Discovery of a Novel, Selective, and Orally Bioavailable Class of Thrombin Inhibitors Incorporating Aminopyridyl Moieties at the P1 Position

Dong-Mei Feng,*[,†] Stephen J. Gardell,[§] S. Dale Lewis,[§] Mark G. Bock,[†] Zhongguo Chen,[‡] Roger M. Freidinger,[†] Adel M. Naylor-Olsen,[∥] Harri G. Ramjit,[†] Richard Woltmann,[⊥] Elizabeth P. Baskin,[⊥] Joseph J. Lynch,[⊥] Robert Lucas,[§] Jules A. Shafer,[§] Kimberley B. Dancheck,[▽] I-Wu Chen,[▽] Shi-Shan Mao,[§] Julie A. Krueger,[§] Timothy R. Hare,[§] Anne M. Mulichak,[‡] and Joseph P. Vacca[†]

Departments of Medicinal Chemistry, Biological Chemistry, X-ray Crystallography Group, Molecular Systems, Drug Metabolism, and General Pharmacology, Merck Research Laboratories, West Point, Pennsylvania 19486

Received July 29, 1997[⊗]

A novel class of thrombin inhibitors incorporating aminopyridyl moieties at the P1 position has been discovered. Four of these thrombin inhibitors (**13b**,**c**,**e** and **14d**) showed nanomolar potency (K_i 0.8−12 nM), 300−1500-fold selectivity for thrombin compared with trypsin, and good oral bioavailability (F = 40−76%) in rats or dogs. The neutral P1 was expected to increase metabolic stability and oral absorption. Identification of this novel aminopyridyl group at P1 was a key step in our search for a clinical candidate.

Introduction

Thrombin plays a central role in hemostasis by mediating the conversion of fibrinogen to fibrin and the activation of platelets.[1] The "fibrinogen-like" sequence D-Phe-Pro-Arg has been widely used in the design of substrates and inhibitors of thrombin.[2] In our laboratories, D-Phe-Pro *trans*-(4-aminocyclohexyl)glycine keto acid derivatives were found to be highly active as transition-state inhibitors of thrombin.[3,4] Recently, a new class of thrombin inhibitor was reported which contains a *trans*-(4-aminocyclohexyl)methyl moiety at the P_1 position (L-371,912), has nanomolar potency, and is selective but exhibits low oral bioavailability (1−10%) in rat and dog.[5] The poor oral bioavailability exhibited by these types of inhibitors has been attributed to highly basic functionalities at P1 which result in metabolic instability and very poor oral absorption.[6] Herein, we report novel aminoaryl P1 substituents varying in size and basicity and the identification of aminopyridyl moieties as important replacements for the *trans*-aminocyclohexyl group at P1 in the L-371,912 template.[7,8] The lipophilic amino acids D-3,3-$(Ph)_2$-Ala,[9,10] D-3,3-$(Chx)_2$-Ala,[11] or D-3,4-Cl_2-Phe[12] and a benzylsulfonyl group on the N-terminus[9,13] were used as P3 ligands. We anticipated that developing potent inhibitors with a neutral P1 would require the use of these lipophilic residues at P3 to add more hydrophobic binding energy to compensate losses at P1.

Chemistry

The requisite P1 precursors, 3-(aminomethyl)-6-aminopyridine (**4**) and 2,4-dimethyl-3-(aminomethyl)-6-aminopyridine (**5**), were synthesized as outlined in Scheme 1[14] where 6-aminonicotinamide and 2,4-dimethyl-3-cyano-6-aminopyridine were reduced rapidly and quantitatively into the corresponding amine by a 7-fold excess of diborane at room temperature. This reaction provides a convenient synthetic procedure for the selective reduction of amide or nitrile into amine in excellent yields (90−100%). In addition, 2-amino-4-(aminomethyl)-1,3-thiazole (**6**) was prepared according to Scheme 1, where 2-acetamido-4-(chloromethyl)-1,3-thiazole reacted with NH_3/MeOH to give a primary amine followed by acid hydrolysis of the acetyl group. The 2-amino-4-(aminomethyl)thiophene (**7**) was prepared starting from the commercially available 2-nitro-4-cyanothiophene which was reduced into the corresponding amine by a 7-fold excess of diborane followed by catalytic hydrogenation of the nitro group as shown in Scheme 1. Hydrogenation of Boc-D-3,3-$(Ph)_2$-Ala with Ir black catalyst gave Boc-D-3,3-$(Chx)_2$-Ala.[11,15] D-3,4-Cl_2-Phe-OH was synthesized by standard procedures.[16] The sulfonamide derivative **1a** was prepared by the treatment of D-3,4-Cl_2-Phe-OH with benzenesulfonyl chloride and 1 N NaOH in dioxane.[9]

The dipeptide esters were prepared by coupling N-protected amino acid with HPro-OMe·HCl using EDC and HOBT activation and then hydrolyzed with lithium hydroxide to give the corresponding dipeptides (**1c**, **2c**, and **3b**) as outlined in Scheme 2. EDC/HOBT-mediated coupling of benzylsulfonyl-D-3,4-Cl_2-Phe-Pro-OH (**1c**), Boc-D-3,3-$(Chx)_2$-Ala-Pro-OH (**2c**), or Boc-D-3,3-$(Ph)_2$-Ala-Pro-OH (**3b**) with intermediate **4**, **5**, **6**, or **7** followed by Boc deprotection with TFA/CH_2Cl_2 afforded the final products.

Biology

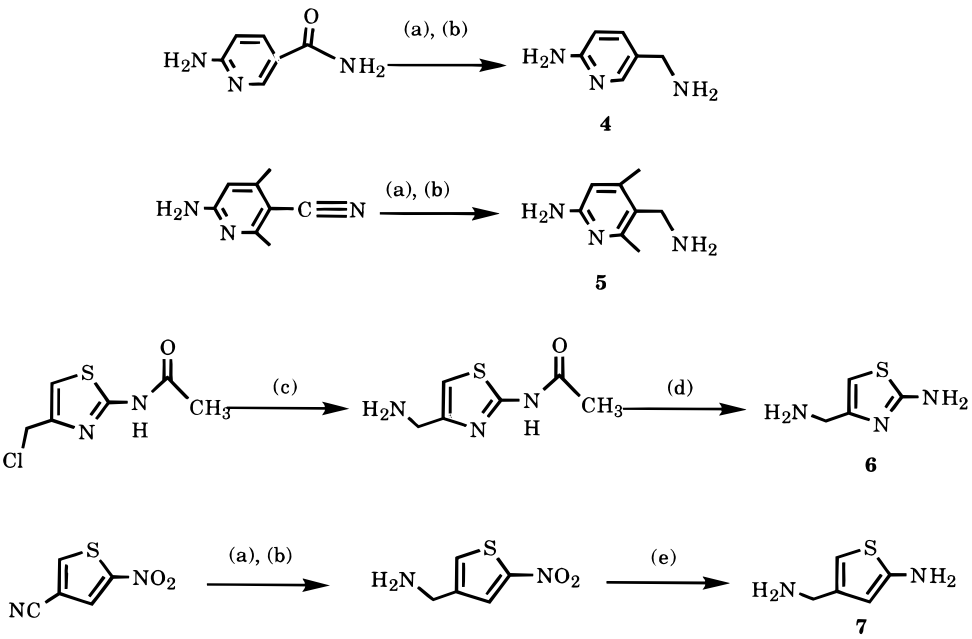
Determination of Inhibition Constants (K_i). Human thrombin (IIa), factor Xa (FXa), and bovine trypsin were used. Values of K_i were determined using methods previously described.[17] Briefly, K_i values were determined from studies of the dependence on inhibitor concentration of the initial rate of release of *p*-nitroaniline from the substrates as determined from the rate of increase of absorbance at 405 nm upon addition of chromogenic substrate to an equilibrated mixture of proteinase and inhibitor.

Determination of Oral Bioavailability in Conscious Rats. Anesthetized rats were instrumented with indwelling left jugular vein and left carotid artery catheters for test agent administration and blood sam-

† Department of Medicinal Chemistry.
§ Department of Biological Chemistry.
‡ Department of X-ray Crystallography Group.
∥ Department of Molecular Systems.
⊥ Department of General Pharmacology.
▽ Department of Drug Metabolism.
⊗ Abstract published in *Advance ACS Abstracts,* October 15, 1997.

S0022-2623(97)00493-7 CCC: $14.00

Scheme 1[a]



[a] (a) BH_3/THF, 0 °C; (b) 6 N HCl, H_2O, MeOH; (c) NH_3, MeOH; (d) 6 N HCl, 100 °C; (e) $H_2/Pd(OH)_2$.

pling, respectively. The following day, blood samples were drawn from the conscious, instrumented rats before and at increasing times after oral (gastric lavage) or intravenous administration of test agents. Plasma inhibitor concentrations were determined as described.[17] Oral bioavailabilities were deduced by comparison of dose-normalized areas under the curve values from the plasma concentration versus time course relationships determined for the orally or intravenously dosed rats.

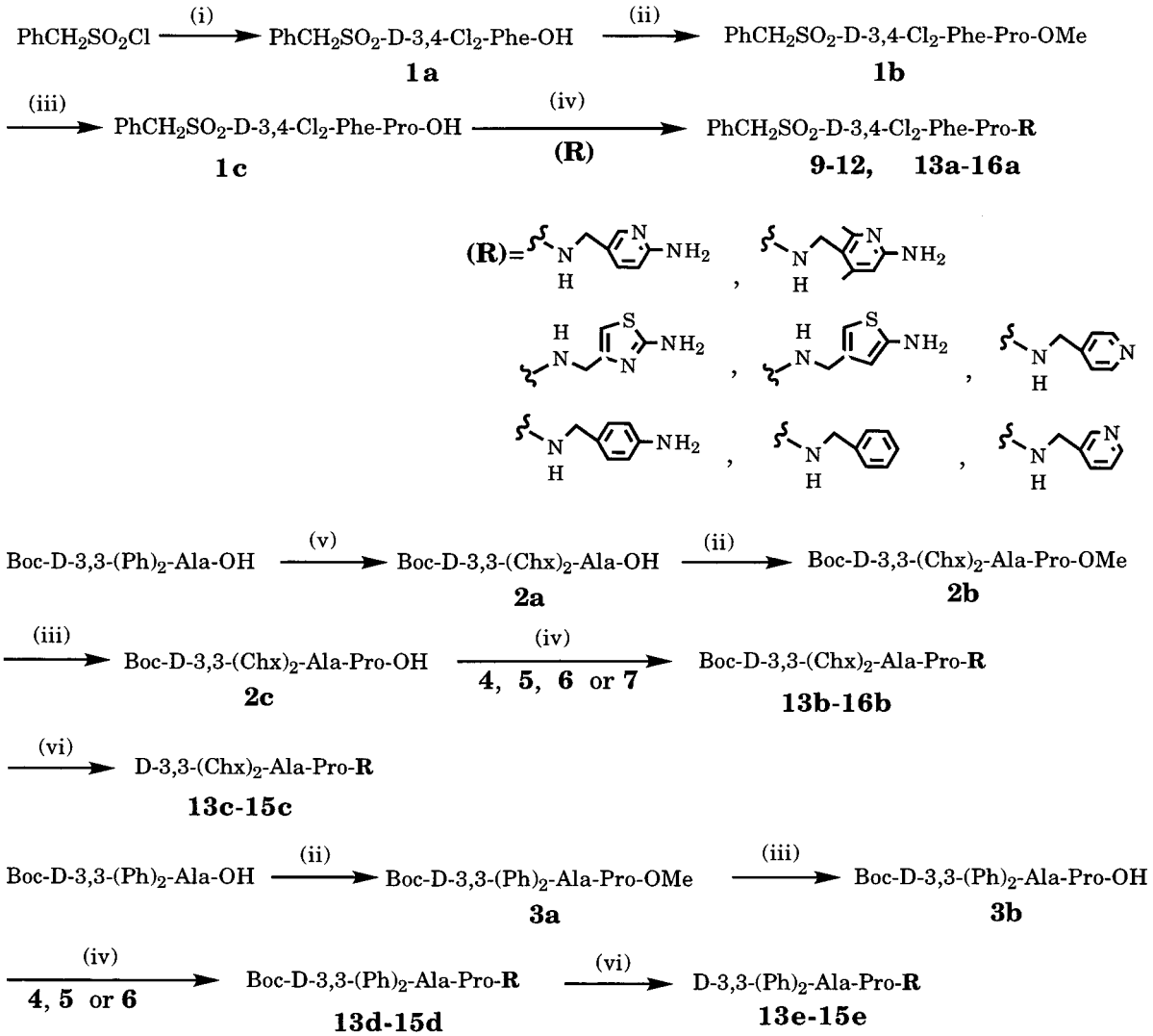
Results and Discussion

As illustrated in Table 1, L-371,912 has a K_i for thrombin of 4.9 nM (pK_a 10.2, log P −1.68) but exhibits low oral bioavailability (1−10%) in rat and dog. Aniline analogue **8** was a low-potency thrombin inhibitor with a K_i of 3.7 μM (log P 2.34). The loss in potency compared to L-371,912 is probably due to the P1 moiety. Presumably, there are two reasons: (1) The planar-shaped aniline ring occupies less volume than the chair-shaped cyclohexyl ring which reduced hydrophobic binding at P1 of analogue **8**. (2) The less basic the P1 group, the weaker the interaction with Asp189 in the specificity pocket of thrombin. Analogue **9**, which has the benzylsulfonyl on the N-terminus and D-3,4-Cl_2-Phe in the P3 position, displayed a K_i for thrombin of 9 nM (pK_a 4.8, log P >3.68), a 400-fold increase in potency over the corresponding analogue **8**. The increase in affinity is due to the longer bond length of the sulfonamide bond which allows one of sulfonamide oxygens to form an H-bond with Gly219, and the hydrophobic interaction between the benzyl group and the side chain Glu192 also increases the potency of the inhibitor.[9] The analogue **9** also showed 35% oral bioavailability in rats (Table 2). The greatly reduced pK_a of P1 in analogue **9** might facilitate penetration of biological membranes by the uncharged form.[6b] Analogue **13a** that contained the aminopyridine was even more potent than the aniline, displaying a K_i for thrombin of 3 nM (pK_a 6.3, log P 2.57). Pursuant to this interesting result, a structure−activity relationship (SAR) study has been initiated to incorporate less basic 5- and 6-membered amino-heteroaryl moieties such as aminopyridyl, aminodimethylpyridyl, aminothiazolyl, or aminothiopheneyl moieties at P1 and append lipophilic groups at P3 offering an opportunity for improved inhibitory activity and structural diversity to achieve better oral bioavailability. There appears to be a strong preference for the $-NH_2$ substituent of the aryl ring at the P1 position, since replacement with benzyl (**10**) or pyridyl (**11** and **12**) results in a severe loss of potency. In the aminopyridine series, the most potent analogue **13c**, which featured D-3,3-$(Chx)_2$-Ala at P3, displayed a K_i for thrombin of 0.82 nM (pK_a 6.2, log P 2.21), 1500-fold selectivity against trypsin, and 75% oral bioavailability in rats. The corresponding *N*-Boc analogue **13b**, with a K_i for thrombin of 3.0 nM (pK_a 6.5, log P >3.35), has 60% oral bioavailability in rats. Analogues **13d**,**e**, featuring D-3,3-$(Ph)_2$-Ala at P3, displayed K_i's for thrombin of 7.6 and 12 nM, respectively. As shown in Figure 1 and Table 3, **13e** displayed reasonably good absorption kinetics in dogs. Following oral administration (5 mg/kg), the drug in plasma reached peak concentration (C_{max}) at 7000 nM in 20 min. The oral bioavailability was estimated to be 52%. When the compound was given intravenously (1 mg/kg), the concentrations of **13e** in plasma declined in a polyphasic manner (Figure 1). The plasma clearance, terminal $t_{1/2}$, and volume of distribution were 7.3 mL/min/kg, 151 min, and 1.20 L/kg, respectively.

In the aminodimethylpyridine series, the bioassay results show that analogue **14d** (K_i 3.6 nM, log P 1.65) featuring Boc-D-3,3-$(Ph)_2$-Ala in the P3 position was 40% orally bioavailable in the rats. The K_i of the corresponding free amino analogue **14e** was 10 nM. Analogues **14b**,**c** featuring Boc-D-3,3-$(Chx)_2$-Ala or its corresponding free amino in the P3 position have a similar inhibitory activity (K_i's 2.2−3.9 nM), but replacement of Boc-D-3,3-$(Chx)_2$-Ala by benzylsulfonyl-D-3,4-Cl_2-Phe led to less potent analogue **14a** (K_i 11 nM).

It is known from X-ray crystal structures of thrombin and trypsin that the thrombin P1 pocket is slightly larger than that of trypsin. The Ser191 in trypsin narrows the P1 pocket.[18] We envisioned utilizing the pocket size differences between thrombin and trypsin

Scheme 2[a]

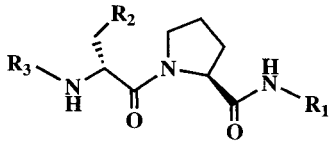


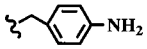
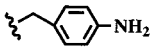
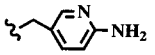
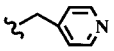
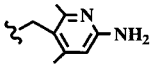
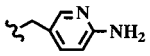
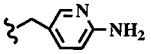
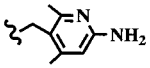
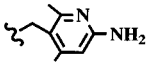
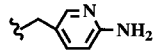
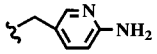
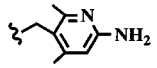
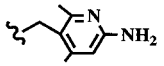
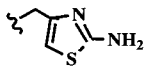
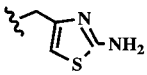
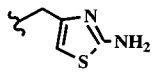
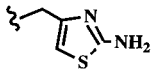
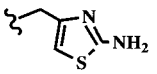
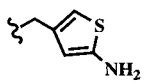
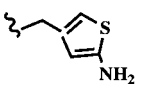
[a] (i) H-D-3,4-Cl_2-Phe-OH, dioxane, 4 N NaOH; (ii) EDC, HOBT, H-Pro-OMe, DIEA, NMP; (iii) 2.2 N LiOH/MeOH, H_2O; (iv) EDC, HOBT, DIEA, NMP; (v) Ir black, HOAc/H_2; (vi) TFA/CH_2Cl_2.

to obtain selectivity over trypsin. Derivatives with dimethyl-substituted aminopyridyl groups, as seen in analogues **14a**−**e** appear to be more selective for thrombin versus trypsin than corresponding nonsubstituted ones (**13a**−**e**), and the nanomolar potency was maintained. However, analogues with 5-membered heteroaryl moieties, as in aminothiazolyl analogues **15a**−**e** (K_i's 70−900 nM) and aminothiophenes **16a,b** (K_i's 130−560 nM), for thrombin are much less potent. Presumably, a 6-membered aryl ring is preferred for hydrophobic binding in the S1 specificity pocket of thrombin.

X-ray Crystallographic Structure. Crystals of the α-thrombin−**13a** complex were derived by the vapor diffusion method and soaking technique. The crystal structure was determined with the difference Fourier method as described elsewhere.[18] After refinement, the *R*-factor was 0.175 at 1.8-Å resolution, and the rms deviations for the ideal bond distances and angles were 0.015 Å and 3.0°, respectively. As illustrated in Figure 2, the thrombin crystal structures of inhibitor **13a** show that the compound binds to the subsidiary recognition sites of this enzyme. The side chain of the P2 Pro is nestled within a lipophilic S2 binding pocket, defined by the insertion peptide sequence Tyr60aPro60bPro60c-Trp60d. The aminopyridine moiety is located in the specificity S1 pocket of thrombin. The amino nitrogen atom interacts directly with Asp189 Od1 (2.91 Å), Gly219 O (2.76 Å), and Ala190 O (3.01 Å) as well as indirectly with Asp189 Od2 via a bridging water molecule.[5] In comparison with L-372,912, the aminopyridine ring shifts 0.6 Å downward to the bottom of this pocket and rotates 30° arond its Cα−Cβ bond. The planar-shaped aminopyridyl ring occupies less volume than the chair form cyclohexyl ring of L-372,912 and runs almost parallel to the backbone of residues Trp215−Glu217 and Ala190−Glu192. There are fewer hydrophobic interactions of the P1 of **13a** with the S1 pocket in comparison with those of L-372,912. The dichlorophenyl of the P3 D-3,4-Cl_2-Phe forms an edge-to-face stacking interaction with Trp215 in an adjacent aryl-binding P3 pocket, and a hydrogen-bonding network is formed between the carbonyl oxygen of D-3,4-Cl_2-Phe with the amide nitrogen of thrombin Gly216, the amide nitrogen of D-3,4-Cl_2-Phe with the carbonyl oxygen of thrombin Gly216, and the carbonyl oxygen of thrombin Ser214 with the amide nitrogen of aminopyridyl P1, to form antiparallel β-sheet. The extra two chloro atoms cause the whole dichlorophenyl group to move outward. Two chlorine atoms are within hydrogen bond distance with Glu97a O (3.2 Å) and Asn98 O (3.0 Å). These interactions will add more binding energy for **13a**.

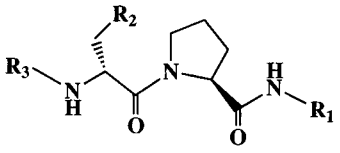
Table 1. In Vitro 6- or 5-Membered Heteroaryl Thrombin Inhibitors

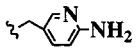
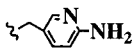
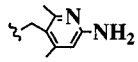
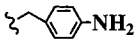


NO.	R_2	R_1	R_3	Ki[a] (nM) Thrombin[b]	Trypsin[c]	FXa[b]
L-371,912	Ph		CH_3	5.0	11000	>1000000
8	Ph		Boc	3700	233000	420000
9	3,4-Cl_2-Ph		SO_2CH_2Ph	9.0	12000	10000
13a	3,4-Cl_2-Ph		SO_2CH_2Ph	3.0	430	7600
10	3,4-Cl_2-Ph		SO_2CH_2Ph	110	384000	14000
11	3,4-Cl_2-Ph		SO_2CH_2Ph	380	284000	230000
12	3,4-Cl_2-Ph		SO_2CH_2Ph	510	31000	>200000
14a	3,4-Cl_2-Ph		SO_2CH_2Ph	11.0	8900	52000
13b	$(Chx)_2$		Boc	3.0	657	4400
13c	$(Chx)_2$		H	0.82	1200	8300
14b	$(Chx)_2$		Boc	2.2	1500	16000
14c	$(Chx)_2$		H	3.9	3000	160000
13d	$(Ph)_2$		Boc	7.6	1300	>200000
13e	$(Ph)_2$		H	12.0	5400	94000
14d	$(Ph)_2$		Boc	3.6	2500	160000
14e	$(Ph)_2$		H	10.0	8300	240000
15a	3,4-Cl_2-Ph		SO_2CH_2Ph	360	767000	>100000
15b	$(Chx)_2$		Boc	350	52000	>100000
15c	$(Chx)_2$		H	70	144000	>100000
15d	$(Ph)_2$		Boc	900	>100000	>100000
15e	$(Ph)_2$		H	350	277000	>100000
16a	3,4-Cl_2-Ph		SO_2CH_2Ph	130	56000	>100000
16b	$(Chx)_2$		BOC	560	45000	27000

[a] K_i determination described in ref 13. [b] Human thrombin and FXa were used. [c] Bovine trypsin was used.

Table 2. Oral Bioavailability and Pharmacokinetic Parameters in Rats



Comp.	R_2	R_1	R_3	I.V. (2 mpk) CL ml/ kg /min	I.V. (2 mpk) t 1/2 (min)	P.O. (10 mpk) Cmax (μM)	P.O. (10 mpk) Tmax (min)	PKa	LogP	F (%)
L-371,912	Ph		CH_3	ND	ND	ND	ND	10.2	-1.68	1.0
13c	$(Chx)_2$		H	>200	102	0.53	26	6.2	2.21	76
13b	$(Chx)_2$		Boc	27	35	1.43	120	6.5	>3.35	60
14d	$(Ph)_2$		Boc	76	50	0.61	53	ND	1.65	40
9	3,4-Cl_2-Ph		SO_2CH_2Ph	71	20	0.66	53	4.8	>3.68	35

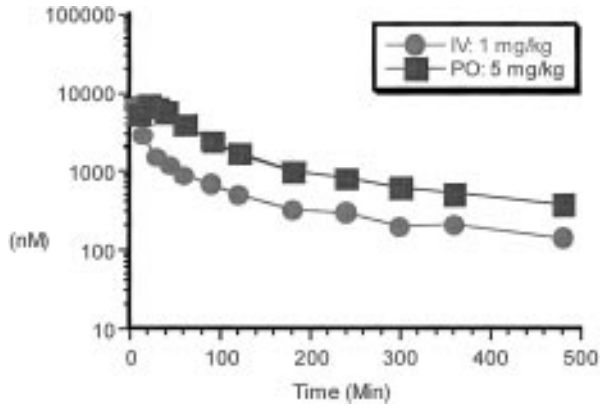


Figure 1. Plasma concentrations of **13e** in beagle dogs following oral and iv administrations.

Further increase in affinity is due to the longer bond length of the sulfonamide bond which allows one of sulfonamide oxygens to form a H-bond with Gly219 N (2.82 Å), and the hydrophobic interaction between the benzyl group and the side chain Glu192 also increases the potency of the inhibitor. This mode of benzylsulfonamide binding has been previously described.[9]

Conclusions

We have designed and evaluated a novel class of thrombin inhibitors incorporating neutral aminopyridyl moieties at P1. The neutral P1 was expected to increase metabolic stability and oral absorption. The aminopyridyl P1 motif also improved pharmacokinetic properties. These targets express a nanomolar potent thrombin inhibitory activity, selectivity, and good oral bioavailability. Discovery of this novel, selective, and orally bioavailable class of thrombin inhibitors incorporating aminopyridyl moieties at P1 was a key step in our search for a clinical candidate. Subsequent communications from our laboratories will describe further results in this area.

Experimental Section

General Experimental. Boc-D-3,3-$(Ph)_2$-Ala was purchased from Synthetech, Inc., Albany, OR. Boc-D-Phe-Pro-*O*-succinimide and H-Pro-OtBu·HCl were purchased from Bachem, Inc., CA. Most chemical reagents were purchased from Aldrich, Inc., MO, except amino-2,4-dimethyl-3-pyridinecarbonitrile was purchased from Lancaster, NH. The solvents employed in these experiments were purchased and were not further purified unless otherwise specified. Some solvents, if necessary, were dried and distilled over appropriate drying agents, e.g., CH_2Cl_2 and DMF were dried and distilled over calcium hydride (CaH_2) and stored over 4A molecular sieves; tetrahydrofuran (THF) was distilled over Na/benzophenone and used immediately. The silica gel for column chromatography was Merck Kieselgel 60 (230−400 mesh). TLC was done on Merck Kieselgel 60-F254. Purification was achieved by preparative HPLC on a Delta-Pak C18 column, 100-Å pore size, 15 μM with 0.1% trifluoroacetic acid (TFA)−aqueous acetonitrile solvent systems using various linear gradients. The purity was further checked by HPLC analysis on a Vydac C_{18} column with 0.1% aqueous TFA−acetonitrile and 0.1 M ammonium acetate−acetonitrile solvent systems using various linear gradients. The flow rate was 1.5 mL/min, and the absorbance was recorded at 210 nm. All compounds showed 98−99% purity in the two HPLC systems. FABMS, with the molecular ion peak clusters at $(M + H)^+$ and $(M - H)^-$ in the negative ion mode, were observed for each compound on a VG Micromas Autospec-Q instrument. Elemental analyses, performed by the analytical group, Merck & Co., Inc., West Point, PA, were within 0.4% of the theoretical values calculated for C, H, and N. Analytic data are shown in Table 4.

Abbreviations: Ph, phenyl; SO_2CH_2Ph, benzylsulfonyl; 3,4-Cl_2-Phe, 3,4-dichloro-Phe; 3,3-$(Chx)_2$-Ala, 3,3-dicyclohexyl-Ala; 3,3-$(Ph)_2$-Ala, 3,3-diphenyl-Ala.

***N*-(Benzylsulfonyl)-D-3,4-Cl_2-Phe-OH (1a).** D-3,4-Cl_2-Phe-OH (1.4 g, 6.0 mmol) was dissolved in 48 mL of dioxane by addition of 6 mL of 1 N NaOH. The resulting solution was treated dropwise with phenylmethanesulfonyl chloride with

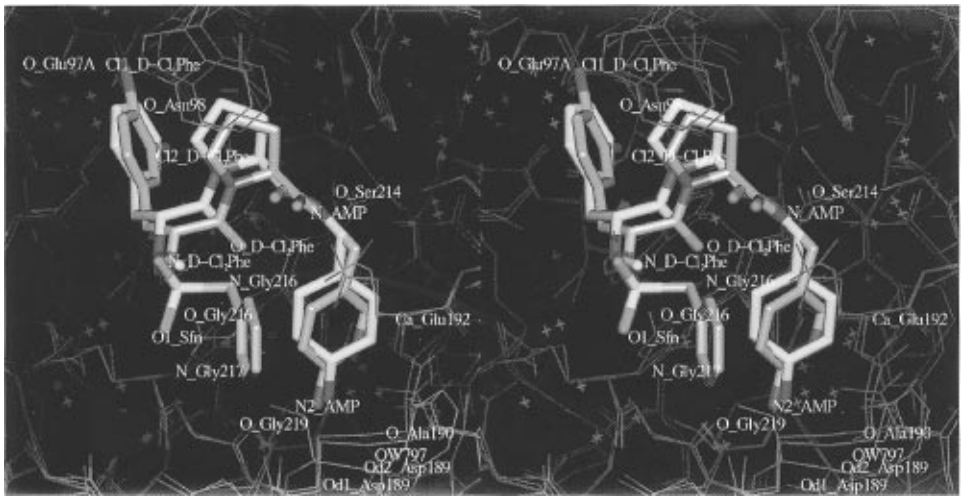
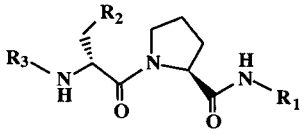


Figure 2. Stereoview of the overlap of **13a** (green) and L-371,912 (yellow) in their complex structures. Surrounding thrombin residues are represented by thin lines with the same color code as their inhibitors.

Table 3. Oral Bioavailability and Pharmacokinetic Parameters in Dogs



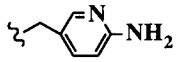
				I.V. (1 mpk)		P.O. (5 mpk)		
NO.	R_2	R_1	R_3	CL (ml/ kg /min)	t 1/2 (min)	Cmax (nM)	Tmax (min)	F (%)
13e	$(Ph)_2$		H	7.3	151	7000	20	52

Table 4. Chromatographic and Amino Analytic Data on Heteroaryl Thrombin Inhibitors

compd	HPLC[a] retention time, min	purity,[b] %	FABMS	formula[c]	analysis
8	16.6	98	467	$C_{26}H_{34}N_4O_4S_1Cl_2 \cdot 1.20CF_3CO_2H \cdot 0.30H_2O$	C,H,N
9	19.5	99	589	$C_{28}H_{30}N_4O_4S_1Cl_2 \cdot 1.15CF_3CO_2H$	C,H,N
10	23.5	99	574	$C_{28}H_{29}N_3O_4S_1Cl_2 \cdot 2.90CF_3CO_2H \cdot 1.35H_2O$	C,H,N
11	18.7	99	575	$C_{27}H_{28}N_4O_4S_1Cl_2 \cdot 1.40CF_3CO_2H \cdot 0.50H_2O$	C,H,N
12	18.6	98	575	$C_{27}H_{28}N_4O_4S_1Cl_2 \cdot 1.45CF_3CO_2H \cdot 0.40H_2O$	C,H,N
13a	18.8	99	590	$C_{27}H_{29}N_5O_4S_1Cl_2 \cdot 2.35CF_3CO_2H \cdot 0.95H_2O$	C,H,N
13b	22.7	99	556	$C_{31}H_{49}N_5O_4 \cdot 1.75CF_3CO_2H \cdot 0.90H_2O$	C,H,N
13c	16.5	99	456	$C_{26}H_{41}N_5O_2 \cdot 2.70CF_3CO_2H \cdot 0.55H_2O$	C,H,N
13d	18.7	99	544	$C_{31}H_{37}N_5O_4 \cdot 1.75CF_3CO_2H \cdot 1.05H_2O$	C,H,N
13e	13.9	99	444	$C_{26}H_{29}N_5O_2 \cdot 2.80CF_3CO_2H \cdot 1.35H_2O$	C,H,N
14a	19.5	99	618	$C_{29}H_{33}N_5O_4 \cdot 1.65CF_3CO_2H \cdot 0.60H_2O$	C,H,N
14b	23.2	99	584	$C_{33}H_{53}N_5O_4 \cdot 1.55CF_3CO_2H \cdot 0.80H_2O$	C,H,N
14c	16.7	99	484	$C_{28}H_{45}N_5O_2 \cdot 2.50CF_3CO_2H \cdot 1.35H_2O$	C,H,N
14d	19.3	99	572	$C_{33}H_{41}N_5O_4 \cdot 1.35CF_3CO_2H \cdot 1.55H_2O$	C,H,N
14e	14.2	99	472	$C_{28}H_{33}N_5O_2 \cdot 2.75CF_3CO_2H \cdot 2.90H_2O$	C,H,N
15a	18.9	99	596	$C_{25}H_{27}N_5O_4S_1Cl_2 \cdot 1.50CF_3CO_2H \cdot 0.25H_2O$	C,H,N
15b	23.2	99	562	$C_{29}H_{47}N_5O_4 \cdot 2.05CF_3CO_2H \cdot 0.70H_2O$	C,H,N
15c	16.6	99	462	$C_{26}H_{40}N_5O_4 \cdot 2.50CF_3CO_2H \cdot 0.25H_2O$	C,H,N
15d	19.0	99	550	$C_{29}H_{35}N_5O_4 \cdot 1.30CF_3CO_2H \cdot 0.50H_2O$	C,H,N
15e	14.1	99	450	$C_{26}H_{28}N_5O_4 \cdot 2.80CF_3CO_2H \cdot 0.50H_2O$	C,H,N
16a	18.9	99	595	$C_{26}H_{28}N_4O_4S_1Cl_2 \cdot 1.95CF_3CO_2H \cdot 0.95H_2O$	C,H,N
16b	22.9	99	561	$C_{30}H_{48}N_4O_4 \cdot 1.50CF_3CO_2H \cdot 1.25H_2O$	C,H,N

[a] HPLC on a column of m-Vadex C_{18} (3.9 cm × 30 cm); 95% A to 5% A over 30 min, A = 0.1% TFA−H_2O, B = 0.1% TFA−CH_3C. [b] Purity was determined by HPLC. [c] All compounds analyzed correctly (±0.4%) for C,H,N.

rapid stirring at room temperature. After 2.5 h, the aqueous layer was further adjusted to pH 2 with $KHSO_4$ solution; 150 mL of EtOAc was added, and the organic layer was separated, washed twice with saturated NaCl solution, dried over Na_2SO_4, and evaporated in vacuo to give **1a** (2.31 g, 80% yield). FABMS: M + 1 = 485. HPLC: 97% pure at 214, retention time = 20.1 min (Vydac C_{18}, gradient of 95% A/B to 5% A/B over 30 min, A = 0.1% TFA−H_2O, B = 0.1% TFA−CH_3CN).

Boc-D-3,3-(Chx)$_2$-Ala-OH (2a). A solution of Boc-D-3,3-$(Ph)_2$-Ala-OH (2.0 g, 5.8 mmol) in 50 mL of acetic acid/10 mL of H_2O was hydrogenated at 62 psi on a Parr apparatus over 400 mg of Ir black catalyst. After 24 h, a second portion of catalyst was added, and the reaction continued for a second 24-h interval. The reaction mixture was filtered through a Celite pad, and the filtrate was added to 150 mL of H_2O and filtered again to give 2.0 g of Boc-D-3,3-$(Chx)_2$-Ala-OH as a

white solid (97% yield) of **2a**. FABMS: 354. HPLC: retention time = 24.3 min (C_{18}, 95% A to 5% A over 30 min, A = 0.1% TFA−H_2O, B = 0.1% TFA−CH_3CN).

Boc-D-3,3-(Chx)$_2$-Ala-Pro-OMe (2b). To a solution of Boc-D-3,3-(Chx)$_2$-Ala-OH (1.77 g, 5.0 mmol) (**2a**) and H-Pro-OMe·HCl (0.91 g, 5.5 mmol) in 12 mL of DMF was added 4.6 g (6.0 mmol) of HOBt, the pH of the solution was adjusted to 8 (moist narrow pH paper), and EDC (6.47 g, 6.76 mmol) was added with magnetic stirring. After 3.5 h the reaction was quenched by the addition of 10 mL of water. After the mixture was kept at room temperature for 5 h, the solvents were evaporated at reduced pressure and the residue was dissolved in EtOAc−H_2O. Aqueous $KHSO_4$ was added to this two-phase mixture, and the layers were separated. The organic layer was extracted with $NaHCO_3$ and saturated NaCl and dried over $MgSO_4$. The solvent was evaporated to give product as a white solid which was further purified by chromatography using two columns of 600 g of silica gel 60 (E. Merck) each and eluting with EtOAc−hexane (2:8). Fractions containing product were combined to give 2.26 g (97% yield) of **2b**.

In a similar manner were prepared the following: ***N*-(benzylsulfonyl)-D-3,4-Cl$_2$-Phe-Pro-OMe (1b)**, by coupling of *N*-(benzylsulfonyl)-D-3,4-Cl_2-Phe-OH (**1a**) with H-Pro-OMe·HCl, and **Boc-D-3,3-(Ph)$_2$-Ala-Pro-OMe (3a)**, by coupling of Boc-D-3,3-(Ph)$_2$-Ala-OH with H-Pro-OMe·HCl.

Boc-D-3,3-(Chx)$_2$-Ala-Pro-OH (2c). A sample of Boc-D-3,3-(Chx)$_2$-Ala-Pro-OMe (**2b**) (1.76 g, 3.8 mmol) dissolved in 100 mL of 1:1 (v/v) MeOH/H_2O was treated with 2.2 N LiOH (2.2 mL) in portions over 1.5 h keeping the pH at 12−13. After 3.5 h, the reaction solution was adjusted to pH 7 with dilute $KHSO_4$ solution, 100 mL of EtOAc and 50 mL of H_2O were added, and the aqueous layer was further adjusted to pH 2 with $KHSO_4$ solution. The organic layer was separated, washed twice with 50% saturated NaCl solution, dried over Na_2SO_4, and evaporated in vacuo to give **2c** (1.64 g, 96% yield). FABMS: 451, HPLC: retention time = 26.4 min (C_{18}, 95% A to 5% A over 30 min, A = 0.1% TFA−H_2O, B = 0.1% TFA−CH_3CN).

In a similar manner were prepared the following: ***N*-(benzylsulfonyl)-D-3,4-Cl$_2$-Phe-Pro-OH (1c)**, by hydrolysis of *N*-(benzylsulfonyl)-D-3,4-Cl_2-Phe-OMe (**1b**) with LiOH, and **Boc-D-3,3-(Ph)$_2$-Ala-Pro-OH (3b)**, by hydrolysis of Boc-D-3,3-(Ph)$_2$-Ala-Pro-OMe (**3a**) with LiOH.

Preparation of 6-Amino-3-pyridinemethylamine (4). A 300-mL flask was dried in an oven and cooled in a dry nitrogen atmosphere. The flask was equipped with a rubber syringe cap and a magnetic stirring bar. The flask was immersed in an ice−water bath, and 21 mL (21 mmol) of 1.0 M borane solution in THF was introduced. Then 6-aminonicotinamide (420 mg, 3.06 mmol) in 10 mL of THF was introduced. The mixture was allowed to warm to room temperature and stir for 5 h, 15 mL of 6 N HCl was added slowly, then 15.0 mL of H_2O and 100 mL of MeOH were introduced, and stirring continued overnight. The reaction mixture was filtered, and the filtrate was evaporated in vacuo to give product as a white solid which was further purified by chromatography using a column of 40 g of silica gel 60 (E. Merck) and eluting with *n*-butanol−HOAc−H_2O (4:1:2). Fractions containing product were combined to give 330 mg (88% yield) of **4**. ES^+: 123. TLC: R_f = 0.51, silica gel, *n*-butanol−HOAc−H_2O (4:1:2).

6-Amino-3-pyridinylmethyl *N*-(Benzylsulfonyl)-D-3,4-dichlorophenylalanyl-L-prolinamide (13a). A solution of 121 mg (0.25 mmol) of *N*-(benzylsulfonyl)-D-3,4-Cl_2-Phe-Pro-OH (**1c**), 62 mg (0.50 mmol) of 6-amino-3-pyridinemethylamine (**4**), 43 mg (0.28 mmol) of HOBT, and 54 mg (0.28 mmol) of EDC in 1.7 mL of anhydrous NMP was treated with DIEA to pH 8.5, and the resulting solution was stirred at room temperature in a N_2 atmosphere for 8 h. The reaction was quenched with 0.3 mL of HOAc and the mixture purified by preparative HPLC using a trifluoroacetic acid (0.1%)−CH_3CN gradient. Lyophilization of pure fractions gave 140 mg (95%) of product **13a** as a trifluoroacetic acid hydrate salt.

6-Amino-3-pyridinylmethyl *N*-Boc-D-3,3-dicyclohexylalanyl-prolinamide (13b). A solution of 113 mg (0.25 mmol) of Boc-D-3,3-(Chx)$_2$-Ala-Pro-OH (**2c**), 62 mg (0.50 mmol) of 6-amino-3-pyridinemethylamine (**4**), 43 mg (0.28 mmol) of HOBT, and 54 mg (0.28 mmol) of EDC in 2.0 mL of anhydrous NMP was treated with DIEA to pH 8.5, and the resulting solution was stirred at room temperature in a N_2 atmosphere for 5 h. The reaction was quenched with 0.3 mL of HOAc and the mixture purified by preparative HPLC using a trifluoroacetic acid (0.1%)−CH_3CN gradient. Lyophilization of pure fractions gave 135 mg (97%) of product **13b** as a trifluoroacetic acid hydrate salt.

6-Amino-3-pyridinylmethyl D-3,3-Dicyclohexylalanyl-L-prolinamide (13c). A solution of 122 mg (0.22 mmol) of 6-amino-3-pyridinylmethyl *N*-Boc-D-3,3-dicyclohexylalanyl-L-prolinamide (**13b**) in 10 mL of 50% TFA/CH_2Cl_2 was stirred for 20 min, and the TFA was removed under reduced pressure. The product was purified by preparative HPLC using a TFA (0.1%)−CH_3CN gradient. Lyophilization of pure fractions gave 96 mg (96%) of the title compound **13c** as a trifluoroacetic acid hydrate salt.

6-Amino-3-pyridinylmethyl *N*-Boc-D-3,3-diphenylalanyl-L-prolinamide (13d). An amount of 110 mg (0.25 mmol) of Boc-D-3,3-(Ph)$_2$-Ala-Pro-OH (**3b**) and 130 mg (0.50 mmol) of 6-amino-3-pyridinemethylamine (**4**) were coupled with hydroxybenzotriazole hydrate (43 mg, 0.28 mmol) and EDC (54 mg, 0.28 mmol) in 1.5 mL of DMF at pH 8.5 with DIEA. The mixture was stirred under N_2 at room temperature overnight, then diluted with 10 mL of 10% aqueous citric acid, and extracted with CH_2Cl_2. The CH_2Cl_2 extracts were washed with aqueous Na_2CO_3, dried (Na_2SO_4), filtered, and concentrated in vacuo to give the crude Boc derivative of the title compound, and the product was purified by preparative HPLC using a TFA (0.1%)−CH_3CN gradient. Lyophilization of pure fractions gave 122 mg (90%) of the white powder **13d** as a trifluoroacetic acid hydrate salt.

6-Amino-3-pyridinylmethyl D-3,3-Diphenylalanyl-L-prolinamide (13e). A solution of 109 mg (0.20 mmol) of 6-amino-3-pyridinylmethyl *N*-Boc-D-3,3-diphenylalanyl-L-prolinamide (**13d**) in 10 mL of 50% TFA/CH_2Cl_2 was stirred for 20 min, and the TFA was removed under reduced pressure. The product was purified by preparative HPLC using a TFA (0.1%)−CH_3CN gradient. Lyophilization of pure fractions gave 87 mg (98%) of the title compound **13e** as a trifluoroacetic acid hydrate salt.

Preparation of 6-Amino-2,4-dimethyl-3-pyridinemethylamine (5). A 300-mL flask was dried in an oven and cooled in a dry nitrogen atmosphere. The flask was equipped with a rubber syringe cap and a magnetic stirring bar. The flask was immersed in an ice−water bath, and 21 mL (21 mmol) of 1.0 M borane solution in THF was introduced. Then 6-amino-2,4-dimethyl-3-pyridinecarbonitrile (442 mg, 3.0 mmol) in 10 mL of THF was introduced. The resulting mixture was stirred for 5 h, 15 mL of 6 N HCl was added slowly, and then 15.0 mL of H_2O and 100 mL of MeOH were introduced. The reaction mixture was stirred continually overnight and filtered, and the filtrate was evaporated in vacuo to give product as a white solid which was further purified by chromatography using columns of 40 g of silica gel 60 (E. Merck) and eluting with *n*-butanol−HOAC−H_2O (4:1:2). Fractions containing product were combined to give 407 mg (90% yield) of **5**. ES^+: 151. TLC: R_f = 0.73, silica gel, *n*-butanol−HOAC−H_2O (4:1:2).

6-Amino-2,4-dimethyl-3-pyridinylmethyl *N*-(Benzylsulfonyl)-D-3,4-dichlorophenylalanyl-L-proinamide (14a). A solution of 121 mg (0.25 mmol) of *N*-(benzylsulfonyl)-D-3,4-Cl_2-Phe-Pro-OH (**1c**), 75.5 mg (0.50 mmol) of 6-amino-2,4-dimethyl-3-pyridinemethylamine (**5**), 43 mg (0.28 mmol) of HOBT, and 54 mg (0.28 mmol) of EDC in 1.7 mL of anhydrous NMP was treated with DIEA to pH 8.5, and the resulting solution was stirred at room temperature in a N_2 atmosphere for 8 h. The reaction mixture was diluted with 3× its volume of water, and the suspension stirred vigorously at room temperature for 15 min. The suspension was filtered and the residue purified by preparative HPLC using a trifluoroacetic acid (0.1%)−CH_3CN gradient. Lyophilization of pure fractions gave 140 mg (95%) of white solid **14a** as a trifluoroacetic acid hydrate salt.

6-Amino-2,4-dimethyl-3-pyridinylmethyl *N*-Boc-D-3,3-dicyclohexylalanyl-L-prolinamide (14b). A solution of 113 mg (0.25 mmol) of Boc-D-3,3-(Chx)$_2$-Ala-Pro-OH (**2c**), 75.5 mg (0.50 mmol) of 6-amino-2,4-dimethyl-3-pyridinemethylamine

(**5**), 43 mg (0.28 mmol) of HOBT, and 54 mg (0.28 mmol) of EDC in 1.7 mL of anhydrous NMP was treated with DIEA to pH 8.5, and the resulting solution was stirred at room temperature in a N_2 atmosphere for 8 h. The reaction mixture was diluted with 3× its volume of water, and the suspension stirred vigorously at room temperature for 15 min. The suspension was filtered, and the residue was purified by preparative HPLC using a trifluoroacetic acid (0.1%)−CH_3CN gradient. Lyophilization of pure fractions gave 135 mg (93%) of product **14b** as a trifluoroacetic acid hydrate salt.

6-Amino-2,4-dimethyl-3-pyridinylmethyl D-3,3-Dicyclohexylalanyl-L-prolinamide (14c). A solution of 128 mg (0.22 mmol) of 6-amino-2,4-dimethyl-3-pyridinylmethyl *N*-Boc-D-3,3-dicyclohexylalanyl-L-prolinamide (**14b**) in 10 mL of 50% TFA/CH_2Cl_2 was stirred for 20 min, and the TFA was removed under reduced pressure. The product was purified by preparative HPLC using a TFA (0.1%)−CH_3CN gradient. Lyophilization of pure fractions gave 102 mg (96%) of the title compound **14c** as a trifluoroacetic acid hydrate salt.

6-Amino-2,4-dimethyl-3-pyridinylmethyl *N*-Boc-D-3,3-diphenylalanyl-L-prolinamide (14d). An amount of 108 mg (0.25 mmol) of Boc-D-3,3-$(Ph)_2$-Ala-Pro-OH (**3b**) and 75.5 mg (0.50 mmol) of 6-amino-2,4-dimethyl-3-pyridinemethylamine (**5**) were coupled with hydroxybenzotriazole hydrate (43 mg, 0.28 mmol) and EDC (54 mg, 0.28 mmol) in 1.5 mL of DMF at pH 8.5 with DIEA. The mixture was stirred under N_2 at room temperature overnight, then diluted with 10 mL of 10% aqueous citric acid, and extracted with CH_2Cl_2. The CH_2Cl_2 extracts were washed with aqueous Na_2CO_3, dried (Na_2SO_4), filtered, and concentrated in vacuo to give the crude Boc derivative of the title compound. The product was purified by preparative HPLC using a TFA (0.1%)−CH_3CN gradient. Lyophilization of pure fractions gave 128 mg (90%) of the white powder **14d** as a trifluoroacetic acid hydrate salt.

6-Amino-2,4-dimethyl-3-pyridinylmethyl D-3,3-Diphenylalanyl-L-prolinamide (14e). A solution of 114 mg (0.20 mmol) of 6-amino-2,4-dimethyl-3-pyridinylmethyl *N*-Boc-D-3,3-diphenylalanyl-L-prolinamide (**14d**) in 10 mL of 50% TFA/CH_2Cl_2 was stirred for 20 min, the TFA was removed under reduced pressure, and the product was purified by preparative HPLC using a TFA (0.1%)−CH_3CN gradient. Lyophilization of pure fractions gave 92 mg (98%) of the title compound **14e** as a trifluoroacetic acid hydrate salt.

2-Amino-4-(aminomethyl)-1,3-thiazole (6). A solution of 2.0 g (10.5 mmol) of 2-acetamido-4-(chloromethyl)thiazole in 35 mL of anhydrous MeOH in an ice−water bath was stirred under NH_3 gas until volume increased to 70 mL. The reaction mixture was allowed to warm to room temperature, stirred for 48 h, and evaporated to dryness under reduced pressure. The residue was hydrolyzed with 6 N HCl at 100 °C for 4 h and then evaporated to provide 1.2 g (88%) of crude precursor **6** as a HCl salt.

2-Aminothiophene-4-methylamine (7). A 300-mL flask was dried in an oven and cooled in a dry nitrogen atmosphere. The flask was equipped with a rubber syringe cap and a magnetic stirring bar. The flask was immersed in an ice−water bath, and 10.5 mL (10.5 mmol) of 2.0 M borane solution in THF was introduced. Then 5-nitrothiophene-2-carbonitrile (468 mg, 3.06 mmol) in 10 mL of THF was introduced. The resulting mixture was allowed to stir for 4 h at room temperature, 15 mL of 6 N HCl was added slowly, and then 15.0 mL of H_2O and 100 mL of MeOH were introduced. The reaction mixture was stirred continually overnight, filtered, and evaporated in vacuo to give 435 mg of product as a white solid (FABMS: 159). A solution of 435 mg of 5-nitrothiophene-2-methylamine in 35 mL of EtOH/4.0 mL of acetic acid/8.0 mL of H_2O was hydrogenated at 60 psi on a Parr apparatus over 500 mg of 10% palladium hydroxide on carbon as catalyst. After 24 h the reaction mixture was filtered through a Celite pad, the residue was washed with ethanol, MeOH, and H_2O, and the combined filtrates were concentrated to provide 370 mg (94%) of amine intermediate **7** ($FABMS^+$: 129).

In a similar manner as **13a**−**e** were prepared the following compounds: **9**−**12**, **15a**−**e**, and **16a,b**.

Acknowledgment. The authors wish to thank Dr. A. Coddington for the fast atom bombardment mass spectroscopy and Dr. G. M. Smith, M. Zrada, and K. Anderson for chemical characterization. We also thank Dr. Joel R. Huff for his support and encouragement.

References

(1) Edit, J. F.; Allison, P.; Noble, S.; Ashton, J.; Golino, P.; McNard, J.; Buja, L. M.; Willerson, J. T. Thrombin is an important mediator of platelet aggregation in stenosed canine coronary arteries with endothelial injury. *J. Clin. Invest.* **1989**, *84*, 18.

(2) Claeson, G.; Aurell, L. Small synthetic peptides with affinity for proteases in coagulation and fibrinolysis. An overview. *Ann. N. Y. Acad. Sci.* **1981**, *370*, 789.

(3) Brady, S. F.; Lewis, S. D.; Colton, C. D.; Stauffer, K. J.; Sisko, J. T.; Ng, A. S.; Homnick, C. F.; Bogusky, M. J.; Shafer, J. A.; Veber, D. F.; Nutt, R. F. Development of highly potent and selective a-keto carbonyl thrombin inhibitors with novel P1 side chains: Synthesis and biological profile of L-370,518. In *Peptides: Chemistry, Structure and Biology (Proceedings of the Fourteenth American Peptide Symposium)*; Kaumaya, P. T. P., Hodges, R. S., Eds.; Mayflower Scientific Ltd.: England, 1995; pp 331−333.

(4) Veber, D. F.; Lewis, S. D.; Shafer, J. A.; Feng, D. M.; Nutt, R. F.; Brady, S. F. Thrombin inhibitors. WO 94-US4539 950607, 1994.

(5) Lyle, T. A.; Chen, Z.; Appleby, S. D.; Freidinger, R. M.; Gardell, S. J.; Lewis, S. D.; Li, Y.; Lyle, E. A.; Lynch, J. J., Jr.; Mulichak, A. M.; Ng, A. S.; Naylor-Olsen, A. M.; Sanders, W. M. Synthesis, evaluation, and crystallographic analysis of L-371,912: A potent and selective active-site thrombin inhibitor. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 67−72.

(6) (a) Hifijikata-Okkunomiya, A.; Okamoto, S. *Thromb. Haemostasis* **1992**, *101*, 440. (b) Angliker, H.; Wilstrom, P.; Shaw, E. Pseudoarginine: Synthesis and properties of derivatives of d-(1-imidazolyl)norvaline. *Biochem. J.* **1990**, *266*, 829−834.

(7) Feng, D.-M.; Bock, M. G.; Freidinger, R. M.; Vacca, J. P.; Dorsey, B. D. Thrombin inhibitors. PCT Patent Application WO 96/31504.

(8) After this work was finished, related lead structures were disclosed; see: Smith, G. F.; Wiley, M. R.; Schacht, A. L.; Shuman, R. T. Antithrombotic Agents. PCT Patent Application WO 95/23609.

(9) Tucker, T. J.; Lumma, W. C.; Mulichak, A. M.; Chen, Z.; Naylor-Olsen, A. M.; Lewis, S. D.; Lucas, R.; Freidinger, R. M.; Kuo, L. C. Design of highly potent noncovalent thrombin inhibitors that utilize a novel lipophilic binding pocket in the thrombin active site. *J. Med. Chem.* **1997**, *40*, 830−832.

(10) TegerNilsson, A. C. E.; Bylund, R. E. WO 9311152-A1, 1995.

(11) Tucker, T. J.; Lumma, W. C.; Lewis, S. D.; Gardell, S. J.; Lucas, B. J.; Baskin, E. P.; Woltmann, R.; Lynch, J. J.; Lyle, E. A.; Appleby, S. D.; Chen, I-W.; Dencheck, K. B.; Vacca, J. P. Potent noncovalent thrombin inhibitors that utilize the unique amino acid D-dicyclohexylalanine in the P3 position. Implications on oral bioavailability and antithrombotic efficacy. *J. Med. Chem.* **1997**, *40*, 1565−1569.

(12) Feng, D.-M.; et al. Unpublished results.

(13) Weir, L. C.; Wiley, M. R.; Borromeo, P. S.; Coffman, W. J.; Craft, T. J.; Gifford-Moore, D.; Lugor, C. W.; Schacht, A. L.; Shuman, R. T.; Smith, J. T.; Wikel, J. H. MEDI 127, Book of Abstracts of 211th ACS National Meeting, New Orleans, LA, March 24−28, 1996.

(14) Brown, H. C.; Heim, P. Selective reductions. XVIII. The fast reaction of primary, secondary, and tertiary amides with diborane. A simple, convenient procedure for the conversion of amides to the corresponding amines. *J. Org. Chem.* **1973**, *38* (5), 912−916.

(15) Lumma, W. C.; Freidinger, R. M.; Brady, S. F.; Sanderson, P. E.; Feng, D.-M.; Lyle, T. A.; Stauffer, K. J.; Tucker, T. J.; Vacca, J. P. Pyrrolidine thrombin inhibitors. US 5,510,369, 1996.

(16) Petermann, C.; Fauchere, J.-L. Synthesis of β-Pyrazinyl-L-alanine (Paa)[1] and Peptide Derivatives. *Helv. Chim. Acta* **1983**, *66*, 1513−1518.

(17) Lewis, S. D.; Ng, A. S.; Lyle, E. A.; Mellott, M. J.; Appleby, S. D.; Brady, S. F.; Stauffer, K. J.; Sisko, J. T.; Mao, S.-S.; Veber, D. F.; Nutt, R. F.; Lynch, J. J.; Cook, J. J.; Gardell, S. J.; Shafer, J. A. Inhibition of thrombin by peptides containing lysyl-α keto carbonyl derivatives. *Thromb. Haemostasis* **1995**, *74* (4), 1107−1112.

(18) Chen, Z.; Li, Y.; Mulichak, A. M.; Lewis, S. D.; Shafer, J. A. Crystal structure of human α-thrombin complexed with hirugen and p-amidinophenylpyruvate at 1.6 Å resolution. *Arch. Biochem. Biophys.* **1995**, *332* (1), 198−203.

JM970493R