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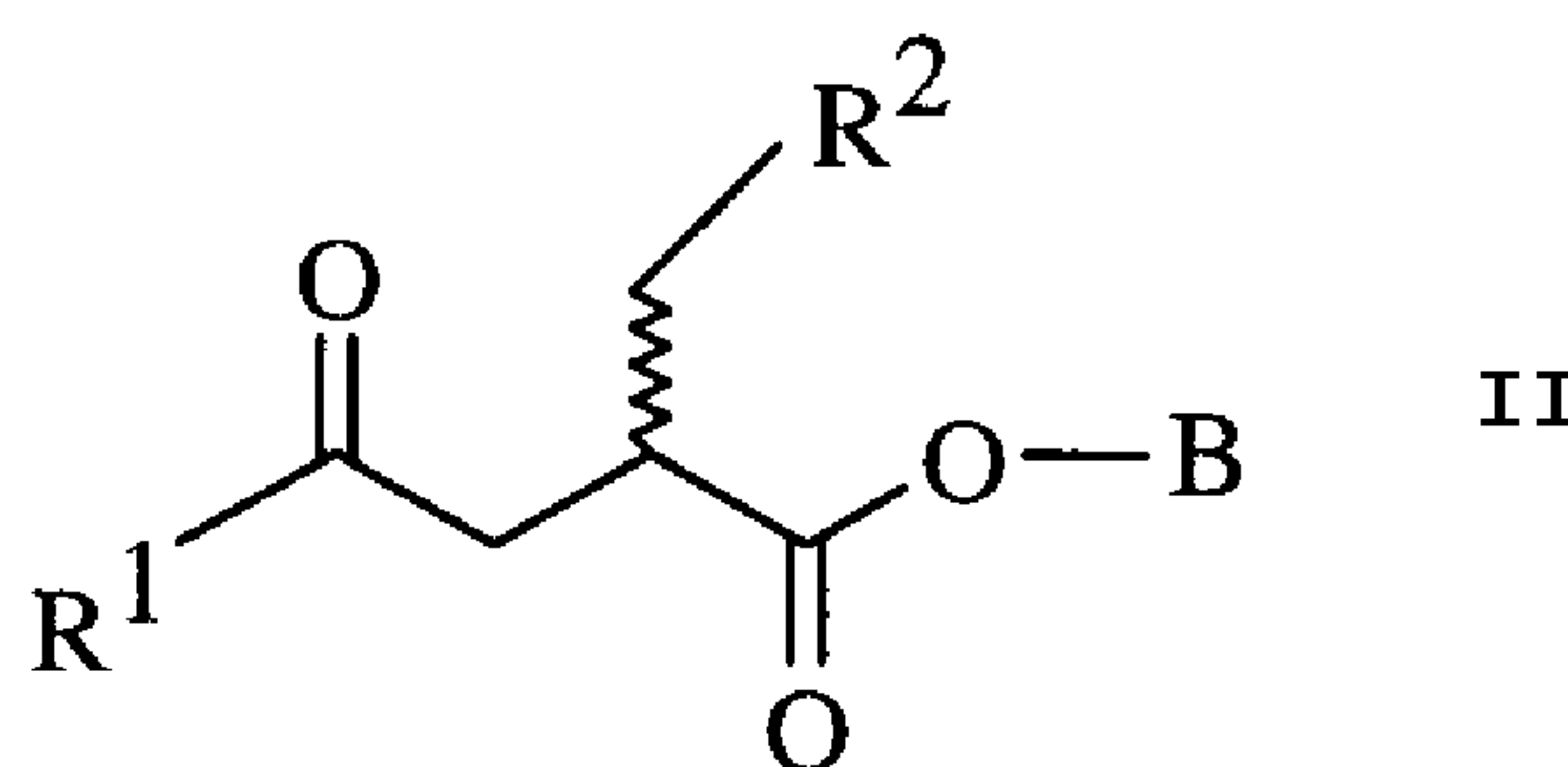
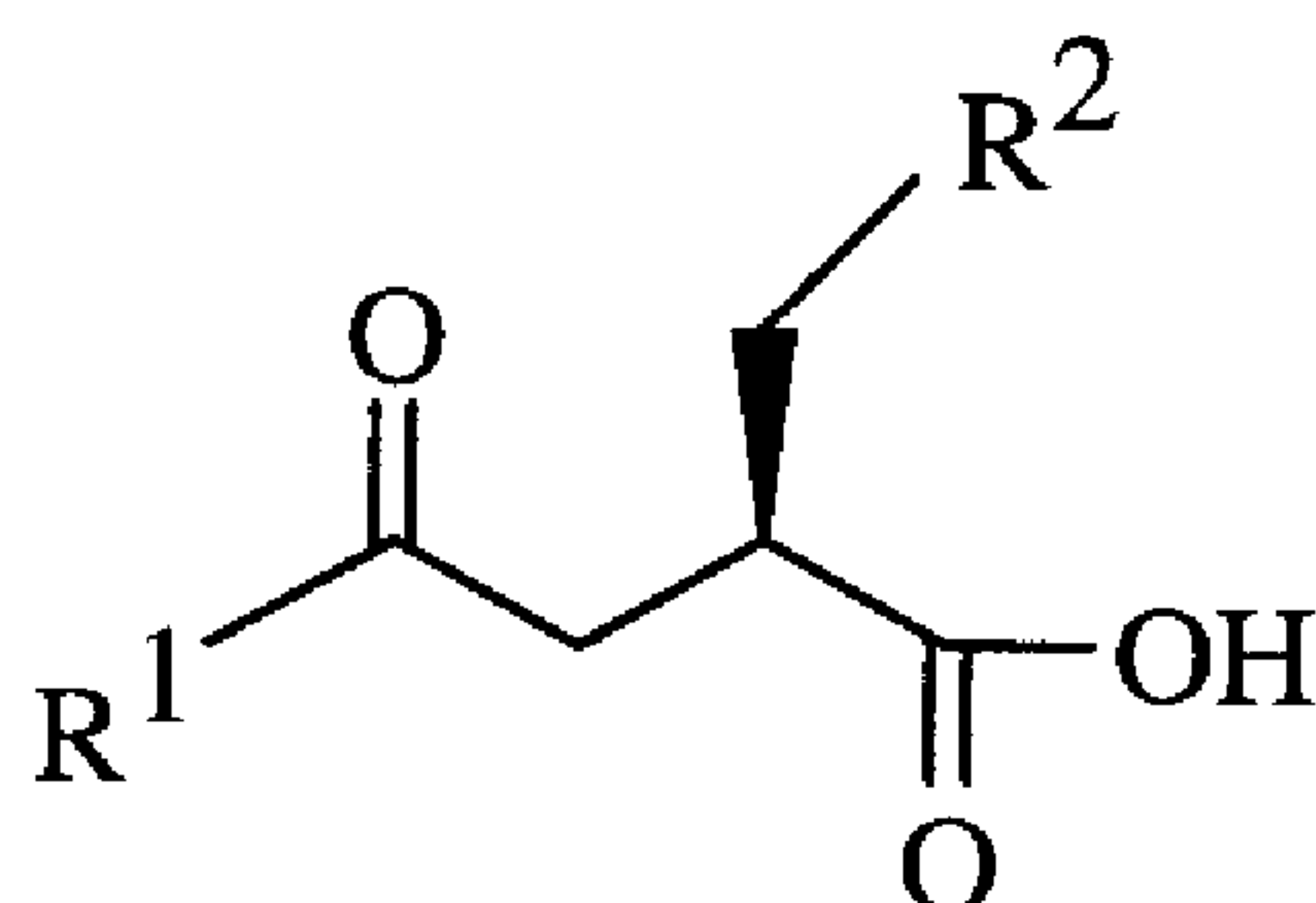
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(54) Titre : PREPARATION STEREOSELECTIVE DE COMPOSES DE TYPE SUCCINIQUE AVEC SUBSTITUTION EN 2

(54) Title: STEREOSELECTIVE PREPARATION OF 2-SUBSTITUTED SUCCINIC DERIVATIVES



(57) **Abrégé/Abstract:**

A highly efficient and practical means has been developed which enables compounds of the formula: (see formula I) wherein R¹ is lower alkoxy, lower alkylamino, di-(loweralkyl)amino, or the monovalent radical A-NR³, wherein A is lower alkyl or A is R⁴R⁵NC(O)CH² wherein, for example, R⁴ is hydrogen or alkyl and R⁵ is hydrogen, alkyl or a substituted alkyl, or R⁵ is R⁶R⁷N-Alk wherein R⁶ and R⁷ each is hydrogen or lower alkyl and Alk is a divalent alkyl radical; R³ is, for example, benzyl, alkyl or a substituted alkyl; and R² is, inter alia, alkyl, cycloalkyl, 1H-imidazol-4-yl, 4-thiazolyl or 2-amino-4-thiazolyl; to be prepared through the kinetic resolution of a compound of the formula: (see formula II) wherein R¹ is as defined herein, R² is as defined herein, and B is lower alkyl. These compounds are useful intermediates in the synthesis of renin inhibiting compounds.

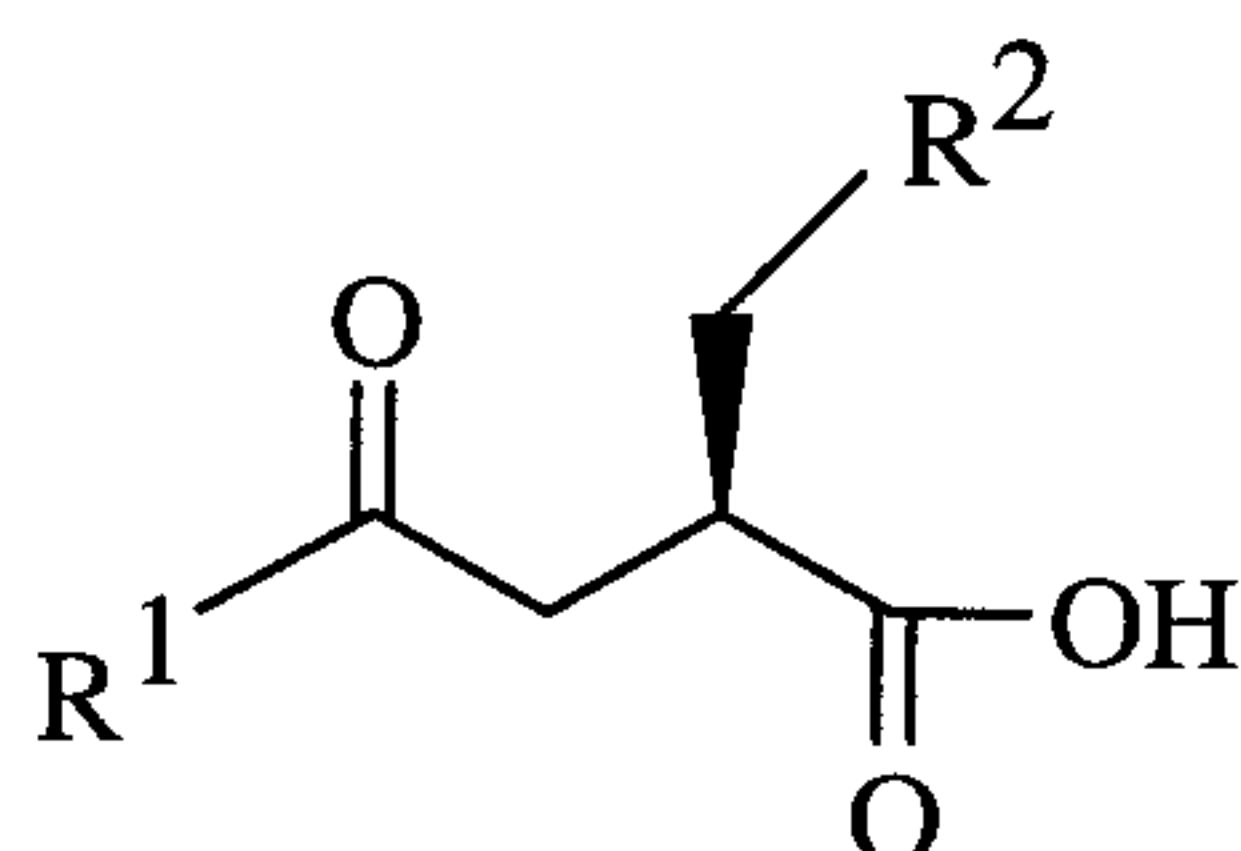


Stereoselective Preparation of 2-Substituted Succinic Derivatives

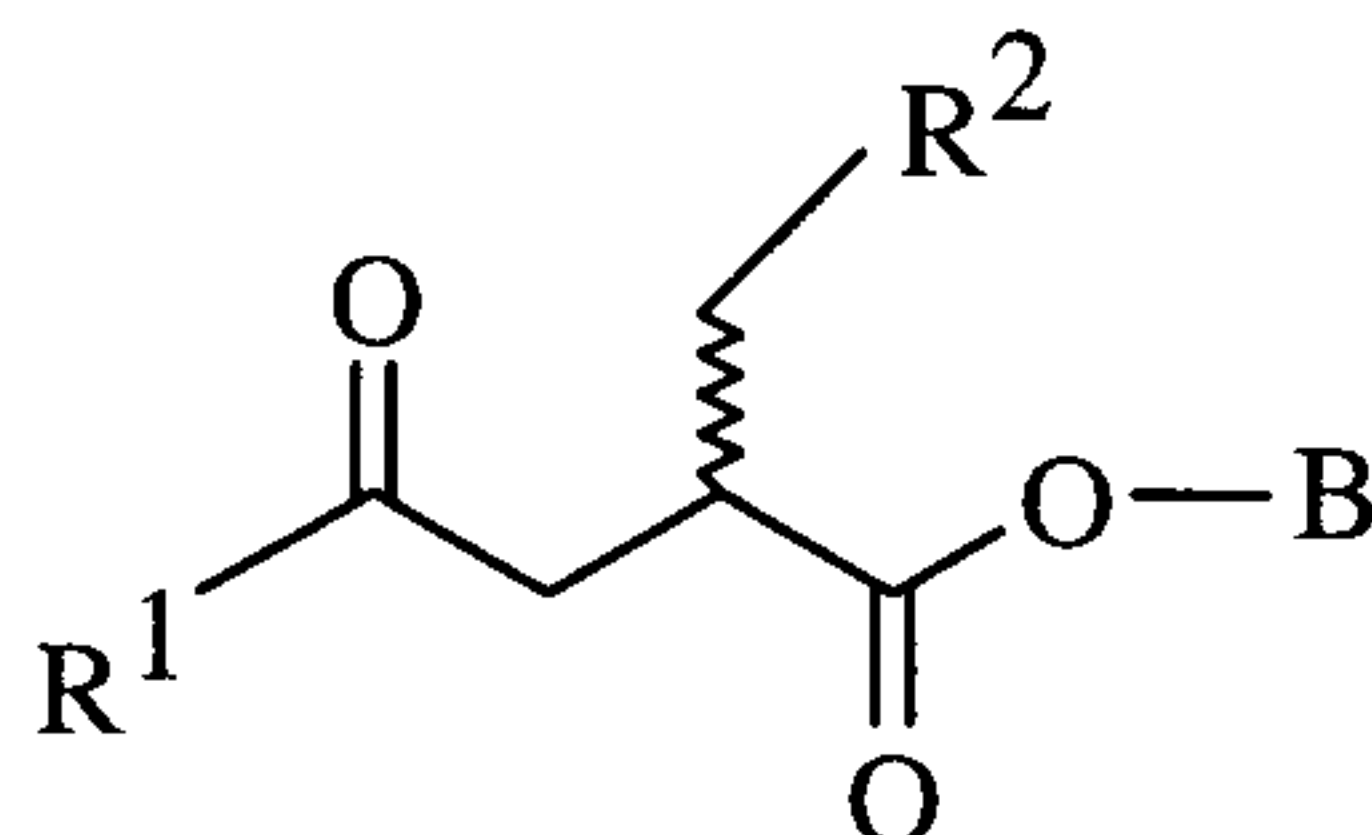
Abstract of the Disclosure

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A highly efficient and practical means has been developed which enables compounds of the formula:



- 10 wherein R^1 is lower alkoxy, lower alkylamino, di-(loweralkyl)amino, or the monovalent radical $A-NR^3$, wherein A is lower alkyl or A is $R^4R^5NC(O)CH_2$ wherein, for example, R^4 is hydrogen or alkyl and R^5 is hydrogen, alkyl or a substituted alkyl, or R^5 is
- 15 R^6R^7N-Alk wherein R^6 and R^7 each is hydrogen or lower alkyl and Alk is a divalent alkyl radical; R^3 is, for example, benzyl, alkyl or a substituted alkyl; and R^2 is, *inter alia*, alkyl, cycloalkyl, 1H-imidazol-4-yl, 4-thiazolyl or 2-amino-4-thiazolyl;
- 20 to be prepared through the kinetic resolution of a compound of the formula:



- 25 wherein R^1 is as defined herein, R^2 is as defined herein, and B is lower alkyl. These compounds are useful intermediates in the synthesis of renin inhibiting compounds.

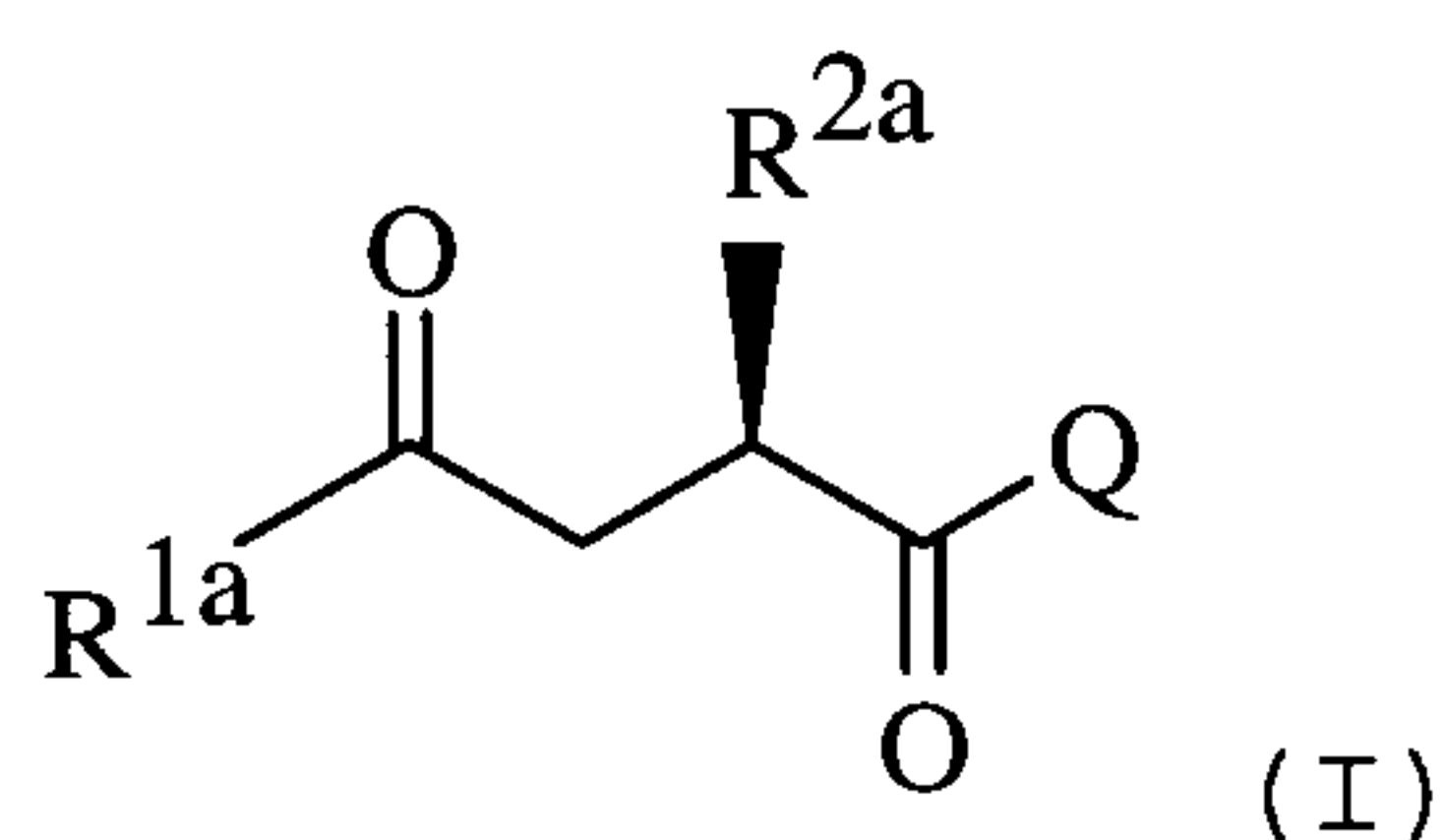
Field of the Invention

This invention relates to renin inhibiting compounds. More specifically, this invention
5 relates to a process for the preparation of compounds with high enantiomeric purity which are useful intermediates in the synthesis of renin inhibiting compounds, and to the compounds with high enantiomeric purity prepared by this process.

10

Background of the Invention

Compounds which inhibit the enzyme renin, an aspartyl protease which cleaves angiotensinogen to
15 AI, have been the subject of much research interest in recent years, and a great deal of effort has been devoted to designing renin inhibitors which mimic the natural renin substrate angiotensinogen. Much of this effort has been focused on the design of
20 analogous substrates incorporating therein a non-cleavable mimic (i.e. a transition state analog) of the renin cleavage site (i.e. Leu-Val) of human angiotensinogen. As a result, a number of potent renin inhibitors have been identified in the
25 laboratory, and the ability of renin inhibitors to lower blood pressure and to reduce plasma renin activity has now been demonstrated in the clinic (for a recent review on renin inhibitors, see W. J. Greenlee, Medical Research Reviews **1990**, 10, 173).
30 Renin inhibiting compounds which have been found to be of particular interest are the compounds of formula I:

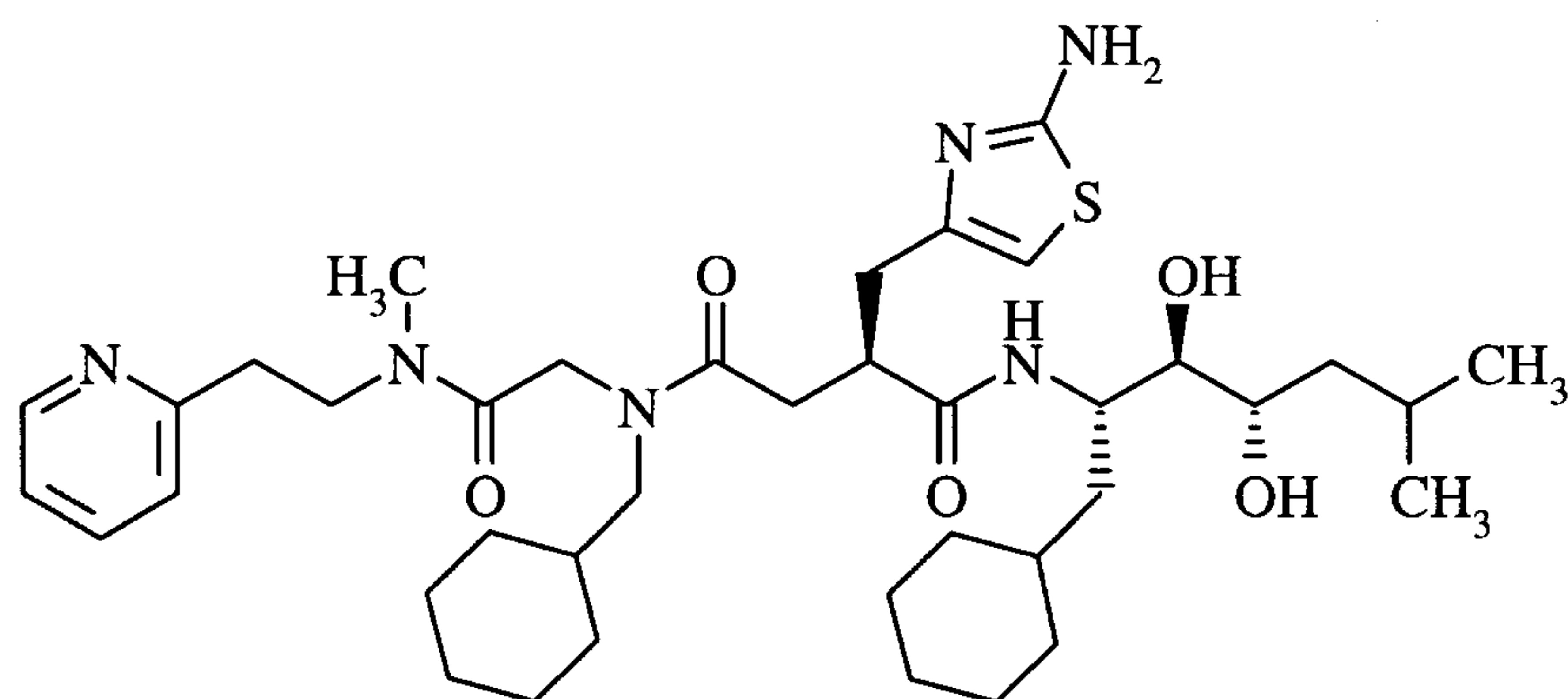


wherein R^{1a} is $A-NR^3$, wherein A is $R^4R^5NC(O)CH_2$ wherein, for example, R^4 is hydrogen or alkyl and R^5 is hydrogen, alkyl or a substituted alkyl such as 2-(2-pyridinyl)ethyl, or R^4 and R^5 together with the nitrogen atom to which they are attached form a pyrrolidino, piperidino, morpholino or thiomorpholino; R^3 is, for example, benzyl, alkyl or a substituted alkyl such as cyclohexylmethyl; R^{2a} is, for example, alkyl, cycloalkyl, 1H-imidazol-4-yl, 4-thiazolyl or 2-amino-4-thiazolyl; and Q is a renin substrate transition state analog, for example, 1(S)-(2-methylpropyl)-2(S)-hydroxy-5-methylhexyl-amino, 1(S)-(cyclohexylmethyl)-2(S)-hydroxy-5-methylhexylamino, 1(S)-{(4-methoxyphenyl)methyl}-2(S)-hydroxy-5-methylhexylamino, 1(S)-(cyclohexylmethyl)-2(S)-hydroxy-4-methylpentylamino, 1(S)-(cyclohexylmethyl)-2(S)-hydroxy-3-cyclopropylpropyl-amino, 1(S)-(2-methylpropyl)-2(R),3(S)-dihydroxy-5-methylhexylamino, 1(S)-(cyclohexylmethyl)-2(R),3(S)-dihydroxy-5-methylhexylamino, 1(S)-{(4-methoxyphenyl)methyl}-2(R),3(S)-dihydroxy-5-methylhexyl-amino, 1(S)-(2-methylpropyl)-2(R),3(S)-dihydroxy-3-cyclopropylpropylamino, 1(S)-(cyclohexylmethyl)-2(R),3(S)-dihydroxy-3-cyclopropylpropylamino, 1(S)-(phenylmethyl)-2(R),3(S)-dihydroxy-3-cyclopropylpropylamino, 1(S)-{(4-methoxyphenyl)methyl}-2(R),3(S)-dihydroxy-3-cyclopropylpropylamino, 1(S)-(cyclohexylmethyl)-2(R)-hydroxy-3-(1-methylethoxy)-

3-oxopropylamino or 1(*S*)-(cyclohexylmethyl)-2(*S*)-hydroxy-2-(1,5,5-trimethyl-2-oxopyrrolidin-3(*S*)-yl)ethylamino; or a therapeutically acceptable acid addition salt thereof.

5

A renin inhibiting compound of most interest is the compound of formula Ia:



(Ia)

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The central portion of these renin inhibiting compounds is a chiral succinoyl derivative with an (*R*)-configuration. The original synthesis of the compounds of formula I and Ia employed succinoyl intermediates derived from an asymmetric synthesis utilizing Evans' chiral auxiliary chemistry to introduce the chiral center with the proper configuration (Lavallée et al., European Patent application EP 589445, published March 30, 1994).

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This methodology employs expensive chiral auxiliaries, as well as several cryogenic steps, making this last procedure difficult for the large scale preparations of these compounds. Therefore, efficient and low cost methods which are amenable to scale-up are needed for the preparation of

25

intermediates useful for the preparation of compounds of formula I and Ia.

The preparation of compounds with high enantiomeric
5 purity has been reported previously, for example:

R.W. Dugger, PCT patent application

WO 95/04043, published February 9, 1995

D.A. Dickman et al., US Patent 5,275,950,
issued January 4, 1995

10 P. Zaderenko et al., Tetrahedron:Asymmetry
1995, 6, 381

L. Blanco et al., Tetrahedron:Asymmetry **1993**,
4, 783

H. Jendralla et al., SYNLETT **1993**, 155

15 M.R. Leanna et al., Tetrahedron Letters **1993**,
34, 4485

H. Mazdiyasni et al., Tetrahedron Letters **1993**,
34, 435

20 L. Shao et al., J. CHEM. SOC. PERKIN TRANS. 1
1990, 1441

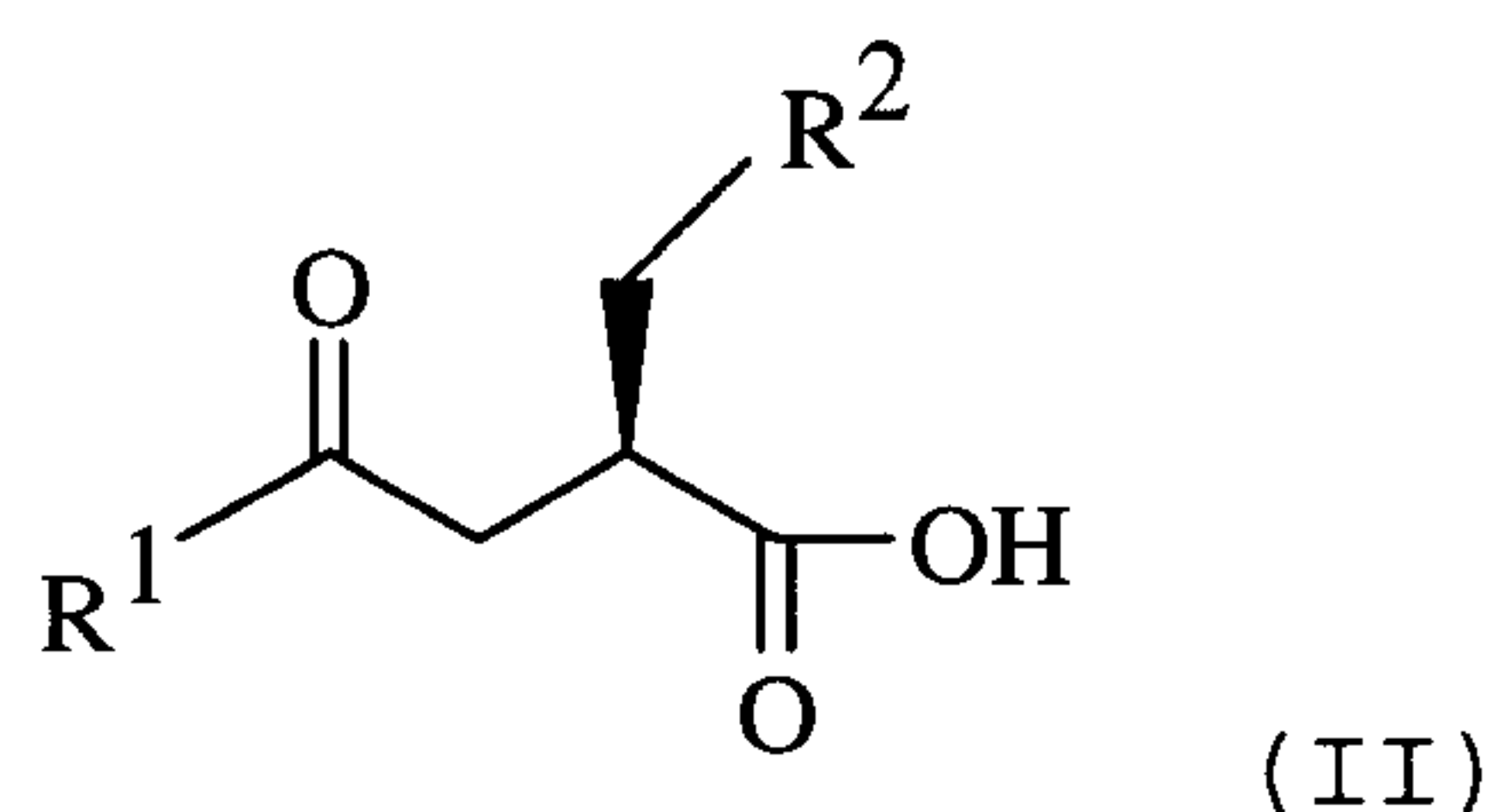
S.G. Cohen et al., J. Am. Chem. Soc. **1968**, 90,
3495

The present process and compounds prepared by the
25 present process can be distinguished readily from
the prior art. The compounds prepared by the
present process possess a different chemical
structure than those in the prior art, and can be
used as intermediates in the preparation of renin
30 inhibiting compounds of formula I and Ia.

Summary of the Invention

In accordance with the present invention, there is disclosed a process for the preparation of renin
5 inhibiting compounds of formula I and Ia, as well as intermediates useful for the preparation of renin inhibiting compounds of formula I and Ia.

10 In particular, the present invention relates to a process for the preparation of compounds with high enantiomeric purity of formula II:



15 wherein R^1 is lower alkoxy, lower alkylamino, di(lower alkyl)amino or R^1 is $A-NR^3$, wherein A is $R^4R^5NC(O)CH_2$ wherein,
(a) R^4 is hydrogen or lower alkyl and R^5 is hydrogen, lower alkyl or lower alkyl monosubstituted
20 with lower cycloalkyl, phenyl, or a heterocyclic ring (hereinafter designated as "Het") which is an unsubstituted, monosubstituted or disubstituted, five- or six-membered ring containing one or two heteroatoms selected from the group consisting of N,
25 O and S, and wherein each substituent is selected independently from the group consisting of lower alkyl, lower alkoxy, halo, amino or lower alkylamino; or
(b) R^4 is lower alkyl and R^5 is R^6R^7N-Alk wherein R^6
30 and R^7 each is hydrogen or lower alkyl and Alk is a

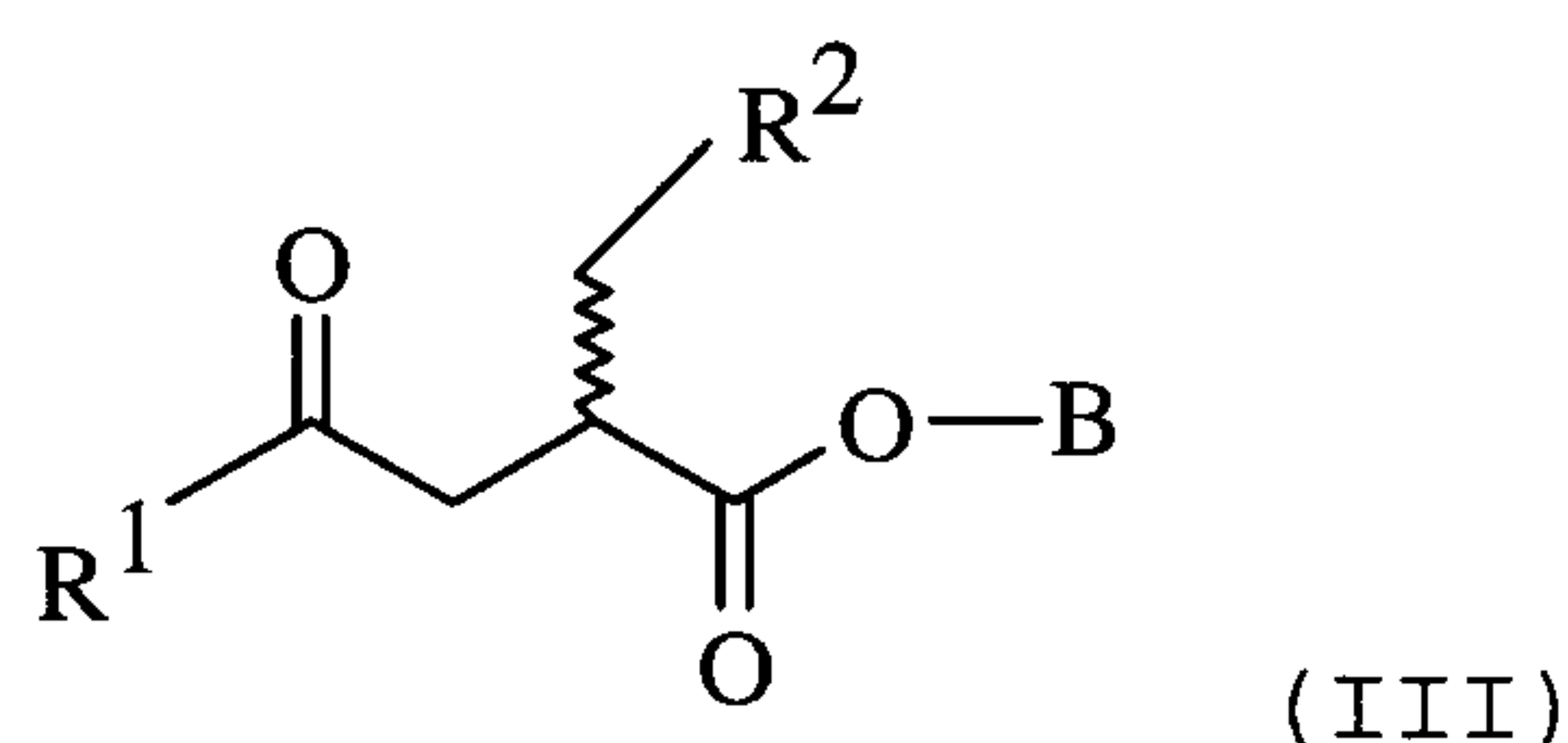
divalent alkyl radical derived by the removal of two hydrogen atoms of a straight or branched chain hydrocarbon containing from one to six carbon atoms; or

- 5 (c) R^4 is lower alkyl and R^5 is lower alkoxy; or
 (d) R^4 and R^5 together with the nitrogen atom to which they are attached form a pyrrolidino, piperidino, 4-((lower alkoxy)-(lower alkoxy))-1-piperidinyl, morpholino or thiomorpholino;

- 10 R^3 is lower alkyl or lower alkyl monosubstituted with lower cycloalkyl, phenyl, 4-(lower alkyl)phenyl, 4-(lower alkoxy)phenyl, 4-halophenyl, 3,4-methylenedioxyphenyl, 1-naphthyl, 2-naphthyl or Het wherein Het is as defined herein; and

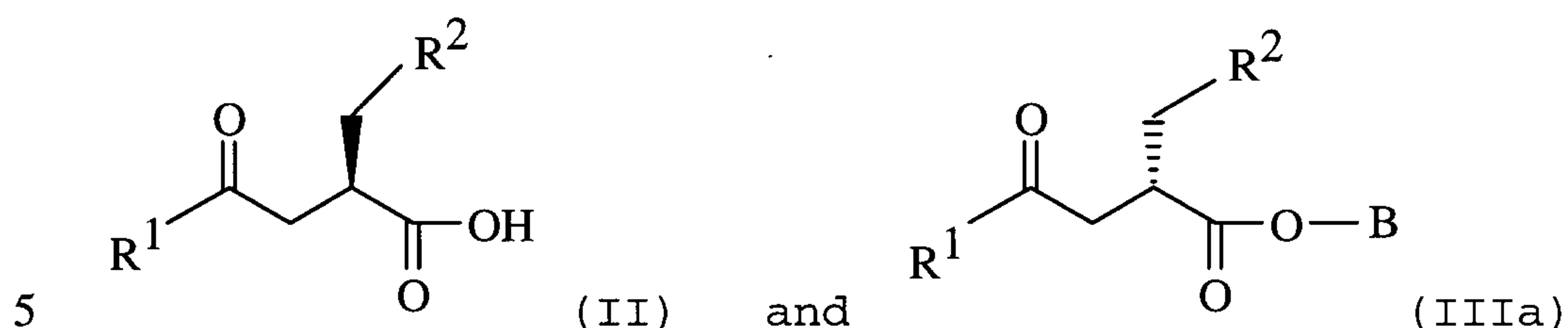
- 15 R^2 is lower alkyl, $CCl=CH_2$, $CBr=CH_2$, lower cycloalkyl, 2-amino-4-thiazolyl, 2-(amino protecting group)amino-4-thiazolyl or Het wherein Het is as defined herein, said process comprising:

- (i) selectively hydrolyzing one enantiomer of a
 20 racemic mixture of an ester of formula III:



- wherein R^1 is as defined herein, R^2 is as defined
 25 herein, and B is lower alkyl, with an effective amount of an esterase selected from Subtilisin Carlsberg or α -chymotrypsin, in a reaction medium comprising H_2O or a mixture of H_2O and an H_2O miscible inert organic solvent, in the presence of
 30 an alkali metal hydroxide to maintain the pH of the

medium at about 7.0-8.5, to form a reaction mixture containing compounds of formula II (as an alkali metal salt) and IIIa:



and

(ii) separating from the mixture resulting from step (i) the compound of formula II.

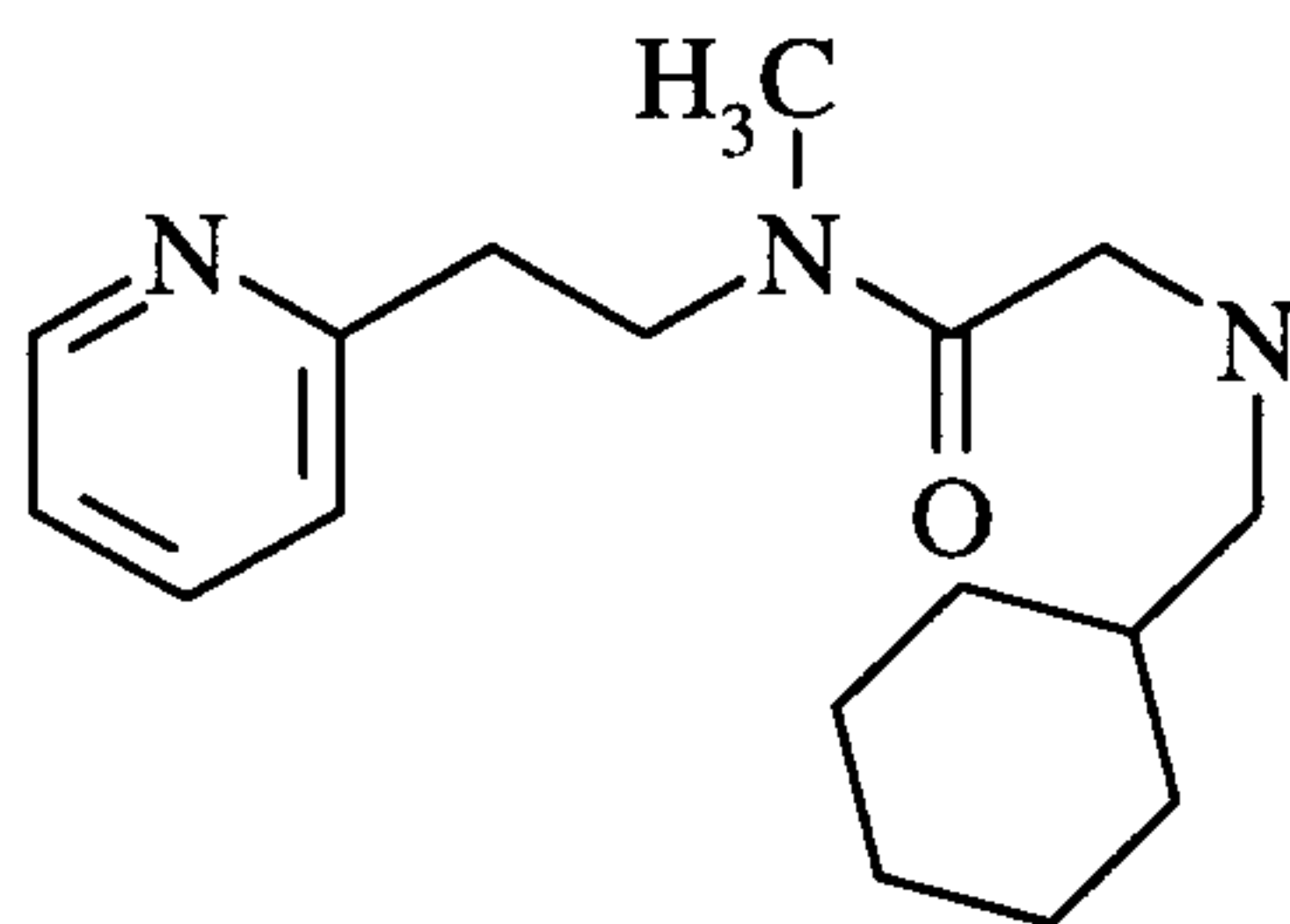
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In a preferred process for the preparation of compounds of formula II, the ester of formula III is selected from the group of compounds represented by formula IIa wherein R^1 is lower alkoxy, lower alkylamino, di(lower alkyl)amino or R^1 is $\text{A}-\text{NR}^3$, wherein A is $\text{R}^4\text{R}^5\text{NC}(\text{O})\text{CH}_2$ wherein R^4 is methyl and R^5 is methyl, 2-(2-pyridinyl)ethyl or 2-(3-pyridinyl)ethyl; R^3 is 1-ethylpropyl, cyclopentylmethyl, cyclohexylmethyl, 1(S)-cyclohexylethyl, cycloheptylmethyl, phenylmethyl, 1(S)-phenylethyl, (1-methylcyclohexyl)methyl; R^2 is $\text{CCl}=\text{CH}_2$, $\text{CBr}=\text{CH}_2$, 2-amino-4-thiazolyl, 2-(amino protecting group)amino-4-thiazolyl or a five-membered heterocyclic ring containing one or two heteroatoms selected from the group consisting of N, O and S; and B is methyl or ethyl. Most preferably, R^1 is 1,1-dimethylethoxy or the monovalent radical:

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R^2 is $\text{CCl}=\text{CH}_2$, $\text{CBr}=\text{CH}_2$, or 2-amino-4-thiazolyl, and B is methyl.

- 5 The process of the instant invention is preferably carried out at a temperature of 20 to 55°C, more preferably at a temperature of 20 to 37°C. The alkali metal hydroxide used in the instant process is preferably selected from NaOH, KOH and LiOH, and
- 10 is added to the reaction mixture in a sufficient amount to maintain the pH of the reaction at about 7.0 to 8.5, more preferably 7.0 to 8.0. The process of the instant invention is carried out in H_2O or a mixture of H_2O and an H_2O miscible inert organic
- 15 solvent, preferably in H_2O or a mixture of H_2O and 0 to 30% (v/v) of an H_2O miscible inert organic solvent. The inert organic solvent is preferably selected from acetone or acetonitrile.
- 20 This invention also relates to compounds of formula II wherein R^1 and R^2 are as defined herein. The compounds of formula II prepared by the instant process can be subjected to standard transformations as disclosed by Lavallée et al., European Patent
- 25 application EP 589445, published March 30, 1994, to prepare renin inhibiting compounds of formula I and Ia.

Details of the InventionGeneral

5 With reference to the instances where (R) or (S) is used to designate the configuration of a radical, e.g. R² of the compound of formula I, the designation is done in the context of the compound and not in the context of the radical alone.

10

The term "halo" as used herein means a halo radical selected from bromo, chloro, fluoro or iodo.

15

The term "lower alkyl" as used herein, either alone or in combination with another radical, means straight chain alkyl radicals containing one to four carbon atoms and branched chain alkyl radicals containing three to four carbon atoms and includes methyl, ethyl, propyl, butyl, 1-methylethyl, 1-methylpropyl, 2-methylpropyl and 1,1-dimethylethyl.

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The term "lower alkoxy" as used herein means straight chain alkoxy radicals containing one to four carbon atoms and branched chain alkoxy radicals containing three to four carbon atoms and includes methoxy, ethoxy, propoxy, 1-methylethoxy, butoxy and 1,1-dimethylethoxy.

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The term "lower cycloalkyl" as used herein, either alone or in combination with another radical, means saturated cyclic hydrocarbon radicals containing from three to seven carbon atoms and includes cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl and cycloheptyl.

30

The term "Het" as used herein means a monovalent radical derived by removal of a hydrogen from a five- or six-membered saturated or unsaturated heterocycle containing from one to two heteroatoms selected from nitrogen, oxygen and sulfur. Optionally, the heterocycle may bear one or two substituents; for example, N-oxido, lower alkyl, lower alkoxy, halo, amino or lower alkylamino. Examples of suitable heterocycles and optionally substituted heterocycles include pyrrolidine, tetrahydrofuran, thiazolidine, pyrrole, 1H-imidazole, 1-methyl-1H-imidazole, pyrazole, furan, thiophene, oxazole, isoxazole, thiazole, 2-methylthiazole, 2-aminothiazole, 2-(methylamino)-thiazole, piperidine, 1-methylpiperazine, 1,4-dioxane, morpholine, pyridine, pyridine N-oxide, pyrimidine and 2,4-dimethylpyrimidine.

The term "amino" as used herein means an amino radical of formula -NH_2 . The term "lower alkylamino" as used herein means alkylamino radicals containing one to six carbon atoms and includes methylamino, propylamino, (1-methylethyl)amino and (2-methylbutyl)amino. The term "di(lower alkyl)amino" means an amino radical having two lower alkyl substituents each of which contains one to six carbon atoms and includes dimethylamino, diethylamino, ethylmethylamino and the like.

The term "amino protecting group" as used herein means a group intended to protect an amino group or the N-terminus of an amino acid against undesirable reactions during synthetic procedures. Commonly used amino protecting groups are disclosed in

general textbooks of peptide chemistry; for instance, "The Peptides: Analysis, Synthesis, Biology", E. Gross et al., Eds., Academic Press, New York, NY, USA, 1979-1987, Volumes 1 to 9, and M. Bodanszky, "Peptide Chemistry", 2nd rev ed, Springer-Verlag, Berlin, Germany, 1993. Examples of amino protecting groups include benzyloxycarbonyl, 4-methoxybenzyloxycarbonyl or 2,2,2-trichloroethoxycarbonyl and 1,1-dimethylethoxycarbonyl.

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The term "high enantiomeric purity" as used herein refer to a compound having at least 75% enantiomeric excess (ee) of one enantiomer, preferably at least 85% ee, more preferably at least 95% ee and most preferably at least 99% ee.

15

Process for Preparing the Compounds of Formula 1

Racemic mixtures of compounds of formula III, which are used in the synthesis of compounds of formula II, can be prepared by a variety of processes involving known methods. Description of the methods are found in standard textbooks such as "Annual Reports In Organic Synthesis-1994", P.M. Weintraub et al., Eds., Academic Press, Inc., San Diego, CA, USA, 1994 (and the preceding annual reports), "Vogel's Textbook of Practical Organic Chemistry", B.S. Furniss et al., Eds., Longman Group Limited, Essex, UK, 1986, and "Comprehensive Organic Synthesis", B.M. Trost and I. Fleming, Eds., Pergaman Press, Oxford, UK, 1991, Volumes 1 to 8.

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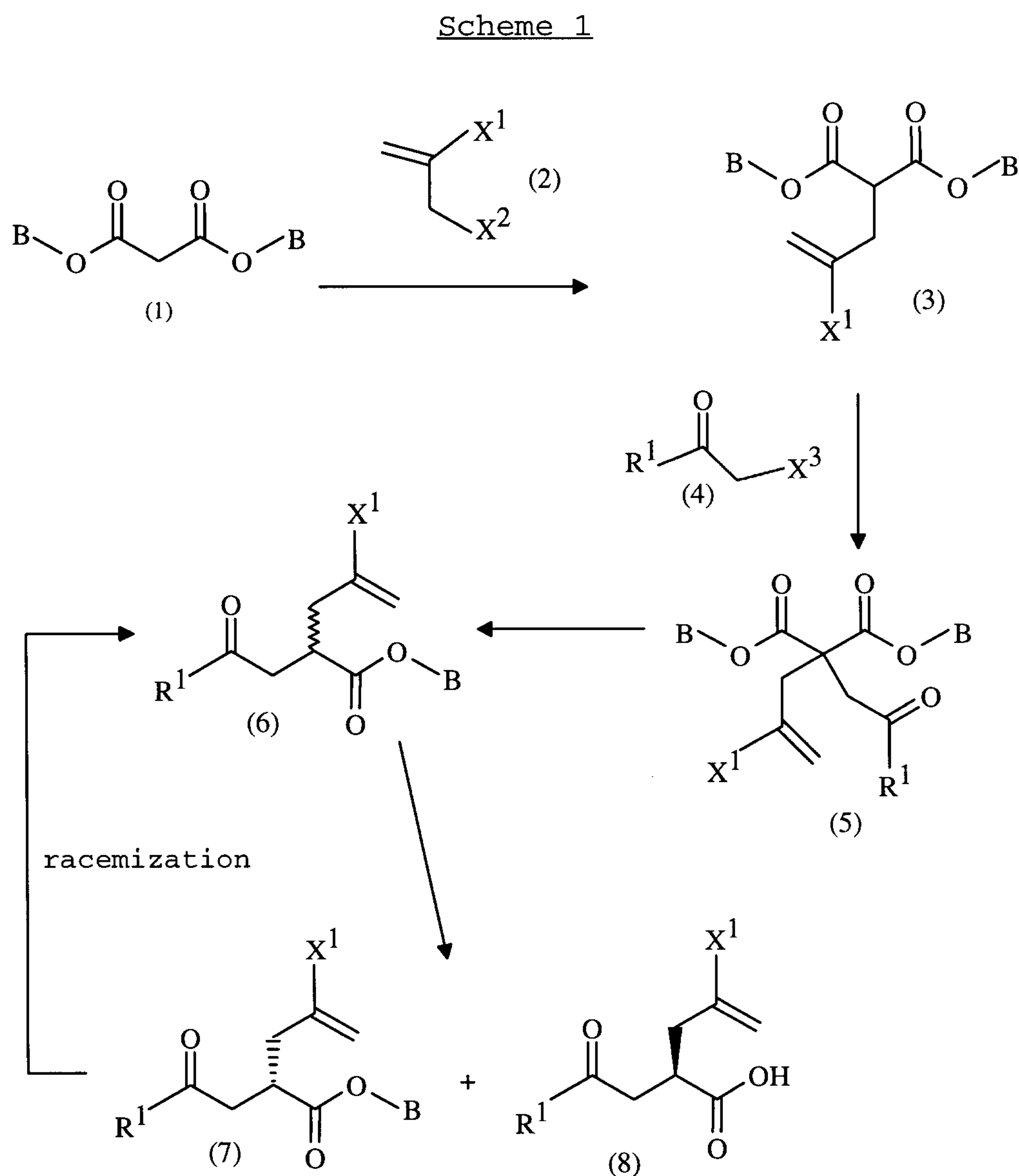
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A general procedure for the preparation of compounds with high enantiomeric purity of formula

II, which are useful intermediates in the synthesis of renin inhibiting compounds of formula I and Ia, is outlined in scheme 1.

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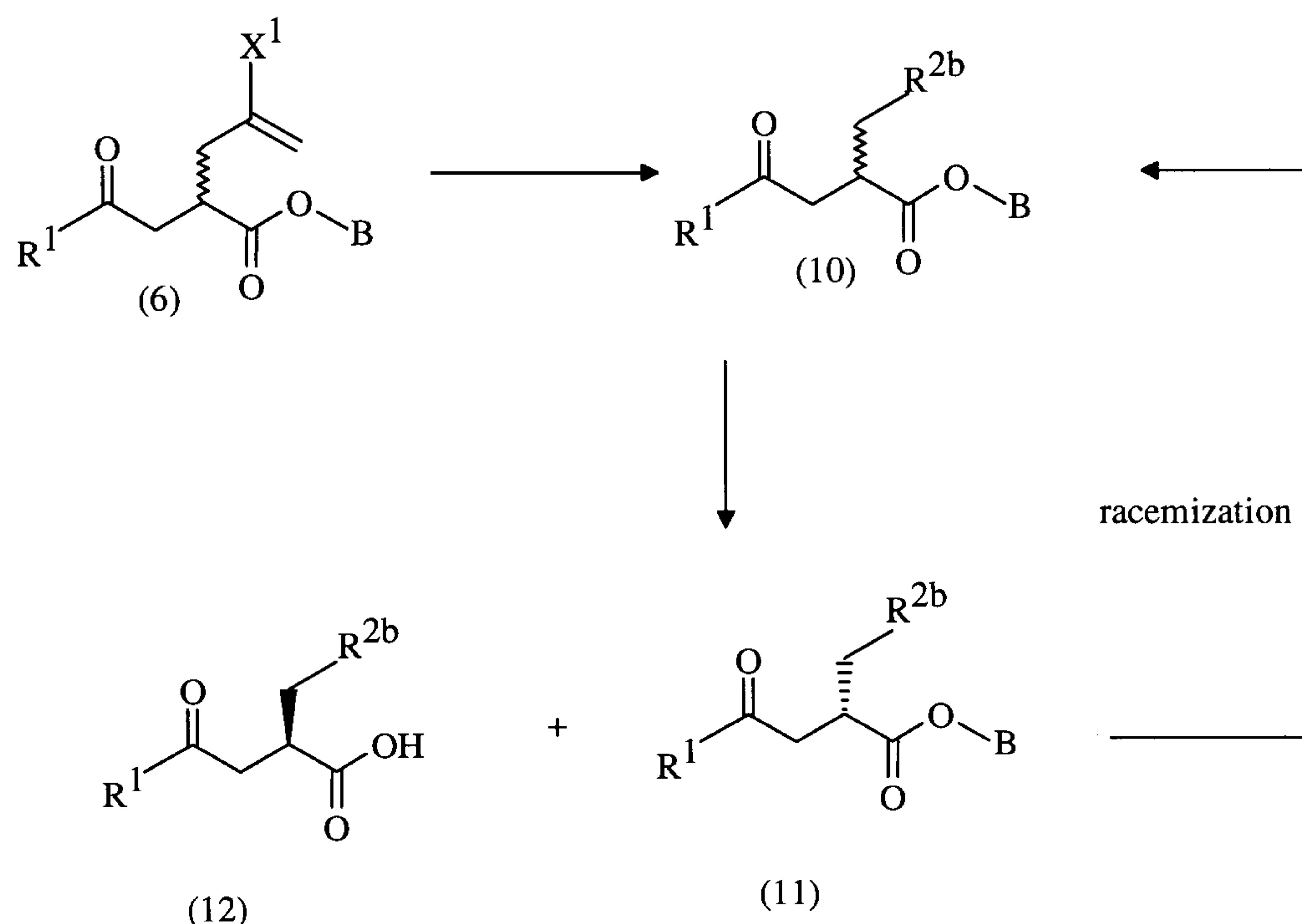
10 According to scheme 1, a mixture of malonate (1), wherein B is lower alkyl, and 2,3-disubstituted propene (2), wherein X^1 and X^2 are selected from Br,

Cl or I, in an inert solvent (for example MeOH, THF and the like) at a temperature of from about 0°C to 100°C is treated with a base (for example NaOMe when the solvent is MeOH, or NaH, NaHMDS or potassium *tert*-butoxide when the solvent is THF, and the like), to give the corresponding mono-alkylated malonate derivative of formula 3. The mono-alkylated malonate derivative of formula 3 is reacted with the compound of formula 4, wherein X³ is selected from Br, Cl or I, and R¹ is as defined herein, in the presence of base (for example NaOH, NaH, KOtBu and the like) to give the corresponding di-alkylated malonate derivative of formula 5. Selective monosaponification of the dialkylated malonate of formula 5 by treatment with an aqueous base (for example KOH, LiOH, NaOH and the like) in a mixture of H₂O and an H₂O miscible inert solvent (for example, THF/H₂O/MeOH, MeOH/H₂O, and the like), followed by acidification and then refluxing in toluene and the like to induce decarboxylation gives the corresponding racemic succinyl derivative of formula 6. Treatment of the racemic succinyl derivative of formula 6 with an esterase (for example, Subtilisin Carlsberg or α -chymotrypsin) in either H₂O or a mixture of H₂O and a H₂O miscible solvent (for example, acetone and the like) in the presence of an alkali metal hydroxide (for example, NaOH, KOH, LiOH and the like) to maintain the pH at about 7 to 8.5, gives a mixture of the corresponding enantiomers of formula 7 and 8. The unreacted (*R*)-ester of formula 7 is extracted from the mixture of the enantiomers of formula 7 and 8 with an organic solvent (for example, EtOAc and the like). The (*R*)-ester of formula 7 can be recycled by racemization

under basic conditions to give the corresponding racemic succinyl derivative of formula 6. The (*S*)-acid of formula 8 is recovered by acidifying the aqueous phase and then extracting with an organic solvent (for example, EtOAc and the like). By using procedures disclosed by Lavallée et al. (EP 589445, published March 30, 1994), the (*S*)-acid of formula 8, which is a compound of formula II, prepared in this manner can be used directly in the preparation of renin inhibiting compounds of formula I and Ia.

Another general procedure for the preparation of compounds of formula II is outlined in scheme 2.

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Scheme 2

According to scheme 2, the succinyl derivative of formula 6 prepared as in scheme 1 is converted to

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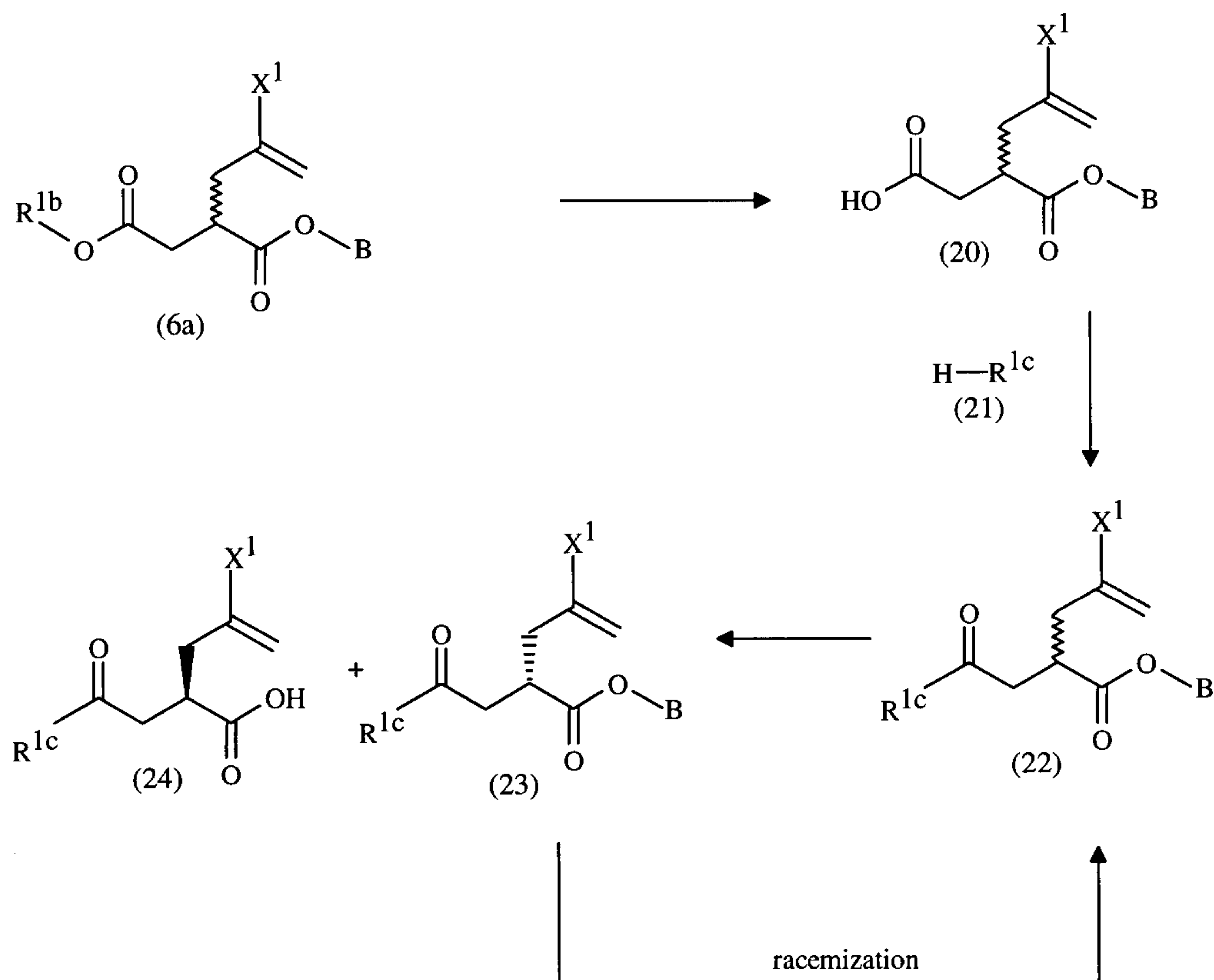
the corresponding 2-aminothiazolyl derivative of formula 10 wherein R^{2b} is 2-amino-4-thiazolyl, 2-(1,1-dimethylethyloxycarbonyl)amino-4-thiazolyl or 2-(amino protecting group)amino-4-thiazolyl according to the method of Hsaio et al. (Synthetic Comm. **1990**, 20, 33507) and Morton et al. (Tetrahedron Letters **1993**, 34, 4481). Briefly, the vinyl halide moiety of compound 6 is converted to an α -haloketone moiety by reacting the compound of formula 6 with *N*-bromosuccinimide or *N*-chlorosuccinimide or the like in a mixture of H_2O and an inert solvent (for example, EtOAc/ H_2O , acetonitrile/ H_2O and the like). Treatment of this latter α -haloketone derivative with thiourea gives the corresponding 2-aminothiazolyl derivative which can be further elaborated to 2-(amino protecting group)amino-4-thiazolyl by standard techniques. Treatment of the 2-aminothiazole derivative of formula 10 with an esterase (for example, Subtilisin Carlsberg or α -chymotrypsin) in either H_2O or a mixture of H_2O and a H_2O miscible solvent (for example, acetone and the like) at a pH of about 7 to 8.5 gives a mixture of the (*R*)-acid of formula 12 and the (*S*)-ester of formula 11, which can be separated as described in scheme 1. The (*S*)-ester of formula 11 can be recycled by racemization under basic conditions to give the aminothiazole derivative of formula 10. By using procedures disclosed by Lavallée et al. (EP 589445, published March 30, 1994), the (*R*)-acid of formula 12, which is a compound of formula II, prepared in this manner can be used directly in the preparation of renin inhibiting compounds of formula I and Ia.

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Another general procedure for the preparation of compounds of formula II is outlined in scheme 3.

Scheme 3

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The starting material in scheme 3 is the racemic compound 6a wherein R^{1b} and B are lower alkyl, which is prepared according to scheme 1 (when R¹ of scheme 1 is lower alkoxy). The substituents R^{1b} and B are selected such that R^{1b} can be selectively removed from compound 6a. For example, when R^{1b} is 1,1-dimethylethyl and B is methyl, acid hydrolysis of 6a (for example HCl, TFA and the like) will selectively remove R^{1b} to give the free carboxylic

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acid derivative of formula 20. The free caboxylic acid derivative of formula 20 is coupled with the compound of formula 21 wherein R^{1c} is selected from lower alkylamino, di(loweralkyl)amino or the monovalent radical $A-NR^3$ wherein A and R^3 are as defined herein, to give the racemic amide derivative of formula 22. The coupling of the carboxylic acid derivative of formula 20 and the amine derivative of formula 21 is effected by the classical dehydrative coupling of a free carboxyl of one reactant with the free amino group of the other reactant in the presence of coupling agent to form a linking amide bond. Description of such coupling agents are found in general textbooks on peptide chemistry; for example, M. Bodanszky, "Peptide Chemistry", 2nd rev ed, Springer-Verlag, Berlin, Germany, 1993. Examples of suitable coupling agents are N,N' -dicyclohexylcarbodiimide, 1-hydroxybenzotriazole in the presence of N,N' -dicyclohexylcarbodiimide, N -ethyl- N' -[(3-dimethylamino)propyl]-carbodiimide or the like. Treatment of the racemic amide derivative of formula 22 with an esterase as in scheme 1 or 2 results in a mixture of the (*S*)-acid of formula 24 and the (*R*)-ester of formula 23, which can be separated as described in scheme 1. The (*R*)-ester of formula 23 can be recycled by racemization under basic conditions to give the amide derivative of formula 22. By using procedures disclosed by Lavallée et al. (EP 589445, published March 30, 1994), the (*S*)-acid of formula 24, which is a compound of formula II, prepared in this manner can be used directly in the preparation of renin inhibiting compounds of formula I and Ia.

Other starting materials for the preceding processes are known or can be readily prepared from known starting materials. The chemical reactions described above are generally disclosed in terms of their broadest application to the preparation of the compounds of this invention. Occasionally, the reactions may not be applicable as described to each compound included within the disclosed scope. The compounds for which this occurs will be readily recognized by those skilled in the art. In all such cases, the reaction can be successfully performed by conventional modification known to those skilled in the art, e.g. by appropriate protection of interfering groups, by changing to alternative conventional reagents, by routine modification of reaction conditions, or by modification illustrated in the examples herein.

The following examples illustrate further this invention. Temperatures are given in degrees Celsius. Solution percentages or ratios express a volume to volume relationship, unless stated otherwise. Nuclear magnetic resonance spectra were recorded on a Bruker 200 or 400 MHz spectrometer; the chemical shifts (δ) are reported in parts per million. The concentrations for the optical rotations are expressed in grams of the compound per 100 mL of solution. Enantiomeric purities were determined using chiral HPLC columns, and are indicated as Condition A: Chiralpak® AS 25 cm column, 0.25% ethanol in hexane, 0.5 mL/min, isocratic; or Condition B: Chiralcel® OD-H 15 cm + 25 cm columns, 2% ethanol in hexane, 0.5 mL/min, isocratic. Abbreviations or symbols used in the

examples include: DBU: 1,8-diazabicyclo[5.4.0]undec-7-ene; DCC: dicyclohexylcarbodiimide; DCU: dicyclohexylurea; DMF: dimethylformamide; EtOH: ethanol; EtOAc: ethyl acetate; Et₂O: diethyl ether; 5 Hex: hexane; HOBT: 1-hydroxybenzotriazole; MeOH: methanol; NMM: *N*-methylmorpholine; THF: tetrahydrofuran.

Example 1

10 **2(*S*)-(2-Bromo-2-propenyl)-1,4-butanedioic Acid 4-(1,1-Dimethylethyl) Ester**

Example 1A

15 **2-(2-Bromo-2-propenyl)-1,3-propanedioic Acid Dimethyl Ester**

To a flask under nitrogen was added MeOH (67 mL), and sodium metal (1.15 g, 0.05 g atom) was added in portions over 30 min. To the resulting solution 20 dimethyl malonate (13.2 g, 0.1 mol) in MeOH (10 mL) was added from a dropping funnel over 30 min. The solution was cooled to 0°, and 2,3-dibromopropene (12 g, 0.05 mol) in MeOH (15 mL) was added over 40 min. After a further 45 min at 0°, the pH of the 25 mixture was neutral to litmus paper. The reaction medium was then concentrated under reduced pressure and the residue was dissolved in EtOAc (100 mL) and the resulting solution washed with distilled H₂O (100 mL). The aqueous phase was re-extracted with 30 Et₂O (100 mL) and the combined organic phases were washed with brine, dried (MgSO₄), concentrated under reduced pressure and purified by distillation (82-84°/1 Torr) to give the title compound as a colorless liquid (8.3 g, 66% yield): ¹H NMR (400

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MHz, CDCl₃) δ 5.69 (m, 1H, olefinic), 5.48 (d, J = 1.9 Hz, 1H, olefinic), 3.81 (t, J = 7.63 Hz, 1H), 3.75 (s, 6H), 3.02 (dd, J = 0.95, 7.63 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 168.30, 129.18, 119.68, 52.59, 50.28, 40.36.

Example 1B

2-(2-Bromo-2-propenyl)-2-(methoxycarbonyl)-1,4-butanedioic Acid 4-(1,1-Dimethylethyl) 1-Methyl Ester

The title compound of Example 1A (0.5 g, 2 mmol) dissolved in THF (20 mL) was cooled to 0° under nitrogen. Potassium tert-butoxide (0.25 g, 2.2 mmol) was added all at once. After a further 20 min, tert-butyl bromoacetate (0.39 g, 2.2 mmol) was added dropwise from a syringe. The reaction mixture was stirred 16 h at room temperature. The excess base was destroyed by the addition of wet THF (2 mL) followed by the addition of H₂O (40 mL) and EtOAc (40 mL). The organic phase was dried (MgSO₄) and concentrated under reduced pressure. The crude oil was purified by column chromatography (SiO₂) to give the title compound (0.619 g, 85% yield) as a colorless oil: ¹H NMR (400 MHz, CDCl₃) δ 5.64 (broad s, 1H), 5.59 (broad s, 1H), 3.75 (s, 6H), 3.35 (s, 2H), 3.07 (s, 2H), 1.43 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) δ 169.42, 168.43, 127.16, 122.35, 81.29, 54.64, 52.91, 43.53, 37.25, 27.92.

Example 1C**2(*R,S*)-(2-Bromo-2-propenyl)-1,4-butanedioic Acid 4-(1,1-Dimethylethyl) 1-Methyl Ester**

5 To a solution of LiBr (12.51 g, 0.144 mol) dissolved
in H₂O (5.2 mL) and DMF (20 mL) was added the title
compound of Example 1B (52.6 g, 0.144 mol) in DMF
(20 mL). This solution was heated to 135° for 6 h.
The DMF was then removed under reduced pressure and
10 the resulting residue diluted with H₂O (60 mL),
extracted twice with EtOAc (2x 60 mL) and twice with
Et₂O (2x 50 mL). The combined organic phases were
washed with brine, dried (MgSO₄) and concentrated
under reduced pressure to give a pale yellow oil
15 (27.6 g, 62% yield). A sample of this material
(9.55 g) was further purified by flash
chromatography (SiO₂, eluent: 5% EtOAc/Hex) to give
the title compound as a clear colorless oil (6.8 g),
which was used in the following Examples 1D and 1E.
20 HPLC analysis using a chiral column (Condition A:
(*R*)-isomer, *t_R* 9.2 min, (*S*)-isomer, *t_R* 9.9 min)
showed this sample to be a 1:1 ratio of the (*R*)- and
(*S*)- enantiomers: ¹H NMR (400 MHz, CDCl₃) δ 5.64 (s,
1H), 5.48 (d, *J* = 1.6 Hz, 1H), 3.71 (s, 3H), 3.14
25 (m, 1H), 2.85 (dd, *J* = 6.4, 14.6 Hz, 1H), 2.61 (dd,
J = 8.3, 14.6 Hz, 1H), 2.57 (dd, *J* = 8.6, 16.5 Hz,
1H), 2.48 (dd, *J* = 5.4, 16.5 Hz, 1H), 1.44 (s, 9H);
¹³C NMR (100 MHz, CDCl₃) δ 173.90, 170.38, 130.46,
119.55, 80.91, 51.87, 42.75, 39.75, 35.82, 27.96.

Example 1D**Kinetic Resolution of 2(*R,S*)-(2-Bromo-2-propenyl)-1,4-butanedioic Acid 4-(1,1-Dimethylethyl) 1-Methyl Ester Using α -Chymotrypsin**

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The purified title compound of Example 1C (0.513 g, 1.67 mmol) was dissolved in acetone (2 mL) and diluted with distilled H₂O (5 mL). To this rapidly stirred solution was added α -chymotrypsin (5 mg) 10 (type I-S from Bovine Pancreas, crystallized 3x, obtained from the Sigma Chemical Co.). Aqueous NaOH (0.1 N), added dropwise from a burette, was used to maintain the reaction at pH 8-9. The uptake of the NaOH solution was extremely slow (< 2 drops of NaOH 15 over 1 h) and after 1 h KCl salt (500 mg) was added as well as an extra 15 mg of the α -chymotrypsin. In this specific reaction the pH was kept at 8-9 and after 4 days the reaction was not proceeding further (pH stable at pH 8). The reaction mixture was then 20 diluted with distilled H₂O (15 mL) and saturated aqueous NaHCO₃ solution (2 mL), extracted twice with methylene chloride (2x 25 mL), dried (MgSO₄) and concentrated under reduced pressure to give the unreacted ester (300 mg), which was shown by HPLC 25 using a chiral column (Condition A) to be a mixture of the (*R*)- and (*S*)-enantiomers in a ratio of 1.4:1.

The basic aqueous phase was rendered acidic (pH 5) by the addition of 10% (w/v) aqueous citric acid 30 (~10 mL) and extracted three times with methylene chloride (3x 30 mL). The methylene chloride phase was dried (MgSO₄) and concentrated under reduced pressure to give 2(*S*)-(2-bromo-2-propenyl)-1,4-butanedioic acid 4-(1,1-dimethylethyl) ester (66 mg,

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28% yield). An aliquot of this material was reacted with diazomethane to give the corresponding methyl ester and analyzed by HPLC using a chiral column (Condition A) which indicated a ratio of 8.4:1 of (S)- to (R)-enantiomers (79% ee).

Example 1E**Kinetic Resolution of 2(R,S)-(2-Bromo-2-propenyl)-1,4-butanedioic Acid 4-(1,1-Dimethylethyl) 1-Methyl Ester Using Subtilisin Carlsberg**

The title compound of Example 1C (5.01 g, 16.3 mmol) was dissolved in acetone (10 mL) and deionized H₂O (30 mL). The system was equipped with an automatic pH titrator and a peristaltic pump connected to a 0.4M aqueous NaOH solution. The pH of the suspension was adjusted to 7.8. A crude preparation of Subtilisin Carlsberg (Alcalase® 2.4L "food grade" enzyme preparation, Novo Nordisk Bioindustrials, USA), was added (0.2 g) and the automatic titrating system started with the pH set at 7.2. After 12 h at pH 7.2, the pH on the automatic titrator was reset to 7.5 and the reaction continued until the pH stabilized. The acetone was removed under reduced pressure and saturated aqueous NaHCO₃ (10 mL) was added to the aqueous phase. The aqueous phase was extracted twice with EtOAc (2x 80 mL). The combined organic phases were dried (MgSO₄) and concentrated under reduced pressure to give the unreacted ester (2.67 g), which was shown by HPLC using a chiral column (Condition A) to be a mixture of the (R)- and (S)-enantiomers in a ratio of 68:1 (>97% ee). This material was saved and used for racemization and recycling (Example 1F).

The aqueous phase from the above extraction was rendered acidic by the addition of 10% aqueous HCl (20 mL) and then extracted twice with a 1:1 mixture of EtOAc/Et₂O (2x 80 mL). The latter extracts were washed with brine and dried (MgSO₄). The solvent was removed under reduced pressure and the residue dried under vacuo to give 2(*S*)-(2-bromo-2-propenyl)-1,4-butanedioic acid 4-(1,1-dimethylethyl) ester (2.246 g, 47% yield). An aliquot of this material was reacted with diazomethane to give the corresponding methyl ester and analyzed by HPLC using a chiral column (Condition A) which indicated a ratio of 214:1 of (*S*)- to (*R*)-enantiomers (>99% ee).

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Example 1F**Racemization of 2(*R*)-(2-Bromo-2-propenyl)-1,4-butanedioic Acid 4-(1,1-Dimethylethyl) 1-Methyl Ester**

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To the unreacted ester of Example 1E (1.64 g, 5.34 mmol), which is a mixture of the (*R*)- and (*S*)-enantiomers in a ratio of 68:1, was added toluene (15 mL) and DBU (0.85 mL). This mixture was heated at reflux for 18 h and then diluted with H₂O and extracted with EtOAc. The organic phase was washed serially with 5% aqueous HCl and brine, dried (MgSO₄) and concentrated under reduced pressure to yield material (1.57 g, 96% yield) identical to that obtained in Example 1C, which was shown by HPLC using a chiral column (Condition A) to be a mixture of the (*R*)- and (*S*)-enantiomers in a ratio of 1.1:1.

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Example 2

***N*⁴-(Cyclohexylmethyl)-*N*⁴-{2-{methyl{2-(2-pyridinyl)ethyl}-amino}-2-oxoethyl}-*N*¹-{1(*S*)-(cyclohexylmethyl)-2(*R*),3(*S*)-dihydroxy-5-methylhexyl}-2(*R*)-{(2-amino-4-thiazolyl)methyl}butanediamide**

Example 2A

**2-(2-Chloro-2-propenyl)-1,3-propanedioic Acid
Dimethyl Ester**

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A solution of dimethylmalonate (2381 g, 18.02 mol) in MeOH (500 mL) was added to a stirred solution of 25% (w/w) NaOMe in MeOH (1071 g, 4.95 mol) and MeOH (500 mL). The mixture was heated to an internal temperature of 70°. 2,3-Dichloropropene (511 g, 4.60 mol) in MeOH (500 mL) was added dropwise over 12 h. Thereafter the mixture was stirred at 70° for 8 h. The MeOH was removed under reduced pressure. The residue was partitioned between Et₂O (2 L) and H₂O (2 L). The organic phase was separated and the aqueous phase was extracted with two additional portions of Et₂O (2x 1 L). The combined organic phases were washed serially with 1N aqueous HCl (500 mL), H₂O (2x 1L) and brine (2x 500 mL), dried (MgSO₄) and concentrated under reduced pressure to give a dark orange oil consisting of unreacted malonate, the desired monoalkylated product and some corresponding dialkylated material. Most of the unreacted dimethyl malonate was removed by rotary evaporation under high vacuum (1 Torr) with a bath temperature of 70°. Dimethylmalonate (1381 g) contaminated with 6% of monoalkylated product (by ¹H NMR) was recovered and recycled in future runs.

The residual material (800 g) was fractionally distilled under vacuum to give a number of fractions as indicated in the following table:

Bp°C/Torr	Weight	%Malonate	% Mono-alkylated	% Di-alkylated
80-90/2	25 g	37	63	0
90-100/2	39 g	16	84	0
100/2	651 g	1	97.5	1.5
105-110/.25	44 g	0	12.5	87

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The yield of the title compound was 651 g (97 % pure by HPLC; 68% yield based on 2,3-dichloropropene): ¹H NMR (400 MHz, CDCl₃) δ 5.24 (m, 2H), 3.81 (t, J = 7.6 Hz, 1H), 3.76 (s, 6H), 2.95 (m, 2H); IR (neat) ν 1740, 1635 cm⁻¹.

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Example 2B

2-(2-Chloro-2-propenyl)-2-(methoxycarbonyl)-1,4-butanedioic Acid 4-(1,1-Dimethylethyl) 1-Methyl Ester

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A mixture of the title compound of Example 2A (519.6 g, 2.564 mol) and *tert*-butyl bromoacetate (500 g, 2.564 mol) was added dropwise over 30 min to a stirred mixture of 10N aqueous NaOH (2.56 L, 25.6 mol) and benzyltriethylammonium chloride (2.56 g, 11.2 mmol) at 10°. The reaction mixture was stirred at room temperature for 2 h and then diluted with Hex (750 mL). The organic phase was separated and washed serially with H₂O (100 mL) and brine (2x 100 mL), dried (MgSO₄), and concentrated under reduced pressure to give the title compound (822 g, 100%

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yield) which was used without further purification for the next step (Example 2C). ¹H NMR (400 MHz, CDCl₃) δ 5.32 (d, J = 1.4 Hz, 1H), 5.20 (broad s, 1H), 3.75 (s, 6H), 3.25 (broad s, 2H), 3.06 (s, 2H), 1.43 (s, 9H). MS (FAB) m/z 321 (MH⁺).

Example 2C

2(R,S)-(2-Chloro-2-propenyl)-1,4-butanedioic Acid 4-(1,1-Dimethylethyl) 1-Methyl Ester

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The title compound of Example 2B (1502 g, 4.18 mol) was dissolved in MeOH (2650 mL). While maintaining a temperature of 30° or below for the ensuing mixture, a cold solution of KOH (329 g, 5.85 mol) in H₂O (1335 mL) was added dropwise over 20 min. The mixture was stirred vigorously for 24 h. Thereafter, the MeOH was evaporated under reduced pressure. The resulting aqueous phase was extracted first with Hex (3x 750 mL), rendered acidic (pH 1) by the addition of concentrated HCl (~500 mL) and then extracted with toluene (3x 1 L). The latter extract was dried (MgSO₄) and then heated at reflux for 24 h to effect decarboxylation. At the completion of this period, the reaction mixture was concentrated under reduced pressure. The residue was distilled under reduced pressure (128-132°/0.75 Torr) to give the title racemic compound (867 g, 79% yield) as a colorless liquid: ¹H NMR (400 MHz, CDCl₃) δ 5.25 (d, J = 1.3 Hz, 1H), 5.20 (broad s, 1H), 3.71 (s, 3H), 3.14 (m, 1H), 2.76 (ddd, J = 14.3, 6.4, 1.0 Hz, 1H), 2.59 (dd, J = 16.5, 8.3 Hz, 1H), 2.53 (dd, J = 14.9, 8.4 Hz, 1H), 2.48 (dd, J = 16.5, 5.1 Hz, 1H), 1.44 (s, 9H); ¹³C NMR (100 MHz,

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CDCl₃) δ 173.9, 170.4, 139.1, 114.9, 80.8, 51.8, 40.6, 39.1, 35.9, 27.9. MS (FAB) m/z 263 (MH⁺).

Example 2D

5 Kinetic Resolution of 2(*R,S*)-(2-Chloro-2-propenyl)-1,4-butanedioic Acid 4-(1,1-Dimethylethyl) 1-Methyl Ester Using Subtilisin Carlsberg

The title compound of Example 2C (860.00 g, 3.273
10 mol) was placed in a 5 L flask and suspended in acetone (15 mL) and deionized H₂O (1.5 L). The system was equipped with an automatic pH titrator and a peristaltic pump connected to a 3N aqueous NaOH solution (550 mL, $\pm$ 0.5 equivalents). The pH
15 of the suspension was adjusted to 7.5. A crude preparation of Subtilisin Carlsberg (Alcalase® 2.4L "food grade" enzyme preparation, Novo Nordisk Bioindustrials, USA) was added (10 g) and the automatic titrating system started with the pH set
20 at 7.5. After 22 h at pH 7.5, the pH on the automatic titrator was then set to 8.0 and the reaction continued for another 96 h. The unreacted (*R*)-ester was extracted with EtOAc (3x 700 mL), washed serially with H₂O and brine, dried (MgSO₄)
25 and concentrated under reduced pressure to give a colorless oil (442 g), which was shown by HPLC using a chiral column (Condition A) to be a mixture of the (*R*)- and (*S*)-enantiomers in a ratio of 21:1. This material was saved for racemization and recycling.

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The aqueous phase from the above extraction was rendered acidic (pH 1) by the addition of 6N aqueous HCl (~800 mL) and then extracted with EtOAc (3x 1 L). The latter extracts were washed serially with

H₂O and brine and then dried (MgSO₄). The solvent was removed under reduced pressure and the residue was dried under vacuo to give 2(*S*)-(2-chloro-2-propenyl)-1,4-butanedioic acid 4-(1,1-dimethylethyl) ester (390 g, 96% of theoretical amount) which was >96% homogeneous by HPLC. An aliquot of this material was reacted with diazomethane to give the corresponding methyl ester and analyzed by HPLC using a chiral column (Condition A) which indicated a ratio of 109:1 of (*S*)- to (*R*)-enantiomers (99% ee): $[\alpha]_D^{25}$ -4.58° (c 1, MeOH), $[\alpha]_{Hg436}^{25}$ -6.11° (c 1, MeOH); ¹H NMR (400 MHz, CDCl₃) δ 11.5 (broad s, 1H), 5.27 (d, J = 1.3 Hz, 1H), 5.22 (broad s, 1H), 3.16 (m, 1H), 2.81 (dd, J = 14.3, 6.0 Hz, 1H), 2.63-2.49 (m, 3H), 1.47 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) δ 179.9, 170.4, 138.9, 115.3, 81.2, 40.2, 39.1, 35.5, 27.9; MS (FAB) m/z 249 (MH⁺). This crude material is of sufficient purity to be used in the coupling reactions of Examples 2E and 2F without further purification.

Example 2E

Alternative Kinetic Resolution of 2(*R,S*)-(2-Chloro-2-propenyl)-1,4-butanedioic Acid 4-(1,1-Dimethylethyl) 1-Methyl Ester Using Subtilisin Carlsberg

In this example, the enzymatic hydrolysis of the title compound of Example 2C is carried out without acetone as a co-solvent.

The title compound of Example 2C (0.505 g, 1.92 mmol) was suspended in H₂O (6 mL) and rapidly stirred. The system was equipped with an automatic pH titrator and a peristaltic pump connected to a

0.1N aqueous NaOH solution. The pH of the biphasic solution was adjusted to 7.6 and a crude preparation of Subtilisin Carlsberg (Alcalase® 2.4L "food grade" enzyme preparation, Novo Nordisk Bioindustrials, USA) was added (0.112 g) and the automatic titrating system started with the pH set at 7.5. After 3 h, the reaction mixture was diluted with 5 mL saturated aqueous NaHCO₃, EtOAc (40 mL) and H₂O (20 mL). The organic phase was separated, dried (MgSO₄) and concentrated under reduced pressure to give the unreacted (*R*)-ester (0.25 g, 50% yield). Analysis by HPLC using a chiral column (Condition A) showed that the optical purity of the (*R*)-enantiomer was >98% ee.

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The aqueous phase from the above extraction was rendered acidic (pH 3) by the addition of 5% aqueous HCl, and extracted with EtOAc (50 mL). The organic phase was dried (MgSO₄) and concentrated under reduced pressure to give 2(*S*)-(2-chloro-2-propenyl)-1,4-butanedioic acid 4-(1,1-dimethylethyl) ester (0.215 g, 45% yield). An aliquot of this material was reacted with diazomethane to give the corresponding methyl ester and analyzed by HPLC using a chiral column (Condition A). The optical purity of the (*S*)-enantiomer was 97.5% ee.

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Example 2F

4-{{1(*S*)-(Cyclohexylmethyl)-2(*R*),3(*S*)-dihydroxy-5-methylhexyl}amino}-3(*S*)-(2-chloro-2-propenyl)-4-oxo-butanoic Acid (1,1-Dimethylethyl) Ester

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The title compound of Example 2D (41.69 g, 167.6 mmol), 2(*S*)-2-amino-1-cyclohexyl-6-methyl-3(*R*),4(*S*)-heptanediol (40.00 g, 164.4 mmol) (J.R. Luly et al., J. Org. Chem. **1988**, 53, 6109), HOBt (25.92 g, 169.3 mmol) and *N*-methylmorpholine (16.29 g, 161.1 mmol) were dissolved in THF (450 mL) and the solution cooled in an ice-water bath. DCC (34.59 g, 167.6 mmol) in THF (50 mL) was added and the mixture stirred 30 min at 5° and then for a further 17 h at room temperature. The precipitated solid was removed by filtration and the filtrate concentrated under reduced pressure. The residue was dissolved in EtOAc (1 L), the suspension filtered to remove precipitated DCU and the filtrate washed serially with 1N aqueous HCl (3x 300 mL), H₂O (250 mL), 3N aqueous NaOH (3x 300 mL) and brine. After drying (MgSO₄), the solvent was removed under reduced pressure to give a white solid. Recrystallisation from EtOAc/Hex gave the title compound (99.3% homogeneity by HPLC) as a white solid (77.91 g, 71% yield): mp 144-146°; $[\alpha]_D^{25}$ -41.39° (c 1, MeOH); ¹H NMR (400 MHz, CDCl₃) δ 5.91 (broad d, J = 8.6 Hz, 1H), 5.27 (m, 2H), 4.29 (dt, J = 9.0, 4.7 Hz, 1H), 4.19 (m, 1H), 3.21 (m, 2H), 3.04 (m, 1H), 2.73 (dd, J = 14.0, 8.9 Hz, 1H), 2.63 (dd, J = 17.2, 9.4 Hz, 1H), 2.43 (dd, J = 14.3, 6.0 Hz, 1H), 2.38 (dd, J = 17.1, 4.2 Hz, 1H), 1.89 (m, 1H), 1.8-1.1 (m, 15H), 1.46 (s, 9H), 1.0-0.77 (m, 2H), 0.94 (d, J = 6.7 Hz, 3H), 0.87 (d, J = 6.4 Hz, 3H); ¹³C NMR (100 MHz,

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CDCl₃) δ 175.0, 171.4, 139.4, 115.6, 81.5, 77.7, 69.8, 47.5, 42.8, 41.7, 40.8, 39.7, 37.2, 34.1, 33.9, 32.8, 28.3, 26.6, 26.3, 26.2, 24.8, 24.1, 22.0. MS (FAB) m/z 474 (MH⁺). Anal. Calcd. for C₂₅H₄₄ClNO₅: C, 63.34; H, 9.35; N, 2.95. Found: C, 63.50; H, 9.62; N, 3.00.

Example 2G

4-({1(*S*)-(Cyclohexylmethyl)-2(*R*),3(*S*)-dihydroxy-5-methylhexyl}amino)-3(*S*)-(2-chloro-2-propenyl)-4-oxo-butanoic Acid

The title compound of Example 2F (34.20 g, 72.1 mmol) was added to TFA (100 mL) and the mixture stirred 24 h at room temperature. The volatiles were removed under reduced pressure and the residue coevaporated twice with MeOH (100 mL). The residue was dissolved in MeOH (100 mL) and 3N aqueous NaOH (100 mL) was added. The mixture was stirred 4 h at room temperature. The MeOH was removed under reduced pressure and the residue rendered acidic (pH 1) with concentrated HCl. The precipitated product was collected by filtration, washed with H₂O and dried to give the title compound (29.36 g, 97% yield): mp 193-196°; $[\alpha]_D^{25}$ -49.2° (c 1, MeOH); ¹H NMR (400 MHz, DMSO-d₆) δ 7.83 (d, J = 8.7 Hz, 1H), 5.34 (s, 1H), 5.20 (s, 1H), 4.61 (broad d, J = 5.7 Hz, 1H), 4.48 (broad s, 1H), 4.09 (m, 1H), 3.07 (t, J = 7.2 Hz, 2H), 2.90 (broad m, 1H), 2.65 (dd, J = 14.7, 7.6 Hz, 1H), 2.48 (dd, J = 15.4, 7.0 Hz, 1H), 2.42 (dd, J = 14.7, 6.6 Hz, 1H), 2.28 (dd, J = 16.6, 5.8 Hz, 1H), 1.77 (m, 1H), 1.7-1.5 (m, 6H), 1.45 (m, 1H), 1.38-1.03 (m, 6H), 0.96-0.7 (m, 2H), 0.87 (d, J = 6.6 Hz, 3H), 0.70 (d, J = 6.6 Hz, 3H); MS (FAB)

m/z 418 (MH⁺). Anal. Calcd. for C₂₁H₃₆ClNO₅: C, 60.35; H, 8.68; N, 3.35. Found: C, 60.15; H, 8.79; N, 3.40.

5 **Example 2H**

N⁴-(Cyclohexylmethyl)-N⁴-{2-{methyl{2-(2-pyridinyl)-ethyl}amino}-2-oxoethyl}-N¹-{1(*S*)-(cyclohexylmethyl)-2(*R*),3(*S*)-dihydroxy-5-methylhexyl}-2(*R*)-(2-chloro-2-propenyl)butanediamide

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The title compound of Example 2G (10.00 g, 23.92 mmol) and *N*-methylmorpholine (5.8 mL, 52.6 mmol) were suspended in cold (5°) THF (75 mL) and the mixture stirred until all solids were dissolved.

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Pivaloyl chloride (2.94 mL, 23.92 mmol) was added and the mixture stirred 10 min in an ice-water bath. *N*-{2-(2-pyridinyl)ethyl}-*N*-methyl-2-[(cyclohexylmethyl)amino]acetamide (8.00 g, 27.5 mmol) (Lavallée et al, EP 589445, published March 30, 1994) in THF

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(15 mL) was added and the cooling bath removed. The mixture was stirred at room temperature for 2 h. The reaction was quenched with H₂O (50 mL) and the THF removed under reduced pressure. The residue was extracted with EtOAc and the organic phase washed serially with 1N aqueous NaOH and H₂O, dried

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(MgSO₄), concentrated under reduced pressure and the residue crystallized twice from hot EtOAc/Hex. The title compound was obtained as a white solid (12.25 g, 74% yield, >97% homogeneity by RP-HPLC): R_f 0.49 (9:1 CHCl₃/MeOH); mp 139.5-140.5°; [α]_D²³ -32.3° (c 1, MeOH); ¹H NMR (400 MHz, CDCl₃) exists as a mixture of rotamers: δ 8.60, 8.57 and 8.52 (broad d, J = 4.8 Hz, total 1H), 7.62 (m, 1H), 7.25 (m, 1H), 7.16 (m, 1H), 6.78, 6.62 and 6.57 (d, J = 8.3 Hz,

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total 1H), 5.26 (m, 2H), 4.33 (m, 2H), 4.11 (m, 1H),
4.05-3.6 (m, 3H), 3.3-2.8 (m, 9H), 2.94 (s, 3H),
2.69-2.4 (m, 3H), 1.90 (m, 1H), 1.8-1.1 (m, 25H), 1-
0.8 (m, 10H); MS (FAB) m/z 689 (MH⁺). Anal. Calcd.
5 for C₃₈H₆₁ClN₄O₅: C, 66.21; H, 8.92; N, 8.13.
Found: C, 66.07; H, 9.06; N, 8.10.

Example 2I

**N⁴-(Cyclohexylmethyl)-N⁴-{2-{methyl{2-(2-pyridinyl)-
10 ethyl}amino}-2-oxoethyl}-N¹-{1(S)-(cyclohexyl-
methyl)-2(R),3(S)-dihydroxy-5-methylhexyl}-2(R)-{(2-
amino-4-thiazolyl)methyl}butanediamide**

The title compound of Example 2H (1.069 g, 1.55
15 mmol) was dissolved in dichloromethane (30 mL), and
para-toluenesulfonic acid monohydrate (0.019 g, 0.1
mmol) was added followed by 2-methoxypropene (0.745
mL, 7.75 mmol). The mixture was stirred for 60 h at
room temperature. Volatiles were removed under
20 reduced pressure and the residue dissolved in a
mixture of 2:1 EtOAc:H₂O (60 mL). After cooling in
an ice-water bath, N-bromosuccinimide (0.32 g, 1.8
mmol) was added and the mixture stirred 3 h at 5°
and 3 h at room temperature. Thiourea (0.142 g,
25 1.86 mmol) was added and the reaction mixture
immersed in a preheated oil bath at 70°. After 1 h,
the reaction was judged complete by RP-HPLC. The
suspension was rendered basic (pH 11) by the
addition of 3N aqueous NaOH and extracted with
30 EtOAc. The organic phase was washed serially with
1N aqueous NaOH and H₂O, dried (MgSO₄), and
concentrated under reduced pressure. The residue
was dissolved in a small amount of EtOAc and the
product precipitated by the addition of Hex. The

cream colored precipitate was collected, washed with Hex and dried under reduced pressure (0.95 g, 84% yield, 96% homogeneity by RP-HPLC). An analytical sample was obtained by a second precipitation from EtOAc/hexane to give a 97% homogeneous preparation of the title compound identical in all respect to material previously prepared by the chiral auxiliary route (Lavallée et al, EP 589445): R_f 0.21 (9:1 $CHCl_3/MeOH$); mp 84-90° (not well defined); precipitated sample from $MeOH/H_2O$: $[\alpha]_D^{25} -21.6^\circ$ (c 1 $MeOH$); ^1H NMR (400 MHz, $DMSO-d_6$) exists as a mixture of rotamers: δ 8.3 (m, 1H), 7.69 (m, 1H), 7.52 (m, 1H), 7.3-7.18 (m, 2H), 6.73 (broad s, 1H), 6.69 (s, 1H), 6.16, 6.15, 6.09 and 6.04 (s, total 1H), 4.62 (m, 1H), 4.5 (m, 1H), 4.2-4.0 (m, 2H), 3.85 (t, $J = 17.4$ Hz, 0.7H), 3.7-3.5 (m, 2.3H), 3.1-2.6 (m, 11H), 2.5-2.0 (m, 2.7H), 1.8-1.0 (m, 24H), 0.9-0.7 (m, 4H), 0.86 (d, $J = 6.6$ Hz, 3H), 0.78 (d, $J = 6.3$ Hz, 3H); MS (FAB) m/z 727 (MH^+). Anal. Calcd. for $C_{39}H_{62}N_6O_5S$: C, 64.43; H, 8.60; N, 11.56. Found: C, 64.04; H, 8.76; N, 11.35.

Example 3

4-((Cyclohexylmethyl)-{2-(methyl(2-(2-pyridinyl)-ethyl)amino)-2-oxoethyl}amino)-2(*S*)-(2-chloro-2-propenyl)-4-oxobutanoic Acid

Example 3A

2(*R,S*)-(2-Chloro-2-propenyl)-1,4-butanedioic Acid 1-Methyl Ester

The title compound of Example 2C (607.76 g, 2.31 mol) was added to an ice-cold solution of aqueous 4N HCl in dioxane (1600 mL). The mixture was allowed

to warm up to room temperature and stirred for 40 h. Volatiles were removed under vacuo to give a yellow oil which was dissolved in ether (250 mL). Hex was added until the solution became cloudy (~650 mL).
5 After seeding, the product crystallized at room temperature. After cooling overnight to 5°, the product was collected, washed with 10% ether in Hex, then with Hex. After drying in vacuo, the title compound was obtained as a white crystalline solid
10 (407.4 g, 85% yield): mp 61-64°; ¹H NMR (400 MHz, CDCl₃) δ 11.5 (broad s, 1 H), 5.27 (d, J = 1.3 Hz, 1 H), 5.22 (broad s, 1 H), 3.72 (s, 3 H), 3.24-3.16 (m, 1 H), 2.84-2.71 (m, 2 H), 2.65-2.54 (m, 2 H); ¹³C NMR (100 MHz, CDCl₃) δ 177.3, 173.7, 138.6,
15 115.2, 52.0, 40.3, 38.5, 33.9; MS (FAB) m/z 207 (MH⁺). Anal. Calcd. for C₈H₁₁ClO₄: C, 46.50; H, 5.37. Found: C, 46.12; H, 5.35.

Example 3B

20 **4-((Cyclohexylmethyl)-{2-{methyl{2-(2-pyridinyl)ethyl}amino}-2-oxoethyl}amino)-2(R,S)-(2-chloro-2-propenyl)-4-oxobutanoic Acid 1-Methyl Ester**

The title compound of Example 3A (20.00 g, 96.8 mmol), N-{2-(2-pyridinyl)ethyl}-N-methyl-2-((cyclohexylmethyl)amino)acetamide (26.68 g, 92.2 mmol) (Lavallée et al., EP 589445, published March 30, 1994), HOBt (15.00g, 98.0 mmol) and NMM (17.00g, 168 mmol) were suspended in THF (150 mL) and the mixture
30 stirred at room temperature until all solids dissolved. DCC (20.00 g, 97 mmol) in THF (10 mL) was added and the mixture stirred overnight at room temperature. Volatiles were removed under reduced pressure and the residue dissolved in EtOAc. The

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solution was filtered to remove insoluble DCU, then washed serially with water (2x), 3N NaOH (3x), water (2x) and brine (2x). After drying over anhydrous sodium sulfate, volatiles were removed under reduced pressure to give an oily orange residue which was dried in vacuo to yield the title compound (42.28 g, 96% yield, crude product): ^1H NMR (400 MHz, CDCl_3 ; exists as a mixture of rotamers): δ 8.60-8.50 (m, 1H), 7.60 (m, 1H), 7.20-7.10 (m, 2H), 5.30-5.15 (m, 2H), 4.20-3.70 (m, 4H), 3.70 and 3.68 (two s, 3H), 3.30-2.20 (m, 10H), 2.90 (s, 3H), 1.80-1.40 (m, 4H), 1.25-1.10 (m, 2H), 0.98-0.80 (m, 2H).

Example 3C

4-((Cyclohexylmethyl)-{2-(methyl{2-(2-pyridinyl)-ethyl}amino)-2-oxoethyl}amino)-2(S)-(2-chloro-2-propenyl)-4-oxobutanoic Acid

The title compound of Example 3B (196.2 g, 0.41 mole), was dissolved in warm acetone (150 mL) and diluted with H_2O (350 mL). The solution was adjusted to pH 7 by the addition of aqueous KHSO_4 and a crude preparation of Subtilisin Carlsberg (Alcalase® 2.4L "food grade" enzyme preparation, Novo Nordisk Bioindustrials, USA) (5 g) was added. The solution was stirred vigorously and the pH of the solution was maintained between 7 and 8 using a pH titrator and a peristaltic pump connected to a 1N aqueous NaOH solution and the reaction was continued until the pH stabilized. The reaction mixture was concentrated under reduced pressure and extracted with EtOAc (4x 200 mL). The combined organic phase was washed serially with H_2O (2x 100 mL) and brine (100 mL), dried (MgSO_4), concentrated under reduced

pressure and the residue partially purified by passage through a 3 cm pad of silica gel using EtOAc (1 L) as the eluent. Removal of the solvent gave the (*R*)-ester as a dark brown oil (103.69 g, 52 %
5 yield), which was saved for racemization and recycling (described in Example 3D). HPLC analysis was performed on an aliquot of this material using a chiral column (Condition B: (*R*)-isomer, t_R 154.1 min (98.8%); (*S*)-isomer, t_R 170.8 min (1.2%)): ^1H NMR
10 (methyl ester, mixture of rotamers; 400 MHz, $CDCl_3$) δ 8.63-8.50 (m, 1H), 7.67-7.57 (m, 1H), 7.24-7.10 (m, 2H), 5.27-5.15 (m, 2H), 4.23-3.67 (m, 6H), 3.31-2.2 (m, 9H), 1.8-1.1 (m, 9H), 1.0-0.8 (m, 2H); MS(FAB) m/z 478 (MH^+).

15

The aqueous phase from the above extraction was rendered acidic (pH 4) with 20% aqueous $KHSO_4$ and extracted with EtOAc (4x 200 mL). The combined organic phase was filtered to remove some insoluble
20 particles, washed serially with H_2O (200 mL) and brine (2x 200 mL), dried ($MgSO_4$), and concentrated under reduced pressure to give the title compound as a yellow-brown foam (67.35 g, 34.3% yield): ^1H NMR (mixture of rotamers; 400 MHz, $CDCl_3$) δ 12-11 (broad
25 s, 1H), 8.63-8.5 (m, 1H), 7.70-7.63 (m, 1H), 7.30-7.14 (m, 2H), 5.32-5.18 (m, 2H), 4.08 and 4.0 (AB quartets, major rotamers, 2H), 3.80-3.61 (m, 2H), 3.25-2.5 (m, 12H), 1.8-1.4 (m, 6H), 1.25-1.10 (m, 3H), 0.98-0.80 (m, 2H); MS(FAB) m/z 464 (MH^+).

30

The enantiomeric purity of the (*S*)-acid was determined by HPLC using a chiral column (Condition B: (*R*)-isomer, t_R 157.4 min (3%); (*S*)-isomer, t_R 166.8 min (97%)) after conversion of an aliquot to

the methyl ester using diazomethane in Et₂O: ¹H NMR (methyl ester, mixture of rotamers; 400 MHz, CDCl₃)
δ 8.63-8.50 (m, 1H), 7.67-7.57 (m, 1H), 7.24-7.10 (m, 2H), 5.27-5.15 (m, 2H), 4.23-3.67 (m, 6H), 3.31-
5 2.2 (m, 9H), 1.8-1.1 (m, 9H), 1.0-0.8 (m, 2H).

Example 3D

Racemization of 4-((Cyclohexylmethyl)-{2-{methyl{2-(2-pyridinyl)ethyl}-amino}-2-oxoethyl}amino)-2(R,S)-(2-chloro-2-propenyl)-4-oxobutanoic Acid 1-Methyl Ester

The unreacted (R)-ester obtained from the first extraction in Example 3C was racemized for recycling
15 as follows. The (R)-ester (3.19 g, 6.67 mmol) was dissolved in MeOH (10 mL) and a 25% (w/w) solution of NaOMe in MeOH (152 μL, 0.66 mmol) was added. The reaction mixture was stirred 17 h at 60°. HPLC analysis using a chiral column (Conditon B: (R)-
20 isomer, t_R 163.2 min (50%); (S)-isomer, t_R 176.6 min (50%)) indicated complete racemization.

Example 4

2(S)-(2-Bromo-2-propenyl)-1,4-butanedioic Acid 4-(1,1-Dimethylethyl) Ester

The title compound can be prepared following the procedures described in Example 1 with the following modifications: NaOMe/MeOH was replaced with NaHMDS
30 (1 M in THF), and dimethyl malonate was replaced with diethyl malonate.

Alternatively, the title compound was also prepared in the following manner. The title compound of

Example 1C (3.0 g, 9.77 mmol) was dissolved in THF (12 mL) and H₂O (4 mL) before being treated with LiOH (0.82 g, 19.5 mmol). The mixture was rapidly stirred for 15 h before being diluted with EtOAc (100 mL) and saturated aqueous NaHCO₃ (30 mL). The phases were separated and the aqueous phase rendered acidic by the addition of 6N aqueous HCl. The aqueous phase was then extracted with EtOAc (2x 80 mL). The combined organic phases were washed with saturated brine (1x 60 mL) and dried (MgSO₄). Concentration under reduced pressure gave the carboxylic acid as a colourless oil which solidified upon standing (2.34 g, 82% yield): ¹H NMR (200 MHz, CDCl₃) δ 5.7 (broad d, 1H), 5.55 (dd, 1H), 3.2 (m, 1H), 2.9 (m, 1H); MS (FAB) m/z 293, 295 (M⁺). Exact mass; calc. 293.0403, found 293.0388.

The carboxylic acid (1.09 g, 3.72 mmol) from above was dissolved in acetonitrile (15 mL) and cooled to 0°. DBU (0.63 mL, 4.09 mmol) was then added and incubated for 10 min after which iodoethane (0.36 mL, 4.46 mmol) was added. The reaction was allowed to stir for 16 h before being concentrated. The residue was dissolved in EtOAc (80 mL) and washed serially with saturated aqueous NaHCO₃ (1x 50 mL), 5% aqueous HCl (1x 40 mL), and saturated brine (1x 50 mL). The organic phase was dried (MgSO₄) and concentrated under reduced pressure. Purification by silica chromatography with 10% EtOAc/Hex gave 0.45 g (40% yield) of racemic 2(R,S)-(2-bromo-2-propenyl)-1,4-butanedioic acid 4-(1,1-dimethylethyl)-1-ethyl ester: MS (FAB) m/z 322.8 (M⁺). Anal. Calcd. for C₁₃H₂₁O₄Br: C, 48.61; H, 6.59. Found: C, 48.75; H, 6.68.

The racemic compound from above was submitted to a kinetic resolution using Subtilisin Carlsberg following the procedure of Example 2E. The title compound was obtained with 75% ee (analysis performed by HPLC using a chiral column (Conditon A) after derivatization of an aliquot with diazomethane to the corresponding methyl ester) and 39.5% yield.

Example 5

10 **2(R)-[(2-Amino-4-thiazolyl)methyl]-1,4-butanedioic Acid 4-(1,1-Dimethylethyl) Ester**

The title compound from Example 2C (5.0 g, 16.3 mmol) was dissolved in acetonitrile (20 mL) and deionized water (7 mL). N-bromosuccinimide (4.06 g, 23 mmol) was added in one portion. The mixture was stirred for 35 min before the excess N-bromosuccinimide was quenched with 2-methoxypropene (0.63 mL, 6.5 mmol). After 3 min the reaction mixture was treated with thiourea (1.5 g, 19.6 mmol) in one portion. The reaction was stirred for 1 h before being concentrated under reduced pressure. The residue was dissolved in EtOAc (150 mL) and washed serially with water (100 mL) and saturated aqueous NaHCO₃ (30 mL). The organic phase was dried (MgSO₄) and concentrated under reduced pressure. The crude extract was purified by flash silica chromatography (5% MeOH/CHCl₃) to give 2.0 g (41% yield) of racemic 2(R,S)-[(2-amino-4-thiazolyl)methyl]-1,4-butanedioic acid 4-(1,1-dimethylethyl) 1-methyl ester: ¹H NMR (400MHz, CDCl₃) δ 6.17 (s, 1H), 4.89 (bs, 2H), 3.68 (s, 3H), 3.2 (m, 1H), 2.95 (m, 1H), 2.55-2.8 (m, 2H), 2.45 (m, 1H), 1.43 (s, 9H); MS (FAB) m/z 301.4 (MH)⁺;

HRMS (FAB) Calcd. for (MH)⁺, 301.12219. Found 301.12320.

The racemic compound from above was submitted to a kinetic resolution using Subtilisin Carlsberg following the procedure of Example 2E. The title compound was obtained with 97% ee (analysis performed by HPLC using a chiral column (Condition A) after derivatization of an aliquot with diazomethane to the corresponding methyl ester) and 26.5% yield.

Example 6

2(R)-{(2-(1,1-dimethylethoxycarbonyl)amino-4-thiazolyl)methyl}-1,4-butanedioic Acid 4-(1,1-Dimethylethyl) Ester

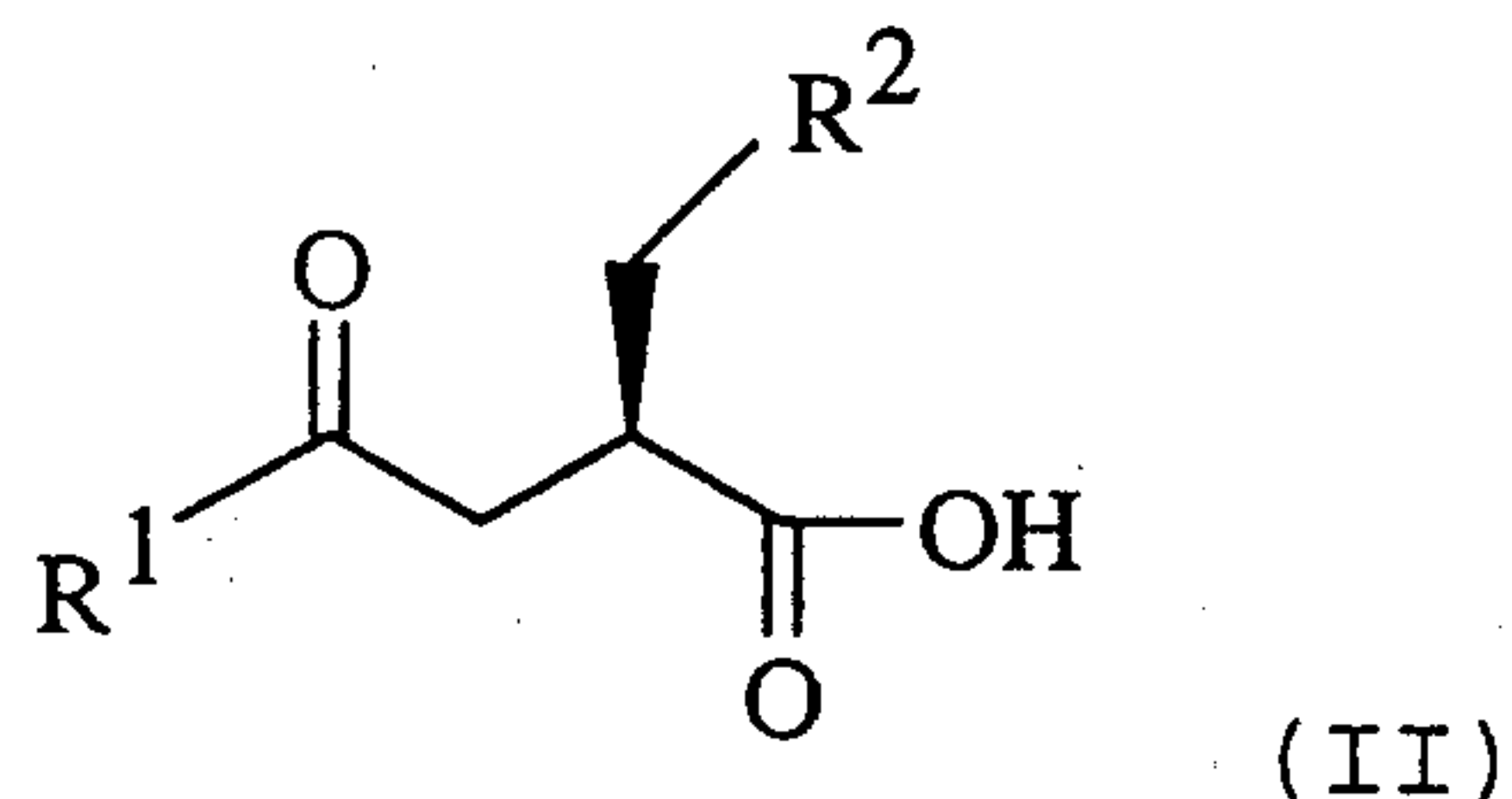
The amino group of the racemic 2(R,S)-{(2-amino-4-thiazolyl)methyl}-1,4-butanedioic acid 4-(1,1-dimethylethyl) 1-methyl ester from Example 5 was protected as a t-butyl carbamate group using (Boc)₂O and a suitable base in THF. Purification by silica chromatography gave the desired racemic 2(R,S)-{(2-(1,1-dimethylethoxycarbonyl)amino-4-thiazolyl)methyl}-1,4-butanedioic acid 4-(1,1-dimethylethyl) 1-methyl ester: ¹H NMR (400MHz, CDCl₃) δ 8.85 (bs, 1H), 6.55 (s, 1H), 3.66 (s, 3H), 3.17 (m, 1H), 3.05 (m, 1H), 2.84 (m, 1H), 2.57 (m, 1H), 2.41 (m, 1H), 1.6 (s, 9H), 1.4 (s, 9H); MS (FAB) m/z 401.1 (MH)⁺; HRMS (FAB) Calcd. for (MH)⁺, 401.17462. Found 401.17570.

The racemic compound from above was submitted to a kinetic resolution using Subtilisin Carlsberg

following the procedure of Example 2E. The title compound was obtained with 82.5% ee (analysis performed by HPLC using a chiral column (Condition A) after derivatization of an aliquot with
5 diazomethane to give the corresponding methyl ester) and 35.5% yield. The methyl ester derivative was characterized: ^1H NMR (400MHz, CDCl_3) δ 8.85 (bs, 1H), 6.55 (s, 1H), 3.66 (s, 3H), 3.17 (m, 1H), 3.05 (m, 1H), 2.84 (m, 1H), 2.57 (m, 1H), 2.41 (m, 1H),
10 1.6 (s, 9H), 1.4 (s, 9H); MS (FAB) m/z 401.1 (MH) $^+$.

The embodiments of the invention, in which an exclusive property or privilege is claimed are defined as follows:

1. A process for the preparation of compounds with high enantiomeric purity of formula II:



wherein R¹ is lower alkoxy, lower alkylamino, di(lower alkyl)amino or R¹ is A-NR³, wherein A is R⁴R⁵NC(O)CH₂ wherein,

10 (a) R⁴ is hydrogen or lower alkyl and R⁵ is hydrogen, lower alkyl or lower alkyl monosubstituted with lower cycloalkyl, phenyl, or a heterocyclic ring (hereinafter designated as "Het") which is an
15 unsubstituted, monosubstituted or disubstituted, five- or six-membered ring containing one or two heteroatoms selected from the group consisting of N, O and S, and wherein each substituent is selected independently from the group consisting of lower
20 alkyl, lower alkoxy, halo, amino or lower alkylamino; or

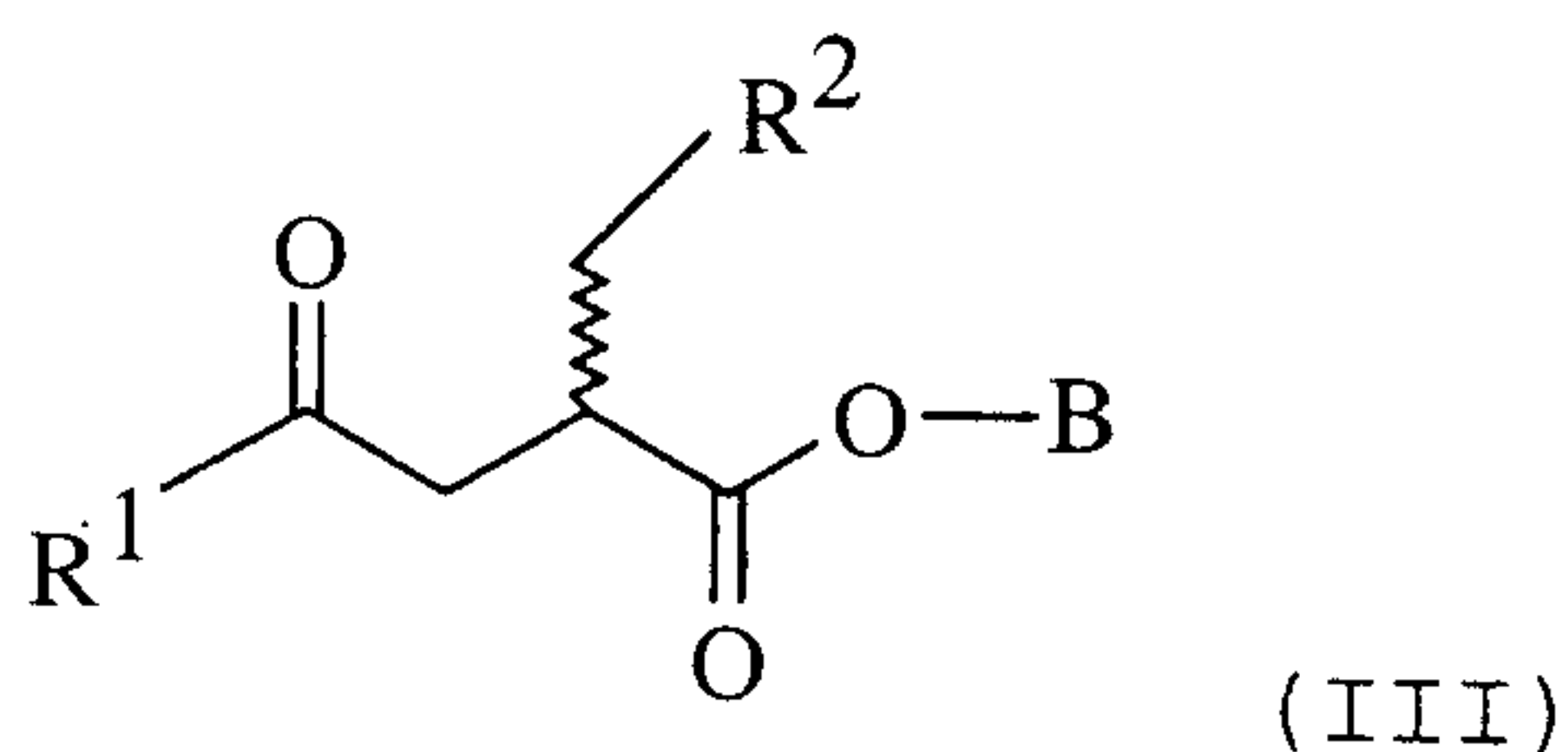
(b) R⁴ is lower alkyl and R⁵ is R⁶R⁷N-Alk wherein R⁶ and R⁷ each is hydrogen or lower alkyl and Alk is a
25 divalent alkyl radical derived by the removal of two hydrogen atoms of a straight or branched chain hydrocarbon containing from one to six carbon atoms;
or

(c) R⁴ is lower alkyl and R⁵ is lower alkoxy; or

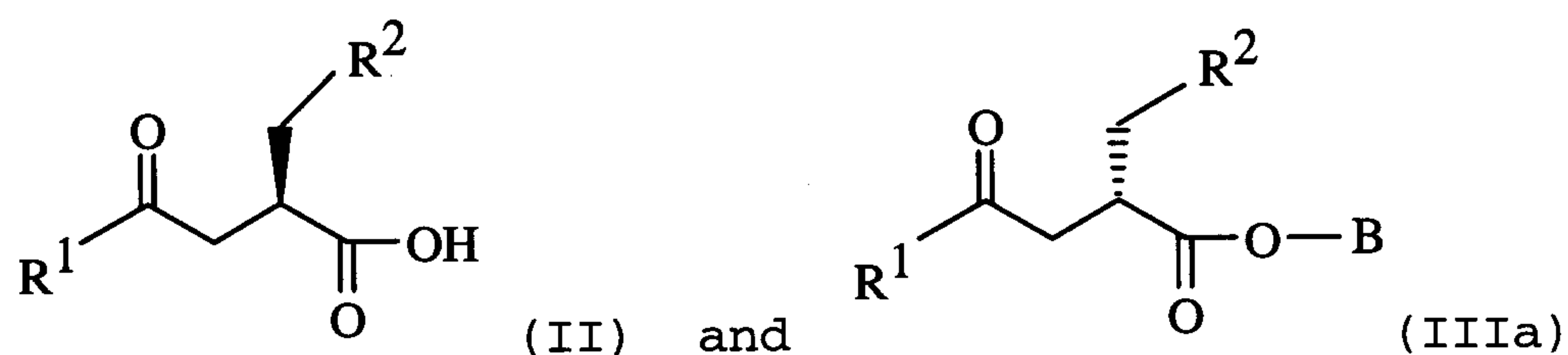
(d) R⁴ and R⁵ together with the nitrogen atom to
30 which they are attached form a pyrrolidino,

piperidino, 4-{(lower alkoxy)-(lower alkoxy)}-1-piperidinyl, morpholino or thiomorpholino;
 R^3 is lower alkyl or lower alkyl monosubstituted with lower cycloalkyl, phenyl, 4-(lower
 5 alkyl)phenyl, 4-(lower alkoxy)phenyl, 4-halophenyl, 3,4-methylenedioxyphenyl, 1-naphthyl, 2-naphthyl or Het wherein Het is as defined in this claim; and
 R^2 is lower alkyl, $CCl=CH_2$, $CBr=CH_2$, lower cycloalkyl, 2-amino-4-thiazolyl, 2-(amino protecting
 10 group)amino-4-thiazolyl or Het wherein Het is as defined herein, said process comprising:

(i) selectively hydrolyzing one enantiomer of a racemic mixture of an ester of formula III:



wherein R^1 is as defined in this claim, R^2 is as defined in this claim; and B is lower alkyl, with an effective amount of an esterase selected from
 20 Subtilisin Carlsberg or α -chymotrypsin, in a reaction medium comprising H_2O or a mixture of H_2O and an H_2O miscible inert organic solvent, in the presence of an alkali metal hydroxide to maintain the pH of the medium at about 7.0-8.5, to form a
 25 reaction mixture containing compounds of formula II (as an alkali metal salt) and IIIa:



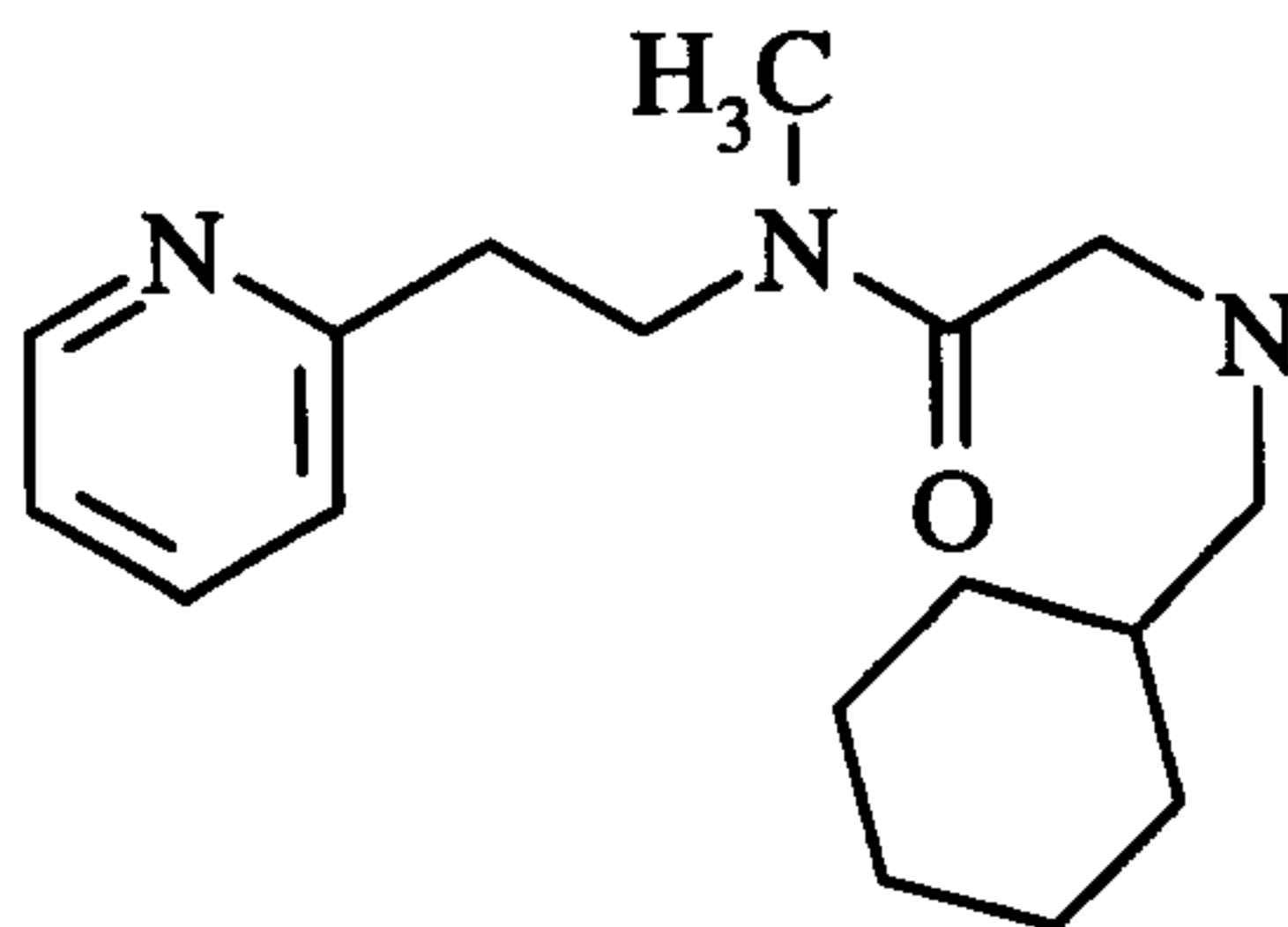
and

(ii) separating from the mixture resulting
5 from step (i) the compound of formula II.

2. A process according to claim 1 wherein R^1 is lower alkoxy, lower alkylamino, di(lower alkyl)amino or R^1 is $A-NR^3$, wherein A is $R^4R^5NC(O)CH_2$ wherein
10 R^4 is methyl and R^5 is methyl, 2-(2-pyridinyl)ethyl or 2-(3-pyridinyl)ethyl; R^2 is $CCl=CH_2$, $CBr=CH_2$, 2-amino-4-thiazolyl, 2-(amino protecting group)amino-4-thiazolyl or a five-membered heterocyclic ring containing one or two heteroatoms selected from the
15 group consisting of N, O and S; R^3 is 1-ethylpropyl, cyclopentylmethyl, cyclohexylmethyl, 1(S)-cyclohexylethyl, cycloheptylmethyl, phenylmethyl, 1(S)-phenylethyl, (1-methylcyclohexyl)methyl; and B is methyl or ethyl.

20

3. A process according to claim 1 wherein R^1 is 1,1-dimethylethoxy or the monovalent radical:



R^2 is $CCl=CH_2$, $CBr=CH_2$, 2-amino-4-thiazolyl, or 2-(1,1-dimethylethoxycarbonyl)amino-4-thiazolyl and B is methyl.

5 4. A process according to claim 1 wherein R^2 is $CBr=CH_2$, $CCl=CH_2$ or 2-amino-4-thiazolyl.

5. A process according to claim 4 wherein R^2 is $CBr=CH_2$ or $CCl=CH_2$.

10

6. A process according to claim 1 wherein the esterase is Subtilisin Carlsberg.

7. A process according to claim 1 wherein the
15 reaction medium in step (i) contains from 0 to 30% (v/v) of a H_2O miscible inert organic solvent.

8. A process according to claim 7 wherein the H_2O miscible inert organic solvent is selected from
20 acetone and acetonitrile.

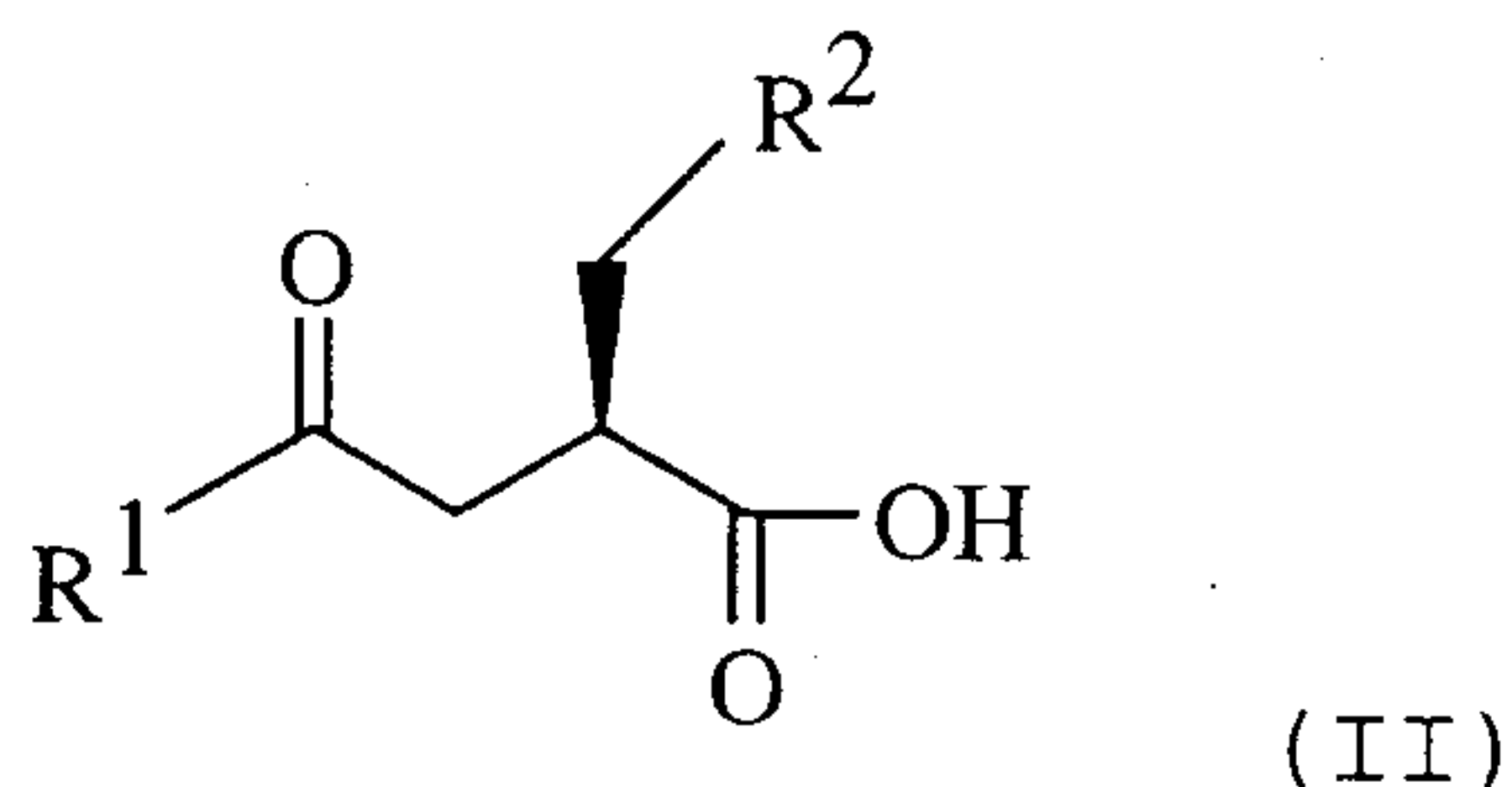
9. A process according to claim 1 wherein the alkali metal hydroxide is selected from NaOH, KOH and LiOH.

25

10. A process according to claim 1 wherein in step (i) a temperature of between 20-55°C is maintained.

11. A process according to claim 10 wherein in step
30 (i) a temperature of between 20-37°C is maintained.

12. A compound of formula II:

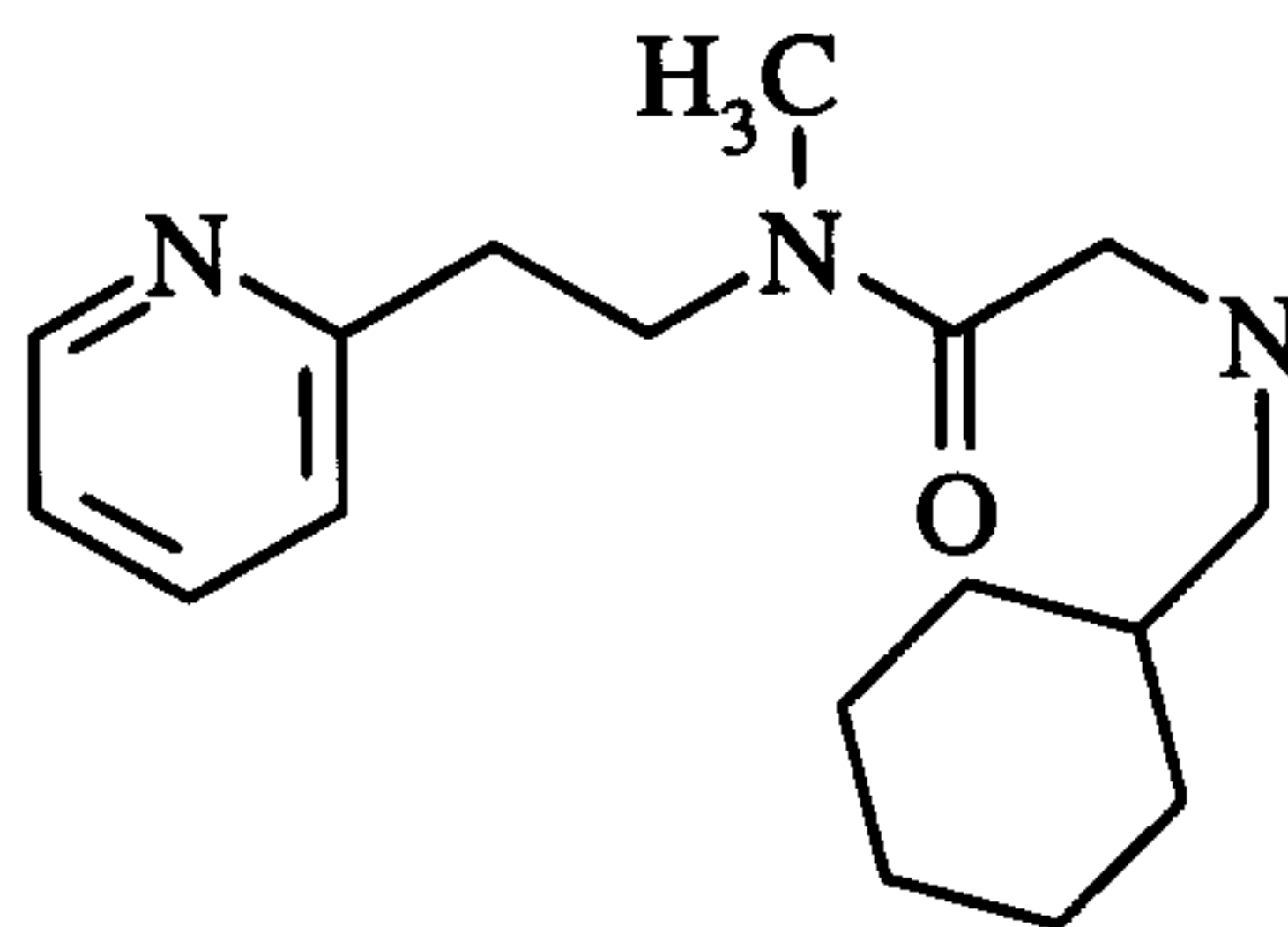


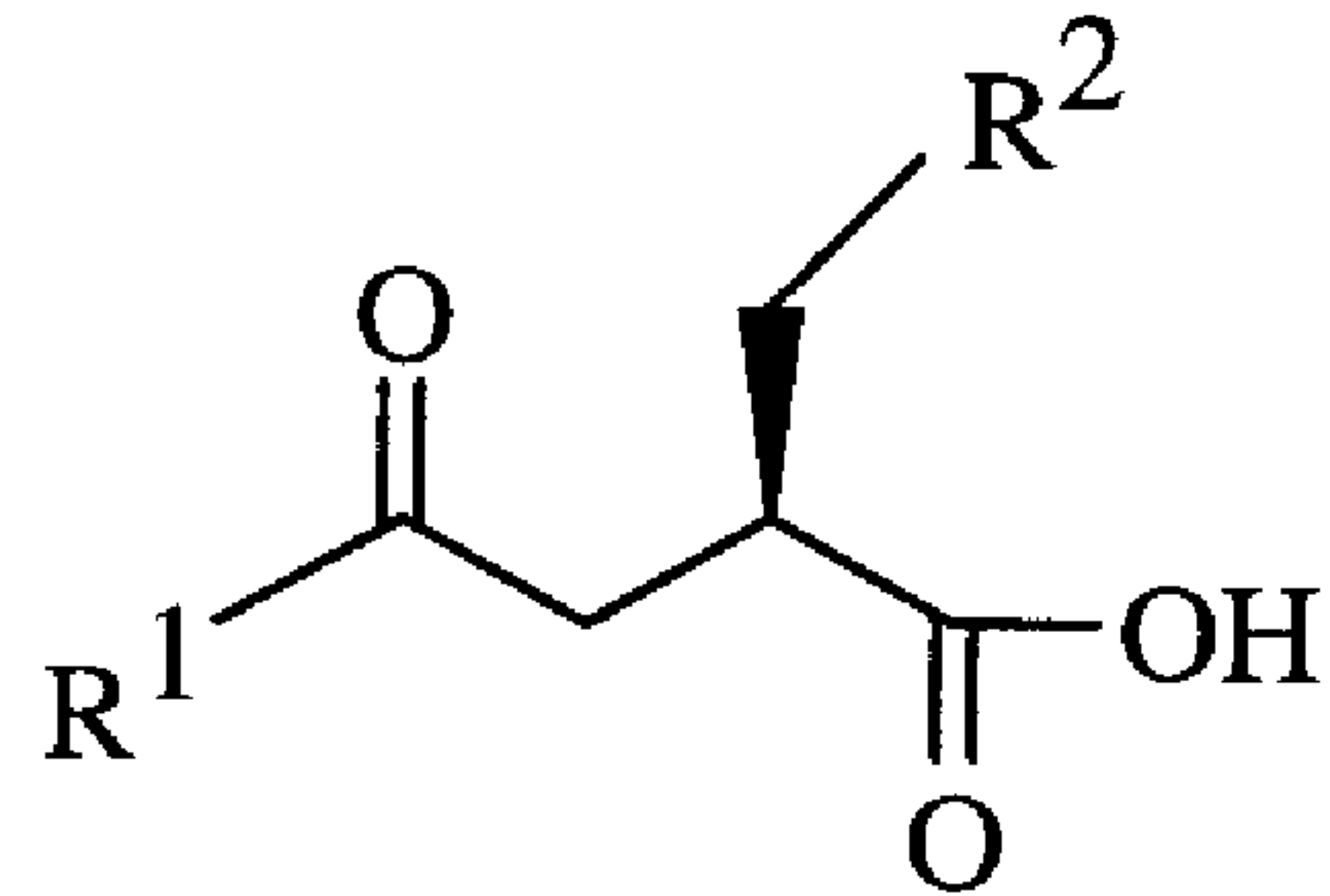
- 5 wherein R^1 is lower alkoxy, lower alkylamino, or di(loweralkyl)amino or R^1 is $A-NR^3$, wherein A is $R^4R^5NC(O)CH_2$ wherein,
- (a) R^4 is hydrogen or lower alkyl and R^5 is hydrogen, lower alkyl or lower alkyl monosubstituted
- 10 with lower cycloalkyl, phenyl, or a heterocyclic ring (hereinafter designated as "Het") which is an unsubstituted, monosubstituted or disubstituted, five- or six-membered ring containing one or two heteroatoms selected from the group consisting of N,
- 15 O and S, and wherein each substituent is selected independently from the group consisting of lower alkyl, lower alkoxy, halo, amino or lower alkylamino; or
- (b) R^4 is lower alkyl and R^5 is R^6R^7N-Alk wherein R^6
- 20 and R^7 each is hydrogen or lower alkyl and Alk is a divalent alkyl radical derived by the removal of two hydrogen atoms of a straight or branched chain hydrocarbon containing from one to six carbon atoms; or
- 25 (c) R^4 is lower alkyl and R^5 is lower alkoxy; or
- (d) R^4 and R^5 together with the nitrogen atom to which they are attached form a pyrrolidino, piperidino, 4-((lower alkoxy)-(lower alkoxy))-1-piperidinyl, morpholino or thiomorpholino;

R^3 is is lower alkyl or lower alkyl monosubstituted with lower cycloalkyl, phenyl, 4-(lower alkyl)phenyl, 4-(lower alkoxy)phenyl, 4-halophenyl, 3,4-methylenedioxyphenyl, 1-naphthyl, 2-naphthyl or
5 Het wherein Het is as defined in this claim; and R^2 is $\text{CCl}=\text{CH}_2$ or $\text{CBr}=\text{CH}_2$.

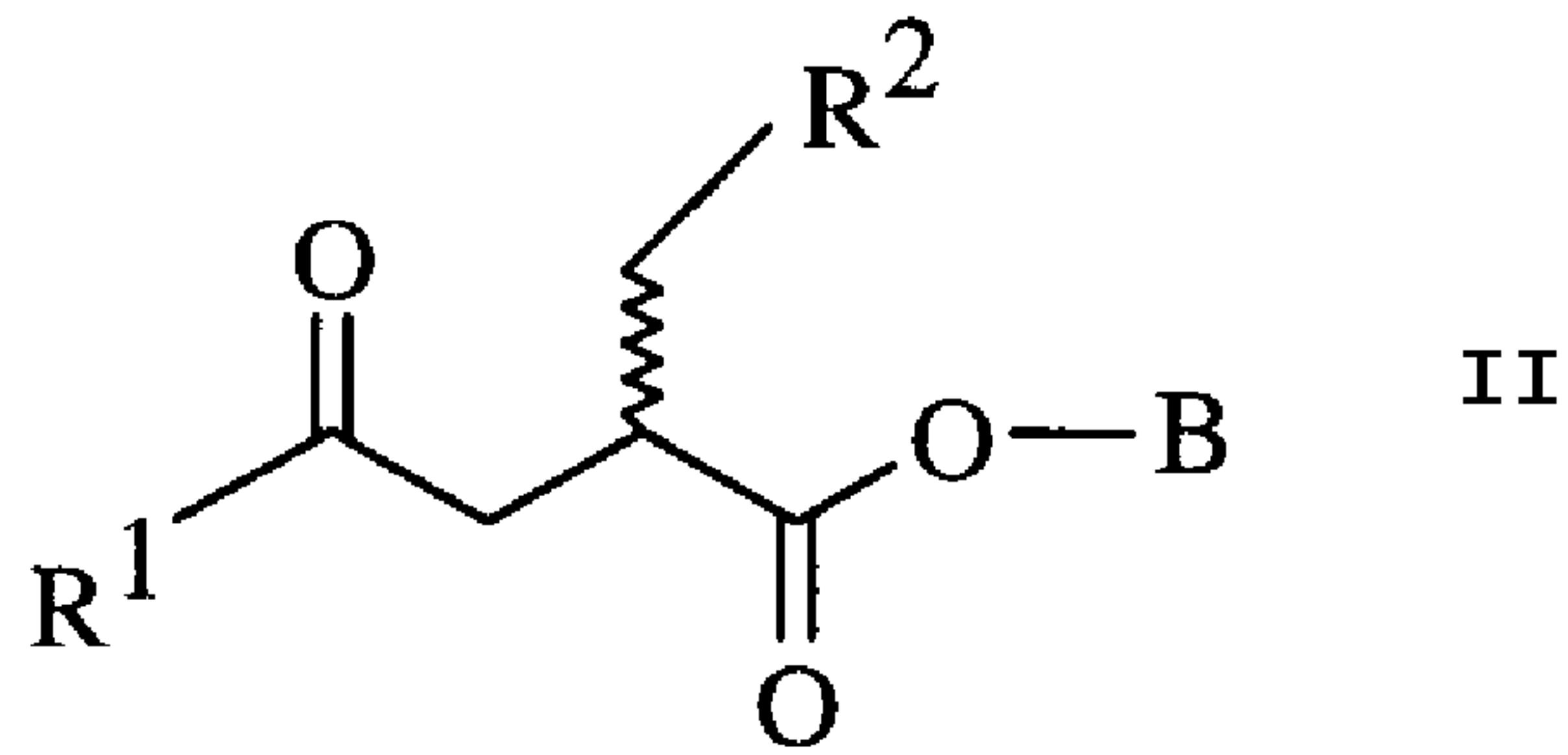
13. A compound as defined in claim 12 wherein R^1 is lower alkoxy, lower alkylamino, di(lower alkyl)amino
10 or R^1 is A-NR^3 , wherein A is $\text{R}^4\text{R}^5\text{NC(O)CH}_2$ wherein R^4 is methyl and R^5 is methyl, 2-(2-pyridinyl)ethyl or 2-(3-pyridinyl)ethyl; and R^3 is 1-ethylpropyl, cyclopentylmethyl, cyclohexylmethyl, 1(S)-cyclohexylethyl, cycloheptylmethyl, phenylmethyl,
15 1(S)-phenylethyl or (1-methylcyclohexyl)methyl.

14. A compound as defined in claim 12 wherein R^1 is 1,1-dimethylethoxy or the monovalent radical:





I



II