Structure–Activity Study of Tripeptide Thrombin Inhibitors Using α-Alkyl Amino Acids and Other Conformationally Constrained Amino Acid Substitutions†

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In our continuing effort to design novel thrombin inhibitors, a series of conformationally constrained amino acids (e.g. α-alkyl, *N*-alkyl cyclic, etc.) were utilized in a systematic structure–activity study of the P3, P2, and P1 positions of tripeptide arginal thrombin inhibitors. Early examples of this effort include: D-MePhe-Pro-Arg-H (**15**), Boc-D-Phg-Pro-Arg-H (**18**), D-1-Tiq-Pro-Arg-H (**23**, D-1-Tiq = D-1,2,3,4-tetrahydroisoquinolin-1-ylcarbonyl), and Boc-D-Phe-Pro-Arg-H (**25**).[10a,20] The current work clarifies the contribution of each residue of the tripeptide arginals toward the potent and selective inhibition of thrombin relative to that of t-PA and plasmin. The α-methylarginal modification in the P1 residue resulted in analogs **30** (D-MePhe at P3) and **32** (D-1-Tiq at P3) which had lower potency toward thrombin while exhibiting improved selectivity. Analogs modified at the P2 site were found to be very sensitive to the conformational changes induced by variations in side chain ring size with the flexible pipecolinic acid **31** being 2 orders of magnitude less potent at thrombin inhibition than the conformationally constrained azetidine analog **20**. Examination of the P3 binding region indicated that α-alkylphenylglycine residues resulted in a tendency to exhibit substantial improvements in selectivity over the nonalkylated residues. Combinations of optimal P3 and P2 changes led to compounds TFA-D-Phg(αEt)-Azt-Arg-H (**16**), TFA-D-Phg(αMe)-Azt-Arg-H (**17**), Ac-D-Phg(αMe)-Azt-Arg-H (**21**), TFA-D-Phg(αMe)-Pro-Arg-H (**27**), **30**, and **32**, which are clearly more selective for thrombin versus plasmin than the nonconformationally constrained compounds.

Thrombosis has emerged in the last several years as one of the most important areas for drug discovery. The process of thrombosis is triggered by a complex proteolytic cascade leading to the formation of fibrin and platelet aggregation. Thrombin converts fibrinogen to fibrin and activates platelets, the major constituents of a thrombus. The enzyme thrombin is the activation product of prothrombin and has numerous bioregulatory functions in hemostasis.[1–3] It is also important to note that the most potent agonist of platelet aggregation is thrombin.[4,5] Thrombin is the central mediator of thrombus formation in the pathogenesis of thrombotic diseases.[6] Due to the diverse functions of this enzyme and its central role in the coagulation cascade, many researchers are becoming cognizant of the importance of thrombin inhibition. The search for synthetic thrombin inhibitors for clinical use is progressing in numerous laboratories. A recently reported tripeptide aldehyde D-1-Tiq-Pro-Arg-H (**23**),[7] contains a confromationally constrained P3 residue and exhibits potent and direct inhibition of thrombin. In our continuing effort to design novel thrombin inhibitors, a series of conformationally constrained amino acids (e.g. α-alkyl, *N*-alkyl cyclic, etc.) were utilized in a systematic structure–activity study of the P3, P2, and P1 positions of tripeptide arginal thrombin inhibitors.

Chemistry

All compounds were synthesized by standard solution-phase peptide synthesis. The properties of the intermediate dipeptides not reported in the experimental section are described in Table 1. Three different synthetic approaches (Schemes 1–3) were employed for the preparation of the desired derivatives. As shown in Scheme 1, the key intermediate α-alkylphenylglycines were prepared in three steps from benzophenone imine and phenylglycine methyl ester.[8] The alkylation of the Schiff base using potassium hydride under anhydrous conditions[9] and subsequent hydrolysis gave the unnatural amino acids **3** and **4**. The dipeptides were constructed in two steps from the 2,4,5-trichlorophenyl active ester using a previously reported methodology.[10a] The diastereomeric tripeptide lactams **9a**, **9b**, **9f**, **9i**, and **9l** were constructed by mixed anhydride coupling and chromatographed over silica gel to obtain the compound with the desired stereochemistry. The protected tripeptide lactams were reduced by $LiAlH_4$ to give the tripeptide aldehydes. Finally, the protecting groups were removed by catalytic hydrogenation to afford compounds **16**, **17**, **21**, **24**, and **27**.

The syntheses of peptides **22** and **26** are outlined in Scheme 2. The unnatural amino acids incorporated into the dipeptides **8g** and **8k** were prepared using a modified procedure of Shuman et al.[11] This procedure employed a catalytic hydrogenation with rhodium on aluminum oxide from isoquinoline-1-carboxylic acid or D-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid.[12] Elaboration to the target compounds **22** and **26** was

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† Symbols and abbreviations are in accordance with the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature (*Eur. J. Biochem.* **1984**, *138*, 9). All amino acids are in the L configuration unless otherwise noted. Other abbreviations: Bz, benzoyl; 2,4,5-TCP, 2,4,5-trichlorophenol; Boc, tert-butyloxycarbonyl; TFA, trifluoroacetic acid; DCC, dicyclohexylcarbodiimide; DCHA, dicyclohexylamine; 1-Tiq, 1-carboxy-1,2,3,4-tetrahyroisoquinoline; Phg, phenylglycine; 3-Piq, 3-carboxyperhydroisoquinoline; 1-Piq, 1-carboxyperhydroisoquinoline; Pip, homoproline; Azt, azetidine-2-carboxylic acid; Thz, thiazolidine-4-carboxylic acid; Phg(αEt), α-ethylphenylglycine.

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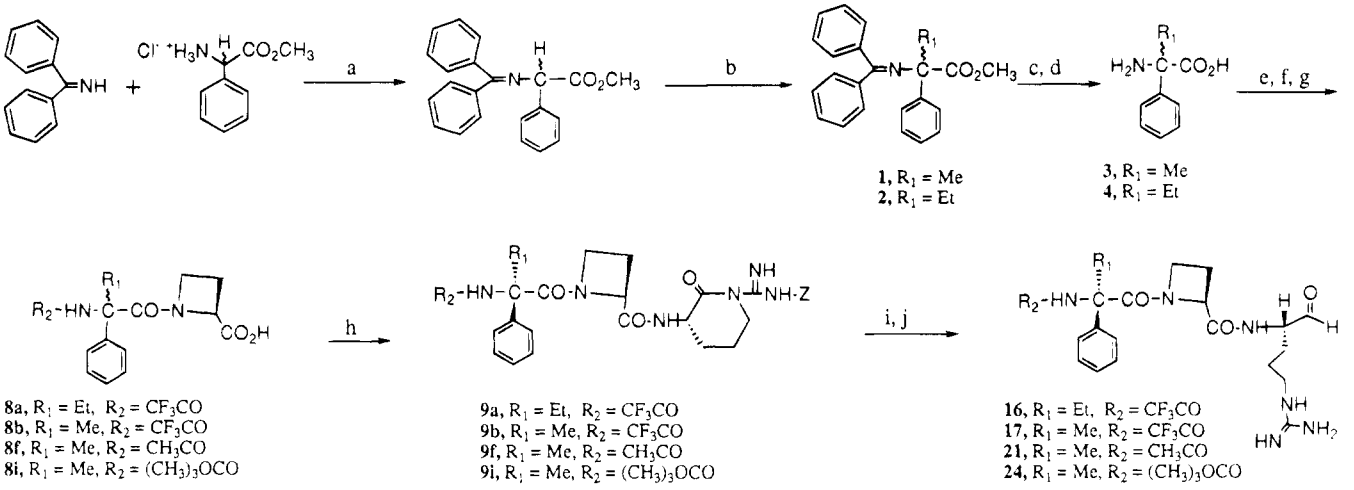
Table 1. Physiochemical Properties of the Dipeptides Fragments[a]

no. peptide	method	formula	anal.[b]	FAB MS[c]	$[\alpha]^{25}_D$,[d] deg	TLC[e] R_f
8a TFA-DL-Phg(αEt)-Azt-OH	A	$C_{16}H_{17}N_2O_4F_3$	C,H,N	359	−41.9	0.33
8c Boc-D-Phg-Azt-OH		$C_{17}H_{22}N_2O_5$	C,H,N	335	−58.4	0.68
8e Boc-D-Phe-Azt-OH		$C_{18}H_{24}N_2O_5$	C,H,N	349	−89.4	0.32
8f Ac-DL-Phg(αMe)-Azt-OH	A	$C_{15}H_{18}N_2O_4$	H,N	291	−36.4	0/12
8i Boc-DL-Phg(αMe)-Azt-OH	A	$C_{18}H_{24}N_2O_5$	C,H,N	349	−73.2	0.36
8k Z-R-(4aS,8aS)-3-Piq-Pro-OH	B	$C_{23}H_{30}N_2O_5$	C,H,N	415	−56.8	NA[f]
8l TFA-DL-Phg(αMe)-Pro-OH	A	$C_{16}H_{17}N_2O_4F_3$	H,N	359	−10.3	0.41
8m Boc-D-Phe-Thz-OH		$C_{18}H_{24}N_2O_5S$	C,N[g]	381	−56.1	0.67
8n Boc-D-Phe-Pip-OH		$C_{20}H_{28}N_2O_5$	C,H,N	377	−17.2	0.39

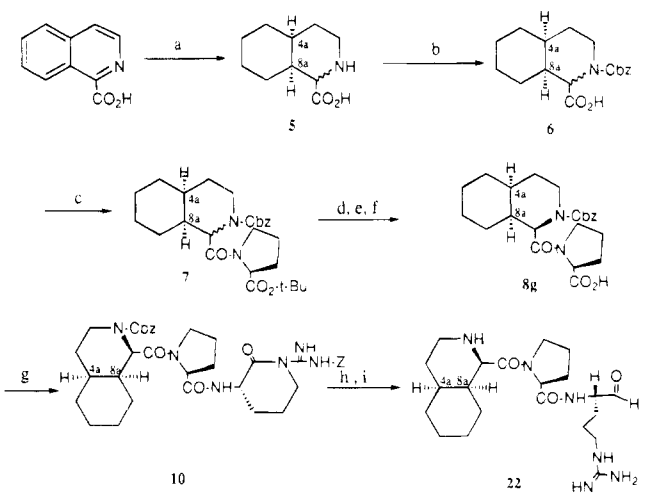
[a] Method used for the synthesis of the unnatural amino acids are described in the Experimental Section; all other dipeptides were prepared from commercially available amino acids. [b] Compounds gave satisfactory analyses (±0.4%). [c] *m/z* (MH^+). [d] c = 0.5. MeOH. [e] Solvent system used $CHCl_3$/MeOH/HOAc (135:15:1). [f] NA = not available. [g] Compound gave satisfactory analyses for 1.5 waters.

Scheme 1[a]



[a] Conditions: (a) CH_2Cl_2; (b) THF/KH/18-crown-6/MeI or EtI; (c) HCl/0 °C; (d) NaOH/reflux; (e) trifluoroacetic anhydride/TFA; or acetic anhydride/TFA; or $(Boc)_2O$/*t*-BuOH/NaOH; (f) DCC/EtOAc/2,4,5-TCP; (g) pyridine/L-azetidine-2-carboxylic acid/Et_3N; (h) HCl Arg(Z) lactam/mixed anhydride/chromatography; (i) $LiAlH_4$/THF/−70 °C; (j) H_2/5% Pd−C/THF/H_2O/1 N HCl, RP-HPLC.

Scheme 2[a]



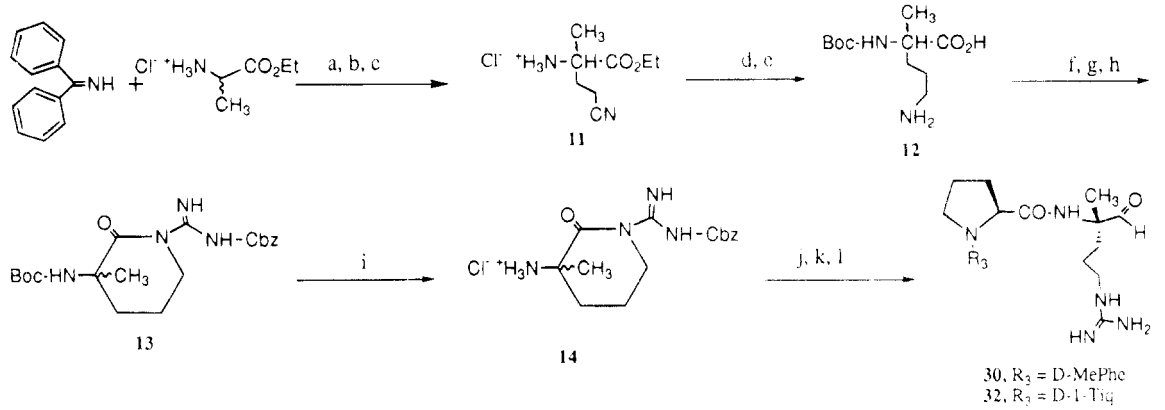
[a] Conditions: (a) EtOH/Rh/$Al_2O_3H_2$/750 psi of H_2/HCl; (b) $PhCH_2OCO$-Cl, NaOH/THF−H_2O; (c) DCC/HOBt/L-proline *tert*-butyl ester/DMF; (d) TFA/anisole; (e) L-(−)-MBA/Et_2O/5 days; (f) neutralization of mother liquor and crystallization; (g) HCl Arg(Z) lactam/mixed anhydride; (h) $LiAlH_4$/THF/−70 °C; (i) H_2/5% Pd−C/THF/H_2O, 1 N HCl, RP-HPLC.

accomplished using standard conditions. Structural assignments of the ring fusion hydrogens for compound **5** (Figure 1) were made from analysis of ^1H and ^{13}C NMR and compared to those of a similar class of compounds.[13] The NMR analysis produces a spectrum at ambient temperature which has broad resonances; however, at 100 °C in DMSO-d_6 the ^1H NMR resonances sharpen, allowing interpretation of the NMR spectrum. Decoupling experiments on compound **5** (Figure 1) show a large (11.0 Hz) coupling between H-4_{ax} and H-4a; the magnitude of this coupling fixes an axial stereochemistry for H-4a. A ROESY experiment at ambient temperature shows NOEs between H-1 and H-3_{ax} and H-1 and H-4a. These NOEs can only exist if H-1 is axial. Assuming a chairlike conformation for the piperidine ring, the stereochemistry for the ring fusion hydrogens in compound **5** would be as shown in Figure 1.

Hydrogenation of the D-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid and incorporation of a Cbz protecting group afforded compounds **33** and **34** (Figure 1) in a 3.5:1 mixture (estimated from integration of the ^1H NMR). The NMR data on a compound (**3a**) reported by Ornstein et al. in a similar series of perhydroisoquinolines compares favorably with NMR data generated for compound **33**.[13] The NMR chemical shifts for compound **33** (the major component) H-1, H-3, and H-4 are 3.67/3.27, 4.52, and 2.02/1.93 ppm, respectively. The NMR chemical shifts for compound **34** (the minor component) H-1, H-3, and H-4 are 3.75/3.18, 4.81, and 2.02/(not recorded) ppm, respectively, and compare favorably to Ornstein and co-workers' report on compound **3b**. Again, assuming a chairlike conformation for the piperidine ring, the stereochemistry for **34** would be as shown in Figure 1. The dipeptide (**8k**) prepared from the mixture of **33** and **34** gave a single component material after crystallization from diethyl ether. The NMR analysis of this dipeptide (**8k**) shows only the amino acid associated with structure **33**.

The DL-C^α-methylarginine containing peptides (**30** and **32**) were synthesized by the procedure outlined in

Scheme 3[a]



[a] Conditions: (a) CH_2Cl_2; (b) KH/18-crown-6/$Br(CH_2)_2CN$; (c) HCl/0 °C; (d) $(Boc)_2O$/THF/DIEA; (e) 1 N NaOH/60 psi of H_2/PtO_2; (f) $CH_3OC(=NH)NH_2 \cdot H_2SO_4$/2 N NaOH; (g) Cbz-Cl/NaOH; (h) *i*-BuOCOCl/NMM; (i) HCl/EtOAc; (j) Z-R$_3$-Pro-OH/DCC/HOBt, chromatography; (k) $LiAlH_4$/THF/−70 °C; (l) H_2/5% Pd−C/THF/H_2O, Rp-HPLC.

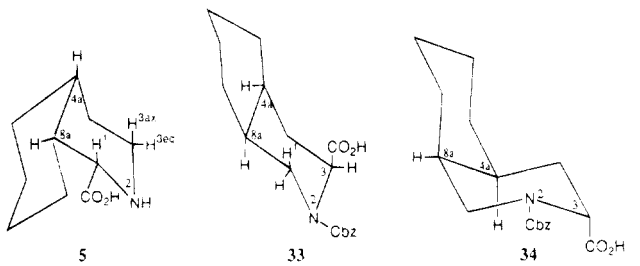


Figure 1. The different conformations of 1- and 3-perhydroisoquinolinecarboxylic acids.

Scheme 3. The Schiff base resulting from the reaction of alanine ethyl ester and benzophenone imine was prepared, and subsequent alkylation using potassium hydride and 3-bromopropionitrile was accomplished in good yields. The protected DL-C^{α}-methylornithine was obtained after hydrolysis of the Schiff base, introduction of a *tert*-butyloxycarbonyl group, and catalytic hydrogenation of the nitrile in the presence of platinum oxide. The ornithine derivative was elaborated into the doubly protected C^{α}-methylarginine with *O*-methylisourea and introduction of a single Cbz protecting group using a standard literature procedure.[14] After cyclization to the lactam, the diastereomeric tripeptides were constructed using a DCC/HOBt coupling of the dipeptide fragment to the DL-C^{α}-methylarginine(Cbz) lactam and chromatographed to obtain the protected tripeptides with the desired stereochemistry. Elaboration to the target compounds **30** and **32** was accomplished using standard conditions.

Enzyme Assays

The biological activities of the compounds reported, herein, were determined by methods previously described[10a,b] and are shown in Table 2. All enzymes and substrates used were obtained from commercial sources and used without additional purification. Amidolytic assays for serine protease inhibition were carried out at 25 °C in 0.1 **M** Tris-HCl buffer (pH 7.4) using a Thermomax plate reader, Molecular Devices, San Francisco, CA. The enzyme and substrate concentrations for each protease assay are in parentheses following the respective substrates: Bz-Phe-Val-Arg-pNA (0.15 mg/mL or 0.125 mg/mL, respectively, with bovine thrombin at 0.77 unit/mL and bovine trypsin at 93 ng/mL), Bz-Ile-Glu-Gly-Arg-pNA (0.135 mg/mL with bovine factor Xa at 73.8 ng/mL), H-D-Val-Leu-Lys-pNA (0.278 mg/mL with human plasmin at 270 ng/mL) or H-D-Ile-Pro-Arg-pNA (0.472 mg/mL with human recombinant tissue plasminogen activator at 81.6 ng/mL). Experiments were conducted in 96-well polystyrene plates, and rates of hydrolysis were determined from the release of *p*-nitroaniline by monitoring the reactions at 405 nm. The following protocol was used for all enzymes studied: 50 μL of buffer (0.1 M Tris-HCl, 0.15 M NaCl, pH 7.4) were added to each well of the 96-well plates, followed by 25 μL of inhibitor solution (in water) and 25 μL enzyme; within 2 min 150 μL of chromogenic substrate (in water) was added to start the enzymatic reactions. The amide hydrolysis rates were shown to be linear with respect to enzyme concentration and were shown to provide linear relationships between inhibitor concentration and percent inhibition for the enzyme concentrations used in the assays. Inhibitor concentrations were varied over a range to provide inhibition higher and lower than 50% for each protease tested. Percent inhibition data points were obtained in triplicate with standard deviations less than 10% of the mean. For each inhibitor concentration which produced between 20% and 80% inhibition an extrapolation was performed to determine an IC_{50} value. For each test compound four inhibitor concentrations were typically used for four such IC_{50} value determinations. Standard deviations from the mean IC_{50} values were in the ranges as follows: 18 ± 11% for thrombin, 13 ± 10% for factor Xa, 15 ± 6% for trypsin, 6 ± 4% for plasmin, and 5 ± 2% for t-PA.

Results and Discussion

The determination of the optimal biologically active conformation of a peptide is critical in the design of new highly potent pharmaceuticals. This determination is usually hindered by the flexibility of the backbone and the side chains of peptides. The number of conformational possibilities in peptides can be reduced by introducing constraints. Thus, one possible approach is to introduce conformational constraints to stabilize the backbone conformation and to control side chain orientation. In an effort to exploit conformationally rigid amino acids to obtain improved enzyme inhibitory potency and selectivity over known tripeptide aldehydes, the evaluation of the structural and conformational role of the amino acid residue in positions P3, P2, and P1 of tripeptide arginals was undertaken.

Table 2. *In Vitro* Enzyme Inhibitory Activity[a] of Tripeptide Arginals

compd	structure[b]	thrombin	trypsin	plasmin	factor X_a	t-PA[c]
15	D-MePhe-Pro-Arg-H	0.0089	0.013	0.67	7.6	21
16	TFA-D-Phg(αEt)-Azt-Arg-H	0.0097	0.0052	4.3	32	62
17	TFA-D-Phg(αMe)-Azt-Arg-H	0.012	0.0096	6.1	98	130
18	Boc-D-Phg-Azt-Arg-H	0.013	0.011	0.15	0.27	6.6
19	Boc-D-Phg-Pro-Arg-H	0.016	0.0098	0.098	0.26	8.7
20	Boc-D-Phe-Azt-Arg-H	0.018	0.0097	0.36	1.3	0.90
21	Ac-D-Phg(αMe)-Azt-Arg-H	0.019	0.011	7.5	86	250
22	R-1-Piq-Pro-Arg-H	0.019	0.053	0.71	0.55	34
23	D-1-Tiq-Pro-Arg-H	0.019	0.023	1.5	0.64	430
24	Boc-D-Phg(αMe)-Azt-Arg-H	0.022	0.015	3.9	0.89	120
25	Boc-D-Phe-Pro-Arg-H	0.045	0.014	0.19	1.6	0.95
26	R-3-Piq-Pro-Arg-H	0.046	0.0091	0.23	3.3	30
27	TFA-D-Phg(αMe)-Pro-Arg-H	0.064	0.18	45	190	>30000
28	Boc-D-Phe-Thz-Arg-H	0.088	0.0088	0.34	0.86	23
29	Boc-D-Phe-DL-Pro(5,5Me)-Arg-H	0.29	0.17	11	18	>30000
30	D-MePhe-Pro-Arg(αMe)-H	1.0	240	850	1800	>30000
31	Boc-D-Phe-Pip-Arg-H	1.7	0.72	8.1	32	48
32	D-1-Tiq-Pro-Arg(αMe)-H	4.9	88	740	500	>30000

[a] IC_{50} (μM). [b] 1-Tiq = 1-carboxy-1,2,3,4-tetrahydroisoquinoline, Phg(αEt) = α-ethylphenylglcine, 1-Piq = (4a*S*,8a*S*)-perhydroisoquinolin-1-ylcarboxy, 3-Piq = (4a*S*,8a*S*)-perhydroisoquinolin-3-ylcarboxy, 3-Tiq = 3-carboxy-1,2,3,4-tetrahydroisoquinoline, Pro(5,5Me) = 5,5-dimethylproline, Arg-H = arginine aldehyde, TFA = trifluoroacetyl, Pip = homoproline, Thz = thiazolidine-4-carboxylate, Azt = azetidine-2-carboxylate, Arg(αMe)-H = α-methylarginine aldehyde. [c] Tissue plasminogen activator.

Table 3. *In Vitro* Enzyme Selectivity[a] of Peptide Arginals

compd	structure	plasmin/ thrombin	t-PA[b]/ thrombin
15	D-MePhe-Pro-Arg-H	80	2400
16	TFA-D-Phg(αEt)-Azt-Arg-H	440	6400
17	TFA-D-Phg(αMe)-Azt-Arg-H	510	11000
18	Boc-D-Phg-Azt-Arg-H	12	510
19	Boc-D-Phg-Pro-Arg-H	6	540
20	Boc-D-Phe-Azt-Arg-H	20	50
21	Ac-D-Phg(αMe)-Azt-Arg-H	400	13000
22	R-1-Piq-Pro-Arg-H	40	1800
23	D-1-Tiq-Pro-Arg-H	80	23000
24	Boc-D-Phg(αMe)-Azt-Arg-H	180	5500
25	Boc-D-Phe-Pro-Arg-H	4	20
26	R-3-Piq-Pro-Arg-H	5	650
27	TFA-D-Phg(αMe)-Pro-Arg-H	700	>50000
28	Boc-D-Phe-Thz-Arg-H	4	260
29	Boc-D-Phe-DL-Pro(5,5Me)-Arg-H	38	>50000
30	D-MePhe-Pro-Arg(αMe)-H	850	>30000
31	Boc-D-Phe-Pip-Arg-H	5	30
32	D-1-Tiq-Pro-Arg(αMe)-H	150	>6000

[a] Ratio of IC_{50}'s. [b] Tissue plasminogen activator.

Several series of analogs were prepared and evaluated for their ability to inhibit various serine proteases (Table 2). In order for these compounds to be therapeutically useful, it is important that they do not inhibit the fibrinolytic processes through inhibition of the enzymes plasmin and tissue plasminogen activator (t-PA). A measure of the predicted therapeutic usefulness of these inhibitors might be obtained by examination of the plasmin to thrombin or the t-PA to thrombin IC_{50} ratios, where higher values denote greater selectivity (Table 3).

Substitution at the P1 Position of Thrombin Inhibitor. Increasing the conformational rigidity of the peptide in the P1 position was examined by replacement of arginine aldehyde with C^{α}-methylarginine aldehyde. The incorporation of C^{α}-methylarginine has led to peptides which exhibited unexpected biological activity.[14] However, in the present example, analogs (**30** and **32**) were prepared and exhibited <100-fold loss in thrombin inhibition but with an improved selectivity for all the other serine proteases measured. This loss in potency caused by the C^{α}-methylarginine in **30** and **32** (versus compounds **15** and **23**, respectively) could be the result of steric effects which cause the peptide backbone to be perturbed. Analog **30** was evaluated for its ability to bind to the active site of bovine thrombin using molecular modeling techniques. An important intermolecular hydrogen bond that normally exists between the amide bond at the P2 residue of the peptide aldehyde inhibitors and the Glu 192 binding site on thrombin is perturbed by the presence of the α-methyl moiety.[15]

Substitution at the P2 Position of Thrombin Inhibitor. There are several types of amino acids that alter backbone conformation upon incorporation into a peptide. One of these conformationally constrained amino acids is proline. In this amino acid the side chain is linked to the amino group creating a rigid five-membered pyrrolidine ring. The role of proline and its analogs in bioactive peptides must be taken into account, because they can result in a β-turn which can dramatically influence the overall conformation of the peptide. The proline residue in **25** was replaced by azetidine-2-carboxylic acid (**20**), thiazolidine-4-carboxylic acid (**28**), 5,5-dimethylproline[16] (**29**), or pipecolinic acid (**31**) in order to investigate the influence of proline on enzyme selectivity and potency.

Since the role of the γ sulfur atom in the thiazolidine ring of thiazolidine-4-carboxylic acid (Thz) on the orientations of the carbonyl amide moiety in a peptide is unknown, analog **28** was prepared. Compound **28** exhibited a 2-fold decrease in potency with respect to its ability to inhibit the enzyme thrombin along with a 10-fold increase in its t-PA to thrombin selectivity. This loss in potency could be a result of the added steric effects caused by the bulky sulfur atom in the pyrrolidine ring, resulting in unfavorable interaction with thrombin.

The 5,5-dimethylproline analog of proline is reported by Magaard and co-workers to exist nearly 90% in the cis peptide bond form with no significant alterations in bond angle and internal torsion angle.[16] Substitution of the proline residue in compound **25** with the 5,5-dimethylproline residue resulted in compound **29**, which exhibited a 6-fold loss in potency with respect to its ability to inhibit the enzyme thrombin. However, this analog exhibited approximately a 1000-fold increase in its t-PA to thrombin selectivity. These results may imply that the *cis* conformation about the amide bond between the P3 and P2 positions may be advantageous for high selectivity between t-PA and thrombin. The

consequence of the previous statement is that the *trans* conformation may be favorable for increasing inhibition of t-PA.

The six-membered ring analog of proline is pipecolinic acid. The substitution of the proline residue in **25** with a pipecolinic acid residue gave compound **31**, which exhibited a 40-fold decrease in potency with respect to its ability to inhibit the enzyme thrombin with no significant improvement in selectivity. These results may be due to the fact that pipecolinic acid induces alterations in bond angles and internal torsion angles from those attainable by proline, resulting in a missalignment of the functional binding sites within the molecule. Additionally, Toniolo[17] observed that the *trans* tertiary amide conformers of pipecolinic acid are slightly more stable than the *cis* conformers, thereby reducing the possibility of the *cis* conformer about the amide linkage.

The four-membered ring analog of proline is azetidine-2-carboxylic acid (Azt). In published structures the Azt ring is either nearly planar or slightly buckled.[17] Thus, substitution of Azt for Pro in a peptide should be expected to affect the structure mainly through side group steric effects and not directly through effects on the backbone conformation.[18] In addition, reduction to the four-membered azetidine ring has significant effects on bond angles and internal torsion angle, but not on *cis–trans* isomerism according to the calculations of Zagari et al.[19] The substitution of the proline residue in **25** with Azt resulted in compound **20**, which exhibited a 2-fold increase in potency with respect to its ability to inhibit the enzyme thrombin and demonstrated a slight increases in selectivity. Thus, the difference between the larger pipecolinic acid analog **31** and the smaller azetidine-2-carboxylic acid analog **20** in ability to inhibit thrombin was approximately 2 orders of magnitude.

The previously reported[10a] substitution of phenylglycine (**19**) for phenylalanine (**25**) in the P3 position caused an increase in potency for inhibition of thrombin. The above results, therefore, prompted the incorporation of Azt in P2 and Phg in P3. The resulting analog (**18**) demonstrated a slight increase in thrombin inhibitory potency and little improvement in selectivity.

Substitution at the P3 Position of Thrombin Inhibitor. Replacement of the D-1-Tiq in **23**, an already constrained phenylglycine amino acid, with a constrained amino acid which adds lipophilicity to the molecule by saturation of the aromatic ring (*cis*-perhydroisoquinoline-3-carbonyl, D-3-Piq) resulted in analog **26**. Compound **26** had a 2-fold loss in its ability to inhibit thrombin; however, it also exhibited a 8-fold loss in plasmin to thrombin and 2-fold loss in t-PA to thrombin selectivity. By contrast, substitution with *cis*-perhydroisoquinoline-1-carboxylic acid (D-1-Piq) in **23** resulted in compound **22** which exhibited no loss in potency. However, when the thrombin to t-PA ratios are compared, compound **22** has suffered a 10-fold loss in selectivity as compared to compound **23**. However, compound **22** was the only analog that maintained potency for inhibition of thrombin while losing potency for inhibition of trypsin. Thus, when the thrombin to trypsin ratio for analog **22** is compared to compound **23**, a 3-fold improvement in selectivity is observed for analog **22**.

The replacement of the phenylglycine residue in **18** with the conformationally constrained α-methylphenylglycine resulted in compound **24** exhibiting a 2-fold loss in potency toward thrombin but a 10-fold increase in selectivity for thrombin versus both plasmin and t-PA. This result prompted the incorporation of α-ethylphenylglycine in the P3 position of compound **17**. The analog obtained (**16**) exhibited a slight increase in thrombin inhibition potency with no significant loss in selectivity. Replacement of the Boc group in the phenylglycine analog **24** with either acetyl (**21**) or trifluoroacetyl (**17**) gave analogs with increases in potency toward thrombin and improved plasmin to thrombin selectivity. When the Azt in compound **17** was replaced with proline (**27**) there was a loss in potency and a substantial increase in selectivity.

Conclusion

This work has demonstrated the critical importance of each residue of the tripeptide arginals toward the potent and selective inhibition of thrombin relative to that of t-PA and plasmin. However, none of the analogs generated, with the possible exception of analog **22**, exhibited improvements in selectivity of thrombin versus trypsin, while maintaining reasonable potency. The α-methylarginal modification in the P1 residue resulted in analogs **30** and **32** which had lower potency while exhibiting improved selectivity for all measured enzymes. The potency toward thrombin inhibition of analogs modified at the P2 site was found to be very sensitive to the conformational changes induced by variations in side chain ring size with the pipecolinic acid **31** being 2 orders of magnitude less than the azetidine analog **20**. Examination of the P3 binding region indicated that α-alkylphenylglycine residues resulted in a tendency to exhibit substantial improvements in selectivity over the nonalkylated residues. Combinations of optimal P3 and P2 changes led to compounds **16**, **17**, **21**, and **27**, which are clearly more selective for thrombin versus plasmin than the unmodified compounds. A systematic investigation of SAR has resulted not only in the development of more potent agents but also in substantial differences in the specificity in this class of compounds. The findings from this series of thrombin inhibitors provide useful clues for the design of more potent and selective serine protease inhibitors. It is important to point out that proper selectivity for a thrombin inhibitor requires that a candidate not interfere with endogenous or exogenous plasminogen activator mediated fibrinolysis. The wide range of inhibitory effects toward plasmin and t-PA shown in Table 2 suggests that certain of the arginals would not interfere with t-PA-mediated fibrinolysis (for example, compounds **20**, **23**, and **27**), but other compounds like **20**, **25**, and **31** could produce such interference. The next step in the process is to evaluate selected compounds in animal models of thrombosis to correlate the relationship between *in vitro* enzyme activity and anticoagulation.

Experimental Section

Chemistry. The unnatural amino acids used in this study were obtained from commercial sources or prepared according to methods A or B and used without detailed characterization. Compounds **19**, **23**, and **25** were prepared according to a previously reported literature procedure.[10a] Compound **15** was a gift from Dr. S. Bajusz at the Hungarian Institute for Drug Research.[20] The unnatural amino acid in the P2 position of compound **29** was prepared according to a literature procedure.[16] Reactions were monitored, and the homogeneity of the products was checked by TLC on Kieselgel-60 F_{254} plates

(Merck, Darmstadt, BRG) with the following eluents (all v/v): (A) $CHCl_3$/MeOH/HOAc (135:15:1); (B) (18:6:1); (C) EtOAc/hexane (3:2). Analytical RP-HPLC was performed on a Pharmacia FPLC liquid chromatography instrument (LCC-500) with UV−visible detector at 214 nm, utilizing a Vydac C_{18} 5 μm particle size, 0.46- × 15-cm column, with eluent system 0.1% TFA (pH 2.0)/CH_3CN under gradient condition at a flow rate of 0.5 mL/min. Molecular weights of peptides were determined by fast atom bombardment (FAB) mass spectra on an VG Analytical Zab 2 SE mass spectrometer. Amino acid analyses were performed on a Beckman System 6300 high-performance amino acid analyzer equipped with a 3 mm × 20 cm column of cation exchange resin (Na^+ form). Elemental analysis indicated by symbols of the elements refer to data within ±0.4% of the theoretical values. All NMR spectra were obtained on a Bruker AM500 spectrometer operating at 500 MHz. Spectra were recorded in the temperature range of 25−100 °C, as is further described in the text. All reagents used were obtained from commercial sources and used without additional purification.

Preparation of Amino Acids (General Procedure, Method A): (Ph)$_2$C=DL-Phg(αMe)-OMe (1). Methyl N^{α}-(diphenylmethylene)-DL-phenylglycinate was prepared by the method of O'Donnel et al. (cf. ref 8). A solution of methyl N^{α}-(diphenylmethylene)-DL-phenylglycinate (14.8 g, 44.8 mmol) in anhydrous THF (200 mL) was added to a stirred mixture of 18-crown-6 (11.8 g, 44.8 mmol), KH (11.2g, 67.3 mmol), and THF (100 mL) under a N_2 atmosphere. A solution of methyl iodide (6.0 mL, 89.7 mmol) in THF (20 mL) was added slowly to the reaction mixture. After the reaction mixture was stirred for 1.5 h at room temperature, the reaction was quenched by adding a solution of glacial acetic acid (7.0 mL), water, and THF (30 mL) cautiously. The reaction mixture was diluted with EtOAc (300 mL) and water (200 mL). The EtOAc layer was separated, washed with water (2 × 100 mL), dried ($MgSO_4$), and filtered. The organic solvent was removed *in vacuo* to an oil. The oil was dissolved in hexane (200 mL), and after standing at 4 °C (4 h), the precipitate was filtered, washed with hexane, and dried *in vacuo* to afford pure **1** (10.2 g, 66%): MS (FAB) *m/e* 344 (MH^+). Anal. ($C_{23}H_{21}NO_2$) C, H, N.

DL-Phg(αMe) (3). A suspension of **1** (72.4 g, 211 mmol) in 5 N HCl (400 mL) was refluxed (24 h). The reaction mixture was cooled to room temperature, and filtered. The pH of the filtrate was raised to 5.8 with dilute NH_4OH solution and concentrated *in vacuo* until crystallization began. The reaction mixture was stored overnight at 5 °C and the resulting precipitate filtered and dried to give pure **3** (22 g, 63%): MS (FAB) *m/e* 166 (MH^+).

CF_3CO-DL-Phg(αMe)-Azt-OH (8b). To a stirred solution of **3** (21.9 g, 133 mmol) in TFA (250 mL) was added trifluoroacetic anhydride (33.5 g, 159 mmol). The reaction mixture was stirred for 2 h at reflux. The solvent was removed *in vacuo*, and the residue was dissolved in EtOAc (200 mL) and washed with water (3 × 100 mL). The organic layer was dried ($MgSO_4$), filtered, and evaporated to give crude TFA-DL-Phg(αMe)-OH (25.3 g, 73%) as a white solid. The crude trifluoroacetyl amino acid (8.0 g, 31 mmol) was prepared using the method of Shuman et al. (cf. ref 10a) to afford pure **8b** (9.3 g, 88%): MS (FAB) *m/e* 345 (MH^+); $[\alpha]^{25}_D$ −80° (*c* 0.5, $CHCl_3$). Anal. ($C_{15}H_{15}N_2O_4F_3$) C, H, N.

TFA-D-Phg(αMe)-Azt-Arg(Z) lactam (9b). In flask 1, **8b** (6.7 g, 19.9 mmol) was dissolved in DMF (50 mL) and cooled to −15 °C, and *N*-methylmorpholine (2.5 mL, 21.9 mmol) was added followed by isobutyl chloroformate (2.6 mL, 19.9 mmol). The reaction mixture was stirred at −15 °C for 2 min. In flask 2, HCl·Arg(Z) lactam[14] (6.5 g, 19.9 mmol) was dissolved in DMF (40 mL) and cooled to 0 °C, diisopropylethylamine (7.0 mL, 39.9 mmol) was added to the solution, and the mixture was stirred at 0 °C for 2 min. The contents of flask 2 was added to flask 1, and the reaction mixture was stirred for 4 h (−15 °C) followed by 24 h at room temperature. A solution of 5% $NaHCO_3$ (18 mL) was added, and the mixture was concentrated *in vacuo*. The residue was dissolved in EtOAc (200 mL) and water (100 mL). The organic layer was separated and washed sequentially with 1 N $NaHCO_3$, water, and 0.01 N HCl. The EtOAc layer was dried ($MgSO_4$), filtered, and evaporated to give 11.5 g of crude **9b**. The crude solid (11.5 g) was purified by chromatography on silica gel using a step gradient elution (CH_2Cl_2 (100) to CH_2Cl_2−EtOAc, 80:20) to yield pure **9b** (3.02 g, 25%): TLC R_f (A) 0.45; MS (FAB) *m/e* 617 (MH^+); $[\alpha]^{25}_D$ −95.9° (*c* 0.5, $CHCl_3$).

TFA-D-Phg(αMe)-Azt-Arg-H·HCl (17). Compound **17** was prepared from **9b** using the LAH reduction and hydrogenation procedures reported by Shuman et al. (cf. ref 10a) to give pure **17** (71%): MS (FAB) *m/e* 485 (MH^+); $[\alpha]^{25}_D$ −77.6° (*c* 0.5, 0.1 N HCl). Anal. ($C_{21}H_{27}N_6O_4F_3$·HCl) C, H.

Preparation of Amino Acids (General Procedure, Method B): Cbz-(4aS*,8aS*)-1,2,3,4,4a,5,6,7,8,8a-Decahydroisoquinoline-1(*RS*)-carboxylic Acid (6). A solution of 1-isoquinolinecarboxylic acid (50 g, 0.288 mol) in EtOH (150 mL) and 5 N HCl (60 mL) was reacted with hydrogen over 5% Rh/Al_2O_3 (14 g) at 750 psi in a stainless steel autoclave at 50 °C for 17 h. The reaction mixture was filtered through a Celite pad, and the filtrate was concentrated *in vacuo*. The solid triturated with water, filtered, and dried to give the amino acid (30g, 48%): ^{1}H NMR (DMSO-d_6) δ 3.98 (bs, 1 H), 3.18 (bd, 1 H), 2.38 (dd, 1 H), 2.14 (m, 1 H), 1.97 (m, 1 H), 1.86 (m, 1 H), 1.69 (bd, 1 H), 1.59 (m, 1 H), 1.52 (m, 2 H), 1.38 (m, 1 H), 1.34 (bd, 1 H), 1.20 (m, 1 H), 1.17 (m, 1 H), 1.08 (m, 1 H); ^{13}C NMR (DMSO-d_6) δ 59.61, 43.10, 20.87, 31.99, 30.35, 19.68, 25.21, 20.26, 35.70, 169.60.

The solid (30.2 g, 137 mmol) was suspended in THF (150 mL) and water (150 mL). The pH of the reaction mixture was adjusted to 9.8 with 5 N NaOH, and the mixture was stirred at room temperature. A solution of benzyl chloroformate (21.6 mL, 151 mmol) in THF (50 mL) was added slowly to the reaction mixture while the pH was maintained at 9.5 with 2 N NaOH. After 2 h at room temperature the organic solvent was evaporated, and the resulting aqueous solution was extracted once with diethyl ether (150 mL). The aqueous layer was separated and acidified with 5 N HCl to pH 2.5 and extracted with ethyl acetate (200 mL). The organic solution was dried ($MgSO_4$), filtered, and concentrated *in vacuo* to an oil. The oil was crystallized from diethyl ether (150 mL) to afford pure **6** (32 g, 75%): MS (FAB) *m/e* 318 (MH^+).

Cbz-R-*cis*-(4aS,8aS)-1-Piq-Pro-OH (8g). To a stirred, cooled (0 °C) solution of **6** (31.8 g, 100 mmol) in DMF (100 mL) were added L-proline *tert*-butyl ester (17.1 g, 100 mmol), HOBt (13.5 g, 100 mmol), and DCC (20.6 g, 100 mmol). The reaction mixture was stirred for 3 h at 0 °C, warmed to room temperature, and stirred (24 h). The reaction mixture was cooled (0 °C), the precipitate was removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was dissolved in EtOAc and washed sequentially with 1 N $NaHCO_3$, water, 1.5 N citric acid, and water. The EtOAc layer was dried ($MgSO_4$), filtered, and evaporated to give compound **7** (47.0 g, 100%) as an oil. The oil (47.0 g, 100 mmol) was dissolved in TFA (100 mL), CH_2Cl_2 (35 mL), and anisole (5 mL) and stirred at room temperature (1 h). The reaction solvent was removed *in vacuo* without heating. The resultant oil was dissolved in diethyl ether (100 mL) and water (100 mL) and the pH adjusted to 9.8 with 5 N NaOH. The aqueous layer was separated, EtOAc (200 mL) was added, and the solution was acidified with 5 N HCl to pH 2.5. The organic layer was separated, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The resultant oil was dissolved in diethyl ether (700 mL), and L-(-)-α-methylbenzylamine was added to the solution. After standing at room temperature for 5 days the precipitate was filtered. The filtrate was suspended in EtOAc, washed with 1.5 N citric acid and water, dried ($MgSO_4$), and filtered. The EtOAc was evaporated *in vacuo* to an oil which was crystallized from diethyl ether (400 mL) to give pure **8g** (5.86 g, 36%): TLC R_f (A) 0.47; MS (FAB) *m/e* 415 (MH^+); $[\alpha]^{25}_D$ −27.3° (*c* 0.5, MeOH). Anal. ($C_{23}H_{30}N_2O_5$) C, H, N.

Cbz-R-*cis*-(4aS,8aS)-3-Piq-Pro-OH (8k). A solution of D-1,2,3,4-tetrahydro-3-isoquinolinecarboxylic acid (17 g, 96 mmol) in water (200 mL) and 5 N HCl (20 mL) was reacted with hydrogen over 5% Rh/Al_2O_3 (8.5 g) at 2000 psi in a stainless steel autoclave at 120 °C (16 h). The reaction mixture was filtered through a Celite pad, and the filtrate was concentrated *in vacuo*. The solid triturated with water, filtered, and dried to give the amino acid (21 g, 100%): MS (FD) *m/e* 184 (MH^+). The solid (21.0 g, 95.8 mmol) was

suspended in THF (75 mL) and water (50 mL). The pH of the reaction mixture was adjusted to 9.8 with 5 N NaOH and stirred at room temperature. A solution of benzyl chloroformate (16.4 mL, 115 mmol) in THF (50 mL) was added slowly to the reaction mixture while the pH was maintained at 9.5 with 2 N NaOH. After 2 h at room temperature, the organic solvent was evaporated, and the resulting aqueous solution was extracted once with diethyl ether (150 mL). The aqueous layer was separated and acidified with 5 N HCl to pH 2.5 and extracted with ethyl acetate (200 mL). The organic solution was dried ($MgSO_4$), filtered, and concentrated *in vacuo* to an oil (25.8 g, 85%): MS (FAB) *m/e* 318 (MH^+); $[\alpha]^{25}_D = -5.1°$ (*c* 0.5, MeOH). To a stirred, cooled (0 °C) solution of the oil (17.2 g, 54 mmol) in DMF (50 mL) was added L-proline *tert*-butyl ester (19.2 g, 54 mmol), HOBt (7.3 g, 54 mmol), and DCC (11.1 g, 54 mmol). The reaction mixture was stirred for 3 h at 0 °C, warmed to room temperature, and stirred (24 h). The reaction was cooled (0 °C), the precipitate was removed by filtration, and the filtrate was concentrated *in vacuo*. The residue was dissolved in EtOAc and washed sequentially with 1 N $NaHCO_3$, water, 1.5 N citric acid, and water. The EtOAc layer was dried ($MgSO_4$), filtered, and evaporated to give an amorphous solid (23.8 g, 94%): MS (FAB) *m/e* 471 (MH^+); $[\alpha]_D = -40.0°$ (*c* 0.5, MeOH). The solid (31.2 g, 66.3 mmol) was dissolved in TFA (100 mL), CH_2Cl_2 (35 mL), and anisole (5 mL) and stirred at room temperature (1 h). The reaction solvent was removed *in vacuo* without heating. The resultant oil was dissolved in diethyl ether (150 mL) and water (100 mL) and the pH adjusted to 9.8 with 5 N NaOH. The aqueous layer was separated, EtOAc (200 mL) was added, and the solution was acidified with 5 N HCl to pH 2.8. The organic layer was separated, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The resultant oil was dissolved in diethyl ether (300 mL) and after standing at room temperature for 5 days the precipitate was filtered and dried to give pure **8k** (13.5 g, 49%): MS (FAB) *m/e* 415 (MH^+); $[\alpha]^{25}_D$ −57° (*c* 0.5, MeOH). Anal. ($C_{23}H_{30}N_2O_5$) C, H, N. The NMR spectra of **8k** has resonances that are broad and nondescript. This is due to isomerism about the two nitrogen−carbonyl bonds. An analytical sample of **8k** was deblocked under catalytic hydrogenation condition in the presence of 5% Pd/C and a stoichiometric amount of 1 N HCl, and the HCl salt was isolated by evaporation of the reaction solvent. The NMR spectra were obtained on the HCl salt of the dipeptide: ^1H NMR (DMSO-d_6) δ 1.23 (m, 1 H), 1.28 (m, 1 H), 1.32 (m, 1 H), 1.39 (m, 1 H), 1.56 (m, 1 H), 1.65 (m, 1 H), 1.68 (m, 1 H), 1.71 (m, 2 H), 1.77 (m, 1 H), 1.82 (m, 1 H), 1.87 (m, 1 H), 1.91 (ddd, 1H), 2.01 (m, 1 H), 2.23 (m, 1 H), 2.74 (dd, 1 H), 3.31 (ddd, 1 H), 3.63 (ddd, 1 H), 3.83 (dd, 1 H), 4.20 (m, 1 H), 4.24 (dd, 1 H); ^{13}C NMR (DMSO-d_6) δ 46.29, 60.72, 28.63, 33.74, 30.27, 19.48, 25.22, 24.02, 35.58, 57.01, 28.63, 20.73, 44.15, 164.32, 163.49.

Cbz-R-*cis*-(4a*S*,8a*S*)-1-Piq-Pro-Arg(Z) Lactam (10). **10** was prepared from **8g** in a similar manner to that for **9b**. The pure **10** was isolated as an amorphous solid (76%): TLC R_f (A) 0.66; MS (FAB) *m/e* 687 (MH^+); $[\alpha]^{25}_D$ −34.1° (*c* 0.5, THF).

R-*cis*-(4a*S*,8a*S*)-1-Piq-Pro-Arg-H·2HCl (22). Compound **22** was prepared from **10** using the LAH reduction and hydrogenation procedures reported by Shuman et al. (cf. ref 10a) to give pure **22** (92%): MS (FAB) *m/e* 421 (MH^+); $[\alpha]^{25}_D$ −86.3° (*c* 0.5, 0.01 N HCl). Anal. ($C_{21}H_{36}N_6O_3 \cdot 2HCl \cdot 2H_2O$) C, H, N.

Preparation of Amino Acid: DL-Ala(α-propionitrile)-OEt (11). Ethyl N^{α}-(diphenylmethylene)-L-alaninate was prepared by the method of O'Donnel et al. (cf. ref 8). Ethyl (N^{α}-(diphenylmethylene)-L-alaninate (20 g, 71.2 mmol) dissolved in anhydrous THF (300 mL) was added slowly to a stirred solution of 18-crown-6 (18.8 g, 71.2 mmol) and KH (17.8 g, 106.8 mmol) in THF (100 mL) under a N_2 atmosphere. To the stirred, cooled (0 °C) reaction mixture was added a solution of bromopropionitrile (8.9 mL, 106.8 mmol) in THF (20 mL). The resulting mixture was stirred at 0 °C for 30 min and warmed to room temperature for 2 h. The reaction mixture was quenched by adding a solution of glacial acetic acid (6.5 mL), water (25 mL), and THF (20 mL) dropwise. The reaction mixture was diluted with EtOAc (200 mL) and water (200 mL). The organic layer was separated, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The crude oil was purified by chromatography on silica gel using a step gradient elution (hexanes (100) to hexanes−EtOAc, 20:80) to yield 15.5 g (65%) of the Schiff base of DL-Ala(α-propionitrile) ethyl ester as an oil. A stirred, cooled (0 °C) solution of the oil (15.2 g, 45.4 mmol) in diethyl ether (90 mL) was reacted with 1 N HCl (54 mL) for 2 h. The reaction mixture was warmed slowly to room temperature and stirred (24 h). The aqueous layer was separated, extracted three times with diethyl ether, and concentrated *in vacuo* to give a clear oil of pure **11** (9.7 g, 100%): MS (FAB) *m/e* 171 (MH^+).

Boc-DL-Orn(αMe) (12). To a stirred solution of **11** (7.8 g, 37.8 mmol) in THF (50 mL) was added diisopropylethylamine (6.6 mL, 37.8 mmol) and di-*tert*-butyl dicarbonate (9.6 mL, 41.6 mmol). After 24 h at room temperature the bulk of the THF was evaporated. The reaction was diluted with EtOAc/water. The EtOAc layer separated and was washed two times with 0.1 N HCl, dried ($MgSO_4$), and filtered. The organic solvent was removed *in vacuo* to afford an oil. To a stirred, cooled (0 °C) solution of the oil (12.6 g, 46.4 mmol) in THF (100 mL) and water (58 mL) was added 1 N NaOH (47 mL, 47 mmol). The reaction mixture was stirred for 30 min at 0 °C and warmed to room temperature. After 4 h at room temperature the organic solvent was evaporated, and the resulting aqueous solution was extracted once with EtOAc (100 mL). The aqueous layer was separated, EtOAc (150 mL) was added, and the solution was acidified with 3 N HCl to pH 2.8. The organic solution was separated, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to afford a clear oil. A solution of the oil (10.8 g, 44.4 mmol) in EtOH (135 mL) was reacted with hydrogen over platinum oxide (3 g) at 60 psi in a Parr shaker apparatus at 60 °C for 24 h. The reaction mixture was filtered through a Celite pad, and the filtrate was concentrated *in vacuo*. The solid was triturated with a mixture of equal amounts of THF, diethyl ether, and pentane, filtered, and dried to afford pure **12** (8.2 g, 88%): MS (FAB) *m/e* 247 (MH^+). Anal. ($C_{11}H_{22}N_2O_4$) H, N; C: calcd, 53.64; found, 52.71.

Boc-DL-Arg(αMe)(Cbz) Lactam (13). The pH of compound **12** (7.6 g, 30.9 mmol) in water (80 mL) was raised to 10.5 with 2 N NaOH. To the reaction mixture was added *O*-methylisourea hydrogen sulfate (10.6 g, 61.7 mmol). After 48 h at room temperature the reaction mixture was cooled to 0 °C. The precipitate was filtered and dried *in vacuo* to afford a white solid (5.8 g). The white solid (5.7 g, 18.4 mmol) was dissolved in water (50 mL) and the pH raised to 13.4 with 5 N NaOH. To the stirred, cooled (−5 °C) solution was added benzyl chloroformate (11 mL, 73.5 mmol) slowly while the pH was maintained between 13.2 and 13.5 with 5 N NaOH. After 1 h at −5 °C the reaction was diluted with water (100 mL) and diethyl ether (100 mL). The aqueous layer was separated, EtOAc (200 mL) was added, and the solution was acidified with 4 N HCl to pH 3.0. The EtOAc solution was separated, dried ($MgSO_4$), filtered, and concentrated to dryness *in vacuo* to give Boc-DL-Arg(αMe)(Cbz)-OH as an amorphous solid (3.9 g, 50%): MS (FAB) *m/e* 423 (MH^+); Anal. ($C_{20}H_{30}N_4O_6$) C, H, N. To a stirred, cooled (−10 °C) solution of Boc-DL-Arg(αMe)(Cbz)-OH (3.76 g, 8.9 mmol) in THF (80 mL) was added triethylamine (1.3 mL, 9.3 mmol) followed by isobutyl chloroformate (1.22 mL, 9.3 mmol). The reaction mixture was stirred for 5 min at −10 °C, and an additional amount of triethylamine (1.3 mL, 9.3 mmol) was added. After 1 h at −10 °C the reaction mixture was warmed to room temperature and stirred (24 h). The reaction mixture was poured into 200 mL of ice−water, and the resulting precipitate was filtered, washed with cold water, and dried *in vacuo*. The solid was crystallized from diethyl ether to afford pure **13** (3.4 g, 95%): MS (FAB) *m/e* 405 (MH^+). Anal. ($C_{20}H_{28}N_4O_5$) C, H, N.

HCl·DL-Arg(αMe)(Z) Lactam (14). A solution of **13** (3.03 g, 7.5 mmol) in CH_2CL_2 (10 mL) was reacted with HCl(g) saturated in EtOAc (20 mL). After 30 min at room temperature the precipitate was filtered, washed with diethyl ether, and dried *in vacuo* to afford pure **14** (2.58 g, 100%): MS (FAB) *m/e* 305 (MH^+).

D-MePhe-Pro-Arg(αMe)-H·2HCl (30). To a stirred, cooled (0 °C) solution of Cbz-MePhe-Pro-OH[20] (1.64 g, 3.2 mmol) in DMF (40 mL) was added HOBt (0.44 g, 3.2 mmol), DCC (0.67 g, 3.2 mmol), diisopropylethylamine (0.84 mL, 4.8 mmol), and **14** (1.1 g, 3.2 mmol). The reaction mixture was stirred for 1

h at 0 °C, warmed to room temperature, and stirred (48 h). The reaction mixture was cooled (0 °C), the precipitate was removed by filtration, and the mother liquor was concentrated *in vacuo*. The resultant oil was dissolved in EtOAc (200 mL) and washed sequentially with 1 N $NaHCO_3$, water, 1.5 N citric acid, and water. The EtOAc layer was dried ($MgSO_4$), filtered, and evaporated to an amorphous solid. The crude solid was purified by chromatography on silica gel using a step gradient elution ($CHCl_3$ (100) to $CHCl_3$–CH_3CN, 50:50) to yield the protected tripeptide lactam with the desired diastereomer as a clear oil (0.71 g, 32%): MS (FAB) *m/e* 697 (MH^+). Compound **30** was prepared from the clear oil in a manner similar to that for **22.** The pure **30** isolated as a lyophilized white solid (0.436 g, 90%): MS (FAB) *m/e* 431 (MH^-); $[\alpha]^{25}_D$ −102.9° (*c* 0.5, 0.01 N HCl). Anal. ($C_{22}H_{34}N_6O_3 \cdot 2HCl \cdot H_2O$) C, H, N.

TFA-D-Phg(αEt)-Azt-Arg-H·HCl (16). 16 was prepared from **8a** in a manner similar to that for **17** as a lyophilized white solid (90%): MS (FAB) *m/e* 499 (MH^+); $[\alpha]^{25}_D$ −106.6° (*c* 0.5, 0.01 N HCl). Anal. ($C_{22}H_{29}N_6O_4F_3 \cdot HCl \cdot H_2O$) C, H, N.

Boc-D-Phg-Azt-Arg-H·2HOAc (18). 18 was prepared by the method of Shuman et al.[10a] from **8c** as a lyophilized white solid (38%): $[\alpha]^{25}_D$ −146° (*c* 0.5, 1 **M** HOAc); MS (FAB) *m/e* 475 (MH^+). Anal. ($C_{23}H_{34}N_6O_5 \cdot 2C_2H_4O_2$) C, H, N.

Boc-D-Phe-Azt-Arg-H·2HOAc (20). 20 was prepared by the method of Shuman et al.[10a] from **8e** as a lyophilized white solid (27%): MS (FAB) *m/e* 489 (MH^+). Anal. ($C_{24}H_{36}N_6O_5 \cdot 2C_2H_4O_2$) C, H, N.

Ac-D-Phg(αMe)-Azt-Arg-H·0.5H_2SO_4 (21). 21 was prepared from **8f** in a manner similar to that for **17** as a lyophilized white solid (70%): MS (FAB) *m/e* 431 (MH^-); $[\alpha]^{25}_D$ −64.8° (*c* 0.5, 0.01 N H_2SO_4). Anal. ($C_{21}H_{30}N_6O_4 \cdot 0.5H_2SO_4 \cdot 2H_2O$) C, H, N.

Boc-D-Phg(αMe)-Azt-Arg-H·0.5H_2SO_4 (24). 24 was prepared from **8i** in a manner similar to that for **17** as a lyophilized white solid (39%): MS (FAB) *m/e* 489 (MH^+); $[\alpha]^{25}_D$ −61.2° (*c* 0.5, 0.01 N H_2SO_4). Anal. ($C_{24}H_{36}N_6O_5 \cdot 0.5H_2SO_4$) C, H, N.

R-*cis*-(4aS,8aS)-3-Piq-Pro-Arg-H·H_2SO_4 (26). 26 was prepared from **8k** in a manner similar to that for **22.** The pure **26** isolated as a lyophilized white solid (76%): MS (FAB) *m/e* 421 (MH^-); $[\alpha]^{25}_D$ −41° (*c* 0.5, 0.01 N H_2SO_4). Anal. ($C_{21}H_{36}N_6O_3 \cdot H_2SO_4 \cdot 3H_2O$) C, H, N.

TFA-D-Phg(αMe)-Pro-Arg-H·HCl (27). 27 was prepared from **8m** in a manner similar to that for **17** as a lyophilized white solid (85%): MS (FAB) *m/e* 499 (M^+); $[\alpha]^{25}_D$ −32.7° (*c* 0.5, 0.01 N HCl). Anal. ($C_{22}H_{29}N_6O_4F_3 \cdot HCl \cdot 2H_2O$) C, H, N.

Boc-D-Phe-Thz-Arg-H·HCl (28). 28 was prepared from **8m** in a manner similar to that for **18** as a lyophilized white solid (44%): MS (FAB) *m/e* 521 (M^-); $[\alpha]^{25}_D$ −65.8° (*c* 0.5, THF). Anal. ($C_{24}H_{36}N_6O_5S \cdot HCl \cdot 2H_2O$) C, H, N.

Boc-D-Phe-DL-Pro(5,5Me)-Arg-H·HCl (29). 29 was prepared in a manner similar to that for **18** from the amino acid published in ref 17 as a lyophilized white solid (50%): MS (FAB) *m/e* 531 (MH^+); $[\alpha]^{25}_D$ −72° (*c* 0.5, 0.01 N HCl). Anal. ($C_{27}H_{42}N_6O_5 \cdot HCl \cdot 2.5H_2O$) C, H, N.

Boc-D-Phe-Pip-Arg-H·H_2SO_4 (31). 31 was prepared from **8n** in a manner similar to that for **18** as a lyophilized white solid (27%): MS (FAB) *m/e* 517 (MH^-). Amino acid analysis: Phe 0.98, Pip 1.02. Anal. ($C_{26}H_{40}N_6O_5 \cdot H_2SO_4 \cdot 4H_2O$) C, H, N.

D-1-Tiq-Pro-Arg(αMe)-H·2HCl (32). The experimental procedure is essentially the same as that described for **30.** The dipeptide portion was prepared by the method of Shuman et al.[10a] to give a lyophilized white solid (91%): MS (FAB) *m/e* 429 (MH^-); $[\alpha]^{25}_D$ −36.1° (*c* 0.5, 0.01 N HCl). Anal. ($C_{22}H_{32}N_6O_3 \cdot 2HCl$) C, H, N.

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