carbon atom and/or the nitrogen indicated by * in the product compound of formula **(III)** can be chiral. As described further herein, the engineered polypeptides having imine reductase activity exhibit stereoselectivity, thus, an imine reductase reaction of **Scheme 1** can be used to establish one, two, or more, chiral centers of a product compound of formula **(III)** in a single biocatalytic reaction.

[0009] In some embodiments, the present disclosure provides an engineered polypeptide having imine reductase activity, comprising an amino acid sequence having at least 80% sequence identity to a naturally occurring opine dehydrogenase amino acid sequence selected from the group consisting of SEQ ID NO: **2, 102, 104, 106, 108,** and **110**, and further comprising one or more residue differences as compared to the amino sequence of selected naturally occurring opine dehydrogenase. In some embodiments of the engineered polypeptide derived from an opine dehydrogenase, the imine reductase activity is the activity of **Scheme 1**, optionally, a reaction as disclosed in **Table 2**, and optionally, the reaction of converting compound **(1b)** and compound **(2b)** to product compound **(3d)**.

[0009a] In one aspect, there is provided an engineered polypeptide having imine reductase activity, comprising an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 2 and one or more residue differences as compared to the sequence of SEQ ID NO: 2 at residue positions selected from X198, X111, X136, X156, X197, X201, X259, X280, X292, and X293.

[0010] In some embodiments, the present disclosure provides an engineered polypeptide having imine reductase activity, comprising an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 2 and one or more residue differences as compared to the sequence of SEQ ID NO: 2 at residue positions selected from: **X111, X136, X156, X197, X198, X201, X259, X280, X292,** and **X293**. In some embodiments, the residue differences are selected from **X111M/Q/S, X136G, X156G/I/Q/S/T/V, X197I/P, X198A/E/H/P/S, X201L, X259E/H/I/L/M/S/T, X280L, X292C/G/I/P/S/T/V/Y,** and **X293H/I/K/L/N/Q/T/V**. In some embodiments, the engineered polypeptide comprises a residue difference as compared to the sequence of SEQ ID NO: 2 at residue position **X198**, wherein optionally the residue difference at position **X198** is selected from **X198A, X198E, X198H, X198P,** and **X198S.** In some embodiments, the engineered polypeptide comprises an amino acid sequence having a residue difference at position **X198** that is selected from **X198E,** and **X198H.** In some embodiments, the amino acid sequence of the engineered polypeptides comprises at least a combination of residue differences selected from: (a) **X111M, X156T, X198H, X259M, X280L, X292V**, and **X293H**; (b) **X156T, X197P, X198H, X259H, X280L, X292P,** and **X293H**; (c) **X111M, X136G, X156S, X197I, X198H, X201L, X259H, X280L, X292V,** and **X293H**; (d) **X197I, X198E, X259M,** and **X280L**; (e) **X156T, X197I, X198E, X201L, X259H, X280L, X292V,** and **X293H**; (f) **X111M, X136G, X198H, X259M, X280L, X292S,** and **X293H**; and (g) **X156V, X197P, X198E, X201L, X259M, X280L,** and **X292T**.

[0011] In some embodiments, the present disclosure provides an engineered polypeptide having imine reductase activity, comprising an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 2 and one or more residue differences as compared to the sequence of SEQ ID NO: 2 at residue positions selected from **X111, X136, X156, X197, X198, X201, X259, X280, X292,** and **X293** (as described above), and further comprising one or more residue differences as compared to the sequence of SEQ ID NO: 2 at residue positions selected from **X4, X5, X14, X20, X29, X37, X67, X71, X74, X82, X94, X97, X100, X111, X124, X137, X141, X143, X149, X153, X154, X157, X158, X160, X163, X177, X178, X183, X184, X185, X186, X220, X223, X226, X232, X243, X246, X256, X258, X259, X260, X261, X265, X266, X270, X273, X274, X277, X279, X283, X284, X287, X288, X294, X295, X296,**

[0014] In some embodiments, the engineered polypeptide having imine reductase activity comprises an amino acid sequence having 70%, 80%, 85%, 90%, 95%, 97%, 98%, 99%, or greater identity to a sequence of even-numbered sequence identifiers SEQ ID NO: **4 – 100** and **112 - 750**.

[0014a] In one aspect, there is provided a polynucleotide encoding the engineered polypeptide as described herein.

[0015] In another ~~aspect~~embodiment, the present disclosure provides polynucleotides encoding any of the engineered polypeptides having imine reductase activity disclosed herein. Exemplary polynucleotide sequences are provided in the Sequence Listing incorporated by reference herein and include the sequences of odd-numbered sequence identifiers SEQ ID NO: **3 – 99** and **111-749**.

[0015a] In one aspect, there is provided an expression vector comprising the polynucleotide as described herein.

[0015b] In one aspect, there is provided a host cell comprising the polynucleotide as described herein or the expression vector as described herein.

[0016] In another embodiment~~aspect~~, the polynucleotides encoding the engineered polypeptides having imine reductase activity of the disclosure can be incorporated into expression vectors and host cells for expression of the polynucleotides and the corresponding encoded polypeptides.

[0016a] In one aspect, there is provided a method of preparing the engineered polypeptide having imine reductase activity, comprising culturing the host cell as described herein under conditions suitable for expression of the polypeptide, optionally further comprising isolating the engineered polypeptide.

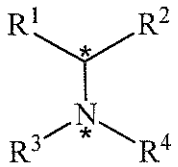
As such, in some

embodiments, the present disclosure provides methods of preparing the engineered polypeptides having imine reductase activity by culturing a host cell comprising the polynucleotide or expression vector capable of expressing an engineered polypeptide of the disclosure under conditions suitable for expression of the polypeptide. In some embodiments, the method of preparing the imine reductase polypeptide can comprise the additional step of isolating the expressed polypeptide.

[0017] In some embodiments, the present disclosure also provides methods of manufacturing an engineered polypeptide having imine reductase activity, where the method can comprise: (a) synthesizing a polynucleotide encoding a polypeptide comprising an amino acid sequence selected from the even-numbered sequence identifiers of SEQ ID NO: **4 – 100** and **112 – 750**, and having one or more residue differences as compared to SEQ ID NO:2 at residue positions selected from: **X4, X5, X14, X20, X29, X37, X67, X71, X74, X82, X94, X97, X100, X111, X124, X136, X137, X141, X143, X149, X153, X154, X156, X157, X158, X160, X163, X177, X178, X183, X184, X185, X186, X197, X198, X201, X220, X223, X226, X232, X243, X246, X256, X258, X259, X260, X261, X265, X266, X270, X273, X274, X277, X279, X280, X283, X284, X287, X288, X292, X293, X294, X295, X296, X297, X308, X311, X323, X324, X326, X328, X332, X353,** and **X356**, and (b) expressing the engineered polypeptide encoded by the polynucleotide. As noted above, the residue differences at these positions can be selected from **X4H/L/R; X5T; X14P; X20T; X29R/T; X37H; X67A/D; X71C/V; X74R; X82P; X94K/R/T; X97P; X100W; X111M/Q/R/S; X124L/N; X136G; X137N; X141W; X143W; X149L; X153V/Y;**

X154F/M/Q/Y; X156G/I/Q/S/T/V; X157D/H/L/M/N/R; X158K; X160N; X163T; X177C/H; X178E; X183C; X184K/Q/R; X185V; X186K/R; X197I/P; X198A/E/H/P/S; X201L; X220D/H; X223T; X226L; X232A/R; X243G; X246W; X256V; X258D; X259E/H/I/L/M/S/T/V/W; X260G; X261A/G/I/K/R/S/T; X265G/L/Y; X266T; X270G; X273W; X274M; X277A/I; X279F/L/V/Y; X280L; X283V; X284K/L/M/Y; X287S/T; X288G/S; X292C/G/I/P/S/T/V/Y; X293H/I/K/L/N/Q/T/V; X294A/I/V; X295R/S; X296L/N/V/W; X297A; X308F; X311C/T/V; X323C/I/M/T/V; X324L/T; X326V; X328A/G/E; X332V; X353E; and **X356R**. As further provided in the detailed description, additional variations can be incorporated during the synthesis of the polynucleotide to prepare engineered imine reductase polypeptides with corresponding differences in the expressed amino acid sequences.

[0019a] In one aspect, there is provided a process for preparing an amine compound of formula **(III)**,



(III)

wherein

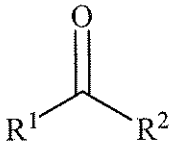
R^1 and R^2 groups are independently selected from a hydrogen atom, and optionally substituted alkyl, alkenyl, alkynyl, alkoxy, carboxy, aminocarbonyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carboxyalkyl, aminoalkyl, haloalkyl, alkylthioalkyl, cycloalkyl, aryl, arylalkyl, heterocycloalkyl, heteroaryl, and heteroarylalkyl; and optionally R^1 and R^2 are linked to form a 3-membered to 10-membered ring;

R^3 and R^4 groups are independently selected from a hydrogen atom, and optionally substituted alkyl, alkenyl, alkynyl, alkoxy, carboxy, aminocarbonyl, heteroalkyl, heteroalkenyl, heteroalkynyl, carboxyalkyl, aminoalkyl, haloalkyl, alkylthioalkyl, cycloalkyl, aryl, arylalkyl, heterocycloalkyl, heteroaryl, and heteroarylalkyl, with the proviso that both R^3 and R^4 cannot be hydrogen; and optionally R^3 and R^4 are linked to form a 3-membered to 10-membered ring; and

optionally, the carbon atom and/or the nitrogen indicated by * is chiral;

the process comprising contacting

a compound of formula **(I)**,



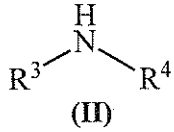
(I)

wherein

R^1, and R^2 are as defined above;

and

a compound of formula **(II)**,



(II)

wherein

R^3, and R^4 are as defined above;

with an engineered polypeptide having imine reductase activity in presence of a cofactor under suitable reaction conditions, optionally wherein the engineered polypeptide is:

(a) derived from a naturally occurring opine dehydrogenase selected from: opine dehydrogenase from *Arthrobacter sp. strain 1C* (SEQ ID NO: 2), D-octopine dehydrogenase from *Pecten maximus* (SEQ ID NO: 102), ornithine dehydrogenase from *Lactococcus lactis K1* (SEQ ID NO: 104), N-methyl-*L*-amino acid dehydrogenase from *Pseudomonas putida* (SEQ ID NO: 106), β-alanopine dehydrogenase from *Cellana grata* (SEQ ID NO: 108), and tauropine dehydrogenase from *Suberites domuncula* (SEQ ID NO: 110); or

(b) an engineered polypeptide as described herein.

[0020] In some embodiments of the process for preparing a product compound of formula **(III)** using an engineered imine reductase, the process further comprises a cofactor regeneration system capable of converting $NADP^+$ to NADPH, or NAD^+ to NADH. In some embodiments, the cofactor recycling system comprises formate and formate dehydrogenase (FDH), glucose and glucose dehydrogenase (GDH), glucose-6-phosphate and glucose-6-phosphate dehydrogenase, a secondary alcohol and alcohol dehydrogenase, or phosphite and phosphite dehydrogenase. In some embodiments, the process can be carried out, wherein the engineered imine reductase is immobilized on a solid support.

5. DETAILED DESCRIPTION

[0021] As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the context clearly indicates otherwise. Thus, for example, reference to "a polypeptide" includes more than one polypeptide. Similarly, "comprise," "comprises," "comprising" "include," "includes," and "including" are interchangeable and not intended to be limiting. It is to be understood that where descriptions of various embodiments use the term "comprising," those skilled in the art would understand that in some specific instances, an embodiment can be alternatively described using language "consisting essentially of" or "consisting of." It is to be further understood that where descriptions of various embodiments use the term "optional" or "optionally" the subsequently described event or circumstance may or may not occur, and that the description includes instances where the event or circumstance occurs and instances in which it does not. It is to be understood that both the foregoing general description, and the following detailed description are exemplary and explanatory only and are not restrictive of this disclosure. The section headings used herein are for organizational purposes only and not to be construed as limiting the subject matter described.

5.1 Abbreviations

[0022] The abbreviations used for the genetically encoded amino acids are conventional and are as follows:

Amino Acid	Three-Letter Abbreviation	One-Letter Abbreviation
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartate	Asp	D
Cysteine	Cys	C
Glutamate	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	HIS	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K