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(54) **RESOLUTION D'AMINES CHIRALES**

(54) **RESOLUTION OF CHIRAL AMINES**

(57) Pour résoudre les amines chirales, l'invention consiste à faire réagir sélectivement un énantiomère de l'amine avec un ester d'alkyle. Cette réaction se fait en présence d'une lipase énantiosélective de façon à produire un amide de cet énantiomère. On sépare ensuite l'ester de l'énantiomère qui n'a pas réagi. Le groupe alkyle de l'ester est un groupe isoalkyle. Les groupes préférés sont l'isobutyle et plus spécialement l'isopropyle.

(57) Chiral amines are resolved by selectively reacting an enantiomer of the amine with an alkyl ester in the presence of an enantioselective lipase enzyme to produce an amide of that enantiomer and separating it from the unreacted enantiomer, the alkyl group of the ester being an isoalkyl group. Isobutyl and especially isopropyl groups are preferred.

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(21) International Application Number: PCT/GB98/03679 (22) International Filing Date: 9 December 1998 (09.12.98) (30) Priority Data: 9726229.9 12 December 1997 (12.12.97) GB (71) Applicant (for all designated States except US): ZENECA LIMITED [GB/GB]; 15 Stanhope Gate, London W1Y 6LN (GB). (72) Inventor; and (75) Inventor/Applicant (for US only): REEVE, Christopher, David [GB/GB]; Belasis Avenue, P.O. Box 2, Billingham TS23 1YN (GB). (74) Agents: LOCKE, Timothy, John; Zeneca Specialties, Intellectual Property Group, Belasis Avenue, P.O. Box 2, Billingham TS23 1YN (GB) et al.		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: RESOLUTION OF CHIRAL AMINES		
(57) Abstract <p>Chiral amines are resolved by selectively reacting an enantiomer of the amine with an alkyl ester in the presence of an enantioselective lipase enzyme to produce an amide of that enantiomer and separating it from the unreacted enantiomer, the alkyl group of the ester being an isoalkyl group. Isobutyl and especially isopropyl groups are preferred.</p>		

RESOLUTION OF CHIRAL AMINES

THIS INVENTION relates to the resolution of chiral amines.

It is known for example from WO95/08636, Kitaguchi et al (J.Am. Chem. Soc., 1989, 111, 3094-3095), Gotor et al (J.Chem. Soc. Perkin Trans. 1, 1993, 2453-2456), Ohmer et al (Enzyme and Microbial Technology, 1996, 19, 328-331) and Sanchez et al (Tetrahedron Asymmetry, 1997, 8, 37-40) and Chiou et al, Bio-organic & Medicinal Chemistry Letters Vol. 7, No 4, pp 433-436 (1977) to resolve racemic amines by acylating one enantiomer by reaction with an alkyl ester in the presence of an enantioselective enzyme as catalyst. However, the results obtained have in many cases been disappointing.

10 We have found that such reactions of attractive stereospecificity occur if the alkyl group of the ester is an isoalkyl group preferably an isopropyl group.

The invention therefore comprises a process of resolution of chiral amines which comprises selectively reacting one enantiomer of the amine with an alkyl ester in the presence of a enantioselective lipase enzyme to produce an amide of one enantiomer and separating it from an unreacted enantiomer optionally after further reaction characterised in that the alkyl group of the ester is an isoalkyl group and preferably an isopropyl group. A lipase is an enzyme capable of catalysing the esterification of aliphatic acids with glycerol and the hydrolysis of esters of glycerol and aliphatic acids.

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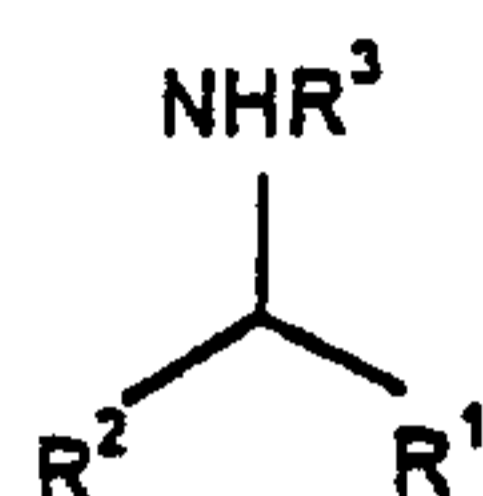
Either or both enantiomers may be recovered. The untreated enantiomer may be recovered as such. The reacted enantiomer may be converted to the original amine enantiomer suitably by hydrolysis. It may of course be utilised as the amide if desired. Suitably such hydrolysis may be carried out using as catalyst an amidase of the same stereospecificity and/or hydrolysing any unwanted stereoisomer present using an amidase of opposite stereospecificity, separating the unwanted amide and hydrolysing that, thus providing a second stage of resolution and enhancing the enantiomeric excess of the product, but if the first stage provides sufficient specificity a non selective hydrolysis may be employed.

The acid component of the ester may have 1 to 10 for example 1 to 5 carbon atoms. It is preferably of formula RCOOH in which R is a hydrocarbyl group, for example an aryl group such as a phenyl, naphthyl or benzyl group, an alkyl or cycloalkyl group or a chloro or bromo substituted derivative thereof, the substitution being preferably on a carbon atom adjacent to the C=O group or one next to it. It may suitably be an unsubstituted alkyl group suitably having 1 to 4 carbon atoms as these are often of

moderate cost tend not to be involved in unwanted side reactions and tend not to be aggressive to metal reaction vessels.

The process is suitable for the resolution of primary and secondary amines for example amines of formula

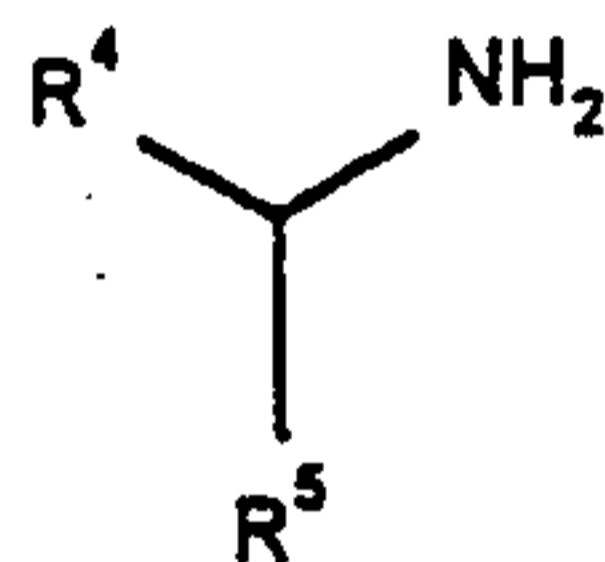
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in which R^1 and R^2 are alkyl, cycloalkyl, alkenyl or alkynyl, or an aryl group or such a group, which is substituted with for example NO_2 , SO_3H , COOR^4 , Cl, Br, F, I, OH, SO, SO_2 , CN, alkoxy and in the case of aryl substitution NH_2 in which R^1 and R^2 are different and R^3 is H, alkyl, cycloalkyl, alkenyl, alkynyl, or an aryl group or such a group which is substituted with for example, NO_2 , SO_3H , COOH , Cl, Br, F, I, OH, SO, SO_2 , CN and R^4 is alkyl, cycloalkyl, alkenyl, alkynyl or an aryl group optionally substituted as described above. The process is suitable for the resolution of amino acids and their esters.

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The amine preferably has the formula



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in which R^4 is an alkyl group having for example 1 to 12 and preferably 1 to 6 carbon atoms and R^5 is an aryl preferably a naphthyl group, an alkyl group or cycloalkyl group in each case optionally substituted by one or more alkoxy, hydroxy, halogen and/or -CN group or in the case of aryl groups, amine groups, groups which preferably have at most 12 and more preferably at most 6 carbon atoms in total in all of the said substituents.

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The amount of lipase present is preferably 10 to 50% by weight of the amine. The lipase is preferably supported on a solid support to enable it to be removed mechanically, for example by filtration or centrifugation, after reaction.

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Separation of the amide of the reacted amine from unreacted amine may be accomplished by known methods, for example, distillation or crystallisation.

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The reaction may be carried out in the presence of a solvent which may be the ester, an ether (for example methyl tert butyl ether, dimethoxy ethane or tetrahydrofuran) or a hydrocarbon, for example toluene or an alkane or cycloalkane having 5 to 10 carbon atoms or a halogenated hydrocarbon solvent. It is preferably free from -OH and NH_2 groups.

The reaction may be carried out at 20-60°C for example at 20-40°C. At least one mole of the ester should be provided per two moles of the amine so as to permit stoichiometric reaction of an enantiomer, but it is preferred that an excess be provided. The excess should normally be sufficient to provide preferably at least 90% and more preferably at least 95% for example 99% reaction of the most reactive enantiomer. In judging the appropriate excess, conditions should not be such as to cause unacceptable conversion of the less reactive enantiomer, and if a very high selectivity for the more reactive enantiomer is needed it may be preferred to convert only part thereof, thus requiring little or no excess; indeed operation with less than the stoichiometric amount may be desirable in some cases.

The step of converting the amine to the amide is preferably carried out in the substantial absence of water and other hydroxy compounds.

Example 1

2-Amino-3, 3-dimethylbutane (125mg) was added to 3ml of acyl donor (e.g. ethyl acetate or isopropyl acetate) and incubated at 28°C in the presence of 60mg of immobilised lipase from *Candida antarctica* (NOVO SP 435). The extent of reaction was followed by quantitative gas chromatography on a Perkin Elmer 8500 system fitted with a J&W 30m x 0.32mm fused silica capillary GC column. The gas chromatograph was operated with helium carrier gas ^{at 5.5×10^4 Pa (8 psi)} using a temperature gradient starting at 80°C and rising to 200°C at a rate of 20° min⁻¹ followed by 6 minutes held at 200°C. Under such conditions 2-amino-3, 3-dimethylbutane was eluted with a retention time of 3.0 minutes and the corresponding acetamide at 6.1 minutes. The enantiomeric purity of unreacted 2-amino-3, 3-dimethylbutane (derivatised as the N-butyramide) and the acetamide product of the aminolysis reaction was determined by chiral phase gas chromatography using a 25m x 0.32mm Chrompack CP-Chirasil-Dex CB column. The gas chromatograph was operated with a helium carrier gas ^{at 5.5×10^4 Pa (8 psi)} and at 120°C. Under such conditions the two enantiomers of the acetamide derivative of 2-amino-3, 3-dimethylbutane were eluted with retention times of 4.78 minutes (S) and 4.95 minutes (R) and the two enantiomers of the butyramide derivative 8.38 minutes (S) and 8.51 minutes (R). The results are summarised in Table 1.

Table 1. Effect of structural changes to the alcohol component of the acyl donor on the enantioselectivity of the resolution of 2-amino-3, 3-dimethylbutane by *Candida antarctica* lipase.

Acyl donor	Reaction time (h)	Conversion (%)	E.e. unreacted amine (as butyramide) (%)	E.e. product (%)	Enantiomeric ratio (E)
Ethyl acetate	216	39.2	50	78	13
Isopropyl acetate	216	46.5	83	95	104

Example 2

5 1-(1-Naphthyl) ethylamine (100mg) was added to 5ml of acyl donor e.g. ethyl acetate or isopropyl acetate) and incubated at 28°C in the presence of 50mg of immobilised *Candida antarctica* lipase (NOVO SP 435). The extent of conversion was determined from measurements of the enantiomeric excess of the unreacted amine (derivatised as its butyramide) and product acetamide using the mathematical expression described by

10 Chen *et. al.* (J. Am. Chem. Soc., 1982, Vol 104, pp 7294-7299). Enantiomeric excess measurements were made by chiral phase HPLC on a Hewlett Packard HP 1050 system fitted with a 250mm x 4.6mm Daicel Chiralcel OD column. The column was eluted isocratically with a mixture of 92.5% hexane and 7.5% ethanol in 1ml min⁻¹. Compounds were detected by UV absorbance at 254nm. The retention times of the unreacted

15 amine (derivatised as its butyramide) were 7.65 minutes (R) and 15.2 minutes (S) and the product acetamide 8.9 minutes (R) and 17.9 minutes (S). The results are summarised in Table 2.

Table 2. Effect of structural changes to the alcohol component of the acyl donor on the enantioselectivity of the resolution of 1-(1-naphthyl) ethylamine by *Candida antarctica* lipase.

Acyl donor	Reaction time (h)	Conversion (%)	E.e. unreacted amine (as butyramide) (%)	E.e. product (%)	Enantiomeric ratio (E)
Ethyl acetate	70	52.5	66	60	8
Isopropyl acetate	66	48.9	88	92	70

Example 3

5 Racemic 1-(1-naphthyl)ethylamine (100mg) was added to 5ml of acyl donor (see Table 3) and incubated at ambient temperature in the presence of 20mg of Chirazyme L2 (immobilised *Candida antarctica* lipase). At intervals 0.5ml samples were removed and diluted to 1ml with a solution of hexane/ethanol (92.5:7.5). The unreacted amine was converted to its corresponding butyramide by the addition of 10 μ l of butyric

10 anhydride. Each sample was analysed by chiral phase HPLC on a Hewlett Packard HP1050 system using a Daicel Chiralcel OD analytical column (250mm x 4.6mm) eluted with hexane/ethanol (92.5:7.5) at a flow rate of 1 ml min⁻¹. Compounds were detected by UV absorbance at 254nm. The retention times of the two enantiomers of the unreacted amine (derivatised as its butyramide) were 7.9 minutes (R) and 15.0 minutes (S) and the

15 product acetamide 9.3 minutes (R) and 17.9 minutes (S). The extent of conversion and enantiomeric ratio was determined from measurements of the enantiomeric excess of unreacted amine and product acetamide using the mathematical expression described by Chen *et. al.* (J. Am. Chem. Soc., 1982, Vol 104, pp 7294-7299). The results are summarised in Table 3.

Table 3. Effect of structural changes to the alcohol component of the acyl donor on the enantiospecificity of the resolution of 1-(1-naphthyl)ethylamine by Chirazyme L2 lipase.

Acyl donor	Time (h)	Conversion (%)	E.e of unreacted amine (%)	E.e of product acetamide (%)	Enantiomeric ratio (E)
Methyl acetate	120	30	6	13	1
Ethyl acetate	120	43	59	79	15
n-Propyl acetate	120	40	54	81	16
n-Butyl acetate	120	32	40	77	18
Isopropyl acetate	72	45	78	95	100
Isobutyl acetate	72	50	86	88	37
Isoamyl acetate	72	44	68	88	28

Example 4

5 Racemic 1,2,3,4-tetrahydro-1-naphthylamine (100mg) was added to 5ml of acyl donor (see Table 4) and incubated at ambient temperature in the presence of 20mg of Chirazyme L2 (immobilised *Candida antarctica* lipase). At intervals 0.5ml samples were removed and diluted to 1ml with dichloromethane. The unreacted amine was converted to its corresponding butyramide by the addition of 10 μ l of butyric anhydride. Each

10 sample was analysed by chiral phase GC on a Perkin Elmer 8700 system using a Chrompack CP-Chirasil-Dex CB column (25m x 0.32mm). The gas chromatograph was operated isothermally at 175°C with helium carrier gas ^{at 5.5 x 10⁴ Pa (8 psi)} ~~at 8 psi~~. Compounds were detected by flame ionisation. The retention times of the two enantiomers of the unreacted amine (derivatised as its butyramide) were 23.8 minutes (S) and 25.8 minutes

15 (R) and the product acetamide 14.4 minutes (S) and 15.9 minutes (R). The extent of conversion and enantiomeric ratio was determined from measurements of the enantiomeric excess of unreacted amine and product acetamide using the mathematical expression described by Chen *et. al.* (J. Am. Chem. Soc., 1982, Vol 104, pp 7294-7299). The results are summarised in Table 4.

M O D I F I E D

Table 4. Effect of structural changes to the alcohol component of the acyl donor on the enantiospecificity of the resolution of 1,2,3,4-tetrahydro-1-naphthylamine by Chirazyme L2 lipase.

Acyl donor	Time (h)	Conversion (%)	E.e of unreacted amine (%)	E.e of product acetamide (%)	Enantiomeric ratio (E)
Methyl acetate	72	57	16	12	1
Ethyl acetate	72	54	82	69	14
n-Propyl acetate	72	52	78	74	14
n-Butyl acetate	72	26	14	42	3
Isopropyl acetate	72	50	98	97	458
Isobutyl acetate	72	53	95	83	43
Isoamyl acetate	72	46	70	81	21

Example 5

5 Racemic 2-amino-3,3-dimethylbutane (100mg) was added to 5ml of acyl donor (see Table 5) and incubated at ambient temperature in the presence of 40mg of Chirazyme L2 (immobilised *Candida antarctica* lipase). At intervals 0.5ml samples were removed and diluted to 1ml with dichloromethane. The unreacted amine was converted to its corresponding butyramide by the addition of 10 μ l of butyric anhydride. Each

10 sample was analysed by chiral phase GC on a Perkin Elmer 8700 system using a Chrompack CP-Chirasil-Dex CB column (25m x 0.32mm). The gas chromatograph was operated isothermally at 120°C with helium carrier gas at ~~8 psi~~ ^{5.5 x 10⁴ Pa (8 psi)}. Compounds were detected by flame ionisation. The retention times of the two enantiomers of the unreacted amine (derivatised as its butyramide) were 10.3 minutes (S) and 10.5 minutes

15 (R) and the product acetamide 5.6 minutes (S) and 5.9 minutes (R). The extent of conversion and enantiomeric ratio was determined from measurements of the enantiomeric excess of unreacted amine and product acetamide using the mathematical expression described by Chen *et. al.* (J. Am. Chem. Soc., 1982, Vol 104, pp 7294-7299). The results are summarised in Table 5.

Table 5. Effect of structural changes to the alcohol component of the acyl donor on the enantiospecificity of the resolution of 2-amino-3,3-dimethylbutane by Chirazyme L2 lipase.

Acyl donor	Time (h)	Conversion (%)	E.e of unreacted amine (%)	E.e of product acetamide (%)	Enantiomeric ratio (E)
Methyl acetate	168	29	2	5	1
Ethyl acetate	168	34	36	69	8
n-Propyl acetate	168	26	24	69	7
n-Butyl acetate	168	17	13	62	5
Isopropyl acetate	168	41	67	98	109
Isobutyl acetate	168	33	44	88	27
Isoamyl acetate	168	25	26	79	10

Example 6

5 Racemic 1-(1-naphthyl)ethylamine (100mg) was added to a solution of 4ml of dimethoxyethane and 1ml of acyl donor (see Table 6) and incubated at ambient temperature in the presence of 20mg of Chirazyme L2 (immobilised *Candida antarctica* lipase). At intervals 0.5ml samples were removed and diluted to 1ml with a solution of hexane/ethanol (92.5:7.5). The unreacted amine was converted to its corresponding

10 butyramide by the addition of 10 μ l of butyric anhydride. Each sample was analysed by chiral phase HPLC as described in Example 3. The results are summarised in Table 6.

Table 6. Effect of structural changes to the alcohol component of the acyl donor on the enantiospecificity of the resolution of 1-(1-naphthyl)ethylamine in dimethoxyethane by Chirazyme L2 lipase.

Acyl donor	Time (h)	Conversion (%)	E.e of unreacted amine (%)	E.e of product acetamide (%)	Enantiomeric ratio (E)
Methyl acetate	168	26	276	77	10
Ethyl acetate	168	37	53	91	33
n-Propyl acetate	168	34	47	92	35
n-Butyl acetate	168	36	52	94	43
n-amyl acetate	168	22	26	93	32
Isopropyl acetate	168	44	78	>98	650
Isobutyl acetate	168	40	64	95	95
Isoamyl acetate	168	35	51	94	61

Example 7

5 Racemic 1,2,3,4-tetrahydro-1-naphthylamine (100mg) was added to a solution of 4ml of dimethoxyethane and 1ml of acyl donor (see Table 7) and incubated at ambient temperature in the presence of 20mg of Chirazyme L2 (immobilised *Candida antarctica* lipase). At intervals 0.5ml samples were removed and diluted to 1ml with dichloromethane. The unreacted amine was converted to its corresponding butyramide by the addition of 10µl of butyric anhydride. Each sample was analysed by chiral phase GC as described in Example 4. The results are summarised in Table 7.

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Table 7. Effect of structural changes to the alcohol component of the acyl donor on the enantiospecificity of the resolution of 1,2,3,4-tetrahydro-1-naphthylamine in dimethoxyethane by Chirazyme L2 lipase.

Acyl donor	Time (h)	Conversion (%)	E.e of unreacted amine (%)	E.e of product acetamide (%)	Enantiomeric ratio (E)
Methyl acetate	120	41	49	69	9
Ethyl acetate	120	47	85	95	129
n-Propyl acetate	120	47	83	47 94	79
n-Butyl acetate	120	42	68	95	65
n-Amyl acetate	120	34	46	90	28
Isopropyl acetate	120	50	96	98	194
Isobutyl acetate	120	50	97	96	278
Isoamyl acetate	120	42	69	96	86

Example 8

- 5 Racemic 2-amino-3,3-dimethylbutane (100mg) was added to a solution of 4ml of dimethoxyethane and 1ml of acyl donor (see Table 8) and incubated at ambient temperature in the presence of 40mg of Chirazyme L2 (immobilised *Candida antarctica* lipase). At intervals 0.5ml samples were removed and diluted to 1ml with dichloromethane. The unreacted amine was converted to its corresponding butyramide
- 10 by the addition of 10 μ l of butyric anhydride. Each sample was analysed by chiral phase GC as described in Example 5. The results are summarised in Table 8.

Table 8. Effect of structural changes to the alcohol component of the acyl donor on the enantiospecificity of the resolution of 2-amino-3,3-dimethylbutane in dimethoxyethane by Chirazyme L2 lipase.

Acyl donor	Time (h)	Conversion (%)	E.e of unreacted amine (%)	E.e of product acetamide (%)	Enantiomeric ratio (E)
Methyl acetate	336	22	17	59	5
Ethyl acetate	336	29	37	90	29
n-Propyl acetate	336	27	34	91	33
n-Butyl acetate	336	25	30	91	26
n-Amyl acetate	336	17	18	86	19
Isopropyl acetate	336	36	56	>98	791
Isobutyl acetate	336	30	41	96	68
Isoamyl acetate	336	27	35	94	51

Example 9

5 Racemic 1-(1-naphthyl)ethylamine (100mg) was added to 5ml of acyl donor (see Table 9) and incubated at ambient temperature in the presence of 50mg of Chirazyme L6 (*Pseudomonas species* lipase). At intervals 0.2ml samples were removed and diluted to 1ml with a solution of hexane/ethanol (92.5:7.5). The unreacted amine was converted to its corresponding butyramide by the addition of 4 μ l of butyric anhydride. Each sample
 10 was analysed by chiral phase HPLC as described in Example 3. The results are summarised in Table 9.

Table 9. Effect of structural changes to the alcohol component of the acyl donor on the Enantiospecificity of the resolution of 1-(1-naphthyl)ethylamine by Chirazyme L6 lipase.

Acyl donor	Time (h)	Conversion (%)	E.e of unreacted amine (%)	E.e of product acetamide (%)	Enantiomeric ratio (E)
Ethyl acetate	266	21	12	47	3
Isopropyl acetate	266	12	12	87	18

Example 10

Racemic 1,2,3,4-tetrahydro-1-naphthylamine (100mg) was added to 5ml of acyl donor (see Table 10) and incubated at ambient temperature in the presence of 50mg of Chirazyme L6 (*Pseudomonas* species lipase). At intervals 0.2ml samples were removed and diluted to 1 μ l with MTBE. The unreacted amine was converted to its corresponding butyramide by the addition of 6ml of butyric anhydride. Each sample was analysed by chiral phase GC as described in Example 4. The results are summarised in Table 10.

Table 10. Effect of structural changes to the alcohol component of the acyl donor on the Enantiospecificity of the resolution of 1,2,3,4-tetrahydro-1-naphthylamine by Chirazyme L6 lipase.

Acyl donor	Time (h)	Conversion (%)	E.e of unreacted amine (%)	E.e of product acetamide (%)	Enantiomeric ratio (E)
Ethyl acetate	244	43	18	24	2
Isopropyl acetate	244	36	50	90	28

Chirazyme is a trade mark of Boehringer Mannheim GmbH

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15 97TJL04S - MS - 04 December 1998

CLAIMS - SBI 60265 - OCTOBER 1999

1 A process of resolution of chiral amines which comprises selectively reacting an enantiomer of the amine with an alkyl ester in the presence of a enantioselective lipase enzyme to produce an amide of that enantiomer and separating it from an unreacted enantiomer optionally after further reaction characterised in that the acid component of the ester has 1 to 10 carbon atoms and the parent acid is of formula RCOOH in which R is a hydrocarbyl group and the alkyl group of the ester is an isoalkyl group.

2 A process as claimed in Claim 1 in which the isoalkyl group is an isobutyl or isopropyl group.

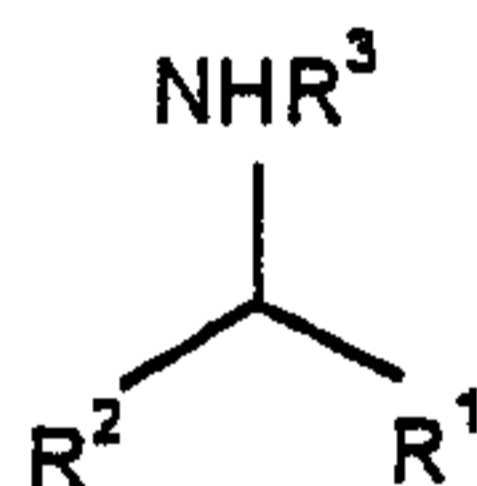
3 A process as claimed in Claim 1 or 2 in which the unreacted enantiomer is recovered as such.

4 A process as claimed in Claims 1 or 2 in which the reacted enantiomer is converted to the original amine enantiomer by hydrolysis.

5 A process as claimed in Claim 1 or 2 in which the reacted enantiomer is utilised in its amide form.

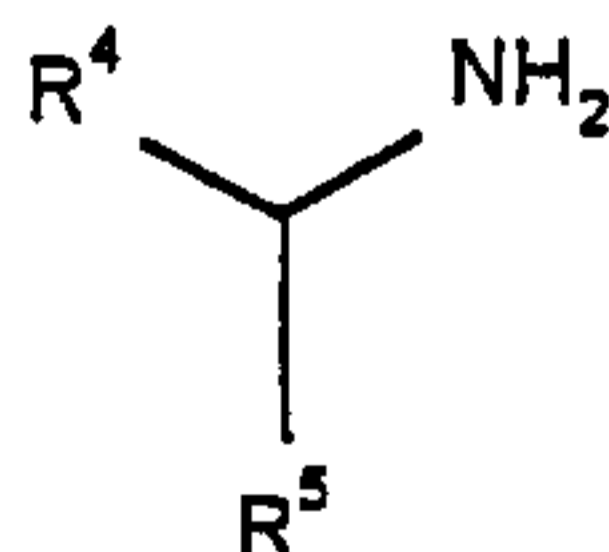
6 A process as claimed in any preceding claim in which the hydrocarbyl group R is an unsubstituted alkyl group having 1 to 4 carbon atoms.

7 A process as claimed in any preceding claim in which the amine has the formula



in which R¹ and R² are alkyl, cycloalkyl, alkenyl or alkynyl, or aryl groups or such a groups, substituted with for example NO₂, SO₃H, COOR⁴, Cl, Br, F, I, OH, SO, SO₂, CN, alkoxy and, in the case of aryl substitution, NH₂ in which R¹ and R² are different and R³ is H, alkyl, cycloalkyl, alkenyl, alkynyl, or an aryl group or such a group which is substituted with for example, NO₂, SO₃H, COOH, Cl, Br, F, I, OH, SO, SO₂, CN and R⁴ is an alkyl, cycloalkyl, alkenyl, alkynyl or aryl group optionally substituted by one or more NO₂, SO₃H, COOR³ Cl, Br, F, I, OH, SO, SO₂, CN or alkoxy groups.

8 A process as claimed in Claim 7 in which the amine has the formula



CLAIMS

in which R⁴ is an alkyl group having for example 1 to 12 and preferably 1 to 6 carbon atoms and R⁵ is an aryl preferably a naphthyl group, an alkyl group or cycloalkyl group in each case optionally substituted by one or more alkoxy, hydroxy, halogen and/or -CN groups or in the case of naphthyl groups, amine groups, groups which preferably have at most 12 and more preferably at most 6 carbon atoms in total in all of the said substituents.

9 A process as claimed in any preceding claim in which 10 to 50% by weight of lipase is present based on the amine.

10 A process as claimed in any preceding claim in which the lipase is supported on a solid support.

11 A process as claimed in any preceding claim which is carried out in the presence of a solvent which is an ester, ether or hydrocarbon or a halogenated hydrocarbon which is free from OH and NH₂ groups.

12 A process as claimed in any preceding claim which is carried out at a temperature of 20 to 60°C.