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(54) Title: METHODS AND PRECURSORS FOR THE PRODUCTION OF CHIRAL VICINAL AMINOALCOHOLS

(57) Abstract

The disclosure describes a method for the preparation of chiral vicinal aminoalcohols in high optical purity. The method combines the stereoselective reduction of the keto group of a β-ketoacid, β-ketoester, or derivative with the stereospecific rearrangement of the corresponding amide, hyroxamic acid, or hydrazide to produce chiral vicinal aminoalcohols with control of stereochemistry at both chiral centers. The method involves novel precursor compounds.

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METHODS AND PRECURSORS FOR THE PRODUCTION OF CHIRAL VICINAL AMINOALCOHOLS

Introduction: Field of the Invention

This invention relates to a method for the production of chiral vicinal aminoalcohols, and more specifically to the production of chemical compounds bearing both amino and alcohol functional groups on adjacent carbon atoms and which contain one or more chiral centers, as well as precursors useful in the method.

Background

Chiral vicinal aminoalcohols are important intermediates in the synthesis of various pharmaceutical products and product candidates, yet the preparation of these compounds remains a significant synthetic challenge to chemists. Gaining control over the stereochemistry of chiral centers at both the alcohol and amine (or in the simplest cases in which only the alcohol- or amine- bearing carbon is chiral, a single chiral center) at reasonable cost is the key to the successful production of these important chemical intermediates.

One example of such an intermediate is found in the compound SCH 56592, described in Tetrahedron Letters 37,5657 (1996) and references therein, hereby incorporated by reference. SCH 56592 is a potent antifungal compound. At the far right side of the molecule as depicted in the reference is found a chiral vicinal aminoalcohol moiety (3S,4S)-3-amino-4-hydroxypentane; this chiral vicinal aminoalcohol is a key part of SCH 56592 and critical to its biological activity. No efficient and cost effective route for its synthesis exists.

Another example of an important chiral vicinal aminoalcohol is found in the drug Crixivan, an HIV-protease inhibitor produced by Merck & Co. This compound is one of the most potent inhibitors of the AIDS virus yet discovered. A key intermediate in its synthesis is the chiral vicinal aminoalcohol (1S,2R)-1-amino-2-indanol (1997 Physicians' Desk Reference, Medical Economics Company, Montvale, NJ, pp. 1670-1673).

There are a number of other examples of important molecules which contain chiral aminoalcohols, including ephedrine, pseudoephedrine, norephedrine, pseudo-norephedrine, epinephrine, norepinephrine, isoserinol, isoleucinol, histidinol, 2-aminocyclopentanol, 2-aminocyclohexanol, and many others. Methods for the production of compounds which contain chiral aminoalcohol functionality tend to be specific for a given molecule or small group of related molecules. For example, several routes exist for the production of ephedrine (see, for example, Fodor, *Recent Develop. Chem. Nat. Carbon Compounds* **1**, 15-160 (1965)). However, these methods are not broadly generalizable to many other chiral vicinal aminoalcohols. The enzyme serine hydroxymethyltransferase can catalyze the production of certain chiral vicinal

aminoalcohols such as threonine and phenylserine, but only with severe structural limitations; there is an absolute requirement for glycine as a substrate, limiting carbon-1 to being only a carboxyl group. In addition, only certain aldehydes are accepted as substrates to condense with glycine. Furthermore, a mixture of stereoisomers is invariably obtained, making the production and recovery of highly pure chiral vicinal aminoalcohols difficult. (See C. Bull et al. in Biocatalytic *Production of Amino Acids and Derivatives*, D. Rozzell and F. Wagner, Eds., Hanser Publishers, Munich, (1992) pp. 255-256.) Often, classical resolution procedures are used due to the absence of any better method, resulting in the loss of 50% or more of the starting material (see, for example, Tullar, *J. Am. Chem. Soc.* **70**, 2067 (1948), which describes the resolution of D,L-epinephrine). A general method for the production of molecules of high optical purity incorporating a chiral vicinal aminoalcohol would facilitate the production of this important class of pharmaceutical intermediates and would be greatly desired.

Summary of the Invention

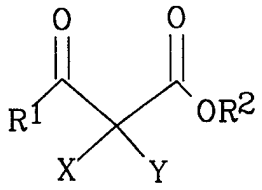
In one embodiment, the invention is directed to a method for producing a chiral vicinal aminoalcohol comprising:

(a) contacting a β-ketoester capable of being converted to a chiral vicinal aminoalcohol with a microorganism in the presence of (i) a carbon source or (ii) a dehydrogenase in combination with a nicotinamide cofactor, under conditions sufficient to permit the stereoselective reduction of the keto group to form a chiral ß-hydroxyester;

(b) converting the chiral ß-hydroxyester to a corresponding amide, hydroxamic acid, or hydrazide derivative, and

(c) exposing the amide, hydroxamic acid, or hydrazide derivative to conditions permitting stereospecific rearrangement to the corresponding chiral vicinal aminoalcohol. Preferably the method further comprises recovering the chiral vicinal aminoalcohol.

Preferably the β-ketoester has the formula 1:



(1)

wherein:

X is selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen-substituted alkyl, aryl, halogen-substituted aryl, benzyl, halogen-substituted benzyl, thiophenyl, halogen-substituted thiophenyl, hydroxy-substituted alkyl, hydroxy-substituted aryl, carboxy-substituted alkyl, carboxy-substituted alkyl, thio-substituted alkyl, thio-substituted aryl and heterocyclic;

Y is H;

R^1 is selected from the group consisting of alkyl, alkenyl, alkynyl, halogen-substituted alkyl, aryl, halogen-substituted aryl, benzyl, halogen-substituted benzyl, thiophenyl, halogen-substituted thiophenyl, hydroxy-substituted alkyl, hydroxy-substituted aryl, carboxy-substituted alkyl, carboxy-substituted aryl, thio-substituted alkyl, thio-substituted aryl, and heterocyclic; or wherein R^1, together with X and the carbon atoms to which they are attached, forms a ring; and

R^2 is selected from the group consisting of alkyl, alkenyl, alkynyl, halogen-substituted alkyl, aryl, halogen-substituted aryl, benzyl, halogen-substituted benzyl, thiophenyl, halogen-substituted thiophenyl, hydroxy-substituted alkyl, hydroxy-substituted aryl, carboxy-substituted alkyl, carboxy-substituted aryl, thio-substituted alkyl, thio-substituted aryl, and heterocyclic.

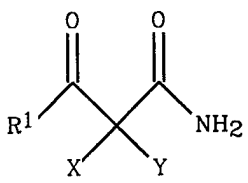
In another embodiment, the invention is directed to a method for producing a chiral vicinal aminoalcohol comprising:

a) contacting a ß-ketoamide capable of being converted to a chiral vicinal amino alcohol, a ß-ketohydroxamic acid capable of being converted to a chiral vicinal amino alcohol, or a ß-ketohydrazide capable of being converted to a chiral vicinal amino alcohol with a microorganism in the presence of (i) a carbon source or (ii) a dehydrogenase in combination with a nicotinamide cofactor, under conditions sufficient for stereoselective reduction of the keto group to form a ß-hydroxyamide, ß-hydroxyhydroxamic acid, or ß-hydroxyhydrazide; and

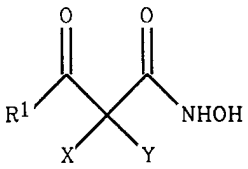
(b) exposing the ß-hydroxyamide, ß-hydroxyhydroxamic acid, or ß-hydroxyhydrazide to conditions sufficient to permit stereospecific rearrangement to the corresponding chiral vicinal

aminoalcohol.

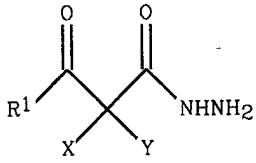
In the above-described method, preferably the ß-ketoamide has the formula 2, the ß-ketohydroxamic acid has the formula 3, and the ß-ketohydrazide has the formula 4:



(2)



(3)



(4)

wherein:

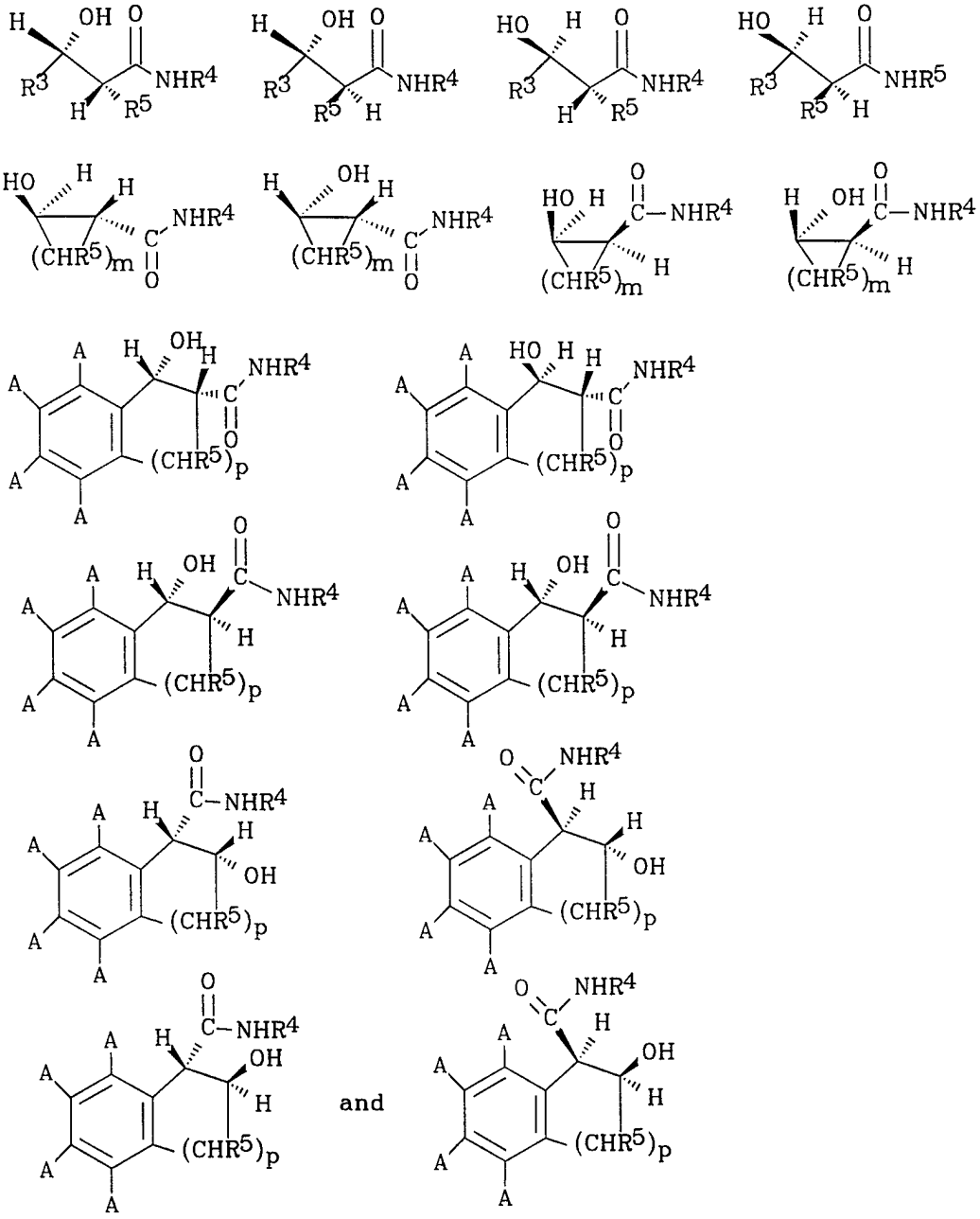
X is selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen-substituted alkyl, aryl, halogen-substituted aryl, benzyl, halogen-substituted benzyl, thiophenyl, halogen-substituted thiophenyl, hydroxy-substituted alkyl, hydroxy-substituted aryl, carboxy-substituted alkyl, carboxy-substituted alkyl, thio-substituted alkyl, thio-substituted aryl and heterocyclic;

Y is H; and

R^1 is selected from the group consisting of alkyl, alkenyl, alkynyl, halogen-substituted alkyl, aryl, halogen-substituted aryl, benzyl, halogen-substituted benzyl, thiophenyl, halogen-substituted thiophenyl, hydroxy-substituted alkyl, hydroxy-substituted aryl, carboxy-substituted alkyl, carboxy-substituted aryl, thio-substituted alkyl, thio-substituted aryl, and heterocyclic; or wherein R^1, together with X and the carbon atoms to which they are attached, forms a ring.

Preferably the above-methods further comprise recovering the chiral vicinal aminoalcohol.

In another embodiment, the invention is directed to a composition having one of the following formulae:



and

wherein:

R^3 is selected from alkyl, alkenyl, alkynyl, hydroxy-substituted alkyl, hydroxy-substituted alkenyl, halogen-substituted alkyl, halogen-substituted alkenyl, carboxy-substituted alkyl, thio-

substituted alkyl, thio-substituted alkenyl, aryl, halogen-substituted aryl, hydroxy-substituted aryl, carboxy-substituted aryl, thio-substituted aryl, benzyl, halogen-substituted benzyl, thiophenyl, halogen-substituted thiophenyl, and heterocyclic;

R^4 is selected from H, OH, and NH_2;

R^5 is selected from hydrogen, alkyl, alkenyl, alkynyl, hydroxy-substituted alkyl, hydroxy-substituted alkenyl, halogen-substituted alkyl, halogen-substituted alkenyl, carboxy-substituted alkyl, thio-substituted alkyl, thio-substituted alkenyl, aryl, halogen-substituted aryl, hydroxy-substituted aryl, carboxy-substituted aryl, thio-substituted aryl, benzyl, halogen-substituted benzyl, thiophenyl, halogen-substituted thiophenyl, and heterocyclic;

A is selected from H, F, Cl, Br, I, OH, OCH_3, alkyl, carboxy-substituted alkyl, hydroxy-substituted alkyl, halogen-substituted alkyl, aryl, carboxy-substituted aryl, hydroxy-substituted aryl, halogen-substituted aryl, and heterocyclic;

m is a number ranging from 1 to 6; and

p is a number ranging from 0 to 6, such that when p is 0, the compound contains a four-membered ring. Preferably p is 1 or 2 and m is 3 or 4. Preferably the composition comprises at least 75%, more preferably at least 90%, still more preferably at least 98%, of single stereoisomer.

Detailed Description of the Invention

This invention is directed to an efficient method for the production of chiral vicinal aminoalcohols. An important aspect of this invention is the generality with which this method described herein may be employed to produce a range of chiral vicinal aminoalcohols, both cyclic and acyclic, with the ability to produce any of the 4 possible stereoisomers in high stereochemical purity.

This invention is also directed to key intermediates useful for the production of chiral vicinal aminoalcohols. An important aspect of this invention is the generality with which these intermediates described herein may be employed to produce a range of chiral vicinal aminoalcohols, both cyclic and acyclic, with the ability to produce any of the 4 possible stereoisomers in high stereochemical purity.

Central to this invention is the novel combination of two steps, each of which proceeds with a well-defined and controllable stereochemical outcome. The first step is the stereoselective reduction of the keto group of a ß-ketoacid, ß-ketoester, ß-ketocarboxamide, ß-ketocarboxylic hydroxamic acid, or ß-ketocarboxylic hydrazide (and, in the case of the ß-ketoester, conversion to the corresponding carboxamide, hydroxamic acid or hydrazide derivative); this reaction provides for control of stereochemistry at both the C-2 and C-3 positions of the ß-ketoester or derivative, producing a product having two chiral centers. Stereoselective reduction of the ß-ketoacid or derivative is effected by any of a range of microorganisms which are able to reduce

carbonyl groups in the presence of a carbon source such as glucose or other carbohydrates. This reaction may be carried out to generate a single diastereomer of the four possibilities in high optical purity, depending on the choice of organism or enzyme for the reduction. In the case of β-ketoacid or β-ketoester, the chiral β-hydroxyacid or β-hydroxyester is converted to a corresponding amide, hydrazide, or hydroxamic acid. The second step is the stereospecific rearrangement of the resulting β-hydroxycarboxamide, β-hydroxyhydrazide, or β-hydroxy hydroxamic acid to the corresponding aminoalcohol, resulting in a chiral vicinal aminoalcohol with control of stereochemistry at one or two chiral centers. When the original β-ketoacid or β-ketoester is substituted in the 2-position, two chiral centers are generated in the resulting chiral vicinal aminoalcohol. This rearrangement occurs with retention of stereochemistry at the carbon bearing the carbonyl group.

The ß-ketoacid or its derivative may be derived from an inexpensive precursor such as ethyl acetoacetate or another acetoacetic ester, or the esters of related ß-ketoacids such as a 2-alkyl substituted acetoacetate, cyclohexanone-2-carboxylate, cyclopentanone-2-carboxylate, 1-indanone-2-carboxylate, 2-indanone-1-carboxylate, 1-tetralone-2-carboxylate, 2-tetralone-1-carboxylate, and the like. Both simple ß-ketoesters such as esters of acetoacetate, 3-ketopentanoate, 4-phenylacetoacetate and various 2-alkyl-substituted ß-ketoesters may be reduced with control of stereochemistry at, depending on the starting material, either the 3-position or both the 2 and 3 positions. Substituents which may be present at the 2-position in the practice of this invention include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, isopentyl, hexyl, octyl, vinyl, propargyl, allyl, thiophenyl, thioalkyl, phenyl, benzyl, furoyl, imidazoyl, carboxymethyl, carboxyethyl, halomethyl, haloethyl, halopropyl, phosphoalkyl, and the like. Cyclic ß-ketoacid derivatives, which upon reduction give rise to two chiral centers, are also useful intermediates for the production of chiral vicinal aminoalcohols. Useful cyclic ß-ketoacid derivatives include cyclohexanone-2-carboxylate, cyclopentanone-2-carboxylate, 1-indanone-2-carboxylate, 2-indanone-1-carboxylate, 1-tetralone-2-carboxylate, 2-tetralone-1-carboxylate, and the like.

Conversion of the chiral ß-hydroxyester or ß-hydroxy acid to the corresponding amide, hydroxamic acid or hydrazide derivative may be accomplished by straightforward chemical methods well-known to those skilled in the art. For example, heating of a chiral ß-hydroxyester with ammonia, hydrazine, or hydroxylamine in ethanol produces the corresponding amide, hydrazide, or hydroxamic acid in high yield. Alternatively, conversion of the ester to the amide, hydroxamic acid or hydrazide may be accomplished by enzymatic catalysis. Esterase, lipase, protease, and amidase enzymes, which can catalyze the hydrolysis of esters in the presence of water, will catalyze conversion of the ester to the amide, hydroxamic acid or hydrazide when ammonia, hydroxylamine or hydrazine are present as nucleophiles. The enzymatic conversion

has the added advantage that it often can be carried out under very mild conditions (e.g. ambient temperature and pressure). Further, the enzyme can provide additional stereoselection in the conversion of the ester to its corresponding amide, hydroxamic acid or hydrazide derivative, further improving the enantiopurity of the final product in cases where this is desired.

As a further embodiment of this invention, the ß-ketoacid or ß-ketoester may be first converted into its amide, hydroxamic acid, or hydrazide derivative, and the stereoselective reduction carried out directly on the corresponding amide, hydroxamic acid, or hydrazide derivative to produce the optically-active, chiral ß-hydroxycarboxamide, ß-hydroxy hydrazide, or ß-hydroxy hydroxamic acid. For example, acetoacetamide or a 2-substituted acetoacetamide may be subjected to the stereoselective reduction by a microorganism or a dehydrogenase, followed by the Hofmann rearrangement on the 2-substituted-3-hydroxybutyramide to give the chiral vicinal aminoalcohol. Similarly, the hydroxamic acid or hydrazide derivatives of a 2-substituted (or unsubstituted) ß-ketoacid may be stereoselectively reduced and then converted to the desired chiral vicinal aminoalcohol using the Lossen or Curtius rearrangements, respectively.

Stereoselective reduction may be conveniently carried out using whole cells or isolated enzymes. In the case of whole cells, organisms useful in the practice of this invention are described in Preparative Biotransformations (S.M. Roberts, editor), Chapter 2, John Wiley & Sons, Chichester, U.K. (1996)and references therein; D. Buisson and R Azerad, Tetrahedron Lett., (1986) 27, 2631, and references therein; S. Servi, *Synthesis*, 1 (1990) and references therein; D. Seebach et al., *Organic Synthesis* **63**, 1 (1985) and references therein; D. W. Brooks and K. W. Woods, *J. Org. Citent.* **52**, 2036 (1987) and references therein; A. Fauve and H Veschambre, *J. Org. Chem.* **53**, 5215 (1988) and references therein; Bucciarelli et al., *J. Chem. Soc. Citent. Comnt.*, 456 (1978) and references therein; K. Kieslich in *Biotransformations*, Eds. H. J. Rehm and G. R. Reed, volume 6a, VCH, Weinheim (1984) and references therein; all hereby incorporated by reference; and include *Saccharomyces cerevisiae, Geotrichum candidum, Colletorichum gloeosporioides, Rhizopus arrhizus, Aspergillus niger, Mortierella isabellina*, and other microorganisms.

It is also possible to use isolated dehydrogenase enzymes, either as crude, partially purified, or pure preparations in the practice of this invention. Dehydrogenases useful in the practice of this invention may be isolated and purified, if desired, from microorganisms capable of effecting the stereoselective reduction. The purification of the dehydrogenase enzymes may be accomplished by techniques well known to those skilled in the art. Some examples of purification methods for enzymes may be found in *Methods in Enzymology*, **22** (1971) and references therein, hereby incorporated by reference. In the case of isolated enzymes, the nicotinamide cofactor is recycled using any of a number of recycling schemes known in the prior art [See, for example, Preparative *Biotransformations* (S.M. Roberts, editor), 3.1.13.1.6, John

Wiley & Sons, Chichester, U.K. (1996) and references therein; Z. Shaked and G. M. Whitesides, *J. Am. Chem. Soc.* **102**, 7104-5 (1980) and references therein; *J. B. Jones and T. Takamura, Can. J. Chem.* **62**, 77 (1984); all hereby incorporated by reference.] These enzymes may be used in solution or, if desired, as immobilized enzymes in accord with the practice of this invention. A number of methods of immobilization for both whole cells containing enzymes and for isolated enzymes are known in the prior art and may be used in the practice of this invention. One example of an immobilized enzyme system is described by Weetall et al., *Methods in Enzymology* **34**, 59-72 (1974) which is hereby incorporated by reference. In this method enzymes may be immobilized on a porous glass or ceramic support which has been activated with glutaraldehyde. Other methods for immobilization of both cells and enzymes which may be used in the practice of this invention are described in *Methods in Enzymology* **44** (1976), K. Mosbach editor, *Immobilization of Enzymes and Cells,* Gordon F. Bickerstaff, ed., Humana Press, Totowa, NJ (1997) and in *Biocatalytic Production of Amino Acids and Derivatives,* D. Rozzell and F. Wagner, Eds., Hanser Publishers, Munich, (1992) pp. 279-319.

An important application of the optically-active, chiral β-hydroxycarboxamide, β-hydroxyhydrazide, and β-hydroxyhydroxamic acid compounds, which are the subject of this invention, derives from their ability to be converted into optically-active chiral vicinal aminoalcohols. For example, the chiral β-hydroxycarboxamide may be converted into a chiral vicinal aminoalcohol via the Hofmann rearrangement. Similarly, the β-hydroxyhydrazide and β-hydroxyhydroxamic acid may be converted to the corresponding chiral vicinal aminoalcohols using the Curtius or Lossen rearrangements, respectively.

The stereospecific rearrangement may be carried out on the carboxamide via the Hofmann-type rearrangement [E. S. Wallis and J. F. Lane, *Organic Reactions* **III**, 267 (1949) and references therein; P. A. S. Smith, *Trans. N.Y. Acad. Sci.* **31**, 504 (1969) and references therein; S. Simons, *J. Org Chem.* **38**, 414 91973) and references therein; W. L. F. Armarego et al, *J. Chem. Soc. Perkin Trans.* **I**, 2229 (1976) and references therein; all hereby incorporated by reference]; on the hydroxamic acid via the Lossen rearrangement [S. Bittner et al (*Tet. Lett.* **23**, 1965-8 (1974) and references therein; L. Bauer and O. Exner, *Angew. Chem. Int. Ed.* **13**, 376 (1974) and references therein; all hereby incorporated by reference]; or on the hydrazide via the Curtius rearrangement [P. A. S. Smith, *Organic Reactions* **III**, 337 (1946) and references therein; J. H. Saunders and R. I. Slocombe, *Chem. Rev.* **43**, 205 (1948) and references therein; D. V. Banthorpe in *The Chemistry of the Azido Group,* S. Patai Ed., Interscience, New York, 1971, pp. 397-405 and references therein; J. D. Warren and J. D. Press, *Synth. Comm.* **10**, 107 (1980) and references therein; all hereby incorporated by reference].

As an example of the practice of this invention, (3S,4S)-3-amino-4-hydroxypentane, the key component of SCH 56592, may be produced either from the precursor (2S,3S)-2-ethyl-3-

hydroxybutyric hydrazide via the Curtius rearrangement, the precursor (2S,3S)-2-ethyl-3-hydroxybutyric hydroxamic acid via the Lossen rearrangement, or the precursor (2S,3S)-2-ethyl-3-hydroxybutyramide via the Hofmann rearrangement. The hydrazide, hydroxamic acid, or amide compounds permit efficient and cost effective synthesis of the corresponding chiral vicinal aminoalcohol. The desired chiral vicinal aminoalcohol is produced from the following simple and inexpensive chemical building blocks: ethyl 2-ethylacetoacetate (produced from ethyl acetoacetate and ethyl bromide), ammonia (or hydroxylamine or hydrazine), and bromine (or benzoyl chloride or sodium nitrite).

Similarly, the chiral vicinal aminoalcohol (1S,2R)-l-amino-2-indanol, a key intermediate in the production of the HIV-inhibitor Crixivan, may be produced from the hydrazide of (1S,2R)-l-carboxy-2-hydroxyindane by reaction with a solution of sodium nitrite in 5% H_2S0_4 via the Curtius rearrangement.

The following example is illustrative of the straightforward application of this invention. Ethyl 2-ethylacetoacetate is added to a culture of *Colletorichum gloeosporioides* ATCC 16330, which had been cultivated on glucose as a carbon source. After 48 hours of agitation, the culture broth is filtered, extracted with ethyl acetate, and evaporated to leave (2S,3S)-ethyl 2-ethyl-3-hydroxybutyrate as a yellowish oil. This product is warmed with hydrazine in ethanol to produce the hydrazide derivative. The resulting hydrazide is isolated and treated with sodium nitrite in 5% sulfuric acid to produce (2S,3S)-2-amino-3-hydroxypentane via the Curtius rearrangement. The aminoalcohol may be recovered conveniently by extraction into an organic solvent such as ethyl acetate or methyl t-butyl ether after basification of the reaction mixture.

In cases where the alcohol is the only chiral center, the sequence is similarly effective, maintaining complete control over the chirality of the alcohol after stereospecific reduction through the rearrangement of the amide, hydrazide, or hydroxamic acid.

The invention will now be further illustrated by the following examples which are given here for illustrative purposes only and are not intended to limit the scope of the invention.

Example 1

Production of ethyl (2R,3S)-2-ethyl-3-hydroxybutyrate

Twenty grams of bakers' yeast (Sigma Chemical Company, *Saccharomyces cerevisiae,* type II) are suspended in a solution of 30 grams of sucrose in water in a conical flask, and the mixture was placed in an orbital shaker chamber maintained at 220 rpm and 30°C for 30 minutes to initiate fermentation. Two grams of ethyl 2-ethyl acetoacetate is dissolved in 2 ml of 95% ethanol, the resulting solution was added to the fermenting yeast, and shaking was resumed. The reaction is followed by TLC (staining with phosphomolybdic acid in ethanolic sulfuric acid) to

monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction was terminated by removing the flask from the shaker and adding 20-30 grams of Celite to the reaction mixture. The resulting suspension is suction filtered through a pad of Celite, and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.6 grams of a yellowish oil containing ethyl (2R,3S)-2-methyl-3-hydroxybutyrate as the major product (80%) and ethyl (2S,3S)-2-methyl-3-hydroxybutyrate (20%) as the minor product as judged by chiral chromatography.

Example 2

Production of octyl (2R,3S)-2-ethyl-3-hydroxybutyrate

Twenty grams of bakers' yeast (Sigma Chemical Company, type II) are suspended in an aqueous solution containing 30 grams of sucrose in a conical flask, and the mixture is placed on an orbital shaker (220 rpm) at 30°C for 30 minutes to initiate fermentation. Two grams of octyl 2-ethyl acetoacetate is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the fermenting yeast, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction was judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.8 grams of octyl (2R,3S)-2-ethyl-3-hydroxybutyrate as a yellowish oil (>96% enantiomeric excess as judged by chiral chromatography).

Example 3

Production of (2R,3S)-ethyl 2-allyl-3-hydroxybutyrate

Twenty grams of bakers' yeast (Sigma Chemical Company, type II) are suspended in an aqueous solution containing 30 grams of sucrose in a conical flask, and the mixture is placed on an orbital shaker (220 rpm) at 30 °C for 30 minutes to initiate fermentation. Two grams of ethyl 20-methyl acetoacetate is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the fermenting yeast, and shaking was resumed. The reaction was followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is

terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate was extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.6 grams of a yellow oil containing (2R,3S)-2-ethyl-3-hydroxybutyrate as the major product (75%) and (2S,3S)-2-ethyl-3-hydroxybutyrate (25%) as the minor product as judged by chiral chromatography.

Example 4

Production of ethyl (2S,3S)-2-ethyl-3-hydroxybutyrate

Colletotrichum gloeosporioides (MMP 3233) is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4 (1 gram), K2HPO4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), FeSO4.7H20 (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of ethyl 2-ethyl acetoacetate are dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking was resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.7 grams of (2S,3S)-2-ethyl-3-hydroxybutyrate as a yellow oil. The chiral purity of the product is greater than 98% as judged by chiral chromatography.

Example 5

Alternative production of ethyl (2S,3S)-2-ethyl-3-hydroxybutyrate

Rhizopus arrhizus (ATCC 11145) is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4 (1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KC1 (0.5 gram) with rotary shaking at 25°C. Two grams of ethyl 2-ethyl acetoacetate are dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction was followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the

reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.6 grams of (2S,3S)-2-ethyl-3-hydroxybutyrate as a yellow oil. The chiral purity of the product is shown to be greater than 98% as judged by chiral chromatography.

Example 6

Alternative production of ethyl (2S,3S)-2-ethyl-3-hydroxybutyrate

Two grams of ethyl 2-ethyl acetoacetate are dissolved in 2 ml of 95% ethanol, and the resulting solution is added to a solution of alcohol dehydrogenase (500 units from *Rhizopus arrhizus* (ATCC 11145) containing potassium phosphate buffer, 100 mM, pH 7.0. NAD+ (100 mg) is added to the solution along with 1 gram of sodium formate and 100 units of formate dehydrogenase (Boehringer Mannhelm) for recycling of the NAD+ cofactor. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction was terminated by removing from the shaker. The resulting solution is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.8 grams of (2S,3S)-2-ethyl-3-hydroxybutyrate as a light yellow oil. The chiral purity of the product is greater than 99% as judged by chiral chromatography.

Example 7

Production of (1S,2R)-ethyl 2-hydroxycyclopentanecarboxylate

Twenty-five grams of bakers' yeast (*Saccharomyces cerevisiae*, Sigma Chemical Company, type II) are suspended in 100 ml of sterilized tap water in a conical flask, and the mixture is placed on an orbital shaker (220 rpm) at 30°C for 1 hour to activate the yeast. One gram of ethyl 2-oxocyclopentanecarboxylate is added, shaking is resumed, and progress of the reaction is monitored by TLC (staining with anisaldehyde). After approximately 100 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with diethyl ether (4 x 100 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.7 grams of octyl (1R,2S)-ethyl 2-hydroxycyclopentanecarboxylate as a yellowish oil (70% yield).

Example 8

Production of (1R,2S)-ethyl 2-hydroxycyclohexanecarboxylate

Twenty-five grams of bakers' yeast (*Saccharomyces cerevisiae*, Sigma Chemical Company, type II) are suspended in 100 m of sterilized tap water in a conical flask, and the mixture is placed on an orbital shaker (220 rpm) at 30°C for 1 hour to activate the yeast. One gram of ethyl 2-oxocyclohexanecarboxylate is added, shaking is resumed, and progress of the reaction is monitored by TLC (staining with anisaldehyde). After approximately 100 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with diethyl ether (4 x 100 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.6 grams of octyl (1R,2S)-ethyl 2-hydroxycyclohexanecarboxylate as a yellowish oil (60% yield).

Example 9

Production of (1S,2S)-ethyl 2-hydroxycyclopentanecarboxylate

Geotrichum candidum (ATCC 34614) is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4 (1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of ethyl 2-oxocyclopentanecarboxylate are dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.5 grams of (1S,2S)-2-hydroxycyclopentanecarboxylate as a yellow oil. The chiral purity of the product is greater than 99% as judged by chiral chromatography.

Example 10

Production of (1S,2S)-ethyl 2-hydroxycyclohexanecarboxylate

Geotrichum candidum (ATCC 34614) is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4 (1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KC1 (0.5 gram) with rotary shaking at 25°C. Two grams of ethyl 2-oxocyclohexanecarboxylate are dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.4 grams of (1S,2S)-ethyl 2-hydroxycyclohexanecarboxylate as a yellow oil. The chiral purity of the product is greater than 99% as judged by chiral chromatography.

Example 11

Production of ethyl 3(S)-hydroxybutyrate

Twenty grams of bakers' yeast (Sigma Chemical Company, type II) are suspended in an aqueous solution containing 30 grams of sucrose in a conical flask, and the mixture is placed on an orbital shaker (220 rpm) at 30°C for 30 minutes to initiate fermentation. Two grams of ethyl acetoacetate are dissolved in 2 ml of 95% ethanol, the resulting solution was added to the fermenting yeast, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.5 grams of a light yellow oil containing ethyl 3(S)-hydroxybutyrate as the major product as judged by chiral chromatography.

Example 12

Production of (R)-ethyl 3-hydroxybutyrate

Geotrichum candidum (ATCC 34614) is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4 (1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KC1 (0.5 gram) with rotary shaking at 25°C. Two grams of ethyl acetoacetate are dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.4 grams of (R)-ethyl 3-hydroxybutyrate as a yellow oil.

Example 13

Production of (2S,3S)-2 ethyl-3-hydroxybutyramide by microbial reduction of the corresponding 2-ethylacetoacetamide

Geotrichum candidum (ATCC 34614) is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4 (1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KC1 (0.5 gram) with rotary shaking at 25°C. Two grams of 2-ethyl-3-ketobutyramide are dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After 48-72 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1 gram of (2S,3S)-2 ethyl-3-hydroxybutyramide as a yellowish solid.

Example 14

Production of S-3 hydroxybutyramide

Twenty grams of bakers' yeast (Sigma Chemical Company, type II) are suspended in an aqueous solution containing 30 grams of sucrose in a conical flask, and the mixture is placed on an orbital shaker (220 rpm) at 30°C for 30 minutes to initiate fermentation. Two grams of acetoacetamide are dissolved in 2 ml of 95% ethanol, the resulting solution was added to the fermenting yeast, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.4 grams of (S)-3-hydroxybutyramide as a light yellow solid.

Example 15

Production of the hydroxamic acid of (2S,3S)-2 ethyl-3-hydroxybutyrate

(2S,3S)-Ethyl 2 ethyl-3-hydroxybutyrate (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of hydroxylamine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydroxylamine is removed by extraction with 1% HCl, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.8 grams of the hydroxamic acid derivative of (2S,3S)-2 ethyl-3-hydroxybutyrate.

Example 16

Enzymatic production of the hydroxamic acid of (2S,3S)-2 ethyl-3-hydroxybutyrate

(2S,3S)-Ethyl 2 ethyl-3-hydroxybutyrate (1 gram) is dissolved in 5 ml of t-butyl methyl ether, followed by the addition of 0.5 gram of hydroxylamine. Lipase from *Candida rugosa* (0.5 g, Sigma L1754) is added, and the progress of the reaction was followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydroxylamine is removed by extraction with 1% HCl, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.8 grams of the hydroxamic acid derivative of (2S,3S)-2 ethyl-3-hydroxybutyrate.

Example 17

Alternative production of the hydroxamic acid of (2S,3S)-2 ethyl-3-hydroxybutyrate by microbial reduction of the corresponding hydroxamic acid of 2-ethylacetoacetate

Geotrichum candidum (ATCC 34614) is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4 (1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KC1 (0.5 gram) with rotary shaking at 25°C. Two grams of 2-ethylacetoacetate hydroxamic acid, produced by the reaction of ethyl acetoacetate with hydroxylamine, are dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After 48-72 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1 gram of (2S,3S)-2 ethyl-3-hydroxybutyrate hydroxamic acid as a yellowish solid.

Example 18

Conversion of (2S,3S)-ethyl 2-ethyl-3-hydroxybutyrate to the hydrazide derivative

(2S,3S)-Ethyl 2 ethyl-3-hydroxybutyrate (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of hydrazine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydrazine is removed by extraction with 1% HCl, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.9 grams of the hydrazide of (2S,3S)2 ethyl-3-hydroxybutyrate.

Example 19

Microbial production of the hydrazide of (2S,3S)-2 ethyl-3-hydroxybutyrate by stereospecific reduction of 2-ethylacetoacetate hydrazide

Geotrichum candidum (ATCC 34614) is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a

medium of glucose (30 grams), KH_2PO_4 (1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KC1 (0.5 gram) with rotary shaking at 25°C. Two grams of 2-ethylacetoacetate hydrazide, produced by the reaction of ethyl 2-ethylacetoacetate with hydrazine, are dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After 48-72 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1 gram of (2S,3S)-2 ethyl-3-hydroxybutyrate hydrazide as a yellowish solid.

Example 20

Conversion of (2S,3S)-ethyl 2-ethyl-3-hydroxybutyrate to the amide derivative

(2S,3S)-Ethyl 2 ethyl-3-hydroxybutyrate (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of gaseous ammonia. The solution is kept in a stoppered flask, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Ammonia is removed by extraction with 1% HCl, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.7 grams of (2S,3S)-2 ethyl-3-hydroxybutyramide.

Example 21

Production of (2R,3S)-2-amino-3-hydroxybutane by Hofmann Reaction

Ten grams of (2R,3S)-2-methyl-3 hydroxybutyramide are dissolved in 250 ml of 0.03 M NaOH and added slowly to a solution 25 grams of bromine in 300 ml of 0.03 M NaOH. The mixture is warmed with stirring until the reddish brown color disappeared. The solution is then cooled, extracted with methyl t-butyl ether x 250 ml), and the extracts dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. The product (2R,3S)-2-amino-3 hydroxy-butane is isolated as a light yellow oil.

Example 22

Production of (2R,3S)-2-amino-3-hydroxybutane by Lossen Rearrangement

Ten grams of (2R,3S)-2-methyl-3 hydroxy-butyrohydroxamic acid are reacted with benzoyl chloride under Schotten-Bauman conditions, followed by warming to reflux. Reaction progress is monitored by thin layer chromatography. The solution is then cooled to room temperature, extracted with methyl t-butyl ether x 250 ml), and the extracts dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. The product (2R,3S)-2-amino-3 hydroxy-butane is isolated as a light yellow oil.

Example 23

Production of (3S,4S)-3-amino-4-hydroxypentane by Lossen Rearrangement

Ten grams of (2S,2S)-2-methyl-3-hydroxypentanohydroxamic acid is reacted with benzoyl chloride under Schotten-Bauman conditions, followed by warming to reflux. Reaction progress is monitored by thin layer chromatography. The solution is then cooled to room temperature, extracted with methyl t-butyl ether x 250 ml), and the extracts dried over $MgSO_4$, filtered, and the solvent removed by rotary evaporation. The product (3S,4S)-3-amino-4-hydroxypentane is isolated as a light yellow oil.

Example 24

Production of (3S,4S)-3-amino-4-hydroxypentane by a modified Lossen Rearrangement

Ten grams of (2S,3S)-2-methyl-3-hydroxypentanohydroxamic acid is reacted with equimolar amounts of diethyl azodicarboxylate and triphenylphosphine in tetrahydrofuran at room temperature using the procedure of Bittner, Grinberg and Kartoon (Tet. Lett. 23, 1965-8 (1974)). Reaction takes place rapidly to produce the product (3S,4S)-3-amino-4-hydroxypentane. The product is isolated by acidification and extraction of the reaction mixture with ethyl acetate, followed by basification of the resulting aqueous solution with NaOH, extraction with methyl t-butyl ether, drying of the extracts over $MgSO_4$, filtration, and the removal of solvent by rotary evaporation. The product (3S,4S)-3-amino-4-hydroxypentane is isolated as a light yellow oil.

Example 25

Production of (2S,3S)-2-amino-3-hydroxybutane

Five grams of (2S,3S)-2-methyl-3-hydroxybutyrate hydrazide are reacted with a solution of 5 grams of sodium nitrite in 100 ml of 5% H_2SO_4. Reaction takes place rapidly to produce the product (2S,3S)-2-amino-3-hydroxybutane. The product is isolated by acidification and extraction of the reaction mixture with ethyl acetate, followed by basification of the resulting aqueous solution with NaOH, extraction with methyl t-butyl ether, drying of the extracts over $MgSO_4$, filtration, and the removal of solvent by rotary evaporation. The product (2S,3S)-2-amino-3-hydroxybutane is isolated as a light yellow oil.

Example 26

Production of (1S,2S)-l-hydroxy-2-carboethoxyindane

Colletorichum gloeospiorioides is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4 (1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_2O$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_2O$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25 ^{o}C. Two grams of 2-carboethoxy-1-indanone is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.7 grams of (1S,2S)-1-hydroxy-2-carboethoxyindane as a yellow oil. The chiral purity of the product is greater than 98% as judged by chiral chromatography.

Example 27

Production of (1S,2S)-l-hydroxy-2-carboethoxy-5 fluoroindane

Colletorichum gloeosporioides is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4 (1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with

rotary shaking at 25°C. Two grams of 5-fluoro-2-carboethoxy-l-indanone is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite, and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.7 grams of (1S,2S)-l-hydroxy-2-carboethoxy-5-fluoroindane as a yellow oil. The chiral purity of the product is greater than 98% as judged by chiral chromatography.

Example 28

Production of (1S,2S)-l-hydroxy-2-carboethoxy-5-methoxyindane

Colletorichum gloeosporioides is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4(1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of 5-methoxy-2-carboethoxy-l-indanone is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.7 grams of (1S,2S)-l-hydroxy-2-carboethoxy-5-methoxyindane as a yellow oil. The chiral purity of the product is greater than 98% as judged by chiral chromatography.

Example 29

Alternative production of (1S,2S)-l-hydroxy-2-carboethoxyindane

Rhizopus arrhizus (ATCC 11145) is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4(1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5

gram) with rotary shaking at 25°C. Two grams of 2-carboethoxy-1-indanone is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.6 grams of (1S,2S)-1-hydroxy-2-carboethoxyindane as a yellow oil. The chiral purity of the product is shown to be greater than 95% as judged by chiral chromatography.

Example 30

Production of (1S,2R)-l-hydroxy-2-carboethoxyindane

Twenty grams of bakers' yeast (Sigma Chemical Company, type II) are suspended in an aqueous solution containing 30 grams of sucrose in a conical flask, and the mixture is placed on an orbital shaker (220 rpm) at 30°C for 30 minutes to initiate fermentation. Two grams of 2-carboethoxy-l-indanone is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the fermenting yeast, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.8 grams of a yellowish oil containing (1S,2R)-l-hydroxy-2-carboethoxyindane (>95% enantiomeric excess as judged by chiral chromatography).

Example 31

Production of (1S,2R)-l-hydroxy-2-carboethoxy-5-fluoroindane

Twenty grams of bakers' yeast (Sigma Chemical Company, type II) are suspended in an aqueous solution containing 30 grams of sucrose in a conical flask, and the mixture is placed on an orbital shaker (220 rpm) at 30°C for 30 minutes to initiate fermentation. Two grams of 5-fluoro-2-carboethoxy-l-indanone are dissolved in 2 ml of 95% ethanol, the resulting solution is added to the fermenting yeast, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production

of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite, and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave a yellowish oil containing 1.5 grams of (1S,2R)-l-hydroxy-2-carboethoxy-5-fluoroindane (>95% enantiomeric excess as judged by chiral chromatography).

Example 32

Production of (1S,2R)-l-hydroxy-2-carboethoxy-5-hydroxyindane

Twenty grams of bakers' yeast (Sigma Chemical Company, type II) are suspended in an aqueous solution containing 30 grams of sucrose in a conical flask, and the mixture is placed on an orbital shaker (220 rpm) at 30°C for 30 minutes to initiate fermentation. Two grams of 5-hydroxy-2-carboethoxy-l-indanone are dissolved in 2 ml of 95% ethanol, the resulting solution is added to the fermenting yeast, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite, and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.5 grams of (1S,2R)-l-hydroxy-2-carboethoxy-5-hydroxyindane as a yellowish oil (>95% enantiomeric excess as judged by chiral chromatography).

Example 33

Production of (1S,2R)-l-hydroxy-2-carboethoxy-5-carboxyethylindane

Twenty grams of bakers' yeast (Sigma Chemical Company, type II) are suspended in an aqueous solution containing 30 grams of sucrose in a conical flask, and the mixture is placed on an orbital shaker (220 rpm) at 30°C for 30 minutes to initiate fermentation. Two grams of 5-carboxyethyl-2-carboethoxy-l-indanone is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the fermenting yeast, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite, and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered,

and rotary evaporated to leave 1.5 grams of (1S,2R)-l-hydroxy-2-carboethoxy-5-carboxyethylindane as a yellowish oil (>95% enantiomeric excess as judged by chiral chromatography).

Example 34

Conversion of (1S,2S)-l-hydroxy-2-carboethoxyindane to the hydrazide derivative

(1S,2S)-l-hydroxy-2-carboethoxyindane (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of hydrazine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydrazine is removed by extraction with 1% HCl, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.9 grams of the hydrazide of (1S,2S)-l-hydroxy-2-carboxyindane.

Example 35

Conversion of (1S,2S)-l-hydroxy-2-carboethoxy-5-fluoroindane to the hydrazide derivative

(1S,2S)-l-hydroxy-2-carboethoxy-5-fluoroindane (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of hydrazine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydrazine is removed by extraction with 1% HCl, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.9 grams of the hydrazide of (1S,2S)-l-hydroxy-2-carboxy-5-fluoroindane.

Example 36

Conversion of (1S,2S)-l-hydroxy-2-carboethoxy-5-chloroindane to the hydrazide derivative

(1S,2S)-l-hydroxy-2-carboethoxy-5-chloroindane (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of hydrazine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydrazine is removed by extraction with 1% HCl, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.9 grams of the hydrazide of (1S,2S)-l-hydroxy-2-carboxy-5-chloroindane.

Example 37

Conversion of (1S,2S)-l-hydroxy-2-carboethoxy-4,5,6,7-tetrafluoroindane to the hydrazide derivative

(1S,2S)-l-hydroxy-2-carboethoxy-4,5,6,7-tetrafluoroindane (1 gram) is dissolved in 5 ml of absolute ethanol followed by the addition of 0.5 gram of hydrazine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydrazine is removed by extraction with 1% HC1, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.9 grams of the hydrazide of (1S,2S)-l-hydroxy-2-carboxy-4,5,6,7-tetrafluoroindane.

Example 38

Conversion of (1S,2S)-l-hydroxy-2-carboethoxy-5-hydroxyindane to the hydrazide derivative

(1S,2S)-l-hydroxy-2-carboethoxy-5-hydroxyindane (1 gram) is dissolved in 5 ml of absolute ethanol followed by the addition of 0.5 gram of hydrazine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydrazine is removed by extraction with 1% HCl, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.9 grams of the hydrazide of (1S,2S)-l-hydroxy-2-carboxy-5-hydroxyindane.

Example 39

Conversion of (1S,2S)-t-hydroxy-2-carboethoxy-5-methoxyindane to the hydrazide derivative

(1S,2S)-l-hydroxy-2-carboethoxy-6-methoxytetralin (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of hydrazine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydrazine is removed by extraction with 1% HC1, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.9 grams of the hydrazide of (1S,2S)-l-hydroxy-2-carboxy-5-methoxyindane.

Example 40

Conversion of (lS,2R)-l-hydroxy-2-carboethoxyindane to the hydrazide derivative

(1S,2R)-l-hydroxy-2-carboethoxyindane (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of hydrazine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydrazine is removed by extraction with 1% HC1, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.9 grams of the hydrazide of (1S,2R)-l-hydroxy-2-carboxyindane.

Example 41

Conversion of (1S,2R)-l-hydroxy-2-carboethoxy-5-hydroxyindane to the hydrazide derivative

(1S,2R)-l-hydroxy-2-carboethoxy-5-hydroxyindane (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of hydrazine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydrazine is removed by extraction with 1% HC1, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.9 grams of the hydrazide of (1S,2S)-1-hydroxy-2-carboxy-5-hydroxyindane.

Example 42

Conversion of (1S,2R)-l-hydroxy-2-carboethoxy-5-fluoroindane to the hydrazide derivative

(1S,2R)-l-hydroxy-2-carboethoxy-5-fluoroindane (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of hydrazine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydrazine is removed by extraction with 1% HC1, and the ethyl acetate solution is dried over $MgSO_4$, filtered; and rotary evaporated to leave 0.9 grams of the hydrazide of (1S,2S)-l-hydroxy-2-carboxy-5-fluoroindane.

Example 43

Microbial production of the hydrazide of (1S,2S)-l-hydroxy-2-carboxyindane by stereospecific reduction of 2-carboxy-l-indanone hydrazide

Colletorichum gloeosporioides is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4(1 gram),KH_2PO_4(2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25 °C. Two grams of 2-carboxy-1-indanone hydrazide, produced by the reaction of 2-carboethoxy-l-tetralone with hydrazine, is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After 48-72 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1 gram of a yellowish solid containing (1S,2S)-l-hydroxy-2-carboxyindane hydrazide.

Example 44

Production of (1S,2S)-l-hydroxy-2-carboxamidoindane by microbial reduction of the corresponding 2-carboxamido- 1-indanone

Colletorichum gloeosporioides is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4(1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of 2-carboxamido-l-indanone are dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After 48-72 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the dear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1 gram of a yellowish solid containing (1S,2S)-l-hydroxy-2-carboxamidoindane.

Example 45

Production of (1S,2R)-l-hydroxy-2-carboxamidoindane

Twenty grams of bakers' yeast (Sigma Chemical Company, type II) is suspended in an aqueous solution containing 30 grams of sucrose in a conical flask, and the mixture is placed on an orbital shaker (220 rpm) at 30°C for 30 minutes to initiate fermentation. Two grams of 2-carboxamido-l-indanone are dissolved in 2 ml of 95% ethanol, the resulting solution is added to the fermenting yeast, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The. resulting suspension is suction filtered through a pad of Celite and the dear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.4 grams of a light yellow solid containing (1S,2R)-l-hydroxy-2-carboxamidoindane.

Example 46

Production of the hydroxamic acid of (1S,2R)-l-hydroxy-2-carboxyindane

(1S,2R)-l-hydroxy-2-carboethoxyindane (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of hydroxylamine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydroxylamine is removed by extraction with 1% HC1, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.8 grams of the hydroxamic acid derivative of (1S,2R)-l-hydroxy-2-carboxyindane.

Example 47

Enzymatic production of the hydroxamic acid of (1S,2R)-l-hydroxy-2-carboxyindane

(1S,2R)-l-Hydroxy-2-carboethoxyindane (1 gram) is dissolved in 5 ml of t-butyl methyl ether, followed by the addition of 0.5 gram of hydroxylamine. Lipase from *Candida rugosa* (0.5g, Sigma L1754) is added, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydroxylamine is removed by extraction with 1%

HCl, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.8 grams of the hydroxamic acid derivative of (1S,2R)-l-hydroxy-2-carboxyindane.

Example 48

Alternative production of the hydroxamic acid of (1S,2R)-l-hydroxy-2-carboxyindane by microbial reduction of the corresponding hydroxamic acid of 2-carboethoxy-1-indanone.

Rhizopus arrhizus (ATCC 11145) is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4 (1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_47H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of 2-carboxy-1-indanone hydroxamic acid, produced by the reaction of 2-carboethoxy-l-tetralone with hydroxylamine, is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After 48-72 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1 gram of a yellowish solid containing as the major product (1S,2R)-l-hydroxy-2-carboxyindane hydroxamic acid.

Example 49

Microbial production of the hydrazide of (2S,3S)-2 ethyl-3-hydroxybutyrate by stereospecific reduction of 2-ethylacetoacetate hydrazide

Geotrichum candidum (ATCC 34614) is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4(1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25 °C. Two grams of 2-ethylacetoacetate hydrazide, produced by the reaction of ethyl acetoacetate with hydrazine, is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After 48-72 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The

resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1 gram of (2S,3S)-2 ethyl-3-hydroxybutyrate hydrazide as a yellowish solid.

Example 50

Production of (2S,3S)-2 ethyl-3-hydroxybutyramide by microbial reduction of the corresponding 2-ethylacetoacetamide

Geotrichum candidum (ATCC 34614) is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4(1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of 2-ethyl-3-ketobutyramide is dissolved in 2 ml of 95% ethanol the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After 48-72 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams -of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1 gram of (2S,3S)-2 ethyl-3-hydroxybutyramide as a yellowish solid.

Example 51

Production of S-3-hydroxybutyramide

Twenty grams of bakers' yeast (Sigma Chemical Company, type II) are suspended in an aqueous solution containing 30 grams of sucrose in a conical flask, and the mixture is placed on an orbital shaker (220 rpm) at 30°C for 30 minutes to initiate fermentation. Two grams of acetoacetamide is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the fermenting yeast, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted

with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.4 grams of (S)-3-hydroxybutyramide as a light yellow solid.

Example 52

Production of the hydroxamic acid of (2S,3S)-2 ethyl-3-hydroxybutyrate

(2S,3S)-Ethyl 2 ethyl-3-hydroxybutyrate (1 gram) is dissolved in 5 ml of absolute ethanol followed by the addition of 0.5 gram of hydroxylamine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydroxylamine is removed by extraction with 1% HCl, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.8 grams of the hydroxamic acid derivative of (2S,3S)-2-ethyl-3-hydroxybutyrate.

Example 53

Enzymatic production of the hydroxamic acid of (2S,3S)-2 ethyl-3-hydroxybutyrate

(2S,3S)-Ethyl 2 ethyl-3-hydroxybutyrate (1 gram) is dissolved in 5 ml of t-butyl methyl ether, followed by the addition of 0.5 gram of hydroxylamine. Lipase from *Candida rugosa* (0.5 g, Sigma L1754) is added, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydroxylamine is removed by extraction with 1% HCl, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.8 grams of the hydroxamic acid derivative of (2S,3S)-2 ethyl-3-hydroxybutyrate.

Example 54

Alternative production of the hydroxamic acid of (2S,3S)-2 ethyl-3-hydroxybutyrate by microbial reduction of the corresponding hydroxamic acid of 2-ethylacetoacetate

Geotrichum candidum (ATCC 34614) is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one-liter of a medium of glucose (30 grams), KH_2PO_4(1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_47H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of 2-ethylacetoacetate hydroxamic acid, produced by the reaction of ethyl acetoacetate with hydroxylamine, is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed

by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After 48-72 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1 gram of (2S,3S)-2-ethyl-3-hydroxybutyrate hydroxamic acid as a yellowish solid.

Example 55

Alternative production of ethyl (2S,3S)-2-ethyl-3-hydroxybutyrate

Two grams of ethyl 2-ethyl acetoacetate is dissolved in 2 ml of 95% ethanol, and the resulting solution is added to a solution of alcohol dehydrogenase (500 units from *Rhizopus arrhizus* (ATCC 11145)) containing potassium phosphate buffer, 100 mm, pH 7.0. NAD+ (100 mg) is added to the solution along with 1 gram of sodium formate and 100 units of formate dehydrogenase (Boehringer Mannheim) for recycling of the NAD+ cofactor. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker. The resulting solution is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.8 grams of (2S,3S)-2-ethyl-3-hydroxybutyrate as a light yellow oil. The chiral purity of the product is greater than 99% as judged by chiral chromatography.

Example 56

Production of (1S,2R)-ethyl 2-hydroxycyclopentanecarboxylate

Twenty-five grams of bakers' yeast (*Saccharomyces cerevisiae*, Sigma Chemical Company, type II) is suspended in 100 ml of sterilized tap water in a conical flask, and the mixture is placed on an orbital shaker (220 rpm) at 30°C for 1 hour to activate the yeast. One gram of ethyl 2-oxocyclopentanecarboxylate is added, shaking is resumed, and progress of the reaction is monitored by TLC (staining with anisaldehyde). After approximately 100 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with diethyl ether (4 x 100 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.7 grams of octyl (1R,2S)-ethyl 2-hydroxycyclopentanecarboxylate as a yellowish oil (70% yield).

Example 57

Production of (1S,2S)-l-hydroxy-2-carboethoxytetralin

Colletorichum gloeosporioides is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4(1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), NaNO3 (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of 2-carboethoxy-1-tetralone is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.7 grams of a yellow oil containing (1S,2S)-l-hydroxy-2-carboethoxytetralin. The chiral purity of the product is greater than 95% as judged by chiral chromatography.

Example 58

Production of (1S,2S)-l-hydroxy-2,carboethoxy-6-fluorotetralin

Colletorichum gloeosporioides is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4(1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of 6-fluoro-2- carboethoxy-l-tetralone is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.7 grams of a yellow oil containing (1S,2S)-l-hydroxy-2-carboethoxy-6- fluorotetralin. The chiral purity of the product is greater than 98% as judged by chiral chromatography.

Example 59

Production of (1S,2S)-l-hydroxy-2-carboethoxy-6-chlorotetralin

Colletorichum gloeosporioides is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4(1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of 6-chloro-2-carboethoxy-l-tetralone is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $Mg2O_4$, filtered, and rotary evaporated to leave 1.7 grams of (1S,2S)-1-hydroxy-2-carboethoxy-6-chlorotetralin as a yellow oil. The chiral purity of the product is greater than 98% as judged by chiral chromatography.

Example 60

Production of (1S,2S)-l-hydroxy-2-carboethoxy-5,6,7,8-tetrafluorotetralin

Colletorichum gloeosporioides is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4(1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of 5,6,7,8-tetrafluoro-2-carboethoxy-l-tetralone is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.7 grams of a yellow oil containing (1S,2S)-1-hydroxy-2-carboethoxy-5,6,7,8-tetrafluorotetralin. The chiral purity of the product is greater than 98% as judged by chiral chromatography.

Example 61

Production of (1S,2S)-l-hydroxy-2-carboethoxy-6-hydroxytetralin

Colletorichum gloeosporioides is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4(1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), NaNO3 (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of 6-hydroxy-2-carboethoxy-l-tetralone is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.7 grams of a yellow oil containing (1S,2S)-l-hydroxy-2-carboethoxy-6-hydroxytetralin. The chiral purity of the product is greater than 95% as judged by chiral chromatography.

Example 62

Production of (1S,2S)-l-hydroxy-2-carboethoxy-6-methoxytetralin

Colletorichum gloeosporioides is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4(1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of 6-methoxy-2-carboethoxy-l-tetralone is dissolved in 2 ml of 95% ethanol the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.7 grams of a yellow oil containing (1S,2S)-1-hydroxy-2-carboethoxy-6-methoxytetralin. The chiral purity of the product is greater than 95% as judged by chiral chromatography.

Example 63

Alternative production of (1S,2S)-l-hydroxy-2-carboethoxytetralin

Rhizopus arrhizus (ATCC 11145) is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4(1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of 2-carboethoxy-1-tetralone is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined; dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.6 grams of a yellow oil containing (1S,2S)-l-hydroxy-2-carboethoxytetralin. The chiral purity of the product is shown to be greater than 95% as judged by chiral chromatography.

Example 64

Production of (1S,2R)-l-hydroxy-2-carboethoxytetralin

Twenty grams of bakers' yeast (Sigma Chemical Company, type II) are suspended in an aqueous solution containing 30 grams of sucrose in a conical flask, and the mixture is placed on an orbital shaker (220 rpm) at 30°C for 30 minutes to initiate fermentation. Two grams of 2-carboethoxy-l-tetralone is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the fermenting yeast, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After-approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.8 grams of a yellow oil containing (1S,2R)-l-hydroxy-2-carboethoxytetralin (>95% enantiomeric excess as judged by chiral chromatography).

Example 65

Production of (1S,2R)-l-hydroxy-2-carboethoxy-6-fluorotetralin

Twenty grams of bakers' yeast (Sigma Chemical Company, type II) are suspended in an aqueous solution containing 30 grams of sucrose in a conical flask, and the mixture is placed on an orbital shaker (220 rpm) at 30°C for 30 minutes to initiate fermentation. Two grams of 6-fluoro-2-carboethoxy-l-tetralone is dissolved in 2 ml of 95% ethanol the resulting solution is added to the fermenting yeast, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.8 grams of a yellow oil containing (1S,2R)-l-hydroxy-2-carboethoxy-6-fluorotetralin (>95% enantiomeric excess as judged by chiral chromatography).

Example 66

Production of (1S,2R)-l-hydroxy-2-carboethoxy-6-hydroxytetralin

Twenty grams of bakers' yeast (Sigma Chemical Company, type II) are suspended in an aqueous solution containing 30 grams of sucrose in a conical flask, and the mixture is placed on an orbital shaker (220 rpm) at 30°C for 30 minutes to initiate - fermentation. Two grams of 6-hydroxy-2-carboethoxy-l-tetralone is dissolved in 2 ml of 95% ethanol the resulting solution is added to the fermenting yeast, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.8 grams of a yellow oil containing (1S,2R)-l-hydroxy-2-carboethoxy-6-hydroxytetralin (>95% enantiomeric excess as judged by chiral chromatography).

Example 67

Production of (1S,2R)-l-hydroxy-2-carboethoxy-6-carboxyethyltetralin

Twenty grams of bakers' yeast (Sigma Chemical Company, type II) are suspended in an aqueous solution containing 30 grams of sucrose in a conical flask, and the mixture is placed on an orbital shaker (220 rpm) at 30°C for 30 minutes to initiate fermentation. Two grams of 6-carboxyethyl-2-carboethoxy-l-tetralone is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the fermenting yeast, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the dear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.8 grams of a yellow oil containing (1S,2R)-l-hydroxy-2-carboethoxy-6-carboxyethyltetralin (>95% enantiomeric excess as judged by chiral chromatography).

Example 68

Conversion of (1S,2S)-l-hydroxy-2-carboethoxytetralin to the hydrazide derivative (1S,2S)

l-hydroxy-2-carboethoxytetralin (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of hydrazine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydrazine is removed by extraction with 1% HCl, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.9 grams of the hydrazide of (1S,2S)-l-hydroxy-2-carboxytetralin.

Example 69

Conversion of (1S,2S)-l-hydroxy-2-carboethoxy-6-fluorotetralin to the hydrazide derivative

(1S,2S)-l-hydroxy-2-carboethoxy-6-fluorotetralin (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of hydrazine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydrazine is removed by extraction with 1% HCI and the ethyl acetate solution is dried over

$MgSO_4$, filtered, and rotary evaporated to leave 0.9 grams of the hydrazide of (1S,2S)-l-hydroxy-2-carboxy-6-fluorotetralin.

Example 70

Conversion of (lS,2S)-l-hydroxy-2-carboethoxy-6-chlorotetralin to the hydrazide derivative

(1S,2S)-l-hydroxy-2-carboethoxy-6-chlorotetralin (1 gram) is dissolved in 5 ml of absolute ethanol followed by the addition of 0.5 gram of hydrazine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydrazine is removed by extraction with 1% HCl, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.9 grams of the hydrazide of (1S,2S)-l-hydroxy-2-carboxy-6-chlorotetralin.

Example 71

Conversion of (1S,2S)-l-hydroxy-2-carboethoxy-5,6,7,8-tetrafluorotetralin to the hydrazide derivative

(1S,2S)-l-hydroxy-2-carboethoxy-5,6,7,8-tetrafluorotetralin (1 gram) is dissolved in 5 ml of absolute ethanol followed by the addition of 0.5 gram of hydrazine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydrazine is removed by extraction with 1% HC1, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.9 grams of the hydrazide of (1S,2S)-l-hydroxy-2-carboxy-5,6,7,8-tetrafluorotetralin.

Example 72

Conversion of (1S,2S)-l-hydroxy-2-carboethoxy-6-hydroxytetralin to the hydrazide derivative

(1S,2S)-l-hydroxy-2-carboethoxy-6-hydroxytetralin (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of hydrazine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydrazine is removed by extraction with 1% HC1, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.9 grams of the hydrazide of (1S,2S)-l-hydroxy-2-carboxy-6-hydroxytetralin.

Example 73

Conversion of (1S,2S)-l-hydroxy-2-carboethoxy-6-methoxytetralin to the hydrazide derivative

(1S,2S)-l-hydroxy-2-carboethoxy-6-methoxytetralin (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of hydrazine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydrazine is removed by extraction with 1% HCl, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.9 grams of the hydrazide of (1S,2S)-l-hydroxy-2-carboxy-6-methoxytetralin.

Example 74

Conversion of (1S,2R)-l-hydroxy-2-carboethoxytetralin to the hydrazide derivative

(1S,2R)-l-hydroxy-2-carboethoxytetralin (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of hydrazine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydrazine is removed by extraction with 1% HC1, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.9 grams of the hydrazide of (1S,2R)-l-hydroxy-2-carboxytetralin.

Example 75

Conversion of (1S,2R)-l-hydroxy-2-carboethoxy-6-hydroxytetralin to the hydrazide derivative

(1S,2R)-l-hydroxy-2-carboethoxy-6-hydroxytetralin (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of hydrazine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydrazine is removed by extraction with 1% HCl, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.9 grams of the hydrazide of (1S,2S)-l-hydroxy-2-carboxy-6-hydroxytetralin.

Example 76

Conversion of (1S,2R)-l-hydroxy-2-carboethoxy-6-fluorotetralin to the hydrazide derivative

(1S,2R)-l-hydroxy-2-carboethoxy-6-fluorotetralin (1 gram) is dissolved in 5 ml of absolute ethanol followed by the addition of 0.5 gram of hydrazine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydrazine is removed by extraction with 1% HCl, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.9 grams of the hydrazide of (1S,2S)-l-hydroxy-2-carboxy-6-fluorotetralin.

Example 77

Microbial production of the hydrazide of (1S,2S)-l-hydroxy-2-carboxytetralin by stereospecific reduction of 2-carboxy-l-tetralone hydrazide

Colletorichum gloeosporioides is cultured according to the method of Buisson and Azerad (Tet. Lett. 7, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4(1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of 2-carboxy-1-tetralone hydrazide, produced by the reaction of 2-carboethoxy-l-tetralone with hydrazine, are dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After 48-72 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1 gram of a yellowish solid containing (1S,2S)-l-hydroxy-2-carboxytetralin hydrazide.

Example 78

Production of (1S,2S)-l-hydroxy-2-carboxamidotetralin by microbial reduction of the corresponding 2-carboxamido-l-tetralone

Colletorichum gloeosporioides is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of

glucose (30 grams), KH_2PO_4(1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of 2-carboxamido-l-tetralone is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After 48-72 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1 gram of a yellowish solid containing (1S,2S)-l-hydroxy-2-carboxamidotetralin.

Example 79

Production of (1S,2R)-l-hydroxy-2-carboxamidotetralin

Twenty grams of bakers' yeast (Sigma Chemical Company, type II) are suspended in an aqueous solution containing 30 grams of sucrose in a conical flask, and the mixture is placed on an orbital shaker (220 rpm) at 30°C for 30 minutes to initiate fermentation. Two grams of 2-carboxamido-l-tetralone are dissolved in 2 ml of 95%ethanol, the resulting solution is added to the fermenting yeast, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.4 grams of a light yellow solid containing (1S,2R)-l-hydroxy-2-carboxamidotetralin.

Example 80

Production of the hydroxamic acid of (1S,2R)-l-hydroxy-2-carboxytetralin

(1S,2R)-l-hydroxy-2-carboethoxytetralin (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of hydroxylamine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydroxylamine is removed by extraction with 1% HCl, and the ethyl acetate solution is dried

over $MgSO_4$, filtered, and rotary evaporated to leave 0.8 grams of the hydroxamic acid derivative of (1S,2R)-l-hydroxy-2-carboxytetralin.

Example 81

Enzymatic production of the hydroxamic acid of (1S,2R)-l-hydroxy-2-carboxytetralin

(1S,2R)-l-hydroxy-2-carboethoxytetralin (1 gram) is dissolved in 5 ml of t-butyl methyl ether, followed by the addition of 0.5 gram of hydroxylamine. Lipase from *Candida rugosa* (0.5 g, Sigma L1754) is added, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydroxylamine is removed by extraction with 1% HCl, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.8 grams of the hydroxamic acid derivative of (1S,2R)-l-hydroxy-2-carboxytetralin.

Example 82

Alternative production of the hydroxamic acid of (1S,2R)-l-hydroxy-2-carboxytetralin by microbial reduction of the corresponding hydroxamic acid of 2-carboethoxy-l-tetralone

Rhizopus arrhizus (ATCC 11145) is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4(1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of 2-carboxy-1-tetralone hydroxamic acid, produced by the reaction of 2-carboethoxy-l-tetralone with hydroxylamine, is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After 48-72 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over MgSO, filtered, and rotary evaporated to leave 1 gram of a yellowish solid containing as the major product (1S,2R)-1-hydroxy-2-carboxytetralin hydroxamic acid.

Example 83

Alternative production of ethyl (2S,3S)-2-ethyl-3-hydroxybutyrate

Two grams of ethyl 2-ethyl acetoacetate is dissolved in 2 ml of 95% ethanol, and the resulting solution is added to a solution of alcohol dehydrogenase (500 units from *Rhizopus arrhizus* (ATCC 11145) containing potassium phosphate buffer, 100 mM, pH 7.0. NAD+ (100 mg) is added to the solution along with 1 gram of sodium formate and 100 units of formate deydrogenase (Boehringer Mannheim) for recycling of the NAD+ cofactor. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker. The resulting solution is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over MgSO, filtered, and rotary evaporated to leave 1.8 grams of (2S,3S)-2-ethyl-3-hydroxybutyrate as a light yellow oil. The chiral purity of the product is greater than 99% as judged by chiral chromatography.

Example 84

Production of (1S,2R)-ethyl 2-hydroxycyclopentanecarboxylate

Twenty-five grams of bakers' yeast (*Saccharomyces cerevisiae*, Sigma Chemical Company, type II) is suspended in 100 ml of sterilized tap water in a conical flask, and the mixture is placed on an orbital shaker (220 rpm) at 30°C for 1 hour to activate the yeast. One gram of ethyl 2-oxocyclopentanecarboxylate is added, shaking is resumed, and progress of the reaction is monitored by TLC (staining with anisaldehyde). After approximately 100 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with diethyl ether (4 x 100 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.7 grams of octyl (1R,2S)-ethyl 2-hydroxycyclopentanecarboxylate as a yellowish oil (70% yield).

Example 85

Conversion of ethyl (1R,2S)-2-hydroxycyclopentanecarboxylate to the hydrazide derivative

Ethyl (1R,2S)-2-hydroxycyclopentanecarboxylate (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of hydrazine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate.

Hydrazine is removed by extraction with 1% HCl, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.9 grams of the hydrazide of (1R,2S)-2-hydroxycyclopentanecarboxylate.

Example 86

Microbial production of the hydrazide of (1R,2S)-2-hydroxycyclopentanecarboxylate by stereospecific reduction of 2-oxocyclopentanecarboxylate hydrazide

Colletorichum gloeosporioides is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4(1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of 2-oxocyclopentanecarboxylate hydrazide, produced by the reaction of ethyl 2 oxocyclopentanecarboxylate with hydrazine, is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After 48-72 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1 gram of a yellowish solid containing (1R,25)-2-hydroxycyclopentanecarboxylate hydrazide.

Example 87

Production of (1S,2S)-2-hydroxy-l-carboxamidocyclopentane by stereospecific reduction of 2-carboxamido-l-cyclopentanone

Colletorichum gloeosporioides is cultured according to the method of Buisson and. Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter-of a medium of glucose (30 grams), KH_2PO_4(1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of 2-carboxamido-l-cyclopentanone is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After 48-72 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The

resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1 gram of a yellowish solid containing (1S,2S)-2-hydroxy-1-carboxamidocyclopentane.

Example 88

Production of (1S,2R)-2-hydroxy-l-carboxamidocyclopentane

Twenty grams of bakers' yeast (Sigma Chemical Company, type II) are suspended in an aqueous solution containing 30 grams of sucrose in a conical flask, and the mixture is placed on an orbital shaker (220 rpm) at 30°C for 30 minutes to initiate fermentation. Two grams of 2-carboxamido-l-cyclopentanone are dissolved in 2 ml of 95% ethanol, the resulting solution is added to the fermenting yeast, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.4 grams of a light yellow solid containing (1S,2S)-2-hydroxy-l-carboxamidocyclopentane.

Example 89

Production of the hydroxamic acid of (1S,2R)-l-hydroxy-2-carboxycyclopentane

(1S,2R)-l-hydroxy-2-carboethoxycyclopentane (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of hydroxylamine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydroxylamine is removed by extraction with 1% HC1, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.8 grams of the hydroxamic acid derivative of (1S,2R)-l-hydroxy-2-carboxycyclopentane.

Example 90

Enzymatic production of the hydroxamic acid of (1S,2R)-l-hydroxy-2-carboxycyclopentane

(1S,2R)-l-hydroxy-2-carboethoxycyclopentane (1 gram) is dissolved in 5 ml of t-butyl methyl ether, followed by the addition of 0.5 gram of hydroxylamine. Lipase from *Candida rugosa* (0.5g, Sigma L1754) is added, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydroxylamine is removed by extraction with 1% HCl, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.8 grams of the hydroxamic acid derivative of (1S,2R)-l-hydroxy-2-carboxycyclopentane.

Example 91

Alternative production of the hydroxamic acid of (1S,2R)-l-hydroxy-2-carboxycyclopentane by microbial reduction of the hydroxamic acid of 2-carboxy-1-cyclopentanone

Rhizopus arrhizus (ATCC 11145) is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4(1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of 2-carboxy-1- cyclopentanone hydroxamic acid is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After 48-72 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1 gram of a yellowish solid containing as the major product (1S,2R)-l-hydroxy-2-carboxycyclopentane hydroxamic acid.

Example 92

Conversion of ethyl (1R,2S)-2-hydroxycyclohexanecarboxylate to the hydrazide derivative

Ethyl (1R,2S)-2-hydroxycyclohexanecarboxylate (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of hydrazine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate.

Hydrazine is removed by extraction with 1% HCl, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.9 grams of the hydrazide of (1R,2S)-2-hydroxycyclohexanecarboxylate.

Example 93

Microbial production of the hydrazide of (1S,2S)-2-hydroxycyclohexanecarboxylate by stereospecific reduction of 2-oxocyclohexanecarboxylate hydrazide

Colletorichum gloeosporioides is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4(1 gram), K_2HPO_4(2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of 2-oxocyclopentanecarboxylate hydrazide is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After 48-72 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1 gram of a yellowish solid containing (1S,2S)-2-hydroxycyclohexanecarboxylate hydrazide.

Example 94

Production of (1S,2S)-2-hydroxy-l-carboxamidocyclohexane by stereospecific reduction of 2-carboxamido-l-cyclohexanone

Colletorichum gloeosporioides is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4(1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of 2-carboxamido-l-cyclohexanone are dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After 48-72 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20- 30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is

extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1 gram of a yellowish solid containing (1S,2S)-2-hydroxy-1-carboxamidocyclohexane.

Example 95

Production of (1S,2R)-2-hydroxy-l-carboxamidocyclohexane

Twenty grams of bakers' yeast (Sigma Chemical Company, type II) are suspended in an aqueous solution containing 30 grams of sucrose in a conical flask, and the mixture is placed on an orbital shaker (220 rpm) at 30°C for 30 minutes to initiate fermentation. Two grams of 2-carboxamido-l-cyclohexanone is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the fermenting yeast, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The, resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.4 grams of a light yellow solid containing (1S,2S)-2-hydroxy-l-carboxamidocyclohexane.

Example 96

Production of the hydroxamic acid of (1S,2R)-l-hydroxy-2-carboxycyclohexane

(1S,2R)-1-hydroxy-2-carboethoxycyclohexane (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of hydroxylamine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydroxylamine is removed by extraction with 1% HC1, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.8 grams of the hydroxamic acid derivative of (1S,2R)-l-hydroxy-2-carboxycyclohexane.

Example 97

Enzymatic production of the hydroxamic acid of (1S,2R)-l-hydroxy-2-carboxycyclohexane

(1S,2R)-1-hydroxy-2-carboethoxycyclohexane (1 gram) is dissolved in 5 ml of t-butyl methyl ether, followed by the addition of 0.5 gram of hydroxylamine. Lipase from *Candida*

rugosa (0.5 g, Sigma L1754) is added, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydroxylamine is removed by extraction with 1% HCl, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.8 grams of the hydroxamic acid derivative of (1S,2R)-l-hydroxy-2-carboxycyclohexane.

Example 98

Alternative production of the hydroxamic acid of (1S,2R)-l-hydroxy-2-carboxycyclohexane by microbial reduction of the corresponding hydroxamic acid of 2-carboethoxy-l-cyclohexanone

Rhizopus arrhizus (ATCC 11145) is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4(1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of 2-carboxy-.1-cyclohexanone hydroxamic acid is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After 48-72 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1 gram of a yellowish solid containing as the major product (1S,2R)-l-hydroxy-2-carboxycyclohexane hydroxamic acid.

Example 99

Conversion of (1S,2R)-l-carboethoxy-2-hydroxyindane to the hydrazide derivative

(1S,2R)-l-carboethoxy-2-hydroxyindane (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of hydrazine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydrazine is removed by extraction with 1% HC1, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.9 grams of the hydrazide of (1S,2R)-l-carboxy-2-hydroxyindane.

Example 100

Production of (1S,2S)-2-hydroxy-l-carboxamidoindane by stereospecific reduction of 1-carboxamido-2-indanone

Colletorichum gloeosporioides is cultured according to the method of Buisson and Azerad (Tet. Lett. 27, 2631-2634 (1986), herein incorporated by reference) in one liter of a medium of glucose (30 grams), KH_2PO_4(1 gram), K_2HPO_4 (2 grams), corn steep liquor (10 grams) $MgSO_4.7H_20$ (0.5 gram), $NaNO_3$ (2 grams), $FeSO_4.7H_20$ (0.02 gram), and KCl (0.5 gram) with rotary shaking at 25°C. Two grams of 1-carboxamido-2-indanone is dissolved in 2 ml of 95% ethanol, the resulting solution is added to the culture, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After 48-72 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1 gram of a yellowish solid containing (1S,2S)-l-carboxamido-2-hydroxyindane.

Example 101

Production of (1R,2S)-l-carboxamido-2-hydroxyindane

Twenty grams of bakers' yeast (Sigma Chemical Company, type II) are suspended in an aqueous solution containing 30 grams of sucrose in a conical flask, and the mixture is placed on an orbital shaker (220 rpm) at 30°C for 30 minutes to initiate fermentation. Two grams of 1-carboxamido-2-indanone are dissolved in 2 ml of 95% ethanol, the resulting solution is added to the fermenting yeast, and shaking is resumed. The reaction is followed by TLC (staining with anisaldehyde) to monitor the consumption of starting material and the production of product alcohol. After approximately 48 hours the reaction is judged complete, and the reaction is terminated by removing from the shaker and adding 20-30 grams of Celite. The resulting suspension is suction filtered through a pad of Celite and the clear yellow filtrate is extracted with ethyl acetate (4 x 200 ml). The extracts are combined, dried over $MgSO_4$, filtered, and rotary evaporated to leave 1.4 grams of a light yellow solid containing (1S,2S)-l-carboxamido-2-hydroxyindane as the major product.

Example 102

Production of the hydroxamic acid of (1R,2S)-l-carboxy-2-hydroxyindane

(1R,2S)-l-carboethoxy-2-hydroxyindane (1 gram) is dissolved in 5 ml of absolute ethanol, followed by the addition of 0.5 gram of hydroxylamine. The solution is heated to reflux, and the progress of the reaction is followed by thin layer chromatography. After the reaction is judged complete, the ethanol is evaporated and the resulting residue redissolved in ethyl acetate. Hydroxylamine is removed by extraction with 1% HC1, and the ethyl acetate solution is dried over $MgSO_4$, filtered, and rotary evaporated to leave 0.8 grams of the hydroxamic acid derivative of (1R,2S)-1-carboxy-2-hydroxyindane.

Example 103

Production of (1S,2R)-l-amino-2-indanol

The hydrazide of (1S,2R)-l-carboxy-2-hydroxyindane (0.5 gram) is reacted with a solution of 0.5 grams of sodium nitrite in 10 ml of 5% H_2SO_4. The reaction mixture is maintained for 1 hour at 0-5 °C, followed extraction of the reaction mixture with ethyl acetate, followed by basification of the resulting aqueous solution with NAOH, extraction with methyl t-butyl ether, drying of the extracts over $MgSO_4$, filtration, and the removal of solvent by rotary evaporation. The product (1S,2R)-l-amino-2-indanol is isolated as a light yellow oil.

Example 104

Production of (1S,2R)-l-amino-2-indanol by a modified Lossen Rearrangement

One gram of the hydroxamic acid of (1R,2S)-l-carboxy-2-hydroxyindane is reacted with equimolar amounts of diethyl azodicarboxylate and triphenylphosphine in tetrahydrofuran at room temperature using the procedure of Bittner, Grinberg and Kartoon (Tet. Lett. 23, 1965-8 (1974), the disclosure of which is incorporated herein by reference). The product is isolated by acidification and extraction of the reaction mixture with ethyl acetate, followed by basification of the resulting aqueous solution with NaOH, extraction with methyl t-butyl ether, drying of the extracts over $MgSO_4$, filtration, and the removal of solvent by rotary evaporation. The product (1S,2R)-l-amino-2-indanol is isolated as a light yellow oil.

Example 105

Production of (1S,2S)-2-amino-l-indanol by a modified Lossen Rearrangement

One gram of the hydroxamic add of (1S,2S)-2-carboxy-l-hydroxyindane is reacted with equimolar amounts of diethyl azodicarboxylate and triphenylphosphine in tetrahydrofuran at room temperature using the procedure of Bittner, Grinberg and Kartoon (Tet. Lett. 23, 1965-8 (1974)). The product is isolated by acidification and extraction of the reaction mixture with ethyl acetate, followed by basification of the resulting aqueous solution with NaOH, extraction with methyl t-butyl ether, drying of the extracts over $MgSO_4$, filtration, and the removal of solvent by rotary evaporation. The product (1S,2S)-2-amino-l-indanol is isolated as a light yellow oil.

Example 106

Production of (1S,2S)-l-hydroxy-2-aminotetralin by a modified Lossen Rearrangement

One gram of the hydroxamic acid of (1S,2S)-l-hydroxy-2-carboxyteralin is reacted with equimolar amounts of diethyl azodicarboxylate and triphenylphosphine in tetrahydrofuran at room temperature using the procedure of Bittner, Grinberg and Kartoon (Tet. Lett. 23, 1965-8 (1974)). The product is isolated by acidification and extraction of the reaction mixture with ethyl acetate, followed by basification of the resulting aqueous solution with NaOH, extraction with methyl t-butyl ether, drying of the extracts over $MgSO_4$, filtration, and the removal of solvent by rotaryevaporation. The product (1S,2S)-l-hydroxy-2-aminotetralin is isolated as a light yellow oil.

Example 107

Production of (1S,2R)-l-hydroxy-2-aminotetralin by a modified Lossen Rearrangement

One gram of the hydroxamic acid of (1S,2R)-2-carboxy-l-hydroxytetralin is reacted with equimolar amounts of diethyl azodicarboxylate and triphenylphosphine in tetrahydrofuran at room temperature using the procedure of Bittner, Grinberg and Kartoon (Tet. Lett. 23, 1965-8 (1974)). The product is isolated by acidification and extraction of the reaction mixture with ethyl acetate, followed by basification of the resulting aqueous solution with NaOH, extraction with methyl t-butyl ether, drying of the extracts over $MgSO_4$, filtration, and the removal of solvent by rotary evaporation. The product (1S,2R)-1-hydroxy-2-aminotetralin is isolated as a light yellow oil.

Example 108

Production of (1S,2R)-l-hydroxy-2-aminotetralin

The hydrazide of (1S,2R)-1-hydroxy-2-carboxyindane (0.5 gram) is reacted with a solution of 0.5 grams of sodium nitrite in 10 ml of 5% H_2SO_4. The reaction mixture is maintained for 1 hour at 0-5°C, followed extraction of the reaction mixture with ethyl acetate, followed by basification of the resulting aqueous solution with NaOH, extraction with methyl t-butyl ether, drying of the extracts over $MgSO_4$, filtration, and the removal of solvent by rotary evaporation. The product (1S,2R)-l-hydroxy-2-aminotetralin is isolated as a light yellow oil.

WHAT IS CLAIMED:

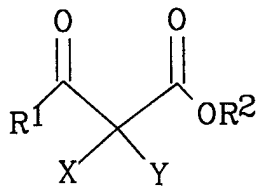
1. A method for producing a chiral vicinal aminoalcohol comprising:

(a) contacting a β-ketoester capable of being converted to a chiral vicinal aminoalcohol with a microorganism in the presence of (i) a carbon source or (ii) a dehydrogenase in combination with a nicotinamide cofactor, under conditions sufficient to permit the stereoselective reduction of the keto group to form a chiral ß-hydroxyester;

(b) converting the chiral ß-hydroxyester to a corresponding amide, hydroxamic acid, or hydrazide derivative, and

(c) exposing the amide, hydroxamic acid, or hydrazide derivative to conditions permitting stereospecific rearrangement to the corresponding chiral vicinal aminoalcohol.

2. The method according to claim 1, wherein the β-ketoester has the formula 1:



(1)

wherein:

X is selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen-substituted alkyl, aryl, halogen-substituted aryl, benzyl, halogen-substituted benzyl, thiophenyl, halogen-substituted thiophenyl, hydroxy-substituted alkyl, hydroxy-substituted aryl, carboxy-substituted alkyl, carboxy-substituted alkyl, thio-substituted alkyl, thio-substituted aryl and heterocyclic;

Y is H;

R^1 is selected from the group consisting of alkyl, alkenyl, alkynyl, halogen-substituted alkyl, aryl, halogen-substituted aryl, benzyl, halogen-substituted benzyl, thiophenyl, halogen-substituted thiophenyl, hydroxy-substituted alkyl, hydroxy-substituted aryl, carboxy-substituted alkyl, carboxy-substituted aryl, thio-substituted alkyl, thio-substituted aryl, and heterocyclic; or wherein R^1, together with X and the carbon atoms to which they are attached, forms a ring; and

R^2 is selected from the group consisting of alkyl, alkenyl, alkynyl, halogen-substituted alkyl, aryl, halogen-substituted aryl, benzyl, halogen-substituted benzyl, thiophenyl, halogen-substituted

thiophenyl, hydroxy-substituted alkyl, hydroxy-substituted aryl, carboxy-substituted alkyl, carboxy-substituted aryl, thio-substituted alkyl, thio-substituted aryl, and heterocyclic.

3. The method of claim 1, wherein the microorganism is selected from the group consisting of *Saccharomyces cerevisiae, Geotrichum candidum, Colletorichum gloeosporioides, Rhizopus arrhizus, Aspergillus niger,* and *Mortierella isabellina.*

4. The method of claim 1, wherein the source of the dehydrogenase is a microorganism selected from the group consisting of *Saccharomyces cerevisiae, Geotrichum candidum, Colletorichum gloeosporioides, Rhizopus arrhizus, Aspergillus niger,* and *Mortierella isabellina.*

5. The method of claim 1, wherein the nicotinamide cofactor for the dehydrogenase is recycled.

6 The method of claim 1, wherein the conversion of the ester to the corresponding amide, hydroxamic acid or hydrazide derivative is catalyzed by an esterase, lipase, protease, or amidase.

7 The method of claim 1, further comprising recovering the chiral vicinal aminoalcohol.

8. The method of claim 1, wherein the yield of the β-hydroxyester is at least 60 percent, based on the amount of starting β-ketoester.

9. The method of claim 1, wherein the yield of the β-hydroxyester is at least 80 percent, based on the amount of starting β-ketoester.

10. The method of claim 2, comprising converting the chiral ß-hydroxyester of step (a) to a corresponding amide.

11. The method of claim 10, wherein X is selected from the group consisting of alkyl, aryl, benzyl and alkenyl, or wherein X and R^1, together with the carbon atoms to which they are attached, form a ring.

12. The method of claim 10, wherein R^1 is selected from the group consisting of alkyl, aryl, benzyl and alkenyl, or wherein R^1 and X, together with the carbon atoms to which they are attached, form a ring.

13. The method of claim 10, wherein R^2 is selected from the group consisting of alkyl, aryl and benzyl.

14. The method of claim 2, comprising converting the chiral ß-hydroxyester of step (a) to a corresponding hydroxamic acid.

15. The method of claim 14, wherein X is selected from the group consisting of alkyl, aryl, benzyl and alkenyl, or wherein X and R^1, together with the carbon atoms to which they are attached, form a ring.

16. The method of claim 14, wherein R^1 is selected from the group consisting of alkyl, aryl, benzyl and alkenyl, or wherein R^1 and X, together with the carbon atoms to which they are attached, form a ring.

17. The method of claim 14, wherein R^2 is selected from the group consisting of alkyl, aryl and benzyl.

18. The method of claim 2, comprising converting the chiral ß-hydroxyester of step (a) to a corresponding hydrazide derivative.

19. The method of claim 18, wherein X is selected from the group consisting of alkyl, aryl, benzyl and alkenyl, or wherein X and R^1, together with the carbon atoms to which they are attached, form a ring.

20. The method of claim 18, wherein R^1 is selected from the group consisting of alkyl, aryl, benzyl and alkenyl, or wherein R^1 and X, together with the carbon atoms to which they are attached, form a ring.

21. The method of claim 18, wherein R^2 is selected from the group consisting of alkyl, aryl and benzyl.

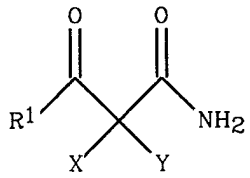
22. A method for producing a chiral vicinal aminoalcohol comprising:

a) contacting a ß-ketoamide capable of being converted to a chiral vicinal amino alcohol, a ß-ketohydroxamic acid capable of being converted to a chiral vicinal amino alcohol, or a ß-ketohydrazide capable of being converted to a chiral vicinal amino alcohol with a microorganism in the presence of (i) a carbon source or (ii) a dehydrogenase in combination with a nicotinamide

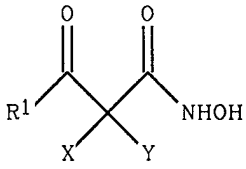
cofactor, under conditions sufficient for stereoselective reduction of the keto group to form a ß-hydroxyamide, ß-hydroxyhydroxamic acid, or ß-hydroxyhydrazide; and

(b) exposing the ß-hydroxyamide, ß-hydroxyhydroxamic acid, or ß-hydroxyhydrazide to conditions sufficient to permit stereospecific rearrangement to the corresponding chiral vicinal aminoalcohol.

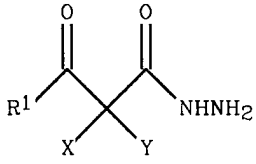
23. The method according to claim 22, wherein the ß-ketoamide has the formula 2, the ß-ketohydroxamic acid has the formula 3, and the ß-ketohydrazide has the formula 4:



(2)



(3)



(4)

wherein:

X is selected from the group consisting of H, alkyl, alkenyl, alkynyl, halogen-substituted alkyl, aryl, halogen-substituted aryl, benzyl, halogen-substituted benzyl, thiophenyl, halogen-substituted thiophenyl, hydroxy-substituted alkyl, hydroxy-substituted aryl, carboxy-substituted alkyl, carboxy-substituted alkyl, thio-substituted alkyl, thio-substituted aryl and heterocyclic;

Y is H; and

R^1 is selected from the group consisting of alkyl, alkenyl, alkynyl, halogen-substituted alkyl, aryl, halogen-substituted aryl, benzyl, halogen-substituted benzyl, thiophenyl, halogen-substituted thiophenyl, hydroxy-substituted alkyl, hydroxy-substituted aryl, carboxy-substituted alkyl, carboxy-substituted aryl, thio-substituted alkyl, thio-substituted aryl, and heterocyclic; or wherein R^1, together with X and the carbon atoms to which they are attached, forms a ring.

24. The method of claim 22, wherein the microorganism is selected from the group consisting of *Saccharomyces cerevisiae, Geotrichum candidum, Colletorichum gloeosporioides, Rhizopus arrhizus, Aspergillus niger,* and *Mortierella isabellina.*

25. The method of claim 22, wherein the source of the dehydrogenase is a microorganism selected from the group consisting of *Saccharomyces cerevisiae, Geotrichum candidum, Colletorichum gloeosporioides, Rhizopus arrhizus, Aspergillus niger,* and *Mortierella isabellina.*

26. The method of claim 22, wherein the nicotinamide cofactor for the dehydrogenase is recycled.

27. The method of claim 22, further comprising recovering the chiral vicinal aminoalcohol.

28. The method of claim 22, wherein yield of the β-hydroxyamide, β-hydroxyhydroxamic acid, or β-hydroxyhydrazide is at least 50 percent, based on the amount of starting ß-ketoamide, ß-ketohydroxamic acid, or ß-ketohydrazide, respectively.

29. The method of claim 22, wherein yield of the β-hydroxyamide is at least 70 percent, based on the amount of starting ß-ketoamide.

30. The method of claim 23, comprising contacting a ß-ketoamide of formula 2 with a microorganism in the presence of a carbon source or a dehydrogenase in combination with a nicotinamide cofactor under conditions sufficient for stereoselective reduction of the keto group to form a ß-hydroxyamide.

31. The method of claim 30, wherein X is selected from the group consisting of alkyl, aryl, benzyl and alkenyl, or wherein X and R^1, together with the carbon atoms to which they are attached, form a ring.

32. The method of claim 30, wherein R^1 is selected from the group consisting of alkyl, aryl, benzyl and alkenyl, or wherein R^1 and X, together with the carbon atoms to which they are attached, form a ring.

33. The method of claim 30, wherein R^2 is selected from the group consisting of alkyl, aryl and benzyl.

34. The method of claim 23, comprising contacting a ß-ketohydroxamic acid of formula 3 with a microorganism in the presence of a carbon source or a dehydrogenase in combination with a nicotinamide cofactor under conditions sufficient for stereoselective reduction of the keto group to form a ß-hydroxyhydroxamic acid.

35. The method of claim 34, wherein X is selected from the group consisting of alkyl, aryl, benzyl and alkenyl, or wherein X and R^1, together with the carbon atoms to which they are attached, form a ring.

36. The method of claim 34, wherein R^1 is selected from the group consisting of alkyl, aryl, benzyl and alkenyl, or wherein R^1 and X, together with the carbon atoms to which they are attached, form a ring.

37. The method of claim 34, wherein R^2 is selected from the group consisting of alkyl, aryl and benzyl.

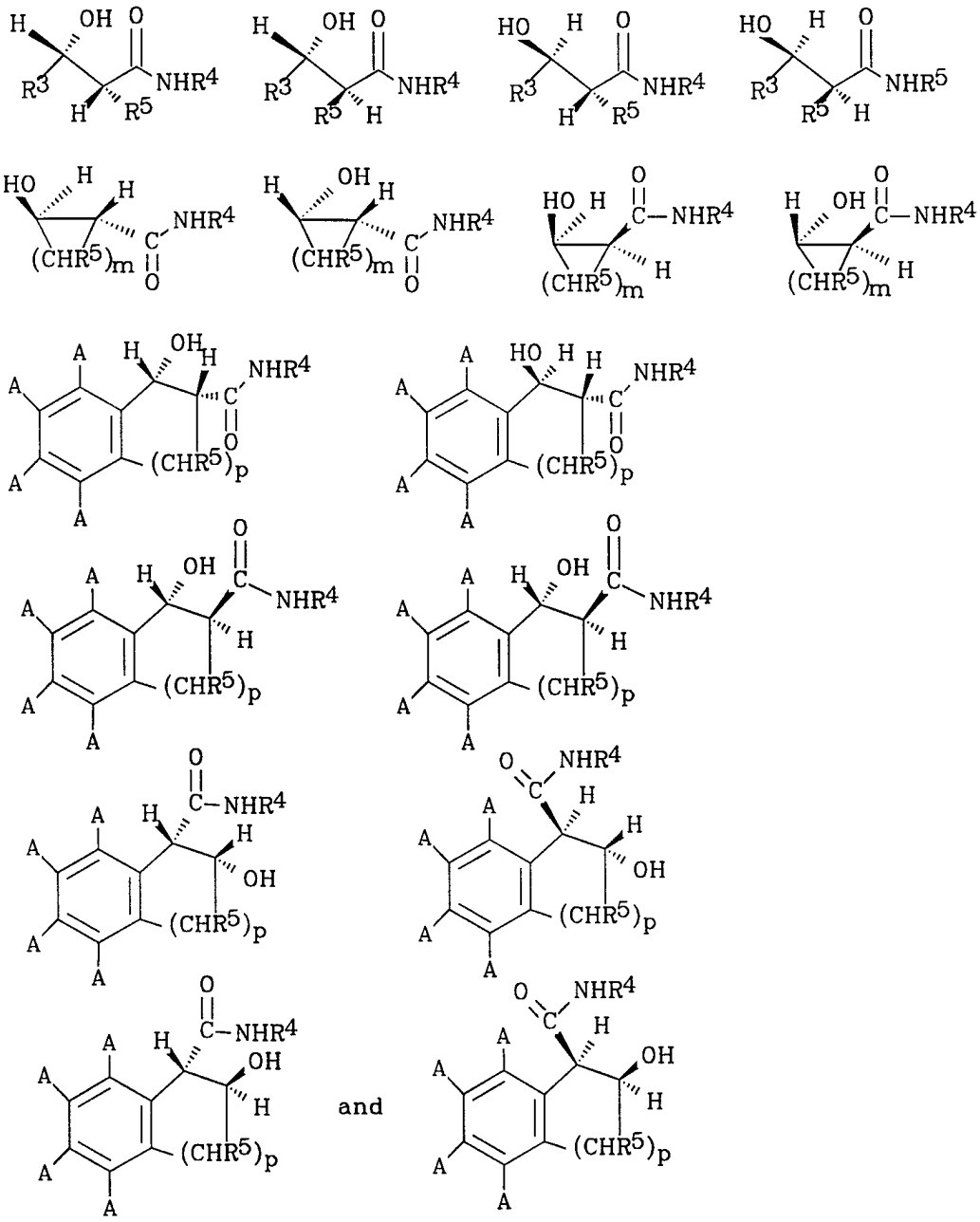
38. The method of claim 23 comprising contacting a ß-ketohydrazide of formula 4 with a microorganism in the presence of a carbon source or a dehydrogenase in combination with a nicotinamide cofactor under conditions sufficient for stereoselective reduction of the keto group to form a ß-hydroxyhydrazide.

39. The method of claim 38, wherein X is selected from the group consisting of alkyl, aryl, benzyl and alkenyl, or wherein X and R^1, together with the carbon atoms to which they are attached, form a ring.

40. The method of claim 38, wherein R^1 is selected from the group consisting of alkyl, aryl, benzyl and alkenyl, or wherein R^1 and X, together with the carbon atoms to which they are attached, form a ring.

41. The method of claim 38, wherein R^2 is selected from the group consisting of alkyl, aryl and benzyl.

42. A composition having a formula selected from the group consisting of:



and

wherein:

R^3 is selected from the group consisting of alkyl, alkenyl, alkynyl, hydroxy-substituted alkyl, hydroxy-substituted alkenyl, halogen-substituted alkyl, halogen-substituted alkenyl, carboxy-substituted alkyl, thio-substituted alkyl, thio-substituted alkenyl, aryl, halogen-substituted aryl, hydroxy-substituted aryl, carboxy-substituted aryl, thio-substituted aryl, benzyl, halogen-substituted benzyl, thiophenyl, halogen-substituted thiophenyl, and heterocyclic;

R^4 is selected from the group consisting of H, OH, and NH_2;

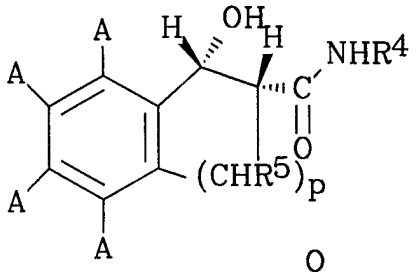
R^5 is selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, hydroxy-substituted alkyl, hydroxy-substituted alkenyl, halogen-substituted alkyl, halogen-substituted alkenyl, carboxy-substituted alkyl, thio-substituted alkyl, thio-substituted alkenyl, aryl, halogen-substituted aryl, hydroxy-substituted aryl, carboxy-substituted aryl, thio-substituted aryl, benzyl, halogen-substituted benzyl, thiophenyl, halogen-substituted thiophenyl, and heterocyclic;

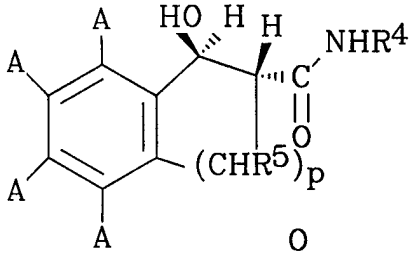
A is selected from the group consisting of H, F, Cl, Br, I, OH, OCH_3, alkyl, carboxy-substituted alkyl, hydroxy-substituted alkyl, halogen-substituted alkyl, aryl, carboxy-substituted aryl, hydroxy-substituted aryl, halogen-substituted aryl, and heterocyclic;

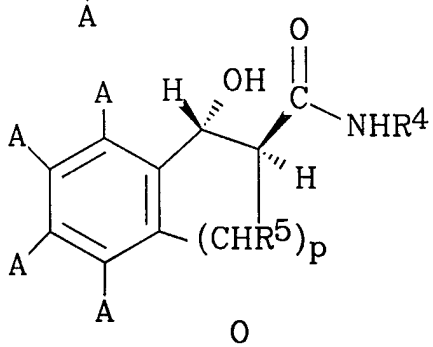
m is a number ranging from 1 to 6; and

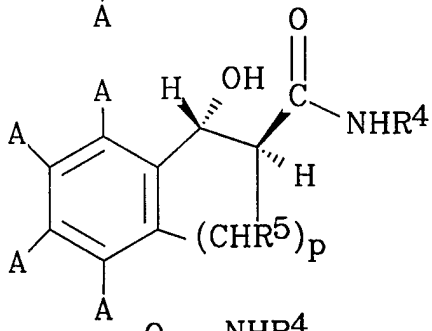
p is a number ranging from 0 to 6.

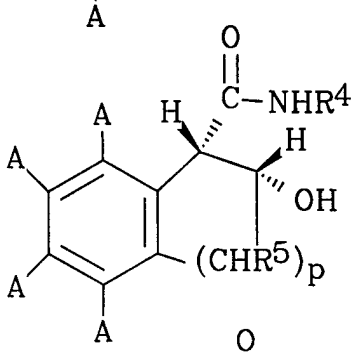
43. The composition of claim 42 comprising a compound having a formula selected from the group consisting of:

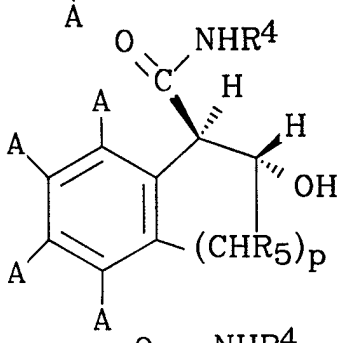


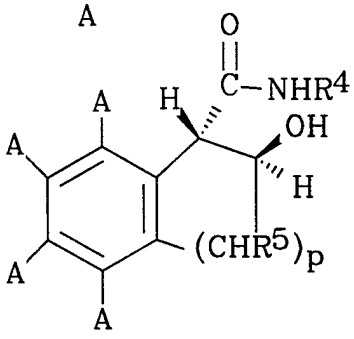




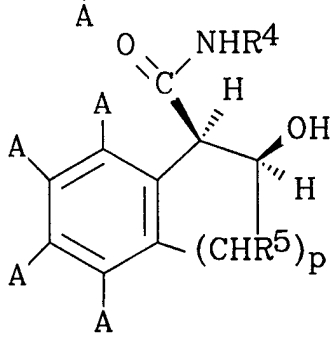






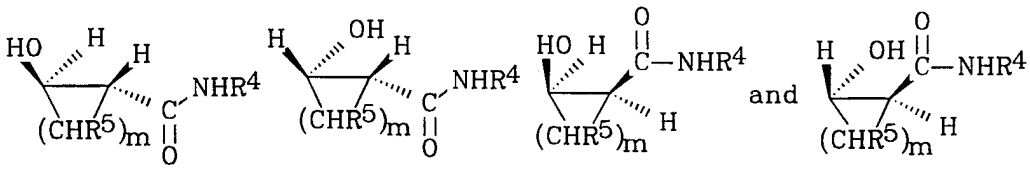


and



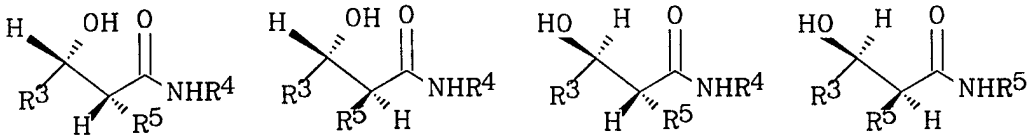
44. The composition of claim 43, wherein p is 1 or 2.

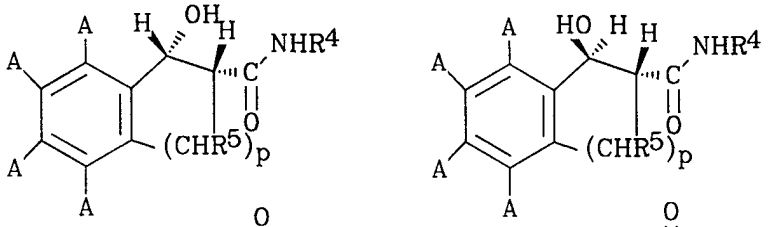
45. The composition of claim 42 comprising a compound having a formula selected from the group consisting of:

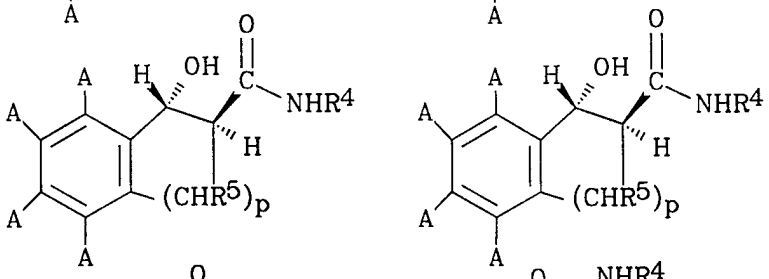


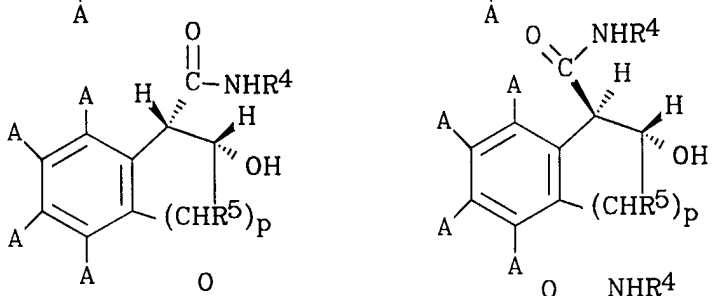
46. The composition of claim 42, wherein m is 3 or 4.

47. The composition of claim 42 comprising a compound having a formula selected from the group consisting of:

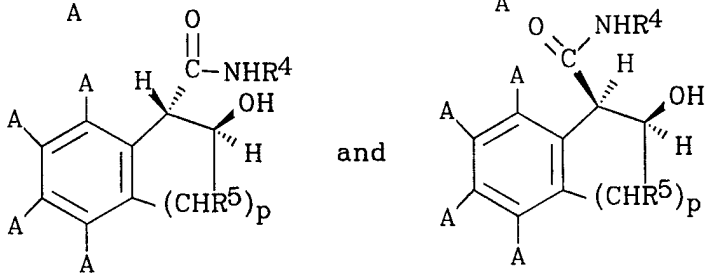








and



48. The composition of claim 42, wherein R^5 is selected from the group consisting of alkyl, aryl, benzyl and alkenyl.

49. The composition of claim 42 wherein R^3 is selected from the group consisting of alkyl, aryl, benzyl and alkenyl.

50. The composition of claim 42, wherein R^4 is H.

51. The composition of claim 42, wherein R^4 is OH.

52. The composition of claim 42, wherein R^4 is NH_2.

53. The composition of claim 42, comprising at least 75% of single stereoisomer.

54. The composition of claim 42, comprising at least 90% of a single stereoisomer.

55. The composition of claim 42, comprising at least 98% of a single stereoisomer.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/10792

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12P 13/00, 41/00
US CL :435/128, 280

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/128, 280

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, Ca-online

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DIDIER et al. Chemo-enzymatic synthesis of 1,2- and 1,3-amino-alcohols and their use in the enantioselective reduction of acetophenone and anti-acetophenone oxime methyl ether with borane. Tetrahedron. 1991, Vol. 47, No. 27, pages 4941-4958.	1-21
A	QUIROS et al. Enantioselective reduction of β-keto amides by the fungus Mortierella isabellina. Tetrahedron: Asymmetry. 1997, Vol. 8, No. 18, pages 3035-3038, especially abstract.	1-21

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	
"A" document defining the general state of the art which is not considered to be of particular relevance	
"B" earlier document published on or after the international filing date	
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention	
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone	
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	
"&" document member of the same patent family	

Date of the actual completion of the international search

29 JULY 1998

Date of mailing of the international search report

24 AUG 1998

Name and mailing address of the ISA/US
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Form PCT/ISA/210 (second sheet)(July 1992)★

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/10792

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-21

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

Group I, claims 1-21, drawn to an enzymatic or microbial method of producing a chiral vicinal aminoalcohol from a beta-keto ester comprising stereoselectively reducing the beta-keto ester with a microbe or a dehydrogenase, converting the beta hydroxy ester to an amide, rearranging the amide to a chiral vicinal aminoalcohol.

Group II, claims 22-41, drawn to an enzymatic or microbial method of producing a chiral vicinal aminoalcohol from a beta-keto amide comprising stereoselectively reducing the beta-keto amide with a microbe or a dehydrogenase, and converting the beta hydroxy amide to a chiral vicinal aminoalcohol.

Group III, claims 42-55, drawn to compositions containing any one of sixteen (16) separate and distinct compounds.

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:

Each structure in claim 42 is a distinct species because each structure is a distinct family composed of many compounds.

The inventions listed as Groups I, II and III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The method of using a microbe or enzyme to synthesize compounds and the compounds, per se, are not a single inventive concept because they lack a special technical feature. The substrates used in the reactions of Group I and II are not the same, and the claimed methods are not directed to the use of any specific microorganism or source of dehydrogenase. Further, 3(R)-hydroxy-2(S),4-dimethyl pentanamide, one the compounds produced by the claimed methods, is known in the prior art as disclosed by Ito et al., Tetrahedron Lett. 26 (38):4643-6 (1985). Thus, the methods as claimed do not have a common special technical feature, and the compounds as claimed are known in the prior art and have been produced by methods other than the claimed methods.

The species of Group III do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons: The species do not share a significant structural element and all of the claimed compounds are not alleged to possess the same utility or function. Further, one of the claimed compounds, 3(R)-hydroxy-2,4-dimethyl pentanamide is known in the prior art, thus the claimed group of compounds does not share a special technical feature.