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(71) Demandeur/Applicant:
CB THERAPEUTICS, INC., US

(72) Inventeurs/Inventors:
PEIFFER, LAURA FLATAUER, US;
VOGAN, JACOB MICHAEL, US;
WADE, JAMES LEE, US;
YACOUB, TYRONE JACOB, US;
TANG, KIRSTEN, US;
BURNETT, RACHEL NADINE, US

(74) Agent: GOWLING WLG (CANADA) LLP

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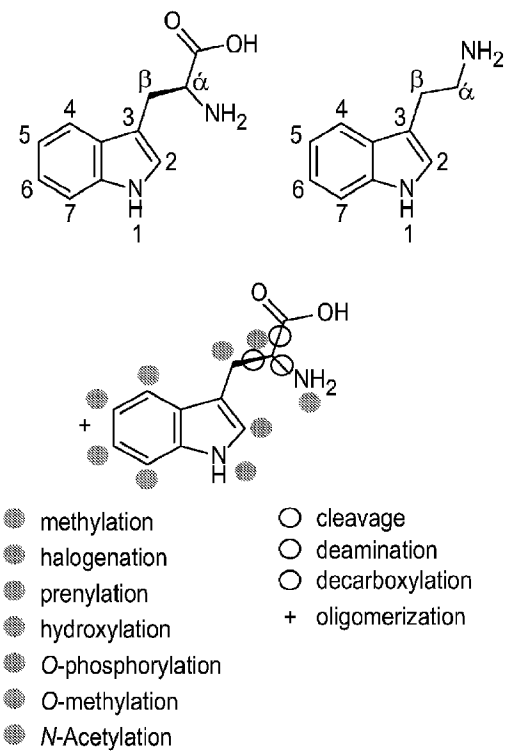

FIG. 1

(57) Abrégé/Abstract:
Provided are non-naturally occurring nucleic acids comprising a sequence encoding an enzyme or regulatory protein in tryptamine metabolism. Also provided are a recombinant microorganisms expressing the enzyme or regulatory protein. Methods of expressing the enzyme or regulatory protein are additionally provided.

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Abstract:

Provided are non-naturally occurring nucleic acids comprising a sequence encoding an enzyme or regulatory protein in tryptamine metabolism. Also provided are a recombinant microorganisms expressing the enzyme or regulatory protein. Methods of expressing the enzyme or regulatory protein are additionally provided.

ENZYMES AND REGULATORY PROTEINS IN TRYPTAMINE METABOLISM

CROSS-REFERENCE TO RELATED APPLICATION

This application claims the benefit of U.S. Provisional Application No. 63/035,692, filed June 6, 2020, and incorporated by reference herein in its entirety.

INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED ON A COMPACT DISC

The Sequence Listing, which is a part of the present disclosure, includes a computer readable form and a written sequence listing comprising nucleotide and/or amino acid sequences of the present invention. The sequence listing information recorded in computer readable form is identical to the written sequence listing. The subject matter of the Sequence Listing is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

(1) Field of the Invention

The present invention generally relates to the production of substituted indoles, e.g. N-methyl-L-tryptophan (NMTP), N,N-dimethyl-L-tryptophan (DMTP), and N,N,N-trimethyl-L-tryptophan (TMTP), and related tryptamines, e.g. N-methyltryptamine (NMT), N,N-dimethyltryptamine (DMT), and N,N,N-trimethyltryptamine (TMT), in a modified heterologous microorganism.

(2) Description of the related art

Mental health problems, which may also be referred to as mental illness or psychiatric disorder, are behavioral or mental patterns which impair the functioning of individuals across the world. Such mental health disorders include: personality disorders, anxiety disorders, major depressions, and various addictions. Indolic and tryptamine-based compounds similar in structure to the endogenous neurotransmitter serotonin have been increasingly evaluated for treating mental health problems. In contrast to anxiolytic medicines, usage of substituted indoles and methylated tryptamines, such as N,N-dimethyltryptamine does not lead to physical dependence.

The chemical synthesis of hydroxy, methoxy, phosphorylated, prenylated, and halogenated substituted tryptamines and indoles typically involve tedious techniques of organic chemistry.

Often, reproducibility is elusive and the solvents used during the syntheses of substituted tryptamines are environmentally toxic. Decarboxylations and selective methylations can be difficult to obtain via the techniques of organic chemistry. Further, the yields and purity of the intermediates for obtaining the target molecules can be low, where, for example, the starting molecule is L-tryptophan and the target molecule is N,N-dimethyltryptophan (DMTP), bufotenine, 5-MeO-dimethyltryptamine (5-MeO-DMT), 7-dimethylallyltryptophan, psilocybin, aeruginascin, among others.

The present invention provides for producing substituted tryptamines and indoles in recombinant microorganisms, providing for a more environmentally benign and higher yielding processes for production of those compounds.

BRIEF SUMMARY OF THE INVENTION

In some embodiments, provided is a non-naturally occurring nucleic acid comprising a sequence encoding an enzyme or regulatory protein in tryptamine metabolism, where the enzyme or regulatory protein is an N-methyltransferase (INMT, PsiM, TrpM), a tryptophan decarboxylase (AADC), a tryptophan hydroxylase (TPH), a tryptamine 4' hydroxylase (T4H), a tryptamine 5' hydroxylase (T5H), a truncated cytochrome p450 reductase (T4H-CPR, T5H-CPR), an hydroxytryptamine O-methyltransferase (IOMT or CaffOMT), an N-acetyltransferase (NAT), a deacetylase (DAC), a hydroxyl tryptamine kinase (PsiK), a tryptophan synthase (TrpS), a toluene monooxygenase (TMO), an aminotransferase/methyltransferase fusion (ATMT), a phosphatase, an oxidase, a dimethylallyltryptophan synthase (DMAT), an isopentenyl-diphosphate isomerase (IDI1), a tryptophan halogenase (TrpHalo), an aspartate oxidase/quinolinic acid synthase fusion (AOQS), a tryptophan importer (TAT2), a methionine importer (MUP1), or a SAMe importer (SAM3).

Also provided is an expression cassette comprising any of the above nucleic acids with a promoter functional in a recombinant microorganism.

Additionally provided is a recombinant microorganism comprising the above expression cassette, that expresses the enzyme or regulatory protein encoded therein.

Further provided is a non-naturally occurring enzyme or regulatory protein comprising an amino acid sequence encoded by any of the above-identified nucleic acids.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

FIG. 1 depicts the chemical structures of tryptophan and tryptamine, including various modifications which are performed by the enzymes disclosed within.

FIG. 2 depicts various substituted indole compounds in the tryptamine and tryptophan pathways utilized in the present invention. Panel A depicts the indole ring structure with positional numbering, and tryptophan and tryptamine. Panel B depicts examples of hydroxy modified tryptophan and tryptamine. Panel C depicts the 5-hydroxy indole ring structure with positional numbering, and examples of modified 5-hydroxy tryptamines. Panel D depicts the 4-hydroxy indole ring structure with positional numbering, and examples of modified 4-hydroxy tryptamines.

FIG. 3 depicts biosynthetic pathways utilized herein. Panel A depicts the biosynthetic pathways to tryptophan and genetic manipulations to increase tryptophan flux toward modified indoles and tryptamines. Panel B depicts the biosynthetic pathways to the methyl donor, SAMe and genetic manipulations to increase SAMe flux toward modified indoles and tryptamines.

FIG. 4 depicts enzymatic reactions utilized herein. Panel A depicts SAMe usage by INMT for methyltransferase activity. Panel B depicts BH4 usage by TPH for hydroxylase activity. Panel C depicts SAMe usage by INMT for methyltransferase activity on hydroxy tryptamine. Panel D depicts SAMe usage by IOMT (or CaffOMT) for methyltransferase activity. Panel E depicts NAD(P)H usage by T5H for hydroxylase activity. Panel F depicts acetyl-CoA usage by NAT for acetylation activity.

FIG. 5 depicts routes of modification of tryptamine by combinatorial usage of INMT, T5H, and IOMT enzymes.

FIG. 6 depicts routes of modification of tryptophan by combinatorial usage of TrpM, TPH, and IOMT enzymes (Panel A) and example branch points where modified tryptophan becomes modified tryptamine via use of the AADC enzyme (Panel B).

FIG. 7 depicts (A) routes of modification of serotonin by combinatorial usage of INMT and IOMT enzymes; (B) conversion of 5-HTP to serotonin by the AADC enzyme; (C) conversion of serotonin to N-acetylserotonin by the NAT enzyme, and N-acetylserotonin conversion to melatonin via the IOMT enzyme; (D) conversion of serotonin .to 5-MT by the IOMT enzyme, and 5-MT conversion to melatonin via the NAT enzyme; and (E) conversion of melatonin to 5-MeO-tryptamine by the DAC enzyme, and subsequent N-methylation by INMT to generate compounds such as 5-MeO-DMT.

FIG. 8 depicts (A) halogenation of tryptophan and tryptamine on the indole ring by the TrpHalo enzyme; (B) example route to halogenated DMT via combinatorial use of TrpHalo,

AADC, and INMT enzymes; (C) prenylation of tryptophan and tryptamine on the indole ring by the DMAT-IDI1 fusion enzyme; and (D) example route to prenylated DMT via combinatorial use of DMAT-IDI1, AADC, and INMT enzymes.

FIG. 9 depicts (A) a modified host organism expressing gene combinations with TPH, AADC, and TrpM enzymes to convert tryptophan into various hydroxy tryptamines; (B) a modified host organism expressing gene combinations with TPH, AADC, TrpM, and IOMT enzymes to convert tryptophan into various methoxy tryptamines; (C) a modified host organism expressing gene combinations with AADC, T5H, and INMT enzymes to convert tryptophan into various hydroxy tryptamines; and (D) a modified host organism expressing gene combinations with AADC, T5H, INMT, and IOMT enzymes to convert tryptophan into various methoxy tryptamines.

FIG. 10 depicts (A) a modified host organism which can generate various hydroxy tryptamines through bioconversion of serotonin provided exogenously or generated within the host organism; and (B) a modified host organism which can generate various methoxy tryptamines through bioconversion of melatonin provided exogenously or generated within the host organism.

FIG. 11 depicts (A) a scaffolded biosynthesis pathway of colocalized AADC, T5H-CPR fusion, IOMT, and NAT enzymes for conversion of tryptophan to melatonin; and (B) a modified host organism expressing the biosynthesis pathway from FIG. 11A to convert tryptophan to melatonin and related products.

FIG. 12 depicts (A) a scaffolded biosynthesis pathway of colocalized AADC, T4H-CPR fusion, PsiK, and PsiM enzymes for conversion of tryptophan to psilocybin related products; and (B) a modified host organism expressing the biosynthesis pathway from FIG. 12A to convert tryptophan to psilocybin and related products.

FIG. 13 depicts (A) example routes to halogenated, prenylated, and N-methylated alpha-methyl-tryptamine (AMT); and (B) a modified host organism expressing gene combinations to modify exogenously provided AMT to generate alpha-methylated-tryptamine variants.

FIG. 14 depicts (A) a heterologous tryptophan synthase (TrpS) route to combine synthetically modified indole with serine or threonine to generate indole modified tryptophan or indole modified beta-methyl tryptophan; and (B) a host organism expressing gene combinations to generate variants of indole modified tryptophan or indole modified beta-methyl tryptophan.

FIG. 15 depicts (A) the ATMT fusion enzyme converted tryptophan to beta-methyl tryptophan; and (B) a host organism expressing the ATMT fusion enzyme with gene combinations to generate beta-methyl tryptophan variants.

FIG. 16 depicts (A) the conversion of phosphorylated tryptamines to the corresponding hydroxy tryptamines by dephosphorylation; and (B) the oxidation of example hydroxy tryptamines which can catalyze polymerization.

FIG. 17 depicts HPLC chromatograms and UV-vis spectral matching of fermentation derived tryptamine via expression of the AADC enzyme.

FIG. 18 depicts HPLC chromatograms of fermentation derived methylated tryptamine via expression of the TrpM enzyme.

FIG. 19 depicts HPLC chromatograms of fermentation derived 4-OH tryptamine with improvements in yield via an optimal T4H-CPR fusion.

FIG. 20 depicts HPLC chromatograms of fermentation derived 5-OH-NMT via bioconversion of exogenous serotonin.

FIG. 21 depicts (A) a biosynthetic route to serotonin and 5-OH-NMT with a T5H enzyme or with a T5H-CPR fusion enzyme; and HPLC chromatograms of fermentation derived serotonin and 5-OH-NMT with improvements in yield via an optimal T5H-CPR fusion.

FIG. 22 depicts HPLC chromatograms of fermentation derived serotonin and melatonin.

FIG. 23 depicts HPLC chromatograms of fermentation derived 5-OH NMT and bufotenine.

FIG. 24 depicts HPLC chromatograms of fermentation derived psilocybin.

FIG. 25 depicts a synthetic route to methylate various tryptamines.

FIG. 26 depicts HPLC chromatograms and UV-vis spectral matching of fermentation derived DMT.

DETAILED DESCRIPTION OF THE INVENTION

Abbreviations and Definitions

To facilitate understanding of the invention, a number of terms and abbreviations as used herein are defined below as follows:

Conservative amino acid substitutions: As used herein, when referring to mutations in a protein, "conservative amino acid substitutions" are those in which at least one amino acid of the polypeptide encoded by the nucleic acid sequence is substituted with another amino acid having similar characteristics. Examples of conservative amino acid substitutions are ser for ala, thr, or

cys; lys for arg; gln for asn, his, or lys; his for asn; glu for asp or lys; asn for his or gln; asp for glu; pro for gly; leu for ile, phe, met, or val; val for ile or leu; ile for leu, met, or val; arg for lys; met for phe; tyr for phe or trp; thr for ser; trp for tyr; and phe for tyr.

Functional variant: The term "functional variant," as used herein, refers to a recombinant enzyme such as an INMTenzyme that comprises a nucleotide and/or amino acid sequence that is altered by one or more nucleotides and/or amino acids compared to the nucleotide and/or amino acid sequences of the parent protein and that is still capable of performing an enzymatic function (e.g., synthesis of DMT) of the parent enzyme. In other words, the modifications in the amino acid and/or nucleotide sequence of the parent enzyme may cause desirable changes in reaction parameters without altering fundamental enzymatic function encoded by the nucleotide sequence or containing the amino acid sequence. The functional variant may have conservative change including nucleotide and amino acid substitutions, additions and deletions. These modifications can be introduced by standard techniques known in the art, such as site-directed mutagenesis and random PCR-mediated mutagenesis, and may comprise natural as well as non-natural nucleotides and amino acids. Also envisioned is the use of amino acid analogs, e.g. amino acids not DNA or RNA encoded in biological systems, and labels such as fluorescent dyes, radioactive elements, electron dense agents, or any other protein modification, now known or later discovered.

Recombinant nucleic acid and recombinant protein: As used herein, a recombinant nucleic acid or protein is a nucleic acid or protein produced by recombinant DNA technology, e.g., as described in Green and Sambrook (2012).

Polypeptide, protein, and peptide: The terms “polypeptide,” “protein,” and “peptide” are used herein interchangeably to refer to amino acid chains in which the amino acid residues are linked by peptide bonds or modified peptide bonds. The amino acid chains can be of any length of greater than two amino acids. Unless otherwise specified, the terms “polypeptide,” “protein,” and “peptide” also encompass various modified forms thereof. Such modified forms may be naturally occurring modified forms or chemically modified forms. Examples of modified forms include, but are not limited to, glycosylated forms, phosphorylated forms, myristoylated forms, palmitoylated forms, ribosylated forms, acetylated forms, and the like. Modifications also include intra-molecular crosslinking and covalent attachment of various moieties such as lipids, flavin, biotin, polyethylene glycol or derivatives thereof, and the like. In addition, modifications may also include protein cyclization, branching of the amino acid chain, and cross-linking of the

protein. Further, amino acids other than the conventional twenty amino acids encoded by genes may also be included in a polypeptide.

The term "protein" or "polypeptide" may also encompass a "purified" polypeptide that is substantially separated from other polypeptides in a cell or organism in which the polypeptide naturally occurs (e.g., 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 100% free of contaminants).

Primer, probe and oligonucleotide: The terms "primer," "probe," and "oligonucleotide" may be used herein interchangeably to refer to a relatively short nucleic acid fragment or sequence. They can be DNA, RNA, or a hybrid thereof, or chemically modified analogs or derivatives thereof. Typically, they are single-stranded. However, they can also be double-stranded having two complementing strands that can be separated apart by denaturation. In certain aspects, they are of a length of from about 8 nucleotides to about 200 nucleotides. In other aspects, they are from about 12 nucleotides to about 100 nucleotides. In additional aspects, they are about 18 to about 50 nucleotides. They can be labeled with detectable markers or modified in any conventional manners for various molecular biological applications.

Vector: As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is an episome, i.e., a nucleic acid capable of extra-chromosomal replication. Various vectors are those capable of autonomous replication and/expression of nucleic acids to which they are linked. Vectors capable of directing the expression of genes to which they are operatively linked are referred to herein as "expression vectors."

Linker: The term "linker" refers to a short amino acid sequence that separates multiple domains of a polypeptide. In some embodiments, the linker prohibits energetically or structurally unfavorable interactions between the discrete domains.

Codon optimized: As used herein, a recombinant gene is "codon optimized" when its nucleotide sequence is modified to accommodate codon bias of the host organism to improve gene expression and increase translational efficiency of the gene.

Expression cassette: As used herein, an "expression cassette" is a nucleic acid that comprises a gene and a regulatory sequence operatively coupled to the gene such that the promoter drives the expression of the gene in a cell. An example is a gene for an enzyme with a promoter functional in yeast, where the promoter is situated such that the promoter drives the expression of the enzyme in a yeast cell.

Compounds and abbreviations in use of or contained within systems and methods herein are provided in Table 1.

Table 1

Compounds
tryptamine
L-tryptophan
L-methionine
bufotenin
4-hydroxy-tryptamine
norbaeocystin
norpsilocin
baeocystin
psilocybin
psilocin
aeruginascin
NMT = N-methyltryptamine
DMT = N,N-dimethyltryptamine
TMT = N,N,N-trimethyltryptamine
NMTP = N-methyltryptophan or L-Abrine
DMTP = N,N-dimethyltryptophan
TMTP = N,N,N-trimethyltryptophan or Hypaphorine or Lenticin
5-HTP = 5-hydroxytryptophan
SAMe = S-Adenosyl-L-methionine
SAH = S-Adenosyl-L-homocysteine
DMC = dimethylcarbonate
DMAPP = dimethylallyl diphosphate
DMSO = dimethyl sulfoxide
5-HT = 5-hydroxytryptamine or Serotonin
NAS = N-acetylserotonin or Normelatonin
NA-MeO-T = N-acetyl-5-methoxy-tryptamine or Melatonin

5-MT = 5-methoxy-tryptamine or Mexamine
5-MeO-NMT = 5-methoxy-N-methytryptamine
5-MeO-DMT = 5-methoxy-N,N-Dimethyltryptamine
5-MeO-TMT = 5-methoxy-N,N,N-trimethyltryptamine
5-HO-NMT = 5-hydroxy-methyltryptamine
5-HO-DMT = 5-hydroxy-dimethyltryptamine or Bufotenine
5-HO-TMT = 5-hydroxy-trimethyltryptamine or Bufotenidine
NMT = N-methyltryptamine
DMT = N,N-dimethyltryptamine
TMT = N,N,N-trimethyltryptamine
NMTP = N-methyltryptophan or L-abrine
DMTP = N,N-dimethyltryptophan
TMTP = N,N,N-trimethyltryptophan or Hypaphorine or Lenticin
5-HO-NMTP = 5-hydroxy-methyltryptophan
5-HO-DMTP = 5-hydroxy-dimethyltryptophan
5-HO-TMTP = 5-hydroxy-methyltryptophan
5-MeO-NMTP = 5-methoxy-methyltryptophan
5-MeO-DMTP = 5-methoxy-dimethyltryptophan
5-MeO-TMTP = 5-methoxy-methyltryptophan
BH4 = Tetrahydrobiopterin
BH2 = Dihydrobiopterin
NADPH = Reduced nicotinamide adenine dinucleotide phosphate
NADP+ = Nicotinamide adenine dinucleotide phosphate
Acetyl-CoA = Acetyl coenzyme A
β-Methyltryptophan (β-mTrp)
N-acetyl-4-hydroxy-tryptamine
N-acetyl-psilocybin
N-acetyl-psilocin

Enzymes and regulatory proteins, and abbreviations, in use of or contained within systems and methods herein are provided in Table 2.

Table 2

Enzymes and Regulatory Proteins
INMT = Indolethylamine-N-methyltransferase; tryptamine N-methyltransferase
IOMT = indole-O-methyltransferase; hydroxytryptamine O-methyltransferase

CaffOMT = caffeic acid-O-methyltransferase
T5H = tryptamine 5’ hydroxylase
TrpM = tryptophan N-methyltransferase
PsiM = psilocybin synthase
AADC = Aromatic amino acid decarboxylase; tryptophan decarboxylase
TPH – tryptophan hydroxylase
T4H = tryptamine 4’ hydroxylase
T4H-CPR, T5H-CPR = chimeras with cytochrome p450 reductase
NAT = N-acetyltransferase
DAC = deacetylase
BH4syn = Tetrahydrobiopterin synthesis
BH4reg = Tetrahydrobiopterin regeneration
PsiK = hydroxy tryptamine kinase
TrpS = tryptophan synthase
TMO = toluene monooxygenase
ΛTMT = aminotransferase/methyltransferase fusion
oxidase = multi-copper oxidase
DMATS = dimethylallyltryptophan synthase
IDI1 = isopentenyl-diphosphate isomerase
TrpHalo = tryptophan halogenase
T5H-IOMT fusion polypeptide
AOQS = aspartate oxidase/quinolinic acid synthase fusion
TAT2 = tryptophan importer
MUP1 = methionine importer
SAM3 = SAMe importer
FEX1 = fluoride exporter

The present invention is directed to biosynthetic production of molecules that are analogs of indoles, tryptophans, and tryptamines, which can also serve as precursors to larger tryptamine

alkaloids, such as tryptamines and tryptophans modified by hydroxylation, halogenation, methylation, phosphorylation, prenylation, and halogenation in recombinant organisms.

FIG. 1 shows the chemical structures of tryptophan (top left) and tryptamine (top right), along with enzyme modifications at specific reaction sites of the tryptophan molecule. Tryptophan is the precursor to a wide array of complex natural products. The electron-rich indole of tryptophan is a weak base. These properties allow for enhanced reactivity as a substrate for numerous enzymes that perform but are not limited to the following activities: methylation, halogenation, prenylation, hydroxylation, isonitrile synthesis, nitration, O-phosphorylation, O-methylation, O-acetylation, N-acetylation, glycosylation, sulfation, cleavage, deamination, decarboxylation, and oligomerization of the molecule. This diverse array of indole intermediates provides a way to tune psychedelic effects. For example, 5-MeO-DMT is reported to be more potent than DMT in neural rodent studies (Lima da Cruz, Rafael Vitor, et al.).

FIG. 2 shows examples of various substituted indole compounds in the tryptamine and tryptophan pathways utilized in the present invention. Panel A depicts the indole ring structure with positional numbering, and tryptophan and tryptamine. Examples of 5-hydroxy modified tryptophan and tryptamine compounds are shown in Panel B; Panel C shows examples of modified 5-hydroxy tryptamines. Additionally, Panel D shows examples of modified 4-hydroxy tryptamines.

By engineering various enzymes and regulatory proteins into a microorganism, tryptophan, tryptamine and other substituted indoles can be modified into a large array of useful compounds, which can be harvested from cultures of the microorganisms.

As depicted in FIG. 3, the *de novo* biosynthesis pathway of L-tryptophan and SAMe are utilized as directing molecules in the systems and methods herein. The directing molecules lead to target molecules of the substituted indoles and tryptamine pathways, when on-pathway. In the systems and methods herein, glycolysis leads to chorismate via the shikimate pathway; glutamate biosynthesis pathway leads to L-glutamine via L-glutamate; and L-serine biosynthesis pathway leads to L-serine via 3-phospho-L-serine (i.e., dephosphorylation). Chorismate, glutamine, and L-serine are combined to form L-tryptophan as a directing molecule to be steered on-pathway for yielding substituted indoles and tryptamine pathways. In the systems and methods herein, L-methionine is a direct precursor leading to SAMe, when combined with ATP in the presence of Sam2 and Adk1 enzymes. A conversion cycle for yielding SAMe as a directing molecule also

involves the formation of S-adenyl-L-homocysteine; S-ribosyl-L-homocysteine; 4-5-dihydroxy-2,3-pentanedione; and homocysteine.

Nucleic acids

Thus, in some embodiments, provided is a non-naturally occurring nucleic acid comprising a sequence encoding an enzyme or regulatory protein in tryptamine metabolism, where the enzyme or regulatory protein is an N-methyltransferase (INMT, PsiM, TrpM), a tryptophan decarboxylase (AADC), a tryptophan hydroxylase (TPH), a tryptamine 4' hydroxylase (T4H), a tryptamine 5' hydroxylase (T5H), a truncated cytochrome p450 reductase (T4H-CPR, T5H-CPR), an hydroxytryptamine O-methyltransferase (IOMT or CaffOMT), an N-acetyltransferase (NAT), a deacetylase (DAC), a hydroxyl tryptamine kinase (PsiK), a tryptophan synthase (TrpS), a toluene monooxygenase (TMO), an aminotransferase/methyltransferase fusion (ATMT), a phosphatase, an oxidase, a dimethylallyltryptophan synthase (DMAT or DMATS), an isopentenyl-diphosphate isomerase (IDI1), a tryptophan halogenase (TrpHalo), an aspartate oxidase/quinolinic acid synthase fusion (AOQS), a tryptophan importer (TAT2), a methionine importer (MUP1), or a SAMe importer (SAM3).

These enzymes and regulatory proteins are further characterized as follows.

Indolethylamine N-methyltransferase (INMT) catalyzes the alkylation (i.e., adding a methyl (CH_3) group) of the primary amine on a tryptamine substrate. The methylation reaction uses up the methyl donor cofactor, SAMe (see FIG. 4, Panels A and C). As an example of INMT activity, INMT can act on serotonin to create 5-OH-DMT (bufotenine) or tryptamine to create DMT (FIG. 4, Panels A and C; FIG. 10, Panel A).

Indole-O-methyltransferase (IOMTor CaffOMT) catalyzes the alkylation of the primary amine on the 5-hydroxy moiety on an indole ring. The methylation reaction uses up the methyl donor cofactor, SAMe (FIG. 4, Panel D). As an example of IOMT activity, IOMT can act on bufotenine (5-OH-DMT) to create 5-MeO-DMT, or N-acetylserotonin to create melatonin (FIG. 4, Panel D).

Tryptamine 5' hydroxylase (T5H) is a p450 tryptamine hydroxylase which prefers hydroxylation at the 5' position of the indole ring, such as generating serotonin from tryptamine (FIG. 4, Panel E), in conjunction with the cofactors NAD(P)H, FMN, and FAD+. P450s such as the T5Hs are generally membrane-associated, with the N-termini imparting an effect on the efficiency of the p450 enzymatic function, including a p450's interaction with an associated CPR, which assists with electron transfer.

FIG. 5 shows a matrix of various compounds that can be made with INMT, IOMT and T5H.

Tryptophan methyltransferase (TrpM) catalyzes the alkylation of the primary amine of L-tryptophan to produce N-methyltryptophan (NMTP, also called L-abrine), the mono-methylated product; N,N-dimethyltryptophan (DMTP), the di-methylated product; and N,N,N-trimethyltryptophan (TMTP), the tri-methylated product. See FIG. 6, Panel A.

Psilocybin synthase (PsiM) is an N-methyltransferase that prefers a substituted tryptamine, such as the phosphorylated tryptamine, norbaeocystin. Novel chimeric PsiMs, were generated to remove potentially deleterious regulatory regions of the enzymes by swapping PsiM domains with the related small rRNA methyltransferases from Ascomycota, the phylum of *S. cerevisiae*.

Aromatic amino acid decarboxylase or tryptophan decarboxylase (AADC) catalyzes the decarboxylation of an aliphatic carboxylic acid (i.e., releases carbon dioxide) from compounds such as L-tryptophan to create tryptamine, 5-HTP to create serotonin; 5-OH-DMTP to create bufotenine; and 5-MeO-DMTP to create 5-MeO-DMT, as depicted in FIG. 6, Panel B.

Tryptophan hydroxylase (TPH), adds a hydroxy group to the 5-carbon of L-tryptophan. The L-tryptophan hydroxylase can catalyze the OH addition to the 5-carbon with the cofactor BH4 and oxygen (Biotechnol J. 2016 May;11(5):717-24) (FIG. 6, Panel A). BH4 is synthesized and regenerated in the cell with the BH4syn and BH4reg heterologous enzymes described herein. The BH4syn genes are enzymes that function as a GTP hydroxylase I, a 6-pyruvoyl-tetrahydropterin synthase, and a sepiapterin reductase to generate the BH4 cofactor necessary for TPH enzyme function. The BH4reg genes are enzymes that function as a 4a-hydroxytetrahydropterin dehydratase and a 6-pyruvoyl-tetrahydropterin synthase to regenerate the BH4 cofactor after conversion to HTHB by the TPH enzyme. As an example of TPH activity, TPH can act on L-tryptophan to generate 5-hydroxy-L-tryptophan (5-HTP), and 5-HTP can then be acted on by an AADC to generate serotonin.

Tryptamine 4' hydroxylase (T4H) is a p450 tryptamine hydroxylase which prefers hydroxylation at the 4' position of the indole ring, in conjunction with the cofactors NAD(P)H, FMN, and FAD+. When derived from psychedelic mushrooms, these are also called PsiH. The T4H enzyme can convert tryptamine to 4-OH-tryptamine, which is a part of the psilocybin pathway. P450s such as the T4Hs are generally membrane-associated, with the N-termini imparting an effect on the efficiency of the p450 enzymatic function, including a p450's interaction with an associated CPR, which assists with electron transfer.

From psychedelic a mushroom derived PsiH and CPR, we generated chimeric p450s and CPRs to better match a heterologous host **(SEQ ID NO:162, 179-180, and 451, 468-469)**, where the N termini of a yeast p450 and CPR replaced the N terminus. Due to the enhancing action of CPRs on p450 enzymatic activity, we determined an optimal fusion between T4H and T4H_CPR, where the T4H_CPR listed are truncated at the N termini and replaced with a linker region. In some embodiments, the T4H nucleic acids have, at the 3' end, an optimized nucleic acid encoding a T4H_CPR, e.g., having **SEQ ID NOs:171-180**, joining the sequences together to form a fusion polypeptide, e.g., having the amino acid sequence of **SEQ ID NOs:460-469** fused at the C terminus of the enzyme polypeptide, generating recombinant T4H-CPR fusion polypeptides.

Similar to the T4H CPR fusions, we generated T5H CPR fusions to enhance the hydroxylation activity. In those embodiments, the T5H nucleic acids have, at the 3' end, an optimized nucleic acid encoding a T5H-CPR, e.g., having **SEQ ID NOs:181-192**, joining the sequences together to form a fusion polypeptide, e.g., having the amino acid sequence of **SEQ ID NOs:470-481** fused at the C terminus of the enzyme polypeptide, generating recombinant T5H-CPR fusion polypeptides.

Examples of the utilization of the T4H-CPR and T5H-CPR in recombinant cells are shown in FIG. 9, Panels C and D; FIGS. 11 and 12; FIG. 13, Panel B; FIG. 14, Panel B; and FIG. 15, Panel B.

Localizing O-methyltransferase activity to hydroxylation can be beneficial for generating methoxytryptamines, such as 5-MT, 5-MeO-DMT, and melatonin. In some embodiments, the T5H nucleic acids have, at the 3' end, an optimized nucleic acid encoding an IOMT e.g., having **SEQ ID NOs:99-130**, joining the sequences together to form a fusion polypeptide, e.g., having the amino acid sequence of **SEQ ID NOs:388-419** fused at the C terminus of the enzyme polypeptide, generating recombinant T5H-IOMT fusion polypeptides.

In all of the fusions described herein, the N-terminal coding sequence has any STOP codon removed, if present, before fusion to a C-terminal coding sequence. If the N-terminal coding sequence does not have a START (ATG) codon, a START codon is added.

N-acetyltransferase (NAT) adds an acetyl group from acetyl-CoA to the terminal amino group of e.g., a tryptamine such as serotonin (FIG. 4, Panel F; FIG. 7, Panels C and D). As an example of NAT activity, NAT can act on serotonin to generate N-acetylserotonin, which in turn can be acted on by an IOMT to generate melatonin (FIG. 11).

Deacetylase (DAC) removes an acetyl group from the terminal amino group of a tryptamine such as melatonin. As an example of DAC activity, DAC can act on melatonin to create 5-MeO-tryptamine, which in turn can be acted on by an INMT to generate 5-MeO-DMT (FIG. 10, Panel B).

Hydroxy tryptamine kinase (PsiK) phosphorylates a hydroxy-indole, in conjunction with ATP. For example, PsiK can act on 4-OH tryptamine to generate norbaeocystin as part of the psilocybin pathway. PsiKs are found in certain mushrooms and parasitic fungi. For psychedelic mushroom derived PsiKs, we generated chimeric PsiKs based on yeast choline kinase to better match a heterologous host.

Non-natural tryptamine analogs can be created with the addition of a synthetic precursor to the fermentation of a recombinant host expressing enzymes capable of utilizing the substrate. For example, the addition of an alpha-methylated amino acid such as alpha-methyl tryptophan to a fermentation where an organism expresses a an indole-N-methyltransferase (INMT) leads to the generation of alpha-methylated DMT (e.g., FIG. 13).

For certain indole ring modifications, such as non-natural indoles, bacterial tryptophan synthases (TrpS) can be used to combine an indole with L-serine or L-threonine to create variants of tryptophan and beta-methyl tryptophan, respectively (FIG. 14, Panel A). While previous groups have made use of the flexibility of versions of bacterial tryptophan synthases to generate exotic tryptamines (De novo Biosynthesis of "Non-Natural" Thaxtomin Phytotoxins. Angew Chem Int Ed Engl. 2018 Jun 4;57(23):6830-6833), efficient bioproduction is limited by the toxic nature of indole. In one embodiment, TrpS is expressed as a modified secreted fusion polypeptide version of the *Salmonella* tryptophan synthase that is able to combine indole or a modified indole with L-serine or L-threonine in the extracellular space, allowing indole conversion away from the cell host. In some embodiments, a multidrug efflux exporter such as mdtEF (accessions: P37636, P37637) can be coexpressed with TrpS with exogenous indole, to enable the host cell to export indole and continue bioproduction of tryptophan and tryptamine analogs.

Alternatively to T4H, T5H, and TPH enzymes, hydroxylation of the indole ring of tryptamines and related indole-like compounds can be carried out by complexes known as toluene-monooxygenases (TMO) typically found in bacteria within the genus *Pseudomonas*. The polypeptides that form this complex can be expressed in a modified host as an alternative to P450-based hydroxylation for compounds such as psilocybin and aeruginascin, whose biosynthetic pathway involves 4'OH hydroxylation. Other non-P450 monooxygenases from genuses of

Pseudomonas and *Burkeholderia* can be optimized and expressed in a modified host for hydroxylation of different indole positions, such as the 3' carbon of the indole ring. TMO complexes are made up of several subunits. For efficient expression of TMOs in a recombinant heterologous host, we generated fusion polypeptide pairs of the four core subunits.

Beta-methylated tryptamine analogs are created by combined expression of a recombinant aminotransferase-methyltransferase (ATMT) fusion polypeptide and an aromatic amino acid decarboxylase (AADC) (FIG. 15). In nature, organisms which produce beta-methyl tryptophan typically express the aminotransferase (AT) and the methyltransferase (MT) as separate genes. Recombinant ATMT genes herein encode both domains as a single polypeptide. Combinatorial expression of ATMTs and other tryptamine modifying genes can be used to create compounds such as beta-methylated DMT and beta-methylated psilocybin.

In some embodiments, recombinant phosphatases and oxidases are used to generate hydroxylated tryptamine dimers such as one psilocin or bufotenine molecule conjugated to another psilocin or bufotenine molecule (FIG. 16). When certain psychedelic mushrooms which contain compounds such as psilocybin are damaged and cellular compartments compromised, phosphatases and oxidases, such as laccases or laccase-like multi-copper oxidases, can then come in contact with tryptamine substrate to dephosphorylate and catalyze hydroxy tryptamine polymerization. Similar polymerization which leads to 'blueing' can occur when psilocybin comes into contact with mitochondria. (Levine, Walter G), In some embodiments, the phosphatase is a recombinant alkaline phosphatase, which dephosphorylates phosphorylated tryptamines and tryptophans (FIG. 16, Panel A), such as psilocybin to psilocin. In some embodiments, the oxidase is a non-laccase member of the multi-copper oxidase superfamily, which creates hydroxy tryptamine radicals which catalyze polymerization (FIG. 16, Panel B). This dimer example and oligomerization of hydroxylated tryptamines can generate a blue color, lending the effect to colorimetric readout for compound production. Dimer variants and other oligomerized tryptamines can be separated from each other through chromatographic methods for purification. Efficient heterologous expression of certain oxidases such as laccases presents several challenges, such as N and C termini processing which may fail in a heterologous host. In some embodiments, to improve heterologous oxidase expression to biosynthetically produce tryptamine dimers and oligomers, we engineered chimeric oxidase yeast oxidase. Example includes SEQ ID NO:274,563 In one embodiment, the oxidases are also coexpressed with the yeast t-SNARE, SSO2, to improve protein expression, processing, and secretion for active enzyme SEQ ID NO:170,459.

Dimethylallyl tryptophan synthase (DMATS or DMAT) generates prenylated tryptophans and tryptamines. DMATS is a prenyltransferase that prefers the dimethylallyl diphosphate (DMAPP) prenyl donor to prenylate tryptophan and tryptamine compounds.

Localizing DMAPP generation to the DMATS enzyme can be beneficial for generating prenylated tryptophans, such as 7-dimethylallyltryptophan. In yeast, IDI1 is the enzyme which generates DMAPP as part of the mevalonate pathway. In some embodiments, the DMATS nucleic acids have, at the 3' end, an optimized nucleic acid encoding IDI1 e.g., having SEQ ID NO:67, joining the sequences together to form a fusion polypeptide, e.g., having the amino acid sequence of SEQ ID NO:356 fused at the C terminus of the enzyme polypeptide, generating recombinant DMATS-IDI1 fusion polypeptides (FIG. 8, Panel C).

Tryptophan halogenase (TrpHalo) is a flavin-associated halogenase that adds fluorine (F), chlorine (Cl), bromine (Br), and/or iodine (I) to various indoles and biogenic amines (FIG. 8, Panel A). In some embodiments, TrpHalo nucleic acids have, at the 5'end, a nucleic acid encoding an vacuolar localization tag to localize TrpHalo to a yeast vacuole, where Cl ions are stored, e.g., having SEQ ID NOs:287-289 , joining the sequences together to form a fusion polypeptide, e.g., having the amino acid sequence of SEQ ID NOs:576-578 fused at the N terminus of the enzyme polypeptide, generating recombinant fusion polypeptides.

In other embodiments, TrpHalo nucleic acids have, at the 5'end, a nucleic acid encoding a secretion tag with or without a 6xHIS tag for purification, e.g., having SEQ ID NO:1, joining the sequences together to form a fusion polypeptide, e.g., having the amino acid sequence of SEQ ID NO:290 fused at the N terminus of the enzyme polypeptide, generating recombinant fusion polypeptides. In one embodiment, TrpHalo is also coexpressed with the yeast fluoride exporter, Fex1, SEQ ID NO:66,355, to limit halide toxicity on the heterologous host.

To improve the yield of tryptophan and tryptamine variants discussed herein, modifying the heterologous host which expresses these genes and enzymes, in various combinatorial ways, to prevent tryptophan and tryptamine compound degradation is beneficial. Replacing the yeast pathway which degrades certain tryptamine and tryptophan compounds for *de novo* NAD+ production, which is an important source of cofactors for cell viability, with an alternative route to NAD+ production can preserve tryptophan as a precursor and increase product yields. In some embodiments, a new *de novo* pathway is expressed in a heterologous host, where the pathway is composed of a fusion protein containing the two enzymatic functions required to convert the amino

acid aspartate into quinolinic acid (AOQS), SEQ ID NO: 26-27,315-316, which replaces the endogenous use of tryptophan for generating quinolinic acid in the pathway for NAD+.

In some embodiments, the nucleic acids have, at the 5' end, a nucleic acid encoding codon optimized cofolding peptides to create a fusion protein, e.g., having SEQ ID NOs:256-269, joining the sequences together to form a fusion polypeptide, e.g., having the amino acid sequence of SEQ ID NOs:554-558 fused at the N terminus of the enzyme polypeptide, generating recombinant fusion polypeptides.

In some embodiments, the nucleic acids have, at the 5' end, a nucleic acid encoding a secretion signal, creating a secreted protein, e.g., having SEQ ID NOs:282-286, joining the sequences together to form a fusion polypeptide, e.g., having the amino acid sequence of SEQ ID NOs:571-575 fused at the N terminus of the enzyme polypeptide, generating recombinant fusion polypeptides.

In some embodiments, the nucleic acids have, at the 5' or 3' end, an optimized nucleic acid encoding a localization scaffold composed of multiple domains where proteins tagged with affibodies can bind and colocalize together (for example, FIG. 11, Panel A; FIG. 12, Panel A), creating a protein scaffold fusion, e.g., having SEQ ID NO:281, joining the sequences together to form a fusion polypeptide, e.g., having the amino acid sequence of SEQ ID NO:570 fused at the N or C terminus of the enzyme polypeptide, generating recombinant fusion polypeptides.

In some embodiments, the nucleic acids have, at the 5' or 3' end, an optimized nucleic acid encoding an affibody tag that can bind one of the domains of the localization scaffold, thereby colocalizing multiple enzymes and creating protein scaffold fusion, e.g., having SEQ ID NOs:259-264, joining the sequences together to form a fusion polypeptide, e.g., having the amino acid sequence of SEQ ID NOs:548-553 fused at the N or C terminus of the enzyme polypeptide, generating recombinant fusion polypeptides.

The initial substrates for DMTP, DMT, and related compound production are L-tryptophan and S-Adenosyl-L-methionine (SAMe). The initial substrate can be produced endogenously in a recombinant host as described and/or provided exogenously to a fermentation involving a recombinant host, whereby the host uptakes the starting substrates to feed into the biosynthetic pathway for indoles and tryptamines. The recombinant hosts herein described that are expressing all, one, or multiple combinations of the engineered INMT, AADC, TPH, T4H, T5H, T4H-CPR, T5H-CPR, IOMT, NAT, DAC, PsiK, TrpS, TMO, ATMT, DMATS, IDI1, and TrpHalo genes can produce tryptamine, NMTP, DMTP, TMTP, NMT, DMT, TMT, psilocybin, bufotenine, 5-MeO

DMT, 4-bromo-tryptamine, 4-dimethylallyl tryptamine, alpha-methylated DMTP, beta-methylated DMTP, melatonin, etc.

As depicted in FIGS. 4 and 9, the engineered INMT, IOMT and TrpM and INMT enzymes require a methyl donor in the form of SAMe to act on substrates in the biosynthetic pathway for substituted indoles and tryptamines such as DMTP, DMT, intermediates, and analogs. The methyltransferase activity of TrpM and INMT subsequently convert the methyl donor cofactor SAMe to SAH. Methylations can occur successively with multiple rounds of methyl donor usage. For instance, TrpM can methylate L-tryptophan to produce NMTP and continue to methylate NMTP to DMTP, and then TMTP (FIG. 6, Panel A). Similarly, an INMT can methylate tryptamine to produce NMT, and then continue to methylate NMT to DMT, and then TMT (FIG. 5).

The methylation occurs selectively at the primary amine of L-tryptophan and tryptamine in the presence of TrpM and INMT enzymes. The nitrogen in the heterocycle and hydroxyl group in the carboxylic acid of L-tryptophan are also sites of alkylation, as SAMe is a highly reactive methylating agent. The TrpM enzyme directs methylation such that di-methylation of the primary amine occurs. Using the traditional techniques of organic chemistry where robust methylating agents, such as methyl iodide, trimethyl sulfonium iodide, and dimethyl sulfate, are employed, a mixture of products is formed. The mixture of products may include: mono, di, and tri-methylation of the amine; O-methylation of the carboxylic acid (i.e., the methyl ester), and N-methylation of the indole ring. Separation of these products are tedious and reduces the yield of a desired product. Additionally, SAMe has a primary amine group which may readily undergo intramolecular methylation at the amine. The systems and methods herein in the recombinant host with TrpM and INMT enzymes maintain the structure of SAMe without methylation of the amine of the SAMe prior to methylating the amine of L-tryptophan and tryptamine.

Heterologous pathway enzymes that are expressed to produce substituted indole and tryptamine compounds such as DMTP and DMT use L-tryptophan as a directing molecule. Tryptophan production in cells is normally tightly regulated. Tryptophan accumulation in a recombinant host is increased by: (a) overexpressing feedback-resistant versions of the endogenous tryptophan-producing enzymes; (b) knocking out off-pathway tryptophan-consuming genes and enzymes; and (c) overexpressing a recombinant L-tryptophan transporter. This allows for exogenous tryptophan to be fed to the cells and transported in the recombinant host. These modifications, genes, and methods are disclosed in U.S. Patent Publication 2021/0147888, incorporated by reference.

On-pathway genes and enzymes can be overexpressed for L-tryptophan accumulation. The immediate precursors for L-tryptophan include chorismate, L-serine, and L-glutamine. To increase the on-pathway flux to L-tryptophan and the substituted indole and tryptamine pathway, off-pathway genes which consume L-tryptophan are deleted. The genes that encode the enzymes, Pdc5 and Aro10 are deleted to reduce pathway flux through the pathways that produce aromatic alcohols. The gene encoding the Aro7 enzyme is deleted to reduce production of tyrosine and phenylalanine from L-tryptophan. The genes that encode the enzymes Pdz1 and Pdz2 are also deleted to reduce pathway flux through the pABA production pathway. The gene encoding the enzyme Bna2 is deleted to reduce consumption of L-tryptophan by the kynurenine pathway.

In some embodiments, a recombinant host is modified to increase the accumulation of the methyl donor, SAMe, which is used by the recombinant TrpM and INMT enzymes to methylate indole and tryptamine molecules, such as L-tryptophan and NMT. SAMe accumulation in the recombinant host cell is increased by: (a) overexpressing enzymes to promote conversion of L-methionine to SAMe; (b) deleting off-pathway genes which encode for enzymes that deplete SAMe for unwanted side products; and (c) overexpressing a permease. This enables exogenous L-methionine to be fed to and transported into the cells.

The TrpM and INMT methyltransferase reactions consume one equivalent of adenosine triphosphate (ATP) and of SAMe. SAMe is a robust methyl donor synthesized from methionine and ATP via the L-methionine adenosyltransferase enzyme, Sam2. In various embodiments, Sam2 is overexpressed in a recombinant host to increase the conversion of L-methionine to SAMe. In other embodiments, to support the increased pathway flux and generate more ATP, the adenylate kinase enzyme, Adk1, is overexpressed. In additional embodiments, to increase the uptake of exogenous L-methionine fed into the SAMe pathway, recombinant Mup1 is overexpressed, which is a methionine transporter. SAMe is a precursor molecule for spermidine production and glycogen biosynthesis. To keep SAMe levels high in the pathways of the recombinant host and decrease off-pathway usage of SAMe, the SPE2 gene can be deleted in the recombinant host, thereby blocking the conversion of SAMe to spermidine. Glycogen biosynthesis consumes ATP, which is required for the conversion of L-methionine to SAMe. The gene encoding the enzyme Glc3 can be deleted in the recombinant host, thereby reducing production of glycogen, maintaining higher levels of ATP in the host cell, and increasing on-pathway flux of SAMe for methyltransferase activity.

As depicted in FIG. 5, the engineered INMT, T5H, and IOMT enzymes act on tryptamine substrates to generate hydroxy and methoxy tryptamine analogs such as serotonin, bufotenine (5-

OH-DMT) and 5-MeO-DMT. The initial substrates for this series of reactions includes compounds such as tryptamine and serotonin, which can be produced within a modified cell or added exogenously, in addition to L-tryptophan and S-Adenosyl-L-methionine (SAMe). The initial substrate can be produced endogenously in a recombinant host as described and/or provided exogenously to a fermentation involving a recombinant host, whereby the host uptakes the starting substrates to feed into the biosynthetic pathway for indoles and tryptamines.

As depicted in FIG. 3, de novo biosynthesis pathway of L-tryptophan and SAMe utilize L-tryptophan and SAMe as directing molecules in the systems and methods herein. The directing molecules lead to target molecules of substituted indoles and tryptamine pathways, when on-pathway. In the systems and methods herein, glycolysis leads to chorismate via the shikimate pathway; glutamate biosynthesis pathway leads to L-glutamine via L-glutamate; and L-serine biosynthesis pathway leads to L-serine via 3-phospho-L-serine (i.e., dephosphorylation). Chorismate, glutamine, and L-serine are combined to form L-tryptophan as a directing molecule to be steered on-pathway for yielding substituted indoles and tryptamine pathways. In the systems and methods herein, L-methionine is a direct precursor leading to SAMe, when combined with ATP in the presence of Sam2 and Adk1 enzymes. A conversion cycle for yielding SAMe as a directing molecule also involves the formation of S-adenyl-L-homocysteine; S-ribosyl-L-homocysteine; 4-5-dihydroxy-2,3-pentanedione; and homocysteine.

Heterologous pathway enzymes that are expressed to produce substituted indole and tryptamine compounds such as DMTP and DMT use L-tryptophan as a directing molecule. Tryptophan production in cells is normally tightly regulated. Tryptophan accumulation in a recombinant host is increased by: (a) overexpressing feedback-resistant versions of the endogenous tryptophan-producing enzymes; (b) knocking out off-pathway tryptophan-consuming genes and enzymes; and (c) overexpressing a recombinant L-tryptophan transporter. This allows for exogenous tryptophan to be fed to the cells and transported in the recombinant host. See also U.S. Patent Publication 2021/0147888.

On-pathway genes and enzymes can be overexpressed for L-tryptophan accumulation. The immediate precursors for L-tryptophan include chorismate, L-serine, and L-glutamine. To increase the on-pathway flux to L-tryptophan and the substituted indole and tryptamine pathway, off-pathway genes which consume L-tryptophan may be deleted. In some embodiments, the genes that encode the enzymes Pdc5 and Aro10 are deleted to reduce pathway flux through the pathways that produce aromatic alcohols. In other embodiments, the gene encoding the Aro7 enzyme is deleted

to reduce production of tyrosine and phenylalanine from L-tryptophan. In additional embodiments, the genes that encode the enzymes Pdz1 and Pdz2 are also deleted to reduce pathway flux through the pABA production pathway. In further embodiments, the gene encoding the enzyme Bna2 is deleted to reduce consumption of L-tryptophan by the kynurenine pathway.

In some embodiments, the nucleic acids described herewith encode a polypeptide or oligopeptide having an amino acid sequence that is naturally occurring. In other embodiments, the nucleic acids encode a polypeptide or oligopeptide having an amino acid sequence that is not naturally occurring. The encoded polypeptides or oligopeptides that are not naturally occurring can vary from a naturally occurring polypeptide or oligopeptide, or portion thereof, by a small amount (e.g., one conservative amino acid substitution or a histidine tag) or extensively (e.g., further comprising a fusion peptide, a substituted or added domain from another protein, a scaffold, etc.).

The nucleic acids can be derived from a naturally occurring gene from any source, e.g., any microorganism, protist, plant, or animal.

In some embodiments, the gene for the enzyme or regulatory protein is derived from a bacterium. It is envisioned that an enzyme or regulatory protein derived from any bacterium now known or later discovered can be utilized in the present invention. For example, the bacterium can be from phylum Abditibacteriota, including class Abditibacteria, including order Abditibacteriales; phylum Abyssubacteria or Acidobacteria, including class Acidobacteriia, Blastocatellia, Holophagae, Thermoanaerobaculia, or Vicinamibacteria, including order Acidobacteriales, Bryobacterales, Blastocatellales, Acanthopleuribacterales, Holophagales, Thermotomaculales, Thermoanaerobaculales, or Vicinamibacteraceae; phylum Actinobacteria, including class Acidimicrobiia, Actinobacteria, Actinomarinidae, Coriobacteriia, Nitriliruptoria, Rubrobacteria, or Thermoleophilia, including orders Acidimicrobiales, Acidothermales, Actinomycetales, Actinopolysporales, Bifidobacteriales, Nanopelagicales, Catenulisporales, Corunebacteriales, Cryptosporangiales, Frankiales, Geodermatophilales, Glycomycetales, Jiangellales, Micrococcales, Micromonosporales, Nakamurellales, Propionibacteriales, Pseudonocardiales, Sporichthyales, Streptomycetales, Streptosporangiales, Actinomarinales, Coriobacteriales, Eggerthellales, Egibacterales, Egicoccales, Euzebyales, Nitriliruptorales, Gaiellales, Rubrobacterales, Solirubrobacterales, or Thermoleophilales; phylum Aquificae, including class Aquificae, including order Aquificales or Desulfurobacteriales; phylum Armatimonadetes, including class Armatimonadia, including order Armatimonadales,

Capsulimonadales, Chthonomonadetes, Chthonomonadales, Fimbriimonadia, or Fimbriimonadales; phylum Aureabacteria or Bacteroidetes, including class Armatimonadia, Bacteroidia, Chitinophagia, Cytophagia, Flavobacteria, Saprospiria or Sphingobacteriia, including order Bacteroidales, Marinilabiliales, Chitinophagales, Cytophagales, Flavobacteriales, Saprospirales, or Sphingopacteriales; phylum Balneolaeota, Caldiserica, Calditrichaeota, or Chlamydiae, including class Balneolia, Caldisericia, Calditrichae, or Chlamydia, including order Balneolales, Caldisericales, Calditrichales, Anoxychlamydiales, Chlamydiales, or Parachlamydiales; phylum Chlorobi or Chloroflexi, including class Chlorobia, Anaerolineae, Ardenticatenia, Caldilineae, Thermofonsia, Chloroflexia, Dehalococcoidia, Ktedonobacteria, Tepidiformia, Thermoflexia, Thermomicrobia, or Sphaerobacteridae, including order Chlorobiales, Anaerolineales, Ardenticatenales, Caldilineales, Chloroflexales, Herpetosiphonales, Kallotenuales, Dehalococcoidales, Dehalogenimonas, Ktedonobacterales, Thermogemmatisporales, Tepidiformales, Thermoflexales, Thermomicrobiales, or Sphaerobacterales; phylum Chrysiogenetes, Cloacimonetes, Coprothermobacterota, Cryosericota, or Cyanobacteria, including class Chrysiogenetes, Coprothermobacteria, Gloeobacteria, or Oscillatoriophycideae, including order Chrysiogenales, Coprothermobacterales, Chroococcidiopsidales, Gloeoemargaritales, Nostocales, Pleurocapsales, Spirulinales, Synechococcales, Gloeobacterales, Chroococcales, or Oscillatoriales; phyla: Eferribacteres, Deinococcus-thermus, Dictyoglomi, Dormibacteraeota, Elusimicrobia, Eremiobacteraeota, Fermentibacteria, or Fibrobacteres, including class Deferribacteres, Deinococci, Dictyoglomia, Elusimicrobia, Endomicrobia, Chitinispirillia, Chitinivibrionia, or Fibrobacteria, including order Deferribacterales, Deinococcales, Thermales, Dictyoglomales, Elusimicrobiales, Endomicrobiales, Chitinspirillales, Chitinvibrionales, Fibrobacterales, or Fibromonadales; phylum Firmicutes, Fusobacteria, Gemmatimonadetes, or Hydrogenedentes, including class Bacilli, Clostridia, Erysipelotrichia, Limnochordia, Negativicutes, Thermolithobacteria, Tissierellia, Fusobacteriia, Gemmatimonadetes, Longimicrobia, including order Bacillales, Lactobacillales, Borkfalkiales, Clostridiales, Halanaerobiales, Natranaerobiales, Thermoanaerobacterales, Erysipelotrichales, Limnochordales, Acidaminococcales, Selenomonadales, Veillonellales, Thermolithobacterales, Tissierellales, Fusobacteriales, Gemmatimonadales, or Longimicrobia; phylum Hydrogenedentes, Ignavibacteriae, Kapabacteria, Kiritimatiellaeota, Krumholzibacteriota, Kryptonia, Latescibacteria, LCP-89, Lentisphaerae, Margulisbacteria, Marinimicrobia, Melainabacteria, Nitrospinae, or Omnitrophica, including class

Ignavibacteria, Kiritimatiellae, Krumholzibacteria, Lentisphaeria, Oligosphaeria, or Nitrospinae, including order Ignavibacteriales, Kiritimatiellales, Krumholzibacteriales, Lentisphaerales, Victivallales, Oligosphaerales, or Nitrospinia; phylum Omnitrophica or Planctomycetes, including class Brocadiae, Phycisphaerae, Planctomycetia, or Phycisphaerales, including order Sedimentisphaerales, Tepidisphaerales, Gemmatales, Isosphaerales, Pirellulales, or Planctomycetales; phylum Proteobacteria including class Acidithiobacillia, Alphaproteobacteria, Betaproteobacteria, Lambdaproteobacteria, Muproteobacteria, Deltaproteobacteria, Epsilonproteobacteria, Gammaproteobacteria, Hydrogenophilalia, Oligoflexia, or Zetaproteobacteria, including order Acidithiobacillales, Caulobacterales, Emcibacterales, Holosporales, Iodidimonadales, Kiloniellales, Kopriimonadales, Kordiimonadales, Magnetococcales, Micropepsales, Minwuiales, Parvularculales, Pelagibacterales, Rhizobiales, Rhodobacterales, Rhodospirillales, Rhodothalassiales, Rickettsiales, Sneathiellales, Sphingomonadales, Burkholderiales, Ferritrophicales, Ferrovales, Neisseriales, Nitrosomonadales, Procabacteriales, Rhodocyclales, Bradymonadales, Acidulodesulfobacterales, Desulfarculales, Desulfobacterales, Desulfovibrionales, Desulfurellales, Desulfuromonadales, Myxococcales, Syntrophobacterales, Campylobacterales, Nautiliales, Acidiferrobacterales, Aeromonadales, Alteromonadales, Arenicellales, Cardiobacteriales, Cellvibrionales, Chromatiales, Enterobacterales, Immundisolibacterales, Legionellales, Methylococcales, Nevskiales, Oceanospirillales, Orbales, Pasteurellales Pseudomonadales, Salinisphaerales, Thiotrichales, Vibrionales, Xanthomonadales, Hydrogenophilales, Bacteriovoracales, Bdellovibrionales, Oligoflexales, Silvanigrellales, or Mariprofundales; phylum Rhodothermaeota, Saganbacteria, Sericytochromatia, Spirochaetes, Synergistetes, Tectomicrobia, or Tenericutes, including class Rhodothermia, Spirochaetia, Synergistia, Izimaplasma, or Mollicutes, including order Rhodothermales, Brachyspirales, Brevinematales, Leptospirales, Spirochaetales, Synergistales, Acholeplasmatales, Anaeroplasmatales, Entomoplasmatales, or Mycoplasmatales; phylum Thermodesulfobacteria, Thermotogae, Verrucomicrobia, or Zixibacteria, including class Thermodesulfobacteria, Thermotogae, Methylacidiphilae, Opitutae, Spartobacteria, or Verrucomicrobiae, including order Thermodesulfobacteriales, Kosmotogales, Mesoaciditogales, Petrotogales, Thermotogales, Methylacidiphilales, Opitutales, Puniceicoccales, Xiphinematobacter, Chthoniobacterales, Terrimicrobium, or Verrucomicrobiales.

In other embodiments, the gene for the enzyme or regulatory protein is derived from an archaeon. It is envisioned that an enzyme or regulatory protein derived from any archaeon now

known or later discovered can be utilized in the present invention. For example, the archaeon can be from phylum Euryarchaeota, including class Archaeoglobi, Hadesarchaea, Halobacteria, Methanobacteria, Methanococci, Methanofastidiosa, Methanomicrobia, Methanopyri, Nanohaloarchaea, Theionarchaea, Thermococci, or Thermoplasmata, including order Archaeoglobales, Hadesarchaeales, Halobacteriales, Methanobacteriales, Methanococcales, Methanocellales, Methanomicrobiales, Methanophagales, Methanosarcinales, Methanopyrales, Thermococcales, Methanomassiliicoccales, Thermoplasmatales, or Nanoarchaeales; DPANN superphylum, including subphyla Aenigmarcheota, Altiarchaeota, Diapherotrites, Micrarchaeota, Nanoarchaeota, Pacearchaeota, Parvarchaeota, or Woesearchaeota; TACK superphylum, including subphylum Korarchaeota, Crenarchaeota, Aigarchaeota, Geoarchaeota, Thaumarchaeota, or Bathyarchaeota; Asgard superphylum including subphylium Odinarchaeota, Thorarchaeota, Lokiarchaeota, Helarchaeota, or Heimdallarchaeota.

In additional embodiments, the gene for the enzyme or regulatory protein is derived from a fungus. It is envisioned that an enzyme or regulatory protein derived from any fungus now known or later discovered can be utilized in the present invention. This includes but is not limited to the phyla Chytridiomycota, Basidiomycota, Ascomycota, Blastocladiomycota, Ascomycota, Microsporidia, Basidiomycota, Glomeromycota, Symbiomycota, and Neocallimastigomycota. For example, the fungus can be from the phylum Ascomycota, including classes and orders Pezizomycotina, Arthoniomycetes, Coniocybomycetes, Dothideomycetes, Eurotiomycetes, Geoglossomycetes, Laboulbeniomycetes, Lecanoromycetes, Leotiomycetes, Lichinomycetes, Orbiliomycetes, Pezizomycetes, Sordariomycetes, Xylonomycetes, Lahmiales, Itchiclahmadion, Triblidiales, Saccharomycotina, Saccharomycetes, Taphrinomycotina, Archaeorhizomyces, Neolectomycetes, Pneumocystidomycetes, Schizosaccharomycetes, Taphrinomycetes; phylum Basidiomycota including subphyla or classes Pucciniomycotina, Ustilaginomycotina, Wallemiomycetes, and Entorrhizomycetes; subphylum Agaricomycotina including classes Tremellomycetes, Dacrymycetes, and Agaricomycetes; phylum Symbiomycota, including class Entorrhizomycota; subphylum Ustilaginomycotina including classes Ustilaginomycetes and Exobasidiomycetes; phylum Glomeromycota including classes Archaeosporomycetes, Glomeromycetes, and Paraglomeromycetes; subphylum Pucciniomycotina including orders and classes: Pucciniomycotina, Cystobasidiomycetes, Agaricostilbomycetes, Microbotryomycetes, Atractiellomycetes, Classiculomycetes, Mixiomycetes, and Cryptomycocolacomycetes; subphylum incertae sedis Mucoromyceta including orders Calcarisporiellomycota and

Mucoromycota; phylum Mortierellomyceta including class Mortierellomycota; subphylum incertae sedis Entomophthoromycotina including order Entomophthorales; phylum Zoopagomyceta including classes Basidiobolomycota, Entomophthoromycota, Kickxellomycota, and Zoopagomycotina; subphylum incertae sedis Mucoromycotina including orders Mucorales, Endogonales, and Mortierellales; phylum Neocallimastigomycota including class Neocallimastigomycetes; phylum Blastocladiomycota including classes Physodermatomycetes and Blastocladiomycetes; phylum Rozellomyceta including classes Rozellomycota and Microsporidia; phylum Aphelidiomyceta including class Aphelidiomycota; Chytridiomyceta including classes Chytridiomycetes and Monoblepharidomycetes; and phylum Oomycota including classes or orders Leptomitales, Myzocytiopsidales, Olpidiopsidales, Peronosporales, Pythiales, Rhipidiales, Salilagenidiales, Saprolegniales, Sclerosporales, Anisolpidiales, Lagenismatales, Rozellopsidales, and Haptoglossales.

In additional embodiments, the gene for the enzyme or regulatory protein is derived from the organism below. This includes but is not limited to: Acanthurus tractus, Aplysina aerophoba, Bos Taurus, Bufo bufo, Bufotes viridis, Chrysochloris asiatica, Fukomys damarensis, Homo sapiens, Rattus norvegicus, Rhinella marina, Rhinella spinulosa, Schistosoma mansoni, Xenopus laevis, Xenopus tropicalis, Acacia koa, Arabidopsis thaliana, Brassica oleracea, Citrus sinensis, Hordeum vulgare, Juglans cinereal, Lophophora williamsii, Nymphaea colorata, Oryza sativa, Ricinus communis, Solanum lycopersicum, Sorghum bicolor, Theobroma cacao, and Triticum aestivum.

In some embodiments, the nucleic acids are codon optimized to improve expression, e.g., using techniques as disclosed in US Patent No. 10,435,727. More specifically, optimized nucleotide sequences are generated based on a number of considerations: (1) For each amino acid of the recombinant polypeptide to be expressed, a codon (triplet of nucleotide bases) is selected based on the frequency of each codon in the Saccharomyces cerevisiae genome; the codon can be chosen to be the most frequent codon or can be selected probabilistically based on the frequencies of all possible codons. (2) In order to prevent DNA cleavage due to a restriction enzyme, certain restriction sites are removed by changing codons that cover those sites. (3) To prevent low-complexity regions, long repeats (sequences of any single base longer than five bases) are modified. (2) and (3) are performed recursively to ensure that codon modification does not lead to additional undesirable sequences. (4) A ribosome binding site is added to the N-terminus. (5) A stop codon is added. (6) A localization signal is removed or replaced.

In some of the above embodiments, the nucleic acid provided herein comprises the sequence of any one of SEQ ID NOs:1-289.

In various embodiments, the nucleic acids further comprise additional nucleic acids encoding amino acids that are not part of the included enzymes or regulatory proteins herein. In some of these embodiments, the additional sequences encode additional amino acids present when the nucleic acid is translated, encoding, for example, a cofolding peptide, as previously discussed, or an additional protein domain, with or without a linker sequence, creating a fusion protein. Other examples are localization sequences, i.e., signals directing the localization of the folded protein to a specific subcellular compartment or membrane. Additional nonlimiting examples are an affibody tag, a localization scaffold, a vacuolar localization tag, a secretion signal, and a 6xhis tag.

In some embodiments, the nucleic acid comprises additional nucleotide sequences that are not translated. Nonlimiting examples include promoters, terminators, barcodes, Kozak sequences, targeting sequences, and enhancer elements. Particularly useful here are promoters that are functional in yeast.

Expression of a gene encoding an enzyme or regulatory protein is determined by the promoter controlling the gene. In order for a gene to be expressed, a promoter must be present within 1,000 nucleotides upstream of the gene. A gene is generally cloned under the control of a desired promoter. The promoter regulates the amount of enzyme expressed in the cell and also the timing of expression, or expression in response to external factors such as sugar source.

Any promoter now known or later discovered can be utilized to drive the expression of the enzymes and regulatory proteins described herein. See e.g. http://parts.igem.org/Yeast for a listing of various yeast promoters. Exemplary promoters listed in Table 3 below drive strong expression, constant gene expression, medium or weak gene expression, or inducible gene expression. Inducible or repressible gene expression is dependent on the presence or absence of a certain molecule. For example, the *GAL1, GAL7,* and *GAL10* promoters are activated by the presence of the sugar galactose and repressed by the presence of the sugar glucose. The *HO* promoter is active and drives gene expression only in the presence of the alpha factor peptide. The *HXT1* promoter is activated by the presence of glucose while the *ADH2* promoter is repressed by the presence of glucose.

Table 3: Exemplary yeast promoters

Strong constitutive promoters	Medium and weak constitutive promoters	Inducible/repressible promoters
TEF1	*STE2*	*GAL1*
PGK1	*TPI1*	*GAL7*
PGI1	*PYK1*	*GAL10*
TDH3		*HO*
		HXT1
		ADH2

In various embodiments, the nucleic acid is in an expression cassette, e.g., a yeast expression cassette. Any yeast expression cassette capable of expressing the enzyme in a yeast cell can be utilized.

Additional regulatory elements can also be present in the expression cassette, including restriction enzyme cleavage sites, antibiotic resistance genes, integration sites, auxotrophic selection markers, origins of replication, and degrons.

The expression cassette can be present in a vector that, when transformed into a host cell, either integrates into chromosomal DNA or remains episomal in the host cell. Such vectors are well-known in the art. See e.g. http://parts.igem.org/Yeast for a listing of various yeast vectors.

A nonlimiting example of a yeast vector is a yeast episomal plasmid (YEp) that contains the pBluescript II SK(+) phagemid backbone, an auxotrophic selectable marker, yeast and bacterial origins of replication and multiple cloning sites enabling gene cloning under a suitable promoter (see Table 3). Other exemplary vectors include pRS series plasmids.

Host cells

The present invention is also directed to genetically engineered host cells that comprise the above-described nucleic acids. Such cells may be, e.g., any species of filamentous fungus, including but not limited to any species of *Aspergillus*, which have been genetically altered to produce precursor molecules, intermediate molecules, or cannabinoid molecules. Host cells may also be any species of bacteria, including but not limited to *Escherichia*, *Corynebacterium*, *Caulobacter*, *Pseudomonas*, *Streptomyces*, *Bacillus*, or *Lactobacillus*.

In some embodiments, the genetically engineered host cell is a yeast cell, which may comprise any of the above-described expression cassettes, and capable of expressing the recombinant enzyme encoded therein.

Any yeast cell capable of being genetically engineered can be utilized in these embodiments. Nonlimiting examples of such yeast cells include species of *Saccharomyces*, *Candida*, *Pichia*, *Schizosaccharomyces*, *Scheffersomyces*, *Blakeslea*, *Rhodotorula*, or *Yarrowia*.

These cells can achieve gene expression controlled by inducible promoter systems; natural or induced mutagenesis, recombination, and/or shuffling of genes, pathways, and whole cells performed sequentially or in cycles; overexpression and/or deletion of single or multiple genes and reducing or eliminating parasitic side pathways that reduce precursor concentration.

The host cells of the recombinant organism may also be engineered to produce any or all precursor molecules necessary for the biosynthesis of substituted indoles, tryptophans and tryptamines.

Construction of *Saccharomyces cerevisiae* strains expressing the enzymes and regulatory proteins provided herein is carried out via expression of a gene which encodes for the enzyme. The gene encoding the enzyme can be cloned into vectors with the proper regulatory elements for gene expression (e.g. promoter, terminator) and the derived plasmid can be confirmed by DNA sequencing. As an alternative to expression from an episomal plasmid, the gene encoding the enzyme may be inserted into the recombinant host genome. Integration may be achieved by a single or double cross-over insertion event of a plasmid, or by nuclease-based genome editing methods, as are known in the art e.g. CRISPR, TALEN and ZFR. Strains with the integrated gene can be screened by rescue of auxotrophy and genome sequencing. See, e.g., Green and Sambrook (2012).

FIGS. 9-15 provide nonlimiting examples of host cells utilizing the nucleic acids provided herein.

In some embodiments, the recombinant microorganism expresses TPH, TrpM, and AADC, where the recombinant microorganism produces at least one hydroxy substituted tryptamine compound, e.g., bufotenine, 5-OH-NMT, or 5-OH-TMT (FIG. 9, Panel A).

In other embodiments, the recombinant microorganism expresses TPH, TrpM, AADC, and IOMT, where the recombinant microorganism produces at least one methoxy substituted tryptamine compound, e.g., 5-MeO-NMT, 5-MeO-DMT, or 5-MeO-TMT (FIG. 9, Panel B).

In additional embodiments, the recombinant microorganism expresses AADC, T5H or T5H-CPR and INMT, where the recombinant microorganism produces at least one hydroxy substituted tryptamine compound, e.g., bufotenine, 5-OH-NMT, or 5-OH-TMT (FIG. 9, Panel C).

In further embodiments, the recombinant microorganism expresses AADC, T5H or T5H-CPR, INMT, and IOMT, where the recombinant microorganism produces at least one methoxy substituted tryptamine compound, e.g., 5-MeO-NMT, 5-MeO-DMT, or 5-MeO-TMT (FIG. 9, Panel D).

In other embodiments, the recombinant microorganism expresses TrpM and TPH, where the recombinant microorganism produces at least one hydroxy substituted tryptophan compound, e.g., 5-HTP, 5-OH-NMTP, 5-OH-DMTP or 5-OH-TMTP.

In additional embodiments, the recombinant microorganism expresses TrpM, TPH and IOMT, where the recombinant microorganism produces at least one methoxy substituted tryptophan compound, e.g., 5-MeO-NMTP, 5-MeO-DMTP or 5-MeO-TMTP.

In further embodiments, the recombinant microorganism expresses INMT and T5H, where the recombinant microorganism produces at least one hydroxy substituted tryptamine compound, e.g., bufotenine, 5-OH-NMT, or 5-OH-TMT.

In other embodiments, the recombinant microorganism expresses INMT, T5H and IOMT, where the recombinant microorganism produces at least one methoxy substituted tryptamine compound, e.g., 5-MeO-NMT, 5-MeO-DMT, or 5-MeO-TMT.

In additional embodiments, the recombinant microorganism expresses INMT, where the recombinant microorganism produces at least one hydroxy substituted tryptophan compound, e.g., 5-OH-NMTP, 5-OH-DMTP or 5-OH-TMTP.

In further embodiments, the recombinant microorganism expresses INMT and IOMT, where the recombinant microorganism produces at least one methoxy substituted tryptophan compound, e.g., 5-MeO-NMTP, 5-MeO-DMTP or 5-MeO-TMTP.

In other embodiments, the recombinant microorganism expresses INMT and AADC, where the recombinant microorganism produces at least one hydroxy substituted tryptamine compound, e.g., bufotenine, 5-OH-NMT, or 5-OH-TMT.

In additional embodiments, the recombinant microorganism expresses INMT, AADC and IOMT, where the recombinant microorganism produces at least one methoxy substituted tryptamine compound, e.g., 5-MeO-NMT, 5-MeO-DMT, or 5-MeO-TMT.

In further embodiments, the recombinant microorganism expresses INMT, where the recombinant microorganism produces at least one hydroxy substituted tryptamine compound, e.g., bufotenine, 5-OH-NMT, or 5-OH-TMT.

In other embodiments, the recombinant microorganism expresses INMT and IOMT, where the recombinant microorganism produces at least one methoxy substituted tryptamine compound, e.g., 5-MeO-NMT, 5-MeO-DMT, or 5-MeO-TMT.

In additional embodiments, the recombinant microorganism expresses INMT, where the recombinant microorganism produces at least one methoxy substituted tryptamine compound, e.g., 5-MeO-NMT, 5-MeO-DMT, or 5-MeO-TMT.

As depicted in FIG. 11, in some embodiments, the recombinant microorganism expresses AADC, IOMT, T5H or T5H-CPR, and NAT, where the recombinant microorganism produces a compound in the melatonin pathway, e.g., serotonin or melatonin. In some of these embodiments, the enzymes are on a scaffold to facilitate pathway throughput.

As depicted in FIG. 12, in some embodiments, the recombinant microorganism expresses AADC, T4H or T4H-CPR, PsiK and INMT (PsiM), where the recombinant microorganism produces a compound in the psilocybin pathway, e.g., baeocystin, psilocybin or aeruginascin. In some of these embodiments, the enzymes are on a scaffold to facilitate pathway throughput.

In accordance with the present invention, a recombinant host may also be modified to increase the accumulation of the methyl donor, SAMe, which is used by the recombinant TrpM and INMT enzymes to methylate indole and tryptamine molecules such as L-tryptophan and NMT. SAMe accumulation in the recombinant host cell may be increased by: (d) overexpressing enzymes to promote conversion of L-methionine to SAMe; (e) deleting off-pathway genes that encode for enzymes that deplete SAMe for unwanted side products; and (f) overexpressing a permease, which enables exogenous L-methionine to be fed to and transported into the cells.

The TrpM and INMT methyltransferase reactions consume one equivalent of adenosine triphosphate (ATP) and of SAMe. SAMe is a robust methyl donor synthesized from methionine and ATP via the L-methionine adenosyltransferase enzyme, Sam2. Sam2 may be overexpressed in a recombinant host to increase the conversion of L-methionine to SAMe. To support the increased pathway flux and generate more ATP, the adenylate kinase enzyme, Adk1, may also be overexpressed. To increase the uptake of exogenous L-methionine to feed into the SAMe pathway, recombinant Mup1, which is a methionine transporter, may be overexpressed.

SAMe is a precursor molecule for spermidine production and glycogen biosynthesis. To keep SAMe levels high in the pathways of the recombinant host and decrease off-pathway usage of SAMe, the SPE2 gene may be deleted in the recombinant host, thereby blocking the conversion of SAMe to spermidine. Glycogen biosynthesis consumes ATP, which is required for the conversion of L-methionine to SAMe. The gene encoding the enzyme Glc3 may be deleted in the recombinant host, thereby reducing production of glycogen, maintaining higher levels of ATP in the host cell, and increasing on-pathway flux of SAMe for methyltransferase activity. FIG. 10 depicts a recombinant host modified to express the enzymes enabling uptake and biosynthesis of indole and tryptamine precursors and the enzymes to create tryptamine, DMTP, DMT, and related substituted indole and tryptamine compounds.

Recombinant enzymes and regulatory proteins

The present invention is also directed to a non-naturally occurring enzyme or regulatory protein comprising an amino acid sequence encoded by any of the nucleic acids described above. In some embodiments, the amino acid sequence is 85%, 90%, 95%, 98%, or 100% identical to any one of SEQ ID NO:290-578. In these embodiments, the enzyme or regulatory protein can be isolated *in vitro* and used *in vitro* to provide enzyme activity. Alternatively, as discussed above, the enzyme can be expressed in a recombinant organism, e.g., a microorganism or a plant. In some of these embodiments, the recombinant microorganism is a bacterium, for example an *E. coli*. In other embodiments, the recombinant microorganism is a yeast cell, e.g., a species *of Saccharomyces* (for example *S. cerevisiae*), *Candida, Pichia, Schizosaccharomyces, Schеffersomyces, Blakeslea, Rhodotorula, Aspergillus* or *Yarrowia*.

Methods

The systems and methods herein include: (i) growing modified recombinant host cells and thereby yielding a recombinant host organism; (ii) expressing engineered indole and tryptamine biosynthesis genes and enzymes in the recombinant host organism; (iii) producing or synthesizing substituted indoles and tryptamines in the recombinant host organism; (iv) fermenting the recombinant host organism; and (v) isolating the substituted indoles and tryptamines from the recombinant host organism. Endogenous pathways of the recombinant host can be modified by the systems and methods herein to produce high purity substituted indoles and tryptamines.

To produce the desired substituted indole, the nucleic acid encoding the enzymes and/or regulatory proteins are introduced into a host cell using standard cell (e.g., yeast) transformation techniques (Green and Sambrook, 2012). Cells are subjected to fermentation under conditions that activate the promoter controlling the synthesis of the enzyme and/or regulatory protein. The broth may be subsequently subjected to HPLC analysis to determine the presence or yield of the desired substituted indole, as in FIGS. 17-24 and 26.

In various embodiments, the host cells are provided with various feedstocks to drive production of the desired substituted indole, e.g., glucose, fructose, sucrose, ethanol, fatty acids, glycerol, molasses, corn steep liquor, dairy, fish waste, etc. for example as discussed in US Patent Application 17/078636.

In some embodiments, for recombinant enzyme purification, the gene encoding the enzyme and/or regulatory protein is cloned into an expression vector such as the pET expression vectors from Novagen, transformed into a protease deficient strain of *E. coli* such as BL21 and expressed by induction with IPTG. The protein of interest may be tagged with an affinity tag to facilitate purification, e.g. hexahistidine, GST, calmodulin, TAP, AP, CAT, HA, FLAG, MBP etc. Coexpression of a bacterial chaperone such as dnaK, GroES/GroEL or SecY may help facilitate protein folding. See Green and Sambrook (2012).

Any of the enzymes and/or regulatory proteins described above can also be produced in transgenic plants, using techniques known in the art (see, e.g., Keshavareddy et al., 2018). In these embodiments, the above-described nucleic acid encoding the enzyme and/or regulatory protein further comprises a promoter functional in a plant. In various embodiments, the nucleic acid is in a plant expression cassette. Any plant capable of being transformed with the nucleic acid can be utilized here. In some embodiments, the plant is a tobacco or cannabis.

Preferred embodiments are described in the following examples. Other embodiments within the scope of the claims herein will be apparent to one skilled in the art from consideration of the specification or practice of the invention as disclosed herein. It is intended that the specification, together with the examples, be considered exemplary only, with the scope and spirit of the invention being indicated by the claims, which follow the examples.

In the examples below, genetically engineered host cells may be any species of yeast herein, including but not limited to any species of *Saccharomyces*, *Candida*, *Schizosaccharomyces*, *Yarrowia*, etc.,which have been genetically altered to produce precursor

molecules, intermediate molecules, and psilocybin molecules. Additionally, genetically engineered host cells may be any species of filamentous fungus, including but not limited to any species of *Aspergillus*, which have been genetically altered to produce precursor molecules such as L-tryptophan and substituted indole and tryptamine molecules. Some of the species of yeast herein for the recombinant host organism include but are not limited to: *Schizosaccharomyces cerevisiae, Schizosaccharomyces japonicus, Schizosaccharomyces pombe, Schizosaccharomyces cryophilus, Saccharomyces cerevisiae, Kluyveromyces lactis, Kluyveromyces dobzhanskii,* and *Yarrowia lipolytica*.

In these examples, the gene sequences from gene source organisms are codon optimized to improve expression using techniques disclosed in U.S. Patent 10,435,727.

DNA sequences are synthesized and cloned using techniques known in the art. Gene expression can be controlled by inducible or constitutive promoter systems using the appropriate expression vectors. Genes are transformed into an organism using standard yeast or fungus transformation methods to generate modified host strains (i.e., the recombinant host organism). The modified strains express genes for: (i) producing L-tryptophan, SAMe and precursor molecules to L-tryptophan and SAMe; (ii) increasing an output of L-tryptophan molecules and precursor molecules to L-tryptophan and SAMe molecules; (iii) increasing the import of exogenous L-tryptophan, L-methionine, SAMe and TMG into the host strain; and (iv) the genes for biosynthetic pathways that generate DMT, DMTP, bufotenine, 5-MeO-DMT and all intermediate indole and tryptamine compounds synthesized and described herein. In the presence or absence of exogenous L-tryptophan, L-methionine, SAMe, TMG, 5-HTP, melatonin, and serotonin, fermentations are run to determine if the cell will convert the fed precursors into tryptamine, serotonin, methylated versions of serotonin, melatonin, or methylated versions of melatonin. The L-tryptophan, SAMe, hydroxylation, decarboxylation, and methylation pathway genes herein can be integrated into the genome of the cell or maintained as an episomal plasmid. Samples are: (i) prepared and extracted using a combination of fermentation, dissolution, and purification steps; and (ii) analyzed by HPLC for the presence of directing molecules (e.g., SAMe and L-tryptophan), precursor molecules, intermediate molecules, and target molecules such as bufotenine and 5-MeO-DMT.

Using the systems and methods herein, the genes which can be expressed to encode for a corresponding enzyme or other type of proteins include but are not limited to: ENO2, TAL1, ARO1, ADK1, MUP1, SAM2, MHT1, SAM4, SAM3, TAT2, AADC, TRPM, INMT, TPH, genes

encoding enzymes for the BH4 biosynthesis pathway, genes encoding enzymes for the BH4 regeneration pathway, T5H, IOMT, caffOMT, NAT, DAC, T4H, PsiK, oxidase, phosphatase, TrpHalo, DMAT, T4H-CPR, T5H-CPR, TrpS, and ATMT. For example, the AADC gene is expressed, or overexpressed, to encode for the aromatic amino decarboxylase enzyme; the TRPM gene is expressed to encode for the TrpM enzyme; and so forth. Gene sequences can be determined using standard techniques known in the art, e.g., the techniques disclosed in U.S. Patent 10,671,632.

EXAMPLES

Example 1 - Construction of Saccharomyces cerevisiae platform strains with elevated indole and tryptamine precursors.

The construction of *Saccharomyces cerevisiae* platform strains with elevated metabolic flux towards L-tryptophan is carried out by overexpressing five optimized enzymes in or upstream of the shikimate pathway to make the aromatic compound intermediate, chorismate, and one optimized enzyme in the tryptophan pathway to make L-tryptophan. Further, tryptophan levels in the cell are enhanced with the expression of TAT2, a tryptophan importer, and L-tryptophan supplementation in the media up to 1% mass to volume. Finally, five enzymes are deleted in the cell to decrease off-pathway consumption of the L-tryptophan. The genetically modified host described herein can be the same host used for production of psilocybin and DMT as both production pathways use the precursor, L-tryptophan. A specific description of the strain with elevated L-tryptophan is disclosed in U.S. Patent Publication 2021/0147888.

Example 2 - Construction of *Saccharomyces cerevisiae* platform strains with synthesis of methyl donor

Construction of *Saccharomyces cerevisiae* platform strains with elevated SAMe production is carried out via expression of SAM2, a SAMe synthetase gene. The SAM2 gene is cloned from *Saccharomyces cerevisiae* using techniques known in the art. The gene can be cloned into vectors with the proper regulatory elements for gene expression (e.g. promoter, terminator) and the derived plasmid can be confirmed by DNA sequencing. As an alternative to expression from an episomal plasmid, the SAM2 gene is inserted into the recombinant host genome. Integration is achieved by a single cross-over insertion event of the plasmid. Strains with the integrated gene can be screened by rescue of auxotrophy and genome sequencing.

Example 3 – Construction of *Saccharomyces cerevisiae* platform strains with elevated methyl donor production

Construction of *Saccharomyces cerevisiae* platform strains with elevated SAMe production via expression of the ADK1, adenylate kinase gene. The ADK1 gene is cloned from *Saccharomyces cerevisiae* using techniques known in the art. The gene can be cloned into vectors with the proper regulatory elements for gene expression (e.g. promoter, terminator) and the derived plasmid can be confirmed by DNA sequencing. As an alternative to expression from an episomal plasmid, the ADK1 gene is inserted into the recombinant host genome. Integration is achieved by a single cross-over insertion event of the plasmid. Strains with the integrated gene can be screened by rescue of auxotrophy and genome sequencing.

Further SAM accumulation for methyl donor availability is achieved herein by engineering the homocysteine to methionine side of the methylation pathway. SAH is generated after methylation of serotonin and other intermediates to produce bufotenine and other compounds described herein. SAH is recycled back to methionine after methyl donation by TMG (trimethylglycine) or betaine. TMG is fed to the cells up to 1% (v/v) in the growth media. Two *Saccharomyces cerevisiae* genes, MHT1 and SAM4 encode the enzymes, Mht1 and Sam4, that are responsible for homocysteine re-methylation using TMG as a methyl donor. MHT1 and SAM4 are overexpressed from a high copy vector with a strong promoter.

Example 4 – Construction of *Saccharomyces cerevisiae* platform strains with enhanced uptake of methyl donor precursors.

Construction of *Saccharomyces cerevisiae* platform strains with elevated SAMe production is carried out via expression of MUP1, the methionine permease gene. The MUP1 gene is cloned from *Saccharomyces cerevisiae* using techniques known in the art. The gene can be cloned into vectors with the proper regulatory elements for gene expression (e.g. promoter, terminator) and the derived plasmid can be confirmed by DNA sequencing. As an alternative to expression from an episomal plasmid, the MUP1 gene is inserted into the recombinant host genome. Integration is achieved by a single cross-over insertion event of the plasmid. Strains with the integrated gene can be screened by rescue of auxotrophy and genome sequencing.

Example 5 – Construction of *Saccharomyces cerevisiae* platform strains with enhanced uptake of methyl donors.

Herein we describe a strategy to increase the SAM accumulation by increasing transport of exogenous SAM into the cell. SAM levels are increased by overexpressing the gene, SAM3. SAM3 encodes for the Sam3 protein, the predominant *Saccharomyces cerevisiae* transporter that is responsible for SAM import. SAM3 is expressed from a high-copy vector with a strong promoter and media is supplemented with 0.5 – 1.0 mM SAMe.

Example 6 – Construction of *Saccharomyces cerevisiae* platform strains with decreased off-pathway flux of methyl donors

Construction of *Saccharomyces cerevisiae* platform strains with elevated metabolic flux towards SAMe is carried out via deletion of SPE2 to reduce SAMe decarboxylation. Deletion of SPE2 is performed by replacement of the SPE2 gene with the URA3 cassette in the recombinant host. The SPE2 URA3 knockout fragment, carrying the marker cassette, URA3, and homologous sequence to the targeted gene, SPE2, can be generated by bipartite PCR amplification. The PCR product is transformed into a recombinant host and transformants can be selected on synthetic URA drop-out media. Further verification of the modification in said strain can be carried out by genome sequencing, then analyzed by the techniques disclosed in U.S. Patent 10,671,632.

Example 7 – Construction of *Saccharomyces cerevisiae* platform strains with decreased off-pathway flux of methyl donor precursors

Saccharomyces cerevisiae platform strains are constructed with elevated metabolic flux towards SAMe via deletion of GLC3 to reduce ATP consumption. Deletion of GLC3 is performed by replacement of the GLC3 gene with the URA3 cassette in the recombinant host. The GLC3 URA3 knockout fragment, carrying the marker cassette, URA3, and homologous sequence to the targeted gene, GLC3, can be generated by bipartite PCR amplification. The PCR product is transformed into a recombinant host and transformants can be selected on synthetic URA drop-out media. Further verification of the modification in said strain can be carried out by genome sequencing and analyzed by the techniques disclosed in U.S. Patent 10,671,632.

Example 8 – Construction of *Saccharomyces cerevisiae* platform strains with increased Tryptophan accumulation

Saccharomyces cerevisiae platform strains with accumulation of tryptophan are generated by deletion of BNA2. Bna2 is an enzyme necessary for *de novo* NAD+ production from

tryptophan. Deletion of BNA2 is performed by replacement of the BNA2 gene with the URA3 cassette in the recombinant host. The BNA2 URA3 knockout fragment, carrying the marker cassette, URA3, and homologous sequence to the targeted gene, BNA2, can be generated by bipartite PCR amplification. The PCR product is transformed into a recombinant host and transformants can be selected on synthetic URA drop-out media. Further verification of the modification in said strain can be carried out by genome sequencing and analyzed by the techniques disclosed in U.S. Patent 10,671,632.

Example 9 – Expression of recombinant L-tryptophan methyltransferases in a modified host organism

Construction of *Saccharomyces cerevisiae* NMTP, DMTP, and TMTP production strains is carried out via expression of the TrpM methyltransferase gene. The optimized TrpM gene is synthesized using DNA synthesis techniques known in the art. The optimized gene can be cloned into vectors with the proper regulatory elements for gene expression (e.g. promoter, terminator) and the derived plasmid can be confirmed by DNA sequencing. As an alternative to expression from an episomal plasmid, the optimized TrpM gene is inserted into the recombinant host genome. Integration is achieved by a single cross-over insertion event of the plasmid. Strains with the integrated gene can be screened by rescue of auxotrophy and genome sequencing.

Example 10 – Expression of recombinant aromatic amino acid decarboxylases in a modified host organism

Construction of *Saccharomyces cerevisiae* tryptamine production strains is carried out via expression of the AADC gene which encodes the enzyme that converts L-tryptophan to tryptamine. AACD also encodes the enzyme that converts 5HTP to serotonin. This specific conversion may be carried out by the same enzyme encoded by the AADC gene that converts L-tryptophan to tryptamine. It also may be carried out by the gene product of a novel AADC described herein. The optimized AADC gene is synthesized using DNA synthesis techniques known in the art. The optimized gene can be cloned into vectors with the proper regulatory elements for gene expression (e.g. promoter, terminator) and the derived plasmid can be confirmed by DNA sequencing. As an alternative to expression from an episomal plasmid, the optimized AADC gene is inserted into the recombinant host genome. Integration is achieved by a single

cross-over insertion event of the plasmid. Strains with the integrated gene can be screened by rescue of auxotrophy and genome sequencing.

Example 11 – Expression of recombinant L-tryptophan hydroxylases in a modified host organism

Construction of the *Saccharomyces cerevisiae* 5-HTP production strains is carried out via expression of the gene that encodes tryptophan hydroxylase. 5-HTP is a precursor compound for production of serotonin and variants described herein. Tryptophan hydroxylase activity is dependent on the availability of the BH4 cofactor. The optimized TPH, BH4 biosynthesis and BH4 regeneration genes are synthesized using DNA synthesis techniques known in the art. The optimized genes can be cloned into vectors with the proper regulatory elements for gene expression (e.g. promoter, terminator) and the derived plasmid can be confirmed by DNA sequencing. As an alternative to expression from an episomal plasmid, the optimized TPH, BH4 biosynthesis and BH4 regeneration genes are inserted into the recombinant host genome. Integration is achieved by a single cross-over insertion event of the plasmid. Strains with the integrated gene can be screened by rescue of auxotrophy and genome sequencing.

Example 12 – Expression of recombinant tryptamine 5-hydroxylases in a modified host organism

Construction of the *Saccharomyces cerevisiae* serotonin production strains is carried out via expression of the gene that encodes tryptamine 5-hydroxylase (T5H). 5-HT or serotonin is a precursor compound for production of bufotenine and variants described herein. T5H activity is dependent on the availability of the intermediate indole compound, tryptamine, production of which is disclosed in U.S. Patent Publication 2021/0147888 and further described herein.

T5H, as a cytochrome p450-containing monooxygenase, is also dependent on the cytochrome p450 reductase enzyme (CPR) for full activity. The CPR facilitates electron transfer from the NAD(P)H. The optimized T5H and CPR genes are synthesized using DNA synthesis techniques known in the art. The optimized genes can be cloned into vectors with the proper regulatory elements for gene expression (e.g. promoter, terminator) and the derived plasmid can be confirmed by DNA sequencing. As an alternative to expression from an episomal plasmid, the optimized T5H and CPR genes are inserted into the recombinant host genome. Integration is

achieved by a single cross-over insertion event of the plasmid. Strains with the integrated gene can be screened by rescue of auxotrophy and genome sequencing.

Example 13 – Expression of recombinant indolethylamine-N-methyltransferase (INMT) in a modified host organism

Construction of *Saccharomyces cerevisiae* DMT production strains is carried out via expression of the INMT gene which encodes the enzyme that methylates tryptamine to DMT. INMT also encodes the enzyme that converts serotonin to bufotenine. Finally, INMT encodes the enzyme that converts 5-MeO-tryptamine to 5-Meo-DMT. These unique conversions may be carried out by the same enzyme encoded by the INMT gene that converts tryptamine to DMT. It also may be carried out by the gene product of a novel INMT described herein. The optimized INMT gene is synthesized using DNA synthesis techniques known in the art. The optimized gene can be cloned into vectors with the proper regulatory elements for gene expression (e.g. promoter, terminator) and the derived plasmid can be confirmed by DNA sequencing. As an alternative to expression from an episomal plasmid, the optimized INMT gene is inserted into the recombinant host genome. Integration is achieved by a single cross-over insertion event of the plasmid. Strains with the integrated gene can be screened by rescue of auxotrophy and genome sequencing.

Example 14 – Expression of 5-hydroxyindole-O-methyltransferase (IOMT) or caffeic acid-O-methyltransferase (CaffOMT) in a modified host organism

Construction of *Saccharomyces cerevisiae* 5-MeO-DMT production strains is carried out via expression of the IOMT gene which encodes the enzyme that methylates the 5-OH in bufotenine, an intermediate derived from the INMT conversion of serotonin, described herein. The IOMT gene also encodes for the enzyme that converts serotonin to 5-MeO-tryptamine in the first intermediate to make melatonin. The IOMT enzyme also methylates the 5-OH of N-acetyl-serotonin to generate melatonin as an intermediate to make 5-MeO-tryptamine and further, 5-MeO-DMT. Alternatively, the enzyme that converts serotonin to 5-MeO-tryptamine can be carried out with a CaffOMT enzyme, an enzyme shared with the phenylpropanoid biosynthesis pathway. This same CaffOMT enzyme can also methylate N-acetyl-serotonin to generate melatonin. The optimized IOMT or CaffOMT gene is synthesized using DNA synthesis techniques known in the art. The optimized gene can be cloned into vectors with the proper regulatory elements for gene expression (e.g. promoter, terminator) and the derived plasmid can be confirmed by DNA

sequencing. As an alternative to expression from an episomal plasmid, the optimized IOMT or CaffOMT gene is inserted into the recombinant host genome. Integration is achieved by a single cross-over insertion event of the plasmid. Strains with the integrated gene can be screened by rescue of auxotrophy and genome sequencing.

Example 15 – Expression of recombinant N-acetyl transferase (NAT) and melatonin deacetylase (NAD) in a modified host organism

Construction of *Saccharomyces cerevisiae* 5-MeO-DMT production strains can alternatively be carried out via expression of the two more enzymes, NAT and NAD. NAT acetylation of serotonin produces the intermediate N-acetyl-serotonin or NAS. NAS is converted to melatonin with the IOMT (or CaffOMT) enzyme described herein. DAC deacetylases melatonin to 5-MeO-tryptamine which is converted to 5-MeO-DMT via the INMT enzyme described herein. The optimized NAT and DAC genes are synthesized using DNA synthesis techniques known in the art. The optimized gene can be cloned into vectors with the proper regulatory elements for gene expression (e.g. promoter, terminator) and the derived plasmid can be confirmed by DNA sequencing. As an alternative to expression from an episomal plasmid, the optimized NAT and DAC genes are inserted into the recombinant host genome. Integration is achieved by a single cross-over insertion event of the plasmid. Strains with the integrated gene(s) can be screened by rescue of auxotrophy and genome sequencing.

Example 16 – Construction of *Saccharomyces cerevisiae* platform strains with accumulation of serotonin

Serotonin is the precursor molecule for both bufotenine and 5-MeO-DMT. Construction of a *Saccharomyces cerevisiae* serotonin strain is carried out by expression of AADC and TPH or AADC and T5H genes described herein for the enzymatic conversion of L-tryptophan to serotonin. Exogenous serotonin is also fed to the strains to increase precursor levels at concentrations of 0.5 mM to 2 mM. Exogenous 5-HTP with expression of the AADC gene is fed to the cells as a mechanism to increase the serotonin precursor.

In order to accumulate serotonin in the cell and prevent off pathway conversion of serotonin to unwanted products, the endogenous Saccharomyces cerevisiae gene, PAA1 (YDR071C) is deleted. PAA1 is a polyamine acetyltransferase that would acetylate serotonin and use up valuable acetyl-CoA.

Example 17 – Method of Growth

Modified host cells that yield substituted indoles and tryptamine compounds, such as a bufotenine-producing strain herein, express engineered bufotenine biosynthesis genes and enzymes. More specifically, the bufotenine-producing strain herein is grown in a minimal, complete culture media containing yeast nitrogen base, amino acids, vitamins, ammonium sulfate, and a carbon source of glucose and galactose. The recombinant host cells are grown in 24-well plates or shake flasks in a volume range of 2 mL to 100 mL of media starting from an inoculation density of OD_{600nm}=1. Exogenous serotonin, melatonin, tryptamine, 5HTP, SAMe and TMG can be added to media to supplement the precursor pool for final compound production or support methyl donor accumulation.

Example 18 - Conversion of melatonin to 5-methoxy-tryptamine using a bio-based enzyme factory

Herein we describe a strategy for 5-methoxy-tryptamine (5-MT) production by recombinant expression and secretion of the melatonin deacetylase, DAC in BL21(DE3)pLysS *E. coli*. The DAC enzyme is cloned into a high-copy vector with key features that allow 1) tight induction by the lactose analog, **β**-D-thiogalactoside (IPTG) 2) an N-terminal secretory signal peptide [MKKTAIAIAVALAGFATVAQA (**SEQ ID NO:286,575)**] and 3) C-terminal fusion to a HIS tag for purification. *E. coli* cells harboring the NAD-expression vector are grown in M9 minimal media with 1% glucose for 18h at 37 °C and shaking at 300 rpm. Concentrated cell culture is diluted to an OD_{600} =1 in fresh M9 minimal media with 1% glucose and 0.2 mM IPTG. After a 3h induction at 18 °C and 300 rpm shaking, melatonin is added to the media at a final concentration of 1-2 mM. Cells are grown at room temperature for 48h, shaking at 300 rpm. Media is collected at 24h and 48h and analyzed by HPLC as described herein.

Alternatively, we describe a strategy for production of the compound, 5-methoxy-tryptamine (5-MT) by recombinant expression and secretion of the melatonin deacetylase, DAC in *Saccharomyces cerevisiae*. The DAC enzyme is cloned into a high-copy vector with key features that allow 1) tight induction by the sugar, galactose 2) an N-terminal alpha factor secretion leader sequence, [MEGVSLEKREAEA (SEQ ID NO:574] and 3) c-terminal fusion to a HIS tag for purification. *Saccharomyces cerevisiae* cells harboring the DAC-expression vector are grown in CM minimal media with 2% glucose for 18h at 30 degrees C and shaking at 300 rpm. Concentrated

cell culture is diluted to an OD_{600} =1 in fresh CM minimal media with 2% galactose. After 24h of induction at 30 degrees C and 300 rpm shaking, melatonin is added to the media at a final concentration of 1-2 mM. Cells are grown at 30 °C and 300 rpm shaking for 48h. Media is collected at 24h and 48h and analyzed by HPLC as described herein.

Alternatively, we describe a strategy for production of the compound, 5-methoxy-tryptamine (5-MT) by recombinant expression and secretion of the melatonin deacetylase (DAC) in *Komagataella phaffii* (*Pichia pastoris*). The DAC enzyme is cloned into a high-copy vector with key features that allow 1) induction by methanol with the AOX1 promoter and 2) a secretion signal consisting of the α-factor pro region. *K. phaffii* cells harboring the DAC enzyme are inoculated into 5 mL of YPD in a 15-mL culture tube. After a day of incubation at 30 °C with shaking at 220 rpm, an aliquot of the culture is diluted to an OD_{600}=0.2 in 5 mL of BMG (buffered minimal glycerol media) in a 15-mL culture tube. This tube is incubated under the same conditions as before. The following day, the culture is centrifuged at 3000 rpm (2000×*g*) for 5 min and resuspended in 25 mL BMM (buffered minimal methanol media) to attain an OD_{600} = 1.0. 25 mL of this culture is placed in a 250-mL baffled flask, and during this induction phase, the cells are incubated at 25 °C with shaking at 150 rpm to reduce loss of methanol. After 1 day of induction, an additional dose of 125 μL methanol is added (yielding a final concentration of 0.5%), melatonin is added to the media at a final concentration of 1-2 mM, and the incubation is continued for another day. After 48 h of induction, media is collected at at 24h and 48h and analyzed by HPLC as described herein.

Example 19 - Conversion of melatonin to 5-methoxy-NMT, 5-methoxy-DMT, and 5-methoxy-TMT using a bio-based enzyme factory

Herein we describe a strategy for 5-MeO-NMT, 5-MeO-DMT, and 5-MeO-TMT production by recombinant expression and secretion of the indolethylamine-*N*-methyltransferase (INMT) in in BL21(DE3)pLysS *E. coli*. The INMT enzyme is cloned into a high-copy vector with key features that allow 1) tight induction by the lactose analog, **β**-D-thiogalactoside (IPTG) 2) an N-terminal secretory signal peptide [MKKTAIAIAVALAGFATVAQA (SEQ ID NO:574)] and 3) C-terminal fusion to a HIS tag for purification. *E. coli* cells harboring the INMT-expression vector are grown in M9 minimal media with 1% glucose for 18h at 37 °C and shaking at 300 rpm. Concentrated cell culture is diluted to an OD_{600} =1 in fresh M9 minimal media with 1% glucose and 0.2 mM IPTG. After a 3h induction at 18 °C and 300 rpm shaking, melatonin is added to the

media at a final concentration of 1-2 mM and SAMe is added to the media at a final concentration of 1-2 mM. Cells are grown at room temperature for 48h, shaking at 300 rpm. Media is collected at 24h and 48h and analyzed by HPLC as described herein.

Alternatively, we describe a strategy for production of the compounds, 5-MeO-NMT, 5-MeO-DMT, and 5-MeO-TMT by recombinant expression and secretion of the indolethylamine-*N*-methyltransferase (INMT) in *Saccharomyces cerevisiae*. The INMT enzyme is cloned into a high-copy vector with key features that allow 1) tight induction by the sugar, galactose 2) an N-terminal alpha factor secretion leader sequence, [MEGVSLEKREAEA (SEQ ID NO:574)] and 3) c-terminal fusion to a HIS tag for purification. *Saccharomyces cerevisiae* cells harboring the INMT-expression vector are grown in CM minimal media with 2% glucose for 18h at 30 °C and shaking at 300 rpm. Concentrated cell culture is diluted to an OD_{600} =1 in fresh CM minimal media with 2% galactose. After 24h of induction at 30 °C and 300 rpm shaking, melatonin is added to the media at a final concentration of 1-2 mM and SAMe is added to the media at a final concentration of 1-2 mM. Cells are grown at 30 °C and 300 rpm shaking for 48h. Media is collected at 24h and 48h and analyzed by HPLC as described herein.

Alternatively, we describe a strategy for production of the compounds, 5-MeO-NMT, 5-MeO-DMT, and 5-MeO-TMT by recombinant expression and secretion of the indolethylamine-*N*-methyltransferase (INMT) in *Komagataella phaffii*. The INMT enzyme is cloned into a high-copy vector with key features that allow 1) induction by methanol with the AOX1 promoter and 2) a secretion signal consisting of the α-factor pro region. *K. phaffii* cells harboring the DAC enzyme are inoculated into 5 mL of YPD in a 15-mL culture tube. After a day of incubation at 30 °C with shaking at 220 rpm, an aliquot of the culture is diluted to an OD_{600}=0.2 in 5 mL of BMG (buffered minimal glycerol media) in a 15-mL culture tube. This tube is incubated under the same conditions as before. The following day, the culture is centrifuged at 3000 rpm (2000×*g*) for 5 min and resuspended in 25 mL BMM (buffered minimal methanol media) to attain an OD_{600} = 1.0. 25 mL of this culture is placed in a 250-mL baffled flask, and during this induction phase, the cells are incubated at 25 °C with shaking at 150 rpm to reduce loss of methanol. After 1 day of induction, an additional dose of 125 μL methanol is added (yielding a final concentration of 0.5%), melatonin is added to the media at a final concentration of 1-2 mM, SAMe is added to the media at a final concentration of 1-2 mM, and the incubation is continued for another day. After 48 h of induction, media is collected at 24h and 48h and analyzed by HPLC as described herein.

Example 20 - Conversion of tryptamine to NMT, DMT, and TMT using a bio-based enzyme factory

Herein we describe a strategy for NMT, DMT, and TMT production by recombinant expression and secretion of the indolethylamine-*N*-methyltransferase (INMT) in BL21(DE3)pLysS *E. coli*. The INMT enzyme is cloned into a high-copy vector with key features that allow 1) tight induction by the lactose analog, **β**-D-thiogalactoside (IPTG) 2) an N-terminal secretory signal peptide [MKKTAIAIAVALAGFATVAQA (SEQ ID NO:574)] and 3) C-terminal fusion to a HIS tag for purification. *E. coli* cells harboring the INMT-expression vector are grown in M9 minimal media with 1% glucose for 18h at 37 °C and shaking at 300 rpm. Concentrated cell culture is diluted to an OD_{600} =1 in fresh M9 minimal media with 1% glucose and 0.2 mM IPTG. After a 3h induction at 18 °C and 300 rpm shaking, tryptamine is added to the media at a final concentration of 1-2 mM and SAMe is added to the media at a final concentration of 1-2 mM. Cells are grown at room temperature for 48h, shaking at 300 rpm. Media is collected at 24h and 48h and analyzed by HPLC as described herein.

Alternatively, we describe a strategy for production of the compounds, NMT, DMT, and TMT by recombinant expression and secretion of the indolethylamine-*N*-methyltransferase (INMT) in *Saccharomyces cerevisiae*. The INMT enzyme is cloned into a high-copy vector with key features that allow 1) tight induction by the sugar, galactose 2) an N-terminal alpha factor secretion leader sequence, [MEGVSLEKREAEA (SEQ ID NO:574)] and 3) c-terminal fusion to a HIS tag for purification. *Saccharomyces cerevisiae* cells harboring the INMT-expression vector are grown in CM minimal media with 2% glucose for 18h at 30 °C and shaking at 300 rpm. Concentrated cell culture is diluted to an OD_{600} =1 in fresh CM minimal media with 2% galactose. After 24h of induction at 30 °C and 300 rpm shaking, tryptamine is added to the media at a final concentration of 1-2 mM and SAMe is added to the media at a final concentration of 1-2 mM. Cells are grown at 30 °C and 300 rpm shaking for 48h. Media is collected at 24h and 48h and analyzed by HPLC as described herein.

Alternatively, we describe a strategy for production of the compounds, NMT, DMT, and TMT by recombinant expression and secretion of the indolethylamine-*N*-methyltransferase (INMT) in *Komagataella phaffii*. The INMT enzyme is cloned into a high-copy vector with key features that allow 1) induction by methanol with the AOX1 promoter and 2) a secretion signal consisting of the α-factor pro region. *K. phaffii* cells harboring the DAC enzyme are inoculated into 5 mL of YPD in a 15-mL culture tube. After a day of incubation at 30 °C with shaking at 220

rpm, an aliquot of the culture is diluted to an OD_{600}=0.2 in 5 mL of BMG (buffered minimal glycerol media) in a 15-mL culture tube. This tube is incubated under the same conditions as before. The following day, the culture is centrifuged at 3000 rpm (2000×*g*) for 5 min and resuspended in 25 mL BMM (buffered minimal methanol media) to attain an OD_{600} = 1.0. 25 mL of this culture is placed in a 250-mL baffled flask, and during this induction phase, the cells are incubated at 25 °C with shaking at 150 rpm to reduce loss of methanol. After 1 day of induction, an additional dose of 125 µL methanol is added (yielding a final concentration of 0.5%), tryptamine is added to the media at a final concentration of 1-2 mM, SAMe is added to the media at a final concentration of 1-2 mM, and the incubation is continued for another day. After 48 h of induction, media is collected at at 24h and 48h and analyzed by HPLC as described herein.

Example 21 - Conversion of serotonin to 5-OH-NMT, 5-OH-DMT, and 5-OH-TMT using a bio-based enzyme factory

Herein we describe a strategy for 5-OH-NMT, 5-OH-DMT, and 5-OH-TMT production by recombinant expression and secretion of the indolethylamine-*N*-methyltransferase (INMT) in BL21(DE3)pLysS *E. coli*. The INMT enzyme is cloned into a high-copy vector with key features that allow 1) tight induction by the lactose analog, **β**-D-thiogalactoside (IPTG) 2) an N-terminal secretory signal peptide [MKKTAIAIAVALAGFATVAQA (SEQ ID NO:574)] and 3) C-terminal fusion to a HIS tag for purification. *E. coli* cells harboring the INMT-expression vector are grown in M9 minimal media with 1% glucose for 18h at 37 °C and shaking at 300 rpm. Concentrated cell culture is diluted to an OD_{600} =1 in fresh M9 minimal media with 1% glucose and 0.2 mM IPTG. After a 3h induction at 18 °C and 300 rpm shaking, serotonin is added to the media at a final concentration of 5 mM and SAMe is added to the media at a final concentration of 1-2 mM. Cells are grown at room temperature for 48h, shaking at 300 rpm. Media is collected at 24h and 48h and analyzed by HPLC as described herein.

Alternatively, we describe a strategy for production of the compounds, 5-OH-NMT, 5-OH-DMT, and 5-OH-TMT by recombinant expression and secretion of the indolethylamine-*N*-methyltransferase (INMT) in *Saccharomyces cerevisiae*. The INMT enzyme is cloned into a high-copy vector with key features that allow 1) tight induction by the sugar, galactose 2) an N-terminal alpha factor secretion leader sequence, [MEGVSLEKREAEA (SEQ ID NO:574)] and 3) c-terminal fusion to a HIS tag for purification. *Saccharomyces cerevisiae* cells harboring the INMT-expression vector are grown in CM minimal media with 2% glucose for 18h at 30 °C and shaking

at 300 rpm. Concentrated cell culture is diluted to an OD_{600} =1 in fresh CM minimal media with 2% galactose. After 24h of induction at 30 °C and 300 rpm shaking, serotonin is added to the media at a final concentration of 5 mM and SAMe is added to the media at a final concentration of 1-2 mM. Cells are grown at 30 °C and 300 rpm shaking for 48h. Media is collected at 24h and 48h and analyzed by HPLC as described herein.

Alternatively, we describe a strategy for production of the compounds, 5-OH-NMT, 5-OH-DMT, and 5-OH-TMT by recombinant expression and secretion of the indolethylamine-*N*-methyltransferase (INMT) in *Komagataella phaffii*. The INMT enzyme is cloned into a high-copy vector with key features that allow 1) induction by methanol with the AOX1 promoter and 2) a secretion signal consisting of the α-factor pro region. *K. phaffii* cells harboring the DAC enzyme are inoculated into 5 mL of YPD in a 15-mL culture tube. After a day of incubation at 30 °C with shaking at 220 rpm, an aliquot of the culture is diluted to an OD_{600}=0.2 in 5 mL of BMG (buffered minimal glycerol media) in a 15-mL culture tube. This tube is incubated under the same conditions as before. The following day, the culture is centrifuged at 3000 rpm (2000×*g*) for 5 min and resuspended in 25 mL BMM (buffered minimal methanol media) to attain an OD_{600} = 1.0. 25 mL of this culture is placed in a 250-mL baffled flask, and during this induction phase, the cells are incubated at 25 °C with shaking at 150 rpm to reduce loss of methanol. After 1 day of induction, an additional dose of 125 μL methanol is added (yielding a final concentration of 0.5%), serotonin is added to the media at a final concentration of 5 mM, SAMe is added to the media at a final concentration of 1-2 mM and the incubation is continued for another day. After 48 h of induction, media is collected at 24h and 48h and analyzed by HPLC as herein.

Example 22 - Purification of recombinant INMT enzyme to use for *in vitro* reactions

The INMT enzyme is cloned into a high-copy vector with key features that allow 1) tight induction by the lactose analog, **β**-D-thiogalactoside (IPTG) 2) an N-terminal secretory signal peptide [MKKTAIAIAVALAGFATVAQA (SEQ ID NO:574] and 3) C-terminal fusion to a HIS tag for purification. *E. coli* cells harboring the INMT-expression vector are grown in M9 minimal media with 1% glucose for 18h at 37 °C and shaking at 300 rpm. Concentrated cell culture is diluted to an OD_{600} =1 in fresh M9 minimal media with 1% glucose and 0.2 mM IPTG and grown for 48h.

The supernatant containing the recombinant proteins is equilibrated in binding buffer (50 mM sodium phosphate, 0.5 M NaCl, 20 mM imidazole, 10% glycerol, 10 mM 2-mercaptoethanol,

1 mM PMSF, Complete EDTA-free (1 tablet/100 ml), 20 mM 1-phenyl-2- thio-urea; pH 7.4) and centrifuged at 2,500g for 5 min to remove insoluble matter. Then the supernatant is filtered through a 0.45 μm filter (Millipore, MA, USA) and applied onto a HisTrap HP column (GE Healthcare Bioscience). The recombinant proteins are eluted with a step gradient of imidazole (concentrations of 5, 20, 40 and 300 mM). Fractions are analyzed by SDS-PAGE and stored at -80 °C before use.

Example 23 - *In vitro* reactions with purified INMT enzyme or INMT lysate

Purified INMT protein is resuspended in activity buffer [100 mM sodium phosphate buffer, pH 6.55, PMSF (1mM), EDTA-free protease inhibitor] cocktail at working concentration (Roche, Meylan, France) for use in *in vitro* assays. 0.1 mg/mL of INMT protein is added to a tube with a final volume of 600 uL per sample and added to 100 mM sodium phosphate buffer (pH 7.5), 2 mM tryptamine, serotonin, or melatonin, 2 mM S-adenosylmethionine, and 5 mM $MgCl_2$.

Alternatively, 0.1 mg/mL BSA protein-equivalent of INMT lysate is used in the same reaction. INMT lysate is derived from *E. coli* cells harboring the INMT-expression vector. They are grown in M9 minimal media with 1% glucose for 18h at 37 °C and shaking at 300 rpm. Concentrated cell culture is diluted to an OD_{600} =1 in fresh M9 minimal media with 1% glucose and 0.2 mM IPTG and grown for 48h. Cell pellets are resuspended in 100 mM sodium phosphate buffer at pH 7.5 and lysed using sonication. After lysis, samples are pelleted by centrifugation (16,000g, 4 °C, 20 min) and supernatant containing INMT is harvested.

Example 24 – Method of Growth

Modified host cells that yield substituted indoles and tryptamine compounds, such as the DMTP-producing strain herein, express engineered DMTP biosynthesis genes and enzymes. More specifically, the DMTP-producing strain herein is grown in a minimal, complete culture media containing yeast nitrogen base, amino acids, vitamins, ammonium sulfate, and a carbon source of glucose and galactose. The recombinant host cells are grown in 24-well plates or shake flasks in a volume range of 2 mL to 100 mL of media starting from an inoculation density of OD600nm=1. Exogenous L-tryptophan and L-methionine up to 1% can be added to media to supplement the precursor pool for DMTP production. Exogenous L-tryptophan can be taken up by strains expressing the TAT2 L-tryptophan importer protein. Exogenous L-methionine can be taken up by strains expressing the MUP1 L-methionine permease protein. The strains herein can be harvested

during a fermentation period ranging from 12 hours onward from the start of pathway enzyme induction.

Example 25 – Detection of Isolated Product

To identify fermentation-derived tryptamine, DMTP, NMT, DMT, and all other products of a recombinant host expressing an engineered biosynthetic pathway for substituted indoles (see Fig. 11), an Agilent 1100 series liquid chromatography (LC) system equipped with a HILIC column (Primesep 100, SIELC, Wheeling, IL USA) is used. A gradient is used of mobile phase A (ultraviolet (UV) grade H_2O+0.2% TFA) and mobile phase B (UV grade acetonitrile+0.2% TFA). Column temperature is set at 40 °C. Compound absorbance is measured at 270 nm using a diode array detector (DAD) and spectral analysis from 200nm to 400nm wavelengths. A secondary wavelength of 315 nm is used to selectively detect 4-hydroxy and 4-methoxy substituted indoles. A 0.1 milligram (mg)/milliliter (mL) analytical standard is made from certified reference material for each of the substituted indoles (Cayman Chemical Company, USA). Each sample is prepared by diluting fermentation biomass from a recombinant host expressing the engineered biosynthesis pathway 1:1 in 100% ethanol and filtered in 0.2 um nanofilter vials. The retention time and UV-visible absorption spectrum (i.e., spectral fingerprints) of the samples are compared to the analytical standard retention time and UV-visible spectra (i.e. spectral fingerprint) when identifying the substituted indole compounds. For example, FIG. 17 depicts the detection of tryptamine isolated from a fermentation with a recombinant host expressing enzymes for L-tryptophan to tryptamine conversion. Detection and isolation is depicted by retention time matching of fermentation derived tryptamine with a tryptamine analytical standard, along with a matching UV-vis spectral fingerprint (i.e. spectral fingerprint) of the fermentation derived tryptamine with the tryptamine analytical standard. This also corroborates that the recombinant host is able to successfully convert L-tryptophan to tryptamine, which further validates that the systems and methods herein direct molecules into tryptamine pathways.

As another example, Fig. 18 depicts the production, detection, and isolation of the substituted indole, DMTP, from a fermentation of a modified recombinant host expressing the DMTP pathway. The retention time and UV-vis spectral absorption (i.e. spectral fingerprint) of the DMTP isolated from fermentation is identical to the retention time and UV-vis spectral absorption (i.e. spectral fingerprint) of the DMTP analytical standard. FIG. 18 also depicts a negative control fermentation from a host strain not expressing the TrpM enzyme or the DMTP

pathway, and this strain does not produce DMTP. The modified host strain expressing the TrpM and DMTP producing pathway, highlighted in FIG. 18, is able to produce DMTP. FIG. 26 depicts the production, isolation, and identification of the dimethylated tryptamine, DMT, derived from a fermentation of a recombinant host expressing the pathway for substituted indoles and tryptamines. The fermentation derived DMT is identified by matching retention times with the DMT analytical standard. Spectral library identification of the fermentation derived DMT matches the UV-vis absorption spectrum (i.e. spectral fingerprint) of the DMT analytical standard.

Example 26 - Synthetic preparation of substituted indoles from recombinant host products

In some instances, it may be preferable, for reasons of either cost or product quality, to utilize recombinant host pathways to accomplish the first part of a substituted indole synthesis and complete the remaining steps synthetically. The tryptamine, as obtained from the recombinant organism, is of a particular grade such that methylations with robust methylating agents selectively leads to mono- or di-methylation. One of ordinary skill in the art would appreciate this as improvement when a primary amine subjected to robust methylating agents as a mixture of alkylation products are not obtained, while obviating the need for tedious chromatography.

One example would be the production of tryptamine via fermentation of a recombinant host organism, followed by N,N-methylation via methylation chemistry to yield DMT. In one embodiment, the reaction of tryptamine would proceed with a 30-fold molar excess of dimethyl carbonate (DMC) under an inert atmosphere, utilizing a Y-type zeolite catalyst (see Fig. 25. This reaction is carried out at 190°C for 6 hours in a pressurized reactor vessel or autoclave; another embodiment would utilize a microwave oven for 15-60 minutes. The DMT product is recovered from the volatile DMC reactant via distillation. Another embodiment of the combined biosynthetic and chemical synthesis route is the production of tryptamine via recombinant host organism, followed by its reaction with DMC in the presence of the catalyst: 1,8-Diazabicyclo[5.4.0]undec-7-one (DBU). This catalyst can be used in a thermally heated reactor system at 90 °C for 6-24 hours or used in a pressurized microwave reactor system for less than one hour. Another embodiment of the combined biosynthetic and chemical synthesis route is the production of tryptamine via recombinant host organism, followed by its methylation to DMT using dimethylsulfoxide (DMSO). The catalysts for this system is acetic acid, and the reaction is carried out in a thermally heated reactor at 150 °C for 6-15 hours.

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http://parts.igem.org/Yeast.

In view of the above, it will be seen that several objectives of the invention are achieved and other advantages attained.

As various changes could be made in the above methods and compositions without departing from the scope of the invention, it is intended that all matter contained in the above description and shown in the accompanying drawings shall be interpreted as illustrative and not in a limiting sense.

All references cited in this specification, including but not limited to patent publications and non-patent literature, and references cited therein, are hereby incorporated by reference. The discussion of the references herein is intended merely to summarize the assertions made by the authors and no admission is made that any reference constitutes prior art. Applicants reserve the right to challenge the accuracy and pertinence of the cited references.

As used herein, in particular embodiments, the terms "about" or "approximately" when preceding a numerical value indicates the value plus or minus a range of 10%. Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the disclosure. That the upper and lower limits of these smaller ranges can independently be included in the smaller ranges is also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure.

The indefinite articles "a" and "an," as used herein in the specification and in the embodiments, unless clearly indicated to the contrary, should be understood to mean "at least one."

The phrase "and/or," as used herein in the specification and in the embodiments, should be understood to mean "either or both" of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with "and/or" should be construed in the same fashion, i.e., "one or more" of the elements so conjoined. Other elements can optionally be present other than the elements specifically identified by the "and/or" clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to "A and/or B", when used in conjunction with open-ended language such as "comprising" can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment, to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the embodiments, "or" should be understood to have the same meaning as "and/or" as defined above. For example, when separating items in a list, "or" or "and/or" shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as "only one of" or "exactly one of," or, when used in the embodiments, "consisting of," will refer to the inclusion of exactly one element of a number or list of elements. In general, the term "or" as used herein shall only be interpreted as indicating exclusive alternatives (i.e. "one or the other but not both") when preceded by terms of exclusivity, such as "either," "one of," "only one of," or "exactly one of." "Consisting essentially of," when used in the embodiments, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the embodiments, the phrase "at least one," in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements can optionally be present other than the elements specifically identified within the list of elements to which the phrase "at least one" refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, "at least one of A and B" (or,

equivalently, "at least one of A or B," or, equivalently "at least one of A and/or B") can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

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FDCSAMWVKKRTDLTGAFRLDPTYLKHSHQDSGLITDYRHWQIPLGRRFRSLKMWFVFRMYGVKGLQAYIRKHVQLS
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Seq. ID No: 295
>AADC_5
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Seq. ID No: 296
>AADC_6
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KFVGCPMPCGVQITRLEHINALSRNVEYLASRDATIMGSRNGHAPLFLWYTLNRKGYRGFQKEVQKCLRNAHYLKGR
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Seq. ID No: 297
>AADC_7
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Seq. ID No: 298
>AADC_8
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MALLAARTKVTRRLQAENPDLSEAEIISRMVAYSSDQAHSSVERAGLISGVRMKKIPSDENFTARGEALKKALEEDK
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Seq. ID No: 299
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Seq. ID No: 300
>AADC_10
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Seq. ID No: 301
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LKLWMVMRTYGTAKLQEHIRSDVAMAKMFEDSVRADNRFEVVVPRNFALVCFRIKARGDMTEEDADEVNRLLMENLN
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Seq. ID No: 302
>AADC_12
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Seq. ID No: 303
>AADC_13
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Seq. ID No: 304
>AADC_14
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Seq. ID No: 305
>AADC_15
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DALRAAIQADADAGLVPFFLCTTVGTTSTAAVDPVALLCEVTKDYGMWVHIDAAYAGNACICPEFRHMINGVENADS
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Seq. ID No: 306

>AADC_16
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Seq. ID No: 307
>AADC_17
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Seq. ID No: 308
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Seq. ID No: 309
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Seq. ID No: 310
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Seq. ID No: 311
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Seq. ID No: 312
>AADC_22

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Seq. ID No: 313
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Seq. ID No: 314
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Seq. ID No: 315
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Seq. ID No: 316
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Seq. ID No: 317
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Seq. ID No: 318
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Seq. ID No: 319
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Seq. ID No: 320
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Seq. ID No: 321
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Seq. ID No: 322
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Seq. ID No: 323
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Seq. ID No: 324
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Seq. ID No: 325
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Seq. ID No: 326
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Seq. ID No: 327
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Seq. ID No: 328
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Seq. ID No: 329
>BH4syn_5
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Seq. ID No: 330
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Seq. ID No: 331
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Seq. ID No: 332
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Seq. ID No: 333
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VKRR

Seq. ID No: 334
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Seq. ID No: 335
>DAC_6
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Seq. ID No: 336
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Seq. ID No: 337
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Seq. ID No: 338
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SKGC

Seq. ID No: 339
>DAC_10

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Seq. ID No: 340
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Seq. ID No: 341
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PHPHPERPDRLRAIAASLSTAGIFPGKCHPIAAREITQEELLKVHSLEHVEAVEVTRQMLSSYFTPDTYANEHSAQA
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ISAGFDAARGDPLGGCDVTPAGYACMTHMLSALAGGKMLVILEGGYNLRSISSSATAVIKVLLGEKPKCQFENIEPS
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Seq. ID No: 342
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Seq. ID No: 343
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Seq. ID No: 344
>DAC_15
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Seq. ID No: 345
>DAC_16

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SAGFDAAAGDELGECLVSPAGYAHMTHMLAGLAGGRMVVALEGGYNLDSISQSALAVTKVLLGEPPDELPPLKANEE
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DIFESKTLVIFVHEFGNLRLELESSTTCDVHLERSYLIDFSKELVGWVKSEGYSLLDANLYPKPSTTPTPNLRHKTM
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Seq. ID No: 346
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Seq. ID No: 347
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Seq. ID No: 348
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Seq. ID No: 349
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Seq. ID No: 350
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Seq. ID No: 351
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SLKYGNTLFQQDAGENVFDPEDWYFLSEWNYVLGRHEASLVPRSKDIKLGRPLDKIVVGKHEKVRFGCSYKYDSEER
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Seq. ID No: 352
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Seq. ID No: 353
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Seq. ID No: 354
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Seq. ID No: 355
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Seq. ID No: 356
>IDI1_for_fusion
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Seq. ID No: 357
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Seq. ID No: 358
>INMT_2
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Seq. ID No: 359
>INMT_3
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Seq. ID No: 360
>INMT_4
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Seq. ID No: 361
>INMT_5
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VDCLLSCLCLEGACKDFESYITALKNMTTLLKIGGYLVMTGDLGNTYYMVGDVKFSGLNLNENFLREAITGAGYVIE
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Seq. ID No: 362
>INMT_6
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PPADCVLSTLCLDAACPDLPTYCRALRNLGSLLKPGGFLVIMDALKSSYYMIGEQKFSSLPLGREAVEAAVKEAGYT
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Seq. ID No: 363
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Seq. ID No: 364
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Seq. ID No: 365
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CLITALCLETACTDIDTYFCSLRNITTLLKPGGHLVLIGVLGNSFYKVGEKKFYCLSLDEQTVRNAVIDAGYSIKDL
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Seq. ID No: 366
>INMT_10
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LTDCLLTASCLSATCKTFTDFKMSLKIIVSLIKPGGHLILIDYLRASYYWVGEVKLPILSLDEHVVREAVVESGCKI
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Seq. ID No: 367
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Seq. ID No: 368
>INMT_12
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PRADCVTCIWGLETISRDHDEWKTTLRKISDLVKLGGHVLIHADINASYFKIGEDKYHLFNFDDAFLRKTLTDGGFA
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Seq. ID No: 369
>INMT_13
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Seq. ID No: 370
>INMT_14
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FTDYKGSIFIAAHKEH

Seq. ID No: 371
>INMT_15
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ADCLLTTVCLEAACKNYESYGTALKNLSNLLKPKGHLLMAGDLGANYYEVGSNKVFSLPVNEKFLKKVISESGYEII
QLVSFGKPENADFDTSDYEGFYFVHAQKV

Seq. ID No: 372
>INMT_16
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PADGLISSLCLEAACPTPQACRDALRHLRTLLRPGGHLVLSGGFETTFFMVGDKRFSTLPLNEKFLREALQEAGFII
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Seq. ID No: 373
>INMT_17
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AEAVTAGGPSAFERTQKWPYYEYMGKNQRLGTLFDNAMAQHSVILVTKMLERFKGFDGVQRLVDVGGGTGSTLGMIT
SKYKHMTGINYDLPHVIAQGLPLPGVEHVAGDMYESIPTGDAVLLQWITLMLNDDEFVKILSNCHNALPKDGKVIVV
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Seq. ID No: 374
>INMT_18
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SICRVGDDLVPKSKSPHLSPILGTKN

Seq. ID No: 375
>INMT_19
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LEEDLKYKFCR

Seq. ID No: 376
>INMT_20
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SELIEIRRVDVDDPASSSGTVESSGGSRMEDSSQGQCDVVELASLEMKEFCDVGVTCKGGTDKNQRRYDEAKHSNVA
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Seq. ID No: 377
>INMT_21
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Seq. ID No: 378
>INMT_22
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WSFADTRLPDTMARIQSISPKHALYPCMPPKNTLVQAFPGPATHLVSTKLIETLHGIEGVSYTTTSLNSFFVEARQN
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RKVGMGLRDVK

Seq. ID No: 379
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FVAESITPSLKTWEEVVNNICSTLKPSGTLIMTAIQGASFYCVENHRYPAIAVTPEDVIRVLSYQGFDVDNLLMRHI
PAEITDISAKDYKGYQGMLFVKATR

Seq. ID No: 380
>INMT_24
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PPADCLLSTLCLDAACPDLPAYRTALRNLGSLLKPGGFLVMVDALKSSYYMIGEQKFSSLSLDREAVRDAVEEAGYT
IEQFEVISQSYSSTTSNNEGLFSLVGRKPVGSE

Seq. ID No: 381
>INMT_25
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FEKLYLTDYFQGNLDEIKKWLNSENDAFDWTPYIRFVCDLENNGSTPREKKEKIRRCVSLMKCDVNLSNPLHPHSLP
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Seq. ID No: 382
>INMT_26
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GKSDNWEKKEETLRRKVTKVLKCDALAEKPYDPVPMPEADCLISCLCLEVACKDLEDF

Seq. ID No: 383
>INMT_27
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DKIITSDYLEQNRAELEKWLKKDPSAFDWTPIIKFVCELEGNRNYEKKAEKLRNKVKEVLKCDALKRNPFDPIVLQP
ADCLLTCLCLEAPCEDMKSYFNVLKNFKDLIKPGGHLVILSVLDATFYYVGDKYFSSMTTRKEELEQALKEAGFEIE
KAVYTTRKDRSQMDIADYQGFYYIHARNPK

Seq. ID No: 384
>INMT_28
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PPADCVLTLLAMECACCSLDAYRAALCNLASLLKPGGHLVTTVTLGISSYMVGKREFSCVVLEKEGVEQAVLDAGFD
IQQFLHIPKCYSATIAANNGVCFIVARKKPAP

Seq. ID No: 385
>INMT_29
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FSDITLSDFTDRNREELQKWLRKDAGAFDWTPVLKFACELEGNSSHWQEKAEKLRATVKRVLKCDVNLGKPLAPVEL
PAADCVLTLLAMECACCSLAAYRAALCNLGSLLKPGGHLVTSITLQISSYMVGKHQFSCLYITKEEVERAILDAGFD
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Seq. ID No: 386
>INMT_30
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WCEEITLFERSPANVRYLKSQVDSYDANWDQFWDALCAHEAYNSLGTDPRERFGKVVWVEQGDLFDLARYERRWSMG
TMFFVAESMTTSYQEFMLGVERFMRALSPGAPFAAAFMEHSKGYHAGEHFFPACDVGESEVRASLEGFAGDFKVQRL
ESAAQLRDGYSGMIVAY

Seq. ID No: 387
>INMT_31

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DITVTWYTNRELQELQKWLNKDPGAFDWSSTVKHVWELEGKRGMLEEKEEKLRGMIRQVLLCDVSKKNPLEPVTLPK
ADCLISTVCLEAACRNYDSYRTALKNLSTLLKPGGHLLLAGDLGANYYEVGSNKVFSLPVNETFLRKAVNESGYVIN
KLVSFGKPEDAGYDTSDYEGFYFIHAQKC

Seq. ID No: 388
>IOMT_1
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LVKAYGMDLFELAAKNDEISGKFNNTMGNQTAIIMKKVLEIYKGFEGINQLVDVGGGLGINLKLIVSKYPQIKGINF
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Seq. ID No: 389
>IOMT_2
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VIDDAPQFPGVEHVGGDMFASVPTAEAILLKLILHDWGDEHCVKLLKNCCKALPEDGKVVVVEAILPEGIDHSYASA
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Seq. ID No: 390
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Seq. ID No: 391
>IOMT_4
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YGMSAFEYHGTDPRFNRVFNEGMKNHSIIITKKLLEVYKGFEGLGTIVDVGGGVGATVGAITAAYPAIKGINFDLPH
VISEAQPFPGVTHVGGDMFQKVPSGDAILMKWILHDWSDEHCATLLKNCYDALPAHGKVVLVECILPVNPEATPKAQ
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Seq. ID No: 392
>IOMT_5
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NKAHGMTSFEYHGKDARFNKVFNKGMADHSTITMKKILETYTGFEGLKSLVDVGGGTGAVISMIVSKYPSIKGFNFD
LPHVIEEAPSYPGVEHVGGDMFVSVPKADAVFMKWICHDWSDEHCVKFLKNCYDALPENGKVIVAECILPVAPDSSL
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Seq. ID No: 393
>IOMT_6
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KAYGMSAFEYHGKDLRFNTVFNNGMSNHSTITMKKILETYKGFEGLTSLVDVGGGIGATLKMIVSKYPDLKGINFDL

PHVIEEATSHPGIDHVGGDMFVSVPKGDAIFMKWICHDWSDEHCVKFLKNCYEALPEDGKVILAECILPETPDSSLS
TKQVVHVDCIMLAHNPGGKERTEKEFEALAKGSGFKGINVACNAFGVYVIELLKKM

Seq. ID No: 394
>IOMT_7
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QQIHFIEGDLFNDPIPEADLFIMARIIHDWTEEKCLELLRKIYQSCRPGGGVLLVEVLLNEDKSGPLMSQLFSLNML
VQTEGRERTPSEYTKLLTDSGFRDIQVKITGKIYDA

Seq. ID No: 395
>IOMT_8
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ISSKDLFEALYRSEEDTLAFMYFMNSTWSICGKYVVQAFDLSEFHTIYDLGGCTGALAKQLVSTYKESTVTIMDMPN
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Seq. ID No: 396
>IOMT_9
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Seq. ID No: 397
>IOMT_10
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GRQFKASELRAMLLEAGFAGAEVVSSPLTPYSLVVGTKGEANPVASKPEAAAAAESESITPLMDVLFSAQHSAVLIV
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Seq. ID No: 398
>IOMT_11
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SSPTWPVDDSAFDVVLMSYISGSVPEPIIGSLYANAMKALRPGGRLLVHDFMVNDSLDGPALGALWGLQHVTVNADG
LGLCPKEVIARMGAAGFDTSKCEAMEMIHGMTKLIVGHKG

Seq. ID No: 399
>IOMT_12
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TTFMHFMDSTWNLCGKDIVQAFDLSEFHTVYDLGGCSGSLAKQLVSTYKESTVTIMDLPKVVQAAKKHFVTDKEQQI
HFLEGDFFNDPLPEADLFIVARIIHDWTEETCIKLLKKMYHSCRPGGGVVIVELLLNEDKSGPVISQVYSLYMLVQA
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Seq. ID No: 400
>IOMT_13

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QFMQGLQDVWRLEGATVLAAFDLSPFPLICDLGGGSGALAKACVSLYPGCRAIVFDIPGVVQIAKRHFSASEDERIS
FHEGDFFKDALPEADLYILARVLHDWTDAKCSHLLQRVYRACRTGGGILVIESLLDTDGRGPLTTLLYSLNMLVQTE
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Seq. ID No: 401
>IOMT_14
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GERFALSALTEGFLVREAETSFCGVLASARGQAAAFSYDFFKASLLKGESQLFGGRDLFDNNAQDPEHCEIFTRAMH
SKSKGPAQAWVEKIDLSAHACLLDVGGGSGVHAISALARWPNLNAVVFDLPPVCAIADTFIERYQMTARAQTHGGDI
WYTDYPFADAHFYSDIFHDWPLERCRFLARKSFDALPSGGRIILHEMLFNAQKTGPRNVAAYNANMLLWTQGQQLSE
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Seq. ID No: 402
>IOMT_15
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GMTVYEYNKTDARMKRVFSQAMNNYSTIINRKLVEMYMGFHDIAFLVDVGGGVGTTIRAITSKYPHIKGINFDLPHV
IADAPQCPGVQHVAGDMFRNVPSGDAIILKWMLHNWTDEHCTTLLRNCYDALPPHGKVFIVENILPLKPDATSRGQQ
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Seq. ID No: 403
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QRAVTMRAALCVMQRVAPFADGNTPLRLLDAGGGPGWVAIALAQAHAGVHGCVFDWPETVAVAAANIAHAQLSDRLE
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Seq. ID No: 404
>IOMT_17
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RVERLYGVGPVCKFLTKNEDGVSIAALCLMNQDKVLMESWYYLKDAVLEGGIPFNKAHGMTSFEYHGKDLRFNKVFN
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SVPKGDAIFMKWICHDWSDEHCLKFLKNCYEALPDNGKVIVAECILPVAPDTSLAAKGVIHIDVIMLAHNPGGKERT
EKEFEALAKGAGFQGFRVMCCAFNTYIMEFIKKL

Seq. ID No: 405
>IOMT_18
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PKADAIFMKWILHDWSDEHCVKLLKNCYAAIPNDGNVIVVDAVLPKMPEVSTSMRCTSQLDVLMLTQNPGGKERTEE
EFMALATKAGFKGIRYQECFVNTFWLMEFFK

Seq. ID No: 406
>IOMT_19
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RNPEFNKIFNDAMACTTKILMGVLLAEYKDGFGSIGSLVDVGGGTGEMIAEIIKQHPHIKGMN

Seq. ID No: 407

>IOMT_20
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KAYGMTAFEYHGTDPRFNKVFNNGMSNHSTITMKKLLENYKGFEGVSTLVDVGGGTGATLNMIISKHPTIKGINFDL
PHVIEDAPTYPGVEHIGGDMFVSVPKGDAIFMKWICHDWSDEHCLRFLKNCYAALADHGKVIVCEYILPVAPETNHA
ARTVFHVDAIMLAHNPGGKERTEQEFESLAKGAGFEGFRVAFFF

Seq. ID No: 408
>IOMT_21
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HGTTLFEYLGTDMRFSKVFNDAMSSSSTITMKKMLENYNGFDGLSTLVDVGGGTGETLNMIIAKYPTIRGINFDLPH
VINDAPNYDGVEHVVGDMFVSVPKGDAIFMKWICHDWSDKLCLKLLKNCYTALPNHGKVIVCECILPVAPETSHSAR
VASNLDMHMLAYCRGGKERTEQEFEALAKGAGFESFRVVCSAYDLKLYMC

Seq. ID No: 409
>IOMT_22
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QDQAVLASWSKLKEAVLEGGIPFNKVHGMDAFEYQGTNPRFNEIFNKAMYDQSTYIIKKIVRRYKGFENIQRLVDVG
GGLGHTLRVITSNYPSIKGINFDLPHVIQHAPTIPGVEHVGGDMFESIPHGDAIFMKCILHDWSDEHCLKTLKNCYK
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Seq. ID No: 410
>IOMT_23
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AQGAGTYVIRHVLHDWDDAQVVTILKHVRNAMLGSPASTPPKLLLVEMMLNETSSRFTRTTSLQLLSLNGGITRTEV
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Seq. ID No: 411
>IOMT_24
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TMDTGKPLDKLISEPDRKYDDTGFPALISHFMDVDQKCGAVGWDVLKDPVLGHSCDLTETIFSRAFNTKSKYWDFFD
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CKMFADKDAVSIASARYYSEAPEPLLPNYTHKNVISDSDMYVFQMMFHYNSQEHTYLSLKSLLDASGWRLVRLRAID
PRNDYFQSIECKILA

Seq. ID No: 412
>IOMT_25
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LDTGKPYEELVKNPGEKLIGTNGIAAYISRSTDESVKSSGFLYEALTYSSSEKVPLPPSPFNLAFNTELHIFSWLAQ
KGNEHRLQRFGIAFDGFDKMLPVNGVTKGYRWGSLPKGSIVVDVGGGVGSESMKIAKTFPDLKVIIQDAEGVVANGV
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LHKLVAVPQPE

Seq. ID No: 413
>IOMT_26
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EAGQHFDNAMTNTSVMTNQALPTAYNFGAFKTLVDVGGGQGSFLSALFHQWDHLHGILFDLPPVIESAEQQNLLSGF
EKRTTLAAGDFFKAVPDGADAYLLKTIIHDWDDASAIAILKTCRRAMNHDSKLLLVELIVPSGNAPSLSKILDLEML
AVFGGVERTEAEYRSLLLSAGLKLTRIYDSPCPWSVIEAIPV

Seq. ID No: 414
>IOMT_27
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IYVLKRKGLKGSFFDWMKADAKRRENYHYAMIALGPVMGSLSILHHYPWNDVATVCDVGASVGSVSIPLSKAHPHLK
ITDQDLPEVLEAARSVWEKEAFEALREKRVEFLTLDFFKEAPVPGKDVYYLRHIIHDWPDAEAAVILRNISKAMEPH
SRLLIHNYVIAGANRRPDEEQRAPEPMLPNFGAGDSRKYRQDLNMWILHNAKERTVDDQITLA

Seq. ID No: 415
>IOMT_28
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AGDGSYTNSALSSTFLVSGSPQSQRCMLLYLAGTTYGCWAHLAAGVREGRNQYSRAVGISAEDPFSAIYRSEPERLL
FMRGLQETWSLCGGRVLTAFDLSRFRVICDLGGGSGALAQEAARLYPGSSVCVFDLPDVIAAARTHFLSPGARPSVR
FVAGDFFRSRLPRADLFILARVLHDWADGACVELLGRLHRACRPGGALLLVEAVLAKGGAGPLRSLLLSLNMMLQAE
GWERQASDYRNLATRAGFPRLQLRRPGGPYHAMLARRGPRPGIITGVGSNTTGTGSFVTGIRRDVPGARSDAAGTGS
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Seq. ID No: 416
>IOMT_29
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SRLSGKEHSVFTGVVIVHCRSKEENHLETDIIDFYEETKVKFADLSEDLLWEYIDSGEPMDKAGGYGIQSLGGMLVE
SVHGDFLNVVGFPLNHFCRKLTEIYYPPPKQAICRVKHDSIPYVESFENLSDVETDCTSTSKACEAKKAVQDGVCKA
DGSGSAVLQNGIEERPVHCAQQLSKITQLLDGFKASQTLFAASKLKVFDKLKDKGALKAMEIAEKINASVHGTERLL
DACVALGLLEKTHQVYSNTELANTYLVSDGAFSIHEYITYSSDHLWSHFTHLDSAVVEGGGQHQTAVKKACDNRNGS
EVKERFMRAMHCMLKITARDLVTAFDLSKYSSACDLGGCTGALAHELVWTYPEMKVNVFDLPEVIKHTSQFQPESFD
SSRVTFSSGNFMEDTLPEADLYILSRVLHDLPEGKLNHLLKKVSEACCPGRSALLVAEIVLDEDKKESRGLLQSLSM
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Seq. ID No: 417
>IOMT_30
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PYEKAHGVPIYEHISSDTESVKGFSQAMDSISSFIMKKVLENYSGFKGLGSLVDVGGGSGFALNMITSEYPSISCIN
FDLPHVVQEAPYHPGVKHVGGDMFLDIPSADAIMIKEVLHNWGNEDCVKVLKNCYEALPKGGKVIVVSHVMPEVVGS
SNAAAKYVCQLDVMMLLFGGGKERTEKEFKALGKAAGFSGFQLICFAAYNAVAVMEFYK

Seq. ID No: 418
>IOMT_31

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LLASYDVVKCQMEDKDGKYERRYSAAPVGKWLTPNEDGVSMAALALMNQDKVLMESWYYLKDAVLDGGIPFNKAYGM
TAFEYHGTDPRFNRVFNEGMKNHSVIITKKLLEFYTGFDESVSTLVDVGGGIGATLHAITSHHSHIRGVNFDLPHVI
SEAPPFPGVQHVGGDMFKSVPAGDAILMKWILHDWSDAHCATLLKNCYDALPEKGGKVIVVECVLPVTTDAVPKAQG
VFHVDMIMLAHNPGGRERYEREFRDLAKAAGFSGFKATYIYANAWAIEFIK

Seq. ID No: 419
>IOMT_32
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MKNNEAFFSNTDVSDVYLVQLSPRSLYHMMMYYSQTLYKCWHFLPDAIREGKSQYERAFGVSSGDIFKALYRSEEEM
VTFMHHMDSVWNICGKDIIAAFDLSSFNEVCDLGGCSGGLAKQLLSIYPSSSVTILDLPEVVQTAKKHFITDADCNI
AFLQGNFFNDPIPEADLYIMARIIHDWTQEKCLQLLNKIYKSCRPGGGVLLVEVLLNEDRSGPLTSQLYSLNMLVQT
EGRERSPCEYTKLLAHSGFRDIQVKATGKIYDAILGRK

Seq. ID No: 420
>MUP1
MSEGRTFLSQLNVFNKENYQFSSSTTKKEVSNSTVDADNGASDFEAGQQFATELDQGEKQLGILSCIGLICNRMLGT
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WAAGNSINTAIMFLTAADTEVTKWNQRGIGVAVVFFAFLINSLNVKIGLYLQNILGIFKIGIVLFISITGWVALGGG
LKDGYQSHNFRNAFEGTETATAYGIVNALYSVIWSFVGYSNVNYALGEVKNPVRTLKIAGPTSMVFLAIIYIFVNIA
YFAVVPKDKLISSKLILAADFFDIVFGGQAKRAAAALVGLSALGNVLSVIFSQGRIIQQLGREGVLPFSNFFASSKP
FNSPMVGLFQHFIVCTVTILAPPPGDAYLLVQNLISYPMNIINFAISAGLLWIYWQRRQGKIEWNPPIKAGVFVTGF
FTLSNLYLIIAPYVPPSNGESVYSSMPYWIHCVIAWGIFFFGGVYYVVWAQLLPRWGHYKLVSKDVLGEDGFWRVKI
AKVYDDTIGDVDTQEDGVIETNIIEHYKSEQEKSL

Seq. ID No: 421
>NAT_1
MAPIEEEEPLPEELVLLERTLADGSTEQIIFSSAGDVNVYDLQALCDKVGWPRRPLTKIAASLRNSYLVATLHSVTM
PSKAEGEERKQLIGMARATSDHAFNATIWDVLVDPSYQGQGLGKALMEKVIRTLLQRDISNITLFADNKVVDFYKNL
GFEADPQGIKGMFWYPRF

Seq. ID No: 422
>NAT_2
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PELSLGWFVEGRLVAFIIGSLWDEERLTQESLALHRPRGHSAHLHALAVHRSFRQQGKGSVLLWRYLHHVGAQPAVR
RAVLMCEDALVPFYQRFGFHPAGPCAIVVGSLTFTEMHCSLRGHAALRRNSDR

Seq. ID No: 423
>NAT_3
MTSDVGADEHATTEAGGGRLQAGGHSSAEEASERCPPAAAPPSGMKGAADCGPQDSSARDDVSFIPYKDETDMPGIV
ELIEKDLSEPYSIFTYRYFINNWPELCFLTMRGDSCVGAIVCKLDVHRCRNTNRGYIAMLAVEKGLRGKGIGSTLVR
LCLDKMREMGADECVLETEVTNKGALGLYRNMGFVKEKRLHKYYLNGNDAFRLKFLFKLPEGFDRGEGCLGPLCEVP
PVTT

Seq. ID No: 424
>NAT_4
MVSIRPATVDDLLAMQACNLCCLPENYQMKYYFYHMLSWPQLLYVAEDYGKKIVGYVLAKMEEDSSEVHGHITSLAV
LRSHRKLGLASKLMRAAMAAMEETFGAEHVSLHVRVTNRAAFTLYSETLGFEINDVEHKYYADKEDAYDMRKMFETG
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Seq. ID No: 425
>NAT_5
MLPRPPVGAAKEGHLTLFYRELRWLCPGTRFYFVVRDPAENVRSIADRLALGPEGLRRPPRIVARADLGWREVLNMS
YAGVREESALGTLVGRWNLMARLYLDAPKGAMALVRYEDLVAEATWEAEVRRVAAAETLDLRERVLWPGRPDLCTLP
GDESALHFGAVAAGKVLGVISVFLSPEPGGRAQFRKFAVDPEVQGRGLGRRLLEQAVAAAREAGAGSLFCHARADQQ
GFYERRGLHVVGEPFEKYGGKPYVEMEVPFQ

Seq. ID No: 426
>NAT_6
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GCLAPYRRLGIGTKMLNHVLNICEKDGTFDNIYLHVQISNESAIDFYRKFGFEIIETKKNYYKRIEPADAHVLQKNL
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Seq. ID No: 427
>NAT_7
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PELSLGWFVEGRLVAFIIGSLWDEERLTQESLTLHRPGGRTAHLHALAVHHSFRQQGKGSVLLWRYLQHAGGQPAVR
RAVLMCEDALVPFYQRFGFHPAGPCAVVVGSLTFTEMHCSLRGHAALRRNSDR

Seq. ID No: 428
>NAT_8
MSSGGVIVDLHRNSTNWAKVVDDIVKLERKIFPKHESLARSFDEELGKKNTGLIYMEVDGEVVGYAMYSWPSSMYAC
VTKLAVKENCRRQGHGEALLKAAIKKCRTRNVHRISLHVDPLRNPAISLYKKFGFQVDNLIDGYYSSDRNAYRMYLD
FDAD

Seq. ID No: 429
>NAT_9
MDERVVVELKKSLADYPKVLEELVRIEKKVFPKHESLSRSFDEELGKKNSGLLYICSNGEVAGYVMYSWPSALLAVI
TKLAVKEKYRRQGYGEALLRAAIQKCKTRNIQRISLHVDPSRTPAANLYKKLGFRIDSLVEKYYAADRDAYRMYLDF
DADV

Seq. ID No: 430
>NAT_10
MMEGAQEDEETEEKAEFDASEIEYVSYGGEHHLPLIMCLVDHELSEPYSIFTYRYFVYLWPQLCFLAFHKGRCVGTV
VCKMGDHRHTFRGYIAMLVVIKPYRGRGIATELVTRAIKVMMESGCDEVTLEAEVTNNGALALYGRLGFIRAKRLFR
YYLNGVDAFRLKLLFPRSEMHPSLHLLADQDGHDDQIAMEGEA

Seq. ID No: 431
>NAT_11
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LGVLAPYRGLGIGTELLNHVLEQCSKQNISEIYLHVQTNNDDAINFYKKFGFEVTETIQNYYTNITPPDCYVVSKRL
EAQPKK

Seq. ID No: 432
>NAT_12
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SISVLRSYRRLGLAKKLMLLSQEAMSSIYKASYVSLHVRKSNKAAIALYKDTLGFEVAKVEKKYYGDGEDALSMRLS
LKNP

Seq. ID No: 433
>NAT_13

MSDFQVAPLTARELARVRDLHAKLLPVQYPVSFFIHLLVIPSRACYVAYSHGSPVGFISAALHNPTRCFISGDSEVS
PRLEILTLGVLPAFQHRGLARRLIMSLVNAFKQDPATPILIYANVSTTNTRALQFYERMGILVSSDIITNLYRTLSY
GSRDAYLVVGAL

Seq. ID No: 434
>NAT_14
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LSLGWFEEGCLVAFIIGSLWDKERLTQESLTLHRPGGRTAHLHVLAVHRTFRQQGKGSVLLWRYLHHLGSQPAVRRA
VLMCENALVPFYEKFGFQAMGPCAITMGSLTFTELQCSLRCHTFLRRNSGC

Seq. ID No: 435
>NAT_15
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PHDERTGAPNGLERLQDLEEEEAVLASRMGACSLHPGDGSIRYVRYESELQMPDIMRLITKDLSEPYSIYTYRYFIH
NWPQLCFLAMVEEECVGAIVCKLDMHKKMFRRGYIAMLAVDSKYRRKGIGTNLVKKAIYAMVEGDCDEVVLETEITN
KSALKLYENLGFVRDKRLFRYYLNGVDALRLKLWLR

Seq. ID No: 436
>NAT_16
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EEFKELVGHDPAAPNAVIMSVVVDPAEQGKGYSKLLMQHFIARMKAMDKKTIHLMCKEAHVPLYARMGYRYTRPSAS
DHGGMAWHEMVMEL

Seq. ID No: 437
>NAT_17
MEGLHSEWEVGAELKALGAVPKPFIGSHVSGKLIQRLKQDLRQSWDRGQSQARPTCTLPQPLPAPLGSSVPSASAQT
QVSRLVPVAPPQPDPAMSVLNAVPFMRPIHLRSPRQQRRHTLPASEFRCLSPEDAVSVFEIEREAFISVSGDCPLHL
NEVRHFLTLCPELSLGWFEEGRLVAFIIGSLWNQDRLSQDALTLHKAEGSSVHIHVLAVHRTFRQQGKGSILLWRYL
QYLRCLPFARRAVLMCEDFLVPFYSKCGFKAVGPCDITVGPLTFIEMQCPVQGHAFMRRNSGC

Seq. ID No: 438
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DSSLNVAVERLKFEYDSLKMIGSEPSIAGEDALISVPSVYHHDNIKHVLIMQDVGTMSTLRDFMGASPPPPTDMAAL
IGCQLATFIAGLHNWGRNNESARAGLSANAYGRTVMDLCGYQTVVPNATASGILDPLLSTAMAALAERDKTSEETAI
MGDFWALNVLVDIDMSASGEKALKNIWIVDWEACRYGSPAVDVATFAGDCYLISRIHNETATDAMRRNFLGTYVALA
KVDPMEVVIGMGTMWIMWTKYQEDIGEAEKRERVAKGVEYIHKGWERSREWLPVSLAQELIA

Seq. ID No: 439
>PSIK_2
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RNNLPAHTRLSANTVGRTAMKKLCYETIVPKAAKSGVVDPLLPMVVAALSEEAMTNDETLVMGDFWTANVLIDVQES
HTGEQVLKKLWVIDWESCRYGNPATDIASFAGDSYLVSRFQDHGLGEALRHSFLETYAALAKVDPLRVALGLGAHWI
MWTDDLGQGGEAETRECVDKGLEYIQRAWDQSAEWVSLSLAKELVVL

Seq. ID No: 440
>PSIK_3
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WGRDPQTSNHVSFDQNQQGRTISGYVTYGRLVSTLTGKDNIPALSDPPLDIAQSKLDTISALSSEKIHAINTSHQTL

TMGDFWPGNIMVRLNPAGDSLERAYVLDWEVAKPGVAGLDIGQFCAEMHSLRRFSPACDASATTVLDAFLKTYRDAA
GVDVGVAKDAMVHVGAHLVAWTPRVPWGSKERTREVVEEGVGYLVEGYAATQEWLRGSLVGRLV

Seq. ID No: 441
>PSIK_4
MEIEWCDLDLTSESRPPTHKYTYFATALMPFDLTTRDGVRMYLTAYLALDVMSVERLSGGYCNFSWRAKLESPYEGQ
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LRNNALPPVPMAEKISNELAAFIAGIHNWGRNNQEARANLSQNLVGRTAIRKLCYETLVPKAEKSGVDDPLLQQVAA
ALSEEVMNSEETLVMGDFWTANVMVDIQETGAGVRSLRKIWVIDWEGCRYGSPAADIASFAGDSYLVARFHHHDLGE
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Seq. ID No: 442
>PSIM_1
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ALDISVTVGNHFLTAEVKDPTWTRAWRRSSKVTSKITPFSFSGQFSDPPERLLVLQLLVDESQTSEDILLSFQSL

Seq. ID No: 443
>PSIM_2
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NLVAADPKGSIFGPLESKHDTTFEFTMCNPPFYSSEEDIAQSAAVKKLASNAVCTGAAVEMITPGGEAAFVVRMVRE
SLALKMRCQWYTSMLGKMSSLTEIVGLLRENSIDNYAITEFVQGKTRRWAIAWSFGHVRLLDSLARLSGGPLQSLMP
TRNTCRRSFAVPRMVLHKHLILVLDGIEGTSQTPMSIPVGAGDADGLYGLQISASRDTWSRAARRKRQHGAMDISLD
NDEVGMKCLIKVLSVEEAREGAEAVVLECTWVYGHERALFESFWGHVCRKIGEANG

Seq. ID No: 444
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RQAKTTTILGGGHEGVSSELFTTGGELLFLSQMVEESFLYKDKVGLVSSYFFVLKCIVEILRLLGQHKIQETIASKL
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Seq. ID No: 445
>PSIM_4
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Seq. ID No: 446
>PSIM_5
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Seq. ID No: 447
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Seq. ID No: 448
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Seq. ID No: 449
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Seq. ID No: 450
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Seq. ID No: 451
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Seq. ID No: 452
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Seq. ID No: 453
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Seq. ID No: 454
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Seq. ID No: 455
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TSIDGPILVPIFEATEEYEYEFTMCNPPFYDGAADMQTSDAAKGFGFGVGAPHSGTVIEMSTEGGESAFVAQMVRES
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Seq. ID No: 456
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LETSIDGPILVPIFEATEEYEYEFTMCNPPFYDGAADMQTSDAAKGFGFGVGAPHSGTVIEMSTEGGESAFVAQMVR
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Seq. ID No: 457
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DARRDGSLPWLRPDTKTQVTVEYEDDNGRWVPKRIDTVVISAQHADEISTADLRTQLQKDIVEKVIPKDMLDENTKY
FIQPSGRFVIGGPQGDAGLTGRKIIVDAYGGASSVGGGAFSGKDYSKVDRSAAYAARWVAKSLVAAGLCKRVQVQFS
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Seq. ID No: 458
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Seq. ID No: 459
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Seq. ID No: 460
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Seq. ID No: 461
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Seq. ID No: 462
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VAPFRGFVQERVALARRTIEKNGPDALADWGRITLFYGCRKSTEDFLYKDEWPQYTEELKGKFTMHSAFSREPPYKA

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Seq. ID No: 463
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DVWS

Seq. ID No: 464
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Seq. ID No: 465
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ARPSFAQQCLQNMDPNIDTAYQERVIKDTSAAMYGGGSDTSVSFLATFVLAMLQYPSVQRRAQVELDSVLGRDRLPT
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Seq. ID No: 466
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Seq. ID No: 467
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Seq. ID No: 468
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Seq. ID No: 469
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Seq. ID No: 470
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IRRSNFRLPTSPKIPVIMVGPGTGVAPFRSFVQERVCSAQKTCDKVNQSPAEALQDWGNIWLFYGCRRSNEDFLYKD
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Seq. ID No: 471
>T5H-CPR_2
SSSSSSLFSTTDVILFSLIVGVMTYWFLFRKKKEEVPEFTKIQTTTSSVKDRSFVEKMKKTGRNIIVFYGSQTGTAE
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VFALGNKTYEHFNAMGKYVDKRLEQLGAQRIFDLGLGDDDGNLEEDFITWREQFWPAVCEHFGVEATGEESSIRQYE
LMVHTDMDMAKVYTGEMGRLKSYENQKPPFDAKNPFLAVVTTNRKLNQGTERHLMHLELDISDSKIRYESGDHVAVY
PANDSALVNQLGEILGADLDIIMSLNNLDEESNKKHPFPCPTSYRTALTYYLDITNPPRTNVLYELAQYASEPTEHE
QLRKMASSSGEGKELYLRWVLEARRHILAILQDYPSLRPPIDHLCELLPRLQARYYSIASSSKVHPNSVHICAVAVE

YETKTGRINKGVATSWLRAKEPAGENGGRALVPMYVRKSQFRLPFKATTPVIMVGPGTGVAPFIGFIQERAWLRQQG
KEVGETLLYYGCRRSDEDYLYREELAGFHKDGALTQLNVAFSREQPQKVYVQHLLKKDKEHLWKLIHEGGAHIYVCG
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Seq. ID No: 472
>T5H-CPR_3
SSSSSSEAVAEEVSLFSMTDMILFSLIVGLLTYWFLFRKKKEEVPEFTKIQTLTSSVRESSFVEKMKKTGRNIIVFY
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DLSGVKFAVFGLGNKTYEHFNAMGKYVDKRLEQLGAQRIFELGLGDDDGNLEEDFITWREQFWPAVCEHFGVEATGE
ESSIRQYELVVHTDIDAAKVYMGEMGRLKSYENQKPPFDAKNPFLAAVTTNRKLNQGTERHLMHLELDISDSKIRYE
SGDHVAVYPANDSALVNQLGKILGADLDVVMSLNNLDEESNKKHPFPCPTSYRTALTYYLDITNPPRTNVLYELAQY
ASEPSEQELLRKMASSSGEGKELYLSWVVEARRHILAILQDCPSLRPPIDHLCELLPRLQARYYSIASSSKVHPNSV
HICAVVVEYETKAGRINKGVATNWLRAKEPAGENGGRALVPMFVRKSQFRLPFKATTPVIMVGPGTGVAPFIGFIQE
RAWLRQQGKEVGETLLYYGCRRSDEDYLYREELAQFHRDGALTQLNVAFSREQSHKVYVQHLLKQDREHLWKLIEGG
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Seq. ID No: 473
>T5H-CPR_4
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KLLRVEDNSSAAQSPYTAAIPQYRIVLTKPEDATHINKSFSLSNGHVVYDSQHPCRANVAVRRELHTPASDRSCIHL
EFDIAGTSLTYETGDHVGVYAENSTETVEEAEKLLDYSPDTYFSIYADQEDGTPLFGGSLPPPFPPCTVRVALARYA
DLLNSPKKSVLLALAAHASDPKEAERLRHLASPAGKKEYSQWIIASQRSLLEVISEFPSAKPPLGVFFAAIAPRLQP
RYYSISSSPRMAPTRIHVTCSLVHGQSPTGRIHKGVCSTWMKNSTPSEEESEECSWAPIFVRQSNFKLPADPTVPII
MVGPGTGLAPFRGFLQERLALKETGVELGRAILFFGCRNRQMDFIYEDELNNFTESGALSELVVAFSREGPTKEYVQ
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Seq. ID No: 474
>T5H-CPR_5
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ISSSPKMAPSRIHVTCALVYGPTPTGRIHQGVCSTWMKNAIPSEYSEECSWAPIYVRQSNFKLPADPTTPIIMIGPG
TGLAPFRGFLQERLALKQSGVELGNSVLFFGCRNRNMDYIYEDELQNFIQEGALSELIVAFSREGPAKEYVQHKMTE
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Seq. ID No: 475
>T5H-CPR_6
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KTYEHYNAIGRKVDTLLTDMGATKIGERGEGDDDKSMEEDYLEWKDGMWKAFSEAMGVEEGQGGDTPDFAVTELDSH
PPEKVYLGELSARALTRTKGIYDGKNPYPSAVKHSRELFQAGAERNCVHAELDIEGSGITYQHGDHVGVWPSNPDVE
VDRMLYVLGLYGKKDAVINIDSLDPALAKVPFPVPTTYATVLRHYIDICAVAGRQMLGVLSKFAPHPKAEAFLKSLN
SDKEEYSNIVTNGCFKLGEVLQLAAGDDIKLCPTPDNTTAWAIPFDIIVSSIPRLQPRFYSISSSPKLYPNAIHLTA
VVLKYDSIPNRLVESRFVYGVATNFLLNVKYAANGETAPFIAEPVISEPAHVSLPKYAIEGPRGAHIEDNIYKIPIH
VRRSTFRLPANPKIPVIMVGPGTGVAPFRGFVQERVALAKRSIEKNGPDALADWGSITLFYGCRKSNEDFLYKEEWP

QYAEELKGKFKMHCAFSREPPYKPDGSKIYVQDLIWEERETIAKAILEGKAYVYICGDAKAMSRAVEDTLARILGEA
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Seq. ID No: 476
>T5H-CPR_7
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ELVVHEDMDVAKVYTGEMGRLKSYENQKPPFDAKNPFLAAVTANRKLNQGTERHLMHLELDISDSKIRYESGDHVAV
YPANDSALVNQIGEILGADLDVIMSLNNLDEESNKKHPFPCPTTYRTALTYYLDITNPPRTNVLYELAQYASEPSEQ
EHLHKMASSSGEGKELYLSWVVEARRHILAILQDYPSLRPPIDHLCELLPRLQARYYSIASSSKVHPNSVHICAVAV
EYEAKSGRVNKGVATSWLRAKEPAGENGGRALVPMFVRKSQFRLPFKSTTPVIMVGPGTGIAPFMGFIQERAWLREQ
GKEVGETLLYYGCRRSDEDYLYREELARFHKDGALTQLNVAFSREQAHKVYVQHLLKRDREHLWKLIHEGGAHIYVC
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Seq. ID No: 477
>T5H-CPR_8
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DDVNGASTPYTAAIPEYRVVIHDASATSCEDKSVLENGNTSIDIHHPCRVNVAVQKELHKPESDRSCIHLEFDISGT
GIIYETGDHVGVYAENFEENVEEAGKLLGQPLDLLFSIHADNEDGAPLGSSLAPPFPGPCTLRTALSHYADLLNPPR
KAALIALAAHASEPSEAERLKYLSSPEGKDEYSQWIVGSQRSLLEVMAEFPSARPPLGVFFAAIAPRLQPRYYSISS
SPRFALSRVHVTCALVYGPTPTGRIHKGVCSTWMKNAVPLEKSHDSSWAPVFIRTSNFKLPTDPSIPIIMVGPGTGL
APFRGFLQERMALKEDGAQLGPALLFFGCRNRRMDFIYEDELNYFVEQGVISELIVAFSREGPQKEYVQHKMMDKAA
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Seq. ID No: 478
>T5H-CPR_9
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IGDLLNIDLNEMISLDAVDPDSLTKHPFPCPCTYRHAFMHFVDITGPPGKSLLSACLDSVTNPEESQFVQLLISDSE
DGKKLYSKWILEDHRGLVDVLQDLKSFRPPADLLLELLNPLKPRLYSISSSSLVHTNRIHITASIVKYKTNSGRIFK
GLATNWLKSLQSTNTERHLKIPVAIHTSNFNLPRSRTIPVIMIASGTGLAPFRAFIQERLKVAHDKVGKTGQMVLFF
GCRHENKDFIYSDELKQACSTGLLEMFTAFSRDCLDGNKVYVQHKVLEMGNMVWKLLDECYAYIYVCGDAAGMVRDV
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Seq. ID No: 479
>T5H-CPR_10
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ATYGDGEPTDNAARFYKWFEEGKERGDCFKNLQYGVFGLGNRQYEHFNKIAKVVDELLAEQGGQRLVPVGLGDDDQC
IEDDFAAWRELVWPELDKLLLDGDDATATTPYTAAVLEYRVVTYDKSNFDNDLTNTNGHANGHVIVDAQHPVRANVA
VRKELHTPASDRSCTHLEFDISCTGLTYETGDHVGVYCENFVETVEEAERLLNISPDTFFSIHTDKEDGTPLGGSSL
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LVVAFSREGPNKEYVQHKMTQKADDIWNMISQGGYVYVCGDAKGMARDVHRTLHTIAQDQGSLDSSKAESFVKNLQT
TGRYLRDVW

Seq. ID No: 480
>T5H-CPR_11
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KWFTEGNERGVWLNDFEYAVFGLGNRQYEHFNKVAKVVDEILTEQGGKRLVPVGLGDDDQCIEDDFNAWKEALWPEL
DRLLRDENDASTGTTYTAAIPEYRVEFIKPEEAAHLERNFSLANGHAVHDAQHPCQANVAVRRELHTPASDRSCTHL
EFDIAGTGLTYETGDHVGVYTENCPEVVEEAERLLGYSPDTFFTIHADKEDGTPLSGSSLAPPFPSPITVRNALARY
ADLLNSPKKTSLVALATYASDPAEADRLRFLASAAGKDEYAQWVVASQRSLLEVMAEFPSAKPPLGVFFAAVAPRLQ
PRYYSISSSPSMAATRIHVTCALVHETTPAGRVHKGVCSTWIKNAVPSEESKDCSWAPIFVRQSNFKLPADPSVPII
MIGPGTGLAPFRGFLQERLAQKESGAELGPSVFFFGCRNSKMDFIYEDELNNFLEQGALSELVLAFSRQGPTKEYVQ
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Seq. ID No: 481
>T5H-CPR_12
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LEEDFITWREQFWPAVCEHFGVEATGEDSSIRQYELVVHTDENMNKVYTGEMGRLKSYETQKPPFDAKNPFLANATV
NRKLNEGGDRHFMHLELDITGSKIRYESGDHVAVYPANDAALVNKLGEILGADLETVISLNNLDEESNKKHPFPCPT
TYRTALTYYLDITNPPRTNVLYELAQYATDSKEQENLRKMASSAQDGKALYLSWVVESRRNILAILEDIPSLRPPLD
HLCELLPRLQARYYSIASSSKVHPNSIHVCAVLVEYETKTGRENKGVATNWLKNKQPSDNGHKSSVPMFVRKSQFRL
PFKPSTPVIMIGPGTGIAPFMGFIQEREWLKQQGKDVGETVLYYGCRHEHEDFLYINELKRYHKEGVLTQLNVAFSR
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DVWS

Seq. ID No: 482
>T5H_1
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Seq. ID No: 483
>T5H_2
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GQETTANQLAFTIMELARNPEILEKAQAEVDEVIGVKRDIEYDDLGKLQYLSQVLKESLRLYPTAPGTSRAIEEETI
IEGFRIPPKVPLMFNSYIMGRMQQFYPDPLTFNPDRFHPDAPKPYYSYFPFSLGPRSCIGQVFAQMEAKVIMAKLLQ
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Seq. ID No: 484
>T5H_3
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GHFNPYRYVVVSVTNVICAICFGRRYDHNHQELLSLVNLNNNFGEVVGSGNPADFIPILRYLPNPSLNAFKDLNEKF
YSFMQKMVKEHYKTFEKGHIRDITDSLIEHCQEKQLDENANVQLSDEKIINIVLDLFGAGFDTVTTAISWSLMYLVM

NPRVQRKIQEELDTVIGRSRRPRLSDRSHLPYMEAFILETFRHSSLVPFTIPHSTTRDTSLKGFYIPKGRCVFVNQW
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Seq. ID No: 485
>T5H_4
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WKAIEANTAKVGRILERSEGTLDMFNLMNRFTLDTIGRIGFSKDIGSLEDPSSPFLRSFDRAQQILILRFWTNPAWK
VLRWLGVGWERELKEHLGRLDGYARGIVRELRQKAEAGQDDSFVGLFMKEEQAAPAARSPELQEKFMRDLVLNFLIA
GRDTTAQCISWTLFELTQHPAVAAKARQEVLDVCGEGPVTFEHLKSLQYVRAILDEGLRLHPSVPYDGKLCLGKDTL
PDGTVVPAGCIIQYIPYAQGRCKDIWGEDACSFRPERWLEMPRRPSSFAFAAFNAGPRECLGRRLAEAEMAALVSTV
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Seq. ID No: 486
>T5H_5
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YKQVRDIVLNPARKRTRALDGWISGFARHYPNLPVFFNTGSNMHTTFRQIFFANFTKTDFVLRALEDEGAGLAKMAA
PILQRWLAGSFRESKSGEGNLYMVEPVAPLILFLLFEVEVESIPPELLTAFSDVVTVGASYFLLPPHSPYWLLSGKV
KAIALLKDFLLEHCNAARPESLKGRAVDWRSLAAQMPAFLPKDECRCPCSGTPAVDPVDAYLEVISVMVCVAGVTGT
TNGFTSVIRKFADVPVGPTKSRWPSAPVQWRPDADDMVRLYRRDPLGFILEALRLGTPVAGTHQVLEEELTCPFLHK
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KPQ

Seq. ID No: 487
>T5H_6
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VGVMSDCVNVMLDKWERLVPDKKPVELFHYISLMTLDTIMKCAFSYQSNCQNDSENEYIKAVYELSYLVDHRTRCPP
YHNDFIFYWSPHGFRLRRALKTAHQHTEKVIKLRKESLKQETELEKIKQKRRLDFLDILLCARDENGQGLSDEDLRA
EVDTFMFEGHDTTASGVSWTFYCLAKNPEHQEKCREEIRQVLGDRRTVEWEDLSKLPYTTMCIKESMRLYPPVPEVA
RELKEPITFCDGRSVPKGSIVFLCIYAINRCPGIWEDPEVFDPLRFSPENSSTRHSHAFLPFSAGGRNCIGQNFAMN
EMKIATALTLQRFELQLETKREPVKRAQLVLRSMNGIYINLKKIHSDKTKII

Seq. ID No: 488
>T5H_7
MGLWTFMTGALILLLILVVLCFLLYCGYIYYMHMKYDHIPGPPRDSFFFGHSPTIMKLMRNNVIMYDTFLEWVKTYG
PVVRVNLSCSTIVFVISQEAVKEFLMSPKYTKDNFYECVETLFGVRYMGKGLLTDRDYEHWHKQRRIMDPAFSRNYL
IGLMGTFNEIAEDLVDILGDKADGKCQVGMHDMMGRVTLDIIAKVAFGMELNSLHDDQTPFTRAITTVMRGMVETRN
PLARYIPGKQALIRDIKESLKFLRKTGRECILQRRKAIQDGEDIPRDILTQILKGAETEGDCSLENLIDNFVTFFIA
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IEGVRVPPGTTLMFNAYIMGRMEKYYHDPLVFNPDRFHPDAPKISYAYLPFSLGPRSCIGQVFAQMEAKVVMAKLLQ
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Seq. ID No: 489
>T5H_8
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SQNIINAQINKLIRQLRIRQGKPVNVLQLMRCTVWRIIWNLIFGKECQLTDKQISDTLDDISSNNLQNQLFQIRQLL
PRFCVNIFKHSQFARKLFEIEEIIYKYKTVRQLIDNNVGEMHNSDSLLGQLINDLKLNLTKNDISRLSFEFMAAGTD

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TPHKSMYIFVTKLNGNRRTSI

Seq. ID No: 490
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QYEPIQEREARIATLHLGRLGQADGGGGDFQRVLHRYAASVIMQVTYDYQVQTLDDPLVRSVAQRGHALAMCIRPGA
SVLDRYPLLEHVPTWLNPWKQEGLRLRKLEQELYLGQVIKVRERMERGECAPCFVSKMTERQQELGLTDLDVAGMSG
SLFGAGSDTTASALSIFVMAVCRYPAVLARLHEELDRVVSRDRMPTFDDIPQMPYVRATVQEVLRWRPVSAGGFQHS
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LDRIAASRGAT

Seq. ID No: 491
>T5H_10
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LEERIQEEASYLIQTIREENGQPFDPHLTINNAVSNIICSITFGERFDYQDDQFQELLRMLDEILNLQTSMCCQLYN
VFPRIMNFLPGPHQALFSNMEKMKMFVARMIENHKRDWNPAEARDFIDAYLQEIEKHKGDATSSFQEENLIYNTLDL
FLAGTETTSTSLRWGLLFMALNPEIQEKVQAEIDRVLGQSQQPSMAARESMPYTNAVIHEVLRMGNIIPLNVPREVA
VDTTLAGYHLPKGTMVMTNLTALHRDPTEWATPDTFNPEHFLENGQFKKRESFLPFSIGKRMCLGEQLARTELFIFF
TSLLQKFTFRPPENEQLSLKFRVSLTLAPVSHRLCAVPRG

Seq. ID No: 492
>T5H_11
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Seq. ID No: 493
>T5H_12
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LQLMMYNDMFRIMFDRRFETVADPLFNQLKALNAERSILSQSFDYNYGDFIPVLRPFLRRYLNRCTNLKTKRMKVFE
DHFVQQRKEALEKTGEIKCAMDHILEAERKGEINHDNVLYIVENINVAAIETTLWSIEWGLAELVNHPEIQQKLREE
IVAVLGPGTPVTEPDLERLPYLQSVVKETLRLRMAIPLLVPHMNLSDAKLAGYDIPAESKILVNAWFLANDPKRWVR
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Seq. ID No: 494
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Seq. ID No: 495
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Seq. ID No: 496
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Seq. ID No: 497
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Seq. ID No: 498
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Seq. ID No: 499
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VAGTDTTFATLEWVMTELVRHPRILKKAQEEVRRVVGDSGRVEESHLGELHYMRAIIKETFRLHPAVPLLVPRESVA

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Seq. ID No: 500
>T5H_19
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VAALESFFLAMSLFPEAQKKAQAEMDRVIGNKRLPTTDDRPLLPYLEAVYRELMRWAPVVPLNAAHTTIADDIYKGY
YIPKGTAVYANTWALTRNEEKYPNPDIFNPDRFFTETGELNDDDTVLTFGFGRRICPGRHMASTTVWLTIASVLSNF
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Seq. ID No: 501
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Seq. ID No: 502
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PIQNLTLGVILQAVFGVPSGERLSRLQQLMSTLLDSFAYPISASFLFFPALQKDLGEWSPWGKFIRLREEVRSLIYA
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YLPEVQQKLRAELDALGPDPDPMAIAQLPYLTAVCQEALRIYPITPTTFIRRLREPMTLAGYRFKAGTALMPATYII
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Seq. ID No: 503
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VASVLLVGSAPGHDETDNTIGTAESAETEAQIAQLASWFADLTNGLFTLPIRWGPTTYRKALRGRDRLLSYIEQEIT
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Seq. ID No: 504
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Seq. ID No: 505
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SRCVG

Seq. ID No: 506
>T5H_25
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EINGVTIPKGVVTVIPAFVLHRDPELWPEPDEFRPERFSKENRETQDPYTFLPFGAGPRNCIGMRFALINMKSVITL
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Seq. ID No: 507
>T5H_26
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ISTVMKGMVETRNPLARYIPGKQAFIREVKESIKLLRETGRECILQRRKEIQDGEDIPMDILTQILKGAEIEDGCSL
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Seq. ID No: 508
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Seq. ID No: 509
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Seq. ID No: 510
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Seq. ID No: 511
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Seq. ID No: 512
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Seq. ID No: 513
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Seq. ID No: 514
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Seq. ID No: 515
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Seq. ID No: 516
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QTARLVGSTDITHDLREFRFRSAAGASFLPGQFAMLDLPGLASARAYSMSNTANDDGEWHFQVRRVPHGQGTHVLFD
RLGVGDEIGLDGPYGVAWLRTGAPRDIVCVAGGSGLAPMVSIARGAAAAGMLKDRKLYFFYGARTPRDVCGAEMLAQ
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RFF

Seq. ID No: 517
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FAQFSQEIGLASLGAPDEYIERLATLYWFTVEFGLCKQDDKIKAYGAGLLSSFGELQYCLTDKPELKPFEPEKTSLQ
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Seq. ID No: 518
>TPH_2
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YKHGDPIPKVEFTEEEIKTWGTVFQELNKLYPTHACREYLKNLPLLSKYCGYREDNIPQLEDVSNFLKERTGFSIRP
VAGYLSPRDFLSGLAFRVFHCTQYVRHSSDPFYTPEPDTCHELLGHVPLLAEPSFAQFSQEIGLASLGASEEAVQKL
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MREFTKTIKRPFGVKYNPYTRSIQILKDTKSITSAMNELQHDLDVVSDALAKVSRKPSI

Seq. ID No: 519
>TPH_3
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AAKEHRHGDRIARVQYLEHEVETWRAVYEQLRECHSRWACTEYLEMLPQMERFCGYAPGNIPQLADISDFLQQRTGF
TLRPITGLLSARDFLNALAFRVFYSTQYIRHHGNPFYTPEPDICHELMGHVPLFANAAFADFSQEIGLASLAASDDD
IARLAAVYWFTVEFGLVRQGGEVKAYGAGLLSSFGEMEWSCSREPSTTCREMGSVAELQAPSIVPLDPTQAGKQAYP
ITTYQPLYFCAESMQDAKAKISQFCDTLTRPFFPQYDPLTQNIRVTKAVRRARRISTVEMQMAKQLDYFEKQ

Seq. ID No: 520
>TPH_4
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ELKLLYPTHACYEHNHVFPLLEKYCGYNENNIPQLEDVSNFLQTCTGFRLRPVAGLLSSRDFLAGLAFRVFHSTQYI
RHWSKPMYTPEPDICHELLGHAPLFADPSFAQFSQEIGLASLGAPDEYIERLATLYWFTIEFGLCKQDDKIKAYGAG
LLSSFGELQYCLTDKPDLKPFEPEKTSLQKYPITEFQPVYFIAESFEDAKEKVRKFATTIPRPFSVRYNPYTQSIEV
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Seq. ID No: 521
>TPH_5
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LPLLVKHCGYREDNIPQLEDVSRFLKERSGFTIRPVAGYLSPRDFLAGLAFRVFHCTQYVRHSSDPLYTPEPDTCHE
LLGHVPLLAEPSFAQFSQEIGLASLGASDEAVQKLATCYFFTVEFGLCKQEGKLKVYGAGLLSSISELKHSLSGNAN
VKPFDPMVTCSQECIITSFQEVYFYSESFEEAKEKMREFAKTIKRPFGLKYNPYTQSVQMLKDTQSITTLVSELRHE
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Seq. ID No: 522
>TPH_6
MHSPEPDCCHELLGHVPMLADKTFAQFSQDIGLASLGVTDEEIEKLSTLYWFTVEFGLCKQDGEVKAYGAGLLSSYG
ELLHALSDKPEVRPFDPDEAAIQPYQDQNYQPVYFVSESFTDAKEKLRNYASRIKRPFAARYDPYTVSIEVLDSPGQ
IQSSLEELKDELQTLTTALNILS

Seq. ID No: 523
>TPH_7
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FQKNLGLLQDKAGYNEFDLPQLQVVSDFLKARTGFCLRPVAGYLSARDFLSGLAFRVFYCTQYIRHQADPFYTPEPD
CCHELLGHVPMLADPKFARFSQEIGLASLGTSDEEIKKLATCYFFTIEFGLCRQDNQLKAYGAGLLSSVAELQHALS
DKAVIKPFIPMKVINEECLVTTFQNGYFETSSFEDATRQMREFVRTIKRPFDVHYNPYTQSIEIIKTPKSVAKLVQD
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Seq. ID No: 524
>TrpHalo_1
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AVFDSSTGLWTISIEGSDVKHMGRVLVCADGAPSKLATQLGIVKQAPQGVCSRAYIKEGTHRFRADGVVFYPRNILP
AYAALFRHIDDTVAYCTYILPFNPKVTTDDLSYWHHRLLEEDPSISQAVGKNADMERMKAWGLRMGGEPVTYGNHVL
VVGDAAGMIDPLTGEGIHHAMDGGRIAAHFLCEAIAVGNFDKEVMKEYQNRWLYTFGNDYKWSQAICHFLYRFPIFI
DATAAAAQRRGNNFLALYADIMTGRIPKANIFRPDISLPIAFEVLVLLWKMMFTGGGGNNKMKSQ

Seq. ID No: 525
>TrpHalo_2
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GYVALLRHIDDQLTYCTYILPGNPRATTKDLSYWHHRLLEEDPNISQAVGKNAELEKMKAWDLRVGGEPVTYGNHVL
VVGDAAGMIDPLTGEGIHHAMDGGRIAAHFLCEAIAVGNFDKEVMKEYQNRWLKAFGNDFRWSQAIGNFLYRYPIFI
DATAAVAEKKGDRFLARWADIMAGRIPKISVLRPQFLLAVGFQALLLFYKKIFKGGYGKKTKIL

Seq. ID No: 526
>TrpHalo_3
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AIEILMDMGVYGGLIREQKAYMIDYGGLVSPSGLSFVGHTHELFGEIPGAVVCKRVVLDKVISRTAQSAGAELLENS
PVTDAVFDSSTGLWTISIEGSDVKHMGRVLVCADGAPSRLAMQLGIVKGTPKCVCSRAYIKGGTHRFKEDGMVFYVP
SILPGYVALLRHIDDQLTYCTYILPGNPRATTKDLSYWHHRLLEEDPNISQAVGKNAELEKMKAWDLRVGGEPVTYG
NHVLVVGDAAGMIDPLTGEGIHHAMDGGRIAAHFLCEAIAVGNFDKEVMKEYQNRWLKAFGNDFRWSQAIGNFLYRY
PIFIDATAAVAEKKGDRFLARWADIMAGRIPKISVLRPQFLLAVGFQALLLFYKKIFKGGYGKKTKIL

Seq. ID No: 527
>TrpHalo_4
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YKVAIKFINWRTAGEGTSEARELDGGPDHFYHSFGLLKYHEQIPLSHYWFDRSYRGKTVEPFDYACYKEPVILDANR
SPRRLDGSKVTNYAWHFDAHLVADFLRRFATEKLGVRHVEDRVEHVQRDANGNIESVRTATGRVFDADLFVDCSGFR
GLLINKAMEEPFLDMSDHLLNDSAVATQVPHDDDANGVEPFTSAIAMKSGWTWKIPMLGRFGTGYVYSSRFATEDEA
VREFCEMWHLDPETQPLNRIRFRVGRNRRAWVGNCVSIGTSSCFVEPLESTGIYFVYAALYQLVKHFPDKSLNPVLT
ARFNREIETMFDDTRDFIQAHFYFSPRTDTPFWRANKELRLADGMQEKIDMYRAGMAINAPASDDAQLYYGNFEEEF
RNFWNNSNYYCVLAGLGLVPDAPSPRLAHMPQATESVDEVFGAVKDRQRNLLETLPSLHEFLRQQHGR

Seq. ID No: 528
>TrpHalo_5
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TQATCKLGIRFLDWSGPGSDFIHPFGLYGQDVKGIGFHHYWLKQRRAGDAAPLAAYSLGAALAAGGKFTLPSPHPPS
QLSVFDWALHLDAGLFAQHLRAYAEAGGCARIDARIRSVELRPEDGFVRALTLDDGREVEGDLFVDCSGFKGLVIGE
ALGVGFEDWGRWLPCDAAYAVQSENRPGDAPAPFTRVTARSAGWQWGIPLRHRAGNGLVFSSAHLSDDQALAELMPH
LLGDPLTEPRRIPFRPGRRSQAWAKNCVAIGLSSGFLEPLESTSIALIETGIERLKALFPDRRFAQPILDEFNDQTA
REMERVRDFIILHYKLNRRTDTDFWRDCREMPVPETLERKIALWTARGQFVRYRWEMFHPASWLAIYDGFGLYPDHH
DPAVDAMDPAYLARSLAEMRANIADLVARTPEHAQFLAGLDPAASAA

Seq. ID No: 529
>TrpHalo_6
MIRSVVIVGGGTAGWMTASYLKAAFDDRIDVTLVESGNVRRIGVGEATFSTVRHFFDYLGLDEREWLPRCAGGYKLG
IRFENWSEPGEYFYHPFERLRVVDGFNMAEWWLAVGDRRTSFSEACYLTHRLCEAKRAPRMLDGSLFASQVDESLGR
STLAEQRAQFPYAYHFDADEVARYLSEYAIARGVRHVVDDVQHVGQDERGWISGVHTKQHGEISGDLFVDCTGFRGL
LINQTLGGRFQSFSDVLPNNRAVALRVPRENDEDMRPYTTATAMSAGWMWTIPLFKRDGNGYVYSDEFISPEEAERE
LRSTVAPGRDDLEANHIQMRIGRNERTWINNCVAVGLSAAFVEPLESTGIFFIQHAIEQLVKHFPGERWDPVLISAY
NERMAHMVDGVKEFLVLHYKGAQREDTPYWKAAKTRAMPDGLARKLELSASHLLDEQTIYPYYHGFETYSWITMNLG
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Seq. ID No: 530
>TrpHalo_7
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IRFENWRAPGHHFYHPFERQRVVDGFTLPDWWLADGGATERFDKECFLVGTLCDTMRSPRHMDGALFEGDLTDRPAG
RSTLAEQGTQFPYAYHFDAALLADFLRDYAVARGVLHVVDDVVHVARDERGWISHVATRGSGDLAGDLFVDCTGFRG
LLINDALDEPFESYQDTLPNDSAVALRVPVDMEREGLRPCTTSTAQAAGWIWTIPLFGRVGTGYVYARDYCTPEEAE
RTLRRFVGPAADDLEANHIRMRIGRSRRSWVNNCVAVGLSSGFVEPLESTGIFFIQHAIEQLVKHFPDADWDPALRS
AYNTLVNRCMDGVREFLVLHYYGAARADNEYWRDTKTRKIPDSLAERVEQWRTKLPHPESVYPHYHGFEAYSYVCMV
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Seq. ID No: 531
>TrpHalo_8

MNKPIKNIVIVGGGTAGWMAASYLVRALQQQANITLIESAAIPRIGVGEATIPSLQKVFFDFLGIPEREWMPQVNGA
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QMSHAWHFDAHLVADFLKRWAVERGVNRVVDEVVDVRLNNRGYISNLLTKEGRTLEADLFIDCSGMRGLLINQALKE
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SDNQPLNQIKFRVGRNKRAWVNNCVSIGLSSCFLEPLESTGIYFIYAALYQLVKHFPDTSFDPRLSDAFNAEIVHMF
DDCRDFVQAHYFTTSRDDTPFWLANRHDLRLSDAIKEKVQRYKAGLPLTTTSFDDSTYYETFDYEFKNFWLNGNYYC
IFAGLGMLPDRSLPLLQHRPESIEKAEAMFASIRREAERLRTSLPTNYDYLRSLRDGDAGLSRGQRGPKLAAQESL

Seq. ID No: 532
>TrpM_1
MSPVALSPKRVDIVDIRGNDMQYSLVNEIHKGLNPPNGTRRSLPTMLLYDSEGLKLFEKITYVDEYYLTNAEIEVLE
KHSRRLVEKIPSNAQLLELGSGNLRKIEILLREFERVGKPVDYYALDLSLSELERTFSNVSLEEYKSVGFHGLHGTY
DDAHTWLSDPKNRERPTVVLSMGSSLGNFSPPDAAAFLAGFATLLKPSDFMVIGLDACEDPDRVYKAYNDSAGITRK
FYENGLANANKTLGHEVFRPDEWEVVTEYDAVNGRHQVFYVPTKDVSVGDVLLRRGEKIIFAEAFKYGCQAREKLWH
DAGLIEAAEFGSGSEDYRTYI*

Seq. ID No: 533
>TrpM_10
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LRLFEQITYQPDYYLTRLEIDILSRHAHQIANSVPDGAILLELGAGALRKTALILDALEAQGKDVTYFALDLDKPEL
LRTLAEVKGRYTHVSLAGLWGTYDDGCTWLKQVKDRPRIILWLGSSVGNMSRKEAGQFIRTFGDILAPRDRFIVAID
SKNHKLNDIRAAYDDRAGVTRRFALNALGNINDLFNADVVDVSSFDYNPYYNEVQGRNEAYFRCLKDTQVRIPSETP
ILVHEGEYIRFAFSHKYDRVERQVLWTAAGAYPVQEWMSQDGDYALTMLSWSS*

Seq. ID No: 534
>TrpM_11
MTYSIVDIRKTDTCLKNSIINGINQSTKSIPAIVLYDELGLQYYEKVTYLKEYYLTEAEIDILKNKADQISDYIPEG
SSLIELGSGALRKTRLLLDSIEKQKKKVIYYALDLMEGELKRTLSSLGKFQYVKLVGLWGVYEDGIDYASNLPGDSH
KTILWMGSSIGNFNRDEAANFVKTIQDKAMNPGDLFLIGIDRRKNPDKITAAYNDPKGINAKFIMNGLNHVNAIFDQ
PIFDSNNFEHVTMYNDDVGRHEAYCKVKNDTTLEFKESKDNPKTIIKLNKNELINIGYSHKYNKAETDALFDFSLLS
YMESWTDSQSLYDLHLVYKSPFHFTRKFDSHK*

Seq. ID No: 535
>TrpM_12
MSKDVQVLDIRASPQSKGSIPNLRTAILDGLQKAPGMRTLPSEILYDDRGLKIYNDCIRSWSEWYYPISAETEILEI
NGKDIARVFSTSDRGEAVLIELGAGSLDKTSKILVSLSETVQNVSDSQPPITYYALDLERSELQRTLSELQKNIGEK
IAGKIATKGMWGTYDDGIRSVENNELHLDAAVPVHFLFLGGTIGNFSKGEGDVTFLRNLPLNAQRGDTILLGIDREK
SKEIIERAYNFPAAREWIMNGLNVSGHLLSGDKDLFQLDNWDRYAMYDEKLGRLEAGYRSKIDQIIEVTANYSIPFK
KDESVMAIFSNKYTDDELNFLISKANLKTINSWVDHKALYYIFSLRKV*

Seq. ID No: 536
>TrpM_13
MPRIQVLDIRGSKESVGSTPHLRAAILEGLLKPPGSRTLPSETLYDEVGLKMYNDGMKAWAEWYYPVEAERQILERY
GRDIAKLFTTSAKGKAVLIELGAGSLDKTSQVLLSAAEITRTTGPMNNIAYYALDLERGELERTIGRLQEVIGDQIA
GKISTAGMWGTYDDGIRVIEKNELELEPDIPVHILFLGGTIGNFSKQDGDVAFLKSLPLDHKRGDTLLVGMDRHKSA
DAIERSYGFAAAKDWIMNGLKVSGRVLTGDEGLFEIGNWERYAKYNEELGRYEAGYKSQKEHALKISEGVDITFLKD
EVVLVMFSNKYTDAEMDSVVDSAGLVKNGSWMDEKAQYCLLSLRANNGPV*

Seq. ID No: 537
>TrpM_14

MSQIEVLDIRGSKEATGSTPHLRAEILQGLSKSPGHRTIPGETLFDETGLKMYDEGMKTWRKWYYPFEAEKEILEVR
GLEIAKLLKTSSKGEAVLIELGAGSLEKTSQILLSAAQIAETADNSTTNPITYYALDLEHRELERTLAALQDAIGPR
IAGKITTKGMWGTYEDGIRVVERNDLKFPSDVPLHILFLGGTIGNFSKADGDIAFLKSLPLNRKRGDTLLLGVDRAK
AVELIERAYGFAAATGWIMNGLKVSGRVLTGDEELFESGNWERYSKYNEELGRYEAGYKSRKDQTIKVAKDVDIVFS
KDEVILVTYSNKYTDAEIKTVFDGAGLEIVESWMDKKAQYCLFLLKA*

Seq. ID No: 538
>TrpM_2
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GADTLVELGSGTSEKTRMLLDAMRDADLLRRFIPFDVDAGVLRSAGAAIGAEYPGIEIDAVCGDFEEHLGKIPRVGR
RLVVFLGSTIGNLTPQPRAEFLATLADTLQPGDSLLLGTDLVKDTGRLVRAYDDAAGVTAAFNRNVLAVVNRELSAD
FDLDAFEHIAKWNDDEERIEVWLRARTAQHVRIPALDLEIDFAAGEQMLTAVSCKFRPDSVAAELAEAGLRQTHWWT
DPAGDFGLSLAVR*

Seq. ID No: 539
>TrpM_3
MTLSLANYLAADSAAEALRRDVRAGLTAAPKSLPPKWFYDAVGSDLFDQITRLPEYYPTRTEAQILRTRSAEIIAAA
GADTLVELGSGTSEKTRMLLDAMRDAELLRRFIPFDVDAGVLRSAGAAIGAEYPGIEIDAVCGDFEEHLGKIPHVGR
RLVVFLGSTIGNLTPAPRAEFLSTLADTLQPGDSLLLGTDLVKDTGRLVRAYDDAAGVTAAFNRNVLAVVNRELSAD
FDLDAFEHVAKWNSDEERIEVWLRARTAQHVRVAALDLEVDFAAGEEMLTAVSCKFRPENVVAELAEAGLRQTHWWT
DPAGDFGLSLAVR*

Seq. ID No: 540
>TrpM_4
MRVSGANHLGEDAGHLALRRDVYSGLQKTPKSLPPKWFYDTVGSELFDQITRLPEYYPTRAEAEILRARSAEVASAC
RADTLVELGSGTSEKTRMLLDALRHRGSLRRFVPFDVDASVLSATATAIQREYSGVEINAVCGDFEEHLTEIPRGGR
RLFVFLGSTIGNLTPGPRAQFLTALAGVMRPGDSLLLGTDLVKDAARLVRAYDDPGGVTAQFNRNVLAVINRELEAD
FDVDAFQHVARWNSAEERIEVWLRADGRQRVRVGALDLTVDFDAGEEMLTAVSCKFRPQAVGAELAAAGLHRIRWWT
DEAGDFGLSLAAK*

Seq. ID No: 541
>TrpM_5
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RLVAFLGSTIGNLTPGPRADFLASLAETLQPGDSVLLGTDLVKDTGRLVSAYDDSAGVTAAFNRNVLSVVNRELDAD
FDLDAFAHVAKWNAEEERIEVWLRADAPQQVRIAGLDLDVAFGAGEEMLTAVSCKFRADGVADELAKAGLRQTHWWT
DEAGDFGLSLAVK*

Seq. ID No: 542
>TrpM_6
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GTYDDGRRWLSENTSDSPRTILWLGSSIGNVKRDDAGDFIRSFGDVLSSKDRFVVAIDSRYHEVDTICRAYNDREGF
AERFCLNGIDSFNQLFGRAIIDISCAKYRTVYNEVKGRHEVYYRCTHDFEIRLPGDYPPTFLYEGELILLAHSYKYA
AVERETLWLRAGARPEKEWMTDGSYTVTMLSWP*

Seq. ID No: 543
>TrpM_7
MSPSTVNKIASSPVFDIRSDETKGFAKAPIEDELAGLQAVYNEKTLPNVLLYDAKGLQLFEKITYTNDYYLTGLEMD
LLGEHADEMAEWIKDGAALVELGAGALRKTAILLDAIERQGKRITFYALDLDHSELTRTLAELEGRYRHITLCGLWG
TYDDGRAWLASTNEEQRVLLWLGSSIGNLSRQEAKDFLHSFGRALRPGIDKFIVAMDSKYNAVSSMTRAYNDSEGVT

ASFALNLLDAFNAKVGFKALPPSSFCYSPFFNQAQGRNEAYLRARHGVRFEVNGIAVEVRDEELIRFAYSHKYDNAE
RDLLWRAAEANVEQEWLHSPQSGRARYSISLLSFRD*

Seq. ID No: 544
>TrpM_8
MTLSLSNHLPANSAARVLRRDVLDGLTQTPKALPPKWFYDSVGSDLFDQITRLPEYYPTRTEAQILRTRSAEIAEAS
GADTLVELGSGTSEKTRMLLDALRDNGTLRRFIPFDVDAGVLNAAGAAIQKEYPGVEVDAVCGDFEEHLGEIPRVGR
RLIAFLGSTIGNLTPQPRARFLTALAQTMRPGDSLLLGTDLVKDTERLVRAYDDSAGVTARFNRNVLAVINRELDAD
FDLAAFDHVARFNAAEERIEVWLRARGAQRVYYVRELDLTVDFADGEEMLTAVSCKFRPDGVAAELAAAGLRRTHWWT
DPAGDFGLSLSTK*

Seq. ID No: 545
>TrpM_9
MTISIANYLAADSAATALRRDVREGLAGTPKSLPPKWFYDSVGSDLFDQITRLPEYYPTRAEAQILRTHAVDVAAAS
GADTLVELGSGTSEKTRLLLDALHRADSLRRFIPFDVDASILQSAGAAISQEYPDVEIEAVCGDFEEHLGKIPLQGR
RLVVFLGSTIGNLTSGPRATFLSALADSLQPGDTLLLGTDLVKDVDRLKRAYDDAAGVTARFNKNVLTVVNRELGAD
FDLDAFEHVCKWNADEERIEVWLRANTLQRVHISGLELDVEYAAGEEMLTAVSCKFRPEGIAAELAAVGLNRTHWWT
DDAGDFGLSLAVK*

Seq. ID No: 546
>TrpS_1
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EVIPVHSGSATVKDACNEALRDWSGSYETAHYMLGTAAGPHPYPTIVREFQRMIGEETKAQILDKEGRLPDAVIACV
GGGSNAIGMFADFINDTSVGLIGVEPGGHGIETGEHGAPLKHGRVGIYFGMKAPMMQTADGQIEESYSISAGLDFPS
VGPQHAYLNSIGRADYVSITDDEALEAFKTLCRHEGIIPALESSHALAHALKMMREQPEKEQLLVVNLSGRGDKDIF
TVHDILKARGEI

Seq. ID No: 547
>TrpS_2
MWFGEFGGQYVLETLIGPLKELEKAYKRFKDDEEFNRQLNYYLKTWAGRPTPLYYAKRLTEKIGGAKVYLKREDLVH
GGAHKTNNAIGQALLAKFMGKTRLIAETGAGQHGVATAMAGALLGMKVDIYMGAEDVERQKMNVFRMKLLGANVIPV
NSGSRTLKDAINEALRDWVATFEYTHYLIGSVVGPHPYPTIVRDFQSVIGREAKAQILEAEGQLPDVIVACVGGGSN
AMGIFYPFVNDKKVKLVGVEAGGKGLESGKHSASLNAGQVGVSHGMLSYFLQDEEGQIKPSHSIAPGLDYPGVGPEH
AYLKKIQRAEYVAVTDEEALKAFHELSRTEGIIPALESAHAVAYAMKLAKEMSRDEIIIVNLSGRGDKDLDIVLKVS
GNV

Seq. ID No: 548
>affibody_tag_1
MVDNKFNKETIQASQEIRLLPNLNGRQKLAFIHSLLDDPSQSANLLAEAKKLNDAQAPKNAAIRSSSASSGGSGGSS
SS

Seq. ID No: 549
>affibody_tag_2
NAAIRSSSASSGGSGGSSSSVDNKFNKETIQASQEIRLLPNLNGRQKLAFIHSLLDDPSQSANLLAEAKKLNDAQAP
K

Seq. ID No: 550
>affibody_tag_3
NAAIRSSSASSGGSGGSSSSGVDNKFNKELGWATWEIFNLPNLNGVQVKAFIDSLRDDPSQSANLLAEAKKLNDAQA
PK

Seq. ID No: 551
>affibody_tag_4
MVDNKFNKELGWATWEIFNLPNLNGVQVKAFIDSLRDDPSQSANLLAEAKKLNDAQAPKGNAAIRSSSASSGGSGGS
SSS

Seq. ID No: 552
>affibody_tag_5
MVDNKFNKEMRNAYWEIALLPNLNNQQKRAFIRSLYDDPSQSANLLAEAKKLNDAQAPKSSNAAIRSSSASSGGSGG
SSSS

Seq. ID No: 553
>affibody_tag_6
NAAIRSSSASSGGSGGSSSSGVDNKFNKEMRNAYWEIALLPNLNNQQKRAFIRSLYDDPSQSANLLAEAKKLNDAQA
PK

Seq. ID No: 554
>cofold_1
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AEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQE
PYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAMTINGPW
AWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSY
EEELAKDPRIAATMENAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQTVDEALKDAQTNSSSNNNNNNNNNNLGIE
GR

Seq. ID No: 555
>cofold_2
MVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKLICTTGKLPVPWPTLVTTLGYGLQCFARYPD
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What is claimed is:

1. A non-naturally occurring nucleic acid comprising a sequence encoding an enzyme or regulatory protein in tryptamine metabolism,

wherein the enzyme or regulatory protein is an N-methyltransferase (INMT, PsiM, TrpM), a tryptophan decarboxylase (AADC), a tryptophan hydroxylase (TPH), a tryptamine 4' hydroxylase (T4H), a tryptamine 5' hydroxylase (T5H), a truncated cytochrome p450 reductase (T4H-CPR, T5H-CPR), an hydroxytryptamine O-methyltransferase (IOMT or CaffOMT), an N-acetyltransferase (NAT), a deacetylase (DAC), a hydroxyl tryptamine kinase (PsiK), a tryptophan synthase (TrpS), a toluene monooxygenase (TMO), an aminotransferase/methyltransferase fusion (ATMT), a phosphatase, an oxidase, a dimethylallyltryptophan synthase (DMATS), an isopentenyl-diphosphate isomerase (IDI1), a tryptophan halogenase (TrpHalo), an aspartate oxidase/quinolinic acid synthase fusion (AOQS), a tryptophan importer (TAT2), a methionine importer (MUP1), or a SAMe importer (SAM3).

2. The nucleic acid of claim 1, encoding a methyltransferase or hydroxylase.

3. The nucleic acid of claim 2, wherein the methyltransferase or hydroxylase is a tryptamine N-methyltransferase (INMT), a hydroxytryptamine O-methyltransferase (IOMT), a tryptamine 5' hydroxylase (T5H) or a tryptophan N-methyltransferase (TrpM).

4. The nucleic acid of claim 1, encoding an amino acid sequence that is naturally occurring.

5. The nucleic acid of claim 1, encoding an amino acid sequence that is not naturally occurring.

6. The nucleic acid of claim 1, wherein the sequence is codon-optimized for yeast expression.

7. The nucleic acid of claim 1, further comprising nucleotides encoding amino acids that are not part of the enzyme or regulatory protein.

8. The nucleic acid of claim 7, having a 5' end, wherein the additional nucleotides are at the 5' end of the nucleic acid and encode a codon optimized cofolding peptide.

9. The nucleic acid of claim 8, wherein the codon optimized cofolding peptide comprises an amino acid sequence of any one of SEQ ID NO:554-558.

10. The nucleic acid of claim 9, wherein the codon optimized cofolding peptide is encoded by any one of SEQ ID NOs:265-269.

11. The nucleic acid of claim 7, wherein the amino acids that are not part of the enzyme or regulatory protein are an affibody tag, a localization scaffold, a vacuolar localization tag, a secretion signal, a 6xhis tag, or any combination thereof.

12. The nucleic acid of claim 1, comprising the sequence of SEQ ID NOs:1-289

13. The nucleic acid of claim 1, further comprising a promoter functional in a recombinant microorganism.

14. The nucleic acid of claim 13, wherein the recombinant microorganism is a yeast.

15. An expression cassette comprising the nucleic acid of claim 13.

16. The expression cassette of claim 15, which is a yeast expression cassette.

17. A recombinant microorganism comprising the expression cassette of claim 15, that expresses the enzyme or regulatory protein encoded therein.

18. The recombinant microorganism of claim 17, which is an E. coli.

19. The recombinant microorganism of claim 17, which is a yeast cell.

20. The yeast cell of claim 19, which is a species of Saccharomyces, Candida, Pichia, Schizosaccharomyces, Scheffersomyces, Blakeslea, Rhodotorula, Aspergillus or Yarrowia.

21. The yeast cell of claim 19, which is a Saccharomyces cerevisiae.

22. A non-naturally occurring enzyme or regulatory protein comprising an amino acid sequence encoded by the nucleic acid of any one of claims 1-12.

23. A recombinant microorganism expressing at least one enzyme or regulatory protein of claim 22.

24. The recombinant microorganism of claim 23, which is an E. coli.

25. The recombinant microorganism of claim 23, which is a yeast cell.

26. The yeast cell of claim 25, which is a species of Saccharomyces, Candida, Pichia, Schizosaccharomyces, Scheffersomyces, Blakeslea, Rhodotorula, Aspergillus or Yarrowia.

27. The yeast cell of claim 25, which is a Saccharomyces cerevisiae.

28. The recombinant microorganism of claim 23, expressing INMT, wherein the recombinant microorganism produces at least one hydroxy substituted tryptophan compound.

29. The recombinant microorganism of claim 28, wherein the at least one hydroxy substituted tryptophan compound is 5-OH-NMTP, 5-OH-DMTP or 5-OH-TMTP.

30. The recombinant microorganism of claim 23, expressing INMT, wherein the recombinant microorganism produces at least one hydroxy substituted tryptamine compound.

31. The recombinant microorganism of claim 30, wherein the at least one hydroxy substituted tryptamine compound is bufotenine, 5-OH-NMT, or 5-OH-TMT.

32. The recombinant microorganism of claim 23, expressing INMT, wherein the recombinant microorganism produces at least one methoxy substituted tryptamine compound.

33. The recombinant microorganism of claim 32, wherein the at least one methoxy substituted tryptamine compound is 5-MeO-NMT, 5-MeO-DMT, or 5-MeO-TMT.

34. The recombinant microorganism of claim 23, expressing IOMT, wherein the recombinant microorganism methylates the primary amine on the 5-hydroxy moiety on an indole ring.

35. The recombinant microorganism of claim 34, wherein the microorganism acts on (a) bufotenine to create 5-MeO-DMT, or (b) N-acetylserotonin to create melatonin.

36. The recombinant microorganism of claim 23, expressing T5H, wherein the recombinant microorganism hydroxylates at the 5' position of an indole ring.

37. The recombinant microorganism of claim 36, wherein the microorganism generates serotonin from tryptamine.

38. The recombinant microorganism of claim 36, wherein the T5H is a fusion polypeptide with a cytochrome P450 reductase (CPR).

39. The recombinant microorganism of claim 36, wherein the T5H is a fusion polypeptide with an IOMT.

40. The recombinant microorganism of claim 23, expressing TrpM, wherein the recombinant microorganism catalyzes the alkylation of the primary amine of L-tryptophan to produce NMTP, DMTP, TMTP, or any combination thereof.

41. The recombinant microorganism of claim 23, expressing PsiM, wherein the recombinant microorganism methylates norbaeocystin.

42. The recombinant microorganism of claim 41, wherein the PsiM comprises a domain from an rRNA methyltransferase from Ascomycota.

43. The recombinant microorganism of claim 23, expressing AADC, wherein the recombinant microorganism decarboxylates an aliphatic carboxylic acid.

44. The recombinant microorganism of claim 43, wherein the recombinant microorganism creates tryptamine from L-tryptophan, creates serotonin from 5-HTP, creates bufotenine from 5-OH-DMTP, creates 5-MeO-DMT from 5-MeO-DMTP, or any combination thereof.

45. The recombinant microorganism of claim 23, expressing TPH, wherein the recombinant microorganism adds a hydroxy group to the 5-carbon of L-tryptophan.

46. The recombinant microorganism of claim 23, expressing T4H, wherein the recombinant microorganism hydroxylates the 4' position of an indole ring.

47. The recombinant microorganism of claim 46, wherein the recombinant microorganism converts tryptamine to 4-OH-tryptamine.

48. The recombinant microorganism of claim 46, wherein the T4H is a chimera of sequences from T4H from different species, wherein the T4H comprises a yeast p450 N terminus.

49. The recombinant microorganism of claim 46, wherein the T4H is a chimera of a mushroom PsiH and a yeast p450 N terminus.

50. The recombinant microorganism of claim 23, expressing NAT, wherein the recombinant microorganism adds an acetyl group from acetyl-CoA to the terminal amino group of a tryptamine.

51. The recombinant microorganism of claim 50, wherein the recombinant microorganism acts on serotonin to generate N-acetylserotonin.

52. The recombinant microorganism of claim 23, expressing DAC, wherein the recombinant microorganism removes an acetyl group from the terminal amino group of a tryptamine.

53. The recombinant microorganism of claim 52, wherein the recombinant microorganism acts on melatonin to create 5-MeO-tryptamine.

54. The recombinant microorganism of claim 23, expressing PsiK, wherein the recombinant microorganism phosphorylates a hydroxy-indole.

55. The recombinant microorganism of claim 54, wherein the PsiK is a chimera of a PsiK and a yeast kinase.

56. The recombinant microorganism of claim 23, expressing TrpS, wherein the recombinant microorganism combines an indole with L-serine or L-threonine to create variants of tryptophan or beta-methyl tryptophan, respectively.

57. The recombinant microorganism of claim 56, wherein the TrpS is coexpressed with a multidrug exporter, wherein the recombinant microorganism exports indole while continuing bioproduction of tryptophan and/or tryptamine analogs.

58. The recombinant microorganism of claim 23, expressing TMO, wherein the recombinant microorganism hydroxylates the indole ring of tryptamines.

59. The recombinant microorganism of claim 58, wherein the TMO comprises four subunits that are fused into a fusion polypeptide.

60. The recombinant microorganism of claim 23, expressing a phosphatase, wherein the recombinant microorganism dephosphorylates a phosphorylated tryptamine.

61. The recombinant microorganism of claim 60, wherein the recombinant microorganism dephosphorylates psilocybin into psilosin.

62. The recombinant microorganism of claim 23, expressing an oxidate, wherein the recombinant microorganism creates a tryptamine radical which reacts with another tryptamine to form a dimer or oligomer.

63. The recombinant microorganism of claim 62, wherein the oxidase is a chimera with a yeast laccase.

64. The recombinant microorganism of claim 63, wherein the oxidase is coexpressed with a yeast t-SNARE.

65. The recombinant microorganism of claim 23, expressing a DMATS, wherein the recombinant microorganism prenylates a tryptophan and/or a tryptamine.

66. The recombinant microorganism of claim 65, wherein the DMATS is a fusion polypeptide with IDI1.

67. The recombinant microorganism of claim 23, expressing a TrpHalo, wherein the recombinant microorganism adds fluorine (F), chlorine (Cl), bromine (Br), and/or iodine (I) to an indole or biogenic amine.

68. The recombinant microorganism of claim 67, wherein the TrpHalo further comprises a secretion tag.

69. The recombinant microorganism of claim 68, wherein the TrpHalo further comprises a 6xhis tag.

70. The recombinant microorganism of claim 67, wherein the TrpHalo is coexpressed with a fluoride exporter.

71. The recombinant microorganism of claim 23, expressing more than one of the enzyme and/or regulatory protein.

72. The recombinant microorganism of claim 71, expressing TPH, TrpM, and AADC, wherein the recombinant microorganism produces at least one hydroxy substituted tryptamine compound.

73. The recombinant microorganism of claim 72, wherein the at least one hydroxy substituted tryptamine compound is bufotenine, 5-OH-NMT, or 5-OH-TMT.

74. The recombinant microorganism of claim 71, expressing TPH, TrpM, AADC, and IOMT, wherein the recombinant microorganism produces at least one methoxy substituted tryptamine compound.

75. The recombinant microorganism of claim 74, wherein the at least one methoxy substituted tryptamine compound is 5-MeO-NMT, 5-MeO-DMT, or 5-MeO-TMT.

76. The recombinant microorganism of claim 71, expressing AADC, T5H and INMT, wherein the recombinant microorganism produces at least one hydroxy substituted tryptamine compound.

77. The recombinant microorganism of claim 76, wherein the at least one hydroxy substituted tryptamine compound is bufotenine, 5-OH-NMT, or 5-OH-TMT.

78. The recombinant microorganism of claim 71, expressing AADC, T5H, INMT, and IOMT, wherein the recombinant microorganism produces at least one methoxy substituted tryptamine compound.

79. The recombinant microorganism of claim 78, wherein the at least one methoxy substituted tryptamine compound is 5-MeO-NMT, 5-MeO-DMT, or 5-MeO-TMT.

80. The recombinant microorganism of claim 71, expressing TrpM and TPH, wherein the recombinant microorganism produces at least one hydroxy substituted tryptophan compound.

81. The recombinant microorganism of claim 80, wherein the at least one hydroxy substituted tryptophan compound is 5-HTP, 5-OH-NMTP, 5-OH-DMTP or 5-OH-TMTP.

82. The recombinant microorganism of claim 71, expressing TrpM, TPH and IOMT, wherein the recombinant microorganism produces at least one methoxy substituted tryptophan compound.

83. The recombinant microorganism of claim 82, wherein the at least one methoxy substituted tryptophan compound is 5-MeO-NMTP, 5-MeO-DMTP or 5-MeO-TMTP.

84. The recombinant microorganism of claim 71, expressing INMT and T5H, wherein the recombinant microorganism produces at least one hydroxy substituted tryptamine compound.

85. The recombinant microorganism of claim 84, wherein the at least one hydroxy substituted tryptamine compound is bufotenine, 5-OH-NMT, or 5-OH-TMT.

86. The recombinant microorganism of claim 71, expressing INMT, T5H and IOMT, wherein the recombinant microorganism produces at least one methoxy substituted tryptamine compound.

87. The recombinant microorganism of claim 86, wherein the at least one methoxy substituted tryptamine compound is 5-MeO-NMT, 5-MeO-DMT, or 5-MeO-TMT.

88. The recombinant microorganism of claim 71, expressing INMT and IOMT, wherein the recombinant microorganism produces at least one methoxy substituted tryptophan compound.

89. The recombinant microorganism of claim 88, wherein the at least one methoxy substituted tryptophan compound is 5-MeO-NMTP, 5-MeO-DMTP or 5-MeO-TMTP.

90. The recombinant microorganism of claim 71, expressing INMT and AADC, wherein the recombinant microorganism produces at least one hydroxy substituted tryptamine compound.

91. The recombinant microorganism of claim 90, wherein the at least one hydroxy substituted tryptamine compound is bufotenine, 5-OH-NMT, or 5-OH-TMT.

92. The recombinant microorganism of claim 71, expressing INMT, AADC and IOMT, wherein the recombinant microorganism produces at least one methoxy substituted tryptamine compound.

93. The recombinant microorganism of claim 92, wherein the at least one methoxy substituted tryptamine compound is 5-MeO-NMT, 5-MeO-DMT, or 5-MeO-TMT.

94. The recombinant microorganism of claim 71, expressing INMT and IOMT, wherein the recombinant microorganism produces at least one methoxy substituted tryptamine compound.

95. The recombinant microorganism of claim 94, wherein the at least one methoxy substituted tryptamine compound is 5-MeO-NMT, 5-MeO-DMT, or 5-MeO-TMT.

96. The recombinant microorganism of claim 71, expressing TPH and AADC, wherein the recombinant microorganism generates serotonin from L-tryptophan through a 5-HTP intermediate.

97. The recombinant microorganism of claim 71, expressing NAT and IOMT, wherein the recombinant microorganism generates melatonin from serotonin through an N-acetylserotonin intermediate.

98. The recombinant microorganism of claim 71, expressing DAC and INMT, wherein the recombinant microorganism generates 5-MeO-DMT from melatonin through a 5-MeO-tryptamine intermediate.

99. The recombinant microorganism of claim 71, expressing ATMT and AADC, wherein ATMT is a fusion polypeptide of aminotransferase and methyltransferase, wherein the recombinant microorganism produces meta-methylated tryptamine analogs.

100. The recombinant microorganism of claim 71, wherein the recombinant microorganism does not express Pdc5, Aro10, Aro7, Pdz1, Pdz2, Bna2, SPE2, Glc3 or any combination thereof.

101. The recombinant microorganism of claim 71, wherein the recombinant microorganism overexpresses an enzyme that promotes conversion of L-methionine to SAMe, does not express an off-pathway gene that encodes for enzymes that deplete SAMe for unwanted side products, overexpresses a permease, or any combination thereof.

102. The recombinant microorganism of claim 71, wherein the recombinant microorganism overexpresses a Sam2, Adk1, Mup1, Sam3.

103. The nucleic acid of claim 1, further comprising a promoter functional in a plant.

104. A plant expression cassette comprising the nucleic acid of claim 103.

105. A recombinant plant comprising the plant expression cassette of claim 104, capable of expressing the enzyme or regulatory protein encoded therein.

106. The recombinant plant of claim 105, expressing TrpM and TPH, wherein the recombinant plant produces at least one hydroxy substituted tryptophan compound.

107. The recombinant plant of claim 106, wherein the at least one hydroxy substituted tryptophan compound is 5-HTP, 5-OH-NMTP, 5-OH-DMTP or 5-OH-TMTP.

108. The recombinant plant of claim 105, expressing TrpM, TPH and IOMT, wherein the recombinant plant produces at least one methoxy substituted tryptophan compound.

109. The recombinant plant of claim 105, which is a tobacco or *Arabadopsis* plant.

110. A method of producing a substituted indole, the method comprising

(i) growing the recombinant microorganism of any one of claims 23-102 or the recombinant plant of any one of claims 105-109;

(ii) expressing the at least one enzyme or regulatory protein in the recombinant microorganism;

(iii) producing or synthesizing substituted indoles and tryptamines in the recombinant microorganism; and

(iv) isolating the substituted indole from the recombinant microorganism.

111. The method of claim 110, wherein precursor chemicals are added to the growing recombinant microorganism or plant, wherein the precursor chemicals are utilized by the at least one enzyme or regulatory protein.

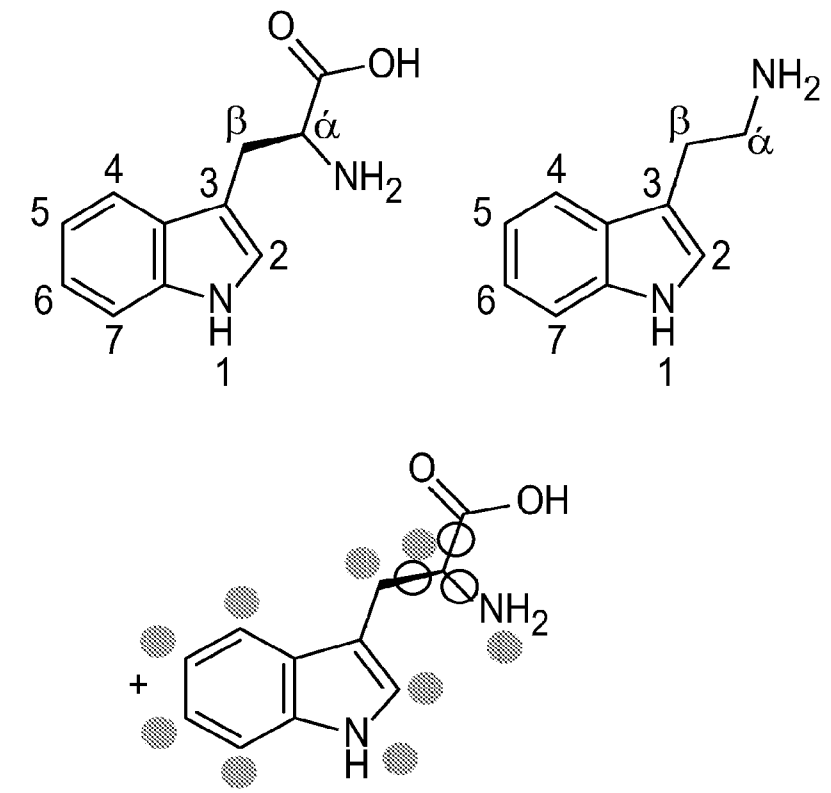

O
OH
β
α
NH2
4
3
5
2
6
N
H
7
1
NH2
β
α
4
3
5
2
6
N
H
7
1
O
OH
NH2
+
N
H
methylation
halogenation
prenylation
hydroxylation
O-phosphorylation
O-methylation
N-Acetylation
cleavage
deamination
decarboxylation
+ oligomerization

FIG. 1

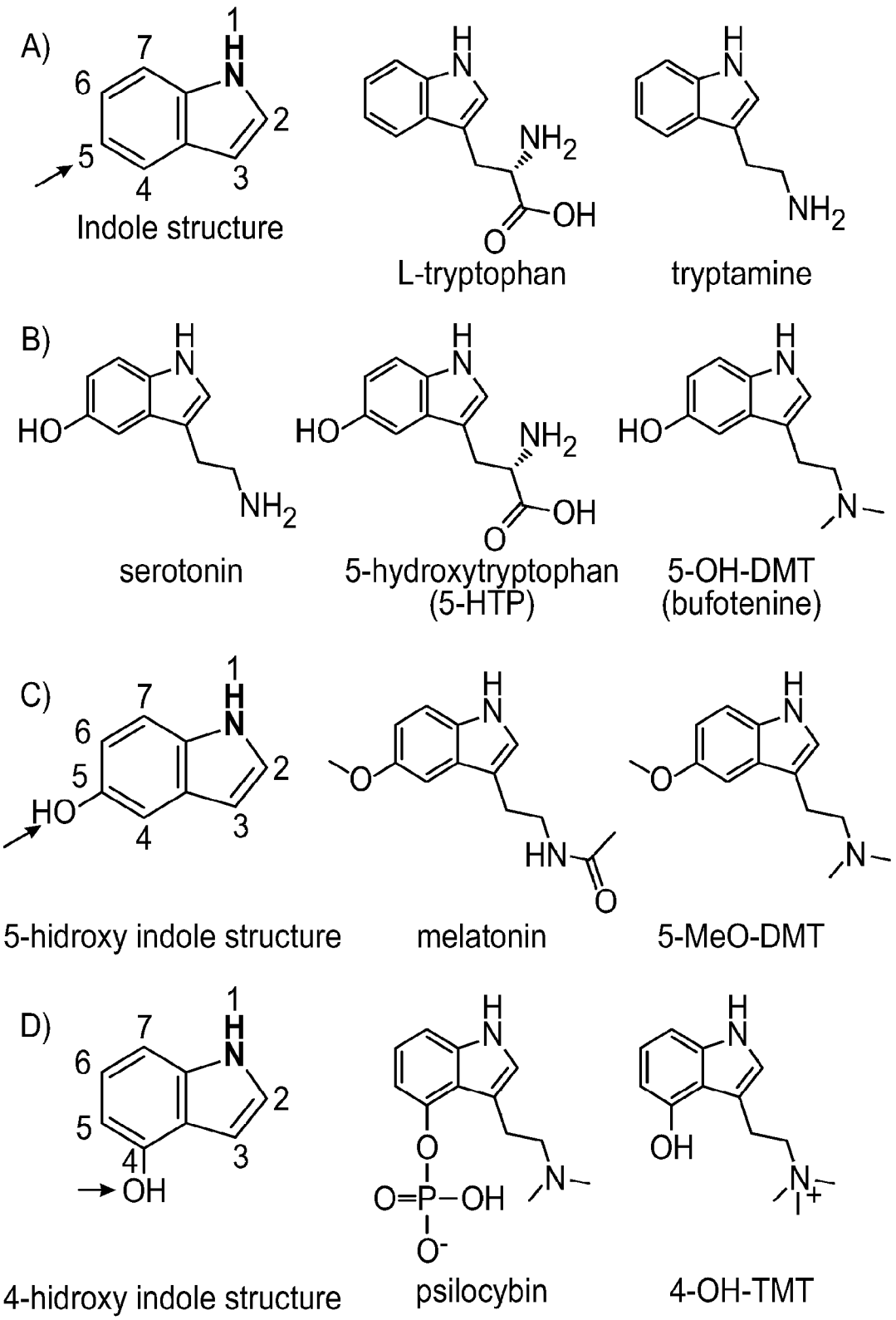

A)
Indole structure
L-tryptophan
tryptamine
B)
serotonin
5-hydroxytryptophan
(5-HTP)
5-OH-DMT
(bufotenine)
C)
5-hidroxy indole structure
melatonin
5-MeO-DMT
D)
4-hidroxy indole structure
psilocybin
4-OH-TMT

FIG. 2

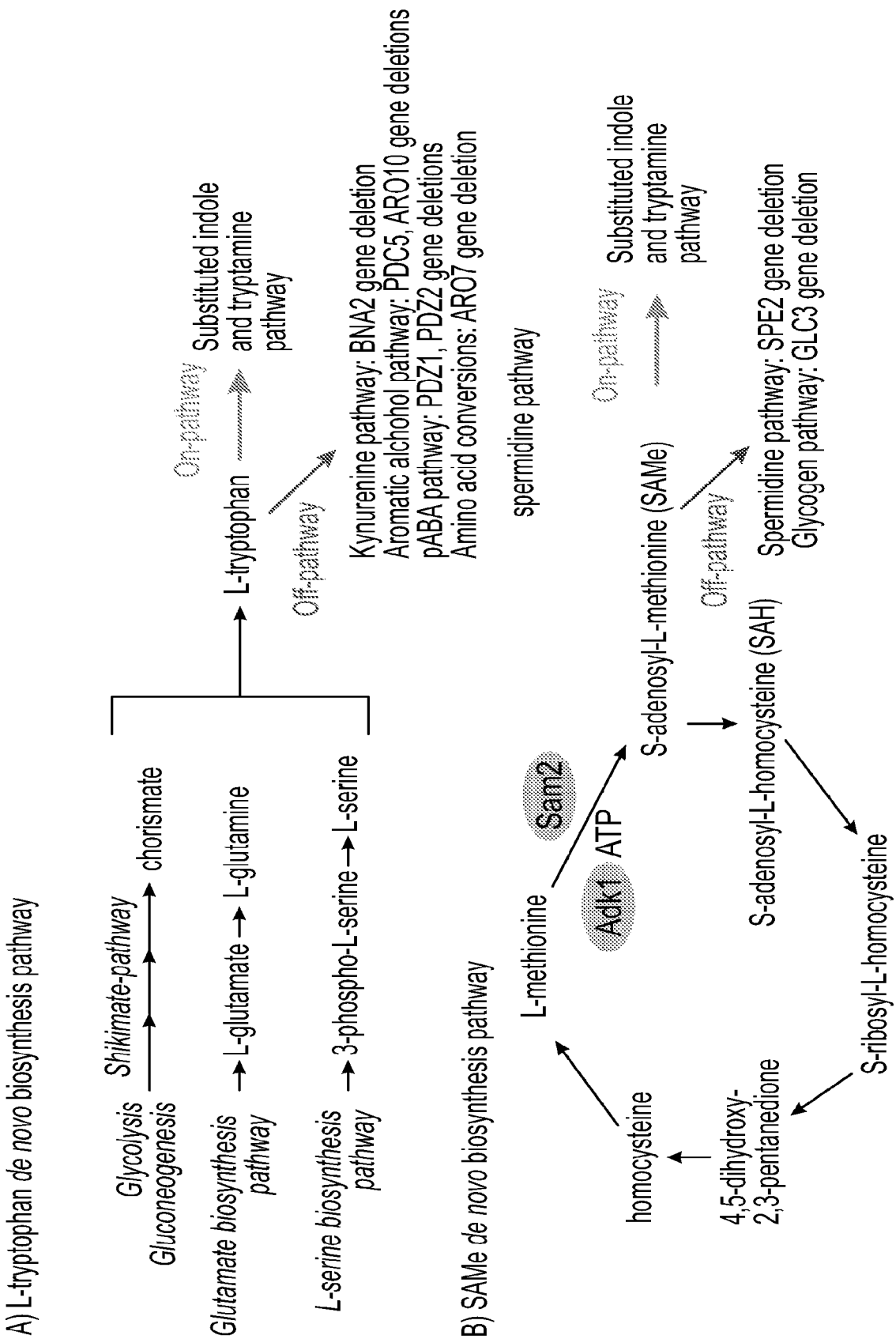

A) L-tryptophan de novo biosynthesis pathway
Glycolysis
Gluconeogenesis
Shikimate-pathway
chorismate
Glutamate biosynthesis pathway
L-glutamate
L-glutamine
L-serine biosynthesis pathway
3-phospho-L-serine
L-serine
L-tryptophan
On-pathway
Substituted indole and tryptamine pathway
Off-pathway
Kynurenine pathway: BNA2 gene deletion
Aromatic alchohol pathway: PDC5, ARO10 gene deletions
pABA pathway: PDZ1, PDZ2 gene deletions
Amino acid conversions: ARO7 gene deletion
B) SAMe de novo biosynthesis pathway
spermidine pathway
L-methionine
Sam2
Adk1
ATP
S-adenosyl-L-methionine (SAMe)
On-pathway
Substituted indole and tryptamine pathway
Off-pathway
homocysteine
S-adenosyl-L-homocysteine (SAH)
Spermidine pathway: SPE2 gene deletion
Glycogen pathway: GLC3 gene deletion
4,5-dihydroxy-2,3-pentanedione
S-ribosyl-L-homocysteine

FIG. 3

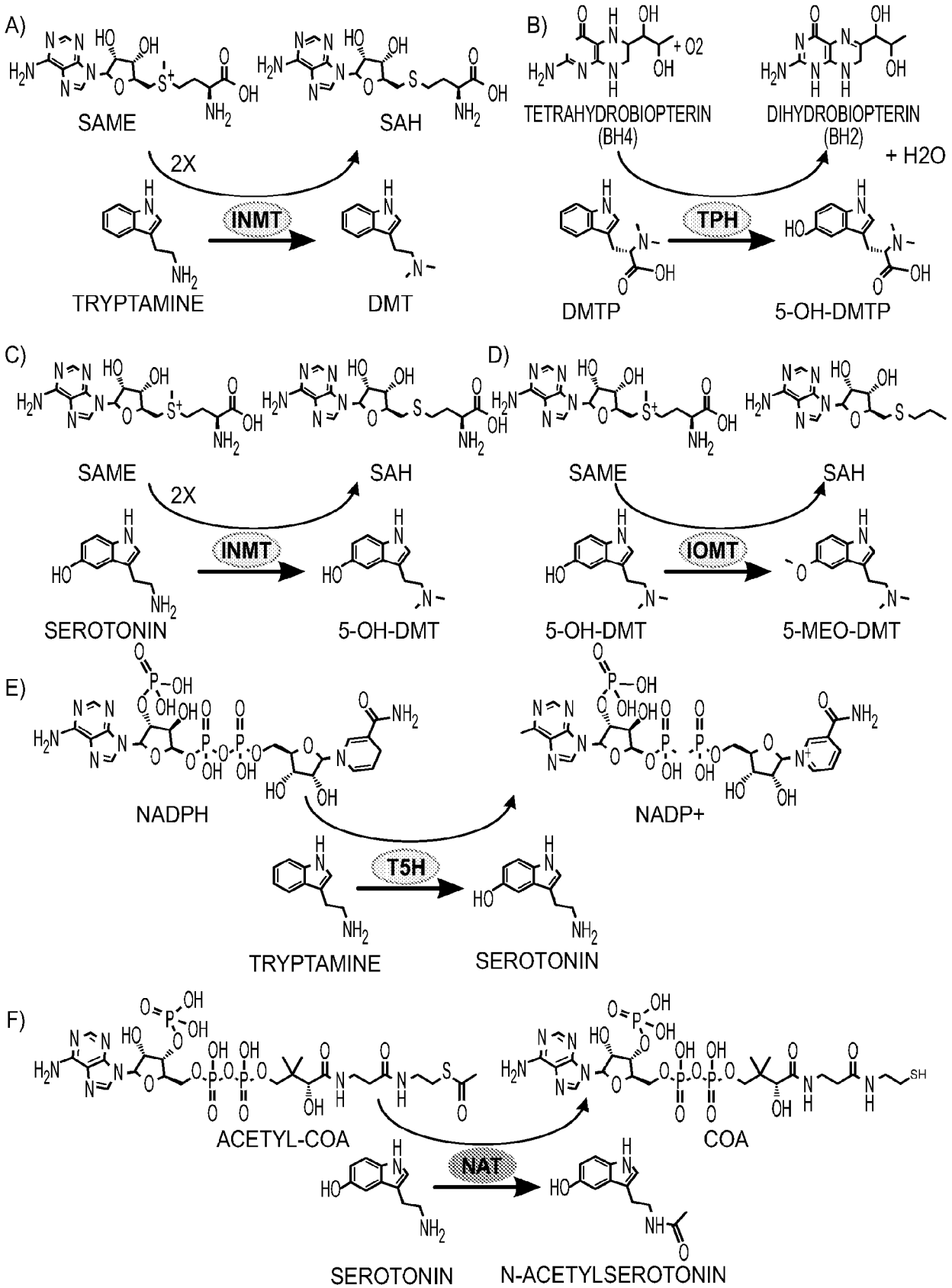

A)
SAME
SAH
2X
INMT
TRYPTAMINE
DMT
B)
TETRAHYDROBIOPTERIN (BH4)
DIHYDROBIOPTERIN (BH2)
+ O2
+ H2O
TPH
DMTP
5-OH-DMTP
C)
SAME
SAH
2X
INMT
SEROTONIN
5-OH-DMT
D)
SAME
SAH
IOMT
5-OH-DMT
5-MEO-DMT
E)
NADPH
NADP+
T5H
TRYPTAMINE
SEROTONIN
F)
ACETYL-COA
COA
NAT
SEROTONIN
N-ACETYLSEROTONIN

FIG. 4

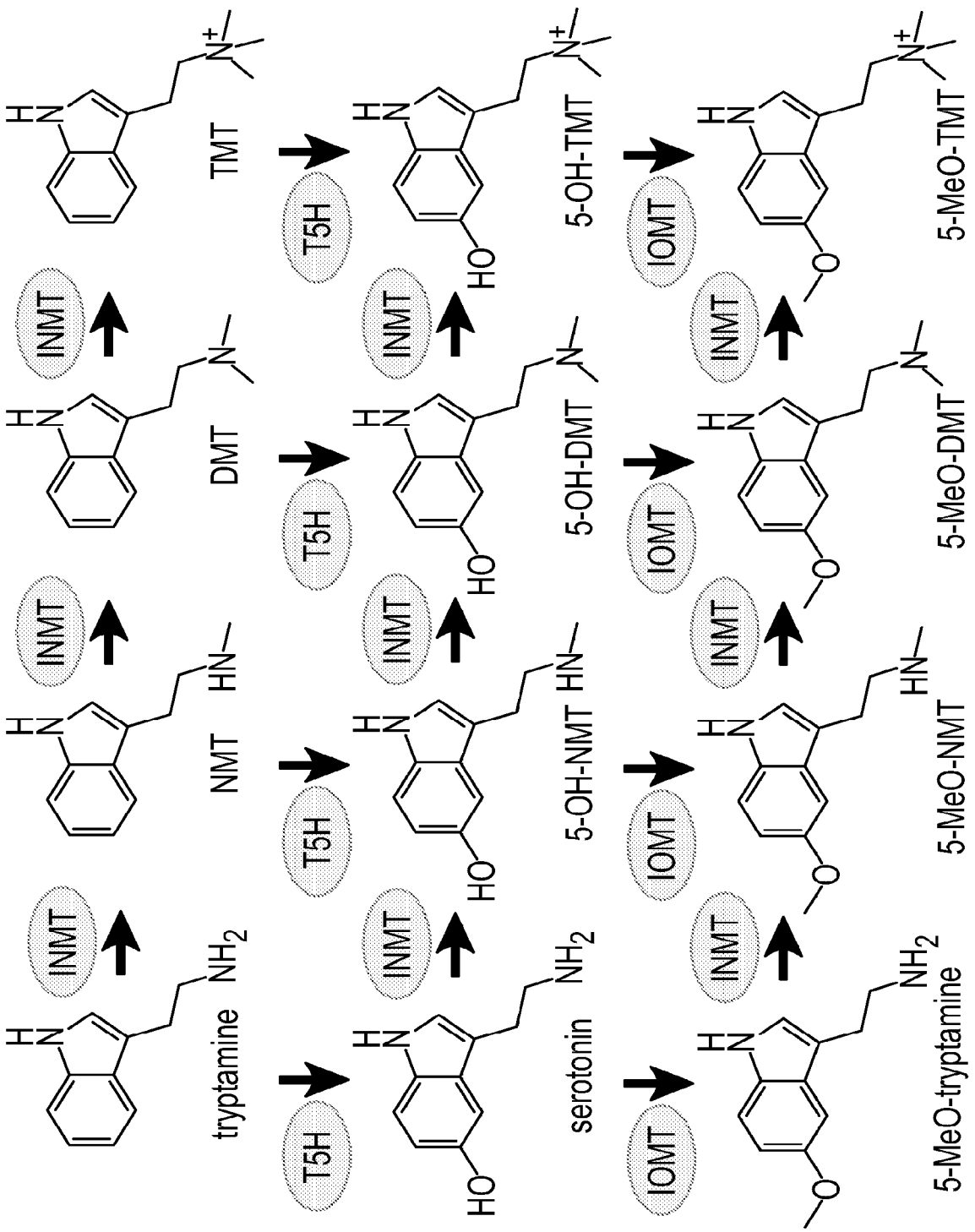

tryptamine
NMT
DMT
TMT
serotonin
5-OH-NMT
5-OH-DMT
5-OH-TMT
5-MeO-tryptamine
5-MeO-NMT
5-MeO-DMT
5-MeO-TMT
INMT
T5H
IOMT

FIG. 5

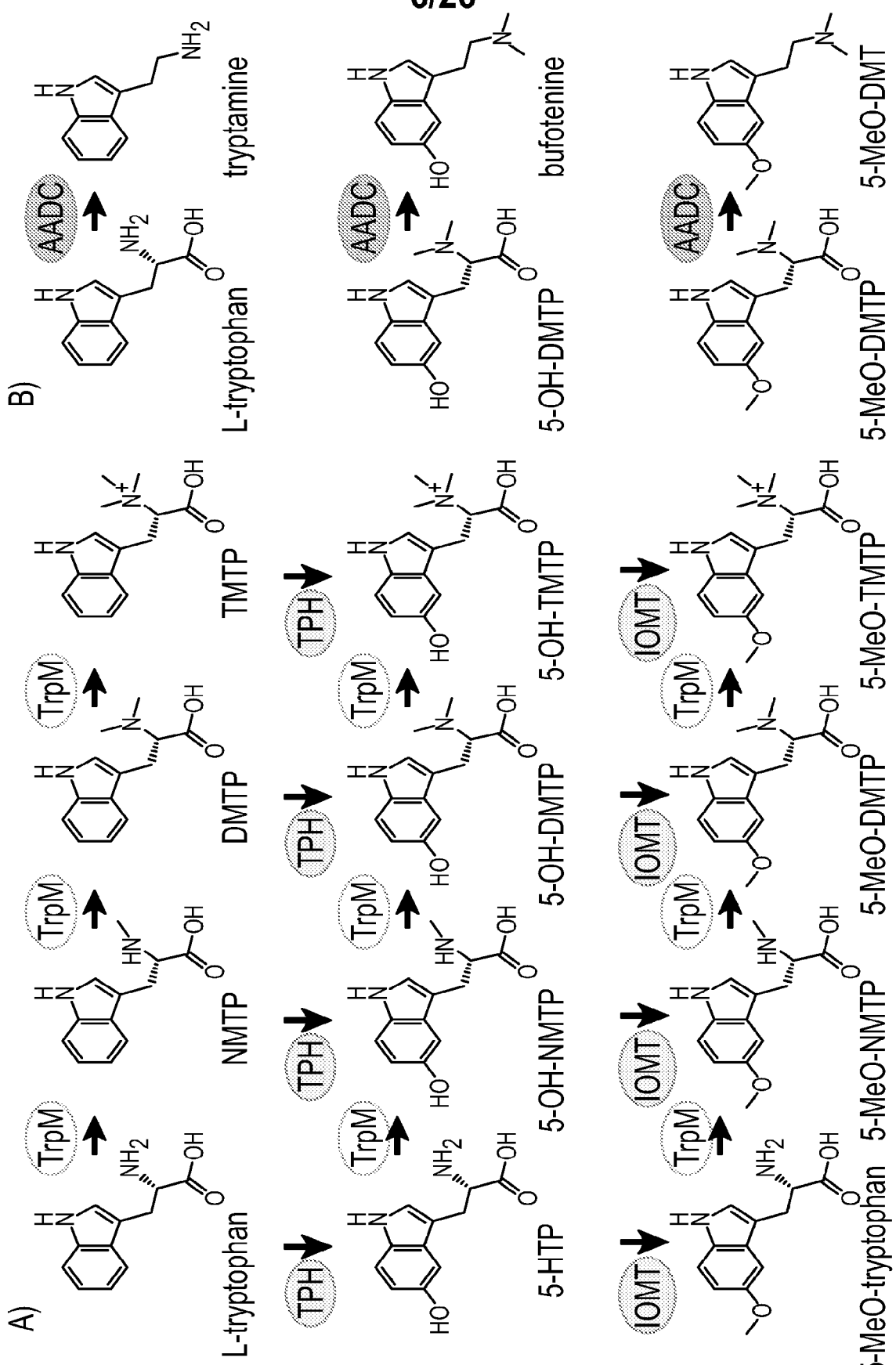

A)
L-tryptophan
TrpM
NMTP
TrpM
DMTP
TrpM
TMTP
TPH
5-HTP
TrpM
5-OH-NMTP
TrpM
5-OH-DMTP
TrpM
5-OH-TMTP
IOMT
5-MeO-tryptophan
TrpM
5-MeO-NMTP
TrpM
5-MeO-DMTP
TrpM
5-MeO-TMTP
B)
L-tryptophan
AADC
tryptamine
5-OH-DMTP
AADC
bufotenine
5-MeO-DMTP
AADC
5-MeO-DMT

FIG. 6

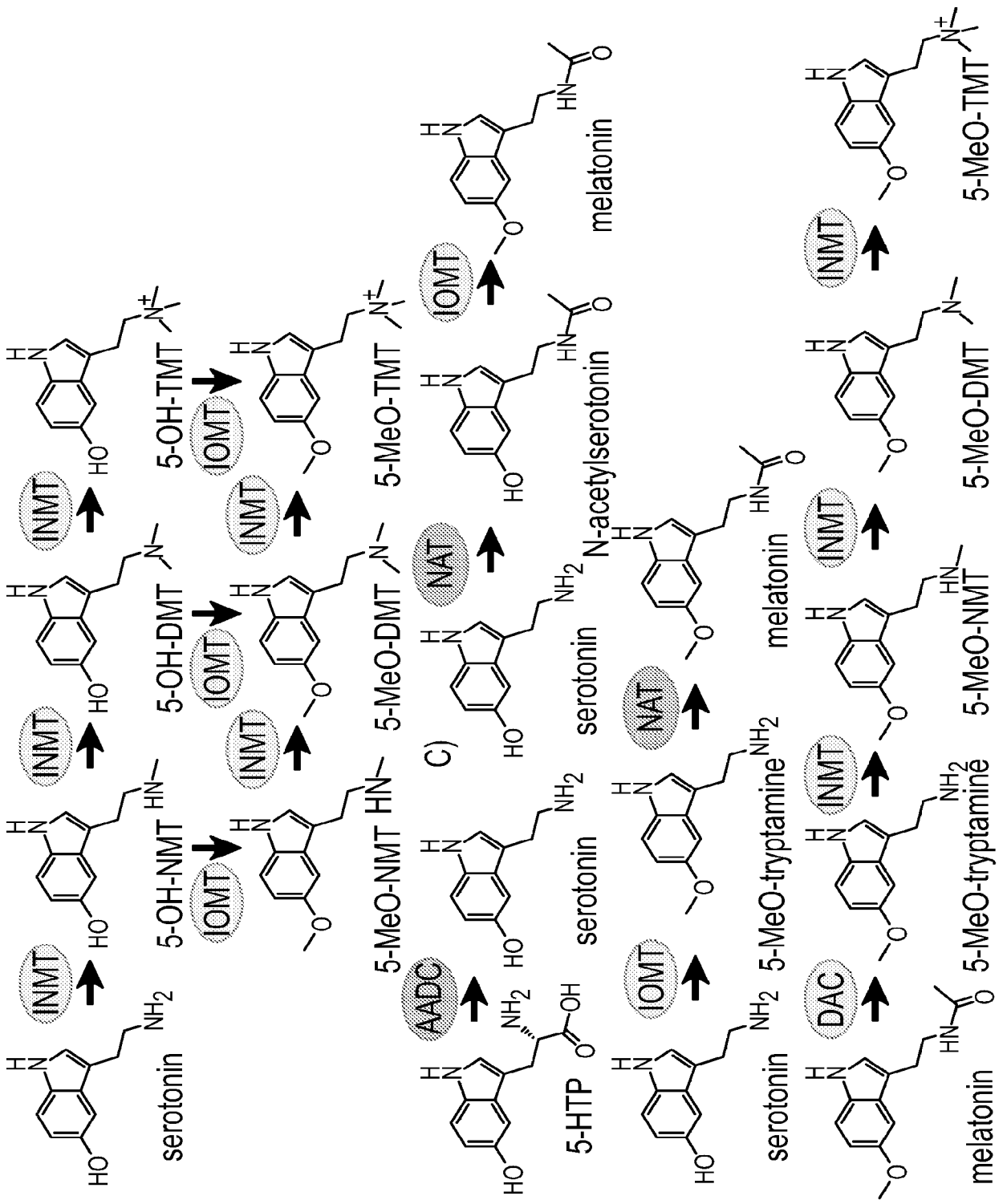

A)
serotonin
INMT
5-OH-NMT
INMT
5-OH-DMT
INMT
5-OH-TMT
IOMT
5-MeO-NMT
IOMT
5-MeO-DMT
IOMT
5-MeO-TMT
B)
5-HTP
AADC
serotonin
NAT
N-acetylserotonin
IOMT
melatonin
C)
D)
serotonin
IOMT
5-MeO-tryptamine
NAT
melatonin
E)
serotonin
DAC
5-MeO-tryptamine
INMT
5-MeO-NMT
INMT
5-MeO-DMT
INMT
5-MeO-TMT
melatonin

FIG. 7

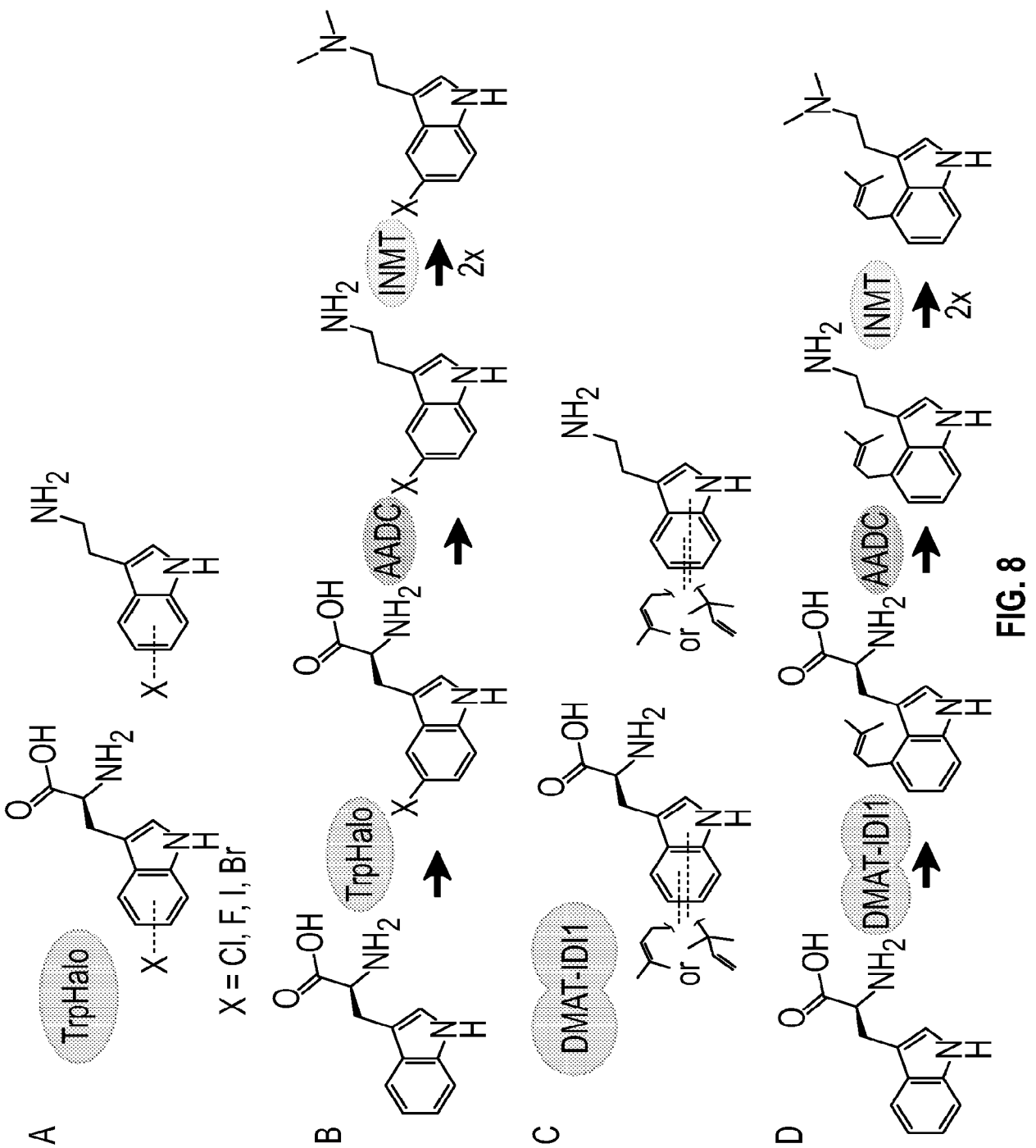

A
TrpHalo
X = Cl, F, I, Br
B
TrpHalo
AADC
INMT
2x
C
DMAT-IDI1
or
D
DMAT-IDI1
AADC
INMT
2x

FIG. 8

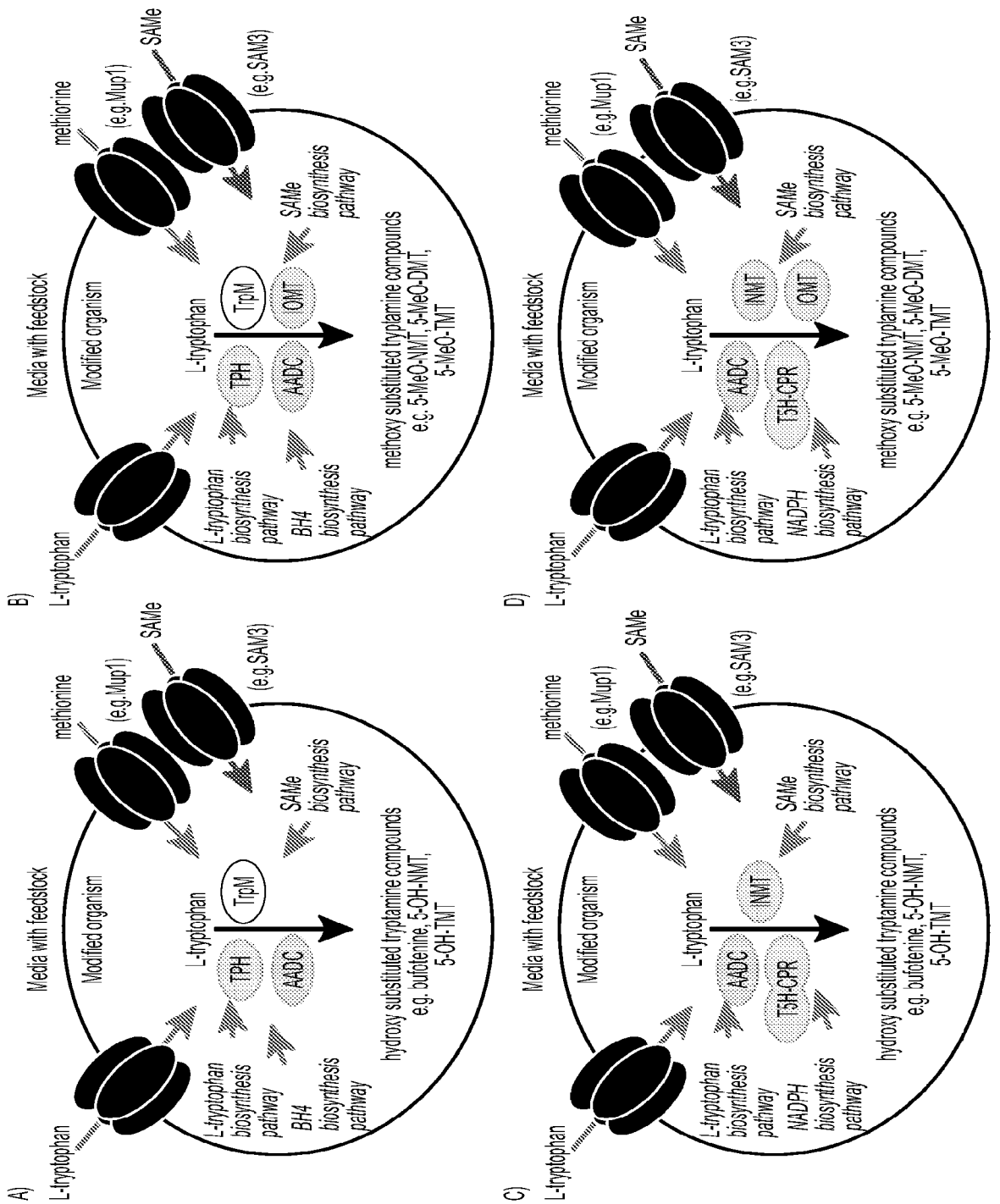

A)
L-tryptophan
Media with feedstock
Modified organism
methionine
(e.g.Mup1)
SAMe
(e.g.SAM3)
L-tryptophan
TPH
TrpM
AADC
L-tryptophan biosynthesis pathway
BH4 biosynthesis pathway
SAMe biosynthesis pathway
hydroxy substituted tryptamine compounds
e.g. bufotenine, 5-OH-NMT,
5-OH-TMT
B)
L-tryptophan
Media with feedstock
Modified organism
methionine
(e.g.Mup1)
SAMe
(e.g.SAM3)
L-tryptophan
TPH
TrpM
AADC
OMT
L-tryptophan biosynthesis pathway
BH4 biosynthesis pathway
SAMe biosynthesis pathway
methoxy substituted tryptamine compounds
e.g. 5-MeO-NMT, 5-MeO-DMT,
5-MeO-TMT
C)
L-tryptophan
Media with feedstock
Modified organism
methionine
(e.g.Mup1)
SAMe
(e.g.SAM3)
L-tryptophan
AADC
INMT
T5H-CPR
L-tryptophan biosynthesis pathway
NADPH biosynthesis pathway
SAMe biosynthesis pathway
hydroxy substituted tryptamine compounds
e.g. bufotenine, 5-OH-NMT,
5-OH-TMT
D)
L-tryptophan
Media with feedstock
Modified organism
methionine
(e.g.Mup1)
SAMe
(e.g.SAM3)
L-tryptophan
AADC
INMT
IOMT
T5H-CPR
L-tryptophan biosynthesis pathway
NADPH biosynthesis pathway
SAMe biosynthesis pathway
methoxy substituted tryptamine compounds
e.g. 5-MeO-NMT, 5-MeO-DMT,
5-MeO-TMT

FIG. 9

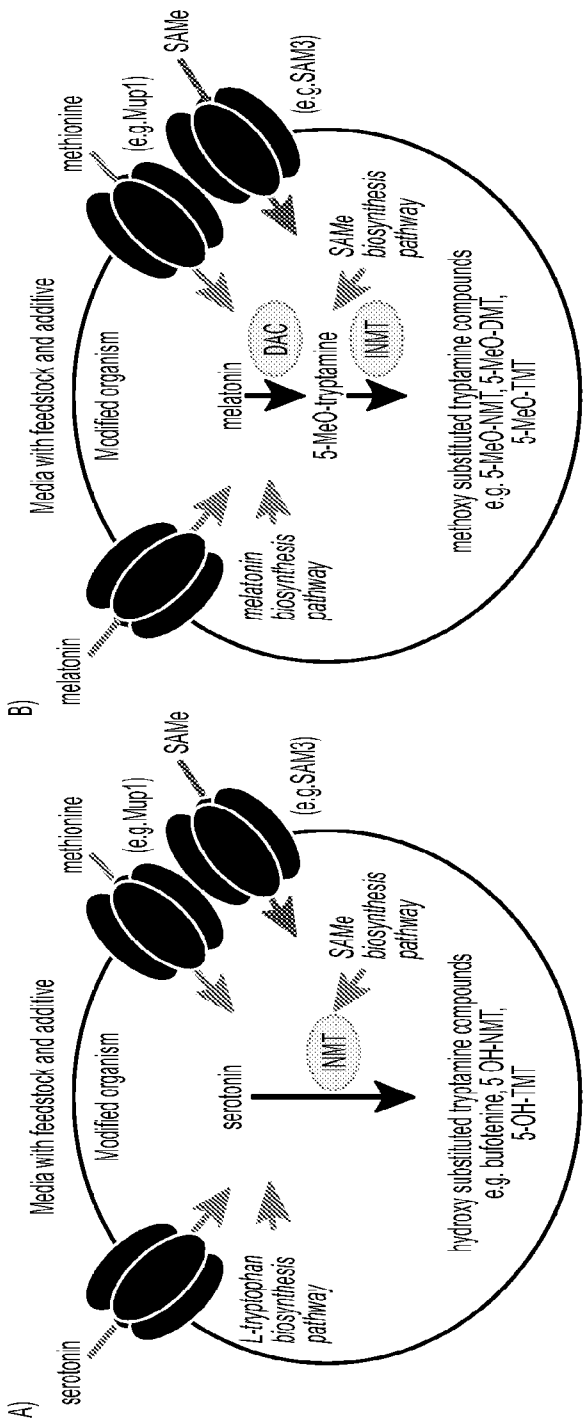

A)
serotonin
L-tryptophan biosynthesis pathway
Media with feedstock and additive
Modified organism
serotonin
NMT
hydroxy substituted tryptamine compounds
e.g. bufotenine, 5 OH-NMT,
5-OH-TMT
SAMe biosynthesis pathway
methionine
(e.g.Mup1)
SAMe
(e.g.SAM3)
B)
melatonin
melatonin biosynthesis pathway
Media with feedstock and additive
Modified organism
melatonin
DAC
5-MeO-tryptamine
NMT
methoxy substituted tryptamine compounds
e.g. 5-MeO-NMT, 5-MeO-DMT,
5-MeO-TMT
SAMe biosynthesis pathway
methionine
(e.g.Mup1)
SAMe
(e.g.SAM3)

FIG. 10

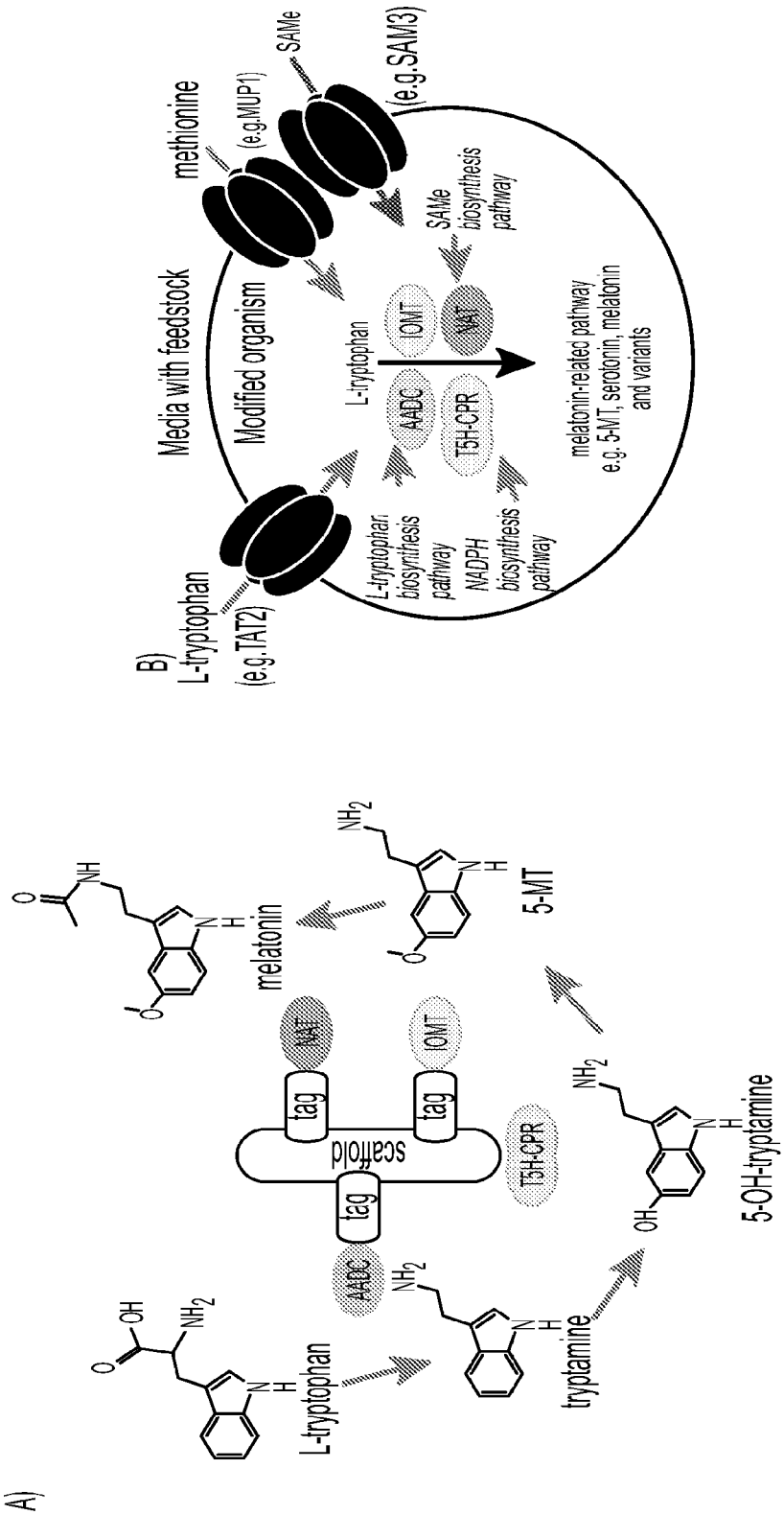

A)
L-tryptophan
tryptamine
5-OH-tryptamine
5-MT
melatonin
AADC
T5H-CPR
IOMT
NAT
tag
scaffold
B)
L-tryptophan
(e.g.TAT2)
Media with feedstock
Modified organism
methionine
(e.g.MUP1)
SAMe
(e.g.SAM3)
L-tryptophan biosynthesis pathway
NADPH biosynthesis pathway
SAMe biosynthesis pathway
melatonin-related pathway
e.g. 5-MT, serotonin, melatonin and variants

FIG. 11

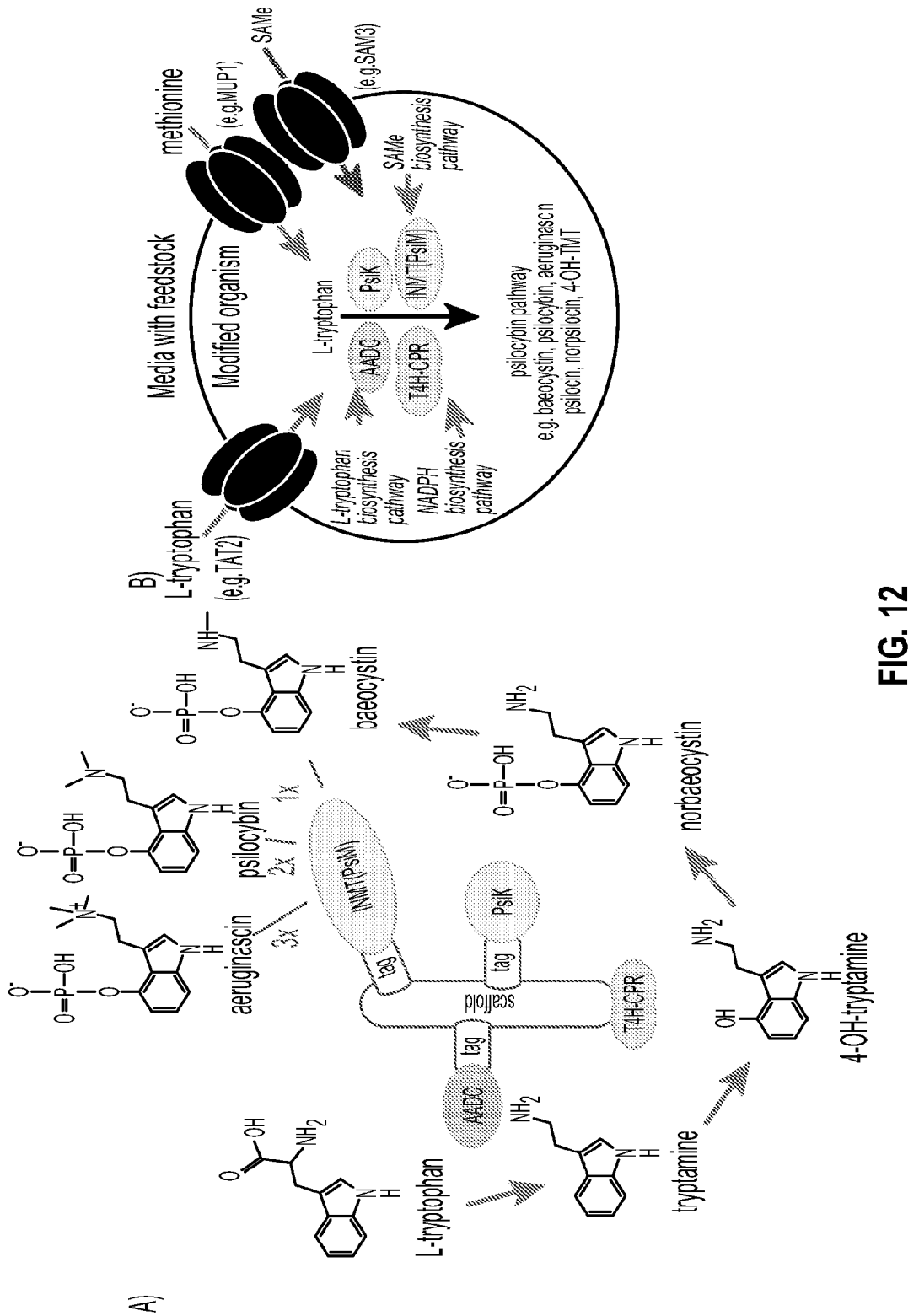

A)
L-tryptophan
AADC
tryptamine
4-OH-tryptamine
T4H-CPR
scaffold
tag
PsiK
INMTPsiM
norbaeocystin
baeocystin
psilocybin
2x
1x
aeruginascin
3x
B)
L-tryptophan
(e.g.TAT2)
Media with feedstock
Modified organism
methionine
(e.g.MUP1)
SAMe
(e.g.SAM3)
L-tryptophan biosynthesis pathway
NADPH biosynthesis pathway
SAMe biosynthesis pathway
psilocybin pathway
e.g. baeocystin, psilocybin, aeruginascin
psilocin, norpsilocin, 4-OH-TMT

FIG. 12

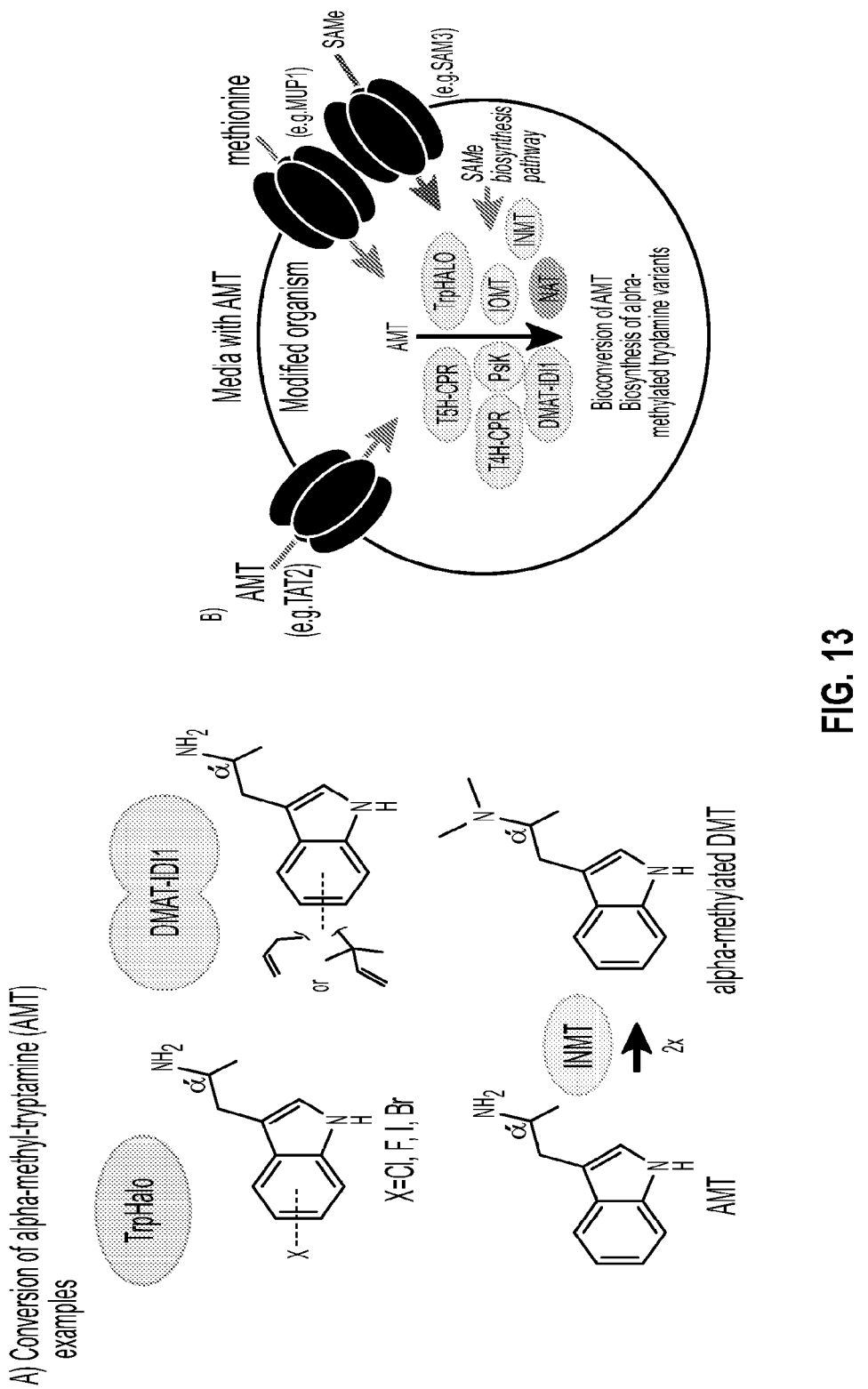
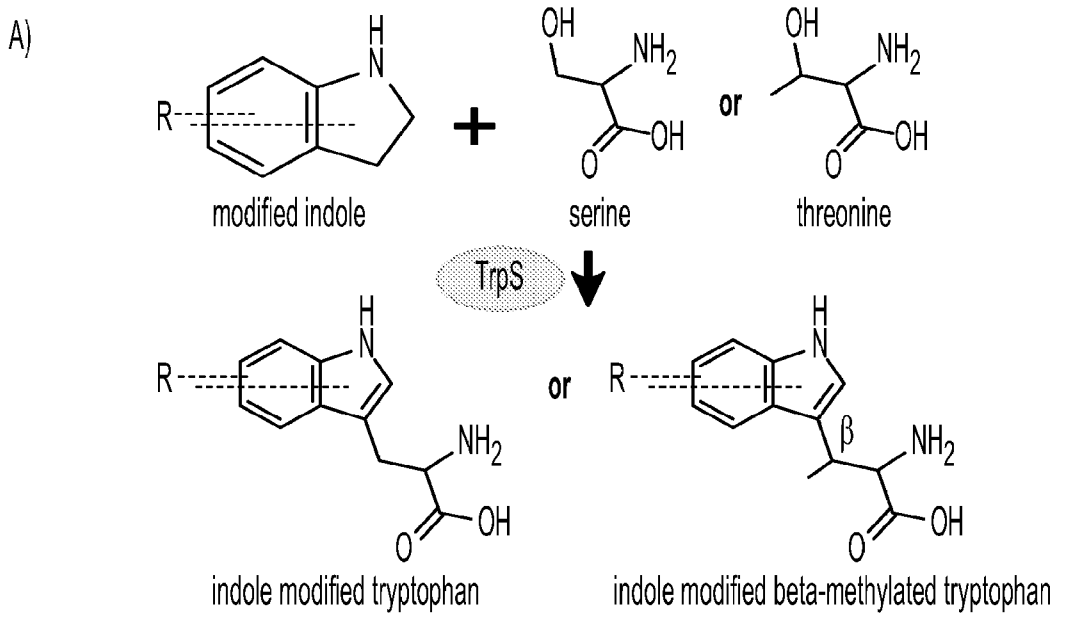

A) Conversion of alpha-methyl-tryptamine (AMT) examples
TrpHalo
DMAT-ID1
X=Cl, F, I, Br
or
INMT
2x
AMT
alpha-methylated DMT
B)
AMT
(e.g.TAT2)
Media with AMT
Modified organism
methionine
(e.g.MUP1)
SAMe
(e.g.SAM3)
SAMe biosyntheis pathway
AMT
TrpHALO
OMT
INMT
NMT
T5H-CPR
PsiK
T4H-CPR
DMAT-ID11
Bioconversion of AMT
Biosynthesis of alpha-methylated tryptamine variants

FIG. 13


A)
R
H
N
modified indole
+
OH
NH2
O
OH
serine
or
OH
NH2
O
OH
threonine
TrpS
R
H
N
NH2
O
OH
indole modified tryptophan
or
R
H
N
β
NH2
O
OH
indole modified beta-methylated tryptophan

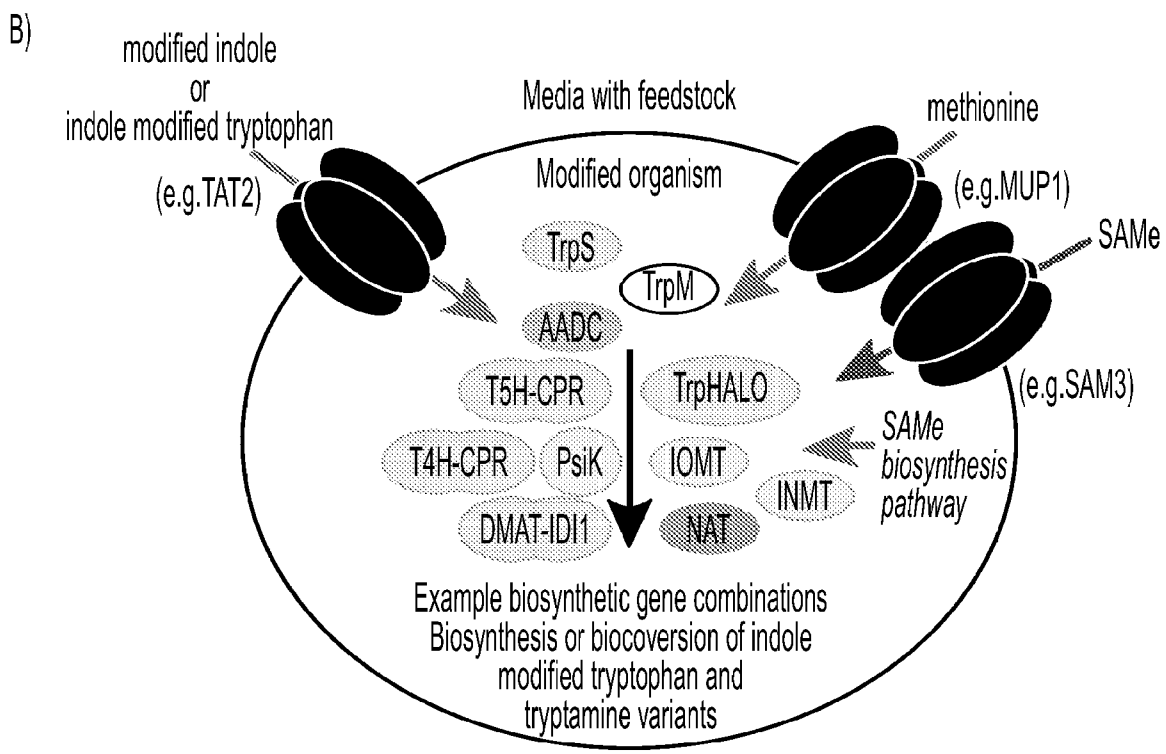
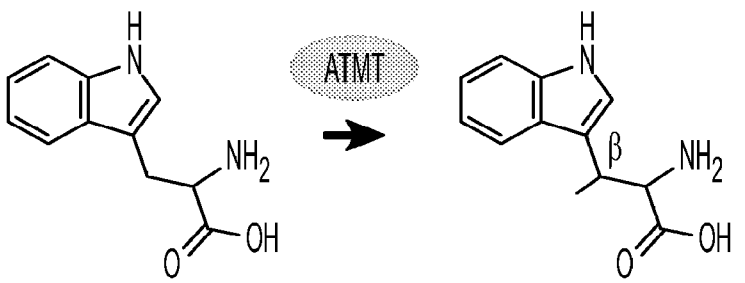

B)
modified indole
or
indole modified tryptophan
(e.g.TAT2)
Media with feedstock
Modified organism
methionine
(e.g.MUP1)
SAMe
(e.g.SAM3)
TrpS
TrpM
AADC
T5H-CPR
TrpHALO
T4H-CPR
PsiK
IOMT
INMT
DMAT-IDI1
NAT
SAMe biosynthesis pathway
Example biosynthetic gene combinations
Biosynthesis or biocoversion of indole modified tryptophan and tryptamine variants

FIG. 14

A)


H
N
NH2
OH
O
ATMT
β
tryptophan
beta-methylated tryptophan

B)

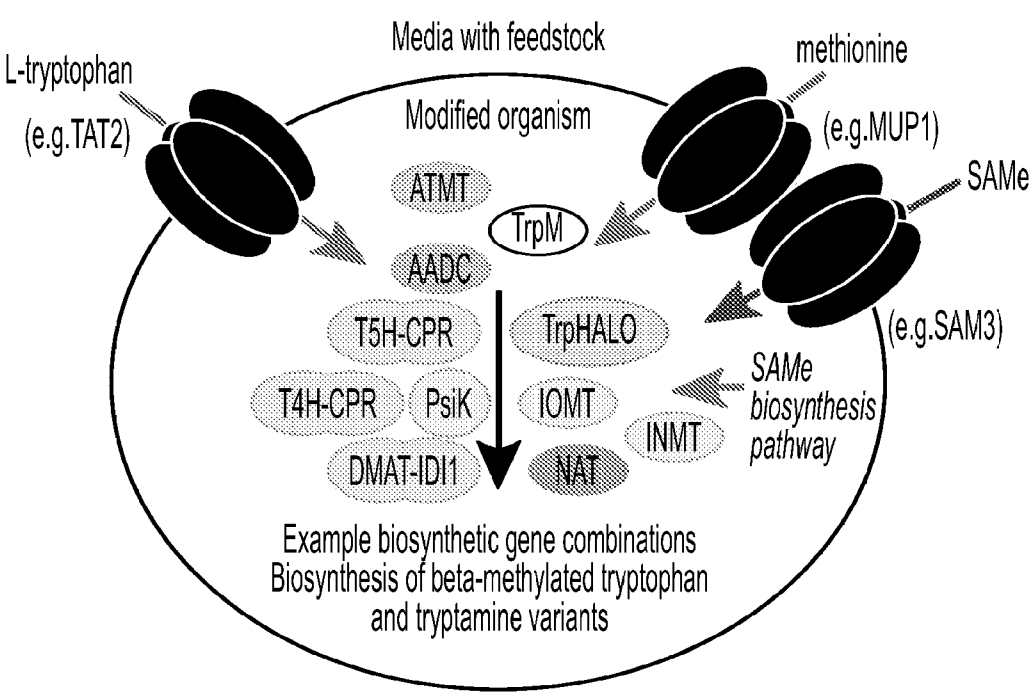

Media with feedstock
L-tryptophan
(e.g.TAT2)
Modified organism
methionine
(e.g.MUP1)
SAMe
(e.g.SAM3)
ATMT
TrpM
AADC
T5H-CPR
TrpHALO
T4H-CPR
PsiK
IOMT
INMT
DMAT-IDI1
NAT
SAMe biosynthesis pathway
Example biosynthetic gene combinations
Biosynthesis of beta-methylated tryptophan
and tryptamine variants

FIG. 15

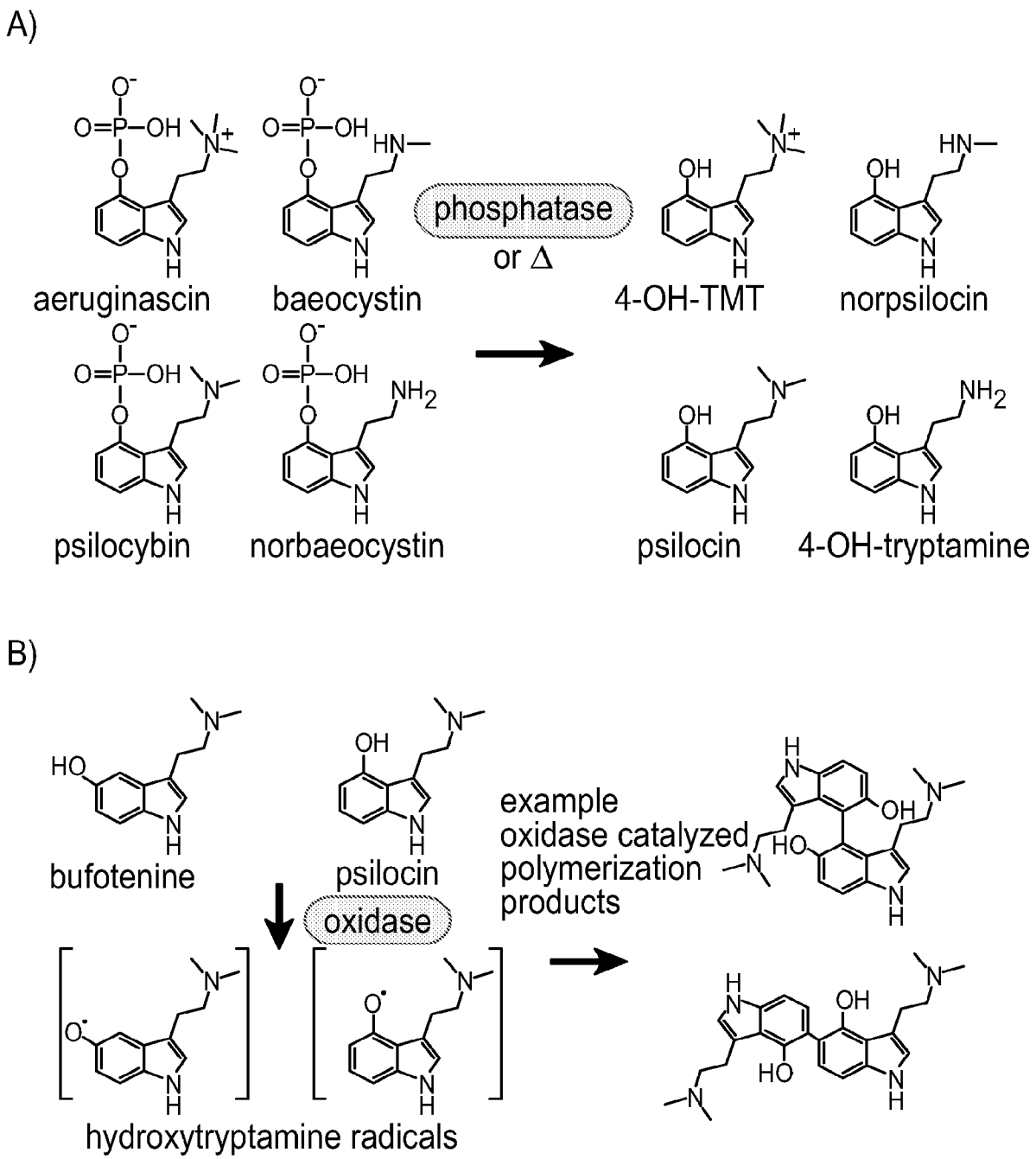

A)
aeruginascin
baeocystin
phosphatase
or Δ
4-OH-TMT
norpsilocin
psilocybin
norbaeocystin
psilocin
4-OH-tryptamine
B)
bufotenine
psilocin
oxidase
example
oxidase catalyzed
polymerization
products
hydroxytryptamine radicals

FIG. 16

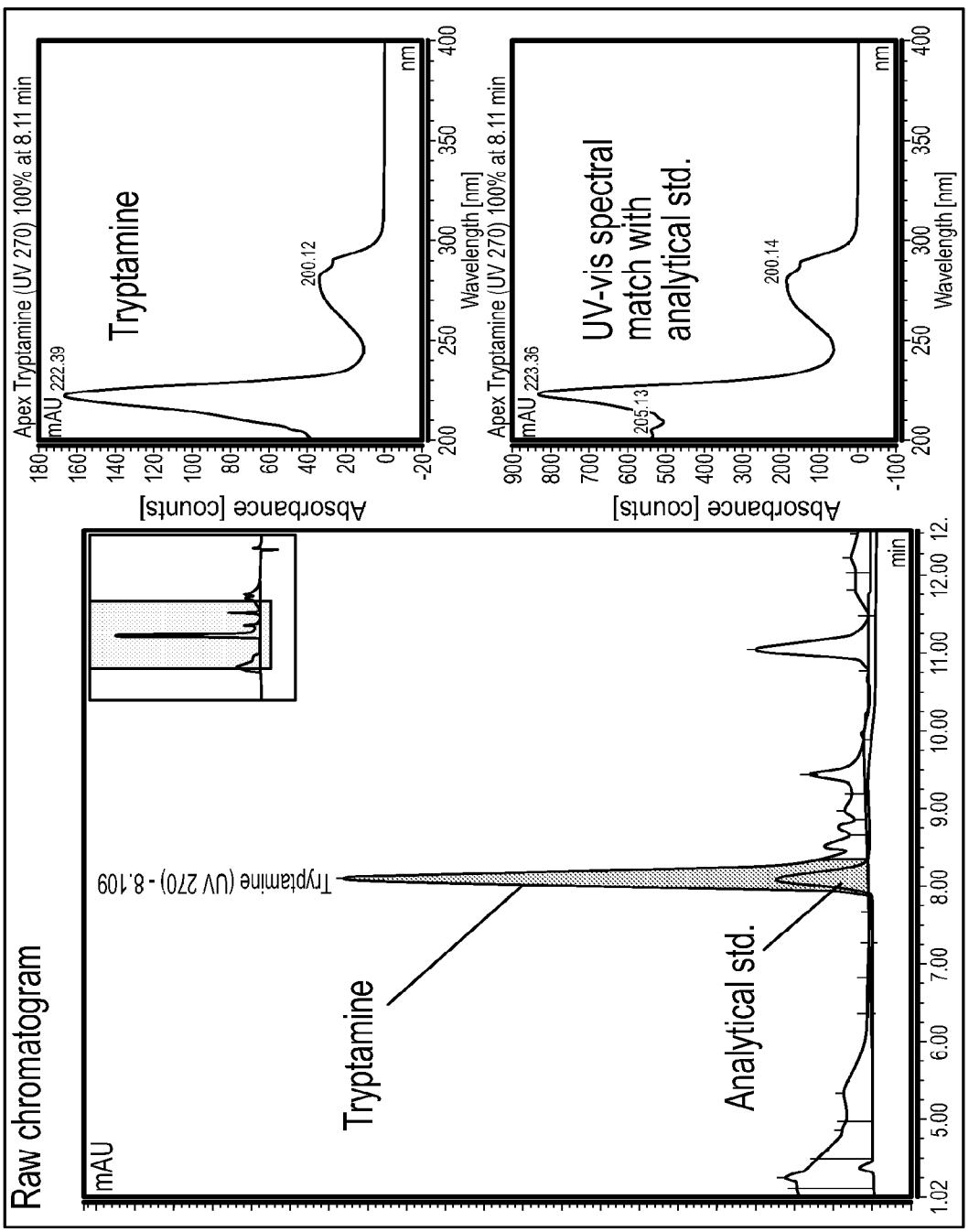

Raw chromatogram
mAU
Tryptamine (UV 270) - 8.109
Tryptamine
Analytical std.
min
1.02
5.00
6.00
7.00
8.00
9.00
10.00
11.00
12.00
12.
Absorbance [counts]
Apex Tryptamine (UV 270) 100% at 8.11 min
mAU
222.39
Tryptamine
200.12
nm
Wavelength [nm]
Absorbance [counts]
Apex Tryptamine (UV 270) 100% at 8.11 min
mAU
223.36
UV-vis spectral match with analytical std.
205.13
200.14
nm
Wavelength [nm]

FIG. 17

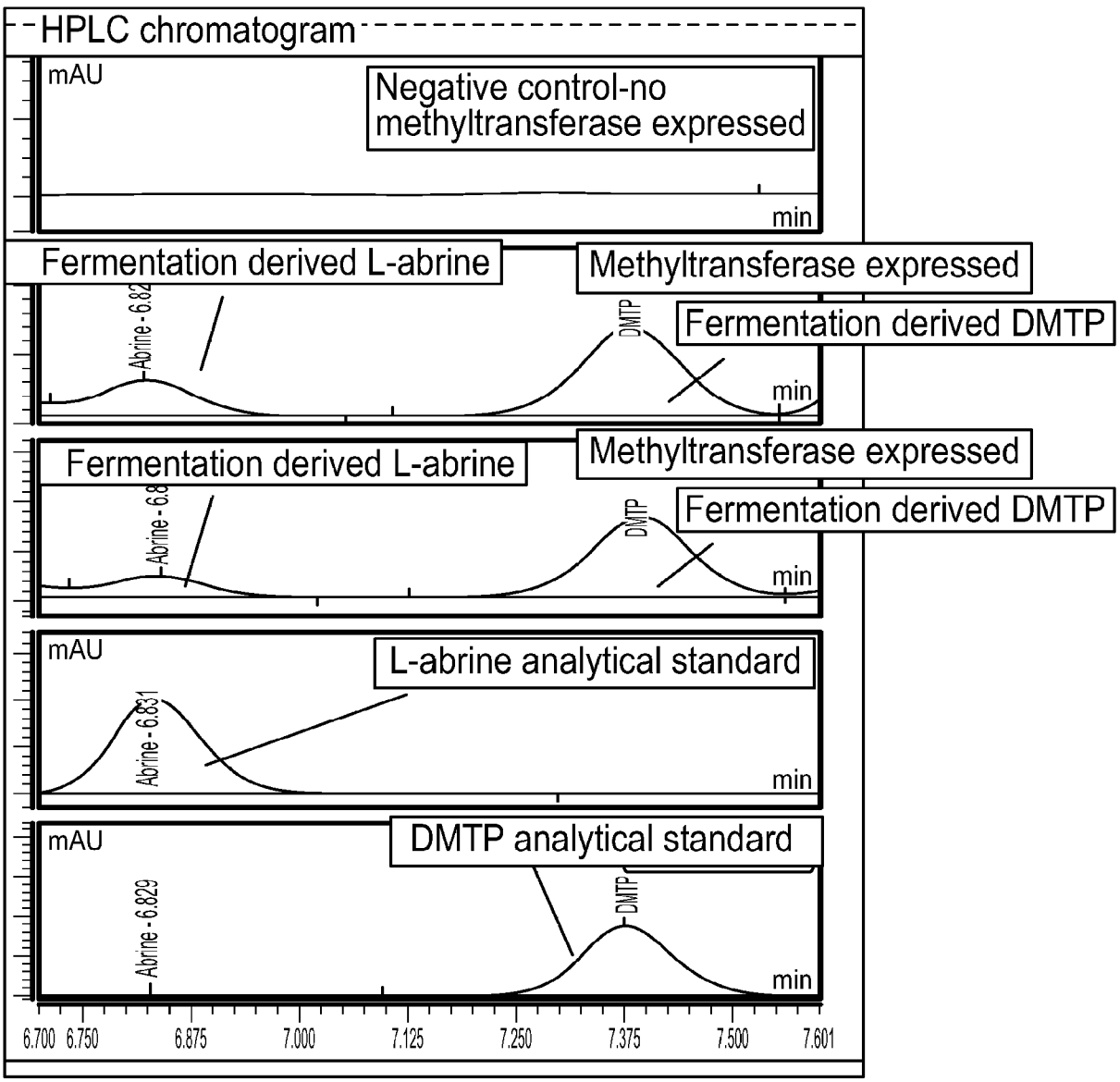

HPLC chromatogram
mAU
Negative control-no methyltransferase expressed
min
Fermentation derived L-abrine
Methyltransferase expressed
Fermentation derived DMTP
Abrine - 6.82
DMTP
min
Fermentation derived L-abrine
Methyltransferase expressed
Fermentation derived DMTP
Abrine - 6.8
DMTP
min
mAU
L-abrine analytical standard
Abrine - 6.831
min
mAU
DMTP analytical standard
Abrine - 6.829
DMTP
min
6.700 6.750 6.875 7.000 7.125 7.250 7.375 7.500 7.601

FIG. 18

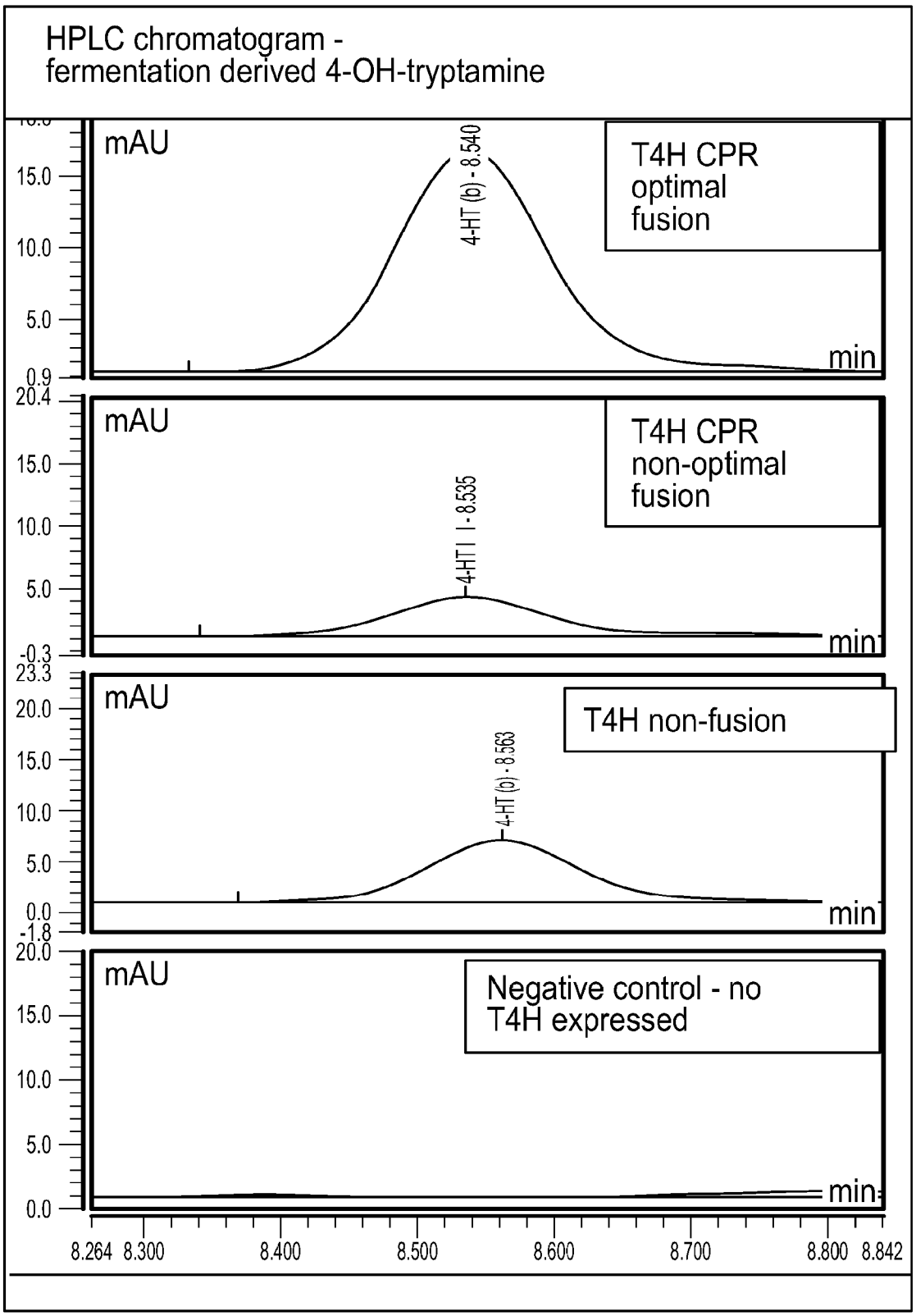

HPLC chromatogram -
fermentation derived 4-OH-tryptamine
mAU
4-HT (b) - 8.540
T4H CPR
optimal
fusion
min
mAU
4-HT I - 8.535
T4H CPR
non-optimal
fusion
min
mAU
4-HT (b) - 8.563
T4H non-fusion
min
mAU
Negative control - no
T4H expressed
min
8.264 8.300 8.400 8.500 8.600 8.700 8.800 8.842

FIG. 19

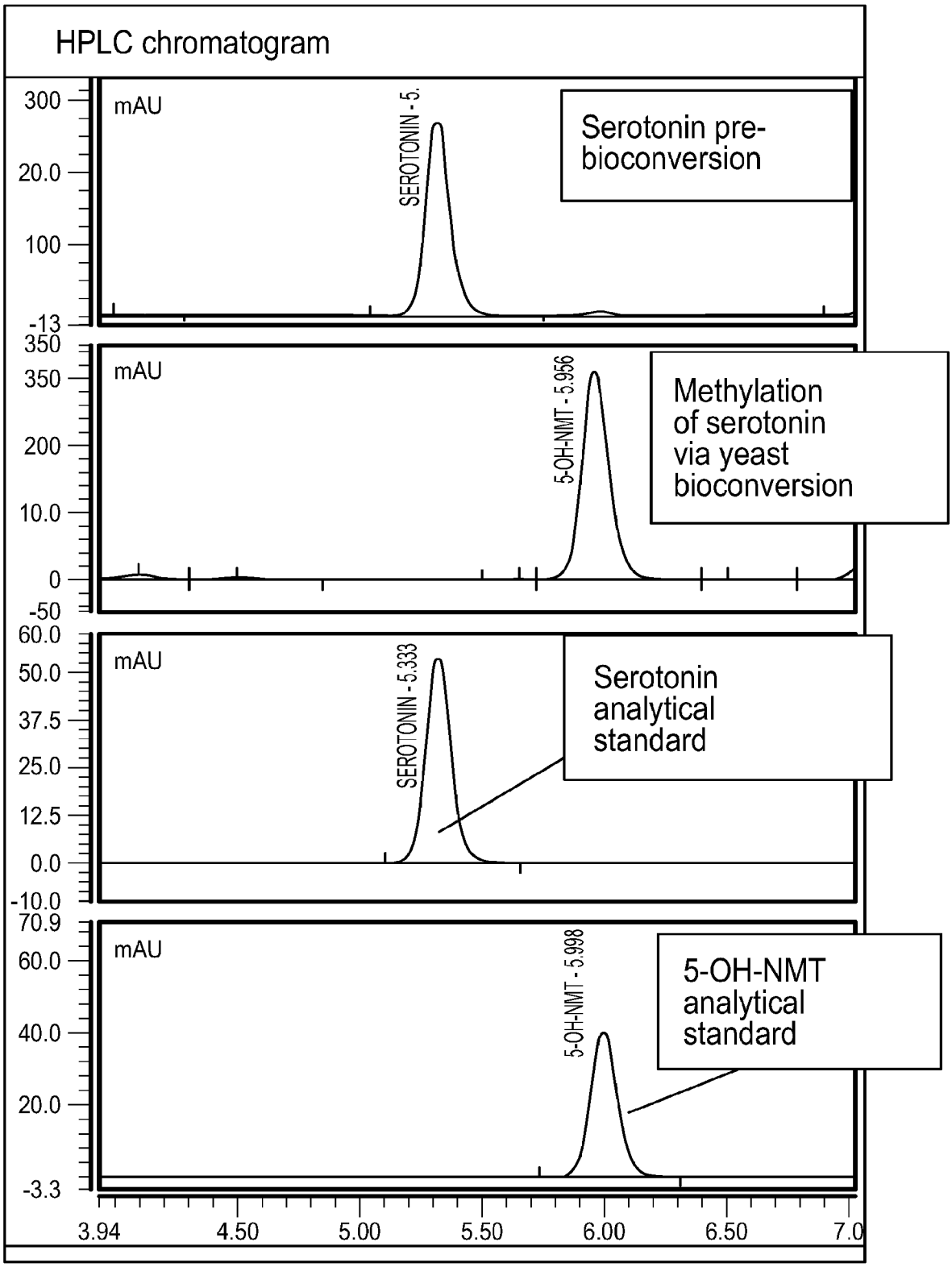

HPLC chromatogram
mAU
SEROTONIN - 5.
Serotonin pre-bioconversion
Methylation of serotonin via yeast bioconversion
5-OH-NMT - 5.956
Serotonin analytical standard
SEROTONIN - 5.333
5-OH-NMT analytical standard
5-OH-NMT - 5.998
3.94
4.50
5.00
5.50
6.00
6.50
7.0

FIG. 20

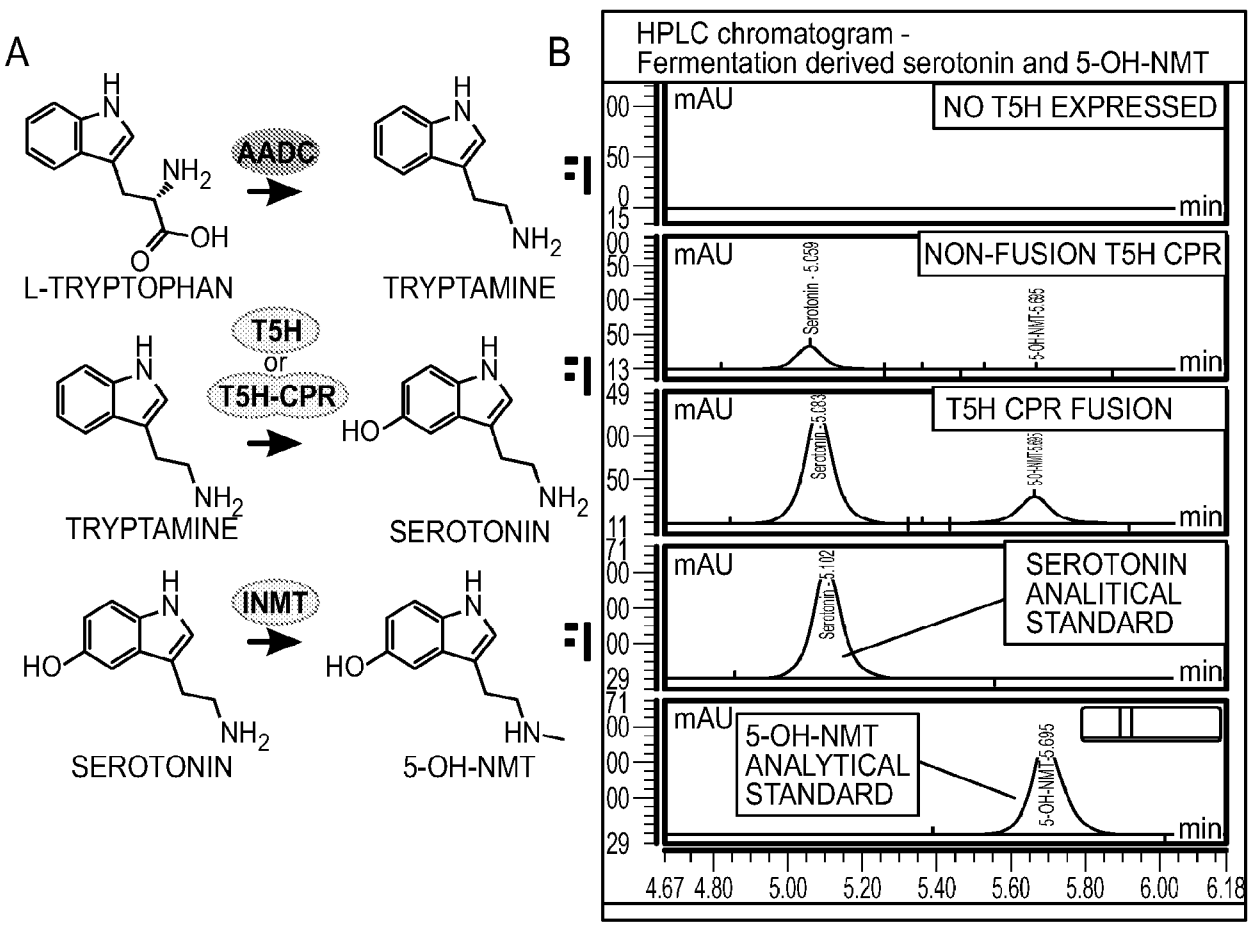

A
B
L-TRYPTOPHAN
AADC
TRYPTAMINE
T5H
or
T5H-CPR
TRYPTAMINE
SEROTONIN
INMT
SEROTONIN
5-OH-NMT
HPLC chromatogram -
Fermentation derived serotonin and 5-OH-NMT
NO T5H EXPRESSED
NON-FUSION T5H CPR
T5H CPR FUSION
SEROTONIN ANALITICAL STANDARD
5-OH-NMT ANALYTICAL STANDARD
mAU
min
4.67 4.80 5.00 5.20 5.40 5.60 5.80 6.00 6.18

FIG. 21

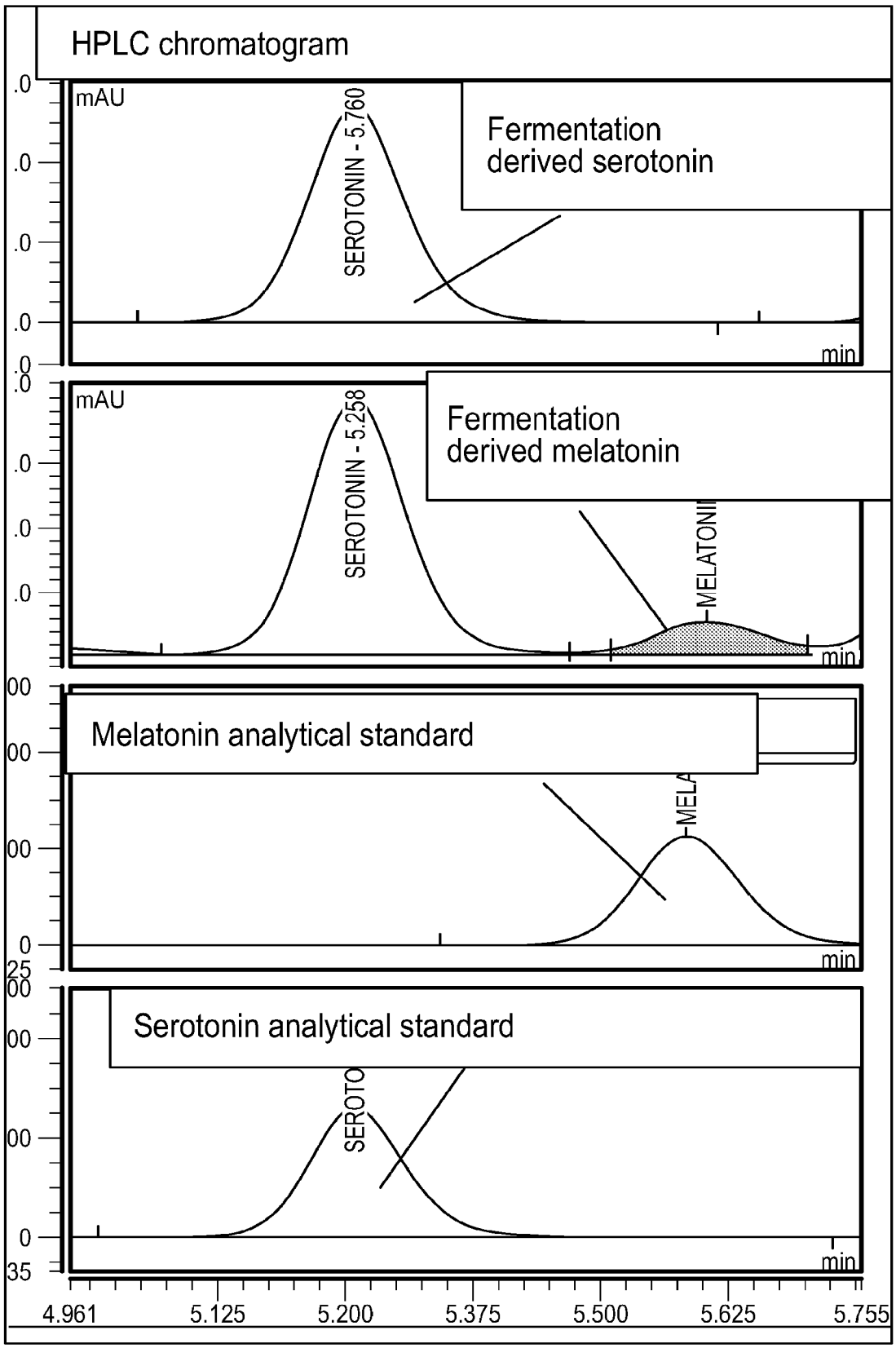

HPLC chromatogram
mAU
SEROTONIN - 5.760
Fermentation derived serotonin
min
mAU
SEROTONIN - 5.258
Fermentation derived melatonin
min
Melatonin analytical standard
min
Serotonin analytical standard
min
4.961
5.125
5.200
5.375
5.500
5.625
5.755

FIG. 22

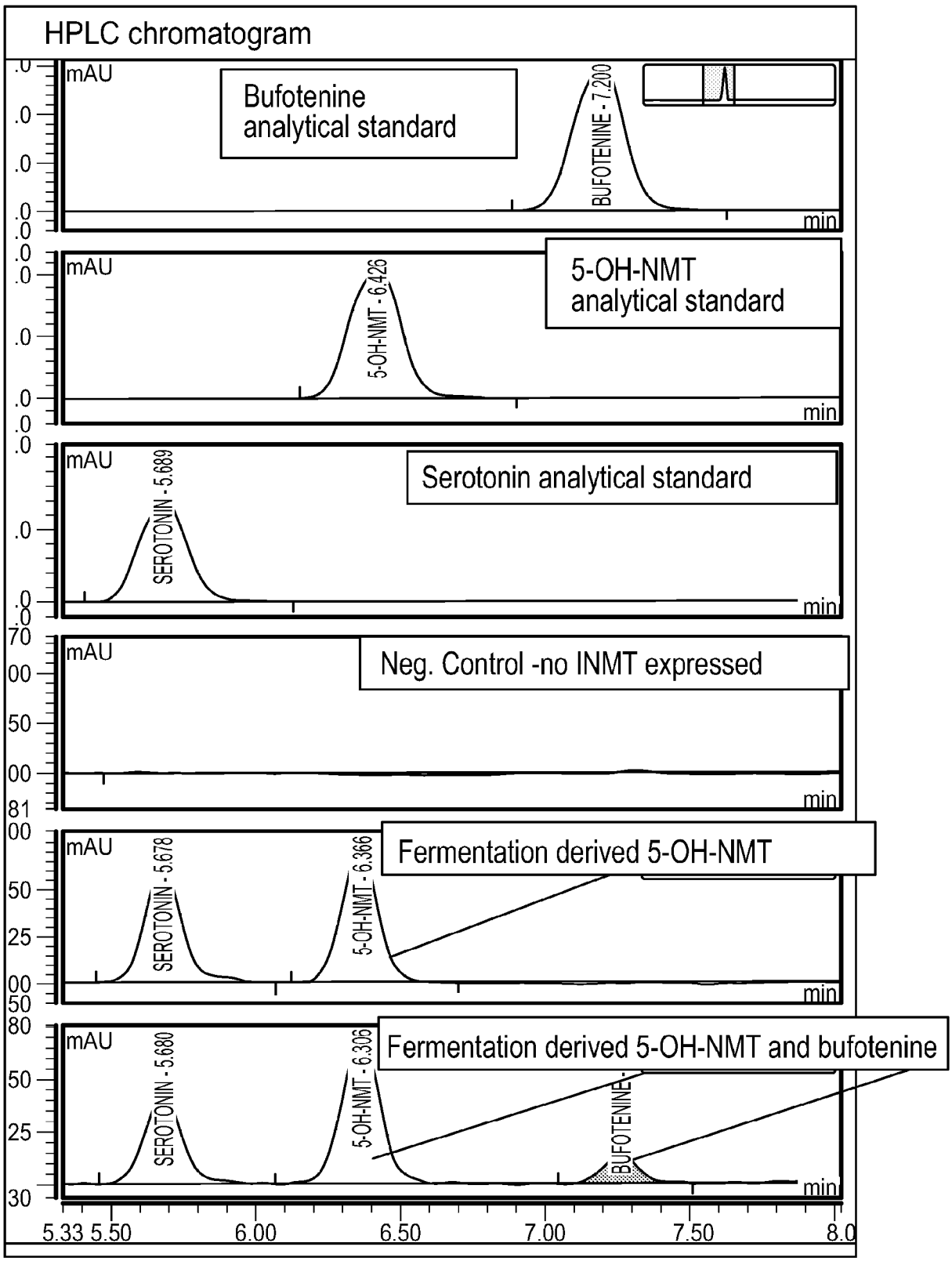

HPLC chromatogram
mAU
Bufotenine analytical standard
BUFOTENINE - 7.200
min
mAU
5-OH-NMT - 6.426
5-OH-NMT analytical standard
min
mAU
SEROTONIN - 5.689
Serotonin analytical standard
min
mAU
Neg. Control -no INMT expressed
min
mAU
SEROTONIN - 5.678
5-OH-NMT - 6.366
Fermentation derived 5-OH-NMT
min
mAU
SEROTONIN - 5.680
5-OH-NMT - 6.306
BUFOTENINE -
Fermentation derived 5-OH-NMT and bufotenine
min
5.33 5.50 6.00 6.50 7.00 7.50 8.0

FIG. 23

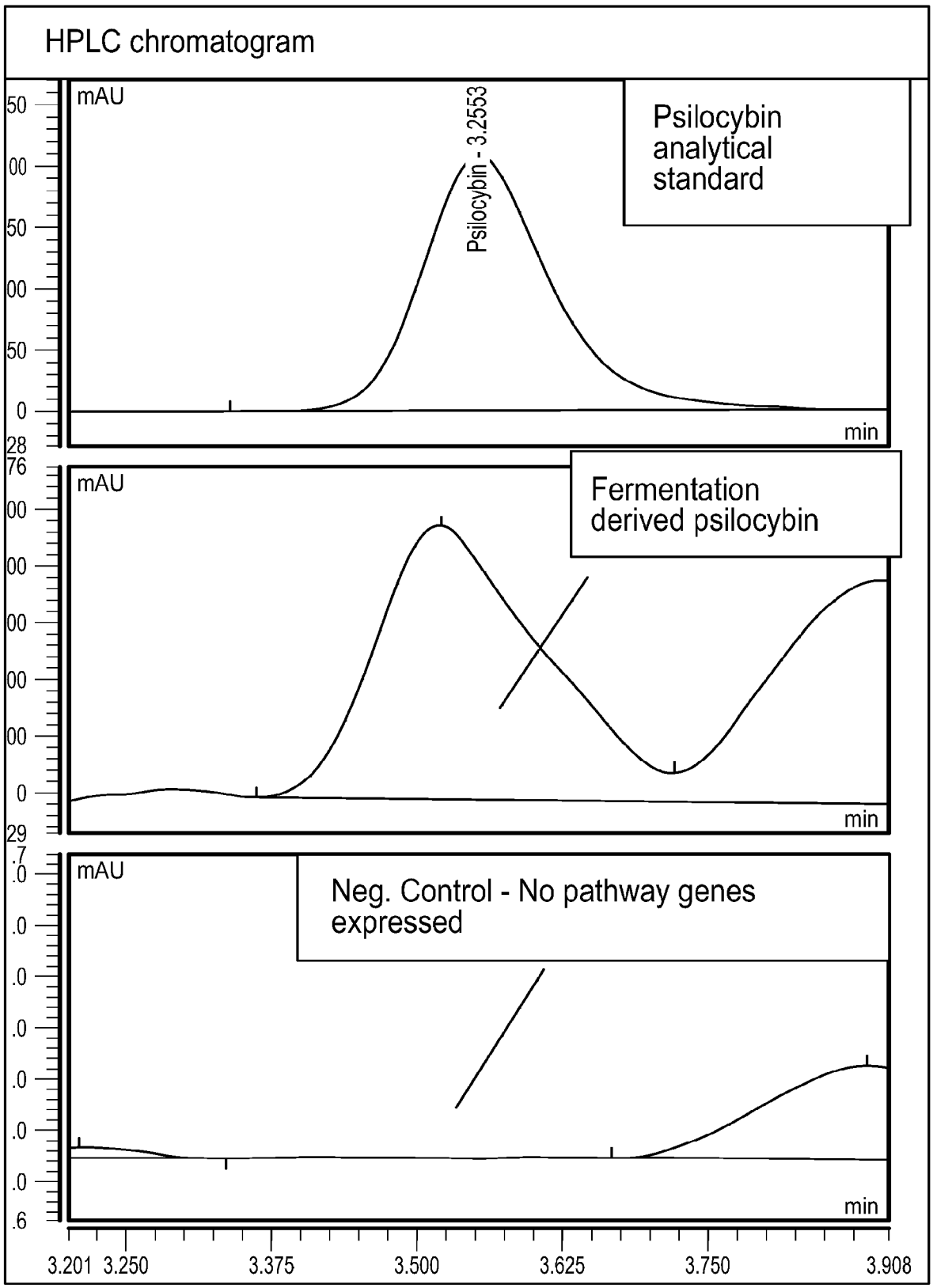

HPLC chromatogram
mAU
Psilocybin - 3.2553
Psilocybin analytical standard
min
mAU
Fermentation derived psilocybin
min
mAU
Neg. Control - No pathway genes expressed
min
3.201
3.250
3.375
3.500
3.625
3.750
3.908

FIG. 24

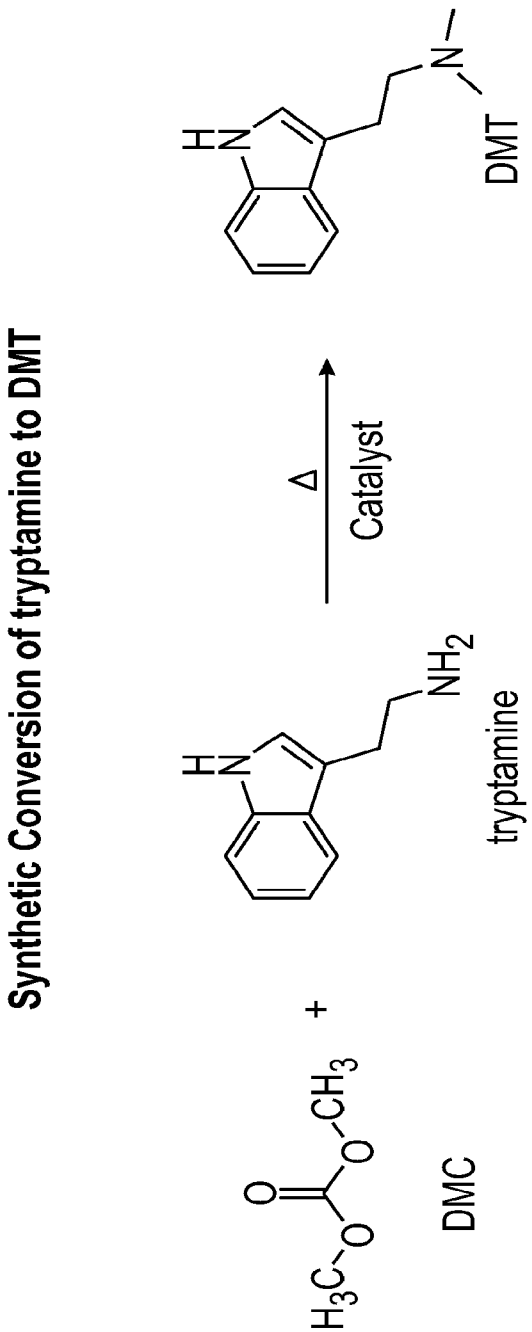

Synthetic Conversion of tryptamine to DMT
DMC
tryptamine
Catalyst
Δ
DMT

FIG. 25

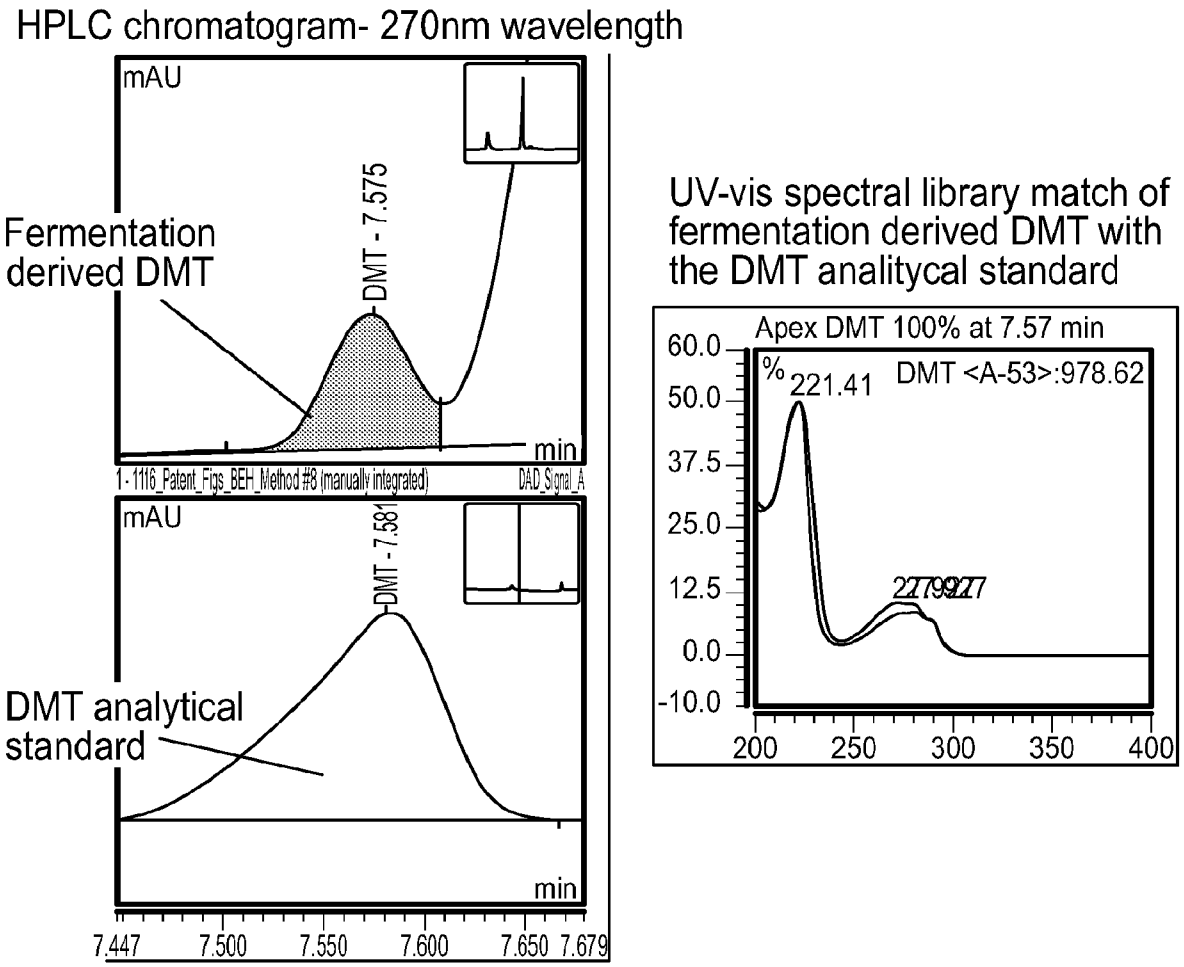

HPLC chromatogram- 270nm wavelength
mAU
Fermentation derived DMT
DMT - 7.575
min
1-1116_Patent_Figs_BEH_Method #8 (manually integrated)
DAD_Signal_A
mAU
DMT - 7.581
DMT analytical standard
min
7.447
7.500
7.550
7.600
7.650
7.679
UV-vis spectral library match of fermentation derived DMT with the DMT analitycal standard
Apex DMT 100% at 7.57 min
%
221.41
DMT <A-53>:978.62
60.0
50.0
37.5
25.0
12.5
0.0
-10.0
200
250
300
350
400

FIG. 26

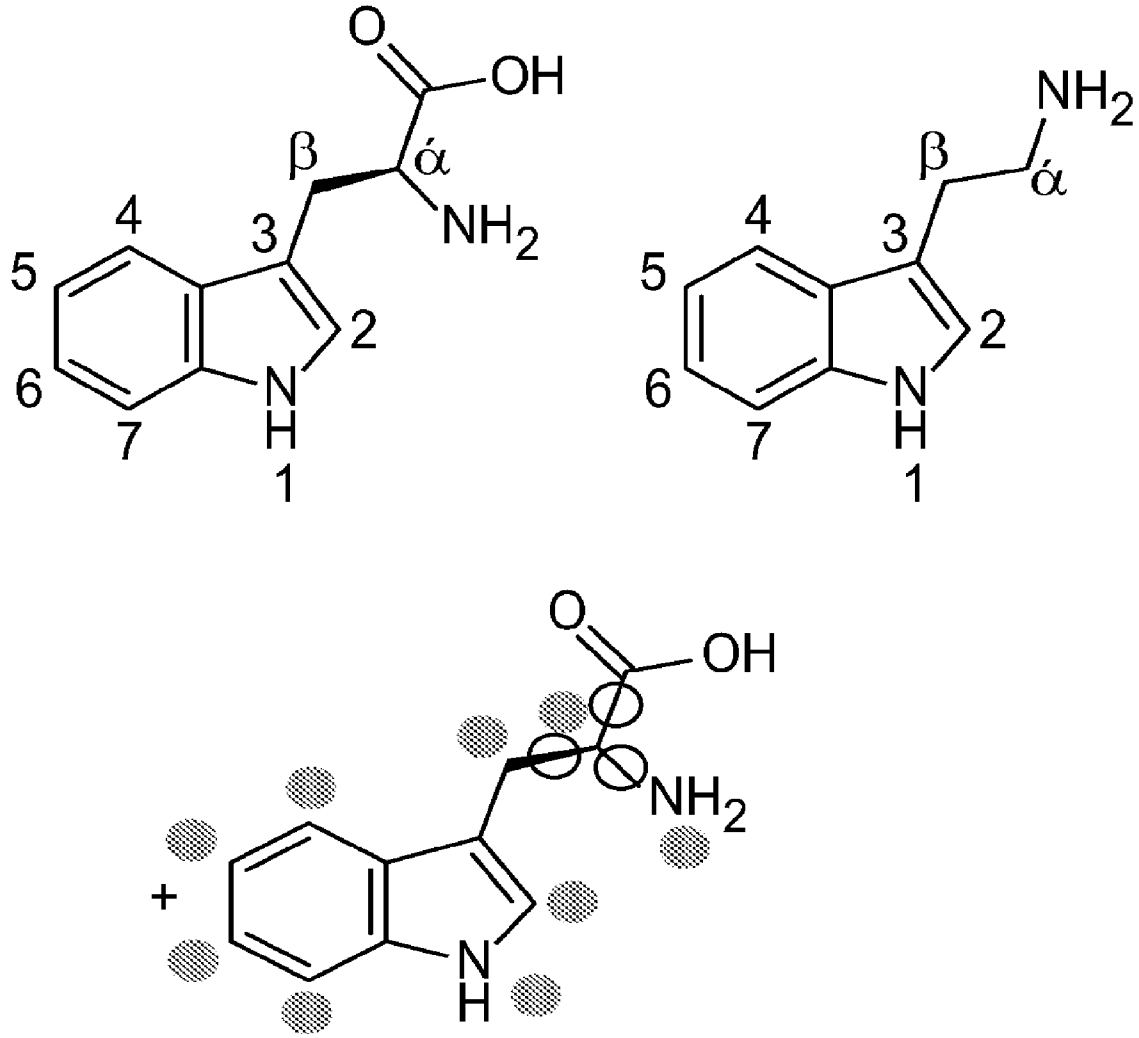

O
OH
β
α
NH2
4
3
5
2
6
N
7
H
1
NH2
β
α
4
3
5
2
6
N
7
H
1
O
OH
NH2
+
N
H
methylation
halogenation
prenylation
hydroxylation
O-phosphorylation
O-methylation
N-Acetylation
cleavage
deamination
decarboxylation
+ oligomerization

FIG. 1