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[Continued on next page]

(54) Title: PTERIN-DEPENDENT BIOCATALYSTS AND USES THEREOF

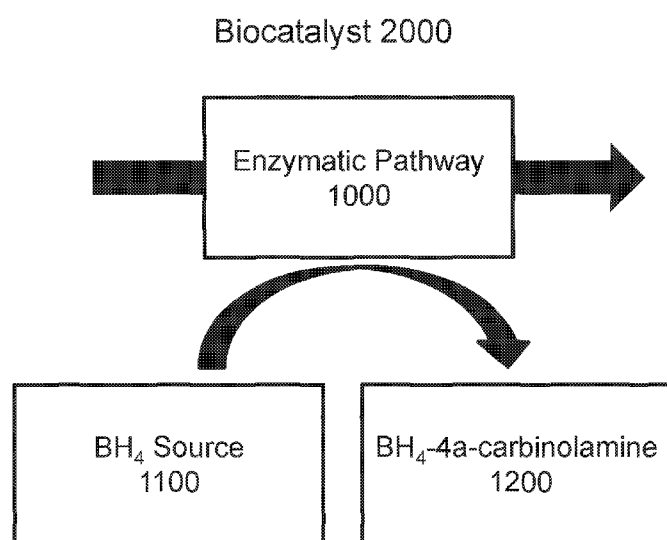


FIG. 1

(57) Abstract: Provided herein are biocatalysts and systems thereof for pterin-dependent enzymes and pathways and methods of making and using the same. Provided herein in some embodiments are biocatalysts having a pterin source and a pterin-dependent enzymatic pathway biologically coupled to the pterin source. Tetrahydrobiopterin (referred to herein as BH₄ or BH₄) can be the pterin source. The BH₄ can be synthesized by a tetrahydrobiopterin synthesis pathway. The tetrahydrobiopterin synthesis pathway can include a GTP cyclohydrolase; a pyruvoyl tetrahydropterin synthase; a sepiapterin reductase, and/or any combination thereof. The biocatalyst can further contain a pterin-dependent enzymatic pathway. The pterin-dependent enzymatic pathway can be amino acid mono-oxygenase, phenylalanine hydroxylase, tryptophan hydroxylase, tyrosine hydroxylase, nitric oxide synthase, alkylglycerol monooxygenase, and/or any combination thereof.

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PTERIN-DEPENDENT BIOCATALYSTS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Serial No. 62/099,309 filed on January 2, 2015, having the title Microbial Synthesis of Monoterpene Indole Alkaloids via Pterin-Dependent Amino Acid Hydroxylation, and U.S. Provisional Application Serial No. 62/130257 filed on March 9, 2015, having the title Pterin Dependent Aromatic Mono-Oxidation for Lignin Valorization, the entirety of which is incorporated herein by reference.

SUMMARY

10 Provided herein in some embodiments are biocatalysts having a pterin source and a pterin-dependent enzymatic pathway biologically coupled to the pterin source. Tetrahydrobiopterin (referred to herein as BH₄ or BH₄) can be the pterin source. The BH₄ can be synthesized by a tetrahydrobiopterin synthesis pathway. The tetrahydrobiopterin synthesis pathway can include a GTP cyclohydrolase; a pyruvoyl tetrahydropterin synthase; a
15 sepiapterin reductase, and/or any combination thereof. The biocatalyst can further contain a pterin-dependent enzymatic pathway. The pterin-dependent enzymatic pathway can be amino acid mono-oxygenase, phenylalanine hydroxylase, tryptophan hydroxylase, tyrosine hydroxylase, nitric oxide synthase, alkylglycerol monooxygenase, and/or any combination thereof. The enzymatic pathway of the biocatalyst can further contain a decarboxylase
20 and/or modified decarboxylase. The decarboxylase can be aromatic-L-amino acid decarboxylase. The biocatalyst can further contain a synthase. The synthase can be a terpene alkaloid synthase. The synthase can be a strictosidine synthase. The biocatalyst can optionally contain a tetrahydrobiopterin recycling pathway, where the tetrahydrobiopterin recycling pathway can be biologically coupled to the enzymatic pathway. The
25 tetrahydrobiopterin recycling pathway can contain a pterin-4a-carbinolamine dehydratase and a dihydropterin reductase.

 The biocatalyst can be contained in a cell. The cell can be an engineered cell. The biocatalyst can contain a tetrahydrobiopterin source and a pterin-dependent enzymatic pathway described previously. The tetrahydrobiopterin source in the cell can be a
30 tetrahydrobiopterin synthesis pathway. The tetrahydrobiopterin synthesis pathway can contain a GTP cyclohydrolase, a pyruvoyl tetrahydropterin synthase, a sepiapterin reductase, and/or any combination thereof. The pterin-dependent enzymatic pathway in the cell can contain at least one element selected from the group of an amino acid mono-oxygenase, a modified amino acid mono-oxygenase, phenylalanine hydroxylase, tryptophan hydroxylase,
35 tyrosine hydroxylase, nitric oxide synthase, and alkylglycerol monooxygenase. The pterin-

dependent enzymatic pathway in the cell can further contain a decarboxylase and/or a modified decarboxylase. the decarboxylase can be aromatic-l-amino acid decarboxylase. The enzymatic pathway of the biocatalyst in the cell can further include a synthase. The synthase can be a modified or unmodified synthase. The synthase can be a terpene alkaloid synthase. The synthase can be a strictosidine synthase. The synthase can be a deacetylisoipecoside synthase. The biocatalyst of the cell can further contain a tetrahydrobiopterin recycling pathway. The tetrahydrobiopterin recycling pathway can contain a pterin-4a-carbinolamine dehydratase, a dihydrofolate reductase and/or a dihydropterin reductase.

Also described herein are methods of biocatalysis that can be carried out by a biocatalyst. The methods can produce direct and/or selective biocatalysis. The biocatalyst can be contained in a cell. The method can include the steps of providing a biocatalyst as previously set forth and providing a substrate to the biocatalyst. The biocatalyst can be contained within a cell. The cell can be a eukaryotic or prokaryotic cell. In embodiments, the cell is a yeast cell. In embodiments, the biocatalyst is not contained in a cell. The substrate can be a carbohydrate. The substrate can be glucose. The substrate can be galactose. The substrate can be lignin or a derivative or metabolite thereof.

BACKGROUND

Carbohydrates, sugars and lignins, are abundant sources for a variety of compounds products that have applications ranging from pharmaceuticals to biofuels. While direct isolation of the compounds, which are often plant metabolites, produced from the carbohydrate, sugar, and/or lignin source, directly from the plant is a way to obtain the desired compounds, isolation often proves laborious and modification of the compound is difficult or impossible. As such, there exists a need for improved compositions and methods for obtaining compounds derived from carbohydrates, sugars, and lignins.

BRIEF DESCRIPTION OF THE DRAWINGS

Further aspects of the present disclosure will be readily appreciated upon review of the detailed description of its various embodiments, described below, when taken in conjunction with the accompanying drawings.

Figure 1 shows a representative diagram of some embodiments of a biocatalyst 2000 containing an enzymatic pathway 1000 coupled to a BH4 source 1100.

Figure 2 shows a representative diagram of some embodiments of a biocatalyst 2000 containing an enzymatic pathway 1000 coupled to a BH4 source 1100 and a BH4

recycling pathway 1300.

Figure 3 shows a representative diagram of an embodiment cell 1400, which can contain a biocatalyst 2000. The biocatalyst can contain an enzymatic pathway 1000 biologically coupled to a BH₄ source 1100.

5 Figure 4 shows a representative diagram of a cell 1400, which can contain an enzymatic pathway 1000 biologically coupled to a BH₄ source 1100 and an optional BH₄ recycling pathway 1300.

Figures 5A-5C demonstrate pterin-dependent synthesis of monoterpene indole alkaloids (MIAs) in *Saccharomyces cerevisiae*. Embodiments of cells, such as of *S.*
 10 *cerevisiae*, described herein can contain one or more of the following: I) the BH₄ biosynthetic pathway, II) the BH₄ recycling pathway, III) a pterin-dependent mono-oxygenase, IV) a decarboxylase, or V) a Pictet-Spenglerase. Grey arrows represent future potential of the system. GTPCH: GTP cyclohydrolase; PTPS: pyruvoyl tetrahydropterin synthase; SR: sepiapterin reductase; PCD: pterin-4a-carbinolamine dehydratase; DHPR: dihydropteridine
 15 reductase; TPH: tryptophan hydroxylase; TH: tyrosine hydroxylase; DDC: aromatic-L-amino-acid decarboxylase; STR: strictosidine synthase.

Figure 6 shows a scheme demonstrating embodiments of a modified MIAs for the semi-synthesis of pharmaceuticals.

Figures 7A-7F shows embodiments of a synthesis scheme and data demonstrating.
 20 production of tetrahydrobiopterin (BH₄) in *S. cerevisiae*. (a) BH₄ biosynthetic pathway from guanosine triphosphate (GTP), which is endogenously made by *S. cerevisiae*. (b) Heat map of biopterin titers (mg/L), as a proxy for BH₄ titers, from the 49 BH₄-production yeast strains. Except for *S. cerevisiae* chromosomal (c), where the chromosomal copy of *S. cerevisiae* GTPCH was used, each enzyme was expressed from a multicopy plasmid from an inducible
 25 galactose promoter (P_{GAL1}). Biopterin was quantified using liquid chromatography/mass spectrometry (LC/MS). Bio[pterin tires reporter as (-) were not determined. Production levels reported as 0.00 were either too low to quantify or undetectable. A control strain expressing green fluorescent protein in a three-plasmid system showed no biopterin production. The experiments were run in triplicate and shown are the means. Standard
 30 deviations can be found in Fig. 13A. (c) LC/MS traces (extracted ion chromatograms) of BH₄ (m/z 242) and oxidation products dihydrobiopterin (m/z 240) and biopterin (m/z 238) found intracellularly and in the production medium. Standard retention times: BH₄= 2.6 min, dihydrobiopterin= 5.3 min, biopterin= 4.6 min. Only biopterin was observed in the production medium. Full windows of the spectra can be found in Fig 13B. (d) Pterin-dependent mono-
 35 oxidation of tyrosine to L-DOPA using the original BH₄ synthesis strain (*S.cerevisiae* GTPCH, *M. alpina* PTPS and SR) and the combinatorially optimized BH₄ synthesis strain (*E. coli* GTPCH, *M. alpina* PTPS and SR), both strains carrying tyrosine mono-oxygenase.

Improving BH₄ production improves amino acid mono-oxidation. (e) Optimization of BH₄ biosynthesis. BH₄ synthesis pathway: *E. coli* GTPCH, *M. alpina* PTPS and SR. For the glucose system, all three enzymes were expressed from a single multicopy plasmid under control of constitutive promoters (P_{ADH1}, P_{TEF1}, and P_{HXT7}). (f) BH₄ biosynthetic pathway bottleneck identification. Biopterin production from galactose in yeast expressing each BH₄ biosynthetic enzyme from either a single-copy (s) or a multicopy (m) plasmid. All experiments were run in triplicate and shown are the mean and standard deviation. GTPCH: GTP cyclohydrolase; PTPS: pyruvoyl tetrahydropterin synthase; SR: sepiapterin reductase.

Figures 8A-8D demonstrate embodiments of a synthesis scheme and data demonstrating microbial synthesis of L-DOPA via pterin-dependent tyrosine hydroxylation. (a) Schematic representation of the BH₄ recycling pathway. (b) Representative LC traces for various production strains (extracted ion chromatograms for L-DOPA= m/z 198). Traces represent strains expressing: i) only tyrosine hydroxylase (TH), ii) TH and the BH₄ synthesis pathway, and iii) TH, the BH₄ synthesis pathway, and the BH₄ recycling pathway. Trace iv is commercial L-DOPA standard.. Full windows of the spectra can be found in Fig. 21a. (c) Production levels of L-DOPA from galactose in the presence (+) or absence (-) of the BH₄ synthesis pathway and/or BH₄ recycling pathway. (d) Production levels of biopterin from galactose in the presence (+) or absence (-) of the BH₄ recycling pathway. All experiments were run in triplicate and shown are the mean and standard deviation. Strains carried four multicopy plasmids in which each gene was expressed from galactose inducible promoters (P_{GAL1} or P_{GAL10}). PCD: pterin-4a-carbinolamine dehydratase; DHPR: dihydropteridine reductase.

Figures 9A-9H shows embodiments of a synthesis scheme and data demonstrating microbial synthesis of biogenic amines via pterin-dependent mono-oxidation. (a) Schematic representation of dopamine biosynthesis. (b) Representative LC traces for various production strains (extracted ion chromatograms (EIC) for dopamine = m/z 154). Traces represent strains expressing: i) tyrosine hydroxylase (TH), aromatic-L-amino-acid decarboxylase (DDC), the BH₄ synthesis pathway, and the BH₄ recycling pathway, ii) TH and DDC, and iii) TH, DDC, and the BH₄ synthesis pathway. Trace iv is commercial dopamine standard. Full windows of the spectra can be found in Fig. 18b. (c) Production levels of dopamine in the presence (+) or absence (-) of the BH₄ synthesis pathway, and/or the BH₄ recycling pathway. (d) Production levels of biopterin in the presence (+) or absence (-) of the BH₄ recycling pathway. (e) Schematic representation of serotonin biosynthesis. (f) Representative LC traces for various production strains (EIC for serotonin = m/z 177). Traces represent strains expressing: i) tryptophan hydroxylase (TPH), DDC, the BH₄ synthesis pathway, and the BH₄ recycling pathway, ii) TPH and DDC, and iii) TPH, DDC, and the BH₄ synthesis pathway. Trace iv is commercial serotonin standard. Full windows of

the spectra can be found in Fig. 18c. (g) Production levels of serotonin in the presence (+) or absence (-) of the BH₄ synthesis pathway and/or BH₄ recycling pathway. (h) Production levels of biopterin in the presence (+) or absence (-) of the BH₄ recycling pathway. All experiments were run in triplicate and shown are the mean and standard deviation. Strains carried four multicopy plasmids in which each gene was expressed from galactose inducible promoters (P_{GAL1} or P_{GAL10}).

Figures 10A-10E show embodiments of a synthesis scheme and data demonstrating microbial synthesis of the modified MIA 10-hydroxystrictosidine. (a) Schematic of hydroxystrictosidine biosynthesis. (b) Representative LC trace (Multiple Reaction Monitoring hydroxystrictosidine 547.60 → 530.00 transition) for the yeast strain (PPY650) carrying tryptophan hydroxylase, aromatic-L-amino-acid decarboxylase, strictosidine synthase, and the BH₄ biosynthesis and recycling pathways in the presence or absence of 0.4mM secologanin. S= (S)-Hydroxystrictosidine; R= (R)-Hydroxystrictosidine. Full window of the spectra can be found in Fig. 21. (c) Tandem mass spectrum of microbially-produced S-hydroxystrictosidine. (d) High-resolution mass spectrum of microbially-produced S-hydroxystrictosidine. (e) (S) and (R)-Hydroxystrictosidine production in *S. cerevisiae*. DDC: aromatic-L-amino-acid decarboxylase; TPH: tryptophan hydroxylase; STR: strictosidine synthase.

Figures 11A-11D show graphs demonstrating the analysis of the 10-hydroxystrictosidine isomer ratio. All reactions contain 0.4mM each of secologanin and serotonin. LC traces trace (extracted ion chromatograms for hydroxystrictosidine = m/z 547) for (a) the chemical reaction in phosphate buffer at pH=3 (black) or pH=7 (red), (b) the reaction in cell lysate of yeast expressing strictosidine synthase (PPY827) adjusted to pH=3 (black) or pH=7 (red), (c) *in vivo* reaction using intact yeast cells expressing strictosidine synthase (PPY827) in standard yeast media (black) or pH=7 buffered media (red), and (d) *in vivo* reaction using intact yeast cells expressing either strictosidine synthase (PPY827, solid red line) or yeast expressing a blank plasmid (PPY828, dotted red line) in pH=7 buffered media. Full windows of the spectra can be found in Fig. 25. Multiple reaction monitoring of Figure 7d can be found in Fig. 27. STR: strictosidine synthase.

Figure 12 shows the chemical structures and stereochemistry of BH₄ and MH₄. Chemical structures of tetrahydrobiopterin (BH₄), the natural amino acid monooxygenase co-factor, and tetrahydromonapterin (MH₄), the BH₄ analogue found in *E. coli*. BH₄ and MH₄ vary in stereochemistry and composition.

Figure 13 shows a graph demonstrating combinatorial production levels of biopterin. Production levels of biopterin were quantified using LC-MS. Production levels reported as 0.00 were either too low to quantify or undetectable. Strain PPY810 represents a control

strain expressing green fluorescent protein in a three-plasmid system. The experiments were run in triplicate and shown are the mean and standard deviation.

Figure 14 shows a structural alignment of *Salinibacter ruber* and *Salmo salar* pyruvoyl tetrahydropterin synthase (PTPS). Structural alignment of homology models of *S. salar* PTPS (cyan) and *S. ruber* (green) PTPS obtained via structural homology to rat PTPS (PDB:1B66) using SWISS-MODEL³⁻⁵. Presented is a monomer of the active site of PTPS (which is composed of three monomers) showing the catalytic cysteine residue of *S. salar* PTPS and corresponding aspartate residue of *S. ruber* PTPS. Biopterin (blue) and Zn(II) (purple) were obtained from the crystal structure from rat. Alignment was completed with PyMOL.

Figure 15 shows a structural alignment of *Mortirella alpina* and *Thalassiosira pseudonana* sepiapterin reductase (SR). Structural alignment of homology models of *M. alpina* SR (green) and *T. pseudonana* (cyan) SR obtained via structural homology to PDB:1Z6Z and 3ICC, respectively, using SWISS-MODEL³⁻⁵. NADPH (yellow) and biopterin (dark blue) were obtained from the crystal structure of mouse SR (PDB:1SEP). While arginine residues are present in the *M. alpina* structure to stabilize the phosphate group of NADPH, there are no stabilizing residues present in the *T. pseudonana* structure. Alignment was completed with PyMOL.

Figures 16A-16B show the purine biosynthetic pathway from the Kyoto Encyclopedia of Genes and Genomes (KEGG)⁶. The enzyme encoded by *Ade2* is boxed in red. GTP is boxed in green. Figure 16B shows an enlarged portion of Figure 16A.

Figure 17 shows a graph demonstrating GTPCH, PTPS and SR mRNA levels. Multi-copy: multi-copy plasmid. Single-copy: single-copy plasmid. Values represent the mean of two reactions.

Figures 18A-18C shows full windows of LC traces in Figures 4b, 5b, 5f. L-DOPA, dopamine and serotonin highlighted with a pink (L-DOPA, dopamine) or green diamond (serotonin). (a) L-DOPA (13.8 min), (b) dopamine (12.8 min), and (c) serotonin (17.2 min).

Figure 19 shows a graph demonstrating the effect of tyrosine on L-DOPA production. In our experiments, 30 mg/L of tyrosine is present when producing L-DOPA or dopamine.

Figure 20 shows a graph demonstrating the effect of tryptophan on serotonin production. In our experiments, tryptophan is not supplemented when producing serotonin or hydroxystrictosidine.

Figure 21 shows a full window of multiple reaction monitoring for Figure 6B. Shown hydroxystrictosidine transition: 547.60 → 530.00 transition.

Figures 22A-22E shows the graphical results from mass spectral characterization of hydroxystrictosidine isomers. (a) LC trace (extracted ion chromatogram corresponding to hydroxystrictosidine, *m/z* 547, extracted from full scan data) from high resolution mass

spectrometry analysis. (b) High-resolution mass spectrum of microbially-produced S-hydroxystrictosidine and R-hydroxystrictosidine. (c) Theoretical high-resolution mass spectrum of hydroxystrictosidine. (d) LC trace for product ions of m/z 547 from tandem mass spectrometry analysis. (e) Tandem mass spectra of microbially-produced S-hydroxystrictosidine and R-hydroxystrictosidine.

Figure 23 shows a graph demonstrating isomer ratios produced in the presence (STR +, PPY650) or absence (STR -, PPY649) of strictosidine synthase using yeast synthetic media (pH=5-3). Green: S-hydroxystrictosidine; Yellow: R-hydroxystrictosidine.

Figures 24A-24D show full windows of LC traces in Figure 7 (extracted ion chromatograms for hydroxystrictosidine, m/z 547). S-hydroxystrictosidine, rt =6.1 min. R-hydroxystrictosidine, rt =6.8 min. Isomer identification was determined due to the fact that strictosidine synthase is known to form only the S-isomer. A single isomer is formed *in vivo* in buffered synthetic media (pH=7 $\rightarrow$ 5).

Figures 25A-25B shows graphs of pH of media over time and cell growth. (a) pH of wild-type W303 yeast grown in synthetic complete media (black) or buffered synthetic complete media (25mM K_2HPO_4 , red). (b) Cell growth monitored by absorption at OD_{600} .

Figure 26 shows a full window for multiple reaction monitoring (MRM) analysis for Figure 7d. Only one peak can be found in the MRM spectrum corresponding to the characteristic hydroxystrictosidine transition 547.60 $\rightarrow$ 530.00. All other peaks in the Figure 7d (and SI Fig 13d) LC trace have m/z value of 547, but do not have the characteristic hydroxystrictosidine transition 547.60 $\rightarrow$ 530.

Figure 27 demonstrates current techniques for the breakdown of lignin lead to the production of low-molecular weight monomeric aromatic units such as those shown here. Many of these are commonly used and, due to their ready availability, they tend to be very cheap, with many been only cents per gram.

Figures 28A-28B show schemes demonstrating upgrading lignin-derived aromatic monomers into value-added chemicals via pterin-based mono-oxygenases. 28A. Pterin-dependent amino acid monooxygenases (AMOs) carry out the hydroxylation of aromatic amino acids. Shown tyrosine monooxygenases hydroxylating tyrosine into L-DOPA. 28B. The structure of lignin-derived aromatic amino acids are similar to tyrosine.

Figures 29A-29B demonstrate embodiments of a synthesis scheme (FIG. 29A) and data (FIG. 29B) demonstrating production of 2,6,-dimethoxy-4-methoxyphenol.

Figure 30 demonstrates an embodiment of a cell engineered to produce BH₄ as described herein. BH₄ can be synthesized by a BH₄ synthesis pathway 1500 comprising GTP cyclohydrolase (GTPCH), pyruvate tetrahydrobiopterin synthase (PTPS), and sepiapterin reductase (SR). A yeast cell is engineered to express said BH₄ synthesis pathway. Said engineered yeast cell is incubated with media and a carbohydrate for an amount of time to

produce BH₄. The carbohydrate can be glucose or galactose. Products of the endogenous yeast carbohydrate metabolic pathway are then used by (or coupled to) the engineered BH₄ pathway to produce BH₄ in the engineered yeast cell.

Figure 31 demonstrates an embodiment of a cell engineered to produce serotonin using a biocatalyst as described herein. The biocatalyst 2100 is comprised of a BH₄ synthesis pathway 1500 and an enzymatic pathway 1000 comprised of tryptophan hydroxylase and aromatic-L-amino acid decarboxylase. The engineered yeast cell can be incubated with media and a carbohydrate for an amount of time to produce serotonin. The carbohydrate can be glucose or galactose. Products of the endogenous yeast carbohydrate metabolic pathway are then used by (or coupled to) the biocatalyst 2100 to produce serotonin in the engineered yeast cell.

Fig. 32 demonstrates an embodiment of a cell engineered to produce dopamine from a carbohydrate using a biocatalyst as described herein.

Fig. 33 demonstrates an embodiment of a cell engineered to produce hydroxystrictosidine from a carbohydrate as described herein.

Figure 34 shows a table demonstrating the overview of the microbial synthesis of L-DOPA, dopamine, serotonin and 10-hydroxystrictosidine. Arrows represent presence of the enzyme. nd = not detectable. Amount produced is represented by the mean $\pm$ standard deviation for samples run in triplicate. GTPCH: GTP cyclohydrolase; PTPS: pyruvoyl tetrahydropterin synthase; SR: sepiapterin reductase; PCD: pterin-4a-carbinolamine dehydratase; DHPR: dihydropteridine reductase; TPH: tryptophan hydroxylase; TH: tyrosine hydroxylase; DDC: aromatic-L-amino-acid decarboxylase; STR: strictosidine synthase.

DETAILED DESCRIPTION

Before the present disclosure is described in greater detail, it is to be understood that this disclosure is not limited to particular embodiments described, and as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

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. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are 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.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure, the preferred methods and materials are now described.

All publications and patents cited in this specification are herein incorporated by reference as if each individual publication or patent were specifically and individually indicated to be incorporated by reference and are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. The citation of any publication is for its disclosure prior to the filing date and should not be construed as an admission that the present disclosure is not entitled to antedate such publication by virtue of prior disclosure. Further, the dates of publication provided could be different from the actual publication dates that may need to be independently confirmed.

As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present disclosure. Any recited method can be carried out in the order of events recited or in any other order that is logically possible.

Embodiments of the present disclosure will employ, unless otherwise indicated, techniques of molecular biology, microbiology, nanotechnology, organic chemistry, synthetic biology, chemistry, biochemistry, botany and the like, which are within the skill of the art. Such techniques are explained fully in the literature.

Definitions

As used herein, "about," "approximately," and the like, when used in connection with a numerical variable, generally refers to the value of the variable and to all values of the variable that are within the experimental error (e.g., within the 95% confidence interval for the mean) or within $\pm 10\%$ of the indicated value, whichever is greater.

As used herein, "control" is an alternative subject or sample used in an experiment for comparison purposes and included to minimize or distinguish the effect of variables other than an independent variable.

As used herein, "overexpressed" or "overexpression" refers to an increased expression level of an RNA or protein product encoded by a gene as compared to the level of expression of the RNA or protein product in a normal or control cell.

As used herein, "expression" refers to the process by which polynucleotides are transcribed into RNA transcripts. In the context of mRNA and other translated RNA species,

“expression” also refers to the process or processes by which the transcribed RNA is subsequently translated into peptides, polypeptides, or proteins.

As used herein, “nucleic acid” and “polynucleotide” generally refer to a string of at least two base-sugar-phosphate combinations and refers to, among others, single-and
5 double-stranded DNA, DNA that is a mixture of single-and double-stranded regions, single-and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, polynucleotide as used herein refers to triple-stranded regions comprising RNA or DNA or
10 both RNA and DNA. The strands in such regions may be from the same molecule or from different molecules. The regions may include all of one or more of the molecules, but more typically involve only a region of some of the molecules. One of the molecules of a triple-helical region often is an oligonucleotide. “Polynucleotide” and “nucleic acids” also encompasses such chemically, enzymatically or metabolically modified forms of
15 polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including simple and complex cells, inter alia. For instance, the term polynucleotide includes DNAs or RNAs as described above that contain one or more modified bases. Thus, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein.
20 “Polynucleotide” and “nucleic acids” also includes PNAs (peptide nucleic acids), phosphorothioates, and other variants of the phosphate backbone of native nucleic acids. Natural nucleic acids have a phosphate backbone, artificial nucleic acids may contain other types of backbones, but contain the same bases. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are “nucleic acids” or “polynucleotide” as that term
25 is intended herein.

As used herein, “deoxyribonucleic acid (DNA)” and “ribonucleic acid (RNA)” generally refer to any polyribonucleotide or polydeoxyribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. RNA may be in the form of a tRNA (transfer RNA), snRNA (small nuclear RNA), rRNA (ribosomal RNA), mRNA (messenger RNA), anti-sense RNA,
30 RNAi (RNA interference construct), siRNA (short interfering RNA), or ribozymes.

As used herein, “nucleic acid sequence” and “oligonucleotide” also encompasses a nucleic acid and polynucleotide as defined above.

As used herein, “DNA molecule” includes nucleic acids/polynucleotides that are made of DNA.

As used herein, “wild-type” is the typical form of an organism, variety, strain, gene, protein, or characteristic as it occurs in nature, as distinguished from mutant forms that may result from selective breeding or transformation with a transgene.

As used herein, "identity," is a relationship between two or more polypeptide or polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also refers to the degree of sequence relatedness between polypeptide as determined by the match between strings of such sequences. "Identity" can be readily calculated by known methods, including, but not limited to, those described in Computational Molecular Biology, Lesk, A. M., Ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., Ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., Eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribnikov, M. and Devereux, J., Eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM J. Applied Math. 1988, 48: 1073. Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity are codified in publicly available computer programs. The percent identity between two sequences can be determined by using analysis software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, Madison Wis.) that incorporates the Needleman and Wunsch (J. Mol. Biol., 1970, 48: 443-453) algorithm (e.g., NBLAST, and XBLAST). The default parameters are used to determine the identity for the polypeptides or polynucleotides of the present disclosure.

As used herein, "heterologous" refers to compounds, molecules, nucleotide sequences (including genes), and polypeptide sequences (including peptides and proteins) that are different in both activity (function) and sequence or chemical structure. As used herein, "heterologous" can also refer to a gene or gene product that is from a different organism. Foreexample, a human GTP cyclohydrolase or a synthase can be said to be heterologous when expressed in yeast.

As used herein, "homologue" refers to a polypeptide sequence that shares a threshold level of similarity and/or identity as determined by alignment of matching amino acids. Two or more polypeptides determined to be homologues are said to be homologues. Homology is a qualitative term that describes the relationship between polypeptide sequences that is based upon the quantitative similarity.

As used herein, "paralog" refers to a homologue produced via gene duplication of a gene. In other words, paralogs are homologues that result from divergent evolution from a common ancestral gene.

As used herein, "orthologues" refers to homologues produced by speciation followed by divergence of sequence but not activity in separate species. When speciation follows duplication and one homologue sorts with one species and the other copy sorts with the other species, subsequent divergence of the duplicated sequence is associated with one or the other species. Such species specific homologues are referred to herein as orthologues.

As used herein, "xenologs" are homologues resulting from horizontal gene transfer.

As used herein, "similarity" is a quantitative term that defines the degree of sequence match between two compared polypeptide sequences.

As used herein, "cell," "cell line," and "cell culture" include progeny. It is also
5 understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Variant progeny that have the same function or biological property, as screened for in the originally transformed cell, are included.

As used herein, "culturing" refers to maintaining cells under conditions in which they can proliferate and avoid senescence as a group of cells. "Culturing" can also include
10 conditions in which the cells also or alternatively differentiate.

As used herein, "organism", "host", and "subject" refers to any living entity comprised of at least one cell. A living organism can be as simple as, for example, a single isolated eukaryotic cell or cultured cell or cell line, or as complex as a mammal, including a human being, and animals (e.g., vertebrates, amphibians, fish, mammals, e.g., cats, dogs, horses,
15 pigs, cows, sheep, rodents, rabbits, squirrels, bears, primates (e.g., chimpanzees, gorillas, and humans). "Subject" may also be a cell, a population of cells, a tissue, an organ, or an organism, preferably to human and constituents thereof.

As used herein, "gene" refers to a hereditary unit corresponding to a sequence of DNA that occupies a specific location on a chromosome and that contains the genetic
20 instruction for a characteristic(s) or trait(s) in an organism.

As used herein, the term "recombinant" generally refers to a non-naturally occurring nucleic acid, nucleic acid construct, or polypeptide. Such non-naturally occurring nucleic acids may include natural nucleic acids that have been modified, for example that have deletions, substitutions, inversions, insertions, *etc.*, and/or combinations of nucleic acid
25 sequences of different origin that are joined using molecular biology technologies (e.g., a nucleic acid sequences encoding a fusion protein (e.g., a protein or polypeptide formed from the combination of two different proteins or protein fragments), the combination of a nucleic acid encoding a polypeptide to a promoter sequence, where the coding sequence and promoter sequence are from different sources or otherwise do not typically occur together
30 naturally (e.g., a nucleic acid and a constitutive promoter), *etc.*). Recombinant also refers to the polypeptide encoded by the recombinant nucleic acid. Non-naturally occurring nucleic acids or polypeptides include nucleic acids and polypeptides modified by man.

As used herein, "plasmid" as used herein refers to a non-chromosomal double-stranded DNA sequence including an intact "replicon" such that the plasmid is replicated in a
35 host cell.

As used herein, the term "vector" or is used in reference to a vehicle used to introduce an exogenous nucleic acid sequence into a cell. A vector may include a DNA

molecule, linear or circular (e.g. plasmids), which includes a segment encoding a polypeptide of interest operatively linked to additional segments that provide for its transcription and translation upon introduction into a host cell or host cell organelles. Such additional segments may include promoter and terminator sequences, and may also include one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal, etc. Expression vectors are generally derived from yeast or bacterial genomic or plasmid DNA, or viral DNA, or may contain elements of both.

As used herein, "operatively linked" or "operatively coupled" indicates that the regulatory sequences useful for expression of the coding sequences of a nucleic acid are placed in the nucleic acid molecule in the appropriate positions relative to the coding sequence so as to effect expression of the coding sequence. This same definition can also be applied to the arrangement of coding sequences and transcription control elements (e.g. promoters, enhancers, and termination elements), and/or selectable markers in an expression vector.

As used herein, "cDNA" refers to a DNA sequence that is complementary to a RNA transcript in a cell. It is a man-made molecule. Typically, cDNA is made in vitro by an enzyme called reverse-transcriptase using RNA transcripts as templates.

As used herein, the term "transfection" refers to the introduction of an exogenous and/or recombinant nucleic acid sequence into the interior of a membrane enclosed space of a living cell, including introduction of the nucleic acid sequence into the cytosol of a cell as well as the interior space of a mitochondria, nucleus, or chloroplast. The nucleic acid may be in the form of naked DNA or RNA, it may be associated with various proteins or regulatory elements (e.g., a promoter and/or signal element), or the nucleic acid may be incorporated into a vector or a chromosome.

As used herein, "transformation" or "transformed" refers to the introduction of a nucleic acid (e.g., DNA or RNA) into cells in such a way as to allow expression of the coding portions of the introduced nucleic acid.

As used herein, "stable expression," "stable incorporation," "stable transfection" and the like refer to the integration of an exogenous gene into the genome of a host cell, which can allow for long term expression of the exogenous gene.

As used herein, "transient expression," "transient transfection," and the like refer to the introduction of an exogenous gene into a host cell that does not result in stable incorporation of the gene into the host cell.

As used herein "chemical" refers to any molecule, compound, particle, or other substance that can be a substrate for an enzyme in the enzymatic pathway described herein and/or a pterin (e.g. BH4) synthesis enzyme or biochemical pathway. A "chemical" can also be used to refer to a metabolite of a carbohydrate or lignin. As such, "chemical" can refer to

nucleic acids, proteins, organic compounds, inorganic compounds, metabolites etc. "Chemical" can also refer to the product produced by the biocatalyst.

As used herein "biologically coupled" refers to the association of or interaction between two or more physically distinct molecules, groups of molecules compounds, organisms, or particles where the association is directly or indirectly mediated between the two or more physically distinct molecules, groups of molecules compounds, organisms or particles via a biologic molecule or compound. This can include direct binding between two biologic molecules and signal transduction pathways.

As used herein, "biological communication" refers to the communication between two or more molecules, compounds, or objects that is mediated by a biologic molecule or biologic interaction.

As used herein, "biologic molecule," "biomolecule," and the like refer to any molecule that is present in a living organism and includes without limitation, macromolecules (e.g. proteins, polysaccharides, lipids, and nucleic acids) as well as small molecules (e.g. metabolites and other products produced by a living organism).

As used herein, "regulation" refers to the control of gene or protein expression or function.

As used herein, "promoter" refers to the DNA sequence(s) that control or otherwise modify transcription of a gene and can include binding sites for transcription factors, RNA polymerases, and other biomolecules and substances (e.g. inorganic compounds) that can influence transcription of a gene by interaction with the promoter. Typically these sequences are located at the 5' end of the sense strand of the gene, but can be located anywhere in the genome.

As used herein, "native" refers to the endogenous version of a molecule or compound relative to the host cell or population being described.

As used herein, "non-naturally occurring" refers to a non-native version of a molecule or compound or non-native expression or presence of a molecule or compound within a host cell or other composition. This can include where a native molecule or compound is influenced to be expressed or present at a different location within a host, at a non-native period of time within a host, or is otherwise in an altered environment, even when considered within the host. Non-limiting examples include where a protein that is expressed only in the nucleus of a cell is expressed in the cytoplasm of the cell or when a protein that is only normally expressed during the embryonic stage of development is expressed during the adult stage.

As used herein, "encode" refers to the biologic phenomena of transcribing DNA into an RNA that, in some cases, can be translated into a protein product. As such, when a protein is said herein to be encoded by a particular nucleotide sequence, it is to be

understood that this refers to this biologic relationship between DNA and protein. It is well established that RNA can be translated into protein based on the triplet code where 3 nucleotides represent an amino acid. This term also includes the idea that DNA can be transcribed into RNA molecules with biologic functions, such as ribozymes and interfering RNA species. As such, when a RNA molecule is said to be encoded by a particular nucleotide sequence it is to be understood that this is referring to the transcriptional relationship between the DNA and RNA species in question. As such "encoding nucleotide" refers to herein as the nucleotide which can give rise through transcription, and in the case of proteins, translation a functional RNA or protein.

As used herein, biocatalyst can refer to a single enzyme or pathway containing one or more enzymes and/or other proteins or other components that is configured to carryout, initiate, and/or modify the rate of a chemical or biochemical reaction in an organism, cell, or in-vitro cell free system.

As used herein, biocatalysis can refer to the catalysis carried out in an organism or cells, or in-vitro cell free system, by a biocatalyst.

As used herein, "pterin-dependent" can refer to the requirement of an enzyme for a pterin co-factor for the enzymatic catalysis that is mediated by the enzyme to occur. "Pterin-dependent" can thus also refer to a biochemical pathway that contains an enzyme that is pterin-dependent.

As used herein "codon optimized" or "codon optimization" refers to a codon modification or making modifications to the codons for amino acids in a polypeptide such that they reflect the codon usage bias of the cell type that the polypeptide is expressed in. Modifications to the codons can be made using techniques generally known in the art.

Discussion

Current techniques for synthesis of complex pharmaceutical compounds rely on isolation of enantiopure starting or intermediate compounds from plants, a challenging and expensive endeavor due to low tissue accumulation and purification complexities. Compounds from plants are also high in aromatic content, presenting additional challenges for the synthesis of target compounds. For example, lignin derived aromatic compounds are extremely difficult to modify, which significantly increases their cost and reduces their applications.

With the limitations of current techniques in mind, described herein is a biocatalyst that can contain a pterin source (which can be tetrahydrobiopterin, or BH_4) and a pterin-dependent enzymatic pathway. the biocatalyst can optionally a pterin recycling pathway. The biocatalyst described herein can synthesize non-natural amino acids, hydroxylated aromatics, neurotransmitters, neurotransmitter metabolites, and alkaloids using a direct and selective reaction step. The synthetic products of the system can be produced from a carbohydrate substrate, such as glucose or galactose. The biocatalyst can be optionally

expressed and/or exist in an artificial cell-free system. The biocatalyst can be expressed and operate within a cell. Other compositions, compounds, methods, features, and advantages of the present disclosure will be or become apparent to one having ordinary skill in the art upon examination of the following drawings, detailed description, and examples. It is intended that all such additional compositions, compounds, methods, features, and advantages be included within this description, and be within the scope of the present disclosure.

Biocatalysts

Described herein is a biocatalyst 2000 that can contain physically distinct components that are biologically coupled to and/or in biologically communication with each other. The biocatalyst can be expressed and operate independently in a cell-free environment and can be expressed and operate within a cell. The cell can be engineered to express one or more of the components, individually or in combination, of the biocatalyst system 2000 described herein.

An overview of the biocatalyst is presented in Figures 1-4. The biocatalyst can contain an enzymatic pathway 1000 coupled to a BH4 source 1100 (Figure 1). The enzymatic pathway 1000 can utilize tetrahydrobiopterin (herein referred to as BH4 or BH₄) from a BH4 source 1100 as a cofactor. As the enzymatic reaction of the enzymatic pathway 1000 progresses, BH4 used by the enzymatic pathway 1000 is converted into BH4-4a-carbinolamine 1200. Tetrahydromonapterin (MH₄), is another pterin that can be used. The enzymatic pathway 1000 can optionally be omitted for a biocatalyst where BH4 is the only desired product.

The biocatalyst 2000 can contain an enzymatic pathway 1000 coupled to a BH4 source 1100 and a BH4 recycling pathway 1300 (Figure 2). The enzymatic pathway 1000 can utilize BH4 from a BH4 source 1100 as a cofactor. As the enzymatic reaction of the enzymatic pathway 1000 progresses, BH4 used by the enzymatic pathway 1000 can be converted into BH4-4a-carbinolamine 1200. The BH4 recycling pathway 1300 can convert BH4-4a-carbinolamine back into BH4.

The biocatalyst 2000 can be contained in or expressed in an cell 1400, which can contain an enzymatic pathway 1000 coupled to a BH4 source 1100 (Figure 3). In other words, a cell can be engineered to contain a biocatalyst as previously described. The enzymatic pathway 1000 can utilize BH4 from a BH4 source 1100 as a cofactor. As the enzymatic reaction of the enzymatic pathway 1000 progresses, BH4 used by the enzymatic pathway can be converted into BH4-4a-carbinolamine 1200. The enzymatic pathway 1000 can optionally be omitted from the engineered cell for a biocatalytic engineered cell that only produces BH4. In some embodiments, the enzymatic pathway can be contained in a first cell and the BH4 source 1100 can be contained in a second

cell. The first cell and the second cell can be contained in the same environment (e.g. a cell culture system) such that the BH4 produced by the second cell can be utilized by the enzymatic pathway 1000 of the second cell.

5 In some embodiments, the cell 1400, can contain an enzymatic pathway 1000 coupled to a BH4 source 1100 and an optional BH4 recycling pathway 1300 (Figure 4). The enzymatic pathway 1000 can utilize BH4 from a BH4 source 1100 as a cofactor. As the enzymatic reaction of the enzymatic pathway 1000 progresses, BH4 can be converted into BH4-4a-carbinolamine 1200. The BH4 recycling pathway 1300 can convert BH4-4a-carbinolamine back into BH4. The enzymatic pathway 1000 can again be
10 omitted if an engineered cell that produces and recycles BH4 only is desired. In some of these embodiments, a cell that only expresses the BH4 source 1100 and a BH4 recycling pathway 1300 can be contained in a population of cells that further contains a cell that contains an enzymatic pathway 1000, such that the BH4 produced by the cell expressing the BH4 source 1100 and recycling pathway 1300 can be utilized by the enzymatic
15 pathway 1000 of the other cell

The physically distinct components (e.g. enzymes and biochemical pathways) can be expressed within a whole cell. The whole cell can be a prokaryotic cell or an eukaryotic cell. In some embodiments, the cell is a yeast cell. In other embodiments, the physically distinct components can be expressed in a synthetic *in vitro* system. The physically distinct
20 components can be considered modular components where each one can be independently manipulated and changed without alteration of the other components. This modular configuration can allow for efficient and rapid tuning and customization of system based on the desired synthetic output of the biocatalyst. The individual modular components are discussed in further detail below. *Tetrahydrobiopterin (BH4) Source*

25 The biocatalyst 2000 described herein can include a enzymatic pathway 1000 that is dependent on and/or biologically coupled to a pterin for catalysis of the enzymatic reaction of the enzymatic pathway 1000. The biocatalyst 2000 can be expressed in an cell 1400 as previously described in relation to e.g. Figs. 3-4. Microbes, such as yeast, do not endogenously express pterins that can be appropriately coupled to an enzymatic pathway
30 1000. Therefore, exogenous pterin must be provided to a microbe (or other pterin deficient cell) that expresses a pterin-dependent enzyme or pathway in order for the pterin-dependent enzyme or pathway to properly function. In embodiments, exogenous pterin can be provided directly or a pterin source 1100 can be included in the biocatalyst system for proper functioning.

35 The biocatalyst 2000 can contain a pterin source. The pterin source can provide tetrahydrobiopterin (herein referred to as BH4 or BH₄). The BH4 source 1100 can be a BH4 synthesis pathway. A BH4 source 1100 that can contain a BH4 synthesis pathway can

include one or more enzymes that are biologically coupled to each other and/or in biological communication with each other. In some embodiments, the BH4 source can contain one or more of the enzymes GTP cyclohydrolase, pyruvoyl tetrahydropterin synthase, and/or sepiapterin reductase. In embodiments, the BH4 source can be a BH4 synthesis pathway that contains enzymes that, in operation, biologically communicate with each other to produce BH4 from a purine, such as guanosine triphosphate (GTP). In embodiments where the BH4 source 1100 is a BH4 synthesis pathway recombinant expressed in a cell, GTP or other suitable purines that fuel the BH4 synthesis pathway can be synthesized by endogenous pathways already present in a cell from a carbohydrate or sugar substrate, such as glucose or galactose.

In some embodiments, the BH4 source can contain a polypeptide with a sequence or a part thereof that is 90% to 100% identical to or corresponds to a sequence that is 90% to 100% identical to SEQ ID NOs.: 1-11. In other embodiments, the BH4 source can contain a polypeptide with a sequence or part thereof that is a homologue, orthologue, xenologue, or paralogue to a sequence or a part thereof that is 90% to 100% identical to or corresponds to a sequence that is 90% to 100% identical to SEQ ID NOs.: 1-11. The sequences can be codon optimized. The sequences can be codon optimized for yeast.

Other pterins and modified pterins may be suitable as a pterin source, such as tetrahydromonopterin (MH₄) or other modified pterins. BH4 can be accepted with higher efficiency by amino acid mono-oxygenases from higher eukaryotes than MH₄, such as those in the present disclosure. The BH4 synthesis pathway can be configured to catalyze a substrate such as a carbohydrate or a sugar, such as glucose or galactose, to synthesize BH4.

Tetrahydrobiopterin (BH4) Recycling Pathway

The biocatalyst can optionally include a pterin recycling pathway 1300. The pterin recycling pathway 1300 can be biologically coupled to the BH4 source 1100. This can provide a constant pterin supply for the enzymatic pathway 1000. A suitable pterin can be BH4 that is provided by a BH4 source 1100. In the presence of a BH4 recycling pathway 1300, BH₄-4a-carbinolamine can be converted back to BH₄ via the intermediate quinoid dihydrobiopterin through consecutive reactions by pterin-4a-carbinolamine dehydratase (PCD) and dihydropteridine reductase (DHPR). Other suitable pterin recycling components can be substituted depending on the desired pterin utilized. In some embodiments, the BH4 recycling pathway can contain a polypeptide with a sequence or a part thereof that is 90% to 100% identical to or corresponds to a sequence that is 90% to 100% identical to SEQ ID NOs.: 12-13. In other embodiments, the BH4 recycling pathway can contain a polypeptide with a sequence or part thereof that is a homologue, orthologue, xenologue, or paralogue to a sequence or a part thereof that is 90% to 100% identical to or corresponds to a sequence

that is 90% to 100% identical to SEQ ID NOs.: 12-13. The sequences can be codon optimized. The sequences can be codon optimized for yeast.

Enzymatic Pathway

The biocatalyst 2000 can have an enzymatic pathway 1000 that can or is configured to utilize BH₄ from a BH₄ source 1100. The enzymatic pathway 1000 can use MH₄ or another modified pterin. The enzymatic pathway 1000 can be a direct and selective enzymatic hydroxylation reaction. The enzymatic pathway 1000 can be a direct and selective enzymatic hydroxylation reaction comprising a natural or modified amino acid mono-oxygenase. The enzymatic pathway 1000 can be comprised of nitric oxide synthase or alkylglycerol monooxygenase. The enzymatic pathway 1000 can be an alkaloid synthesis pathway. An enzymatic pathway 1000 comprising an alkaloid synthesis pathway can be comprised of a pterin-dependent oxidation component that is coupled to a pterin, a decarboxylation component, and/or a condensation component. The enzymatic pathway 1000 can be configured to receive a carbohydrate, glucose, galactose, and/or lignin or a derivative or a metabolite thereof. In some embodiments, the enzymatic pathway can contain a polypeptide having a sequence or a part thereof that is 90% to 100% identical to or corresponds to a sequence that is 90% to 100% identical to SEQ ID NOs.: 14-17. In other embodiments, the enzymatic pathway can contain polypeptide having a sequence or part thereof that is a homologue, orthologue, xenologue, or paralogue to a sequence or a part thereof that is 90% to 100% identical to or corresponds to a sequence that is 90% to 100% identical to SEQ ID NOs.: 14-17. The sequences can be codon optimized. The sequences can be codon optimized for yeast.

Biocatalyst-Expressing Vectors and Cells

Enzymes and other components of the biocatalyst 2000 herein can be present as DNA sequences in an expression vector. The expression vector can be a plasmid. The DNA sequences can be coding sequences and/or codon optimized for suitable expression in a host. The DNA sequences in the vector can be expressed downstream of a promoter. A promoter can be a generic constitutive promoter, generic species-specific promoter, host-specific promoter, or inducible promoter. A promoter can be naturally occurring or artificial, codon optimized, and/or modified. In embodiments, a vector can contain a sequence containing all or part of a sequence having about 90% to 100% identity to any one of SEQ ID NOs: 1-17. The vector can contain all or part of a sequence that is a homologue, orthologue, xenologue, paralogue to any sequence having about 90%-100% identity to any one of SEQ ID NOs: 1-17. The sequences can be codon optimized. The sequences can be codon optimized for yeast.

The BH₄ synthesis pathway, BH₄ recycling pathway, and enzymatic pathway can be expressed or otherwise contained within a single host cell. The host cell can be eukaryotic or

prokaryotic. In some embodiments, the host cell can be a fungal cell or a bacterial cell. In some embodiments the host cell is a yeast cell. A yeast platform for amino acid mono-oxidation can facilitate the synthesis of complex plant alkaloids, as expression of downstream alkaloid pathway enzymes are thought to be mainly transmembrane cytochrome P450s, which are difficult to functionally express in bacteria such as *E.coli* without protein engineering. In addition, *S. cerevisiae*'s robustness, tolerance to industrial conditions, including low pH and high sugar concentrations, and insusceptibility to phage infection can make it a suitable host for chemical production. Yeast species for the host cell can include but are not limited to *S. cerevisiae*, *Pichia Pastoris*, *Saccharomyces Pombe*. Suitable strains of *S. cerevisiae* include, but are not limited to the W303 strain (ATCC), PPY810, PPY752, PPY753, PPY754, PPY755, PPY756, PPY757, PPY758, PPY759, PPY760, PPY761, PPY762, PPY764, PPY764, PPY765, PPY766, PPY767, PPY768, PPY797, PPY798, PPY799, PPY800, PPY801, PPY802, PPY803, PPY769, PPY804, PPY805, PPY806, PPY807, PPY808, PPY809, PPY770, PPY771, PPY772, PPY773, PPY774, PPY775, PPY776, PPY77, PPY778, PPY779, PPY780, PPY781, PPY782, PPY783, PPY784, PPY785, and PPY786.

The biocatalyst system or any component thereof can be introduced into the host cell via a single or multiple plasmid system (transient expression) or integrated into the genome (for stable expression). The biocatalyst system can be introduced via electroporation, nucleofection, transfection, transformation, or any otherwise suitable technique for introducing exogenous genetic sequences into a prokaryotic or eukaryotic cell. There can be single and/or multiple copies of each system component present. The biocatalyst can be stably or transiently expressed within the host cell. Stable or transient expression of the biocatalyst system within the host cell can be accomplished under generic constitutive promoters, generic species-specific promoters, host-specific promoters, or inducible promoters. Promoters can be naturally occurring or artificial, codon optimized, and/or modified. Components of the biocatalysts and systems thereof described herein can also be introduced into a cell by a virus. The virus can be an adeno-associated virus, a lentivirus, a baculovirus, or any other viral host suitable for delivering a genetic sequence into a host.

Systems and Methods of Using the Pterin-dependent biocatalysts

Also described herein are systems and methods of using the biocatalyst 2000. As described above the modular components of the biocatalyst 2000 can be expressed within a host cell (also referred to herein as a cell or an engineered cell). The biocatalyst (either in a cell-free system_) or as contained within an engineered cell can be used in a method to produce amino acids, non-natural amino acids, hydroxylated aromatics, and other compounds of interest. The method can include providing a biocatalyst and/or cell containing a biocatalyst as described elsewhere herein and a substrate. The method can further contain

the step of incubating the biocatalysts and/or the cell containing a biocatalyst 2000 as described herein in with the substrate for a length of time. The length of time can range from about 1 hour to about 1, 2, 3, 4 or more weeks. Suitable substrates include, but are not limited to carbohydrates and sugars. In some embodiments, the substrate is glucose and/or galactose. Suitable cell culture techniques will be appreciated by those of skill in the art.

After incubation a suitable assay or other suitable measurement technique can be performed to confirm and/or measure the product or amount of product produced by the biocatalyst. One of skill in the art will appreciate that the particular assays or measurement technique used will depend on the type of substrate and the enzymatic components of the enzymatic pathway. Suitable assays and measurement techniques include, but are not limited to, mass spectrometry, nuclear magnetic resonance, UV-Vis evaluation, flow cytometry, FACS, luciferase assays (single and dual), β -galactosidase assays, microtiter plate reader, and CAT assays, antibiotic selection, auxotrophic forward and counter selection. Other assays and techniques will be readily appreciated by those of ordinary skill in the art.

The biocatalyst can produce BH₄ from a carbohydrate substrate. The biocatalyst can directly and/or selectively hydroxylate aromatic rings. The biocatalyst can produce alkaloids. In some embodiments, the biocatalyst can produce non-natural amino acids. In embodiments, the biocatalyst can convert tyrosine to L-DOPA, which is an example of an unnatural amino acid. It will be appreciated that unnatural amino acids can be incorporated into polypeptide using appropriate tRNA/aminoacyl-tRNA synthase pairs. In other embodiments, the biocatalyst contained in an engineered cell can produce L-dopa, dopamine, 5-hydroxytryptophan and serotonin from a carbohydrate substrate, this carbohydrate can be glucose or galactose.

Additional embodiments of the biocatalyst can modify monoterpene indole alkaloids or modified monoterpene indole alkaloids produced by condensation to produce more advanced complex monoterpene indole alkaloid products or benzisoquinoline alkaloids (Figure 5). Modifications of hydroxystrictosidine and norlaudanoline can produce valuable pharmaceutical compounds such as camptothecin, vinblastine, berberine, and morphine. In some embodiments, the biocatalyst can be used to produce pharmaceutical compounds. Figure 6 shows one embodiment of pharmaceutical semisynthesis wherein hydroxycamptothecin 31 can be converted from hydroxystrictosidine 30 via the MIA biosynthetic pathway. Hydroxycamptothecin can then enable rapid access to the anticancer drugs topotecan and irinotecan. As such, in embodiments, the biocatalyst can be used to produce chemotherapeutics, including but not limited to topotecan and irinotecan.

Kits

Also provided herein are kits containing a biocatalyst, a cell or population thereof containing a biocatalyst as described herein, and/or one or more vectors configured to express one or more components of the biocatalyst described herein. The kit can contain one or more substrates suitable for use with the biocatalyst described herein. The kit can
5 further contain additional reagents, diluents, cell culture media, cell culture plates or other container, syringes, and other components (cells, vectors, transfection reagents, etc.) that can be used with the biocatalyst, a cell or population thereof containing a biocatalyst as described herein, and/or one or more vectors.

10

EXAMPLES

Now having described the embodiments of the present disclosure, in general, the following examples describe some additional embodiments of the present disclosure. While embodiments of the present disclosure are described in connection with the following examples and the corresponding text and figures, there is no intent to limit embodiments of
15 the present disclosure to this description. On the contrary, the intent is to cover all alternatives, modifications, and equivalents included within the spirit and scope of embodiments of the present disclosure.

Example 1: Alkaloid Synthesis.

20 Alkaloids are the largest group of nitrogen-containing secondary metabolites, with more than 20,000 structures¹, are present in roughly 20% of plant species², and are important because of their medicinal use³. Of particular importance are monoterpene indole alkaloids (MIAs) (Figure 5), which include anticancer agents such as camptothecin (1) and vinblastine (2), antimalarial agents such as quinine, and antiarrhythmic agents such as
25 ajmalicine. Another important alkaloid family is benzylisoquinoline alkaloids (BIAs), which include the antibiotic berberine (3) and the analgesic morphine (4)⁴. Due to their chemical complexity, alkaloids often require multi-step chemical syntheses, which, coupled with the necessity of enantiopure material, make them a challenging synthetic target⁵⁻⁸. Therefore, medically important alkaloids, or advanced intermediates thereof, are frequently isolated
30 directly from the native plants^{5,7,9}. Although effective, isolation of alkaloids from plants is often limited by their low accumulation in plant tissue and their difficult separation from other natural products, thus resulting in the high cost of alkaloid-derived pharmaceuticals, especially those based on MIAs^{5,7}. Via plant breeding and, more recently, plant metabolic engineering, the production of BIAs and MIAs *in planta* has been increased¹⁰⁻¹³. Further,
35 plants have been engineered to produce halogenated MIAs, with potentially higher bioactivity, which can serve as late intermediates in the semi-synthesis of other alkaloids¹⁴⁻¹⁸.

Nevertheless, limited understanding of plant secondary metabolite regulation and slow plant growth rate cloud the future of engineering plants to overproduce alkaloids^{2,5,7}. These limitations result in the underrepresentation of alkaloid-derived compounds in pharmaceutical drug screenings¹⁷. The synthesis of plant alkaloids in microbes would enable the rapid and scalable production of known alkaloids and open the door to the biosynthesis of novel alkaloids using engineered enzymes or combinatorial enzyme assembly. Plant alkaloid production in microbes has the advantages of short doubling time, rapid extraction of the alkaloid from the culture medium, easier isolation of the desired alkaloid due to the absence of similar natural products, and a lack of endogenous pathway regulation, which allows for deregulation of alkaloid biosynthesis.

Many alkaloids are obtained via the hydroxylation and decarboxylation of amino acids¹⁸. Specifically, BIAs are derived from tyrosine (5) and MIAs are derived from tryptophan (6). In the last ten years, full elucidation of many BIA biosynthetic pathways⁴, in conjunction with advances in synthetic biology, have allowed the reconstruction of BIA pathways in both *Escherichia coli* and *Saccharomyces cerevisiae*¹⁹⁻²⁵. Although MIA biosynthetic pathways have been extensively engineered *in planta*, the engineering of MIA alkaloids in microbes has been limited. A major problem in engineering microbes for the synthesis of plant alkaloid is the amino acid hydroxylation step. Tyrosinase (also known as tyrosine hydroxylase), the most common enzyme used to hydroxylate tyrosine, not only oxidizes tyrosine into L-3,4-dihydroxyphenylalanine (L-DOPA, 7), but its o-diphenolase activity also results in further oxidation of L-DOPA to L-dopaquinone, a melanin precursor²⁶⁻²⁸, thus reducing the availability of L-DOPA for alkaloid production. Nevertheless, tyrosinase has been used to produce the BIA reticuline from glycerol^{21 and 25}. Recently, a P450 enzyme from beet was engineered for reduced o-diphenolase activity to increase the specificity of tyrosine hydroxylation to L-DOPA²⁹. There is no equivalent to tyrosinase for tryptophan hydroxylation. To circumvent this problem, microbial production of 5-hydroxytryptophan (8) can be achieved by indole hydroxylation followed by coupling to serine³⁰ or using an engineered phenylalanine hydroxylase with changed substrate specificity³¹. Hydroxytryptophan has not been converted to serotonin (9) or to MIAs microbially from glucose. Specific mono-oxygenases for tyrosine and tryptophan exist in higher eukaryotes; however, they require the pterin co-factor tetrahydrobiopterin (BH₄, 10), which is not present in *E. coli* or *S. cerevisiae*.

A pterin-dependent oxidation strategy to specifically mono-oxidize of tyrosine or tryptophan can provide an alternative biosynthetic route for MIA and BIA biosynthesis (Fig. 5). The difficulty in this strategy is that neither *E. coli* nor *S. cerevisiae* endogenously produce BH₄, which is necessary for the activity of mono-oxygenases found in higher eukaryotes^{32,33}. Although BH₄ production has been previously accomplished in *E. coli*³⁴, it

was not coupled to amino acid mono-oxidation. The endogenous *E. coli* BH₄ analog, tetrahydromapterin (MH₄), can be used as a co-factor for BH₄-dependent mono-oxygenases in the production of hydroxytyrosol³⁵ and 5-hydroxytryptophan³¹. However, MH₄ has a different composition and stereochemistry when compared to BH₄ (Figure 13). To
5 establish a microbial platform for the synthesis of plant alkaloids via the pterin-dependent mono-oxidation strategy, *S. cerevisiae* can be engineered for BH₄ production. A yeast platform for amino acid mono-oxidation can further facilitate the synthesis of complex plant alkaloids.

A pterin-dependent mono-oxidation strategy for the microbial synthesis of the
10 biogenic amines dopamine and serotonin is described herein. Serotonin synthesis can further be leveraged to produce a modified MIA. An engineered BH₄-producing yeast can mono-oxidize tryptophan to 5-hydroxytryptophan, which, after decarboxylation to serotonin, can be condensed with the monoterpene secologanin (11) to produce the modified MIA hydroxystrictosidine (12). BH₄ biosynthetic enzymes can be combinatorially screened to
15 produce different levels of BH₄. A BH₄ recycling pathway can optionally be present to guarantee supply of BH₄ to the amino acid mono-oxygenases. Pterin-dependent oxidation of tryptophan is shown herein, followed by decarboxylation results in the biogenic amine serotonin, a key MIA intermediate. The MIA biosynthetic pathway can further be introduced to ultimately produce hydroxystrictosidine from glucose and secologanin. The generality of
20 the pterin-dependent mono-oxidation strategy for the microbial synthesis of alkaloids is shown herein by using the example of a tyrosine mono-oxygenase to convert tyrosine into L-DOPA, which can subsequently decarboxylated to dopamine (13), a key BIA intermediate. The microbial strains presented herein can be used for the scalable production of MIAs, as well as the production of modified MIAs to serve as late intermediates in the semisynthesis
25 of known and novel therapeutics (Fig. 34). Further, the microbial strains in this work can be used as plant pathway discovery tools to elucidate known MIA biosynthetic pathways or to identify pathways leading to novel MIAs.

Target choice: hydroxystrictosidine. While the natural branch point in MIA biosynthesis is strictosidine⁴⁴, the biosynthesis of 10-hydroxystrictosidine can be pursued
30 instead, a minor MIA produced by *Camptotheca acuminata*⁴⁵, the major producer of the anticancer agent camptothecin. The biosynthesis of 10' functionalized strictosidine can provide a chemical handle for the rapid derivatization of strictosidine-derived MIAs. 10-hydroxystrictosidine can be synthesized via the condensation of 5-hydroxytryptamine (serotonin) and secologanin, rather than tryptamine and secologanin as in the case of
35 strictosidine. Modifications at the 5' position of tryptophan have been shown to be processed by MIA enzymes in *Catheranthus roseus* to produce 10' modified ajmalicine, serpentine, and tabersonine⁴⁶. In this spirit, 10-hydroxystrictosidine can enable the

biosynthesis of modified MIAs, such as 10-hydroxycamptothecin (14), which has higher anticancer activity than camptothecin¹⁸ (Fig. 6). Modified MIAs such as 10-hydroxycamptothecin can serve as better semi-synthesis intermediates than camptothecin for the chemical synthesis of more water soluble derivatives¹⁸, such as the colon anticancer drug irinotecan (15) and the ovarian and lung cancer drug topotecan (16). More generally, modified MIAs can serve as synthons for the semisynthesis of novel complex alkaloids with potential therapeutic activities.

Microbial synthesis of tetrahydrobiopterin in S. cerevisiae. *S. cerevisiae* does not produce BH₄, but guanosine triphosphate (GTP, 17) can be re-routed to produce BH₄ through the intermediates dihydroneopterin triphosphate (18) and pyruvoyl tetrahydropterin (19) using three enzymes: GTP cyclohydrolase I (GTPCH), pyruvoyl tetrahydropterin synthase (PTPS), and sepiapterin reductase (SR) (Fig. 7a). Given that BH₄ oxidizes to dihydrobiopterin and, subsequently, to biopterin in water⁴⁷, the presence of biopterin in the medium can be used to monitor BH₄ in cells. GTPCH is the first committed step in BH₄ biosynthesis⁴⁸. *S. cerevisiae* has an endogenous GTPCH as part of the folate biosynthetic pathway and thus requires only expression of heterologous PTPS and SR to produce BH₄. *Mortierella alpina* is the only fungus shown to carry the full BH₄ biosynthetic pathway from GTP⁴⁹, and, given that *S. cerevisiae* is also a fungus, *M. alpina* enzymes can be efficiently expressed in this organism. Overexpression of *M. alpina* PTPS and SR in *S. cerevisiae* produced 0.74 mg/L of BH₄, measured as biopterin (Fig. 7b, Fig. 13A-B). BH₄ can be exogenously fed to yeast cells expressing only a pterin-dependent tyrosine mono-oxygenase to convert tyrosine into L-DOPA. No L-DOPA was seen when feeding 0-50 mg/L of BH₄, suggesting that BH₄ oxidizes to dihydrobiopterin or biopterin before reaching the tyrosine mono-oxygenase. BH₄ can be synthesized intracellularly rather than exogenously fed to determine whether 0.74 mg/L BH₄ limits alkaloid biosynthesis.

Four GTPCHs, four PTPSs, and three SRs can be combinatorially expressed in *S. cerevisiae* to identify yeast strains that produce different amounts of BH₄. Among GTPCHs, enzymes from *E. coli*, *M. alpina*, *Homo sapiens*, and *S. cerevisiae* can be expressed. *E. coli* GTPCH has a low K_M (0.02⁵⁰-100⁵¹ μM) and has been previously expressed in *S. cerevisiae*⁵². *H. sapiens* GTPCH has a pI of 5.6⁵³, which could aid in its solubility, and it has also been expressed in *S. cerevisiae*⁵². Among PTPSs, the enzymes from *M. alpina*, *Salmo salar*, the halophile *Salinibacter ruber*, and the bacteria *Phycisphaera mikurensis* can be screened. *S. salar* PTPS has a specific activity that is fifty times higher, and a K_M that is five times lower, than that of the canonical human PTPS⁵⁴. The putative PTPS from *S. ruber* may be suitable because a structural homology model alignment with *S. salar* PTPS revealed that these enzymes have an almost identical active site, except that *S. ruber* PTPS has a catalytic aspartate rather than a cysteine residue⁵⁵ (Fig. 14). The aspartate's

carboxylate can function as a better acid-base catalyst compared to cysteine's thiol group. The predicted PTPS from *P. mikurensis* can be suitable because its active site is almost identical to the well-studied *Rattus rattus* PTPS⁵⁶; however, the *P. mikurensis* PTPS N-terminus has an additional ~100 amino acids. A bioinformatics search revealed that *S. cerevisiae* lacked any PTPS homolog. Among SRs, the SR from *M. alpina*, a predicted SR from the diatom *Thalassiosira pseudonana*, which, based on structural homology models is hypothesized to be NADH- rather than NADPH-dependent (Fig. 15), and a putative SR from *S. cerevisiae* found using bioinformatics can be screened.

Optimization of tetrahydrobiopterin biosynthesis. Increasing the flux through the purine pathway can increase GTP levels and, in turn, BH₄ production. The parent yeast strain, W303, has a non-functional phosphoribosylaminoimidazole carboxylase (*ade2*) gene located upstream of GTP in the purine biosynthetic pathway (Fig. 16). A functional *Ade2* yeast strain can be generated and introduced the BH₄ synthesis pathway. To identify bottlenecks within the BH₄ pathway, the expression of each gene in the combinatorially optimized BH₄ synthesis pathway can be monitored by expressing each gene from a single- or multi-copy plasmid using galactose-inducible promoters. GTPCH, PTPS and SR mRNA levels can be measured when expressed from single- or multi-copy plasmids (Fig. 17) to determine a suitable expression level. To produce BH₄ from glucose and to reduce plasmid burden^{37,58}, an enzyme combination can be expressed from a multicopy plasmid using constitutive promoters.

Tetrahydrobiopterin recycling for pterin-dependent amino acid mono-oxidation. Upon amino acid mono-oxidation, BH₄ can be converted to BH₄-4a-carbinolamine (20). Previous work in *E. coli* has shown the BH₄ recycling pathway to be critical to ensure continuous supply of the BH₄ analog MH₄ in pterin-dependent amino acid mono-oxidation^{31,35}. To provide a continuous supply of BH₄ to the amino acid mono-oxygenases, a BH₄ recycling pathway in *S. cerevisiae* can optionally be included. In the recycling pathway, BH₄-4a-carbinolamine is converted back to BH₄ via the intermediate quinoid dihydrobiopterin (21) through consecutive reactions by pterin-4a-carbinolamine dehydratase (PCD) and dihydropteridine reductase (DHPR) (Fig. 8a). The effect of the *H. sapiens* BH₄ recycling pathway on the pterin-dependent mono-oxidation of tyrosine to L-DOPA can be measured, using the codon optimized *Mus musculus* tyrosine hydroxylase, in 1) the absence of the BH₄ biosynthetic and recycling pathways, 2) the presence of only the BH₄ biosynthetic pathway, and 3) the presence of both the BH₄ biosynthetic and recycling pathways (Fig. 8b, c, Fig. 18a). The media can be optionally supplemented with tyrosine to increase L-DOPA production. High-level BH₄ synthesis can eliminate the necessity of the BH₄ recycling pathway at the current rate of tyrosine mono-oxidation. The media can be supplemented with tyrosine to improve tyrosine biosynthesis.

Microbial synthesis of biogenic amines via pterin-dependent mono-oxidation.

Biogenic amines can be the immediate precursors to both MIAs and BIAs⁵. To microbially synthesize the BIA biogenic amine precursor dopamine from galactose, a strain carrying the BH₄ biosynthetic and recycling pathways, tyrosine mono-oxygenase, and the codon optimized *Sus scrofa* aromatic L-amino-acid decarboxylase (DDC) can be engineered (Figs 9a-d). To microbially synthesize the MIA biogenic amine serotonin from galactose, a yeast strain carrying the BH₄ biosynthetic and recycling pathways, a truncated codon optimized *H. sapiens* tryptophan hydroxylase⁵⁹, and aromatic-L-amino acid decarboxylase (also known herein as DDC) (Figs. 9e-h) can be engineered. The BH₄ recycling pathway may not affect biogenic amine production. The media can be optionally supplemented with tryptophan to increase serotonin production.

Microbial synthesis of the modified MIA hydroxystrictosidine. Although several microbial strains have been engineered for the production of BIAs¹⁹⁻²⁵, engineering of MIA microbial platforms has lagged behind⁶⁰. Further, to our knowledge no modified alkaloid has been produced microbially to date. Microbial synthesis of modified alkaloids can generate more amenable intermediates for chemical derivatization to obtain final therapeutics. The pterin-dependent biogenic amine-producing strain can be used for the production of the modified MIA hydroxystrictosidine. To microbially synthesize hydroxystrictosidine from galactose and secologanin, the serotonin-producing strain, carrying the BH₄ recycling pathway and expressing the *Ophiorrhiza pumila* strictosidine synthase⁶¹ with the vacuolar tag removed so as to avoid enzyme secretion⁶² (Fig.10a) can be utilized. The strain produced both R- and S-hydroxystrictosidine. (Fig. 10b, Fig. 21). Given that hydroxystrictosidine is not commercially available, the compounds using tandem mass spectrometry and high resolution mass spectrometry (Fig. 10c-d, Fig. 22) can be characterized. To improve upon the inducible four-plasmid system, a three-plasmid system using multicopy plasmids with the enzymes under control of constitutive promoters can be used instead of an inducible four plasmid system. The BH₄ recycling pathway can be optionally expressed in both the four- and three-plasmid systems. A two-plasmid system without the BH₄ recycling pathway can be utilized. The hydroxystrictosidine-producing yeast strains can result in a mixture of R- and S-hydroxystrictosidine isomers. Removing strictosidine synthase from the hydroxystrictosidine-producing strain resulted in similar R- and S-hydroxystrictosidine levels (Fig. 23). This indicated that, at the low pH of the yeast medium (pH=5-3), secologanin and serotonin can couple chemically rather than enzymatically to produce a mixture of hydroxystrictosidine isomers⁶³. Indeed, strictosidine synthase can retain less than one tenth of its activity at pH=4-3⁶⁴ and has three orders of magnitude less catalytic activity with serotonin when compared to tryptamine⁶⁵.

Determining strictosidine synthase functionality. To determine if strictosidine synthase was functionally expressed in the hydroxystrictosidine-producing strain, the level of spontaneous and enzymatic hydroxystrictosidine synthesis can be examined under different conditions. The spontaneous condensation of serotonin and secologanin may not occur at pH=7, but can occur at pH=3, producing both hydroxystrictosidine isomers (Fig. 11a, Fig. 24a). Next, hydroxystrictosidine formation can be examined using the lysate of yeast cells expressing strictosidine synthase. When the lysate was placed at pH=3 and fed serotonin and secologanin, both hydroxystrictosidine isomers can be formed, while the same experiment at pH=7 resulted in only the S-isomer (Fig. 11b, Fig. 24b), demonstrating that strictosidine synthase can be functionally expressed in yeast. Isomer identification can be determined due to the fact that strictosidine synthase is known to form only the S-isomer⁸¹ while the spontaneous chemical condensation produces both R- and S-isomers, with the R-isomer being the major product⁶³. Intact yeast cells expressing strictosidine synthase, can be fed exogenous serotonin and secologanin, and cultured the cells for 136 hours using standard (pH= 4-2.5, Fig. 25) or buffered (pH=7-5, Fig.25) synthetic complete media. Yeast cells in the buffered media can result in the synthesis of only S-hydroxystrictosidine (Fig. 11c, Fig. 24c). Finally, strictosidine synthase can be necessary for S-hydroxystrictosidine production in yeast and that the reaction was not catalyzed by an endogenous yeast enzyme (Fig. 11d, Fig. 24d, Fig. 26). These results demonstrate that strictosidine synthase can be functionally expressed in a hydroxystrictosidine-producing yeast strain, that the enzymatic reaction leading to the S-isomer can take place intracellularly, and that secologanin can cross the yeast cell membrane. In standard yeast synthetic complete media, however, the spontaneous coupling of serotonin and secologanin can be the predominant hydroxystrictosidine forming reaction.

Example 2: Pterin-dependent direct and selective hydroxylation of lignin-derived aromatic monomers

The utility of plant-derived compounds is not limited to the synthesis of advanced pharmaceuticals. Lignins are a class of complex cross-linked phenol polymers that are important structural components of vascular plants and some algae. Lignocellulosic feedstocks, high in lignin content, supply sources of energy, chemicals, and fuel. Most of the products we obtain from feedstocks come from cellulose and hemicellulose, while lignin, which is the second most abundant carbon source on earth, is largely unutilized, with the majority of lignin being burned for energy. Lignin is the only large-volume renewable source that's composed of aromatic units, so there is a great potential for conversion of lignin to products in a variety of industries, including plastics, fuels, and various chemicals.

Current techniques for the breakdown of lignin lead to the production of low-molecular weight monomeric aromatic units such as those shown here (Figure 27). Many of these are commonly used and, due to their ready availability, they tend to be very cheap, with many being only cents per gram. These monomeric units can then be upgraded, or
5 valorized, to create more costly molecules by various reactions including repolymerization, alkylation, oxidation to quinones, reduction, or oxidation, specifically hydroxylation.

Hydroxylation is a useful tool in chemical synthesis, however it is very challenging to obtain direct and selective hydroxylation onto aromatic rings. Various reactions exist for chemical hydroxylation, however they each have their own drawbacks. These include metal
10 catalysis, which can be unpredictable in the number and selectivity of the hydroxylations, the use of super critical CO₂, which hasn't been used on aromatic substrates, and the Heck process, which represents a multi-step hydroxylation process. One solution to these problems is to utilize biology and take advantage of enzymes already found throughout nature.

Enzymatic hydroxylation as described previously and further described herein can also be applied to monomeric aromatic compounds that are the degradation products of lignins. This yields an environmentally-friendly solution to the direct and selective hydroxylation of aromatic rings. Enzymatic hydroxylation is catalyzed by many classes of enzymes, a few of which are described herein. The first class includes dioxygenases, which
20 use molecular oxygen to hydroxylate either the substrate twice, such as the case in indigo production by naphthalene dioxygenase, creating a cis-diol, or hydroxylate the substrate and a co-substrate, such as is the case with flavone 6 hydroxylase. The most well-known class of oxygenases are cytochrome P450s, which are typically membrane-bound enzymes which hydroxylate their substrates using a heme-iron and a reductase partner. Non-heme
25 monooxygenases include many subclasses. Flavin mono-oxygenases, which utilize reducing cofactors NADH or NADPH, have been used in the production of muconic acid. A further type of oxygenases are aromatic amino acid mono-oxygenases.

The side chain of tyrosine resembles many of the major products obtained during lignin pyrolysis, having the phenol scaffold (Figure 27). A whole-cell biocatalysis system where
30 various monomeric aromatics (which can be products of lignin degradation, Figure 27) are fed to a cell expressing an amino acid mono-oxygenase coupled to a pterin source (exemplified in Figures 28A-28B) to obtain selectively hydroxylated products with a single step reaction is described in further detail in an example below. The active site of an amino-acid mono oxygenase can be modified to improve substrate specificity for aromatic
35 monomers or other aromatic compounds. These hydroxylated products could then be further used in industry or in the creation of industrially-used chemicals, used as pharmaceutical precursors, or used as fuel precursors (Figure 28A-28B).

Example 3: Biosynthesis of hydroxylated 2,6-dimethoxy-4-methylphenol via pterin-dependent tyrosine mono-oxidation. Figure 29A. shows a schematic representation of the mono-oxidation of 2,6-dimethoxy-4-methylphenol. 1: Engineered yeast carrying the BH4
 5 synthesis pathway to provide a BH4 source and tyrosine hydroxylase. Figure 29b. shows representative LC traces for 2,6-dimethoxy-4-methylphenol mono-oxidation. i) Wild type yeast with 2,6-dimethoxy-4-methylphenol. ii) Media with 2,6-dimethoxy-4-methylphenol. iii). Engineered yeast carrying the BH4 synthesis pathway (BH4 pathway), tyrosine hydroxylase and 2,6-dimethoxy-4-methylphenol result in the biosynthesis of a hydroxylated 2,6-
 10 dimethoxy-4-methylphenol ($m/z=184$) at retention time 8.5min (black diamond).

References for Examples 1,2, and 3.

- 1 Schafer, H., and Wink, M. (2009) Medicinally important secondary metabolites in recombinant microorganisms or plants: progress in alkaloid biosynthesis. *Biotechnol. J.* 4, 1684-1703.
 15
- 2 Minami, H. (2013) Fermentative Production of Plant Benzylisoquinoline Alkaloids in Microbes. *Biosci. Biotech. Bioch.* 77, 1617-1622.
- 3 Yang, L. Q., and Stockigt, J. (2010) Trends for diverse production strategies of plant medicinal alkaloids. *Nat. Prod. Rep.* 27, 1469-1479.
- 4 Hagel, J. M., and Facchini, P. J. (2013) Benzylisoquinoline Alkaloid Metabolism: A Century of Discovery and a Brave New World. *Plant Cell Physiol.* 54, 647-672.
 20
- 5 Leonard, E., Runguphan, W., O'Connor, S., and Prather, K. J. (2009) Opportunities in metabolic engineering to facilitate scalable alkaloid production. *Nat. Chem. Biol.* 5, 292-300.
- 6 Winter, J. M., and Tang, Y. (2012) Synthetic biological approaches to natural product biosynthesis. *Curr. Opin. Biotechnol.* 23, 736-743.
 25
- 7 Chemler, J. A., and Koffas, M. A. (2008) Metabolic engineering for plant natural product biosynthesis in microbes. *Curr. Opin. Biotechnol.* 19, 597-605.
- 8 Kuboyama, T., Yokoshima, S., Tokuyama, H., and Fukuyama, T. (2004) Stereocontrolled total synthesis of (+)-vincristine. *Proc. Natl. Acad. Sci. U. S. A.* 101, 11966-11970.
 30
- 9 Murata, J., Roepke, J., Gordon, H., and De Luca, V. (2008) The leaf epidermome of *Catharanthus roseus* reveals its biochemical specialization. *Plant Cell* 20, 524-542.
- 10 Sato, F., Hashimoto, T., Hachiya, A., Tamura, K., Choi, K. B., Morishige, T., Fujimoto, H., and Yamada, Y. (2001) Metabolic engineering of plant alkaloid biosynthesis. *Proc. Natl. Acad. Sci. U. S. A.* 98, 367-372.
 35

11 Frick, S., Kramell, R., and Kutchan, T. M. (2007) Metabolic engineering with a morphine biosynthetic P450 in opium poppy surpasses breeding. *Metab. Eng.* 9, 169-176.

12 Allen, R. S., Millgate, A. G., Chitty, J. A., Thisleton, J., Miller, J. A. C., Fist, A. J., Gerlach, W. L., and Larkin, P. J. (2004) RNAi-mediated replacement of morphine with the
5 nonnarcotic alkaloid reticuline in opium poppy. *Nat. Biotechnol.* 22, 1559-1566.

13 Glenn, W. S., Runguphan, W., and O'Connor, S. E. (2013) Recent progress in the metabolic engineering of alkaloids in plant systems. *Curr. Opin. Biotechnol.* 24, 354-365.

14 Runguphan, W., and O'Connor, S. E. (2009) Metabolic reprogramming of periwinkle plant culture. *Nat. Chem. Biol.* 5, 151-153.

10 15 Runguphan, W., Qu, X. D., and O'Connor, S. E. (2010) Integrating carbon-halogen bond formation into medicinal plant metabolism. *Nature* 468, 461-464.

16 Glenn, W. S., Nims, E., and O'Connor, S. E. (2011) Reengineering a Tryptophan Halogenase To Preferentially Chlorinate a Direct Alkaloid Precursor. *J. Am. Chem. Soc.* 133, 19346-19349.

15 17 Harvey, A. L. (2008) Natural products in drug discovery. *Drug Discovery Today* 13, 894-901.

18 Dewick, P. M. (2002) *Medicinal natural products: a biosynthetic approach*, 2nd ed., John Wiley & Sons Ltd.

19 Hawkins, K. M., and Smolke, C. D. (2008) Production of benzyloquinoline alkaloids
20 in *Saccharomyces cerevisiae*. *Nat. Chem. Biol.* 4, 564-573.

20 Minami, H., Kim, J. S., Ikezawa, N., Takemura, T., Katayama, T., Kumagai, H., and Sato, F. (2008) Microbial production of plant benzyloquinoline alkaloids. *Proc. Natl. Acad. Sci. U. S. A.* 105, 7393-7398.

21 Nakagawa, A., Minami, H., Kim, J. S., Koyanagi, T., Katayama, T., Sato, F., and
25 Kumagai, H. (2011) A bacterial platform for fermentative production of plant alkaloids. *Nat. Commun.* 2:326 doi: 10.1038/ncomms1327.

22 Fossati, E., Ekins, A., Narcross, L., Zhu, Y., Falgueyret, J. P., Beaudoin, G. A. W., Facchini, P. J., and Martin, V. J. J. (2014) Reconstitution of a 10-gene pathway for synthesis of the plant alkaloid dihydrosanguinarine in *Saccharomyces cerevisiae*. *Nat. Commun.*
30 5:3283 doi: 10.1038/ncomms4283.

23 Thodey, K., Galanie, S., and Smolke, C. D. (2014) A microbial biomanufacturing platform for natural and semisynthetic opioids. *Nat. Chem. Biol.* 10, 837-844

24 Fossati, E., Narcross, L., Ekins, A., Falgueyret, J. P., and Martin, V. J. J. (2015) Synthesis of Morphinan Alkaloids in *Saccharomyces cerevisiae*. *PloS one* 10, DOI: 10.1371/journal.pone.0124459.
35

25 Nakagawa, A., Matsuzaki, C., Matsumura, E., Koyanagi, T., Katayama, T., Yamamoto, K., Sato, F., Kumagai, H., and Minami, H. (2014) (R, S)-Tetrahydropapaveroline

production by stepwise fermentation using engineered *Escherichia coli*. *Sci Rep* 4
doi:10.1038/srep06695.

26 Hernandez-Romero, D., Sanchez-Amat, A., and Solano, F. (2006) A tyrosinase with
an abnormally high tyrosine hydroxylase/dopa oxidase ratio - Role of the seventh histidine
and accessibility to the active site. *Febs J.* 273, 257-270.

27 Luetke-Eversloh, T., Santos, C. N. S., and Stephanopoulos, G. (2007) Perspectives
of biotechnological production of L-tyrosine and its applications. *Appl. Microbiol. Biotechnol.*
77, 751-762.

28 Land, E. J., Ramsden, C. A., and Riley, P. A. (2003) Tyrosinase autoactivation and
the chemistry of ortho-quinone amines. *Accounts Chem. Res.* 36, 300-308.

29 DeLoache, W. C., Russ, Z. N., Narcross, L., Gonzales, A. M., Martin, V. J., and
Dueber, J. E. (2015) An enzyme-coupled biosensor enables (S)-reticuline production in
yeast from glucose. *Nat. Chem. Biol.* doi:10.1038/nchembio.1816.

30 Sun, X., Lin, Y., Yuan, Q., and Yan, Y. (2014) Precursor-Directed Biosynthesis of 5-
Hydroxytryptophan Using Metabolically Engineered *E. coli*. *ACS Synth. Biol.* 4, 554-558.

31 Lin, Y. H., Sun, X. X., Yuan, Q. P., and Yan, Y. J. (2014) Engineering Bacterial
Phenylalanine 4-Hydroxylase for Microbial Synthesis of Human Neurotransmitter Precursor
5-Hydroxytryptophan. *ACS Synth. Biol.* 3, 497-505.

32 Fitzpatrick, P. F. (1999) Tetrahydropterin-dependent amino acid hydroxylases. *Annu.*
Rev. Biochem. 68, 355-381.

33 Werner-Felmayer, G., Golderer, G., and Werner, E. R. (2002) Tetrahydrobiopterin
biosynthesis, utilization and pharmacological effects. *Curr. Drug Metab.* 3, 159-173.

34 Yamamoto, K., Kataoka, E., Miyamoto, N., Furukawa, K., Ohsuye, K., and Yabuta,
M. (2003) Genetic engineering of *Escherichia coli* for production of tetrahydrobiopterin.
Metab. Eng. 5, 246-254.

35 Satoh, Y., Tajima, K., Munekata, M., Keasling, J. D., and Lee, T. S. (2012)
Engineering of L-tyrosine oxidation in *Escherichia coli* and microbial production of
hydroxytyrosol. *Metab. Eng.* 14, 603-610.

36 Leiros, H. K. S., Pey, A. L., Innselset, M., Moe, E., Leiros, I., Steen, I. H., and
Martinez, A. (2007) Structure of phenylalanine hydroxylase from *Colwellia psychrerythraea*
34H, a monomeric cold active enzyme with local flexibility around the active site and high
overall stability. *J. Biol. Chem.* 282, 21973-21986.

37 Siddiqui, M. S., Thodey, K., Trenchard, I., and Smolke, C. D. (2012) Advancing
secondary metabolite biosynthesis in yeast with synthetic biology tools. *Fems Yeast Res.* 12,
144-170.

38 Chang, M. C. Y., Eachus, R. A., Trieu, W., Ro, D. K., and Keasling, J. D. (2007) Engineering *Escherichia coli* for production of functionalized terpenoids using plant P450s. *Nat. Chem. Biol.* 3, 274-277.

39 Miki, Y., and Asano, Y. (2014) Biosynthetic pathway for the cyanide-free production
5 of phenylacetonitrile in *Escherichia coli* by utilizing plant cytochrome P450 79A2 and bacterial aldoxime dehydratase. *Appl. Environ. Microbiol.* 80, 6828-6836.

40 Barnes, H. J., Arlotto, M. P., and Waterman, M. R. (1991) Expression and Enzymatic-Activity of Recombinant Cytochrome-P450 17-Alpha-Hydroxylase in *Escherichia-Coli*. *Proc. Natl. Acad. Sci. U. S. A.* 88, 5597-5601.

10 41 Hong, K. K., and Nielsen, J. (2012) Metabolic engineering of *Saccharomyces cerevisiae*: a key cell factory platform for future biorefineries. *Cell Mol. Life Sci.* 69, 2671-2690.

42 Peralta-Yahya, P. P., Zhang, F. Z., del Cardayre, S. B., and Keasling, J. D. (2012) Microbial engineering for the production of advanced biofuels. *Nature* 488, 320-328.

15 43 Da Silva, N. A., and Srikrishnan, S. (2012) Introduction and expression of genes for metabolic engineering applications in *Saccharomyces cerevisiae*. *Fems Yeast Res.* 12, 197-214.

44 O'Connor, S. E., and Maresh, J. J. (2006) Chemistry and biology of monoterpene indole alkaloid biosynthesis. *Nat. Prod. Rep.* 23, 532-547.

20 45 Silvestrini, A., Pasqua, G., Botta, B., Monacelli, B., van der Heijden, R., and Verpoorte, R. (2002) Effects of alkaloid precursor feeding on a *Camptotheca acuminata* cell line. *Plant Physiol. Bioch.* 40, 749-753.

46 Friedrich, A., Brase, S., and O'Connor, S. E. (2009) Synthesis of 4-, 5-, 6-, and 7-azidotryptamines. *Tetrahedron Lett.* 50, 75-76.

25 47 Davis, M. D., Kaufman, S., and Milstien, S. (1988) The auto-oxidation of tetrahydrobiopterin. *Eur. J. Biochem.* 173, 345-351.

48 Thony, B., Auerbach, G., and Blau, N. (2000) Tetrahydrobiopterin biosynthesis, regeneration and functions. *Biochem. J.* 347, 1-16.

30 49 Wang, H. C., Yang, B., Hao, G. F., Feng, Y., Chen, H. Q., Feng, L., Zhao, J. X., Zhang, H., Chen, Y. Q., Wang, L., and Chen, W. (2011) Biochemical characterization of the tetrahydrobiopterin synthesis pathway in the oleaginous fungus *Mortierella alpina*. *Microbiology.* 157, 3059-3070.

50 Yim, J. J., and Brown, G. M. (1976) Characteristics of Guanosine Triphosphate Cyclohydrolase-I Purified from *Escherichia-Coli*. *J. Biol. Chem.* 251, 5087-5094.

35 51 Schoedon, G., Redweik, U., Frank, G., Cotton, R. G. H., and Blau, N. (1992) Allosteric Characteristics of Gtp Cyclohydrolase-I from *Escherichia-Coli*. *Eur. J. Biochem.* 210, 561-568.

- 52 Mancini, R., Saracino, F., Buscemi, G., Fischer, M., Schramek, N., Bracher, A., Bacher, A., Gutlich, M., and Carbone, M. L. A. (1999) Complementation of the fol2 deletion in *Saccharomyces cerevisiae* by human and *Escherichia coli* genes encoding GTP cyclohydrolase I. *Biochem. Biophys. Res. Commun.* 255, 521-527.
- 5 53 Blau, N., and Niederwieser, A. (1986) The application of 8-aminoguanosine triphosphate, a new inhibitor of GTP cyclohydrolase I, to the purification of the enzyme from human liver. *Biochim. Biophys. Acta* 880, 26-31.
- 54 Hasler, T., and Curtius, H. C. (1989) Purification and characterization of 6-pyruvoyl tetrahydropterin synthase from salmon liver. *Eur. J. Biochem. / FEBS* 180, 205-211.
- 10 55 Burgisser, D. M., Thony, B., Redweik, U., Hess, D., Heizmann, C. W., Huber, R., and Nar, H. (1995) 6-Pyruvoyl Tetrahydropterin Synthase, an Enzyme with a Novel Type of Active-Site Involving Both Zinc-Binding and an Intersubunit Catalytic Triad Motif - Site-Directed Mutagenesis of the Proposed Active-Center, Characterization of the Metal-Binding Site and Modeling of Substrate-Binding. *J. Mol. Biol.* 253, 358-369.
- 15 56 Ploom, T., Thony, B., Yim, J., Lee, S., Nar, H., Leimbacher, W., Richardson, J., Huber, R., and Auerbach, G. (1999) Crystallographic and kinetic investigations on the mechanism of 6-pyruvoyl tetrahydropterin synthase. *J. Mol. Biol.* 286, 851-860.
- 57 Glick, B. R. (1995) Metabolic Load and Heterologous Gene-Expression. *Biotechnol. Adv.* 13, 247-261.
- 20 58 Jones, K. L., Kim, S. W., and Keasling, J. D. (2000) Low-Copy Plasmids can Perform as Well as or Better Than High-Copy Plasmids for Metabolic Engineering of Bacteria. *Metab. Eng.* 2, 328-338.
- 59 Carkaci-Salli, N., Flanagan, J. M., Martz, M. K., Salli, U., Walther, D. J., Bader, M., and Vrana, K. E. (2006) Functional domains of human tryptophan hydroxylase 2 (hTPH2). *J. Biol. Chem.* 281, 28105-28112.
- 25 60 Brown, S., Clastre, M., Courdavault, V., and O'Connor, S. E. (2015) De novo production of the plant-derived alkaloid strictosidine in yeast. *Proc. Natl. Acad. Sci. U. S. A.* 112, 3205-3210.
- 61 Bernhardt, P., Usera, A. R., and O'Connor, S. E. (2010) Biocatalytic asymmetric formation of tetrahydro-beta-carbolines. *Tetrahedron Lett.* 51, 4400-4402.
- 30 62 Geerlings, A., Redondo, F. J., Contin, A., Memelink, J., van der Heijden, R., and Verpoorte, R. (2001) Biotransformation of tryptamine and secologanin into plant terpenoid indole alkaloids by transgenic yeast. *Appl. Microbiol. Biotechnol.* 56, 420-424.
- 63 Battersby A.R., Burnett, A. R., and Parsons, P. G. (1969) Alkaloid Biosynthesis Part XV. Partial Synthesis and Isolation of Vincoside and Isovincoside - Biosynthesis of 3 Major Classes of Indole Alkaloids from Vincoside. *J. Chem. Soc. C* 1193-1200.
- 35

64 Maresh, J. J., Giddings, L. A., Friedrich, A., Loris, E. A., Panjikar, S., Trout, B. L., Stockigt, J., Peters, B., and O'Connor, S. E. (2008) Strictosidine synthase: mechanism of a Pictet-Spengler catalyzing enzyme. *J. Am. Chem. Soc.* 130, 710-723.

65 McCoy, E., Galan, M. C., and O'Connor, S. E. (2006) Substrate specificity of
5 strictosidine synthase. *Bioorg. Med. Chem. Lett.* 16, 2475-2478.

66 Miettinen, K., Dong, L. M., Navrot, N., Schneider, T., Burlat, V., Pollier, J., Woittiez, L., van der Krol, S., Lugan, R., Ilc, T., Verpoorte, R., Oksman-Caldentey, K. M., Martinoia, E., Bouwmeester, H., Goossens, A., Memelink, J., and Werck-Reichhart, D. (2014) The seco-iridoid pathway from *Catharanthus roseus*. *Nat. Commun.* 5:3606 doi:
10 10.1038/ncomms460.

67 Yerkes, N., Wu, J. X., McCoy, E., Galan, M. C., Chen, S., and O'Connor, S. E. (2008) Substrate specificity and diastereoselectivity of strictosidine glucosidase, a key enzyme in monoterpene indole alkaloid biosynthesis. *Bioorg. Med. Chem. Lett.* 18, 3095-3098.

68 Bernhardt, P., Yerkes, N., and O'Connor, S. E. (2009) Bypassing stereoselectivity in
15 the early steps of alkaloid biosynthesis. *Org. Biomol. Chem.* 7, 4166-4168.

69 Dittrich, H. Kutcham, T.M. (1991) Molecular cloning, expression, and induction of berberine bridge enzyme, and enzyme essential to the formation of benzylophenanthridine alkaloids in the response of plants to pathogenic attack. *Proc. Natl. Acad. Sci. U.S.A.* 88, 9969-9973.

70 Luttik, M. A. H., Vuralhan, Z., Suij, E., Braus, G. H., Pronk, J. T. & Daran, J. M. (2008) Alleviation of feedback inhibition in *Saccharomyces cerevisiae* aromatic amino acid biosynthesis: Quantification of metabolic impact. *Metab Eng* 10, 141-153.

71 Gold, N. D., Gowen, C. M., Lussier, F. X., Cautha, S. C., Mahadevan, R. & Martin, V. J. (2015) Metabolic engineering of a tyrosine-overproducing yeast platform using targeted
25 metabolomics. *Microb Cell Fact* 14, 73.

72 Sarria, S., Wong, B., Martin, H. G., Keasling, J. D. & Peralta-Yahya, P. (2014) Microbial Synthesis of Pinene. *ACS Synth. Biol.*, 3, 466-475.

73 Mukherjee, K., Bhattacharyya, S. & Peralta-Yahya, P. GPCR-Based Chemical Biosensors for Medium-Chain Fatty Acids. *ACS Synth. Biol.*, doi:10.1021/sb500365m
30 (2015).

74 Belanger-Quintana, A., Burlina, A., Harding, C. O., and Muntau, A. C. (2011) Up to date knowledge on different treatment strategies for phenylketonuria. *Mol. Genet. Metab.* 104, S19-S25.

75 Byerley, W. F., Judd, L. L., Reimherr, F. W., and Grosser, B. I. (1987) 5-Hydroxytryptophan - a Review of Its Antidepressant Efficacy and Adverse-Effects. *J. Clin. Psychopharm.* 7, 127-137.

76 Birdsall, T. C. (1998) 5-Hydroxytryptophan: a clinically-effective serotonin precursor. *Alt. Med. Rev. : a journal of clinical therapeutic* 3, 271-280.

77 Bloom, F., and Kupfer, D. (1995) *Psychopharmacology, the fourth generation of progress* pp 933-944, Raven Press.

5 78 Connolly, B. S., and Lang, A. E. (2014) Pharmacological Treatment of Parkinson Disease A Review. *JAMA, J. Am. Med. Assoc.* 311, 1670-1683.

Example 4: Experimental Methods for Examples 1, 2 and 3.

Fig. 34 shows an overview of the microbial synthesis of L-DOPA, dopamine, serotonin and 10-hydroxystrictosidine. Arrows represent presence of the enzyme. nd = not detectable. Amount produced is represented by the mean $\pm$ standard deviation for samples run in triplicate. GTPCH: GTP cyclohydrolase; PTPS: pyruvoyl tetrahydropterin synthase; SR: sepiapterin reductase; PCD: pterin-4 α -carbinolamine dehydratase; DHPR: dihydropteridine reductase; TPH: tryptophan hydroxylase; TH: tyrosine hydroxylase; DDC: aromatic-L-amino-acid decarboxylase; STR: strictosidine synthase

Abbreviations used in Examples 1-2: GTPCH- GTP cyclohydrolase I; PTPS- pyruvoyl tetrahydropterin synthase; SR- sepiapterin reductase; PCD- pterin-4- α -carbinolamine dehydratase; DHPR- dihydropteridine reductase; TH- tyrosine hydroxylase; TPH- tryptophan hydroxylase; DDC- aromatic-L-amino-acid decarboxylase; STR- strictosidine synthase

20 Yeast strain construction.

Construction of W303-Ade2⁺ strain. *S. cerevisiae* W303 were transformed with AME245 and AME246 via an adapted electroporation protocol. Transformed cells were plated and subsequently patched on synthetic complete media with 2% glucose lacking adenine (SD (Ade⁻)). To confirm the presence of a functional Ade2, genomic DNA from multiple patches was isolated, the mutation was amplified by PCR using primers AME128/AME247, and the PCR product sequenced with AME247.

Yeast transformation. A modified electroporation method² was utilized to transform *S. cerevisiae* W303 or W303-Ade2⁺. Modifications included no DNA precipitation step and immediately after electroporation, cells were rescued with YPD and left at room temperature overnight before plating on selection media plates.

Strains used are shown in Table 1.

Table 1: Table of Strains

Strain #	Strain name	Description	Source
PPY11	W303	<i>S. cerevisiae</i> MATa ade2-1 ura3-1 his3-11 trp1-1 leu2-3 leu2-112 can1-100	ATCC® 20835
PPY568	W303-Ade2 ⁺	<i>S. cerevisiae</i> W303 with a T190G mutation in <i>Ade2</i> gene	This study
PPY752	W303-172226	W303 transformed with pAME17, pAME22, and pAME26	This study

PPY753	W303-172227	W303 transformed with pAME17, pAME22, and pAME27	This study
PPY754	W303-172228	W303 transformed with pAME17, pAME22, and pAME28	This study
PPY755	W303-172326	W303 transformed with pAME17, pAME23, and pAME26	This study
PPY756	W303-172327	W303 transformed with pAME17, pAME23, and pAME27	This study
PPY757	W303-172328	W303 transformed with pAME17, pAME23, and pAME28	This study
PPY758	W303-172426	W303 transformed with pAME17, pAME24, and pAME26	This study
PPY759	W303-172427	W303 transformed with pAME17, pAME24, and pAME27	This study
PPY760	W303-172428	W303 transformed with pAME17, pAME24, and pAME28	This study
PPY761	W303-172526	W303 transformed with pAME17, pAME25, and pAME26	This study
PPY762	W303-172527	W303 transformed with pAME17, pAME25, and pAME27	This study
PPY763	W303-172528	W303 transformed with pAME17, pAME25, and pAME28	This study
PPY764	W303-182226	W303 transformed with pAME18, pAME22, and pAME26	This study
PPY765	W303-182227	W303 transformed with pAME18, pAME22, and pAME27	This study
PPY766	W303-182228	W303 transformed with pAME18, pAME22, and pAME28	This study
PPY767	W303-182326	W303 transformed with pAME18, pAME23, and pAME26	This study
PPY768	W303-182327	W303 transformed with pAME18, pAME23, and pAME27	This study
PPY797	W303-182328	W303 transformed with pAME18, pAME23, and pAME28	This study
PPY798	W303-182426	W303 transformed with pAME18, pAME24, and pAME26	This study
PPY799	W303-182427	W303 transformed with pAME18, pAME24, and pAME27	This study
PPY800	W303-182428	W303 transformed with pAME18, pAME24, and pAME28	This study
PPY801	W303-182526	W303 transformed with pAME18, pAME25, and pAME26	This study
PPY802	W303-182527	W303 transformed with pAME18, pAME25, and pAME27	This study
PPY769	W303-182528	W303 transformed with pAME18, pAME25, and pAME28	This study
PPY803	W303-192226	W303 transformed with pAME19, pAME22, and pAME26	This study
PPY804	W303-192227	W303 transformed with pAME19, pAME22, and pAME27	This study
PPY805	W303-192228	W303 transformed with pAME19, pAME22, and pAME28	This study
PPY806	W303-192326	W303 transformed with pAME19, pAME23, and pAME26	This study
PPY807	W303-192327	W303 transformed with pAME19, pAME23, and pAME27	This study
PPY808	W303-192328	W303 transformed with pAME19, pAME23, and pAME28	This study
PPY809	W303-192426	W303 transformed with pAME19, pAME24, and pAME26	This study
PPY770	W303-192427	W303 transformed with pAME19, pAME24, and pAME27	This study
PPY771	W303-192428	W303 transformed with pAME19, pAME24, and pAME28	This study
PPY772	W303-192526	W303 transformed with pAME19, pAME25, and pAME26	This study
PPY773	W303-192527	W303 transformed with pAME19, pAME25, and pAME27	This study
PPY774	W303-192528	W303 transformed with pAME19, pAME25, and pAME28	This study
PPY775	W303-202226	W303 transformed with pAME20, pAME22, and pAME26	This study
PPY776	W303-202227	W303 transformed with pAME20, pAME22, and pAME27	This study
PPY777	W303-202228	W303 transformed with pAME20, pAME22, and pAME28	This study
PPY778	W303-202326	W303 transformed with pAME20, pAME23, and pAME26	This study
PPY779	W303-202327	W303 transformed with pAME20, pAME23, and pAME27	This study
PPY780	W303-202328	W303 transformed with pAME20, pAME23, and pAME28	This study
PPY781	W303-202426	W303 transformed with pAME20, pAME24, and pAME26	This study
PPY782	W303-202427	W303 transformed with pAME20, pAME24, and pAME27	This study
PPY783	W303-202428	W303 transformed with pAME20, pAME24, and pAME28	This study
PPY784	W303-202526	W303 transformed with pAME20, pAME25, and pAME26	This study
PPY785	W303-202527	W303 transformed with pAME20, pAME25, and pAME27	This study
PPY786	W303-202528	W303 transformed with pAME20, pAME25, and pAME28	This study
PPY810	W303-032930	W303 transformed with pAME3, pAME29, and pAME30	This study
PPY751	W303-2226	W303 transformed with pAME22, and pAME26	This study
PPY787	W303A-172226	W303-Ade2 ⁺ transformed with pAME17, pAME22, and pAME26	This study
PPY749	W303A-57	W303-Ade2 ⁺ transformed with pAME57	This study
PPY788	W303A-172252	W303-Ade2 ⁺ transformed with pAME17, pAME22, and pAME52	This study
PPY789	W303A-175426	W303-Ade2 ⁺ transformed with pAME17, pAME54, and pAME26	This study
PPY790	W303A-552226	W303-Ade2 ⁺ transformed with pAME55, pAME22, and pAME26	This study
PPY793	W303A-175453	W303-Ade2 ⁺ transformed with pAME17, pAME54, and pAME53	This study
PPY792	W303A-552253	W303-Ade2 ⁺ transformed with pAME55, pAME22, and pAME53	This study
PPY791	W303A-555426	W303-Ade2 ⁺ transformed with pAME55, pAME54, and pAME26	This study

PPY750	W303A-555453	W303-Ade2 ⁺ transformed with pAME55, pAME54, and pAME53	This study
PPY949	W303A-17	W303-Ade2 ⁺ expressing pAME17	This study
PPY950	W303A-55	W303-Ade2 ⁺ expressing pAME55	This study
PPY951	W303A-22	W303-Ade2 ⁺ expressing pAME22	This study
PPY952	W303A-54	W303-Ade2 ⁺ expressing pAME54	This study
PPY953	W303A-26	W303-Ade2 ⁺ expressing pAME26	This study
PPY954	W303A-53	W303-Ade2 ⁺ expressing pAME53	This study
PPY946	W303A-946	W303-Ade2 ⁺ transformed with pSS68, pESC-Leu2, pESC-His3, and pESC-Trp1	This study
PPY646	W303A-646	W303-Ade2 ⁺ transformed with pAME17, pAME22PCD, pAME26DHPR, and pSS68	This study
PPY679	W303A-679	W303-Ade2 ⁺ transformed with pAME17, pAME22, pAME26, and pSS68	This study
PPY947	W303A-947	W303 Ade2 ⁺ transformed with pAME63, pSS68, pESC-His3, and pESC-Trp1	This study
PPY658	W303A-658	W303-Ade2 ⁺ transformed with pSS66, pAME22PCD, pAME26DHPR, and pSS68	This study
PPY743	W303A-743	W303 Ade2 ⁺ transformed with pSS66, pAME22, pAME26, and pSS68	This study
PPY948	W303A-948	W303 Ade2 ⁺ transformed with pAME63, pSS70, pESC-His3, and pESC-Trp1	This study
PPY649	W303A-649	W303-Ade2 ⁺ transformed with pSS66, pAME22PCD, pAME26DHPR, and pSS70	This study
PPY741	W303A-741	W303-Ade2 ⁺ transformed with pSS66, pAME22, pAME26, and pSS70	This study
PPY650	W303A-650	W303-Ade2 ⁺ transformed with pSS66, pAME22PCD, pAME26DHPR, and pSS71	This study
PPY955	W303A-955	W303-Ade2 ⁺ transformed with pSS66, pAME22, pAME26, and pSS71	This study
PPY744	W303A-744	W303 Ade2 ⁺ transformed with pAME56, pAME57, and pAME58	This study
PPY748	W303A-748	W303Ade2 ⁺ transformed with pAME56, pAME57, and pESC-Ura3	This study
PPY740	W303A-740	W303 Ade2 ⁺ transformed with pAME56 and pAME57	This study
PPY827	W303A-64	W303 Ade2 ⁺ transformed with pAME64	This study
PPY828	W303A-ura	W303 Ade2 ⁺ transformed with pESC-Ura3	This study
PPY835	W303-835	W303 transformed with pAME22, pAME26, and pSS68	This study
PPY836	W303-836	W303 transformed with pAME17, pAME22, pAME26, and pSS68	This study

Vector construction.

Construction of multi-copy vectors expressing the BH₄ synthetic pathway. To construct pAME18, 20, 22-26, and 28, genes were amplified from plasmids carrying codon-
5 optimized nucleotide sequences of *M. alpina* GTPCH, *H. sapiens* GTPCH, *M. alpina* PTPS, *S. salar* PTPS, *S. ruber* PTPS, *P. mikurensis* PTPS, *M. alpina* SR, and *T. pseudonana* SR with primers AME143/AME144, AME141/AME139, AME149/AME150, AME147/AME148, AME151/AME152, AME153/AME154, AME165/AME166, or AME165/AME167, respectively, and cloned into pESC-Leu2 at *Bam*HI/*Hind*III (pAME18, 20), pESC-Trp1 at *Bam*HI/*Sac*II
10 (pAME22-25), or pESC-His3 at *Bam*HI/*Sac*II (pAME26, 28). To construct pAME17, *E. coli* GTPCH was amplified from the *E. coli* DH10B genome with primers AME163/164. The gene product was re-amplified with primers AME135/140 and cloned into pESC-Leu2 at *Bam*HI/*Hind*III. To construct pAME19, *S. cerevisiae* GTPCH was amplified from *S.*

S. cerevisiae W303 genome with primers AME161/162, and re-amplified with primers AME137/142. The gene product was cloned into pESC-Leu2 at *Bam*HI/*Hind*III. To construct pAME27, *S. cerevisiae* SR was amplified from *S. cerevisiae* W303 genome with primers AME168/169, and re-amplified with primers AME180/183. The gene product was cloned
 5 into pESC-His3 at *Bam*HI/*Sac*I. To construct pAME3, 29-30, green fluorescent protein (GFP) was amplified from pEGFP with primers AME123/124 and cloned into pESC-Leu2, pESC-Trp1, or pESC-His3, respectively, at *Bam*HI/*Hind*III (Leu2) or *Bam*HI/*Sac*I (Trp1, His3). Constructs were sequence verified using primers AME104 and AME105.

Construction of single-copy vectors expressing BH₄ synthetic pathway. To construct
 10 pAME53-55, the region between terminators T_{ADH1} and T_{CYC1} was amplified from pAME26, 22, or 17 using primers AME184/AME185 and cloned into pRS413, pRS414, or pRS415, respectively, at *Bam*HI/*Hind*III. Constructs were sequence verified using primers MH100 and MH101.

Construction of multi-copy vectors expressing BH₄ recycling pathway. To construct
 15 pAME22PCD and pAME26DHPR, PCD and DHPR genes were amplified from plasmids carrying the codon optimized genes with primers SS152/SS153 or AME241/AME242, respectively, and cloned into pAME22 or pAME26, respectively, at *Not*I/*Sac*I. Constructs were sequence verified using primers AME229 and AME104.

*Construction of multicopy vectors expressing alkaloid pathway enzymes from
 20 inducible promoters.* To construct pSS61, the STR gene was amplified from pSS42 with primers SS159/SS160 and cloned into pESC-Ura3 at *Bam*HI/*Hind*III. To construct pSS66, the DDC gene was amplified from pSS62 with primers SS157/SS158 and cloned into pAME17 at *Not*I/*Sac*I. To construct pSS68, the TH gene was amplified from pSS64 with primers
 25 SS179/SS180 and cloned into pESC-Ura3 at *Not*I/*Sac*I. To construct pSS70, the TPH gene was amplified from pSS44 with primers SS207/SS208 and cloned into pESC-Ura3 at *Bam*HI/*Hind*III. To construct pSS71, the TPH gene was amplified from pSS44 with primers SS177/SS178 and cloned into pSS61 at *Not*I/*Sac*I. Constructs were sequence verified using primer SS112. To construct pAME63, the DDC gene was amplified from pSS62 with primers
 30 SS157/SS158 and cloned into pESC-Leu2 at *Not*I/*Sac*I. To construct pAME64, the STR gene was amplified from pSS42 with primers SS159/AME406 and cloned into pESC-Ura3 at *Bam*HI/*Hind*III. Constructs were sequence verified using primers AME229/AME230 (pAME63) or AME104/AME105 (pAME64).

*Construction of multicopy vectors expressing alkaloid pathway enzymes from
 35 constitutive promoters.* To construct pAME56-58, assembly similar to sewing PCR was utilized. Fragments were amplified from template plasmids using primers as follows (fragment-primer/primer/template):

- SR-AME373/AME374/pAME26;
P_{TEF1}_P_{HXT7}-AME365/AME366/pSS102;
PTPS-AME363/AME364/pAME22;
T_{HXT7}-AME369/AME370/pSS102
- 5 P_{ADH1g}-AME371/AME372/pSS43;
P_{ADH1ng}-AME371/AME383/pSS43;
GTPCH-AME367/AME368/pAME17;
PCD-AME375/AME376/pAME22PCD;
DHPR-AME377/AME378/pAME26DHPR;
- 10 DDC-AME384/AME385/pSS66;
TPH-AME386/AME387/pSS70;
STR-AME388/AME389/pSS61;
vector-
AME394/AME395/pAME26DHPR,pAME22
- 15 PCD,pSS67.

After amplification, PCR products were gel purified. To create pAME56-58, fragments were sewn together using primers AME384/AME389 ($P_{TEF1_P_{HXT7}}$, T_{HXT7} , P_{ADH1g} , DDC, TPH, STR), AME363/AME374 ($P_{TEF1_P_{HXT7}}$, T_{HXT7} , P_{ADH1g} , GTPCH, PTPS, SR), and AME375/AME383 ($P_{TEF1_P_{HXT7}}$, T_{HXT7} , P_{ADH1g} , PCD, DHPR), respectively, using a typical PCR protocol and equimolar amounts of fragments. Resulting products were gel purified and combined with respective vector fragments (from pAME22PCD, pAME26DHPR, pSS67, respectively) via Gibson assembly¹. Sequencing was obtained using primers AME105/AME229/AME396/AME397/AME369/AME370/AME372.

Table 2 shows plasmids used in Examples 1-2 and Table 2 shows primers and primer sequences.

Table 2: Table of Plasmids

Strain #	Plasmid	Description	Source
PPY34	pESC-His3	Yeast shuttle vector with divergent Gal1/Gal10 promoter	Agilent #217451
PPY35	pESC-Ura3	Yeast shuttle vector with divergent Gal1/Gal10 promoter	Agilent #217454
PPY36	pESC-Trp1	Yeast shuttle vector with divergent Gal1/Gal10 promoter	Agilent #217453
PPY39	pESC-Leu2	Yeast shuttle vector with divergent Gal1/Gal10 promoter	Agilent #217452
PPY13	pRS413	YC-type (centromeric) shuttle vector	ATCC® 87518
PPY14	pRS414	YC-type (centromeric) shuttle vector	ATCC® 87519
PPY15	pRS415	YC-type (centromeric) shuttle vector	ATCC® 87520
PPY154	pCR2.1 HGTPCH	Codon optimized* GTPCH from <i>H. sapiens</i>	This study
PPY156	pCR2.1 MaGTPCH	Codon optimized* GTPCH from <i>M. alpinas</i>	This study
PPY171	pCR2.1 MaPTS	Codon optimized* PTPS from <i>M. alpinas</i>	This study
PPY172	pCR2.1 SPTS	Codon optimized* PTPS from <i>S. salar</i>	This study
PPY173	pCR2.1 RubPTS	Codon optimized* PTPS from <i>S. ruber</i>	This study
PPY174	pCR2.1 PmPTS	Codon optimized* PTPS from <i>P. mikurensis</i>	This study
PPY181	pCR2.1_MaSR	Codon optimized* SR from <i>M. alpina</i> with N-terminal His ₆ -tag	This study
PPY182	pCR2.1_PseudoSR	Codon optimized* SR from <i>T. pseudonana</i> with N-terminal His ₆ -tag	This study
PPY435	pCR2.1 DHPR	Codon optimized* DHPR from <i>H. sapiens</i>	This study
PPY465	pSS48	Codon optimized* PCD from <i>H. sapiens</i>	This study
PPY539	pSS62	Codon optimized* DDC from <i>S. serofa</i>	This study
PPY563	pSS64	Codon optimized* TH from <i>M. musculus</i>	This study
PPY444	pSS44	Codon optimized* TPH from <i>H. sapiens</i>	This study
PPY442	pSS42	Codon optimized (for <i>E. coli</i>) STR from <i>O. pumila</i>	Commercially synthesized for this study. Sequence from Bernhardt et al.
PPY38	pEGFP	Enhanced green fluorescent protein	F. Storici lab
PPY40	pAME3	pESC-Leu2- P_{GAL1} -eGFP	This study
PPY242	pAME29	pESC-Trp1- P_{GAL1} -eGFP	This study
PPY243	pAME30	pESC-His3- P_{GAL1} -eGFP	This study
PPY183	pAME17	pESC-Leu2- P_{GAL1} -His ₆ - <i>E. coli</i> GTPCH	This study
PPY168	pAME18	pESC-Leu2- P_{GAL1} -His ₆ - <i>M. alpina</i> GTPCH	This study
PPY184	pAME19	pESC-Leu2- P_{GAL1} -His ₆ - <i>S. cerevisiae</i> GTPCH	This study
PPY166	pAME20	pESC-Leu2- P_{GAL1} -His ₆ - <i>H. sapiens</i> GTPCH	This study

PPY186	pAME22	pESC-Trp1-P _{GAL1} -His ₆ - <i>M.alpina</i> PTPS	This study
PPY187	pAME23	pESC-Trp1-P _{GAL1} -His ₆ - <i>S.salar</i> PTPS	This study
PPY188	pAME24	pESC-Trp1-P _{GAL1} -His ₆ - <i>S.ruber</i> PTPS	This study
PPY189	pAME25	pESC-Trp1-P _{GAL1} -His ₆ - <i>P.mikurensis</i> PTPS	This study
PPY190	pAME26	pESC-His3-P _{GAL1} -His ₆ - <i>M.alpina</i> SR	This study
PPY241	pAME27	pESC-His3-P _{GAL1} -His ₆ - <i>S.cerevisiae</i> SR	This study
PPY191	pAME28	pESC-His3-P _{GAL1} -His ₆ - <i>T.pseudonana</i> SR	This study
PPY670	pAME53	pRS413-His3-P _{GAL1} -His ₆ - <i>M.alpina</i> SR	This study
PPY667	pAME54	pRS414-Trp1-P _{GAL1} -His ₆ - <i>M.alpina</i> PTPS	This study
PPY668	pAME55	pRS415-Leu2-P _{GAL1} -His ₆ - <i>E.coli</i> GTPCH	This study
PPY520	pAME22PCD	pESC-Trp1-P _{GAL1} -His ₆ - <i>M.alpina</i> PTPS-P _{GAL10} - <i>H.sapiens</i> PCD	This study
PPY555	pAME26DHP	pESC-His3-P _{GAL1} -His ₆ - <i>M.alpina</i> SR- P _{GAL10} - <i>H.sapiens</i> DHP	This study
PPY538	pSS61	pESC-Ura3-P _{GAL1} -STR	This study
PPY572	pSS66	pESC-Leu2-P _{GAL1} -His ₆ - <i>E.coli</i> GTPCH-P _{GAL10} -DDC	This study
PPY574	pSS68	pESC-Ura3-P _{GAL10} -TH	This study
PPY630	pSS70	pESC-Ura3-P _{GAL1} -TPH	This study
PPY631	pSS71	pESC-Ura3-P _{GAL1} -STR- P _{GAL10} -TPH	This study
PPY700	pAME56	pESC-Trp1-P _{HXT7} -DDC- P _{TEF1} -TPH- P _{ADH1} -STR	This study
PPY704	pAME57	pESC-His3-P _{HXT7} -PTPS-P _{TEF1} -GTPCH-P _{ADH1} -SR	This study
PPY701	pAME58	pESC-Ura3-P _{HXT7} -PCD- P _{TEF1} -DHP	This study
PPY723	pAME63	pESC-Leu2-P _{GAL10} -DDC	This study
PPY338	pSS102	pESC-Ura3-P _{HXT7} / P _{TEF1}	This study
PPY443	pSS43	pESC-Trp1-P _{TEF1} /P _{ADH1}	This study
PPY573	pSS67	pESC-Ura3-P _{GAL10} -TH-P _{GAL1} -NCS	This study
PPY822	pAME64	pESC-Ura3-P _{GAL1} -STR-His ₆	This study

Table 3: Table of Primers

Name	Sequence (5'->3')
AME104	CACTTTAACTAATACTTTCAAC
AME105	TAAATAACGTTCTTAATACTAAC
AME123	CGTCAAGGAGAAAAAACCCGGATCCATCACGTGCACCATGGTGAGCAAGGGCGAG
AME124	TCTTAGCTAGCCGCGGTACCAAGCTTTTACTTGTACAGCTCGTCC
AME128	CTGGAGAAGGGTAAATTTTTA
AME135	TCTTAGCTAGCCGCGGTACCAAGCTTTTAGTTGTGATGACGCACAGC
AME137	TCTTAGCTAGCCGCGGTACCAAGCTTTTAAATACTTCTTCTTCTTAAAG
AME139	TCTTAGCTAGCCGCGGTACCAAGCTTTTAAAGATCTAATCAAAGTCAAG
AME140	CGTCAAGGAGAAAAAACCCGGATCCATCACGTGCACCATGCATCACCATCACCATCAC CCATCACTCAGTAAAGAAGC
AME141	CGTCAAGGAGAAAAAACCCGGATCCATCACGTGCACCATGCATCACCATCACCATCAC GAGAAAGGTCCAGTTAGAG
AME142	CGTCAAGGAGAAAAAACCCGGATCCATCACGTGCACCATGCATCACCATCACCATCAC CATAACATCCAATTAGTGCAA
AME143	CGTCAAGGAGAAAAAACCCGGATCCATCACGTGCACCATGCATCACCATCACCATCAC TCCCATACTCCAACCTCTC
AME144	TCTTAGCTAGCCGCGGTACCAAGCTTTTAAACACCTCTTCTTCTAATC
AME147	CGTCAAGGAGAAAAAACCCGGATCCATCACGTGCACCATGCATCACCATCACCATCAC GCTCAAGCTGATTCCAGAA
AME148	TCTTAGCTAGCCGCGGTACCAAGCTTTTATTCACCTCTGTAGACAAC
AME149	CGTCAAGGAGAAAAAACCCGGATCCATCACGTGCACCATGCATCACCATCACCATCAC ACCTCCTCAACTCCAGTTA

AME150	TCTTAGCTAGCCGCGGTACCAAGCTTTTATTACCTCTGTAAACGAC
AME151	CGTCAAGGAGAAAAAACCCGGATCCATCACGTGCACCATGCATCACCATCACCATCAC TCCACCGTTTACATTACCAG
AME152	TCTTAGCTAGCCGCGGTACCAAGCTTTTATTACCTCTGTATTCAAC
AME153	CGTCAAGGAGAAAAAACCCGGATCCATCACGTGCACCATGCATCACCATCACCATCAC TTTGAATTGACTAGAACTTTAAG
AME154	TCTTAGCTAGCCGCGGTACCAAGCTTTTAACCACCTCTATAAGCAC
AME161	CATAACATCCAATTAGTGCAA
AME162	AATACTTCTTCTTCTAAAAG
AME163	CCATCACTCAGTAAAGAAGC
AME164	GTTGTGATGACGCACAGC
AME165	CGTCAAGGAGAAAAAACCCGGATCCATCACGTGCACCATGCATCACCATCACCATC
AME166	TCTTAGCTAGCCGCGGTACCAAGCTTTTATTATCGTAGAAATCAATAT
AME167	TCTTAGCTAGCCGCGGTACCAAGCTTTTAAACATCGAAGTAATCAACA
AME168	GGTAAAGTTATTTTAGTTACAG
AME169	AGGCATAAAGTCCGCCAAG
AME180	TCTTAGCTAGCCGCGGTACCAAGCTTTTAAGGCATAAAGTCCGCCAAG
AME183	CGTCAAGGAGAAAAAACCCGGATCCATCACGTGCACCATGCATCACCATCACCATCAC GGTAAAGTTATTTTAGTTACAG
AME184	TCGAGGTCGACGGTATCGATAAGCTTGAGCGACCTCATGCTATAC
AME185	GCGGCCGCTCTAGAACTAGTGGATCCCTTCGAGCGTCCCAAAC
AME229	ACGTATCTACCAACGATTTG
AME230	GTATATGGATATGTATATGGTG
AME241	AATTCAACCCTCACTAAAGGGCGGCCGCATGGCAGCTGCTGCAGC
AME242	GGCGAAGAATTGTTAATTAAAGAGCTCTTAGAAATAAGCTGGAGTCAA
AME245	GGCTCCTTTTCCAATCCTCTTGATATCGAAAACTAGCTGAAAAATGTGATGTGCTAACG ATTGAGATTGAGCATGTTGA
AME246	TCAACATGCTCAATCTCAATCGTTAGCACATCACATTTTTTCAGCTAGTTTTTCGATATCA AGAGGATTGGAAAAGGAGCC
AME247	AAGACGGTAATACTAGATGC
AME363	TGTAATCCATCGATACTAGTTTATTACCTCTGTAAACGAC
AME364	ATTTTAATCAAAAAGCGACCATGACCTCCTCAACTCCAG
AME365	GGTCGCTTTTTGATTAAAATTAAAAAACTTT
AME366	GGTGGCTGTAATTAAAACTTAGATTAGATT
AME367	AAGTTTTAATTACAGCCACCATGCCATCACTCAGTAAAGA
AME368	TTAATAAAAGTGTTTCGCAAATTAGTTGTGATGACGCACAG
AME369	TTTGCGAACACTTTTATTAATTC
AME370	TCTTTAAAGTTTCTTTGTCTCC
AME371	AGACAAAGAACTTTAAAGAATCCTTTTGTGTTTCCGGG
AME372	GGTCGGTGTATATGAGATAGTTGATTGT
AME373	CTATCTCATATACACCGACCATGTCATCCAAAGAACATCAT
AME374	CCTATAGTGAGTCGTATTACTTATTTCATCGTAGAAATCAATATG
AME375	TGTAATCCATCGATACTAGTTTAAAGTCATGGAGACAGCG
AME376	ATTTTAATCAAAAAGCGACCATGGCTGGTAAAGCTCATAG
AME377	AAGTTTTAATTACAGCCACCATGGCAGCTGCTGCAGC
AME378	TTAATAAAAGTGTTTCGCAAATTAGAAATAAGCTGGAGTCAA
AME383	CCTATAGTGAGTCGTATTACTGTATATGAGATAGTTGATTGT
AME384	TGTAATCCATCGATACTAGTTTAAAGATTTAATTTTCAGCTTTAC
AME385	ATTTTAATCAAAAAGCGACCATGAATGCTTCTGATTTTAGAA
AME386	AAGTTTTAATTACAGCCACCATGGAAGAATTGGAAGATGTT
AME387	TTAATAAAAGTGTTTCGCAAATTAAAGTATCCTTCAAAATTTCAA
AME388	CTATCTCATATACACCGACCATGGGCTCTCCTGAGTTTT
AME389	CCTATAGTGAGTCGTATTACTTAAAGATCCAAACGAAGAGAA

AME394	GTAATACGACTCACTA
AME395	ACTAGTATCGATGGATTACAA
AME396	CATTGTCAGCTATTGTAAAATA
AME397	CTCAAGTTTCAGTTTCATTTTT
AME406	TCTTAGCTAGCCGCGGTACCTTAGTGATGGTGATGGTGATGAGATCCAAACGAAGAGAA C
AME441	CACAAGGGTCCATAACAGC
AME442	ACGGTCATAATTACAAGGTTG
AME443	CGATGAAATGGTCACCGTG
AME444	GACAGACCGATCACCGAAT
AME445	CTGGCAAGAAGCTAGATCC
AME446	GTTCTATGATCTGGATATTGTT
SS112	GACAACCTTGATTGGAGA
SS152	GGCGAAGAATTGTTAATTAATTAAGTCATGGAGACAGC
SS153	AATTCAACCCTCACTAAAGGATGGCTGGTAAAGCTC
SS157	TGGCGAAGAATTGTTAATTAATTAAGATTTAATTCAGCTTTACCTTC
SS158	GAATTCAACCCTCACTAAAGGATGAATGCTTCTGATTTTAGAAG
SS159	CGTCAAGGAGAAAAAACCCCATGGGCTCTCCTGAG
SS160	TCTTAGCTAGCCGCGGTACCTTAAGATCCAAACGAAGAGA
SS177	GGCGAAGAATTGTTAATTAATTAAGTATCCTTCAAAATTTCAATG
SS178	AATTCAACCCTCACTAAAGGATGGAAGAATTGGAAGATGT
SS179	GGCGAAGAATTGTTAATTAATTAAGAAATAGCAGACAATGCT
SS180	AATTCAACCCTCACTAAAGGATGCCAACTCCATCC
SS207	CGTCAAGGAGAAAAAACCCCATGGAAGAATTGGAAGATGT
SS208	TCTTAGCTAGCCGCGGTACCTTAAGTATCCTTCAAAATTTCAATG
MH100	ACGTTGTAAACGACGGCC
MH101	CTATGACCATGATTACGCC
ACT1-F	TTGGATTCCGGTGATGGTGT
ACT1-R	CGGCCAAATCGATTCTCAAA

Below are sequences for various components of the biocatalyst described herein. Underlining within a sequences demonstrates the nucleotides corresponding to a His₆ Tag. The sequences below are codon-optimized for yeast. Where a UniProt database code is provided, this is referencing the sequence that was used as the input sequence for the yeast codon-optimization and does not necessarily refer to the specific sequences below.

SEQ ID NO: 1. *Escherichia coli* GTP cyclohydrolase I (UniProtKB- P0A6T5)

ATGCATCACCATCACCATCACCATCACTCAGTAAAGAAGCGGCCCTGGTTCATGAAGCGTTAGTTGCGC
 GAGGACTGGAAACACCGCTGCGCCCGCCGTCATGAAATGGATAACGAAACGCGCAAAAGCCTTATTGC
 TGGTCATATGACCGAAATCATGCAGCTGCTGAATCTCGACCTGGCTGATGACAGTTTGATGGAAACGCCG
 CATCGCATCGCTAAATGTATGTCGATGAAATTTCTCCGGTCTGGATTACGCCAATTTCCCGAAAATCA
 CCCTCATTGAAAACAAAATGAAGGTCGATGAAATGGTCACCGTGCGCGATATCACTCTGACCAGCACCTG
 TGAACACCATTTTGTACCATCGATGGCAAAGCGACGGTGGCCTATATCCCGAAAGATTCTGGTGATCGGT
 CTGTCAAAAATTAACCGCATTGTGCAATTCTTTGCCAGCGTCCGCAGGTGCAGGAACGTCTGACGCAGC
 AAATTCTTATTGCGCTACAAACGCTGCTGGGCACCAATAACGTGGCTGTCTCGATCGACGCGGTGCATTA
 CTGCGTGAAGGCGCGTGGCATCCGCGATGCAACCAGTGCCACGACAACGACCTCTCTGGTGATTGTTT
 AAATCCAGTCAGAATACGCGCCACGAGTTTCTGCGCGCTGTGCGTCATCACAACTAA

SEQ ID NO: 2 *Mortierella alpina* GTP cyclohydrolase I (UniProtKB- G3FNL6)

ATGCATCACCATCACCATCACTCCCATACTCCAACCTCTCCAAAGACCGCTTCCTCTGTTGAATTGGTTC
 ATCCAACCGCAAAGCAAGCATTGTTGAACCACGCTTTGACTGGTCATTCCCATTCTCTGGTAGATCCTA
 CTTGAAGTCCGAATCTCCAGAAGGTAGATCCGCTACTCCAATTGATTTGACGTTTTATCCTTTCCATCC
 ATTGGTGCTAGAGATAGAAGAGAAGATACCGAAGAACAAGAGCTGCTAGAATTGAGAAGATAGCTGGTT
 5 CCGTTAGAACCATTGTTGGAGTGTATTGGTGAAGATCCAGATAGAGAAGTTTTGTTGAAGACTCCAGAAAG
 ATACGCTAAGGCATTGATGTTCTTCTCCAAGGTTACGAAGAATCCGTTACTCATTGATGAATAAGGCA
 TTATTTCAAGAAGATCACGACGAAATGGTTATTGTTAAAGATATTGACGTTTTCTCCTTGTGTGAACATC
 ATATGGTTCATTACTGGTAAGATTCATATTGGTTACATTCCAAGAACGGTAAGGTTGTTGGTTTTGTC
 CAAAATTGCTAGATTGGCTGAAATGTTTTCCAGAAGATTGCAAGTTCAAGAAAGATTGACCAAACAAGTT
 10 GCTATGGCTTTGCAAGAATTGTTAGATCCATTGGGTGTTGCTGTTGTTATGGAAGCATCTCATTTCTGTA
 TGGTTATGAGAGGTGTTCAAAGCCAGGTTCTCAAACCATTACCTCCTCTATGTTTGGTTGTTTTAGAGA
 TCAAGGTAAAACCAGAGAAGAGTTCTTGTCCTTGATTAGAAGAAGAGGTGTTTAA

SEQ ID NO: 3 *Saccharomyces cerevisiae* GTP cyclohydrolase I (UniProtKB- P51601)

ATGCATCACCATCACCATCACCATAACATCCAATTAGTGCAAGAGATAGAAAGACATGAAACCCCGTTAA
 ACATTAGACCTACCTCTCCATACACTTTAAACCTCCTGTGCGAGAGAGATGGGTTTTCTTGGCCAAGTGT
 GGGTACAAGACAACGTGCAGAGGAACTGAAGAGGAGGAAAAGGAACGAATTCACGCATTTACAGGCGCT
 ATCAAGACAATTTTGACCGAAGTGGTGAAGATGTCAACAGAGAAGGTCTACTAGATACTCCACAAAGAT
 ACGCTAAAGCCATGCTTTATTTCACTAAAGGTTACCAAACGAACATTATGGACGATGTCATTAAGAATGC
 20 TGTCTTTGAAGAAGATCATGATGAAATGGTTATTGTTCTGATATTGAAATTTACTCGTTATGTGAACAT
 CATTTGGTGCCATTTTTTCGGCAAGGTTTCATATCGGGTATATACCAAATAAAAAAGTCATCGGGTTAAGTA
 AGTTGGCCAGATTGGCAGAAATGTATGCGAGAAGGCTCCAAGTTCAAGAAAGACTTACAAAGCAAATTGC
 AATGGCCCTAAGTGATATTCTAAAACCATTAGGTGTAGCCGTTGTTATGGAAGCTTCTCATATGTGCATG
 GTTTCAGAGGCATTCAAAAAACGGGATCTTCTACGGTAACTTCTTGTATGCTTGGAGGGTTTAGGGCTC
 25 ATAAAACAAGAGAAGAGTTTTTAACTCTTTTAGGAAGAAGAAGTATTTAA

SEQ ID NO: 4 *Homo sapiens* GTP cyclohydrolase I (UniProtKB- P30793-1)

ATGCATCACCATCACCATCACGAGAAAGGTCCAGTTAGAGCTCCAGCAGAGAAGCCAAGAGGTGCTAGAT
 GTTCTAACGGATTTCCAGAAAGAGATCCTCCAAGACCAGGTCCTTCTAGACCAGCTGAGAAACCACCTAG
 30 ACCAGAAGCTAAATCTGCTCAACCAGCTGACGGTTGGAAAGGTGAAAGACCAAGATCTGAAGAGGACAAC
 GAATTGAATCTACCAAATCTAGCTGCCGCTTATTCATCTATCTTGTCTTCTTGGGAGAGAATCCACAAA
 GACAAGGTCTATTGAAGACTCCTTGGAGAGCTGCCTCTGCTATGCAATTCTTACTAAAGGTTATCAAGA
 AACTATTTCTGACGTTTTGAACGACGCAATCTTCGACGAGGATCACGACGAGATGGTTATTGTCAAAGAT
 ATTGATATGTTCTCTATGTGTGAACACCACTTGGTTCCATTTGTTGGTAAAGTTCACATTGGTTATTTGC
 35 CTAATAAGCAAGTTTTGGGTTTGTCTAAATTGGCTAGAATTGTTGAAATCTATTCTAGAAGATTGCAAGT
 TCAAGAAAGATTGACTAAACAAATTGCTGTTGCTATTACTGAAGCATTGAGACCAGCAGGTGTTGGTGT
 GTCGTTGAAGCTACTCACATGTGTATGGTTATGAGAGGTGTTGAGAAGATGAACTCTAAGACTGTTACTT
 CTACTATGTTGGGTGTCTTTAGAGAAGATCCAAAGACTAGAGAAGAGTTCTTGACTTTGATTAGATCTTA
 A

SEQ ID NO: 5 *Mortierella alpina* 6-pyruvoyl tetrahydrobiopterin synthase (UniProtKB- G3FNL7)

ATGCATCACCATCACCATCACACCTCCTCAACTCCAGTTAGAACTGCTTACGTTACCAGAATTGAACATT
 TCTCCGCTGCTCATAGATTGAATTCCGTTTCAATTTGTCTCCTGCTGAAAACGTTAAGTTGTTCCGGTAAGTG
 TAATCATACTTCCGGTCACGGTCATAATTACAAGGTTGAAGTTACCATTAAAGGTCAAATTAATCCACAA
 45 TCCGGTATGGTTATTAACATTACCGATTTGAAGAAGACCTTGCAAGTTGCTGTTATGGACCCTTGTGATC
 ATAGAAATTTGGATATTGATGTTCCATACTTCGAATCCAGACCATCCACCACTGAAAACCTGGCTGTCTT
 CTTGTGGGAAAACATTAAAGTCCCATTTGCCACCATCCGATGCTTACGATTTGTACGAAATTAAGTTGCAC
 GAAACCGATAAGAACGTTGTCGTTTACAGAGGTGAATAA

SEQ ID NO: 6 *Salmo salar* 6-pyruvoyl tetrahydrobiopterin synthase (UniProtKB- B5XE18)

ATGCATCACCATCACCATCACGCTCAAGCTGATTCCAGAAACGAAGTTGCTGAAAGAATTGGTTACATTA
 CCAGAGTTCAATCCTTCTCCGCTTGTCATAGATTGCATTCCCCAACCTTGTCGATGAAGTCAACAAGAG
 AATCTTCGGTAAGTGTAACAATCCAAACGGTCACGGTCATAACTACAAGGTTGAAGTCACCGTCAGAGGT
 AAGATTGATAGACATACTGGTATGGTCATGAACATTACCGATTGAAGCAACATATTGAAGAAGTCATTA
 5 TGATTCCATTGGATCATAAGAATTTGGATAAGGACGTTCCATACTTTGCTAACGTTGTCTCTACTACCGA
 AAACGTTGCTGTCTACATTTGGGATAACATGGTTAAGCAATTGCCAGCTAACTTGTTGTACGAAGTTAAG
 ATTCACGAAACCGATAAGAACATTGTTGTCTACAGAGGTGAATAA

SEQ ID NO: 7 *Salinibacter ruber* 6-carboxy-5,6,7,8-tetrahydropterin synthase (UniProtKB- Q2RYU6)

ATGCATCACCATCACCATCACTCCACCGTTTACATTACCAGAAAGGTTCAATTCAACGCTGCTCATAGAT
 TGCATAATCCAAATAAGTCCGATGCTTGGAAACGAAGATACCTACGGTAAGGATAACAATCCAACTGGCA
 TGGTCATAACTACGAATTGGAAAGTCACCGTTGCTGGTGAACCAGATCCAGAAACCGGTTACGTTGTCTGAT
 TTGGGTGCTTGAAGGATATTTTGCATGATAGAGTTTGGATAAGGTTGATCATAAGAACTTGAAGTTGG
 AAGTCGATTTTCATGGATGGTGTATTTCCTTCCTCTGAAAACCTTCGCTATTGCTATTTGGAATGAAATTGA
 15 AGATGCTTTGCCAAACGGTGAATTGCATTGTGTCTCAGATTGTACGAAACTCCAAGAACTTCGTTGAATAC
 AGAGGTGAATAA

SEQ ID NO: 8 *Phycisphaera mikurensis* Putative 6-pyruvoyl tetrahydrobiopterin synthase (UniProtKB- I0IIJ5)

ATGCATCACCATCACCATCACTTTGAATTGACTAGAACTTTAAGATTTTGTCCATCTGGTGATCCAGGTG
 CTCCAAGAGATAACGCTCATGCTGCTTGGCCACCACCAAGAGGTTTAGCAGGTGTATTATCTTTAGATTT
 GACTATTGCTGGTAGACCAGATCCAGGTACTGGTGTTTTATTGAACGTTAAAGATTTAGATGCAGCTTTT
 GCTGCCGCTGCATTACCAAGATTCAGAGCAGCTGCAGGTGCTGAACCAGCAGGTTTATTGAGAGGTGTTG
 CTCAAGCATTAGCTCCTACTTTACCATTTCCATTGTAAAGATTGAGATTATCTGCATCTGCTTCAGCTTC
 25 TACTGAATTGAGACCAGCTGATATGTCTAGAGTTATTTTGGACAAAGATTCTCTTTCTCTGCTGCTCAT
 AGATTACAAGCTGATGCTTTGTCTGAAGAGGAAAATAGAACATTGTTTGGTAAGTGTAATAGACCATCTT
 TTCATGGTCATAATTACGAATTAGAAGTTGCTGCAGCCGCTGCTATTGCTCCAGATGGTAGATCTTTAGA
 ACCAGCTGCATTAGATGCTGCTGTAGAACTAGAGTCATTGATACCTTTAGATCATAGAAATTTGAATACT
 GATGTTGCTGCTTTTGGTACTAGAAATCCAACGTTGAACATATTGCTCAAACCTTGTTGGGATTTGTTAG
 30 CTGGTGGTTTACCAGAAGGTGCAGAATTACAAGAAGTTGTAGTTTGGGAACTGATAGAACATCTTGTGC
 TTATAGAGGTGGTTAA

SEQ ID NO: 9 *Mortierella alpina* Sepiapterin reductase (UniProtKB- G3FNL8)

ATGCATCACCATCACCATCACTCATCCAAAGAACATCATTTGGTTATTATTAACGGTGTTAATAGAGGTT
 35 TTGGTCATTCCGTTGCATTGGATTACATAAGACATTCAGGTGCTCATGCTGTTTCCTTTGTTTTGGTTGG
 TAGAACTCAACATTCCTTGGAAACAAGTTTGAAGTGAATTGCATGAAGCTGCATCTCATGCTGGTGTGTC
 TTCAAGGGTGTCGTTGTCTCCGAAGTTGATTTGGCTCATTTGAACTCTTTGGATTCTAATTTGGCTAGAA
 TACAATCTGCTGCAGCTGATTTGAGAGACGAAGCTGCACAATCTACCAGAACTATTACTAAGTCTGTTTT
 GTTTAATAACGCTGGTTCATTGGGTGATTTGTCCAAGACTGTTAAGGAATTTACCTGGCAAGAAGCTAGA
 40 TCCTACTTGGATTTCAACGTCGTTTCCTTAGTTGGTTTGTGTTCCATGTTCTTGAAGGATACCTTGGAAAG
 CATTTCCAAAGGAACAATATCCAGATCATAGAACTGTTGTGCTTTCATTTCTTCCTTGTAGCTGTTCA
 AGCATTCCCAAATTGGGGTTTGTACGCTGCAGGTAAGGCAGCTAGAGATAGATTGTTAGGTGTTATTGCT
 TTGGAAGAAGCAGCTAATAACGTTAAGACCTTGAATTACGCTCCAGGTCCATTGGATAACGAAATGCAAG
 CTGATGTTAGAAGAACCTTGGGTGATAAAGAACAATTGAAGATTTACGATGATATGCATAAGTCTGGTTC
 45 CTTGGTTAAGATGGAAGATTCTCTAGAAAGTTGATTCATTTGTTAAAGGCTGATACCTTCACCTCCGGT
 GGTCATATTGATTTCTACGATGAATAA

SEQ ID NO: 10 *Saccharomyces cerevisiae* Putative cytoplasmic short-chain dehydrogenase/reductase (UniProtKB- P40579)

ATGCATCACCATCACCATCACGGTAAAGTTATTTTAGTTACAGGTGTTTCCAGAGGTATCGGTAAGTCCA
 TCGTGGATGTTCTTTTCAGTTTGGACAAGGACACGGTTGTTTACGGTGTAGCCAGGTCTGAGGCACCCTT

GAAGAAGTTGAAAGAGAAGTATGGCGACAGGTTTTTTTACGTTGTCGGTGATATTACCGAGGATTCCGTG
 TTGAAGCAGTTGGTTAACGCTGCTGTTAAGGGCCACGGCAAGATCGACTCCTTGGTTGCCAACGCTGGTG
 TCCTAGAGCCCGTGCAAAATGTCAACGAGATTGATGTCAACGCTTGGAAGAAGCTGTATGACATCAACTT
 CTTACAGCATTGTTTTCTTGGTTGGCATTGCGTTACCTGAATTGAAGAAGACCAACGGTAACGTGGTATTC
 5 GTCAGTTCGGACGCCTGTAACATGTACTTCAGCAGTTGGGGAGCTTACGGTTCTTCAAAGCCGCTCTGA
 ACCACTTCGCCATGACTCTGGCCAACGAGGAAAGGCAAGTGAAAGCCATTGCCGTCGCCCCAGGTATTGT
 GGACACAGATATGCAAGTTAACATTAGGGAGAACGTGGGGCCTTCCTCCATGAGTGCAGAGCAATTGAAG
 ATGTTTAGAGGTTTAAAGGAGAATAACAGTTGCTGGATAGCTCTGTGCCAGCTACAGTTTATGCCAAAT
 TGGCCCTTCATGGTATTCTTGACGGTGTTAATGGACAGTACTTGAGCTATAATGACCCTGCCTTGGCGGA
 10 CTTTATGCCTTAA

SEQ ID NO: 11 *Thalassiosira pseudonana* Sepiapterin reductase (UniProtKB- B8BVR3)

ATGCATCACCATCACCATCACCAAAACAAGGAAAACGATGAAACCTCCATTGTTGTGATATTTCATGAAA
 TGGATTTGTCCGATTTGGATATTTTGGCTGTTAACATGAAGTTGTTGTTTGAATTCTACACCAAGGTAC
 15 CAAGTACAATCAATGTTGGTTGTTCAACAATGCTGGTTCCTTGGGTCCATTGGGTCCAACCTTGTCTTG
 TGTAACGGTGATCCATTGAGATTAATGCAAGATTTGAAGAAAGCTGTTGATTTGAACGTTACCTCCGCTA
 CCTGGATTTCTCACAATTCGTTTCCACCTTTGGTTCCTCTCATAAGGACGATACTCCACCATTGGTTAG
 AATTGTTAACATTTCTTCTTGTGTGCTATTGAACCATTCCAAACATATGGCTGTTTACTGTATGGGTAAAG
 GCTGCAAGAGATATGTACCATTTGGTTTTGGCTAAAGAACATAAGGATTCCGATACTATGAAAGTTTTGA
 20 ACTACGCTCCAGGTCTTGTGATACTGAAATGACTGATGTTTTGGCTGGTTCTGCTGTTTTGGATTGGGA
 TTTGCATCAATATTACGCTACATCCAAGAGAGATCAAAGTTGGTTGATCCTTTGGATTCTGCTAAGAAA
 TTGATTGAATTGTTAGAAAAGGATGAATTCACCACAGGTTCCCATGTTGATTACTTCGATGTTTAA

SEQ ID NO: 12 *Homo sapiens* Pterin-4-alpha-carbinolamine dehydratase (UniProtKB- P61457)

ATGGCTGGTAAAGCTCATAGATTGTCTGCTGAAGAAAGAGATCAATTGTTGCCAACTTGAGAGCTGTTG
 GTTGAACGAATTGGAAGGTAGAGATGCTATTTTCAAGCAATTCCATTTCAAAGATTTCAATAGAGCCTT
 25 CGGTTTCATGACTAGAGTTGCCTTGCAAGCTGAAAAGTTAGATCATCATCCAGAATGGTTCAACGTCTAC
 AATAAGGTCCATATTACCTTGTCCACTCATGAATGTGCTGGTTTGTCTGAAAGAGATATTAACCTGGCAT
 CCTTCATTGAACAAGTCGCTGTCTCCATGACTTAA

SEQ ID NO: 13 *Homo sapiens* Dihydropteridine reductase (UniProtKB- P09417-1)

ATGGCAGCTGCTGCAGCCGCTGGTGAAGCTAGAAGAGTTTTGGTTTACGGTGGTAGAGGTGCTTTGGGTT
 CTAGATGTGTCCAAGCATTTCAGAGCTAGAAATTGGTGGGTTGCTTCTGTTGATGTCGTTGAAAACGAAGA
 AGCATCTGCTTCTATTATTGTTAAAATGACTGATTCTTTTACTGAACAAGCTGATCAAGTTACTGCTGAA
 35 GTTGGTAAATTGTTAGGTGAAGAGAAAGTTGATGCTATTTTGTGTGTTGCTGGTGGTTGGGCTGGTGGTA
 ACGCTAAATCTAAATCTTTGTTTAAAGATTGTGATTTGATGTGGAACAATCTATTTGGACTTCTACTAT
 TTCTTCTCATTTGGCTACTAAACATTTGAAAGAAGGTGGTTTGTAACTTTGGCAGGTGCTAAAGCTGCT
 TTGGATGGTACTCCAGGTATGATTGGTTACGGTATGGCTAAAGGTGCAGTTCATCAATTGTGTCAATCTT
 TGGCTGGTAAGAACTCTGGTATGCCACCTGGTGCAGCTGCTATTGCTGTTTTGCCAGTTACTTTGGATAC
 40 ACCAATGAATAGAAAATCTATGCCAGAAGCTGATTTCTTCTTGGACTCCATTGGAATTCTTGGTTGAA
 ACTTTTCATGATTGGATTACTGGAAAGAATAGACCATCTTCTGGTTCTTTGATTCAAGTTGTTACTACTG
 AAGGTAGAATGAATTGACTCCAGCTTATTTCTAA

SEQ ID NO: 14 *Mus musculus* Tyrosine 3-monooxygenase (UniProtKB- P24529)

ATGCCAACTCCATCCGCTTCCTCCCCACAACCAAAGGGTTTCAGACGCGCTGTGTCTGAACAAGATACTA
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 GCTGTTGTCTTCAAGAAAGAGATGGTAATGCTGTTTTGAATTTGTTGTTCTTTGAGAGGTACTAAGC
 CATCTTCTTGTCTAGAGCTCTAAAGGTATTCGAACTTTCAAGCTAAGATTCATCATTTGGAACTAG
 45 ACCTGCACAAAGACCATTGGCTGGTTCCTCCACATTTGGAATACTTCGTTAGATTTGAAGTCCATCCGGT
 GATTTGGCTGCTTTGTTGTCTTCCGTTAGAAGAGTTTCTGATGATGTTAGATCCGCTAGAGAAGATAAGG

TTCCTTGGTTTCCAAGAAAGGTTTCTGAATTGGATAAGTGTGTCATCATTTGGTTACTAAGTTTGATCCAGA
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 GCTTTCCAATACAAGCAAGGTGAACCAATTCCACATGTTGAATACACTAAGGAAGAAATTGCTACTTGGA
 AGGAAGTTTACGCTACTTTGAAGGGTTTGTACGCTACTCATGCTTGTAGAGAACATTTGGAAGCATTTC
 5 ATTGTTGGAAAGATACTGTGGTTACAGAGAAGATTCTATTCCACAATTGGAAGATGTTTCTCATTTCTTG
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 10 CTGTTGAATTTGGTTTGTGTAAGCAAAATGGTGAATTGAAGGCTTACGGTGCCGGATTGTTGTCCTCTTA
 CGGTGAATTTGTTGCATTCTTTGTCTGAAGAACCAGAAGTTAGAGCTTTCGATCCAGATACTGCTGCTGTT
 CAACCATACCAAGATCAAACCTACCAACCAGTTTACTTCGTTTCTGAATCTTCTCTGATGCTAAGGATA
 AGTTGAGAAATTACGCTTCTAGAATCCAAAGACCATTCTCTGTTAAGTTTGATCCATACACTTTGGCTAT
 TGATGTCTTGGATTCTCCACATACTATTAGAAGATCTTTGGAAGGTGTTCAAGATGAATTGCATACTTTG
 15 ACTCAAGCATTGTCTGCTATTTCTTAA

SEQ ID NO: 15 *Homo sapiens* Tryptophan-5-hydroxylase 2 isoform 1 AA145-460 (UniProtKB- Q8IWU9-1)

ATGGAAGAATTGGAAGATGTTCCCTTGGTTCCCAAGAAAGATTTCCGAATTGGATAAGTGTTCATAGAG
 20 TTTTGATGTATGGTTCCGAATTGGATGCTGATCATCCAGGTTTCAAGGATAATGTTTACAGACAAAGAAG
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 AATACTTGAAGAATTTCCCATTTGTTGACTAAGTACTGTGGTTACAGAGAAGATAATGTTCCACAATTGGA
 AGATGTTTCCATGTTCTTGAAGGAAAGATCCGGTTTCACTGTTAGACCAGTTGCTGGTTACTTGTCCCCA
 25 AGAGATTTCTTGGCTGGTTTGGCTTACAGAGTCTTCCATTGTACTCAATACATTAGACATGGTTCCGATC
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 GCTACTTGTACTTCTTCACTATTGAATTCCGTTTGTGTAAGCAAGAAGGTCAATTGAGAGCTTACGGTG
 CTGGTTTGTATCCTCTATTGGTGAATTGAAGCAGCTTTGTCCGATAAGGCTTGTGTTAAGGCTTTTCGA
 30 CCCAAAGACTACTTGTGTTGCAAGAATGTTTGATTACTACTTTCCAAGAAGCATACTTCGTTTCCGAATCC
 TTCGAAGAAGCTAAGGAGAAGATGAGAGATTTGCTAAGTCCATTACTAGACCATTCTCCGTTTACTTCA
 ATCCATACACTCAATCCATTGAAATTTTGAAGGATACTTAA

SEQ ID NO: 16 *Sus scrofa* Aromatic-L-amino-acid decarboxylase (UniProtKB- P80041)

ATGAATGCTTCTGATTTTAGAAGGAGAGGTAAAGAAATGGTTGACTACATGGCTGATTACTTGGAAGGTA
 TTGAAGGTAGACAAGTTTACCCAGATGTTCAACCAGGTTACTTGAGACCATTGATTCCAGCTACTGCTCC
 ACAAGAACCAGATACTTTTGAAGATATTTTGCAAGATGTTGAGAAGATTATTATGCCAGGTGTCACACAT
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 TGTGTGGTGCTATTGGTTGTATTGGTTTCTCCTGGGCTGCTTCCCAGCTTGTACTGAATTGGAACTGT
 40 TATGATGGATTGGTTGGGTAAATGTTGCAATTGCCAGAAGCCTTCTTGGCTGGTGAAGCTGGTGAAGGT
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 45 TGATTCCATTCTTCGTTGTTGCTACTTTGGGTACTACTTCCTGTTGTTCCCTTGATAATTTGTTGGAAGT
 TGGTCCAATTTGTCATGAAGAAGATATTTGGTTGCATGTTGATGCTGCTTACGCTGGTTCCGCTTTCATT
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 50 TTGCCATTGGGTAGAAGATTTAGATCCTTGAAAATGTGGTTTGTCTCAGAATGTACGGTGTTAAAGGTT
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ATTTGAAGTTTGTGCTGAAGTTACTTTGGGTTTGGTTTGTTTTAGATTGAAAGGTTCCGATGGTTTGAAT
 GAGGCTTTGTTGGAAAGAATTAATTCCGCTAGAAAAGATTTCATTTGGTTCCATGTAGATTGAGAGGTCAAT
 TTGTTTTGAGATTTGCTATTTGTTCCAGAAAAGTTGAATCCGGTCATGTTAGATTGGCTTGGGAACATAT
 TAGAGGTTTGGCTGCTGAATTGTTGGCTGCTGAAGAAGGTAAAGCTGAAATTAAATCTTAA

5 SEQ ID NO: 17 *Ophiorrhiza pumila* Strictosidine synthase AA26-350, His₆ only included in pAME64 (UniProtKB- Q94LW9)⁷

ATGGGCTCTCCTGAGTTTTTTCGAATTTATTGAAGCACCGTCTTATGGTCCAAATGCGTATGCGTTCGACA
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 10 CCTGACTCATGCTGTTGCCAGCCCGATCTGGAACAATGCCCTGTGTGAGAATAATACCAACCAAGACCTG
 AAGCCGCTGTGCGGTGCGCTCTACGACTTTGGTTTTCATTATGAAACGCAGCGCCTGTACATTGCAGATT
 GCTACTTCGGCTTGGGCTTTGTTGGTCCGGACGGCGGTACGCGATTCAACTGGCAACCTCCGGTGATGG
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 TCTACTAAGTACGATGATCGTGGTGTTTCAGGACATTATTCGCATTAATGATACCACGGGTGCGCTGATTA
 15 AGTATGACCCTTCGACCGAAGAGGTGACCGTGCTGATGAAAGGCCTGAATATTCGGGGCGGTACCGAGGT
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 TGAGTTTGGCAACATTCTGGAGGTCGTTGCTATTCCGCTGCCGTATAAAGGTGAACATATCGAGCAGGTC
 20 CAAGAACACGACGGCGCCCTGTTGCTGGGTAGCCTGTTTCATGAGTTCGTGCGCATCCTGCATAACTATA
 AGAGCAGCGTTGACCATCATCAGGAAAAGAACTCGGGTGGTCTGAACGCGAGCTTCAAGGAGTTCTCTTC
 GTTTGGATCTCATCACCATCACCATCACTAG

Reagents. Tetrahydrobiopterin, dihydrobiopterin, and biopterin were purchased from
 25 Cayman Chemical (81880, 81882, and 10007662). Dopamine and vanillin were purchased from
 Alfa Aesar (A11136 and A11169). L-DOPA and serotonin were purchased from TCI America
 (D0600 and S0370). Secologanin and tryptophan were purchased from Sigma-Aldrich (50741-
 5MG-F and T0254). 5-chlorotryptamine was purchased from Ark Pharm, Inc. (AK-32281).

Microbial synthesis of tetrahydrobiopterin. Overnight cultures of strains PPY750, 752-
 30 793 and 797-810 in synthetic complete media with 2% glucose lacking histidine, tryptophan, and
 leucine (SD (HWL⁻)) were used to inoculate 5mL of synthetic complete media with 2% galactose
 lacking histidine, tryptophan, and leucine (SCgal (HWL⁻)) to OD₆₀₀=0.1 and incubated for 136
 hours at 30°C (250 rpm). Overnight culture of strain PPY749 in synthetic complete media with
 2% glucose lacking histidine (SD (H⁻)) was used to inoculate 5mL of synthetic complete media
 35 with 2% galactose lacking histidine (SCgal (H⁻)) to OD₆₀₀=0.1 and incubated for 136 hours at
 30°C (250 rpm). Overnight culture of strain PPY751 in synthetic complete media with 2%
 glucose lacking histidine and tryptophan (SD (HW⁻)) was used to inoculate 5mL of synthetic
 complete media with 2% galactose lacking histidine and tryptophan (SCgal (HW⁻)) to OD₆₀₀=0.1
 and incubated for 136 hours at 30°C (250 rpm). After incubation, cultures were centrifuged for 5
 40 min at 3230 x g, the supernatant was filtered, vanillin was added as an internal standard and
 samples were analyzed via LC/MS. For quantification of biopterin in L-DOPA, dopamine, and
 serotonin-producing strains, 5-chlorotryptamine was used as an internal standard.

Microbial synthesis of L-DOPA, dopamine, serotonin, and hydroxystrictosidine.

Overnight cultures of strains PPY646, 649-650, 658, 679, 741, 743, 946-948, and 955 in synthetic complete media with 2% glucose lacking histidine, tryptophan, leucine, and uracil (SD (HWLU⁻)) were used to inoculate 5mL of synthetic complete media with 2% galactose lacking histidine, tryptophan, leucine, and uracil (SCgal (HWLU⁻)) to OD₆₀₀=0.1. Overnight cultures of strains PPY744 and 748 in SD (HWU⁻) were used to inoculate 5mL of fresh SD (HWU⁻) to OD₆₀₀=0.1. Overnight culture of strain PPY740 in SD (HW⁻) was used to inoculate 5mL of fresh SD (HW⁻) to OD₆₀₀=0.1. For hydroxystrictosidine production (strains PPY649, 650, 740, 741, 744, 748, and 955), secologanin (solution in water) was added at the time of inoculation to a final concentration of 0.4mM (150mg/L). After inoculation, all strains were incubated for 136 hours at 30°C (250 rpm). The cultures were then centrifuged for 5 min at 3230 x g, the supernatant was filtered, 5-chlorotryptamine (L-DOPA, dopamine, serotonin) or vanillin (hydroxystrictosidine) was added as an internal standard, and the samples were analyzed via LC/MS.

Bioppterin quantification. LC/MS analysis was completed using an Agilent 1100/1260 series system equipped with a 1260 ALS autosampler and a 6120 Single Quadrupole LC/MS with a Poroshell 120 SB-Aq 3.0 x 100mm x 2.7µM column and an electrospray ion source. LC conditions: Solvent A- 150mM acetic acid with 0.1% formic acid and Solvent B- methanol with 0.1% formic acid. Gradient: 4 min ramp from 95%:5%:0.2 (A:B: flow rate in mL/min) to 70%:30%:0.2, 6 min ramp to 40%:60%:0.2, 2 min ramp to 2%:98%:0.2, 2 min ramp to 2%:98%:0.5, 4 min at 2%:98%:0.5, 1 min ramp to 95%:5%:0.5, 7 min at 95%:5%:0.5, and 1.5 min post time. MS acquisition (positive ion mode) included 25% scan from m/z 100-600, 25% scan from m/z 230-260, 25% scan from m/z 145-165, and 25% Selected Ion Monitoring (SIM) for BH₄ (m/z 242.1), dihydrobiopterin (m/z 240.1), biopterin (m/z 238.1), and vanillin (m/z 153.1). Quantitation was performed by obtaining the area under the peak in the extracted ion chromatogram (EIC) for the desired m/z value from the SIM signal. For biopterin quantification in L-DOPA-, dopamine-, and serotonin-producing strains, 5-chlorotryptamine (m/z 195.1) was used as an internal standard instead of vanillin. Area was converted to concentration using a standard curves produced from commercially available biopterin. Retention times were determined using commercially available standards.

Quantification of L-DOPA, dopamine and serotonin. LC/MS system and solvent composition was the same as the one used in the analysis of biopterin. LC gradient: 8 min ramp from 95%:5%:0.05 to 70%:30%:0.05, 6 min ramp to 40%:60%:0.05, 1 min ramp to 40%:60%:0.1, 9 min ramp to 2%:98%:0.1, 1 min at 2%:98%:0.1, 5 min ramp to 2%:98%:0.3, 0.1

min ramp to 2%:98%:0.5, 3.9 min at 2%:98%:0.5, 1 min ramp to 95%:5%:0.5, 7 min at 95%:5%:0.5, and 3.5 min post time. MS acquisition (positive ion mode) included 33% scan from m/z 100-600, 33% scan from m/z 120-240, and 33% SIM for DOPA (m/z 198.2), dopamine (m/z 154.2), hydroxytryptophan (m/z 221.2), serotonin (m/z 177.2), and 5-chlorotryptamine (m/z 195.1). Quantitation was performed by obtaining the area under the peak in the EIC for the desired m/z value from the SIM signal. Area was converted to concentration using standard curves produced from commercially available L-DOPA, dopamine and serotonin dissolved in media taken from a culture of strain PPY810 grown under the same conditions as production samples. Traces used for the L-DOPA standard curve were background subtracted using just media. Retention times were determined using commercially available standards.

Analysis of hydroxystrictosidine. High resolution mass spectrometry (HRMS) and tandem mass spectrometry (MS/MS) analysis of hydroxystrictosidine was performed at the Mass Spectrometry Facility at Georgia Tech. MS/MS was done using a Waters Quattro LC Mass Spectrometer with a Gemini 2 x 150mm 5µm C18 column from Phenomenex. LC conditions: Solvent A: 95%:5% water:acetonitrile; Solvent B: 5%:95% water:acetonitrile. Gradient: 7 min at 100%:0% (A:B), 37 min ramp to 0%:100%, 8 min at 0%:100%, 1 min ramp to 100%:0%, and 7 min at 100%:0%. Flow rate was 0.2 mL/min. HRMS was done using a Thermo LTQ Orbitrap XL equipped with a Nano ACQUITY UPLC with a BEH130 300µm x 100mm 1.7µm C8 column from Waters. Solvent A: 10mM ammonium acetate in water; Solvent B: acetonitrile. Gradient: 5 min at 95%:5% (A:B), 40 min ramp to 70%:30%, 5 min at 70%:30%, 2 min ramp to 5%:95%, 3 min at 5%:95%, 1 min ramp to 95%:5%, and 4 min at 95%:5%. Flow rate was 8 µL/min. Multiple Reaction Monitoring (MRM) was done on the Waters Quattro LC Mass Spectrometer using the same column and LC gradient using Solvent A- 95%:5%:0.1% water:acetonitrile:formic acid and Solvent B- 5%:95%:0.1%. MRM parameters: hydroxystrictosidine- transition 547.60 → 530.00, cone voltage 20V, collision energy 35eV; transition 547.60 → 298.00, cone voltage 20V, collision energy 35eV; vanillin- transition 152.80 → 92.80, cone voltage 25V, collision energy 15eV; transition 152.80 → 124.80, cone voltage 25V, collision energy 15eV; camptothecin- transition 349.10 → 305.00, cone voltage 45V, collision energy 35eV; transition 349.10 → 220.00, cone voltage 45V, collision energy 40eV. Reported hydroxystrictosidine counts obtained using 547.60 → 530.00 transition.

Hydroxystrictosidine isomer ratios. For the chemical reactions, secologanin and serotonin were mixed to a final concentration of 0.4mM each in pH=3 or pH=7 phosphate buffer (135mM NaCl, 2.7mM KCl, 4.3mM Na₂HPO₄, 1.4mM KH₂PO₄). Solutions were mixed and incubated for 136 hours at 30°C (250 rpm). After incubation, solutions were analyzed using

LC/MS. For lysate experiments, an overnight culture of PPY827 was used to inoculate SCgal (U⁻) to OD₆₀₀=0.1. The culture was incubated for 24 hours at 30°C (250 rpm). After incubation, the culture was centrifuged at 3230 x g for 5 min, the supernatant was removed, and pellet was resuspended in 1.5mL phosphate buffer. The pellet was lysed by sonication using a Misonix
 5 Sonicator 3000 at 5.0 output level for 20 sec, 20 sec rest, for a total 6 pulses. The lysate was centrifuged and supernatant collected. The pH of the lysate was adjusted to either pH=3 or pH=7 and secologanin and serotonin were added to a final concentration of 0.4mM each. After mixing, the lysates were incubated for 136 hours at 30°C (250 rpm), after which the lysates were analyzed using LC/MS. For *in vivo* intact cell experiments, overnight cultures of strains
 10 PPY827 and PPY828 in SD (U⁻) were used to inoculate 5mL of SCgal (U⁻) or SCgal (U⁻) buffered with 25mM K₂HPO₄ (pH=7) to OD₆₀₀=0.1. Secologanin and serotonin were added to a final concentration of 0.4mM each and cultures were incubated for 136 hours at 30°C (250 rpm). After incubation, all cultures were centrifuged for 5 min at 3230 x g, the supernatant was filtered, and analyzed via LC/MS. The column compartment was kept constant at 28°C. LC/MS
 15 analysis was completed on the Agilent system described above. Gradient: 0.25 min ramp from 95%:5% (A:B) to 70%:30%, 4.75 min ramp to 68%:32%, 2 min ramp to 30%:70%, 1 min at 30%:70%, 0.50 min ramp to 95%:5%, and 5.5 min at 95%:5%. Flow rate was 0.4 mL/min. MS acquisition (positive ion mode) included 30% scan from m/z 100-600 and 70% SIM for ions related to alkaloid formation (dopamine- m/z 154; tryptamine- m/z 161; serotonin- m/z 177;
 20 tyrosine- m/z 182; L-DOPA- m/z 198; tryptophan- m/z 205; 5-hydroxytryptophan- m/z 221; strictosidine- m/z 531; hydroxystictosidine- m/z 547).

Yeast cell lysis for intracellular biopterin determination. After 136 h of microbial production, cultures were centrifuged at 3230g for 5 min. The supernatant was removed and filtered with a 0.2µm filter. The pellet was frozen at -80°C, thawed, washed with 1mL water, and
 25 resuspended in 250µL water. 250µL 0.2M NaOH was mixed in and the cells remained at room temperature for 10 minutes. The lysate was centrifuged and filtered. Both supernatant and lysate were analyzed using liquid chromatography/ mass spectrometry (LC-MS).

Statistical analysis. Two-tailed, paired T-tests were performed in Microsoft Excel.

Determining SR open reading frame from T. pseudonana. As only a portion of the
 30 amino acid sequence is known for the predicted SR from *T. pseudonana*, we searched upstream and downstream of the sequence in the genome to obtain a complete open reading frame.

Amino acid limiting experiments. Overnight cultures of strain PPY649 and PPY646 in synthetic media containing 2% glucose and lacking histidine, leucine, uracil, and tryptophan (SD

(HWUL-) was used to inoculate 5mL of synthetic media containing 2% galactose and lacking histidine, leucine, uracil, and tryptophan (SCgal (HWUL)) to OD₆₀₀=0.1. Tryptophan was added to strain PPY649 (final concentrations 0-640mg/L) and tyrosine was added to strain PPY646 (final concentrations 30-960mg/L). Cultures were incubated for 136 hours at 30°C (250 rpm).
 5 After incubation, cultures were centrifuged for 5 min at 3230g. Supernatant was removed, filtered and analyzed via LC-MS analysis. 5-chlorotryptamine was used as an internal standard.

Determination of GTPCH, PTPS, and SR mRNA levels. Overnight cultures of strain PPY949-950 in synthetic complete media with 2% glucose lacking leucine (SD (L)) was used to inoculate 5mL of synthetic complete media with 2% galactose lacking leucine (SCgal (L)) to
 10 OD₆₀₀=0.1 and incubated overnight at 30°C (250 rpm). Overnight cultures of strain PPY951-952 in synthetic complete media with 2% glucose lacking tryptophan (SD (W)) was used to inoculate 5mL of synthetic complete media with 2% galactose lacking tryptophan (SCgal (W)) to OD₆₀₀=0.1 and incubated overnight at 30°C (250 rpm). Overnight cultures of strain PPY953-954 in synthetic complete media with 2% glucose lacking histidine (SD (H)) was used to inoculate
 15 5mL of synthetic complete media with 2% galactose lacking histidine (SCgal (H)) to OD₆₀₀=0.1 and incubated overnight at 30°C (250 rpm). Total RNA for all cultures was extracted using a RNeasy Mini Kit (Qiagen) following the manufacturer's protocol for isolation from yeast using 3x10⁷ cells per culture. RNA quantity was measured using a NanoDrop Lite. 1 µg of total RNA was taken from each strain and converted into cDNA using QuantiTect® reverse transcription kit
 20 (Qiagen) using manufacturer's instructions. Relative expression levels of GFP were quantified using QuantiTect® SYBR Green PCR kit (Qiagen) using manufacturer's instructions for LightCyclers 1.x and 2.0 with 150ng cDNA per reaction. Duplicate reactions were set up for each strain. Quantification was completed using a StepOnePlus Real-time PCR system (Applied Biosystems) with primers AME443/AME444 (GTPCH), AME441/AME442 (PTPS),
 25 AME445/AME446 (SR), and ACT-F/ACT-R. Cycling conditions: 15 min activation at 95°C followed by 40 cycles of 15 sec 95°C, 15 sec 57°C, and 15 sec 72°C. ACT1, a gene that encodes actin, was used to normalize the amount of the mRNA for the gene of interest in all samples.

References for Methods:

- 30 1. Gibson, D.G., Young, L., Chuang, R-Y., Venter, J.C., Hutchison III, C.A. & Smith, H.O. Enzymatic assembly of DNA molecules up to several hundred kilobases. *Nat. Methods* **6**, 343 - 345 (2009).
2. Peralta-Yahya, P., Carter, B.T., Lin, H., Tao, H. & Cornish, V.W High-Throughput Selection for Cellulase Catalysts Using Chemical Complementation *J. Am. Chem. Soc.*

130 (51), 17446–17452 (2008).

3. Arnold K., Bordoli L., Kopp J. & Schwede T. The SWISS-MODEL Workspace: A web-based environment for protein structure homology modelling. *Bioinformatics* **22**, 195-201 (2006).
- 5 4. Kiefer F., Arnold K., Künzli M., Bordoli L. & Schwede T. The SWISS-MODEL Repository and associated resources. *Nucleic Acids Res.* **37**, D387-D392 (2009).
5. Peitsch, M. C. Protein modeling by E-mail *Nature Biotechnol.* **13**, 658-660 (1995).
6. Kanehisa, M. Goto, S. KEGG: Kyoto encyclopedia of genes and genomes *Nucleic Acids Res.* **28**, 27-30 (2000).
- 10 7. Bernhardt, P., Usera, A. R., and O'Connor, S. E. Biocatalytic asymmetric formation of tetrahydro-beta-carbolines. *Tetrahedron Lett.* **51**, 4400–4402 (2010).

Example 4: A method of producing BH₄ in an engineered yeast cell.

BH₄ can be synthesized by a BH₄ synthesis pathway 1500 comprising GTP cyclohydrazase (GTPCH), pyruvate tetrahydrobiopterin synthase (PTPS), and sepiapterin reductase (SR). A yeast cell can be engineered to express said BH₄ synthesis pathway. Said engineered yeast cell can be incubated a carbohydrate for an amount of time to produce BH₄. One skilled in the art will recognize the appropriate analysis measures to determine the incubation parameters for suitable BH₄ production. The carbohydrate can be glucose or galactose (Figure 30). Products of the endogenous yeast carbohydrate metabolic pathway are then used by (or coupled to) the engineered BH₄ pathway to produce BH₄ in the engineered yeast cell.

Example 5: A method of producing serotonin in an engineered yeast cell.

A yeast cell can be engineered to express a biocatalyst 2100. The biocatalyst 2100 is comprised of a BH₄ synthesis pathway 1500 (1500 comprising GTP cyclohydrazase (GTPCH), pyruvate tetrahydrobiopterin synthase (PTPS), and sepiapterin reductase (SR)) and an enzymatic pathway 1000 comprised of tryptophan hydroxylase and aromatic-L-amino acid decarboxylase. Said engineered yeast cell is incubated with a carbohydrate for an amount of time to produce serotonin (Figure 31). One skilled in the art will recognize the appropriate analysis measures to determine the incubation parameters for suitable serotonin production. The carbohydrate can be glucose or galactose. Products of the endogenous yeast carbohydrate metabolic pathway are then used by (or coupled to) the biocatalyst 2100 to produce serotonin in the engineered yeast cell.

Example 6: A method of producing dopamine in an engineered yeast cell.

A yeast cell can be engineered to express a biocatalyst 2100. The biocatalyst 2100 is comprised of a BH4 synthesis pathway 1500 (1500 comprising GTP cyclohydrolase (GTPCH), pyruvyl tetrahydrobiopterin synthase (PTPS), and sepiapterin reductase (SR)) and an enzymatic pathway 1000 comprised of tyrosine hydroxylase and aromatic-L-amino acid decarboxylase.

5 Said engineered yeast cell is incubated with a carbohydrate for an amount of time to produce dopamine (Figure 32). One skilled in the art will recognize the appropriate analysis measures to determine the incubation parameters for suitable serotonin production. The carbohydrate can be glucose or galactose. Products of the endogenous yeast carbohydrate metabolic pathway are then used by (or coupled to) the biocatalyst 2100 to produce dopamine in the engineered

10 yeast cell. This method can be used to produce L-DOPA if aromatic-L-amino acid is omitted from the enzymatic pathway 1000.

Example 7: A method of producing hydroxystrictosidine in an engineered yeast cell.

A yeast cell can be engineered to express a biocatalyst 2100. The biocatalyst 2100 is comprised of a BH4 synthesis pathway 1500 (1500 comprising GTP cyclohydrolase (GTPCH), pyruvyl tetrahydrobiopterin synthase (PTPS), and sepiapterin reductase (SR)) and an enzymatic pathway 1000 comprised of tyrosine hydroxylase, aromatic-L-amino acid decarboxylase, and strictosidine synthase. Said engineered yeast cell is incubated with a carbohydrate for an amount of time to produce dopamine (Figure 33). One skilled in the art will recognize the

20 appropriate analysis measures to determine the incubation parameters for suitable hydroxystrictosidine production. The carbohydrate can be glucose or galactose. Products of the endogenous yeast carbohydrate metabolic pathway are then used by (or coupled to) the biocatalyst 2100 to produce hydroxystrictosidine in the engineered yeast cell.

25 **Example 8: Overview of the microbial synthesis of L-DOPA, dopamine, serotonin and 10-hydroxystrictosidine** (Figure 34). Arrows represent presence of the enzyme. nd = not detectable. Amount produced is represented by the mean $\pm$ standard deviation for samples run in triplicate. GTPCH: GTP cyclohydrolase; PTPS: pyruvyl tetrahydropterin synthase; SR: sepiapterin reductase; PCD: pterin-4a-carbinolamine dehydratase; DHPR: dihydropteridine reductase; TPH: tryptophan hydroxylase; TH: tyrosine hydroxylase; DDC: aromatic-L-amino acid decarboxylase; STR: strictosidine synthase.

30

We claim:

1. A biocatalyst comprising:
a tetrahydrobiopterin source; and
a pterin-dependent enzymatic pathway, where the tetrahydrobiopterin source is biologically coupled to the tetrahydrobiopterin source.
2. The biocatalyst of claim 1, wherein said tetrahydrobiopterin source is a tetrahydrobiopterin synthesis pathway comprising:
a GTP cyclohydrolase;
a pyruvoyl tetrahydropterin synthase; and
a sepiapterin reductase.
3. The biocatalyst of claims 1 or 2, wherein said pterin-dependent enzymatic pathway is at least one enzyme selected from the group consisting of:
an amino acid mono-oxygenase, a modified amino acid mono-oxygenase, phenylalanine hydroxylase, tryptophan hydroxylase, nitric oxide synthase, and tyrosine hydroxylase.
4. The biocatalyst of claims 1 or 2, wherein said pterin-dependent enzymatic comprises a decarboxylase.
5. The biocatalyst of claim 4, wherein the decarboxylase is aromatic-L-amino acid decarboxylase.
6. The biocatalyst of claims 1 or 2, further comprising a synthase.
7. The biocatalyst of claim 6, wherein the synthase is a terpene alkaloid synthase, strictosidine synthase, or a deacetylisoipecoside synthase.
8. The biocatalyst of any of claims 1 or 2, further comprising a tetrahydrobiopterin recycling pathway, the tetrahydrobiopterin pathway comprising:
a pterin-4a-carbinolamine dehydratase; and

a dihydropterin reductase, where the dihydropterin reductase is biologically coupled to the pterin-4a-carbinolamine dehydratase;

where the tetrahydrobiopterin pathway is biologically coupled to the pterin-dependent enzymatic pathway.

9. A cell comprising:

a biocatalyst, the biocatalyst comprising:

a tetrahydrobiopterin source; and

a pterin-dependent enzymatic pathway biologically coupled to the tetrahydrobiopterin source.

10. The cell of claim 10, wherein the tetrahydrobiopterin source is a tetrahydrobiopterin synthesis pathway comprising:

a GTP cyclohydrolase;

a pyruvoyl tetrahydropterin synthase; and

a sepiapterin reductase.

11. The cell of any one of claims 9-10, wherein the pterin-dependent enzymatic pathway comprises at least one enzyme selected from the group consisting of:

an amino acid mono-oxygenase, a modified amino acid mono-oxygenase, phenylalanine hydroxylase, tryptophan hydroxylase, tyrosine hydroxylase, nitric oxide synthase, and alkylglycerol monooxygenase.

12. The cell of any one of claims 9-10, wherein the pterin-dependent enzymatic pathway comprises a decarboxylase.

13. The cell of claim 12, wherein the decarboxylase is aromatic-L-amino acid decarboxylase.

14. The cell of any one of claims 9-10, further comprising a synthase.

15. The engineered cell of claim 14, wherein the synthase is a terpene alkaloid synthase.

16. The engineered cell of claim 14, wherein the synthase is a strictosidine synthase.

17. The engineered cell of any one of claims 9-10, further comprising a tetrahydrobiopterin recycling pathway, the tetrahydrobiopterin pathway comprising:
a pterin-4a-carbinolamine dehydratase; and
a dihydropterin reductase, where the dihydropterin reductase is biologically coupled to the pterin-4a-carbinolamine dehydratase;
where the tetrahydrobiopterin pathway is biologically coupled to the pterin-dependent enzymatic pathway.
18. A method of biocatalysis comprising the steps of:
providing a biocatalyst as in any one of claims 1-2; and
providing a substrate to the biocatalyst.
19. The method of claim 18, wherein the biocatalyst is contained in a cell.
20. The method of claim 18, wherein the biocatalyst further comprises a tetrahydrobiopterin recycling pathway, the tetrahydrobiopterin pathway comprising:
a pterin-4a-carbinolamine dehydratase; and
a dihydropterin reductase, where the dihydropterin reductase is biologically coupled to the pterin-4a-carbinolamine dehydratase;
where the tetrahydrobiopterin pathway is biologically coupled to the pterin-dependent enzymatic pathway.
21. The method of claim 18, wherein the substrate is a carbohydrate.
22. The method of claim 18 wherein the substrate is glucose.
23. The method of claim 18, wherein the substrate is galactose.
24. The method of claim 18, wherein the substrate is an aromatic compound.
25. The method of claim 18, wherein the substrate is a lignin or metabolite thereof.

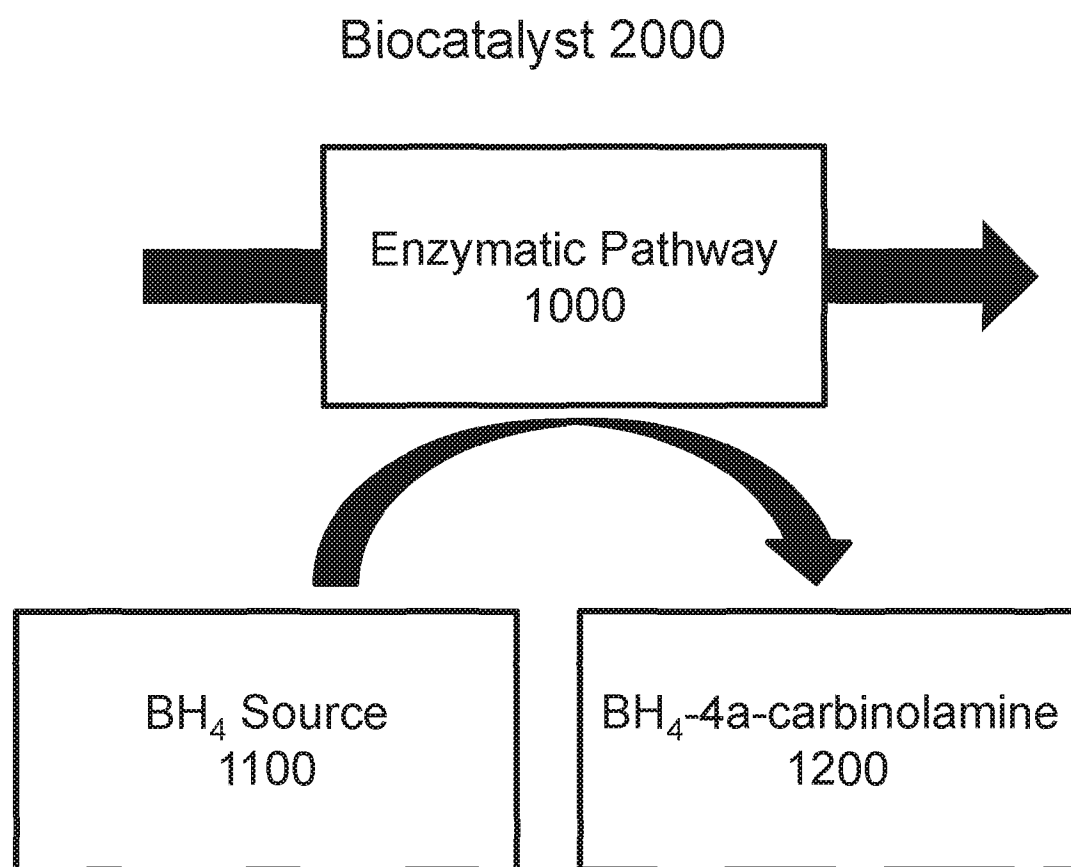


FIG. 1

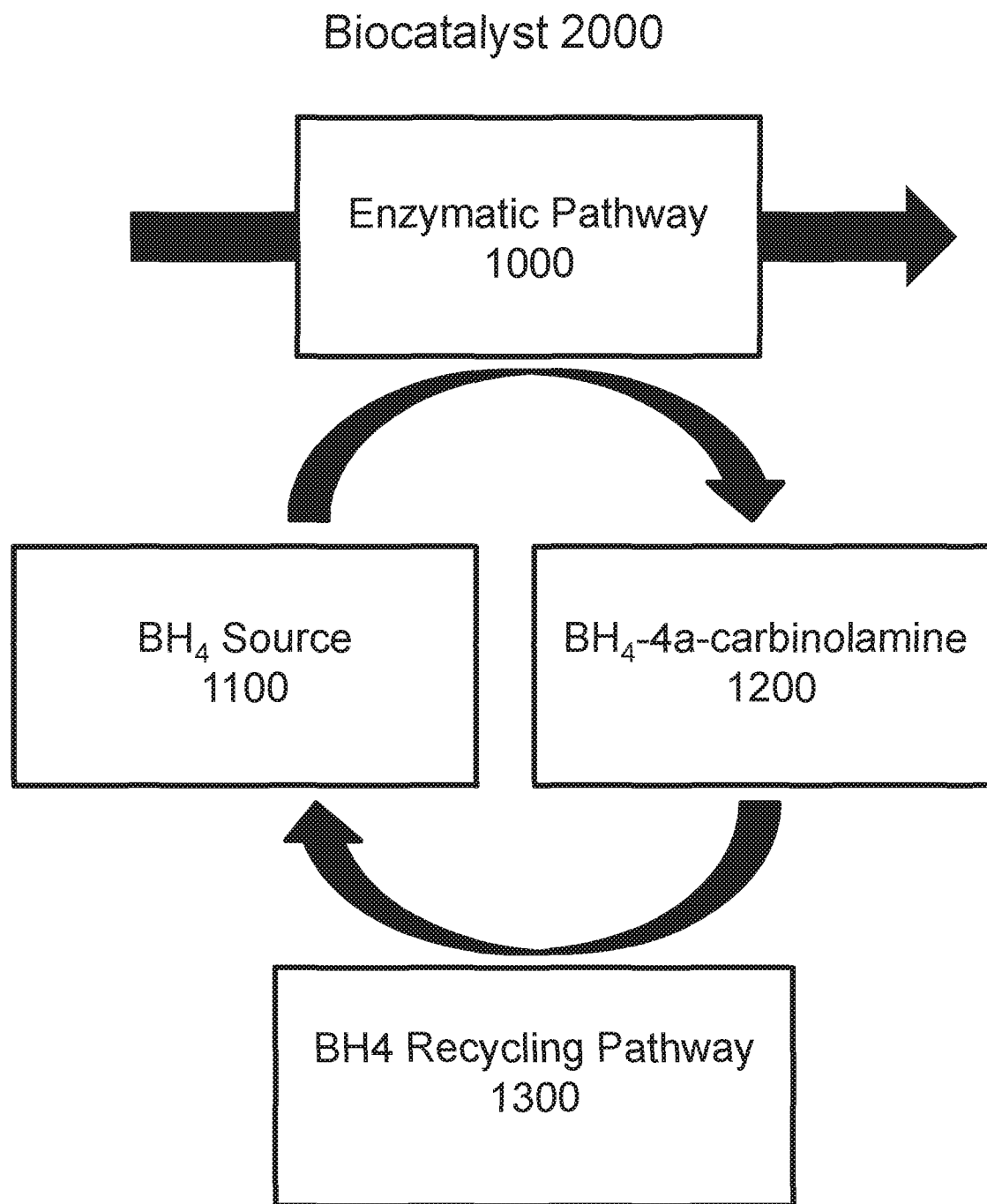


FIG. 2

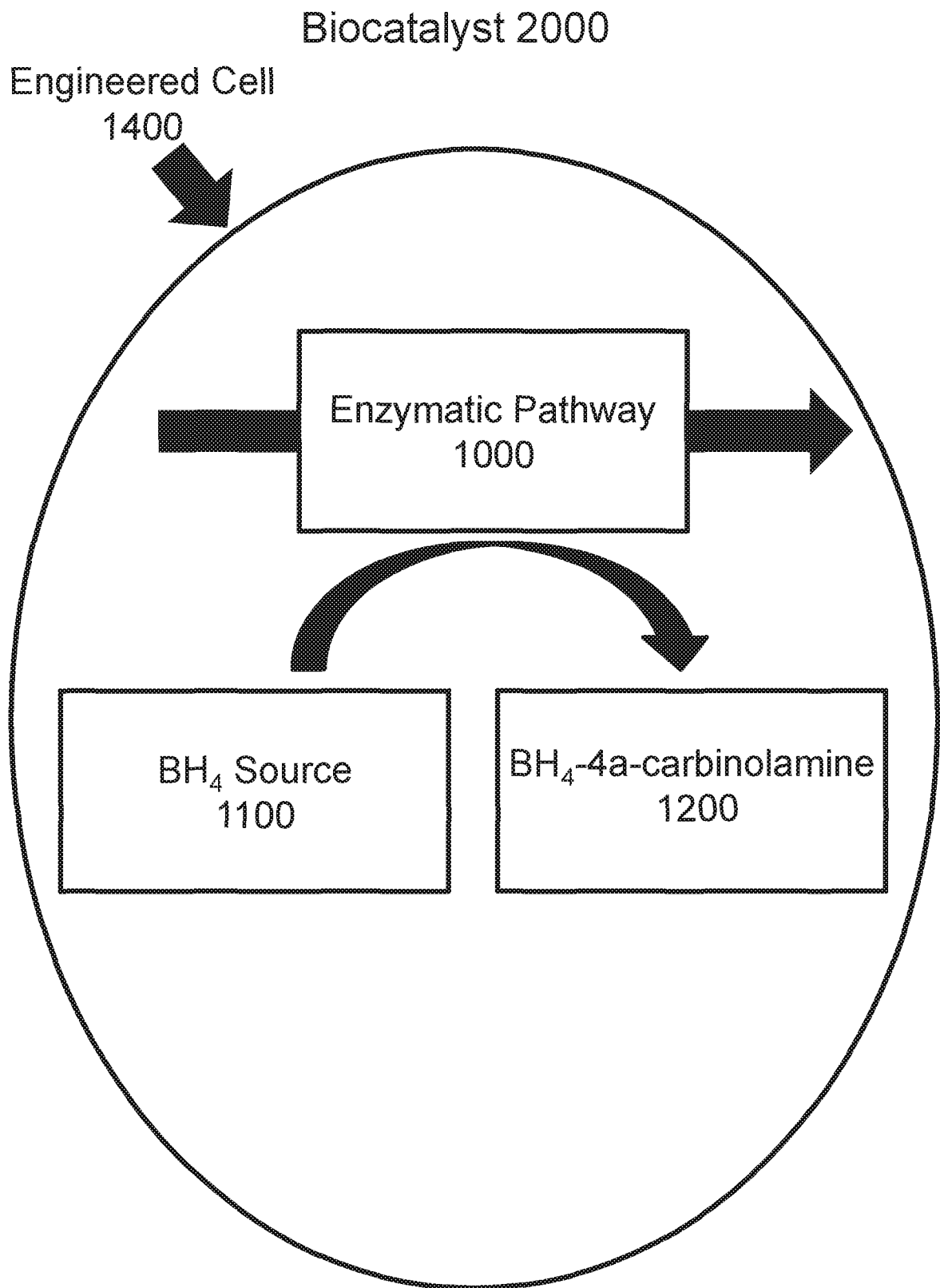


FIG. 3

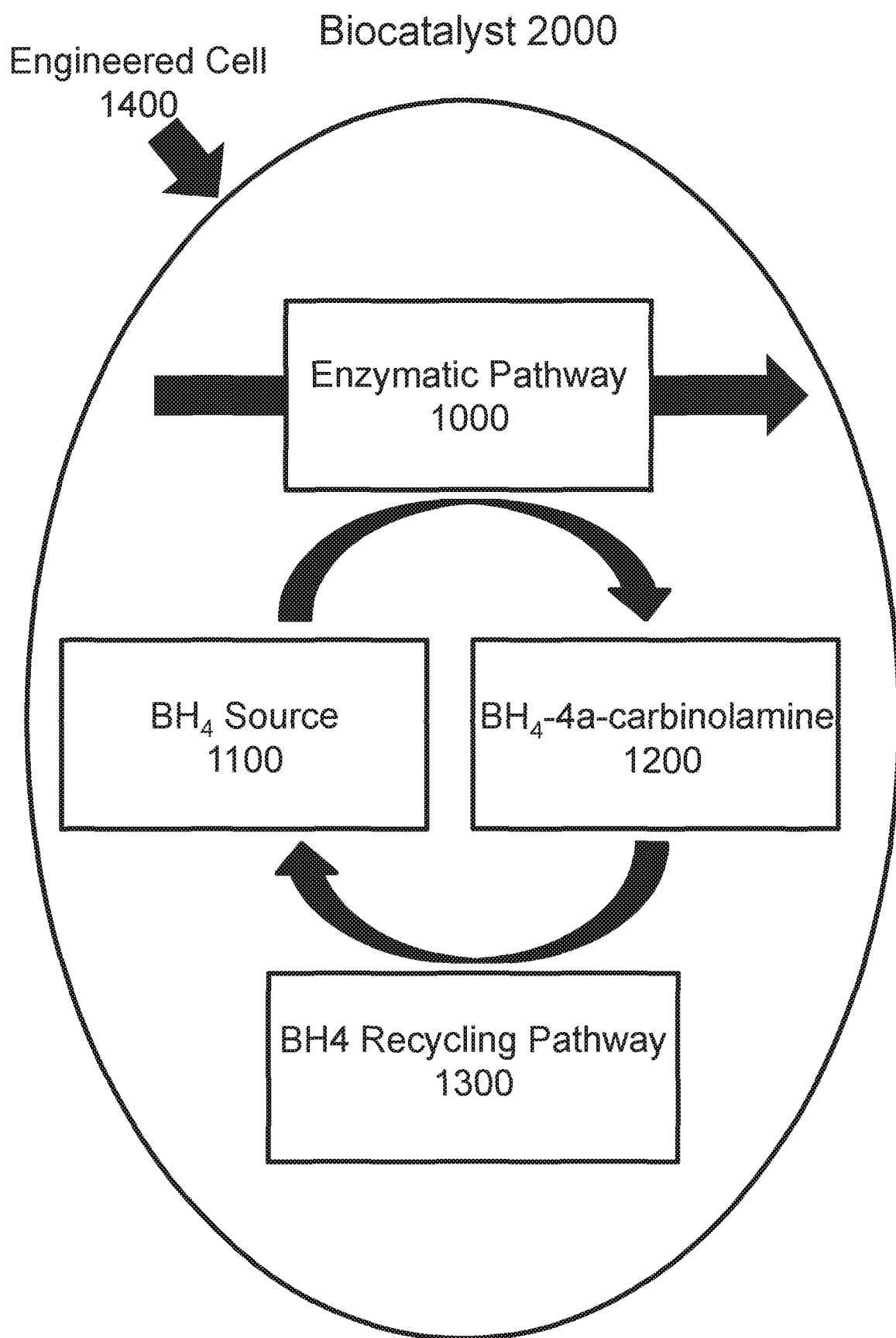


FIG. 4

BH4 Source 1100

1. BH₄ Biosynthesis

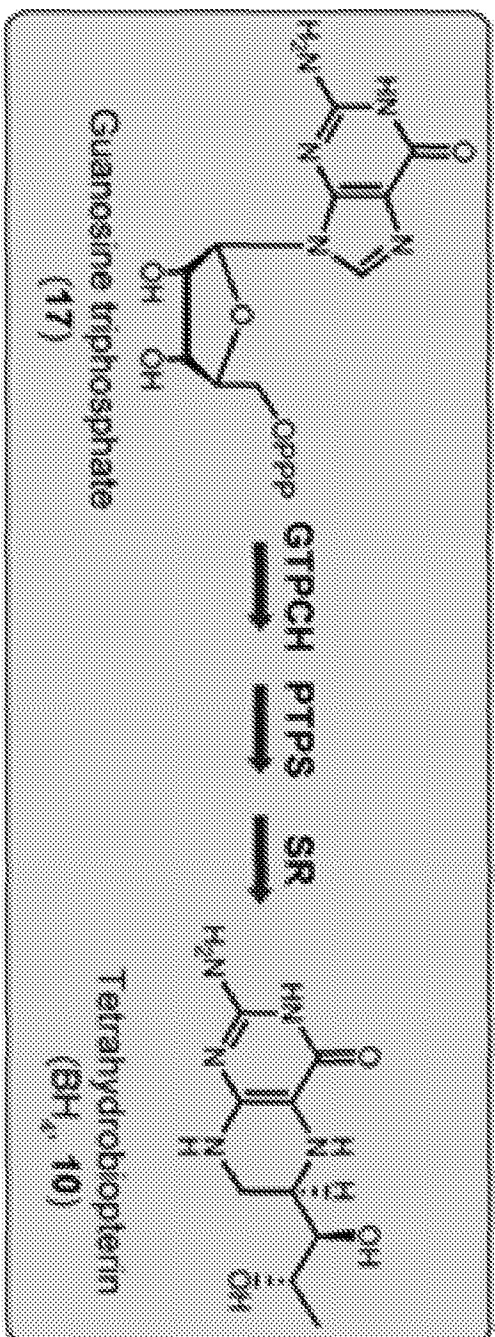


FIG. 5A

Enzymatic Pathway 1000

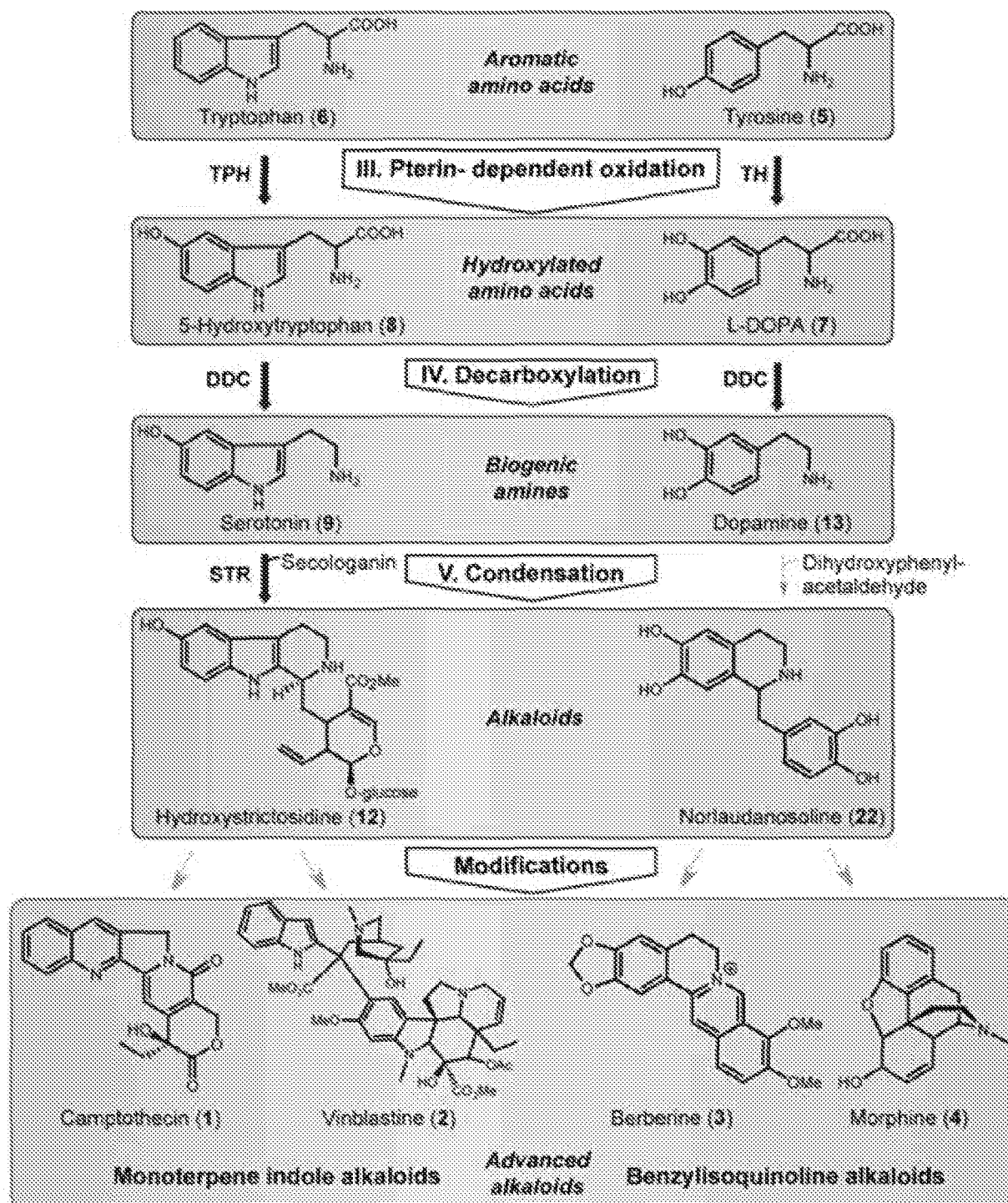


FIG. 5C

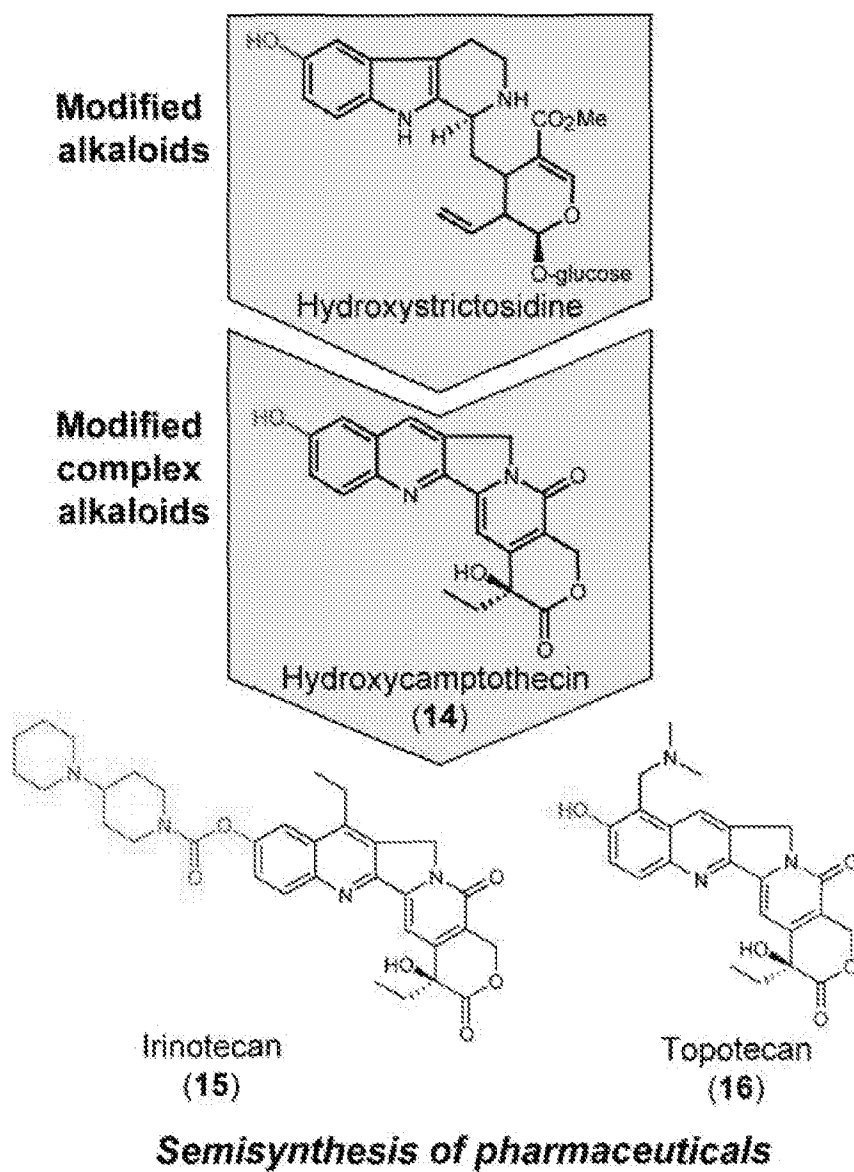


FIG. 6

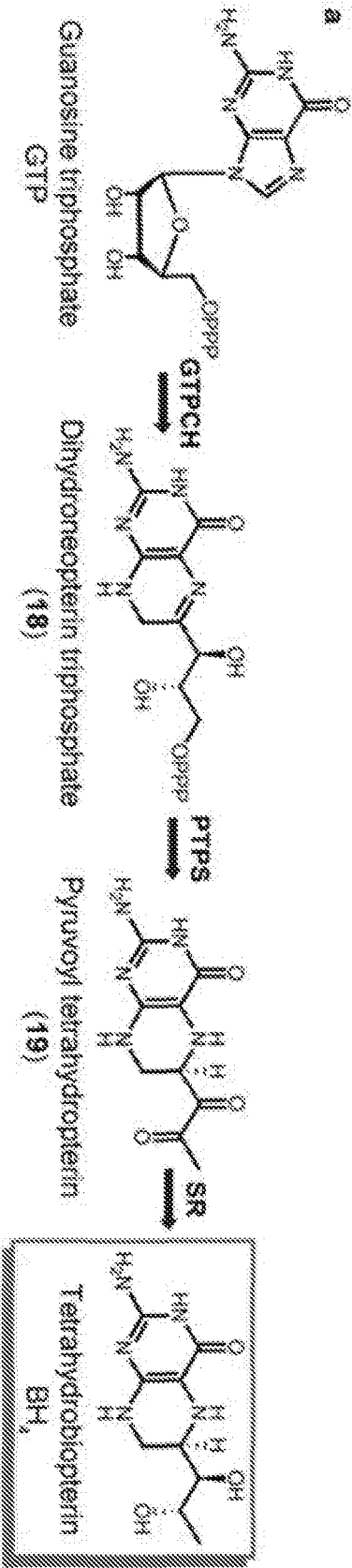


FIG. 7A

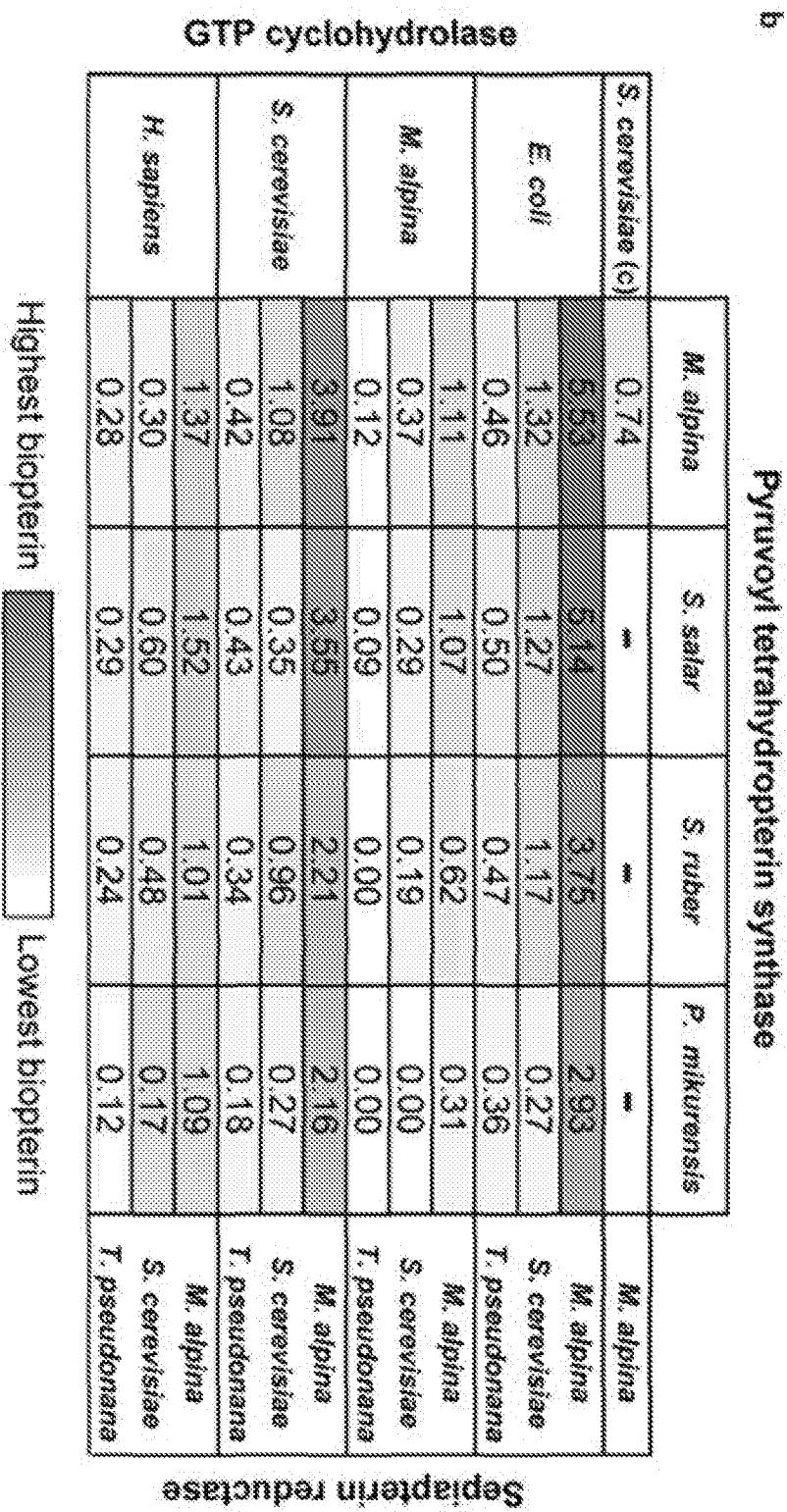


FIG. 7B

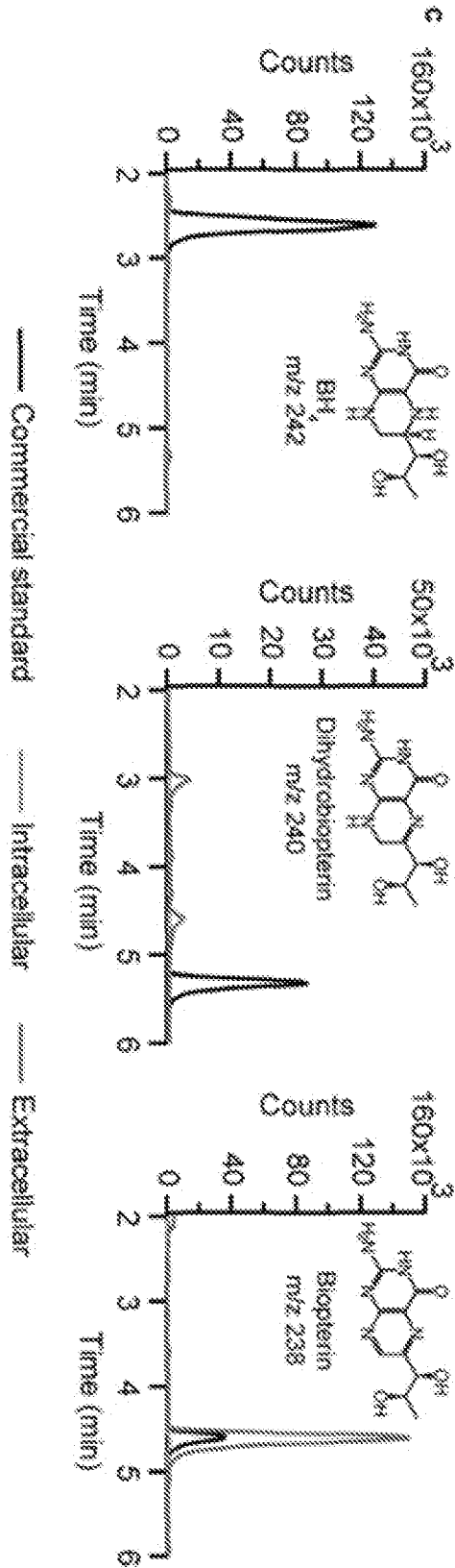


FIG. 7C

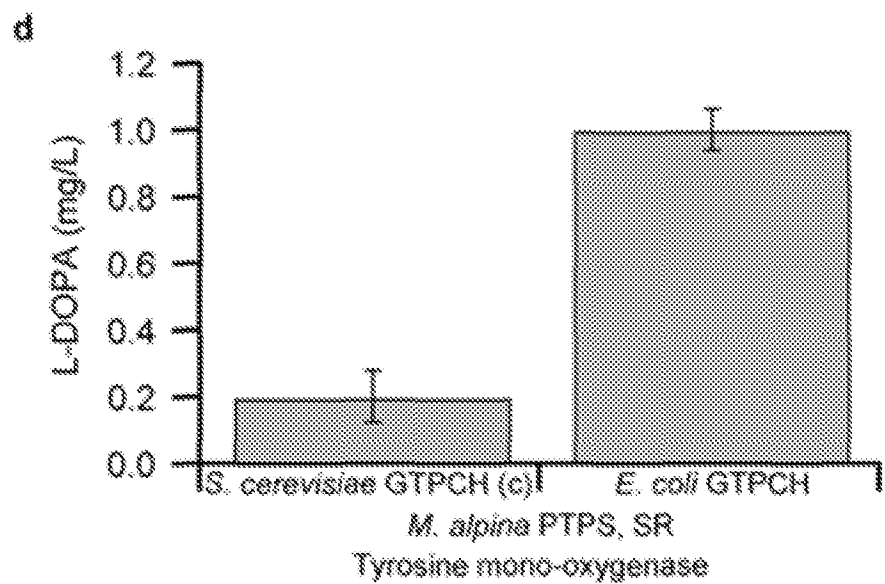


FIG. 7D

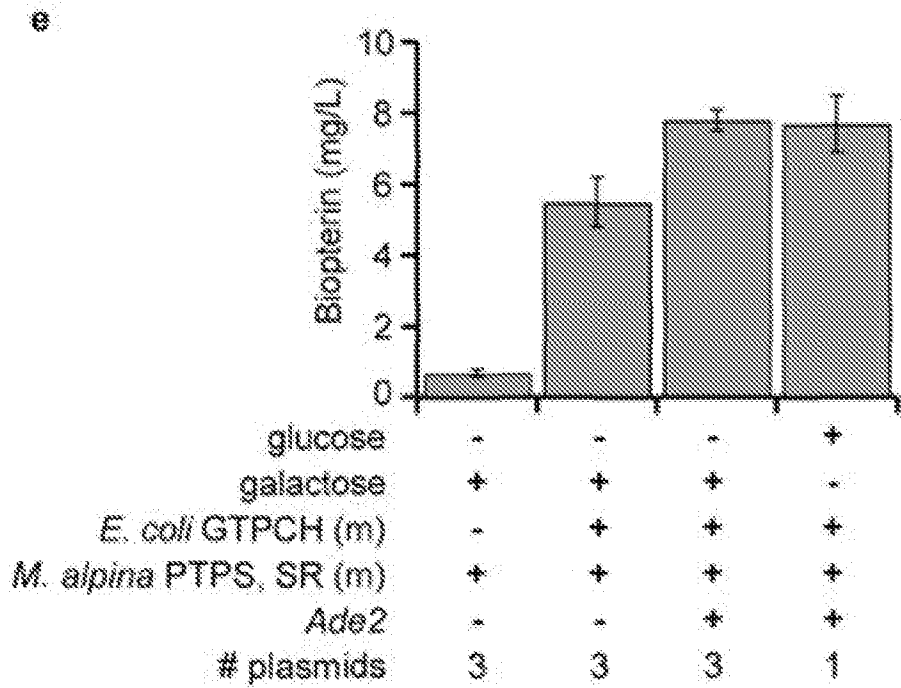


FIG. 7E

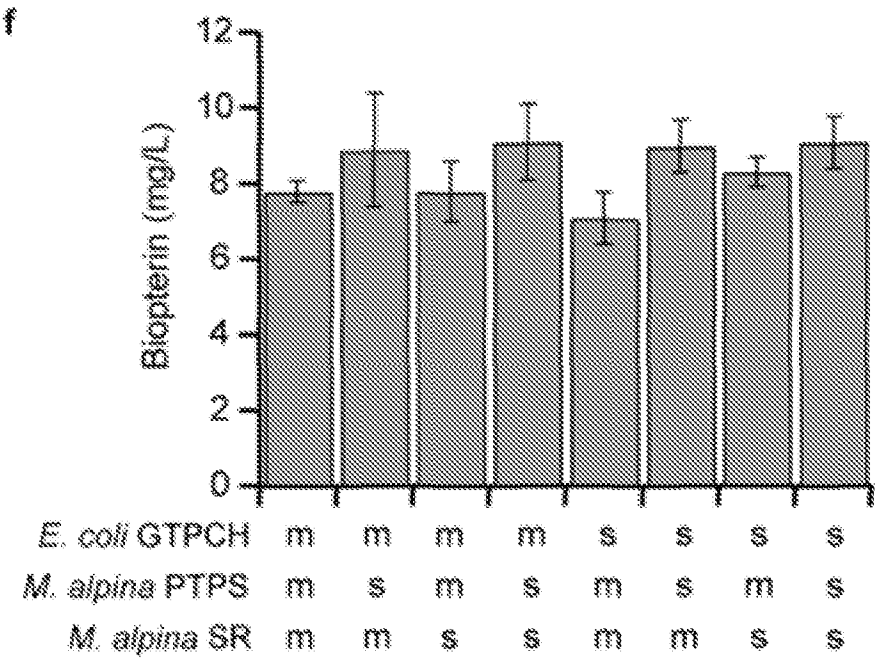


FIG. 7F

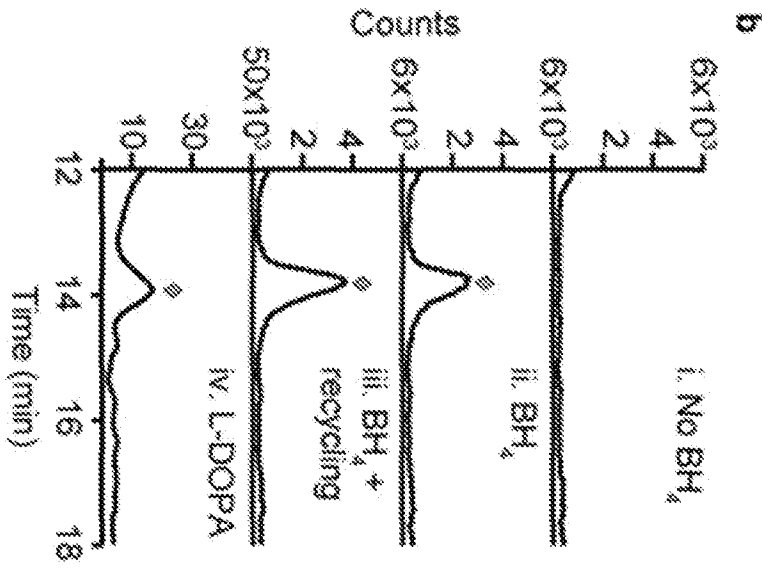
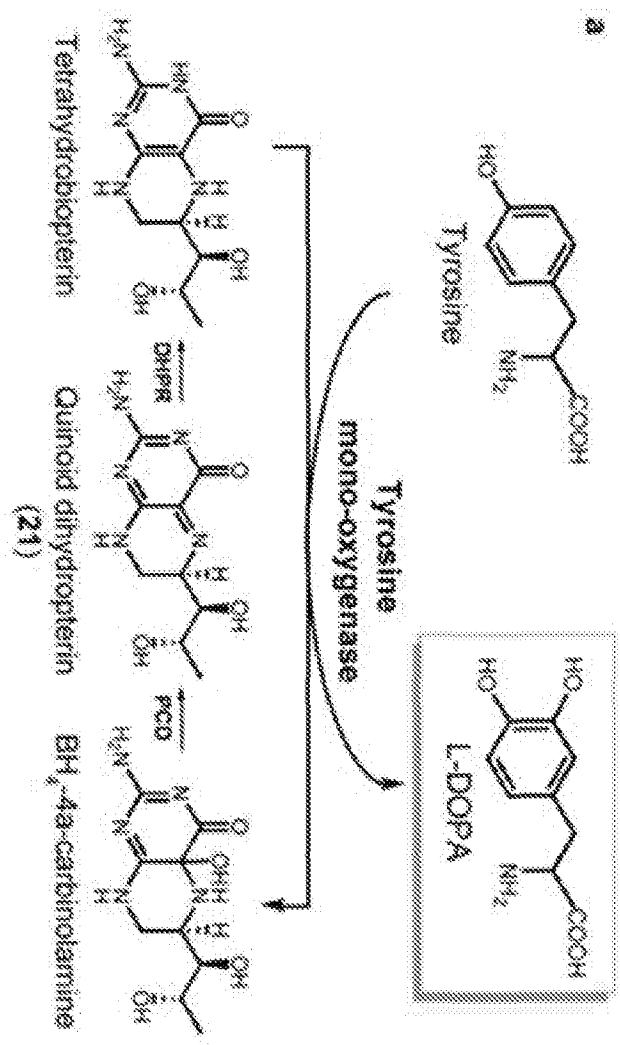


FIG. 8A

FIG. 8B

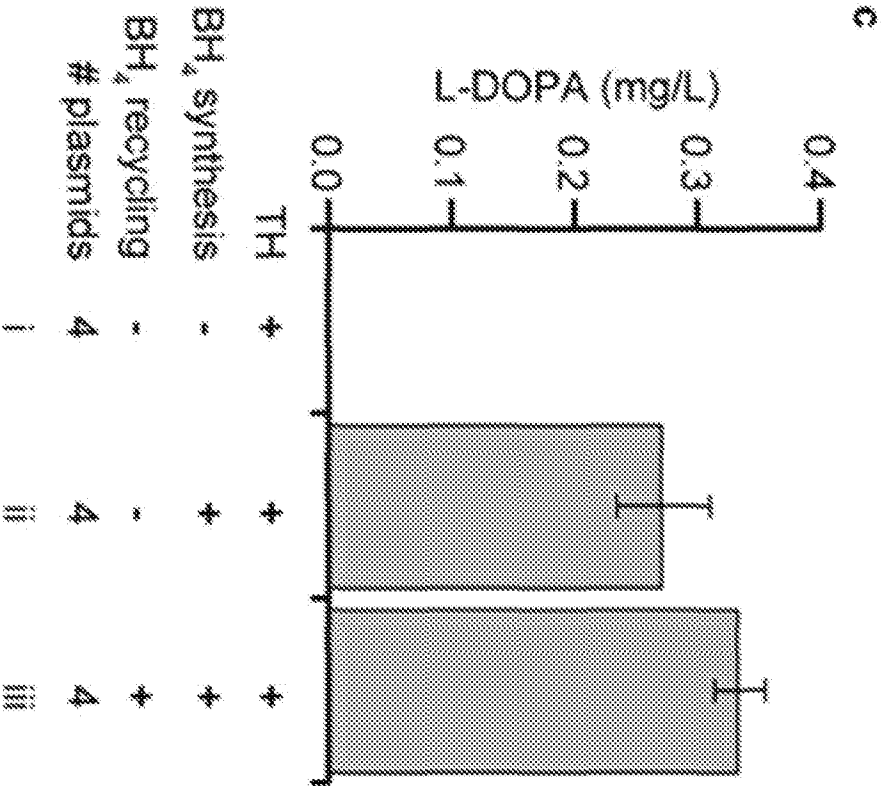


FIG. 8C

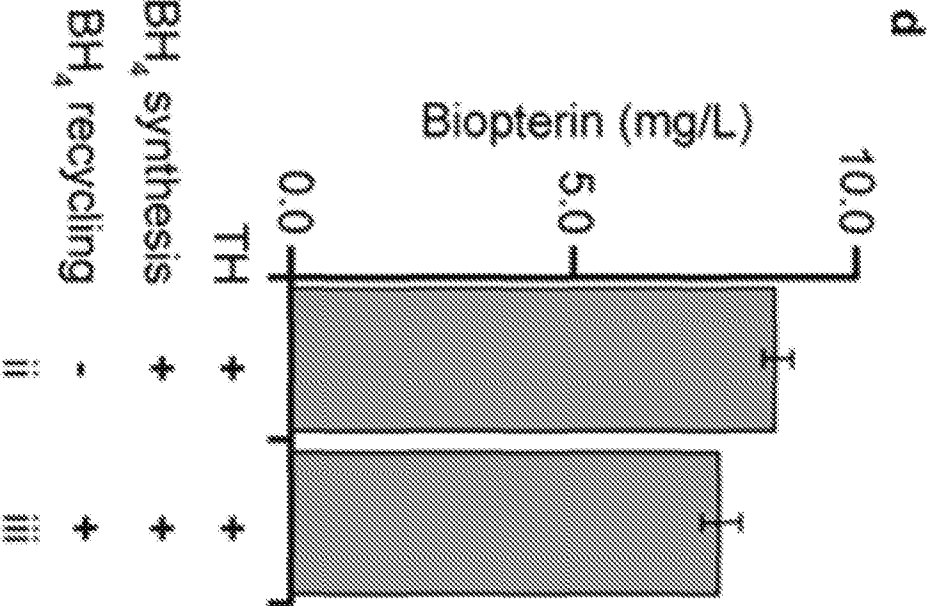


FIG. 8D

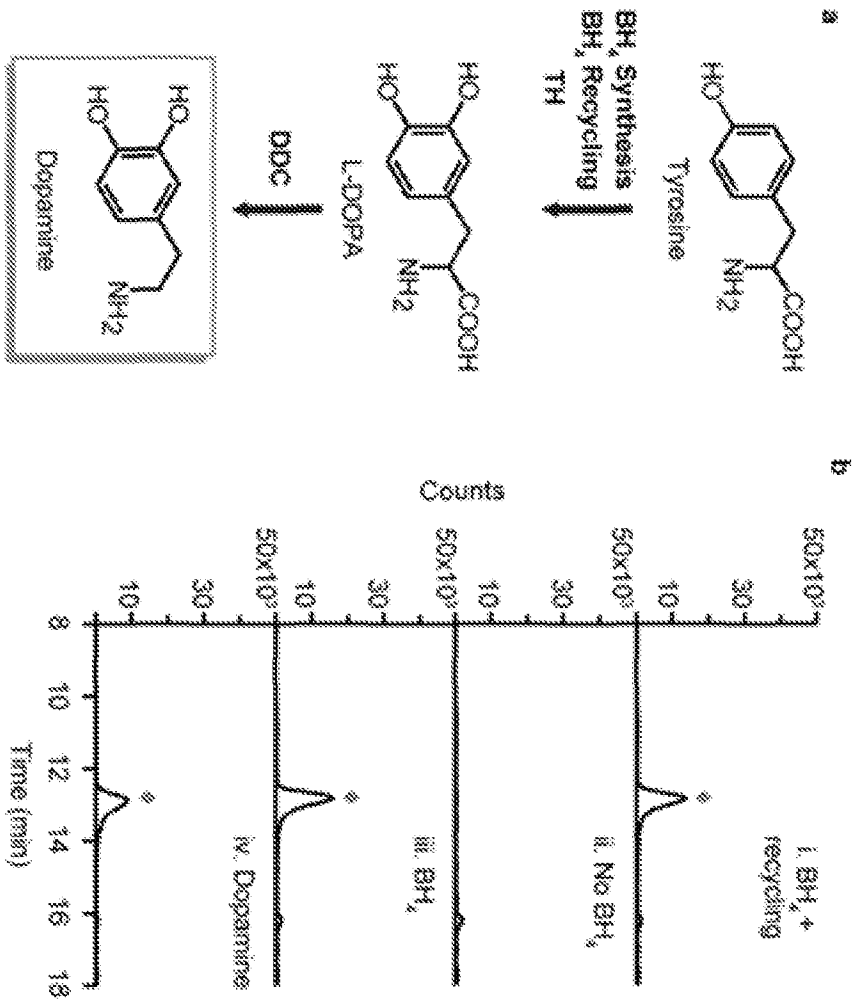


FIG. 9A

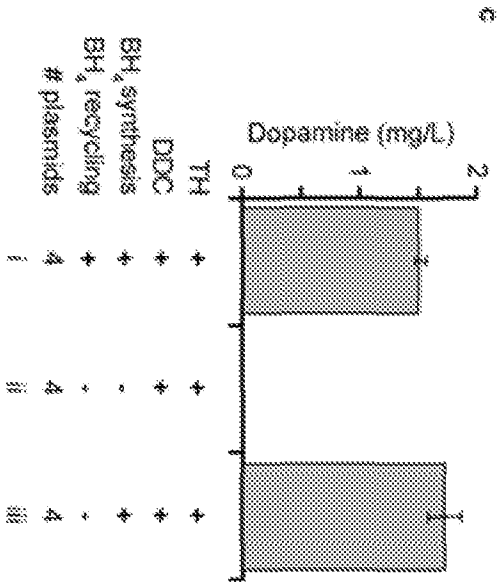


FIG. 9B

FIG. 9C

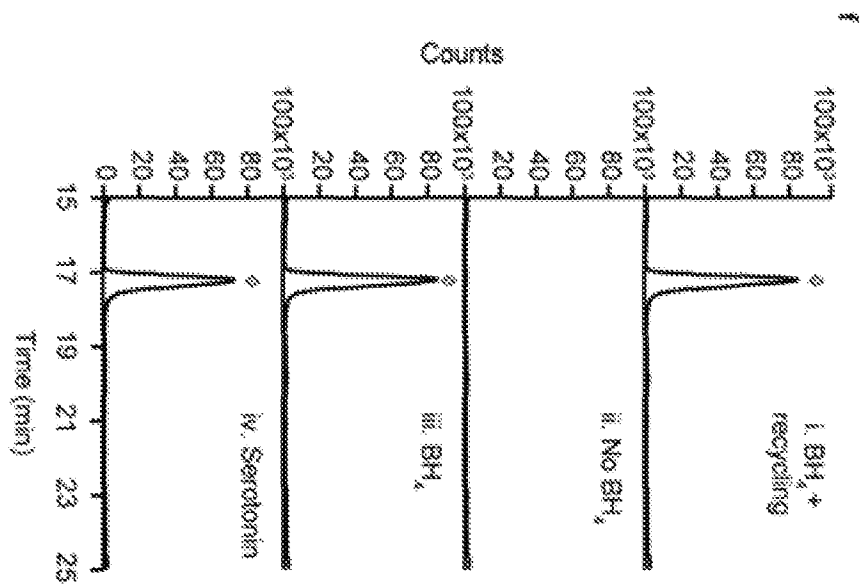
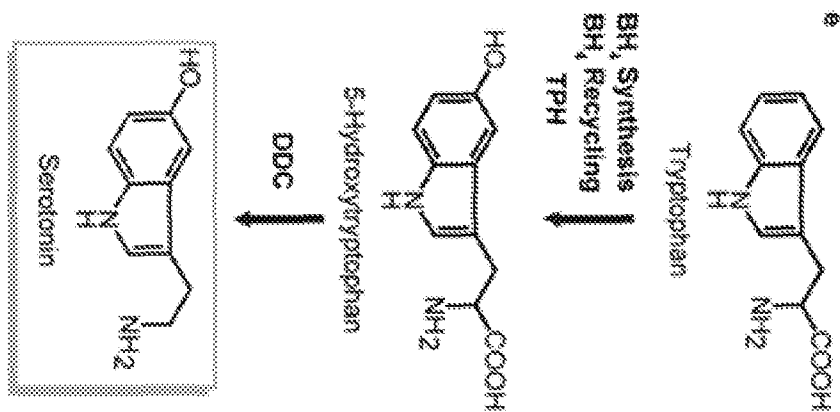
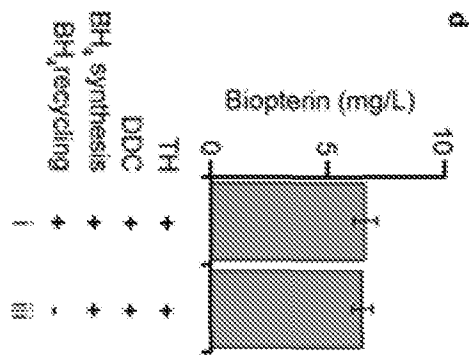


FIG. 9D

FIG. 9E

FIG. 9F

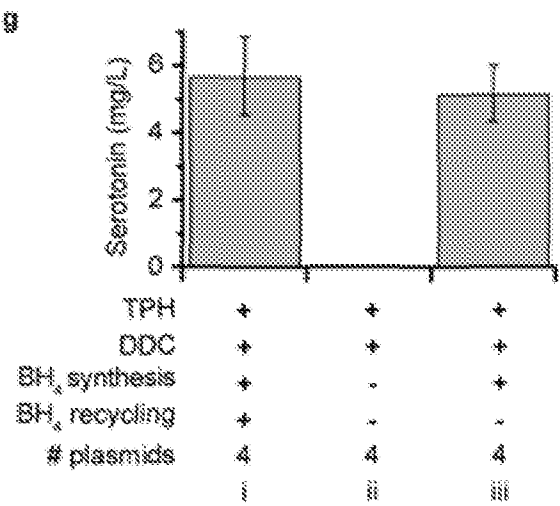


FIG. 9G

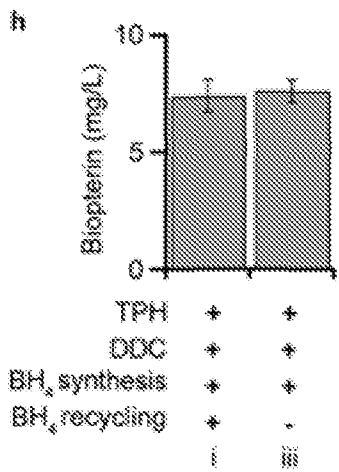


FIG. 9H

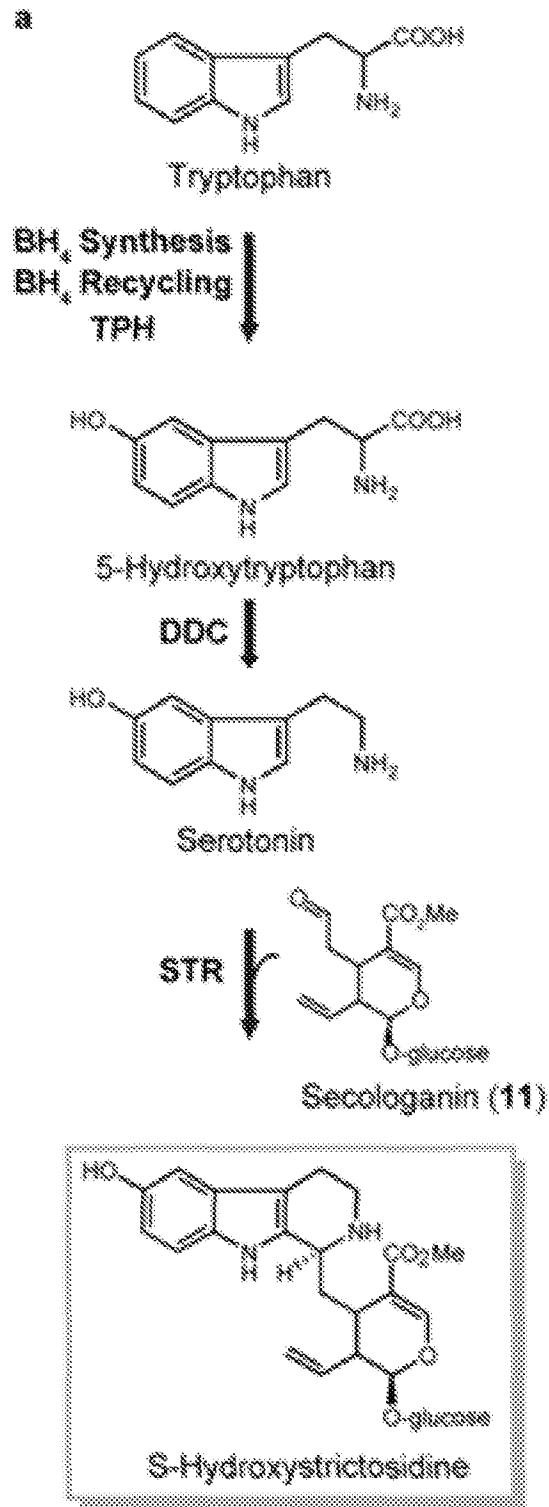


FIG. 10A

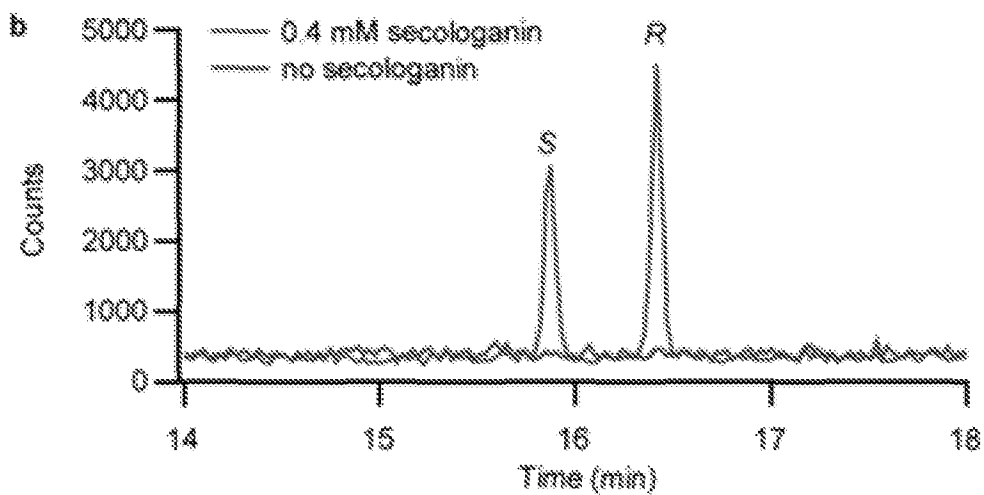


FIG. 10B

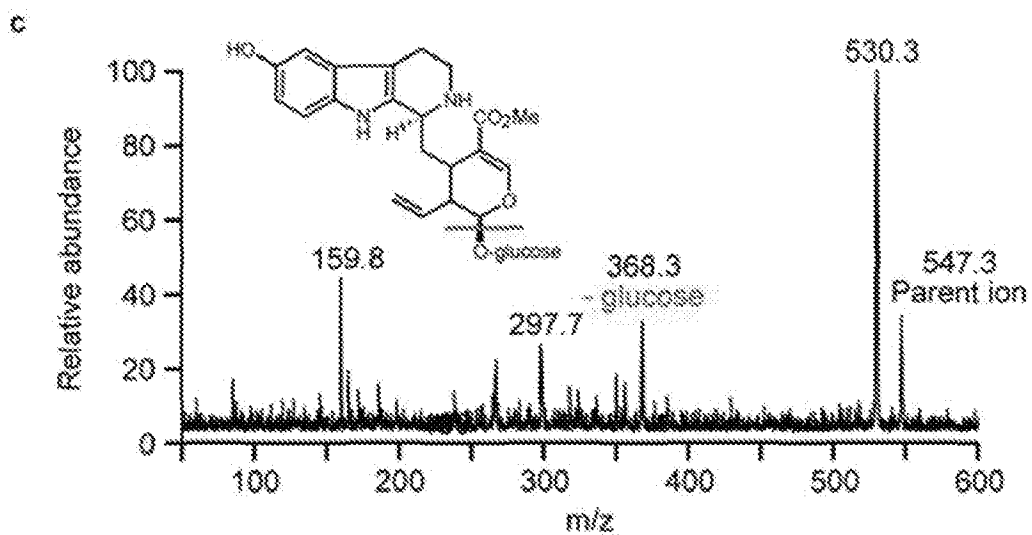


FIG. 10C

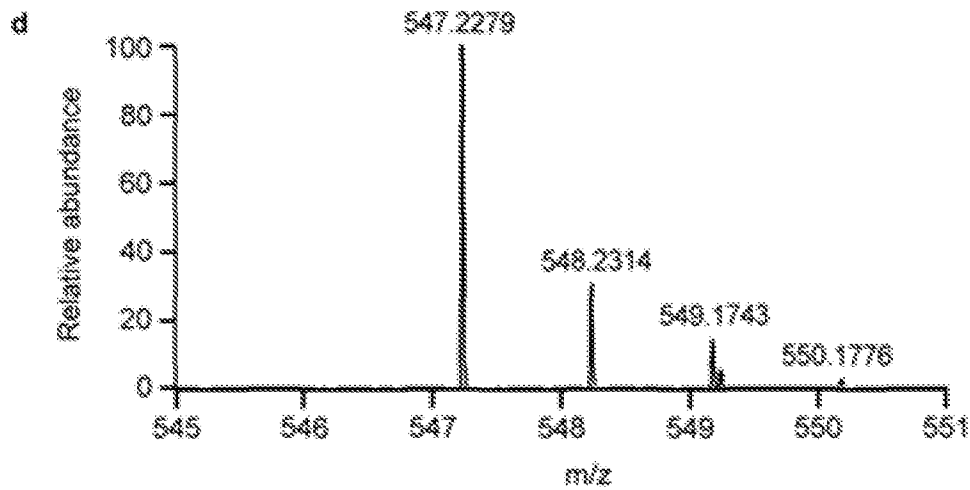


FIG. 10D

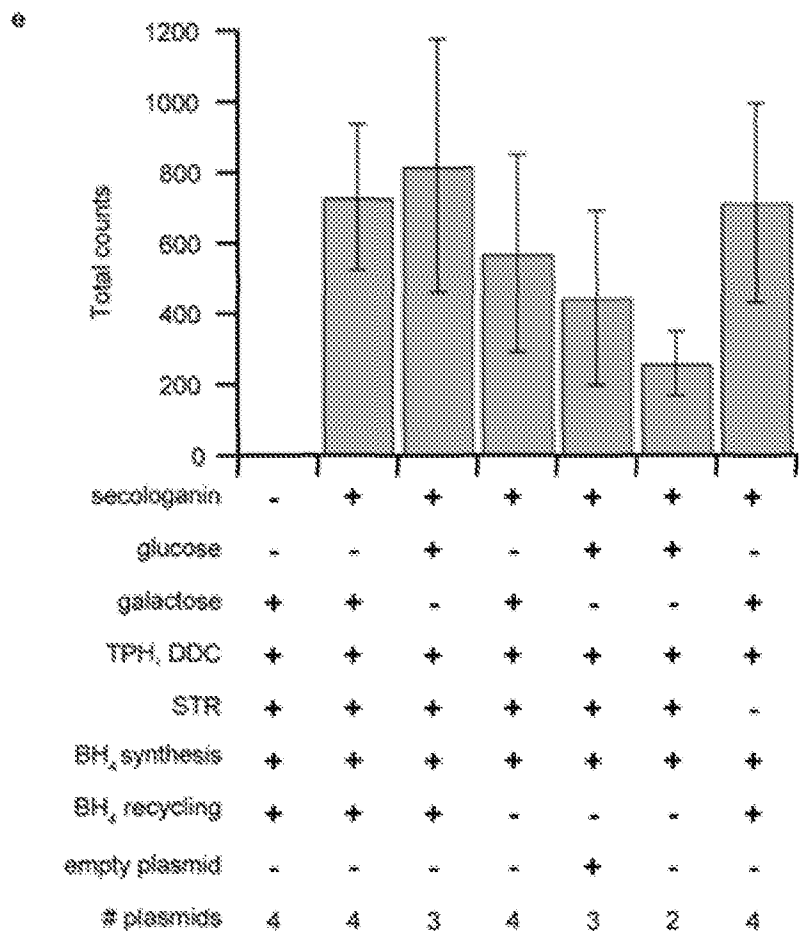


FIG. 10E

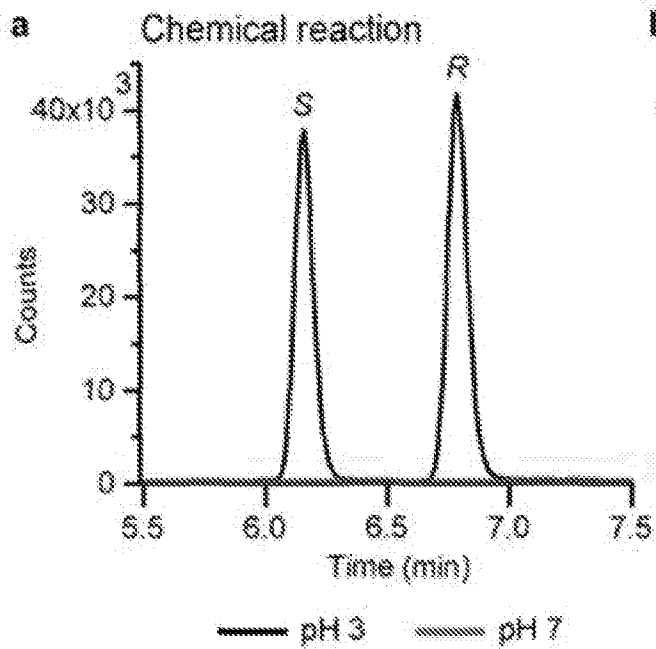


FIG. 11A

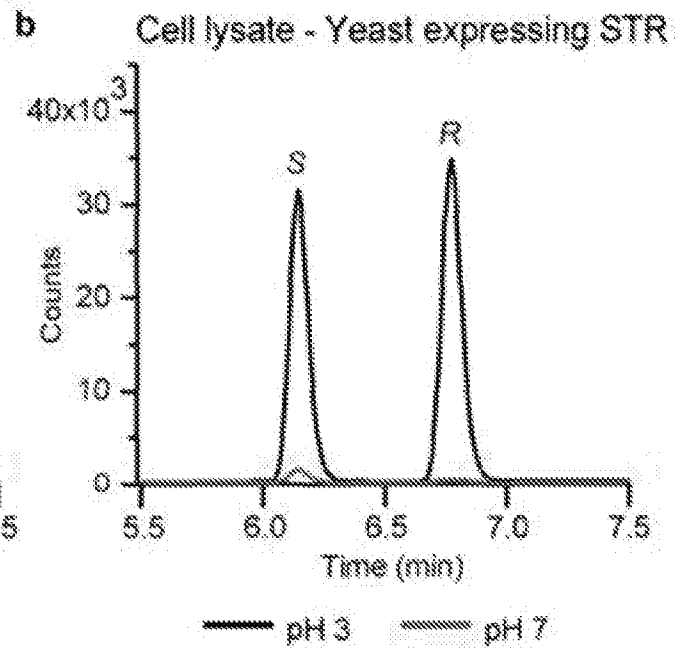


FIG. 11B

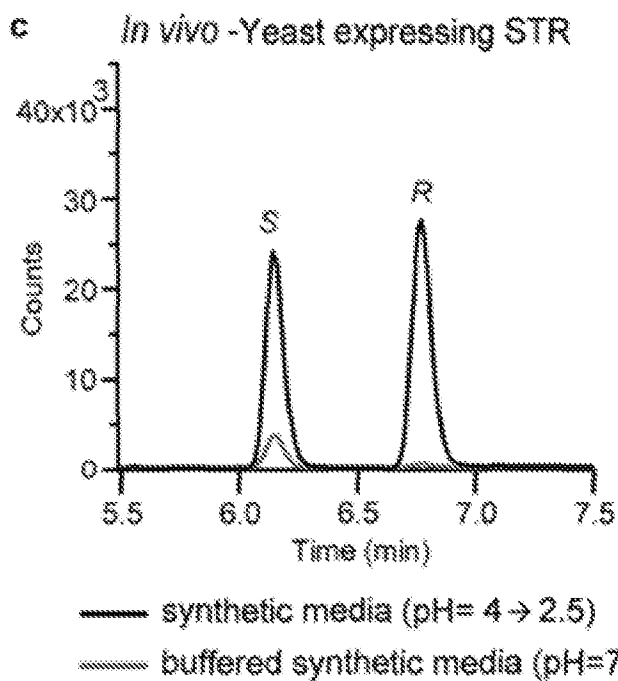


FIG. 11C

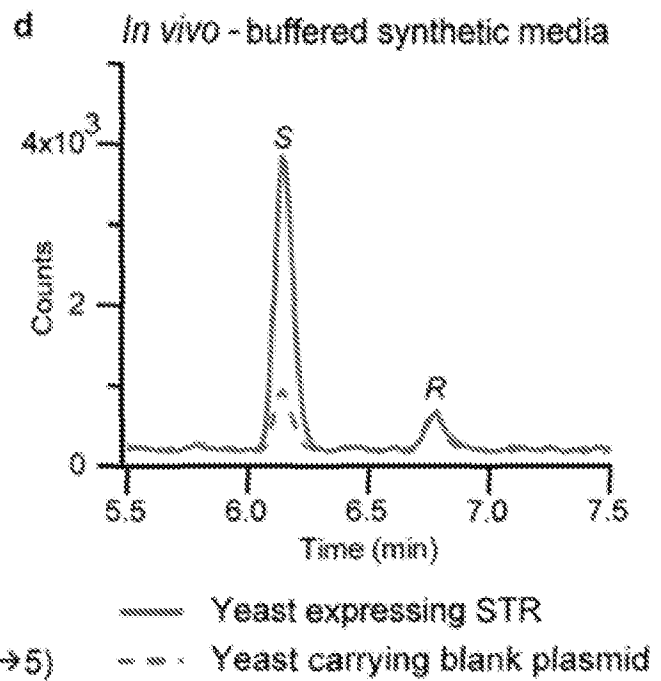


FIG. 11D

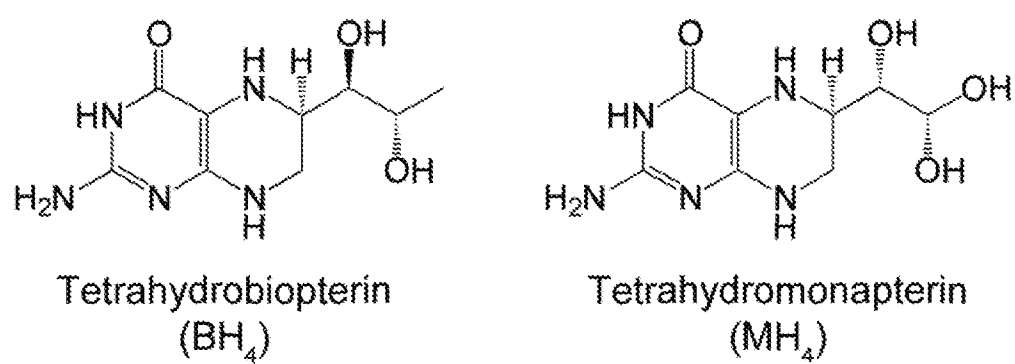
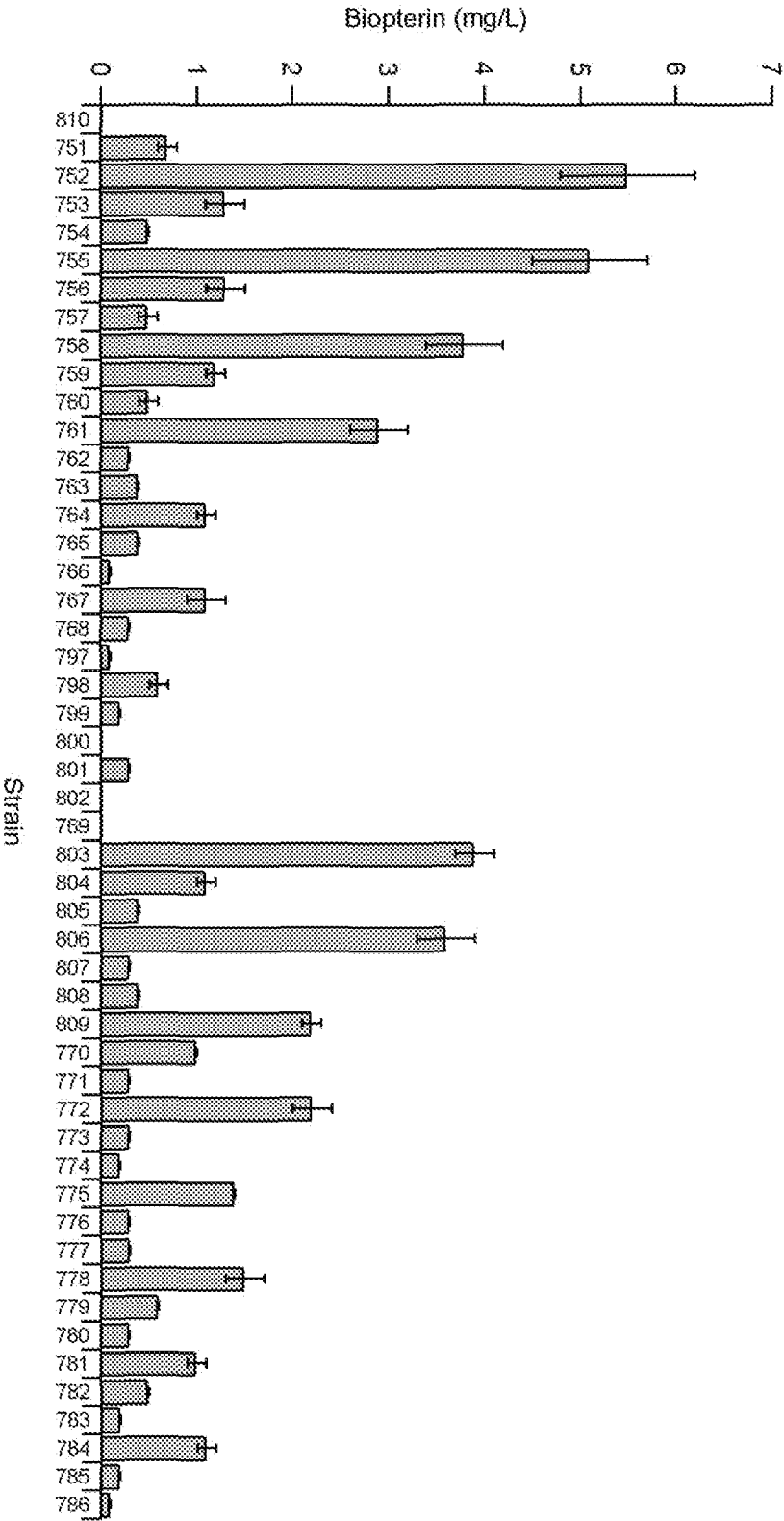


FIG. 12

FIG. 13A



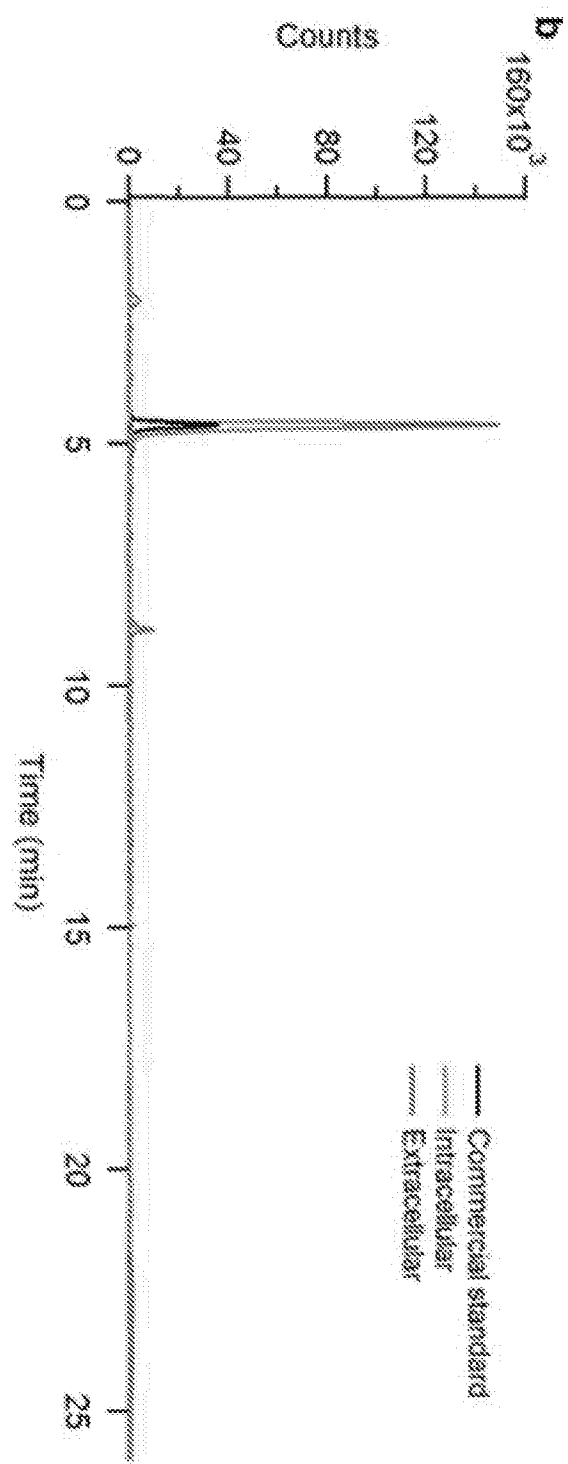


FIG. 13B

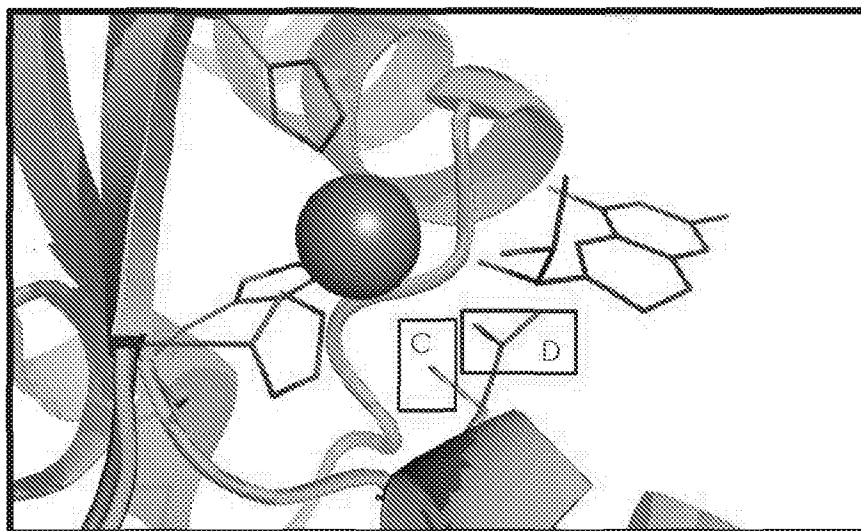


FIG. 14

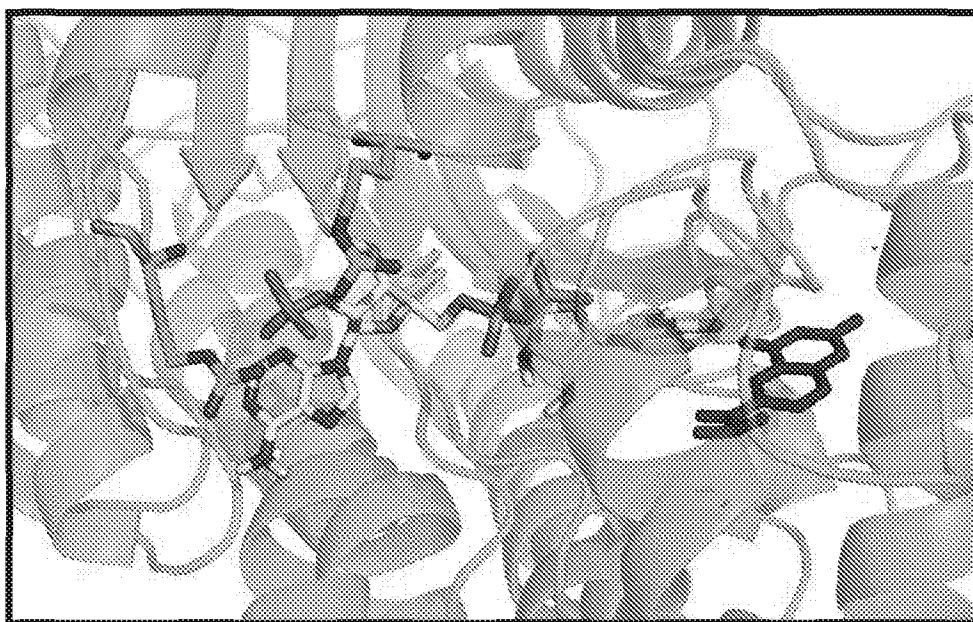


FIG. 15

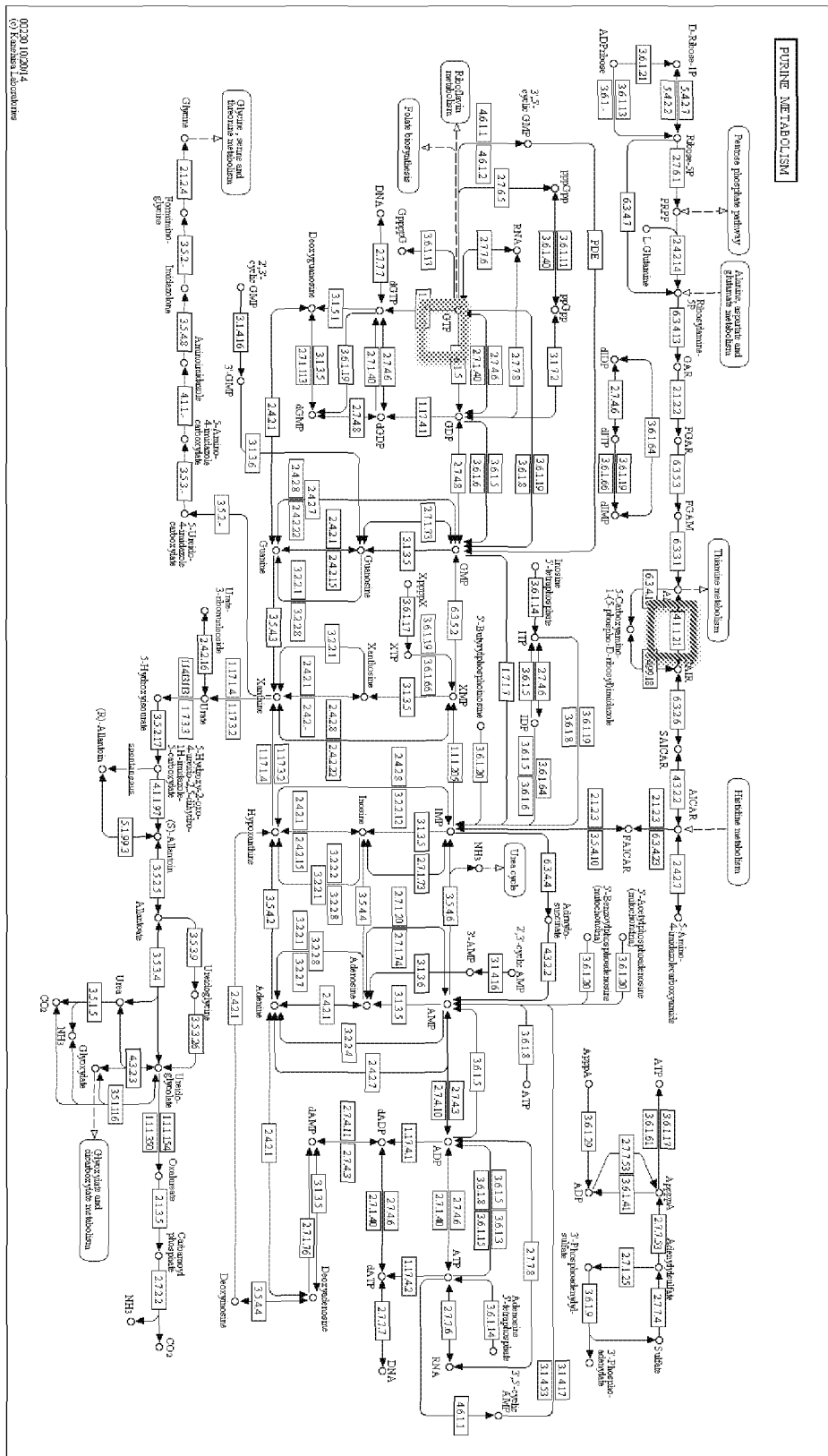


FIG. 16A

Purine metabolism

Pyrimidine metabolism

Nucleotide metabolism

Regulation

Enzymes and Cofactors

Pathway Details:

- Purine Biosynthesis:** PRPP → IMP (Enzyme: PNPase, Cofactor: H₂O) → AMP (Enzyme: PDE, Cofactor: ATP) → GMP (Enzyme: PNPase, Cofactor: H₂O) → UMP (Enzyme: PNPase, Cofactor: H₂O).
- Pyrimidine Biosynthesis:** UTP → CTP (Enzyme: PNPase, Cofactor: H₂O) → GTP (Enzyme: PNPase, Cofactor: H₂O) → ATP (Enzyme: PNPase, Cofactor: H₂O).
- Nucleotide Metabolism:** ATP → ADP (Enzyme: PNPase, Cofactor: H₂O) → AMP (Enzyme: PNPase, Cofactor: H₂O).
- Regulation:** The pathway is regulated by various enzymes and cofactors, including PDE, PNPase, and various nucleotides.

Fig. 16B

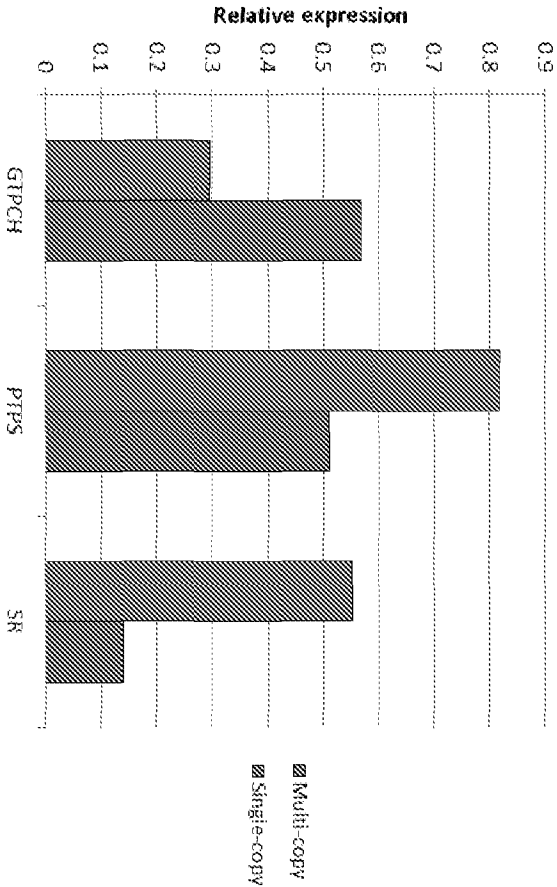


FIG. 17

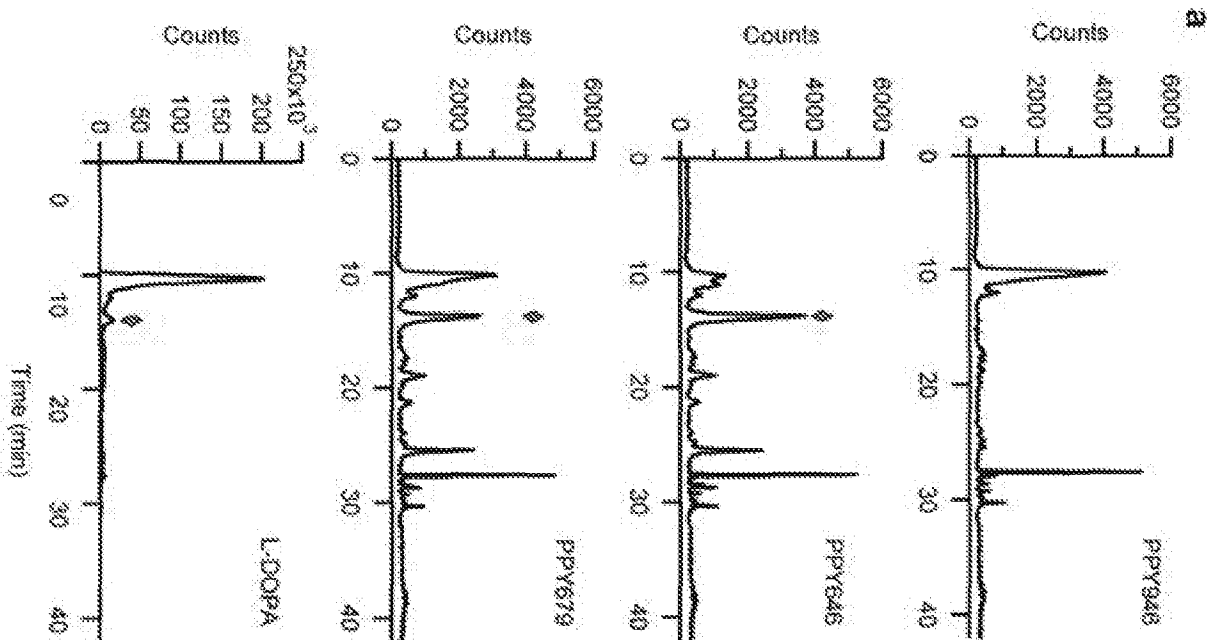


FIG. 18A

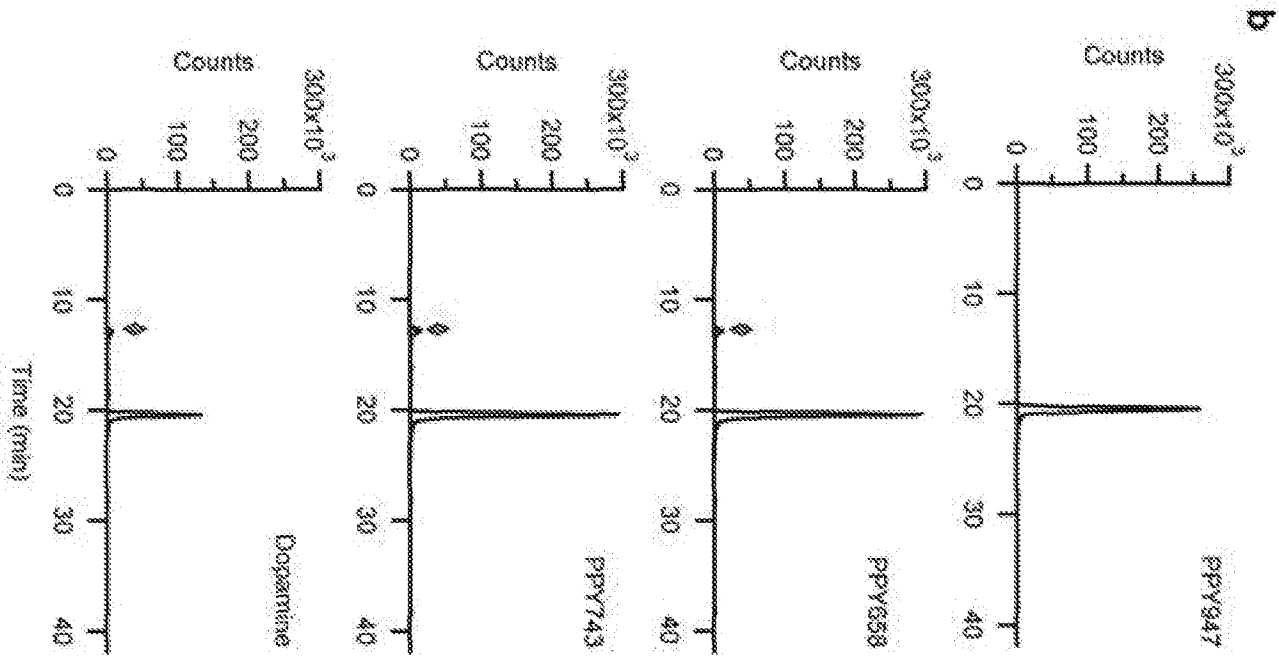


FIG. 18B

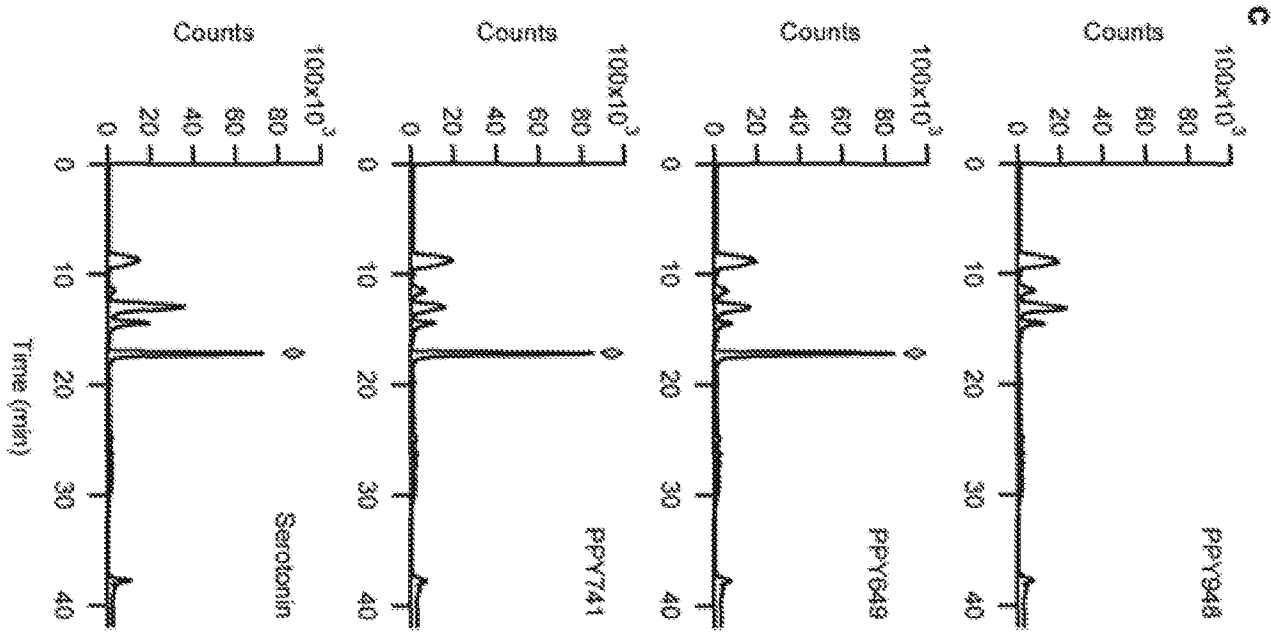


FIG. 18C

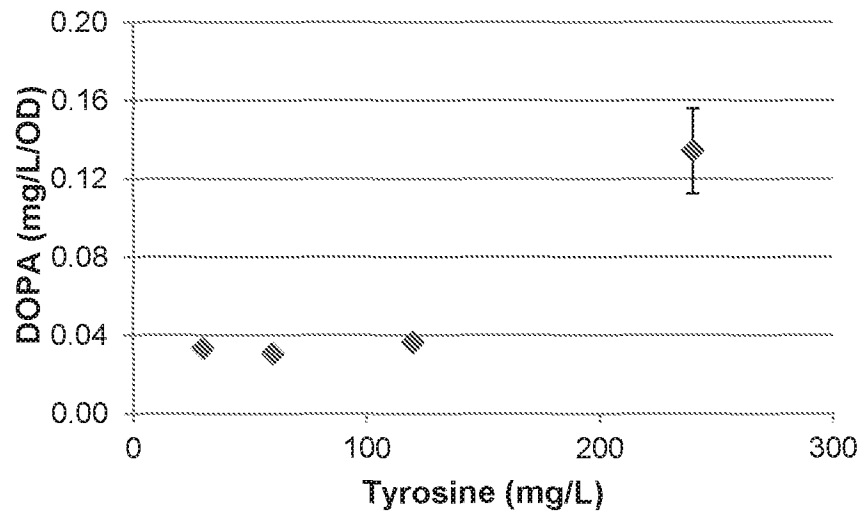


FIG. 19

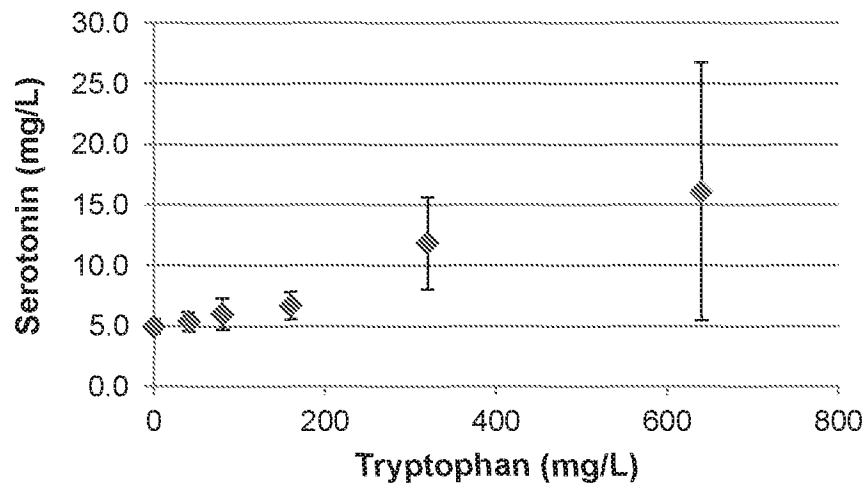


FIG. 20

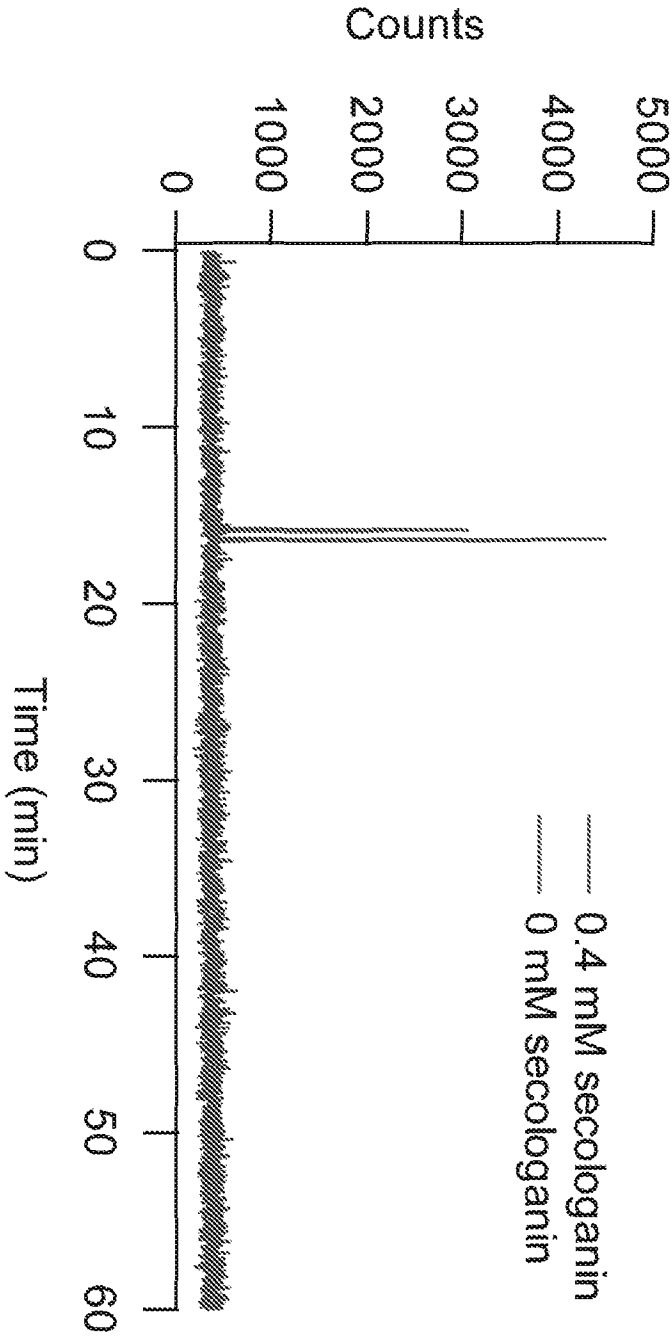


FIG. 21

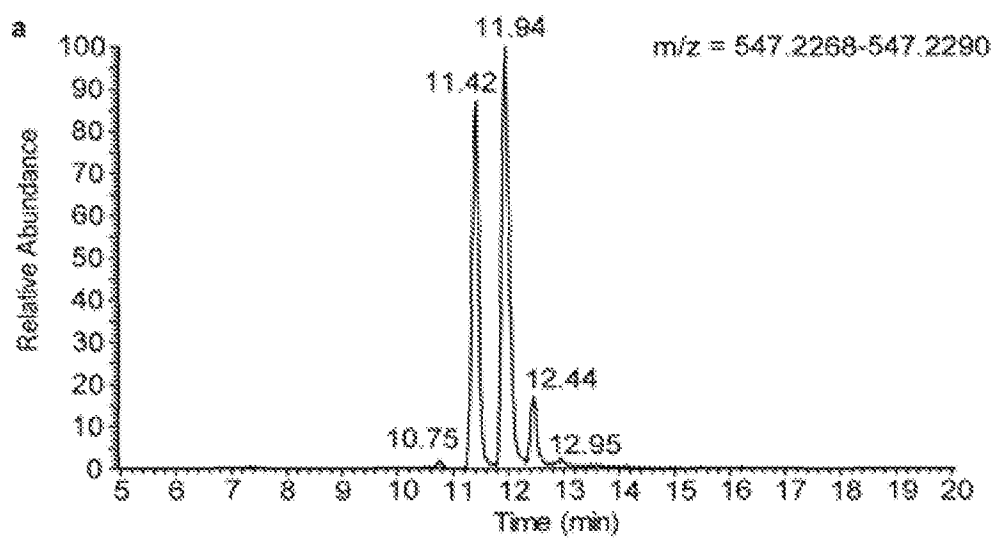


FIG. 22A

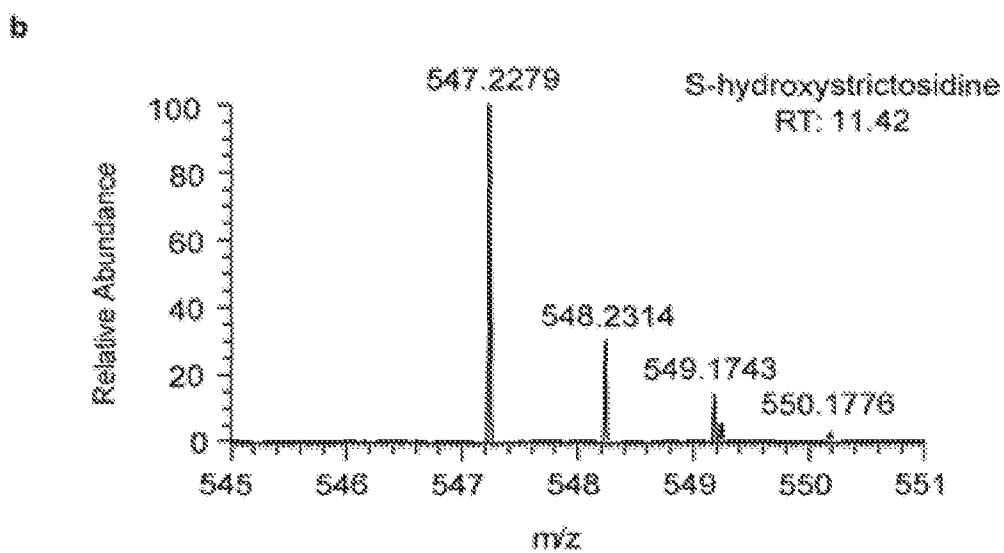


FIG. 22B

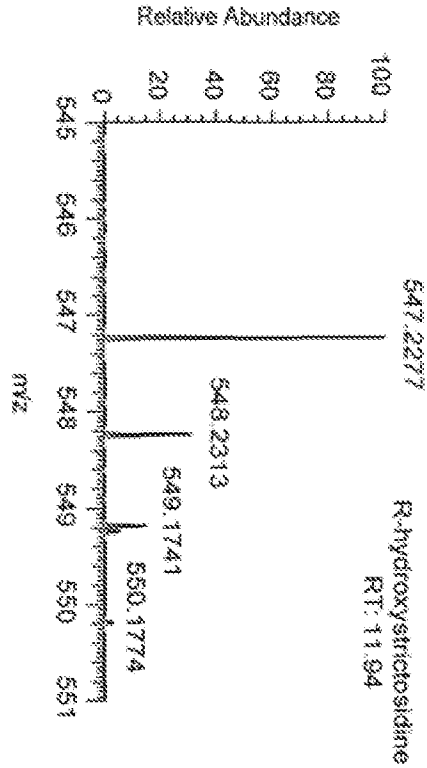
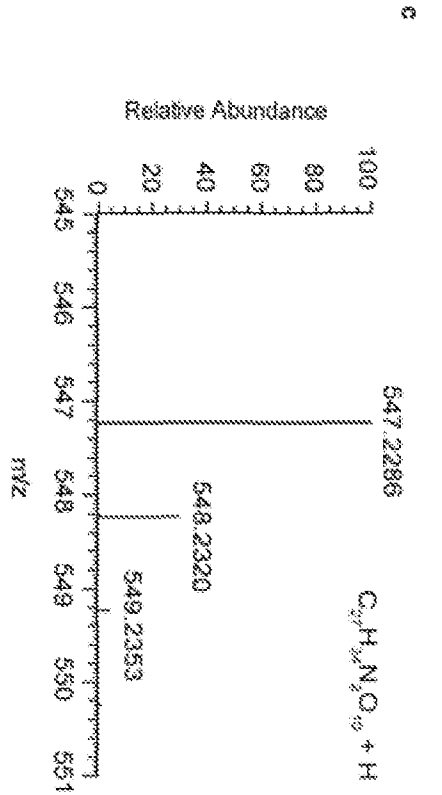


FIG. 22C

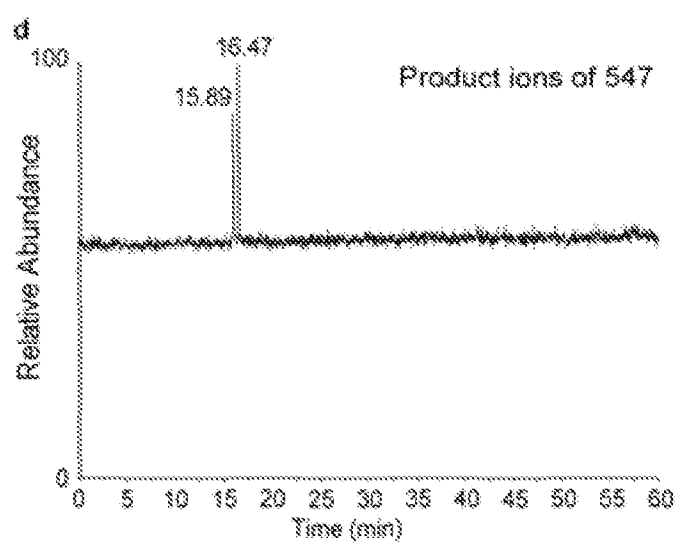


FIG. 22D

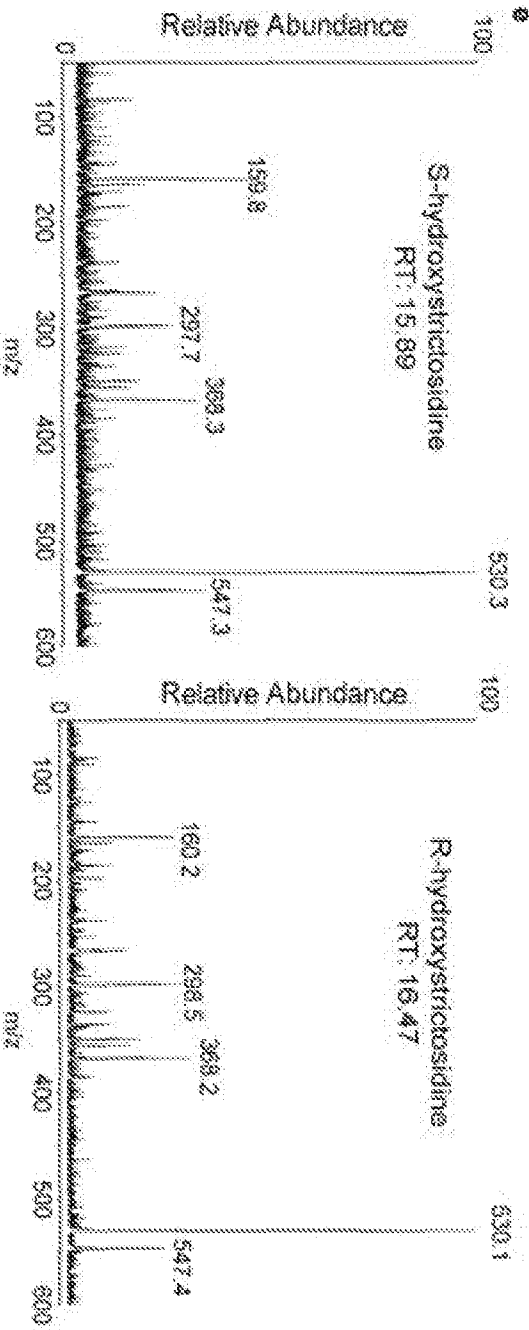


FIG. 22E

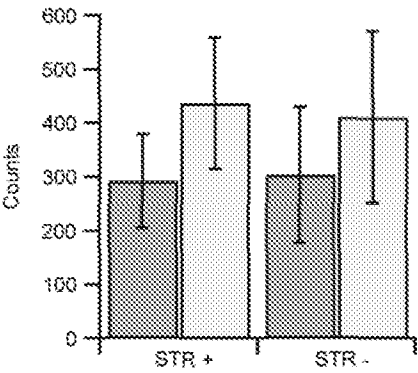


FIG. 23

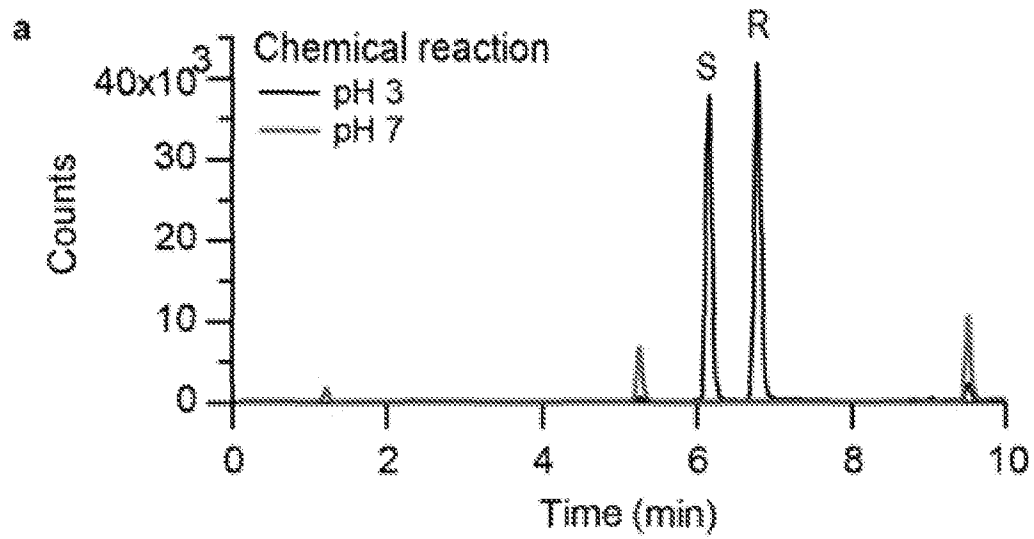


FIG. 24A

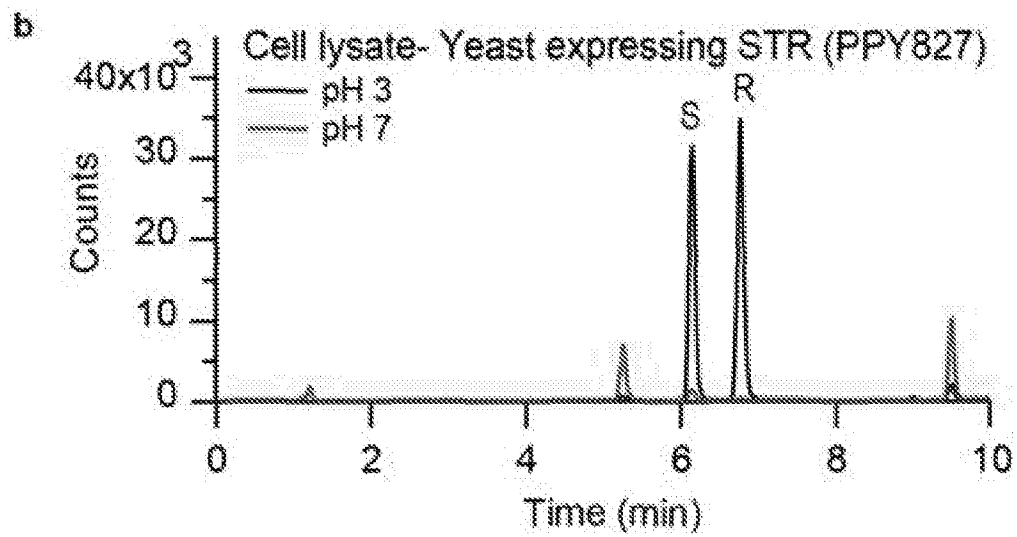


FIG. 24B

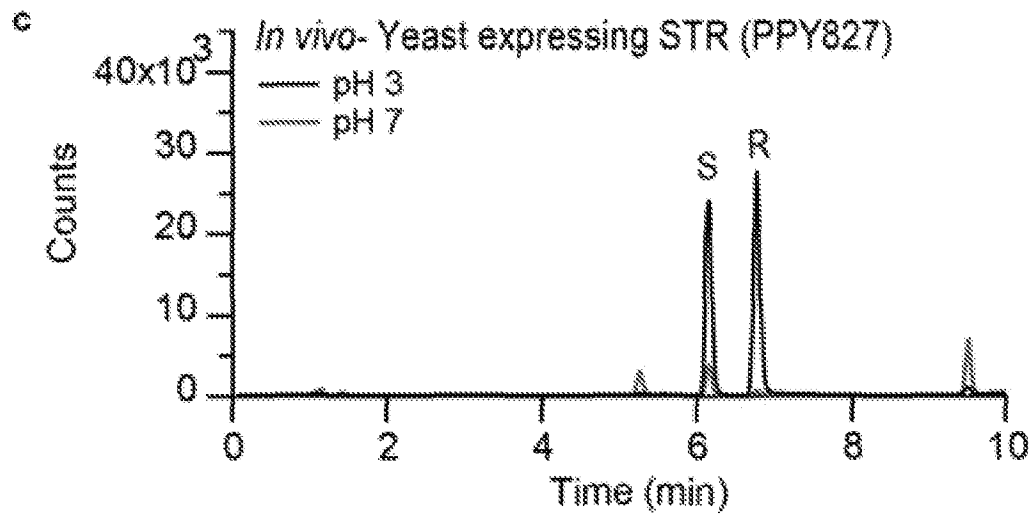


FIG. 24C

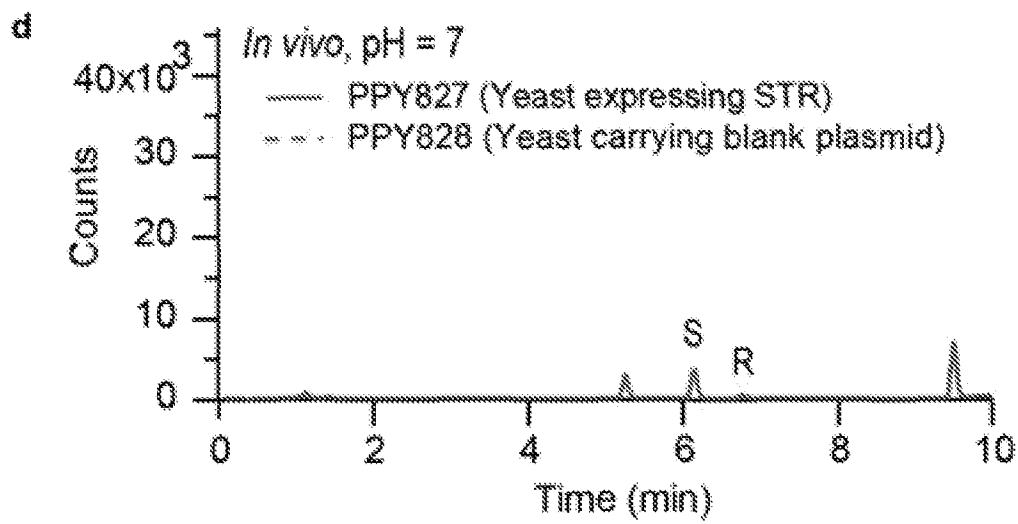


FIG. 24D

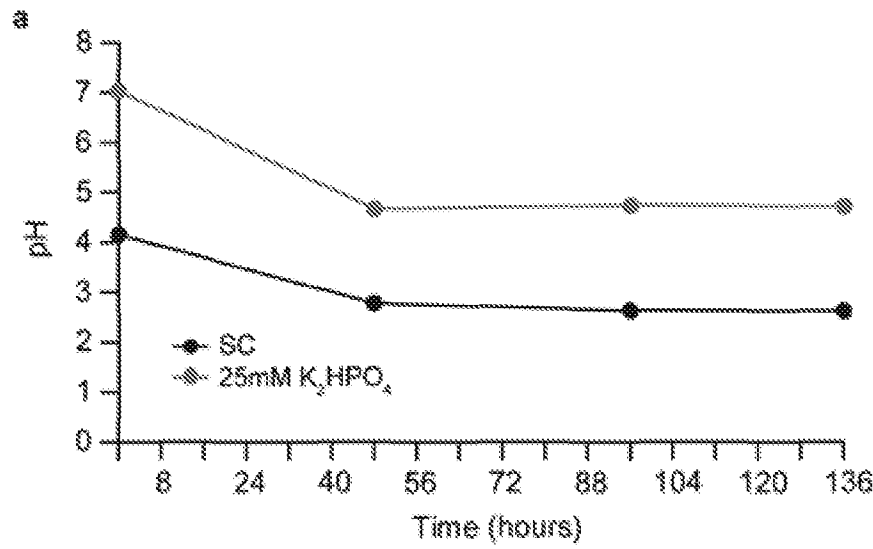


FIG. 25A

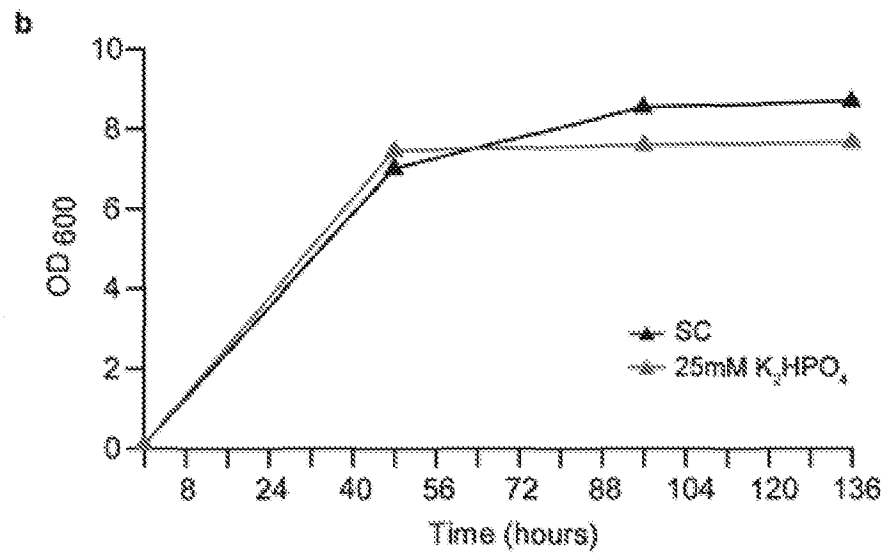


FIG. 25B

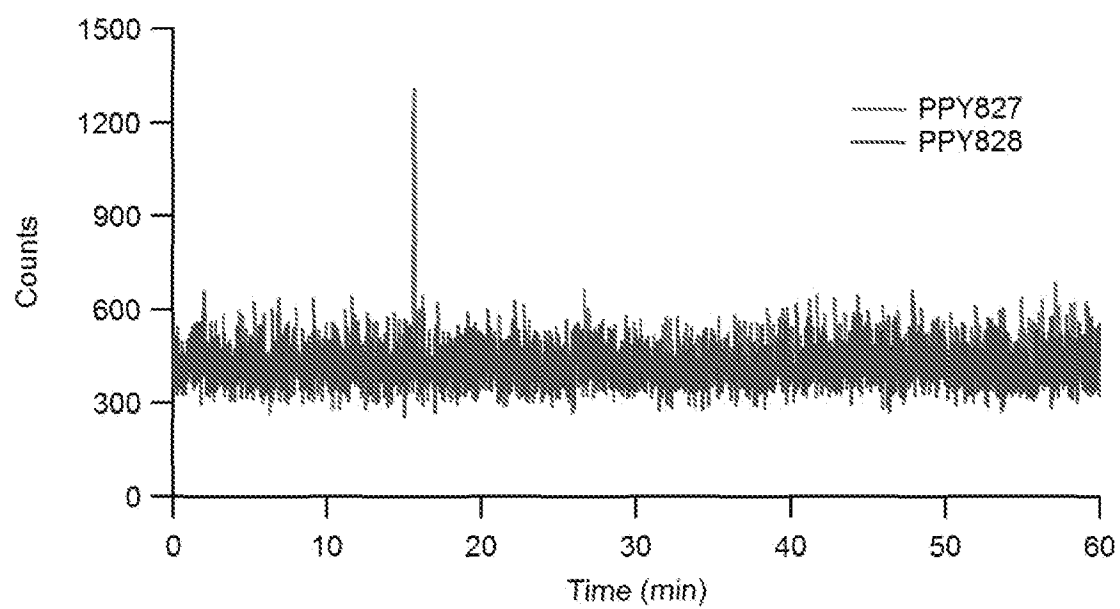


FIG. 26

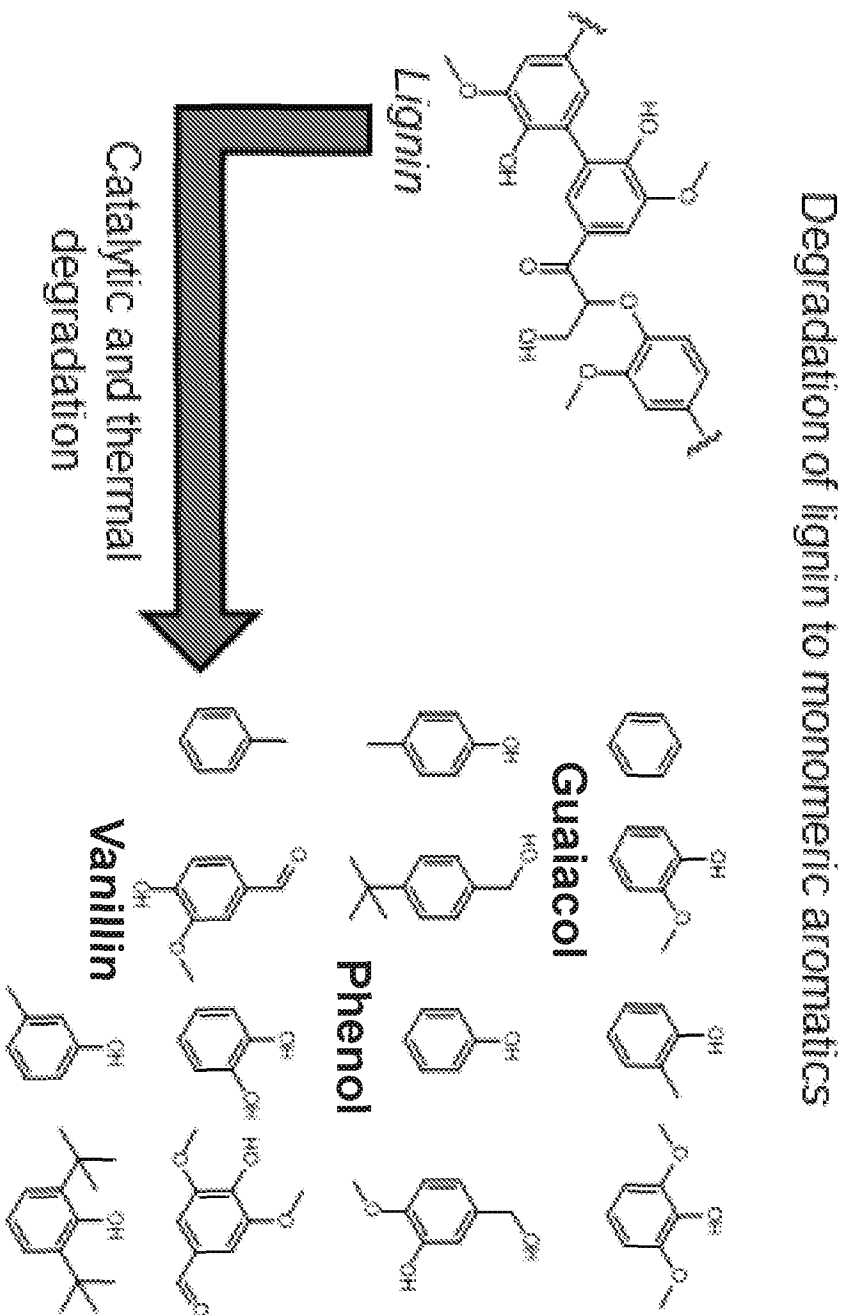


FIG. 27

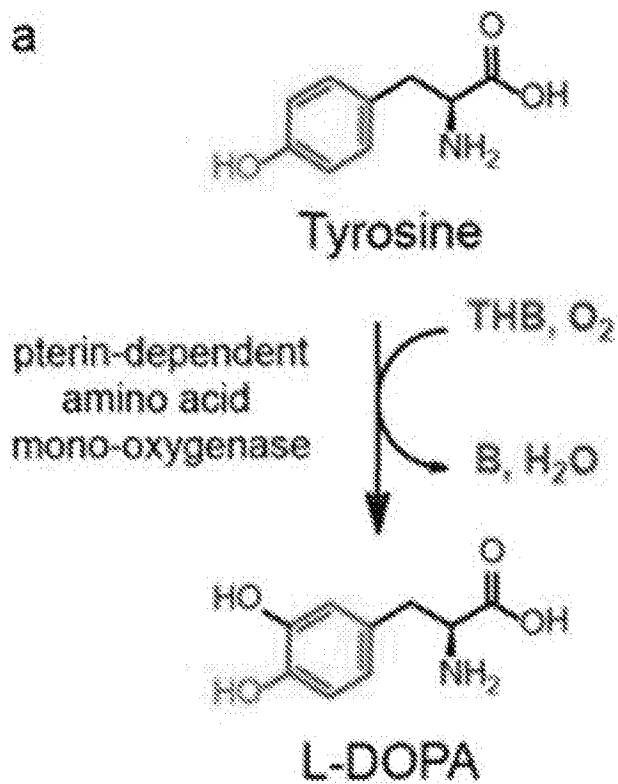
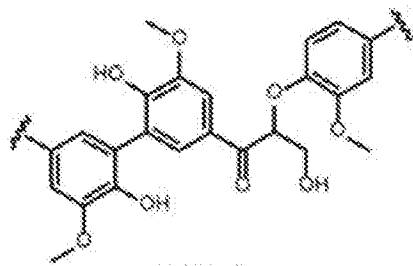


FIG. 28A

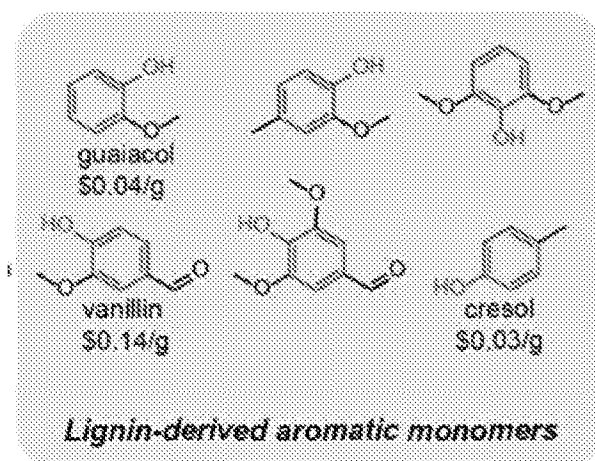
b



Lignin



Breakdown



Upgrading with BH_4 , O , Fe_2^+ , Pterin-dependent amino Acid mono-oxygenase

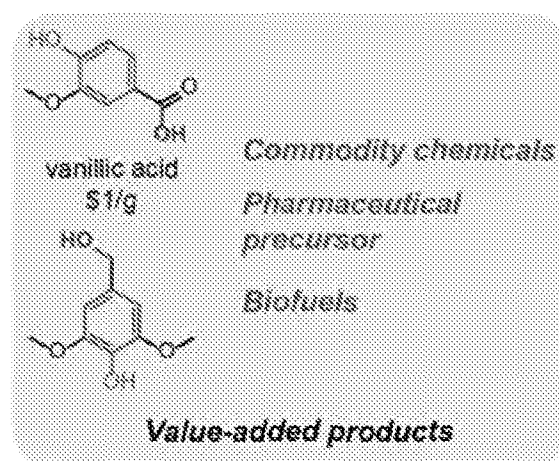


FIG. 28B

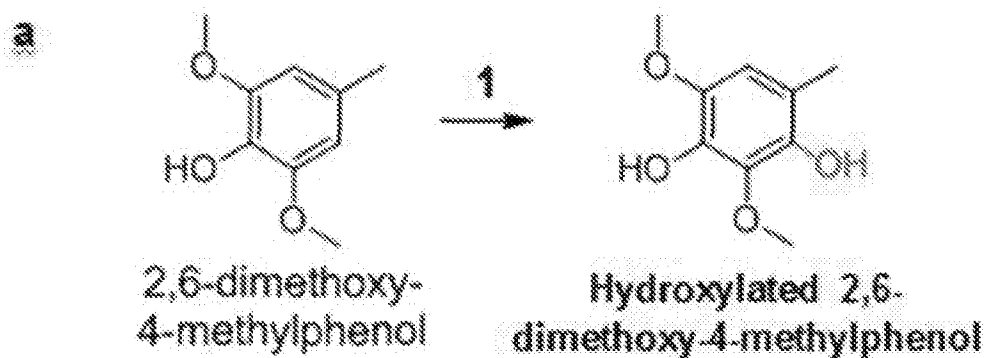


FIG. 29A

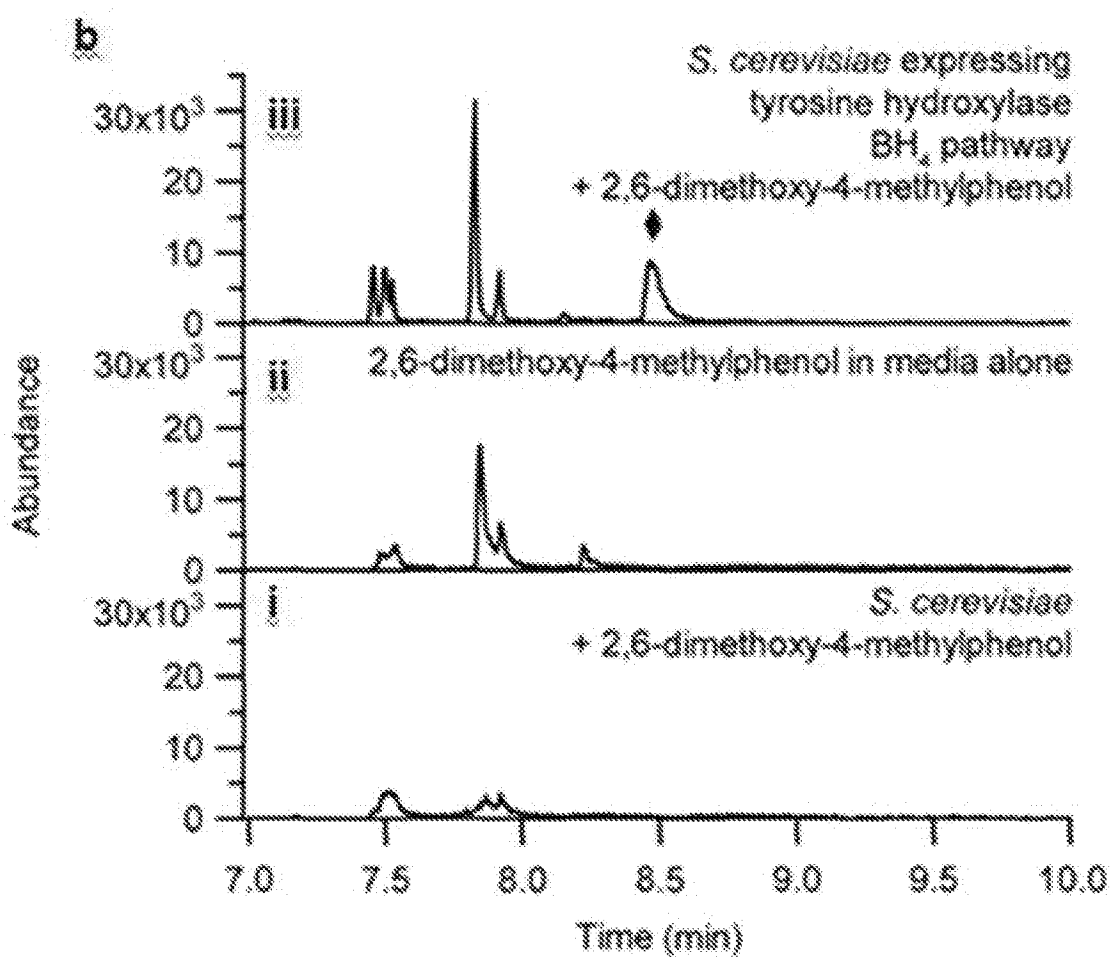


FIG. 29B

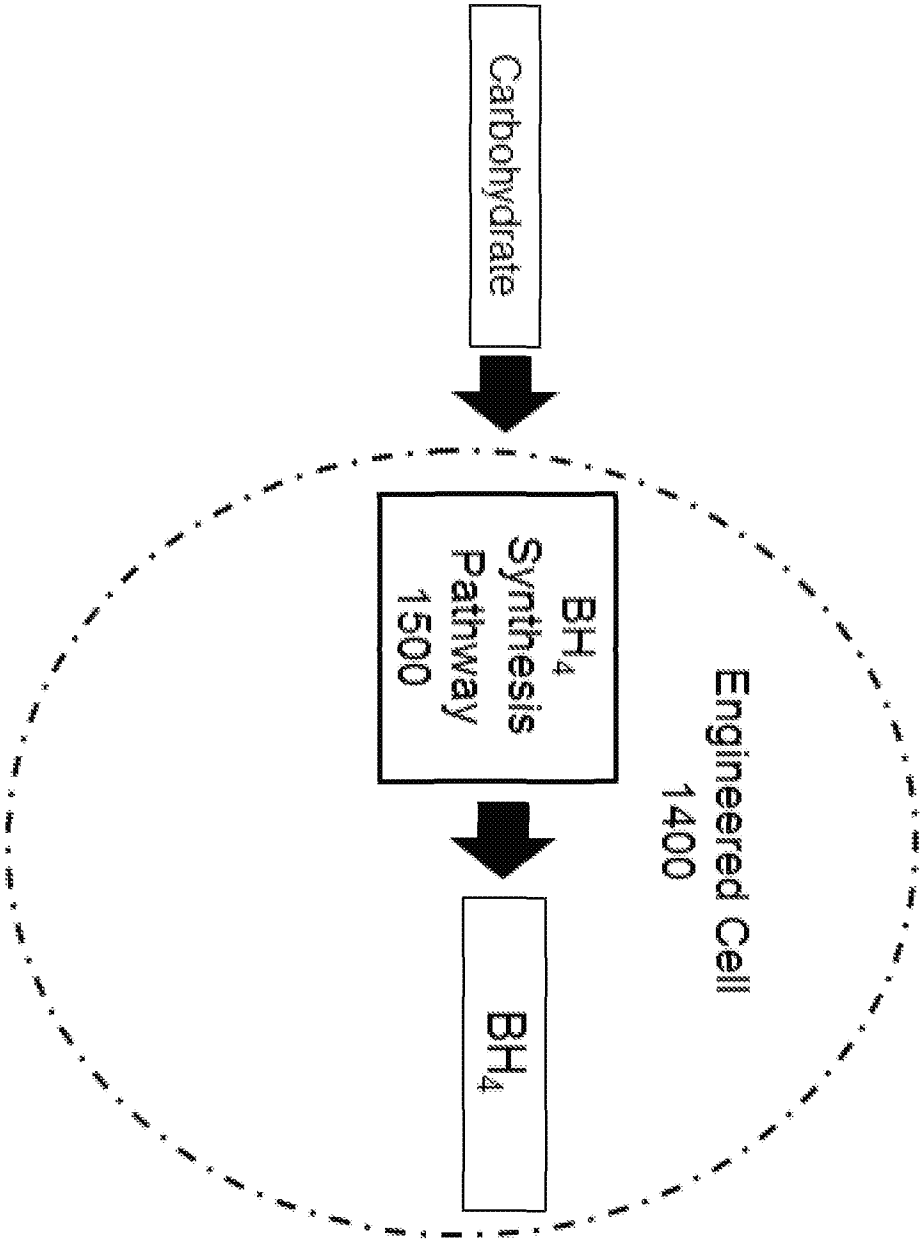


FIG. 30

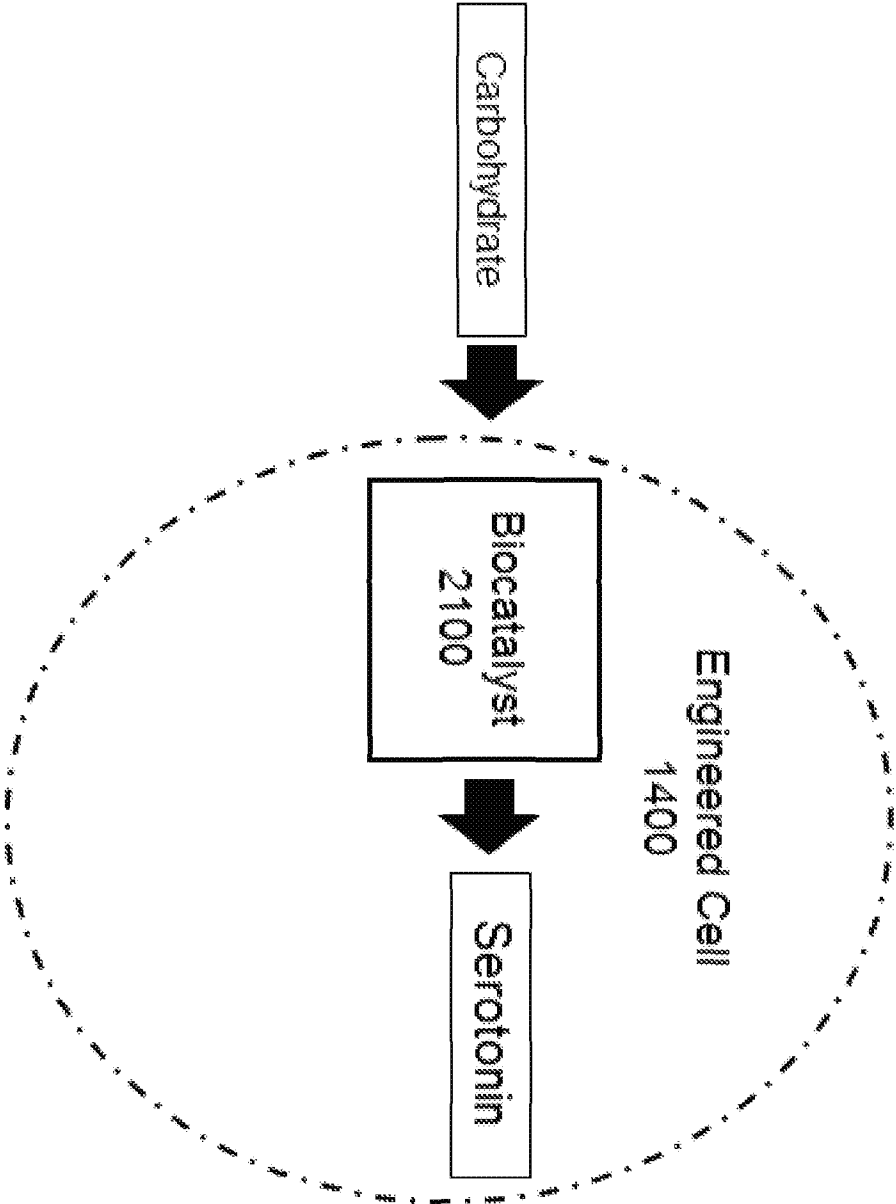


FIG. 31

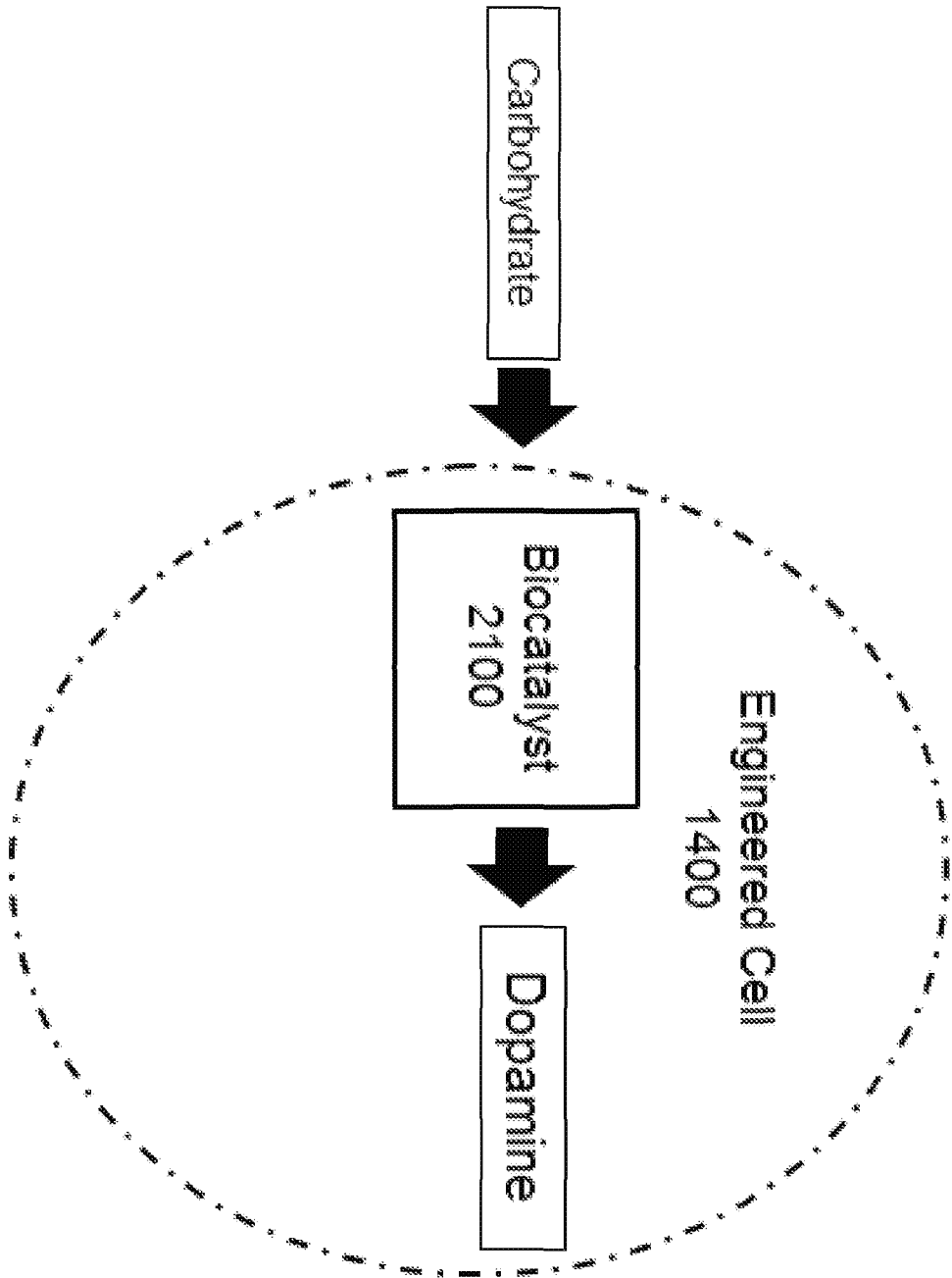


FIG. 32

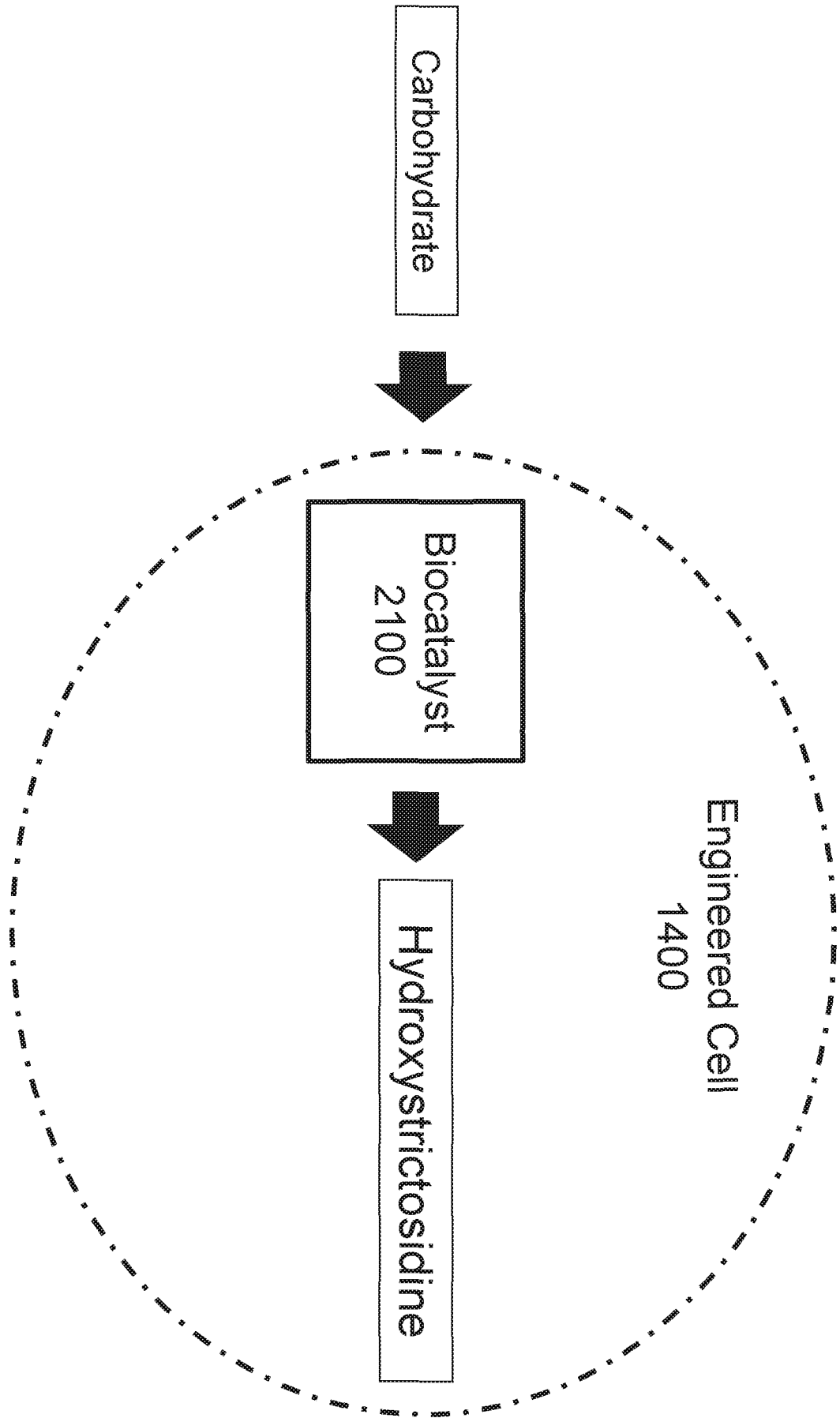


FIG. 33

Strain	BH ₄ Synthesis Pathway	BH ₄ Recycling Pathway	Alkaloid Synthesis Pathway	# Plasmids	Substrate(s)	Product	Production
PPY946	TH			4	Galactose	L-DOPA	nd
PPY646	STPCDH → PTPS → SR	PCDH → DHHR	TH	4	Galactose	L-DOPA	0.33 ± 0.02 mg/L
PPY679	STPCDH → PTPS → SR		TH	4	Galactose	L-DOPA	0.27 ± 0.04 mg/L
PPY947	TH		TH → DDC	4	Galactose	Dopamine	nd
PPY658	STPCDH → PTPS → SR	PCDH → DHHR	TH → DDC	4	Galactose	Dopamine	1.52 ± 0.05 mg/L
PPY743	STPCDH → PTPS → SR		TH → DDC	4	Galactose	Dopamine	1.73 ± 0.14 mg/L
PPY948	TPH		TPH → DDC	4	Galactose	Serotonin	nd
PPY649	STPCDH → PTPS → SR	PCDH → DHHR	TPH → DDC	4	Galactose	Serotonin	5.72 ± 1.18 mg/L
PPY741	STPCDH → PTPS → SR		TPH → DDC	4	Galactose	Serotonin	5.18 ± 0.84 mg/L
PPY650	STPCDH → PTPS → SR	PCDH → DHHR	TPH → DDC → STR	4	Galactose Secologanin	Hydroxytryptosidine	728.8 ± 206.2 counts
PPY955	STPCDH → PTPS → SR		TPH → DDC → STR	4	Galactose Secologanin	Hydroxytryptosidine	570.6 ± 280.6 counts
PPY744	STPCDH → PTPS → SR	PCDH → DHHR	TPH → DDC → STR	3	Glucose Secologanin	Hydroxytryptosidine	819.4 ± 357.3 counts
PPY748	STPCDH → PTPS → SR		TPH → DDC → STR	3	Glucose Secologanin	Hydroxytryptosidine	444.7 ± 246.5 counts
PPY740	STPCDH → PTPS → SR		TPH → DDC → STR	2	Glucose Secologanin	Hydroxytryptosidine	288.7 ± 91.3 counts
PPY649	STPCDH → PTPS → SR	PCDH → DHHR	TPH → DDC	4	Galactose Secologanin	Hydroxytryptosidine	713.9 ± 281.9 counts

FIG. 34

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2015/068228

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12P-017/16 (2016.01)

CPC - C12N-015/52 (2016.02)

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - C07H 21/00; C07K 14/00; C12N 1/15, 1/19, 1/21, 15/00, 15/52, 5/10, 9/00, 9/04, 9/78, 9/88; C12P 17/16, 17/18, (2016.01)
CPC - C12N 15/52, 9/00; C12P 17/10, 17/182; Y02P 20/52 (2016.02)Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 435/18, 25, 11, 190, 227, 232, 252, 252.330, 254, 320, 325; 530/350; 536/23 (keyword delimited)

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

Orbit, Google Patents, Google Scholar

Search terms used: Tetrahydrobiopterin catalyst "GTP cyclohydrazase" "pyruvoyl tetrahydropterin synthase" "sepiapterin reductase" aromatic-L-amino acid decarboxylase" strictosidine synthase "pterin-4a-carbinolamine dehydratase" "dihydropterin reductase" glucose galactose lignin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2006/0008869 A1 (YABUTA et al) 12 January 2006 (12.01.2006) entire document	1-3, 6, 9-11, 14, 18, 19, 21-24
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Y		4, 5, 7, 8, 12, 13, 15-17, 20, 25
Y	WO 2013/127915 A1 (DANMARKS TEKNISKE UNIVERSITET) 06 September 2013 (06.09.2013) entire document	4, 5, 12, 13, 25
Y	FARRE et al. "Engineering Complex Metabolic Pathways in Plants," Annual Review of Plant Biology, 26 February 2014 (26.02.2014), Vol. 65, Pgs. 187-223. entire document	7, 15, 16
Y	THONY et al. "Tetrahydrobiopterin biosynthesis, regeneration and functions," Biochemical Journal, 1 April 2000(01.04.2000), Vol. 347, Pgs. 1-16. entire document	8, 17, 20
P, X	EHRENWORTH et al. "Pterin-dependent mono-oxidation for the microbial synthesis of a modified monoterpene indole alkaloid," ACS Synthetic Biology, 18 December 2015 (27.07.2015), Vol. 4, Pgs. 1295-1307. entire document	1-25
A	WO 2000/042200 A1 (UNIV LEIDEN et al) 20 July 2000 (20.07.2000) entire document	1-25
A	NAPONELLI et al. "Phylogenomic and Functional Analysis of Pterin-4a-Carbinolamine Dehydratase Family (COG2154) Proteins in Plants and Microorganisms," Plant Physiology, 30 April 2008 (30.04.2008), Vol. 146, Pgs. 1515-1527. entire document	1-25

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

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

23 March 2016

Date of mailing of the international search report

15 APR 2016

Name and mailing address of the ISA/

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