Process for the enzymatic preparation of enantiomer-enriched β-amino acids

The present invention relates to a process for preparing enantiomer-enriched N-unprotected β-amino acids.

Optically active β-aminocarboxylic acids occur in natural substances such as alkaloids and antibiotics and their isolation is increasingly acquiring interest, not least because of their increasing importance as essential intermediates in the preparation of pharmaceuticals (see, inter alia: E. Juaristi, H. Lopez-Ruiz, *Curr. Med. Chem.* 1999, *6*, 983-1004). Both the free form of optically active β-aminocarboxylic acids and their derivatives have interesting pharmacological effects and can also be used in the synthesis of modified peptides.

The conventional racemate resolution by means of diastereomeric salts (proposed route in: H. Boesch et al., Org. Proc. Res. Developm. 2001, 5, 23-27) and, in particular, the diastereoselective addition of lithium phenylethylamide (A. F. Abdel-Magid, J. H. Cohen, C. A. Maryanoff, *Curr. Med. Chem.* 1999, *6*, 955-970) have been established as preparation methods for β-aminocarboxylic acids. The latter method is considered as intensively researched and, despite numerous disadvantages occurring in it, is preferably used. On the one hand, stoichiometric amounts of a chiral reagent are needed, which is a big disadvantage compared to catalytic asymmetrical methods. In addition, expensive and, moreover, hazardous auxiliaries, such as, for example, *n*-butyllithium are needed to activate the stoichiometric reagent by deprotonation. Moreover, the performance of the reaction

at low temperatures of approximately -70°C is important for a satisfactory stereoselectivity, which means a high requirement relating to the reactor material, additional costs and a high energy consumption.

Although the preparation of optically active β-aminocarboxylic acids biocatalytically plays only a subordinate role at present, it is desirable, in particular because of the economic and ecological advantages of biocatalytic reactions. The use of stoichiometric amounts of a chiral reagent is unnecessary and, instead, small, catalytic amounts of enzymes are used that are natural and environmentally-friendly catalysts. Moreover, these biocatalysts, which are efficiently used in aqueous medium, have, in addition to their catalytic properties and their high efficiency the advantage, in contrast to a multiplicity of synthetic metal-containing catalysts, that it is not necessary to use metal-containing, in particular heavy-metal-containing and consequently toxic feedstocks.

In the prior art, for instance, the enantioselective *N*-acylation of β-aminocarboxylic acids has already often been reported.

Thus, L.T. Kanerva et al. describe in Tetrahedron: Asymmetry, Vol. 7, No. 6, pages 1707-1716, 1996 the enantioselective *N*-acylation of ethyl esters of various cycloaliphatic β-aminocarboxylic acids with 2,2,2-trifluoroethyl ester in organic solvents and lipase SP 526 from *Candida antarctica* or lipase PS from *Pseudomonas cepacia* as biocatalyst.

V.M. Sánchez et al. studied the biocatalytic racemate resolution of (±)-ethyl 3-aminobutyrate (Tetrahedron: Asymmetry, Vol. 8, No. 1, pages 37-40, 1997) with lipase from *Candida antarctica* via the preparation of *N*-acetylated β-aminocarboxylic esters.

EP-A-8 890 649 discloses a process for preparing optically active amino acid esters from racemic amino acid esters by enantioselective acylation with a carboxylic ester in the presence of a hydrolase selected from the group comprising amidase, protease, esterase and lipase, and subsequent isolation of the unreacted enantiomers of the amino acid esters.

WO-A-98/50575 relates to a process for obtaining a chiral β-aminocarboxylic acid or its corresponding ester by bringing a racemic β-aminocarboxylic acid, an acyl donor and penicillin G acylase into contact under conditions for stereoselectively acylating an enantomer of the racemic β-aminocarboxylic acid, in which the other enantiomer is substantially not reacted and a chiral β-aminocarboxylic acid is thus obtained. The reverse reaction sequence has also been studied (V. A. Soloshonok, V. K. Svedas, V. P. Kukhar, A. G. Kirilenko, A. V. Rybakova, V. A. Solodenko, N. A. Fokina, O. V. Kogut, I. Y. Galaev, E. V. Kozlova, I. P. Shishkina, S. V. Galushko, Synlett 1993, 339-341; V. Soloshonok, A. G. Kirilenko, N. A. Fokina, I. P. Shishkina, S. V. Galushko, V. P. Kukhar, V. K. Svedas, E. V. Kozlova, Tetrahedron: Asymmetry 1994, 5, 1119-1126; V. Soloshonok, N. A. Fokina, A. V. Rybakova, I. P. Shishkina, S. V. Galushko, A. E. Sochorinsky, V. P. Kukhar, M. V. Savchenko, V. K. Svedas, Tetrahedron: Asymmetry 1995, 6, 1601-1610; G. Cardillo, A. Tolomelli, C. Tomasini, Eur. J.

Org. Chem. 1999, 155-161). A disadvantage of this process is the difficult working-up of the product mixture after the enantioselective hydrolysis. After isolating the free β-aminocarboxylic acid, a mixture of phenylacetic acid and *N*-phenylacetyl-β-aminocarboxylic acid is obtained that is difficult to separate.

Their reaction with lipases has already been known for a long time for obtaining enantiomer-enriched carboxylic acids. In US5518903, this principle has been transferred to N-protected β-amino acid esters, but with varying success. Whereas only the corresponding benzyl ester of racemic N-butoxycarbonyl-β-aminobutyric acid was resolved highly enantioselectively by means of a lipase, the remaining methyl esters or n-butyl esters used yielded only ee values in the region of not more than 70% ee. In this connection, it should be stated that, apparently, going over from a corresponding methyl ester to an n-butyl ester is accompanied by an impairment of the ee value of the acid prepared. Thus, starting from the n-butyl ester of N-Boc-β-aminobutyric acid, the ester hydrolysis with the enzyme lipase from Asahi reduces after 8 days an ee value of the corresponding acid of 45% ee in a yield of 37%. With the lipase PS supplied by Amano, a compound enriched to 61% ee is obtained in the same reaction with a yield of 41% at any rate within 7 days. In comparison therewith, the corresponding methyl ester yields 70% ee.

From the results recently published by Faulconbridge et al., it is to be inferred that the ester hydrolysis of aromatic β-amino acid ethyl esters at a pH of 8 with the lipase PS supplied by Amano takes place with acceptable yields and very good enantiomeric excesses (Tetrahedron

Letters 2000, 41, 2679-81). The product is obtained with an enantiomeric purity of up to 99% ee, but the synthesis, which was performed exclusively in aqueous suspension, is associated with some disadvantages. On the one hand, it has been found that, although the crystallization is selective under these conditions, the reaction per se results, as documented in Comparison Example 2, in lower ee values of 85.1% ee. All in all, this means, on the one hand, a yield loss due to the formation of the undesirable enantiomer and, on the other hand, it also entails the problem that the ee value may easily also drop below 99% ee or even below 98% ee as a function of slight process fluctuations on a technical scale because of altered crystallization conditions. As high an ee value as possible of > 98% ee, in particular > 99% ee, is, however, a requirement for pharmaceutical applications. In addition, the performance in purely liquid medium would be desirable as well as the performance in suspension in order, for example, to be able to ensure a good enzyme isolation by means of ultrafiltration. Optimally, a high ee value should likewise be produced in this step, which cannot be achieved with the existing literature processes (in this connection, see also Comparison Examples 1 and 2).

An enzymatic hydrolysis in the presence of single-phase reaction media using organic solvents was reported by Nagata et al. (S. Katayama, N. Ae, R. Nagata, *Tetrahedron: Asymmetry* **1998**, *9*, 4295-4299). In that case, a cyclic β-amino acid ester was used. The best results with enantioselectivities of 94% ee and conversions of 50% with a reaction time of 20 h were achieved using a solvent mixture composed of acetone (90%) and water (10%). Poorer

results were achieved with lower proportions of water. In general, the use of readily water-soluble solvents has proved superior compared with sparingly water-miscible solvents. Thus, the use of diisopropyl ether as organic medium, which was saturated with water and 20% acetone, produces only an ee value of 58% ee. In contrast thereto, a further readily water-soluble solvent proves suitable (95% ee) in addition to acetone with THF, but in this case long reaction times are needed (96 h) in order to achieve a conversion that is to any extent complete.

Hitherto, this successful synthesis having the feature of the presence of organic solvents has, however, remained limited to the preparation of cyclic β-amino acid esters. Under the optimum conditions specified in the literature for cyclic β-amino acid esters (see above), drastically lower yields and unacceptable, long reaction times are obtained in the preparation of the desired target compounds of the open-chain pendants (Comparison Example 1).

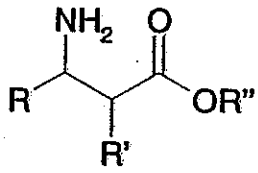
Embodiments of the present invention may therefore provide a further process for the enzymatic preparation of β-amino acids. In particular, the said process should advantageously be usable on an industrial scale economically as well as ecologically, i.e. be particularly outstanding in regard to environmental compatibility, industrial safety, ruggedness of the processing, the space/time yield and selectivity.

These and further embodiments not mentioned in greater detail, but obviously emerging from the prior art are achieved by a process having the features of the subject-matter

Claim 1. Dependent Claims 2 to 8 relate to preferred embodiments of the present invention.

As a result of the fact that a process for preparing enantiomer-enriched N-unprotected β-amino acids, in particular open-chain β-amino acids is performed by enzymatic hydrolysis of an enantiomer mixture of N-unprotected β-amino acids, in particular open-chain, β-amino acid esters with a hydrolase in a two-phase system composed of water and an organic solvent forming two phases under the given reaction conditions, the object set is very surprisingly achieved, but, on the other hand, in a no less advantageous way. In this connection, not only are substantially higher reactivities than in the hitherto known organic/aqueous systems are surprisingly achieved, but also good enantioselectivities. The reaction in the two-phase system can even be optimized in such a way that the product is produced at ≥99% ee (Example 4). Moreover, it is interesting that the ee value in accordance with Example 3 (89% ee) according to the invention turns out to be markedly better compared with the experiment in a purely aqueous system (Comparison Example 2, 81.5% ee). In addition to the processing advantages of organic solvents, this process consequently has, moreover, the advantage of generating higher enantioselectivities in the products compared with the aqueous standard medium.

Preferably, compounds of the general Formula (I)



(I)

where

R, R'' denote, independently of one another, (C_1-C_8)-alkyl, (C_2-C_8)-alkenyl, (C_2-C_8)-alkynyl, (C_3-C_8)-cycloalkyl, (C_6-C_{18})-aryl, (C_7-C_{19})-aralkyl, (C_3-C_{18})-heteroaryl, (C_4-C_{19})-heteroaralkyl, $((C_1-C_8)\text{-alkyl})_{1-3}\text{-}(C_3-C_8)$-cycloalkyl, $((C_1-C_8)\text{-alkyl})_{1-3}\text{-}(C_6-C_{18})$-aryl, and $((C_1-C_8)\text{-alkyl})_{1-3}\text{-}(C_3-C_{18})$-heteroaryl,

R' denotes H, (C_1-C_8)-alkyl, (C_2-C_8)-alkenyl, (C_2-C_8)-alkynyl, (C_3-C_8)-cycloalkyl, (C_6-C_{18})-aryl, (C_7-C_{19})-aralkyl, (C_3-C_{18})-heteroaryl, (C_4-C_{19})-heteroaralkyl, $((C_1-C_8)\text{-alkyl})_{1-3}\text{-}(C_3-C_8)$-cycloalkyl, $((C_1-C_8)\text{-alkyl})_{1-3}\text{-}(C_6-C_{18})$-aryl, and $((C_1-C_8)\text{-alkyl})_{1-3}\text{-}(C_3-C_{18})$-heteroaryl,

in the process of the subject matter.

In principle, the person skilled in the art is free to choose the appropriate ester group. He will base his selection on economic and reaction-engineering aspects. Favourable alcohols for forming the ester are, in particular, those that can easily be removed from the reaction mixture, optionally by distillation. Quite particularly preferred in the method according to the invention is the use of β-amino acid alkyl esters or β-amino acid aryl esters. Extremely preferred is the use of appropriate n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl or tert-butyl esters.

The choice of the reaction parameters is likewise up to the person skilled in the art. He will determine them separately for the individual case on the basis of routine experiments. At all events, a pH value range of between 4 and 10, preferably between 6 and 9 and more preferably between 7 and 8.5, is suitable for the enzymatic process

of the subject matter. The lipase PS supplied by Amano has proved particularly suitable around a pH of 8.

With regard to temperature, the same requirements exist in principle as for the pH. Here, again, as optimum a temperature as possible can be determined for the individual case, depending on which enzyme functions most optimally at which temperature. For enzymes from thermophilic organisms, high temperatures of up to 100°C are possible. Others, again, function optimally only at <0°C to -15°C, possibly in an ice matrix. Preferably, the temperature established during the reaction should be in the range between 15 and 40°C and, more preferably, between 20 and 30°C.

The choice of the enzyme to be used is the responsibility of the person skilled in the art. Many suitable enzymes can be selected from Enzyme Catalysis in Organic Synthesis, Ed.: K. Drauz, H. Waldmann, VCH, 1995, page 165 and the literature cited therein. Preferably, a lipase is taken for the ester hydrolysis, more preferably, the lipase PS supplied by Amano from *Pseudomonas cepacia* is used.

For the application, the polypeptide under consideration can be used in free form as a homogeneously purified compound or as an enzyme prepared as recombinant. Furthermore, the polypeptide may also be used as a constituent of an intact guest organism or in conjunction with the digested cell material of the host organism purified as much as desired.

The use of the enzymes in immobilized form is also possible (Sharma B. P.; Bailey L. F. and Messing R. A.

(1982), Immobilisierte Biomaterialien - Techniken und Anwendungen, Angew. Chem. 94, 836-852). Advantageously, the immobilization takes place through lyophilization (Paradkar, V. M.; Dordick, J. S. (1994), Aqueous-Like Activity of α-Chymotrypsin Dissolved in Nearly Anhydrous Organic Solvents, J. Am. Chem. Soc. 116, 5009-5010; Mori, T.; Okahata, Y. (1997), A variety of lipi-coated glycoside hydrolases as effective glycosyl transfer catalysts in homogeneous organic solvents, Tetrahedron Lett. 38, 1971-1974; Otamiri, M.; Adlercreutz, P.; Matthiasson, B. (1992), Complex formation between chymotrypsin and ethyl cellulose as a means to solubilize the enzyme in active form in toluene, Biocatalysis 6, 291-305). Quite particularly preferable is lyophilization in the presence of surfactant substances, such as Aerosol OT or polyvinylpyrrolidone or polyethylene glycol (PEG) or Brij 52 (diethylene glycol monocetyl ether) (Kamiya, N.; Okazaki, S.-Y.; Goto, M. (1997), Surfactant-horseradish peroxidase complex catalytically active in anhydrous benzene, Biotechnol. Tech. 11, 375-378).

Extremely preferred is immobilization on Eupergit® , in particular Eupergit C® and Eupergit 250L® (Röhm) (for a summary see: E. Katchalski-Katzir, D. M. Kraemer, *J. Mol. Catal. B: Enzym.* **2000**, *10*, 157). Equally preferred is the immobilization on Ni-NTA in combination with the polypeptide modified by attaching a His tag (Hexahistidine) (Petty, K.J. (1996), Metal-chelate affinity chromatography In: Ausubel, F.M. et al. eds. *Current Protocols in Molecular Biology*, Vol. 2, New York: John Wiley and Sons).

Use as CLECs is likewise conceivable (St. Clair, N.; Wang, Y.-F.; Margolin, A. L. (2000), Cofactor-bound cross-linked enzyme crystals (CLEC) of alcohol dehydrogenase, Angew. Chem. Int. Ed. 39, 380-383).

These measures can make it possible to generate, from polypeptides that are unstable due to organic solvents, those that can function in mixtures of aqueous and organic solvents or entirely in organic medium.

The reaction of the subject matter can be performed in any reaction vessel provided for the purpose. In detail, these are normal batch reactors, loop reactors or an enzyme membrane reactor (Bommarius, A. S.; Drauz, K.; Groeger, U.; Wandrey, C.; Membrane Bioreactors for the Production of Enantiomerically Pure α-Amino Acids, in: Chirality in Industry (eds.: Collins, A. N.; Sheldrake, G. N.; Crosby, J.) 1992, John Wiley & Sons, pages 371-397).

Suitable as organic phase that forms two phases with water under the given reaction conditions, that is to say, consequently, is insoluble or poorly water-soluble and can be used in the method according to the invention, are all the types of organic, insoluble or poorly water-soluble solvents and also mixtures thereof. In particular, these are ethers, ketones, esters, saturated or unsaturated, linear or branched-chain hydrocarbons.

In this connection, methyl-tert-butyl ether (MTBE), diisopropyl ether, ethyl acetate, hexane, heptane, cyclohexane, methylcyclohexane and toluene, and also any appropriate desirable mixtures thereof have proved particularly suitable.

If enzymes are used that exist in adsorbed form, optionally on water-insoluble support materials and/or minor constituents or stabilizers, it has proved advantageous to isolate the insoluble support and/or minor constituent or stabilizer prior to the use of the enzyme in the reaction so that contamination of the product produced with the insoluble support material of the enzyme used does not occur, provided the separation of enzyme and support is easily possible. For example, the lipase PS supplied by Amano, which can advantageously be used, is absorbed on silica supports. In this case, the aqueous enzyme solution could therefore be filtered prior to adding the reactants to the reaction medium in order to remove the silicic acids from the reaction system. The activities or processing stability of the enzyme do not, as a rule, adversely affect this procedure.

Within the scope of the invention, "N-unprotected" is to be understood as meaning that the β-nitrogen atom of the acid is not blocked by an N-protective group that is stable under the reaction conditions. Regarded as such are, in particular, the common protected groups, such as Z, Boc, Fmoc, Eoc, Moc, acetyl, etc.

To be regarded as (C_1-C_8)-alkyl are methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, hexyl, heptyl or octyl together with all the bonding isomers. These may be singly or multiply substituted by (C_1-C_8)-alkoxy, (C_1-C_8)-haloalkyl, OH, halogen, NH_2, NO_2, SH or S-(C_1-C_8)-alkyl.

(C_2-C_8)-alkenyl is to be understood as meaning a (C_1-C_8)-alkyl radical as depicted above, with the exception of methyl, containing at least one double bond.

(C_2-C_8)-aklynyl is to be understood as meaning a (C_1-C_8)-alkyl radical as depicted above, with the exception of methyl, containing at least one triple bond.

(C_1-C_8)-acyl is to be understood as meaning a (C_1-C_8)-alkyl radical bonded to the molecule by means of a -C = O function.

(C_3-C_8)-cycloalkyl is to be understood as meaning cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl radicals, etc. These may be substituted by one or more halogens and/or N-, O-, P-, S-atom-containing radicals and/or may have N-, O-, P-, S-atom-containing radicals in the ring, such as, for example, 1-, 2-, 3-, 4-piperidyl, 1-, 2-, 3-pyrrolidinyl, 2-, 3-tetrahydrofuryl, 2-, 3-, 4-morpholinyl. These may be singly or multiply substituted by (C_1-C_8)-alkoxy, (C_1-C_8)-haloalkyl, OH, halogen, NH_2, NO_2, SH, S-(C_1-C_8)-alkyl, (C_1-C_8)-acyl, (C_1-C_8)-alkyl.

A (C_6-C_{18})-aryl radical is understood as meaning an aromatic radical containing 6 to 18 carbon atoms. In particular, these include compounds, such as phenyl, naphthyl, anthryl, phenanthryl, biphenyl radicals. These may be singly or multiply substituted by (C_1-C_8)-alkoxy, (C_1-C_8)-haloalkyl, OH, halogen, NH_2, NO_2, SH, S-(C_1-C_8)-alkyl, (C_1-C_8)-acyl, (C_1-C_8)-alkyl.

A (C_7-C_{19})-aralkyl radical is a (C_6-C_{18})-alkyl radical bonded to the molecule by means of a (C_1-C_8)-alkyl radical.

(C_1-C_8)-alkoxy is a (C_1-C_8)-alkyl radical bonded to the molecule under consideration by means of an oxygen atom.

(C_1-C_8)-alkoxycarbonyl is a (C_1-C_8)-alkyl radical bonded to the molecule under consideration by means of an -OC(O) funktion. This is a synonym for the other oxycarbonyl radicals.

(C_1-C_8)-haloalkyl is a (C_1-C_8)-alkyl radical substituted by one or more halogen atoms.

A (C_3-C_{18})-heteroaryl radical denotes, within the scope of the invention, a five-, six- or seven-member aromatic ring system containing 3 to 18 carbon atoms that contains heteroatoms, such as, for example, nitrogen, oxygen or sulphur in the ring. Regarded as such heteroaromatics are, in particular, radicals, such as 1-, 2-, 3-furyl, such as 1-, 2-, 3-pyrrolyl, 1-,2-,3-thienyl, 2-, 3-, 4-pyridyl, 2-, 3-, 4-, 5-, 6-, 7-indolyl, 3-, 4-, 5-pyrazolyl, 2-,4-, 5-imidazolyl, acridinyl, quinolinyl, phenanthridinyl, 2-, 4-, 5-, 6-pyrimidinyl. These may be singly or multiply substituted by (C_1-C_8)-alkoxy, (C_1-C_8)-haloalkyl, OH, halogen, NH_2, NO_2, SH, S-(C_1-C_8)-alkyl, (C_1-C_8)-acyl, (C_1-C_8)-alkyl.

(C_4-C_{19})-heteroaralkyl is to be understood as meaning a heteroaromatic system corresponding to the (C_7-C_{19})-aralkyl radical.

Suitable as halogens are fluorine, chlorine, bromine and iodine.

The term enantiomer-enriched is to be understood as meaning, within the scope of the invention, the proportion of an enantiomer in the mixture with its optical antipodes in a range of >50 % and <100 %.

The structures shown refer to all the possible diastereomers and enantiomers and their mixtures that are possible.

Experimental Examples:

Comparison Example 1:

9.2 mmol of the racemic compound ethyl *rac*-3-amino-3-phenylpropionate (1.79 g) are taken up in 50 ml of a solvent mixture composed of 25 ml of water and 25 ml of acetone as organic solvent component and the solution is set to a pH of 8.2 by means of automatic pH adjustment through adding 1 M sodium hydroxide solution (obtained from Merck). On reaching a reaction temperature of 20°C, 200 mg of Amano lipase PS (*Pseudomonas cepacia*; obtained through Amano Enzymes, Inc.) are added to start the reaction. After a reaction time of 3, 5 and 24 hours, the conversion rate formed of the (*S*)-3-amino-3-phenylpropionic acid formed is determined. In this process, a conversion of 1.8% after 3 hours, 2.0% after 5 hours and 5.5% after 24 hours is determined. The value of the enantioselectivity was not determined because of the unsatisfactory course of the reaction in view of the low conversion. The conversion was determined by means of HPLC.

Comparison Example 2:

9.2 mmol of the racemic compound ethyl *rac*-3-amino-3-phenylpropionate (1.79 g) are taken up in 50 ml of water and the solution is set to a pH of 8.2 by means of automatic pH adjustment through adding 1 M sodium hydroxide solution (obtained through Merck). In order to dissolve the ester completely, 3 ml of acetone are also added to the solution. On reaching a reaction temperature of 20°C, 200 mg of Amano lipase PS (*Pseudomonas cepacia*; obtained through Amano Enzymes, Inc.) are added to start

the reaction. After a reaction time of 3 and 6 hours, the conversion rate formed and also after 6 hours the enantioselectivity of the (*S*)-3-amino-3-phenylpropionic acid formed are determined. In this process, a conversion of 18.5% after 3 hours or 37.8% after 6 hours and an enantioselectivity of 85.1% ee (after 6 hours) are determined. The conversion and enantioselectivity were determined by means of HPLC.

Example 3:

9.2 mmol of the racemic compound ethyl *rac*-3-amino-3-phenylpropionate (1.79 g) are taken up in 50 ml of a two-phase solvent mixture composed of 25 ml of water and 25 ml of methyl tert-butyl ether (MTBE) as organic solvent component and set to a pH of 8.2 by automatic pH adjustment through adding 1 M sodium hydroxide solution (obtained through Merck). On reaching a reaction temperature of 20°C, 200 mg of Amano lipase PS (*Pseudomonas cepacia*; obtained through Amano Enzymes, Inc.) are added to start the reaction. After a reaction time of 3, 5 and 24 hours, the conversion rate formed of the (*S*)-3-amino-3-phenylpropionic acid formed is determined. In this process, a conversion of 23.5% after 3 hours or a quantitative conversion of approximately ≥50% after 15 hours and an enantioselectivity of 89.0% ee (after 15 hours) are determined. The conversion and enantioselectivity were determined by means of HPLC.

Example 4:

81 ml of water are taken and 1.45 g of Amano lipase PS (*Pseudomonas cepacia*; obtained through Amano Enzymes, Inc.) are added thereto. The undissolved solid is then

filtered off. 81 ml of methyl tert-butyl ether (MTBE) are added as organic solvent component to the aqueous enzyme solution resulting as filtrate. The two-phase system is set to a pH of 8.2 by means of automatic pH adjustment through adding 1 M sodium hydroxide solution (obtained through Merck). On reaching a temperature of 20°C, 188.2 mmol of the racemic compound n-propyl rac-3-Amino-3-phenylpropionate (39.0 g) are then added and the reaction is started. The reaction time is 15 hours, a white precipitate being produced that is composed of the desired product (*S*)-3-amino-3-phenylpropionic acid. After a reaction time of 15 hours, 160 ml of acetone are added to complete the precipitation, stirring is continued for 45 minutes and the solid is filtered off. The solid is washed several times with a little acetone and then dried in vacuo. 12.91 g of the desired (*S*)-3-amino-3-phenylpropionic acid are obtained, equivalent to a yield of 41.6%. The enantioselectivity for the product is 99.6% ee. The enantioselectivity was determined by means of HPLC. 98.8% was determined for the chemical purity (determined by means of titration). The structure of the product was additionally confirmed by means of NMR spectroscopy.

Patent Claims:

1. Process for producing enantiomer-enriched N-unprotected β-amino acids, in particular open chain β-amino acids, by enzymatic hydrolysis of an enantiomeric mixture of N-unprotected β-amino acid esters, in particular open-chain β-amino acid esters, with a hydrolase, wherein the hydrolysis takes place in a two-phase system composed of water and an organic solvent forming two phases with water under the given reaction conditions.

2. Process according to Claim 1, characterized in that a β-amino acid alkyl ester or a β-amino acid aryl ester is used.

3. Process according to Claim 2, characterized in that an appropriate n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl or tert-butyl ester is used.

4. Process according to one or more of the preceding claims, characterized in that the pH of the reaction is between 4 and 10, preferably between 6 and 9 and more preferably between 7 and 8.5.

5. Process according to one or more of the preceding claims, characterized in that the temperature during the reaction is between -15 and +100°C, preferably between +15 and +40°C and more preferably between +20 and +30°C.

6. Process according to one or more of the preceding claims,
characterized in that
a lipase, preferably the lipase PS from *Pseudomonas cepacia* is used.

7. Process according to one or more of the preceding claims,
characterized in that
the reaction is performed in an enzyme membrane reactor.

8. Process according to one or more of the preceding claims,
characterized in that
ethers, ketones, esters, saturated or unsaturated linear or branched-chain hydrocarbons are used as organic solvent.

Process for the enzymatic preparation of enantiomer-enriched β-amino acids

The present invention relates to a process for preparing enantiomer-enriched β-amino acids by enzymatic ester resolution of N-unprotected β-amino acid esters in a two-phase system composed of water and an organic solvent forming two phases with water under the given reaction conditions.