dynamics of the analogous spin-forbidden processes in iron(II) complexes is suggested by our recent studies[9] of the pressure dependence of the $^1A \rightleftharpoons {}^5T$ relaxation. We now plan to measure the corresponding activation volumes for spin relaxation in the present series of iron(III) complexes.

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## Phosphinic Acid Dipeptide Analogues: Potent, Slow-Binding Inhibitors of Aspartic Peptidases

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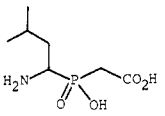
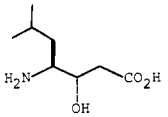
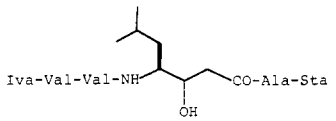
The invention of potent, specific inhibitors for the various classes of peptidases is a topic of intense biochemical as well as medicinal interest. For the zinc and serine peptidases, a number of effective strategies have been developed based on the ability of phosphorus-containing amino acid analogues to mimic the unstable tetrahedral intermediates involved in peptide hydrolysis.[1-3] We now describe a phosphorus-containing analogue (**1**) of the amino acid



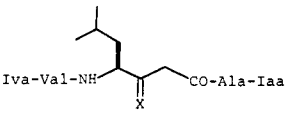
**1**



**2**



**3**



**4**: X = O

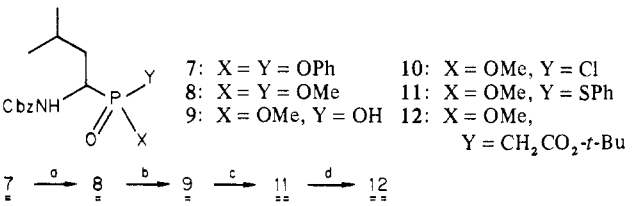
**5**: X = H, OH

**6**: X = HO, OH

statine and show that its incorporation into appropriate oligopeptide sequences affords a very tight, slow-binding inhibitor of the prototypical aspartic peptidase pepsin.

The hydroxymethylene group of statine (**2**) may mimic the tetrahedral intermediate that is involved in peptide hydrolysis by the aspartic peptidases, hence oligopeptides such as pepstatin (**3**) that incorporate this amino acid are considered to be transition state analogue inhibitors of these enzymes.[4] Of particular interest is the demonstration by Rich et al. that the keto analogue **4** is bound to the enzyme as a tetrahedral species,[5] possibly the *gem*-diol **6**. Since the equilibrium ketone $\rightleftharpoons$ hydrate lies far to the left, the binding energy available to the hydrate is in principle significantly greater than that represented by $K_i$ for the ketone.

Scheme I[a]



**7**: X = Y = OPh
**8**: X = Y = OMe
**9**: X = OMe, Y = OH
**10**: X = OMe, Y = Cl
**11**: X = OMe, Y = SPh
**12**: X = OMe, Y = $CH_2CO_2$-*t*-Bu

[a] (a) MeONa, MeOH, 22 °C (84%); (b) NaOH, MeOH, 22 °C (87%); (c) $SOCl_2$, $CH_2Cl_2$, 22 °C; PhSH, $Et_3N$, $CH_2Cl_2$, 22 °C (56%); (d) $LiCH_2CO_2$-*t*-Bu, THF, −78 °C → 0 °C.

**Table I.** Binding of Tripeptide Analogues to Pepsin

| inhibitor | $K_i$, μM |
|---|---|
| Iva-D-Sta$^P$-Ala-Iaa (**15A**) | 25[a] |
| Iva-L-Sta$^P$-Ala-Iaa (**15B**) | 0.9[b] |
| Iva-L-Sta-Ala-Iaa (**18**) | 0.35[c] |

[a] Determined at 37 °C at pH 3.5 (0.1 M NaOAc) with Z-His-*p*$NO_2$Phe-Phe-OMe as substrate. [b] Same as *a* but with Lys-Pro-Ala-Glu-Phe-*p*$NO_2$Phe-Arg-Leu also as substrate. [c] Reference 11.

It was this surmise that led us to prepare the phosphorus analogue **1**, to mimic the tetrahedral hydrate **6** and take advantage of its additional binding energy.

A suitably protected derivative of "phosphastatine" is prepared as depicted in Scheme I, starting with a phosphonate analogue of leucine, **7**.[6,7] The racemic diastereomers of **11** are readily separated and obtained in pure form by crystallization. Displacement of the phenylthio moiety from each of these diastereomers with *tert*-butyl lithioacetate proceeds stereospecifically and provides the two crystalline diastereomers of phosphinate **12**: **12A**, mp 113-114 °C; **12B**, mp 89-90 °C.

To evaluate the amino acid **1** as a mimic of the hydrate **6**, we incorporated the diastereomer **12A** into two oligopeptide sequences (see Scheme II).[7] Although the starting material **12A** is diastereomerically pure, it is racemic and therefore affords oligopeptides that incorporate both D- and L-enantiomers of phosphastatine.[8] These are differentiated in the course of the synthetic sequences by chromatographic separation of the diastereomers of **14** and **16**, respectively. All four phosphorus-containing peptides **15** and **17** are therefore available in stereochemically pure form.

The phosphorus-containing peptides were evaluated as inhibitors of porcine pepsin by using a spectroscopic assay and either Z-His-*p*$NO_2$Phe-Phe-OMe ($K_m$ = 0.5 mM, $k_{cat}$ = 0.29 $s^{-1}$) or the octapeptide substrate Lys-Pro-Ala-Glu-Phe-*p*$NO_2$Phe-Arg-Leu ($K_m$ = 50 μM, $k_{cat}$ = 100 $s^{-1}$).[10] Both diastereomers of the triamide **14** proved to be simple competitive inhibitors of porcine pepsin, with binding affinities comparable to those of the analogous statine-containing compound **18** (Table I). The behavior of the diastereomeric tetramides **17** is markedly different: one of the diastereomers (**17A**) is bound more weakly than either of the triamides, whereas the other (**17B**) is one of the most potent inhibitors of pepsin known. We assume that the more potent inhibitor in each pair is that with the L configuration.[8] The modest difference in binding affinity between the two diastereomers of **15**, in contrast to the differences observed between **17A** and **17B**, suggests that the isovaleramido moiety of **15A** can fit into the binding site occupied by the isobutyl side chain of inhibitors with

(1) (a) Bartlett, P. A.; Marlowe, C. K. *Biochemistry* **1983**, *22*, 4618-4624. (b) Jacobsen, N. E.; Bartlett, P. A. *J. Am. Chem. Soc.* **1981**, *103*, 654-657. Jacobsen, N. E.; Bartlett, P. A. *ACS Symp. Ser.* **1981**, *171*, 221.

(2) Lamden, L. A.; Bartlett, P. A. *Biochem. Biophys. Res. Commun.* **1983**, *112*, 1085 and references cited therein.

(3) Weaver, L. H.; Kester, W. R.; Matthews, B. W. *J. Mol. Biol.* **1977**, *114*, 119-132. Kam, C.-M.; Nishino, N.; Powers, J. C. *Biochemistry* **1979**, *18*, 3032-3038. Nishino, N.; Powers, J. C. *Ibid.* **1979**, *18*, 4340-4347. Petrillo, E. W., Jr.; Ondetti, M. A. *Med. Res. Rev.* **1982**, *2*, 1-41. Thorsett, E. D.; Harris, E. E.; Peterson, E. R.; Greenlee, W. J.; Patchett, A. A.; Ulm, E. H.; Vassil, T. C. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 2176-2180. Galardy, R. E. *Biochemistry* **1982**, *21*, 5777-5781. Galardy, R. E.; Kontoyiannidou-Ostrem, V.; Kortylewicz, Z. P. *Ibid.* **1983**, *22*, 1990-1995. Monzingo, A. F.; Tronrud, D. E.; Matthews, B. W., personal communication.

(4) Marciniszyn, J., Jr.; Hartsuck, J. A.; Tang, J. *J. Biol. Chem.* **1976**, *251*, 7088-1094. Bott, R.; Subramanian, E.; Davies, D. R. *Biochemistry* **1982**, *21*, 6956-6962.

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(6) Oleksyszen, J.; Subotkowska, L.; Mastalerz, P. *Synthesis* **1979**, 985.

(7) All new compounds were fully characterized by high-field $^1H$ and $^{31}P$ NMR; satisfactory combustion analyses were obtained for all neutral compounds.

(8) We have chosen to use the D and L descriptors for the phosphastatine enantiomers to facilitate comparison with the natural amino acids and with statine itself; the L enantiomer of phosphastatine has the *R* configuration at the stereocenter α to phosphorus. The configurations of the phosphastatine moieties in the two series were correlated by separation of the diastereomers of **13** and formation of both **14A** and **16A** from one of them.

(9) Bartlett, P. A.; Johnson, W. S. *Tetrahedron Lett.* **1970**, 4459-4462.

(10) Dunn, B. M.; deLucy, P.; Magazine, H.; Parten, B.; Jimenez, M. In "Peptides: Structure and Function"; Hruby, V. J., Ed.; Pierce Chemical Co.: Rockford, IL, 1984.

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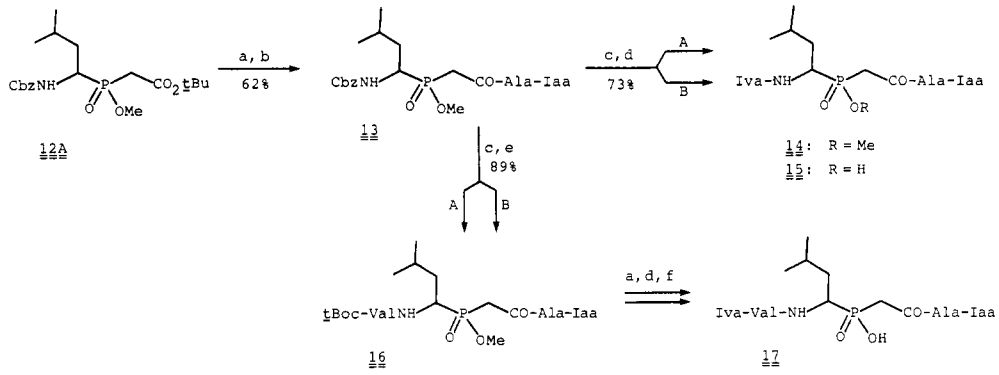
Scheme II[a]




[a] (a) TFA, 21 °C; (b) Ala-Iaa, HOBT, DCC, $CH_2Cl_2$, 21 °C; (c) TsOH, $H_2$/Pd-C, MeOH, 21 °C; (d) IvaOH, DCC, $Et_3N$, $CH_2Cl_2$/DMF, 21 °C; (e) *t*-Boc-ValOH, DCC, $Et_3N$, DMF, $CH_2Cl_2$/DMF, 21 °C; (f) LiS-*n*-Pr, HMPA (ref 9).

**Table II.** Binding of Tetrapeptide Analogues to Pepsin

| inhibitor | $K_D$, nM | $t_{1/2}$ (→) | $K_i$, nM |
|---|---|---|---|
| Iva-Val-D-Sta$^P$-Ala-Iaa (**17A**) | *d* | | 200[a] |
| Iva-Val-L-Sta$^P$-Ala-Iaa (**17B**) | 7 | 115 min | <0.07[b] |
| Iva-Val-L-Sta-Ala-Iaa (**5**) | 60 | <10 s | 1.1[c] |
| Iva-Val-L-Sto-Ala-Iaa (**4**) | *d* | | 56[e] |
| Iva-Val-Val-L-Sta-Ala-Sta (Pepstatin, **3**) | 13 | ~30 s | 0.046[f] |

[a] Determined at 37 °C at pH 3.5 (0.1 M NaOAc) with Z-His-*p*$NO_2$Phe-Phe-OMe as substrate. [b] As *a* with Lys-Pro-Ala-Glu-Phe-*p*$NO_2$Phe-Arg-Leu as substrate (ref 10). [c] Reference 11. [d] Slow binding not observed. [e] Reference 4. [f] Reference 12.

the L configuration; in contrast, the longer IvaValNH moiety appears to be restricted to one extended pocket.

The inhibition observed with **17B** is not of the simple competitive type, however, as a marked increase in the degree of inhibition occurs during the course of a 10- or 15-min assay. Such behavior is frequently observed with tightly-bound inhibitors of pepsin and has been well-characterized by Rich and his co-workers as involving the two-stage association sequence represented by eq 1.[11]

$$E + I \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E{\cdot}I \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E{\cdot}I^* \quad (1)$$

$$K_D = k_2/k_1 \quad (2)$$

$$K_i = \frac{k_2}{k_1}\frac{k_4}{(k_3 + k_4)} \quad (3)$$

From the chemical nature of the phosphinic acid moiety, we expect that **17B** is binding to the enzyme in a reversible and noncovalent fashion. Accurate determination of its binding affinity is not straightforward, however. The binding constant $K_D$ for the "loose" complex E·I can be readily determined by steady-state methods in the case of inhibitor **17B**, since the rate of isomerization to the "tightened" complex is very slow ($t_{1/2} \approx 2$ h). By treatment of the isomerization of E·I ⇌ E·I* as an irreversible process, it is possible to determine a value for $k_3$ of 0.36 $h^{-1}$ ($t_{1/2}$ = 115 min) at 37 °C and a ratio of $k_4/k_3 < 0.01$.[13] If the binding process is in fact reversible, the overall $K_i$ must therefore be less than 70 pM.

Although an accurate determination of $k_4$ (and thus $K_i$) is not possible without radiolabeled material, it is clear that the phosphinate **17B** is an exceedingly potent inhibitor of pepsin, approaching the affinity of pepstatin itself (Table II). The use of phosphorus analogues to mimic tetrahedral intermediates therefore appears to be an effective strategy for inhibition of the aspartic peptidases as well as the zinc and serine peptidases. We hope to be able to extend these results to related enzymes of this class and to probe the nature of the exceedingly slow-binding transition.

**Acknowledgment.** We thank Professor B. M. Dunn and Dr. Robert M. Scarborough for generous gifts of the octapeptide substrate and Professor D. H. Rich for helpful discussions. Support for this research was provided by a grant from the National Institutes of Health (CA-22747).

**Supplementary Material Available:** Experimental procedures for the preparation of compounds **8–17** and description of inhibitor assay procedures (12 pages). Ordering information is given on any current masthead page.

(11) Rich, D. H.; Sun, E. T. O. *Biochem. Pharmacol.* **1980**, *29*, 2205–2212. Rich, D. H.; Sun, E. T. O.; Ulm, E. *J. Med. Chem.* **1980**, *23*, 27–33. Rich, D. H.; Bernatowicz, P. G. *Ibid.* **1982**, *25*, 791.

(12) Workman, R. J.; Burkitt, D. W. *Arch. Biochem. Biophys.* **1979**, *194*, 157–164.

(13) On incubation of the enzyme at 50 nM and inhibitor **17B** at 100 nm (=14$K_D$) concentrations, the enzyme is present initially as E·I, isomerizing with time to an equilibrium mixture of E·I and E·I*. The extent of isomerization can be determined by removing aliquots and diluting them 100-fold into excess octapeptide substrate at 250 μM (=5$K_m$) concentration. E·I dissociates relatively rapidly, whereas E·I* does not, hence the enzymatic activity observed in the diluted aliquot reflects how much of the enzyme has *not* been transformed into E·I*. After 30 h, when equilibrium has been reached between E·I and E·I*, less than 1% of the control activity recovers on dilution, suggesting that $k_4/(k_3 + k_4) \approx k_4/k_3 < 0.01$.

## Iron(II)-Induced Activation of Hydrogen Peroxide to Ferryl Ion ($FeO^{2+}$) and Singlet Oxygen ($^1O_2$) in Acetonitrile: Monoxygenations, Dehydrogenations, and Dioxygenations of Organic Substrates

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Although activation of $H_2O_2$ by iron(II) (Fenton chemistry) has been thoroughly characterized in aqueous media[1] and shown

$$Fe(II) + H_2O_2 \xrightarrow{H_2O} Fe^{III}(OH^-) + {\cdot}OH \quad (1)$$

to have substrate reactions that are identical with those for hydroxyl radical (·OH),[1,2] the nature of this system in an anhydrous, noncomplexing solvent has not been evaluated. Here we report that the slow addition of dilute $H_2O_2$ (in dry acetonitrile (MeCN)) to a solution that contains iron(II) and an organic substrate (RH) in dry MeCN (<0.005% $H_2O$) results in the monooxygenation or dehydrogenation of RH. Table I compares the products that result from the Fe(II)–$H_2O_2$–RH/MeCN system with those from

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(2) Dorfman, L. M.; Adams, G. E. "Reactivity of the Hydroxyl Radical in Aqueous Solutions"; NSRDS-NBS 46, SD Catalog No. 13.48:46, U.S. Department Printing Office: Washington, DC; June, 1978.

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