catalysts. In particular, it would be desirable to explore new transformations that provide optically pure intermediates which minimize the environmental burden by using biocatalysts, such as enzymes. The present disclosure provides such enzymes and a process for making a useful intermediate to prepare belzutifan.

SUMMARY

The present disclosure relates to enzymes derived from the fungi *Fusarium oxysporum* c8D (hereinafter "FoPip4H enzymes") capable of hydroxylating a substituted indanone to provide an optically pure alcohol. In some examples, the subject FoPip4H enzymes described herein are capable of converting 4-fluoro-7-(methylsulfonyl)-2,3-dihydro-1H-inden-1-one (II) to stereomerically pure (R)-4-fluoro-3-hydroxy-7-(methylsulfonyl)-2,3-dihydro-1H-inden-1-one (I), which is useful for the synthesis of belzutifan, 3-[[(1S,2S,3R)-2,3-difluoro-2,3-dihydro-1-hydroxy-7-(methylsulfonyl)-1H-inden-4-yl]oxy]-5-fluorobenzonitrile.

Additional examples describe processes for preparing the subject FoPip4H enzymes and processes for using the subject FoPip4H enzymes.

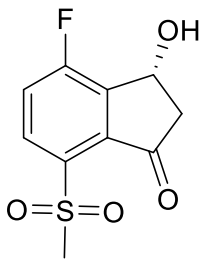
In one aspect, the present disclosure provides a polypeptide comprising an amino acid sequence having at least a 90% sequence identity to SEQ ID NO: 2. In some examples, the amino acid sequence has at least a 95% sequence identity to SEQ ID NO: 2. In certain examples, the amino acid sequence has at least a 98% sequence identity to SEQ ID NO: 2. In particular examples, the the amino acid sequence consists of SEQ ID NO: 2. In a specific example, the polypeptide consists of SEQ ID NO:2.

In another aspect, the present disclosure provides a polynucleotide encoding any of the aforesaid polypeptides. In a particular example, the polynucleotide comprises SEQ ID NO:3.

In another aspect, the present disclosure provides an expression vector comprising any of the aforesaid polynucleotides, operably linked to one or more control sequences suitable for directing expression of the encoded polypeptide in a host cell. In some examples, the control sequence comprises a promoter. In certain examples, the promoter comprises an *E. coli* promoter.

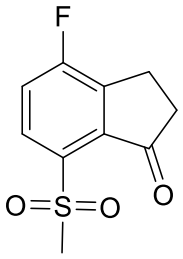
In another aspect, the present disclosure provides a host cell comprising any of the aforesaid expression vectors. In certain examples, the host cell is *E. coli*.

In another aspect, the present disclosure provides a process for preparing a compound according to Formula (I)



(I)

in at least 60% enantiomeric excess (ee), the process comprising contacting the indanone according to Formula (II)



(II)

with α-ketoglutarate in the presence of the aforesaid polypeptides to provide the compound of Formula (I). In specific examples of the process, the compound according to Formula (I) is prepared in at least 80%, 90%, 95%, 98%, or 99% ee.

In some examples, the process further comprises a reductant selected from the group consisting of L-cysteine, ascorbic acid, dithiothreitol, D-cysteine, L-homocysteine, and D-cysteine ethyl ester.

In certain examples, the process is performed with a buffer selected from the group consisting of phosphate buffer, 2-morpholinoethanesulfonic acid, Bis Tris, PIPES, citrate, bicine, and TEOA.

In some examples, the process further comprises an iron salt selected from the group consisting of Mohr's salt ($(NH_4)_2Fe(SO_4)_2\ 6H_2O$), and iron chloride.

Other examples, aspects and features of the present disclosure are either further described in or will be apparent from the ensuing description, examples and appended claims.a

DETAILED DESCRIPTION OF THE INVENTION

Definitions

Certain technical and scientific terms are specifically defined below. Unless