CLAIMS

1. An engineered monoamine oxidase comprising a polypeptide sequence comprising at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NOs: 6 and/or 414, or a functional fragment thereof, wherein said engineered monoamine oxidase polypeptide comprises at least one substitution or substitution set in said polypeptide sequence, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NOs: 6 and/or 414.

2. The engineered monoamine oxidase polypeptide of Claim 1, wherein said polypeptide sequence comprises at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO: 6, wherein said engineered monoamine oxidase polypeptide comprises at least one substitution at one or more positions selected from 429, 149, 198, 246, 260, 266, and 421, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 6; or

wherein said engineered monoamine oxidase polypeptide comprises at least one substitution selected from 429G, 149M, 198D, 246M, 260S, 266L, and 421S, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 6; or

wherein said engineered monoamine oxidase polypeptide comprises at least one substitution selected from A429G, L149M, E198D, I246M, R260S, A266L, and C421S, wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 6.

3. The engineered monoamine oxidase polypeptide of Claim 1, wherein said polypeptide sequence comprises at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO: 6, wherein said engineered monoamine oxidase polypeptide comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 13, 32/65/135/246/284, 32/135/284, 32/284, 47/197, 55/347/348, 61, 65/135, 65/190, 65/246, 65/284, 79, 80, 82, 85, 87, 87/185, 93, 100, 100/144/421/447, 100/144/447, 104, 107, 113, 114, 115, 117, 127, 135, 136, 144, 144/447, 145, 146, 147, 149, 149/157, 153, 155, 161, 162, 166, 167, 169, 170, 174, 177, 181, 184, 185, 187/242, 193, 196, 198, 199, 208, 211, 218, 219, 219/332, 221, 229, 232, 234, 238, 239, 240, 242, 246, 250, 252, 254, 260, 262/266/421, 262/421/447, 266/447, 277, 277/347, 284, 290, 292, 294, 298, 300, 306, 310, 323, 332, 338, 347, 349, 359, 361, 366, 381, 385, 386, 387, 392, 393, 400, 407, 411/445, 413, 418, 421, 425, 429, 433, 434, 441, 444, 445, 447, 449, 452, 475, and 483, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 6; or

wherein said engineered monoamine oxidase polypeptide comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 79, 80, 87, 146, 149, 219/332, 238, 260, 262/266/421, 338, and 429, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 6; or

wherein said engineered monoamine oxidase polypeptide comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 79, 79/80, 79/80/332/429, 79/93, 79/93/238/386/429, 79/93/386, 79/183/284/332, 79/238, 79/238/332/429, 79/238/429, 80, 80/183/284/332/381/386/429, 80/188/190/332/386, 80/237/332, 80/238/332, 80/238/386/429, 80/284, 80/284/386, 80/332, 80/332/381/386, 80/332/386, 80/381, 80/386, 80/386/429, 80/429, 82, 82/87, 82/87/113, 82/87/113/117/218, 82/87/113/117/219, 82/87/113/117/219/418, 82/87/113/117/260/338, 82/87/113/117/338/418, 82/87/113/421, 82/87/117, 82/87/117/218/421, 82/87/117/260/338/421, 82/87/117/338/418, 82/87/218, 82/87/218/219/338, 82/87/218/219/421, 82/87/218/338, 82/87/219, 82/87/219/421, 82/87/338, 82/87/338/418, 82/87/338/421, 82/113/218/338, 82/113/218/338/418, 82/113/219/338, 82/113/338, 82/218/219/421, 82/260/338/421, 82/338/418/421, 82/418, 87, 87/113, 87/113/117/219/338/418/421, 87/113/117/260/338/421, 87/113/117/418/421, 87/113/218/219/260/338, 87/113/219/421, 87/113/260, 87/117, 87/117/218/219, 87/117/219/310/338, 87/218, 87/218/219, 87/218/219/338, 87/218/418, 87/219, 87/219/338, 87/219/418, 87/260, 87/310, 87/310/338, 87/338, 87/418, 87/421, 93/184/429, 93/238, 93/238/284, 93/238/386, 93/238/386/429, 100, 100/143/145/146/149/198/242/347/348/445/447, 100/143/145/149/347/447, 100/145/146/149, 100/145/146/149/154/348/445, 100/145/146/149/198, 100/145/146/149/198/242/250/252/347/348/447, 100/145/146/149/205/347/445/447, 100/145/146/149/242/445, 100/145/146/149/250/277/347/348/447, 100/145/149/154/246/277, 100/145/149/198/242/246/277/348/447, 100/145/149/198/242/246/445/447, 100/145/149/198/242/277/347/348/447, 100/145/149/198/252/348/445, 100/145/149/205, 100/145/149/242/246/277/347/445, 100/145/149/242/246/347/348/445/447, 100/145/149/242/445/447, 100/145/149/246/252/277/347/445/447, 100/145/149/246/347/445, 100/145/149/277/348/445, 100/145/149/347/445/447, 100/145/198/242/246/348, 100/145/198/277/348/445, 100/145/198/300, 100/145/205/246/277/348/445, 100/145/205/277/348, 100/145/242/246/277/445/447, 100/145/246/347/348/445, 100/145/277/347, 100/145/348, 100/146/149/198/242/246/277/347/348/445/447, 100/146/198/347/348/447, 100/146/198/445, 100/146/242/277/447, 100/146/250/447, 100/146/347/348/445/447, 100/149/198, 100/149/242/246/277/347/348/445, 100/149/242/246/277/347/348/447, 100/149/242/347/348/447, 100/149/246/277/348/445, 100/149/246/447, 100/149/250/347/447, 100/198/205/348, 100/198/277/347/348, 100/198/347/348, 100/198/347/348/445, 100/198/347/445, 100/198/447, 100/242/246/277, 100/242/246/347, 100/242/277/348, 100/242/347/348/445, 100/242/347/348/445/447, 100/242/348/445, 100/246/277/348/445, 100/277/347/445/447, 100/277/348, 100/277/348/447,

100/347/348/445, 100/347/447, 100/445/447, 110/338/418, 113/218/338/421, 113/338, 113/338/421, 117/219, 117/260, 121/198/347/445/447, 145/146/149/154/246/277/347/348, 145/146/149/198/242/252/277/347, 145/146/149/198/277/445/447, 145/146/149/205/348/447, 145/146/149/250/277/347/348/447, 145/146/149/277/347, 145/146/149/347/348, 145/146/198/205/242/277/348/447, 145/146/198/445/447, 145/146/246/277/447, 145/146/246/347/348/447, 145/146/277/347, 145/146/347/447, 145/149/198/205/348, 145/149/198/242/246/277/347/447, 145/149/198/242/246/348, 145/149/198/242/277/347, 145/149/198/246/447, 145/149/198/347/447, 145/149/205/242/347/348/445, 145/149/205/246/250/252, 145/149/205/277/445, 145/149/242, 145/149/242/348, 145/149/246, 145/149/246/347/445, 145/149/246/447, 145/149/347/348/445, 145/149/347/447, 145/149/447, 145/155/445/447, 145/198/205/348, 145/246, 145/246/252/347/447, 145/250/252/348, 145/252/348/447, 145/255, 145/277/347/445, 145/277/348/447, 145/347/348/445/447/491, 145/445/447, 146/149/151/246/348, 146/149/242/246/445, 146/149/246/252/447, 146/149/277/348/447, 146/198/246/277, 146/198/277/348/447, 146/242, 146/242/246/277/347/348/445/447, 146/246/277/348/445/447, 146/277, 146/277/347/348, 146/277/347/445/447, 146/277/445/447, 146/347/348/447, 146/348, 146/445/447, 149, 149/198/277/347/348, 149/198/348, 149/198/445, 149/242/246, 149/242/246/348/445, 149/347/348/445/447, 149/445, 184, 184/284, 184/284/429, 184/386, 198/242/348, 198/246, 198/250, 198/277/348/447, 198/445, 205, 205/246/347, 205/252/347/445/447, 205/347/348/445/447, 218, 218/260/338, 219, 219/338/418, 219/421, 238, 238/242/284, 238/284/386/429, 238/332/429, 238/381/429, 238/386, 238/429, 242, 242/277/347/348, 242/284/381/429, 242/348, 242/445/447, 246, 246/277/348/447, 246/445/447, 260, 260/338, 260/338/418/421, 277/347/348/445, 277/347/447, 284, 284/332, 284/386, 284/386/429, 310/338/418, 310/418/421, 332, 332/386, 332/429, 338, 338/418, 347/348/445, 347/348/447, 348, 348/445/447, 348/447, 386, 386/429, 418, 429, and 447, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 6; or

wherein said engineered monoamine oxidase polypeptide comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 80/429, 87/113/117/260/338/421, 87/421, 100/145/146/149/198/242/250/252/347/348/447, 100/145/149/198/242/277/347/348/447, 110/338/418, 145/149/205/246/250/252, 145/246/252/347/447, 145/252/348/447, 238/386/429, and 242/284/381/429, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 6.

4. The engineered monoamine oxidase polypeptide of Claim 1, wherein said polypeptide sequence comprises at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NO: 414, wherein said engineered monoamine oxidase polypeptide comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 87/113/145/198/242/246/262/266, 87/117/149/242, 87/117/242/260/266,

87/145/149/198/447, 87/149/242/260/266, 87/149/260/262/266/381, 87/198, 87/242/246/260/262/266/338, 87/284/338/347, 113/117/146/149/260/262, 113/117/266/381, 113/117/447, 113/145/146/149/242/447, 113/242/347, 113/242/381, 117/145/146/262/284, 117/145/149/284/447, 117/149, 117/198, 117/198/262, 117/242/246/260/266/284/381, 117/242/260/262, 117/246/260/262/381, 117/246/284, 117/266, 145/146/149/260/262/338/347, 145/146/242/260/262/338/347/447, 145/149/198/260/266/338/347, 145/149/260/266/338/347/381/447, 146/149/260/262/347, 149/198/246/260/266/421, 198/447, 242, 242/246, 242/246/338/347, 242/260/262/338, 242/381, 246, 260/262/277/284/447, 260/266, 262, 347/381, and 447, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 414; or

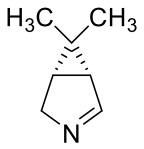
wherein said engineered monoamine oxidase polypeptide comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 87/145/149/198/447, 87/149/260/262/266/381, 113/117/146/149/260/262, 117/145/149/284/447, 145/149/198/260/266/338/347, 146/149/260/262/347, and 149/198/246/260/266/421, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 414.

5. The engineered monoamine oxidase polypeptide of Claim 1, wherein said engineered monoamine oxidase polypeptide comprises an amino acid sequence with at least 80% sequence identity to any even-numbered sequence set forth in SEQ ID NOs: 6 to 1084.

6. The engineered monoamine oxidase polypeptide of Claim 1, wherein said engineered monoamine oxidase polypeptide comprises a polypeptide sequence set forth in the even numbered sequences of SEQ ID NOs: 6 to 1084.

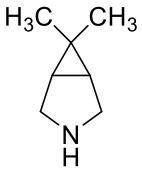
7. The engineered monoamine oxidase polypeptide of any one of Claims 1 to 6, wherein said engineered monoamine oxidase polypeptide comprises a polypeptide sequence that exhibits at least one improved property compared to the engineered monoamine oxidase polypeptide of SEQ ID NOs: 6 and/or 414.

8. The engineered monoamine oxidase polypeptide of Claim 7, wherein said improved property comprises improved production of Compound **2**



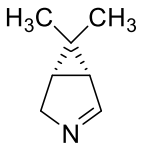
Compound **2** .

9. The engineered monoamine oxidase polypeptide of Claim 7, wherein said improved property comprises reduced enzyme loading for the conversion of Compound **1**



Compound **1**

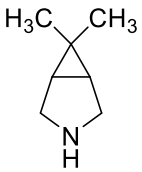
to Compound **2**



Compound **2**

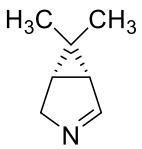
, as compared to a reference sequence of SEQ ID NO: 6 or 414.

10. The engineered monoamine oxidase polypeptide of Claim 7, wherein said improved property comprises 2-fold, 5-fold, 10-fold, or more activity for the conversion of Compound **1**



Compound **1**

to Compound **2**



Compound **2**

, as compared to a reference sequence of SEQ ID NO: 6 or 414.

11. A composition comprising at least one engineered monoamine oxidase polypeptide provided in any one of Claims 1 to 10.

12. An engineered polynucleotide encoding at least one engineered monoamine oxidase polypeptide of any one of Claims 1 to 10.

13. An engineered polynucleotide sequence encoding at least one engineered polypeptide, wherein said polynucleotide sequence comprises at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NOs: 5 and/or 413, wherein the polynucleotide sequence of said polypeptide comprises at least one substitution at one or more positions.

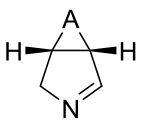
14. An engineered polynucleotide comprising any one of the odd-numbered sequences set forth in SEQ ID NOs: 5 to 1083.

15. A vector comprising the engineered polynucleotide of any one of Claims 12 to 14.

16. A host cell comprising the vector of Claim 15.

17. A method of producing an engineered monoamine oxidase polypeptide in a host cell, comprising culturing the host cell of Claim 16, in a culture medium under suitable conditions, such that at least one engineered monoamine oxidase polypeptide is produced.

18. A method of preparing a substantially stereomerically pure compound according to structural Formula **II**:

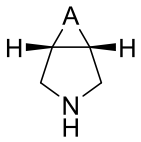


II

including salts and hydrates thereof, wherein:

A is O, C**R**^{1}**R**^{2}, -C=C-, or –CH_2-CH_2-, wherein **R**^{1} and **R**^{2} are each independently selected from -H, -COOH, -**X**, -NH_2, -$CH_2NHC(NH)NH_2$, -C**X**$_3$, -CH_3, -CH_2CH_3, and wherein **X** is selected from F, Cl, and Br;

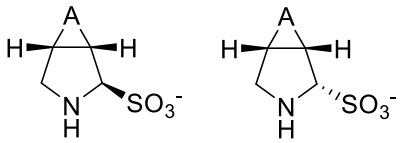
the method comprising contacting an amine compound according to structural Formula **I**



I

wherein **A** is as defined for structural Formula **II**, with oxygen in the presence of an engineered monoamine oxidase enzyme with a co-factor under conditions in which the engineered monoamine oxidase enzyme oxidizes the amine compound of structural Formula **I** to a corresponding imine compound of structural Formula **II,** wherein the engineered monoamine oxidase comprises a polypeptide sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NOs: 6 and/or 414, or a functional fragment thereof, wherein said engineered monoamine oxidase polypeptide comprises at least one substitution or substitution set in said polypeptide sequence, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NOs: 6 and/or 414 .

19. A method of preparing a mixture comprising a substantially enantiomerically pure aminosulfonate compound according to structural Formula **III(a**) and a substantially enantiomerically pure aminosulfonate compound according to structural Formula **III(b)**:

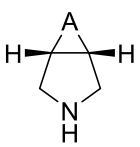


III(a) III(b)

including salts and hydrates thereof, wherein:

A is O, CR1R2, -C=C-, or –CH2-CH2-, wherein R1 and R2 are each independently selected from -H, -COOH, -X, -NH2, -CH2NHC(NH)NH2, -CX3, -CH3, -CH2CH3, and wherein X is selected from F, Cl, and Br;

the method comprising contacting an amine compound according to structural Formula **I**

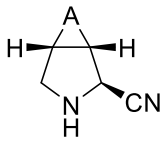


I

wherein **A** is as defined for structural Formula **III** with oxygen in the presence of an engineered monoamine oxidase enzyme associated with a co-factor and with bisulfite, wherein the engineered

monoamine oxidase comprises a polypeptide sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to SEQ ID NOs: 6 and/or 414, or a functional fragment thereof, wherein said engineered monoamine oxidase polypeptide comprises at least one substitution or substitution set in said polypeptide sequence, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NOs: 6 and/or 414 under conditions which yield the mixture comprising a substantially enantiomerically aminosulfonate compound according to structural Formula **III(a)** and a substantially enantiomerically aminosulfonate compound according to structural Formula **III(b)**.

20. A method of preparing a substantially enantiomerically pure aminonitrile compound according to structural Formula **IV(a)**:

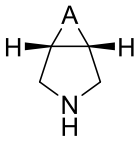


IV(a)

including salts and hydrates thereof, wherein:

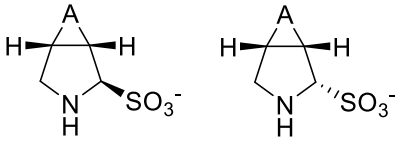
A is O, C**R**^{1}**R**^{2}, -C=C-, or –CH_2-CH_2-, wherein **R**^{1} and **R**^{2} are each independently selected from -H, -COOH, -**X**, -NH_2, -$CH_2NHC(NH)NH_2$, -C**X**$_3$, -CH_3, -CH_2CH_3, and wherein **X** is selected from F, Cl, and Br;

the method comprising contacting an amine compound according to structural Formula **I**



I

wherein **A** is as defined for structural Formula **IV** with oxygen in the presence of an engineered monoamine oxidase enzyme associated with a co-factor and with bisulfite wherein the engineered monoamine oxidase comprises a polypeptide sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to any of the even-numbered sequences of SEQ ID NOs: 8-1084 under conditions which yield a mixture comprising a substantially enantiomerically pure aminosulfonate compound according to structural Formula **III(a)** and a substantially enantiomerically pure aminosulfonate compound according to structural Formula **III(b)**



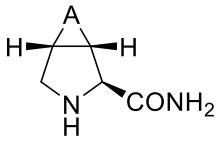
III(a) III(b) ; and

contacting the compounds according to structural Formulae **III(a)** and **III(b)** with cyanide under conditions which yield the substantially enantiomerically pure aminonitrile compound according to structural Formula **IV(a)**.

21. The method of Claim 20, wherein the engineered monoamine oxidase comprises a polypeptide sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to any of the even-numbered sequences of SEQ ID NOs: 8 to 1084.

22. The method of Claim 20, wherein the substantially enantiomerically pure aminonitrile compound according to structural Formula **IV(a)** is contacted with an acid and water under conditions in which the aminonitrile compound is converted to a substantially stereomerically pure amino acid compound.

23. A method of preparing a substantially stereomerically pure amino amide compound according to structural Formula **VII**:

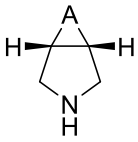


VII

including salts thereof, wherein:

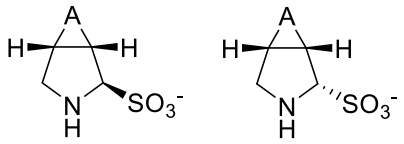
A is O, C**R**^{1}**R**^{2}, -C=C-, or –CH_2-CH_2-, wherein **R**^{1} and **R**^{2} are each independently selected from -H, -COOH, -**X**, -NH_2, -$CH_2NHC(NH)NH_2$, -C**X**$_3$, -CH_3, -CH_2CH_3, and wherein **X** is selected from F, Cl, and Br;

the method comprising contacting an amine compound according to structural Formula **I**



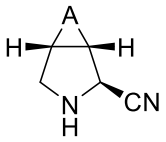
I

wherein **A** is as defined for structural Formula **VII** with oxygen in the presence of an engineered monoamine oxidase enzyme wherein the engineered monoamine oxidase comprises a polypeptide sequence having at least 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% sequence identity to any of the even-numbered sequences of SEQ ID NOs: 8-1084 associated with a co-factor and with bisulfite under conditions which yield a mixture comprising a substantially enantiomerically pure aminosulfonate compound according to structural Formula **III(a)** and a substantially enantiomerically pure aminosulfonate compound according to structural Formula **III(b)**



III(a) III(b) ; and

contacting a stereomerically pure bisulfite amine adduct compound according to structural Formula **III(a)**, substantially stereomerically pure bisulfite amine adduct compound according to structural Formula **III(b)** or a mixture thereof with cyanide under conditions which yield the substantially stereomerically pure aminonitrile compound according to structural Formula **IV(a)**



IV(a)

wherein **A** is as defined for the amino compound of structural Formula **VII**; and

contacting the aminonitrile compound of structural Formula **IV(a)** with an acid under conditions in which the aminonitrile compound is converted to the substantially stereomerically pure amino amide compound according to structural Formula **VII**.