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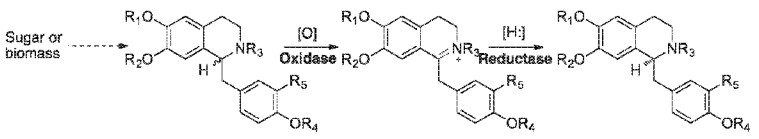


FIG. 3

(57) Abstract: A method of epimerizing an (*S*)-1-benzylisoquinoline alkaloid to an (*R*)-1 -benzylisoquinoline alkaloid is provided. The method comprises contacting the (*S*)-1 -benzylisoquinoline alkaloid with at least one enzyme. Contacting the (*S*)-1 -benzylisoquinoline alkaloid with the at least one enzyme converts the (S)-1 - benzylisoquinoline alkaloid to an (*R*)-1 -benzylisoquinoline alkaloid.

WO 2016/183023 A1

METHODS OF PRODUCING EPIMERASES AND BENZYLISOQUINOLINE ALKALOIDS

CROSS-REFERENCE

[0001] Pursuant to 35 U.S.C. §119(e), this application claims priority to the filing date of United States Provisional Patent Application Serial No. 62/159,122 filed on May 8, 2015 and United States Provisional Patent Application Serial No. 62/174,475 filed on June 11, 2015; the disclosures of which applications are herein incorporated by reference.

[0002] Additionally, this application is related to: United States Patent Application Serial No. 14/211,611 now published as US 2014-0273109, which application was filed on March 14, 2014 and has Attorney Docket No. STAN-1018; PCT Application Serial No. PCT/US2014/027833 now published as WO 2014/143744, which application was filed on March 14, 2014 and has Attorney Docket No. STAN-1018WO; United States Patent Application Serial No. 15/031,618, which application was filed on April 22, 2016 and has Attorney Docket No. STAN-1078; Application Serial No. PCT/US2014/063738 now published as WO 2015/066642, which application was filed on November 3, 2014 and has Attorney Docket No. STAN-1078WO; United States Provisional Patent Application Serial No. 62/080,610, which was filed November 17, 2014 and has Attorney Docket No. STAN-1169PRV; United States Provisional Patent Application Serial No. 62/107,238, which was filed January 23, 2015 and has Attorney Docket No. STAN-1169PRV2; Application Serial No. PCT/US2015/060891 which application was filed on November 16, 2015 and has Attorney Docket No. STAN-1169WO; United States Provisional Patent Application Serial No. 62/156,701, which was filed May 4, 2015 and has Attorney Docket No. STAN-1221PRV; and Application Serial No. PCT/US2016/030808 which application was filed on May 4, 2016 and has Attorney Docket No. STAN-1221WO; the disclosures of which applications are herein incorporated by reference.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

[0003] This invention was made with Government support under contract AT007886 awarded by the National Institutes of Health. The Government has certain rights in the invention.

SUMMARY OF THE INVENTION

[0004] The present disclosure provides methods for the production of diverse benzylisoquinoline alkaloids (BIAs) in engineered host cells. The present disclosure further provides compositions of diverse alkaloids produced in engineered host cells. Additionally, the present disclosure provides methods for the production of an epimerase in engineered host cells. In particular cases, the disclosure provides methods for producing diverse alkaloid products through the epimerization of a *(S)*-1-benzylisoquinoline alkaloid to a *(R)*-1-benyzlisoquinoline alkaloid in an engineered host cell. In further particular cases, the present disclosure

provides methods for producing diverse alkaloid products through the epimerization of *(S)*-reticuline to *(R)*-reticuline.

[0005] An aspect of the invention provides an engineered non-plant cell having increased tyrosine hydroxylase activity relative to a non-engineered cell. Another aspect of the invention provides an engineered non-plant cell having increased tyrosine hydroxylase activity relative to a cell that expresses wild-type TyrH. An additional aspect of the invention provides an engineered non-plant cell having increased tyrosine hydroxylase activity relative to a cell that expresses wild-type TyrH without mutations that increase tyrosine hydroxylase activity as provided herein. In particular, the engineered non-plant cell has at least one modification selected from a group consisting of: a substrate inhibition alleviating mutation; a product inhibition alleviating mutation; and a cofactor recovery promoting mechanism.

[0006] An additional aspect of the invention provides a method of epimerizing an *(S)*-1-benzylisoquinoline alkaloid to an *(R)*-1-benzylisoquinoline alkaloid. The method comprises contacting the *(S)*-1-benzylisoquinoline alkaloid with at least one enzyme. Contacting the *(S)*-1-benzylisoquinoline alkaloid with the at least one enzyme converts the *(S)*-1-benzylisoquinoline alkaloid to an *(R)*-1-benzylisoquinoline alkaloid.

[0007] Another aspect of the invention provides an engineered non-plant cell that produces an epimerase that converts *(S)*-1-benzylisoquinoline alkaloid to *(R)*-1-benzylisoquinoline alkaloid. The epimerase comprises an amino acid sequence selected from the group consisting of: SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15, as listed in **Table 1**.

[0008] A further aspect of the invention provides a process for converting an *(S)*-1-benzylisoquinoline alkaloid to an *(R)*-1-benzylisoquinoline alkaloid comprising contacting the *(S)*-1-benzylisoquinoline alkaloid with an epimerase in an amount sufficient to convert the *(S)*-1-benzylisoquinoline alkaloid to *(R)*-1-benzylisoquinoline alkaloid.

[0009] In another aspect of the invention, a method of epimerizing a stereocenter of a 1-benzylisoquinoline alkaloid is provided. The method comprises contacting the 1-benzylisoquinoline alkaloid with at least one enzyme. Contacting the 1-benzylisoquinoline alkaloid with the at least one enzyme inverts the stereochemistry of the stereocenter of the 1-benzylisoquinoline alkaloid to the opposite stereochemistry of the stereocenter of the 1-benzylisoquinoline alkaloid.

[0010] In some examples, an engineered non-plant cell comprises a plurality of coding sequences each encoding an enzyme that is selected from the group of enzymes listed in **Table 2**. In some examples, the heterologous coding sequences may be operably connected. Heterologous coding sequences that are operably connected may be within the same pathway of producing a particular benzylisoquinoline alkaloid product and/or epimerase product.

[0011] In an additional aspect of the invention, an engineered non-plant cell that produces a bisbenzylisoquinoline alkaloid is provided. The bisbenzylisoquinoline alkaloid is produced using a coupling enzyme that is present within the engineered non-plant cell. Additionally, the engineered non-plant cell comprises at least one heterologous coding sequence encoding at least one enzyme used in the production of at least one benzylisoquinoline alkaloid monomer within the engineered non-plant cell. Further, the at least one coupling enzyme dimerizes two benzylisoquinoline alkaloid monomers within the engineered non-plant cell, thereby forming the bisbenzylisoquinoline alkaloid.

INCORPORATION BY REFERENCE

[0012] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0014] **FIG. 1** illustrates examples of synthesis, recycling, and salvage pathways of tetrahydrobiopterin, in accordance with embodiments of the invention.

[0015] **FIG. 2** illustrates a biosynthetic scheme for conversion of glucose to 4-HPA, dopamine, and 3,4-DHPA, in accordance with embodiments of the invention.

[0016] **FIG. 3** illustrates a schematic example of *(R)*-1-benzylisoquinoline alkaloid formation, in accordance with embodiments of the invention.

[0017] **FIG. 4** illustrates an amino acid sequence of a CYP-COR enzyme, in accordance with embodiments of the invention.

[0018] **FIG. 5** illustrates a biosynthetic scheme for conversion of L-tyrosine to reticuline via norcoclaurine, in accordance with embodiments of the invention.

[0019] **FIG. 6** illustrates a biosynthetic scheme for conversion of L-tyrosine to reticuline via norlaudanosoline, in accordance with embodiments of the invention.

[0020] **FIG. 7** illustrates a biosynthetic scheme for conversion of L-tyrosine to morphinan alkaloids, in accordance with embodiments of the invention.

[0021] **FIG. 8** illustrates a biosynthetic scheme for conversion of L-tyrosine to protoberberine, phthalideisoquinoline, and berberine alkaloid products, in accordance with embodiments of the invention.

[0022] FIG. 9 illustrates a biosynthetic scheme for conversion of L-tyrosine to noscapine, noscapinoid, and phthalideisoquinoline alkaloid products, in accordance with embodiments of the invention.

[0023] FIG. 10 illustrates a biosynthetic scheme for conversion of L-tyrosine to sanguinarine and benzophenanthridine alkaloids, in accordance with embodiments of the invention.

[0024] FIG. 11A illustrates a biosynthetic scheme for conversion of canadine to noscapine, in accordance with embodiments of the invention.

[0025] FIG. 11B illustrates a biosynthetic scheme for production of semi-synthetic opioids, in accordance with embodiments of the invention.

[0026] FIG. 12 illustrates tyrosine hydroxylase mutants that improve norcoclaurine production from sugar in engineered yeast strains, in accordance with embodiments of the invention.

[0027] FIG. 13 illustrates tyrosine hydroxylase mutants that improve reticuline production from sugar in engineered yeast strains, in accordance with embodiments of the invention.

[0028] FIG. 14 illustrates coexpression of dihydrofolate reductase (DHFR) that improves L-DOPA production by tyrosine hydroxylase in engineered yeast strains, in accordance with embodiments of the invention.

[0029] FIG. 15 illustrates (A) the addition of antioxidants to culture media that improves L-DOPA production by tyrosine hydroxylase in engineered yeast strains and (B) the addition of antioxidants to culture media that increase BH_4 levels, in accordance with embodiments of the invention.

[0030] FIG. 16 illustrates (A) a biosynthetic scheme for conversion of L-tyrosine to bisBIAs and (B) yeast strains engineered to biosynthesize bisBIAs, in accordance with embodiments of the invention.

[0031] FIG. 17 illustrates a phylogenetic tree of cytochrome P450 oxidase-codeinone reductase-like (CYP-COR) fusions, in accordance with embodiments of the invention.

[0032] FIGS. 18(A) and **18(B)** illustrate an LC-MS/MS analysis of yeast strains engineered to convert *(S)*-reticuline to salutaridine, in accordance with embodiments of the invention.

[0033] FIG. 19 illustrates a chiral LC-MS/MS analysis of yeast strains engineered to convert racemic norlaudanosoline to *(R)*-reticuline, in accordance with embodiments of the invention.

[0034] FIGS. 20(A) and **20(B)** illustrate engineered fusions of salutaridine synthase that eliminates N-linked glycosylation of the protein observed when heterologously expressed in yeast but not plants, in accordance with embodiments of the invention

[0035] FIGS. 21(A) and **21(B)** illustrate cheilanthifoline synthase-salutaridine synthase fusion designs, in accordance with embodiments of the invention.

[0036] **FIG. 22** illustrates salutaridine synthase codon-optimization and engineered fusions that improve activity in yeast, in accordance with embodiments of the invention.

[0037] **FIGS. 23(A)** and **23(B)** illustrate an LC-MS/MS analysis of small scale batch fermentation in which engineered yeast catalyze the conversion of *(R)*-reticuline to thebaine and the conversion of *rac*-norlaudanosoline to thebaine, in accordance with embodiments of the invention.

[0038] **FIG. 24** illustrates generation of a CODM enzyme variant exhibiting enhanced activity in yeast through random mutagenesis and screening, in accordance embodiments of the invention.

[0039] **FIGS. 25(A), 25(B)** and **25(C)** illustrate fermentation optimization for conversion of *(R)*-reticuline to thebaine by engineered yeast, in accordance with embodiments of the invention.

[0040] **FIG. 26** illustrates yeast platform strains for the production of the key branchpoint intermediate reticuline from L-tyrosine, in accordance with embodiments of the invention.

[0041] **FIG. 27(A)**, **27(B)**, **27(C)** and **27(D)** illustrate thebaine and hydrocodone production in engineered yeast strains, in accordance with embodiments of the invention.

[0042] **FIG. 28** illustrates increased production of the benzylisoquinoline alkaloid reticuline by increasing the copy number of the *NCS* gene from two copies to three copies in an engineered yeast strain, in accordance with embodiments of the invention.

DETAILED DESCRIPTION OF THE INVENTION

[0043] The present disclosure provides methods for the production of diverse benzylisoquinoline alkaloids (BIAs) in engineered host cells. The present disclosure further provides compositions of diverse alkaloids produced in engineered host cells. Additionally, the present disclosure provides methods for the production of an epimerase in engineered host cells. In particular cases, the disclosure provides methods for producing diverse alkaloid products through the epimerization of a *(S)*-1-benzylisoquinoline alkaloid to a *(R)*-1-benyzlisoquinoline alkaloid in an engineered host cell. In further particular cases, the present disclosure provides methods for producing diverse alkaloid products through the epimerization of *(S)*-reticuline to *(R)*-reticuline.

Benzylisoquinoline Alkaloids (BIAs) of Interest

[0044] Host cells which produce BIAs of interest are provided. In some examples, engineered strains of host cells such as the engineered strains of the invention provide a platform for producing benzylisoquinoline alkaloids of interest and modifications thereof across several structural classes including, but not limited to, precursor BIAs, benzylisoquinolines, protoberberines, protopines, benzophenanthridines, promorphinans,

morphinans, secoberberines, phthalideisoquinolines, aporphines, bisbenzylisoquinolines, and others. Each of these classes is meant to include biosynthetic precursors, intermediates, and metabolites thereof, of any convenient member of an engineered host cell biosynthetic pathway that may lead to a member of the class. Non-limiting examples of compounds are given below for each of these structural classes. In some cases, the structure of a given example may or may not be characterized itself as a benzylisoquinoline alkaloid. The present chemical entities are meant to include all possible isomers, including single enantiomers, racemic mixtures, optically pure forms, mixtures of diastereomers, and intermediate mixtures.

[0045] BIA precursors may include, but are not limited to, norcoclaurine (NC) and norlaudanosoline (NL), as well as NC and NL precursors, such as tyrosine, tyramine, 4-hydroxyphenylacetaldehyde (4-HPA), 4-hydroxyphenylpyruvic acid (4-HPPA), L-3,4-dihydroxyphenylalanine (L-DOPA), 3,4-dihydroxyphenylacetaldehyde (3,4-DHPA), and dopamine. In some embodiments, the one or more BIA precursors are 3,4-dihydroxyphenylacetaldehyde (3,4-DHPA) and dopamine. In certain instances, the one or more BIA precursors are 4-hydroxyphenylacetaldehyde (4-HPA) and dopamine. In particular, NL and NC may be synthesized, respectively, from precursor molecules via a Pictet-Spengler condensation reaction, where the reaction may occur spontaneously or may by catalyzed by any convenient enzymes.

[0046] Benzylisoquinolines may include, but are not limited to, norcoclaurine, norlaudanosoline, coclaurine, 3'-hydroxycoclaurine, 4'-*O*-methylnorlaudanosoline, 4'-*O*-methyl-laudanosoline, *N*-methylnorcoclaurine, laudanosoline, *N*-methylcoclaurine, 3'-hydroxy-*N*-methylcoclaurine, reticuline, norreticuline, papaverine, laudanine, laudanosine, tetrahydropapaverine, 1,2-dihydropapaverine, and orientaline.

[0047] Protoberberines may include, but are not limited to, scoulerine, cheilanthifoline, stylopine, nandinine, jatrorrhizine, stepholidine, discretamine, *cis*-*N*-methylstylopine, tetrahydrocolumbamine, palmatine, tetrahydropalmatine, columbamine, canadine, *N*-methylcanadine, 1-hydroxycanadine, berberine, *N*-methyl-ophiocarpine, 1,13-dihydroxy-*N*-methylcanadine, and 1-hydroxy-10-*O*-acetyl-*N*-methylcanadine.

[0048] Protopines may include, but are not limited to, protopine, 6-hydroxyprotopine, allocryptopine, cryptopine, muramine, and thalictricine.

[0049] Benzophenanthridines may include, but are not limited to, dihydrosanguinarine, sanguinarine, dihydrocheilirubine, cheilirubine, dihydromarcapine, marcapine, and chelerythrine.

[0050] Promorphinans may include, but are not limited to, salutaridine, salutaridinol, and salutaridinol-7-*O*-acetate.

[0051] Morphinans may include, but are not limited to, thebaine, codeinone, codeine, morphine, morphinone, oripavine, neopinone, neopine, neomorphine, hydrocodone, dihydrocodeine, 14-hydroxycodeinone, oxycodone, 14-hydroxycodeine, morphinone, hydromorphone, dihydromorphine, dihydroetorphine, ethylmorphine, etorphine, metopon, buprenorphine, pholcodine, heterocodeine, and oxymorphone.

[0052] Secoberberines may include, but are not limited to, 4'-*O*-desmethylmacrantaldehyde, 4'-*O*-desmethylpapaveroxine, 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine, papaveroxine, and 3-*O*-aceteylpapaveroxine.

[0053] Phthalideisoquinolines may include, but are not limited to, narcotolinehemiacetal, narcotinehemiacetal, narcotoline, noscapine, adlumidine, adlumine, (+) or (-)-bicuculline, capnoidine, carlumine, corledine, corlumidine, decumbenine, 5'-*O*-demethylnarcotine, (+) or (-)-α or β-hydrastine, and hypecoumine .

[0054] Aporphines may include, but are not limited to, magnoflorine, corytuberine, apomorphine, boldine, isoboldine, isothebaine, isocorytuberine, and glaufine.

[0055] Bisbenzylisoquinolines may include, but are not limited to, berbamunine, guattgaumerine, dauricine, and liensinine.

[0056] Other compounds that may be produced by the engineered strains of the invention may include, but are not limited to, rhoeadine, pavine, isopavine, and cularine.

[0057] In certain embodiments, the engineered strains of the invention may provide a platform for producing compounds related to tetrahydrobiopterin synthesis including, but not limited to, dihydroneopterin triphosphate, 6-pyruvoyl tetrahydropterin, 5,6,7,8-tetrahydrobiopterin, 7,8-dihydrobiopterin, tetrahydrobiopterin 4a-carbinolamine, quinonoid dihydrobiopterin, and biopterin.

Host Cells

[0058] Any convenient cells may be utilized in the subject host cells and methods. In some cases, the host cells are non-plant cells. In some instances, the host cells may be characterized as microbial cells. In certain cases, the host cells are insect cells, mammalian cells, bacterial cells, or yeast cells. Any convenient type of host cell may be utilized in producing the subject BIA-producing cells, see, e.g., US2008/0176754, and US2014/0273109 the disclosures of which are incorporated by reference in their entirety. Host cells of interest include, but are not limited to, bacterial cells, such as *Bacillus subtilis*, *Escherichia coli, Streptomyces,* and *Salmonella typhimuium* cells, insect cells such as *Drosophila melanogaster* S2 and *Spodoptera frugiperda* Sf9 cells, and yeast cells such as *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, and *Pichia pastoris* cells. In some examples, the host cells are yeast cells or *E. coli* cells. In some cases, the host cell is a yeast cell. In some instances, the host cell is from a strain of yeast engineered to produce a BIA of interest, such as an *(R)*-1-benzylisoquinoline alkaloid. In some instances, the host cell is from a strain of yeast engineered to produce an enzyme of interest. In some instances, the host cell is from a strain of yeast engineered to produce an epimerase. The epimerase may have an oxidase and a reductase. Additionally, the epimerase may be able to convert an *(S)*-1-benzylisoquinoline alkaloid to an *(R)*-1-benzylisoquinoline alkaloid. Further, the

epimerase may be separated into smaller enzymes that retain oxidase or reductase activity so as to be used to convert an *(S)*-1-benzylisoquinoline alkaloid to an *(R)*-1-benzylisoquinoline alkaloid.

[0059] Any of the host cells described in US2008/0176754 and US2014/0273109 by Smolke et al. may be adapted for use in the subject cells and methods. In certain embodiments, the yeast cells may be of the species *Saccharomyces cerevisiae* (*S. cerevisiae*). In certain embodiments, the yeast cells may be of the species *Schizosaccharomyces pombe*. In certain embodiments, the yeast cells may be of the species *Pichia pastoris*. Yeast is of interest as a host cell because cytochrome P450 proteins are able to fold properly into the endoplasmic reticulum membrane so that their activity is maintained. In examples, cytochrome P450 proteins are involved in some biosynthetic pathways of interest. In additional examples, cytochrome P450 proteins are involved in the production of BIAs of interest. In further examples, cytochrome P450 proteins are involved in the production of an enzyme of interest, such as an epimerase having an oxidase and a reductase.

[0060] Yeast strains of interest that find use in the invention include, but are not limited to, CEN.PK (Genotype: *MAT*a/α *ura3-52/ura3-52 trp1-289/trp1-289 leu2-3_112/leu2-3_112 his3 Δ1/his3 Δ1 MAL2-8C/MAL2-8C SUC2/SUC2*), S288C, W303, D273-10B, X2180, A364A, ∑1278B, AB972, SK1, and FL100. In certain cases, the yeast strain is any of S288C (MATα; SUC2 mal mel gal2 CUP1 flo1 flo8-1 hap1), BY4741 (MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0), BY4742 (MATα; his3Δ1; leu2Δ0; lys2Δ0; ura3Δ0), BY4743 (MATa/MATα; his3Δ1/his3Δ1; leu2Δ0/leu2Δ0; met15Δ0/MET15; LYS2/lys2Δ0; ura3Δ0/ura3Δ0), and WAT11 or W*(R)*, derivatives of the W303-B strain (MATa; ade2-1; his3-11, -15; leu2-3,-112; ura3-1; canR; cyr+) which express the *Arabidopsis thaliana* NADPH-P450 reductase ATR1 and the yeast NADPH-P450 reductase CPR1, respectively. In another embodiment, the yeast cell is W303alpha (MATα; his3-11,15 trp1-1 leu2-3 ura3-1 ade2-1). The identity and genotype of additional yeast strains of interest may be found at EUROSCARF (web.uni-frankfurt.de/fb15/mikro/euroscarf/col_index.html).

Genetic Modifications to Host Cells

[0061] The host cells may be engineered to include one or more modifications (such as two or more, three or more, four or more, five or more, or even more modifications) that provide for the production of BIAs of interest. Additionally or alternatively, the host cells may be engineered to include one or more modifications (such as two or more, three or more, four or more, five or more, or even more modifications) that provide for the production of enzymes of interest. In some cases, a modification is a genetic modification, such as a mutation, addition, or deletion of a gene or fragment thereof, or transcription regulation of a gene or fragment thereof. As used herein, the term "mutation" refers to a deletion, insertion, or substitution of an amino acid(s) residue or nucleotide(s) residue relative to a reference sequence or motif. The mutation may be incorporated as a directed mutation to the native gene at the original locus. In some cases, the mutation may be incorporated as an additional copy of the gene introduced as a genetic integration at a separate locus, or as an

additional copy on an episomal vector such as a 2μ or centromeric plasmid. In certain instances, the substrate inhibited copy of the enzyme is under the native cell transcriptional regulation. In some instances, the substrate inhibited copy of the enzyme is introduced with engineered constitutive or dynamic regulation of protein expression by placing it under the control of a synthetic promoter. In some examples, the object of one or more modifications may be a native gene. In some examples, the object of one or more modifications may be a non-native gene. In some examples, a non-native gene may be inserted into a host cell. In further examples, a non-native gene may be altered by one or more modifications prior to being inserted into a host cell.

[0062] An engineered host cell may overproduce one or more BIAs of interest. By overproduce is meant that the cell has an improved or increased production of a BIA molecule of interest relative to a control cell (e.g., an unmodified cell). By improved or increased production is meant both the production of some amount of the BIA of interest where the control has no BIA of interest production, as well as an increase of about 10% or more, such as about 20% or more, about 30% or more, about 40% or more, about 50% or more, about 60% or more, about 80% or more, about 100% or more, such as 2-fold or more, such as 5-fold or more, including 10-fold or more in situations where the control has some BIA of interest production.

[0063] An engineered host cell may overproduce one or more *(S)*-1-benzylisoquinoline alkaloids. In some cases, the engineered host cell may produce some amount of the *(S)*-1-benzylisoquinoline alkaloid of interest where the control has no *(S)*-1-benzylisoquinoline alkaloid production, as well as an increase of about 10% or more, such as about 20% or more, about 30% or more, about 40% or more, about 50% or more, about 60% or more, about 80% or more, about 100% or more, such as 2-fold or more, such as 5-fold or more, including 10-fold or more in situations where the control has some *(S)*-1-benzylisoquinoline alkaloid of interest production.

[0064] An engineered host cell may further overproduce one or more *(R)*-1-benzylisoquinoline alkaloids. In some cases, the engineered host cell may produce some amount of the *(R)*-1-benzylisoquinoline alkaloid of interest where the control has no *(R)*-1-benzylisoquinoline alkaloid production, as well as an increase of about 10% or more, such as about 20% or more, about 30% or more, about 40% or more, about 50% or more, about 60% or more, about 80% or more, about 100% or more, such as 2-fold or more, such as 5-fold or more, including 10-fold or more in situations where the control has some *(R)*-1-benzylisoquinoline alkaloid of interest production. An engineered host cell may further overproduce one or more of morphinan, protoberberine, noscapinoid, and benzophenanthridine alkaloids.

[0065] In some cases, the engineered host cell is capable of producing an increased amount of *(R)*-reticuline relative to a control host cell that lacks the one or more modifications (e.g., as described herein). In certain instances, the increased amount of *(R)*-reticuline is about 10% or more relative to the control host cell, such as about 20% or more, about 30% or more, about 40% or more, about 50% or more, about 60% or more, about 80% or more, about 100% or more, about 2-fold or more, about 5-fold or more, or even about 10-fold or more

relative to the control host cell. In some cases, *(R)*-reticuline is the product of an epimerization reaction within an engineered host cell. In these cases, *(S)*-reticuline may be the substrate of the epimerization reaction.

[0066] Additionally, an engineered host cell may overproduce one or more enzymes of interest. By overproduce is meant that the cell has an improved or increased production of an enzyme of interest relative to a control cell (e.g., an unmodified cell). By improved or increased production is meant both the production of some amount of the enzyme of interest where the control has no production, as well as an increase of about 10% or more, such as about 20% or more, about 30% or more, about 40% or more, about 50% or more, about 60% or more, about 80% or more, about 100% or more, such as 2-fold or more, such as 5-fold or more, including 10-fold or more in situations where the control has some enzyme of interest production.

[0067] An engineered host cell may overproduce one or more CYP-COR enzyme. In some cases, the engineered host cell may produce some amount of the CYP-COR enzyme where the control has no CYP-COR enzyme production, as well as an increase of about 10% or more, such as about 20% or more, about 30% or more, about 40% or more, about 50% or more, about 60% or more, about 80% or more, about 100% or more, such as 2-fold or more, such as 5-fold or more, including 10-fold or more in situations where the control has some CYP-COR enzyme production.

[0068] An engineered host cell may further overproduce one or more enzymes that are derived from the CYP-COR enzyme. In some cases, the engineered host cell may produce some amount of the enzymes that are derived from the CYP-COR enzyme, where the control has no production of enzymes that are derived from the CYP-COR enzyme, as well as an increase of about 10% or more, such as about 20% or more, about 30% or more, about 40% or more, about 50% or more, about 60% or more, about 80% or more, about 100% or more, such as 2-fold or more, such as 5-fold or more, including 10-fold or more in situations where the control has some production of enzymes that are derived from the CYP-COR enzyme.

[0069] Additionally, an engineered host cell may overproduce one or more bisbenzylisoquinoline alkaloids (bisBIAs). In particular, an engineered host cell is capable of producing an increased amount of bisbenzylisoquinoline alkaloids (bisBIAs) relative to a control host cell that lacks the one or more modifications (e.g., as described herein). In certain instances, the increased amount of bisBIAs is about 10% or more relative to the control host cell, such as about 20% or more, about 30% or more, about 40% or more, about 50% or more, about 60% or more, about 80% or more, about 100% or more, about 2-fold or more, about 5-fold or more, or even about 10-fold or more relative to the control host cell. In some cases, the bisBIA is formed from at least one BIA monomer that is the product, or derivative thereof, of an epimerization reaction within an engineered host cell. An engineered host cell may further overproduce one or more of cepharanthine, fangchinoline, liensinine, neferine, tubocurarine, dauricine, tetrandrine, curine, berbamunine, guattegaumerine, 2'-norberbamunine, and berbamine.

[0070] In some cases, the one or more (such as two or more, three or more, or four or more) modifications may be selected from: a substrate inhibition alleviating mutation in a biosynthetic enzyme gene; a product inhibition alleviating mutation in a biosynthetic enzyme gene; a cofactor recovery promoting mechanism; a feedback inhibition alleviating mutation in a biosynthetic enzyme gene; a transcriptional modulation modification of a biosynthetic enzyme gene; an inactivating mutation in an enzyme gene; an epimerization modification; a bisBIA generating modification; and a heterologous coding sequence that encodes an enzyme. A cell that includes one or more modifications may be referred to as an engineered cell.

Substrate Inhibition Alleviating Mutations

[0071] In some instances, the engineered host cells are cells that include one or more substrate inhibition alleviating mutations (such as two or more, three or more, four or more, five or more, or even more) in one or more biosynthetic enzyme genes of the cell. In some examples, the one or more biosynthetic enzyme genes are native to the cell (e.g., is present in an unmodified cell). In some examples, the one or more biosynthetic enzyme genes are non-native to the cell. As used herein, the term "substrate inhibition alleviating mutation" refers to a mutation that alleviates a substrate inhibition control mechanism of the cell.

[0072] A mutation that alleviates substrate inhibition reduces the inhibition of a regulated enzyme in the cell of interest relative to a control cell and provides for an increased level of the regulated compound or a downstream biosynthetic product thereof. In some cases, by alleviating inhibition of the regulated enzyme is meant that the IC_{50} of inhibition is increased by 2-fold or more, such as by 3-fold or more, 5-fold or more, 10-fold or more, 30-fold or more, 100-fold or more, 300-fold or more, 1000-fold or more, or even more. By increased level is meant a level that is 110% or more of that of the regulated compound in a control cell or a downstream product thereof, such as 120% or more, 130% or more, 140% or more, 150% or more, 160% or more, 170% or more, 180% or more, 190% or more, or 200% or more, such as at least 3-fold or more, at least 5-fold or more, at least 10-fold or more or even more of the regulated compound in the engineered host cell or a downstream product thereof.

[0073] A variety of substrate inhibition control mechanisms and biosynthetic enzymes in the engineered host cell that are directed to regulation of levels of BIAs of interest, or precursors thereof, may be targeted for substrate inhibition alleviation. The engineered host cell may include one or more substrate inhibition alleviating mutations in one or more biosynthetic enzyme genes. The one or more mutations may be located in any convenient biosynthetic enzyme genes where the biosynthetic enzyme is subject to regulatory control. In some embodiments, the one or more biosynthetic enzyme genes encode one or more tyrosine hydroxylase enzymes. In certain instances, the one or more substrate inhibition alleviating mutations are present in a biosynthetic enzyme gene that is TyrH. In some embodiments, the engineered host cell may include one or

more substrate inhibition alleviating mutations in one or more biosynthetic enzyme genes such as one of those genes described in **Table 2**.

[0074] In certain embodiments, the one or more substrate inhibition alleviating mutations are present in the TyrH gene. The TyrH gene encodes tyrosine hydroxylase, which is an enzyme that converts tyrosine to L-DOPA. However, TyrH is inhibited by its substrate, tyrosine. Mammalian tyrosine hydroxylase activity, such as that seen in humans or rats, can be improved through mutations to the TyrH gene that relieve substrate inhibition. In particular, substrate inhibition from tyrosine can be relieved by a point mutation W166Y in the TyrH gene. The point mutation W166Y in the TyrH gene may also improve the binding of the cosubstrate of tyrosine hydroxylase, BH_4, to catalyze the reaction of tyrosine to L-DOPA. The mutants of TyrH, when expressed in yeast strains to produce BIAs from sugar (such as those described in United States Provisional Patent Application Serial No. 61/899,496) can significantly improve the production of BIAs.

[0075] Any convenient numbers and types of mutations may be utilized to alleviate a substrate inhibition control mechanism. In certain embodiments, the engineered host cells of the present invention may include 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, or even 15 or more substrate inhibition alleviating mutations, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 substrate inhibition alleviating mutations in one or more biosynthetic enzyme genes within the engineered host cell.

Cofactor Recovery Promoting Mechanisms

[0076] In some instances, the engineered host cells are cells that include one or more cofactor recovery promoting mechanisms (such as two or more, three or more, four or more, five or more, or even more) in one or more biosynthetic enzyme genes of the cell. In some examples, the one or more biosynthetic enzyme genes are native to the cell (e.g., is present in an unmodified cell). In some examples, the one or more biosynthetic enzyme genes are non-native to the cell. As used herein, the term "cofactor recovery promoting mechanism" refers to a mechanism that promotes a cofactor recovery control mechanism of the cell.

[0077] A variety of cofactor recovery control mechanisms and biosynthetic enzymes in the engineered host cell that are directed to regulation of levels of BIAs of interest, or precursors thereof, may be targeted for cofactor recovery promotion. The engineered host cell may include one or more cofactor recovery promoting mechanism in one or more biosynthetic enzyme genes. In examples, the engineered host cell may include a heterologous coding sequence that encodes dihydrofolate reductase (DHFR). When DHFR is expressed, it may convert 7,8-dihydrobiopterin (BH_2) to the tetrahydrobiopterin (BH_4), thereby recovering BH_4 as a TyrH cosubstrate. In some examples, the engineered host cell may include one or more cofactor recovery promoting mechanisms in one or more biosynthetic enzyme genes such as one of those genes described in **Table 2**.

[0078] Any convenient numbers and types of mechanisms may be utilized to promote a cofactor recovery control mechanism. In certain embodiments, the engineered host cells of the present invention may include 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, or even 15 or more cofactor recovery promoting mechanisms such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 cofactor recovery promoting mechanisms in one or more biosynthetic enzyme genes within the engineered host cell.

Product Inhibition Alleviating Mutations

[0079] In some instances, the engineered host cells are cells that include one or more product inhibition alleviating mutations (such as two or more, three or more, four or more, five or more, or even more) in one or more biosynthetic enzyme genes of the cell. In some examples, the one or more biosynthetic enzyme genes are native to the cell (e.g., is present in an unmodified cell). In some examples, the one or more biosynthetic enzyme genes are non-native to the cell. As used herein, the term "product inhibition alleviating mutation" refers to a mutation that alleviates a short term and/or long term product inhibition control mechanism of an engineered host cell. Short term product inhibition is a control mechanism of the cell in which there is competitive binding at a cosubstrate binding site. Long term product inhibition is a control mechanism of the cell in which there is irreversible binding of a compound away from a desired pathway.

[0080] A mutation that alleviates product inhibition reduces the inhibition of a regulated enzyme in the cell of interest relative to a control cell and provides for an increased level of the regulated compound or a downstream biosynthetic product thereof. In some cases, by alleviating inhibition of the regulated enzyme is meant that the IC_{50} of inhibition is increased by 2-fold or more, such as by 3-fold or more, 5-fold or more, 10-fold or more, 30-fold or more, 100-fold or more, 300-fold or more, 1000-fold or more, or even more. By increased level is meant a level that is 110% or more of that of the regulated compound in a control cell or a downstream product thereof, such as 120% or more, 130% or more, 140% or more, 150% or more, 160% or more, 170% or more, 180% or more, 190% or more, or 200% or more, such as at least 3-fold or more, at least 5-fold or more, at least 10-fold or more or even more of the regulated compound in the engineered host cell or a downstream product thereof.

[0081] A variety of product inhibition control mechanisms and biosynthetic enzymes in the engineered host cell that are directed to regulation of levels of BIAs of interest may be targeted for product inhibition alleviation. The engineered host cell may include one or more product inhibition alleviating mutations in one or more biosynthetic enzyme genes. The mutation may be located in any convenient biosynthetic enzyme genes where the biosynthetic enzyme is subject to regulatory control. In some embodiments, the one or more biosynthetic enzyme genes encode one or more tyrosine hydroxylase enzymes. In certain instances, the one or more product inhibition alleviating mutations are present in a biosynthetic enzyme gene that is TyrH. In some

embodiments, the engineered host cell includes one or more product inhibition alleviating mutations in one or more biosynthetic enzyme genes such as one of those genes described in **Table 2**.

[0082] In certain embodiments, the one or more product inhibition alleviating mutations are present in the TyrH gene. The TyrH gene encodes tyrosine hydroxylase, which is an enzyme that converts tyrosine to L-DOPA. TyrH requires tetrahydrobiopterin (BH_4) as a cosubstrate to catalyze the hydroxylation reaction. Some microbial strains, such as *Saccharomyces cerevisiae*, do not naturally produce BH_4, but can be engineered to produce this substrate through a four-enzyme synthesis and recycling pathway, as illustrated in **FIG. 1**. **FIG. 1** illustrates examples of synthesis, recycling, and salvage pathways of tetrahydrobiopterin, in accordance with embodiments of the invention. **FIG. 1** provides the use of the enzymes PTPS, pyruvoyl tetrahydropterin synthase; SepR, sepiapterin reductase; PCD, pterin 4a-carbinolamine dehydratase; QDHPR, dihydropteridine reductase; and DHFR, dihydrofolate reductase. Of the enzymes that are illustrated in **FIG. 1**, yeast synthesizes an endogenous GTP cyclohydrolase I. GTP and dihydroneopterin triphosphate are naturally synthesized in yeast. Additionally, other metabolites in **FIG. 1** are not naturally produced in yeast.

[0083] TyrH is inhibited by its product L-DOPA, as well as other catecholamines, particularly dopamine. Mammalian tyrosine hydroxylase activity, such as from humans or rats, can be improved through mutations that relieve product inhibition. For example, short term product inhibition, such as competitive binding at the cosubstrate binding site, can be relieved by a point mutation W166Y on the TyrH gene. In particular, the point mutation W166Y on the TyrH gene may improve binding of the cosubstrate. Additionally, short term product inhibition to relieve competitive binding at the cosubstrate binding site may be improved by a point mutation S40D on the TyrH gene. Short term product inhibition may also be improved by the joint mutations of R37E, R38E on the TyrH gene. In particular, R37E, R38E mutations may together specifically improve tyrosine hydroxylase activity in the presence of dopamine.

[0084] Additionally, long term product inhibition may be relieved by point mutations on the TyrH gene. Long term product inhibition relief may include the irreversible binding of catecholamine to iron in the active site such that there is less catecholamine present to act as a product inhibitor of tyrosine hydroxylase activity. Long term product inhibition can be relieved by the mutations E332D and Y371F, respectively, in the TyrH gene.

[0085] Combinations of the mutations can be made (such as two or three or more mutations at once) to relieve multiple types of substrate and product inhibition to further improve the activity of TyrH. The mutants of TyrH, when expressed in yeast strains to produce BIAs from sugar (such as those described in United States Provisional Patent Application Serial No. 61/899,496) can significantly improve the production of BIAs.

[0086] Any convenient numbers and types of mutations may be utilized to alleviate a product inhibition control mechanism. In certain embodiments, the engineered host cells of the present invention may include 1

or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, or even 15 or more product inhibition alleviating mutations, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 product inhibition alleviating mutations in one or more biosynthetic enzyme genes within the engineered host cell.

Feedback Inhibition Alleviating Mutations

[0087] In some instances, the engineered host cells are cells that include one or more feedback inhibition alleviating mutations (such as two or more, three or more, four or more, five or more, or even more) in one or more biosynthetic enzyme genes of the cell. In some cases, the one or more biosynthetic enzyme genes are native to the cell (e.g., is present in an unmodified cell). Additionally or alternatively, in some examples the one or more biosynthetic enzyme genes are non-native to the cell. As used herein, the term "feedback inhibition alleviating mutation" refers to a mutation that alleviates a feedback inhibition control mechanism of an engineered host cell. Feedback inhibition is a control mechanism of the cell in which an enzyme in the synthetic pathway of a regulated compound is inhibited when that compound has accumulated to a certain level, thereby balancing the amount of the compound in the cell. A mutation that alleviates feedback inhibition reduces the inhibition of a regulated enzyme in the engineered host cell relative to a control cell. In this way, engineered host cell provides for an increased level of the regulated compound or a downstream biosynthetic product thereof. In some cases, by alleviating inhibition of the regulated enzyme is meant that the IC_{50} of inhibition is increased by 2-fold or more, such as by 3-fold or more, 5-fold or more, 10-fold or more, 30-fold or more, 100-fold or more, 300-fold or more, 1000-fold or more, or even more. By increased level is meant a level that is 110% or more of that of the regulated compound in a control cell or a downstream product thereof, such as 120% or more, 130% or more, 140% or more, 150% or more, 160% or more, 170% or more, 180% or more, 190% or more, or 200% or more, such as at least 3-fold or more, at least 5-fold or more, at least 10-fold or more or even more of the regulated compound in the host cell or a downstream product thereof.

[0088] A variety of feedback inhibition control mechanisms and biosynthetic enzymes that are directed to regulation of levels of BIAs of interest may be targeted for alleviation in the host cell. The host cell may include one or more feedback inhibition alleviating mutations in one or more biosynthetic enzyme genes native to the cell. The one or more mutations may be located in any convenient biosynthetic enzyme genes where the biosynthetic enzyme is subject to regulatory control. In some embodiments, the one or more biosynthetic enzyme genes may encode one or more enzymes selected from a 3-deoxy-d-arabinose-heptulosonate-7-phosphate (DAHP) synthase and a chorismate mutase. In some embodiments, the one or more biosynthetic enzyme genes encode a 3-deoxy-d-arabinose-heptulosonate-7-phosphate (DAHP) synthase. In some instances, the one or more biosynthetic enzyme genes may encode a chorismate mutase. In certain

instances, the one or more feedback inhibition alleviating mutations may be present in a biosynthetic enzyme gene selected from ARO4 and ARO7. In certain instances, the one or more feedback inhibition alleviating mutations may be present in a biosynthetic enzyme gene that is ARO4. In certain instances, the one or more feedback inhibition alleviating mutations are present in a biosynthetic enzyme gene that is ARO7. In some embodiments, the engineered host cell may include one or more feedback inhibition alleviating mutations in one or more biosynthetic enzyme genes such as one of those genes described in **Table 2**.

[0089] Any convenient numbers and types of mutations may be utilized to alleviate a feedback inhibition control mechanism. As used herein, the term "mutation" refers to a deletion, insertion, or substitution of an amino acid(s) residue or nucleotide(s) residue relative to a reference sequence or motif. The mutation may be incorporated as a directed mutation to the native gene at the original locus. In some cases, the mutation may be incorporated as an additional copy of the gene introduced as a genetic integration at a separate locus, or as an additional copy on an episomal vector such as a 2μ or centromeric plasmid. In certain instances, the feedback inhibited copy of the enzyme is under the native cell transcriptional regulation. In some instances, the feedback inhibited copy of the enzyme is introduced with engineered constitutive or dynamic regulation of protein expression by placing it under the control of a synthetic promoter.

[0090] In certain embodiments, the one or more feedback inhibition alleviating mutations may be present in the ARO4 gene. ARO4 mutations of interest may include, but are not limited to, substitution of the lysine residue at position 229 with a leucine, a substitution of the glutamine residue at position 166 with a lysine residue, or a mutation as described by Hartmann M, et al. ((2003) Proc Natl Acad Sci U S A 100(3):862-867) or Fukuda et al. ((1992) J Ferment Bioeng 74(2):117-119). In some instances, mutations for conferring feedback inhibition may be selected from a mutagenized library of enzyme mutants. Examples of such selections may include rescue of growth of o-fluoro-D,L-phenylalanine or growth of aro3 mutant yeast strains in media with excess tyrosine as described by Fukuda et al. ((1990) Breeding of Brewing Yeast Producing a Large Amount of Beta-Phenylethyl Alcohol and Beta-Phenylethyl Acetate. Agr Biol Chem Tokyo 54(1):269-271).

[0091] In certain embodiments, the engineered host cells of the present invention may include 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, or even 15 or more feedback inhibition alleviating mutations, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 feedback inhibition alleviating mutations in one or more biosynthetic enzyme genes within the engineered host cell.

Transcriptional Modulation Modifications

[0092] The host cells may include one or more transcriptional modulation modifications (such as two or more, three or more, four or more, five or more, or even more modifications) of one or more biosynthetic

enzyme genes of the cell. In some examples, the one or more biosynthetic enzyme genes are native to the cell. In some examples, the one or more biosynthetic enzyme genes are non-native to the cell. Any convenient biosynthetic enzyme genes of the cell may be targeted for transcription modulation. By transcription modulation is meant that the expression of a gene of interest in a modified cell is modulated, e.g., increased or decreased, enhanced or repressed, relative to a control cell (e.g., an unmodified cell). In some cases, transcriptional modulation of the gene of interest includes increasing or enhancing expression. By increasing or enhancing expression is meant that the expression level of the gene of interest is increased by 2-fold or more, such as by 5-fold or more and sometimes by 25-, 50-, or 100-fold or more and in certain embodiments 300-fold or more or higher, as compared to a control, i.e., expression in the same cell not modified (e.g., by using any convenient gene expression assay). Alternatively, in cases where expression of the gene of interest in a cell is so low that it is undetectable, the expression level of the gene of interest is considered to be increased if expression is increased to a level that is easily detectable. In certain instances, transcriptional modulation of the gene of interest includes decreasing or repressing expression. By decreasing or repressing expression is meant that the expression level of the gene of interest is decreased by 2-fold or more, such as by 5-fold or more and sometimes by 25-, 50-, or 100-fold or more and in certain embodiments 300-fold or more or higher, as compared to a control. In some cases, expression is decreased to a level that is undetectable. Modifications of host cell processes of interest that may be adapted for use in the subject host cells are described in U.S. Publication No. 20140273109 (14/211,611) by Smolke et al., the disclosure of which is herein incorporated by reference in its entirety.

[0093] Any convenient biosynthetic enzyme genes may be transcriptionally modulated, and include but are not limited to, those biosynthetic enzymes described in **FIG. 2**. In particular, **FIG. 2** illustrates a biosynthetic scheme for conversion of glucose to 4-HPA, dopamine, and 3,4-DHPA, in accordance with embodiments of the invention. Examples of enzymes described in **FIG. 2** include ARO3, ARO4, ARO1, ARO7, TYR1, TYR, TyrH, DODC, MAO, ARO10, ARO9, and TKL. In some instances, the one or more biosynthetic enzyme genes may be selected from ARO10, ARO9, and TKL. In some cases, the one or more biosynthetic enzyme genes may be ARO10. In certain instances, the one or more biosynthetic enzyme genes may be ARO9. In some embodiments, the one or more biosynthetic enzyme genes may be TKL. In some embodiments, the host cell includes one or more transcriptional modulation modifications to one or more genes such as one of those genes described in **Table 2**.

[0094] In some embodiments, the transcriptional modulation modification may include a substitution of a strong promoter for a native promoter of the one or more biosynthetic enzyme genes or the expression of an additional copy(ies) of the gene or genes under the control of a strong promoter. The promoters driving expression of the genes of interest may be constitutive promoters or inducible promoters, provided that the promoters may be active in the host cells. The genes of interest may be expressed from their native promoters. Additionally or alternatively, the genes of interest may be expressed from non-native promoters. Although

not a requirement, such promoters may be medium to high strength in the host in which they are used. Promoters may be regulated or constitutive. In some embodiments, promoters that are not glucose repressed, or repressed only mildly by the presence of glucose in the culture medium, may be used. There are numerous suitable promoters, examples of which include promoters of glycolytic genes such as the promoter of the *B. subtilis* tsr gene (encoding fructose biphosphate aldolase) or GAPDH promoter from yeast *S. cerevisiae* (coding for glyceraldehyde-phosphate dehydrogenase) (Bitter G. A., *Meth. Enzymol.* 152:673 684 (1987)). Other strong promoters of interest include, but are not limited to, the ADHI promoter of baker's yeast (Ruohonen L., et al, *J. Biotechnol.* 39:193 203 (1995)), the phosphate-starvation induced promoters such as the PHO5 promoter of yeast (Hinnen, A., et al, in *Yeast Genetic Engineering,* Barr, P. J., et al. eds, Butterworths (1989), the alkaline phosphatase promoter from *B. licheniformis* (Lee. J. W. K., et al., *J. Gen. Microbiol.* 137:1127 1133 (1991)), GPD1, and TEF1. Yeast promoters of interest include, but are not limited to, inducible promoters such as Gal1-10, Gal1, GalL, GalS, repressible promoter Met25, tetO, and constitutive promoters such as glyceraldehyde 3-phosphate dehydrogenase promoter (GPD), alcohol dehydrogenase promoter (ADH), translation-elongation factor-1-alpha promoter (TEF), cytochrome c-oxidase promoter (CYC1), MRP7 promoter, etc. In some instances, the strong promoter is GPD1. In certain instances, the strong promoter is TEF1. Autonomously replicating yeast expression vectors containing promoters inducible by hormones such as glucocorticoids, steroids, and thyroid hormones are also known and include, but are not limited to, the glucorticoid responsive element (GRE) and thyroid hormone responsive element (TRE), see e.g., those promoters described in U.S. Pat. No. 7,045,290. Vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. Additionally any promoter/enhancer combination (as per the Eukaryotic Promoter Data Base EPDB) could also be used to drive expression of genes of interest. It is understood that any convenient promoters specific to the host cell may be selected, e.g., *E. coli.* In some cases, promoter selection may be used to optimize transcription, and hence, enzyme levels to maximize production while minimizing energy resources.

Inactivating Mutations

[0095] The engineered host cells may include one or more inactivating mutations to an enzyme of the cell (such as two or more, three or more, four or more, five or more, or even more). The inclusion of one or more inactivating mutations may modify the flux of a synthetic pathway of an engineered host cell to increase the levels of a BIA of interest or a desirable enzyme or precursor leading to the same. In some examples, the one or more inactivating mutations are to an enzyme native to the cell. Additionally or alternatively, the one or more inactivating mutations are to an enzyme non-native to the cell. As used herein, by "inactivating mutation" is meant one or more mutations to a gene or regulatory DNA sequence of the cell, where the mutation(s) inactivates a biological activity of the protein expressed by that gene of interest. In some cases, the gene is native to the cell. In some instances, the gene encodes an enzyme that is inactivated and is part of

or connected to the synthetic pathway of a BIA of interest produced by the host cell. In some instances, an inactivating mutation is located in a regulatory DNA sequence that controls a gene of interest. In certain cases, the inactivating mutation is to a promoter of a gene. Any convenient mutations (e.g., as described herein) may be utilized to inactivate a gene or regulatory DNA sequence of interest. By "inactivated" or "inactivates" is meant that a biological activity of the protein expressed by the mutated gene is reduced by 10% or more, such as by 20% or more, 30% or more, 40% or more, 50% or more, 60% or more, 70% or more, 80% or more, 90% or more, 95% or more, 97% or more, or 99% or more, relative to a control protein expressed by a non-mutated control gene. In some cases, the protein is an enzyme and the inactivating mutation reduces the activity of the enzyme.

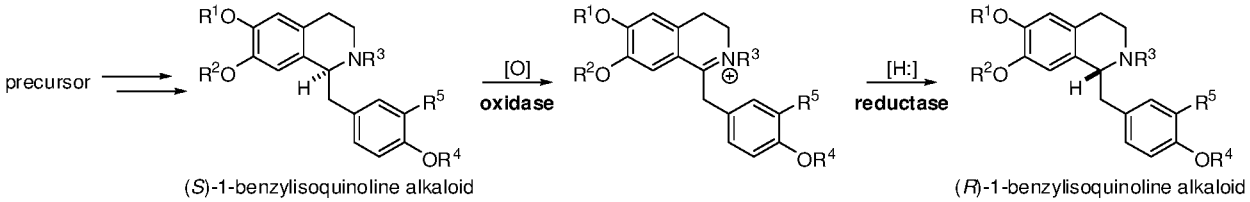
[0096] In some examples, the engineered host cell includes an inactivating mutation in an enzyme native to the cell. Any convenient enzymes may be targeted for inactivation. Enzymes of interest may include, but are not limited to those enzymes, described in **Table 2** whose action in the synthetic pathway of the engineered host cell tends to reduce the levels of a BIA of interest. In some cases, the enzyme has glucose-6-phosphate dehydrogenase activity. In certain embodiments, the enzyme that includes an inactivating mutation is ZWF1. In some cases, the enzyme has alcohol dehydrogenase activity. In some embodiments, the enzyme that includes an inactivating mutation is selected from ADH2, ADH3, ADH4, ADH5, ADH6, ADH7, and SFA1. In certain embodiments, the enzyme that includes an inactivating mutation(s) is ADH2. In certain embodiments, the enzyme that includes an inactivating mutation(s) is ADH3. In certain embodiments, the enzyme that includes an inactivating mutation(s) is ADH4. In certain embodiments, the enzyme that includes an inactivating mutation(s) is ADH5. In certain embodiments, the enzyme that includes an inactivating mutation(s) is ADH6. In certain embodiments, the enzyme that includes an inactivating mutation(s) is ADH7. In some cases, the enzyme has aldehyde oxidoreductase activity. In certain embodiments, the enzyme that includes an inactivating mutation is selected from ALD2, ALD3, ALD4, ALD5, and ALD6. In certain embodiments, the enzyme that includes an inactivating mutation(s) is ALD2. In certain embodiments, the enzyme that includes an inactivating mutation(s) is ALD3. In certain embodiments, the enzyme that includes an inactivating mutation(s) is ALD4. In certain embodiments, the enzyme that includes an inactivating mutation(s) is ALD5. In certain embodiments, the enzyme that includes an inactivating mutation(s) is ALD6. In some embodiments, the host cell includes one or more inactivating mutations to one or more genes described in **Table 2**.

Epimerization Modifications

[0097] Some methods, processes, and systems provided herein describe the conversion of *(S)*-1-benzylisoquinoline alkaloids to *(R)*-1-benzylisoquinoline alkaloids. Some of these methods, processes, and systems may comprise an engineered host cell. In some examples, the conversion of *(S)*-1-benzylisoquinoline

alkaloids to *(R)*-1-benzylisoquinoline alkaloids is a key step in the conversion of a substrate to a diverse range of alkaloids. In some examples, the conversion of *(S)*-1-benzylisoquinoline alkaloids to *(R)*-1-benzylisoquinoline alkaloids comprises an epimerization reaction. In some cases, epimerization of a substrate alkaloid may be performed by oxidizing an *(S)*-substrate to the corresponding Schiff base or imine intermediate, then stereospecifically reducing this intermediate to an *(R)*-product as provided in **FIG. 3** and as represented generally in **Scheme 1**. As provided in **Scheme 1**, R_1, R_2, R_3, and R_4 may be H or CH_3. R_5 may be H, OH, or OCH_3.

Scheme 1



[0098] In some examples, the conversion of the *(S)*-substrate to the *(R)*-product may involve at least one oxidation reaction and at least one reduction reaction. In some cases, an oxidation reaction is optionally followed by a reduction reaction. In some cases, at least one of the oxidation and reduction reactions is carried out in the presence of an enzyme. In some cases, at least one of the oxidation and reduction reactions is catalyzed by an enzyme. In some cases, the oxidation and reduction reactions are both carried out in the presence of at least one enzyme. In some cases, at least one enzyme is useful to catalyze the oxidation and reduction reactions. The oxidation and reduction reactions may be catalyzed by the same enzyme.

[0099] In some methods, processes and systems described herein, an oxidation reaction may be performed in the presence of an enzyme. In some examples, the enzyme may be an oxidase. The oxidase may use an *(S)*-1-benzylisoquinoline as a substrate. The oxidase may convert the *(S)*-substrate to a corresponding imine or Schiff base derivative. The oxidase may be referred to as 1,2-dehydroreticuline synthase (DRS). Non-limiting examples of enzymes suitable for oxidation of *(S)*-1-benzylisoquinoline alkaloids in this disclosure include a cytochrome P450 oxidase, a 2-oxoglutarate-dependent oxidase, and a flavoprotein oxidase. For example, *(S)*-tetrahydroprotoberberine oxidase (STOX, E.C 1.3.3.8) may oxidize *(S)*-norreticuline and other *(S)*-1-benzylisoquinoline alkaloids to 1,2-dehydronorreticuline and other corresponding 1,2-dehydro products. In some examples, a protein that comprises an oxidase domain of any one of the preceding examples may perform the oxidation. In some examples, the oxidase may catalyze the oxidation reaction within a host cell, such as an engineered host cell, as described herein.

[00100] In some examples, a reduction reaction may follow the oxidation reaction. The reduction reaction may be performed by an enzyme. In some examples, the reductase may use an imine or Schiff base derived from a 1-benzylisoquinoline as a substrate. The reductase may convert the imine or Schiff base derivative to an *(R)*-1-benzylisoquinoline. The reductase may be referred to as 1,2-dehydroreticuline reductase (DRR).

Non-limiting examples of enzymes suitable for reduction of an imine or Schiff base derived from an *(S)*-1-benzylisoquinoline alkaloid include an aldo-keto reductase (e.g., a codeinone reductase-like enzyme (EC 1.1.1.247)) and a short chain dehydrogenase (e.g., a salutaridine reductase-like enzyme (EC 1.1.1.248)). In some examples, a protein that comprises a reductase domain of any one of the preceding examples may perform the reduction. In a further embodiment, the reduction is stereospecific. In some examples, the reductase may catalyze the reduction reaction within a host cell, such as an engineered host cell, as described herein.

[00101] An example of an enzyme that can perform an epimerization reaction that converts *(S)*-1-benzylisoquinoline alkaloids to *(R)*-1-benzylisoquinoline alkaloids includes an epimerase having an oxidase domain and a reductase domain. In particular, the epimerase may have a cytochrome P450 oxidase 82Y2-like domain. Additionally, the epimerase may have a codeinone reductase-like domain. Further, an epimerase having a cytochrome P450 oxidase 82Y2-like domain and also having a codeinone reductase-like domain may be referred to as a CYP-COR enzyme. In particular, a CYP-COR enzyme may be a fusion enzyme.

[00102] An example of an amino acid sequence of a CYP-COR enzyme that may be used to perform the conversion of *(S)*-1-benzylisoquinoline alkaloids to *(R)*-1-benzylisoquinoline alkaloids is provided in **FIG. 4**. In particular, **FIG. 4** illustrates an amino acid sequence of a CYP-COR enzyme, in accordance with embodiments of the invention. As seen in **FIG. 4**, underlined text denotes the cytochrome P450 CYP82Y2-like domain (59% identity to AFB74617.1). The dotted underlined text denotes the aldo-keto reductase NADPH-dependent codeinone reductase-like domain (75% identity to ACM44066.1). Additional amino acid sequences of a CYP-COR enzyme are set forth in **Table 1**. An amino acid sequence for an epimerase that is utilized in converting an *(S)*-1-benzylisoquinoline alkaloid to an *(R)*-1-benzylisoquinoline alkaloid may be 75% or more identical to a given amino acid sequence as listed in **Table 1**. For example, an amino acid sequence for such an epimerase may comprise an amino acid sequence that is at least 75% or more, 80% or more, 81% or more, 82% or more, 83% or more, 84% or more, 85% or more, 86% or more, 87% or more, 88% or more, 89% or more, 90% or more, 91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98% or more, or 99% or more identical to an amino acid sequence as provided herein. Additionally, in certain embodiments, an "identical" amino acid sequence contains at least 80%-99% identity at the amino acid level to the specific amino acid sequence. In some cases an "identical" amino acid sequence contains at least about 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94% and more in certain cases, at least 95%, 96%, 97%, 98% and 99% identity, at the amino acid level. In some cases, the amino acid sequence may be identical but the DNA sequence is altered such as to optimize codon usage for the host organism, for example.

[00103] An engineered host cell may be provided that produces an epimerase that converts *(S)*-1-benzylisoquinoline alkaloid to *(R)*-1-benzylisoquinoline alkaloid, wherein the epimerase comprises an amino acid sequence selected from the group consisting of: SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14,

and 15. The epimerase that is produced within the engineered host cell may be recovered and purified so as to form a biocatalyst. In some cases, the epimerase may be split into one or more enzymes. Additionally, one or more enzymes that are produced by splitting the epimerase may be recovered from the engineered host cell. These one or more enzymes that result from splitting the epimerase may also be used to catalyze the conversion of *(S)*-1-benzylisoquinoline alkaloids to *(R)*-1-benzylisoquinoline alkaloids. In particular, the one or more enzymes that are recovered from the engineered host cell that produces the epimerase may be used in a process for converting an *(S)*-1-benzylisoquinoline alkaloid to an *(R)*-1-benzylisoquinoline alkaloid. The process may include contacting the *(S)*-1-benzylisoquinoline alkaloid with an epimerase in an amount sufficient to convert said *(S)*-1-benzylisoquinoline alkaloid to *(R)*-1-benzylisoquinoline alkaloid. In examples, the *(S)*-1-benzylisoquinoline alkaloid may be contacted with a sufficient amount of the one or more enzymes such that at least 5% of said *(S)*-1-benzylisoquinoline alkaloid is converted to *(R)*-1-benzylisoquinoline alkaloid. In further examples, the *(S)*-1-benzylisoquinoline alkaloid may be contacted with a sufficient amount of the one or more enzymes such that at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or 100% of said *(S)*-1-benzylisoquinoline alkaloid is converted to *(R)*-1-benzylisoquinoline alkaloid.

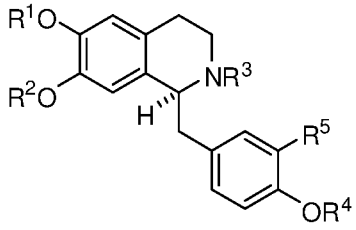
[00104] The one or more enzymes that may be used to convert an *(S)*-1-benzylisoquinoline alkaloid to an *(R)*-1-benzylisoquinoline alkaloid may contact the *(S)*-1-benzylisoquinoline alkaloid in vitro. Additionally, or alternatively, the one or more enzymes that may be used to convert an *(S)*-1-benzylisoquinoline alkaloid to an *(R)*-1-benzylisoquinoline alkaloid may contact the *(S)*-1-benzylisoquinoline alkaloid in vivo. Additionally, the one or more enzymes that may be used to convert an *(S)*-1-benzylisoquinoline alkaloid to an *(R)*-1-benzylisoquinoline alkaloid may be provided to a cell having the *(S)*-1-benzylisoquinoline alkaloid within, or may be produced within an engineered host cell.

[00105] In some examples, the methods provide for engineered host cells that produce an alkaloid product, wherein the epimerization of an *(S)*-substrate to an *(R)*-product may comprise a key step in the production of an alkaloid product. In some examples, the alkaloid produced is an *(R)*-1-benzylisoquinoline alkaloid. In still other embodiments, the alkaloid produced is derived from an *(R)*-1-benzylisoquinoline alkaloid, including, for example, 4-ring promorphinan and 5-ring morphinan alkaloids. In another embodiment, an *(S)*-1-benzylisoquinoline alkaloid is an intermediate toward the product of the engineered host cell. In still other embodiments, the alkaloid product is selected from the group consisting of morphinan, protoberberine, noscapinoid, and benzophenanthridine alkaloids.

[00106] In some examples, the *(S)*-substrate is an *(S)*-1-benzylisoquinoline alkaloid selected from the group consisting of *(S)*-norreticuline, *(S)*-reticuline, *(S)*-tetrahydropapaverine, *(S)*-norcoclaurine, *(S)*-coclaurine, *(S)*-

N-methylcoclaurine, *(S)*-3'-hydroxy-*N*-methylcoclaurine, *(S)*-norisoorientaline, *(S)*-orientaline, *(S)*-isoorientaline, *(S)*-norprotosinomenine, *(S)*-protosinomenine, *(S)*-norlaudanosoline, *(S)*-laudanosoline, *(S)*-4'-*O*-methyllaudanosoline, *(S)*-6-*O*-methylnorlaudanosoline, *(S)*-4'-*O*-methylnorlaudanosoline.

[00107] In some examples, the *(S)*-substrate is a compound of Formula I:



Formula I,

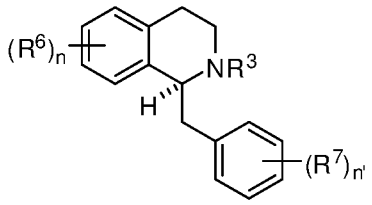
or a salt thereof, wherein:

R^1, R^2, R^3, and R^4 are independently selected from hydrogen and methyl; and

R^5 is selected from hydrogen, hydroxy, and methoxy.

[00108] In some other examples, at least one of R^1, R^2, R^3, R^4, and R^5 is hydrogen.

[00109] In still other examples, the *(S)*-substrate is a compound of Formula II:



Formula II,

or a salt thereof, wherein:

R^3 is selected from hydrogen and C_1-C_4 alkyl;

R^6 and R^7 are independently selected at each occurrence from hydroxy, fluoro, chloro, bromo, carboxaldehyde, C_1-C_4 acyl, C_1-C_4 alkyl, and C_1-C_4 alkoxy;

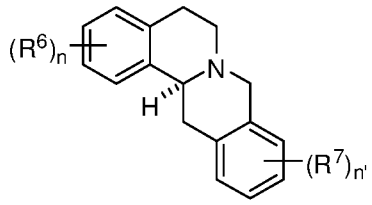
n is 0, 1, 2, 3, or 4; and

n' is 0, 1, 2, 3, 4 or 5.

[00110] When a bond is drawn across a ring, it means substitution may occur at a non-specific ring atom or position. For example, in Formula II shown above, the hydrogen of any –CH– in the 6-membered ring may be replaced with R^7 to form –CR^7–.

[00111] In some examples, R^6 and R^7 are independently methyl or methoxy. In some other examples, n and n' are independently 1 or 2. In still other embodiments, R^3 is hydrogen or methyl.

[00112] In some other examples, the *(S)*-substrate is a compound of Formula III:



Formula III,

or a salt thereof, wherein:

R^6 and R^7 are independently selected at each occurrence from hydroxy, fluoro, chloro, bromo, carboxaldehyde, C_1-C_4 acyl, C_1-C_4 alkyl, and C_1-C_4 alkoxy; and

n and n' are independently 0, 1, 2, 3, or 4.

[00113] In some examples, R^6 and R^7 are independently hydroxy, methyl, or methoxy. In some other examples, n and n' are independently 1 or 2. In still further embodiments, R^6 and R^7 are independently fluoro, hydroxy, methyl, or methoxy.

[00114] In some examples, the methods provide for engineered host cells that produce alkaloid products from *(S)*-reticuline. The epimerization of *(S)*-reticuline to *(R)*-reticuline may comprise a key step in the production of diverse alkaloid products from a precursor. In some examples, the precursor is L-tyrosine or a sugar (e.g., glucose). The diverse alkaloid products can include, without limitation, morphinan, protoberberine, noscapinoid, and benzophenanthridine alkaloids.

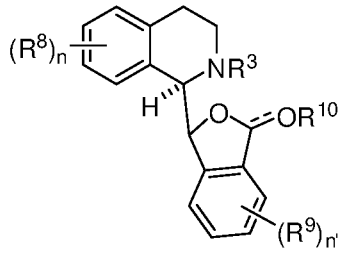
[00115] Any suitable carbon source may be used as a precursor toward an epimerized 1-benzylisoquinoline alkaloid. Suitable precursors can include, without limitation, monosaccharides (e.g., glucose, fructose, galactose, xylose), oligosaccharides (e.g., lactose, sucrose, raffinose), polysaccharides (e.g., starch, cellulose), or a combination thereof. In some examples, unpurified mixtures from renewable feedstocks can be used (e.g., cornsteep liquor, sugar beet molasses, barley malt, biomass hydrolysate). In still other embodiments, the carbon precursor can be a one-carbon compound (e.g., methanol, carbon dioxide) or a two-carbon compound (e.g., ethanol). In yet other embodiments, other carbon-containing compounds can be utilized, for example, methylamine, glucosamine, and amino acids (e.g., L-tyrosine). In some examples, a 1-benzylisoquinoline alkaloid may be added directly to an engineered host cell of the invention, including, for example, norlaudanosoline, laudanosoline, norreticuline, and reticuline. In still further embodiments, a 1-benzylisoquinoline alkaloid may be added to the engineered host cell as a single enantiomer (e.g., an *(S)*-1-benzylisoquinoline alkaloid), or a mixture of enantiomers, including, for example, a racemic mixture.

[00116] In some examples, the methods provide for the epimerization of a stereocenter of a 1-benzylisoquinoline alkaloid, or a derivative thereof. In a further embodiment, the method comprises contacting the 1-benzylisoquinoline alkaloid with at least one enzyme. The at least one enzyme may invert the stereochemistry of a stereocenter of a 1-benzylisoquinoline alkaloid, or derivative thereof, to the opposite

stereochemistry. In some examples, the at least one enzyme converts an *(S)*-1-benzylisoquinoline alkaloid to an *(R)*-1-benzylisoquinoline alkaloid. In some examples of this conversion of an *(S)*-1-benzylisoquinoline alkaloid to an *(R)*-1-benzylisoquinoline alkaloid utilizing the at least one enzyme, the *(S)*-1-benzylisoquinoline alkaloid is selected from the group consisting of *(S)*-norreticuline, *(S)*-reticuline, *(S)*-tetrahydropapaverine, *(S)*-norcoclaurine, *(S)*-coclaurine, *(S)*-*N*-methylcoclaurine, *(S)*-3'-hydroxy-*N*-methylcoclaurine, *(S)*-norisoorientaline, *(S)*-orientaline, *(S)*-isoorientaline, *(S)*-norprotosinomenine, *(S)*-protosinomenine, *(S)*-norlaudanosoline, *(S)*-laudanosoline, *(S)*-4'-*O*-methyllaudanosoline, *(S)*-6-*O*-methylnorlaudanosoline, and *(S)*-4'-*O*-methylnorlaudanosoline.

[00117] In some other examples, the at least one enzyme converts an 5'*R*-phthalideisoquinoline alkaloid to an 5'*S*-phthalideisoquinoline alkaloid. In some examples of this conversion of an 5'*R*-phthalideisoquinoline alkaloid to an 5'*S* -phthalideisoquinoline alkaloid utilizing the at least one enzyme, the 5'*R*-phthalideisoquinoline alkaloid is selected from the group consisting of 5'*R*-narcotolinehemiacetal, 5'*R*-narcotoline, 5'*R*-narcotinehemiacetal, and 5'*R*-noscapine.

In some examples, the 3*S*, 5'*R*-phthalideisoquinoline alkaloid is a compound of Formula IV:



Formula IV,

or a salt thereof, wherein:

R^3 is selected from hydrogen and C_1-C_4 alkyl;

R^8 and R^9 are independently selected at each occurrence from hydroxy, fluoro, chloro, bromo, carboxaldehyde, C_1-C_4 acyl, C_1-C_4 alkyl, and C_1-C_4 alkoxy;

R^{10} is absent, hydrogen, or C_1-C_4 alkyl;

two R^8s and the carbon atoms to which they are attached optionally form a 5- to 8-membered cycloalkyl or heterocycloalkyl moiety;

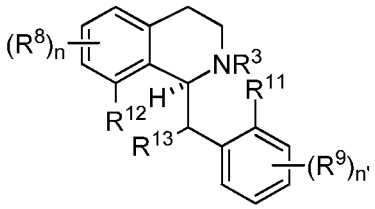
two R^9s and the carbon atoms to which they are attached optionally form a 5- to 8-membered cycloalkyl or heterocycloalkyl moiety; and

n and n' are independently 0, 1, 2, 3, or 4.

[00118] In some other examples, the at least one enzyme converts an 5'*R*-secoberberine alkaloid to an 5'*S*-secoberberine alkaloid. In some examples of this conversion of an 5'*R*-secoberberine alkaloid to an 5'*S*-

secoberberine alkaloid utilizing the at least one enzyme, the 5'*R*-secoberberine alkaloid is selected from the group consisting of 5'*R*-4'-*O*-desmethyl-3-*O*-acetylpapaveroxine, 5'*R*-3-*O*-acetylpapaveroxine, and 5'*R*-papaveroxine

In some examples, the 5'*R*-secoberberine alkaloid is a compound of Formula V:



Formula V,

or a salt thereof, wherein:

R^3 is selected from hydrogen and C_1-C_4 alkyl;

R^8 and R^9 are independently selected at each occurrence from hydroxy, fluoro, chloro, bromo, carboxaldehyde, C_1-C_4 acyl, C_1-C_4 alkyl, and C_1-C_4 alkoxy;

R^{11} is selected from hydrogen and aldehyde;

R^{12} is selected from hydrogen, hydroxyl, and O-methyl;

R^{13} is selected from hydroxyl and O-methyl;

two R^8s and the carbon atoms to which they are attached optionally form a 5- to 8-membered cycloalkyl or heterocycloalkyl moiety;

two R^9s and the carbon atoms to which they are attached optionally form a 5- to 8-membered cycloalkyl or heterocycloalkyl moiety; and

n and n' are independently 0, 1, 2, 3, or 4.

[00119] In still other embodiments, the 1-benzylisoquinoline alkaloid that is epimerized may comprise two or more stereocenters, wherein only one of the two or more stereocenters is inverted to produce a diastereomer of the substrate (e.g., (*S*, *R*)-1-benzylisoquinoline alkaloid converted to (*R*, *R*)-1-benzylisoquinoline alkaloid). In examples where only one stereocenter of a 1-benzylisoquinoline alkaloid is inverted when contacted with the at least one enzyme, the product is referred to as an epimer of the 1-benzylisoquinoline alkaloid.

[00120] In some examples, the 1-benzylisoquinoline alkaloid is presented to the enzyme as a single stereoisomer. In some other examples, the 1-benzylisoquinoline alkaloid is presented to the enzyme as a mixture of stereoisomers. In still further embodiments, the mixture of stereoisomers may be a racemic

mixture. In some other examples, the mixture of stereoisomers may be enriched in one stereoisomer as compared to another stereoisomer.

[00121] In some examples, an 1-benzylisoquinoline alkaloid, or a derivative thereof, is recovered. In some examples, the 1-benzylisoquinoline alkaloid is recovered from a cell culture. In still further embodiments, the recovered 1-benzylisoquinoline alkaloid is enantiomerically enriched in one stereoisomer as compared to the original mixture of 1-benzylisoquinoline alkaloids presented to the enzyme. In still further embodiments, the recovered 1-benzylisoquinoline alkaloid has an enantiomeric excess of at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or 100%.

[00122] "Isomers" are different compounds that have the same molecular formula. "Stereoisomers" are isomers that differ only in the way the atoms are arranged in space. "Enantiomers" are a pair of stereoisomers that are non superimposable mirror images of each other. A 1:1 mixture of a pair of enantiomers is a "racemic" mixture. "Diastereoisomers" or “diastereomers” are stereoisomers that have at least two asymmetric atoms but are not mirror images of each other. The term “epimer” as used herein refers to a compound having the identical chemical formula but a different optical configuration at a particular position. For example, the (*R,S*) and (*S,S*) stereoisomers of a compound are epimers of one another. In some examples, a 1-benzylisoquinoline alkaloid is converted to its epimer (e.g., epi-1-benzylisoquinoline alkaloid). The absolute stereochemistry is specified according to the Cahn-Ingold-Prelog R-S system. When a compound is a pure enantiomer, the stereochemistry at each chiral carbon can be specified by either R or S. Resolved compounds whose absolute configuration is unknown can be designated (+) or (-) depending on the direction (dextro- or levorotatory) in which they rotate plane polarized light at the wavelength of the sodium D line. Certain compounds described herein contain one or more asymmetric centers and can thus give rise to enantiomers, diastereomers, and other stereoisomeric forms that can be defined, in terms of absolute stereochemistry, as *(R)*- or *(S)*-.

Table 1. Example partial and full-length amino acid sequences of CYP-COR fusion enzymes.

Sequence	Description	SEQ. ID NO.
MELQYISYFQPTSSVVALLLALVSILSSVVVLRKTFLNNYSSSPASSTKTA VLSHQRQQSCALPISGLLHIFMNKNGLIHVTLGNMADKYGPIFSFPTGSH RTLVVSSWEMVKECFTGNNDTAFSNRPIPLAFKTIFYACGGIDSYGLSSV PYGKYWRELRKVCVHNLLSNQQLLKFRHLIISQVDTSFNKLYELCKNSE	*P. somniferum* plant source; full-length	SEQ. ID NO. 1

DNHGNYTTTTTTAAGMVRIDDWLAELSFNVIGRIVCGFQSGPKTGAPSR VEQFKEAINEASYFMSTSPVSDNVPMLGWIDQLTGLTRNMKHCGKKLD LVVESIINDHRQKRRFSRTKGGDEKDDEQDDFIDICLSIMEQPQLPGNNN PSQIPIKSIVLDMIGGGTDTTKLTTIWTLSLLLNNPHVLDKAKQEVDAHF RTKRRSTNDAAAAVVDFDDIRNLVYIQAIIKESMRLYPASPVVERLSGE DCVVGGFHVPAGTRLWANVWKMQRDPKVWDDPLVFRPDRFLSDEQK MVDVRGQNYELLPFGAGRRVCPGVSFSLDLMQLVLTRLILEFEMKSPSG KVDMTATPGLMSYKVIPLDILLTHRRIKPCVQSAASERDMESSGVPVITL GSGKVMPVLGMGTFEKVGKGSERERLAILKAIEVGYRYFDTAAAYETE EVLGEAIAEALQLGLVKSRDELFISSMLWCTDAHADRVLLALQNSLRNL KLEYVDLYMLPFPASLKPGKITMDIPEEDICRMDYRSVWAAMEECQNL GFTKSIGVSNFSCKKLQELMATANIPPAVNQVEMSPAFQQKKLREYCNA NNILVSAISVLGSNGTPWGSNAVLGSEVLKKIAMAKGKSVAQVSMRWV YEQGASLVVKSFSEERLRENLNIFDWELTKEDHEKIGEIPQCRILSAYFL VSPNGPFKSQEELWDDEA*	amino acid sequence >RQNK-2062398 (also FPYZ-2037562, BMRX-2007040, and MLPX-2016197)	
MELQYISYFQPTSSVVALLLALVSILSSVVVLRKTFLNNYSSSPASSTKTA VLSHQRQQSCALPISGLLHIFMNKNGLIHVTLGNMADKYGPIFSFPTGSH RTLVVSSWEMVKECFTGNNDTAFSNRPIPLAFKTIFYACGGIDSYGLSSV PYGKYWRELRKVCVHNLLSNQQLLKFRHLIISQVDTSFNKLYELCKNSE DNHGNYTTXLLLPQLAWRQPWKLYYXTTTTAAGMVRIDDWLAELSFN VIGRIVCGFQSGPKTGAPSRVEQFKEAINEASYFMSTSPVSDNVPMLGWI DQLTGLTRNMKHCGKKLDLVVESIINDHRQKRRFSRTKGGDEKDDEQD DFIDICLSIMEQPQLPGNNNPSQIPIKSIVLDMIGGGTDTTKLTTIWTLSLL LNNPHVLDKAKQEVDAHFRTKRRSTNDAAAAVVDFDDIRNLVYIQAII KESMRLYPASPVVERLSGEDCVVGGFHVPAGTRLWANVWKMQRDPK VWDDPLVFRPDRFLSDEQKMVDVRGQNYELLPFGAGRRVCPGVSFSLD LMQLVLTRLILEFEMKSPSGKVDMTATPGLMSYKVIPLDILLTHRRIKPC VQSAASERDMESSGVPVITLGSGKVMPVLGMGTFEKVGKGSERERLAIL KAIEVGYRYFDTAAAYETEEVLGEAIAEALQLGLVKSRDELFISSMLWC TDAHADRVLLALQNSLRNLKLEYVDLYMLPFPASLKPGKITMDIPEEDI CRMDYRSVWAAMEECQNLGFTKSIGVSNFSCKKLQELMATANIPPAVN QVEMSPAFQQKKLREYCNANNILVSAISVLGSNGTPWGSNAVLGSEVL KKIAMAKGKSVAQVSMRWVYEQGASLVVKSFSEERLRENLNIFDWELT KEDHEKIGEIPQCRILSAYFLVSPNGPFKSQEELWDDEA*	*P. somniferum* plant source; full-length amino acid sequence >KKCW-2026866 (also FPYZ-2037562, MLPX-2016197)	SEQ. ID NO. 2
MELQYISYFQPTSSVVALLLALVSILSSVVVLRKTFLNNYSSSPASSTKTA	*P.*	SEQ. ID

VLSHQRQQSCALPISGLLHIFMNKNGLIHVTLGNMADKYGPIFSFPTGSH RTLVVSSWEMVKECFTGNNDTAFSNRPIPLAFKTIFYACGGIDSYGLSSV PYGKYWRELRKVCVHNLLSNQQLLKFRHLIISQVDTSFNKLYELCKNSE DNHGNYTTTTTTAAGMVRIDDWLAELSFNVIGRIVCGFQSGPKTGAPSR VEQFKEAINEASYFMSTSPVSDNVPMLGWIDQLTGLTRNMKHCGKKLD LVVESIINDHRQKRRFSRTKGGDEKDDEQDDFIDICLSIMEQPQLPGNNN PSQIPIKSIVLDMIGGGTDTTKLTTIWTLSLLLNNPHVLDKAKQEVDAHF RTKRRSTNDAAAAVVDFDDIRNLVYIQAIIKESMRLYPASPVVERLSGE DCVVGGFHVPAGTRLWANVWKMQRDPKVWDDPLVFRPDRFLSDEQK MVDVRGQNYELLPFGAGRRVCPGVSFSLDLMQLVLTRLILEFEMKSPSG KVDMTATPGLMSYKVIPLDILLTHRRIKPCVQSAASERDMESSGVPVITL GSGKVMPVLGMGTFEKVGKGSERERLAILKAIEVGYRYFDTAAAYETE EVLGEAIAEALQLGLVKSRDELFISSMLWCTDAHADRVLLALQNSLRNL KLEYVDLYMLPFPASLKPGKITMDIPEEDICRMDYRXVSKPWLH*	*somniferum* plant source; partial-length amino acid sequence >SUFP-2025636	NO. 3
MRWHRXIDSYGLSSVPYGKYWRELRKVCVHNLLSNQQLLKFRHLIISQ VDTSFNKLYELCKNSEDNQGNYPTTTTAAGMVRIDDWLAELSFNVIGRI VCGFQSGPKTGAPSRVEQFKEAINEASYFMSTSPVSDNVPMLGWIDQLT GLTRNMKHCGKKLDLVVESIINDHRQKRRFSRTKGGDEKDDEQDDFIDI CLSIMEQPQLPGNNNPSQIPIKSIVLDMIGGGTDTTKLTTIWTLSLLLNNP HVLDKAKQEVDAHFRTKRRSTNDAAAAVVDFDDIRNLVYIQAIIKESM RLYPASPVVERLSGEDCVVGGFHVPAGTRLWANVWKMQRDPKVWDD PLVFRPDRFLSDEQKMVDVRGQNYELLPFGAGRRVCPGVSFSLDLMQL VLTRLILEFEMKSPSGKVDMTATPGLMSYKVIPLDILLTHRRIKPCVQSA ASERDMESSGVPVITLGSGKVMPVLGMGTFEKVGKGSERERLAILKAIE VGYRYFDTAAAYETEEVLGEAIAEALQLGLVKSRDELFISSMLWCTDA HADRVLLALQNSLRNLKLEYVDLYMLPFPASLKPGKITMDIPEEDICRM DYRSVWAAMEECQNLGFTKSIGVSNFSCKKLQELMATANIPPAVNQVE MSPAFQQKKLREYCNANNILVSAISVLGSNGTPWGSNAVLGSEVLKKIA MAKGKSVAQVSMRWVYEQGASLVVKSFSEERLRENLNIFDWELTKED HEKIGEIPQCRILSAYFLVSPNGPFKSQEELWDDEA*	*P. somniferum* plant source; partial-length amino acid sequence >MIKW-2013651	SEQ. ID NO. 4
MELQYISYFQPTSSVVALLLALVSILSSVVVLRKTFLNNYSSSPASSTKTA VLSHQRQQSCALPISGLLHIFMNKNGLIHVTLGNMADKYGPIFSFPTGSH RTLVVSSWEMVKECFTGNNDTAFSNRPIPLAFKTIFYACGGIDSYGLSSV	*P. setigerum* plant source; full-length	SEQ. ID NO. 5

PYGKYWRELRKVCVHNLLSNQQLLKFRHLIISQVDTSFNKLYELCKNSE DNQGNYTTTTTAAGMVRIDDWLAELSFNVIGRIVCGFQSGPKTGAPSRV EQFKEAINEASYFMSTSPVSDNVPMLGWIDQLTGLTRNMKHCGKKLDL VVESIINDHRQKRRFSRTKGGDEKDDEQDDFIDICLSIMEQPQLPGNNNP SQIPIKSIVLDMIGGGTDTTKLTTIWTLSLLLNNPHVLDKAKQEVDAHFR TKRRSTNDAAAAVVDFDDIRNLVYIQAIIKESMRLYPASPVVERLSGED CVVGGFHVPAGTRLWANVWKMQRDPKVWDDPLVFRPDRFLSDEQKM VDVRGQNYELLPFGAGRRVCPGVSFSLDLMQLVLTRLILEFEMKSPSGK VDMTATPGLMSYKVIPLDILLTHRRIKPCVQSAASERDMESSGVPVITLG SGKVMPVLGMGTFEKVGKGSERERLAILKAIEVGYRYFDTAAAYETEE VLGEAIAEALQLGLVKSRDELFISSMLWCTDAHADRVLLALQNSLRNL KLEYVDLYMLPFPASLKPGKITMDIPEEDICRMDYRSVWAAMEECQNL GFTKSIGVSNFSCKKLQELMATANIPPAVNQVEMSPAFQQKKLREYCNA NNILVSAISVLGSNGTPWGSNAVLGSEVLKKIAMAKGKSVAQVSMRWV YEQGASLVVKSFSEERLRENLNIFDWELTKEDHEKIGEIPQCRILSAYFL VSPNGPFKSQEELWDDEA*	amino acid sequence >EPRK-2027940 (also FPYZ-2037562, STDO-2019715, FNXH-2029312, MLPX-2016196, MLPX-2016197)	
MELQYISYFQPTSSVVALLLALVSILSSVVVLRKTFLNNYSSSPASSTKTA VLSHQRQQSCALPISGLLHIFMNKNGLIHVTLGNMADKYGPIFSFPTGSH RTLVVSSWEMVKECFTGNNDTAFSNRPIPLAFKTIFYACGGIDSYGLSSV PYGKYWRELRKVCVHNLLSNQQLLKFRHLIISQVDTSFNKLYELCKNSE DNQGNYTTTTTAAGMVRIDDWLAELSFNVIGRIVCGFQSGPKTGAPSRV EQFKEAINEASYFMSTSPVSDNVPMLGWIDQLTGLTRNMKHCGKKLDL VVESIINDHRQKRRFSRTKGGDEKDDEQDDFIDICLSIMEQPQLPGNNNP SQIPIKSIVLDMIGGGTDTTKLTTIWTLSLLLNNPHVLDKAKQEVDAHFR TKRRSTNDAAAAVVDFDDIRNLVYIQALYPASPVVERLSGEDCVVGGF HVPAGTRLWANVWKMQRDPKVWDDPLVFRPDRFLSDEQKMVDVRGQ NYELLPFGAGRRVCPGVSFSLDLMQLVLTRLILEFEMKSPSGKVDMTAT PGLMSYKVIPLDILLTHRRIKPCVQSAASERDMESSGVPVITLGSGKVMP VLGMGTFEKVGKGSERERLAILKAIEVGYRYFDTAAAYETEEVLGEAIA EALQLGLVKSRDELFISSMLWCTDAHADRVLLALQNSLRNLKLEYVDL YMLPFPASLKPGKITMDIPEEDICRMDYRSVWAAMEE	*P. setigerum* plant source; partial-length amino acid sequence >QCOU-2000833	SEQ. ID NO. 6
MELQYFSYFQPTSSVVALLLALVSILFSVVVLRKTFSNNYSSPASSTETA VLCHQRQQSCALPISGLLHVFMNKNGLIHVTLGNMADKYGPIFSFPTGS	*P. bracteatum*	SEQ. ID NO. 7

HRTLVVSSWEMVKECFTGNNDTAFSNRPIPLAFQTIFYACGGIDSYGLSS VPYGKYWRELRKVCVHNLLSNQQLLKFRHLIISQVDTSFNKLYELCKNS EDNQGMVRMDDWLAQLSFNVIGRIVCGFQSDPKTGAPSRVEQFKEVIN EASYFMSTSPVSDNVPMLGWIDQLTGLTRNMKHCGKKLDLVVESIIKD HRQKRRFSRTKGGDEKDDEQDDFIDICLSIMEQPQLPGNNSPPQIPIKSIV LDMIGGGTDTTKLTTIWTLSLLLNNPHVLDKAKQEVDAHFRKKRRSTD DAAAAVVDFDDIRNLVYIQAIIKESMRLYPASPVVERLSGEDCVVGGFH VPAGTRLWANVWKMQRDPKVWDDPLVFRPERFLSDEQKMVDVRGQN YELLPFGAGRRICPGVSFSLDLMQLVLTRLILEFEMKSPSGKVDMTATPG LMSYKVVPLDILLTHRRIKSCVQLASSERDMESSGVPVITLSSGKVMPVL GMGTFEKVGKGSERERLAILKAIEVGYRYFDTAAAYETEEVLGEAIAEA LQLGLIESRDELFISSMLWCTDAHPDRVLLALQNSLRNLKLEYLDLYML PFPASLKPGKITMDIPEEDICRMDYRSVWSAMEECQNLGFTKSIGVSNFS SKKLQELMATANIPPAVNQVEMSPAFQQKKLREYCNANNILVSAVSILG SNGTPWGSNAVLGSEVLKQIAMAKGKSVAQVSMRWVYEQGASLVVK SFSEERLRENLNIFDWELTKEDNEKIGEIPQCRILTAYFLVSPNGPFKSQE ELWDDKA*	plant source; full-length amino acid sequence >SSDU-2015634 (also SSDU-2015636, ZSNV-2027701, RRID-2004435)	
MELQYFSYFQPTSSVVALLLALVSILFSVVVLRKTFSNNYSSPASSTETA VLCHQRQQSCALPISGLLHVFMNKNGLIHVTLGNMADKYGPIFSFPTGS HRTLVVSSWEMVKECFTGNNDTAFSNRPIPLAFQTIFYACGGIDSYGLSS VPYGKYWRELRKVCVHNLLSNQQLLKFRHLIISQVDTSFNKLYELCKNS EDNQGMVRMDDWLAQLSFNVIGRIVCGFQSDPKTGAPSRVEQFKEVIN EASYFMSTSPVSDNVPMLGWIDQLTGLTRNMKHCGKKLDLVVESIIKD HRQKRRFSRTKGGDEKDDEQDDFIDICLSIMEQPQLPGNNSPPQIPIKSIV LDMIGGGTDTTKLTTIWTLSLLLNNPHVLDKAKQEVDAHFRKKRRSTD DAAAAVVDFDDIRNLVYIQAIIKESMRLYPASPVVERLSGEDCVVGGFH VPAGTRLWANVWKMQRDPKVWDDPLVFRPERFLSDEQKMVDVRGQN YELLPFGAGRRICPGVSFSLDLMQLVLTRLILEFEMKSPSGKVDMTATPG LMSYKVVPLDILLTHRRIKSCVQLASSERDMESSGVPVITLSSGKVMPVL GMGTFEKVGKGSERERLAILKAIEVGYRYFDTAAAYETEEVLGEAIAEA LQLGLIESRDELFISSMLWCTDAHPDRVLLALQNSLRNLKLEYLDLYML PFPASLKPGKITMDIPEEDICRMDYRSVWSAMEECQNLGFTKSIGVSNFS CKKLQELMATANIPPAVNQVEMSPAFQQKKLREYCNANNILVSAVSILG SNGTPWGSNAVLGSEVLKQIAMAKGKSVAQVSMRWVYEQGASLVVK SFSEERLRENLNIFDWELTKEDNEKIGEIPQCRILTAYFLVSPNGPFKSQE	*P. bracteatum* plant source; full-length amino acid sequence >TMWO-2027322 (also RRID-2004435)	SEQ. ID NO. 8

ELWDDKA*		
SSPASSTETAVLCHQRQQSCALPISGLLHIFMNKNGLIHVTLGNMADKY GPIFSFPTGSHRILVVSSWEMVKECFTGNNDTAFSNRPIPLAFKTIFYACR GIDSYGLSSVPYGKYWRELRKVCVHNLLSNQQLLKFRHLIISQVDTSFN KLYELCKNSEDNQGMVRMDDWLAQLSFSVIGRIVCGFQSDPKTGAPSR VEQFKEAINEASYFMSTSPVSDNVPMLGWIDQLTGLTRNMTHCGKKLD LVVESIINDHRQKRRFSRTKGGDEKDDEQDDFIDICLSIMEQPQLPGNNN PPKIPIKSIVLDMIGAGTDTTKLTIIWTLSLLLNNPNVLAKAKQEVDAHFE TKKRSTNEASVVVDFDDIGNLVYIQAIIKESMRLYPVSPVVERLSSEDCV VGGFHVPAGTRLWANVWKMQRDPKVWDDPLVFRPERFLSDEQKMVD VRGQNYELLPFGAGRRICPGVSFSLDLMQLVLTRLILEFEMKSPSGKVD MTATPGLMSYKVVPLDILLTHRRIKSCVQLASSERDMESSGVPVITLRSG KVMPVLGMGTFEKAGKGSERERLAILKAIEVGYRYFDTAAAYETEEVL GEAIAEALQLGLIKSRDELFISSMLWCTDAHPDRVLLALQNSLRNLKLE YVDLYMLPFPASLKPGKITMDIPEEDICPMDYRSVWSAMEECQNLGLTK SIGVSNFSCKKLEELMATANIPPAVNQVEMSPAFQQKKLREYCNANNIL VSAVSILGSNGTPWGSNAVLGSEVLKKIAMAKGKSVAQVSMRWVYEQ GASLVVKSFSEERLRENLNIFDWQLTKEDNEKIGEIPQCRILSAYFLVSPK GPFKSQEELWDDKA*	*P. bracteatum* plant source; partial-length amino acid sequence >pbr.PBRST1PF_89405	SEQ. ID NO. 9
SSPASSTETAVLCHQRQQSCALPISGLLHIFMNKNGLIHVTLGNMADKY GPIFSFPTGSHRILVVSSWEMVKECFTGNNDTFFSNRPIPLAFKIIFYAGG VDSYGLALVPYGKYWRELRKICVHNLLSNQQLLKFRHLIISQVDTSFNK LYELCKNSEDNQGMVRMDDWLAQLSFSVIGRIVCGFQSDPKTGAPSRV EQFKEAINEASYFMSTSPVSDNVPMLGWIDQLTGLTRNMTHCGKKLDL VVESIINDHRQKRRFSRTKGGDEKDDEQDDFIDICLSIMEQPQLPGNNNP PKIPIKSIVLDMIGGGTDTTKLTTIWTLSLLLNNPHVLDKAKQEVDAHFL TKRRSTNDAAVVDFDDIRNLVYIQAIIKESMRLYPASPVVERLSGEDCV VGGFHVPAGTRLWVNVWKMQRDPNVWADPMVFRPERFLSHGQKKM VDVRGKNYELLPFGAGRRICPGISFSLDLMQLVLTRLILEFEMKSPSGKV DMTATPGLMSYKVVPLDILLTHRRIKSCVQLASSERDMESSGVPVITLRS GKVMPVLGMGTFEKAGKGSERERLAILKAIEVGYRYFDTAAAYETEEV LGEAIAEALQLGLIKSRDELFISSMLWCTDAHPDRVLLALQNSLRNLKLE YVDLYMLPFPASLKPGKITMDIPEEDICPMDYRSVWSAMEECQNLGLTK SIGVSNFSCKKLEELMATANIPPAVNQVEMSPAFQQKKLREYCNANNIL VSAVSILGSNGTPWGSNAVLGSEVLKKIAMAKGKSVAQVSMRWVYEQ	*P. bracteatum* plant source; partial-length amino acid sequence >pbr.PBRST1PF_4328	SEQ. ID NO. 10

GASLVVKSFSEERLRENLNIFDWQLTKEDNEKIGEIPQCRILSAYFLVSPK GPFKSQEELWDDKA*		
SSPASSTETAVLCHQRQQSCALPISGLLHIFMNKNGLIHVTLGNMADKY GPIFSFPTGSHRILVVSSWEMVKECFTGNNDTFFSNRPIPLAFKIIFYAGG VDSYGLALVPYGKYWRELRKICVHNLLSNQQLLNFRHLIISQVDTSFNK LYDLSNKKKNTTTDSGTVRMDDWLAQLSFNVIGRIVCGFQTHTETSATS SVERFTEAIDEASRFMSIATVSDTFPWLGWIDQLTGLTRKMKHYGKKLD LVVESIIEDHRQNRRISGTKQGDDFIDICLSIMEQPQIIPGNNDPPRQIPIKS IVLDMIGGGTDTTKLTTTWTLSLLLNNPHVLEKAREEVDAHFGTKRRPT NDDAVMVEFDDIRNLVYIQAIIKESMRLYPASPVVERLSGEDCVVGGFH VPAGTRLWVNVWKMQRDPNVWADPMVFRPERFLSDEQKMVDVRGQ NYELLPFGAGRRICPGVSFSLDLMQLVLTRLILEFEMKSPSGKVDMTATP GLMSYKVVPLDILLTHRRIKSCVQLASSERDMESSGVPVITLRSGKVMP VLGMGTFEKAGKGSERERLAILKAIEVGYRYFDTAAAYETEEVLGEAIA EALQLGLIKSRDELFISSMLWCTDAHPDRVLLALQNSLRNLKLEYVDLY MLPFPASLKPGKITMDIPEEDICPMDYRSVWSAMEECQNLGLTKSIGVS NFSCKKLEELMATANIPPAVNQVEMSPAFQQKKLREYCNANNILVSAV SILGSNGTPWGSNAVLGSEVLKKIAMAKGKSVAQVSMRWVYEQGASL VVKSFSEERLRENLNIFDWQLTKEDNEKIGEIPQCRILSAYFLVSPKGPFK SQEELWDDKA*	*P. bracteatum* plant source; partial-length amino acid sequence >pbr.PBRST1PF_12180	SEQ. ID NO. 11
VALRKKILKNYYSSSSSTATAVSHQWPKASRALPLIDLLHVFFNKTDLM HVTLGNMADKFGPIFSFPTGSHRTLVVSSWEKAKECFTGNNDIVFSGRP LPLAFKLIFYAGGIDSYGISQVPYGKKWRELRNICVHNILSNQQLLKFRH LMISQVDNSFNKLYEVCNSNKDEGDSATSTTAAGIVRMDDWLGKLAFD VIARIVCGFQSQTETSTTSSMERFTEAMDEASRFMSVTAVSDTVPWLGW IDQLTGLKRNMKHCGKKLNLVVKSIIEDHRQKRRLSSTKKGDENIIDED EQDDFIDICLSIMEQPQLPGNNNPPKIPIKSIVLDMIGGGTDTTKLTTIWTL SLLLNNPHVLDKAKQEVDAHFLTKRRSTNDAAVVDFDDIRNLVYIQAII KESMRLYPASPVVERLSGEDCVVGGFHVPAGTRLWVNVWKMQRDPN VWADPMVFRPERFLSDEQKMVDVRGQNYELLPFGAGRRICPGVSFSLD LMQLVLTRLILEFEMKSPSGKVDMTATPGLMSYKVVPLDILLTHRRIKS CVQLASSERDMESSGVPVITLRSGKVMPVLGMGTFEKAGKGSERERLAI LKAIEVGYRYFDTAAAYETEEVLGEAIAEALQLGLIKSRDELFISSMLWC TDAHPDRVLLALQNSLRNLKLEYVDLYMLPFPASLKPGKITMDIPEEDIC PMDYRSVWSAMEECQNLGLTKSIGVSNFSCKKLEELMATANIPPAVNQ	*P. bracteatum* plant source; partial-length amino acid sequence >pbr.PBRST1PF_4329	SEQ. ID NO. 12

VEMSPAFQQKKLREYCNANNILVSAVSILGSNGTPWGSNAVLGSEVLK KIAMAKGKSVAQVSMRWVYEQGASLVVKSFSEERLRENLNIFDWQLT KEDNEKIGEIPQCRILSAYFLVSPKGPFKSQEELWDDKA*		
MELQYFSYFQPTSSVVALLLALVSILFSVVVLRKTFSNNYSSPASSTETA VLCHQRQQSCALPISGLLHVFMNKNGLIHVTLGNMADKYGPIFSFPTGS HRTLVVSSWEMVKECFTGNNDTAFSNRPIPLAFQTIFYACGGIDSYGLSS VPYGKYWRELRKVCVHNLLSNQQLLKFRHLIISQVDTSFNKLYELCKNS EDNQGMVRMDDWLAQLSFNVIGRIVCGFQSDPKTGAPSRVEQFKEVIN EASYFMSTSPVSDNVPMLGWIDQLTGLTRNMKHCGKKLDLVVESIIKD HRQKRRFSRTKGGDEKDDEQDDFIDICLSIMEQPQLPGNNSPPQIPIKSIV LDMIGGGTDTTKLTTIWTLSLLLNNPHVLDKAKQEVDAHFRKKRRSTD DAAAAVVDFDDIRNLVYIQAIIKESMRLYPASPVVERLSGEDCVVGGFH VPAGTRLWANVWKMQRDPKVWDDPLVFRPERFLSDEQKMVDVRGQN YELLPFGAGRRICPGVSFSLDLMQLVLTRLILEFEMKSPSGKVDMTATPG LMSYKVVPLDILLTHRRIKSCVQLASSERDMESSGVPVITLSSGKVMPVL GMGTFEKVGKGSERERLAILKAIEVGYRYFDTAAAYETEEVLGEAIAEA LQLGLIESRDELFISSMLWCTDAHPDRVLLALQNSLRNLKLEYLDLYML PFPASLKPGKITMDIPEEDICRMDYRSVWSAMEECQNLGFTKSIGVSNFS SKKLQELMATANIPPAVNQVEMSPAFQQKKLREYCNANNILVSAVSILG SNGTPWGSNAVLGSEVLKQIAMAKGKSVAQVSMRWVXKFSAYAIVWS LFFGHRICITLYSFLIRNVAYICITY*	*P. bracteatum* plant source; partial-length amino acid sequence >SSDU-2015635	SEQ. ID NO. 13
MELQYFSYFQPTSSVVALLLALVSILFSVVVLRKTFSNNYSSPASSTETA VLCHQRQQSCALPISGLLHVFMNKNGLIHVTLGNMADKYGPIFSFPTGS HRTLVVSSWEMVKECFTGNNDTAFSNRPIPLAFQTIFYACGGIDSYGLSS VPYGKYWRELRKVCVHNLLSNQQLLKFRHLIISQVDTSFNKLYELCKNS EDNQGMVRMDDWLAQLSFNVIGRIVCGFQSDPKTGAPSRVEQFKEVIN EASYFMSTSPVSDNVPMLGWIDQLTGLTRNMKHCGKKLDLVVESIIKD HRQKRRFSRTKGGDEKDDEQDDFIDICLSIMEQPQLPGNNSPPQIPIKSIV LDMIGGGTDTTKLTTIWTLSLLLNNPHVLDKAKQEVDAHFRKKRRSTD DAAAAVVDFDDIRNLVYIQAIIKESMRLYPASPVVERLSGEDCVVGGFH VPAGTRLWANVWKMQRDPKVWDDPLVFRPERFLSDEQKMVDVRGQN YELLPFGAGRRICPGVSFSLDLMQLVLTRLILEFEMKSPSGKVDMTATPG LMSYKVVPLDILLTHRRIKSCVQLASSERDMESSGVPVITLSSGKVMPVL GMGTFEKVGKGSERERLAILKAIEVGYRYFDTAAAYETEEVLGEAIAEA LQLGLIESRDELFISSMLWCTDAHPDRVLLALQNSLRQVFLMQIRLIYIC	*P. bracteatum* plant source; partial-length amino acid sequence >SSDU-2015637	SEQ. ID NO. 14

TYQQVHLNIYFQINEFVLCDMYRNLKLEY		
LNNYSSSPASSTKTAVLSHQRQQSCALPISGLLHIFMNKNGLIHVTLGNM ADKYGPIFSFPTGSHRTLVVSSWEMVKECFTGNNDTAFSNRPIPLAFKTI FYACGGIDSYGLSSVPYGKYWRELRKVCVHNLLSNQQLLKFRHLIISQV DTSFNKLYELCKNSEDNQGNYPTTTTAAGMVRIDDWLAELSFNVIGRIV CGFQSGPKTGAPSRVEQFKEAINEASYFMSTSPVSDNVPMLGWIDQLTG LTRNMKHCGKKLDLVVESIINDHRQKRRFSRTKGGDEKDDEQDDFIDIC LSIMEQPQLPGNNNPSQIPIKSIVLDMIGGGTDTTKLTTIWTLSLLLNNPH VLDKAKQEVDAHFRTKRRSTNDAAAAVVDFDDIRNLVYIQAIIKESMR LYPASPVVERLSGEDCVVGGFHVPAGTRLWANVWKMQRDPKVWDDP LVFRPDRFLSDEQKMVDVRGQNYELLPFGAGRRVCPGVSFSLDLMQLV LTRLILEFEMKSPSGKVDMTATPGLMSYKVIPLDILLTHRRIKPCVQSAA SERDMESSGVPVITLGSGKVMPVLGMGTFEKVGKGSERERLAFLKAIEV GYRYFDTAAAYETEEFLGEAIAEALQLGLIKSRDELFITSKLWPCDAHPD LVVPALQNSLRNLKLEYVDLYMLPFPASLKPGKITMDIPEEDICRMDYR SVWAAMEECQNLGFTKSIGVSNFSCKKLQELMATANIPPAVNQVEMSP AFQQKKLREYCNANNILVSAISVLGSNGTPWGSNAVLGSEVLKKIAMA KGKSVAQVSMRWVYEQGASLVVKSFSEERLRENLNIFDWELTKEDHEK IGEIPQCRILSAYFLVSPNGPFKSQEELWDDEA*	*C. majus* plant source; partial-length amino acid sequence >chm.CMAST2PF_14984	SEQ. ID NO. 15

BisBIA Generating Modifications

[00123] Some methods, processes, and systems provided herein describe the production of bisbenzylisoquinoline alkaloids (bisBIAs). BisBIAs are dimeric molecules that may be formed by coupling reactions between two BIA monomers. In examples, bisBIAs may be formed by carbon-oxygen coupling reactions. In other examples, bisBIAs may be formed by carbon-carbon coupling reactions. In some examples, the bisBIA dimeric molecule is a homodimer, comprising two identical BIA monomers. In examples, an engineered host cell may produce one BIA monomer. In these examples, the BIA monomers may form homodimers when contacted with one or more coupling enzymes. In other examples, the bisBIA dimeric molecule is a heterodimer, comprising two different BIA monomers. For example, a bisBIA may be a heterodimer that comprises BIA monomers that are enantiomers of each other. In some examples, an engineered host cell may produce two or more BIA monomers. In these examples, the BIA monomers may form homodimers and heterodimers when contacted with one or more coupling enzymes.

[00124] Some of these methods, processes, and systems that describe the production of bisBIAs may comprise an engineered host cell. In some examples, the engineered host cell may be engineered to produce BIA

monomers which, in turn, may be used as building block molecules for forming bisBIAs. Examples of BIA monomers that may be used to form bisBIAs include coclaurine, *N*-methylcoclaurine, laudanine, norcoclaurine, norlaudanosoline, 6-*O*-methyl-norlaudanosoline, 3'-hydroxy-*N*-methylcoclaurine, 3'-hydroxycoclaurine, reticuline, norreticuline, norlaudanine, laudanosine, and papaverine. In particular, engineered host cells may synthesize BIA monomers from norcoclaurine or norlaudanosoline by expression of heterologous enzymes including *O*-methyltransferases, *N*-methyltransferases, and 3'-hydroxylases. Examples of *O*-methyltransferases may include norcoclaurine 6-*O*-methyltransferase (6OMT) from *Thalicrum flavum*, *Nelumbo nucifera*, *Populus euphratica*, or another species. Further examples of *O*-methyltransferases may include catechol *O*-methyltransferase (COMT) from *Homo sapiens*, *Mus musculus*, *Rattus norvegicus*, *Gorilla gorilla*, or another species. Further examples of *N*-methyltransferases may include coclaurine *N*-methyltransferase (CNMT) from *T. flavum*, *N. nucifera*, *Aristolochia fimbriata*, or another species. Examples of 3'hydroxylases may include *N*-methylcoclaurine 3'-hydroxylase (CYP80B1) from *Eschscholzia californica*, *T. flavum*, *N. nucifera*, or another species.

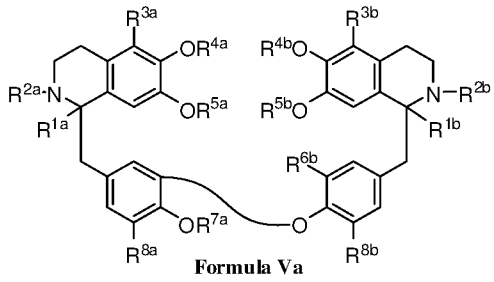
[00125] The engineered host cells may produce either *(S)* or *(R)* enantiomers of any given BIA monomer. Additionally or alternatively, the engineered host cells may produce a mixture of both enantiomers. The ratio of *(S)* and *(R)* enantiomers may be determined by the substrate and product specificities of the one or more enzymes that synthesize the BIA monomers. Alternatively, the amount of each enantiomer present may be modified by the expression of an additional enzyme or enzymes that perform the epimerization of one stereoisomer into another, as discussed above.

[00126] These BIA monomers may be fused into a dimeric bisBIA scaffold. In particular, the BIA monomers may be fused into a dimeric bisBIA scaffold utilizing one or more enzymes that are produced by the engineered host cell. Additionally or alternatively, the BIA monomers may be fused into a dimeric bisBIA scaffold utilizing one or more enzymes that are provided to the BIA monomers from a source that is external to the engineered host cell. The one or more enzymes may be used to form carbon-oxygen and/or carbon-carbon coupling reactions to fuse two BIA monomers at one, two, or three positions. In some examples, two BIA monomers may be linked by an ether bridge. In some examples, a direct carbon-carbon bond may be used to connect the two BIA monomers. In some examples, a bisBIA that is formed by fusing two BIA monomers may comprise one diphenyl ether linkage. In some examples, two BIA monomers may be fused to form a bisBIA that comprises two diphenyl ether linkages. In some examples, a bisBIA that is formed from two BIA monomers may comprise three diphenyl ether linkages. In some examples, the bisBIA may comprise one diphenyl ether linkage and one benzyl phenyl ether linkage. In some cases, the bisBIA may comprise one benzyl phenyl ether linkage and two diphenyl ether linkages.

[00127] In examples, the BIA monomers may be contacted with a sufficient amount of the one or more enzymes that may be used to form coupling reactions to fuse two BIA monomers such that at least 10%, at least 15%, at least 20%, at least 25%, at least 30%, at least 35%, at least 40%, at least 45%, at least 50%, at

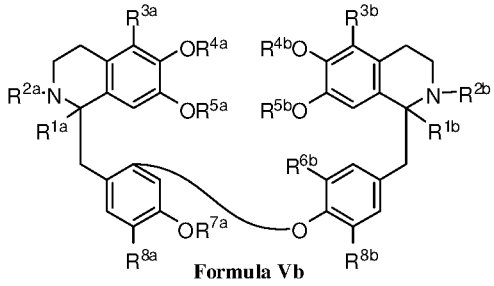
least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 82%, at least 84%, at least 86%, at least 88%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5%, at least 99.7%, or 100% of said BIA monomers are converted to bisBIAs. The one or more enzymes that may be used to dimerize the BIA monomers into bisBIAs may contact the BIA monomers *in vitro*. Additionally, or alternatively, the one or more enzymes that may be used to dimerize the BIA monomers into bisBIAs may contact the BIA monomers *in vivo*. Additionally, the one or more bisBIA dimerizing enzyme may be expressed in a host cell that produces BIA monomers. Alternatively, the BIA monomers may be provided to the engineered host cell that expresses the bisBIA dimerizing enzyme. Alternatively, the one or more bisBIA dimerizing enzymes may be provided to a cell having BIA monomers within.

[00128] In some examples, the bisbenzylisoquinoline alkaloid is a compound of any one of Formulas Va-Vu:



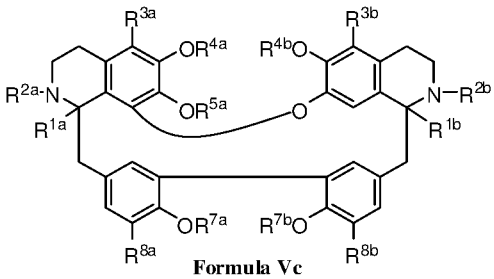
Formula Va

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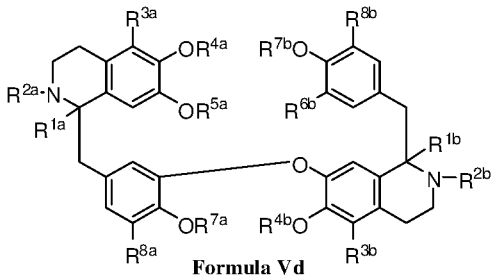
Formula Vb

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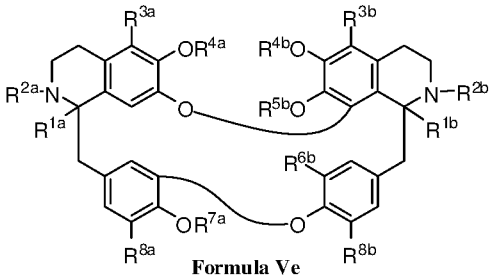
Formula Vc

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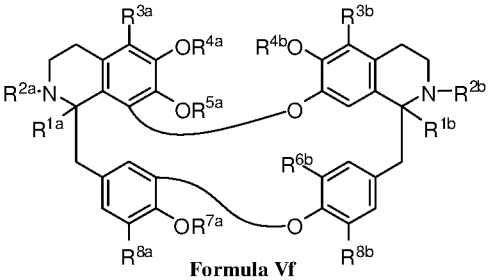
Formula Vd

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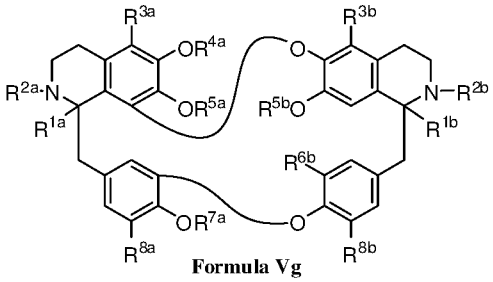
Formula Ve

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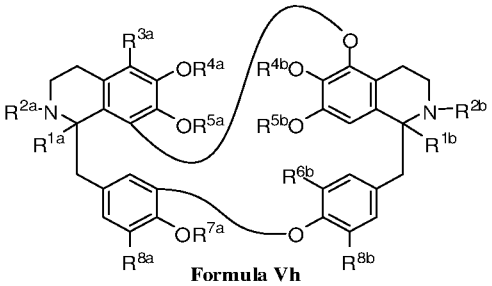
Formula Vf

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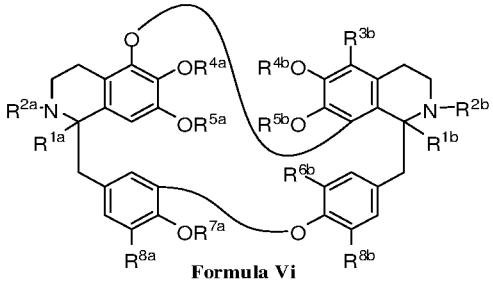
Formula Vg

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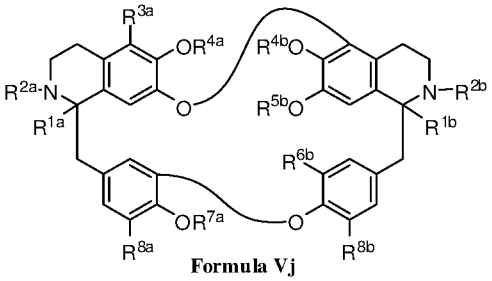
Formula Vh

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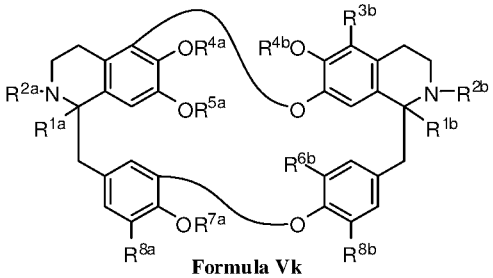
Formula Vi

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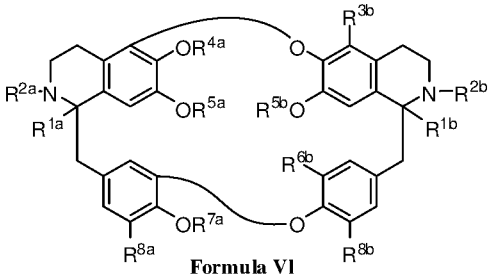
Formula Vj

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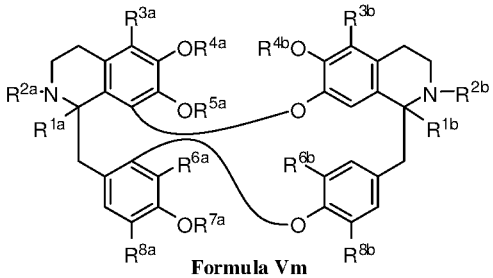
Formula Vk

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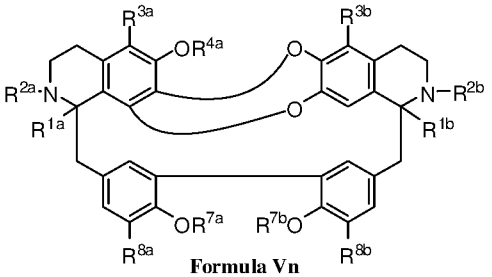
Formula Vl

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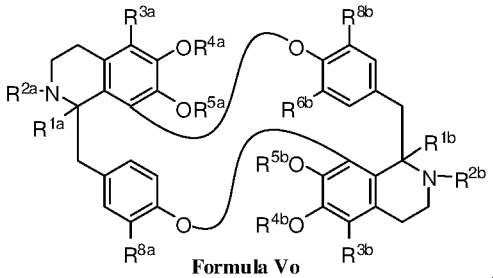
Formula Vm

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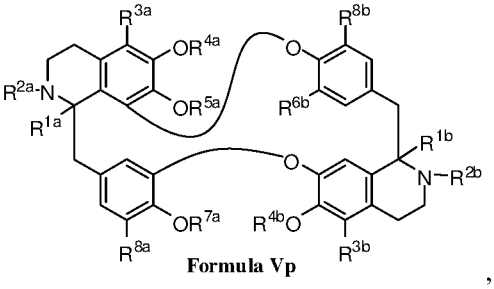
Formula Vn

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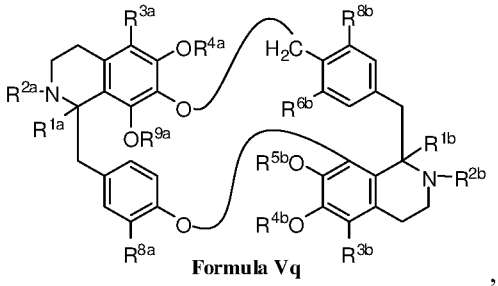
Formula Vo

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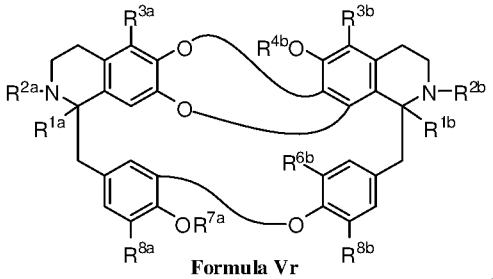
Formula Vp

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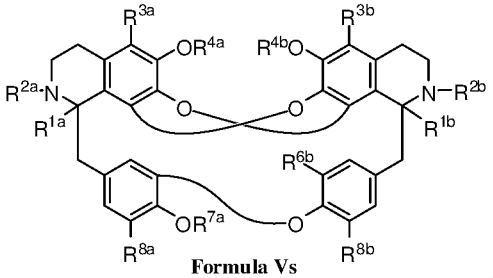
Formula Vq

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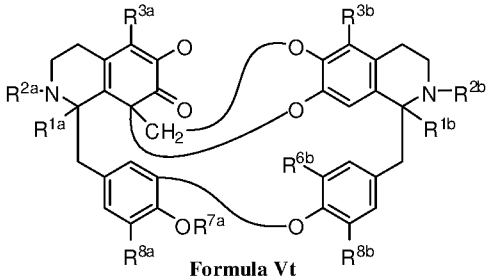
Formula Vr

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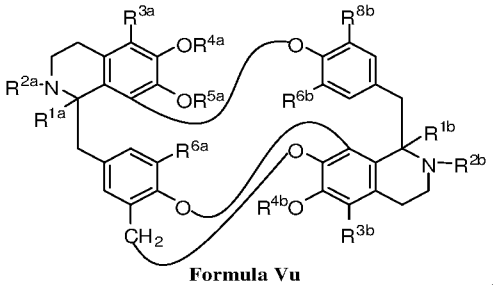
Formula Vs

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Formula Vt

, and



Formula Vu

,

or a salt thereof, wherein:

R^{1a}, R^{1b}, R^{2a}, and R^{2b} are independently selected from hydrogen and C_1-C_4 alkyl;

R^{3a}, R^{3b}, R^{6a}, R^{6b}, R^{8a}, and R^{8b} are independently selected from hydrogen, hydroxy, fluoro, chloro, bromo, carboxaldehyde, C_1-C_4 acyl, C_1-C_4 alkyl, and C_1-C_4 alkoxy;

R^{4a} and R^{5a} are independently selected from hydrogen and C_1-C_4 alkyl, or R^{4a} and R^{5a} together form a methylene bridge;

R^{4b} and R^{5b} are independently selected from hydrogen and C_1-C_4 alkyl, or R^{4b} and R^{5b} together form a methylene bridge; and

R^{7a}, R^{7b}, and R^{9a} are independently selected from hydrogen and C_1-C_4 alkyl.

[00129] In some examples, R^{1a} and R^{1b} are each hydrogen; R^{2a} and R^{2b} are each methyl; R^{3a} and R^{3b} are each hydrogen; R^{4a} and R^{5a} are independently hydrogen or methyl; R^{4b} and R^{5b} are independently hydrogen or methyl, or R^{4b} and R^{5b} together form a methylene bridge; R^{6a}, R^{6b}, R^{8a}, and R^{8b} are each hydrogen; and R^{7a}, R^{7b}, and R^{9a} are independently hydrogen or methyl.

[00130] As illustrated above, the bisBIA compounds of Formulas Va, Vb, and Vd are formed by fusing two BIA monomers using a carbon-oxygen coupling reaction. Additionally, the bisBIA compounds of Formulas Vc, Vf, and Vh are formed by fusing two BIA monomers using both a carbon-oxygen coupling reaction and a carbon-carbon coupling reaction. Further, the bisBIA compounds of Formulas Ve, Vg, Vi, Vj, Vk, Vl, Vm,

Vo, Vp, and Vq are formed by fusing two BIA monomers using two carbon-oxygen coupling reactions. The bisBIA compound of Formula Vn is formed by fusing two BIA monomers using two carbon-oxygen coupling reactions and a carbon-carbon coupling reaction. Additionally, the bisBIA compound of Formula Vr is formed by fusing two BIA monomers using three carbon-oxygen coupling reactions.

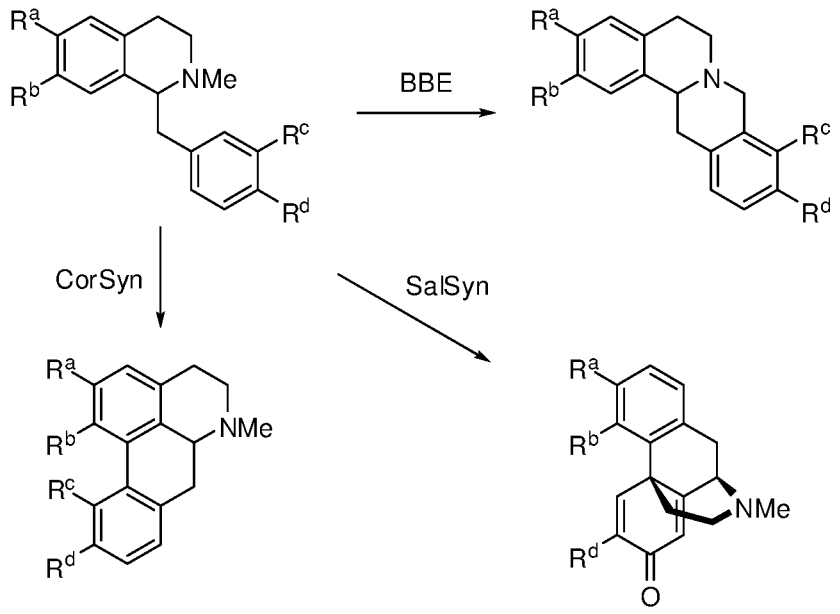
[00131] The one or more enzymes that may be used to form the coupling reactions may include known cytochrome P450s such as *Berberis stolonifera* CYP80A1 or similar cytochrome P450 enzymes from other plants that naturally synthesize these compounds. Alternatively, the coupling reaction may be performed by an enzyme that is not a cytochrome P450. The one or more enzymes that may be used to form the coupling reactions may be engineered to accept non-native substrates. Accordingly, the one or more enzymes that may be used to form the coupling reactions may be used to generate non-natural bisBIA molecules. In examples, the one or more enzymes may fuse a natural BIA monomer with a non-natural BIA monomer to produce a non-natural bisBIA molecule. In other examples, the one or more enzymes may fuse two non-natural BIA monomers to produce a non-natural bisBIA molecule. Enzyme engineered strategies may be used to identify one or more enzymes that may be used to form the coupling reactions that fuse BIA monomers to produce bisBIAs. In examples, enzyme engineering strategies may include site directed mutagenesis, random mutagenesis and screening, DNA shuffling, and screening.

[00132] Once bisBIAs are formed, the bisBIAs may be further derivatized or modified. The bisBIAs may be derivatized or modified utilizing one or more enzymes that are produced by the engineered host cell. In particular, the bisBIAs may be derivatized or modified by contacting the bisBIAs with one or more enzymes that are produced by the engineered host cell. Additionally or alternatively, the bisBIAs may be derivatized or modified by contacting the bisBIAs with one or more enzymes that are provided to the bisBIAs from a source that is external to the engineered host cell. The one or more enzymes that may be used to derivatize or modify the bisBIAs may be used to perform tailoring reactions. Examples of tailoring reactions include oxidation, reduction, *O*-methylation, *N*-methylation, *O*-demethylation, acetylation, methylenedioxybridge formation, and *O,O*-demethylenation. A bisBIA may be derivatized or modified using one or more tailoring reactions.

[00133] Examples of tailoring reactions are provided in **Table 3**. In some examples, tailoring enzymes may be used to catalyze carbon-carbon coupling reactions performed on a bisBIA, or a derivative thereof. Examples of tailoring enzymes that may be used to catalyze carbon-carbon coupling reactions include a Berberine bridge enzyme (BBE) from *Papaver somniferum*, *Eschscholzia californica*, *Coptis japonica*, *Berberis stolonifer*, *Thalictrum flavum*, or another species; Salutaridine synthase (SalSyn) from *Papaver somniferum* or another species; and Corytuberine synthase (CorSyn) from *Coptis japonica* or another species. Non-limiting examples of reactions that can be catalyzed by tailoring enzymes are shown in **Scheme 2**, wherein R^a, R^b, R^c, and R^d are independently selected from hydrogen, hydroxy, fluoro, chloro, bromo, carboxaldehyde, C_1-C_4 acyl, C_1-C_4 alkyl, and C_1-C_4 alkoxy. In some examples, R^a, R^b, and the carbon atoms

to which they are attached optionally form a carbocycle or heterocycle. In some examples, R^c, R^d, and the carbon atoms to which they are attached optionally form a carbocycle or heterocycle.

Scheme 2



[00134] In some examples, tailoring enzymes may be used to catalyze oxidation reactions performed on a bisBIA, or a derivative thereof. Examples of tailoring enzymes that may be used to catalyze oxidation reactions include a Tetrahydroprotoberberine oxidase (STOX) from *Coptis japonica*, *Argemone mexicana*, *Berberis wilsonae*, or another species; Dihydrobenzophenanthridine oxidase (DBOX) from *Papaver somniferum* or another species; Methylstylopine hydroxylase (MSH) from *Papaver somniferum* or another species; and Protopine 6-hydroxylase (P6H) from *Papaver somniferum*, *Eschscholzia californica*, or another species.

[00135] Tailoring enzymes may also be used to catalyze methylenedioxy bridge formation reactions performed on a bisBIA, or a derivative thereof. Examples of tailoring enzymes that may be used to catalyze methylenedioxy bridge formation reactions include a Stylopine synthase (StySyn) from *Papaver somniferum*, *Eschscholzia californica*, *Argemone mexicana*, or another species; Cheilanthifoline synthase (CheSyn) from *Papaver somniferum*, *Eschscholzia californica*, *Argemone mexicana*, or another species; and Canadine synthase (CAS) from *Thalictrum flavum*, *Coptis chinensis*, or another species.

[00136] In other examples, tailoring enzymes may be used to catalyze *O*-methylation reactions performed on a bisBIA, or a derivative thereof. Examples of tailoring enzymes that may be used to catalyze *O*-methylation reactions include a Norcoclaurine 6-*O*-methyltransferase (6OMT) from *Papaver somniferum*, *Thalictrum flavum*, *Coptis japonica*, *Papaver bracteatum*, or another species; 3'hydroxy-*N*-methylcoclaurine 4'-*O*-methyltransferase (4'OMT) from *Papaver somniferum*, *Thalictrum flavum*, *Coptis japonica*, *Coptis chinensis*, or another species; Reticuline 7-*O*-methyltransferase (7OMT) from *Papaver somniferum*, *Eschscholzia*

californica, or another species; and Scoulerine 9-*O*-methyltransferase (9OMT) from *Papaver somniferum*, *Thalictrum flavum*, *Coptis japonica*, *Coptis chinensis*, or another species.

[00137] Additionally, tailoring enzymes may be used to catalyze *N*-methylation reactions performed on a bisBIA, or a derivative thereof. Examples of tailoring enzymes that may be used to catalyze *N*-methylation reactions include Coclaurine *N*-methyltransferase (CNMT) from *Papaver somniferum*, *Thalictrum flavum*, *Coptis japonica*, or another species; Tetrahydroprotoberberine *N*-methyltransferase (TNMT) from *Papaver somniferum*, *Eschscholzia californica*, *Papaver bracteatum*, or another species.

[00138] Further, tailoring enzymes may be used to catalyze *O*-demethylation reactions performed on a bisBIA, or a derivative thereof. Examples of tailoring enzymes that may be used to catalyze *O*-demethylation reactions include Thebaine demethylase (T6ODM) from *Papaver somniferum* or another species; and Codeine demethylase (CODM) from *Papaver somniferum*, or another species.

[00139] Tailoring enzymes may also be used to catalyze reduction reactions performed on a bisBIA, or a derivative thereof. Examples of tailoring enzymes that may be used to catalyze reduction reactions include Salutaridine reductase (SalR) from *Papaver somniferum*, *Papaver bracteatum*, or another species; Codeinone reductase (COR) from *Papaver somniferum* or another species; and Sanguinarine reductase (SanR) from *Eschscholzia californica* or another species. In other examples, tailoring enzymes may be used to catalyze acetylation reactions performed on a bisBIA, or a derivative thereof. An example of a tailoring enzyme that may be used to catalyze acetylation reactions includes Salutaridine acetyltransferase (SalAT) from *Papaver somniferum* or another species.

Heterologous coding sequences

[00140] In some instances, the engineered host cells harbor one or more heterologous coding sequences (such as two or more, three or more, four or more, five or more) which encode activity(ies) that enable the engineered host cells to produce desired enzymes of interest and/or BIAs of interest, e.g., as described herein. As used herein, the term "heterologous coding sequence" is used to indicate any polynucleotide that codes for, or ultimately codes for, a peptide or protein or its equivalent amino acid sequence, e.g., an enzyme, that is not normally present in the host organism and may be expressed in the host cell under proper conditions. As such, "heterologous coding sequences" includes multiple copies of coding sequences that are normally present in the host cell, such that the cell is expressing additional copies of a coding sequence that are not normally present in the cells. The heterologous coding sequences may be RNA or any type thereof, e.g., mRNA, DNA or any type thereof, e.g., cDNA, or a hybrid of RNA/DNA. Coding sequences of interest include, but are not limited to, full-length transcription units that include such features as the coding sequence, introns, promoter regions, 3'-UTRs, and enhancer regions.

[00141] In examples, the engineered host cells may comprise a plurality of heterologous coding sequences each encoding an enzyme, such as an enzyme listed in Table 2. In some examples, the plurality of enzymes encoded by the plurality of heterologous coding sequences may be distinct from each other. In some examples, some of the plurality of enzymes encoded by the plurality of heterologous coding sequences may be distinct from each other and some of the plurality of enzymes encoded by the plurality of heterologous coding sequences may be duplicate copies.

[00142] In some examples, the heterologous coding sequences may be operably connected. Heterologous coding sequences that are operably connected may be within the same pathway of producing a particular benzylisoquinoline alkaloid product and/or epimerase product. In some examples, the operably connected heterologous coding sequences may be directly sequential along the pathway of producing a particular benzylisoquinoline alkaloid product and/or epimerase product. In some examples, the operably connected heterologous coding sequences may have one or more native enzymes between one or more of the enzymes encoded by the plurality of heterologous coding sequences. In some examples, the heterologous coding sequences may have one or more heterologous enzymes between one or more of the enzymes encoded by the plurality of heterologous coding sequences. In some examples, the heterologous coding sequences may have one or more non-native enzymes between one or more of the enzymes encoded by the plurality of heterologous coding sequences.

[00143] The engineered host cells may also be modified to possess one or more genetic alterations to accommodate the heterologous coding sequences. Alterations of the native host genome include, but are not limited to, modifying the genome to reduce or ablate expression of a specific protein that may interfere with the desired pathway. The presence of such native proteins may rapidly convert one of the intermediates or final products of the pathway into a metabolite or other compound that is not usable in the desired pathway. Thus, if the activity of the native enzyme were reduced or altogether absent, the produced intermediates would be more readily available for incorporation into the desired product.

[00144] Heterologous coding sequences include but are not limited to sequences that encode enzymes, either wild-type or equivalent sequences, that are normally responsible for the production of BIAs of interest in plants. In some cases, the enzymes for which the heterologous sequences code may be any of the enzymes in the 1-BIA pathway, and may be from any convenient source. The choice and number of enzymes encoded by the heterologous coding sequences for the particular synthetic pathway may be selected based upon the desired product. In certain embodiments, the host cells of the invention may include 1 or more, 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, or even 15 or more heterologous coding sequences, such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 heterologous coding sequences.

[00145] As used herein, the term "heterologous coding sequences" also includes the coding portion of the peptide or enzyme, i.e., the cDNA or mRNA sequence, of the peptide or enzyme, as well as the coding portion of the full-length transcriptional unit, i.e., the gene including introns and exons, as well as "codon optimized" sequences, truncated sequences or other forms of altered sequences that code for the enzyme or code for its equivalent amino acid sequence, provided that the equivalent amino acid sequence produces a functional protein. Such equivalent amino acid sequences may have a deletion of one or more amino acids, with the deletion being N-terminal, C-terminal, or internal. Truncated forms are envisioned as long as they have the catalytic capability indicated herein. Fusions of two or more enzymes are also envisioned to facilitate the transfer of metabolites in the pathway, provided that catalytic activities are maintained.

[00146] Operable fragments, mutants, or truncated forms may be identified by modeling and/or screening. In some cases, this is achieved by deletion of, for example, N-terminal, C-terminal, or internal regions of the protein in a step-wise fashion, followed by analysis of the resulting derivative with regard to its activity for the desired reaction compared to the original sequence. If the derivative in question operates in this capacity, it is considered to constitute an equivalent derivative of the enzyme proper.

[00147] In examples, some heterologous proteins may show occurrences where they are incorrectly processed when expressed in a recombinant host. For example, plant proteins such as cytochrome P450 enzymes expressed in microbial production hosts may have occurrences of incorrect processing. In particular, salutaridine synthase may undergo N-linked glycosylation when heterologously expressed in yeast. This N-linked glycosylation may not be observed in plants, which may be indicative of incorrect N-terminal sorting of the nascent SalSyn transcript so as to reduce the activity of the enzyme in the heterologous microbial host. In such examples, protein engineering directed at correcting N-terminal sorting of the nascent transcript so as to remove the N-linked glycosylation pattern may result in improved activity of the salutaridine synthase enzyme in the recombinant production host. This is explained further in **Example 10** below.

[00148] Aspects of the invention also relate to heterologous coding sequences that code for amino acid sequences that are equivalent to the native amino acid sequences for the various enzymes. An amino acid sequence that is "equivalent" is defined as an amino acid sequence that is not identical to the specific amino acid sequence, but rather contains at least some amino acid changes (deletions, substitutions, inversions, insertions, etc.) that do not essentially affect the biological activity of the protein as compared to a similar activity of the specific amino acid sequence, when used for a desired purpose. The biological activity refers to, in the example of an epimerase, its catalytic activity. Equivalent sequences are also meant to include those which have been engineered and/or evolved to have properties different from the original amino acid sequence. Mutable properties of interest include catalytic activity, substrate specificity, selectivity, stability, solubility, localization, etc.

[00149] In some instances, the expression of each type of enzyme is increased through additional gene copies (i.e., multiple copies), which increases intermediate accumulation and/or BIA of interest production. Embodiments of the invention include increased BIA of interest production in a host cell through simultaneous expression of multiple species variants of a single or multiple enzymes. In some cases, additional gene copies of a single or multiple enzymes are included in the host cell. Any convenient methods may be utilized including multiple copies of a heterologous coding sequence for an enzyme in the host cell.

[00150] In some examples, the engineered host cell includes multiple copies of a heterologous coding sequence for an enzyme, such as 2 or more, 3 or more, 4 or more, 5 or more, or even 10 or more copies. An example of this is described in **FIG. 28**. In particular, **FIG. 28** illustrates increased production of the benzylisoquinoline alkaloid reticuline by increasing the copy number of the *NCS* gene from two copies to three copies in an engineered yeast strain.

[00151] In certain embodiments, the engineered host cell includes multiple copies of heterologous coding sequences for one or more enzymes, such as multiple copies of two or more, three or more, four or more, etc. In some cases, the multiple copies of the heterologous coding sequence for an enzyme are derived from two or more different source organisms as compared to the host cell. For example, the engineered host cell may include multiple copies of one heterologous coding sequence, where each of the copies is derived from a different source organism. As such, each copy may include some variations in explicit sequences based on inter-species differences of the enzyme of interest that is encoded by the heterologous coding sequence.

[00152] The engineered host cell medium may be sampled and monitored for the production of BIAs of interest. The BIAs of interest may be observed and measured using any convenient methods. Methods of interest include, but are not limited to, LC-MS methods (e.g., as described herein) where a sample of interest is analyzed by comparison with a known amount of a standard compound. Additionally, there are other ways that BIAs of interest may be observed and/or measured. Examples of alternative ways of observing and/or measuring BIAs include GC-MS, UV-vis spectroscopy, NMR, LC-NMR, LC-UV, TLC, capillary electrophoresis, among others. Identity may be confirmed, e.g., by m/z and MS/MS fragmentation patterns, and quantitation or measurement of the compound may be achieved via LC trace peaks of know retention time and/or EIC MS peak analysis by reference to corresponding LC-MS analysis of a known amount of a standard of the compound.

[00153] Additionally, a culture of the engineered host cell may be sampled and monitored for the production of enzymes of interest, such as a CYP-COR enzyme. The enzymes of interest may be observed and measured using any convenient methods. Methods of interest include enzyme activity assays, polyacrylamide gel electrophoresis, carbon monoxide spectroscopy, and western blot analysis.

METHODS

Process Steps

[00154] As summarized above, aspects of the invention include methods of preparing benzylisoquinoline alkaloids (BIAs) of interest. Additionally, aspects of the invention include methods of preparing enzymes of interest. As such, aspects of the invention include culturing an engineered host cell under conditions in which the one or more host cell modifications (e.g., as described herein) are functionally expressed such that the cell converts starting compounds of interest into product enzymes and/or BIAs of interest. Also provided are methods that include culturing an engineered host cell under conditions suitable for protein production such that one or more heterologous coding sequences are functionally expressed and convert starting compounds of interest into product enzymes or BIAs of interest. In examples, the method is a method of preparing a benzylisoquinoline alkaloid (BIA) that includes culturing an engineered host cell (e.g., as described herein); adding a starting compound to the cell culture; and recovering the BIA from the cell culture. In some examples, the method is a method of preparing an enzyme that includes culturing an engineered host cell (e.g., as described herein); adding a starting compound to the cell culture; and recovering the enzyme from the cell culture.

[00155] Fermentation media may contain suitable carbon substrates. The source of carbon suitable to perform the methods of this disclosure may encompass a wide variety of carbon containing substrates. Suitable substrates may include, without limitation, monosaccharides (e.g., glucose, fructose, galactose, xylose), oligosaccharides (e.g., lactose, sucrose, raffinose), polysaccharides (e.g., starch, cellulose), or a combination thereof. In some cases, unpurified mixtures from renewable feedstocks may be used (e.g., cornsteep liquor, sugar beet molasses, barley malt). In some cases, the carbon substrate may be a one-carbon substrate (e.g., methanol, carbon dioxide) or a two-carbon substrate (e.g., ethanol). In other cases, other carbon containing compounds may be utilized, for example, methylamine, glucosamine, and amino acids.

[00156] Any convenient methods of culturing engineered host cells may be employed for producing the enzymes and/or BIAs of interest. The particular protocol that is employed may vary, e.g., depending on the engineered host cell, the heterologous coding sequences, the enzymes of interest, the BIAs of interest, etc. The cells may be present in any convenient environment, such as an environment in which the cells are capable of expressing one or more functional heterologous enzymes. In some embodiments, the cells are cultured under conditions that are conducive to enzyme expression and with appropriate substrates available to allow production of enzymes and/or BIAs of interest *in vivo*. In some embodiments, the functional enzymes are extracted from the engineered host for production of enzymes and/or BIAs of interest under *in vitro* conditions. In some instances, the engineered host cells are placed back into a multicellular host organism. The engineered host cells are in any phase of growth, including, but not limited to, stationary phase and log-

growth phase, etc. In addition, the cultures themselves may be continuous cultures or they may be batch cultures.

[00157] Cells may be grown in an appropriate fermentation medium at a temperature between 14-40°C. Cells may be grown with shaking at any convenient speed (e.g., 200 rpm). Cells may be grown at a suitable pH. Suitable pH ranges for the fermentation may be between pH 5-9. Fermentations may be performed under aerobic, anaerobic, or microaerobic conditions. Any suitable growth medium may be used. Suitable growth media may include, without limitation, common commercially prepared media such as synthetic defined (SD) minimal media or yeast extract peptone dextrose (YEPD) rich media. Any other rich, defined, or synthetic growth media appropriate to the microorganism may be used.

[00158] Cells may be cultured in a vessel of essentially any size and shape. Examples of vessels suitable to perform the methods of this disclosure may include, without limitation, multi-well shake plates, test tubes, flasks (baffled and non-baffled), and bioreactors. The volume of the culture may range from 10 microliters to greater than 10,000 liters.

[00159] The addition of agents to the growth media that are known to modulate metabolism in a manner desirable for the production of alkaloids may be included. In a non-limiting example, cyclic adenosine 2'3'-monophosphate may be added to the growth media to modulate catabolite repression.

[00160] Any convenient cell culture conditions for a particular cell type may be utilized. In certain embodiments, the host cells that include one or more modifications are cultured under standard or readily optimized conditions, with standard cell culture media and supplements. As one example, standard growth media when selective pressure for plasmid maintenance is not required may contain 20 g/L yeast extract, 10 g/L peptone, and 20 g/L dextrose (YPD). Host cells containing plasmids are grown in synthetic complete (SC) media containing 1.7 g/L yeast nitrogen base, 5 g/L ammonium sulfate, and 20 g/L dextrose supplemented with the appropriate amino acids required for growth and selection. Alternative carbon sources which may be useful for inducible enzyme expression include, but are not limited to, sucrose, raffinose, and galactose. Cells are grown at any convenient temperature (e.g., 30°C) with shaking at any convenient rate (e.g., 200 rpm) in a vessel, e.g., in test tubes or flasks in volumes ranging from 1-1000 mL, or larger, in the laboratory.

[00161] Culture volumes may be scaled up for growth in larger fermentation vessels, for example, as part of an industrial process. The industrial fermentation process may be carried out under closed-batch, fed-batch, or continuous chemostat conditions, or any suitable mode of fermentation. In some cases, the cells may be immobilized on a substrate as whole cell catalysts and subjected to fermentation conditions for alkaloid production.

[00162] A batch fermentation is a closed system, in which the composition of the medium is set at the beginning of the fermentation and not altered during the fermentation process. The desired organism(s) are inoculated into the medium at the beginning of the fermentation. In some instances, the batch fermentation is

run with alterations made to the system to control factors such as pH and oxygen concentration (but not carbon). In this type of fermentation system, the biomass and metabolite compositions of the system change continuously over the course of the fermentation. Cells typically proceed through a lag phase, then to a log phase (high growth rate), then to a stationary phase (growth rate reduced or halted), and eventually to a death phase (if left untreated).

[00163] A continuous fermentation is an open system, in which a defined fermentation medium is added continuously to the bioreactor and an equal amount of fermentation media is continuously removed from the vessel for processing. Continuous fermentation systems are generally operated to maintain steady state growth conditions, such that cell loss due to medium being removed must be balanced by the growth rate in the fermentation. Continuous fermentations are generally operated at conditions where cells are at a constant high cell density. Continuous fermentations allow for the modulation of one or more factors that affect target product concentration and/or cell growth.

[00164] The liquid medium may include, but is not limited to, a rich or synthetic defined medium having an additive component described above. Media components may be dissolved in water and sterilized by heat, pressure, filtration, radiation, chemicals, or any combination thereof. Several media components may be prepared separately and sterilized, and then combined in the fermentation vessel. The culture medium may be buffered to aid in maintaining a constant pH throughout the fermentation.

[00165] Process parameters including temperature, dissolved oxygen, pH, stirring, aeration rate, and cell density may be monitored or controlled over the course of the fermentation. For example, temperature of a fermentation process may be monitored by a temperature probe immersed in the culture medium. The culture temperature may be controlled at the set point by regulating the jacket temperature. Water may be cooled in an external chiller and then flowed into the bioreactor control tower and circulated to the jacket at the temperature required to maintain the set point temperature in the vessel.

[00166] Additionally, a gas flow parameter may be monitored in a fermentation process. For example, gases may be flowed into the medium through a sparger. Gases suitable for the methods of this disclosure may include compressed air, oxygen, and nitrogen. Gas flow may be at a fixed rate or regulated to maintain a dissolved oxygen set point.

[00167] The pH of a culture medium may also be monitored. In examples, the pH may be monitored by a pH probe that is immersed in the culture medium inside the vessel. If pH control is in effect, the pH may be adjusted by acid and base pumps which add each solution to the medium at the required rate. The acid solutions used to control pH may be sulfuric acid or hydrochloric acid. The base solutions used to control pH may be sodium hydroxide, potassium hydroxide, or ammonium hydroxide.

[00168] Further, dissolved oxygen may be monitored in a culture medium by a dissolved oxygen probe immersed in the culture medium. If dissolved oxygen regulation is in effect, the oxygen level may be

adjusted by increasing or decreasing the stirring speed. The dissolved oxygen level may also be adjusted by increasing or decreasing the gas flow rate. The gas may be compressed air, oxygen, or nitrogen.

[00169] Stir speed may also be monitored in a fermentation process. In examples, the stirrer motor may drive an agitator. The stirrer speed may be set at a consistent rpm throughout the fermentation or may be regulated dynamically to maintain a set dissolved oxygen level.

[00170] Additionally, turbidity may be monitored in a fermentation process. In examples, cell density may be measured using a turbidity probe. Alternatively, cell density may be measured by taking samples from the bioreactor and analyzing them in a spectrophotometer. Further, samples may be removed from the bioreactor at time intervals through a sterile sampling apparatus. The samples may be analyzed for alkaloids produced by the host cells. The samples may also be analyzed for other metabolites and sugars, the depletion of culture medium components, or the density of cells.

[00171] In another example, a feed stock parameter may be monitored during a fermentation process. In particular, feed stocks including sugars and other carbon sources, nutrients, and cofactors that may be added into the fermentation using an external pump. Other components may also be added during the fermentation including, without limitation, anti-foam, salts, chelating agents, surfactants, and organic liquids.

[00172] Any convenient codon optimization techniques for optimizing the expression of heterologous polynucleotides in host cells may be adapted for use in the subject host cells and methods, see e.g., Gustafsson, C. et al. (2004) *Trends Biotechnol*, *22*, 346-353, which is incorporated by reference in its entirety.

[00173] The subject method may also include adding a starting compound to the cell culture. Any convenient methods of addition may be adapted for use in the subject methods. The cell culture may be supplemented with a sufficient amount of the starting materials of interest (e.g., as described herein), e.g., a mM to μM amount such as between about 1-5 mM of a starting compound. It is understood that the amount of starting material added, the timing and rate of addition, the form of material added, etc., may vary according to a variety of factors. The starting material may be added neat or pre-dissolved in a suitable solvent (e.g., cell culture media, water, or an organic solvent). The starting material may be added in concentrated form (e.g., 10x over desired concentration) to minimize dilution of the cell culture medium upon addition. The starting material may be added in one or more batches, or by continuous addition over an extended period of time (e.g., hours or days).

Methods for Isolating Products from the Fermentation Medium

[00174] The subject methods may also include recovering the enzymes and/or BIAs of interest from the cell culture. Any convenient methods of separation and isolation (e.g., chromatography methods or precipitation methods) may be adapted for use in the subject methods to recover the enzymes and/or BIAs of interest from

the cell culture. Filtration methods may be used to separate soluble from insoluble fractions of the cell culture. In some cases, liquid chromatography methods (e.g., reverse phase HPLC, size exclusion, normal phase chromatography) may be used to separate the BIA of interest from other soluble components of the cell culture. In some cases, extraction methods (e.g., liquid extraction, pH based purification, solid phase extraction, affinity chromatography, ion exchange, etc.) may be used to separate the enzymes and/or BIAs of interest from other components of the cell culture.

[00175] The produced alkaloids may be isolated from the fermentation medium using methods known in the art. A number of recovery steps may be performed immediately after (or in some instances, during) the fermentation for initial recovery of the desired product. Through these steps, the alkaloids (e.g., BIAs) may be separated from the cells, cellular debris and waste, and other nutrients, sugars, and organic molecules may remain in the spent culture medium. This process may be used to yield a BIA-enriched product.

[00176] In an example, a product stream having a benzylisoquinoline alkaloid (BIA) product is formed by providing engineered yeast cells and a feedstock including nutrients and water to a batch reactor. In particular, the engineered yeast cells may be subjected to fermentation by incubating the engineered yeast cells for a time period of at least about 5 minutes to produce a solution comprising the BIA product and cellular material. Once the engineered yeast cells have been subjected to fermentation, at least one separation unit may be used to separate the BIA product from the cellular material to provide the product stream comprising the BIA product. In particular, the product stream may include the BIA product as well as additional components, such as a clarified yeast culture medium. Additionally, a BIA product may comprise one or more BIAs of interest, such as one or more BIA compounds.

[00177] Different methods may be used to remove cells from a bioreactor medium that include an enzyme and/or BIA of interest. In examples, cells may be removed by sedimentation over time. This process of sedimentation may be accelerated by chilling or by the addition of fining agents such as silica. The spent culture medium may then be siphoned from the top of the reactor or the cells may be decanted from the base of the reactor. Alternatively, cells may be removed by filtration through a filter, a membrane, or other porous material. Cells may also be removed by centrifugation, for example, by continuous flow centrifugation or by using a continuous extractor.

[00178] If some valuable enzymes and/or BIAs of interest are present inside the cells, the cells may be permeabilized or lysed and the cell debris may be removed by any of the methods described above. Agents used to permeabilize the cells may include, without limitation, organic solvents (e.g., DMSO) or salts (e.g., lithium acetate). Methods to lyse the cells may include the addition of surfactants such as sodium dodecyl sulfate, or mechanical disruption by bead milling or sonication.

[00179] Enzymes and/or BIAs of interest may be extracted from the clarified spent culture medium through liquid-liquid extraction by the addition of an organic liquid that is immiscible with the aqueous culture

medium. In examples, the use of liquid-liquid extraction may be used in addition to other processing steps. Examples of suitable organic liquids include, but are not limited to, isopropyl myristate, ethyl acetate, chloroform, butyl acetate, methylisobutyl ketone, methyl oleate, toluene, oleyl alcohol, ethyl butyrate. The organic liquid may be added to as little as 10% or as much as 100% of the volume of aqueous medium.

[00180] In some cases, the organic liquid may be added at the start of the fermentation or at any time during the fermentation. This process of extractive fermentation may increase the yield of enzymes and/or BIAs of interest from the host cells by continuously removing enzymes and/or BIAs to the organic phase.

[00181] Agitation may cause the organic phase to form an emulsion with the aqueous culture medium. Methods to encourage the separation of the two phases into distinct layers may include, without limitation, the addition of a demulsifier or a nucleating agent, or an adjustment of the pH. The emulsion may also be centrifuged to separate the two phases, for example, by continuous conical plate centrifugation.

[00182] Alternatively, the organic phase may be isolated from the aqueous culture medium so that it may be physically removed after extraction. For example, the solvent may be encapsulated in a membrane.

[00183] In examples, enzymes and/or BIAs of interest may be extracted from a fermentation medium using adsorption methods. In examples, BIAs of interest may be extracted from clarified spent culture medium by the addition of a resin such as Amberlite® XAD4 or another agent that removes BIAs by adsorption. The BIAs of interest may then be released from the resin using an organic solvent. Examples of suitable organic solvents include, but are not limited to, methanol, ethanol, ethyl acetate, or acetone.

[00184] BIAs of interest may also be extracted from a fermentation medium using filtration. At high pH, the BIAs of interest may form a crystalline-like precipitate in the bioreactor. This precipitate may be removed directly by filtration through a filter, membrane, or other porous material. The precipitate may also be collected by centrifugation and/or decantation.

[00185] The extraction methods described above may be carried out either *in situ* (in the bioreactor) or *ex situ* (e.g., in an external loop through which media flows out of the bioreactor and contacts the extraction agent, then is recirculated back into the vessel). Alternatively, the extraction methods may be performed after the fermentation is terminated using the clarified medium removed from the bioreactor vessel.

Methods for Purifying Products from Alkaloid-Enriched Solutions

[00186] Subsequent purification steps may involve treating the post-fermentation solution enriched with BIA product(s) of interest using methods known in the art to recover individual product species of interest to high purity.

[00187] In one example, BIAs of interest extracted in an organic phase may be transferred to an aqueous solution. In some cases, the organic solvent may be evaporated by heat and/or vacuum, and the resulting powder may be dissolved in an aqueous solution of suitable pH. In a further example, the BIAs of interest may be extracted from the organic phase by addition of an aqueous solution at a suitable pH that promotes extraction of the BIAs of interest into the aqueous phase. The aqueous phase may then be removed by decantation, centrifugation, or another method.

[00188] The BIA-containing solution may be further treated to remove metals, for example, by treating with a suitable chelating agent. The BIA of interest-containing solution may be further treated to remove other impurities, such as proteins and DNA, by precipitation. In one example, the BIA of interest-containing solution is treated with an appropriate precipitation agent such as ethanol, methanol, acetone, or isopropanol. In an alternative example, DNA and protein may be removed by dialysis or by other methods of size exclusion that separate the smaller alkaloids from contaminating biological macromolecules.

[00189] In further examples, the solution containing BIAs of interest may be extracted to high purity by continuous cross-flow filtration using methods known in the art.

[00190] If the solution contains a mixture of BIAs of interest, it may be subjected to acid-base treatment to yield individual BIA of interest species using methods known in the art. In this process, the pH of the aqueous solution is adjusted to precipitate individual BIAs.

[00191] For high purity, small-scale preparations, the BIAs may be purified in a single step by liquid chromatography.

Yeast-Derived Alkaloid APIs Versus Plant-Derived APIs

[00192] The clarified yeast culture medium (CYCM) may contain a plurality of impurities. The clarified yeast culture medium may be dehydrated by vacuum and/or heat to yield an alkaloid-rich powder. This product is analogous to the concentrate of poppy straw (CPS) or opium, which is exported from poppy-growing countries and purchased by API manufacturers. For the purposes of this invention, CPS is a representative example of any type of purified plant extract from which the desired alkaloids product(s) may ultimately be further purified. **Table 4** and **Table 5** highlight the impurities in these two products that may be specific to either CYCM or CPS or may be present in both. While some BIAs may have a pigment as an impurity, other BIAs may be categorized as pigments themselves. Accordingly, these BIAs may be assessed for impurities based on non-pigment impurities. By analyzing a product of unknown origin for a subset of these impurities, a person of skill in the art could determine whether the product originated from a yeast or plant production host.

[00193] API-grade pharmaceutical ingredients are highly purified molecules. As such, impurities that could indicate the plant- or yeast-origin of an API (such as those listed in **Table 4** and **Table 5**) may not be present at the API stage of the product. Indeed, many of the API products derived from yeast strains of the present invention may be largely indistinguishable from the traditional plant-derived APIs. In some cases, however, conventional alkaloid compounds may be subjected to chemical modification using chemical synthesis approaches, which may show up as chemical impurities in plant-based products that require such chemical modifications. For example, chemical derivatization may often result in a set of impurities related to the chemical synthesis processes. In certain situations, these modifications may be performed biologically in the yeast production platform, thereby avoiding some of the impurities associated with chemical derivation from being present in the yeast-derived product. In particular, these impurities from the chemical derivation product may be present in an API product that is produced using chemical synthesis processes but may be absent from an API product that is produced using a yeast-derived product. Alternatively, if a yeast-derived product is mixed with a chemically-derived product, the resulting impurities may be present but in a lesser amount than would be expected in an API that only or primarily contains chemically-derived products. In this example, by analyzing the API product for a subset of these impurities, a person of skill in the art could determine whether the product originated from a yeast production host or the traditional chemical derivatization route.

[00194] Non-limiting examples of impurities that may be present in chemically-derivatized morphinan APIs but not in biosynthesized APIs include a codeine-*O*(6)-methyl ether impurity in API codeine; 8,14-dihydroxy-7,8-dihydrocodeinone in API oxycodone; and tetrahydrothebaine in API hydrocodone. The codeine-*O*(6)-methyl ether may be formed by chemical over-methylation of morphine. The 8,14-dihydroxy-7,8-dihydrocodeinone in API oxycodone may be formed by chemical over-oxidation of thebaine. Additionally, the tetrahydrothebaine in API hydrocodone may be formed by chemical over-reduction of thebaine.

[00195] However, in the case where the yeast-derived compound and the plant-derived compound are both subjected to chemical modification through chemical synthesis approaches, the same impurities associated with the chemical synthesis process may be expected in the products. In such a situation, the starting material (e.g., CYCM or CPS) may be analyzed as described above.

Methods of Engineering Host Cells

[00196] Also included are methods of engineering host cells for the purpose of producing enzymes and/or BIAs of interest. Inserting DNA into host cells may be achieved using any convenient methods. The methods are used to insert the heterologous coding sequences into the engineered host cells such that the host cells functionally express the enzymes and convert starting compounds of interest into product enzymes and/or BIAs of interest.

[00197] Any convenient promoters may be utilized in the subject engineered host cells and methods. The promoters driving expression of the heterologous coding sequences may be constitutive promoters or inducible promoters, provided that the promoters are active in the engineered host cells. The heterologous coding sequences may be expressed from their native promoters, or non-native promoters may be used. Such promoters may be low to high strength in the host in which they are used. Promoters may be regulated or constitutive. In certain embodiments, promoters that are not glucose repressed, or repressed only mildly by the presence of glucose in the culture medium, are used. Promoters of interest include but are not limited to, promoters of glycolytic genes such as the promoter of the *B. subtilis* tsr gene (encoding the promoter region of the fructose bisphosphate aldolase gene) or the promoter from yeast *S. cerevisiae* gene coding for glyceraldehyde 3-phosphate dehydrogenase (GPD, GAPDH, or TDH3), the ADH1 promoter of baker's yeast, the phosphate-starvation induced promoters such as the PHO5 promoter of yeast, the alkaline phosphatase promoter from *B. licheniformis*, yeast inducible promoters such as Gal1-10, Gal1, GalL, GalS, repressible promoter Met25, tetO, and constitutive promoters such as glyceraldehyde 3-phosphate dehydrogenase promoter (GPD), alcohol dehydrogenase promoter (ADH), translation-elongation factor-1-α promoter (TEF), cytochrome c-oxidase promoter (CYC1), MRP7 promoter, etc. Autonomously replicating yeast expression vectors containing promoters inducible by hormones such as glucocorticoids, steroids, and thyroid hormones may also be used and include, but are not limited to, the glucorticoid responsive element (GRE) and thyroid hormone responsive element (TRE). These and other examples are described U.S. Pat. No. 7,045,290, which is incorporated by reference, including the references cited therein. Additional vectors containing constitutive or inducible promoters such as α factor, alcohol oxidase, and PGH may be used. Additionally any promoter/enhancer combination (as per the Eukaryotic Promoter Data Base EPDB) could also be used to drive expression of genes. Any convenient appropriate promoters may be selected for the host cell, e.g., *E. coli*. One may also use promoter selection to optimize transcript, and hence, enzyme levels to maximize production while minimizing energy resources.

[00198] Any convenient vectors may be utilized in the subject engineered host cells and methods. Vectors of interest include vectors for use in yeast and other cells. The types of yeast vectors may be broken up into 4 general categories: integrative vectors (YIp), autonomously replicating high copy-number vectors (YEp or 2μ plasmids), autonomously replicating low copy-number vectors (YCp or centromeric plasmids) and vectors for cloning large fragments (YACs). Vector DNA is introduced into prokaryotic or eukaryotic cells via any convenient transformation or transfection techniques. DNA of another source (e.g. PCR-generated double stranded DNA product, or synthesized double stranded or single stranded oligonucleotides) may be used to engineer the yeast by integration into the genome. Any single transformation event may include one or several nucleic acids (vectors, double stranded or single stranded DNA fragments) to genetically modify the host cell.

UTILITY

[00199] The engineered host cells and methods of the invention, e.g., as described above, find use in a variety of applications. Applications of interest include, but are not limited to: research applications and therapeutic applications. Methods of the invention find use in a variety of different applications including any convenient application where the production of enzymes and/or BIAs is of interest.

[00200] The subject engineered host cells and methods find use in a variety of therapeutic applications. Therapeutic applications of interest include those applications in which the preparation of pharmaceutical products that include BIAs is of interest. The engineered host cells described herein produce BIAs of interest and enzymes of interest. Reticuline is a major branch point intermediate of interest in the synthesis of BIAs including engineering efforts to produce end products such as opioid products. The subject host cells may be utilized to produce BIAs of interest from simple and inexpensive starting materials that may find use in the production of BIAs of interest, including reticuline, and BIA end products. As such, the subject host cells find use in the supply of therapeutically active BIAs of interest.

[00201] In some instances, the engineered host cells and methods find use in the production of commercial scale amounts of BIAs thereof where chemical synthesis of these compounds is low yielding and not a viable means for large-scale production. In certain cases, the host cells and methods are utilized in a fermentation facility that would include bioreactors (fermenters) of e.g., 5,000-200,000 liter capacity allowing for rapid production of BIAs of interest thereof for therapeutic products. Such applications may include the industrial-scale production of BIAs of interest from fermentable carbon sources such as cellulose, starch, and free sugars.

[00202] The subject engineered host cells and methods find use in a variety of research applications. The subject host cells and methods may be used to analyze the effects of a variety of enzymes on the biosynthetic pathways of a variety of enzymes and/or BIAs of interest. In addition, the engineered host cells may be engineered to produce enzymes and/or BIAs of interest that find use in testing for bioactivity of interest in as yet unproven therapeutic functions. In some cases, the engineering of host cells to include a variety of heterologous coding sequences that encode for a variety of enzymes elucidates the high yielding biosynthetic pathways towards enzymes and/or BIAs of interest. In certain cases, research applications include the production of enzymes and/or BIAs of interest for therapeutic molecules of interest that may then be further chemically modified or derivatized to desired products or for screening for increased therapeutic activities of interest. In some instances, host cell strains are used to screen for enzyme activities that are of interest in such pathways, which may lead to enzyme discovery via conversion of BIA metabolites produced in these strains.

[00203] The subject engineered host cells and methods may be used as a production platform for plant specialized metabolites. The subject host cells and methods may be used as a platform for drug library development as well as plant enzyme discovery. For example, the subject engineered host cells and methods

may find use in the development of natural product based drug libraries by taking yeast strains producing interesting scaffold molecules, such as protopine, and further functionalizing the compound structure through combinatorial biosynthesis or by chemical means. By producing drug libraries in this way, any potential drug hits are already associated with a production host that is amenable to large-scale culture and production. As another example, these subject engineered host cells and methods may find use in plant enzyme discovery. The subject host cells provide a clean background of defined metabolites to express plant EST libraries to identify new enzyme activities. The subject host cells and methods provide expression methods and culture conditions for the functional expression and increased activity of plant enzymes in yeast.

KITS AND SYSTEMS

[00204] Aspects of the invention further include kits and systems, where the kits and systems may include one or more components employed in methods of the invention, e.g., engineered host cells, starting compounds, heterologous coding sequences, vectors, culture medium, etc., as described herein. In some embodiments, the subject kit includes an engineered host cell (e.g., as described herein), and one or more components selected from the following: starting compounds, a heterologous coding sequence and/or a vector including the same, vectors, growth feedstock, components suitable for use in expression systems (e.g., cells, cloning vectors, multiple cloning sites (MCS), bi-directional promoters, an internal ribosome entry site (IRES), etc.), and a culture medium.

[00205] Any of the components described herein may be provided in the kits, e.g., host cells including one or more modifications, starting compounds, culture medium, etc. A variety of components suitable for use in making and using heterologous coding sequences, cloning vectors and expression systems may find use in the subject kits. Kits may also include tubes, buffers, etc., and instructions for use. The various reagent components of the kits may be present in separate containers, or some or all of them may be pre-combined into a reagent mixture in a single container, as desired.

[00206] Also provided are systems for producing enzymes and/or BIAs of interest, where the systems may include engineered host cells including one or more modifications (e.g., as described herein), starting compounds, culture medium, a fermenter and fermentation equipment, e.g., an apparatus suitable for maintaining growth conditions for the host cells, sampling and monitoring equipment and components, and the like. A variety of components suitable for use in large scale fermentation of yeast cells may find use in the subject systems.

[00207] In some cases, the system includes components for the large scale fermentation of engineered host cells, and the monitoring and purification of enzymes and/or BIA compounds produced by the fermented host cells. In certain embodiments, one or more starting compounds (e.g., as described herein) are added to the system, under conditions by which the engineered host cells in the fermenter produce one or more desired

BIA products of interest. In some instances, the host cells produce a BIA of interest (e.g., as described herein). In certain cases, the BIA products of interest are opioid products, such as thebaine, codeine, neopine, morphine, neomorphine, hydrocodone, oxycodone, hydromorphone, dihydrocodeine, 14-hydroxycodeine, dihydromorphine, or oxymorphone.

[00208] In some cases, the system includes processes for monitoring and or analyzing one or more enzymes and/or BIAs of interest compounds produced by the subject host cells. For example, a LC-MS analysis system as described herein, a chromatography system, or any convenient system where the sample may be analyzed and compared to a standard, e.g., as described herein. The fermentation medium may be monitored at any convenient times before and during fermentation by sampling and analysis. When the conversion of starting compounds to enzymes and/or BIA products of interest is complete, the fermentation may be halted and purification of the BIA products may be done. As such, in some cases, the subject system includes a purification component suitable for purifying the enzymes and/or BIA products of interest from the host cell medium into which it is produced. The purification component may include any convenient means that may be used to purify the enzymes and/or BIA products of interest produced by fermentation, including but not limited to, silica chromatography, reverse-phase chromatography, ion exchange chromatography, HIC chromatography, size exclusion chromatography, liquid extraction, and pH extraction methods. In some cases, the subject system provides for the production and isolation of enzyme and/or BIA fermentation products of interest following the input of one or more starting compounds to the system.

[00209] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the present invention, and are not intended to limit the scope of what the inventors regard as their invention nor are they intended to represent that the experiments below are all or the only experiments performed. Efforts have been made to ensure accuracy with respect to numbers used (e.g. amounts, temperature, etc.), but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, molecular weight is weight average molecular weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

Discussion of Enzyme List

[00210] The host cells may be engineered to include one or more modifications (such as two or more, three or more, four or more, five or more, or even more modifications) that provide for the production of BIAs of interest and/or enzymes of interest. **Table 2** provides a list of exemplary genes that may be acted upon by one or more modifications so as to provide for the production of BIAs of interest and/or enzymes of interest in an engineered host cell.

[00211] Modifications of genes as provided in **Table 2** may be used to produce BIAs of interest from engineered host cells that are supplied with a medium containing the minimal nutrients required for growth.

This minimal medium may contain a carbon source, a nitrogen source, amino acids, vitamins, and salts. For example, modifications of genes as provided in **Table 2** may be used to produce BIAs of interest from engineered host cells that are fed sugar. Additionally, modifications of one or more genes as provided in **Table 2** may be used to augment the biosynthetic processes of host cells that may be engineered for drug production.

[00212] Additionally, the use of these modifications to provide for the production of BIAs of interest and/or enzymes of interest in engineered host cells is not readily apparent from the mere identification of enzymes that may be produced by the genes. In particular, synthetic pathways that have been reconstructed in host cells, such as yeast cells, as described herein comprise a variety of enzymes that do not act together in nature within a single organism. Additionally, some of the enzymes discussed herein do not act for BIA biosynthesis in their natural context. Further, some of the enzymes described herein are not evolved to function in particular host cells, such as yeast cells, and are not evolved to function together. In these cases, it would not be obvious that the enzymes would exhibit sufficient activity in the context of the synthetic BIA pathway in a host cell, such as yeast, to have sufficient flux through the pathway to produce downstream BIA end products.

[00213] For example, plant enzymes are often difficult to functionally express in heterologous microbial hosts, such as yeast. In many cases the enzymes may be misfolded, not correctly localized within the host cell, and/or incorrectly processed. The differences in protein translation and processing between yeast and plants can lead to these enzymes exhibiting substantially reduced to no detectable activities in the yeast host. These challenges arise commonly for endomembrane localized enzymes, such as cytochrome P450s, which are strongly represented in the BIA pathways. Even reduced enzyme activities may pose a substantial challenge to engineering yeast to produce complex BIAs, which requires sufficient activity at each step to ensure high-level accumulation of the desired BIA products.

[00214] Additionally, there are endogenous enzymes/pathways in some host cells, such as yeast, that may act on many of the early precursors in the BIA pathway (i.e., intermediates from tyrosine to norcoclaurine), and thus it may not be readily apparent that there would be sufficient flux through the heterologous pathway to achieve substantial BIA production given these competing endogenous pathways. For example, the Erlich pathway (Hazelwood, et al. 2008. Appl. Environ. Microbiol. 74: 2259-66; Larroy, et al. 2003. Chem. Biol. Interact. 143-144: 229-38; Larroy, et al. 2002. Eur. J. Biochem. 269: 5738-45) in yeast is the main endogenous pathway that would act to convert many of the intermediates in the early BIA pathway to undesired products and divert flux from the synthetic pathway.

[00215] Further, many of the enzymes as discussed herein, and as provided in **Table 2**, may function under very specific regulation strategies, including spatial regulation, in the native plant hosts, which may be lost upon transfer to the heterologous yeast host. In addition, plants present very different biochemical

environments than yeast cells under which the enzymes are evolved to function, including pH, redox state, and substrate, cosubstrate, coenzyme, and cofactor availabilities. Given the differences in biochemical environments and regulatory strategies between the native hosts and the heterologous yeast hosts, it is not obvious that the enzymes would exhibit substantial activities when in the context of the yeast environment and further not obvious that they would work together to direct simple precursors such as sugar to complex BIA compounds. Maintaining the activities of the enzymes in the yeast host is particularly important as many of the pathways have many reaction steps (>10), such that if these steps are not efficient then one would not expect accumulation of desired downstream products.

[00216] In addition, in the native plant hosts, the associated metabolites in these pathways may be localized across different cell and tissue types. In several examples, there are cell types that may be specialized for biosynthesis and cell types that may be synthesized for metabolite accumulation. This type of cell specialization may be lost when expressing the pathways within a heterologous yeast host, and may play an important role in controlling the toxicity of these metabolites on the cells. Thus, it is not obvious that yeast could be successfully engineered to biosynthesize and accumulate these metabolites without being harmed by the toxicity of these compounds.

[00217] As one example, in the native plant hosts, the enzyme BBE is reported to have dynamic subcellular localization. In particular, the enzyme BBE initially starts in the ER and then is sorted to the vacuole (Bird and Facchini. 2001. Planta. 213: 888-97). It has been suggested that the ER-association of BBE in plants (Alcantara, et al. 2005. Plant Physiol. 138: 173-83) provides the optimal basic pH (pH ~8.8) for BBE activity (Ziegler and Facchini. 2008. Annu. Rev. Plant Biol. 59: 735-69). As another example, there is evidence that sanguinarine biosynthesis occurs in specialized vesicles within plant cells (Amann, et al. 1986. Planta. 167: 310-20), but only some of the intermediates accumulate in the vesicles. This may occur so as to sequester them from other enzyme activities and/or toxic effects.

[00218] As another example, the biosynthetic enzymes in the morphinan pathway branch are all localized to the phloem, which is part of the vascular tissue in plants. In the phloem, the pathway enzymes may be further divided between two cell types: the sieve elements common to all plants, and the laticifer which is a specialized cell type present only in certain plants which make specialized secondary metabolites. The upstream enzymes (i.e., from NCS through to SalAT) are predominantly in the sieve elements, and the downstream enzymes (i.e., T6ODM, COR, CODM) are mostly in the laticifer (Onoyovwe, et al. 2013. Plant Cell. 25: 4110-22). Additionally, it was discovered that the final steps in the noscapine biosynthetic pathway take place in the laticifer (Chen and Facchini. 2014. Plant J. 77: 173-84). This compartmentalization is thought to be highly important for regulating biosynthesis by isolating or trafficking intermediates, providing optimal pH, enhancing supply of cofactors, although the nature of the poppy laticifer microenvironment is still under investigation (Ziegler and Facchini. 2008. Annu. Rev. Plant Biol. 59: 735-69). Further, it is predicted that several of the enzymes may function as multi-enzyme complexes or metabolic channels

common to plant secondary metabolism (Kempe, et al. 2009. Phytochemistry. 70: 579-89; Allen, et al. 2004. Nat. Biotechnol. 22: 1559-66). When biosynthetic enzymes are combined from different hosts and/or expressed recombinantly in a heterologous yeast cell it is not clear that these complexes or channels will form as they would in the native host. In an additional example, in *Coptis japonica*, berberine is biosynthesized in root tissues and then accumulated within the rhizome via the action of specialized ATP-binding cassette transport proteins (Shitan, et al. 2013. Phytochemistry. 91: 109-16). In opium poppy, morphinan alkaloids are accumulated within the latex (cytoplasm of laticifer cells) (Martin, et al. 1967. Biochemistry. 6: 2355-63).

[00219] Further, even without these considerations, it is also the case that the plant enzymes for several of the steps in the pathways described herein have not yet been characterized. For example, the conversion of tyrosine to the early benzylisoquinoline alkaloid scaffold norcoclaurine has not yet been characterized. Additionally, the conversion of *(S)*-reticuline to *(R)*-reticuline has only recently been characterized as described herein. Thus, for several of the steps in the pathways described herein, alternative biosynthetic scheme were produced by bringing together enzyme activities that do not normally occur together in nature for the biosynthesis of BIAs or identifying new enzyme activities from genome sequence information to use in the reconstructed pathways.

[00220] For example, the two-step conversion of tyrosine to dopamine may be achieved by combining at least 5 mammalian enzymes and 1 bacterial enzyme, which do not naturally occur together and were not evolved to function in the context of this pathway or with plant enzymes. In these instances, it may not be obvious to utilize these enzymes for the biosynthesis of compounds they were not evolved for in nature and that they would function effectively in the context of a heterologous microbial host and this pathway. As another example, the enzyme responsible for the conversion of *(S)*-reticuline to *(R)*-reticuline was unknown. Novel enzymes as discussed herein may perform this epimerization reaction in yeast and in the context of the synthetic BIA pathway. As this represents new enzyme discovery, it would not have been obvious to use this enzyme in the context of this pathway for the synthesis of those BIA compounds.

[00221] Examples of the genes that are the object of modifications so as to produce BIAs of interest and/or enzymes of interest are discussed below. Additionally, the genes are discussed in the context of a series of Figures that illustrate pathways that are used in generating BIAs of interest and/or enzymes of interest.

[00222] [TLK1] In some examples, the engineered host cell may modify the expression of the enzyme transketolase. Transketolase is encoded by the TKL1 gene. In examples, transketolase catalyzes the reaction of fructose-6-phosphate + glyceraldehyde-3-phosphate ↔ xylulose-5-phosphate + erythrose-4-phosphate, as referenced in **FIG. 2**. An engineered host cell may be modified to include constitutive overexpression of the TKL1 gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the TKL1 gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the TKL1

gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the TKL1 gene within the engineered host cell. The TKL1 gene may be derived from *Saccharomyces cerevisiae* or another species. In some examples, the TKL1 gene may be 100% similar to the naturally occurring gene.

[00223][ZWF1] In some examples, the engineered host cell may modify the expression of the enzyme glucose-6-phosphate dehydrogenase. Glucose-6-phosphate dehydrogenase is encoded by the ZWF1 gene. In examples, glucose-6-phosphate dehydrogenase catalyzes the reaction of glucose-6-phosphate → 6-phosphogluconolactone, as referenced in **FIG. 2**. An engineered host cell may be modified to delete the coding region of the ZWF1 gene in the engineered host cell. Alternatively, the engineered host cell may be modified to disable the functionality of the ZWF1 gene, such as by introducing an inactivating mutation.

[00224][ARO4] In some examples, the engineered host cell may modify the expression of the enzyme 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) synthase. DAHP synthase is encoded by the ARO4 gene. In examples, DAHP synthase catalyzes the reaction of erythrose-4-phosphate + phosphoenolpyruvic acid → DAHP, as referenced in **FIG. 2**. An engineered host cell may modify the ARO4 gene to incorporate one or more feedback inhibition alleviating mutations. In particular, a feedback inhibition alleviating mutation (e.g., $ARO4^{FBR}$) may be incorporated as a directed mutation to a native ARO4 gene at the original locus; as an additional copy introduced as a genetic integration at a separate locus; or as an additional copy on an episomal vector such as a 2-μm or centromeric plasmid. The identifier "FBR" in the mutation $ARO4^{FBR}$ refers to feedback resistant mutants and mutations. The feedback inhibited copy of the DAHP synthase enzyme may be under a native yeast transcriptional regulation, such as when the engineered host cell is a yeast cell. Alternatively, the feedback inhibited copy of the DAHP synthase enzyme may be introduced to the engineered host cell with engineered constitutive or dynamic regulation of protein expression by placing it under the control of a synthetic promoter. In some cases, the ARO4 gene may be derived from *Saccharomyces cerevisiae*. In some cases, the ARO4 gene may be 100% similar to the naturally occurring gene. Examples of modifications to the ARO4 gene include a feedback inhibition resistant mutation, K229L, or Q166K.

[00225][ARO7] In some examples, the engineered host cell may modify the expression of the enzyme chorismate mutase. Chorismate mutase is encoded by the ARO7 gene. In examples, chorismate mutase catalyzes the reaction of chorismate → prephenate, as referenced in **FIG. 2**. An engineered host cell may modify the ARO7 gene to incorporate one or more feedback inhibition alleviating mutations. In particular, a feedback inhibition alleviating mutation (e.g., $ARO7^{FBR}$) may be incorporated as a directed mutation to a native ARO7 gene at the original locus; as an additional copy introduced as a genetic integration at a separate locus; or as an additional copy on an episomal vector such as a 2-μm or centromeric plasmid. The identifier "FBR" in the mutation $ARO7^{FBR}$ refers to feedback resistant mutants and mutations. The feedback inhibited copy of the chorismate mutase enzyme may be under a native yeast transcriptional regulation, such as when

the engineered host cell is a yeast cell. Alternatively, the feedback inhibited copy of the chorismate mutase enzyme may be introduced to the engineered host cell with engineered constitutive or dynamic regulation of protein expression by placing it under the control of a synthetic promoter. In some cases, the ARO7 gene may be derived from *Saccharomyces cerevisiae*. In some cases, the ARO7 gene may be 100% similar to the naturally occurring gene. Examples of modifications to the ARO7 gene include a feedback inhibition resistant mutation or T226I.

[00226][ARO10] In some examples, the engineered host cell may modify the expression of the enzyme phenylpyruvate decarboxylase. Phenylpyruvate decarboxylase is encoded by the ARO10 gene. In examples, phenylpyruvate decarboxylase catalyzes the reaction of hydroxyphenylpyruvate → 4-hydroxyphenylacetate (4HPA), as referenced in **FIG. 2**. An engineered host cell may be modified to include constitutive overexpression of the ARO10 gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the ARO10 gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the ARO10 gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the ARO10 gene within the engineered host cell. The ARO10 gene may be derived from *Saccharomyces cerevisiae* or another species. In some examples, the ARO10 gene may be 100% similar to the naturally occurring gene.

[00227][ADH2-7, SFA1] In some examples, the engineered host cell may modify the expression of alcohol dehydrogenase enzymes. Alcohol dehydrogenase enzymes may be encoded by one or more of the ADH2, ADH3, ADH4, ADH5, ADH6, ADH7, and SFA1 genes. In examples, alcohol dehydrogenase catalyzes the reaction of 4HPA → tyrosol. An engineered host cell may be modified to delete the coding region of one or more of the ADH2, ADH3, ADH4, ADH5, ADH6, ADH7, and SFA1 genes in the engineered host cell. Alternatively, the engineered host cell may be modified to disable the functionality of one or more of the ADH2, ADH3, ADH4, ADH5, ADH6, ADH7, and SFA1 genes, such as by introducing an inactivating mutation.

[00228][ALD2-6] In some examples, the engineered host cell may modify the expression of aldehyde oxidase enzymes. Aldehyde oxidase enzymes may be encoded by one or more of the ALD2, ALD3, ALD4, ALD5, and ALD6 genes. In examples, aldehyde oxidase catalyzes the reaction of 4HPA → hydroxyphenylacetic acid. An engineered host cell may be modified to delete the coding region of one or more of the ALD2, ALD3, ALD4, ALD5, and ALD6 genes in the engineered host cell. Alternatively, the engineered host cell may be modified to disable the functionality of one or more of the ALD2, ALD3, ALD4, ALD5, and ALD6 genes, such as by introducing an inactivating mutation.

[00229][ARO9] In some examples, the engineered host cell may modify the expression of the enzyme aromatic aminotransferase. Aromatic aminotransferase is encoded by the ARO9 gene. In examples, aromatic

aminotransferase catalyzes the reaction of hydroxyphenylpyruvate + glutamate → tyrosine + alpha-ketogluterate, as referenced in **FIG. 2**. An engineered host cell may be modified to include constitutive overexpression of the ARO9 gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the ARO9 gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the ARO9 gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the ARO9 gene within the engineered host cell. The ARO9 gene may be derived from *Saccharomyces cerevisiae* or another species. In some examples, the ARO9 gene may be 100% similar to the naturally occurring gene.

[00230][TYR] In some examples, the engineered host cell may modify the expression of the enzyme tyrosinase. Tyrosinase is encoded by the TYR gene. In examples, tyrosinase catalyzes the reaction of tyrosine → L-DOPA, as referenced in **FIG. 2**. In other examples, tyrosinase catalyzes the reaction of L-DOPA → dopaquinone. An engineered host cell may be modified to include constitutive expression of the TYR gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the TYR gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the TYR gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the TYR gene within the engineered host cell. The TYR gene may be derived from *Ralstonia solanacearum, Agaricus bisporus*, or another species. In some examples, the TYR gene may be 100% similar to the naturally occurring gene.

[00231] [TyrH] In some examples, the engineered host cell may modify the expression of the enzyme tyrosine hydroxylase. Tyrosine hydroxylase is encoded by the TyrH gene. In examples, tyrosine hydroxylase catalyzes the reaction of tyrosine → L-DOPA, as referenced in **FIGs. 2** and **5**. An engineered host cell may be modified to include constitutive expression of the TyrH gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the TyrH gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the TyrH gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the TyrH gene within the engineered host cell. The TyrH gene may be derived from *Homo sapiens, Rattus norvegicus, Mus musculus,* or another species. In some examples, the TyrH gene may be 100% similar to the naturally occurring gene.

[00232][DODC] In some examples, the engineered host cell may modify the expression of the enzyme L-DOPA decarboxylase. L-DOPA decarboxylase is encoded by the DODC gene. In examples, L-DOPA decarboxylase catalyzes the reaction of L-DOPA → dopamine, as referenced in **FIGs. 2** and **5**. An engineered host cell may be modified to include constitutive expression of the DODC gene in the engineered

host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the DODC gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the DODC gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the DODC gene within the engineered host cell. The DODC gene may be derived from *Pseudomonas putida, Rattus norvegicus,* or another species. In some examples, the DODC gene may be 100% similar to the naturally occurring gene.

[00233][TYDC] In some examples, the engineered host cell may modify the expression of the enzyme tyrosine/DOPA decarboxylase. Tyrosine/DOPA decarboxylase is encoded by the TYDC gene. In examples, tyrosine/DOPA decarboxylase catalyzes the reaction of L-DOPA → dopamine, as referenced in **FIG. 2**. An engineered host cell may be modified to include constitutive expression of the TYDC gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the TYDC gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the TYDC gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the TYDC gene within the engineered host cell. The TYDC gene may be derived from *Papaver somniferum* or another species. In some examples, the TYDC gene may be 100% similar to the naturally occurring gene.

[00234][MAO] In some examples, the engineered host cell may modify the expression of the enzyme monoamine oxidase. Monoamine oxidase is encoded by the MAO gene. In examples, monoamine oxidase catalyzes the reaction of dopamine → 3,4-DHPA, as referenced in **FIG. 2**. An engineered host cell may be modified to include constitutive expression of the MAO gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the MAO gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the MAO gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the MAO gene within the engineered host cell. In some cases, the MAO gene may be codon optimized for expression in *Saccharomyces cerevisiae*. The MAO gene may be derived from *Escherichia coli, Homo sapiens, Micrococcus luteus,* or another species. In some examples, the MAO gene may be 77% similar to the naturally occurring gene.

[00235][NCS] In some examples, the engineered host cell may modify the expression of the enzyme norcoclaurine synthase. Norcoclaurine synthase is encoded by the NCS gene. In examples, norcoclaurine synthase catalyzes the reaction of 4HPA + dopamine → (*S*)-norcoclaurine, as referenced in **FIG. 5**. In particular, **FIG. 5** illustrates a biosynthetic scheme for conversion of L-tyrosine to reticuline via norcoclaurine, in accordance with embodiments of the invention. **FIG. 5** provides the use of the enzymes

TyrH, tyrosine hydroxylase; DODC, DOPA decarboxylase; NCS, norcoclaurine synthase, as discussed herein; 6OMT, 6-*O*-methyltransferase; CNMT, coclaurine *N*-methyltransferase; CYP80B1, cytochrome P450 80B1; CPR, cytochrome P450 NADPH reductase; 4'OMT, 3'hydroxy-*N*-methylcoclaurine 4'-*O*-methyltransferase. L-DOPA, L-3,4-dihydroxyphenylalanine; and 4-HPA, 4-hydroxyphenylacetylaldehyde. Of the enzymes that are illustrated in **FIG. 5**, 4-HPA and L-tyrosine are naturally synthesized in yeast. All other metabolites shown are not naturally produced in yeast. Additionally, although TyrH is depicted as catalyzing the conversion of L-tyrosine to L-DOPA, other enzymes may also be used to perform this step as described in the specification. For example, tyrosinases may also be used to perform the conversion of L-tyrosine to L-DOPA. In addition, other enzymes such as cytochrome P450 oxidases may also be used to perform the conversion of L-tyrosine to L-DOPA. Such enzymes may exhibit oxidase activity on related BIA precursor compounds including L-DOPA and L-tyrosine.

[00236] Additionally, norcoclaurine synthase catalyzes the reaction of 3,4-DHPA + dopamine → (*S*)-norlaudanosoline, as referenced in **FIG. 6**. In particular, **FIG. 6** illustrates a biosynthetic scheme for conversion of L-tyrosine to reticuline via norlaudanosoline, in accordance with embodiments of the invention. **FIG. 6** provides the use of the enzymes TyrH, tyrosine hydroxylase; DODC, DOPA decarboxylase; maoA, monoamine oxidase; NCS, norcoclaurine synthase; 6OMT, 6-*O*-methyltransferase; CNMT, coclaurine *N*-methyltransferase; 4'OMT, 3'hydroxy-*N*-methylcoclaurine 4'-*O*-methyltransferase. L-DOPA, L-3,4-dihydroxyphenylalanine; and 3,4-DHPA, 3,4-dihydroxyphenylacetaldehyde. Of the enzymes that are illustrated in **FIG. 6**, L-tyrosine is naturally synthesized in yeast. Other metabolites that are shown in **FIG. 6** are not naturally produced in yeast.

[00237] An engineered host cell may be modified to include constitutive expression of the NCS gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the NCS gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the NCS gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the NCS gene within the engineered host cell. Additionally, the norcoclaurine synthase may have an N-terminal truncation. In some cases, the NCS gene may be codon optimized for expression in *Saccharomyces cerevisiae*. The NCS gene may be derived from *Coptis japonica, Papaver somniferum, Papver bracteatum, Thalicitum flavum, Corydalis saxicola,* or another species. In some examples, the NCS gene may be 80% similar to the naturally occurring gene.

[00238] [6OMT] In some examples, the engineered host cell may modify the expression of the enzyme norcoclaurine 6-*O*-methyltransferase. Norcoclaurine 6-*O*-methyltransferase is encoded by the 6OMT gene. In some examples, norcoclaurine 6-O-methyltransferase catalyzes the reaction of norcoclaurine → coclaurine, as referenced in **FIG. 5**. In other examples, norcoclaurine 6-*O*-methyltransferase catalyzes the reaction of norlaudanosoline → 3'hydroxycoclaurine, as well as other reactions detailed herein, such as those provided in

FIG. 6. Additionally, the engineered host cell may be modified to include constitutive expression of the 6OMT gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the 6OMT gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the 6OMT gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the 6OMT gene within the engineered host cell. The 6OMT gene may be derived from *P. somniferum, T. flavum, Coptis japonica*, or another species. In some examples, the 6OMT gene may be 100% similar to the naturally occurring gene.

[00239][CNMT] In some examples, the engineered host cell may modify the expression of the enzyme coclaurine-*N*-methyltransferase. Coclaurine-*N*-methyltransferase is encoded by the CNMT gene. In some examples, coclaurine-*N*-methyltransferase catalyzes the reaction of coclaurine → *N*-methylcoclaurine, as referenced in **FIG. 5**. In other examples, the coclaurine-*N*-methyltransferase enzyme may catalyze the reaction of 3'hydroxycoclaurine → 3'hydroxy-*N*-methylcoclaurine. In other examples, coclaurine-*N*-methyltransferase may catalyze other reactions detailed herein, such as those provided in **FIG. 6**. Additionally, the engineered host cell may be modified to include constitutive expression of the CNMT gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the CNMT gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the CNMT gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the CNMT gene within the engineered host cell. The CNMT gene may be derived from *P. somniferum, T. flavum, Coptis japonica*, or another species. In some examples, the CNMT gene may be 100% similar to the naturally occurring gene.

[00240][4'OMT] In some examples, the engineered host cell may modify the expression of the enzyme 4'-*O*-methyltransferase. 4'-*O*-methyltransferase is encoded by the 4'OMT gene. In some examples, 4'-*O*-methyltransferase catalyzes the reaction of 3'-hydroxy-*N*-methylcoclaurine → reticuline, as referenced in **FIG. 5**. In other examples, 4'-*O*-methyltransferase catalyzes other reactions detailed herein, such as those provided in **FIG. 6**. Additionally, the engineered host cell may be modified to include constitutive expression of the 4'OMT gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the 4'OMT gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the 4'OMT gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the 4'OMT gene within the engineered host cell. The 4'OMT gene may be derived from *P. somniferum, T. flavum, Coptis japonica*, or another species. In some examples, the 4'OMT gene may be 100% similar to the naturally occurring gene.

[00241][CYP80B1] In some examples, the engineered host cell may modify the expression of the enzyme cytochrome P450 80B1. Cytochrome P450 80B1 is encoded by the CYP80B1 gene. In examples, cytochrome P450 80B1 catalyzes the reaction of *N*-methylcoclaurine → 3'-hydroxy-*N*-methylcoclaurine, as referenced in **FIG. 5**. An engineered host cell may be modified to include constitutive expression of the cytochrome P450 80B1 gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the cytochrome P450 80B1 gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the cytochrome P450 80B1 gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the cytochrome P450 80B1 gene within the engineered host cell. In some cases, the CYP80B1 gene may be codon optimized for expression in *Saccharomyces cerevisiae*. The cytochrome P450 80B1 gene may be derived from *P. somniferum, E. californica, T. flavum,* or another species. In some examples, the P450 80B1 gene may be 77% similar to the naturally occurring gene.

[00242][FOL2] In some examples, the engineered host cell may modify the expression of the enzyme GTP cyclohydrolase. GTP cyclohydrolase is encoded by the FOL2 gene. In some examples, GTP cyclohydrolase catalyzes the reaction of GTP → dihydroneopterin triphosphate, as referenced in **FIG. 1**. The engineered host cell may be modified to include constitutive overexpression of the FOL2 gene in the engineered host cell. The engineered host cell may also be modified to include native regulation. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the FOL2 gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the FOL2 gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the FOL2 gene within the engineered host cell. The FOL2 gene may be derived from *Saccharomyces cerevisiae, Homo sapiens, Mus musculus*, or another species. In some examples, the FOL2 gene may be 100% similar to the naturally occurring gene.

[00243][PTPS] In some examples, the engineered host cell may modify the expression of the enzyme 6-pyruvoyl tetrahydrobiopterin (PTP) synthase. Pyruvoyl tetrahydrobiopterin synthase is encoded by the PTPS gene. In some examples, 6-pyruvoyl tetrahydrobiopterin synthase catalyzes the reaction of dihydroneopterin triphosphate → PTP, as referenced in **FIG. 1**. The engineered host cell may be modified to include constitutive expression of the PTPS gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the PTPS gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the PTPS gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the PTPS gene within the engineered host cell. In some cases, the PTPS gene may be codon optimized for expression in

Saccharomyces cerevisiae. The PTPS gene may be derived from *Rattus norvegicus, Homo sapiens, Mus musculus*, or another species. In some examples, the PTPS gene may be 80% similar to the naturally occurring gene.

[00244][SepR] In some examples, the engineered host cell may modify the expression of the enzyme sepiapterin reductase. Sepiapterin reductase is encoded by the SepR gene. In some examples, sepiapterin reductase catalyzes the reaction of PTP → BH_4, as referenced in **FIG. 1**. The engineered host cell may be modified to include constitutive expression of the SepR gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the SepR gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the SepR gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the SepR gene within the engineered host cell. In some cases, the SepR gene may be codon optimized for expression in *Saccharomyces cerevisiae*. The SepR gene may be derived from *Rattus norvegicus, Homo sapiens, Mus musculus*, or another species. In some examples, the SepR gene may be 72% similar to the naturally occurring gene.

[00245][PCD] In some examples, the engineered host cell may modify the expression of the enzyme 4a-hydroxytetrahydrobiopterin (pterin-4α-carbinolamine) dehydratase. 4a-hydroxytetrahydrobiopterin dehydratase is encoded by the PCD gene. In some examples, 4a-hydroxytetrahydrobiopterin dehydratase catalyzes the reaction of 4a-hydroxytetrahydrobiopterin → H_2O + quinonoid dihydropteridine, as referenced in **FIG. 1**. The engineered host cell may be modified to include constitutive expression of the PCD gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the PCD gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the PCD gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the PCD gene within the engineered host cell. In some cases, the PCD gene may be codon optimized for expression in *Saccharomyces cerevisiae*. The PCD gene may be derived from *Rattus norvegicus, Homo sapiens, Mus musculus*, or another species. In some examples, the PCD gene may be 79% similar to the naturally occurring gene.

[00246][QDHPR] In some examples, the engineered host cell may modify the expression of the enzyme quinonoid dihydropteridine reductase. Quinonoid dihydropteridine reductase is encoded by the QDHPR gene. In some examples, quinonoid dihydropteridine reductase catalyzes the reaction of quinonoid dihydropteridine → BH_4, as referenced in **FIG. 1**. The engineered host cell may be modified to include constitutive expression of the QDHPR gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the QDHPR gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the

QDHPR gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the QDHPR gene within the engineered host cell. In some cases, the QDHPR gene may be codon optimized for expression in *Saccharomyces cerevisiae*. The QDHPR gene may be derived from *Rattus norvegicus, Homo sapiens, Mus musculus*, or another species. In some examples, the QDHPR gene may be 75% similar to the naturally occurring gene.

[00247][DHFR] In some examples, the engineered host cell may modify the expression of the enzyme dihydrofolate reductase. Dihydrofolate reductase is encoded by the DHFR gene. In some examples, dihydrofolate reductase catalyzes the reaction of 7,8-dihydrobiopterin (BH_2) → 5,6,7,8-tetrahydrobiopterin (BH_4), as referenced in **FIG. 1**. This reaction may be useful in recovering BH_4 as a co-substrate for the converstion of tyrosine to L-DOPA, as illustrated in **FIG. 5**. The engineered host cell may be modified to include constitutive expression of the DHFR gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the DHFR gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the DHFR gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the DHFR gene within the engineered host cell. In some cases, the DHFR gene may be codon optimized for expression in *Saccharomyces cerevisiae*. The DHFR gene may be derived from *Rattus norvegicus, Homo sapiens*, or another species. In some examples, the DHFR gene may be 77% similar to the naturally occurring gene.

[00248][CYP-COR] As discussed above with regard to epimerizing 1-BIAs, the engineered host cell may modify the expression of a BIA epimerase. The BIA epimerase is encoded by the CYP-COR gene (e.g., CYP82Y2-COR gene). In some examples, CYP-COR may also be referred to as DRS-DRR. In some examples, the BIA epimerase catalyzes the conversion of (*S*)-1-BIA → (*R*)-1-BIA, as referenced in **FIG. 7**. In particular, **FIG. 7** illustrates a biosynthetic scheme for conversion of L-tyrosine to morphinan alkaloids, in accordance with embodiments of the invention. **FIG. 7** provides the use of the enzymes CPR, cytochrome P450 reductase; CYP-COR, cytochrome P450 CYP82Y1-like codeinone reductase-like fusion; SalSyn, salutaridine synthase; SalR, salutaridine reductase; SalAT, salutaridinol 7-*O*-acetyltransferase; T6ODM, thebaine 6-*O*-demethylase; COR, codeinone reductase; and CODM, codeine-*O*-demethylase.

[00249] The engineered host cell may be modified to include constitutive expression of the CYP-COR gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the CYP-COR gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the CYP-COR gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the CYP-COR gene within the engineered host cell. The CYP-COR gene may be derived from *Papaver bracteatum, Papaver somniferum, Papaver setigerum,*

Chelidonium majus, or another species. In some examples, the CYP-COR gene may be 77% similar to the naturally occurring gene.

[00250][CPR] In some examples, the engineered host cell may modify the expression of the enzyme cytochrome P450 reductase. The cytochrome P450 reductase is encoded by the CPR gene. In some examples, the cytochrome P450 reductase catalyzes the reaction of *(R)*-reticuline → salutaridine, as referenced in **FIG. 7**. Additionally, the cytochrome P450 reductase catalyzes other reactions such as those described in FIGs. throughout the application. The engineered host cell may be modified to include constitutive expression of the CPR gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the CPR gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the CPR gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the CPR gene within the engineered host cell. The CPR gene may be derived from *E. californica, P. somniferum, H. sapiens, S. cerevisiae, A. thaliana,* or another species. In some examples, the CPR gene may be 100% similar to the naturally occurring gene.

[00251][SalSyn] In some examples, the engineered host cell may modify the expression of the enzyme salutaridine synthase. The salutaridine synthase is encoded by the SalSyn gene. In some examples, the salutaridine synthase catalyzes the reaction of *(R)*-reticuline → salutaridine, as referenced in **FIG. 7**. The engineered host cell may be modified to include constitutive expression of the SalSyn gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the SalSyn gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the SalSyn gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the SalSyn gene within the engineered host cell. In some cases, the SalSyn gene may be codon optimized for expression in *Saccharomyces cerevisiae*. In some examples the SalSyn may be modified at the N-terminus. The SalSyn gene may be derived from *Papaver somniferum, Papaver spp, Chelidonium majus*, or another species. In some examples, the SalSyn gene may be 78% similar to the naturally occurring gene.

[00252][SalR] In some examples, the engineered host cell may modify the expression of the enzyme salutaridine reductase. Salutaridine reductase is encoded by the SalR gene. In some examples, salutaridine reductase reversibly catalyzes the reaction of salutaridinol → salutaridine, as referenced in **FIG. 7**. The engineered host cell may be modified to include constitutive expression of the SalR gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the SalR gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the SalR gene. Additionally or alternatively,

the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the SalR gene within the engineered host cell. In some cases, the SalR gene may be codon optimized for expression in *Saccharomyces cerevisiae*. The SalR gene may be derived from *Papaver somniferum, Papaver bracteatum, Papaver spp., Chelidonium majus*, or another species. In some examples, the SalR gene may be 80-100% similar to the naturally occurring gene.

[00253] [SalAT] In some examples, the engineered host cell may modify the expression of the enzyme acetyl-CoA:salutaridinol 7-*O*-acetyltransferase. Acetyl-CoA:salutaridinol 7-*O*-acetyltransferase is encoded by the SalAT gene. In some examples, acetyl-CoA:salutaridinol 7-*O*-acetyltransferase catalyzes the reaction of acetyl-CoA + salutaridinol → CoA + 7-*O*-acetylsalutaridinol, as referenced in **FIG. 7**. The engineered host cell may be modified to include constitutive expression of the SalAT gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the SalAT gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the SalAT gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the SalAT gene within the engineered host cell. In some cases, the SalAT gene may be codon optimized for expression in *Saccharomyces cerevisiae*. The SalAT gene may be derived from *Papaver somniferum, Papaver bracteatum, Papaver orientale, Papaver spp.*, or another species. In some examples, the SalAT gene may be 77-80% similar to the naturally occurring gene.

[00254] [T6ODM] In some examples, the engineered host cell may modify the expression of the enzyme thebaine 6-*O*-demethylase. Thebaine 6-*O* demethylase is encoded by the T6ODM gene. In some examples, thebaine 6-*O*-demethylase catalyzes the reaction of thebaine→ neopinone, as referenced in **FIG. 7**. Once the neopinone has been produced, the neopinone may be converted to codeinone. The conversion of neopinone → codeinone may occur spontaneously. Alternatively, the conversion of neopinone → codeinone may occur as a result of a catalyzed reaction. In other examples, the T6ODM enzyme may catalyze the *O*-demethylation of substrates other than thebaine. For example, T6ODM may *O*-demethylate oripavine to produce morphinone. Alternatively, T6ODM may catalyze the *O*-demethylation of BIAs within the 1-benzylisoquinoline, protoberberine, or protopine classes such as papaverine, canadine, and allocryptopine, respectively. The engineered host cell may be modified to include constitutive expression of the T6ODM gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the T6ODM gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the T6ODM gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the T6ODM gene within the engineered host cell. In some cases, the T6ODM gene may be codon optimized for expression in *Saccharomyces cerevisiae*. The T6ODM

gene may be derived from *Papaver somniferum,* or another species. In some examples, the T6ODM gene may be 76.2% similar to the naturally occurring gene.

[00255][COR] In some examples, the engineered host cell may modify the expression of the enzyme codeinone reductase. Codeinone reductase is encoded by the COR gene. In some examples, codeinone reductase catalyzes the reaction of codeinone to codeine, as referenced in **FIG. 7**. In some cases, codeinone reductase can catalyze the reaction of neopinone to neopine. In other examples, COR can catalyze the reduction of other morphinans including hydrocodone → dihydrocodeine, 14-hydroxycodeinone → 14-hydroxycodeine, and hydromorphone → dihydromorphine. The engineered host cell may be modified to include constitutive expression of the COR gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the COR gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the COR gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the COR gene within the engineered host cell. In some cases, the COR gene may be codon optimized for expression in *Saccharomyces cerevisiae*. Additionally or alternatively, the COR gene may be modified with the addition of targeting sequences for mitochondria, vacuole, endoplasmic reticulum, or a combination thereof. The COR gene may be derived from *Papaver somniferum,* or another species. In some examples, the COR gene may be 76-78% similar to the naturally occurring gene. In examples, the COR gene may be 76.8%, 77.0%, 77.3%, or 77.7% similar to the naturally occurring gene.

[00256][CODM] In some examples, the engineered host cell may modify the expression of the enzyme codeine *O*-demethylase. Codeine *O*-demethylase is encoded by the CODM gene. In some examples, codeine *O*-demethylase catalyzes the reaction of codeine to morphine, as referenced in **FIG. 7**. Codeine *O*-demethylase can also catalyze the reaction of neopine to neomorphine. Codeine *O*-demethylase can also catalyze the reaction of thebaine to oripavine. In other examples, CODM may catalyze the *O*-demethylation of BIAs within the 1-benzylisoquinoline, aporphine, and protoberberine classes such as reticuline, isocorydine, and scoulerine, respectively. In other examples, the CODM enzyme may catalyze an *O*,*O*-demethylenation reaction to cleave the methylenedioxy bridge structures in protopines. The engineered host cell may be modified to include constitutive expression of the CODM gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the CODM gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the CODM gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the CODM gene within the engineered host cell. In some cases, the CODM gene may be codon optimized for expression in *Saccharomyces cerevisiae*. Additionally or alternatively, the CODM gene may be modified with the addition of targeting sequences for mitochondria. The CODM gene

may be derived from *Papaver somniferum, Papaver spp.*, or another species. In some examples, the CODM gene may be 75% similar to the naturally occurring gene. In examples, the CODM gene may be 75.2% similar to the naturally occurring gene.

[00257][BBE] In some examples, the engineered host cell may modify the expression of the enzyme berberine bridge enzyme. The berberine bridge enzyme is encoded by the BBE gene. In some examples, berberine bridge enzyme catalyzes the reaction of *(S)*-reticuline → *(S)*-scoulerine, as referenced in **FIG. 8**. **FIG. 8** illustrates a biosynthetic scheme for conversion of L-tyrosine to protoberberine alkaloids, in accordance with embodiments of the invention. In particular, **FIG. 8** provides the use of the enzymes BBE, berberine bridge enzyme; S9OMT, scoulerine 9-*O*-methyltransferase; CAS, canadine synthase; CPR, cytochrome P450 reductase; and STOX, tetrahydroprotoberberine oxidase. The engineered host cell may be modified to include constitutive expression of the BBE gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the BBE gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the BBE gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the BBE gene within the engineered host cell. The BBE gene may be derived from *Papaver somniferum, Argemone mexicana, Eschscholzia californica, Berberis stolonifera, Thalictrum flavum subsp. glaucum, Coptis japonica,Papaver spp.*, or another species. In some examples, the BBE gene may be 99% similar to the naturally occurring gene.

[00258][S9OMT] In some examples, the engineered host cell may modify the expression of the enzyme *S*-adenosyl-L-methionine:*(S)*-scoulerine 9-*O*-methyltransferase. *S*-adenosyl-L-methionine:*(S)*-scoulerine 9-*O*-methyltransferase is encoded by the S9OMT gene. In some examples, *S*-adenosyl-L-methionine:*(S)*-scoulerine 9-*O*-methyltransferase catalyzes the reaction of *S*-adenosyl-L-methionine + *(S)*-scoulerine → *S*-adenosyl-L-homocysteine + *(S)*-tetrahydrocolumbamine, as referenced in **FIG. 8**. The engineered host cell may be modified to include constitutive expression of the S9OMT gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the S9OMT gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the S9OMT gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the S9OMT gene within the engineered host cell. In some cases, the S9OMT gene may be codon optimized for expression in *Saccharomyces cerevisiae*. The S9OMT gene may be derived from *Thalictrum flavum subsp. glaucum, Coptis japonica, Coptis chinensis, Papaver somniferum, Thalictrum spp., Coptis spp., Papaver spp.,* or another species. In some examples, the S9OMT gene may be 100% similar to the naturally occurring gene. In examples, the S9OMT gene may be 80% similar to the naturally occurring gene.

[00259][CAS] In some examples, the engineered host cell may modify the expression of the enzyme *(S)*-canadine synthase. *(S)*-canadine synthase is encoded by the CAS gene. In some examples, *(S)*-canadine synthase catalyzes the reaction of *(S)*-tetrahydrocolumbamine → *(S)*-canadine, as referenced in **FIG. 8**. The engineered host cell may be modified to express the CAS gene in the engineered host cell. The engineered host cell may be modified to include constitutive expression of the CAS gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the CAS gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the CAS gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the CAS gene within the engineered host cell. The CAS gene may be derived from *Thalictrum flavum subsp. glaucum, Coptis japonica, Thalictrum spp., Coptis spp.,* or another species. In some examples, the CAS gene may be 100% similar to the naturally occurring gene.

[00260][STOX] In some examples, the engineered host cell may modify the expression of the enzyme *(S)*-tetrahydroprotoberberine oxidase. *(S)*-tetrahydroprotoberberine oxidase is encoded by the STOX gene. In some examples, *(S)*-tetrahydroprotoberberine oxidase catalyzes the reaction of *(S)*-tetrahydroberberine + 2 O_2 → berberine + 2 H_2O_2, as referenced in **FIG. 8**. The engineered host cell may be modified to include constitutive expression of the STOX gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the STOX gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the STOX gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the STOX gene within the engineered host cell. In some examples the STOX may be modified at the N-terminus. In some cases, the STOX gene may be codon optimized for expression in *Saccharomyces cerevisiae*. The STOX gene may be derived from *Berberis wilsonae, Coptis japonica, Berberis spp., Coptis spp.,* or another species. In some examples, the STOX gene may be 78% similar to the naturally occurring gene.

[00261][TNMT] In some examples, the engineered host cell may modify the expression of the enzyme tetrahydroprotoberberine-*N*-methyltransferase. Tetrahydroprotoberberine-*N*-methyltransferase is encoded by the TNMT gene. In some examples, tetrahydroprotoberberine-*N*-methyltransferase catalyzes the reaction of canadine → *N*-methylcanadine, as referenced in **FIG. 9**. **FIG. 9** illustrates a biosynthetic scheme for conversion of L-tyrosine to noscapine, noscapinoid, and phthalideisoquinoline, in accordance with embodiments of the invention. In particular, **FIG. 9** provides the use of the enzymes BBE, berberine bridge enzyme; S9OMT, scoulerine 9-*O*-methyltransferase; CAS, canadine synthase; CPR, cytochrome P450 reductase; TNMT, tetrahydroprotoberberine cis-*N*-methyltransferase; CYP82Y1, *N*-methylcanadine 1-hydroxylase; CYP82X2, 1-hydroxy-*N*-methylcanadine 13-hydroxylase; AT1, 1,13-dihydroxy-*N*-methylcandine 13-*O*-acetyltransferase; CYP82X1, 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine synthase; CXE1,

narcotine hemiacetal synthase; NOS (or SDR1), noscapine synthase; MT2, narcotoline-4'-*O*-methyltrasnferase 1; MT3, narcotoline-4'-*O*-methyltransferase 2; and 6OMT, 6-*O*-methyltransferase.

[00262] In other examples, tetrahydroprotoberberine-*N*-methyltransferase catalyzes the reaction of stylopine → cis-*N*-methylstylopine, as referenced in **FIG. 10**. **FIG. 10** illustrates a biosynthetic scheme for conversion of L-tyrosine to sanguinarine and benzophenanthridine alkaloids, in accordance with embodiments of the invention. In particular, **FIG. 10** provides the use of the enzymes BBE, berberine bridge enzyme; CFS, cheilanthifoline synthase; STS, stylopine synthase; TNMT, tetrahydroberberine *N*-methyltransferase; MSH, cis-*N*-methylstylopine 14-hydroxylase; P6H, protopine 6-hydroxylase; and DBOX, dihydrobenzophenanthride oxidase. The engineered host cell may be modified to include constitutive expression of the TNMT gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the TNMT gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the TNMT gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the TNMT gene within the engineered host cell. In some cases, the TNMT gene may be codon optimized for expression in *Saccharomyces cerevisiae*. The TNMT gene may be derived from *Papaver somniferum, Eschscholzia californica, Papaver bracteatum, Argemone mexicana,* or another species. In some examples, the TNMT gene may be 100% similar to the naturally occurring gene. In examples, the TNMT gene may be 81% similar to the naturally occurring gene.

[00263][CYP82Y1] In some examples, the engineered host cell may modify the expression of the enzyme *N*-methylcanadine 1-hydroxylase. *N*-methylcanadine 1-hydroxylase is encoded by the CYP82Y1 gene. In some examples, *N*-methylcanadine 1-hydroxylase catalyzes the reaction of *N*-methylcanadine → 1-hydroxy-*N*-methylcanadine, as referenced in **FIG. 9**. The engineered host cell may be modified to include constitutive expression of the CYP82Y1 gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the CYP82Y1 gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the CYP82Y1 gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the CYP82Y1 gene within the engineered host cell. In some cases, the CYP82Y1 gene may be codon optimized for expression in *Saccharomyces cerevisiae*. In some examples the CYP82Y1 may be modified at the N-terminus. The CYP82Y1 gene may be derived from *Papaver somniferum, Papaver spp., Plantago arenaria, Rauwolfia heterophylla, Adlumia fungosa, Hydrastis canadensis, Stylomecon heterophylla, Hypecoum,* or another species. In some examples, the CYP82Y1 gene may be 70-78% similar to the naturally occurring gene.

[00264][CYP82X2] In some examples, the engineered host cell may modify the expression of the enzyme 1-hydroxy-*N*-methylcanadine 13-hydroxylase. 1-hydroxy-*N*-methylcanadine 13-hydroxylase is encoded by the

CYP82X2 gene. In some examples, 1-hydroxy-*N*-methylcanadine 13-hydroxylase catalyzes the reaction of 1-hydroxy-*N*-methylcanadine → 1-hydroxy-*N*-methylophiocarpine (i.e. 1,13-dihydroxy-*N*-methylcanadine), as referenced in **FIG. 9**. The engineered host cell may be modified to include constitutive expression of the CYP82X2 gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the CYP82X2 gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the CYP82X2 gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the CYP82X2 gene within the engineered host cell. In some cases, the CYP82X2 gene may be codon optimized for expression in *Saccharomyces cerevisiae*. In some examples the CYP82X2 may be modified at the N-terminus. The CYP82X2 gene may be derived from *P. somniferum, Papaver spp, Plantago arenaria, Rauwolfia heterophylla, Adlumia fungosa, Hydrastis Canadensis, Stylomecon heterophylla, Dactylicapnos torulosa, Glaucium flavum, Berberis laurina, B. Vulgaris, Corydalis spp, Fumaria spp, Dactylicapnos spp.,* or another species. In some examples, the CYP82X2 gene may be 70-77% similar to the naturally occurring gene. In other examples, the CYP82X2 gene may undergo N-terminus engineering. In examples, N-terminus engineering may include N-terminal truncation.

[00265][CYP82X1] In some examples, the engineered host cell may modify the expression of the enzyme 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine synthase. 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine synthase is encoded by the CYP82X1 gene. In some examples, 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine synthase catalyzes the reaction of 1-hydroxy-13-*O*-acetyl-*N*-methylcanadine → 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine, as referenced in **FIG. 9**. Additionally, CYP82X1 catalyzes the reaction of 1-hydroxy-*N*-methylcanadine → 4'-*O*-desmethylmacrantaldehyde. The engineered host cell may be modified to include constitutive expression of the CYP82X1 gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the CYP82X1 gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the CYP82X1 gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the CYP82X1 gene within the engineered host cell. In some cases, the CYP82X1 gene may be codon optimized for expression in *Saccharomyces cerevisiae*. In some examples the CYP82X1 may be modified at the N-terminus. The CYP82X1 gene may be derived from *Papaver somniferum, Papaver spp., Plantago arenaria, Rauwolfia heterophylla, Adlumia fungosa, Hydrastis canadensis, Stylomecon heterophylla, Hypecoum,* or another species. In some examples, the CYP82X1 gene may be 71-77% similar to the naturally occurring gene. In other examples, the CYP82X1 gene may undergo N-terminus engineering. In examples, N-terminus engineering may include N-terminal truncation.

[00266][CFS] In some examples, the engineered host cell may modify the expression of the enzyme cheilanthifoline synthase. Cheilanthifoline synthase is encoded by the CFS gene. In examples, cheilanthifoline synthase catalyzes the reaction of scoulerine → cheilanthifoline, as referenced in **FIG. 10**. An engineered host cell may be modified to include constitutive expression of the CFS gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the CFS gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the CFS gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promotor element for the overexpression of the CFS gene within the engineered host cell. The CFS gene may be derived from *P. somniferum, E. californica, A. mexicana*, or another species. In some examples, the CFS gene may be 77%, 78%, or 79% similar to the naturally occurring gene. Additionally, the CFS gene may be codon optimized for expression in *Saccharomyces cerevisiae*.

[00267][STS] In some examples, the engineered host cell may modify the expression of the enzyme stylopine synthase. Stylopine synthase is encoded by the STS gene. In examples, stylopine synthase catalyzes the reaction of cheilanthifoline → stylopine, as referenced in **FIG. 10**. An engineered host cell may be modified to include constitutive expression of the STS gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the STS gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the STS gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promotor element for the overexpression of the STS gene within the engineered host cell. The STS gene may be derived from *P. somniferum, E. californica, A. mexicana*, or another species. In some examples, the STS gene may be 76%, 78%, or 79% similar to the naturally occurring gene. Additionally, the STS gene may be codon optimized for expression in *Saccharomyces cerevisiae*.

[00268][MSH] In some examples, the engineered host cell may modify the expression of the enzyme cis-*N*-methylstylopine 14-hydroxylase. Cis-*N*-methylstylopine 14-hydroxylase is encoded by the MSH gene. In examples, cis-*N*-methylstylopine 14-hydroxylase catalyzes the reaction of cis-*N*-methylstylopine → protopine, as referenced in **FIG. 10**. An engineered host cell may be modified to include constitutive expression of the MSH gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the MSH gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the MSH gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promotor element for the overexpression of the MSH gene within the engineered host cell. The MSH gene may be derived from *P. somniferum* or another species. In some examples, the MSH

gene may be 79% similar to the naturally occurring gene. Additionally, the MSH gene may be codon optimized for expression in *Saccharomyces cerevisiae*.

[00269] [P6H] In some examples, the engineered host cell may modify the expression of the enzyme protopine-6-hydroxylase. Protopine-6-hydroxylase is encoded by the P6H gene. In examples, protopine-6-hydroxylase catalyzes the reaction of Protopine → 6-hydroxyprotopine, as referenced in **FIG. 10**. An engineered host cell may be modified to include constitutive expression of the P6H gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the P6H gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the P6H gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promotor element for the overexpression of the CFS gene within the engineered host cell. The P6H gene may be derived from *P. somniferum, E. californica,* or another species. In some examples, the P6H gene may be 79% similar to the naturally occurring gene. Additionally, the P6H gene may be codon optimized for expression in *Saccharomyces cerevisiae*.

[00270][DBOX] In some examples, the engineered host cell may modify the expression of the enzyme dihydrobenzophenanthridine oxidase. Dihydrobenzophenanthridine oxidase is encoded by the DBOX gene. In examples, dihydrobenzophenanthridine oxidase catalyzes the reaction of dihydrosanguinarine → sanguinarine, as referenced in **FIG. 10**. An engineered host cell may be modified to include constitutive expression of the DBOX gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the DBOX gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the DBOX gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promotor element for the overexpression of the DBOX gene within the engineered host cell. The DBOX gene may be derived from *P. somniferum* or another species. In some examples, the DBOX gene may be 100% similar to the naturally occurring gene. Additionally, the DBOX gene may be codon optimized for expression in *Saccharomyces cerevisiae*.

[00271][AT1] In some examples, the engineered host cell may modify the expression of the enzyme 1, 13-dihydroxy-*N*-methylcanadine 13-*O* acetyl transferase. 1, 13-dihydroxy-N-methylcanadine 13-*O* acetyltransferase is encoded by the AT1 gene. In some examples, 1, 13-dihydroxy-N-methylcanadine 13-*O* acetyltransferase catalyzes the reaction of 1, 13-dihydroxy-N-methylcanadine → 1-hydroxy-13-*O*-acetyl-*N*-methylcanadine, as referenced in **FIG. 11A**. **FIG. 11A** illustrates a biosynthetic scheme for conversion of canadine to noscapine, in accordance with embodiments of the invention. The engineered host cell may be modified to include constitutive expression of the AT1 gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the AT1 gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a

copy, copies, or additional copies, of the AT1 gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the AT1 gene within the engineered host cell. In some cases, the AT1 gene may be codon optimized for expression in *Saccharomyces cerevisiae*. The AT1 gene may be derived from *P. somniferum, Papaver spp, Plantago arenaria, Rauwolfia heterophylla, Adlumia fungosa, Hydrastis Canadensis, Stylomecon heterophylla, Hypecoum leptocarpum, Dactylicapnos torulosa, Glaucium flavum, Berberis laurina, B. Vulgaris, Corydalis spp, Fumaria spp, Dactylicapnos spp,* or another species. In some examples, the AT1 gene may be 81% similar to the naturally occurring gene.

[00272][CXE1 or CXE2] In some examples, the engineered host cell may modify the expression of the enzyme narcotinehemiacetal synthase. Narcotinehemiacetal synthase is encoded by the CXE1 gene. The enzyme encoded by the CXE2 gene can also function as a narcotinehemiacetal synthase. In some examples, narcotinehemiacetal synthase catalyzes the reaction of 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine → narcotolinehemiacetal and 3-*O*-acetylpapaveroxine → narcotinehemiacetal, as referenced in **FIG. 11A**. The engineered host cell may be modified to include constitutive expression of the CXE1 or CXE2 gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the CXE1 or CXE2 gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the CXE1 or CXE2 gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the CXE1 or CXE2 gene within the engineered host cell. In some cases, the CXE1 or CXE2 gene may be codon optimized for expression in *Saccharomyces cerevisiae*. The CXE1 or CXE2 gene may be derived from *P. somniferum, Papaver spp, Plantago arenaria, Rauwolfia heterophylla, Adlumia fungosa, Hydrastis Canadensis, Stylomecon heterophylla, Hypecoum leptocarpum, Dactylicapnos torulosa, Glaucium flavum, Berberis laurina, B. Vulgaris, Corydalis spp, Fumaria spp, Dactylicapnos spp,* or another species. In some examples, the CXE1 gene or CXE2 gene may be 78% similar to the naturally occurring gene.

[00273][SDR1] In some examples, the engineered host cell may modify the expression of the enzyme noscapine synthase. Noscapine synthase is encoded by the SDR1 gene. In some examples, noscapine synthase catalyzes the reaction of narcotolinehemiacetal → narcotoline, as referenced in **FIG. 11A**. Additionally, noscapine synthase catalyzes the reaction of narcotinehemiacetal → noscapine. The engineered host cell may be modified to include constitutive expression of the SDR1 gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the SDR1 gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the SDR1 gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the SDR1 gene within the engineered host cell. In some cases, the SDR1 gene may be

codon optimized for expression in *Saccharomyces cerevisiae*. The SDR1 gene may be derived from *P. somniferum, Papaver spp, Plantago arenaria, Rauwolfia heterophylla, Adlumia fungosa, Hydrastis Canadensis, Stylomecon heterophylla, Hypecoum leptocarpum, Dactylicapnos torulosa, Glaucium flavum, Berberis laurina, B. Vulgaris, Corydalis spp, Fumaria spp, Dactylicapnos spp,* or another species. In some examples, the SDR1 gene may be 79% similar to the naturally occurring gene.

[00274] [MT2 and MT3] In some examples, the engineered host cell may modify the expression of the enzyme narcotoline 4'-*O*-methylase. Narcotoline 4'-*O*-methylase is a heterodimer formed by the *O*-methyltransferase monomer encoded by the MT2 and MT3 genes. In some examples, narcotoline 4'-*O*-methylase catalyzes the reaction of narcotoline → noscapine, as referenced in **FIG. 11A**. Additionally, narcotoline 4'-*O*-methylase catalyzes the reaction of narcotolinenehemiacetal → narcotinehemiacetal and 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine → 3-*O*-acetylpapaveroxine. The engineered host cell may be modified to include constitutive expression of the MT2 and MT3 genes in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the MT2 and MT3 genes in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the MT2 and MT3 genes. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the MT2 and MT3 genes within the engineered host cell. In some cases, the MT2 and MT3 genes may be codon optimized for expression in *Saccharomyces cerevisiae*. The MT2 and MT3 genes may be derived from *P. somniferum, Papaver spp, Fumaria parviflora, Plantago arenaria, Rauwolfia heterophylla,* or another species. In some examples, the MT2 and MT3 genes may be 80% and 79% similar, respectively, to the naturally occurring genes.

[00275] [morA] In some examples, the engineered host cell may modify the expression of the enzyme morphine dehydrogenase. Morphine dehydrogenase is encoded by the morA gene. In some examples, morphine dehydrogenase catalyzes the reaction of morphine → morphinone, as referenced in **FIG. 11B**. In other examples, morphine dehydrogenase catalyzes the reaction of codeinone → codeine, also as referenced in **FIG. 11B**. **FIG. 11B** illustrates a biosynthetic scheme for production of semi-synthetic opiods, in accordance with embodiments of the invention. In particular, **FIG. 11B** illustrates extended transformations of thebaine in yeast by incorporating morA, morphine dehydrogenase; and morB, morphine reductase.

[00276] The engineered host cell may be modified to include constitutive expression of the morA gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the morA gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the morA gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the morA gene within the engineered host cell. In some cases, the morA gene may be codon optimized for expression in *Saccharomyces cerevisiae*. The morA gene may be derived

from *Pseudomonas putida* or another species. In some examples, the morA gene may be 73.7% similar to the naturally occurring gene.

[00277][morB] In some examples, the engineered host cell may modify the expression of the enzyme morphinone reductase. Morphinone reductase is encoded by the morB gene. In some examples, morphinone reductase catalyzes the reaction of codeinone → hydrocodone, as referenced in **FIG. 11B**. In other examples, morphinone reductase catalyzes the reaction of morphinone → hydromorphone , also as referenced in **FIG. 11B**. In other examples, morphinone reductase catalyzes the reaction 14-hydroxycodeinone → oxycodone. The engineered host cell may be modified to include constitutive expression of the morB gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the morB gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the morB gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the morB gene within the engineered host cell. In some cases, the morB gene may be codon optimized for expression in *Saccharomyces cerevisiae*. The morB gene may be derived from *Pseudomonas putida* or another species. In some examples, the morB gene may be 67.2% similar to the naturally occurring gene.

[00278][CYP80A1] In some examples, the engineered host cell may express the enzyme berbamunine synthase. Berbamunine synthase is encoded by the gene for cytochrome P450 enzyme 80A1 (CYP80A1). In some examples, CYP80A1 catalyzes the reaction *(S)-N*-methylcoclaurine + *(R)-N*-methylcoclaurine → berbamunine. In other examples, CYP80A1 catalyzes the reaction *(R)-N*-methylcoclaurine + *(R)-N*-methylcoclaurine → guattegaumerine. In other examples, CYP80A1 catalyzes the reaction *(R)-N*-methylcoclaurine + *(S)*-coclaurine → 2'norberbamunine. The engineered host cell may be modified to include constitutive expression of the CYP80A1 gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the CYP80A1 gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the CYP80A1 gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the CYP80A1 gene within the engineered host cell. In some cases, the CYP80A1 gene may be codon optimized for expression in *Saccharomyces cerevisiae*. The CYP80A1 gene may be derived from *Berberis stolonifera* or another species. In some examples, the CYP80A1 gene may be 76% similar to the naturally occurring gene.

[00279][PODA] In some example, the engineered host cell may express the enzyme protopine *O*-dealkylase. Protopine *O*-dealkylase is encoded by the gene PODA. In some examples, PODA catalyzes the *O,O*-demethylenation of protoberberines and protopines such as canadine, stylopine, berberine, cryptopine, allocryptopine, and protopine. In some examples, PODA catalyzes the *O*-demethylation of BIAs including tetrahydropapaverine, tetrahydropalmatine, and cryptopine. The engineered host cell may be modified to

include constitutive expression of the PODA gene in the engineered host cell. Additionally or alternatively, the engineered host cell may be modified to synthetically regulate the expression of the PODA gene in the engineered host cell. In examples, the engineered host cell may be modified to incorporate a copy, copies, or additional copies, of the PODA gene. Additionally or alternatively, the engineered host cell may be modified to incorporate the introduction of a strong promoter element for the overexpression of the PODA gene within the engineered host cell. In some cases, the PODA gene may be codon optimized for expression in *Saccharomyces cerevisiae*. The PODA gene may be derived from *Papaver somniferum* or other species. In some examples, the PODA gene may be 70-100% similar to the naturally occurring gene.

[00280] Examples of the aforementioned genes can be expressed from a number of different platforms in the host cell, including plasmid (2μ, ARS/CEN), YAC, or genome. In addition, examples of the aforementioned gene sequences can either be native or codon optimized for expression in the desired heterologous host (e.g., *Saccharomyces cerevisiae*).

EXAMPLES

[00281] The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the invention in any fashion. Where indicated, expression constructs are understood to incorporate a suitable promoter, gene, and terminator, even if the exact terminator sequence used is not specified. The present examples, along with the methods described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses which are encompassed within the spirit of the invention as defined by the scope of the claims will occur to those skilled in the art.

Example 1: Tyrosine hydroxylase mutants improve norcoclaurine production in engineered yeast strains

[00282] Tyrosine hydroxylase from *R. norvegicus* was yeast codon optimized, synthesized, and cloned into a low-copy plasmid. Single mutants (W166Y, E332D, S40D and R37ER38E), double mutants (W166Y and E332D, W166Y and S40D, W166Y and R37ER38E), and one triple mutant (W166Y, R37ER38E, and E332D) were generated through site-directed mutagenesis. Each TyrH mutant was expressed from a low-copy plasmid with the GPD promoter in a yeast strain containing the following mutations to central metabolism (as described in United States Provisional Patent Application Serial No. 61/899,496): $ARO4^{FBR}$, ΔZWF1, and GPD-TKL1 promoter replacement. In addition, the strain expressed a chromosomally integrated copy of DOPA decarboxylase (DODC) from *P. putida*, four chromosomally integrated genes from *R. norvegicus* that generate the cosubstrate tetrahydrobiopterin (pyruvoyl tetrahydropterin synthase, PTPS; sepiapterin reductase, SepR; pterin 4a-carbinolamine dehydratase, PCD; dihydropteridine reductase, QDHPR) and norcoclaurine synthase (NCS) from *C. japonica* expressed from a low-copy plasmid with a GPD promoter. The strains harboring TyrH mutants were grown in selective defined media (YNB) lacking

tyrosine with 2% dextrose for 96 hours, and the production of norcoclaurine was measured in the media via LC-MS/MS in MRM mode with the transition 272 m/z to 107 m/z. **FIG. 12** shows the results of this assay and demonstrates that TyrH mutants can improve norcoclaurine production by as much as 9-fold when compared to wild-type TyrH. As such, **FIG. 12** illustrates tyrosine hydroxylase mutants that improve norcoclaurine production from sugar in engineered yeast strains, in accordance with embodiments of the invention.

Example 2: Tyrosine hydroxylase mutants improve reticuline production in engineered yeast strains

[00283] Tyrosine hydroxylase from *R. norvegicus* was yeast codon optimized, synthesized, and cloned into a low-copy plasmid. Single mutants (W166Y, E332D, S40D and R37ER38E), double mutants (W166Y and E332D, W166Y and S40D, W166Y and R37ER38E), and one triple mutant (W166Y, R37ER38E, and E332D) were generated through site-directed mutagenesis. Each TyrH mutant was expressed from a low-copy plasmid with the GPD promoter in a yeast strain containing the following mutations to central metabolism (as described in United States Provisional Patent Application Serial No. 61/899,496): $ARO4^{FBR}$, ΔZWF1, and GPD-TKL1 promoter replacement. In addition, the strain expressed a chromosomally integrated copy of DOPA decarboxylase (DODC) from *P. putida*, four chromosomally integrated genes from *R. norvegicus* that generate the cosubstrate tetrahydrobiopterin (pyruvoyl tetrahydropterin synthase, PTPS; sepiapterin reductase, SepR; pterin 4a-carbinolamine dehydratase, PCD; dihydropteridine reductase, QDHPR), norcoclaurine synthase (NCS) from *C. japonica* expressed from a low-copy plasmid with a GPD promoter, and five genes for the biosynthesis of reticuline from norcoclaurine (*P. somniferum* 6-*O*-methyltransferase, Ps6OMT; *P. somniferum* coclaurine *N*-methyltransferase, PsCNMT; *E. californica* cytochrome P450 80B1, EcCYP80B1; *P. somniferum* cytochrome P450 NADPH reductase, PsCPR; and *P. somniferum* 3'hydroxy-*N*-methylcoclaurine 4'-*O*-methyltransferase, Ps4'OMT). The strains harboring TyrH mutants were grown in selective defined media (YNB) lacking tyrosine with 2% dextrose for 96 hours, and the production of reticuline was measured in the media via LC-MS/MS in MRM mode with the transition 330 m/z to 137 m/z. **FIG. 13** shows the results of this assay and demonstrates that TyrH mutants can improve reticuline production by as much as 5-fold when compared to wild-type TyrH. As such, **FIG. 13** illustrates tyrosine hydroxylase mutants that improve reticuline production from sugar in engineered yeast strains, in accordance with embodiments of the invention.

Example 3: Expression of DHFR improves tyrosine hydroxylase activity in engineered yeast strains

[00284] Dihydrofolate reductase (DHFR) from *R. norvegicus* was yeast codon optimized, synthesized, and cloned into a low-copy plasmid under the control of a GPD promoter. DHFR was coexpressed with wild-type RnTyrH (low-copy plasmid with a GPD promoter) in a yeast strain containing the following mutations to

central metabolism (as described in United States Provisional Patent Application Serial No. 61/899,496): $ARO4^{FBR}$, ΔZWF1, and GPD-TKL1 promoter replacement. In addition, the strain expressed four chromosomally integrated genes from *R. norvegicus* that generate the cosubstrate tetrahydrobiopterin (pyruvoyl tetrahydropterin synthase, PTPS; sepiapterin reductase, SepR; pterin 4a-carbinolamine dehydratase, PCD; dihydropteridine reductase, QDHPR). The strains expressing DHFR and wild-type RnTyrH were grown in selective defined media (YNB) lacking tyrosine with 2% dextrose for 96 hours, and the production of L-DOPA was measured in the media via LC-MS/MS in MRM mode with the transition 198 m/z to 152 m/z. Expression of DHFR with wild-type RnTyrH increases L-DOPA production by 1.8-fold, as illustrated in **FIG. 14**. As such, **FIG. 14** illustrates coexpression of dihydrofolate reductase (DHFR) that improves L-DOPA production by tyrosine hydroxylase in engineered yeast strains, in accordance with embodiments of the invention.

Example 4: Addition of antioxidants to growth media improve tyrosine hydroxylase activity in engineered yeast strains

[00285] A yeast strain containing the following mutations to central metabolism (as described in United States Provisional Patent Application Serial No. 61/899,496): $ARO4^{FBR}$, ΔZWF1, and GPD-TKL1 promoter replacement and expressing four chromosomally integrated genes from *R. norvegicus* that generate the cosubstrate tetrahydrobiopterin (pyruvoyl tetrahydropterin synthase, PTPS; sepiapterin reductase, SepR; pterin 4a-carbinolamine dehydratase, PCD; dihydropteridine reductase, QDHPR) as well as wild-type RnTyrH from a low-copy plasmid under the control of the GPD promoter was grown in selective defined media (YNB) lacking tyrosine with 2% galactose and 2 mM ascorbic acid for 96 hours.

[00286] The production of L-DOPA was measured in the media via LC-MS/MS in MRM mode with the transition 198 m/z to 152 m/z. The addition of 2 mM ascorbic acid improves L-DOPA production with wild-type RnTyrH by 1.8-fold. In addition, the concentration BH_4 intermediates were measured with LC-MS/MS in MRM mode with the following transitions: B, 238 m/z to 178 m/z; BH2, 240 m/z to 165 m/z and BH4, 242 m/z to 166 m/z. The addition of ascorbic acid also increases BH_4 in the media, which indicates the oxidation of BH_4 to BH_2 is prevented.

[00287] Accordingly, **FIG. 15** illustrates (A) addition of antioxidants to culture media that improves L-DOPA production by tyrosine hydroxylase in engineered yeast strains and (B) addition of antioxidants to culture media that increase BH_4 levels, in accordance with embodiments of the invention. In particular, **FIG. 15A** illustrate a wild-type RnTyrH (expressed from a low-copy plasmid under the control of a GPD promoter) was expressed in a yeast strain containing the following mutations to central metabolism (as described in United States Provisional Patent Application Serial No. 61/899,496): $ARO4^{FBR}$, ΔZWF1, and GPD-TKL1 promoter replacement. In addition, the strain expressed four chromosomally integrated genes from *R. norvegicus* that

generate the cosubstrate tetrahydrobiopterin (pyruvoyl tetrahydropterin synthase, PTPS; sepiapterin reductase, SepR; pterin 4a-carbinolamine dehydratase, PCD; dihydropteridine reductase, QDHPR). The strains expressing wild-type RnTyrH was grown in selective defined media (YNB) lacking tyrosine with 2% dextrose, with and without 2 mM ascorbic acid (aa) for 96 hours. The production of L-DOPA was measured in the media via LC-MS/MS in MRM mode with the transition 198 m/z to 152 m/z. Additionally, **FIG. 15B** illustrates, in the same strain described in **FIG. 15A**, the concentration of the BH_4 intermediate was measured in the media of strains grown with and without 2 mM ascorbic acid (aa) with LC-MS/MS in MRM mode with the following transition: BH_4, 242 m/z to 166 m/z.

Example 5: Yeast strains engineered to produce the bisBIA berbamunine

[00288] **FIG. 16** illustrates (A) a biosynthetic scheme for conversion of L-tyrosine to bisBIAs and (B) yeast strains engineered to biosynthesize bisBIAs, in accordance with embodiments of the invention. In particular, **FIG. 16** illustrates (A) a pathway that is used to produce bisBIAs berbamunine and guattegaumerine. **FIG. 16** provides the use of the enzymes ARO9, aromatic aminotransferase; ARO10, phenylpyruvate decarboxlase; TyrH, tyrosine hydroxylase; DODC, DOPA decarboxylase; NCS, norcoclaurine synthase; 6OMT, 6-*O*-methyltransferase; CNMT, coclaurine *N*-methyltransferase; CYP80A1, cytochrome P450 80A1; CPR, cytochrome P450 NADPH reductase. Of the metabolites provided in **FIG. 16**, 4-HPA, 4-HPP, and L-tyrosine are naturally synthesized in yeast. Other metabolites that are shown in **FIG. 16** are not naturally produced in yeast.

[00289] In examples of the invention, a bisBIA-producing yeast strain, that produces bisBIAs such as those generated using the pathway illustrated in (A), is engineered by integration of a single construct into locus YDR514C. Additionally, **FIG. 16** provides (B) example yeast strains engineered to synthesize bisBIAs. Ps6OMT, PsCNMT, PsCPR, and BsCYP80A1 were integrated into the yeast genome at a single locus (YDR514C). Each enzyme was expressed from a constitutive promoter. The arrangement and orientation of gene expression cassettes is indicated by arrows in the schematic. These strains convert *(R)*- and *(S)*-norcoclaurine to coclaurine and then to *N*-methylcoclaurine. In one example, the strains may then conjugate one molecule of *(R)*-*N*-methylcoclaurine and one molecule of *(S)*-*N*-methylcoclaurine to form berbamunine. In another example, the strains may conjugate two molecules of *(R)*-*N*-methylcoclaurine to form guattegaumerine. In another example, the strains may conjugate one molecule of *(R)*-*N*-methylcoclaurine and one molecule of *(S)*-coclaurine to form 2'-norberbamunine. In another embodiment, the strain may be engineered to supply the precursors *(R)*- and *(S)*-norcoclaurine from L-tyrosine, as provided in **FIG. 5**.

[00290] The construct includes expression cassettes for *P. somniferum* enzymes 6OMT and CNMT expressed as their native plant nucleotide sequences. A third enzyme from *P. somniferum,* CPR, is codon optimized for expression in yeast. The PsCPR supports the activity of a fourth enzyme, *Berberis stolonifera* CYP80A1,

also codon optimized for expression in yeast. The expression cassettes each include unique yeast constitutive promoters and terminators. Finally, the integration construct includes a LEU2 selection marker flanked by loxP sites for excision by Cre recombinase.

[00291] A yeast strain expressing Ps6OMT, PsCNMT, BsCYP80A1, and PsCPR is cultured in selective medium for 16 hours at 30°C with shaking. Cells are harvested by centrifugation and resuspended in 400 µL breaking buffer (100 mM Tris-HCl, pH 7.0, 10 % glycerol, 14 mM 2-mercaptoethanol, protease inhibitor cocktail). Cells are physically disrupted by the addition of glass beads and vortexing. The liquid is removed and the following substrates and cofactors are added to start the reaction: 1 mM (*R,S*)-norcoclaurine, 10 mM *S*-adenosyl methionine, 25 mM NADPH. The crude cell lysate is incubated at 30°C for 4 hours and then quenched by the 1:1 addition of ethanol acidified with 0.1% acetic acid. The reaction is centrifuged and the supernatant analyzed by liquid chromatography mass spectrometry (LC-MS) to detect bisBIA products berbamunine, guattegaumerine, and 2'-norberbamunine by their retention and mass/charge.

Example 6. Identification of an epimerase enzyme

[00292] To identify an epimerase enzyme suitable for performing the epimerization reactions of the methods disclosed herein, a cytochrome P450 oxidase 82Y1-like domain and a codeinone reductase-like domain were identified in a single open reading frame (CYP-COR) in publically available plant transcriptomes. The CYP-COR fusions were identified from a BLAST search of the 1000 Plants Project (Matasci, et al. 2014. Gigascience. 3: 17) and PhytoMetaSyn (Facchini, et al. 2012. Trends Biotechnol. 30: 127-31; Xiao, et al. 2013. J. Biotechnol. 166: 122-34) transcriptomes using blastn with the query being the sequence of a previously published COR-silencing VIGS construct that resulted in reticuline accumulation (Wijekoon and Facchini. 2012. Plant J. 69: 1052-63). Once one CYP-COR fusion sequence was observed as a hit, that sequence was translated and the amino acid sequence was used as the query for a second search of both databases with tblastn. A phylogenetic tree of the CYP-COR fusion enzymes identified from the databases is provided in **FIG. 17**. The sequences were identified from The 1000 Plants Project and PhytoMetaSyn transcriptome databases based on a bioinformatic search. Additionally, an example amino acid sequence is provided in **FIG. 4,** as discussed above. Additionally, **Table 1** lists various examples of amino acid sequences identified for this CYP-COR enzyme, which come from various plants including *Papaver somniferum* (opium poppy), *Papaver setigerum* (poppy of Troy), *Papaver bracteatum* (Iranian poppy), and *Chelidonium majus* (greater celandine).

Example 7. Epimerization of (S)-reticuline to (R)-reticuline in an engineered non-plant host cell

[00293] Non-plant host cells were engineered to heterologously express enzymes described herein. For instance, yeast strains (*Saccharomyces cerevisiae*) were engineered to heterologously express the identified epimerases described in **Example 6** and to verify their function in the context of this microbial host. The yeast-codon optimized DNA coding sequences for the partial amino acid sequences pbr.PBRST1PF_4328 and pbr.PBRST1PF_89405 were synthesized in-frame with the yeast-codon optimized coding sequence for amino acids 1-40 of SSDU-2015634 (**Table 1**) to generate CYP-COR_4328 and CYP-COR_89405, respectively. These CYP-COR coding sequences were cloned into a low-copy plasmid harboring a *URA3* selection marker and expressed from the *TDH3* promoter. The plasmids were transformed into yeast strains that harbored an expression cassette for a cytochrome P450 reductase (P_{TEF1}-*ATR1* or P_{TEF1}-*PsCPRv2*) integrated into the chromosome. These yeast strains harboring the two plasmids were grown in synthetic complete media with the appropriated drop out solution (-Ura-Trp). The yeast strains were fed *(S)*-reticuline and BIA metabolites were analyzed after 72 hours of growth by LC-MS/MS analysis.

Example 8. Production of salutaridine from (S)-reticuline in an engineered yeast cell

[00294] Yeast strains (*Saccharomyces cerevisiae*) were engineered to heterologously express the identified epimerases described in **Example 6** and to verify their function in the context of this microbial host. The yeast-codon optimized DNA coding sequences for the partial amino acid sequences pbr.PBRST1PF_4328 and pbr.PBRST1PF_89405 were synthesized in-frame with the yeast-codon optimized coding sequence for amino acids 1-40 of SSDU-2015634 (**Table 1**) to generate CYP-COR_4328 and CYP-COR_89405, respectively. These CYP-COR coding sequences were cloned into a low-copy plasmid harboring a *URA3* selection marker and expressed from the *TDH3* promoter. The salutaridine synthase (SalSyn) coding sequence was cloned into a low-copy plasmid harboring a *TRP1* selection marker and expressed from the *TDH3* promoter. The plasmids were transformed into yeast strains that harbored an expression cassette for a cytochrome P450 reductase (P_{TEF1}-*ATR1* or P_{TEF1}-*PsCPRv2*) integrated into the chromosome. These yeast strains harboring the two plasmids were grown in synthetic complete media with the appropriated drop out solution (-Ura-Trp). The yeast strains were fed *(S)*-reticuline and BIA metabolites were analyzed after 72 hours of growth by LC-MS/MS analysis. The analysis indicated that the engineered yeast cells were able to convert *(S)*-reticuline to *(R)*-reticuline, which was then acted on by salutaridine synthase to form salutaridine, a 4-ring promorphinan alkaloid (**FIG. 7, FIG. 18**). Salutaridine synthase has been previously shown to act on *(R)*-reticuline and have no observable activity on *(S)*-reticuline (Gesell, et al. 2009. J. Biol. Chem. 284: 24432-42).

[00295] As shown in **FIG. 7**, CYP-COR catalyzes the conversion of *(S)*-reticuline to *(R)*-reticuline, which is then acted on by salutaridine synthase to make the promorphinan alkaloid salutaridine. **FIG. 18** illustrates (A) chromatogram traces showing reticuline and salutaridine for two epimerase variants (CYP-COR_89405,

CYP-COR_4328) and a standard. **FIG. 18** also illustrates (B) the same chromatogram traces for salutaridine in (A) as replotted to demonstrate co-elution with the standard. In this experiment, the yeast contains two low-copy CEN/ARS plasmids with *URA3* and *TRP1* selective markers, *TDH3* promoters, and the CYP-COR and SalSyn coding sequences. Yeast were grown from freshly transformed colonies in 3 mL selective media overnight, back-diluted into 3.5 mL media to OD 0.8, grown 7 hours, pelleted, and then resuspended into pH 7.4 HEPES buffer with 100 µM *(S)*-reticuline (Specs). After 16 hours on a spinner at 30 °C, the yeast were pelleted and the buffer supernatant was analyzed by LC-MS/MS. Each trace is from a single sample representative of 2. Peaks are normalized such that the largest peak in all chromatograms is 100%.

Example 9. Production of (R)-reticuline from racemic norlaudanosoline in an engineered non-plant host cell

[00296] Yeast strains (*Saccharomyces cerevisiae*) were engineered to heterologously express the identified epimerases described in **Example 6** and to verify their function in the context of this microbial host. The yeast-codon optimized DNA coding sequence CYP-COR_89405 described in **Example 7** was cloned into a low-copy plasmid harboring a *URA3* selection marker and expressed from the *TDH3* promoter. This plasmid was transformed into yeast strains that harbored expression cassettes for a cytochrome P450 reductase (P_{TEF1}-*ATR1* or P_{TEF1}-*PsCPRv2*) and three methyltransferases (*Papaver somniferum* norcoclaurine-6-*O*-methyltransferase, coclaurine *N*-methyltransferase, and 3'-hydroxy-*N*-methylcoclaurine 4'-*O*-methyltransferase, all expressed from P_{TEF1}) integrated into the chromosome. This yeast strain harboring the plasmid was grown in synthetic complete media with the appropriated drop out solution (-Ura). The yeast strain was fed racemic norlaudanosoline and BIA metabolites were analyzed after 72 hours of growth by LC-MS/MS analysis. For chiral characterization, reticuline was concentrated from yeast media by pelleting 5 mL yeast culture and adding 120 mg XAD-4 resin to 4 mL supernatant, incubating on rotator overnight at room temperature, and eluting with 0.5 mL methanol. The concentrate was fractionated by reverse-phase HPLC (Pursuit XRs-C18, 5 µm, 50 mm × 10 mm) with isocratic 15% methanol with 0.1% formic acid over 6.5 min with a flow rate of 5 mL/min and injection volume of 40-50 µL. Peak-based fractions were collected at approximately 4.5 min. Fractions were pooled, freeze-dried, and resuspended in 0.5 mL isopropanol. Depending on concentration, 0.5-5 µL were injected onto a chiral column (Phenomenex Lux cellulose-1, 3 µm, 150 mm x 2 mm) and separated with isocratic 72% N-hexane, 28% isopropanol, 0.1% diethylamine with a flow rate of 0.3 mL/min and detection by MS and 250 nm UV. MS detection was performed with an Agilent 6320 Ion Trap mass spectrometer with ESI source gas temperature 350 °C, gas flow of 10 L/min, nebulizer pressure 40 PSI and isolation of *m/z* 330.1 with width 1.0. The retention time of reticuline peaks was compared to that of authentic *(S)*-reticuline and *(R)*-reticuline standards. The analysis indicated that the engineered yeast cells containing the CYP-COR plasmid were able to convert racemic norlaudanosoline to

(R)-reticuline, while engineered yeast cells with an empty plasmid produced exclusively *(S)*-reticuline (**FIG. 19**).

Example 10: Protein engineering of salutaridine synthase to improve its processing and activity when expressed in a microbial host

[00297] Heterologous proteins may be incorrectly processed when expressed in a recombinant host, for example, plant proteins such as cytochrome P450 enzymes expressed in microbial production hosts. For example, salutaridine synthase, which converts *(R)*-reticuline to salutaridine, undergoes N-linked glycosylation when heterologously expressed in yeast (**FIG. 20**). The observed N-linked glycosylation pattern on salutaridine synthase is not observed when the enzyme is expressed in plants and is indicative of incorrect N-terminal sorting of the nascent SalSyn transcript, which reduces the activity of the enzyme in the heterologous microbial host. Thus, protein engineering directed at correcting N-terminal sorting of the nascent transcript and thereby removing the N-linked glycosylation pattern will result in improved activity of the salutaridine synthase enzyme in the recombinant production host.

[00298] For example, N-terminal alpha-helices from cheilanthifoline synthase (CFS) were used to replace N-terminal alpha-helices from salutaridine synthase (SalSyn, **FIG. 21**). Junction points for these fusions were selected based on secondary structure motifs of CFS and SalSyn or based on amino acid alignments of CFS and SalSyn. The fusions were cloned by amplifying the N-terminal fragment from CFS and C-terminal fragment from SalSyn with 15-40 nucleotides of overlap with the other fragment, and then assembled with each other and a vector backbone by Gibson assembly to form the complete fusion open reading frame (Gibson, et al. 2009. Nat Methods. 6: 343-5).

[00299] As another example, the coding sequence for the cytochrome P450 domain from salutaridine synthase was placed directly into the P450 encoding region of other stably expressed cytochrome P450s such as the BM3 enzyme. For example, the conserved cytochrome P450 domain of the salutaridine synthase and the cytochrome P450 domain from an engineered variant of the *Bacillus megaterium* P450 monooxygenase CYP102A1 (BM3, (Michener and Smolke. 2012. Metab. Eng. 14: 306-16)) were identified by NCBI conserved domain search. Primers were designed to fuse the coding sequence of the first few amino acids of the BM3 to the coding sequence for the P450 domain of the salutaridine synthase, followed by the coding sequence for BM3 domains C-terminal to the P450 domain. As before, this construct was assembled via Gibson assembly.

[00300] The engineered salutaridine synthase protein fusions were analyzed by Western Blot analysis to confirm full-length expression and modification to or elimination of N-linked glycosylation patterns in yeast (**FIG. 20**). The salutaridine synthase enzyme and protein fusions were C-terminally tagged with the human influenza hemagglutinin (HA) epitope and cloned into expression plasmids appropriate for yeast and plant

expression. For yeast, the enzyme coding sequences were cloned into a low-copy yeast/*E. coli* shuttle vector harboring a *URA3* selection marker and expressed from the *TDH3* promoter. For plants, the sequences were cloned into an *E. coli*/*Agrobacterium* tumefaciens shuttle vector with kanamycin resistance and the Cauliflower mosaic virus (CaMV) 35S promoter with flanking 5' and 3'-untranslated regions from Cowpea mosaic virus RNA-2 for transient plant expression via *Agrobacterium tumefaciens*-infiltration. Yeast engineered to express salutaridine synthase exhibited a banding pattern indicative of N-linked glycosylation. We confirmed that this pattern was due to N-linked glycosylation by performing site-directed mutagenesis on the glycosylation site. In contrast, plant expression of this enzyme did not result in a banding pattern indicative of N-linked glycosylation, as seen in (A) of **FIG. 20**. Although the N-linked glycosylation sites were unmodified, the engineered salutaridine synthase protein fusions were not N-glycosylated when expressed in yeast, as seen in (B) of **FIG. 20**. By Western blot, we demonstrated that the yeast-expressed fusion enzymes were present as a single band, similar to the expression observed for the plant-expressed parent enzyme, indicating that the mis-processing of the nascent protein in yeast that resulted in N-linked glycosylation was repaired by the engineered fusions.

[00301] The engineered salutaridine synthase protein fusions were analyzed for improved enzyme activity when heterologously expressed in yeast. Coding sequences for salutaridine synthase and the engineered fusions were cloned into a low-copy plasmid harboring a *URA3* selection marker and expressed from the *TDH3* promoter. The yeast have P_{TEF1}-*PsCPRv2* integrated into the *TRP1* locus and contain a single low-copy plasmid with the *URA3* selective marker and the salutaridine synthase coding sequence with the *TDH3* promoter. Yeast were grown from freshly transformed colonies in 1 mL selective media (-Ura) overnight and back-diluted 1:20 into 0.5 mL selective media in 96-well plates with 10 µM *(R)*-reticuline (Toronto Research Chemicals). After 72-96 hours in the shaking incubator, the yeast were pelleted and the media supernatant was analyzed by LC-MS/MS. The analysis indicated that the engineered salutaridine synthase enzymes exhibited improved activity relative to that of the wild-type sequence when heterologously expressed in yeast (**FIG. 22**).

[00302] **FIG. 22** illustrates salutaridine synthase codon-optimization and engineered fusions that improve activity in yeast, in accordance with embodiments of the invention. As seen in **FIG. 22**, a black bar indicates a native wild-type sequence for salutaridine synthase, PsCYP719B1. Grey bars with black borders are yeast codon-optimized variants from *Papaver somniferum* and a newly identified sequence from *Papaver bracteatum*. The diagonally patterned bar indicates the most improved engineered fusion, which is based on the *P. bracteatum* sequence. Error bars indicate the range of at least two biological replicates. Natural, synthetic codon-optimized, and/or protein engineered variants of salutaridine synthase from *P. bracteatum*, *P. somniferum*, or *P. setigerum* (or related plant) may be used in these engineered strains.

[00303] The engineered salutaridine synthase protein fusions can be used in the context of a biosynthetic pathway to increase production of downstream benzylisoquinoline alkaloid products. In one example, yeast

were engineered to heterologously express yeast codon optimized genes encoding an engineered salutaridine synthase fusion, *P. bracteatum* salutaridine reductase, and *P. somniferum* salutaridinol 7-*O*-acetyltransferase. The three expression cassettes (P_{TDH3}-*D94yPsSS*, P_{TPI1}-*yPbSalR*, P_{TEF1}-*yPsSalAT*) were assembled into a yeast artificial chromosome (YAC) with a *TRP1* section marker. The YAC was placed into yeast that harbored an expression cassette for a cytochrome P450 reductase (P_{TEF1}-*ATR1* or P_{TEF1}-y*PsCPRv2*) integrated into the chromosome. The yeast strains were grown in synthetic complete media with the appropriated drop out solution (-Trp) and fed *(R)*-reticuline. BIA metabolites were analyzed after 96 hours of growth through LC-MS/MS analysis. The analysis indicates that yeast strains engineered with the engineered salutaridine synthase enzymes and other pathway enzymes produce the morphinan alkaloid thebaine, as illustrated in (A) of **FIG. 23**.

[00304] Accordingly, **FIG. 23** illustrates (A) an LC-MS/MS analysis of small scale batch fermentation in which engineered yeast catalyze the conversion of *(R)*-reticuline to thebaine, in accordance with embodiments of the invention. As provided in (A) of **FIG. 23**, yeast strains are engineered to have a P_{TEF1}-*ATR1* expression cassette integrated into the *TRP1* locus and contain a single yeast artificial chromosome with the *TRP1* selective marker and three expression cassettes: P_{TDH3}- *yEcCFS*$^{1\text{-}83}$-*yPsSS*$^{95\text{-}505}$, P_{TPI1}-*yPbSalR*, and P_{TEF1}-*yPsSalAT*. Yeast were grown from freshly transformed colonies in 3 mL selective media overnight and back-diluted 1:20 into 0.5 mL selective media (-Trp) in culture tubes with 100 µM *(R)*-reticuline (Toronto Research Chemicals). After 72 hours in the shaking incubator, the yeast were pelleted and the media supernatant was analyzed by LC-MS/MS. Chromatogram traces show thebaine produced by this strain and salutaridinol and salutaridine accumulated, along with standards. These traces are representative of two samples.

Example 11: Protein engineering of enzymes in the downstream morphinan branch to improve production of morphinan products from a heterologous microbial host

[00305] In one embodiment of the invention, pathway enzymes are engineered to exhibit increased activity to increase production of the BIA of interest. In this example, mutations were introduced into the open reading frame of a particular pathway enzyme by amplification with Mutazyme II (see **Table 6**). Sufficient template DNA was included in the amplification reaction to result in a mutation rate of 1-4 nucleotide substitutions per gene. The mutagenized library was cloned into the pYES1L vector by gap repair directly in yeast. In several instances, yeast strains selected for library expression contained integrated copies of genes that generate the substrate of the mutagenized enzyme. For example, a library of CODM variants was transformed into a strain with integrated copies of T6ODM and COR1.3 and fed thebaine in the culture medium. Expression of T6ODM and COR1.3 in these strains ensured that codeine and neopine would be available as substrates for each introduced CODM variant. Individual colonies were inoculated into 96-well plates and cultured 96 hours then assayed for production of their product by liquid chromatograph mass spectrometry (LC-MS). In

the example of the CODM library, the products screened for were morphine and neomorphine. In each screen, variants with enhanced BIA production were sequenced and re-cloned for validation. **Table 6** includes a summary of mutated enzyme variants identified through the screens that resulted in increased BIA production in yeast.

[00306] FIG. 24 shows data of the validated enhanced activity of one of these mutants. In particular, **FIG. 24** illustrates generation of a CODM enzyme variant exhibiting enhanced activity in yeast through random mutagenesis and screening, in accordance with embodiments of the invention. A library of CODM variants was generated by mutagenizing the coding region by error-prone PCR. A variant identified by screening of this library, $CODM^{N35S,G335V}$, was re-cloned and expressed in a yeast strain harboring integrated copies of T6ODM and COR1.3. This strain and another control strain expressing wild-type CODM were cultured in liquid medium with 1 mM thebaine. After 96 hours the culture medium was analyzed for CODM activity by LC-MS. Variant $CODM^{N35S,G335V}$ produced 1.4x more morphine and 2.6x more neomorphine than a strain expressing wild-type CODM.

Example 12: Optimization of expression and growth conditions to improve benzylisoquinoline alkaloid production from a heterologous microbial host

[00307] Bezylisoquinoline alkaloid production from an engineered microbial host can be further improved by optimizing the expression of pathway enzymes and growth conditions. In one example, the expression of salutaridinol 7-*O*-acetyltransferase was altered in yeast by expressing the enzyme from a series of different promoters. The yeast were engineered to heterologously express yeast codon-optimized genes encoding *P. somniferum* salutaridinol 7-*O*-acetyltransferase from different promoters (as provided in (A) of **FIG. 25**). Two expression cassettes (P_{TPI1}-*yPbSalR*, P_X-*yPsSalAT*) were assembled into a yeast artificial chromosome (YAC) with a *TRP1* section marker. The YAC was placed into yeast and cells were grown in synthetic complete media with the appropriated drop out solution (-Trp) and fed salutaridine. BIA metabolites were analyzed after 72 hours of growth by LC-MS/MS analysis. The optimization of pathway enzyme expression level can result in increased production of the morphinan alkaloid thebaine (as provided in (A) of **FIG. 25**).

[00308] Optimization of strain cultivation conditions, including but not limited to sugar source, growth temperature, and pH, can be used to increase production of benzylisoquinoline alkaloids from engineered yeast strains (as provided in (B) and (C) of **FIG. 25**). In one example, pH was varied to increase thebaine production from engineered yeast strains. Two expression cassettes (P_{TPI1}-*yPbSalR*, P_{TEF1}-*yPsSalAT*) were assembled into a yeast artificial chromosome (YAC) with a *TRP1* section marker. The YAC was placed into yeast and cells were grown in synthetic complete media with the appropriated drop out solution (-Trp), resuspended in buffer at pH 5.7-9, and fed salutaridine. BIA metabolites were analyzed after 16 hours of

incubation by LC-MS/MS analysis. Levels of the 4-ring promorphinan alkaloid salutaridinol and the 5-ring morphinan alkaloid thebaine increased as a function of increasing pH (as provided in (B) of **FIG. 25**).

[00309] In another example, temperature, sugar, and media buffer content were varied to increase thebaine production from engineered yeast strains. Three expression cassettes (P_{TDH3}-*D94yPsSS*, P_{TPI1}-*yPbSalR*, P_{TEF1}-*yPsSalAT*) were assembled into a yeast artificial chromosome (YAC) with a *TRP1* section marker. The YAC was placed into yeast that harbored an expression cassette for a cytochrome P450 reductase (P_{TEF1}-*ATR1* or P_{TEF1}-y*PsCPRv2*) integrated into the chromosome. The yeast strains were grown in synthetic complete media with the appropriated drop out solution (-Trp) and fed *(R)*-reticuline. BIA metabolites were analyzed after 72 hours of growth by LC-MS/MS analysis. The analysis indicates the microbial production of the morphinan alkaloid thebaine increases under certain cultivation conditions (buffered media with dextrose at 30 °C, as provided in (C) of **FIG. 25**).

[00310] Accordingly, **FIG. 25** illustrates fermentation optimization for conversion of *(R)*-reticuline to thebaine by engineered yeast, in accordance with embodiments of the invention. LC-MS/MS analysis of whole cell buffered assay of (A) SalAT promoter variants, (B) SalR and SalAT strain grown under different pH conditions, and (C) optimization of sugar source, growth temperature, and media buffer content. (A) Yeast strains engineered to contain a single yeast artificial chromosome with the *TRP1* selective marker and two expression cassettes: P_{TPI1}-*yPbSalR* and P_X-*yPsSalAT* with varied SalAT promoters. Yeast were grown from freshly transformed colonies in 3 mL selective media overnight and back-diluted 1:20 into 0.5 mL media in culture tubes with 100 µM salutaridine (Specs). After 72 hours in the shaking incubator, the yeast were pelleted and the media supernatant was analyzed by LC-MS/MS. (B) Yeast strains engineered to contain a single yeast artificial chromosome with the *TRP1* selective marker and two expression cassettes: P_{TPI1}-*yPbSalR* and P_{TEF1}-*yPsSalAT*. Yeast were grown from freshly transformed colonies in 3 mL selective media overnight, back-diluted into 3.5 mL media to OD 0.8, grown 7 hours, pelleted, and then resuspended into pH 5.7 MOPS, or pH 7, 8, or 9 Tris buffer with 10 µM salutaridine (Specs). After 16 hours on a spinner at 30 °C, the yeast were pelleted and the buffer supernatant was analyzed by LC-MS/MS. Error bars represent the range of two samples. (C) Optimization of sugar source, growth temperature, and media buffer content. In this experiment, the yeast strains are engineered to have P_{TEF1}-*ATR1* integrated into the *TRP1* locus and contain a single yeast artificial chromosome with the *TRP1* selective marker and three expression cassettes: P_{TDH3}-*yEcCFS*$^{1\text{-}83}$-*yPsSS*$^{95\text{-}505}$, P_{TPI1}-*yPbSalR*, and P_{TEF1}-*yPsSalAT*. Yeast were grown from freshly transformed colonies in 3 mL selective media overnight and back-diluted 1:20 into 0.5 mL media in culture tubes with 100 µM *(R)*-reticuline (Toronto Research Chemicals). After 72 hours in the shaking incubator, the yeast were pelleted and the media supernatant was analyzed by LC-MS/MS.

Example 13: Yeast engineered for the production of thebaine from an early 1-benzylisoquinoline alkaloid scaffold

[00311] Yeast strains can be engineered for the production of the morphinan alkaloid thebaine, or morphinan alkaloids derived from thebaine, from early 1-benzylisoquinoline alkaloids. As an example, the engineered yeast strains can produce the morphinan alkaloid products from racemic or *(S)*-norcoclaurine or racemic or *(S)*-norlaudanosoline (**FIGs. 5, 6, 7,** and (B) of **23**). Yeast strains are engineered to produce *(S)*-reticuline from *(S)*-norcoclaurine or racemic or *(S)*-norlaudanosoline by the integration of three or five expression cassettes into the yeast genome. To produce *(S)*-reticuline from racemic or *(S)*-norlaudanosoline, the integrated expression cassettes encode *Papaver somniferum* norcoclaurine 6-*O*-methyltransferase (Ps6OMT, EC 2.1.1.128), 4'-*O*-methyltransferase (Ps4'OMT, EC 2.1.1.116), and coclaurine-*N*-methyltransferase (CNMT, EC 2.1.1.140), each with a *TEF1* promoter (Hawkins and Smolke. 2008. Nat. Chem. Biol. 4: 564-73). To produce *(S)*-reticuline from racemic or *(S)*-norcoclaurine, the strain further harbors integrated expression cassettes for yeast codon-optimized *Eschscholzia californica N*-methylcoclaurine 3'-hydroxylase (yEcCYP80B1, EC 1.14.13.71) and ATR1 or yPsCPRv2 cytochrome P450 reductase expressed from the *TDH3* or *TEF1* promoter (CPR, EC 1.6.2.4). These strains are further engineered to incorporate epimerization-catalyzing enzymes (e.g., CYP-COR), salutaridine synthase, salutaridine reductase, and salutaridinol acetyltransferase to convert racemic or *(S)*-norcoclaurine or racemic or *(S)*-norlaudanosoline to the morphinan alkaloid thebaine, or morphinan alkaloids derived from thebaine (**FIG. 7**). As an alternative to expression of an epimerization-catalyzing enzyme, 6OMT, 4'OMT, CNMT, and/or CYP80B1 may be engineered such that *rac*-reticuline is produced from *rac*-norcoclaurine or *rac*-norlaudanosoline.

[00312] In one example, a yeast strain was engineered to convert *rac*-norlaudanosoline to thebaine. The yeast strain harbors integrated expression cassettes encoding Ps6OMT, Ps4'OMT, CNMT, and yPsCPRv2, each with a *TEF1* promoter. Four expression cassettes (P_{TDH3}-*yEcCFS*$^{1\text{-}83}$-*yPsSS*$^{95\text{-}505}$, P_{TPI1}-*yPbSalR*, P_{TEF1}-*yPsSalAT,* P_{HXT7}- CYP-COR_89405) were assembled into a yeast artificial chromosome (YAC) with a *TRP1* selective marker in this strain. The yeast strain harboring the YAC and integrated cassettes was grown in synthetic complete media with the appropriated drop out solution (-Trp) and 1 mM *rac*-norlaudanosoline substrate. After 96 hours of growth, the media was analyzed for BIA metabolites by LC-MS/MS analysis. Nearly 200 nM thebaine was detected ((B) of **FIG. 23**). Other engineered salutaridine synthase variants may also be used in this strain (**FIG. 22**, **Example 10**).

Example 14: Platform yeast strains engineered for the production of reticuline from L-tyrosine

[00313] A platform yeast strain that produces the key branch point BIA intermediate *(S)*-reticuline from L-tyrosine was constructed (**FIG. 5**). Specifically, four multi-gene expression constructs were integrated into the genome of a yeast strain. The composition of the four constructs is indicated in **FIG. 26**. Each construct

is comprised of 4 or 5 genes expressed from strong constitutive promoters. Genes are positioned at each locus as complete expression cassettes comprising a promoter, gene open reading frame, and terminator as specified in the annotations above the schematic. The schematic shows the orientation of each expression cassette by the direction of the arrow representing a given gene. Selectable markers are italicized in the annotation and represented by grey arrows in the schematic. Each selection marker is flanked by loxP sites to allow removal of the marker from the locus. Additionally, each construct has a selectable marker flanked by loxP sites so that it can be removed by Cre recombinase.

[00314] In the first integration construct, four heterologous genes from *Rattus norvegicus* are integrated into the YBR197C locus together with a G418 selection marker (*KanMX*). *RnPTPS*, *RnSepR*, *RnPCD*, and *RnQDHPR* are required to synthesize and regenerate tetrahydrobiopterin (BH_4) from the yeast endogenous folate synthesis pathway. Each gene is codon optimized for expression in yeast.

[00315] In the second integration construct, four heterologous genes are integrated into the *HIS3* locus together with the HIS5 selection marker. *Rattus norvegicus* tyrosine hydroxylase (*RnTyrH*) converts tyrosine to L-DOPA using the cosubstrate BH_4 generated by the preceding integration construct. The *RnTyrH* gene can be any of the wild-type or improved mutants which confer enhanced activity (e.g., W166Y, R37E, and R38E, **Example 2**). A second *Rattus norvegicus* gene, *RnDHFR*, encodes an enzyme that reduces dihydrobiopterin (an oxidation product of BH_4) to BH_4, in this way increasing the availability of this cosubstrate. Also included in the third construct is *PpDODC* from *Pseudomonas putida*, an enzyme that converts L-DOPA to dopamine. The fourth enzyme is CjNCS from *Coptis japonica,* which condenses 4-HPA and dopamine to make norcoclaurine. Each gene is codon optimized for expression in yeast.

[00316] In the third integration construct, five heterologous genes from plants and the *LEU2* selection marker are integrated into the locus YDR514C. *Ps6OMT*, *Ps4'OMT,* and *PsCNMT* are methyltransferases from *Papaver somniferum* and are expressed as native plant nucleotide sequences. A fourth *P. somniferum* gene, y*PsCPRv2*, is codon optimized for yeast and encodes a reductase that supports the activity of a cytochrome P450 from *Eschscholzia californica*, *EcCYP80A1*. *EcCYP80A1* is expressed as its native plant nucleotide sequence. The enzymes encoded in this construct perform two *O*-methylations, an *N*-methylation, and a hydroxylation to produce reticuline from the norcoclaurine produced by the preceding integration construct.

[00317] In the final integration construct, additional copies of *Saccharomyces cerevisiae* endogenous genes *ARO4* ^{Q166K}, *ARO7* ^{T226I}, *TKL1*, and *ARO10* are integrated into the *ARO4* locus together with a hygromycin resistance selection marker. *ARO4* ^{Q166K} and *ARO7* ^{T226I} are feedback-resistant mutants of *ARO4* and *ARO10* which each encode a single base pair substitution relative to the wild-type sequence. *TKL1* and *ARO10* are identical to the native yeast genes, but are expressed behind strong promoters. Aro4p and Aro7p are enzymes in the biosynthesis of aromatic amino acids including tyrosine. Removing feedback inhibition from these enzymes results in upregulation of endogenous tyrosine biosynthesis. Overexpression of Tkl1p upregulates

the pentose phosphate pathway resulting in enhanced supply of erythrose 4-phosphate (E4P), a precursor for tyrosine. Overexpression of Aro10p increases the production of 4-HPA.

[00318] Platform yeast strains can be constructed with any number of the four expression cassettes. Specifically, platform yeast strains were constructed with integration constructs 1-4 and integration constructs 1-3. In the latter strain in which the tyrosine over-production construct (construct 4) is excluded, additional tyrosine may be supplied in the culture medium to support the biosynthesis of reticuline. Additional genetic modifications may be incorporated into the platform strains to support production of downstream BIAs and increased flux to BIA biosynthesis.

[00319] The yeast strains were grown in synthetic complete media with the appropriated amino acid drop out solution at 25 and 30 °C. BIA metabolites in the media supernatant were analyzed after 48 and 96 hours of growth by LC-MS/MS analysis.

Example 15: Yeast engineered for the production of thebaine and other morphinan alkaloids from L-tyrosine

[00320] Yeast strains can be engineered for the production of the morphinan alkaloid thebaine, or morphinan alkaloids derived from thebaine, from early precursors such as tyrosine. As an example, the platform yeast strains described in **Example 14** can be further engineered to produce the morphinan alkaloid products from L-tyrosine (**FIG. 7**).

[00321] The platform yeast strain producing *(S)*-reticuline from L-tyrosine (see description in **Example 14**) was further engineered to incorporate epimerization-catalyzing enzymes, such as the newly identified CYP-COR, salutaridine synthase, salutaridine reductase, and salutaridinol acetyltransferase to convert the biosynthesized *(S)*-reticuline to the morphinan alkaloid thebaine, or morphinan alkaloids derived from thebaine (**FIG. 7**). Three expression cassettes (P_{TDH3}-*yEcCFS*$^{1-26}$-*yPbSS*$^{33-504}$, P_{TPI1}-*yPbSalR*, P_{TEF1}-*yPsSalAT*) were assembled into a yeast artificial chromosome (YAC) with a *TRP1* selective marker directly in the platform yeast strain. Other engineered salutaridine synthase variants may also be incorporated into the YAC (**FIG. 22**, **Example 10**). The resulting yeast strain was also transformed with a low-copy CEN/ARS plasmid with a *URA3* selective marker, *TDH3* promoter, and a CYP-COR coding sequence.

[00322] The yeast strains harboring the YAC, low-copy plasmid, and integrated cassettes were grown in synthetic complete media with the appropriated drop out solution (-Ura-Trp) at 25 and 30 °C. After 96 hours of growth, the media was analyzed for BIA metabolites by LC-MS/MS analysis. Further culture optimization with respect to temperature, carbon source, pH condition, and media composition was performed to improve BIA production.

[00323] Additional genetic modifications can be introduced into the yeast strains to produce morphinan alkaloids derived from thebaine (**FIG. 7**). In one example, the expression cassettes P_{ADH1}-*T6ODM*-T_{ADH1}, P_{HXT7}-*COR*-T_{PGK1}, and P_{TEF1}-*CODM*-T_{CYC1} were directly assembled and integrated into the *trp1* locus of the thebaine-producing yeast strain (Thodey et al., 2014). In another example, these yeast strains can be further engineered to produce additional morphine alkaloids by directly assembling the expression cassettes P_{GPD}-*morA*-T_{CYC1}, P_{PGK1}-*morB*-T_{PHO5} and integrating this construct into the *ura3* locus on the chromosome (Thodey et al., 2014).

Example 16: Yeast engineered for the production of berberine and other protoberberine alkaloids from L-tyrosine

[00324] Yeast strains can be engineered for the production of the alkaloid berberine, and of protoberberine, phthalideisoquinoline, and berberine alkaloids derived from berberine or the intermediates involved in forming berberine, from L-tyrosine (**FIGs. 5, 8**). For example, three or four expression cassettes (P_{PGK1}-*PsBBE*-T_{PHO5}, P_{TEF1}-*yPsS9OMT*-T_{CYC1}, P_{TDH3}-*yCjCAS*-T_{ADH1}, with or without P_{TPI1}-*yBwSTOX*-T_{STE2}) were assembled into a yeast artificial chromosome (YAC) with a *TRP1* section marker. The YAC was placed into a platform yeast strain that produce *(S)*-reticuline from L-tyrosine (see **Example 14**) and additionally harbors an expression cassette for a cytochrome P450 reductase (P_{TEF1}-*ATR1* or P_{TEF1}-*PsCPRv2*) integrated into the chromosome. An additional high-copy plasmid containing the P_{TEF1}-*S9OMT*-T_{CYC1} expression cassette was transformed into this strain to improve flux to downstream products.

[00325] The yeast strains were grown in synthetic complete media with the appropriated amino acid drop out solution (-Trp) at 25 and 30 °C. BIA metabolites in the media supernatant were analyzed after 48 and 96 hours of growth by LC-MS/MS analysis.

Example 17: Yeast engineered for the production of noscapine and other noscapinoid alkaloids from L-tyrosine

[00326] Yeast strains can be engineered for the production of phthalideisoquinoline alkaloids such as narcotoline and noscapine, the intermediates involved in forming noscapine, or derivatives thereof from L-tyrosine (**FIGs. 5, 9**).

[00327] For example, the expression cassettes P_{ADH1}-*CAS*-T_{ADH1}, P_{HXT7}-*CYP82Y1*-T_{PGK1}, P_{TEF1}-*S9OMT*-T_{CYC1}, and P_{PGK1}-*BBE*-T_{PHO5} were directly assembled and integrated into the *trp1* locus of a platform yeast strain that produces *(S)*-reticuline from L-tyrosine (see **Example 14**) and additionally harbors an expression cassette for a cytochrome P450 reductase (P_{TEF1}-*ATR1* or P_{TEF1}-*PsCPRv2*) integrated into the chromosome. Then, the expression cassettes P_{GPD}-*TNMT*-T_{CYC1}, P_{PGK1}-*PsMT2*-T_{PHO5}, P_{ADH1}-*CYP82X1*-T_{GAP1}, and P_{PYK1}-Ps*CXE1*-T_{Mf1}

were directly assembled and integrated into the *ura3* locus of the yeast strain. Then, the expression cassettes P_{HXT7}-*CYP82X2*-T_{CYC1}, P_{GPD}-*PsAT1*-T_{ADH1}, P_{TPI1}-*PsSDR1*-T_{Ste2}, and P_{PGK1}-*PsMT3*-T_{PHO5} were directly assembled and integrated into the *leu2* locus of the yeast strain. An additional high-copy plasmid containing the P_{TEF1}-*S9OMT*-T_{CYC1} expression cassette and an additional low-copy plasmid containing the P_{HXT7}-CYP82X2-T_{CYC1} expression cassette were transformed into this yeast strain to improve flux to downstream products.

[00328] The yeast strains were grown in synthetic complete media with the appropriated amino acid drop out solution (-Trp, Ura) at 25 and 30 °C. BIA metabolites in the media supernatant were analyzed after 48 and 96 hours of growth by LC-MS/MS analysis.

Example 18: Yeast engineered for the production of sanguinarine and other benzophenanthridine alkaloids from L-tyrosine

[00329] Yeast strains can be engineered for the production of protoberberine, protopine, and benzophenanthridine alkaloids such as the end-product sanguinarine, the intermediates involved in forming sanguinarine, or derivatives thereof from L-tyrosine (**FIGs. 5, 10**).

[00330] For example, the expression cassettes P_{ADH1}-*TNMT*-T_{ADH1}, P_{HXT7}-*EcSTS*-T_{PGK1}, P_{GPD}-*EcCFS*-T_{CYC1}, and P_{PGK1}-*BBE*-T_{PHO5} were directly assembled and integrated into the *trp1* locus of a platform yeast strain that produces *(S)*-reticuline from L-tyrosine (see **Example 14**) and additionally harbors an expression cassette for a cytochrome P450 reductase (P_{TEF1}-*ATR1* or P_{TEF1}-*PsCPRv2*) integrated into the chromosome. Then, the expression cassettes P_{GPD}-*PsMSH*-T_{CYC1}, P_{PGK1}-*PsP6H*-T_{PHO5} were directly assembled and integrated into the *ura3* locus of the yeast strain.

[00331] The yeast strains were grown in synthetic complete media with the appropriated amino acid drop out solution at 25 and 30 °C. BIA metabolites in the media supernatant were analyzed after 48 and 96 hours of growth by LC-MS/MS analysis.

Example 19: Yeast engineered for the production of hydrocodone and other morphinan alkaloids from L-tyrosine

[00332] Yeast strains can be engineered for the production of hydrocodone. As an example, the thebaine-producing yeast strain described in **Example 15** can be further engineered to produce the active pharmaceutical ingredient hydrocodone. **FIG. 27** illustrates thebaine and hydrocodone production in engineered yeast strains, in accordance with embodiments of the invention. In FIG. **27A**, multi-gene constructs were incorporated into engineered yeast strains containing constructs 1-4 (**FIG. 26**). In FIG. **27B**, the integration of construct 5 increased reticuline titers relative to the strain containing constructs 1-4. In

FIG. 27C, the integration of construct 6 leads to thebaine production from sugar. Thebaine produced by the engineered strain was identified in the culture medium by LC-MS/MS analysis and compared to a commercial reference standard. In **FIG. 27D**, the introduction of construct 7 on a YAC leads to hydrocodone production from sugar. Hydrocodone produced by the engineered strain was identified in the culture medium by LC-MS/MS analysis and compared to a commercial reference standard.

[00333] In one example, the yeast strain that produces reticuline from L-tyrosine (see description in **Example 14**) was further engineered to increase reticuline production, then modified further to incorporate the thebaine-producing enzymes described in **Example 15**, and then modified further to incorporate thebaine demethylase and morphinone reductase to convert the biosynthesized thebaine to hydrocodone. Three multi-gene expression constructs were incorporated into the reticuline-producing yeast strain described in **Example 14**; two as chromosomal integrations, and a third as an episomal YAC construct.

[00334] In the first construct, genes were incorporated to increase biosynthesis of (S)-reticuline from the strain described in **Example 14**. To increase (S)-reticuline production, additional copies of *CjNCS* (to increase incorporation of dopamine into norcoclaurine) and *Ps4'OMT* (to increase incorporation of 3'-hydroxy-N-methylcoclaurine into (S)-reticuline) were incorporated into the strain. An additional copy of *RnTyrH* was also included to increase entry of tyrosine into the heterologous pathway for reticuline biosynthesis. The three expression cassettes (P_{PGK1}-*yCjNCS*-T_{PHO5}, P_{TEF1}-*Ps4'OMT*-T_{CYC1}, and P_{GPD}-*RnTyrH*^{WR}-T_{ADH1}) were integrated into locus *YPL250C* together with the ble selection marker for phleomycin resistance (**FIG. 27A**). The resulting yeast strain exhibited a 4-fold increase in reticuline accumulation (**FIG. 27B**).

[00335] In the second construct, genes were incorporated to produce thebaine from biosynthesized (S)-reticuline. Four enzymes for thebaine production described in **Example 15** were assembled as four expression cassettes (P_{HXT7}-*yPbCYP-COR*-T_{CYC1}, P_{GPD}-*yEcCFS*$^{1-83}$-*yPbSalSyn*$^{92-504}$-T_{ADH1}, P_{TPI1}-*yPbSalR*-T_{STE2}, and P_{PGK1}-*yPsSalAT*-T_{PHO5}) and integrated into locus *TRP1* with selection marker *KlURA3* (**FIG. 27A**). The resulting yeast strain produced thebaine when cultured in standard yeast culture medium (**FIG. 27C**).

[00336] In the third construct, genes were incorporated to produce hydrocodone from biosynthesized thebaine. In this example, thebaine demethylase activity was incorporated as the T6ODM enzyme from *P. somniferum* to convert thebaine to neopinone, and morphinone reductase activity was incorporated as the morB enzyme from *P. putida* to convert codeinone to hydrocodone. In this example, the intervening conversion of neopinone to codeinone occurred spontaneously. In another example, an isomerase enzyme is included to enzymatically convert neopinone to codeinone. The *T6ODM* and morB genes were codon optimized for yeast and included as two expression cassettes (P_{GPD}-*yT6ODM*-T_{ADH1} and P_{PGK1}-*yPbmorB*-T_{PHO5}) assembled into a YAC with *TRP1* selection marker (**FIG. 27A**). In this example, the yeast strain is cultured in medium supplemented with 50 mM 2-oxoglutarate to support the activity of T6ODM, a 2-oxoglutarate-dependent dioxygenase. In another example, the yeast host cell is engineered to accumulate 2-oxoglutarate at levels

sufficient to support T6ODM activity. The yeast strain harboring the described multi-gene constructs and cultured in the described culture medium biosynthesized hydrocodone from glucose (**FIG. 27D**).

Example 20: Increasing gene copy number to overcome bottlenecks in pathway flux

[00337] In some instances yeast strains can be optimized to enhance the production of benzylisoquinoline alkaloids by increasing the gene copy number for enzymes whose activity is limiting.

[00338] In one example, reticuline production in a reticuline-producing strain as described in **Example 19** was optimized by addition of a third gene copy of *NCS* (**FIG. 28**). In this example, the parent strain had one copy of *NCS* integrated at the *YMR206W* locus and another at the *YPL250C* locus, each expressed from the *PGK1* promoter. A third copy of *NCS* (expressed from a *TDH3* promoter) was integrated at the *BUD9* locus and this modification resulted in a 2-fold increase in reticuline titers. In another example, additional copies of other enzymes in the pathway are incorporated in to the strain to further increase reticuline titers.

Table 2: Enzyme List

Enzyme	Abbrev	Catalyzed Reactions	Source organisms	Similarity to naturally occurring gene	Modifications*	Genbank #
Transketolase	TKL1	fructose-6-phosphate + glyceraldehyde-3-phosphate ↔ xylulose-5-phosphate + erythrose-4-phosphate (EC 2.2.1.1)	*Saccharomyces cerevisiae*	100%	constitutive overexpression, synthetic regulation	NP_015399.1
Glucose-6-phosphate dehydrogenase	ZWF1	glucose-6-phosphate → 6-phosphogluconolactone (EC 1.1.1.49)	*Saccharomyces cerevisiae*	full deletion of coding region	full deletion of coding region	CAA96146.1
3-deoxy-d-arabinose-heptulosonate-7-	ARO4, DAHP synthase	erythrose-4-phosphate + PEP → DAHP (EC 2.5.1.54)	*Saccharomyces cerevisiae*	100%	Feedback inhibition resistant mutation, K229L, Q166K	CAA85212.1
Chorismate mutase	ARO7	chorismate → prephenate (EC 5.4.99.5)	*Saccharomyces cerevisiae*	100%	Feedback inhibition resistant mutation, T226I	NP_015385.1
Phenylpyruvate decarboxylase	ARO10	hydroxyphenylpyruvate → 4HPA (EC 4.1.1.80)	*Saccharomyces cerevisiae*	100%	constitutive overexpression, synthetic regulation	NP_010668.3
Alcohol dehydrogenase	ADH2-7, SFA1	4HPA → tyrosol (EC 1.1.1.90)	*Saccharomyces cerevisiae*	full deletion of coding region	full deletion of coding region	NP_014032.1, AAT93007.1, NP_011258.2, NP_009703.3, NP_014051.3, NP_010030.1, NP_010113.1
Aldehyde oxidase	ALD2-6	4HPA → hydroxyphenylacetic acid (EC 1.2.1.39)	*Saccharomyces cerevisiae*	full deletion of coding region	full deletion of coding region	NP_013893.1, NP_013892.1, NP_015019.1, NP_010996.2, NP_015264.1
Aromatic aminotransferase	ARO9	hydroxyphenylpyruvate + glutamate → tyrosine + alpha-ketogluterate (EC 2.6.1.57)	*Saccharomyces cerevisiae*	100%	constitutive overexpression, synthetic regulation	AEC14313.1
Tyrosinase	TYR	tyrosine → L-DOPA, L-DOPA → dopaquinone (EC 1.14.18.1)	*Ralstonia solanacearum*, *Agaricus bisporus*	100%	constitutive expression, synthetic regulation	NP_518458.1, AJ223816,
Tyrosine hydroxylase	TyrH	tyrosine → L-DOPA (EC 1.14.16.2)	*Homo sapiens*, *Rattus norvegicus*, *Mus musculus*	100%	constitutive expression, synthetic regulation	NM 012740, NM 000240
L-DOPA decarboxylase	DODC	L-DOPA → dopamine (EC 4.1.1.28)	*Pseudomonas putida*, *Rattus norvegicus*	100%	constitutive expression, synthetic regulation	AE015451.1, NP_001257782.1
Tyrosine/DOPA decarboxylase	TYDC	L-DOPA → dopamine (EC 4.1.1.28) tyrosine →tyramine (EC 4.1.1.25)	*Papaver somniferum*	100%	constitutive expression, synthetic regulation	AAA97535

Monoamine oxidase	MAO	dopamine → 3,4-DHPA (EC 1.4.3.4)	*Escherichia coli, Homo sapiens, Micrococcus luteus*	77%	constitutive expression, synthetic regulation; codon optimized for expression in *Saccharomyces cerevisiae*	J03792, D2367, AB010716.1
Norcoclaurine synthase	NCS	4HPA + dopamine → S- norcoclaurine (EC 4.2.1.78) 3,4-DHPA + dopamine → S-norlaudanosoline	*Coptis japonica, Papaver somniferum, Papaver bracteatum, Thalictrum flavum, Corydalis saxicola*	80%	constitutive expression, synthetic regulation; N-terminal truncation; codon optimized for expression in *Saccharomyces cerevisiae*	BAF45337.1, AB267399.2,A CI45396.1,AC O90258.1, ACO90247.1, AEB71889.1
Norcoclaurine 6-O-methyltransferase	6OMT	Norcoclaurine → coclaurine Norlaudanosoline → 3'hydroxycoclaurine (EC 2.1.1.128)	*P. somniferum* *T. flavum* *Coptis japonica**	100% 100% 100%	constitutive expression, synthetic regulation	AY268894 AY610507 D29811
Coclaurine-N-methyltransferase	CNMT	Coclaurine → N-methylcoclaurine 3'hydroxycoclaurine → 3'-hydroxy-N-methylcoclaurine (EC 2.1.1.140)	*P. somniferum* *T. flavum* *Coptis japonica**	100%	constitutive expression, synthetic regulation	AY217336 AY610508 AB061863
4'-O-methyltransferase	4'OMT	3'-hydroxy-N-methylcoclaurine → Reticuline (EC 2.1.1.116)	*P. somniferum* *T. flavum* *Coptis japonica**	100% 100% 100%	constitutive expression, synthetic regulation	AY217333, AY217334 AY610510 D29812
Cytochrome P450 80B1	CYP80B1	N-methylcoclaurine → 3'-hydroxy-N-methylcoclaurine	*P. somniferum, E. californica, T. flavum*	77%	constitutive expression, synthetic regulation; codon optimized for expression in *Saccharomyces cerevisiae*	AAF61400.1 AAC39453.1 AAU20767.1
GTP cyclohydrolase	FOL2	GTP → dihydroneopterin triphosphate (EC 3.5.4.16)	*Saccharomyces cerevisiae, Homo sapiens, Mus musculus*	100%	native regulation, constitutive overexpression, synthetic regulation	CAA97297.1, NP_001019195.1, NP_032128.1
6-pyruvoyl tetrahydrobiopterin (PTP) synthase	PTPS	dihydroneopterin triphosphate → PTP (EC 4.2.3.12)	*Rattus norvegicus, Homo sapiens, Mus musculus*	80%	constitutive expression, synthetic regulation; codon optimized for expression in *Saccharomyces cerevisiae*	AAH59140.1, BAA04224.1, AAH29013.1
Sepiapterin reductase	SepR	PTP → BH4 (EC 1.1.1.153)	*Rattus norvegicus, Homo sapiens, Mus musculus*	72%	constitutive expression, synthetic regulation; codon optimized for expression in *Saccharomyces cerevisiae*	NP_062054.1, NP_003115.1, NP_035597.2
4a-hydroxytetrahydrobiopterin (pterin-4α-carbinolamine)	PCD	4a-hydroxytetrahydrobiopterin → H2O + quinoid dihydropteridine (EC 4.2.1.96)	*Rattus norvegicus, Homo sapiens, Mus musculus*	79%	constitutive expression, synthetic regulation; codon optimized for expression in *Saccharomyces cerevisiae*	NP_001007602.1, AAB25581.1, NP_079549.1
Quinoid dihydropteridine reductase	QDHPR	quinoid dihydropteridine → BH4 (EC 1.5.1.34)	*Rattus norvegicus, Homo sapiens, Mus musculus*	75%	constitutive expression, synthetic regulation; codon optimized for expression in *Saccharomyces cerevisiae*	AAH72536.1, NP_000311.2, AAH02107.1

Dihydrofolate reductase	DHFR	7,8-Dihydrobiopterin → 5,6,7,8-Tetrahydrobiopterin (BH4) (EC 1.5.1.3)	*Rattus norvegicus*, *Homo sapiens*	77%	constitutive expression, synthetic regulation; codon optimized for expression in *Saccharomyces cerevisiae*	AF318150.1
1-benzylisoquinoline alkaloid epimerase (cytochrome P450 82Y2-codeinone reductase)	CYP-COR	(S)-reticuline -> (R)-reticuline (S)-1-benzylisoquinoline->(R)-1-benzylisoquinoline (EC 1.5.1.27)	*Papaver bracteatum*, *Papaver somniferum*, *Papaver setigerum*, *Chelidonium majus*	77%	constitutive expression, synthetic regulation; Isolated from transcriptome data set and codon optimized for expression in *Saccharomyces cerevisiae*	amino acid sequences listed in Table 1
(R)-reticuline,NADPH:oxygen oxidoreductase (C-C phenol-coupling), also known as salutaridine synthase	SalSyn	(R)-reticuline → salutaridine (EC 1.14.21.4)	*Papaver somniferum*, *Papaver spp* *Chelidonium majus*	78%	Codon-optimized by DNA2.0 for expression in *Saccharomyces cerevisiae*; N-terminus engineering	EF451150 (Farrow, et al. 2012. Phytochemistry. 77: 79-88)
salutaridinol:NADP+ 7-oxidoreductase, also known as salutaridine reductase	SalR	salutaridinol ↔ salutaridine (EC 1.1.1.248)	*Papaver somniferum*, *Papaver bracteatum*, *Papaver spp* *Chelidonium majus*	80-100%	constitutive expression, synthetic regulation ; Codon-optimized by DNA2.0 for expression in *Saccharomyces cerevisiae*; site-directed mutants	DQ316261, EF184229 (Farrow, et al. 2012. Phytochemistry. 77: 79-88)
acetyl-CoA:salutaridinol 7-O-acetyltransferase	SalAT	acetyl-CoA + salutaridinol → CoA + 7-O-acetylsalutaridinol (EC 2.3.1.150)	*Papaver somniferum*, *Papaver bracteatum*, *Papaver orientale*, *Papaver spp*	77-80%	constitutive expression, synthetic regulation ; Codon-optimized by Life Technologies or DNA2.0 for expression in *Saccharomyces cerevisiae*	AF339913, FJ200355, FJ200358, FJ200356, JQ659008
Thebaine 6-O demethylase	T6ODM	thebaine → neopinone (EC 1.14.11.31)	*Papaver somniferium*, *Papaver spp.*	76.2%	constitutive expression, synthetic regulation; Codon optimized for expression in *Saccharomyces cerevisiae*	GQ500139.1
Codeinone reductase	COR	codeinone→ codeine (EC 1.1.1.247), neopinone→ neopine	*Papaver somniferium*, *Papaver spp.*	77.7% 76.8% 77.3% 77.0%	constitutive expression, synthetic regulation; Codon optimized for expression in *Saccharomyces cerevisiae*; addition of targeting sequences for mitochondria, vacuole, ER.	AF108432.1 AF108433.1 AF108434.1 AF108435.1

Codeine O-demethylase	CODM	codeine→ morphine (EC 1.14.11.32), neopine→ neomorphine	*Papaver somniferium*, *Papaver spp.*	75.2%	constitutive expression, synthetic regulation; Codon optimized for expression in *Saccharomyces cerevisiae*; addition of targeting sequence for mitochondria	GQ500141.1
(S)-reticuline:oxygen oxidoreductase (methylene-bridge-forming), also known as berberine bridge enzyme	BBE	(S)-reticuline → (S)-scoulerine (EC 1.21.3.3)	*Papaver somniferum*, *Argemone mexicana*, *Eschscholzia californica*, *Berberis stolonifera*, *Thalictrum flavum subsp. glaucum*, *Coptis japonica*, *Papaver spp*, *Eschscholzia spp*, *Berberis spp*, *Thalictrum spp*, *Coptis spp*	99%	constitutive expression, synthetic regulation	AF025430, EU881889, EU881890, S65550 AF005655, AF049347, AY610511, AB747097
S-adenosyl-L-methionine:(S)-scoulerine 9-O-methyltransferase	S9OMT	S-adenosyl-L-methionine + (S)-scoulerine → S- adenosyl-L-homocysteine + (S)-tetrahydrocolumbamine (EC 2.1.1.117)	*Thalictrum flavum subsp. glaucum*, *Papaver somniferum*, *Coptis japonica*, *Coptis chinensis*, *Thalictrum spp*, *Coptis spp*, *Papaver spp*	100% 80%	constitutive expression, synthetic regulation; codon-optimized by Life Technologies for expression in *Saccharomyces cerevisiae*	AY610512, D29809, EU980450, JN185323
(S)-tetrahydrocolumbamine,NA D PH:oxygen oxidoreductase (methylenedioxy-bridge-forming), also known as (S)-canadine synthase	CAS	(S)-tetrahydrocolumbamine → (S)-canadine (EC 1.14.21.5)	*Thalictrum flavum subsp. glaucum*, *Coptis japonica*, *Thalictrum spp*, *Coptis spp*	100%	constitutive expression, synthetic regulation	AY610513, AB026122, AB374407, AB374408
(S)-tetrahydroprotoberberine oxidase	STOX	(S)-tetrahydroberberine + 2 O_2 → berberine + 2 H_2O_2 (EC 1.3.3.8)	*Berberis wilsonae*, *Coptis japonica*, *Berberis spp*, *Coptis spp*	78%	constitutive expression, synthetic regulation; Codon-optimized by GeneArt for expression in *Saccharomyces cerevisiae*	HQ116697, AB564543
Tetrahydroprotoberberine-N-methyltransferase	TNMT	Stylopine → cis-N-methylstylopine (EC 2.1.1.122) Canadine → N-methylcanadine	*P. somniferum* *E. californica* *P. bracteatum* *A. mexicana*	100% 81%	constitutive expression, synthetic regulation; codon-optimized by Life Technologies for expression in *Saccharomyces cerevisiae*	DQ028579 EU882977 EU882994 HQ116698

N-methylcanadine 14-hydroxylase	CYP82Y1	N-methylcanadine → 1-hydroxy-N-methylcanadine	*P. somniferum*, *Papaver spp, Plantago arenaria, Rauwolfia heterophylla, Adlumia fungosa, Hydrastis Canadensis, Stylomecon heterophylla, Hypecoum leptocarpum, Dactylicapnos torulosa, Glaucium flavum, Berberis laurina, B. Vulgaris, Corydalis spp, Fumaria spp, Dactylicapnos spp*	70 (N-terminus engineering)-78%	constitutive expression, synthetic regulation; Codon-optimized by Life Technologies for expression in *Saccharomyces cerevisiae*; N-terminus engineering	JQ65900651
1-hydroxy-N-methylcanadine 13-hydroxylase	CYP82X2	1-hydroxy-N-methylcanadine → 1, 13-dihydroxy-N-methylcanadine	*P. somniferum*, *Papaver spp, Plantago arenaria, Rauwolfia heterophylla, Adlumia fungosa, Hydrastis Canadensis, Stylomecon heterophylla, Dactylicapnos torulosa, Glaucium flavum, Berberis laurina, B. Vulgaris, Corydalis spp, Fumaria spp, Dactylicapnos spp*	70 (N-terminus engineering)-77%	constitutive expression, synthetic regulation; Codon-optimized by Life Technologies for expression in *Saccharomyces cerevisiae*; N-terminus engineering	JQ659004.1
4'-O-Desmethyl-3-O-acetylpapaveroxine synthase	CYP82X1	1-Hydroxy-13-O-acetyl-N-methylcanadine → 4'-O-Desmethyl-3-O-acetylpapaveroxine 1-hydroxy-N-methylcanadine → 4'-O-desmethylmacrantaldehyde	*P. somniferum*, *Papaver spp, Plantago arenaria, Rauwolfia heterophylla, Adlumia fungosa, Hydrastis Canadensis, Stylomecon heterophylla, Hypecoum leptocarpum, Dactylicapnos torulosa, Glaucium flavum, Berberis laurina, B. Vulgaris, Corydalis spp, Fumaria spp, Dactylicapnos spp*	71(N-terminus engineering)-77%	constitutive expression, synthetic regulation; Codon-optimized by Life Technologies for expression in *Saccharomyces cerevisiae*; N-terminus engineering	JQ659002.1
Cheilanthifoline synthase	CFS	Scoulerine → cheilanthifoline (EC 1.14.21.2)	*P. somniferum* *E. californica* *A. mexicana*	79% 77% 78%	constitutive expression, synthetic regulation; Codon optimized for expression in *Saccharomyces cerevisiae*	GU325749 AB434654 EF451152

Stylopine synthase	STS	Cheilanthifoline → stylopine (EC 1.14.21.1)	*P. somniferum* *E. californica* *A. mexicana*	79% 78% 76%	constitutive expression, synthetic regulation; Codon optimized for expression in *Saccharomyces cerevisiae*	GU325750 AB126257 EF451151
Cis-N-methylstylopine 14-hydroxylase	MSH	cis-N-methylstylopine → protopine (EC 1.14.13.37)	*P. somniferum*	79%	constitutive expression, synthetic regulation; Codon optimized for expression in *Saccharomyces cerevisiae*	KC154003
Protopine-6-hydroxylase	P6H	Protopine → 6-hydroxyprotopine (EC 1.14.13.55)	*E. californica* *P. somniferum*	79%	constitutive expression, synthetic regulation; Codon optimized for expression in *Saccharomyces cerevisiae*	AB598834 AGC92397
Dihydrobenzophenanthridine oxidase	DBOX	Dihydrosanguinarine → sanguinarine (EC 1.5.3.12)	*P. somniferum*	100%	constitutive expression, synthetic regulation; Codon optimized for expression in *Saccharomyces cerevisiae*	[not in genbank]
Narcotoline 14-O-methylase I	MT2	Narcotoline → Noscapine Narcotinehemiacetal → 4'-O-desmethyl-3-O-acetylpapaveroxine → 3-O-acetylpapveroxine	*P. somniferum*, *Papaver spp, Fumaria parviflora, Plantago arenaria, Rauwolfia heterophylla*	80%	constitutive expression, synthetic regulation; Codon-optimized by Life Technologies for expression in *Saccharomyces cerevisiae*	JQ659000.1
1, 13-dihydroxy-N-methylcanadine 13-O acetyl transferase	AT1	1, 13-dihydroxy-N-methylcanadine → 1-hydroxy-13-O-acetyl-N-methycanadine	*P. somniferum*, *Papaver spp, Plantago arenaria, Rauwolfia heterophylla, Adlumia fungosa, Hydrastis Canadensis, Stylomecon heterophylla, Hypecoum leptocarpum, Dactylicapnos torulosa, Glaucium flavum, Berberis laurina, B. Vulgaris, Corydalis spp, Fumaria spp, Dactylicapnos spp*	81%	constitutive expression, synthetic regulation; Codon-optimized by Life Technologies for expression in *Saccharomyces cerevisiae*	JQ659008.1

Narcotinehemiacetal synthase	CXE1	4'-O-desmethyl-3-O-acetylpapaveroxine → Narcotolinehemiacetal Papaveroxine → Narcotinehemiacetal	*P. somniferum, Papaver spp, Plantago arenaria, Rauwolfia heterophylla, Adlumia fungosa, Hydrastis Canadensis, Stylomecon heterophylla, Hypecoum leptocarpum, Dactylicapnos torulosa, Glaucium flavum, Berberis laurina, B. Vulgaris, Corydalis spp, Fumaria spp, Dactylicapnos spp*	78%	constitutive expression, synthetic regulation; Codon-optimized by Life Technologies for expression in *Saccharomyces cerevisiae*	JQ659006.1
Narcotinehemiacetal synthase	CXE2	4'-O-desmethyl-3-O-acetylpapaveroxine → Narcotolinehemiacetal Papaveroxine → Narcotinehemiacetal	*P. somniferum, Papaver spp, Plantago arenaria, Rauwolfia heterophylla, Adlumia fungosa, Hydrastis Canadensis, Stylomecon heterophylla, Hypecoum leptocarpum, Dactylicapnos torulosa, Glaucium flavum, Berberis laurina, B. Vulgaris, Corydalis spp, Fumaria spp, Dactylicapnos spp*	78%	constitutive expression, synthetic regulation; Codon-optimized by Life Technologies for expression in *Saccharomyces cerevisiae*	KJ890443.1
Noscapine synthase	SDR1	Narcotolinehemiacetal → Narcotoline Narcotinehemiacetal → Noscapine	*P. somniferum, Papaver spp, Plantago arenaria, Rauwolfia heterophylla, Adlumia fungosa, Hydrastis Canadensis, Stylomecon heterophylla, Hypecoum leptocarpum, Dactylicapnos torulosa, Glaucium flavum, Berberis laurina, B. Vulgaris, Corydalis spp, Fumaria spp, Dactylicapnos spp*	79%	constitutive expression, synthetic regulation; Codon-optimized by Life Technologies for expression in *Saccharomyces cerevisiae*	JQ659007.1

Narcotoline 14-O-methylase II	MT3	Narcotoline → Noscapine Narcotolinehemiacetal → Narcotinehemiacetal 4'-O-desmethyl-3-O-acetylpapaveroxine → 3-O-acetylpapveroxine	*P. somniferum, Papaver spp, Fumaria parviflora, Plantago arenaria, Rauwolfia heterophylla*	79%	constitutive expression, synthetic regulation; Codon-optimized by Life Technologies for expression in *Saccharomyces cerevisiae*	JQ659001.1
Morphine dehydrogenase	morA	morphine → morphinone (EC 1.1.1.218), codeinone → codeine (EC 1.1.1.247)	*Pseudomonas putida*	73.7%	constitutive expression, synthetic regulation; Codon optimized for expression in *Saccharomyces cerevisiae;* introduced mutation morAC81S	M94775.1
Morphinone reductase	morB	codeinone → hydrocodone morphinone → hydromorphone (EC 1.3.1.)	*Pseudomonas putida*	67.2%	constitutive expression, synthetic regulation; Codon optimized for expression in *Saccharomyces cerevisiae;* introduced mutation morBE160G	U37350.1
NADPH:hemoprotein oxidoreductase, also known as cytochrome P450 reductase	ATR1, CPR	NADPH + H+ + n oxidized hemoprotein → NADP+ + n reduced hemoprotein (EC 1.6.2.4)	*Arabidopsis thaliana*, all plants (*e.g., P. somniferum, E. californica*), yeast (*e.g., S. cerevisiae*), *H. sapiens*	100%	constitutive expression, synthetic regulation at various promoter strengths	NM118585, many others (Jensen and Moller. 2010. Phytochemistry. 71: 132-41)
Cytochrome P450, family 2, subfamily D, polypeptide 6	CYP2D6	Promiscuous oxidase, can perform (R)-reticuline → salutaridine, among other reactions (EC 1.14.14.1)	*Homo sapiens*	73%	constitutive expression, synthetic regulation; Codon-optimized by DNA2.0 for expression in *Saccharomyces cerevisiae*	BC067432
Berbamunine synthase Cytochrome P450 80A1	CYP80A1	(S)-N-methylcoclaurine + (R)-N-methylcociaurine → berbamunine (EC 1.14.21.3)	*Berberis stolonifera*	76%	constitutive overexpression, synthetic regulation; codon-optimized for expression in *Saccharomyces cerevisiae*	UO9610.1

Protopine O-dealkylase	PODA	*O,O-demethylenation of canadine, stylopine and berberine*	*P. somniferum, Papaver spp.*	70-100%	Constitutive overexpression, synthetic regulation; codon-optimized for expression in *Saccharomyces cerevisiae*	GQ500140.1

Table 3. Tailoring enzymes

Reaction Catalyzed	*Enzyme*	*Species*
Carbon-carbon coupling	Berberine bridge enzyme (BBE)	Ps, Ec, Cj, Bs, Tf
	Salutaridine synthase (SalSyn)	Ps
	Corytuberine synthase (CorSyn)	Cj
Oxidation	Tetrahydroprotoberberine oxidase (STOX)	Cj, Am, Bw
	Dihydrobenzophenanthridine oxidase (DBOX)	Ps
	Methylstylopine hydroxylase (MSH)	Ps
	Protopine 6-hydroxylase (P6H)	Ps, Ec
Methylenedioxy bridge formation	Stylopine synthase (StySyn)	Ps, Ec, Am
	Cheilanthifoline synthase (CheSyn)	Ps, Ec, Am
	Canadine synthase (CAS)	Tf, Cc
O-methylation	Norcoclaurine 6-*O*-methyltransferase (6OMT)	Ps, Tf, Cj, Pb
	3'hydroxy-*N*-methylcoclaurine 4'-*O*-methyltransferase (4'OMT)	Ps, Tf, Cj, Cc
	Reticuline 7-*O*-methyltransferase (7OMT)	Ps, Ec
	Scoulerine 9-*O*-methyltransferase (9OMT)	Ps, Tf, Cj, Cc
N-methylation	Coclaurine *N*-methyltransferase (CNMT)	Ps, Tf, Cj
	Tetrahydroprotoberberine *N*-methyltransferase (TMNT)	Ps, Ec, Pb
O-demethylation	Thebaine demethylase (T6ODM)	Ps
	Codeine demethylase (CODM)	Ps, Ga
Reduction	Salutaridine reductase (SalR)	Ps, Pb, Ga
	Codeinone reductase (COR)	Ps
	Sanguinarine reductase (SanR)	Ec
Acetylation	Salutaridine acetyltransferase (SalAT)	Ps

Table 4: Comparison of impurities that may be present in concentrate of poppy straw (or opium) and clarified yeast culture medium.

Impurities:		Concentrate of Poppy Straw	Clarified Yeast Culture Medium
Inorganic	Sodium	✓	✓
	Magnesium	✓	✓
	Silicon	✓	✗ (not in culture medium)
	Phosphorus	✓	✓
	Sulfur	✓	✓
	Chloride	✓	✓
	Potassium	✓	✓
	Calcium	✓	✓
	Copper	✓	✓
	Zinc	✓	✓
	Molybdenum	✓	✓ (residual molybdenum in medium)
	Iron	✓	✓
	Manganese	✓	✓
	Ammonium	✓	✓
	Boron	✓	✓
Organic	Polysaccharides (starch, cellulose, xylan)	✓	✗ (yeast not secrete sugars)
	Lignin (p-coumaryl, coniferyl, sinapyl alcohols)	✓	✗
	Pigments (chlorophyll, anthocyanins, carotenoids)	✓	✗
	Flavonoids	✓	✗
	Phenanthrenoids	✓	✗
	Latex, gum, and wax	✓	✗
	Rubisco	✓	✗
	Meconic acid	✓	✗
	Pseudomorphine	✓	✗
	Narceine	✓	✗
	Thebaol	✓	✗
Other	Pesticides, Fungicides, Herbicides	✓	✗
	Pollen	✓	✗

Table 5: Distinct groups of molecules present in clarified yeast culture medium (CYCM). Unlike concentrate of poppy straw (CPS) or opium, yeast host strains may be engineered to produce molecules of a predetermined class of alkaloids (i.e., only one biosynthesis pathway per strain) such that other classes of alkaloids are not present. Therefore, the CYCM may contain molecules within a single biosynthesis pathway including a subset of molecules spanning one or two columns, whereas the CPS may contain a subset of molecules across many columns.

1-Benzylisoquinoline	Protoberberine and Phthalideisoquinoline	Morphinan	Isoparvine	Aporphine	BisBA
Tetrahydropapaverine	*Scoulerine*	*Salutaridine*	*Parine*	*Magnoflorine*	*Dauricine*
Dihydropapaverine	*Cheilanthifoline*	*Salutaridinol*	*Caryachine*	*Corydaberine*	*Berbamunine*
Papaverine	*Stylopine*	*Salutaridine 7-O-acetate*	*Bionoraragemonine*	*Apocamphine*	*Liguraline*
	Cis-N-methylstylopine	*Thebaine*	*Isoneoraemonine*	*Boldine*	*Fangchinoline*
	Protopine	*Codeinone*			*Tetrandrine*
	Dihydrosanguirarine	*Oripavine*			*Curine*
	Sanguinarine	*Morphinone*			*Cepharanthine*
	Tetrahydrocolumbamine	*Neopinone*			*Berbamine*
	Canadine	*Neopine*			
	N-methylcanadine	*Codeine*			
	Noscapine	*Morphine*			
	Berberine	*Neomorphine*			
	Narcotoline	*Hydrocodone*			
	Narcotolinehemiacetal	*Oxycodone*			
	Narcotinehemiacetal	*14-hydroxycodeinone*			
		14-hydroxycodeine			
		Dihydromorphine			
		Dihydrocodeine			
		Oxymorphone			
		Hydrocodone			

Table 6. Beneficial mutations of various enzymes identified by mutagenesis screening.

Gene	GenBank ID#	Substrate(s)	Product(s)	Mutations
PsCODM	ADD833311	Codeine, Neopine	Morphine, Neomorphine	N33S, G33V (Figure 21)
PsT6ODM	ADD833291	Thebaine, Oripavine	Neopinone, Morphinone	M51T, A202V, P229S, D96H L138M
PsCOR1.3	AAF13738.1	Codeinone, Neopinone	Codeine, Neopine	C220G
PsMOMT	AAP43315.1	Norcoclaurine	Coclaurine	D322N

[00339] Notwithstanding the appended clauses, the disclosure is also defined by the following clauses:

1. An engineered non-plant cell having increased tyrosine hydroxylase activity relative to a non-engineered cell, the engineered non-plant cell having at least one modification selected from the group consisting of: a substrate inhibition alleviating mutation; a product inhibition alleviating mutation; and a cofactor recovery promoting mechanism.

2. The engineered non-plant cell of clause 1, wherein the at least one modification comprises a substrate inhibition alleviating mutation.

3. The engineered non-plant cell of clause 2, wherein the substrate inhibition alleviating mutation comprises a point mutation W166Y.

4. The engineered non-plant cell of clause 1, wherein the at least one modification comprises at least one product inhibition alleviating mutation that relieves competitive binding at a cosubstrate binding site.

5. The engineered non-plant cell of clause 4, wherein the at least one product inhibition alleviating mutation comprises a point mutation S40D.

6. The engineered non-plant cell of clause 4, wherein the at least one product inhibition alleviating mutation comprises joint mutations R37E and R38E.

7. The engineered non-plant cell of clause 6, wherein the joint mutations R37E and R38E improve tyrosine hydroxylase activity in the presence of dopamine.

8. The engineered non-plant cell of clause 1, wherein the at least one modification comprises a product inhibition alleviating mutation that relieves irreversible product inhibition.

9. The engineered non-plant cell of clause 8, wherein the product inhibition alleviating mutation comprises a point mutation E332D.

10. The engineered non-plant cell of clause 8, wherein the product inhibition alleviating mutation comprises a point mutation Y371F.

11. The engineered non-plant cell of clause 8, wherein the product inhibition alleviating mutation relieves irreversible binding of catecholamine to iron in an active site.

12. The engineered non-plant cell of clause 1, wherein the at least one modification comprises a cofactor recovery promoting mechanism.

13. The engineered non-plant cell of clause 12, wherein the cofactor recovery promoting mechanism comprises a heterologous coding sequence that encodes dihydrofolate reductase.

14. The engineered non-plant cell of clause 13, wherein dihydrofolate reductase produced by the engineered non-plant cell catalyzes conversion of dihydrobiopterin to tetrahydrobiopterin within the engineered non-plant cell.

15. A method for forming a product stream having a benzylisoquinoline alkaloid product, comprising:

(a) providing engineered non-plant cells and a feedstock including nutrients and water to a batch reactor, which engineered non-plant cells have at least one modification selected from the group consisting of: a substrate inhibition alleviating mutation; a product inhibition alleviating mutation; and a cofactor recovery promoting mechanism

(b) in said batch reactor, subjecting said engineered non-plant cells to fermentation by incubating said engineered non-plant cells for a time period of at least about 5 minutes to produce a solution comprising said benzylisoquinoline alkaloid product and cellular material; and

(c) using at least one separation unit to separate said benzylisoquinoline alkaloid product from said cellular material to provide said product stream comprising said benzylisoquinoline alkaloid product.

16. The method of clause 15, wherein at least one process parameter of the batch reactor is modifiable to alter a resultant benzylisoquinoline alkaloid product composition.

17. The method of clause 16, wherein the at least one process parameter that is modifiable comprises at least one of dissolved oxygen, pH, stirring speed, aeration rate, and cell density.

18. The method of clause 15, wherein the benzylisoquinoline alkaloid product comprises a benzylisoquinoline alkaloid precursor.

19. The method of clause 18, wherein the benzylisoquinoline alkaloid precursor is selected from the group of norcoclaurine, norlaudanosoline, tyrosine, tyramine, 4-hydroxyphenylacetaldehyde, 4-hydroxyphenylpyruvic acid, L-3,4-dihydroxyphenylalanine, 3,4-dihydroxyphenylacetaldehyde, and dopamine.

20. The method of clause 15, wherein the benzylisoquinoline alkaloid product comprises a benzylisoquinoline alkaloid.

21. The method of clause 20, wherein the benzylisoquinoline alkaloid has a structural class that is selected from the group of benzylisoquinolines, protoberberines, protopines, benzophenanthridines, promorphinans, morphinans, secoberberines, phthalideisoquinolines, aporphines, and bisbenzylisoquinolines.

22. The method of clause 21, wherein the benzylisoquinoline alkaloid is a benzylisoquinoline that is selected from the group of coclaurine, 3'-hydroxycoclaurine, 4'-*O*-methylnorlaudanosoline, 4'-*O*-methyl-laudanosoline, *N*-methylnorcoclaurine, laudanosoline, *N*-methylcoclaurine, 3'-hydroxy-*N*-methylcoclaurine, reticuline, norreticuline, papaverine, laudanine, laudanosine, tetrahydropapaverine, 1,2-dihydropapaverine, and orientaline.

23. The method of clause 21, wherein the benzylisoquinoline alkaloid is a protoberberine that is selected from the group of scoulerine, cheilanthifoline, stylopine, nandinine, jatrorrhizine, stepholidine, discretamine, *cis*-*N*-methylstylopine, tetrahydrocolumbamine, palmatine, tetrahydropalmatine, columbamine,

canadine, *N*-methylcanadine, 1-hydroxycanadine, berberine, *N*-methyl-ophiocarpine, 1,13-dihydroxy-*N*-methylcanadine, and 1-hydroxy-10-*O*-acetyl-*N*-methylcanadine.

24. The method of clause 21, wherein the benzylisoquinoline alkaloid is a protopine that is selected from the group of protopine, 6-hydroxyprotopine, allocryptopine, cryptopine, muramine, and thalictricine.

25. The method of clause 21, wherein the benzylisoquinoline alkaloid is a benzophenanthridine that is selected from the group of dihydrosanguinarine, sanguinarine, dihydrocheilirubine, cheilirubine, dihydromarcapine, marcapine, and chelerythrine.

26. The method of clause 21, wherein the benzylisoquinoline alkaloid is a promorphinan that is selected from the group of salutaridine, salutaridinol, and salutaridinol-7-*O*-acetate.

27. The method of clause 21, wherein the benzylisoquinoline alkaloid is a morphinan that is selected from the group of thebaine, codeinone, codeine, morphine, morphinone, oripavine, neopinone, neopine, neomorphine, hydrocodone, dihydrocodeine, 14-hydroxycodeinone, oxycodone, 14-hydroxycodeine, morphinone, hydromorphone, dihydromorphine, dihydroetorphine, ethylmorphine, etorphine, metopon, buprenorphine, pholcodine, heterocodeine, and oxymorphone.

28. The method of clause 21, wherein the benzylisoquinoline alkaloid is a secoberberine that is selected from the group of 4'-*O*-desmethylmacrantaldehyde, 4'-*O*-desmethylpapaveroxine, 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine, and 3-*O*-aceteylpapaveroxine.

29. The method of clause 21, wherein the benzylisoquinoline alkaloid is a phthalideisoquinoline that is selected from the group of narcotolinehemiacetal, narcotinehemiacetal, narcotoline, and noscapine.

30. The method of clause 21, wherein the benzylisoquinoline alkaloid is an aporphine that is selected from the group of magnoflorine, corytuberine, apomorphine, boldine, isoboldine, isothebaine, isocorytuberine, and glaufine.

31. The method of clause 21, wherein the benzylisoquinoline alkaloid is a bisbenzylisoquinoline that is selected from the group of berbamunine, guattgaumerine, dauricine, and liensinine.

32. A method for forming a product stream having a benzylisoquinoline alkaloid product, comprising:

(a) providing engineered non-plant cells having increased tyrosine hydroxylase activity relative to a cell that expresses wild-type tyrosine hydroxylase without mutations that increase tyrosine hydroxylase activity, a feedstock including nutrients, and water to a reactor;

(b) in said reactor, subjecting said engineered non-plant cells to fermentation by incubating said engineered non-plant cells for a time period of at least about 5 minutes to produce a solution comprising cellular material and said benzylisoquinoline alkaloid product, wherein said solution comprises not more than

one class of molecule selected from the group of protoberberine, morphinan, isopavine, aporphine, and bisbenzylisoquinoline; and

(c) using at least one separation unit to separate said benzylisoquinoline alkaloid product from said cellular material to provide said product stream comprising said benzylisoquinoline alkaloid product.

33. The method of clause 32, wherein the product stream does not contain more than 5ppm of a molecule selected from the group of lignin, flavonoids, phenanthreoids, latex, rubisco, meconic acid, pseudomorphine, narceine, thebaol, and pollen.

34. The method of clause 33, wherein the product stream does not contain more than 5ppm of meconic acid.

35. The method of clause 32, wherein the product stream does not contain a detectable amount of a substance selected from the group consisting of pesticides, fungicides, or herbicides.

36. The method of clause 32, wherein the benzylisoquinoline alkaloid product is recovered from said product stream using at least liquid-liquid extraction.

37. The method of clause 36, wherein the benzylisoquinoline alkaloid product is recovered immediately after a fermentation process has been completed.

38. A method of epimerizing an *(S)*-1-benzylisoquinoline alkaloid to an *(R)*-1-benzylisoquinoline alkaloid, comprising:

contacting the *(S)*-1-benzylisoquinoline alkaloid with at least one enzyme,

wherein contacting the *(S)*-1-benzylisoquinoline alkaloid with the at least one enzyme converts the *(S)*-1-benzylisoquinoline alkaloid to an *(R)*-1-benzylisoquinoline alkaloid.

39. The method of clause 38, wherein the at least one enzyme is produced by culturing an engineered non-plant cell having a coding sequence for encoding the at least one enzyme.

40. The method of clause 39, further comprising:

adding an *(S)*-1-benzylisoquinoline alkaloid to the cell culture.

41. The method of clause 40, further comprising:

recovering the *(R)*-1-benzylisoquinoline alkaloid, or a derivative thereof, from the cell culture.

42. The method of any one of clauses 38-41, wherein the at least one enzyme comprises an oxidase.

43. The method of any one of clauses 38-42, wherein the at least one enzyme comprises a reductase.

44. The method of any one of clauses 38-43, wherein the at least one enzyme comprises an epimerase.

45. The method of clause 44, wherein the epimerase comprises an amino acid sequence selected from the group consisting of: SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15.

46. The method of clause 44 or 45, wherein the epimerase comprises an oxidase domain and a reductase domain.

47. The method of clause 46, wherein the oxidase domain is a cytochrome P450 oxidase-like domain.

48. The method of clause 46, wherein the reductase domain is a codeinone reductase-like domain.

49. The method of any one of clauses 38-48, wherein the *(S)*-1-benzylisoquinoline alkaloid is *(S)*-reticuline.

50. The method of any one of clauses 38-49, wherein the *(R)*-1-benzylisoquinoline alkaloid is *(R)*-reticuline.

51. The method of any one of clauses 38-50, further comprising:

converting the *(R)*-1-benzylisoquinoline alkaloid to a 4-ring promorphinan alkaloid.

52. The method of clause 51, wherein the 4-ring promorphinan alkaloid is salutaridine.

53. The method of any one of clauses 38-50, further comprising:

converting the *(R)*-1-benzylisoquinoline alkaloid to a 5-ring morphinan alkaloid.

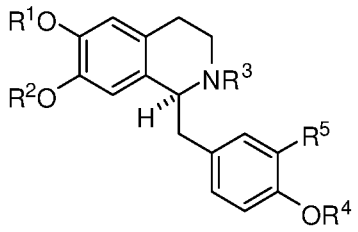
54. The method of any one of clauses 38-53, wherein the *(S)*-1-benzylisoquinoline alkaloid present within the engineered non-plant cell is produced within an engineered microbial cell.

55. The method of any one of clauses 38-54, wherein the *(S)*-1-benzylisoquinoline alkaloid is produced within the engineered non-plant cell by a metabolic pathway starting with L-tyrosine.

56. The method of any one of clauses 38-54, wherein the *(S)*-1-benzylisoquinoline alkaloid is produced within the engineered non-plant cell by a metabolic pathway starting with a carbohydrate and nitrogen source.

57. The method of any one of clauses 38-56, wherein the *(S)*-1-benzylisoquinoline alkaloid is selected from the group consisting of: *(S)*-norreticuline; *(S)*-reticuline; *(S)*-tetrahydropapaverine; *(S)*-norcoclaurine; *(S)*-coclaurine; *(S)*-*N*-methylcoclaurine; *(S)*-3'-hydroxy-*N*-methylcoclaurine; *(S)*-norisoorientaline; *(S)*-orientaline; *(S)*-isoorientaline; *(S)*-norprotosinomenine; *(S)*-protosinomenine; *(S)*-norlaudanosoline; *(S)*-laudanosoline; *(S)*-4'-*O*-methyllaudanosoline; *(S)*-6-*O*-methylnorlaudanosoline; and *(S)*-4'-*O*-methylnorlaudanosoline.

58. The method of any one of clauses 38-57, wherein the *(S)*-1-benzylisoquinoline alkaloid is a compound of Formula I:



Formula I,

wherein:

R^1, R^2, R^3, and R^4 are independently hydrogen or methyl; and

R^5 is hydrogen, hydroxy, or methoxy.

59. An engineered non-plant cell that produces an *(R)*-1-benzylisoquinoline alkaloid from an *(S)*-1-benzylisoquinoline alkaloid present within the engineered non-plant cell, comprising a heterologous coding sequence encoding an epimerase, wherein the epimerase converts the *(S)*-1-benzylisoquinoline alkaloid to the *(R)*-1-benzylisoquinoline alkaloid.

60. The engineered non-plant cell of clause 59, further comprising a heterologous coding sequence encoding salutaridine synthase.

61. The engineered non-plant cell of clause 60, wherein the salutaridine synthase converts the *(R)*-1-benzylisoquinoline alkaloid to salutaridine.

62. An engineered non-plant cell that produces an epimerase that converts *(S)*-1-benzylisoquinoline alkaloid to *(R)*-1-benzylisoquinoline alkaloid, wherein the epimerase comprises an amino acid sequence selected from the group consisting of: SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15.

63. A process for converting an *(S)*-1-benzylisoquinoline alkaloid to an *(R)*-1-benzylisoquinoline alkaloid comprising contacting the *(S)*-1-benzylisoquinoline alkaloid with an epimerase in an amount sufficient to convert the *(S)*-1-benzylisoquinoline alkaloid to *(R)*-1-benzylisoquinoline alkaloid.

64. The process of clause 63, wherein said epimerase comprises an amino acid sequence selected from the group consisting of: SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15.

65. The process of clause 63, wherein at least 5% of the *(S)*-1-benzylisoquinoline alkaloid is converted to *(R)*-1-benzylisoquinoline alkaloid.

66. A method of epimerizing a stereocenter of a 1-benzylisoquinoline alkaloid, comprising:

contacting the 1-benzylisoquinoline alkaloid with at least one enzyme,

wherein the contacting the 1-benzylisoquinoline alkaloid with the at least one enzyme inverts the stereochemistry of the stereocenter of the 1-benzylisoquinoline alkaloid to the opposite stereochemistry of the stereocenter of the 1-benzylisoquinoline alkaloid.

67. The method of clause 66, wherein the at least one enzyme is produced by culturing an engineered non-plant cell having a coding sequence for encoding the at least one enzyme.

68. The method of clause 67, further comprising:

adding the 1-benzylisoquinoline alkaloid to the cell culture.

69. The method of clause 66, wherein the 1-benzylisoquinoline alkaloid is present as a single stereoisomer of the 1-benzylisoquinoline alkaloid.

70. The method of clause 66, wherein the 1-benzylisoquinoline alkaloid is present as a mixture of stereoisomers of the 1-benzylisoquinoline alkaloid.

71. The method of clause 70, wherein the mixture of stereoisomers of the 1-benzylisoquinoline alkaloid is a racemic mixture of enantiomers of the 1-benzylisoquinoline alkaloid.

72. The method of clause 67, further comprising:

recovering a stereoisomer of the 1-benzylisoquinoline alkaloid, or a derivative thereof, from the cell culture.

73. The method of clause 72, wherein the recovered 1-benzylisoquinoline alkaloid has an enantiomeric excess of at least about 80%.

74. The method of clause 72, wherein the recovered 1-benzylisoquinoline alkaloid has an enantiomeric excess of at least about 98%.

75. The method of any one of clauses 66-74, wherein the at least one enzyme comprises an oxidase.

76. The method of any one of clauses 66-75, wherein the at least one enzyme comprises a reductase.

77. The method of any one of clauses 66-74, wherein the at least one enzyme comprises an epimerase.

78. The method of clause 77, wherein the epimerase comprises an amino acid sequence selected from the group consisting of: SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15.

79. The method of clause 77, wherein the epimerase comprises an amino acid sequence that is at least 75% identical to any of the SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15.

80. The method of clause 77, wherein the epimerase comprises an amino acid sequence that is at least 80% identical to any of the SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15.

81. The method of clause 77, wherein the epimerase comprises an amino acid sequence that is at least 85% identical to any of the SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15.

82. The method of clause 77, wherein the epimerase comprises an amino acid sequence that is at least 90% identical to any of the SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15.

83. The method of clause 77, wherein the epimerase comprises an amino acid sequence that is at least 95% identical to any of the SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15.

84. The method of clause 77, wherein the epimerase comprises an amino acid sequence that is at least 98% identical to any of the SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15.

85. The method of any of clauses 77-84, wherein the epimerase comprises an oxidase domain and a reductase domain.

86. The method of clause 85, wherein the oxidase domain is a cytochrome P450 oxidase-like domain.

87. The method of clause 85, wherein the reductase domain is a codeinone reductase-like domain.

88. An engineered non-plant cell that produces a bisbenzylisoquinoline alkaloid from two benzylisoquinoline alkaloid monomers that are present within the engineered non-plant cell, comprising:

a heterologous coding sequence encoding at least one coupling enzyme,

wherein the at least one coupling enzyme dimerizes the two benzylisoquinoline alkaloid monomers, thereby forming the bisbenzylisoquinoline alkaloid.

89. The engineered non-plant cell of clause 88, wherein the at least one coupling enzyme dimerizes the two benzylisoquinoline alkaloid monomers by forming one or more covalent bonds.

90. The engineered non-plant cell of clause 88, wherein the at least one coupling enzyme dimerizes the two benzylisoquinoline alkaloid monomers by forming one or more carbon-carbon bonds.

91. The engineered non-plant cell of clause 88, wherein the at least one coupling enzyme dimerizes the two benzylisoquinoline alkaloid monomers by forming one or more carbon-oxygen bonds.

92. The engineered non-plant cell of clause 88, wherein the at least one coupling enzyme dimerizes identical benzylisoquinoline alkaloid monomers, thereby forming a homodimer.

93. The engineered non-plant cell of clause 88, wherein the at least one coupling enzyme dimerizes distinct benzylisoquinoline alkaloid monomers, thereby forming a heterodimer.

94. The engineered non-plant cell of clause 88, wherein the two benzylisoquinoline alkaloid monomers are stereoisomers of each other.

95. The engineered non-plant cell of clause 88, wherein a first benzylisoquinoline alkaloid monomer of the two benzylisoquinoline alkaloid monomers is selected from the group of: coclaurine, *N*-methylcoclaurine, laudanine, norcoclaurine, norlaudanosoline, 6-*O*-methyl-norlaudanosoline, 3'-hydroxy-*N*-methylcoclaurine, 3'-hydroxycoclaurine, reticuline, norreticuline, norlaudanine.

96. The engineered non-plant cell of clause 95, wherein a second benzylisoquinoline alkaloid monomer of the two benzylisoquinoline alkaloid monomers is selected from the group of: coclaurine, *N*-methylcoclaurine, laudanine, norcoclaurine, norlaudanosoline, 6-*O*-methyl-norlaudanosoline, 3'-hydroxy-*N*-methylcoclaurine, 3'-hydroxycoclaurine, reticuline, norreticuline, norlaudanine.

97. The engineered non-plant cell of clause 88, wherein at least one of the two benzylisoquinoline alkaloid monomers is produced within the engineered non-plant cell.

98. The engineered non-plant cell of clause 88, wherein at least one of the two benzylisoquinoline alkaloid monomers is produced outside the engineered non-plant cell.

99. An engineered non-plant cell that produces a bisbenzylisoquinoline alkaloid using a coupling enzyme that is present within the engineered non-plant cell, comprising:

at least one heterologous coding sequence encoding at least one enzyme used in the production of at least one benzylisoquinoline alkaloid monomer within the engineered non-plant cell,

wherein the at least one coupling enzyme dimerizes two benzylisoquinoline alkaloid monomers within the engineered non-plant cell, thereby forming the bisbenzylisoquinoline alkaloid.

100. The engineered non-plant cell of clause 99, wherein the at least one coupling enzyme dimerizes the two benzylisoquinoline alkaloid monomers by forming one or more covalent bonds.

101. The engineered non-plant cell of clause 99, wherein the at least one coupling enzyme dimerizes the two benzylisoquinoline alkaloid monomers by forming one or more carbon-carbon bonds.

102. The engineered non-plant cell of clause 99, wherein the at least one coupling enzyme dimerizes the two benzylisoquinoline alkaloid monomers by forming one or more carbon-oxygen bonds.

103. The engineered non-plant cell of clause 99, wherein the at least one coupling enzyme dimerizes identical benzylisoquinoline alkaloid monomers, thereby forming a homodimer.

104. The engineered non-plant cell of clause 99, wherein the at least one coupling enzyme dimerizes distinct benzylisoquinoline alkaloid monomers, thereby forming a heterodimer.

105. The engineered non-plant cell of clause 99, wherein the two benzylisoquinoline alkaloid monomers are stereoisomers of each other.

106. The engineered non-plant cell of clause 99, wherein a first benzylisoquinoline alkaloid monomer of the two benzylisoquinoline alkaloid monomers is selected from the group of: coclaurine, *N*-methylcoclaurine, laudanine, norcoclaurine, norlaudanosoline, 6-*O*-methyl-norlaudanosoline, 3'-hydroxy-*N*-methylcoclaurine, 3'-hydroxycoclaurine, reticuline, norreticuline, norlaudanine.

107. The engineered non-plant cell of clause 106, wherein a second benzylisoquinoline alkaloid monomer of the two benzylisoquinoline alkaloid monomers is selected from the group of: coclaurine, *N*-methylcoclaurine, laudanine, norcoclaurine, norlaudanosoline, 6-*O*-methyl-norlaudanosoline, 3'-hydroxy-*N*-methylcoclaurine, 3'-hydroxycoclaurine, reticuline, norreticuline, norlaudanine.

108. The engineered non-plant cell of clause 99, wherein the coupling enzyme is produced within the engineered non-plant cell.

109. The engineered non-plant cell of clause 99, wherein the coupling enzyme is produced outside the engineered non-plant cell.

110. A method for forming a product stream having a bisbenzylisoquinoline alkaloid product, comprising:

(a) providing engineered non-plant cells and a feedstock including nutrients and water to a batch reactor, which engineered non-plant cells comprise at least one heterologous coding sequence encoding at least one coupling enzyme, wherein the engineered non-plant cells further comprise at least one heterologous coding sequence encoding at least one enzyme used in the production of at least one benzylisoquinoline alkaloid monomer;

(b) in said batch reactor, subjecting said engineered non-plant cells to fermentation by incubating said engineered non-plant cells for a time period of at least about 5 minutes to produce a solution comprising said bisbenzylisoquinoline alkaloid product and cellular material; and

(c) using at least one separation unit to separate said bisbenzylisoquinoline alkaloid product from said cellular material to provide said product stream comprising said bisbenzylisoquinoline alkaloid product.

111. The method of clause 108, wherein at least one process parameter of the batch reactor is modifiable to alter a resultant bisbenzylisoquinoline alkaloid product composition.

112. The method of clause 109, wherein the at least one process parameter that is modifiable comprises at least one of dissolved oxygen, pH, stirring speed, aeration rate, and cell density.

113. The method of clause 108, wherein the at least one benzylisoquinoline alkaloid monomer comprises at least one of coclaurine, *N*-methylcoclaurine, laudanine, norcoclaurine, norlaudanosoline, 6-*O*-methyl-norlaudanosoline, 3'-hydroxy-*N*-methylcoclaurine, 3'-hydroxycoclaurine, reticuline, norreticuline, norlaudanine.

114. An engineered non-plant cell that converts a carbohydrate source to a benzylisoquinoline alkaloid product that is derived from *(S)*-reticuline.

115. The engineered non-plant cell of clause 114, wherein the engineered non-plant cell comprises a plurality of multigene expression constructs having a plurality of heterologous coding sequences that encode one or more enzymes used to convert guanosine triphosphate to tetrahydrobiopterin.

116. The engineered non-plant cell of clause 115, wherein the plurality of heterologous coding sequences encode at least one of pyruvoyl tetrahydropterin synthase, sepiapterin reductase, pterin 4a-carbinolamine dehydratase, and dihydropteridine reductase.

117. The engineered non-plant cell of clause 115, wherein the engineered non-plant cell further comprises one or more heterologous coding sequences that encode one or more enzymes used to convert tyrosine to L-DOPA.

118. The engineered non-plant cell of clause 117, wherein one or more heterologous coding sequences encode at least one of tyrosine hydroxylase.

119. The engineered non-plant cell of clause 114, wherein the engineered non-plant cell comprises a plurality of multigene expression constructs having a plurality of heterologous coding sequences that encode one or more enzymes used to convert L-DOPA to *(S)*-reticuline.

120. The engineered non-plant cell of clause 119, wherein the plurality of heterologous coding sequences encode at least one of L-DOPA decarboxylase, norcoclaurine synthase, norcoclaurine-6-O-methyltransferase, coclaurine-N-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, and 4'-O-methyltransferase.

121. The engineered non-plant cell of clause 119, wherein the plurality of heterologous coding sequences encode at least one of L-DOPA decarboxylase, monoamine oxidase, norcoclaurine synthase, norcoclaurine-6-O-methyltransferase, coclaurine-N-methyltransferase, and 4'-O-methyltransferase.

122. The engineered non-plant cell of clause 114, wherein the engineered non-plant cell comprises a plurality of multigene expression constructs having a plurality of heterologous coding sequences that encode one or more enzymes used to convert *(S)*-reticuline to morphinan alkaloids.

123. The engineered non-plant cell of clause 122, wherein the plurality of heterologous coding sequences encode at least one of a 1-benzylisoquinoline alkaloid epimerase, salutaridine synthase, cytochrome P450 reductase, salutaridine reductase, and salutaridinol 7-O-acetyltransferase.

124. The engineered non-plant cell of clause 123, wherein the plurality of heterologous coding sequences additionally encode at least one of thebaine-6-O-demethylase, codeinone reductase, and codeine demethylase.

125. The engineered non-plant cell of clause 122, wherein the one or more enzymes are used to convert *(S)*-reticuline to promorphinan alkaloids.

126. The engineered non-plant cell of clause 122, wherein the one or more enzymes are used to convert *(S)*-reticuline to semi-synthetic morphinan alkaloids.

127. The engineered non-plant cell of clause 114, wherein the engineered non-plant cell comprises a plurality of multigene expression constructs having a plurality of heterologous coding sequences that encode one or more enzymes used to convert *(S)*-reticuline to canadine.

128. The engineered non-plant cell of clause 127, wherein the plurality of heterologous coding sequences encode at least one of berberine bridge enzyme, scoulerine-9-O-methyltransferase, canadine synthase, and cytochrome P450 reductase.

129. The engineered non-plant cell of clause 128, wherein the plurality of heterologous coding sequences additionally encode one or more enzymes to convert canadine to protoberberine products.

130. The engineered non-plant cell of clause 128, wherein the plurality of heterologous coding sequences additionally encode one or more enzymes to convert canadine to phthalideisoquinnoline products.

131. The engineered non-plant cell of clause 128, wherein the plurality of heterologous coding sequences additionally encode one or more enzymes to convert canadine to noscapinoid products.

132. The engineered non-plant cell of clause 114, wherein the engineered non-plant cell comprises a plurality of multigene expression constructs having a plurality of heterologous coding sequences that encode one or more enzymes used to convert *(S)*-reticuline to berberine.

133. The engineered non-plant cell of clause 132, wherein the plurality of heterologous coding sequences encode at least one of berberine bridge enzyme, scoulerine-9-O-methyltransferase, canadine synthase, cytochrome P450 reductase, and tetrahydroprotoberberine oxidase.

134. The engineered non-plant cell of clause 133, wherein the plurality of heterologous coding sequences additionally encode one or more enzymes to convert berberine to berberine products.

135. The engineered non-plant cell of clause 114, wherein the engineered non-plant cell comprises a plurality of multigene expression constructs having a plurality of heterologous coding sequences that encode one or more enzymes used to convert *(S)*-reticuline to protoberberine products.

136. The engineered non-plant cell of clause 138, wherein the plurality of heterologous coding sequences encode berberine bridge enzyme.

137. The engineered non-plant cell of clause 114, wherein the engineered non-plant cell comprises a plurality of multigene expression constructs having a plurality of heterologous coding sequences that encode one or more enzymes used to convert *(S)*-reticuline to protopine.

138. The engineered non-plant cell of clause 137, wherein the plurality of heterologous coding sequences encode at least one of berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, cytochrome P450 reductase, tetrahydroprotoberberine-N-methyltransferase, and cis-N-methylstylopine 14-hydroxylase.

139. The engineered non-plant cell of clause 138, wherein the plurality of heterologous coding sequences additionally encode one or more enzymes to convert protopine to protopine products.

140. The engineered non-plant cell of clause 138, wherein the plurality of heterologous coding sequences additionally encode one or more enzymes to convert protopine to benzophenanthridine products.

141. The engineered non-plant cell of clause 140, wherein the plurality of heterologous coding sequences additionally encode protopine-6-hydroxylase.

142. A method for forming a product stream having a benzylisoquinoline alkaloid product, comprising:

(a) providing engineered non-plant cells and a feedstock including nutrients and water to a batch reactor;

(b) in said batch reactor, contacting the engineered non-plant cells with a carbohydrate source and a nitrogen source, and subjecting said engineered non-plant cells to fermentation by incubating said engineered non-plant cells for a time period of at least about 5 minutes to produce a solution comprising said benzylisoquinoline alkaloid product and cellular material, wherein said benzylisoquinoline alkaloid product is derived from reticuline; and

(c) using at least one separation unit to separate said benzylisoquinoline alkaloid product from said cellular material to provide said product stream comprising said benzylisoquinoline alkaloid product.

143. The method of clause 139, wherein at least one process parameter of the batch reactor is modifiable to alter a resultant benzylisoquinoline alkaloid product composition.

144. The method of clause 140, wherein the at least one process parameter that is modifiable comprises at least one of dissolved oxygen, pH, stirring speed, aeration rate, and cell density.

145. The method of clause 139, wherein the benzylisoquinoline alkaloid product comprises a benzylisoquinoline alkaloid.

146. The method of clause 142, wherein the benzylisoquinoline alkaloid has a structural class that is selected from the group of benzylisoquinolines, protoberberines, protopines, benzophenanthridines, promorphinans, morphinans, secoberberines, phthalideisoquinolines, aporphines, and bisbenzylisoquinolines.

147. The method of clause 143, wherein the benzylisoquinoline alkaloid is a benzylisoquinoline that is selected from the group of papaverine, tetrahydropapaverine, 1,2-dihydropapaverine, and orientaline.

148. The method of clause 143, wherein the benzylisoquinoline alkaloid is a protoberberine that is selected from the group of scoulerine, cheilanthifoline, stylopine, nandinine, jatrorrhizine, stepholidine, discretamine, *cis*-*N*-methylstylopine, tetrahydrocolumbamine, palmatine, tetrahydropalmatine, columbamine, canadine, *N*-methylcanadine, 1-hydroxycanadine, berberine, *N*-methyl-ophiocarpine, 1,13-dihydroxy-*N*-methylcanadine, and 1-hydroxy-10-*O*-acetyl-*N*-methylcanadine.

149. The method of clause 143, wherein the benzylisoquinoline alkaloid is a protopine that is selected from the group of protopine, 6-hydroxyprotopine, allocryptopine, cryptopine, muramine, and thalictricine.

150. The method of clause 143, wherein the benzylisoquinoline alkaloid is a benzophenanthridine that is selected from the group of dihydrosanguinarine, sanguinarine, dihydrocheilirubine, cheilirubine, dihydromarcapine, marcapine, and chelerythrine.

151. The method of clause 143, wherein the benzylisoquinoline alkaloid is a promorphinan that is selected from the group of salutaridine, salutaridinol, and salutaridinol-7-*O*-acetate.

152. The method of clause 143, wherein the benzylisoquinoline alkaloid is a morphinan that is selected from the group of thebaine, codeinone, codeine, morphine, morphinone, oripavine, neopinone, neopine, neomorphine, hydrocodone, dihydrocodeine, 14-hydroxycodeinone, oxycodone, 14-hydroxycodeine, morphinone, hydromorphone, dihydromorphine, dihydroetorphine, ethylmorphine, etorphine, metopon, buprenorphine, pholcodine, heterocodeine, and oxymorphone.

153. The method of clause 143, wherein the benzylisoquinoline alkaloid is a secoberberine that is selected from the group of 4'-*O*-desmethylmacrantaldehyde, 4'-*O*-desmethylpapaveroxine, 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine, and 3-*O*-aceteylpapaveroxine.

154. The method of clause 143, wherein the benzylisoquinoline alkaloid is a phthalideisoquinoline that is selected from the group of narcotolinehemiacetal, narcotinehemiacetal, narcotoline, and noscapine.

155. The method of clause 143, wherein the benzylisoquinoline alkaloid is an aporphine that is selected from the group of magnoflorine, corytuberine, apomorphine, boldine, isoboldine, isothebaine, isocorytuberine, and glaufine.

156. The method of clause 143, wherein the benzylisoquinoline alkaloid is a bisbenzylisoquinoline that is selected from the group of berbamunine, guattgaumerine, dauricine, and liensinine.

157. A method for forming a product stream having a benzylisoquinoline alkaloid product, comprising:

(a) providing engineered non-plant cells and a feedstock including nutrients and water to a reactor;

(b) in said reactor, contacting the engineered non-plant cells with a carbohydrate source and a nitrogen source, thereby producing a solution comprising cellular material and said benzylisoquinoline alkaloid product, wherein said benzylisoquinoline alkaloid product is derived from reticuline, wherein said solution comprises not more than one class of molecule selected from the group of protoberberine, morphinan, isopavine, aporphine, and bisbenzylisoquinoline; and

(c) using at least one separation unit to separate said benzylisoquinoline alkaloid product from said cellular material to provide said product stream comprising said benzylisoquinoline alkaloid product.

158. The method of clause 154, wherein the product stream does not contain more than 5ppm of a molecule selected from the group of lignin, flavonoids, phenanthreoids, latex, rubisco, meconic acid, pseudomorphine, narceine, thebaol, and pollen.

159. The method of clause 155, wherein the product stream does not contain more than 5ppm of meconic acid.

160. The method of clause 154, wherein the product stream does not contain a detectable amount of a substance selected from the group consisting of pesticides, fungicides, or herbicides.

161. The method of clause 154, wherein the benzylisoquinoline alkaloid product is recovered from said product stream using at least liquid-liquid extraction.

162. The method of clause 158, wherein the benzylisoquinoline alkaloid product is recovered immediately after a fermentation process has been completed.

163. The method of any of the previous clauses, wherein the engineered non-plant cell comprises two or more heterologous coding sequences, wherein the two or more heterologous coding sequences encode at least a first enzyme and a second enzyme that are involved in a metabolic pathway that converts a substrate to the benzylisoquinoline alkaloid product, wherein the first enzyme and the second enzyme are operably connected along the metabolic pathway.

164. The method of any of the previous clauses, wherein the engineered non-plant cell comprises three heterologous coding sequences, wherein the three heterologous coding sequences encode at least a first enzyme, a second enzyme, and a third enzyme that are involved in a metabolic pathway that converts a substrate to the benzylisoquinoline alkaloid product, wherein the first enzyme, the second enzyme, and the third enzyme are operably connected along the metabolic pathway.

165. The method of clause 163, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of 4'-*O*-methyltransferase, berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, tetrahydroprotoberberine-*N*-methyltransferase, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine oxidase, protopine *O*-dealkylase, and cytochrome P450 reductase.

166. The method of clause 164, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of 4'-*O*-methyltransferase, berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, tetrahydroprotoberberine-*N*-methyltransferase, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine oxidase, protopine *O*-dealkylase, and cytochrome P450 reductase.

167. The method of clause 165 or 166, wherein the benzylisoquinoline alkaloid product is a protoberberine product.

168. The method of clause 163, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, tetrahydroprotoberberine-*N*-methyltransferase, cis-*N*-methylstylopine 14-hydroxylase, protopine-6-hydroxylase, protopine *O*-dealkylase, and cytochrome P450 reductase.

169. The method of clause 164, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, tetrahydroprotoberberine-*N*-methyltransferase, cis-*N*-methylstylopine 14-hydroxylase, protopine-6-hydroxylase, protopine *O*-dealkylase, and cytochrome P450 reductase.

170. The method of clause 168 or 169, wherein the benzylisoquinoline alkaloid product is a protopine product.

171. The method of clause 163, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, tetrahydroprotoberberine-*N*-methyltransferase, cis-*N*-methylstylopine 14-hydroxylase, protopine-6-hydroxylase, dihydrobenzophenanthridine oxidase, and cytochrome P450 reductase.

172. The method of clause 164, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, tetrahydroprotoberberine-*N*-methyltransferase, cis-*N*-methylstylopine 14-hydroxylase, protopine-6-hydroxylase, dihydrobenzophenanthridine oxidase, and cytochrome P450 reductase.

173. The method of clause 171 or 172, wherein the benzylisoquinoline alkaloid product is a benzophenanthridine product.

174. The method of clause 163, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group

consisting of 4'-*O*-methyltransferase, BIA epimerase, salutaridine synthase, salutaridine reductase, salutaridinol 7-*O*-acetyltransferase, and cytochrome P450 reductase.

175. The method of clause 164, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of 4'-*O*-methyltransferase, BIA epimerase, salutaridine synthase, salutaridine reductase, salutaridinol 7-*O*-acetyltransferase, and cytochrome P450 reductase.

176. The method of clause 174 or 175, wherein the benzylisoquinoline alkaloid product is a promorphinan product.

177. The method of clause 163, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of BIA epimerase, salutaridine synthase, salutaridine reductase, salutaridinol 7-*O*-acetyltransferase, thebaine 6-*O*-demethylase, codeinone reductase, codeine *O*-demethylase, morphine dehydrogenase, morphinone reductase, and cytochrome P450 reductase.

178. The method of clause 164, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of BIA epimerase, salutaridine synthase, salutaridine reductase, salutaridinol 7-*O*-acetyltransferase, thebaine 6-*O*-demethylase, codeinone reductase, codeine *O*-demethylase, morphine dehydrogenase, morphinone reductase, and cytochrome P450 reductase.

179. The method of clause 177 or 178, wherein the benzylisoquinoline alkaloid product is a morphinan product.

180. The method of clause 163, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of berberine bridge enzyme, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine-*N*-methyltransferase, *N*-methylcanadine 1-hydroxylase, 1-hydroxy-*N*-methylcanadine 13-hydroxylase, 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine synthase, 1, 13-dihydroxy-*N*-methylcanadine 13-*O* acetyl transferase, and cytochrome P450 reductase.

181. The method of clause 164, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of berberine bridge enzyme, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine-*N*-methyltransferase, *N*-methylcanadine 1-hydroxylase, 1-hydroxy-*N*-methylcanadine 13-hydroxylase, 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine synthase, 1, 13-dihydroxy-*N*-methylcanadine 13-*O* acetyl transferase, and cytochrome P450 reductase.

182. The method of clause 180 or 181, wherein the benzylisoquinoline alkaloid product is a secoberberine product.

183. The method of clause 163, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of berberine bridge enzyme, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine-*N*-methyltransferase, *N*-methylcanadine 1-hydroxylase, 1-hydroxy-*N*-methylcanadine 13-hydroxylase, 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine synthase, 1, 13-dihydroxy-*N*-methylcanadine 13-*O* acetyl transferase, narcotinehemiacetal synthase, noscapine synthase, narcotoline 4'-*O*-methylase, and cytochrome P450 reductase.

184. The method of clause 164, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of berberine bridge enzyme, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine-*N*-methyltransferase, *N*-methylcanadine 1-hydroxylase, 1-hydroxy-*N*-methylcanadine 13-hydroxylase, 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine synthase, 1, 13-dihydroxy-*N*-methylcanadine 13-*O* acetyl transferase, narcotinehemiacetal synthase, noscapine synthase, narcotoline 4'-*O*-methylase, and cytochrome P450 reductase.

185. The method of clause 183 or 184, wherein the benzylisoquinoline alkaloid product is a phthalideisoquinoline product.

186. The method of clause 163, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, and berbamunine synthase.

187. The method of clause 164, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, and berbamunine synthase.

188. The method of clause 186 or 187, wherein the benzylisoquinoline alkaloid product is a bisbenzylisoquinoline product.

189. The method of clause 163, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group

consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, and BIA epimerase.

190. The method of clause 164, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, and BIA epimerase.

191. The method of clause 189 or 190, wherein the benzylisoquinoline alkaloid product is a benzylisoquinoline product.

192. The method of any of clauses 165-191, wherein the engineered non-plant cell comprises two or more additional heterologous coding sequences, wherein the two or more additional heterologous coding sequences encode at least a first additional enzyme and a second additional enzyme that are involved in a metabolic pathway that converts a substrate to the benzylisoquinoline alkaloid product, wherein the first additional enzyme and the second additional enzyme are operably connected along the metabolic pathway, and wherein each of the first additional and second additional enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, and BIA epimerase.

193. The method of any of clauses 165-191, wherein the engineered non-plant cell comprises three additional heterologous coding sequences, wherein the three heterologous coding sequences encode at least a first additional enzyme, a second additional enzyme, and a third additional enzyme that are involved in a metabolic pathway that converts a substrate to the benzylisoquinoline alkaloid product, wherein the first additional enzyme, the second additional enzyme, and the third additional enzyme are operably connected along the metabolic pathway, and wherein each of the first additional, second additional, and third additional enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, and BIA epimerase.

194. The engineered non-plant cell of any of the previous clauses, wherein the engineered non-plant cell comprises two or more heterologous coding sequences, wherein the two or more heterologous coding sequences encode at least a first enzyme and a second enzyme that are involved in a metabolic pathway that converts a substrate to the benzylisoquinoline alkaloid product, wherein the first enzyme and the second enzyme are operably connected along the metabolic pathway.

195. The engineered non-plant cell of any of the previous clauses, wherein the engineered non-plant cell comprises three heterologous coding sequences, wherein the three heterologous coding sequences encode

at least a first enzyme, a second enzyme, and a third enzyme that are involved in a metabolic pathway that converts a substrate to the benzylisoquinoline alkaloid product, wherein the first enzyme, the second enzyme, and the third enzyme are operably connected along the metabolic pathway.

196. The engineered non-plant cell of clause 194, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of 4'-*O*-methyltransferase, berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, tetrahydroprotoberberine-*N*-methyltransferase, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine oxidase, protopine *O*-dealkylase, and cytochrome P450 reductase.

197. The engineered non-plant cell of clause 195, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of 4'-*O*-methyltransferase, berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, tetrahydroprotoberberine-*N*-methyltransferase, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine oxidase, protopine *O*-dealkylase, and cytochrome P450 reductase.

198. The engineered non-plant cell of clause 196 or 197, wherein the benzylisoquinoline alkaloid product is a protoberberine product.

199. The engineered non-plant cell of clause 194, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, tetrahydroprotoberberine-*N*-methyltransferase, cis-*N*-methylstylopine 14-hydroxylase, protopine-6-hydroxylase, protopine *O*-dealkylase, and cytochrome P450 reductase.

200. The engineered non-plant cell of clause 195, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, tetrahydroprotoberberine-*N*-methyltransferase, cis-*N*-methylstylopine 14-hydroxylase, protopine-6-hydroxylase, protopine *O*-dealkylase, and cytochrome P450 reductase.

201. The engineered non-plant cell of clause 199 or 200, wherein the benzylisoquinoline alkaloid product is a protopine product.

202. The engineered non-plant cell of clause 194, whereineach of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, tetrahydroprotoberberine-*N*-methyltransferase, cis-*N*-methylstylopine 14-hydroxylase, protopine-6-hydroxylase, dihydrobenzophenanthridine oxidase, and cytochrome P450 reductase.

203. The engineered non-plant cell of clause 195, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, tetrahydroprotoberberine-*N*-methyltransferase, cis-*N*-methylstylopine 14-hydroxylase, protopine-6-hydroxylase, dihydrobenzophenanthridine oxidase, and cytochrome P450 reductase.

204. The engineered non-plant cell of clause 202 or 203, wherein the benzylisoquinoline alkaloid product is a benzophenanthridine product.

205. The engineered non-plant cell of clause 194, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of 4'-*O*-methyltransferase, BIA epimerase, salutaridine synthase, salutaridine reductase, salutaridinol 7-*O*-acetyltransferase, and cytochrome P450 reductase.

206. The engineered non-plant cell of clause 195, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of 4'-*O*-methyltransferase, BIA epimerase, salutaridine synthase, salutaridine reductase, salutaridinol 7-*O*-acetyltransferase, and cytochrome P450 reductase.

207. The engineered non-plant cell of clause 205 or 206, wherein the benzylisoquinoline alkaloid product is a promorphinan product.

208. The engineered non-plant cell of clause 194, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of BIA epimerase, salutaridine synthase, salutaridine reductase, salutaridinol 7-*O*-acetyltransferase, thebaine 6-*O*-demethylase, codeinone reductase, codeine *O*-demethylase, morphine dehydrogenase, morphinone reductase, and cytochrome P450 reductase.

209. The engineered non-plant cell of clause 195, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of BIA epimerase, salutaridine synthase, salutaridine reductase, salutaridinol 7-*O*-acetyltransferase, thebaine 6-*O*-demethylase, codeinone reductase, codeine *O*-demethylase, morphine dehydrogenase, morphinone reductase, and cytochrome P450 reductase.

210. The engineered non-plant cell of clause 208 or 209, wherein the benzylisoquinoline alkaloid product is a morphinan product.

211. The engineered non-plant cell of clause 194, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of berberine bridge enzyme, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine-*N*-methyltransferase, *N*-methylcanadine 1-hydroxylase, 1-hydroxy-*N*-

methylcanadine 13-hydroxylase, 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine synthase, 1, 13-dihydroxy-*N*-methylcanadine 13-*O* acetyl transferase, and cytochrome P450 reductase.

212. The engineered non-plant cell of clause 195, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of berberine bridge enzyme, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine-*N*-methyltransferase, *N*-methylcanadine 1-hydroxylase, 1-hydroxy-*N*-methylcanadine 13-hydroxylase, 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine synthase, 1, 13-dihydroxy-*N*-methylcanadine 13-*O* acetyl transferase, and cytochrome P450 reductase.

213. The engineered non-plant cell of clause 211 or 212, wherein the benzylisoquinoline alkaloid product is a secoberberine product.

214. The engineered non-plant cell of clause 194, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of berberine bridge enzyme, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine-*N*-methyltransferase, *N*-methylcanadine 1-hydroxylase, 1-hydroxy-*N*-methylcanadine 13-hydroxylase, 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine synthase, 1, 13-dihydroxy-*N*-methylcanadine 13-*O* acetyl transferase, narcotinehemiacetal synthase, noscapine synthase, narcotoline 4'-*O*-methylase, and cytochrome P450 reductase.

215. The engineered non-plant cell of clause 195, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of berberine bridge enzyme, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine-*N*-methyltransferase, *N*-methylcanadine 1-hydroxylase, 1-hydroxy-*N*-methylcanadine 13-hydroxylase, 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine synthase, 1, 13-dihydroxy-*N*-methylcanadine 13-*O* acetyl transferase, narcotinehemiacetal synthase, noscapine synthase, narcotoline 4'-*O*-methylase, and cytochrome P450 reductase.

216. The engineered non-plant cell of clause 214 or 215, wherein the benzylisoquinoline alkaloid product is a phthalideisoquinoline product.

217. The engineered non-plant cell of clause 194, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, and berbamunine synthase.

218. The engineered non-plant cell of clause 195, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected

from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, and berbamunine synthase.

219. The engineered non-plant cell of clause 217 or 218, wherein the benzylisoquinoline alkaloid product is a bisbenzylisoquinoline product.

220. The engineered non-plant cell of clause 194, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, and BIA epimerase.

221. The engineered non-plant cell of clause 195, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, and BIA epimerase.

222. The engineered non-plant cell of clause 220 or 221, wherein the benzylisoquinoline alkaloid product is a benzylisoquinoline product.

223. The engineered non-plant cell of any of clauses 196-222, wherein the engineered non-plant cell comprises two or more additional heterologous coding sequences, wherein the two or more additional heterologous coding sequences encode at least a first additional enzyme and a second additional enzyme that are involved in a metabolic pathway that converts a substrate to the benzylisoquinoline alkaloid product, wherein the first additional enzyme and the second additional enzyme are operably connected along the metabolic pathway, and wherein each of the first additional and second additional enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, and BIA epimerase.

224. The engineered non-plant cell of any of clauses 196-222, wherein the engineered non-plant cell comprises three additional heterologous coding sequences, wherein the three heterologous coding sequences encode at least a first additional enzyme, a second additional enzyme, and a third additional enzyme that are involved in a metabolic pathway that converts a substrate to the benzylisoquinoline alkaloid product, wherein the first additional enzyme, the second additional enzyme, and the third additional enzyme are operably connected along the metabolic pathway, and wherein each of the first additional, second additional, and third additional enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase,

coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, and BIA epimerase.

225. The method of clause 163, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, 4'-*O*-methyltransferase, berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, tetrahydroprotoberberine-*N*-methyltransferase, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine oxidase, and protopine *O*-dealkylase.

226. The method of clause 164, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, 4'-*O*-methyltransferase, berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, tetrahydroprotoberberine-*N*-methyltransferase, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine oxidase, and protopine *O*-dealkylase.

227. The method of clause 225 or 226, wherein the benzylisoquinoline alkaloid product is a protoberberine product.

228. The method of clause 163, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, tetrahydroprotoberberine-*N*-methyltransferase, cis-*N*-methylstylopine 14-hydroxylase, protopine-6-hydroxylase, and protopine *O*-dealkylase.

229. The method of clause 164, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, tetrahydroprotoberberine-*N*-methyltransferase, cis-*N*-methylstylopine 14-hydroxylase, protopine-6-hydroxylase, and protopine *O*-dealkylase.

230. The method of clause 228 or 229, wherein the benzylisoquinoline alkaloid product is a protopine product.

231. The method of clause 163, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, tetrahydroprotoberberine-*N*-methyltransferase, cis-*N*-methylstylopine 14-hydroxylase, protopine-6-hydroxylase, and dihydrobenzophenanthridine oxidase.

232. The method of clause 164, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, tetrahydroprotoberberine-*N*-methyltransferase, cis-*N*-methylstylopine 14-hydroxylase, protopine-6-hydroxylase, and dihydrobenzophenanthridine oxidase.

233. The method of clause 231 or 232, wherein the benzylisoquinoline alkaloid product is a benzophenanthridine product.

234. The method of clause 163, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, 4'-*O*-methyltransferase, BIA epimerase, salutaridine synthase, salutaridine reductase, and salutaridinol 7-*O*-acetyltransferase.

235. The method of clause 164, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, 4'-*O*-methyltransferase, BIA epimerase, salutaridine synthase, salutaridine reductase, and salutaridinol 7-*O*-acetyltransferase.

236. The method of clause 234 or 235, wherein the benzylisoquinoline alkaloid product is a promorphinan product.

237. The method of clause 163, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, salutaridine synthase, salutaridine reductase, salutaridinol 7-*O*-acetyltransferase, thebaine 6-*O*-demethylase, codeinone reductase, codeine *O*-demethylase, morphine dehydrogenase, and morphinone reductase.

238. The method of clause 164, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, salutaridine synthase, salutaridine reductase, salutaridinol 7-*O*-acetyltransferase, thebaine 6-*O*-demethylase, codeinone reductase, codeine *O*-demethylase, morphine dehydrogenase, and morphinone reductase.

239. The method of clause 237 or 238, wherein the benzylisoquinoline alkaloid product is a morphinan product.

240. The method of clause 163, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, berberine bridge enzyme, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine-*N*-methyltransferase, *N*-methylcanadine 1-hydroxylase, 1-hydroxy-*N*-methylcanadine 13-hydroxylase, 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine synthase, and 1, 13-dihydroxy-*N*-methylcanadine 13-*O* acetyl transferase.

241. The method of clause 164, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, berberine bridge enzyme, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine-*N*-methyltransferase, *N*-methylcanadine 1-hydroxylase, 1-hydroxy-*N*-methylcanadine 13-hydroxylase, 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine synthase, and 1, 13-dihydroxy-*N*-methylcanadine 13-*O* acetyl transferase.

242. The method of clause 240 or 241, wherein the benzylisoquinoline alkaloid product is a secoberberine product.

243. The method of clause 163, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, berberine bridge enzyme, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine-*N*-methyltransferase, *N*-methylcanadine 1-hydroxylase, 1-hydroxy-*N*-methylcanadine 13-hydroxylase, 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine synthase, 1, 13-dihydroxy-*N*-methylcanadine 13-*O* acetyl transferase, narcotinehemiacetal synthase, noscapine synthase, and narcotoline 4'-*O*-methylase.

244. The method of clause 164, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group

consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, berberine bridge enzyme, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine-*N*-methyltransferase, *N*-methylcanadine 1-hydroxylase, 1-hydroxy-*N*-methylcanadine 13-hydroxylase, 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine synthase, 1, 13-dihydroxy-*N*-methylcanadine 13-*O* acetyl transferase, narcotinehemiacetal synthase, noscapine synthase, and narcotoline 4'-*O*-methylase.

245. The method of clause 243 or 244, wherein the benzylisoquinoline alkaloid product is a phthalideisoquinoline product.

246. The method of clause 163, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of tyrosine hydroxylase, dopa decarboxylase, tyrosine/dopa decarboxylase, monoamine oxidase, tyrosinase, norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, and berbamunine synthase.

247. The method of clause 164, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of tyrosine hydroxylase, dopa decarboxylase, tyrosine/dopa decarboxylase, monoamine oxidase, tyrosinase, norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, and berbamunine synthase.

248. The method of clause 246 or 247, wherein the benzylisoquinoline alkaloid product is a bisbenzylisoquinoline product.

249. The method of clause 163, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of tyrosine hydroxylase, dopa decarboxylase, tyrosine/dopa decarboxylase, monoamine oxidase, tyrosinase, norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, and BIA epimerase.

250. The method of clause 164, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of tyrosine hydroxylase, dopa decarboxylase, tyrosine/dopa decarboxylase, monoamine oxidase, tyrosinase, norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, and BIA epimerase.

251. The method of clause 249 or 250, wherein the benzylisoquinoline alkaloid product is a benzylisoquinoline product.

252. The method of any of clauses 225-251, wherein the engineered non-plant cell comprises two or more additional heterologous coding sequences, wherein the two or more additional heterologous coding sequences encode at least a first additional enzyme and a second additional enzyme that are involved in a metabolic pathway that converts a substrate to the benzylisoquinoline alkaloid product, wherein the first additional enzyme and the second additional enzyme are operably connected along the metabolic pathway, and wherein each of the first additional and second additional enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, and BIA epimerase.

253. The method of any of clauses 225-251, wherein the engineered non-plant cell comprises three additional heterologous coding sequences, wherein the three heterologous coding sequences encode at least a first additional enzyme, a second additional enzyme, and a third additional enzyme that are involved in a metabolic pathway that converts a substrate to the benzylisoquinoline alkaloid product, wherein the first additional enzyme, the second additional enzyme, and the third additional enzyme are operably connected along the metabolic pathway, and wherein each of the first additional, second additional, and third additional enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, and BIA epimerase.

254. The engineered non-plant cell of clause 194, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, 4'-*O*-methyltransferase, berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, tetrahydroprotoberberine-*N*-methyltransferase, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine oxidase, and protopine *O*-dealkylase.

255. The engineered non-plant cell of clause 195, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, 4'-*O*-methyltransferase, berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase,

tetrahydroprotoberberine-*N*-methyltransferase, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine oxidase, and protopine *O*-dealkylase.

256. The engineered non-plant cell of clause 254 or 255, wherein the benzylisoquinoline alkaloid product is a protoberberine product.

257. The engineered non-plant cell of clause 194, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, tetrahydroprotoberberine-*N*-methyltransferase, cis-*N*-methylstylopine 14-hydroxylase, protopine-6-hydroxylase, and protopine *O*-dealkylase.

258. The engineered non-plant cell of clause 195, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, tetrahydroprotoberberine-*N*-methyltransferase, cis-*N*-methylstylopine 14-hydroxylase, protopine-6-hydroxylase, and protopine *O*-dealkylase.

259. The engineered non-plant cell of clause 257 or 258, wherein the benzylisoquinoline alkaloid product is a protopine product.

260. The engineered non-plant cell of clause 194, whereineach of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, tetrahydroprotoberberine-*N*-methyltransferase, cis-*N*-methylstylopine 14-hydroxylase, protopine-6-hydroxylase, and dihydrobenzophenanthridine oxidase.

261. The engineered non-plant cell of clause 195, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, berberine bridge enzyme, cheilanthifoline synthase, stylopine synthase, tetrahydroprotoberberine-*N*-methyltransferase, cis-*N*-methylstylopine 14-hydroxylase, protopine-6-hydroxylase, and dihydrobenzophenanthridine oxidase.

262. The engineered non-plant cell of clause 260 or 261, wherein the benzylisoquinoline alkaloid product is a benzophenanthridine product.

263. The engineered non-plant cell of clause 194, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, 4'-*O*-methyltransferase, BIA epimerase, salutaridine synthase, salutaridine reductase, and salutaridinol 7-*O*-acetyltransferase.

264. The engineered non-plant cell of clause 195, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, 4'-*O*-methyltransferase, BIA epimerase, salutaridine synthase, salutaridine reductase, and salutaridinol 7-*O*-acetyltransferase.

265. The engineered non-plant cell of clause 263 or 264, wherein the benzylisoquinoline alkaloid product is a promorphinan product.

266. The engineered non-plant cell of clause 194, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, salutaridine synthase, salutaridine reductase, salutaridinol 7-*O*-acetyltransferase, thebaine 6-*O*-demethylase, codeinone reductase, codeine *O*-demethylase, morphine dehydrogenase, and morphinone reductase.

267. The engineered non-plant cell of clause 195, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, salutaridine synthase, salutaridine reductase, salutaridinol 7-*O*-acetyltransferase, thebaine 6-*O*-demethylase, codeinone reductase, codeine *O*-demethylase, morphine dehydrogenase, and morphinone reductase.

268. The engineered non-plant cell of clause 266 or 267, wherein the benzylisoquinoline alkaloid product is a morphinan product.

269. The engineered non-plant cell of clause 194, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, berberine bridge enzyme, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine-*N*-methyltransferase, *N*-methylcanadine 1-hydroxylase, 1-hydroxy-*N*-methylcanadine 13-hydroxylase, 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine synthase, and 1, 13-dihydroxy-*N*-methylcanadine 13-*O* acetyl transferase.

270. The engineered non-plant cell of clause 195, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, berberine bridge enzyme, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine-*N*-methyltransferase, *N*-methylcanadine 1-hydroxylase, 1-hydroxy-*N*-methylcanadine 13-hydroxylase, 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine synthase, and 1, 13-dihydroxy-*N*-methylcanadine 13-*O* acetyl transferase.

271. The engineered non-plant cell of clause 269 or 270, wherein the benzylisoquinoline alkaloid product is a secoberberine product.

272. The engineered non-plant cell of clause 194, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, berberine bridge enzyme, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine-*N*-methyltransferase, *N*-methylcanadine 1-hydroxylase, 1-hydroxy-*N*-methylcanadine 13-hydroxylase, 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine synthase, 1, 13-dihydroxy-*N*-methylcanadine 13-*O* acetyl transferase, narcotinehemiacetal synthase, noscapine synthase, and narcotoline 4'-*O*-methylase.

273. The engineered non-plant cell of clause 195, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, berberine bridge enzyme, scoulerine 9-*O*-methyltransferase, canadine synthase, tetrahydroprotoberberine-*N*-methyltransferase, *N*-methylcanadine 1-hydroxylase, 1-hydroxy-*N*-methylcanadine 13-hydroxylase, 4'-*O*-desmethyl-3-*O*-acetylpapaveroxine synthase, 1, 13-dihydroxy-*N*-

methylcanadine 13-*O* acetyl transferase, narcotinehemiacetal synthase, noscapine synthase, and narcotoline 4'-*O*-methylase.

274. The engineered non-plant cell of clause 272 or 273, wherein the benzylisoquinoline alkaloid product is a phthalideisoquinoline product.

275. The engineered non-plant cell of clause 194, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of tyrosine hydroxylase, dopa decarboxylase, tyrosine/dopa decarboxylase, monoamine oxidase, tyrosinase, norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, and berbamunine synthase.

276. The engineered non-plant cell of clause 195, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of tyrosine hydroxylase, dopa decarboxylase, tyrosine/dopa decarboxylase, monoamine oxidase, tyrosinase, norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, BIA epimerase, and berbamunine synthase.

277. The engineered non-plant cell of clause 275 or 276, wherein the benzylisoquinoline alkaloid product is a bisbenzylisoquinoline product.

278. The engineered non-plant cell of clause 194, wherein each of the first and second enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of tyrosine hydroxylase, dopa decarboxylase, tyrosine/dopa decarboxylase, monoamine oxidase, tyrosinase, norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, and BIA epimerase.

279. The engineered non-plant cell of clause 195, wherein each of the first, second, and third enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of tyrosine hydroxylase, dopa decarboxylase, tyrosine/dopa decarboxylase, monoamine oxidase, tyrosinase, norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, and BIA epimerase.

280. The engineered non-plant cell of clause 220 or 221, wherein the benzylisoquinoline alkaloid product is a benzylisoquinoline product.

281. The engineered non-plant cell of any of clauses 196-222, wherein the engineered non-plant cell comprises two or more additional heterologous coding sequences, wherein the two or more additional heterologous coding sequences encode at least a first additional enzyme and a second additional enzyme that are involved in a metabolic pathway that converts a substrate to the benzylisoquinoline alkaloid product, wherein the first additional enzyme and the second additional enzyme are operably connected along the metabolic pathway, and wherein each of the first additional and second additional enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, and BIA epimerase.

282. The engineered non-plant cell of any of clauses 196-222, wherein the engineered non-plant cell comprises three additional heterologous coding sequences, wherein the three heterologous coding sequences encode at least a first additional enzyme, a second additional enzyme, and a third additional enzyme that are involved in a metabolic pathway that converts a substrate to the benzylisoquinoline alkaloid product, wherein the first additional enzyme, the second additional enzyme, and the third additional enzyme are operably connected along the metabolic pathway, and wherein each of the first additional, second additional, and third additional enzymes involved in the metabolic pathway that produces the benzylisoquinoline alkaloid product is selected from the group consisting of norcoclaurine synthase, norcoclaurine 6-*O*-methyltransferase, coclaurine-*N*-methyltransferase, 4'-*O*-methyltransferase, cytochrome P450 80B1, cytochrome P450 reductase, and BIA epimerase.

283. The engineered non-plant cell of any of the previous clauses, wherein the engineered non-plant cell is an engineered yeast cell.

284. The method of any of the previous clauses, wherein the engineered non-plant cell is an engineered yeast cell.

285. The engineered non-plant cell of any of clauses 1-282, wherein the engineered non-plant cell is an engineered bacteria cell.

286. The method of any of clauses 1-282, wherein the engineered non-plant cell is an engineered bacteria cell.

[00340] While preferred embodiments of the invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein may be employed in practicing the invention. It is intended that the following claims define the scope

of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of epimerizing an (S)-1-benzylisoquinoline alkaloid to an (R)-1-benzylisoquinoline alkaloid, comprising:

contacting the (S)-1-benzylisoquinoline alkaloid with at least one enzyme,

wherein contacting the (S)-1-benzylisoquinoline alkaloid with the at least one enzyme converts the (S)-1-benzylisoquinoline alkaloid to an (R)-1-benzylisoquinoline alkaloid.

2. The method of claim 1, wherein the at least one enzyme is produced by culturing an engineered non-plant cell having a coding sequence for encoding the at least one enzyme.

3. The method of claim 2, further comprising:

adding an (S)-1-benzylisoquinoline alkaloid to the cell culture.

4. The method of claim 3, further comprising:

recovering the (R)-1-benzylisoquinoline alkaloid, or a derivative thereof, from the cell culture.

5. The method of any one of claims 1-4, wherein the at least one enzyme comprises an oxidase.

6. The method of any one of claims 1-5, wherein the at least one enzyme comprises a reductase.

7. The method of any one of claims 1-6, wherein the at least one enzyme comprises an epimerase.

8. The method of claim 7, wherein the epimerase comprises an amino acid sequence selected from the group consisting of: SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15.

9. The method of claim 7 or 8, wherein the epimerase comprises an oxidase domain and a reductase domain.

10. The method of claim 9, wherein the oxidase domain is a cytochrome P450 oxidase-like domain.

11. The method of claim 9, wherein the reductase domain is a codeinone reductase-like domain.

12. The method of any one of claims 7-11, wherein the engineered non-plant cell is an engineered yeast cell.

13. The method of any one of claims 1-12, wherein the (S)-1-benzylisoquinoline alkaloid is (S)-reticuline.

14. The method of any one of claims 1-13, wherein the (R)-1-benzylisoquinoline alkaloid is (R)-reticuline.

15. The method of any one of claims 1-14, wherein the (S)-1-benzylisoquinoline alkaloid is produced within the engineered non-plant cell by a metabolic pathway starting with L-tyrosine.

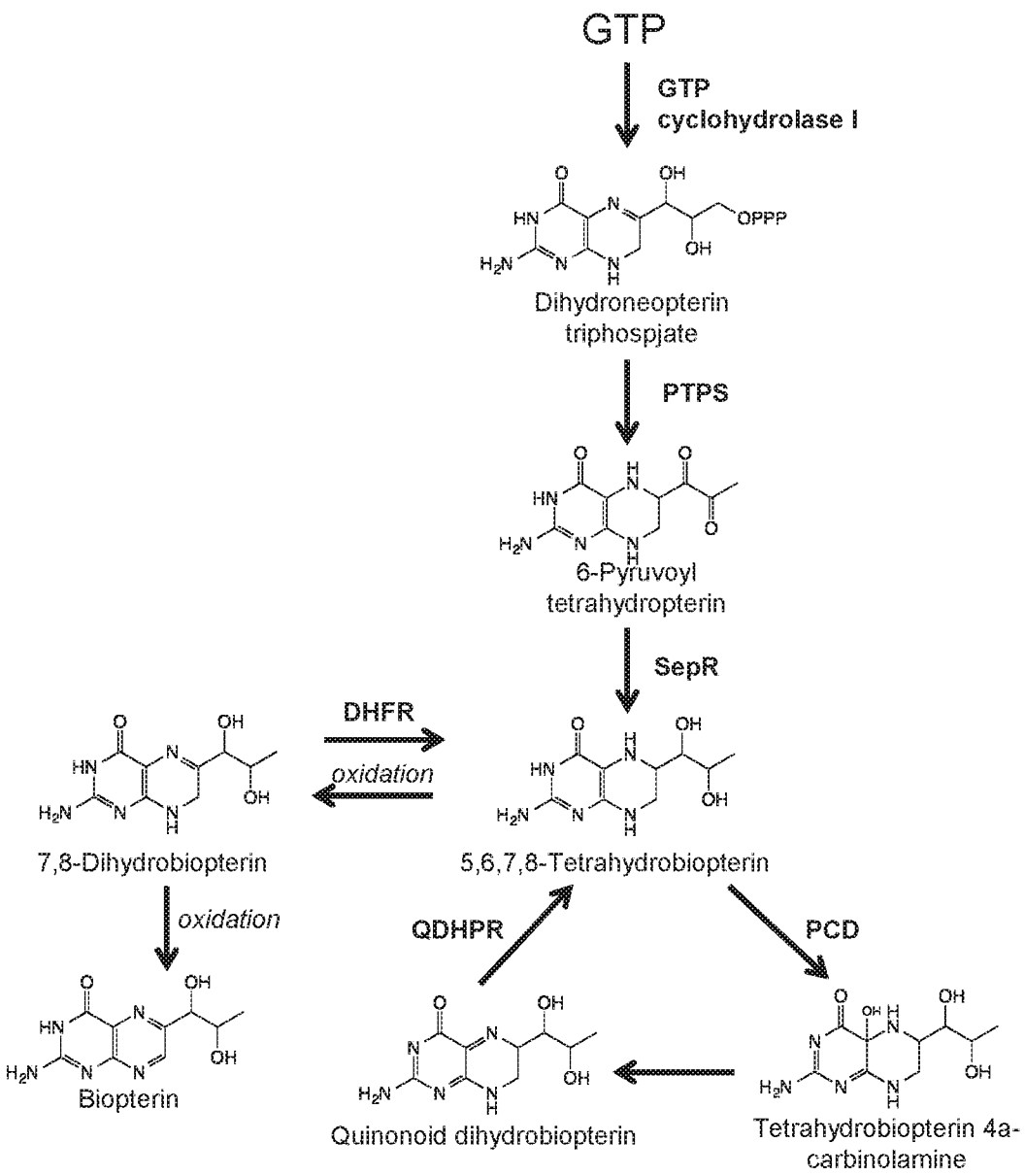

GTP
GTP cyclohydrolase I
Dihydroneopterin triphospjate
PTPS
6-Pyruvoyl tetrahydropterin
SepR
DHFR
oxidation
7,8-Dihydrobiopterin
5,6,7,8-Tetrahydrobiopterin
oxidation
QDHPR
PCD
Biopterin
Quinonoid dihydrobiopterin
Tetrahydrobiopterin 4a-carbinolamine

FIG. 1

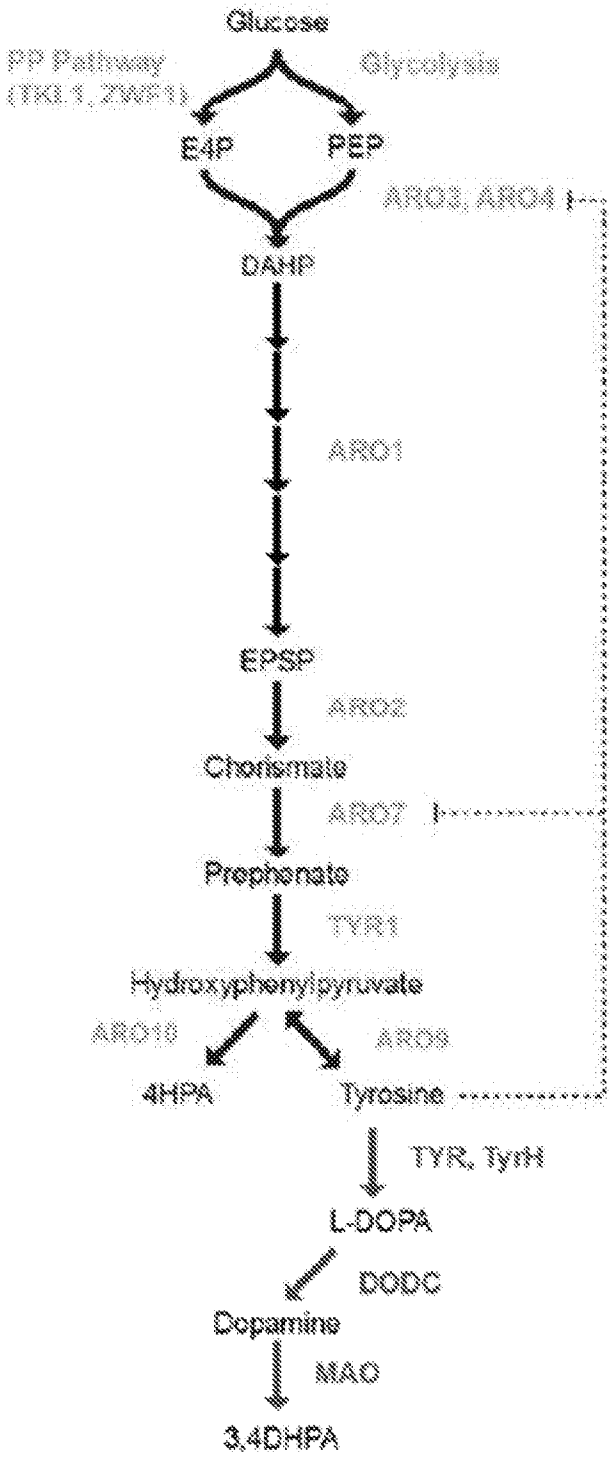

Glucose
PP Pathway
(TKL1, ZWF1)
Glycolysis
E4P
PEP
ARO3, ARO4
DAHP
ARO1
EPSP
ARO2
Chorismate
ARO7
Prephenate
TYR1
Hydroxyphenylpyruvate
ARO10
ARO9
4HPA
Tyrosine
TYR, TyrH
L-DOPA
DODC
Dopamine
MAO
3,4DHPA

FIG. 2

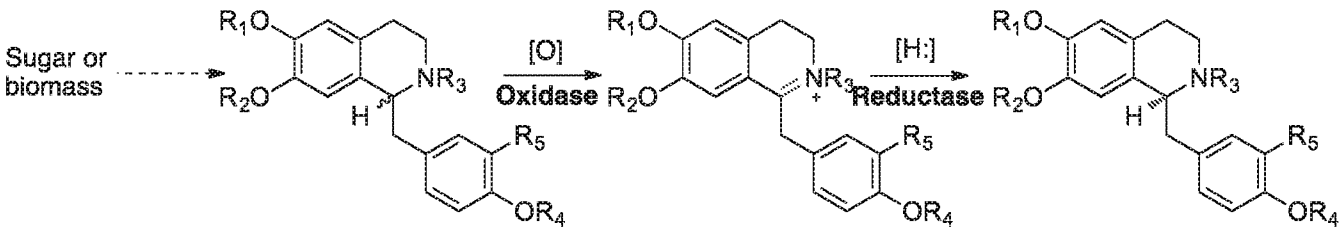


FIG. 3

>RQNK-2062398
MELQYISYFQPTSSVVALLLALVSILSSVVVLRKTFLNNYSSSPASSTKTAVLSH
QRQQSCALPISGLLHIFMNKNGLIHVTLGNMADKYGPIFSFPTGSHRTLVVSS
WEMVKECFTGNNDTAFSNRPIPLAFKTIFYACGGIDSYGLSSVPYGKYWREL
RKVCVHNLLSNQQLLKFRHLIISQVDTSFNKLYELCKNSEDNHGNYTTTTTTA
AGMVRIDDWLAELSFNVIGRIVCGFQSGPKTGAPSRVEQFKEAINEASYFMS
TSPVSDNVPMLGWIDQLTGLTRNMKHCGKKLDLVVESIINDHRQKRRFSRTK
GGDEKDDEQDDFIDICLSIMEQPQLPGNNNPSQIPIKSIVLDMIGGGTDTTKLT
TIWTLSLLLNNPHVLDKAKQEVDAHFRTKRRSTNDAAAAVVDFDDIRNLVYIQ
AIIKESMRLYPASPVVERLSGEDCVVGGFHVPAGTRLWANVWKMQRDPKVW
DDPLVFRPDRFLSDEQKMVDVRGQNYELLPFGAGRRVCPGVSFSLDLMQLV
LTRLILEFEMKSPSGKVDMTATPGLMSYKVIPLDILLTHRRIKPCVQSAASERD
MESSGVPVITLGSGKVMPVLGMGTFEKVGKGSERERLAILKAIEVGYRYFDT
AAAYETEEVLGEAIAEALQLGLVKSRDELFISSMLWCTDAHADRVLLALQNSL
RNLKLEYVDLYMLPFPASLKPGKITMDIPEEDICRMDYRSVWAAMEECQNLG
FTKSIGVSNFSCKKLQELMATANIPPAVNQVEMSPAFQQKKLREYCNANNILV
SAISVLGSNGTPWGSNAVLGSEVLKKIAMAKGKSVAQVSMRWVYEQGASLV
VKSFSEERLRENLNIFDWELTKEDHEKIGEIPQCRILSAYFLVSPNGPFKSQEE
LWDDEA*

FIG. 4

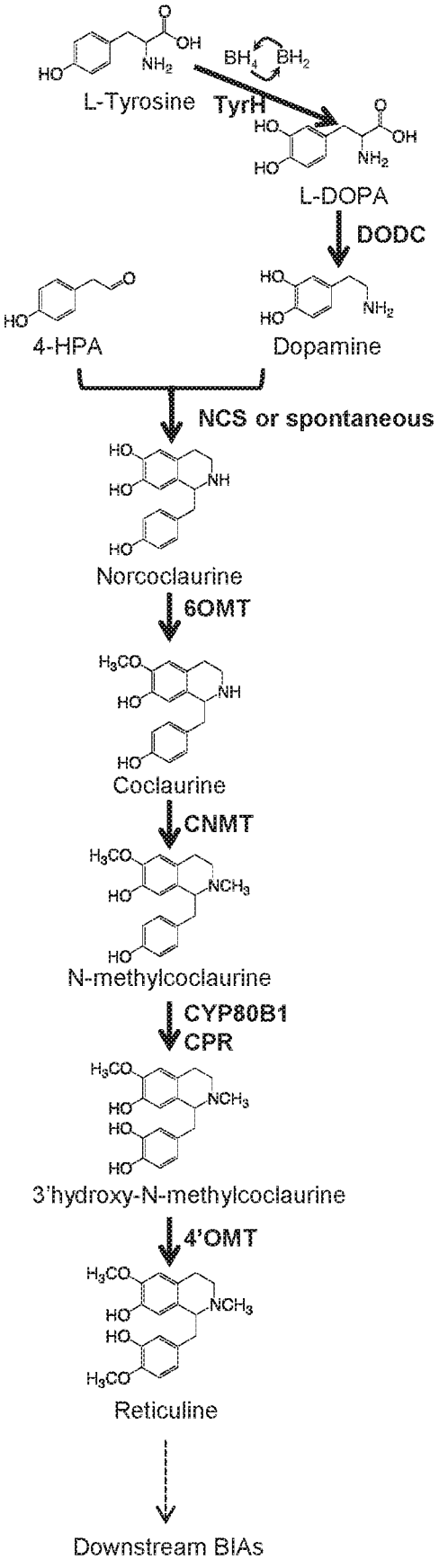

L-Tyrosine
TyrH
L-DOPA
DODC
4-HPA
Dopamine
NCS or spontaneous
Norcoclaurine
6OMT
Coclaurine
CNMT
N-methylcoclaurine
CYP80B1
CPR
3'hydroxy-N-methylcoclaurine
4'OMT
Reticuline
Downstream BIAs

FIG. 5

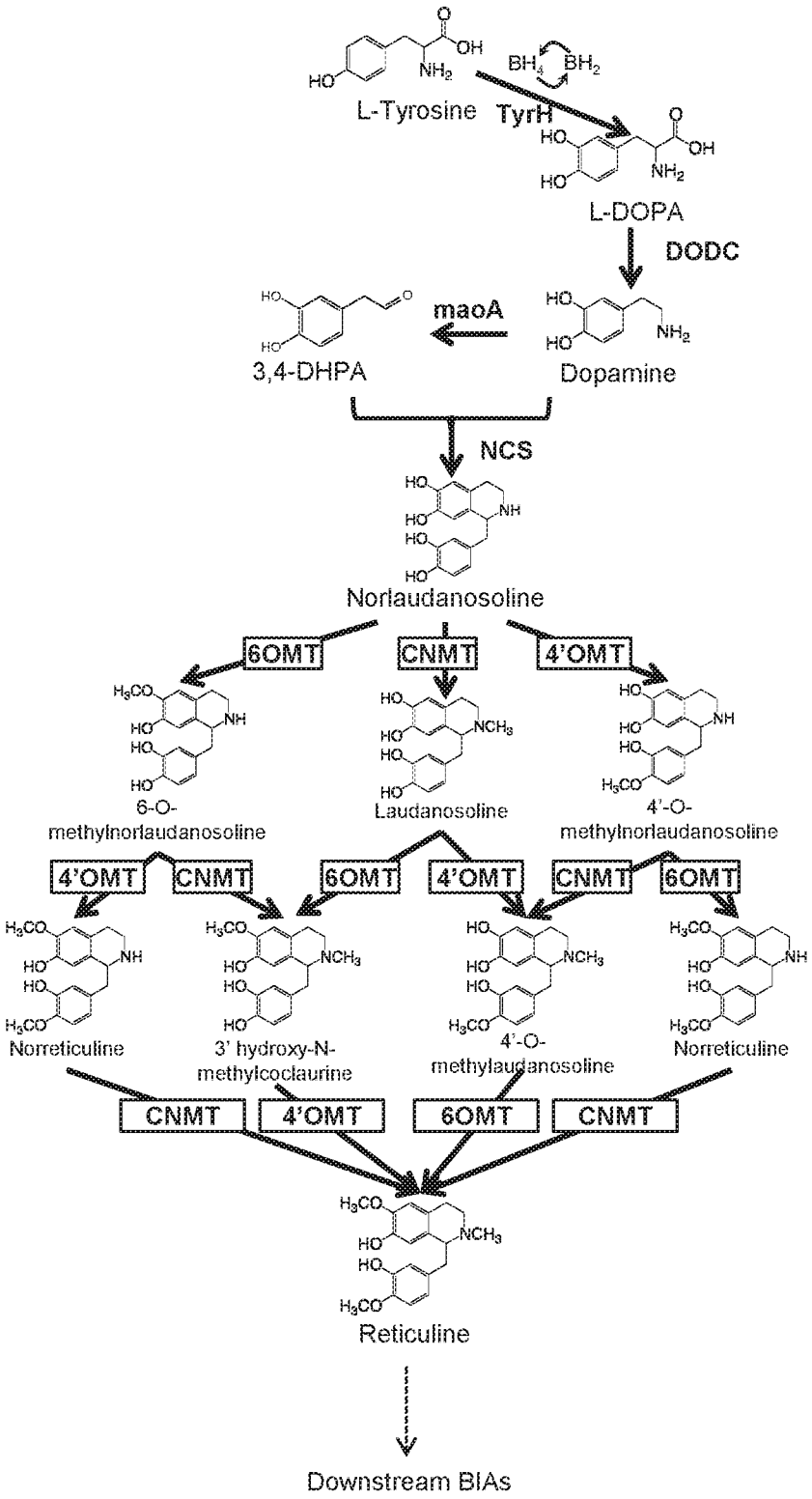

L-Tyrosine
TyrH
L-DOPA
DODC
Dopamine
maoA
3,4-DHPA
NCS
Norlaudanosoline
6OMT
CNMT
4'OMT
6-O-methylnorlaudanosoline
Laudanosoline
4'-O-methylnorlaudanosoline
4'OMT
CNMT
6OMT
4'OMT
CNMT
6OMT
Norreticuline
3' hydroxy-N-methylcoclaurine
4'-O-methylaudanosoline
Norreticuline
CNMT
4'OMT
6OMT
CNMT
Reticuline
Downstream BIAs

FIG. 6

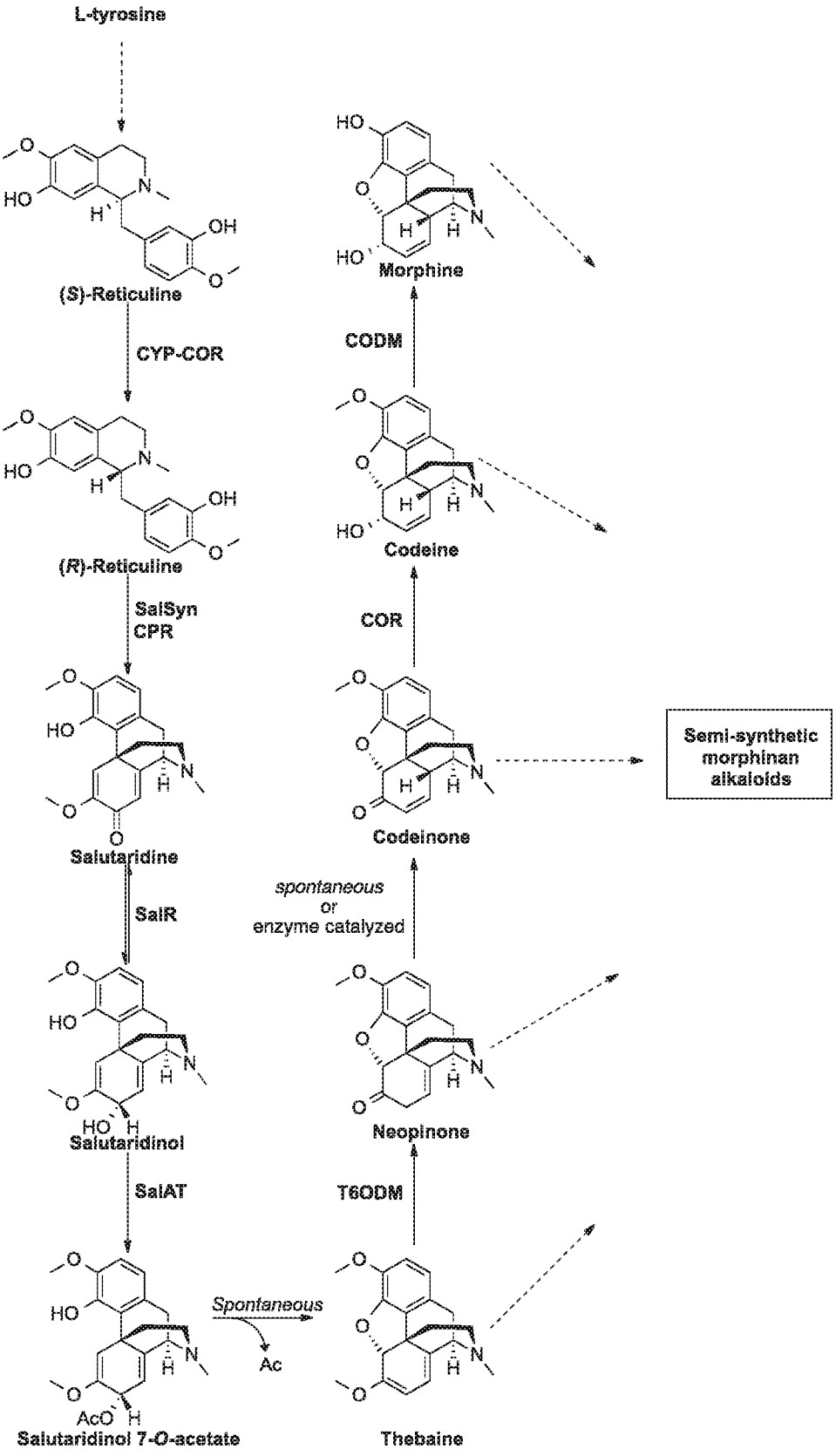

L-tyrosine
(S)-Reticuline
CYP-COR
(R)-Reticuline
SalSyn
CPR
Salutaridine
SalR
Salutaridinol
SalAT
Salutaridinol 7-O-acetate
Spontaneous
Ac
Thebaine
T6ODM
Neopinone
spontaneous
or
enzyme catalyzed
Codeinone
COR
Codeine
CODM
Morphine
Semi-synthetic
morphinan
alkaloids

FIG. 7

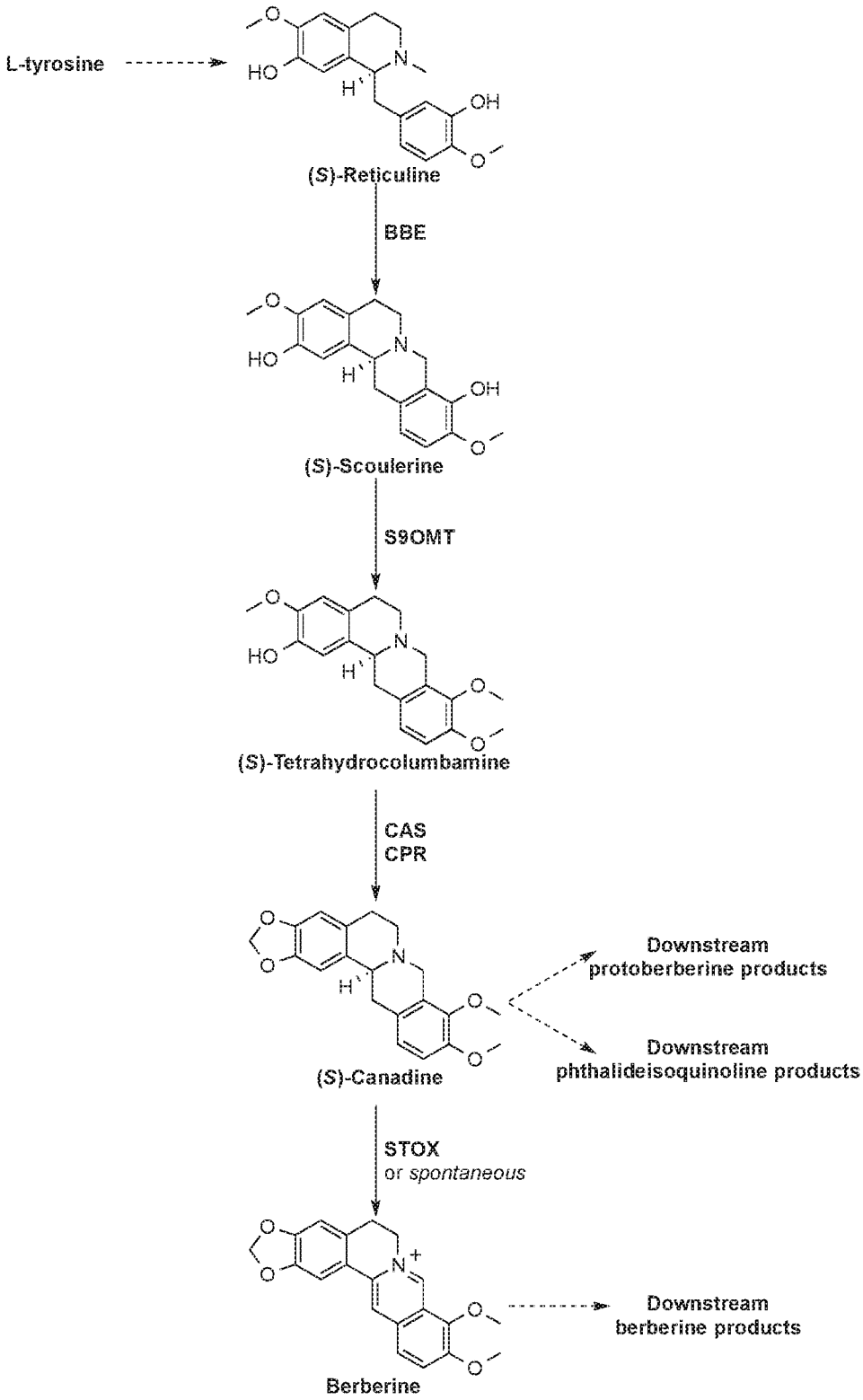

L-tyrosine
(S)-Reticuline
BBE
(S)-Scoulerine
S9OMT
(S)-Tetrahydrocolumbamine
CAS
CPR
(S)-Canadine
Downstream
protoberberine products
Downstream
phthalideisoquinoline products
STOX
or spontaneous
Berberine
Downstream
berberine products

FIG. 8

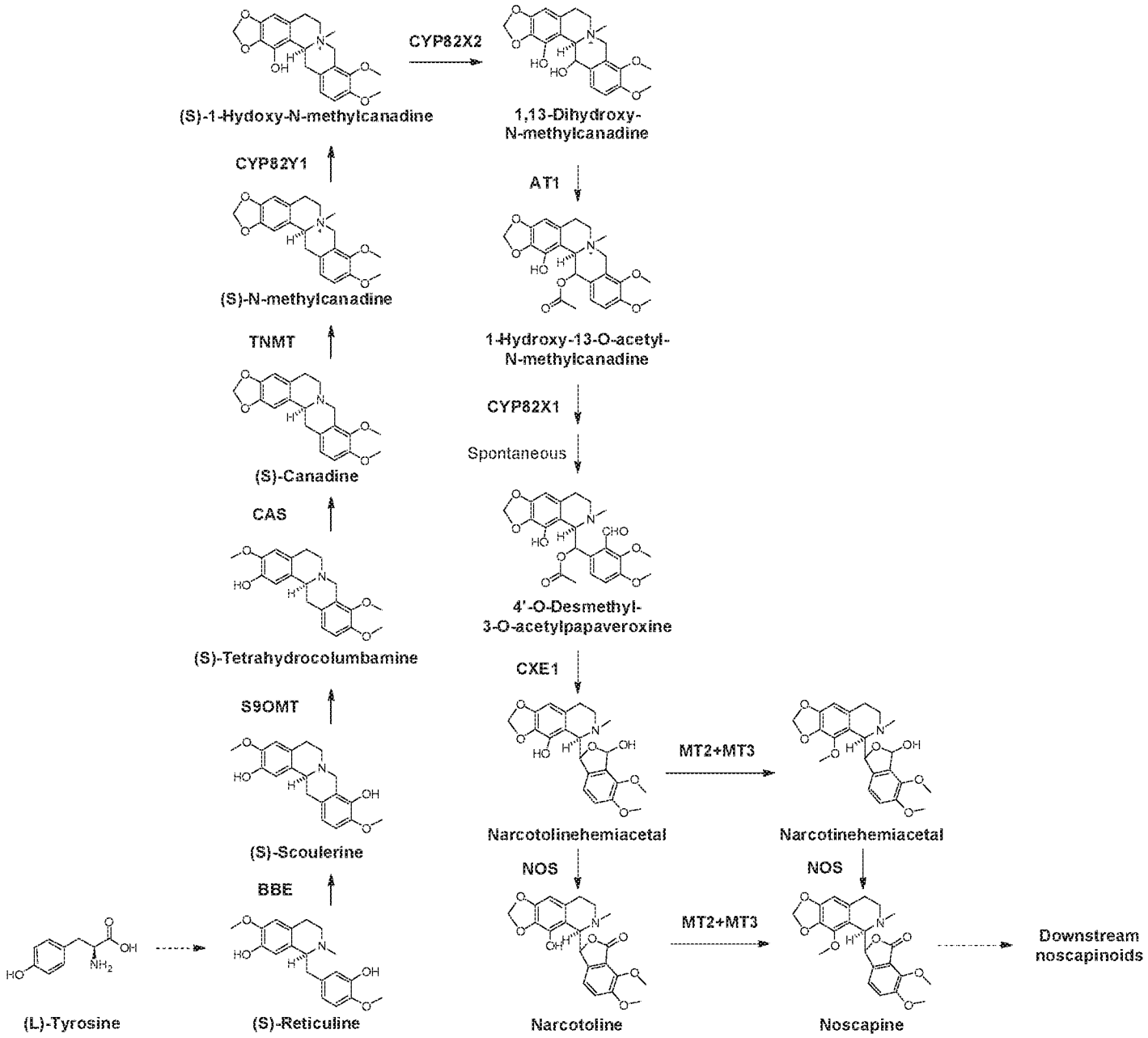

CYP82X2
(S)-1-Hydoxy-N-methylcanadine
1,13-Dihydroxy-N-methylcanadine
CYP82Y1
AT1
(S)-N-methylcanadine
1-Hydroxy-13-O-acetyl-N-methylcanadine
TNMT
CYP82X1
Spontaneous
(S)-Canadine
4'-O-Desmethyl-3-O-acetylpapaveroxine
CAS
CXE1
(S)-Tetrahydrocolumbamine
S9OMT
MT2+MT3
Narcotolinehemiacetal
Narcotinehemiacetal
(S)-Scoulerine
NOS
NOS
BBE
MT2+MT3
Downstream noscapinoids
(L)-Tyrosine
(S)-Reticuline
Narcotoline
Noscapine

FIG. 9

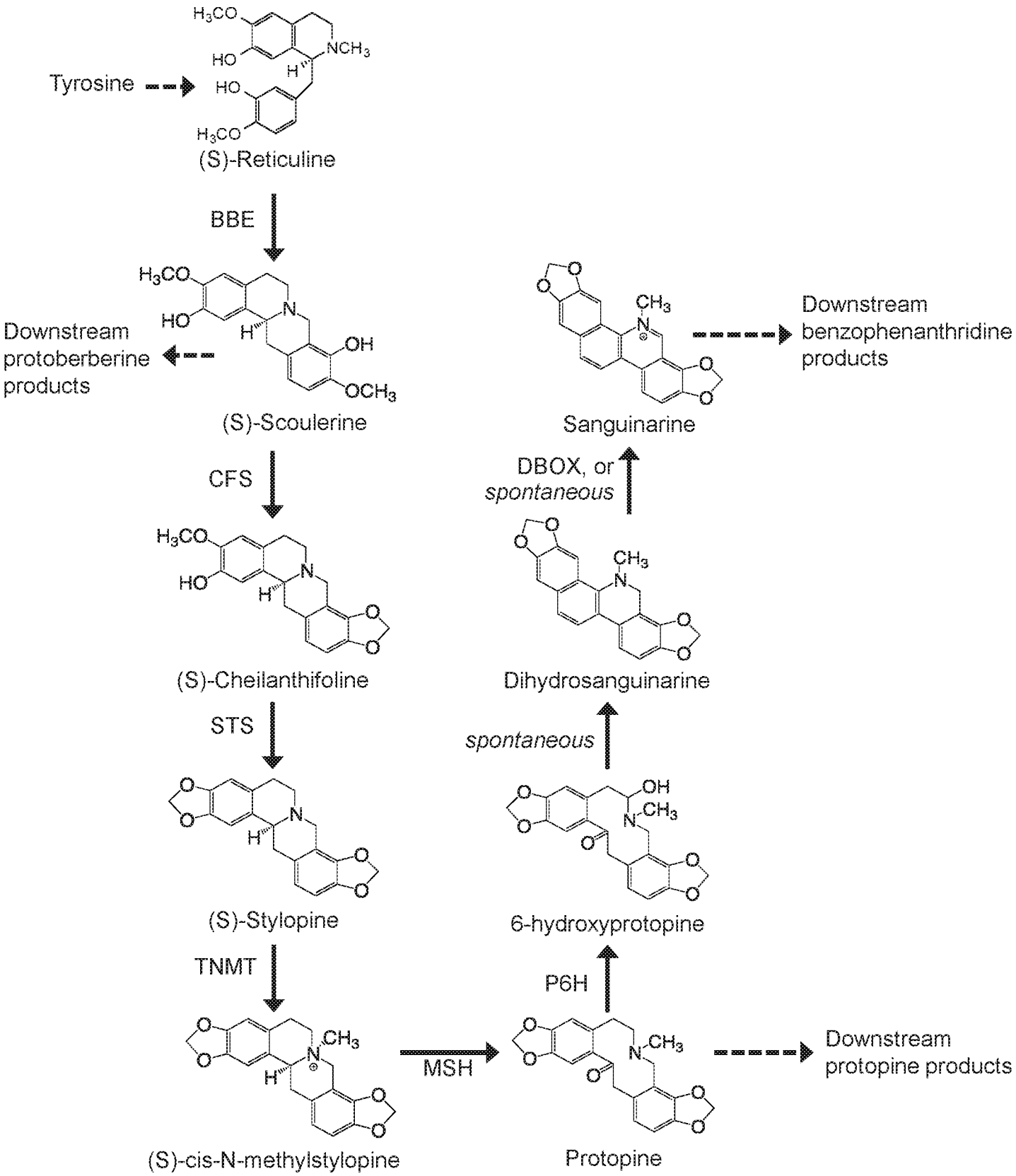

Tyrosine
(S)-Reticuline
BBE
Downstream protoberberine products
(S)-Scoulerine
CFS
(S)-Cheilanthifoline
STS
(S)-Stylopine
TNMT
(S)-cis-N-methylstylopine
MSH
Protopine
P6H
6-hydroxyprotopine
spontaneous
Dihydrosanguinarine
DBOX, or spontaneous
Sanguinarine
Downstream benzophenanthridine products
Downstream protopine products

FIG. 10

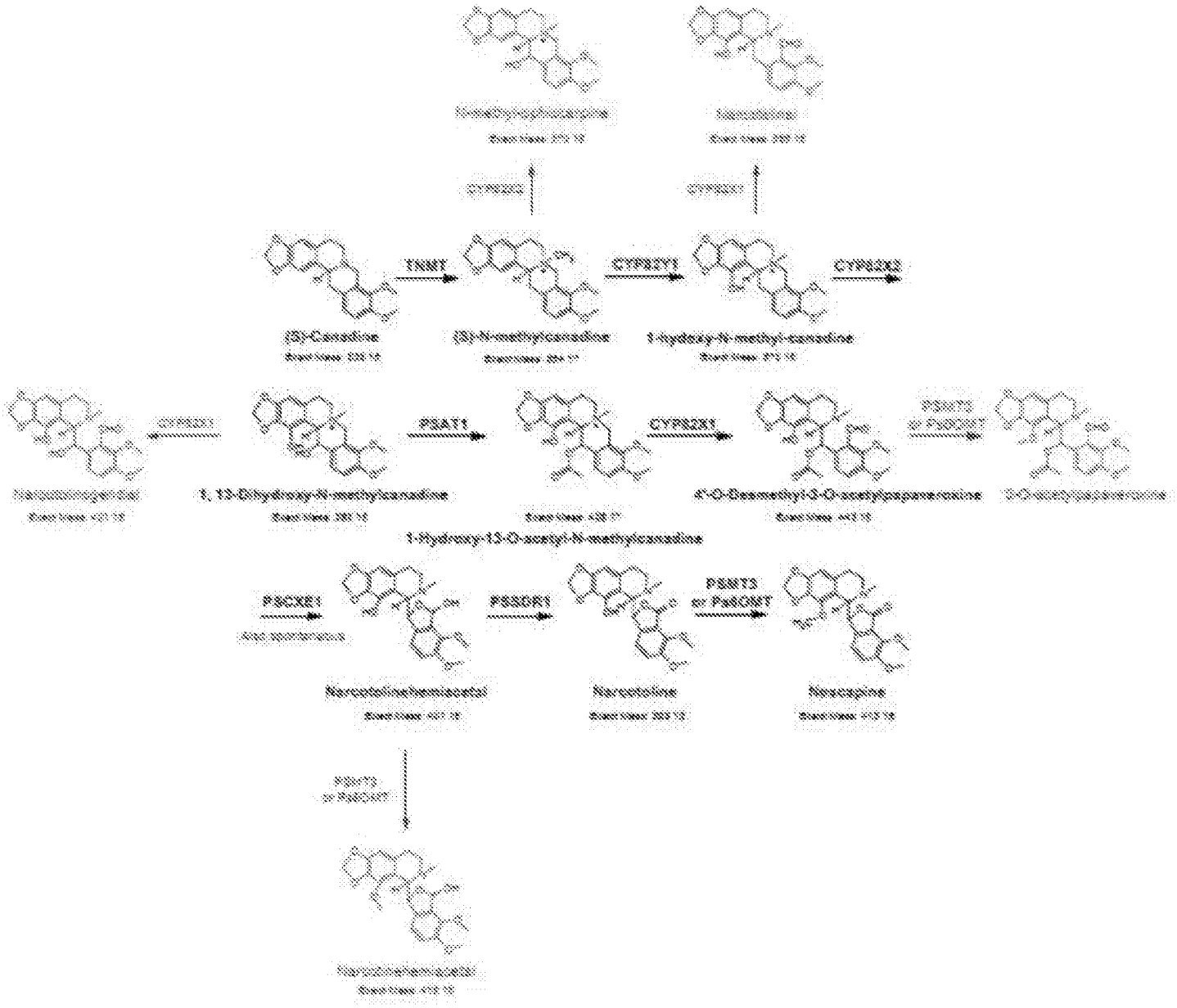

TNMT
CYP82Y1
CYP82X2
PSAT1
CYP82X1
PSCXE1
PSSDR1
PSMT3
or PsSOMT

FIG. 11A

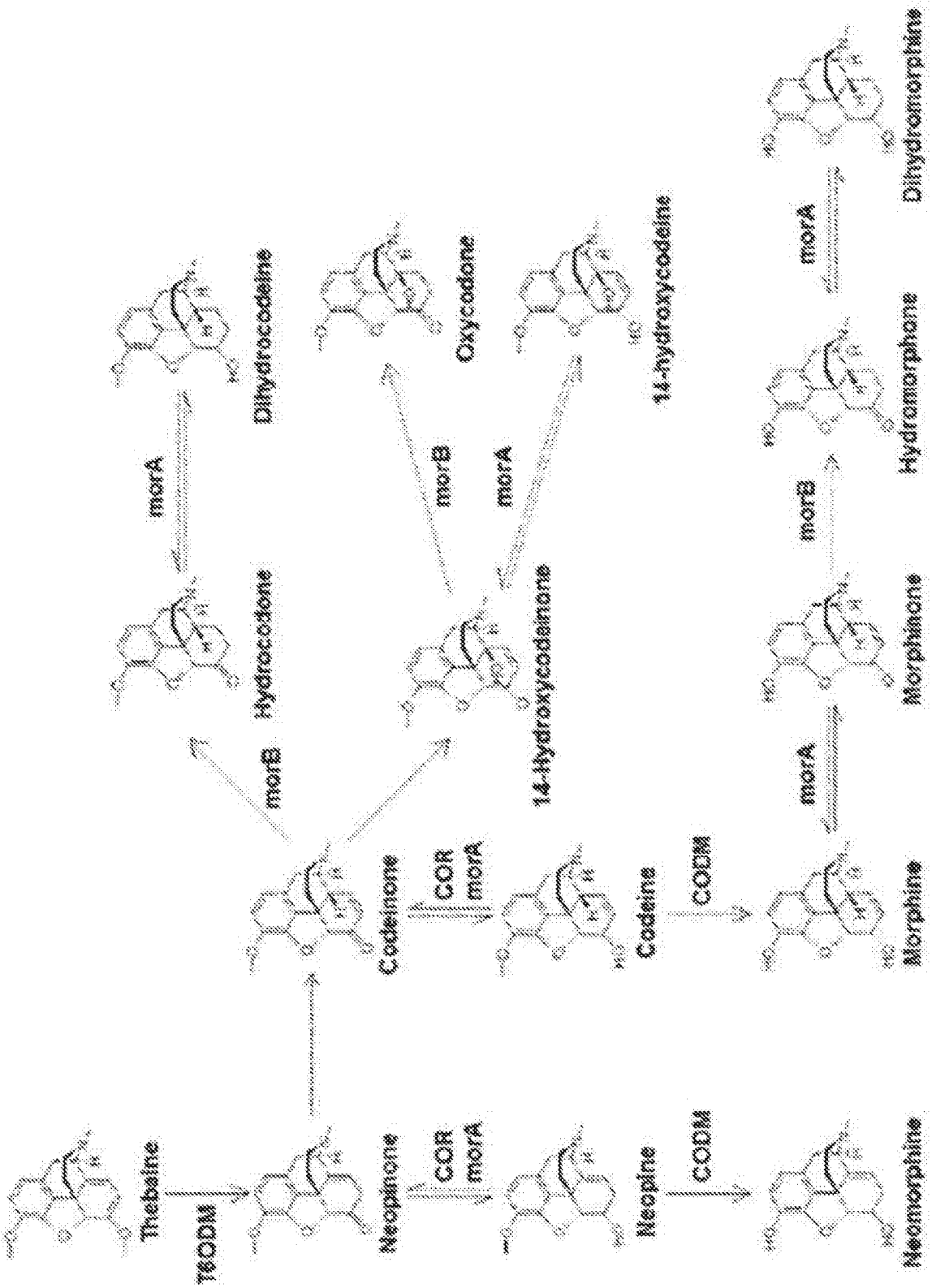

Thebaine
T6ODM
Neopinone
COR
morA
Neopine
CODM
Neomorphine
morB
Hydrocodone
morA
Dihydrocodeine
Codeinone
COR
morA
Codeine
CODM
Morphine
morB
Oxycodone
morA
14-Hydroxycodeinone
14-hydroxycodeine
morA
Morphinone
morB
Hydromorphone
morA
Dihydromorphine

FIG. 11B

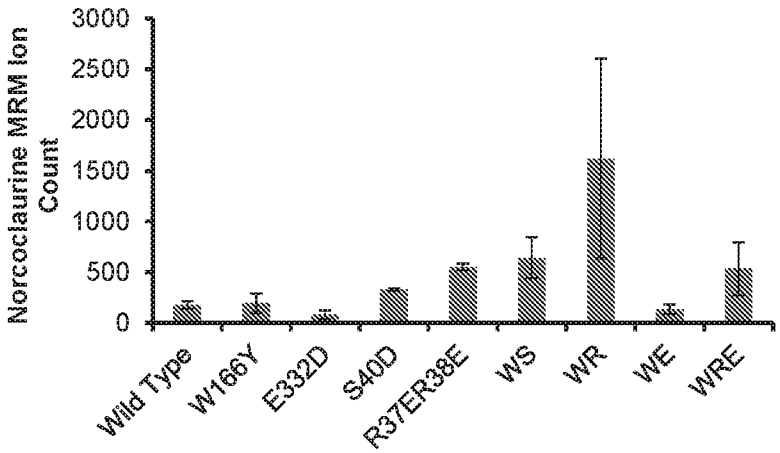

Norcoclaurine MRM Ion Count
3000
2500
2000
1500
1000
500
0
Wild Type
W166Y
E332D
S40D
R37ER38E
WS
WR
WE
WRE

FIG. 12

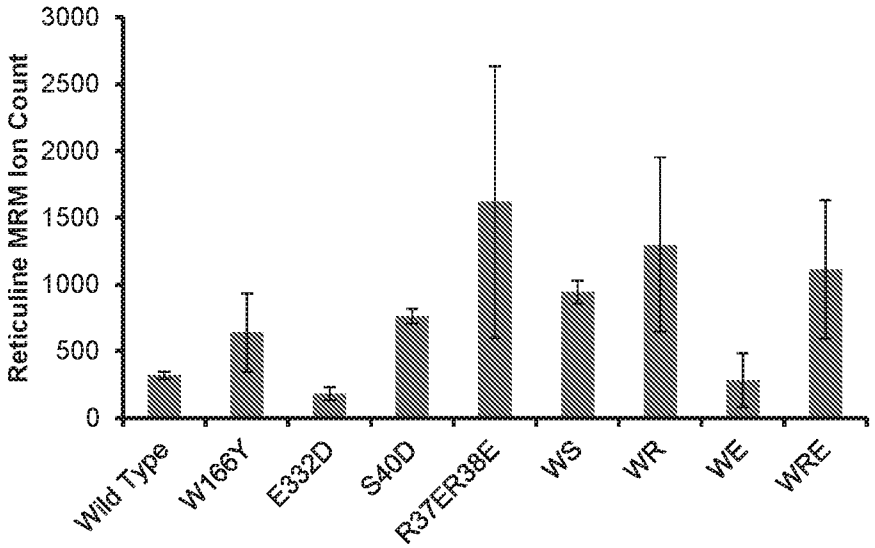

Reticuline MRM Ion Count
3000
2500
2000
1500
1000
500
0
Wild Type
W166Y
E332D
S40D
R37ER38E
WS
WR
WE
WRE

FIG. 13

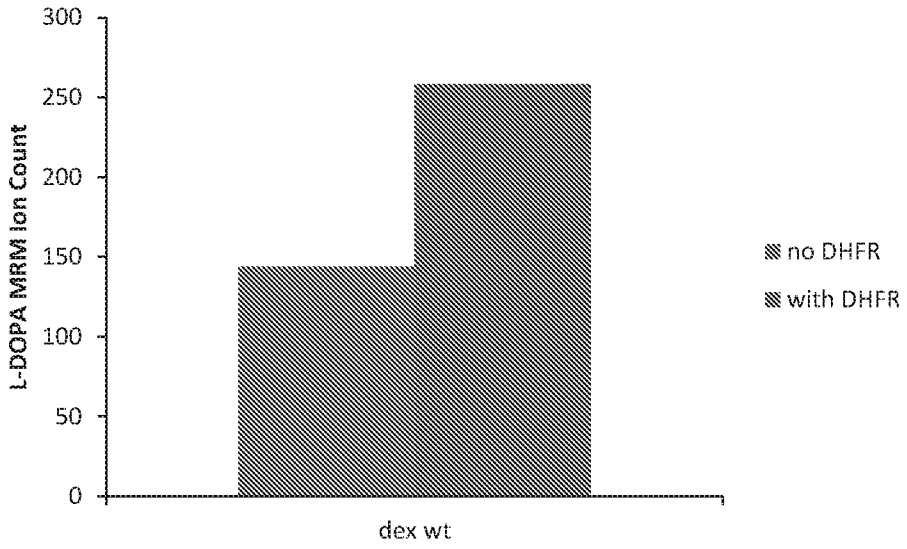

300
250
200
150
100
50
0
L-DOPA MRM Ion Count
dex wt
no DHFR
with DHFR

FIG. 14

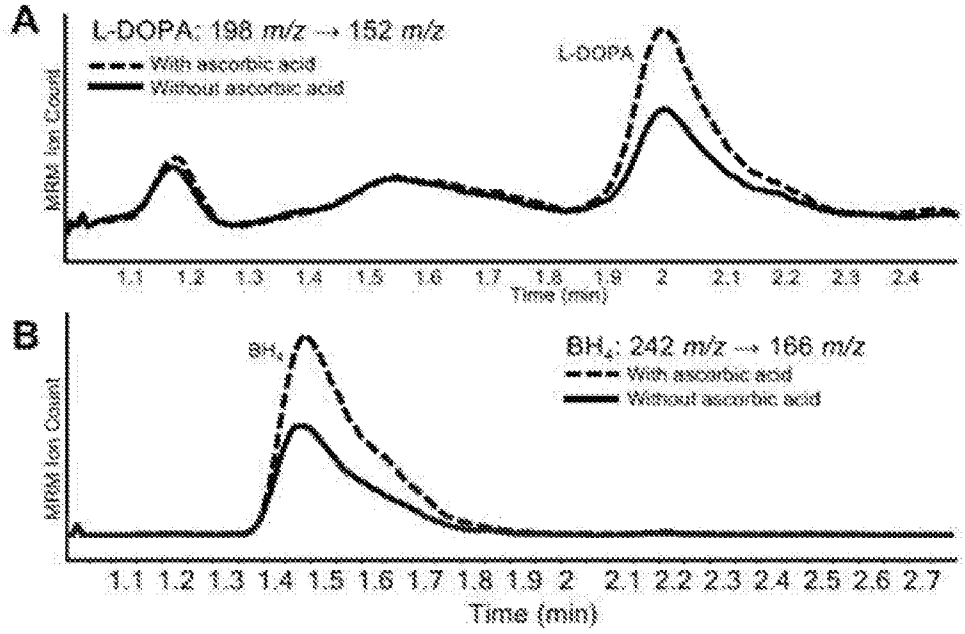
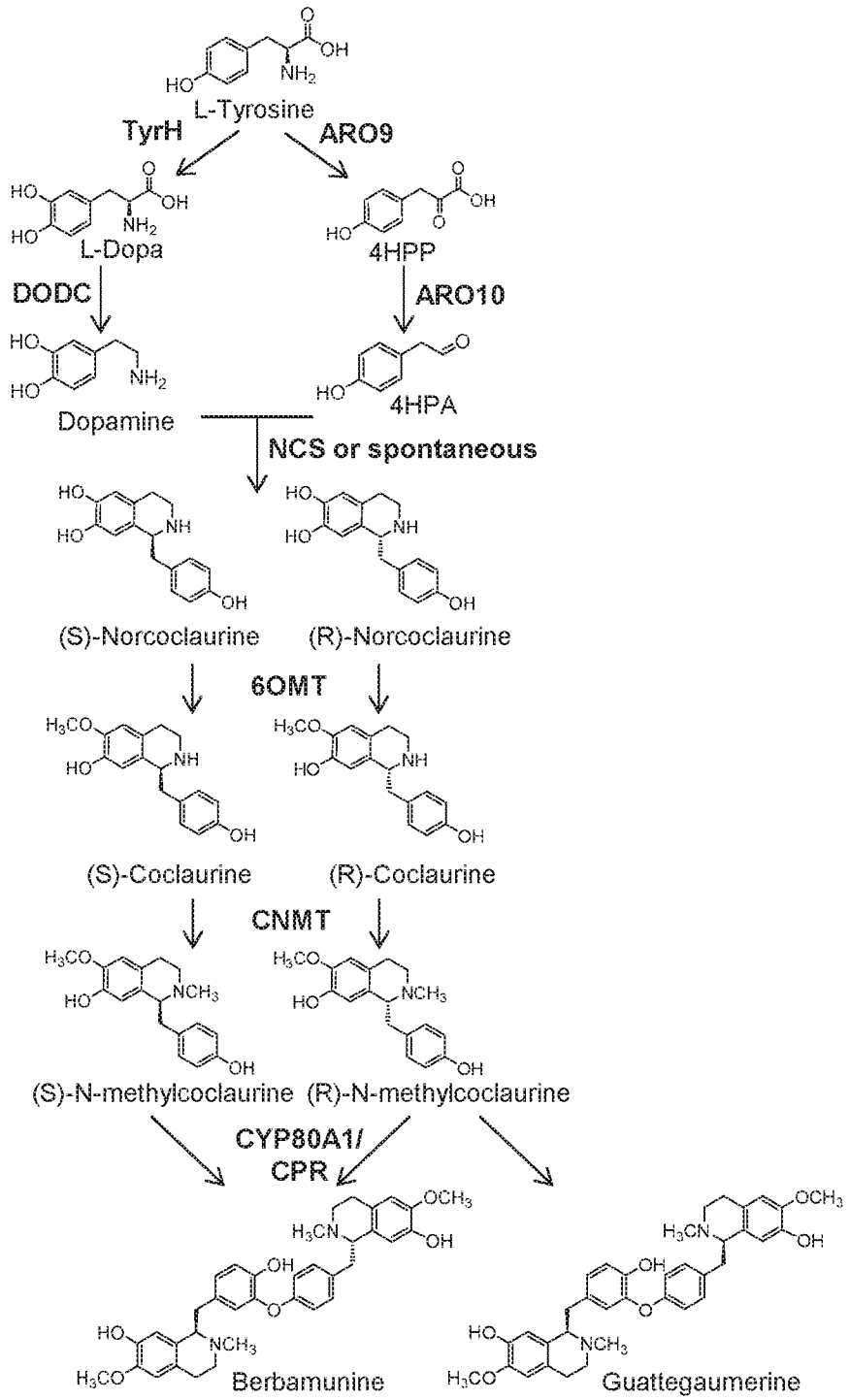

A
L-DOPA: 198 m/z → 152 m/z
With ascorbic acid
Without ascorbic acid
L-DOPA
MRM Ion Count
1.1 1.2 1.3 1.4 1.5 1.6 1.7 1.8 1.9 2 2.1 2.2 2.3 2.4
Time (min)
B
BH4
BH4: 242 m/z → 166 m/z
With ascorbic acid
Without ascorbic acid
MRM Ion Count
1.1 1.2 1.3 1.4 1.5 1.6 1.7 1.8 1.9 2 2.1 2.2 2.3 2.4 2.5 2.6 2.7
Time (min)

FIG. 15

A



B

Genotype:

YDR514CΔ::P_{PYK1}-PsCNMT-T_{MFa1}, P_{PGK1}-Ps6OMT-T_{PHO5}, *LEU2 marker*, P_{TEF1}-PsCPR-T_{CYC1}, P_{GPD}-BsCYP80A1-T_{ADH1}

Schematic representation:

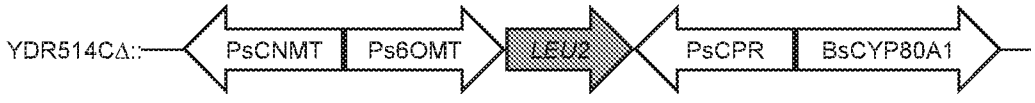


FIG. 16

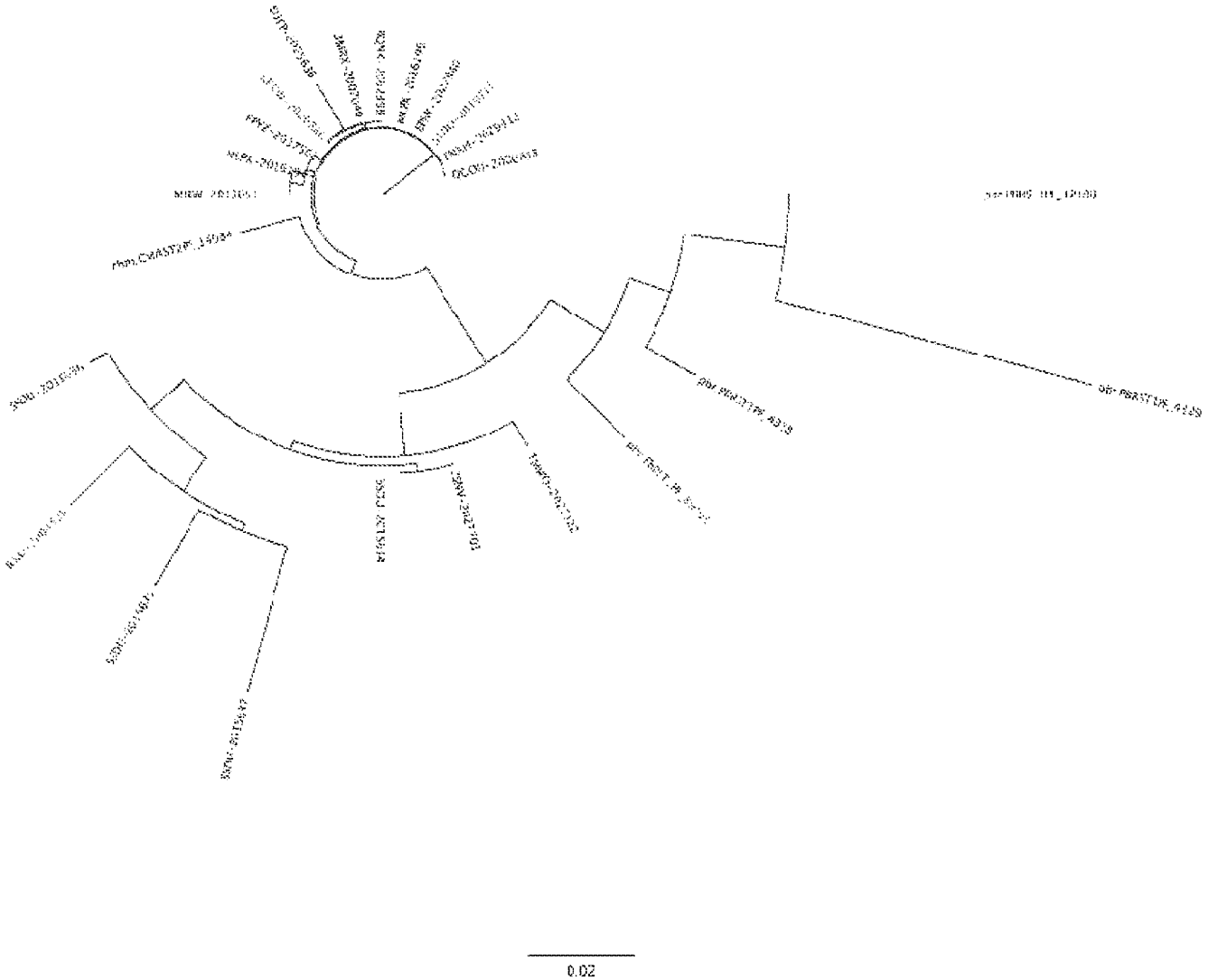


FIG. 17

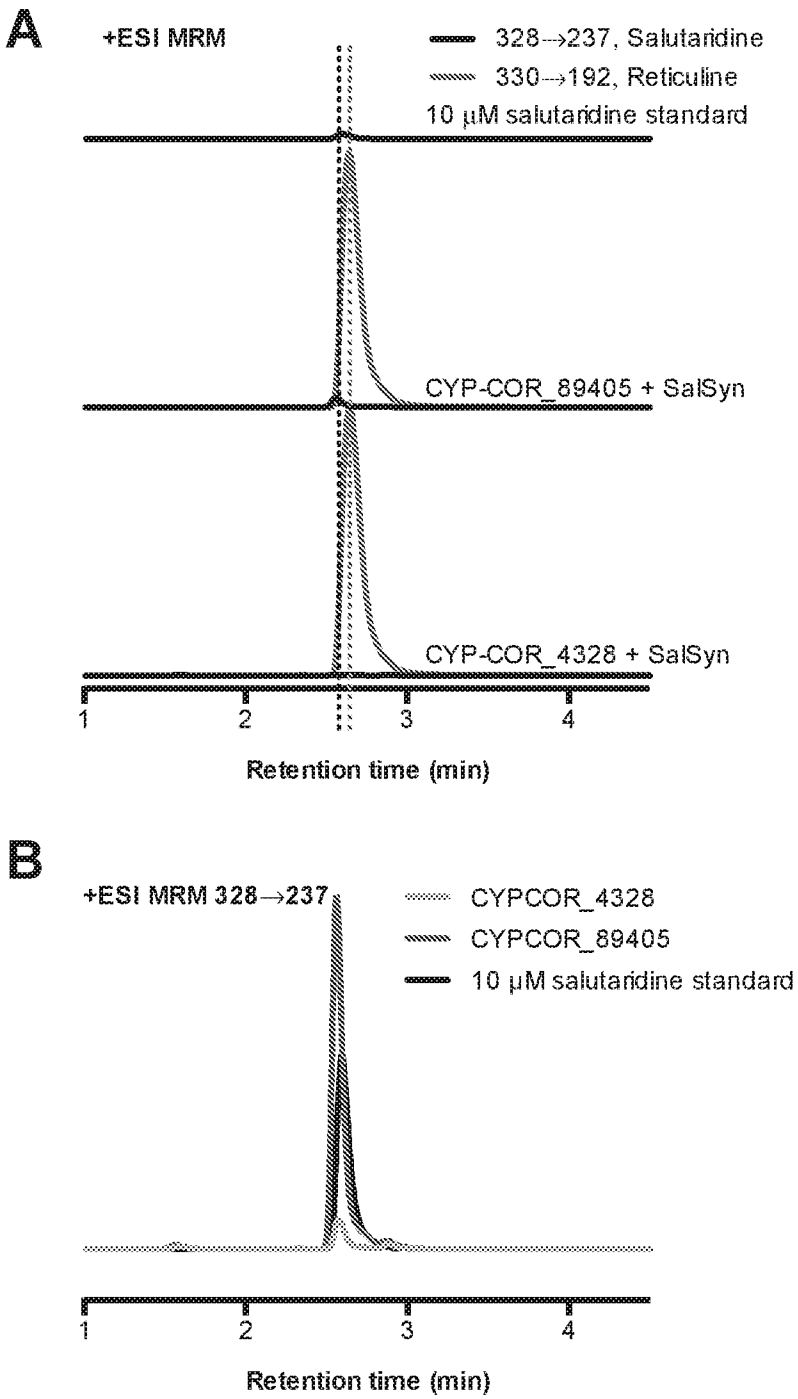

A
+ESI MRM
328→237, Salutaridine
330→192, Reticuline
10 µM salutaridine standard
CYP-COR_89405 + SalSyn
CYP-COR_4328 + SalSyn
1
2
3
4
Retention time (min)
B
+ESI MRM 328→237
CYPCOR_4328
CYPCOR_89405
10 µM salutaridine standard
1
2
3
4
Retention time (min)

FIG. 18

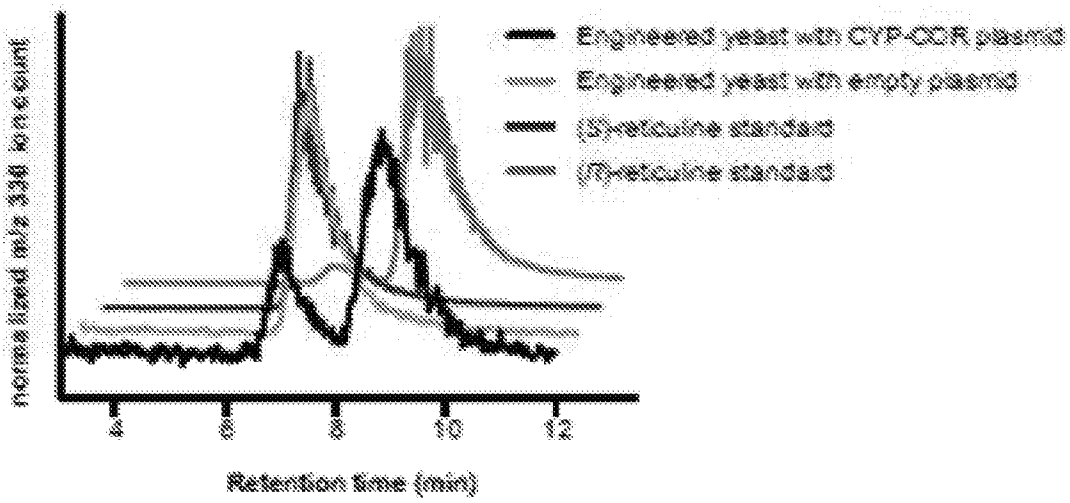

Engineered yeast with CYP-CCR plasmid
Engineered yeast with empty plasmid
(S)-reticuline standard
(R)-reticuline standard
normalized m/z 330 ion count
4
6
8
10
12
Retention time (min)

FIG. 19

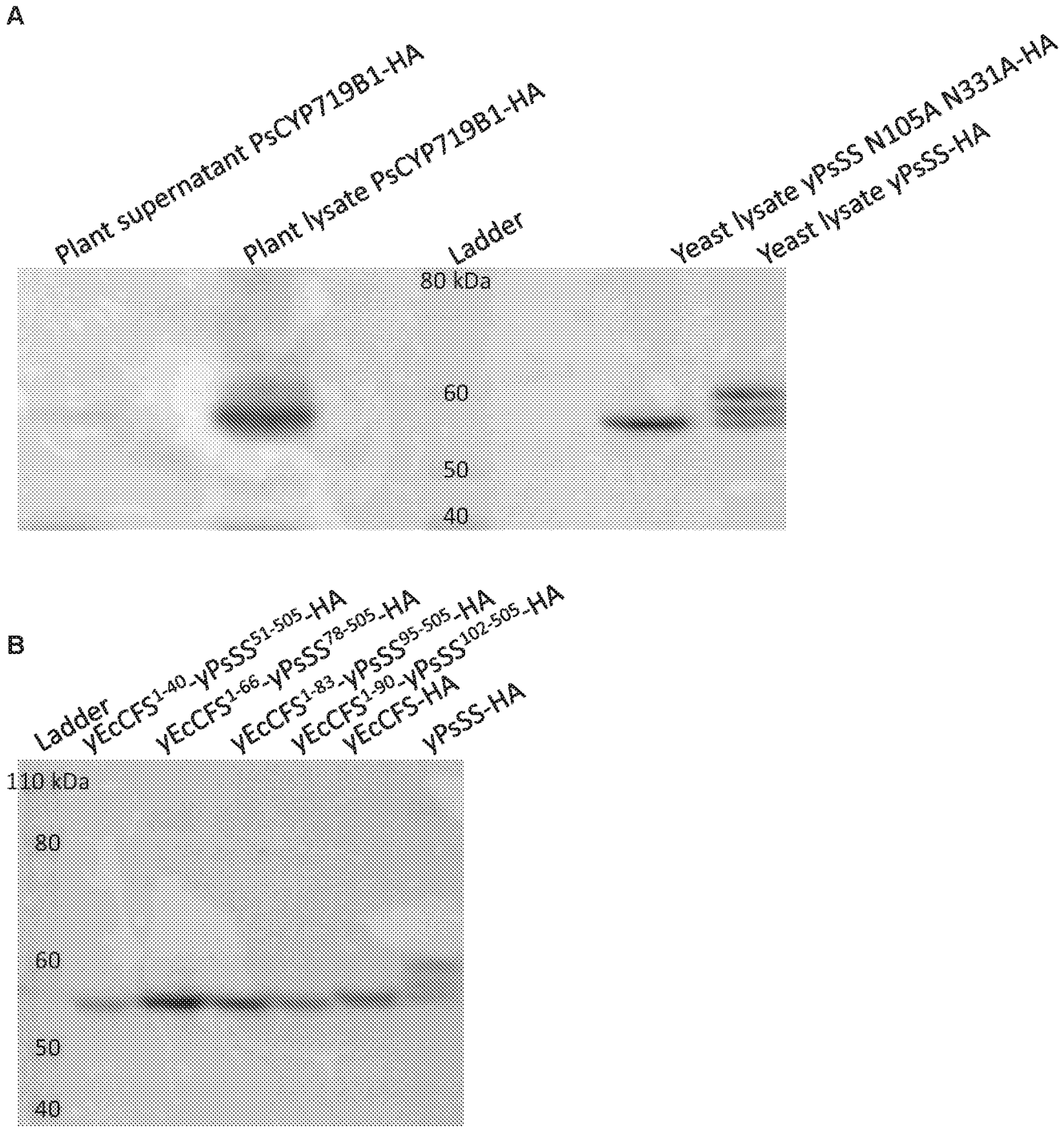

A
Plant supernatant PsCYP719B1-HA
Plant lysate PsCYP719B1-HA
Ladder
Yeast lysate yPsSS N105A N331A-HA
Yeast lysate yPsSS-HA
80 kDa
60
50
40
B
Ladder
yEcCFS1-40-yPsSS51-505-HA
yEcCFS1-66-yPsSS78-505-HA
yEcCFS1-83-yPsSS95-505-HA
yEcCFS1-90-yPsSS102-505-HA
yEcCFS-HA
yPsSS-HA
110 kDa
80
60
50
40

FIG. 20


A
Phyre2
Email sgalanie@stanford.edu
Description Undefined
Date Sat Dec 14 23:54:55 GMT 2013
Unique Job ID 118bf4a9c797fa6
Sequence MEESLWVVTA ... Download FASTA
Sequence
Secondary structure
SS confidence
Disorder
Disorder confidence
B
Phyre2
Email sgalanie@stanford.edu
Description PsSalSyn_
Date Thu Sep 13 22:30:44 BST 2012
Unique Job ID fbb80ba41159fa033
Secondary structure and disorder prediction
Sequence
Secondary structure
SS confidence
Disorder
Disorder confidence

FIG. 21

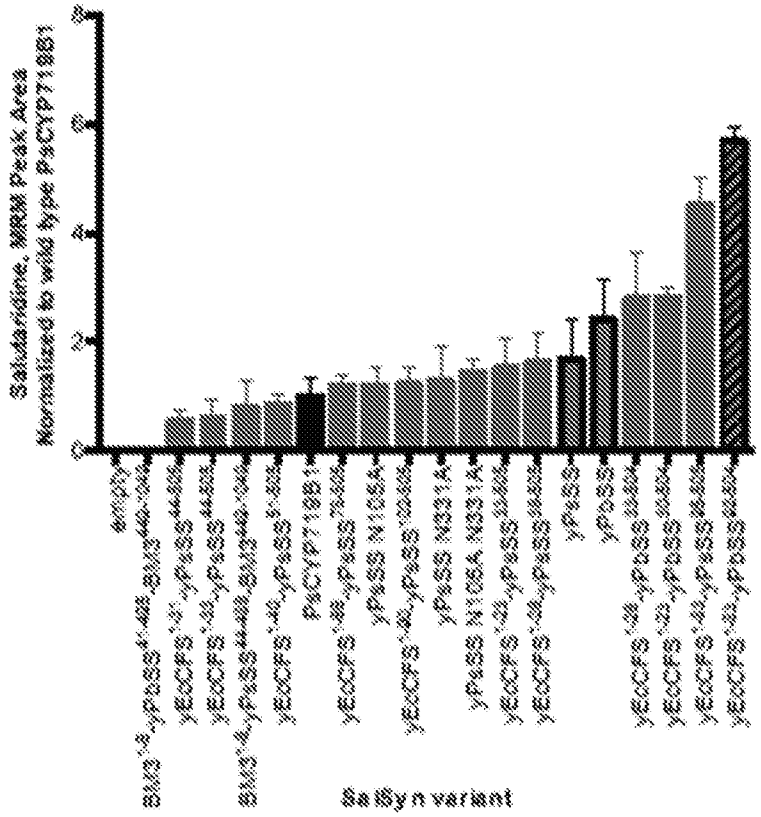

Saludaridine, MRM Peak Area
Normalized to wild type PsCYP71BE1
0
2
4
6
8
empty
BM3(1-3)-yPsSS(4-428)-BM3(442-1014)
yEcFS(1-3)-yPsSS(4-428)
yEcFS(1-40)-yPsSS(4-428)
BM3(1-4)-yPsSS(4-428)-BM3(442-1014)
yEcFS(1-40)-yPsSS(1-428)
PsCYP71BE1
yEcFS(1-46)-yPsSS(7-428)
yPsSS N106A
yEcFS(1-46)-yPsSS(10-428)
yPsSS N331A
yPsSS N106A, N331A
yEcFS(1-23)-yPsSS(4-428)
yEcFS(1-46)-yPsSS(4-428)
yPsSS
yPsSS
yEcFS(1-46)-yPsSS(4-428)
yEcFS(1-23)-yPsSS(4-428)
yEcFS(1-40)-yPsSS(4-428)
yEcFS(1-23)-yPsSS(4-428)
SalSyn variant

FIG. 22

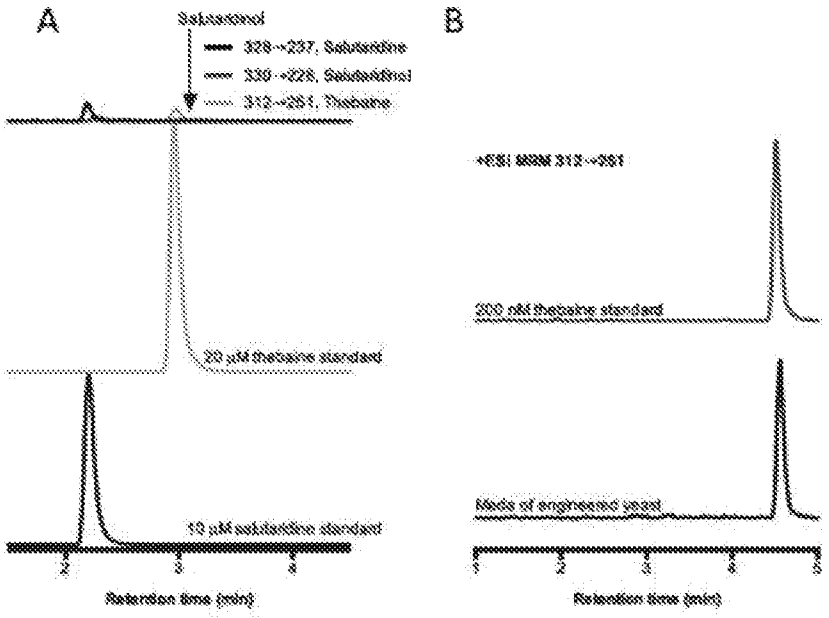

A
B
20 µM thebaine standard
10 µM salutaridine standard
200 nM thebaine standard
Retention time (min)
Retention time (min)

FIG. 23

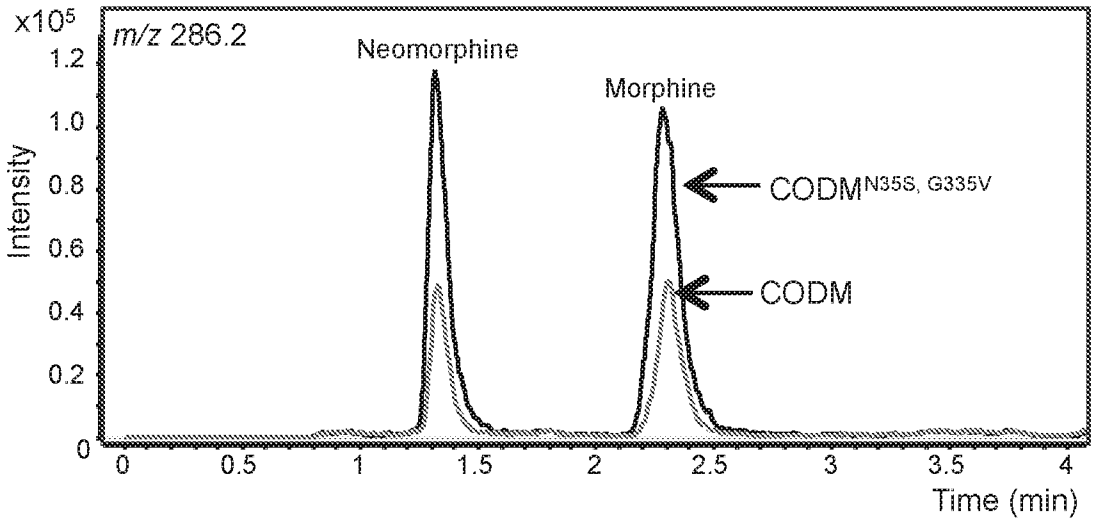

x10^5
m/z 286.2
Neomorphine
Morphine
CODM^N35S, G335V
CODM
Intensity
1.2
1.0
0.8
0.6
0.4
0.2
0
0
0.5
1
1.5
2
2.5
3
3.5
4
Time (min)

FIG. 24

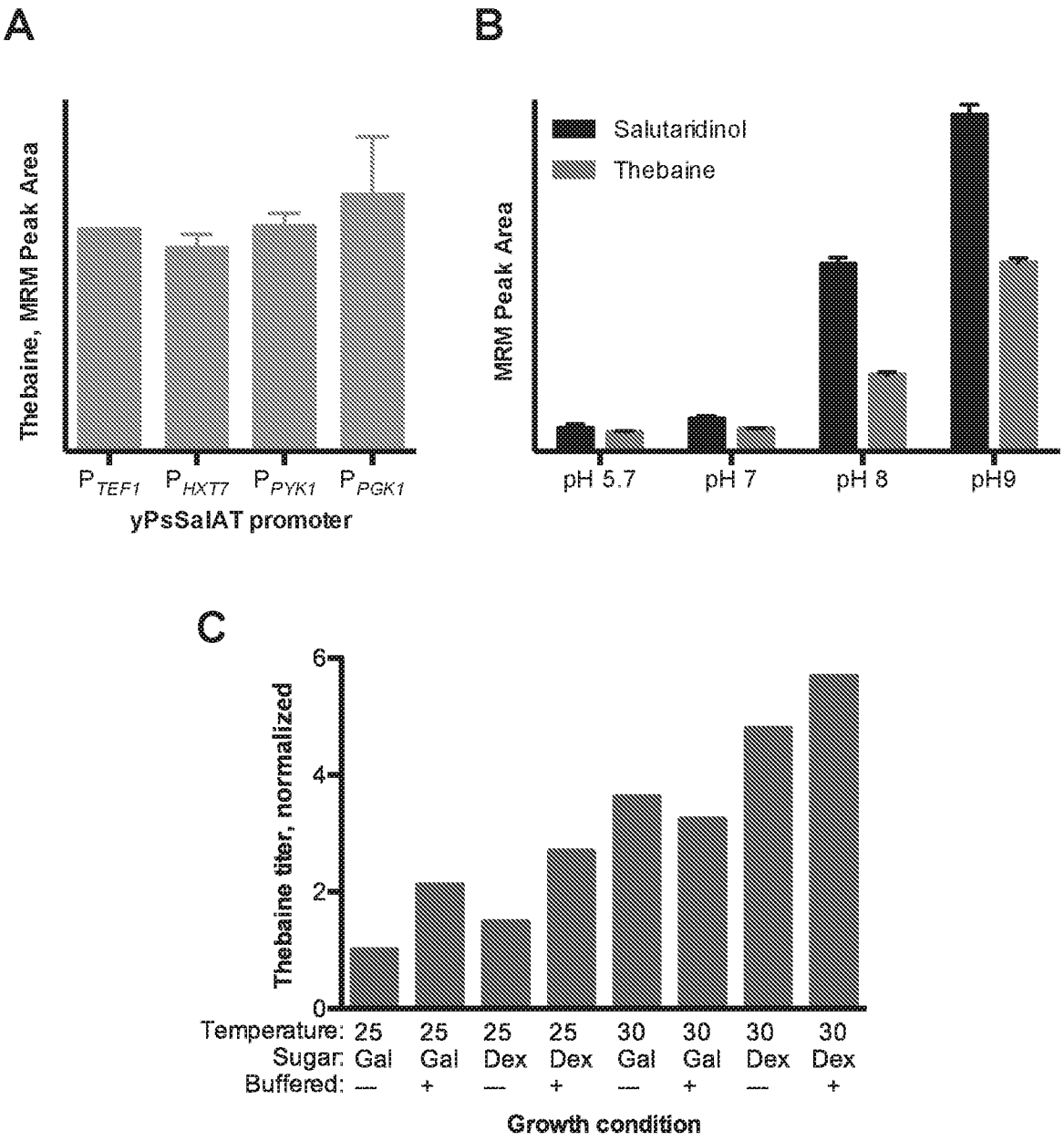

A
Thebaine, MRM Peak Area
P TEF1
P HXT7
P PYK1
P PGK1
yPsSalAT promoter
B
Salutaridinol
Thebaine
MRM Peak Area
pH 5.7
pH 7
pH 8
pH9
C
Thebaine titer, normalized
0
2
4
6
Temperature: 25 25 25 25 30 30 30 30
Sugar: Gal Gal Dex Dex Gal Gal Dex Dex
Buffered: — + — + — + — +
Growth condition

FIG. 25

Four genetic constructs integrated into the yeast genome:

1. YBR197CΔ::P_{TPI1}-RnSepR-T_{STE2}, P_{TEF1}-RnPTPS-T_{CYC1}, *KanMX marker*, P_{GPD}-RnQDHPR-T_{AHD1}, P_{PGK1}-RnPCD-T_{PHO5}

2. HIS3 Δ ::P_{GPD}-RnTyrH-T_{ADH1}, P_{TPI1}-PpDODC-T_{STE2}, *HIS5 marker*, P_{TEF1}-RnDHFR-T_{CYC1}, P_{PGK1}-CjNCS-T_{PHO5}

3. YDR514CΔ::P_{PYK1}-PsCNMT-T_{MFa1}, P_{PGK1}-Ps6OMT-T_{PHO5}, P_{GPD}-EcCYP80B1-T_{ADH1}, *LEU2 marker*, P_{TEF1}-PsCPR-T_{CYC1}, P_{TPI1}-Ps4OMT-T_{STE2}

4. ARO4Δ::P_{TEF1}-$ARO4^{FBR}$-T_{CYC1}, P_{GPD}-$ARO7^{FBR}$-T_{ADH1}, *HygR marker*, P_{PGK1}-TKL1-T_{PHO5}, P_{TPI1}-ARO10-T_{STE2}

Schematic of the integrated constructs indicating the orientation of each expression cassette:

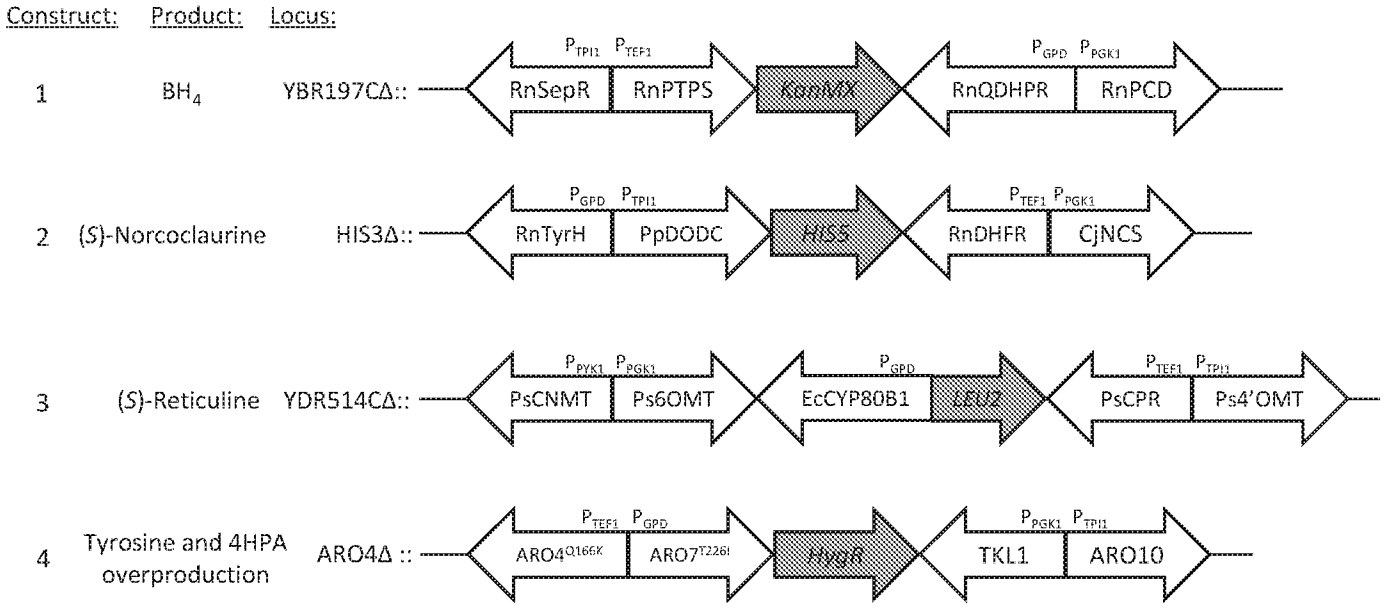
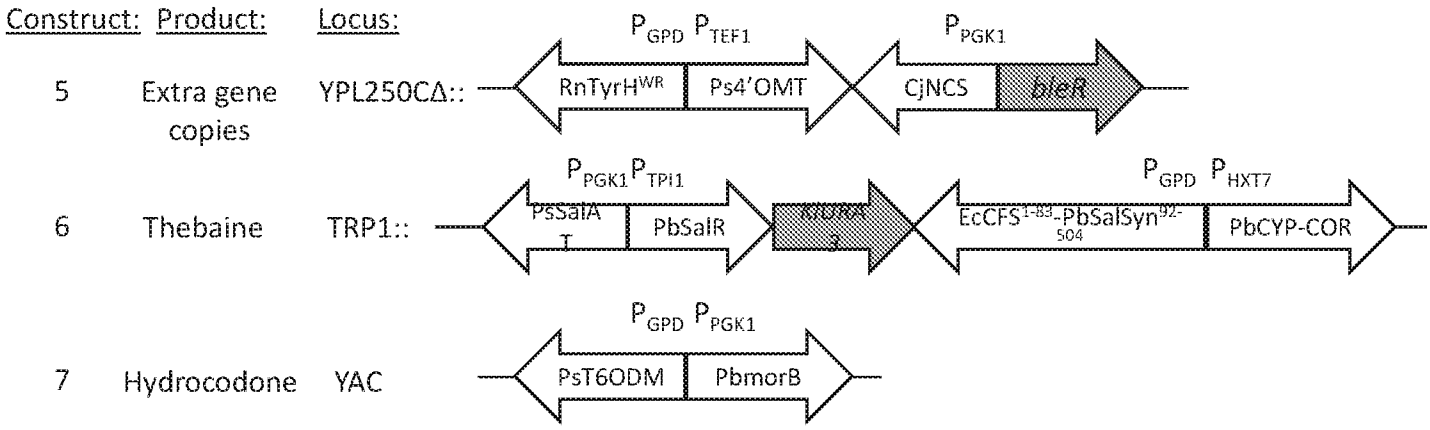


FIG. 26

A

Example genetic constructs:

5. YPL250CΔ::P_{GPD}-RnTyrH-T_{ADH1}, P_{TEF1}-Ps4OMT-T_{CYC1}, P_{PGK1}-CjNCS-T_{PHO5}, bleR marker
6. TRP1::P_{PGK1}-PsSalAT-T_{PHO5}, P_{TPI1}-PbSalR-T_{STE2}, URA3 marker, P_{GPD}-EcCFS$^{1-83}$-PbSalSyn$^{92-504}$-T_{ADH1}, P_{HXT7}-PbCYP-COR-T_{CYC1}
7. YAC::P_{GPD}-PsT6ODM-T_{ADH1}, P_{PGK1}-PbmorB-T_{PHO5}



B

Integration of Multi-Gene Construct 5:

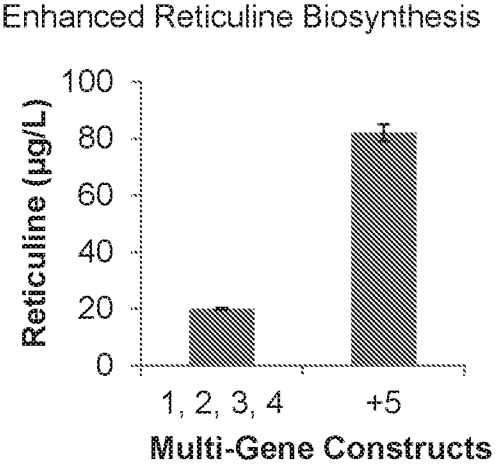
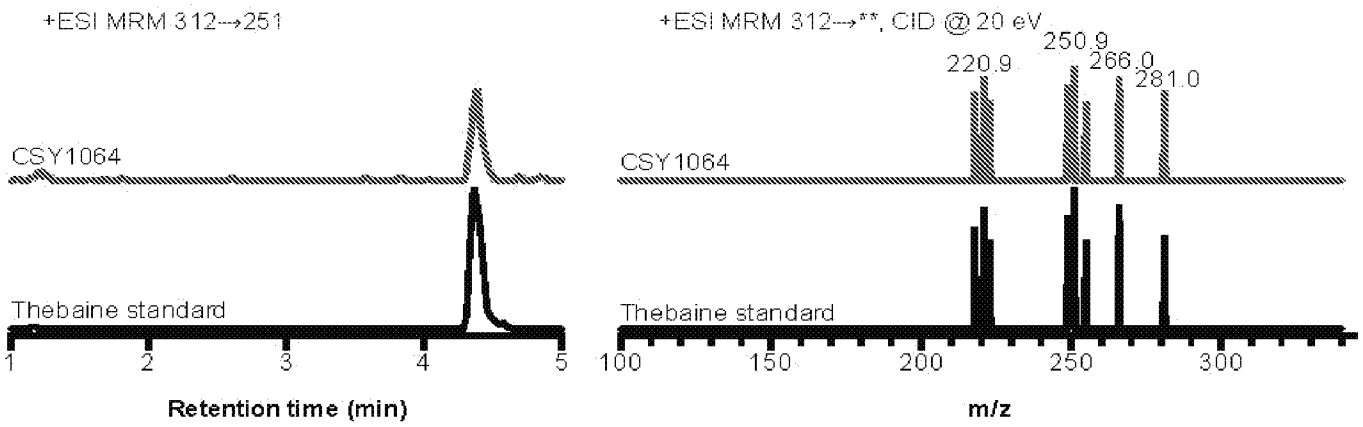


FIG 27

C

Integration of Multi-Gene Construct 6:

Thebaine Biosynthesis



D

Integration of Multi-Gene Construct 7:

Hydrocodone Biosynthesis

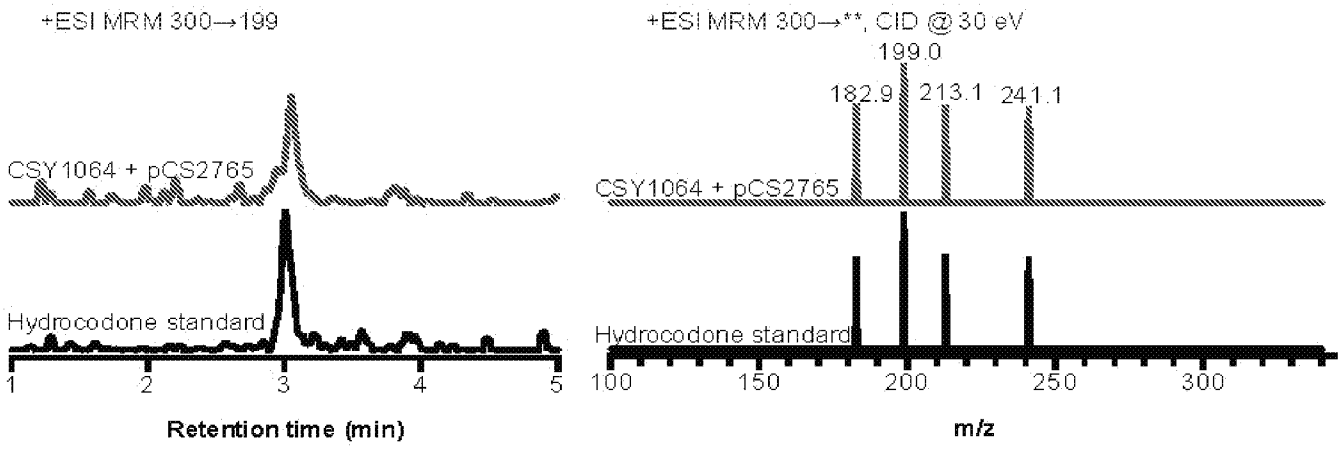


FIG 27

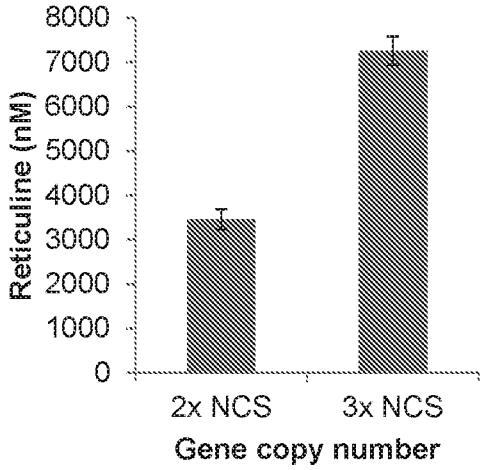

8000
7000
6000
5000
4000
3000
2000
1000
0
Reticuline (nM)
2x NCS
3x NCS
Gene copy number

FIG 28

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/031506

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 1/19; C12N 15/52; C12N 15/53 (2016.01)
CPC - C12N 9/10; C12N 15/52; C12N 15/81 (2016.05)
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC - C12N 1/19; C12N 15/52; C12N 15/53
CPC - C12N 9/10; C12N 15/52; C12N 15/81

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 435/41; 435/117; 435/171 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Patbase, Google Patents, PubMed, Google
Search terms used: BIA benzylisoquinoline reticulin S R enantiomer isomer chiral epimerization yeast express isolate purify oxidase pathway

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MISHRA et al. "Wound Induced Tanscriptional Regulation of Benzylisoquinoline Pathway and Characterization of Wound Inducible PsWRKY Transcription Factor from Papaver somniferum," PLoS One, 30 January 2013 (30.01.2013), Vol. 8, Iss. 1, Pgs. 1-15. entire document	1-5
A	ALCANTARA et al. "Sanguinarine Biosynthesis Is Associated with the Endoplasmic Reticulum in Cultured Opium Poppy Cells after Elicitor Treatment," Plant Physiology, 22 April 2005 (22.04.2005), Vol. 138, Pgs. 173-183. entire document	1-5
A	US 2014/0273109 A1 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD UNIVERSITY) 18 September 2014 (18.09.2014) entire document	1-5
P, X	WO 2015/081437 A1 (EPIMERON INC.) 11 June 2015 (11.06.2015) entire document	1-5
P, X	TRENCHARD et al. "De novo production of the key branch point benzylisoquinoline alkaloid reticuline in yeast," Metab. Eng. 10 July 2015 (10.07.2015), Vol. 31, Pgs. 74-83. entire document	1-5

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

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

Date of the actual completion of the international search	Date of mailing of the international search report
19 July 2016	26 AUG 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/031506

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

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

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

3. ☒ Claims Nos.: 6-15
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

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

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

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

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

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

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

(19)中华人民共和国国家知识产权局

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李嫣然

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代理人 郭放 许伟群

(51)Int.Cl.
C12N 1/19(2006.01)
C12N 15/52(2006.01)
C12N 15/53(2006.01)

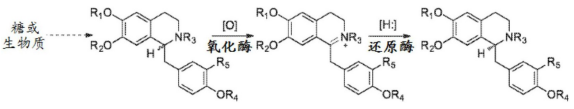
权利要求书1页 说明书118页 附图24页

(54)发明名称

产生差向异构酶和苄基异喹啉生物碱的方法

(57)摘要

本发明提供了使(S)-1-苄基异喹啉生物碱差向异构化为(R)-1-苄基异喹啉生物碱的方法。所述方法包括使所述(S)-1-苄基异喹啉生物碱与至少一种酶接触。使所述(S)-1-苄基异喹啉生物碱与所述至少一种酶接触将所述(S)-1-苄基异喹啉生物碱转化成(R)-1-苄基异喹啉生物碱。



CN 107960102 A

1.一种使(S)-1-苄基异喹啉生物碱差向异构化为(R)-1-苄基异喹啉生物碱的方法,其包括:

使所述(S)-1-苄基异喹啉生物碱与至少一种酶接触,

其中使所述(S)-1-苄基异喹啉生物碱与所述至少一种酶接触将所述(S)-1-苄基异喹啉生物碱转化成(R)-1-苄基异喹啉生物碱。

2.根据权利要求1所述的方法,其中所述至少一种酶通过培养具有编码所述至少一种酶的编码序列的工程化的非植物细胞产生。

3.根据权利要求2所述的方法,其还包括:

向所述细胞培养物中添加(S)-1-苄基异喹啉生物碱。

4.根据权利要求3所述的方法,其还包括:

从所述细胞培养物中回收所述(R)-1-苄基异喹啉生物碱或其衍生物。

5.根据权利要求1-4中任一项所述的方法,其中所述至少一种酶包括氧化酶。

6.根据权利要求1-5中任一项所述的方法,其中所述至少一种酶包括还原酶。

7.根据权利要求1-6中任一项所述的方法,其中所述至少一种酶包括差向异构酶。

8.根据权利要求7所述的方法,其中所述差向异构酶包括选自由以下各项组成的组的氨基酸序列:SEQ ID NO:1、2、3、4、5、6、7、8、9、10、11、12、13、14和15。

9.根据权利要求7或8所述的方法,其中所述差向异构酶包括氧化酶结构域和还原酶结构域。

10.根据权利要求9所述的方法,其中所述氧化酶结构域为细胞色素P450氧化酶样结构域。

11.根据权利要求9所述的方法,其中所述还原酶结构域为可待因酮还原酶样结构域。

12.根据权利要求7-11中任一项所述的方法,其中所述工程化的非植物细胞为工程化的酵母细胞。

13.根据权利要求1-12中任一项所述的方法,其中所述(S)-1-苄基异喹啉生物碱为(S)-牛心果碱。

14.根据权利要求1-13中任一项所述的方法,其中所述(R)-1-苄基异喹啉生物碱为(R)-牛心果碱。

15.根据权利要求1-14中任一项所述的方法,其中通过以L-酪氨酸起始的代谢途径在所述工程化的非植物细胞内产生所述(S)-1-苄基异喹啉生物碱。

产生差向异构酶和苄基异喹啉生物碱的方法

交叉引用

[0001] 按照美国法典第35篇第119条(e)款,本申请要求于2015年5月8日提交的美国临时专利申请序列号62/159,122和于2015年6月11日提交的美国临时专利申请序列号62/174,475的提交日期的优先权,所述申请的公开内容以引用方式并入本文。

[0002] 另外,本申请涉及:美国专利申请序列号14/211,611,现公布为US 2014-0273109,该申请提交于2014年3月14日,代理人案卷号为STAN-1018;PCT申请序列号PCT/US2014/027833,现公布为WO 2014/143744,该申请提交于2014年3月14日,代理人案卷号为STAN-1018WO;美国专利申请序列号15/031,618,该申请提交于2016年4月22日,代理人案卷号为STAN-1078;申请序列号PCT/US2014/063738,现公布为WO 2015/066642,该申请提交于2014年11月3日,代理人案卷号为STAN-1078WO;美国临时专利申请序列号62/080,610,其提交于2014年11月17日,代理人案卷号为STAN-1169PRV;美国临时专利申请序列号62/107,238,其提交于2015年1月23日,代理人案卷号为STAN-1169PRV2;申请序列号PCT/US2015/060891,该申请提交于2015年11月16日,代理人案卷号为STAN-1169WO;美国临时专利申请序列号62/156,701,其提交于2015年5月4日,代理人案卷号为STAN-1221PRV;以及申请序列号PCT/US2016/030808,该申请提交于2016年5月4日,代理人案卷号为STAN-1221WO;所述申请的公开内容均以引用方式并入本文。

关于联邦政府资助研究的声明

[0003] 本发明是根据美国国立卫生研究院(National Institutes of Health)授予的合同AT007886在政府支持下完成的。美国政府拥有本发明的某些权利。

发明内容

[0004] 本公开提供了用于在工程化的宿主细胞中产生各种不同的苄基异喹啉生物碱(BIA)的方法。本公开还提供了在工程化的宿主细胞中产生的各种不同的生物碱的组合物。另外,本公开提供了用于在工程化的宿主细胞中产生差向异构酶的方法。在具体的情况中,本公开提供了用于通过(S)-1-苄基异喹啉生物碱到(R)-1-苄基异喹啉生物碱的差向异构化而在工程化的宿主细胞中产生各种不同的生物碱产物的方法。在另外的具体情况中,本公开提供了用于通过(S)-牛心果碱到(R)-牛心果碱的差向异构化来产生各种不同的生物碱产物的方法。

[0005] 本发明的一个方面提供了一种相对于非工程化细胞而言具有增加的酪氨酸羟化酶活性的工程化的非植物细胞。本发明的另一个方面提供了一种相对于表达野生型TyrH的细胞而言具有增加的酪氨酸羟化酶活性的工程化的非植物细胞。本发明的另一个方面提供了一种相对于表达不具有如本文所提供的增加酪氨酸羟化酶活性的突变的野生型TyrH的细胞而言具有增加的酪氨酸羟化酶活性的工程化的非植物细胞。具体地讲,工程化的非植物细胞具有选自由以下各项组成的组的至少一个修饰:底物抑制减轻突变;产物抑制减轻突变;以及辅因子回收促进机制。

[0006] 本发明的另一个方面提供了一种使(S)-1-苄基异喹啉生物碱差向异构化为(R)-

1-苄基异喹啉生物碱的方法。该方法包括使(S)-1-苄基异喹啉生物碱与至少一种酶接触。使(S)-1-苄基异喹啉生物碱与至少一种酶接触将(S)-1-苄基异喹啉生物碱转化成(R)-1-苄基异喹啉生物碱。

[0007] 本发明的另一个方面提供了一种产生将(S)-1-苄基异喹啉生物碱转化成(R)-1-苄基异喹啉生物碱的差向异构酶的工程化的非植物细胞。差向异构酶包括选自由以下各项组成的组的氨基酸序列:SEQ ID NO:1、2、3、4、5、6、7、8、9、10、11、12、13、14和15,在表1中列出。

[0008] 本发明的另一个方面提供了一种用于将(S)-1-苄基异喹啉生物碱转化成(R)-1-苄基异喹啉生物碱的工艺,其包括使(S)-1-苄基异喹啉生物碱与足以将(S)-1-苄基异喹啉生物碱转化成(R)-1-苄基异喹啉生物碱的量的差向异构酶接触。

[0009] 在本发明的另一个方面,提供了一种使1-苄基异喹啉生物碱的立构中心差向异构化的方法。该方法包括使1-苄基异喹啉生物碱与至少一种酶接触。使1-苄基异喹啉生物碱与至少一种酶接触将1-苄基异喹啉生物碱的立构中心的立体化学翻转成1-苄基异喹啉生物碱的立构中心的相反的立体化学。

[0010] 在一些实例中,工程化的非植物细胞包括多个编码序列,其各自编码选自表2中列出的酶的组的酶。在一些实例中,异源编码序列可以可操作地连接。可操作地连接的异源编码序列可以在产生特定苄基异喹啉生物碱产物和/或差向异构酶产物的相同途径内。

[0011] 在本发明的另一个方面,提供了一种产生双苄基异喹啉生物碱的工程化的非植物细胞。双苄基异喹啉生物碱使用存在于工程化的非植物细胞内的偶合酶产生。另外,工程化的非植物细胞包括编码在工程化的非植物细胞内产生至少一个苄基异喹啉生物碱单体中所用的至少一种酶的至少一个异源编码序列。此外,至少一种偶合酶在工程化的非植物细胞内将两个苄基异喹啉生物碱单体二聚化,从而形成双苄基异喹啉生物碱。

以引用方式并入

[0012] 在本说明书中提及的所有公布、专利和专利申请均以引用方式并入本文至如同每个单独的公布、专利或专利申请被具体且单独地指明为以引用方式并入的相同程度。

附图说明

[0013] 本发明的新型特征在随附权利要求中具体阐述。通过参考对其中利用了本发明原理的例示性实施方案作出阐述的以下详细描述以及所述实施方案的附图,将更好地理解本发明的特征和优点:

[0014] 图1示出根据本发明的实施方案,四氢生物蝶呤的合成、再循环和补救途径的实例。

[0015] 图2示出根据本发明的实施方案,用于将葡萄糖转化成4-HPA、多巴胺和3,4-DHPA的生物合成方案。

[0016] 图3示出根据本发明的实施方案,(R)-1-苄基异喹啉生物碱形成的示意性实例。

[0017] 图4示出根据本发明的实施方案,CYP-COR酶的氨基酸序列。

[0018] 图5示出根据本发明的实施方案,用于通过去甲乌药碱将L-酪氨酸转化成牛心果碱的生物合成方案。

[0019] 图6示出根据本发明的实施方案,用于通过全去甲劳丹碱将L-酪氨酸转化成牛心

果碱的生物合成方案。

[0020] 图7示出根据本发明的实施方案，用于将L-酪氨酸转化成吗啡喃生物碱的生物合成方案。

[0021] 图8示出根据本发明的实施方案，用于将L-酪氨酸转化成原小檗碱、苯酞异喹啉和小檗碱生物碱产物的生物合成方案。

[0022] 图9示出根据本发明的实施方案，用于将L-酪氨酸转化成诺斯卡品、类诺斯卡品和苯酞异喹啉生物碱产物的生物合成方案。

[0023] 图10示出根据本发明的实施方案，用于将L-酪氨酸转化成血根碱和苯菲啶生物碱的生物合成方案。

[0024] 图11A示出根据本发明的实施方案，用于将坎那定转化成诺斯卡品的生物合成方案。

[0025] 图11B示出根据本发明的实施方案，用于产生半合成的阿片类物质的生物合成方案。

[0026] 图12示出根据本发明的实施方案，改善在工程化的酵母菌株中由糖产生去甲乌药碱的酪氨酸羟化酶突变体。

[0027] 图13示出根据本发明的实施方案，改善在工程化的酵母菌株中由糖产生牛心果碱的酪氨酸羟化酶突变体。

[0028] 图14示出根据本发明的实施方案，改善工程化的酵母菌株中通过酪氨酸羟化酶实现的L-DOPA产生的二氢叶酸还原酶(DHFR)的共表达。

[0029] 图15示出根据本发明的实施方案，(A)向培养基中添加抗氧化剂改善工程化的酵母菌株中通过酪氨酸羟化酶实现的L-DOPA产生，以及(B)向培养基中添加增加BH_4水平的抗氧化剂。

[0030] 图16示出根据本发明的实施方案，(A)用于将L-酪氨酸转化成bisBIA的生物合成方案，以及(B)被工程化为生物合成bisBIA的酵母菌株。

[0031] 图17示出根据本发明的实施方案，细胞色素P450氧化酶-可待因酮还原酶样(CYP-COR)融合物的系统发育树。

[0032] 图18(A)和18(B)示出根据本发明的实施方案，被工程化为将(S)-牛心果碱转化成沙罗泰里啶的酵母菌株的LC-MS/MS分析。

[0033] 图19示出根据本发明的实施方案，被工程化为将外消旋的全去甲劳丹碱转化成(R)-牛心果碱的酵母菌株的手性LC-MS/MS分析。

[0034] 图20(A)和20(B)示出根据本发明的实施方案，当在酵母而不是植物中异源表达时观察到的沙罗泰里啶合酶的工程化融合物消除蛋白质的N-连接的糖基化。

[0035] 图21(A)和21(B)示出根据本发明的实施方案，碎叶紫堇碱合酶-沙罗泰里啶合酶融合设计。

[0036] 图22示出根据本发明的实施方案，改善在酵母中的活性的沙罗泰里啶合酶密码子优化和工程化融合物。

[0037] 图23(A)和23(B)示出根据本发明的实施方案，小规模成批发酵的LC-MS/MS分析，其中工程化酵母催化(R)-牛心果碱到蒂巴因的转化和外消旋的全去甲劳丹碱到蒂巴因的转化。

[0038] 图24示出根据本发明的实施方案，通过随机诱变和筛选生成在酵母中表现出增强活性的CODM酶变体。

[0039] 图25(A)、25(B)和25(C)示出根据本发明的实施方案，通过工程化酵母将(R)-牛心果碱转化成蒂巴因的发酵优化。

[0040] 图26示出根据本发明的实施方案，用于由L-酪氨酸产生关键的分支点中间体牛心果碱的酵母平台菌株。

[0041] 图27(A)、27(B)、27(C)和27(D)示出根据本发明的实施方案，工程化的酵母菌株中的蒂巴因和氢可酮产生。

[0042] 图28示出根据本发明的实施方案，在工程化的酵母菌株中，通过将NCS基因的拷贝数从两个拷贝增加至三个拷贝来实现苄基异喹啉生物碱牛心果碱的产生增加。

具体实施方式

[0043] 本公开提供了用于在工程化的宿主细胞中产生各种不同的苄基异喹啉生物碱(BIA)的方法。本公开还提供了在工程化的宿主细胞中产生的各种不同的生物碱的组合物。另外，本公开提供了用于在工程化的宿主细胞中产生差向异构酶的方法。在具体的情况中，本公开提供了用于通过(S)-1-苄基异喹啉生物碱到(R)-1-苄基异喹啉生物碱的差向异构化而在工程化的宿主细胞中产生各种不同的生物碱产物的方法。在另外的具体情况中，本公开提供了用于通过(S)-牛心果碱到(R)-牛心果碱的差向异构化来产生各种不同的生物碱产物的方法。

目标苄基异喹啉生物碱(BIA)

[0044] 提供了产生目标BIA的宿主细胞。在一些实例中，宿主细胞的工程化菌株诸如本发明的工程化菌株提供用于产生目标苄基异喹啉生物碱以及遍及若干结构种类的它们的修饰物的平台，所述种类包括但不限于：前体BIA、苄基异喹啉、原小檗碱、原阿片碱、苯菲啶、原吗啡喃(promorphinan)、吗啡喃、断裂小檗碱、苯酞异喹啉、阿朴啡、双苄基异喹啉以及其他。这些种类中的每一种旨在包括可产生该种类的成员的工程化宿主细胞生物合成途径的任何适宜成员的生物合成前体、中间体，以及其代谢物。对于这些结构种类中的每一种，在下文给出化合物的非限制性实例。在一些情况下，给定实例的结构可以或可以不将自身表征为苄基异喹啉生物碱。本发明的化学实体旨在包括所有可能的异构体，包括单一对映体、外消旋混合物、光学纯形式、非对映体的混合物，以及中间体混合物。

[0045] BIA前体可包括但不限于去甲乌药碱(NC)和全去甲劳丹碱(NL)，以及NC和NL前体，诸如酪氨酸、酪胺、4-羟基苯乙醛(4-HPA)、4-羟基苯丙酮酸(4-HPPA)、L-3,4-二羟基苯丙氨酸(L-DOPA)、3,4-二羟基苯乙醛(3,4-DHPA)以及多巴胺。在一些实施方案中，一种或多种BIA前体为3,4-二羟基苯乙醛(3,4-DHPA)和多巴胺。在某些情况中，一种或多种BIA前体为4-羟基苯乙醛(4-HPA)和多巴胺。具体地讲，NL和NC可通过皮克特-施彭格勒(Pictet-Spengler)缩合反应由前体分子分别合成，其中反应可自发发生或者可通过任何适宜的酶催化。

[0046] 苄基异喹啉类可包括但不限于：去甲乌药碱、全去甲劳丹碱、乌药碱、3'-羟基乌药碱、4'-O-甲基全去甲劳丹碱、4'-O-甲基-劳丹碱、N-甲基去甲乌药碱、劳丹碱、N-甲基乌药碱、3'-羟基-N-甲基乌药碱、牛心果碱、去甲牛心果碱、罂粟碱、劳丹宁、劳丹素、四氢罂粟

碱、1,2-二氢罂粟碱,以及东罂粟灵。

[0047] 原小檗碱类可包括但不限于金黄紫堇碱、碎叶紫堇碱、刺罂粟碱、南丁宁碱、药根碱、千金藤啶碱、离木明碱、顺式-N-甲基刺罂粟碱、四氢非洲防己碱、掌叶防己碱、四氢掌叶防己碱、非洲防己碱、坎那定、N-甲基坎那定、1-羟基坎那定、小檗碱、N-甲基-左旋蛇果黄堇碱、1,13-二羟基-N-甲基坎那定,以及1-羟基-10-O-乙酰基-N-甲基坎那定。

[0048] 原阿片碱类可包括但不限于原阿片碱、6-羟基原阿片碱、别隐品碱、隐品碱、隐掌叶防己碱(muramine)和黄唐松草碱(thalictricine)。

[0049] 苯菲啶类可包括但不限于二氢血根碱、血根碱、dihydrocheilirubine、cheilirubine、dihydromarcapine、marcapine和白屈菜红碱。

[0050] 原吗啡喃类可包括但不限于沙罗泰里啶、沙罗泰里啶醇和沙罗泰里啶醇-7-O-乙酸酯。

[0051] 吗啡喃类可包括但不限于蒂巴因、可待因酮、可待因、吗啡、吗啡酮、东罂粟碱、尼奥品酮、尼奥品、新吗啡(neomorphine)、氢可酮、二氢可待因、14-羟基可待因酮、羟考酮、14-羟基可待因、吗啡酮、氢吗啡酮、二氢吗啡、二氢埃托啡、乙基吗啡、埃托啡、美托酮、丁丙诺啡、福尔可定、异可待因和氧吗啡酮。

[0052] 断裂小檗碱类可包括但不限于4'-O-脱甲基马克兰特醛(4'-O-desmethylmacrantaldehyde)、4'-O-脱甲基罂粟奥辛、4'-O-脱甲基-3-O-乙酰基罂粟奥辛、罂粟奥辛,以及3-O-乙酰基罂粟奥辛。

[0053] 苯肽异喹啉类可包括但不限于那可托灵半缩醛、那可汀半缩醛、那可托灵、诺斯卡品、山缘草定碱、山缘草碱、(+)或(-)-荷包牡丹碱、咖喏定、紫堇明、对叶延胡索碱、紫堇米定碱、夏无碱、5'-O-脱甲基那可汀、(+)或(-)-α或β-白毛茛碱以及角茴香碱。

[0054] 阿朴啡类可包括但不限于木兰花碱、紫堇块茎碱、阿朴吗啡、波尔定碱、异波尔定碱、异蒂巴因、异紫堇块茎碱以及glaufine。

[0055] 双苄基异喹啉类可包括但不限于黄芦木碱、guattgaumerine、蝙蝠葛碱和莲心碱。

[0056] 可通过本发明的工程化菌株产生的其他化合物可包括但不限于丽春花定碱、帕威(pavine)、异帕威(isopavine)和枯拉灵。

[0057] 在某些实施方案中,本发明的工程化菌株可提供用于产生与四氢生物蝶呤合成相关的化合物的平台,所述化合物包括但不限于二氢新蝶呤三磷酸、6-丙酮酰四氢蝶呤、5,6,7,8-四氢生物蝶呤、7,8-二氢生物蝶呤、四氢生物蝶呤4a-甲醇胺、醌型二氢生物喋呤和生物蝶呤。

宿主细胞

[0058] 可在主题宿主细胞和方法中利用任何适宜的细胞。在一些情况下,宿主细胞为非植物细胞。在一些情况下,宿主细胞可表征为微生物细胞。在某些情况下,宿主细胞为昆虫细胞、哺乳动物细胞、细菌细胞或酵母细胞。任何适宜类型的宿主细胞可用于产生主题产BIA细胞,参见例如,US2008/0176754和US2014/0273109,其公开内容以引用方式全文并入。目标宿主细胞包括但不限于:细菌细胞,诸如枯草芽孢杆菌(Bacillus subtilis)、大肠杆菌(Escherichia coli)、链霉菌属(Streptomyces)和鼠伤寒沙门氏菌(Salmonella typhimuium)细胞;昆虫细胞,诸如黑腹果蝇(Drosophila melanogaster)S2和草地夜蛾(Spodoptera frugiperda)Sf9细胞;以及酵母细胞,诸如酿酒酵母(Saccharomyces

cerevisiae)、粟酒裂殖酵母(Schizosaccharomyces pombe)和巴斯德毕赤酵母(Pichia pastoris)细胞。在一些实例中,宿主细胞为酵母细胞或大肠杆菌细胞。在一些情况下,宿主细胞为酵母细胞。在一些情况下,宿主细胞来自被工程化以产生目标BIA,诸如(R)-1-苄基异喹啉生物碱的酵母菌株。在一些情况下,宿主细胞来自被工程化为产生目标酶的酵母菌株。在一些情况下,宿主细胞来自被工程化为产生差向异构酶的酵母菌株。差向异构酶可具有氧化酶和还原酶。另外,差向异构酶可以能够将(S)-1-苄基异喹啉生物碱转化成(R)-1-苄基异喹啉生物碱。此外,差向异构酶可分成保持氧化酶或还原酶活性的更小的酶,以便用于将(S)-1-苄基异喹啉生物碱转化成(R)-1-苄基异喹啉生物碱。

[0059] Smolke等人的US2008/0176754和US2014/0273109中所述的任何宿主细胞可适用于主题细胞和方法。在某些实施方案中,酵母细胞可以是酿酒酵母(S.cerevisiae)物种。在某些实施方案中,酵母细胞可以是粟酒裂殖酵母物种。在某些实施方案中,酵母细胞可以是巴斯德毕赤酵母物种。酵母作为宿主细胞受到关注是因为细胞色素P450蛋白能够正确地折叠到内质网膜中,以使它们的活性得以保持。在实例中,细胞色素P450蛋白参与一些目标生物合成途径。在另外的实例中,细胞色素P450蛋白参与目标BIA的产生。在另外的实例中,细胞色素P450蛋白参与目标酶的产生,所述酶诸如具有氧化酶和还原酶的差向异构酶。

[0060] 可用于本发明的目标酵母菌株包括但不限于CEN.PK(基因型:MATa/αura3-52/ura3-52 trp1-289/trp1-289 leu2-3_112/leu2-3_112 his3 Δ1/his3 Δ1MAL2-8C/MAL2-8C SUC2/SUC2)、S288C、W303、D273-10B、X2180、A364A、∑1278B、AB972、SK1和FL100。在某些情况下,酵母菌株是以下中的任一个:S288C(MATα;SUC2mal mel gal2 CUP1 flo1 flo8-1 hap1)、BY4741(MATα;his3Δ1;leu2Δ0;met15Δ0;ura3Δ0)、BY4742(MATα;his3Δ1;leu2Δ0;lys2Δ0;ura3Δ0)、BY4743(MATa/MATα;his3Δ1/his3Δ1;leu2Δ0/leu2Δ0;met15Δ0/MET15;LYS2/lys2Δ0;ura3Δ0/ura3Δ0),以及WAT11或W(R),W303-B菌株的衍生物(MATa;ade2-1;his3-11、-15;leu2-3、-112;ura3-1;canR;cyr+),其分别表达拟南芥(Arabidopsis thaliana)NADPH-P450还原酶ATR1和酵母NADPH-P450还原酶CPR1。在另一个实施方案中,酵母细胞是W303α(MATα;his3-11,15trp1-1 leu2-3 ura3-1 ade2-1)。另外的目标酵母菌株的身份和基因型可见于EUROSCARF(web.uni-frankfurt.de/fb15/mikro/euroscarf/col_index.html)。

对宿主细胞的基因修饰

[0061] 宿主细胞可被工程化为包括提供目标BIA的产生的一个或多个修饰(诸如两个或更多个、三个或更多个、四个或更多个、五个或更多个、或甚至更多个修饰)。另外或另选地,宿主细胞可被工程化为包括提供目标酶的产生的一个或多个修饰(诸如两个或更多个、三个或更多个、四个或更多个、五个或更多个、或甚至更多个修饰)。在一些情况下,修饰为基因修饰,诸如基因或其片段的突变、添加或缺失,或基因或其片段的转录调控。如本文所用,术语"突变"是指相对于参考序列或基序,一个或多个氨基酸残基或一个或多个核苷酸残基的缺失、插入或取代。突变可作为在原始基因座处的天然基因的定向突变并入。在一些情况下,突变可作为在单独基因座处作为遗传整合引入的基因的另外拷贝或作为附加型载体诸如2μ或着丝粒质粒上的另外拷贝并入。在某些情况下,酶的底物抑制的拷贝在天然细胞转录调控下。在一些情况下,通过将酶的底物抑制的拷贝置于合成启动子的控制下在蛋白质表达的工程化的组成型或动态调控的情况下引入所述拷贝。在一些实例中,一个或多个修

饰的对象可以是天然基因。在一些实例中，一个或多个修饰的对象可以是非天然基因。在一些实例中，非天然基因可插入宿主细胞中。在另外的实例中，可在插入宿主细胞中之前通过一个或多个修饰改变非天然基因。

[0062] 工程化的宿主细胞可过量产生一种或多种目标BIA。所谓的过量产生是指，相对于对照细胞(例如，未修饰细胞)而言，细胞具有改善或增加的目标BIA分子的产生。所谓的改善或增加的产生是指在对照没有目标BIA产生的情况下一定量的目标BIA的产生，以及在其中对照具有一些目标BIA产生的情况下，约10％或更多，诸如约20％或更多，约30％或更多，约40％或更多，约50％或更多，约60％或更多，约80％或更多，约100％或更多，诸如2倍或更多，诸如5倍或更多，包括10倍或更多的增加。

[0063] 工程化的宿主细胞可过量产生一种或多种(S)-1-苄基异喹啉生物碱。在一些情况下，工程化的宿主细胞可在对照没有(S)-1-苄基异喹啉生物碱产生的情况下产生一定量的目标(S)-1-苄基异喹啉生物碱，并且在其中对照具有一些目标(S)-1-苄基异喹啉生物碱产生的情况下，具有约10％或更多，诸如约20％或更多，约30％或更多，约40％或更多，约50％或更多，约60％或更多，约80％或更多，约100％或更多，诸如2倍或更多，诸如5倍或更多，包括10倍或更多的增加。

[0064] 工程化的宿主细胞还可过量产生一种或多种(R)-1-苄基异喹啉生物碱。在一些情况下，工程化的宿主细胞可在对照没有(R)-1-苄基异喹啉生物碱产生的情况下产生一定量的目标(R)-1-苄基异喹啉生物碱，并且在其中对照具有一些目标(R)-1-苄基异喹啉生物碱产生的情况下，具有约10％或更多，诸如约20％或更多，约30％或更多，约40％或更多，约50％或更多，约60％或更多，约80％或更多，约100％或更多，诸如2倍或更多，诸如5倍或更多，包括10倍或更多的增加。工程化的宿主细胞还可过量产生吗啡喃、原小檗碱、类诺斯卡品和苯菲啶生物碱中的一种或多种。

[0065] 在一些情况下，相对于缺乏一个或多个修饰(例如，如本文所述)的对照宿主细胞而言，工程化的宿主细胞能够产生增大量的(R)-牛心果碱。在某些情况下，增大量的(R)-牛心果碱为相对于对照宿主细胞约10％或更多，诸如相对于对照宿主细胞约20％或更多、约30％或更多、约40％或更多、约50％或更多、约60％或更多、约80％或更多、约100％或更多、约2倍或更多、约5倍或更多、或甚至约10倍或更多。在一些情况下，(R)-牛心果碱为工程化宿主细胞内的差向异构化反应的产物。在这些情况下，(S)-牛心果碱可以是差向异构化反应的底物。

[0066] 另外，工程化的宿主细胞可过量产生一种或多种目标酶。所谓的过量产生是指，相对于对照细胞(例如，未修饰细胞)而言，细胞具有改善或增加的目标酶的产生。所谓的改善或增加的产生是指在对照没有产生的情况下一定量的目标酶的产生，以及在其中对照具有一些目标酶产生的情况下，约10％或更多，诸如约20％或更多，约30％或更多，约40％或更多，约50％或更多，约60％或更多，约80％或更多，约100％或更多，诸如2倍或更多，诸如5倍或更多，包括10倍或更多的增加。

[0067] 工程化的宿主细胞可过量产生一种或多种CYP-COR酶。在一些情况下，工程化的宿主细胞可在对照没有CYP-COR酶产生的情况下产生一定量的CYP-COR酶，并且在其中对照具有一些CYP-COR酶产生的情况下，具有约10％或更多，诸如约20％或更多，约30％或更多，约40％或更多，约50％或更多，约60％或更多，约80％或更多，约100％或更多，诸如2倍或更

多，诸如5倍或更多，包括10倍或更多的增加。

[0068] 工程化的宿主细胞还可过量产生衍生自CYP-COR酶的一种或多种酶。在一些情况下，工程化的宿主细胞可在对照没有衍生自CYP-COR酶的酶的产生的情况下产生一定量的衍生自CYP-COR酶的酶，并且在其中对照具有一些衍生自CYP-COR酶的酶产生的情况下，具有约10％或更多，诸如约20％或更多，约30％或更多，约40％或更多，约50％或更多，约60％或更多，约80％或更多，约100％或更多，诸如2倍或更多，诸如5倍或更多，包括10倍或更多的增加。

[0069] 另外，工程化的宿主细胞可过量产生一种或多种双苄基异喹啉生物碱(bisBIA)。具体地讲，相对于缺乏一个或多个修饰(例如，如本文所述)的对照宿主细胞而言，工程化的宿主细胞能够产生增大量的双苄基异喹啉生物碱(bisBIA)。在某些情况下，增大量的bisBIA为相对于对照宿主细胞约10％或更多，诸如相对于对照宿主细胞约20％或更多、约30％或更多、约40％或更多、约50％或更多、约60％或更多、约80％或更多、约100％或更多、约2倍或更多、约5倍或更多、或甚至约10倍或更多。在一些情况下，bisBIA由至少一种BIA单体形成，所述BIA单体为工程化宿主细胞内的差向异构化反应的产物或其衍生物。工程化的宿主细胞还可过量产生以下中的一种或多种：千金藤碱、防己诺林碱、莲心碱、甲基莲心碱、筒箭毒碱、蝙蝠葛碱、粉防己碱、箭毒碱、黄芦木碱、guattegaumerine、2’-去甲黄芦木碱和小檗胺。

[0070] 在一些情况下，一个或多个(诸如两个或更多个、三个或更多个、或四个或更多个)修饰可选自：生物合成酶基因中的底物抑制减轻突变；生物合成酶基因中的产物抑制减轻突变；辅因子回收促进机制；生物合成酶基因中的反馈抑制减轻突变；生物合成酶基因中的转录调节修饰；酶基因中的失活突变；差向异构化修饰；bisBIA生成修饰；以及编码酶的异源编码序列。包括一个或多个修饰的细胞可称为工程化细胞。

底物抑制减轻突变

[0071] 在一些情况下，工程化的宿主细胞为在细胞的一个或多个生物合成酶基因中包括一个或多个底物抑制减轻突变(诸如两个或更多个、三个或更多个、四个或更多个、五个或更多个、或甚至更多个)的细胞。在一些实例中，一个或多个生物合成酶基因对于细胞是天然的(例如，存在于未修饰的细胞中)。在一些实例中，一个或多个生物合成酶基因对于细胞是非天然的。如本文所用，术语“底物抑制减轻突变”是指减轻细胞的底物抑制控制机制的突变。

[0072] 减轻底物抑制的突变相对于对照细胞减少目标细胞中调控酶的抑制并且提供增加水平的调控化合物或其下游生物合成产物。在一些情况下，所谓的减轻调控酶的抑制是指抑制的IC_{50}增加2倍或更多，诸如增加3倍或更多、5倍或更多、10倍或更多、30倍或更多、100倍或更多、300倍或更多、1000倍或更多、或甚至更多。所谓的增加的水平是指为对照细胞中的调控化合物或其下游产物的水平的110％或更多的水平，诸如120％或更多、130％或更多、140％或更多、150％或更多、160％或更多、170％或更多、180％或更多、190％或更多、或200％或更多，诸如至少3倍或更多、至少5倍或更多、至少10倍或更多、或甚至更多的工程化宿主细胞中的调控化合物或其下游产物。

[0073] 工程化宿主细胞中针对目标BIA或其前体的水平的调控的多种底物抑制控制机制和生物合成酶可被靶向以用于底物抑制减轻。工程化宿主细胞可在一个或多个生物合成酶

基因中包括一个或多个底物抑制减轻突变。一个或多个突变可位于其中生物合成酶受到调控控制的任何适宜的生物合成酶基因中。在一些实施方案中，一个或多个生物合成酶基因编码一种或多种酪氨酸羟化酶。在某些情况下，一个或多个底物抑制减轻突变存在于为TyrH的生物合成酶基因中。在一些实施方案中，工程化的宿主细胞可在一个或多个生物合成酶基因(诸如表2中所述的那些基因中的一个)中包括一个或多个底物抑制减轻突变。

[0074] 在某些实施方案中，一个或多个底物抑制减轻突变存在于TyrH基因中。TyrH基因编码酪氨酸羟化酶，其为将酪氨酸转化成L-DOPA的酶。然而，TyrH受其底物酪氨酸的抑制。哺乳动物酪氨酸羟化酶活性，诸如在人或大鼠中所见的，可通过缓解底物抑制的对TyrH基因的突变改善。具体地讲，可通过TyrH基因中的点突变W166Y缓解由酪氨酸造成的底物抑制。TyrH基因中的点突变W166Y也可改善酪氨酸羟化酶的共底物BH_4的结合，以催化酪氨酸至L-DOPA的反应。TyrH的突变体当在酵母菌株中表达以由糖产生BIA时(诸如美国临时专利申请序列号61/899,496中所述的那些)可显著改善BIA的产生。

[0075] 可利用任何适宜数量和类型的突变来减轻底物抑制控制机制。在某些实施方案中，本发明的工程化的宿主细胞可在工程化的宿主细胞内的一个或多个生物合成酶基因中包括1个或多个、2个或更多个、3个或更多个、4个或更多个、5个或更多个、6个或更多个、7个或更多个、8个或更多个、9个或更多个、10个或更多个、11个或更多个、12个或更多个、13个或更多个、14个或更多个、或甚至15个或更多个底物抑制减轻突变，诸如1、2、3、4、5、6、7、8、9、10、11、12、13、14或15个底物抑制减轻突变。

辅因子回收促进机制

[0076] 在一些情况下，工程化的宿主细胞为在细胞的一个或多个生物合成酶基因中包括一个或多个辅因子回收促进机制(诸如两个或更多个、三个或更多个、四个或更多个、五个或更多个、或甚至更多个)的细胞。在一些实例中，一个或多个生物合成酶基因对于细胞是天然的(例如，存在于未修饰的细胞中)。在一些实例中，一个或多个生物合成酶基因对于细胞是非天然的。如本文所用，术语“辅因子回收促进机制”是指促进细胞的辅因子回收控制机制的机制。

[0077] 工程化宿主细胞中针对目标BIA或其前体的水平的调控的多种辅因子回收控制机制和生物合成酶可被靶向以用于辅因子回收促进。工程化宿主细胞可在一个或多个生物合成酶基因中包括一个或多个辅因子回收促进机制。在实例中，工程化的宿主细胞可包括编码二氢叶酸还原酶(DHFR)的异源编码序列。当DHFR表达时，其可将7,8-二氢生物蝶呤(BH_2)转化成四氢生物蝶呤(BH_4)，从而回收BH_4作为TyrH共底物。在一些实例中，工程化的宿主细胞可在一个或多个生物合成酶基因(诸如表2中所述的那些基因中的一个)中包括一个或多个辅因子回收促进机制。

[0078] 可利用任何适宜数量和类型的机制来促进辅因子回收控制机制。在某些实施方案中，本发明的工程化的宿主细胞可在工程化宿主细胞内的一个或多个生物合成酶基因中包括1个或多个、2个或更多个、3个或更多个、4个或更多个、5个或更多个、6个或更多个、7个或更多个、8个或更多个、9个或更多个、10个或更多个、11个或更多个、12个或更多个、13个或更多个、14个或更多个、或甚至15个或更多个辅因子回收促进机制，诸如1、2、3、4、5、6、7、8、9、10、11、12、13、14或15个辅因子回收促进机制。

产物抑制减轻突变

[0079] 在一些情况下，工程化的宿主细胞为在细胞的一个或多个生物合成酶基因中包括一个或多个产物抑制减轻突变(诸如两个或更多个、三个或更多个、四个或更多个、五个或更多个、或甚至更多个)的细胞。在一些实例中，一个或多个生物合成酶基因对于细胞是天然的(例如，存在于未修饰的细胞中)。在一些实例中，一个或多个生物合成酶基因对于细胞是非天然的。如本文所用，术语“产物抑制减轻突变”是指减轻工程化宿主细胞的短期和/或长期产物抑制控制机制的突变。短期产物抑制是其中在共底物结合位点处存在竞争性结合的细胞的控制机制。长期产物抑制是其中存在远离期望途径的化合物的不可逆结合的细胞的控制机制。

[0080] 减轻产物抑制的突变相对于对照细胞减少目标细胞中调控酶的抑制并且提供增加水平的调控化合物或其下游生物合成产物。在一些情况下，所谓的减轻调控酶的抑制是指抑制的IC_{50}增加2倍或更多，诸如增加3倍或更多、5倍或更多、10倍或更多、30倍或更多、100倍或更多、300倍或更多、1000倍或更多、或甚至更多。所谓的增加的水平是指为对照细胞中的调控化合物或其下游产物的水平的110％或更多的水平，诸如120％或更多、130％或更多、140％或更多、150％或更多、160％或更多、170％或更多、180％或更多、190％或更多、或200％或更多，诸如至少3倍或更多、至少5倍或更多、至少10倍或更多、或甚至更多的工程化宿主细胞中的调控化合物或其下游产物。

[0081] 工程化宿主细胞中针对目标BIA的水平的调控的多种产物抑制控制机制和生物合成酶可被靶向以用于产物抑制减轻。工程化宿主细胞可在一个或多个生物合成酶基因中包括一个或多个产物抑制减轻突变。突变可位于其中生物合成酶受到调控控制的任何适宜的生物合成酶基因中。在一些实施方案中，一个或多个生物合成酶基因编码一种或多种酪氨酸羟化酶。在某些情况下，一个或多个产物抑制减轻突变存在于为TyrH的生物合成酶基因中。在一些实施方案中，工程化的宿主细胞在一个或多个生物合成酶基因(诸如表2中所述的那些基因中的一个)中包括一个或多个产物抑制减轻突变。

[0082] 在某些实施方案中，一个或多个产物抑制减轻突变存在于TyrH基因中。TyrH基因编码酪氨酸羟化酶，其为将酪氨酸转化成L-DOPA的酶。TyrH需要四氢生物蝶呤(BH_4)作为共底物来催化羟化反应。一些微生物菌株，诸如酿酒酵母，不天然地产生BH_4，但是可被工程化为通过四酶合成和再循环途径产生此底物，如图1中所示。图1示出根据本发明的实施方案，四氢生物蝶呤的合成、再循环和补救途径的实例。图1提供了酶PTPS(丙酮酰四氢蝶呤合酶)、SepR(墨蝶呤还原酶)、PCD(蝶呤4a-甲醇胺脱水酶)、QDHPR(二氢蝶啶还原酶)和DHFR(二氢叶酸还原酶)的使用。在图1所示的酶中，酵母合成内源性的GTP环化水解酶I。GTP和二氢新蝶呤三磷酸在酵母中天然合成。另外，图1中的其他代谢物不在酵母中天然产生。

[0083] TyrH受到其产物L-DOPA，以及其他儿茶酚胺(尤其是多巴胺)的抑制。哺乳动物酪氨酸羟化酶活性，诸如来自人或大鼠的酪氨酸羟化酶，可通过缓解产物抑制的突变改善。例如，短期产物抑制，诸如在共底物结合位点处的竞争性结合，可通过TyrH基因上的点突变W166Y缓解。具体地讲，TyrH基因上的点突变W166Y可改善共底物的结合。另外，用以缓解共底物结合位点处的竞争性结合的短期产物抑制可通过TyrH基因上的点突变S40D改善。短期产物抑制也可通过TyrH基因上的R37E、R38E的联合突变改善。具体地讲，R37E、R38E突变可在多巴胺的存在下一起特定改善酪氨酸羟化酶活性。

[0084] 另外，长期产物抑制可通过TyrH基因上的点突变缓解。长期产物抑制缓解可包括

儿茶酚胺与铁在活性位点中的不可逆结合，由此使得存在较少的儿茶酚胺充当酪氨酸羟化酶活性的产物抑制剂。长期产物抑制可相应地通过TyrH基因中的突变E332D和Y371F缓解。

[0085] 可制备突变的组合(诸如同时两个或三个或更多个突变)来缓解多种类型的底物和产物抑制以进一步改善TyrH的活性。TyrH的突变体当在酵母菌株中表达以由糖产生BIA时(诸如美国临时专利申请序列号61/899,496中所述的那些)可显著改善BIA的产生。

[0086] 可利用任何适宜数量和类型的突变来减轻产物抑制控制机制。在某些实施方案中，本发明的工程化的宿主细胞可在工程化的宿主细胞内的一个或多个生物合成酶基因中包括1个或多个、2个或更多个、3个或更多个、4个或更多个、5个或更多个、6个或更多个、7个或更多个、8个或更多个、9个或更多个、10个或更多个、11个或更多个、12个或更多个、13个或更多个、14个或更多个、或甚至15个或更多个产物抑制减轻突变，诸如1、2、3、4、5、6、7、8、9、10、11、12、13、14或15个产物抑制减轻突变。

反馈抑制减轻突变

[0087] 在一些情况下，工程化的宿主细胞为在细胞的一个或多个生物合成酶基因中包括一个或多个反馈抑制减轻突变(诸如两个或更多个、三个或更多个、四个或更多个、五个或更多个、或甚至更多个)的细胞。在一些情况下，一个或多个生物合成酶基因对于细胞是天然的(例如，存在于未修饰的细胞中)。另外或另选地，在一些实例中，一个或多个生物合成酶基因对于细胞是非天然的。如本文所用，术语“反馈抑制减轻突变”是指减轻工程化宿主细胞的反馈抑制控制机制的突变。反馈抑制是其中调控化合物的合成途径中的酶在所述化合物积聚到某一水平时受到抑制，从而平衡细胞中化合物的量的细胞控制机制。减轻反馈抑制的突变相对于对照细胞减少工程化宿主细胞中调控酶的抑制。以此方式，工程化宿主细胞提供增大水平的调控化合物或其下游生物合成产物。在一些情况下，所谓的减轻调控酶的抑制是指抑制的IC_{50}增加2倍或更多，诸如增加3倍或更多、5倍或更多、10倍或更多、30倍或更多、100倍或更多、300倍或更多、1000倍或更多、或甚至更多。所谓的增加的水平是指为对照细胞中的调控化合物或其下游产物的水平的110％或更多的水平，诸如120％或更多、130％或更多、140％或更多、150％或更多、160％或更多、170％或更多、180％或更多、190％或更多、或200％或更多，诸如至少3倍或更多、至少5倍或更多、至少10倍或更多、或甚至更多的宿主细胞中的调控化合物或其下游产物。

[0088] 针对目标BIA的水平的调控的多种反馈抑制控制机制和生物合成酶可被靶向以用于宿主细胞中的减轻。宿主细胞可在对于细胞是天然的一个或多个生物合成酶基因中包括一个或多个反馈抑制减轻突变。一个或多个突变可位于其中生物合成酶受到调控控制的任何适宜的生物合成酶基因中。在一些实施方案中，一个或多个生物合成酶基因可编码选自3-脱氧-d-阿拉伯糖-庚酮糖酸-7-磷酸(DAHP)合酶和分支酸变位酶的一种或多种酶。在一些实施方案中，一个或多个生物合成酶基因编码3-脱氧-d-阿拉伯糖-庚酮糖酸-7-磷酸(DAHP)合酶。在一些情况下，一个或多个生物合成酶基因可编码分支酸变位酶。在某些情况下，一个或多个反馈抑制减轻突变可存在于选自ARO4和ARO7的生物合成酶基因中。在某些情况下，一个或多个反馈抑制减轻突变可存在于为ARO4的生物合成酶基因中。在某些情况下，一个或多个反馈抑制减轻突变存在于为ARO7的生物合成酶基因中。在一些实施方案中，工程化的宿主细胞可在一个或多个生物合成酶基因(诸如表2中所述的那些基因中的一个)中包括一个或多个反馈抑制减轻突变。

[0089] 可利用任何适宜数量和类型的突变来减轻反馈抑制控制机制。如本文所用，术语“突变”是指相对于参考序列或基序，一个或多个氨基酸残基或一个或多个核苷酸残基的缺失、插入或取代。突变可作为在原始基因座处的天然基因的定向突变并入。在一些情况下，突变可作为在单独基因座处作为遗传整合引入的基因的另外拷贝或作为附加型载体诸如2μ或着丝粒质粒上的另外拷贝并入。在某些情况下，酶的反馈抑制的拷贝在天然细胞转录调控下。在一些情况下，通过将酶的反馈抑制的拷贝置于合成启动子的控制下在蛋白质表达的工程化的组成型或动态调控的情况下引入所述拷贝。

[0090] 在某些实施方案中，一个或多个反馈抑制减轻突变可存在于ARO4基因中。目标ARO4突变可包括但不限于在229位上用亮氨酸取代赖氨酸残基、在166位上用赖氨酸残基取代谷氨酰胺残基，或如Hartmann M等人((2003)Proc Natl Acad Sci U S A 100(3):862-867)或Fukuda等人((1992)J Ferment Bioeng 74(2):117-119)所述的突变。在一些情况下，赋予反馈抑制的突变可选自酶突变体的诱变文库。此类选择的实例可包括用过量的酪氨酸拯救培养基中的o-氟-D,L-苯丙氨酸的生长或aro3突变酵母菌株的生长，如Fukuda等人((1990)Breeding of Brewing Yeast Producing a Large Amount of Beta-Phenylethyl Alcohol and Beta-Phenylethyl Acetate.Agr Biol Chem Tokyo 54(1):269-271)所述。

[0091] 在某些实施方案中，本发明的工程化的宿主细胞可在工程化的宿主细胞内的一个或多个生物合成酶基因中包括1个或多个、2个或更多个、3个或更多个、4个或更多个、5个或更多个、6个或更多个、7个或更多个、8个或更多个、9个或更多个、10个或更多个、11个或更多个、12个或更多个、13个或更多个、14个或更多个、或甚至15个或更多个反馈抑制减轻突变，诸如1、2、3、4、5、6、7、8、9、10、11、12、13、14或15个反馈抑制减轻突变。

转录调节修饰

[0092] 宿主细胞可包括所述细胞的一个或多个生物合成酶基因的一个或多个转录调节修饰(诸如两个或更多个、三个或更多个、四个或更多个、五个或更多个或甚至更多个修饰)。在一些实例中，一个或多个生物合成酶基因对于细胞是天然的。在一些实例中，一个或多个生物合成酶基因对于细胞是非天然的。所述细胞的任何适宜的生物合成酶基因可被靶向以用于转录调节。所谓的转录调节意指相对于对照细胞(例如，未修饰的细胞)调节修饰的细胞中的目标基因的表达，例如，增加或减少、增强或阻抑。在一些情况下，目标基因的转录调节包括增加或增强表达。所谓的增加或增强表达意指相较于对照，即未修饰的相同细胞中的表达(例如，通过使用任何适宜的基因表达测定)，目标基因的表达水平增加2倍或更多，诸如5倍或更多且有时25、50或100倍或更多，并且在某些实施方案中300倍或更多或更高。另选地，在细胞中的目标基因的表达如此低以至于其不可检测的情况下，如果表达被增加至可容易检测的水平，则目标基因的表达水平被认为增加。在某些情况下，目标基因的转录调节包括减少或阻抑表达。所谓的减少或阻抑表达意指相较于对照，目标基因的表达水平减少2倍或更多，诸如5倍或更多且有时25、50或100倍或更多，并且在某些实施方案中300倍或更多或更高。在一些情况下，表达被减少至不可检测的水平。可适于在主题宿主细胞中使用的目标宿主细胞过程的修改描述于Smolke等人的美国公布20140273109(14/211,611)中，所述公布的公开内容以引用方式全文并入本文。

[0093] 任何适宜的生物合成酶基因可进行转录调节，并且包括但不限于，图2中所述的那

些生物合成酶。具体地讲，图2示出根据本发明的实施方案，用于将葡萄糖转化成4-HPA、多巴胺和3,4-DHPA的生物合成方案。图2中所述的酶的实例包括ARO3、ARO4、ARO1、ARO7、TYR1、TYR、TyrH、DODC、MAO、ARO10、ARO9和TKL。在一些情况下，一个或多个生物合成酶基因可选自ARO10、ARO9和TKL。在一些情况下，一个或多个生物合成酶基因可以是ARO10。在某些情况下，一个或多个生物合成酶基因可以是ARO9。在一些实施方案中，一个或多个生物合成酶基因可以是TKL。在一些实施方案中，宿主细胞包括一个或多个基因(诸如表2中描述的那些基因中的一个)的一个或多个转录调节修饰。

[0094] 在一些实施方案中，转录调节修饰可包括用强启动子取代一个或多个生物合成酶基因的天然启动子或在强启动子的控制下表达一个或多个基因的一个或多个另外的拷贝。驱动目标基因的表达的启动子可以是组成型启动子或诱导型启动子，条件是所述启动子在宿主细胞中可以是活性的。目标基因可由其天然启动子表达。另外或另选地，目标基因可由非天然启动子表达。虽然不是要求，但这类启动子在使用它们的宿主中可为中等至高强度的。启动子可以是调控的或组成型的。在一些实施方案中，可使用不受葡萄糖阻抑的、或者在培养基中存在葡萄糖时仅受轻微阻抑的启动子。存在众多合适的启动子，所述启动子的实例包括糖酵解基因的启动子，诸如枯草芽孢杆菌tsr基因的启动子(编码果糖二磷酸醛缩酶)或来自酵母酿酒酵母的GAPDH启动子(编码甘油醛-磷酸脱氢酶)(Bitter G.A.,Meth.Enzymol.152:673 684(1987))。其他目标强启动子包括但不限于面包酵母的ADHI启动子(Ruohonen L.等人,J.Biotechnol.39:193 203(1995))；磷酸饥饿诱导的启动子，诸如酵母的PHO5启动子(Hinnen,A.等人,Yeast Genetic Engineering,Barr,P.J.等人编辑,Butterworths(1989)；来自地衣芽孢杆菌(B.licheniformis)的碱性磷酸酶启动子(Lee.J.W.K.等人,J.Gen.Microbiol.137:1127 1133(1991))；GPD1和TEF1。目标酵母启动子包括但不限于，诱导型启动子，诸如Gal1-10、Gal1、GalL、GalS；可阻抑启动子Met25、tetO；以及组成型启动子，诸如甘油醛3-磷酸脱氢酶启动子(GPD)、醇脱氢酶启动子(ADH)、翻译延长因子-1-α启动子(TEF)、细胞色素c-氧化酶启动子(CYC1)、MRP7启动子等。在一些情况下，强启动子为GPD1。在某些情况下，强启动子是TEF1。含有可受激素(诸如糖皮质激素、类固醇和甲状腺激素)诱导的启动子的自主复制酵母表达载体也是已知的并且包括但不限于，糖皮质激素反应性元件(GRE)和甲状腺激素反应性元件(TRE)，参见例如在美国专利7,045,290中描述的那些启动子。可使用含有组成型或诱导型启动子诸如α因子、醇氧化酶和PGH的载体。此外，任何启动子/增强子组合(根据真核启动子数据库EPDB)也可用于驱动目标基因的表达。应理解，可选择对宿主细胞具有特异性的任何适宜的启动子，例如大肠杆菌。在一些情况下，启动子选择可用于优化转录，并且因此，优化酶水平以使产生最大化，同时使能源最小化。

失活突变

[0095] 工程化的宿主细胞可包括所述细胞的酶的一个或多个失活突变(诸如两个或更多个、三个或更多个、四个或更多个、五个或更多个或甚至更多个)。包括一个或多个失活突变可修饰工程化宿主细胞的合成途径的通量以增加目标BIA或产生所述BIA的期望的酶或前体的水平。在一些实例中，一个或多个失活突变是针对对细胞天然的酶。另外或另选地，一个或多个失活突变是针对对细胞非天然的酶。如本文所用，“失活突变”意指细胞的基因或调控DNA序列的一个或多个突变，其中所述突变使由所述目标基因表达的蛋白质的生物活

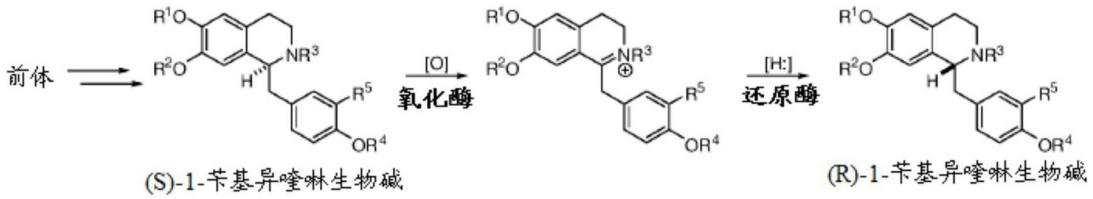
性失活。在一些情况下，基因对于细胞是天然的。在一些情况下，所述基因编码失活的酶，并且是由宿主细胞产生的目标BIA的合成途径的一部分或连接至所述合成途径。在一些情况下，失活突变位于控制目标基因的调控DNA序列中。在某些情况下，失活突变针对基因的启动子。任何适宜的突变(例如，如本文所述)可用于使目标基因或调控DNA序列失活。“失活的”或“失活”意指相对于由未突变对照基因表达的对照蛋白质，由突变基因表达的蛋白质的生物活性被降低10％或更多，诸如20％或更多、30％或更多、40％或更多、50％或更多、60％或更多、70％或更多、80％或更多、90％或更多、95％或更多、97％或更多、或99％或更多。在一些情况下，蛋白质是酶并且失活突变降低酶的活性。

[0096] 在一些实例中，工程化的宿主细胞在对于细胞天然的酶中包括失活突变。任何适宜的酶可被靶向以失活。目标酶可包括但不限于在表2中描述的那些酶，所述酶在工程化的宿主细胞的合成途径中的作用倾向于降低目标BIA的水平。在一些情况下，所述酶具有葡萄糖-6-磷酸脱氢酶活性。在某些实施方案中，包括失活突变的酶是ZWF1。在一些情况下，所述酶具有醇脱氢酶活性。在一些实施方案中，包括失活突变的酶选自ADH2、ADH3、ADH4、ADH5、ADH6、ADH7以及SFA1。在某些实施方案中，包括一个或多个失活突变的酶是ADH2。在某些实施方案中，包括一个或多个失活突变的酶是ADH3。在某些实施方案中，包括一个或多个失活突变的酶是ADH4。在某些实施方案中，包括一个或多个失活突变的酶是ADH5。在某些实施方案中，包括一个或多个失活突变的酶是ADH6。在某些实施方案中，包括一个或多个失活突变的酶是ADH7。在一些情况下，所述酶具有醛氧化还原酶活性。在某些实施方案中，包括失活突变的酶选自ALD2、ALD3、ALD4、ALD5以及ALD6。在某些实施方案中，包括一个或多个失活突变的酶是ALD2。在某些实施方案中，包括一个或多个失活突变的酶是ALD3。在某些实施方案中，包括一个或多个失活突变的酶是ALD4。在某些实施方案中，包括一个或多个失活突变的酶是ALD5。在某些实施方案中，包括一个或多个失活突变的酶是ALD6。在一些实施方案中，宿主细胞包括表2中描述的一个或多个基因的一个或多个失活突变。

差向异构化修饰

[0097] 本文提供的一些方法、工艺和系统描述了(S)-1-苄基异喹啉生物碱到(R)-1-苄基异喹啉生物碱的转化。这些方法、工艺和系统中的一些可包括工程化的宿主细胞。在一些实例中，(S)-1-苄基异喹啉生物碱到(R)-1-苄基异喹啉生物碱的转化是底物到各种不同范围的生物碱的转化的关键步骤。在一些实例中，(S)-1-苄基异喹啉生物碱到(R)-1-苄基异喹啉生物碱的转化包括差向异构化反应。在一些情况下，底物生物碱的差向异构化可通过以下方式进行：将(S)-底物氧化成对应的希夫碱或亚胺中间体，接着将此中间体立体定向地还原为(R)-产物(如图3中所提供和方案1中大致表示的)。如方案1中所提供的，R_1、R_2、R_3和R_4可为H或CH_3。R_5可为H、OH或OCH_3。

方案1



[0098] 在一些实例中，(S)-底物到(R)-产物的转化可涉及至少一个氧化反应和至少一个还原反应。在一些情况下，任选地在氧化反应之后进行还原反应。在一些情况下，氧化反应

和还原反应中的至少一个在酶的存在下进行。在一些情况下，氧化反应和还原反应中的至少一个通过酶催化。在一些情况下，氧化反应和还原反应两者均在至少一种酶的存在下进行。在一些情况下，至少一种酶可用于催化氧化和还原反应。氧化和还原反应可由相同的酶催化。

[0099] 在本文所述的一些方法、工艺和系统中，氧化反应可在酶的存在下进行。在一些实例中，酶可以是氧化酶。氧化酶可使用(S)-1-苄基异喹啉作为底物。氧化酶可将(S)-底物转化成对应的亚胺或希夫碱衍生物。氧化酶可被称为1,2-去氢牛心果碱合酶(DRS)。在本公开中适用于(S)-1-苄基异喹啉生物碱的氧化的酶的非限制性实例包括细胞色素P450氧化酶、2-酮戊二酸依赖性氧化酶，以及黄素蛋白氧化酶。例如，(S)-四氢原小檗碱氧化酶(STOX，E.C 1.3.3.8)可将(S)-去甲牛心果碱和其他(S)-1-苄基异喹啉生物碱氧化成1,2-脱氢去甲牛心果碱和其他对应的1,2-脱氢产物。在一些实例中，包括前述实例中的任一个的氧化酶结构域的蛋白质可进行氧化。在一些实例中，氧化酶可催化宿主细胞(诸如如本文所述的工程化的宿主细胞)内的氧化反应。

[0100] 在一些实例中，还原反应可在氧化反应之后进行。还原反应可通过酶进行。在一些实例中，还原酶可使用衍生自1-苄基异喹啉的亚胺或希夫碱作为底物。还原酶可将亚胺或希夫碱衍生物转化成(R)-1-苄基异喹啉。还原酶可被称为1,2-去氢牛心果碱还原酶(DRR)。适用于衍生自(S)-1-苄基异喹啉生物碱的亚胺或希夫碱的还原的酶的非限制性实例包括醛酮还原酶(例如，可待因酮还原酶样的酶(EC 1.1.1.247))和短链脱氢酶(例如，沙罗泰里啶还原酶样的酶(EC 1.1.1.248))。在一些实例中，包括前述实例中的任一个的还原酶结构域的蛋白质可进行还原。在另一个实施方案中，还原是立体定向的。在一些实例中，还原酶可催化宿主细胞(诸如如本文所述的工程化的宿主细胞)内的还原反应。

[0101] 可进行将(S)-1-苄基异喹啉生物碱转化成(R)-1-苄基异喹啉生物碱的差向异构化反应的酶的实例包括具有氧化酶结构域和还原酶结构域的差向异构酶。具体地讲，差向异构酶可具有细胞色素P450氧化酶82Y2样结构域。另外，差向异构酶可具有可待因酮还原酶样结构域。此外，具有细胞色素P450氧化酶82Y2样结构域且具有可待因酮还原酶样结构域的差向异构酶可被称为CYP-COR酶。具体地讲，CYP-COR酶可以是融合酶。

[0102] 可用于进行(S)-1-苄基异喹啉生物碱到(R)-1-苄基异喹啉生物碱的转化的CYP-COR酶的氨基酸序列的实例提供于图4中。具体地讲，图4示出根据本发明的实施方案的CYP-COR酶的氨基酸序列。如图4中看出，加下划线的文本表示细胞色素P450CYP82Y2样结构域(与AFB74617.1具有59％同一性)。加点划线的文本表示醛酮还原酶NADPH依赖性可待因酮还原酶样结构域(与ACM44066.1具有75％同一性)。CYP-COR酶的另外的氨基酸序列在表1中示出。用于将(S)-1-苄基异喹啉生物碱转化成(R)-1-苄基异喹啉生物碱的差向异构酶的氨基酸序列可与如表1中列出的给定氨基酸序列具有75％或更多的同一性。例如，这一差向异构酶的氨基酸序列可包括与如本文提供的氨基酸序列具有至少75％或更多、80％或更多、81％或更多、82％或更多、83％或更多、84％或更多、85％或更多、86％或更多、87％或更多、88％或更多、89％或更多、90％或更多、91％或更多、92％或更多、93％或更多、94％或更多、95％或更多、96％或更多、97％或更多、98％或更多、或99％或更多同一性的氨基酸序列。另外，在某些实施方案中，“相同的”氨基酸序列在氨基酸水平上与特定的氨基酸序列包含至少80％-99％的同一性。在一些情况下，“相同的”氨基酸序列在氨基酸水平上包含至少约

85％、86％、87％、88％、89％、90％、91％、92％、93％、94％和更多，在某些情况下，至少95％、96％、97％、98％和99％的同一性。在一些情况下，氨基酸序列可以是相同的，但是DNA序列有所改变，诸如例如以优化宿主生物体的密码子使用。

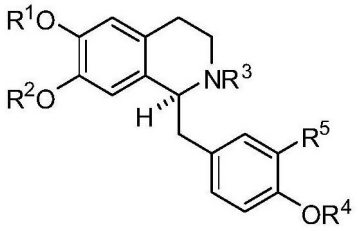
[0103] 可提供工程化的宿主细胞，其产生将(S)-1-苄基异喹啉生物碱转化成(R)-1-苄基异喹啉生物碱的差向异构酶，其中差向异构酶包括选自由以下各项组成的组的氨基酸序列：SEQ ID NO:1、2、3、4、5、6、7、8、9、10、11、12、13、14和15。在工程化的宿主细胞内产生的差向异构酶可回收和纯化，以便形成生物催化剂。在一些情况下，差向异构酶可拆分成一个或多个酶。另外，可从工程化的宿主细胞中回收通过拆分差向异构酶产生的一个或多个酶。由拆分差向异构酶产生的这一个或多个酶也可用于催化(S)-1-苄基异喹啉生物碱到(R)-1-苄基异喹啉生物碱的转化。具体地讲，从产生差向异构酶的工程化宿主细胞中回收的一种或多种酶可用于将(S)-1-苄基异喹啉生物碱转化成(R)-1-苄基异喹啉生物碱的工艺中。所述工艺可包括使(S)-1-苄基异喹啉生物碱与足以将所述(S)-1-苄基异喹啉生物碱转化成(R)-1-苄基异喹啉生物碱的量的差向异构酶接触。在实例中，(S)-1-苄基异喹啉生物碱可与足量的一种或多种酶接触，由此使得至少5％的所述(S)-1-苄基异喹啉生物碱被转化成(R)-1-苄基异喹啉生物碱。在另外的实例中，(S)-1-苄基异喹啉生物碱可与足量的一种或多种酶接触，由此使得至少10％、至少15％、至少20％、至少25％、至少30％、至少35％、至少40％、至少45％、至少50％、至少55％、至少60％、至少65％、至少70％、至少75％、至少80％、至少82％、至少84％、至少86％、至少88％、至少90％、至少91％、至少92％、至少93％、至少94％、至少95％、至少96％、至少97％、至少98％、至少99％、至少99.5％、至少99.7％或100％的所述(S)-1-苄基异喹啉生物碱被转化成(R)-1-苄基异喹啉生物碱。

[0104] 可用于将(S)-1-苄基异喹啉生物碱转化成(R)-1-苄基异喹啉生物碱的一种或多种酶可在体外接触(S)-1-苄基异喹啉生物碱。另外或另选地，可用于将(S)-1-苄基异喹啉生物碱转化成(R)-1-苄基异喹啉生物碱的一种或多种酶可在体内接触(S)-1-苄基异喹啉生物碱。另外，可用于将(S)-1-苄基异喹啉生物碱转化成(R)-1-苄基异喹啉生物碱的一种或多种酶可提供至具有(S)-1-苄基异喹啉生物碱的细胞内，或可在工程化的宿主细胞内产生。

[0105] 在一些实例中，所述方法提供产生生物碱产物的工程化的宿主细胞，其中(S)-底物到(R)-产物的差向异构化可包括生物碱产物的产生中的关键步骤。在一些实例中，所产生的生物碱为(R)-1-苄基异喹啉生物碱。在其他实施方案中，所产生的生物碱衍生自(R)-1-苄基异喹啉生物碱，包括例如，4环原吗啡喃和5环吗啡喃生物碱。在另一个实施方案中，(S)-1-苄基异喹啉生物碱为朝向工程化宿主细胞的产物的中间体。在其他实施方案中，生物碱产物选自由吗啡喃、原小檗碱、类诺斯卡品和苯菲啶生物碱组成的组。

[0106] 在一些实例中，(S)-底物为(S)-1-苄基异喹啉生物碱，其选自由以下各项组成的组：(S)-去甲牛心果碱、(S)-牛心果碱、(S)-四氢罂粟碱、(S)-去甲乌药碱、(S)-乌药碱、(S)-N-甲基乌药碱、(S)-3’-羟基-N-甲基乌药碱、(S)-去甲异东罂粟灵、(S)-东罂粟灵、(S)-异东罂粟灵、(S)-去甲原青藤碱、(S)-原青藤碱、(S)-全去甲劳丹碱、(S)-劳丹碱、(S)-4’-O-甲基劳丹碱、(S)-6-O-甲基全去甲劳丹碱、(S)-4’-O-甲基全去甲劳丹碱。

[0107] 在一些实例中，(S)-底物为式I的化合物：



式 I，

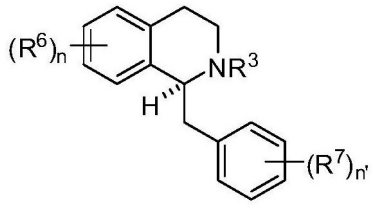
或其盐，其中：

R^1、R^2、R^3和R^4独立地选自氢和甲基；并且

R^5选自氢、羟基和甲氧基。

[0108] 在一些其他实例中，R^1、R^2、R^3、R^4和R^5中的至少一个为氢。

[0109] 在其他实例中，(S)-底物为式II的化合物：



式(II)，

或其盐，其中：

R^3选自氢和C_1-C_4烷基；

R^6和R^7在每次出现时独立地选自羟基、氟代、氯代、溴代、甲醛、C_1-C_4酰基、C_1-C_4烷基和C_1-C_4烷氧基；

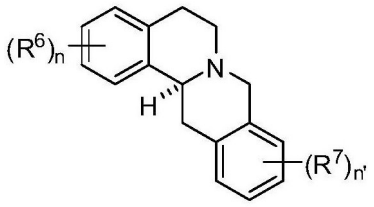
n为0、1、2、3或4；并且

n’为0、1、2、3、4或5。

[0110] 当键画在整个环上时，意指取代可发生在非特异性环原子或位置处。例如，在以上所示的式II中，6元环中的任何-CH-的氢可用R^7代替，形成-CR^7-。

[0111] 在一些实例中，R^6和R^7独立地为甲基或甲氧基。在一些其他实例中，n和n’独立地为1或2。在其他实施方案中，R^3为氢或甲基。

[0112] 在一些其他实例中，(S)-底物为式III的化合物：



式(III)，

或其盐，其中：

R^6和R^7在每次出现时独立地选自羟基、氟代、氯代、溴代、甲醛、C_1-C_4酰基、C_1-C_4烷基和C_1-C_4烷氧基；并且

n和n’独立地为0、1、2、3或4。

[0113] 在一些实例中，R^6和R^7独立地为羟基、甲基或甲氧基。在一些其他实例中，n和n'独立地为1或2。在另外的实施方案中，R^6和R^7独立地为氟代、羟基、甲基或甲氧基。

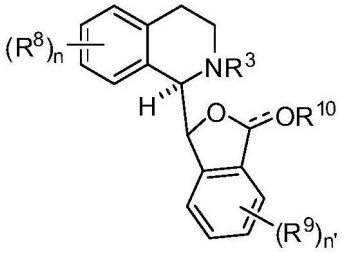
[0114] 在一些实例中，所述方法提供由(S)-牛心果碱产生生物碱产物的工程化宿主细胞。(S)-牛心果碱到(R)-牛心果碱的差向异构化可包括由前体产生各种不同的生物碱产物的关键步骤。在一些实例中，前体为L-酪氨酸或糖(例如，葡萄糖)。各种不同的生物碱产物可包括但不限于吗啡喃、原小檗碱、类诺斯卡品和苯菲啶生物碱。

[0115] 可使用任何合适的碳源作为朝向差向异构化的1-苄基异喹啉生物碱的前体。合适的前体可包括但不限于单糖(例如，葡萄糖、果糖、半乳糖、木糖)、低聚糖(例如，乳糖、蔗糖、棉子糖)、多糖(例如，淀粉、纤维素)，或其组合。在一些实例中，可使用来自可再生原料的未纯化混合物(例如，玉米浆液、糖用甜菜糖蜜、大麦麦芽、生物质水解产物)。在其他实施方案中，碳前体可以是一碳化合物(例如，甲醇、二氧化碳)或二碳化合物(例如，乙醇)。在其他实施方案中，可利用其他含碳化合物，例如，甲胺、葡糖胺和氨基酸(例如，L-酪氨酸)。在一些实例中，可将1-苄基异喹啉生物碱直接添加到本发明的工程化宿主细胞中，包括例如，全去甲劳丹碱、劳丹碱、去甲牛心果碱和牛心果碱。在另外的实施方案中，1-苄基异喹啉生物碱可作为单一对映体(例如，(S)-1-苄基异喹啉生物碱)或对映体的混合物(包括例如外消旋混合物)添加到工程化的宿主细胞中。

[0116] 在一些实例中，所述方法提供1-苄基异喹啉生物碱或其衍生物的立构中心的差向异构化。在另一个实施方案中，该方法包括使1-苄基异喹啉生物碱与至少一种酶接触。至少一种酶可将1-苄基异喹啉生物碱或其衍生物的立构中心的立体化学翻转成相反的立体化学。在一些实例中，至少一种酶将(S)-1-苄基异喹啉生物碱转化成(R)-1-苄基异喹啉生物碱。在利用至少一种酶的(S)-1-苄基异喹啉生物碱到(R)-1-苄基异喹啉生物碱的这种转化的一些实例中，(S)-1-苄基异喹啉生物碱选自由以下各项组成的组：(S)-去甲牛心果碱、(S)-牛心果碱、(S)-四氢罂粟碱、(S)-去甲乌药碱、(S)-乌药碱、(S)-N-甲基乌药碱、(S)-3'-羟基-N-甲基乌药碱、(S)-去甲异东罂粟灵、(S)-东罂粟灵、(S)-异东罂粟灵、(S)-去甲原青藤碱、(S)-原青藤碱、(S)-全去甲劳丹碱、(S)-劳丹碱、(S)-4'-O-甲基劳丹碱、(S)-6-O-甲基全去甲劳丹碱，以及(S)-4'-O-甲基全去甲劳丹碱。

[0117] 在一些其他实例中，至少一种酶将5'R-苯酞异喹啉生物碱转化成5'S-苯酞异喹啉生物碱。在利用至少一种酶的5'R-苯酞异喹啉生物碱到5'S-苯酞异喹啉生物碱的这种转化的一些实例中，5'R-苯酞异喹啉生物碱选自由5'R-那可托灵半缩醛、5'R-那可托灵、5'R-那可汀半缩醛、以及5'R-诺斯卡品组成的组。

在一些实例中，3S,5'R-苯酞异喹啉生物碱为式IV的化合物：



式 IV，

或其盐，其中：

R^3选自氢和C_1-C_4烷基；

R^8和R^9在每次出现时独立地选自羟基、氟代、氯代、溴代、甲醛、C_1-C_4酰基、C_1-C_4烷基和C_1-C_4烷氧基；

R^{10}不存在、为氢或C_1-C_4烷基；

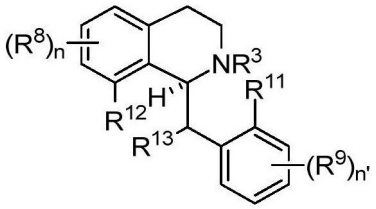
两个R^8及其所附接的碳原子任选形成5至8元环烷基或杂环烷基部分；

两个R^9及其所附接的碳原子任选形成5至8元环烷基或杂环烷基部分；并且

n和n'独立地为0、1、2、3或4。

[0118] 在一些其他实例中，至少一种酶将5'R-断裂小檗碱生物碱转化成5'S-断裂小檗碱生物碱。在利用至少一种酶的5'R-断裂小檗碱生物碱到5'S-断裂小檗碱生物碱的这种转化的一些实例中，5'R-断裂小檗碱生物碱选自由5'R-4'-O-脱甲基-3-O-乙酰基罂粟奥辛、5'R-3-O-乙酰基罂粟奥辛和5'R-罂粟奥辛组成的组。

在一些实例中，5'R-断裂小檗碱生物碱为式V的化合物：



式 V，

或其盐，其中：

R^3选自氢和C_1-C_4烷基；

R^8和R^9在每次出现时独立地选自羟基、氟代、氯代、溴代、甲醛、C_1-C_4酰基、C_1-C_4烷基和C_1-C_4烷氧基；

R^{11}选自氢和醛；

R^{12}选自氢、羟基和O-甲基；

R^{13}选自羟基和O-甲基；

两个R^8及其所附接的碳原子任选形成5至8元环烷基或杂环烷基部分；

两个R^9及其所附接的碳原子任选形成5至8元环烷基或杂环烷基部分；并且

n和n'独立地为0、1、2、3或4。

[0119] 在其他实施方案中，差向异构化的1-苄基异喹啉生物碱可包括两个或更多个立构中心，其中两个或更多个立构中心中的仅一个被翻转以产生底物的非对映体(例如，(S,R)-1-苄基异喹啉生物碱转化成(R,R)-1-苄基异喹啉生物碱)。在其中当与至少一种酶接触时1-苄基异喹啉生物碱的仅一个立构中心翻转的实例中，产物被称为1-苄基异喹啉生物碱的差向异构体。

[0120] 在一些实例中，1-苄基异喹啉生物碱作为单一立体异构体呈现给酶。在一些其他实例中，1-苄基异喹啉生物碱作为立体异构体的混合物呈现给酶。在另外的实施方案中，立体异构体的混合物可以是外消旋混合物。在一些其他实例中，立体异构体的混合物可相较于另一种立体异构体富含一种立体异构体。

[0121] 在一些实例中，回收1-苄基异喹啉生物碱或其衍生物。在一些实例中，从细胞培养物中回收1-苄基异喹啉生物碱。在另外的实施方案中，相较于呈现给酶的1-苄基异喹啉生

物碱的原始混合物，回收的1-苄基异喹啉生物碱对映体地富含一种立体异构体。在另外的实施方案中，回收的1-苄基异喹啉生物碱具有至少50％、至少55％、至少60％、至少65％、至少70％、至少75％、至少80％、至少82％、至少84％、至少86％、至少88％、至少90％、至少91％、至少92％、至少93％、至少94％、至少95％、至少96％、至少97％、至少98％、至少99％、至少99.5％、至少99.7％或100％的对映体过量。

[0122] “异构体”是具有相同分子式的不同化合物。“立体异构体”是仅在原子的空间布置上不同的异构体。“对映体”是一对不可重叠互为镜像的立体异构体。一对对映体的1:1混合物为“外消旋”混合物。“非对映异构体”或“非对映体”是具有至少两个非对称原子但是彼此不互为镜像的立体异构体。如本文所用的术语“差向异构体”是指具有相同的化学式，但是在特定位置上具有不同的光学构型的化合物。例如，化合物的(R,S)和(S,S)立体异构体为彼此的差向异构体。在一些实例中，1-苄基异喹啉生物碱转化成其差向异构体(例如，差向异构-1-苄基异喹啉生物碱)。绝对立体化学根据Cahn-Ingold-Prelog R-S系统指定。当化合物为纯对映体时，每个手性碳处的立体化学可通过R或S指定。其绝对构型未知的拆分化合物可根据它们在钠D线波长处旋转平面偏振光的方向(右旋或左旋)而被命名为(+)或(-)。本文所述的某些化合物包含一个或多个非对称中心并且因此可产生对映体、非对映体，以及可以绝对立体化学定义为(R)-或(S)-的其他立体异构形式。

表1.CYP-COR融合酶的示例性部分和全长氨基酸序列。

序列	说明	SEQ.ID NO.
MELQYISYFQPTSSVVALLLALVSILSSVVVLRKT FLNNYSSSPASSTKTAVLSHQRQQSCALPISGLLHI FMNKNGLIHVTLGNMADKYGPIFSFPTGSHRTLV VSSWEMVKECFTGNNDTAFSNRPIPLAFKTIFYA CGGIDSYGLSSVPYGKYWRELRKVCVHNLLSNQ QLLKFRHLIISQVDTSFNKLYELCKNSEDNHGNY	罂粟(*P. somniferum*)植物来源；全长氨基酸序列	SEQ.ID NO. 1

TTTTTTAAGMVRIDDWLAELSFNVIGRIVCGFQS GPKTGAPSRVEQFKEAINEASYFMSTSPVSDNVP MLGWIDQLTGLTRNMKHCGKKLDLVVESIINDH RQKRRFSRTKGGDEKDDEQDDFIDICLSIMEQPQ LPGNNNPSQIPIKSIVLDMIGGGTDTTKLTTIWTL SLLLNNPHVLDKAKQEVDAHFRTKRRSTNDAAA AVVDFDDIRNLVYIQAIIKESMRLYPASPVVERLS GEDCVVGGFHVPAGTRLWANVWKMQRDPKVW DDPLVFRPDRFLSDEQKMVDVRGQNYELLPFGA GRRVCPGVSFSLDLMQLVLTRLILEFEMKSPSGK VDMTATPGLMSYKVIPLDILLTHRRIKPCVQSAA SERDMESSGVPVITLGSGKVMPVLGMGTFEKVG KGSERERLAILKAIEVGYRYFDTAAAYETEEVLG EAIAEALQLGLVKSRDELFISSMLWCTDAHADRV LLALQNSLRNLKLEYVDLYMLPFPASLKPGKITM DIPEEDICRMDYRSVWAAMEECQNLGFTKSIGVS NFSCKKLQELMATANIPPAVNQVEMSPAFQQKKL REYCNANNILVSAISVLGSNGTPWGSNAVLGSEV LKKIAMAKGKSVAQVSMRWVYEQGASLVVKSFS EERLRENLNIFDWELTKEDHEKIGEIPQCRILSAY FLVSPNGPFKSQEELWDDEA*	>RQNK-2062398（以及FPYZ-2037562、BMRX-2007040和MLPX-2016197)	
MELQYISYFQPTSSVVALLLALVSILSSVVVLRKT FLNNYSSSPASSTKTAVLSHQRQQSCALPISGLLHI FMNKNGLIHVTLGNMADKYGPIFSFPTGSHRTLV VSSWEMVKECFTGNNDTAFSNRPIPLAFKTIFYA CGGIDSYGLSSVPYGKYWRELRKVCVHNLLSNQ QLLKFRHLIISQVDTSFNKLYELCKNSEDNHGNY TTXLLLPQLAWRQPWKLYYXTTTTAAGMVRIDD WLAELSFNVIGRIVCGFQSGPKTGAPSRVEQFKE AINEASYFMSTSPVSDNVPMLGWIDQLTGLTRNM KHCGKKLDLVVESIINDHRQKRRFSRTKGGDEK DDEQDDFIDICLSIMEQPQLPGNNNPSQIPIKSIVL DMIGGGTDTTKLTTIWTLSLLLNNPHVLDKAKQ EVDAHFRTKRRSTNDAAAAVVDFDDIRNLVYIQA IIKESMRLYPASPVVERLSGEDCVVGGFHVPAGT RLWANVWKMQRDPKVWDDPLVFRPDRFLSDEQ KMVDVRGQNYELLPFGAGRRVCPGVSFSLDLM QLVLTRLILEFEMKSPSGKVDMTATPGLMSYKVI PLDILLTHRRIKPCVQSAASERDMESSGVPVITLG SGKVMPVLGMGTFEKVGKGSERERLAILKAIEV GYRYFDTAAAYETEEVLGEAIAEALQLGLVKSRD ELFISSMLWCTDAHADRVLLALQNSLRNLKLEY VDLYMLPFPASLKPGKITMDIPEEDICRMDYRSV WAAMEECQNLGFTKSIGVSNFSCKKLQELMATA NIPPAVNQVEMSPAFQQKKLREYCNANNILVSAIS VLGSNGTPWGSNAVLGSEVLKKIAMAKGKSVAQ VSMRWVYEQGASLVVKSFSEERLRENLNIFDWE LTKEDHEKIGEIPQCRILSAYFLVSPNGPFKSQEEL WDDEA*	罂粟植物来源；全长氨基酸序列 >KKCW-2026866（以及FPYZ-2037562、MLPX-2016197)	SEQ.ID NO. 2
MELQYISYFQPTSSVVALLLALVSILSSVVVLRKT FLNNYSSSPASSTKTAVLSHQRQQSCALPISGLLHI FMNKNGLIHVTLGNMADKYGPIFSFPTGSHRTLV	罂粟植物来源；部分长度氨	SEQ.ID

VSSWEMVKECFTGNNDTAFSNRPIPLAFKTIFYA CGGIDSYGLSSVPYGKYWRELRKVCVHNLLSNQ QLLKFRHLIISQVDTSFNKLYELCKNSEDNHGNY TTTTTTAAGMVRIDDWLAELSFNVIGRIVCGFQS GPKTGAPSRVEQFKEAINEASYFMSTSPVSDNVP MLGWIDQLTGLTRNMKHCGKKLDLVVESIINDH RQKRRFSRTKGGDEKDDEQDDFIDICLSIMEQPQ LPGNNNPSQIPIKSIVLDMIGGGTDTTKLTTIWTL SLLLNNPHVLDKAKQEVDAHFRTKRRSTNDAAA AVVDFDDIRNLVYIQAIIKESMRLYPASPVVERLS GEDCVVGGFHVPAGTRLWANVWKMQRDPKVW DDPLVFRPDRFLSDEQKMVDVRGQNYELLPFGA GRRVCPGVSFSLDLMQLVLTRLILEFEMKSPSGK VDMTATPGLMSYKVIPLDILLTHRRIKPCVQSAA SERDMESSGVPVITLGSGKVMPVLGMGTFEKVG KGSERERLAILKAIEVGYRYFDTAAAYETEEVLG EAIAEALQLGLVKSRDELFISSMLWCTDAHADRV LLALQNSLRNLKLEYVDLYMLPFPASLKPGKITM DIPEEDICRMDYRXVSKPWLH*	基酸序列 >SUFP-2 025636	NO. 3
MRWHRXIDSYGLSSVPYGKYWRELRKVCVHNL LSNQQLLKFRHLIISQVDTSFNKLYELCKNSEDN QGNYPTTTTAAGMVRIDDWLAELSFNVIGRIVCG FQSGPKTGAPSRVEQFKEAINEASYFMSTSPVSD NVPMLGWIDQLTGLTRNMKHCGKKLDLVVESII NDHRQKRRFSRTKGGDEKDDEQDDFIDICLSIME QPQLPGNNNPSQIPIKSIVLDMIGGGTDTTKLTTI WTLSLLLNNPHVLDKAKQEVDAHFRTKRRSTND AAAAVVDFDDIRNLVYIQAIIKESMRLYPASPVVE RLSGEDCVVGGFHVPAGTRLWANVWKMQRDPK VWDDPLVFRPDRFLSDEQKMVDVRGQNYELLPF GAGRRVCPGVSFSLDLMQLVLTRLILEFEMKSPS GKVDMTATPGLMSYKVIPLDILLTHRRIKPCVQS AASERDMESSGVPVITLGSGKVMPVLGMGTFEK VGKGSERERLAILKAIEVGYRYFDTAAAYETEEV LGEAIAEALQLGLVKSRDELFISSMLWCTDAHAD RVLLALQNSLRNLKLEYVDLYMLPFPASLKPGKI TMDIPEEDICRMDYRSVWAAMEECQNLGFTKSI GVSNFSCKKLQELMATANIPPAVNQVEMSPAFQQ KKLREYCNANNILVSAISVLGSNGTPWGSNAVLG SEVLKKIAMAKGKSVAQVSMRWVYEQGASLVV KSFSEERLRENLNIFDWELTKEDHEKIGEIPQCRI LSAYFLVSPNGPFKSQEELWDDEA*	罂粟植物来源；部分长度氨基酸序列 >MIKW-2013651	SEQ.ID NO. 4
MELQYISYFQPTSSVVALLLALVSILSSVVVLRKT FLNNYSSSPASSTKTAVLSHQRQQSCALPISGLLHI FMNKNGLIHVTLGNMADKYGPIFSFPTGSHRTLV VSSWEMVKECFTGNNDTAFSNRPIPLAFKTIFYA CGGIDSYGLSSVPYGKYWRELRKVCVHNLLSNQ QLLKFRHLIISQVDTSFNKLYELCKNSEDNQGNY TTTTTAAGMVRIDDWLAELSFNVIGRIVCGFQSG PKTGAPSRVEQFKEAINEASYFMSTSPVSDNVPM LGWIDQLTGLTRNMKHCGKKLDLVVESIINDHR	渥美罂粟(*P. setigerum*)植物来源；全长氨基酸序列 >EPRK-2	SEQ.ID NO. 5

QKRRFSRTKGGDEKDDEQDDFIDICLSIMEQPQL PGNNNPSQIPIKSIVLDMIGGGTDTTKLTTIWTLS LLLNNPHVLDKAKQEVDAHFRTKRRSTNDAAAA VVDFDDIRNLVYIQAIIKESMRLYPASPVVERLSG EDCVVGGFHVPAGTRLWANVWKMQRDPKVWD DPLVFRPDRFLSDEQKMVDVRGQNYELLPFGAG RRVCPGVSFSLDLMQLVLTRLILEFEMKSPSGKV DMTATPGLMSYKVIPLDILLTHRRIKPCVQSAASE RDMESSGVPVITLGSGKVMPVLGMGTFEKVGKG SERERLAILKAIEVGYRYFDTAAAYETEEVLGEAI AEALQLGLVKSRDELFISSMLWCTDAHADRVLL ALQNSLRNLKLEYVDLYMLPFPASLKPGKITMDI PEEDICRMDYRSVWAAMEECQNLGFTKSIGVSN FSCKKLQELMATANIPPAVNQVEMSPAFQQKKLR EYCNANNILVSAISVLGSNGTPWGSNAVLGSEVL KKIAMAKGKSVAQVSMRWVYEQGASLVVKSFSE ERLRENLNIFDWELTKEDHEKIGEIPQCRILSAYF LVSPNGPFKSQEELWDDEA*	027940(以及FPYZ-2037562、STDO-2019715、FNXH-2029312、MLPX-2016196、MLPX-2016197)	
MELQYISYFQPTSSVVALLLALVSILSSVVVLRKT FLNNYSSSPASSTKTAVLSHQRQQSCALPISGLLHI FMNKNGLIHVTLGNMADKYGPIFSFPTGSHRTLV VSSWEMVKECFTGNNDTAFSNRPIPLAFKTIFYA CGGIDSYGLSSVPYGKYWRELRKVCVHNLLSNQ QLLKFRHLIISQVDTSFNKLYELCKNSEDNQGNY TTTTTAAGMVRIDDWLAELSFNVIGRIVCGFQSG PKTGAPSRVEQFKEAINEASYFMSTSPVSDNVPM LGWIDQLTGLTRNMKHCGKKLDLVVESIINDHR QKRRFSRTKGGDEKDDEQDDFIDICLSIMEQPQL PGNNNPSQIPIKSIVLDMIGGGTDTTKLTTIWTLS LLLNNPHVLDKAKQEVDAHFRTKRRSTNDAAAA VVDFDDIRNLVYIQALYPASPVVERLSGEDCVVG GFHVPAGTRLWANVWKMQRDPKVWDDPLVFRP DRFLSDEQKMVDVRGQNYELLPFGAGRRVCPGV SFSLDLMQLVLTRLILEFEMKSPSGKVDMTATPG LMSYKVIPLDILLTHRRIKPCVQSAASERDMESSG VPVITLGSGKVMPVLGMGTFEKVGKGSERERLA ILKAIEVGYRYFDTAAAYETEEVLGEAIAEALQL GLVKSRDELFISSMLWCTDAHADRVLLALQNSLR NLKLEYVDLYMLPFPASLKPGKITMDIPEEDICR MDYRSVWAAMEE	渥美罂粟植物来源；部分长度氨基酸序列 >QCOU-2000833	SEQ.ID NO. 6
MELQYFSYFQPTSSVVALLLALVSILFSVVVLRKT FSNNYSSPASSTETAVLCHQRQQSCALPISGLLHV FMNKNGLIHVTLGNMADKYGPIFSFPTGSHRTLV VSSWEMVKECFTGNNDTAFSNRPIPLAFQTIFYA CGGIDSYGLSSVPYGKYWRELRKVCVHNLLSNQ QLLKFRHLIISQVDTSFNKLYELCKNSEDNQGMV RMDDWLAQLSFNVIGRIVCGFQSDPKTGAPSRVE QFKEVINEASYFMSTSPVSDNVPMLGWIDQLTGL TRNMKHCGKKLDLVVESIIKDHRQKRRFSRTKG GDEKDDEQDDFIDICLSIMEQPQLPGNNSPPQIPI KSIVLDMIGGGTDTTKLTTIWTLSLLLNNPHVLD KAKQEVDAHFRKKRRSTDDAAAAVVDFDDIRNL	大红罂粟(*P. bracteatum*)植物来源；全长氨基酸序列 >SSDU-2015634(以及	SEQ.ID NO. 7

VYIQAIIKESMRLYPASPVVERLSGEDCVVGGFH VPAGTRLWANVWKMQRDPKVWDDPLVFRPERF LSDEQKMVDVRGQNYELLPFGAGRRICPGVSFS LDLMQLVLTRLILEFEMKSPSGKVDMTATPGLM SYKVVPLDILLTHRRIKSCVQLASSERDMESSGVP VITLSSGKVMPVLGMGTFEKVGKGSERERLAIL KAIEVGYRYFDTAAAYETEEVLGEAIAEALQLGL IESRDELFISSMLWCTDAHPDRVLLALQNSLRNL KLEYLDLYMLPFPASLKPGKITMDIPEEDICRMD YRSVWSAMEECQNLGFTKSIGVSNFSSKKLQEL MATANIPPAVNQVEMSPAFQQKKLREYCNANNIL VSAVSILGSNGTPWGSNAVLGSEVLKQIAMAKGK SVAQVSMRWVYEQGASLVVKSFSEERLRENLNIF DWELTKEDNEKIGEIPQCRILTAYFLVSPNGPFKS QEELWDDKA*	SSDU-2015636 、ZSNV-2027701 、RRID-2004435)	
MELQYFSYFQPTSSVVALLLALVSILFSVVVLRKT FSNNYSSPASSTETAVLCHQRQQSCALPISGLLHV FMNKNGLIHVTLGNMADKYGPIFSFPTGSHRTLV VSSWEMVKECFTGNNDTAFSNRPIPLAFQTIFYA CGGIDSYGLSSVPYGKYWRELRKVCVHNLLSNQ QLLKFRHLIISQVDTSFNKLYELCKNSEDNQGMV RMDDWLAQLSFNVIGRIVCGFQSDPKTGAPSRVE QFKEVINEASYFMSTSPVSDNVPMLGWIDQLTGL TRNMKHCGKKLDLVVESIIKDHRQKRRFSRTKG GDEKDDEQDDFIDICLSIMEQPQLPGNNSPPQIPI KSIVLDMIGGGTDTTKLTTIWTLSLLLNNPHVLD KAKQEVDAHFRKKRRSTDDAAAAVVDFDDIRNL VYIQAIIKESMRLYPASPVVERLSGEDCVVGGFH VPAGTRLWANVWKMQRDPKVWDDPLVFRPERF LSDEQKMVDVRGQNYELLPFGAGRRICPGVSFS LDLMQLVLTRLILEFEMKSPSGKVDMTATPGLM SYKVVPLDILLTHRRIKSCVQLASSERDMESSGVP VITLSSGKVMPVLGMGTFEKVGKGSERERLAIL KAIEVGYRYFDTAAAYETEEVLGEAIAEALQLGL IESRDELFISSMLWCTDAHPDRVLLALQNSLRNL KLEYLDLYMLPFPASLKPGKITMDIPEEDICRMD YRSVWSAMEECQNLGFTKSIGVSNFSCKKLQEL MATANIPPAVNQVEMSPAFQQKKLREYCNANNIL VSAVSILGSNGTPWGSNAVLGSEVLKQIAMAKGK SVAQVSMRWVYEQGASLVVKSFSEERLRENLNIF DWELTKEDNEKIGEIPQCRILTAYFLVSPNGPFKS QEELWDDKA*	大红罂粟植物来源；全长氨基酸序列 >TMWO-2027322（以及RRID-2004435)	SEQ.ID NO. 8
SSPASSTETAVLCHQRQQSCALPISGLLHIFMNKN GLIHVTLGNMADKYGPIFSFPTGSHRILVVSSWE MVKECFTGNNDTAFSNRPIPLAFKTIFYACRGIDS YGLSSVPYGKYWRELRKVCVHNLLSNQQLLKFR HLIISQVDTSFNKLYELCKNSEDNQGMVRMDDW LAQLSFSVIGRIVCGFQSDPKTGAPSRVEQFKEAI NEASYFMSTSPVSDNVPMLGWIDQLTGLTRNMT HCGKKLDLVVESIINDHRQKRRFSRTKGGDEKD DEQDDFIDICLSIMEQPQLPGNNNPPKIPIKSIVLD MIGAGTDTTKLTIIWTLSLLLNNPNVLAKAKQEV	大红罂粟植物来源；部分长度氨基酸序列 >pbr.PBRST1PF_89405	SEQ.ID NO. 9

DAHFETKKRSTNEASVVVDFDDIGNLVYIQAIIKE SMRLYPVSPVVERLSSEDCVVGGFHVPAGTRLWA NVWKMQRDPKVWDDPLVFRPERFLSDEQKMVD VRGQNYELLPFGAGRRICPGVSFSLDLMQLVLTR LILEFEMKSPSGKVDMTATPGLMSYKVVPLDILL THRRIKSCVQLASSERDMESSGVPVITLRSGKVM PVLGMGTFEKAGKGSERERLAILKAIEVGYRYF DTAAAYETEEVLGEAIAEALQLGLIKSRDELFISS MLWCTDAHPDRVLLALQNSLRNLKLEYVDLYM LPFPASLKPGKITMDIPEEDICPMDYRSVWSAME ECQNLGLTKSIGVSNFSCKKLEELMATANIPPAVN QVEMSPAFQQKKLREYCNANNILVSAVSILGSNG TPWGSNAVLGSEVLKKIAMAKGKSVAQVSMRW VYEQGASLVVKSFSEERLRENLNIFDWQLTKEDN EKIGEIPQCRILSAYFLVSPKGPFKSQEELWDDKA *		
SSPASSTETAVLCHQRQQSCALPISGLLHIFMNKN GLIHVTLGNMADKYGPIFSFPTGSHRILVVSSWE MVKECFTGNNDTFFSNRPIPLAFKIIFYAGGVDSY GLALVPYGKYWRELRKICVHNLLSNQQLLKFRH LIISQVDTSFNKLYELCKNSEDNQGMVRMDDWL AQLSFSVIGRIVCGFQSDPKTGAPSRVEQFKEAIN EASYFMSTSPVSDNVPMLGWIDQLTGLTRNMTH CGKKLDLVVESIINDHRQKRRFSRTKGGDEKDD EQDDFIDICLSIMEQPQLPGNNNPPKIPIKSIVLD MIGGGTDTTKLTTIWTLSLLLNNPHVLDKAKQE VDAHFLTKRRSTNDAAVVDFDDIRNLVYIQAIIKE SMRLYPASPVVERLSGEDCVVGGFHVPAGTRLW VNVWKMQRDPNVWADPMVFRPERFLSHGQKK MVDVRGKNYELLPFGAGRRICPGISFSLDLMQLV LTRLILEFEMKSPSGKVDMTATPGLMSYKVVPLD ILLTHRRIKSCVQLASSERDMESSGVPVITLRSGK VMPVLGMGTFEKAGKGSERERLAILKAIEVGYR YFDTAAAYETEEVLGEAIAEALQLGLIKSRDELFI SSMLWCTDAHPDRVLLALQNSLRNLKLEYVDLY MLPFPASLKPGKITMDIPEEDICPMDYRSVWSAM EECQNLGLTKSIGVSNFSCKKLEELMATANIPPAV NQVEMSPAFQQKKLREYCNANNILVSAVSILGSN GTPWGSNAVLGSEVLKKIAMAKGKSVAQVSMR WVYEQGASLVVKSFSEERLRENLNIFDWQLTKE DNEKIGEIPQCRILSAYFLVSPKGPFKSQEELWDD KA*	大红罂粟植物来源；部分长度氨基酸序列 >pbr.PBRST1PF_4328	SEQ.ID NO. 10
SSPASSTETAVLCHQRQQSCALPISGLLHIFMNKN GLIHVTLGNMADKYGPIFSFPTGSHRILVVSSWE MVKECFTGNNDTFFSNRPIPLAFKIIFYAGGVDSY GLALVPYGKYWRELRKICVHNLLSNQQLLNFRH LIISQVDTSFNKLYDLSNKKKNTTTDSGTVRMDD WLAQLSFNVIGRIVCGFQTHTETSATSSVERFTE AIDEASRFMSIATVSDTFPWLGWIDQLTGLTRKM KHYGKKLDLVVESIIEDHRQNRRISGTKQGDDFI DICLSIMEQPQIIPGNNDPPRQIPIKSIVLDMIGGG TDTTKLTTTWTLSLLLNNPHVLEKAREEVDAHF	大红罂粟植物来源；部分长度氨基酸序列 >pbr.PBRST1PF_12180	SEQ.ID NO. 11

GTKRRPTNDDAVMVEFDDIRNLVYIQAIIKESMR LYPASPVVERLSGEDCVVGGFHVPAGTRLWVNV WKMQRDPNVWADPMVFRPERFLSDEQKMVDVR GQNYELLPFGAGRRICPGVSFSLDLMQLVLTRLI LEFEMKSPSGKVDMTATPGLMSYKVVPLDILLTH RRIKSCVQLASSERDMESSGVPVITLRSGKVMPV LGMGTFEKAGKGSERERLAILKAIEVGYRYFDT AAAYETEEVLGEAIAEALQLGLIKSRDELFISSML WCTDAHPDRVLLALQNSLRNLKLEYVDLYMLPF PASLKPGKITMDIPEEDICPMDYRSVWSAMEECQ NLGLTKSIGVSNFSCKKLEELMATANIPPAVNQVE MSPAFQQKKLREYCNANNILVSAVSILGSNGTPW GSNAVLGSEVLKKIAMAKGKSVAQVSMRWVYEQ GASLVVKSFSEERLRENLNIFDWQLTKEDNEKIG EIPQCRILSAYFLVSPKGPFKSQEELWDDKA*		
VALRKKILKNYYSSSSSTATAVSHQWPKASRALPL IDLLHVFFNKTDLMHVTLGNMADKFGPIFSFPTG SHRTLVVSSWEKAKECFTGNNDIVFSGRPLPLAF KLIFYAGGIDSYGISQVPYGKKWRELRNICVHNI LSNQQLLKFRHLMISQVDNSFNKLYEVCNSNKDE GDSATSTTAAGIVRMDDWLGKLAFDVIARIVCGF QSQTETSTTSSMERFTEAMDEASRFMSVTAVSDT VPWLGWIDQLTGLKRNMKHCGKKLNLVVKSIIE DHRQKRRLSSTKKGDENIIDEDEQDDFIDICLSIM EQPQLPGNNNPPKIPIKSIVLDMIGGGTDTTKLTT IWTLSLLLNNPHVLDKAKQEVDAHFLTKRRSTN DAAVVDFDDIRNLVYIQAIIKESMRLYPASPVVER LSGEDCVVGGFHVPAGTRLWVNVWKMQRDPNV WADPMVFRPERFLSDEQKMVDVRGQNYELLPFG AGRRICPGVSFSLDLMQLVLTRLILEFEMKSPSG KVDMTATPGLMSYKVVPLDILLTHRRIKSCVQLA SSERDMESSGVPVITLRSGKVMPVLGMGTFEKA GKGSERERLAILKAIEVGYRYFDTAAAYETEEVL GEAIAEALQLGLIKSRDELFISSMLWCTDAHPDR VLLALQNSLRNLKLEYVDLYMLPFPASLKPGKIT MDIPEEDICPMDYRSVWSAMEECQNLGLTKSIGV SNFSCKKLEELMATANIPPAVNQVEMSPAFQQKK LREYCNANNILVSAVSILGSNGTPWGSNAVLGSEV LKKIAMAKGKSVAQVSMRWVYEQGASLVVKSFS EERLRENLNIFDWQLTKEDNEKIGEIPQCRILSAY FLVSPKGPFKSQEELWDDKA*	大红罂粟植物来源；部分长度氨基酸序列 >pbr.PBRST1PF_4329	SEQ.ID NO. 12
MELQYFSYFQPTSSVVALLLALVSILFSVVVLRKT FSNNYSSPASSTETAVLCHQRQQSCALPISGLLHV FMNKNGLIHVTLGNMADKYGPIFSFPTGSHRTLV VSSWEMVKECFTGNNDTAFSNRPIPLAFQTIFYA CGGIDSYGLSSVPYGKYWRELRKVCVHNLLSNQ QLLKFRHLIISQVDTSFNKLYELCKNSEDNQGMV RMDDWLAQLSFNVIGRIVCGFQSDPKTGAPSRVE QFKEVINEASYFMSTSPVSDNVPMLGWIDQLTGL TRNMKHCGKKLDLVVESIIKDHRQKRRFSRTKG GDEKDDEQDDFIDICLSIMEQPQLPGNNSPPQIPI KSIVLDMIGGGTDTTKLTTIWTLSLLLNNPHVLD	大红罂粟植物来源；部分长度氨基酸序列 >SSDU-2015635	SEQ.ID NO. 13

KAKQEVDAHFRKKRRSTDDAAAAVVDFDDIRNL VYIQAIIKESMRLYPASPVVERLSGEDCVVGGFH VPAGTRLWANVWKMQRDPKVWDDPLVFRPERF LSDEQKMVDVRGQNYELLPFGAGRRICPGVSFS LDLMQLVLTRLILEFEMKSPSGKVDMTATPGLM SYKVVPLDILLTHRRIKSCVQLASSERDMESSGVP VITLSSGKVMPVLGMGTFEKVGKGSERERLAIL KAIEVGYRYFDTAAAYETEEVLGEAIAEALQLGL IESRDELFISSMLWCTDAHPDRVLLALQNSLRNL KLEYLDLYMLPFPASLKPGKITMDIPEEDICRMD YRSVWSAMEECQNLGFTKSIGVSNFSSKKLQEL MATANIPPAVNQVEMSPAFQQKKLREYCNANNIL VSAVSILGSNGTPWGSNAVLGSEVLKQIAMAKGK SVAQVSMRWVVXKFSAYAIVWSLFFGHRICITLYSF LIRNVAYICITY*		
MELQYFSYFQPTSSVVALLLALVSILFSVVVLRKT FSNNYSSPASSTETAVLCHQRQQSCALPISGLLHV FMNKNGLIHVTLGNMADKYGPIFSFPTGSHRTLV VSSWEMVKECFTGNNDTAFSNRPIPLAFQTIFYA CGGIDSYGLSSVPYGKYWRELRKVCVHNLLSNQ QLLKFRHLIISQVDTSFNKLYELCKNSEDNQGMV RMDDWLAQLSFNVIGRIVCGFQSDPKTGAPSRVE QFKEVINEASYFMSTSPVSDNVPMLGWIDQLTGL TRNMKHCGKKLDLVVESIIKDHRQKRRFSRTKG GDEKDDEQDDFIDICLSIMEQPQLPGNNSPPQIPI KSIVLDMIGGGTDTTKLTTIWTLSLLLNNPHVLD KAKQEVDAHFRKKRRSTDDAAAAVVDFDDIRNL VYIQAIIKESMRLYPASPVVERLSGEDCVVGGFH VPAGTRLWANVWKMQRDPKVWDDPLVFRPERF LSDEQKMVDVRGQNYELLPFGAGRRICPGVSFS LDLMQLVLTRLILEFEMKSPSGKVDMTATPGLM SYKVVPLDILLTHRRIKSCVQLASSERDMESSGVP VITLSSGKVMPVLGMGTFEKVGKGSERERLAIL KAIEVGYRYFDTAAAYETEEVLGEAIAEALQLGL IESRDELFISSMLWCTDAHPDRVLLALQNSLRQV FLMQIRLIYICTYQQVHLNIYFQINEFVLCDMYR NLKLEY	大红罂粟植物来源；部分长度氨基酸序列 >SSDU-2015637	SEQ.ID NO. 14
LNNYSSSPASSTKTAVLSHQRQQSCALPISGLLHIF MNKNGLIHVTLGNMADKYGPIFSFPTGSHRTLV VSSWEMVKECFTGNNDTAFSNRPIPLAFKTIFYA CGGIDSYGLSSVPYGKYWRELRKVCVHNLLSNQ QLLKFRHLIISQVDTSFNKLYELCKNSEDNQGNY PTTTTAAGMVRIDDWLAELSFNVIGRIVCGFQSG PKTGAPSRVEQFKEAINEASYFMSTSPVSDNVPM LGWIDQLTGLTRNMKHCGKKLDLVVESIINDHR QKRRFSRTKGGDEKDDEQDDFIDICLSIMEQPQL PGNNNPSQIPIKSIVLDMIGGGTDTTKLTTIWTLS LLLNNPHVLDKAKQEVDAHFRTKRRSTNDAAAA VVDFDDIRNLVYIQAIIKESMRLYPASPVVERLSG EDCVVGGFHVPAGTRLWANVWKMQRDPKVWD DPLVFRPDRFLSDEQKMVDVRGQNYELLPFGAG RRVCPGVSFSLDLMQLVLTRLILEFEMKSPSGKV	白屈菜(*C. majus*)植物来源；部分长度氨基酸序列 >chm.CMAST2PF_14984	SEQ.ID NO. 15

DMTATPGLMSYKVIPLDILLTHRRIKPCVQSAASE RDMESSGVPVITLGSGKVMPVLGMGTFEKVGKG SERERLAFLKAIEVGYRYFDTAAAYETEEFLGEAI AEALQLGLIKSRDELFITSKLWPCDAHPDLVVPA LQNSLRNLKLEYVDLYMLPFPASLKPGKITMDIP EEDICRMDYRSVWAAMEECQNLGFTKSIGVSNFS CKKLQELMATANIPPAVNQVEMSPAFQQKKLRE YCNANNILVSAISVLGSNGTPWGSNAVLGSEVLK KIAMAKGKSVAQVSMRWVYEQGASLVVKSFSEE RLRENLNIFDWELTKEDHEKIGEIPQCRILSAYFL VSPNGPFKSQEELWDDEA*		

BisBIA生成修饰

[0123] 本文提供的一些方法、工艺和系统描述了双苄基异喹啉生物碱(bisBIA)的产生。BisBIA是可通过两个BIA单体之间的偶合反应形成的二聚分子。在实例中,bisBIA可通过碳-氧偶合反应形成。在其他实例中,bisBIA可通过碳-碳偶合反应形成。在一些实例中,bisBIA二聚分子为同二聚体,包括两个相同的BIA单体。在实例中,工程化的宿主细胞可产生一个BIA单体。在这些实例中,当与一种或多种偶合酶接触时,BIA单体可形成同二聚体。在其他实例中,bisBIA二聚分子为异二聚体,包括两个不同的BIA单体。例如,bisBIA可以是包括为彼此的对映体的BIA单体的异二聚体。在一些实例中,工程化的宿主细胞可产生两个或更多个BIA单体。在这些实例中,当与一种或多种偶合酶接触时,BIA单体可形成同二聚体和异二聚体。

[0124] 描述bisBIA的产生的这些方法、工艺和系统中的一些可包括工程化的宿主细胞。在一些实例中,工程化的宿主细胞可被工程化以产生BIA单体,所述BIA单体继而可用作用于形成的bisBIA的构件分子。可用于形成bisBIA的BIA单体的实例包括乌药碱、N-甲基乌药碱、劳丹宁、去甲乌药碱、全去甲劳丹碱、6-O-甲基-全去甲劳丹碱、3'-羟基-N-甲基乌药碱、3'-羟基乌药碱、牛心果碱、去甲牛心果碱、去甲劳丹宁、劳丹素和罂粟碱。具体地讲,工程化的宿主细胞可通过包括O-甲基转移酶、N-甲基转移酶和3'-羟化酶的异源酶的表达,由去甲乌药碱或全去甲劳丹碱合成BIA单体。O-甲基转移酶的实例可包括来自黄唐松草、莲藕(Nelumbo nucifera)、胡杨(Populus euphratica)或另外的物种的去甲乌药碱6-O-甲基转移酶(6OMT)。O-甲基转移酶的另外的实例可包括来自智人(Homo sapiens)、小家鼠(Mus musculus)、褐家鼠(Rattus norvegicus)、大猩猩(Gorilla gorilla)或另外的物种的儿茶酚O-甲基转移酶(COMT)。N-甲基转移酶的另外的实例可包括来自黄唐松草、莲藕、弱茎马兜铃(Aristolochia fimbriata)或另外的物种的乌药碱N-甲基转移酶(CNMT)。3'羟化酶的实例可包括来自花菱草(Eschscholzia californica)、黄唐松草、莲藕或另外物种的N-甲基乌药碱3'-羟化酶(CYP80B1)。

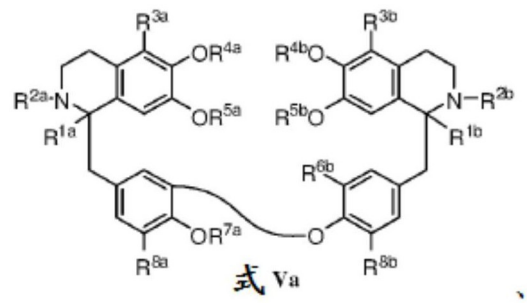
[0125] 工程化的宿主细胞可产生任何给定BIA单体的(S)或(R)对映体。另外或另选地,工程化的宿主细胞可产生两种对映体的混合物。(S)和(R)对映体的比率可由合成BIA单体的一种或多种酶的底物和产物特异性确定。另选地,存在的每种对映体的量可通过进行一种立体异构体到另一种立体异构体的差向异构化的一种或多种另外的酶的表达而改变,如上文所论述。

[0126] 这些BIA单体可融合到二聚bisBIA支架中。具体地讲,BIA单体可利用工程化宿主

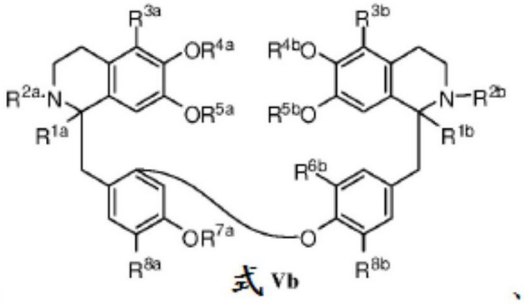
细胞产生的一种或多种酶融合到二聚bisBIA支架中。另外或另选地,BIA单体可利用从工程化宿主细胞外部的来源提供给BIA单体的一种或多种酶融合到二聚bisBIA支架中。一种或多种酶可用于形成碳-氧和/或碳-碳偶合反应,以在一个、两个或三个位置处融合两个BIA单体。在一些实例中,两个BIA单体可通过醚桥连接。在一些实例中,可使用直接的碳-碳键连接两个BIA单体。在一些实例中,通过融合两个BIA单体形成的bisBIA可包括一个二苯基醚键。在一些实例中,两个BIA单体可融合形成包括两个二苯基醚键的bisBIA。在一些实例中,由两个BIA单体形成的bisBIA可包括三个二苯基醚键。在一些实例中,bisBIA可包括一个二苯基醚键和一个苄基苯基醚键。在一些情况下,bisBIA可包括一个苄基苯基醚键和两个二苯基醚键。

[0127] 在实例中,BIA单体可与足量的一种或多种酶接触,所述酶可用于形成偶合反应,以融合两个BIA单体,由此使得至少10%、至少15%、至少20%、至少25%、至少30%、至少35%、至少40%、至少45%、至少50%、至少55%、至少60%、至少65%、至少70%、至少75%、至少80%、至少82%、至少84%、至少86%、至少88%、至少90%、至少91%、至少92%、至少93%、至少94%、至少95%、至少96%、至少97%、至少98%、至少99%、至少99.5%、至少99.7%或100%的所述BIA单体被转化成bisBIA。可用于将BIA单体二聚化成bisBIA的一种或多种酶可在体外接触BIA单体。另外或另选地,可用于将BIA单体二聚化成bisBIA的一种或多种酶可在体内接触BIA单体。另外,一种或多种bisBIA二聚化酶可在产生BIA单体的宿主细胞中表达。另选地,可向表达bisBIA二聚化酶的工程化宿主细胞中提供BIA单体。另选地,可向其内具有BIA单体的细胞中提供一种或多种bisBIA二聚化酶。

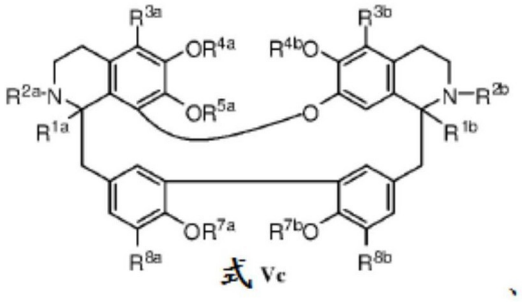
[0128] 在一些实例中,双苄基异喹啉生物碱是式Va-Vu中的任一个的化合物:



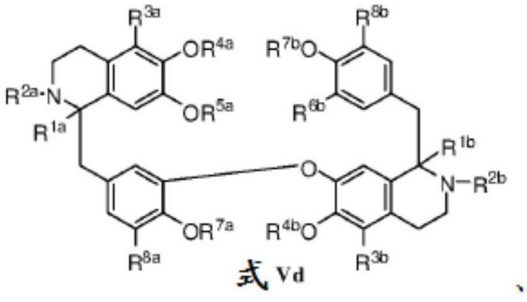
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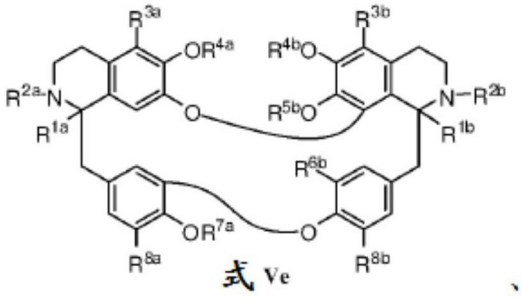
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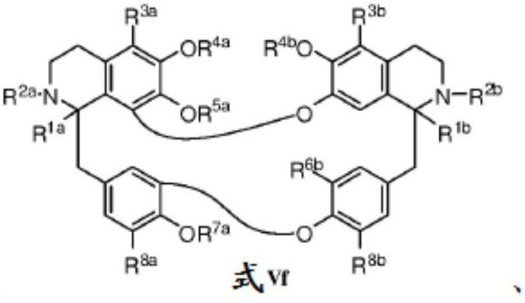
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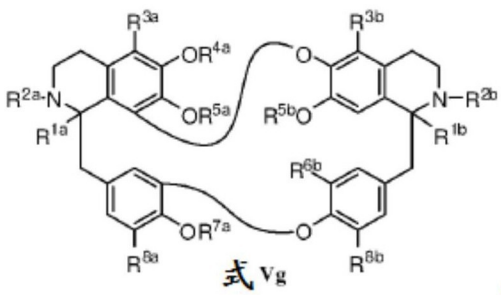
式 Vd



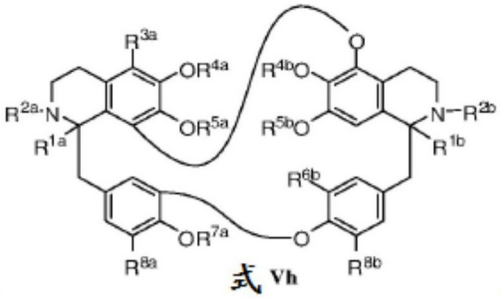
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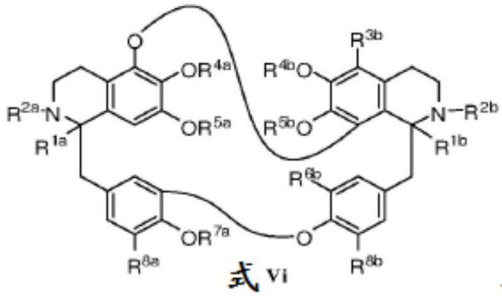
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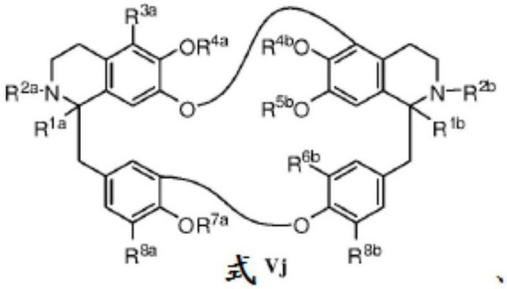
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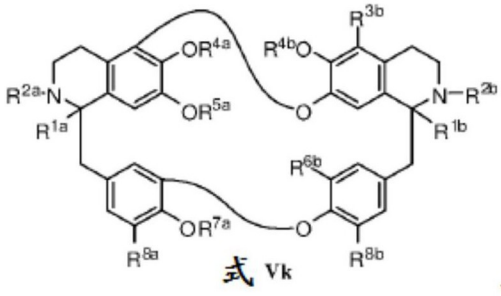
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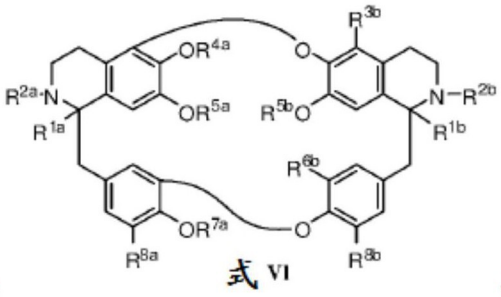
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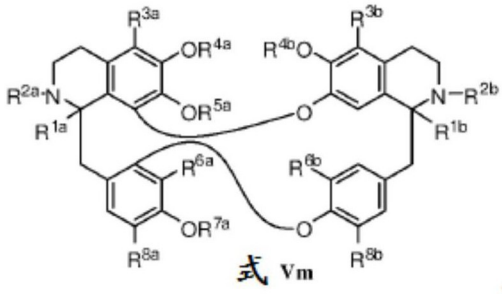
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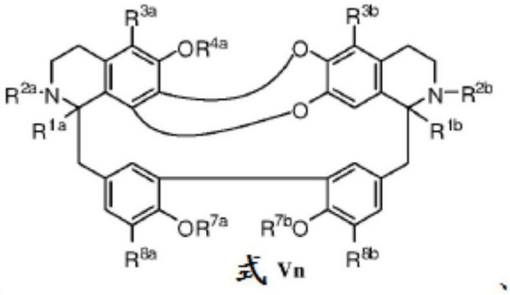
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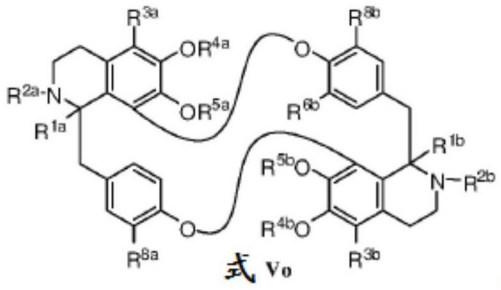
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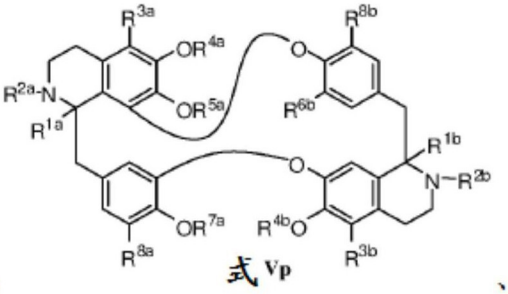
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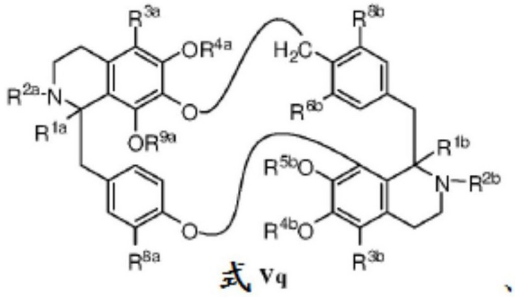
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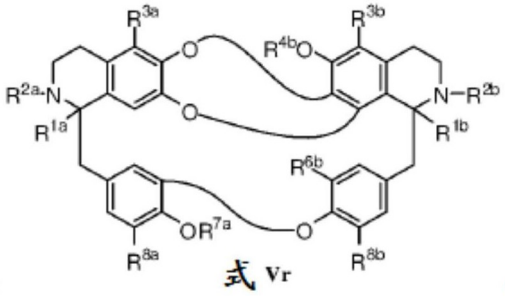
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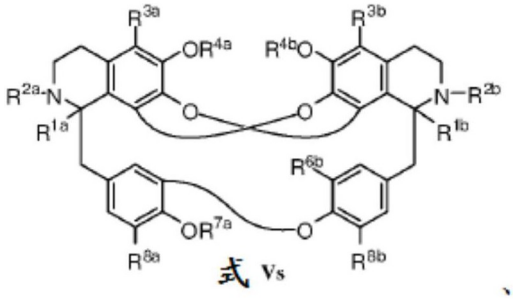
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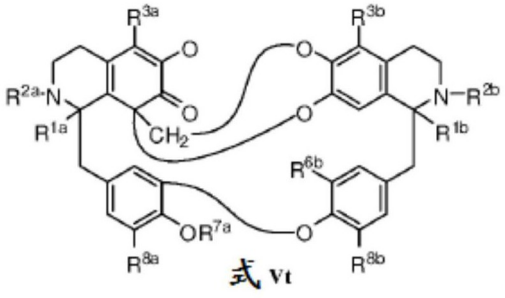
式 Vq、



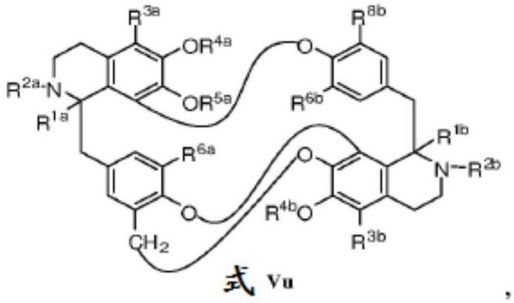
式 Vr、



式 Vs、



式 Vt 和



式 Vu，

或其盐，其中：

R^{1a}、R^{1b}、R^{2a}和R^{2b}独立地选自氢和C_1-C_4烷基；

R^{3a}、R^{3b}、R^{6a}、R^{6b}、R^{8a}和R^{8b}独立地选自氢、羟基、氟代、氯代、溴代、甲醛、C_1-C_4酰基、C_1-C_4烷基和C_1-C_4烷氧基；

R^{4a}和R^{5a}独立地选自氢和C_1-C_4烷基，或R^{4a}和R^{5a}一起形成亚甲基桥；

R^{4b}和R^{5b}独立地选自氢和C_1-C_4烷基，或R^{4b}和R^{5b}一起形成亚甲基桥；并且

R^{7a}、R^{7b}和R^{9a}独立地选自氢和C_1-C_4烷基。

[0129] 在一些实例中，R^{1a}和R^{1b}各自为氢；R^{2a}和R^{2b}各自为甲基；R^{3a}和R^{3b}各自为氢；R^{4a}和R^{5a}独立地为氢或甲基；R^{4b}和R^{5b}独立地为氢或甲基，或R^{4b}和R^{5b}一起形成亚甲基桥；R^{6a}、R^{6b}、R^{8a}和R^{8b}各自为氢；并且R^{7a}、R^{7b}和R^{9a}独立地为氢或甲基。

[0130] 如上文所示，式Va、Vb和Vd的bisBIA化合物通过使用碳-氧偶合反应融合两个BIA单体形成。另外，式Vc、Vf和Vh的bisBIA化合物通过使用碳-氧偶合反应和碳-碳偶合反应两者融合两个BIA单体形成。此外，式Ve、Vg、Vi、Vj、Vk、Vl、Vm、Vo、Vp和Vq的bisBIA化合物通过使用碳-氧偶合反应融合两个BIA单体形成。式Vn的bisBIA化合物通过使用两个碳-氧偶合反应和一个碳-碳偶合反应融合两个BIA单体形成。另外，式Vr的bisBIA化合物通过使用三个碳-氧偶合反应融合两个BIA单体形成。

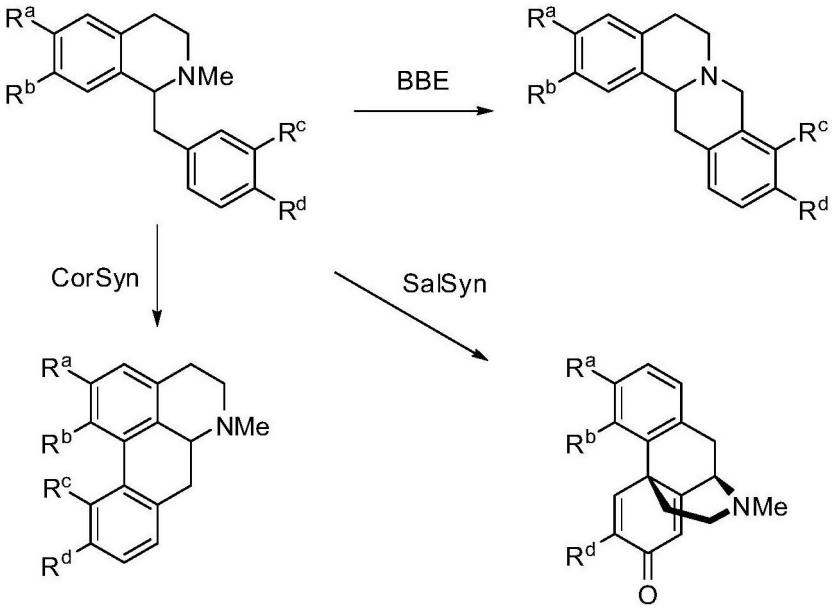
[0131] 可用于形成偶合反应的一种或多种酶可包括已知的细胞色素P450，诸如伏牛花(Berberis stolonifera)CYP80A1或来自天然合成这些化合物的其他植物的类似的细胞色素P450酶。另选地，偶合反应可通过不是细胞色素P450的酶进行。可用于形成偶合反应的一

种或多种酶可被工程化为接受非天然底物。因此，可用于形成偶合反应的一种或多种酶可用于生成非天然bisBIA分子。在实例中，一种或多种酶可将天然BIA单体与非天然BIA单体融合，以产生非天然bisBIA分子。在其他实例中，一种或多种酶可将两个非天然BIA单体融合，以产生非天然bisBIA分子。可使用酶工程化策略鉴定可用于形成融合BIA单体产生bisBIA的偶合反应的一种或多种酶。在实例中，酶工程化策略可包括定点诱变、随机诱变和筛选、DNA改组和筛选。

[0132] 一旦形成bisBIA，bisBIA可进一步衍生或修饰。可利用工程化宿主细胞产生的一种或多种酶衍生或修饰bisBIA。具体地讲，可通过使bisBIA与工程化宿主细胞产生的一种或多种酶接触来衍生或修饰bisBIA。另外或另选地，可通过使bisBIA与从工程化宿主细胞外部的来源提供给bisBIA的一种或多种酶接触来衍生或修饰bisBIA。可用于衍生或修饰bisBIA的一种或多种酶可用于进行裁剪反应。裁剪反应的实例包括氧化、还原、O-甲基化、N-甲基化、O-脱甲基化、乙酰化、亚甲基二氧桥形成以及O,O-脱亚甲基化。可使用一种或多种裁剪反应衍生或修饰bisBIA。

[0133] 裁剪反应的实例提供于表3中。在一些实例中，裁剪酶可用于催化在bisBIA或其衍生物上进行的碳-碳偶合反应。可用于催化碳-碳偶合反应的裁剪酶的实例包括来自罂粟、花菱草、日本黄连(Coptis japonica)、匍枝小檗(Berberis stolonifer)、黄唐松草或另外物种的小檗碱桥酶(BBE)；来自罂粟或另外物种的沙罗泰里啶合酶(SalSyn)；以及来自日本黄连或另外物种的紫堇块茎碱合酶(CorSyn)。可通过裁剪酶催化的反应的非限制性实例在方案2中示出，其中R^a、R^b、R^c和R^d独立地选自氢、羟基、氟代、氯代、溴代、甲醛、C_1-C_4酰基、C_1-C_4烷基和C_1-C_4烷氧基。在一些实例中，R^a、R^b及其所附接的碳原子任选形成碳环或杂环。在一些实例中，R^c、R^d及其所附接的碳原子任选形成碳环或杂环。

方案2



[0134] 在一些实例中，裁剪酶可用于催化在bisBIA或其衍生物上进行的氧化反应。可用于催化氧化反应的裁剪酶的实例包括来自日本黄连、蓟罂粟(Argemone mexicana)、金花小檗(Berberis wilsonae)或另外物种的四氢原小檗碱氧化酶(STOX)；来自罂粟或另外物种的二氢苯菲啶氧化酶(DBOX)；来自罂粟或另外物种的甲基刺罂粟碱羟化酶(MSH)；以及来自

罂粟、花菱草或另外物种的原阿片碱6-羟化酶(P6H)。

[0135] 裁剪酶也可用于催化在bisBIA或其衍生物上进行的亚甲基二氧桥形成反应。可用于催化亚甲基二氧桥形成反应的裁剪酶的实例包括来自罂粟、花菱草、蓟罂粟或另外物种的刺罂粟碱合酶(StySyn);来自罂粟、花菱草、蓟罂粟或另外物种的碎叶紫堇碱合酶(CheSyn);以及来自黄唐松草、黄连(Coptis chinensis)或另外物种的坎那定合酶(CAS)。

[0136] 在其他实例中,裁剪酶可用于催化在bisBIA或其衍生物上进行的O-甲基化反应。可用于催化O-甲基化反应的裁剪酶的实例包括来自罂粟、黄唐松草、日本黄连、大红罂粟或另外物种的去甲乌药碱6-O-甲基转移酶(6OMT);来自罂粟、黄唐松草、日本黄连、黄连或另外物种的3'羟基-N-甲基乌药碱4'-O-甲基转移酶(4'OMT);来自罂粟、花菱草或另外物种的牛心果碱7-O-甲基转移酶(7OMT);以及来自罂粟、黄唐松草、日本黄连、黄连或另外物种的金黄紫堇碱9-O-甲基转移酶(9OMT)。

[0137] 另外,裁剪酶可用于催化在bisBIA或其衍生物上进行的N-甲基化反应。可用于催化N-甲基化反应的裁剪酶的实例包括来自罂粟、黄唐松草、日本黄连或另外物种的乌药碱N-甲基转移酶(CNMT);来自罂粟、花菱草、大红罂粟或另外物种的四氢原小檗碱N-甲基转移酶(TNMT)。

[0138] 此外,裁剪酶可用于催化在bisBIA或其衍生物上进行的O-脱甲基化反应。可用于催化O-脱甲基化反应的裁剪酶的实例包括来自罂粟或另外物种的蒂巴因脱甲基酶(T6ODM);以及来自罂粟或另外物种的可待因脱甲基酶(CODM)。

[0139] 裁剪酶也可用于催化在bisBIA或其衍生物上进行的还原反应。可用于催化还原反应的裁剪酶的实例包括来自罂粟、大红罂粟或另外物种的沙罗泰里啶还原酶(SalR);来自罂粟或另外物种的可待因酮还原酶(COR);以及来自花菱草或另外物种的血根碱还原酶(SanR)。在其他实例中,裁剪酶可用于催化在bisBIA或其衍生物上进行的乙酰化反应。可用于催化乙酰化反应的裁剪酶的实例包括来自罂粟或其他物种的沙罗泰里啶乙酰转移酶(SalAT)。

异源编码序列

[0140] 在一些情况中,工程化的宿主细胞携带一个或多个异源编码序列(诸如两个或更多个、三个或更多个、四个或更多个、五个或更多个),所述异源编码序列编码使工程化的宿主细胞能够产生期望的目标酶和/或目标BIA(例如,如本文所述)的活性。如本文所用,术语"异源编码序列"用于指示编码或最终编码通常不存在于宿主生物体中且可在适当条件下在宿主细胞中表达的肽或蛋白质或其等效氨基酸序列(例如,酶)的任何多核苷酸。因而,"异源编码序列"包括通常存在于宿主细胞中的编码序列的多个拷贝,以使所述细胞表达通常不存在于所述细胞中的编码序列的另外拷贝。异源编码序列可以是RNA或其任何类型(例如,mRNA)、DNA或其任何类型(例如cDNA)或RNA/DNA的杂合体。目标编码序列包括但不限于包括此类特征如编码序列、内含子、启动子区、3'-UTR和增强子区的全长转录单位。

[0141] 在实例中,工程化的宿主细胞可包括各自编码酶的多个异源编码序列,所述酶诸如表2中列出的酶。在一些实例中,由多个异源编码序列编码的多种酶可以彼此不同。在一些实例中,由多个异源编码序列编码的多种酶中的一些可彼此不同,并且由多个异源编码序列编码的多种酶中的一些可以是重复拷贝。

[0142] 在一些实例中,异源编码序列可以可操作地连接。可操作地连接的异源编码序列

可以在产生特定苄基异喹啉生物碱产物和/或差向异构酶产物的相同途径内。在一些实例中，可操作地连接的异源编码序列可以直接顺序性地沿着产生特定的苄基异喹啉生物碱产物和/或差向异构酶产物的途径。在一些实例中，在由多个异源编码序列编码的一种或多种酶之间，可操作地连接的异源编码序列可具有一种或多种天然酶。在一些实例中，在由多个异源编码序列编码的一种或多种酶之间，异源编码序列可具有一种或多种异源酶。在一些实例中，在由多个异源编码序列编码的一种或多种酶之间，异源编码序列可具有一种或多种非天然酶。

[0143] 工程化的宿主细胞还可被修饰为具有一个或多个遗传改变以适应异源编码序列。天然宿主基因组的改变包括但不限于修饰基因组以减少或消除可能干扰期望途径的特定蛋白质的表达。此类天然蛋白质的存在可将途径的中间体或最终产物中的一种迅速转化成代谢物或在期望途径中不可用的其他化合物。因此，如果天然酶的活性被降低或完全不存在，则所产生的中间体将可更容易地用于并入期望产物中。

[0144] 异源编码序列包括但不限于编码通常负责植物中的目标BIA产生的酶的序列(野生型序列或等效序列)。在一些情况下，异源序列所编码的酶可以是1-BIA途径中的任何酶，并且可来自任何适宜的来源。用于特定合成途径的由异源编码序列编码的酶的选择和数目可基于期望产物进行选择。在某些实施方案中，本发明的宿主细胞可包括1个或多个、2个或更多个、3个或更多个、4个或更多个、5个或更多个、6个或更多个、7个或更多个、8个或更多个、9个或更多个、10个或更多个、11个或更多个、12个或更多个、13个或更多个、14个或更多个或甚至15个或更多个异源编码序列，诸如1、2、3、4、5、6、7、8、9、10、11、12、13、14或15个异源编码序列。

[0145] 如本文所用，术语“异源编码序列”还包括肽或酶的编码部分(即，肽或酶的cDNA或mRNA序列)以及全长转录单位的编码部分(即，包括内含子和外含子的基因)，以及编码酶或编码其等效氨基酸序列的“密码子优化的”序列、截短序列或改变的序列的其他形式，条件是等效氨基酸序列产生功能蛋白质。此类等效氨基酸序列可具有一个或多个氨基酸的缺失，其中缺失是N末端、C末端或内部。设想了截短形式，只要它们具有本文所指示的催化能力即可。还设想了两种或更多种酶的融合物以有助于途径中的代谢物的转移，条件是催化活性得以维持。

[0146] 可操作的片段、突变体或截短形式可通过建模和/或筛选进行鉴定。在一些情况下，通过以逐步方式缺失例如蛋白质的N末端、C末端或内部区域、接着相较于原始序列关于所得衍生物针对期望反应的活性对所得衍生物进行分析来实现这一点。如果讨论中的衍生物在这种能力方面操作，则认为正确构成酶的等效衍生物。

[0147] 在实例中，一些异源蛋白当在重组宿主中表达时可出现它们被不正确地加工的情况。例如，植物蛋白，诸如在微生物产生宿主中表达的细胞色素P450酶，可出现不正确加工。具体地讲，沙罗泰里啶合酶当在酵母中异源表达时可发生N-连接的糖基化。这种N-连接的糖基化在植物中可能观察不到，这可指示初生SalSyn转录物的不正确的N末端分选，以便降低在异源微生物宿主中的酶活性。在此类实例中，针对校正初生转录物的N末端分选以便去除N连接的糖基化模式的蛋白质工程化可导致沙罗泰里啶合酶在重组产生宿主中的活性得到改善。这在下文的实施例10中进一步解释。

[0148] 本发明的方面还涉及编码与各种酶的天然氨基酸序列等效的氨基酸序列的异源

编码序列。为“等效”的氨基酸序列被定义为与特定氨基酸序列不相同、而是包含至少一些氨基酸变化(缺失、取代、翻转、插入等)的氨基酸序列,当用于期望目的时,与特定氨基酸序列的类似活性相比,所述变化不会实质影响蛋白质的生物活性。在差向易构酶的实例中,生物活性是指其催化活性。等效序列还意在包括已被工程化和/或进化为具有与原始氨基酸序列不同的特性的那些。目标易突变特性包括催化活性、底物特异性、选择性、稳定性、溶解性、定位等。

[0149] 在一些情况下,每种类型的酶的表达通过另外的基因拷贝(即,多个拷贝)增加,其增加中间体积聚和/或目标BIA产生。本发明的实施方案包括通过同时表达单一或多种酶的多个物种变体,宿主细胞中的目标BIA产生增加。在一些情况下,单一或多种酶的另外基因拷贝包括于宿主细胞中。可利用任何适宜的方法,包括宿主细胞中的酶的异源编码序列的多个拷贝。

[0150] 在一些实例中,工程化的宿主细胞包括酶的异源编码序列的多个拷贝,诸如2个或更多个、3个或更多个、4个或更多个、5个或更多个,或甚至10个或更多个拷贝。这一点的实例描述于图28中。具体地讲,图28示出在工程化的酵母菌株中,通过将NCS基因的拷贝数从两个拷贝增加至三个拷贝来实现苄基异喹啉生物碱牛心果碱的产生增加。

[0151] 在某些实施方案中,工程化的宿主细胞包括一种或多种酶的异源编码序列的多个拷贝,诸如两种或更多种、三种或更多种、四种或更多种酶等的多个拷贝。在一些情况下,酶的异源编码序列的多个拷贝源自两种或更多种相较于宿主细胞不同的来源生物体。例如,工程化的宿主细胞可包括一个异源编码序列的多个拷贝,其中所述拷贝中的每一个源自不同的来源生物体。因此,每个拷贝可基于由异源编码序列编码的目标酶的种间差异包括显式序列中的一些变异。

[0152] 可对工程化的宿主细胞培养基取样并且监测目标BIA的产生。可使用任何适宜的方法观察和测量目标BIA。目标方法包括但不限于LC-MS方法(例如,如本文所述),其中通过与已知量的标准化合物比较来分析目标样品。另外,存在可观察和/或测量目标BIA的其他方式。观察和/或测量BIA的另选方式的实例包括GC-MS、紫外-可见光谱法、NMR、LC-NMR、LC-UV、TLC、毛细管电泳,以及其他方法。身份可例如通过m/z和MS/MS片段化模式确认,并且定量或测量化合物可通过已知保留时间的LC迹线峰和/或参考已知量的化合物标准品的对应LC-MS分析进行的EIC MS峰分析来实现。

[0153] 另外,可对工程化宿主细胞的培养物取样并且监测目标酶诸如CYP-COR酶的产生。可使用任何适宜的方法观察和测量目标酶。目标方法包括酶活性测定、聚丙烯酰胺凝胶电泳、一氧化碳光谱法以及蛋白质印迹分析。

方法

工艺步骤

[0154] 如上文所总结,本发明的方面包括制备目标苄基异喹啉生物碱(BIA)的方法。另外,本发明的方面包括制备目标酶的方法。因而,本发明的方面包括在功能性地表达一种或多种宿主细胞修饰(例如,如本文所述)的条件下培养工程化的宿主细胞,以使所述细胞将目标起始化合物转化成目标产物酶和/或BIA。还提供以下方法,所述方法包括在适于蛋白质产生的条件下培养工程化的宿主细胞以使得功能性地表达一个或多个异源编码序列并且将目标起始化合物转化成目标产物酶或BIA。在实例中,所述方法是一种制备苄基异喹啉

生物碱(BIA)的方法,其包括培养工程化的宿主细胞(例如,如本文所述);将起始化合物添加至细胞培养物;以及从细胞培养物中回收BIA。在一些实例中,所述方法是一种制备酶的方法,其包括培养工程化的宿主细胞(例如,如本文所述);将起始化合物添加至细胞培养物;以及从细胞培养物中回收酶。

[0155] 发酵培养基可包含合适的碳底物。适于进行本公开的方法的碳源可涵盖广泛多种含碳底物。合适的底物可包括但不限于单糖(例如,葡萄糖、果糖、半乳糖、木糖)、低聚糖(例如,乳糖、蔗糖、棉子糖)、多糖(例如,淀粉、纤维素),或其组合。在一些情况下,可使用来自可再生原料的未纯化混合物(例如,玉米浆液、糖用甜菜糖蜜、大麦麦芽)。在一些情况下,碳底物可以是一碳底物(例如,甲醇、二氧化碳)或二碳底物(例如,乙醇)。在其他情况下,可利用其他含碳化合物,例如,甲胺、葡糖胺和氨基酸。

[0156] 培养工程化的宿主细胞的任何适宜的方法可用于产生目标酶和/或BIA。所采用的具体方案可例如根据工程化的宿主细胞、异源编码序列、目标酶、目标BIA等而变化。细胞可存在于任何适宜的环境中,诸如其中细胞能够表达一种或多种功能性异源酶的环境。在一些实施方案中,将细胞在有利于酶表达的条件下并且用可用于允许体内产生目标酶和/或BIA的适当底物进行培养。在一些实施方案中,功能性酶从工程化宿主中提取,以用于在体外条件下产生目标酶和/或BIA。在一些情况下,工程化的宿主细胞被放回到多细胞宿主生物体中。工程化的宿主细胞处于任何生长期,包括但不限于稳定期和对数生长期等。此外,培养物本身可以是连续培养物或它们可以是分批培养物。

[0157] 细胞可在介于14-40℃之间的温度下的适当的发酵培养基中生长。细胞可在任何适宜速度(例如200rpm)的振荡下生长。细胞可在合适的pH下生长。适用于发酵的pH范围可介于pH 5-9之间。发酵可在有氧、缺氧或微氧条件下进行。可使用任何合适的生长培养基。合适的生长培养基可包括但不限于常见的商业制备的培养基,诸如合成限定的(SD)基本培养基或富含酵母提取物蛋白胨右旋糖(YEPD)的培养基。可使用适于微生物的任何其他丰富、限定或合成的生长培养基。

[0158] 可在基本上任何尺寸和形状的容器中培养细胞。适用于进行本公开的方法的容器的实例可包括但不限于多孔摇板、试管、烧瓶(带挡板和不带挡板的)以及生物反应器。培养物的体积可在10微升至大于10,000升的范围内。

[0159] 可包括向生长培养基中加入已知以生物碱的产生所期望的方式调节代谢的试剂。在非限制性实例中,可向生长培养基中加入环腺苷2’3’-单磷酸以调节分解代谢物阻抑。

[0160] 可利用针对特定细胞类型的任何适宜的细胞培养条件。在某些实施方案中,使用标准细胞培养基和补充剂,将包括一个或多个修饰的宿主细胞在标准或容易优化的条件下培养。作为一个实例,当不需要用于质粒维持的选择性压力时,标准生长培养基可包含20g/L酵母提取物、10g/L蛋白胨和20g/L右旋糖(YPD)。使含有质粒的宿主细胞在含有1.7g/L酵母氮源、5g/L硫酸铵和20g/L右旋糖、补充有生长和选择所需的适当氨基酸的合成完全(SC)培养基中生长。可用于诱导酶表达的替代碳源包括但不限于蔗糖、棉子糖和半乳糖。在实验室中将细胞在任何适宜的温度(例如,30℃)下在任何适宜速率(例如,200rpm)的振荡下生长于容器中,例如在1-1000mL或更大范围体积的试管或烧瓶中。

[0161] 培养体积可按比例放大以用于在例如作为工业过程的一部分的较大发酵容器中生长。工业发酵工艺可在封闭补料、分批补料或连续恒化器条件下或任何合适的发酵模式

下进行。在一些情况下，可以将细胞作为完整的细胞催化剂固定在底物上并使其经受发酵条件以产生生物碱。

[0162] 分批发酵是封闭系统，其中培养基的组成在发酵开始时设定并且在发酵过程中不改变。在发酵开始时将一种或多种期望的生物体接种到培养基中。在一些情况下，分批发酵在对系统做出改变以控制诸如pH和氧浓度(而不是碳)的因素的情况下进行。在这种类型的发酵系统中，系统的生物质和代谢物组成在整个发酵过程中持续变化。细胞通常经历了迟滞期，接着到对数期(高生长速率)，接着到稳定期(生长速率降低或停止)，并且最终到死亡期(如果保持未处理的话)。

[0163] 连续发酵是一种开放式系统，其中将设定好的发酵培养基连续加入生物反应器中，并从容器中连续移除等量的发酵培养基用于加工。连续发酵系统通常操作以维持稳态生长条件，由此使得由于移除培养基造成的细胞损失必须通过发酵中的生长速率进行平衡。连续发酵通常在细胞处于恒定高细胞密度的条件下操作。连续发酵允许调节影响目标产物浓度和/或细胞生长的一个或多个因素。

[0164] 液体培养基可包括但不限于具有上述添加剂组分的丰富或合成限定培养基。培养基组分可溶解于水中并且通过加热、压力、过滤、辐射、化学物质或其任何组合进行灭菌。若干培养基组分可单独制备并灭菌，然后在发酵容器中配混。培养基可以被缓冲，以便有助于在整个发酵过程中保持恒定的pH。

[0165] 可在整个发酵过程中监测或控制工艺参数，包括温度、溶解氧、pH、搅拌、通气速率和细胞密度。例如，可通过浸入培养基中的温度探针监测发酵过程的温度。可通过调控夹套温度将培养温度控制在设定点。可将水在外部冷却器中冷却，接着使其流动到生物反应器控制塔中并且循环至容器中的处于保持设定点温度所需温度的夹套。

[0166] 另外，可监测发酵过程中的气体流参数。例如，气体可通过喷射器流动到培养基中。适用于本公开的方法的气体可包括压缩空气、氧气和氮气。气体流可处于固定的速率或被调控以保持溶解氧设定点。

[0167] 还可监测培养基的pH。在实例中，可通过浸入容器内部的培养基中的pH探针监测pH。如果pH控制起作用，那么可通过酸碱泵调整pH，所述酸碱泵以所需速率向培养基中加入每种溶液。用于控制pH的酸溶液可以是硫酸或盐酸。用于控制pH的碱溶液可以是氢氧化钠、氢氧化钾或氢氧化铵。

[0168] 此外，可通过浸入培养基中的溶解氧探针监测培养基中的溶解氧。如果溶解氧调控起作用，那么可通过增大或减小搅拌速度来调整氧水平。也可通过增大或减小气体流动速率来调整溶解氧水平。气体可以是压缩空气、氧气或氮气。

[0169] 也可监测发酵过程中的搅拌速度。在实例中，搅拌机马达可驱动搅动器。搅拌机速度可在整个发酵过程中设定为恒定rpm或者可动态调控以保持设定的溶解氧水平。

[0170] 另外，可监测发酵过程中的浊度。在实例中，可使用浊度探针测量细胞密度。另选地，可通过从生物反应器中取样并且在分光光度计中对它们进行分析来测量细胞密度。此外，可通过无菌取样装置以时间间隔从生物反应器中移除样品。可针对宿主细胞产生的生物碱对样品进行分析。也可针对其他代谢物和糖、培养基组分的耗尽或细胞的密度对样品进行分析。

[0171] 在另一个实例中，可监测发酵过程期间的原料参数。具体地讲，可使用外部泵向发

酵中加入原料，所述原料包括糖和其他碳源、营养素和辅因子。也可在发酵过程中加入其他组分，包括但不限于消泡剂、盐、螯合剂、表面活性剂和有机液体。

[0172] 用于优化宿主细胞中的异源多核苷酸的表达的任何适宜的密码子优化技术可适用于在主题宿主细胞和方法中使用，参见例如，Gustafsson,C.等人(2004)Trends Biotechnol,22,346-353，其以引用的方式全文并入。

[0173] 主题方法还可包括将起始化合物添加至细胞培养物。任何适宜的添加方法可适于在主题方法中使用。细胞培养物可补充有足够量的目标起始材料(例如，如本文所述)，例如mM至μM量，诸如约1-5mM之间的起始化合物。应当理解，所添加的起始材料的量、添加定时和速率、添加的材料的形式等可根据多种因素而变化。可纯净地添加起始材料或可将起始材料预先溶解于合适的溶剂(例如，细胞培养基、水或有机溶剂)中。起始材料可以浓缩形式(例如，超过期望浓度10x)添加以使在添加时细胞培养基的稀释最小化。起始材料可以一个或多个批次添加，或通过在延长的时间段(例如，数小时或数天)内连续添加而添加。

用于从发酵培养基中分离产物的方法

[0174] 主题方法还可包括从细胞培养物中回收目标酶和/或BIA。任何适宜的分开和分离方法(例如，色谱方法或沉淀方法)可适于在主题方法中使用以从细胞培养物中回收目标酶和/或BIA。过滤方法可用于分开细胞培养物的可溶性部分与不溶性部分。在一些情况下，液相色谱方法(例如，反相HPLC、尺寸排阻、正相色谱法)可用于使目标BIA与细胞培养物的其他可溶性组分分开。在一些情况下，萃取方法(例如，液体萃取、基于pH的纯化、固相萃取、亲和色谱法、离子交换等)可用于使目标酶和/或BIA与细胞培养物的其他组分分开。

[0175] 可使用本领域中已知的方法将所产生的生物碱从发酵培养基中分离。可在发酵之后立即(或在一些情况下，在发酵期间)进行多个回收步骤，以用于初始回收期望产物。通过这些步骤，可将生物碱(例如，BIA)与细胞、细胞碎片和废物分开，并且其他营养素、糖和有机分子可保留在废培养基中。可使用这一过程产生富含BIA的产物。

[0176] 在一个实例中，通过向间歇式反应器中提供工程化的酵母细胞以及包括营养素和水的原料来形成具有苄基异喹啉生物碱(BIA)产物的产物流。具体地讲，可通过将工程化的酵母细胞孵育至少约5分钟的时间段来使工程化的酵母细胞进行发酵，以产生包含BIA产物和细胞材料的溶液。一旦工程化的酵母细胞已经进行发酵，可使用至少一个分离装置将BIA产物与细胞材料分离以提供包含BIA产物的产物流。具体地讲，产物流可包括BIA产物，以及另外的组分，诸如澄清的酵母培养基。另外，BIA产物可包括一种或多种目标BIA，诸如一种或多种BIA组分。

[0177] 可使用不同的方法从生物反应器培养基中移除包括目标酶和/或BIA的细胞。在实例中，通过随时间推移的沉降移除细胞。这种沉降过程可通过冷却或通过添加澄清剂诸如二氧化硅来加速。然后可从反应器顶部虹吸废培养基或者可从反应器基部滗析细胞。另选地，可通过用过滤器、膜或其他多孔材料过滤来移除细胞。也可通过离心，例如通过连续流动离心或通过使用连续萃取器来移除细胞。

[0178] 如果一些有价值的目标酶和/或BIA存在于细胞内部，那么可将细胞透化或裂解，并且可通过上述方法中的任一种移除细胞碎片。用于透化细胞的试剂可包括但不限于有机溶剂(例如DMSO)或盐(例如乙酸锂)。用以裂解细胞的方法可包括添加表面活性剂诸如十二烷基硫酸钠，或通过珠磨或超声处理进行机械破坏。

[0179] 可通过添加与水性培养基不混溶的有机液体，通过液-液萃取从澄清的废培养基中萃取目标酶和/或BIA。在实例中，除了其他处理步骤之外，可使用液-液萃取的使用。合适的有机液体的实例包括但不限于肉豆蔻酸异丙酯、乙酸乙酯、氯仿、乙酸丁酯、甲基异丁基酮、油酸甲酯、甲苯、油醇、丁酸乙酯。可以水性培养基的体积的少至10％或多至100％添加有机液体。

[0180] 在一些情况下，可在发酵开始时或在发酵期间的任何时间添加有机液体。这一萃取发酵过程可通过将酶和/或BIA连续地移除至有机相而增加来自宿主细胞的目标酶和/或BIA的收率。

[0181] 搅拌可使有机相与水性培养基形成乳液。促进两个相分成不同层的方法可包括但不限于破乳剂或成核剂的添加或pH的调整。还可例如通过连续锥形板离心将乳液离心以分开两个相。

[0182] 另选地，可将有机相与水性培养基分离，以使在萃取后可以物理方式移除所述有机相。例如，可将溶剂包封在膜中。

[0183] 在实例中，可使用吸附方法从发酵培养基中萃取目标酶和/或BIA。在实例中，可通过添加树脂诸如**Amberlite®**XAD4或通过吸附移除BIA的另一种试剂而从澄清的废培养基中萃取目标BIA。然后可使用有机溶剂从树脂中释放目标BIA。合适的有机溶剂的实例包括但不限于甲醇、乙醇、乙酸乙酯或丙酮。

[0184] 也可使用过滤从发酵培养基中萃取目标BIA。在高pH下，目标BIA可在生物反应器中形成结晶状沉淀。可通过用过滤器、膜或其他多孔材料过滤直接移除这种沉淀。还可通过离心和/或滗析收集沉淀。

[0185] 上述萃取方法可原位(在生物反应器中)或非原位(例如，在外部回路中，培养基通过所述外部回路流出生物反应器并接触萃取剂，接着再循环回到容器中)进行。另选地，萃取方法可在发酵结束之后，使用从生物反应器容器中移除的澄清培养基进行。

用于纯化来自富含生物碱的溶液的产物的方法

[0186] 后续纯化步骤可涉及使用本领域中已知的方法处理富含一种或多种目标BIA产物的发酵后溶液，以回收高纯度的各目标产物物质。

[0187] 在一个实例中，可将萃取于有机相中的目标BIA转移到水溶液中。在一些情况下，可通过加热和/或真空蒸发有机溶剂，并且可将所得粉末溶解于合适pH的水溶液中。在另一个实例中，可通过添加有利于将目标BIA萃取到水相中的合适pH的水溶液来从有机相中萃取目标BIA。然后可通过滗析、离心或另外的方法移除水相。

[0188] 可对含BIA的溶液进一步处理以移除金属，例如，通过用合适的螯合剂处理。含目标BIA的溶液可通过沉淀进一步处理，以移除其他杂质，诸如蛋白质和DNA。在一个实例中，用适当的沉淀剂诸如乙醇、甲醇、丙酮或异丙醇处理含目标BIA的溶液。在另选的实例中，可通过渗析或通过将较小的生物碱与污染的生物大分子分开的其他尺寸排阻方法移除DNA和蛋白质。

[0189] 在另外的实例中，可使用本领域已知的方法通过连续交叉流过滤将包含目标BIA的溶液萃取至高纯度。

[0190] 如果溶液包含目标BIA的混合物，那么可使用本领域已知的方法使其受到酸-碱处理，以得到各目标BIA物质。在此过程中，调整水溶液的pH以沉淀各BIA。

[0191] 对于高纯度的小规模制备,可通过液相色谱法在单一步骤中纯化BIA。

酵母来源的生物碱API相对于植物来源的API

[0192] 澄清的酵母培养基(CYCM)可包含多种杂质。澄清的酵母培养基可通过真空和/或加热脱水,以得到富含生物碱的粉末。此产物类似于由罂粟生长国出口且购自API制造商的罂粟杆的浓缩物(CPS)或鸦片。出于本发明的目的,CPS是任何类型的纯化的植物萃取物的代表性实例,可由其最终进一步纯化一种或多种期望的生物碱产物。表4和表5强调了这两种产物中的杂质,它们对于CYCM或CPS是特异性的或可存在于两者中。虽然一些BIA可能具有作为杂质的颜料,但是其他BIA本身可被归类为颜料。因此,可基于非颜料杂质,针对杂质对这些BIA进行评估。通过针对这些杂质的子组对未知来源的产物进行分析,本领域技术人员可确定产物来源于酵母还是植物产生宿主。

[0193] API级药物成分是高度纯化的分子。因而,可指示API的植物或酵母来源的杂质(诸如表4和表5中列出的那些)可能不存在于API级的产物中。实际上,源自本发明的酵母菌株的许多API产物可能非常难以与传统的植物来源的API区分开。然而,在一些情况下,可使用化学合成方法使常规的生物碱化合物经受化学修饰,其可在需要此类化学修饰的基于植物的产物中表现为化学杂质。例如,化学衍生化通常可导致与化学合成工艺相关的一组杂质。在某些情况下,这些修饰可在酵母产生平台中以生物学方式进行,从而避免与化学衍生相关联的一些杂质存在于酵母来源的产物中。具体地讲,来自化学衍生产物的这些杂质可存在于使用化学合成工艺产生的API产物中,但是在使用酵母来源的产物产生的API产物中可能不存在。另选地,如果将酵母来源的产物与化学衍生的产物混合,那么可能存在所得杂质,但是其量少于在仅或主要包含化学衍生产物的API中将预期的量。在此实例中,通过针对这些杂质的子组对API产物进行分析,本领域技术人员可确定产物来源于酵母产生宿主还是传统的化学衍生化路径。

[0194] 可存在于化学衍生化的吗啡喃API中,但不存在于生物合成的API中的杂质的非限制性实例包括API可待因中的可待因-O(6)-甲基醚杂质;API羟考酮中的8,14-二羟基-7,8-二氢可待因酮;以及API氢可酮中的四氢蒂巴因。可待因-O(6)-甲基醚可通过吗啡的化学过度甲基化形成。API羟考酮中的8,14-二羟基-7,8-二氢可待因酮可通过蒂巴因的化学过度氧化形成。另外,API氢可酮中的四氢蒂巴因可通过蒂巴因的化学过度还原形成。

[0195] 然而,在其中酵母来源的化合物和植物来源的化合物均通过化学合成方法受到化学修饰的情况下,在产物中可预期与化学合成过程相关联的相同杂质。在这一情况下,可如上所述对起始材料(例如,CYCM或CPS)进行分析。

工程化宿主细胞的方法

[0196] 还包括出于产生目标酶和/或BIA的目的工程化宿主细胞的方法。将DNA插入宿主细胞中可使用任何适宜的方法来实现。所述方法用于将异源编码序列插入工程化的宿主细胞中,以使得宿主细胞功能性地表达酶并且将目标起始化合物转化成目标产物酶和/或BIA。

[0197] 任何适宜的启动子可用于主题工程化的宿主细胞和方法中。驱动异源编码序列的表达的启动子可以是组成型启动子或诱导型启动子,条件是所述启动子在工程化的宿主细胞中是活性的。异源编码序列可由其天然启动子表达,或可使用非天然启动子。这类启动子可在使用它们的宿主中为低至高强度。启动子可以是调控的或组成型的。在某些实施方案

中，使用不受葡萄糖阻抑的、或者在培养基中存在葡萄糖时仅受轻微阻抑的启动子。目标启动子包括但不限于，糖酵解基因的启动子，诸如枯草芽孢杆菌tsr基因的启动子(编码果糖二磷酸醛缩酶基因的启动子区)或来自酵母酿酒酵母基因的编码甘油醛3-磷酸脱氢酶的启动子(GPD、GAPDH或TDH3)；面包酵母的ADH1启动子；磷酸饥饿诱导的启动子，诸如酵母的PHO5启动子；来自地衣芽孢杆菌的碱性磷酸酶启动子；酵母诱导型启动子，诸如Gal1-10、Gal1、GalL、GalS；可阻抑启动子Met25、tetO；以及组成型启动子，诸如甘油醛3-磷酸脱氢酶启动子(GPD)、醇脱氢酶启动子(ADH)、翻译延长因子-1-α启动子(TEF)、细胞色素c-氧化酶启动子(CYC1)、MRP7启动子等。含有可受激素(诸如糖皮质激素、类固醇和甲状腺激素)诱导的启动子的自主复制酵母表达载体也可使用并且包括但不限于，糖皮质激素反应性元件(GRE)和甲状腺激素反应性元件(TRE)。这些和其他实例描述于美国专利7,045,290中，其以引用的方式并入，包括其中引用的参考文献。可使用包含组成型或诱导型启动子诸如α因子、醇氧化酶和PGH的另外的载体。此外，任何启动子/增强子组合(根据真核启动子数据库EPDB)也可用于驱动基因的表达。任何适宜的适当启动子可被选择用于宿主细胞，例如大肠杆菌。还可使用启动子选择以优化转录，并且因此，优化酶水平以使产生最大化，同时使能源最小化。

[0198] 任何适宜的载体可用于主题工程化的宿主细胞和方法中。目标载体包括用于在酵母和其他细胞中使用的载体。酵母载体的类型可分成4种一般类别：整合型载体(YIp)、自主复制高拷贝数目载体(YEp或2μ质粒)、自主复制低拷贝数目载体(YCp或着丝粒质粒)以及用于克隆大片段的载体(YAC)。经由任何适宜转化或转染技术将载体DNA引入原核或真核细胞中。另一来源的DNA(例如，PCR生成的双链DNA产物，或合成的双链或单链寡核苷酸)可通过整合到基因组中来用于工程化酵母。任何单转化事件可包括一种或若干核酸(载体、双链或单链DNA片段)以对宿主细胞进行基因修饰。

效用

[0199] 本发明的工程化的宿主细胞和方法(例如，如上文所述)适用于多种应用。目标应用包括但不限于：研究应用和治疗应用。本发明的方法适用于多种不同的应用中，包括其中酶和/或BIA的产生令人感兴趣的任何适宜的应用。

[0200] 主题工程化的宿主细胞和方法适用于多种治疗应用。目标治疗应用包括其中制备包含BIA的药物产品令人感兴趣的那些应用。本文所述的工程化的宿主细胞产生目标BIA和目标酶。牛心果碱是包括工程化努力以产生最终产物诸如阿片类产物的BIA合成中的目标主要分支点中间体。主题宿主细胞可用于由简单且廉价的起始材料产生目标BIA，所述材料可适用于产生包括牛心果碱的目标BIA和BIA最终产物。因此，主题宿主细胞适用于供应治疗活性目标BIA。

[0201] 在一些情况下，工程化的宿主细胞和方法适用于产生商业规模量的其BIA，其中这些化合物的化学合成是低产量的并且不是用于大规模生产的可行手段。在某些情况下，宿主细胞和方法用于发酵设施中，所述发酵设施将包括例如5,000-200,000升容量的生物反应器(发酵罐)，从而允许快速产生用于治疗性产物的其目标BIA。这类应用可包括由可发酵碳源诸如纤维素、淀粉和游离糖工业规模产生目标BIA。

[0202] 主题工程化的宿主细胞和方法适用于多种研究应用。主题宿主细胞和方法可用于分析多种酶对多种目标酶和/或BIA的生物合成途径的作用。此外，工程化的宿主细胞可被

工程化以产生目标酶和/或BIA，所述酶和/或BIA适用于测试在尚未证实的治疗功能方面的目标生物活性。在一些情况下，工程化宿主细胞以包括编码多种酶的多种异源编码序列阐明针对目标酶和/或BIA的高产量生物合成途径。在某些情况下，研究应用包括产生用于目标治疗分子的目标酶和/或BIA，所述目标酶和/或BIA随后可进一步化学修饰或衍生化为期望产物或针对增加的目标治疗活性进行筛选。在一些情况下，宿主细胞菌株用于筛选在这类途径中感兴趣的酶活性，其可导致经由在这些菌株中产生的BIA代谢物的转化发现酶。

[0203] 主题工程化的宿主细胞和方法可用作植物特化代谢物的产生平台。主题宿主细胞和方法可用作药物库开发以及植物酶发现的平台。例如，主题工程化的宿主细胞和方法可适用于通过采用酵母菌株产生令人感兴趣的支架分子(诸如原阿片碱)并且通过组合生物合成或通过化学手段进一步功能化化合物结构来开发基于天然产物的药物库。通过以这种方式产生药物库，任何潜在药物命中已经与易于大规模培养和产生的产生宿主相关。作为另一个实例，这些主题工程化的宿主细胞和方法可适用于植物酶发现。主题宿主细胞提供限定代谢物的干净背景以表达植物EST库来鉴定新的酶活性。主题宿主细胞和方法提供用于酵母中的植物酶的功能表达和活性增加的表达方法和培养条件。

试剂盒和系统

[0204] 本发明的方面还包括试剂盒和系统，其中所述试剂盒和系统可包括在本发明的方法中采用的一种或多种组分，例如，工程化的宿主细胞、起始化合物、异源编码序列、载体、培养基等，如本文所述。在一些实施方案中，主题试剂盒包括工程化的宿主细胞(例如，如本文所述)，以及一种或多种选自以下的组分：起始化合物、异源编码序列和/或包括所述异源编码序列的载体、载体、生长原料、适合用于表达系统的组分(例如，细胞、克隆载体、多克隆位点(MCS)、双向启动子、内部核糖体进入位点(IRES)等)以及培养基。

[0205] 本文所述的任何组分可提供于试剂盒中，例如，包括一个或多个修饰的宿主细胞、起始化合物、培养基等。适合用于制备和使用异源编码序列的多种组分、克隆载体和表达系统可适用于主题试剂盒中。试剂盒还可包括管、缓冲剂等，以及使用说明书。根据需要，试剂盒的各种试剂组分可存在于单独容器中，或所述组分中的一些或全部可预组合于单一容器中的试剂混合物中。

[0206] 还提供了用于产生目标酶和/或BIA的系统，其中所述系统可包括包含一个或多个修饰(例如，如本文所述)的工程化的宿主细胞、起始化合物、培养基、发酵罐和发酵设备，例如，适于维持宿主细胞的生长条件的装置、取样和监测设备和部件等。适合用于酵母细胞的大规模发酵的多种组分可适用于主题系统中。

[0207] 在一些情况下，所述系统包括用于工程化的宿主细胞的大规模发酵以及监测和纯化由发酵的宿主细胞产生的酶和/或BIA化合物的部件。在某些实施方案中，在发酵罐中的工程化的宿主细胞产生一种或多种期望的目标BIA产物的条件下将一种或多种起始化合物(例如，如本文所述)添加至所述系统。在一些情况下，宿主细胞产生目标BIA(例如，如本文所述)。在某些情况下，目标BIA产物是阿片类产物，诸如蒂巴因、可待因、尼奥品、吗啡、新吗啡、氢可酮、羟考酮、氢吗啡酮、二氢可待因、14-羟基可待因、二氢吗啡或羟吗啡酮。

[0208] 在一些情况下，所述系统包括用于监测和或分析通过主题宿主细胞产生的一种或多种目标酶和/或BIA化合物的工艺。例如，如本文所述的LC-MS分析系统、色谱系统或任何适宜的系统，其中样品可进行分析且与标准品比较，例如，如本文所述。可通过取样和分析

在发酵之前和期间的任何适宜的时间监测发酵培养基。当起始化合物到目标酶和/或BIA产物的转化完成时，发酵可停止并且可进行BIA产物的纯化。因此，在一些情况下，主题系统包括适用于纯化来自宿主细胞培养基的目标酶和/或BIA产物的纯化部件，所述目标酶和/或BIA产物在宿主细胞培养基中产生。纯化部件可包括可用于纯化发酵产生的目标酶和/或BIA产物的任何适宜的手段，包括但不限于，二氧化硅色谱法、反相色谱法、离子交换色谱法、HIC色谱法、尺寸排阻色谱法、液体萃取和pH萃取方法。在一些情况下，主题系统提供在将一种或多种起始化合物输入至所述系统之后目标酶和/或BIA发酵产物的产生和分离。

[0209] 给出以下实施例以便为本领域的普通技术人员提供如何制备和使用本发明的完整公开和描述，并且既不旨在限制发明人所认为的他们的发明的范围，也不旨在表示以下实验是所进行的全部或仅有的实验。尽管已经努力确保所用数字(例如，量、温度等)的精确性，但是应考虑到一些实验误差和偏差。除非另外指明，否则份为重量份，分子量为重均分子量，温度为摄氏度，并且压力为大气压或接近大气压。

酶列表的讨论

[0210] 宿主细胞可被工程化为包括提供目标BIA和/或目标酶的产生的一个或多个修饰(诸如两个或更多个、三个或更多个、四个或更多个、五个或更多个、或甚至更多个修饰)。表2提供了可受到一个或多个修饰的作用以便在工程化的宿主细胞中提供目标BIA和/或目标酶的产生的示例性基因的列表。

[0211] 如表2中提供的基因的修饰可用于由工程化的宿主细胞产生目标BIA，所述工程化的宿主细胞被供应有包含生长所需的基本营养素的培养基。这种基本培养基可包含碳源、氮源、氨基酸、维生素和盐。例如，如表2中提供的基因的修饰可用于由进料糖的工程化的宿主细胞产生目标BIA。另外，如表2中提供的一个或多个基因的修饰可用于加强可被工程化用于药物产生的宿主细胞的生物合成过程。

[0212] 另外，使用这些修饰在工程化的宿主细胞中提供目标BIA和/或目标酶的产生仅由可由所述基因产生的酶的鉴定不容易明白。具体地讲，在宿主细胞(诸如如本文所述的酵母细胞)中已经重构的合成途径包括在自然界中不在单个生物体内一起起作用的多种酶。另外，本文讨论的一些酶在它们的天然背景中不作用于BIA生物合成。此外，本文所述的一些酶不被进化成在特定的宿主细胞诸如酵母细胞中行使功能，并且不被进化成一起行使功能。在这些情况下，将不太清楚酶是否将在宿主细胞诸如酵母中的合成BIA途径的背景中表现出足够的活性，以具有通过所述途径的足够通量以产生下游BIA最终产物。

[0213] 例如，植物酶通常难以在异源微生物宿主诸如酵母中功能性地表达。在许多情况下，酶可能错误折叠、不正确定位在宿主细胞内和/或不正确加工。酵母与植物之间的蛋白质翻译和加工差异可能导致这些酶在酵母宿主中表现出基本上降低至不可检测的活性。通常针对内膜定位的酶诸如细胞色素P450(其强烈表示于BIA途径中)产生这些质疑。甚至降低的酶活性也可对工程化酵母产生复杂的BIA提出基本的挑战，因为其在每个步骤均需要足够的活性以确保期望BIA产物的高水平积聚。

[0214] 另外，在一些宿主细胞诸如酵母中存在内源酶/途径，它们可作用于BIA途径中的许多早期前体(即，从酪氨酸至去甲乌药碱的中间体)上，因此可能不容易明白由于这些竞争性内源途径，是否将存在通过异源途径的足够通量，以实现大量的BIA产生。例如，酵母中的Erlich途径(Hazelwood等人2008.Appl.Environ.Microbiol.74:2259-66;Larroy等人

2003.Chem.Biol.Interact.143-144:229-38;Larroy等人2002.Eur.J.Biochem.269:5738-45)是作用于将早期BIA途径中的许多中间体转化成非期望产物并且使来自合成途径的通量转向的主内源途径。

[0215] 此外,如本文所讨论和如表2中所提供的许多酶在天然植物宿主中可在非常特定的调控策略下行使功能,包括部分调控,其在转移至异源酵母宿主中时可能丢失。此外,与酵母细胞相比,植物提供了非常难的生化环境,酶在所述生化环境下进化以行使功能,所述环境包括pH、氧化还原态,以及底物、共底物、辅酶和辅因子可利用性。鉴于天然宿主与异源酵母宿主之间的生化环境和调控策略差异,不太清楚酶在处于酵母环境的背景中时是否将表现出显著活性,并且还不清楚它们是否将一起作用以将简单的前体诸如糖引导成复杂的BIA化合物。在酵母宿主中保持酶的活性是尤其重要的,因为许多途径具有许多反应步骤(>10),由此使得如果这些步骤无效,那么将不可预期期望下游产物的积聚。

[0216] 此外,在天然植物宿主中,这些途径中的相关代谢物可遍布不同的细胞和组织类型定位。在若干实例中,存在可专用于生物合成的细胞类型和可被合成用于代谢物积聚的细胞类型。当在异源酵母宿主内表达所述途径时,可能失去这种类型的细胞特化,并且这种类型的细胞特化在控制这些代谢物对细胞的毒性方面可能起到重要作用。因此,不太清楚酵母是否可成功地被工程化以生物合成和积聚这些代谢物,而不受到这些化合物的毒性的伤害。

[0217] 例如,在天然植物宿主中,酶BBE被报道为具有动态亚细胞定位。具体地讲,酶BBE初始起始于ER中,然后被分选至液泡(Bird和Facchini.2001.Planta.213:888-97)。已经提出,植物中BBE的ER相关(Alcantara等人2005.Plant Physiol.138:173-83)提供对于BBE活性最佳的碱性pH(pH～8.8)(Ziegler和Facchini.2008.Annu.Rev.Plant Biol.59:735-69)。又如,有证据显示,血根碱生物合成发生在植物细胞内的特化囊泡中(Amann等人1986.Planta.167:310-20),但是仅一些中间体积聚在囊泡中。这可能发生,以便将它们与其他酶活性和/或毒性作用隔离开。

[0218] 又如,吗啡喃途径分支中的生物合成酶全部定位至韧皮部,这是植物中的维管组织的一部分。在该问题中,途径酶可在两种细胞类型之间进一步划分:所有植物共有的筛管成分;以及乳汁管,它是仅存在于某些植物中的特化细胞类型,其制备特化的次生代谢物。上游酶(即,从NCS一直至SalAT)主要在筛管成分中,并且下游酶(即,T6ODM、COR、CODM)大部分处于乳汁管中(Onoyovwe等人2013.Plant Cell.25:4110-22)。另外,据发现,诺斯卡品生物合成途径中的最终步骤发生在乳汁管中(Chen和Facchini.2014.Plant J.77:173-84)。这种区域化被认为对于通过分离或运输中间体来调控生物合成、提供最佳pH、增强辅因子供应是高度重要的,但是罂粟乳汁管微环境的性质尚在研究中(Ziegler和Facchini.2008.Annu.Rev.Plant Biol.59:735-69)。此外,据推测,几种酶可充当多酶复合物或植物次生代谢常见的代谢通道(Kempe等人2009.Phytochemistry.70:579-89;Allen等人2004.Nat.Biotechnol.22:1559-66)。当将来自不同宿主的生物合成酶组合和/或将所述生物合成酶在异源酵母细胞中重组表达时,这些复合物或通道是否将像它们在天然宿主中那样形成尚不清楚。在另一个实例中,在日本黄连中,小檗碱在根组织中生物合成,然后通过特化的ATP结合盒转运蛋白的作用积聚在根茎内(Shitan等人2013.Phytochemistry.91:109-16)。在鸦片罂粟中,吗啡喃生物碱积聚在乳汁(乳汁管细胞的细胞质)内(Martin等人

1967.Biochemistry.6:2355-63)。

[0219] 此外,即使不考虑这些,还存在的问题是用于本文所述的途径中的若干步骤的植物酶尚未得到表征。例如,酪氨酸到早期苄基异喹啉生物碱支架去甲乌药碱的转化尚未得到表征。另外,如本文所述,(S)-牛心果碱到(R)-牛心果碱的转化仅仅近期才得到表征。因此,对于本文所述的途径中的若干步骤,通过使在自然界中通常不一起发生的酶活性一起用于BIA的生物合成或由基因组序列信息鉴定新酶活性以用于重构的途径来产生另选的生物合成方案。

[0220] 例如,可通过组合至少5种哺乳动物酶和1种细菌酶来实现酪氨酸到多巴胺的两步转化,所述至少5种哺乳动物酶和1种细菌酶并非天然地一起存在并且未进化成在此途径的背景中或与植物酶一起行使功能。在这些情况下,可能不太清楚是否可利用这些酶进行它们在自然界中并未进化进行的化合物的生物合成,并且它们在异源微生物宿主的背景中和此途径中是否将等效地行使功能也不太清楚。又如,负责(S)-牛心果碱到(R)-牛心果碱的转化的酶是未知的。如本文所讨论的新型酶可在酵母和在合成BIA途径的背景中进行这一差向异构化反应。由于这代表了新酶发现,因而尚不清楚是否可在这一途径的背景中使用这种酶进行那些BIA化合物的合成。

[0221] 下文讨论了作为修饰对象以便产生目标BIA和/或目标酶的基因的实例。另外,在示出用于生成目标BIA和/或目标酶的途径的一系列附图的上下文中对所述基因进行了讨论。

[0222] [TLK1]在一些实例中,工程化的宿主细胞可改变酶转酮醇酶的表达。转酮醇酶由TKL1基因编码。在实例中,转酮醇酶催化果糖-6-磷酸+甘油醛-3-磷酸↔木酮糖-5-磷酸+赤藓糖-4-磷酸的反应,参考图2。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括TKL1基因的组成型过表达。另外或另选地,工程化的宿主细胞可被修饰成合成性地调控TKL1基因在工程化的宿主细胞中的表达。在实例中,工程化的宿主细胞可被修饰成并入TKL1基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地,工程化的宿主细胞可被修饰成包括用于TKL1基因在工程化的宿主细胞内的过表达的强启动子元件的引入。TKL1基因可来源于酿酒酵母或另外的物种。在一些实例中,TKL1基因可与天然存在的基因具有100%的相似性。

[0223] [ZWF1]在一些实例中,工程化的宿主细胞可改变酶葡萄糖-6-磷酸脱氢酶的表达。葡萄糖-6-磷酸脱氢酶由ZWF1基因编码。在实例中,葡萄糖-6-磷酸脱氢酶催化葡萄糖-6-磷酸→6-磷酸葡糖酸内酯的反应,参考图2。工程化的宿主细胞可被修饰成在工程化的宿主细胞中缺失ZWF1基因的编码区。另选地,工程化的宿主细胞可被修饰成使ZWF1基因的功能性失效,诸如通过引入失活突变。

[0224] [ARO4]在一些实例中,工程化的宿主细胞可改变酶3-脱氧-D-阿拉伯糖-庚酮糖酸-7-磷酸(DAHP)合酶的表达。DAHP合酶由ARO4基因编码。在实例中,DAHP合酶催化赤藓糖-4-磷酸+磷酸烯醇式丙酮酸→DAHP的反应,参考图2。工程化的宿主细胞可修饰ARO4基因以并入一个或多个反馈抑制减轻突变。具体地讲,反馈抑制减轻突变(例如,$ARO4^{FBR}$)可作为对原始基因座处的天然ARO4基因的定向突变,作为在单独基因座处的基因整合引入的另一个拷贝,或作为附加型载体(诸如2-μm或着丝粒质粒)上的另一个拷贝并入。突变$ARO4^{FBR}$中的标识“FBR”称为反馈抗性突变体和突变。DAHP合酶的反馈抑制拷贝可在天然酵母转录调控

下,诸如当工程化的宿主细胞为酵母细胞时。另选地,可通过将DAHP合酶的反馈抑制拷贝置于合成启动子的控制下,在蛋白质表达的工程化的组成型或动态调控的情况下向工程化的宿主细胞中引入DAHP合酶的所述拷贝。在一些情况下,ARO4基因可来源于酿酒酵母。在一些情况下,ARO4基因可与天然存在的基因具有100%的相似性。ARO4基因的修饰的实例包括反馈抑制抗性突变、K229L或Q166K。

[0225] [ARO7]在一些实例中,工程化的宿主细胞可改变酶分支酸变位酶的表达。分支酸变位酶由ARO7基因编码。在实例中,分支酸变位酶催化分支酸酯→预苯酸酯的反应,参考图2。工程化的宿主细胞可修饰ARO7基因以并入一个或多个反馈抑制减轻突变。具体地讲,反馈抑制减轻突变(例如,$ARO7^{FBR}$)可作为对原始基因座处的天然ARO7基因的定向突变,作为在单独基因座处的基因整合引入的另一个拷贝,或作为附加型载体(诸如2-μm或着丝粒质粒)上的另一个拷贝并入。突变$ARO7^{FBR}$中的标识"FBR"称为反馈抗性突变体和突变。分支酸变位酶的反馈抑制拷贝可在天然酵母转录调控下,诸如当工程化的宿主细胞为酵母细胞时。另选地,可通过将分支酸变位酶的反馈抑制拷贝置于合成启动子的控制下,在蛋白质表达的工程化的组成型或动态调控的情况下向工程化的宿主细胞中引入分支酸变位酶的所述拷贝。在一些情况下,ARO7基因可来源于酿酒酵母。在一些情况下,ARO7基因可与天然存在的基因具有100%的相似性。ARO7基因的修饰的实例包括反馈抑制抗性突变或T226I。

[0226] [ARO10]在一些实例中,工程化的宿主细胞可改变酶苯丙酮酸脱羧酶的表达。苯丙酮酸脱羧酶由ARO10基因编码。在实例中,苯丙酮酸脱羧酶催化羟苯基丙酮酸酯→4-羟苯基乙酸酯(4HPA)的反应,参考图2。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括ARO10基因的组成型过表达。另外或另选地,工程化的宿主细胞可被修饰成合成性地调控ARO10基因在工程化的宿主细胞中的表达。在实例中,工程化的宿主细胞可被修饰成并入ARO10基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地,工程化的宿主细胞可被修饰成包括用于ARO10基因在工程化的宿主细胞内的过表达的强启动子元件的引入。ARO10基因可来源于酿酒酵母或另外的物种。在一些实例中,ARO10基因可与天然存在的基因具有100%的相似性。

[0227] [ADH2-7、SFA1]在一些实例中,工程化的宿主细胞可改变醇脱氢酶的表达。醇脱氢酶可由ADH2、ADH3、ADH4、ADH5、ADH6、ADH7和SFA1基因中的一个或多个编码。在实例中,醇脱氢酶催化4HPA→酪醇的反应。工程化的宿主细胞可被修饰成在工程化的宿主细胞中缺失ADH2、ADH3、ADH4、ADH5、ADH6、ADH7和SFA1基因中的一个或多个的编码区。另选地,工程化的宿主细胞可被修饰成使ADH2、ADH3、ADH4、ADH5、ADH6、ADH7和SFA1基因中的一个或多个的功能性失效,诸如通过引入失活突变。

[0228] [ALD2-6]在一些实例中,工程化的宿主细胞可改变醛氧化酶的表达。醛氧化酶可由ALD2、ALD3、ALD4、ALD5和ALD6基因中的一个或多个编码。在实例中,醛氧化酶催化4HPA→羟基苯乙酸的反应。工程化的宿主细胞可被修饰成在工程化的宿主细胞中缺失ALD2、ALD3、ALD4、ALD5和ALD6基因中的一个或多个的编码区。另选地,工程化的宿主细胞可被修饰成使ALD2、ALD3、ALD4、ALD5和ALD6基因中的一个或多个的功能性失效,诸如通过引入失活突变。

[0229] [ARO9]在一些实例中,工程化的宿主细胞可改变酶芳族转氨酶的表达。芳族转氨酶由ARO9基因编码。在实例中,芳族转氨酶催化羟苯基丙酮酸酯+谷氨酸酯→酪氨酸+α-酮戊二酸酯的反应,参考图2。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括ARO9

基因的组成型过表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控ARO9基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入ARO9基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于ARO9基因在工程化的宿主细胞内的过表达的强启动子元件的引入。ARO9基因可来源于酿酒酵母或另外的物种。在一些实例中，ARO9基因可与天然存在的基因具有100％的相似性。

[0230] [TYR]在一些实例中，工程化的宿主细胞可改变酶酪氨酸酶的表达。酪氨酸酶由TYR基因编码。在实例中，酪氨酸酶催化酪氨酸→L-DOPA的反应，参考图2。在其他实例中，酪氨酸酶催化L-DOPA→多巴醌的反应。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括TYR基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控TYR基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入TYR基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于TYR基因在工程化的宿主细胞内的过表达的强启动子元件的引入。TYR基因可来源于青枯雷尔氏菌(Ralstonia solanacearum)、双孢蘑菇(Agaricus bisporus)或另外的物种。在一些实例中，TYR基因可与天然存在的基因具有100％的相似性。

[0231] [TyrH]在一些实例中，工程化的宿主细胞可改变酶酪氨酸羟化酶的表达。酪氨酸羟化酶由TyrH基因编码。在实例中，酪氨酸羟化酶催化酪氨酸→L-DOPA的反应，参考图2和5。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括TyrH基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控TyrH基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入TyrH基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于TyrH基因在工程化的宿主细胞内的过表达的强启动子元件的引入。TyrH基因可来源于智人、褐家鼠、小家鼠或另外的物种。在一些实例中，TyrH基因可与天然存在的基因具有100％的相似性。

[0232] [DODC]在一些实例中，工程化的宿主细胞可改变酶L-DOPA脱羧酶的表达。L-DOPA脱羧酶由DODC基因编码。在实例中，L-DOPA脱羧酶催化L-DOPA→多巴胺的反应，参考图2和5。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括DODC基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控DODC基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入DODC基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于DODC基因在工程化的宿主细胞内的过表达的强启动子元件的引入。DODC基因可来源于恶臭假单胞菌(Pseudomonas putida)、褐家鼠或另外的物种。在一些实例中，DODC基因可与天然存在的基因具有100％的相似性。

[0233] [TYDC]在一些实例中，工程化的宿主细胞可改变酶酪氨酸/DOPA脱羧酶的表达。酪氨酸/DOPA脱羧酶由TYDC基因编码。在实例中，酪氨酸/DOPA脱羧酶催化L-DOPA→多巴胺的反应，参考图2。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括TYDC基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控TYDC基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入TYDC基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于TYDC基因在工程化的宿主细胞内的过表达的强启动子元件的引入。TYDC基因可来源于罂粟或另外的物种。在一些实例中，TYDC基因可与天然存在的基因具有100％的相似性。

[0234] [MAO]在一些实例中，工程化的宿主细胞可改变酶单胺氧化酶的表达。单胺氧化酶由MAO基因编码。在实例中，单胺氧化酶催化多巴胺→3,4-DHPA的反应，参考图2。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括MAO基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控MAO基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入MAO基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于MAO基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下，MAO基因可经密码子优化以在酿酒酵母中表达。MAO基因可来源于大肠杆菌、智人、藤黄微球菌(Micrococcus luteus)或另外的物种。在一些实例中，MAO基因可与天然存在的基因具有77％的相似性。

[0235] [NCS]在一些实例中，工程化的宿主细胞可改变酶去甲乌药碱合酶的表达。去甲乌药碱合酶由NCS基因编码。在实例中，去甲乌药碱合酶催化4HPA+多巴胺→(S)-去甲乌药碱的反应，参考图5。具体地讲，图5示出根据本发明的实施方案，用于通过去甲乌药碱将L-酪氨酸转化成牛心果碱的生物合成方案。图5提供了酶TyrH，酪氨酸羟化酶；DODC，DOPA脱羧酶；NCS，去甲乌药碱合酶，如本文所论述；6OMT，6-O-甲基转移酶；CNMT，乌药碱N-甲基转移酶；CYP80B1，细胞色素P450 80B1；CPR，细胞色素P450NADPH还原酶；4’OMT，3’羟基-N-甲基乌药碱4’-O-甲基转移酶的使用。L-DOPA，L-3,4-二羟基苯丙氨酸；以及4-HPA，4-羟基苯乙醛。在图5所示的酶中，4-HPA和L-酪氨酸在酵母中天然合成。所示出的所有其他代谢物不在酵母中天然产生。另外，尽管TyrH被描绘为催化L-酪氨酸到L-DOPA的转化，也可使用其他酶进行这一步骤，如说明书中所描述。例如，也可使用酪氨酸酶进行L-酪氨酸到L-DOPA的转化。此外，也可使用其他酶诸如细胞色素P450氧化酶进行L-酪氨酸到L-DOPA的转化。此类酶可在包括L-DOPA和L-酪氨酸的相关BIA前体化合物上表现出氧化酶活性。

[0236] 另外，去甲乌药碱合酶催化3,4-DHPA+多巴胺→(S)-全去甲劳丹碱的反应，参考图6。具体地讲，图6示出根据本发明的实施方案，用于通过全去甲劳丹碱将L-酪氨酸转化成牛心果碱的生物合成方案。图6提供了酶TyrH，酪氨酸羟化酶；DODC，DOPA脱羧酶；maoA，单胺氧化酶；NCS，去甲乌药碱合酶；6OMT，6-O-甲基转移酶；CNMT，乌药碱N-甲基转移酶；4’OMT，3’羟基-N-甲基乌药碱4’-O-甲基转移酶的使用。L-DOPA，L-3,4-二羟基苯丙氨酸；以及3,4-DHPA，3,4-二羟基苯乙醛。在图6所示的酶中，L-酪氨酸在酵母中天然合成。图6所示的其他代谢物不在酵母中天然产生。

[0237] 工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括NCS基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控NCS基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入NCS基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于NCS基因在工程化的宿主细胞内的过表达的强启动子元件的引入。另外，去甲乌药碱合酶可具有N末端截短。在一些情况下，NCS基因可经密码子优化以在酿酒酵母中表达。NCS基因可来源于日本黄连、罂粟、大红罂粟、黄唐松草、岩黄连(Corydalis saxicola)或另外的物种。在一些实例中，NCS基因可与天然存在的基因具有80％的相似性。

[0238] [6OMT]在一些实例中，工程化的宿主细胞可改变酶去甲乌药碱6-O-甲基转移酶的表达。去甲乌药碱6-O-甲基转移酶由6OMT基因编码。在一些实例中，去甲乌药碱6-O-甲基转移酶催化去甲乌药碱→乌药碱的反应，参考图5。在其他实例中，去甲乌药碱6-O-甲基转移

酶催化全去甲劳丹碱→3'羟基乌药碱的反应，以及本文详述的其他反应，诸如图6中提供的那些。另外，工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括6OMT基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控6OMT基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入6OMT基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于6OMT基因在工程化的宿主细胞内的过表达的强启动子元件的引入。6OMT基因可来源于罂粟、黄唐松草、日本黄连或另外的物种。在一些实例中，6OMT基因可与天然存在的基因具有100％的相似性。

[0239] [CNMT]在一些实例中，工程化的宿主细胞可改变酶乌药碱-N-甲基转移酶的表达。乌药碱-N-甲基转移酶由CNMT基因编码。在一些实例中，乌药碱-N-甲基转移酶催化乌药碱→N-甲基乌药碱的反应，参考图5。在其他实例中，乌药碱-N-甲基转移酶可催化3'羟基乌药碱→3'羟基-N-甲基乌药碱的反应。在其他实例中，乌药碱-N-甲基转移酶可催化本文详述的其他反应，诸如图6中提供的那些。另外，工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括CNMT基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控CNMT基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入CNMT基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于CNMT基因在工程化的宿主细胞内的过表达的强启动子元件的引入。CNMT基因可来源于罂粟、黄唐松草、日本黄连或另外的物种。在一些实例中，CNMT基因可与天然存在的基因具有100％的相似性。

[0240] [4'OMT]在一些实例中，工程化的宿主细胞可改变酶4'-O-甲基转移酶的表达。4'-O-甲基转移酶由4'OMT基因编码。在一些实例中，4'-O-甲基转移酶催化3'-羟基-N-甲基乌药碱→牛心果碱的反应，参考图5。在其他实例中，4'-O-甲基转移酶催化本文详述的其他反应，诸如图6中提供的那些。另外，工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括4'OMT基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控4'OMT基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入4'OMT基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于4'OMT基因在工程化的宿主细胞内的过表达的强启动子元件的引入。4'OMT基因可来源于罂粟、黄唐松草、日本黄连或另外的物种。在一些实例中，4'OMT基因可与天然存在的基因具有100％的相似性。

[0241] [CYP80B1]在一些实例中，工程化的宿主细胞可改变酶细胞色素P450 80B1的表达。细胞色素P450 80B1由CYP80B1基因编码。在实例中，细胞色素P450 80B1催化N-甲基乌药碱→3'-羟基-N-甲基乌药碱的反应，参考图5。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括细胞色素P450 80B1基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控细胞色素P450 80B1基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入细胞色素P450 80B1基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于细胞色素P450 80B1基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下，CYP80B1基因可经密码子优化以在酿酒酵母中表达。细胞色素P450 80B1基因可来源于罂粟、花菱草、黄唐松草或另外的物种。在一些实例中，P450 80B1基因可与天然存在的基因具有77％的相似性。

[0242] [FOL2]在一些实例中，工程化的宿主细胞可改变酶GTP环化水解酶的表达。GTP环

化水解酶由FOL2基因编码。在一些实例中，GTP环化水解酶催化GTP→二氢新蝶呤三磷酸的反应，参考图1。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括FOL2基因的组成型过表达。工程化的宿主细胞也可被修饰成包括天然调控。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控FOL2基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入FOL2基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于FOL2基因在工程化的宿主细胞内的过表达的强启动子元件的引入。FOL2基因可来源于酿酒酵母、智人、小家鼠或另外的物种。在一些实例中，FOL2基因可与天然存在的基因具有100％的相似性。

[0243] [PTPS]在一些实例中，工程化的宿主细胞可改变酶6-丙酮酰四氢生物蝶呤(PTP)合酶的表达。丙酮酰四氢生物蝶呤合酶由PTPS基因编码。在一些实例中，6-丙酮酰四氢生物蝶呤合酶催化二氢新蝶呤三磷酸→PTP的反应，参考图1。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括PTPS基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控PTPS基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入PTPS基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于PTPS基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下，PTPS基因可经密码子优化以在酿酒酵母中表达。PTPS基因可来源于褐家鼠、智人、小家鼠或另外的物种。在一些实例中，PTPS基因可与天然存在的基因具有80％的相似性。

[0244] [SepR]在一些实例中，工程化的宿主细胞可改变酶墨蝶呤还原酶的表达。墨蝶呤还原酶由SepR基因编码。在一些实例中，墨蝶呤还原酶催化PTP→BH_4的反应，参考图1。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括SepR基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控SepR基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入SepR基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于SepR基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下，SepR基因可经密码子优化以在酿酒酵母中表达。SepR基因可来源于褐家鼠、智人、小家鼠或另外的物种。在一些实例中，SepR基因可与天然存在的基因具有72％的相似性。

[0245] [PCD]在一些实例中，工程化的宿主细胞可改变酶4a-羟基四氢生物蝶呤(蝶呤-4α-甲醇胺)脱水酶的表达。4a-羟基四氢生物蝶呤脱水酶由PCD基因编码。在一些实例中，4a-羟基四氢生物蝶呤脱水酶催化4a-羟基四氢生物蝶呤→H_2O+醌型二氢蝶啶的反应，参考图1。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括PCD基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控PCD基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入PCD基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于PCD基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下，PCD基因可经密码子优化以在酿酒酵母中表达。PCD基因可来源于褐家鼠、智人、小家鼠或另外的物种。在一些实例中，PCD基因可与天然存在的基因具有79％的相似性。

[0246] [QDHPR]在一些实例中，工程化的宿主细胞可改变酶醌型二氢蝶啶还原酶的表达。醌型二氢蝶啶还原酶由QDHPR基因编码。在一些实例中，醌型二氢蝶啶还原酶催化醌型二氢

蝶啶→BH_4的反应，参考图1。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括QDHPR基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控QDHPR基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入QDHPR基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于QDHPR基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下，QDHPR基因可经密码子优化以在酿酒酵母中表达。QDHPR基因可来源于褐家鼠、智人、小家鼠或另外的物种。在一些实例中，QDHPR基因可与天然存在的基因具有75％的相似性。

[0247] [DHFR]在一些实例中，工程化的宿主细胞可改变酶二氢叶酸还原酶的表达。二氢叶酸还原酶由DHFR基因编码。在一些实例中，二氢叶酸还原酶催化7,8-二氢生物蝶呤(BH_2)→5,6,7,8-四氢生物蝶呤(BH_4)的反应，参考图1。该反应可用于回收BH_4作为共底物，以用于将酪氨酸转化成L-DOPA，如图5中所示。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括DHFR基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控DHFR基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入DHFR基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于DHFR基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下，DHFR基因可经密码子优化以在酿酒酵母中表达。DHFR基因可来源于褐家鼠、智人或另外的物种。在一些实例中，DHFR基因可与天然存在的基因具有77％的相似性。

[0248] [CYP-COR]如上文关于差向异构化1-BIA所论述的，工程化的宿主细胞可改变BIA差向异构酶的表达。BIA差向异构酶由CYP-COR基因(例如，CYP82Y2-COR基因)编码。在一些实例中，CYP-COR也可称为DRS-DRR。在一些实例中，BIA差向异构酶催化(S)-1-BIA→(R)-1-BIA的转化，参考图7。具体地讲，图7示出根据本发明的实施方案，用于将L-酪氨酸转化成吗啡喃生物碱的生物合成方案。图7提供了酶CPR，细胞色素P450还原酶；CYP-COR，细胞色素P450CYP82Y1-可待因酮还原酶样融合物；SalSyn，沙罗泰里啶合酶；SalR，沙罗泰里啶还原酶；SalAT，沙罗泰里啶醇7-O-乙酰转移酶；T6ODM，蒂巴因6-O-脱甲基酶；COR，可待因酮还原酶；以及CODM，可待因-O-脱甲基酶的使用。

[0249] 工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括CYP-COR基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控CYP-COR基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入CYP-COR基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于CYP-COR基因在工程化的宿主细胞内的过表达的强启动子元件的引入。CYP-COR基因可来源于大红罂粟、罂粟、渥美罂粟、白屈菜或另外的物种。在一些实例中，CYP-COR基因可与天然存在的基因具有77％的相似性。

[0250] [CPR]在一些实例中，工程化的宿主细胞可改变酶细胞色素P450还原酶的表达。细胞色素P450还原酶由CPR基因编码。在一些实例中，细胞色素P450还原酶催化(R)-牛心果碱→沙罗泰里啶的反应，参考图7。另外，细胞色素P450还原酶催化其他反应，诸如整篇申请的附图中所述的那些。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括CPR基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控CPR基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入CPR基因的一个拷贝、

多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于CPR基因在工程化的宿主细胞内的过表达的强启动子元件的引入。CPR基因可来源于花菱草、罂粟、智人、酿酒酵母、拟南芥或另外的物种。在一些实例中，CPR基因可与天然存在的基因具有100％的相似性。

[0251] [SalSyn]在一些实例中，工程化的宿主细胞可改变酶沙罗泰里啶合酶的表达。沙罗泰里啶合酶由SalSyn基因编码。在一些实例中，沙罗泰里啶合酶催化(R)-牛心果碱→沙罗泰里啶的反应，参考图7。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括SalSyn基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控SalSyn基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入SalSyn基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于SalSyn基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下，SalSyn基因可经密码子优化以在酿酒酵母中表达。在一些实例中，SalSyn可以在N末端处进行修饰。SalSyn基因可来源于罂粟、罂粟属物种、白屈菜或另外的物种。在一些实例中，SalSyn基因可与天然存在的基因具有78％的相似性。

[0252] [SalR]在一些实例中，工程化的宿主细胞可改变酶沙罗泰里啶还原酶的表达。沙罗泰里啶还原酶由SalR基因编码。在一些实例中，沙罗泰里啶还原酶可逆地催化沙罗泰里啶醇→沙罗泰里啶的反应，参考图7。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括SalR基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控SalR基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入SalR基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于SalR基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下，SalR基因可经密码子优化以在酿酒酵母中表达。SalR基因可来源于罂粟、大红罂粟、罂粟属物种、白屈菜或另外的物种。在一些实例中，SalR基因可与天然存在的基因具有80-100％的相似性。

[0253] [SalAT]在一些实例中，工程化的宿主细胞可改变酶乙酰-CoA:沙罗泰里啶醇7-O-乙酰转移酶的表达。乙酰-CoA:沙罗泰里啶醇7-O-乙酰转移酶由SalAT基因编码。在一些实例中，乙酰-CoA:沙罗泰里啶醇7-O-乙酰转移酶催化乙酰-CoA+沙罗泰里啶醇→CoA+7-O-乙酰基沙罗泰里啶醇的反应，参考图7。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括SalAT基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控SalAT基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入SalAT基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于SalAT基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下，SalAT基因可经密码子优化以在酿酒酵母中表达。SalAT基因可来源于罂粟、大红罂粟、鬼罂粟(Papaver orientale)、罂粟属物种或另外的物种。在一些实例中，SalAT基因可与天然存在的基因具有77-80％的相似性。

[0254] [T6ODM]在一些实例中，工程化的宿主细胞可改变酶蒂巴因6-O-脱甲基酶的表达。蒂巴因6-O-脱甲基酶由T6ODM基因编码。在一些实例中，蒂巴因6-O-脱甲基酶催化蒂巴因→尼奥品酮的反应，参考图7。一旦已经产生尼奥品酮，尼奥品酮可转化成可待因酮。尼奥品酮→可待因酮的转化可自发发生。另选地，尼奥品酮→可待因酮的转化可由于催化反应而发

生。在其他实例中，T6ODM酶可催化除蒂巴因之外的底物的O-脱甲基化。例如，T6ODM可使东罂粟碱O-脱甲基化，产生吗啡酮。另选地，T6ODM可催化1-苄基异喹啉、原小檗碱或原阿片碱种类之内的BIA(诸如分别为罂粟碱、坎那定和别隐品碱)的O-脱甲基化。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括T6ODM基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控T6ODM基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入T6ODM基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于T6ODM基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下，T6ODM基因可经密码子优化以在酿酒酵母中表达。T6ODM基因可来源于罂粟或另外的物种。在一些实例中，T6ODM基因可与天然存在的基因具有76.2％的相似性。

[0255] [COR]在一些实例中，工程化的宿主细胞可改变酶可待因酮还原酶的表达。可待因酮还原酶由COR基因编码。在一些实例中，可待因酮还原酶催化可待因酮至可待因的反应，参考图7。在一些情况下，可待因酮还原酶可催化尼奥品酮至尼奥品的反应。在其他实例中，COR可催化其他吗啡喃的还原，包括氢可酮→二氢可待因、14-羟基可待因酮→14-羟基可待因，以及氢吗啡酮→二氢吗啡。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括COR基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控COR基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入COR基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于COR基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下，COR基因可经密码子优化以在酿酒酵母中表达。另外或另选地，COR基因可通过添加针对线粒体、液泡、内质网或其组合的靶序列进行修饰。COR基因可来源于罂粟或另外的物种。在一些实例中，COR基因可与天然存在的基因具有76-78％的相似性。在实例中，COR基因可与天然存在的基因具有76.8％、77.0％、77.3％或77.7％的相似性。

[0256] [CODM]在一些实例中，工程化的宿主细胞可改变酶可待因O-脱甲基酶的表达。可待因O-脱甲基酶由CODM基因编码。在一些实例中，可待因O-脱甲基酶催化可待因至吗啡的反应，参考图7。可待因O-脱甲基酶还可催化尼奥品至新吗啡的反应。可待因O-脱甲基酶还可催化蒂巴因至东罂粟碱的反应。在其他实例中，CODM可催化1-苄基异喹啉、阿朴啡和原小檗碱种类之内的BIA(诸如分别为牛心果碱、异可利定和金黄紫堇碱)的O-脱甲基化。在其他实例中，CODM酶可催化O,O-脱亚甲基化反应，以裂解原阿片碱中的亚甲基二氧桥结构。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括CODM基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控CODM基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入CODM基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于CODM基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下，CODM基因可经密码子优化以在酿酒酵母中表达。另外或另选地，CODM基因可通过添加针对线粒体的靶序列进行修饰。CODM基因可来源于罂粟、罂粟属物种或另外的物种。在一些实例中，CODM基因可与天然存在的基因具有75％的相似性。在实例中，CODM基因可与天然存在的基因具有75.2％的相似性。

[0257] [BBE]在一些实例中，工程化的宿主细胞可改变酶小檗碱桥酶的表达。小檗碱桥酶由BBE基因编码。在一些实例中，小檗碱桥酶催化(S)-牛心果碱→(S)-金黄紫堇碱的反应，

参考图8。图8示出根据本发明的实施方案，用于将L-酪氨酸转化成原小檗碱生物碱的生物合成方案。具体地讲，图8提供了酶BBE，小檗碱桥酶；S9OMT，金黄紫堇碱9-O-甲基转移酶；CAS，坎那定合酶；CPR，细胞色素P450还原酶；以及STOX，四氢原小檗碱氧化酶的使用。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括BBE基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控BBE基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入BBE基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于BBE基因在工程化的宿主细胞内的过表达的强启动子元件的引入。BBE基因可来源于罂粟、蓟罂粟、花菱草、伏牛花、黄唐松草粉绿亚种(Thalictrum flavum subsp.glaucum)、日本黄连、罂粟属物种或另外的物种。在一些实例中，BBE基因可与天然存在的基因具有99％的相似性。

[0258] [S9OMT]在一些实例中，工程化的宿主细胞可改变酶S-腺苷-L-甲硫氨酸:(S)-金黄紫堇碱9-O-甲基转移酶的表达。S-腺苷-L-甲硫氨酸:(S)-金黄紫堇碱9-O-甲基转移酶由S9OMT基因编码。在一些实例中，S-腺苷-L-甲硫氨酸:(S)-金黄紫堇碱9-O-甲基转移酶催化S-腺苷-L-甲硫氨酸+(S)-金黄紫堇碱→S-腺苷-L-高半胱氨酸+(S)-四氢非洲防己碱的反应，参考图8。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括S9OMT基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控S9OMT基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入S9OMT基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于S9OMT基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下，S9OMT基因可经密码子优化以在酿酒酵母中表达。S9OMT基因可来源于黄唐松草粉绿亚种、日本黄连、黄连、罂粟、唐松草属物种、黄连属物种、罂粟属物种或另外的物种。在一些实例中，S9OMT基因可与天然存在的基因具有100％的相似性。在一些实例中，S9OMT基因可与天然存在的基因具有80％的相似性。

[0259] [CAS]在一些实例中，工程化的宿主细胞可改变酶(S)-坎那定合酶的表达。(S)-坎那定合酶由CAS基因编码。在一些实例中，(S)-坎那定合酶催化(S)-四氢非洲防己碱→(S)-坎那定的反应，参考图8。工程化的宿主细胞可被修饰成在工程化的宿主细胞中表达CAS基因。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括CAS基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控CAS基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入CAS基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于CAS基因在工程化的宿主细胞内的过表达的强启动子元件的引入。CAS基因可来源于黄唐松草粉绿亚种、日本黄连、唐松草属物种、黄连属物种或另外的物种。在一些实例中，CAS基因可与天然存在的基因具有100％的相似性。

[0260] [STOX]在一些实例中，工程化的宿主细胞可改变酶(S)-四氢原小檗碱氧化酶的表达。(S)-四氢原小檗碱氧化酶由STOX基因编码。在一些实例中，(S)-四氢原小檗碱氧化酶催化(S)-四氢小檗碱+$2O_2$→小檗碱+$2H_2O_2$的反应，参考图8。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括STOX基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控STOX基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入STOX基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的

宿主细胞可被修饰成包括用于STOX基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些实例中，STOX可以在N末端处进行修饰。在一些情况下，STOX基因可经密码子优化以在酿酒酵母中表达。STOX基因可来源于金花小檗(Berberis wilsonae)、日本黄连、小檗属物种、黄连属物种或另外的物种。在一些实例中，STOX基因可与天然存在的基因具有78％的相似性。

[0261] [TNMT]在一些实例中，工程化的宿主细胞可改变酶四氢原小檗碱-N-甲基转移酶的表达。四氢原小檗碱-N-甲基转移酶由TNMT基因编码。在一些实例中，四氢原小檗碱-N-甲基转移酶催化坎那定→N-甲基坎那定的反应，参考图9。图9示出根据本发明的实施方案，用于将L-酪氨酸转化成诺斯卡品、类诺斯卡品和苯酞异喹啉的生物合成方案。具体地讲，图9提供了酶BBE，小檗碱桥酶；S9OMT，金黄紫堇碱9-O-甲基转移酶；CAS，坎那定合酶；CPR，细胞色素P450还原酶；TNMT，四氢原小檗碱顺式-N-甲基转移酶；CYP82Y1，N-甲基坎那定1-羟化酶；CYP82X2，1-羟基-N-甲基坎那定13-羟化酶；AT1，1,13-二羟基-N-甲基坎那定13-O-乙酰转移酶；CYP82X1，4'-O-脱甲基-3-O-乙酰罂粟奥辛合酶；CXE1，那可汀半缩醛合酶；NOS(或SDR1)，诺斯卡品合酶；MT2，那可托灵-4'-O-甲基转移酶1；MT3，那可托灵-4'-O-甲基转移酶2；以及6OMT，6-O-甲基转移酶的使用。

[0262] 在其他实例中，四氢原小檗碱-N-甲基转移酶催化刺罂粟碱→顺式-N-甲基刺罂粟碱的反应，参考图10。图10示出根据本发明的实施方案，用于将L-酪氨酸转化成血根碱和苯菲啶生物碱的生物合成方案。具体地讲，图10提供了酶BBE，小檗碱桥酶；CFS，碎叶紫堇碱合酶；STS，刺罂粟碱合酶；TNMT，四氢小檗碱N-甲基转移酶；MSH，顺式-N-甲基刺罂粟碱14-羟化酶；P6H，原阿片碱6-羟化酶；以及DBOX，二氢苯菲啶氧化酶的使用。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括TNMT基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控TNMT基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入TNMT基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于TNMT基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下，TNMT基因可经密码子优化以在酿酒酵母中表达。TNMT基因可来源于罂粟、花菱草、大红罂粟、蓟罂粟或另外的物种。在一些实例中，TNMT基因可与天然存在的基因具有100％的相似性。在实例中，TNMT基因可与天然存在的基因具有81％的相似性。

[0263] [CYP82Y1]在一些实例中，工程化的宿主细胞可改变酶N-甲基坎那定1-羟化酶的表达。N-甲基坎那定1-羟化酶由CYP82Y1基因编码。在一些实例中，N-甲基坎那定1-羟化酶催化N-甲基坎那定→1-羟基-N-甲基坎那定的反应，参考图9。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括CYP82Y1基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控CYP82Y1基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入CYP82Y1基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于CYP82Y1基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下，CYP82Y1基因可经密码子优化以在酿酒酵母中表达。在一些实例中，CYP82Y1可以在N末端处进行修饰。CYP82Y1基因可来源于罂粟、罂粟属物种、沙生车前(Plantago arenaria)、Rauwolfia heterophylla、覃状山缘草(Adlumia fungosa)、金印草(Hydrastis canadensis)、异叶罂粟(Stylomecon heterophylla)、角茴

香属(Hypecoum)或另外的物种。在一些实例中,CYP82Y1基因可与天然存在的基因具有70-78%的相似性。

[0264] [CYP82X2]在一些实例中,工程化的宿主细胞可改变酶1-羟基-N-甲基坎那定13-羟化酶的表达。1-羟基-N-甲基坎那定13-羟化酶由CYP82X2基因编码。在一些实例中,1-羟基-N-甲基坎那定13-羟化酶催化1-羟基-N-甲基坎那定→1-羟基-N-甲基左旋蛇果黄堇碱(即1,13-二羟基-N-甲基坎那定)的反应,参考图9。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括CYP82X2基因的组成型表达。另外或另选地,工程化的宿主细胞可被修饰成合成性地调控CYP82X2基因在工程化的宿主细胞中的表达。在实例中,工程化的宿主细胞可被修饰成并入CYP82X2基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地,工程化的宿主细胞可被修饰成包括用于CYP82X2基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下,CYP82X2基因可经密码子优化以在酿酒酵母中表达。在一些实例中,CYP82X2可以在N末端处进行修饰。CYP82X2基因可来源于罂粟、罂粟属物种、沙生车前、Rauwolfia heterophylla、覃状山缘草、金印草、异叶罂粟、扭果紫金龙(Dactylicapnos torulosa)、黄海罂粟(Glaucium flavum)、月桂小檗(Berberis laurina)、刺檗(B.Vulgaris)、紫堇属物种(Corydalis spp.)、球果紫堇属物种(Fumaria spp.)、紫金龙属物种(Dactylicapnos spp.)或另外的物种。在一些实例中,CYP82X2基因可与天然存在的基因具有70-77%的相似性。在其他实例中,CYP82X2基因可发生N末端工程化。在实例中,N末端工程化可包括N末端截短。

[0265] [CYP82X1]在一些实例中,工程化的宿主细胞可改变酶4'-O-脱甲基-3-O-乙酰基罂粟奥辛合酶的表达。4'-O-脱甲基-3-O-乙酰基罂粟奥辛合酶由CYP82X1基因编码。在一些实例中,4'-O-脱甲基-3-O-乙酰基罂粟奥辛合酶催化1-羟基-13-O-乙酰基-N-甲基坎那定→4'-O-脱甲基-3-O-乙酰基罂粟奥辛的反应,参考图9。另外,CYP82X1催化1-羟基-N-甲基坎那定→4'-O-脱甲基马克兰特醛的反应。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括CYP82X1基因的组成型表达。另外或另选地,工程化的宿主细胞可被修饰成合成性地调控CYP82X1基因在工程化的宿主细胞中的表达。在实例中,工程化的宿主细胞可被修饰成并入CYP82X1基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地,工程化的宿主细胞可被修饰成包括用于CYP82X1基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下,CYP82X1基因可经密码子优化以在酿酒酵母中表达。在一些实例中,CYP82X1可以在N末端处进行修饰。CYP82X1基因可来源于罂粟、罂粟属物种、沙生车前、Rauwolfia heterophylla、覃状山缘草、金印草、异叶罂粟、角茴香属或另外的物种。在一些实例中,CYP82X1基因可与天然存在的基因具有71-77%的相似性。在其他实例中,CYP82X1基因可发生N末端工程化。在实例中,N末端工程化可包括N末端截短。

[0266] [CFS]在一些实例中,工程化的宿主细胞可改变酶碎叶紫堇碱合酶的表达。碎叶紫堇碱合酶由CFS基因编码。在实例中,碎叶紫堇碱合酶催化金黄紫堇碱→碎叶紫堇碱的反应,参考图10。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括CFS基因的组成型表达。另外或另选地,工程化的宿主细胞可被修饰成合成性地调控CFS基因在工程化的宿主细胞中的表达。在实例中,工程化的宿主细胞可被修饰成并入CFS基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地,工程化的宿主细胞可被修饰成包括用于CFS基因在工程化的宿主细胞内的过表达的强启动子元件的引入。CFS基因可来源于罂粟、花菱草、蓟罂粟或

另外的物种。在一些实例中，CFS基因可与天然存在的基因具有77％、78％或79％的相似性。另外，CFS基因可经密码子优化以在酿酒酵母中表达。

[0267] [STS]在一些实例中，工程化的宿主细胞可改变酶刺罂粟碱合酶的表达。刺罂粟碱合酶由STS基因编码。在实例中，刺罂粟碱合酶催化碎叶紫堇碱→刺罂粟碱的反应，参考图10。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括STS基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控STS基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入STS基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于STS基因在工程化的宿主细胞内的过表达的强启动子元件的引入。STS基因可来源于罂粟、花菱草、蓟罂粟或另外的物种。在一些实例中，STS基因可与天然存在的基因具有76％、78％或79％的相似性。另外，STS基因可经密码子优化以在酿酒酵母中表达。

[0268] [MSH]在一些实例中，工程化的宿主细胞可改变酶顺式-N-甲基刺罂粟碱14-羟化酶的表达。顺式-N-甲基刺罂粟碱14-羟化酶由MSH基因编码。在实例中，顺式-N-甲基刺罂粟碱14-羟化酶催化顺式-N-甲基刺罂粟碱→原阿片碱的反应，参考图10。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括MSH基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控MSH基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入MSH基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于MSH基因在工程化的宿主细胞内的过表达的强启动子元件的引入。MSH基因可来源于罂粟或另外的物种。在一些实例中，MSH基因可与天然存在的基因具有79％的相似性。另外，MSH基因可经密码子优化以在酿酒酵母中表达。

[0269] [P6H]在一些实例中，工程化的宿主细胞可改变酶原阿片碱-6-羟化酶的表达。原阿片碱-6-羟化酶由P6H基因编码。在实例中，原阿片碱-6-羟化酶催化原阿片碱→6-羟基原阿片碱的反应，参考图10。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括P6H基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控P6H基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入P6H基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于CFS基因在工程化的宿主细胞内的过表达的强启动子元件的引入。P6H基因可来源于罂粟、花菱草或另外的物种。在一些实例中，P6H基因可与天然存在的基因具有79％的相似性。另外，P6H基因可经密码子优化以在酿酒酵母中表达。

[0270] [DBOX]在一些实例中，工程化的宿主细胞可改变酶二氢苯菲啶氧化酶的表达。二氢苯菲啶氧化酶由DBOX基因编码。在实例中，二氢苯菲啶氧化酶催化二氢血根碱→血根碱的反应，参考图10。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括DBOX基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控DBOX基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入DBOX基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于DBOX基因在工程化的宿主细胞内的过表达的强启动子元件的引入。DBOX基因可来源于罂粟或另外的物种。在一些实例中，DBOX基因可与天然存在的基因具有100％的相似性。另外，DBOX基因可经密码子优化以在酿酒酵母中表达。

[0271] [AT1]在一些实例中，工程化的宿主细胞可改变酶1,13-二羟基-N-甲基坎那定13-

O乙酰转移酶的表达。1,13-二羟基-N-甲基坎那定13-O乙酰转移酶由AT1基因编码。在一些实例中,1,13-二羟基-N-甲基坎那定13-O乙酰转移酶催化1,13-二羟基-N-甲基坎那定→1-羟基-13-O-乙酰基-N-甲基坎那定的反应,参考图11A。图11A示出根据本发明的实施方案,用于将坎那定转化成诺斯卡品的生物合成方案。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括AT1基因的组成型表达。另外或另选地,工程化的宿主细胞可被修饰成合成性地调控AT1基因在工程化的宿主细胞中的表达。在实例中,工程化的宿主细胞可被修饰成并入AT1基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地,工程化的宿主细胞可被修饰成包括用于AT1基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下,AT1基因可经密码子优化以在酿酒酵母中表达。AT1基因可来源于罂粟、罂粟属物种、沙生车前、Rauwolfia heterophylla、覃状山缘草、金印草、异叶罂粟、细果角茴香(Hypecoum leptocarpum)、扭果紫金龙、黄海罂粟、月桂小檗、刺檗、紫堇属物种、球果紫堇属物种、紫金龙属物种或另外的物种。在一些实例中,AT1基因可与天然存在的基因具有81%的相似性。

[0272] [CXE1或CXE2]在一些实例中,工程化的宿主细胞可改变酶那可汀半缩醛合酶的表达。那可汀半缩醛合酶由CXE1基因编码。由CXE2基因编码的酶也可用作那可汀半缩醛合酶。在一些实例中,那可汀半缩醛合酶催化4'-O-脱甲基-3-O-乙酰基罂粟奥辛→那可托灵半缩醛和3-O-乙酰基罂粟奥辛→那可汀半缩醛的反应,参考FIG.11A。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括CXE1或CXE2基因的组成型表达。另外或另选地,工程化的宿主细胞可被修饰成合成性地调控CXE1或CXE2基因在工程化的宿主细胞中的表达。在实例中,工程化的宿主细胞可被修饰成并入CXE1或CXE2基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地,工程化的宿主细胞可被修饰成包括用于CXE1或CXE2基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下,CXE1或CXE2基因可经密码子优化以在酿酒酵母中表达。CXE1或CXE2基因可来源于罂粟、罂粟属物种、沙生车前、Rauwolfia heterophylla、覃状山缘草、金印草、异叶罂粟、细果角茴香、扭果紫金龙、黄海罂粟、月桂小檗、刺檗、紫堇属物种、球果紫堇属物种、紫金龙属物种或另外的物种。在一些实例中,CXE1基因或CXE2基因可与天然存在的基因具有78%的相似性。

[0273] [SDR1]在一些实例中,工程化的宿主细胞可改变酶诺斯卡品合酶的表达。诺斯卡品合酶由SDR1基因编码。在一些实例中,诺斯卡品合酶催化那可托灵半缩醛→那可托灵的反应,参考图11A。另外,诺斯卡品合酶催化那可汀半缩醛→诺斯卡品的反应。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括SDR1基因的组成型表达。另外或另选地,工程化的宿主细胞可被修饰成合成性地调控SDR1基因在工程化的宿主细胞中的表达。在实例中,工程化的宿主细胞可被修饰成并入SDR1基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地,工程化的宿主细胞可被修饰成包括用于SDR1基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下,SDR1基因可经密码子优化以在酿酒酵母中表达。SDR1基因可来源于罂粟、罂粟属物种、沙生车前、Rauwolfia heterophylla、覃状山缘草、金印草、异叶罂粟、细果角茴香、扭果紫金龙、黄海罂粟、月桂小檗、刺檗、紫堇属物种、球果紫堇属物种、紫金龙属物种或另外的物种。在一些实例中,SDR1基因可与天然存在的基因具有79%的相似性。

[0274] [MT2和MT3]在一些实例中,工程化的宿主细胞可改变酶那可托灵4'-O-甲基化酶

的表达。那可托灵4'-O-甲基化酶是由MT2和MT3基因编码的O-甲基转移酶单体形成的异二聚体。在一些实例中，那可托灵4'-O-甲基化酶催化那可托灵→诺斯卡品的反应，参考图11A。另外，那可托灵4'-O-甲基化酶催化那可托灵半缩醛→那可汀半缩醛和4'-O-脱甲基-3-O-乙酰基罂粟奥辛→3-O-乙酰基罂粟奥辛的反应。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括MT2和MT3基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控MT2和MT3基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入MT2和MT3基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于MT2和MT3基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下，MT2和MT3基因可经密码子优化以在酿酒酵母中表达。MT2和MT3基因可来源于罂粟、罂粟属物种、小花球果紫堇(Fumaria parviflora)、对叶车前(Plantago arenaria)、Rauwolfia heterophylla或另外的物种。在一些实例中，MT2和MT3基因可分别与天然存在的基因具有80％和79％的相似性。

[0275] [morA]在一些实例中，工程化的宿主细胞可改变酶吗啡脱氢酶的表达。吗啡脱氢酶由morA基因编码。在一些实例中，吗啡脱氢酶催化吗啡→吗啡酮的反应，参考图11B。在其他实例中，吗啡脱氢酶催化可待因酮→可待因的反应，也参考图11B。图11B示出根据本发明的实施方案，用于产生半合成的阿片类物质的生物合成方案。具体地讲，图11B示出通过并入morA吗啡脱氢酶和morB吗啡还原酶，蒂巴因在酵母中的转化延长。

[0276] 工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括morA基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控morA基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入morA基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于morA基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下，morA基因可经密码子优化以在酿酒酵母中表达。morA基因可来源于恶臭假单胞菌或另外的物种。在一些实例中，morA基因可与天然存在的基因具有73.7％的相似性。

[0277] [morB]在一些实例中，工程化的宿主细胞可改变酶吗啡酮还原酶的表达。吗啡酮还原酶由morB基因编码。在一些实例中，吗啡酮还原酶催化可待因酮→氢可酮的反应，参考图11B。在其他实例中，吗啡酮还原酶催化吗啡酮→氢吗啡酮的反应，还参考图11B。在其他实例中，吗啡酮还原酶催化14-羟基可待因酮→羟考酮的反应。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括morB基因的组成型表达。另外或另选地，工程化的宿主细胞可被修饰成合成性地调控morB基因在工程化的宿主细胞中的表达。在实例中，工程化的宿主细胞可被修饰成并入morB基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地，工程化的宿主细胞可被修饰成包括用于morB基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下，morB基因可经密码子优化以在酿酒酵母中表达。morB基因可来源于恶臭假单胞菌或另外的物种。在一些实例中，morB基因可与天然存在的基因具有67.2％的相似性。

[0278] [CYP80A1]在一些实例中，工程化的宿主细胞可表达酶黄芦木碱合酶。黄芦木碱合酶由细胞色素P450酶80A1(CYP80A1)的基因编码。在一些实例中，CYP80A1催化(S)-N-甲基乌药碱+(R)-N-甲基乌药碱→黄芦木碱的反应。在其他实例中，CYP80A1催化(R)-N-甲基乌药碱+(R)-N-甲基乌药碱→guattegaumerine的反应。在其他实例中，CYP80A1催化(R)-N-甲

基乌药碱+(S)-乌药碱→2'去甲黄芦木碱的反应。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括CYP80A1基因的组成型表达。另外或另选地,工程化的宿主细胞可被修饰成合成性地调控CYP80A1基因在工程化的宿主细胞中的表达。在实例中,工程化的宿主细胞可被修饰成并入CYP80A1基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地,工程化的宿主细胞可被修饰成包括用于CYP80A1基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下,CYP80A1基因可经密码子优化以在酿酒酵母中表达。CYP80A1基因可来源于伏牛花或另外的物种。在一些实例中,CYP80A1基因可与天然存在的基因具有76%的相似性。

[0279] [PODA]在一些实例中,工程化的宿主细胞可表达酶原阿片碱O-脱烷基酶。原阿片碱O-脱烷基酶由基因PODA编码。在一些实例中,PODA催化原小檗碱类和原阿片碱类诸如坎那定、刺罂粟碱、小檗碱、隐品碱、别隐品碱和原阿片碱的O,O-脱亚甲基化。在一些实例中,PODA催化包括四氢罂粟碱、四氢非洲防己碱和隐品碱的BIA的O-脱甲基化。工程化的宿主细胞可被修饰成在工程化的宿主细胞中包括PODA基因的组成型表达。另外或另选地,工程化的宿主细胞可被修饰成合成性地调控PODA基因在工程化的宿主细胞中的表达。在实例中,工程化的宿主细胞可被修饰成并入PODA基因的一个拷贝、多个拷贝或另外的拷贝。另外或另选地,工程化的宿主细胞可被修饰成包括用于PODA基因在工程化的宿主细胞内的过表达的强启动子元件的引入。在一些情况下,PODA基因可经密码子优化以在酿酒酵母中表达。PODA基因可来源于罂粟或其他物种。在一些实例中,PODA基因可与天然存在的基因具有70-100%的相似性。

[0280] 前述基因的实例可在宿主细胞中由多种不同的平台表达,包括质粒(2μ,ARS/CEN)、YAC或基因组。此外,前述基因序列的实例可以是天然的或经密码子优化以在期望的异源宿主(例如,酿酒酵母)中表达。

实施例

[0281] 以下实施例出于说明本发明的各种实施方案的目的给出并且不旨在以任何方式限制本发明。在指出时,表达构建体应理解为并入合适的启动子、基因和终止子,即使未指明所使用的准确终止子序列。本发明的实施例,连同本文所述的方法一起目前代表优选实施方案,是示例性的,并且不旨在限制本发明的范围。本领域技术人员会发现涵盖在如通过权力要求的范围所限定的本发明的精神之内的其中的变化及其他用途。

实施例1:酪氨酸羟化酶突变体改善工程化酵母菌株中的去甲乌药碱产生

[0282] 将来自褐家鼠的酪氨酸羟化酶进行酵母密码子优化、合成并且克隆到低拷贝质粒中。通过定点诱变生成单突变体(W166Y、E332D、S40D和R37ER38E)、双突变体(W166Y和E332D、W166Y和S40D、W166Y和R37ER38E)以及一种三突变体(W166Y、R37ER38E和E332D)。每种TyrH突变体由具有GPD启动子的低拷贝质粒在酵母菌株中表达,所述酵母菌株包含对中心代谢的以下突变(如美国临时专利申请序列号61/899,496中所述):$ARO4^{FBR}$、ΔZWF1和GPD-TKL1启动子替换。此外,所述菌株表达来自恶臭假单胞菌的DOPA脱羧酶(DODC)的染色体整合的拷贝;来自褐家鼠的生成共底物四氢生物蝶呤(丙酮酰四氢蝶呤合酶,PTPS;墨蝶呤还原酶,SepR;蝶呤4a-甲醇胺脱水酶,PCD;二氢蝶啶还原酶,QDHPR)的四种染色体整合的基因;以及由具有GPD启动子的低拷贝质粒表达的来自日本黄连的去甲乌药碱合酶(NCS)。使携带TyrH突变体的菌株在缺乏酪氨酸的具有2%右旋糖的选择性限定培养基(YNB)中生

长96小时，并且通过LC-MS/MS以MRM模式利用272m/z至107m/z的跃迁测量培养基中去甲乌药碱的产生。图12示出该测定的结果并且展示了在与野生型TyrH进行比较时，TyrH突变体可使去甲乌药碱产生改善多至9倍。因而，图12示出根据本发明的实施方案，改善在工程化的酵母菌株中由糖产生去甲乌药碱的酪氨酸羟化酶突变体。

实施例2：酪氨酸羟化酶突变体改善工程化酵母菌株中的牛心果碱产生

[0283] 将来自褐家鼠的酪氨酸羟化酶进行酵母密码子优化、合成并且克隆到低拷贝质粒中。通过定点诱变生成单突变体(W166Y、E332D、S40D和R37ER38E)、双突变体(W166Y和E332D、W166Y和S40D、W166Y和R37ER38E)以及一种三突变体(W166Y、R37ER38E和E332D)。每种TyrH突变体由具有GPD启动子的低拷贝质粒在酵母菌株中表达，所述酵母菌株包含对中心代谢的以下突变(如美国临时专利申请序列号61/899,496中所述)：ARO4^{FBR}、ΔZWF1和GPD-TKL1启动子替换。此外，所述菌株表达来自恶臭假单胞菌的DOPA脱羧酶(DODC)的染色体整合的拷贝；来自褐家鼠的生成共底物四氢生物蝶呤(丙酮酰四氢蝶呤合酶，PTPS；墨蝶呤还原酶，SepR；蝶呤4a-甲醇胺脱水酶，PCD；二氢蝶啶还原酶，QDHPR)的四种染色体整合的基因；由具有GPD启动子的低拷贝质粒表达的来自日本黄连的去甲乌药碱合酶(NCS)；以及用于由去甲乌药碱生物合成牛心果碱的五种基因(罂粟6-O-甲基转移酶，Ps6OMT；罂粟乌药碱N-甲基转移酶，PsCNMT；花菱草细胞色素P450 80B1，EcCYP80B1；罂粟细胞色素P450NADPH还原酶，PsCPR；以及罂粟3'羟基-N-甲基乌药碱4'-O-甲基转移酶，Ps4'OMT)。使携带TyrH突变体的菌株在缺乏酪氨酸的具有2％右旋糖的选择性限定培养基(YNB)中生长96小时，并且通过LC-MS/MS以MRM模式利用330m/z至137m/z的跃迁测量培养基中的牛心果碱的产生。图13示出该测定的结果并且展示了在与野生型TyrH进行比较时，TyrH突变体可使牛心果碱产生改善多至5倍。因而，图13示出根据本发明的实施方案，改善在工程化的酵母菌株中由糖产生牛心果碱的酪氨酸羟化酶突变体。

实施例3：DHFR的表达改善工程化酵母菌株中的酪氨酸羟化酶活性

[0284] 将来自褐家鼠的二氢叶酸还原酶(DHFR)进行酵母密码子优化、合成并且克隆到GPD启动子控制下的低拷贝质粒中。DHFR与野生型RnTyrH(具有GPD启动子的低拷贝质粒)在酵母菌株中共表达，所述酵母菌株包含对中心代谢的以下突变(如美国临时专利申请序列号61/899,496中所述)：ARO4^{FBR}、ΔZWF1和GPD-TKL1启动子替换。此外，所述菌株表达来自褐家鼠的生成共底物四氢生物蝶呤(丙酮酰四氢蝶呤合酶，PTPS；墨蝶呤还原酶，SepR；蝶呤4a-甲醇胺脱水酶，PCD；二氢蝶啶还原酶，QDHPR)的四种染色体整合的基因。使表达DHFR和野生型RnTyrH的菌株在缺乏酪氨酸的具有2％右旋糖的选择性限定培养基(YNB)中生长96小时，并且通过LC-MS/MS以MRM模式利用198m/z至152m/z的跃迁测量培养基中L-DOPA的产生。DHFR与野生型RnTyrH的表达使L-DOPA产生增加1.8倍，如图14中所示。因而，图14示出根据本发明的实施方案，改善工程化的酵母菌株中通过酪氨酸羟化酶实现的L-DOPA产生的二氢叶酸还原酶(DHFR)的共表达。

实施例4：向生长培养基中添加抗氧化剂改善工程化的酵母菌株中的酪氨酸羟化酶活性

[0285] 使酵母菌株在缺乏酪氨酸的具有2％半乳糖和2mM抗坏血酸的选择性限定培养基(YNB)中生长96小时，所述酵母菌株包含对中心代谢的以下突变(如美国临时专利申请序列号61/899,496中所述)：ARO4^{FBR}、ΔZWF1和GPD-TKL1启动子替换并且由GPD启动子控制下的

低拷贝质粒表达来自褐家鼠的生成共底物四氢生物蝶呤(丙酮酰四氢蝶呤合酶,PTPS;墨蝶呤还原酶,SepR;蝶呤4a-甲醇胺脱水酶,PCD;二氢蝶啶还原酶,QDHPR)的四种染色体整合的基因以及野生型RnTyrH。

[0286] 通过LC-MS/MS以MRM模式,利用198m/z至152m/z的跃迁测量培养基中L-DOPA的产生。2mM抗坏血酸的添加使利用野生型RnTyrH的L-DOPA产生改善1.8倍。此外,利用LC-MS/MS以MRM模式,利用以下跃迁测量BH_4中间体浓度:B,238m/z至178m/z;BH2,240m/z至165m/z;以及BH4,242m/z至166m/z。抗坏血酸的添加还增加培养基中的BH_4,这表明防止了BH_4氧化成BH_2。

[0287] 因此,图15示出根据本发明的实施方案,(A)向培养基中添加抗氧化剂改善工程化的酵母菌株中通过酪氨酸羟化酶实现的L-DOPA产生,以及(B)向培养基中添加增加BH_4水平的抗氧化剂。具体地讲,图15A示出野生型RnTyrH(由GPD启动子控制下的低拷贝质粒表达)在酵母菌株中表达,所述酵母菌株包含对中心代谢的以下突变(如美国临时专利申请序列号61/899,496中所述):$ARO4^{FBR}$、ΔZWF1和GPD-TKL1启动子替换。此外,所述菌株表达来自褐家鼠的生成共底物四氢生物蝶呤(丙酮酰四氢蝶呤合酶,PTPS;墨蝶呤还原酶,SepR;蝶呤4a-甲醇胺脱水酶,PCD;二氢蝶啶还原酶,QDHPR)的四种染色体整合的基因。使表达野生型RnTyrH的菌株在缺乏酪氨酸的具有2%右旋糖、具有和不具有2mM抗坏血酸(aa)的选择性限定培养基(YNB)中生长96小时。通过LC-MS/MS以MRM模式,利用198m/z至152m/z的跃迁测量培养基中L-DOPA的产生。另外,图15B示出,在图15A中所述的相同菌株中,利用LC-MS/MS以MRM模式,利用以下跃迁:BH_4,242m/z至166m/z测量菌株在具有和不具有2mM抗坏血酸(aa)的情况下生长的培养基中的BH_4中间体的浓度。

实施例5:被工程化为产生bisBIA黄芦木碱的酵母菌株

[0288] 图16示出根据本发明的实施方案,(A)用于将L-酪氨酸转化成bisBIA的生物合成方案,以及(B)被工程化为生物合成bisBIA的酵母菌株。具体地讲,图16示出(A)用于产生bisBIA黄芦木碱和guattegaumerine的途径。图16提供了酶ARO9,芳族转氨酶;ARO10,苯丙酮酸脱羧酶;TyrH,酪氨酸羟化酶;DODC,DOPA脱羧酶;NCS,去甲乌药碱合酶;6OMT,6-O-甲基转移酶;CNMT,乌药碱N-甲基转移酶;CYP80A1,细胞色素P450 80A1;CPR,细胞色素P450NADPH还原酶的使用。在图16提供的代谢物中,4-HPA、4-HPP和L-酪氨酸在酵母中天然合成。图16所示的其他代谢物不在酵母中天然产生。

[0289] 在本发明的实例中,产生bisBIA(诸如使用(A)所示的途径生成的那些)的产bisBIA酵母菌株通过将单个构建体整合到基因座YDR514C中而被工程化。另外,图16提供了(B)被工程化为合成bisBIA的示例性酵母菌株。在单个基因座(YDR514C)处将Ps6OMT、PsCNMT、PsCPR和BsCYP80A1整合到酵母基因组中。每种酶由组成型启动子表达。基因表达盒的布置和取向在示意图中通过箭头指示。这些菌株将(R)-和(S)-去甲乌药碱转化成乌药碱,接着转化成N-甲基乌药碱。在一个实例中,菌株可接着缀合一个分子的(R)-N-甲基乌药碱和一个分子的(S)-N-甲基乌药碱以形成黄芦木碱。在另一个实例中,菌株可缀合两个分子的(R)-N-甲基乌药碱,以形成guattegaumerine。在另一个实例中,菌株可缀合一个分子的(R)-N-甲基乌药碱和一个分子的(S)-乌药碱以形成2'-去甲黄芦木碱。在另一个实施方案中,菌株可被工程化为由L-酪氨酸供应前体(R)-和(S)-去甲乌药碱,如图5中所提供。

[0290] 构建体包括作为它们的天然植物核苷酸序列表达的罂粟酶6OMT和CNMT的表达盒。

来自罂粟的第三种酶CPR经密码子优化以在酵母中表达。PsCPR支持第四种酶伏牛花CYP80A1的活性，所述伏牛花CYP80A1也经密码子优化以在酵母中表达。表达盒各自包括唯一的酵母组成型启动子和终止子。最终，整合构建体包括侧接通过Cre重组酶切除的loxP位点的LEU2选择标记。

[0291] 将表达Ps6OMT、PsCNMT、BsCYP80A1和PsCPR的酵母菌株在选择培养基中在30℃下振荡培养16小时。通过离心收获细胞并且将其重悬于400μL破碎缓冲液(100mM Tris-HCl，pH 7.0;10％甘油;14mM 2-巯基乙醇;蛋白酶抑制剂混合液)中。通过添加玻璃珠并且涡旋物理破坏细胞。移除液体并且添加以下底物和辅因子以起始反应:1mM(R,S)-去甲乌药碱、10mM S-腺苷甲硫氨酸、25mM NADPH。将粗细胞裂解物在30℃下孵育4小时，然后通过1:1添加用0.1％乙酸酸化的乙醇淬灭。将反应物离心并且通过液相色谱-质谱(LC-MS)分析上清液以通过它们的保留和质量/电荷检测bisBIA产物黄芦木碱、guattegaumerine和2'-去甲黄芦木碱。

实施例6.差向异构酶的鉴定

[0292] 为了鉴定适用于进行本文所公开的方法的差向异构化反应的差向异构酶，在公共可用的植物转录组中的单个开放阅读框(CYP-COR)中鉴定细胞色素P450氧化酶82Y1样结构域和可待因酮还原酶样结构域。CYP-COR融合物使用blastn由1000Plants Project(Matasci等人2014.Gigascience.3:17)和PhytoMetaSyn(Facchini等人2012.Trends Biotechnol.30:127-31;Xiao等人2013.J.Biotechnol.166:122-34)转录组的BLAST搜索鉴定，其中查询为导致牛心果碱积聚的先前公布的COR沉默的VIGS构建体的序列(Wijekoon和Facchini.2012.Plant J.69:1052-63)。一旦观察到一个CYP-COR融合物序列作为命中，就翻译所述序列并且使用氨基酸序列作为查询，以使用tblastn进行两个数据库的二次搜索。由数据库鉴定的CYP-COR融合酶的系统发育树提供于图17。基于生物信息学搜索由The 1000Plants Project和PhytoMetaSyn转录组数据库鉴定序列。另外，示例性氨基酸序列提供于图4中，如上文所讨论的。另外，表1列出了针对这种CYP-COR酶鉴定的氨基酸序列的各种实例，它们来自各种植物，包括罂粟(鸦片罂粟)、渥美罂粟(特洛伊罂粟(poppy of Troy))、大红罂粟(伊朗罂粟(Iranian poppy))，以及白屈菜(白屈菜(greater celandine))。

实施例7.工程化的非植物宿主细胞中(S)-牛心果碱至(R)-牛心果碱的差向异构化

[0293] 将非植物宿主细胞工程化为异源表达本文所述的酶。例如，将酵母菌株(酿酒酵母)工程化为异源表达实施例6中所述的所鉴定的差向异构酶并且在此微生物宿主的背景中验证它们的功能。将部分氨基酸序列pbr.PBRST1PF_4328和pbr.PBRST1PF_89405的酵母密码子优化的DNA编码序列与SSDU-2015634(表1)的氨基酸1-40的酵母密码子优化的编码序列框内合成，以分别生成CYP-COR_4328和CYP-COR_89405。将这些CYP-COR编码序列克隆到携带URA3选择标记的低拷贝质粒中并且由TDH3启动子表达。将质粒转化到携带整合到染色体中的细胞色素P450还原酶(P_{TEF1}-ATR1或P_{TEF1}-PsCPRv2)的表达盒的酵母菌株中。使携带两种质粒的这些酵母菌株在具有适当的缺陷性溶液(drop out solution)(-Ura-Trp)的合成完全培养基中生长。向酵母菌株进料(S)-牛心果碱并且在生长72小时之后通过LC-MS/MS分析对BIA代谢物进行分析。

实施例8.在工程化的酵母细胞中由(S)-牛心果碱产生沙罗泰里啶

[0294] 将酵母菌株(酿酒酵母)工程化为异源表达实施例6中所述的所鉴定的差向异构酶并且在此微生物宿主的背景中验证它们的功能。将部分氨基酸序列pbr.PBRST1PF_4328和pbr.PBRST1PF_89405的酵母密码子优化的DNA编码序列与SSDU-2015634(表1)的氨基酸1-40的酵母密码子优化的编码序列框内合成,以分别生成CYP-COR_4328和CYP-COR_89405。将这些CYP-COR编码序列克隆到携带URA3选择标记的低拷贝质粒中并且由TDH3启动子表达。将沙罗泰里啶合酶(SalSyn)编码序列克隆到携带TRP1选择标记的低拷贝质粒中并且由TDH3启动子表达。将质粒转化到携带整合到染色体中的细胞色素P450还原酶(P_{TEF1}-ATR1或P_{TEF1}-PsCPRv2)的表达盒的酵母菌株中。使携带两种质粒的这些酵母菌株在具有适当的缺陷性溶液(drop out solution)(-Ura-Trp)的合成完全培养基中生长。向酵母菌株进料(S)-牛心果碱并且在生长72小时之后通过LC-MS/MS分析对BIA代谢物进行分析。分析表明,工程化的酵母细胞能够将(S)-牛心果碱转化成(R)-牛心果碱,(R)-牛心果碱接着受到沙罗泰里啶合酶的作用形成沙罗泰里啶,一种4环原吗啡喃生物碱(图7、图18)。沙罗泰里啶合酶先前已经显示出作用于(R)-牛心果碱并且对(S)-牛心果碱没有可观察到的活性(Gesell等人2009.J.Biol.Chem.284:24432-42)。

[0295] 如图7所示,CYP-COR催化(S)-牛心果碱到(R)-牛心果碱的转化,(R)-牛心果碱接着受到沙罗泰里啶合酶的作用以制备原吗啡喃生物碱沙罗泰里啶。图18示出(A)示出两种差向异构酶变体(CYP-COR_89405、CYP-COR_4328)的牛心果碱和沙罗泰里啶以及标准品的色谱图迹线。图18还示出(B)重新作图以展示与标准品的共洗脱的(A)中的沙罗泰里啶的相同色谱图迹线。在此实验中,酵母包含具有URA3和TRP1选择性标记、TDH3启动子以及CYP-COR和SalSyn编码序列的两种低拷贝CEN/ARS质粒。使酵母从新鲜转化的菌落开始在3mL选择培养基中生长过夜、反向稀释到3.5mL培养基中至OD 0.8,生长7小时,沉淀,接着重悬于具有100μM(S)-牛心果碱(Specs)的pH 7.4HEPES缓冲液中。在30℃的旋转器上16小时之后,将酵母沉淀并且通过LC-MS/MS分析缓冲液上清液。每条迹线来自代表2个样品的单个样品。将峰归一化,以使所有色谱图中最大的峰为100%。

实施例9.在工程化的非植物宿主细胞中由外消旋的全去甲劳丹碱产生(R)-牛心果碱

[0296] 将酵母菌株(酿酒酵母)工程化为异源表达实施例6中所述的所鉴定的差向异构酶并且在此微生物宿主的背景中验证它们的功能。将实施例7中所述的经酵母密码子优化的DNA编码序列CYP-COR_89405克隆到携带URA3选择标记的低拷贝质粒中并且由TDH3启动子表达。将此质粒转化到携带整合到染色体中的细胞色素P450还原酶(P_{TEF1}-ATR1或P_{TEF1}-PsCPRv2)和三种甲基转移酶(罂粟去甲乌药碱-6-O-甲基转移酶、乌药碱N-甲基转移酶和3'-羟基-N-甲基乌药碱4'-O-甲基转移酶,均由P_{TEF1}表达)的表达盒的酵母菌株中。使携带该质粒的这种酵母菌株在具有适当的缺陷性溶液(-Ura)的合成完全培养基中生长。向酵母菌株进料外消旋的全去甲劳丹碱并且在生长72小时之后通过LC-MS/MS分析对BIA代谢物进行分析。对于手性表征,通过沉淀5mL酵母培养物由酵母培养基浓缩牛心果碱并且向4mL上清液中添加120mg XAD-4树脂,在室温下在旋转机上孵育过夜,并且用0.5mL甲醇洗脱。通过反相HPLC(Pursuit XRs-C18,5μm,50mm×10mm),利用等度15%甲醇与0.1%甲酸经6.5min将浓缩物分级,其中流速为5mL/min并且进样体积为40-50μL。在大约4.5min时收集基于峰的级分。将级分汇集、冷冻干燥并且重悬于0.5mL异丙醇中。根据浓度,将0.5-5μL进样到手性柱(Phenomenex Lux纤维素-1,3μm,150mm x 2mm)上,利用等度的72%N-己烷、28%异丙

醇、0.1％二乙胺分离，其中流速为0.3mL/min，并且通过MS和250nm UV进行检测。通过Agilent6320Ion Trap质谱仪，利用ESI源气体温度350℃、10L/min的气体流、喷雾器压力40PSI以及m/z 330.1的隔离(宽度为1.0)进行MS检测。将牛心果碱峰的保留时间与真正的(S)-牛心果碱和(R)-牛心果碱标准品的保留时间进行比较。分析表明，包含CYP-COR质粒的工程化的酵母细胞能够将外消旋的全去甲劳丹碱转化成(R)-牛心果碱，而具有空质粒的工程化的酵母细胞仅产生(S)-牛心果碱(图19)。

实施例10:沙罗泰里啶合酶的蛋白质工程化当在微生物宿主中表达时改善其加工和活性

[0297] 异源蛋白质当在重组宿主中表达时可能不正确加工，例如，植物蛋白，诸如在微生物产生宿主中表达的细胞色素P450酶。例如，将(R)-牛心果碱转化成沙罗泰里啶的沙罗泰里啶合酶当在酵母中异源表达时发生N-连接的糖基化(图20)。在沙罗泰里啶合酶上所观察到的N-连接的糖基化模式当酶在植物中表达时观察不到，并且指示了初生SalSyn转录物的不正确的N末端分选，这降低了酶在异源微生物宿主中的活性。因此，针对校正初生转录物的N末端分选从而去除N连接的糖基化模式的蛋白质工程化将导致沙罗泰里啶合酶在重组产生宿主中的活性得到改善。

[0298] 例如，使用来自碎叶紫堇碱合酶(CFS)的N末端α-螺旋替换来自沙罗泰里啶合酶(SalSyn，图21)的N末端α-螺旋。这些融合物的接合点基于CFS和SalSyn的二级结构基序或基于CFS和SalSyn的氨基酸比对进行选择。通过用与另一个片段重叠的15-40个核苷酸扩增来自CFS的N末端片段和来自SalSyn的C末端片段，然后通过Gibson组装将它们与彼此以及载体主链组装以形成完整的融合物开放阅读框来克隆融合物(Gibson等人2009.Nat Methods.6:343-5)。

[0299] 又如，将来自沙罗泰里啶合酶的细胞色素P450结构域的编码序列直接置于其他稳定表达的细胞色素P450诸如BM3酶的P450编码区中。例如，沙罗泰里啶合酶的保守细胞色素P450结构域和来自巨大芽胞杆菌(Bacillus megaterium)P450单加氧酶CYP102A1(BM3，(Michener和Smolke.2012.Metab.Eng.14:306-16))的工程化变体的细胞色素P450结构域通过NCBI保守结构域搜索进行鉴定。设计引物将BM3的前几个氨基酸的编码序列与沙罗泰里啶合酶的P450结构域的编码序列融合，之后将BM3结构域C末端的编码序列与P450结构域融合。与之前一样，通过Gibson组装来组装此构建体。

[0300] 通过蛋白质印迹分析来分析工程化的沙罗泰里啶合酶蛋白融合物，以确认在酵母中的全长表达以及N-连接的糖基化模式的改变或消除(图20)。将沙罗泰里啶合酶和蛋白质融合物用人流感血凝素(HA)表位在C末端标记并且克隆到适用于酵母和植物表达的表达质粒中。对于酵母，将酶编码序列克隆到携带URA3选择标记的低拷贝酵母/大肠杆菌穿梭载体中并且由TDH3启动子表达。对于植物，通过根癌农杆菌浸润将序列克隆到具有卡那霉素抗性和花椰菜花叶病毒(CaMV)35S启动子的大肠杆菌/根癌农杆菌穿梭载体中以用于瞬时植物表达，所述花椰菜花叶病毒(CaMV)35S启动子具有侧接的来自豇豆花叶病毒RNA-2的5’和3’-非翻译区。被工程化为表达沙罗泰里啶合酶的酵母表现出指示了N-连接的糖基化的带型。通过在糖基化位点上进行定点诱变，我们确认了这种图谱是由N-连接的糖基化造成的。相比之下，该酶的植物表达不导致指示N-连接的糖基化的带型，如图20的(A)中所见。虽然N-连接的糖基化位点未修饰，但是工程化的沙罗泰里啶合酶蛋白融合物当在酵母中表达时

不被N-糖基化，如图20的(B)中所见。通过蛋白质印迹，我们展示了酵母表达的融合酶呈现为单个条带，类似于对于植物表达的亲本酶所观察到的表达，这表明了导致N-连接的糖基化的在酵母中的初生蛋白质错误加工通过工程化的融合物修复。

[0301] 针对当在酵母中异源表达时改善的酶活性对工程化的沙罗泰里啶合酶蛋白融合物进行分析。将沙罗泰里啶合酶和工程化融合物的编码序列克隆到携带URA3选择标记的低拷贝质粒中并且由TDH3启动子表达。酵母具有整合到TRP1基因座中的P_{TEF1}-PsCPRv2并且包含具有URA3选择标记和沙罗泰里啶合酶编码序列以及TDH3启动子的单个低拷贝质粒。使酵母从新鲜转化的菌落开始在1mL选择培养基(-Ura)中生长过夜并且反向稀释1:20到具有10μM(R)-牛心果碱(Toronto Research Chemicals)的96孔板中的0.5mL选择培养基中。在振荡培养箱中72-96小时之后，将酵母沉淀并且通过LC-MS/MS分析培养基上清液。分析表明，当在酵母中异源表达时，相对于野生型序列，工程化的沙罗泰里啶合酶表现出改善的活性(图22)。

[0302] 图22示出根据本发明的实施方案，改善在酵母中的活性的沙罗泰里啶合酶密码子优化和工程化融合物。如图22中所见，黑色条柱指示沙罗泰里啶合酶的天然野生型序列PsCYP719B1。具有黑色边界的灰色条柱为来自罂粟的经酵母密码子优化的变体和来自大红罂粟的新鉴定的序列。有斜线图案的条柱指示基于大红罂粟序列的具有最大改善的工程化融合物。误差线指示至少两个生物学重复的范围。实质上，来自大红罂粟、罂粟或渥美罂粟(或相关植物)的沙罗泰里啶合酶的合成的密码子优化的和/或蛋白质工程化的变体可用于这些工程化菌株中。

[0303] 工程化的沙罗泰里啶合酶蛋白融合物可在生物合成途径的背景中使用，以增加下游苄基异喹啉生物碱产物的产生。在一个实例中，将酵母工程化为异源表达编码工程化的沙罗泰里啶合酶融合物、大红罂粟沙罗泰里啶还原酶以及罂粟沙罗泰里啶醇7-O-乙酰转移酶的经酵母密码子优化的基因。将三个表达盒(P_{TDH3}-D94yPsSS、P_{TPI1}-yPbSalR、P_{TEF1}-yPsSalAT)组装到具有TRP1区段标记的酵母人工染色体(YAC)中。将YAC置于携带整合到染色体中的细胞色素P450还原酶(P_{TEF1}-ATR1或P_{TEF1}-yPsCPRv2)的表达盒的酵母中。使酵母菌株在具有适当的缺陷性溶液(-Trp)的合成完全培养基中生长并且进料(R)-牛心果碱。在生长96小时后通过LC-MS/MS分析对BIA代谢物进行分析。分析表明用工程化的沙罗泰里啶合酶和其他途径酶工程化的酵母菌株产生吗啡喃生物碱蒂巴因，如图23的(A)中所示。

[0304] 因此，图23示出(A)根据本发明的实施方案，小规模成批发酵的LC-MS/MS分析，其中工程化酵母催化(R)-牛心果碱到蒂巴因的转化。如图23的(A)中所提供的，酵母菌株被工程化为具有整合到TRP1基因座中的P_{TEF1}-ATR1表达盒并且包含单一酵母人工染色体，所述染色体具有TRP1选择标记和三个表达盒：P_{TDH3}-yEcCFS$^{1-83}$-yPsSS$^{95-505}$、P_{TPI1}-yPbSalR和P_{TEF1}-yPsSalAT。使酵母从新鲜转化的菌落开始在3mL选择培养基中生长过夜并且反向稀释1:20到具有100μM(R)-牛心果碱(Toronto Research Chemicals)的培养管中的0.5mL选择培养基中(-Trp)。在振荡培养箱中72小时之后，将酵母沉淀并且通过LC-MS/MS分析培养基上清液。色谱图迹线显示出由此菌株产生的蒂巴因以及积聚的沙罗泰里啶醇和沙罗泰里啶，以及标准品。这些迹线代表了两个样品。

实施例11：下游吗啡喃分支中的酶的蛋白质工程化改善异源微生物宿主的吗啡喃产物的产生

[0305] 在本发明的一个实施方案中，将途径酶工程化为表现出增加的活性，以增加目标BIA的产生。在该实施例中，通过用Mutazyme II(参见表6)扩增向特定途径酶的开放阅读框中引入突变。在扩增反应中包括足够的模板DNA，以产生每个基因1-4核苷酸取代的突变率。通过缺口修复直接在酵母中将诱变文库克隆到pYES1L载体中。在若干情况下，选择用于文库表达的酵母菌株包含生成诱变酶的底物的基因的整合拷贝。例如，将CODM变体的文库转化到具有T6ODM和COR1.3的整合拷贝的菌株中并且在培养基中进料蒂巴因。T6ODM和COR1.3在这些菌株中的表达确保了可待因和尼奥品将可用作各引入的CODM变体的底物。将各个菌落接种到96孔板中并且培养96小时，然后通过液相色谱-质谱(LC-MS)针对它们产物的产生进行测定。在CODM文库的实例中，所筛选的产物为吗啡和新吗啡。在每次筛选中，对具有增强的BIA产生的变体进行测序并且重新克隆以进行验证。表6包括通过筛选鉴定的在酵母中导致增加的BIA产生的突变的酶变体的汇总。

[0306] 图24示出这些突变体中的一个的经过验证的增强活性的数据。具体地讲，图24示出根据本发明的实施方案，通过随机诱变和筛选生成在酵母中表现出增强活性的CODM酶变体。CODM变体的文库通过利用易错PCR诱变编码区生成。将通过该文库的筛选鉴定的变体$CODM^{N35S,G335V}$重新克隆在携带T6ODM和COR1.3的整合拷贝的酵母菌株中并且在其中表达。将该菌株和另一种表达野生型CODM的对照菌株在具有1mM蒂巴因的液体培养基中培养。96小时后，针对CODM活性，通过LC-MS对培养基进行分析。变体$CODM^{N35S,G335V}$产生比表达野生型CODM的菌株多1.4x的吗啡和多2.6x的新吗啡。

实施例12：用以改善异源微生物宿主的苄基异喹啉生物碱产生的表达和生长条件的优化

[0307] 工程化的微生物宿主的苄基异喹啉生物碱产生可通过优化途径酶的表达和生长条件而进一步改善。在一个实例中，通过由一系列不同的启动子表达酶来改变沙罗泰里啶醇7-O-乙酰转移酶在酵母中的表达。将酵母工程化为由不同的启动子异源表达编码罂粟沙罗泰里啶醇7-O-乙酰转移酶的酵母密码子优化的基因(如图25的(A)中所提供的)。将两个表达盒(P_{TPI1}-yPbSalR、P_X-yPsSalAT)组装到具有TRP1区段标记的酵母人工染色体(YAC)中。将YAC置于酵母中并且使细胞在具有适当的缺陷性溶液(-Trp)的合成完全培养基中生长并且进料沙罗泰里啶。在生长72小时后通过LC-MS/MS分析对BIA代谢物进行分析。途径酶表达水平的优化可导致吗啡喃生物碱蒂巴因的产生增加(如图25的(A)中所提供的)。

[0308] 菌株培养条件(包括但不限于糖源、生长温度和pH)的优化可用于增加工程化的酵母菌株的苄基异喹啉生物碱的产生(如图25的(B)和(C)中所提供的)。在一个实例中，改变pH来增加工程化的酵母菌株的蒂巴因产生。将两个表达盒(P_{TPI1}-yPbSalR、P_{TEF1}-yPsSalAT)组装到具有TRP1区段标记的酵母人工染色体(YAC)中。将YAC置于酵母中并且使细胞在具有适当的缺陷性溶液(-Trp)的合成完全培养基中生长，重悬于pH5.7-9的缓冲液中并且进料沙罗泰里啶。在孵育16小时后通过LC-MS/MS分析对BIA代谢物进行分析。4环原吗啡喃生物碱沙罗泰里啶醇和5环吗啡喃生物碱蒂巴因的水平随着pH的增加而增加(如图25的(B)中所提供的)。

[0309] 在另一个实例中，改变温度、糖和培养基缓冲剂含量以增加工程化的酵母菌株的蒂巴因产生。将三个表达盒(P_{TDH3}-D94yPsSS、P_{TPI1}-yPbSalR、P_{TEF1}-yPsSalAT)组装到具有TRP1区段标记的酵母人工染色体(YAC)中。将YAC置于携带整合到染色体中的细胞色素P450

还原酶(P_{TEF1}-ATR1或P_{TEF1}-yPsCPRv2)的表达盒的酵母中。使酵母菌株在具有适当的缺陷性溶液(-Trp)的合成完全培养基中生长并且进料(R)-牛心果碱。在生长72小时后通过LC-MS/MS分析对BIA代谢物进行分析。分析表明,吗啡喃生物碱蒂巴因的微生物产生在某些培养条件下(30℃的具有右旋糖的缓冲培养基,如图25的(C)中所提供的)增加。

[0310] 因此,图25示出根据本发明的实施方案,通过工程化酵母将(R)-牛心果碱转化成蒂巴因的发酵优化。(A)SalAT启动子变体的全细胞缓冲测定的LC-MS/MS分析;(B)在不同pH条件下生长的SalR和SalAT菌株的全细胞缓冲测定的LC-MS/MS分析;以及(C)糖源、生长温度和培养基缓冲剂含量的优化。(A)被工程化为包含具有TRP1选择标记和两个表达盒的单一酵母人工染色体的酵母菌株,所述表达盒为:具有变化的SalAT启动子的P_{TPI1}-yPbSalR和P_X-yPsSalAT。使酵母从新鲜转化的菌落开始在3mL选择培养基中生长过夜并且反向稀释1:20到具有100μM沙罗泰里啶(Specs)的培养管中的0.5mL培养基中。在振荡培养箱中72小时之后,将酵母沉淀并且通过LC-MS/MS分析培养基上清液。(B)被工程化为包含具有TRP1选择标记和两个表达盒的单一酵母人工染色体的酵母菌株,所述表达盒为:P_{TPI1}-yPbSalR和P_{TEF1}-yPsSalAT。使酵母从新鲜转化的菌落开始在3mL选择培养基中生长过夜,反向稀释到3.5mL培养基中至OD 0.8,生长7小时,沉淀,接着重悬于具有10μM沙罗泰里啶(Specs)的pH 5.7的MOPS或pH 7、8或9的Tris缓冲液中。在30℃的旋转器上16小时之后,将酵母沉淀并且通过LC-MS/MS分析缓冲液上清液。误差线代表两个样品的范围。(C)糖源、生长温度和培养基缓冲剂含量的优化。在该实验中,酵母菌株被工程化为具有整合到TRP1基因座中的P_{TEF1}-ATR1并且包含单一酵母人工染色体,所述染色体具有TRP1选择标记和三个表达盒:P_{TDH3}-yEcCFS$^{1-83}$-yPsSS$^{95-505}$、P_{TPI1}-yPbSalR和P_{TEF1}-yPsSalAT。使酵母从新鲜转化的菌落开始在3mL选择培养基中生长过夜并且反向稀释1:20到具有100μM(R)-牛心果碱(Toronto Research Chemicals)的培养管中的0.5mL培养基中。在振荡培养箱中72小时之后,将酵母沉淀并且通过LC-MS/MS分析培养基上清液。

实施例13:被工程化用于由早期1-苄基异喹啉生物碱支架产生蒂巴因的酵母

[0311] 酵母菌株可被工程化用于由早期1-苄基异喹啉生物碱产生吗啡喃生物碱蒂巴因或衍生自蒂巴因的吗啡喃生物碱。例如,工程化的酵母菌株可由外消旋或(S)-去甲乌药碱或者外消旋或(S)-全去甲劳丹碱产生吗啡喃生物碱产物(图5、6、7以及图23的(B))。通过将三个或五个表达盒整合到酵母基因组中,酵母菌株被工程化为从(S)-去甲乌药碱或外消旋或(S)-全去甲劳丹碱生产(S)-牛心果碱。为了由外消旋或(S)-全去甲劳丹碱产生(S)-牛心果碱,整合的表达盒编码罂粟去甲乌药碱6-O-甲基转移酶(Ps6OMT,EC 2.1.1.128)、4'-O-甲基转移酶(Ps4'OMT,EC 2.1.1.116)以及乌药碱-N-甲基转移酶(CNMT,EC 2.1.1.140),所述表达盒各自具有TEF1启动子(Hawkins和Smolke.2008.Nat.Chem.Biol.4:564-73)。为了由外消旋或(S)-去甲乌药碱产生(S)-牛心果碱,菌株还携带用于酵母密码子优化的花菱草N-甲基乌药碱3'-羟化酶(yEcCYP80B1,EC 1.14.13.71)和由TDH3或TEF1启动子表达的ATR1或yPsCPRv2细胞色素P450还原酶(CPR,EC 1.6.2.4)的整合的表达盒。这些菌株被进一步工程化为并入差向异构化催化酶(例如,CYP-COR)、沙罗泰里啶合酶、沙罗泰里啶还原酶和沙罗泰里啶醇乙酰转移酶,以将外消旋或(S)-去甲乌药碱或者外消旋或(S)-全去甲劳丹碱转化成吗啡喃生物碱蒂巴因,或衍生自蒂巴因的吗啡喃生物碱(图7)。作为差向异构化催化酶的表达的替代形式,6OMT、4'OMT、CNMT和/或CYP80B1可被工程化,使得由外消旋的去甲乌药

碱或外消旋的全去甲劳丹碱产生外消旋的牛心果碱。

[0312] 在一个实例中，酵母菌株被工程化为将外消旋的全去甲劳丹碱转化成蒂巴因。酵母菌株携带编码Ps6OMT、Ps4'OMT、CNMT和yPsCPRv2的整合的表达盒，所述表达盒各自具有TEF1启动子。在此菌株中将四个表达盒(P_{TDH3}-yEcCFS$^{1-83}$-yPsSS$^{95-505}$、P_{TPI1}-yPbSalR、P_{TEF1}-yPsSalAT、P_{HXT7}-CYP-COR_89405)组装到具有TRP1选择标记的酵母人工染色体(YAC)中。使携带YAC和整合的盒的酵母菌株在具有适当的缺陷性溶液(-Trp)和1mM外消旋的全去甲劳丹碱底物的合成完全培养基中生长。生长96小时后，通过LC-MS/MS分析针对BIA代谢物对培养基进行分析。检测到将近200nM蒂巴因(图23的(B))。其他工程化的沙罗泰里啶合酶变体也可用于此菌株(图22，实施例10)。

实施例14：被工程化用于由L-酪氨酸产生牛心果碱的平台酵母菌株

[0313] 构建由L-酪氨酸产生关键分支点BIA中间体(S)-牛心果碱的平台酵母菌株(图5)。具体地讲，将四种多基因表达构建体整合到酵母菌株的基因组中。四种构建体的组成在图26中指出。每种构建体包括由强组成型启动子表达的4或5种基因。基因定位在每个基因座处作为包括启动子、基因开放阅读框和终止子的完整表达盒，如示意图上方的注释中所指明的。示意图通过代表给定基因的箭头的方向示出每个表达盒的取向。选择性标记在注释中是斜体的并且在示意图中通过灰色箭头表示。每个选择标记侧接loxP位点，以允许从基因座中移除标记。另外，每个构建体具有侧接loxP位点的选择性标记，以使通过Cre重组酶将其移除。

[0314] 在第一整合构建体中，将来自褐家鼠的四个异源基因与G418选择标记(KanMX)一起整合到YBR197C基因座中。需要RnPTPS、RnSepR、RnPCD和RnQDHPR来由酵母内源性叶酸合成途径合成和再生四氢生物蝶呤(BH_4)。每个基因经密码子优化以在酵母中表达。

[0315] 在第二整合构建体中，将四个异源基因与HIS5选择标记一起整合到HIS3基因座中。褐家鼠酪氨酸羟化酶(RnTyrH)使用由先前的整合构建体生成的共底物BH_4将酪氨酸转化成L-DOPA。RnTyrH基因可以是野生型或赋予增强活性的改善的突变体(例如，W166Y、R37E和R38E，实施例2)中的任一个。第二褐家鼠基因RnDHFR编码将二氢生物蝶呤(BH_4的氧化产物)还原为BH_4的酶，以此方式增加该共底物的可利用性。第三构建体中还包括来自恶臭假单胞菌的PpDODC，一种将L-DOPA转化成多巴胺的酶。第四种酶为来自日本黄连的CjNCS，其缩合4-HPA和多巴胺以制备去甲乌药碱。每个基因经密码子优化以在酵母中表达。

[0316] 在第三整合构建体中，将来自植物的五种异源基因和LEU2选择标记整合到基因座YDR514C中。Ps6OMT、Ps4'OMT和PsCNMT为来自罂粟的甲基转移酶并且作为天然植物核苷酸序列表达。第四罂粟基因yPsCPRv2针对酵母进行密码子优化并且编码支持来自花菱草的细胞色素P450的活性的还原酶EcCYP80A1。EcCYP80A1作为其天然植物核苷酸序列表达。在此构建体中编码的酶进行两种O-甲基化、一种N-甲基化以及一种羟基化，以由先前的整合构建体产生的去甲乌药碱产生牛心果碱。

[0317] 在最后的整合构建体中，将酿酒酵母内源性基因ARO4^{Q166K}、ARO7^{T226I}、TKL1和ARO10的另外拷贝与潮霉素抗性选择标记一起整合到ARO4基因座中。ARO4^{Q166K}和ARO7^{T226I}为ARO4和ARO10的反馈抗性突变体，相对于野生型序列而言，其各自编码单个碱基对取代。TKL1和ARO10与天然酵母基因相同，但是在强启动子后面表达。Aro4p和Aro7p为芳族氨基酸(包括酪氨酸)的生物合成中的酶。从这些酶中移除反馈抑制导致内源性酪氨酸生物合成的上调。

Tkl1p的过表达使戊糖磷酸途径上调，从而导致酪氨酸的前体赤藓糖4-磷酸(E4P)的供应增强。Aro10p的过表达增加4-HPA的产生。

[0318] 可用任何数目的所述四个表达盒构建平台酵母菌株。具体地讲，利用整合构建体1-4和整合构建体1-3构建平台酵母菌株。在其中不包括酪氨酸过量产生构建体(构建体4)的后一菌株中，可在培养基中供应另外的酪氨酸以支持牛心果碱的生物合成。可将另外的基因修饰并入平台菌株中，以支持下游BIA的产生和至BIA生物合成的增加的流量。

[0319] 使酵母菌株在具有适当的氨基酸缺陷性溶液的合成完全培养基中在25和30℃下生长。在生长48和96小时之后通过LC-MS/MS分析对培养基上清液中的BIA代谢物进行分析。

实施例15：被工程化用于由L-酪氨酸产生蒂巴因和其他吗啡喃生物碱的酵母

[0320] 酵母菌株可被工程化用于由早期前体诸如酪氨酸产生吗啡喃生物碱蒂巴因或衍生自蒂巴因的吗啡喃生物碱。例如，实施例14中所述的平台酵母菌株可进一步工程化以由L-酪氨酸产生吗啡喃生物碱产物(图7)。

[0321] 将由L-酪氨酸产生(S)-牛心果碱的平台酵母菌株(参见实施例14中的描述)进一步工程化以并入差向易构化催化酶，诸如新鉴定的CYP-COR、沙罗泰里啶合酶、沙罗泰里啶还原酶以及沙罗泰里啶醇乙酰转移酶，以将生物合成的(S)-牛心果碱转化成吗啡喃生物碱蒂巴因或衍生自蒂巴因的吗啡喃生物碱(图7)。直接在平台酵母菌株中将三个表达盒(P_{TDH3}-yEcCFS$^{1-26}$-yPbSS$^{33-504}$、P_{TPI1}-yPbSalR、P_{TEF1}-yPsSalAT)组装到具有TRP1选择标记的酵母人工染色体(YAC)中。其他工程化的沙罗泰里啶合酶变体也可并入YAC中(图22，实施例10)。所得酵母菌株也用具有URA3选择标记、TDH3启动子和CYP-COR编码序列的低拷贝CEN/ARS质粒转化。

[0322] 使携带YAC、低拷贝质粒和整合盒的酵母菌株在具有适当的缺陷性溶液(-Ura-Trp)的合成完全培养基中在25和30℃生长。生长96小时后，通过LC-MS/MS分析针对BIA代谢物对培养基进行分析。进行关于温度、碳源、pH条件和培养基组成的进一步培养基优化来改善BIA产生。

[0323] 可将另外的基因修饰引入酵母菌株中以产生衍生自蒂巴因的吗啡喃生物碱(图7)。在一个实例中，将表达盒P_{ADH1}-T6ODM-T_{ADH1}、P_{HXT7}-COR-T_{PGK1}和P_{TEF1}-CODM-T_{CYC1}直接组装并且整合到产蒂巴因酵母菌株的trp1基因座中(Thodey等人，2014)。在另一个实例中，可通过直接组装表达盒P_{GPD}-morA-T_{CYC1}、P_{PGK1}-morB-T_{PHO5}并且将此构建体整合到染色体上的ura3基因座中来将这些酵母菌株进一步工程化以产生另外的吗啡生物碱(Thodey等人，2014)。

实施例16：被工程化用于由L-酪氨酸产生小檗碱和其他原小檗碱生物碱的酵母

[0324] 酵母菌株可被工程化用于由L-酪氨酸产生生物碱小檗碱和原小檗碱、苯酞异喹啉，以及衍生自小檗碱或参与形成小檗碱的中间体的小檗碱生物碱(图5、8)。例如，将三个或四个表达盒(P_{PGK1}-PsBBE-T_{PHO5}、P_{TEF1}-yPsS9OMT-T_{CYC1}、P_{TDH3}-yCjCAS-T_{ADH1}，具有或不具有P_{TPI1}-yBwSTOX-T_{STE2})组装到具有TRP1区段标记的酵母人工染色体(YAC)中。将YAC置于由L-酪氨酸产生(S)-牛心果碱(参见实施例14)并且另外携带整合到染色体中的细胞色素P450还原酶(P_{TEF1}-ATR1或P_{TEF1}-PsCPRv2)的表达盒的平台酵母菌株中。将另一种包含P_{TEF1}-S9OMT-T_{CYC1}表达盒的高拷贝质粒转化到该菌株中以改善至下游产物的通量。

[0325] 使酵母菌株在具有适当的氨基酸缺陷性溶液(-Trp)的合成完全培养基中在25和30℃下生长。在生长48和96小时之后通过LC-MS/MS分析对培养基上清液中的BIA代谢物进

行分析。

实施例17:被工程化用于由L-酪氨酸产生诺斯卡品和其他类诺斯卡品生物碱的酵母

[0326] 酵母菌株可被工程化用于由L-酪氨酸产生苯酞异喹啉生物碱诸如那可托灵和诺斯卡品、参与形成诺斯卡品的中间体,或其衍生物(图5、9)。

[0327] 例如,将表达盒P_{ADH1}-CAS-T_{ADH1}、P_{HXT7}-CYP82Y1-T_{PGK1}、P_{TEF1}-S9OMT-T_{CYC1}和P_{PGK1}-BBE-T_{PHO5}直接组装并且整合到由L-酪氨酸产生(S)-牛心果碱(参见实施例14)并且另外携带整合到染色体中的细胞色素P450还原酶(P_{TEF1}-ATR1或P_{TEF1}-PsCPRv2)的表达盒的平台酵母菌株的trp1基因座中。接着,将表达盒P_{GPD}-TNMT-T_{CYC1}、P_{PGK1}-PsMT2-T_{PHO5}、P_{ADH1}-CYP82X1-T_{GAP1}和P_{PYK1}-PsCXE1-T_{Mf1}直接组装并且整合到酵母菌株的ura3基因座中。接着,将表达盒P_{HXT7}-CYP82X2-T_{CYC1}、P_{GPD}-PsAT1-T_{ADH1}、P_{TPI1}-PsSDR1-T_{Ste2}和P_{PGK1}-PsMT3-T_{PHO5}直接组装并且整合到酵母菌株的leu2基因座中。将包含P_{TEF1}-S9OMT-T_{CYC1}表达盒的另外的高拷贝质粒和包含P_{HXT7}-CYP82X2-T_{CYC1}表达盒的另外的低拷贝质粒转化到此酵母菌株中以改善至下游产物的通量。

[0328] 使酵母菌株在具有适当的氨基酸缺陷性溶液(-Trp、Ura)的合成完全培养基中在25和30℃下生长。在生长48和96小时之后通过LC-MS/MS分析对培养基上清液中的BIA代谢物进行分析。

实施例18:被工程化用于由L-酪氨酸产生血根碱和其他苯菲啶生物碱的酵母

[0329] 酵母菌株可被工程化用于由L-酪氨酸产生原小檗碱、原阿片碱以及苯菲啶生物碱诸如最终产物血根碱、参与形成血根碱的中间体,或其衍生物(图5、10)。

[0330] 例如,将表达盒P_{ADH1}-TNMT-T_{ADH1}、P_{HXT7}-EcSTS-T_{PGK1}、P_{GPD}-EcCFS-T_{CYC1}和P_{PGK1}-BBE-T_{PHO5}直接组装并且整合到由L-酪氨酸产生(S)-牛心果碱(参见实施例14)并且另外携带整合到染色体中的细胞色素P450还原酶(P_{TEF1}-ATR1或P_{TEF1}-PsCPRv2)的表达盒的平台酵母菌株的trp1基因座中。接着,将表达盒P_{GPD}-PsMSH-T_{CYC1}、P_{PGK1}-PsP6H-T_{PHO5}直接组装并且整合到酵母菌株的ura3基因座中。

[0331] 使酵母菌株在具有适当的氨基酸缺陷性溶液的合成完全培养基中在25和30℃下生长。在生长48和96小时之后通过LC-MS/MS分析对培养基上清液中的BIA代谢物进行分析。

实施例19:被工程化用于由L-酪氨酸产生氢可酮和其他吗啡喃生物碱的酵母

[0332] 酵母菌株可被工程化用于产生氢可酮。例如,实施例15中所述的产蒂巴因酵母菌株可被进一步工程化以产生活性药物成分氢可酮。图27示出根据本发明的实施方案,工程化的酵母菌株中的蒂巴因和氢可酮产生。在图27A中,将多基因构建体并入包含构建体1-4的工程化的酵母菌株中(图26)。在图27B中,相对于包含构建体1-4的菌株而言,构建体5的整合增加牛心果碱滴度。在图27C中,构建体6的整合导致由糖产生蒂巴因。工程化菌株所产生的蒂巴因通过LC-MS/MS分析在培养基中鉴定并且与商业参考标准进行比较。在图27D中,在YAC上引入构建体7导致由糖产生氢可酮。工程化菌株所产生的氢可酮通过LC-MS/MS分析在培养基中鉴定并且与商业参考标准进行比较。

[0333] 在一个实例中,将由L-酪氨酸产生牛心果碱的酵母菌株(参见实施例14中的描述)进一步工程化以增加牛心果碱产生,接着进一步修饰以并入实施例15中所述的产蒂巴因的酶,然后进一步修饰以并入蒂巴因脱甲基酶和吗啡酮还原酶以将生物合成的蒂巴因转化成氢可酮。将三种多基因表达构建体并入实施例14中所述的产牛心果碱的酵母菌株中;两种

作为染色体整合，并且第三种作为附加型YAC构建体。

[0334] 在第一构建体中，将基因并入以增加实施例14中所述的菌株的(S)-牛心果碱的生物合成。为了增加(S)-牛心果碱产生，将另外拷贝的CjNCS(用以增加多巴胺到去甲牛心果碱中的并入)和Ps4'OMT(用以增加3'-羟基-N-甲基乌药碱到(S)-牛心果碱中的并入)并入菌株中。还包括了另一拷贝的RnTyrH，以增加进入用于牛心果碱生物合成的异源途径的酪氨酸。将三个表达盒(P_{PGK1}-yCjNCS-T_{PHO5}、P_{TEF1}-Ps4'OMT-T_{CYC1}和P_{GPD}-RnTyrH^{WR}-T_{ADH1})与用于腐草霉素抗性的选择标记一起整合到基因座YPL250C中(图27A)。所得的酵母菌株表现出牛心果碱积聚的4倍增加(图27B)。

[0335] 在第二构建体中，并入由生物合成的(S)-牛心果碱产生蒂巴因的基因。将用于实施例15中所述的蒂巴因产生的四种酶组装为四个表达盒(P_{HXT7}-yPbCYP-COR-T_{CYC1}、P_{GPD}-yEcCFS$^{1-83}$-yPbSalSyn$^{92-504}$-T_{ADH1}、P_{TPI1}-yPbSalR-T_{STE2}和P_{PGK1}-yPsSalAT-T_{PHO5})并且整合到具有选择标记KlURA3的基因座TRP1中(图27A)。所得的酵母菌株当在标准酵母培养基中培养时产生蒂巴因(图27C)。

[0336] 在第三构建体中，并入由生物合成的蒂巴因产生氢可酮的基因。在此实例中，蒂巴因脱甲基酶活性作为来自罂粟的T6ODM酶并入，以将蒂巴因转化成尼奥品酮，并且吗啡酮还原酶活性作为来自恶臭假单胞菌的morB酶并入以将可待因酮转化成氢可酮。在此实例中，尼奥品酮至可待因酮的居间转化自发发生。在另一个实例中，包括了异构酶以将尼奥品酮酶促转化为可待因酮。T6ODM和morB基因针对酵母进行密码子优化并且作为组装到具有TRP1选择标记的YAC中的两个表达盒(P_{GPD}-yT6ODM-T_{ADH1}和P_{PGK1}-yPbmorB-T_{PHO5})被包括在内(图27A)。在此实例中，将酵母菌株在补充有50mM 2-酮戊二酸以支持T6ODM、2-酮戊二酸依赖性双加氧酶的活性的培养基中培养。在另一个实例中，将酵母宿主细胞工程化为以足以支持T6ODM活性的水平积聚2-酮戊二酸。携带所述多基因构建体且在所述培养基中培养的酵母菌株由葡萄糖生物合成氢可酮(图27D)。

实施例20:增加基因拷贝数以克服途径通量中的瓶颈

[0337] 在一些情况下，可通过增加活性有限的酶的基因拷贝数优化酵母菌株以提高苄基异喹啉生物碱的产生。

[0338] 在一个实例中，如实施例19中所述的产牛心果碱菌株的牛心果碱产生通过添加NCS的第三基因拷贝进行优化(图28)。在此实例中，亲本菌株具有整合在YMR206W基因座处的NCS的一个拷贝和整合在YPL250C基因座处的另一个拷贝，其各自由PGK1启动子表达。NCS的第三拷贝(由TDH3启动子表达)整合在BUD9基因座处并且这种修饰导致牛心果碱滴度增加2倍。在另一个实例中，将途径中的其他酶的另外拷贝并入菌株中以进一步增加牛心果碱滴度。

表 2：酶列表

酶	缩写	催化的反应	来源生物体	与天然存在的基因的相似性	修饰	Genbank #
转酮醇酶	TKL1	果糖-6-磷酸 + 甘油醛-3-磷酸 ↔ 木酮糖-5-磷酸 + 赤藓糖-4-磷酸(EC 2.2.1.1)	*酿酒酵母*	100%	组成型过表达、合成调控	NP_015399.1
葡萄糖-6-磷酸脱氢酶	ZWF1	葡萄糖-6-磷酸 → 6-磷酸葡糖酸内酯 (EC 1.1.1.49)	*酿酒酵母*	编码区的完全缺失	编码区的完全缺失	CAA96146.1
3-脱氧-d-阿拉伯糖-庚酮糖酸-7-	ARO4，DAHP 合酶	赤藓糖-4-磷酸+ PEP → DAHP (EC 2.5.1.54)	*酿酒酵母*	100%	反馈抑制抗性突变、K229L 、Q166K	CAA85212.1
分支酸变位酶	ARO7	分支酸酯→预苯酸酯(EC 5.4.99.5)	*酿酒酵母*	100%	反馈抑制抗性突变、T226I	NP_015385.1
苯丙酮酸脱羧酶	ARO10	羟苯基丙酮酸酯 → 4HPA (EC 4.1.1.80)	*酿酒酵母*	100%	组成型过表达、合成调控	NP_010668.3
醇脱氢酶	ADH2-7、SFA1	4HPA → 酪醇 (EC 1.1.1.90)	*酿酒酵母*	编码区的完全缺失	编码区的完全缺失	NP_014032.1、AAT93007.1、NP_011258.2、NP_009703.3、NP_014051.3、NP 010030.1、NP_010113.1
醛氧化酶	ALD2-6	4HPA→羟基苯乙酸(EC 1.2.1.39)	*酿酒酵母*	编码区的完全	编码区的完全缺失	NP_013893.1、NP_013892.1、

				缺失		NP_015019.1、NP_010996.2、NP_015264.1
芳族转氨酶	ARO9	羟苯基丙酮酸酯+谷氨酸酯→酪氨酸+α-酮戊二酸酯 (EC 2.6.1.57)	*酿酒酵母*	100%	组成型过表达、合成调控	AEC14313.1
酪氨酸酶	TYR	酪氨酸→L-DOPA、L-DOPA →多巴醌(EC 1.14.18.1)	*青枯雷尔氏菌、双孢蘑菇*	100%	组成型表达、合成调控	NP_518458.1、AJ223816
酪氨酸羟化酶	TyrH	酪氨酸→L-DOPA (EC 1.14.16.2)	*智人、褐家鼠、小家鼠*	100%	组成型表达、合成调控	NM 012740、NM 000240
L-DOPA 脱羧酶	DODC	L-DOPA → 多巴胺(EC 4.1.1.28)	*恶臭假单胞菌、褐家鼠*	100%	组成型表达、合成调控	AE015451.1、NP_001257782.1
酪氨酸/DOPA 脱羧酶	TYDC	L-DOPA → 多巴胺(EC 4.1.1.28) 酪氨酸 -> 酪胺(EC 4.1.1.25)	*罂粟*	100%	组成型表达、合成调控	AAA97535
单胺氧化酶	MAO	多巴胺→ 3,4-DHPA (EC 1.4.3.4)	*大肠杆菌、智人、藤黄微球菌*	77%	组成型表达、合成调控；经密码子优化以在酿酒酵母中表达	J03792、D2367、AB010716.1
去甲乌药碱合酶	NCS	4HPA +多巴胺→ S-去甲乌药碱(EC 4.2.1.78) 3,4-DHPA +多巴胺→ S-全去甲劳丹碱	*日本黄连、罂粟、大红罂粟、黄唐松草、岩黄连*	80%	组成型表达、合成调控；N末端截短；经密码子优化以在酿酒	BAF45337.1、AB267399.2、ACI45396.1、ACO90258.1、ACO90247.1、AEB71889.1

					酵母中表达	
去甲乌药碱6-O-甲基转移酶	6OMT	去甲乌药碱→ 乌药碱 全去甲劳丹碱 → 3'羟基乌药碱 (EC 2.1.1.128)	*罂粟*、*黄唐松草*、*日本黄连**	100% 100% 100%	组成型表达、合成调控	AY268894 AY610507 D29811
乌药碱-N-甲基转移酶	CNMT	乌药碱 → N-甲基乌药碱 3'羟基乌药碱→ 3'-羟基-N-甲基乌药碱 (EC 2.1.1.140)	*罂粟*、*黄唐松草*、*日本黄连**	100%	组成型表达、合成调控	AY217336 AY610508 AB061863
4'-O-甲基转移酶	4'OMT	3'-羟基-N-甲基乌药碱→牛心果碱 (EC 2.1.1.116)	*罂粟*、*黄唐松草*、*日本黄连**	100% 100% 100%	组成型表达、合成调控	AY217333、 AY217334 AY610510 D29812
细胞色素 P450 80B1	CYP80B1	N-甲基乌药碱→ 3'-羟基-N-甲基乌药碱	*罂粟*、*花菱草*、*黄唐松草*	77%	组成型表达、合成调控；经密码子优化以在酿酒酵母中表达	AAF61400.1 AAC39453.1 AAU20767.1
GTP 环化水解酶	FOL2	GTP → 二氢新蝶呤三磷酸 (EC 3.5.4.16)	*酿酒酵母*、*智人*、*小家鼠*	100%	天然调控、组成型过表达、合成调控	CAA97297.1、 NP_001019195.1、 NP_032128.1
6-丙酮酰四氢生物蝶呤(PTP)合酶	PTPS	二氢新蝶呤三磷酸→ PTP (EC 4.2.3.12)	*褐家鼠*、*智人*、*小家鼠*	80%	组成型表达、合成调控；经密码子优化以在酿酒酵母中表达	AAH59140.1、 BAA04224.1、 AAH29013.1
墨蝶呤还原酶	SepR	PTP → BH4 (EC 1.1.1.153)	*褐家鼠*、	72%	组成型表	NP_062054.1、

			智人、小家鼠		达、合成调控；经密码子优化以在酿酒酵母中表达	NP_003115.1、NP_035597.2
4a-羟基四氢生物蝶呤(蝶呤-4a-甲醇胺)	PCD	4a-羟基四氢生物蝶呤→ H2O + 醌型二氢蝶啶 (EC 4.2.1.96)	褐家鼠、智人、小家鼠	79%	组成型表达、合成调控；经密码子优化以在酿酒酵母中表达	NP_00100760 2.1、AAB25581.1 、NP 079549.1
醌型二氢蝶啶还原酶	QDHPR	醌型二氢蝶啶→ BH4 (EC 1.5.1.34)	褐家鼠、智人、小家鼠	75%	组成型表达、合成调控；经密码子优化以在酿酒酵母中表达	AAH72536.1、NP_000311.2、AAH02107.1
二氢叶酸还原酶	DHFR	7,8-二氢生物蝶呤→ 5,6,7,8- 四氢生物蝶呤(BH4) (EC 1.5.1.3)	褐家鼠、智人	77%	组成型表达、合成调控；经密码子优化以在酿酒酵母中表达	AF318150.1
1-苄基异喹啉生物碱差向异构酶(细胞色素 P450 82Y2-可待因酮还原酶)	CYP-COR	(S)-牛心果碱→ (R)-牛心果碱 (S)-1-苄基异喹啉->(R)-1-苄基异喹啉 (EC 1.5.1.27)	大红鳜粟、鳜粟、渥美鳜粟、白屈菜	77%	组成型表达、合成调控；分离自转录组数据组并且经密码子优化以在	表 1 中列出的氨基酸序列

					酿酒酵母中表达	
(R)-牛心果碱，NADPH:氧气氧化还原酶(C-C苯酚-偶合)，也称为沙罗泰里啶合酶	SalSyn	(R)-牛心果碱→ 沙罗泰里啶 (EC 1.14.21.4)	*罂粟*、*罂粟属物种*、*白屈菜*	78%	通过DNA2.0进行密码子优化以在酿酒酵母中表达；N末端工程化	EF451150 (Farrow 等人 2012. Phytochemistry. 77:79-88)
沙罗泰里啶醇:NADP+ 7- 氧化还原酶，也称为沙罗泰里啶还原酶	SalR	沙罗泰里啶醇 ↔沙罗泰里啶(EC 1.1.1.248)	*罂粟*、*大红罂粟*、*罂粟属物种*、*白屈菜*	80-100%	组成型表达、合成调控；通过DNA2.0进行密码子优化以在酿酒酵母中表达；定点突变体	DQ316261 、EF184229 (Farrow 等人 2012. Phytochemistry. 77:79-88)
乙酰-CoA: 沙罗泰里啶醇7-O-乙酰转移酶	SalAT	乙酰-CoA +沙罗泰里啶醇→CoA + 7-O-乙酰基沙罗泰里啶醇 (EC 2.3.1.150)	*罂粟*、*大红罂粟*、*鬼罂粟*、*罂粟属物种*	77-80%	组成型表达、合成调控；通过Life Technologies 或DNA2.0进行密码子优化以在酿酒酵母中表达	AF339913、FJ200355、FJ200358、FJ200356、JQ659008

蒂巴因 6-O 脱甲基酶	T6ODM	蒂巴因→尼奥品酮(EC 1.14.11.31)	*罂粟*、*罂粟属物种*	76.2%	组成型表达、合成调控；经密码子优化以在酿酒酵母中表达	GQ500139.1
可待因酮还原酶	COR	可待因酮→ 可待因 (EC 1.1.1.247)、尼奥品酮→ 尼奥品	*罂粟*、*罂粟属物种*	77.7% 76.8% 77.3% 77.0%	组成型表达、合成调控；经密码子优化以在酿酒酵母中表达；添加针对线粒体、液泡、ER 的靶序列	AF108432.1 AF108433.1 AF108434.1 AF108435.1
可待因 O-脱甲基酶	CODM	可待因→ 吗啡(EC 1.14.11.32), 尼奥品→新吗啡	*罂粟*、*罂粟属物种*	75.2%	组成型表达、合成调控；经密码子优化以在酿酒酵母中表达；添加针对线粒体的靶序列	GQ500141.1
(S)-牛心果碱:氧气氧化还原酶(亚甲基桥形成)，也称为小檗碱桥酶	BBE	(S)-牛心果碱→(S)-金黄紫堇碱(EC 1.21.3.3)	*罂粟*、*蓟罂粟*、*花菱草*、*伏牛花*、*黄唐松草粉*	99%	组成型表达、合成调控	AF025430、EU881889、EU881890、S65550 AF005655、

			绿亚种、日本黄连、罂粟属物种、花菱草属物种、小檗属物种、唐松草属物种、黄连属物种			AF049347、AY610511、AB747097
		S-腺苷-L-甲硫氨酸 + (S)- 金黄紫堇碱→S-腺苷-L-高半胱氨酸+ (S)- 四氢非洲防己碱(EC 2.1.1.117)	*黄唐松草粉绿亚种、罂粟、日本黄连、黄连、唐松草属物种、黄连属物种、罂粟属物种*	100% 80%	组成型表达、合成调控；通过Life Technologies进行密码子优化以在酿酒酵母中表达	AY610512、D29809、EU980450、JN185323
	S9OMT					
	CAS	(S)- 四氢非洲防己碱→ (S)- 坎那定 (EC 1.14.21.5)	*黄唐松草粉绿亚种、日本黄连、唐松草属物种、黄连属物种*	100%	组成型表达、合成调控	AY610513、AB026122、AB374407、AB374408
(S)- 四氢非洲防己碱, NAD PH:氧气氧化还原酶(亚甲基二氧桥形成)，也称为(S)-坎那定合酶	STOX	(S)- 四氢小檗碱+ 2 O_2→小檗碱+ 2 H_2O_2 (EC 1.3.3.8)	*金花小檗、日本*	78%	组成型表达、合成调	HQ116697、AB564543
(S)- 四氢原小檗碱氧化酶						

			黄连、小檗属物种、黄连属物种			
		刺罂粟碱→顺式-N-甲基刺罂粟碱(EC 2.1.1.122) 坎那定→N-甲基坎那定	*罂粟、花菱草、大红罂粟、蜀罂粟*	100% 81%	组成型表达、合成调控；通过Life Technologies进行密码子优化以在酿酒酵母中表达	DQ028579 EU882977 EU882994 HQ116698
		N-甲基坎那定→1-羟基-N-甲基坎那定	*罂粟、罂粟属物种、沙生车前、Rauwolfia heterophylla、覃状山缘草、金印草、异叶罂粟、细果角茴香、扭果紫金龙、黄海罂粟、月桂小檗、*	70 (N末端工程化)-78%	组成型表达、合成调控；通过Life Technologies进行密码子优化以在酿酒酵母中表达；N末端工程化	JQ65900651
	TNMT	CYP82Y1				
四氢原小檗碱-N-甲基转移酶		N-甲基坎那定14-羟化酶				

			刺蒺藜、紫堇属物种、球果紫堇属物种、紫金龙属物种	70 (N末端工程化)-77%	组成型表达、合成调控；通过Life Technologies进行密码子优化以在酿酒酵母中表达；N末端工程化	JQ659004.1
1-羟基-13-O-乙酰基-N-甲基坎那定→4'-O-脱甲基-3-O-乙酰基罂粟奥辛 1-羟基-N-甲基坎那定→ 4'-O- 脱甲基马克兰特醛	CYP82X1	1-羟基-N-甲基坎那定→1,13-二羟基-N-甲基坎那定	*罂粟、罂粟属物种、沙生车前、Rauwolfia heterophylla、蔓状山缘草、金印草、异叶罂粟、扭果紫金龙、黄海罂粟、月桂小蘗、刺蘗、紫堇属物种、球果紫堇属物种、紫金龙属物种*	71(N末端工程化)-77%	组成型表达、合成调控；通过Life	JQ659002.1
4'-O- 脱甲基-3-O-乙酰基罂粟奥辛合酶	1-羟基-N-甲基坎那定 13-羟化酶	CYP82X2				

				Technologies进行密码子优化以在酿酒酵母中表达；N末端工程化	
			79% 77% 78%	组成型表达、合成调控；经密码子优化以在酿酒酵母中表达	GU325749 AB434654 EF451152
		Rauwolfia heterophylla、*蔓状山绿草*、*金印草*、*异叶罂粟*、*细果角茴香*、*扭果紫金龙*、*黄海罂粟*、*月桂小檗*、*刺檗*、*紫堇属物种*、*球果紫堇属物种*、*紫金龙属物种*	*罂粟*、*花菱草*、*蓟罂粟*	金黄紫堇碱→碎叶紫堇碱（EC 1.14.21.2）	碎叶紫堇碱合酶
			79% 78% 76%	组成型表达、合成调控；经密码子优化以在酿酒酵母中表达	GU325750 AB126257 EF451151
		罂粟、*花菱草*、*蓟罂粟*	碎叶紫堇碱→刺罂粟碱(EC 1.14.21.1)	STS	刺罂粟碱合酶
			79%	组成型表	KC154003
		罂粟	顺式-N-甲基刺罂粟碱→原阿片碱	MSH	顺式-N-甲基刺

罂粟碱 14-羟化酶	(EC 1.14.13.37)				
原阿片碱-6-羟化酶	P6H	原阿片碱→6-羟基原阿片碱(EC 1.14.13.55)	*花菱草*、*罂粟*	79%	组成型表达、合成调控；经密码子优化以在酿酒酵母中表达	AB598834 AGC92397
二氢苯菲啶氧化酶	DBOX	二氢血根碱→血根碱 (EC 1.5.3.12)	*罂粟*	100%	组成型表达、合成调控；经密码子优化以在酿酒酵母中表达	[genbank 中没有]
那可托灵 14-O-甲基化酶 I	MT2	那可托灵→诺斯卡品 那可托灵半缩醛→那可汀半缩醛 4'-O-脱甲基-3-O-乙酰基罂粟奥辛→3-O-乙酰基罂粟奥辛	*罂粟*、*罂粟属物种*、*小花球果紫堇*、*对叶车前*、*Rauwolfia heterophylla*	80%	组成型表达、合成调控；通过 Life Technologies 进行密码子优化以在酿酒酵母中表达	JQ659000.1
1,13-二羟基-N-甲基坎那定 13-O 乙酰转移酶	AT1	1,13-二羟基-N-甲基坎那定→ 1-羟基-13-O-乙酰基-N-甲基坎那定	*罂粟*、*罂粟属物种*、*沙生车前*、*Rauwolfia*	81%	组成型表达、合成调控；通过 Life Technologi	JQ659008.1

				es进行密码子优化以在酿酒酵母中表达	JQ659006.1
			78%	组成型表达、合成调控；通过Life Technologies进行密码子优化以在酿酒酵母中表达	
		*4'-O-*脱甲基*-3-O-*乙酰基罂粟奥辛→那可托灵半缩醛 罂粟奥辛→那可汀半缩醛	*heterophylla*、*Rauwolfia heterophylla*、*Rauwolfia*属物种、*Rauwolfia*种、*Rauwolfia*属物种、*Rauwolfia*种、*Rauwolfia*属物种、*Rauwolfia*种、*Rauwolfia*属物种、*Rauwolfia*种、*Rauwolfia*属物种		
	CXE1				
那可汀半缩醛合酶					

					KJ890443.1
			组成型表达、合成调控；通过Life Technologies进行密码子优化以在酿酒酵母中表达		
罂粟、月桂小檗、刺檗、紫堇属物种、球果紫堇属物种、紫金龙属物种	78%	*罂粟、罂粟属物种、沙生车前、Rauwolfia heterophylla、覆状山缘草、金印草、异叶罂粟、细果角茴香、扭果紫金龙、黄海罂粟、月桂小檗、刺檗、紫堇属物种、球果紫堇属物种、紫金龙属物种*	79%	组成型表	JQ659007.1
		4'-O-脱甲基-3-O-乙酰基罂粟奥辛→那可托灵半缩醛 罂粟奥辛→那可汀半缩醛			
	CXE2				
那可汀半缩醛合酶	SDR1	那可托灵半缩醛→那可托灵那可汀半	*罂粟、罂*		
诺斯卡品合酶					

		缩醛→诺斯卡品	*罂粟属物种、沙生车前、Rauwolfia heterophylla、蕈状山缘草、金印草、异叶罂粟、细果角茴香、扭果紫金龙、黄海罂粟、月佳小檗、刺檗、紫堇属物种、球果紫堇属物种、紫金龙属物种*	79%	组成型表达、合成调控；通过Life Technologies进行密码子优化以在酿酒酵母中表达	JQ659001.1
那可托灵 14-O-甲基化酶 II	MT3	那可托灵→诺斯卡品 那可托灵半缩醛→那可汀半缩醛 4'-O-脱甲基-3-O-乙酰基罂粟奥辛→3-O-乙酰基罂粟奥辛	*罂粟、罂粟属物种、小花球果紫堇、对叶车前、Rauwolfia heterophylla*	73.7%	组成型表达、合成调控；通过Life Technologies进行密码子优化以在酿酒酵母中表达	M94775.1
吗啡脱氢酶	morA	吗啡→吗啡酮(EC 1.1.1.218)	*恶臭假单*			

	可待因酮→可待因(EC 1.1.1.247)	***胞菌***		达、合成调控；经密码子优化以在酿酒酵母中表达；引入的突变 morAC81S	
吗啡酮还原酶	morB	可待因酮→氢可酮 吗啡酮→氢吗啡酮(EC 1.3.1.)	***恶臭假单胞菌***	67.2%	组成型表达、合成调控；经密码子优化以在酿酒酵母中表达；引入的突变 morBE160G	U37350.1
NADPH：血红素蛋白氧化还原酶，也称为细胞色素 P450 还原酶	ATR1 、CPR	NADPH + H+ + n 氧化的血红素蛋白→NADP+ + n 还原的血红素蛋白(EC 1.6.2.4)	***拟南芥、所有植物(例如，罂粟、花菱草)、酵母(例如，酿酒酵母)、智人***	100%	组成型表达、在各种启动子强度下的合成调控	NM118585、许多其他序列(Jensen 和 Moller. 2010. Phytochemistry. 71:132-41)
细胞色素 P450，家族 2，亚家族 D，多肽 6	CYP2D6	混杂氧化酶可进行(R)-牛心果碱 → 沙罗泰里啶，以及其他反应(EC 1.14.14.1)	***智人***	73%	组成型表达、合成调控；通过 DNA2.0 进行密码子	BC067432

						优化以在酿酒酵母中表达	
黄芦木碱合酶细胞色素 P450 80A1	CYP80A1	(S)-N-甲基乌药碱 + (R)-N-甲基乌药碱→ 黄芦木碱(EC 1.14.21.3)	*伏牛花*		76%	组成型表达、合成调控；经密码子优化以在酿酒酵母中表达	UO9610.1
原阿片碱 O-脱烷基酶	PODA	坎那定、刺罂粟碱和小檗碱的 O,O-脱亚甲基化	***罂粟*、*罂粟属物种***		70-100 %	组成型过表达、合成调控；经密码子优化以在酿酒酵母中表达	GQ500140.1

表 3：裁剪酶

催化的反应	*酶*	*物种*
碳-碳	小檗碱桥酶(BBE)	罂粟、花菱草、日本黄连、匍枝小檗、黄唐松草
偶合	沙罗泰里啶合酶(SalSyn)	罂粟
	紫堇块茎碱合酶(CorSyn)	日本黄连
氧化	四氢原小檗碱氧化酶(STOX)	日本黄连、蓟罂粟、金花小檗
	二氢苯菲啶氧化酶(DBOX)	罂粟
	甲基刺罂粟碱羟化酶(MSH)	罂粟
	原阿片碱 6-羟化酶(P6H)	罂粟、花菱草
亚甲基二氧桥	刺罂粟碱合酶(StySyn)	罂粟、花菱草、蓟罂粟
形成	碎叶紫堇碱合酶(CheSyn)	罂粟、花菱草、蓟罂粟
	坎那定合酶(CAS)	黄唐松草、黄连
O-甲基化	去甲乌药碱 6-O-甲基转移酶(6OMT)	罂粟、黄唐松草、日本黄连、大红罂粟
	3’羟基-N-甲基乌药碱 4’-O-甲基转移酶(4’OMT)	罂粟、黄唐松草、日本黄连、黄连
	牛心果碱 7-O-甲基转移酶(7OMT)	罂粟、花菱草
	金黄紫堇碱 9-O-甲基转移酶(9OMT)	罂粟、黄唐松草、日本黄连、黄连
N-甲基化	乌药碱 N-甲基转移酶(CNMT)	罂粟、黄唐松草、日本黄连
	四氢原小檗碱 *N*-甲基转移酶(TMNT)	罂粟、花菱草、大红罂粟
O-脱甲基化	蒂巴因脱甲基酶(T6ODM)	罂粟
	可待因脱甲基酶(CODM)	罂粟、Ga
还原	沙罗泰里啶还原酶(SalR)	罂粟、大红罂粟、Ga
	可待因酮还原酶(COR)	罂粟
	血根碱还原酶(SanR)	花菱草
乙酰化	沙罗泰里啶乙酰转移酶(SalAT)	罂粟

表 4：罂粟杆浓缩物(或鸦片)和澄清的酵母培养基中可能存在的杂质的比较。

杂质：		罂粟杆浓缩物	澄清的酵母培养基
无机	钠	✓	✓
	镁	✓	✓
	硅	✓	×(培养基中不存在)
	磷	✓	✓
	硫	✓	✓
	氯化物	✓	✓
	钾	✓	✓
	钙	✓	✓
	铜	✓	✓
	锌	✓	✓
	钼	✓	✓(培养基中的钼酸钠)
	铁	✓	✓
	锰	✓	✓
	铵	✓	✓
	硼	✓	✓
有机	多糖(淀粉、纤维素、木聚糖)	✓	×(酵母饲喂单糖)
	木质素(对香豆醇、松柏醇、芥子醇)	✓	×
	颜料(叶绿素、花青素、类胡萝卜素)	✓	×
	类黄酮	✓	×
	类菲	✓	×
	乳胶、胶和蜡	✓	×
	1,5-二磷酸核酮糖羧化酶／加氧酶	✓	×
	袂康酸	✓	×
	假吗啡	✓	×
	那碎因	✓	×
	蒂巴酚	✓	×
其他	杀虫剂、杀真菌剂、除草剂	✓	×
	花粉	✓	×

表 5：澄清的酵母培养基(CYCM)中存在的各种不同组的分子。与罂粟杆浓缩物(CPS)或鸦片不同，酵母宿主菌株可被工程化以产生预定种类的生物碱的分子(即，每种菌株仅一个生物合成途径)，使得不存在其他种类的生物碱。因此，CYCM 可包含单个生物合成途径内的分子，包括跨越一个或两个列的分子的子组，而 CPS 可包含遍布许多列的分子的子组。

1-苄基异喹啉	原小檗碱和苯酞异喹啉	吗啡喃	异帕威	阿朴啡	BisBIA
四氢罂粟碱	金黄紫堇碱	沙罗泰里啶	帕威	木兰花碱	蝙蝠葛碱
二氢罂粟碱	碎叶紫堇碱	沙罗泰里啶醇	华厚壳桂碱	紫堇块茎碱	黄芦木碱
罂粟碱	刺罂粟碱	沙罗泰里啶-7-O-乙酸酯	双去甲蓟罂粟碱	阿朴吗啡	莲心碱
	顺式-N-甲基刺罂粟碱	蒂巴因	Isonoraremonine	波尔定碱	防己诺林碱
	原阿片碱	可待因酮			粉防己碱
	二氢血根碱	东罂粟碱			箭毒碱
	血根碱	吗啡酮			千金藤碱
	四氢非洲防己碱	尼奥品酮			小檗胺
	坎那定	尼奥品			
	N-甲基坎那定	可待因			
	诺斯卡品	吗啡			
	小檗碱	新吗啡			
	那可托灵	氢可酮			
	那可汀半缩醛	羟考酮			
	那可托灵半缩醛	14-羟基可待因酮			
		14-羟基可待因			
		二氢吗啡			
		二氢可待因			
		氧吗啡酮			
		氢吗啡酮			

表6：通过诱变筛选鉴定的各种酶的有益突变。

基因	GenBank ID#	底物	产物	突变
PsCODM	ADD853311.1	可待因、尼奥品	吗啡、新吗啡	N35S, G335V (图 21)
PsT6ODM	ADD853291.1	蒂巴因、东罂粟碱	尼奥品酮、吗啡酮	M151T, A202V, P229S, D86H L158M
PsCOR1.3	AAF13738.1	可待因酮、尼奥品酮	可待因、尼奥品	C220G
Ps6OMT	AAP45315.1	去甲乌药碱	乌药碱	D322N

[0339] 尽管具有随附条款，但本公开也由以下条款限定：

1.一种工程化的非植物细胞，其相对于非工程化细胞而言具有增加的酪氨酸羟化酶活性，所述工程化的非植物细胞具有选自由以下各项组成的组的至少一个修饰：底物抑制减轻突变；产物抑制减轻突变；以及辅因子回收促进机制。

2.根据条款1所述的工程化的非植物细胞，其中所述至少一个修饰包括底物抑制减轻突变。

3.根据条款2所述的工程化的非植物细胞，其中所述底物抑制减轻突变包括点突变

W166Y。

4.根据条款1所述的工程化的非植物细胞，其中所述至少一个修饰包括缓解在共底物结合位点处的竞争性结合的至少一个产物抑制减轻突变。

5.根据条款4所述的工程化的非植物细胞，其中所述至少一个产物抑制减轻突变包括点突变S40D。

6.根据条款4所述的工程化的非植物细胞，其中所述至少一个产物抑制减轻突变包括联合突变R37E和R38E。

7.根据条款6所述的工程化的非植物细胞，其中所述联合突变R37E和R38E在多巴胺存在下改善酪氨酸羟化酶活性。

8.根据条款1所述的工程化的非植物细胞，其中所述至少一个修饰包括缓解不可逆的产物抑制的产物抑制减轻突变。

9.根据条款8所述的工程化的非植物细胞，其中所述产物抑制减轻突变包括点突变E332D。

10.根据条款8所述的工程化的非植物细胞，其中所述产物抑制减轻突变包括点突变Y371F。

11.根据条款8所述的工程化的非植物细胞，其中所述产物抑制减轻突变缓解儿茶酚胺与铁在活性位点中的不可逆结合。

12.根据条款1所述的工程化的非植物细胞，其中所述至少一个修饰包括辅因子回收促进机制。

13.根据条款12所述的工程化的非植物细胞，其中所述辅因子回收促进机制包括编码二氢叶酸还原酶的异源编码序列。

14.根据条款13所述的工程化的非植物细胞，其中通过所述工程化的非植物细胞产生的二氢叶酸还原酶在所述工程化的非植物细胞内催化二氢生物蝶呤到四氢生物蝶呤的转化。

15.一种用于形成具有苄基异喹啉生物碱产物的产物流的方法，包括：

(a)向间歇式反应器中提供工程化的非植物细胞以及包括营养素和水的原料，所述工程化的非植物细胞具有选自由以下各项组成的组的至少一个修饰：底物抑制减轻突变；产物抑制减轻突变；以及辅因子回收促进机制

(b)在所述间歇式反应器中，通过将所述工程化的非植物细胞孵育至少约5分钟的时间段而使所述工程化的非植物细胞进行发酵，以产生包括所述苄基异喹啉生物碱产物和细胞材料的溶液；以及

(c)使用至少一个分离装置将所述苄基异喹啉生物碱产物与所述细胞材料分离，以提供包括所述苄基异喹啉生物碱产物的所述产物流。

16.根据条款15所述的方法，其中所述间歇式反应器的至少一个工艺参数可修改，以改变所得的苄基异喹啉生物碱产物组成。

17.根据条款16所述的方法，其中可修改的所述至少一个工艺参数包括溶解氧、pH、搅拌速度、通气速率和细胞密度中的至少一个。

18.根据条款15所述的方法，其中所述苄基异喹啉生物碱产物包括苄基异喹啉生物碱前体。

19.根据条款18所述的方法，其中所述苄基异喹啉生物碱前体选自下组：去甲乌药碱、全去甲劳丹碱、酪氨酸、酪胺、4-羟基苯乙醛、4-羟基苯丙酮酸、L-3,4-二羟基苯丙氨酸、3,4-二羟基苯乙醛以及多巴胺。

20.根据条款15所述的方法，其中所述苄基异喹啉生物碱产物包括苄基异喹啉生物碱。

21.根据条款20所述的方法，其中所述苄基异喹啉生物碱具有选自下组的结构种类：苄基异喹啉类、原小檗碱类、原阿片碱类、苯菲啶类、原吗啡喃类、吗啡喃类、断裂小檗碱类、苯酞异喹啉类、阿朴啡类，以及双苄基异喹啉类。

22.根据条款21所述的方法，其中所述苄基异喹啉生物碱为选自下组的苄基异喹啉：乌药碱、3’-羟基乌药碱、4’-O-甲基全去甲劳丹碱、4’-O-甲基-劳丹碱、N-甲基去甲乌药碱、劳丹碱、N-甲基乌药碱、3’-羟基-N-甲基乌药碱、牛心果碱、去甲牛心果碱、罂粟碱、劳丹宁、劳丹素、四氢罂粟碱、1,2-二氢罂粟碱以及东罂粟灵。

23.根据条款21所述的方法，其中所述苄基异喹啉生物碱为选自下组的原小檗碱：金黄紫堇碱、碎叶紫堇碱、刺罂粟碱、南丁宁碱、药根碱、千金藤啶碱、离木明碱、顺式-N-甲基刺罂粟碱、四氢非洲防己碱、掌叶防己碱、四氢掌叶防己碱、非洲防己碱、坎那定、N-甲基坎那定、1-羟基坎那定、小檗碱、N-甲基-左旋蛇果黄堇碱、1,13-二羟基-N-甲基坎那定，以及1-羟基-10-O-乙酰基-N-甲基坎那定。

24.根据条款21所述的方法，其中所述苄基异喹啉生物碱为选自下组的原阿片碱：原阿片碱、6-羟基原阿片碱、别隐品碱、隐品碱、隐掌叶防己碱和黄唐松草碱。

25.根据条款21所述的方法，其中所述苄基异喹啉生物碱为选自下组的苯菲啶：二氢血根碱、血根碱、dihydrocheilirubine、cheilirubine、dihydromarcapine、marcapine和白屈菜红碱。

26.根据条款21所述的方法，其中所述苄基异喹啉生物碱为选自下组的原吗啡喃：沙罗泰里啶、沙罗泰里啶醇，以及沙罗泰里啶醇-7-O-乙酸酯。

27.根据条款21所述的方法，其中所述苄基异喹啉生物碱为选自下组的吗啡喃：蒂巴因、可待因酮、可待因、吗啡、吗啡酮、东罂粟碱、尼奥品酮、尼奥品、新吗啡、氢可酮、二氢可待因、14-羟基可待因酮、羟考酮、14-羟基可待因、吗啡酮、氢吗啡酮、二氢吗啡、二氢埃托啡、乙基吗啡、埃托啡、美托酮、丁丙诺啡、福尔可定、异可待因和氧吗啡酮。

28.根据条款21所述的方法，其中所述苄基异喹啉生物碱为选自下组的断裂小檗碱：4’-O-脱甲基马克兰特醛、4’-O-脱甲基罂粟奥辛、4’-O-脱甲基-3-O-乙酰基罂粟奥辛和3-O-乙酰基罂粟奥辛。

29.根据条款21所述的方法，其中所述苄基异喹啉生物碱为选自下组的苯酞异喹啉：那可托灵半缩醛、那可汀半缩醛、那可托灵和诺斯卡品。

30.根据条款21所述的方法，其中所述苄基异喹啉生物碱为选自下组的阿朴啡：木兰花碱、紫堇块茎碱、阿朴吗啡、波尔定碱、异波尔定碱、异蒂巴因、异紫堇块茎碱以及glaufine。

31.根据条款21所述的方法，其中所述苄基异喹啉生物碱为选自下组的双苄基异喹啉：黄芦木碱、guattgaumerine、蝙蝠葛碱和莲心碱。

32.一种用于形成具有苄基异喹啉生物碱产物的产物流的方法，包括：

(a)向反应器中提供工程化的非植物细胞、包括营养素和水的原料，所述工程化的非植物细胞相对于表达不具有增加酪氨酸羟化酶活性的突变的野生型酪氨酸羟化酶的细胞而

言具有增加的酪氨酸羟化酶活性;

(b)在所述反应器中,通过将所述工程化的非植物细胞孵育至少约5分钟的时间段而使所述工程化的非植物细胞进行发酵,以产生包括细胞材料和所述苄基异喹啉生物碱产物的溶液,其中所述溶液包括不超过一个种类的选自下组的分子:原小檗碱、吗啡喃、异帕威、阿朴啡以及双苄基异喹啉;以及

(c)使用至少一个分离装置将所述苄基异喹啉生物碱产物与所述细胞材料分离,以提供包括所述苄基异喹啉生物碱产物的所述产物流。

33.根据条款32所述的方法,其中所述产物流不包含超过5ppm的选自下组的分子:木质素、类黄酮、类菲(phenanthreoid)、乳胶、1,5-二磷酸核酮糖羧化酶/加氧酶(rubisco)、袂康酸、假吗啡、那碎因、蒂巴酚和花粉。

34.根据条款33所述的方法,其中所述产物流不包含超过5ppm的袂康酸。

35.根据条款32所述的方法,其中所述产物流不包含可检测量的选自由以下各项组成的组的物质:杀虫剂、杀真菌剂或除草剂。

36.根据条款32所述的方法,其中使用至少液-液萃取从所述产物流中回收所述苄基异喹啉生物碱产物。

37.根据条款36所述的方法,其中在发酵过程完成之后立即回收所述苄基异喹啉生物碱产物。

38.一种使(S)-1-苄基异喹啉生物碱差向异构化为(R)-1-苄基异喹啉生物碱的方法,包括:

使所述(S)-1-苄基异喹啉生物碱与至少一种酶接触,

其中使所述(S)-1-苄基异喹啉生物碱与所述至少一种酶接触将所述(S)-1-苄基异喹啉生物碱转化成(R)-1-苄基异喹啉生物碱。

39.根据条款38所述的方法,其中所述至少一种酶通过培养具有编码所述至少一种酶的编码序列的工程化的非植物细胞产生。

40.根据条款39所述的方法,还包括:

向所述细胞培养物中添加(S)-1-苄基异喹啉生物碱。

41.根据条款40所述的方法,还包括:

从所述细胞培养物中回收所述(R)-1-苄基异喹啉生物碱或其衍生物。

42.根据条款38-41中任一项所述的方法,其中所述至少一种酶包括氧化酶。

43.根据条款38-42中任一项所述的方法,其中所述至少一种酶包括还原酶。

44.根据条款38-43中任一项所述的方法,其中所述至少一种酶包括差向异构酶。

45.根据条款44所述的方法,其中所述差向异构酶包括选自由以下各项组成的组的氨基酸序列:SEQ ID NO:1、2、3、4、5、6、7、8、9、10、11、12、13、14和15。

46.根据条款44或45所述的方法,其中所述差向异构酶包括氧化酶结构域和还原酶结构域。

47.根据条款46所述的方法,其中所述氧化酶结构域为细胞色素P450氧化酶样结构域。

48.根据条款46所述的方法,其中所述还原酶结构域为可待因酮还原酶样结构域。

49.根据条款38-48中任一项所述的方法,其中所述(S)-1-苄基异喹啉生物碱为(S)-牛心果碱。

50.根据条款38-49中任一项所述的方法，其中所述(R)-1-苄基异喹啉生物碱为(R)-牛心果碱。

51.根据条款38-50中任一项所述的方法，还包括：

将所述(R)-1-苄基异喹啉生物碱转化成4-环原吗啡喃生物碱。

52.根据条款51所述的方法，其中所述4环原吗啡喃生物碱为沙罗泰里啶。

53.根据条款38-50中任一项所述的方法，还包括：

将所述(R)-1-苄基异喹啉生物碱转化成5-环吗啡喃生物碱。

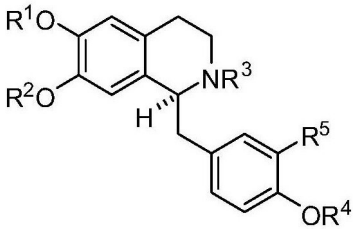
54.根据条款38-53中任一项所述的方法，其中在工程化的微生物细胞内产生存在于所述工程化的非植物细胞内的所述(S)-1-苄基异喹啉生物碱。

55.根据条款38-54中任一项所述的方法，其中通过以L-酪氨酸起始的代谢途径在所述工程化的非植物细胞内产生所述(S)-1-苄基异喹啉生物碱。

56.根据条款38-54中任一项所述的方法，其中通过以碳水化合物和氮源起始的代谢途径在所述工程化的非植物细胞内产生所述(S)-1-苄基异喹啉生物碱。

57.根据条款38-56中任一项所述的方法，其中所述(S)-1-苄基异喹啉生物碱选自由以下各项组成的组：(S)-去甲牛心果碱、(S)-牛心果碱、(S)-四氢罂粟碱、(S)-去甲乌药碱、(S)-乌药碱、(S)-N-甲基乌药碱、(S)-3'-羟基-N-甲基乌药碱、(S)-去甲异东罂粟灵、(S)-东罂粟灵、(S)-异东罂粟灵、(S)-去甲原青藤碱、(S)-原青藤碱、(S)-全去甲劳丹碱、(S)-劳丹碱、(S)-4'-O-甲基劳丹碱、(S)-6-O-甲基全去甲劳丹碱，以及(S)-4'-O-甲基全去甲劳丹碱。

58.根据条款38-57中任一项所述的方法，其中所述(S)-1-苄基异喹啉生物碱为式I的化合物：



式I，

其中：

R^1、R^2、R^3和R^4独立地为氢或甲基；并且

R^5为氢、羟基或甲氧基。

59.一种工程化的非植物细胞，其由存在于所述工程化的非植物细胞内的(S)-1-苄基异喹啉生物碱产生(R)-1-苄基异喹啉生物碱，其包括编码差向异构酶的异源编码序列，其中所述差向异构酶将所述(S)-1-苄基异喹啉生物碱转化成所述(R)-1-苄基异喹啉生物碱。

60.根据条款59所述的工程化的非植物细胞，还包括编码沙罗泰里啶合酶的异源编码序列。

61.根据条款60所述的工程化的非植物细胞，其中所述沙罗泰里啶合酶将所述(R)-1-苄基异喹啉生物碱转化成沙罗泰里啶。

62.一种产生将(S)-1-苄基异喹啉生物碱转化成(R)-1-苄基异喹啉生物碱的差向异构酶的工程化的非植物细胞，其中所述差向异构酶包括选自由以下各项组成的组的氨基酸序

列:SEQ ID NO:1、2、3、4、5、6、7、8、9、10、11、12、13、14和15。

63.一种用于将(S)-1-苄基异喹啉生物碱转化成(R)-1-苄基异喹啉生物碱的工艺,其包括使所述(S)-1-苄基异喹啉生物碱与足以将所述(S)-1-苄基异喹啉生物碱转化成(R)-1-苄基异喹啉生物碱的量的差向异构酶接触。

64.根据条款63所述的工艺,其中所述差向异构酶包括选自由以下各项组成的组的氨基酸序列:SEQ ID NO:1、2、3、4、5、6、7、8、9、10、11、12、13、14和15。

65.根据条款63所述的工艺,其中至少5%的所述(S)-1-苄基异喹啉生物碱被转化成(R)-1-苄基异喹啉生物碱。

66.一种使1-苄基异喹啉生物碱的立构中心差向异构化的方法,包括:

使所述1-苄基异喹啉生物碱与至少一种酶接触,

其中所述1-苄基异喹啉生物碱与所述至少一种酶的所述接触将所述1-苄基异喹啉生物碱的所述立构中心的立体化学翻转成所述1-苄基异喹啉生物碱的所述立构中心的相反的立体化学。

67.根据条款66所述的方法,其中所述至少一种酶通过培养具有编码所述至少一种酶的编码序列的工程化的非植物细胞产生。

68.根据条款67所述的方法,还包括:

向所述细胞培养物中添加所述1-苄基异喹啉生物碱。

69.根据条款66所述的方法,其中所述1-苄基异喹啉生物碱呈现为所述1-苄基异喹啉生物碱的单一立体异构体。

70.根据条款66所述的方法,其中所述1-苄基异喹啉生物碱呈现为所述1-苄基异喹啉生物碱的立体异构体的混合物。

71.根据条款70所述的方法,其中所述1-苄基异喹啉生物碱的立体异构体的所述混合物为所述1-苄基异喹啉生物碱的对映体的外消旋混合物。

72.根据条款67所述的方法,还包括:

从所述细胞培养物中回收所述1-苄基异喹啉生物碱的立体异构体或其衍生物。

73.根据条款72所述的方法,其中所回收的1-苄基异喹啉生物碱具有至少约80%的对映体过量。

74.根据条款72所述的方法,其中所回收的1-苄基异喹啉生物碱具有至少约98%的对映体过量。

75.根据条款66-74中任一项所述的方法,其中所述至少一种酶包括氧化酶。

76.根据条款66-75中任一项所述的方法,其中所述至少一种酶包括还原酶。

77.根据条款66-74中任一项所述的方法,其中所述至少一种酶包括差向异构酶。

78.根据条款77所述的方法,其中所述差向异构酶包括选自由以下各项组成的组的氨基酸序列:SEQ ID NO:1、2、3、4、5、6、7、8、9、10、11、12、13、14和15。

79.根据条款77所述的方法,其中所述差向异构酶包括与以下中的任一个具有至少75%同一性的氨基酸序列:SEQ ID NO:1、2、3、4、5、6、7、8、9、10、11、12、13、14和15。

80.根据条款77所述的方法,其中所述差向异构酶包括与以下中的任一个具有至少80%同一性的氨基酸序列:SEQ ID NO:1、2、3、4、5、6、7、8、9、10、11、12、13、14和15。

81.根据条款77所述的方法,其中所述差向异构酶包括与以下中的任一个具有至少

85%同一性的氨基酸序列:SEQ ID NO:1、2、3、4、5、6、7、8、9、10、11、12、13、14和15。

82.根据条款77所述的方法,其中所述差向异构酶包括与以下中的任一个具有至少90%同一性的氨基酸序列:SEQ ID NO:1、2、3、4、5、6、7、8、9、10、11、12、13、14和15。

83.根据条款77所述的方法,其中所述差向异构酶包括与以下中的任一个具有至少95%同一性的氨基酸序列:SEQ ID NO:1、2、3、4、5、6、7、8、9、10、11、12、13、14和15。

84.根据条款77所述的方法,其中所述差向异构酶包括与以下中的任一个具有至少98%同一性的氨基酸序列:SEQ ID NO:1、2、3、4、5、6、7、8、9、10、11、12、13、14和15。

85.根据条款77-84中任一项所述的方法,其中所述差向异构酶包括氧化酶结构域和还原酶结构域。

86.根据条款85所述的方法,其中所述氧化酶结构域为细胞色素P450氧化酶样结构域。

87.根据条款85所述的方法,其中所述还原酶结构域为可待因酮还原酶样结构域。

88.一种工程化的非植物细胞,其由存在于所述工程化的非植物细胞内的两个苄基异喹啉生物碱单体产生双苄基异喹啉生物碱,其包括:

编码至少一种偶合酶的异源编码序列,

其中所述至少一种偶合酶将所述两个苄基异喹啉生物碱单体二聚化,从而形成所述双苄基异喹啉生物碱。

89.根据条款88所述的工程化的非植物细胞,其中所述至少一种偶合酶通过形成一个或多个共价键将所述两个苄基异喹啉生物碱单体二聚化。

90.根据条款88所述的工程化的非植物细胞,其中所述至少一种偶合酶通过形成一个或多个碳-碳键将所述两个苄基异喹啉生物碱单体二聚化。

91.根据条款88所述的工程化的非植物细胞,其中所述至少一种偶合酶通过形成一个或多个碳-氧键将所述两个苄基异喹啉生物碱单体二聚化。

92.根据条款88所述的工程化的非植物细胞,其中所述至少一种偶合酶将相同的苄基异喹啉生物碱单体二聚化,从而形成同二聚体。

93.根据条款88所述的工程化的非植物细胞,其中所述至少一种偶合酶将不同的苄基异喹啉生物碱单体二聚化,从而形成异二聚体。

94.根据条款88所述的工程化的非植物细胞,其中所述两个苄基异喹啉生物碱单体为彼此的立体异构体。

95.根据条款88所述的工程化的非植物细胞,其中所述两个苄基异喹啉生物碱单体中的第一苄基异喹啉生物碱单体选自下组:乌药碱、N-甲基乌药碱、劳丹宁、去甲乌药碱、全去甲劳丹碱、6-O-甲基-全去甲劳丹碱、3'-羟基-N-甲基乌药碱、3'-羟基乌药碱、牛心果碱、去甲牛心果碱、去甲劳丹宁。

96.根据条款95所述的工程化的非植物细胞,其中所述两个苄基异喹啉生物碱单体中的第二苄基异喹啉生物碱单体选自下组:乌药碱、N-甲基乌药碱、劳丹宁、去甲乌药碱、全去甲劳丹碱、6-O-甲基-全去甲劳丹碱、3'-羟基-N-甲基乌药碱、3'-羟基乌药碱、牛心果碱、去甲牛心果碱、去甲劳丹宁。

97.根据条款88所述的工程化的非植物细胞,其中所述两个苄基异喹啉生物碱单体中的至少一个在所述工程化的非植物细胞内产生。

98.根据条款88所述的工程化的非植物细胞,其中所述两个苄基异喹啉生物碱单体中

的至少一个在所述工程化的非植物细胞外产生。

99.一种工程化的非植物细胞，其使用存在于所述工程化的非植物细胞内的偶合酶产生双苄基异喹啉生物碱，其包括：

编码在所述工程化的非植物细胞内产生至少一种苄基异喹啉生物碱单体中所用的至少一种酶的至少一个异源编码序列，

其中所述至少一种偶合酶在所述工程化的非植物细胞内将两个苄基异喹啉生物碱单体二聚化，从而形成所述双苄基异喹啉生物碱。

100.根据条款99所述的工程化的非植物细胞，其中所述至少一种偶合酶通过形成一个或多个共价键将所述两个苄基异喹啉生物碱单体二聚化。

101.根据条款99所述的工程化的非植物细胞，其中所述至少一种偶合酶通过形成一个或多个碳-碳键将所述两个苄基异喹啉生物碱单体二聚化。

102.根据条款99所述的工程化的非植物细胞，其中所述至少一种偶合酶通过形成一个或多个碳-氧键将所述两个苄基异喹啉生物碱单体二聚化。

103.根据条款99所述的工程化的非植物细胞，其中所述至少一种偶合酶将相同的苄基异喹啉生物碱单体二聚化，从而形成同二聚体。

104.根据条款99所述的工程化的非植物细胞，其中所述至少一种偶合酶将不同的苄基异喹啉生物碱单体二聚化，从而形成异二聚体。

105.根据条款99所述的工程化的非植物细胞，其中所述两个苄基异喹啉生物碱单体为彼此的立体异构体。

106.根据条款99所述的工程化的非植物细胞，其中所述两个苄基异喹啉生物碱单体中的第一苄基异喹啉生物碱单体选自下组：乌药碱、N-甲基乌药碱、劳丹宁、去甲乌药碱、全去甲劳丹碱、6-O-甲基-全去甲劳丹碱、3'-羟基-N-甲基乌药碱、3'-羟基乌药碱、牛心果碱、去甲牛心果碱、去甲劳丹宁。

107.根据条款106所述的工程化的非植物细胞，其中所述两个苄基异喹啉生物碱单体中的第二苄基异喹啉生物碱单体选自下组：乌药碱、N-甲基乌药碱、劳丹宁、去甲乌药碱、全去甲劳丹碱、6-O-甲基-全去甲劳丹碱、3'-羟基-N-甲基乌药碱、3'-羟基乌药碱、牛心果碱、去甲牛心果碱、去甲劳丹宁。

108.根据条款99所述的工程化的非植物细胞，其中所述偶合酶在所述工程化的非植物细胞内产生。

109.根据条款99所述的工程化的非植物细胞，其中所述偶合酶在所述工程化的非植物细胞外产生。

110.一种用于形成具有双苄基异喹啉生物碱产物的产物流的方法，包括：

(a)向间歇式反应器中提供工程化的非植物细胞以及包括营养素和水的原料，所述工程化的非植物细胞包括编码至少一种偶合酶的至少一个异源编码序列，其中所述工程化的非植物细胞还包括编码在至少一个苄基异喹啉生物碱单体的产生中使用的至少一种酶的至少一个异源编码序列；

(b)在所述间歇式反应器中，通过将所述工程化的非植物细胞孵育至少约5分钟的时间段而使所述工程化的非植物细胞进行发酵，以产生包括所述双苄基异喹啉生物碱产物和细胞材料的溶液；以及

(c)使用至少一个分离装置将所述双苄基异喹啉生物碱产物与所述细胞材料分离，以提供包括所述双苄基异喹啉生物碱产物的所述产物流。

111.根据条款108所述的方法，其中所述间歇式反应器的至少一个工艺参数可修改，以改变所得的双苄基异喹啉生物碱产物组成。

112.根据条款109所述的方法，其中可修改的所述至少一个工艺参数包括溶解氧、pH、搅拌速度、通气速率和细胞密度中的至少一个。

113.根据条款108所述的方法，其中所述至少一个苄基异喹啉生物碱单体包括以下各项中的至少一个：乌药碱、N-甲基乌药碱、劳丹宁、去甲乌药碱、全去甲劳丹碱、6-O-甲基-全去甲劳丹碱、3'-羟基-N-甲基乌药碱、3'-羟基乌药碱、牛心果碱、去甲牛心果碱、去甲劳丹宁。

114.一种工程化的非植物细胞，其将碳水化合物源转化成衍生自(S)-牛心果碱的苄基异喹啉生物碱产物。

115.根据条款114所述的工程化的非植物细胞，其中所述工程化的非植物细胞包括具有编码用于将鸟苷三磷酸转化成四氢生物蝶呤的一种或多种酶的多个异源编码序列的多个多基因表达构建体。

116.根据条款115所述的工程化的非植物细胞，其中所述多个异源编码序列编码以下各项中的至少一个：丙酮酰四氢蝶呤合酶、墨蝶呤还原酶、蝶呤4a-甲醇胺脱水酶以及二氢蝶啶还原酶。

117.根据条款115所述的工程化的非植物细胞，其中所述工程化的非植物细胞还包括编码用于将酪氨酸转化成L-DOPA的一种或多种酶的一个或多个异源编码序列。

118.根据条款117所述的工程化的非植物细胞，其中一个或多个异源编码序列编码酪氨酸羟化酶中的至少一种。

119.根据条款114所述的工程化的非植物细胞，其中所述工程化的非植物细胞包括具有编码用于将L-DOPA转化成(S)-牛心果碱的一种或多种酶的多个异源编码序列的多个多基因表达构建体。

120.根据条款119所述的工程化的非植物细胞，其中所述多个异源编码序列编码以下各项中的至少一个：L-DOPA脱羧酶、去甲乌药碱合酶、去甲乌药碱-6-O-甲基转移酶、乌药碱-N-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶，以及4'-O-甲基转移酶。

121.根据条款119所述的工程化的非植物细胞，其中所述多个异源编码序列编码以下各项中的至少一个：L-DOPA脱羧酶、单胺氧化酶、去甲乌药碱合酶、去甲乌药碱-6-O-甲基转移酶、乌药碱-N-甲基转移酶，以及4'-O-甲基转移酶。

122.根据条款114所述的工程化的非植物细胞，其中所述工程化的非植物细胞包括具有编码用于将(S)-牛心果碱转化成吗啡喃生物碱的一种或多种酶的多个异源编码序列的多个多基因表达构建体。

123.根据条款122所述的工程化的非植物细胞，其中所述多个异源编码序列编码以下各项中的至少一个：1-苄基异喹啉生物碱差向异构酶、沙罗泰里啶合酶、细胞色素P450还原酶、沙罗泰里啶还原酶，以及沙罗泰里啶醇7-O-乙酰转移酶。

124.根据条款123所述的工程化的非植物细胞，其中所述多个异源编码序列另外编码以下各项中的至少一个：蒂巴因-6-O-脱甲基酶、可待因酮还原酶，以及可待因脱甲基酶。

125.根据条款122所述的工程化的非植物细胞，其中所述一种或多种酶用于将(S)-牛心果碱转化成原吗啡喃生物碱。

126.根据条款122所述的工程化的非植物细胞，其中所述一种或多种酶用于将(S)-牛心果碱转化成半合成的吗啡喃生物碱。

127.根据条款114所述的工程化的非植物细胞，其中所述工程化的非植物细胞包括具有编码用于将(S)-牛心果碱转化成坎那定的一种或多种酶的多个异源编码序列的多个多基因表达构建体。

128.根据条款127所述的工程化的非植物细胞，其中所述多个异源编码序列编码以下各项中的至少一个：小檗碱桥酶、金黄紫堇碱-9-O-甲基转移酶、坎那定合酶，以及细胞色素P450还原酶。

129.根据条款128所述的工程化的非植物细胞，其中所述多个异源编码序列另外编码用以将坎那定转化成原小檗碱产物的一种或多种酶。

130.根据条款128所述的工程化的非植物细胞，其中所述多个异源编码序列另外编码用以将坎那定转化成苯酞异喹啉产物的一种或多种酶。

131.根据条款128所述的工程化的非植物细胞，其中所述多个异源编码序列另外编码用以将坎那定转化成类诺斯卡品产物的一种或多种酶。

132.根据条款114所述的工程化的非植物细胞，其中所述工程化的非植物细胞包括具有编码用于将(S)-牛心果碱转化成小檗碱的一种或多种酶的多个异源编码序列的多个多基因表达构建体。

133.根据条款132所述的工程化的非植物细胞，其中所述多个异源编码序列编码以下各项中的至少一个：小檗碱桥酶、金黄紫堇碱-9-O-甲基转移酶、坎那定合酶、细胞色素P450还原酶，以及四氢原小檗碱氧化酶。

134.根据条款133所述的工程化的非植物细胞，其中所述多个异源编码序列另外编码用以将小檗碱转化成小檗碱产物的一种或多种酶。

135.根据条款114所述的工程化的非植物细胞，其中所述工程化的非植物细胞包括具有编码用于将(S)-牛心果碱转化成原小檗碱产物的一种或多种酶的多个异源编码序列的多个多基因表达构建体。

136.根据条款138所述的工程化的非植物细胞，其中所述多个异源编码序列编码小檗碱桥酶。

137.根据条款114所述的工程化的非植物细胞，其中所述工程化的非植物细胞包括具有编码用于将(S)-牛心果碱转化成原阿片碱的一种或多种酶的多个异源编码序列的多个多基因表达构建体。

138.根据条款137所述的工程化的非植物细胞，其中所述多个异源编码序列编码以下各项中的至少一个：小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、细胞色素P450还原酶、四氢原小檗碱-N-甲基转移酶，以及顺式-N-甲基刺罂粟碱14-羟化酶。

139.根据条款138所述的工程化的非植物细胞，其中所述多个异源编码序列另外编码用以将原阿片碱转化成原阿片碱产物的一种或多种酶。

140.根据条款138所述的工程化的非植物细胞，其中所述多个异源编码序列另外编码用以将原阿片碱转化成苯菲啶产物的一种或多种酶。

141.根据条款140所述的工程化的非植物细胞，其中所述多个异源编码序列另外编码原阿片碱-6-羟化酶。

142.一种用于形成具有苄基异喹啉生物碱产物的产物流的方法，包括：

(a)向间歇式反应器中提供工程化的非植物细胞以及包括营养素和水的原料；

(b)在所述间歇式反应器中，使所述工程化的非植物细胞与碳水化合物源和氮源接触，并且通过将所述工程化的非植物细胞孵育至少约5分钟的时间段而使所述工程化的非植物细胞进行发酵，以产生包括所述苄基异喹啉生物碱产物和细胞材料的溶液，其中所述苄基异喹啉生物碱产物衍生自牛心果碱；以及

(c)使用至少一个分离装置将所述苄基异喹啉生物碱产物与所述细胞材料分离，以提供包括所述苄基异喹啉生物碱产物的所述产物流。

143.根据条款139所述的方法，其中所述间歇式反应器的至少一个工艺参数可修改，以改变所得的苄基异喹啉生物碱产物组成。

144.根据条款140所述的方法，其中可修改的所述至少一个工艺参数包括溶解氧、pH、搅拌速度、通气速率和细胞密度中的至少一个。

145.根据条款139所述的方法，其中所述苄基异喹啉生物碱产物包括苄基异喹啉生物碱。

146.根据条款142所述的方法，其中所述苄基异喹啉生物碱具有选自下组的结构种类：苄基异喹啉类、原小檗碱类、原阿片碱类、苯菲啶类、原吗啡喃类、吗啡喃类、断裂小檗碱类、苯酞异喹啉类、阿朴啡类，以及双苄基异喹啉类。

147.根据条款143所述的方法，其中所述苄基异喹啉生物碱为选自下组的苄基异喹啉：罂粟碱、四氢罂粟碱、1,2-二氢罂粟碱，以及东罂粟灵。

148.根据条款143所述的方法，其中所述苄基异喹啉生物碱为选自下组的原小檗碱：金黄紫堇碱、碎叶紫堇碱、刺罂粟碱、南丁宁碱、药根碱、千金藤啶碱、离木明碱、顺式-N-甲基刺罂粟碱、四氢非洲防己碱、掌叶防己碱、四氢掌叶防己碱、非洲防己碱、坎那定、N-甲基坎那定、1-羟基坎那定、小檗碱、N-甲基-左旋蛇果黄堇碱、1,13-二羟基-N-甲基坎那定，以及1-羟基-10-O-乙酰基-N-甲基坎那定。

149.根据条款143所述的方法，其中所述苄基异喹啉生物碱为选自下组的原阿片碱：原阿片碱、6-羟基原阿片碱、别隐品碱、隐品碱、隐掌叶防己碱和黄唐松草碱。

150.根据条款143所述的方法，其中所述苄基异喹啉生物碱为选自下组的苯菲啶：二氢血根碱、血根碱、dihydrocheilirubine、cheilirubine、dihydromarcapine、marcapine和白屈菜红碱。

151.根据条款143所述的方法，其中所述苄基异喹啉生物碱为选自下组的原吗啡喃：沙罗泰里啶、沙罗泰里啶醇，以及沙罗泰里啶醇-7-O-乙酸酯。

152.根据条款143所述的方法，其中所述苄基异喹啉生物碱为选自下组的吗啡喃：蒂巴因、可待因酮、可待因、吗啡、吗啡酮、东罂粟碱、尼奥品酮、尼奥品、新吗啡、氢可酮、二氢可待因、14-羟基可待因酮、羟考酮、14-羟基可待因、吗啡酮、氢吗啡酮、二氢吗啡、二氢埃托啡、乙基吗啡、埃托啡、美托酮、丁丙诺啡、福尔可定、异可待因和氧吗啡酮。

153.根据条款143所述的方法，其中所述苄基异喹啉生物碱为选自下组的断裂小檗碱：4'-O-脱甲基马克兰特醛、4'-O-脱甲基罂粟奥辛、4'-O-脱甲基-3-O-乙酰基罂粟奥辛和3-

O-乙酰基罂粟奥辛。

154.根据条款143所述的方法，其中所述苄基异喹啉生物碱为选自下组的苯酞异喹啉：那可托灵半缩醛、那可汀半缩醛、那可托灵和诺斯卡品。

155.根据条款143所述的方法，其中所述苄基异喹啉生物碱为选自下组的阿朴啡：木兰花碱、紫堇块茎碱、阿朴吗啡、波尔定碱、异波尔定碱、异蒂巴因、异紫堇块茎碱以及glaufine。

156.根据条款143所述的方法，其中所述苄基异喹啉生物碱为选自下组的双苄基异喹啉：黄芦木碱、guattgaumerine、蝙蝠葛碱和莲心碱。

157.一种用于形成具有苄基异喹啉生物碱产物的产物流的方法，包括：

(a)向反应器中提供工程化的非植物细胞以及包括营养素和水的原料；

(b)在所述反应器中，使所述工程化的非植物细胞与碳水化合物源和氮源接触，从而产生包括细胞材料和所述苄基异喹啉生物碱产物的溶液，其中所述苄基异喹啉生物碱产物衍生自牛心果碱，其中所述溶液包括不多于一个种类的选自下组的分子：原小檗碱、吗啡喃、异帕威、阿朴啡，以及双苄基异喹啉；以及

(c)使用至少一个分离装置将所述苄基异喹啉生物碱产物与所述细胞材料分离，以提供包括所述苄基异喹啉生物碱产物的所述产物流。

158.根据条款154所述的方法，其中所述产物流不包含超过5ppm的选自下组的分子：木质素、类黄酮、类菲、乳胶、1,5-二磷酸核酮糖羧化酶/加氧酶、袂康酸、假吗啡、那碎因、蒂巴酚和花粉。

159.根据条款155所述的方法，其中所述产物流不包含超过5ppm的袂康酸。

160.根据条款154所述的方法，其中所述产物流不包含可检测量的选自由以下各项组成的组的物质：杀虫剂、杀真菌剂或除草剂。

161.根据条款154所述的方法，其中使用至少液-液萃取从所述产物流中回收所述苄基异喹啉生物碱产物。

162.根据条款158所述的方法，其中在发酵过程完成之后立即回收所述苄基异喹啉生物碱产物。

163.根据前述条款中任一项所述的方法，其中所述工程化的非植物细胞包括两个或更多个异源编码序列，其中所述两个或更多个异源编码序列编码参与将底物转化成苄基异喹啉生物碱产物的代谢途径的至少第一酶和第二酶，其中所述第一酶和所述第二酶沿着所述代谢途径可操作地连接。

164.根据前述条款中任一项所述的方法，其中所述工程化的非植物细胞包括三个异源编码序列，其中所述三个异源编码序列编码参与将底物转化成苄基异喹啉生物碱产物的代谢途径的至少第一酶、第二酶和第三酶，其中所述第一酶、所述第二酶和所述第三酶沿着所述代谢途径可操作地连接。

165.根据条款163所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：4'-O-甲基转移酶、小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、四氢原小檗碱-N-甲基转移酶、金黄紫堇碱9-O-甲基转移酶、坎那定合酶、四氢原小檗碱氧化酶、原阿片碱O-脱烷基酶，以及细胞色素P450还原酶。

166.根据条款164所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：4'-O-甲基转移酶、小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、四氢原小檗碱-N-甲基转移酶、金黄紫堇碱9-O-甲基转移酶、坎那定合酶、四氢原小檗碱氧化酶、原阿片碱O-脱烷基酶，以及细胞色素P450还原酶。

167.根据条款165或166所述的方法，其中所述苄基异喹啉生物碱产物为原小檗碱产物。

168.根据条款163所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、四氢原小檗碱-N-甲基转移酶、顺式-N-甲基刺罂粟碱14-羟化酶、原阿片碱-6-羟化酶、原阿片碱O-脱烷基酶，以及细胞色素P450还原酶。

169.根据条款164所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、四氢原小檗碱-N-甲基转移酶、顺式-N-甲基刺罂粟碱14-羟化酶、原阿片碱-6-羟化酶、原阿片碱O-脱烷基酶，以及细胞色素P450还原酶。

170.根据条款168或169所述的方法，其中所述苄基异喹啉生物碱产物为原阿片碱产物。

171.根据条款163所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、四氢原小檗碱-N-甲基转移酶、顺式-N-甲基刺罂粟碱14-羟化酶、原阿片碱-6-羟化酶、二氢苯菲啶氧化酶，以及细胞色素P450还原酶。

172.根据条款164所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、四氢原小檗碱-N-甲基转移酶、顺式-N-甲基刺罂粟碱14-羟化酶、原阿片碱-6-羟化酶、二氢苯菲啶氧化酶，以及细胞色素P450还原酶。

173.根据条款171或172所述的方法，其中所述苄基异喹啉生物碱产物为苯菲啶产物。

174.根据条款163所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：4'-O-甲基转移酶、BIA差向异构酶、沙罗泰里啶合酶、沙罗泰里啶还原酶、沙罗泰里啶醇7-O-乙酰转移酶，以及细胞色素P450还原酶。

175.根据条款164所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：4'-O-甲基转移酶、BIA差向异构酶、沙罗泰里啶合酶、沙罗泰里啶还原酶、沙罗泰里啶醇7-O-乙酰转移酶，以及细胞色素P450还原酶。

176.根据条款174或175所述的方法，其中所述苄基异喹啉生物碱产物为原吗啡喃产物。

177.根据条款163所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：BIA差向异构酶、沙罗泰里啶合酶、沙罗泰里啶还原酶、沙罗泰里啶醇7-O-乙酰转移酶、蒂巴因6-O-脱甲基酶、

可待因酮还原酶、可待因O-脱甲基酶、吗啡脱氢酶、吗啡酮还原酶，以及细胞色素P450还原酶。

178.根据条款164所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：BIA差向异构酶、沙罗泰里啶合酶、沙罗泰里啶还原酶、沙罗泰里啶醇7-O-乙酰转移酶、蒂巴因6-O-脱甲基酶、可待因酮还原酶、可待因O-脱甲基酶、吗啡脱氢酶、吗啡酮还原酶，以及细胞色素P450还原酶。

179.根据条款177或178所述的方法，其中所述苄基异喹啉生物碱产物为吗啡喃产物。

180.根据条款163所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：小檗碱桥酶、金黄紫堇碱9-O-甲基转移酶、坎那定合酶、四氢原小檗碱-N-甲基转移酶、N-甲基坎那定1-羟化酶、1-羟基-N-甲基坎那定13-羟化酶、4'-O-脱甲基-3-O-乙酰基罂粟奥辛合酶、1,13-二羟基-N-甲基坎那定13-O乙酰转移酶，以及细胞色素P450还原酶。

181.根据条款164所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：小檗碱桥酶、金黄紫堇碱9-O-甲基转移酶、坎那定合酶、四氢原小檗碱-N-甲基转移酶、N-甲基坎那定1-羟化酶、1-羟基-N-甲基坎那定13-羟化酶、4'-O-脱甲基-3-O-乙酰基罂粟奥辛合酶、1,13-二羟基-N-甲基坎那定13-O乙酰转移酶，以及细胞色素P450还原酶。

182.根据条款180或181所述的方法，其中所述苄基异喹啉生物碱产物为断裂小檗碱产物。

183.根据条款163所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：小檗碱桥酶、金黄紫堇碱9-O-甲基转移酶、坎那定合酶、四氢原小檗碱-N-甲基转移酶、N-甲基坎那定1-羟化酶、1-羟基-N-甲基坎那定13-羟化酶、4'-O-脱甲基-3-O-乙酰基罂粟奥辛合酶、1,13-二羟基-N-甲基坎那定13-O乙酰转移酶、那可汀半缩醛合酶、诺斯卡品合酶、那可托灵4'-O-甲基化酶，以及细胞色素P450还原酶。

184.根据条款164所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：小檗碱桥酶、金黄紫堇碱9-O-甲基转移酶、坎那定合酶、四氢原小檗碱-N-甲基转移酶、N-甲基坎那定1-羟化酶、1-羟基-N-甲基坎那定13-羟化酶、4'-O-脱甲基-3-O-乙酰基罂粟奥辛合酶、1,13-二羟基-N-甲基坎那定13-O乙酰转移酶、那可汀半缩醛合酶、诺斯卡品合酶、那可托灵4'-O-甲基化酶，以及细胞色素P450还原酶。

185.根据条款183或184所述的方法，其中所述苄基异喹啉生物碱产物为苯酞异喹啉产物。

186.根据条款163所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶，以及黄芦木碱合酶。

187.根据条款164所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢

途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4’-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶，以及黄芦木碱合酶。

188.根据条款186或187所述的方法，其中所述苄基异喹啉生物碱产物为双苄基异喹啉产物。

189.根据条款163所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4’-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶，以及BIA差向异构酶。

190.根据条款164所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4’-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶，以及BIA差向异构酶。

191.根据条款189或190所述的方法，其中所述苄基异喹啉生物碱产物为苄基异喹啉产物。

192.根据条款165-191中任一项所述的方法，其中所述工程化的非植物细胞包括两个或更多个另外的异源编码序列，其中所述两个或更多个另外的异源编码序列编码参与将底物转化成所述苄基异喹啉生物碱产物的代谢途径的至少第一附加酶和第二附加酶，其中所述第一附加酶和所述第二附加酶沿着所述代谢途径可操作地连接，并且其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一附加酶和所述第二附加酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4’-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶，以及BIA差向异构酶。

193.根据条款165-191中任一项所述的方法，其中所述工程化的非植物细胞包括三个另外的异源编码序列，其中所述三个异源编码序列编码参与将底物转化成所述苄基异喹啉生物碱产物的代谢途径的至少第一附加酶、第二附加酶和第三附加酶，其中所述第一附加酶、所述第二附加酶和所述第三附加酶沿着所述代谢途径可操作地连接，并且其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一附加酶、所述第二附加酶和所述第三附加酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4’-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶，以及BIA差向异构酶。

194.根据前述条款中任一项所述的工程化的非植物细胞，其中所述工程化的非植物细胞包括两个或更多个异源编码序列，其中所述两个或更多个异源编码序列编码参与将底物转化成苄基异喹啉生物碱产物的代谢途径的至少第一酶和第二酶，其中所述第一酶和所述第二酶沿着所述代谢途径可操作地连接。

195.根据前述条款中任一项所述的工程化的非植物细胞，其中所述工程化的非植物细胞包括三个异源编码序列，其中所述三个异源编码序列编码参与将底物转化成所述苄基异喹啉生物碱产物的代谢途径的至少第一酶、第二酶和第三酶，其中所述第一酶、所述第二酶和所述第三酶沿着所述代谢途径可操作地连接。

196.根据条款194所述的工程化的非植物细胞，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：4’-O-甲基转移酶、小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、四氢原小檗碱-N-甲基转移酶、金黄紫堇碱9-O-甲基转移酶、坎那定合酶、四氢原小檗碱氧化酶、原阿片碱O-脱烷基酶，以及细胞色素P450还原酶。

197.根据条款195所述的工程化的非植物细胞，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：4’-O-甲基转移酶、小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、四氢原小檗碱-N-甲基转移酶、金黄紫堇碱9-O-甲基转移酶、坎那定合酶、四氢原小檗碱氧化酶、原阿片碱O-脱烷基酶，以及细胞色素P450还原酶。

198.根据条款196或197所述的工程化的非植物细胞，其中所述苄基异喹啉生物碱产物为原小檗碱产物。

199.根据条款194所述的工程化的非植物细胞，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、四氢原小檗碱-N-甲基转移酶、顺式-N-甲基刺罂粟碱14-羟化酶、原阿片碱-6-羟化酶、原阿片碱O-脱烷基酶，以及细胞色素P450还原酶。

200.根据条款195所述的工程化的非植物细胞，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、四氢原小檗碱-N-甲基转移酶、顺式-N-甲基刺罂粟碱14-羟化酶、原阿片碱-6-羟化酶、原阿片碱O-脱烷基酶，以及细胞色素P450还原酶。

201.根据条款199或200所述的工程化的非植物细胞，其中所述苄基异喹啉生物碱产物为原阿片碱产物。

202.根据条款194所述的工程化的非植物细胞，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、四氢原小檗碱-N-甲基转移酶、顺式-N-甲基刺罂粟碱14-羟化酶、原阿片碱-6-羟化酶、二氢苯菲啶氧化酶，以及细胞色素P450还原酶。

203.根据条款195所述的工程化的非植物细胞，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、四氢原小檗碱-N-甲基转移酶、顺式-N-甲基刺罂粟碱14-羟化酶、原阿片碱-6-羟化酶、二氢苯菲啶氧化酶，以及细胞色素P450还原酶。

204.根据条款202或203所述的工程化的非植物细胞，其中所述苄基异喹啉生物碱产物为苯菲啶产物。

205.根据条款194所述的工程化的非植物细胞，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：4’-O-甲基转移酶、BIA差向异构酶、沙罗泰里啶合酶、沙罗泰里啶还原酶、沙罗泰里啶醇7-O-乙酰转移酶，以及细胞色素P450还原酶。

206.根据条款195所述的工程化的非植物细胞，其中参与产生所述苄基异喹啉生物碱

产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组:4'-O-甲基转移酶、BIA差向异构酶、沙罗泰里啶合酶、沙罗泰里啶还原酶、沙罗泰里啶醇7-O-乙酰转移酶,以及细胞色素P450还原酶。

207.根据条款205或206所述的工程化的非植物细胞,其中所述苄基异喹啉生物碱产物为原吗啡喃产物。

208.根据条款194所述的工程化的非植物细胞,其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组:BIA差向异构酶、沙罗泰里啶合酶、沙罗泰里啶还原酶、沙罗泰里啶醇7-O-乙酰转移酶、蒂巴因6-O-脱甲基酶、可待因酮还原酶、可待因O-脱甲基酶、吗啡脱氢酶、吗啡酮还原酶,以及细胞色素P450还原酶。

209.根据条款195所述的工程化的非植物细胞,其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组:BIA差向异构酶、沙罗泰里啶合酶、沙罗泰里啶还原酶、沙罗泰里啶醇7-O-乙酰转移酶、蒂巴因6-O-脱甲基酶、可待因酮还原酶、可待因O-脱甲基酶、吗啡脱氢酶、吗啡酮还原酶,以及细胞色素P450还原酶。

210.根据条款208或209所述的工程化的非植物细胞,其中所述苄基异喹啉生物碱产物为吗啡喃产物。

211.根据条款194所述的工程化的非植物细胞,其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组:小檗碱桥酶、金黄紫堇碱9-O-甲基转移酶、坎那定合酶、四氢原小檗碱-N-甲基转移酶、N-甲基坎那定1-羟化酶、1-羟基-N-甲基坎那定13-羟化酶、4'-O-脱甲基-3-O-乙酰基罂粟奥辛合酶、1,13-二羟基-N-甲基坎那定13-O乙酰转移酶,以及细胞色素P450还原酶。

212.根据条款195所述的工程化的非植物细胞,其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组:小檗碱桥酶、金黄紫堇碱9-O-甲基转移酶、坎那定合酶、四氢原小檗碱-N-甲基转移酶、N-甲基坎那定1-羟化酶、1-羟基-N-甲基坎那定13-羟化酶、4'-O-脱甲基-3-O-乙酰基罂粟奥辛合酶、1,13-二羟基-N-甲基坎那定13-O乙酰转移酶,以及细胞色素P450还原酶。

213.根据条款211或212所述的工程化的非植物细胞,其中所述苄基异喹啉生物碱产物为断裂小檗碱产物。

214.根据条款194所述的工程化的非植物细胞,其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组:小檗碱桥酶、金黄紫堇碱9-O-甲基转移酶、坎那定合酶、四氢原小檗碱-N-甲基转移酶、N-甲基坎那定1-羟化酶、1-羟基-N-甲基坎那定13-羟化酶、4'-O-脱甲基-3-O-乙酰基罂粟奥辛合酶、1,13-二羟基-N-甲基坎那定13-O乙酰转移酶、那可汀半缩醛合酶、诺斯卡品合酶、那可托灵4'-O-甲基化酶,以及细胞色素P450还原酶。

215.根据条款195所述的工程化的非植物细胞,其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组:小檗碱桥酶、金黄紫堇碱9-O-甲基转移酶、坎那定合酶、四氢原小檗碱-N-甲基转移酶、N-甲基坎那定1-羟化酶、1-羟基-N-甲基坎那定13-羟化酶、4'-O-脱甲基-3-O-乙酰基

罂粟奥辛合酶、1,13-二羟基-N-甲基坎那定13-O乙酰转移酶、那可汀半缩醛合酶、诺斯卡品合酶、那可托灵4'-O-甲基化酶，以及细胞色素P450还原酶。

216.根据条款214或215所述的工程化的非植物细胞，其中所述苄基异喹啉生物碱产物为苯酞异喹啉产物。

217.根据条款194所述的工程化的非植物细胞，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶，以及黄芦木碱合酶。

218.根据条款195所述的工程化的非植物细胞，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶，以及黄芦木碱合酶。

219.根据条款217或218所述的工程化的非植物细胞，其中所述苄基异喹啉生物碱产物为双苄基异喹啉产物。

220.根据条款194所述的工程化的非植物细胞，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶，以及BIA差向异构酶。

221.根据条款195所述的工程化的非植物细胞，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶，以及BIA差向异构酶。

222.根据条款220或221所述的工程化的非植物细胞，其中所述苄基异喹啉生物碱产物为苄基异喹啉产物。

223.根据条款196-222中任一项所述的工程化的非植物细胞，其中所述工程化的非植物细胞包括两个或更多个另外的异源编码序列，其中所述两个或更多个另外的异源编码序列编码参与将底物转化成所述苄基异喹啉生物碱产物的代谢途径的至少第一附加酶和第二附加酶，其中所述第一附加酶和所述第二附加酶沿着所述代谢途径可操作地连接，并且其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一附加酶和所述第二附加酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶，以及BIA差向异构酶。

224.根据条款196-222中任一项所述的工程化的非植物细胞，其中所述工程化的非植物细胞包括三个另外的异源编码序列，其中所述三个异源编码序列编码参与将底物转化成所述苄基异喹啉生物碱产物的代谢途径的至少第一附加酶、第二附加酶和第三附加酶，其中所述第一附加酶、所述第二附加酶和所述第三附加酶沿着所述代谢途径可操作地连接，并且其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一附加酶、所述第二附加酶和所述第三附加酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细

胞色素P450还原酶，以及BIA差向异构酶。

225.根据条款163所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、4'-O-甲基转移酶、小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、四氢原小檗碱-N-甲基转移酶、金黄紫堇碱9-O-甲基转移酶、坎那定合酶、四氢原小檗碱氧化酶，以及原阿片碱O-脱烷基酶。

226.根据条款164所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、4'-O-甲基转移酶、小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、四氢原小檗碱-N-甲基转移酶、金黄紫堇碱9-O-甲基转移酶、坎那定合酶、四氢原小檗碱氧化酶，以及原阿片碱O-脱烷基酶。

227.根据条款225或226所述的方法，其中所述苄基异喹啉生物碱产物为原小檗碱产物。

228.根据条款163所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、四氢原小檗碱-N-甲基转移酶、顺式-N-甲基刺罂粟碱14-羟化酶、原阿片碱-6-羟化酶，以及原阿片碱O-脱烷基酶。

229.根据条款164所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、四氢原小檗碱-N-甲基转移酶、顺式-N-甲基刺罂粟碱14-羟化酶、原阿片碱-6-羟化酶，以及原阿片碱O-脱烷基酶。

230.根据条款228或229所述的方法，其中所述苄基异喹啉生物碱产物为原阿片碱产物。

231.根据条款163所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、四氢原小檗碱-N-甲基转移酶、顺式-N-甲基刺罂粟碱14-羟化酶、原阿片碱-6-羟化酶，以及二氢苯菲啶氧化酶。

232.根据条款164所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、小檗碱桥酶、碎叶紫堇碱合酶、刺罂

粟碱合酶、四氢原小檗碱-N-甲基转移酶、顺式-N-甲基刺罂粟碱14-羟化酶、原阿片碱-6-羟化酶，以及二氢苯菲啶氧化酶。

233.根据条款231或232所述的方法，其中所述苄基异喹啉生物碱产物为苯菲啶产物。

234.根据条款163所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、4'-O-甲基转移酶、BIA差向异构酶、沙罗泰里啶合酶、沙罗泰里啶还原酶，以及沙罗泰里啶醇7-O-乙酰转移酶。

235.根据条款164所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、4'-O-甲基转移酶、BIA差向异构酶、沙罗泰里啶合酶、沙罗泰里啶还原酶，以及沙罗泰里啶醇7-O-乙酰转移酶。

236.根据条款234或235所述的方法，其中所述苄基异喹啉生物碱产物为原吗啡喃产物。

237.根据条款163所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、沙罗泰里啶合酶、沙罗泰里啶还原酶、沙罗泰里啶醇7-O-乙酰转移酶、蒂巴因6-O-脱甲基酶、可待因酮还原酶、可待因O-脱甲基酶、吗啡脱氢酶，以及吗啡酮还原酶。

238.根据条款164所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、沙罗泰里啶合酶、沙罗泰里啶还原酶、沙罗泰里啶醇7-O-乙酰转移酶、蒂巴因6-O-脱甲基酶、可待因酮还原酶、可待因O-脱甲基酶、吗啡脱氢酶，以及吗啡酮还原酶。

239.根据条款237或238所述的方法，其中所述苄基异喹啉生物碱产物为吗啡喃产物。

240.根据条款163所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、小檗碱桥酶、金黄紫堇碱9-O-甲基转移酶、坎那定合酶、四氢原小檗碱-N-甲基转移酶、N-甲基坎那定1-羟化酶、1-羟基-N-甲基坎那定13-羟化酶、4'-O-脱甲基-3-O-乙酰基罂粟奥辛合酶，以及1,13-二羟基-N-甲基坎那定13-O乙酰转移酶。

241.根据条款164所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、小檗碱桥酶、金黄紫堇碱9-O-甲基

转移酶、坎那定合酶、四氢原小檗碱-N-甲基转移酶、N-甲基坎那定1-羟化酶、1-羟基-N-甲基坎那定13-羟化酶、4'-O-脱甲基-3-O-乙酰基罂粟奥辛合酶，以及1,13-二羟基-N-甲基坎那定13-O乙酰转移酶。

242.根据条款240或241所述的方法，其中所述苄基异喹啉生物碱产物为断裂小檗碱产物。

243.根据条款163所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、小檗碱桥酶、金黄紫堇碱9-O-甲基转移酶、坎那定合酶、四氢原小檗碱-N-甲基转移酶、N-甲基坎那定1-羟化酶、1-羟基-N-甲基坎那定13-羟化酶、4'-O-脱甲基-3-O-乙酰基罂粟奥辛合酶、1,13-二羟基-N-甲基坎那定13-O乙酰转移酶、那可汀半缩醛合酶、诺斯卡品合酶，以及那可托灵4'-O-甲基化酶。

244.根据条款164所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、小檗碱桥酶、金黄紫堇碱9-O-甲基转移酶、坎那定合酶、四氢原小檗碱-N-甲基转移酶、N-甲基坎那定1-羟化酶、1-羟基-N-甲基坎那定13-羟化酶、4'-O-脱甲基-3-O-乙酰基罂粟奥辛合酶、1,13-二羟基-N-甲基坎那定13-O乙酰转移酶、那可汀半缩醛合酶、诺斯卡品合酶，以及那可托灵4'-O-甲基化酶。

245.根据条款243或244所述的方法，其中所述苄基异喹啉生物碱产物为苯酞异喹啉产物。

246.根据条款163所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：酪氨酸羟化酶、dopa脱羧酶、酪氨酸/dopa脱羧酶、单胺氧化酶、酪氨酸酶、去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶，以及黄芦木碱合酶。

247.根据条款164所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：酪氨酸羟化酶、dopa脱羧酶、酪氨酸/dopa脱羧酶、单胺氧化酶、酪氨酸酶、去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶，以及黄芦木碱合酶。

248.根据条款246或247所述的方法，其中所述苄基异喹啉生物碱产物为双苄基异喹啉产物。

249.根据条款163所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：酪氨酸羟化酶、dopa脱羧酶、酪氨酸/dopa脱羧酶、单胺氧化酶、酪氨酸酶、去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶，以及BIA差向异构酶。

250.根据条款164所述的方法，其中参与产生所述苄基异喹啉生物碱产物的所述代谢

途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组:酪氨酸羟化酶、dopa脱羧酶、酪氨酸/dopa脱羧酶、单胺氧化酶、酪氨酸酶、去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶,以及BIA差向异构酶。

251.根据条款249或250所述的方法,其中所述苄基异喹啉生物碱产物为苄基异喹啉产物。

252.根据条款225-251中任一项所述的方法,其中所述工程化的非植物细胞包括两个或更多个另外的异源编码序列,其中所述两个或更多个另外的异源编码序列编码参与将底物转化成所述苄基异喹啉生物碱产物的代谢途径的至少第一附加酶和第二附加酶,其中所述第一附加酶和所述第二附加酶沿着所述代谢途径可操作地连接,并且其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一附加酶和所述第二附加酶中的每一个选自由以下各项组成的组:去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶,以及BIA差向异构酶。

253.根据条款225-251中任一项所述的方法,其中所述工程化的非植物细胞包括三个另外的异源编码序列,其中所述三个异源编码序列编码参与将底物转化成所述苄基异喹啉生物碱产物的代谢途径的至少第一附加酶、第二附加酶和第三附加酶,其中所述第一附加酶、所述第二附加酶和所述第三附加酶沿着所述代谢途径可操作地连接,并且其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一附加酶、所述第二附加酶和所述第三附加酶中的每一个选自由以下各项组成的组:去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶,以及BIA差向异构酶。

254.根据条款194所述的工程化的非植物细胞,其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组:去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、4'-O-甲基转移酶、小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、四氢原小檗碱-N-甲基转移酶、金黄紫堇碱9-O-甲基转移酶、坎那定合酶、四氢原小檗碱氧化酶,以及原阿片碱O-脱烷基酶。

255.根据条款195所述的工程化的非植物细胞,其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组:去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、4'-O-甲基转移酶、小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、四氢原小檗碱-N-甲基转移酶、金黄紫堇碱9-O-甲基转移酶、坎那定合酶、四氢原小檗碱氧化酶,以及原阿片碱O-脱烷基酶。

256.根据条款254或255所述的工程化的非植物细胞,其中所述苄基异喹啉生物碱产物为原小檗碱产物。

257.根据条款194所述的工程化的非植物细胞,其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组:去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细

胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、四氢原小檗碱-N-甲基转移酶、顺式-N-甲基刺罂粟碱14-羟化酶、原阿片碱-6-羟化酶，以及原阿片碱O-脱烷基酶。

258.根据条款195所述的工程化的非植物细胞，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、四氢原小檗碱-N-甲基转移酶、顺式-N-甲基刺罂粟碱14-羟化酶、原阿片碱-6-羟化酶，以及原阿片碱O-脱烷基酶。

259.根据条款257或258所述的工程化的非植物细胞，其中所述苄基异喹啉生物碱产物为原阿片碱产物。

260.根据条款194所述的工程化的非植物细胞，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、四氢原小檗碱-N-甲基转移酶、顺式-N-甲基刺罂粟碱14-羟化酶、原阿片碱-6-羟化酶，以及二氢苯菲啶氧化酶。

261.根据条款195所述的工程化的非植物细胞，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、小檗碱桥酶、碎叶紫堇碱合酶、刺罂粟碱合酶、四氢原小檗碱-N-甲基转移酶、顺式-N-甲基刺罂粟碱14-羟化酶、原阿片碱-6-羟化酶，以及二氢苯菲啶氧化酶。

262.根据条款260或261所述的工程化的非植物细胞，其中所述苄基异喹啉生物碱产物为苯菲啶产物。

263.根据条款194所述的工程化的非植物细胞，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、4'-O-甲基转移酶、BIA差向异构酶、沙罗泰里啶合酶、沙罗泰里啶还原酶，以及沙罗泰里啶醇7-O-乙酰转移酶。

264.根据条款195所述的工程化的非植物细胞，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、4'-O-甲基转移酶、BIA差向异构酶、沙罗泰里啶合酶、沙罗泰里啶还原酶，以及沙罗泰里啶醇7-O-乙酰转移酶。

265.根据条款263或264所述的工程化的非植物细胞，其中所述苄基异喹啉生物碱产物为原吗啡喃产物。

266.根据条款194所述的工程化的非植物细胞，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：去

甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、沙罗泰里啶合酶、沙罗泰里啶还原酶、沙罗泰里啶醇7-O-乙酰转移酶、蒂巴因6-O-脱甲基酶、可待因酮还原酶、可待因O-脱甲基酶、吗啡脱氢酶，以及吗啡酮还原酶。

267.根据条款195所述的工程化的非植物细胞，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、沙罗泰里啶合酶、沙罗泰里啶还原酶、沙罗泰里啶醇7-O-乙酰转移酶、蒂巴因6-O-脱甲基酶、可待因酮还原酶、可待因O-脱甲基酶、吗啡脱氢酶，以及吗啡酮还原酶。

268.根据条款266或267所述的工程化的非植物细胞，其中所述苄基异喹啉生物碱产物为吗啡喃产物。

269.根据条款194所述的工程化的非植物细胞，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、小檗碱桥酶、金黄紫堇碱9-O-甲基转移酶、坎那定合酶、四氢原小檗碱-N-甲基转移酶、N-甲基坎那定1-羟化酶、1-羟基-N-甲基坎那定13-羟化酶、4'-O-脱甲基-3-O-乙酰基罂粟奥辛合酶，以及1,13-二羟基-N-甲基坎那定13-O乙酰转移酶。

270.根据条款195所述的工程化的非植物细胞，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、小檗碱桥酶、金黄紫堇碱9-O-甲基转移酶、坎那定合酶、四氢原小檗碱-N-甲基转移酶、N-甲基坎那定1-羟化酶、1-羟基-N-甲基坎那定13-羟化酶、4'-O-脱甲基-3-O-乙酰基罂粟奥辛合酶，以及1,13-二羟基-N-甲基坎那定13-O乙酰转移酶。

271.根据条款269或270所述的工程化的非植物细胞，其中所述苄基异喹啉生物碱产物为断裂小檗碱产物。

272.根据条款194所述的工程化的非植物细胞，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、小檗碱桥酶、金黄紫堇碱9-O-甲基转移酶、坎那定合酶、四氢原小檗碱-N-甲基转移酶、N-甲基坎那定1-羟化酶、1-羟基-N-甲基坎那定13-羟化酶、4'-O-脱甲基-3-O-乙酰基罂粟奥辛合酶、1,13-二羟基-N-甲基坎那定13-O乙酰转移酶、那可汀半缩醛合酶、诺斯卡品合酶，以及那可托灵4'-O-甲基化酶。

273.根据条款195所述的工程化的非植物细胞，其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶、小檗碱桥酶、金黄紫

堇碱9-O-甲基转移酶、坎那定合酶、四氢原小檗碱-N-甲基转移酶、N-甲基坎那定1-羟化酶、1-羟基-N-甲基坎那定13-羟化酶、4'-O-脱甲基-3-O-乙酰基罂粟奥辛合酶、1,13-二羟基-N-甲基坎那定13-O乙酰转移酶、那可汀半缩醛合酶、诺斯卡品合酶,以及那可托灵4'-O-甲基化酶。

274.根据条款272或273所述的工程化的非植物细胞,其中所述苄基异喹啉生物碱产物为苯酞异喹啉产物。

275.根据条款194所述的工程化的非植物细胞,其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组:酪氨酸羟化酶、dopa脱羧酶、酪氨酸/dopa脱羧酶、单胺氧化酶、酪氨酸酶、去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶,以及黄芦木碱合酶。

276.根据条款195所述的工程化的非植物细胞,其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组:酪氨酸羟化酶、dopa脱羧酶、酪氨酸/dopa脱羧酶、单胺氧化酶、酪氨酸酶、去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶、BIA差向异构酶,以及黄芦木碱合酶。

277.根据条款275或276所述的工程化的非植物细胞,其中所述苄基异喹啉生物碱产物为双苄基异喹啉产物。

278.根据条款194所述的工程化的非植物细胞,其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶和所述第二酶中的每一个选自由以下各项组成的组:酪氨酸羟化酶、dopa脱羧酶、酪氨酸/dopa脱羧酶、单胺氧化酶、酪氨酸酶、去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶,以及BIA差向异构酶。

279.根据条款195所述的工程化的非植物细胞,其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一酶、所述第二酶和所述第三酶中的每一个选自由以下各项组成的组:酪氨酸羟化酶、dopa脱羧酶、酪氨酸/dopa脱羧酶、单胺氧化酶、酪氨酸酶、去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶,以及BIA差向异构酶。

280.根据条款220或221所述的工程化的非植物细胞,其中所述苄基异喹啉生物碱产物为苄基异喹啉产物。

281.根据条款196-222中任一项所述的工程化的非植物细胞,其中所述工程化的非植物细胞包括两个或更多个另外的异源编码序列,其中所述两个或更多个另外的异源编码序列编码参与将底物转化成所述苄基异喹啉生物碱产物的代谢途径的至少第一附加酶和第二附加酶,其中所述第一附加酶和所述第二附加酶沿着所述代谢途径可操作地连接,并且其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一附加酶和所述第二附加酶中的每一个选自由以下各项组成的组:去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶,以及BIA差向异构酶。

282.根据条款196-222中任一项所述的工程化的非植物细胞,其中所述工程化的非植

物细胞包括三个另外的异源编码序列，其中所述三个异源编码序列编码参与将底物转化成所述苄基异喹啉生物碱产物的代谢途径的至少第一附加酶、第二附加酶和第三附加酶，其中所述第一附加酶、所述第二附加酶和所述第三附加酶沿着所述代谢途径可操作地连接，并且其中参与产生所述苄基异喹啉生物碱产物的所述代谢途径的所述第一附加酶、所述第二附加酶和所述第三附加酶中的每一个选自由以下各项组成的组：去甲乌药碱合酶、去甲乌药碱6-O-甲基转移酶、乌药碱-N-甲基转移酶、4'-O-甲基转移酶、细胞色素P450 80B1、细胞色素P450还原酶，以及BIA差向异构酶。

283.根据前述条款中任一项所述的工程化的非植物细胞，其中所述工程化的非植物细胞为工程化的酵母细胞。

284.根据前述条款中任一项所述的方法，其中所述工程化的非植物细胞为工程化的酵母细胞。

285.根据条款1-282中任一项所述的工程化的非植物细胞，其中所述工程化的非植物细胞为工程化的细菌细胞。

286.根据条款1-282中任一项所述的方法，其中所述工程化的非植物细胞为工程化的细菌细胞。

[0340] 虽然本文已经示出和描述了本发明的优选实施方案，但是将对本领域技术人员显而易见的是，此类实施方案仅以举例的方式提供。本领域的技术人员可以对其做出许多变形、改变和替换而不脱离本发明。应当理解，本文所述的本发明的实施方案的各种替代方案可在本发明的实践中采用。以下权利要求旨在限定本发明的范围并且从而覆盖在这些权利要求及其等同物范围内的方法和结构。

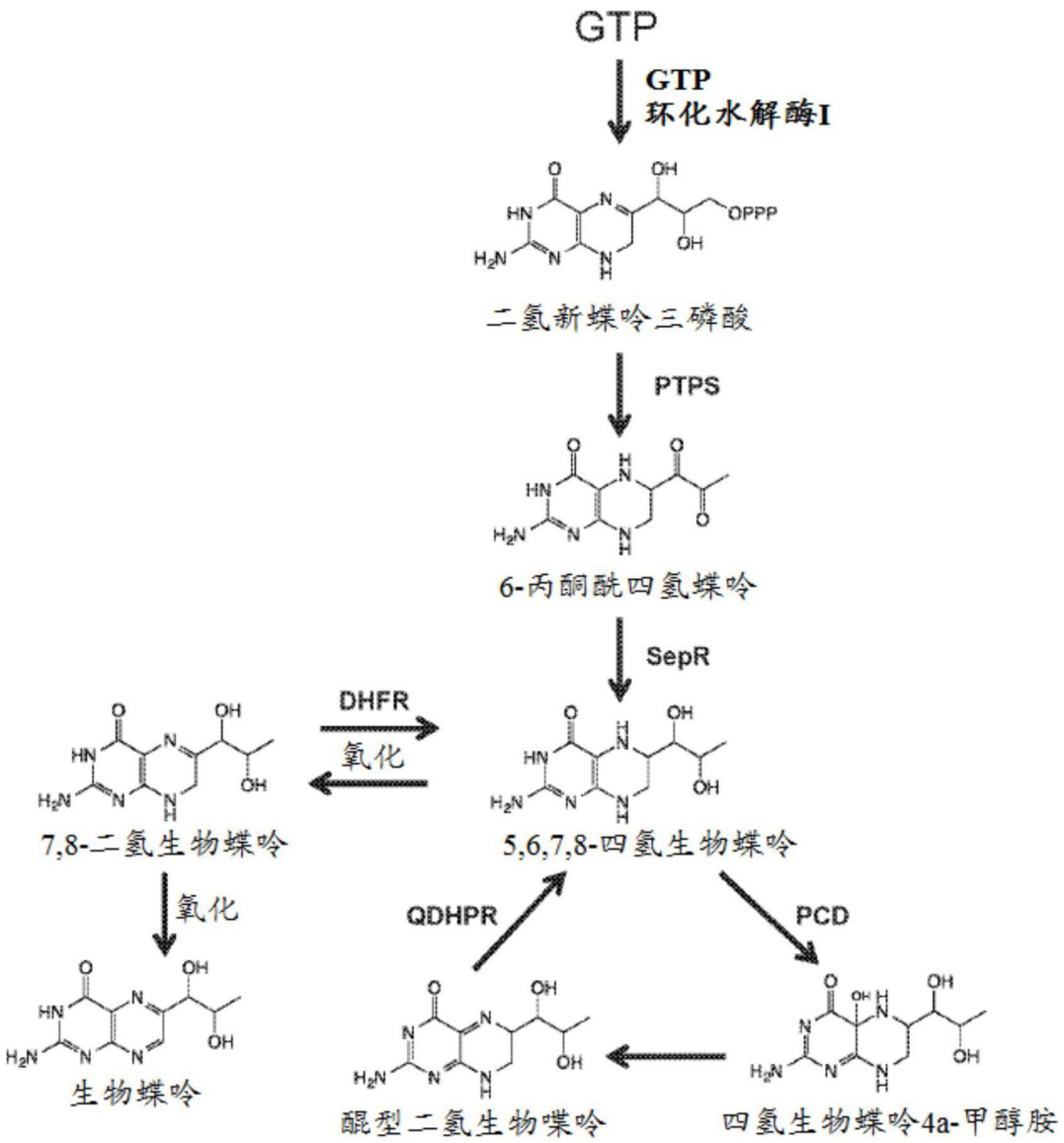

GTP
GTP
环化水解酶I
二氢新蝶呤三磷酸
PTPS
6-丙酮酰四氢蝶呤
SepR
DHFR
氧化
7,8-二氢生物蝶呤
5,6,7,8-四氢生物蝶呤
氧化
QDHPR
PCD
生物蝶呤
醌型二氢生物喋呤
四氢生物蝶呤4a-甲醇胺

图1

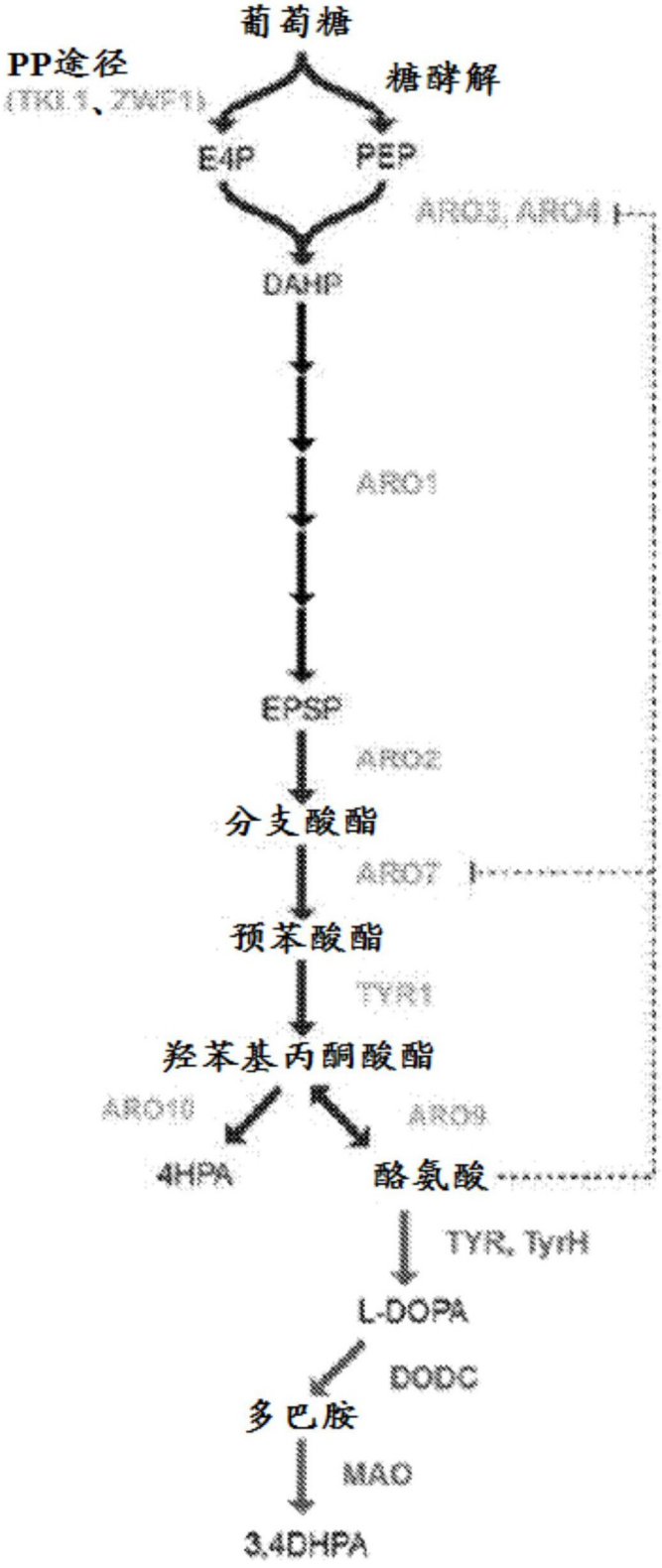

葡萄糖
PP途径
(TKL1、ZWF1)
糖酵解
E4P
PEP
ARO3, ARO4
DAHP
ARO1
EPSP
ARO2
分支酸酯
ARO7
预苯酸酯
TYR1
羟苯基丙酮酸酯
ARO10
ARO9
4HPA
酪氨酸
TYR, TyrH
L-DOPA
DODC
多巴胺
MAO
3,4DHPA

图2

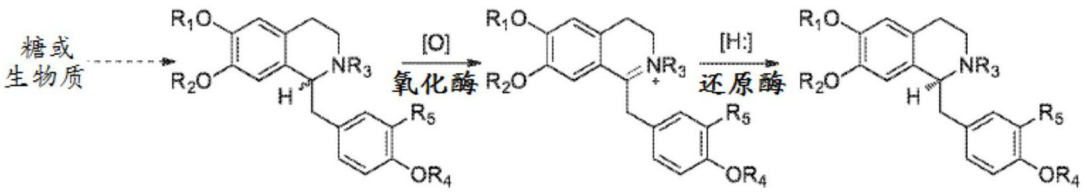


图3

>RQNK-2062398
MELQYISYFQPTSSVVALLLALVSILSSVVVLRKTFLNNYSSSPASSTKTAVLSH
QRQQSCALPISGLLHIFMNKNGLIHVTLGNMADKYGPIFSFPTGSHRTLVVSS
WEMVKECFTGNNDTAFSNRPIPLAFKTIFYACGGIDSYGLSSVPYGKYWREL
RKVCVHNLLSNQQLLKFRHLIISQVDTSFNKLYELCKNSEDNHGNYTTTTTTA
AGMVRIDDWLAELSFNVIGRIVCGFQSGPKTGAPSRVEQFKEAINEASYFMS
TSPVSDNVPMLGWIDQLTGLTRNMKHCGKKLDLVVESIINDHRQKRRFSRTK
GGDEKDDEQDDFIDICLSIMEQPQLPGNNNPSQIPIKSIVLDMIGGGTDTTKLT
TIWTLSLLLNNPHVLDKAKQEVDAHFRTKRRSTNDAAAAVVDFDDIRNLVYIQ
AIIKESMRLYPASPVVERLSGEDCVVGGFHVPAGTRLWANVWKMQRDPKVW
DDPLVFRPDRFLSDEQKMVDVRGQNYELLPFGAGRRVCPGVSFSLDLMQLV
LTRLILEFEMKSPSGKVDMTATPGLMSYKVIPLDILLTHRRIKPCVQSAASERD
MESSGVPVITLGSGKVMPVLGMGTFEKVGKGSERERLAILKAIEVGYRYFDT
AAAYETEEVLGEAIAEALQLGLVKSRDELFISSMLWCTDAHADRVLLALQNSL
RNLKLEYVDLYMLPFPASLKPGKITMDIPEEDICRMDYRSVWAAMEECQNLG
FTKSIGVSNFSCKKLQELMATANIPPAVNQVEMSPAFQQKKLREYCNANNILV
SAISVLGSNGTPWGSNAVLGSEVLKKIAMAKGKSVAQVSMRWVYEQGASLV
VKSFSEERLRENLNIFDWELTKEDHEKIGEIPQCRILSAYFLVSPNGPFKSQEE
LWDDEA*

图4

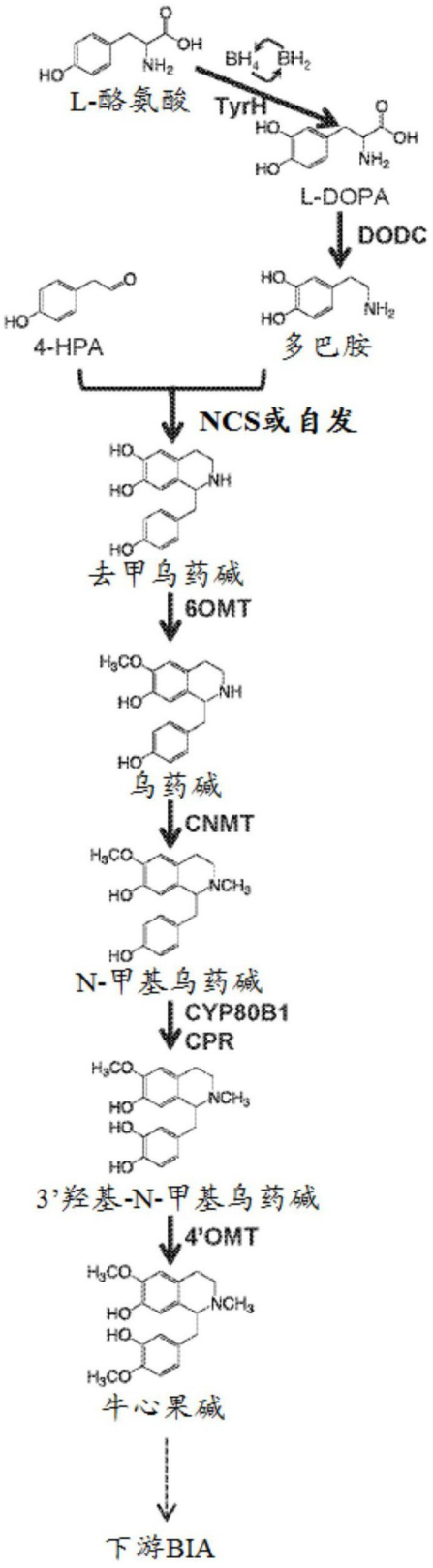

L-酪氨酸
TyrH
L-DOPA
DODC
4-HPA
多巴胺
NCS或自发
去甲乌药碱
6OMT
乌药碱
CNMT
N-甲基乌药碱
CYP80B1
CPR
3'羟基-N-甲基乌药碱
4'OMT
牛心果碱
下游BIA

图5

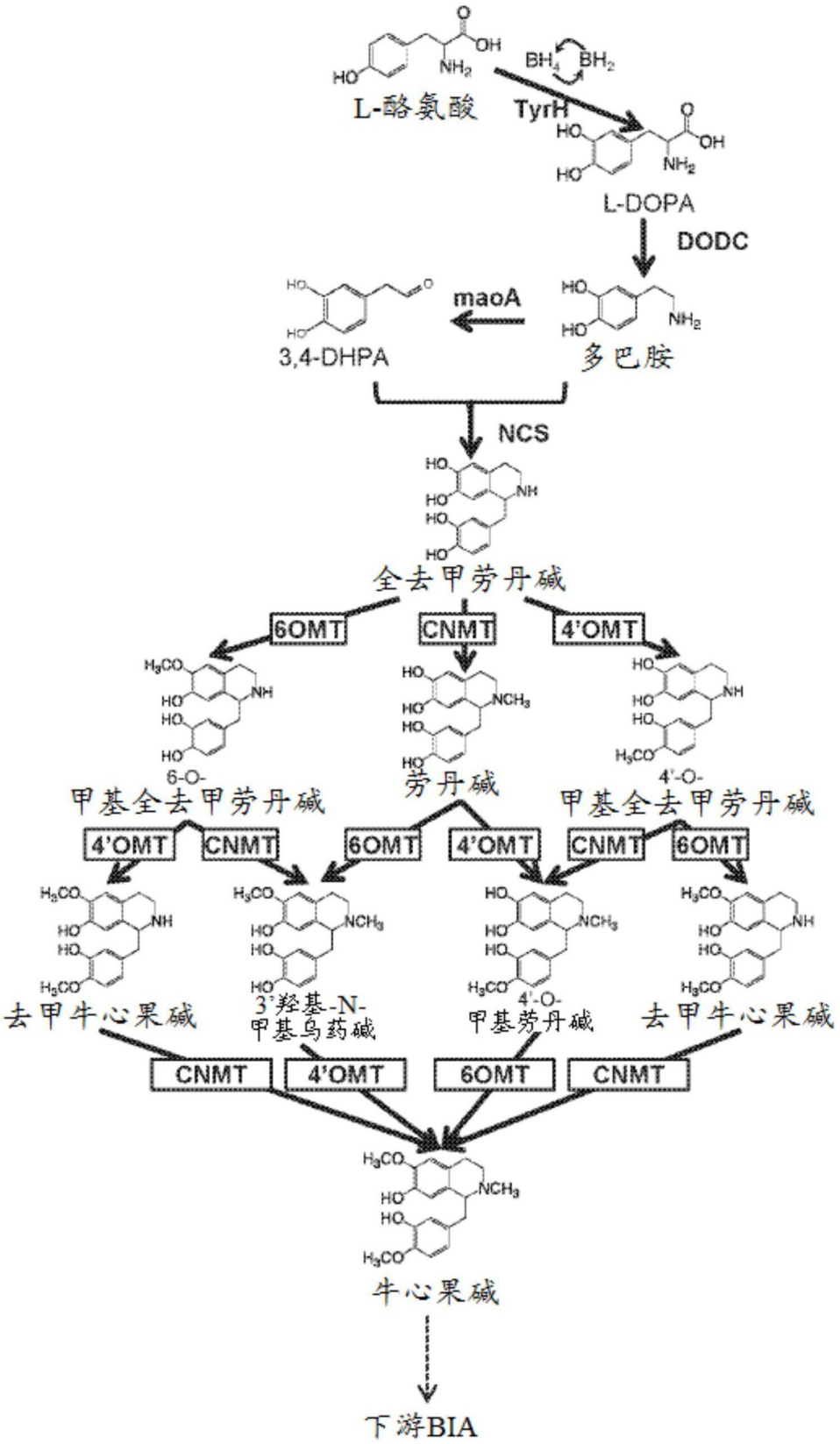

L-酪氨酸
TyrH
L-DOPA
DODC
多巴胺
maoA
3,4-DHPA
NCS
全去甲劳丹碱
6OMT
CNMT
4'OMT
6-O-甲基全去甲劳丹碱
劳丹碱
4'-O-甲基全去甲劳丹碱
去甲牛心果碱
3'羟基-N-甲基乌药碱
4'-O-甲基劳丹碱
去甲牛心果碱
牛心果碱
下游BIA

图6

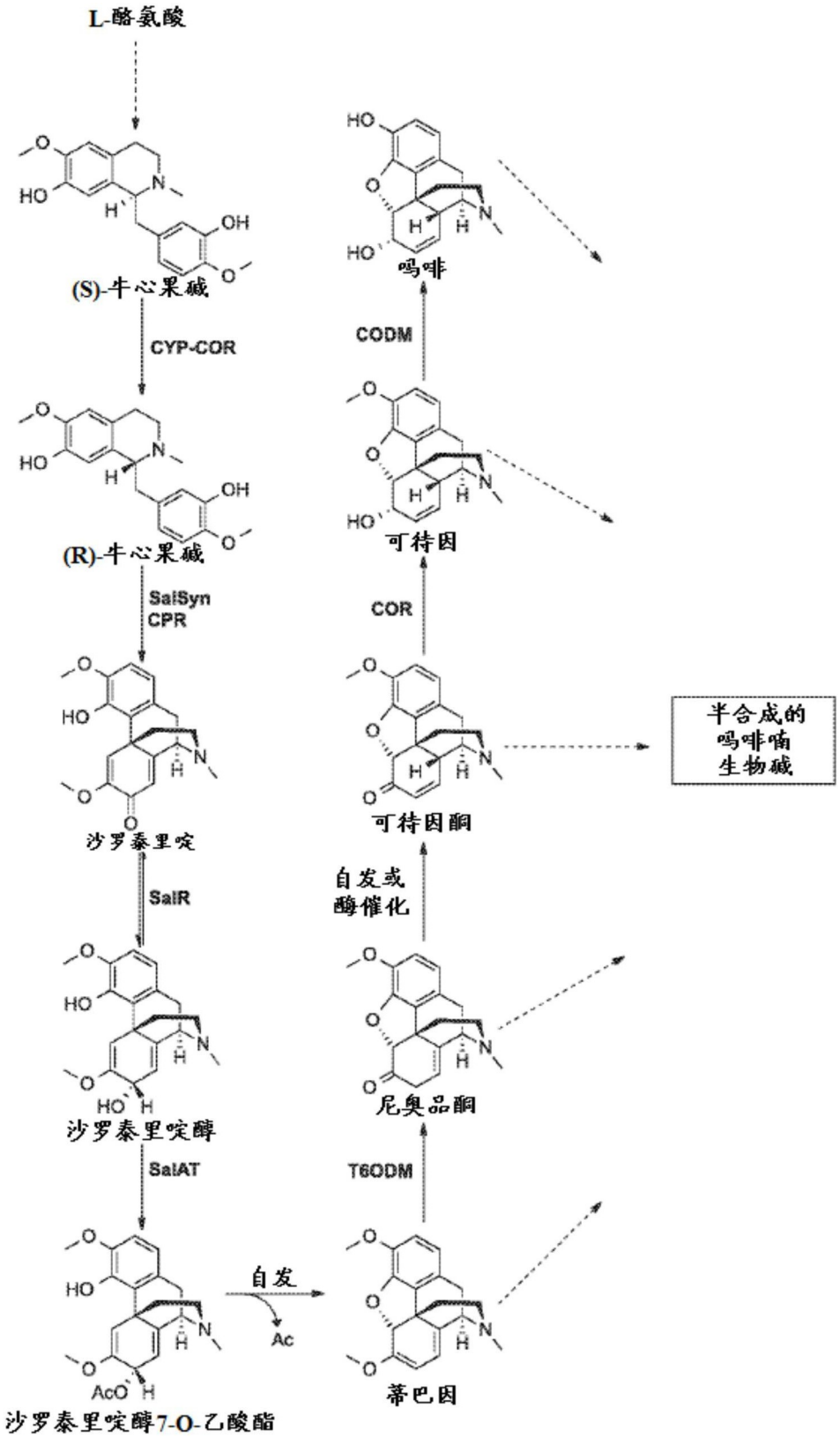

L-酪氨酸
(S)-牛心果碱
CYP-COR
(R)-牛心果碱
SalSyn
CPR
沙罗泰里啶
SalR
沙罗泰里啶醇
SalAT
沙罗泰里啶醇7-O-乙酸酯
自发
Ac
蒂巴因
T6ODM
尼奥品酮
自发或
酶催化
可待因酮
COR
可待因
CODM
吗啡
半合成的
吗啡喃
生物碱

图7

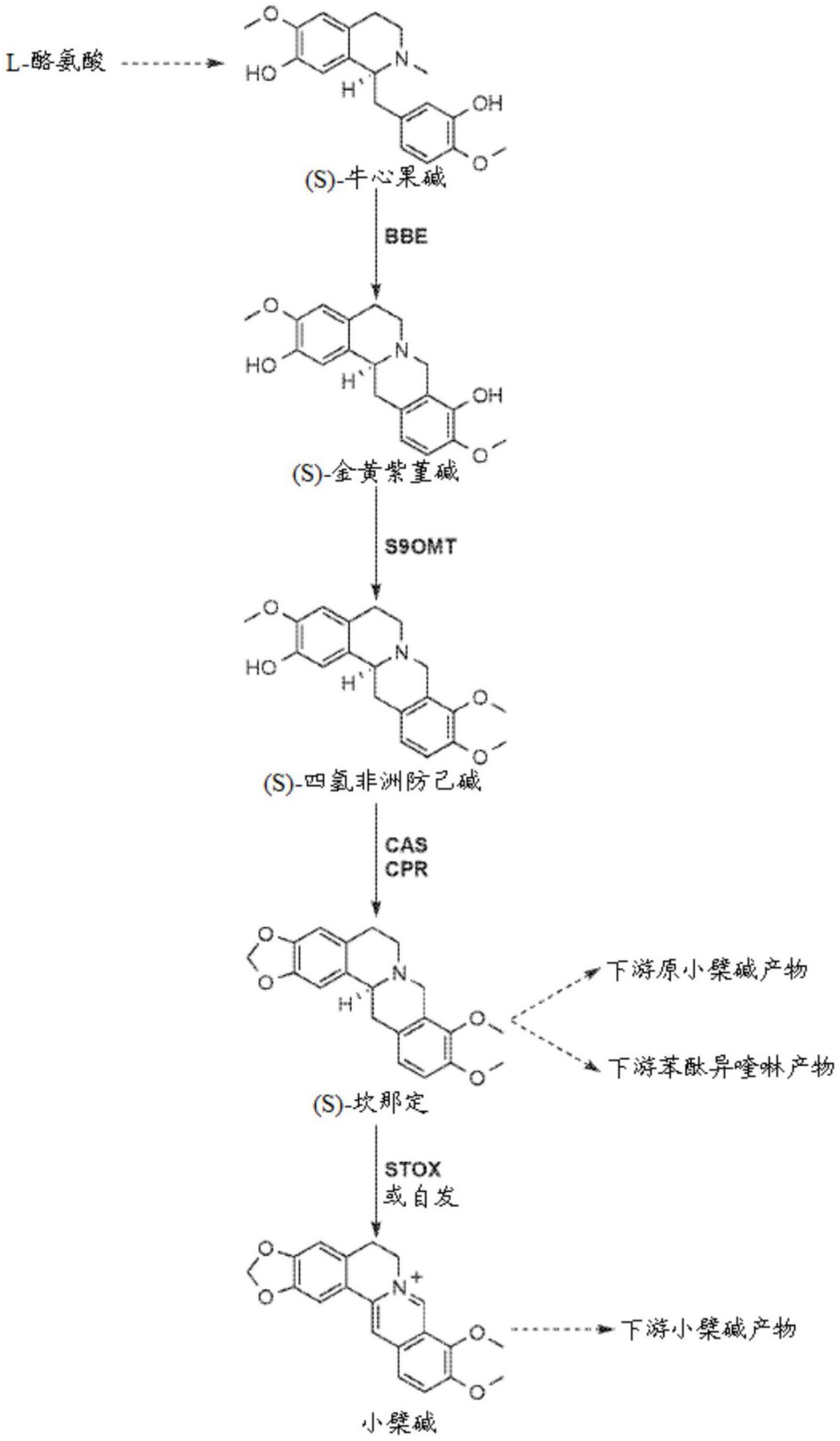

L-酪氨酸
(S)-牛心果碱
BBE
(S)-金黄紫堇碱
S9OMT
(S)-四氢非洲防己碱
CAS
CPR
下游原小檗碱产物
下游苯酞异喹啉产物
(S)-坎那定
STOX
或自发
下游小檗碱产物
小檗碱

图8

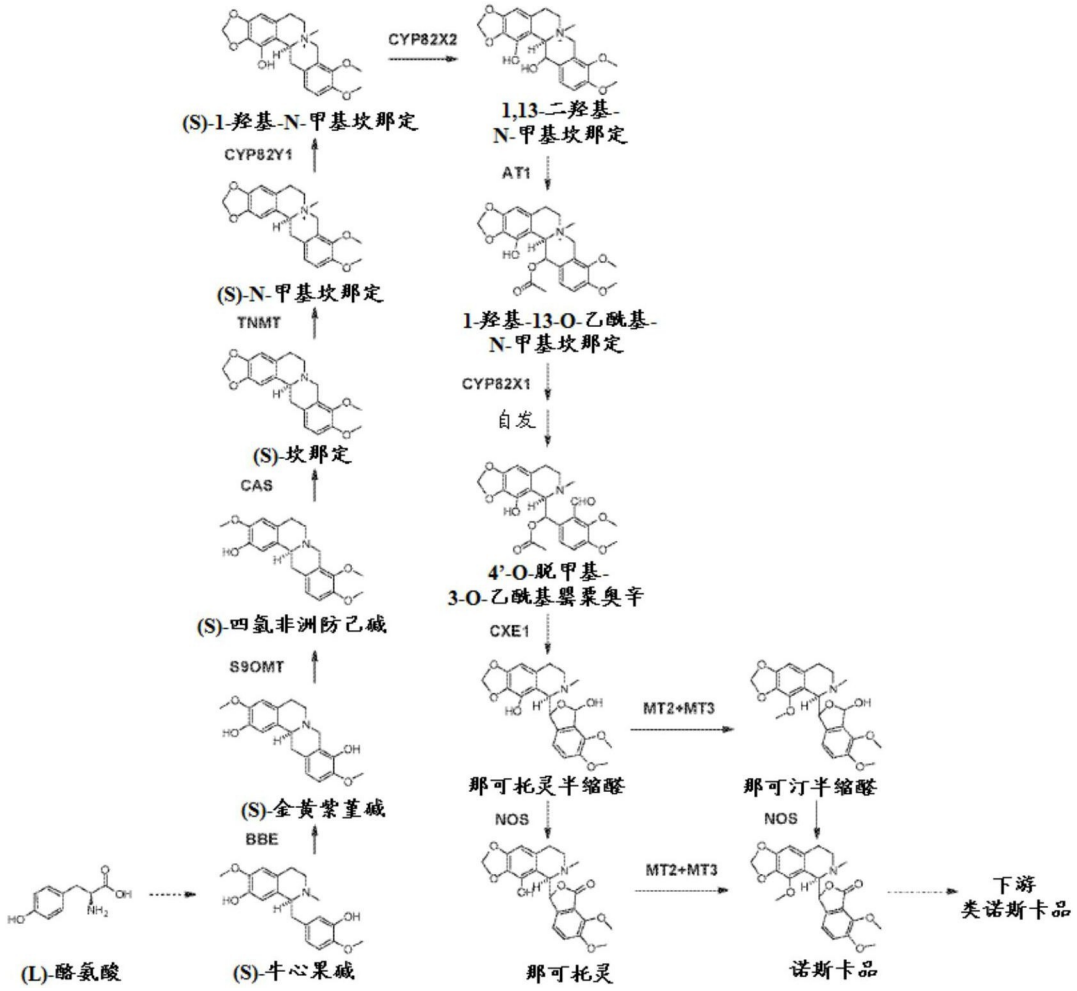

CYP82X2
(S)-1-羟基-N-甲基坎那定
1,13-二羟基-N-甲基坎那定
CYP82Y1
AT1
(S)-N-甲基坎那定
1-羟基-13-O-乙酰基-N-甲基坎那定
TNMT
CYP82X1
自发
(S)-坎那定
CAS
4'-O-脱甲基-3-O-乙酰基罂粟奥辛
(S)-四氢非洲防己碱
CXE1
S9OMT
MT2+MT3
(S)-金黄紫堇碱
那可托灵半缩醛
那可汀半缩醛
NOS
NOS
BBE
MT2+MT3
下游类诺斯卡品
(L)-酪氨酸
(S)-牛心果碱
那可托灵
诺斯卡品

图9

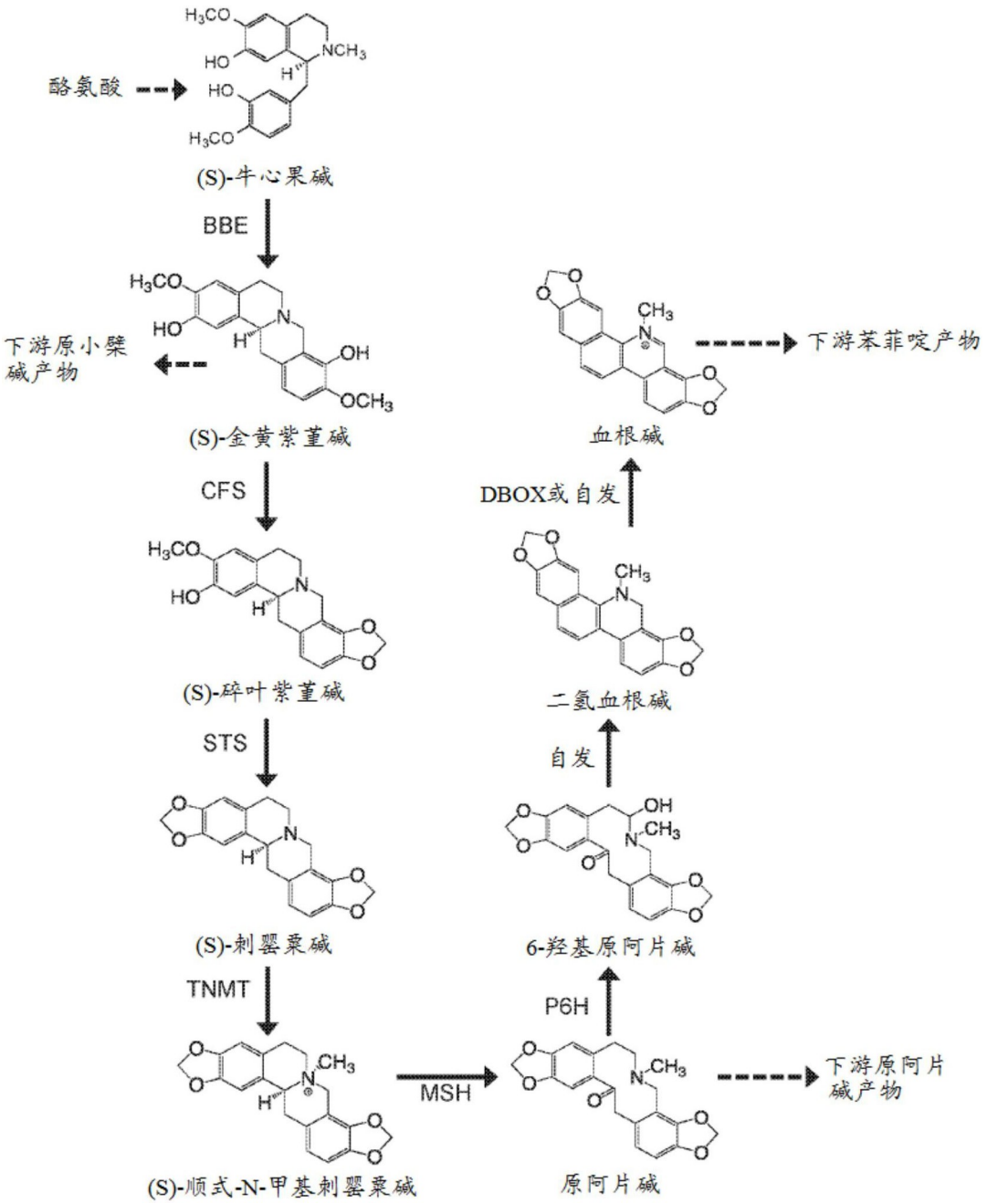

酪氨酸
(S)-牛心果碱
BBE
下游原小檗碱产物
(S)-金黄紫堇碱
CFS
(S)-碎叶紫堇碱
STS
(S)-刺罂粟碱
TNMT
(S)-顺式-N-甲基刺罂粟碱
MSH
原阿片碱
P6H
6-羟基原阿片碱
自发
二氢血根碱
DBOX或自发
血根碱
下游苯菲啶产物
下游原阿片碱产物

图10

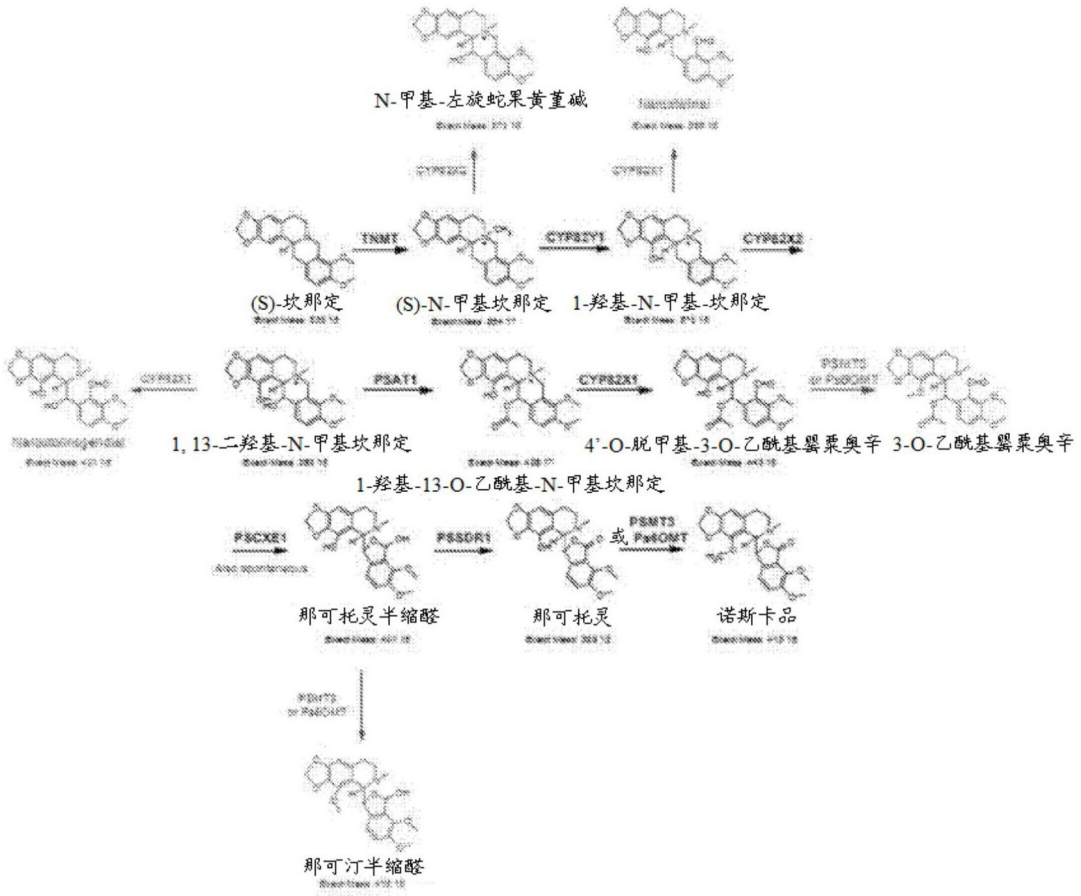

N-甲基-左旋蛇果黄堇碱
TNMT
CYP82Y1
CYP82X2
(S)-坎那定
(S)-N-甲基坎那定
1-羟基-N-甲基-坎那定
CYP82X1
PSAT1
CYP82X1
1,13-二羟基-N-甲基坎那定
4'-O-脱甲基-3-O-乙酰基罂粟奥辛
3-O-乙酰基罂粟奥辛
1-羟基-13-O-乙酰基-N-甲基坎那定
PSCXE1
PSSDR1
PSMT3
或 PsNOMT
那可托灵半缩醛
那可托灵
诺斯卡品
那可汀半缩醛

图11A

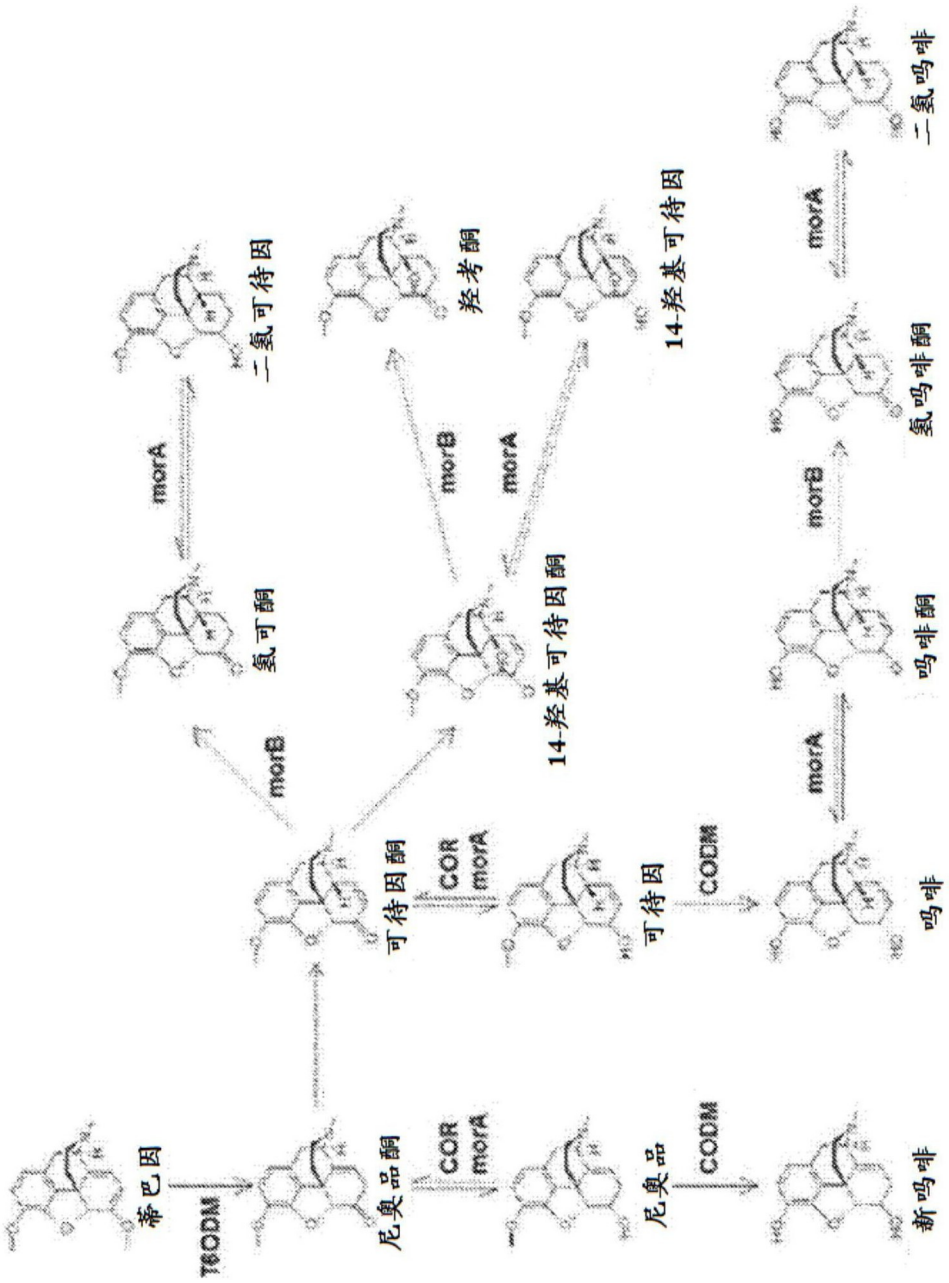

蒂巴因
T6ODM
尼奥品酮
COR
morA
尼奥品
CODM
新吗啡
可待因酮
COR
morA
可待因
CODM
吗啡
morB
氢可酮
morA
二氢可待因
morB
羟考酮
morA
14-羟基可待因酮
14-羟基可待因
morA
吗啡酮
morB
氢吗啡酮
morA
二氢吗啡

图11B

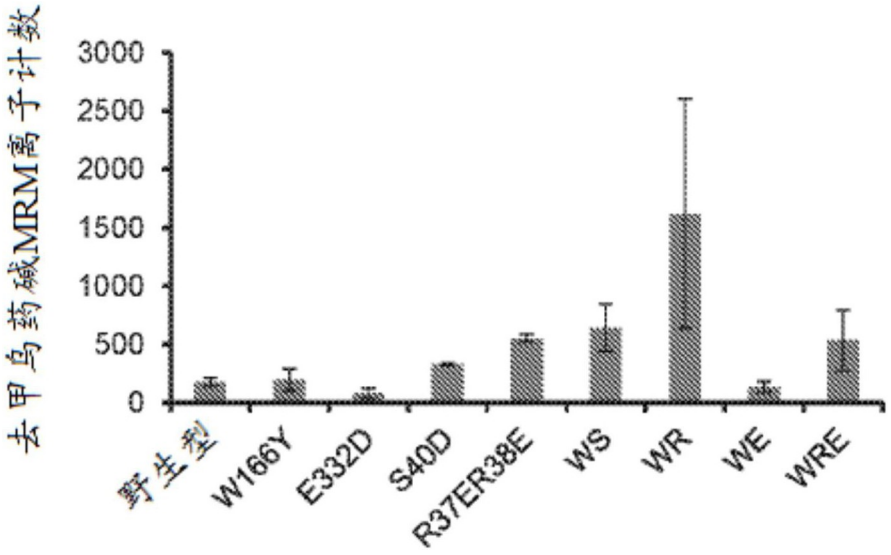

去甲乌药碱MRM离子计数
3000
2500
2000
1500
1000
500
0
野生型
W166Y
E332D
S40D
R37ER38E
WS
WR
WE
WRE

图12

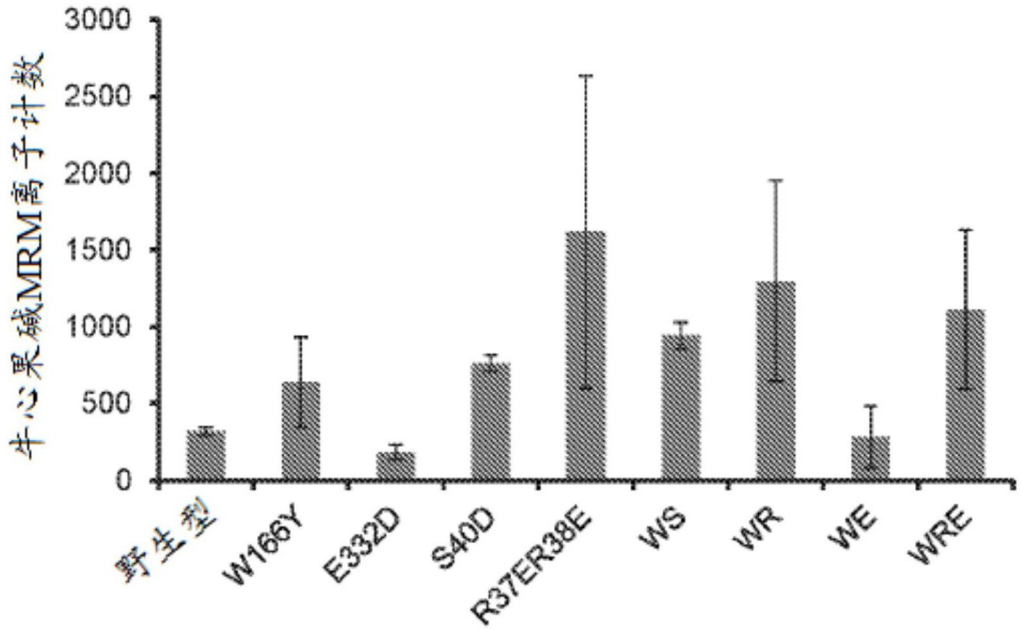

牛心果碱MRM离子计数
3000
2500
2000
1500
1000
500
0
野生型
W166Y
E332D
S40D
R37ER38E
WS
WR
WE
WRE

图13

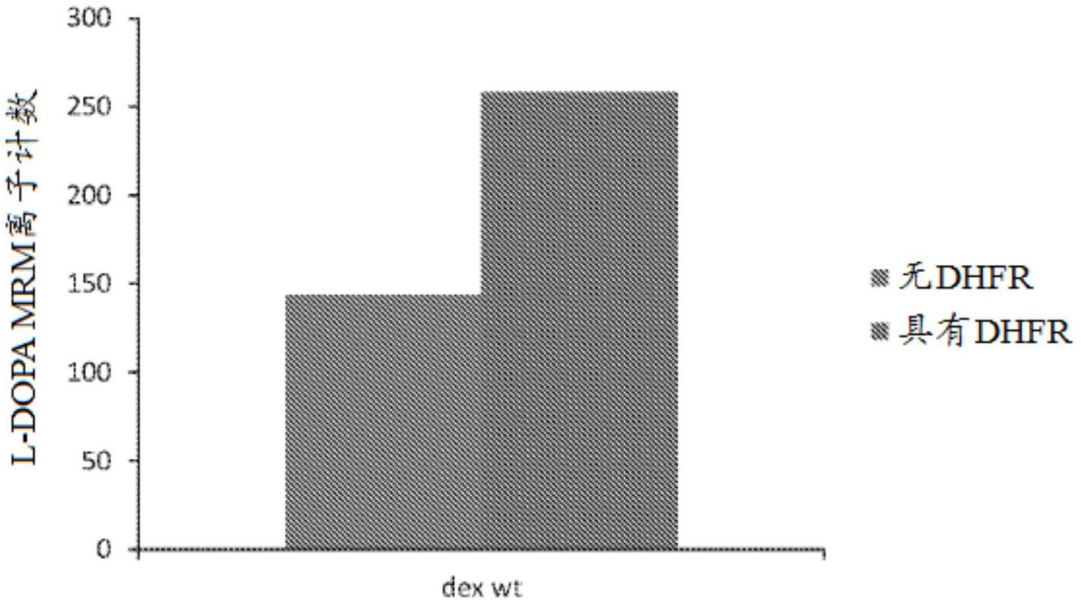

300
250
200
150
100
50
0
L-DOPA MRM离子计数
dex wt
无DHFR
具有DHFR

图14

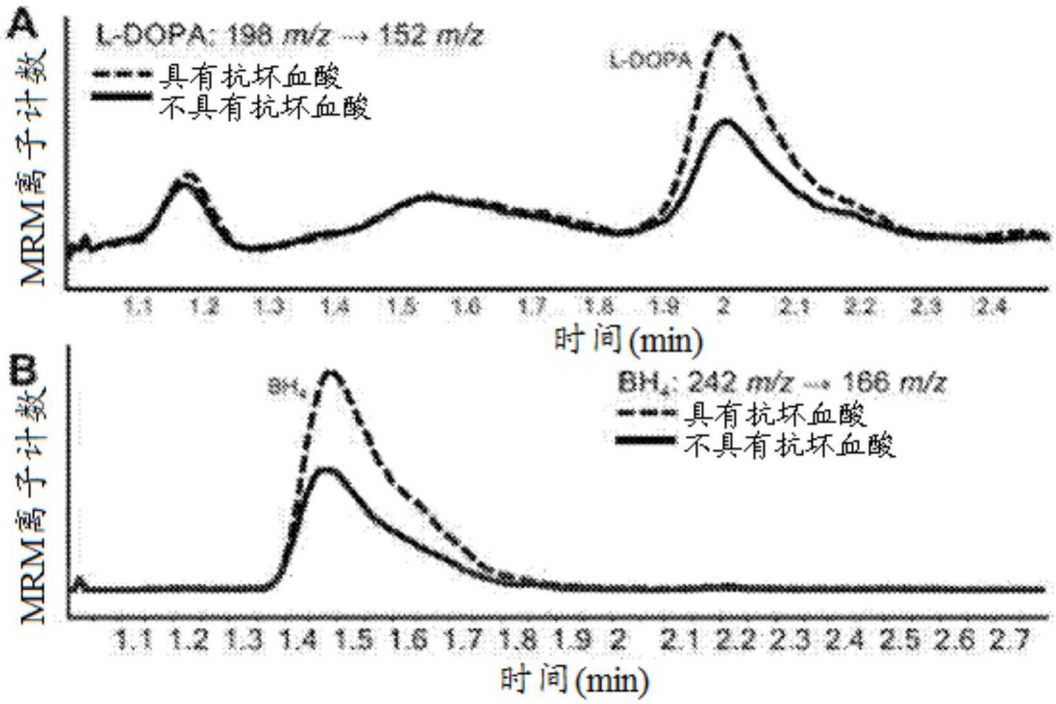
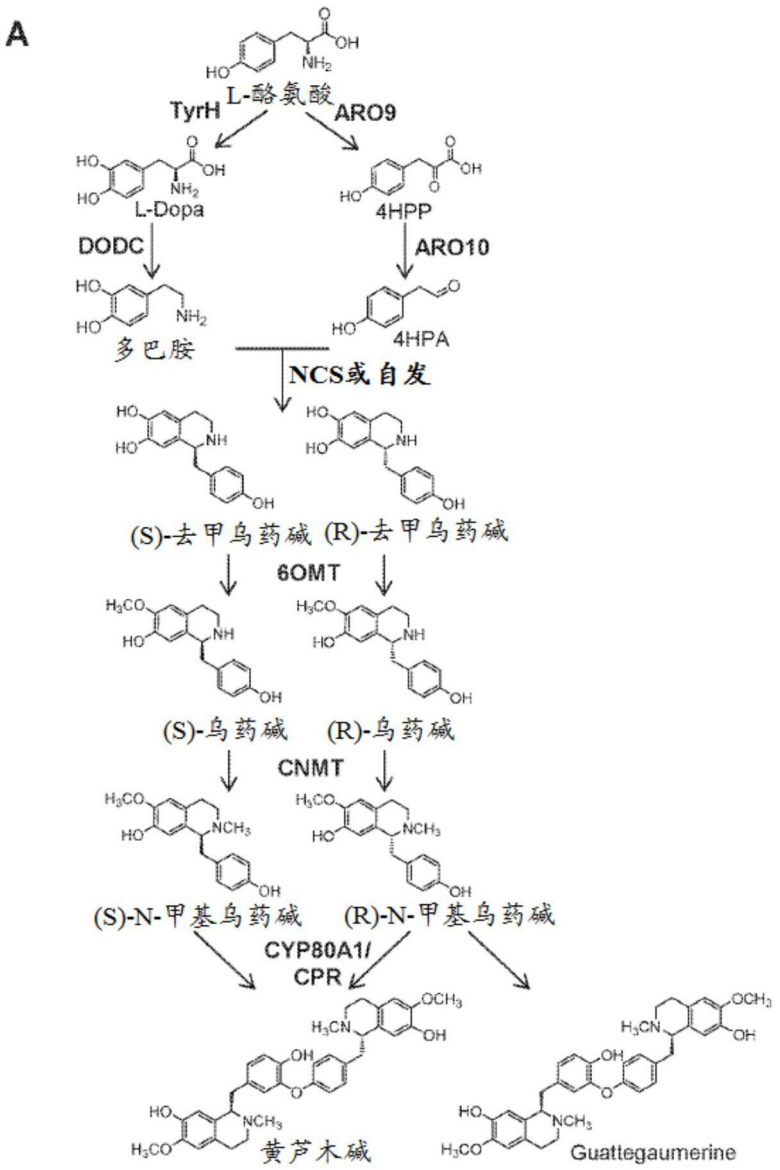

A
L-DOPA: 198 m/z → 152 m/z
具有抗坏血酸
不具有抗坏血酸
L-DOPA
MRM离子计数
1.1 1.2 1.3 1.4 1.5 1.6 1.7 1.8 1.9 2 2.1 2.2 2.3 2.4
时间(min)
B
BH4
BH4: 242 m/z → 166 m/z
具有抗坏血酸
不具有抗坏血酸
MRM离子计数
1.1 1.2 1.3 1.4 1.5 1.6 1.7 1.8 1.9 2 2.1 2.2 2.3 2.4 2.5 2.6 2.7
时间(min)

图15



B

基因型：

YDR514CΔ::P_{PYK1}-PsCNMT-T_{MFa1}, P_{PGK1}-Ps6OMT-T_{PHO5}, *LEU2* 标记, P_{TEF1}-PsCPR-T_{CYC1}, P_{GPD}-BsCYP80A1-T_{ADH1}

示意性表示：

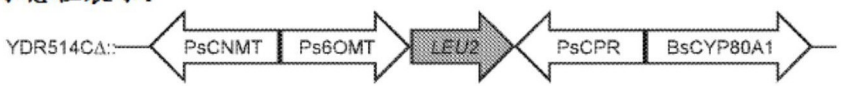


图16

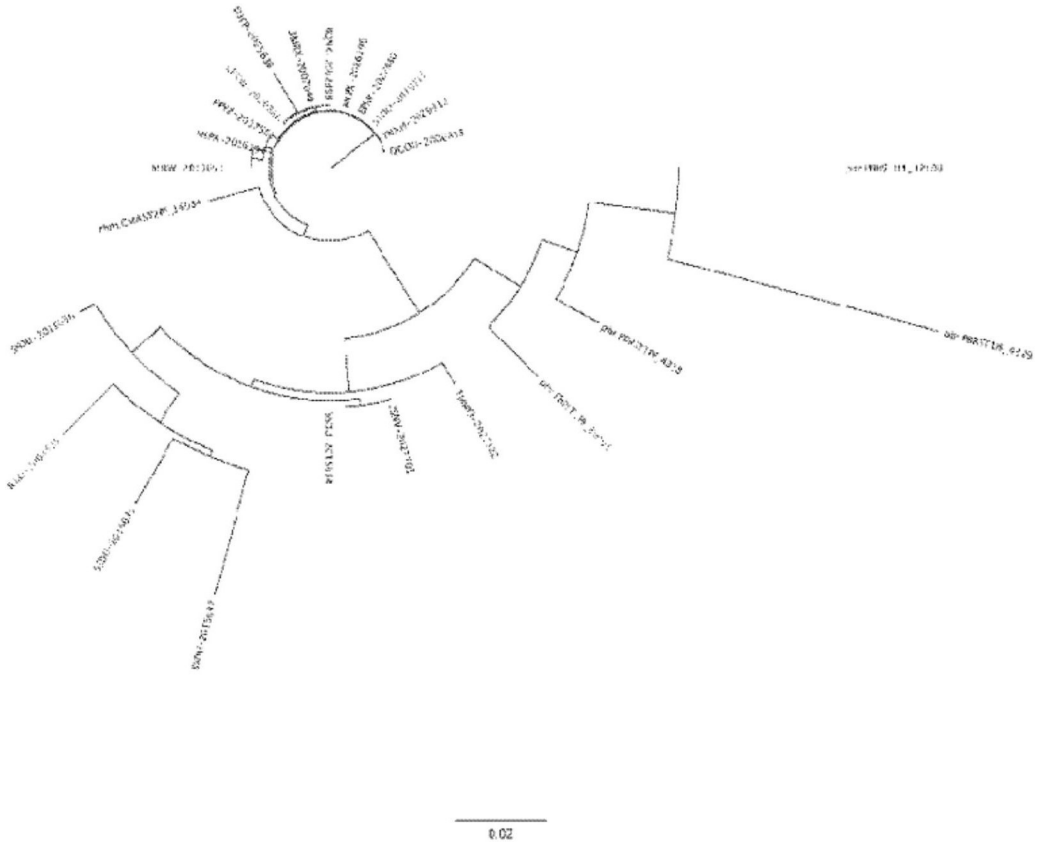


图17

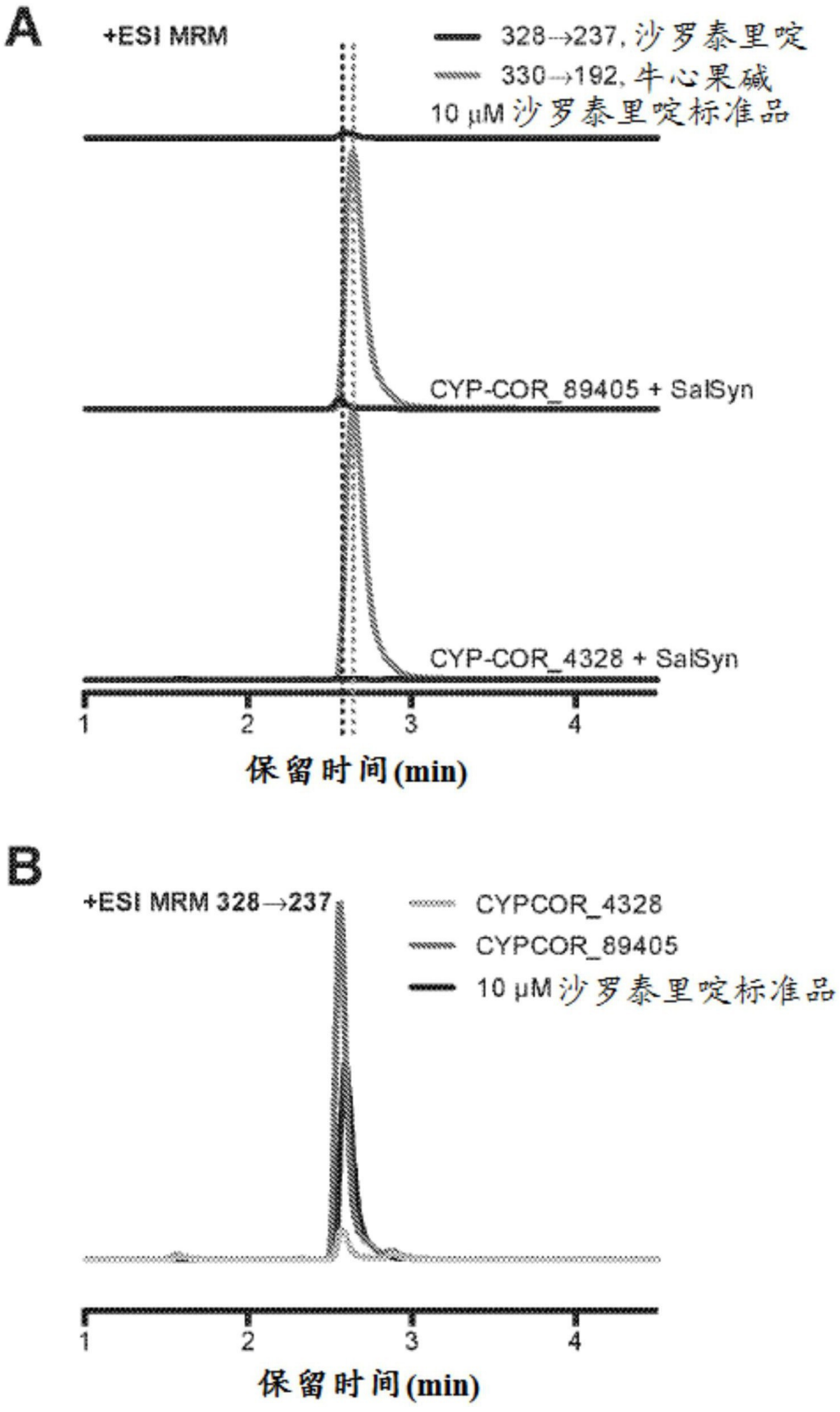

A
+ESI MRM
328→237, 沙罗泰里啶
330→192, 牛心果碱
10 μM 沙罗泰里啶标准品
CYP-COR_89405 + SalSyn
CYP-COR_4328 + SalSyn
1
2
3
4
保留时间(min)
B
+ESI MRM 328→237
CYPCOR_4328
CYPCOR_89405
10 μM 沙罗泰里啶标准品
1
2
3
4
保留时间(min)

图18

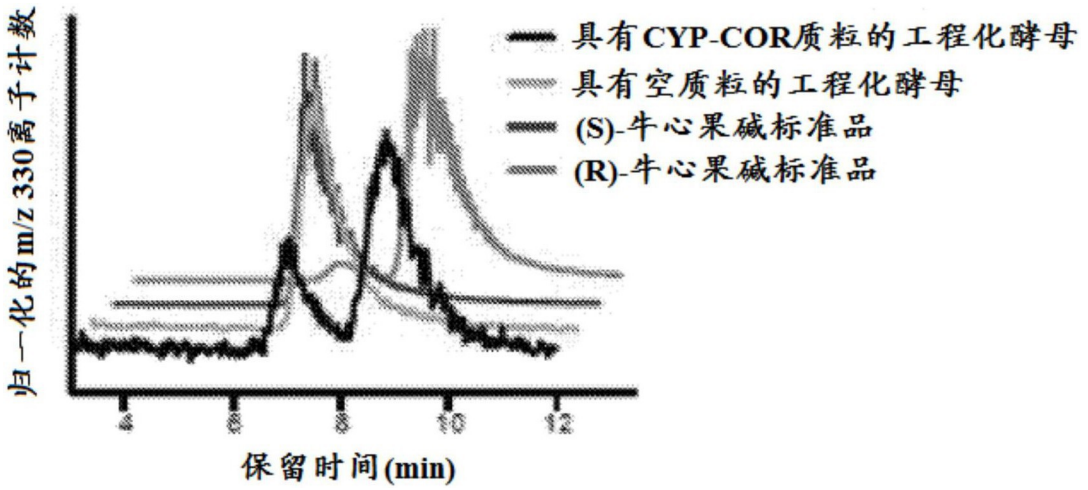

具有CYP-COR质粒的工程化酵母
具有空质粒的工程化酵母
(S)-牛心果碱标准品
(R)-牛心果碱标准品
归一化的m/z 330离子计数
4
6
8
10
12
保留时间(min)

图19

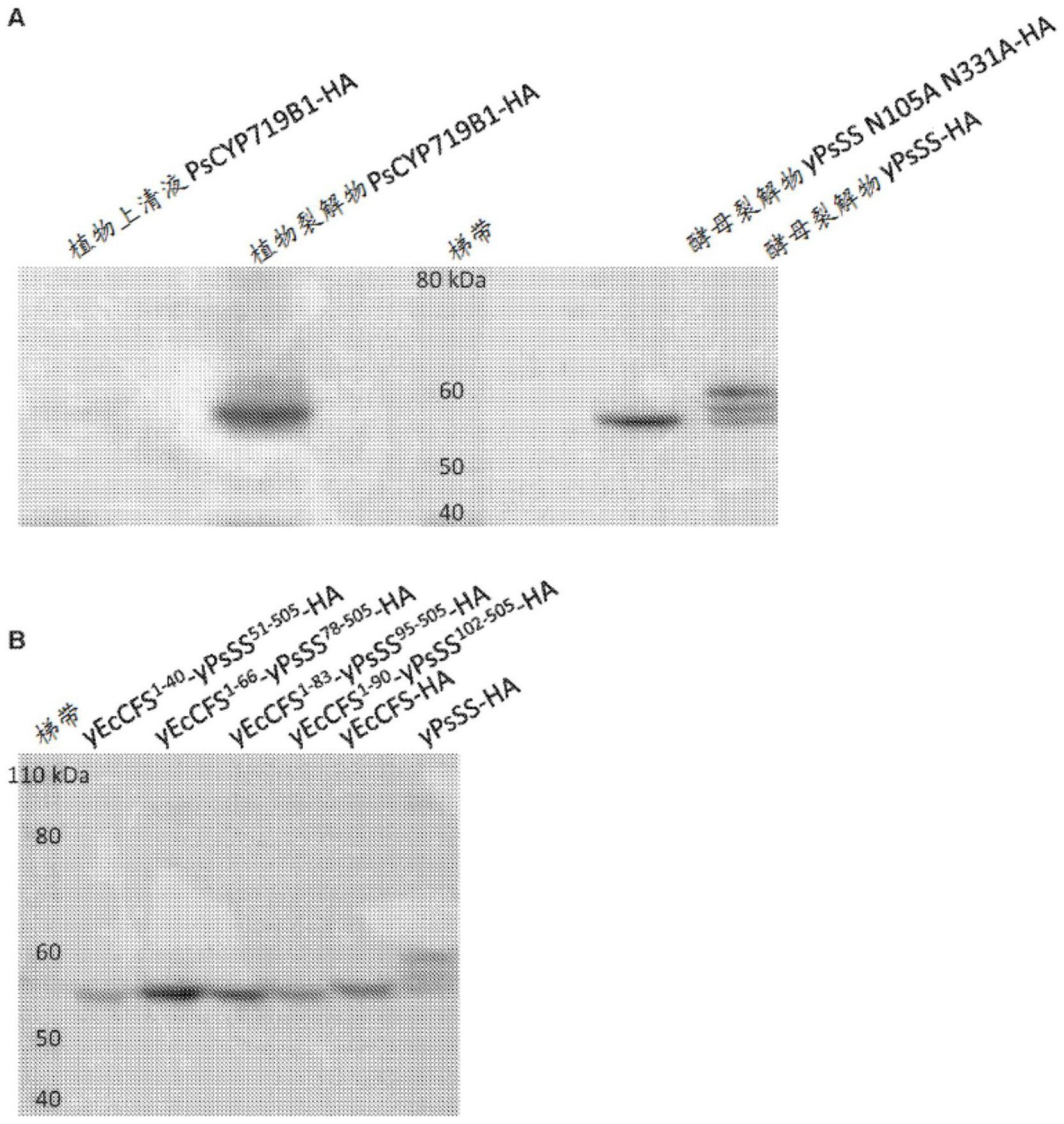

A
植物上清液 PsCYP719B1-HA
植物裂解物PsCYP719B1-HA
梯带
酵母裂解物 yPsSS N105A N331A-HA
酵母裂解物 yPsSS-HA
80 kDa
60
50
40
B
梯带
yEcCFS1-40-yPsSS51-505-HA
yEcCFS1-66-yPsSS78-505-HA
yEcCFS1-83-yPsSS95-505-HA
yEcCFS1-90-yPsSS102-505-HA
yEcCFS-HA
yPsSS-HA
110 kDa
80
60
50
40

图20

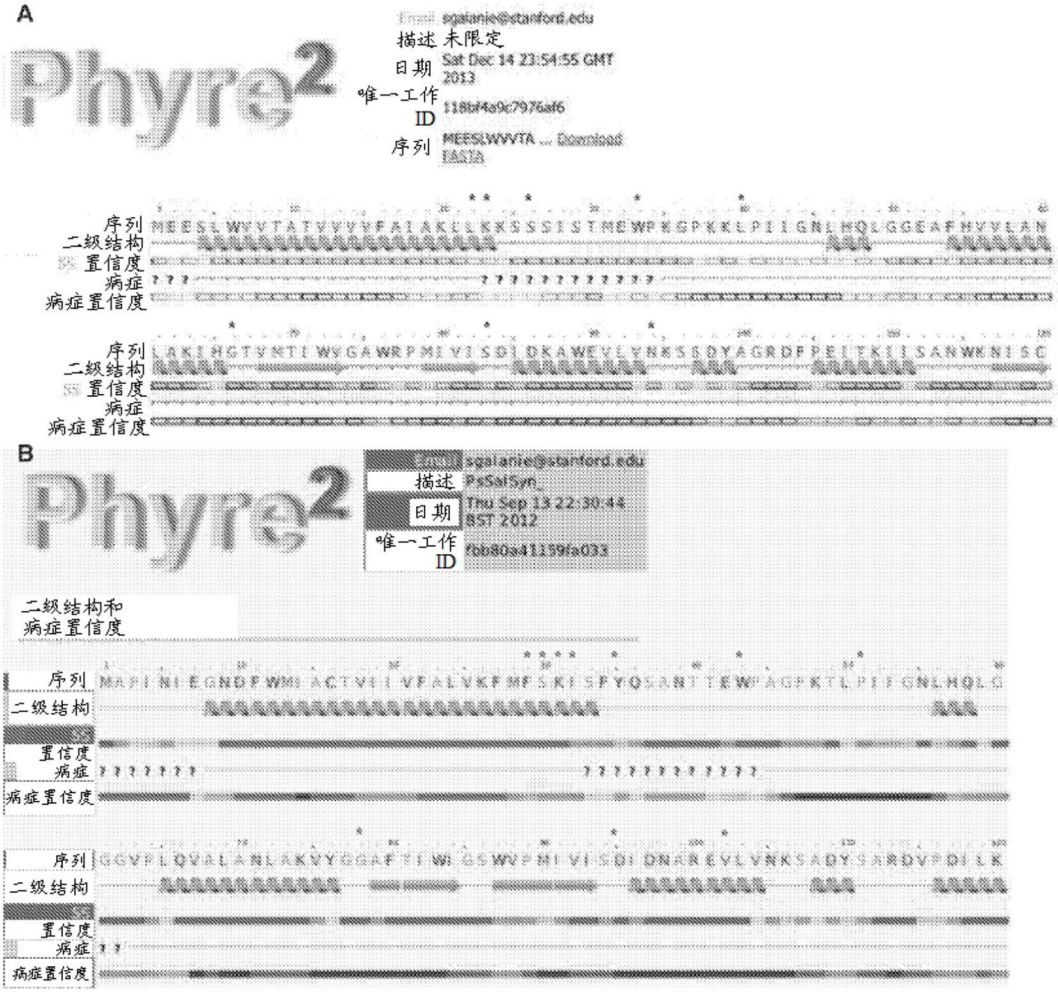

A
Phyre2
描述 未限定
日期 Sat Dec 14 23:54:55 GMT 2013
唯一工作ID 118bf4a9c7976af6
序列 MEESLWVVTATVVVVFAIAKELKKSSSISTMEWPKGPKKLPIIGNLHQLGGEAFHVVLAN
二级结构
置信度
病症
病症置信度
B
Phyre2
描述 PsSalSyn_
日期 Thu Sep 13 22:30:44 BST 2012
唯一工作ID fbb80a41159fa033
二级结构和病症置信度
序列
二级结构
置信度
病症
病症置信度

图21

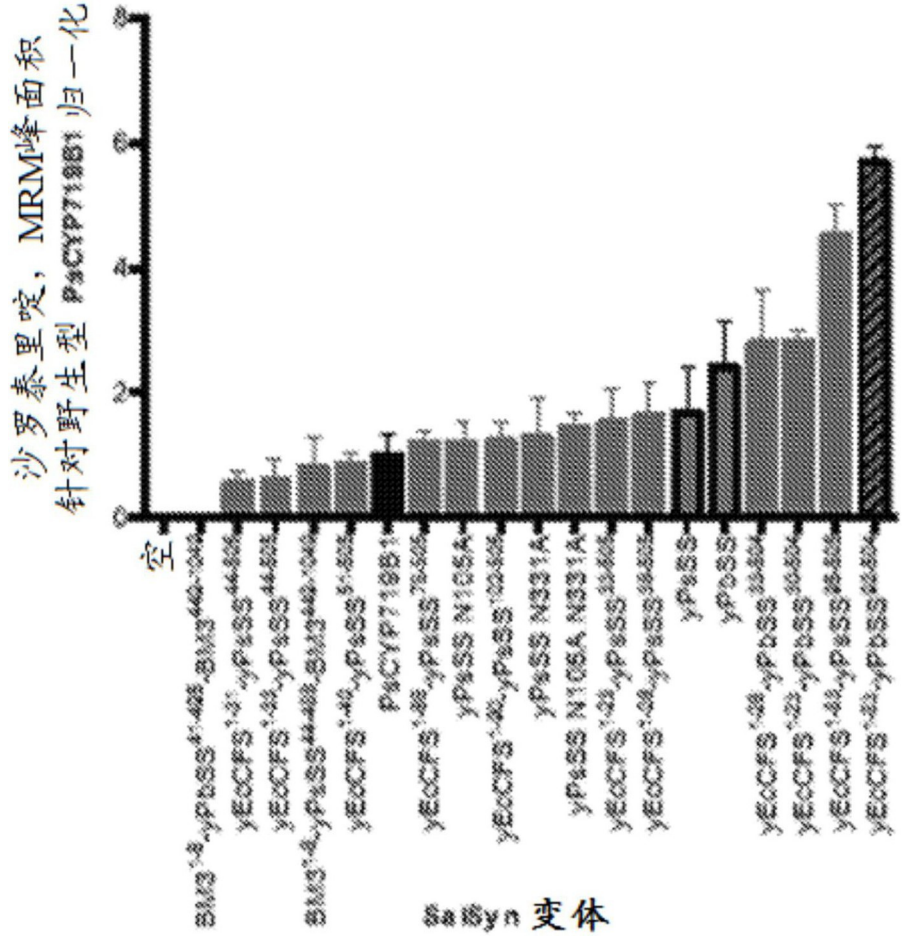


图22

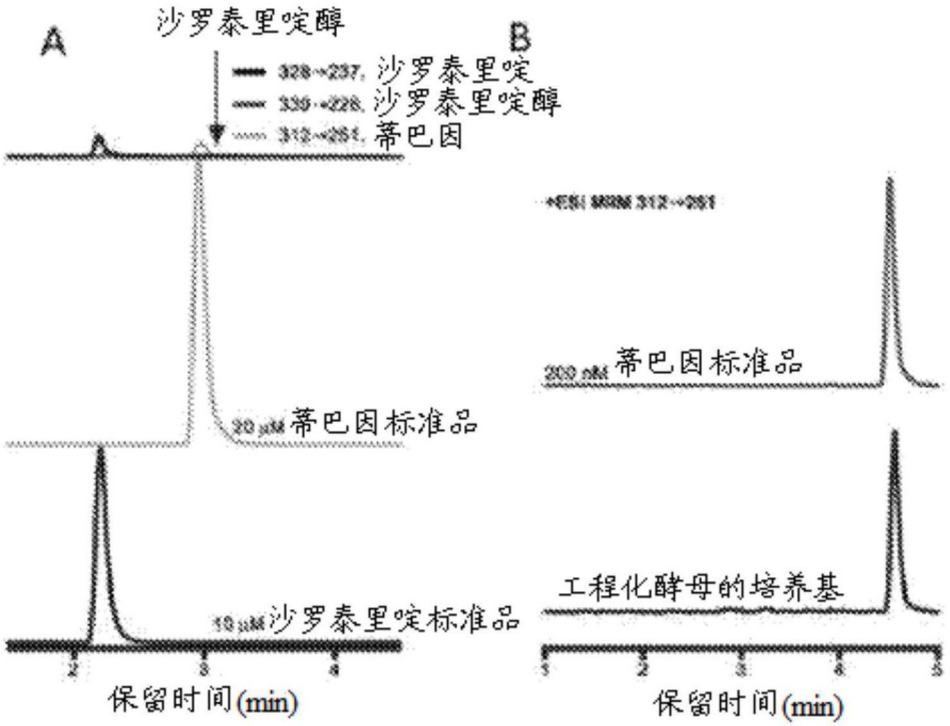

A
沙罗泰里啶醇
B
328→237, 沙罗泰里啶
339→228, 沙罗泰里啶醇
312→251, 蒂巴因
20 μM 蒂巴因标准品
10 μM 沙罗泰里啶标准品
保留时间(min)
200 nM 蒂巴因标准品
工程化酵母的培养基
保留时间(min)

图23

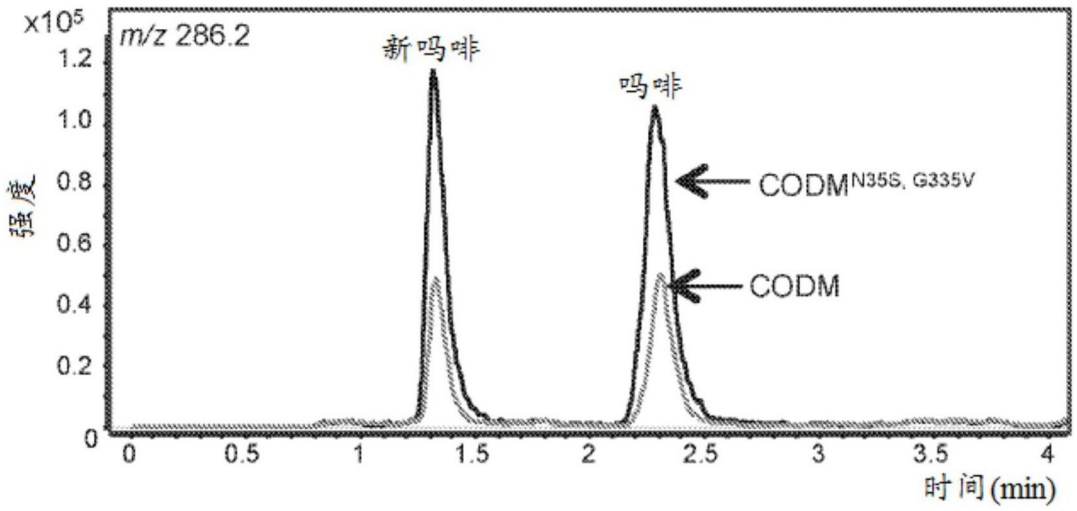

x10^5
m/z 286.2
新吗啡
吗啡
CODM^N35S, G335V
CODM
强度
时间(min)

图24

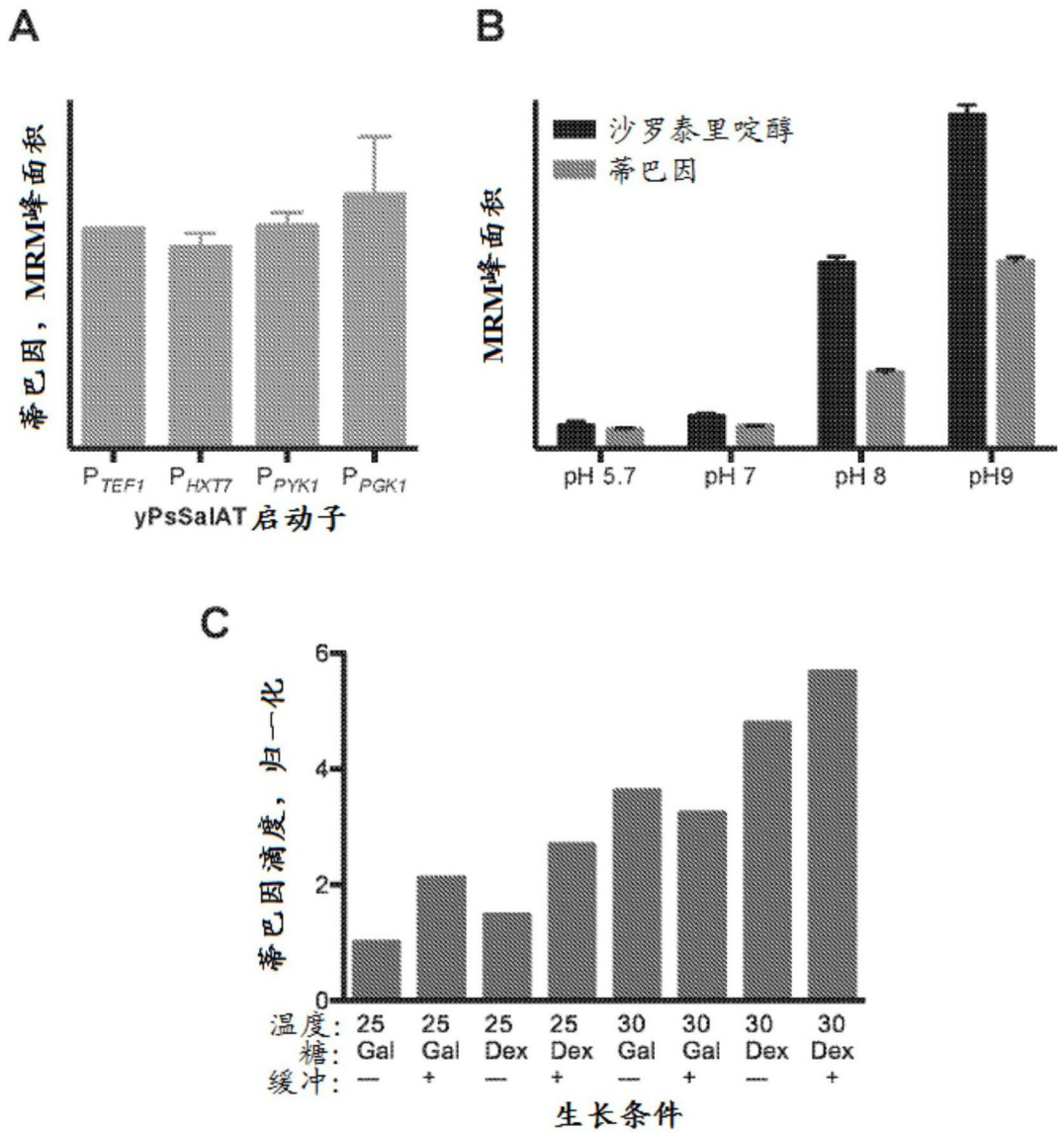

A
蒂巴因，MRM峰面积
P_{TEF1} P_{HXT7} P_{PYK1} P_{PGK1}
yPsSalAT启动子
B
MRM峰面积
沙罗泰里啶醇
蒂巴因
pH 5.7
pH 7
pH 8
pH9
C
蒂巴因滴度，归一化
0
2
4
6
温度: 25 25 25 25 30 30 30 30
糖: Gal Gal Dex Dex Gal Gal Dex Dex
缓冲: — + — + — + — +
生长条件

图25

整合到酵母基因组中的四个基因构建体:

1. YBR197CΔ::P_{TPI1}-RnSepR-T_{STE2}, P_{TEF1}-RnPTPS-T_{CYC1}, *KanMX* 标记, P_{GPD}-RnQDHPR-T_{AHD1}, P_{PGK1}-RnPCD-T_{PHO5}
2. HIS3 Δ ::P_{GPD}-RnTyrH-T_{ADH1}, P_{TPI1}-PpDODC-T_{STE2}, *HIS5* 标记, P_{TEF1}-RnDHFR-T_{CYC1}, P_{PGK1}-CjNCS-T_{PHO5}
3. YDR514CΔ::P_{PYK1}-PsCNMT-T_{MFa1}, P_{PGK1}-Ps6OMT-T_{PHO5}, P_{GPD}-EcCYP80B1-T_{ADH1}, *LEU2* 标记, P_{TEF1}-PsCPR-T_{CYC1}, P_{TPI1}-Ps4OMT-T_{STE2}
4. ARO4Δ::P_{TEF1}-$ARO4^{FBR}$-T_{CYC1}, P_{GPD}-$ARO7^{FBR}$-T_{ADH1}, *HygR* 标记, P_{PGK1}-TKL1-T_{PHO5}, P_{TPI1}-ARO10-T_{STE2}

指示每个表达盒的取向的整合的构建体的示意图:

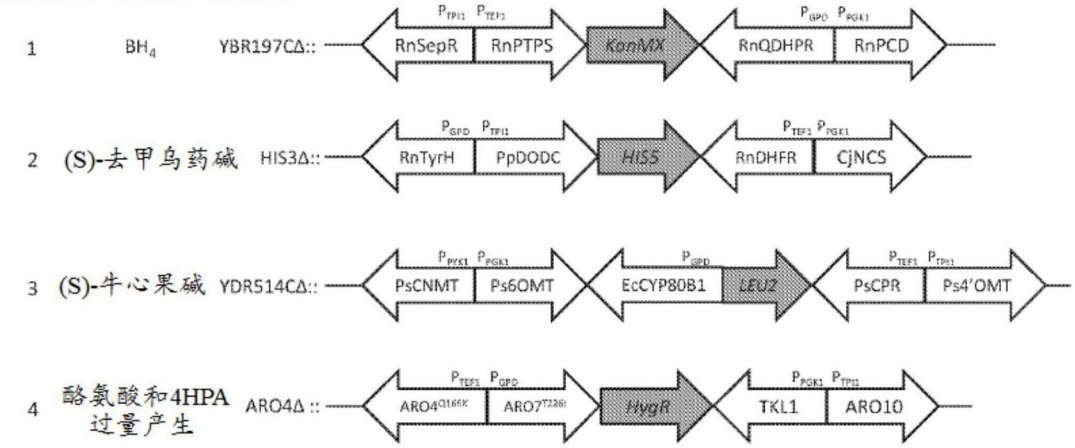


图26

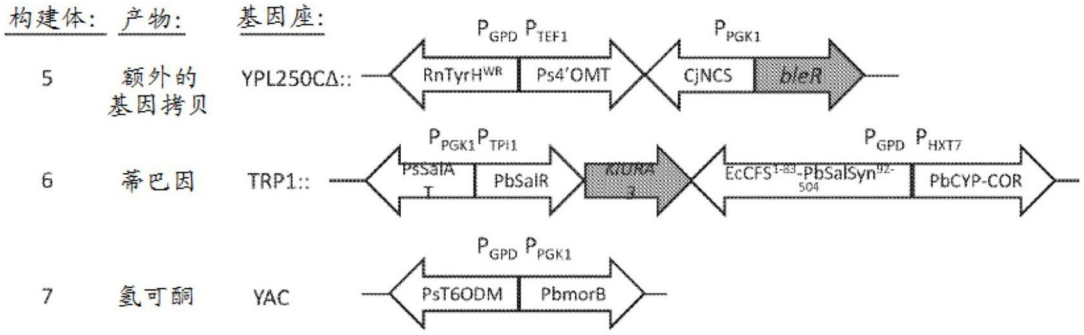
A

示例性基因构建体：

5. YPL250CΔ::P_{GPD}-RnTyrH-T_{ADH1}, P_{TEF1}-Ps4OMT-T_{CYC1}, P_{PGK1}-CjNCS-T_{PHO5}, bleR 标记

6. TRP1::P_{PGK1}-PsSalAT-T_{PHO55}, P_{TPI1}-PbSalR-T_{STE2}, URA3 标记 , P_{GPD}-EcCFS$^{1-83}$-PbSalSyn$^{92-504}$-T_{ADH1}, P_{HXT7}-PbCYP-COR-T_{CYC1}

7. YAC::P_{GPD}-PsT6ODM-T_{ADH1}, P_{PGK1}-PbmorB-T_{PHO5}



B

多基因构建体5的整合：

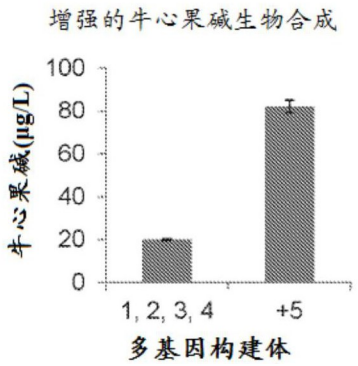


图27

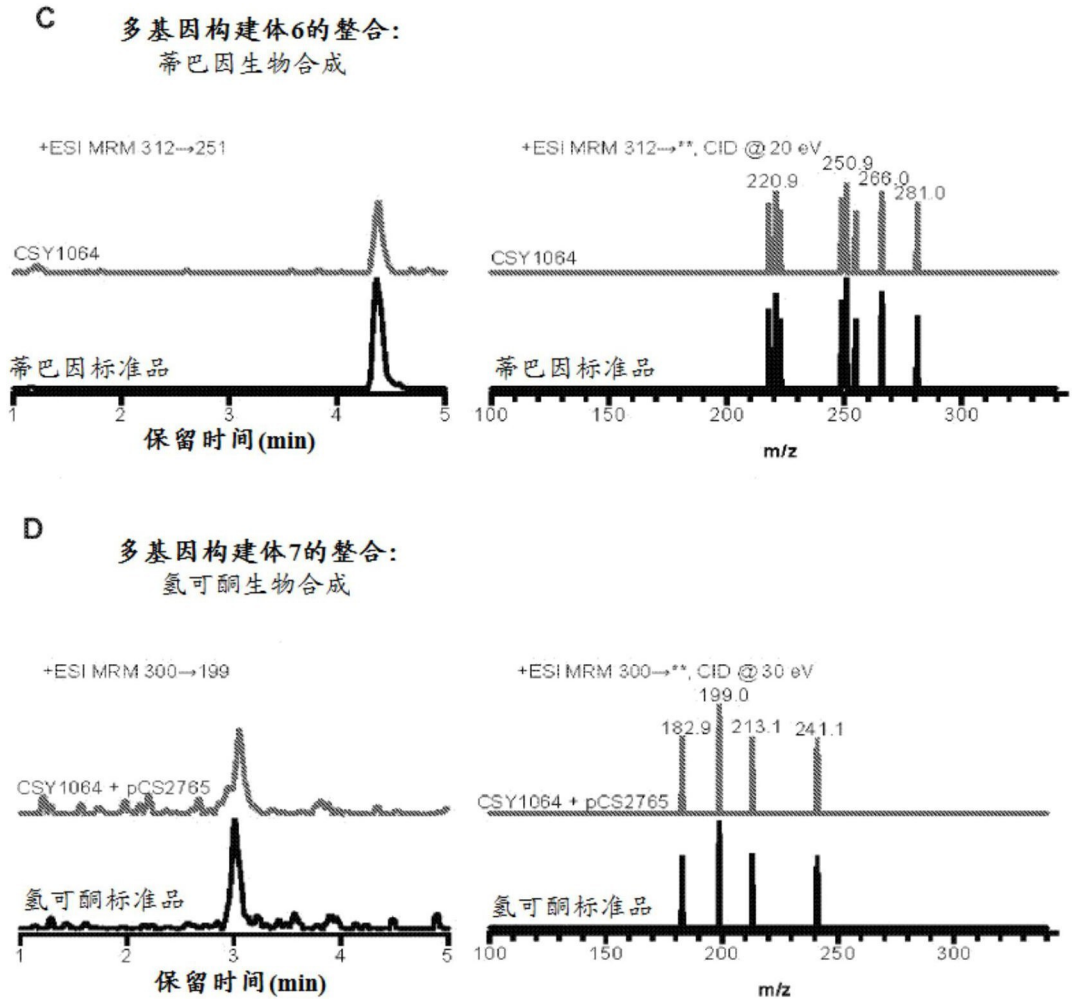

C
多基因构建体6的整合:
蒂巴因生物合成
+ESI MRM 312→251
CSY1064
蒂巴因标准品
保留时间(min)
+ESI MRM 312→**, CID @ 20 eV
220.9
250.9
266.0
281.0
CSY1064
蒂巴因标准品
m/z
D
多基因构建体7的整合:
氢可酮生物合成
+ESI MRM 300→199
CSY1064 + pCS2765
氢可酮标准品
保留时间(min)
+ESI MRM 300→**, CID @ 30 eV
199.0
182.9
213.1
241.1
CSY1064 + pCS2765
氢可酮标准品
m/z

图27

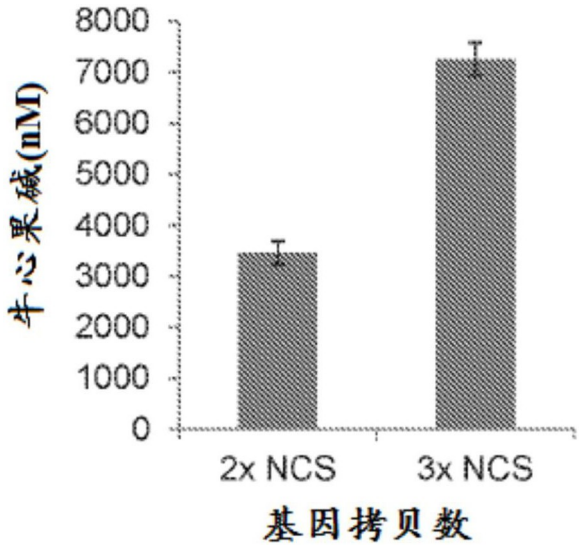

牛心果碱(mM)
8000
7000
6000
5000
4000
3000
2000
1000
0
2x NCS
3x NCS
基因拷贝数

图28