Acylamino Boronic Acids and Difluoroborane Analogues of Amino Acids: Potent Inhibitors of Chymotrypsin and Elastase

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A series of 1-acylamino boronic acids (IA–VA), analogues of the amino acids phenylalanine, phenylglycine, alanine, valine, and isoleucine, were prepared as potential transition-state inhibitors of the serine proteases α-chymotrypsin and elastase, by a boronate homologation reaction. The corresponding difluoroboranes (IB–VB), produced from the boronic acids by treatment with HF, were more easily purified than the boronic acids. Since the difluoroboranes readily hydrolyze in water, they proved to be convenient precursors for the boronic acids. The phenylalanine and phenylglycine analogues I and II were good competitive inhibitors of α-chymotrypsin (K_i = 0.3–8 μM), and the alanine, valine, and isoleucine analogues (III–IV) proved to be good inhibitors of elastase (K_i = 0.1–35 μM). On the basis of their high affinity and the tendency of boronic acids to form borate complexes, these acylamino boronic acids may be behaving as transition-state inhibitors.

Certain boronic acids have been investigated as inhibitors of serine hydrolase enzymes.[1] Their inhibitory activity has been ascribed to their potential to act as "transition-state" analogues:[2,3] the boronic acid, being isoelectronic with a protonated carboxyl group, is presumed to block enzymatic activity by engaging in nonproductive tetrahedral borate complexes with the active site serine, analogous to the productive tetrahedral carbonyl adducts that are involved in the hydrolysis of carboxy derivatives.[4] While these earlier studies have been provocative and have suggested that boronic acids may be useful as potent, specific inhibitors, they have been relatively limited in scope.

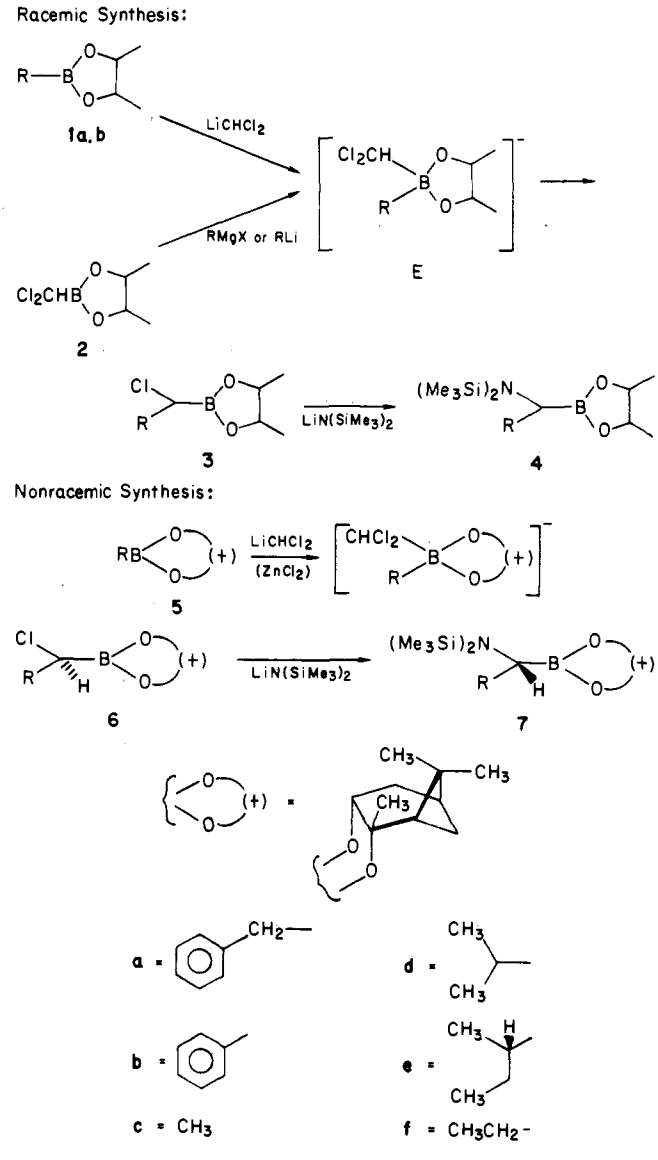
In this report, we describe the preparation and biological activity of several 1-acylamino boronic acids (IA–VA) (in many cases as single enantiomers) that are analogues of different amino acids and dipeptides and are designed to act as transition-state inhibitors of the serine proteases α-chymotrypsin and elastase. In the course of preparing these derivatives, we have found that the difluoroborane analogues (IB–VB) of these boronic acids are stable and more easily purified than the acids. Since they hydrolyzed readily in aqueous buffer, they are convenient precursor forms for the boronic acid amino acid analogue type transition-state inhibitors. These boronic acid and difluoroborane analogues are good inhibitors of chymotrypsin (K_i = 0.3–8 μM) and elastase (K_i = 0.1–35 μM).

During the course of this work, several publications by Matteson[5a–c] appeared, in which the preparation of various precursors 1-chloro boronic esters and the 1-acetylamino boronic acids (*R*)- and (*S*)-Ac-IA, both racemic and nonracemic, was described. Preliminary enzyme-inhibition studies on Ac-IA have also been reported.[5d,e] In addition, a very recent publication by Kettner and Shenvi[6] describes the preparation of a number of tetrapeptide boronic acid analogues and the demonstration that some of them are potent inhibitors of chymotrypsin, cathepsin G, and both leukocyte and pancreatic elastase.

Results and Discussion

Chemical Synthesis. General Approach. Boronic acid and difluoroborane analogues of five amino acids (IA–VA, IB–VB) were prepared (Figure 1).[7] The phenylalanine and phenylglycine analogues (I and II) were designed as α-chymotrypsin inhibitors, and the alanine, valine, and isoleucine analogues (III–V), as inhibitors of elastase. As will be described below, some of the derivatives of these chiral species were prepared in nonracemic form (in the same configuration as the L amino acids), and in some cases, several different *N*-acyl derivatives were

Scheme I. Synthesis of 1-Amino Boronates by Homologation-Amination



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(1) (a) Koehler, K. A.; Lienhard, G. E. *Biochemistry* **1971**, *10*, 2477. (b) Lindquist, R. N.; Terry, G. *Arch. Biochem. Biophys.* **1974**, *160*, 135.

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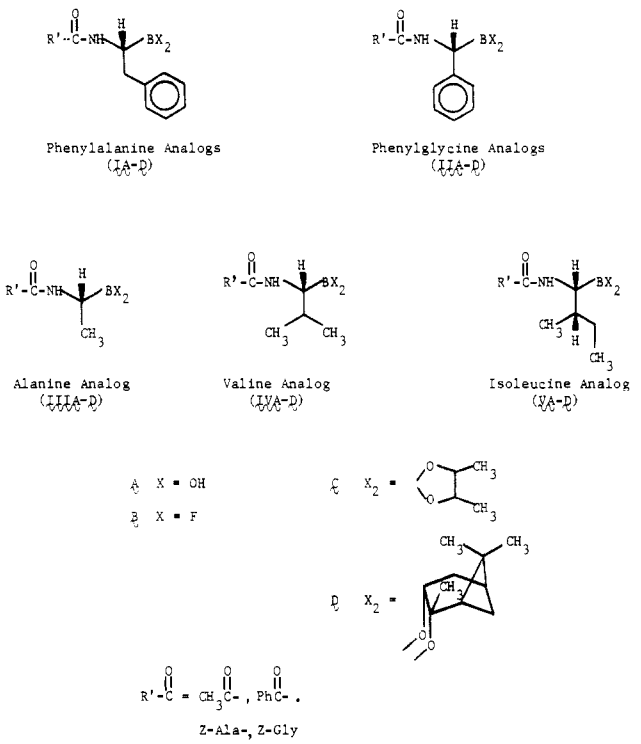
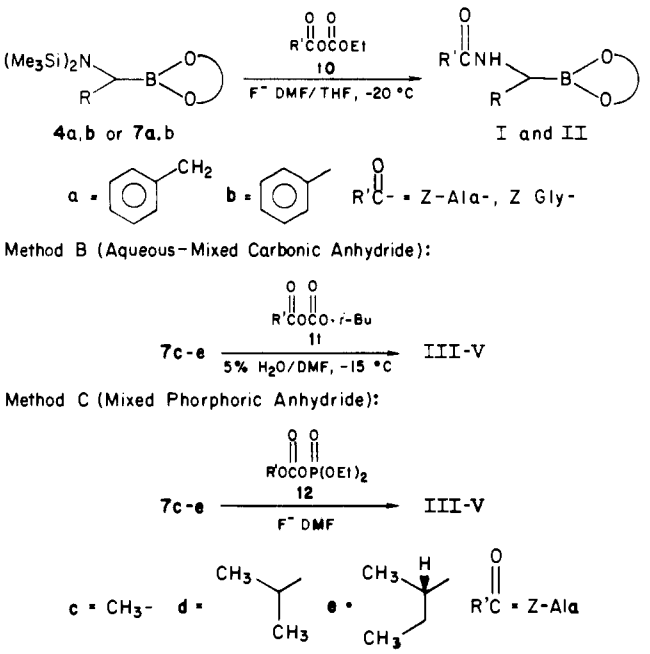


Figure 1. 1-Acylamino boronic acids, difluoroboranes, and related esters (*R* enantiomers are shown).

prepared. The synthetic methods were patterned after those of Matteson.[5a-d]

The 1-[bis(trimethylsilyl)amino] boronic esters are precursors to the acylamino boronates. The general approach to the 1-[bis(trimethylsilyl)amino] boronic ester derivative **4** is shown in Scheme I. A precursor boronate,

Scheme II. Deprotection-Acylation of !-Amino Boronates



as either the *d,l-threo*-2,3-butanediol ester **1** or the (+)-pinanediol ester **5** for the nonracemic synthesis,[8] is treated with (dichloromethyl)lithium.[5a] In the unstable dichloromethyl borate complex E, migration of the original carbon substituent results in the formation of the "homologated" 1-chloro boronate **3**. The 1-amino group is introduced by displacement of the chloride with lithium hexamethyldisilylamide ($LiN(SiMe_3)_2$).[5d] While the 1-[bis(trimethylsilyl)amino] boronic esters can be hydrolyzed to the unstable 1-amino boronates, desilylation is generally accomplished in situ during the acylation reaction. The stable 1-acylamino boronates can then be hydrolyzed to the acid and converted further to the difluoroborane.

Preparation of 1-Chloro- and 1-Bis(Trimethylsilyl)amino Boronates. The 1-chloro boronates were prepared by the "homologation" (chloromethyl insertion)[9] of the next lower boronic ester **1** or **5** and (dichloromethyl)lithium ($LiCHCl_2$) (Scheme I).[8,9] The *d,l-threo*-2,3-butanediol 1-chloro boronic ester could also be prepared by the treatment of 2,3-butanediyl (dichloromethyl)boronate (**2**) with a Grignard or lithium reagent.[10] The 1-chloro boronic esters (**3** and **6**) are sufficiently stable

(2) (a) Wolfenden, R. *Ann. Rev. Biophys. Bioeng.* **1976**, *5*, 271. (b) Lienhard, G. E. *Science (Washington, D.C.)* **1973**, *180*, 149. (c) Wolfenden, *R. Acc. Chem. Res.* **1972**, *5*, 10. (d) Wolfenden, R. *Nature* **1969**, *223*, 704. (e) Rawn, J. D.; Lienhard, G. E. *Biochemistry* **1974**, *13*, 3124. (f) Koehler, K. A.; Hess, B. P. *Ibid.* **1974**, *13*, 5545. (g) Ckrna, J.; Rychlik, I. *FEBS Lett.* **1980**, *119*, 343. (h) Garner, C. W. *J. Biol. Chem.* **1980**, *255*, 5064. (i) Westerlik, J. O.; Wolfenden, R. *Ibid.* **1972**, *247*, 8195.

(3) The term "transition-state" analogue is a designation given to principles of design rather than actual function, and as such, this term should be understood in more of a figurative than a literal sense. Transition-state mimics, in fact, have often been more like mimics of high-energy intermediates.

(4) A crystal structure of (phenylethyl)boronic acid or phenylboronic acid with subtilisin shows the boron engaged in a tetrahedral borate complex with the active site serine: Matthews, D. A.; Alden, R. A.; Birktoft, J. J.; Freer, S. T.; Kraut, J. *J. Biol. Chem.* **1975**, *250*, 7120.

(5) (a) Matteson, D. S.; Majumdar, D. *Organometallics* **1983**, *2*, 1959 and references cited therein. (b) Matteson, D. S.; Ray, R.; Rocks, R. R.; Tsai, D. J. *Ibid.* 1536 and references therein. (c) Matteson, D. S.; Sadhu, K. M. *Organometallics* **1984**, *3*, 614. (d) Matteson, D. S.; Sadhu, K. M.; Lienhard, G. E. *J. Am. Chem. Soc.* **1981**, *103*, 5241. (e) Amiri, P.; Lindquist, R. N.; Matteson, D. S.; Sadhu, K. M.. *Arch. Biochem. Biophys.* **1984**, *234*, 531.

(6) Kettner, C. A.; Shenvi, A. B. *J. Biol. Chem.* **1984**, *259*, 15106.

(7) To facilitate discussion, we have used a numerical designation system for these boronic acid derivatives that is indicative of their structure (cf. Figure 1): (a) The basic boronate structure is indicated by a Roman numeral (I = phenylalanine analogue; II = phenylglycine analogue; III = alanine analogue; IV = valine analogue; V = isoleucine analogue). (b) The appropriate *N*-acyl group is shown as a prefix to the Roman numeral. (c) The form of the boronic acid is designated with a suffix letter (A = boronic acid; B = difluoroborane; C = *dl*-2,3-butanediol ester; D = (+)-pinanediol ester).

(8) The (+)-pinanediol ester directs rearrangement of the -ate complex to produce the (*S*)-chloroboronates (cf. ref 5a). The chloride is displaced by $LiN(SiMe_3)_2$ with inversion to produce the *R* configuration in the amino boronate.

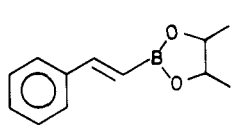
(9) The nonracemic phenylalanine analogues (I) were prepared from 95% enantiomeric excess (ee) pinanediol, and the elastase inhibitors (III–V) were prepared from 92% ee material. The nonracemic phenylglycine analogues (II) were prepared from 100% ee pinanediol phenylboronate. This phenylboronate was recrystallized from moist ethanol, with 85% recovery.

(10) Tsai et al. have recently reported similar homologations from (dichloromethyl)boronic esters. He found, as we also found, that rearrangement of the -ate complex from (+)-pinanediol (dichloromethyl)boronate and alkyl anion did not proceed stereospecifically to produce a single enantiomeric product: Tsai, D. J. S.; Jesthi, P. K.; Matteson, D. S. *Organometallics* **1983**, *2*, 143. However, Sadhu et al. reports that (*R,R*)-2,3-butanediol (dichloromethyl)boronate does rearrange stereospecifically when treated with alkyl anion to produce a single enantiomeric chloroboronate: Sadhu, K. M.; Matteson, D. S.; Hurst, G. D.; Kurosky, J. M. *Organometallics* **1984**, *3*, 804.

to be purified by flash chromatography on silica. The 2,3-butanediol esters **3** could also be distilled.

The "homologation" of the boronic esters is facilitated by $ZnCl_2$ as catalyst,[11] and the yields of some of the pinanediol 1-chloro esters are improved. Thus, compounds **6c** and **6e** were prepared with $ZnCl_2$; the yield for the reaction to produce **6c** increased from 50% for the uncatalyzed to 70% for the catalyzed reaction, and the amount of **6e** increased from a few percent to 86% with catalyst.

The 1-[bis(trimethylsilyl)amino] boronates **4** and **7** were prepared by treating the 1-chloro boronates with lithium hexamethyldisilylamide ($LiN(SiMe_3)_2$).[5c] When **3a** was treated with $LiN(SiMe_3)_2$ at −78 °C, in addition to the amino boronate **4a**, about 3% of the vinyl boronate **8** was



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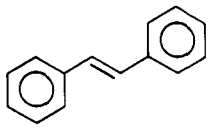

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isolated after chromatography of the acylamino product. When **3b** was treated in a similar manner, about 30% stilbene (**9**) was isolated.[12] However, there was no detectable stilbene from the reaction of **6b** with $LiN(SiMe_3)_2$, indicating increased steric bulk of the boronic ester can suppress the formation of stilbene.[13] The 1-[bis(trimethylsilyl)amino] boronates **4** and **7** were isolated by separating them from the salts, and they were acylated without further purification.

Acylation of the 1-Amino Boronates. In preliminary studies, we attempted to introduce the amino group by treatment of chloro boronates with ammonia in methanol or tetrahydrofuran (THF), but we found that the products had undergone extensive protiodeboronation, indicating the free amino boronates were prone to decompose even under mild conditions.[5c] This led us to perform acylation directly, with in situ deprotection (desilylation) of the amine with fluoride salts. We also found that triethylamine hydrochloride leads to protiodeboronation of either protected or free amino boronate (but not the acylamino products). Thus, acylation methods that produced trialkylamine hydrochlorides were unacceptable.

The methods that were used to effect the desired acylation, under neutral conditions, are outlined in Scheme II. The acyl carbonate method (mixed anhydride, methods A and B), traditionally used in peptide synthesis, was modified by use of the cesium salt of the acid,[14] thereby eliminating the need for a tertiary amine base. An acyl phosphonate method (method C),[15] that has been used to couple bulky peptides in dimethylformamide (DMF) solutions, was also modified by use of the cesium salt of the acid and diethyl chlorophosphonate.

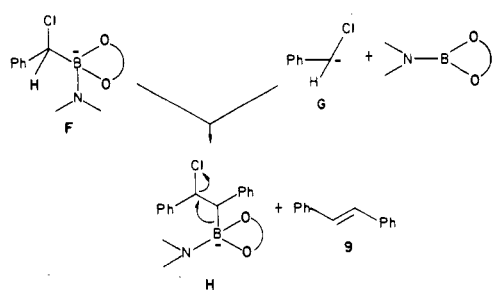
In situ deprotection of the silylamines could be accomplished with fluoride ion or water. Anhydrous fluoride salts were prepared as either tetrabutylammonium fluoride (n-Bu_4NF), the dihydrogen fluoride salt (n-$Bu_4NF{\cdot}2HF$), or an anion-exchange resin in the fluoride form.[16] Commercially available fluoride salts that contained 5% water could not be successfully employed in the deprotection–acylation sequences. Apparently, the combination of water and fluoride ion leads to decomposition of the 1-amino boronate. Water was found to be useful for deprotection of the pinanediol esters **7c–e** when it was noted that acylation of amino acids with symmetrical anhydrides[17] could be successfully accomplished in high yields in aqueous DMF solutions and that acyl carbonates are only slowly hydrolyzed in aqueous DMF.[18]

The fluoride deprotection–acylation sequence was used to produce PhCO-IA and PhCO-IIA: **4a** and **4b** were treated with the benzoyl ethyl carbonate (**10**) in 1:1 DMF/THF at −20 °C, followed by 2 equiv of fluoride ion, as either n-Bu_4NF or the anion-exchange resin. Acylamido boronic acid PhCO-IA was produced in 50–70% yield, whereas PhCO-IIA was produced in 10% yield (three steps), due in part to competing stilbene production.[19] Since the butanediol esters are hydrolyzed in the basic aqueous workup, the 1-acylamino boronic acids are isolated directly. The analogous difluoroboranes PhCO-IB and -IIB could be produced directly from **4a** and **4b** by using n-$Bu_4NF{\cdot}2HF$ for the deprotection (see below). The Z-Gly analogues IA and IIA were produced in the same manner and in similar yields as the benzoyl analogues from Z-Gly-ethyl carbonate **10**. For comparison, the acetyl analogues Ac-IA and (*R*)-Ac-IA were prepared by a method similar to the literature[5d] and then converted into the difluoroboranes Ac-IB.

The nonracemic analogues **7a,b** were acylated under similar conditions as **4a,b** with PhCO- and Z-Ala-ethyl carbonate **10**. Benzoic anhydride could also be substituted for $PhCOOCO_2Et$ (**10**), since it is produced to some degree during the formation of **10**, and excess benzoic acid does not complicate purification. Since the pinanediol esters are stable to product-isolation conditions (and must be cleaved with BCl_3 to produce the final boronic acids),[20] the

(11) Matteson, D. S.; Sadhu, K. M. *J. Am. Chem. Soc.* **1983**, *105*, 2078.

(12) Stilbene probably arises as a result of α-elimination in the -ate complex to form a chlorobenzyl anion G, which can displace chloride in F. The adduct H can then undergo β-elimination to **9**.



(13) Ethylene glycol (1-chlorobenzyl)boronate produced stilbene exclusively.

(14) To our knowledge, use of the cesium salt of the amino acid in reaction with chloroformate to form acyl carbonates (mixed anhydrides) has not been reported. The method provides neutral conditions for coupling of amino acids, and yields were equivalent to those obtained with tertiary amines as base. The ethylbenzoyl carbonate, however, was subject to nucleophilic attack by cesium benzoate, forming benzoic anhydride, although at −20 °C the amount of anhydride produced was minimal. Anhydride production was also noted with ethyl chloroformate, triethylamine, and benzoic acid.

(15) (a) Kumar, A. A.; Fresheim, J. H.; Kempton, R. J.; Anstead, G. M.; Black, A. M.; Judge, L. *J. Med. Chem.* **1983**, *26*, 111. (b) Yamada, S.; Kasai, Y.; Shioiri, T. *Tetrahedron Lett.* **1973**, 1595.

(16) Gelbard, G.; Colona, S. *Synthesis* **1977**, 13.

(17) Benoiton, N. L.; Chen, F. M. F. *FEBS Lett.* **1981**, *125*, 104.

(18) Chen, F. M. F.; Steinauer, R.; Benoiton, N. L. *J. Org. Chem.* **1983**, *48*, 2941.

(19) The phenylglycine analogues II were converted to the difluoroboranes for purification when the boronic acids could not be induced to crystallize.

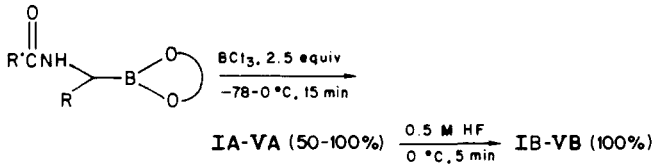
(20) Matteson, D. S.; Majumdar, D. *J. Am. Chem. Soc.* **1980**, *102*, 7588.

Table I. Competitive Inhibition of α-Chymotrypsin by 1-Acylamino Boronic Acids and Difluoroboranes IA, IB, and IIB[a]

compd	R'CO gp					
	K_i(rac), μM			K_i(R enant), μM		
	CH_3CO	PhCO	Z-Gly	CH_3CO	PhCO	Z-Ala
phenylalanine boronic acid (IA) analog	6.5	0.65		2.1[b]	0.36	0.32
phenylalanine difluoroborane (IB) analog	7.4	0.65	1.4	1.7	0.27	0.29
phenylglycine difluoroborane (IIB) analog		5.0	2.0			6.5

[a] Assay was conducted at 22 °C with 50 nM α-chymotrypsin, inhibitor concentrations 10–100-fold enzyme concentration, 0.01–0.5 mM BTEE, in pH 7.8, 0.08 M Tris/0.1 M $CaCl_2$ buffer. BTEE and inhibitor were premixed, and enzyme solution was added just prior to assay. Increase in absorbance at 256 nm was read with time. For further details, see the Experimental Section. Absent values correspond to compounds that were not prepared; the phenylglycine boronic acid analogues (IIA) were not studied because they were difficult to purify. [b] Literature value, see ref 5d.

Scheme III. Preparation of Difluoroboranes



pinanediol esters of the amido boronates were isolated after acylation and were recrystallized to constant rotation. PhCO-ID, Z-Ala, ID, and Z-Ala-IID were produced in 25–35% yield (three steps).

The acylation of **7c–e** could not be achieved under the conditions used to acylate **7a,b**, and conditions that enhanced the nucleophilicity of the amino boronates were required to produce reasonable amounts of the desired acylamino product. Acylation could be achieved in 5% water/DMF at −15 °C with the acyl carbonate 11. The water also served to deprotect the amino group. Thus, when **7d,e** were treated with Z-Ala isobutyl carbonate 11, a 42% yield (three steps) of Z-Ala-IVD was produced, and a 21% yield (two steps) of Z-Ala-VD. When **7c** was acylated under these conditions, only 7% yield (two steps) of Z-Ala-IIID could be obtained. No conditions were found that improved the yield.

The acylation of **7c–e** could also be accomplished with Z-Ala acyl phosphate 12 in DMF at 0 °C, with concomitant fluoride deprotection. The yields were slightly lower than for the isobutyl acyl carbonates: Z-Ala-IVD in 35% (three steps), Z-Ala-VD in 10–15% (two steps), and Z-Ala-IIID in 6% yields. It is unclear why Z-Ala-IIID is produced in such low yield.

All of the esters could be purified by flash chromatography on silica gel. However, the butanediol esters were usually hydrolyzed with aqueous base and the acids purified by recrystallization. The pinanediol esters could often be triturated with hexane–ethyl acetate and recrystallized to constant rotation before hydrolysis (see below).

Hydrolysis of the Pinanediol Esters. The butanediol esters were easily hydrolyzed in aqueous base during workup of the acylation reactions. Conversely, the pinanediol esters required destruction of the pinanediol moiety[20] to free the boronic acid (Scheme III). This cleavage could be effected by treating the esters with BCl_3 at low temperatures. However, the benzyloxycarbonyl protecting group is sensitive to this Lewis acid. Loss of the protecting group was minimized by mixing equal volumes of the precooled (−78 °C) CH_2Cl_2 solutions of 0.5 M BCl_3 (2–5-fold excess) and the boronic ester. After the reaction was warmed to 0 °C, the hydrolysis was complete within 15 min. (The disappearance of starting ester was followed by TLC.) The reaction was stopped by the addition of water at 0 °C. The final boronic acids IA–VA were produced in 50–87% yield (100% for the benzoyl analogue). The acids were recrystallized from acetone-/H_2O by dissolving the acids in acetone, adding water until the solution was almost saturated, and evaporating the more volatile acetone under vacuum. The combination of agitation and cooling caused crystals to form.

Table II. Competitive Inhibition of Porcine Elastase by Chiral Boronic Acids (IVA, VA) and Difluoroboranes (IIB–VB)

Z-Ala/X	K_i, μM	
	OH	F
alanine analogue (IIIB)		34.5
valine analogues (IVA, IVB)	1.1	1.0
isoleucine analogues (VA, VB)	0.12	0.10
phenylglycine analogue (IIB)		17.6

[a] The assay was conducted at 23 °C with 1 μg/mL of porcine elastase (10 units/mg) and inhibitor concentrations 10–100-fold enzyme concentrator, 0.12–0.4 mM Ac-Ala-Ala-Ala-*p*-nitroanaline, in Tris buffer, 0.1 M, pH 8.0. The inhibitor and substrate were premixed, and enzyme was added just prior to assay. Increase in absorbance at 385 nm was read with time. For further details, see the Experimental Section. Absent values correspond to compounds not tested.

Difluoroboranes. The boronic acids IA–VA were converted quantitatively to the difluoroboranes IB–VB by brief exposure to a slight excess of 0.5 M aqueous HF at 0 °C, extraction of the product into ethyl acetate, and purification by recrystallization from acetone/water. (Alcohol did not produce analytically pure material.) The difluoroboranes behave as acids, being extracted into aqueous base. Attempts to regenerate the boronic acid by treatment of the difluoroborane with trimethylsilyl chloride or attempts to regenerate the esters by treatment with butanediol or pinanediol were unsuccessful. The major advantage of the difluoroboranes is their ease of purification relative to the boronic acids. However, there is evidence that the fluoroboranes are completely hydrolyzed in aqueous Tris buffer, pH 6.5. This is discussed in the next section.

Enzyme-Inhibition Study. Structure–Inhibition Potency Relationships. The dissociation constants, K_i, for the inhibitors of α-chymotrypsin and porcine elastase are shown in Tables I and II. They were determined by competitive assay using the substrates benzoyltyrosine ethyl ester (BTEE) at pH 7.8 for chymotrypsin,[21] and Ac-$(Ala)_3$-*p*-nitroanilide at pH 8.0 for elastase.[22] As can be seen in the representative double-reciprocal plot of initial velocity and substrate concentration (Figure 2), the inhibition was purely competitive. Several aspects of these data are immediately obvious. All of the compounds are good inhibitors of their respective enzymes, being bound by enzyme several orders of magnitude more tightly than substrates, the difluoroborane analogues (IB-VB) having

(21) "Worthington Enzyme Manual"; Worthington Biochemical Corp., 1972; p 129.
(22) Feinstein, G.; Kupfer, A.; Sokolovsky, M. *Biochem. Biophys. Res. Commun.* **1973**, *50*, 1020.

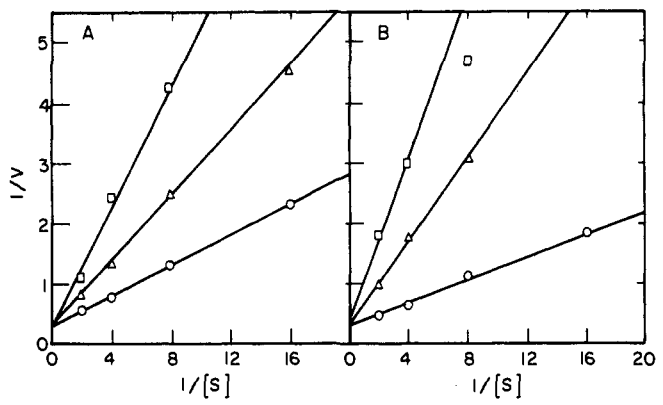


Figure 2. Competitive-inhibition assay of the hydrolysis of various concentrations of benzoyltyrosine ethyl ester by chymotrypsin, shown as double-reciprocol plots of velocity (V arbitrary units) and substrate concentration ([S], mM). K_i values were calculated according to a literature method.[30] (A) Z-Alanylphenylalanine difluoroborane analog ((*R*)-Z-Ala-IB): I = 0.0 (⊙), 0.43 (▲), 0.86 (⊡) μM; K_i = 0.29 μM. (B) Z-Alanylphenylalanine boronic acid analog ((*R*)-Z-Ala-IA): I = 0.0 (⊙), 0.87 (▲), 1.7 (⊡) μM; K_i = 0.32 μM.

binding constants similar to those of the boronic acids (IA-VB).

The difluoroboranes were easily prepared from the boronic acids, and they were more easily purified. The near identity of the K_i values for the corresponding boronic acids and difluoroboranes suggested that in aqueous solution they might be hydrolyzing and that the observed inhibition resulted from the boronic acid. If the boron–fluoride bond was hydrolyzing, then there should be fluoride ion in solution that could be detected by fluoride-specific electrode. The results of fluoride ion determination[23] showed that the fluoroboranes are completely hydrolyzed in aqueous Tris buffer at pH 6.5 and are thus biologically equivalent to the boronic acids; so, for the remainder of the discussion, no distinction will be made between the difluoroboranes and the boronic acids.

Of the chymotrypsin inhibitors, the Z-Ala and benzoyl analogues of phenylalanine were the most effective inhibitors. There is a fairly constant 2-fold difference in binding between racemic compounds and the *R* enantiomers. This is reasonable if the *S* enantiomer of these boronic acid derivatives binds with lower affinity than the *R*. (Some D amino acid derivatives are substrates or inhibitors of chymotrypsin[24] and the (*S*)-acetylphenylalanine analogue was found to be a competitive inhibitor, but their binding affinities are generally lower than the enantiomers with the normal configuration.[5d]) In the phenylalanine analogue series, a 5-fold increase in binding was observed going from acetyl to Z-glycinyl, and a 3-fold increase from Z-glycinyl to Z-alanyl, taking into account the 2-fold difference between single enantiomer and racemic mixtures.

The elastase inhibitors exhibited an increase in affinity proceeding from the alanine analogue to the isoleucine analogue. The phenylglycine analogue was twice as effective as the alanine, and the valine analogue was 34 times as effective. The isoleucine analogue was the most tightly bound, being 10-fold more effective than the valine analogue and about 300-fold more than the alanine analogue.

Conclusion

The boronic acid analogues of amino acids, whose preparation is described in this report, are potent competitive inhibitors of α-chymotrypsin and porcine elastase. This is a feature expected of transition-state analogues, but such binding alone does not necessarily prove the inhibitor is behaving as a transition-state analogue, since tight binding might result from opportunistic interactions that have nothing to do with the specific interactions that develop between enzyme and substrate during the transition state.[2a]

An approach to ascertaining to what degree transition-state inhibitors are in fact binding in a way that mimics transition-state interactions is to compare the dissociation constants (K_i) of these inhibitors with the kinetic parameters of related substrates.[2i,25] However, the compounds we have studied as inhibitors of chymotrypsin and elastase do not constitute a set of data sufficient for such a comparison to be made in rigorous fashion. Therefore, we can only presume, on the basis of analogy,[4,6] that these boronic acids bind to the proteases with the boron in the form of an –ate complex involving the hydroxyl group of the active site serine in a way that mimics the transition states or high-energy tetrahedral intermediate involved in ester or amide hydrolysis. Still, regardless of their mechanism of interaction, these boronic acids are unusually high-affinity inhibitors of chymotrypsin and elastase. Further refinement of the structure of these boronic acid inhibitors should enable yet more potent inhibitors of serine proteases to be developed.[6]

Experimental Section

General Procedures. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was performed using 0.25-mm silica gel 60 glass-backed plates with fluorescent indicator UV_{254} (Merck). Optical rotations were obtained using a Rudolph Autopol III automatic polarimeter. All column chromatography was done by the flash chromatography technique.[26] The column packing was Woelm 32–63-μm silica gel.

Proton magnetic resonance (^{1}H NMR) spectra were recorded on Varian EM 390 (90-MHz), Varian XL 220 (220-MHz), or Nicolet NT 360 (360-MHz) spectrometers in the indicated solvents. Chemical shifts are reported in parts per million downfield from tetramethylsilane as internal standard (δ scale); only selected signals are reported. Pinanediol esters all had resonances at δ 4.2 (OCH, 1 H, dd, J = 3, 9 Hz) and 1.6–2.4 (m, 6 H) and singlets at δ 1.5, 1.3, 0.8 (CH_3, 9 H), and butanediol esters all has resonances at δ 4.0 (OCH, 2 H, m) and 1.35 ($CHCH_3$, 6 H, d, J = 6 Hz). Infrared (IR) spectra were obtained with Perkin-Elmer Model 137 or Nicolet 7000 FT IR spectrometers, and data are presented in reciprocal centrimeters for important diagnostic absorptions. Mass spectra were obtained on a Varian Associates MAT CH-5 spectrometer at 10 or 70 eV. High-resolution mass spectra were obtained on a Varian 731 high-resolution mass spectrometer. Data are presented in the form m/z (intensity relative to base peak 100). Unless indicated otherwise, ionization is by electron impact. Microanalytical data were provided by the Microanalytical Service Laboratory of the University of Illinois. Biological assays were performed on a Varian Model 635 UV–vis spectrophotometer.

n-Butyllithium was purchased from Alfa (Ventron) and titrated prior to use.[27] 2,3-Butanediol was the *d,l*-threo isomer and thus

(23) Fluoride ion concentration was determined for solutions of PhCO-IB (0.1 and 0.01 mM), with and without enzyme present, and various standard fluoride concentration (KF, 0.1 and 0.04 mM) at pH 6.5, Tris-HCl (0.1 M). After subtraction of the blank values, the values determined were in excellent agreement with calculated values. PhCO-IA was used as a control with and without added fluoride and/or enzyme present. There was no indication that the fluoroborane was forming.

(24) Baker, J. O.; Wilkes, S. H.; Bayliss, M. E.; Prescott, J. M.. *Biochemistry* **1983**, *22*, 2098.

(25) (a) Bartlett, P. A.; Marlowe, C. K. *Biochemistry* **1983**, *22*, 4618. (b) Thompson, R. C. *Biochemistry* **1973**, *12*, 47. (c) Thompson, R. C.; Bauer, C. A. *Biochemistry* **1979**, *18*, 1552.

(26) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

(27) Watson, S. C.; Eastham, J. F. *J. Organomet. Chem.* **1967**, *9*, 1651.

produced diastereomeric 1-substituted boronic esters. Other reagents and solutions were purchased as analytical reagent grade or purified according to literature procedures as noted. Commercial sources included: Aldrich Chemical Co., Mallinckrodt, Inc., Alfa (Ventron); Eastman Chemical Co., Sigma Chemical Co., Worthington Biochemical Corp.

Pinanediol was synthesized according to an osmium tetroxide/Me_3NO procedure in 90–95% yield.[28] Material used was 95% ee for the phenylalanine analogues and 92% ee for the elastase inhibitors. Alanine and glycine were protected with the benzyloxycarbonyl group (2 M NaOH/dioxane) in 70–90% yields.[29] All amino acids were of the L configuration; synthetically produced derivatives had properties consistent with those reported in the literature. The cesium salts of the acids were formed by treating the acid with 0.5 equiv of Cs_2CO_3 in methanol and evaporating solvents. The salts could be recrystallized from ethanol/ethyl acetate.

Tetrahydrofuran (THF) was distilled from sodium/benzophenone immediately prior to use. Dimethylformamide (DMF) and methylene chloride were dried over 4-Å molecular sieves. All other solvents were used as received.

General Synthetic Methods. Boronic Esters. Except for pinanediol methylboronate (**5c**) and 2,3-butanediol (dichloromethyl)boronate (**2**), the boronic acids were synthesized by mixing equimolar quantities of the glycol and boronic acid, drying the ethereal solution over $MgSO_4$, and purifying by distillation. Pinanediol phenylboronate was recrystallized from moist ethanol and was 98–100% optically pure. 2,3-Butanediol (dichloromethyl)boronate was prepared as described below.

Pinanediol methylboronates (**5c**) was prepared in situ by treating the aqueous workup mixture from the reaction of MeMgBr and $B(OMe)_3$ with 0.9 equiv of pinanediol. The product was extracted into ether and purified by distillation.

1-Chloroboronates (Chloromethyl Homologation). (Dichloromethyl)lithium was generated at −100 °C, and the homologations to produce the 1-chloroboronic esters were carried out in a manner similar to those recently published.[5] Spectral characteristics of the 1-chloroboronic esters not listed here can be found in those references.

1-[Bis(trimethylsilyl)amino] Boronates. The 1-[bis(trimethylsilyl)amino] boronates were prepared by treating the 1-chloro boronates with lithium hexamethyldisilylamide (LiN-$(SiMe_3)_2$) in a manner similar to a published procedure.[5c] These compounds were isolated from salts but were not purified.

Acyl Carbonate and Phosphonate Generation: Conditions for Acylation of the Protected Amino Boronates. The PhCO-, Z-Ala-, and Z-Gly-acyl carbonates were prepared on a 1–10-mmol scale from equimolar quantities of the cesium salt of the acid and the chloroformate (ethyl chloroformate in DMF/THF for the phenylalanine and phenylglycine analogues and isobutyl chloroformate in DMF for the remaining analogues) at −15 °C, for 0.2–0.5 h, under nitrogen; Z-Ala-phosphonate was prepared from cesium Z-alanate and diethyl chlorophosphate for 10 min at 0 °C, under nitrogen. The [bis(trimethylsilyl)amino] boronates were treated with 1.5 equiv of acylating agents. This was followed by 2 equiv of fluoride salt as either Bu_4NF or the resin fluoride. The pinanediol aminoboronates (**7a,b**) were treated with 2 equiv of acylating agent (DMF/THF (2:1)), followed by 2 equiv of fluoride as $Bu_4N\cdot 2HF$; amino boronates **7c,d** were treated with 0.5 equiv of acylating agent in DMF, followed by water (5%). The reaction was quenched after about 2 h by the addition of 1 M HCl, and the organic layer was washed with acid. The butanediol esters hydrolyze upon treatment with base; thus, the boronic esters were hydrolyzed by extraction into 1 M NaOH. The aqueous layer was washed with ethyl acetate, and after acidification the acid was extracted into ethyl acetate, dried, and purified by recrystallization as described below.

The pinanediol esters do not hydrolyze readily upon exposure into base and remain in the organic layer. The organic layer is then washed with base, acid, and brine, and the products are then either triturated with hexane/ethyl acetate or purified by flash column chromatography (ethyl acetate/hexane). The product esters are recrystallized to constant rotation.

Boronic Acid Purification. The boronic acids were purified by dissolving the acid in cold acetone and adding water to saturation. The volatile solvents were rapidly removed under vacuum, where the combination of cooling and agitation caused crystallization to occur.

Pinanediol Ester Cleavage. Pinanediol ester (0.5 mmol) was dissolved in 5 mL of CH_2Cl_2 and the resultant mixture cooled to −78 °C. This solution was slowly added (ca. 1 min) to 5 mL of a 0.5 M solution of BCl_3 in CH_2Cl_2, cooled to −78 °C. The dry ice bath was removed after 5 min and replaced with an ice bath. The reaction was followed by TLC and was usually complete within 15 min. The reaction was quenched with 5 mL of H_2O and the solution diluted with ethyl acetate. The aqueous layer was separated, and the organic layer was extracted with 1 M NaOH (3 × 5 mL). After acidification, the product was extracted into ethyl acetate, and the residue, after evaporation of solvent, was recrystalized from water/acetone as was described above. Deviations from this procedure are listed with the specific compound.

Difluoroborane Synthesis. The boronic acid was dissolved in a minimal amount of acetone and placed in a plastic container that was then cooled in an ice bath. A slight excess of aqueous HF (0.5 M) was added. After 5 min, water was added and product was extracted into ethyl acetate, solvent removed, and product then recrystallized from acetone/H_2O.

Fluoride Salts. Anion-exchange resin in the fluoride form was prepared from Dowex AG MP1 chloride form resin according to the literature method.[16] Fluoride content was estimated by fluoride ion analysis after total combustion and was 2.0–2.3 mequiv/g. Tetrabutylammonium fluoride and the dihydrogen fluoride salts were prepared by neutralizing 20 g of n-$Bu_4N^+OH^-$ (as a 40% solution in water) with 1 or 3 equiv of 1 M aqueous hydrogen fluoride, evaporating water, and removing water of hydration by azeotropic distillation from benzene/acetonitrile in a Soxhlet extractor charged with 4-Å molecular sieves, at ca. 100 mm pressure and 40–50 °C (the monofluoride salt is temperature sensitive). Water content was estimated by total combustion analysis. Alternatively, the dihydrogen fluoride form could be dried on a high-vacuum line, 3 days, at room temperature; any solid material was removed by filtration of a benzene solution.

Alkyl Boronic Esters. 2,3-Butanediol Benzylboronate (1a): prepared from benzyl Grignard and trimethyl borate; yield 80–85%; bp 53–54 °C (0.05 torr); NMR δ (90 MHz, $CDCl_3$) 7.2 (Ar, 5 H, s), 4.1 (OCH, 2 H, m), 2.4 (ArCH_2, 2 H, s), 1.35 ($CHCH_3$, 6 H, d, J = 6 Hz). Anal. ($C_{11}H_{15}BO_2$) C, H, B.

2,3-Butanediol Phenylboronate (1b): prepared from phenylboronic acid; yield 76%: bp 35–37 °C (1.0 torr); NMR (90 MHz, $CDCl_3$) δ 7.8 (ortho to amide, 2 H, m), 7.3 (m, p-Ar, 3 H, m); mol wt ($C_{10}H_{13}BO_2$) calcd 176.1009, obsd 176.1005.

2,3-Butanediol (Dichloromethyl)boronate (2). (Dichloromethyl)lithium was generated according to the general method[5a] (0.1-mol scale) and quenched with 11.5 g (1.1 equiv) of $B(OMe)_3$ at −100 °C. When the solution reached −40 °C, 30 mL of 3 M HCl (ca. 1.2 equiv) was added, and the cold bath was removed. (The low-temperature quench avoided decomposition that occurred upon warming to room temperature.) Extraction into ether followed by removal of solvent left to oily residue that was dissolved in a minimal amount of ethyl acetate and treated with 9.1 g (1.1 equiv) of 2,3-butanediol. The solid that formed immediately was collected by filtration and recrystallized from ethyl acetate to give 19 g (98%) (based on butanediol) of **2** as a white solid: mp (hydrate) 76–78 °C; bp 50–56 °C (2 torr), 25–26.5 °C (0.2 torr); NMR (90 MHz, $CDCl_3$) δ 5.2 (Cl_2CHB, 1 H, s); mass spectrum m/z 184 (M^+, 22), 183 (10), 182 (44), 181 (14), 140 (82), 138 (100). Anal. ($C_5H_9BCl_2O_2\cdot H_2O$) C, H, Cl.

(+)-Pinanediol Ethylboronate (5f): from EtMgBr and $B(OMe)_3$ product distilled to give 7.25 g (86%) of a colorless oil; bp 51–52 °C (0.1 torr); NMR (90 MHz, $CDCl_3$) δ 0.95 (CH_3CH_2, 5 H, m); mass spectrum m/z 208 (M^+, 13), 193 (37), 166 (23), 153 (28), 138 (100), 112 (71), 83 (93). Anal ($C_{12}H_{21}BO_2$) C, H.

(+)-Pinanediol (2S)-Butylboronate (5e). Ethylboronate (**5f**) (8.00 g, 38 mmol) in 10 mL of THF was added to a solution of $LiCHCl_2$ (3.23 g, of CH_2Cl_2, 16.75 mL, 2.3 M n-BuLi, −100 °C, 38 mmol, 75 mL of THF) followed by 0.65 equiv (3.3 g in 20 mL

(28) Ray, R.; Matteson, D. S. *Tetrahedron Lett.* **1980**, *21*, 449.
(29) "Organic Syntheses"; Wiley: New York, 1955; Collect. Vol. III, p 167.

of THF) of fused $ZnCl_2$. The solution was allowed to warm to room temperature, and TLC (Et_2O/silica I_2 visualization) at 2 h showed reaction was complete. The solution was cooled to −78 °C, 1.1 equiv of CH_3MgBr (3.9 M, 10.7 mL) was added, and the cooling bath was removed. The solution was stirred for 8 h or longer, after which time saturated NH_4Cl was added. The product was extracted with ether, and the extracts were washed with 1 M HCl, water, and brine and then dried over $MgSO_4$. After removal of the ether, the product was isolated by distillation: 89% yield; bp 62–64 °C (0.1 torr); NMR (90 MHz, $CDCl_3$) δ 0.95 (2-butyl, 9 H, M); mass spectrum m/z 236 (M^+, 8), 221 (30), 195 (15), 180 (10), 167 (73), 140 (41), 83 (100), 67 (82). A sample for rotation was obtained by preparative GLC, 15-m OV 1701 column $[\alpha]^{21}_D$ +27.63 (*C* 26, CH_3OH). Anal. ($C_{14}H_{25}BO_2$) C, H, B.

(+)-Pinanediol Isopropylboronate (5d): from *i*-PrMgBr and $B(OMe)_3$; yield 10.33 g (91%) of a colorless oil: bp 49–51 °C (0.1 torr); NMR (90 MHz, $CDCl_3$) δ 1.05 ($(CH_3)_2CH$, 7 H, s); mass spectrum m/z 222 (M^+, 12), 207 (42), 181 (23), 153 (100), 138 (30), 83 (99). Anal. ($C_{13}H_{23}BO_2$) C, H.

2,3-Butanediol (1-Chlorobenzyl)boronate (3b). Method A. Homologation of **1b** with $LiCHCl_2$ and purification by distillation gave 70% yield: bp 34–35 °C (0.1 torr); NMR (90 MHz, $CDCl_3$) δ 7.3 (Ar, 5 H, m), 4.50 (CHCl, 1 H, s); mol wt ($C_{11}H_{14}BClO_2$) calcd 224.0775, obsd 224.0773.

Method B. (Dichloromethyl)boronate hydrate (6.0 g, 30 mmol) was added to a flask containing ca. 100 mL of benzene, and water was removed by azeotropic distillation. Most of the benzene was removed by distillation, ether was added, and the solution was cooled to −78 °C. Phenyllithium (1.1 equiv) was added slowly, and once the addition was complete, the cooling bath was removed. The solution was stirred 1 h at room temperature, solvent was removed in vacuo and ether was added, whereupon LiCl precipitated and was removed by filtration. The product was isolated by distillation in yields comparable to method A.

2,3-Butanediol (1-Chloro-2-phenylethyl)boronate (3a): synthesis performed as described above for **3b**, both methods A and B giving 70–85% yields; bp 89–90 °C (0.1 torr); NMR δ (360 MHz, $CDCl_3$) 6.52 (Ar, 5 H, m), 3.68 (OCH, 2 H, m), 3.30 (CHB, 1 H, t, J = 8 Hz), 2.84 (CH_2CH, 2 H, m), 1.15 (CH_3, 6 H, d, J = 6 Hz); IR (CCl_4) 1068, 1378; mol wt ($C_{12}H_{16}BClO_2$) calcd 238.0932, obsd 238.0932.

(+)-Pinanediol [1(*S*)-Chloro-2-methylpropyl]boronate (6d): by method A from **5d**; product partially purified by flash chromatography; NMR (90 MHz, $CDCl_3$) δ 3.5 (ClCHB, 1 H, d, J = 5 Hz), 1.0 ($CH(CH_3)_2$, 7 H, m); converted to acylamine product to complete characterization.

(+)-Pinanediol [1(*S*)-Chloro-2(*S*)-methylbutyl]boronate (6e): by method A from **5e**: product purified by flash chromatography (Et_2O, silica) to give a colorless oil (81–85%); NMR (90 MHz, $CDCl_3$) δ 3.5 (ClCHB, 1 H, d, J = 5 Hz), 1.2 (2-butyl, ca. 9 H, m); mass spectrum m/z 285 (M^+ + 4), 286 (1), 221 (15), 214 (24), 199 (37), 145 (96), 124 (100). ($C_{15}H_{26}BClO_2$) calcd 284.1712. Observed: 284.1713.

Acylamino Boronic Esters. (+)-Pinanediol [1(*R*)-Benzamido-2-phenylethyl]boronate ((*R*)-PhCO-ID): from **3a**, via **4a**; solid recrystallized from hexane/ethyl acetate; yield 35%; mp 161–162 °C; NMR (90 MHz, acetone-d_6) δ 8.7 (NH, 1 H), 7.8 (*o*-benzamide, 2 H, m), 7.4 (*m,p*-benzamide, 3 H, m), 7.2 (Ph, 5 H, s), 4.15 (CHB, 1 H, dd), 2.9 (CH_2CH, 3 H, m); mass spectrum m/z 403 (M^+, 6.3), 402 (5.0), 312 (14.8), 105 (100); $[\alpha]^{22}_D$ −62.50 (c 10, CH_3OH). Anal. ($C_{25}H_{30}BNO_3$) C, H, N.

(+)-Pinanediol [1(*R*)-(Z-Alanylamino)-2-phenylethyl]boronate ((*R*)-Z-Ala-ID): from **6a** via **7a**; solid recrystallized from either hexane/ethyl acetate or ether; yield 25–35%; mp 126–127 °C; NMR (220 MHz, acetone-d_6) δ 7.25 (Ar, 5 H, s), 7.1 (Ar, 5 H, s), 6.5 (NH, ~1 H, b), 5.0 (CH_2O, 2 H, s), 4.2 (OCH and NCH, 2 H), 2.8 ($BCHCH_2$, 3 H, m), 1.3 (alanyl CH_3, 3 H, d); mass spectrum m/z 504 (M^+, 1.82), 396 (10.4), 91 (100); $[\alpha]^{22}_D$ −78.5 (*c* 16, CH_3OH). Anal. ($C_{29}H_{37}BN_2O_3$) C, H, N.

(+)-Pinanediol [α-(Z-Alanylamino)benzyl]boronate ((*R*)-Z-Ala-IID): from **6b** via **7b**; product recrystallized from hexane/ethyl acetate; yield (three steps) 25%; mp 129–130 °C; NMR (CD_3OD) δ 7.2 (Ar, 10 H, m), 5.1 ($ArCH_2$, 2 H, s), 4.5 (CHN, 1 H, q, J = 7 Hz), 4.0 (OCH, 1 H, dd, J = 9, 3 Hz), 3.75 (NCHB, 1 H, s), 1.4 ($NHCHCH_3$, 3 H, d, J = 7 Hz), pinanediol resonances; mass spectrum m/z 382 (4), 284 (10), 106 (55), 91 (100); $[\alpha]^{25}_D$ −70.75 (c 10, CH_3OH). Anal. ($C_{28}H_{35}BN_2O_5$) C, H, B, N.

(+)-Pinanediol [1(*R*)-(Z-Alanylamino)ethyl]boronate ((*R*)-Z-Ala-IIID): from **6c** via **7c**; purified by flash chromatography (15% CH_3OH/ethyl acetate) and recrystallized from ethyl acetate/CCl_4; yield varied from 4 to 7%; mp 118–119 °C; NMR (360 MHz, $CDCl_3$) δ 7.25 (Ph, 5 H, s), 6.3 (NH, 1 H, s), 5.4 (NH, 1 H, s), 5.0 ($PhCH_2$, 2 H, s), 4.2 (alanyl CHN + CHO, 2 H, m), 3.1 (NCHB, 1 H, dq, J = 7 Hz), 1.3 (alanyl CH_3, 3 H, d, J = 7 Hz), 1.08 ($BCHCH_3$, 3 H, d, J = 7); mass spectrum m/z 428 (M^+, 14), 359 (13), 320 (17), 266 (2), 222 (96), 55 (100), 41 (100); $[\alpha]^{22}_D$ −14.40 (c 3, CH_3OH); mol wt ($C_{23}H_{33}BN_2O_5$) calcd 428.2483, obsd 428.2488.

(+)-Pinanediol [1(*R*)-(Z-Alanylamino)-2-methylpropyl]boronate ((*R*)-Z-Ala-IVD): from **6d** via **7d**; after recrystallization from ethyl acetate/hexane, 0.98 g of white needles (42%) isolated; mp 125–126 °C; NMR (90 MHz, $CDCl_3$) δ 7.2 (Ph, 5 H, s), 6.2 (NH, 1 H, brs), 5.4 (NH, 1 H, brd), 5.0 ($PhCH_2$, 2 H, s), 4.2 (alanyl CHN, pinanediol, HCO, 2 H, m), 3.1 (NCHB, 1 H, dd, J = 6, 6 Hz), 1.3 (CH_3CHN, 3 H, d), 0.95 (CH_5CH, 6 H, d, J = 7 Hz), ca. 1.3 (CH_3CH); mass spectrum m/z 456 (M^+, 3), 441 (1), 387 (3), 348 (6), 250 (19), 223 (10), 208 (14), 135 (24), 91 (100), 88 (46); $[\alpha]^{23}_D$ −56.50 (c 2, CH_3OH). Anal. ($C_{25}H_{37}N_2O_5B$) C, H, B, N.

(+)-Pinanediol [1(*R*)-(Z-Alanylamino)-2(*S*)-methylbutyl]boronate ((*R*)-Z-Ala-VD): from **6e** via **7e**; product isolated (0.65 g, 21%) recrystallized from hexane with a trace of ethyl acetate; mp 101–102 °C; NMR (360 MHz, $CDCl_3$) δ 7.3 (Ph, 5 H, s), 6.2 (NH, 1 H, brs), 5.4 (NH, 1 H, brd), 5.05 ($PhCH_2$, 2 H, s), 4.3 (alanyl CHN + HCO, 2 H, m), 3.3 (CHB, 1 H, dd, J = 5.5 Hz), 1.7 ($CH(CH_3)CH_2$, 1 H, brm), 1.39 (CH_3CHN, 3 H, d, J = 7), 0.85 ($CH_3CHCH_2CH_3$, 8 H, m); mass spectrum m/z 470 (M^+, 3), 455 (2), 362 (27), 347 (14), 333 (49), 293 (54), 211 (65), 181 (25), 135 (88), 108 (100), 91 (70), 79 (97). Anal. ($C_{26}H_{39}BN_2O_5$) C, H, B, N.

Acylamino Boronic Acids. (1-Benzamido-2-phenylethyl)boronic Acid (PhCO-IA): from **3a** via **4a**; yield 50–70%; mp 98–100 °C; NMR (360 MHz, acetone-d_6) δ 7.7 (*o*-benzamide, 2 H, m), 7.4 (*m,p*-benzamide, 3 H, m), 7.2 (Ar, 5 H, m), 3.0 ($ArCH_2$, 2 H, m), 2.8 (CHB, 1 H, m); IR (KBr) 1610, 1578 cm^{-1}; FAB mass spectrum, positive ion M^+ + 1, m/e 252. Anal. ($C_{15}H_{14}BNO_2$): C, H.

[1(*R*)-Benzamido-2-phenylethyl]boronic Acid ((*R*)-PhCO-IA: from (R)-PhCO-ID; after hydrolysis of the pinanediol ester crude yield quantitative; mp 117–118 °C; NMR (220 MHz, acetone-d_6) δ 8.7 (NH, ~1 H, br), 7.85 (*o*-benzamide, 2 H, d, J = 9 Hz), 7.45 (*m,p*-benzamide, 3 H, m), 7.15 (Ph, 5 H, m), 3.15 (CHB + BOH, 2 $^1/_2$ H, m), 2.85 ($PhCH_2$, 2 H, m); $[\alpha]^{23}_D$ −112 (c 8, CH_3OH). Anal. ($C_{15}H_{16}BNO_3 \cdot {}^1/_2H_2O$) C, H. N.

[1(*R*)-(Z-Alanylamino)-2-phenylethyl]boronic Acid ((*R*)-Z-Ala-IA): from (*R*)-Z-Ala-ID; crude acid triturated with water and recrystallized from CH_3OH/H_2O to furnish the hydrate in 50% yield; mp 85–90 °C; NMR (220 MHz, acetone-d_6) δ 7.9 (NH, ~1 H, br), 7.25 (Z-Ph, 5 H, s), 7.1 (Ph, 5 H, m), 6.7 (NH, ~1 H, brd), 4.9, 5.05 (CH_2, 2 H, 2 d, J = 10 Hz), 4.3 (CHN, 1 H, m), 2.9 (Ph CH_2 + BOH-H_2O, brs), 2.65 (BCH, 1 H, m), 1.35 ($CHCH_3$, 3 H, d, J = 7 Hz) [nonhydrated material: δ 7.3 (Z-Ph, s), 7.15 (Ph, m), 5.02 (CH_2, s), 3.25 (BOH, s), 2.5–3.0 ($CHCH_2$, m), 1.4 (CH_3, d, J = 7 Hz)]; mass spectrum m/z 353 (0.47, M^+ − H_2O) 282 (4.3), 108 (79.6), 91 (42.4), 79 (100); $[\alpha]^{23}_D$ −104.5 (c 12, CH_3OH). Anal. ($C_{19}H_{23}BN_2O_5 \cdot H_2O$) C, H. B. N.

[1(*R*)-(Z-Alanylamino)-2-methylpropyl]boronic Acid ((*R*)-Z-Ala-VA): from (*R*)-Z-Ala-IVD; yield 87%; mp 80–82 °C; NMR (360 MHz, acetone-d_6) δ 8.0 (NH, 1 H, s), 7.4 (Ph, 5 H, m), 6.7 (NH, 1 H, d), 5.1 ($PhCH_2$, 2 H, 2 d, J = 8 Hz), 4.4 (CH_3CHN, 1 H, m), 2.9 (H_2O, BOH, ca. 3 H, s), 2.6 (CHB, 1 H, Br), 1.95 ($(CH_3)_2CH$, 1 H, m), 1.4 ($(CH_3CHN$, 3 H, d, J = 7 Hz), 0.95 ($(CH_3)_2CH$, 6 H, d, J = 6 Hz); mass spectrum m/z 321 (M^+, 6), 305 (10), 250 (13), 208 (15), 135 (17), 108 (38), 91 (100), 78 (50); $[\alpha]^{22}_D$ −56.50 (c 2, CH_3OH). Anal. ($C_{15}H_{23}BN_2O_5 \cdot H_2O$) C, H, B, N.

[1(*R*)-(Z-Alanylamino)-2(*S*)-methylbutyl]boronic Acid ((*R*)-Z-Ala-VA): from (*R*)-Z-Ala-VD; yield (61%; mp 65–70 °C; NMR (360 MHz, acetone-d_6) δ 8.0 (NH, 1 H, brs), 7.4 (Ph, 5 H, m), 6.7 (NH, 1 H, brd), 5.1 ($PhCH_2$, 2 H, 2 d, J = 8 Hz), 4.4 (CH_3CHN, 1 H, m), 2.75 (CHB, 1 h, brm), 1.75 ($CH_2(CH_3)CH$, 1 H, m), 1.4 (CH_3CHN, 3 H, d, J = 7), 0.95 ($CH_3CH_2(CH_3)CH$,

8 H, m); mass spectrum m/z 335 (M^+, 1), 332 (10), 318 (3), 208 (12), 178 (4), 154 (3), 135 (10), 120 (70), 118 (100), 116 (100), 91 (86), 81 (43); $[\alpha]^{22}_D$ −61.11 (c 6, CH_3OH). Anal. ($C_{16}H_{25}BN_2O_5 \cdot {}^3/_4H_2O$) C, H, B, N.

Acylamino Difluoroboranes. **(1-Benzamido-2-phenylethyl)difluoroborane (PhCO-IB).** Boronic acid PhCO-IA was converted to the difluoroborane according to the general procedure and recrystallized from H_2O/acetone: mp 199–200 °C; NMR (90 MHz, Me_2SO-d_6) δ 8.05 (o-benzamide, 2 H, m), 7.6 (m,p-benzamide, 3 H, m), 7.2 (Ph, 5 H, s), 2.85 ($CHCH_2$, 3 H m); IR (KBr) 1243, 1638 cm^{-1}; mass spectrum m/z 273 (M^+, 18), 272 (18), 181 (32), 104 (100). Anal. ($C_{15}H_{14}BF_2NO$) C, H, F, N.

[1-(Z-Glycinylamino)-2-phenylethyl]difluoroborane (Z-Gly-IB): from **3a** via **4a** and Z-Gly-IA; recrystallized from H_2O/acetone; yield 35%; mp 157–158 °C; NMR (360 MHz, acetone-d_6) δ 9.8 (NH, 1 H, br), 7.35 (Z-Ph, 5 H, m), 7.2 (Ph, 5 H, m), 7.0 (NH, 1 H, br), 5.0 ($PhCH_2$, 2 H, dd), 4.3 (NCH_2, 2 H, d, J = 6 Hz), 2.9 (($PhCH_2CH$, 2 H, m), 2.65 ($CHBF_2$, 1 H, m). Anal. ($C_{18}H_{19}BF_2N_2O_3$) C, H, F, N.

(1-Acetamido-2-phenylethyl)difluoroborane (Ac-IB): from Ac-IA; recrystallized from water; quantitative yield: mp 109–110 °C; NMR (90 MHz, CD_3OD) δ 7.25 (Ph, 5 H, s), 2.8 (CH_2CH, 3 H, m), 2.2 (CH_3CO, 3 H, s); mass spectrum m/z 211 (M^+, 16), 104 (100); IR (KBr) 1050, 1120, 1213, 1640 cm^{-1}. Anal. ($C_{10}H_{12}BF_2NO$) C, H, F, N.

(1-Benzamidobenzyl)difluoroborane (PhCO-IIB): From PhCO-IIA; recrystallized from H_2O/CH_3OH; mp 186–189 °C; NMR (360 MHz, CD_3OD) δ 8.3 (NH, 1 H, br), 7.8 (ortho to amide, 2 H, m), 7.5 (meta), para to amide, 2 H, m), 7.3 (Ar, 5 H, m), 4.1 (CHB, 1 H, brt); mass spectrum m/z 259 (M^+, 1.14), 211 (31.4), 105 (100), 77 (79); IR (KBr) 1614, 1192 cm^{-1}. Anal. ($C_{14}H_{12}BF_2NO$) calcd 259.0980, obsd 259.0985.

[1-(Z-Glycinylamino)benzyl]difluoroborane (Z-Gly-IIB): from **3b** via **4b** and Z-Gly-IIA; crystals recrystallized from H_2O/CH_3OH; mp 147–149 °C; NMR (90 MHz, acetone-d_6) δ 7.4 (Ar, 5 H, s), 7.2 (Ph, 5 H, m), 5.1 ($ArCH_2$, 2 H, s), 4.4 (NCH_2CO, 2 H, d, J = 7 Hz), 3.85 (CHB, 1 H, br); mass spectrum m/z 297 (M^+-BF_2, 2.9), 106 (62), 91 (100). Anal. ($C_{17}H_{17}N_2O_3$) C, H, F, N.

[1(*R*)-(Z-Alanylamino)benzyl]difluoroborane ((*R*)-Z-Ala-IIB). Pinanediol ester (*R*)-Z-Ala-IID was hydrolyzed with 3 equiv of BCl_3 in CH_2Cl_2 at −40 °C, 5 min. Water was added to the −40 °C solution, and the mixture was allowed to warm to room temperature. The organic layer was separated and the aqueous layer extracted with ether. The organic layers were combined and extracted with 1 M NaOH solution. The basic extract was neutralized, and the product was extracted with ethyl acetate. Solvents were removed in vacuo, and the resulting solid was dissolved in acetone/water and converted to the difluoroborane by treatment with a slight excess of HF: yield 50% (quantitative yield from acid to difluoroborane); mp 135–140 °C; NMR (90 MHz, acetone-d_6) δ 7.3 (Z-Ph, 5 H, s), 7.0 (Ar, 5 H, m), 5.05 ($ArCH_2$, 2 H, s), 4.6 ($NCHCH_3$, 1 H, m), 3.7 ($ArCHBF_2$, 1 H, br m), 1.5 ($CHCH_3$, 3 H, d, J = 7 Hz); mass spectrum m/z 360 (M^+, 0.4), 341 (1), 269 (10), 154 (27), 106 (100), 91 (89); $[\alpha]^{22}_D$ 56.3 (c 2, CH_3OH). Anal. ($C_{18}H_{19}BF_2O_3N_2$) C, H, N.

[1(*R*)-Benzamido-2-phenylethyl]difluoroborane ((*R*)-PhCO-IB): from (*R*)-PhCO-ID; mp 199–200 °C; NMR (90 MHz, acetone-d_6) identical with racemic material PhCO-IB; $[\alpha]^{23}_D$ −82.9 (c, 5.1, CH_3OH). Anal. ($C_{15}H_{14}BF_2NO$) C, H, F, N.

[1(*R*)-(Z-Alanylamino)-2-phenylethyl]difluoroborane ((*R*)-Z-Ala-IB): from (*R*)-Z-Ala-ID; mp 115–115 °C; NMR (90 MHz, CD_3OD) δ 7.3 (CBzPh, 5 H, s), 7.15 (Ph, 5 H, s), 5.1 ($PhCH_2$, 2 H, s), 4.4 (CH_3CH, 1 H, q, J = 7 Hz), 2.75 (CH_2CH, 3 H, m), 1.4 ($CHCH_3$, 3 H, d, J = 7 Hz); mass spectrum m/z (percent of base peak) 283 (3.0), 239 (3.2), 168 (22.8), 120 (93), 91 (100); $[\alpha]^{23}_D$ −98.0 (c 7, CH_3OH). Anal. ($C_{19}H_{21}BF_2O_3N_2$) C, H, F, N.

[1(*R*)-Acetamino-2-phenylethyl]difluoroborane ((*R*)-Ac-IB): from (*R*)-Ac-ID; mp 104–105 °C; NMR (identical with racemic material Ac-IB); mass spectrum m/z 211 (M^+, 16.3), 104 (100); $[\alpha]^{23}_D$ −141.7 (c 3, CH_3OH). Anal. ($C_{10}H_{12}BFNO$) C, H, F, N.

[1(*R*)-(Z-Alanylamino)ethyl]difluoroborane ((*R*)-Z-Ala-IIIB): from (*R*)-Z-Ala-IIID: yield 60%; mp 118 °C; NMR (360 MHz, CD_3OD) δ 7.4 (Ph, 5 H, m), 5.15 ($PhCH_2$, 2 H, m), 4.45 (CHNC, 1 H, m), 2.65 (NCHB, 1 H, m), 1.5 (CH_3CH. 3 H, d, J = 7 Hz), 1.1 (CH_3CHB, 3 H, m); mass spectrum m/z 298 (M^+, 0.5); $[\alpha]^{21}_D$ −58.0 (c 3, CH_3OH). Anal. ($C_{13}H_{17}BF_2N_2O_3$) calcd 298.1300, obsd 298.1300.

[1(*R*)-(Z-Alanylamino)-2-methylpropyl]difluoroborane ((*R*)-Z-Ala-IVB): from (*R*)-Z-Ala-IVD; mp 167–168 °C; NMR (360 MHz, acetone-d_6) δ 9.8 (NH, 1 H, brs), 7.4 (Ph, 5 H, m), 7.0 (NH, 1 H, brs), 5.15 ($PhCH_2$, 2 H, 2 d, J = 6 Hz), 4.6 (CH_3CHN, 1 H, m), 2.4 (CHB, 1 H, m), 1.9 (($CH_3)_2CH$, 1 H, m), 1.55 (CH_3CHN, 3 H, d, J = 7 Hz), 0.95 (($CH_3)_2CH$, 6 H, m); mass spectrum m/z 326 (M^+, 5), 306 (4), 235 (2), 191 (5), 157 (3), 136 (4), 120 (14), 91 (100), 72 (61), 44 (21); $[\alpha]^{22}_D$ −60.0 (c 4, CH_3OH). Anal. ($C_{15}H_{21}BF_2N_2O_3$) C, H, B, F, N.

[1(*R*)-(Z-Alanylamino)-2(*S*)-methylbutyl]difluoroborane ((*R*)-Z-Ala-VB): from (*R*)-Z-Ala-VD; mp 122–124 °C; NMR (360 MHz, acetone-d_6) δ 7.4 (Ph, 5 H, m), 7.0 (NH, 1 H, brs), 5.17 ($PhCH_2$, 2 H, 2 d, J = 6 Hz), 4.6 (CH_3CHN, 1 H, m), 2.5 (CHB, 1 H, m), 1.65 (CH_3CH_2, 2 H, m), 1.2 (CH_2CHCH_3, 1 H, m), 0.95 ($CH_3CH_2CHCH_3$, 6 H, m); mass spectrum m/z 340 (M^+, 1), 320 (3), 205 (5), 132 (13), 91 (100), 79 (7), 65 (10), 44 (18); $[\delta]^{22}_D$ −62.25 (c 4, CH_3OH). Anal. ($C_{16}H_{23}BF_2N_2O_3$) C, H, B, F, N.

Biochemical Assays. Assay of Inhibitors of α-Chymotrypsin. The procedure is similar to the Worthington method[21] for assaying activity of α-chymotrypsin. A stock benzoyltyrosine ethyl ester (BTEE) solution (1.0 mM) was made by dissolving 31.2 mg (0.1 mmol) of BTEE in 1:1 water/methanol. Concentrations of 0.67, 0.50, 0.33, and 0.25 mM were made by serial dilution. For the assay, the stock solutions were diluted 1:1 with 0.08 M Tris/0.1 M $CaCl_2$ buffer, pH 7.8. Stock enzyme solution of 3 μM was made by dissolving 3.5 mg of chymotrypsin in 50 mL of Tris/$CaCl_2$ buffer, pH 7.8 solution. Inhibition concentration was made such that the final concentration would be in the 0.1–10 μM range.

The assay was carried out by placing 1.50 mL of BTEE solution in a small test tube and adding 25 μL of inhibitor solution. Stock enzyme solution, 25 μL, was added, and the contents were thoroughly mixed. The solution was placed in a 1.5-mL cuvette, and the increase in absorbance with time was read at 256 nm against a reference sample of an equivalent concentration of BTEE.

The velocity of the reaction was calculated (M min^{-1}) as $\Delta A/(\Delta t\epsilon)$ (M min^{-1}) where ϵ = 9000. A double-recipricol plot of the substrate concentrations and velocities was made. The K_i for the inhibitor was calculated from the relationship K_i = [I]-$slope_a/(slope_i - slope_a)$, where [I] is inhibitor concentration, $slope_a$ is the slope of the line of the uninhibited reaction, and $slope_i$ is the slope of the line of the inhibited reaction.[30]

Assay of Inhibitors of Procine Elastase. The inhibitors were tested by competitive assay against acetylalanylalanylalanyl-p-nitroanaline as substrate, pH 8.0, Tris buffer, 0.08 M, and ambient temperature.[22] Inhibitor stock solutions were made by dissolving 1–3 mg in 1–0 mL of CH_3OH. Lower concentrations were made by serial dilution. A stock enzyme solution (10 units/mg) was made to give 1 mg/mL of protein in pH 8.0 Tris buffer. Enzyme was stable for at least 48 h when refrigerated.

Substrate at 2.0, 1.5, and 1.0 mg/mL was dissolved in Me_2SO. The assay was performed by adding 0.89 mL of buffer to a 1.5-mL cuvette followed by 75 μL of substrate, 25 μL of inhibitor, and finally by 10 μL of enzyme stock solution. The released p-nitroanaline was measured as the increase in absorbance at 385 nm against an equivalent concentration of substrate in buffer as blank. The extinction coefficient was assumed to be 12 900, and velocities were calculated from the increase in absorbance with time. The K_i's were calculated from double-recipricol plots as described for the chymotrypsin inhibitors.

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(30) (a) Segal, D. *Biochemistry* **1972**, *11*, 349. (b) Morihara, K.; Oka, T. *FEBS Lett.* **1973**, *33*, 54.

struments supported by a grant from the National Institutes of Health (Grant PHS GM 27029).

Registry No. 1a, 98541-32-3; 1b, 98541-33-4; 2, 98632-84-9; 3a, 98632-85-0; 3b, 98632-86-1; 4a, 98541-34-5; 4b, 98541-35-6; 5c, 84110-38-3; 5d, 90084-26-7; 5e, 98541-36-7; 5f, 98541-37-8; 6a, 78902-03-1; 6b, 76110-79-7; 6c, 85167-12-0; 6d, 89618-77-9; 6e, 98541-38-9; 7a, 88824-38-8; 7b, 98541-39-0; 7c, 98541-40-3; 7d, 94242-79-2; 7e, 98541-41-4; (*R*)-PhCO-ID, 98541-42-5; (*R*)-Z-Ala-ID, 98541-43-6; (*R*)-Z-Ala-IID, 98541-44-7; (*R*)-Z-Ala-IIID, 98541-45-8; (*R*)-Z-Ala-IVD, 98541-46-9; (*R*)-Z-Ala-VD, 98541-47-0; Rh-CO-IA, 98541-48-1; (*R*)-PhCO-IA, 98632-87-2; (*R*)-Z-Ala-IA, 98541-49-2; (*R*)-Z-Ala-IVA, 98541-50-5; (*R*)-Z-Ala-Va, 98541-51-6; PhCO-IB, 98541-52-7; Z-Gly-IB, 98541-53-8; Ac-IB, 98541-54-9; PhCO-IIB, 98541-55-0; Z-Gly-IIB, 98541-56-1; (*R*)-Z-Ala-IIB, 98541-57-2; (*R*)-PhCO-IB, 98632-88-3; (*R*)-Z-Ala-IB, 98541-58-3; (*R*)-Ac-IB, 98632-89-4; (*R*)-Z-Ala-IIIB, 98541-59-4; (*R*)-Z-Ala-IVB, 98576-62-6; (*R*)-Z-Ala-VB, 98576-63-7; Z-Gly-IA, 98541-60-7; Ac-IA, 98632-90-7; Ph-CO-IIA, 98541-61-8; Z-Gly-IIA, 98541-62-9; (*R*)-Z-Ala-IIA, 98541-63-0; (*R*)-Ac-ID, 78902-05-3; MeBr, 74-83-9; $B(OMe)_3$, 121-43-7; $LiN(SiMe_3)_2$, 4039-32-1; $PhCH_2Br$, 100-39-0; $LiCHCl_2$, 2146-67-0; *i*PrBr, 75-26-3; EtBr, 74-96-4; $PhCO_2Cs$, 17265-04-2; Z-Ala-OH·Cs, 61543-49-5; *dil-threo*-2,3-butanediol, 6982-25-8; pinanediol, 18680-27-8; phenylboronic acid, 98-80-6; phenyllithium, 591-51-5; chymotrypsin, 9004-07-3; elastase, 9004-06-2.

New Antihistaminic N-Heterocyclic 4-Piperidinamines. 1. Synthesis and Antihistaminic Activity of *N*-(4-Piperidinyl)-1*H*-benzimidazol-2-amines

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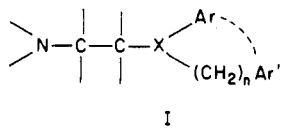
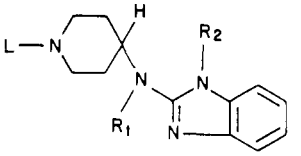
The synthesis of a series *N*-(4-piperidinyl)-1*H*-benzimidazol-2-amines and the preliminary evaluation of their in vitro and in vivo antihistaminic activity are described. Cyclodesulfurization of (2-aminophenyl)thioureas with mercury(II) oxide resulted in 2-aminobenzimidazole intermediates, which were monoalkylated on the *endo*-nitrogen atom. After deprotection of the piperidine nitrogen atom with 48% aqueous hydrobromic acid solution, the title compounds were obtained by three different methods, viz. alkylation, reductive amination, or oxirane ring-opening reactions. The in vivo antihistaminic activity was evaluated by the compound 48/80 induced lethality test in rats and histamine-induced lethality test in guinea pigs after oral and/or subcutaneous administration. The duration of action, for a selected number of compounds, was studied in the guinea pig. The phenylethyl derivatives showed the most potent antihistamine properties after oral administration in both animal species.

Since the beginning of antihistamine research in the Pasteur Institute in France in 1937, many new antihistamines have been discovered.[1] Nearly all the antihistamine drugs that have been developed may be represented by the general formula I, where Ar and Ar′ represent an



I



II

aryl group, $n = 0$ or 1, and X represents a nitrogen, oxygen, or carbon atom connecting the aminoalkyl chain to the aromatic nucleus. In I X–C< may also be replaced by a carbon–carbon double bond. The mean N–X distance is 4.1 ± 0.6 Å.[2]

The terminal nitrogen can be part of a tertiary acyclic or alicyclic amine, and the two aromatic nuclei may be bridged to form tricyclic derivatives.[3] These antihistamines exhibit, in varying degrees, local anaesthetic, adrenergic blocking, antispasmodic, sympathomimetic, analgesic, and antiserotonin activity.[4,5] Moreover, many antihistamines exert some depressant activity on the central nervous system and cause sedation.[6] Peripheral side effects such as gastrointestinal complaints or dry mouth may be due to anticholinergic properties.

Finally, most antihistamines have a short duration of action of only a few hours.[4] In some cases, this drawback can be remedied with a galenic sustained-release form. The side effects and short duration of action limit the use of high oral doses for the treatment of asthma where high tissue levels of antihistamines may be required to prevent allergen-induced bronchospasm.

In light of these facts, it was reasoned that compounds with a long duration of action, a high safety index, and a very low risk of provoking central and anticholinergic side effects would have a broad range of therapeutic applications in diseases, characterized by a prolonged stimulation of histamine H_1 receptors.

In this paper, we report the synthesis of a series of new *N*-(4-piperidinyl)-1*H*-benzimidazol-2-amines (II), which are structurally unrelated to any known antihistamine.

Some of these compounds are long-acting antihistamines by either subcutaneous or oral administration.

Chemistry. The synthesis and chemical properties of 2-aminobenzimidazoles were recently reviewed.[7,8] The cyclodesulfurization (Scheme I) of the (2-aminophenyl)-thioureas **2** and **9** resulted in the 2-aminobenzimidazoles **3** and **10** in moderate to excellent yields. Although many cyclodesulfurization agents are available, e.g. alkyl halides, dialkyl sulfates, mercury(II) chloride or acetate, lead(II)

(1) Bovet, D.; Staub, A. M. *C. R. Hebd. Seances Acad. Sci.* **1937**, *124*, 547.
(2) Tollenaere, J. P.; Moereels, H.; Raymaekers, L. A. "Atlas of Three-Dimensional Structure of Drugs", Janssen Research Foundation Series; Elsevier: Amsterdam, 1979; Vol. 1, p 4.
(3) Casy, A. F. "Handbook of Experimental Pharmacology"; Rocha, E., Silva, M., Eds.; Springer-Verlag: Berlin, 1978; Vol. XVIII/2, pp 175–214.
(4) Bovet, D.; Bovet-Nitti, F. "Medicaments du Systeme Nerveux Vegetatif"; Karger, S., Ed.; Basel, 1948; p 741.
(5) Wade, A., Ed. "The Extra Pharmacopeia", 27th ed; The Pharmaceutical Press: London, 1978; pp 1287–1309.
(6) Green, A. F.; Garland, L. G.; Hodson, H. F. "Antiinflammatory Drugs"; Vane, J. R., Ferreira, S. H., Eds.; Springer-Verlag: Berlin, 1979; pp 415–466.
(7) Simonov, A. M.; Anisimova, V. A. *Chem. Heterocycl. Compd.* **1979**, *15*, 705.
(8) Rastogi, R.; Sharma, S. *Synthesis* **1983**, 861.

0022-2623/85/1828-1925$01.50/0