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Enzymatic Formation of Lactams in Organic Solvents

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Abstract: Porcine pancreatic lipase in organic solvents catalyses the intramolecular cyclisation of aminoesters and the formation of macrocyclic bislactams from diesters and diamines.

In this Paper we report the enzyme-catalysed formation of lactams in organic solvents. In particular we have looked at (a) the formation of small ring lactams by intramolecular aminolysis of the corresponding aminoesters; and (b) the formation of macrocyclic bislactams *via* a bimolecular condensation of a diamine and a diester. The studies extend the use of enzymes in organic solvents for amide bond formation.

It is now well established that hydrolytic enzymes are highly stable in organic solvents and can be used for certain types of transformations which are difficult or impossible to do in water.[1] The most common reactions are lipase-catalysed stereoselective esterifications and transesterifications, which have been extensively used for the preparative resolution of chiral acids and alcohols.[1] Recently we reported the lipase-catalysed preparation of γ- and δ–lactones by intramolecular transesterification of hydroxyesters,[2] and two other groups have described the formation of macrocyclic lactones by condensation between diacids (or diesters) and diols under carefully controlled kinetic conditions.[3] Proteases in organic solvents have previously been used to catalyse the enzymatic resolution of racemic amines by aminolysis.[4]

Intramolecular aminolysis

1 – 8 see Table

Scheme 1

Amino esters (**2-8**, Scheme 1) undergo intramolecular aminolysis in organic solvents to give lactams of ring size 5-7. This reaction was found to be catalysed by several enzymes including: liver acetone powder horse, liver acetone powder porcine, subtilisin, the protease from *Streptomyces griseus,* lipases from *Pseudomonas* species, and porcine pancreatic lipase.

The Table shows the results of a study using crude pancreatic porcine lipase (Sigma). The cyclisations to form five and six-membered rings proceed at similar rates. Formation of a seven membered ring is much

slower, and significantly the amino ester **1** did not cyclise to give the four-membered β-lactam ring. Also the secondary amines **5** and **7** cyclised more slowly than the corresponding primary amines **3** and **6**.

For this study of intramolecular cyclisation of aminoesters it was necessary to suppress the significant uncatalysed cyclisation.[5] This was achieved by using isopropyl esters rather than the less sterically hindered methyl or ethyl esters, and by performing the reaction in tertiary amyl alcohol where very low rates of intramolecular cyclisation are found. For example, methyl γ-aminobutyrate cyclises in isooctane in 85 % yield in 12 hours whereas less than 2% of isopropyl γ-aminobutyrate cyclises in tertiary amyl alcohol in 7 days.

Intramolecular Aminolysis of Aminoesters Catalysed by Crude Pancreatic Porcine Lipase

No	R^1	R^2	R^3	n	time (days)	enzymatic conversion (%)
1	H	H	CH_2CH_3	1	7	0
2	H	CO_2Et	CH_2CH_3	2	2	50
3	H	H	$CH(CH_3)_2$	2	3	45
4	H	CH_3	$CH(CH_3)_2$	2	6	40
5	CH_3	H	$CH(CH_3)_2$	2	6	30
6	H	H	$CH(CH_3)_2$	3	4	80
7	CH_3	H	$CH(CH_3)_2$	3	5	40
8	H	H	$CH(CH_3)_2$	4	7	10

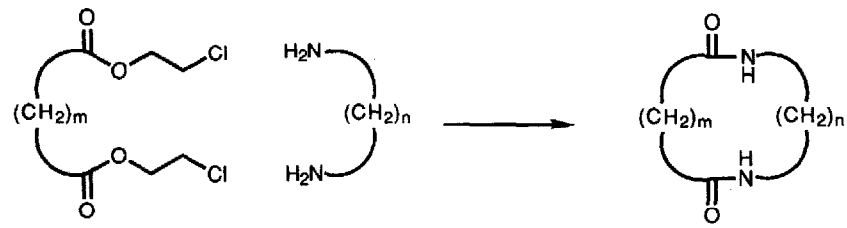
In order to investigate the enantioselectivity of the reaction, the racemates of the chiral compounds **2** and **4** were cyclised using the different enzymes listed above. The reactions were stopped at low conversion (40 %) to increase the chance of seeing any selectivity. The products were then examined by NMR spectroscopy in the presence of a chiral shift reagent.[6] Enantioselection was only seen in one case, the subtilisin catalysed cyclisation of **4**, and the measured enantiomeric excess was low (23 %). This lack of enantioselectivity contrasts with the high levels seen in intramolecular lactonisations of hydroxy esters.[2]

In a typical reaction the enzyme (1.1 g crude porcine pancreatic lipase, or 180 mg of subtilisin lyophylised after adjustment to pH 7.8)[7] is added as a powder to a solution of the aminoester (1 mmole) in tertiary amyl alcohol (12 ml, dried over 3 Å molecular sieves). The suspension is shaken vigorously at 200 rpm at 40 °C. After the reaction, the enzyme is removed by filtration, and the products purified chromatographically.

Formation of bislactams

The formation of macrocyclic bislactams provides a route to large host molecules for use in host guest chemistry.[8] We have performed preliminary studies on the enzymatic formation of macrocyclic bislactams from diesters and diamines (Scheme 2). The reaction is catalysed by porcine pancreatic lipase (either crude or purified) but not by BPN XXVII, subtilisin VIII, PSL (K10) lipase type XIII, or *Candida cylindracea* lipase. This reaction proceeds with the activated monochloroethyl diester, but not with the ethyl ester or free acid. Unlike the intramolecular aminolysis to form 5 and 6 membered rings, control experiments showed that no product was formed in the absence of enzyme . There is also a markedly different solvent requirement, as the

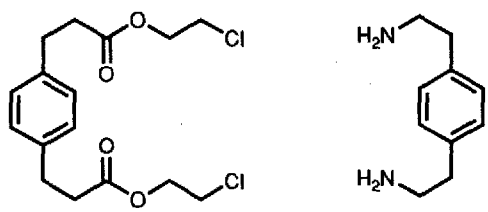
bislactams are formed enzymically in dichloromethane or chloroform at reflux but not in ether or tertiary amyl alcohol.



9a m = 8
9b m = 10

10a n = 10
10b n = 12

11a m = 8, n = 10
11b m = 10, n = 12



12 **13**

Scheme 2

The formation of bislactam is followed over several days by NMR spectroscopy, increasing to about 40 % conversion after 4 days. In a typical procedure porcine pancreatic lipase (1 g crude or 1.5 mg purified) is added to a 1:1 mixture of diamine and diester (1 mmole) in dichloromethane (10 ml) in the presence of 4 Å molecular sieves. The resulting suspension is heated at reflux and shaken at 200 rpm. After 3-4 days the enzyme is removed by filtration and the products purified chromatographically. The identity of the cyclic products was confirmed spectroscopically and by comparision with bislactams prepared non-enzymically from the appropriate diacid chloride and diamine.

Preliminary studies on the substrate specificity of this reaction have been carried out. Most of the experiments to determine the reaction conditions were performed with the diester (**9a**) and diamine (**10a**). Using the conditions described above, this reaction gave the corresponding bislactam (**11a**) in 35-45 % as well as some uncyclised oligimers.[9] A similar yield of (**11b**) was obtained using the longer diester (**9b**) and diamine (**10b**). No cyclised products were isolated from the reaction of the aromatic diester (**12**) and aromatic diamine (**13**),[10] but the reaction of the aromatic diamine (**13**) and the diester (**9a**) formed the corresponding cyclic bislactam (25 % by NMR spectroscopy) as well as linear oligomers.

The novel reactivity of lipases in organic solvents described in this Paper complements reports of lipase-catalysed lactone[2] and bislactone formation.[3]

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6. Enantiomeric excesses were determined by NMR spectroscopy in CCl_4: $CDCl_3$ 2:1 in the presence of the chiral shift reagent *tris*[3-(trifluoromethyl-hydroxymethylene)-(+)-camphorato] europium III [Eu(tfc)$_3$].

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9. In most experiments varying amounts of linear oligomers are formed. Only the lower oligomers (A-E, E-A-E) were characterised.

10. The diamine (**13**) was made by reduction of the corresponding dinitrile by Raney Nickel under hydrogen (90 atmos) at 100 °C in ethanol.

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