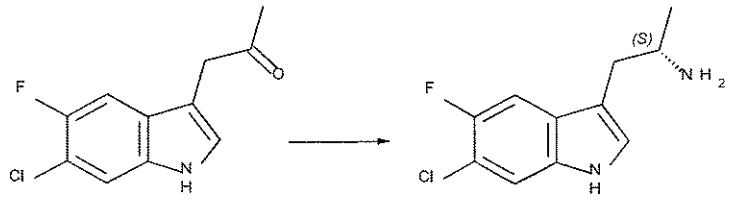
[0009] The present disclosure provides engineered polypeptides having transaminase activity, polynucleotides encoding the polypeptides, methods of the making the polypeptides, and methods of using the polypeptides for the biocatalytic conversion of ketone substrates to amine products. The present enzymes have been engineered to have one or more residue differences as compared to the amino acid sequence of the naturally occurring transaminase of *Vibrio fluvialis* (SEQ ID NO:2) and a reference engineered transaminase with enhanced solvent and thermal stability (SEQ ID NO:4), where the residue differences occur at residue positions affecting various enzyme properties, including among others, activity, stereoselectivity, stability, expression, and product tolerance. In particular, the transaminases of the present disclosure have been engineered for efficient formation of chiral tryptamine derivatives from its corresponding prochiral ketone substrates. Thus, the engineered polypeptides disclosed herein display, among others, increased activity, solvent and thermal stability, increased product tolerance, and high stereoselectivity in the formation of tryptamine derivatives from the corresponding prochiral ketone substrates, particularly in the conversion of substrate compound (**2**), 1-(1*H*-5-fluoro-6-chloro-indol-3-yl)propan-2-one, to product compound (**1**), (*S*)-1-(1*H*-5-fluoro-6-chloro-indol-3-yl)propan-2-amine,



(2) **(1)**

[0010] Accordingly, in one ~~aspect~~embodiment, the present disclosure provides engineered polypeptides having transaminase activity, where the engineered polypeptide comprises an amino acid sequence having at least 80% identity to SEQ ID NO: 2 or 4 and one or more residue differences as compared to SEQ ID NO:4 at residue positions selected from: X14; X26; X31; X33; X41; X47; X57; X70; X86; X88; X107; X132; X148; X163; X168; X173; X203; X250; X284; X314; X315; X324; X346; X395; X398; X400; X417; X419; X423; X448; and X451, wherein the residue differences at residue positions X31; X57; X86; X163; X168; X314; X324; X398; and X417 are selected from: X31S; X57Y; X86D; X163I; X163L; X163R; X163V; X168S; X314N; X324H; X398L; X398V; X398W; and X417M.

[0011] In some embodiments, the residue differences at the residue positions X14; X26; X33; X41; X47; X70; X88; X107; X132; X148; X173; X203; X250; X284; X315; X346; X395; X400; X419; X423; X448; and X451 are selected from X14V; X26R; X33T; X41L; X47N; X70A; X88A; X88L; X107P; X132F; X148Q; X148R; X173A; X203S; X250A; X284A; X315G; X346L; X395P; X400G; X419S; X423I; X448E; and X451D.

[0012] As provided herein, in some embodiments, the disclosed residue differences can be used singly or in various combinations to generate the engineered polypeptides having the improved

enzyme properties. Guidance on the choice of residue differences and effects on enzyme properties are provided in the detailed description herein.

[0012a] In one aspect, there is provided an engineered polypeptide having transaminase activity, comprising an amino acid sequence having at least 80% identity to SEQ ID NO: 4 and one or more residue differences as compared to SEQ ID NO:4 at residue positions selected from: X14; X26; X31; X33; X41; X47; X57; X70; X86; X88; X107; X132; X148; X163; X168; X173; X203; X250; X284; X314; X315; X324; X346; X395; X398; X400; X417; X419; X423; X448; and X451, wherein the residue differences at residue positions X31; X57; X86; X163; X168; X314; X324; X398; and X417 are selected from: X31S; X57Y; X86D; X163I; X163L; X163R; X163V; X168S; X314N; X324H; X398L; X398V; X398W; and X417M.

[0012b] In some embodiments, the engineered transaminase polypeptide comprises an amino acid sequence having at least 80% sequence identity to reference sequence SEQ ID NO:4 and at least a residue difference as compared to SEQ ID NO:4 at residue positions selected from X14, X86, X163, or X400. In some embodiments, the amino acid sequence has at least a residue difference at position X163, where the amino acid residues are selected from I, L, R and V.

[0013] In some embodiments, the engineered transaminase polypeptide comprises an amino acid sequence having a combination of residue differences as compared to SEQ ID NO:4 selected from: (a) X14V and X163I/L/R/V; (b) X86D and X400G; (c) X57F/Y and X163I/L/R/V; (d) X57F/Y and X398L/V/W; (e) X14V, X113L/V, X163I/L/R/V, X284A, and X424V; and (f) X31S, X57F/Y, X163I/L/R/V, X315G, X346L, and X398L/V/W.

[0014] In some embodiments, the engineered transaminase polypeptide comprises an amino acid sequence having a combination of residue differences as compared to SEQ ID NO:4 selected from: (a) X14V, X113L, X163L, X284A, and X424V; (b) X14V, X26R, X163L, X284A, and X400G; (c) X14V, X26R, X88L, and X113L; (d) X57F, X163L, X168K, X314N, X315G, X346L, and X398V; (e) X14V, X163L, X173A, X400G, and X420N; (f) X14V, X113L, X163L, and X284A; (g) X14V, X26R, X163L, X284A, and X400G; and (h) X14V, X33T, X57F, X113L, and X163L.

[0015] In some embodiments, the engineered transaminase polypeptide is capable of converting substrate compound (**2**), 1-(1*H*-5-fluoro-6-chloro-indol-3-yl)propan-2-one, to product compound (**1**), (*S*)-1-(1*H*-5-fluoro-6-chloro-indol-3-yl)propan-2-amine, in at least 90% enantiomeric excess under suitable reaction conditions.

[0016] In some embodiments, the engineered transaminase polypeptide has at least 1.2 fold the activity of SEQ ID NO:4 in converting substrate compound **(2)** to product compound **(1)**, wherein the amino acid sequence comprises one or more residue differences selected from: X14V, X26R; X31S; X33T; X41L; X70A; X86D; X88A/L; X163I/L; X284A; and X419S. In some embodiments, the engineered transaminase polypeptide has at least 5 fold the activity of SEQ ID NO:4 in converting

substrate compound **(2)** to product compound **(1)**, wherein the amino acid sequence comprises one or more residue differences selected from: X14V, X26R; X33T; X88A/L; X163I/L; and X284A.

[0017] In some embodiments, the engineered transaminase polypeptide has at least 1.2 fold increased refractoriness to product compound (**1**) inhibition as compared to SEQ ID NO:4 in the conversion of substrate compound **(2)** to product compound (**1**), under suitable reaction conditions, wherein the amino acid sequence comprises one or more residue differences selected from: X26R; X70A; X86D; X88A/L; X132F; X163L; X315G; X395P; X398L; and X419S. In some embodiments, the engineered transaminase polypeptide has at least 5 fold increased refractoriness to product compound

(**1**) inhibition as compared to SEQ ID NO:4 in the conversion of substrate compound (**2**) to product compound (**1**), under suitable reaction conditions, wherein the amino acid sequence comprises one or more residue differences selected from: X26R; X88L; and X163L.

[0018] In some embodiments, the engineered transaminase polypeptides can have additional residue differences at other residue positions. In some embodiments, the engineered transaminases can have 1-2, 1-3, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9, 1-10, 1-15, 1-20, 1-21, 1-22, 1-23, 1-24, 1-25, 1-30, 1-35, 1-40, 1-45, or 1-50 additional residue differences as compared to SEQ ID NO:2 or 4. In some embodiments, the engineered transaminases can have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 30, 35, 40, 45, or 50 additional residue differences. In some embodiments, the amino acid sequence has additionally 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 20, 21, 22, 23, 24, or 25 residue differences as compared to SEQ ID NO: 2 or 4.

[0019] Exemplary engineered polypeptides incorporating the residue differences, including various combinations thereof, and having improved properties (*e.g.*, capable of converting compound (**2**) to compound (**1**) in at least 90% enantiomeric excess under suitable reaction conditions) are disclosed in **Tables 2A** and **2B**, and the Examples. The amino acid sequences are provided in the Sequence Listing and include SEQ ID NO: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, and 154.

[0019a] In one aspect, there is provided a polynucleotide encoding a engineered transaminase polypeptide as described herein.

[0019b] In one aspect, there is provided an expression vector comprising a polynucleotide as described herein, optionally further comprising a control sequence.

[0019c] In one aspect, there is provided a host cell comprising a polynucleotide or an expression vector as described herein.

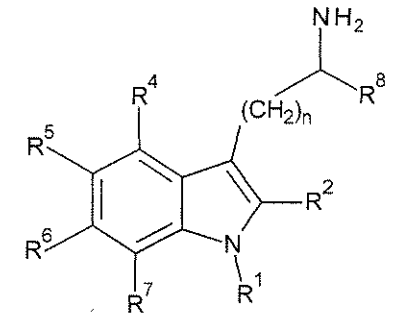
[0020] In another ~~aspect~~embodiment, the present disclosure provides polynucleotides encoding the engineered polypeptides having transaminase activity, as well as expression vectors comprising the polynucleotides, and host cells capable of expressing the polynucleotides encoding the engineered polypeptides. Exemplary polynucleotide sequences are provided in the Sequence Listing incorporated by reference herein and include SEQ ID NO: 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, and 153.

[0020a] In one aspect, there is provided a method of preparing the engineered polypeptide comprising culturing a host cell as described herein under conditions suitable for expression of the polypeptide, optionally further comprising isolating the engineered polypeptide.

[0021] In some embodiments, the present disclosure also provides methods of manufacturing the engineered transaminase polypeptides, where the method can comprise culturing a host cell capable of expressing a polynucleotide encoding the engineered transaminase polypeptide under conditions suitable for expression of the polypeptide. In some embodiments, the method for manufacturing the engineered transaminase polypeptide can also include: (a) synthesizing a polynucleotide encoding a polypeptide comprising an amino acid sequence selected from SEQ ID NO: 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118,

120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, and 154 and having one or more residue differences as compared to SEQ ID NO:4 at residue positions selected from: X14; X26; X31; X33; X41; X47; X57; X70; X86; X88; X107; X132; X148; X163; X168; X173; X203; X250; X284; X314; X315; X324; X346; X395; X398; X400; X417; X419; X423; X448; and X451, wherein the residue differences at residue positions X31; X57; X86; X163; X168; X314; X324; X398; and X417 are selected from: X31S; X57Y; X86D; X163I; X163L; X163R; X163V; X168S; X314N; X324H; X398L; X398V; X398W; and X417M; and (b) expressing the transaminase polypeptide encoded by the polynucleotide. As noted above, the residue differences at residue positions X14; X26; X33; X41; X47; X70; X88; X107; X132; X148; X173; X203; X250; X284; X315; X346; X395; X400; X419; X423; X448; and X451 can be selected from X14V; X26R; X33T; X41L; X47N; X70A; X88A; X88L; X107P; X132F; X148Q; X148R; X173A; X203S; X250A; X284A; X315G; X346L; X395P; X400G; X419S; X423I; X448E; and X451D. As further provided in the detailed description, additional variations can be incorporated during the synthesis of the polynucleotide to prepare engineered transaminases with corresponding differences in the expressed amino acid sequences.

[0021a] In one aspect, there is provided a process for preparing a compound of formula (I),



(I)

wherein

R^1 is selected from the group consisting of hydrogen, carboxy, carboxy(C_1-C_6)alkyl, optionally substituted (C_1-C_6)alkyl, optionally substituted (C_2-C_6)alkenyl, optionally substituted alkyloxycarbonyl, optionally substituted arylcarbonyl, optionally substituted arylsulfonyl, and a protecting group;

R^2 is selected from the group consisting of hydrogen, oxo, halo, hydroxy, amino, thio, optionally substituted (C_1-C_6)alkyl, (C_1-C_6)alkylamino, (C_1-C_6)dialkylamino, (C_1-C_6)alkylthio, (C_1-C_6)alkylsulfonyl, (C_1-C_6)alkylsulfinyl, and optionally substituted (C_1-C_6)alkyloxy;

R^4, R^6 and R^7 are each, independently of the others, selected from the group consisting of hydrogen, halo, hydroxy, amino, carboxy, cyano, nitro, thio, optionally substituted (C_1-C_6)alkyl, hydroxy(C_1-C_6)alkyl, optionally substituted(C_1-C_6)alkyloxy, optionally substituted (C_1-C_6)alkylamino, optionally substituted (C_1-C_6)dialkylamino, optionally substituted (C_1-C_6)alkylthio, optionally

substituted (C_1-C_6)alkylsulfonyl, optionally substituted (C_1-C_6)alkylsulfinyl, carboxy(C_1-C_6)alkyl, (C_1-C_6)alkyloxycarbonyl, (C_1-C_6)alkylcarbonyloxy, optionally substituted aminocarbonyl, aminocarbonyl(C_1-C_6)alkyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted aryloxy, optionally substituted arylamino, optionally substituted arylthio, optionally substituted arylsulfonyl, optionally substituted arylsulfinyl, optionally substituted aryloxycarbonyl, optionally substituted arylcarbonyloxy, optionally substituted heteroaryloxy, optionally substituted heteroarylamino, optionally substituted heteroarylthio, optionally substituted heteroarylsulfonyl, optionally substituted heteroarylsulfinyl, optionally substituted heteroaryloxycarbonyl, optionally substituted heteroarylcarbonyloxy, alkylaminosulfonyl(C_1-C_6)alkyl, arylsulfonyl(C_1-C_6)alkyl, and heteroarylsulfonyl(C_1-C_6)alkyl;

R^5 is selected from the group consisting of hydrogen, halo, hydroxy, amino, carboxy, cyano, nitro, thio, optionally substituted (C_1-C_6)alkyl, hydroxy(C_1-C_6)alkyl, optionally substituted(C_1-C_6)alkyloxy, optionally substituted (C_1-C_6)alkylamino, optionally substituted (C_1-C_6)dialkylamino, optionally substituted (C_1-C_6)alkylthio, optionally substituted (C_1-C_6)alkylsulfonyl, optionally substituted (C_1-C_6)alkylsulfinyl, carboxy(C_1-C_6)alkyl, (C_1-C_6)alkyloxycarbonyl, (C_1-C_6)alkylcarbonyloxy, optionally substituted aminocarbonyl, aminocarbonyl(C_1-C_6)alkyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted aryloxy, optionally substituted arylamino, optionally substituted arylthio, optionally substituted arylsulfonyl, optionally substituted arylsulfinyl, optionally substituted aryloxycarbonyl, optionally substituted arylcarbonyloxy, optionally substituted heteroaryloxy, optionally substituted heteroarylamino, optionally substituted heteroarylthio, optionally substituted heteroarylsulfonyl, optionally substituted heteroarylsulfinyl, optionally substituted heteroaryloxycarbonyl, optionally substituted heteroarylcarbonyloxy, alkylaminosulfonyl(C_1-C_6)alkyl, arylsulfonyl(C_1-C_6)alkyl, and heteroarylsulfonyl(C_1-C_6)alkyl, or together with R^4 forms a 5 to 8 membered optionally substituted cycloalkyl or optionally substituted heterocyclic ring;

R^8 is selected from the group consisting of optionally substituted (C_1-C_6) alkyl, optionally substituted (C_1-C_6)alkyloxy, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, and optionally substituted heteroaryl, or together with R^2 forms an optionally substituted 5 to 8 membered cycloalkyl or heterocyclic ring; and

n is 1 or 2;

with the provisos that

(a) when R^2 is hydrogen, then at least one of the following applies:

(i) R^1 is not hydrogen, methyl, 4-(methyloxy)phenylcarbonyl-, 4-(trifluoromethyloxy)phenylsulfonyl-, 3-bromophenylcarbonyl-, 3-aminopropyl-, or 3-(methylcarbonylamino)propyl-;

(ii) R^4 and R^7 are each, independently of the other, not hydrogen or chloro;

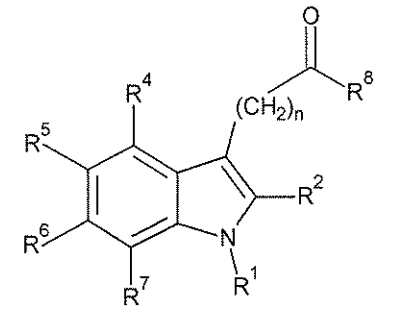
(iii) R^5 is not hydrogen, hydroxy, methyl, methyloxy, fluoro, chloro, trifluoromethyl, or cyano;

(iv) R^6 is not hydrogen, hydroxy, methyloxy, fluoro or chloro; or

(v) R^8 is not methyl, ethyl, hydroxymethyl, or trifluoromethyl-; and

(b) when n is 1, R^2 and R^8 together form a cyclohexyl ring, and R^1, R^4, R^6, and R^7 are hydrogen, then R^5 is not fluoro;

comprising contacting the substrate compound of formula (II),

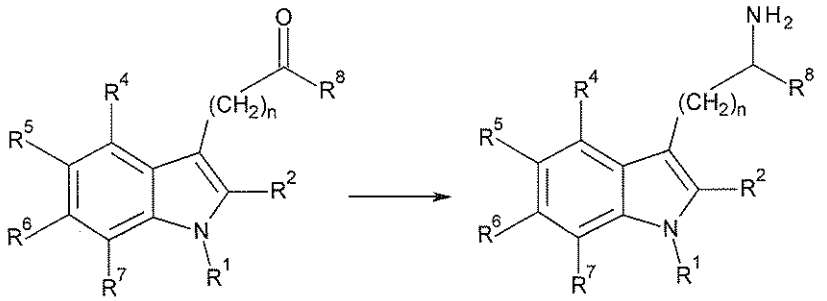


(II)

wherein R^1, R^2, R^4, R^5, R^6, R^7, R^8 and n are as defined above,

in presence of an amino donor under suitable reaction conditions with an engineered transaminase polypeptide as described herein.

[0022] In another ~~aspect~~embodiment, the engineered transaminase polypeptides can be used in a process for preparing various derivatives of tryptamine and tryptamine analogs, such as substituted homotryptamine, from their corresponding ketone substrates. Accordingly, in some embodiments, the engineered transaminase polypeptides can be used in a process for the conversion of substrate compound of formula **(II)** to product compound of formula **(I)**, as shown below:



(II) **(I)**

wherein

R^1 is selected from the group consisting of hydrogen, carboxy, carboxy(C_1-C_6)alkyl, optionally substituted (C_1-C_6)alkyl, optionally substituted (C_2-C_6)alkenyl, optionally substituted alkyloxycarbonyl, optionally substituted arylcarbonyl, optionally substituted arylsulfonyl, and a protecting group;

R^2 is selected from the group consisting of hydrogen, oxo, halo, hydroxy, amino, thio, optionally substituted (C_1-C_6)alkyl, (C_1-C_6)alkylamino, (C_1-C_6)dialkylamino, (C_1-C_6)alkylthio, (C_1-C_6)alkylsulfonyl, (C_1-C_6)alkylsulfinyl, and optionally substituted (C_1-C_6)alkyloxy;

(a) when R^2 is hydrogen, then at least one of the following applies:

(i) R^1 is not hydrogen, methyl, 4-(methyloxy)phenylcarbonyl-, 4-(trifluoromethyloxy)phenylsulfonyl-, 3-bromophenylcarbonyl-, 3-aminopropyl-, or 3-(methylcarbonylamino)propyl-;

(ii) R^4 and R^7 are each, independently of the other, not hydrogen or chloro;

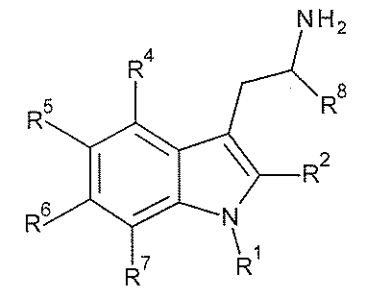
(iii) R^5 is not hydrogen, hydroxy, methyl, methyloxy, fluoro, chloro, trifluoromethyl, or cyano;

(iv) R^6 is not hydrogen, hydroxy, methyloxy, fluoro or chloro; or

(v) R^8 is not methyl, ethyl, hydroxymethyl, or trifluoromethyl-; and

(b) when n is 1, R^2 and R^8 together form a cyclohexyl ring, and R^1, R^4, R^6, and R^7 are hydrogen, then R^5 is not fluoro.

[0022a] In one aspect, there is provided a process for preparing a compound of formula (Ia):



(Ia)

wherein,

R^1 is selected from the group consisting of hydrogen, carboxy, carboxy(C_1-C_6)alkyl, optionally substituted (C_1-C_6)alkyl, optionally substituted (C_2-C_6)alkenyl, optionally substituted alkyloxycarbonyl, optionally substituted arylcarbonyl, optionally substituted arylsulfonyl, and a protecting group;

R^2 is selected from the group consisting of hydrogen, oxo, halo, hydroxy, amino, thio, optionally substituted (C_1-C_6)alkyl, (C_1-C_6)alkylamino, (C_1-C_6)dialkylamino, (C_1-C_6)alkylthio, (C_1-C_6)alkylsulfonyl, (C_1-C_6)alkylsulfinyl, and optionally substituted (C_1-C_6)alkyloxy;

R^4, R^5, R^6 and R^7 are each, independently of the others, selected from the group consisting of hydrogen, halo, hydroxy, amino, carboxy, cyano, nitro, thio, optionally substituted (C_1-C_6)alkyl, hydroxy(C_1-C_6)alkyl, optionally substituted(C_1-C_6)alkyloxy, optionally substituted (C_1-C_6)alkylamino, optionally substituted (C_1-C_6)dialkylamino, optionally substituted (C_1-C_6)alkylthio, optionally substituted (C_1-C_6)alkylsulfonyl, optionally substituted (C_1-C_6)alkylsulfinyl, carboxy(C_1-C_6)alkyl, (C_1-C_6)alkyloxycarbonyl, (C_1-C_6)alkylcarbonyloxy, optionally substituted aminocarbonyl, aminocarbonyl(C_1-C_6)alkyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted aryloxy, optionally

substituted arylamino, optionally substituted arylthio, optionally substituted arylsulfonyl, optionally substituted arylsulfinyl, optionally substituted aryloxycarbonyl, optionally substituted arylcarbonyloxy, optionally substituted heteroaryloxy, optionally substituted heteroarylamino, optionally substituted heteroarylthio, optionally substituted heteroarylsulfonyl, optionally substituted heteroarylsulfinyl, optionally substituted heteroaryloxycarbonyl, optionally substituted heteroarylcarbonyloxy, alkylaminosulfonyl(C_1-C_6)alkyl, arylsulfonyl(C_1-C_6)alkyl, and heteroarylsulfonyl(C_1-C_6)alkyl; and

R^8 is selected from the group consisting of optionally substituted (C_1-C_6) alkyl, optionally substituted (C_1-C_6)alkyloxy, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, and optionally substituted heteroaryl;

with the proviso that when $R^2$is hydrogen, then at least one of the following applies:

(i) R^1 is not hydrogen, methyl, 4-(methyloxy)phenylcarbonyl-, 4-(trifluoromethyloxy)phenylsulfonyl-, 3-bromophenylcarbonyl-, 3-aminopropyl-, or 3-(methylcarbonylamino)propyl-;

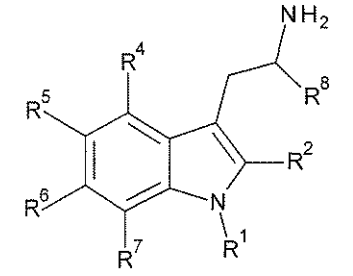
(ii) R^4 and R^7 are each, independently of the other, not hydrogen or chloro;

(iii) R^5 is not hydrogen, hydroxy, methyl, methyloxy, fluoro, chloro, trifluoromethyl or cyano;

(iv) R^6 is not hydrogen, hydroxy, methyloxy, fluoro or chloro; or

(v) R^8 is not methyl, ethyl, hydroxymethyl-, or trifluoromethyl-;

comprising contacting the substrate compound of formula (**IIa**)

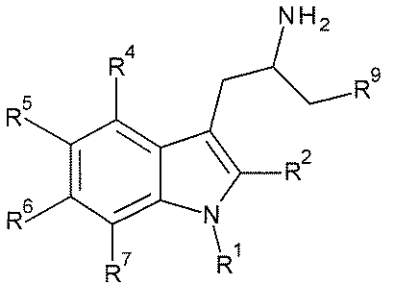


(IIa)

wherein R^1, R^2, R^4, R^5, R^6, R^7, and R^8 are as defined above,

in presence of an amino donor under suitable reaction conditions with an engineered transaminase polypeptide as described herein.

[0022b] In one aspect, there is provided a process for preparing a compound of formula (**Ia1**)



(Ia1)

wherein

R^1 is selected from the group consisting of hydrogen and (C_1-C_6)alkyl;

R^2 is selected from the group consisting of hydrogen, halo, and (C_1-C_6)alkyl;

R^4, R^5, R^6, and R^7 are each, independently of the others, selected from the group consisting of hydrogen, halo, hydroxy, optionally substituted (C_1-C_6)alkyl, hydroxy(C_1-C_6)alkyl, (C_1-C_6)alkyl; amino, (C_1-C_6)alkylamino, and (C_1-C_6)dialkylamino; and

R^9 is selected from the group consisting of halo, hydroxy, hydroxy(C_1-C_6)alkyl, amino, (C_1-C_6)alkylamino, and (C_1-C_6)dialkylamino;

with the proviso that when R^2 is hydrogen, then at least one of the following applies:

(i) R^1 is not hydrogen or methyl;

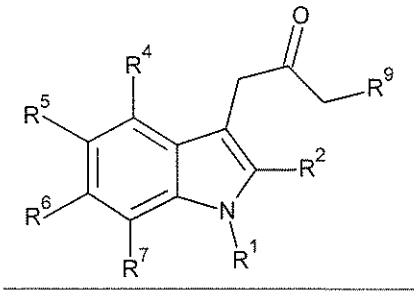
(ii) R^4 and R^7 are each, independently of the other, not hydrogen or chloro;

(iii) R^5 is not hydrogen, hydroxy, methyl, methyloxy, fluoro, chloro, or trifluoromethyl;

(iv) R^6 is not hydrogen, hydroxy, methyloxy, fluoro or chloro; or

(v) R^9 is not hydroxy;

comprising contacting the substrate compound of formula (**IIa1**)



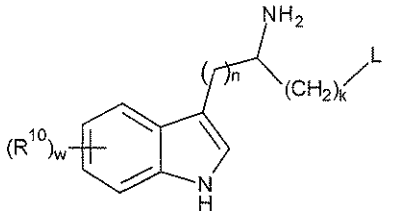
(IIa1)

wherein

R^1, R^2, R^4, R^5, R^6, R^7 and R^9 are as defined above,

in presence of an amino donor under suitable reaction conditions with an engineered transaminase polypeptide as described herein.

[0022c] In one aspect, there is provided a process for preparing a compound of formula (**Ia2**)



(Ia2)

wherein

L is a leaving group;

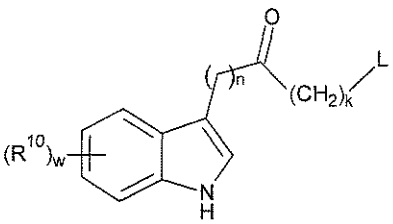
each occurrence of R^{10} is, independently of the others, selected from the group consisting of halo, hydroxy, amino, carboxy, cyano, nitro, thio, optionally substituted (C_1-C_6)alkyl, hydroxy(C_1-C_6)alkyl, optionally substituted(C_1-C_6)alkyloxy, optionally substituted (C_1-C_6)alkylamino, optionally substituted (C_1-C_6)dialkylamino, optionally substituted (C_1-C_6)alkylthio, optionally substituted (C_1-C_6)alkylsulfonyl, optionally substituted (C_1-C_6)alkylsulfinyl, carboxy(C_1-C_6)alkyl, (C_1-C_6)alkyloxycarbonyl, (C_1-C_6)alkylcarbonyloxy, optionally substituted aminocarbonyl, aminocarbonyl(C_1-C_6)alkyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted aryloxy, optionally substituted arylamino, optionally substituted arylthio, optionally substituted arylsulfonyl, optionally substituted arylsulfinyl, optionally substituted aryloxycarbonyl, optionally substituted arylcarbonyloxy, optionally substituted heteroaryloxy, optionally substituted heteroarylamino, optionally substituted heteroarylthio, optionally substituted heteroarylsulfonyl, optionally substituted heteroarylsulfinyl, optionally substituted heteroaryloxycarbonyl, optionally substituted heteroarylcarbonyloxy, alkylaminosulfonyl(C_1-C_6)alkyl, arylsulfonyl(C_1-C_6)alkyl, and heteroarylsulfonyl(C_1-C_6)alkyl;

k is an integer from 3 to 5;

n is 1 or 2; and

w is an integer from 0 to 4;

comprising contacting the substrate compound of formula **(IIa2)**



(IIa2)

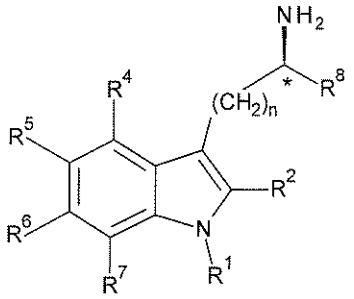
wherein

L, R^{10}, k, n and w are as defined above;

in presence of an amino donor under suitable reaction conditions with an engineered transaminase polypeptide as described herein.

[0023] Accordingly, in some embodiments, a process for preparing the compound of structural formula **(I)** can comprise contacting the compound of structural formula **(II)** with any of the engineered transaminases of the present disclosure in presence of an amine donor under suitable reaction conditions.

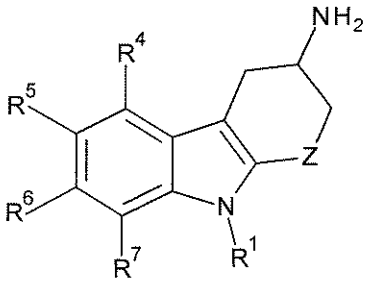
[0024] In some embodiments, the stereoselectivity of the transaminases can be used to prepare the chiral compound of formula **(*IS*),**



(*IS*)

wherein R^1, R^2, R^4, R^5, R^6, R^7, R^8 and n are as defined above, and having the indicated stereochemistry at the carbon atom marked with an *, wherein compound of formula **(*IS*)** is formed in enantiomeric excess. In some embodiments of the process, the compound of formula **(*IS*)** can be formed in at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater enantiomeric excess.

[0024a] In one aspect, there is provided a process for preparing a compound of formula (Ib)



(Ib)

wherein

Z is selected from the group consisting of O, S, NH or $-(CH_2)_m-$, wherein m is 0, 1, 2 or 3;

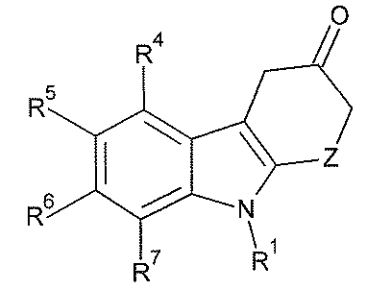
R^1 is selected from the group consisting of hydrogen, carboxy, carboxy(C_1-C_6)alkyl, optionally substituted (C_1-C_6)alkyl, optionally substituted (C_2-C_6)alkenyl, optionally substituted alkyloxycarbonyl, optionally substituted arylcarbonyl, optionally substituted arylsulfonyl, and a

protecting group;

R^4, R^5, R^6 and R^7 are each, independently of the others, selected from the group consisting of hydrogen, halo, hydroxy, amino, carboxy, cyano, nitro, thio, optionally substituted (C_1-C_6)alkyl, hydroxy(C_1-C_6)alkyl, optionally substituted(C_1-C_6)alkyloxy, optionally substituted (C_1-C_6)alkylamino, optionally substituted (C_1-C_6)dialkylamino, optionally substituted (C_1-C_6)alkylthio, optionally substituted (C_1-C_6)alkylsulfonyl, optionally substituted (C_1-C_6)alkylsulfinyl, carboxy(C_1-C_6)alkyl, (C_1-C_6)alkyloxycarbonyl, (C_1-C_6)alkylcarbonyloxy, optionally substituted aminocarbonyl, aminocarbonyl(C_1-C_6)alkyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted aryloxy, optionally substituted arylamino, optionally substituted arylthio, optionally substituted arylsulfonyl, optionally substituted arylsulfinyl, optionally substituted aryloxycarbonyl, optionally substituted arylcarbonyloxy, optionally substituted heteroaryloxy, optionally substituted heteroarylamino, optionally substituted heteroarylthio, optionally substituted heteroarylsulfonyl, optionally substituted heteroarylsulfinyl, optionally substituted heteroaryloxycarbonyl, optionally substituted heteroarylcarbonyloxy, alkylaminosulfonyl(C_1-C_6)alkyl, arylsulfonyl(C_1-C_6)alkyl, and heteroarylsulfonyl(C_1-C_6)alkyl;

with the proviso that when Z is $-(CH_2)_m-$, m is 1, and R^1, R^4, R^6 and R^7 are hydrogen, then R^5 is not fluoro;

comprising contacting the substrate compound of formula (**IIb**)

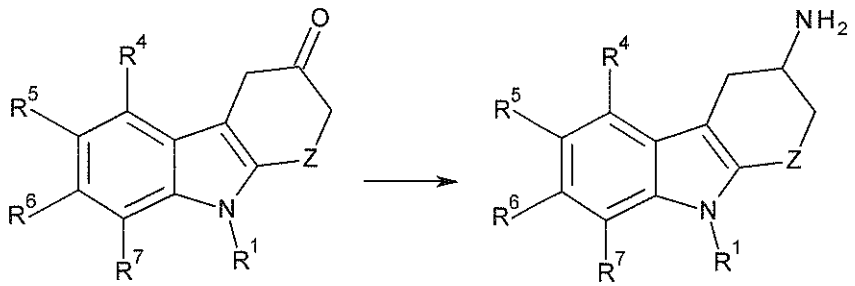


(**IIb**)

wherein Z, R^1, R^4, R^5, R^6, and R^7 are as defined above;

in presence of an amino donor under suitable reaction conditions with an engineered transaminase polypeptide as described herein.

[0025] In some embodiments, the engineered transaminase polypeptides can be used in a process for the conversion of substrate compounds of formula **(IIb)** to product compounds of structural formula **(Ib)**, as shown below:



(IIb) **(Ib)**

wherein,

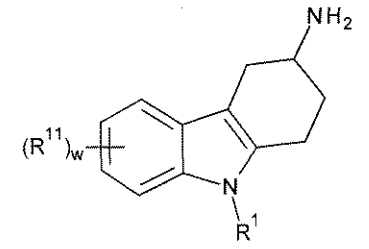
Z is selected from the group consisting of O, S, NH, or $-(CH_2)_m-$, wherein m is 0, 1, 2 or 3;

R^1 is selected from the group consisting of hydrogen, carboxy, carboxy(C_1-C_6)alkyl, optionally substituted (C_1-C_6)alkyl, optionally substituted (C_2-C_6)alkenyl, optionally substituted alkyloxycarbonyl, optionally substituted arylcarbonyl, optionally substituted arylsulfonyl, and a protecting group;

R^4, R^5, R^6 and R^7 are each, independently of the others, selected from the group consisting of hydrogen, halo, hydroxy, amino, carboxy, cyano, nitro, thio, optionally substituted (C_1-C_6)alkyl, hydroxy(C_1-C_6)alkyl, optionally substituted(C_1-C_6)alkyloxy, optionally substituted (C_1-C_6)alkylamino, optionally substituted (C_1-C_6)dialkylamino, optionally substituted (C_1-C_6)alkylthio, optionally substituted (C_1-C_6)alkylsulfonyl, optionally substituted (C_1-C_6)alkylsulfinyl, carboxy(C_1-C_6)alkyl, (C_1-C_6)alkyloxycarbonyl, (C_1-C_6)alkylcarbonyloxy, optionally substituted aminocarbonyl, aminocarbonyl(C_1-C_6)alkyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted aryloxy, optionally substituted arylamino, optionally substituted arylthio, optionally substituted arylsulfonyl, optionally substituted arylsulfinyl, optionally substituted aryloxycarbonyl, optionally substituted arylcarbonyloxy, optionally substituted heteroaryloxy, optionally substituted heteroarylamino, optionally substituted heteroarylthio, optionally substituted heteroarylsulfonyl, optionally substituted heteroarylsulfinyl, optionally substituted heteroaryloxycarbonyl, optionally substituted heteroarylcarbonyloxy, alkylaminosulfonyl(C_1-C_6)alkyl, arylsulfonyl(C_1-C_6)alkyl, and heteroarylsulfonyl(C_1-C_6)alkyl;

with the proviso that when Z is $-(CH_2)_m-$, m is 1, and R^1, R^4, R^6 and R^7 are hydrogen, then R^5 is not fluoro.

[0025a] In one aspect, there is provided a process for preparing a compound of formula (Ib1)



(Ib1)

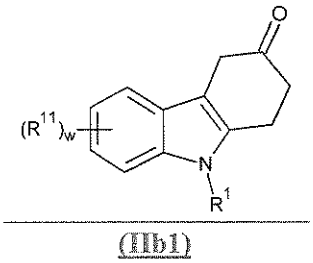
wherein

R^1 is selected from the group consisting of hydrogen, carboxy(C_1-C_6)alkyl, and (C_1-C_6)alkyl;

each occurrence of R^{11} is, independently of the others, selected from the group consisting of chloro, bromo, iodo, hydroxy, amino, carboxy, cyano, nitro, thio, optionally substituted (C_1-C_6)alkyl, hydroxy(C_1-C_6)alkyl, optionally substituted(C_1-C_6)alkyloxy, optionally substituted (C_1-C_6)alkylamino, optionally substituted (C_1-C_6)dialkylamino, optionally substituted (C_1-C_6)alkylthio, optionally substituted (C_1-C_6)alkylsulfonyl, optionally substituted (C_1-C_6)alkylsulfinyl, carboxy(C_1-C_6)alkyl, (C_1-C_6)alkyloxycarbonyl, (C_1-C_6)alkylcarbonyloxy, optionally substituted aminocarbonyl, aminocarbonyl(C_1-C_6)alkyl, optionally substituted cycloalkyl, optionally substituted heterocycloalkyl, optionally substituted aryl, optionally substituted heteroaryl, optionally substituted aryloxy, optionally substituted arylamino, optionally substituted arylthio, optionally substituted arylsulfonyl, optionally substituted arylsulfinyl, optionally substituted aryloxycarbonyl, optionally substituted arylcarbonyloxy, optionally substituted heteroaryloxy, optionally substituted heteroarylamino, optionally substituted heteroarylthio, optionally substituted heteroarylsulfonyl, optionally substituted heteroarylsulfinyl, optionally substituted heteroaryloxycarbonyl, optionally substituted heteroarylcarbonyloxy, alkylaminosulfonyl(C_1-C_6)alkyl, arylsulfonyl(C_1-C_6)alkyl, and heteroarylsulfonyl(C_1-C_6)alkyl; and

w is an integer from 0 to 4;

comprising contacting the substrate compound of formula **(IIb1)**



(IIb1)

wherein

R^1, R^{11} and w are as defined above;

in presence of an amino donor under suitable reaction conditions with an engineered transaminase polypeptide as described herein.

[0026] Accordingly, in some embodiments, a process for preparing the compound of structural formula **(Ib)** can comprise contacting the compound of structural formula **(IIb)** with any of the engineered transaminases of the present disclosure in presence of an amine donor under suitable reaction conditions.

[0027] In some embodiments, the stereoselectivity of the transaminases can be used to prepare the chiral compound of formula **(Ib*S*),**