Design of Selective Thrombin Inhibitors Based on the (R)-Phe-Pro-Arg Sequence

John C. Danilewicz,[†,‡] Stuart M. Abel,[§] Alan D. Brown,[‡] Paul V. Fish,*[,‡] Edward Hawkeswood,[∥] Stephen J. Holland,[∥] Keith James,[‡] Andrew B. McElroy,[‡] John Overington,[⊥] Michael J. Powling,[∥] and David J. Rance[§]

Departments of Discovery Chemistry, Drug Metabolism, Discovery Biology, and Molecular Informatics Structure and Design, Pfizer Global Research and Development, Sandwich, Kent CT13 9NJ, United Kingdom

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Potent and selective inhibitors of thrombin were sought based on the (*R*)-Phe-Pro-Arg sequence. The objective was to generate similar binding interactions to those achieved by potent competitive inhibitors of the argatroban type, so eliminating the need for covalent interaction with the catalytic serine function, as utilized by aldehyde and boronic acid type inhibitors. Improving the S_1 subsite interaction by substitution of arginine with a 4-alkoxybenzamidine residue provided potent lead **2** (K_i = 0.37 nM). Though an amide bond, which H-bonds to the active site, is lost, modeling indicated that a new H-bond is generated between the alkoxy oxygen atom and the catalytic Ser-195 hydroxyl group. Substitution of the benzamidine system by 1-amidinopiperidine then gave compound **4**, which provided a further gain in selectivity over trypsin. However, previous work had shown that these compounds were likely to be too lipophilic (Log *D* +0.4 and +0.2, respectively) and to suffer rapid hepatic extraction, presumably via biliary elimination. Accordingly, both proved short-acting when administered intravenously to rats and showed poor activity when given intraduodenally. The aim was then to reduce lipophilicity below a log *D* of −1.2, which in a previously reported series had been effective in preventing rapid clearance. It was anticipated that compounds of this type would rely on the cation selective paracellular route of absorption from the gastrointestinal tract. Potent polar analogues with selectivity >1000 over trypsin were obtained. The best in vivo activity was shown by compound **12**. However, in the final analysis, its oral bioavilability proved poor, relative to analogues with similar physicochemical properties derived from argatroban, consistent with the hypothesis that molecular shape is an additional important determinant of paracellular absorption.

Introduction

The search for potent selective and orally active thrombin inhibitors has gathered momentum in recent years.[1] Thrombin is the last in a cascade of trypsin-like plasma serine proteases, which by catalyzing the conversion of fibrinogen to fibrin, activation of FXIII and inducing platelet aggregation is a key enzyme in haemostasis and thrombus formation. The inhibition of a single enzyme in the cascade, and in particular thrombin, has been an attractive goal in that it could also provide superior antithrombotic therapy by increasing efficacy and safety as compared to heparin and the coumarins. Additionally, by keeping molecular size small, the opportunity exists for obtaining oral activity.[1,2]

Two small molecular weight inhibitor types are emerging as structure−activity relationships are explored. The first is of the argatroban[3] and NAPAP[4] type (Chart 1), where lipophilic groups on either side of the basic P_1 side chain pack together to interact with the hydrophobic S_2 site.[5−7] Napsagatran (Ro 46-6240), developed by Hilpert et al.,[8] though having a more complex P_1 residue, can nevertheless be viewed as belonging to this group. The only interaction with the catalytic serine residue is via a hydrogen bond to the carboxylate function in both argatroban and napsagatran. Unfortunately, none of these compounds is orally active due to either poor absorption from the gastrointestinal tract and/or rapid clearance via the bile.[4,9]

A second inhibitor type is based on the substrate-derived irreversible chloromethyl ketone inhibitor PPACK and includes compounds such as DuP-714[10,11] and efegatran (GYKI-14 766).[12] These compounds interact covalently with the hydroxyl group of the catalytic serine residue. The neighboring proline ring and (*R*)-Phe side chain cooperate to fill the S_2 site in a similar fashion as the two distal lipophilic groups of the first series.[5] Though oral activity has been claimed for these compounds, we were concerned that high enzyme selectivity might not be obtainable when substantial affinity is derived by interacting covalently with the ubiquitous active site serine function. In the case of aldehyde type inhibitors, there is also the potential problem of achieving adequate optical and chemical stability.

* To whom correspondence should be addressed. Tel.: 44 1304 644589. Fax: 44 1304 656433. E.mail: paul_fish@sandwich.pfizer.com.
† Senior author.
‡ Department of Discovery Chemistry.
§ Department of Drug Metabolism.
∥ Department of Discovery Biology.
⊥ Molecular Informatics Structure and Design.

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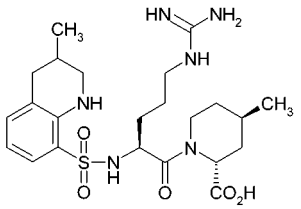
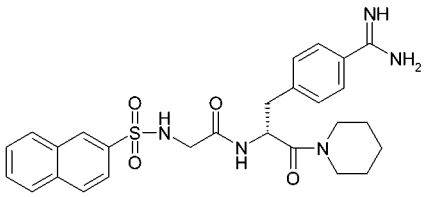
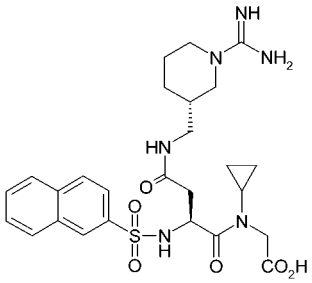
Chart 1



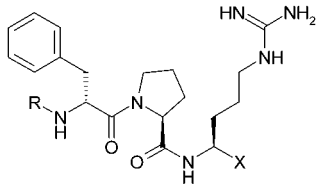
argatroban



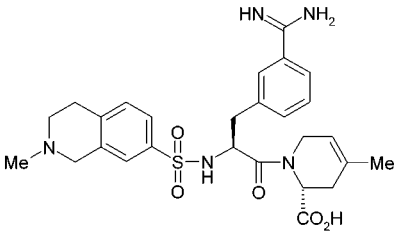
NAPAP



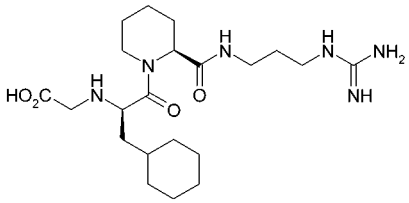
napsagratran



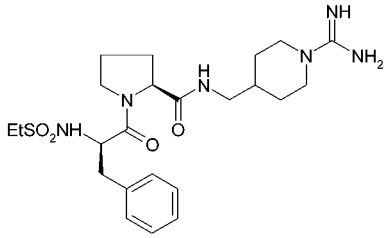
PPACK	R = H; X = -$COCH_2Cl$
DuP-714	R = Ac; X = -$B(OH)_2$
efegatran	R = Me; X = -CHO



UK-156,406



inogatran



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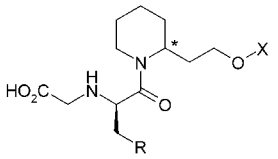
Subnanomolar potency and oral bioavailability have been obtained with "folded" inhibitors of the argatroban type, e.g., UK-154 606.[13] We considered that these properties might be equally well-combined in "linear" PPACK-derived structures but ones in which the reactive functionality was deleted to ensure competitive kinetics and to enhance the potential for high selectivity.

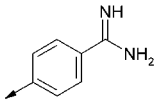
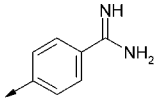
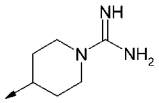
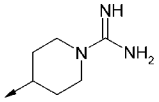
While our work was in progress,[14] two series, which included inogatran[15] and compound **1**,[16] were published. Inogatran is reported to have a K_i of 15 nM against human thrombin, which gave encouragement that the above objective should be achievable. We describe here our own work on this approach.

Compound Design

In their early studies on aryl amidines, Markwardt et al.[17] showed that simple 4-alkoxyphenylamidines possess significant thrombin and trypsin inhibitory activity, with K_i values in the 5×10^{-5} M region. We, therefore, sought to determine how such a P_1 side chain might be incorporated into a linear inhibitor type. The published complex of human thrombin with PPACK[18] (PDB[19] code 1PPB) was used as a basis for modeling studies. The covalent linkage to Ser 195 and His 57 along with the bridging methylene was removed by modeling, and the resulting noncovalently bound com-

Table 1



Compound	*	R	X	Thrombin Ki (nM)	Trypsin Ki (nM)	Selectivity Tryp/Throm	Log D
2	*S*	Cyclohexyl		0.37	89	242	+0.4
3	*R*	Cyclohexyl		120	7300	60	
4	*RS*	Cyclohexyl		0.96	370	385	+0.2 [a]
5	*RS*	Phenyl		370	7900	21	-1.1

[a] Calculated from the measured value for **5** using Rekker fragmental constants.

plex was minimized using the CHARMM program.[20] The histidine and serine residues adopted conformations closer to those observed for the remainder of the serine proteinase family and to those observed in thrombin complexes with noncovalently bound inhibitors,[5,6] while the inhibitor backbone and side chains maintained similar interactions with the enzyme. When the complex of benzamidine with human thrombin (PDB code 1DWB[18]) was examined with respect to this model, it showed that a 4-oxy substituent would be best joined via a two carbon link to the proline pyrrolidine ring. Such a modification would result in loss of an amide linking group, the NH of which, in PPACK, is H-bonded to the Ser-214 carbonyl function of thrombin. Initially, we argued that loss of this interaction might, in large measure, be compensated for by a reduction in desolvation energy on active site entry. However, it appeared from our model that a new H-bond between the alkoxy oxygen atom and the catalytic Ser-195 hydroxyl group was also possible. These considerations led to the design of compound **2**, but because racemic 2-(2-hydroxyethyl)-piperidine was used as starting material, the diastereoisomer **3** was also obtained. The more active isomer **2** proved highly potent and was assigned the *S* configuration on the basis of the modeling studies.

Very interestingly, Hilpert et al.[8] had shown that 1-amidinopiperidine is intrinsically more selective than benzamidine for thrombin and used this finding to produce their novel napsagatran series, which has high thrombin/trypsin selectivity. 1-Amidinopiperidine was therefore docked into the P_1 pocket, which showed that this system could effectively replace the benzamidine fragment. This resulted in the design of compound **4**. High potency was indeed retained, with a further small gain in selectivity, taking into account that **4** is a mixture of diastereoisomers (Table 1). It is of note that a related alkoxy 1-amidinopiperidine P_1 group has been successfully employed by Soll et al. in a different inhibitor template.[21]

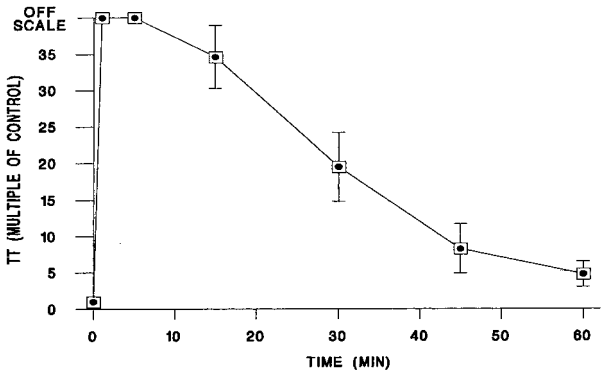


Figure 1. Prolongation in TT on iv administration of compound **2** to rats at 1 mg/kg; means ± SEM, $n = 4$.

In a previous paper,[13] it was shown that compounds of this type require a degree of polarity, with log *D* values less than or equal to −1.2, to minimize hepatic extraction and so allow some oral bioavailability. Absorption of such polar compounds from the gastrointestinal tract is then presumably achieved by the paracellular route. When given intravenously (iv) to rats, compound **2** showed only a moderate duration of action, as indicated by the relatively rapid decline in thrombin time (TT) in Figure 1, when compared with similarly potent inhibitors that have good pharmacokinetics.[13] Prolongation of TT was routinely used for in vivo compound evaluation as a more sensitive measurement of activity than the more commonly used activated partial thromboplastin time (APTT). Little activity was also evident at a standard dose of 10 mg/kg given intraduodenally (idd), as shown in Figure 2. Thus, the in vivo profile was indeed indicative of too rapid a clearance, which was consistent with a measured log *D*

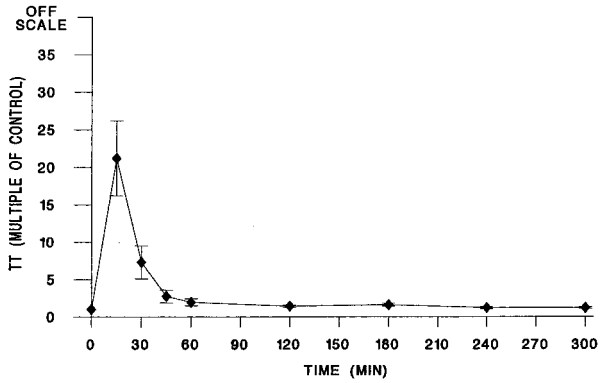


Figure 2. Prolongation in TT on idd administration of compound **2** to rats at 10 mg/kg; means ± SEM, $n = 4$.

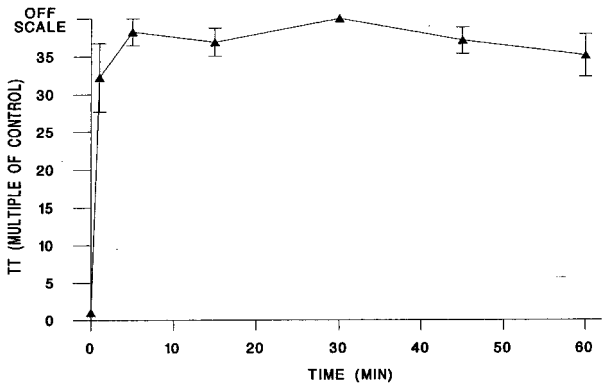


Figure 3. Prolongation in TT on iv administration of compound **12** to rats at 1 mg/kg; means ± SEM, $n = 4$.

of +0.4. Compound **4**, with a log *D* of +0.2, showed a similarly poor duration of action when given iv to rats at 1 mg/kg. Replacement of the cyclohexyl ring by a phenyl ring as in compound **5** reduced log *D* to −1.1, which is in our desired range, but potency fell sharply showing the importance of the hydrophobic interaction with the S_2 site.

Our objective was then to modify the N-carboxymethyl-(*R*)-cyclohexylalanine (Cha) fragment so as to achieve a substantial reduction in lipophilicity. N-alkyl aspartic acid derivatives were selected for study (Table 2), as this residue satisfied four requirements. First, the N atom would provide a proton (even when tertiary, if protonated), which modeling predicted could still interact with the Gly-216 residue. The thrombin−PPACK X-ray crystal structure[5,18] shows that the (*R*)-Phe carbonyl and amino groups form antiparallel H bonds to the corresponding amido and carbonyl functions of Gly-216. Second, the total lipophilicity contribution of the alkyl substituent would be reduced if brought under the close influence of a protonated N atom. Third, a free carboxyl group, which appears to be important for good in vivo toleration, as discussed below, would be retained, and fourth, a net positive charge would be maintained, so favoring absorption by the paracellular route, which is cation selective.[22] An important consideration, however, is that it would be necessary to invert stereochemistry and to use (*S*)-aspartic acid, so that the relative orientation of lipophilic and carboxyl groups in compound **4** would be preserved.

The N-cyclohexyl analogue **7** was the first to be synthesized and showed a promising level of activity. The cyclopentyl analogue **8** was inferior, but further small gains in potency were possible by increasing ring size as in compounds **9** and **10**, taking into account that some compounds were prepared as mixtures of diastereoisomers, one of which was assumed to be only weakly active (cf. compounds **2** and **3**). Activity was retained on "opening" the ring as with the 3-pentyl analogue **11**. Because the N substituent is presumed to extend into the hydrophobic S_2 site, the effect of further substitution was examined. N-methylation gave a small increace in potency (compounds **12** and **13**), but introduction of an ethyl group resulted in loss of activity (compound **17**). Large polar groups such as dimethylaminoethyl were detrimental. The introduction of a double bond into the cycloalkyl group maintained potency as in compound **14**. Attempts to further extend the hydrophobic interaction, as in the cyclohexylmethyl and benzyl analogues **15** and **16**, reduced potency. Similarly, introduction of a heteroatom as in compounds **19**−**21** was detrimental. Extension of the carboxylic acid side chain as in the glutamic acid analogue **22** (Table 3), which more closely mimicked compound **4**, reduced selectivity as compared to the corresponding aspartic acid analogue **12** and offered no in vivo advantage.

In their pioneering work on thrombin inhibitors, the group at Mitsubishi[23,24] showed that the incorporation of a carboxyl group, as in argatroban, was important to improve compound toleration. We showed a similar effect in a related series of phenylamidine-derived inhibitors,[13] the carboxyl group being in the same relative position in both series, forming an H bond to the catalytic serine residue. The same phenomenon appeared to operate in this current series. Compound **6** (Table 3) was lethal when given iv to rats at 1 mg/kg. In contrast, compounds **2** and **12** were tolerated at 30 mg/kg when given iv to mice. It is apparent that the carboxyl group in these latter compounds is positioned quite differently than in the argatroban-derived series. Thus, all that may be required is the mere physical presence of a carboxyl group anywhere in the molecule, that is compatible with thrombin inhibitory activity, to improve toleration.

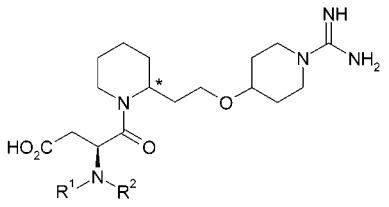
The more active compounds were evaluated in vivo, and the best responses, shown in Figures 3 and 4, were obtained with compound **12**. Compound **13** gave a less-sustained response when given idd to rats. Therefore, despite reduced in vitro potency, compound **12** exhibited an in vivo profile that is clearly superior to that of compound **4** (Log *D* +0.2). The log *D* of **12** was found to be −1.8. Thus, again, a reduction in lipophilicity is seen to be beneficial in improving in vivo activity.

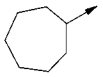
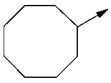
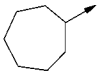
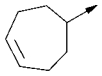
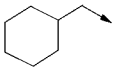
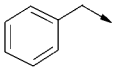
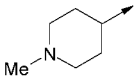
The selectivity of compound **12** was examined against a representative selection of thrombin-related enzymes, as can be seen from Table 4. Relatively low activity was evident against factor (F) Xa, plasmin, and FVIIa, with selectivities of >1000 being achieved. Selectivity against trypsin was slightly lower (900×), though values >1000× were achieved with compounds **13** and **14**.

Enzyme−Inhibitor Binding

Details of the interactions of PPACK with thrombin have been described in detail.[18] However, briefly, as shown in Figure 5, aside from the covalent bonds formed to Ser 195 and His 57, a substratelike, antiparallel

Table 2

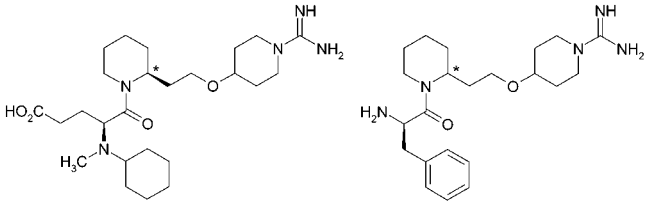


Compound	*	R^1	R^2	Thrombin Ki (nM)	Trypsin Ki (nM)	Selectivity Tryp/Throm
7	*RS*		H	7.1	2900	408
8	*RS*		H	32	5000	150
9	*S*		H	1.1	700	640
10	*RS*		H	1.3	720	550
11	*S*		H	9.8	1400	140
12	*S*		Me	2.17	1930	890
13	*S*		Me	0.53	810	1530
14	*S*		Me	0.8	1100	1375
15	*RS*		H	16	1600	100
16	*S*		H	24%[a]	9200	
17	*RS*		Et	16	5000	312
18	*RS*		$CH_2CH_2NMe_2$	0%[a]	0%[c]	
19	*RS*		H	42%[a]	23%[b]	
20	*S*		Me	16%[a]	48%[c]	
21	*RS*		H	90	4800	50

[a] Inhibition at 10^{-6} M. [b] Inhibition at 10^{-5} M. [c] Inhibition at 10^{-4} M.

β-sheet type interaction is formed between the peptide backbone of PPACK and the mainchain of the enzyme (NH of P_1 Arg to carbonyl of Ser 214, carbonyl of P_3 (*R*)-Phe to amide of Gly 216, and amino terminus of P_3 with carbonyl of Gly 216). The carbonyl of P_2 Pro is exposed to solvent in the complex. The guanidine of the P_1 Arg residue forms an "end-on" salt bridge to Asp 189 of the enzyme and, further, hydrogen bonds to the carbonyl of Gly 219 and also to a "conserved" buried water molecule at the base of the P_1 pocket. The methylenes of the pyrrolidine ring of P_2 Pro are cradled under a hydrophobic lid formed by the aryl portions of Tyr 60A and Trp 60D, from the loop insertion unique to thrombin. It is this unique loop that is thought to be

Table 3



22 **6**

compd	*	thrombin K_i (nM)	trypsin K_i (nM)	selectivity tryp/throm
22	*S*	1.8	550	305
6	*RS*	56	1600	28

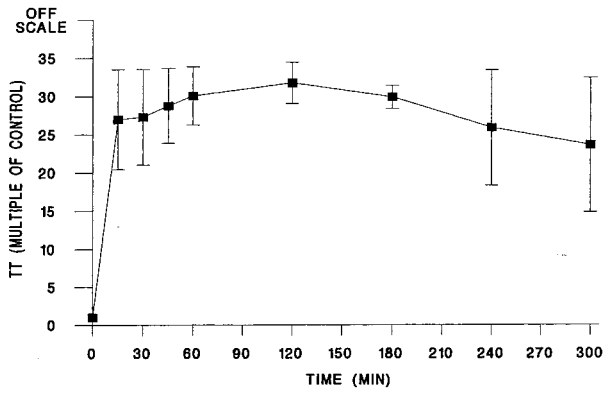


Figure 4. Prolongation in TT on idd administration of compound **12** to rats at 10 mg/kg; means ± SEM, $n = 3$.

responsible for a large part of the selectivity of thrombin in vivo. The (*R*)-Phe residue binds into a hydrophobic pocket formed by Leu 99, Ile 174, and Trp 215.

A model of compound **12** bound to the active site of human thrombin was built using PPACK as the template and the modeling program QUANTA.[25] The model was refined by energy minimization techniques and restrained molecular dynamics simulations, selected water molecules from the thrombin−PPACK complex structure that were included in the calculations (Figure 6). As in PPACK, a series of important "backbone" interactions are formed, despite the fact that **12** only contains one peptide bond. The proton associated with the tertiary amine (which we assume is protonated at physiological pH) interacts with the carbonyl of Gly 216, while the amide carbonyl of **12** forms a hydrogen bond with the amide proton of Gly 216. The hydrogen bond corresponding to that from the P_2 residue of PPACK is absent in **12**, with the carbonyl of Ser 214 now partially exposed to solvent; however, a new hydrogen bond would appear to be formed between the ether oxygen of **12** and the active site residue, Ser 195. As discussed above, it is possible that the formation of this hydrogen bond compensates for the loss of the preceding main chain interaction. A further interaction to the main chain of thrombin is formed by one of the carboxylate oxygens, corresponding to a substrate-like main chain interaction for a P_4 residue.

Table 4. Enzyme Inhibition Profile of Compound **12**

enzyme	calcd K_i
thrombin	$2.17 \pm 0.09 \times 10^{-9}$ M
trypsin	$1.93 \pm 0.12 \times 10^{-6}$ M
FXa	$4.97 \pm 0.24 \times 10^{-6}$ M
plasmin	$3.30 \pm 0.15 \times 10^{-5}$ M
FVIIa	38.7 ± 1.9% inhibition at 10^{-4} M

The guanidine of **12** forms an end-on salt bridge interaction with Asp 189 at the base of the P_1 pocket, with further required hydrogen bonds observed to the backbone carbonyl of Gly 219 and a water molecule bound at the base of the P_1 pocket, as with PPACK. The methylene atoms of the piperidine ring, however, form extensive van der Waals interactions with the remaining hydrophobic parts of the P_1 pocket. It is interesting to note that there is a sequence difference between

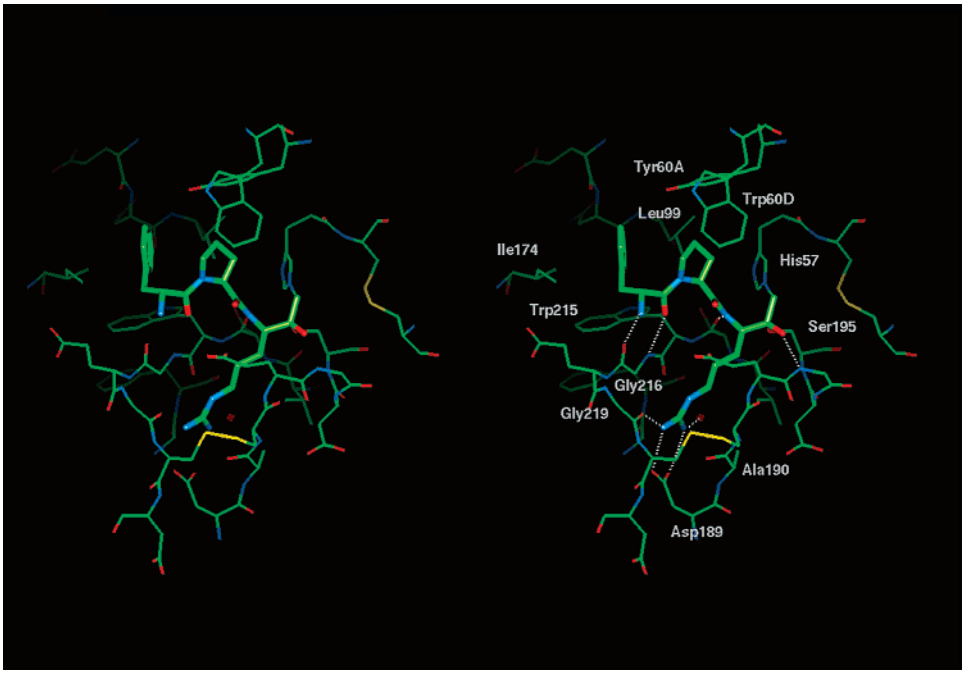


Figure 5. Stereoview of active site of human thrombin−PPACK thrombin complex (PDB code 1PPB), showing binding mode of PPACK (thick lines). Hydrogen bonds are indicated by dotted gray lines. Residues in thrombin are labeled according to established numbering conventions.

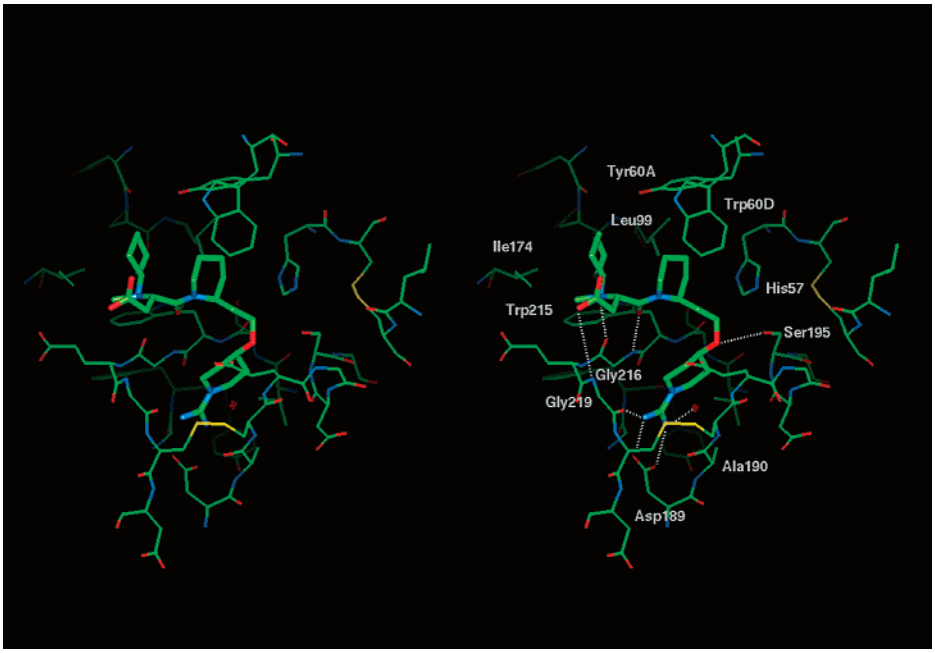


Figure 6. Stereoview of modeled binding of **12** to active site of human thrombin. See legend to Figure 5 for further details.

trypsin and thrombin (Ser 190 in trypsin is Ala in thrombin) in the P_1 pocket that favors the binding of more lipophillic ligands in the P_1 pocket of thrombin, and this gives rise to some of the observed thrombin selectivity of **12**.

The binding in the aryl pocket is quite distinct to that observed in PPACK. The cyclohexyl group shows extensive hydrophobic contacts with Trp 215, Leu 99, and Ile 174, and the *N*-methyl group extends this pattern of hydrophobic interactions, primarily increasing interactions with Ile 174. This area is more polar in trypsin (the residue corresponding in position to Ile 174 is a glutamine residue), and so, the additional hydrophobic interactions possible in thrombin contribute to the observed enhanced potency and selectivity. Extending the methyl group to larger alkyl groups would affect the conformation of the essential acid moiety and start to shield polar atoms of thrombin from contact with solvent, consistent with the observed loss of potency on extending this group.

Chemistry

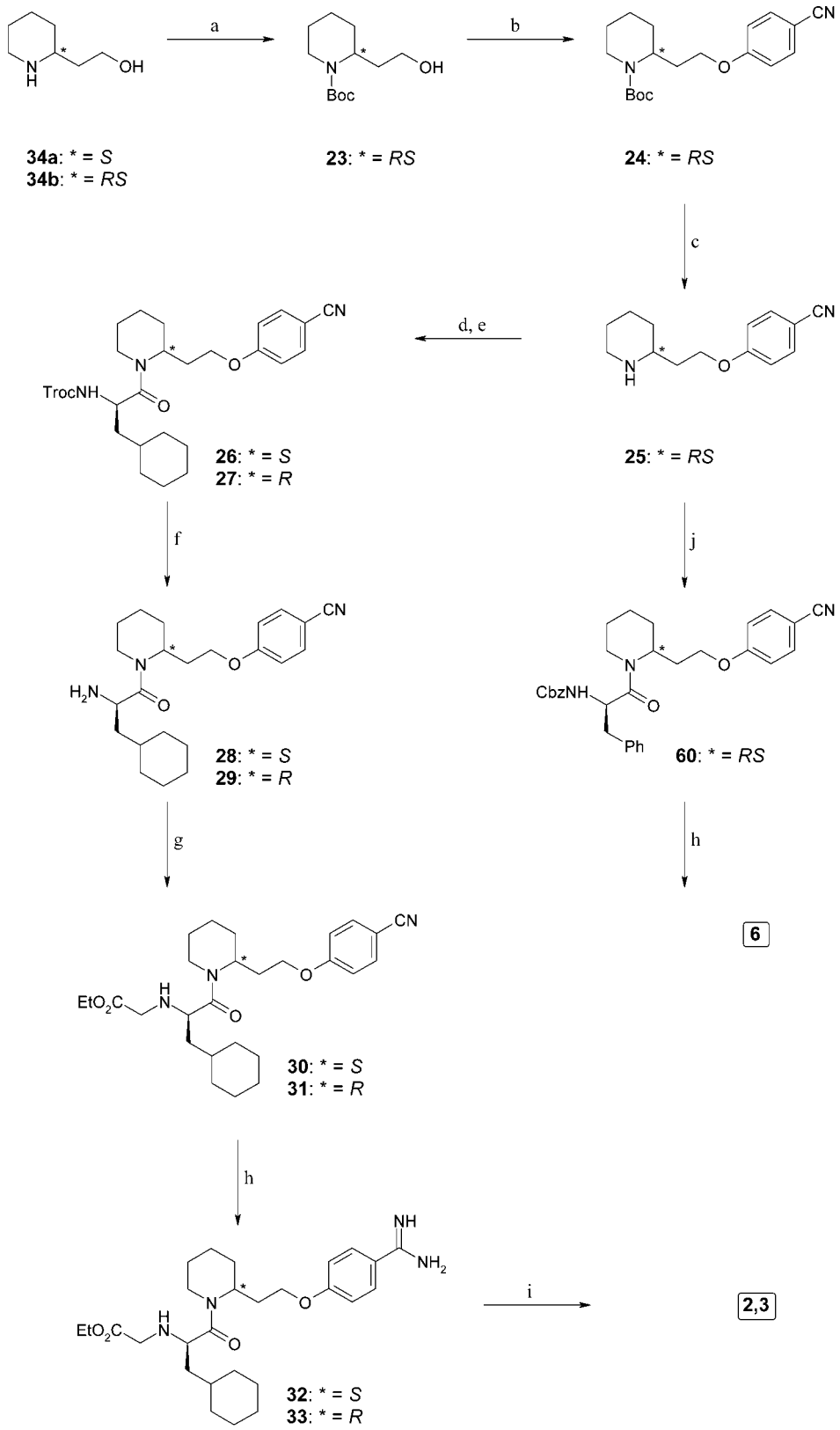
The methods for the synthesis of the inhibitors disclosed in Tables 1−3 (compounds **2**−**22**) are described in Schemes 1−6. Phenylamidines **2**, **3**, and **6** were prepared as outlined in Scheme 1. Compound **23** was reacted with 4-cyanophenol under Mitsunobu conditions[26] to give the phenyl ether **24**. Removal of the Boc-protecting group using trifluoroacetyl (TFA) in the presence of anisole, as a carbonium ion scavenger, gave amine **25**. Coupling with the acid chloride of N-Troc-(R)-Cha then gave the diastereoisomers **26** and **27**, which were readily separated by chromatography on silica. Removal of the Troc-protecting group with zinc[27] afforded amines **28** and **29**. Reaction with ethyl bromoacetate, followed by the sequential treatment with ethanolic HCl and ethanolic ammonia (Pinner reaction[28]), gave amidines **32** and **33**. Hydrolysis with NaOH followed by acidification then gave the diastereoisomers **2** and **3**. The more active was assigned the *S* configuration on the basis of the computer modeling studies described above, the *R* diastereoisomer showing the much poorer fit. Diimide coupling of **25** with N-Cbz-(*R*)-Phe-OH afforded compound **60**. Treatment of **60** with ethanolic HCl, to form the imidate, resulted only in partial simultaneous removal of the Cbz-protecting group. Thus, when the product was treated with ammonia, a mixture of **6** and its N-Cbz derivative, which were readily separable by chromatography, was obtained.

The guanidine analogues **4** and **5** were prepared as shown in Schemes 2 and 3. *N*-Cbz-2-(2-hydroxyethyl)-piperidine **35b** was converted to the methanesulfonate **36b**, which was reacted with the Na alkoxide of N-Boc-4-hydroxypiperidine in dimethylformamide (DMF) to give the ether **37b** (Scheme 2). Hydrogenolysis of the Cbz-protecting group and reaction of amine **41** with the acid chloride of N-Troc-(*R*)-Cha gave **42** (Scheme 3). Removal of the Boc-protecting group and reaction of amine **43** with *N*,*N*′-di-*t*-butoxycarbonyl-*S*-methylisothiourea[29] then gave bis-Boc-protected guanidine **44**. We found, however, that inclusion of mercuric chloride greatly facilitated this reaction and improved yields. Removal of the Troc-protecting group with zinc, as described above, gave **45**, which on reaction with *tert*-butyl bromoacetate and acid treatment, gave compound **4** as a mixture of diastereoisomers.

Removal of the Boc-protecting group from **37b** (Scheme 2), followed by reaction of the resultant amine **38b** with *N*,*N*′-di-*t*-butoxycarbonyl-*S*-methylisothiourea gave bis-Boc-protected guanidine **39b** in good yield. Hydrogenolysis of the Cbz-protecting group gave **40b**, which on coupling with N-Fmoc-(*R*)-Phe-OH, using PyBroP,[30] gave **47**. Removal of the Fmoc-protecting group with piperidine and reaction of **48** with *tert*-butyl bromoacetate, followed by acid treatment, gave **5** as the hydrochloride salt.

Compound **34b** was resolved as the (1*S*)-(+)-10-camphorsulfonic acid salt,[31,32] the absolute configuration

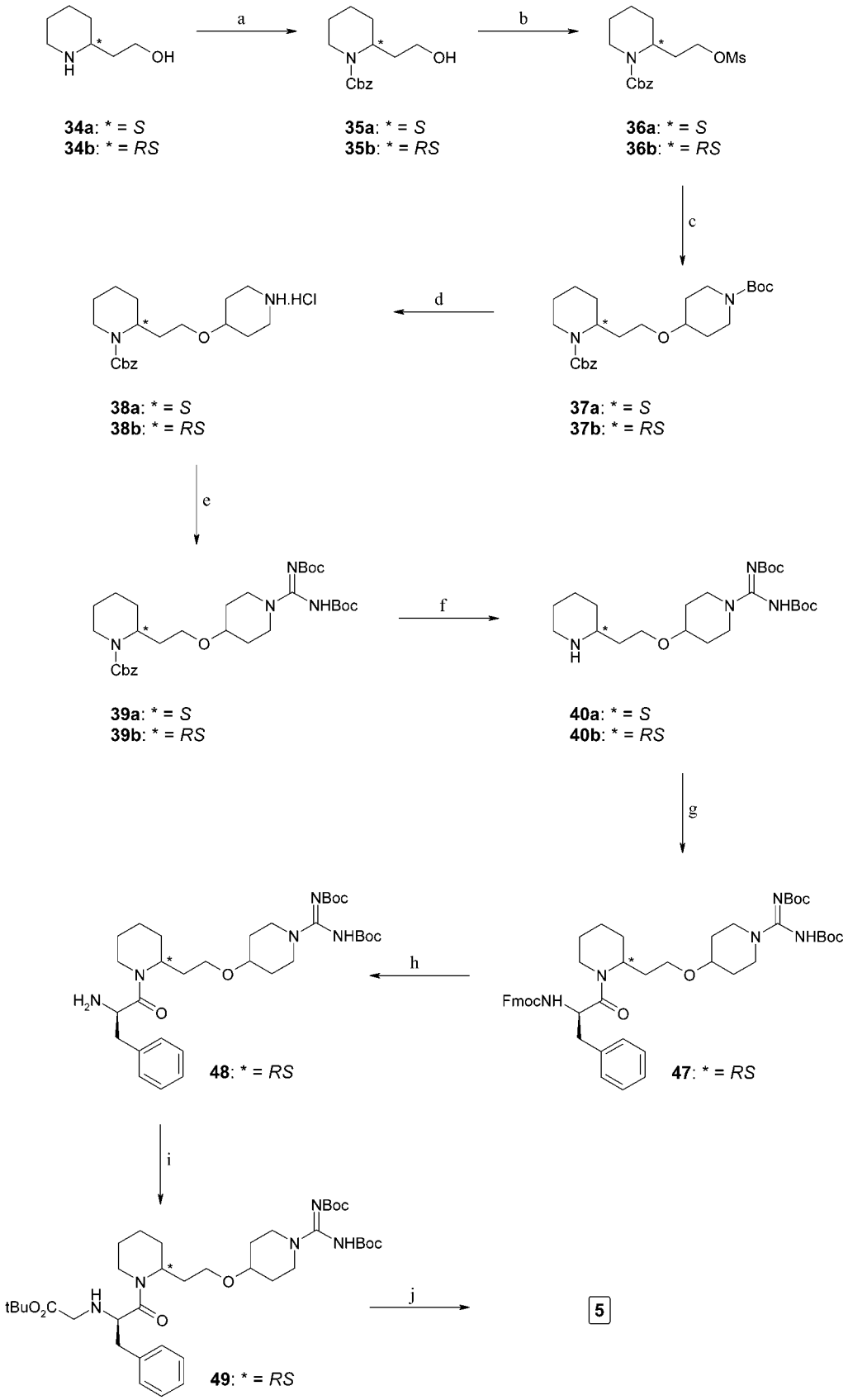
Scheme 1[a]



[a] Reagents: (a) Boc_2O, EtOAc; (b) 4-cyanophenol, DEAD, PPh_3, THF; (c) TFA, PhOMe, CH_2Cl_2; (d) Troc-D-Cha-Cl, DIPEA, CH_2Cl_2; (e) chromatographic separation of diastereoisomers; (f) Zn, KH_2PO_4, THF; (g) $BrCH_2CO_2Et$, K_2CO_3, MeCN; (h) HCl and then NH_3; (i) NaOH then HCl; (j) Cbz-D-Phe-OH, WSCDI, HOBt, NMM, CH_2Cl_2.

of which was confirmed as *S* by single-crystal X-ray crystallographic analysis. The free base **34a** was then elaborated, in a manner similar to **34b**, to give **40a**. Both **40a**,**b** were coupled with N-Fmoc-(*S*)-aspartic acid *β-tert*-butyl ester to give **50a**,**b** (Scheme 4). Removal of the Fmoc-protecting group, followed by reductive amination with a range of ketones and aldehydes, gave **52a**−**l**. Acid treatment then gave **7**−**11**, **15**, **16**, **19**, and

Scheme 2[a]

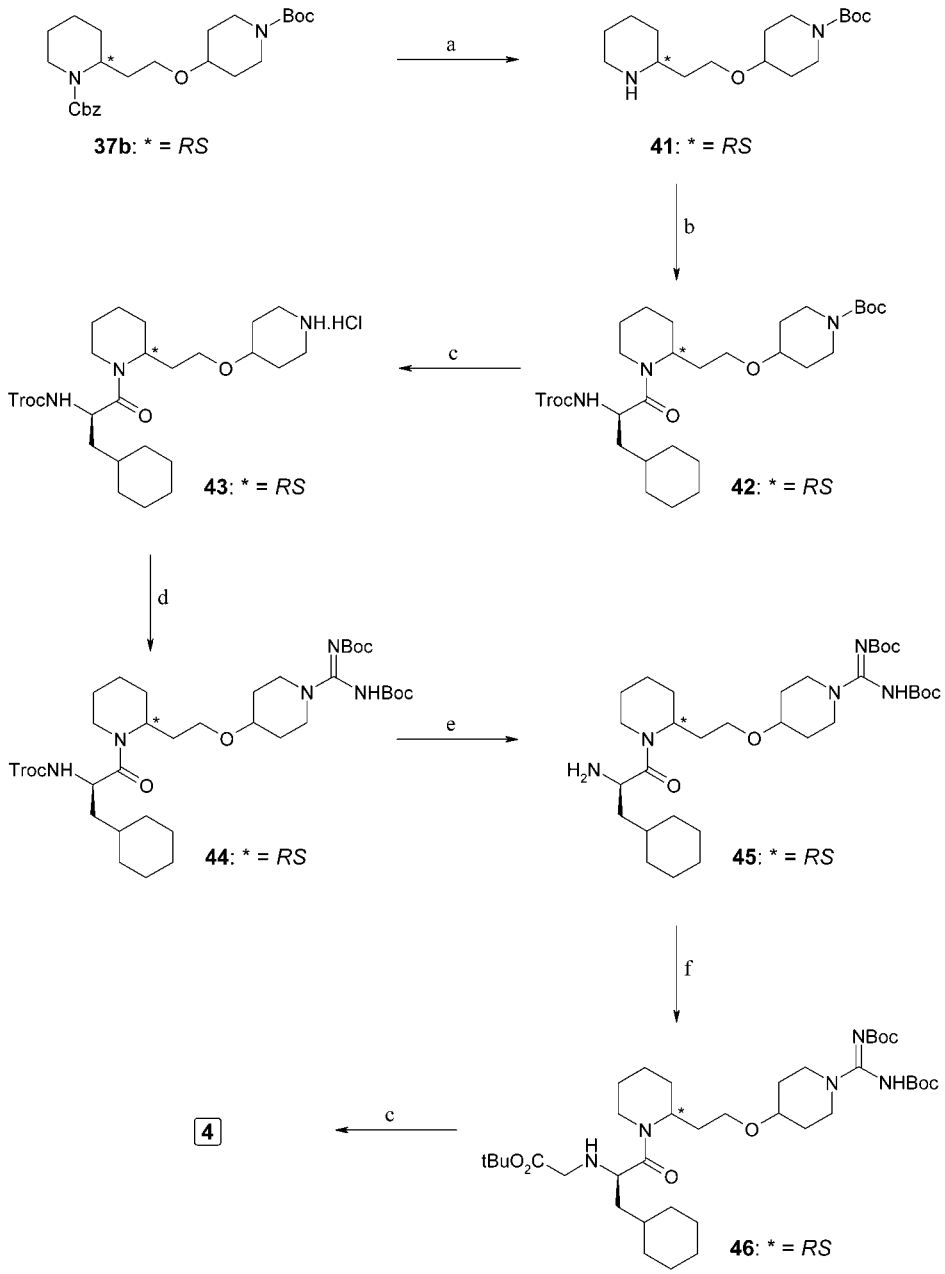


[a] Reagents: (a) CbzOSuc, TEA; (b) MsCl, TEA, CH_2Cl_2; (c) *N*-Boc-4-hydroxypiperidine, NaH, DMF; (d) HCl, CH_2Cl_2; (e) MeSC(=NBoc)NHBoc, $HgCl_2$, TEA, CH_2Cl_2; (f) H_2, Pd/C, EtOH; (g) Fmoc-D-Phe-OH, PyBroP, DIPEA, CH_2Cl_2; (h) piperidine, THF; (i) $BrCH_2CO_2tBu$, K_2CO_3, MeCN; (j) HCl, CH_2Cl_2.

21. Methylation of **52c**,**f**,**g**,**k**, followed by acid treatment, gave **12**−**14** and **20**.

Compound **52a** was also alkylated with acetaldehyde and 2-chloroethyldimethylamine to give **54** and **55**,

Scheme 3[a]



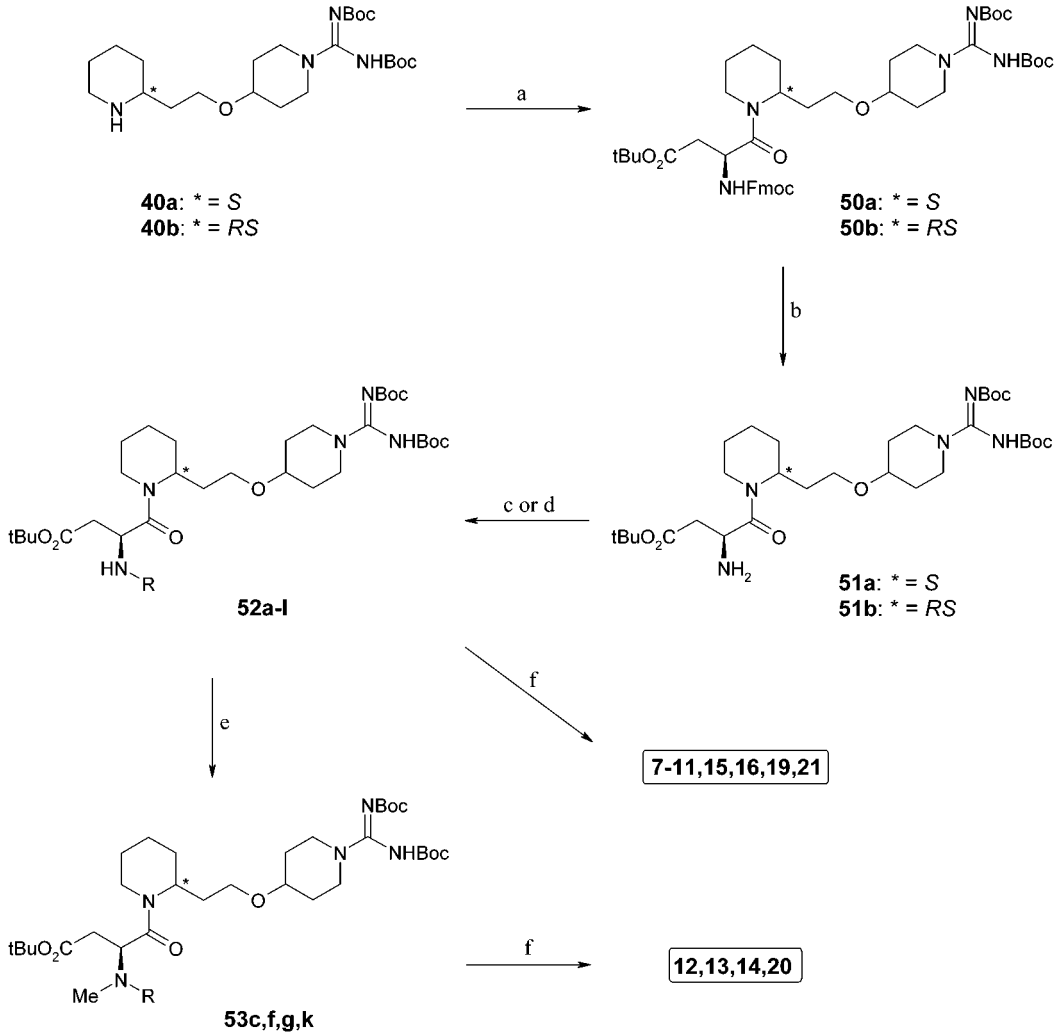
[a] Reagents: (a) Pd/C, 1,4-cyclohexadiene, EtOH; (b) Troc-D-Cha-Cl, DIPEA, CH_2Cl_2; (c) HCl, CH_2Cl_2; (d) MeSC(=NBoc)NHBoc, $HgCl_2$, TEA, CH_2Cl_2; (e) Zn, AcOH; (f) $BrCH_2CO_2tBu$, K_2CO_3, MeCN.

respectively, which on acid treatment afforded the acids **17** and **18** (Scheme 5). Reaction of **40a** with N-Fmoc-(*S*)-glutamic acid γ-*tert*-butyl ester gave **56** as shown in Scheme 6. Reaction of **56** in a manner similar to that shown in Scheme 4 then gave the glutamic acid analogue **22**.

Pharmacokinetics and Disposition

The pharmacokinetics of compound **12** were examined in detail and compared with one of our best benzamidine analogues, **61** (UK-179 094).[13] As can be seen in Table 5, following iv administration of **12**, volumes of distribution at steady state were 0.31 and 0.53 L/kg for rat and dog, respectively. Corresponding plasma clearance values were 21.1 and 6.4 mL/min/kg, and β-elimination half-life values were 0.8 and 1.0 h in rat and dog, respectively. Renal elimination of unchanged drug was substantial in both rat (39% of the iv dose) and dog (21%). The overall iv pharmacokinetic profiles for **12** and **61** are therefore very similar (Table 5). In particular, non-renal clearance is low with respect to hepatic blood flow for both compounds in both species, indicating very little first-pass extraction. However, after oral administration, the bioavailability of compound **12** was found to be only 2% in the rat and 9% in the dog, indicative of very low absorption. These poor results contrasted markedly with the corresponding values of 16 and 37% obtained with compound **61**. We were particularly concerned as our experience indicated that for polar compounds absorbed by the paracellular route, the rat is likely to be the more predictive species for man. Compounds **12** and **61** have similar physicochemical properties [**12**: M_w 465.6, Log $D_{7.4}$ −1.8, pK_a 8.8 (second basic center); **61**: M_w 553.7, Log $D_{7.4}$ −1.8, pK_a 8.0

Scheme 4[a]



52 or **53**	*	R
a	*RS*	cyclohexyl
b	*RS*	cyclopentyl
c	*S*	cycloheptyl
d	*RS*	cyclooctyl
e	*S*	3-pentyl
f	*S*	cyclohexyl
g	*S*	1-cyclohept-4-enyl
h	*RS*	cyclohexylmethyl
i	*S*	benzyl
j	*RS*	4-tetrahydropyranyl
k	*S*	3-tetrahydropyranyl
l	*RS*	*N*-methyl-4-piperidinyl

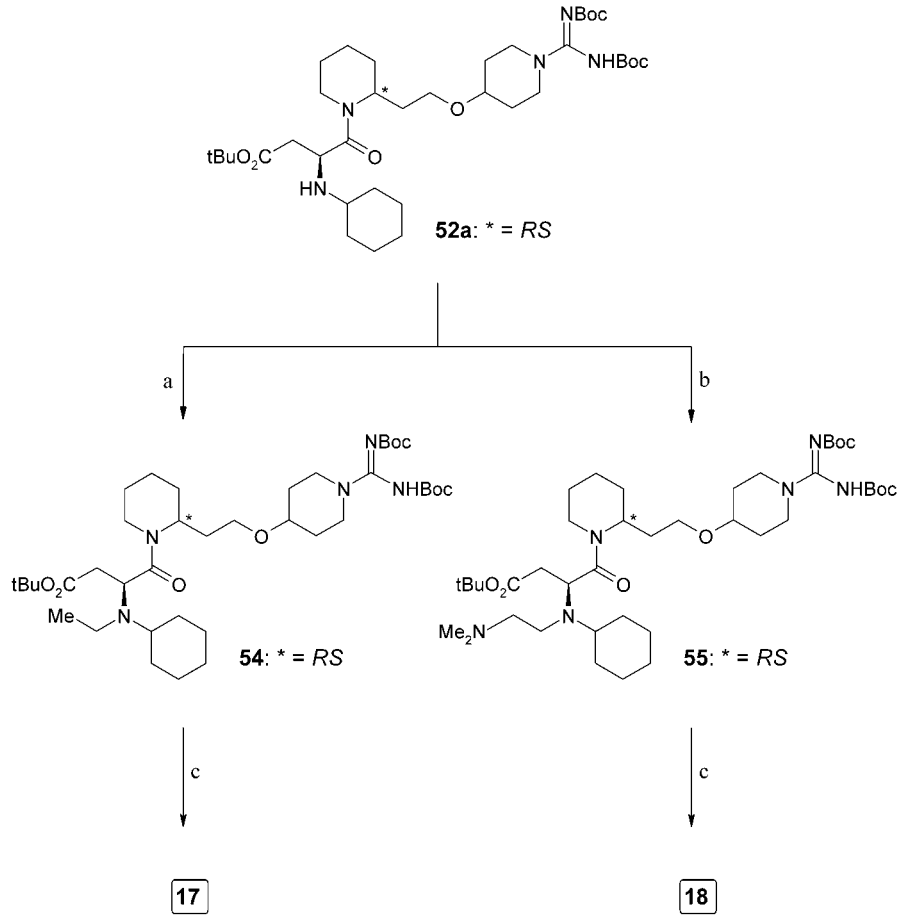
[a] Reagents: (a) Fmoc-L-Asp(OtBu)-OH, PyBroP, DIPEA, CH_2Cl_2; (b) piperidine, THF; (c) ketone, $NaBH(OAc)_3$, AcOH, THF; (d) aldehyde, $NaBH(OAc)_3$, THF; (e) 37% HCHO(aq), $NaBH(OAc)_3$, CH_2Cl_2; (f) HCl, CH_2Cl_2.

(second basic center)]; they have the same Log *D* values, and **12** even has the potential advantage of the lower molecular weight. However, the second basic pK_a of **12** is a little greater than that of **61**, but because we believe that these inhibitors are absorbed by the paracellular route, that is through aqueous channels, we would not expect that small differences in the degree of ionization would influence absorption from the gastrointestinal tract. We conclude that molecular shape, which is influenced by folding, may be the determining factor.

Conclusion

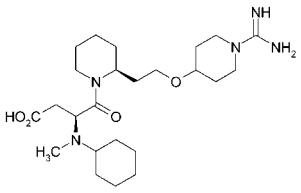
We have successfully developed a series of highly potent and selective thrombin inhibitors, based on the (*R*)-Phe-Pro-Arg sequence, without recourse to covalent receptor interaction. By adjusting polarity and the incorporation of a carboxyl group, presystemic clearance has been minimized and toleration has been improved. However, the best compound of the series, **12** (UK-285 954), showed low oral bioavailability as compared

Scheme 5[a]

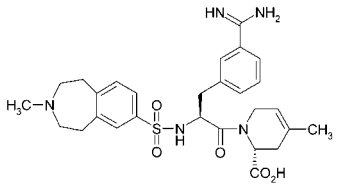


[a] Reagents: (a) MeCHO, $NaBH(OAc)_3Cl_2$, THF; (b) $HCl{\cdot}Me_2NCH_2CH_2Cl$, K_2CO_3, MeCN; (c) HCl, CH_2Cl_2.

Table 5. Pharmacokinetic Parameters for Compounds **12** and **61** in Rat and Dog



12



61

parameter	rat		dog	
compd	**12**	**61**	**12**	**61**
iv dose level (mg/kg)	10	10	1	1
clearance (mL/min/kg)	21.2	11	6.4	6.9
volume of dist (L/kg)	0.31	0.60	0.53	0.53
α-elim half-life (h)	0.12		0.16	
β-elim half-life (h)	0.8	0.8	1.0	0.90
po dose level (mg/kg)	50	50	10	2
oral bioavailability (%)	2	16	9	37

to our most successful examples of benzamidine type inhibitors, such as **61** (UK-179 094). We conclude that the optimization of physicochemical parameters is insufficient and that molecular size and shape also appear to be important in allowing absorption of polar compounds from the gastrointestinal tract by the paracellular route.

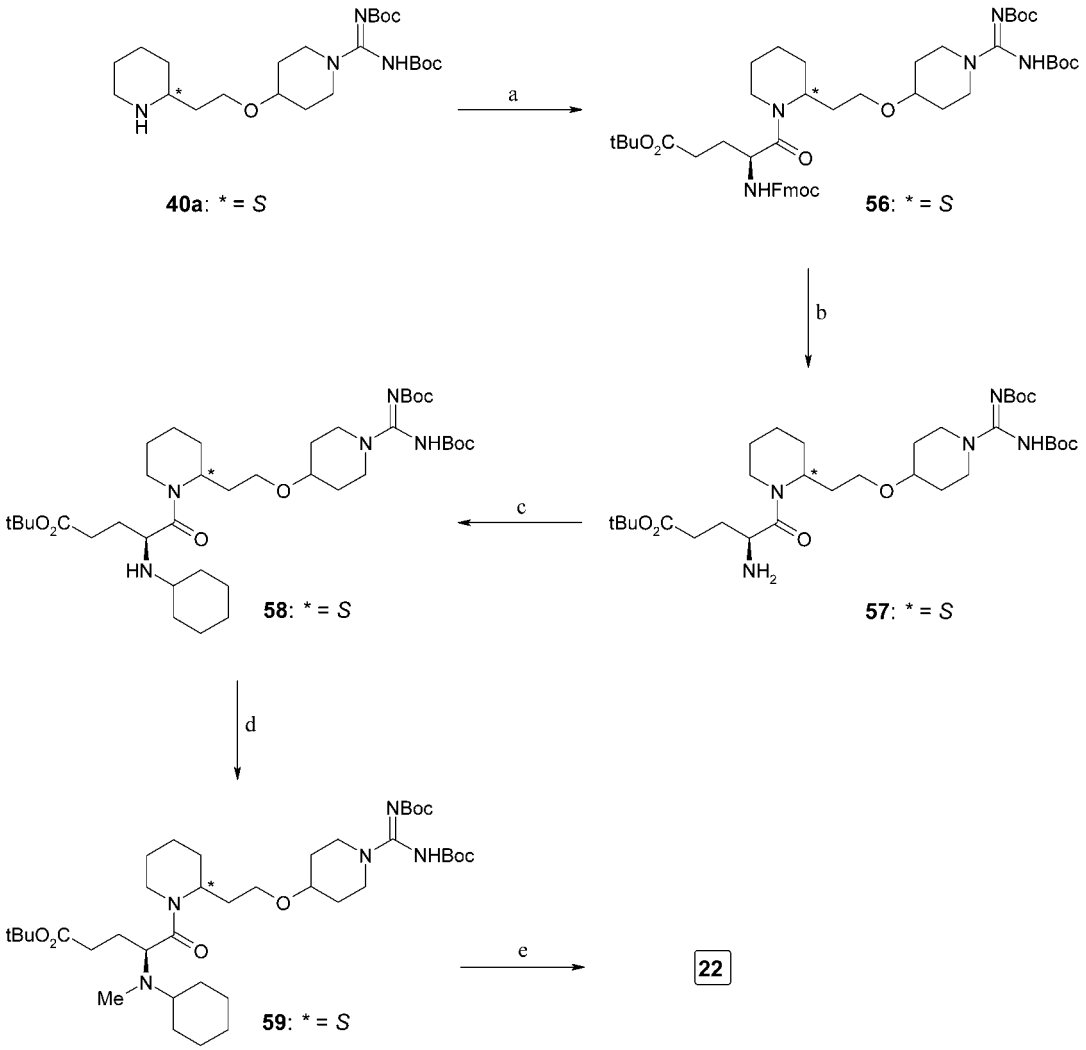
Experimental Section

Chemistry. The abbreviations used are as follows: Boc_2O, di-*tert*-butyl dicarbonate; CbzOSuc, *N*-(benzyloxycarbonyloxy)-succinimide; Cbz-D-Phe-OH, *N*-benzyloxycarbonyl-(*R*)-phenylalanine; DEAD, diethyl azodicarboxylate; DIPEA, diisopropylethylamine; DMAP, 4-(dimethylamino)pyridine; Fmoc-Asp(OtBu)-OH, *N*-α-fluorenylmethoxycarbonyl-(*S*)-aspartic acid *β*-*tert*-butyl ester; Fmoc-Glu(OtBu)-OH, *N*-α-fluorenylmethoxycarbonyl-(*S*)-glutamic acid *β*-*tert*-butyl ester; Fmoc-D-Phe-OH, *N*-α-fluorenylmethoxycarbonyl-(*R*)-phenylalanine; HOBt, *N*-hydroxybenzotriazole; MsCl, methanesulfonyl chloride; NMM, *N*-methylmorpholine; PyBroP, bromotris(pyrrolidino)phosphonium hexafluorophosphate; TEA, triethylamine; TFA, trifluoroacetic acid; Troc-D-Cha-OH, *N*-(2,2,2-trichloroethoxycarbonyl)-(*R*)-Cha; WSCDI, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; $0.88NH_3$, ammonia solution specific gravity 0.88.

Melting points were determined on a Buchi 510 apparatus and are uncorrected. ^1H nuclear magnetic resonance (NMR) spectroscopic data were recorded on a Varian Unity 300 or a Bruker AC-300 NMR instrument. The ^1H NMR spectra of advanced intermediates were somewhat complex due to the occurrence of rotamers, and in some cases diastereoisomers, but were thoroughly consistent with the assigned structure. Mass spectra were obtained with a Fisons Trio-1000 spectrometer using thermospray ionization. Column chromatography was accomplished on Kieselgel 60 (230−400 mesh) obtained from E. Merck, Darmstadt. Kieselgel 60 F_{254} plates, also from E. Merck, were used for thin-layer chromatography (TLC), and compounds were visualized with UV light and sprayed sequentially with iodoplatinate reagent (chloroplatinic acid, KI, and H_2O), Dragendorff's reagent (bismuth oxynitrate, $AcOH-H_2O$; KI, H_2O), and aqueous $NaNO_2$. Analar or high-performance liquid chromatography (HPLC) grade solvents were used, and evaporation was conducted on a rotary evaporator at 25−50 °C under vacuum.

***N*-*t*-Butyloxycarbonyl-2(*RS*)-(2-hydroxyethyl)piperidine (23).** A solution of Boc_2O (10.91 g, 50 mmol) in EtOAc

Scheme 6[a]



[a] Reagents: (a) Fmoc-L-Glu(OtBu)-OH, PyBroP, DIPEA, CH_2Cl_2; (b) piperidine, THF; (c) cyclohexanone, $NaBH(OAc)_3$, AcOH, THF; (d) 37% HCHO(aq), $NaBH(OAc)_3$, CH_2Cl_2; (e) HCl, CH_2Cl_2.

(15 mL) was added to a stirred solution of 2(*RS*)-(2- hydroxyethyl)piperidine (**34b**) (5.06 g, 50 mmol) in EtOAc (25 mL) at 0 °C, and the mixture was then warmed to 23 °C. After a further 1.5 h, the reaction mixture was evaporated under reduced pressure and the residue was purified by chromatography on silica gel using hexanes−Et_2O (1:1) as eluant to provide BOC-amine **23** (8.46 g, 74%) as a clear oil. R_f 0.25 (hexane:Et_2O, 1:1). ^{1}H NMR ($CDCl_3$): δ 1.30−1.85 (m, 8 H), 1.48 (s, 9 H), 1.92 (t, 1 H), 2.68 (t, 1 H), 3.16 (m, 1 H), 3.56−3.66 (m, 1 H), 3.94 (d, 1 H), 4.22 (s, 1 H).

***N*-*t*-Butoxycarbonyl-2(*RS*)-[2-(4-cyanophenoxy)ethyl]-piperidine (24).** DEAD (1.73 mL, 11 mmol) was added to a stirred, ice-cooled solution of alcohol **23** (2.29 g, 10 mmol), triphenylphosphine (2.88 g, 11 mmol), and 4-cyanophenol (1.31 g, 11 mmol) in tetrahydrofuran (THF; 75 mL), and then, the cooling bath was removed. After an additional 18 h, the reaction mixture was evaporated under reduced pressure and the residue was dissolved in ether. The resulting solution was washed with aqueous NaOH solution (1 M, ×2) and brine, dried (Na_2SO_4), and evaporated under reduced pressure to give crude product, which was purified by chromatography on silica gel, using hexanes−Et_2O (1:1) as eluant, to furnish the ether **24** (2.86 g, 87%) as a clear oil, which solidified on standing. R_f 0.30 (hexane:Et_2O, 1:1). ^{1}H NMR ($CDCl_3$): δ 1.20−1.80 (m, 6 H), 1.35 (s, 9 H), 1.78−1.94 (m, 1 H), 2.20−2.34 (m, 1 H), 2.80 (t, 1 H), 3.90−4.10 (m, 3 H), 4.44−4.56 (m, 1 H), 6.90 (d, 2 H), 7.58 (d, 2 H). Anal. ($C_{19}H_{26}N_2O_3$) C, H, N.

2(*RS*)-[2-(4-Cyanophenoxy)ethyl]piperidine (25). TFA (10 mL) was added to a stirred, ice-cooled solution of BOC-amine **24** (2.83 g, 8.56 mmol) and anisole (1.88 mL, 17.3 mmol) in dry CH_2Cl_2 (15 mL). After a further 1 h, the reaction mixture was evaporated under reduced pressure, the residue was dissolved in water, and the solution was washed with Et_2O, basified with aqueous NaOH (2 M), and extracted with Et_2O (×3). The combined extracts were washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure to afford amine **25** (1.43 g, 75%) as an oil, which solidified on standing. R_f 0.15 (CH_2Cl_2:MeOH:0.88NH_3, 93:7:1). ^{1}H NMR ($CDCl_3$): δ 1.06−1.28 (m, 1 H), 1.28−1.51 (m, 3 H), 1.51−1.76 (m, 2 H), 1.76−1.94 (m, 3 H), 2.56−2.80 (m, 2 H), 3.07 (d, 1 H), 4.02−4.10 (m, 2 H), 6.94 (d, 2 H), 7.58 (d, 2 H). LRMS: *m*/*z* 231.3 $(M + H)^+$.

***N*-(2,2,2-Trichloroethoxycarbonyl)-(*R*)-Cha.** Aqueous $NaHCO_3$ (82 mL, 1 M) was added to an ice cold stirred solution of H-D-Cha-OH (11.91 g, 41 mmol) dissolved in aqueous NaOH (41 mL, 1 M). The resulting thick suspension was stirred vigorously as a solution of *N*-(2,2,2-trichloroethoxycarbonyloxy)succinimide (7.02 g, 41 mmol) in dioxan (82 mL) was added. After 1 h, most of the dioxan was evaporated and the residue was extracted with EtOAc. The aqueous phase was then acidified with concentrated HCl and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). Evaporation gave an oil (13.79 g), which was dissolved in ether, and the solution was filtered through a short column of silica to give Troc-D-Cha-OH (12.3 g, 87%) as a white foam, which solidified on standing. R_f 0.20 (Et_2O:hexane, 6:4). ^{1}H NMR ($CDCl_3$) (85:15 distribution of rotamers): δ 0.96 (m, 2 H), 1.22

(m, 3 H), 1.45 (m, 1 H), 1.55−1.90 (m, 7 H), 4.48 (m, 1 H), 4.69 (d, 1 H), 4.82 (d, 1 H), 5.32 (d, 0.85 H), 5.58 (d, 0.15 H).

***N*-[*N*-(2,2,2-Trichloroethoxycarbonyl)-(*R*)-cyclohexylalanyl]-2(*S*)-[2-(4-cyanophenoxy)ethyl]piperidine (26) and *N*-[*N*-(2,2,2-Trichloroethoxycarbonyl)-(*R*)-cyclohexylalanyl]-2(*R*)-[2-(4-cyanophenoxy)ethyl]piperidine (27).** Oxalyl chloride (1.5 mL, 17.2 mmol) was added to a stirred solution of Troc-D-Cha-OH (1.49 g, 4.3 mmol) in CH_2Cl_2 (20 mL) followed by one drop of DMF. After 1.5 h, the solution was evaporated, azeotroped with CH_2Cl_2 (×2), and dried under high vacuum. The residual oil was dissolved in CH_2Cl_2 (10 mL) and stirred with ice-cooling, and the amine **25** (1.0 g, 4.3 mmol) was added dissolved in CH_2Cl_2 (10 mL), followed by DIPEA (1.5 mL, 8.6 mmol). After 1 h, the solvent was evaporated and the residue was partitioned between EtOAc and H_2O. The organic phase was washed with HCl (2 M), H_2O, saturated aqueous $NaHCO_3$, and brine. Drying over $MgSO_4$ and evaporation gave a foam (2.60 g). The above reaction was repeated on twice the scale, and the crude products were combined (7.55 g) and chromotographed on silica. Elution with increasing proportions of Et_2O−hexane (1:1 to 6:4) gave initially the *S*,*R* diastereoisomer **26** (3.04 g, 42%) as a foam. R_f 025 (Et_2O:hexane, 6:4). ^{1}H NMR ($CDCl_3$) (90:10 distribution of rotamers): δ 0.60−1.85 (mm, 18 H), 1.92 (m, 2 H), 2.75 (m, 1 H), 2.70 (t, 0.1 H), 3.22 (t, 0.9 H), 3.70 (d, 0.9 H), 3.98 (m, 2.1 H), 4.44 (m, 0.1 H), 4.57 (d, 2 H), 4.68 (m, 1H), 4.79 (m, 1.1 H), 4.97 (m, 0.9 H), 5.63 (d, 0.1 H), 5.71 (d, 0.9 H). LRMS: *m*/*z* 559 $(M + H)^+$. Anal. ($C_{26}H_{34}Cl_3N_3O_4$) C, H, N. Continued elution gave the *R*,*R* diastereoisomer **27** (3.17 g, 44%) as a foam. R_f 0.20 (Et_2O:hexane, 6:4). ^{1}H NMR ($CDCl_3$) (1:1 distribution of rotamers): δ 0.72−1.85 (mm, 8 H), 2.00 (m, 2 H), 2.28 (m, 1 H), 2.70 (t, 0.5 H), 3.17 (t, 0.5 H), 3.68 (d, 0.5 H), 3.88−4.23 (mm, 2.5 H), 4.47 (d, 0.5 H), 4.53 (dd, 0.5 H), 4.62 (d, 0.5 H), 4.79 (m, 2 H), 5.00 (m, 0.5 H), 5.77 (t, 1 H), 6.89 (d, 1 H), 7.57 (d, 2 H). LRMS: *m*/*z* 559 $(M + H)^+$. Anal. ($C_{26}H_{34}Cl_3N_3O_4$) C, H, N.

***N*-[(*R*)-Cyclohexylalanyl]-2(*S*)-[2-(4-cyanophenoxy)-ethyl]piperidine (28).** This compound was prepared from **26** in a similar manner as **29**. Amine **28** (71%) was obtained as a gum. R_f 0.50 (CH_2Cl_2:MeOH:0.88NH_3, 93:7:1). ^{1}H NMR ($CDCl_3$) (80:20 distribution of rotamers): δ 0.63−2.07 (mm, 22 H), 2.23 (m, 1 H), 2.68 (t, 0.2H), 316 (t, 0.8 H), 3.70 (m, 1.8 H), 4.03 (m, 2 H), 4.37 (m, 0.2 H), 4.58 (d, 0.2 H), 4.98 (m, 0.8 H), 6.91 (d, 2 H), 7.56 (d, 2 H). LRMS: *m*/*z* 384 $(M + H)^+$, 767 $(2M + H)^+$.

***N*-[(*R*)-Cyclohexylalanyl]-2(*R*)-[2-(4-cyanophenoxy)-ethyl]piperidine (29).** Zn dust (18 g, 0.28 mol) was added to a stirred solution of Troc-amine **27** (3.1 g, 55 mmol) in THF (56 mL) followed by KH_2PO_4 (18 mL, 1 M). After 1.5 h, the mixture was filtered and evaporated to a small volume. The pH was lowered to 2 with HCl (2 M), H_2O was added, and the mixture was extracted with EtOAc (100 mL). The aqueous phase was separated, and the organic phase was extracted with H_2O (×8). The combined aqueous extracts were basified to pH 11 with NaOH (2 M) and extracted with CH_2Cl_2. The extract was washed with brine and dried over Na_2SO_4 to give amine **29** (1.63 g, 77%) as a gum. R_f 0.43 (CH_2Cl_2:MeOH: 0.88NH_3, 93:7:1). ^{1}H NMR ($CDCl_3$) (1:1 distribution of rotamers): δ 0.7−2.08 (mm, 22 H), 2.29 (m, 1 H), 2.65 (t, 0.5 H), 3.13 (t, 0.5 H), 3.65 (d, 0.5 H), 3.80 (m, 1 H), 4.00 (m, 2 H), 4.23 (m, 0.5 H), 4.60 (d, 0.5 H), 5.03 (m, 0.5 H), 6.89 (d, 1 H), 6.95 (d, 1 H), 7.57 (t, 2 H). LRMS: *m*/*z* 384 $(M + H)^+$.

***N*-[*N*-(Ethoxycarbonylmethyl)-(*R*)-cyclohexylalanyl]-2(*S*)-[2-(4-cyanophenoxy)ethyl]piperidine (30).** K_2CO_3 (1.1 g, 8 mmol) was added to a solution of amine **28** (1.53 g, 4 mmol) in CH_3CN (20 mL), followed by ethyl bromoacetate (0.49 mL, 4.4 mmol). The suspension was stirred at 23 °C for 19 h, and most of the solvent was evaporated under vacuum. The residue was partitioned between EtOAc and H_2O, and the organic phase was washed with brine and dried over Na_2SO_4. Evaporation gave a gum that was chromotographed on silica, with EtOAc−hexane as eluant (6:4), giving ester **30** (1.79 g, 95%) as a gum. R_f 0.40 (EtOAc:hexane, 6:4). ^{1}H NMR ($CDCl_3$) (85: 15 distribution of rotamers): δ 0.62−1.05 (m, 2 H), 1.05−2.0 (m, 21 H), 2.24 (m, 2 H), 2.68 (t, 0.15 H), 3.12 (m, 2.7 H), 3.38 (d, 0.15 H), 3.59 (dd, 0.85 H), 3.72 (m, 1.14 H), 4.05 (m, 3.85 H), 4.37 (m, 0.15 H), 4.60 (d, 0.15 H), 5.04 (m, 0.85 H), 6.92 (d, 2 H), 7.55 (dd, 2 H). LRMS: *m*/*z* 470 $(M + H)^+$.

***N*-[*N*-(Ethoxycarbonylmethyl)-(*R*)-cyclohexylalanyl]-2(*R*)-[2-(4-cyanophenoxy)ethyl]piperidine (31).** This compound was prepared from **29** in a manner similar to **30**. Ester **31** (94%) was obtained a a gum. R_f 0.28 (EtOAc:hexane, 6:4). ^{1}H NMR ($CDCl_3$) (1:1 distribution of rotamers): δ 2.83 (m, 2 H), 1.05−2.11 (mm, 21 H), 2.22 (m, 2 H), 2.63 (t, 0.5 H), 2.74 (d, 0.5 H), 3.05 (d, 0.5 H), 3.11 (t, 0.5 H), 3.20 (d, 0.5 H), 3.36 (d, 0.5 H), 3.62 (m, 1.5 H), 3.85−4.25 (mm, 4.5 H), 4.60 (dd, 0.5 H), 5.04 (q, 0.5 H), 6.90 (d, 1 H), 6.94 (d, 1 H), 7.56 (d, 1 H), 7.60 (d, 1 H). LRMS: *m*/*z* 470 $(M + H)^+$.

***N*-[*N*-(Ethoxycarbonylmethyl)-(*R*)-cyclohexylalanyl]-2(*S*)-[2-(4-amidinophenoxy)ethyl]piperidine Dihydrochloride (32).** An ice cold solution of nitrile **30** (780 mg, 1.66 mmol) in absolute EtOH (10 mL, dried over 3 Å molecular sieves) was saturated with HCl gas and left at 0 °C for 18 h. The solvent was evaporated under vacuum, and the residue was azeotroped with EtOH to give the intermediate ethyl imidate, R_f 0.6 (CH_2Cl_2:MeOH:0.88NH_3, 93:7:1). A solution of NH_3 in EtOH (3.4 mL, 1.96 M, 6.6 mmol) was added to the foregoing imidate, and the resulting suspension was heated at 50 °C for 2 h. A second portion of ethanolic ammonia (2 mL, 3.9 mM) was added, and heating was continued for a further 1 h. The solvent was evaporated, and the residue was partitioned between ether and H_2O. The aqueous phase was then basified with NaOH (1 M) and extracted with CH_2Cl_2. The extract was washed with brine, dried over Na_2SO_4, and filtered. The filtrate was acidified with etherial HCl and evaporated to dryness to give amidine **32** (804 mg, 79%) as a white powder. R_f 0.33 (CH_2Cl_2:MeOH:0.88NH_3, 80:20:5). ^{1}H NMR (DMSO-d_6) (90:10 distribution of rotamers): δ 0.84 (m, 2 H), 1.00 (mm, 20 H), 1.93 (m, 1 H), 2.22 (m, 1 H), 2.79 (t, 0.1 H), 3.20 (m, 0.9 H), 3.29−3.91 (mm, 5 H), 4.11 (m, 4 H), 4.30 (d, 0.1 H), 4.48 (m, 0.9 H), 4.60 (m, 0.1 H), 4.79 (m, 0.9 H), 7.08 (d, 2 H), 7.88 (d, 2 H), 9.15 (s, 2 H), 9.30 (s, 2 H). LRMS: *m*/*z* 487 $(M + H)^+$, 470 $(M + H - NH_3)^+$. Anal. ($C_{27}H_{42}N_4O_4$·2HCl·3.2H_2O) C, N; H: calcd, 8.23; found, 7.78.

***N*-[*N*-(Ethoxycarbonylmethyl)-(*R*)-cyclohexylalanyl]-2(*R*)-[2-(4-amidinophenoxy)ethyl]piperidine Dihydrochloride (33).** This compound was prepared from **31** in a manner similar to **32**. Amidine **33** (83%) was obtained as a white powder. R_f 0.35 (CH_2Cl_2:MeOH:0.88NH_3, 80:20:5). ^{1}H NMR (DMSO-d_6) (6:4 distribution of rotamers): δ 0.83 (m, 2 H), 1.00−2.29 (mm, 22 H), 2.76 (t, 0.4 H), 3.22 (t, 0.6 H), 2.28−4.25 (mm, 9 H), 4.30 (br s, 0.4 H), 4.34 (br s, 0.4 H), 4.55 (br s, 0.6 H), 4.90 (br s, 0.6 H), 7.05 (d, 1.2 H), 7.17 (d, 0.8 H), 7.87 (t, 2 H), 9.05 (s, 2 H), 9.27 (d, 2 H). LRMS: *m*/*z* 487 $(M + H)^+$, 470 $(M + H - NH_3)^+$. Anal. ($C_{27}H_{42}N_4O_4$·2HCl·1.0H_2O·0.1-CH_2Cl_2) C, H, N.

2(*S*)-(2-Hydroxyethyl)piperidine (34a). 2(*RS*)-(2-Hydroxyethyl)piperidine (**34b**) (110 g) was resolved using (1*S*)-(+)-10-camphorsulfonic acid as described[31] via the intermediate (*S*,*S*)-10-camphor-sulfonate salt, mp 167 °C (Literature[31] 166−167 °C); $[\alpha]_D$ 25 + 32.5° (*c* = 2.2, $CHCl_3$) (Literature[32] $[\alpha]_D$ + 32.4° (*c* = 2, $CHCl_3$)). The absolute configuration of the salt was confirmed by single-crystal X-ray crystallographic analysis. The amine **34a** (13.56 g) was obtained as fine needles, mp 69−70 °C (Literature[31] 68−69 °C), R_f 0.25 (isobutyl methyl ketone: AcOH:H_2O, 2:1:1, upper phase). GC analysis of the bis-TFA derivative, employing a Chiraldex B-TA No C70 column, showed an enantiomeric excess (ee) of >98%.

***N*-Benzyloxycarbonyl-2(*S*)-(2-hydroxyethyl)piperidine (35a).** To a stirred, ice-cooled solution of amine **34a** (13.4 g, 0.104 mol) in CH_2Cl_2 (250 mL) were added, sequentially, Et_3N (15.9 mL, 0.114 mol) and CbzOSuc (27.21 g, 0.109 mol). The cooling bath was removed, and the reaction mixture was stirred at 23 °C for 18 h, washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure to give an oil (30.6 g), which was purified by chromatography on silica gel, using hexanes−EtOAc (1:1) as eluant, to provide carbamate **35a** (27.5 g, 100%) as an oil. R_f 0.48 (hexane:EtOAc, 1:1);

$[\alpha]_D25-24.5°$ (c = 1.06, MeOH). ^{1}H NMR ($CDCl_3$): δ 1.50−1.86 (m, 8 H), 1.86−2.05 (m, 1 H), 2.70−2.85 (m, 1 H), 3.14−3.50 (br s, 2 H), 3.50−3.68 (m, 1 H), 4.06(d, 1 H), 4.40−4.58 (m, 1 H), 5.16 (s, 2 H), 7.20−7.43 (m, 5 H).

***N*-Benzyloxycarbonyl-2(*RS*)-(2-hydroxyethyl)piperidine (35b).** Protection of 2(*RS*)-(2-hydroxyethyl)piperidine (**34b**) (25.0 g, 193 mmol) with CbzOSuc (48.1 g, 193 mmol) and Et_3N (29.6 mL, 212 mmol) by the procedure described for **35a** gave Cbz-amine **35b** (22.0 g, 43%) as a colorless oil. R_f 0.48 (hexane:EtOAc, 1:1). ^{1}H NMR ($CDCl_3$): consistent with that of **35a**. LRMS: m/z 264.4 $(M + H)^+$. Anal. ($C_{15}H_{21}NO_3$) C, H, N.

***N*-Benzyloxycarbonyl-2(*S*)-(2-methanesulfonyloxyethyl)piperidine (36a).** MsCl (16.1 mL, 0.208 mol) was added dropwise over 15 min to a stirred, ice-cooled solution of alcohol **35a** (27.43 g, 0.104 mol) and Et_3N (29 mL, 0.208 mol) in CH_2Cl_2 (350 mL); the temperature of the reaction mixture was allowed to rise to 17 °C during the addition. After an additional 25 min, the reaction mixture was washed with aqueous citric acid solution (1 M), water, and saturated aqueous $NaHCO_3$ solution, dried (Na_2SO_4), and evaporated under reduced pressure to give a light yellow oil (40 g), which was purified by chromatography on silica gel, using hexanes−EtOAc (1:1) as eluant, to furnish mesylate **36a** (33.5 g, 93%) as a clear oil, which solidified on standing. R_f 0.59 (hexane:EtOAc, 1:1). ^{1}H NMR ($CDCl_3$): δ 1.34−1.86 (m, 6 H), 1.86−1.95 (m, 1 H), 2.16−2.32 (m, 1 H), 2.74−2.95 (m, 4 H), 4.01−4.18 (m, 1 H), 4.06(t, 2 H), 4.41−4.56 (m, 1 H), 5.10 (s, 2 H), 7.22−7.42 (m, 5 H). A trace of EtOAc was removed from the product azeotropically, using hexane (×2), before the next step of the reaction sequence.

***N*-Benzyloxycarbonyl-2(*RS*)-(2-methanesulfonyloxyethyl)piperidine (36b).** Mesylation of alcohol **35b** (21.3 g, 80.9 mmol) with MsCl (12.5 mL, 0.162 mol) and Et_3N (22.6 mL, 0.162 mol) by the procedure described for **36a** gave **36b** (23.24 g, 84%) as a colorless oil. R_f 0.5 (hexane:EtOAc, 1:1). ^{1}H NMR ($CDCl_3$): consistent with that of **36a**. Anal. ($C_{16}H_{23}NO_5S\cdot0.15CH_2Cl_2$) C, H, N.

***N*-*t*-Butoxycarbonyl-4-hydroxypiperidine.** Boc_2O (35.58 g, 0.163 mol) was added to a stirred, ice-cooled solution of 4-hydroxypiperidine (15.0 g, 0.148 mol) in CH_2Cl_2 (250 mL). The cooling bath was removed, and then, the reaction mixture was stirred at 23 °C for 56 h, washed with aqueous citric acid solution (1 M), dried ($MgSO_4$), and evaporated under reduced pressure to give a yellowish oil; treatment of which with hexane (20 mL), followed by chilling, promoted crystallization. Filtration and washing of the product with cold hexane afforded *N*-*t*-butoxycarbonyl-4-hydroxypiperidine (25.72 g, 86%). R_f 0.37 (hexane:EtOAc, 1:1). ^{1}H NMR ($CDCl_3$): δ 1.28−1.56 (m, 2 H), 1.42 (s, 9 H), 1.72−1.83 (m, 2 H), 2.25 (br s, 1 H), 2.92−3.08 (m, 2 H), 3.74−3.88 (m, 3 H). Anal. ($C_{10}H_{19}NO_3$) H, N; C: calcd, 59.68; found, 59.27.

***N*-Benzyloxycarbonyl-2(*S*)-[2-(*N*-*t*-butoxycarbonyl-4-piperidyloxy)ethyl]piperidine (37a).** *N*-*t*-Butoxycarbonyl-4-hydroxypiperidine (19.67 g, 97.5 mmol) was added to a stirred suspension of NaH (3.9 g, 60% dispersion in oil, 97.5 mmol) in dry DMF (150 mL) under N_2. After 2 h, a solution of mesylate **36a** (32.9 g, 96.4 mmol) in dry DMF (100 mL) was added and the resulting reaction mixture was stirred for 18 h. The bulk of the solvent was removed under reduced pressure, the residue was diluted with water, and the resulting oily suspension was extracted with EtOAc (×3). The combined extracts were washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure to give an oil, which was purified by chromatography on silica gel, using hexanes−EtOAc (7:3) as eluant, to afford ether **37a** (26.26 g, 61%) as an oil. R_f 0.35 (hexane:EtOAc, 7:3); $[\alpha]_D25-12.3°$ (c = 1.01, MeOH). ^{1}H NMR ($CDCl_3$): δ 1.30−1.80 (m, 11 H), 1.39 (s, 9 H), 1.94−2.12 (m, 1 H), 2.86 (t, 1 H), 2.94−3.10 (m, 2 H), 3.20−3.45 (m, 3 H), 3.60−3.77 (m, 2 H), 4.07 (d, 1 H), 4.43 (br s, 1 H), 5.10 (s, 2 H), 7.23−7.40 (m, 5 H). Anal. ($C_{25}H_{38}N_2O_5$) C, H, N.

***N*-Benzyloxycarbonyl-2(*RS*)-[2-(*N*-*t*-butoxycarbonyl-4-piperidyloxy)ethyl]piperidine (37b).** Reaction of the mesylate **36b** (3.41 g, 10.0 mmol) with the sodium alkoxide (12.0 mmol) derived from *N*-*t*-butoxycarbonyl-4-hydroxypiperidine (2.42 g, 12.0 mmol) and NaH (0.48 g, 60% dispersion in oil, 12.0 mmol) by the procedure described for **37a** gave **37b** (2.62 g, 59%) as a colorless oil. R_f 0.30 (hexane:EtOAc, 7:3). ^{1}H NMR ($CDCl_3$): consistent with that of **37a**.

***N*-Benzyloxycarbonyl-2(*S*)-[2-(4-piperidyloxy)ethyl]-piperidine hydrochloride (38a).** A stirred, ice-cooled solution of BOC-amine **37a** (26.16 g, 58.6 mmol) in CH_2Cl_2 (300 mL) was saturated with hydrogen chloride gas. After a further 1.25 h, the solvent was removed by evaporation under reduced pressure and residual HCl was removed azeotropically using CH_2Cl_2 (×3) to give amine hydrochloride **38a** (22.59 g, 100%) as a white foam. R_f 0.60 (CH_2Cl_2:MeOH:0.88NH_3, 85:15:2). ^{1}H NMR ($CDCl_3$): δ 1.28−2.15 (m, 12 H), 2.85 (t, 1 H), 2.95−3.54 (m, 7 H), 4.06 (d, 1 H), 4.43 (br s, 1 H), 5.10 (dd, 2 H), 7.20−7.40 (m, 5 H), 9.41 (br s, 2 H). LRMS: m/z 347.3 $(M + H)^+$.

***N*-Benzyloxycarbonyl-2(*RS*)-[2-(4-piperidyloxy)ethyl]-piperidine Hydrochloride (38b).** HCl gas deprotection of BOC-amine **37b** (15.27 g, 34.2 mmol) by the procedure described for **38a** gave **38b** (13.4 g, 100%) as a white foam. R_f 0.50 (CH_2Cl_2:MeOH:0.88NH_3, 84:14:2). ^{1}H NMR ($CDCl_3$): consistent with that of **38a**. LRMS: m/z 347.0 $(M + H)^+$. Anal. ($C_{20}H_{30}N_2O_3\cdot$HCl) C, H, N.

***N*-Benzyloxycarbonyl-2(*S*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxycarbonylamidino)-4-piperidyloxy]ethyl}piperidine (39a).** Et_3N (24.5 mL, 0.176 mol) was added to a stirred, ice-cooled solution of amine hydrochloride **38a** (22.59 g, 58.6 mmol) in CH_2Cl_2 (280 mL), the mixture was allowed to warm to 23 °C, and then, *N*,*N*′-di-*t*-butoxycarbonyl-*S*-methylisothiourea[29] (18.7 g, 64.4 mmol) and $HgCl_2$ (15.91 g, 58.6 mmol) were added sequentially. The reaction mixture was stirred for 18 h, for an additional 2 h under reflux, and then filtered through Celite. The filtrate was washed with water, (further filtration of this two phase mixture was necessary to remove precipitated material), dried (Na_2SO_4), and evaporated under reduced pressure to give crude product, which was purified by chromatography on silica gel, using an elution gradient of hexanes−EtOAc (7:3 to 1:1), to provide guanidine **39a** (30.37 g, 88%) as a sticky foam. R_f 0.20 (hexane:EtOAc, 1:1); $[\alpha]_D25-5.7°$ (c = 1.1, MeOH). ^{1}H NMR ($CDCl_3$): δ 1.30−1.87 (m, 11 H), 1.45 (s, 18 H), 1.92−2.09 (m, 1 H), 2.85 (t, 1 H), 3.23−3.45 (m, 5 H), 3.68 (br s, 2 H), 4.06 (d, 1 H), 4.42 (br s, 1 H), 5.11 (dd, 2 H), 7.25−7.38 (m, 5 H), 10.09 (s, 1 H). LRMS: m/z 589.4 $(M + H)^+$. Anal. ($C_{31}H_{48}N_4O_7\cdot$0.15EtOAc) C, H, N.

***N*-Benzyloxycarbonyl-2(*RS*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxycarbonylamidino)-4-piperidyloxy]ethyl}piperidine (39b).** Guanidinylation of **38b** (13.09 g, 34.2 mmol) with *N*,*N*′-di-*t*-butoxycarbonyl-*S*-methylisothiourea (10.86 g, 37.4 mmol), $HgCl_2$ (9.29 g, 34.2 mmol), and Et_3N (14.3 mL, 0.103 mol) by the procedure described for **39a** gave **39b** (15.7 g, 78%) as a sticky foam. R_f 0.35 (hexane:EtOAc, 6:4). ^{1}H NMR ($CDCl_3$): consistent with that of **39a**. LRMS: m/z 589.4 $(M + H)^+$.

2(*S*)-{2-[*N*-(*N*,*N*′-Di-*t*-butoxycarbonylamidino)-4-piperidyloxy]ethyl}piperidine (40a). A solution of Cbz-amine **39a** (26.82 g, 45.55 mmol) in absolute EtOH (400 mL) was hydrogenated (60 psi) over 10% palladium on charcoal (5.0 g) at 23 °C for 2.5 h. The resulting mixture was filtered through a pad of Arbocel, and the filtrate was evaporated under reduced pressure, azeotroping with EtOAc (×2), to furnish amine **40a** (20.36 g, 94%) as a gum. R_f 0.13 (CH_2Cl_2:MeOH:0.88NH_3, 93:7:1); $[\alpha]_D25-1.69°$ (c = 1.3, MeOH). ^{1}H NMR ($CDCl_3$): δ 1.00−1.81 (m, 11 H), 1.44 (s, 18 H), 1.81−1.94 (m, 2 H), 2.52−2.66 (m, 2 H), 3.05 (d, 1 H), 3.39 (br s, 1 H), 3.44−3.57 (m, 3 H), 3.72 (br s, 1 H), 9.8 (vbr s, 1 H). LRMS: m/z 455 $(M + H)^+$. Anal. ($C_{23}H_{42}N_4O_5\cdot$0.15EtOAc) C, H, N.

2(*RS*)-{2-[*N*-(*N*,*N*′-Di-*t*-butoxycarbonylamidino)-4-piperidyloxy]ethyl}piperidine (40b). Hydrogenation of amine **39b** (15.68 g, 26.6 mmol) by the procedure described for **40a** gave **40b** (12.0 g, 99%) as a gum. R_f 0.22 (CH_2Cl_2:MeOH:0.88NH_3, 93:7:1). ^{1}H NMR ($CDCl_3$): consistent with that of **40a**.

2(*RS*)-[2-(*N*-*t*-Butoxycarbonyl-4-piperidyloxy)ethyl]-piperidine (41). To a stirred solution of Cbz-amine **37b** (5.39 g, 12.1 mmol) in absolute EtOH (300 mL) was added 10% palladium on charcoal (5.4 g), followed by 1,4-cyclohexadiene (11.4 mL, 121.1 mmol). The reaction mixture was stirred for 1 h under an atmosphere of N_2 and then filtered through a pad of Arbocel. The filtrate was evaporated under reduced pressure to give crude product, which was purified by chromatography on silica gel, using CH_2Cl_2−MeOH−0.88NH_3 (90:10:1) as eluent, to give piperidine **41** (2.75 g, 73%) as a brown oil. R_f 0.63 (CH_2Cl_2:MeOH:0.88NH_3, 90:10:1). ^{1}H NMR ($CDCl_3$): δ 1.00−1.86 (m, 20 H), 2.05 (br s, 1 H), 2.58−2.65 (m, 2 H), 3.00−3.13 (m, 3 H), 3.38−3.45 (m, 1 H), 3.45−3.58 (t, 2 H), 3.62−3.80 (m, 2 H). LRMS: *m*/*z* 313.3 $(M + H)^+$. Anal. ($C_{17}H_{32}N_2O_3 \cdot 0.1CH_2Cl_2$) C, N; H: calcd, 10.11; found, 10.76.

***N*-[*N*-(2,2,2-Trichloroethoxycarbonyl)-(*R*)-cyclohexylalanyl]-2(*RS*)-[2-(*N*-*t*-butoxy-carbonyl-4-piperidinyloxy)ethyl]piperidine (42).** Reaction of amine **41** (1.85 g, 5.91 mmol) with the acid chloride prepared from Troc-D-Cha-OH (2.05 g, 5.91 mmol) and DIPEA (2.06 mL, 11.82 mmol) by the procedure described for **26** furnished amide **42** (3.29 g, 87%) as a yellow gum. R_f 0.73 (CH_2Cl_2:MeOH:0.88NH_3, 90:10:1). ^{1}H NMR ($CDCl_3$): δ 0.80−2.18 (m, 34 H), 2.62−3.25 (m, 3 H), 3.30−3.57 (m, 3 H), 3.60−3.83 (m, 2 H), 4.45−4.88 (m, 5 H), 5.68−5.92 (m, 1 H). LRMS: *m*/*z* 644.1 $(M + H)^+$. Anal. ($C_{29}H_{48}N_3O_6Cl_3 \cdot 0.4CH_2Cl_2$) C, H, N.

***N*-[*N*-(2,2,2-Trichloroethoxycarbonyl)-(*R*)-cyclohexylalanyl]-2(*RS*)-[2-(4-piperidinyl-oxy)ethyl]piperidine Hydrochloride (43).** HCl gas deprotection of BOC-amine **42** (3.23 g, 5.04 mmol) by the procedure described for **38a** gave **43** (3.00 g, 100%) as a yellow foam. R_f 0.83 (CH_2Cl_2:MeOH:0.88NH_3, 80:20:5). ^{1}H NMR ($CDCl_3$): δ 0.80−2.19 (m, 25 H), 2.58−3.68 (m, 8 H), 4.42−4.90 (m, 5 H), 5.81−5.88 (m, 1 H), 9.20−9.68 (br s, 2 H). LRMS: *m*/*z* 540.1 $(M + H)^+$. Anal. ($C_{24}H_{40}N_3O_4Cl_3 \cdot HCl \cdot 0.4CH_2Cl_2$) C, H, N.

***N*-[*N*-(2,2,2-Trichloroethoxycarbonyl)-(*R*)-cyclohexylalanyl]-2(*RS*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxycarbonylamidino)-4-piperidyloxy]ethyl}piperidine (44).** Guanidinylation of **43** (2.97 g, 5.15 mmol) with *N*,*N*′-di-*t*-butoxycarbonyl-*S*-methylisothiourea (1.49 g, 5.15 mmol), $HgCl_2$ (3.53 g, 10.29 mmol), and Et_3N (2.15 mL, 15.44 mmol) by the procedure described for **39a** gave **44** (3.76 g, 83%) as a beige foam. R_f 0.63 (CH_2Cl_2:MeOH, 95:5). ^{1}H NMR ($CDCl_3$): δ 0.79−2.18 (m, 43 H), 2.60−3.85 (m, 8 H), 4.42−4.90 (m, 5 H), 5.82−5.92 (m,1 H), 10.02−10.19 (br s, 1 H). Anal. ($C_{35}H_{58}N_5O_8Cl_3 \cdot 1.1CH_2Cl_2$) C, H, N.

***N*-[(*R*)-Cyclohexylalanyl]-2(*RS*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxycarbonylamidino)-4-piperidyloxy]ethyl}piperidine (45).** Zn dust (1.25 g, 19.02 mmol) was added to a stirred solution of Troc-amine **44** (745 mg, 0,95 mmol) in AcOH (35 mL), and the resulting heterogeneous solution was stirred at room temperature for 4 h. The reaction mixture was then neutralized by the cautious addition of saturated aqueous $NaHCO_3$ solution. The resulting aqueous solution was extracted with ethyl acetate (×1), the organic extract was washed with brine and dried ($MgSO_4$), and the solvent was evaporated under reduced pressure to give the crude product. Purification by chromatography on silica gel, using an elution gradient of CH_2Cl_2:MeOH (100:0 to 95:5), gave amine **45** (367 mg, 63%). R_f 0.52 (CH_2Cl_2:MeOH:0.88NH_3, 90:10:1). ^{1}H NMR ($CDCl_3$): δ 0.75−2.13 (m, 43 H), 3.05−3.65 (m, 5 H), 3.66−3.88 (m, 2 H), 3.90−4.33 (m, 2 H), 4.46−4.90 (m, 2 H). LRMS: *m*/*z* 608.7 $(M + H)^+$.

***N*-[*N*-(*t*-Butoxycarbonylmethyl)-(*R*)-cyclohexylalanyl]-2(*RS*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxycarbonylamidino)-4-piperidyloxy]ethyl}piperidine (46).** *tert*-Butyl bromoacetate (94 μL, 0.64 mmol) was added to a stirred suspension of K_2CO_3 (0.16 g, 1.16 mmol) in a solution of amine **45** (352 mg, 0.58 mmol) in MeCN (5.0 mL). The reaction mixture was stirred at room temperature under N_2 for 30 h. The solvent was then evaporated under reduced pressure, and the resultant semisolid was partitioned between water and EtOAc. The organic layer was separated, washed with brine, and dried ($MgSO_4$), and the solvent was evaporated under reduced pressure to give the crude product. Purification by chromatography on silica gel, using an elution gradient of CH_2Cl_2−MeOH (100:0 to 95:5), gave *tert*-butyl ester **46** (299 mg, 72%) as a white foam. R_f 0.55 (CH_2Cl_2:MeOH:0.88NH_3, 90:10:1). ^{1}H NMR ($CDCl_3$): δ 0.73−1.02 (m, 2 H), 1.05−2.12 (m, 50 H), 3.00−3.86 (m, 12 H), 4.08−4.23 (m, 1 H), 4.51−4.62 (m, 1 H), 4.85−4.96 (m, 1 H), 10.10−10.20 (m, 1H). Anal. ($C_{38}H_{67}N_5O_8$) C, H, N.

***N*-[*N*-(α-Fluorenylmethoxycarbonyl)-(*R*)-phenylalanyl]-2(*RS*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxy-carbonylamidino)-4-piperidyloxy]ethyl}piperidine (47).** DIPEA (0.53 mL, 3 mmol) was added to an ice cold stirred solution of amine **40b** (681 mg, 1.5 mmol), Fmoc-D-Phe-OH (620 mg, 1.6 mmol), and PyBroP (769 mg, 1.65 mmol) in CH_2Cl_2 (5 mL). After 2 h, a second portion of PyBroP (100 mg, 0.21 mmol) was added and the reaction was stirred for a further 2 h. The solvent was evaporated, and the residue was partitioned between EtOAc and H_2O. The organic phase was washed with citric acid (1 M), H_2O, saturated aqueous $NaHCO_3$, and brine and then dried over $MgSO_4$. Evaporation gave a foam, which was purified by chromotography on silica, using EtOAc−hexane (1:1) as eluant, to give amide **47** (790 mg, 64%) as a white foam. R_f 0.3 (EtOAc:hexane, 1:1). ^{1}H NMR ($CDCl_3$): δ 1.30−1.96 (mm, ∼30 H), 2,62 (m, 0.75 H), 3.00 (m, 2 H), 3.36 (m, 5 H), 3.71 (m, 2.25 H), 3.95 (m, 0.25 H), 4.11−4.57 (mm, 3.75 H), 4.88 (m, 1.25 H), 5.71 (m, 0.75 H), 7.14−7.44 (mm, ∼10 H), 7.57 (d, 2 H), 7.77 (d, 2 H), 10.11 (d, 1 H). LRMS: *m*/*z* 602 (M + H − Fmoc)$^+$. Anal. ($C_{47}H_{61}N_5O_8$) C, H, N.

***N*-[(*R*)-Phenylalanyl]-2(*RS*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxycarbonylamidino)-4-piperidyloxy]ethyl}piperidine (48).** Piperidine (0.85 mL, 9 mmol) was added to a solution of Fmoc-amine **47** (750 mg, 0.91 mmol) in THF (4 mL). After 35 min, the solvent was evaporated and the residue was purified by chromatography on silica, using CH_2Cl_2−MeOH−0.88NH_3 (193:7:1) as eluant, to give amine **48** (480 mg, 88%) as a white foam. R_f 0.5 (CH_2Cl_2:MeOH:0.88NH_3, 93:7:1). ^{1}H NMR ($CDCl_3$): δ 0.78 (m, 0.25 H), 0.96 (m, 0.25 H), 1.32−1.78 (m, ∼29 H), 1.86 (m, 3 H), 2.45−3.17 (mm, 3 H), 3.17−3.87 (mm, ∼7.5 H), 3.73 (t, 0.6 H), 4.08 (m, 0.6 H), 4.29 (m, 0.15 H), 4.55 (m, 0.5 H), 4.78 (m, 0.15 H), 4.86 (m, 0.5 H), 5.03 (m, 0.15 H), 7.24 (m, 5 H), 10.12 (m, 1 H). LRMS: *m*/*z* 602 $(M + H)^+$.

***N*-[*N*-(*t*-Butoxycarbonylmethyl)-(*R*)-phenylalanyl]-2(*RS*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxy-carbonylamidino)-4-piperidyloxy]ethyl}piperidine (49).** *tert*-Butyl bromoacetate (126 μL, 0.86 mmol) was added to a stirred suspension of K_2CO_3 (215 mg, 1.56 mmol) and amine **48** (470 mg, 0.78 mmol) in CH_3CN (5 mL). After 14 h at 23 °C, the solvent was evaporated and the residue was partitioned between EtOAc and H_2O. The organic phase was washed with brine and dried over $MgSO_4$. Purification by chromatography on silica, using EtOAc−hexane (7:3) as eluant, gave ester **49** (480 mg, 86%) as a white foam. R_f 0.28 (EtOAc:hexane, 7:3). ^{1}H NMR ($CDCl_3$): δ 0.24 (m, 0.32 H), 0.51 (m, 0.28 H), 1.03−1.97 (mm, ∼38 H), 2.33 (br s, 1 H), 2.52 (q, 0.6 H), 2.73−3.58 (mm, ∼10 H), 3.72 (m, 2 H), 3.90 (m, 1 H), 4.57 (d, 0.4 H), 4.85 (m, 0.6 H), 7.20 (m, 5 H), 10.15 (d, 1 H). LRMS: *m*/*z* 716 $(M + H)^+$.

***N*-[*N*-(α-Fluorenylmethoxycarbonyl)-*O*-*t*-butyl-(*S*)-α-aspartyl]-2(*S*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxycarbonylamidino)-4-piperidyloxy]ethyl}piperidine (50a).** DIPEA (25 mL, 143 mmol) was added to a stirred solution of amine **40a** (22.9 g, 47.7 mmol), Fmoc-Asp(OtBu)-OH (19.6 g, 47.7 mmol), and PyBroP (23.4 g, 50.1 mmol) in dry CH_2Cl_2 (160 mL) under N_2 at 0 °C, and the mixture was stirred for 3 h. After it was stirred for an additional 1 h at 23 °C, the reaction mixture was diluted with EtOAc (1 L) and washed sequentially with water, aqueous citric acid (1 M), saturated aqueous $NaHCO_3$ solution, water, and brine (200 mL each). The organic solution was dried ($MgSO_4$) and evaporated under reduced pressure to give crude product (2.87 g), which was purified by chromatography on silica gel, using hexanes−EtOAc (6:4 to 1:1) as eluant, to furnish amide **50a** (33.0 g, 77%) as a white foam. R_f 0.38 (hexanes:EtOAc, 1:1). ^{1}H NMR ($CDCl_3$): δ 1.47 (2xs, 29 H), 1.57−2.12 (mm, 10 H), 2.48 (m, 0.8 H), 2.70 (m, 1.2 H), 3.15 (m, 0.4 H), 3.23−3.60 (mm, 5.6 H), 3.95 (m, 3 H), 4.13−4.58

(mm, 3.4 H), 4.85 (m, 0.6 H), 5.01 (m, 1 H), 5.65 (d, 0.6 H), 5.85 (d, 0.4 H), 7.30 (t, 2 H), 7.40 (t, 2 H), 7.59 (d, 2 H), 7.76 (d, 2 H), 10.10 (d, 1 H). LRMS: *m/z* 849.3 $(M + H)^+$, 670, 626.7 $(M\text{-Fmoc} + 2H)^+$. Anal. ($C_{46}H_{65}N_5O_{10}$·0.4EtOAc) C, H, N.

***N*-[*N*-(α-Fluorenylmethoxycarbonyl)-*O*-*t*-butyl-(*S*)-α-aspartyl]-2(*RS*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxycarbonylamidino)-4-piperidyloxy]ethyl}piperidine (50b)**. Coupling of amine **40b** (5.00 g, 11.0 mmol) with Fmoc-Asp(OtBu)-OH (4.75 g, 11.5 mmol) in the presence of PyBroP (5.38 g, 11.5 mmol) and DIPEA (3.83 mL, 22 mmol) by the procedure described for **50a** gave **50b** (6.05 g, 65%) as a gum. R_f 0.68 (CH_2Cl_2:MeOH, 90:10). ^{1}H NMR ($CDCl_3$): consistent with that of **50a**. LRMS: *m/z* 849 $(M + H)^+$.

***N*-(*O*-*t*-Butyl-(*S*)-α-aspartyl)-2(*S*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxycarbonylamidino)-4-piperidyloxy]ethyl}piperidine (51a)**. Piperidine (1.7 mL, 18.3 mmol) was added to a stirred solution of Fmoc-amine **50a** (1.55 g, 1.83 mmol) in THF (7.5 mL). After 1 h, the reaction mixture was evaporated under reduced pressure and the residue was purified by chromatography on silica gel, using CH_2Cl_2−MeOH−0.88NH_3 (193:7:1) as eluant, to afford amine **51a** (1.05 g, 92%) as a white foam. R_f 0.50 (CH_2Cl_2:MeOH:0.88NH_3, 93:7:1). ^{1}H NMR ($CDCl_3$): 1.30−2.17 (mm, 41 H), 2.42 (m, 1 H), 2.55 (m, 1.5 H), 3.12 (t, 0.5 H), 3.36 (m, 3 H), 3.47 (m, 2 H), 3.72 (m, 2.5 H), 4.08 (m, 0.5 H), 4.23 (m, 1 H), 4.52 (d, 0.5 H), 4.85 (m, 0.5 H), 10.09 (s, 1 H). LRMS: *m/z* 626 $(M + H)^+$. Anal. ($C_{31}H_{55}N_5O_8$·0.3H_2O) C, H, N.

***N*-(*O*-*t*-Butyl-(*S*)-α-aspartyl)-2(*RS*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxycarbonylamidino)-4-piperidyloxy]ethyl}piperidine (51b).** Treatment of Fmoc-amine **51b** (6.05 g, 7.10 mmol) with piperidine by the procedure described for **51a** gave **51b** (3.71 g, 84%) as a foam. R_f 0.52 (CH_2Cl_2:MeOH, 90:10). ^{1}H NMR ($CDCl_3$): consistent with that of **51a**. LRMS: *m/z* 626 $(M + H)^+$.

General Procedure for the Preparation of Amines 52. $NaBH(OAc)_3$ (15.0 mmol) was added, in one portion, to a stirred solution of amine **51** (10.0 mmol), ketone/aldehyde (12.0 mmol), and AcOH (11.0 mmol) in THF (65 mL) under N_2 at 23 °C, and the mixture was stirred for 2 h (monitored by TLC). The reaction mixture was evaporated in vacuo, diluted with EtOAc (100 mL), washed with saturated aqueous $NaHCO_3$ solution and brine (100 mL each), dried ($MgSO_4$), and evaporated under reduced pressure to leave the crude product. Purification by chromatography on silica gel, using CH_2Cl_2−MeOH or hexanes−EtOAc as eluant, gave amine **52**.

***N*-(*N*-Cyclohexyl-*O*-*t*-butyl-(*S*)-α-aspartyl)-2(*RS*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxycarbonyl-amidino)-4-piperidyloxy]ethyl}piperidine (52a).** Alkylation of amine **51b** (420 mg, 0.67 mmol) with cyclohexanone (73 mg, 0.74 mmol) in the presence of $NaBH(OAc)_3$ (213 mg, 1.01 mmol) and AcOH (0.038 mL, 0.67 mmol) by the general procedure gave *sec*-amine **52a** (412 mg, 87%) as a white foam. R_f 0.70 (CH_2Cl_2:MeOH:0.88NH_3, 90:10:1). ^{1}H NMR ($CDCl_3$): δ 0.95−2.04 (mm, 20 H), 1.41 (s, 9 H), 1.45 (s, 18 H), 2.12−2.75 (mm, 4 H), 3.10 (m, 0.6 H), 3.24−3.90 (mm, 9 H), 4.03−4.31 (mm, 2 H), 4.50 (br d, 0.4 H), 4.87 (br s, 0.6 H), 10.12 (br s, 1 H). LRMS: *m/z* 708.7 $(M + H)^+$.

***N*-(*N*-Cyclopentyl-*O*-*t*-butyl-(*S*)-α-aspartyl)-2(*RS*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxycarbonyl-amidino)-4-piperidyloxy]ethyl}piperidine (52b).** Alkylation of amine **51b** (200 mg, 0.32 mmol) with cyclopentanone (60 mg, 0.70 mmol) in the presence of $NaBH(OAc)_3$ (102 mg, 0.48 mmol) and AcOH (0.018 mL, 0.32 mmol) by the general procedure gave *sec*-amine **52b** (185 mg, 83%) as a gum. R_f 0.56 (CH_2Cl_2:MeOH:0.88NH_3, 90:10:1). ^{1}H NMR ($CDCl_3$): δ 1.20−2.05 (mm, 21 H), 1.41 (2xs, 9 H), 1.46 (s, 18 H), 2.24−4.37 (mm, 13 H), 4.50 (br d, 0.4 H), 4.88 (br s, 0.6 H), 10.12 (br s, 1 H). LRMS: *m/z* 694.5 $(M + H)^+$.

***N*-(*N*-Cycloheptyl-*O*-*t*-butyl-(*S*)-α-aspartyl)-2(*S*)-{2-[*N*-(*N*,*N*′-di-*tert*-butoxycarbonyl-amidino)-4-piperidyloxy]ethyl}piperidine (52c).** Alkylation of amine **51a** (700 mg, 1.12 mmol) with cycloheptanone (376 mg, 3.36 mmol) in the presence of $NaBH(OAc)_3$ (711 mg, 3.36 mmol) and AcOH (0.192 mL, 3.36 mmol) by the general procedure gave *sec*-amine **52c** (740 mg, 92%). R_f 0.56 (CH_2Cl_2:MeOH:0.88NH_3, 90:10:1); $[\alpha]_D$ −5.7° (*c* = 2.45, MeOH). ^{1}H NMR ($CDCl_3$): δ 1.20−2.05 (mm, 26 H), 1.44 (s, 9 H), 1.49 (2xs, 18 H), 2.26−2.52 (mm, 3 H), 2.68 (t, 0.4 H), 3.10 (t, 0.6 H), 3.30−3.58 (mm, 5 H), 3.66−4.04 (mm, 4 H), 4.18 (br s, 0.4 H), 4.53 (br d, 0.4 H), 4.88 (br s, 0.6 H), 10.1 (br d, 1 H). LRMS: *m/z* 722.9 $(M + H)^+$.

***N*-(*N*-Cyclooctyl-*O*-*t*-butyl-(*S*)-α-aspartyl)-2(*RS*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxycarbonyl-amidino)-4-piperidyloxy]ethyl}piperidine (52d).** Alkylation of amine **51b** (280 mg, 0.45 mmol) with cyclooctanone (282 mg, 2.24 mmol) in the presence of $NaBH(OAc)_3$ (474 mg, 2.24 mmol) and AcOH (0.128 mL, 2.24 mmol) by the general procedure gave *sec*-amine **52d** (160 mg, 49%) as an oil. R_f 0.58 (CH_2Cl_2:MeOH:0.88NH_3, 90:10:1). ^{1}H NMR ($CDCl_3$): δ 1.20−2.08 (mm, 27 H), 1.42 (s, 9 H), 1.45 (s, 18 H), 2.10−2.73 (mm, 3 H), 3.01−4.37 (mm, 10 H), 4.50 (br d, 0.4 H), 4.88 (br s, 0.6 H), 10.1 (br s, 1 H). LRMS: *m/z* 737 $(M + H)^+$.

***N*-(*N*-3-Pentyl-*O*-*t*-butyl-(*S*)-α-aspartyl)-2(*S*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxycarbonyl-amidino)-4-piperidyloxy]ethyl}piperidine (52e).** Alkylation of amine **51a** (300 mg, 0.479 mmol) with 3-pentanone (273 mg, 3.16 mmol) in the presence of $NaBH(OAc)_3$ (456 mg, 2.15 mmol) and AcOH (0.081 mL, 1.44 mmol) by the general procedure gave *sec*-amine **52e** (68 mg, 20%). ^{1}H NMR ($CDCl_3$): δ 0.88 (m, 6 H), 1.2−2.46 (mm, 19 H), 1.43 (s, 9 H), 1.49 (18 H), 2.67 (t, 0.4 H), 3.11 (t, 0.6 H), 3.26−4.28 (mm, 10 H), 4.51 (br d, 0.4 H), 4.86 (br s, 0.6 H), 10.06 (br d, 1 H). LRMS: *m/z* 696.2 $(M + H)^+$.

***N*-(*N*-Cyclohexyl-*O*-*t*-butyl-(*S*)-α-aspartyl)-2(*S*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxycarbonyl-amidino)-4-piperidyloxy]ethyl}piperidine (52f).** Alkylation of amine **51a** (688 mg, 1.10 mmol) with cyclohexanone (0.137 mL, 1.32 mmol) in the presence of $NaBH(OAc)_3$ (350 mg, 1.65 mmol) and AcOH (0.069 mL, 1.21 mmol) by the general procedure gave *sec*-amine **52f** (745 mg, 96%) as a white foam. R_f 0.80 (CH_2Cl_2:MeOH:0.88NH_3, 93:7:1). ^{1}H NMR ($CDCl_3$): δ 0.94−2.10 (mm, ∼50 H), 2.10−2.52 (mm, 2.6 H), 2.68 (t, 0.4 H), 3.11 (t, 0.6 H), 3.40 (2xm, 5 H), 3.55 (2xm, 2.6 H), 4.03 (t, 1 H), 4.20 (m, 0.4 H), 4.52 (d, 0.4 H), 4.88 (m, 0.6 H), 10.11 (d, 1 H). LRMS: *m/z* 708 $(M + H)^+$.

***N*-(*N*-Cyclohept-4-enyl-*O*-*t*-butyl-(*S*)-α-aspartyl)-2(*S*)-{2-[*N*-(*N*,*N*′-di-*tert*-butoxy-carbonylamidino)-4-piperidyloxy]ethyl}piperidine (52g).** Alkylation of amine **51a** (600 mg, 0.959 mmol) with 4-cycloheptenone[33] (320 mg, 2.90 mmol) in the presence of $NaBH(OAc)_3$ (610 mg, 2.90 mmol) and AcOH (0.165 mL, 2.90 mmol) by the general procedure gave *sec*-amine **52g** (600 mg, 87%) as a foam. R_f 0.39 (hexanes:EtOAc, 1:1); $[\alpha]_D$ −0.2° (*c* = 0.50, MeOH). ^{1}H NMR ($CDCl_3$): δ 1.20−2.06 (m, 20 H), 1.40 (s, 9 H), 1.50 (s, 18 H), 2.14−2.66 (m, 4 H), 2.68 (t, 0.4 H), 3.13 (t, 0.6 H), 3.31−3.58 (m, 5 H), 3.75 (br s, 2 H), 3.86 (d, 0.6 H), 4.04 (t, 1 H), 4.21 (br s, 0.4 H), 4.53 (d, 0.4 H), 4.87 (br s, 0.6 H), 5.72 (s, 2 H), 10.02 (vbr s, 1 H). LRMS: *m/z* 720.4 $(M + H)^+$, 578.4, 503.0, 478.3. Anal. ($C_{38}H_{65}N_5O_8$) C, H, N.

***N*-[(*N*-Cyclohexylmethyl)-*O*-*t*-butyl-(*S*)-α-aspartyl]-2(*RS*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxy-carbonylamidino)-4-piperidyloxy]ethyl}piperidine (52h).** Alkylation of amine **51b** (276 mg, 0.441 mmol) with cyclohexanecarboxaldehyde (0.107 mL, 0.88 mmol) in the presence of $NaBH(OAc)_3$ (185 mg, 0.88 mmol) by the general procedure gave cyclohexylmethylamines **52h** (137 mg, 43%) as a foam. R_f 0.30 (hexanes:EtOAc, 1:1). ^{1}H NMR ($CDCl_3$): δ 0.78−0.97 (m, 2 H), 1.07−2.07 (m, 23 H), 1.44 (s, 9 H), 1.48 (s, 18 H), 2.12−2.72 (m, 3.4 H), 3.13 (q, 0.6 H), 3.28−3.60 (m, 4.8 H), 3.66−3.97 (m, 3.2 H), 4.22 (br s, 0.4 H), 4.36 (br s, 0.4 H), 4.55 (br s, 0.6 H), 4.87 (br s, 0.6 H), 10.10 (vbr s, 1 H). LRMS: *m/z* 722.3 $(M + H)^+$, 622.7, 580.6, 480.6.

***N*-(*N*-Benzyl-*O*-*t*-butyl-(*S*)-α-aspartyl)-2(*S*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxycarbonylamidino)-4-piperidyloxy]ethyl}piperidine (52i).** Alkylation of amine **51a** (502 mg, 0.802 mmol) with benzaldehyde (0.90 mL, 0.88 mmol) in the presence of $NaBH(OAc)_3$ (258 mg, 1.22 mmol) by the general procedure gave benzylamine **52i** (394 mg, 66%) as a foam. R_f 0.31 (hexanes:EtOAc, 1:1); $[\alpha]_D$ −11.3° (*c* = 0.16, MeOH). ^{1}H NMR ($CDCl_3$): δ 1.30−2.02 (m,13 H), 1.44 (s, 9 H), 1.47 (s, 18 H), 2.33−2.54 (m, 2 H), 2.67 (t, 0.4 H), 3.05 (t, 0.6 H), 3.28−3.83

(m, 9.4 H), 3.93 (t, 0.6 H), 4.05 (dd, 0.4 H), 4.15 (br s, 0.4 H), 4.55 (d, 0.6 H), 4.88 (br s, 0.6 H), 7.15−7.38 (m, 5 H), 10.10 (vbr s, 1 H). LRMS: *m*/*z* 716.6 $(M + H)^+$, 616.6, 574.6, 499.6, 322.3, 238.3. Anal. ($C_{38}H_{61}N_5O_8 \cdot 0.3CH_2Cl_2$) C, H, N.

***N*-[*N*-(4-Tetrahydropyranyl)-*O*-*t*-butyl-(*S*)-α-aspartyl]-2(*RS*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxy-carbonylamidino)-4-piperidyloxy]ethyl}piperidine (52j).** Alkylation of amine **51b** (200 mg, 0.32 mmol) with 4-tetrahydropyranone (35 mg, 0.48 mmol) in the presence of $NaBH(OAc)_3$ (102 mg, 0.48 mmol) and AcOH (0.018 mL, 0.32 mmol) by the general procedure gave *sec*-amine **52j** (180 mg, 79%). R_f 0.60 (CH_2Cl_2:MeOH:0.88NH_3, 90:10:1). ^{1}H NMR ($CDCl_3$): δ 1.22−2.08 (mm, 16 H), 1.42 (s, 9 H), 1.47 (s, 18 H), 2.1−2.74 (mm, 4 H), 3.03−4.32 (mm, 14 H), 4.5 (br d, 0.25 H), 4.8 (br s, 0.75 H), 10.1 (br s, 1 H). LRMS: *m*/*z* 710.5 $(M + H)^+$.

***N*-[*N*-(3-Tetrahydropyranyl)-*O*-*t*-butyl-(*S*)-α-aspartyl]-2(*S*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxycarbonylamidino)-4-piperidyloxy]ethyl}piperidine (52k).** Alkylation of amine **51a** (300 mg, 0.479 mmol) with 3-tetrahydropyranone[34] (116 mg, 1.15 mmol) in the presence of $NaBH(OAc)_3$ (297 mg, 1.40 mmol) and AcOH (0.027 mL, 0.32 mmol) by the general procedure gave *sec*-amine **52k** (221 mg, 65%) as a white foam. R_f 0.79 (CH_2Cl_2:MeOH, 90:10). ^{1}H NMR ($CDCl_3$): δ 1.20−2.05 (m, 43 H), 2.17−2.75 (m, 3 H), 2.92−3.20 (m, 2 H); 3.22−3.60 (m, 6 H), 3.68−4.08 (m, 5 H), 4.10−4.56 (m, 1 H), 4.82−4.92 (m, 1 H), 10.08 (br s, 1 H). Anal. ($C_{36}H_{63}N_5O_9 \cdot 0.2CH_2Cl_2$) C, H, N.

***N*-[*N*-(*N*-Methyl-4-piperidyl)-*O*-*t*-butyl-(*S*)-α-aspartyl]-2(*RS*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxy-carbonylamidino)-4-piperidyloxy]ethyl}piperidine (52l).** Alkylation of amine **51b** (500 mg, 0.80 mmol) with *N*-methyl-4-piperidone (0.11 mL, 0.90 mmol) in the presence of $NaBH(OAc)_3$ (254 mg, 1.12 mmol) and AcOH (96 μL, 1.66 mmol) by the general procedure gave *sec*-amine **52l** (510 mg, 86%) as a white foam. R_f 0.50 (CH_2Cl_2:MeOH:0.88NH_3, 93:7:2). ^{1}H NMR ($CDCl_3$) δ 1.20−1.98 (m, 45 H), 2.25−2.35 (m, 3 H), 2.50−4.90 (m, 15 H). Anal. ($C_{37}H_{66}N_6O_8 \cdot 0.2CH_2Cl_2$) C, H, N.

General Procedure for the Preparation of *N*-Methyl Amines 53. A solution of amine **52** (1.04 mmol) in CH_2Cl_2 (15 mL) was vigorously stirred with aqueous formaldehyde (37%, w/v; 0.34 mL, 4.15 mmol) for 1 h. $NaBH(OAc)_3$ (2.08 mmol) was added, and stirring was continued for 1 h. The reaction mixture was diluted with CH_2Cl_2 (25 mL), washed sequentially with saturated aqueous $NaHCO_3$ solution (10 mL), dried ($MgSO_4$), and evaporated under reduced pressure to leave the crude product. Purification by chromatography on silica gel, using CH_2Cl_2−MeOH or hexanes−EtOAc as eluant, gave amine **53**.

***N*-(*N*-Cycloheptyl-*N*-methyl-*O*-*t*-butyl-(*S*)-α-aspartyl)-2(*S*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxy-carbonylamidino)-4-piperidyloxy]ethyl}piperidine (53c).** Methylation of amine **52c** (420 mg, 0.58 mmol) with 37% aqueous formaldehyde (0.065 mL, 0.80 mmol) in the presence of $NaBH(OAc)_3$ (185 mg, 0.87 mmol) by the general procedure gave *tert*-amine **53c** (390 mg, 91%). R_f 0.74 (CH_2Cl_2:MeOH:0.88NH_3, 90:10:1). ^{1}H NMR ($CDCl_3$): δ 1.2−2.1 (mm, 24 H), 1.42 (s, 9 H), 1.48 (s, 18 H), 2.20 (s, 3 H), 2.34 (dd, 1 H), 2.65 (br s, 1 H), 2.89 (dd, 1 H), 3.05 (t, 1 H), 3.29−3.56 (mm, 5 H), 3.74 (br s, 2 H), 4.00 (m, 2 H), 4.79 (br s, 1 H), 10.08 (br s, 1 H). LRMS: *m*/*z* 736.6 $(M + H)^+$.

***N*-(*N*-Cyclohexyl-*N*-methyl-*O*-*t*-butyl-(*S*)-α-aspartyl)-2(*S*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxy-carbonylamidino)-4-piperidyloxy]ethyl}piperidine (53f).** Methylation of amine **52f** (735 mg, 1.04 mmol) with 37% aqueous formaldehyde (0.34 mL, 4.15 mmol) in the presence of $NaBH(OAc)_3$ (440 mg, 2.08 mmol) by the general procedure gave *tert*-amine **53f** (720 mg, 96%) as a white foam. R_f 0.32 (CH_2Cl_2:MeOH:0.88NH_3, 193:7:1). ^{1}H NMR ($CDCl_3$): δ 1.00−1.94 (mm, ∼50 H), 2.02 (m, 1 H), 2.19 (s, 3 H), 2.31 (m, 2 H), 2.64 (m, 0.2 H), 2.97 (m, 1.8 H), 3.37 (m, 4 H), 3.48 (m, 1 H), 3.73 (m, 2 H), 4.08 (m, 2 H), 4.30 (m, 0.2 H), 4.48 (m, 0.2 H), 4.80 (m, 1 H), 10.00 (s, 1 H). LRMS: *m*/*z* 722 $(M + H)^+$. Anal. ($C_{38}H_{67}N_5O_8 \cdot 0.5H_2O$) C, H, N.

***N*-(*N*-Cyclohept-4-enyl-*N*-methyl-*O*-*t*-butyl-(*S*)-α-aspartyl)-2(*S*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxycarbonylamidino)-4-piperidyloxy]ethyl}piperidine (53g).** Methylation of amine **52g** (346 mg, 0.481 mmol) with 37% aqueous formaldehyde (0.065 mL, 0.80 mmol) in the presence of $NaBH(OAc)_3$ (204 mg, 0.96 mmol) by the general procedure gave *tert*-amine **53g** (309 mg, 87%). R_f 0.66 (CH_2Cl_2:MeOH, 90:10); $[\alpha]_D$ −20.6° (*c* = 0.53, MeOH). ^{1}H NMR ($CDCl_3$): δ 1.30−2.4 (mm, ∼23 H), 1.44 (s, 9 H), 1.50 (s, 18 H), 2.25 (s, 3 H), 2.68 (m, 0.6 H), 2.92 (dd, 0.6 H), 3.05 (t, 0.6 H), 3.3−3.54 (mm, 5 H), 3.74 (m, 2 H), 4.05 (br d, 1.6 H), 4.7 (br s, 0.6 H), 5.73 (s, 2 H), 10.04 (br s, 1 H). LRMS: *m*/*z* 734 $(M + H)^+$. Anal. ($C_{39}H_{67}N_5O_8 \cdot 0.5H_2O$) C, H, N.

***N*-[*N*-Methyl-*N*-(3-tetrahydropyranyl)-*O*-*t*-butyl-(*S*)-α-aspartyl]-2(*S*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxycarbonylamidino)-4-piperidyloxy]ethyl}piperidine (53k).** Methylation of amine **52k** (208 mg, 0.293 mmol) with 37% aqueous formaldehyde (0.095 mL, 1.17 mmol) in the presence of $NaBH(OAc)_3$ (124 mg, 0.586 mmol) by the general procedure gave *tert*-amine **53k** (185 mg, 87%) as a white solid. R_f 0.83 (CH_2Cl_2:MeOH:0.88NH_3, 90:10:1). ^{1}H NMR ($CDCl_3$): δ 1.22−2.12 (m, 43 H), 2.21−2.23 (m, 3 H), 2.50−2.68 (m, 1 H), 2.90−3.55 (m, 9 H), 3.68−3.90 (m, 4 H), 3.91−4.53 (m,3 H), 4.76−4.88 (m, 1 H), 10.02 (br s, 1 H). LRMS: *m*/*z* 723.8 $(M + H)^+$. Anal. ($C_{37}H_{65}N_5O_9 \cdot 0.2CH_2Cl_2$) C, H, N.

***N*-(*N*-Cyclohexyl-*N*-ethyl-*O*-*t*-butyl-(*S*)-α-aspartyl)-2(*RS*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxy-carbonylamidino)-4-piperidyloxy]-ethyl}piperidine (54).** A solution of acetaldehyde in THF (1 M; 0.97 mL, 0.97 mmol) was added to a stirred solution of amine **52a** (328 mg, 0.463 mmol) in THF (7 mL) at 23 °C, and after 30 min, $NaBH(OAc)_3$ (216 mg, 1.02 mmol) was added. After 18 h, a second portion of the acetaldehyde solution (1 M; 0.34 mL, 0.34 mmol) and $NaBH(OAc)_3$ (72 mg, 0.34 mmol) were added, the reaction mixture was stirred for 3 h and then evaporated under reduced pressure. The residual solid was partitioned between EtOAc and water, and the organic phase was separated, washed sequentially with saturated aqueous $NaHCO_3$ solution and brine, dried ($MgSO_4$), and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel, using hexanes−EtOAc (1:1) as eluant, to provide ethylamine **54** (193 mg, 56%) as a white foam. R_f 0.87 (EtOAc). ^{1}H NMR ($CDCl_3$): δ 1.04 (t, 3 H), 1.12−2.18 (m, 49 H), 2.28−3.11 (m, 5 H), 3.26−3.88 (m, 7 H), 4.08−4.19 (m, 2H), 4.43−4.86 (m, 1 H). LRMS: *m*/*z* 736.3 $(M + H)^+$. Anal. ($C_{39}H_{69}N_5O_8 \cdot 0.2CH_2Cl_2$) C, H, N.

***N*-{*N*-Cyclohexyl-*N*-[2-(dimethylamino)ethyl]-*O*-*t*-butyl-(*S*)-α-aspartyl}-2(*RS*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxycarbonyl-amidino)-4-piperidyloxy]ethyl}piperidine (55).** Solid K_2CO_3 (228 mg, 1.64 mmol) and then 2-(dimethylamino)ethyl chloride hydrochloride (119 mg, 0.826 mmol) were added to a solution of amine **52a** (328 mg, 0.55 mmol) in MeCN (10 mL) at 23 °C, and the mixture was heated at 50 °C for 18 h and then evaporated under reduced pressure. The residual solid was partitioned between EtOAc and water, and the organic phase was separated, washed with brine, dried ($MgSO_4$), and evaporated under reduced pressure. The crude product was purified by chromatography on silica gel, using an elution gradient of CH_2Cl_2−MeOH (100:0 to 95:5), to provide diamine **55** (400 mg, 93%) as an oil. R_f 0.39 (CH_2Cl_2:MeOH:0.88NH_3, 95:5:0.1). ^{1}H NMR ($CDCl_3$): δ 0.95−2.18 (m, 49 H), 2.20−2.75 (m, 10 H), 3.03−4.98 (m, 14 H). LRMS: *m*/*z* 779 $(M + H)^+$.

***N*-[*N*-(α-Fluorenylmethoxycarbonyl)-*O*-*t*-butyl-(*S*)-α-glutamyl]-2(*S*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxycarbonylamidino)-4-piperidyloxy]ethyl}piperidine (56).** Coupling of amine **40a** (1.074 g, 2.36 mmol) with Fmoc-Glu(OtBu)-OH (1.012 g, 2.38 mmol) in the presence of PyBroP (1.171 g, 2.51 mmol) and DIPEA (1.23 mL, 7.06 mmol) by the procedure described for **50a** gave **56** (1.368 g, 67%) as a white foam. R_f 0.47 (hexanes:EtOAc, 1:1); $[\alpha]_D$ −8.6° (*c* = 0.78, MeOH). ^{1}H NMR ($CDCl_3$): δ 1.30−2.14 (m, 15 H), 1.43 (s, 18 H), 1.50 (s, 9 H), 2.18−2.42 (m, 2 H), 2.68 (t, 0.5 H), 3.15 (t, 0.5 H), 3.24−3.55 (m, 4 H), 3.63−3.88 (m, 2 H), 4.15−4.54 (m, 4 H), 4.73 (br s, 1 H), 4.87 (br s, 1 H), 5.75 (br s, 1 H), 7.30 (t, 2 H), 7.39 (t, 2 H), 7.60 (d, 2 H), 7.75 (d, 2 H), 10.02 (br s, 1 H). LRMS: *m*/*z* 864, 862 $(M + H)^+$, 721, 640 (M-Fmoc + 2H)$^+$. Anal. ($C_{47}H_{67}N_5O_{10} \cdot 0.2CH_2Cl_2$) C, H, N.

***N*-(*O*-*t*-Butyl-(*S*)-α-glutamyl)-2(*S*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxycarbonylamidino)-4-piperidyloxy]ethyl}piperidine (57).** Treatment of Fmoc-amine **56** (1.325 g, 1.54 mmol) with piperidine by the procedure described for **51a** gave **57** (0.90 g, 91%) as a white foam. R_f 0.34 (CH_2Cl_2:MeOH, 90:10); $[\alpha]_D$ +11.4° (c = 0.71, MeOH). ^{1}H NMR ($CDCl_3$): δ 1.30−2.10 (m, 16 H), 1.42 (s, 9 H), 1.50 (s, 18 H), 2.25−2.68 (m, 2.5 H), 3.10 (t, 0.5 H), 3.25−3.55 (m, 5 H), 3.65−3.88 (m, 3.5 H), 4.21 (br s, 0.5 H), 4.52 (d, 0.5 H), 4.86 (br s, 0.5 H), 10.0 (vbr s, 1 H). LRMS: m/z 640 $(M + H)^+$. Anal. ($C_{32}H_{57}N_5O_8 \cdot 0.1CH_2Cl_2$) C, H, N.

***N*-(*N*-Cyclohexyl-*O*-*t*-butyl-(*S*)-α-glutamyl)-2(*S*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxycarbonyl-amidino)-4-piperidyloxy]-ethyl}piperidine (58).** Alkylation of amine **57** (853 mg, 1.33 mmol) with cyclohexanone (0.414 mL, 3.99 mmol) in the presence of $NaBH(OAc)_3$ (861 mg, 3.99 mmol) and AcOH (0.233 mL, 3.99 mmol) by the general procedure gave amine **58** (847 mg, 88%) as a white foam. R_f 0.32 (hexanes:EtOAc, 1:1); $[\alpha]_D$ −6.3° (c = 0.79, MeOH). ^{1}H NMR ($CDCl_3$): δ 0.94−2.02 (m, 23 H), 1.42 (s, 9 H), 1.46 (s, 18 H), 2.08−2.73 (m, 4 H), 3.10 (t, 0.5 H), 3.28−3.63 (m, 7 H), 3.66−3.88 (m, 3 H), 4.22 (br s, 0.5 H), 4.53 (d, 0.5 H), 4.87 (br s, 0.5 H), 10.0 (vbr s, 1 H). LRMS: m/z 723 $(M + H)^+$. Anal. ($C_{38}H_{67}N_5O_8$) H, N; C: calcd, 63.22; found, 62.75.

***N*-(*N*-Cyclohexyl-*N*-methyl-*O*-*t*-butyl-(*S*)-α-glutamyl)-2(*S*)-{2-[*N*-(*N*,*N*′-di-*t*-butoxy-carbonylamidino)-4-piperidyloxy]ethyl}piperidine (59).** Methylation of amine **58** (598 mg, 0.828 mmol) with 37% aqueous formaldehyde (0.12 mL, 1.46 mmol) in the presence of $NaBH(OAc)_3$ (0.350 mg, 1.66 mmol) by the general procedure gave amine **59** (0.572 mg, 94%) as a white foam. R_f 0.42 (hexanes:EtOAc, 1:1); $[\alpha]_D$ +0.5° (c = 0.65, MeOH). ^{1}H NMR ($CDCl_3$): δ 0.95−2.15 (m, 22 H), 1.42 (s, 9 H), 1.46 (s, 18 H), 2.20−2.53 (m, 3 H), 2.28 (s, 3 H), 2.62-(t, 0.2 H), 3.00 (t, 0.8 H), 3.30−3.82 (m, 10 H), 3.99 (d, 0.8 H), 4.23 (br s, 0.2 H), 4.51 (d, 0.2 H), 4.86 (br s, 0.8 H), 10.0 (br s, 1 H). LRMS: m/z 735 $(M + H)^+$. Anal. ($C_{39}H_{69}N_5O_8$) C, H, N.

***N*-[*N*-(Benzyloxycarbonyl)-(*R*)-phenylalanyl]-2(*RS*)-[2-(4-cyanophenoxy)ethyl]piperidine (60).** WSCDI (1.53 g, 8 mmol) was added to an ice cold stirred solution of amine **25** (921 mg, 4 mmol), Cbz-D-Phe-OH (1.32 g, 4 mmol), HOBt (540 mg, 4 mmol), and NMM (809 mg, 8 mmol) in CH_2Cl_2 (20 mL). The mixture was allowed to warm to 23 °C, and after 18 h, the solvent was evaporated. The residue was partitioned between EtOAc and H_2O, and the organic phase was washed with HCl (2 M), brine, saturated aqueous $NaHCO_3$, and brine, and then dried over $MgSO_4$. Evaoporation gave a foam, which was purified by chromatography on silica, using Et_2O−hexane (2:1) as eluant, to give amide **60** (910 mg, 44%) as a white solid. R_f 0.38 and 0.46 (diastereoisomers) (Et_2O:hexane, 4:1). ^{1}H NMR ($CDCl_3$): δ 0.6 (m, 0.6 H), 1.10−1.75 (mm, 6 H), 1.75−2.35 (mm, 2 H), 2.60 (m, 0.4 H), 2.97 (m, 2.6 H), 3.56 (m, 0.6 H), 3.90 (m, 2.4 H), 4.50 (m, 0.4 H), 4.77−5.14 (m, 4 H), 5.50 (4xd, 1 H), 6.89 (m, 2 H), 7.20 (m, 10 H), 7.48 (m, 2 H). Anal. ($C_{31}H_{33}N_3O_4 \cdot 0.2Et_2O$) C, H, N.

***N*-[*N*-Carboxymethyl-(*R*)-cyclohexylalanyl]-2(*S*)-[2-(4-amidinophenoxy)ethyl]piperidine Dihydrochloride (2).** NaOH (8 mL, 1 M) was added to a suspension of the ester **32** (740 mg, 1.3 mmol) in dioxan (8 mL). After 35 min, the resulting solution was acidified to pH 2 with HCl (1 M) and evaporated to dryness, and the residue was dried azeotropically with *i*-PrOH. The residue was then extracted with hot *i*-PrOH, the suspension was filtered, and the filtrate was evaporated to dryness. The residue on trituration with Et_2O gave **2** (630 mg, 86%) as a white powder. R_f 0.15 (CH_2Cl_2:MeOH:0.88NH_3, 80:20:5). ^{1}H NMR (DMSO-d_6) (75:25 rotamer ratio): δ 0.65−1.84 (mm, ∼20 H), 1.94 (m, 0.75 H), 2.21 (m, 0.75 H), 2.79 (t, 0.25 H), 3.12−3.89 (mm, ∼6 H), 4.07 (m, 2 H), 4.33 (m, 0.25 H), 4.45 (m, 0.75 H), 4.55 (m, 0.25 H), 4.79 (m, 0.75 H), 7.08 (dd, 2 H), 7.85 (dd, 2 H), 9.06 (s, 2 H), 9.26 (s, 2 H). LRMS: m/z 459 $(M + H)^+$, 442 $(M + H - NH_3)^+$. Anal. ($C_{25}H_{38}N_4O_4 \cdot 2HCl \cdot 0.3H_2O \cdot 0.41i$-PrOH) C, H, N.

***N*-[*N*-Carboxymethyl-(*R*)-cyclohexylalanyl]-2(*R*)-[2-(4-amidinophenoxy)ethyl]piperidine Dihydrochloride (3).** This compound was prepared from **33** in a manner similar to **2**. Amidine **3** (96%) was obtained as a white powder. R_f 0.1 (CH_2Cl_2:MeOH:0.88NH_3, 80:20:5). ^{1}H NMR (DMSO-d_6) (6:4 distribution of rotamers): δ 0.8 (m, 2 H), 0.94−1.83 (mm, ∼19 H), 1.99 (m, 0.8 H), 2.15 (m, 1.2 H), 2.74 (t, 0.4 H), 3.12−3.84 (mm, ∼3.6 H), 3.96 (m, 1.4 H), 4.13 (m,1 H), 4.30 (m, 1 H), 4.50 (m, 0.6 H), 4.90 (m, 0.6 H), 7.04 (d, 1.2 H), 7.16 (m, 0.8 H), 7.86 (t, 2 H), 9.10 (s, 2 H), 9.25 (s, 2 H). LRMS: m/z 459 $(M + H)^+$, 442 $(M + H - NH_3)^+$. Anal. ($C_{25}H_{38}N_4O_4 \cdot 2HCl \cdot 0.4i$-PrOH) C, H, N.

General Procedure of the Preparation of Targets 4, 5, 7−22. A stirred, ice-cooled solution of the *tert*-butyl ester (0.5 mmol) in CH_2Cl_2 (10 mL) was saturated with HCl gas, the cooling bath was removed, and the resulting solution was stirred at 23 °C until total deprotection was complete (typically 2−6 h at 23 °C). The solvent was removed by evaporation under reduced pressure, and the residual HCl was removed azeotropically using CH_2Cl_2 (×3) to give the amine di(tri)-hydrochloride.

***N*-[*N*-Carboxymethyl-(*R*)-cyclohexylalanyl]-2(*RS*)-[2-(*N*-amidino-4-piperidyloxy)ethyl]piperidine Dihydrochloride (4).** HCl gas deprotection of *tert*-butyl ester **46** (286 mg, 0.40 mmol) by the general procedure gave **4** (209 mg, 90%) as a white solid. R_f 0.15, 0.12 (consistent with two diastereoisomers) (CH_2Cl_2:MeOH:0.88NH_3, 80:20:5). ^{1}H NMR (DMSO-d_6): δ 0.78−2.00 (m, 25 H), 2.95−4.06 (m, integral obscured by HOD), 4.12−4.85 (m, 3 H), 7,52 (s, 4 H), 8.80−9.58 (br s, 2 H). LRMS: m/z 466.5 $(M + H)^+$. Anal. ($C_{24}H_{43}N_5O_4 \cdot 2HCl \cdot 0.45CH_2Cl_2 \cdot 0.5H_2O$) C, H, N.

***N*-[*N*-Carboxymethyl-(*R*)-phenylalanyl]-2(*RS*)-[2-(*N*-amidino-4-piperidyloxy)ethyl]piperidine Dihydrochloride (5).** An ice cold solution of ester **49** (470 mg, 0.66 mmol) in CH_2Cl_2 (15 mL) was saturated with HCl gas over 0.5 h. The solution was then left at 23 °C for 2 h, and the solvent was evaporated initially with a stream of N_2 and then under vacuum. The residue was azeotroped with CH_2Cl_2 to give **5** (336 mg, 89%) as a white powder. R_f 0.48 (MeOH:EtOAc:AcOH: 0.88NH_3:H_2O, 60:12:4:4:8). ^{1}H NMR (DMSO-d_6): δ 0.18 (m, 0.25 H), 0.14 (m, 0.15 H), 0.92−1.90 (mm, ∼12 H), 2.21 (m, 0.25 H), 2.92 (m, 1 H), 3.10−3.68 (m, ∼10 H), 2.81 (m, 2 H), 4.27 (m, 0.25 H), 4.64 (mm, 1.5 H), 7.27 (m, 5 H), 7.54 (m, 4 H). LRMS: m/z 460 $(M + H)^+$. Anal. ($C_{24}H_{37}N_5O_4 \cdot 2HCl \cdot 1.6H_2O \cdot 0.25CH_2Cl_2$) C, H, N.

***N*-[(*R*)-Phenylalanyl]-2(*RS*)-[2-(4-amidinophenoxy)ethyl]piperidine Dihydrochloride (6).** This compound was prepared from **60** in a manner similar to **32**. The crude reaction product was chromotographed on silica. Elution with mixtures of CH_2Cl_2−MeOH−0.88NH_3 (92.5:7.5:1 to 85:15:2) gave the Cbz derivative of compound **6** (30%). R_f 0.47 (CH_2Cl_2:MeOH: 0.88NH_3, 80:20:5). LRMS: m/z 520 $(M + H)^+$, followed by **6** (21%). R_f 0.3 (CH_2Cl_2:MeOH:0.88NH_3, 80:20:5). LRMS: m/z 395 $(M + H)^+$. Compound **6** was treated with etherial HCl to give the hydrochloride as a white powder. ^{1}H NMR (DMSO-d_6): δ 0.11 (m, 0.5 H), 1.00−1.71 (mm, 5 H), 1.83 (m, 1 H), 2.12 (m, 1 H), 2.62 (m, 0.5 H), 3.00 (mm, 2.5 H), 3.45 (m, 1 H), 3.90 (m, 0.5 H), 4.04 (m, 2 H), 4.57 (m, 1 H), 4.72 (m, 1 H), 7.08 (m, 2 H), 7.23 (m, 5 H), 7.81 (m, 2 H), 8.42 (br s, 3 H), 9.04 (d, 2 H), 9.25 (d, 2 H). Anal. ($C_{23}H_{30}N_4O_2 \cdot 2HCl \cdot 1.4H_2O \cdot 0.2Et_2O \cdot 0.2$ EtOAc) C, H, N.

***N*-(*N*-Cyclohexyl-(*S*)-α-aspartyl)-2(*RS*)-[2-(*N*-amidino-4-piperidyloxy)ethyl]piperidine dihydrochloride (7).** HCl gas deprotection of *tert*-butyl ester **52a** (280 mg, 0.39 mmol) by the general procedure gave **7** (170 mg) as a white foam. R_f 0.57 (MeOH:EtOAc:AcOH:0.88NH_3:H_2O, 60:12:4:4:8). ^{1}H NMR (DMSO-d_6): δ 0.95−2.10 (mm, 20 H), 2.62−3.82 (mm, 13 H), 3.92−4.36 (mm, 1 H), 4.57−4.83 (m, 2 H), 7.50 (s, 4 H), 8.75 (vbr s, 1 H), 9.35 (vbr s, 1 H), 12.9 (vbr s, 1 H). LRMS: m/z 452 $(M + H)^+$. Anal. ($C_{23}H_{41}N_5O_4 \cdot 2HCl \cdot CH_2Cl_2$) C, H, N.

***N*-(*N*-Cyclopentyl-(*S*)-α-aspartyl)-2(*RS*)-[2-(*N*-amidino-4-piperidyloxy)ethyl]piperidine Dihydrochloride (8).** HCl gas deprotection of *tert*-butyl ester **52b** (143 mg, 0.21 mmol) by the general procedure gave **8** (95 mg) as a white foam. R_f 0.44 (MeOH:EtOAc:AcOH: 0.88NH_3:H_2O, 60:12:4:4:8). ^{1}H NMR (DMSO-d_6): δ 1.20−2.04 (mm, 16 H), 2.64−3.80 (mm, 15 H), 3.94−4.86 (mm, 3 H), 7.43 (s, 4 H), 8.92 (vbr s, 1 H),

9.34 (vbr s, 1 H), 12.8 (vbr s, 1 H). LRMS: *m/z* 438 $(M + H)^+$. Anal. ($C_{22}H_{39}N_5O_4 \cdot 2HCl \cdot 1.5H_2O \cdot 0.5CH_2Cl_2$) C, H, N.

***N*-(*N*-Cycloheptyl-(*S*)-α-aspartyl)-2(*S*)-[2-(*N*-amidino-4-piperidyloxy)ethyl]piperidine Dihydrochloride (9).** HCl gas deprotection of *tert*-butyl ester **52c** (320 mg, 0.44 mmol) by the general procedure gave **9** (200 mg) as a white powder. R_f 0.50 (MeOH:EtOAc:AcOH:0.88NH_3:H_2O, 60:12:4:4:8); $[\alpha]_{578}$ +6.3° (*c* = 0.8, MeOH). ^{1}H NMR (DMSO-d_6): δ 1.19−2.15 (mm, 22 H), 2.64−3.05 (mm, 3 H), 3.05−3.8 (mm, 10 H), 3.92−4.07 (mm, 3 H), 7.50 (s, 4 H), 8.6 (vbr s, 1 H), 9.2 (vbr s, 1 H), 13.0 (vbr s, 1 H). LRMS: *m/z* 466.5 $(M + H)^+$. Anal. ($C_{24}H_{43}N_5O_4 \cdot 2HCl \cdot 1.1H_2O \cdot 0.6CH_2Cl_2$) C, H, N.

***N*-(*N*-Cyclooctyl-(*S*)-α-aspartyl)-2(*RS*)-[2-(*N*-amidino-4-piperidyloxy)ethyl]piperidine Dihydrochloride (10).** HCl gas deprotection of *tert*-butyl ester **52d** (160 mg, 0.22 mmol) by the general procedure gave **10** (89 mg) as a white powder. R_f 0.58 (MeOH:EtOAc:AcOH:0.88NH_3:H_2O, 60:12:4:4:8). ^{1}H NMR (DMSO-d_6): δ 1.1−2.06 (mm, 24 H), 2.61−3.8 (mm, 13 H), 3.92−4.93 (mm, 3 H), 7.48 (s, 4 H), 8.48−9.25 (br, 2 H), 13.0 (vbr s, 1 H). LRMS: *m/z* 480 $(M + H)^+$. Anal. ($C_{25}H_{45}N_5O_4 \cdot 2HCl \cdot 2.0H_2O \cdot 0.25CH_2Cl_2$) C, H, N.

***N*-(*N*-3-Pentyl-(*S*)-α-aspartyl)-2(*S*)-[2-(*N*-amidino-4-piperidyloxy)ethyl]piperidine Dihydrochloride (11).** HCl gas deprotection of *tert*-butyl ester **52e** by the general procedure gave **11** as a white foam. ^{1}H NMR (DMSO-d_6): δ 0.82−0.99 (m, 6 H), 1.20−2.08 (mm, 16 H), 2.6−4.34 (mm, 12 H), 4.59−4.80 (m, 2 H), 7.50 (s, 4 H), 8.38 (vbr s, 1 H), 9.10 (vbr s, 1 H), CO_2H not detected. LRMS: *m/z* 460.6 $(M + H)^+$. Anal. ($C_{22}H_{41}N_5O_4 \cdot 2HCl \cdot 3.0H_2O \cdot 0.5CH_2Cl_2$) C, H, N.

***N*-(*N*-Cyclohexyl-*N*-methyl-(*S*)-α-aspartyl)-2(*S*)-[2-(*N*-amidino-4-piperidyloxy)ethyl]piperidine Dihydrochloride (12).** HCl gas deprotection of *tert*-butyl ester **53f** (690 mg, 0.95 mmol) by the general procedure gave **12** (523 mg) as a white powder. R_f 0.50 (MeOH:EtOAc:AcOH:0.88NH_3:H_2O, 60:12:4:4:8). ^{1}H NMR (DMSO-d_6): δ 1.00−2.30 (mm, 22 H), 2.55−4.40 (mm, ∼15 H), 4.80 (m, 2 H), 7.52 (d, 4 H), 9.50−10.40 (br m, 1 H), 13.00 (vbr s, 1 H). LRMS: *m/z* 466 $(M + H)^+$. Anal. ($C_{24}H_{43}N_5O_4 \cdot 2HCl \cdot 1.3H_2O \cdot 0.8CH_2Cl_2$) C, H, N.

***N*-(*N*-Cycloheptyl-*N*-methyl-(*S*)-α-aspartyl)-2(*S*)-[2-(*N*-amidino-4-piperidyloxy)ethyl]piperidine Dihydrochloride (13).** HCl gas deprotection of *tert*-butyl ester **53c** (310 mg, 0.42 mmol) by the general procedure gave **13** (117 mg) as a white solid. R_f 0.52 (MeOH:EtOAc:AcOH:0.88NH_3:H_2O, 60:12:4:4:8). ^{1}H NMR (DMSO-d_6): δ 1.20−2.05 (mm, 22 H), 2.68 (s, 3 H), 2.74−4.4 (mm, 12 H), 4.64−4.94 (m, 2 H), 7.42 (s, 4 H), 9.43 (vbr s, 1 H), 13.02 (vbr s, 1 H). LRMS: *m/z* 480.2 $(M + H)^+$. Anal. ($C_{25}H_{45}N_5O_4 \cdot 2HCl \cdot 2.0H_2O \cdot 0.25CH_2Cl_2$) C, H, N.

***N*-(*N*-Cyclohept-4-enyl-*N*-methyl-(*S*)-α-aspartyl)-2(*S*)-[2-(*N*-amidino-4-piperidyloxy)ethyl]piperidine Dihydrochloride (14).** HCl gas deprotection of *tert*-butyl ester **53g** (281 mg, 0.38 mmol) by the general procedure gave **14** (231 mg) as a white solid. R_f 0.54 (MeOH:EtOAc:AcOH:0.88NH_3:H_2O, 60:12:4:4:8); $[\alpha]_D$ −19.1° (*c* = 0.79, H_2O). ^{1}H NMR (DMSO-d_6): δ 1.2−2.4 (mm, 16 H), 2.68 (s, 3 H), 2.7−4.35 (mm, 16 H), 4.74−4.94 (m, 2 H), 5.75 (s, 2 H), 7.6 (s, 4 H), 9.5−10.1 (vbr s, 1 H), 13.0 (vbr s, 1 H). LRMS: *m/z* 478 $(M + H)^+$. Anal. ($C_{25}H_{43}N_5O_4 \cdot 2HCl \cdot 0.5H_2O \cdot 1.0CH_2Cl_2$) C, H, N.

***N*-(*N*-Cyclohexylmethyl-(*S*)-α-aspartyl)-2(*RS*)-[2-(*N*-amidino-4-piperidyloxy)ethyl]piperidine Dihydrochloride (15).** HCl gas deprotection of *tert*-butyl ester **52h** (132 mg, 0.183 mmol) by the general procedure gave **15** (100 mg) as a white foam. R_f 0.56 (MeOH:EtOAc:AcOH:0.88NH_3:H_2O, 60:12:4:4:8). ^{1}H NMR (DMSO-d_6): δ 0.8−2.0 (mm, 23 H), 2.6−3.8 (mm, 13 H), 3.96−4.48 (mm, 2 H), 7.52 (s, 4 H), 8.68 (vbr s, 1 H), 9.48 (vbr s, 1 H), 13.0 (vbr s, 1 H). LRMS: *m/z* 466 $(M + H)^+$, 424, 335, 255. Anal. ($C_{24}H_{43}N_5O_4 \cdot 2HCl \cdot 1.5H_2O \cdot 0.4CH_2Cl_2$) C, H, N.

***N*-(*N*-Benzyl-(*S*)-α-aspartyl)-2(*S*)-[2-(*N*-amidino-4-piperidyloxy)ethyl]piperidine Dihydrochloride (16).** HCl gas deprotection of *tert*-butyl ester **52i** (173 mg, 0.24 mmol) by the general procedure gave **16** (107 mg) as a white solid. R_f 0.55 (MeOH:EtOAc:AcOH: 0.88NH_3:H_2O, 60:12:4:4:8); $[\alpha]_D$ −5.3° (*c* = 0.15, MeOH). ^{1}H NMR (DMSO-d_6): δ 1.14−1.94 (mm, 12 H), 2.62−3.70 (mm, 10 H), 3.88−4.37 (mm, 3 H), 4.55−4.80 (m, 2 H), 7.4 (s, 4 H), 7.5 (s, 5 H), 9.3 (br s, 1 H), 9.98 (br s, 1 H), 12.9 (br s, 1 H). LRMS: *m/z* 460 $(M + H)^+$, 418, 255, 213, 108. Anal. ($C_{24}H_{37}N_5O_4 \cdot 2HCl \cdot 0.5H_2O \cdot 0.66CH_2Cl_2$) C, H, N.

***N*-(*N*-Cyclohexyl-*N*-ethyl-(*S*)-α-aspartyl)-2(*RS*)-[2-(*N*-amidino-4-piperidyloxy)ethyl]piperidine Dihydrochloride (17).** HCl gas deprotection of *tert*-butyl ester **54** (186 mg, 0.253 mmol) by the general procedure gave **17** (156 mg) as a white foam. R_f 0.54 (MeOH:EtOAc:AcOH:0.88NH_3:H_2O, 60:12:4:4:8). ^{1}H NMR (DMSO-d_6): δ 0.85−2.20 (m, 25 H), 2.58−4.40 (m, integral obscured by solvent), 4.62−4.93 (m, 2 H), 7.35−7.63 (m, 4 H), 9.30−9.90 (br m, 1 H). LRMS: *m/z* 480.7 $(M + H)^+$. Anal. ($C_{25}H_{45}N_5O_4 \cdot 2HCl \cdot 0.1H_2O \cdot 1.0CH_2Cl_2$) C, H, N.

***N*-{*N*-Cyclohexyl-*N*-[2-(dimethylamino)ethyl]-(*S*)-α-aspartyl}-2(*RS*)-[2-(*N*-amidino-4-piperidyloxy)ethyl]piperidine Trihydrochloride (18).** HCl gas deprotection of *tert*-butyl ester **55** (390 mg, 0.501 mmol) by the general procedure gave **18** (0.31 mg) as a sticky white foam. R_f 0.15 (MeOH:EtOAc:AcOH:0.88NH_3:H_2O, 60:12:4:4:8). ^{1}H NMR (DMSO-d_6): δ 0.98−2.20 (m, 24 H), 2.60−4.82 (m, integral obscured by HOD), 7.85−8.20 (m, 4 H). Anal. ($C_{27}H_{50}N_6O_4 \cdot 3HCl \cdot 1.0H_2O \cdot 0.7CH_2Cl_2$) C, H, N.

***N*-[*N*-(4-Tetrahydropyranyl)-(*S*)-α-aspartyl]-2(*R,S*)-[2-(*N*-amidino-4-piperidyloxy)ethyl]piperidine Dihydrochloride (19).** HCl gas deprotection of *tert*-butyl ester **52j** (180 mg, 0.253 mmol) by the general procedure gave **19** (125 mg) as a white foam. R_f 0.48 (MeOH:EtOAc:AcOH:0.88NH_3:H_2O, 60:12:4:4:8). ^{1}H NMR (DMSO-d_6): δ 1.19−2.05 (mm, 16 H), 2.68−4.37 (mm, 16 H), 4.58−4.82 (m, 2 H), 7.37 (s, 4 H), 8.92 (vbr s, 1 H), 9.25 (vbr s, 1 H), CO_2H not detected. LRMS: *m/z* 454 $(M + H)^+$. Anal. ($C_{22}H_{39}N_5O_5 \cdot 2HCl \cdot 0.5H_2O \cdot 1.0CH_2Cl_2$) C, H, N.

***N*-[*N*-Methyl-*N*-(3-tetrahydropyranyl)-(*S*)-α-aspartyl]-2(*S*)-[2-(*N*-amidino-4-piperidyl-oxy)ethyl]piperidine Dihydrochloride (20).** HCl gas deprotection of *tert*-butyl ester **53k** (165 mg, 0.228 mmol) by the general procedure gave **20** (124 mg) as an off-white solid. R_f 0.52 (MeOH:EtOAc:AcOH:0.88NH_3:H_2O, 60:12:4:4:8). ^{1}H NMR (DMSO-d_6): δ 1.08−2.18 (m, 21 H), 2.68 (br s, 3 H), 3.70−4.94 (m, integral obscured by HOD), 7.38−7.59 (m, 5 H). Anal. ($C_{23}H_{41}N_5O_5 \cdot 2HCl \cdot 3.1H_2O \cdot 0.6CH_2Cl_2$) C, H, N.

***N*-[*N*-(*N*-Methyl-4-piperidyl)-(*S*)-α-aspartyl]-2(*RS*)-[2-(*N*-amidino-4-piperidyloxy)ethyl]piperidine Trihydrochloride (21).** HCl gas deprotection of *tert*-butyl ester **52l** (230 mg, 0.32 mmol) by the general procedure gave **21** (130 mg) as a white foam. R_f 0.13 (MeOH:EtOAc:AcOH:0.88NH_3:H_2O, 60:12:4:4:8). ^{1}H NMR (DMSO-d_6): δ1.20−2.32 (m, 17 H), 2.63 (s, 3 H), 2.64−4.35 (m, integral obscured by HOD), 4.58−4.83 (m, 1 H). LRMS: *m/z* 467.5 $(M + H)^+$. Anal. ($C_{23}H_{42}N_6O_4 \cdot 3HCl \cdot 1.4H_2O \cdot 0.6CH_2Cl_2$) C, H, N.

***N*-(*N*-Cyclohexyl-*N*-methyl-(*S*)-α-glutamyl)-2(*S*)-[2-(*N*-amidino-4-piperidyloxy)ethyl]piperidine Dihydrochloride (22).** HCl gas deprotection of *tert*-butyl ester **59** (538 mg, 0.730 mmol) by the general procedure gave **22** (412 mg) as a white foam. R_f 0.67 (MeOH:EtOAc:AcOH:0.88 NH_3:H_2O, 60:12:4:4:8); $[\alpha]_D$ −12.7° (*c* = 0.55, H_2O). ^{1}H NMR (DMSO-d_6): δ 1.0−2.5 (m, 23 H), 2.5−2.95 (m, 1 H), 2.66 (s, 3 H), 3.02−3.68 (m, 11.5 H), 3.75−4.9 (m, 2.5 H), 7.45 (s, 4 H), 9.75−9.05 (m, 1 H), 12.3 (br s, 1 H). LRMS: *m/z* 480.2 $(M + H)^+$, 438.2 $(M-CH_3N_2 + 2H)^+$. Anal. ($C_{25}H_{45}N_5O_4 \cdot 2HCl \cdot 1.9H_2O \cdot 0.35CH_2Cl_2$) C, H, N.

Biology

Determination of Inhibitor Potency and Selectivity. The inhibition of thrombin, trypsin, FXa, FVIIa, and plasmin were measured spectrophotometrically in 96 well plates using chromogenic substrates. All assays were carried out in a total reaction volume of 200 μL. Compound dilutions in water were preincubated with enzyme at room temperature for 15 min prior to addition of chromogenic substrate. After 30 min incubation at 30 °C, the optical density was measured at 405 nm in a Thermomax plate reader (Molecular Devices,

Inc). The percentage inhibition and IC_{50} were calculated from triplicate assays of an eight concentration response curves. From the substrate K_m (previously determined by standard methods) and the IC_{50}, the K_i for each inhibitor was calculated from the formula $K_i = IC_{50}/\{(1 + S/K_m)\}$ according to the method of Cheng and Prusoff.[35]

Enzyme and chromogenic substrate concentration and supplier for each assay were as follows: thrombin (human or bovine plasma; Sigma) at final concentrations of 0.04 and 0.08 U/mL, respectively; thrombin substrate—S2238 (H-D-Phe-Pip-Arg-pNA, Quadratech, U.K.), final concentration 0.1 mM; trypsin (bovine pancreas; Sigma), final concentration 0.5 U/mL; trypsin substrate—S2222 (Benz-Ile-Glu-Gly-Arg-pNA, Kabi, Quadratech), final concentration 0.1 mM; plasmin (bovine plasma; Boehringer Mannheim, U.K.), final concentration 0.01 U/mL; plasmin substrate—chromozyme PL (tosyl-Gly-Pro-Lys-pNA; Boehringer Mannheim), final concentration 0.2 mM; FXa (bovine plasma; Boehringer Mannheim), final concentration 0.02 U/mL; FXa substrate—S2222, final concentration 0.2 mM; FVIIa (human plasma; Diagnostica Stago, Shield Diagnostics, U.K.), final concentration 0.06 μg/mL; FVIIa substrate—chromozyme t-PA (*N*-methylsulfonyl-D-Phe-Pro-Arg-pNA; Boehringer Mannheim). Recombinant tissue factor (American Diagnostica, Alpha Labs, U.K.) was added to the FVIIa assay at a final concentration of 0.12 μg/mL. The thrombin, trypsin, and plasmin assays were performed in 50 mM HEPES and 150 mM NaCl buffer (pH 8.0) and at pH 7.5 for the FXa assay. For the FVIIa assay, 50 mM TRIS and 100 mM NaCl buffer (pH 7.5) was used.

Measured K_i values for human thrombin and human trypsin (pancreas; Calbiochem, U.K.) were determined using the same substrates/buffers as described above. Kinetic assays were performed over 15 min, after the addition of substrate (at four different concentrations) to start the reaction. K_i was determined from a plot of $1/V$ against $1/S$.

Determination of Ex Vivo Anticoagulant Activity. Anaesthetized male Sprague—Dawley rats were given compound either by bolus iv injection dissolved in saline or by delivery directly idd dissolved in water. Appropriate vehicle controls were performed. Serial arterial blood samples were collected into one-tenth volume of 3.2% trisodium citrate and centrifuged, and plasma was collected and stored frozen until assay. Thrombin clotting times were determined using an Automated Coagulation Laboratory (ACL-300; Instrumentation Laboratories [IL], U.K.) and the IL Test TT reagent at a thrombin concentration of 6 U/mL. The change in clotting time induced by the inhibitor was recorded as a multiple of the predose TT.

Pharmacokinetics. Male rats (Sprague—Dawley, $n = 3$ per sampling time) and dogs (Beagle, $n = 2$) were used to determine in vivo pharmacokinetic parameters. Drugs were formulated in saline for iv administration (1 mL/kg) by rapid bolus via the caudal vein in rat and by constant rate infusion (over 15 min) via the saphenous vein in dog. Drug solutions in water were administered orally by gavage to rat (10 mL/kg) and dog (1 mL/kg). Plasma was prepared from blood collected into lithium heparin tubes from an indwelling cannula in the cotralateral saphenous vein in conscious dogs and from the vena cava in rats anaesthetized by halothane. Urine samples from dog (0—7 h) were collected via a urethral bladder catheter and from rat (0—24 h) by housing in metabolism cages. Plasma and urine drug concentrations were quantified utilizing LCMS (Perkin-Elmer Sciex API III$^+$ mass spectrometer equipped with an articulated IonSpray interface). Pharmacokinetic parameters were derived by computational analysis using the programs PIVKIN and POPKIN (Dr. B. A. Wood, Sandwich) for intravenous and oral data, respectively.

Drug Assay. Plasma or urine (1 mL) was fortified with compound **8** (100 ng) as internal standard. Calibration standards were prepared covering the range 0.005—1.0 μg 12/mL. Drug and internal standards were extracted from plasma or urine by mixing with pH 6.0 potassium phosphate buffer (1 mL) and percolation through an activated Certify Bond Elut cartridge [3 mL cartridge, prerinsed with methanol (2 mL) and pH 6.0 potassium phosphate buffer (2 mL)]. Following washing with methanol (2 mL) and then water (2 mL) and aspiration to dryness, the cartridge was eluted with 5% methanolic ammonia (3 mL) and the residue was evaporated to dryness. Samples were reconstituted in 50:50 methanol/water, vortexed, centrifuged (13 000 rpm), and submitted for mass spectral analysis using LCMS.

Log $D_{7.4}$ Determinations. These were carried out using the method developed by Stopher and McClean.[36] Concentrations of drug in the organic and aqueous phases were determined by HPLC using a Kromasil Silica column (25 cm × 4.6 mm id), with ultraviolet detection at 210 nm. A mixture of ammonium phospate buffer (pH 3.0, 0.05 M) and acetonitrile (3:1, v/v) was used as the mobile phase with a flow rate of 1 mL/min.

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