

METHODS OF SYNTHESIS

Technical Field

- 5 This present disclosure relates to methods of synthesis of intermediate compounds in the synthesis of muscarinic acetylcholine receptor (mAChR) agonists; in particular, the present disclosure relates to enzymatic processes utilising a ketoreductase to perform a stereoselective reduction of a ketone to an alcohol.

10 Sequence Listing

- The instant application contains a Sequence Listing which has been submitted electronically as an XML file named "46696-0184WO1_ST26_SL.xml." The XML file, created on July 31, 2024, is 3,828 bytes in size. The material in the XML file is hereby incorporated by
15 reference in its entirety.

Technical Background

- Muscarinic acetylcholine receptors (mAChRs) are members of the G protein-coupled
20 receptor superfamily which mediate the actions of the neurotransmitter acetylcholine in both the central and peripheral nervous system. Five mAChR subtypes have been cloned, M1 to M5.

- Muscarinic receptors in the central nervous system, especially the M1 mAChR, play a critical
25 role in mediating higher cognitive processing. Diseases associated with cognitive impairments, such as Alzheimer's disease, are accompanied by loss of cholinergic neurons in the basal forebrain (Whitehouse et al., 1982 Science). In schizophrenia, which is also characterised by cognitive impairments, mAChR density is reduced in the pre-frontal cortex, hippocampus and caudate putamen of schizophrenic subjects (Dean et al., 2002 Mol
30 Psychiatry). Furthermore, in animal models, blockade or lesion of central cholinergic pathways results in profound cognitive deficits and non-selective mAChR antagonists have been shown to induce psychotomimetic effects in psychiatric patients. Cholinergic replacement therapy has largely been based on the use of acetylcholinesterase inhibitors to prevent the breakdown of endogenous acetylcholine. These compounds have shown
35 efficacy versus symptomatic cognitive decline in the clinic, but give rise to dose-limiting side

effects resulting from stimulation of peripheral M2 and M3 mAChRs including disturbed gastrointestinal motility, bradycardia, nausea and vomiting.

Alzheimer's disease (AD) is the most common neurodegenerative disorder (26.6 million people worldwide in 2006) that affects the elderly, resulting in profound memory loss and cognitive dysfunction. The aetiology of the disease is complex, but is characterised by two hallmark brain sequelae: aggregates of amyloid plaques, largely composed of amyloid- β peptide ($A\beta$), and neurofibrillary tangles, formed by hyperphosphorylated tau proteins. The accumulation of $A\beta$ is thought to be the central feature in the progression of AD and, as such, many putative therapies for the treatment of AD are currently targeting inhibition of $A\beta$ production. $A\beta$ is derived from proteolytic cleavage of the membrane bound amyloid precursor protein (APP). APP is processed by two routes, non-amyloidogenic and amyloidogenic. Cleavage of APP by γ -secretase is common to both pathways, but in the former APP is cleaved by an α -secretase to yield soluble APP α . The cleavage site is within the $A\beta$ sequence, thereby precluding its formation. However, in the amyloidogenic route, APP is cleaved by β -secretase to yield soluble APP β and also $A\beta$. In vitro studies have shown that mAChR agonists can promote the processing of APP toward the soluble, non-amyloidogenic pathway.

Preclinical studies have suggested that mAChR agonists display an atypical antipsychotic-like profile in a range of pre-clinical paradigms. Muscarinic receptors have also been implicated in the neurobiology of addiction. The reinforcing effects of cocaine and other addictive substances are mediated by the mesolimbic dopamine system where behavioural and neurochemical studies have shown that the cholinergic muscarinic receptor subtypes play important roles in regulation of dopaminergic neurotransmission.

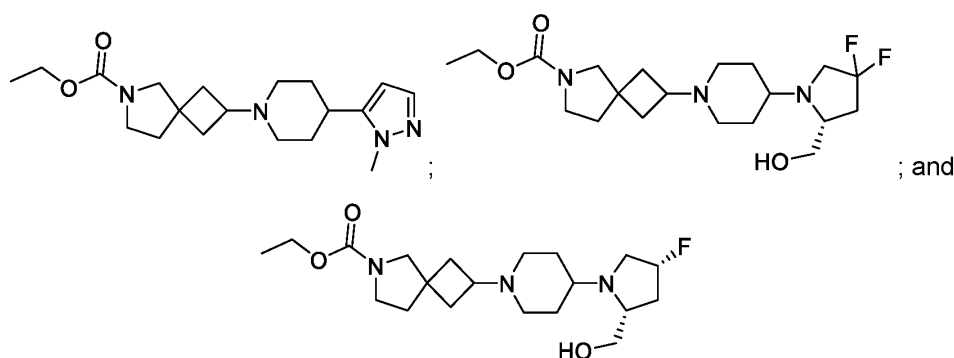
Muscarinic receptors are also involved in the control of movement and potentially represent novel treatments for movement disorders such as Parkinson's disease, ADHD, Huntington's disease, Tourette's syndrome and other syndromes associated with dopaminergic dysfunction as an underlying pathogenetic factor driving disease.

The mAChR agonists xanomeline, sabcomeline, milameline and cevimeline have all progressed into various stages of clinical development for the treatment of Alzheimer's disease and/or schizophrenia. However, in all clinical studies xanomeline and other related mAChR agonists have displayed an unacceptable safety margin with respect to cholinergic side effects, including nausea, gastrointestinal pain, diarrhoea, diaphoresis (excessive sweating), hypersalivation (excessive salivation), syncope and bradycardia.

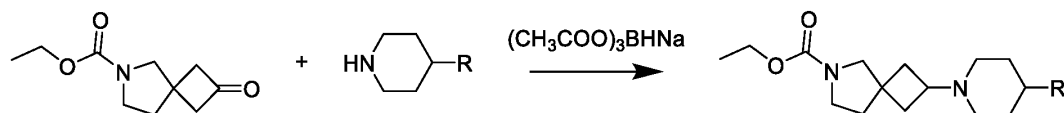
Muscarinic receptors are involved in central and peripheral pain. Pain can be divided into three different types: acute, inflammatory, and neuropathic. Activation of muscarinic receptors has been shown to be analgesic across a number of pain states through the activation of receptors in the spinal cord and higher pain centres in the brain. Increasing endogenous levels of acetylcholine through acetylcholinesterase inhibitors, direct activation of muscarinic receptors with agonists or allosteric modulators has been shown to have analgesic activity. In contrast blockade of muscarinic receptors with antagonists or using knockout mice increases pain sensitivity. Evidence for the role of the M1 receptor in pain is reviewed by D. F. Fiorino and M. Garcia-Guzman, 2012.

More recently, a small number of compounds have been identified which display improved selectivity for the M1 mAChR subtype over the peripherally expressed mAChR subtypes (Bridges et al., 2008 Bioorg Med Chem Lett; Johnson et al., 2010 Bioorg Med Chem Lett; Budzik et al., 2010 ACS Med Chem Lett). Despite increased levels of selectivity versus the M3 mAChR subtype, some of these compounds retain significant agonist activity at both this subtype and the M2 mAChR subtype.

WO2015/118342 describes certain mAChR agonist compounds including the compounds having the following structures:

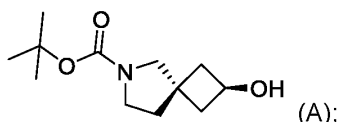


In WO2015/118342 these compounds are produced via a reaction between a spiroketone and a secondary amine in the presence of sodium triacetoxyborohydride ((CH₃COO)₃BHNa):



This synthetic route results in a mixture of isomers which are then separated via preparative HPLC chromatography. Such an approach may not be suitable for large-scale synthesis of these compounds.

- 5 Herein we describe synthesis of a trans-alcohol compound of Formula (A):

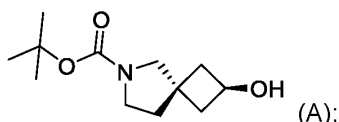


which is an intermediate for an improved synthetic route to mAChR agonist compounds which display high levels of selectivity for the M1 and/or M4 mAChR over the M2 and M3 receptor subtypes.

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Summary

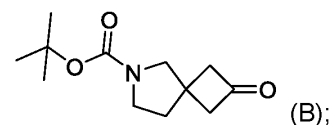
One aspect of the present disclosure is a process for preparing a trans-alcohol compound of Formula (A):



15

wherein:

the process comprises the step of reducing a compound of Formula (B):



20 in the presence of a ketoreductase enzyme having SEQ ID NO: 1 to provide the compound of Formula (A).

In one embodiment, the step of reducing a compound of Formula (B) is performed in the presence of NADPH as a cofactor.

- 25 In one embodiment, the NADPH cofactor is provided by a cofactor regeneration system comprising NADP⁺, glucose dehydrogenase, and glucose.

In one embodiment:

- 30 the NADP⁺ is present in a concentration of about 5 mM;
the glucose dehydrogenase is present in a concentration of about 1 g/L; and

the glucose is present in a concentration of about 178 mM.

In one embodiment:

the NADP⁺ is present in a concentration of about 15 mM;

5 the glucose dehydrogenase is present in a concentration of about 0.45 g/L;

and

the glucose is present in a concentration of about 247 mM.

10 In one embodiment, the compound of Formula (B) is present in a concentration of about 88 mM. In one embodiment, the compound of Formula (B) is present in a concentration of about 198 mM.

15 In one embodiment, the ketoreductase enzyme having SEQ ID NO: 1 is present in a concentration of about 1 g/L. In one embodiment, the ketoreductase enzyme is present in a concentration of about 2 g/L.

20 In one embodiment, the step of reducing a compound of Formula (B) is performed in the presence of potassium phosphate buffer. In one embodiment, the step of reducing a compound of Formula (B) is performed in the presence of about 100 mM potassium phosphate buffer. In one embodiment, the step of reducing a compound of Formula (B) is performed in the presence of about 100 mM potassium phosphate buffer at about pH 7.0.

In one embodiment:

the step of reducing a compound of Formula (B) is performed in the presence of:

25 (a) NADPH as a cofactor, wherein the NADPH cofactor is provided by a cofactor regeneration system comprising NADP⁺, glucose dehydrogenase, and glucose; and
(b) about 100 mM potassium phosphate buffer about pH 7.0.

In one embodiment:

30 the NADP⁺ is present in a concentration of about 5 mM;

the glucose dehydrogenase is present in a concentration of about 1 g/L;

the glucose is present in a concentration of about 178 mM;

the compound of Formula (B) is present in a concentration of about 88 mM;

and

35 the ketoreductase enzyme having SEQ ID NO: 1 is present in a concentration of about 1 g/L.

In one embodiment:

the NADP⁺ is present in a concentration of about 15 mM;

the glucose dehydrogenase is present in a concentration of about 0.45 g/L;

the glucose is present in a concentration of about 247 mM;

5 the compound of Formula (B) is present in a concentration of about 198 mM;

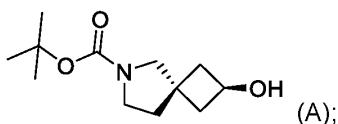
and

the ketoreductase enzyme having SEQ ID NO: 1 is present in a concentration of about 1 g/L.

10 In one embodiment, the step of reducing a compound of Formula (B) comprising heating at about 30 °C. In one embodiment, the step of reducing a compound of Formula (B) comprises stirring for about 20-24 h.

Another aspect of the present disclosure is a composition comprising a compound of

15 Formula (A):



wherein the compound of Formula (A) is present in a diastereomeric excess of about 96%de or about 97%de.

20 In one embodiment, the compound of Formula (A) is present in a diastereomeric excess of about 96%de.

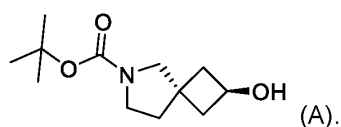
In one embodiment, the compound of Formula (A) is present in a diastereomeric excess of about 97%de.

Detailed DescriptionProcesses for Preparing Compounds

- 5 The present application provides processes for preparing intermediate compounds useful in the preparation of mAChR agonist compounds (e.g., the mAChR agonist compounds described in WO2015/118342), and salts thereof.

The trans-alcohol intermediate described herein has the structure of the following

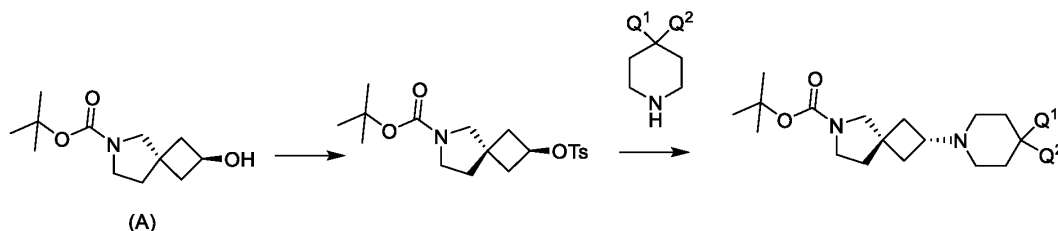
- 10 Formula (A):



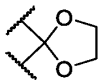
- A synthetic route to the mAChR agonist compounds utilising the trans-alcohol intermediate compound described herein, avoids the need to separate stereoisomers by preparative HPLC and therefore has improved developability for large scale synthesis.

- For example, mAChR agonist compounds (e.g., the mAChR agonist compounds described in WO2015/118342) may be produced via a synthetic route comprising the steps outlined in the following scheme:

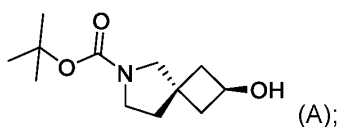
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wherein Q¹ is  and Q² is -H; or Q¹ and Q², together with the carbon atom to which

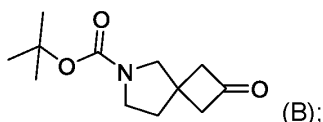
they are attached form a cyclic ketal, e.g., .

Accordingly, provided herein are processes for preparing a trans-alcohol compound of Formula (A):



wherein:

- 5 the process comprises the step of reducing a compound of Formula (B):



in the presence of a ketoreductase enzyme having SEQ ID NO: 1 to provide the compound of Formula (A).

- 10 In one embodiment, the step of reducing a compound of Formula (B) is performed in the presence of NADPH as a cofactor.

In one embodiment, the NADPH cofactor is provided by a cofactor regeneration system comprising NADP⁺, glucose dehydrogenase, and glucose.

- 15 In one embodiment, the NADP⁺ is present in a concentration of from about 5 mM to about 15 mM. In one embodiment, the NADP⁺ is present in a concentration of about 5 mM. In one embodiment, the NADP⁺ is present in a concentration of about 15 mM.

- 20 In one embodiment, the glucose dehydrogenase is present in a concentration of from about 1 g/L to about 0.45 g/L. In one embodiment, the glucose dehydrogenase is present in a concentration of about 1 g/L. In one embodiment, the glucose dehydrogenase is present in a concentration of about 0.45 g/L.

- 25 In one embodiment, the glucose is present in a concentration of from about 178 mM to about 247 mM. In one embodiment, the glucose is present in a concentration of about 178 mM. In one embodiment, the glucose is present in a concentration of about 247 mM.

In one embodiment:

- 30 the NADP⁺ is present in a concentration of about 5 mM;
the glucose dehydrogenase is present in a concentration of about 1 g/L; and
the glucose is present in a concentration of about 178 mM.

In one embodiment:

the NADP⁺ is present in a concentration of about 15 mM;

the glucose dehydrogenase is present in a concentration of about 0.45 g/L;

and

5 the glucose is present in a concentration of about 247 mM.

In one embodiment, the compound of Formula (B) is present in a concentration of from about 88 mM to about 198 mM. In one embodiment, the compound of Formula (B) is present in a concentration of about 88 mM. In one embodiment, the compound of Formula
10 (B) is present in a concentration of about 198 mM.

In one embodiment, the ketoreductase enzyme having SEQ ID NO: 1 is present in a concentration of about 1 g/L.

15 In one embodiment, the step of reducing a compound of Formula (B) is performed in the presence of potassium phosphate buffer. In one embodiment, the step of reducing a compound of Formula (B) is performed in the presence of about 100 mM potassium phosphate buffer. In one embodiment, the step of reducing a compound of Formula (B) is performed in the presence of about 100 mM potassium phosphate buffer about pH 7.0.

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In one embodiment:

the step of reducing a compound of Formula (B) is performed in the presence of:

(a) NADPH as a cofactor wherein the NADPH cofactor is provided by a cofactor regeneration system comprising NADP⁺, glucose dehydrogenase, and glucose; and

25 (b) about 100 mM potassium phosphate buffer about pH 7.0.

In one embodiment:

the NADP⁺ is present in a concentration of about 5 mM;

the glucose dehydrogenase is present in a concentration of about 1 g/L;

30 the glucose is present in a concentration of about 178 mM;

the compound of Formula (B) is present in a concentration of about 88 mM;

and

the ketoreductase enzyme having SEQ ID NO: 1 is present in a concentration of about 1 g/L.

In one embodiment:

the NADP⁺ is present in a concentration of about 15 mM;

the glucose dehydrogenase is present in a concentration of about 0.45 g/L;

the glucose is present in a concentration of about 247 mM;

5 the compound of Formula (B) is present in a concentration of about 198 mM;

and

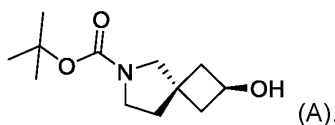
the ketoreductase enzyme having SEQ ID NO: 1 is present in a concentration of about 1 g/L.

10 In one embodiment, the step of reducing a compound of Formula (B) comprises heating at about 30 °C. In one embodiment, the step of reducing a compound of Formula (B) comprises stirring for about 20-24 h.

The compound of Formula (A) is an intermediate compound which is useful in the synthesis
15 of the mAChR agonist compounds described in WO2015/118342.

The processes described herein also provide the compound of Formula (A) in an especially high level of diastereomeric purity.

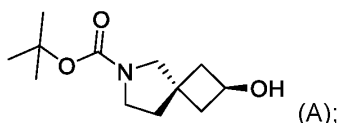
20 Accordingly, also described herein is a compound of Formula (A), as prepared by any one of the processes described hereinabove:



In one embodiment, the compound of Formula (A) has a diastereomeric excess of about
25 96%de.

In one embodiment, the compound of Formula (A) has a diastereomeric excess of about 97%de.

Also described herein is a composition comprising a compound of Formula (A):



wherein the compound of Formula (A) is present in a diastereomeric excess of about 96%de or about 97%de.

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In one embodiment, the compound of Formula (A) is present in a diastereomeric excess of about 96%de.

10 In one embodiment, the compound of Formula (A) is present in a diastereomeric excess of about 97%de.

One or more of the embodiments of the present disclosure may be combined with other embodiments of the present disclosure.

15 Definitions

When values described herein are expressed as approximations, by the use of the antecedent “about”, it is understood that the term “about” means $\pm 10\%$ of the stated value. Additionally, it will be understood that the particular value forms another embodiment.

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As used herein, the term “reacting” is used as known in the art and generally refers to the bringing together of chemical reagents in such a manner so as to allow their interaction at the molecular level to achieve a chemical or physical transformation. Certain types of reaction may be more specifically described, for example a reduction reaction (e.g., the formal addition of dihydrogen across a double bond) may be described using the term “reducing”, e.g., reducing a ketone ($>C=O$) to a secondary alcohol ($>C(H)-OH$).

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Cis-trans isomerism

30 The compounds described herein (e.g., intermediate compounds) comprise one or more areas of restricted rotation giving rise to *cis-trans* isomerism. *Cis-trans* isomers may also be described herein as diastereomers.

Accordingly, the compounds (e.g., intermediate compounds), produced by the methods described herein may be produced in a manner wherein at least 55% (e.g., at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, or 98%) of the compound is present as a single stereoisomer of the *cis-trans* pair.

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In one general embodiment, 99% or more (e.g., substantially all) of the total amount of the compound (e.g., mAChR agonist compound, intermediate compound) is present as a single *cis-trans* isomer.

- 10 For example, in one embodiment the compound is present as a *trans*-isomer (e.g., an intermediate compound).

Also described herein are mixtures of *cis-trans* isomers produced by the processes described herein.

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Isotopes

- 20 The compounds described herein (e.g., intermediate compounds) may contain one or more isotopic substitutions, and a reference to a particular element includes within its scope all isotopes of the element. For example, a reference to hydrogen includes within its scope ^1H , ^2H (D), and ^3H (T). Similarly, references to carbon and oxygen include within their scope respectively ^{12}C , ^{13}C and ^{14}C and ^{16}O and ^{18}O .

- 25 In an analogous manner, a reference to a particular functional group also includes within its scope isotopic variations, unless the context indicates otherwise. For example, a reference to an alkyl group such as an ethyl group also covers variations in which one or more of the hydrogen atoms in the group is in the form of a deuterium or tritium isotope, e.g., as in an ethyl group in which all five hydrogen atoms are in the deuterium isotopic form (a perdeuteroethyl group).

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The isotopes may be radioactive or non-radioactive. The compounds may contain no radioactive isotopes. Such compounds are preferred for therapeutic use. However, the compound may contain one or more radioisotopes. Compounds containing such radioisotopes may be useful in a diagnostic context.

Solvates

The compounds described herein may form solvates. Preferred solvates are solvates formed by the incorporation into the solid-state structure (e.g. crystal structure) of the compounds of the present disclosure of molecules of a non-toxic pharmaceutically acceptable solvent (referred to below as the solvating solvent). Examples of such solvents include water, alcohols (such as ethanol, isopropanol and butanol) and dimethylsulfoxide. Solvates can be prepared by recrystallising the compounds of the present disclosure with a solvent or mixture of solvents containing the solvating solvent. Whether or not a solvate has been formed in any given instance can be determined by subjecting crystals of the compound to analysis using well known and standard techniques such as thermogravimetric analysis (TGE), differential scanning calorimetry (DSC) and X-ray crystallography. The solvates can be stoichiometric or non-stoichiometric solvates. Particularly preferred solvates are hydrates, and examples of hydrates include hemihydrates, monohydrates and dihydrates.

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Accordingly, also described herein is:

An intermediate compound in the form of a solvate.

An intermediate compound in the form of a solvate wherein the solvate is a hydrate.

For a more detailed discussion of solvates and the methods used to make and characterise them, see Bryn et al., Solid-State Chemistry of Drugs, Second Edition, published by SSCI, Inc of West Lafayette, IN, USA, 1999, ISBN 0-967-06710-3.

Alternatively, rather than existing as a hydrate, the compounds described herein may be anhydrous. Therefore, also described herein is an intermediate compound described herein in an anhydrous form (e.g., anhydrous crystalline form).

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Ketoreductase Enzymes

Ketoreductases (KREDs, also called 'alcohol dehydrogenases' ADHs, or 'carbonyl reductases') catalyze the reduction of aldehydes and ketones to the corresponding primary and secondary alcohols, respectively.

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The reduction catalyzed by KREDs requires a reduced cofactor as electron donor. Some KREDs use reduced nicotinamide adenine dinucleotide (NADH) as a cofactor, other KREDs

35

use reduced nicotinamide adenine dinucleotide phosphate (NADPH) and some ketoreductases accept both, NADH and NADPH.

5 The in vitro use of KREDs in reduction processes requires a cofactor regeneration system to regenerate NADPH from NADP⁺ or NADH from NAD⁺. Common cofactor regeneration systems are glucose dehydrogenase (GDH) which takes glucose as a feedstock, or formate dehydrogenase which takes formate as a feedstock. These cofactor regeneration systems may be used in conjunction with KREDs.

10 KREDs are ubiquitous enzymes found in all kingdoms of life. Well-known, commercially available KREDs are derived from horse liver (HLADH), baker's yeast (YADH) and from bacteria, such as *Thermoanaerobium brockii* (TBADH) and *Lactobacillus kefir* (LKADH).

15 For industrial applications it is desirable to employ KREDs with a high specific activity and stereoselectivity. Another important criterion in the industrial use of KREDs is a long process stability, which often correlates with a high stability at elevated temperatures and a high solvent stability. It may also be desirable to employ KREDs with a high stereospecificity.

20 Enzyme A, described herein and used in the processes described herein is a ketoreductase enzyme (KRED) having an amino acid sequence of SEQ ID NO: 1:

MNSVQSQGTALITGASSGIGAIYAERLAARGFDLLLVARDKARLDSAASQLRDAHGVQVEVW
KADLTQKDDVIKLEQRLRSDSSISLLINNAGVAADGPLANADMDQLERLIQLNITAVTRLAS
AAAASFAKAGRGTIINIASVVALFPERFNATYTASKAYVLSLTQSLNAELEGSGVQIQAVLP
25 GVTRTEIWERSGIDASGIPAEMVMDAGEMVDAALAGLDQGELVTIPSLPDAGEWQSFVAARH
VMAPNLSRSAAAQRYKSGH.

30 Enzyme A may be produced by methods known in the art. For instance, methods for expression of enzymes in cellular (e.g., microbial) expression systems are well known and routine to those skilled in the art.

35 Enzyme A may be provided by or generated from a host cell (e.g., a microbial cell, such as *E. Coli*) comprising a polynucleotide and/or expression vector that encodes Enzyme A. The host cell can be TOP10 *E. Coli*. Enzyme A may be produced using the method disclosed in the Examples.

The polynucleotide may be DNA or RNA. The polynucleotide may be single-stranded or double-stranded. The polynucleotide may be provided in isolated/purified form, or within a host cell.

- 5 Because of the knowledge of the codons corresponding to the various amino acids, availability of a polypeptide sequence provides a description of all the polynucleotides capable of encoding the subject polypeptide. The degeneracy of the genetic code, where the same amino acids are encoded by alternative or synonymous codons allows an extremely large number of nucleic acids to be made, all of which encode a disclosed enzyme. Thus,
- 10 having identified a particular amino acid sequence, those skilled in the art could make any number of different nucleic acids by simply modifying the sequence of one or more codons in a way which does not change the amino acid sequence of the protein. In this regard, the present disclosure specifically contemplates each and every possible variation of polynucleotides that could be made by selecting combinations based on the possible codon
- 15 choices.

Enzyme A may be encoded by a polynucleotide and/or expression vector comprising a nucleotide sequence according to SEQ ID NO: 2:

20 ATGAATTCTGTGCAGTCTCAAGGTACGGCTCTGATCACTGGCGCCTCGTCCGGTATCGGTGC
GATTTACGCCGAGCGTCTGGCGGCGCGTGTTTGGATCTGTTGCTGGTGGCCCGTGACAAGG
CCCGTCTGGACAGCGCCGCCAGCCAATTGCGCGACGCTCACGGTGTGCAGGTGAGGTGTGG
AAAGCGGATCTGACCCAAAAGGACGACGTGATCAAACAGCGCTTGCGCAGCGATTG
GAGCATCAGCCTGCTGATTAACAATGCCGGCGTGGCCGCCGACGGCCCGCTGGCCAATGCCG

25 ACATGGATCAACTGGAACGCCTGATCCAGTTGAACATCACCGCCGTCACGCGTCTGGCGTCG
GCCGCCGCTGCCAGTTTCGCCAAGGCAGGTGCGGGCACGATCATCAACATCGCCTCGGTCTG
GGCGCTGTTCCCCGAGCGTTTCAATGCGACCTACACCGCCAGCAAGGCCTATGTGTTGAGTC
TGACCCAATCGTTGAACGCGGAGCTCGAAGGCTCCGGCGTGCAGATCCAGGCCGTGCTGCCG
GGCGTGACCCGCACTGAAATCTGGGAGCGTTCGGGGATCGACGCCAGTGGCATTCCGGCGGA

30 AATGGTCATGGACGCCGGGGAGATGGTGGATGCCGCCCTGGCCGGTCTGGATCAGGGCGAAC
TGGTCACCATTCATCGCTGCCCGATGCCGGCGAATGGCAGTCGTTTGTGGCGGCGCGCCAT
GTCATGGCGCCGAACCTTTCGCGCAGCGCTGCAGCCCAGCGCTACAAATCAGGTCATTGA.

The polynucleotide may consist of SEQ ID NO: 2.

The polynucleotide may be operatively linked to one or more heterologous regulatory or control sequences that control gene expression to create a recombinant polynucleotide capable of expressing the polypeptide. Expression constructs containing a heterologous polynucleotide encoding the engineered ketoreductase can be introduced into appropriate host cells to express the corresponding ketoreductase. The polynucleotides encoding the ketoreductase enzymes may be codon optimized for optimal production from the host organism selected for expression. For example, preferred codons used in bacteria are used to express the gene in bacteria; preferred codons used in yeast are used for expression in yeast; and preferred codons used in mammals are used for expression in mammalian cells.

A polynucleotide encoding a disclosed enzyme may be manipulated in a variety of ways to provide for expression of the polypeptide. Manipulation of the isolated polynucleotide prior to its insertion into an expression vector may be desirable or necessary depending on the expression vector. The techniques for modifying polynucleotides and nucleic acid sequences utilizing recombinant DNA methods are well known in the art. Guidance is provided in Sambrook et al., 2001, *Molecular Cloning: A Laboratory Manual*, 3rd Ed., Cold Spring Harbor Laboratory Press; and *Current Protocols in Molecular Biology*, Ausubel, F. ed., Greene Pub. Associates, 1998, updates to 2006.

The control sequence may be an appropriate promoter sequence, which can be obtained from genes encoding extracellular or intracellular polypeptides either homologous or heterologous to the host cell. For bacterial host cells, suitable promoters for directing transcription of the nucleic acid constructs of the present disclosure, include the promoters obtained from the *E. coli* lac operon, *Streptomyces coelicolor* agarase gene (*dagA*), *Bacillus subtilis* levansucrase gene (*sacB*), *Bacillus licheniformis* alpha-amylase gene (*amyL*), *Bacillus stearothermophilus* maltogenic amylase gene (*amyM*), *Bacillus amyloliquefaciens* alpha-amylase gene (*amyQ*), *Bacillus licheniformis* penicillinase gene (*penP*), *Bacillus subtilis* *xylA* and *xylB* genes, and prokaryotic beta-lactamase gene (Villa-Kamaroff et al., 1978, *Proc. Natl Acad. Sci. USA* 75: 3727-3731), as well as the *tac* promoter (DeBoer et al., 1983, *Proc. Natl Acad. Sci. USA* 80: 21-25). Further promoters are described in "Useful proteins from recombinant bacteria" in *Scientific American*, 1980, 242:74-94; and in Sambrook et al., *supra*.

The control sequence may also be a signal peptide coding region that codes for an amino acid sequence linked to the amino terminus of a polypeptide and directs the encoded polypeptide into the cell's secretory pathway. The 5' end of the coding sequence of the

nucleic acid sequence may inherently contain a signal peptide coding region naturally linked in translation reading frame with the segment of the coding region that encodes the secreted polypeptide. Alternatively, the 5' end of the coding sequence may contain a signal peptide coding region that is foreign to the coding sequence. The foreign signal peptide coding
5 region may be required where the coding sequence does not naturally contain a signal peptide coding region.

Alternatively, the foreign signal peptide coding region may simply replace the natural signal peptide coding region in order to enhance secretion of the polypeptide. However, any signal
10 peptide coding region which directs the expressed polypeptide into the secretory pathway of a host cell of choice may be used.

Any suitable vectors, promoters, enhancers and termination codons known in the art may be used to express a polypeptide from a vector according to the present disclosure. The vector
15 may be a plasmid, phage, MAC, virus, etc. The plasmid may be a pET28a plasmid (see Nature 2020; <https://doi.org/10.1038/s42003-020-0939-8>).

The polynucleotide and/or expression vector may be synthesised by convention DNA synthesis techniques. Where the sequence of the engineered polypeptide is known, the
20 polynucleotides encoding the enzyme can be prepared by standard solid-phase methods, according to known synthetic methods. Fragments of up to about 100 bases can be individually synthesized, then joined (e.g., by enzymatic or chemical ligation methods, or polymerase mediated methods) to form any desired continuous sequence. For example, the disclosed polynucleotides can be prepared by chemical synthesis using, e.g., the classical
25 phosphoramidite method described by Beaucage et al., 1981, Tet Lett 22:1859-69, or the method described by Matthes et al., 1984, EMBO J. 3:801-05, e.g., as it is typically practiced in automated synthetic methods. According to the phosphoramidite method, oligonucleotides are synthesized, e.g., in an automatic DNA synthesizer, purified, annealed, ligated and cloned in appropriate vectors. In addition, essentially any nucleic acid can be obtained from
30 any of a variety of commercial sources, such as The Midland Certified Reagent Company, Midland, TX, The Great American Gene Company, Ramona, CA, ExpressGen Inc. Chicago, IL, Operon Technologies Inc., Alameda, CA, and many others.

Protecting Groups

In the processes described herein, it may be necessary to protect one or more groups to prevent reaction from taking place at an undesirable location on the molecule. Examples of
5 protecting groups, and methods of protecting and deprotecting functional groups, can be found in *Protective Groups in Organic Synthesis* (T. Greene and P. Wuts; 3rd Edition; John Wiley and Sons, 1999).

Purification

10 Compounds made by the methods described herein may be isolated and purified by any of a variety of methods well known to those skilled in the art and examples of such methods include recrystallisation and chromatographic techniques such as column chromatography (e.g., flash chromatography) and HPLC.

15

Reaction solvents and conditions

As discussed hereinabove, the term "reacting" is used as known in the art. In some embodiments, the reacting involves two reagents, wherein one or more equivalents of the
20 second reagent are used with respect to the first reagent. The reacting steps of the processes described herein can be conducted for a time and under conditions suitable for preparing the identified product.

The reactions of the processes described herein can be carried out in suitable solvents
25 which can be readily selected by one of skill in the art of organic synthesis. Suitable solvents can be substantially nonreactive with the starting materials (reactants), the intermediates, or products at the temperatures at which the reactions are carried out, e.g., temperatures which can range from the solvent's freezing temperature to the solvent's boiling temperature. A given reaction can be carried out in one solvent or a mixture of more than one solvent.
30 Depending on the particular reaction step, suitable solvents for a particular reaction step can be selected.

Suitable solvents can include halogenated solvents such as carbon tetrachloride, bromodichloromethane, dibromochloromethane, bromoform, chloroform,
35 bromochloromethane, dibromomethane, butyl chloride, dichloromethane, tetrachloroethylene, trichloroethylene, 1,1,1-trichloroethane, 1,1,2-trichloroethane, 1,1-

dichloroethane, 2-chloropropane, 1,2-dichloroethane, 1,2-dibromoethane, hexafluorobenzene, 1,2,4-trichlorobenzene, 1,2-dichlorobenzene, chlorobenzene, fluorobenzene, mixtures thereof and the like.

- 5 Suitable ether solvents include: dimethoxymethane, tetrahydrofuran, 1,3-dioxane, 1,4-dioxane, furan, diethyl ether, ethylene glycol dimethyl ether, ethylene glycol diethyl ether, diethylene glycol dimethyl ether, diethylene glycol diethyl ether, triethylene glycol dimethyl ether, anisole, t-butyl methyl ether, mixtures thereof and the like.
- 10 Suitable protic solvents can include, by way of example and without limitation, water, methanol, ethanol, 2-nitroethanol, 2-fluoroethanol, 2,2,2-trifluoroethanol, ethylene glycol, 1-propanol, 2-propanol, 2-methoxyethanol, 1-butanol, 2-butanol, i-butyl alcohol, t-butyl alcohol, 2-ethoxyethanol, diethylene glycol, 1-, 2-, or 3- pentanol, neo-pentyl alcohol, t-pentyl alcohol, diethylene glycol monomethyl ether, diethylene glycol monoethyl ether, cyclohexanol, benzyl
- 15 alcohol, phenol, or glycerol.

- Suitable aprotic solvents can include, by way of example and without limitation, tetrahydrofuran (THF), N,N-dimethylformamide (DMF), N,N-dimethylacetamide (DMA), 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone (DMPU), 1,3-dimethyl-2-imidazolidinone
- 20 (DMI), N methylpyrrolidinone (NMP), formamide, N-methylacetamide, N-methylformamide, acetonitrile, dimethyl sulfoxide (DMSO), propionitrile, ethyl formate, methyl acetate, hexachloroacetone, acetone, ethyl methyl ketone, ethyl acetate, sulfolane, N,N-dimethylpropionamide, tetramethylurea, nitromethane, nitrobenzene, or hexamethylphosphoramide.
- 25 Suitable hydrocarbon solvents include benzene, cyclohexane, pentane, hexane, toluene, cycloheptane, methylcyclohexane, heptane, ethylbenzene, m-, o-, or p-xylene, octane, indane, nonane, or naphthalene.

- 30 Suitable aqueous buffer solvents include phosphate buffers, tris buffers, barbital buffers, BES (N, N-Bis-(2-hydroxyethyl)-2-aminoethanesulphonic acid) buffers, and MOPS (3-(N-morpholino) propane sulphonic acid) buffers.

- The reactions of the processes described herein can be carried out at appropriate
- 35 temperatures which can be readily determined by the skilled artisan. Reaction temperatures will depend on, for example, the melting and boiling points of the reagents and solvent, if

present; the thermodynamics of the reaction (e.g., vigorously exothermic reactions may need to be carried out at reduced temperatures); the kinetics of the reaction (e.g., a high activation energy barrier may need elevated temperatures); and the temperature range over which any enzymatic component is active / the temperature at which any enzymatic component is

5 denaturated.

The expressions, “ambient temperature” and “room temperature” or “rt” as used herein, are understood in the art, and refer generally to a temperature, e.g., a reaction temperature, that is about the temperature of the room in which the reaction is carried out, for example, a

10 temperature from about 20 °C to about 30 °C.

The reactions of the processes described herein can be carried out in air or under an inert atmosphere. Typically, reactions containing reagents or products that are substantially reactive with air can be carried out using air-sensitive synthetic techniques that are well

15 known to the skilled artisan.

EXAMPLES

The present disclosure will now be illustrated, but not limited, by reference to the specific embodiments described in the following example compounds and methods of synthesis.

5

General Methods and Materials

The following general methods and materials are exemplary for the methods and materials used during the processes described hereinbelow.

10 Chiral RP-HPLC method:

System: Shimadzu LC-40

Column: Phenomenex Lux i-Amylose3 250 x 4.6mm 5 μ m

15 Mobile phase: water and acetonitrile mixture (60/40 water/acetonitrile) with 0.1% phosphoric acid.

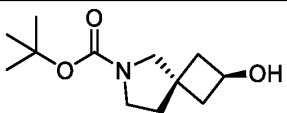
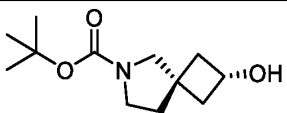
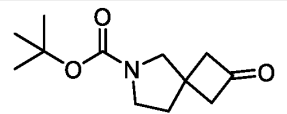
Flow rate: 1 mL/min.

Column temperature: 40°C.

Run time: 15 min.

Detection: refractive index detector (RID) was used.

20 Retention times:

Compound	Retention time
	5.6 min
	7.1 min
	9.9 min

Sample preparation: To 0.5 mL reaction sample 0.5 mL acetonitrile was added and mixed until homogeneity. The sample was placed into an ultrasonic bath for 5 min and then centrifuged at 12,000 x g for 5 min. An aliquot of the prepared sample was transferred to a
25 glass vial and analyzed using the chiral RP-HPLC method.

Calculation of diastereomeric excess:

Diastereomeric excess (%de) was calculated using the following equation:

$$\%de = \frac{C_{trans\ isomer}}{(C_{trans\ isomer} + C_{cis\ isomer})} * 100$$

5

Where $C_{trans\ isomer}$ corresponds to the %Area of the peak in an HPLC chromatograph generated using the Chiral RP-HPLC method, and having a retention time of 5.6 min; and $C_{cis\ isomer}$ corresponds to the %Area of the peak in the HPLC chromatograph generated using the Chiral RP-HPLC method, and having a retention time of 7.1 min.

10

Example 1: Screening of KRED enzymes

A photometric assay was used to screen a range of KRED enzymes for their utility in the transformation of Formula (B) to Formula (A). 288 diverse KRED enzymes were provided as dried enzyme formulation in 96 microtiter plates. The enzymes were solubilized in an appropriate buffer solution to generate a clear solution, necessary for photometric measurement and screening. The photometric measurement recorded the decrease of the absorption at 340 nm, which corresponds with an enzyme consuming the NADPH cofactor for a reductive reaction.

20

The collected data was analyzed and enzymes selected, which either showed a negative slope (lowest numbers chosen first) over 10 min of reaction time or an overall absolute absorbance of lower than 0.7 and decreasing absorption over 10 min. A low starting absorbance is an indication for a highly active enzyme, that consumes the cofactor faster, than the technician can transfer the MTP to the MTP-reader.

25

Those enzymes meeting the selection criteria were submitted to a 0.5 mL biocatalysis reaction and HPLC verification using the chiral RP-HPLC method.

30 Biocatalysis conditions:

- Ketone 20 g/L (88 mM)
- NADP 5 mM
- DMSO 5% (v/v)
- $MgCl_2$ 2 mM
- 35 • Glucose 178 mM (2 equiv.)
- GDH (glucose dehydrogenase, CDX-901, Codexis Inc.) 1 g/L

- Potassium phosphate buffer 100 mM pH 7.0
- 0.5 mL, 30°C, 1000 rpm shaking, 24 h reaction time

As shown in Table 1, below, Enzyme A significantly out-performed the next best enzymes (Enzymes B-F) for conversion of starting material, while maintaining the highest level of stereoselectivity (diastereomeric excess) observed for the trans-alcohol (Formula (A)).

Table 1

Enzyme	conversion	%de
Enzyme A	100% (98.7*)	97% (97*)
Enzyme B	41.2%	97%
Enzyme C	37.0%	94%
Enzyme D	46.1%	94%
Enzyme E	42.8%	94%
Enzyme F	19.1%	91%

10 Example 2: Production of synthetic DNA encoding Enzyme A

Synthetic DNA having SEQ ID NO: 2 and encoding Enzyme A was synthesised with codon optimisation for E. coli expression.

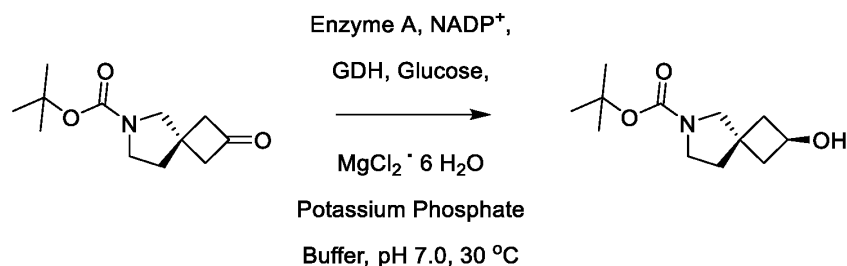
15 SEQ ID NO: 2

ATGAATTCTGTGCAGTCTCAAGGTACGGCTCTGATCACTGGCGCCTCGTCCGGTATCGGTGC
 GATTTACGCCGAGCGTCTGGCGGCGCGTGTTTGGATCTGTTGCTGGTGGCCCGTGACAAGG
 CCCGTCTGGACAGCGCCGCCAGCCAATTGCGCGACGCTCACGGTGTGCAGGTCGAGGTGTGG
 AAAGCGGATCTGACCCAAAAGGACGACGTGATCAAACCTCGAACAGCGCTTGCGCAGCGATTC
 20 GAGCATCAGCCTGCTGATTAACAATGCCGGCGTGGCCGCCGACGGCCCGCTGGCCAATGCCG
 ACATGGATCAACTGGAACGCCTGATCCAGTTGAACATCACCGCCGTCACGCGTCTGGCGTCG
 GCCGCCGCTGCCAGTTTCGCCAAGGCAGGTCGCGGCACGATCATCAACATCGCCTCGGTCTGT
 GCGCTGTTCCCCGAGCGTTTCAATGCGACCTACACGCCAGCAAGGCCTATGTGTTGAGTC
 TGACCCAATCGTTGAACGCGGAGCTCGAAGGCTCCGGCGTGCAGATCCAGGCCGTGCTGCCG
 25 GCGTGACCCGCACTGAAATCTGGGAGCGTTCGGGGATCGACGCCAGTGGCATTCCGGCGGA
 AATGGTCATGGACGCCGGGAGATGGTGGATGCCGCCCTGGCCGGTCTGGATCAGGGCGAAC
 TGGTCACCATTCATCGCTGCCCGATGCCGGCGAATGGCAGTCGTTTGTGGCGGCGCGCCAT
 GTCATGGCGCCGAACCTTTCGCGCAGCGCTGCAGCCCAGCGCTACAAATCAGGTCATTGA.

Example 3: Production of Enzyme A

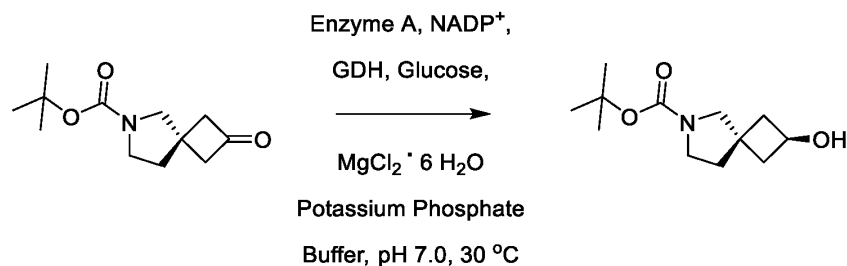
The synthetic DNA (SEQ ID No: 2) and pET28a were digested with *NdeI* and *XhoI* restriction endonucleases, purified, and ligated together employing T4 DNA ligase. The resultant
5 recombinant molecule was subsequently transformed into *E. coli* strain TOP10 and the desired expression construct verified by Sanger DNA sequencing. After transforming sequence verified plasmid DNA into the *E. coli* protein expression host BL21(DE3), a single colony was used to inoculate 10 mL of LBP growth medium (10 g/L plant peptone, 10 g/L NaCl and 5 g/L yeast extract, supplemented with 35 µg/mL kanamycin), with culturing
10 performed at 37 °C for 16 h with shaking at 200 rpm. This starter culture was then used to inoculate 1 L of TB growth medium (12 g/L plant peptone, 12 g/L glycerol, 9.96 g/L sodium phosphate dibasic, 20.4 g/L sodium phosphate monobasic, supplemented with 35 µg/mL kanamycin) in a 2 L baffled glass Erlenmeyer flask, which was incubated at 28 °C for 16 h to effect expression of Enzyme A. The culture was then transferred into 2 x 500 mL centrifuge
15 pots (Nalgene®) and centrifuged at 6000 g / 4 °C in a SLA3000 rotor using a Sorvall RC-5C centrifuge. The bacterial pellets were then resuspended within their pots with 5 x pellet weight of 50 mM sodium phosphate buffer, pH 7.5. Subsequent to thorough re-suspension, the bacterial suspension was transferred into a 100 mL glass beaker for cell disruption, that was achieved using an MSE Soniprep 150 sonicator, configured with a 9.5mm sonication
20 probe at 0 °C, for a total of 8 min (4 x 2 min intervals at 16 microns, with 4 min incubation time between cycles). The disrupted cell suspension was then transferred into a 50 mL (Nalgene®) centrifuge pot and centrifuged at 13000 x g in a Fiberlite™ F13-1 X60cy rotor for 45 min. Following centrifugation, the supernatant (approx. 40 mL), was carefully transferred into a 100 mL plastic container and placed at -20 °C for 16 h. The frozen Enzyme A solution
25 was then subjected to lyophilisation by freeze drying (Edwards SuperModulo) over 2 days, maintaining a pressure below 1 mbar throughout. The dried enzyme cake was pulverised thoroughly by crushing with a steel spatula into a free-flowing homogenous powder and stored at -20 °C between use.

Example 4: Analytical scale preparation of tert-butyl trans-2-hydroxy-6-azaspiro[3.4]octane-6-carboxylate



- 5 1 mg of Enzyme A was placed into a 2 mL reaction tube. Into the tube was added 0.5 mL of a reaction mix consisting of 5 mM nicotinamide adenine dinucleotide phosphate (NADP⁺), 2 mM magnesium chloride hexahydrate, 178 mM glucose anhydrous, 1 g/L glucose dehydrogenase (CDX-901, Codexis Inc.), 5% (v/v) dimethyl sulfoxide (DMSO) and 20 g/L tert-butyl 2-oxo-6-azaspiro[3.4]octane-6-carboxylate in 100 mM potassium phosphate buffer.
- 10 The reaction tube was shaken at 1000 rpm and 30°C for 24 h. The reaction was analyzed using the chiral HPLC method showing a conversion in a range of 98.7% - 100% and a diastereomeric excess of 97%de for tert-butyl trans-2-hydroxy-6-azaspiro[3.4]octane-6-carboxylate.

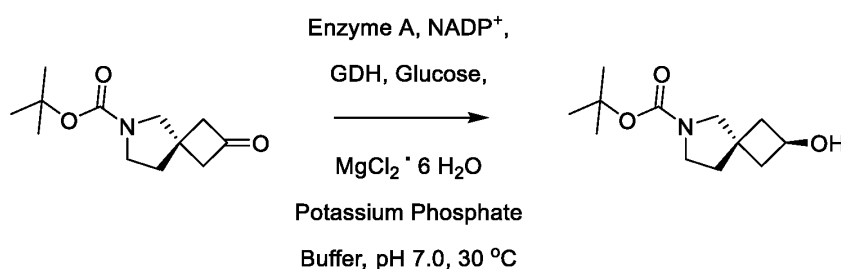
15 Example 5: Lab-bench scale preparation of tert-butyl trans-2-hydroxy-6-azaspiro[3.4]octane-6-carboxylate (20 g/L substrate)



- To a 100 mL Schott-bottle was added 50 mg Enzyme A (1 g/L), 50 mg glucose dehydrogenase (1 g/L, CDX-901, Codexis Inc.), 197 mg nicotinamide adenine dinucleotide phosphate (5 mM, NADP⁺), 20 mg magnesium chloride hexahydrate (2 mM) and 1.6 g glucose anhydrous (178 mM). The bottle was filled under magnetic stirring at 300 rpm with 47.5 mL of 100 mM potassium phosphate buffer pH 7.0, tempered to 30°C and the pH adjusted to 7, if necessary. To the stirred solution, 1 g of tert-butyl 2-oxo-6-azaspiro[3.4]octane-6-carboxylate (20 g/L, 88 mM) in 2.5 mL dimethyl sulfoxide (5% v/v,

DMSO) was added. After 20 h the reaction was analyzed using the chiral RP-HPLC method showing a conversion of 100% and a diastereomeric excess of 96%de for tert-butyl trans-2-hydroxy-6-azaspiro[3.4]octane-6-carboxylate.

5 Example 6: Lab-bench scale preparation of tert-butyl trans-2-hydroxy-6-azaspiro[3.4]octane-6-carboxylate (45 g/L substrate)



In a 100 mL Schott-bottle the following materials were added: 50 mg Enzyme A (1 g/L),
10 22.5 mg glucose dehydrogenase (0.45 g/L, CDX-901, Codexis Inc.), 0.56 g nicotinamide adenine dinucleotide phosphate (11.3 g/L, 15 mM, NADP⁺), 20 mg magnesium chloride hexahydrate (2 mM) and 2.2 g glucose anhydrous (247 mM). The bottle was filled under magnetic stirring at 300 rpm with 47.5 mL of 100 mM potassium phosphate buffer pH 7.0, tempered to 30°C and the pH adjusted to 7 if necessary. To the stirred solution 2.25 g of tert-
15 butyl 2-oxo-6-azaspiro[3.4]octane-6-carboxylate (45 g/L, 198 mM) in 2.5 mL dimethyl sulfoxide (5% v/v, DMSO) was added. The pH of the reaction was kept constant at 7 by adding sodium hydroxide solution. After 24 h the reaction was analyzed using the chiral HPLC method showing a conversion of 67% and a diastereomeric excess of 97%de for tert-butyl trans-2-hydroxy-6-azaspiro[3.4]octane-6-carboxylate.

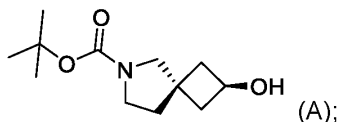
20

Equivalents

The foregoing examples are presented for the purpose of illustrating the present disclosure
25 and should not be construed as imposing any limitation on the scope of the present disclosure. It will readily be apparent that numerous modifications and alterations may be made to the specific embodiments of the present disclosure described above and illustrated in the examples without departing from the principles underlying the present disclosure. All such modifications and alterations are intended to be embraced by this application.

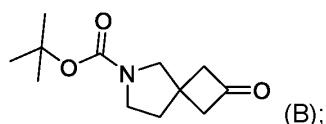
CLAIMS

1. A process for preparing a trans-alcohol compound of Formula (A):



wherein:

the process comprises the step of reducing a compound of Formula (B):

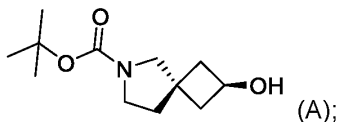


in the presence of a ketoreductase enzyme having SEQ ID NO: 1 to provide the compound of Formula (A).

2. The process of claim 1, wherein the step of reducing a compound of Formula (B) is performed in the presence of NADPH as a cofactor.
3. The process of claim 2, wherein the NADPH cofactor is provided by a cofactor regeneration system comprising NADP^+ , glucose dehydrogenase, and glucose.
4. The process of claim 3, wherein:
- the NADP^+ is present in a concentration of about 5 mM;
 - the glucose dehydrogenase is present in a concentration of about 1 g/L; and
 - the glucose is present in a concentration of about 178 mM.
5. The process of any one of claims 1-4, wherein the compound of Formula (B) is present in a concentration of about 88 mM.
6. The process of claim 3, wherein:
- the NADP^+ is present in a concentration of about 15 mM;
 - the glucose dehydrogenase is present in a concentration of about 0.45 g/L;
- and
- the glucose is present in a concentration of about 247 mM.
7. The process of any one of claims 1-3 or 6, wherein the compound of Formula (B) is present in a concentration of about 198 mM.

8. The process of any one of claims 1-7, wherein in the step of reducing a compound of Formula (B), the ketoreductase enzyme having SEQ ID NO: 1 is present in a concentration of about 1 g/L.
9. The process of any one of claims 1-9, wherein the step of reducing a compound of Formula (B) is performed in the presence of about 100 mM potassium phosphate buffer at about pH 7.0.
10. The process of claim 1, wherein:
the step of reducing a compound of Formula (B) is performed in the presence of:
 - (a) NADPH as a cofactor, wherein the NADPH cofactor is provided by a cofactor regeneration system comprising:
 - (i) NADP⁺;
 - (ii) glucose dehydrogenase; and
 - (iii) glucose; and
 - (b) about 100 mM potassium phosphate buffer at about pH 7.0.
11. The process of claim 10, wherein:
the NADP⁺ is present in a concentration of about 5 mM;
the glucose dehydrogenase is present in a concentration of about 1 g/L;
the glucose is present in a concentration of about 178 mM;
the compound of Formula (B) is present in a concentration of about 88 mM;
and
the ketoreductase enzyme having SEQ ID NO: 1 is present in a concentration of about 1 g/L.
12. The process of claim 10, wherein:
the NADP⁺ is present in a concentration of about 15 mM;
the glucose dehydrogenase is present in a concentration of about 0.45 g/L;
the glucose is present in a concentration of about 247 mM;
the compound of Formula (B) is present in a concentration of about 198 mM;
and
the ketoreductase enzyme having SEQ ID NO: 1 is present in a concentration of about 1 g/L.

13. The process of any one of claims 1-12, wherein the step of reducing a compound of Formula (B) comprises heating at about 30 °C.
14. The process of any one of claims 1-13, wherein the step of reducing a compound of Formula (B) comprises stirring for about 20-24 h.
15. A composition comprising a compound of Formula (A):



- wherein the compound of Formula (A) is present in a diastereomeric excess of about 96%de or about 97%de.
16. The composition of claim 15, wherein the compound of Formula (A) is present in a diastereomeric excess of about 96%de.
17. The composition of claim 15, wherein the compound of Formula (A) is present in a diastereomeric excess of about 97%de.