Potentially Macrocyclic Peptidyl Boronic Acids as Chymotrypsin Inhibitors

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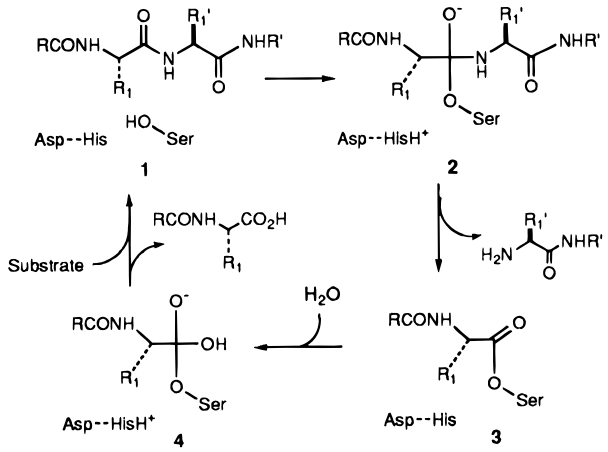
The possibility of forming a peptide boronate adduct in a serine protease active site that mimics the first tetrahedral intermediate in the peptide hydrolysis mechanism was explored with the complex boronic acid analogs **7, 8-OH**, and **8-NH$_2$.** In these structures, the P_1 and P_2 residues and the P_1'-P_3' residues are connected through the P_2 and P_1' side chains, to encourage formation of the diester or amide-ester adducts via macrocyclization. These inhibitors were assembled from suitably protected derivatives of 2,4-diaminobutanoic acid or 2,4-diaminopentanoic acid (**11**), borophenylalanine (**12**), aspartic acid, malic acid or the substituted malic acid analog **13**, and Leu-Arg dipeptide. Stereoselective syntheses were developed for the (*S*,*S*)-2,4-diaminopentanoate **11** and for the (*S*,*S*)-β-isobutylmalate **13** derivatives. The complex peptidyl boronates **7** (K_i = 26 nM) and **8-OH** (68 nM) are potent inhibitors of α-chymotrypsin; however, the affinity of **7** is neither time- nor pH-dependent, and it is only moderately greater than that found for comparison compounds like **8-H** (114 nM), **9** (356 nM), and **10** (219 nM) that cannot cyclize or form a diester adduct.

Serine proteases, a large and functionally diverse class of proteolytic enzymes, are prominent therapeutic targets because of their involvement in a host of physiological processes.[1] They catalyze peptide bond cleavage by acylation and deacylation of the active site serine residue in a sequence that involves two tetrahedral intermediates, as illustrated in Scheme 1.[3,4] Most small-molecule inhibitors of these enzymes form covalent adducts with the active site serine that mimic to some degree these tetrahedral intermediates. Peptide derivatives with electron-deficient ketones and aldehydes, boronic acids, and phosphonylating agents have been devised as analogs of the second tetrahedral intermediate **4**,[5] with their selectivity among the various proteases related to the substrate specificity these enzymes manifest at the S_1, S_2, and higher, binding sites.[6] Inhibitor motifs that reach into the S_1', S_2', etc., sites and mimic the first tetrahedral intermediate **2** are less common,[7−10] although significant additional binding energy is potentially available from these regions of the active sites. For example, Imperiali and Abeles found that the extended difluoro ketone Ac-Leu-Phe-$CF_2CH_2CH_2$CO-Leu-Arg-OMe (K_i = 9 nM) is more than 3 orders of magnitude more potent as an inhibitor of chymotrypsin than the simple difluoromethyl ketone Ac-Leu-Phe-CF_2H (25 μM).[7]

Peptide boronic acids are among the most potent inhibitors of serine proteases known,[2,11−16] achieving subnanomolar affinity from interaction with the S-subsites alone. For example, MeO-Suc-Ala-Ala-Pro-boroPhe-OH inhibits α-chymotrypsin with a K_i value of 0.16 nM.[11] We were intrigued by the possibility of using a boronic acid to mimic the first tetrahedral intermediate in the enzymatic sequence (Figure 1c), in order to enhance binding affinity and specificity and to provide a structural model for this transition state. However, in view of the lability of boronate esters and amides in aqueous solution, the desired serine adduct requires formation of a ternary complex. The feasibility of forming a boronate diester in the active site was demonstrated recently by Katz et al., who showed that simple alcohols present in the mother liquor were incorporated in the crystalline adduct of trypsin with a boronate inhibitor.[2] We sought to overcome the inherent disadvantage of ternary adduct formation by tethering the P′ components

Scheme 1



[⊗] Abstract published in *Advance ACS Abstracts,* January 15, 1997.

(1) Proteases as Therapeutic Targets. Craik, C. S.; Debouck, C. In *Perspectives in Drug Discovery and Design*; Anderson, P. S., Kenyon, G. L., Marshall, G. R., Eds., ESCOM Science Publishers BV.: The Netherlands, 1995; Vol. 2, No. 3. See also the following citation for recent leading references.

(2) Katz, B. A.; Finer-Moore, J.; Mortezaei, R.; Rich, D. H.; Stroud, R. M. *Biochemistry* **1995**, *34*, 8264.

(3) Kraut, J. *Annu. Rev. Biochem.* **1977**, *46*, 331.

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(6) Nomenclature of: Schecter, I.; Berger, A. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157.

(7) Imperiali, B.; Abeles, R. H. *Biochemistry* **1987**, *26*, 4474.

(8) Sampson, N. S.; Bartlett, P. A. *Biochemistry* **1991**, *30*, 2255.

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

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(14) Zhong, S.; Haghjoo, K.; Kettner, C.; Jordan, F. *J. Am. Chem. Soc.* **1995**, *117*, 7048.

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(16) Cacciola, J.; Fevig, J. M.; Alexander, R. S.; Brittelli, D. R.; Kettner, C. A.; Knabb, R. M.; Weber, P. C. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 301.

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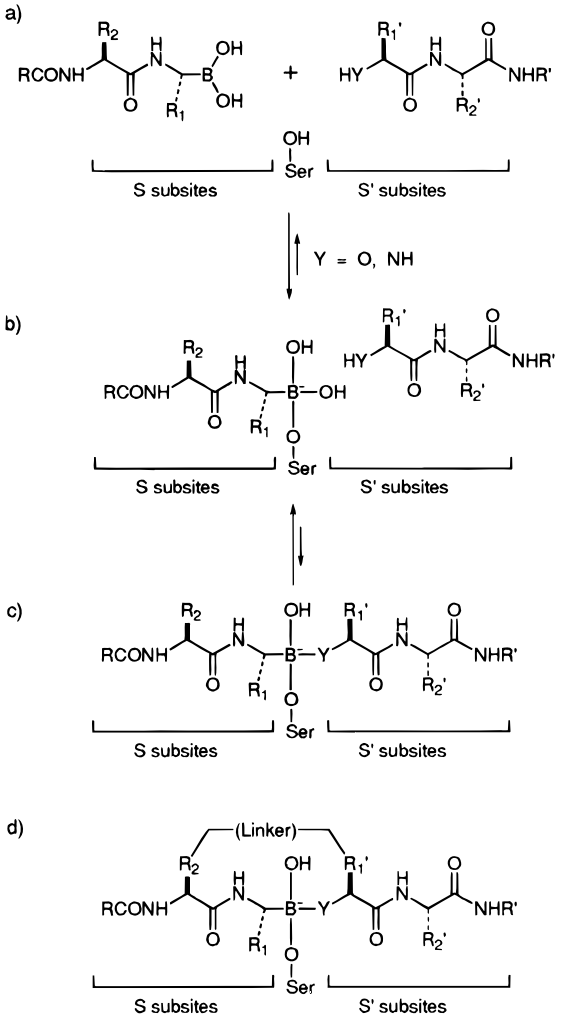


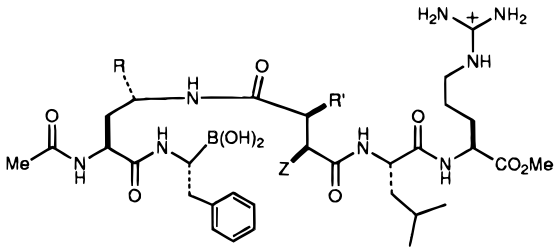
Figure 1. Proposed binding of ternary and macrocyclic boronate inhibitors: (a) P- and P′-components; (b) binary adduct mimicking **4**; (c) ternary adduct mimicking **2**; (d) macrocyclic mimic of **2**.

to the peptidyl boronic acid, making formation of the desired diester an intramolecular process (Figure 1d). In this report, we describe the design, synthesis, and evaluation of chymotrypsin inhibitors that embody this concept.

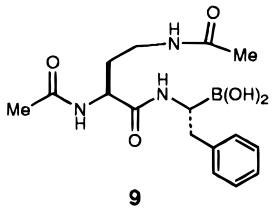
Design

The starting point for our design was the complex between bovine α-chymotrypsin and the secretory leukocyte protease inhibitor (SLPI).[17] The binding loop of this inhibitor, Gln-70 to Arg-75, extends from the S_3 to the S_3' subsites and is configured such that the Cys-71 and Met-73 side chains are in close proximity (Figure 2). The program CAVEAT[18] was used to identify chains of linking atoms that could span the $C_\alpha-C_\beta$ bonds of the P_2 and P_1' residues of SLPI without interfering with the enzyme or introducing eclipsing interactions. A five-atom linker with two sp^2-hybridized atoms was identified from a number of hits from the Cambridge Structural Database;[19] one example, ICYSPA, is depicted in Figure 2b. The linker was combined with the SLPI loop and modeled in the active site of chymotrypsin as the tetrahedral adduct **5a**. The design was improved by reversing the orientation of the amide unit to avoid steric interference with the enzyme and by incorporating the alkyl side chains from the ICYSPA structure. These substituents were expected to bias the conformation of the linking chain and favor the cyclic form, and they appeared to add favorable hydrophobic contacts with the enzyme. Modeling studies of **5a** inside the chymotrypsin active site and of the hydrate **5b** in solution indicated that the macrocyclic ring is well-defined conformationally and that ring closure would be essentially strain-free.

The boronic acid adducts **6** that mimic the macrocyclic tetrahedral intermediate would arise from complexation and cyclization of acyclic precursors. Analogs were synthesized both with and without the alkyl substituents (**7** and **8**), and with hydroxyl or amino groups as the nucleophile Z (**8-NH$_2$** and **8-OH**). Several inhibitors incapable of cyclizing were also prepared as comparison compounds (**8-H, 9**, and **10**).



7: R = Me, R' = *i*-Bu, Z = OH
8-NH$_2$: R = R' = H, Z = NH$_2$
8-OH: R = R' = H, Z = OH
8-H: R = R' = Z = H



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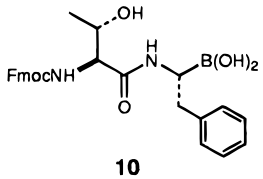

10

Synthesis

Synthesis of the peptide boronic acids, as outlined for the substituted analog **7** in Scheme 2, required preparation of the boronic acid analog of phenylalanine, **12**, assembly of a protected derivative of (*S*,*S*)-2,4-diaminopentanoic acid **11**, and synthesis of the substituted malate derivative **13**. A direct route was available to the pinanediol ester of L-borophenylalanine, **12**, using methods developed by Matteson and his co-workers,[20−23] and stereoselective routes to the substituted amino and hydroxy acids **11** and **13** were devised, as shown in Schemes 3 and 4, respectively.

α-*tert*-Butyl γ-methyl *N*-9-phenylfluorenyl-L-glutamate was methylated at the γ-position to give **15** (Scheme 3) with moderate diastereoselectivity (3.5:1) following procedures of Rapoport and co-workers.[24,25] After saponification of the methyl ester, the amino protecting group

(17) Grütter, M. G.; Gabriele, F.; Huber, R.; Bode, W. *EMBO J.* **1988**, *7*, 345.
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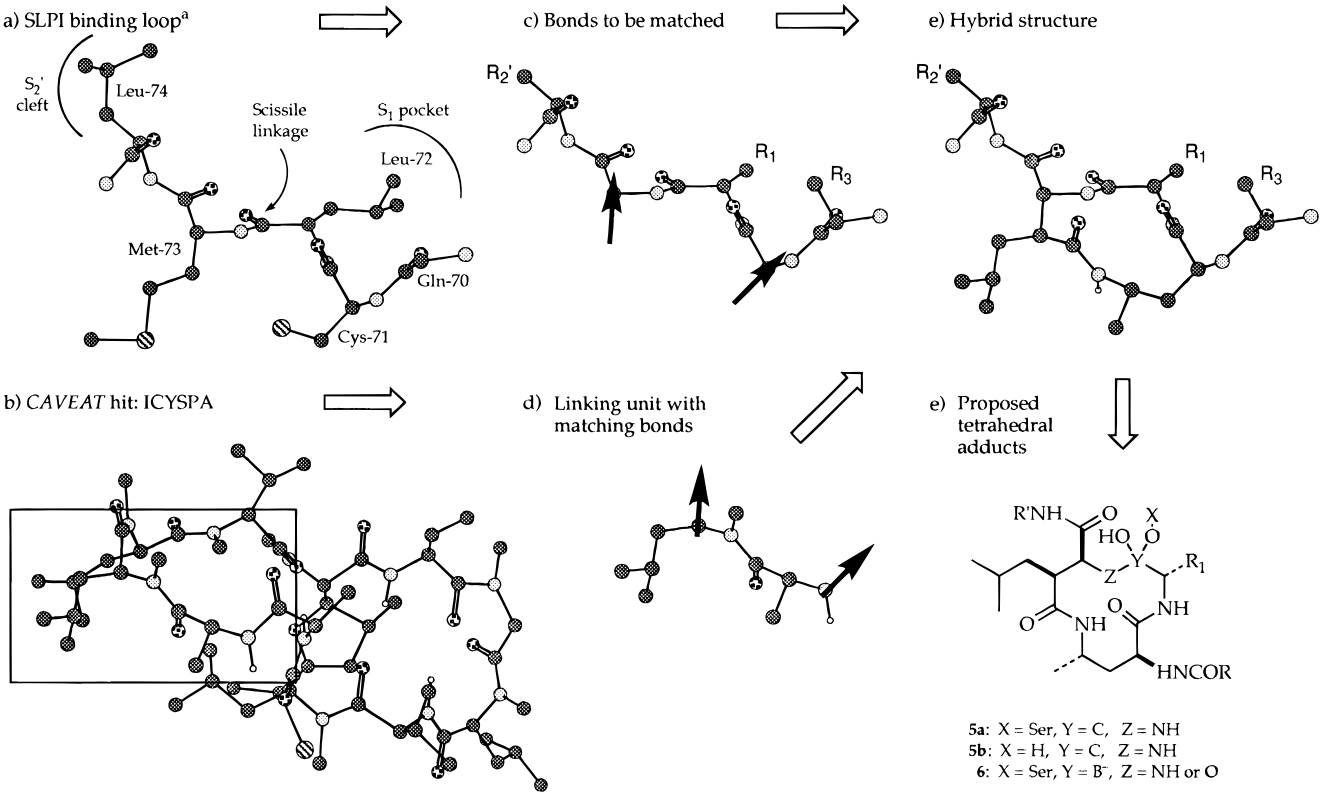
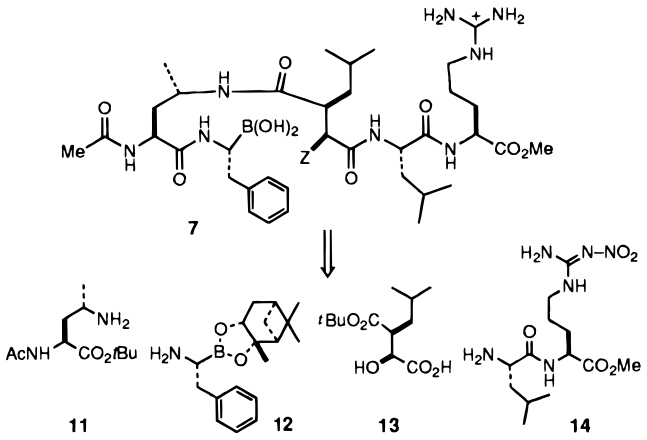
(23) Matteson, D. S.; Jesthi, P. K.; Sadhu, K. M. *Organometallics* **1984**, *3*, 1284.



[a] From complex of SLPI with chymotrypsin (reference 17)

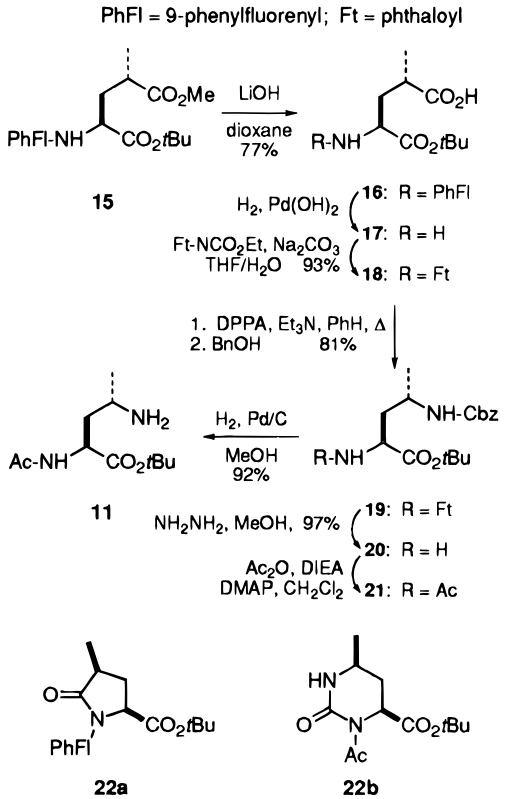
Figure 2. The design of a macrocyclic mimic of the chymotrypsin tetrahedral adduct.

Scheme 2



was switched to the phthalimide (**16** → **18**),[26] otherwise the subsequent Curtius rearrangement led to undesired cyclization reactions (e.g., to the lactam **22a** with the phenylfluorenyl derivative or the urea **22b** from the acetamide). The desired rearrangement of phthalimide **18** proceeded smoothly with diphenylphosphoryl azide (DPPA)[27] in the presence of benzyl alcohol to introduce the C-4 nitrogen functionality (**19**). Removal of the phthaloyl protecting group using anhydrous hydrazine,[28] acetylation with acetic anhydride, and hydrogenolysis proceeded in good yield to give the desired precursor **11**.

Scheme 3



The hydroxy acid **13** was synthesized from (*R*)-malic acid (Scheme 4). The C-1 carboxy and the 2-hydroxyl groups were blocked as the dioxolanone **23**,[29] which facilitated selective *tert*-butylation of the C-4 carboxy group. Methanolysis of **24** then gave a differentially

(24) Koskinen, A. M. P.; Rapoport, H. *J. Org. Chem.* **1989**, *54*, 1859.
(25) Bergmeier, S. C.; Cobas, A. A.; Rapoport, H. *J. Org. Chem.* **1993**, *58*, 2369.
(26) Nefkins, G. H. L.; Tesser, G. I.; Nivard, R. J. F. *Recl. Trav. Chim. Pays. Bas.* **1960**, *79*, 688.
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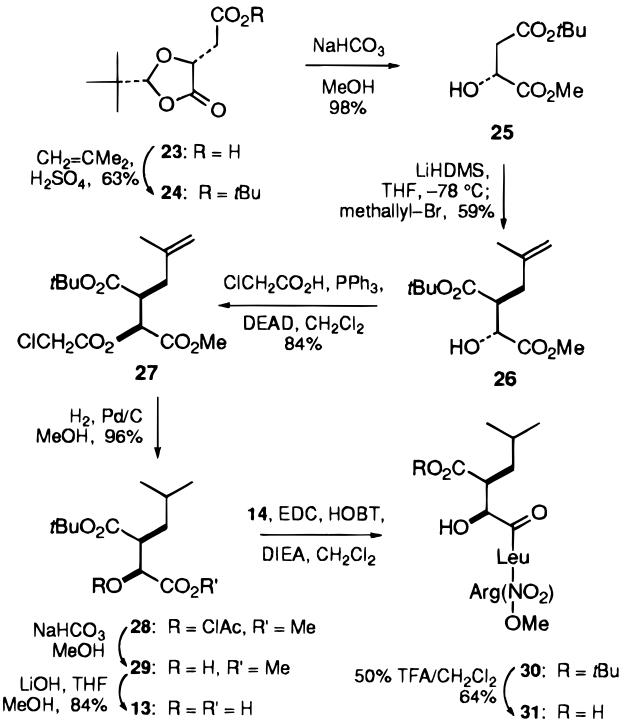
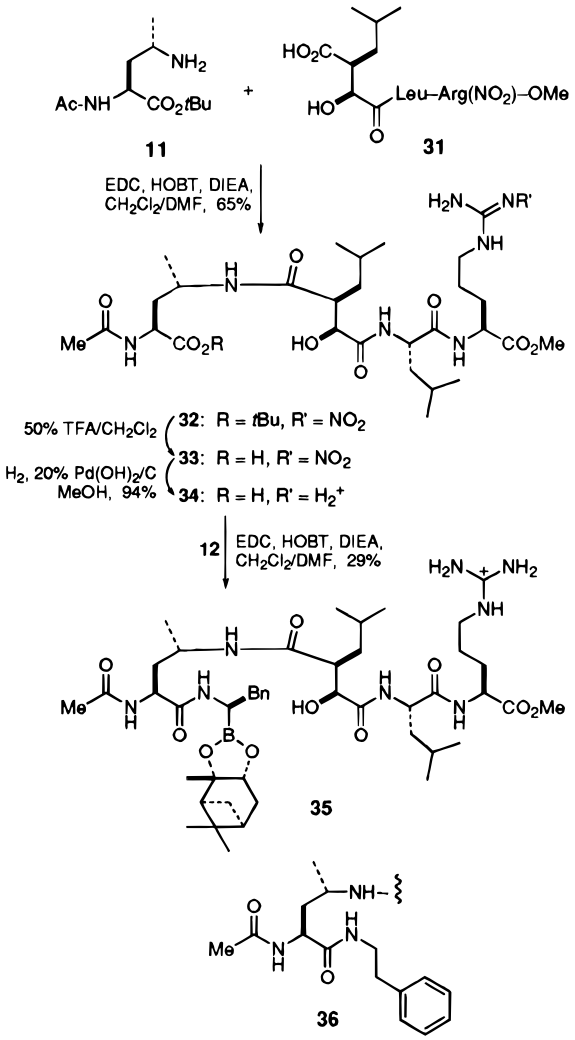
Scheme 4



protected malate, **25**. The lithium dianion of **25** was alkylated with methallyl bromide to give the 2*R*,3*S*-diastereomer **26** with high (20:1) selectivity.[30−32] Inversion of the C-2 hydroxyl stereocenter to the desired *S*-configuration was accomplished using a modified Mitsunobu displacement, as described by Martin et al.[33] Catalytic hydrogenation of the double bond, followed by hydrolysis of the chloroacetate and methyl esters, afforded the desired intermediate **13**. Coupling of this material to the Leu-Arg dipeptide and removal of the *tert*-butyl ester afforded **31**, in preparation for assembly of the inhibitor **7**.

The amine **11** and hydroxy acid **31** were coupled under standard EDC/HOBT conditions to afford the amide **32** in 65% yield (Scheme 5). Removal of the *tert*-butyl and the nitro protecting groups then set the stage for introduction of the borophenylalanine unit **12**. This coupling reaction was the last synthetic step in the sequence, affording the water-labile pinanediol ester, **35**, of the target inhibitor, **7**. Unfortunately, neither the mixed anhydride method (IBCF, NMM, THF, −20 to 10 °C)[11,34,35] nor carbodiimide coupling conditions (EDC, HOBT, DIEA, CH_2Cl_2/DMF) gave very much of the product **35**; following

Scheme 5



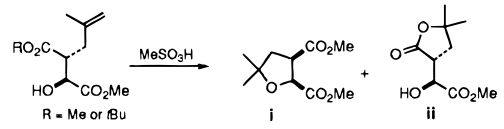
purification by reverse phase HPLC, a 29% yield was obtained from EDC coupling and <20% from the mixed anhydride protocol. The major byproduct was identified as the deboronated material, **36**. Isolation of the inhibitors as the boronic acids themselves was not necessary, since the pinanediol ester is hydrolyzed by incubating in pH 7.5 phosphate buffer prior to the enzymatic assay.[11]

Syntheses of the pinanediol esters of the simpler boronic acids, **8-OH** and **8-NH$_2$**, and the control compound **8-H** followed similar routes as described for the substituted analog **7**. The α-acetyl *tert*-butyl ester of L-2,4-diaminobutanoic acid, **42**, a common building block for these compounds, was prepared from the Cu(II) chelate **37**, which was protected selectively on the γ-amino group (Scheme 6).[36]

Inhibition of Chymotrypsin

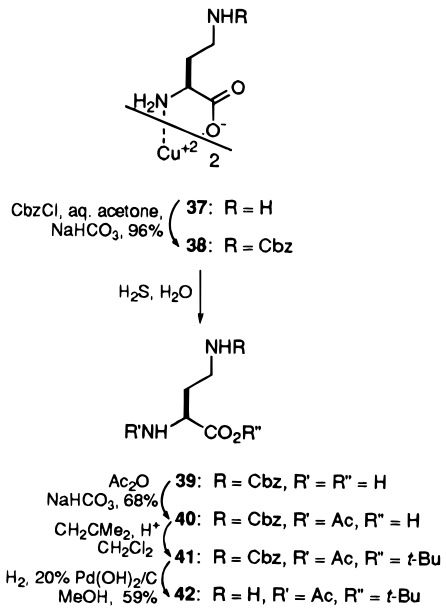
The boronate esters were incubated in phosphate buffer at pH 7.5 for 1−2 h prior to enzyme assay to ensure conversion to the boronic acids.[11] Inhibition of bovine α-chymotrypsin was evaluated at 25 °C at pH 7.0 by incubating the enzyme with inhibitor for 10−15 min and initiating the reaction by adding substrate (succinyl-Ala-Ala-Pro-Phe-*p*-nitroanilide).[37,38] Reciprocal plots (1/V vs 1/[S]) at several concentrations of boronate **7** gave a

(29) Hoye, T. R.; Peterson, B. H.; Miller, J. D. *J. Org. Chem.* **1987**, *52*, 1351.
(30) Seebach, D.; Wasmuth, D. *Helv. Chim. Acta* **1980**, *63*, 197.
(31) Frater, G. *Helv. Chim. Acta* **1979**, *62*, 2825.
(32) The 2*R*,3*S*-stereochemistry was assigned to **26** in closely related work by Seebach and Wasmuth,[30] but it was also determined unambiguously by correlation, in the enantiomeric series, with the cyclic ether **i** formed by acid catalysis.



(33) Martin, S. F.; Dodge, J. A. *Tetrahedron Lett.* **1991**, *32*, 3017.
(34) Deadman, J. J.; Elgendy, S.; Goodwin, C. A.; Green, D.; Baban, J. A.; Patel, G.; Skordalakes, E.; Chino, N.; Claeson, G.; Kakkar, V. V.; Scully, M. F. *J. Med. Chem.* **1995**, *38*, 1511.
(35) Wityak, J.; Earl, R. A.; Abelman, M. M.; Bethel, Y. B.; et al. *J. Org. Chem.* **1995**, *60*, 3717.
(36) Rosowsky, A.; Wright, J. E. *J. Org. Chem.* **1983**, *48*, 1539.
(37) Nakajima, K.; Powers, J. C.; Ashe, B. M.; Zimmerman, M. *J. Biol. Chem.* **1979**, *254*, 4027.

Scheme 6



similar 1/*V* intercept (Figure 3a), indicative of competitive inhibition. Dixon analysis at several substrate concentrations gave a K_i value of 26 ± 3 nM for this inhibitor (Figure 3b). The other boronic acids were analyzed similarly and their inhibition constants are given in Table 1.

The possibility of time- and pH-dependent development of a greater degree of inhibition, as might be expected for the transition from monoester to diester adduct, was investigated for the cyclizable boronates **7** and **8-NH₂** and the simple analog **10**. Solutions of inhibitor (ca. 20 × K_i) were incubated with enzyme at pH 5.0 (acetate buffer), 7.0 (HEPES), or 7.5 (phosphate) at 25 °C; aliquots were removed over time, diluted 50-fold into assay buffer, and assayed for activity. No significant change was observed over a period of 24 h.

Discussion

The extended inhibitors are more potent than the dipeptidyl boronates **9** and **10** that can only interact with the S-subsites. However, the advantage is about an order of magnitude at most, less than anticipated for occupancy of the S′-subsites.[7] Nevertheless, there are differences among the extended analogs that could relate to their substitution pattern (**7** vs **8-OH**) and their ability to undergo the desired cyclization (**8-OH** vs **8-H** and **8-NH₂**). The cyclization reaction requires that the initial serine−boronate adduct lose hydroxide ion to give the trivalent intermediate (Scheme 7). Since this process is pH-dependent and potentially slow, we determined the inhibition constants after extended incubation of enyzme and inhibitor and at pH's down to 5.5. No significant difference was seen, indicating either that cyclization does not occur or that it is complete during the normal preincubation period. Cyclization of the amino derivative **8-NH₂** may be impeded by the conflicting requirements that the trivalent intermediate be formed by loss of hydroxide (favored at low pH) and that the amine be nucleophilic (favored at high pH). Since boronic acid−amine adducts are formed readily within six-membered rings from the trivalent boronic acid form, even at neutral pH,[39] the hurdle of Scheme 7 may be conversion of the tetrahedral boronate to the trigonal boronic acid.

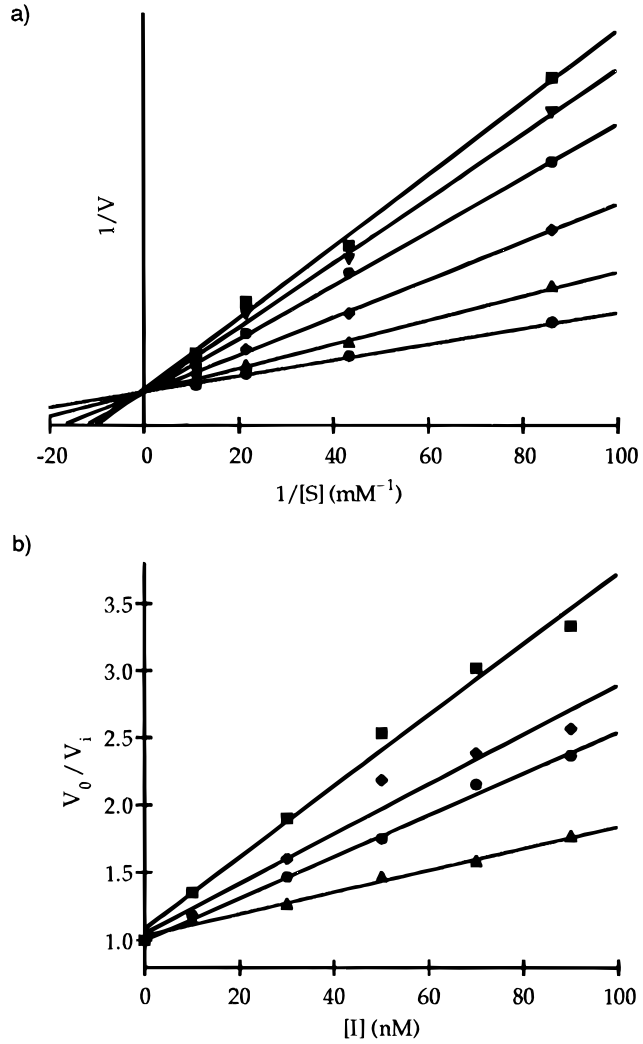


Figure 3. (a) Lineweaver−Burk plot for inhibition of α-chymotrypsin by boronic acid **7**; [I] (nM) = 0 (●), 10 (▲), 30 (◆), 50 (●), 70 (▼), and 90 (■); (b) Dixon plot for inhibition by **7**; [S] (μM) = 11.6 (■), 23.2 (◆), 46.4 (●), and 92.8 (▲).

Table 1. Inhibition of α-Chymotrypsin by Peptidyl Boronic Acids[a]

inhibitor	K_i (nM)
9	356 ± 50
10	219 ± 90
8-NH₂	133 ± 4
8-H	114 ± 5
8-OH	68 ± 5
7	26 ± 3

[a] Values are reported with standard deviations from multiple determinations.

The simplest interpretation of these results is that the extended boronic acids do not undergo the desired cyclization, with the modest differences in affinity between the inhibitors explained by fortuitous rather than specific interactions in the enzyme active site. An alternative explanation is that occupancy of the S_1'-S_3' subsites and formation of the boronate diester do not confer enough additional binding affinity to overcome the flexibility or

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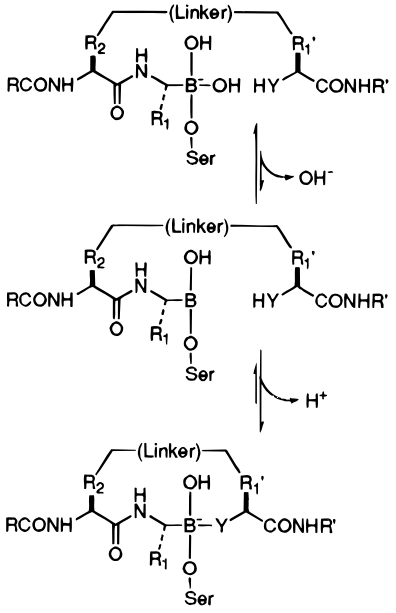
Scheme 7



binding characeristics of the linking group. A more detailed structural investigation is needed to confirm this interpretation, but it calls into question the strategy underlying the design of cyclizable boronic acids. Since macrocyclic structures are effective for other inhibitor motifs, and since ternary enzyme−boronate−alcohol complexes have been demonstrated for serine proteases,[2] the question narrows to that of linker design. The primary considerations in designing the linkers in **7** and **8** were to avoid steric and torsional strain in the macrocyclic complexes and to devise targets that were relatively accessible synthetically. While the amide moiety achieves these goals, it is a very polar group whose strong solvation outside the enzyme is not replaced with complementary hydrogen bonds in the active site. In hindsight, a less polar linker such as an ester or an alkene might have proven to be more effective.

Experimental Section[40]

Preparation of Compounds. Procedure A: General Procedure for Peptide Coupling Using EDC/HOBT. To a solution (∼0.1 M) containing the free acid and the free amine (1.1 equiv) in CH_2Cl_2 at room temperature were added EDC (1.5 equiv) and HOBT (2.0 equiv). The mixture was stirred for 16 h, diluted with CH_2Cl_2, and washed with 3 M NaCl. The aqueous layer was extracted twice with CH_2Cl_2, and the combined organic layers were dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give the crude product. This material was purified by chromatography on silica gel with 95:5 CH_2Cl_2/MeOH.

Procedure B: General Procedure for Peptide Coupling Using EDC/HOBT/DIEA. To a solution (∼0.1 M) containing the free acid and the salt of the amine (1.1 equiv) in CH_2Cl_2 or 1:1 (v) DMF/CH_2Cl_2 were added EDC (1.3 equiv) and HOBT (1.5 equiv). DIEA (1.1 equiv) was added, and the reaction was stirred under N_2 at room temperature for 12−16 h. The mixture was worked up as described for procedure A.

Procedure C: General Procedure for Removal of *tert*-Butyl Ester Protecting Group. To a solution (∼1.0 M) of the *tert*-butyl ester in CH_2Cl_2 was added TFA/CH_2Cl_2 over a period of 1 h to a final concentration of about 0.1 M. The mixture was stirred at room temperature for 1−5 h or until TLC analysis indicated that no starting material remained. The reaction mixture was concentrated *in vacuo* and twice dissolved in 1,2-dichloroethane and evaporated to remove TFA. The product was collected in CH_2Cl_2, evaporated, and dried in high vacuum.

***tert*-Butyl (*S,S*)-2-Acetamido-4-aminopentanoate, 11.** A slurry of 10% Pd/C (12 mg, 0.01 mmol) and carbamate **21** (see below) (42 mg, 0.11 mmol) in anhydrous MeOH (2 mL) in a Parr bottle was evacuated and vented twice with N_2 before hydrogen was added via a balloon. After stirring for 4 h, the slurry was filtered through a 0.2−μ nylon filter and concentrated, and the crude product was chromatographed (96:4 CH_2Cl_2/MeOH) to give 25 mg (96%) of amine **11**: TLC R_f 0.09 (96:4 CH_2Cl_2/MeOH); ^{1}H NMR (CD_3OD) δ 4.30 (dd, 1, J = 10.5, 4.6), 2.96 (m, 1), 1.95 (s, 3), 1.76 (m, 2), 1.43 (m, 9), 1.14 (d, 3, J = 6.7); LRMS (FAB) calcd for $C_{11}H_{23}N_2O_3$: m/z 231.3 (MH^+), found 231.3.

[(2*S*)-(*R,*R**)-2-Hydroxy-3-(2-methylpropyl)butandioic Acid, 4-*tert*-Butyl Ester, 13.** A solution of hydroxy diester **29** (see below) (0.13 g, 0.5 mmol) in 3:1:1 THF/MeOH/1.0 M aqueous LiOH (5 mL, 0.1 M) was stirred for 15 h at 25 °C. The solution was diluted with hexanes (15 mL) and the organic layer was extracted with H_2O (15 mL). The combined aqueous layers were acidified to pH = 5 with 1.0 M H_3PO_4 and extracted with three 20-mL portions of EtOAc, and the organic phase was dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give 0.12 g (99%) of acid **13** that was pure by ^{1}H NMR: [α] = −10.5° (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 4.47 (d, 1, J = 4.1), 2.85 (ddd, 1, J = 4.1, 4.5, 10.2), 1.76 (ddd, 1, J = 5.1, 10.2, 13.7), 1.58 (m, 1), 1.46 (s, 9), 1.27 (ddd, 1, J = 4.5, 9.1, 13.7), 0.91 (d, 3, J = 6.3), 0.90 (d, 3, J = 6.3); ^{13}C NMR ($CDCl_3$) δ 175.6, 173.6, 82.1, 71.4, 47.2, 36.1, 27.9, 26.0, 23.2, 21.5; IR 3432 (br), 2959, 2866, 1736, 1716, 1469, 1369, 1253, 1156, 845 cm^{-1}; HRMS (FAB) calcd for $C_{12}H_{23}O_5$: m/z = 247.1545 (MH^+), found 247.1550.

***threo*-4-Methyl-*N*-(9-phenyl-9*H*-fluoren-9-yl)-L-glutamic Acid, 1-*tert*-Butyl Ester, 16.** A solution of the mixed diester **15**[24,25] (0.45 g, 0.95 mmol) and 1.0 M aqueous LiOH (8.5 mL, 90 equiv) in dioxane (50 mL) was stirred for 15 h at 65 °C, cooled, and diluted with THF (15 mL) and 2 N NaOH (5 mL). The aqueous layer was washed three times with hexanes, acidified to pH = 5 with 1.0 M H_3PO_4, and extracted with EtOAc (3 × 50 mL). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give 0.34 g (77%) of acid **16** that was pure by ^{1}H NMR: TLC R_f 0.61 (1:1 hexanes/EtOAc); [α] = −204.1° (*c* 1.5, $CHCl_3$); ^{1}H NMR (CD_3OD) δ 7.76−7.73 (m, 2), 7.39−7.13 (m, 11), 2.58 (m, 1), 2.48 (dd, 1, J = 10.6, 4.2), 1.88 (m, 1), 1.30 (m, 1), 1.14 (m, 9), 0.81 (d, 3, J = 7.1); partial ^{13}C NMR 180.6 (C), 176.9 (C), 82.0 (C), 74.1 (C), 55.9 (CH), 39.9 (CH_2), 37.7 (CH), 28.2 (CH_3), 17.7 (CH_3); IR 3302 (br), 2976, 2934, 1725, 1703, 1599, 1449, 1368, 1280, 1153, 753 cm^{-1}; HRMS (FAB) calcd for $C_{29}H_{32}NO_4$: m/z 458.2331 (MH^+), found 458.2331.

***threo*-4-Methyl-L-glutamic Acid, 1-*tert*-Butyl Ester, 17.** A slurry of 20% $Pd(OH)_2$/C (0.08 g, 0.3 mmol) and the phenylfluorenyl derivative **16** (0.27 g, 0.6 mmol) in 4:1 anhydrous MeOH/EtOAc (12 mL) in a Parr bottle was placed under hydrogen at 50 psi. After 18 h at room temperature, the slurry was filtered through Celite and concentrated on a rotary evaporator. The residue was washed with hexanes (5 mL) to give 0.13 g (99%) of amino acid **17**: mp 140−142 °C;

(40) **General.** Reagents and solvents were obtained from commercial suppliers and used as received. Bovine pancreatic α-amylase (EC 3.4.21.1, type II; 47 units/mg) and Suc-Ala-Ala-Pro-Phe-*p*-nitroanilide were obtained from Sigma. Leu-Arg(NO_2)-OMe (**14**) was prepared by standard peptide coupling protocol. All moisture- or air-sensitive reactions were conducted under nitrogen in dried solvents. Unless otherwise indicated, chromatography was performed on silica gel with the indicated solvent as eluant. NMR spectra were recorded at 400 MHz for ^{1}H (100 MHz for ^{13}C); ^{1}H NMR spectral data are reported as chemical shift (multiplicity, number of hydrogens, coupling constants in Hz). ^{1}H NMR chemical shifts are referenced to tetramethylsilane (0 ppm) or CHD_2OD (3.30 ppm); ^{13}C NMR spectra were proton decoupled and chemical shifts are referenced to $CDCl_3$ (77.0 ppm) or CD_3OD (49.0 ppm). Mass spectra were obtained by the Mass Spectrometry Laboratory of the College of Chemistry, University of California, Berkeley. Abbreviations used: HOBT: 1-hydroxybenzotriazole; EDC: 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide; DIEA: diisopropylethylamine; DMAP: 4-(dimethylamino)pyridine; HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

(41) Leatherbarrow, R. J. *ENZFITTER*; Elsevier Science: New York, 1987.

[α] = +39.6° (*c* 1.1, MeOH); ^{1}H NMR (CD_3OD) δ 3.78 (br t, 1, *J* = 6.3), 2.49 (m, 1), 1.89 (m, 2), 1.51 (m, 9), 1.17 (d, 3, *J* = 7.1); ^{13}C NMR (CD_3OD) δ 181.2, 169.3, 83.2, 52.9, 40.2, 35.3, 26.6, 18.2; HRMS (FAB) calcd for $C_{10}H_{20}NO_4$: *m*/*z* 218.1392 (MH^+), found 218.1386.

***threo*-4-Methyl-*N*-(phthaloyl)-L-glutamic Acid, 1-*tert*-Butyl Ester, 18.** A mixture of amino acid **17** (0.15 g, 0.7 mmol), crushed *N*-carboethoxyphthalimide (0.31 g, 1.4 mmol), and Na_2CO_3 (0.08 g, 0.8 mmol) in 4:1 THF/H_2O (2.7 mL, 0.25 M) was stirred for 18 h at room temperature and then diluted with EtOAc (10 mL). The aqueous layer was acidified to pH 5 with 1.0 M H_3PO_4 and extracted with three 10-mL portions of EtOAc. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated *in vacuo*, and the product was chromatographed (97:3 CH_2Cl_2/MeOH) to give 0.23 g (94%) of phthalimide **18**: TLC R_f 0.31 (98:2 CH_2Cl_2/MeOH); [α] = +11.6° (*c* 0.8, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.86 (m, 2), 7.84 (m, 2), 4.82 (dd, 1, *J* = 4.7, 11.0), 2.60 (ddd, 1, *J* = 6.2, 11.0, 14.2), 2.40 (ddq, 1, *J* = 6.2, 8.1, 7.0), 2.25 (ddd, 1, *J* = 4.7, 8.1, 14.2), 1.42 (s, 9), 1.22 (d, 3, *J* = 7.0); ^{13}C NMR ($CDCl_3$) δ 180.7, 167.7, 167.5, 134.1, 131.7, 123.4, 82.8, 50.9, 36.6, 32.0, 27.7, 16.4; IR 3388 (br), 2977, 1776, 1716 (br), 1467, 1386, 1257, 1156, 874, 720 cm^{-1}; HRMS (FAB) calcd for $C_{18}H_{22}NO_6$: *m*/*z* 348.1447 (MH^+), found 348.1454.

***threo*-N^4-(Benzyloxycarbonyl)-4-methyl-N^2-phthaloyl-L-ornithine, *tert*-Butyl Ester, 19.** Carboxylic acid **18** (0.22 g, 0.63 mmol) was dissolved in anhydrous benzene (8.0 mL, 0.08 M) under N_2, and the solution was heated to a gentle reflux before triethylamine (0.1 mL, 0.72 mmol) and diphenyl phosphorazidate (0.15 mL, 0.66 mmol) were added. The clear solution was refluxed for 1 h, benzyl alcohol (0.23 mL, 2.2 mmol) was added, and reflux was continued for an additional 16 h. The reaction mixture was diluted with EtOAc (35 mL) and washed with saturated NH_4Cl and 0.5 M $NaHCO_3$. The organic extract was dried ($MgSO_4$), filtered, and concentrated *in vacuo*, and the product was chromatographed (5:1 hexanes/EtOAc) to give 0.23 g (81%) of carbamate **19**: TLC R_f 0.48 (3:1 hexanes/EtOAc); [α] = −8.1° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.85 (m, 2), 7.84 (m, 2), 7.70 (m, 5), 4.96 (d, 1, *J* = 12.1), 4.85 (m, 1), 4.84 (d, 1, *J* = 12.1), 4.68 (br s, 1), 3.80 (m, 1), 2.33 (m, 2), 1.41 (m, 9), 1.21 (d, 3, *J* = 6.7); ^{13}C NMR ($CDCl_3$) δ 167.8, 167.6, 155.5, 136.4, 134.1, 131.8, 128.4, 127.9, 123.5, 82.8, 66.5, 50.4, 45.3, 35.9, 27.8, 20.9; IR 3373 (br), 2976, 1776, 1715, 1698, 1530, 1454, 1389, 1257, 1157, 940, 844, 721 cm^{-1}; HRMS (FAB) calcd for $C_{25}H_{29}N_2O_6$: *m*/*z* 453.2026 (MH^+), found 453.2017.

***threo*-N^4-(Benzyloxycarbonyl)-4-methyl-L-ornithine, *tert*-Butyl Ester, 20.** A solution of phthalimide **19** (0.22 g, 0.48 mmol) and anhydrous hydrazine (0.1 mL, 2.9 mmol) in anhydrous MeOH (5 mL) was stirred for 18 h, during which period a white precipitate formed. The mixture was concentrated *in vacuo*, and the resultant sticky solid was slurried with 2:1 Et_2O/hexanes, dissolved in CH_2Cl_2, and filtered through glass wool to give 0.15 g (97%) of amine **19** that proved to be pure by ^{1}H NMR: TLC R_f 0.24 (98:2 CH_2Cl_2/MeOH); ^{1}H NMR (CD_3OD) δ 7.33 (br m, 5), 5.05 (s, 2), 3.82 (m, 1), 3.40 (t, 1, *J* = 6.1), 1.76 (m, 2), 1.45 (m, 9), 1.15 (d, 3, *J* = 6.6); LRMS (FAB) calcd for $C_{17}H_{27}N_2O_4$: *m*/*z* 323.2 (MH^+), found 323.2.

***threo*-N^2-Acetyl-N^4-(benzyloxycarbonyl)-4-methyl-L-ornithine, *tert*-Butyl Ester, 21.** To a solution of amine **20** (0.15 mg, 0.46 mmol) in CH_2Cl_2 (5.0 mL, 0.1 M) were added, in sequence, acetic anhydride (0.07 mL, 0.71 mmol), DIEA (0.24 mL, 1.38 mmol), and DMAP (0.01 g, 0.07 mmol). The mixture was stirred for 16 h and then concentrated *in vacuo*, and the crude mixture was chromatographed (98:2 CH_2Cl_2/MeOH) to give 0.16 g (96%) of acetamide **21**: TLC R_f 0.33 (98:2 CH_2Cl_2/MeOH); [α] = +6.1° (*c* 1.4, $CHCl_3$); ^{1}H NMR (CD_3OD) δ 7.32 (br m, 5), 5.03 (s, 2), 4.34 (t, 1, *J* = 6.4), 3.75 (m, 1),1.92 (s, 3), 1.81 (m, 2), 1.41 (m, 9), 1.14 (d, 3, *J* = 6.6); ^{13}C NMR (CD_3OD) δ 171.7, 171.2, 156.6, 138.8, 127.9, 127.4, 127.3, 81.4, 65.8, 50.6, 43.9, 37.7, 26.6, 20.8, 19.2; IR 3310 (br), 2976, 1737, 1716, 1661, 1537, 1455, 1370, 1251, 1147, 1062, 845, 751 cm^{-1}; HRMS (FAB) calcd for $C_{19}H_{29}N_2O_5$: *m*/*z* 365.2076 (MH^+), found 365.2066.

***tert*-Butyl (*R*,*R*)-2-*tert*-Butyl-5-oxo-1,3-dioxole-4-acetate, 24.** To a −10 °C solution of **23**[29] (2.5 g, 12.4 mmol) in CH_2Cl_2 (25 mL) in a Parr shaker bottle was added isobutylene (25 mL) via a −78 °C cold finger apparatus. A catalytic amount of concd H_2SO_4 (0.3−0.5 mL) was added, the bottle was sealed under N_2 and warmed to 25 °C, and the mixture was stirred for 18 h before being recooled to 0 °C and opened. The mixture was diluted with CH_2Cl_2 (50 mL), washed with saturated NH_4Cl and brine, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The crude product was chromatographed (8:1 hexanes/Et_2O) to give 1.70 g (63%) of ester **24** as a white solid: mp 62−63 °C; TLC R_f 0.35 (4:1 hexanes/Et_2O); [α] = +3.0° (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.15 (d, 1, *J* = 1.1), 4.60 (q, 1), 2.84 (dd, 1, *J* = 16.7, 3.7), 2.63 (dd, 1, *J* = 16.7, 7.7), 1.45 (s, 9), 0.96 (s, 9); IR ($CHCl_3$) 2960, 1790, 1731, 1478, 1363, 1307, 1222, 1157, 1111, 1052, 963, 910, 848, 753 cm^{-1}; HRMS (EI) calcd for $C_{13}H_{23}O_5$: *m*/*z* = 259.1545 (MH^+), found 259.1539.

D-Malic Acid, 1-Methyl 4-*tert*-Butyl Ester, 25. A solution containing acetal **24** (0.70 g, 2.71 mmol) and $NaHCO_3$ (0.05 g, 0.60 mmol) in anhydrous MeOH (1.0 M) was stirred at 25 °C for 1 h, diluted with EtOAc (30 mL), and washed with H_2O and brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo*, and the crude product was chromatographed (1:1 hexanes/Et_2O) to give 0.54 g (98%) of alcohol **25**: TLC R_f 0.46 (4:3 hexanes/Et_2O); [α] = +3.1° (*c* 0.55, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 4.43 (q, 1), 3.80 (s, 3), 2.74 (m, 2), 1.45 (s, 9); ^{13}C NMR ($CDCl_3$) δ 173.8, 169.6, 81.5, 67.4, 52.5, 39.6, 27.9; IR 3492 (br OH) 2979, 1732 (br) 1440, 1393, 1258, 1154, 1105, 1044, 960, 845 cm^{-1}; HRMS (FAB) calcd for $C_9H_{17}O_5$: *m*/*z* = 205.1070 (MH^+), found 205.1030.

[(*S*)-(*R,*R**)]-2-Hydroxy-3-(2-methyl-2-propenyl)butanedioic Acid, 1-Methyl 4-*tert*-Butyl Ester, 26.** To a solution of hydroxy diester **25** (1.5 g, 7.3 mmol) in anhydrous THF (75 mL, 0.1 M) at −78 °C was added 1.0 M lithium hexamethyldisilazide (29.4 mL, 29.4 mmol) under N_2 over a 1-h period. The mixture was stirred for 3 h before 3-bromo-2-methylpropene (2.25 mL, 21.9 mmol) was added. After 13 h at −78 °C, additional lithium hexamethyldisilazide (3.6 mL, 0.75 mmol) and 3-bromo-2-methylpropene (0.75 mL, 7.3 mmol) were added, and the mixture was stirred for 16 h before being diluted with water (30 mL) and extracted with three 50-mL portions of EtOAc. The combined organic layers were dried ($MgSO_4$), filtered, and concentrated *in vacuo*. ^{1}H NMR analysis of the crude reaction mixture revealed **26** as the major product (≥90% diastereomeric purity). The crude mixture was chromatographed (3:1 hexanes/Et_2O) to give 1.12 g of the major diastereomer **26** (59%; 73% based on recovered starting material), 0.06 g (3%) of a minor diastereomer, and 0.28 g (19%) of recovered **25**. Data for **26**: TLC R_f 0.54 (1:1 hexanes/Et_2O); [α] = −2.4° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 4.85 (br s, 1), 4.81 (br s, 1), 4.22 (d, 1, *J* = 2.7), 3.80 (s, 3), 3.01 (ddd, 1, *J* = 2.7, 6.4, 9.2), 2.52 (dd, 1, *J* = 6.4, 14.5), 2.39 (dd, 1, *J* = 9.2, 14.5), 1.76 (s, 3), 1.41 (s, 9); ^{13}C NMR ($CDCl_3$) δ 174.2, 171.2, 142.0, 113.0, 81.6, 70.2, 52.4, 46.7, 35.9, 27.8, 22.0; IR ($CHCl_3$) 3506 (br), 3078, 2977, 2935, 1754, 1716, 1653, 1455, 1256, 1113, 1015, 896 cm^{-1}; HRMS (FAB) calcd for $C_{13}H_{23}O_5$: *m*/*z* = 259.1545 (MH^+), found 259.1542.

[(*S*)-(*R,*R**)]-2-[(Chloroacetyl)oxy]-3-(2-methyl-2-propenyl)butanedioic Acid, 1-Methyl 4-*tert*-Butyl Ester, 27.** To a solution of **26** (1.2 g, 4.5 mmol) in anhydrous benzene (46 mL, 0.1 M) at 10 °C were added triphenylphosphine (4.9 g, 17.8 mmol), chloroacetic acid (1.75 g, 17.8 mmol), and DEAD (2.9 mL, 17.8 mmol), sequentially. This mixture was allowed to warm to 25 °C and stirred for 46 h under N_2 before hexanes (10 mL) were added, and the slurry was filtered through Celite. Concentration of the filtrate provided a viscous oil, which was chromatographed (3:1 hexanes/Et_2O) to give 1.30 g (84%) of the desired chloroacetate **27**: TLC R_f 0.48 (2:1 hexanes/Et_2O); [α] = +5.1° (*c* 1.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.50 (d, 1, *J* = 5.0), 4.80 (br s, 1), 4.74 (br s, 1), 4.16 (d, 1, *J* = 15.5), 4.15 (d, 1, *J* = 15.5), 3.74 (s, 3), 3.10 (ddd, 1, *J* = 5.0, 6.0, 8.9), 2.50 (dd, 1, *J* = 8.9, 14.6), 2.29 (dd, 1, *J* = 14.6, 6.0), 1.73 (s, 3), 1.42 (s, 9); ^{13}C NMR 169.9, 168.2, 166.4, 141.6, 113.0, 81.9, 73.1, 52.5, 45.6, 40.4, 35.4, 27.9, 22.0; IR 3058, 2978, 2935, 1753, 1730 (br), 1649, 1437, 1369, 1257, 1153, 1064, 914 cm^{-1}; HRMS (FAB) calcd for $C_{15}H_{24}ClO_6$: *m*/*z* = 334.1261 (MH^+), found 334.1262.

[(*S*)-(*R,*R**)]-2-[(Chloroacetyl)oxy]-3-(2-methylpropyl)-butanedioic Acid, 1-Methyl 4-*tert*-Butyl Ester, 28.** A slurry of 10% Pd/C (0.67 g, 1.8 mmol) and ester **27** (1.0 g, 3.1 mmol) in anhydrous MeOH (31 mL, 0.1 M) in a Parr bottle was placed under hydrogen at 45 psi. After 18 h at 25 °C, the slurry was filtered through Celite and concentrated to give 1.02 g (96%) of crude **28**, which was used without further purification: TLC R_f 0.49 (2:1 hexanes/Et_2O); $[\alpha] = -8.9°$ (*c* 2.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.47 (d, 1, $J = 5.0$), 4.16 (d, 1, $J = 15.5$), 4.15 (d, 1, $J = 15.5$), 3.77 (s, 3), 2.93 (ddd, 1, $J = 4.0$, 5.0, 10.2), 1.79 (ddd, 1, $J = 4.9$, 10.2, 13.7), 1.63 (m, 1), 1.45 (s, 9), 1.22 (ddd, 1, $J = 4.0$, 9.2, 13.7), 0.94 (d, 3, $J = 6.6$), 0.88 (d, 3, $J = 6.5$); ^{13}C NMR ($CDCl_3$) δ 170.5, 168.3, 166.5, 81.6, 73.9, 52.6, 45.7, 40.4, 36.2, 27.9, 26.0, 23.2, 21.4; IR 3056, 2958, 2872, 1754, 1736 (br), 1469, 1437, 1368, 1251, 1158, 1050, 914 cm^{-1}; HRMS (FAB) calcd for $C_{15}H_{26}ClO_6$: m/z = 336.1418 (MH^+), found 336.1419.

[(*S*)-(*R,*R**)]-2-Hydroxy-3-(2-methylpropyl)butanedioic Acid, 1-Methyl 4-*tert*-Butyl Ester, 29.** A mixture of crude **28** (1.02 g, ≤3.1 mmol) and $NaHCO_3$ (53 mg, 0.6 mmol) in anhydrous MeOH (10 mL) was stirred at room temperature for 4 h, diluted with EtOAc (35 mL), and washed with H_2O (15 mL) and brine (15 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated *in vacuo*, and the residue was chromatographed (3:1 hexanes/Et_2O) to give 0.69 g (85%) of the alcohol **29**: TLC R_f 0.36 (1:1 hexanes/Et_2O); ^1H NMR ($CDCl_3$) δ 4.38 (d, 1, $J = 4.6$), 3.79 (s, 3), 3.10 (br s, 1), 2.73 (ddd, 1, $J = 4.3$, 4.8, 10.2), 1.74 (ddd, 1, $J = 5.0$, 10.2, 13.7), 1.54 (m, 1), 1.45 (s, 9), 1.24 (ddd, 1, $J = 4.3$, 9.1, 13.7), 0.90 (d, 3, $J = 6.6$), 0.87 (d, 3, $J = 6.6$); ^{13}C NMR ($CDCl_3$) δ 173.8, 172.5, 81.2, 71.7, 52.5, 48.2, 36.3, 28.0, 26.0, 23.2, 21.6; IR 3491 (br), 2957, 2871, 1737 (br), 1455, 1368, 1250, 1155, 1102, 847 cm^{-1}; HRMS (FAB) calcd for $C_{13}H_{25}O_5$: m/z = 260.1702 (MH^+), found 261.1698.

***N*²-[*N*-[(2*S*,3*S*)-3-(*tert*-Butoxycarbonyl)-2-hydroxy-5-methyl-1-oxohexyl]-L-leucyl]-*N*⁸-nitro-L-arginine, Methyl Ester, 30.** Acid **13** (0.15 g, 0.47 mmol) and the TFA salt of amine **14** (0.20 g, 0.56 mmol) were coupled in CH_2Cl_2 according to procedure B. The crude product was chromatographed (97:3 CH_2Cl_2/MeOH) to give 0.18 g (65%) of peptide **30**: TLC R_f 0.29 (95:5 CH_2Cl_2/MeOH); ^1H NMR (CD_3OD) δ 4.49−4.43 (m, 2), 4.31 (d, 1, $J = 4.4$), 3.71 (s, 3), 3.23 (br m, 2), 2.74 (m, 1), 1.91 (m, 1), 1.77−1.52 (m, 9), 1.46 (s, 9), 1.13 (ddd, 1, $J = 3.2$, 9.4, 13.6), 0.97 (d, 3, $J = 6.5$), 0.95 (d, 3, $J = 6.5$), 0.88 (d, 3, $J = 6.5$), 0.84 (d, 3, $J = 6.5$); HRMS (FAB) calcd for $C_{25}H_{47}N_6O_9$: m/z 575.3405 (MH^+), found 575.3407.

***N*²-[*N*-[(2*S*,3*S*)-3-Carboxy-2-hydroxy-5-methyl-1-oxohexyl]-L-leucyl]-*N*⁸-nitro-L-arginine, Methyl Ester, 31.** *tert*-Butyl ester **30** (77 mg, 0.13 mmol) was deprotected according to procedure C to give 68 mg (99%) of acid **31**, which was used without further purification. ^1H NMR (CD_3OD) δ 4.48−4.43 (m, 2), 4.41 (d, 1, $J = 3.9$), 3.72 (s, 3), 3.28 (br m, 2), 2.87 (m, 1), 1.92 (m, 1), 1.81−1.54 (m, 9), 1.14 (ddd, 1, $J = 3.5$, 9.3, 13.7), 0.98 (d, 3, $J = 6.5$), 0.95 (d, 3, $J = 6.5$), 0.89 (d, 3, $J = 6.6$), 0.84 (d, 3, $J = 6.6$); HRMS (FAB) calcd for $C_{21}H_{39}N_6O_9$: m/z 519.2779 (MH^+), found 519.2784.

***N*²-[*N*-[(2*S*,3*S*)-3-[[[(1*S*,3*S*)-3-(Acetylamino)-4-*tert*-butoxy-1-methyl-4-oxobutyl]amino]carbonyl]-2-hydroxy-5-methyl-1-oxohexyl]-L-leucyl]-*N*⁸-nitro-L-arginine, Methyl Ester, 32.** Hydroxy acid **31** (38 mg, 0.07 mmol) and amine **11** (17 mg, 0.07 mmol) were coupled according to procedure A to give 35 mg (66%) of the intermediate **32**: ^1H NMR (CD_3OD) δ 4.46 (m, 2), 4.30 (dd, 1, $J = 8.7$, 6.2), 4.22 (d, 1, $J = 4.1$), 3.98 (m, 1), 3.72 (s, 3), 3.25 (m, 2), 2.71 (m, 1), 1.98 (s, 3), 1.93−1.48 (m, 11), 1.45 (s, 9), 1.28 (m, 1), 1.14 (d, 3, $J = 6.6$), 0.98 (d, 3, $J = 6.4$), 0.95 (d, 3, $J = 6.4$), 0.88 (d, 3, $J = 6.8$), 0.87 (d, 3, $J = 6.8$); HRMS (FAB) calcd for $C_{32}H_{59}N_8O_{11}$: m/z 731.4303 (MH^+), found 731.4304.

***N*²-[*N*-[(2*S*,3*S*)-3-[[[(1*S*,3*S*)-3-(Acetylamino)-4-hydroxy-1-methyl-4-oxobutyl]amino]carbonyl]-2-hydroxy-5-methyl-1-oxohexyl]-L-leucyl]-*N*⁸-nitro-L-arginine, Methyl Ester, 33.** *tert*-Butyl ester **32** (23 mg, 0.03 mmol) was deprotected according to procedure C to give 20 mg (99%) of acid **33**. ^1H NMR (CD_3OD) δ 4.38 (m, 3), 4.23 (d, 1, $J = 4.2$), 4.05 (m, 1), 3.71 (s, 3), 3.24 (m, 2), 2.69 (m, 1), 1.99 (s, 3), 1.96−1.46 (m, 11), 1.28 (m, 1), 1.16 (d, 3, $J = 6.6$), 0.98 (d, 3, $J = 6.4$), 0.94 (d, 3, $J = 6.4$), 0.88 (d, 3, $J = 6.9$), 0.87 (d, 3, $J = 6.9$); ^{13}C NMR (CD_3OD) δ 175.66, 175.33, 174.73, 174.61, 173.53, 173.25, 160.75, 74.06, 53.09, 52.81, 51.24, 48.66, 45.09, 43.63, 42.00, 41.64, 39.03, 36.87, 29.53, 27.08, 25.78, 24.07, 23.35, 22.50, 22.14, 22.04, 20.45; HRMS (FAB) calcd for $C_{28}H_{51}N_8O_{11}$: m/z 675.3677 (MH^+), found 675.3695.

***N*²-[*N*-[(2*S*,3*S*)-3-[[[(1*S*,3*S*)-3-(Acetylamino)-4-hydroxy-1-methyl-4-oxobutyl]amino]carbonyl]-2-hydroxy-5-methyl-1-oxohexyl]-L-leucyl]-L-arginine, Methyl Ester, 34.** A slurry of crude **33** (20 mg, 0.03 mmol) and 20% $Pd(OH)_2$ (2 mg, 0.003 mmol) in anhydrous MeOH (1.0 mL) was stirred under a hydrogen atmosphere for 17 h, filtered through a 0.2-μm nylon filter, and concentrated *in vacuo*. The residue was washed with hexanes (0.5 mL) to give 18 mg (100%) of **34**. ^1H NMR (CD_3OD) δ 4.48−4.19 (m, 3), 4.20 (d, 1, $J = 4.2$), 4.02 (m, 1), 3.70 (s, 3), 3.18 (m, 2), 2.66 (d, 1, $J = 13.9$), 2.62 (m, 1), 2.51 (d, 1, $J = 13.9$), 1.98 (s, 3), 1.99−1.44 (m, 9), 1.26 (m, 1), 1.15 (d, 3, $J = 6.6$), 0.98 (d, 3, $J = 6.4$), 0.94 (d, 3, $J = 6.4$), 0.88 (d, 3, $J = 6.9$), 0.87 (d, 3, $J = 6.9$); ^{13}C NMR (CD_3OD) δ 177.88, 175.31, 174.81, 174.64, 173.43, 172.75, 158.66, 74.12, 52.83, 52.74, 52.63, 49.85, 48.71, 45.35, 43.44, 41.86, 41.65, 39.36, 36.49, 29.59, 27.15, 26.22, 25.79, 24.09, 23.39, 22.80, 22.17, 22.06, 21.08; HRMS (FAB) calcd for $C_{28}H_{52}N_7O_9$: m/z 630.3827 (MH^+), found 630.3829.

[3a*S*-[2[*S[1*R**(2*R**,3*R**),3*R**]],3aα,4*β*,6*β*,7aα]]-*N*²-[*N*-[(2*S*,3*S*)-3-[[[(1*S*,3*S*)-3-(Acetylamino)-4-[[1-hexahydro-3a,5,5-trimethyl-4,6-methano-1,3,2-benzodioxaborol-2-yl)-2-phenylethyl]amino]-1-methyl-4-oxobutyl]amino]-carbonyl]-2-hydroxy-5-methyl-1-oxohexyl]-L-leucyl]-L-arginine, Methyl Ester, 35.** To a solution containing acid **34** (18 mg, 0.03 mmol) and the hydrochloride of amino boronate **12**[20−23] (11 mg, 0.03 mmol) in CH_2Cl_2 (0.9 mL) and DMF (0.1 mL) were added EDC (7 mg, 0.04 mmol), HOBT (5 mg, 0.04 mmol), and DIEA (6 μL, 0.03 mmol), and the mixture was stirred under N_2 at 25 °C for 16 h. The mixture was diluted with CH_2Cl_2, filtered through glass wool, and concentrated *in vacuo*. The crude product was washed with EtOAc (2 × 1 mL) and purified by reverse phase HPLC (gradient of 9:1 $H_2O/CH_3CN \rightarrow$ 9:1 CH_3CN/H_2O) to give 7 mg (29%) of the boronate ester **35**: ^1H NMR (CD_3OD) δ 9.69 (s, NH), 8.62 (d, $J = 7.3$, NH), 8.57 (d, $J = 6.8$, NH), 7.28−7.10 (m, 5), 4.35 (m, 1), 4.27 (m, 1), 4.15 (d, 1, $J = 8.6$), 4.08 (d, 1, $J = 9.0$), 4.07 (m, 1), 3.69 (s, 3), 3.07 (m, 2), 2.91−2.75 (m, 4), 2.39 (m, 1), 2.27 (m, 1), 1.93 (s, 3), 2.04−1.44 (m, 16), 1.34 (s, 3), 1.24 (s, 3), 1.14 (d, 3, $J = 6.4$), 1.09 (d, 1, $J = 10.2$), 0.95 (d, 3, $J = 6.4$), 0.92 (d, 3, $J = 6.4$), 0.89 (m, 6), 0.85 (s, 3); ^{13}C NMR (CD_3OD) δ 177.66, 175.26, 174.96 174.93, 173.43, 172.71, 158.6, 142.04, 130.20, 129.29, 127.10, 84.45, 77.44, 73.81, 53.51, 53.38, 53.28, 53.01, 52.72, 50.85, 49.50, 48.91, 42.79, 41.90, 41.80, 41.33, 39.16, 39.00, 38.27, 37.20, 29.67, 29.51, 27.73, 27.14, 26.87, 26.15, 25.64, 24.54, 24.44, 22.88, 22.85, 22.41, 21.90, 21.85; HRMS (FAB) calcd for $C_{46}H_{76}BN_8O_{10}$: m/z = 911.5777 (MH^+), found 911.5793.

Enzyme Assays. Inhibitor and buffer solutions were prepared using doubly distilled water and filtered through 0.45-μm nylon filters. Bovine α-chymotrypsin stock solutions were prepared in 0.1 M sodium acetate buffer, pH 5.0, containing 0.5 M NaCl, and stored at −20 °C. Dilutions were made with the pH 5.0 acetate buffer containing 0.2 mg/mL bovine serum albumin (BSA). Substrate (Suc-Ala-Ala-Pro-Phe-*p*-nitroanilide) solutions were made in 0.1 M HEPES buffer, pH 7.0, containing 0.5 M NaCl. The substrate concentration was determined from the absorbance at 315 nm (ϵ = 14000 cm^{-1} mol^{-1} L). Boronic acid inhibitor solutions were made by incubating the corresponding pinanediol ester in potassium phosphate buffer, pH 7.5, containing 0.5 M NaCl, for 1−2 h, and dilutions were made with the pH 7.0 HEPES buffer. Assays were conducted at 25 °C in the pH 7.0 HEPES buffer.

The initial rate of hydrolysis of Suc-Ala-Ala-Pro-Phe-*p*-nitroanilide was monitored by observing the formation of *p*-nitroaniline at 410 nm. Six substrate concentrations between 5 and 300 μM were used with several independent determinations carried out at each concentration to determine the K_m value of 24 μM. Initial rates and K_m values were calculated using the Enzfitter program.[41]

For K_i measurements, typically, ~0.8 nM α-chymotrypsin was equilibrated with the inhibitor for 10−15 min at 25 °C before initiation of the reaction with substrate. After substrate addition, 30 absorption points were recorded in a 10-min period, during which time linear kinetic behavior was observed and less than 10% reaction occurred. Initial rates (V_i) were determined at several inhibitor concentrations, [I], and the data were analyzed according to eqs 1 and 2 (V_0 is the initial rate in the absence of inhibitor). Competitive inhibition was demonstrated for inhibitor **7** (Figure 3) and assumed for the others, so the data were analyzed by fitting to straight lines using the least squares method.

$$\frac{V_0}{V_i} = 1 + \frac{[I]}{\left(1 + \frac{[S]}{K_m}\right)K_i} \quad (1)$$

$$K_i = \frac{1}{\frac{1}{\text{slope}}\left(1 + \frac{[S]}{K_m}\right)} \quad (2)$$

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Supporting Information Available: Experimental procedures and characterization of intermediates for syntheses of **8-NH$_2$**, **8-OH**, **8-H**, **9**, and **10**; kinetic plots for K_i determinations for **8-OH**, **8-H**, **9**, and **10**; ^{1}H and ^{13}C NMR spectra for compounds **11**−**35** (42 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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