Enzyme Models

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Primary-Amine-Specific Lactamization of ω-Amino Acids by an Artificial Cyclotransferase Based on [18]Crown-6**

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In memory of Kiyoshi Tanaka

Enzyme models are of practical importance for the development of efficient molecular catalysts for organic reactions and of theoretical importance in elucidating mechanisms of enzymatic reactions.[1] In comparison with hydrolase models, an artificial acyltransferase that promotes the reverse reaction of dehydrocondensation is particularly difficult to design.[2] As a representative host compound, [18]crown-6 has received much attention in biomimetic chemistry and, although specific binding of ammonium ions to [18]crown-6 has been extensively investigated,[3–5] application of the complex as an artificial acyltransferase is very limited.[6,7] Among acyltransferase models, cyclotransferase models that catalyze intramolecular dehydrocondensation to lead to the formation of lactams have not been developed. Herein, we report a novel enzyme model of cyclotransferase,[8] which utilizes the specific binding of a primary ammonium ion and [18]crown-6. The artificial enzyme substrate specifically activates the carboxylic acids that possess a primary amino group at the γ-, δ-, or ε-positions, thus leading to the formation of the corresponding lactams.

The reaction system is outlined in Scheme 1. Methanol is employed as the solvent as it dissolves the amino acid substrates and stabilizes the complex of the primary ammonium salt and the [18]crown-6.[9–11] A combination system of triazines and tertiary amines which is available in alcohols was attached to the [18]crown-6 and employed for the activation of the substrate carboxyl groups.[12,13] When CDMT was added to a methanolic solution of 5-aminovaleric acid (**3a**) and crown derivative **1**, which can be compared to an apoenzyme,

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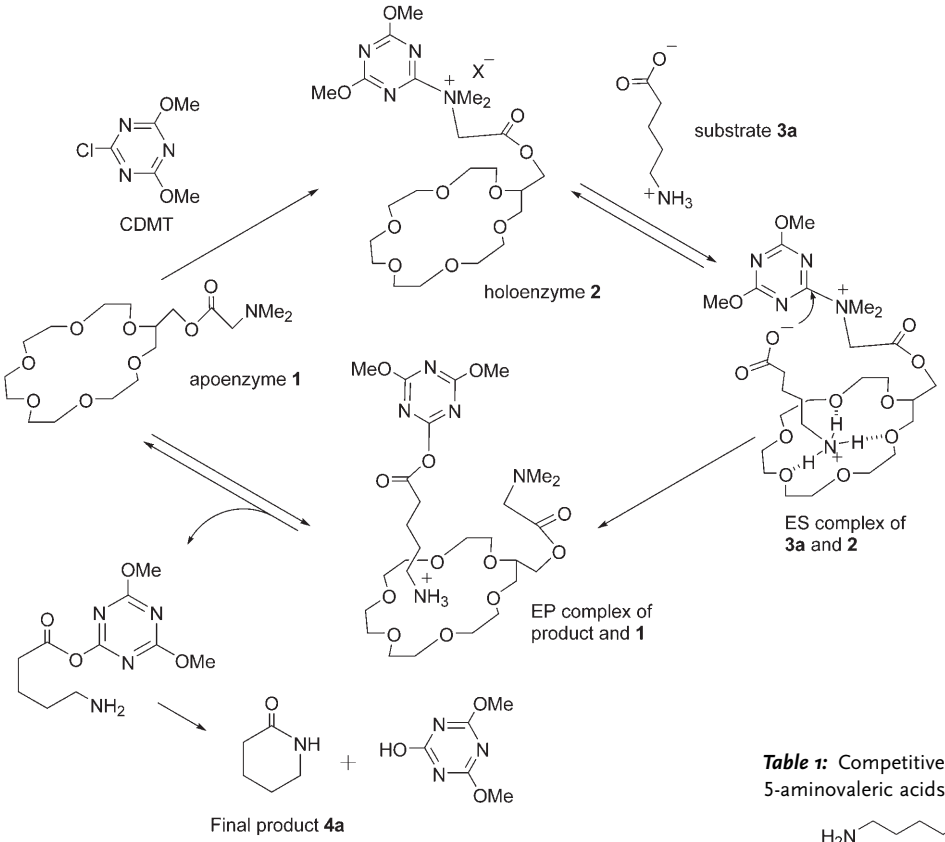
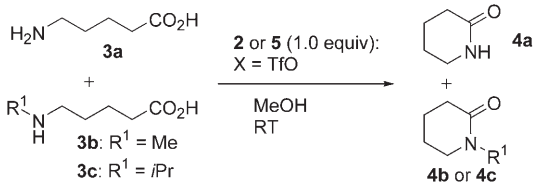


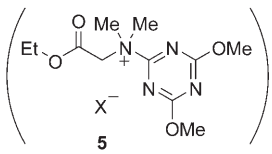
Scheme 1. Substrate-specific lactamization by artificial cyclotransferase. CDMT = 2-chloro-4,6-dimethoxy-1,3,5-triazine, ES = enzyme–substrate, EP = enzyme–product.

a reactive dehydrocondensing reagent **2** (similarly, compared to holoenzyme; X = Cl) was generated by coupling **1** with CDMT through the dimethylamino group. Upon introduction of substrate **3a**, the primary amino group in its ionized form preferentially interacts with **2** to form a so-called ES complex. In the resulting complex, the carboxylate ion of the substrate is brought into proximity with the triazino group and attacks it preferentially, thus giving an EP complex. Intramolecular aminolysis of the resulting acyloxytriazine takes place quickly to give δ-valerolactam with concomitant liberation of **1**, which can be reused in the next catalytic cycle. Since the product **4a** lacks a free amino group, it no longer binds to the crown ring, and thus there is no competitive binding of the product with the catalyst to inhibit the next reaction.

First, to verify that the substrate-specific lactamization takes place through such molecular recognition, we attempted the reaction under stoichiometric conditions using **2** (X = OTf; Tf = trifluoromethanesulfonyl). A competitive reaction between **3a** and *N*-methyl-5-aminovaleric acid (**3b**) was performed. Since the primary amino group of **3a** has a much greater affinity for [18]crown-6 than the secondary amino group of **3b**,[11] **3a** can be expected to react preferentially (Table 1). When a mixture of 1.1 equivalents of both the amino acids was treated with 1.0 equivalents of **2** in methanol at room temperature for 10 min, **4a** was obtained predominantly (**4a**/**4b** = 92:8) in 89% yield (run 1). No significant improvement in either the selectivity or the yield was observed by prolonging the reaction time to 2 h (run 2). The selectivity slightly increased with an excess of **3a** and **3b** (2.0 equiv each; run 3). In the control reaction using **5**, which has the dehydrocondensing group attached to an ethyl group instead of the crown ether, both the selectivity and the yield dropped (54:46, 50% yield; run 4). Prolongation of the reaction time increased the yield to 80%, whereas the selectivity was completely lost (run 5). Similar results were obtained with **3c**, which has an isopropyl group at the nitrogen atom (runs 6 and 7). The reaction rates uniformly decreased when either the methyl or isopropyl group was introduced to the terminal amino group, thus indicating that the mere existence of

Table 1: Competitive lactamization between primary and secondary 5-aminovaleric acids (**3a** vs **3b** or **3c**).





Entry	Substrate[a]	Condensing agent	*t* [min]	Product[b] Yield [%]	Product[b] Ratio (**4a**/**4b** or **4c**)
1	**3a** vs **3b** (1.1:1.1)	**2**	10	89	92:8
2	**3a** vs **3b** (1.1:1.1)	**2**	120	92	90:10
3	**3a** vs **3b** (2.0:2.0)	**2**	10	100	94:6
4	**3a** vs **3b** (1.1:1.1)	**5**[c]	10	50	54:46
5	**3a** vs **3b** (1.1:1.1)	**5**[c]	120	80	51:49
6	**3a** vs **3c** (3.0:3.0)	**2**	20	100	94:6
7	**3a** vs **3c** (3.0:3.0)	**5**[c]	120	88	55:45
8[d]	**3a** vs **3b** (1.1:1.1)	**2**	10	93	94:6

[a] The molar ratios given in parenthesis are based on the amount of **2** or **5** (1.0 equiv). [b] Determined by HPLC. [c] Control. [d] Reaction was carried out in the presence of 2-piperidone (**4a**; 0.85 equiv).

a substituent at the amino group (rather than size or steric hindrance) was the main factor in decreasing the reaction rate.

To confirm that the observed selectivity is attributable to the supramolecular interaction between the protonated primary amino group of **3a** and the crown ether of **2**, we examined the effect of alkaline-metal ions on the selectivity because the affinity of these cations toward [18]crown-6 correlates with the match of their ionic radii to the diameter of the host.[10,14,15] Competitive reactions under the same conditions as run 2 in Table 1 were conducted in the presence of 2 equivalents of various alkaline-metal triflates. As shown in Table 2, the lithium ion, which has the smallest binding

Table 2: Effect of alkali-metal ions on substrate selectivity.

Entry	CF_3SO_3M (2 equiv)		Product[a]	
		pK_a[b]	Yield [%]	Ratio (**4a**/**4b**)
1	CF_3SO_3Li	<0.5	96	90:10
2	CF_3SO_3Na	4.38	93	84:16
3	CF_3SO_3K	6.20	69	55:45
4	CF_3SO_3Cs	4.55	84	85:15

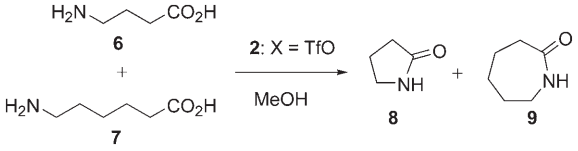
[a] Determined by HPLC. [b] The pK_a values in MeOH/benzene (8:2) are cited from reference [15].

constant ($pK_a = <0.5$),[15] was found to have almost no effect on selectivity, whereas the potassium ion, well known to have the highest affinity for the crown ring, nearly abolished the selectivity and generated a lower yield (runs 1 and 3). The moderate decrease in the selectivity for **4a** was observed with sodium and cesium salts. The extent to which alkaline-metal ions lowered selectivity correlated very well with their affinity toward [18]crown-6, and we therefore conclude that the observed substrate selectivity for **3a** resulted from the formation of a host–guest complex of the primary ammonium group within the crown ring.

In general, reactions using host compounds suffer from product inhibition because the products retain a high affinity for the host.[1b] In the present case, the primary amino group of **3a**, which is recognized by the crown at the initial stage of the reaction, disappears as it is converted into the amide nitrogen atom of **4a**, which should no longer bind to **2**. In fact, when the competitive reaction was carried out in the presence of **4a**, no decrease in selectivity was observed (Table 1, run 8). Thus, properties intrinsic to this reaction circumvent the problem of product inhibition and, in principle, this benefit applies to all reactions that modify the guest moiety to one with significantly less affinity for the host.[16]

It should be noted that the substrate-specific lactamization proceeds under catalytic conditions. The competitive reaction between **3a** and **3b** was performed using a combination of a catalytic amount of **2** (0.2 equiv) and CDMT (1.0 equiv). To neutralize HCl generated during the reaction, *N*-cyclohexylmorpholine was slowly added by a syringe pump over a period of 5 h. As a result, **4a** and **4b** were obtained in a ratio of 96:4 (yield = 78%). The control reaction using **5** instead of **2** under the same conditions resulted in a low selectivity (**4a**/**4b** = 53:47, 70% yield).

Interestingly, the chain length between the amino group and carboxyl group can be discriminated by **2**. The reactivity of 4-aminobutyric acid (**6**), **3a**, and 6-aminocaproic acid (**7**) toward **2** at room temperature was estimated to be 1:1.5:2.1, respectively, based on their competitive reactions, thus indicating that the kinetically most unfavorable seven-membered ε-caprolactam **9** was formed faster than five- or six-membered lactams (**8** and **4a**). Since the order of observed reactivity of the amino acids does not correlate with their affinity for [18]crown-6,[17] the number of the methylene groups that link the amino group and the carboxyl group could be important. The selectivity of **9**/**8** was improved with decreasing reaction temperature; the ratio of **9** increased up to 85:15 (88% yield) at −40 °C [Eq. (1)].



Conditions	**8**/**9**	Yield [%]
RT, 10 min	32:68	90
0°C, 2 h	28:72	88
-40°C, 48 h	15:85	88

In conclusion, we have succeeded in developing a novel artificial enzyme based on [18]crown-6, which is capable of converting ω-amino acids into their cyclized lactams in a substrate-specific manner. The catalyst can recognize the distance and the degree of substitution of the amino group farthest from the carboxyl group undergoing reaction. Furthermore, although reaction yield and selectivity are sometimes inversely related in organic reactions, both increase in the present system because the reaction rate is enhanced by the formation of the ES complex, which, in turn, is based on molecular recognition. Finally, the present system offers a new concept for circumventing product inhibition.

Experimental Section

General procedure for competitive lactamization using a stoichiometric amount of dehydrocondensing agent (**2** or **5**): A solution of **2** (95.1 mg, 0.142 mmol) in MeOH was added at room temperature to a stirred solution of **3a** (18.3 mg, 0.157 mmol) and **3b** (20.5 mg, 0.157 mmol) in MeOH (the total amount of MeOH = 26 mL). The reaction mixture was stirred at room temperature, and the products were quantified by HPLC at a definite time (1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone was used as the internal standard).

General procedure for competitive lactamization under the catalytic system of **2** and CDMT: A solution of **2** (19.0 mg, 0.029 mmol) and CDMT (20.0 mg, 0.114 mmol) in MeOH at room temperature was added to a stirred solution of **3a** (18.3 mg, 0.157 mmol) and **3b** (20.5 mg, 0.157 mmol) in MeOH. *N*-cyclohexylmorpholine (0.142 mmol, 0.6 mL of 0.237 M) dissolved in MeOH was added over a period of 5 h by a syringe pump (the total amount of MeOH = 8.5 mL). The reaction mixture was stirred at room temperature, and the products were quantified by HPLC at a definite time.

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