acceleration factor may be obtainable with a shorter, less flexible tether between the catalytic group and the binding pocket.

These experiments are a first step toward the development of selective catalysts which combine the high binding affinity and specificity of the immune system with the diverse, efficient catalytic groups available from synthetic chemistry.

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Supplementary Material Available: The synthesis and characterization of compounds **1a–d** (2 pages). Ordering information is given on any current masthead page.

(19) Sodium acetate (0.1 M) was used as the buffer in the range of pH 4.5–6.0, morpholineethanesulfonic acid (0.1 M) in the range of pH 5.0–7.0, sodium phosphate (0.1 M) in the range of pH 6.0–8.0 and tris·HCl (0.1 M) in the range of pH 7–9. These experiments were carried out at 30 °C in the presence of 1 μM modified antibody and 20 μM ester **1b**.

^{13}C NMR Evidence for an Enzyme-Induced Lossen Rearrangement in the Mechanism-Based Inactivation of α-Chymotrypsin by 3-Benzyl-*N*-((methylsulfonyl)oxy)succinimide

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There has been considerable interest in recent years in the development of mechanism-based inhibitors and their subsequent utilization as enzyme probes and as potential therapeutic agents.[1,2] A novel type of mechanism-based inhibitor is one that generates an electrophilic species via an enzyme-induced *rearrangement*. The one and only example reported so far[3] involves an enzyme-induced allyl sulfoxide–allyl sulfenate ester 2,3-sigmatropic rearrangement. We now present evidence that 3-benzyl-*N*-((methylsulfonyl)oxy)succinimide **1** and related compounds[4] inactivate α-chymotrypsin and human leukocyte elastase (HLE), an enzyme of considerable clinical interest,[5,6] via an enzyme-induced Lossen rearrangement and according to the mechanism depicted in Scheme I.[7]

In earlier biochemical studies[8] we demonstrated that compound **1** is a time-dependent irreversible inactivator of α-chymotrypsin and HLE and that the inactivation involves the active site. The chemical competence of the steps shown in Scheme I was also established. Thus, reaction of equivalent amounts of **1** and $NaOCH_3/CH_3OH$ (room temperature/1 h) resulted in the formation of a mixture of two isomeric Lossen rearrangement products.[4] Furthermore, amino acid-derived isocyanates such as, L-norvaline methyl ester isocyanate, for example, have been shown to inactivate HLE and chymotrypsin rapidly and irreversibly.[9,10]

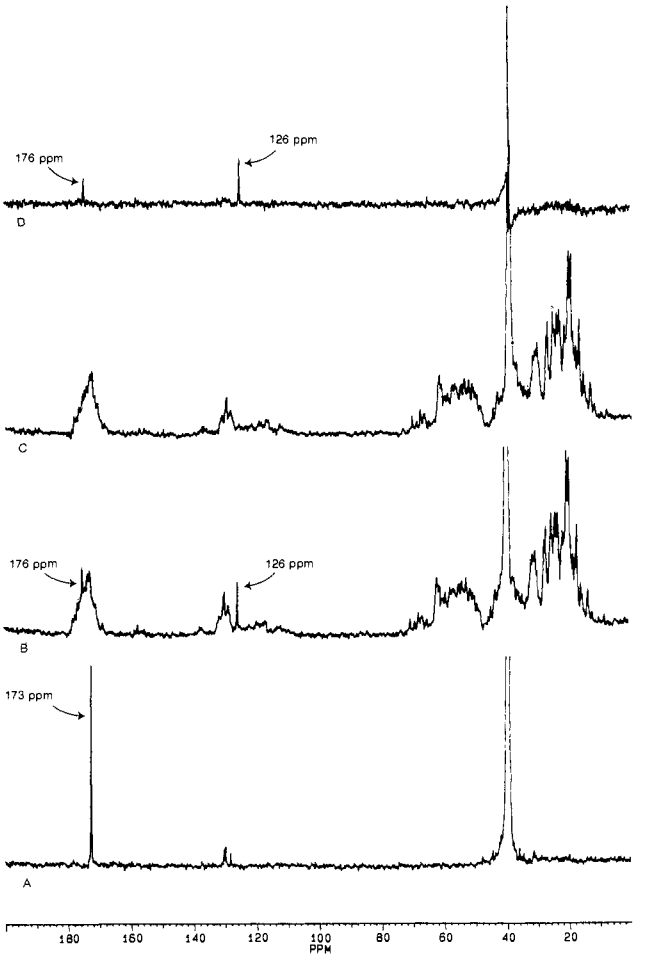
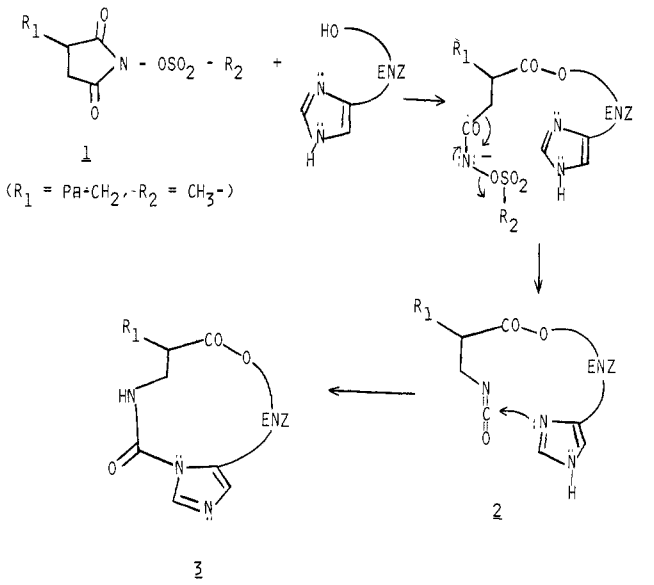


Figure 1. ^{13}C NMR spectra of labeled **1** and chymotrypsin. A: 2 mM **1** in D_2O (7.5% DMSO); B: 2 mM **1** plus 2 mM chymotrypsin in D_2O (7.5% DMSO); C: 2 mM unlabeled **1** plus 2 mM chymotrypsin in D_2O (7.5% DMSO); D: difference spectrum of B and C. All spectra were run on a Bruker 500 MHz instrument using the following conditions: 54° pulse, 0.6 s repetition period, 14 000 scans, broad band ^1H decoupling, and 20 Hz line broadening. In all spectra the large multiplet at 39.5 ppm is due to DMSO.

Scheme I



(1) Silverman, R. B. *Mechanism-Based Inactivation: Chemistry and Enzymology*; CRC Press, Inc.: Boca Raton, FL, 1988; Vol. 1 and 2.
(2) Silverman, R. B. *J. Enz. Inh.* **1988**, *2*, 73.
(3) Johnston, M.; Raines, R.; Walsh, C.; Firestone, R. *J. Am. Chem. Soc.* **1980**, *102*, 4241.
(4) Groutas, W. C.; Brubaker, M. J.; Stanga, M. A.; Castrisos, J. C.; Crowley, J. P.; Schatz, E. J., submitted for publication.
(5) Groutas, W. C. *Med. Res. Rev.* **1987**, *7*, 227.
(6) *Pulmonary Emphysema and Proteolysis*; Taylor, J. C., Mittman, C., Eds.; Academic Press: FL, 1987.
(7) See, also: (a) Fischer, G.; Demuth, H.-U.; Barth, A. *Pharmazie* **1983**, *38*, 249. (b) Demuth, H. U.; Baumgrass, R.; Schaper, C.; Fischer, G.; Barth, A. *J. Enz. Inh.* **1988**, *2*, 129. We thank Professor Demuth for bringing ref 7a to our attention.
(8) Groutas, W. C.; Giri, P. K.; Crowley, J. P.; Castrisos, J. C.; Brubaker, M. J. *Biochem. Biophys. Res. Commun.* **1986**, *141*, 741.

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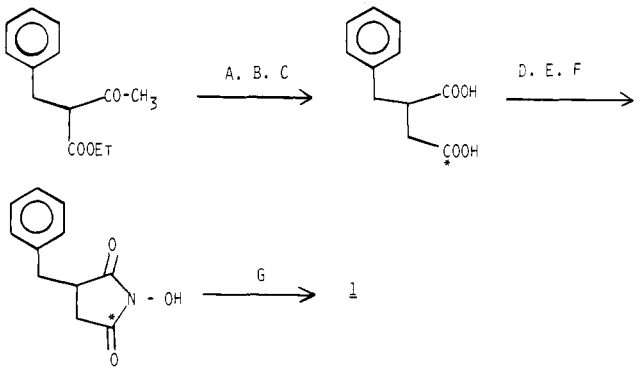
Scheme II. Synthesis of Compound **1**[a]



[a] (a) $NaOEt/EtOH$; (b) $Br\text{-}CH_2\text{-}^*COOEt$; (c) $KOH/EtOH/H_2O$; (d) Ac_2O/heat; (e) $PHCH_2ONH_2$/toluene/heat; (f) 10% Pd-C/H_2/THF; (g) CH_3SO_2Cl/pyridine.

In order to obtain direct evidence in support of the proposed tentative mechanism of Scheme I, high resolution ^{13}C NMR was utilized.[11] Thus, compound **1**, labeled at C-5 (99%), was synthesized according to Scheme II.[8] Incubation of equivalent amounts of **1** with α-chymotrypsin led to the appearance of two new signals at 176 and 126 ppm (Figure 1 (parts B and D)). The ^{13}C NMR spectrum of the inhibitor shows a peak at 173 ppm in the *same* solvent system (Figure 1A). The signal at 176 ppm is interpreted to arise from enzyme-inhibitor adduct **3**, while the signal at 126 ppm arises from an enzyme-generated isocyanate. It appears that the sharp signal at 126 ppm is due to *free* isocyanate, formed by deacylation of intermediate **2** (Scheme I). This assignment is supported by the fact that incubation of chymotrypsin or HLE with unlabeled inhibitor **1** in the presence of an external nucleophile results in partial protection of the enzyme.[8] Futhremore, imidazole-*N*-carboxamides and isocyanates give rise to signals at around 170 and 126 ppm, respectively. For example, the signal for the imidazole-*N*-carboxamide obtained from the reaction of ethyl 3-isocyanatopropionate with imidazole appears at 171 ppm (DMSO-d_6), while the signal of the isocyanate carbon of L-norvaline methyl ester isocyanate appears at 126.5 ppm.

The spectrum of the 1 mM solution of chymotrypsin in D_2O shows, among other signals, signals at 129-132 ppm. Hence, the signals appearing at 129-132 ppm in Figure 1 (parts B and C) are due to the enzyme.

In order to eliminate the likelihood of any extraneous interferences, the spectrum of the enzyme with unlabeled **1** was also recorded under identical conditions (Figure 1C). Lastly, inhibitor **1** is stable indefintely under the conditions used to record the NMR spectra (as monitored by HPLC).

In summary, the chemical shift data presented establish unequivocally that inhibitor **1** is a novel type of mechanism-based inhibitor that inactivates chymotrypsin and other serine proteases via an enzyme-induced Lossen rearrangement. The data also validate the biochemical rationale involved in the design of this class of inhibitors.[8]

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(9) (a) Groutas, W. C.; Abrams, W. R.; Theodorakis, M. C.; Kasper, A. M.; Rude, S. A.; Badger, R. C.; Ocain, T. D.; Miller, K. E.; Moi, M. K.; Brubaker, M. J.; Davis, K. S.; Zandler, M. E. *J. Med. Chem.* **1985**, *28*, 204. (b) Groutas, W. C.; Brubaker, M. J.; Zandler, M. E.; Mazo-Gray, V. Rude, S. A.; Crowley, J. P.; Castrisos, J. C.; Dunshee, D. A.; Giri, P. K. *Ibid.* **1986**, *29*, 1302.

(10) The inhibition of serine proteases by aliphatic isocyanates has been repported by Brown and Wold: *Biochemistry* **1973**, *12*, 828, 835.

(11) Mackenzie, N. E.; Matlhouse, J. P. G.; Scott, A. I. *Science* **1984**, *225*, 883.

(12) The appearance of the isocyanate signal at 126 ppm (Figures 1, part B) is somewhat surprising, considering the high hydrolytic instability of isocyanates. We have observed that the admixture of L-norvaline methyl ester isocyanate with water (7% DMSO) does result in appreciable hydrolysis of the isocyanate; nevertheless, a residual amount of isocyanate can be readily detected by infrared spectroscopy hours after mixing. See also ref 10.

Flash Photolysis Studies of $RhCl(CO)L_2$ (L = Trimethyl- or Tritolylphosphine). Evidence for Intermediates in the Photocatalytic Carbonylation of Hydrocarbons[1]

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Recently, Kunin and Eisenberg[2] then Tanaka[3] and others[4] have reported that various *trans*-$RhCl(CO)L_2$ (L = a trialkyl- or triarylphosphine) serve as photocatalysts for carbonylation and other C-H activation pathways of certain hydrocarbons (e.g., eq 1). Of these the trialkylphosphine complexes have been shown

$$\text{Ph-H} + \text{CO} \xrightarrow[\text{RhCl(CO)L}_2]{h\nu} \text{Ph-CHO} \qquad (1)$$

to be effective even for alkane activation.[3,4] Herein are reported results of the flash photolysis investigation of two representative complexes, *trans*-$RhCl(CO)(PMe_3)_2$ (I) and *trans*-$RhCl(CO)(P(tolyl)_3)_2$ (II, tolyl = p-$CH_3C_6H_4$-). In benzene under argon, I and II each gave transients with spectral properties and kinetics behavior implying photoinduced CO dissociation followed by reversible insertion of the tricoordinate intermediate into the solvent C-H bond. In cyclohexane only I showed such behavior. These observations contrast sharply with those described previously for the case where L = PPh_3 (III),[5] for which the initial transients formed under analogous flash photolysis conditions do not undergo observable reaction with benzene.

Flash photolysis ($\lambda_{irr} > 330$ nm) of I in deaerated benzene solution under argon[6] led to the immediate formation[7] of a transient (A) with increased absorption in the spectral region 400-500 nm. This species decayed exponentially ($k_a = (6.2 \pm 2.0) \times 10^3\ s^{-1}$) to a second species with a smaller absorbance than I over the same spectral region. Finally, this bleached transient (B) underwent slow, first-order decay to the initial spectrum with $k_b = (3.8 \pm 0.6) \times 10^{-2}\ s^{-1}$. Under these conditions, analogous temporal spectral changes were observed for flash photolysis of II with the exceptions that $k_a((5.9 \pm 1.5) \times 10^2\ s^{-1})$ proved to be an order of magnitude smaller and $k_b(4.4 \pm 0.8\ s^{-1})$ two orders of magnitude faster.

In contrast, the behaviors of the two systems differed markedly when flashed in deaerated cyclohexane. For I the sequential formation of absorbing and bleached transients were again seen,

(1) Reported in part at the International Conference on Organometallic Chemistry Turin, Italy, September 1988, at the 196th National American Chemical Society Meeting, Los Angeles, CA, September 1988, and at the 1987 Pacific Conference on Chemistry and Spectroscopy, Irvine, CA, October 1987.

(2) Kunin, A. J.; Eisenberg, R. *J. Am. Chem. Soc.* **1986**, *108*, 535-536.

(3) (a) Sakakura, T.; Tanaka, M. *Chem. Lett.* **1987**, 249-252; 1113-1116; *Chem. Soc., Chem. Commun.* **1987**, 758-759. (b) Sakakura, T.; Sasaki, K.; Tokunaga, Y.; Wada, K.; Tanaka, M. *Chem. Lett.* **1988**, 155-158.

(4) Nomura, K.; Saito, Y. *J. Chem. Soc., Chem. Commun.* **1988**, 161-162.

(5) (a) Wink, D. A.; Ford, P. C. *J. Am. Chem. Soc.* **1985**, *107*, 1794-1796. (b) Wink, D. A; Ford, P. C. *J. Am. Chem. Soc.* **1986**, *108*, 4838-4842. (c) Wink, D. A.; Ford, P. C. *J. Am. Chem. Soc.* **1987**, *109*, 436-442.

(6) (a) Extensive continuous photolysis of II under the same conditions shows no net photodecomposition of the metal complex. (b) The flash photolysis apparatus is that described previously (ref 5c). A methyl ethyl ketone solution was used as a UV-vis filter. All solvents used were scrupulously deaerated by freeze/pump/thaw cycles and dried by distillation from a Na/K amalgam. All solutions were prepared by vacuum manifold techniques.

(7) The formation of A has been studied by picosecond flash photolysis in the laboratory of T. L. Netzel of Amoco Research Corp. (Netzel, T. L.; Pourreau, D. B., manuscript in preparation. Netzel, T., private communication). These studies demonstrated that the decay of excited states and/or other intermediates to give A occurs on a subnanosecond time scale.

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