Expedited Articles

Design and Evaluation of Nonpeptide Fibrinogen γ-Chain Based GPIIb/IIIa Antagonists

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Two series of nonpeptide turn mimetics were designed by analysis of the solution NMR structure of the 385−411 sequence of the γ-chain of fibrinogen. These compounds, based on the KQAGD (Lys-Gln-Ala-Gly-Asp, 406−410) sequence, were synthesized and studied *in vitro*. The most interesting compound from our study, RWJ 50042 (**25**), exhibits potent inhibition of fibrinogen binding to GPIIb/IIIa (IC_{50} = 0.009 μM), as well as thrombin- or collagen-induced platelet aggregation (IC_{50} = 0.76, 0.14 μM). Since the 400−411 sequence is required for γ-chain bioactivity and is a unique recognition sequence among ligands for integrins, vis-á-vis other RGD (Arg-Gly-Asp)-presenting proteins, these turn mimetics may represent a new, selective approach to antagonism of the fibrinogen receptor.

Platelet aggregation constitutes an important response to curtail bleeding induced by vascular injury. However, pathological extension of this normal hemostatic process can lead to total occlusion of a vessel. Platelet aggregation is mediated by fibrinogen binding to platelet receptor glycoprotein IIb/IIIa (GPIIb/IIIa). Because this binding is the final common pathway leading to platelet aggregation, a fibrinogen receptor antagonist has become well-recognized as a potentially useful drug in the treatment of thrombotic disorders.[1] Fibrinogen interacts with binding sites on GPIIb/IIIa through peptide domains present on the α- and γ-chains: RGDF (Arg-Gly-Asp-Phe, α95−98), RGDS (Arg-Gly-Asp-Ser, α572−575), and HHLGGAKQAGDV (His-His-Leu-Gly-Gly-Ala-Lys-Gln-Ala-Gly-Asp-Val, γ400−411).[2] Although the RGD motifs and the γ-chain dodecapeptide appear to have unique binding sites on GPIIb/IIIa (i.e., 109−171 of IIIa and 294−314 of IIb, respectively), the RGD binding site is not mutually exclusive from the dodecapeptide site, which implies either cross-binding or close proximity of these two sites.[3] In our biochemical assays, RGDS and HHLGGAKQAGDV have comparable IC_{50} values for the inhibition of fibrinogen binding to immobilized GPIIb/IIIa, 1.0 and 4.1 μM, respectively (Figure 1).

The RGD sequences found in fibrinogen's α-chain have provided a basis for the successful design of various potent and selective fibrinogen receptor antagonists.[1] Cyclic RGD-based peptides have demonstrated *in vivo* activity as antithrombotics, and their three-dimensional structures were subsequently used as tools for the design of second generation nonpeptide antagonists.[1] However, the RGD sequence is ubiquitous among the adhesive proteins and widely recognized by integrin receptors,[4,5] while the γ-chain dodecapeptide is unique to fibrinogen.[6] Farrell and co-workers have shown that the HHLGGAKQAGDV sequence is a vital component of platelet aggregation, whereas the RGD sequence is "neither necessary nor sufficient."[7] Therefore, an agent that mimics the γ-chain of fibrinogen represents an attractive mechanistic approach to antithrombotic therapy.

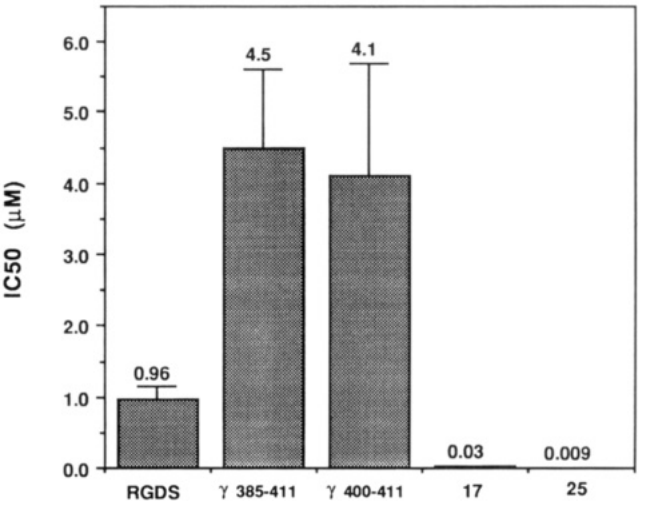

Figure 1. Inhibition of biotinylated Fg binding to isolated GPIIb/IIIa. A comparison of Fg peptides with nipecotamides **17** and **25**.

Our goal was to design low molecular weight, nonpeptide mimics of the C-terminal sequence of γ-fibrin-

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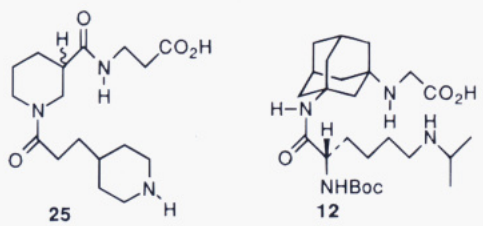
ogen. Our small molecule design efforts were guided by both the solution NMR-derived conformations of the γ385−411 peptide and the peptide structure−activity relationships (SAR).[6,8] This γ385−411 peptide, initially isolated by cyanogen bromide degradation of fibrinogen, is more suitable for NMR studies than bioactive γ400−411 since its longer sequence is more likely to give rise to larger conformer populations with defined secondary structural features. Furthermore, although all of the biological activity lies within the 400−411 region, γ385−411 (IC_{50} = 4.5 μM) is equipotent to γ400−411 (IC_{50} = 4.1 μM) in our binding assay (*vide infra*). Studies of the γ-chain peptide SAR show that substitutions or modifications at His^{400}, Lys^{406}, Ala^{408}, Asp^{410}, and Val^{411} have detrimental effects on bioactivity.[6,8] In our NMR-derived structures, the spatial arrangements and intramolecular interactions of these SAR-critical amino acid side chains provided ideas about the potential binding conformations of the peptide ligand.

In the absence of a three-dimensional structure of the complex between γ385−411 and GPIIb/IIIa, representatives from the NMR-derived peptide ligand family of conformations were used as starting structures for the design, synthesis, and testing of target mimetics. Thus, potent antagonists of platelet aggregation and the binding of fibrinogen to immobilized GPIIb/IIIa were obtained. Our first design hypothesis produced aminoadamantane substrates such as **12**.[9] In this series, the Gln-Ala (406−407) sequence was replaced by an aminoadamantane spacer while the adjacent Lys^{406} and Gly^{409} residues were retained. This adamantane scaffold, a weakly bioactive template, afforded a premise to design additional motifs, as exemplified by highly active nipecotamide **25** (Figure 1).



25 **12**

NMR Studies and Mimetic Design

NMR studies of the fibrinogen γ-chain C-terminal sequence (γ385−411) in water provided solution conformations which displayed two general features: (1) multiple-turn or helix-like structure in the 390−402 region and (2) a considerably less constrained conformation in the 403−411 region.[10] Further NMR investigation of γ385−411 in TFE/water solution gave rise to conformations possessing more defined secondary structural features within the γ400−411 receptor binding domain. Under these conditions, α-helix-like characteristics were observed throughout the 385−405 sequence, with a relatively well-defined turn encompassing Lys-Gln-Ala-Gly (406−409) as shown in Figure 2. Furthermore, from NOE-based distance geometry calculations, the average $C_\alpha-C_\alpha$ distance between the i and $i+3$ residues in the Lys-Gln-Ala-Gly turn region ranged between 4.5 and 5.0 Å, suggesting a type II β-turn. Other type II β-turn evidence included a strong Gln^{407} α-CH to Ala^{408} NH NOE, and an *ca.* 5 Hz $^3J_{\alpha N}$ coupling constant for Ala^{408}. Blumenstein and co-workers have reported a 60% incidence of a type II β-turn spanning

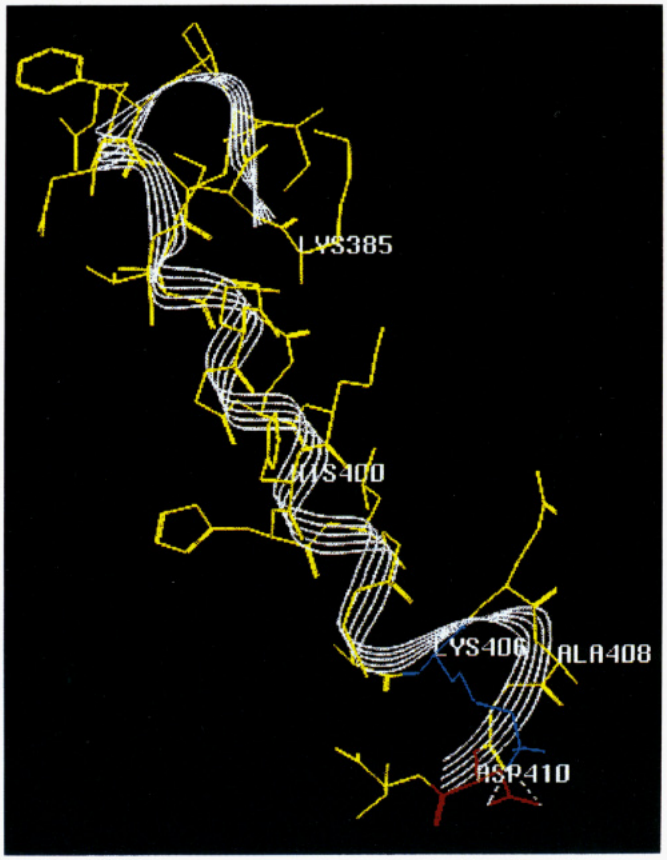


Figure 2. A representative from the family of conformers constituting the distance geometry calculated solution structure of Fg γ385−411.

residues 407−410 (Gln to Asp) in fibrinogen peptide γ392−411.[11] Other turns or structural families were not observed in their NMR conformational analysis. In our structural analysis of γ385−411, the average $C_{\alpha Gln}-C_{\alpha Asp}$ distance was greater than that expected for a β-turn. Recently, transfer NOE studies of γ385−411 and γ400−411 in the presence of purified GPIIb/IIIa receptor have supported our conformational findings (unpublished results).

Numerous literature examples of β-turn-mediated protein binding have been reported,[12] and many have successfully mimicked the conformational features of the β-turn in small nonpeptide molecules. Since our goal was to design peptide mimetics to interrupt C-terminal γ-fibrinogen binding to platelets, this putative β-turn region (406−409) seemed to be an obvious focal point. Peptide SAR data for γ400−411 suggested the importance of four residues which could have an impact on the secondary structure/binding of the C-terminal region: Lys^{406} and Ala^{408} within the turn, and Asp^{410} and Val^{411} immediately outside the turn region.[6] Additionally, NMR studies indicated a potential ionic interaction between Lys^{406} ε-NH_3^+ and Asp^{410} β-CO_2^- (Figure 2) which we targeted in our mimetic design. We decided to replace the $i+1$ (Gln) and $i+2$ (Ala) residues with a spacer ring system, i.e. 1-aminoadamantane or nipecotic acid, while retaining the natural residues of i (Lys) and $i+3$ (Gly). While certainly crucial for β-turn induction, the two replaced residues were deemed uncritical for direct binding interactions with the receptor. The side chains which were presumed to be important for binding were those of Lys and Asp. Incorporation of a lysine side chain and a carboxylic acid

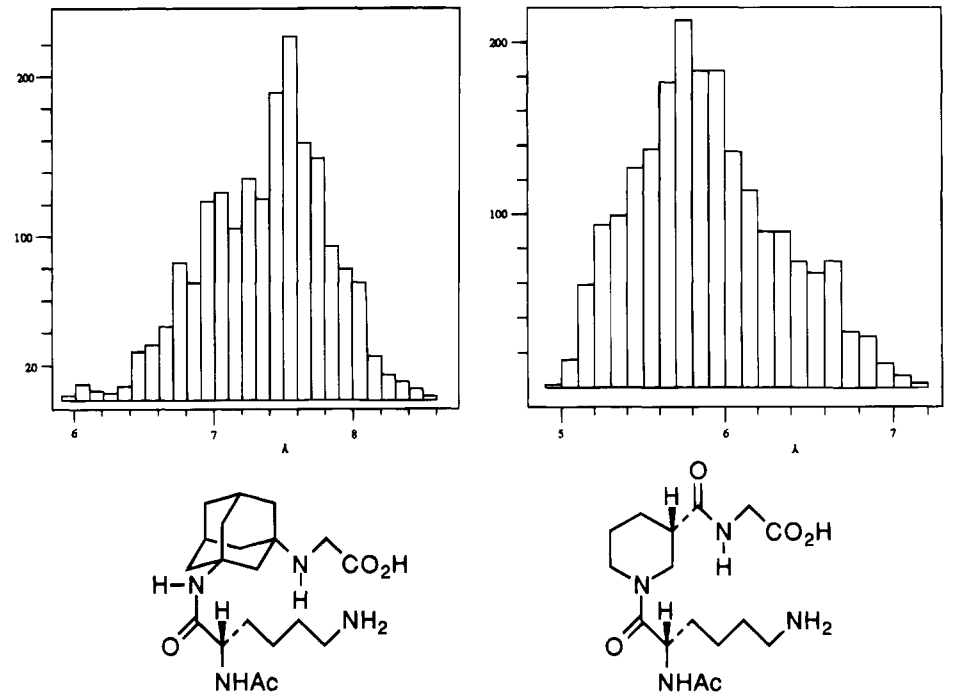
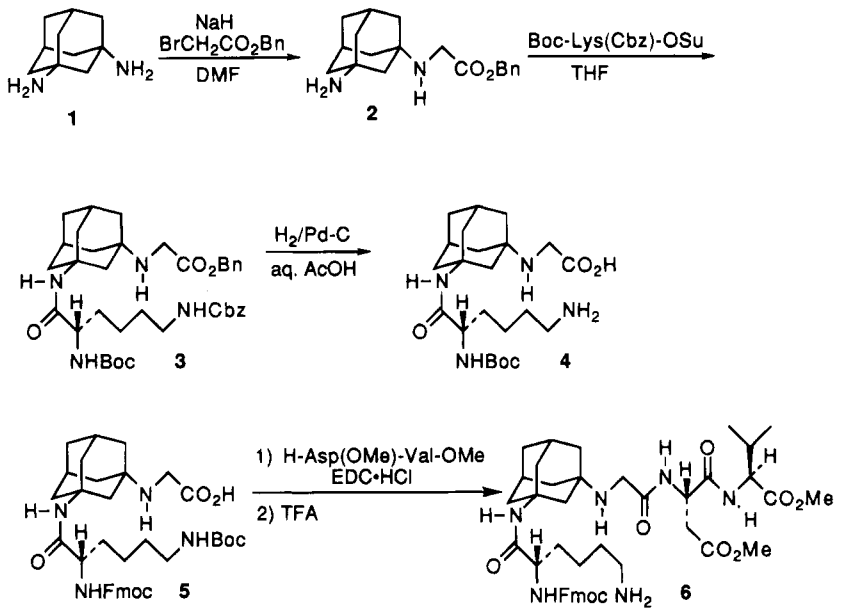


Figure 3. $C_{\alpha K}-C_{\alpha G}$ distance distribution resulting from molecular dynamics simulations of prototypic KQAG turn mimetics. Distance in angstroms is plotted versus conformer population.

Scheme 1

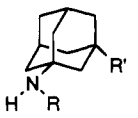


side chain into both the 1,3-adamantanediamine and nipecotic acid systems could be readily achieved, since both already contain 1,3-diamino or 1-amino-3-carboxyl functionality (and are commercially-available). In addition, these two ring systems, as shown by the histograms in Figure 3, impose a highly constrained relationship between the Lys and Gly sites, with median $C_{\alpha K}-C_{\alpha G}$ distances (after minimization and dynamics) ranging from 6.8 to 7.6 Å for the adamantane system, and from 5.5 to 6.1 Å for the nipecotamide system. The $C_{\alpha K}-C_{\alpha G}$ distance values calculated for the nipecotamide system are closer to those measured in the NMR-derived structures of fibrinogen γ385–411 (4.5–5.0 Å).[9]

Synthetic Chemistry

The 1-aminoadamantane-type mimetics were prepared by a combination of solution phase peptide and synthetic organic techniques. 1,3-Adamantanediamine (**1**) was alkylated with sodium hydride/benzyl 2-bromoacetate to affix the glycine portion of the molecule and give diamino ester **2** in modest yield (Scheme 1/Table 1). Acylation of **2** with the appropriate diprotected lysine succinimide ester required longer reaction times than standard peptide couplings. Presumably, the adamantane's steric bulk, an asset in rigidly positioning the lysine and glycine residues as in the native peptide's turn, also contributed to the diminished accessibility of primary amine **2**. Amide **3** was deprotected by hydrogenolysis to afford diamino acid **4**. For the N^{ϵ}-isopropyl amine **12** (Table 1), bis(2-oxo-3-oxazolidinyl)-phosphinic chloride (BOP-Cl)[13] was employed to activate *N*-Boc-Lys(Cbz)(*i*-Pr)-OH toward coupling with **2** (21% yield). Glycine amides (e.g., **6**) in this series were

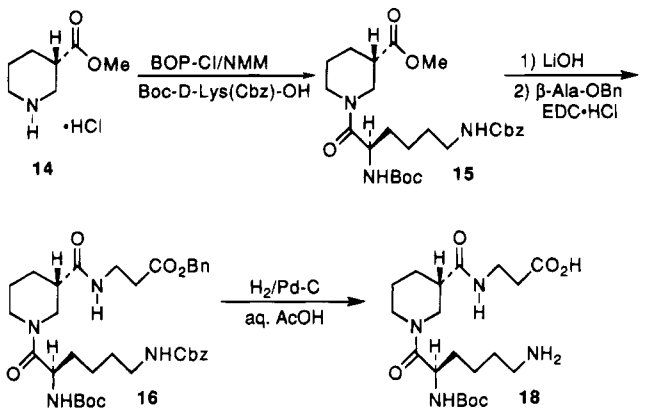
Table 1. Aminoadamantane Inhibition of Biotinylated Fg Binding to Isolated GPIIb/IIIa and Inhibition of Thrombin-Induced Platelet Aggregation



compd	R	R′	Fg binding[a] (%)	platelet aggr[a] (%)
4	Boc-Lys	Gly-OH	44	40 at 20 μM
6	Fmoc-Lys	Gly-Asp(OMe)-Val(OMe)	0	15
7	Z-Lys	Gly-OH	26	24
8	Fmoc-Ala-Lys	Gly-OH	42	37
9	Z-Lys	N-PhOAc-Gly-OH	33	2
10	Fmoc-Lys	Gly-OMe	1	25
11	Boc-Arg	Gly-OH	28	9
12	Boc-Lys(iPr)	Gly-OH	29.9 μM[b]	3
13	$CO(CH_2)_2$-4-piperidine	Gly-OH	49	5

[a] Percent inhibition at 50 μM. IC_{50}'s for RGDS were 17 μM for fibrinogen binding and 30 μM for thrombin-induced platelet aggregation. [b] IC_{50}.

Scheme 2



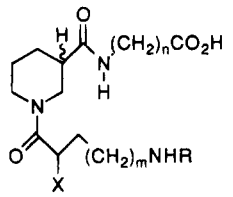
prepared by using Boc-ε-amino- and Fmoc-α-amino (or Cbz)-protected lysine. Hydrogenolysis of the benzyl ester, ethyl [(dimethylamino)propyl]carbodiimide (EDC)-mediated coupling of the glycine acid **5** with the appropriate amine, and then Boc group removal with TFA, sequentially, gave **6**. Piperidine **13** was synthesized via BOP-Cl/3-(4-pyridyl)acrylic acid coupling with **2**, followed by hydrogenation with platinum oxide to reduce the pyridine, olefin, and benzyl ester moieties.

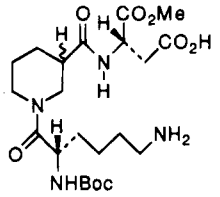
The nipecotic acid derivatives are a second family of γ-fibrinogen-based inhibitors prepared in this study. The preparation of nipecotamide **18** represents a typical synthetic sequence (Scheme 2/Table 2). (*S*)-(+)-Methyl nipecotate hydrochloride (**14**), isolated by resolution from racemic material using tartaric acid,[14] was acylated with BOP-Cl activated *N*-Boc-D-Lys(CBz)-OH to afford amido ester **15**. Saponification of methyl ester **15** and carbodiimide-mediated coupling with β-Ala-OBn gave protected intermediate **16**. For the second coupling transformation, EDC activation resulted in higher yields than did use of BOP-Cl. Hydrogenolysis of dibenzylated material afforded amino acid **18** as an inseparable mixture with its antipode **19** (83:17), based on reverse phase HPLC and ^1H NMR (*tert*-butyl resonances) analyses. The racemate **17** was also prepared by this method starting with racemic methyl nipecotate. Presumably, racemization at the 3-nipecotyl position may have occurred during any of the first three base-mediated steps of Scheme 2. This racemization, also observed in the intended preparation of **19** (**19/18**, 87:13), displays a disadvantage of this class of chiral β-amino acids in solution phase peptide synthesis. The two mixtures of compounds **18** and **19** both co-elute on HPLC with racemate **17**. All other entries in Table 2 reflect use of racemic methyl nipecotate hydrochloride as starting material; final products were 1:1 mixtures based on NMR and/or HPLC evidence (diastereomeric cases). Intermediates in this series variably showed Lys-Nip tertiary amide bond rotamers (typically 1:1). To prepare piperidine **25**, methyl nipecotate was acylated with *trans*-3-(4-pyridine)acrylic acid in the presence of BOP-Cl to give methyl [3-(4-pyridyl)acryloyl]nipecotate. The methyl ester was converted to the corresponding acid, which was then used to acylate β-Ala-OBn, again with BOP-Cl, in a manner similar to Scheme 2. Hydrogenolysis of resultant [3-(4-pyridyl)acryloylnipecotyl-β-alanine benzyl ester in the presence of platinum oxide afforded piperidino acid **25**. Amino acid **35**, the descarbonyl analog of potent straight-chain antagonist **24**, was prepared in the following five-step sequence. Acylation of racemic methyl nipecotate with Boc-6-aminocaproic acid *N*-oxysuccinimide ester and saponification of the methyl ester gave Boc-6-aminocaproylnipecotic acid. Hydride reduction of its intermediate imidazolide (1,1-carbonyldiimidazole/DIBAL/THF) to the aldehyde, reductive amination of the aldehyde (sodium cyanoborohydride/β-Ala-OBn), and deprotection (aqueous HCl and hydrogenolysis) gave **35**.

Biological Results and Discussion

The aminoadamantanes and nipecotamides were evaluated in both biotinylated fibrinogen binding and platelet aggregation assays, and the results are summarized in Tables 1 and 2. The binding assay measures inhibition of biotinylated fibrinogen binding to isolated GPIIb/IIIa, while platelet aggregation determines the functional effects of the fibrinogen binding inhibition. In the aminoadamantane series, the glycine acids (especially **4** and **12**) are more active than their ester or amide analogs in the more sensitive binding assay. Interestingly, compounds containing a secondary amine moiety (**12** and **13**) proved to be slightly more active than **4** which contains a primary amine moiety; *N*-isopropyl amino acid **12** is the most active aminoadamantane with an IC_{50} of approximately 30 μM. The weak inhibition of fibrinogen binding in this series is corroborated by weak inhibition of platelet aggregation (generally < 50% inhibition at 50 μM). With weak but authentic activity in the fibrinogen binding assay established using this approach, we turned our attention

Table 2. Nipecotamide Inhibition of Biotinylated Fg Binding to Isolated GPIIb/IIIa and Inhibition of Thrombin-Induced Platelet Aggregation





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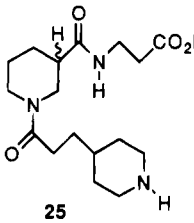

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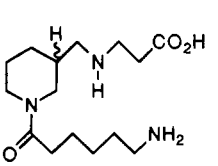

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compd	R	*m*	*n*	X	IC_{50} Fg binding[a]	% Inhibn of platelet aggregation[b] 50 μM	10 μM	1.0 μM	0.1 μM
17	H	3	2	D-NHBoc	0.029	88	87	21	(0.35 μM[c])
18	3-*S*-isomer of **17**				0.028	91	87	11	
19	3-*R*-isomer of **17**				0.008	91	87	45	2 (0.22 μM[c])
20	Z	3	2	L-NHBoc(CO_2Bn)	20	-			
21	H	3	2	L-NHBoc	0.74	45	12		
22					0.029	87	32	2	(2.63 μM[c])
23	H	3	2	L-NH_2	2.6	8	1		
24	H	3	2	H	0.013	88	87	29	
25					0.009	91	89	89	4 (0.14 μM[c])
26	H	3	1	L-NHAc	24% at 50 μM	4			
27	H	3	2	L-NHAc	0.074	88	30	4	
28	C(NH)NH_2	2	2	L-NHBoc	2.7	17	0		
29	H	3	3	L-NHBoc	59% at 50 μM	0	0		
30	H	3	2	D-NH_2	0.76	87	48	0	
31	H	3	3	D-NHBoc	7.6	43	7		
32	H	3	1	D-NHBoc	50	24	7		
33	H	3	2	D-NHAc	0.34	87	67	1	
34	iPr	3	2	L-NHBoc	20% at 5 μM	4	6		
35					5.8	63	24		

[a] IC_{50} of biotinylated fibrinogen binding to isolated GPIIb/IIIa (μM). [b] Percent inhibition of thrombin-induced platelet aggregation at 50, 10, 1, 0.1 μM. All reported results (duplicate) were obtained using the same platelet preparation. Variability of the assay is less than 10% in all cases. [c] IC_{50} for collagen-induced platelet aggregation. Statistical error (triplicate) for these compounds: **17**, ±0.05; **19**, ±0.085; **22**, ±0.946; **25**, ±0.015.

to the less-bulky nipecotic acid-based β-turn mimics. The nipecotic acid scaffold was employed to optimize the positioning of the Lys- and Asp-type side chains spatially as in the NMR structure of γ385–411.

Application of the limited structure–activity relationships of the aminoadamantanes to the nipecotic acid scaffold rendered remarkable results (Table 2). Although the nipecotamide glycine acid **26** shows no improvement compared to aminoadamantane glycine acids **4** and **12** in fibrinogen binding inhibition, extension of the carboxyl functionality in nipecotamide **26** via a β-alanine unit (**27**) results in significant increases in binding potency (submicromolar range).[15] Additional extension of the carboxyl via the γ-aminobutyric acid reduces potency significantly in the nipecotamides as compared to the β-alanine case (**31** vs **17**). The observation in both adamantane and nipecotamide series that the free acid and amine side chains are important for binding potency reinforces our hypothesis that the Lys^{406} and Asp^{410} side chain orientations in the NMR-derived structures of the native peptide have great importance. In a preliminary modeling study of the conformational features of representatives from the nipecotamide series, the COO^- and NH_3^+ are proximal to each other, with observed heavy atom distances of <10 Å (predominant conformer families resulting from a systematic search)—an interaction that was unobserved in relatively-unconstrained model systems.[9b] Thus, the nipecotamide may serve as a template to anchor the amine and carboxylic acid side chains in orientations where they can interact with each other in either an ionic or hydrogen bonding fashion, possibly as a preliminary event to receptor recognition of each moiety.

Although biologically-active RGD oligopeptides have been reported to assume type I or II β-turns at the receptor recognition site,[16] an elegant study of potent cyclic RGD peptides has shown that extended secondary structure through the RGD sequence ("turn-extended-turn") and a negative/positive charge separation of 14–15 Å is representative of the most potent inhibitors.[17] It is possible that potent Boc-D-Lys **19** could attain this negative/positive charge separation if it were to adopt extended conformations (both amides trans), with $C_{\alpha K}$–

$C_{\beta A}$ distances measuring *ca.* 9 Å. However, in a systematic conformational search of **19**, the vast majority of low-energy conformers were observed in the 6.0–7.5 Å range ($C_{\alpha K}$–$C_{\beta A}$, all conformations of relevant energies were 5.0–8.0 Å). Since these distances were considerably less than the *ca.* 9 Å distance required for the extended conformers, the extended conformers are less likely.

An unexpected observation is the enhanced potency of the D-Lys inhibitors as compared to their "L" counterparts (**17** vs **21**) in binding. As in the previous series, a secondary amine in this series offers an advantage over primary amines. Piperidine **25** is more potent than enantiomerically-enriched Boc-D-Lys analog **19**. The pronounced activity of piperidine **25** suggests that the α-Boc-amino group of Lys is not required for activity and that the degrees of rotational freedom exhibited by the Lys side chain have been reduced by the six-membered ring. This result has also been observed with nonpeptide RGD mimetics,[18] yet conversely arginine analog **28** is inferior to the primary and secondary amines of this series (e.g., vs **21**) in both binding and aggregation. Also, Boc-arginyl-aminoadamantane **11** gives a nonlinear dose–response curve which indicates nonspecific binding at high concentrations and affords very low inhibition of platelet aggregation.

Although unnecessary for good potency, the bulky, hydrophobic α-Boc-amino group of highly-potent **19** may steer the amine side chain into an optimal position for receptor recognition when in the D-configuration. Removal of the Boc group to provide an unmasked α-amine gives an inferior inhibitor (**30**), whereas α-acetyl substitution produces a suitable replacement for Boc (**33**). While clearly more potent than its racemate **17**, exact superiority of **19** could not be assessed due to contamination with **18** (83:17). The separation of **19** from **18**, either at the protected or final synthetic step, is a subject of further investigation.

Most of the binding results in the nipecotamide series correlate well with observed inhibition of platelet aggregation. For instance, piperidine **25** (binding IC_{50} = 9 nM) is also the most potent compound in the functional assay (IC_{50} = 0.14 μM). Without exception, compounds with binding potency in the submicromolar range give >65% inhibition of platelet aggregation at 50 μM. Generally, weak antagonists of fibrinogen binding (IC_{50} > 1 μM) cause <50% inhibition of platelet aggregation at 50 μM. A single exception to the latter observation is descarbonyl compound **35**, which inhibits aggregation equally as well as some of the more binding-active analogs (63% at 50 μM), although it has a binding IC_{50} of only 5.8 μM.

To investigate the mechanistic selectivity of the nipecotic acid-based mimetics for GPIIb/IIIa against a representative RGD-binding integrin, four diverse, relatively potent compounds were selected for binding study in the presence of the vitronectin (Vn) receptor: Boc-D-Lys-based **19**, Asp-containing **22**, piperidine **25**, and Boc-L-Arg-based **28**. Gratifyingly, this assay indicates that none of these four nipecotamides inhibit biotinylated vitronectin binding to isolated vitronectin receptor at concentrations up to 100 μM.[19] These results suggest that this class of fibrinogen receptor antagonists are quite specific for GPIIb/IIIa over the vitronectin receptor.

Conclusion

A new class of high-affinity GPIIb/IIIa antagonists was designed from the NMR-derived structural and conformational characteristics of fibrinogen's γ-chain C-terminal domain. Compound **25**, the most potent representative of the nipecotamide series (IC_{50} = 9 nM in binding to isolated GPIIb/IIIa), incorporates a piperidinepropionyl group and a β-amido carboxylic acid at the N-1 and C-3 positions of the nipecotic acid spacer, respectively. The spacer ring serves to place these two groups to approximate the Lys and Asp side chain positions in the native peptide. When compared to the RGD-based antagonists, these putative γ-chain mimetics show remarkably similar structural features in terms of Lys or Arg and Asp-type composition. However, there is one distinct difference. The separation of positive and negative charges of cyclic RGD peptides has been reported to be greater than 12 Å,[17] and numerous examples of these kinds of antagonists have been published.[1] Our NMR studies of the γ-chain, and our antagonist results, suggest that this turn-induced charge separation may prefer to be less than 10 Å in our series. Additionally, while guanidine-containing antagonists have shown good activity in the RGD-based antagonist arena, arginine incorporation into our antagonists afforded inferior activity in one case (e.g., Arg-containing **28**).[20] An interesting issue is the cross-reactivity of the two receptor sites. Whether or not GPIIb/IIIa-binding species that are conformationally mobile can bind at both receptor sites as proposed by Peishoff *et al.* and others remains an open question.[17] It might be that some RGD-based antagonists are also capable of accessing the smaller positive/negative charge separation (<10 Å) and therefore act as γ-chain (and RGD) mimetics. As mentioned previously, RGD-based antagonists which mimick a β-turn have been identified.[16] While fully-extended conformers in our antagonist series could, in principle, access the larger positive/negative charge separation (>12 Å), modeling results herein indicate that this pronounced extension is unlikely.

The γ-chain dodecapeptide is relatively selective for GPIIb/IIIa versus the vitronectin receptor (and possibly other receptors of the integrin class).[6a] RGD-based antagonists have been prepared which exhibit this selectivity profile as well.[19,21,22] Nevertheless, some RGD peptides do not share this selectivity. The lack of affinity of the nipecotamides for the vitronectin receptor (an RGD-binding integrin) indicates that one of our selectivity milestones was achieved and seems to imply the γ-chain's structural exigence for GPIIb/IIIa binding has been adopted by our mimetics. The C-terminal γ-chain of fibrinogen is a unique recognition sequence among the disintegrins. Mimetics of this sequence may represent a novel approach toward antagonism of the fibrinogen receptor.

Experimental Section

In Vitro Solid Phase-Purified Glycoprotein IIb/IIIa Binding Assay. A 96-well Immulon-2 microtiter plate (Dynatech-Immulon) is coated with 50 μL/well of RGD-affinity purified GPIIb/IIIa (effective range 0.5–10 μg/mL) in 10 mM HEPES, 150 mM NaCl, 1 mM at pH 7.4. The plate is covered and incubated overnight at 4 °C. The GPIIb/IIa solution is discarded, 150 μL of 5% BSA is added, and the mixture is incubated at room temperature for 1–3 h. The plate is washed

extensively with modified Tyrodes buffer. Biotinylated fibrinogen (25 μL/well) at twice the final concentration is added to the wells that contain the test compounds (25 μL/well) at twice the final concentration. The plate is covered and incubated at room temperature for 2–4 h. Twenty minutes prior to incubation completion, one drop of reagent A (Vecta Stain ABC Horse Radish Peroxidase kit, Vector Laboratories, Inc.) and one drop reagent B are added with mixing to 5 mL of modified Tyrodes buffer mix and left to stand. The ligand solution is discarded and the plate washed (5 × 200 μL/well) with modified Tyrodes buffer. Vecta Stain HRP-Biotin-Avidin reagent (50 μL/well, as prepared above) is added and incubated at room temperature for 15 min. The Vecta Stain solution is discarded and the wells washed (5 × 200 μL/well) with modified Tyrodes buffer. Developing buffer (10 mL of 50 mM citrate/phosphate buffer at pH 5.3, 6 mg *o*-phenylenediamine, 6 μL 30% H_2O_2; 50 μL/well) is added and incubated at room temperature for 3–5 min, and then 2 N H_2SO_4 (50 μL/well) is added. The absorbance is read at 490 nM. Reported results were run in duplicate. Compounds of special interest were run in pairs of duplicates.

In Vitro Inhibition of Gel-Filtered Platelet Aggregation Assay. The percentage of platelet aggregation is calculated as an increase in light transmission of compound-treated platelet concentrate vs control-treated platelet concentrate. Blood is obtained from drug-free, normal donors into tubes containing 0.13 M sodium citrate. Platelet-rich plasma (PRP) is collected by centrifugation of whole blood at 200*g* for 10 min at 25 °C. The PRP (5 mL) is gel filtered through Sepharose 2B (bed volume 50 mL), and the platelet count is adjusted to 2×10^7 platelets per sample. The following constituents are added to a siliconized cuvette: concentrated platelet filtrate and Tyrode's buffer (0.14 M NaCl, 0.0027 M KCl, 0.012 M $NaHCO_3$, 0.76 mM Na_2HPO4, 0.0055 M glucose, 2 mg/mL BSA, and 5.0 mM HEPES at pH 7.4) in an amount equal to 350 μL, 50 μL of 20 mM calcium and 50 μL of the test compound. Aggregation is monitored in a BIODATA aggregometer for the 3 min following the addition of agonist (thrombin 50 μL of 1 unit/mL, or collagen 1 μg/mL final concentration). Reported results were run in duplicate.

Computer Calculations. NOE's observed for fibrinogen peptide γ385–411 were used in Biosym Technologies' distance geometry calculation suite of programs run on an SGI Challenge-L computer.[9] Small molecule minimization and dynamics runs were performed using an IBM-550. The differences in the various dynamics runs reflects something of the history of how the systems were examined. Initially, the structures were treated to a 10 ps (10 000 fs) dynamics run using the Tripos force field in the molecular modeling software package, SYBYL. The time step was 1 fs, using a Boltzmann starting factor, and a temperature of 300 K, with all other settings as the default choices. Additionally, all bonds to hydrogen were constrained with SHAKE. Those systems that appeared promising were treated to additional, more extensive dynamics simulations (promising is defined as having some maximum population of $C_{\alpha 1}$–$C_{\alpha 4}$ distances around 6.8 Å which was the maximum population value obtained from dynamics calculations on the NMR-derived structure of γ385–411). Also, systems that resulted from some minor structural variations of the original set were considered. More extensive dynamics runs typically involved the same conditions as just described with the exception that the length of the run was 100 ps. A minimized structure was used as a starting point in all cases (minimization also involved the use of the Tripos force field). In both minimization and dynamics runs, all atoms were explicitly examined.

Chemical Procedures. High-field ^{1}H NMR spectra were recorded on a Bruker AC-360 spectrometer at 360 MHz with tetramethylsilane as an internal standard, and coupling constants are given in hertz. All mass spectra were obtained by fast atom bombardment (MH^+) on a Finnegan TSQ-70 or a VG 7070E spectrometer. Melting points were determined on a MEL-TEMP II melting point apparatus and are uncorrected. Microanalyses were performed at Robertson Microlit Laboratories, Inc., Madison, NJ, or The R. W. Johnson Pharmaceutical Research Institute Analytical R&D Department. Final compounds were purified by recrystallization/precipitation from common organic solvents and/or column chromatography using Merck silica gel 60. Purities were assessed on a combination Waters/Beckman HPLC System and a Phenomenex Ultra-carb column (100 × 4.6 mm) using an aqueous acetonitrile mobile phase (typically 10% MeCN/90% water).

Materials. Protected amino acids and the peptide γ400–411 were purchased from Bachem Bioscience. Amino acid residues are L-configured unless noted otherwise. Nipecotic acid (or its methyl ester) was used in racemic form unless noted otherwise. The peptide γ385–411 was synthesized and purified according to the procedure in ref 9. All other reagents were purchased from Aldrich.

***N*-(3-Amino-1-adamantyl)glycine Benzyl Ester (2).** To a suspension of 1,3-adamantanediamine dihydrochloride (15.0 g, 0.063 mol) and DMF (300 mL) at room temperature was added NaH (5.64 g, 0.19 mol, 80% mineral oil suspension). The mixture was warmed at 55 °C for 2 h, cooled to room temperature, and treated with benzyl 2-bromoacetate (14.4 g, 0.063 mol) dropwise over a 1 h period. The mixture was stirred for 18 h at room temperature, diluted with saturated NH_4Cl (50 mL), saturated $NaHCO_3$ (150 mL), and CH_2Cl_2 (200 mL). The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (100 mL). The combined organic layers were washed with water (150 mL), filtered through $MgSO_4$, and evaporated to an oil. The oil was purified by flash chromatography (1% NH_4OH/10–40% $iPrOH/CH_2Cl_2$) to give **2** (13.0 g, 65%) as a white powder: mp 172–174 °C; ^{1}H NMR (DMSO-d_6) δ 8.05 (m, 3 H), 7.40 (m, 5 H), 5.15 (s, 2 H), 3.37 (s, 2 H), 2.18 (s, 2 H), 1.2-1.9 (m, 12 H); MS *m/e* 315 (MH^+).

***N*-[1-[*N*-(*N*$^\alpha$-Boc-*N*$^\epsilon$-Cbz-L-lysyl)amino]-3-adamantyl]glycine Benzyl Ester (3).** A mixture of **2** (0.50 g, 1.29 mmol), Boc-L-Lys(Cbz)-OSu (0.62 g, 1.29 mmol), and THF (25 mL) was stirred for 21 h at room temperature and diluted with saturated NH_4Cl (10 mL) and CH_2Cl_2 (100 mL), and the resultant layers were separated. The organic layer was filtered through $MgSO_4$, evaporated, and purified by flash chromatography (0.5% NH_4OH/2–8% $EtOH/CH_2Cl_2$) to give **3** (0.40 g, 46%) as a tan powder: ^{1}H NMR (DMSO-d_6) δ 7.33 (m, 10 H), 6.59 (d, J = 8, 1 H), 5.72 (s, 1 H), 5.10 (s, 3 H), 4.97 (s, 2 H), 3.80 (m, 1 H), 3.37 (d, J = 5, 2 H), 3.30 (m, 1 H), 2.95 (m, 2 H), 2.07 (m, 2 H), 1.71 (m, 4 H), 1.4–1.6 (m, 14 H), 1.32 (s, 9 H); MS *m/e* 677 (MH^+).

***N*-[1-[*N*-(*N*$^\alpha$-Boc-L-lysyl)amino]-3-adamantyl]glycine (4).** To a solution of **3** (0.08 g, 0.12 mmol) in EtOH (5 mL) in a Parr bottle under nitrogen atmosphere was added AcOH (3 mL), water (10 mL), and Pd/C (10%, 0.02 g). This mixture was hydrogenated at 50 psi and room temperature for 20 h, filtered through Celite, and evaporated to a volume of *ca.* 3 mL. This solution was treated with Et_2O (20 mL) and filtered to give **4** (0.05 g, 72%) as a tan powder: ^{1}H NMR (DMSO-d_6) δ 7.48 (br s, 1 H), 6.64 (d, J = 7, 1 H), 3.86 (m, 1 H), 3.03 (br s, 4 H), 2.62 (m, 1 H), 2.03 (m, 2 H), 1.99 (m, 1 H), 1.7–1.9 (m, 10 H), 1.62 (br s, 2 H), 1.4-1.6 (m, 7 H), 1.37 (s, 9 H), 1.26 (m, 3 H); MS *m/e* 453 (MH^+); $[\alpha]^{25}_D$ −26.2° (*c* 0.08, MeOH). Anal. ($C_{23}H_{40}N_4O_5 \cdot 2C_2H_4O_2 \cdot H_2O$) C, H, N.

***N*-[1-[*N*-(*N*$^\alpha$-Fmoc-*N*$^\epsilon$-Boc-L-lysyl)amino]-3-adamantyl]glycine (5).** Compound **5**, prepared by hydrogenolysis (see **4**) of *N*-[1-[*N*-(*N*$^\alpha$-Fmoc-*N*$^\epsilon$-Boc-L-lysyl)amino]-3-adamantyl]-glycine benzyl ester, was isolated as a white foam: ^{1}H NMR ($CDCl_3$) δ 7.75 (d, J = 8, 2 H), 7.58 (m, 2 H), 7.34 (t, J = 8, 2H), 7.24 (t, J = 8, 2 H), 6.25 (m, 1 H), 4.90 (m, 1 H), 4.35 (m, 1 H), 4.23 (m, 1 H), 4.10 (m, 3 H), 3.47 (m, 2 H), 3.06 (m, 2 H), 2.22 (m, 2 H), 1.4–2.1 (m, 20 H), 1.38 (s, 9 H); MS *m/e* 675 (MH^+).

***N*-[1-[*N*-(*N*$^\alpha$-Fmoc-L-lysyl)amino]-3-adamantyl]glycyl-L-aspartyl(OMe)-L-valine Methyl Ester (6).** A solution of **5** (2.2 g, 3.3 mmol), H-L-Asp(OMe)-L-Val-OMe·HCl (0.96 g, 3.3 mmol), ethyl [(dimethylamino)propyl]carbodiimide hydrochloride (0.93 g, 5.0 mmol), and $CHCl_3$ (150 mL) at room temperature was treated with NMM (0.35 mL, 3.3 mmol) and stirred for 16 h. The reaction was diluted with saturated NH_4Cl (40 mL), and the layers were separated. The organic layer was filtered through $MgSO_4$, evaporated, and purified by flash chromatography (0.2% NH_4OH/3% $EtOH/CH_2Cl_2$) to give a glass. The glass was dissolved in $CHCl_3$ (10 mL), treated with

HCl (1.5 mL, 4.0 M in dioxane) at room temperature, and stirred for 5 h. The solution was evaporated to *ca.* 5 mL, treated with Et_2O (20 mL), filtered, and dried to afford **6** (0.49 g, 17%) as a white foam: ^{1}H NMR (DMSO-d_6) δ 8.75 (d, J = 7, 1 H), 8.33 (m, 1 H), 7.88 (d, J = 7, 2 H), 7.80 (br s, 1 H), 7.71 (m, 2 H), 7.38 (q, J = 7, 2 H), 7.30 (t, J = 7, 2 H), 7.28 (m, 1 H), 4.20 (m, 5 H), 3.93 (m, 1 H), 3.72 (m, 1 H), 3.66 (s, 3 H), 3.58 (s, 3 H), 2.83 (m, 1 H), 2.71 (m, 2 H), 2.33 (m, 2 H), 2.20 (br s, 2 H), 2.06 (m, 2 H), 1.6–2.0 (m, 10 H), 1.50 (m, 8 H), 1.26 (m, 4 H), 0.90 (d, J = 6, 3 H), 0.83 (t, J = 6, 3 H); MS m/e 817 (MH^+). Anal. ($C_{44}H_{60}N_6O_9 \cdot 2HCl \cdot H_2O$) C, H, N.

***N*-[1-[*N*-(*N*$^\alpha$-Cbz-L-lysyl)amino]-3-adamantyl]glycine (7).** To a solution of *N*-[1-[*N*-(*N*$^\alpha$-Cbz-*N*$^\epsilon$-Boc-L-lysyl)amino]-3-adamantyl]glycine *tert*-butyl ester (0.40 g, 0.62 mmol, prepared from Boc-L-Lys(Cbz)-OSu and *N*-(3-amino-1-adamantyl)glycine *tert*-butyl ester as in Scheme 1) and CH_2Cl_2 (5 mL) at room temperature was added TFA (5 mL). This solution was stirred for 2 h, evaporated to a volume of *ca.* 3 mL, treated with Et_2O (30 mL), and filtered to give **7** (0.43 g, 95%) as a white powder: ^{1}H NMR (DMSO-d_6) δ 8.90 (m, 1 H), 7.69 (br s, 3 H), 7.36 (m, 5 H), 7.29 (d, J = 7, 2 H), 5.03 (s, 2 H), 3.84 (m, 1 H), 3.80 (br s, 2 H), 2.72 (m, 2 H), 2.22 (br s, 4 H), 2.00 (m, 3 H), 1.6–1.9 (m, 7 H), 1.4–1.6 (m, 6 H), 1.31 (m, 2 H); MS m/e 487 (MH^+); $[\alpha]^{25}_D$ −6.8° (*c* 6.4, MeOH). Anal. ($C_{26}H_{38}N_4O_5 \cdot 2C_2HF_3O_2 \cdot 0.5H_2O$) C, H, N.

***N*-[1-[*N*-(*N*$^\alpha$-Fmoc-L-alanyl-L-lysyl)amino]-3-adamantyl]glycine (8).** Compound **8**, prepared from *N*-[1-[*N*-(*N*$^\alpha$-Fmoc-L-alanyl-L-lysyl(Boc))amino]-3-adamantyl]glycine *tert*-butyl ester and TFA (see **7**), was isolated as a white powder: ^{1}H NMR (DMSO-d_6) δ 8.89 (br s, 2 H), 7.91 (d, J = 7, 2 H), 7.80 (m, 1 H), 7.70 (m, 2 H), 7.66 (br s, 3 H), 7.44 (t, J = 7, 2 H), 7.33 (t, J = 7, 2 H), 4.24 (m, 5 H), 4.19 (m, 3 H), 3.91 (m, 3 H), 2.73 (t, J = 7, 2 H), 2.0–2.3 (m, 5 H), 1.7–2.0 (m, 7 H), 1.4–1.6 (m, 6 H), 1.31 (m, 2 H), 1.21 (d, J = 6, 3 H); IR (KBr) 3500–2840, 1674, 1202 cm^{-1}; MS m/e 646 (MH^+); $[\alpha]^{25}_D$ −36.9° (*c* 0.23, MeOH). Anal. ($C_{36}H_{47}N_5O_6 \cdot 3C_2HF_3O_2$) C, H, N.

***N*-[1-[*N*-(*N*$^\alpha$-Cbz-L-lysyl)amino]-3-adamantyl](*N*-phenoxyacetyl)glycine (9).** To a solution of *N*-[1-[*N*-(*N*$^\alpha$-Cbz-*N*$^\epsilon$-Boc-L-lysyl)amino]-3-adamantyl]glycine *tert*-butyl ester (0.18 g, 0.28 mmol), CH_2Cl_2 (30 mL), and Et_3N (0.04 mL, 0.28 mmol) at room temperature was added phenoxyacetyl chloride (0.04 mL, 0.28 mmol). This mixture was stirred for 3 h, treated with NH_4Cl (0.10 g), filtered, and evaporated to give an oil. The oil was purified by flash chromatography (0.5% NH_4OH/2% EtOH/CH_2Cl_2) to give a glass. This glass was deprotected with TFA (see **7**) to afford **9** (0.11 g, 44%) as a white powder: ^{1}H NMR (DMSO-d_6) δ 8.88 (br s, 1 H), 7.70 (br s, 3 H), 7.32 (m, 7 H), 6.94 (t, J = 7, 2 H), 6.85 (d, J = 7, 1 H), 5.00 (s, 2 H), 3.80 (m, 5 H), 2.72 (br s, 2 H), 2.42 (m, 2 H), 2.16 (br s, 2 H), 1.6–2.0 (m, 10 H), 1.4–1.6 (m, 6 H), 1.24 (m, 3 H); MS m/e 621 (MH^+); $[\alpha]^{25}_D$ −11.2° (*c* 0.16, MeOH). Anal. ($C_{34}H_{44}N_4O_7 \cdot 2C_2HF_3O_2 \cdot 3H_2O$) C, H, N.

***N*-[1-[*N*-(*N*$^\alpha$-Fmoc-L-lysyl)amino]-3-adamantyl]glycine Methyl Ester (10).** Compound **10**, prepared from *N*-[1-[*N*-(*N*$^\alpha$-Fmoc-*N*$^\epsilon$-Boc-L-lysyl)amino]-3-adamantyl]glycine methyl ester and TFA (see **7**), was isolated as a white powder: ^{1}H NMR (DMSO-d_6) δ 9.08 (br s, 2 H), 7.91 (d, J = 7, 2 H), 7.72 (m, 2 H), 7.68 (br s, 3 H), 7.40 (t, J = 7, 2 H), 7.29 (t, J = 7, 2 H), 4.26 (m, 3 H), 3.98 (br s, 2 H), 3.90 (m, 1 H), 3.73 (s, 3 H), 2.74 (br s, 2 H), 2.23 (br s, 3 H), 2.08 (d, J = 11, 1 H), 1.95 (d, J = 11, 1 H), 1.84 (d, J = 11, 1 H), 1.6–1.8 (m, 7 H), 1.52 (m, 7 H), 1.28 (m, 3 H); MS m/e 589 (MH^+). Anal. ($C_{34}H_{44}N_4O_5 \cdot 3C_2HF_3O_2 \cdot H_2O$) C, H, N.

***N*-[1-[*N*-(*N*$^\alpha$-Boc-L-arginyl)amino]-3-adamantyl]glycine (11).** Compound **11**, prepared from Boc-L-Arg(Cbz$_2$)-OSu as in Scheme 1 (see **4**), was isolated as a white powder: ^{1}H NMR (DMSO-d_6) δ 9.27 (m, 1 H), 7.8–8.0 (m, 3 H), 7.44 (s, 1 H), 6.80 (d, J = 7, 1 H), 3.86 (m, 1 H), 3.02 (m, 4 H), 2.19 (br s, 2 H), 2.02 (m, 2 H), 1.6–1.9 (m, 10 H), 1.4–1.6 (m, 8 H), 1.35 (s, 9 H); MS m/e 481 (MH^+); $[\alpha]^{25}_D$ −20.0° (*c* 0.12, MeOH). Anal. ($C_{23}H_{40}N_6O_5 \cdot 2C_2H_4O_2$) C, H, N.

***N*-[1-[*N*-(*N*$^\alpha$-Boc-*N*$^\epsilon$-isopropyl-L-lysyl)amino]-3-adamantyl]glycine (12).** Compound **12**, prepared from Boc-L-Lys(*i*-Pr)(Cbz)-OSu as in Scheme 1 (see **4**), was isolated as a glass: ^{1}H NMR (DMSO-d_6) δ 7.92 (s, 1 H), 7.37 (br s, 2 H), 6.58 (d, J = 7, 1 H), 3.81 (m, 1 H), 2.95 (m, 3 H), 2.70 (m, 3 H), 2.20 (br s, 2 H), 1.6–2.0 (m, 12 H), 1.4–1.6 (m, 5 H), 1.34 (s, 9 H), 1.20 (m, 3 H), 0.94 (d, J = 6, 6 H); MS m/e 495 (MH^+); $[\alpha]^{25}_D$ −10.4° (*c* 0.52, MeOH). Anal. ($C_{26}H_{46}N_4O_5 \cdot 2C_2H_4O_2$) C, H, N.

N-[1-(4-Piperidinopropionamido)-3-adamantyl]glycine (13). Compound **13**, prepared from **2** and 3-(4-pyridyl)-acrylic acid (for method, see compound **25**), was isolated as a white foam: ^{1}H NMR (DMSO-d_6) δ 9.17 (br s, 2 H), 8.85 (m, 2 H), 7.62 (s, 1 H), 3.79 (br s, 1 H), 3.19 (d, J = 10, 2 H), 2.71 (d, J = 10, 2 H), 2.0–2.4 (m, 6 H), 1.6–1.9 (m, 9 H), 1.3–1.6 (m, 9 H), 1.15 (m, 2 H); MS m/e 364 (MH^+). Anal. ($C_{20}H_{33}N_3O_3 \cdot 3HCl \cdot H_2O$) C, H, N.

***N*$^\alpha$-Boc-D-Lys(Cbz)-(*S*)-(+)-Nip-OMe (15).** To a mixture of *N*$^\alpha$-Boc-D-Lys(Cbz)-OH (2.9 g, 7.74 mmol) and CH_2Cl_2 (80 mL) at 5 °C was added BOP-Cl (1.96 g, 7.7 mmol) and NMM (0.83 mL, 7.7 mmol). This mixture was stirred for 30 min, treated with (*S*)-(+)-nipecotic acid methyl ester hydrochloride (1.39 g, 7.7 mmol) and NMM (0.83 mL), stirred for 2 h at 5 °C, and diluted with saturated NH_4Cl (50 mL). The organic layer was separated from the aqueous layer, dried with $MgSO_4$, and evaporated to a glassy solid. This solid was purified by flash chromatography (4% EtOH/CH_2Cl_2) to afford **15** (3.52 g, 90%) as a white foam: ^{1}H NMR ($CDCl_3$) δ 7.30 (m, 5 H), 5.50 (m, 1 H), 5.09 (s, 2 H), 4.61 (m, 1 H), 3.92 (m, 1 H), 3.66 (s, 3 H), 3.20 (m, 4 H), 2.79 (m, 1 H), 2.51 (m, 1 H), 2.12 (m, 1 H), 1.50–1.80 (m, 10 H), 1.39 (s, 9 H); MS m/e 506 (MH^+).

***N*$^\alpha$-Boc-D-Lys(Cbz)-(*S*)-(+)-Nip-β-Ala-OBn (16).** To a solution of **15** (3.52 g, 7.0 mmol) in THF (25 mL) at room temperature was added aqueous lithium hydroxide (0.19 g in 15 mL water) dropwise over a 3 min period. This solution was stirred for 6 h and evaporated to give a white foam. This foam was slurried with CH_2Cl_2 (80 mL) at room temperature and treated sequentially with β-Ala-OBn·PTSA (2.43 g, 7.0 mmol), HOBT (5 mg), EDC·HCl (1.98 g, 10.4 mmol), and NMM (0.76 mL, 7.0 mmol). This mixture was stirred for 13 h diluted with saturated NH_4Cl (50 mL), and the layers were separated. The organic layer was dried with $MgSO_4$ and evaporated to give a white foam. The foam was purified by flash chromatography (3–4% EtOH/CH_2Cl_2) to give **16** (1.41 g, 31%) as a white foam: ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 10 H), 6.29 (m, 1 H), 5.45 (m, 1 H), 5.12 (s, 2 H), 5.05 (s, 2 H), 5.00 (m, 1 H), 4.55 (m, 1 H), 4.32 (m, 1 H), 3.48 (m, 2 H), 3.19 (m, 4 H), 2.53 (m, 3 H), 2.21 (m, 1 H), 1.84 (m, 1 H), 1.48–1.72 (m, 9 H), 1.42 (s, 9 H); MS m/e 653 (MH^+).

***N*$^\alpha$-Boc-D-Lys-Nip-β-Ala-OH (17).** Compound **17**, prepared from racemic nipecotic acid methyl ester as in Scheme 2 (see **18**), was isolated as colorless flakes: mp 48–54 °C; ^{1}H NMR (DMSO-d_6) δ 7.96 (m, 1 H), 6.82 (m, 1 H), 4.30 (m, 2 H), 3.81 (m, 1 H), 3.12 (m, 4 H), 2.69 (m, 2 H), 2.56 (m, 1 H), 2.33 (m, 2 H), 2.14 (m, 2 H), 1.80 (m, 2 H), 1.4–1.7 (m, 9 H), 1.32 and 1.34 (br s, 1:1, 9 H), 1.22 (m, 2 H); IR (KBr) 3580–2770, 1711, 1642 cm^{-1}; MS m/e 429 (MH^+); $[\alpha]^{25}_D$ −7.8° (*c* 1.7, MeOH). Anal. ($C_{20}H_{36}N_4O_6 \cdot 2C_2H_4O_2 \cdot 0.5H_2O$) C, H, N. Reverse phase HPLC, purity >97.3% as a 1:1 mixture of **18/19**.

***N*$^\alpha$-Boc-D-Lys-(*S*)-(+)-Nip-β-Ala-OH (18).** To a solution of **16** (0.80 g, 1.22 mmol) in THF (15 mL) in a Parr bottle under nitrogen atmosphere was added AcOH (5 mL), water (10 mL), and Pd/C (10%, 0.09 g). This mixture was hydrogenated at 50 psi and room temperature for 21 h, filtered through Celite, and evaporated to *ca.* 5 mL. This solution was treated with Et_2O (60 mL) to give a white precipitate which was filtered and lyophilized to afford **18** (0.47 g, 92%) as colorless flakes: mp 52–60 °C; ^{1}H NMR (DMSO-d_6) δ 7.85 (m, 1 H), 6.83 (d, J = 7, 1 H), 4.34 (d, J = 12, 1 H), 4.22 (m, 1 H), 3.60 (m, 2 H), 3.41 (m, 2 H), 2.98 (t, J = 11, 1 H), 2.88 (m, 1 H), 2.69 (m, 2 H), 2.35 (m, 2 H), 2.12 (m, 1 H), 2.03 (m, 1 H), 1.70 (m, 2 H), 1.4–1.6 (m, 8 H), 1.35 and 1.38 (br s, 8.5:1, 9 H), 1.16 (m, 2 H); IR (KBr) 3450–2860, 1709, 1641 cm^{-1}; MS m/e 429 (MH^+); $[\alpha]^{25}_D$ −15.2° (*c* 0.63, MeOH). Anal. ($C_{20}H_{36}N_4O_6 \cdot C_2H_4O_2 \cdot 0.5C_4H_8O$) C, H, N. Reverse phase HPLC, purity >96.4% as a 84.0:12.4 mixture of **18/19**.

***N*$^\alpha$-Boc-D-Lys-(*R*)-(−)-Nip-β-Ala-OH (19).** Compound **19**, prepared from (*R*)-(−)-nipecotic acid methyl ester as in Scheme 2 (see **18**), was isolated as colorless flakes: mp 42–51 °C; ^{1}H NMR (DMSO-d_6) δ 7.95 (m, 1 H), 6.82 (d, J = 7, 1 H), 4.33 (m, 1 H), 4.19 (m, 1 H), 3.79 (m, 1 H), 3.25 (m, 1 H), 3.04 (t, J = 10, 2 H), 2.69 (m, 2 H), 2.34 (m, 1 H), 2.21 (m, 1 H), 2.14 (m,

2 H), 1.78 (m, 2 H), 1.71 (m, 2 H), 1.4–1.6 (m, 9 H), 1.34 and 1.38 (br s, 1:8, 9 H), 1.20 (m, 2 H); MS *m/e* 429 (MH^+); $[\alpha]^{25}_D$ −4.8° (*c* 0.33, DMSO). Anal. ($C_{20}H_{36}N_4O_6 \cdot 2.5C_2H_4O_2$) C, H, N. Reverse phase HPLC purity, >92.8% as a 76.9:15.9 mixture of **19**/**18**.

N^{α}-Boc-L-Lys(Cbz)-Nip-β-Ala-OBn (20). Compound **20**, prepared from Boc-L-Lys(Cbz)-OH and racemic nipecotic acid methyl ester as in Scheme 2, was isolated as a glass: ^{1}H NMR ($CDCl_3$) δ 7.29 (m, 10 H), 6.51 (m, 1 H), 5.39 (m, 1 H), 5.11 (s, 2 H), 5.06 (s, 2 H), 4.94 (m, 1 H), 4.54 (m, 2 H), 4.18 (m, 1 H), 4.02 (d, *J* = 10, 1 H), 3.61 (m, 1 H), 3.48 (m, 2 H), 3.17 (m, 4 H), 2.54 (m, 3 H), 2.20 (m, 1 H), 1.83 (m, 1 H), 1.67 (m, 2 H), 1.51 (m, 4 H), 1.39 (s, 9 H); MS *m/e* 653 (MH^+). Anal. ($C_{35}H_{48}N_4O_8 \cdot 1.5H_2O$) C, H, N.

N^{α}-Boc-L-Lys-Nip-β-Ala-OH (21). Compound **21**, prepared by hydrogenolysis of **20** (see **18**), was isolated as a white foam: ^{1}H NMR (DMSO-d_6) δ 8.00 (m, 1 H), 7.86 (m, 1 H), 4.29 (m, 2 H), 3.82 (m, 1 H), 3.11 (m, 3 H), 2.70 (m, 2 H), 2.53 (m, 1 H), 2.31 (m, 2 H), 2.17 (m, 2 H), 1.4–1.9 (m, 10 H), 1.34 and 1.36 (br s, 1:1, 9 H), 1.23 (m, 2 H); MS *m/e* 429 (MH^+); $[\alpha]^{25}_D$ +0.8° (*c* 0.82, MeOH). Anal. ($C_{20}H_{36}N_4O_6 \cdot 1.5C_2H_4O_2$) C, H, N. Reverse phase HPLC purity, >94.5% as a 1:1 mixture of nipecotyl C-3 epimers.

N^{α}-Boc-D-Lys-Nip-L-Asp-OMe (22). Compound **22**, prepared from L-Asp(OBn)-OMe and Boc-D-Lys(Cbz)-Nip-OH as in Scheme 2, was isolated as a white foam: ^{1}H NMR (DMSO-d_6) δ 8.33 (m, 1 H), 6.77 (d, *J* = 7, 1 H), 4.32 (m, 3 H), 3.82 (m, 1 H), 3.59 (s, 3 H), 2.96 (m, 2 H), 2.73 (m, 3 H), 2.46 (m, 2 H), 2.34 (m, 1 H), 1.79 (m, 3 H), 1.4-1.7 (m, 8 H), 1.34 and 1.37 (br s, 1:1, 9 H), 1.27 (m, 2 H); MS *m/e* 487 (MH^+); $[\alpha]^{25}_D$ −3.6° (*c* 0.56, MeOH). Anal. ($C_{22}H_{38}N_4O_8 \cdot C_2H_4O_2 \cdot H_2O$) C, H, N.

H-L-Lys-Nip-β-Ala-OH (23). To a solution of **21** (0.30 g, 0.70 mmol) in MeOH (10 mL) and water (10 mL) at room temperature was added HCl (0.50 mL, concentrated). This solution was stirred for 1 h and evaporated to *ca.* 2 mL of oil. This oil was treated with MeCN (20 mL), filtered, washed with Et_2O (3 × 20 mL), and dried to afford **23** (0.25 g, 82%) as a white powder: mp 65–75 °C; ^{1}H NMR (DMSO-d_6) δ 8.23 (m, 3 H), 8.06 (m, 3 H), 4.33 (m, 2 H), 3.73 (m, 4 H), 3.25 (m, 2 H), 3.01 (m, 1 H), 2.72 (m, 2 H), 2.44 (m, 1 H), 2.34 (m, 2 H), 1.5–1.8 (m, 6 H), 1.35 (m, 4 H); MS *m/e* 329 (MH^+); $[\alpha]^{25}_D$ +9.8° (*c* 1.60, MeOH). Anal. ($C_{15}H_{28}N_4O_4 \cdot 2HCl \cdot 2H_2O$) C, H, N.

N-(N^{ϵ}-Aminocaproyl)-Nip-β-Ala-OH (24). Compound **24**, prepared from racemic nipecotic acid methyl ester and Boc-6-aminocaproic acid *N*-oxysuccinimide ester as in Scheme 2, was isolated as a glass: ^{1}H NMR (DMSO-d_6) δ 8.18 (t, *J* = 5, 1 H), 8.04 (br s, 3 H), 4.28 (m, 2 H), 3.78 (m, 2 H), 3.20 (m, 3 H), 2.99 (t, *J* = 12, 1 H), 2.71 (d, *J* = 6, 2 H), 2.39 (m, 2 H), 2.31 (m, 2 H), 2.16 (m, 1 H), 1.79 (m, 1 H), 1.61 (m, 4 H), 1.42 (t, *J* = 6, 2 H), 1.28 (m, 2 H), 1.19 (m, 1 H); MS *m/e* 314 (MH^+). Anal. ($C_{15}H_{27}N_3O_4 \cdot 2HCl$) C, H, N.

N-[3-(4-Piperidyl)propionyl]-Nip-β-Ala-OH (25). To a solution of *N*-[3-(4-pyridyl)acryloyl]-Nip-β-Ala-OBn (0.56 g, 1.33 mmol; prepared from 3-(4-pyridyl)acrylic acid and methyl nipecotate as in Scheme 2) in EtOH (20 mL) and water (10 mL) under nitrogen atmosphere was added HCl (0.66 mL, 4.0 M in dioxane) and platinum (IV) oxide (0.060 g). This mixture was hydrogenated at 50 psi and room temperature for 22 h, filtered through Celite, and evaporated to *ca.* 5 mL. This solution was treated with MeCN (30 mL), and the precipitate was filtered, washed with Et_2O (3 × 20 mL), and dried to give **25** (0.34 g, 63%) as a pale yellow foam: ^{1}H NMR (DMSO-d_6) δ 9.02 (br s, 2 H), 8.03 (m, 1 H), 7.46 (m, 1 H), 4.28 (t, *J* = 7, 1 H), 4.11 (m, 1 H), 3.79 (m, 1 H), 3.44 (t, *J* = 7, 1 H), 3.19 (m, 3 H), 3.06 (t, *J* = 12, 1 H), 2.75 (d, *J* = 11, 1 H), 2.53 (m, 1 H), 2.32 (m, 4 H), 2.12 (m, 1 H), 1.77 (m, 2 H), 1.4-1.7 (m, 7 H), 1.27 (m, 2 H), 1.18 (t, *J* = 6, 1 H); MS *m/e* 340 (MH^+). Anal. Calcd for $C_{17}H_{29}N_3O_4 \cdot 2HCl$ (412.4): H, 7.58. Found: H, 7.02. Accurate protonated mass calcd for $C_{17}H_{29}N_3O_4$: 340.2236 amu. Found: 340.2245 amu.

N^{α}-Ac-L-Lys-Nip-Gly-OH (26). Compound **26**, prepared by coupling Ac-L-Lys(Boc)-Nip-OH and H-Gly-OBn (Scheme 2) followed by hydrogenolysis (see **18**) and TFA deprotection (see **7**), was isolated as a tan powder: mp 40–55 °C; ^{1}H NMR (DMSO-d_6) δ 8.24 (t, *J* = 6, 1 H), 8.03 (d, *J* = 8, 1 H), 7.75 (br s, 3 H), 4.24 (m, 1 H), 3.72 (t, *J* = 6, 2 H), 3.61 (m, 2 H), 2.72 (m, 2 H), 1.83 (s, 3 H), 1.78 (m, 2 H), 1.63 (m, 2 H), 1.4–1.6 (m, 8 H), 1.28 (m, 4 H); MS *m/e* 357 (MH^+). Anal. ($C_{16}H_{28}N_4O_5 \cdot 3C_2HF_3O_2$) C, H, N.

N^{α}-Ac-L-Lys-Nip-β-Ala-OH (27). Compound **27**, prepared by coupling Ac-L-Lys(Boc)-Nip-OH and β-Ala-OBn (Scheme 2) followed by hydrogenolysis (see **18**) and HCl deprotection (see **23**), was isolated as a white foam: mp 53–67 °C; ^{1}H NMR (DMSO-d_6) δ 8.13 (m, 1 H), 8.00 (m, 1 H), 7.91 (d, *J* = 15, 3 H), 4.64 (m, 1 H), 4.36 (m, 1 H), 3.87 (m, 1 H), 3.66 (m, 2 H), 3.23 (m, 3 H), 2.99 (m, 1 H), 2.68 (m, 2 H), 2.59 (m, 1 H), 2.38 (m, 2 H), 2.11 (m, 1 H), 1.80 (s, 3 H), 1.63 (m, 1 H), 1.4–1.6 (m, 5 H), 1.24 (m, 3 H); MS *m/e* 371 (MH^+); $[\alpha]^{25}_D$ −5.6° (*c* 0.25, MeOH). Anal. ($C_{17}H_{30}N_4O_5 \cdot 2HCl \cdot C_2H_4O_2 \cdot H_2O$) C, H, N.

N^{α}-Boc-L-Arg-Nip-β-Ala-OH (28). Compound **28**, prepared from Boc-L-Arg(Cbz_2)-OSu and racemic nipecotic acid methyl ester (Scheme 2), was isolated as a white foam: mp 47–55 °C; ^{1}H NMR (DMSO-d_6) δ 9.53 (m, 1 H), 7.85 (m, 2 H), 6.96 (m, 1 H), 4.32 (m, 2 H), 3.84 (m, 1 H), 3.38 (m, 2 H), 3.03 (m, 4 H), 2.20 (m, 3 H), 1.74 (m, 2 H), 1.4–1.7 (m, 8 H), 1.35 (s, 9 H), 1.24 (m, 2 H); MS *m/e* 457 (MH^+). Anal. ($C_{20}H_{36}N_6O_6 \cdot 1.5C_2H_4O_2$) C, H, N.

N^{α}-Boc-L-Lys-Nip-γ-aminobutyric Acid (29). Compound **29**, prepared by coupling Boc-L-Lys(Cbz)-Nip-OH and γ-aminobutyric acid benzyl ester (Scheme 2), was isolated as a white foam: mp 65-71 °C; ^{1}H NMR (DMSO-d_6) δ 8.25 (m, 1 H), 6.87 (m, 1 H), 4.31 (m, 3 H), 3.74 (m, 2 H), 3.15 (m, 2 H), 2.98 (m, 3 H), 2.69 (m, 2 H), 2.10 (m, 3 H), 1.76 (m, 3 H), 1.4–1.7 (m, 9 H), 1.31 (s, 9 H), 1.21 (m, 2 H); MS *m/e* 443 (MH^+); $[\alpha]^{25}_D$ −9.1° (*c* 1.83, MeOH). Anal. ($C_{21}H_{38}N_4O_6 \cdot 2C_2H_4O_2$) C, H, N.

H-D-Lys-Nip-β-Ala-OH (30). Compound **30**, prepared from **17** by HCl deprotection (see **23**), was isolated as a cream-colored powder: mp 108–128 °C; ^{1}H NMR (DMSO-d_6) δ 8.28 (m, 3 H), 8.05 (m, 3 H), 4.31 (m, 2 H), 3.84 (m, 2 H), 3.25 (m, 2 H), 3.09 (m, 2 H), 2.72 (m, 3 H), 2.37 (m, 3 H), 1.80 (m, 1 H), 1.5–1.7 (m, 6 H), 1.33 (m, 4 H); MS *m/e* 329 (MH^+); $[\alpha]^{25}_D$ −22.7° (*c* 0.26, MeOH). Anal. ($C_{15}H_{28}N_4O_4 \cdot 2HCl \cdot C_2H_4O_2$) C, H, N.

N^{α}-Boc-D-Lys-Nip-γ-aminobutyric Acid (31). Compound **31**, prepared by coupling Boc-D-Lys(Cbz)-Nip-OH and γ-aminobutyric acid benzyl ester (Scheme 2), was isolated as a tan powder: mp 50-57 °C; ^{1}H NMR (DMSO-d_6) δ 7.97 (m, 1 H), 6.91 (m, 1 H), 4.32 (m, 1 H), 4.22 (m, 1 H), 3.82 (m, 1 H), 3.02 (m, 3 H), 2.71 (m, 2 H), 2.52 (m, 1 H), 2.29 (m, 1 H), 2.17 (m, 2 H), 1.84 (m, 5 H), 1.4–1.7 (m, 9 H), 1.33 (s, 9 H), 1.19 (m, 2 H); MS *m/e* 443 (MH^+); $[\alpha]^{25}_D$ +3.6° (*c* 3.0, MeOH). Anal. ($C_{21}H_{38}N_4O_6 \cdot 2C_2H_4O_2 \cdot 0.5H_2O$) C, H, N.

N^{α}-Boc-D-Lys-Nip-Gly-OH (32). Compound **32**, prepared by coupling Boc-D-Lys(Cbz)-Nip-OH and H-Gly-OBn (Scheme 2), was isolated as white flakes: mp 66–80 °C; ^{1}H NMR (DMSO-d_6) δ 7.82 (m, 1 H), 6.81 (d, *J* = 7, 1 H), 4.34 (m, 2 H), 4.09 (m, 1 H), 3.77 (m, 1 H), 3.48 (m, 1 H), 3.16 (m, 2 H), 2.70 (m, 3 H), 2.44 (m, 2 H), 2.28 (m, 1 H), 1.78 (m, 2 H), 1.4–1.7 (m, 8 H), 1.32 and 1.35 (br s, 1:1, 9 H), 1.23 (m, 2 H); MS *m/e* 415 (MH^+). Anal. ($C_{19}H_{34}N_4O_6 \cdot 2C_2H_4O_2$) C, H, N.

N^{α}-Ac-D-Lys-Nip-β-Ala-OH (33). Compound **33**, prepared by coupling Ac-D-Lys(Cbz)-Nip-OH and β-Ala-OBn (Scheme 2), was isolated as a glass: mp 46–59 °C; ^{1}H NMR (DMSO-d_6) δ 8.11 (m, 3 H), 4.70 (m, 1 H), 4.33 (m, 1 H), 3.74 (m, 1 H), 3.38 (m, 1 H), 3.19 (m, 4 H), 3.00 (m, 1 H), 2.68 (m, 2 H), 2.21 (m, 4 H), 1.82 (s, 3 H), 1.76 (m, 2 H), 1.4–1.7 (m, 7 H), 1.24 (m, 2 H); MS *m/e* 371 (MH^+); $[\alpha]^{25}_D$ +3.4° (*c* 3.23, MeOH). Anal. ($C_{17}H_{30}N_4O_5 \cdot 2.5C_2H_4O_2$) C, H, N.

N^{α}-Boc-L-Lys(*i*Pr)-Nip-β-Ala-OH (34). Compound **34**, prepared by coupling Boc-L-Lys(Cbz)(*i*Pr)-Nip-OH and β-Ala-OBn followed by hydrogenolysis (Scheme 2), was isolated as white flakes: mp 90–123 °C; ^{1}H NMR (DMSO-d_6) δ 7.93 (m, 1 H), 6.81 (d, *J* = 7, 1 H), 4.36 (m, 1 H), 4.24 (m, 1 H), 3.60 (m, 1 H), 3.37 (m, 1 H), 3.10 (m, 1 H), 2.91 (m, 3 H), 2.62 (m, 3 H), 2.39 (m, 2 H), 2.14 (m, 1 H), 2.05 (m, 1 H), 1.4–1.8 (m, 9 H), 1.34 and 1.37 (br s, 1:1, 9 H), 1.26 (m, 3 H), 1.13 (d, *J* = 5, 6 H); IR (KBr) 3500–2830, 1704, 1638 cm^{-1}; MS *m/e* 471 (MH^+). Anal. ($C_{23}H_{42}N_4O_6 \cdot 2C_2H_4O_2$) C, H, N.

3-[[[1-(6-Aminocaproyl)piperid-3-yl]methyl]amino]propionic Acid (35). To a solution of 1-(6-Boc-aminocaproyl)-nipecotic acid (3.1 g, 9.0 mmol, prepared in two steps from 6-Boc-aminocaproic acid *N*-oxysuccinimide ester and methyl

nipecotate as in Scheme 2) and THF (50 mL) was added 1,1-carbonyldiimidazole (1.45 g, 9.0 mmol). This solution was stirred for 1 h, cooled to −10 °C, treated with DIBAL (36.0 mL, 1.0 *M* in toluene) dropwise over a 20 min period, and stirred for an additional 2 h. This solution was treated with aqueous citric acid (5.0 g in 40 mL water) and diluted with $CHCl_3$ (200 mL), and the resultant layers were separated. The aqueous layer was extracted with $CHCl_3$ (100 mL), and the combined organic layers were dried, evaporated, and purified by flash chromatography (4% EtOH/CH_2Cl_2) to afford 1-(6-Boc-aminocaproyl)piperidine-3-carboxaldehyde (0.69 g, 23%) as a glass: ^{1}H NMR ($CDCl_3$) δ 9.65 (d, J = 8, 1 H), 4.58 (m, 1 H), 4.10 (m, 1 H), 3.65 (m, 1 H), 3.45 (m, 1 H), 3.22 (m, 1 H), 3.14 (m, 2 H), 2.46 (m, 2 H), 2.33 (t, J = 7 Hz, 1 H), 2.09 (m, 1 H), 1.5−1.8 (m, 7 H), 1.39 (s, 9 H), 1.33 (m, 2 H); MS *m/e* 327 (MH^+).

To a solution of 1-(6-Boc-aminocaproyl)piperidine-3-carboxaldehyde (0.69 g, 2.12 mmol) in MeOH (10 mL) at room temperature was added β-Ala-OBn·PTSA (0.74 g, 2.12 mmol) and $NaCNBH_3$ (0.13 g, 2.12 mmol). This mixture was stirred for 2.5 h and evaporated to give a white solid. This solid was partitioned between saturated $NaHCO_3$ (10 mL) and CH_2Cl_2 (50 mL), and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (2 × 50 mL), and the combined organic layers were dried, evaporated, and purified by flash chromatography (0.5% NH_4OH/4−10% EtOH/CH_2Cl_2) to give [[[1-(6-Boc-aminocaproyl)piperid-3-yl]methyl]amino]propionic acid benzyl ester (0.28 g, 27%) as a glass: ^{1}H NMR ($CDCl_3$) δ 7.33 (m, 5 H), 5.13 (s, 2 H), 4.61 (m, 1 H), 4.28 (m, 1 H), 3.70 (m, 1 H), 3.11 (m, 3 H), 2.85 (m, 3 H), 2.53 (m, 4 H), 2.31 (t, J = 7, 2 H), 1.5−1.9 (m, 8 H), 1.42 (s, 9 H), 1.29 (m, 3 H), 0.89 (m, 1 H); MS *m/e* 490 (MH^+).

To a solution of [[[1-(6-Boc-aminocaproyl)piperid-3-yl]-methyl]amino]propionic acid benzyl ester (0.28 g, 0.57 mmol) and THF (10 mL) at room temperature was added aqueous HCl (3.4 mL, 1.0 *N*). This mixture was stirred for 22 h, evaporated to a glassy solid, triturated with Et_2O (3 × 25 mL), and dried to give a white powder. This powder was dissolved in THF (5 mL) and water (10 mL), transferred to a Parr bottle under nitrogen atmosphere, and treated with Pd/C (0.04 g, 10%). This mixture was hydrogenated at 50 psi/room temperature for 20 h, filtered through Celite, and evaporated to *ca.* 5 mL. This solution was treated with MeCN (25 mL), and the precipitate was filtered, washed with Et_2O (2 × 25 mL), and dried to give **35** (0.23 g, 90%) as a colorless glass: mp 65−74 °C; ^{1}H NMR (DMSO-d_6) δ 9.31 (m, 2 H), 8.12 (br s, 3 H), 4.18 (m, 2 H), 3.70 (m, 1 H), 3.04 (m, 2 H), 2.67 (m, 5 H), 2.51 (m, 1 H), 2.35 (m, 3 H), 1.87 (m, 2 H), 1.58 (m, 4 H), 1.42 (m, 2 H), 1.30 (m, 4 H); MS *m/e* 300 (MH^+). Anal. Calcd for $C_{15}H_{29}N_3O_3$·4HCl (445.3): C, 40.46; H, 7.47; N, 9.44; Cl, 31.85. Found: C, 35.01; H, 6.32; N, 7.01; Cl, 32.38. Accurate protonated mass calcd for $C_{15}H_{29}N_3O_3$: 300.2287 amu. Found: 300.2306 amu. Reverse phase HPLC, purity >94.7%.

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