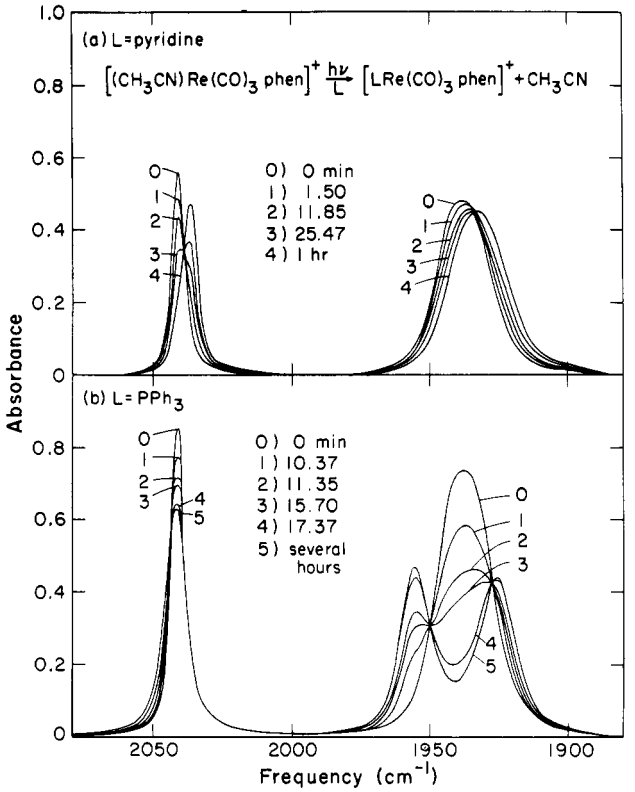

Figure 2. Infrared spectral changes accompanying light-induced formation of $[LRe(CO)_3phen]^+$ from $[(CH_3CN)Re(CO)_3phen]^+$ for (a) L = pyridine and (b) L = PPh_3.

relevance in imaging, since many metal complexes undergo significant optical spectral changes upon ligand substitution.

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(*R*)-1-Acetamido-2-phenylethaneboronic Acid. A Specific Transition-State Analogue for Chymotrypsin

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We report the synthesis of (*R*)-1-acetamido-2-phenylethaneboronic acid (**5a**), the boronic acid analogue of *N*-acetyl-L-phenylalanine, by the unambiguous route outlined in Scheme I, and its potent competitive inhibition of chymotrypsin, with a dissociation constant of 2.1×10^{-6} M at 25.0 °C and pH 7.5.

Aryl and arylalkylboronic acids bind strongly to the serine proteases chymotrypsin[1,2] and subtilisin.[2,3] The reason for this affinity is that the boronic acid group reversibly forms a tetrahedral adduct with the active site serine hydroxyl group, and the adduct crudely resembles the transition state for ester or amide hydrolysis.[2,4] It was anticipated that boronic acids corresponding to the specific amino acid substrates for these proteases would be even more potent inhibitors than the compounds tested to date, which only partially satisfy the specificity requirements of the enzymes. Previous attemtps to synthesize α-amino or α-amido boronic acids have been unsuccessful, except for the alkylated amino series $R_2NCH_2B(OR')_2$ and R_3N^+–$CH_2B(OR')_2$.[5,6] Esters and amides of *N*-acetyl-L-phenylalanine are specific substrates for chymotrypsin,[7] and the compound described herein provides the first example of a transition-state analogue of the boronic acid type corresponding to a specific substrate for a serine protease.

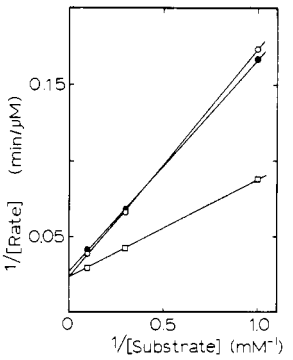
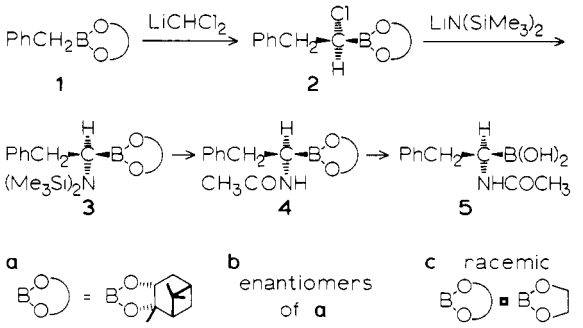


Figure 1. Inhibition of the chymotrypsin-catalyzed hydrolysis of methyl hippurate at pH 7.5 and 25.0 °C by 4×10^{-6} M (*R*)-1-acetamido-2-phenylethaneboronic acid (**5a**) (●) and 5×10^{-5} M *S* enantiomer (**5b**) (○). (□) Values obtained without inhibitor. The concentration of chymotrypsin, determined by active site titration of the stock solution,[23] was 2.8×10^{-6} M in each assay. The details of the assay are given in ref 1. Each rate of hydrolysis was constant for at least 5 min after initiation. The points give the averages of duplicate determinations, which agreed to within ±5%.

Scheme I



The recently reported homologation of ethylene glycol benzylboronate (**1c**) by (dichloromethyl)lithium to yield the 1-chloro-2-phenylethaneboronate (**2c**)[8] made this material easily available. The key to completion of the synthesis was the reaction of **2c** with lithiohexamethyldisilazane, which yielded 85% of the silylated amino boronic ester **3c**, a stable, distillable liquid that

(1) (a) Koehler, K. A.; Lienhard, G. E. *Biochemistry* **1971**, *10*, 2477–2483. (b) Rawn, J. D.; Lienhard, G. E. *Ibid.* **1974**, *13*, 3124–3130.
(2) Philipp, M.; Bender, M. L. *Proc. Natl. Acad. Sci. U.S.A.* **1971**, *68*, 478–480.
(3) Lindquist, R. N.; Terry, C. *Arch. Biochem. Biophys.* **1974**, *160*, 135–144.
(4) (a) Matthews, D. A.; Alden, R. A.; Birktoft, J. J.; Freer, S. T.; Kraut, J. *J. Biol. Chem.* **1975**, *250*, 7120–7126. (b) Robillard, G.; Shulman, R. G. *J. Mol. Biol.* **1974**, *86*, 541–558.
(5) Lindquist, R. N.; Nguyen, A. C. *J. Am. Chem. Soc.* **1977**, *99*, 6435–6437 reported the preparation of benzamidomethaneboronic acid and its inhibition of chymotrypsin. However, it now appears likely that the compound obtained by them was an isomer, $PhC(=NH)OCH_2B(OH)_2$ (probably with B–N chelation), from O-alkylation of the amide anion by the α-halo boronic ester, which has been observed by D.S.M. in analogous reactions. Their compound did not exhibit the expected pK of ~ 9 upon potentiometric titration.
(6) (a) Matteson, D. S.; Cheng, T. C. *J. Org. Chem.* **1968**, *33*, 3055–3060. (b) Matteson, D. S.; Jesthi, P. K. *J. Organomet. Chem.* **1976**, *114*, 1–7. (c) Matteson, D. S.; Majumdar, D. *Ibid.* **1979**, *170*, 259–264. (d) Matteson and Arne (Matteson, D. S.; Arne, K. *J. Am. Chem. Soc.* **1978**, *100*, 1325–1326) reported $PhCH_2CHBO_2C_2(CH_3)_4$, which with ammonia yields 2-phenylethylamine. Matteson, D. S., unpublished.
(7) (a) Blow, D. M. *Enzymes, 3rd Ed.* **1971**, *3*, 185–212. (b) Hess, G. P. *Ibid.* **1971**, *3*, 213–248.
(8) Matteson, D. S.; Majumdar, D. *J. Am. Chem. Soc.* **1980**, *102*, 7588–7590.

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was fully characterized.[9,10] Precedent could be cited for expecting dehydrohalogenation of **2c** by hindered base,[11] but there are other precedents for boron-assisted displacements by highly hindered nucleophiles.[8,11,12]

Desilylation of **3c** with methanol gave impure ethylene glycol 1-amino-2-phenylethaneboronate, $PhCH_2CH(NH_2)BO_2C_2H_4$, unstable to distillation.[13] Its isolation was bypassed by treating **3c** with acetic anhydride and acetic acid, which yielded 86% ethylene glycol 1-acetamido-2-phenylethaneboronate (**4c**),[10,14] a distillable solid, too water soluble to be extracted into ether.

The directed chiral synthesis of pinanediol α-chloro boronic esters[15] was used to make the separate enantiomers of 1-acetamido-2-phenylethaneboronic acid (**5a** and **5b**). Optically pure (+)-pinanediol benzylboronate (**1a**)[10,16] was homologated with (dichloromethyl)lithium[15] to **2a**, which was treated in situ with lithiohexamethyldisilazane followed by acetic anhydride and acetic acid to yield (+)-pinanediol (*R*)-1-acetamido-2-phenylethaneboronate (**4a**), 63% isolated by chromatography, recrystallized from dichloromethane to constant rotation.[10,17] Destructive cleavage of the pinanediol ester with boron trichloride[15] yielded (*R*)-1-acetamido-2-phenylethaneboronic acid (**5a**), which was characterized as its reversibly formed boronic anhydride.[10,18] The *S* enantiomers were similarly prepared.[19]

The affinity of chymotrypsin for the *R* and *S* isomers of 1-acetamido-2-phenylethaneboronic acid (**5a** and **5b**) was determined by examining the effects of these compounds on the rates of hydrolysis of methyl hippurate.[1] The reciprocal plots of initial velocity against substrate concentration given in Figure 1 show the pattern characteristic of competitive inhibition.[20] The competitive nature of the inhibition indicates that the compounds bind at the active site. The values of the dissociation constants, which were obtained from the data by standard equations,[20,21] are 2.1×10^{-6} M for the *R* isomer (**5a**) and 5.3×10^{-5} M (or greater if optical purity < 100%) for the *S* isomer (**5b**). The dissociation constant for 2-phenylethaneboronic acid, which lacks the acetamido substituent, was previously found to be 4×10^{-5} M.[1] The fact that (*R*)-1-acetamido-2-phenylethaneboronic acid (**5a**) binds the most tightly agrees with expectations[1] based upon the properties of the corresponding carbon compounds: L-phenylalanine derivatives are hydrolyzed by chymotrypsin much more rapidly than are 3-phenylpropionic acid and D-phenylalanine derivatives, although the three classes bind to the enzyme with about the same strength.[7,22] The affinity of chymotrypsin for the (*R*)-boronic acid **5a** is about 14 000 times greater than that for *N*-acetyl-L-phenylalanine amide.[7]

Acknowledgment. D.S.M. thanks the National Institutes of Health for financial support (Grant GM-27109).[24]

(9) One equivalent of **2c** was added to $LiN(SiMe_3)_2$ in THF at −78 °C, the mixture kept overnight at 20 °C, and the product distilled from LiCl; bp 103–104 °C (0.03 torr), ^1H NMR ($CDCl_3$) δ 0.15 (s, 18, $SiCH_3$), 2.95 (m, 3, CH_2CH), 4.23 (s, 4, OCH_2), 7.40 (s, 5, C_6H_5).

(10) Satisfactory analyses (±0.3%) were obtained for all elements except oxygen.

(11) (a) Matteson, D. S.; Mah, R. W. H. *J. Org. Chem.* **1963**, *28*, 2174–2176. (b) Reaction of $PhCH_2CHIBO_2C_2(CH_3)_4$[6d] with $LiN(COCH_3)_2$ yielded $PhCH{=}CHBO_2C_2(CH_3)_4$. Matteson, D. S., unpublished.

(12) Brown, H. C.; De Lue, N. R.; Yamamoto, Y.; Maruyama, K.; Kasahara, T.; Murahashi, S.; Sonoda, A. *J. Org. Chem.* **1977**, *42*, 4088–4092.

(13) Treatment of **3c** with methanol at 0 °C followed by vacuum concentration and washing the residue with ether gave impure $PhCH_2CH(NH_2)BO_2C_2H_4$, mp 143–149 °C. ^1H NMR in D_2O/CF_3CO_2H showed a single CH_2CH peak at δ 3.1, distinctly different from the multiplet of added $PhCH_2CH_2NH_2$.

(14) A THF solution of **3c** at −78 °C was treated with 3 equiv of acetic anhydride and 1 equiv of acetic acid, kept at 20 °C for 15 h, and distilled; bp 143–145 °C (0.03 torr), resublimed, mp 128 °C; H NMR ($CDCl_3$) δ 2.04 (s, 3, $COCH_3$), 2.75 (m, 3, CH_2CH, resolved to doublet and triplet by Eu(fod)$_3$), 3.90 (s, 4, OCH_2), 7.40 (s, 5, C_6H_5), 7.10, concentration dependent (br s, 1, N*H*); ^{13}C NMR consistent with assigned structure.

(15) Matteson, D. S.; Ray, R. *J. Am. Chem. Soc.* **1980**, *102*, 7590–7591.

(16) Bp 108 °C (0.1 torr); $[\alpha]^{22}_D$ + 31.30° (*c* 18, toluene); ^1H NMR consistent with assigned structure.

(17) Eluted from silica gel with ether, recrystallized three times (CH_2Cl_2), mp 185–186 °C; $[\alpha]^{20}_D$ −82.4° (*c* 3–5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 0.8–2.5 (m, 18, pinane + $COCH_3$), 2.9 (m, 3, $CHCH_2$), 4.32 (m, 1, OCH), 7.36 (s, 5, C_6H_5); ^{13}C NMR consistent with assigned structure.

(18) The mixture was concentrated under vacuum and the residue was washed with ether, treated with methanol, concentrated, dissolved in water, and neutralized with Dowex 1-X8 ion exchange resin bicarbonate, concentrated, and crystallized from THF/water, 80–85%; $[\alpha]^{22}_D$ −196° (*c* 0.6, H_2O) for the boronic anhydride; ^1H NMR (CD_3OD) δ 2.20 (s, 3, $COCH_3$), 2.82 (m, 3, CH_2CH), 5.2 (s, ~2.5, OH + NH), 7.40 (s, 5, C_6H_5); ^{13}C NMR (D_2O) δ 176.36 ($COCH_3$); 140.57, 128.68 (2), 128.55 (2), 128.55 (2), 126.21 (C_6H_5); 49.56 (br, CHNB); 36.18 ($PhCH_2$); 16.11 ($COCH_3$). Potentiometric titration showed 1 mol of boronic acid, pK = 8.85.

(19) **4b**: $[\alpha]^{22}_D$ + 83.3° (*c* 3, $CHCl_3$). **5b**: $[\alpha]^{22}_D$ + 195° (*c* 0.7, H_2O) for sample of composition $C_{10}H_{12}BNO_2{\cdot}0.25H_2O$.[10]

(20) Dixon, M.; Webb, E. C. "Enzymes", 3rd ed.; Academic Press: New York, 1979; pp 332–381.

(21) In the case of the *R* isomer, eq VIII.89 of ref 20 was used, since significant fractions of the added inhibitor (20–35%) are bound to the enzyme.

(22) Ingles, D. W.; Knowles, J. R. *Biochem. J.* **1968**, *108*, 561–569.

(23) Kezdy, F. J.; Clement, G. E.; Bender, M. L. *J. Am. Chem. Soc.* **1964**, *86*, 3690–3696.

(24) After completing this work, we learned of the interest of Dr. Manfred Philipp in boronic acids as enzyme inhibitors and sent him a sample of **5a**. He has found **5a** to be a competitive inhibitor of subtilisin, k_i(lim) = 1.7×10^{-6} M. We thank Dr. Philipp for informing us of these results: Philipp, M.; Sreenivasulu, M., manuscript submitted for publication.

Flavoprotein Monooxygenases: A Chemical Model

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Among the metabolic functions of the flavin-dependent monooxygenases[2] is the ortho hydroxylation of phenolic substrates such as salicylate and *p*-hydroxybenzoate. The unique position of the flavoproteins among biological hydroxylases follows from the reactivity of the metal-free, reduced isoalloxazine (dihydroflavin) nucleus with molecular oxygen. Evidence points to a subsequently formed 4α-hydroperoxide (**10**, Scheme II) as the molecular species responsible for, or leading to, flavin monooxygenase activity.[3] Herein we present a chemical model which suggests a flavin-based nitroxyl radical as the hydroxylating agent in the flavin monooxygenase ortho hydroxylation of phenolic substrates. Possible in vivo routes from the putative 4α-(hydroperoxy)flavin to an N^5-nitroxyl radical are discussed.

The flavin model work of Bruice et al.[4,5] has demonstrated the extremely facile oxidation of sulfides and amines and the dioxygenation of phenolate anions by 5-ethyl-3-methyl-4α-(hydroxyperoxy)lumiflavin. However, no flavin *mono*oxygenase model system has adequately explained flavin-mediated hydroxylation of phenols.[6] Our earlier work[7] with flavin N^5-oxide **1a** (Scheme I) showed the photolytic transfer of the N^5-oxygen

(1) Alfred P. Sloan Fellow, 1980–1982.

(2) (a) Walsh, C. *Acc. Chem. Res.* **1980**, *13*, 148. (b) Massey, V.; Hemmerich, P. *Enzymes, 3rd Ed.* **1976**, *12*, 191. (c) Hemmerich, P. *Fortschr. Chem. Org. Naturst.* **1976**, *33*, 451. (d) Dagley, S. In "Essays in Biochemistry", Campbell, P. N., Aldridge, W. N., Eds.; Academic Press: New York, 1975; Vol. 11, p 81. (e) Flashner, M. S.; Massey, V. In "Molecular Mechanisms of Oxygen Activation", Hayaishi, O., Ed.; Academic Press: New York, 1974; p 245.

(3) (a) Spector, T.; Massey, V. *J. Biol. Chem.* **1972**, *247*, 7123. (b) Strickland, S.; Massey, V. *Ibid.* **1973**, *248*, 2953. (c) Entsch, B.; Massey, V.; Ballou, D. P. *Biochem. Biophys. Res. Commun.* **1974**, *57*, 1018. (d) Entsch, B.; Ballou, D. P.; Massey, V. *J. Biol. Chem.* **1976**, *251*, 2550. (e) Kemal, C.; Bruice, T. C. *Proc. Natl. Acad. Sci. U.S.A.* **1976**, *73*, 995. (f) Presswood, R.; Kamin, H. In "Flavins and Flavoproteins, Proceedings of the Fifth International Symposium", Singer, T. P., Ed.; Elsevier: New York, 1976; p 145. (g) Ghisha, S.; Entsch, B.; Massey, V.; Husein, M. *Eur. J. Biochem.* **1977**, *76*, 139.

(4) (a) Ball, S.; Bruice, T. C. *J. Am. Chem. Soc.* **1979**, *101*, 4017. (b) *Ibid.* **1980**, *102*, 6498.

(5) (a) Kemal, C.; Bruice, T. C. *J. Am. Chem. Soc.* **1979**, *101*, 1635. (b) Bruice, T. C.; Muto, S. *Ibid.* **1980**, *102*, 4472. (c) Iwata, M.; Bruice, T. C.; Carrell, H. L.; Glusker, J. P. *Ibid.* **1980**, *102*, 5036.

(6) Bruice has suggested that flavoprotein monooxygenase activity may be explained by initial dioxygenation of phenolic substrates.[5a]

(7) Rastetter, W. H.; Gadek, T. R.; Tane, J. R.; Frost, J. W. *J. Am. Chem. Soc.* **1979**, *101*, 2228.

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