Claims

1. An engineered oxynitrilase polypeptide comprising an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 606, which is, under suitable reaction conditions, capable of coupling 1,1,1-trifluoropropan-2-one with nitromethane to produce (*S*)-1,1,1-trifluoro-2-methyl-3-nitropropan-2-ol in an enantiomeric excess of at least 60%, 70%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more.

2. The oxynitrilase polypeptide of claim 1, wherein the suitable reaction conditions include about 5 g/L to about 150 g/L 1,1,1-trifluoropropan-2-one, nitromethane loading about 2 times the molar amount of 1,1,1-trifluoropropan-2-one, at least 3 g/L oxynitrilase polypeptide, isopropyl acetate concentration of about 20% (v/v) to about 60% (v/v), pH of about 4.0 to 8.0, and temperature of about 10 ℃ to 30 ℃.

3. A polypeptide immobilized on a solid material by chemical bond or a physical adsorption method, wherein the polypeptide is an engineered oxynitrilase polypeptide of (a) or (b) below:
(a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 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, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458, 460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 492, 494, 496, 498, 500, 502, 504, 506, 508, 510, 512, 514, 516, 518, 520, 522, 524, 526, 528, 530, 532, 534, 536, 538, 540, 542, 544, 546, 548, 550, 552, 554, 556, 558, 560, 562, 564, 566,

568, 570, 572, 574, 576, 578, 580, 582, 584, 586, 588, 590, 592, 594, 596, 598, 600, 602, 604, 608, 610, 612, 614, 616, 618, 620, 622, 624, 626, 628, 630, 632, 634, 636, 638, and 640;

or

(b) a polypeptide having oxynitrilase activity, which comprises an amino acid sequence having (i) at least 80% sequence identity to one of the polypeptides recited in (a), and (ii) a substitution, deletion, addition or insertion of one or more amino acid residues relative to said one amino acid sequence recited in (a).

4. Use of a polynucleotide for providing the polypeptide of any one of claims 1 to 3.

5. Use of the polynucleotide of claim 4, wherein the polynucleotide sequence is 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, 197, 199, 201, 203, 205, 207, 209, 211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233, 235, 237, 239, 241, 243, 245, 247, 249, 251, 253, 255, 257, 259, 261, 263, 265, 267, 269, 271, 273, 275, 277, 279, 281, 283, 285, 287, 289, 291, 293, 295, 297, 299, 301, 303, 305, 307, 309, 311, 313, 315, 317, 319, 321, 323, 325, 327, 329, 331, 333, 335, 337, 339, 341, 343, 345, 347, 349, 351, 353, 355, 357, 359, 361, 363, 365, 367, 369, 371, 373, 375, 377, 379, 381, 383, 385, 387, 389, 391, 393, 395, 397, 399, 401, 403, 405, 407, 409, 411, 413, 415, 417, 419, 421, 423, 425, 427, 429, 431, 433, 435, 437, 439, 441, 443, 445, 447, 449, 451, 453, 455, 457, 459, 461, 463, 465, 467, 469, 471, 473, 475, 477, 479, 481, 483, 485, 487, 489, 491, 493, 495, 497, 499, 501, 503, 505, 507, 509, 511, 513, 515, 517, 519, 521, 523, 525, 527, 529, 531, 533, 535, 537, 539, 541, 543, 545, 547, 549, 551, 553, 555, 557, 559, 561, 563, 565, 567, 569, 571, 573, 575, 577, 579, 581, 583, 585, 587, 589, 591, 593, 595, 597, 599, 601, 603, 607, 609, 611, 613, 615, 617, 619, 621, 623, 625, 627, 629, 631, 633, 635, 637 or 639.

6. Use of an expression vector comprising the polynucleotide according to any one of claims 4 and 5 for providing the polypeptide of any one of claims 1 to 3.

7. Use of the expression vector of claim 6, which comprises a plasmid, a cosmid, a bacteriophage or a viral vector.

8. A method of preparing an oxynitrilase polypeptide, which comprises the steps of culturing a host cell comprising the expression vector of any one of claims 6 and 7 and obtaining an oxynitrilase polypeptide from the culture, wherein the host cell is preferably E. coli.

9. An oxynitrilase catalyst comprising cells or culture fluid containing the oxynitrilase polypeptides of any one of claims 1 to 3, or an article processed therewith, wherein the article refers to an extract obtained from the culture of transformant cell, an isolated product obtained by isolating or purifying an oxynitrilase from the extract, or an immobilized product obtained by immobilizing transformant cell, an extract thereof, or isolated product of the extract.

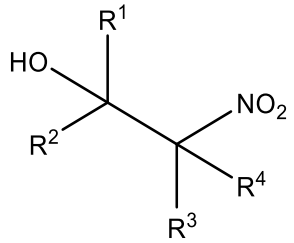
10. A process for the asymmetric synthesis of a β-nitro alcohol, the process comprising the step of contacting a nitroalkane and an aldehyde or ketone substrate with the oxynitrilase polypeptide of (a) or (b) below:
(a) a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 4, 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, 154, 156, 158, 160, 162, 164, 166, 168, 170, 172, 174, 176, 178, 180, 182, 184, 186, 188, 190, 192, 194, 196, 198, 200, 202, 204, 206, 208, 210, 212, 214, 216, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, 250, 252, 254, 256, 258, 260, 262, 264, 266, 268, 270, 272, 274, 276, 278, 280, 282, 284, 286, 288, 290, 292, 294, 296, 298, 300, 302, 304, 306, 308, 310, 312, 314, 316, 318, 320, 322, 324, 326, 328, 330, 332, 334, 336, 338, 340, 342, 344, 346, 348, 350, 352, 354, 356, 358, 360, 362, 364, 366, 368, 370, 372, 374, 376, 378, 380, 382, 384, 386, 388, 390, 392, 394, 396, 398, 400, 402, 404, 406, 408, 410, 412, 414, 416, 418, 420, 422, 424, 426, 428, 430, 432, 434, 436, 438, 440, 442, 444, 446, 448, 450, 452, 454, 456, 458, 460, 462, 464, 466, 468, 470, 472, 474, 476, 478, 480, 482, 484, 486, 488, 490, 492, 494, 496, 498, 500, 502, 504, 506, 508, 510, 512, 514, 516, 518, 520, 522, 524, 526, 528, 530, 532, 534, 536, 538, 540, 542, 544, 546, 548, 550, 552, 554, 556, 558, 560, 562, 564, 566, 568, 570, 572, 574, 576, 578, 580, 582, 584, 586, 588, 590, 592, 594, 596, 598, 600, 602, 604, 608, 610, 612, 614, 616, 618, 620, 622, 624, 626, 628, 630, 632, 634, 636, 638, and 640;

or

(b) a polypeptide having oxynitrilase activity, which comprises an amino acid sequence having (i) at least 80% sequence identity to one of the polypeptides recited in (a), and (ii) a substitution, deletion, addition or insertion of one or more amino acid residues relative to said one amino acid sequence recited in (a).

11. The process according to claim 10, wherein the aldehyde or ketone substrate comprises an electron withdrawing substituent.

12. The process according to any one of claims 10 and 11, wherein the resulting β-nitro alcohol has the structure shown in formula (I):



(I),

wherein:

R^1 and R^2 are each independently selected from H, alkyl, *e.g.*, C_1-C_{20}alkyl, alkenyl, *e.g.*, C_2-C_{20}alkenyl, alkynyl, *e.g.*, C_2- C_{20}alkynyl, cycloalkyl, *e.g.*, C_3-C_{10}cycloalkyl, aryl, *e.g.*, C_6-C_{14}aryl, arylalkyl, *e.g.*, C_7-C_{20}arylalkyl, heterocyclyl, *e.g.*, 3-14 membered heterocyclyl, and heteroaryl, *e.g.*, 5-20 membered heteroaryl,

wherein the alkyl, alkenyl, and alkynyl are each optionally substituted by one or more R^a, *e.g.*, one to six R^a,

wherein the cycloalkyl, aryl, arylalkyl, heterocyclyl, and heteroaryl are each optionally substituted by one or more R^b, *e.g.*, one to six R^b;

R^3 and R^4 are each independently selected from H and alkyl, *e.g.*, C_1-C_{20}alkyl, wherein the alkyl, *e.g.*, C_1-C_{20}alkyl, is optionally substituted by one or more R^a, *e.g.*, one to six R^a;

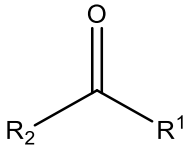
each R^a is at each occurrence independently selected from cycloalkyl, *e.g.*, C_3-C_{10}cycloalkyl, aryl, *e.g.*, C_6-C_{14}aryl, arylalkyl, *e.g.*, C_7-C_{20}arylalkyl, heterocyclyl, *e.g.*, 3-14 membered heterocyclyl, and heteroaryl, *e.g.*, 5-20 membered heteroaryl, halogen, *e.g.*, F, haloalkyl, *e.g.*, C_1-C_{20}haloalkyl, *e.g.*, $-CF_3$, -CN, $-OR^c$, $-NR^cR^c$, $-(CH_2)_nCOOR^c$, $-(CH_2)_n$-$C(=O)R^c$, $-(CH_2)_n$-$C(=O)NR^cR^c$;

each R^b is at each occurrence independently selected from halogen, *e.g.*, F, haloalkyl, *e.g.*, C_1-C_{20}haloalkyl, *e.g.*, $-CF_3$, -CN, $-NO_2$, $-OR^c$, $-NR^cR^c$, $-(CH_2)_nCOOR^c$, $-(CH_2)_n-C(=O)R^c$, $-(CH_2)_n-C(=O)NR^cR^c$, C_1-C_{20}alkyl, C_2-C_{20}alkenyl, or C_2-C_{20}alkynyl;

each R^c is at each occurrence independently selected from H, C_1-C_{20}alkyl, C_2-C_{20}alkenyl, and C_2-C_{20}alkynyl, each optionally substituted by one or more R^b, *e.g.*, one to six R^b; and

n is 0, 1, 2, 3, 4, 5 or 6, *e.g.*, 0, 1, 2 or 3;

the process comprising the step of contacting a nitroalkane of formula (II) which is $R^3R^4CHNO_2$ and an aldehyde or ketone substrate with the oxynitrilase polypeptide according to any one of claims 1 to 3, to obtain a β-nitro alcohol product of formula (I), the aldehyde or ketone substrate having the formula (III),



(III).

13. The process according claim 12, wherein R^3 and R^4 are each independently selected from H and C_1-C_{20}alkyl, wherein the C_1-C_{20}alkyl is optionally substituted by one to six R^a,

wherein each R^a is at each occurrence independently selected from C_3-C_{10}cycloalkyl, C_6-C_{14}aryl, 3-14 membered heterocyclyl, 5-20 membered heteroaryl, halogen, *e.g.*, F, haloalkyl, *e.g.*, C_1-C_{20}haloalkyl, *e.g.*, $-CF_3$, $-OR^c$, and $-NR^cR^c$;

wherein each R^c is at each occurrence independently selected from H, C_1-C_{20}alkyl, C_2-C_{20}alkenyl, and C_2-C_{20}alkynyl.

14. The process according to any one of claims 12 and 13, wherein R^3 and R^4 are each independently selected from H and C_1-C_{20}alkyl, wherein the C_1-C_{20}alkyl is optionally substituted by one to six R^a,

wherein each R^a is at each occurrence independently selected from halogen, *e.g.*, F, C_1-C_{20}haloalkyl, *e.g.*, $-CF_3$, $-OR^c$, and $–NR^cR^c$;

wherein each R^c is at each occurrence independently selected from H, C_1-C_{20}alkyl, C_2-C_{20}alkenyl, and C_2-C_{20}alkynyl.

15. The process according to any one of claims 12 to 14, wherein R^3 and R^4 are each independently selected from H and C_1-C_{20}alkyl, wherein the C_1-C_{20}alkyl is optionally substituted by one to six R^a,

wherein each R^a is at each occurrence independently selected from halogen, *e.g.*, F, C_1-C_{20}haloalkyl, *e.g.*, -CF_3, -OR^c, and –NR^cR^c;

wherein each R^c is at each occurrence independently selected from H, and C_1-C_{20}alkyl.