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(57) Abstract

A process for carrying out enzymatically catalyzed conversions of organic compounds comprises reacting an ester of an organic acid in a liquid organic phase with ammonia in the presence of a hydrolytic enzyme such as a lipase, esterase or protease. The process permits the highly enantioselective preparation of enantiomers of amides, esters and/or alcohols in good yield from racemic starting material under very mild reaction conditions.
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A PROCESS FOR CARRYING OUT ENZYMATICALLY CATALYZED CONVERSIONS OF ORGANIC COMPOUNDS

The present invention relates to a process for carrying out enzymatically catalyzed conversions of organic compounds.

An article by Alexander M. Klibanov, entitled "Asymmetric Transformations Catalyzed by Enzymes in Organic Solvents", in Acc. Chem. Res. 1990, 23, 114-120, gives an overview of the state of the art in the field of enzymatically catalyzed asymmetric conversions of organic compounds in organic solvents. It is also mentioned in passing that the asymmetric hydrolysis of chiral esters in water under the catalytic activity of hydrolases, in particular lipases and proteases, has been employed successfully for years. When it is desired to resolve a racemic alcohol or a racemic acid, that alcohol or that acid is chemically esterified, whereafter a more or less selective hydrolysis of only one enantiomer is carried out using a hydrolase.

An article by V. Gotor, entitled "Enzymatic Aminolysis, Hydrazinolysis and Oximolysis Reactions", in Microbiological Reagents in Organic Synthesis, S. Serui, Kluwer Academic Publishers, Dordrecht, 1992, pp. 199-208, reports, among other things, on the conversion of racemic ethyl 2-chloropropionate with a number of aliphatic and aromatic amines in the presence of catalytically active enzymes. The lipase from Candida cylindracea proves to give the best results. Depending on the starting amine, amides are obtained with a high or sometimes only a minor enantioselectivity.

It has now been found that enzymatically catalyzed conversions of particular organic compounds can also be carried out with ammonia and that such conversions afford important application possibilities (vide infra).

Accordingly, the present invention provides a process for carrying out enzymatically catalyzed conversions of organic compounds, wherein an ester of an organic acid is reacted with ammonia in a liquid organic phase in the presence of a hydrolytic

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enzyme. This conversion reaction with ammonia is referred to herein as ammonolysis, and leads to the obtaining of amides.

In preferred embodiments of the invention, the organic acid ester is a carboxylic acid ester. However, it is believed that the invention may also be applied to conversions of, e.g., phosphoric and sulfonic acid esters.

In cases where the acid component (organic acid ester) is derived - in accordance with a preferred embodiment of the invention - from a carboxylic acid, the conversion according to the invention can be generally represented as follows:

\[
\text{RCO}_2R' + \text{NH}_3 \longrightarrow \rightarrow \text{RCONH}_2 + R'O\text{H}
\]

The carboxylic acid (RCOOH) corresponding to the ester in question may be a simple carboxylic acid (such as a straight- or branched-chain alkanoic or alkenoic acid) or a carboxylic acid of more complex structure. Thus, it may, for example, be selected among substituted and unsubstituted aliphatic, cycloaliphatic (alicyclic), aromatic and heterocyclic carboxylic acids, including aliphatic and cycloaliphatic carboxylic acids containing aromatic and/or heterocyclic rings. Aliphatic or cycloaliphatic (alicyclic) carboxylic acids of the types in question, as well as substituents therein, may further be saturated or unsaturated (i.e. contain ethylenic and/or acetylenic double bonds). Carboxylic acids belonging to the above-mentioned types and containing two or more carboxylic acid (carboxy) functionalities are also of relevance in the context of the present invention.

Relevant examples of esters of simple carboxylic acids are to be found among esters of saturated C_{1}-C_{10} carboxylic acids, e.g. propionic, butyric, isobutyric, valeric, isovaleric, caproic (hexanoic), heptanoic, caprylic (octanoic), nonanoic and decanoic acids. Among examples of suitable esters of carboxylic acids of somewhat more complex structure may be mentioned esters of the chiral acids 2-(6-methoxy-2-naphthyl)propionic acid (an enantiomer of which is marketed as an
antiinflammatory/analgesic drug under the name, *inter alia*, of "Naproxen"), 2-(4-isobutylphenyl)propionic acid (the racemic form of which is marketed as an antiinflammatory drug under the name, *inter alia*, of "Ibuprofen") and trans-(4-methoxyphenyl)glycidic acid (derivatives of which are useful, for example, in the synthesis of a vasodilatory drug marketed under the name, *inter alia*, of "Diltiazem").

The ester function (alcohol component) in the organic acid ester employed in the process of the invention can be derived from a wide variety of substituted and unsubstituted alcohols, including lower aliphatic alcohols such as methanol, ethanol, propanol and higher homologues with, e.g., 4, 5, 6 or more carbon atoms, and may in this connection contain aromatic rings (such as substituted or unsubstituted phenyl or naphthyl rings) and/or heterocyclic rings (e.g. substituted or unsubstituted pyridyl rings). However, some ester functions have been found to be more reactive than others. Thus, for example, a substituted ethanol such as 2-chloroethanol provides a quite reactive ester function. More generally, it appears that the more reactive ester functions, notably when using lipases as enzymes, will be derived from relatively lipophilic alcohols, including moderately long-chain (e.g. C₆-C₁₃) alkanols.

In carboxylic acid esters of the above-mentioned general formula RCO₂R′, R and R′ may also be attached to each other. In such cases the starting material is a lactone, and the reaction product then obtained is a hydroxy-substituted amide.

According to an interesting variant of the invention, a carboxylic acid ester RCO₂R′ to be used as starting material for the ammonolysis procedure can be prepared enzymatically (i.e. using an enzyme - e.g. an esterase or a lipase - as catalyst) from the acid (RCOOH) in question and the alcohol (R′OH) in question, whereafter the ester obtained is subjected to the present ammonolysis using the same or a different enzyme. When using a liquid alcohol, the alcohol itself can then serve as solvent at the same time. In this protocol, the water which is released during the ester formation should be removed, preferably continuously, from the reaction medium in order to maximise the degree of ester formation and, notably, the subsequent conversion of the resulting ester to the amide. This variant of the process of the
invention has a number of important advantages: Firstly, e.g., the ester formed need not be separated from the reaction medium (although it may, of course, if so desired), and it is therefore possible [cf. the working examples provided herein (vide infra)] to carry out the ester synthesis in situ (i.e. in the reaction vessel system subsequently used for the ammonolysis reaction) prior to performing the ammonolysis procedure, i.e. to carry out the entire sequence of reactions (i.e. starting from acid + ester and ending up with the amide) as a "one-pot" procedure. Secondly, e.g., it is possible to use as starting materials the acid and the alcohol, both of which are typically more readily available starting materials than the ester derived therefrom. Acids and alcohols suitable for use in this variant of the process of the invention may be selected among those already mentioned above.

When particular hydroxycarboxylic acids are used as starting material, hydroxy-substituted amides can be prepared without requiring the presence of a separate alcohol.

Numerous hydrolytic enzymes can be used as catalytic components in the practice of the present conversions. Enzymes belonging to the group of the esterases and in particular of the lipases have been found to be particularly suitable. Proteases have, however, also proved to be well suited for certain purposes, such as the ammonolysis of protected (notably N-protected) amino acid esters. These enzymes are commercially available, sometimes immobilized on a support. If they are not provided in supported form by the supplier, it is generally preferable that they be immobilized on a support before use in the process of the invention, since it is then easier to separate the enzyme from the reaction medium afterwards and to obtain the desired reaction product. The preparation of the supported enzyme can be carried out by any appropriate known technique. A suitable technique is disclosed, for instance, in WO 90/15868.

As examples of suitable enzymes for use in accordance with the invention, the following can be mentioned:
Lipases:

lipase SP 435 (a supported form of *Candida antarctica* lipase B, disclosed in EP 0 287 634), available from Novo Nordisk A/S, Denmark, under the name Novozym® 435;

5 lipase SP 398 and lipase SP 523, both available from Novo Nordisk A/S;

lipase SP 524 (from *Rhizomucor miehei*), available in supported form from Novo Nordisk A/S;

lipoprotein lipase from *Pseudomonas*, available from Boehringer Mannheim GmbH, Germany, catalogue No. 734 286;

10 lipase from *Rhizopus arrhizus*, available from Boehringer Mannheim GmbH, catalogue No. 186 791;

porcine pancreas lipase (type II, 70 units/mg), available from Sigma Chemical Company, St. Louis, USA; and

Amano PS lipase, unimmobilized preparation available from Amano, Japan (possibly cross-linked, since it displays low solubility in water);

Esterases:

cholesterol esterase from *Candida rugosa* (*C. cylindracea*), available from Boehringer Mannheim GmbH, catalogue No. 129 046.

Proteases:

20 protease SP 539, available from Novo Nordisk A/S; and
Subtilisin A, available from Novo Nordisk A/S.

The reaction medium, i.e. the liquid organic phase, for practicing the present process may contain one or more organic solvents. Solvents are required only in certain cases where one of the starting materials or one of the reaction products is a solid substance. When an ester is converted, it is usually dissolved in a solvent such as an alcohol, for instance tert-butyl alcohol. When the starting materials are an organic acid and an alcohol, according to the variant of the process of the invention described above, then an excess of the alcohol in question can often serve as solvent, optionally in combination with one or more other suitable solvents.

In general, solvents suitable for use in the process of the invention are solvents which are of moderate to low polarity, display limited to low miscibility with water and are good solvents for ammonia (i.e. substantially anhydrous ammonia). Solvents meeting these requirements are to be found, for example, among alcohols, suitably certain lower aliphatic alcohols, and in particular tertiary aliphatic alcohols such as tert-butyl alcohol, tert-amyl alcohol and the like, which are sterically relatively hindered with respect to nucleophilic reaction of the oxygen atom of the alcoholic hydroxy group and therefore have relatively little tendency to compete as nucleophiles with ammonia (in ammonolysis) or with ester-forming alcohols (in ester formation from an acid and an alcohol). Secondary alcohols, and in some cases primary alcohols, which generally speaking are less sterically hindered in this respect than corresponding tertiary alcohols, may nevertheless be satisfactory solvents for certain embodiments of the process of the invention. Other types of organic solvents which appear to be suitable for use in the context of the invention include, e.g., certain ethers.

Typically, the concentration of the starting material(s) to be converted is between 3 and 50%, calculated on the basis of the liquid organic phase. However, higher concentrations are quite possible, and concentrations of, e.g., up to about 60% or even about 70% appear to be feasible.
The typical procedure will then be to add the enzyme - preferably immobilized on a support - to the reaction medium, which will typically be a solution (as discussed above). The amount of enzyme to be used is partly determined by the extent to which the enzyme is active towards effecting the conversion contemplated. This can easily be determined experimentally by methods well known per se. For some enzymes, the required amount of enzyme is larger, sometimes even considerably larger, than for others. For any selected enzyme, of course, the extent of conversion can also be increased by prolonging the reaction time.

The reaction may then be carried out by allowing ammonia to act on the reaction medium. For this purpose, gaseous ammonia at atmospheric pressure (obtained, e.g. from a "lecture bottle" or the like) can be bubbled through the reaction medium. However, and particularly when using an organic solvent (or mixture of solvents) in which the solubility of ammonia at atmospheric pressure is relatively poor, ammonia can also be allowed to act under pressure, so that the reaction velocity can be increased. The use of liquid ammonia (necessitating an appropriately low reaction temperature) may in some cases be advantageous, especially if ester substrates of low thermal stability are involved.

In embodiments of the process of the invention in which tert-butyl alcohol is employed as solvent, it is often advantageous to use a saturated solution of ammonia in tert-butyl alcohol, prepared by bubbling ammonia gas through the solvent for a moderately extended period of time. Such a saturated solution at ambient temperature contains approximately 2.5 mol of ammonia per liter, and it may suitably be used directly to perform the ammonolysis in an essentially closed system; alternatively, the saturated solution may be diluted with tert-butyl alcohol to achieve a desired concentration of ammonia lower than the saturation concentration. An ammonia concentration less than 2.5 mol/l is advantageous for the stability of certain lipases and proteases.

The conversion is typically carried out at room temperature (i.e. ambient temperature), although a higher temperature may be employed to increase the
velocity, or a lower temperature to improve the selectivity and/or (as mentioned above) to minimize thermal degradation of thermolabile reaction components. A temperature in the range of about 15-50°C will generally be suitable. Temperatures in the range of about 20-40°C, e.g. 20-25°C, will often be very suitable for the ammonolysis procedure, whereas it may be appropriate to employ a rather higher temperature, such as a temperature in the range of about 25-50°C, e.g. around 40°C, for enzyme-catalyzed ester formation from an acid and an alcohol. In any case, the overall reaction conditions can be classified as particularly mild. After the desired conversion has been achieved, the enzyme can be separated from the reaction medium, typically by filtration, whereafter the filtrate can be subjected to further treatment using conventional techniques in order to obtain the intended product.

The invention affords a number of interesting advantages and application possibilities, some of which will be discussed in the following:

The first to be mentioned is the provision of a process for preparing amides under very mild conditions. This is of importance in cases where it is desired to perform conversions on very sensitive molecules which are not resistant to the temperature at which amides are formed in previously known chemical procedures. The present process, by contrast, can be carried out at room temperature (or at least at a temperature not deviating greatly therefrom), and the other conditions are also such that even rather unstable starting materials are not at risk of being broken down or undergoing other undesired conversions.

Secondly, and very importantly, it has further been found that the ammonolysis procedure of the present invention exhibits high stereoselectivity when the starting material is a racemate, i.e. one of the enantiomers is converted with a high selectivity. The invention thus provides a process for separating the enantiomers from each other. The asymmetry (chirality) can reside in the acid component or in the alcohol component or even in both. It is both surprising and of great importance that the present ammonolysis has a higher enantioselectivity than the enzymatically catalyzed hydrolysis which is employed on an industrial scale, for instance in the
preparation of one enantiomer of the drug substance ibuprofen (*vide supra*) in pure form.

Thirdly, the present ammonolysis procedure can be used for carrying out regioselective and/or enantioselective conversions in compounds containing, for example, two ester groups, so that only one of those ester groups is converted to amide, whilst the other one is maintained in unmodified condition.

The invention will now be further illustrated and explained by means of the working examples given below. The enzymes investigated in the examples were in all cases used in immobilized form, i.e. immobilized on a support. As already mentioned, lipase SP 435 (a supported lipase) was supplied by the manufacturer (Novo Nordisk A/S), and the other enzymes were immobilized on a support consisting of ACCUREL EP 100. ACCUREL™ is an AKZO trade name for a particulate polypropene. The different types of ACCUREL are described in a datasheet on ACCUREL systems from AKZO, Fibers and Polymers Division, obtainable from Enka AG, Postfach D-8753 Obenbourg, Germany. The immobilization of the enzymes on ACCUREL EP 100 was performed by the method described in Example 3 of WO 90/15868, utilizing an enzyme-to-support ratio of 1:1. The amounts of enzyme specified in the Examples relate to the total amount of enzyme + support.
Abbreviations used in the following are as follows:

GC: gas chromatography
HPLC: high-performance liquid chromatography
HOAc: acetic acid
NaOAc: sodium acetate

EXAMPLE 1

Ammonolysis of ethyl octanoate

A solution of 0.5 ml ethyl octanoate and 100 μl diethylene glycol dibutyl ether (internal HPLC standard) in 5 ml anhydrous tert-butyl alcohol was agitated using an electrically driven agitating apparatus (referred to hereafter as a top agitator). Ammonia (from a lecture bottle) was bubbled through the solution. After 5 minutes 10 mg lipase SP 435 was added. The reaction was monitored by HPLC.

Sampling: 50 μl reaction mixture + 500 μl mobile phase.

HPLC: Novapak™ C18; mobile phase 60/40 acetonitrile/water; NaOAc/HOAc 0.01M; pH 4.5.

After 24 hours (100% conversion; 95% octanamide; 5% octanoic acid) the reaction mixture was processed further.

The isolation was carried out as follows. The enzyme was filtered off and washed with 1,2-dichloroethane. The washing liquid and the filtrate were combined, and 1,2-dichloroethane and tert-butyl alcohol were evaporated under vacuum. The resulting crude octanamide was then recrystallized from 40/60 petroleum ether at -20°C. The solid octanamide was filtered off and washed with cold 40/60 petroleum ether.
EXAMPLE 2

Ammonolysis of ethyl octanoate using other enzymes

The enzymatic activity of other enzymes was investigated in the same manner as in Example 1. The various immobilized enzymes were added in an amount of 100 mg. The following results were obtained:

<table>
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<th>Enzyme</th>
<th>Amide (%)</th>
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<tr>
<td>lipase SP 398</td>
<td>60% in 24 hours</td>
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<tr>
<td>lipase SP 523</td>
<td>85% in 24 hours</td>
</tr>
<tr>
<td>lipoprotein lipase from <em>Pseudomonas</em></td>
<td>15% in 24 hours</td>
</tr>
<tr>
<td>lipase from <em>Rhizopus arrhizus</em></td>
<td>10% in 72 hours</td>
</tr>
<tr>
<td>cholesterol esterase from <em>C. cylindracea</em></td>
<td>5% in 72 hours</td>
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EXAMPLE 3

Ammonolysis of ethyl butyrate

The reaction and further treatment of the reaction mixture were carried out as in Example 1, but employing ethyl butyrate as starting material instead of ethyl octanoate. After 24 hours (100% conversion; 95% butanamide; 5% butanoic acid) the reaction mixture was treated further.
EXAMPLE 4

Enzyme-catalyzed conversions starting from octanoic acid

A solution of 5 ml 96% ethanol, 0.5 ml octanoic acid and 100 µl diethylene glycol dibutyl ether (internal HPLC standard) was agitated at 40°C. To the solution was added 100 mg lipase SP 435. After agitation at 40°C for 24 hours (95% conversion to ethyl octanoate), the enzyme was filtered off and 5 ml anhydrous tert-butyl alcohol was added to the filtrate. The ethanol (together with water formed in the esterification) was removed by evaporation under vacuum. To the residue was added a further 2 ml anhydrous tert-butyl alcohol as well as 100 mg lipase SP 435. Ammonia was then bubbled through the reaction medium at room temperature. The ammonolysis was monitored by HPLC (see Example 1). After ammonia had been bubbled through the reaction medium for 24 hours, the conversion was 90% (80% octanamide; 10% octanoic acid).

This protocol is important when the starting material is a chiral acid, since in that case an enantioselective conversion takes place twice in succession.

EXAMPLE 5

Enantioselective ammonolysis of Ibuprofen 2-chloroethyl ester

A solution of 5 ml tert-butyl alcohol, 500 µl Ibuprofen 2-chloroethyl ester [prepared from Ibuprofen (Sigma, catalogue No. 1 4883) by standard esterification procedure] and 100 µl diethylene glycol dibutyl ether (internal HPLC standard) was agitated with a top agitator at room temperature. Ammonia was bubbled through the solution. After 5 minutes 50 mg lipase SP 435 was added. The reaction was monitored by HPLC (see Example 1). The enantioselectivity in the reaction [given as enantiomeric excess (ee)] was calculated (vide infra) from the enantiomeric excess of the
remaining ester, determined by HPLC using a chiral HPLC column material (Chiralcel™ OD).

Sampling: for Chiralcel™ OD: 5 µl reaction mixture + 1 ml mobile phase; for Novapak™ C18: 50 µl reaction mixture + 500 µl mobile phase.

HPLC determination of conversion: Novapak™ C18; mobile phase 60/40 acetonitrile/water; NaOAc/HOAc 0.01 M; pH 4.3.

HPLC determination of ee (separation of the enantiomers of the ester): Chiralcel™ OD; mobile phase 85/15 hexane/2-propanol.

It was determined that 98% of the ester consisted of S enantiomer. From this the ee was calculated according to the formula: \( \text{ee}_{\text{ester}} = \frac{(S-R)}{(S+R)} \times 100 \). The amide formed, consisting substantially of R enantiomer, gave only one peak. The ee of the amide was calculated according to the formula:

\[ \text{ee}_{\text{amide}} = \text{ee}_{\text{ester}} \times \left(100 - \text{conversion}\right)/\text{conversion}. \]

The results are summarized below:

<table>
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<tr>
<th>Time (hours)</th>
<th>Conversion (%)</th>
<th>Amide (%)</th>
<th>ee_{amide} (%)</th>
<th>ee_{ester} (%)</th>
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<td>48</td>
<td>56</td>
<td>53</td>
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**COMPARATIVE EXAMPLE**

Enantioselective hydrolysis of ibuprofen 2-chloroethyl ester

To a mixture of 5 ml tert-butyl alcohol, 400 µl ibuprofen 2-chloroethyl ester, 270 µl water and 100 µl diethylene glycol dibutyl ether (internal HPLC standard) was added 50 mg lipase SP 435. The monitoring of the reaction and the determination of
enantioselectivity was carried out in the same manner as described in Example 5. It was determined that 79% of the residual ester consisted of S enantiomer. The ee of the acid was calculated in a manner analogous to that specified in Example 5 for the calculation of the ee of the amide.

The results are summarized below:

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<th>Time (hours)</th>
<th>Conversion (%)</th>
<th>Acid (%)</th>
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It is clear from a comparison of the latter results for hydrolysis with those for ammonolysis (see Example 5) that the ammonolysis is considerably more enantioselective. Thus:

- hydrolysis: conversion 63%; ee<sub>ester</sub> 58%; E = 3.5
- ammonolysis: conversion 56%; ee<sub>ester</sub> 96%; E = 28

"E" is the "enantiomeric ratio" and is calculated according to the formula given in "Biotransformations in Organic Chemistry" Kurt Faber, Springer Verlag, 1992, page 33.

EXAMPLE 6

Enantioselective ammonolysis of ethyl 2-chloropropanoate

A solution of 5 ml tert-butyl alcohol, 200 µl ethyl 2-chloropropanoate and 100 µl diethylene glycol dibutyl ether (internal standard) was agitated with a top agitator at room temperature. Ammonia was bubbled through the solution. After 5 minutes 50 mg lipase SP 435 was added. The reaction was monitored by GC. As in Example 5, the enantioselectivity in the reaction [given as enantiomeric excess (ee)] was
calculated (*vide infra*) from the enantiomeric excess of the remaining ester, determined by GC using a chiral GC column material (Astec Chiraldex$^\text{TM}$ G-TA).

Sampling: 50 $\mu$l reaction mixture + 500 $\mu$l dichloromethane.

GC determination of conversion: CP Sil5 CB, 25 m.

GC determination of ee (separation of the enantiomers of the ester): Astec Chiraldex$^\text{TM}$ G-TA; 50$^\circ$C for 8 minutes, followed by heating to 110$^\circ$C at a rate of 10$^\circ$C per minute.

The results are summarized below:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Conversion (%)</th>
<th>ee$_{\text{ester}}$ (%)</th>
<th>ee$_{\text{amide}}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>30</td>
<td>49</td>
<td>40</td>
</tr>
<tr>
<td>100</td>
<td>76</td>
<td>90</td>
<td>28</td>
</tr>
</tbody>
</table>

**COMPARATIVE EXAMPLE**

Enantioselective hydrolysis of ethyl 2-chloropropanoate

To a mixture of 5 ml tert-butyl alcohol, 200 $\mu$l ethyl 2-chloropropanoate, 270 $\mu$l water and 100 $\mu$l diethylene glycol dibutyl ether (internal standard) was added 10 mg lipase SP 435. The monitoring of the reaction and the determination of enantioselectivity was carried out in the same manner as described in Example 6. The ee of the ester was calculated from the percentage of one of the ester enantiomers. The ee of the acid was calculated in a manner analogous to that specified in Example 5 for the calculation of the ee of the amide.
The results are summarized below:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Conversion (%)</th>
<th>ee_{ester} (%)</th>
<th>ee_{acid} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>45</td>
<td>31</td>
<td>37</td>
</tr>
<tr>
<td>1440</td>
<td>80</td>
<td>60</td>
<td>15</td>
</tr>
</tbody>
</table>

When the results for the ammonolysis of ethyl 2-chloropropanoate (see Example 6) are compared with the latter results for the hydrolysis, it is apparent that the ammonolysis is more enantioselective. Thus: \( E = 6.1 \) for the ammonolysis and 2.7 for the hydrolysis.

**EXAMPLE 7**

**Ammonolysis of an ester of a chiral alcohol**

A solution of 5 ml tert-butyl alcohol, 200 \( \mu l \) \( \alpha \)-methylbenzyl \( n \)-butyrate [prepared from \( \alpha \)-methylbenzyl alcohol (Jansen Chimica, catalogue No. 13.018.20) by standard esterification] and 100 \( \mu l \) diethylene glycol dibutyl ether (internal standard) was agitated with a top agitator at room temperature. Ammonia was bubbled through the solution. After 5 minutes 100 mg lipase SP 435 was added. The reaction was monitored by GC.

The enantioselectivity in the reaction [given as enantiomeric excess (ee) of \( \alpha \)-methylbenzyl alcohol] was determined by GC using a chiral GC column material (Astec Chiraldex® G-TA).

**Sampling:** 50 \( \mu l \) reaction mixture + 500 \( \mu l \) dichloromethane.

**GC determination of conversion:** CP Si5 CB, 25 m.
GC determination of ee (separation of the enantiomers of α-methylbenzyl alcohol): Astec ChiralDEX™ G-TA; 80°C for 10 minutes, followed by heating to 110°C at a rate of 5°C per minute.

It was determined that 99% of the alcohol consisted of R enantiomer. The ee of the alcohol was calculated according to the formula \( (R-S)/(R+S) \times 100 \).

The results are summarized below:

<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Conversion (%)</th>
<th>( \text{ee}_{\text{α-methylbenzyl alcohol}} ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>144</td>
<td>45</td>
<td>98</td>
</tr>
</tbody>
</table>

EXAMPLE 8

10 Enantioselective ammonolysis of methyl trans-(4-methoxyphenyl)glycidate

A mixture of 9 ml 1,4-dioxan, 1 ml tert-butyl alcohol saturated with ammonia (ca. 2.5 mol/l), 200 mg of the glycidic acid ester and 25 µl 1,3-dimethoxybenzene (internal HPLC standard) was agitated at 40°C. After 30 minutes 100 mg lipase SP 523 was added. The reaction was monitored by HPLC.

Sampling: 10 µl reaction mixture + 500 µl mobile phase.

Conversion and ee were determined using a Chiralcel™ OD column. The enantiomers of the ester and of the amide were separated from each other in one analysis. The ee values were determined according to the formula:

\[ \text{ee} = \frac{(S-R)}{(S+R)} \times 100. \]

The results are shown below:
<table>
<thead>
<tr>
<th>Time (hours)</th>
<th>Conversion (%)</th>
<th>Amide (%)</th>
<th>ee&lt;sub&gt;ester&lt;/sub&gt; (%)</th>
<th>ee&lt;sub&gt;amide&lt;/sub&gt; (%)</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>35</td>
<td>31</td>
<td>48</td>
<td>90</td>
<td>28</td>
</tr>
<tr>
<td>110</td>
<td>43</td>
<td>38</td>
<td>65</td>
<td>87</td>
<td>26</td>
</tr>
</tbody>
</table>

(E is the enantiomeric ratio, calculated as indicated in Example 5).

5 EXAMPLE 9

Ammonolysis of amino acid esters

It has been found that amino acid esters can be converted to amino acid amides by ammonolysis; amide compounds of this type are important, for example, in the enzymatic synthesis of penicillins such as Ampicillin and Amoxicillin (see, e.g. WO 92/01061). The reaction is catalyzed by, for example, lipases and proteases. With lipases, there is a strong preference for the use of unprotected amino acid esters; with proteases, there is a preference for protected (N-protected) amino acid esters, for instance N-benzyloxycarbonyl-protected esters. The present example illustrates the use of lipases and proteases, respectively, in the enantioselective ammonolysis of amino acid esters:

A) Lipase-catalysed ammonolysis

To 5 ml tert-butyl alcohol saturated with ammonia (ca. 2.5 mol NH$_3$/l) was added 50 μl 1,3-dimethoxybenzene (internal HPLC standard). To this solution was then added 200 mg (0.99 mmol) racemic phenylglycine methyl ester hydrochloride, followed by 50 mg lipase SP 435, whereafter the reaction mixture was agitated at 40°C. 50 μl samples were withdrawn at intervals and diluted ten times for analysis. The degree of conversion and the ee were determined by HPLC on Chiralcel™ OD, eluent: 80/20 hexane/2-propanol, using UV detection at 254nm.
The results were as follows:

Reaction time: 4 hours
conversion: 30%
$e_{ee, \text{product}}$: 93%
5 $E$: 41.

b) Protease-catalyzed ammonolysis

To 125 mg (0.4 mmol) racemic N-benzylxocarbonyl-protected phenylalanine methyl ester in 5 ml tert-butyl alcohol were added 200 µl tert-butyl alcohol saturated with ammonia (ca. 0.5 mmol NH$_3$) and 50 µl dimethoxybenzene (internal HPLC standard). 20 mg protease immobilized on ACCUREL EP100 was added and the entire reaction mixture was agitated at 40°C. 50 µl samples were withdrawn at intervals and diluted ten times for analysis. The degree of conversion and the $ee$ were determined by HPLC on Chiralcel™ OD, eluent: 93/7 hexane/2-propanol, using UV detection at 254 nm.

The results are summarized below:

<table>
<thead>
<tr>
<th>Protease</th>
<th>Conversion (%)</th>
<th>$e_{ee, \text{ester}}$ (%)</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>SP 539</td>
<td>40</td>
<td>61</td>
<td>88</td>
</tr>
<tr>
<td>Subtilisin A</td>
<td>37</td>
<td>52</td>
<td>22</td>
</tr>
</tbody>
</table>
CLAIMS

1. A process for carrying out enzymatically catalyzed conversions of organic compounds, characterized in that an ester of an organic acid is reacted with ammonia in a liquid organic phase in the presence of a hydrolytic enzyme.

2. The process according to Claim 1, wherein said organic acid is a carboxylic acid.

3. The process according to Claim 2, wherein said ester is prepared in situ, prior to said reaction of said ester with ammonia, from said carboxylic acid and the appropriate alcohol in a liquid organic phase in the presence of a further hydrolytic enzyme.

4. The process according to Claim 3, wherein said further hydrolytic enzyme and said hydrolytic enzyme are the same.

5. The process according to any one of the preceding claims, wherein the liquid organic phase comprises an organic solvent or a mixture of organic solvents.

6. The process according to Claim 5, wherein the liquid organic phase comprises a tertiary aliphatic alcohol as organic solvent.

7. The process according to Claim 6, wherein said alcohol is tert-butyl alcohol.

8. The process according to any one of the preceding claims, wherein the enzyme is immobilized on a solid support.

9. The process according to any one of the preceding claims, wherein the enzyme is selected from the group consisting of lipases, esterases and proteases.

10. The process according to any one of the preceding claims, wherein the enzyme is Candida antarctica lipase B.
11. Use of the process according to any one of Claims 1 - 10 for the preparation of amides, esters and/or alcohols under mild conditions.

12. The use according to Claim 11 for the enantioselective preparation of amides, esters and/or alcohols.

13. The use according to Claim 12 for the enantioselective preparation of Ibuprofen amide and/or an Ibuprofen ester.

14. The use according to Claim 11 for the regioselective and/or enantioselective conversion of organic compounds with at least two ester functions.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12P13/02 C12P41/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
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<tbody>
<tr>
<td>X</td>
<td>CHEMICAL ABSTRACTS, vol. 70, no. 17, 28 April 1969, Columbus, Ohio, US; abstract no. 74630x, S.E.BRESLER ET AL. 'Mechanisms of the tryptic hydrolysis of ester and amide bonds.' page 40; see abstract &amp; MOL.BIOL., vol.3, no.1, 1969, USSR page 15-28</td>
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</tbody>
</table>

Further documents are listed in the continuation of box C.

Date of the actual completion of the international search
17 January 1995

Date of mailing of the international search report
01.02.95

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European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3916

Authorized officer
Delanghe, L

Form PCT/ISA/218 (second sheet) (July 1993)

page 1 of 2
<table>
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<th>Relevant to claim No.</th>
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<td>CHEMICAL ABSTRACTS, vol. 97, no. 11, 13 September 1982, Columbus, Ohio, US; abstract no. 87938m, D.PETKOV 'Enzyme peptide synthesis and semisynthesis: kinetic and thermodynamic aspects.' page 378; see abstract &amp; INT.CONF.CHEM.BIOTECHNOL.BIOL.ACT.NAT.PR OD.,(PROC.),1ST, vol.2, 1981 pages 469 - 479</td>
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<tr>
<td>A</td>
<td>TETRAHEDRON, (INCL. TETRAHEDRON REPORTS), vol.47, no.44, 1991, OXFORD GB pages 9207 - 9214 VICENTE GOTOR ET AL. 'Enzymatic aminolysis and transamination reactions' see the whole document</td>
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<td>A</td>
<td>TETRAHEDRON, (INCL. TETRAHEDRON REPORTS), vol.49, no.19, 1993, OXFORD GB pages 4007 - 4014 SUSANA PUERTAS ET AL. 'Lipase catalyzed aminolysis of ethyl propiolate and acrylic esters. Synthesis of chiral acrylamides.' see the whole document</td>
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</tr>
<tr>
<td>A</td>
<td>TETRAHEDRON LETTERS., vol.29, no.52, 1988, OXFORD GB pages 6973 - 6974 VICENTE GOTOR ET AL. 'A simple procedure for the preparation of chiral amides.' see the whole document</td>
<td>1</td>
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<td>T</td>
<td>TETRAHEDRON LETTERS., vol.34, no.38, 17 September 1993, OXFORD GB pages 6141 - 6142 MARIA JESUS GARCIA ET AL. 'Chemoenzymatic aminolysis and ammonolysis of beta-ketoesters' see the whole document</td>
<td>1</td>
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