4. SUMMARY

[0011] The present disclosure provides transaminase polypeptides having the ability to catalyze the transfer of an amino group from a donor amine to an amine acceptor molecule, polynucleotides encoding such polypeptides, and methods for using the polypeptides. Generally, transaminase polypeptides are useful for the enantiomeric enrichment and stereoselective synthesis of chiral amines.

[0011a] In one aspect, there is provided an engineered transaminase enzyme, comprising: a polypeptide comprising an amino acid sequence that has a T residue at position X9 and is at least 90% identical to SEQ ID NO: 6, wherein the polypeptide has transaminase activity.

[0012] In one embodiment~~aspect~~, the transaminase polypeptides described herein have altered properties as compared to a reference enzyme, such as the naturally occurring transaminase obtained with *Vibrio fluvialis* (e.g., SEQ ID NO:2) or an engineered transaminase, such as the polypeptide of SEQ ID NO:18. Changes to enzyme properties can include, among others, improvements in enzymatic activity, stereoselectivity, sterospecificity, thermostability, solvent stability, and/or reduced substrate or product inhibition. In the embodiments herein, the altered properties are based on engineered transaminase polypeptides having residue differences at specific residue positions as compared to a reference sequence of a naturally occurring *Vibrio fluvialis* transaminase or a reference engineered transaminase, such as the sequence of SEQ ID NO:18. In some embodiments, the residue differences are present at one or more of the following residue positions: X4, X6, X9, X12, X21, X30, X31, X44, X45, X, 56, X57, X81, X82, X85, X86, X95, X112, X113, X127, X147, X153, X157, X166, X177, X181, X208, X211, X228, X233, X253, X272, X294, X297, X302, X311, X314, X316, X317, X318, X319, X320, X321, X324, X385, X391, X398, X407, X408, X409, X415, X417, X418, X420, X431, X434, X438, X444, and X446. Amino acid residues that can be present at the specified residue positions and the associated changes to enzyme properties are provided in the detailed description.

[0013] In some embodiments, the transaminase polypeptides of the disclosure are characterized by increased thermostability as compared to the wild-type polypeptide under the same reaction conditions. Thus, the polypeptides are capable of mediating transamination reactions (e.g., reaction schemes I, II or III), as indicated by the continued formation of products, at higher temperatures and for longer times than the wild-type polypeptide. In some embodiments, the transaminase polypeptides of the disclosure~~invention~~ are improved in being able to retain activity under higher concentrations of the donor amine, such as 2 M concentration of isopropylamine. In some embodiments, the engineered transaminases with increased thermostability and/or increased isopropylamine stability comprises an amino acid sequence that is at least about 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the reference sequence of SEQ ID NO: 10, 16 or 18.

[0014] In some embodiments, the engineered transaminase polypeptide can have increased enzymatic activity as compared to the wild type transaminase enzyme or a reference engineered transaminase for transforming the substrates to the products. The amount of the improvement can range from more than 1.1 times (or fold) the enzymatic activity of the corresponding wild-type or reference transaminase enzyme, to as much as 2 times, 5 times, 10 times, 20 times, or more. In some embodiments, the engineered transaminase enzyme exhibits improved enzymatic activity that is at least 1.1-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 25-fold, 50-fold, 100-fold, 500-fold, or 1000-fold

greater than that of the wild-type transaminase of SEQ ID NO:2 or a reference transaminase enzyme of SEQ ID NO:18. Improvements in enzyme activity may also have associated increases in stereoselectivity, stereospecificity, thermostability, solvent stability, and/or substrate binding, or reduced substrate and/or product inhibition.

[0015] In some embodiments, the engineered transaminases are characterized by activity on a variety of structurally different amine acceptor substrates. In some embodiments, the engineered transaminases are capable of stereoselectively converting substrates 3,4-dihydronaphthalen-1(2*H*)-one, 1-phenylbutan-2-one, 3,3-dimethylbutan-2-one, octan-2-one, 1-(4-bromophenyl)ethanone, 4-phenylbutan-2-one, ethyl 3-oxobutanoate, 1-(6-methoxynaphthalen-2-yl)ethanone, 1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)ethanone, 1-(4-phenoxyphenyl)ethanone, (*R*)-4-oxotetrahydro-2*H*-pyran-3-yl-benzoate and/or (*R*)-3-(benzyloxy)dihydro-2H-pyran-4(3H)-one to the corresponding amine product at a rate that is greater than the polypeptides of SEQ ID NO:2 and/or SEQ ID NO:18.

[0016] In some embodiments, the improved transaminase polypeptide comprises an amino acid sequence corresponding to SEQ ID NO: 4, 6, 8, 10, 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, 154, 156, 158, 160, 162, 164, 166, 168, 170. 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, or 198.

[0017] In some embodiments, the engineered transaminase enzymes described herein can be obtained by mutagenizing a gene encoding a naturally-occurring wild-type transaminase enzyme that is at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the amino acid sequence of *Vibrio fluvialis* transaminase (SEQ ID NO:2).

[0018] ~~In another aspect, t~~The present disclosure also provides polynucleotides encoding the engineered transaminases 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 engineered transaminase, and can utilize codons optimized for specific desired expression systems.

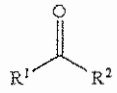
[0019] In some embodiments, the polynucleotide encoding the improved transaminases comprises a sequence corresponding to SEQ ID NO: 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 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, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, or 197.

[0020] ~~In another aspect, t~~The present disclosure further provides host cells comprising the polynucleotides and/or expression vectors described herein. The host cells can be *Vibrio fluvialis* or they may be a different organism, such as *E. coli*. The host cells can be used for the expression and isolation of the

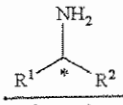
engineered transaminase enzymes described herein, or, alternatively, they can be used directly for the conversion of the substrate to the stereoisomeric product.

[0020a] In one aspect, there is provided a method for the conversion of a substrate of Formula I to a chiral amine product of Formula III in stereomeric excess, the method comprising contacting the substrate of Formula I with the engineered transaminase polypeptide as defined above in the presence of an amino donor under suitable reaction conditions for the conversion of the substrate of Formula I to the chiral amine product of Formula III

Formula I



Formula III



wherein

the chiral carbon atom of the chiral amine product is marked with an *;

each of R^1, and R^2, when taken independently, is an unsubstituted or substituted alkyl, alkylaryl, or aryl group, wherein R^1 is different from R^2 in structure or chirality, or R^1 and R^2, taken together, is a hydrocarbon chain of 4 or more carbon atoms containing a center of chirality.

[0021] ~~In a further aspect,~~ also provided are methods for carrying out reaction Schemes I, II or III (below) using any of the engineered transaminases described herein, which method comprises contacting or incubating the amino acceptor substrate with a transaminase polypeptide of the disclosure in presence of an amino donor under reaction conditions suitable for the conversion of the substrate to the amine product, thereby transforming the substrates to the product compounds. Whether carrying out the method with whole cells, cell extracts or purified transaminase enzymes, a single transaminase enzyme can be used or, alternatively, mixtures of two or more transaminase enzymes can be used.

5. BRIEF DESCRIPTION OF THE FIGURES

[0022] FIG. 1 illustrates the role of transaminases in the conversion of an amino acceptor (ketone) substrate of general formula I and an amino donor of general formula II to the chiral amine (III) product and an amino acceptor (ketone) byproduct (IV). This reaction uses a transaminase described herein and a co-factor such as pyridoxal-5'-phosphate.

6. DETAILED DESCRIPTION

6.1 Abbreviations

[0023] 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
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P

WHAT IS CLAIMED IS:

1. A transaminase enzyme, comprising:

a polypeptide having transaminase activity with increased thermostability at 50°C as compared to the polypeptide of SEQ ID NO:2, wherein the polypeptide comprises an amino acid sequence that is at least 80% identical to SEQ ID NO:18 and has one or more residue differences as compared to SEQ ID NO:2 at residue positions associated with increased thermostability.

2. The transaminase enzyme of claim 1 in which the polypeptide has at least about 10% residual activity in the conversion of pyruvate to L-alanine in presence of amino donor isopropylamine after treatment of the polypeptide at 50°C for 23 h.

3. The transaminase enzyme of claim 1 or 2 in which the residue positions associated with increased thermostability are selected from: X4, X9, X12, X21, X45, X86, X157, X177, X208, X211, X253, X272, X294, X302, X316, X324, X391, X398, X418, X420, X431, X444, and X446.

4. The transaminase enzyme of claim 3 in which the amino acid sequence comprises one or more of the features selected from:

X4 is R, Q, or L; X9 is T; X12 is K; X21 is N; X45 is H; X86 is Y; X157 is T; X177 is L; X208 is I; X211 is K; X253 is M; X272 is A; X294 is V; X302 is K; X316 is K; X324 is G; X391 is A; X398 is R; X418 is V; X420 is N; X431 is D; X444 is V; and X446 is V.

5. The transaminase enzyme of claim 4, in which the amino acid sequence has at least one of the following features selected from:

X45 is H, X86 is Y, X177 is L, X211 is K, X294 is V, X324 is G; X391 is A, X398 is R, and X420 is N;

X9 is T, X45 is H, X86 is Y, X177 is L, X211 is K, X294 is V, X324 is G, and X391 is A;

X21 is N, X45 is H, X177 is L, X208 is I, X211 is K, X324 is G, and X391 is A; and

X9 is T, X21 is N, X86 is Y, X208 is I, and X294 is V.

6. The transaminase enzyme of claim 5, which comprises an amino acid sequence corresponding to SEQ ID NO: 10, 16, or 18.

7. The transaminase enzyme of any one of claims 1 to 5, further having increased transaminase activity as compared to SEQ ID NO:2 for conversion of an amino acceptor substrate to the corresponding chiral amino product.

8. The transaminase enzyme of claim 7 in which the amino acid sequence has additionally one or more of the features selected from: X30 is A; X31 is A; X44 is A; X45 is N; X56 is V; X57 is, A, C, F, I, L, or S; X81 is D; X82 is H; X85 is A, C, S, N, T, G, or V; X86 is F, S, N, A, G, or

H; X95 is T; X112 is I; X113 is C or H; X127 is L; X147 is G; X153 is A, C, G, N, M, Q, S, or T; X166 is S; X177 is V; X181 is R; X208 is I; X211 is R; X228 is G or T; X233 is L, S, I, V, N, G, or T; X294 is M; V297 is A, S, T, I, M, Q, C, or G; X311 is V; X314 is T or V; X317 is L, M, or Y; X318 is G, F, C, K, W, or R; X319 is Q, G, M, N, or V, X320 is A or K; X321 is L, M, or I; X324 is S; X385 is R; X398 is R; X407 is S; X408 is A; X409 is G; X415 is M; X417 is A, C, E, F, I, N, Q, Y, S, T, or V; X420 is N; X434 is V; and X438 is L.

9. The transaminase enzyme of claim 7 in which the amino acid sequence additionally has one or more of the features selected from: X30 is A; X31 is A; X56 is V; X81 is D; X82 is H; X95 is T; and X113 is C or H.

10. The transaminase enzyme of claim 7, in which the amino acid sequence has additionally at least one of the following features selected from:

X12 is G and X434 is V; X44 is A and X166 is S; X57 is I and X153 is S; X81 is D and X86 is H; X82 is H and X417 is F; X85 is A and X317 is L; X85 is S and X153 is A; X85 is A and X153 is A; X85 is S and X153 is S; X86 is S and X153 is S; X86 is H and X153 is A; X112 is I and X317 is L; X113 is H and X407 is S; X153 is S and X233 is S; X311 is V and X314 is T; X314 is V and X409 is G; and X318 is G and X408 is A.

11. The transaminase enzyme of claim 7, in which the amino acid sequence has additionally at least one of the following features:

X57 is A, X153 is S and X318 is G; X57 is F, X86 is H and X153 is Q; X57 is I, X86 is F and X320 is A; X57 is F, X86 is F and X153 is Q; X57 is A, X153 is C and X321 is L; X57 is C, X86 is S and X417 is T; X57 is C, X86 is A and X317 is L; X57 is F, X318 is F and X417 is S; X57 is L, X86 is S and X153 is A; X57 is F, X318 is G and X417 is I. X57 is L, X86 is F and X318 is F; X57 is L, X417 is C and X438 is L; X57 is F, X127 is L and X417 is C; X57 is S, X233 is L and X417 is V; X57 is S, X86 is G and X417 is C; X85 is A, X147 is G and X153 is A; X85 is S, X153 is A and X233 is T; X85 is A, X153 is S and X417 is S; X86 is H, X153 is A and X417 is C; X86 is H, X153 is S and X417 is C; X86 is F, X153 is C and X297 is A; X86 is S, X153 is T and X297 is A; X86 is H, X233 is L and X417 is A; X86 is F, X318 is R and X417 is A; X86 is N, X228 is G and X317 is L; X95 is T, X153 is A and X417 is C; X113 is C, X385 is R and X417 is C; X153 is A, X317 is L and X318 is G; X153 is A, X233 is T and X417 is C; X153 is S, X318 is R and X417 is E; X153 is C, X233 is L and X318 is R; X153 is T, X228 is G and X321 is L; X153 is S, X317 is L and X417 is C; X153 is T, X319 is V and X417 is I; X153 is S, X228 is G and X417 is V; X153 is C, X317 is Y and X319 is Q; X153 is T, X228 is G and X417 is A; X228 is G, X317 is L and X417 is C; X228 is G, X318 is G and X417 is C; X233 is L, X321 is L and X417 is I; and X317 is L, X318 is R and X417 is T.

12. The transaminase enzyme of claim 7, in which the amino acid sequence has additionally one of the following features:

X57 is L and X86 is F and X153 is S and X233 is T and X417 is T;
X86 is H and X153 is A and XA228 is G and X417 is I; and
X86 is H and X153 is S and X181 is R and X417 is T.

13. The transaminase enzyme of claim 7, which comprises an amino acid sequence corresponding to SEQ ID NO: 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, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, or 192.

14. The transaminase enzyme of any one of claims 1 to 5 in which the polypeptide is capable of converting the amino acceptor substrate to the corresponding amino product with improved *R*-enantioselectivity as compared to SEQ ID NO:18.

15. The transaminase enzyme of claim 14 in which the amino acid sequence has additionally one or more of the following features selected from:

X57 is L; X81 is D; X82 is H; X85 is A ,C, N, T, or V; X86 is S,F, or H; X95 is T; X112 is I; X147 is G; X153 is A,S, N, G, or T; X233 is S or T; X317 is L, X318 is G or R; and X319 is V.

16. The transaminase enzyme of claim 14 in which the amino acid sequence has additionally one of the following features selected from:

X85 is A; X85 is A and X153 is A; and
X85 is S and X153 is A.

17. The transaminase enzyme of claim 14, which comprises an amino acid sequence corresponding to SEQ ID NO: 22, 30, 32, 34, 36, 40, 44, 48, 56, 58, 60, 62, 64, 66, 68, 70, 72, 76, 84, 88, 90, 92, 104, 108, 110, 120, 122, 124, 126, 128, 132, 160, or 176.

18. The transaminase enzyme of any one of claims 1 to 5 which is capable of converting the amino acceptor substrate selected from 3,4-dihydronaphthalen-1(2*H*)-one, 1-phenylbutan-2-one, 3,3-dimethylbutan-2-one, octan-2-one, ethyl 3-oxobutanoate, 4-phenylbutan-2-one, and 1-(4-bromophenyl)ethanone to the corresponding amine product at a rate that is greater than the polypeptide of SEQ ID NO:2 or 18.

19. The transaminase enzyme of claim 18 in which the amino acid sequence has additionally at least one of the following features selected from:

X30 is A; X31 is A; X57 is I; X82 is H; X85 is V; X85 is C; X153 is S; X317 is M; X317 is Y; X417 is A; X319 is Q; X320 is A; X417 is I; X113 is H and X407 is S; X44 is A and X166 is S; X314 is

V and X409 is G; X153 is S and X233 is S; X311 is V and X314 is T; X153 is S, X318 is R and X417 is E; X153 is C, X233 is L and X318 is R; X153 is S, X317 is L and X417 is C; X233 is L, X321 is L, and X417 is I; and X153 is C, X317 is Y and X319 is Q.

20. The transaminase enzyme of claim 18, which comprises an amino acid sequence corresponding to SEQ ID NO: 38, 42, 44, 52, 56, 66, 74, 76, 78, 80, 82, 84, 94, 96, 98, 100, 104, 118, 160, 164, 172, 184, or 188.

21. The transaminase enzyme of claim 18 in which the polypeptide is capable of converting the amino acceptor substrate 1-phenylbutan-2-one to the amino product (*S*)-1-phenylbutan-2-amine with improved S-enantioselectivity as compared to SEQ ID NO:2 or 18.

22. The transaminase enzyme of claim 21 in which the amino acid sequence has additionally at least one of the following features selected from:

X81 is D and X86 is H; X86 is H and X153 is A; X86 is H, X153 is A and X417 is C; X86 is H, X153 is S and X417 is C; X86 is H, X153 is A, X228 is G and X417 is I; X86 is H, X153 is S, X181 is R and X417 is T; X228 is G, X317 is L and X417 is C; X57 is A, X153 is S and X318 is G; X57 is F, X86 is H, and X153 is Q; X57 is A X153 is C and X321 is L; X86 is F, X318 is R and X417 is A; X228 is G, X318 is G and X417 is C; X153 is S, X318 is R and X417 is E; X153 is C, X233 is L and X318 is R; X86 is N, X228 is G and X317 is L; X153 is T, X228 is G and X321 is L; X153 is S, X317 is L, X417 is C; X57is L, X86 is S and X153 is A; X153 is S, X228 is G and X417 is V; X153 is C , X317 is Y and X319 is Q; and X153 is T, X228 is G and X417 is A.

23. The transaminase enzyme of claim 21, which comprises an amino acid sequence corresponding to SEQ ID NO: 22, 88, 90, 92, 106, 110, 138, 140, 142, 148, 150, 152, 160, 164, 168, 170, 172, 178, 182, 188, or 190.

24. The transaminase enzyme of claim 18 in which the polypeptide is capable of converting the amino acceptor substrate 3,3-dimethylbutan-2-one to the amino product (*S*)-3,3-dimethylbutan-2-amine with improved S-enantioselectivity as compared to SEQ ID NO:2 or 18.

25. The transaminase enzyme of claim 24 in which the amino acid sequence comprises additionally at least one of the following features selected from: X30 is A; X31 is A; X57 is L; X57 is I; X82 is H; X85 is V; X85 is S; X85 is T; X85 is A; X85 is N; X85 is C; X86 is S; X86 is N; X86 is F; X153 is T; X153 is N; X153 is G; X153 is S; X317 is M; X317 is Y; X319 is Q; X320 is A; X417 is A; X417 is I; X417 is C; X12 is G and X434 is V; X44 is A and X166 is S; X57 is I and X153 is S; X81 is D and X86 is H; X82 is H and X417 is F; X85 is S and X153 is A; X85 is A and X153 is A; X85 is A and X317 is L; X86 is H and X153 is A; X86 is S and X153 is S; X112 is I and X317 is L; X113 is H and X407 is S; X153 is S and X233 is S; X311 is V and X314 is T; X314 is V and X409 is G; X318 is

G and X408 is A; X57 is L, X417 is C and X438 is L; X57 is F, X127 is L and X417 is C; X57 is S, X233 is L and X417 is V; X57 is A, X153 is S and X318 is G; X57 is F, X86 is H and X153 is Q; X57 is I, X86 is F and X320 is A; X57 is F, X86 is F and X153 is Q; X57 is C, X86 is S and X417 is T; X57 is F, X318 is F and X417 is S; X57 is L, X86 is S and X153 is A; X57 is L, X86 is F and X318 is F; X57 is F, X318 is G and X417 is I; X86 is H, X153 is S and X417 is C; X86 is H, X153 is A and X417 is C; X86 is F, X153 is C and X297 is A; X86 is H, X233 is L and X417 is A; X86 is N, X228 is G and X317 is L; X95 is T, X153 is A, and X417 is C; X113 is C, X385 is R and X417 is C; X153 is A, X317 is L and X318 is G; X153 is A, X233 is T and X417 is C; X153 is C, X233 is L and X318 is R; X153 is T, X228 is G, and X321 is L; X153 is S, X317 is L, and X417 is C; X153 is T, X319 is V and X417 is I; X153 is S, X228 is G and X417 is V; X153 is C, X317 is Y, and X319 is Q; X153 is T, X228 is G and X417 is A; X233 is L, X321 is L and X417 is I; X228 is G, X318 is G and X417 is C; X317 is L, X318 is R and X417 is T; X86 is H, X153 is A, X228 is G and X417 is I; and X86 is H, X153 is S, X181 is R and X417 is T.

26. The transaminase enzyme of claim 24, which comprises an amino acid sequence corresponding to SEQ ID NO: 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 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, 134, 140, 142, 144, 146, 152, 154, 156, 158, 162, 164, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, or 192.

27. The transaminase enzyme of claim 18 in which the polypeptide is capable of converting the amino acceptor substrate octan-2-one to the amino product (*S*)-octan-2-amine with improved S-enantioselectivity as compared to SEQ ID NO:2 or 18.

28. The transaminase enzyme of claim 27 in which the amino acid sequence has additionally at least one of the following features selected from:

X30 is A; X31 is A; X57 is I; X57 is L; X82 is H; X85 is V; X85 is S; X85 is T; X85 is A; X85 is N; X85 is C; X86 is S; X86 is N; X86 is F; X153 is S; X153 is T; X153 is N; X153 is G; X317 is M; X317 is Y; X417 is A; X319 is Q; X320 is A; X417 is I; X417 is C; X12 is G and X434 is V; X44 is A and X166 is S; X57 is I and X153 is S; X81 is D and X86 is H; X82 is H and X417 is F; X85 is A and X317 is L; X85 is S and X153 is A; X85 is A and X153 is A; X86 is H and X153 is A; X86 is S and X153 is S; X112 is I and X317 is L; X113 is H and X407 is S; X153 is S and X233 is S; X311 is V and X314 is T; X314 is V and X409 is G; X318 is G and X408 is A; X57 is L, X417 is C and X438 is L; X57 is F, X127 is L and X417 is C; X57 is S, X233 is L and X417 is V; X57 is S, X86 is G and X417 is C; X57 is A, X153 is S and X318 is G; X57 is F, X86 is H and X153 is Q; X57 is I, X86 is F and X320 is A; X57 is F, X86 is F and X153 is Q; X57 is A, X153 is C and X321 is L; X57 is C, X86 is S and X417 is T; X57 is C, X86 is A and X317 is L; X57 is F, X318 is F and X417 is S; X57 is L, X86 is S and X153 is A; X57 is L, X86 is F and X318 is F; X57 is F, X318 is G and X417 is I; X86 is F, X318 is R and X417 is A; X86 is F, X153 is C and X297 is A; X86 is S, X153 is T and X297 is A; X86 is H, X233 is L

and X417 is A; X86 is N, X228 is G, and X317 is L; X86 is H, X153 is A and X417 is C; X86 is H, X153 is S and X417 is C; X95 is T, X153 is A and X417 is C; X113 is C, X385 is R and X417 is C; X153 is A, X233 is T and X417 is C; X153 is S, X318 is R and X417 is E; X153 is C, X233 is L and X318 is R; X153 is S, X228 is G and X417 is V; X153 is C, X317 is Y and X319 is Q; X153 is T, X228 is G, and X417 is A; X153 is T, X319 is V and X417 is I; X153 is T, X228 is G and X321 is L; X153 is S, X317 is L and X417 is C; X153 is A, X317 is L and X318 is G; X228 is G, X318 is G and X417 is C; X228 is G, X317 is L and X417 is C; X233 is L, X321 is L and X417 is I; X317 is L, X318 is R and X417 is T; X86 is H, X153 is A, X228 is G and X417 is I; and X86 is H, X153 is S, X181 is R and X417 is T.

29. The transaminase enzyme of claim 27, which comprises an amino acid sequence corresponding to SEQ ID NO: 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 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, 134, 136, 138, 140, 142, 144, 146, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 178, 180, 182, 184, 186, 188, 190, or 192.

30. The transaminase enzyme of claim 18 in which the polypeptide is capable of converting the amino acceptor substrate 1-(4-bromophenyl)ethanone, to the amino product (*S*)-1-(4-bromophenyl)ethanamine with improved S-enantioselectivity as compared to SEQ ID NO:2 or 18.

31. The transaminase enzyme of claim 30 in which the amino acid sequence has additionally at least one of the following features selected from:

X30 is A; X57 is L; X57 is I; X82 is H; X85 is V; X85 is T; X85 is A; X85 is N; X86 is N; X86 is F; X86 is S; X153 is S; X153 is T; X153 is N; X153 is G; X317 is M; X317 is Y; X319 is Q; X320 is A; X417 is A; X417 is I; X417 is C; X12 is G and X434 is V; X44 is A and X166 is S; X57 is I and X153 is S; X81 is D and X86 is H; X82 is H and X417 is F; X85 is S and X153 is A; X85 is A and X153 is A; X86 is S and X153 is S; X86 is H and X153 is A; X112 is I and X317 is L; X113 is H and X407 is S; X153 is S and X233 is S; X314 is V and X409 is G; X318 is G and X408 is A; X31 is A, X311 is V and X314 is T; X57 is L, X417 is C and X438 is L; X57 is F, X127 is L and X417 is C; X57 is I, X86 is F and X320 is A; X57 is S, X86 is G and X417 is C; X57 is C, X86 is S and X417 is T; X57 is C, X86 is A and X317 is L; X57 is F, X318 is F and X417 is S; X57 is L, X86 is S and X153 is A; X57 is F, X318 is G and X417 is I; X86 is H, X153 is A and X417 is C; X86 is H, X153 is S and X417 is C; X86 is F, X318 is R and X417 is A; X86 is F, X153 is C and X297 is A; X86 is S, X153 is T and X297 I is A; X86 is N, X228 is G and X317 is L; X86 is H, X233 is L and X417 is A; X95 is T, X153 is A and X417 is C; X113 is C, X385 is R and X417 is C; X153 is A, X233 is T and X417 is C; X153 is A, X317 is L and X318 is G; X153 is S, X318 is R and X417 is E; X153 is T, X228 is G and X321 is L; X153 is S, X317 is L and X417 is C; X153 is C, X233 is L and X318 is R; X153 is S, X228 is G and X417 is V; X153 is T, X319 is V and X417 is I; X153 is C, X317 is Y and X319 is Q; X153 is T, X228 is G and X417 is A; X228 is G, X317 is L and X417 is C; X228 is G, X318 is G and X417 is C; X233 is L, X321 is L and

X417 is I; X317 is L, X318 is R and X417 is T; X86 is H, X153 is A, X228 is G and X417 is I; and X86 is H, X153 is S, X181 is R and X417 is T.

32. The transaminase enzyme of claim 30, which comprises an amino acid sequence corresponding to SEQ ID NO: 20, 22, 24, 26, 28, 30, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 60, 62, 64, 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, 136, 138, 144, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 182, 184, 186, 188, 190, and 192.

33. The transaminase enzyme of claim 18 which is capable of stereoselectively converting 4-phenylbutan-2-one to (*R*)-4-phenylbutan-2-amine at a rate that is greater than the polypeptide of SEQ ID NO:2 or 18.

34. The transaminase enzyme of claim 33 in which the amino acid sequence has additionally at least one of the following features selected from:

X30 is A; X31 is A; X57 is L; X57 is I; X82 is H; X85 is V; X85 is S; X85 is T; X85 is A; X86 is S; X86 is N; X86 is F; X153 is S; X153 is T; X153 is N; X153 is G; X319 is Q; X317 is M; X317 is Y; X320 is A; X417 is A; X417 is I; X417 is C; X44 is A and X166 is S; X57 is I and X153 is S; X81 is D and X86 is H; X82 is H and X417 is F; X85 is A and X153 is A; X85 is A and X317 is L; X85 is S and X153 is A; X86 is S and X153 is S; X86 is H and X153 is A; X113 is H and X407 is S; X318 is G and X408 is A; X153 is S and X233 is S; X57 is A, X153 is S and X318 is G; X57 is F, X86 is H and X153 is Q; X57 is I, X86 is F and X320 is A; X57 is F, X86 is F, and X153 is Q; X57 is A, X153 is C and X321 is L; X57 is L, X417 is C and X438 is L; X57 is S, X233 is L and X417 is V; X57 is C, X86 is S, and X417 is T; X57 is S, X86 is G and X417 is C; X57 is C; X86 is A and X317 is L; X57 is F, X318 is F and X417 is S; X57 is L, X86 is S and X153 is A; X57 is L, X86 is F and X318 is F; X57 is F, X318 is G and X417 is I; X86 is H, X153 is S and X417 is C; X86 is H, X153 is A and X417 is C; X86 is F, X318 is R and X417 is A; X86 is F, X153 is C and X297 is A; X86 is S, X153 is T and X297 is A; X86 is H, X233 is L and X417 is A; X86 is N, X228 is G and X317 is L; X113 is C, X385 is R and X417 is C; X153 is A, X233 is T and X417 is C X153 is A, X317 is L and X318 is G; X153 is S, X318 is R and X417 is E; X153 is C, X233 is L and X318 is R; X153 is T, X228 is G and X321 is L; X153 is S, X317 is L and X417 is C; X153 is S, X228 is G and X417 is V; X153 is C, X317 is Y and X319 is Q; X153 is T, X228 is G and X417 is A; X228 is G, X317 is L and X417 is C; X228 is G, X318 is G and X417 is C; X233 is L, X321 is L and X417 is I; X317 is L, X318 is R and X417 is T; X86 is H, X153 is A, X228 is G and X417 is I; and X86 is H, X153 is S, X181 is R and X417 is T.

35. The transaminase enzyme of claim 33 which comprises an amino acid sequence corresponding to SEQ ID NO: 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, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 178, 182, 184, 186, 188, 190, or 192.

36. The transaminase enzyme of any one of claims 1 to 5 which is capable of stereoselectively converting 1-(6-methoxynaphthalen-2-yl)ethanone, to the product (*S*)-1-(6-methoxynaphthalen-2-yl)ethanamine at a rate that is greater than the polypeptide of SEQ ID NO:2 or 18.

37. The transaminase enzyme of claim 36 in which the amino acid sequence has additionally at least one of the following features selected from: X57 is L; X86 is F or N; X417 is C, I or A; X57 is L, X86 is F, X153 is S, X233 is T and X417 is T; X57 is C, X86 is S and X417 is T; X57 is S, X233 is L, and X417 is V; X57 is A, X153 is S and X318 is G; X57 is A, X153 is C, and X321 is L; X57 is L, X86 is S, and X153 is A; X57 is S, X86 is G and X417 is C; X57 is L, X86 is F, X318 is F; X57 is F, X86 is F, and X153 is Q; X57 is I; X86 is F and X320 is A; X57 is C, X86 is A and X317 is L; X57 is L, X417 is C and X438 is L; X86 is F, X318 is R and X417 is A; X57 is F. X86 is H and X153 is Q; X57 is F, X318 is F and X417 is S; X86 is H, X153 is S, X181 is R and X417 is T; X153 is S, X317 is L and X417 is C; X153 is T, X228 is G and X417 is A; X57 is F, X127 is L and X417 is C; X228 is G, X317 is L and X417 is C; X233 is L, X321 is L and X417 is I; X228 is G, X318 is G and X417 is C; X86 is H, X153 is S and X417 is C; X86 is H, X233 is L and X417 is A; X86 is S and X153 is S; X86 is F, X153 is C and X297 is A; X153 is S, X228 is G and X417 is V; X57 is F, X318 is G and X417 is I; X153 is T, X228 is G and X321 is L; X317 is L, X318 is R and X417 is T; X153 is C, X317 is Y, and X319 is Q; X57 is I and X153 is S; X86 is H, X153 is A and X417 is C; X153 is A, X233 is T and X417 is C; X95 is T, X153 is A and X417 is C; X86 is N, X228 is G and X317 is L; and X86 is S, X153 is T, X297 is A.

38. The transaminase enzyme of claim 36 which comprises an amino acid sequence corresponding to SEQ ID NO: 24, 26, 28, 46, 48, 78, 84, 86, 88, 92, 102, 108, 110, 114, 116, 126, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 162, 166, 168, 170, 172, 174, 178, 180, 182, 184, 186, 188, 190, or 192.

39. The transaminase enzyme of any one of claims 1 to 5 which is capable of stereoselectively converting 1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)ethanone, to the product (*S*)-1-(2,3-dihydrobenzo[*b*][1,4]dioxin-6-yl)ethanamine at a rate that is greater than the polypeptide of SEQ ID NO:2 or 18.

40. The transaminase enzyme of claim 39 in which the amino acid sequence has additionally at least one of the following features selected from: X31 is A; X57 is C, I or L; X86 is S or F; X153 is G or S; X417 is T or I; X57 is F, X86 is F, and X153 is Q; X57 is I and X153 is S; X57 is L, X86 is F, X153 is S, X233 is T and X417 is T; X86 is F, X153 is C, and X297 is A; X57 is L, X417 is C,

and X438 is L; X57 is S, X233 is L, and X417 is V; X57 is L, X86 is S, X153 is A; X57 is S, X86 is G, and X417 is C; X57 is A, X153 is S, X318 is G; X57 is I, X86 is F and X320 is A; X57 is L, X86 is F, X318 is F; X153 is S, X228 is G, and X417 is V; X57 is C, X86 is A, and X317 is L; X153 is S and X233 is S; X57 is A, X153 is C and X321 is L; X86 is S and X153 is S; X86 is H, X153 is S, X181 is R, and X417 is T; X21 is N, X45 is N, X177 is V, X208 is I, X211 is R, X324 is S, and X391 is T; X86 is H, X153 is A, X228 is G, and X417 is I; and X153 is C, X233 is L and X318 is R.

41. The transaminase enzyme of claim 39 which comprises an amino acid sequence corresponding to SEQ ID NO: 16, 26, 28, 42, 44, 46, 48, 72, 84, 98, 104, 106, 110, 114, 126, 134, 136, 140, 144, 146, 148, 152, 156, 164, 166, 178, 180, or 182.

42. The transaminase enzyme of any one of claims 1 to 5 which is capable of stereoselectively converting 1-(4-phenoxyphenyl)ethanone, to the product (*S*)-1-(4-phenoxyphenyl)ethanamine at a rate that is greater than the polypeptide of SEQ ID NO:2 or 18.

43. The transaminase enzyme of claim 42 in which the amino acid sequence has additionally at least one of the following features selected from: X31 is A; X86 is S or F; X57 is L or I; X153 is S or G; X417 is A or I; X57 is I and X153 is S; X86 is S and X153 is S; X153 is S and X233 is S; X86 is H and X153 is A; X86 is H; X153 is A and X417 is C; X95 is T, X153 is A and X417 is C; X86 is H, X153 is S and X417 is C; X57 is L, X417 is C and X438 is L; X57 is S, X233 is L and X417 is V; X57 is S, X86 is G and X417 is C; X57 is A, X153 is S, X318 is G; X57 is F, X86 is H and X153 is Q; X57 is I, X86 is F and X320 is A; X57 is F, X86 is F and X153 is Q; X57 is C, X86 is S and X417 is T; X86 is F, X153 is C and X297 is A; X86 is H, X233 is L and X417 is A; X153 is C, X233 is L and X318 is R; X57 is C, X86 is A and X317 is L; X57 is L, X86 is S and X153 is A; X57 is L, X86 is F and X318 is F; X153 is S, X228 is G and X417 is V; X153 is T, X228 is G and X417 is A; X86 is H, X153 is A, X228 is G and X417 is I; X86 is H, X153 is S, X181 is R, and X417 is T; and X57 is L, X86 is F, X153 is S, X233 is T and X417 is T.

44. The transaminase enzyme of claim 42 which comprises an amino acid sequence corresponding to SEQ ID NO: 16, 20, 26, 28, 42, 44, 46, 48, 72, 78, 84, 88, 90, 92, 98, 104, 106, 108, 110, 114, 126, 134, 136, 140, 142, 144, 146, 154, 156, 162, 164, 166, 178, 180, 182, or 190.

45. The transaminase enzyme of any one of claims 1 to 5 which is capable of stereoselectively converting (*R*)-4-oxotetrahydro-2*H*-pyran-3-yl-benzoate to the product (3*S*,4*S*)-3-aminotetrahydro-2*H*-pyran-3-yl benzoate at a rate that is greater than the polypeptide of SEQ ID NO:2 or 18.

46. The transaminase enzyme of claim 45 in which the amino acid sequence has additionally at least one of the following features selected from: X57 is I and X153 is S; X57 is A,

X153 is S and X318 is G; X57 is A, X153 is C and X321 is L; X57 is L, X86 is S and X153 is A; X57 is I, X86 is F, X320 is A; X57 is S, X86 is G, and X417 is C; X57 is C, X86 is S and X417 is T; X57 is C, X86 is A and X317 is L; X57 is L, X86 is F, X153 is S. X233 is T and X417 is T.

47. The transaminase enzyme of claim 45 which comprises an amino acid sequence corresponding to SEQ ID NO: 46, 126, 136, 140, 144, 148, 154, 166, and 178.

48. The transaminase enzyme of any one of claims 1 to 5 which is capable of stereoselectively converting (*R*)-3-benzyloxydihydro-2*H*-pyran-4(3*H*)-one, to the product (3*S*,4*S*)-3-benzyloxytetrahydro-2*H*-pyran-4-amine at a rate that is greater than the polypeptide of SEQ ID NO:2 or 18.

49. The transaminase enzyme of claim 48 in which the amino acid sequence has additionally at least one of the following features selected from: X30 is A; X31 is A; X57 is I or L; X82 is H; X85 is S, T, A; X86 is S, F; X153 is N, G, S, T; X317 is M or Y; X319 is Q; X320 is A; X417 is A, I or C; X57 is I and X153 is S; X85 is S and X153 is S; X81 is D and X86 is H; X86 is H and X153 is A; X86 is S and X153 is S; X85 is S and X153 is A; X85 is A and X153 is A; X314 is V and X409 is G; X311 is V and X314 is T; X82 is H and X417 is F; X85 is A and X317 is L; X318 is G and X408 is A; X153 is S and X233 is S; X113 is H and X407 is S; X12 is G and X434 is V; X112 is I and X317 is L; X44 is A and X166 is S; X86 is H, X153 is S and X417 is C; X57 is F. X127 is L and X417 is C; X57 is S, X86 is G and X417 is C; X228 is G, X317 is L, X417 is C; X57 is F, X86 is H and X153 is Q; X86 is F, X318 is R and X417 is A; X86 is F, X153 is C and X297 is A; X86 is N, X228 is G and X317 is L; X57 is L, X86 is F and X318 is F; X153 is S, X228 is G and X417 is V; X57 is F, X86 is F and X153 is Q; X57 is L, X417 is C and X438 is L; X57 is A, X153 is C and X321 is L; X57 is S, X233 is L and X417 is V; X86 is S, X153 is T and X297 is A; X57 is F, X318 is F and X417 is S; X153 is T, X319 is V and X417 is I; X57 is S, X233 is L and X417 is V; X57 is I, X86 is F and X320 is A; X57 is F, X318 is G and X417 is I; X153 is C, X317 is Y and X319 is Q; X57 is L, X86 is S and X153 is A; X233 is L, X321 is L and X417 is I; X95 is T, X153 is A; and X417 is C; X86 is H, X153 is A and X417 is C; X228 is G, X318 is G and X417 is C; X85 is S, X153 is A and X233 is T; X57 is A, X153 is S and X318 is G; X153 is S, X318 is R and X417 is E; X153 is C, X233 is L and X318 is R; X153 is A; X233 is T and X417 is C; X85 is A, X147 is G and X153 is A; X153 is S, X317 is L and X417 is C; X317 is L, X318 is R and X417 is T; X153 is T, X228 is G and X417 is A; X57 is C, X86 is A and X317 is L; X153 is A, X317 is L and X318 is G; X57 is C, X86 is S and X417 is T; X86 is H, X233 is L and X417 is A; X153 is T, X228 is G, X321 is L; X113 is C, X385 is R and X417 is C; X86 is H, X153 is S, X181 is R and X417 is T; X86 is H, X153 is A, X228 is G and X417 is I; X57 is I, X85 is A, X86 is H and X417 is C; and X57 is L, X86 is F, X153 is S, X233 is T and X417 is T.

50. The transaminase enzyme of claim 48 which comprises an amino acid sequence corresponding to SEQ ID NO: 20, 22, 26, 28, 30, 32, 34, 36, 40, 42, 44, 46, 48, 50, 52, 58, 60, 68, 70,

72, 78, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 114, 116, 118, 122, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172; 174, 176, 178, 180, 182, 184, 186, 188, 190, or 192.

51. The transaminase enzyme of any one of claims 1-50, in which the polypeptide has improved expression in a host cell as compared to SEQ ID NO:2.

52. The transaminase enzyme of claim 51 in which the amino acid sequence has additionally one or more of the following features selected from:

X4 is R; X6 is R or I or N; X9 is T or G; or X9 is T and X86 is Y and X294 is V.

53. A polynucleotide encoding the polypeptide of any one of claims 1-52.

54. The polynucleotide of claim 53 which comprises a polynucleotide sequence corresponding to SEQ ID NO: 3, 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, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 181, 183, 185, 187, 189, 191, 193, 195, or 197.

55. An expression vector comprising the polynucleotide of claim 53 or 54.

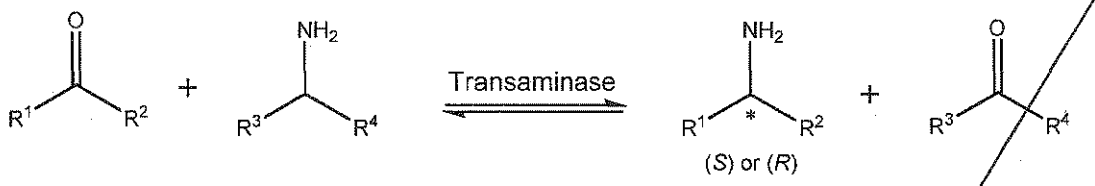
56. The expression vector of claim 55, further comprising a control sequence.

57. The expression vector of claim 56 in which the control sequence comprises a promoter.

58. A host cell comprising the polynucleotide of claim 53 or 54.

59. The host cell of claim 58 which is E. coli.

60. A method for the conversion of a substrate of Formula I to the chiral amine product of Formula III in stereomeric excess,



I. Amino acceptor II. Amino donor III. Chiral Amine Product IV. Amino acceptor byproduct

wherein

the chiral carbon atom of the chiral amine product is marked with an *;

each of R^1, R^2, R^3 and R^4, when taken independently, is an unsubstituted or substituted alkyl, alkylaryl, or aryl group, wherein R^1 is different from R^2 in structure or chirality, or R^1 and R^2, taken together, is a hydrocarbon chain of 4 or more carbon atoms containing a center of chirality,

the method comprising contacting the substrate of Formula I with a transaminase polypeptide of any one of claims 1 to 52 in presence of an amino donor under suitable reaction conditions for the conversion of the substrate to the product of Formula III in stereomeric excess.

61. The method of claim 60 in which the substrate of Formula I is 3,4-dihydronaphthalen-1(2*H*)-one, which is converted to the product (S)-1,2,3,4-tetrahydronaphthalen-1-amine in stereomeric excess.

62. The method of claim 61 in which the transaminase polypeptide comprises an amino acid sequence corresponding to SEQ ID NO: 38, 42, 44, 52, 56, 66, 74, 76, 78, 80, 82, 84, 94, 96, 98, 100, 104, 118, 160, 164, 172, 184, or 188.

63. The method of claim 60 in which the substrate of Formula I is 1-phenylbutan-2-one , which is converted to the product (*S*)-1-phenylbutan-2-amine in stereomeric excess.

64. The method of claim 63 in which the transaminase polypeptide comprises an amino acid sequence corresponding to SEQ ID NO: 22, 88, 90, 92, 106, 110, 138, 140, 142, 148, 150, 152, 160, 164, 168, 170, 172, 178, 182, 188, or 190.

65. .The method of claim 60 in which the substrate of Formula I is 3,3-dimethylbutan-2-one, which is converted to the product (*S*)-3,3-dimethylbutan-2-amine in stereomeric excess.

66. The method of claim 65 in which the transaminase polypeptide comprises an amino acid sequence corresponding to SEQ ID NO: 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 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, 134, 140, 142, 144, 146, 152, 154, 156, 158, 162, 164, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, or 192.

67. The method of claim 60 in which the substrate of Formula I is octan-2-one, which is converted to the product (*S*)-octan-2-amine in stereomeric excess.

68. The method of claim 67 in which the transaminase polypeptide comprises an amino acid sequence corresponding to SEQ ID NO: 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 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, 134, 136, 138, 140, 142, 144, 146, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 178, 180, 182, 184, 186, 188, 190, or 192.

69. The method of claim 60 in which the substrate of Formula I is 1-(4-bromophenyl)ethanone, which is converted to the product (*S*)-1-(4-bromophenyl)ethanamine in stereomeric excess.

70. The method of claim 69 in which the transaminase polypeptide comprises an amino acid sequence corresponding to SEQ ID NO: 20, 22, 24, 26, 28, 30, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 60, 62, 64, 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, 136, 138, 144, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 182, 184, 186, 188, 190, or 192.

71. The method of claim 60 in which the substrate of Formula I is 4-phenylbutan-2-one, which is converted to the product (*R*)-4-phenylbutan-2-amine in stereomeric excess.

72. The method of claim 71 in which the transaminase polypeptide comprises an amino acid sequence corresponding to SEQ ID NO: 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, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 178, 182, 184, 186, 188, 190, or 192.

73. The method of claim 60 in which the substrate of Formula I is ethyl 3-oxobutanoate, which is converted to the product (*R*)-ethyl 3-aminobutanoate in stereomeric excess.

74. The method of claim 73 in which the transaminase polypeptide comprises an amino acid sequence corresponding to SEQ ID NO: 32, 34, 36, 40, 44, 56, 58, 60, 62, 64, 66, 68, 70, 72, 86, 88, 90, 92, 122, 126, 128, 130, or 132.

75. The method of claim 60 in which the substrate of Formula I is ethyl 3-oxobutanoate, which is converted to the product (*S*)-ethyl 3-aminobutanoate in stereomeric excess.

76. The method of claim 75 in which the transaminase polypeptide comprises an amino acid sequence corresponding to SEQ ID NO: 20, 22, 24, 26, 28, 30, 38, 42, 46, 48, 50, 52, 54, 74, 78, 80, 82, 94, 96, 98, 100, 102, 106, 112, 114, 116, 118, 120, 124, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 162, 164, 166, 168, 170, 172, 174, 178, 180, 182, 184, 186, 188, 190 or 192.

77. The method of claim 60 in which the substrate of Formula I is 1-(6-methoxynaphthalen-2-yl)ethanone, which is converted to the product (*S*)-1-(6-methoxynaphthalen-2-yl)ethanamine in stereomeric excess.

78. The method of claim 77 in which the transaminase polypeptide comprises an amino acid sequence corresponding to SEQ ID NO: 24, 26, 28, 46, 48, 78, 84, 86, 88, 92, 102, 108, 110, 114, 116, 126, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 162, 166, 168, 170, 172, 174, 178, 180, 182, 184, 186, 188, 190, or 192.

79. The method of claim 60 in which the substrate of Formula I is 1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)ethanone, which is converted to the product (*S*)-1-(6-methoxynaphthalen-2-yl)ethanamine in stereomeric excess.

80. The method of claim 79 in which the transaminase polypeptide comprises an amino acid sequence corresponding to SEQ ID NO: 16, 26, 28, 42, 44, 46, 48, 72, 84, 98, 104, 106, 110, 114, 126, 134, 136, 140, 144, 146, 148, 152, 156, 164, 166, 178, 180, or 182.

81. The method of claim 60 in which the substrate of Formula I is 1-(4-phenoxyphenyl)ethanone, which is converted to the product (*S*)-1-(4-phenoxyphenyl)ethanamine in stereomeric excess.

82. The method of claim 81 in which the transaminase polypeptide comprises an amino acid sequence corresponding to SEQ ID NO: 16, 20, 26, 28, 42, 44, 46, 48, 72, 78, 84, 88, 90, 92, 98, 104, 106, 108, 110, 114, 126, 134, 136, 140, 142, 144, 146, 154, 156, 162, 164, 166, 178, 180, 182, or 190.

83. The method of claim 60 in which the substrate of Formula I is (*R*)-4-oxotetrahydro-2*H*-pyran-3-yl-benzoate, which is converted to the product (3*S*,4*S*)-3-aminotetrahydro-2*H*-pyran-3-yl benzoate in stereomeric excess.

84. The method of claim 83 in which the transaminase polypeptide comprises an amino acid sequence corresponding to SEQ ID NO: 46, 126, 136, 140, 144, 148, 154, 166, or 178.

85. The method of claim 60 in which the substrate of Formula I is (*R*)-3-benzyloxydihydro-2*H*-pyran-4(3*H*)-one, which is converted to the product (3*S*,4*S*)-3-benzyloxytetrahydro-2*H*-pyran-4-amine in stereomeric excess.

86. The method of claim 85 in which the transaminase polypeptide comprises an amino acid sequence corresponding to SEQ ID NO: 20, 22, 26, 28, 30, 32, 34, 36, 40, 42, 44, 46, 48, 50, 52, 58, 60, 68, 70, 72, 78, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 114, 116, 118, 122, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172; 174, 176, 178, 180, 182, 184, 186, 188, 190, or 192.

87. The method of claim 60 in which the amino donor is selected from the group consisting of isopropylamine, α-phenethylamine, D-alanine or L-alanine.

88. The method of claim 60 in which the reaction condition is 20°C to about 55°C.

89. The method of claim 60 in which the reaction condition is 35°C to 50°C.

90. The method of claim 60 in which the amino acceptor byproduct is removed.

91. The method of claim 60 which is carried out in presence of reduced nicotinamide adenine dinucleotide (NADH).

WHAT IS CLAIMED IS:

1. An engineered transaminase enzyme, comprising:
a polypeptide comprising an amino acid sequence that has a T residue at position X9 and is at least 90% identical to SEQ ID NO: 6, wherein the polypeptide has transaminase activity.

2. The engineered transaminase enzyme of claim 1, wherein the polypeptide has at least 10% residual activity in the conversion of pyruvate to L-alanine in presence of amino donor isopropylamine after treatment of the polypeptide at 50° C for 23 h.

3. The engineered transaminase enzyme of claim 1, wherein the amino acid sequence further comprises one or more of the amino acid substitutions selected from the group consisting of: X45 is H; X86 is Y, F, S, N, A, G, or H; X177 is L; X211 is K; X294 is V; X324 is G; and X391 is A.

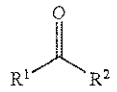
4. The engineered transaminase enzyme of claim 3, wherein the amino acid sequence comprises the amino acid substitutions: X45 is H, X86 is Y, X177 is L, X211 is K, X294 is V, X324 is G, and X391 is A.

5. The engineered transaminase enzyme of claim 3, wherein the enzyme further comprises increased transaminase activity for conversion of an amino acceptor substrate to the corresponding chiral amino product as compared to the enzyme of SEQ ID NO: 2.

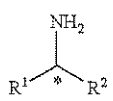
6. The engineered transaminase enzyme of claim 1, which comprises an amino acid sequence corresponding to SEQ ID NO: 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148, 150, 152, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, or 192.

7. A method for the conversion of a substrate of Formula I to a chiral amine product of Formula III in stereomeric excess, the method comprising contacting the substrate of Formula I with the engineered transaminase polypeptide of claim 1 in the presence of an amino donor under suitable reaction conditions for the conversion of the substrate of Formula I to the chiral amine product of Formula III

Formula I



Formula III



wherein

the chiral carbon atom of the chiral amine product is marked with an *;
each of R^1, and R^2, when taken independently, is an unsubstituted or substituted alkyl, alkylaryl, or aryl group, wherein R^1 is different from R^2 in structure or chirality, or R^1 and R^2, taken together, is a hydrocarbon chain of 4 or more carbon atoms containing a center of chirality.