Amide Cleavage

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Zinc-Catalyzed Amide Cleavage and Esterification of β-Hydroxyethylamides**

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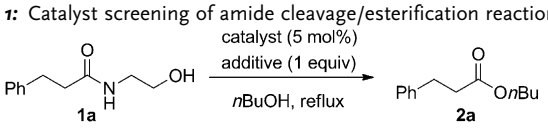
Amides are ubiquitous in nature and have extremely stable bonds, as their half-life for spontaneous hydrolysis is estimated to be 350 to 600 years at neutral pH and room temperature.[1] In the biological metabolic cycle in nature, in contrast to the relatively easy formation of amides, the cleavage (hydrolysis) of amide bonds is an energy-consuming, ATP-dependent event that requires enzymes.[2] In chemical evolution in the pre-enzyme era, non-enzymatic catalytic systems played a role in the chemical scenario of development, such as hydrolysis processes. In various proteases, water can either be activated by ligation to a mononuclear zinc ion (metalloproteases) or by binding in a small cleft defined by two aspartic acid residues (aspartate proteases). Among these, we specifically focused our attention on zinc-containing metalloproteases such as carboxypeptidase A (CPA)[3] and thermolysin (TLN).[4] In the context of the structure and function of metalloproteases in which mononuclear zinc species are active sites, we studied mononuclear zinc compounds as potential precursors for catalytic scission of the amide bond and found that $Zn(OTf)_2$ acts as a catalyst for the selective cleavage of amides bearing a β-hydroxyethyl group. In addition, to the best of our knowledge, this new catalyst system can be applied to sequence-specific peptide bond scission as the first artificial catalyst, which cleaves peptide bonds using catalytic amounts of metal complex. This finding provides insight into the chemical processes in nature wherein fatty-acid ethanolamides were selected in the pre-enzyme stage as controllable chemicals because of their chemical advantages of easy formation and degradation, and, in particular, used as special molecules that regulate the endogenous signaling function in the mammalian central nervous system.[5]

We began by screening efficient catalysts for amide the cleavage/esterification process, in which the amide bond was cleaved by alcohols to give the corresponding esters and free amines. Recently, amide alcoholysis has become the subject of increasing interest,[6] partly because the produced esters can be converted easily into a variety of functional groups compared with carboxylic acids derived from hydrolysis of amides. We used a test reaction of *N*-(2-hydroxyethyl)-3-phenylpropionamide (**1a**) with 1-butanol in the presence of 5 mol% of various zinc catalysts as well as some other transition-metal precursors to give butyl 3-phenylpropionate (**2a**), and the results are summarized in Table 1. Mononuclear

Table 1: Catalyst screening of amide cleavage/esterification reaction.[a]



Entry	Catalyst	Additive	*t* [h]	Yield [%][b]
1	none	–	18	<1
2	$ZnCl_2$	–	18	15
3	$Zn(OAc)_2$	–	3	20
4	$Zn(OAc)_2$	–	18	11
5	$Zn(OCOCF_3)_2$	–	18	17
6	$Zn(OTf)_2$	–	3	22
7	$Zn(OTf)_2$	–	18	20
8	$Zn(OTf)_2$	benzaldehyde (**3a**)	18	52
9	$Zn(OTf)_2$	acetophenone (**3b**)	18	52
10	$Zn(OTf)_2$	benzophenone (**3c**)	18	50
11	$Zn(OTf)_2$	diethylcarbonate (**3d**)	18	61
12[c]	$Zn(OTf)_2$	diethylcarbonate (**3d**)	18	70
13[c]	$Zn(OTf)_2$	diethylcarbonate (**3d**)	45	85
14	$Cu(OAc)_2{\cdot}H_2O$	–	18	12
15	$Pd(OAc)_2$	–	18	<1
16	$Ni(OAc)_2{\cdot}4H_2O$	–	18	<1

[a] Reaction conditions: A mixture of amide (1.0 mmol), catalyst (0.0050 mmol), and additive (1.0 mmol) in 1-butanol (2.0 mL) was refluxed for 18 h. [b] Determined by GC analysis based on the produced butyl ester. [c] 2.0 mmol of diethylcarbonate was used. Tf = trifluoromethanesulfonyl.

zinc complexes such as $ZnCl_2$, $Zn(OAc)_2$, $Zn(OCOCF_3)_2$, and $Zn(OTf)_2$ were used for an 18 h reaction period and produced 11 to 22% yields (entries 2, 3, 5, and 6). In addition, μ-oxo-tetranuclear zinc clusters $Zn_4(OCOCH_3)_6O$ and $Zn_4(OCOCF_3)_6O$, which are reported to catalyze the transesterification much better than the corresponding mononuclear compound,[7] showed almost the same catalytic activity (17–20% yield) as the corresponding mononuclear zinc complexes. Exclusion of the zinc catalyst led to minimal product yield (entry 1). Because some copper, palladium, and nickel complexes mediate stoichiometric hydrolysis of peptide bonds as model systems for mimicking metalloenzymes,[8] we used acetate complexes of copper, palladium, and nickel as catalysts, which resulted in a low yield and almost no activity (entries 14–16). Among the mononuclear zinc precursors examined, $Zn(OTf)_2$ was the best catalyst (entries 4 and 7) for reactions conducted with a shorter reaction time

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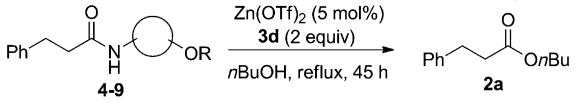
(3 h). Although we previously demonstrated that amines and N-heteroaromatic compounds increased the catalytic activity of the zinc cluster for transesterification,[9] such positive effects of amines were not observed in this transformation of an amide into an ester (see the Supporting Information).

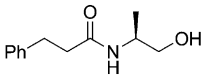
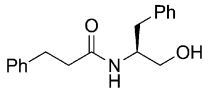
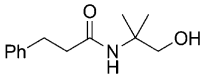
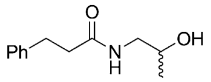
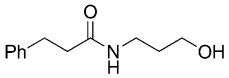
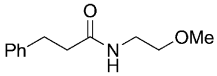
The chemical yield of the desired butyl ester **2a** was limited to a maximum yield of approximately 20% when using any zinc complex, thus suggesting that this catalytic reaction is reversible. In fact, the reversibility of this transformation was confirmed by the following control experiments: 1) amidation of the butyl ester **2a** using aminoalcohol to give **1a**, a reverse reaction, proceeded under the same reaction conditions, and 2) addition of aminoalcohol to the reaction mixture of the amide cleavage/esterification reaction suppressed the yield of the butyl ester **2a** (20–9%). Thus, it is reasonable to anticipate that any aminoalcohol-capture reagent efficiently shifts the equilibrium of the reversible reaction to afford the esters in high yield. At the outset, carbonyl compounds, such as benzaldehyde (**3a**), acetophenone (**3b**), and benzophenone (**3c**), were added as aminoalcohol-capture reagents, because aminoalcohol can react with these carbonyl compounds to afford the corresponding imines. As expected, the yield of the ester increased up to 52% (entries 8–10). Finally, diethylcarbonate (**3d**) was found to be the best trapping reagent of aminoalcohol, thus accelerating the amide cleavage/esterification of **1a** to give **2a** in 61% yield (entry 11) together with a trace amount of the corresponding ethyl ester as the product of the transesterification of the ester with ethanol derived from diethylcarbonate. The increase in the amount of carbonate (2 equivalents to amide) improved the yield of the ester **2a** from 61 to 70% (entry 12), and increasing the reaction time (45 h) led to an increase in the yield of **2a** to 85% (entry 13). Other alcohols were then examined under these optimized reaction conditions. As a result, 1-butanol and 1-pentanol indicated high reactivity, probably because of its suitable refluxing temperature (see the Supporting Information).

With the optimized reaction conditions in hand, the scope of the present amide cleavage/esterification reaction was investigated with respect to the aminoalcohol moiety (Table 2). Substrates having methyl, benzyl, and dimethyl groups adjacent to the nitrogen atom participated in this catalytic reaction to form the ester **2a** in high yield (entries 1–3), and the methyl substituent next to the oxygen atom was also applicable to this catalytic system (entry 4). Amide **8**, having a three-carbon chain, was transformed into the corresponding ester **2a** in a relatively lower yield (entry 5), and the O-protected hydroxyamide **9** elicited almost no reaction under the same reaction conditions, thus suggesting a plausible mechanism involving N,O-acyl rearrangement[10] (see below).

We then examined the generality of amides with a β-hydroxyethyl group (Table 3). The amide cleavage/esterification of a series of benzamide derivatives (**1b**–**1k**) afforded the corresponding butyl esters **2b**–**2k** (entries 1–10). The results obtained using the substituted benzamides indicated that an electronic effect influenced this catalytic transformation. The use of benzamide with an electron-donating group at the *para* position retarded the reaction (entries 2 and 3), whereas

Table 2: Scope of the substituent on the aminoalcohol moiety.[a]



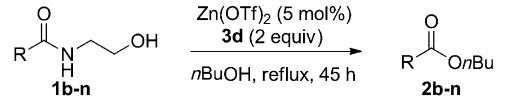
Entry	Amide		Yield [%][b]
1		**4**	85
2		**5**	86
3		**6**	87
4		**7**	84
5		**8**	36
6		**9**	trace

[a] Reaction conditions: A mixture of amide (1.0 mmol), $Zn(OTf)_2$ (0.050 mmol), and diethylcarbonate (2.0 mmol) in 1-butanol (1.0 mL) was refluxed for 45 h. [b] Determined by GC analysis based on the produced butyl ester.

benzamide with an electron-withdrawing group at the *para* position successfully underwent the reaction (entries 4 and 5). The reaction was also sensitive to the steric environment of the amide moiety, and thus sterically congested substrates afforded moderate yield (entries 7, 8, and 12). A rate-accelerating effect resulting from the electron-withdrawing group could compensate for this unfavorable steric effect (entry 9). Aliphatic amides **1l** and **1m** were also applicable to this catalytic system (entries 11 and 12). Notably, the amide **1n**, bearing both a hexylcarbamoyl group and a hydroxyethylcarbamoyl group, afforded only butyl 4-(hexylcarbamoyl)benzoate, in which the hydroxyamide moiety was selectively converted into a butyl ester and the hexyl amide moiety remained intact (entry 13), thus demonstrating that hydroxyamide could be selectively converted under this catalytic system.

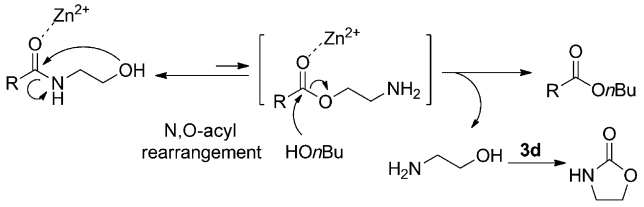
Consequently, as shown in Scheme 1, an initial intramolecular attack of the hydroxy group on the carbonyl carbon atom at the amide bond affords an ester intermediate (N,O-acyl rearrangement). Subsequent transesterification with 1-butanol gives the butyl ester. Thus, a two-step reaction achieves the amide–ester exchange. The dissociated ethanolamines react with diethylcarbonate (**3d**) to afford the corresponding carbamate, which was isolated in the reaction of amide **5**. The coordination of the amide to the zinc complex increased the electrophilicity enough to facilitate the intramolecular attack of the hydroxy group.[11] On the basis of the previous report on metal-assisted transesterification,[7,12] we assume that the zinc catalyst spontaneously mediated the transesterification of a nascent β-aminoethanolate. In addi-

Table 3: Scope of the amide substrate.[a]



Entry	Amide		Yield [%][b]
1		**1b**: R′ = H	88
2		**1c**: R′ = OMe	82
3		**1d**: R′ = NMe_2	45
4		**1e**: R′ = CF_3	90
5		**1f**: R′ = F	86
6		**1g**: R′ = Br	89
7		**1h**: R′ = OMe	32
8		**1i**: R′ = Me	43
9		**1j**: R′ = F	70
10		**1k**	87
11		**1l**	71
12		**1m**	34
13		**1n**	84[c]

[a] Reaction conditions: A mixture of amide (1.0 mmol), $Zn(OTf)_2$ (0.050 mmol), and diethylcarbonate (2.0 mmol) in 1-butanol (1.0 mL) was refluxed for 45 h. [b] Determined by NMR analysis based on the produced butyl ester. [c] Butyl 4-(hexylcarbamoyl)benzoate was obtained.

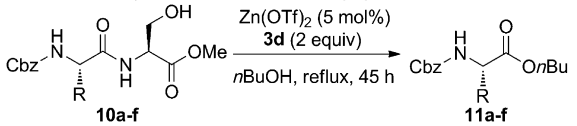


Scheme 1. Proposed mechanism.

tion, our proposed mechanism is consistent with the known in vivo phenomena observed for serine residue in proteins, such as protein splicing.[13] Scheme 1 also explains the low yield of the amide **8**, that is, a nucleophilic attack proceeded through the less favorable six-membered transition state compared with five-membered transition state.

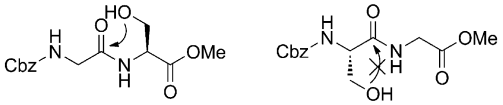
It is of great interest that hydroxyamide cleavage allows selective peptide bond scission at the amine side of a serine residue, even though more than stoichiometric amounts of metal complexes have been used to mediate peptide bond hydrolysis.[8] First, we achieved cleavage of some dipeptides including Ser residues (Table 4). The peptide bond of Gly-Ser

Table 4: Zinc-catalyzed esterification of dipeptides.[a]



Entry	Dipeptide	Yield [%][b]
1	Cbz-Gly-Ser-OMe (**10a**)	84
2	Cbz-Ala-Ser-OMe (**10b**)	64
3	Cbz-Pro-Ser-OMe (**10c**)	63
4	Cbz-Met-Ser-OMe (**10d**)	55
5	Cbz-Gly-Gly-OMe (**10e**)	trace
6	Cbz-Ser-Gly-OMe (**10f**)	trace

[a] Reaction conditions: A mixture of peptide (1.0 mmol), $Zn(OTf)_2$ (0.050 mmol), and diethylcarbonate (2.0 mmol) in 1-butanol (0.5 mL) was refluxed for 45 h. [b] Yield of the isolated Cbz-protected butyl esters. Cbz = benzyloxycarbonyl.



was cleaved under the optimized reaction conditions (entry 1). Alanine, proline, and methionine were also applicable for use in this catalytic system (entries 2–4). In no case did we observe any racemization during the reaction. As mentioned above, a hydroxyethyl group is necessary for the present amide cleavage/esterification reaction. In fact, Gly-Gly afforded only trace amounts of the corresponding ester (entry 5). In addition, the position of the cleavage was confirmed by the examination with Ser-Gly (entry 6), thus indicating that the cleavage occurred at the amine side of the serine residue because in the Ser-Gly case the intramolecular attack of hydroxy group would result in an unfavorable four-membered transition state. This work is the first example of an artificial catalyst for the cleavage of peptide bonds at a specific residue. Selective cleavage of peptide bonds has been rarely achieved, except in two types of metal-assisted stoichiometric reactions: one is cleavage induced by metal complexes which coordinate to organic moieties to recognize the target proteins,[14] and the other is cleavage mediated by metal complexes which directly bind to the specific residues (e.g., Ser, Thr, Met, His, etc.) to be cleaved.[15]

In summary, we developed an esterification of β-hydroxyethylamides in the presence of the zinc catalyst $Zn(OTf)_2$ together with carbonates to capture ethanolamines. Notable features of this catalyst system are that the amide bond can be cleaved under neutral conditions and, more attractively, sequence-selective peptide bonds can be cleaved at the amine side of serine residues, thus demonstrating the first selective peptide bond-cleavage reaction using a catalytic amount of a metal complex, through a mechanism including N,O-acyl rearrangement and subsequent transesterification of ester intermediates.

Experimental Section

An oven-dried Schlenk tube was equipped with $Zn(OTf)_2$ (0.050 mmol). Diethylcarbonate (2.0 mmol), amide (1.0 mmol), and 1-butanol (1.0 mL) were then added and the resulting mixture was refluxed under an argon atmosphere. The yield of the produced ester was determined by ^1H NMR analysis using phenanthrene as an internal standard or by GC analysis using dodecane as an internal standard.

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