Ester Ammoniolysis: a New Enzymatic Reaction

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A new enzymatic reaction of carboxylic esters and ammonia (ammoniolysis) provides a synthetically useful and mild procedure for the enantioselective synthesis of amides.

Lipases and esterases (EC 3.1.1) comprise a versatile group of enzymes that catalyse the hydrolysis of esters, esterifications and transesterifications *via* an acylenzyme intermediate.[1] Recently, other unnatural nucleophiles, *e.g.* amines[2], oximes[2], hydrazine[3] and hydrogen peroxide[4] have been added to the repertoire of nucleophiles that lipases can accommodate, according to the general Scheme 1.

Surprisingly, the enzymatic ammoniolysis of esters (NuH = NH_3) has hitherto not been described. We now report that certain lipases are efficient catalysts for this reaction at

$$RCO_2R' + Enz\text{–}H \underset{R'OH}{\overset{-R'OH}{\rightleftharpoons}} RCO\text{–}Enz \underset{-NuH}{\overset{NuH}{\rightleftharpoons}} RCONu + Enz\text{–}H$$

$NuH = H_2O, ROH, H_2O_2, RNH_2, N_2H_4, R_2C{=}NOH$

Scheme 1 General lipase-catalysed reaction

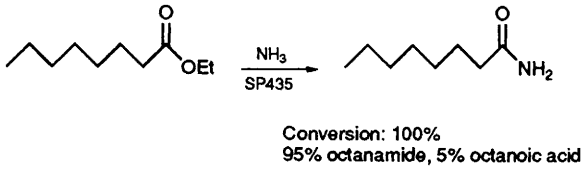

Fig. 1 Ammoniolysis of ethyl octanoate

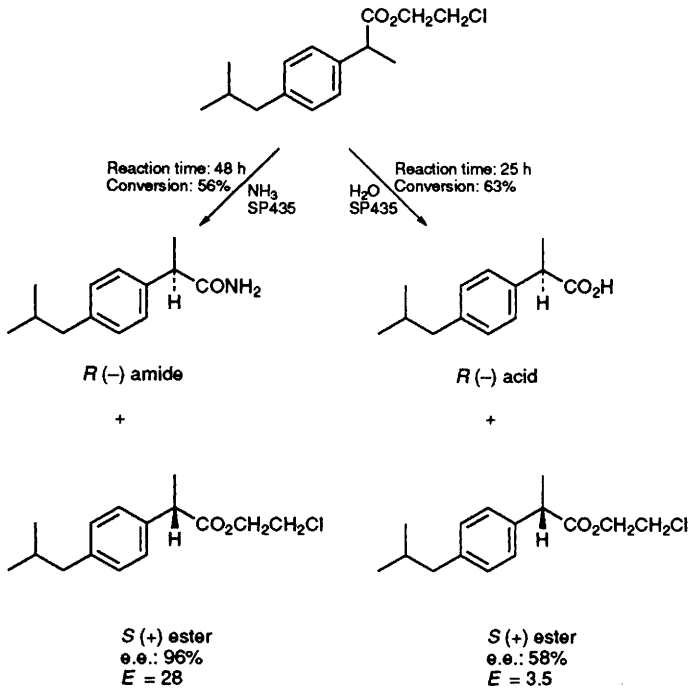

Fig. 2 Ammoniolysis and hydrolysis of Ibuprofen 2-chloroethyl ester

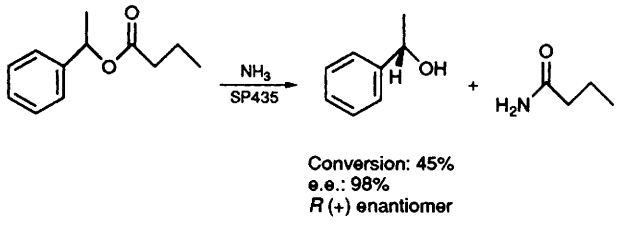

Fig. 3 Ammoniolysis of α-methylbenzyl *n*-butyrate

ambient temperature. It provides a mild procedure for the conversion of esters to the corresponding amides. Moreover, the synthetic utility of the method is greatly enhanced by taking advantage of the regio- and/or enantio-selective capabilities of enzymes.

An initial feasibility study was carried out using the ammoniolysis of ethyl octanoate (Fig. 1) as the test reaction. The standard procedure was as follows: gaseous NH_3 was bubbled through a solution of ethyl octanoate (0.5 ml) in dry *tert*-butanol (5 ml) at room temp. After 5 min 100 mg of enzyme preparation (except for SP435 where 10 mg was used) was added. The mixture was stirred at room temp. under NH_3 and the course of the reaction followed by HPLC analysis using diethylene glycol dibutyl ether as an internal standard. Of a wide variety of lipases, esterases and proteases tested (details will be reported elsewhere), only the *Candida antarctica* lipase SP435 and the lipases SP398 and SP523 showed high activity. After 24 h reaction time the octanamide yields with SP435, SP523 and SP398 were 95, 85 and 60%, respectively. The enzyme preparations of SP398 and SP523 were immobilized on Accurel EP100.

Since lipases also catalyse esterifications we reasoned that it should be possible to carry out the conversion of a carboxylic acid to the corresponding amide *via in situ* formation of the ester (direct ammoniolysis is not feasible because the ammonium salt of the carboxylic acid is not a suitable substrate). This indeed proved to be the case. Thus, when 100 mg of SP435 was added to a solution of octanoic acid (0.5 ml) in 96% ethanol (5 ml) and the mixture stirred for 24 h at 40 °C, 95% of the octanoic acid was converted to ethyl octanoate. The mixture was filtered, 5 ml of *tert*-butanol added and the ethanol removed by vacuum distillation. A further 2 ml of *tert*-butanol was added and 100 mg of SP435. Ammonia was bubbled through the solution at room temp. for 24 h to give a mixture containing 80% octanamide, 10% ethyl octanoate and 10% octanoic acid.

Although the above-described enzymatic ammoniolysis is of synthetic value as such its utility would be greatly enhanced if it exhibited enantioselectivities superior to conventional hydrolytic processes. In order to compare the enantioselectivity of ammoniolysis *vs.* hydrolysis we chose the 2-chloroethyl ester of ibuprofen **1** as a model substrate (Fig. 2).

Ammonia was bubbled through a solution of **1** (0.5 ml) in *tert*-butanol (5 ml), together with 50 mg of SP435 lipase at room temp. After 48 h 56% of **1** was converted and the remaining *S*-ester had an enantiomeric excess (e.e.) of 96% (HPLC on a Chiralcel OD column). By comparison SP435-catalysed hydrolysis of **1** under the same conditions (0.27 ml water instead of ammonia) gave 63% conversion after 25 h and the remaining *S*-ester had an e.e. of 58%. Based on these results the enantiomeric ratios (*E*) of the two reactions were calculated to be 28 and 3.5 for ammoniolysis and hydrolysis, respectively. In other words, lipase-catalysed ammoniolysis is an order of magnitude more enantioselective than the corresponding hydrolysis.

Similarly, enzymatic ester ammoniolysis also can be used for the kinetic resolution of chiral alcohols. Thus, ammoniolysis of *R*/*S*-α-methylbenzyl *n*-butyrate (Fig. 3) under the same conditions as described above gave 45% conversion in 120 h and the e.e. of *R*-α-methylbenzyl alcohol product was 98% (GC on an Astec Chiraldex G-TA column).

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