

Formula **(XIVb)**,

wherein R_1 is C_1-C_3 alkyl, C_1-C_3 deuteroalkyl, or C_1-C_3 haloalkyl, R_2 is a nitrogen protecting group, n is an integer from 1 to 20, and * is a stereocenter; or

4) Crystallizing a compound of Formula (I) or a salt thereof from one or more solvents, optionally chloroform and hexanes.

[0009] In some embodiments, the compound of Formula (XIb) is crystallized in a mixture of tetrahydrofuran and methyl t-butyl ether. In some embodiments, the ratio of tetrahydrofuran and methyl t-butyl ether is between: 1:10 and 3:10. For example, the ratio is 1.5:10.

[0010] In some embodiments, the compound of Formula (I) or a salt thereof is crystallized in a mixture of chloroform and hexanes. In some embodiments, the ratio of chloroform to hexanes is between 1:5 and 1:1. For example, the ratio is 1:3 or 1:2. Also provided herein are methods of preparing a polypeptide, comprising making the polypeptide with one or more crystalline compounds of Formula (I) or their crystalline salts.

[0010a] In some embodiments, there is provided a method of making a polypeptide, comprising making the polypeptide with one or more crystalline compounds of Formula (I) or their crystalline salts as described herein.

[0010b] In some embodiments, there is provided a method of making a peptide, the method comprising reacting a crystalline compound of Formula (I), or its crystalline salt with an amino acid building block in presence of a coupling agent, thereby forming a peptide bond.

BRIEF DESCRIPTION OF THE DRAWINGS

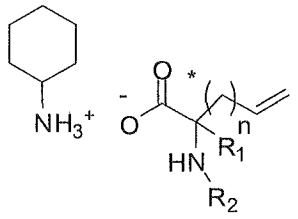
[0011] Fig. 1 is a chiral HPLC trace of N-Fmoc-(S)-alpha-methyl-alpha-amino-6-enoic acid.

[0012] Fig. 2 is a chiral HPLC trace of N-Fmoc-(S)-alpha-methyl-alpha-amino-6-enoic acid spiked with N-Fmoc-(R)-alpha-methyl-alpha-amino-6-enoic acid .

[0013] Fig. 3 is an HPLC trace of N-Fmoc-(S)-alpha-methyl-alpha-amino-6-enoic acid with the detector set to 215 nm.

[0014] Fig. 4 is an HPLC trace of N-Fmoc-(S)-alpha-methyl-alpha-amino-6-enoic acid with the detector set to 254 nm.

[0015] Fig. 5 is an HPLC trace of an N-Fmoc-(S)-alpha-methyl-alpha-amino-6-enoic acid standard.



Formula **(XIVb)**,

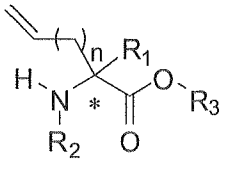
wherein R_1 is C_1-C_3 alkyl, C_1-C_3 deuteroalkyl, or C_1-C_3 haloalkyl, R_2 is a nitrogen protecting group, n is an integer from 1 to 20, and * is a stereocenter; or

4) Crystallizing a compound of Formula (I) or a salt thereof from one or more solvents, optionally chloroform and hexanes.

[0009] In some embodiments, the compound of Formula (XIb) is crystallized in a mixture of tetrahydrofuran and methyl t-butyl ether. In some embodiments, the ratio of tetrahydrofuran and methyl t-butyl ether is between: 1:10 and 3:10. For example, the ratio is 1.5:10.

[0010] In some embodiments, the compound of Formula (I) or a salt thereof is crystallized in a mixture of chloroform and hexanes. In some embodiments, the ratio of chloroform to hexanes is between 1:5 and 1:1. For example, the ratio is 1:3 or 1:2. Also provided herein are methods of preparing a polypeptide, comprising making the polypeptide with one or more crystalline compounds of Formula (I) or their crystalline salts.

[0010a] In some embodiments, there is provided a method of preparing the crystalline compound of Formula (I) or its crystalline salt:



Formula (I)

wherein:

R_1 is C_1-C_3 alkyl, C_1-C_3 deuteroalkyl, or C_1-C_3 haloalkyl;

* is a stereocenter;

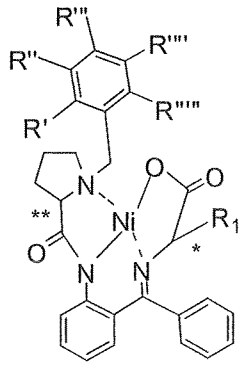
n is an integer from 1 to 20;

R_2 is –H or a nitrogen protecting group; and

R_3 is –H or a protecting or activating group,

comprising performing at least one purification step selected from the group consisting of the steps:

1) Crystallizing a metal complex of Formula (XIb)



(XIb)

from one or more solvents, wherein R_1 is C_1-C_3 alkyl, C_1-C_3 deuteroalkyl, or C_1-C_3 haloalkyl, * and ** are each independently stereocenters, and R', R'', R''', R'''', and R''''' are, in the order going around the aromatic ring from R' to R''''', selected from the following combinations:

H, H, Cl, Cl, H;

F, F, F, F, F;

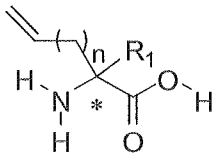
F, F, OiPr, F, F;

F, F, OMe, F, F;

Cl, H, H, H, H; or

H, H, Me, Me, H;

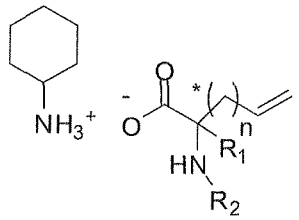
2) Precipitating a compound of Formula (Ia) as its HCl salt:



Formula **(Ia)**

wherein R_1 is C_1-C_3 alkyl, C_1-C_3 deuteroalkyl, or C_1-C_3 haloalkyl, n is an integer from 1 to 20, and * is a stereocenter;

3) Forming an addition salt of Formula (XIVb):



Formula (XIVb),

wherein R_1 is C_1-C_3 alkyl, C_1-C_3 deuteroalkyl, or C_1-C_3 haloalkyl, R_2 is a nitrogen protecting group, n is an integer from 1 to 20, and * is a stereocenter; and/or

4) crystallizing a compound of Formula (I) or a salt thereof from one or more solvents.

[0010b] In some embodiments, there is provided a method of making a polypeptide, comprising making the polypeptide with one or more crystalline compounds of Formula (I) or their crystalline salts as described herein.

[0010c] In some embodiments, there is provided a method of making a peptide, the method comprising reacting a crystalline compound of Formula (I), or its crystalline salt with an amino acid building block in presence of a coupling agent, thereby forming a peptide bond.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Fig. 1 is a chiral HPLC trace of N-Fmoc-(S)-alpha-methyl-alpha-amino-6-enoic acid.

[0012] Fig. 2 is a chiral HPLC trace of N-Fmoc-(S)-alpha-methyl-alpha-amino-6-enoic acid spiked with N-Fmoc-(R)-alpha-methyl-alpha-amino-6-enoic acid.

[0013] Fig. 3 is an HPLC trace of N-Fmoc-(S)-alpha-methyl-alpha-amino-6-enoic acid with the detector set to 215 nm.

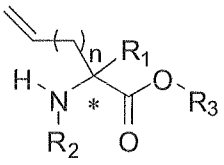
[0014] Fig. 4 is an HPLC trace of N-Fmoc-(S)-alpha-methyl-alpha-amino-6-enoic acid with the detector set to 254 nm.

[0015] Fig. 5 is an HPLC trace of an N-Fmoc-(S)-alpha-methyl-alpha-amino-6-enoic acid standard.

CLAIMS

WHAT IS CLAIMED IS:

1. A crystalline compound of Formula (I) or its crystalline salt:



Formula (I)

wherein:

R_1 is C_1-C_3 alkyl, C_1-C_3 deuteroalkyl, or C_1-C_3 haloalkyl;

* is a stereocenter;

n is an integer from 1 to 20;

R_2 is –H or a nitrogen protecting group; and

R_3 is –H or a protecting or activating group.

2. The crystalline compound of Formula (I) or its crystalline salt of claim 1, wherein R_1 is C_1-C_3 alkyl.
3. The crystalline compound of Formula (I) or its crystalline salt of any one of the preceding claim, wherein R_1 is methyl.
4. The crystalline compound of Formula (I) or its crystalline salt of any one of the preceding claim, wherein R_2 is selected from the group consisting of:

-H,

9-Fluorenylmethoxycarbonyl (Fmoc),

Trityl (Trt),

4-Methoxytrityl (Mmt),

2-(3,5-Dimethoxyphenyl)propan-2-yloxycarbonyl (Ddz),

2-(p-Biphenylyl)-2-propyloxycarbonyl (Bpoc),

2-(4-Nitrophenylsulfonyl)ethoxycarbonyl (NSC),
(1,1-Dioxobenzo[b]thiophene-2-yl)methyloxycarbonyl (Bsmoc),
(1,1-Dioxonaphtho[1,2-b]thiophene-2-yl)methyloxycarbonyl (α-Nsmoc),
1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl (ivDde),
2,7-Di-tert-butyl-Fmoc (Fmoc*),
2-Fluoro-Fmoc (Fmoc(2F)),
2-Monoisooctyl-Fmoc (mio-Fmoc),
2,7-Diisooctyl-Fmoc (dio-Fmoc),
2-[Phenyl(methyl)sulfonio]ethyloxy carbonyl tetrafluoroborate (Pms),
Ethanesulfonylethoxycarbonyl (Esc),
2-(4-Sulfophynylsulfonyl)ethoxy carbonyl (Sps),
Tert-butyloxycarbonyl (Boc),
Benzyloxycarbonyl (Z),
Allyloxycarbonyl (Alloc),
2,2,2-Trichloroethyloxycarbonyl (Troc),
p-Nitrobenzyloxycarbonyl (pNZ),
Propargyloxycarbonyl (Poc),
o-Nitrobenzenesulfonyl (oNBS),
2,4-Dinitrobenzenesulfonyl (dNBS),
Benzothiazole-2-sulfonyl (Bts),
o-Nitrobenzyloxycarbonyl (oNz),
4-Nitroveratryloxycarbonyl (NVCO),
2-(2-Nitrophenyl)propyloxycarbonyl (NPPOC),
2-(3, 4-Methylethenedioxy-6-nitrophenyl)propyloxycarbonyl (MNPPOC),
9-(4-Bromophenyl)-9-fluorenyl (BrPhF),
Azidomethoxycarbonyl (Azoc),
Hexafluoroacetone (HFA),
2-Chlorobenzyloxycarbonyl (Cl-Z),
4-Methyltrityl (Mtt),
Trifluoroacetyl (tfa),
(Methylsulfonyl)ethoxycarbonyl (Msc),
Phenyldisulphanylethyloxycarbonyl (Phdec),

2-Pyridyldisulphanylethyloxycarbonyl (Pydec), and
o-Nitrobenzenesulfonyl (O-NBS).

5. The crystalline compound of Formula (I) or its crystalline salt of any one of the preceding claim, wherein R_2 is selected from the group consisting of:

9-Fluorenylmethoxycarbonyl (Fmoc),
Trityl (Trt),
4-Methoxytrityl (Mmt),
2-(3,5-dimethoxyphenyl)propan-2-yloxycarbonyl (Ddz),
2-(p-biphenylyl)-2-propyloxycarbonyl (*Bpoc*),
2-(4-Nitrophenylsulfonyl)ethoxycarbonyl (NSC),
1,1-Dioxobenzo[b]thiophene-2-yl)methyloxycarbonyl (Bsmoc),
1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl (ivDde),
Tert-butyloxycarbonyl (Boc),
Benzyloxycarbonyl (Z),
Allyloxycarbonyl (Alloc),
2,2,2-Trichloroethyloxycarbonyl (Troc),
p-Nitrobenzyloxycarbonyl (pNZ),
o-Nitrobenzenesulfonyl (oNBS),
2,4-Dinitrobenzenesulfonyl (dNBS),
o-Nitrobenzyloxycarbonyl (oNz),
4-Nitroveratryloxycarbonyl (NVCO),
2-(2-Nitrophenyl)propyloxycarbonyl (NPPOC),
Hexafluoroacetone (HFA),
2-Chlorobenzyloxycarbonyl (Cl-Z),
4-Methyltrityl (Mtt),
Trifluoroacetyl (tfa),
(Methylsulfonyl)ethoxycarbonyl (Msc), and
o-Nitrobenzenesulfonyl (O-NBS).

6. The crystalline compound of Formula (I) or its crystalline salt of any one of the preceding claim, wherein R_2 is selected from the group consisting of:

9-Fluorenylmethoxycarbonyl (Fmoc),
4-Methoxytrityl (Mmt),
2-(3,5-dimethoxyphenyl)propan-2-yloxycarbonyl (Ddz),

2-(p-biphenylyl)-2-propyloxycarbonyl (*Bpoc*),

Tert-butyloxycarbonyl (Boc),

Benzyloxycarbonyl (Z),

Allyloxycarbonyl (Alloc),

2,2,2-Trichloroethyloxycarbonyl (Troc),

o-Nitrobenzenesulfonyl (oNBS),

Trityl (Trt),

4-Methyltrityl (Mtt), and

o-Nitrobenzenesulfonyl (O-NBS).

7. The crystalline compound of Formula (I) or its crystalline salt of any one of the preceding claim, wherein R_2 is:

9-Fluorenylmethoxycarbonyl (Fmoc).

8. The crystalline compound of Formula (I) or its crystalline salt of any one of the preceding claim, wherein R_3 is selected from the group consisting of:

-H,

tert-Butyl (^{t}Bu),

2-Chlorotrityl (2-Cl-Trt),

2,4-Dimethoxybenzyl (DMB),

Benzyl (Bn),

2-Phenylisopropyl (2-Ph^{i}Pr),

5-Phenyl-3,4-ethylenedioxythenyl,

9-Fluorenylmethyl (Fm),

4-(N-[1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl]-amino)benzyl (Dmab),

Methyl (Me),

Ethyl (Et),

Carbamoylmethyl (Cam),

Allyl (Al),

Phenacyl (Pac),

p-Nitrobenzyl (pNB),

2-Trimethylsilylethyl (TMSE),

(2-Phenyl-2-trimethylsilyl)ethyl (PTMSE),

2-(Trimethylsilyl)isopropyl (Tmsi),

Trimethylsilyl (TMS),

2,2,2-Trichloroethyl (Tce),
p-Hydroxyphenacyl (pHP),
4,5-Dimethoxy-2-nitrobenzyl (Dmnb),
1,1-Dimethylallyl (Dma),
Pentaamine cobalt (III),
Succinimide,
p-Nitrophenyl,
Pentaflurophenyl, and
2, 4, 5-trichlorophenyl.

9. The crystalline compound of Formula (I) or its crystalline salt of any one of the preceding claim, wherein R_3 is:
-H.

10. The crystalline compound of Formula (I) or its crystalline salt of any one of the preceding claim, wherein n is an integer from 3 to 11.

11. The crystalline compound of Formula (I) or its crystalline salt of any one of the preceding claim, wherein n is selected from the group consisting of: 3 and 6.

12. The crystalline compound of Formula (I) or its crystalline salt of any one of claims 1-11, wherein the stereocenter * is (S).

13. The crystalline compound of Formula (I) or its crystalline salt of any one of claims 1-11, wherein the stereocenter * is (R).

14. The crystalline compound of Formula (I) or its crystalline salt of any one of claims 1-13, having a chemical purity ranging from about 90% to 100%.

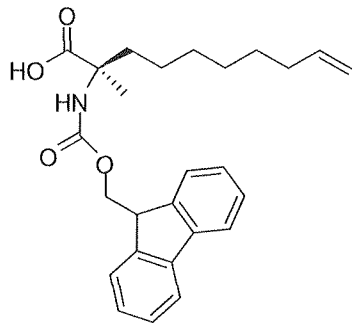
15. The crystalline compound of Formula (I) or its crystalline salt of any one of claims 1-13, having an optical purity ranging from about 90% to 100%.

16. The crystalline compound of Formula (I) or its crystalline salt of any one of claims 1-13, having an optical purity ranging from about 95% to 100%.

17. The crystalline compound of Formula (I) or its crystalline salt of any one of claims 1-13, having an enantiomeric excess ranging from about 90% to 100%.

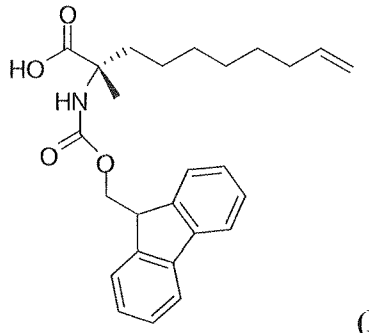
18. The crystalline compound of Formula (I) or its crystalline salt of any one of claims 1-13, having an enantiomeric excess ranging from about 95% to 100%.

19. The crystalline compound of Formula (I) or its crystalline salt of claim 1, having the Formula (IIa):



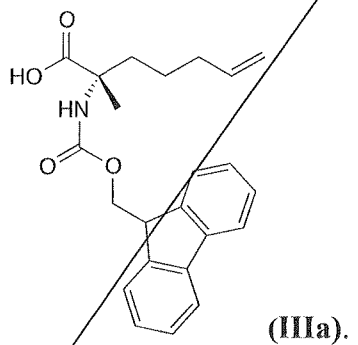
(IIa).

20. The crystalline compound of Formula (I) or its crystalline salt of claim 1, having the Formula **(IIb)**:



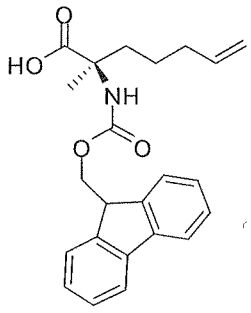
(IIb).

21. The crystalline compound of Formula (I) or its crystalline salt of claim 1, having the Formula (IIIa):



(IIIa).

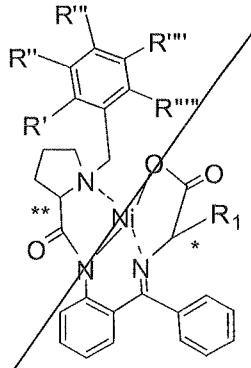
22. The crystalline compound of Formula (I) or its crystalline salt of claim 1, having the Formula (IIIb):



(IIIb).

23. A method of preparing the crystalline compound of Formula (I) or its crystalline salt of claim 1, comprising performing at least one purification step selected from the group consisting of the steps:

1) Crystallizing a metal complex of Formula (XIb)



(XIb)

from one or more solvents, wherein R_1 is C_1-C_3 alkyl, C_1-C_3 deuteroalkyl, or C_1-C_3 haloalkyl, * and ** are each independently stereocenters, and R', R'', R''', R'''', and R''''' are, in the order going around the aromatic ring from R' to R''''', selected from the following combinations:

H, H, Cl, Cl, H;

F, F, F, F, F;

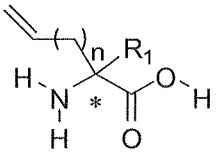
F, F, OiPr, F, F;

F, F, OMe, F, F;

Cl, H, H, H, H; or

H, H, Me, Me, H;

2) Precipitating a compound of Formula (Ia) as its HCl salt:

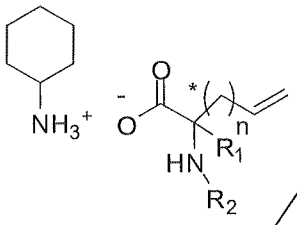


Formula **(Ia)**

,

wherein R_1 is C_1-C_3 alkyl, C_1-C_3 deuteroalkyl, or C_1-C_3 haloalkyl, n is an integer from 1 to 20, and * is a stereocenter;

3) Forming an addition salt of Formula (XIVb):



Formula **(XIVb)** ,

wherein R_1 is C_1-C_3 alkyl, C_1-C_3 deuteroalkyl, or C_1-C_3 haloalkyl, R_2 is a nitrogen protecting group, n is an integer from 1 to 20, and * is a stereocenter; and/or

4) Crystallizing a compound of Formula (I) or a salt thereof from one or more solvents.

24. The method of claim 23, comprising performing steps 2), 3), and 4).

25. The method of claim 23, comprising steps 2) and 4).

26. A method of making a polypeptide, comprising making the polypeptide with one or more crystalline compounds of Formula (I) or their crystalline salts of claim 1.

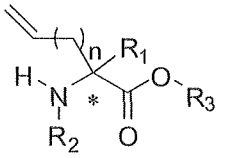
27. The method of claim 23, wherein crystallization of the compound of Formula XIb in step 1 is conducted with methyl tertiary-butyl ether.

28. The method of claim 23, wherein crystallization of the compound Formula XIb in step 1 is conducted with tetrahydrofuran and methyl tertiary-butyl ether.
29. The method of claim 23, wherein crystallization of the compound of Formula XIb in step 1 is conducted with isopropyl acetate.
30. The method of claim 23, wherein the precipitated HCl salt of Formula (Ia) in step 2 is further crystallized with acetonitrile.
31. The method of claim 23, wherein the precipitated HCl salt of Formula (Ia) in step 2 is further crystallized with acetonitrile and methyl tertiary-butyl ether.
32. The method of claim 23, wherein the precipitated HCl salt of Formula (Ia) in step 2 is further crystallized with isopropyl acetate.
33. The method of claim 23, wherein step 3 is conducted in methyl tertiary-butyl ether.
34. The method of claim 23, wherein step 3 is conducted in acetonitrile.
35. The method of claim 23, wherein step 4 comprises crystallizing a cyclohexyl amine (CHA) salt of Formula (I) with tetrahydrofuran and methyl tertiary-butyl ether.
36. The crystalline compound of Formula (I) or its crystalline salt of claim 14, wherein the chemical purity is determined by HPLC.
37. The crystalline compound of Formula (I) or its crystalline salf of any one of claim 19-22, having an enantiomeric excess of about 95% to 100%.
38. The method of claim 23, wherein step 4 comprises crystallizing the compound of Formula (I) or a salt thereof from chloroform and/or hexanes.
39. A method of making a peptide, the method comprising reacting a crystalline compound of Formula (I), or its crystalline salt with an amino acid building block in presence of a coupling agent, thereby forming a peptide bond.
40. The method of claim 40, wherein the peptide comprises an α-helix.
41. The method of claim 40, further comprising crosslinking a pair of terminal alkene side chains of at least two amino acids of the peptide.
42. The method of claim 42, wherein the crosslinking comprises a metal-catalyzed olefin metathesis reaction.

CLAIMS

WHAT IS CLAIMED IS:

1. A method of preparing the crystalline compound of Formula (I) or its crystalline salt:



Formula (I)

wherein:

R_1 is C_1-C_3 alkyl, C_1-C_3 deuteroalkyl, or C_1-C_3 haloalkyl;

* is a stereocenter;

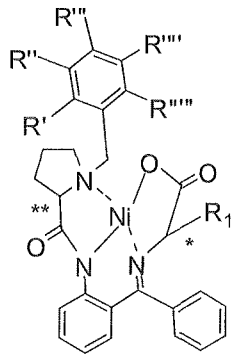
n is an integer from 1 to 20;

R_2 is –H or a nitrogen protecting group; and

R_3 is –H or a protecting or activating group,

comprising performing at least one purification step selected from the group consisting of the steps:

1) Crystallizing a metal complex of Formula (XIb)



(XIb)

from one or more solvents, wherein R_1 is C_1-C_3 alkyl, C_1-C_3 deuteroalkyl, or C_1-C_3 haloalkyl, * and ** are each independently stereocenters, and R', R'', R''', R'''', and R''''' are, in the order going around the aromatic ring from R' to R''''', selected from the following combinations:

H, H, Cl, Cl, H;

F, F, F, F, F;

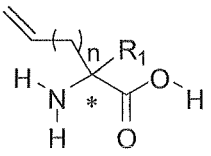
F, F, OiPr, F, F;

F, F, OMe, F, F;

Cl, H, H, H, H; or

H, H, Me, Me, H;

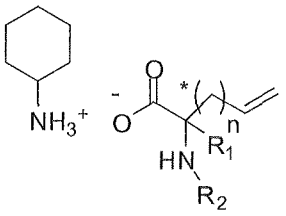
2) Precipitating a compound of Formula (Ia) as its HCl salt:



Formula **(Ia)**

wherein R_1 is C_1-C_3 alkyl, C_1-C_3 deuteroalkyl, or C_1-C_3 haloalkyl, n is an integer from 1 to 20, and * is a stereocenter;

3) Forming an addition salt of Formula (XIVb):



Formula (XIVb),

wherein R_1 is C_1-C_3 alkyl, C_1-C_3 deuteroalkyl, or C_1-C_3 haloalkyl, R_2 is a nitrogen protecting group, n is an integer from 1 to 20, and * is a stereocenter; and/or

4) crystallizing a compound of Formula (I) or a salt thereof from one or more solvents.

2. The method of claim 1, wherein crystallization of the compound of Formula XIb in step 1 is conducted with methyl tertiary-butyl ether.

3. The method of claim 1, wherein crystallization of the compound Formula XIb in step 1 is conducted with tetrahydrofuran and methyl tertiary-butyl ether.

4. The method of claim 1, wherein crystallization of the compound of Formula XIb in step 1 is conducted with isopropyl acetate.

5. The method of claim 1, wherein the precipitated HCl salt of Formula (Ia) in step 2 is further crystallized with acetonitrile.

6. The method of claim 1, wherein the precipitated HCl salt of Formula (Ia) in step 2 is further crystallized with acetonitrile and methyl tertiary-butyl ether.

7. The method of claim 1, wherein the precipitated HCl salt of Formula (Ia) in step 2 is further crystallized with isopropyl acetate.

8. The method of claim 1, wherein step 3 is conducted in methyl tertiary-butyl ether.

9. The method of claim 1, wherein step 3 is conducted in acetonitrile.

10. The method of claim 1, wherein step 4 comprises crystallizing a cyclohexyl amine (CHA) salt of Formula (I) with tetrahydrofuran and methyl tertiary-butyl ether.