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Synthesis of lactams using enzyme-catalyzed aminolysis

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ABSTRACT

The formation of ϵ -caprolactam from 6-aminocaproic acid catalyzed by CALB (N435) is reported. Different lactam ring sizes can be prepared starting from 4-aminobutanoic acid, 5-aminovaleric acid, and 8-aminooctanoic acid. Experiments with mixtures of aminocarboxylic acids have shown that CALB prefers homocyclization of the individual aminocarboxylic acids.

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Lactams are widely used monomers for the synthesis of polyamides/Nylons, which are polymeric materials used extensively in our daily life. Industrial synthesis of most lactams involves step-wise reactions using aggressive chemicals and high temperatures.¹ However, biocatalytic approaches might be superior alternative synthetic routes that can be performed under mild reaction conditions. Currently, there is significant interest in the development of milder and more efficient methods to prepare ϵ -caprolactam and other lactams, for example, by using enzymatic catalysis. Reactions using enzymes are more environmentally friendly. Enzymes are non-toxic natural catalysts and reactions using enzymes can be carried out under mild conditions.²

The most commonly used biocatalyst in esterification or transesterification reactions and polymerizations³ is immobilized *Candida antarctica* lipase B (CALB), commercially available as N435. CALB is extremely useful due to its stability at high temperatures and its acceptance of various substrates.^{4,3a} CALB has a broad specificity toward straight chain fatty acids from butanoic acid to octadecanoic acid. On the other hand, CALB shows high activity toward carboxylic acids shorter than hexanoic acid.⁵

Lactams have previously been synthesized via enzymatic processes from aliphatic α,ω -dinitriles⁶ and amino esters.⁷ In the latter case the cyclization of amino esters catalyzed by crude pancreatic porcine lipase to form five-, six-, and seven-membered lactams was reported. However, in that study, the formation of seven membered rings proceeded slowly with low conversion, resulting in only 10% enzymatic conversion after 7 days.

There are no reports on the in vitro cyclization of aminocarboxylic acids of any length catalyzed by CALB. Therefore, in this study, we present the enzymatic formation of ϵ -caprolactam from 6-aminocaproic acid using CALB as the catalyst. Enzymatic syntheses were carried out in concentrated and dilute systems.⁸ We optimized the reaction conditions to obtain ϵ -caprolactam in a high yield. Furthermore, other aminocarboxylic acids such as β -alanine, 4-aminobutanoic acid (GABA), 5-aminovaleric acid, 8-aminooctanoic acid, and 12-aminododecanoic acid were used to synthesize lactams of different ring sizes (Scheme 1).

In both the concentrated⁹ and dilute systems,¹⁰ ϵ -caprolactam was successfully synthesized from 6-aminocaproic acid via intramolecular aminolysis catalyzed by N435. In the concentrated system, a low conversion (14%) for the formation of ϵ -caprolactam was observed (calculated from the ¹H NMR spectrum in D₂O). Furthermore, no oligomers were observed in the ¹H NMR spectrum in TFA-*d* of the reaction mixture. In the N435-catalyzed reaction using 8-aminooctanoic acid, in the concentrated system, no lactams or oligomers were formed. Therefore, it can be concluded that aminocarboxylic acids cannot be used as monomers for polymerization.

In the dilute system, enzymatic formation of ϵ -caprolactam occurred with a higher conversion (40%) compared with the concentrated system. This is in accordance with the theory of Jacobson and Stockmayer¹¹ that increased dilution increases ring formation.

To increase the formation of amide bonds (yield) the reaction conditions were optimized (water content of the solvent and the enzyme, etc.). The water content of N435 was reduced by drying under vacuum for 24–96 h at 55 °C, over P₂O₅. The dried N435 was subsequently used in the enzymatic formation of ϵ -caprolactam at 55 and 90 °C for 3 days. The highest conversions were obtained with N435 that had been dried for 24 h and employing a reaction

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Scheme 1. Cyclization of aminocarboxylic acids catalyzed by CALB in toluene.

temperature of 90 °C for the ϵ -caprolactam synthesis. Drying the enzyme for more than 24 h decreased the synthetic activity of N435 as shown in Figure 1. This reduction in activity is probably due to partial denaturation and to excessive removal of water from the enzyme; it is known that a certain amount of water is necessary to keep enzymes catalytically active.¹²

The formation of ϵ -caprolactam increased from 3 to 5 day reaction time; after this time, the conversion decreased (see Fig. 2). The optimal conditions are therefore reactions for 5 days at 90 °C using dried N435 under a nitrogen atmosphere.

Control reactions¹³ were carried out to determine whether formation of ϵ -caprolactam from 6-aminocaproic acid also occurred in the absence of CALB. The control reaction, which contained only 6-aminocaproic acid showed no formation of ϵ -caprolactam after 5 days stirring under exactly the same reaction conditions. Reactions using deactivated N435 resulted in an 11% conversion into ϵ -caprolactam. From activity tests,^{13b} we know that N435 cannot be deactivated completely by heat treatment for 4 or 24 h at 150 °C (Table S1 in Supplementary data), as N435 still showed residual transesterification and synthetic activity after this treatment. Therefore, we also tested macroporous acrylic polymer beads without immobilized enzymes (identical to the N435 resins) for control reactions. From these it became obvious that the carrier for N435 did not catalyze the formation of ϵ -caprolactam. Thus we can conclude that the formation of ϵ -caprolactam occurs only due to the synthetic activity of CALB.

Besides 6-aminocaproic acid, we found that N435 was able to catalyze the formation of five- and six-membered lactams with good conversions from 4-aminobutanoic acid and 5-aminovaleric acid,¹⁴ as shown in Table 1. We found that N435 did not catalyze the formation of a lactam from β -alanine and aminocarboxylic acids longer than 8-aminooctanoic acid, such as 12-aminododecanoic acid. These results are in agreement with previous reports, in which N435 showed poor activity in esterification reactions with straight chain fatty acids shorter than butanoic acid and longer than octanoic acid.⁵

Figure 1. Influence of drying N435 at 55 °C under vacuum over P₂O₅ for 0–96 h on the enzymatic formation of ϵ -caprolactam at 55 and 90 °C.

Figure 2. Influence of the reaction time on the formation of ϵ -caprolactam.

Table 1
Lactam formation using different aminocarboxylic acids

Aminocarboxylic acid	Conversion (%)	
	Control reaction ^a	Reaction with N435 ^a
4-Aminobutanoic acid	0	30
5-Aminovaleric acid	6	70
6-Aminocaproic acid	0	83
8-Aminooctanoic acid	0	41

^a Reaction was carried out in toluene at 90 °C for 5 days.

Unlike the formation of ϵ -caprolactam, the formation of δ -valerolactam can occur in the absence of N435, as δ -valerolactam was formed in the control reaction without enzyme. However, addition of N435 to the reaction increased the conversion of δ -valerolactam up to 11 times compared to the reaction without enzymes, which makes the enzymatic route a superior alternative to the chemical synthesis.

For reactions using 8-aminooctanoic acid as the substrate, N435 did not catalyze the formation of caprylolactam. Instead, the corresponding dimer and trimer lactams were formed,¹⁵ see Scheme 2. The formation of macrocyclic bislactams from reactions between diesters and diamines using porcine pancreatic lipase as the catalyst was previously reported.⁷ However, in the enzymatic reaction of 8-aminooctanoic acid using N435, different macrocyclic dimer and trimer lactams were formed in a ratio of 3:2. The ratio was calculated from the mass spectrometric peak intensities of the product.

Scheme 2. Formation of macrocyclic dimer and trimer lactams catalyzed by CALB in toluene.

Reactions between two different aminocarboxylic acids were performed to determine whether mixed macrocyclic lactams could be formed as well.¹⁶ However, instead of forming macrocycles comprised of the two aminocarboxylic acids, in all cases, mixtures of two or three different lactams were formed. In fact, the products resemble the lactams formed in the reactions with individual aminocarboxylic acids as outlined above. The products were formed in a ratio of 1:1 (calculated from the ¹H NMR spectra of the crude products) which perfectly matched the feed ratio. Thus it is obvious that CALB has a preference for homocyclization of the individual aminocarboxylic acids.

Other enzymes such as CALA (immobilized on imobead 150 and in form of CLEA) and cutinase in the form of CLEA were tested for the synthesis of homocyclic and macrocyclic lactams as well. However, these enzymes did not catalyze the cyclization reactions and no lactams were formed.

In summary, this study has shown that dried CALB is able to catalyze the cyclization of 6-aminocaproic acid to ϵ -caprolactam in moderate yields of up to 60% after 5 days. The anhydrous reaction conditions play a crucial role in increasing the yield of ϵ -caprolactam formation. Other lactams such as butyrolactam, δ -valerolactam, and dimer and trimer lactams can be formed from enzymatic reactions of 4-aminobutanoic acid, 5-aminovaleric acid, and 8-aminooctanoic acid using N435 as the catalyst. Reactions with mixtures of the individual aminocarboxylic acids showed that CALB prefers homocyclization of the aminocarboxylic acids thus preventing the formation of mixed macrocyclic lactams.

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Supplementary data

Supplementary data (¹H NMR spectra, ESI-MS chromatogram, and synthetic and transesterification procedures) associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.tetlet.2012.10.133>.

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- Enzymatic syntheses of lactams were carried out in concentrated (~1 M) and dilute systems.
- Synthesis of lactams in concentrated systems (~1 M): A mixture of aminocarboxylic acid (2.5 mmol) (6-aminocaproic acid or 8-aminooctanoic acid), N435 (100 mg), and dry toluene (2.5 mL) was stirred for 120 h at 90 °C under an N₂ atmosphere. Subsequently, the toluene was removed by rotary evaporation. An aliquot from the crude reaction mixture was analyzed by ¹H NMR in D₂O or in TFA-*d*.
- (a) Synthesis of lactams in dilute systems: A mixture of 6-aminocaproic acid (100 mg), N435 (100 mg), and dry toluene (5 mL) was stirred for 72–120 h, at 90 °C under an N₂ atmosphere. After cooling to room temperature, the toluene was removed by rotary evaporation. CHCl₃ (20 mL) was added to the residue to extract the lactam product. After filtration and evaporation of the CHCl₃, the remaining product (ϵ -caprolactam) was purified using short path distillation (Kugelrohr); yielding a white crystalline product (60%); mp 68.68 °C (Lit. 68–71 °C).
(b) ϵ -Caprolactam (yield 60%); ¹H NMR (400 MHz, CDCl₃): δ = 5.89 (s, 1H, NH); 3.21 (dd, *J* = 9.67, 5.69 Hz, 2H, CH₂); 2.47 (m, 2H, CH₂); 1.70 (m, 6H, CH₂). (C₆H₁₁NO) (113.16): Calcd C 63.68, H 9.80, N 12.38. Found C 63.60, H 9.97, N 12.33.
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- (a) Control reactions were conducted without enzyme, by stirring the aminocarboxylic acid in toluene for 72–120 h at 90 °C under an N₂ atmosphere. Using the same procedure, other control reactions were performed using deactivated N435 and macroporous acrylic polymer beads.
(b) The determination of the activity from deactivated N435 was accomplished by the transesterification of 4-nitrophenyl acetate (transesterification assay) and by enzymatic ring-opening polymerization of ϵ -caprolactone (synthetic activity assay).^{3c} These assays are required to determine whether there is any residual activity of N435 left after the thermal deactivation process. N435 was deactivated by heating at 150 °C under vacuum for 4 and 24 h, and subsequently used in the control reactions.
- (a) Using similar procedures, β -alanine, 4-aminobutanoic acid, 5-aminovaleric acid, 8-aminooctanoic acid, and 12-aminododecanoic acid were used to synthesize the corresponding lactams.
(b) Butyrolactam (conversion 30%); ¹H NMR (400 MHz, CDCl₃): δ = 6.32 (s, 1H, NH); 3.39 (t, *J* = 6.94 Hz, 2H, CH₂); 2.29 (t, *J* = 8.07 Hz, 2H, CH₂); 2.13 (m, 2H, CH₂).
(c) δ -Valerolactam (conversion 70%); ¹H NMR (400 MHz, CDCl₃): δ = 6.54 (s, 1H, NH); 3.29 (t, *J* = 5.95 Hz, 2H, CH₂); 2.34 (t, *J* = 6.46 Hz, 2H, CH₂); 1.76 (m, 4H, CH₂).
- Dimer and trimer lactams (conversion 41%); ¹H NMR (400 MHz, CDCl₃): δ = 5.43 (s, 2H, NH); 3.30 (dd, *J* = 12.36, 6.09 Hz, 4H, CH₂); 2.16 (m, 4H, CH₂); 1.63 (m, 4H, CH₂); 1.47 (m, 4H, CH₂); 1.30 (m, 12H, CH₂). MS (ESI) dimer: *m/z* = 283.24 [M+H]⁺ and 305.22 [M+Na]⁺; trimer: *m/z* = 424.35 [M+H]⁺ and 446.33 [M+Na]⁺.
- The syntheses of macrocyclic lactams were performed using two different aminocarboxylic acids in equimolar amounts (0.485 mmol in 5 mL of toluene).