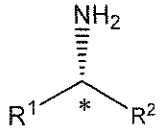
Biotech. 69:499-505, Yun et al., 2004, Appl. Environ. Microbiol. 70:2529-2534; and Hwang et al., 2004, Enzyme Microbiol. Technol. 34: 429-426). Chiral amines play an important role in the pharmaceutical, agrochemical and chemical industries. Chiral amines are frequently used as intermediates or synthons for the preparation of various pharmaceuticals, such as cephalosporin or pyrrolidine derivatives. Examples of the use of aminotransferases to generate useful chemical compounds include: preparation of intermediates and precursors of pregabalin (e.g., WO 2008/127646); the stereospecific synthesis and enantiomeric enrichment of β-amino acids (e.g., WO 2005/005633); the enantiomeric enrichment of amines (e.g., US Patent No. 4,950,606, US Patent No. 5,300,437, and US Patent No. 5,169,780); and the production of amino acids and derivatives (e.g., US Patent No. 5,316,943, US Patent No. 4,518,692, US Patent No. 4,826,766, US Patent No. 6,197,558, and US Patent No. 4,600,692). Hence, transaminases are useful for the enantiomeric enrichment and stereoselective synthesis of chiral amines.

4. SUMMARY

[0006] The present disclosure provides processes for the biocatalytic conversion of prochiral keto substrates to chiral amines in presence of an amino group donor, and transaminase biocatalysts and corresponding polynucleotides for use in the processes. Disclosed is~~In one aspect, the present invention comprise~~ a process for preparing an amine product of structural formula (I):



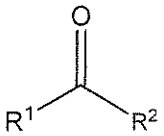
(I)

having the indicated stereochemical configuration at the stereogenic center marked with an *; in enantiomeric excess over the opposite enantiomer, wherein

R^1 is optionally substituted aryl or heteroaryl; and

R^2 is an optionally substituted C_1-C_6 alkyl, $-R^3C(O)R^4$, or $-R^3OC(O)R^5$;

wherein R^3 is an optionally substituted C_1-C_4 alkyl; and R^4 is H, an optionally substituted C_1-C_4 alkyl, NR^6R^7, or OR^8, where R^5, R^6, R^7, and R^8 are independently H or C_1-C_4 alkyl, which process comprises contacting a ketone substrate of structural formula (II):



(II)

with a transaminase polypeptide in presence of an amino donor under reaction conditions suitable for converting the ketone substrate to the amine product. In some embodiments, the transaminase polypeptide has at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,

97%, 98%, 99% or more amino acid sequence identity to SEQ ID NO:4 and is capable of converting the ketone substrate to the amine product at a rate that is improved as compared to the transaminase of SEQ ID NO:2.

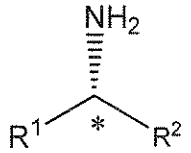
[0007] In some embodiments, the transaminase polypeptide has at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more amino acid sequence identity to SEQ ID NO: 58, 72, 74, 80, 86, 96, 98, 100, or 102 and is capable of converting the ketone substrate to the amine product at a rate that is improved as compared to the transaminase of SEQ ID NO:2. Specific embodiments of the engineered transaminases for use in the processes are further provided in the detailed description.

[0008] In some embodiments, the engineered transaminase has at least 5%, 10%, 20%, 30%, 40%, 50% or more activity of SEQ ID NO:74.

[0009] In any of the embodiments of the processes disclosed herein, the process can be carried out wherein the transaminase polypeptide is capable of converting the sitagliptin ketoamide substrate to sitagliptin at a rate that is improved as compared to the transaminase of SEQ ID NO:2, and has at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more amino acid sequence identity to SEQ ID NO:4.

[0010] In some embodiments, the amine product is produced in at least 70%, 80%, 85%, 90%, 95%, 96%, 97%, 96%, or 99% or more enantiomeric excess. In some embodiments of the processes described above, the amine product is produced in at least 99% enantiomeric excess.

[0010a] In one aspect, there is provided a process for preparing an amine product of structural formula (I):



(I)

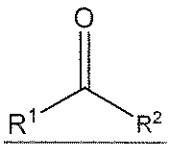
having the indicated stereochemical configuration at the stereogenic center marked with an *; in an enantiomeric excess over the opposite enantiomer, wherein

R^1 is optionally substituted aryl or heteroaryl;

R^2 is an optionally substituted C_1-C_6 alkyl, $-R^3C(O)R^4$, or $-R^3OC(O)R^5$

R^3 is an optionally substituted C_1-C_4 alkyl, and R^4 is H, an optionally substituted C_1-C_4 alkyl, NR^6R^7, or OR^8, where R^5, R^6, R^7, and R^8 are independently H or C_1-C_4 alkyl;

the process comprising contacting a ketone substrate of structural formula (II):



(II)

with a transaminase polypeptide in presence of an amino donor under reaction conditions suitable for converting the ketone substrate to the amine product, wherein the transaminase polypeptide has at least 80% sequence identity to SEQ ID NO:74 and is capable of converting the ketone substrate to the amine product at a rate that is improved as compared to SEQ ID NO:2, and wherein the transaminase polypeptide comprises an amino acid sequence having a residue difference as compared to SEQ ID NO: 2 at one or more positions, wherein the amino acid residue at the position of the residue difference is selected from: X61 is Y; X62 is T, Y or F, X65 is A; X69 is C or T; X94 is I or L; X122 is I or M; X136 is Y or F; X199 is I; X209 is L; X215 is C; X223 is P; X282 is S; and X284 is G.

[0011] In some embodiments of the process, R^1 is an optionally substituted phenyl. In some embodiments, R^1 is an optionally substituted pyridinyl. In some embodiments, the substitutions on the aryl or heteroaryl are selected from C_1-C_4 alkyl, -OR', -SR', -NR'R', $-NO_2$, -NO, -CN, $-CF_3$, halogen (e.g., -F, -Cl, -Br and –I), -C(O)R', -C(O)OR', -C(O)NR', $-S(O)_2R'$, $-S(O)_2NR'R''$, where each R' and R'' are independently selected from the group consisting of hydrogen and (C_1-C_4) alkyl. In some embodiments R^2 is methyl or halo substituted methyl. In some embodiments, R^2 is CF_2H or CF_3.

[0012] In some embodiments, the substitution on C_1-C_6 alkyl and R^3 of group R^2 are selected from halogen, NR^5R^6 or $-OR^8$, where R^5 and R^6 are defined above and R^8 is H or C_1-C_4 alkyl. In some embodiments, the compounds of formula (I) produced in the process described above are substantially chirally pure compounds. In certain embodiments, the compounds of formula (I) produced in the process described above are chirally pure.

[0013] In some embodiments of the process, the amine product of formula (I) is: