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Enantioselective synthesis of amino acid amides via enzymatic ammoniolysis of amino acid esters

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Abstract

Ammoniolysis of D,L-phenylglycine methyl ester catalysed by *Candida antarctica* B lipase (Novozym 435) gave D-phenylglycine amide with 78% ee at 47% conversion. The combination of this reaction with racemisation of the unconverted ester in a single step was investigated. Pyridoxal and salicylaldehyde efficiently catalysed the racemisation of the ester. Because the solubility of the amide was low under the reactions conditions, its racemisation was slow. In-process racemisation of the reactant gave D-phenylglycine amide with 73% ee at 85% conversion.

Keywords: Ammoniolysis; Deracemisation; *Candida antarctica*; lipase; D-Phenylglycine amide; Dynamic kinetic resolution

1. Introduction

Lipase catalysed ammoniolysis is an attractive method for the transformation of carboxylic esters into amides due to its mildness and selectivity [1–8]. Esters of chiral alcohols or chiral carboxylic acids can be resolved in high enantioselectivities via lipase-catalysed ammoniolysis [1,2]. Ammoniolysis of amino acid esters is also efficiently catalysed by lipases and several proteases [9]. Recent developments in the industrial synthesis of semisynthetic penicillins and cefalosporins prompted us to develop the enzyme catalysed ammoniolysis into a practical synthesis of D-phenylglycine amide from the racemic amino acid ester (see Fig. 1).

Ammoniolysis would become much more attractive if it could be developed into a dynamic kinetic resolution by combining the ammoniolysis step with racemisation of the unconverted ester; this would make a 100% yield—theoretically—possible. For such a scheme to be effective, the product should racemise much more slowly than the reactant. In the present paper we present a preliminary account of the in-process racemisation catalysed by pyridoxal and salicylaldehyde.

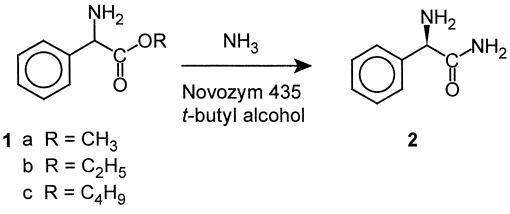

Fig. 1. Enantioselective ammoniolysis of D,L-phenylglycine esters.

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2. Experimental

Candida antarctica B lipase (Novozym 435) was kindly donated by Novo Nordisk (Bagsværd, Denmark) and was used as received.

Reactions were performed [9] by adding 0.2 g (0.99 mmol) of phenylglycine methyl ester hydrochloride to 5 ml ammonia-saturated *tert*-butyl alcohol (12.5 mmol ammonia). About 50 mg Novozym 435 was added and dry ammonia gas was bubbled through the reaction mixture; the reaction temperature was maintained at 40°C by immersion in a thermostated oil bath. Samples were periodically withdrawn and analysed by HPLC on a 4×150 mm 5 μ Crownpak CR column from Daicel Chemical Industries. Aqueous perchloric acid at 0.6–1.0 ml/min was used as eluant. Full experimental details will be reported elsewhere.

3. Results and discussion

3.1. Ammoniolysis

Because *C. antarctica* lipase B (Novozym 435) had emerged from our initial study [9] as the catalyst of choice, it was used throughout the present work. The influence of the leaving (alcohol) group size on the course of the reaction was briefly investigated. The data in Table 1 show that the reaction rate decreases from methanol to butanol, but the effect on the enantioselectivity ratio E is negligible [10]. [1,2] In view of these results we used phenylglycine methyl ester for further experiments.

Table 1
Ammoniolysis of D,L-phenylglycine esters; effect of the leaving group size[a]

Ester	Conversion (%)	ee_e (%)	ee_a (%)	E_e	E_a
1a	47	69	78	16	17
1b	36	47	84	18	18
1c	16	17	89	19	20

[a] Reaction conditions: D,L-phenylglycine ester, HCl (1.0 mmol), 2.5 M ammonia and Novozym 435 (50 mg) in *tert*-butyl alcohol (5 ml) at 40°C for 4 h.

3.2. Racemisation via Schiff-base intermediates

D-Phenylglycine methyl ester was subjected to pyridoxal catalysed racemisation under ammoniolysis conditions (*tert*-butyl alcohol, 2.5 M ammonia). The ee decreased to 0% according to first order reaction kinetics. The initial racemisation rate was found to obey Michaelis–Menten kinetics according to:

$$r = \frac{(10.4 \times 10^{-3})\,[\text{D-Phg-OMe}]}{0.178 + [\text{D-Phg-OMe}]}\,[\text{Pyridoxal}]. \quad (1)$$

We conclude that the reaction takes place via a substrate binding step with dissociation con-

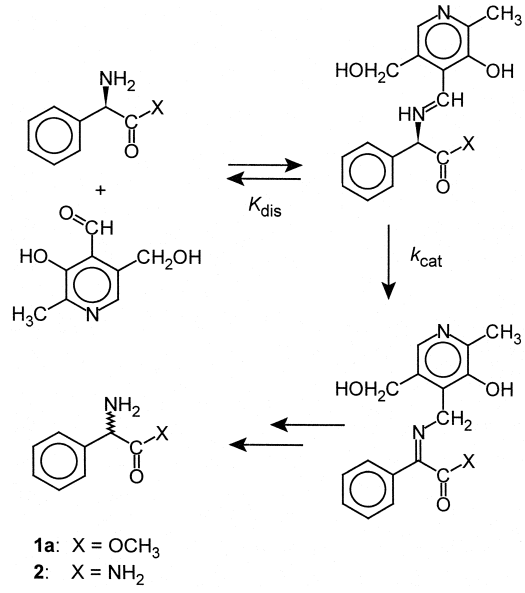

Fig. 2. Pyridoxal catalysed racemisation of D-**1a**.

[1] Enantiomeric ratios for the ester (E_e) and the amide (E_a) were calculated from the conversion (c) and the optical purities of the ester (ee_e) and the amide (ee_a) as follows:For the ester:

$$E_e = \frac{\ln[(1-c)(1-ee_e)]}{\ln[(1-c)(1+ee_e)]}.$$

For the amide:

$$E_a = \frac{\ln[(1-c(1+ee_a)]}{\ln[(1-c(1-ee_a)]}.$$

See: Ref. [10].

[2] Experimental values for E were lower than those found previously [9] because the enantiomeric analysis has been improved considerably.

Table 2
Ammoniolysis with in-process racemisation of D,L-phenylglycine methyl ester[a]

Racemisation catalyst	Ratio ester/racemisation catalyst	Time (h)	Conversion (%)	ee_a (%)
Pyridoxal	200	4	59	78
		5.7	68	75
	300	5.5	53	77
		19	80	68
Salicylaldehyde	100	19	85	73

[a]Reaction conditions as described in Table 1.

stant K_{dis} 0.178 M and catalytic rate constant k_{cat} 21×10^{-3} s^{-1} (see Fig. 2, the racemisation rate constant is half the kinetic rate constant). The racemisation of D-phenylglycine amide (D-**2**), which was much slower, similarly obeyed Michaelis–Menten kinetics with K_{dis} 0.045 M and k_{cat} 0.74×10^{-3} s^{-1}.

The high cost of pyridoxal would be an obstacle for its practical application as a racemisation catalyst. Salicylaldehyde (2-hydroxybenzaldehyde) could be a suitable replacement, although it is less active than pyridoxal by a factor of 10. Measurement of its kinetic parameters is in progress.

3.3. In-process racemisation

The results of preliminary ammoniolysis experiments with pyridoxal- or salicylaldehyde-catalysed in-process racemisation are given in Table 2. It was found that the ratio of **1a** and racemisation catalyst is critical, as might be expected. The conversion of D,L-**1a** into D-**2** could be increased considerably at a small penalty in ee. Further work is in progress to improve these results.

4. Conclusion

Lipase catalysed ammoniolysis of D,L-phenylglycine methyl ester and in-process racemisation of the slow-reacting L-ester via a Schiff base intermediate can be combined in one step. D-Phenylglycine amide with 73% ee was obtained at 85% conversion.

Acknowledgements

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