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(72) Inventeurs/Inventors:
BALKENHOHL, FRIEDHELM, DE;
HAUER, BERNHARD, DE;
LADNER, WOLFGANG, DE;
PRESSLER, UWE, DE;
NUBLING, CHRISTOPH, DE

(73) Propriétaire/Owner:
BASF AKTIENGESELLSCHAFT, DE

(74) Agent: ROBIC

(54) Titre : RESOLUTION DE RACEMIQUES D'AMINES PRIMAIRES ET SECONDAIRES PAR ACYLATION EN
PRESENCE D'ENZYME CATALYSEUR

(54) Title: RESOLUTION OF THE RACEMATES OF PRIMARY AND SECONDARY AMINES BY ENZYME-CATALYZED
ACYLATION

(57) **Abrégé/Abstract:**

A process for producing optically active primary and secondary amines from the corresponding racemates is characterised in that (a) a racemic amine is enantioselectively acylated in the presence of a hydrolase with an ester whose acid component bears a fluorine, nitrogen, oxygen or sulphur atom at the proximity of the carbonyl carbon atom; (b) the mixture of optically active amine and optically active acylated amine is separated so that an enantiomer of amine is produced; (c) if desired the other enantiomer of the amine is extracted from the acylated amine by amide cleavage.



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(54) Title: **RACEMATE CLEAVAGE OF PRIMARY AND SECONDARY AMINES BY ENZYME-CATALYSED ACYLATION**

(54) Bezeichnung: **RACEMATSPALTUNG PRIMÄRER UND SEKUNDÄRER AMINE DURCH ENZYM-KATALYSIERTE ACYLIERUNG**

(57) Abstract

A process for producing optically active primary and secondary amines from the corresponding racemates is characterised in that (a) a racemic amine is enantioselectively acylated in the presence of a hydrolase with an ester whose acid component bears a fluorine, nitrogen, oxygen or sulphur atom at the proximity of the carbonyl carbon atom; (b) the mixture of optically active amine and optically active acylated amine is separated so that an enantiomer of amine is produced; (c) if desired the other enantiomer of the amine is extracted from the acylated amine by amide cleavage.

(57) Zusammenfassung

Beschrieben wird ein Verfahren zur Herstellung von optisch aktiven primären und sekundären Aminen aus den entsprechenden Racematen, das dadurch gekennzeichnet ist, daß man a) ein racemisches Amin mit einem Ester, dessen Säurekomponente ein Fluor-, Stickstoff-, Sauerstoff- oder Schwefelatom in Nachbarschaft des Carbonylkohlenstoffatoms trägt, in Gegenwart einer Hydrolase enantioselektiv acyliert, b) das Gemisch aus optisch aktivem Amin und optisch aktivem acylierten Amin trennt und somit ein Enantiomer des Amins erhält, c) gewünschtenfalls aus dem acylierten Amin das andere Enantiomer des Amins durch Amidspaltung gewinnt.

Resolution of the racemates of primary and secondary amines by enzyme-catalyzed acylation

- 5 The present invention relates to a novel process for resolving the racemates of primary and secondary amines by reacting with an ester in the presence of a hydrolase and subsequently separating one amine which has been enantioselectively acylated from the other, unreacted, enantiomer of the amine.
- 10
- Resolution of the racemates of amines by enzyme-catalyzed reaction with esters is known. Kitaguchi et al. (J. Amer. Chem. Soc. 111 (1989) 3094-3095) describe the resolution of racemates of amines with trifluoroethyl butyrate with catalysis by subtilisin.
- 15 The enantioselectivity of this reaction is, however, greatly dependent on the solvent. Even with the most suitable of the solvents described (3-methyl-3-pentanol) only moderate selectivity is achieved.
- 20 WO 91/19002 describes a process for the chiral enrichment of asymmetric primary amines, in which the amines are reacted with ethyl acetate or ethyl butyrate with catalysis by subtilisin. The enantiomeric excesses achieved thereby are, however, unsatisfactory; in addition, long reaction times of up to several weeks are
- 25 required.
- Gotor et al. (J. Chem. Soc. Chem. Commun. (1988) 957-958) describe the enantioselective acylation of 2-amino-1-butanol with ethyl acetate with catalysis by pig pancreatic lipase (PPL). In
- 30 this case, the ester used (ethyl acetate) is also employed as a solvent. No satisfactory results are obtained on use of other solvents or other enzymes.
- Brieva et al. (J. Chem. Soc. Chem. Commun. (1990) 1386-1387)
- 35 describe the enantioselective synthesis of amides from racemic primary amines by reaction with 2-chloropropionate with catalysis by subtilisin in hexane or *Candida cylindracea* lipase in 3-methyl-3-pentanol.
- 40 Quiros et al. (Tetrahedron: Asymmetry 4 (1993) 1105-1112) describe the lipase-catalyzed synthesis of optically active amides from racemic α -halo-substituted ethyl propionates and primary amines. However, the enantioselectivity achieved with this reaction is unsatisfactory.
- 45

Asensio et al. (Tetrahedron Letters 32 (1991) 4197-4198) describe the lipase-catalyzed enantioselective acylation of secondary amines. However, this reaction takes place enantioselectively only with one amine and even there only with moderate success. Other amines show absolutely no enantioselectivity.

The processes hitherto disclosed for enzyme-catalyzed racemate resolution either have too little enantioselectivity or can be carried out only under very specific conditions (solvent, enzyme). In addition, they require long reaction times and enormous amounts of enzyme for the catalysis so that a process based thereon is uneconomic.

- 10 It is an object of the present invention to provide a process for the enzyme-catalyzed resolution of racemates of primary and secondary amines which ensures high enantioselectivity, can be employed in a wide range of reaction conditions and moreover makes do with minimal amounts of catalyst.

More specifically, the invention as broadly disclosed is a process for resolving the racemates of primary and secondary amines by reacting with an ester with specific catalysis by a hydrolase and subsequently separating one amine which has been enantioselectively acylated from the other, unreacted, enantiomer of the amine, which process functions particularly well when the acid component of
20 the ester has an electron-rich heteroatom selected from the group comprising fluorine, nitrogen, oxygen and sulphur atoms in the vicinity of the carbonyl carbon atom.

The invention as claimed is however restricted to a process for resolving racemates of primary and secondary amines by reacting with an ester in the presence of a protease or lipase and subsequently separating one amine which has been enantioselectively acylated from the other, unreacted, enantiomer of the amine, wherein the acid component of the ester has an oxygen atom in the α , β or γ position relative to the carbonyl carbon.

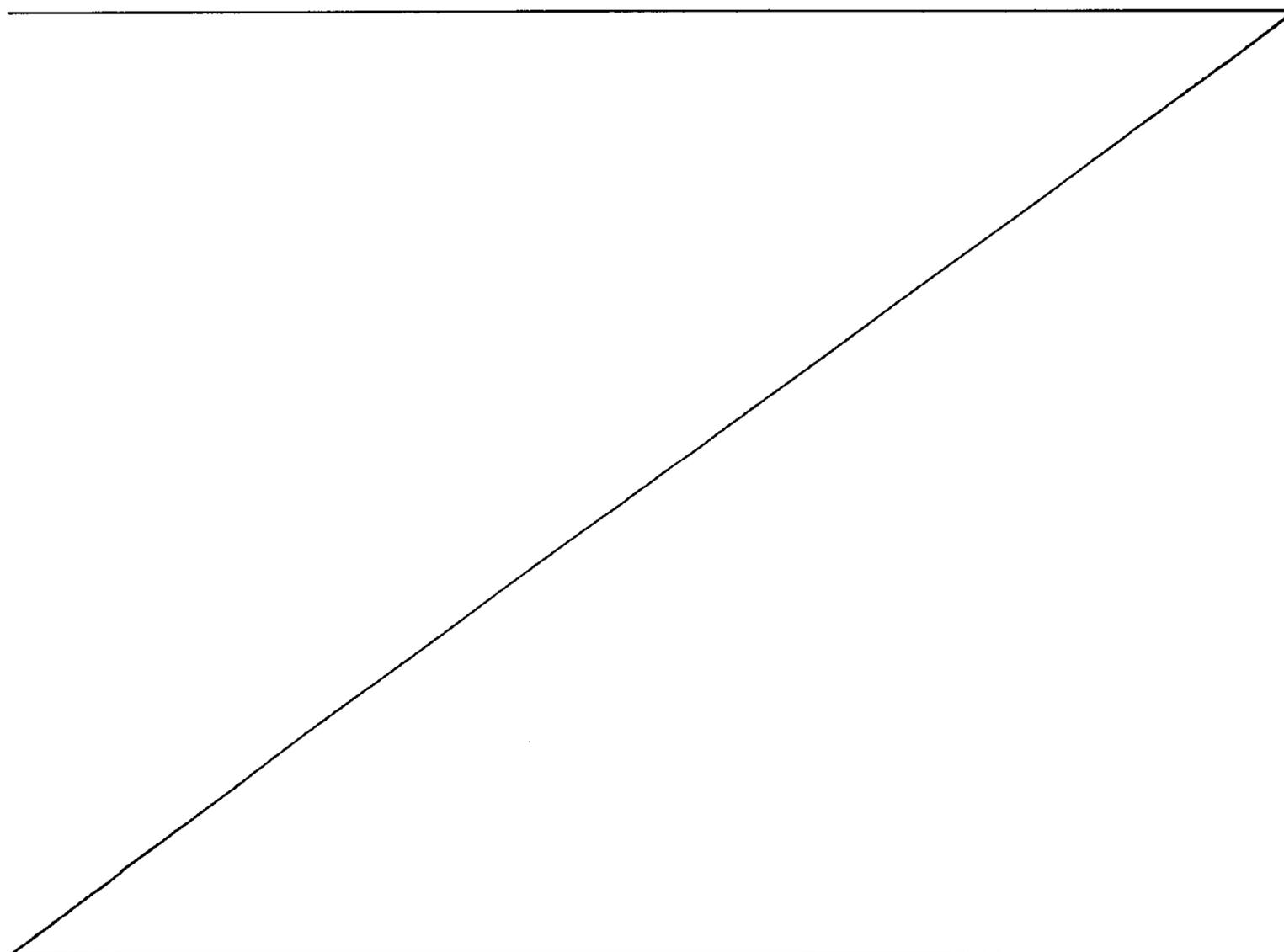
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The invention as claimed is also directed to a process for preparing acylated primary and secondary amines by reacting the amines with an ester in the presence of a protease or lipase, wherein the acid component of the ester has an oxygen atom in the α , β or γ position relative to the carbonyl carbon.

Esters suitable for the process according to the invention are those which have an electron-rich heteroatom in the vicinity of the carbonyl carbon in the acid component of the ester.

The heteroatom must have at least one free pair of electrons. It can be a fluorine, nitrogen, oxygen or sulphur atom. In the invention as claimed, the
10 heteroatom is exclusively oxygen.

It should be located in the vicinity of the carbonyl carbon. This means that the heteroatom is bonded to a carbon atom in the position alpha, beta or gamma to the carbonyl carbon. Preferred acid



components of the ester are those in which the heteroatom is bonded to the C-alpha atom. Oxygen is the preferred heteroatom.

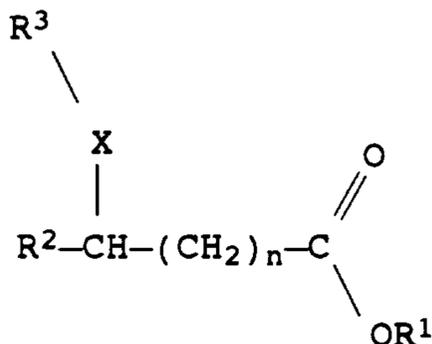
The heteroatom may be linked to other groups, eg. alkyl groups.

5 If the heteroatom is oxygen, for example, an ether moiety is present.

The alcohol component of the ester is not so crucial for the process according to the invention. It is possible to use for this
10 purpose branched and unbranched C₁-C₁₀-alcohols, which may also be substituted.

Particularly suitable esters are those having the structure

15



20

where

25 R¹ is C₁-C₁₀-alkyl,

R² is C₁-C₁₀-alkyl, H,

30 R³ is H, C₁-C₁₀-alkyl, or phenyl which is unsubstituted or substituted by NH₂, OH, C₁-4-alkoxy or halogen,

X is O, S, NR⁴,

35 R⁴ is H, C₁-C₁₀-alkyl, or phenyl which is unsubstituted or substituted by NH₂, OH, C₁-4-alkoxy or halogen,

n is 0, 1 or 2.

40 Among these, the C₁-4-alkyl esters of C₁-4-alkoxyacetic acids, such as ethyl methoxyacetate, are preferred.

A large number of enzymes can be employed as hydrolases in the process according to the invention. Proteases and, in particular, lipases are preferably used. Especially suitable lipases are
45 microbial lipases which can be isolated, for example, from yeasts or bacteria. Particularly suitable lipases are those from

Pseudomonas, eg. Amano P or the lipase from Pseudomonas spec. DSM 8246.

Furthermore the lipases Chirazymes L1 to L8, which are
5 commercially available (Boehringer Mannheim), can be advantageously used in the process according to the invention.

The enzyme can be used in native or immobilized form.

10 Suitable solvents are in general organic solvents. The reaction takes place especially well in ethers, for example in MTBE or THF, or in hydrocarbons such as hexane, cyclohexane, toluene or halohydrocarbons such as methylene chloride.

15 The primary and secondary amines used can also be amino alcohols.

The reaction of the ester with the racemic amine or amino alcohol with enzyme catalysis is normally carried out at room temperature. The reaction times depend on the substrate and are from 1
20 to 48 hours. As a rule, longer reaction times are required for secondary amines/amino alcohols than for primary amines/amino alcohols. The lower reactivity of secondary amines may also be compensated by increasing the amount of catalyst compared with the primary amines.

25

From 1 to 3 mol of ester are added per mole of substrate to be reacted. From 1 to 3 mol of ester are added even when racemic substrates are used.

30 The amount of enzyme to be added depends on the nature of the hydrolase and the activity of the enzyme preparation. The optimal amount of enzyme for the reaction can easily be established by simple preliminary tests. As a rule, 1000 units of lipase are added per mmol of amine or amino alcohol.

35

The course of the reaction can easily be followed by conventional methods, for example gas chromatography. In the case of racemate resolution, it is sensible to terminate the reaction when 50% of the racemic amine or amino alcohol has reacted. This usually
40 takes place by removing the catalyst from the reaction medium, for example by filtering off the enzyme.

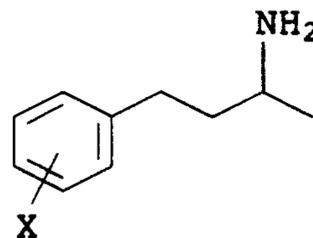
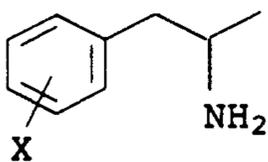
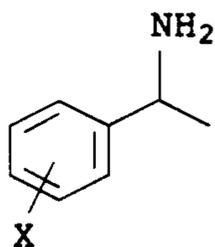
The enantioselective reaction of the racemic substrate with the ester results in the correspondingly acylated product (amide) of
45 one enantiomer, whereas the other enantiomer remains unchanged. The resulting mixture of amine and amide can easily be separated by conventional methods. Examples of very suitable methods for

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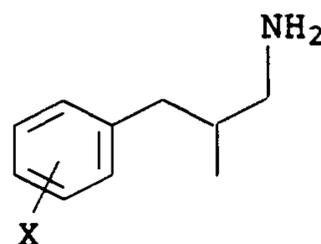
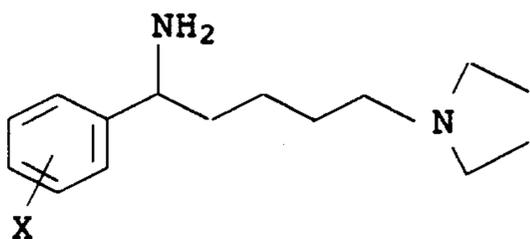
separating the mixture of amine and amide are extraction or distillation.

The process according to the invention is suitable for acylating all primary and secondary amines. It can also be used to resolve the racemates of virtually all primary and secondary amines. It takes place particularly well in the case of primary arylalkylamines, for example those of the following structures:

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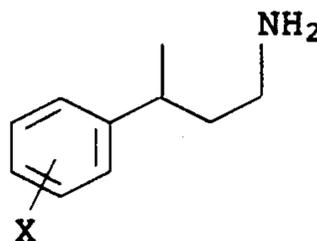


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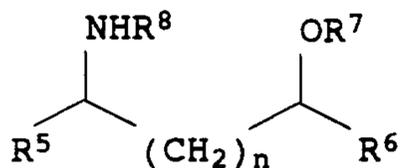
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30 where X is any conventional aromatic substituent, especially halogen, nitro, cyano, C₁-C₄-alkyl, C₁-C₄-alkoxy or C₁-C₄-alkylthio.

The process according to the invention is furthermore suitable for the enantioselective acylation of amino alcohols of the general formula

40



where

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- R⁵, R⁶ = independently of one another H, branched and unbranched C₁-C₁₀-alkyl, C₁-C₄-alkoxycarbonyl, phenyl, phenyl-C₁-C₄-alkyl, it being possible for the phenyl groups to be substituted by halogen, nitro, cyano, C₁-C₄-alkyl, C₁-C₄-alkoxy and C₁-C₄-alkylthio. R⁵ and R⁶ may also be closed to a mono-, bi- or tricyclic system by a carbon chain which may be interrupted by oxygen, sulfur or nitrogen and in turn substituted
- 10 R⁷ = H, C₁-C₁₀-alkyl, C₁-C₄-alkoxycarbonyl
 R⁸ = H, C₁-C₁₀-alkyl
 n = 0 or 1.

When the carbon atoms substituted by OR⁷ or NHR⁸ are stereogenic centers, the process according to the invention relates both to the syn and to the anti isomers.

Examples of amino alcohols of the above general structure are:

- 20 2-amino-1-butanol; ephedrine; pseudoephedrine; norephedrine; norpseudoephedrine; tert-leucinol; phenylglycidol; 1,2-diphenyl-aminoethanol; cis- and trans-2-aminocyclopentanol; cis- and trans-1-amino-2-hydroxyindane; cis- and trans-2-aminocyclohexanol, statine, 2-hydroxy-3-amino-phenylpropionic acid.

25

Preferred amino alcohols are cis- and trans-1-amino-2-hydroxyindane.

The invention is also suitable for preparing optically active primary and secondary amines from the corresponding racemates by

- a) enantioselectively acylating a racemic amine or a racemic amino alcohol with an ester whose acid component has a fluorine, nitrogen, oxygen or sulfur atom in the vicinity of the carbonyl carbon atom, in the presence of a hydrolase,
- b) separating the mixture of optically active amine and optically active acylated amine and obtaining one enantiomer of the amine,
- c) if required obtaining the other enantiomer of the amine or amino alcohol from the acylated amine by amide cleavage.

The process according to the invention can be made even more economic if, after removal of the required enantiomer, the remaining unwanted enantiomer is racemized and reused in the process. This

recycling makes it possible to obtain overall more than 50% of the required enantiomer from the racemic amine.

The processes according to the invention not only are suitable as processes for producing optically active primary and secondary amines and amino alcohols but can also form part of complicated multistage chemical syntheses, for example in the preparation of pharmaceutical active ingredients or crop protection agents. The following examples illustrate the invention.

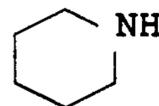
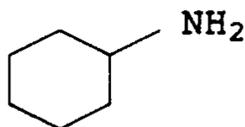
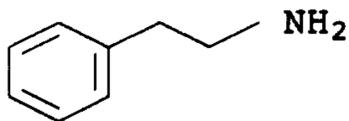
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Example 1: General method for the lipase-catalyzed acylation of amines

10 mmol of the primary or secondary amine are dissolved in MTBE (methyl tert-butyl ether) to give an approximately 10% strength solution. 11 mmol of ethyl methoxyacetate are added to the solution, and the reaction is started by adding 100 mg of lipase (about 1000 U/mg, *Pseudomonas spec.* DSM 8246). When the reaction is complete (12-48 h depending on the amine), the enzyme is filtered off and the solution is concentrated under reduced pressure. The methoxyacetamides are obtained in a yield of more than 90 percent.

Amines employed:

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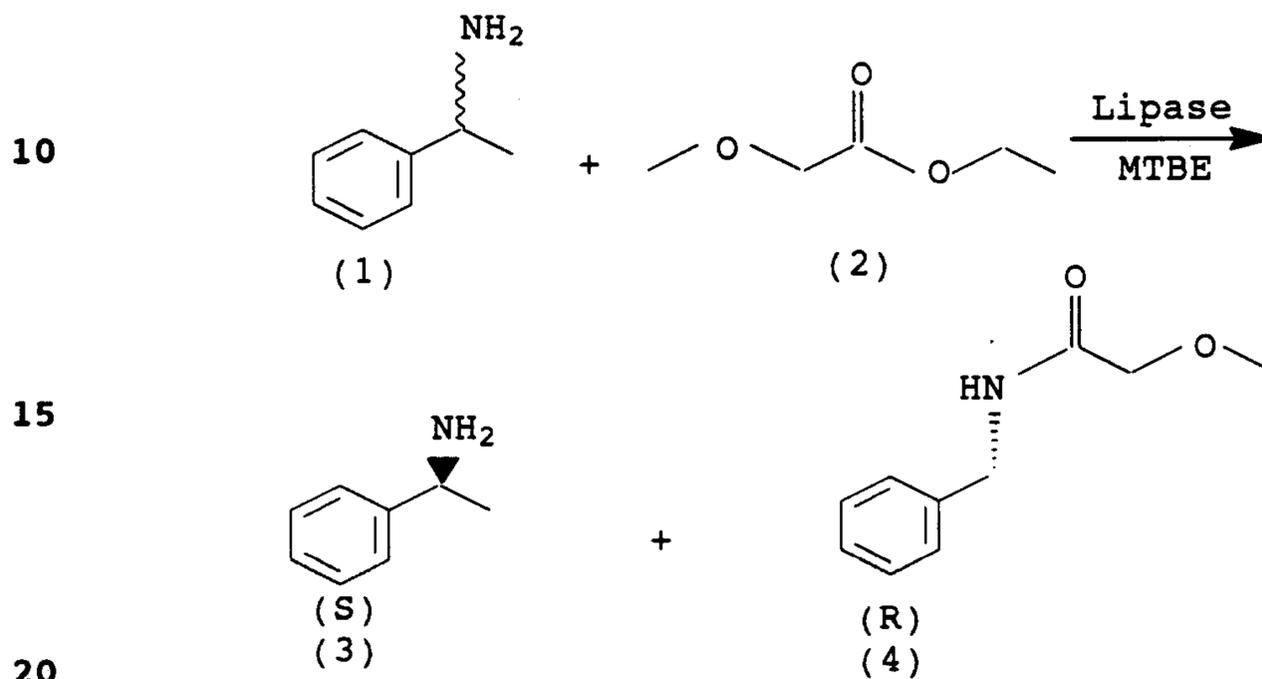
Example 2: Method for racemate resolution

The primary or secondary amine is dissolved in MTBE (about 10% by weight). 1 mole of ethyl methoxyacetate is added per 1 mole of racemic amine and then *Pseudomonas lipase* (DSM 8246) is added and the suspension is stirred at room temperature. About 10,000 units of lipase (10 mg) are added per mmol of amine. After 50% reaction has been reached (checked by gas chromatography), which takes 1-48 h depending on the amine, the enzyme is filtered off. The mixture of amine and acylated amine (amide) is separated by distillation or extraction.

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Example 3: Racemate resolution

20 g of (1) were reacted with 1 equivalent of (2) in the presence of 2 g of *Pseudomonas* lipase in MTBE at room temperature as in Example 2. 50% reaction was reached after 22 h.



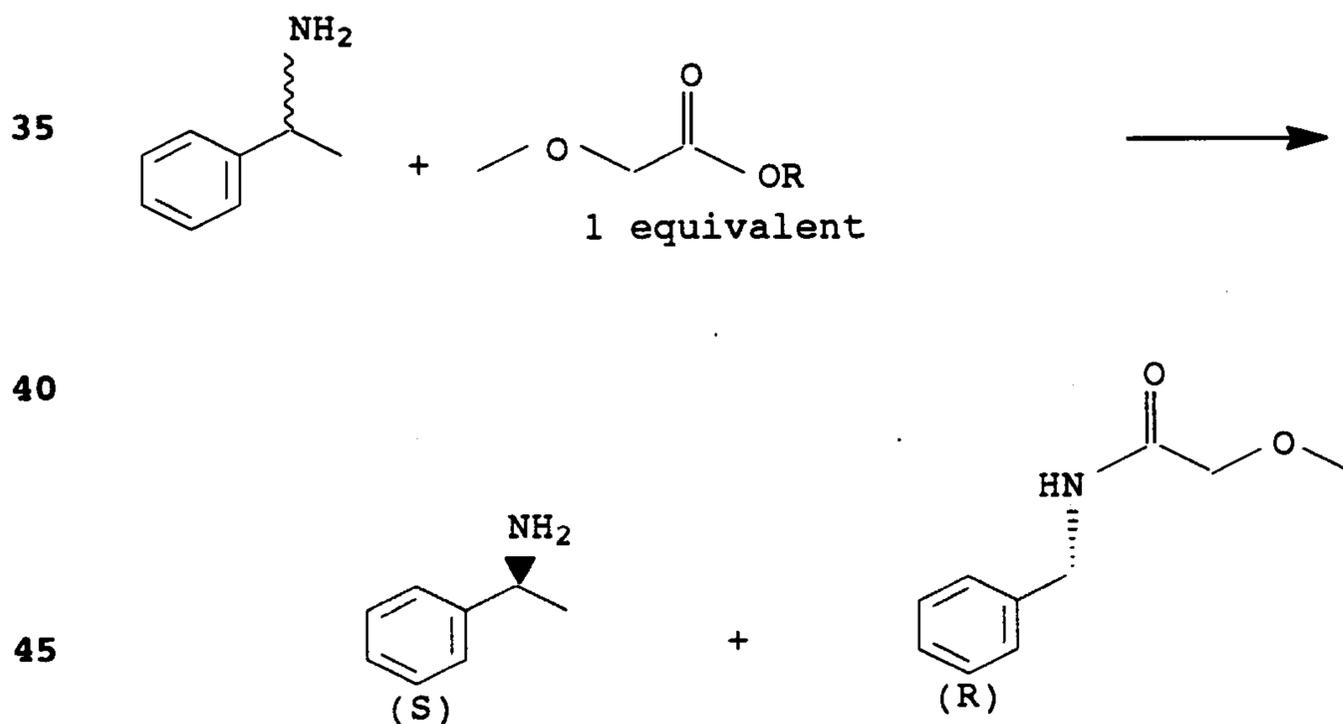
The yield of (3) was 45% (ee > 99%),
the yield of (4) was 45% (ee > 90%).

25 Example 4: Racemate resolution

Various racemate resolutions were carried out as in Example 2. Various amines were employed with diverse reaction conditions. The details are to be found in the table.

30

Table



No.	Racemic amine (g)	R	MTBE (ml)	Lipase (g;U/mg)	Time (h)	Reaction by GC (%)	ee (amine) (%)	ee (amide) (%)																																							
5	20	Et	180	2; 1000	7	47	> 99	93																																							
					72	57																																									
	5	Et	45	0.5;1000	3	43.6	95	96																																							
					22	56.1																																									
10	5	Me	45	0.5;1000	23	38.5	79	97																																							
					23	51.4																																									
	2.5	Et	20	0.5;1000	1	39.6	> 99	87																																							
					3	50.1																																									
					24	59																																									
					48	59																																									
15	2.5	Et	20	0.5;1000	1	41	85	97																																							
					4	52																																									
20	2.5	Et	25	0.125;1000	27	56.1	87	97																																							
					7	25			0.5;1000	21	60.1	93	98																																		
										8	2.5			Et	10	0.25;1000	22	50.6	82	98																											
																					9	2.5	Et	25	0.25;1000	21	55.7	87	99																		
																														10*)	2.5	Et	25	0.25;1000	4.5	40	50	97									
																																							11**)	2.5	Et	25	0.25;1000	21	56.1	92	98
25	2.5	Me	25	0.5; 200	4	54.3	69	99																																							
									14	2.5	Bu	25	0.5; 200	4	54.2	68	> 99																														
																		15***)	2.5	Et	25	0.5; 200	3	54.8	57	> 99																					
30	§) Amine 1	Et	20	0.5;1000	15.5	56	48	52																																							
									17	§) Amine 2	Et	20	0.5;1000	3	53.6	70	75																														
																		18	§) Amine 3	Et	25	0.25;1000	2	53.9	83	> 99																					
																											19	§) Amine 4	Et	25	0.5;1000	1.5	54.6	97	> 99												

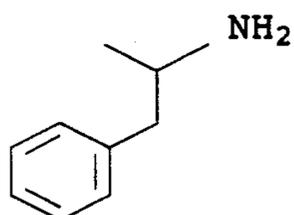
*) as Experiment No. 9 but reaction at 50°C

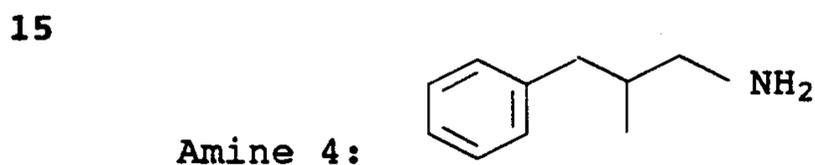
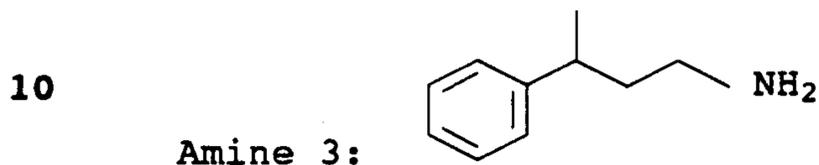
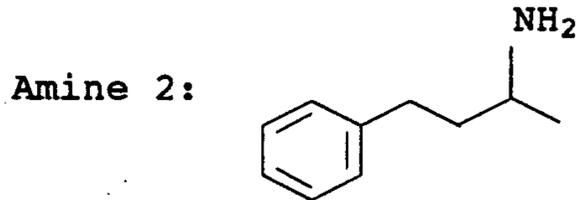
35 **) The reaction mixture was shaken not stirred

***) Ethyl butoxyacetate was used as ester in place of the methoxyacetate.

40 §) 20.6 mmol of each

45 Amine 1:





20 Example 5: Resolution of racemic amino alcohols

a) to a suspension of 150 mg (1 mmol) of trans-1-amino-2-hydroxyindane and 350 mg (3 mmol) of ethyl methoxyacetate in 10 ml of methyl-tert.-butyl ether were added 9.6 mg of Chirazyme L2, and the mixture was stirred at room temperature. 49% conversion was reached after 41 h. After adding 3 ml of ethanol, the enzyme was filtered off, the filtrate was evaporated to dryness and the residue was taken up in 10 ml of 1N HCl. Extraction (CH_2Cl_2), drying (MgSO_4) of the organic phase and concentration resulted in the hydroxy amide in the form of white crystals.

Crude yield: 100 mg (42%)

35 $[\alpha]_D^{20} = -53.9^\circ$ ($c = 1.65$; CH_2Cl_2), ee > 95%.

b) 150 mg (1 mmol) of cis-1-amino-2-hydroxyindane were reacted with 350 mg (3 mmol) of ethyl methoxyacetate and 50 mg of Chirazyme L1 as in Example 5a). 50% conversion was reached after 48 h. The enantiomeric excess of the hydroxy amide obtained by workup similar to that above was 71%.

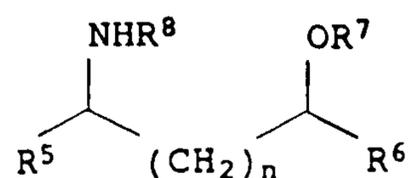
WHAT IS CLAIMED IS:

1. A process for preparing acylated primary and secondary amines by reacting the amines with an ester in the presence of a protease or lipase, wherein the acid component of the ester has an oxygen atom in the α , β or γ position relative to the carbonyl carbon.
2. A process for resolving racemates of primary and secondary amines by reacting with an ester in the presence of a protease or lipase and subsequently separating one amine which has been enantioselectively acylated from the
10 other, unreacted, enantiomer of the amine, wherein the acid component of the ester has an oxygen atom in the α , β or γ position relative to the carbonyl carbon.
3. A process for preparing optically active primary and secondary amines from the corresponding racemates, which comprises:
 - a) enantioselectively acylating a racemic amine with an ester whose acid component has an oxygen atom in the α , β or γ position relative to the carbonyl carbon, in the presence of a protease or lipase,
 - b) separating the mixture of optically active amine and optically active acylated amine and obtaining one enantiomer of the amine,
 - 20 c) if required obtaining the other enantiomer of the amine from the acylated amine by amide cleavage.
4. A process as claimed in claim 3, wherein step b) or c) is followed by another step in which the unwanted enantiomer is racemized and then returned to the process for resolving the racemate.
5. A process for preparing optically active compounds, which comprises a process as claimed in any one of claims 2 to 4.

6. A process as claimed in claim 2 or 3, wherein the primary or secondary amine used is an amino alcohol.

7. A process as claimed in claim 6, wherein the amino alcohol is cis- or trans-1-amino-2-hydroxyindane.

8. A process as defined in claim 6, wherein the amino alcohol has the formula:



10 where:

R^5 and R^6 independently of one another are H, branched and unbranched C_1 - C_{10} -alkyl, C_1 - C_4 -alkoxycarbonyl, phenyl, phenyl- C_1 - C_4 -alkyl, it being possible for the phenyl groups to be substituted by halogen, nitro, cyano, C_1 - C_4 -alkyl, C_1 - C_4 -alkoxy and C_1 - C_4 -alkylthio, where R^5 and R^6 may also be closed to a mono-, bi- or tricyclic system by a carbon chain which may be interrupted by oxygen, sulfur or nitrogen,

R^7 is H, C_1 - C_{10} -alkyl or C_1 - C_4 -alkoxycarbonyl,

R^8 is H or C_1 - C_{10} -alkyl, and

n is 0 or 1.

20 9. A process as claimed in any one of claims 1 to 8, wherein the ester is ethyl methoxyacetate.