, 2H), 3.1 (m, 2H), 1.85 (m, 2H), 1.4 (m, 13H), 1.3 (m, 6H).

4-[*N*-[(*tert*-Butyloxy)carbonyl]piperidin-4-yl]butyl Bromide (9). 4-Pyridineacetic acid (50 g, 0.314 mol) was dissolved in H_2O (50 mL) and HOAc (100 mL), PtO_2 (1 g) was added, and the mixture was hydrogenated at 55 psi for 72 h. An additional 1 g of PtO_2 was added after 48 h. The solution was filtered through Celite, the cake was washed with H_2O, and the filtrate was evaporated, using heptane to azeotrope off excess HOAc. The 4-piperadineacetic acid (51.6 g, 87%) was obtained as a white solid. R_f (9:1 EtOH/H_2O): 0.11. NMR (400 MHz, CD_3OD): δ 3.36 (b d, 2H), 3.30 (b t, 2H), 2.3 (d, J = 7 Hz, 2H), 2.08 (m, 1H), 1.99 (b d, 2H), 1.47 (m, 2H). This solid was dissolved in H_2O (200 mL) and dioxane (200 mL), treated with triethylamine (76 mL, 0.546 mol) and di-*tert*-butyl dicarbonate (59.5 g, 0.273 mol), and stirred for 96 h. After 72 h, an additional equivalent of triethylamine and 10 g of di-*tert*-butyl dicarbonate were added. The organic solvent was evaporated, and the remaining aqueous solution was brought to pH 10 with saturated $NaHCO_3$, washed with 3 × 50 mL of EtOAc, acidified with 10% $KHSO_4$ to pH 2–3, extracted with 3 × 100 mL of EtOAc, dried with $MgSO_4$, filtered, and evaporated to give *N*-[(*tert*-butyloxy)carbonyl]-4-piperidineacetic acid (67 g, >100%). R_f (9:1 EtOH/H_2O): 0.68. NMR ($CDCl_3$): δ 4.1 (b s, 2H), 2.7 (b t, 2H), 2.27 (d, J = 7 Hz, 1.95 (m, 1H), 2.7 (b d, 2H), 1.45 (s, 9H), 1.18 (m, 2H). This acid was reduced with borane as described for compounds **8a–d** to yield *N*-[(*tert*-butyloxy)carbonyl]-4-piperidineethanol (99%) as an oil. R_f (1:1 EtOAc/hexanes): 0.38. NMR (400 MHz, $CDCl_3$): δ 4.08 (b d, J = 12 Hz, 2H), 3.7 (t, J = 6.5 Hz, 2H), 2.68 (b t, 12 Hz, 2H), 1.67 (b d, 2H), 1.6–1.45 (m, 3H), 1.45 (s, 9H), 1.12 (m, 2H). This alcohol (102 g, 0.45 mol) was added dropwise to a solution of oxalyl chloride (55.8 mL, 0.64 mol) and DMSO (54.2 mL, 0.76 mol) in CH_2Cl_2 (1 L) at −78 °C under argon. After 20 min, triethylamine (213 mL, 1.53 mol) was added dropwise and the cooling bath was removed for 1.5 h. The intermediate 2-[*N*-[(*tert*-butyloxy)carbonyl]piperidinyl-4-yl]ethyl aldehyde was not isolated but treated with (carbomethoxymethylene)triphenylphosphorane (179 g, 0.536 mol) in CH_2Cl_2 (350 mL). The reaction mixture was stirred at room temperature overnight and then diluted with 300 mL of Et_2O, extracted with 800 mL of H_2O and 2 × 300 mL of 10% $KHSO_4$, filtered, and evaporated. Column chromatography (SiO_2, 5% EtOAc/hexanes) yielded methyl 4-[*N*-[(*tert*-butyloxy)carbonyl]-piperidin-4-yl]but-2-enoate (78.4 g, 62%) as a clear oil. R_f (5% EtOAc/hexanes): 0.35. NMR ($CDCl_3$): δ 6.99 (ddd, J = 15.6, 7.6, 7.6 Hz, 1H), 5.8 (d, J = 15.6 Hz, 1H), 4.0 (b s, 2H), 3.7 (s, 3H), 2.6 (t, J = 12.6 Hz, 2H), 2.1 (t, J = 7.4 Hz, 2H), 1.7–1.4 (m, 3H), 1.4 (s, 9H), 1.1 (m, 2H). This butenoate (36.2 g, 0.128 mol) was dissolved in EtOAc (500 mL), 10% palladium on carbon (10 g) was added as a slurry in EtOAc, and the reaction mixture was hydrogenated under balloon pressure overnight. The mixture was filtered through Solka-Floc, and the cake was washed with EtOAc. The solution was evaporated to give methyl 4-[*N*-[(*tert*-butyloxy)carbonyl]piperidin-4-yl]butanoate (34.7 g, 90%). R_f (30% EtOAc/hexanes): 0.69. NMR ($CDCl_3$): δ 4.0 (b s, 2H), 3.6 (s, 3H), 2.60 (t, J = 12.2 Hz, 2H), 2.20 (t, J = 7.4 Hz, 2H), 1.6 (m, 4H), 1.4 (s, 9H), 1.2 (m, 2H), 1.0 (m, 2H). This methyl ester (45.3 g, 0.159 mol) was dissolved in CH_3OH (150 mL) and treated with 1 N NaOH (500 mL, 0.5 mol) overnight. The solvent was removed *in vacuo*, water was added, and the resulting solution was washed with ether. The water layer was then acidified with 10% $KHSO_4$ solution and extracted with ether. The ether layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo* to give 4-[4-[(*tert*-butyloxy)carbonyl]piperidinyl)butanoic acid (41.8 g, 97%) as a clear oil. NMR ($CDCl_3$): δ 4.0 (b s, 2H), 2.6 (m, 2H), 2.25 (m, 2H), 1.6 (b s, 4H), 1.4 (s, 9H), 1.3–0.9 (m, 9H). This acid (20.4 g, 0.077 mol) was reduced with borane as described for compounds **8a–d** to give 4-[4-[(*tert*-butyloxy)carbonyl]piperidinyl]butanol (19.7 g, 98%) as a colorless oil. This alcohol (19.7 g, 76.5 mmol) was treated with triphenylphosphine and carbon tetrabromide as described for compounds **8a–d** and chromatographed (SiO_2, 5% EtOAc/hexanes) to yield **9** (20.7 g, 85%) as

a colorless oil. R_f (1:4 EtOAc/hexanes): 0.6. NMR ($CDCl_3$): δ 4.1 (b s, 2H), 3.4 (t, 2H), 2.65 (t, 2H), 1.85 (m, 2H), 1.65 (b d, 2H), 1.4 (s, 9H), 1.35 (m, 2H), 1.3 (m, 3H), 1.1 (m, 2H).

3-[*N*-[(*tert*-Butyloxy)carbonyl]piperidin-4-yl]propyl Iodide (10). 4-Pyridinepropanol (15 g, 0.11 mol) was dissolved in HOAc (100 mL), 10% palladium on carbon (2 g) was added, and the reaction mixture was hydrogenated at 50 psi on a Parr apparatus for 48 h. The reaction mixture was filtered through Celite, and the cake was washed with HOAc. The solution was evaporated to give 3-[piperidin-4-yl]propyl alcohol (42.7 g, 95%) which contained 2.5 equiv of HOAc. R_f (5% MeOH/$CHCl_3$ saturated with NH_3): 0.08. NMR (400 MHz, $CDCl_3$): δ 8.0 (b s, 2H), 3.63 (t, J = 7 Hz, 2H), 3.35 (b d, 2H), 2.8 (b t, 2H), 2.0 (s, HOAc), 1.82 (b d, 2H), 1.55 (m, 2H), 1.47 (m, 2H), 1.38 (m, 2H). This alcohol (41.8 g, 0.146 mol) was dissolved in dioxane (300 mL) and treated with 3 N NaOH (150 mL) and di-*tert*-butyl dicarbonate (32.1 g, 0.147 mol) for 18 h. The dioxane was removed by evaporation, and the remaining solution was extracted with 3 × 100 mL of Et_2O. The organic layer was washed with 3 × 25 mL of 10% $KHSO_4$ and brine, dried over $MgSO_4$, filtered, and evaporated. The residue was chromatographed (SiO_2, 30–50% EtOAc/hexanes) to give 3-[*N*-[(*tert*-butyloxy)carbonyl]piperidin-4-yl]propyl alcohol (27.7 g, 73%). R_f (20% EtOAc/hexanes): 0.07. NMR (400 MHz, $CDCl_3$): δ 4.06 (b d, 2H), 3.61 (t, J = 7 Hz, 2H), 2.65 (b t, 2H), 1.65 (b d, 2H), 1.6–1.5 (m, 2H), 1.44 (s, 9H), 1.38 (m, 1H), 1.3 (m, 2H), 1.08 (m, 2H). This alcohol (22.7 g, 93.4 mmol) was dissolved in toluene (800 mL), cooled to 0 °C, and treated sequentially with triphenylphosphine chloride (26.7 g, 0.121 mol), imidazole (19 g, 0.28 mol), and iodine (30.6 g, 0.121 mol) for 1.5 h. The solution was washed with 2 × 500 mL of saturated Na_2CO_3, 2 × 250 mL of 5% $Na_2S_2O_3$, 2 × 100 mL of $KHSO_4$, and 2 × 100 mL of brine. The solution was dried over $MgSO_4$, filtered, and evaporated. The residue was chromatographed (SiO_2, 30% EtOAc/hexanes) to give **10** (25.6 g, 78%). R_f (10% EtOAc/hexanes): 0.41. NMR (400 MHz, $CDCl_3$): δ 4.05 (b s, 2H), 3.15 (t, J = 7 Hz, 2H), 2.65 (b t, 2H), 1.82 (m, 2H), 1.63 (b d, 2H), 1.3 (s, 9H), 1.4 (m, 1H), 1.35 (m, 2H), 1.1 (m, 2H).

4-[*N*-[(*tert*-Butyloxy)carbonyl]piperidin-4-yl]but-2-enyl Bromide (11). The intermediate methyl 4-[*N*-[(*tert*-butyloxy)carbonyl]piperidin-4-yl]butanoate described in the preparation of **9** (0.66 g, 2.22 mmol) was dissolved in $CHCl_3$ (25 mL), cooled to −78 °C under argon, treated with DIBAL (4.44 mL, 4.44 mmol), warmed to −30 °C, diluted with ether (130 mL), and treated with H_2O (3 mL). The mixture was stirred overnight and then $MgSO_4$ was added, and the solution was filtered and evaporated to give 0.32 g (56%) 4-[*N*-[(*tert*-butyloxy)carbonyl]piperidin-4-yl]but-2-enol after chromatography (SiO_2, 60% Et_2O/hexanes). R_f (50% Et_2O/hexanes): 0.18. NMR ($CDCl_3$): δ 5.8 (m, 2H), 4.05 (m, 4H), 2.64 (b t, 3H), 2.0 (t, 2H), 1.8 (m, 1H), 1.65 (b d, 2H), 1.53 (s, 9H), 1.15 (m, 2H). This alcohol (0.46 g, 1.82 mmol) was treated at −20 °C with triphenylphosphine and carbon tetrabromide as described for compounds **8a–d** to give **11** (0.295 g, 75%) after chromatography (SiO_2, 10% EtOAc/hexanes). R_f (10% EtOAc/hexanes): 0.27. NMR ($CDCl_3$): δ 5.7 (m, 2H), 4.05 (b s, 2H), 3.96 (d, J = 6 Hz, 2H), 2.62 (b t, 2H), 2.0 (t, J = 7 Hz, 2H), 1.6 (b d, 2H), 1.43 (s, 10H), 1.1 (m, 2H).

5-[*N*-[(*tert*-Butyloxy)carbonyl]piperidin-4-yl]pent-3-en-1-yne (12). [(Trimethylsilyl)propargyl]triphenylphosphonium bromide (3.0 g, 6.6 mmol) was suspended in THF (20 mL) under argon, cooled to −78 °C, and treated with *n*-butyllithium (1.6 M, 4.13 mL, 9.5 mmol). The solution was allowed to warm to −40 °C for 0.5 h and then recooled to −78 °C and treated with the intermediate 2-[*N*-[(*tert*-butyloxy)-carbonyl]piperidin-4-yl]ethyl aldehyde described in the preparation of **9** (1.07 g, 4.7 mmol). The reaction mixture was allowed to warm to 0 °C for 1 h and then diluted with water and EtOAc, and the layers were separated. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and evaporated to give 0.96 g (47%) of crude 1-(trimethylsilyl)-5-[*N*-[(*tert*-butyloxy)carbonyl]piperidin-4-yl]pent-3-en-1-yne. R_f (5% EtOAc/hexanes): 0.20. NMR ($CDCl_3$): δ 6.0 (m, 1H), 5.31 (d, J = 4.5 Hz, 1H), 3.85 (b s, 2H), 2.45 (b t, 2H), 1.82 (t, J = 7 Hz, 1H), 1.5 (b d, 2H), 1.26 (s, 9H), 0.93 (m, 2H), 0.0 (s, 9H). This TMS-enyne (0.815 g, 2.5 mmol) was dissolved in THF/H_2O (60 mL/12 mL) and treated with $LiOH·H_2O$ (0.96 g, 23 mmol) at room temperature for 6 h. The reaction mixture was diluted with ether, and the water layer was separated and washed with ether. The ether layers were combined, washed with brine, dried over $MgSO_4$, filtered, and evaporated. The residue was purified (SiO_2, 10% EtOAc/hexanes) to give 0.3 g (47%) of **12** which was used immediately (see **26**). R_f (10% EtOAc/hexanes): 0.30. NMR ($CDCl_3$): δ 6.2 (m, 1H), 5.43 (m, 1H), 4.06 (b s, 2H), 2.65 (b t, 2H), 2.3 (t, J = 5 Hz, 1H), 2.04 (t, J = 7 Hz, 2H), 1.6 (b s, 3H), 1.45 (s, 9H), 1.05 (m, 2H).

Ethyl 2-(*S*)-Methyl-2-[*N*-[(benzyloxy)carbonyl]amino]-3-(4-hydroxyphenyl)propionic Acid Ester (13a). Ethyl α-methyltyrosine hydrochloride (3.9 g, 15 mmol) was dissolved in DMF (100 mL), cooled to 0 °C, and treated with Et_3N (1.52 g, 15 mmol) and benzyl chloroformate (2.81 g, 16.5 mmol). The solution was allowed to warm to room temperature and stir for 72 h. The solvent was removed *in vacuo* and the residue dissolved in 150 mL of ether and washed with 2 × 50 mL of H_2O, 2 × 50 mL of 10% $KHSO_4$, and brine. The solution was dried (Na_2SO_4) and concentrated. Column chromatography (SiO_2, 98:2 $CHCl_3$/MeOH) gave **13a** (2.75 g, 44%) as an oil. R_f (98:2 $CHCl_3$/MeOH): 0.3. NMR (CD_3OD): 7.34 (m, 5H), 6.83 (d, J = 7 Hz, 2H), 6.62 (d, J = 7 Hz, 2H), 5.11 (d, J = 11 Hz, 1H), 5.06 (d, J = 11 Hz, 1H), 4.1 (m, 2H), 3.15 (d, J = 11.5 Hz, 1H), 3.0 (d, J = 11.5 Hz, 1H), 1.35 (s, 9H), 1.2 (b t, J = 5.5 Hz, 3H).

Ethyl 2-(*S*)-Methyl-2-[*N*-[(benzyloxy)carbonyl]amino]-3-[4-[[[*N*-[(*tert*-butyloxy)carbonyl]piperidin-4-yl]butyl]-oxy]phenyl]propionic Acid Ester (14a, n = 4, R^1 = CH_2CH_3, R^2 = CH_3). The method was the same as that for **7a**, except using **13a** and column chromatography (SiO_2 1:4 EtOAc/hexanes) gave **14a** in 69% yield. R_f (1:4 EtOAc/hexanes): 0.43. NMR ($CDCl_3$): δ 7.35 (m, 5H), 6.88 (d, J = 6 Hz, 2H), 6.7 (d, J = 6 Hz, 2H), 5.48 (b s, 1H), 5.18 (d, J = 10 Hz, 1H), 5.08 (d, J = 10 Hz, 1H), 4.2 (m, 2H), 4.08 (b d, J = 12 Hz, 2H), 3.9 (t, J = 6 Hz, 2H), 3.35 (b d, 1H), 3.1 (d, J = 11.5 Hz, 1H), 2.65 (t, J = 11 Hz, 2H), 1.7 (m, 2H), 1.6 (b s, 5H), 1.45 (s, 9H), 1.3 (m, 8H), 1.1 (m, 2H).

2-(*S*)-Methyl-2-[*N*-[(benzyloxy)carbonyl]amino]-3-[4-[[(piperidin-4-yl)butyl]oxy]phenyl]propionic Acid (15a). A solution of **14a** (n = 4, R_1 = CH_2CH_3, R_2 = CH_3) (0.24 g, 0.4 mmol) in 1:1:1 THF/MeOH/H_2O (10 mL) was treated with LiOH (0.048 g, 2 mmol) for 18 h. The solution was diluted with H_2O (75 mL), acidified with 10% $KHSO_4$, and extracted with EtOAc. The organic layer was dried (brine, Na_2SO_4), filtered, and evaporated. The residue was chromatographed (SiO_2, 97:3:1 $CHCl_3$/MeOH/HOAc) to give the acid in 89% yield. R_f (97:3:1 $CHCl_3$/MeOH/HOAc): 0.45. NMR (CD_3OD): δ 7.35 (m, 5H), 6.9 (d, J = 6 Hz, 2H), 6.7 (d, J = 6 Hz, 2H), 5.1 (s, 2H), 4.05 (b d, J = 11 Hz, 2H), 3.9 (t, J = 6 Hz, 2H), 3.2 (d, J = 11 Hz, 1H), 3.1 (d, J = 11 Hz, 1H), 2.7 (m, 2H), 1.7 (m, 4H), 1.5 (m, 4H), 1.45 (s, 9H), 1.3 (m, 3H), 1.05 (m, 2H). BOC deprotection as described for **7c** gave the hydrochloride **15a** (71%) as an amorphous white solid. R_f (9:1:1 EtOH/H_2O/NH_4OH): 0.48. NMR (CD_3OD): δ 7.35 (m, 5H), 6.93 (d, J = 7 Hz, 2H), 6.7 (d, J = 7 Hz, 2H), 5.1 (s, 2H), 3.94 (t, J = 6 Hz, 2H), 3.38 (b d, J = 11 Hz, 2H), 3.22 (d, J = 12 Hz, 1H), 3.1 (d, J = 12 Hz, 1H), 2.95 (b t, J = 11 Hz, 2H), 1.95 (b d, J = 12 Hz, 2H), 1.75 (m, 2H), 1.6–1.45 (m, 4H), 1.4 (s, 3H), 1.4–1.25 (m, 5H). Anal. ($C_{27}H_{36}N_2O_5·HCl·H_2O$) C,H,N.

Methyl 2-(*S*)-[*N*-[(Benzyloxy)carbonyl]amino]-3-[4-[[[*N*-[(*tert*-butyloxy)carbonyl]piperidin-4-yl]propyl]oxy]-phenyl]propionic Acid Ester (14b, n = 3, R_1 = CH_3, R_2 = H). A solution of **13b** (1 g, 3 mmol) and **10** (1.1 g, 3.3 mmol) in DMF (40 mL) was treated with cesium carbonate (0.4 g, 1.35 mmol), and the resulting solution was stirred at room temperature for 20 h. The solvent was removed *in vacuo*, and the residue was taken up in EtOAc, washed with water and brine, dried (Na_2SO_4), and concentrated. The residue was purified (SiO_2, 1:4 EtOAc/hexanes) to give **14b** (0.45 g, 27%) as a clear oil. R_f (1:4 EtOAc/hexanes): 0.23. NMR ($CDCl_3$): δ 7.35 (b s, 5H), 7.0 (d, 2H), 6.79 (d, 2H), 5.18 (m, 1H), 5.10 (s, 1H), 4.61 (m, 1H), 4.08 (b d, 2H), 3.9 (t, 2H), 3.71 (s, 3H), 3.03 (m, 2H), 2.68 (m, 2H), 1.65–1.82 (m, 4H), 1.37–1.45 (m, 11H), 1.1 (m, 2H).

2-(*S*)-[*N*-[(Benzyloxy)carbonyl]amino]-3-[4-[[[*N*-[(*tert*-butyloxy)carbonyl]piperidin-4-yl]propyl]oxy]phenyl]propionic Acid (14c, $n = 3$, $R_1 = H$, $R_2 = H$). A solution of **14b** ($n = 3$, $R_1 = CH_3$, $R_2 = H$) (5.5 g, 9.9 mmol) in methanol (140 mL) was treated with 1 N NaOH (35 mL) for 16 h. The solvent was removed *in vacuo*; the residue was acidified with 5% $KHSO_4$ and extracted with EtOAc. The organic layers were dried (brine, Na_2SO_4) and evaporated to give **14c** (100%) as a clear oil. NMR ($CDCl_3$): 7.34 (b s, 5H), 7.03 (d, 2H), 6.79 (d, 2H), 5.19 (m, 1H), 5.09 (s, 1H), 4.62 (m, 1H), 4.10 (m, 4H), 4.89 (t, 2H), 3.10 (m, 2H), 2.66 (t, 2H), 1.62–1.85 (m, 5H), 1.37–1.52 (m, 12H), 1.1 (m, 2H).

2-(*S*)-[*N*-[(Benzyloxy)carbonyl]amino]-3-[4-[[(piperidin-4-yl)propyl]oxy]phenyl]propionic Acid (15b). A solution of **14c** (160 mg, 0.3 mmol) in CH_2Cl_2 was treated with anisole (0.1 mL) and trifluoroacetic acid (1 mL) for 1.5 h. Evaporation of the solution and chromatography (SiO_2, 10:0.8:0.8 EtOH/H_2O/NH_4OH) gave **15b** (28 mg, 20%) as an amorphous white solid. R_f (10:1:1 EtOH/H_2O/NH_4OH): 0.2. NMR (D_2O): δ 6.88 (b s, 3H), 6.72 (d, 2H), 6.65 (d, 2H), 6.38 (d, 2H), 4.6 (s, 2H), 3.95 (dd, 1H), 3.5 (t, 2H), 2.88 (b d, 2H), 2.7 (dd, 1H), 2.4 (m, 3H), 1.43 (b d, 2H), 1.25 (m, 2H), 1.1 (m, 1H), 0.95–0.8 (m, 4H). Exact mass (FAB, $m + 1$): calcd, 441.2389; found, 441.2375. Anal. ($C_{24}H_{28}N_4O_4 \cdot 1.5H_2O$) C,H,N.

Methyl 3-(*S*)-[*N*-[(Benzyloxy)carbonyl]amino]-4-[4-[[[*N*-[(*tert*-butyloxy)carbonyl]piperidin-4-yl]propyl]oxy]-phenyl]butanoate (16, $n = 3$, $R_1 = CH_3$, $R_2 =$ BOC). To a stirred solution of **14c** (1.6 g, 2.9 mmol) in EtOAc at −15 °C was added isobutyl chloroformate (0.37 mL, 2.9 mmol) and *N*-methylmorpholine (0.32 mL, 2.9 mmol), and the resulting solution was stirred for 0.5 h at −15 °C. Diazomethane (5 mmol in Et_2O) was added, and the reaction mixture was stirred at 0 °C for 20 min. The reaction mixture was purged with argon, diluted with EtOAc, and washed with water. The organic phase was dried ($MgSO_4$) and the solvent removed to provide the desired diazoketone. R_f (30% EtOAc/hexanes): 0.175. NMR ($CDCl_3$): δ 7.35 (b s, 5H), 7.06 (d, 2H), 6.8 (d, 2H), 5.35 (m, 1H), 5.2 (m, 1H), 5.06 (m, 1H), 4.42 (m, 1H), 4.09 (m, 3H), 3.90 (t, 2H), 2.95 (d, 2H), 2.68 (b t, 2H), 1.55–1.85 (m, 6H), 1.35–1.5 (m, 12H), 1.1 (m, 2H). The diazo ketone (1.63 g, 2.9 mmol) was dissolved in methanol (20 mL) and treated at room temperature with a methanol solution (5 mL) of silver benzoate (0.22 g, 0.96 mmol) and triethylamine (1.25 mL). After a few minutes, the reaction mixture became black with gas evolution apparent. After 0.5 h, the solvent was removed and the residue was purified (SiO_2, 1:4 EtOAc/hexanes) to give **16** (0.3 g, 24%). R_f (30% EtOAc/hexanes): 0.32. NMR ($CDCl_3$): δ 7.32 (b s, 5H), 7.05 (d, 2H), 6.79 (d, 2H), 5.24 (m, 1H), 5.08 (s, 2H), 4.03–4.20 (m, 4H), 3.90 (t, 2H), 3.67 (s, 3H), 2.62–2.91 (m, 4H), 2.49 (m, 2H), 1.65–1.83 (m, 4H), 1.60 (s, 2H), 1.37–1.47 (m, 12H), 1.12 (m, 2H).

3-(*S*)-[*N*-[(Benzyloxy)carbonyl]amino]-4-[4-[[(piperidin-4-yl)propyl]oxy]phenyl]butanoate (17, $n = 3$, $R_1 = H$, $R_2 = H$). Hydrolysis of **16** ($n = 3$, $R_1 = CH_3$, $R_2 =$ BOC) (0.3 g, 0.53 mmol) as described for **14c** gave the desired acid, which underwent BOC deprotection as described for **15b** followed by chromatography (SiO_2, 10:1:1 EtOH/H_2O/NH_4OH) to give **17** (50 mg, 27%) as an amorphous white solid. R_f (10:1:1 EtOH/H_2O/NH_4OH): 0.25. NMR (CD_3OD): δ 7.25 (m, 5H), 7.05 (d, 2H), 6.75 (d, 2H), 4.95 (m, 2H), 4.11 (m, 1H), 3.92 (t, 2H), 3.32 (m, 2H), 2.93 (t, 2H), 2.72 (m, 2H), 2.54 (m, 2H), 1.95 (d, 2H), 1.75–1.85 (m, 2H), 1.6 (m, 1H), 1.3–1.5 (m, 4H). Exact mass (FAB, $m + 1$) calcd, 455.2545; found, 455.2535. Anal. ($C_{26}H_{34}N_2O_5 \cdot 0.5H_2O$) C,H,N.

3-[4-[[(Piperidin-4-yl)butyl]oxy]phenyl]propionic Acid (19b). A solution **18** (R = CH_3) (2.06 g, 11.3 mmol) in DMF (65 mL) was treated with Cs_2CO_3 (1.86 g, 5.7 mmol) at room temperature for 10 min, and then a solution of **9** (3.66 g, 11.3 mmol) in DMF (75 mL) was added dropwise and heated to 79 °C for 36 h. Removal of the solvent *in vacuo* gave a residue which was dissolved in H_2O and extracted with EtOAc. The EtOAc layers were combined, washed with H_2O and brine, dried (Na_2SO_4), and concentrated. Chromatography (SiO_2, 15:85 EtOAc/hexanes) gave the alkylated ester (2.28 g, 48%) as a viscous oil. R_f (15:85 EtOAc/hexanes): 0.34. NMR ($CDCl_3$): δ 7.1 (d, 2H), 6.81 (d, 2H), 4.08 (b d, 2H), 3.92 (t, 2H), 3.66 (s, 3H), 2.88 (t, 2H), 2.67 (b t, 2H), 2.6 (t, 2H), 1.75 (m, 2H), 1.65 (b d, 2H), 1.48 (b s, 10H), 1.3 (m, 3H), 1.1 (m, 2H). This oil (0.4 g, 0.9 mmol) was hydrolyzed and BOC deprotected as described for **15a** to give **19b** (0.26 g, 80% for two steps) as an amorphous solid. R_f (9:1:1 EtOH/H_2O/NH_4OH): 0.42. NMR (CD_3OD): δ 7.13 (d, 2H), 6.81 (d, 2H), 3.95 (t, 2H), 3.35 (b d, 2H), 2.96 (b t, 2H), 2.82 (t, 2H), 2.55 (t, 2H), 1.96 (b d, 2H), 1.78 (m, 2H), 1.65–1.45 (m, 3H), 1.4–1.3 (m, 4H). Anal. ($C_{18}H_{27}NO_3 \cdot HCl$) C,H,N.

3-[4-[(6-Aminohexyl)phenyl]propionic Acid (19a). See method for **19b**. NMR (D_2O): δ 7.21 (d, $J = 8.5$ Hz, 2H), 6.92 (d, $J = 8.5$ Hz, 2H), 4.02 (t, $J = 6$ Hz, 2H), 2.75 (m, 4H), 2.4 (t, 2H), 1.73 (m, 2H), 1.5–1.3 (m, 6H). Anal. ($C_{15}H_{23}NO_3$) C,H,N.

General Procedure for Scheme 5 Compounds. Method A. 2-(*S*)-Amino-3-[4-[[(piperidin-4-yl)butyl]oxy]phenyl]-propionic Acid (20b). A solution of **5b** (13.5 g, 26.3 mmol) in absolute EtOH (250 mL) was treated with 10% Pd/C (1.5 g) and hydrogenated under balloon pressure for 12 h. The reaction mixture was diluted with 1000 mL of 9:1:1 absolute EtOH/H_2O/NH_4OH and stirred for 10 min and then filtered and concentrated *in vacuo* to give a yellowish solid. Trituration of this solid with Et_2O provided pure **20b** (8.7 g, 87%) as an amorphous solid. NMR (CD_3OD): δ 7.2 (d, 2H), 6.88 (d, 2H), 3.94 (t, 2H), 3.7 (dd, 1H), 3.23 (dd, 1H), 3.02 (t, 2H), 2.93 (dd, 1H), 1.75 (m, 2H), 1.55–1.3 (m, 16H).

2-(*S*)-Amino-3-[4-[(6-aminohexyl)oxy]phenyl]propionic Acid (22a). This compound was prepared via a method similar to that described for compound **6b**. Anal. ($C_{15}H_{24}N_2O_3$: HCl) C,H,N.

2-(*S*)-[(Phenethylcarbonyl)amino]-3-[4-[(6-aminohexyl)oxy]phenyl]propionic Acid (22b). To a suspension of **20a** (0.457 g, 1.2 mmol) in 1.2 mL of 1 N NaOH and 15 mL of H_2O at 0 °C were added 3-phenylpropanoyl chloride (0.223 g, 1.32 mmol) and solid sodium carbonate (0.111 g, 1.32 mmol). The suspension was stirred for 1.5 h and then diluted with 40 mL of H_2O, acidified to pH 2–3 with 10% $KHSO_4$ solution, and extracted with 4 × 50 mL portions of EtOAc. The EtOAc layers were washed with 50 mL of H_2O and 50 mL of brine, dried (Na_2SO_4), and evaporated to give 0.5 g of crude product. Chromatography (SiO_2, 95:5 $CHCl_3$/MeOH) gave 0.3 g (51%) of the acylated product as a clear viscous gum. R_f (95:5 $CHCl_3$/MeOH): 0.35. NMR ($CDCl_3$): δ 7.29 (m, 2H), 7.20 (m, 3H), 6.88 (m, 2H), 6.72 (d, 2H), 5.85 (b s, 1H), 4.82 (m, 1H), 4.65 (b s, 1H), 3.91 (m, 2H), 3.02 (m, 6H), 2.5 (m, 2H), 1.72 (b s, 2H), 1.4 (m, 14H). A solution of this acylated product (0.3 g, 0.6 mmol) in EtOAc (15 mL) was cooled to −15 °C, and HCl gas was bubbled in for 10 min. The stoppered reaction mixture was stirred for 2 h at 0 °C and then evaporated, and the resulting foam was triturated with 40 mL of Et_2O to give **22b** (0.22 g, 82%) as an amorphous white solid. R_f (9:1:1 EtOH/H_2O/NH_4OH): 0.59. NMR (CD_3OD): δ 7.22 (m, 2H), 7.15 (m, 3H), 7.06 (d, 2H), 6.79 (d, 2H), 4.6 (dd, 1H), 3.95 (t, 2H), 3.1 (dd, 1H), 2.90 (m, 2H), 2.80 (m, 3H), 2.46 (m, 2H), 1.80 (m, 2H), 1.67 (m, 2H), 1.48 (m, 4H). Anal. ($C_{24}H_{32}N_2O_4 \cdot HCl \cdot H_2O$) C,N; H: calcd, 7.55; observed, 7.11.

2-(*S*)-[(Phenylacetyl)amino]-3-[4-[(6-aminohexyl)oxy]-phenyl]propionic acid (22c): method A; 33% yield for two steps. NMR (CD_3OD): δ 7.30 (m, 3H), 7.13 (d, 2H), 7.02 (d, 2H), 6.75 (d, 2H), 4.61 (dd, 1H), 3.94 (m, 2H), 3.47 (m, 2H), 3.12 (dd, 1H), 2.88 (m, 3H), 1.65 (m, 2H), 1.5 (m, 6H). Anal. ($C_{23}H_{30}N_2O_4 \cdot HCl \cdot H_2O$) C,N; H: calcd, 7.34; observed, 6.92.

2-(*S*)-[(Phenylcarbonyl)amino]-3-[4-[(6-aminohexyl)-oxy]phenyl]propionic acid (22d): method A; 90% yield for two steps. R_f (1:1:1 EtOH/H_2O/NH_4OH): 0.67. NMR (CD_3OD): δ 7.72 (d, 2H), 7.5 (m, 1H), 7.42 (m, 2H), 7.19 (d, 2H), 6.8 (d, 2H), 4.80 (dd, 1H), 3.94 (t, 2H), 3.21 (dd, 1H), 3.05 (dd, 1H), 2.90 (t, 2H), 1.78 (m, 2H), 1.70 (m, 2H), 1.5 (m, 4H). Anal. ($C_{22}H_{28}N_2O_4 \cdot HCl \cdot 0.75H_2O$) C,H,N.

General Procedure for Scheme 5 Compounds. Method B. Methyl 2-(*S*)-Amino-3-[4-[[6-[*N*-(*tert*-butyloxy)amino]-hexyl]oxy]phenyl]propionic Acid (21a). A solution of **5a** (10 g, 19.4 mmol) in DMF (75 mL) was treated with Cs_2CO_3 (3.16 g, 9.7 mmol) at room temperature for 1 h. Methyl iodide (2.76 g, 19.4 mmol) was added dropwise, and the solution was stirred for 18 h. The DMF was removed *in vacuo*, and the residue was redissolved in EtOAc (300 mL), washed with 2 ×

50 mL of saturated $NaHCO_3$ and 40 mL of brine, and concentrated to a volumes of 50 mL. The solution was passed through a plug of SiO_2 and eluted with 900 mL of EtOAc. The solvent was removed to give the methyl ester (10.5 g, 100%) as a viscous, yellow oil. NMR ($CDCl_3$): δ 7.32 (m, 5H), 6.98 (d, 2H), 6.88 (d, 2H), 5.19 (m, 1H), 5.10 (m, 2H), 4.61 (m, 1H), 4.5 (b s, 1H), 3.90 (t, 2H), 3.71 (s, 3H), 2.96–3.17 (m, 4H), 1.76 (m, 2H), 1.25–1.53 (m, 13H). This methyl ester (10.5 g, 19.4 mmol) in absolute EtOH (150 mL) was treated with 10% Pd/C (1.0 g) and hydrogenated under balloon pressure for 12 h. The reaction mixture was filtered and evaporated to give **21a** (5.62 g, 73%) as a viscous oil. R_f (95:5 $CHCl_3$/MeOH): 0.37. NMR ($CDCl_3$): δ 7.09 (d, 2H), 6.82 (d, 2H), 4.5 (b s, 1H), 3.93 (t, 2H), 3.71 (s, 3H), 3.68 (m, 1H), 3.12 (m, 2H), 3.03 (dd, 1H), 2.80 (dd, 1H), 1.70 (m, 2H), 1.30–1.55 (m, 16H).

2-(*S*)-(Acetylamino)-3-[4-[(6-aminohexyl)oxy]phenyl]propionic Acid (22e). A solution of **21a** (0.56 g, 1.4 mmol) in EtOAc (15 mL) was treated with solid $NaHCO_3$ (0.36 g, 4.3 mmol) and acetyl chloride (0.112 g, 1.4 mmol) at room temperature for 18 h. The resulting suspension was filtered, concentrated, and chromatographed (SiO_2, 98:2 $CHCl_3$/MeOH) to give the acylated product (0.58 g, 95%) as a viscous oil. R_f (95:5 $CHCl_3$/MeOH): 0.54. NMR ($CDCl_3$): δ 7.0 (d, 2H), 6.8 (d, 2H), 5.88 (b d, 1H), 4.84 (dd, 1H), 4.5 (b s, 1H), 3.92 (t, 2H), 3.73 (s, 3H), 3.12 (m, 2H), 3.07 (m, 2H), 1.98 (s, 3H), 1.75 (m, 2H), 1.6–1.3 (m, 15H). This oil (0.58 g, 1.3 mmol) was hydrolyzed and BOC deprotected as described for **15a** to give **22e** (0.4 g, 86% for two steps) as an amorphous solid. NMR (CD_3OD): δ 7.12 (d, 2H), 6.82 (d, 2H), 4.61 (dd, 1H), 3.96 (t, 2H), 3.13 (dd, 1H), 2.92 (t, 2H), 2.85 (dd, 1H), 2.9 (s, 3H), 1.8 (m, 2H), 1.7 (m, 2H), 1.45 (m, 4H). Anal. ($C_{17}H_{26}N_2O_4$·HCl·H_2O) C,H,N.

2-(*S*)-(Propanoylamino)-3-[4-[(6-aminohexyl)oxy]phenyl]propionic acid (22f): method A. NMR (CD_3OD): δ 8.1 (d, 1H), 7.12 (d, 2H), 6.81 (d, 2H), 4.6 (dd, 1H), 3.95 (t, 2H), 3.14 (dd, 1H), 3.92 (t, 2H), 3.85 (dd, 1H), 2.13 (t, 2H), 1.78 (m, 2H), 1.68 (m, 2H), 1.6–1.4 (m, 5H). Anal. ($C_{19}H_{30}N_2O_4$·HCl·H_2O) C,H,N.

2-(*S*)-(Hexanoylamino)-3-[4-[(6-aminohexyl)oxy]phenyl]propionic acid (22g): method A. NMR (CD_3OD): δ 7.12 (d, 2H), 6.8 (d, 2H), 4.6 (m, 1H), 3.93 (t, 2H), 3.14 (dd, 1H), 3.91 (t, 2H), 3.84 (dd, 1H), 2.14 (m, 2H), 1.8 (m, 2H), 1.7 (m, 2H), 1.6–1.4 (m, 6H), 1.3–1.2 (m, 6H), 1.87 (t, 3H). Anal. ($C_{22}H_{36}N_2O_4$·HCl·0.75H_2O) C,H,N.

2-(*S*)-Methanesulfonamido-3-[4-[(6-aminohexyl)oxy]phenyl]propionic acid (22h): method B. R_f (9:1:1 EtOH/H_2O/NH_4OH): 0.67. NMR (CD_3OD): δ 7.19 (d, 2H), 6.85 (d, 2H), 4.16 (dd, 1H), 3.97 (t, 2H), 3.11 (dd, 1H), 2.92 (t, 2H), 2.83 (dd, 1H), 2.67 (s, 3H), 1.8 (m, 2H), 1.65 (m, 2H), 1.6–1.4 (m, 4H). Anal. ($C_{16}H_{26}N_2O_5S$·HCl·0.25H_2O) C,H,N.

2-(*S*)-Phenylmethanesulfonamido-3-[4-[(6-aminohexyl)oxy]phenyl]propionic acid (22i): method B. NMR (CD_3OD): δ 7.28 (m, 5H), 7.14 (d, 2H), 6.84 (d, 2H), 4.08 (m, 3H), 3.96 (t, 2H), 3.03 (dd, 1H), 2.91 (t, 2H), 3.81 (dd, 1H), 1.8 (m, 2H), 1.7 (m, 2H), 1.5 (m, 4H). Anal. ($C_{22}H_{30}N_2O_5S$·HCl·0.5H_2O) C,H,N.

2-(*S*)-Butanesulfonamido-3-[4-[(6-aminohexyl)oxy]phenyl]propionic acid (22j): method B. R_f (9:1:1 EtOH/H_2O/NH_4OH): 0.59. NMR (CD_3OD): δ 7.2 (d, 2H), 6.85 (d, 2H), 4.1 (dd, 1H), 3.97 (t, 2H), 3.11 (dd, 1H), 2.92 (t, 2H), 2.80 (dd, 1H), 2.67 (t, 2H), 1.8 (m, 2H), 1.7 (m, 2H), 1.6–1.4 (m, 6H), 1.23 (m, 2H), 0.85 (t, 3H). Anal. ($C_{19}H_{32}N_2O_5S$·HCl) C,H,N.

2-(*S*)-(Acetylamino)-3-[4-[[(piperidin-4-yl)butyl]oxy]phenyl]propionic acid (23a): method A starting from **5b**. R_f (9:1:1 EtOH/H_2O/NH_4OH): 0.33. NMR (CD_3OD): δ 7.21 (d, 2H), 6.8 (d, 2H), 4.43 (dd, 1H), 3.94 (t, 2H), 3.31 (b d, 2H), 3.1 (dd, 1H), 2.86 (m, 3H), 1.9 (s, 5H), 1.75 (m, 2H), 1.55 (m, 3H), 1.45 (m, 4H). Anal. ($C_{20}H_{30}N_2O_4$·1.5H_2O) C,N; H: calcd, 8.54; observed, 7.93.

2-(*S*)-(Propanoylamino)-3-[4-[[(piperidin-4-yl)butyl]oxy]phenyl]propionic acid (23b): method A starting from **5b**. R_f (9:1:1 EtOH/H_2O/NH_4OH): 0.31. NMR (CD_3OD): δ 8.05 (d, 1H), 7.13 (d, 2H), 6.81 (d, 2H), 4.6 (dd, 1H), 3.94 (t, 2H), 3.35 (b d, 2H), 3.11 (dd, 1H), 2.94 (t, 2H), 2.85 (dd, 1H), 2.16 (q, 2H), 1.95 (b d, 2H), 1.75 (m, 2H), 1.65–1.45 (m, 3H), 1.38 (m, 4H), 1.03 (t, 3H). Anal. ($C_{21}H_{32}N_2O_4$·HCl·H_2O) C,H,N.

2-(*S*)-(Pentanoylamino)-3-[4-[[(piperidin-4-yl)butyl]oxy]phenyl]propionic acid (23c): method A starting from **5b**. NMR (CD_3OD): δ 7.12 (d, 2H), 6.8 (d, 2H), 4.61 (dd, 1H), 3.95 (t, 2H), 3.36 (b d, 2H), 3.14 (dd, 1H), 2.95 (t, 2H), 2.82 (dd, 1H), 2.15 (t, 2H), 1.96 (b d, 2H), 1.75 (m, 2H), 1.65–1.3 (m, 9H), 1.2 (m, 2H), 1.84 (t, 3H). Anal. ($C_{22}H_{36}N_2O_4$·HCl·0.75H_2O) C,H,N.

2-(*S*)-(Hexanoylamino)-3-[4-[[(piperidin-4-yl)butyl]oxy]phenyl]propionic acid (23d): method A starting from **5b**. NMR (CD_3OD): δ 7.12 (d, 2H), 6.81 (d, 2H), 4.61 (dd, 1H), 3.93 (t, 2H), 3.36 (b d, 2H), 3.15 (dd, 1H), 2.95 (b t, 2H), 2.85 (dd, 1H), 2.15 (t, 2H), 1.95 (b d, 2H), 1.75 (m, 2H), 1.65–1.4 (m, 5H), 1.4–1.3 (m, 4H), 1.2 (m, 2H), 0.85 (t, 3H). Mass spectroscopy (FAB, m + 1): 419. Anal. ($C_{24}H_{38}N_2O_4$·1.3TFA·0.55H_2O) C,H,N.

2-(*S*)-[(3-Phenylpropanoyl)amino]-3-[4-[[(piperidin-4-yl)butyl]oxy]phenyl]propionic acid (23e): method A starting from **5b**. NMR (D_2O): δ 7.23 (b s, 2H), 7.1 (b s, 5H), 6.9 (b s, 2H), 4.63 (b s, 1H), 3.95 (b s, 2H), 3.41 (b d, 2H), 3.15 (m, 1H), 3.0–2.75 (m, 5H), 2.5 (b s, 2H), 1.9 (b d, 2H), 1.83 (b s, 2H), 1.4–1.2 (m, 7H). Exact mass (FAB, m + 1): calcd, 453.2753; found, 453.2754. Anal. ($C_{27}H_{36}N_2O_4$·HCl·0.70H_2O) C,H,N.

2-(*S*)-[(*trans*-β-Styrylsulfonyl)amino]-3-[4-[[(piperidin-4-yl)butyl]oxy]phenyl]propionic acid (23f): method B starting from **5b**. R_f (9:1:1 EtOH/H_2O/NH_4OH): 0.34. NMR (CD_3OD): δ 7.43 (m, 5H), 7.23 (d, 1H), 7.17 (d, 2H), 6.78 (d, 2H), 6.51 (d, 1H), 4.04 (dd, 1H), 3.87 (t, 2H), 3.36 (b d, 2H), 3.09 (dd, 1H), 2.96 (b t, 2H), 2.81 (dd, 1H), 1.95 (b d, 2H), 1.75 (m, 2H), 1.6–1.45 (m, 4H), 1.4–1.3 (m, 4H). Anal. ($C_{26}H_{34}N_2O_5S$·HCl·0.50H_2O) C,H,N.

2-(*S*)-[[(2-Phenylethyl)sulfonyl]amino]-3-[4-[[(piperidin-4-yl)butyl]oxy]phenyl]propionic Acid (23g). Reduction of 2-(*S*)-[(3-phenyl-2-propenoyl)amino]-3-[4-[[[*N*-[(*tert*-butyloxy)carbonyl]piperidin-4-yl]butyl]oxy]phenyl]propionic acid (see preparation of **23f**) as described for **21a** followed by BOC deprotection as described for **7c** gave **23g**. R_f (9:1:1 EtOH/H_2O/NH_4OH): 0.33. NMR (CD_3OD): δ 7.22 (m, 5H), 7.19 (d, 2H), 6.82 (d, 2H), 4.17 (dd, 1H), 3.87 (t, 2H), 3.35 (b d, 2H), 3.13 (dd, 1H), 2.91 (m, 4H), 2.85–2.7 (m, 3H), 1.95 (b d, 2H), 1.75 (m, 2H), 1.6 (m, 1H), 1.5 (m, 2H), 1.4–1.3 (m, 4H). Anal. ($C_{25}H_{36}N_2O_5S$·HCl·1.5H_2O) C,H,N.

2-(*S*)-[(Benzylsulfonyl)amino]-3-[4-[[(piperidin-4-yl)butyl]oxy]phenyl]propionic acid (23h): method B starting from **5b**. R_f (9:1:1 EtOH/H_2O/NH_4OH): 0.21. NMR (CD_3OD): δ 7.3 (m, 5H), 7.15 (d, 2H), 6.85 (d, 2H), 4.12–4.0 (m, 2H), 3.96 (t, 2H), 3.33 (b d, 2H), 3.04 (dd, 1H), 2.92 (b t, 2H), 2.81 (dd, 1H), 1.91 (b d, 2H), 1.75 (m, 2H), 1.6–1.4 (m, 3H), 1.35 (m, 4H). Mass spectroscopy (FAB, m + 1): 475. Anal. ($C_{25}H_{34}N_2O_5S$·HCl·1.05H_2O) C,H,N.

2-(*S*)-[(Phenylsulfonyl)amino]-3-[4-[[(piperidin-4-yl)butyl]oxy]phenyl]propionic acid (23i): method B starting from **5b**. R_f (9:1:1 EtOH/H_2O/NH_4OH): 0.37. NMR (CD_3OD): δ 7.66 (d, 2H), 7.52 (m, 1H), 7.42 (t, 2H), 7.02 (d, 2H), 6.72 (d, 2H), 3.95 (m, 3H), 3.36 (b d, 2H), 2.96 (b t, 3H), 2.75 (dd, 1H), 1.95 (b d, 2H), 1.75 (m, 2H), 1.6–1.4 (m, 3H), 1.4 (m, 4H). Anal. ($C_{24}H_{32}N_2O_5S$·HCl·0.5H_2O) C,H,N.

2-(*S*)-[(2-Thienylsulfonyl)amino]-3-[4-[[(piperidin-4-yl)butyl]oxy]phenyl]propionic acid (23j): method B starting from **5b**. R_f (9:1:1 EtOH/H_2O/NH_4OH): 0.38. NMR (CD_3OD): δ 7.68 (d, 1H), 7.4 (d, 1H), 7.05 (m, 3H), 6.78 (d, 2H), 4.05 (dd, 1H), 3.96 (t, 2H), 3.48 (b d, 2H), 2.98 (m, 3H), 2.8 (dd, 1H), 1.95 (b d, 2H), 1.78 (m, 2H), 1.6–1.4 (m, 3H),1.4 (m, 4H). Anal. ($C_{22}H_{30}N_2O_5S_2$·HCl·0.5H_2O) C,H,N.

2-(*S*)-[(Methylsulfonyl)amino]-3-[4-[[(piperidin-4-yl)butyl]oxy]phenyl]propionic acid (23k): method A starting from **5b**. R_f (9:1:1 EtOH/H_2O/NH_4OH): 0.54. NMR (D_2O + NaOD): δ 7.37 (d, J = 8.5 Hz, 2H), 7.03 (d, J = 8.5 Hz, 2H), 4.14 (t, J = 6.5 Hz, 2H), 3.90 (t, J = 7.5 Hz, 1H), 3.05 (b d, 2H), 2.86 (dd, J = 7.6, 13 Hz, 1H), 2.7 (s, 3H), 2.6 (b t, J = 12 Hz, 2H), 1.8 (m, 3H), 1.5 (m, 2H), 1.35 (m, 2H), 1.17 (m, 2H). Exact mass (FAB, m + 1): calcd, 399.1953; observed, 399.1941. Anal. ($C_{19}H_{30}N_2O_5S$) C,H,N.

2-(*S*)-[(Propylsulfonyl)amino]-3-[4-[[(piperidin-4-yl)-butyl]oxy]phenyl]propionic acid (23l): method B starting from **5b**. R_f (9:1:1 EtOH/H_2O/NH_4OH): 0.30. NMR (CD_3-OD): δ 7.2 (d, 2H), 6.85 (d, 2H), 4.11 (dd, 1H), 3.98 (t, 2H), 3.36 (b d, 2H), 3.1 (dd, 1H), 2.96 (b t, 2H), 2.8 (dd, 1H), 2.65 (t, 2H), 1.95 (b d, 2H), 1.78 (m, 2H), 1.6–1.5 (m, 5H), 1.4–1.3 (m, 5H), 0.86 (t, 3H). Anal. ($C_{21}H_{34}N_2O_5S$·HCl·0.5H_2O) C,N; H: calcd, 7.69; observed, 7.23.

2-(*S*)-[(Butylsulfonyl)amino]-3-[4-[[(piperidin-4-yl)butyl]oxy]phenyl]propionic acid (23m): method A, 23% yield for three steps starting from **5b**. Nitrogen was bubbled through the EtOAc/HCl solution at 0 °C for 0.5 h to remove most of the excess HCl. The resulting solution was seeded with some previously prepared product and allowed to stir for 2 h. The resulting **23m** hydrochloride salt had a melting point of 132–134 °C. R_f (9:1:1 EtOH/H_2O/NH_4OH): 0.64. NMR (CD_3OD): δ 7.2 (d, 2H), 6.85 (d, 2H), 4.1 (dd, 1H), 3.95 (t, 2H), 3.36 (b d, 2H), 3.11 (dd, 1H), 2.95 (b t, 2H), 2.79 (dd, 1H), 2.64 (t, 2H), 1.96 (b d, 2H), 1.75 (m, 2H), 1.6–1.4 (m, 4H), 1.4 (m, 5H), 1.25 (m, 2H), 0.85 (t, 3H). Exact mass (FAB, m + 1): calcd, 441.2423; observed, 441.2427. Anal. ($C_{22}H_{36}N_2O_5S$·HCl) C,H,N. Chiral HPLC demonstrated that this material had an enantiomeric excess of 98%.[48] Trituration with CH_3-CN resulted in material with an enantiomeric excess of 99.6%. Product **23m** that had been prepared via method B was found to have an enantiomeric excess of 85.7% using the above method of detection.

2-(*R*)-[(Butylsulfonyl)amino]-3-[4-[[(piperidin-4-yl)butyl]oxy]phenyl]propionic acid (23n): method A starting with D-(*R*)-tyrosine. R_f (10:1:1 EtOH/H_2O/NH_4OH): 0.55. NMR (400 MHz, DMSO): δ 9.1 (b s, 1H), 8.2 (b s, 1H), 7.84 (d, J = 9 Hz, 2H), 7.49 (d, J = 9 Hz, 2H), 4.56 (t, J = 6 Hz, 2H), 4.53 (m, 1H), 3.88 (b d, 2H), 3.62 (dd, 1H), 3.46 (b t, 2H), 3.34 (dd, 1H), 3.2 (b s, 2H), 2.44 (b d, 2H), 2.34 (m, 2H), 2.18 (b, 1H), 2.06 (m, 2H), 1.96–1.84 (m, 4H), 1.8 (m, 2H), 1.4 (t, J = 7 Hz, 3H). Anal. ($C_{22}H_{36}N_2O_5S$·1.70HCl) C,H,N. Chiral HPLC demonstrated that this material had an enantiomeric excess of 93.4%.[48]

3-(*S*)-[*N*-(Butylsulfonyl)amino]-4-[4-[[(piperidin-4-yl)-propyl]oxy]phenyl]butanoate (24a). Compound **16** (n = 3, R_1 = CH_3, R_2 = BOC) was treated utilizing method B to give **24a**. R_f (10:1:1 EtOH/H_2O/NH_4OH): 0.21. NMR (CD_3-OD + CF_3CO_2D): δ 7.05 (d, J = 8.6 Hz, 2H), 6.74 (d, J = 8.6 Hz, 2H), 3.84 (t, J = 6 Hz, 2H), 3.72 (m, 1H), 3.28 (b d, J = 13 Hz, 2H), 2.87 (b t, 2H), 2.67 (dd, 1H), 2.6 (dd, 1H), 2.5 (m, 2H), 2.4 (dd, J = 4, 7 Hz, 2H), 1.88 (b d, 2H), 1.7 (m, 2H), 1.55 (m, 1H), 1.4–1.2 (m, 5H), 1.2–1.0 (m, 2H), 0.73 (t, J = 7 Hz, 3H). Exact mass (FAB, m + 1): calcd, 441.2423; observed, 441.2412. Anal. ($C_{22}H_{38}N_2O_5S$·0.25H_2O) C,H,N.

3-(*S*)-[*N*-(Butylsulfonyl)amino]-4-[4-[[(piperidin-4-yl)-butyl]oxy]phenyl]butanoate (24b). Compound **16** (n = 4, R_1 = CH_3, R_2 = BOC) was treated utilizing method B and BOC deprotected as described for **15b** to give **24b** (13% yield from **16**). R_f (10:1:1 EtOH/H_2O/NH_4OH): 0.32. NMR (CD_3OD + CF_3CO_2D): δ 7.3 (d, 2H), 7.02 (d, 2H), 4.1 (t, 2H), 3.95 (m, 1H), 3.44 (b d, 2H), 3.0 (m, 3H), 2.8–2.5 (m, 3H), 2.48 (b t, 2H), 1.98 (b d, 2H), 1.8 (m, 2H), 1.64 (m, 1H), 1.5 (m, 2H), 1.45–1.3 (m, 6H), 1.2 (m, 2H), 0.82 (t, 3H). Exact mass (FAB, m + 1): calcd, 455.2503; observed, 445.2580. Anal. ($C_{22}H_{36}$-N_2O_5S·1.25H_2O·1.25TFA) C,H,N.

2-(*S*)-(Hexanoylamino)-3-[4-[(piperidin-4-yl)pentyl]-phenyl]propionic Acid (27). Methyl 4-iodophenylalanine (**25**) (1.01 g, 2.96 mmol) was suspended in $CHCl_2$ (20 mL) and treated with pyridine (1.4 mL, 1.77 mmol) and hexanoyl chloride (1.25 mL, 8.88 mmol) at 0 °C. After 20 min, the solution was diluted with H_2O (20 mL) and extracted with EtOAc. The EtOAc layer was washed with 10% $KHSO_4$ and brine, dried ($MgSO_4$), filtered, and evaporated. The crude material was chromatographed (SiO_2, 5% Et_2O/$CHCl_3$), and **26** (1.07 g, 89%) was isolated as a white solid. R_f (5% Et_2O/$CHCl_3$): 0.36. NMR ($CDCl_3$): δ 7.6 (d, J = 8 Hz, 2H), 6.83 (d, J = 8 Hz, 2H), 5.9 (b d, 1H), 4.86 (dd, J = 5.7, 11.6 Hz, 1H), 3.10 (dd, J = 5.7, 14 Hz, 1H), 3.00 (dd, J = 5.7, 14 Hz, 1H), 2.16 (t, J = 7 Hz, 2H), 1.6 (m, 2H), 1.23 (m, 6H), 0.85 (t, J = 7 Hz, 3H). A solution of **26** (0.58 g, 1.4 mmol) in diethylamine (6 mL) was treated with **12** (0.3 g, 1.2 mmol), $Pd(Ph_3)_2Cl_2$ (0.049 g, 0.007 mmol), and CuI (0.007 g, 0.0035 mmol) under N_2 for 18 h. The solvent was removed *in vacuo*; the residue was dissolved in 10% $KHSO_4$ and extracted with Et_2O. The organic layer was dried and concentrated and the residue chromatographed (SiO_2, 20% EtOAc/hexanes) to give the alkylated product (0.28 g, 44%). R_f (35% EtOAc/hexanes): 0.27. NMR ($CDCl_3$): δ 7.35 (m, 2H), 7.04 (m, 2H), 6.2 (m, 1H), 5.85 (m, 1H), 5.7 (m, 1H), 4.9 (m, 1H), 4.1 (b d, 2H), 3.13 (m, 2H), 2.7 (m, 2H), 2.18 (m, 3H), 1.6 (m, 4H), 1.45 (s, 9H), 1.3 (m, 6H), 0.9 (m, 3H). This alkylated product (0.275 g, 0.52 mmol) was dissolved in EtOH (12 mL) and H_2O (2 mL), treated with HOAc (5 drops) and 10% palladium on carbon (0.1 g), and hydrogenated at 50 psi for 4 h. The solution was filtered and concentrated to give an oil which was chromatographed (SiO_2, 35% EtOAc/hexanes) to give the product (0.22 g, 79%). R_f (35% EtOAc/hexanes): 0.28. NMR ($CDCl_3$): δ 7.06 (d, J = 8 Hz, 2H), 6.97 (d, J = 8 Hz, 2H), 5.93 (d, J = 8 Hz, 1H), 4.85 (dd, J = 6, 14 Hz, 1H), 4.01 (b s, 2H), 3.70 (s, 3H), 3.10 (dd, J = 6, 14 Hz, 1H), 3.02 (dd, J = 6, 14 Hz, 1H), 2.65 (b t, 2H), 2.55 (t, J = 7 Hz, 2H), 2.14 (t, J = 7 Hz, 2H), 1.56 (m, 6H), 1.42 (s, 9H), 1.3–1.1 (m, 10H), 1.03 (m, 2H), 0.8 (t, 3H). This BOC ester (0.17 g, 0.32 mmol) was deprotected as described for **7c** to give **27** (0.13 g, 90%). R_f (9:1 EtOH/H_2O): 0.16. NMR (CD_3OD): δ 7.0 (d, J = 8 Hz, 2H), 6.91 (d, J = 8 Hz, 2H), 4.36 (dd, J = 5, 7 Hz, 1H), 3.2 (b d, 2H), 3.05 (dd, J = 5, 14 Hz, 1H), 2.8–2.7 (m, 3H), 2.43 (t, J = 7 Hz, 2H), 2.03 (m, 2H), 1.74 (b d, J = 10 Hz, 2H), 1.5–1.4 (m, 4H), 1.25–1.0 (m, 8H), 0.75 (t, J = 7 Hz, 3H). Exact mass (FAB, m + 1): calcd, 417.3117; found, 417.3128. Anal. ($C_{25}H_{40}N_2O_3$·0.5H_2O) C,H,N.

Computational Chemistry. Compound **23m** (L-700,462) was compared to the previously reported structure of cyclic Ac-CRGDC-OH (L-366,690).[52] All molecular modeling was done using the Merck Advanced Modeling Facility.[61] The distance geometry algorithm JIGGLE[62] was used to produce aligned pairs of structures. For these calculations, the conformation of the cyclic portion of the peptide was held fixed in the two geometries consistent with the NMR data while the Arg and Asp side chain positions were allowed to vary. The conformation of **23m** was randomly varied within the limits set for alignment. Three sites for matching were selected. The piperidine nitrogen atom and the carboxylic acid carbon atom of **23m** were matched to within 0.1 Å of the midpoint of the Arg guanidino group and the Asp carboxylic acid carbon, respectively, of the cyclic peptide. The ether oxygen atom of the non-peptide was superposed to within 1.0 Å of the Arg carbonyl oxygen atom. The stereochemical centers of the ligands were maintained. Structure pairs were visualized with C_View.[63]

***In Vitro* Pharmacology—Inhibition of HUVEC Attachment to Fibrinogen, Vitronectin, and Fibronectin. Growth of Human Umbilical Vein Endothelial Cells.** Human umbilical vein endothelial cells (HUVEC), isolated and pooled from 10 to 15 umbilical cords, were received weekly from Dr. D. Cines of the University of Pennsylvania. HUVEC were grown as monolayers on rat type I collagen matrix (Collagen Corp.) in tissue culture flasks. Cultures were maintained in MCDB-107 medium (Sigma), supplemented with heparin (90 μg/mL; BTI), fetal calf serum (15%; Flow), and gentamycin (50 μg/mL; Gibco), in a humidified 37 °C incubator with 2.2% CO_2 in air. HUVEC were subcultured biweekly by 30 s treatment with trypsin (0.05%)–EDTA (0.53 mM; Gibco) to detach the cells followed by the addition of 10% fetal calf serum in medium to neutralize the trypsin.

Cell Attachment Studies. HUVEC cells were detached from confluent monolayers with trypsin–EDTA and then treated with an equal volume of Dulbecco's phosphate-buffered saline (DPBS; Gibco), containing 10% FCS, to neutralize the trypsin. Cells were then pelleted by centrifugation at 1100 rpm for 10 min, washed twice with MCDB 107, counted by trypan blue exclusion, and diluted to 10^5 viable cells/mL in MCDB-107 supplemented with 5 mg/mL bovine serum albumin (BSA; Calbiochem; low fatty acid, endotoxin-free). Microtiter plates (Nunc Immuno 1) were coated with 0.1 mL/well of human fibrinogen (Sigma or Calbiochem; 5–10 nm in DPBS), human vitronectin (Calbiochem; 5–10 nM in 0.5 M sodium carbonate buffer, pH 9.76), human fibronectin (Cal-

biochem; 2.5–5.0 nM in carbonate buffer, or BSA (Calbiochem; 2% wt/vol in DPBS) by incubating either 16 h at 4 °C (fibrinogen and vitronectin), 1 h at ambient temperature (fibronectin), or 1 h at 4 °C (BSA). After the coating period, the plates were washed three times with DPBS, treated with 200 μl of DPBS–2% BSA/well for 1 h at 4 °C, and washed as above just before use.

Stock solutions of test compounds were prepared, usually in distilled water. For each compound to be assayed, a series of five 2× dilutions was prepared in the HUVEC suspension, using poly(propylene) tubes. Dilution or control suspension (50 μL) was then added to individual wells of the prepared microtiter plates, each containing 50 μL of MCDB-107. Test dilutions were assayed in quadruplicate against fibrinogen, fibronectin, and vitronectin. The highest test concentration of each compound and control suspension was tested against BSA alone. The plates were covered and incubated at 37 °C with 2.2% CO_2 for 75 min. Compound-containing wells were then examined microscopically for signs of gross cytotoxicity. Next, the plates were flicked and washed twice with DPBS to remove nonadherent cells. (*p*-Nitrophenyl)-*N*-acetyl-β-D-glucosaminide (100 μL of 3.75 mM; Sigma) in 0.1 M citrate buffer (pH 5.0, containing 0.25% Triton-X-100; Sigma) was then added to each well. To quantitate HUVEC glucosaminidase activity, samples of control HUVEC suspension were seeded to wells containing 100 μL of the glucosaminide solution. Plates were then incubated in the dark at ambient temperature for approximately 24 h. Finally, 150–200 μL of 50 mM glycine and 5 mM EDTA (Sigma), pH 10.4, was added to each well.

Well contents were then analyzed spectrophotometrically by reading absorbance at 405 nm, using an automated plate reader. Average test absorbance values were divided by the control value and subtracted from 1.0 to determine the percent inhibition. These values were then graphed versus the compound concentrations to allow extrapolation of an IC_{50} for each inhibitory compound.

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