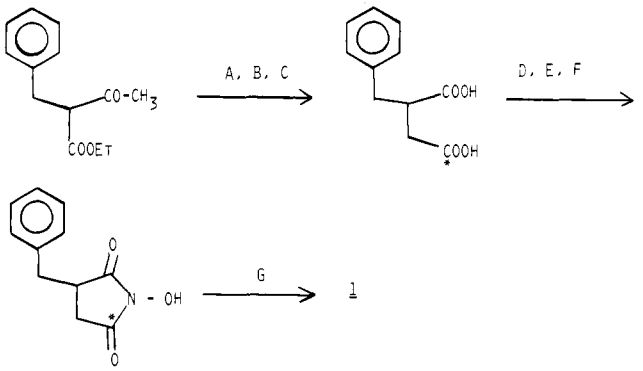
Scheme II. Synthesis of Compound 1[a]



[a] (a) NaOEt/EtOH; (b) Br-CH_2-*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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(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.

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(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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the respective rate constants being $k_a = (3.3 \pm 0.8) \times 10^3\ s^{-1}$ and $k_b = (3.6 \pm 0.4) \times 10^{-2}\ s^{-1}$. However, for II, A decayed by second-order kinetics to give another long-lived transient C which is absorbing relative to II at $\lambda > 425$ nm and underwent slow first-order decay to regenerate the initial spectrum.

These observations can be compared to those for III (L = PPh_3).[5] In benzene III was shown[5] to undergo CO photodissociation (eq 2) to give A, which undergoes second-order recombination with CO (eq 3) and competing dimerization to $[RhClL_2]_2$.

$$trans\text{-}RhCl(CO)L_2 \xrightarrow{h\nu} \underset{A}{RhClL_2} + CO \qquad (2)$$

$$A \xrightarrow{k_{CO}[CO]} trans\text{-}RhCl(CO)L_2 \qquad A \xrightarrow{+A} [RhClL_2]_2 \qquad (3)$$

The dimer displayed spectral properties and kinetics similar to those noted above for C. No bleached transient was observed except under H_2, in which case, the formation of the Rh(III) dihydride H_2RhClL_2 was concluded on the basis of spectral properties plus dihydrogen concentration and deuterium isotope effects on the reaction kinetics.

These observations and comparisons indicate that flash photolysis of I or II leads first to CO dissociation (eq 2). This view is reinforced by the observation that when I was flashed in benzene under 1% CO (the balance being argon), the disappearance of A was markedly accelerated ($k_{obsd} = (1.8 \pm 0.5) \times 10^4\ s^{-1}$),[8] but formation of B was about 35% that seen in the absence of CO. However, the decay rate of B back to I was unaffected. Similarly, for II in benzene, added CO accelerated the disappearance of A and suppressed formation of B. In contrast, there was little effect of adding $P(tolyl)_3$ (10^{-3} mol L^{-1}).

Formation of the bleached transient B is consistent with reaction of the highly unsaturated "tricoordinate" 14-electron species A[9] with the hydrocarbon solvent to form Rh(III) alkyl or aryl halide species (eq 4),[10] which are logical intermediates for photocatalytic

$$RhClL_2 + R\text{—}H \xrightarrow{k_a} \begin{matrix} R \\ \diagdown \\ H \end{matrix}\!\!>\!RhClL_2 \qquad (4)$$

functionalization of hydrocarbons above). For L = $P(tolyl)_3$, oxidative addition of benzene to the photogenerated intermediate A is slower and correspondingly B is less stable than for L = PMe_3, both observations consistent with the lower basicity and greater steric bulk of the triarylphosphine ligand. The same factors are consistent with the apparent failure of $RhCl(P(tolyl)_3)_2$ to react with cyclohexane. Remarkably, $P(tolyl)_3$ is enough more electron donating than PPh_3 that oxidative addition of solvent to A (eq 4) is the predominant decay pathway in benzene for L = $P(tolyl)_3$, while eq 3 is predominant for L = PPh_3 under analogous conditions. However, the differences may be rather subtle given that photocatalyzed carbonylation of benzene has also been observed to be a minor pathway in the photolysis of III.[2]

In summary, the flash photolysis of the Rh(I) complexes *trans*-$RhCl(CO)L_2$ (L = PMe_3, $P(tolyl)_3$, or PPh_3) leads to the following observations. (a) In each case, photodissociation of CO occurs to give the tricoordinate intermediate $RhClL_2$. (b) This species undergoes rapid recombination with CO, but, for L = PMe_3, it reacts with either solvent cyclohexane or solvent benzene via C–H activation at rates sufficient to compete with the second-order trapping by the low concentrations of CO generated in the flash.[11] (c) For L = $P(tolyl)_3$, similar, but slower, reaction with solvent is also the predominant decay pathway for A in benzene under these conditions; however, analogous reaction with cyclohexane was not seen. (d) The substituent effect of the *p*-Me group of $P(tolyl)_3$ is apparently sufficient to make C–H activation the predominant reaction of A in benzene. In contrast, the predominant reactions of this intermediate for the PPh_3 complex are second-order recombination with photogenerated CO plus competing dimerization. Continuing studies here regarding Rh(I) complexes are concerned with quantitatively characterizing ligand substituent effects on the photocatalyzed C–H activation of various substrates as well as examining the dynamics of the reactive intermediates formed by such reactions.

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(8) Given the solubility of CO in benzene (6.9×10^{-3} mol L^{-1} atm^{-1}, ref 5a), this k_{obsd} allows the estimate of a k_{CO} value of about 2×10^8 L mol^{-1} s^{-1} for the tricoordinate species $RhCl(PMe_3)_2$. This value is consistent with those measured for other $MClL_2$ (M = Ir or Rh) (ref 5).

(9) In this paper we will follow the practice as referring to the reactive $RhClL_2$ intermediate as being tricoordinate, although it seems likely that the vacant coordination site may be occupied by a weakly coordinating solvent molecule.

(10) The spectral and kinetic behavior of the $RhClL_2$ intermediates (L = PMe_3 or $P(tolyl)_3$) would also be consistent with the formation of orthometalated phosphine ligand complexes. However, this appears to be an unlikely explanation given that the $P(tolyl)_3$ complex shows distinctly different behavior in benzene and in cyclohexane solutions. If orthometalation were the principal reaction of the tricoordinate complexes, the solvent would not be expected to have such an effect.

(11) (a) Notably, the intermediate generated by photodissociation of CO from *trans*-$RhCl(CO)(PMe_3)_2$ appears to be relatively unselective toward reaction with either cyclohexane or benzene. Earlier studies by Bergman and co-workers (ref 11b) suggested a similar low selectivity for the proposed intermediate $Ir(\eta^5\text{-}C_5Me_5)(PMe_3)$ but a significantly higher ratio of the reactivity toward a phenyl C–H bond vs a secondary C–H of an alkane has been suggested (ref 11c) for the proposed intermediate $Re(\eta^5\text{-}C_5H_5)(PPh_3)H_2$. (b) Buchanan, J. M.; Stryker, J. M.; Bergman, R. G. *J. Am. Chem. Soc.* **1986**, *108*, 1537-1550. (c) Jones, W. D.; Maguire, J. A. *Organometallics* **1986**, *5*, 590-591.

Enzymatic Peptidyl α-Amidation Proceeds through Formation of an α-Hydroxyglycine Intermediate

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The peptidyl α-amidating enzyme plays a critical role in the posttranslational bioactivation of many peptide hormones by catalyzing the oxidation of a carboxyl terminal glycine-extended precursor to yield both an α-amidated peptide and glyoxylic acid.[1] The mechanism of this N–C bond cleavage, which requires enzyme-bound copper ions, L-ascorbate, and molecular oxygen, is unknown; however, formation of a carbinolamine intermediate has been suggested.[2] Carbinolamine formation could occur either directly, as favored for dopamine-β-hydroxylase (DBM)[3] and cytochrome P450 catalyzed N-dealkylations,[4] or indirectly from hydrolysis of an imine-type intermediate.[1a] The capacity of a homogeneous α-amidating enzyme to convert either the model substrate *N*-dansyl-Tyr-Val-Gly (**1**) or its *N*- and α-hydroxyglycine analogues to *N*-dansyl-Tyr-Val-NH_2 (**2**) was examined in this

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