of dimensions $0.28 \times 0.13 \times 0.07$ mm was used. A total of 6403 independent reflections were measured on a Siemens P4/PC diffractometer with graphite-monochromated $Cu_{K\alpha}$ radiation using ω scans. The structure was solved by the heavy atom (Patterson) method and all the major occupancy non-hydrogen atoms were refined anisotropically with absorption corrected (lamina [100]) data using full-matrix least-squares based on F^2 to give $R_1 = 0.071$, $wR_2 = 0.190$ for 5193 independent observed reflections ($|F_o| > 4\sigma(|F_o|)$, $2\theta \leq 120^\circ$) and 443 parameters. Crystallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-102341. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk).

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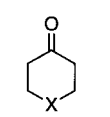
Hydrolysis of Amides Catalyzed by 4-Heterocyclohexanones: Small Molecule Mimics of Serine Proteases**

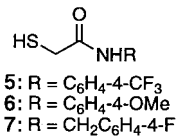
Mousumi Ghosh, Jeffrey L. Conroy, and Christopher T. Seto*

One of the long-standing problems in bioorganic chemistry is the design of catalysts that hydrolyze amide bonds under mild conditions.[1, 2] Amides are stable species; the half-life for peptide hydrolysis under neutral conditions at 25 °C has been estimated to be seven years.[3] However, nature has been able to develop four different classes of proteases that are capable of sequence-specific hydrolysis of peptides with tremendous rate accelerations. Therefore, the design of artificial catalysts that begin to approach the activity and specificity of protein-based catalysts is a fascinating and challenging problem. Here we report that the cyclohexanone **1** and the 4-heterocyclohexanones **2**–**4** are efficient catalysts for the base-promoted hydrolysis of amides.

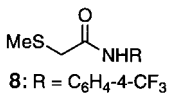
We have shown previously that 4-heterocyclohexanones can be used to synthesize inhibitors of cysteine proteases.[4] These compounds inhibit the protease by reaction of the 4-heterocyclohexanone carbonyl group with the active-site cysteine nucleophile of the enzyme with reversible formation of a hemithioacetal adduct.[5] In our current studies we are interested in developing catalysts of amide hydrolysis, and we reasoned that the amide substrates **5**–**7** could be anchored reversibly to a 4-heterocyclohexanone catalyst to form similar hemithioacetal adducts. Hydrolysis of the amide could then occur through a series of reactions that mimic the mechanism used by serine proteases to catalyze hydrolysis of peptides, as discussed below. These reactions serve as a model for hydrolysis of peptides specifically on the C-terminal side of cysteine residues.



1: X = CH_2
2: X = NH
3: X = S
4: X = O



5: R = C_6H_4-4-CF_3
6: R = C_6H_4-4-OMe
7: R = $CH_2C_6H_4$-4-F



8: R = C_6H_4-4-CF_3

We have monitored the hydrolysis of **5**–**7** catalyzed by 4-heterocyclohexanones by ^1H or ^{19}F NMR spectroscopy, or reverse-phase HPLC.[6] The reactions were performed under pseudo-first-order conditions, and they showed an exponential decrease in the substrate concentration as a function of time. Table 1 shows the observed rate constants for several reactions with a variety of substrates and catalysts. The most efficient catalysis that we have measured is shown in entry 5. In this reaction the hydrolysis of **5** is accelerated by more than four orders of magnitude relative to the background reaction when it is carried out in the presence of 600 mM tetrahydropyranone (THP, **4**).

Table 1. Observed rate constants for hydrolysis of amides catalyzed by 4-heterocyclohexanones.[a]

Entry	Catalyst	Substrate	k_{obs} [s^{-1}]	k_{rel}[b]
1	none	**5**	1.5×10^{-8}	
2	**1**	**5**	2.5×10^{-8}	2
3	**2**	**5**	5.9×10^{-8}	4
4	**3**[c]	**5**	3.7×10^{-8}	2
5	**4**	**5**	2.2×10^{-4}	14700
6	**4**	**6**	1.5×10^{-4}	10000
7	none	**6**	1.5×10^{-8}	
8	**4**	**7**	1.2×10^{-4}	3900
9	none	**7**	3.1×10^{-8}	
10	**4**[c]	**8**	1.0×10^{-7}	

[a] All reactions were performed at 25 °C with 20 mM substrate, 200 mM NaOD, and 600 mM catalyst (where present) D_2O/CD_3OD (4/1) unless otherwise specified. [b] Rate constant relative to the background reaction (no catalyst) with the same substrate. [c] Reaction was performed in D_2O/CD_3OD (1/1) because of low solubility of the catalyst or substrate in aqueous solution.

The efficiency of the hydrolysis reaction is highly dependent on the heteroatom in the 4-heterocyclohexanone catalyst (compare entries 2–5, Table 1). The reactivity of the carbonyl group in the catalyst is controlled by a through-space electrostatic repulsion between the dipoles of the ketone and the heteroatom. We have demonstrated previously that the equilibrium constant for addition of a thiol to 4-heterocyclo-

[*] Prof. Dr. C. T. Seto, M. Ghosh, J. L. Conroy
Department of Chemistry
Brown University
324 Brook Street, Box H, Providence, RI 02912 (USA)
Fax: (+1) 401-863-2594
E-mail: christopher_seto@brown.edu

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hexanones is correlated with the strength of this electrostatic repulsion.[4] Thus THP, which has the strongest through-space interaction, is the most substrate bound and has the most effective catalyst at nonsaturating concentrations of substrate and catalyst.[7] In addition to simple binding of the substrate to the catalyst, this type of through-space electrostatic interaction can also exert other effects which significantly influence the rate of the hydrolysis reaction. For example, electrostatic interactions should alter the pK_a of the hemithioacetal hydroxyl group which is involved in catalysis. A combination of these effects causes THP to be a much better catalyst than the corresponding carbon, nitrogen, and sulfur analogues. It is interesting to note that benzaldehyde, acetophenone, and trifluoromethyl ketone derivatives are not effective catalysts for these hydrolysis reactions.

To investigate the mechanism of this reaction we have examined the kinetic order for hydrolysis of **5** catalyzed by THP by varying the NaOD and catalyst concentrations under pseudo-first order conditions (Figure 1). The observed rate

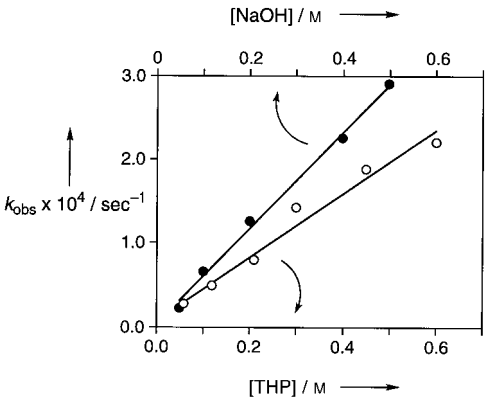


Figure 1. Hydrolysis of **5** catalyzed by tetrahydropyranone (THP, **4**) in D_2O/CD_3OD 4/1. ●: reactions with 20 mM substrate, 300 mM tetrahydropyranone, and NaOD in concentrations from 50 to 500 mM; slope = $5.72 \times 10^{-3}\,M^{-1}s^{-1}$. ○: reactions with 20 mM substrate, 200 mM NaOD, and THP in concentrations from 60 to 600 mM; slope = $3.77 \times 10^{-3}\,M^{-1}s^{-1}$.

constant increases linearly with the concentration of NaOD or THP. These results indicate that the rate expression for the hydrolysis reaction is defined by Equation (1).

$$\text{rate} = k_{hydr}[\text{substrate}][\text{catalyst}][\text{NaOH}] \quad (1)$$

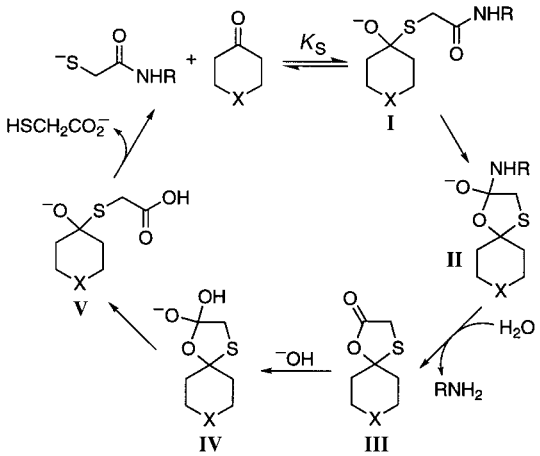
We have used the slopes of the plots shown in Figure 1 to calculate the value of the third-order rate constant k_{hydr}. The experiments in which the catalyst concentration was varied (open circles) give a calculated rate constant of $k_{hydr} = 1.88 \times 10^{-3}\,M^{-2}s^{-1}$, while the experiments in which the NaOD concentration was varied (closed circles) give $k_{hydr} = 1.91 \times 10^{-3}\,M^{-2}s^{-1}$. The excellent agreement between these values provides further evidence that the rate expression formulated in Equation (1) is correct.

The observed rate constant for these reactions is a linear function of the catalyst concentration over the range of 60–600 mM THP (Figure 1). However, at concentrations at which the substrate is fully bound by catalyst, the rate constant should become independent of catalyst. These observations indicate that the association constant K_S for the binding of **5** to THP is less than $1.7\,M^{-1}$.[7]

To explore the scope of this reaction, we have measured the hydrolysis rates for the three substrates **5**–**7**. Interestingly, there is less than a twofold difference in the rate constants between **5**, which is an relatively activated substrate, and **7**, which is an example of an unactivated amide. These results indicate that THP can accelerate significantly the rate of hydrolysis of unactivated amides, and suggest that it may serve as a useful catalyst for the cleavage of peptide bonds.

Under strongly basic conditions, the rate of uncatalyzed amide hydrolysis typically shows a small dependence on the nature of the leaving group.[8] This observation can be rationalized because substituents on the leaving group have opposite effects on the rate of hydroxide addition to the amide carbonyl group and the rate of departure of the leaving group, which must be partially or fully protonated during this step. The small dependence of the rate of the catalyzed reaction on the nature of the leaving group suggests a similarity between the mechanisms of the catalyzed and uncatalyzed reactions.

Scheme 1 shows a plausible mechanism for the hydrolysis of amides catalyzed by 4-heterocyclohexanones. This mechanism mimics the series of reactions that occur during the



Scheme 1. Proposed mechanism for the hydrolysis of amides catalyzed by 4-heterocyclohexanones.

hydrolysis of peptides by serine proteases. Three important features of the enzymatic reaction are replicated in the proposed mechanism: 1) The substrate binds to the catalyst in a reaction that reaches equilibrium faster than amide hydrolysis. In Scheme 1 this entails nucleophilic attack by the substrate thiolate on the carbonyl group of the 4-heterocyclohexanone to yield hemithioacetal anion **I**. This strategy, which involves formation of a reversible covalent bond, provides a reliable method for anchoring the substrate to the catalyst in a well-defined geometry. 2) The substrate reacts with a catalyst nucleophile to generate an acyl-enzyme intermediate. In the small-molecule system, the anion in hemithioacetal **I** is positioned for nucleophilic attack on the amide carbonyl group through formation of a five-membered ring to give tetrahedral intermediate **II**. Breakdown of this tetrahedral intermediate releases the amine leaving group and

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generates acyl-catalyst intermediate **III**. Similar mechanisms have been observed in the neighboring group participation by carbonyl hydrates during the hydrolysis of carboxylate and phosphate esters. However these examples are stoichiometric reactions that are promoted by intramolecular carbonyl groups.[9–11] 3) Deacylation of the acyl-enzyme intermediate regenerates the catalysts. This process is mimicked by reaction of **III** with hydroxide to give tetrahedral intermediate **IV**, which then breaks down to yield hemithioacetal **V**. Dissociation of **V** releases the carboxylate anion and regenerates the 4-heterocyclohexanone catalyst.

We have performed two additional experiments to probe the validity of this proposed mechanism. First, we have synthesized control compound **8** in which the thiol group is blocked as the methyl thioether in order to determine if a thiol functionality in the substrate is necessary for catalysis. Comparison of entries 1 and 10 in Table 1 shows that the rate of hydrolysis of **8** in the presence of 600 mM catalyst is only sevenfold faster than the rate of hydrolysis of substrate **5** in the absence of catalyst. This comparison shows that a free thiol group in the substrate is required for catalysis. In addition, the results shows that the mechanism of the catalyzed reaction cannot involve simple intermolecular nucleophilic attack by the anion of the 4-heterocyclohexanone hydrate on the carbonyl of the amide substrate.

In a second experiment we have independently synthesized the acyl-catalyst intermediate **III** (Scheme 1) in which X = S, and we monitored its rate of hydrolysis under the reaction conditions. We find that this intermediate is hydrolyzed much faster than the amide substrates in any of the catalyzed reactions. These two observations are consistent with the mechanism proposed in Scheme 1, and they suggest that the rate-limiting step for the catalyzed reaction occurs before hydrolysis of intermediate **III**.

In conclusion, we have demonstrated that tetrahydropyranone (**4**) is an effective catalyst for the hydrolysis of amide substrates that contain an adjacent thiol functionality. The reaction displays two features that are most often associated with enzymatic systems. First, the substrate is bound to the catalyst through a preliminary equilibrium in order to decrease the entropic barrier to reaction. The catalysts employ reversible formation of a hemithioacetal to establish this equilibrium. We believe that formation of reversible covalent bonds of this type will prove to be a useful method for mediating the molecular recognition processes that are involved in catalysis and self-assembly. Reversible covalent bonds are complementary to the noncovalent interactions—such as hydrogen bonds, hydrophobic interactions, and electrostatic interactions—that are typically observed in biological recognition processes. A second similarity to enzymatic catalysis is that the reaction is catalyzed through the participation of neighboring groups. We are currently conducting experiments to characterize further the mechanism of the reaction, and also to explore the possibility of using 4-heterocyclohexanones to catalyze the cysteine-specific hydrolysis of peptides.

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Highly Enantioselective Hydrogenation of Cyclic Enol Acetates Catalyzed by a Rh – PennPhos Complex**

Qiongzhong Jiang, Dengming Xiao, Zhaoguo Zhang, Ping Cao, and Xumu Zhang*

The growing demand for practical and effective chiral ligands and/or catalysts has fueled much recent progress in ligand design. Although benchmark ligands such as 2,2′-

[*] Prof. X. Zhang, Dr. Q. Jiang, D. Xiao, Dr. Z. Zhang, P. Cao
Department of Chemistry
The Pennsylvania State University
University Park, PA 16802 (USA)
Fax: (+1) 814-863-8403
E-mail: xumu@chem.psu.edu

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