

**Process for the enzymatic preparation of
enantiomerically enriched β -amino acids**

The present invention relates to a process for the preparation of enantiomerically enriched β -amino acids. The
5 invention relates also to advantageous esters of β -amino acids and to the use thereof in a process for the enzymatic preparation of enantiomerically enriched β -amino acids.

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

As preparation methods for β -aminocarboxylic acids there have hitherto become established conventional racemate
20 cleavage via diastereoisomeric salts (proposed route in: H. Boesch *et al.*, *Org. Proc. Res. Developm.* 2001, 5, 23-27) and, especially, the diastereoselective addition of lithium phenylethylamide (A. F. Abdel-Magid, J. H. Cohen, C. A. Maryanoff, *Curr. Med. Chem.* 1999, 6, 955-970). The latter
25 method has been intensively researched and is preferably employed, despite numerous disadvantages that occur therewith. On the one hand, stoichiometric amounts of a chiral reagent are required, which represents a major disadvantage compared with catalytic asymmetric methods.
30 Furthermore, expensive and, additionally, dangerous auxiliary substances, such as, for example, *n*-butyllithium, are required to activate the stoichiometric reagent by deprotonation. In addition, for satisfactory stereo-selectivity it is important to carry out the reaction at

low temperatures of about -70°C , which means high demands on the reactor material, additional costs and high energy consumption.

Although the preparation of optically active β -amino-carboxylic acids by biocatalytic means plays only a secondary role at present, it is desirable especially on account of the economic and ecological advantages of biocatalytic reactions. It is not necessary to use stoichiometric amounts of a chiral reagent, and there are used instead small, catalytic amounts of enzymes, which are natural and environmentally friendly catalysts. In addition to their catalytic properties and their high activity, such biocatalysts, which are used efficiently in an aqueous medium, have the advantage, in contrast to a large number of synthetic metal-containing catalysts, that it is possible to dispense with the use of metal-containing, especially heavy-metal-containing and hence toxic substances.

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

For example, in *Tetrahedron: Asymmetry*, Vol. 7, No. 6, p. 1707-1716, 1996, L.T. Kanerva et al. describe the enantioselective *N*-acylation of ethyl esters of various alicyclic 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 cleavage of ($\pm$)-ethyl-3-aminobutyrate (*Tetrahedron: Asymmetry*, Vol. 8, No. 1, p. 37-40, 1997) with lipase from *Candida antarctica* via the preparation of the *N*-acetylated β -aminocarboxylic acid ester.

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

WO-A-98/50575 relates to a process for obtaining a chiral β -aminocarboxylic acid or its corresponding esters by bringing a racemic β -aminocarboxylic acid, an acyl donor and penicillin G acylase into contact under conditions in order to acylate an enantiomer of the racemic β -aminocarboxylic acid stereoselectively, wherein the other enantiomer is substantially unconverted, 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 that process is that the product mixture is difficult to work up following the enantioselective hydrolysis. After separating off the free β -aminocarboxylic acid there is obtained a mixture of phenylacetic acid and *N*-phenylacetyl- β -aminocarboxylic acid which is difficult to separate.

In order to obtain enantiomerically enriched carboxylic acids, it has long been known to react them with lipases.

In US5518903, that principle has been transferred to N-protected β -amino acid esters, but with varying degrees of success. While only the corresponding benzyl ester of racemic N-butoxycarbonyl- β -aminobutyric acid could be
5 cleaved in a highly enantioselective manner by means of a lipase, the remaining methyl esters and n-butyl esters used yielded ee values only in the region of 70%. It is to be noted that a transition from a corresponding methyl ester to an n-butyl ester is evidently accompanied by a fall in
10 the ee value of the acid prepared. For example, ester hydrolysis starting from the n-butyl ester of N-Boc- β -aminobutyric acid with the enzyme lipase from Asahi gives an ee value of the corresponding acid of 45%ee after 8 days in 37% yield. Using Lipase PS from Amano, a compound
15 enriched to 61%ee is obtained in the same reaction within a period of 7 days with a yield of 41%. By comparison, the corresponding methyl ester yields 70%ee.

The results recently published by Faulconbridge *et al.* show that the ester hydrolysis of aromatic β -amino acid ethyl
20 esters at pH 8 with Lipase PS from 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%, but the synthesis, which was carried out solely in suspension, is
25 associated with some disadvantages. On the one hand, it has been found that, although the crystallisation is selective under those conditions, the reaction itself, as documented in Comparison Example 1, leads to lower ee values of 85.1% ee. Overall, this means, on the one hand, a loss of yield
30 owing to the formation of the undesired enantiomer and, on the other hand, that the ee value, in dependence on slight process variations, may easily fall below 99% ee or even below 98% ee on a commercial scale owing to changed crystallisation conditions. However, as high an ee value as
35 possible, of >98% ee, especially >99% ee, is a requirement for pharmaceutical applications. Moreover, it would also be

desirable to carry out the reaction in a homogeneous medium (no suspension!) in order to be able to ensure good enzyme separation by ultrafiltration. Optimally, a high ee value should also be obtained in that step, which cannot be achieved with the literature processes known hitherto. The object of the present invention was, therefore, to provide a further process for the enzymatic preparation of β -amino acids. In particular, it should be possible to use the process advantageously on a commercial scale from economic and also ecological points of view, that is to say the process should stand out especially in respect of environmental compatibility, safety in the workplace and the robustness of the process, and also the space/time yield and selectivity.

The present invention provides a process for preparing enantiomer-enriched N-unprotected β -amino acids by enzymatic hydrolysis of an enantiomeric mixture of N-unprotected β -amino acid alkyl esters of β -amino acid aryl esters with a lipase provided that no corresponding methyl or ethyl ester is employed.

Disclosed herein is also a process for the preparation of enantiomerically enriched N-unprotected β -amino acids by enzymatic hydrolysis of an enantiomeric mixture of N-unprotected β -amino acid esters with a hydrolase, with the proviso that no corresponding methyl or ethyl ester is used.

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Because a process for the preparation of enantiomerically enriched N-unprotected β -amino acids by enzymatic hydrolysis of an enantiomeric mixture of N-unprotected β -amino acid esters with a hydrolyase is carried out with the proviso that no corresponding methyl or ethyl ester is used, the object which has been set out is achieved in a very surprising, but no less advantageous, manner. Hitherto, only methyl or ethyl esters of the N-unprotected β -amino acids have been used for the reaction in question, but such esters are associated with the disadvantages already mentioned above. A better result both in terms of the space/time yield and in terms of selectivity is obviously obtained when bulkier ester groups having, for

example, (C₃-C₈)-alkyl radicals are used for the enzymatic hydrolysis. That is to be regarded on the one hand as surprising in view of US 5518903 (sic) discussed above, on the other hand the trend is unexpected because, with a more rapid reaction, the probability of enantiodifferentiation generally decreases. This logical connection is illustrated, for example, if the generally lower enantioselectivities at higher reaction temperatures - at which the reaction accordingly proceeds more rapidly - are considered.

In principle, the person skilled in the art is free to choose the ester group. When making his choice, he will be guided by economic and reaction-related considerations. Advantageous alcohols for forming the ester are especially those which can readily be removed from the reaction mixture. They are alcohols such as alkyl alcohols or aryl alcohols, optionally low-boiling phenol or benzyl alcohol. The β -amino acid alkyl esters or β -amino acid aryl esters obtainable with those alcohols are therefore preferably used in the hydrolysis. Very particular preference is given to the use of corresponding n-propyl, isopropyl, n-butyl, sec-butyl, isobutyl or tert-butyl esters of the β -amino acids.

The person skilled in the art is also free to choose the reaction parameters. They will be determined separately for each individual case by means of routine experiments. In any case, a suitable pH range for the enzymatic process according to the subject-matter is from 4 to 10, preferably from 6 to 9 and more preferably from 7 to 8.5. Lipase PS from Amano has proved to be particularly suitable at about pH 8.

With regard to the temperature, the requirements are in principle the same as for the pH value. In this case too, an optimum temperature can be determined for each individual case, according to which enzyme works optimally

at which temperature. For enzymes from thermophilic organisms, high temperatures up to 100°C are possible. Other enzymes work optimally only at <0°C to -15°C, optionally in an ice matrix. The temperature that is set during the reaction should preferably be in the range from 15 to 40°C, more preferably from 20 to 30°C.

The person skilled in the art is responsible for choosing the enzyme to be used. Many suitable enzymes can be chosen from Enzyme Catalysis in Organic Synthesis, Ed.: K. Drauz, H. Waldmann, VCH, 1995, p. 165 and the literature cited therein. A lipase is preferably employed for the ester hydrolysis, more preferably Lipase PS from Amano from *Pseudomonas cepacia* is used.

The polypeptide in question can be used in free form as a homogeneously purified compound, or in the form of an enzyme prepared by recombinant methods. The polypeptide may also be used as part of an intact host organism or in conjunction with the cell mass of the host organism broken down and highly purified as desired.

It is also possible to use the enzymes in immobilised form (Sharma B. P.; Bailey L. F. and Messing R. A. (1982), Immobilisierte Biomaterialien - Techniken und Anwendungen, Angew. Chem. 94, 836-852). Immobilisation is preferably effected by lyophilisation (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). Very particular preference is given to lyophilisation in the presence of surface-active substances, such as Aerosol OT or polyvinylpyrrolidone or

polyethylene glycol (PEG) or Brij 52 (diethylene glycol mono-cetyl ether) (Kamiya, N.; Okazaki, S.-Y.; Goto, M. (1997), Surfactant-horseradish peroxidase complex catalytically active in anhydrous benzene, *Biotechnol. Tech.* 11, 375-378).

Most preferred is immobilisation on Eupergit®, especially Eupergit C® and Eupergit 250L® (Röhm) (as overview see: E. Katchalski-Katzir, D. M. Kraemer, *J. Mol. Catal. B: Enzym.* 2000, 10, 157). Also preferred is immobilisation on Ni-NTA in combination with the polypeptide modified by attachment of a His tag (hexa-histidine) (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 in the form of CLECs is also possible (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).

By means of these measures it may be possible to generate from polypeptides that become unstable owing to organic solvents, polypeptides that are able to work in mixtures of aqueous and organic solvents or in wholly organic media.

The reaction according to the subject-matter can be carried out in any reaction vessel provided therefor. Such reaction vessels are, in detail, 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, p. 371-397).

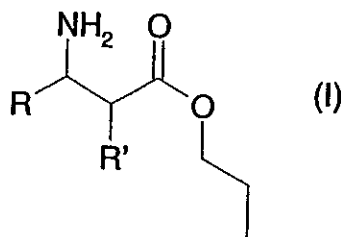
The esters to be used in the reaction according to the invention sometimes exhibit poor solubility in an aqueous reaction medium. In such cases it may be advantageous, depending on the solvent tolerance of the enzymes used, to add water-soluble organic solvents to the reaction mixture

in order to obtain a homogeneous reaction phase. Suitable as such water-soluble organic solvents are, *inter alia*: acetone, DMF, ethanol, methanol.

However, the reaction is also possible at higher substrate concentrations with formation of a suspension.

In the case of the use of enzymes which are optionally adsorbed on water-insoluble carrier materials or accompanying substances or stabilisers, the insoluble carrier or accompanying substance or the stabiliser can be separated off before the enzyme is used in the reaction, so that there is no contamination of the product that is to be precipitated by the insoluble carrier material of the enzyme used, provided separation of the enzyme and the carrier is possible in a simple manner. For example, Lipase PS from Amano is adsorbed on silica carriers. Before the reactants are added to the reaction medium, filtration of the aqueous enzyme solution should therefore be carried out in order to remove the silicas from the reaction system. This procedure does not adversely affect the activity or process stability of the enzyme.

Disclosed herein is also β -amino acid n-propyl esters having the following structure (I)



wherein

R represents (C₁-C₈)-alkyl, (C₂-C₈)-alkenyl, (C₂-C₈)-alkynyl, (C₃-C₈)-cycloalkyl, (C₆-C₁₈)-aryl, (C₇-C₁₉)-aralkyl, (C₃-C₁₈)-heteroaryl, (C₄-C₁₉)-heteroaralkyl, ((C₁-C₈)-alkyl)₁₋₃-(C₃-C₈)-cycloalkyl, ((C₁-C₈)-alkyl)₁₋₃-(C₆-C₁₈)-aryl, ((C₁-C₈)-alkyl)₁₋₃-(C₃-C₁₈)-heteroaryl,

R' represents H, (C₁-C₈)-alkyl, (C₂-C₈)-alkenyl, (C₂-C₈)-alkynyl, (C₃-C₈)-cycloalkyl, (C₆-C₁₈)-aryl, (C₇-C₁₉)-aralkyl, (C₃-C₁₈)-heteroaryl, (C₄-C₁₉)-heteroaralkyl, ((C₁-C₈)-alkyl)₁₋₃-(C₃-C₈)-cycloalkyl, ((C₁-C₈)-alkyl)₁₋₃-(C₆-C₁₈)-aryl, 5 ((C₁-C₈)-alkyl)₁₋₃-(C₃-C₁₈)-heteroaryl.

Disclosed are compounds in which R represents an aromatic radical, especially phenyl, thienyl, furyl, pyridyl, and the derivatives thereof mono-or poly-substituted on the aromatic ring, especially with (C₁-C₈)-alkyl or (C₁-C₈)-alkoxy as substituents and in which R' represents a H.

3-Amino-3-phenylpropionic acid n-propyl ester may be mentioned as an example of a representative of this type of compound that is of particular interest.

Disclosed further is the use of the above-mentioned compounds in a process as disclosed.

"N-unprotected" is

20 understood to mean that the β -nitrogen atom of the acid is not blocked by a N-protecting group that is stable under the reaction conditions. Such protecting groups are to be regarded as being especially the usual protecting groups such as Z, Boc, Fmoc, Eoc, Moc, acetyl, etc..

25 (C₁-C₈)-Alkyl is to be regarded as being methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl, tert-butyl, pentyl, hexyl, heptyl or octyl, including all isomers due to different positions of the double bond. They may be mono- or poly-substituted by (C₁-C₈)-alkoxy, (C₁-C₈)-haloalkyl, OH, halogen, NH₂, NO₂, SH, S-(C₁-C₈)-alkyl.

30 (C₃-C₈)-Alkyl is to be regarded accordingly.

(C₂-C₈)-Alkenyl is to be understood as being a (C₁-C₈)-alkyl radical as described above, with the exception of methyl, that contains at least one double bond.

(C₂-C₈)-Alkynyl is to be understood as being a (C₁-C₈)-alkyl radical as described above, with the exception of methyl, that contains at least one triple bond.

5 (C₁-C₈)-Acyl is understood as being a (C₁-C₈)-alkyl radical bonded to the molecule via a -C=O- function.

(C₃-C₈)-Cycloalkyl is understood as being cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl or cycloheptyl radicals, etc.. They may be substituted by one or more
10 halogens and/or radicals containing N, O, P, S atoms, and/or may have in the ring radicals containing N, O, P, S atoms, such as, for example, 1-, 2-, 3-, 4-piperidyl, 1-, 2-, 3-pyrrolidinyl, 2-, 3-tetrahydrofuryl, 2-, 3-, 4-morpholinyl. Such a radical may be mono- or poly-substituted by (C₁-C₈)-alkoxy, (C₁-C₈)-haloalkyl, OH,
15 halogen, NH₂, NO₂, SH, S-(C₁-C₈)-alkyl, (C₁-C₈)-acyl, (C₁-C₈)-alkyl.

A (C₆-C₁₈)-aryl radical is understood as being an aromatic radical having from 6 to 18 carbon atoms. Such radicals include especially compounds such as phenyl, naphthyl,
20 anthryl, phenanthryl, biphenyl radicals. Such a radical may be mono- or poly-substituted by (C₁-C₈)-alkoxy, (C₁-C₈)-haloalkyl, OH, halogen, NH₂, NO₂, SH, S-(C₁-C₈)-alkyl, (C₁-C₈)-acyl, (C₁-C₈)-alkyl.

25 A (C₇-C₁₉)-aralkyl radical is a (C₆-C₁₈)-aryl radical bonded to the molecule via a (C₁-C₈)-alkyl radical.

(C₁-C₈)-Alkoxy is a (C₁-C₈)-alkyl radical bonded to the molecule in question via an oxygen atom.

(C₁-C₈)-Alkoxycarbonyl is a (C₁-C₈)-alkyl radical bonded to the molecule in question via a -OC(O)- function. The same
30 applies analogously to the other oxycarbonyl radicals.

(C₁-C₈)-Haloalkyl is a (C₁-C₈)-alkyl radical substituted by one or more halogen atoms.

A (C₃-C₁₈)-heteroaryl radical denotes a five-, six- or seven-membered aromatic ring system having from 3 to 18 carbon atoms and containing hetero atoms, such as, for example, nitrogen, oxygen or sulfur, in the ring. Such heteroaromatic compounds are regarded as being especially 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. Such a radical may be mono- or poly-substituted by (C₁-C₈)-alkoxy, (C₁-C₈)-haloalkyl, OH, halogen, NH₂, NO₂, SH, S-(C₁-C₈)-alkyl, (C₁-C₈)-acyl, (C₁-C₈)-alkyl.

A (C₄-C₁₉)-heteroaralkyl is understood as being a heteroaromatic system corresponding to the (C₇-C₁₉)-aralkyl radical.

Suitable halogens are fluorine, chlorine, bromine and iodine.

The expression enantiomerically enriched is understood to mean the proportion of an enantiomer in admixture with its optical antipode in a range of from >50 % to <100 %.

The structures shown relate to all possible diastereoisomers and enantiomers and mixtures thereof which are possible.

Experimental examples:**Example 1 (= comparison example):**

9.2 mmol of the racemic compound *rac*-3-amino-3-phenyl-propionic acid ethyl ester (1.79 g) are taken up in 50 ml of water, and the solution is adjusted to a pH value of pH 8.2 by means of automatic pH metering by addition of 1 M sodium hydroxide solution (obtained from Merck). In order to dissolve the ester without residue, 3 ml of acetone are also added for dissolution. When a reaction temperature of 20°C is reached, 200 mg of Amano Lipase PS (*Pseudomonas cepacia*; obtained from Amano Enzymes, Inc.) are added in order to start the reaction. After a reaction time of 3 and 6 hours, the rate of conversion and, after 6 hours, the enantioselectivity of the resulting (*S*)-3-amino-3-phenyl-propionic acid are determined. A conversion of 18.5% after 3 hours and 37.8% after 6 hours and an enantioselectivity of 85.1% ee (after 6 hours) are determined. The conversion and enantioselectivity were determined by HPLC.

Example 2:

9.2 mmol of the racemic compound *rac*-3-amino-3-phenyl-propionic acid *n*-propyl ester (1.91 g) are taken up in 50 ml of water, and the solution is adjusted to a pH value of pH 8.2 by means of automatic pH metering by addition of 1 M sodium hydroxide solution (obtained from Merck). In order to dissolve the ester without residue, 3 ml of acetone are also added for dissolution. When a reaction temperature of 20°C is reached, 200 mg of Amano Lipase PS (*Pseudomonas cepacia*; obtained from Amano Enzymes, Inc.) are added in order to start the reaction. After a reaction time of one hour, the rate of conversion and, after 3 hours, the enantioselectivity of the resulting (*S*)-3-amino-3-phenylpropionic acid are determined. A conversion of

48.7 % after one hour and an enantioselectivity of 96.4% ee (after 3 hours) are determined. The conversion and enantioselectivity were determined by HPLC.

5 **Example 3:**

8.63 mmol of the racemic compound *rac*-3-amino-3-phenylpropionic acid *n*-butyl ester (1.91 g) are taken up in 50 ml of water, and the solution is adjusted to a pH value of pH 8.2 by means of automatic pH metering by addition of 1 M sodium hydroxide solution (obtained from Merck). In order to dissolve the ester without residue, 3 ml of acetone are also added for dissolution. When a reaction temperature of 20°C is reached, 200 mg of Amano Lipase PS (*Pseudomonas cepacia*; obtained from Amano Enzymes, Inc.) are added in order to start the reaction. After a reaction time of 3 hours, both the rate of conversion and the enantioselectivity of the resulting (*S*)-3-amino-3-phenylpropionic acid are determined. A conversion of 45.2 % and an enantioselectivity of 96.8% ee are determined. The conversion and enantioselectivity were determined by HPLC.

Example 4:

81 ml of water are placed in a vessel, 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) as organic solvent component are added to the aqueous enzyme solution obtained as filtrate. The resulting two-phase system is adjusted to pH 8.2 by means of automatic pH metering by addition of 1 M sodium hydroxide solution (obtained through Merck). When a temperature of 20°C is reached, 188.2 mmol of the racemic compound *rac*-3-amino-3-phenylpropionic acid *n*-propyl ester (39.0 g) are added, and

the reaction is started. The reaction time is 15 hours, during which a white precipitate consisting of the desired product (S)-3-amino-3-phenylpropionic acid forms. After a reaction time of 15 hours, 160 ml of acetone are added to
5 complete the precipitation, stirring is then carried out for about 45 minutes, and the solid is filtered off. The solid is washed several times with a small amount of acetone and is then dried *in vacuo*. 12.91 g of the desired (S)-3-amino-3-phenylpropionic acid are obtained,
10 corresponding to a yield of 41.6%. The enantioselectivity for the product is 99.6% ee. The enantioselectivity was determined by HPLC. The chemical purity was determined as 98.8% (determined by titration). The structure of the product was additionally confirmed by NMR spectroscopy.

Claims

1. Process for preparing enantiomer-enriched N-unprotected β -amino acids by enzymatic hydrolysis of an enantiomeric mixture of N-unprotected β -amino acid alkyl esters or β -amino acid aryl esters with a lipase provided that no corresponding methyl or ethyl ester is employed.
2. Process according to Claim 1, characterized in that a corresponding n-propyl, i-propyl, n-butyl, sec-butyl, i-butyl or tert-butyl ester is employed.
3. Process according to one or more of the preceding claims, characterized in that the pH of reaction is between 4 and 10.
4. Process according to one or more of the preceding claims, characterized in that the temperature during the reaction is between -15 and $+100^{\circ}\text{C}$.
5. Process according to one or more of the preceding claims, characterized in that the lipase PS from *Pseudomonas cepacia* is employed.
6. Process according to one or more of the preceding claims, characterized in that the reaction is carried out in an enzyme membrane reactor.

7. Process according to one or more of the preceding claims, characterized in that the hydrolysis is carried out in aqueous medium to which water-soluble organic solvents may be admixed.
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8. Process according to one or more of the preceding claims, characterized in that the hydrolysis is carried out in aqueous medium to which water-soluble organic solvents may be admixed.