Asymmetric Synthesis of Chiral Amines from Ketones Applied to Sitagliptin Manufacture, Science, Vol. 329, pgs. 305-309, 16 July 2010.

Though advances in producing chiral amines using transaminases have been highly regarded, there still exits some drawbacks to the enzymatic process. Currently enzymatic processes can only be run in aqueous solvent systems as the transaminases are not stable in 100% organic solvents. Additionally, during product amine isolation, the transaminase catalyst is deactivated and discarded resulting in the inability to reuse the catalyst.

Thus, though attempts have been made to immobilize transaminases none have been successful in overcoming their lack of stability, more specifically their lack of stability in organic solvents.

SUMMARY ~~OF THE INVENTION~~

Described herein, are immobilized transaminases comprising a recombinant transaminase physically attached to a resin. The immobilized transaminases described herein include recombinant transaminases that are capable of converting 4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-one to (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine in the presence of an amino group donor to levels measurable by an analysis technique. In certain embodiments the immobilized transaminases described herein are used to make (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine.

In certain embodiments, described herein are immobilized transaminases comprising a recombinant transaminase that is capable of converting 4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-one to (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine in the presence of an amino group donor to levels measurable by an analysis technique; and a resin, wherein the recombinant transaminase is attached to the resin by physical interactions, and wherein the immobilized transaminase is stable in a solvent system that comprises at least 90% of organic solvents.

For example in one embodiment, the recombinant transaminase is attached to a resin by hydrophilic interactions.

The immobilized transaminases described herein are stable in organic solvents systems. As used herein stable immobilized transaminases mean that the immobilized transaminase retains its structural conformation or its activity, in organic solvent systems. In one embodiment described herein, the immobilized transaminase is stable in a solvent system that comprises at least 95% of organic solvents.

In certain embodiments described herein, the recombinant transaminase is a transaminase that is capable of converting 4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-one to (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine in the presence of an amino group donor to levels measurable by HPLC-UV absorbance.

In yet another embodiment the recombinant transaminase is selected from the group consisting of: SEQ ID NO: 1.

In certain embodiments of the immobilized transaminases described herein, the resin comprises methacrylates. In one aspect, there is provided an immobilized transaminase comprising: a recombinant transaminase that is SEQ ID NO: 1; and a resin comprising methacrylate and wherein the recombinant transaminase is attached to the resin. For example in one embodiment, the resin is synthesized from methacrylates, wherein the monomer and the crosslinker are methacrylates. In certain embodiments, the resin does not contain any aromatic compounds. In other embodiments, the resin is DIAION HP2MG (Mistubishi). DIAION HP2MG is a high porous, methacrylate based adsorption resin which is synthesized only from methacrylates (monomer and crosslinker are methacrylates). DIAION HP2MG (Mistubishi) does not contain any aromatic compounds, and is considered an intermediate polarity adsorption resin. DIAION HP2MG (Mistubishi)is suitable for desalting and adsorption of organic compounds of relatively high polarity by using the more hydrophilic characteristics of the polymer matrix.

In one embodiment, of the immobilized transaminase, transaminase SEQ ID NO: 1 is attached to the resin DIAION HP2MG (Mitsubishi). In another embodiment, the transaminase SEQ ID NO: 1 is attached to the resin DIAION HP2MG (Mitsubishi) via hydrophilic interactions. In another aspect, there is provided an immobilized transaminase comprising the transaminase SEQ ID NO: 1 physically attached to the resin DIAION HP2MG (Mitsubishi).

Also, described herein are methods of making and using the immobilized transaminases. The immobilized transaminases described herein can be used in batch reactions, wherein the immobilized transaminases can be filtered out after the reaction is complete and reused in other reactions. Alternatively, the immobilized transaminases described herein can be used in a continuous reaction system wherein the starting material is continuously passed over the immobilized transaminase and the product is collected.

In one embodiment the process described herein is a process for making (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine comprising ~~comprising~~ the steps of:

1) dissolving 4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-one in an organic solvent;

2) contacting 4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-one with an immobilized transaminase that is capable of converting 4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-one to (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-

dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine in presence of an amino group.

In another aspect, there is provided a process for making (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine comprising the steps of:

1) dissolving 4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-one in an organic solvent;

2) contacting 4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-one with an immobilized transaminase as defined herein in the presence of an amino group.

Preferably in such a process the immobilized transaminase is the transaminase SEQ ID NO: 1 physically attached to the resin DIAION (Mitsubishi).

Also described herein is a process of making an immobilized transaminase comprising:

1) incubating a solution of tranaminase with a resin and an enzyme solution to form an immobilized transaminase;

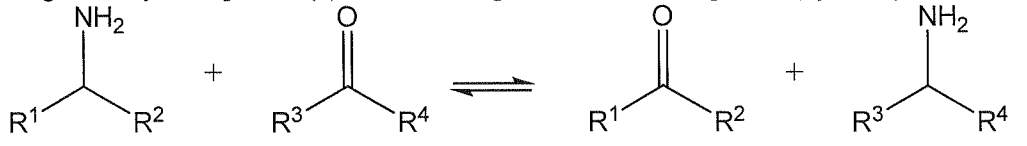
2) filtering and rinsing the immobilized transaminase;

3) drying the immobilized transaminase.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

"Transaminase", also called "aminotransferase", is used herein to refer to a polypeptide having an enzymatic capability of transferring an amino group (NH_2) and a hydrogen atom from a primary amine (3) to an acceptor carbonyl compound (2), converting the amine donor into its corresponding carbonyl compound (4) and the acceptor into its corresponding primary amine (1):



(3) (2) (4) (1)

With regard to the immobilized transaminases described herein, the transaminase polypeptides are capable of converting the substrate of formula (2a), 4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-one (the "ketoamide substrate"), to the product of formula (1a) (2*R*)-4-oxo-4-[3-(trifluoromethyl)-5,6-dihydro[1,2,4]triazolo[4,3-*a*]pyrazin-7(8*H*)-yl]-1-(2,4,5-trifluorophenyl)butan-2-amine (the "product) in presence of an amino group donor of formula (3), as follows: