

(2)

[0007] The disclosure provides engineered polypeptides having ketoreductase activity, polynucleotides encoding the polypeptides, and methods of using the polypeptides for the synthesis of enantiospecific compounds.

[0007a] In one aspect, there is provided an engineered polypeptide capable of converting 1-(3-hydroxyphenyl)-2 (methylamino)ethanone to (R)-phenylephrine, wherein the amino acid sequence of the polypeptide has at least 70% identity to SEQ ID NO: 4 and comprises the residue difference M206C.

[0008] The engineered ketoreductase polypeptides of the disclosure are capable of catalyzing the conversion of compound **(2)** to compound **(1)** with a relative activity at least 10-fold greater than the wild-type ketoreductase polypeptide of SEQ ID NO: 2, and with an improved stereoselectivity capable of providing compound **(1)** in >99 % e.e. Accordingly, in some embodiments, the disclosure provides methods of using the engineered polypeptides for synthesizing (*R*)-phenylephrine of compound **(1)**. The methods include pH ranges, isopropyl alcohol (IPA) concentrations, and buffer substances that are useful for maintaining substrate stability and providing improved catalysis (*e.g.*, higher conversion with higher substrate loading at lower enzyme concentrations).

[0009] In one embodiment, the engineered polypeptides have improved enzyme properties as compared to the naturally occurring ketoreductase of *L. kefir*, the sequence of which is represented by SEQ ID NO: 2, in particular the engineered polypeptides are capable of converting 1-(3-hydroxyphenyl)-2-(methylamino)ethanone to (*R*)-phenylephrine with improved activity and enantioselectivity. In some embodiments, the engineered polypeptides having ketoreductase activity are improved in activity and enantioselectivity for converting compound (**2**) to compound (**1**) as compared to another engineered ketoreductase polypeptide, such as a polypeptide of SEQ ID NO: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34. The improved properties of the engineered polypeptides provide for improved processes for preparing compound **(1)** and related compounds of Formula I (see below). In addition to improved enzyme activity (*e.g.*, conversion rate), the engineered polypeptides of the disclosure comprise improved pH stability, improved solvent characteristics (*e.g.*, activity in 50% isopropyl alcohol), and reduced non-enzymatic decomposition of substrate (*i.e.*, compound (**2**)). In some embodiments, the engineered polypeptides having ketoreductase activity are characterized by a combination of improved properties, such as increased enzymatic activity, pH stability, and reduced compound (**2**) decomposition compared to a wild-type ketoreductase (*e.g.*, an *L. kefir* ketoreductase).

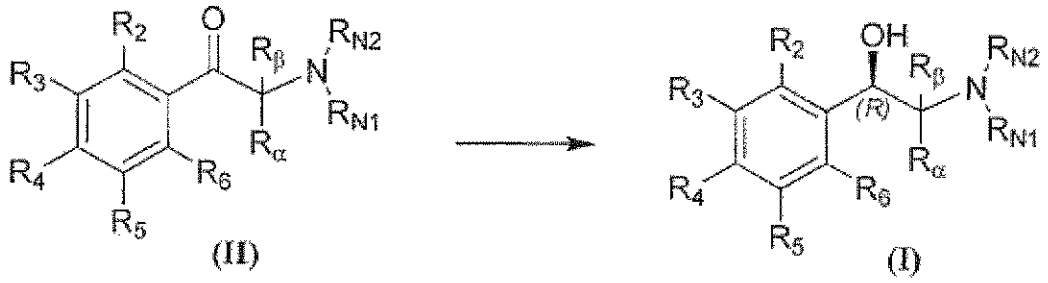
[0010] In some embodiments, the engineered polypeptides having ketoreductase activity are improved with respect to enzyme activity as compared to the activity of the polypeptide comprising SEQ ID NO: 4 in the synthesis of compound (**1**). In some embodiments, the engineered polypeptides having ketoreductase activity have at least 7-fold greater than the activity of SEQ ID NO: 4 at a reaction condition of pH about 6.0-7.5 and temperature of about 25-35°C. In some embodiments, the

carried out wherein the reaction conditions comprise a temperature of about 25°C to about 35°C (*e.g.*, at about 30°C). In certain embodiments, the method can be carried out wherein the mixture comprises a solvent comprising a buffer and 50% (v/v) isopropyl alcohol. In some embodiments, the buffer is selected from triethanolamine (*e.g.*, about 0.05 M to about 0.25 M TEA, or about 0.1 M TEA) and potassium phosphate (*e.g.*, about 0.025 M to about 0.1 M phosphate, or about 0.05 M phosphate). In certain embodiments, the method can be carried out wherein the mixture comprises about 0.03-0.1 g/L NADP (*e.g.*, about 0.05 g/L NADP). In certain embodiments, the method can be carried out wherein the reaction conditions comprise an inert atmosphere (*e.g.*, N_2, Ar, etc.).

[0028] Accordingly, in some embodiments, the methods for preparing (*R*)-phenylephrine of the present disclosure can be carried out using a combination of any of the mixture and reaction conditions disclosed above (and elsewhere herein) *e.g.*, (1) a pH of about 6.75-7.0; (2) a temperature of about 30 °C; (3) about 50% isopropyl alcohol; (4) about 0.05 g/L NADP; (5) about 100 g/L 1-(3-hydroxyphenyl)-2-(methylamino)ethanone; (5) and about 0.7-1.1 g/L of the polypeptide; and (6) N_2 atmosphere.

[0029] In some embodiments, the method can reaction conditions comprise a pH of about 6.75-7.0, a temperature of about 30°C, about 100 g/L of compound (**2**) (or 130 g/L of the hydrosulfate of compound (**2a**)), and about 1 g/L of an polypeptide having a sequence as set forth in SEQ ID NO: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34 in a reaction time of about 19-24 hrs, wherein at least 50%-99% of the substrate is converted to *(R)*-phenylephrine.

[0029a] In one aspect, there is provided a method for producing a compound of Formula I from a compound of Formula II:



wherein R_2 is a group selected from: -H, -Cl, -Br, -I, -F, $-CH_3$, -OH, $-OCH_3$, -SH, $-SCH_3$, $-NH_2$, $-NHCH_3$, or a long chain alkyl;

wherein R_3 is a group selected from: -H, -Cl, -Br, -I, -F, $-CH_3$, -OH, $-OCH_3$, -SH, $-SCH_3$, $-S(O)CH_3$, $-NH_2$, $-NHCH_3$, $-N(CH_3)_2$, -OR, -SR, $-NR_2$, $-SO_2NR_2$ (wherein R = -H, $-CH_3$, or alkyl), ethyl, propyl, isopropyl, cyclopropyl, or a long chain alkyl;

wherein R_4 is a group selected from: -H, -Cl, -Br, -I, -F, $-CH_3$, -OH, $-OCH_3$, -SH, $-SCH_3$, $-S(O)CH_3$, $-SO_2CH_3$, $-NH_2$, $-NHCH_3$, $-N(CH_3)_2$, SO_2NR_2 (wherein R = -H, $-CH_3$);

wherein R_5 is a group selected from: -H, -Cl, -Br, -I, -F, $-CH_3$, -OH, $-OCH_3$, -SH, $-SCH_3$, $-S(O)CH_3$, $-SO_2CH_3$, $-NH_2$, $-NHCH_3$, $-N(CH_3)_2$, -OR, -SR, $-NR_2$, $-SO_2NR_2$ (wherein R = -H, $-CH_3$, or alkyl), ethyl, propyl, isopropyl, or cyclopropyl;

wherein R_6 is a group selected from: -H, -Cl, -Br, -I, -F, $-CH_3$, -OH, -SH, or $-NH_2$;

wherein R_2 and R_3, R_3 and R_4 or R_4 and R_5 can optionally be connected as part of a 5 or 6 membered ring;

wherein Rα is a group selected from: -H, $-CH_3$, ethyl, propyl, isopropyl, cyclopropyl, or a long chain alkyl; wherein R_β is a group selected from: -H, $-CH_3$, ethyl, propyl, isopropyl, or cyclopropyl;

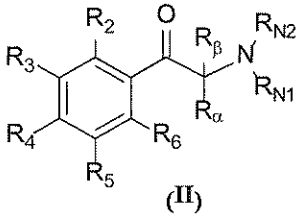
wherein Rα and Rβ can form a ring, or wherein the R_α-R_β unit is a carbonyl or imino functional group;

wherein R_{N1} and R_{N2} can be independently a group selected from: -H, $-CH_3$, -OH, $-OCH_3$, -OR, -C(O)R (wherein R = -H, $-CH_3$, or alkyl), ethyl, propyl, isopropyl, cyclopropyl, long chain alkyl, carbonyl, or carboxy;

the method comprising contacting a mixture comprising the compound of Formula II with a polypeptide as defined herein, under reaction conditions suitable to convert the compound of Formula II to the compound of Formula I.

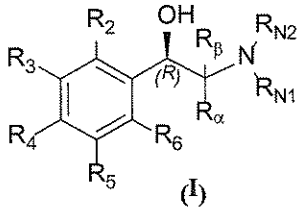
[0029b] In one aspect, there is provided a method as defined herein for preparing (R)-phenylephrine, the method comprising: contacting a polypeptide as defined herein with a mixture comprising a 1-(3-hydroxyphenyl)-2-(methylamino)ethanone substrate and a buffer under reaction conditions suitable to convert 1-(3-hydroxyphenyl)-2-(methylamino)ethanone to (R)-phenylephrine.

[0030] In another ~~aspect~~embodiment, the disclosure provide a method for the stereoselective conversion of a substrate compound of Formula **II**:



(II)

to a product compound of Formula **I**:



(I)

wherein R_2 is a group selected from: -H, -Cl, -Br, -I, -F, $-CH_3$, -OH, $-OCH_3$, -SH, $-SCH_3$, $-NH_2$, $-NHCH_3$, or a long chain alkyl; R_3 is a group selected from: -H, -Cl, -Br, -I, -F, $-CH_3$, -OH, $-OCH_3$, -SH, $-SCH_3$, $-S(O)CH_3$, $-NH_2$, $-NHCH_3$, $-N(CH_3)_2$, -OR, -SR, $-NR_2$, $-SO_2NR_2$ (wherein R = -H, $-CH_3$, or alkyl), ethyl, propyl, isopropyl, cyclopropyl, or a long chain alkyl; R_4 is a group selected from: -H,

-Cl, -Br, -I, -F, $-CH_3$, -OH, $-OCH_3$, -SH, $-SCH_3$, $-S(O)CH_3$, $-SO_2CH_3$, $-NH_2$, $-NHCH_3$, $-N(CH_3)_2$, SO_2NR_2 (wherein R = -H, $-CH_3$); R_5 is a group selected from: -H, -Cl, -Br, -I, -F, $-CH_3$, -OH, $-OCH_3$, -SH, $-SCH_3$, $-S(O)CH_3$, $-SO_2CH_3$, $-NH_2$, $-NHCH_3$, $-N(CH_3)_2$, -OR, -SR, $-NR_2$, $-SO_2NR_2$ (wherein R = -H, $-CH_3$, or alkyl), ethyl, propyl, isopropyl, or cyclopropyl; R_6 is a group selected from: -H, -Cl, -Br, -I, -F, $-CH_3$, -OH, -SH, or $-NH_2$; wherein R_2 and R_3, R_3 and R_4, or R_4 and R_5 can optionally be connected as part of a 5 or 6 membered ring; wherein R_α is a group selected from: -H, $-CH_3$, ethyl, propyl, isopropyl, cyclopropyl, or a long chain alkyl; wherein R_β is a group selected from: -H, $-CH_3$, ethyl, propyl, isopropyl, or cyclopropyl; wherein R_α and R_β can form a ring, or wherein the R_α-R_β unit is a carbonyl or imino functional group; wherein R_{N1} and R_{N2} can be independently a group selected from: -H, $-CH_3$, -OH, $-OCH_3$, -OR, -C(O)R (wherein R = -H, $-CH_3$, or alkyl), ethyl, propyl, isopropyl, cyclopropyl, long chain alkyl, carbonyl, or carboxy.

[0031] In some embodiments of the method, the substrate compound of Formula II is 1-(3-hydroxyphenyl)-2-(methylamino)ethanone, compound **(2)**, and the product of Formula I is compound **(1)**, (*R*)-phenylephrine.

[0031a] In one aspect, there is provided an isolated polynucleotide encoding the polypeptide as defined herein, optionally wherein the sequence is selected from SEQ ID NO: 19, 5, 7, 9, 11, 13, 15, 17, 21, 23, 25, 27, 29, 31, and 33.

[0032] In another ~~aspect~~embodiment, the disclosure provides polynucleotides encoding the engineered polypeptides described herein or polynucleotides that hybridize to such polynucleotides under highly stringent conditions. The polynucleotide can include promoters and other regulatory elements useful for expression of the encoded polypeptide having ketoreductase activity, and can utilize codons optimized for specific desired expression systems. Exemplary polynucleotides include, but are not limited to SEQ ID NO: 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, and 33.

[0032a] In one aspect, there is provided a host cell comprising the expression vector as defined herein, optionally wherein the host cell is *E. coli*.

[0032b] In one aspect, there is provided an expression vector comprising the polynucleotide as defined herein operably linked to a control sequence suitable for directing expression in a host cell, optionally wherein the control sequence comprises a secretion signal.

[0033] In another embodiment, the disclosure provides host cells comprising the polynucleotides and/or expression vectors described herein. The host cells may be prokaryotic or eukaryotic. In one embodiment, the host cell can be *E. coli,* or a different prokaryotic organism. In another embodiment, the host cell may be a yeast cell. The host cells can be used for the expression and isolation of the engineered polypeptides described herein, or, alternatively, they can be used directly for the conversion of the substrate to, for example, a phenylephrine product.

[0033a] In one aspect, there is provided a method for preparing an engineered polypeptide as defined herein comprising culturing a host cell as defined herein and optionally isolating the polypeptide from the cell.

5. DETAILED DESCRIPTION

[0034] As used herein and in the appended claims, the singular forms "a," "and," and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a substrate" includes a plurality of such substrates and reference to "the enzyme" includes reference to one or more enzymes, and so forth.

[0035] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this disclosure belongs. Although any methods and reagents similar or equivalent to those described herein can be used in the practice of the disclosed methods and compositions, the exemplary methods and materials are now described.

CLAIMS

1. An engineered polypeptide capable of converting 1-(3-hydroxyphenyl)-2-(methylamino)ethanone to (*R*)-phenylephrine, wherein the amino acid sequence of the polypeptide has at least 70% identity to SEQ ID NO: 4 and comprises the residue difference M206C.

2. The engineered polypeptide of claim 1, wherein the amino acid sequence further comprises at least one residue difference at a position of SEQ ID NO: 4 corresponding to T2, I11, A64, T76, V95, V99, V148, T152, L153, S159, D197, or E200, optionally wherein the residue difference is V95M.

3. The engineered polypeptide of claim 1 or claim 2, wherein the amino acid sequence comprises the residue differences: V95M, A202F, and M206C, optionally wherein the amino acid sequence comprises the residue differences: V95M, C190G, A202F, and M206C, for example wherein the amino acid sequence comprises the residue differences: V95M, C190G, A202F, M206C, and Y249F.

4. The engineered polypeptide of any one of claims 1 to 3, wherein the amino acid sequence comprises the combination of residue differences of any one of SEQ ID NO: 20, 4, 6, 8, 10, 12, 14, 16, 18, 22, 24, 26, 28, 30, 32, or 34 as compared to SEQ ID NO: 2, optionally wherein the amino acid sequence comprises the combination of residue differences of SEQ ID NO: 4 as compared to SEQ ID NO: 2.

5. The engineered polypeptide of any one of claims 1 to 4, wherein the amino acid sequence further comprises at least one of the following residue differences:

I11, A64, T76, and/or V148 is substituted with an amino acid selected from alanine (A), leucine (L), isoleucine (I), and valine (V);

V99, T152, L153, and/or D197 is substituted with an amino acid selected from alanine (A), valine (V), leucine (L), isoleucine (I), glycine (G), or methionine (M);

S159 is substituted with an amino acid selected from asparagine (N), glutamine (Q), serine (S) or threonine (T); or

E200 is substituted with an amino acid selected from proline (P) or histidine (H).

6. The engineered polypeptide of any one of claims 1 to 5, wherein:

(i) the amino acid sequence comprises at least one residue difference selected from T2S, I11L, A64V, T76I, V95M, S96L,V99L, V148L, T152A, L153M, S159T, D197A, and E200P; and/or

(ii) the amino acid sequence further comprises at least 1-60 conservative amino acid substitutions at positions of SEQ ID NO: 4 other than T2, I11, A64, T76, V95, S96, V99, A145, L147, V148, T152, L153, S159, C190, D197, E200, A202, M206, and Y249.

7. The engineered polypeptide of any one of claims 1 to 6, wherein the engineered polypeptide is capable of converting 1-(3-hydroxyphenyl)-2-(methylamino)ethanone to (*R*)-phenylephrine at pH 6.5 and 25°C with an activity at least 7.3-fold greater than a polypeptide of SEQ ID NO: 4.

8. The engineered polypeptide of any one of claims 1 to 7, wherein the polypeptide comprises a sequence selected from the group consisting of SEQ ID NO: 20, 6, 8, 10, 12, 14, 16, 18, 22, 24, 26, 28, 30, 32, and 34.

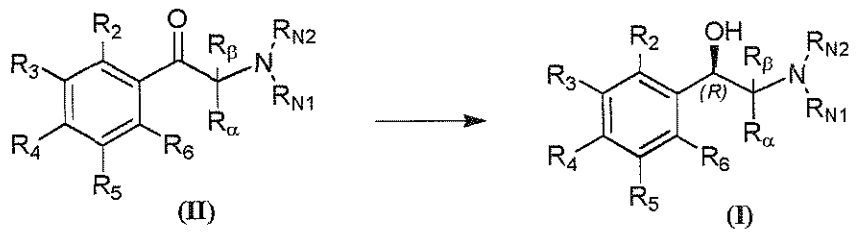
9. An isolated polynucleotide encoding the polypeptide of any one of claims 1 to 8, optionally wherein the sequence is selected from SEQ ID NO: 19, 5, 7, 9, 11, 13, 15, 17, 21, 23, 25, 27, 29, 31, and 33.

10. An expression vector comprising the polynucleotide of claim 9 operably linked to a control sequence suitable for directing expression in a host cell, optionally wherein the control sequence comprises a secretion signal.

11. A host cell comprising the expression vector of claim 10, optionally wherein the host cell is *E. coli.*

12. A method for preparing an engineered polypeptide of any one of claims 1 to 8 comprising culturing a host cell of claim 11 and optionally isolating the polypeptide from the cell.

13. A method for producing a compound of Formula I from a compound of Formula II:



wherein R_2 is a group selected from: -H, -Cl, -Br, -I, -F, $-CH_3$, -OH, $-OCH_3$, -SH,

-SCH_3, -NH_2, -$NHCH_3$, or a long chain alkyl;

wherein R_3 is a group selected from: -H, -Cl, -Br, -I, -F, -CH_3, -OH, -OCH_3, -SH, -SCH_3, -$S(O)CH_3$, -NH_2, -$NHCH_3$, -$N(CH_3)_2$, -OR, -SR, -NR_2, -SO_2NR_2 (wherein R = -H, -CH_3, or alkyl), ethyl, propyl, isopropyl, cyclopropyl, or a long chain alkyl;

wherein R_4 is a group selected from: -H, -Cl, -Br, -I, -F, -CH_3, -OH, -OCH_3, -SH, -SCH_3, -$S(O)CH_3$, -SO_2CH_3, -NH_2, -$NHCH_3$, -$N(CH_3)_2$, SO_2NR_2 (wherein R = -H, -CH_3);

wherein R_5 is a group selected from: -H, -Cl, -Br, -I, -F, -CH_3, -OH, -OCH_3, -SH, -SCH_3, -$S(O)CH_3$, -SO_2CH_3, -NH_2, -$NHCH_3$, -$N(CH_3)_2$, -OR, -SR, -NR_2, -SO_2NR_2 (wherein R = -H, -CH_3, or alkyl), ethyl, propyl, isopropyl, or cyclopropyl;

wherein R_6 is a group selected from: -H, -Cl, -Br, -I, -F, -CH_3, -OH, -SH, or -NH_2;

wherein R_2 and R_3, R_3 and R_4, or R_4 and R_5 can optionally be connected as part of a 5 or 6 membered ring;

wherein R_α is a group selected from: -H, -CH_3, ethyl, propyl, isopropyl, cyclopropyl, or a long chain alkyl; wherein R_β is a group selected from: -H, -CH_3, ethyl, propyl, isopropyl, or cyclopropyl;

wherein R_α and R_β can form a ring, or wherein the R_α-R_β unit is a carbonyl or imino functional group;

wherein R_{N1} and R_{N2} can be independently a group selected from: -H, -CH_3, -OH, -OCH_3, -OR, -C(O)R (wherein R = -H, -CH_3, or alkyl), ethyl, propyl, isopropyl, cyclopropyl, long chain alkyl, carbonyl, or carboxy;

the method comprising contacting a mixture comprising the compound of Formula II with a polypeptide of any one of claims 1 to 8, under reaction conditions suitable to convert the compound of Formula II to the compound of Formula I.

14. A method according to claim 13 for preparing (*R*)-phenylephrine, the method comprising:

contacting a polypeptide of any one of claims 1 to 8 with a mixture comprising a 1-(3-hydroxyphenyl)-2-(methylamino)ethanone substrate and a buffer under reaction conditions suitable to convert 1-(3-hydroxyphenyl)-2-(methylamino)ethanone to (*R*)-phenylephrine.

15. The method of claim 14, wherein:

(i) the reaction conditions comprise a temperature of about 25°C to about 35°C;

(ii) the reaction conditions comprise a temperature of about 30°C;

(iii) the reaction conditions comprise a pH of about 6.5 to about 7.0;

(iv) the reaction conditions comprise an initial pH of about 7.0 and then adjusting the initial pH to about 6.75 after about 2 hours;

(v) the mixture comprises 50% isopropyl alcohol;

(vi) the buffer is selected from 0.1 M triethanolamine and 0.05 M potassium phosphate;

(vii) the mixture comprises about 0.05 g/L NADP;

(viii) the reaction conditions comprise an inert atmosphere; and/or

(ix) the reaction conditions comprise a pH of about 6.75-7.0, a temperature of about 30 °C, about 50% isopropyl alcohol, about 0.05 g/L NADP, about 100 g/L 1-(3-hydroxyphenyl)-2-(methylamino)ethanone, and about 0.9-1.1 g/L of the polypeptide, wherein the reaction is carried out under N_2 for about 19-24 hours.

~~What is claimed is:~~

1. An engineered polypeptide capable of converting 1-(3-hydroxyphenyl)-2-(methylamino)ethanone to (*R*)-phenylephrine, wherein the amino acid sequence of the polypeptide has at least 70% identity to SEQ ID NO: 4 and comprises at least one residue difference selected from C190G, A202F, M206C, and Y249F and at least one residue difference at a position of SEQ ID NO: 4 corresponding to T2, I11, A64, T76, V95, V99, V148, T152, L153, S159, D197, or E200.
2. The engineered polypeptide of claim 1, wherein the at least one residue difference is A202F.
3. The engineered polypeptide of claim 1, wherein the at least one residue difference is M206C.
4. The engineered polypeptide of any one of claims 1 to 3, wherein the amino acid sequence comprises the residue difference: V95M.
5. The engineered polypeptide of any one of claims 1 to 4, wherein the amino acid sequence comprises at least two residue differences selected from V95M, S96L, L147I, C190G, A202F, M206C, and Y202F.
6. The engineered polypeptide of any one of claims 1 to 5, wherein the amino acid sequence comprises the residue differences: V95M, A202F, and M206C.
7. The engineered polypeptide of any one of claims 1 to 6, wherein the amino acid sequence comprises the residue differences: V95M, C190G, A202F, and M206C.
8. The engineered polypeptide of any one of claims 1 to 7, wherein the amino acid sequence comprises the residue differences: V95M, C190G, A202F, M206C, and Y249F.
9. The engineered polypeptide of any one of claims 1 to 8, wherein the amino acid sequence comprises the combination of residue differences of any one of SEQ ID NO: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34 as compared to SEQ ID NO: 2.
10. The engineered polypeptide of any one of claims 1 to 9, wherein the amino acid sequence comprises the combination of residue differences of SEQ ID NO: 4 as compared to SEQ ID NO: 2.
11. The engineered polypeptide of any one of claims 1 to 10, wherein the amino acid sequence further comprises at least one of the following residue differences:

    I11, A64, T76, and/or V148 is substituted with an amino acid selected from alanine (A), leucine (L), isoleucine (I), and valine (V);

    V99, T152, L153, and/or D197 is substituted with an amino acid selected from (A), valine (V), leucine (L), isoleucine (I), glycine (G), or methionine (M);

    S159 is substituted with an amino acid selected from asparagine (N), glutamine (Q), serine (S) or threonine (T);

~~E200 is substituted with an amino acid selected from proline (P) or histidine (H).~~

12. The engineered polypeptide of any one of claims 1 to 11, wherein the amino acid sequence comprises at least one residue difference selected from T2S, I11L, A64V, T76I, V95M, S96L,V99L, V148L, T152A, L153M, S159T, D197A, and E200P.

13. The engineered polypeptide of any one of claims 1 to 12, wherein the amino acid sequence further comprises at least 1-60 conservative amino acid substitutions at positions of SEQ ID NO: 4 other than T2, I11, A64, T76, V95, S96, V99, A145, L147, V148, T152, L153, S159, C190, D197, E200, A202, M206, and Y249.

14. The engineered polypeptide of any one of claims 1 to 13, wherein the engineered polypeptide is capable of converting 1-(3-hydroxyphenyl)-2-(methylamino)ethanone to (*R*)-phenylephrine at pH 6.5 and 25°C with an activity at least 7.3-fold greater than a polypeptide of SEQ ID NO: 4.

15. The engineered polypeptide of any one of claims 1 to 14, wherein the polypeptide is capable of stereospecifically converting 1-(3-hydroxyphenyl)-2-(methylamino)ethanone to (*R*)-phenylephrine in a enantiomeric excess of at least 99%.

16. The engineered polypeptide of any one of claims 1 to 15, wherein about 0.7-1.1 g/L of the polypeptide is capable of converting at least about 40% of about 100 g/L of 1-(3-hydroxyphenyl)-2-(methylamino)ethanone to *(R)*-phenylephrine under reaction conditions of about pH 6.5-7.2 and about 30°C in about 19-24 hrs.

17. The engineered polypeptide of any one of claims 1 to 16, wherein the polypeptide comprises a sequence selected from the group consisting of SEQ ID NO: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, and 34.

18. An isolated polypeptide comprising a sequence selected from the group consisting of SEQ ID NO: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, and 34.

19. An isolated polynucleotide encoding the polypeptide of any one of claims 1 to 18.

20. The polynucleotide of claim 16, wherein the sequence is selected from SEQ ID NO: 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, and 33.

21. An expression vector comprising the polynucleotide of any one of claims 19 to 20 operably linked to a control sequence suitable for directing expression in a host cell.

22. The expression vector of claim 21, wherein the control sequence comprises a secretion signal.

23. A host cell comprising the expression vector of any one of claims 21 to 22.

24. The host cell of claim 23, wherein the host cell is *E. coli.*

25. A method for preparing an engineered polypeptide of any one of claims 1 to 18 comprising culturing a host cell of any one of claims 23 to 24 and optionally isolating the polypeptide from the cell.

26. A method for preparing (*R*)-phenylephrine the method comprising:

contacting a polypeptide of any one of claims 1 to 18 with a mixture comprising a 1-(3-hydroxyphenyl)-2-(methylamino)ethanone substrate and a buffer under reaction conditions suitable to convert 1-(3-hydroxyphenyl)-2-(methylamino)ethanone to (*R*)-phenylephrine.

27. The method of claim 26, wherein the reaction conditions comprise a temperature of about 25°C to about 35°C.

28. The method of claim 26, wherein the reaction conditions comprise a temperature of about 30°C.

29. The method of claim 26, wherein the reaction conditions comprise a pH of about 6.5 to about 7.0.

30. The method of claim 26, wherein the reaction conditions comprise an initial pH of about 7.0 and then adjusting the initial pH to about 6.75 after about 2 hours.

31. The method of claim 26, wherein the mixture comprises 50% isopropyl alcohol.

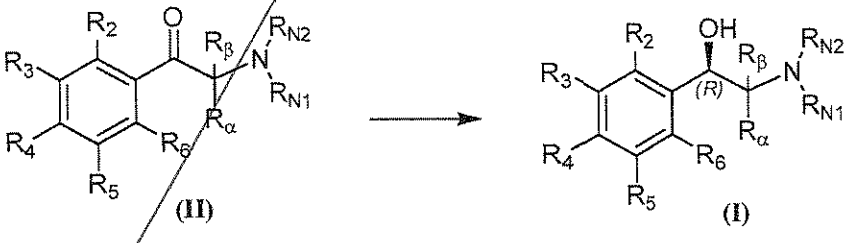
32. The method of claim 26, wherein the buffer is selected from 0.1 M triethanolamine and 0.05 M potassium phosphate.

33. The method of claim 26, wherein the mixture comprises about 0.05 g/L NADP.

34. The method of claim 26, wherein the reaction conditions comprise an inert atmosphere.

35. The method of claim 26, wherein the reaction conditions comprise a pH of about 6.75-7.0, a temperature of about 30 °C, about 50% isopropyl alcohol, about 0.05 g/L NADP, about 100 g/L 1-(3-hydroxyphenyl)-2-(methylamino)ethanone, and about 0.9-1.1 g/L of the polypeptide, wherein the reaction is carried out under N_2 for about 19-24 hours.

36. A method for producing a compound of Formula I from a compound of Formula II:



wherein R_2 is a group selected from: -H, -Cl, -Br, -I, -F, $-CH_3$, -OH, $-OCH_3$, -SH, $-SCH_3$, $-NH_2$, $-NHCH_3$, or a long chain alkyl;

wherein R_3 is a group selected from: -H, -Cl, -Br, -I, -F, $-CH_3$, -OH, $-OCH_3$, -SH, $-SCH_3$, $-S(O)CH_3$, $-NH_2$, $-NHCH_3$, $-N(CH_3)_2$, -OR, -SR, $-NR_2$, $-SO_2NR_2$ (wherein R = -H, $-CH_3$, or alkyl), ethyl, propyl, isopropyl, cyclopropyl, or a long chain alkyl;

wherein R_4 is a group selected from: -H, -Cl, -Br, -I, -F, $-CH_3$, -OH, $-OCH_3$, -SH, $-SCH_3$, $-S(O)CH_3$, $-SO_2CH_3$, $-NH_2$, $-NHCH_3$, $-N(CH_3)_2$, SO_2NR_2 (wherein R = -H, $-CH_3$);

wherein R_5 is a group selected from: -H, -Cl, -Br, -I, -F, $-CH_3$, -OH, $-OCH_3$, -SH, $-SCH_3$, $-S(O)CH_3$, $-SO_2CH_3$, $-NH_2$, $-NHCH_3$, $-N(CH_3)_2$, -OR, -SR, $-NR_2$, $-SO_2NR_2$ (wherein R = -H, $-CH_3$, or alkyl), ethyl, propyl, isopropyl, or cyclopropyl;

wherein R_6 is a group selected from: -H, -Cl, -Br, -I, -F, $-CH_3$, -OH, -SH, or $-NH_2$;

wherein R_2 and R_3, R_3 and R_4, or R_4 and R_5 can optionally be connected as part of a 5 or 6 membered ring;

wherein R_α is a group selected from: -H, $-CH_3$, ethyl, propyl, isopropyl, cyclopropyl, or a long chain alkyl; wherein R_β is a group selected from: -H, $-CH_3$, ethyl, propyl, isopropyl, or cyclopropyl;

wherein R_α and R_β can form a ring, or wherein the R_α-R_β unit is a carbonyl or imino *functional group*;

wherein R_{N1} and R_{N2} can be independently a group selected from: -H, $-CH_3$, -OH, $-OCH_3$, -OR, -C(O)R (wherein R = -H, $-CH_3$, or alkyl), ethyl, propyl, isopropyl, cyclopropyl, long chain alkyl, carbonyl, or carboxy;

the method comprising contacting a mixture comprising the compound of Formula II with a polypeptide of any one of claims 1 to 18, under reaction conditions suitable to convert the compound of Formula **II** to the compound of Formula **I**.