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(54) Title: A PROCESS FOR THE PREPARATION OF NICOTINE COMPRISING THE ENZYMATIC REDUCTION OF 4-(METHYLAMINO) -1- (PYRIDIN-3- YL) BUTAN-1-ONE

(57) Abstract: The present invention is directed to the preparation of nicotine comprising the enzymatic reduction of 4- (methyl-amino) -1- (pyridin-3-yl) butan-1-one.

A PROCESS FOR THE PREPARATION OF NICOTINE COMPRISING THE ENZYMATIC REDUCTION OF 4-(METHYLAMINO)-1-(PYRIDIN-3-YL)BUTAN-1-ONE

FIELD OF THE INVENTION

The present invention is directed to the preparation of nicotine comprising the enzymatic reduction of 4-(methylamino)-1-(pyridin-3-yl)butan-1-one.

BACKGROUND OF THE INVENTION

Nicotine is a parasympathomimetic alkaloid found in plants of the *Nicotiana* genus of the Solanaceae plant family, such as tobacco, and functions as an antiherbivore chemical in the plant. The naturally occurring enantiomer is (S)-nicotine, which is more physiologically active and toxic than the corresponding (R)-enantiomer. Nicotine is an addictive substance and is the primary contributing factor to the dependence-forming properties of cigarette smoking. The main therapeutic use of nicotine is in nicotine replacement therapy, as part of smoking cessation; such replacement therapy comprises the administration of controlled levels of nicotine through gums, dermal patches, lozenges, electronic cigarettes or nasal sprays in an effort to wean off smoking.

Dunsmore et al [J. Am. Chem. Soc., 2006, 128(7), 2224-2225] describe the chemo-enzymatic deracemization of racemic nicotine using borohydride reduction in combination with oxidation by a monoamine oxidase; (S)-nicotine is oxidized in a stereoselective manner to a non-chiral intermediate which is concurrently reduced to

nicotine, thus causing an accumulation of the (R)-enantiomer.

The reduction of 4-(methylamino)-1-(pyridin-3-yl)butan-1-one to 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol in the preparation of racemic nicotine was described in Spath et al [Berichte der Deutschen Chemischen Gesellschaft [Abteilung] B: Abhandlungen (1928), 61B, 327-34]; the reduction was carried out using zinc dust in boiling alkaline ethanol solution.

SUMMARY OF THE INVENTION

The present invention provides a process for the preparation of nicotine comprising the enzymatic reduction of 4-(methylamino)-1-(pyridin-3-yl)butan-1-one [abbreviated herein pseudooxynicotine].

It has now been found that nicotine can be prepared by the enzymatic reduction of 4-(methylamino)-1-(pyridin-3-yl)butan-1-one using ketoreductase enzymes.

Accordingly, the present invention provides a process for the preparation of nicotine comprising the step of reacting 4-(methylamino)-1-(pyridin-3-yl)butan-1-one, or any of its forms which are in equilibrium in solution, with a ketoreductase enzyme.

In one embodiment, provided by the invention is a process for the preparation of 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol comprising reacting pseudooxynicotine, or any of its forms which are in equilibrium in solution, with a ketoreductase enzyme, wherein the ketoreductase is capable of converting the pseudooxynicotine to the corresponding alcohol. The 4-(methylamino)-1-(pyridin-3-

yl)butan-1-ol obtained is subsequently converted to nicotine.

In another embodiment, the invention provides a process for the direct preparation of nicotine from pseudooxynicotine comprising the step of reacting pseudooxynicotine, or any of its forms which are in equilibrium in solution, with a ketoreductase enzyme, wherein the ketoreductase is capable of converting the pseudooxynicotine directly to nicotine.

It has further been found that nicotine can be prepared directly from pseudooxynicotine by the enzymatic reduction of pseudooxynicotine using imine reductase enzymes.

Accordingly, in another embodiment, the invention provides a process for the direct preparation of nicotine from pseudooxynicotine comprising the step of reacting pseudooxynicotine, or any of its forms which are in equilibrium in solution, with an imine reductase enzyme, wherein the imine reductase is capable of converting the pseudooxynicotine directly to nicotine.

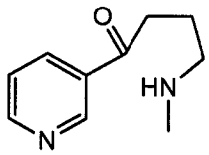
DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a process for the preparation of nicotine comprising the step of reacting 4-(methylamino)-1-(pyridin-3-yl)butan-1-one [pseudooxynicotine], or any of its forms which are in equilibrium in solution, with an enzyme, wherein said enzyme is capable of catalyzing the reduction of the pseudooxynicotine.

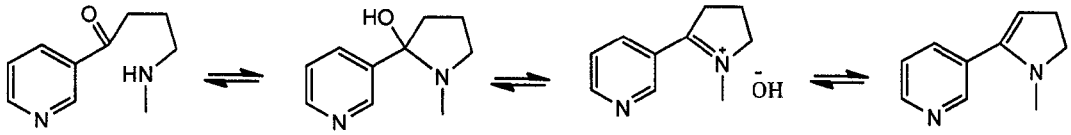
The invention provides a process for the preparation of nicotine comprising the step of reacting 4-(methylamino)-

1-(pyridin-3-yl)butan-1-one [pseudooxynicotine], or any of its forms which are in equilibrium in solution, with a ketoreductase enzyme.

It is noted that pseudooxynicotine, having the following chemical structure:



is present in solution in the following four different forms which are all in equilibrium:



Where used herein, the term "pseudooxynicotine" refers to pseudooxynicotine *per se* [i.e., 4-(methylamino)-1-(pyridin-3-yl)butan-1-one] or to any of its forms which are in equilibrium in solution.

As used herein, the term "ketoreductase" or "KRED" refers to an enzyme that catalyzes the reduction of a ketone, optionally in a stereoselective manner. Ketoreductase enzymes include, for example, those classified under EC 1.1.1. Kedoreductase enzymes are commercially available, for example, from Codexis, Inc.

The ketoreductase may be either a wild type or a variant enzyme, and may be either isolated from a natural source or synthesized, e.g. with recombinant technology.

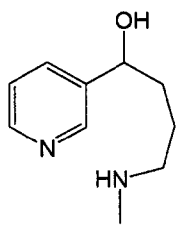
Examples of ketoredutases which may suitably be employed in the process of the invention include, but are not limited to, Codexis Inc's products with the following catalog numbers: KRED-101, KRED-119, KRED-130, KRED-NADH-

101, KRED-NADH-110, KRED-P1-B02, KRED-P1-B05, KRED-P1-B10, KRED-P1-B12, KRED-P1-C01, KRED-P1-H08, KRED-P1-H10, KRED-P2-B02, KRED-P2-C02, KRED-P2-C11, KRED-P2-D11, KRED-P2-D12, KRED-P2-G03, KRED-P3-B03, KRED-P3-G09, KRED-P3-H12 and mixtures thereof.

The use of enzymes as catalysts for the reduction reaction is typically environmentally advantageous and lower in cost in comparison to metallic reducing agents used in the prior art. Moreover, enzymes are selective towards the substrate and their use generally results in less by-products. Advantageously, the enzymatic reduction process of the invention may be carried out in a stereoselective manner, such that an optically active product is obtained.

In one embodiment of the invention, the ketoreductase reduces the pseudooxynicotine to the corresponding alcohol, namely 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol. The 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol obtained is useful as an intermediate in the preparation of nicotine.

Accordingly, in one aspect, the invention provides a process for the preparation of 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol, having the following chemical structure:



comprising reacting 4-(methylamino)-1-(pyridin-3-yl)butan-1-one [pseudooxynicotine], or any of its forms which are in equilibrium in solution, with a

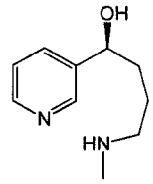
ketoreductase enzyme, wherein the ketoreductase is capable of converting the pseudooxynicotine to the corresponding alcohol. The 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol is subsequently converted to nicotine.

The reduction of pseudooxynicotine to the corresponding alcohol by the ketoreductase is advantageously carried out in a stereoselective manner, such that either (S)- or (R)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol is obtained in an enantiomeric excess. Preferably, (S)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol is obtained in an enantiomeric excess.

As used herein, the term "enantiomeric excess" (abbreviated "ee") refers to the excess of one enantiomer compared to that of the other enantiomer, and is defined as [F(R)-F(S)], wherein F(R) refers to the mole or weight fraction of the (R)-enantiomer and F(S) refers to the mole or weight fraction of the (S)-enantiomer. Correspondingly, the term "percent enantiomeric excess" or "%ee" is defined as [F(R)-F(S)]×100. Alternatively, the percent enantiomeric excess can be calculated using the following equation: %ee=([R]-[S])/([R]+[S])×100, wherein [R] and [S] are the molar concentration of the (R)-enantiomer and the (S)-enantiomer, respectively.

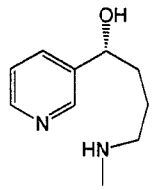
The term "optically active" refers to a chiral compound the enantiomeric excess of which differs from zero.

In one embodiment, the above process for the preparation of 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol provides (S)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol, having the following formula:



in an enantiomeric excess. The (S)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol preferably has an enantiomeric excess greater than about 95%, and more preferably greater than 99%, as determined by HPLC.

In another embodiment, the above process for the preparation of 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol provides (R)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol, having the following formula:



in an enantiomeric excess. The (R)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol preferably has an enantiomeric excess greater than about 95%, and more preferably greater than 99%, as determined by HPLC.

The starting material of the process, i.e. pseudooxynicotine, can be prepared according to any method known in the art, for example, according to the procedure disclosed in Spath et al [Berichte der Deutschen Chemischen Gesellschaft [Abteilung] B: Abhandlungen (1928), 61B, 327-34], which is **incorporated herein** by reference **in its entirety**. The procedure described in Spath et al comprises reacting ethyl nicotinate with **1-methyl-2-pyrrolidone in the presence of sodium ethoxide to give 1-methyl-3-nicotinoylpyrrolidin-2-one; the latter is then heated with fuming HCl at 130°C to afford pseudooxynicotine.**

The process for the preparation of 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol comprises reacting pseudooxynicotine, or any of its forms which are in equilibrium in solution, with a ketoreductase enzyme. The ketoreductase catalyzes the reduction of the ketone to the corresponding alcohol, advantageously in a stereoselective manner. Preferably, the ketoreductase is one that is capable of producing the product alcohol with a yield greater than about 50% and more preferably greater than about 90%.

Examples of ketoredutases which may be used for the reduction of pseudooxynicotine to 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol include, but are not limited to, Codexis Inc's products with the following catalog numbers: KRED-101, KRED-119, KRED-130, KRED-NADH-110, KRED-P1-B02, KRED-P1-B05, KRED-P1-B10, KRED-P1-B12, KRED-P1-C01, KRED-P1-H08, KRED-P1-H10, KRED-P2-B02, KRED-P2-C02, KRED-P2-C11, KRED-P2-D11, KRED-P2-D12, KRED-P2-G03 and combinations thereof.

For the preparation of (S)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol in an enantiomeric excess, the ketoreductase may be chosen from the group consisting of KRED-101, KRED-P1-B02, KRED-P1-B05, KRED-P1-B10, KRED-P1-B12, KRED-P1-C01, KRED-P1-H10, KRED-P2-B02, KRED-P2-C02, KRED-P2-D11 and combinations thereof. Preferably, the ketoreductase is chosen from the group consisting of KRED-P1-B02, KRED-P1-B05, KRED-P1-B10, KRED-P1-B12 and KRED-P2-D11, with KRED-P1-B10 and KRED-P1-B12 being especially preferred.

For the preparation of (R)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol in an enantiomeric excess, KRED-P2-C11 is conveniently used as the ketoreductase.

The reduction of pseudooxynicotine by kedoreductase is carried out in the presence of a co-factor. As used herein, the term "co-factor" refers to a compound that operates in combination with the enzyme that catalyzes the reaction of interest. The co-factor, in its reduced form, reacts as a hydride donor. Examples for suitable co-factors for use in the process of the invention include, but are not limited to, nicotinamide co-factors such as reduced nicotinamide adenine dinucleotide ("NADH"), nicotinamide adenine dinucleotide ("NAD^+"), reduced nicotinamide adenine dinucleotide phosphate ("NADPH"), nicotinamide adenine dinucleotide phosphate ("$NADP^+$") and any salts, derivatives or analogs thereof.

When the ketoreductase is NADH-dependent, the co-factor is suitably selected from NADH, NAD^+ and mixtures thereof. When the ketoreductase is NADPH-dependent, the co-factor is suitably selected from NADPH, $NADP^+$ and mixtures thereof.

The process of the invention may advantageously be carried out, further to the co-factor, in the presence of a co-factor regenerating system, capable of regenerating the oxidized form of the co-factor to its reduced form. The co-factor regenerating system comprises a dehydrogenase and a corresponding substrate. Non-limiting examples of suitable regenerating systems include glucose dehydrogenase and D-glucose, glucose phosphate dehydrogenase and glucose-6-phosphate, formate dehydrogenase and formate, and ketoreductase or alcohol dehydrogenase and a secondary alcohol. Dehydrogenases are commercially available from e.g. Codexis Inc.

In some embodiments, the ketoreductase enzyme which catalyzes the reduction of pseudooxynicotine is also capable of converting a secondary alcohol, such as

isopropanol, to its corresponding ketone, thereby regenerating the oxidized form of the co-factor to its reduced form, in which case the addition of a further co-factor regenerating dehydrogenase may be omitted. In such embodiments, the process of the invention is carried out, in addition to the co-factor, in the presence of a co-factor regenerating substrate, such as a secondary alcohol, and preferably isopropanol.

The enzymatic reduction of pseudooxynicotine is carried out in a solvent, which is preferably water (as known in the art, an aqueous salt solution or a buffer may be used as needed in order to avoid denaturation of the reductase enzymes). A water-miscible co-solvent may be added, typically in an amount of up to about 90%. In such cases where isopropanol is used as a co-factor regenerating substrate, the solvent consists of a mixture of isopropanol and water. The reduction may be carried out in a buffer, which is preferably a solution of one or more salts, such as magnesium sulfate, potassium phosphate and mixtures thereof. Preferably, the buffer has a pH from about 4 to about 9, more preferably from about 6 to about 8.

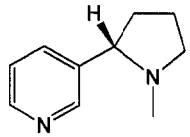
The concentration of the pseudooxynixotine starting material in the solution is typically within the range of 1 to 1000 mM, and preferably 10 to 100 mM. The ketoreductase is added in a catalytically effective amount (typically between about 0.01% and 20% weight enzyme/ weight pseudooxynicotine, and preferably between about 0.1% and 10%).

The process of the invention may be carried out at a temperature between about 10°C and 50°C, more preferably between about 25°C and 30°C.

The pseudooxynicotine is allowed to contact the ketoreductase for a duration of from about 2 to 48 hours, and preferably from about 20 to 24 hours. Upon completion of the reaction, the obtained 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol may be isolated and purified by means of conventional techniques such as extraction, evaporation, filtration and chromatography.

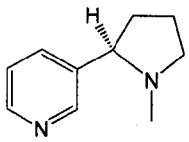
The 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol is useful as an intermediate in the preparation of nicotine. Accordingly, the present invention provides the use of 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol prepared by the above-described enzymatic reduction process in the preparation of nicotine. Additionally provided is a process for the preparation of nicotine comprising preparing 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol from pseudooxynicotine by the above-described enzymatic reduction process and converting it to nicotine.

In a preferred embodiment, optically active 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol is converted to (S)-nicotine [i.e. 3-[(2S)-1-methylpyrrolidin-2-yl]pyridine], corresponding to the following formula:



The (S)-nicotine obtained has an enantiomeric excess, which is preferably above 50%, 80%, 90%, 95% and most preferably above 99%, as determined by HPLC.

In another embodiment, optically active 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol is converted to (R)-nicotine, [i.e. 3-[(2R)-1-methylpyrrolidin-2-yl]pyridine], corresponding to the following formula:



The (R)-nicotine obtained has an enantiomeric excess, which is preferably above 50%, 80%, 90%, 95% and most preferably above 99%, as determined by HPLC.

The conversion of 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol to nicotine may be carried out by reacting the 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol with a compound of formula RSO_2X, wherein R represents an alkyl or optionally-substituted aryl, and X represents a halogen, and subsequently adding a base to the reaction mixture. Preferably, R is selected from methyl and toluene, and X is chloride.

It has been found that the above-described reaction of optically active 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol with RSO_2X to give nicotine results in an inversion of the stereochemistry. Accordingly, the use of (S)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol as starting material will lead to the formation of (R)-nicotine, while the use of (R)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol as starting material will lead to the formation of (S)-nicotine. Preferably, the 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol starting material is optically active, such that an optically active nicotine product is obtained.

More specifically, 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol is allowed to react with RSO_2X, wherein R and X have the above-mentioned meanings, in a suitable solvent, following which a base is added to the reaction mixture to afford nicotine. The solvent used is preferably propylene carbonate. It has been generally observed that

the stereoselectivity of the reaction is temperature dependent, i.e. at a higher reaction temperature, a higher enantiomeric excess is obtained (in the case that the starting material and, accordingly, the desired nicotine product are optically active). The reaction of the alcohol with RSO_2X is thus suitably carried out under heating, wherein the reaction temperature is maintained at about 20°C to 80°C for about 2 to 96 hours. Optionally, a nucleophilic catalyst, such as dimethylaminopyridine, may be added to the reaction mixture. Subsequently, a base is added to the reaction mixture. Bases operable in the reaction are preferably inorganic, with the hydroxides of alkali metals (e.g., potassium hydroxide or sodium hydroxide) being especially preferred. Upon completion of the reaction (typically, between 10 and 30 minutes after the addition of the base) the obtained nicotine is isolated from the reaction mixture by means of extractive procedure, following which the solvent used for the extraction is removed by evaporation. Other conventional methods of isolation and purification may be applied in order to collect the product nicotine.

As an alternative to the above reaction, the 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol may conveniently be transformed to nicotine through a Mitsunobu reaction. To this end, 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol is dissolved in a suitable solvent, such as dichloromethane, propylene carbonate or solvents promoting S_N2 reaction [i.e. polar aprotic solvents]. Triphenylphosphine is added and the mixture cooled to 0°C. Optionally, a basic amine, such as triethylamine, may be added to prevent the formation of undesired by-products. An azodicarboxylate compound, such as diethyl azodicarboxylate or diisopropyl azodicarboxylate, is

dissolved in a suitable solvent such as dichloromethane, propylene carbonate or solvents promoting S_N2 reaction [i.e. polar aprotic solvents], and added dropwise to the reaction mixture. The mixture is maintained under stirring at 0°C for, typically, between about 2 to 24 hours. The obtained nicotine is subsequently isolated from the reaction mixture by conventional means. Most conveniently, the nicotine is isolated by means of extractive procedure, following which the solvent used for the extraction is removed by evaporation. Other conventional methods of isolation and purification may be applied in order to collect the product nicotine.

In the course of the Mitsunobu reaction, the stereochemistry is inverted. Therefore, the use of (S)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol as starting material will lead to the formation of (R)-nicotine, while the use of (R)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol as starting material will lead to the formation of (S)-nicotine. Preferably, the 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol starting material is optically active, such that an optically active nicotine product is obtained.

A third suitable route for the formation of nicotine from 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol, in which route the stereochemistry of the starting material is retained, comprises chlorination of the aminoalcohol followed by cyclization, which is conveniently accomplished as follows.

4-(methylamino)-1-(pyridin-3-yl)butan-1-ol is reacted with thionylchloride [$SOCl_2$] in a suitable solvent, such as propylene carbonate. The weight ratio between thionylchloride and the 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol starting material in the reaction mixture

is typically in the range between 1:1 and 10:1, and preferably between 1.5:1 and 4:1. The reaction is suitably carried out at a temperature between about 20°C to 80°C for about 2 to 96 hours. Subsequently, a base is added to the reaction mixture. Bases operable in the reaction are preferably inorganic, with the hydroxides of alkali metals (e.g., potassium hydroxide or sodium hydroxide) being especially preferred. Upon completion of the reaction (typically, between 10 and 30 minutes after the addition of the base) the obtained nicotine is isolated from the reaction mixture by means of extractive procedure, following which the solvent used for the extraction is removed by evaporation. Other conventional methods of isolation and purification may be applied in order to collect the product nicotine.

The preparation of nicotine by the above route will result in (S)-nicotine when (S)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol is used as the starting material, and (R)-nicotine when (R)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol is used as the starting material. Preferably, the 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol starting material is optically active, such that an optically active nicotine product is obtained.

It has been found that some ketoreductase enzymes are capable of converting pseudooxynicotine directly to nicotine, thereby omitting the need for further reaction steps in the preparation of nicotine using pseudooxynicotine as starting material.

Thus, in another aspect, the present invention provides a process for the direct preparation of nicotine from pseudooxynicotine comprising the step of reacting pseudooxynicotine, or any of its forms which are in equilibrium in solution, with a ketoreductase enzyme,

wherein the ketoreductase is capable of converting the pseudooxynicotine directly to nicotine.

By the terms "direct preparation of nicotine from pseudooxynicotine" and "converting the pseudooxynicotine directly to nicotine" is meant that nicotine is obtained by the above one-step process [i.e., the reduction of pseudooxynicotine by the enzyme] without the need for carrying out further reaction steps.

Examples of ketoreductases which may be used for the direct preparation of nicotine from pseudooxynicotine include Codexis Inc's products with the following catalog numbers: KRED-101, KRED-119, KRED-130, KRED-NADH-101, KRED-NADH-110, KRED-P3-B03, KRED-P3-G09, KRED-P3-H12 and combinations thereof, with KRED-P3-G09 being especially preferred.

In a preferred embodiment, the conversion of pseudooxynicotine to nicotine by the ketoreductase is carried out in a stereoselective manner, such that either (R)- or (S)-nicotine is obtained in an enantiomeric excess. The enantiomeric excess of the product nicotine is preferably above 50%, 80%, 90%, 95% and most preferably above 99%, as determined by HPLC.

For the direct preparation of (R)-nicotine in an enantiomeric excess, KRED-P3-G09 is conveniently used as the ketoreductase.

The above reaction is carried out in the presence of a co-factor, such as nicotinamide co-factors (e.g. NADH, NAD^+, NADPH, $NADP^+$ and mixtures thereof), and is advantageously further carried out in the presence of a co-factor regenerating system, comprising a dehydrogenase and a corresponding substrate, such as, for example, glucose dehydrogenase and D-glucose. In cases where the

ketoreductase which catalyzes conversion of pseudooxynicotine to nicotine is also capable of converting a secondary alcohol, such as isopropanol, to its corresponding ketone, thereby regenerating the oxidized form of the co-factor to its reduced form, the addition of a further co-factor regenerating dehydrogenase may be omitted; in such cases the reaction is carried out, in addition to the co-factor, in the presence of a co-factor regenerating substrate, such as a secondary alcohol, preferably isopropanol.

The above reaction is typically carried out by dissolving the pseudooxynicotine starting material in a suitable solvent, which is preferably water (as known in the art, an aqueous salt solution or a buffer may be used as needed in order to avoid denaturation of the reductase enzymes). A water-miscible co-solvent may be added, typically in an amount of up to about 90%. In such cases where isopropanol is used as a co-factor regenerating substrate, the solvent consists of a mixture of isopropanol and water. The reaction may be carried out in a buffer, which is preferably a solution of one or more salts, such as magnesium sulfate, potassium phosphate and mixtures thereof. Preferably, the buffer has a pH from about 4 to about 9, more preferably from about 6 to about 8.

The concentration of the pseudooxynixotine starting material in the solution is generally within the range of 1 to 1000 mM, and preferably 10 to 100 mM. The ketoreductase is added in a catalytically effective amount (typically between about 0.01% and 20% weight enzyme/ weight pseudooxynicotine, and preferably between about 0.1% and 10%).

The pseudooxynicotine is allowed to contact the ketoreductase for a duration of from about 1 to 4 days, at a temperature of, for example, between about 10°C and 50°C, and preferably between about 20°C and 30°C. Upon completion of the reaction, the obtained nicotine may be isolated and purified by means of conventional techniques such as extraction, evaporation, filtration and chromatography.

It has been found that nicotine can be prepared directly from pseudooxynicotine by the enzymatic reduction of pseudooxynicotine using imine reductase enzymes.

Accordingly, in another aspect, the present invention provides a process for the direct preparation of nicotine from pseudooxynicotine comprising the step of reacting pseudooxynicotine, or any of its forms which are in equilibrium in solution, with an imine reductase enzyme, wherein the imine reductase is capable of converting the pseudooxynicotine directly to nicotine.

As used herein, the term "imine reductase" or "IRED" refers to an enzyme that catalyzes the reduction of an imine, or of an iminium cation, optionally in a stereoselective manner. Imine reductase enzymes include, for example, those classified under EC 1.5.1. Some imine reductase enzymes are commercially available, for example, from Enzymicals AG (Greifswald, Germany). The imine reductase may be either a wild type or a variant enzyme.

As an example, imine reductase from Streptomyces sp. GF3546 or imine reductase from Streptomyces sp. GF3587 may be employed as the imine reductase in the process of the invention.

The imine reductase may be isolated from a natural source, such as microorganisms having the ability of reducing imines. Examples of microorganisms comprising imine reductases which may be employed in the process of the invention include Streptomyces sp. GF3546 and Streptomyces sp. GF3587. As an alternative, microorganisms having the ability of reducing imines may be employed in the process of the invention without isolation and purification of the imine reductase enzymes.

Alternatively, imine reductases which may be employed in the process of the invention may be synthesized, e.g. with recombinant technology. In this case, a recombinant DNA construct encoding the imine reductase is transplanted into a host organism and expressed.

The production by recombinant technology of the imine reductase comprised in Streptomyces sp. GF3546 may be carried out by the process described in Leipold et al [ChemCatChem, 2013, 5(12), 3505-3508], which is incorporated herein by reference in its entirety (including its supporting information). According to Leipold, the enzyme is produced by expression in E. coli bearing a plasmid with an inserted codon-optimized gene of the imine reductase; following cultivation, biomass is harvested by centrifugation and cell disruption is carried out using ultrasonication; the crude enzyme extract is obtained by centrifugation and subsequently purified by chromatography.

The isolation of the imine reductase from Streptomyces sp. GF3546, and its production by recombinant technology, can be carried out, for example, according to the procedures described in US2011/0262977, which is incorporated herein by reference in its entirety. The

isolation of the imine reductase from Streptomyces sp. GF3587, and its production by recombinant technology, can be carried out, for example, according to the procedures described in US2011/0287494, which is incorporated herein by reference in its entirety.

In a preferred embodiment, the conversion of pseudooxynicotine to nicotine by the imine reductase is carried out in a stereoselective manner, such that either (R)- or (S)-nicotine is obtained in an enantiomeric excess. As an example, imine reductase from Streptomyces sp. GF3546 or imine reductase from Streptomyces sp. GF3587 may be used to obtain (R)-nicotine in an enantiomeric excess. The enantiomeric excess of the product nicotine is preferably above 50%, 70%, 80%, 90%, 95% and most preferably above 99%, as determined by HPLC.

The above reaction is carried out in the presence of a co-factor, such as nicotinamide co-factors (e.g. NADH, NAD^+, NADPH, $NADP^+$ and mixtures thereof), and is advantageously further carried out in the presence of a co-factor regenerating system, comprising a dehydrogenase and a corresponding substrate, such as, for example, glucose dehydrogenase and D-glucose. In cases where the imine reductase which catalyzes conversion of pseudooxynicotine to nicotine is also capable of converting a secondary alcohol, such as isopropanol, to its corresponding ketone, thereby regenerating the oxidized form of the co-factor to its reduced form, the addition of a further co-factor regenerating dehydrogenase may be omitted; in such cases the reaction is carried out, in addition to the co-factor, in the presence of a co-factor regenerating substrate, such as a secondary alcohol, preferably isopropanol.

The above reaction is typically carried out by dissolving the pseudooxynicotine starting material in a suitable solvent, which is preferably water (as known in the art, an aqueous salt solution or a buffer may be used as needed in order to avoid denaturation of the reductase enzymes). A water-miscible co-solvent may be added, typically in an amount of up to about 90%. In such cases where isopropanol is used as a co-factor regenerating substrate, the solvent consists of a mixture of isopropanol and water. The reaction may be carried out in a buffer, which is preferably a solution of one or more salts, such as magnesium sulfate, potassium phosphate and mixtures thereof. Preferably, the buffer has a pH from about 4 to about 9, more preferably from about 6 to about 8.

The concentration of the pseudooxynixotine starting material in the solution is generally within the range of 1 to 1000 mM, and preferably 10 to 100 mM. The imine reductase is added in a catalytically effective amount (typically between about 0.01% and 20% weight enzyme/ weight pseudooxynicotine, and preferably between about 0.1% and 10%).

The pseudooxynicotine is allowed to contact the imine reductase for a duration of from about 2 hours to 4 days, at a temperature of, for example, between about 10°C and 50°C, and preferably between about 20°C and 30°C). Upon completion of the reaction, the obtained nicotine may be isolated and purified by means of conventional techniques such as extraction, evaporation, filtration and chromatography.

The invention is further illustrated by the following examples, which are not to be construed as limiting.

EXAMPLES

All percentages herein are weight percentages unless otherwise indicated.

Where used herein, the term "room temperature" refers to a temperature in the range from about 20°C to 30°C, such as, for example, 25°C.

MATERIALS

4-(methylamino)-1-(pyridin-3-yl)butan-1-one hydrochloride was supplied by Chemagis. All enzymes used in examples 1-5 were purchased from Codexis Inc. All other chemicals were of analytical grade and were purchased from Sigma Aldrich.

METHODS

HPLC analysis was performed on a Shimadzu Prominence LC20 system consisting of a LC-20AT gradient pump, DGU-20A solvent degasser, SIL-20AC autosampler, SPD-20A UV detector, RID-10A RI detector and a Jasco CO1560 column oven at 21 °C.

Non-chiral HPLC method (used in Examples 1-5): Analyses were performed using two columns in series; phenomenex Gemini C18 150 x 4.6 mm x 5 micron, followed by Varian Polaris C18A 100 x 3 mm x 5 micron. The eluent consisted of pure water (+0.1 % TFA) with a flowrate of 0.6 ml/min. Detection was performed using a UV detector at 254 nm. 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol elutes at 4.5 minutes, 4-(methylamino)-1-(pyridin-3-yl)butan-1-one at 8.5 minutes and nicotine at 6.1 minutes.

Non-chiral HPLC method (used in Example 6): Analyses were carried out in gradient elution mode, using a Waters XBridge Shield RP18 150 x 4.6 mm x 5 micron column. The eluent profile is presented in Table 1 below, wherein Eluent A was prepared by mixing 900 mL water, 25 mL of a 60 g/L acetic acid solution and 6 mL concentrated ammonia, adjusting the pH to 10 with dilute ammonia or dilute acetic acid, and adding water to a final volume of 1000 mL. Eluent B consists of acetonitrile.

Table 1

Time (minutes)	Mobile Phase A (%v/v)	Mobile Phase B (%v/v)
0-3	100	0
3-3.01	100 → 95	0 → 5
3.01-28	95 → 74	5 → 26
28-32	74 → 60	26 → 40

The eluent flow rate was 1.0 mL/minute. Detection was performed using a UV detector at 254 nm; 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol elutes at 9 minutes, 4-(methylamino)-1-(pyridin-3-yl)butan-1-one at 10 minutes and nicotine at 20 minutes.

Chiral HPLC method for 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol*:* Samples of 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol were analyzed for enantiopurity using a Phenomenex Lux cellulose 2 column 150 x 4.6 mm x 5 micron. The eluent consisted of acetonitrile (+0.1% DEA)/2-propanol in a volume ratio of 95/5 with a flow rate of 1.5 ml/minute. Detection was performed using a UV detector at 254 nm. The alcohol peaks elute at 9 minutes (R enantiomer) and 14 minutes (S enantiomer).
The absolute chiral configuration of the alcohol was determined by having the product of sample no. 5 of Table

1, Example 1 (see below) undergo a Mitsunobu reaction (see Example 5 below for the general procedure), which is known to cause an inversion of stereochemistry, and comparing the product obtained in a chiral HPLC with S-nicotine.

Chiral HPLC method for nicotine: Samples of nicotine were analyzed for enantiopurity using a Phenomenex Lux amylose 2 column 250 x 4.6 mm x 3 micron. The eluent consisted of heptane (+0.1% DEA)/2-propanol in a volume ratio of 95/5 with a flow rate of 1.0 ml/minute. Detection was performed using the UV detector at 254 nm. The nicotine peaks elute at 14 minutes (S enantiomer) and 15.5 minutes (R enantiomer). The absolute chiral configuration was determined using *S*-nicotine as reference material.

Preparation 1

Synthesis of racemic 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol

Racemic 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol was synthesized from pseudooxynicotine for use as a reference material for the HPLC analytical analyses.

Protection of pseudooxynicotine: Pseudooxynicotine dihydrochloride (250 mg, 1 mmol) was added to a mixture of dioxane (10 ml) and water (10 ml). Sodium hydroxide (400 mg, 10 mmol) was added and the reaction mixture stirred until full dissolution. Di-*tert*-butyl dicarbonate (abbreviated BOC anhydride, 250 mg, 1.15 mmol) was added and the reaction mixture maintained under stirring at 20°C overnight.

Reduction of the protected pseudooxynicotine: NaBH4 (75 mg, 2eq) was added to the reaction mixture. After 2

hours, 35 additional mg of NaBH4 were added and the reaction mixture maintained for 4 additional hours at room temperature. The protected alcohol obtained was isolated from the reaction mixture and purified by column chromatography using a silica column and heptane/EtOAc/EtOH in a volume ratio of 4:4:2 as eluent.

Removal of the protection group: Toluene (10 mL) and aqueous HCl (37%) were added to the purified protected alcohol; gas formation was observed (indicative of the formation of CO_2). After one hour the reaction mixture was subjected to evaporation; the remaining liquid was identified by 1H-NMR and 13C-NMR as 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol.

Example 1

Preparation of 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol by the enzymatic reduction of pseudooxynicotine

Reaction systems were prepared to provide for the regeneration of the enzymes. Two different systems were used, depending on the enzyme/cofactor regenerating system:

a) KRED/GDH/glucose system (hereinafter, System A):

A stock solution was prepared, consisting of 5.5 mL water, 250 mM potassium phosphate, 2 mM magnesium sulfate, 1.1 mM NADP+, 1.1 mM NAD+, 10 U/ml glucose dehydrogenase and 80 mM glucose. 137 mg of pseudooxynicotine dihydrochloride was added to the solution. 1 mL potassium hydroxide solution (1 M) was added to neutralize the pH of the solution.

5 mg of ketoreductase was added to a vial comprising 1 mL of the above-described stock solution and the mixture was stirred at room temperature for a duration of 21 hours to

give a crude product mixture. Non-chiral analyses were performed by diluting 20 microliter samples of the mixture to 1 mL with water (containing 0.1% trifluoroacetic acid) and injecting the diluted samples to the non-chiral HPLC (injection volume 5 microliter). Chiral analyses were performed by diluting 200 microliter samples of the mixture to 1 mL with ethanol (containing 0.1% diethyl amine) and injecting the diluted samples to the chiral HPLC (injection volume 5 microliter).

b) KRED/2-propanol system (hereinafter, System B):

A stock solution was prepared, consisting of 9.2 mL water, 125 mM potassium phosphate, 1.25 mM magnesium sulfate and 1.0 mM NADP+. 178 mg of pseudooxynicotine dihydrochloride was added to the solution. 1.8 mL potassium hydroxide solution (1 M) was added to neutralize the pH of the solution.

5 mg of ketoreductase was added to a vial comprising 1 mL of the above-described stock solution and 3.8 mL isopropanol, and the mixture was stirred at room temperature for a duration of 21 hours to give a product mixture. Non-chiral analyses were performed by diluting 20 microliter samples of the mixture to 1 mL with water (containing 0.1% trifluoroacetic acid) and injecting the diluted samples to the non-chiral HPLC (injection volume 5 microliter). Chiral analyses were performed by diluting 500 microliter samples of the mixture to 1 mL with ethanol (containing 0.1% diethyl amine) and injecting the diluted samples to the chiral HPLC (injection volume 5 microliter).

The results are presented in Table 1 below.

Table 1

Sampl	Ketoreductas	syste	Pseudooxynicotin	ee, R-	ee, S-

e no.	e (Codexis catalog no.)	m	e conversion	enantiomer	enantiomer
1	KRED-101	A	2.5%		83%
2	KRED-NADH-110	A	14.9%		0%
3	KRED-P1-B02	B	60.3%		>99%
4	KRED-P1-B05	B	6.5%		>99%
5	KRED-P1-B10	B	94.3%		>99%
6	KRED-P1-B12	B	100%		>99%
7	KRED-P1-C01	B	46.4%		89%
8	KRED-P1-H10	B	3.6%		52%
9	KRED-P2-B02	B	9.4%		66%
10	KRED-P2-C02	B	13.0%		25%
11	KRED-P2-C11	B	3.1%	>99%	
12	KRED-P2-D11	B	45.8%		96%

Example 2

Synthesis of nicotine by the direct reduction of pseudooxynicotine

A stock solution was prepared, consisting of 5.5 mL water, 125 mM potassium phosphate, 1.25 mM magnesium sulfate and 1.0 mM NADP+. 81 mg of pseudooxynicotine dihydrochloride was added to the solution. Potassium hydroxide solution (1 M) was added to neutralize the pH of the solution.

25 mg of ketoreductase KRED-P3-G09 was added to a vial comprising 2.5 mL of the above-described stock solution and 9.5 mL isopropanol, and the mixture was stirred at room temperature for a duration of 4 days. Subsequently, the reaction mixture was evaporated and the residue redissolved in 3 mL of DCM/diethylether in a volume ratio of 1:2. The solution was washed with an HCl solution (1M, 2 x 4 mL). Sodium hydroxide solution (1 M) was added to the aqueous layer to neutralize its pH, as indicated by pH paper. The aqueous layer was then subjected to

extraction using dichloromethane (3 x 10 mL). The solvent of the organic layer was evaporated to give a residue.
Non-chiral analysis was performed by adding 1 mL of water (containing 0.1% trifluoroacetic acid) to the residue and injecting to the non-chiral HPLC (injection volume 5 microliter). Chiral analysis was performed by adding 1 mL of ethanol (containing 0.1% diethyl amine) to the residue and injecting to the chiral HPLC (injection volume 5 microliter).
The HPLC analyses revealed that (R)-nicotine was obtained with an enantiomeric excess of 92%.

Example 3

Preparation of (R)-nicotine from (S)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol by mesylchloride mediated cyclisation

(S)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol was prepared according to Example 1 using KRED-P1-B12 as the ketoreductase. 1 mL of the crude product mixture was evaporated to dryness under vacuum over P_2O_5. The residue obtained was dissolved in 2 mL of propylene carbonate. Mesylchloride (10 microliters) was added and the mixture maintained at a temperature of 50°C for 72 hours. 2 mL of potassium hydroxide solution (10% weight/volume) was added and the reaction mixture stirred for 30 minutes. The mixture was then extracted with dichloromethane, the layers separated and the organic layer was evaporated to dryness.
Non-chiral analysis was performed by adding 1 mL of water (containing 0.1% trifluoroacetic acid) to the obtained residue and injecting to the non-chiral HPLC (injection volume 5 microliter). Chiral analysis was performed by adding 1 mL of ethanol (containing 0.1% diethyl amine) to

the obtained residue and injecting to the chiral HPLC (injection volume 5 microliter).
The HPLC analyses revealed that (R)-nicotine was obtained with an enantiomeric excess of 92%.

Example 4

Preparation of (S)-nicotine from (S)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol by thionylchloride mediated cyclisation

(S)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol was prepared according to Example 1 using KRED-P1-B12 as the ketoreductase. 1 mL of the crude product mixture was evaporated to dryness under vacuum over P_2O_5. The residue obtained was dissolved in 2 mL of propylene carbonate.
Thionylchloride (50 microliters) was added and the mixture maintained at 20°C for 20 hours. 2 mL of potassium hydroxide solution (10% weight/volume) was added and the reaction mixture stirred for 30 minutes. The mixture was then extracted with dichloromethane, the layers separated and the organic layer was evaporated to dryness.
Non-chiral analysis was performed by adding 1 mL of water (containing 0.1% trifluoroacetic acid) to the obtained residue and injecting to the non-chiral HPLC (injection volume 5 microliter). Chiral analysis was performed by adding 1 mL of ethanol (containing 0.1% diethyl amine) to the obtained residue and injecting to the chiral HPLC (injection volume 5 microliter).
According to the HPLC analyses, (S)-nicotine was obtained with an enantiomeric excess of 93%.

Example 5

Preparation of nicotine from 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol by Mitsunobu reaction

(S)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol and (R)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol were prepared according to Example 1 using KRED-P1-B10 and KRED-P2-C11 as the ketoreductases, respectively. Following is a general procedure for the preparation of nicotine by the Mitsunobu reaction from each of the two 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol enantiomers.

2 mL of the crude product mixture was filtered to remove solids and evaporated to dryness under vacuum over P_2O_5. The residue obtained was dissolved in 2 mL of dichloromethane. Triethylamine (25 microliters, 0.18 mmol) and triphenylphosphine (47 mg, 0.18 mmol) were added and the mixture cooled to 0°C. Diisopropyl azodicarboxylate (35 microliters) dissolved in 1 mL dichloromethane was added dropwise and the mixture kept under stirring at 0°C for 2 hours. Subsequently, the reaction mixture was subjected to evaporation and the residue redissolved in 3 mL of dichloromethane/diethylether in a volume ratio of 1:2, following which the solution was washed with an HCl solution (1 M, 2 x 4 mL).

Sodium hydroxide solution (1 M) was added to the combined aqueous fractions until their pH became alkaline, as indicated by pH paper. The combined fractions were subsequently extracted with dichloromethane (3 x 10 ml), the layers separated and the organic layer evaporated to dryness.

Non-chiral analysis was performed by adding 1 mL of water (containing 0.1% trifluoroacetic acid) to the obtained residue and injecting to the non-chiral HPLC (injection volume 5 microliter). Chiral analysis was performed by adding 1 mL of ethanol (containing 0.1% diethyl amine) to the obtained residue and injecting to the chiral HPLC (injection volume 5 microliter).

The results are presented in Table 2 below.

Table 2

Sample no.	Starting material	Product	Enantiomeric excess
1	(S)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol	(R)-nicotine	98%
2	(R)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol	(S)-nicotine	93%

Example 6

Synthesis of nicotine by the direct reduction of pseudooxynicotine

To a vial were added 2.5 mg magnesium sulfate, 4 mg NADP+, 1 mg glucose dehydrogenase, 180 mg glucose and 5 mL phosphate buffer (pH 7.5, 250 mM). Pseudooxynicotine hydrochloride (130 mg) was added and the pH was checked to be 7.

500 mg of isolated, lyophilized imine reductase from Streptomyces sp. GF3546 were added to the vial, and the mixture was stirred at room temperature for a duration of 24 hours.

Non-chiral analysis was performed by adding 1 mL of water (containing 0.1% trifluoroacetic acid) to a 20 microliter sample of the reaction mixture and injecting the diluted sample to the non-chiral HPLC (injection volume 5 microliters). Chiral analysis was performed by adding 500 microliters of ethanol (containing 0.1% trifluoroacetic acid) to a 500 microliter sample of the reaction mixture, centrifuging and injecting the diluted sample to the chiral HPLC (injection volume 5 microliters).

HPLC analysis revealed a 23% conversion of the pseudooxynicotine starting material (calculated according to HPLC peak area). According to chiral HPLC analysis, R-nicotine was obtained in an enantiomeric excess of 70%. The formation of 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol was not observed.

The above procedure was repeated using 500 mg isolated, lyophilized imine reductase from Streptomyces sp. GF3587 (instead of imine reductase from Streptomyces sp. GF3546). According to chiral HPLC analysis, R-nicotine was obtained in an enantiomeric excess of 73%. The formation of 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol was not observed.

CLAIMS

1. A process for the preparation of nicotine comprising the step of reacting pseudooxynicotine, or any of its forms which are in equilibrium in solution, with a ketoreductase enzyme.
2. The process according to claim 1 wherein the ketoreductase is selected from the group consisting of KRED-101, KRED-119, KRED-130, KRED-NADH-101, KRED-NADH-110, KRED-P1-B02, KRED-P1-B05, KRED-P1-B10, KRED-P1-B12, KRED-P1-C01, KRED-P1-H08, KRED-P1-H10, KRED-P2-B02, KRED-P2-C02, KRED-P2-C11, KRED-P2-D11, KRED-P2-D12, KRED-P2-G03, KRED-P3-B03, KRED-P3-G09, KRED-P3-H12 and mixtures thereof.
3. A process for the preparation of 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol, comprising reacting pseudooxynicotine, or any of its forms which are in equilibrium in solution, with a ketoreductase enzyme, wherein the ketoreductase is capable of converting the pseudooxynicotine to 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol.
4. The process according to claim 3 wherein (S)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol is obtained in an enantiomeric excess.
5. The process according to claim 3 wherein (R)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol is obtained in an enantiomeric excess.
6. The process according to claim 3 wherein the ketoreductase is selected from the group consisting of KRED-101, KRED-119, KRED-130, KRED-NADH-110, KRED-P1-B02, KRED-P1-B05, KRED-P1-B10, KRED-P1-B12, KRED-P1-C01, KRED-P1-H08, KRED-P1-H10, KRED-P2-B02, KRED-P2-C02, KRED-P2-C11, KRED-P2-D11, KRED-P2-D12, KRED-P2G03 and combinations thereof.

7. The process according to claim 4 wherein the ketoreductase is selected from the group consisting of KRED-101, KRED-P1-B02, KRED-P1-B05, KRED-P1-B10, KRED-P1-B12, KRED-P1-C01, KRED-P1-H10, KRED-P2-B02, KRED-P2-C02, KRED-P2-D11 and combinations thereof.
8. The process according to claim 7 wherein the ketoreductase is selected from the group consisting of KRED-P1-B02, KRED-P1-B05, KRED-P1-B10, KRED-P1-B12 and KRED-P2-D11 and combinations thereof.
9. The process according to claim 5 wherein the ketoreductase is KRED-P2-C11.
10. The process according to claim 4, wherein the (S)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol has an enantiomeric excess of greater than about 99 percent as determined by HPLC.
11. The process according to claim 5, wherein the (R)-4-(methylamino)-1-(pyridin-3-yl)butan-1-ol has an enantiomeric excess of greater than about 99 percent as determined by HPLC.
12. The process according to claim 3, wherein said process is carried out in the presence of a co-factor.
13. The process according to claim 12, wherein the co-factor is a nicotinamide co-factor.
14. The process according to claim 13, wherein the co-factor is selected from the group consisting of nicotinamide adenine dinucleotide (NAD^+), reduced nicotinamide adenine dinucleotide (NADH), nicotinamide adenine dinucleotide phosphate ($NADP^+$), reduced nicotinamide adenine dinucleotide phosphate (NADPH) and mixtures thereof.
15. The process according to claim 12, wherein said process is carried out in the presence of a co-factor regenerating substrate.

16. The process according to claim 15, wherein the co-factor regenerating substrate is 2-propanol.

17. The process according to claim 12, wherein said process is carried out in the presence of a co-factor regenerating system comprising a dehydrogenase and a substrate.

18. The process according to claim 17, wherein the dehydrogenase is glucose dehydrogenase and the substrate is D-glucose.

19. The process according to claim 3, wherein the process is carried out in water.

20. The process according to claim 3 wherein the pseudooxynicotine, or any of its forms which are in equilibrium in solution, is reacted with the ketoreductase for a duration of from about 2 to 48 hours.

21. The process according to claim 3 wherein said process is carried out at a temperature of from about 10 to 50°C.

22. Use of the 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol prepared according to the process of any of claims 3 to 21 in the preparation of nicotine.

23. A process for the preparation of nicotine comprising preparing 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol according to the process of any of claims 3 to 21, and converting said 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol to nicotine.

24. The process according to claim 1, wherein said process comprises preparing 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol according to any of claims 3 to 21, and converting said 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol to nicotine.

25. The process according to any of claims 23-24 wherein (S)-nicotine is prepared in an enantiomeric excess.

26. The process according to any of claims 23-24 wherein (R)-nicotine is prepared in an enantiomeric excess.

27. The process according to any of claims 23 to 26, wherein the conversion to nicotine comprises an inversion of stereochemistry.

28. The process according to any of claims 23 to 26, wherein the conversion to nicotine comprises retention of stereochemistry.

29. The process according to claim 27 wherein the conversion to nicotine comprises the steps of:

a) reacting the 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol with RSO_2X, wherein R represents an alkyl or optionally substituted aryl, and X represents a halogen; and

b) adding a base to the reaction mixture.

30. The process according to claim 27 wherein the conversion to nicotine is carried out through a Mitsunobu reaction.

31. The process according to claim 28 wherein the conversion to nicotine comprises the steps of:

a) reacting the 4-(methylamino)-1-(pyridin-3-yl)butan-1-ol with thionylchloride; and

b) adding a base to the reaction mixture.

32. The process according to claim 1, wherein nicotine is prepared directly from pseudooxynicotine, comprising the step of reacting pseudooxynicotine, or any of its forms which are in equilibrium in solution, with a ketoreductase enzyme, wherein the ketoreductase is capable of

converting the pseudooxynicotine directly to nicotine.

33. The process according to claim 32 wherein optically active nicotine is prepared.

34. The process according to claim 32 wherein (S)-nicotine is prepared in an enantiomeric excess.

35. The process according to claim 32 wherein (R)-nicotine is prepared in an enantiomeric excess.

36. The process according to claim 32 wherein the ketoreductase is selected from the group consisting of KRED-101, KRED-119, KRED-130, KRED-NADH-101, KRED-NADH-110, KRED-P3-B03, KRED-P3-G09, KRED-P3-H12 and combinations thereof.

37. The process according to claim 35 wherein the ketoreductase is KRED-P3-G09.

38. The process according to claim 35, wherein the (R)-nicotine has an enantiomeric excess of greater than about 99 percent as determined by HPLC.

39. The process according to claim 32 or claim 50, wherein said process is carried out in the presence of a co-factor.

40. The process according to claim 39, wherein the co-factor is a nicotinamide co-factor.

41. The process according to claim 40, wherein the co-factor is selected from the group consisting of nicotinamide adenine dinucleotide (NAD^+), reduced nicotinamide adenine dinucleotide (NADH), nicotinamide adenine dinucleotide phosphate ($NADP^+$), reduced nicotinamide adenine dinucleotide phosphate (NADPH) and mixtures thereof.

42. The process according to claim 39, wherein said process is carried out in the presence of a co-factor regenerating substrate.

43. The process according to claim 42, wherein the co-factor regenerating substrate is 2-propanol.

44. The process according to claim 39, wherein said process is carried out in the presence of a co-factor regenerating system comprising a dehydrogenase and a substrate.

45. The process according to claim 44, wherein the dehydrogenase is glucose dehydrogenase and the substrate is D-glucose.

46. The process according to claim 32 or claim 50, wherein the process is carried out in water.

47. The process according to claim 32 wherein the pseudooxynicotine, or any of its forms which are in equilibrium in solution, is reacted with the ketoreductase for a duration of from about 1 to 4 days.

48. The process according to claim 32 or claim 50 wherein said process is carried out at a temperature of from about 10 to 50°C.

49. Optically active nicotine prepared according to the process of any of claims 1-2, 23-48 and 50-57.

50. A process for the preparation of nicotine comprising reacting pseudooxynicotine, or any of its forms which are in equilibrium in solution, with an imine reductase enzyme, wherein the imine reductase is capable of converting the pseudooxynicotine to nicotine.

51. The process according to claim 50 wherein optically active nicotine is prepared.

52. The process according to claim 50 or 51 wherein the imine reductase is the imine reductase from Streptomyces sp. GF3546.

53. The process according to claim 50 or 51 wherein the imine reductase is the imine reductase from Streptomyces sp. GF3587.

54. The process according to any of claims 51-53 wherein (R)- nicotine is prepared in an enantiomeric excess.

55. The process according to claim 54, wherein the (R)-nicotine has an enantiomeric excess of greater than about 70 percent as determined by HPLC.

56. The process according to claim 51 wherein (S)-nicotine is prepared in an enantiomeric excess.

57. The process according to claim 50 wherein the pseudooxynicotine, or any of its forms which are in equilibrium in solution, is reacted with the imine reductase for a duration of from about 2 hours to 4 days.