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# DESIGN AND SYNTHESIS OF RING-CONSTRAINED BOROPEPTIDE THROMBIN INHIBITORS

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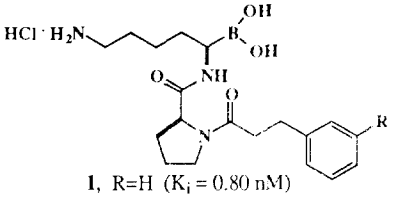
**Abstract** Ring-constrained boropeptide thrombin inhibitors were designed using information from the X-ray crystal structure of **1** (3-Phenylpropionyl-Pro-boroLys-OH · HCl) bound to thrombin. The constraints utilized cyclohexane and pyrrolidine rings to preorganize an aromatic ring in an orientation allowing optimum edge-to-face interaction with the tryptophan 215 side chain located in the S3 specificity pocket of thrombin.

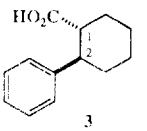
The serine protease thrombin catalyzes the conversion of fibrinogen to fibrin, the last enzyme-mediated step in the blood coagulation cascade. Fibrin contributes to clot formation by cross-linking platelets which aggregate at the point of blood vessel injury. Additionally, thrombin is a potent activator of platelets and also potentiates its own production by activation of factor V and factor VIII. These and other biological activities demonstrate the central role of thrombin in hemostasis and thrombosis and have led to an intense pursuit of thrombin inhibitors as potential antithrombotic agents.[1]

We have previously demonstrated that boronic acid derivatives of peptides can be effective inhibitors of serine proteases.[2a,b] Recently, the peptide boronic acids **1** (3-Phenylpropionyl-Pro-boroLys-OH · HCl) and **2** (3-[3-Trifluoromethyl]phenylpropionyl-Pro-boroLys-OH · HCl) were identified as potent, orally active inhibitors of thrombin (Figure 1).[2c] An X-ray crystal structure analysis of the complex of **1** bound to thrombin showed the expected interaction of the amino side chain with Asp189 in the S1 specificity pocket and the tetrahedral complex between the hydroxyl of the active site serine (Ser195) and the boron of the inhibitor.[3] This complex mimics the transition state formed during natural substrate hydrolysis and strongly contributes to the observed potency of this class of inhibitors. Another important interaction involves the 3-phenylpropionyl (P3) residue, which is kinked out of its extended conformation, placing the phenyl ring in the vicinity of the proline residue and allowing it to form a favorable edge-to face interaction[4] with Trp215 located at the base of the S3 specificity pocket.

Figure 1. Lead Compounds and Ring-Constrained 3-Arylpropionic Acid Mimics

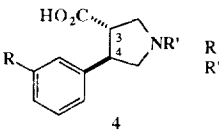



**1**, R=H ($K_i$ = 0.80 nM)
**2**, R=$CF_3$ ($K_i$ = 0.78 nM)




**3**

(1*R*, 2*R*)-2-phenylcyclohexane carboxylic acid




**4**

(3*S*, 4*R*)-4-arylpyrrolidine-3-carboxylic acid and analogs

The similarity between **1** and its 3α-amino analogue **3** was apparent from their $^{1}H$ and $^{13}C$ nmr spectra recorded in deuterium oxide (*Figure*); both **1** and **3** show six resonances in their $^{1}H$ (two of which overlap in the spectrum of **3**) and seven signals in their $^{13}C$ spectra. Spectral data (coupling constants) showed the solution structures of **1** and **3** in $D_2O$ to be similar; replacement of the 3α-hydroxyl functionality of **1** with an amino group in **3** therefore has little effect upon preferred molecular conformation. Noticeably in amino acid **3** the $^{1}H$ and $^{13}C$ resonances corresponding to H-3 and C-3 (the point of substitution) are shifted upfield relative to those in **1** (by 0·43 and 14·7 ppm respectively) as a result of the lower electonegativity of nitrogen when compared to oxygen, similarly H-2 and C-2 are somewhat deshielded in **3** relative to **1** although to a lesser extent.

Previously reported attempts to introduce nitrogen functionality at C-3 of the shikimate nucleus have centred around the formation of 3-imino species by the addition of primary amines to both 3-dehydroshikimic and 3-dehydroquinic acids.[19] These studies proved inappropriate for the synthesis of 3-amino derivatives of shikimate and quinate as aromatization of the carbocyclic ring invariably occurred under the reaction conditions; our studies thus provide the first examples of methods suitable for the introduction of nitrogen functionality at C-3 of the shikimate nucleus.

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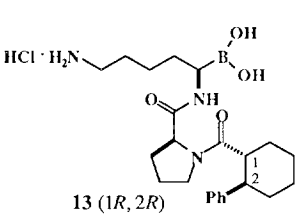
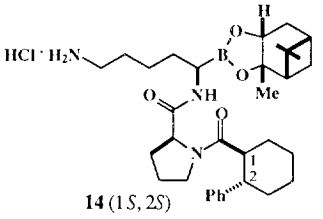
Stereochemical assignment required the resolution of the enantiomers of (±)-**8**, which was accomplished using (*R*)-α-phenylethylamine, giving the desired (1*R*, 2*R*) enantiomer **3** with good optical purity.[8] Coupling of **3** with proline methyl ester hydrochloride and saponification cleanly produced diastereomer **10**, allowing for the definitive stereochemical assignments of **9** and **10**. The elaboration of **9** and **10** to their respective boropeptide derivatives **14** and **13** followed an established synthesis route[9] involving initial mixed anhydride coupling with optically pure (+)-pinanediol (*S*)-1-amino-5-bromopentyl boronate · hydrochloride **12**, which was prepared from 4-bromo-1-butene by the asymmetric homologation procedure of Matteson.[10] Figure 2 shows **13** and **14** along with a comparison of their inhibitory activities relative to **1**.

Figure 2. Binding Results of Cyclohexane-constrained Inhibitors vs. **1***



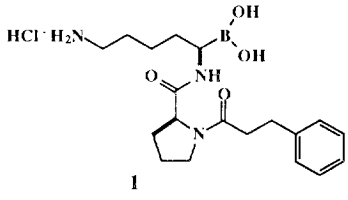

**13** (1*R*, 2*R*)

$K_i$ = 0.46 nM




**14** (1*S*, 2*S*)

$K_i$ = 30 nM




**1**

$K_i$ = 0.80 nM

*The (+)-pinanediol ester of **14** is hydrolyzed to the free boronic acid under the assay conditions. Throughout this manuscript, the inhibitory constant ($K_i$) assays were performed as described in reference 2a and the $K_i$ values reported are averages from multiple experiments.

It was gratifying that the desired (1*R*, 2*R*) isomer **13** is about two orders of magnitude more potent in its affinity for thrombin than the (1*S*, 2*S*) isomer **14**. Presumably, the P3 aromatic ring of **14** is locked into a conformation that does not allow effective edge-to-face interaction with Trp215 and, given the magnitude of the potency difference, may be locked into a conformation which results in destabilizing steric interactions with the enzyme. The desired (1*R*, 2*R*) isomer **13** showed a modest increase in binding affinity relative to **1**. We were able to obtain an X-ray crystal structure of **13** bound to thrombin which confirmed our proposed binding hypothesis. This is shown in Figure 3, where the thrombin-bound conformations of **1** and **13** are superimposed.[11] The figure shows the excellent overlap of the P3 phenyl rings between the two inhibitors and also how they are engaged in an edge-to-face interaction with Trp215. We might have expected a greater increase in affinity for **13**, given the apparent flexibility of the 3-phenylpropionyl P3 residue in **1**. However, recent NMR evidence of related boropeptides which contain (D)-Phe-Pro as the P2P3 residue indicate that these inhibitors adopt a solution conformation that is similar to the thrombin-bound conformation.[12] This preorganization appears to involve stacking between the phenyl and proline rings which is stabilized by π-π interactions between the phenyl ring and the (D)-Phe-Pro amide bond. It is conceivable that **1** may be preorganized in the same manner, resulting in the observed small difference in binding potency between **13** and **1**.

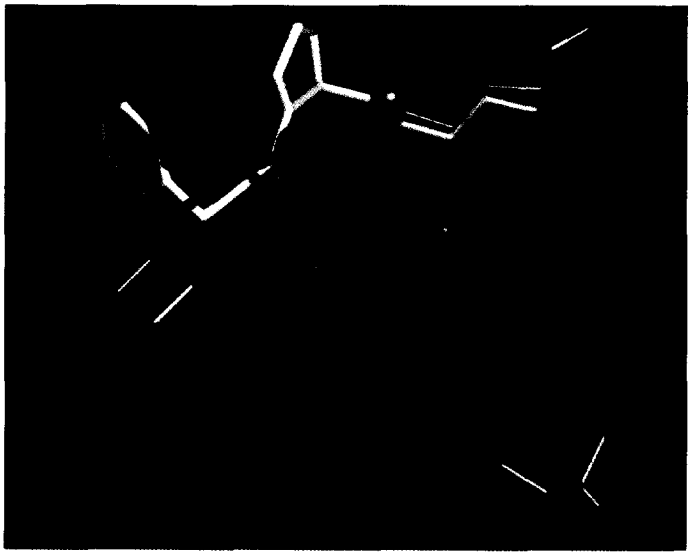
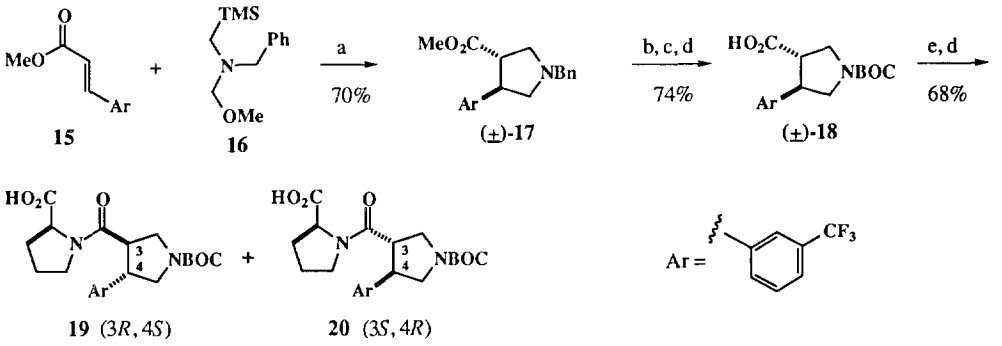


Figure 3. Superposition of the thrombin binding conformations of **1** (yellow) and **13** (red). Highlighted residues of thrombin, clockwise from upper right, are Ser195, Asp189 and Trp215.

The pyrrolidine-based inhibitors were prepared as shown for the 3-trifluoromethyl analog in Scheme 2. The key step involved the [3+2] cyclization of the azomethine ylide derived from **16**[13] with the methyl cinnamate **15** to establish the racemic 3,4-*trans*-disubstituted pyrrolidine (±)-**17**. N-protecting group exchange and saponification led to the acid (±)-**18**. Coupling with proline methyl ester hydrochloride and saponification produced the expected 1:1 mixture of diastereomers **19** and **20**. These diastereomers were separately elaborated to their respective borolysine derivatives as described for the cyclohexane series.

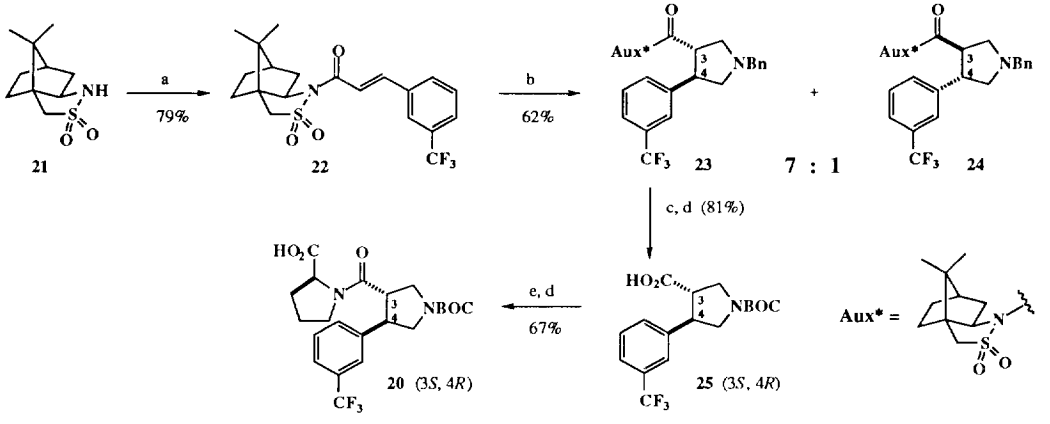
Scheme 2




Reagents: (a) TFA, $CH_2Cl_2$ (b) $H_2$ (1 atm), 10% Pd/C, HCl, MeOH (c) $(BOC)_2O$, diisopropylethylamine, $CH_2Cl_2$ (d) $LiOH \cdot H_2O$, $THF/H_2O$ (e) Pro-OMe hydrochloride, HBTU, diisopropylethylamine, DMF

Stereochemical assignment of the diastereomers **19** and **20** was accomplished as outlined in Scheme 3. Cuprous chloride-mediated[14] reaction of (1*S*)-(-)-2,10-camphorsultam **21**[15] with *trans*-3-trifluoromethylcinnamoyl chloride afforded the sulfonamide **22**, which underwent [3 + 2] azomethine ylide cyclization to produce an unoptimized 7:1 mixture of readily separable diastereomers **23** and **24**. An X-ray crystal structure analysis of **23** confirmed the desired (3*S*, 4*R*) stereochemistry.[16,17] Subsequently, **23** was easily converted into **20**, which allowed for the definitive stereochemical assignments of diastereomers **19** and **20**.

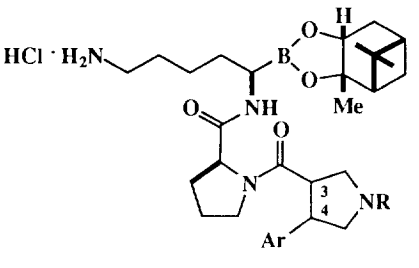
Scheme 3




Reagents: (a) i) NaH, toluene ii) *trans*-3-(trifluoromethyl)cinnamoyl chloride, CuCl (b) **16**, TFA, $CH_2Cl_2$ (c) $H_2$ (1 atm), 10% Pd/C, $(BOC)_2O$, MeOH (d) LiOH · $H_2O$, THF/$H_2O$ (e) Pro-OMe hydrochloride, EDC, HOBT, $Et_3N$, THF

The binding results for the pyrrolidine-constrained inhibitors are presented in Table 1. The desired (3*S*, 4*R*) isomers **27** and **29** were more active than the (3*R*, 4*S*) isomers **26** and **28**, although in this series the binding difference between isomers was not as dramatic as in the cyclohexane series (one order of magnitude vs. two orders of magnitude). These results may reflect the greater flexibility of the pyrrolidine series in that the undesired

Table 1. Binding Results of Pyrrolidine-constrained Inhibitors vs. **2**




| CMPD# | isomer | R | Ar | $K_i$ (nM) |
|---|---|---|---|---|
| **2** | - | - | - | 0.78 |
| **26** | (3*R*,4*S*) | BOC | 3-$CF_3C_6H_4$ | 3.1 |
| **27** | (3*S*,4*R*) | BOC | 3-$CF_3C_6H_4$ | 0.32 |
| **28** | (3*R*,4*S*) | H · HCl | 3-$CF_3C_6H_4$ | 5.6 |
| **29** | (3*S*,4*R*) | H · HCl | 3-$CF_3C_6H_4$ | 0.5 |

isomers may be better able to adopt a conformation which minimizes severe steric interactions. Both the N-BOC-protected **27** and the pyrrolidine **29** are about two-fold more potent than the reference compound **2**. Given the opposite polarities of the added functionality in the pyrrolidine **29** and the cyclohexane **13**, that both constraints result in a two-fold increase in potency relative to their unconstrained counterparts suggests that the potency increase is a preorganization effect and is not due to interactions of the added functionality in the S3 pocket.

In summary, we have prepared ring-constrained boropeptide thrombin inhibitors which were designed to optimize an aromatic edge-to-face interaction observed in the X-ray crystal structure of **1** bound to thrombin. The results are an illustration of the effectiveness of using X-ray crystallography data in drug design, as the targeted compounds showed improved binding potency relative to the unconstrained lead compounds. This increase in binding affinity is notable because of the evidence that compounds like **1** and **2** may already be preorganized for binding as a result of the stacking between the P3 aromatic moiety and the P2 proline residue.

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## References and Notes

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