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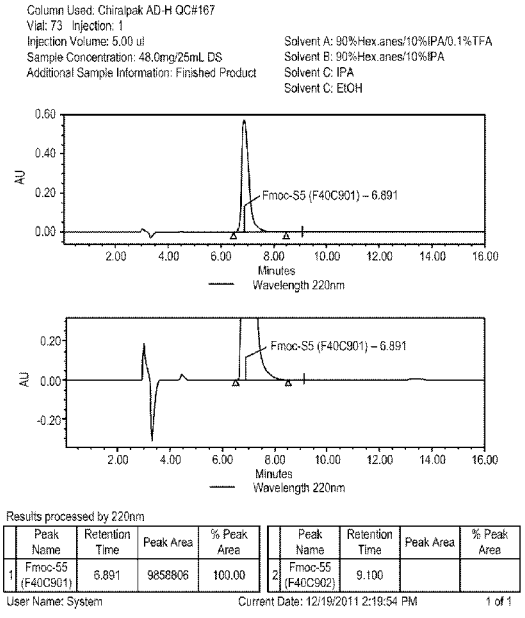

FIG. 1

(57) Abstract: Provided are crystalline α, α-disubstituted amino acids and their crystalline salts containing a terminal alkene on one of their side chains, as well as optionally crystalline halogenated and deuterated analogs of the a, a-disubstituted amino acids and their salts; methods of making these, and methods of using these.

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DISUBSTITUTED AMINO ACIDS AND METHODS OF PREPARATION AND USE THEREOF

BACKGROUND OF THE INVENTION

[0001] α, α-Disubstituted amino acids bearing a terminal alkene on one of their side chains and their salts ("alkene α, α-disubstituted amino acids") are useful for making cross-linked macrocyclic peptides. For example, International Application No. PCT/US2004/038403 ("the '403 application") discloses incorporating into a peptide two α, α-disubstituted amino acids that each contain a side-chain bearing a terminal alkene, and cross-linking the terminal alkene groups to form a cross-linked ("stapled") macrocyclic peptide. The cross-link can, for example, stabilize a secondary structure (*e.g.*, an α-helix) present in the stapled macrocyclic peptide.

[0002] International Application Publication No. WO2008/121767 ("the '767 publication") discloses using alkene α, α-disubstituted amino acids to form stitched polypeptides (*e.g.*, multiple and tandem crosslinked polypeptides) having secondary structures stabilized by stitching. The '403 application, the '767 publication, and other applications, publications, and patents, disclose that stapled and stitched macrocyclic peptides are useful for treating and preventing various diseases including cancer.

[0003] Alkene α, α-disubstituted amino acids are thus important and useful building blocks for forming stitched and stapled polypeptides and their precursors. The use of alkene α, α-disubstituted amino acids, however, has been limited by an inability to provide these important molecules in crystalline form. For example, commercially available preparations of alkene α, α-disubstituted amino acids are typically sold as pre-made solutions. The pre-made solutions limit the amount of α, α-disubstituted amino acid that can be shipped per unit volume, limit the chemical reactions that are available to be run with the alkene α, α-disubstituted amino acids, subject the alkene α, α-disubstituted amino acids to an enhanced degradation rate, and are environmentally unfriendly. Thus, there remains a compelling need for crystalline alkene α, α-disubstituted amino acids and their crystalline salts, and processes for producing and using these crystalline amino acids.

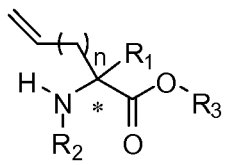
[0004] In addition, substituting one or more hydrogen atoms of an alkene α, α-disubstituted amino acid with deuterium or a halogen atom can change one or more of the amino acid's properties. For example dipole moment, hydrophobicity, hydrophilicity, steric bulk, or reactivity of an alkene α, α-disubstituted amino acid can be changed by

substituting one or more hydrogen atoms thereon with one or more deuterium or halogen atoms. Thus, there also remains a need for optionally crystalline alkene α, α-disubstituted amino acids and their optionally crystalline salts having one or more hydrogen atoms thereon substituted with deuterium or halogen, and methods of making and using these.

SUMMARY OF THE INVENTION

[0005] The above needs, and others, are addressed herein. The inventive embodiments provided in this Summary of the Invention are meant to be illustrative only and to provide an overview of selected inventive embodiments disclosed herein. The Summary of the Invention, being illustrative and selective, does not limit the scope of any claim, does not provide the entire scope of inventive embodiments disclosed or contemplated herein, and should not be construed as limiting or constraining the scope of this disclosure or any claimed inventive embodiment.

[0006] Provided herein are crystalline compounds of Formula (I) and crystalline salts thereof:



Formula **(I)**

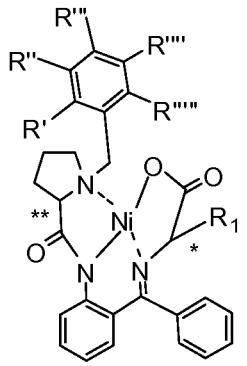
,

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.

[0007] 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.

[0008] Further provided herein are methods of making crystalline compounds of Formula (I) or their crystalline salts, comprising at least one of the following purifications:

1) Crystallizing a metal complex of Formula (XIb)



(XIb)

from one or more solvents, optionally a cyclic ether, optionally tetrahydrofuran and methyl tert-butyl ether, or optionally an alcohol, optionally isopropyl alcohol, optionally an ester, optionally isopropyl acetate, optionally ethyl acetate, 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

H, H, Cl, Cl, H;

F, F, F, F, F;

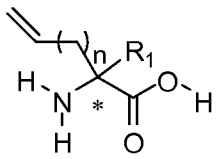
F, F, OiPr, F, F;

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:

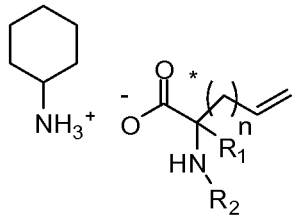


Formula **(Ia)**

,

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; 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.

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.

[0016] Fig. 6 is a chiral HPLC trace of N-Fmoc-(R)-alpha-methyl-alpha-aminodec-9-enoic acid.

[0017] Fig. 7 is a chiral HPLC trace of N-Fmoc-(R)-alpha-methyl-alpha-aminodec-9-enoic acid spiked with N-Fmoc-(S)-alpha-methyl-alpha-aminodec-9-enoic acid.

[0018] Fig. 8 is an HPLC trace of N-Fmoc-(R)-alpha-methyl-alpha-aminodec-9-enoic acid with the detector set to 215 nm.

[0019] Fig. 9 is an HPLC trace of N-Fmoc-(R)-alpha-methyl-alpha-aminodec-9-enoic acid with the detector set to 254 nm.

INCORPORATION BY REFERENCE

[0020] All publications, patents, and patent applications referenced herein are incorporated by reference in their entireties. In the event of a conflict between a term herein and a term incorporated by reference, the term herein controls.

DETAILED DESCRIPTION OF THE INVENTION

[0021] The details of one or more inventive embodiments are set forth in the accompanying drawings, the claims, and in the description herein. Other features, objects, and advantages of inventive embodiments disclosed and contemplated herein will be apparent from the description and drawings, and from the claims.

Initial Definitions

[0022] As used herein, unless otherwise indicated, the article "a" means one or more unless explicitly otherwise provided for.

[0023] As used herein, unless otherwise indicated, terms such as "contain," "containing," "include," "including," and the like mean "comprising."

[0024] As used herein, unless otherwise indicated, the term "or" can be conjunctive or disjunctive.

[0025] Herein, unless otherwise indicated, any embodiment can be combined with any other embodiment.

[0026] Herein, unless otherwise indicated, some inventive embodiments herein contemplate numerical ranges. When ranges are present, the ranges include the range endpoints. Additionally, every subrange and value within the range is present as if explicitly written out.

[0027] Herein, unless otherwise indicated, the symbol "D" stands for deuterium or a radical thereof.

[0028] Herein, unless otherwise indicated, the term "halo" or the term "halogen" each refer to fluorine, chlorine, bromine or iodine, or a radical thereof.

[0029] Herein, unless otherwise indicated, the term "alkyl" refers to a hydrocarbon chain that is a straight chain or branched chain, containing the indicated number of carbon atoms. For example, C_1-C_3 alkyl group indicates that the group has from 1 to 3 (inclusive) carbon atoms in it.

[0030] "Deuteroalkyl" refers to a deuterated alkyl chain, where the alkyl chain hydrogen atoms are replaced at least the 90% level with deuterium atoms.

[0031] Herein, unless otherwise indicated, the term "haloalkyl" refers to a halogenated alkyl chain where the alkyl chain hydrogen atoms are replaced with halogen atoms. In some embodiments, the halogen atoms are all the same (*e.g.*, all F or all Cl).

[0032] Herein, unless otherwise indicated, $\diagup\!\!\diagup$ is a double (*e.g.*, alkene) bond.

[0033] As used herein, unless otherwise indicated, the term "peptidomimetic macrocycle" or "crosslinked polypeptide" refers to a compound comprising a plurality of amino acid residues joined by a plurality of peptide bonds and at least one macrocycle-forming linker which forms a macrocycle between a first naturally-occurring or non-naturally-occurring amino acid residue (or analog) and a second naturally-occurring or non-naturally-occurring amino acid residue (or analog) within the same molecule. Peptidomimetic macrocycles include embodiments where the macrocycle-forming linker connects an α-carbon of the first amino acid residue (or analog) to the α-carbon of the second amino acid residue (or analog) in the peptide. Peptidomimetic macrocycles include one or more non-peptide bonds between one or more amino acid residues and/or amino acid analog residues, and optionally include one or more non-naturally-occurring amino acid residues or amino acid analog residues in addition to any which form the macrocycle.

[0034] As used herein, unless otherwise indicated, a "corresponding uncrosslinked polypeptide" when referred to in the context of a peptidomimetic macrocycle is understood to relate to a polypeptide of the same length as the macrocycle and comprising the equivalent natural amino acids of the wild-type sequence corresponding to the macrocycle.

[0035] As used herein, unless otherwise indicated, the term "amino acid" refers to a molecule containing both an amino group and a carboxyl group. Suitable amino acids include, for example, both the D-and L-isomers of the naturally-occurring amino acids, as well

as non-naturally occurring amino acids prepared by organic synthesis or other metabolic routes. The term amino acid contemplates, for example, α-amino acids, natural amino acids, non-natural amino acids, and amino acid analogs.

[0036] As used herein, unless otherwise indicated, the term "α-amino acid" refers to a molecule containing both an amino group and a carboxyl group bound to a carbon atom which is designated the α-carbon atom.

[0037] As used herein, unless otherwise indicated, the term "naturally occurring amino acid" refers to any one of the twenty amino acids commonly found in peptides synthesized in nature, and known by the one letter abbreviations A, R, N, C, D, Q, E, G, H, I, L, K, M, F, P, S, T, W, Y and V.

[0038] Herein, unless otherwise indicated, the term "amino acid side chain" refers to a moiety attached to the α-carbon atom (or another backbone atom) in an amino acid. For example, the amino acid side chain for alanine is methyl, the amino acid side chain for phenylalanine is phenylmethyl, the amino acid side chain for cysteine is thiomethyl, the amino acid side chain for aspartate is carboxymethyl, the amino acid side chain for tyrosine is 4-hydroxyphenylmethyl, etc. Other non-naturally occurring amino acid side chains are also included, for example, those that occur in nature (*e.g.*, an amino acid metabolite) or those that are made synthetically (*e.g.*, an α,α di-substituted amino acid).

[0039] Herein, unless otherwise indicated, the term "α,α di-substituted amino" acid refers to a molecule or moiety containing both an amino group and a carboxyl group bound to a carbon atom (*e.g.*, the α-carbon atom) that is also attached a natural and non-natural, to two natural, or to two non-natural amino acid side chains.

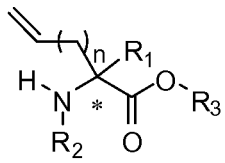
[0040] Herein, unless otherwise indicated, the term "polypeptide" can encompass two or more naturally or non-naturally-occurring amino acids joined by a covalent bond (*e.g.*, an amide bond). Polypeptides, as described herein can include full length proteins (*e.g.*, fully processed proteins) as well as shorter amino acid sequences (*e.g.*, fragments of naturally-occurring proteins or synthetic polypeptide fragments).

[0041] Herein, unless otherwise indicated, the term "macrocyclization reagent" or "macrocycle-forming reagent" can refer to any reagent which can be used to prepare a peptidomimetic macrocycle by mediating the reaction between two reactive olefinic groups thereon. The reactive groups that, once reacted, close the linker, can be for example terminal olefins (alkenes), deuterated or non-deuterated.

[0042] Macrocyclization reagents or macrocycle-forming reagents can be metathesis catalysts including, but not limited to, stabilized, late transition metal carbene complex catalysts such as Group VIII transition metal carbene catalysts. For example, such catalysts can contain Ru and Os metal centers having a +2 oxidation state, an electron count of 16 and pentacoordinated. The catalysts can have W or Mo centers. Various catalysts are disclosed in Grubbs *et al.*, "Ring Closing Metathesis and Related Processes in Organic Synthesis" *Acc. Chem. Res.* 1995, *28*, 446-452; U.S. Pat. No. 5,811,515; U.S. Pat. No. 7,932,397; U.S. Pat. Application Pub. No. 2011/0065915; U.S. Pat. Application Pub. No. 2011/0245477; Yu *et al.*, "Synthesis of Macrocyclic Natural Products by Catalyst-Controlled Stereoselective Ring-Closing Metathesis," *Nature* 2011, *479*, 88; and Peryshkov *et al.*, "Z-Selective Olefin Metathesis Reactions Promoted by Tungsten Oxo Alkylidene Complexes," *J. Am. Chem. Soc.* 2011, *133*, 20754.

[0043] Herein, unless otherwise indicated, the term "treatment" is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease.

[0044] Provided herein are crystalline compounds of Formula (I) or their crystalline salts:



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.

R_1

[0045] In the crystalline compound of Formula (I) or its crystalline salt, R_1 can be C_1-C_3 alkyl. R_1 can be, for example, methyl, ethyl, n-propyl, or isopropyl.

[0046] In the crystalline compound of Formula (I) or its crystalline salt, R_1 can be C_1-C_3 deuteroalkyl. R_1 can be, for example, -CD_3, -CD_2CD_3, -$CD_2CD_2CD_3$, or –$CD(CD_3)_2$.

[0047] In the crystalline compound of Formula (I) or its crystalline salt, R_1 can be C_1-C_3 haloalkyl. The halogen can be, for example, -F, -Cl, -Br, or –I. R_1 can be, for example, -CX_3, -CX_2CX_3, -$CX_2CX_2CX_3$, or -$CX(CX_3)_2$, wherein X is a halogen.

R_2

[0048] In the crystalline compound of Formula (I) or its crystalline salt, R_2 can be, for example, -H, or a nitrogen protecting group 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), (1,1-Dioxonaphtho[1,2-b]thiophene-2-yl)methyloxycarbonyl (α-Nsmoc), 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-3-methylbutyl (ivDde),

2,-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).

[0049] Nitrogen protecting groups can be found, for example, in Isidro-Llobet, A., *et al.*, "Amino Acid-Protecting Groups," *Chem. Rev.* 2455-2504 (2009).

[0050] In the crystalline compound of Formula (I) or its crystalline salt, R_2 can be, for example, a nitrogen protecting group 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).

[0051] In the crystalline compound of Formula (I) or its crystalline salt, R_2 can be a nitrogen protecting group 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), 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).

[0052] In the crystalline compound of Formula (I) or its crystalline salt, R_2 can be, for example, the nitrogen protecting group 9-Fluorenylmethoxycarbonyl (Fmoc).

R_3

[0053] In the crystalline compound of Formula (I) or its crystalline salt, R_3 can be, for example, -H or a protecting or activating group selected from the group consisting of: tert-Butyl (tBu),

2-Chlorotrityl (2-Cl-Trt), 2,4-Dimethoxybenzyl (DMB), Benzyl (Bn), 2-Phenylisopropyl (2-PhiPr),

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.

[0054] In the crystalline compound of Formula (I) or its crystalline salt, R_3 can be, for example –H.

n

[0055] In the crystalline compound of Formula (I) or its crystalline salt, n can range, for example, from 1-20, from 3-11, or from 3-6. n can be, for example 3 or 6 or 11. n can be 3. n can be 6. n can be 11.

*

[0056] In the crystalline compound of Formula (I) or its crystalline salt, the stereocenter * can be (R). In the crystalline compound of Formula (I) or its crystalline salt, the stereocenter * can be (S).

[0057] In one embodiment, in the crystalline compound of Formula (I) or it crystalline salt, R_1 can be C_1-C_3 alkyl; R_2 can be 9-Fluorenylmethoxycarbonyl (Fmoc); R_3 can be selected from the group consisting of -H tert-Butyl (tBu), 2-Chlorotrityl (2-Cl-Trt), 2,4-Dimethoxybenzyl (DMB), Benzyl (Bn), 2-Phenylisopropyl (2-PhiPr), 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; n can be an integer ranging from 3 to 11; and the stereocenter * can be (R).

[0058] In one embodiment, in the crystalline compound of Formula (I) or it crystalline salt, R_1 can be C_1-C_3 alkyl; R_2 can be 9-Fluorenylmethoxycarbonyl (Fmoc); R_3 can be selected from the group consisting of -H tert-Butyl (tBu), 2-Chlorotrityl (2-Cl-Trt), 2,4-Dimethoxybenzyl (DMB), Benzyl (Bn), 2-Phenylisopropyl (2-PhiPr), 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; n can be an integer ranging from 3 to 11; and the stereocenter * can be (S).

[0059] In one embodiment, in the crystalline compound of Formula (I) or its crystalline salt, R_1 can be methyl, R_2 can be 9-Fluorenylmethoxycarbonyl (Fmoc); R_3 can be –H, n can be 3, 6, or 11, and the stereocenter * can be (R).

[0060] In one embodiment, in the crystalline compound of Formula (I) or its crystalline salt, R_1 can be methyl, R_2 can be 9-Fluorenylmethoxycarbonyl (Fmoc); R_3 can be –H, n can be 3, 6, or 11, and the stereocenter * can be (S).

Chemical Purity

[0061] Herein, unless otherwise indicated, any compound, its salt, crystalline compound, or crystalline salt of a compound, can have a chemical purity. Chemical purity can be

defined, for example, as the degree to which a substance is undiluted or unmixed with extraneous material, and can be typically expressed as a percentage. Any compound, salt thereof, crystalline compound, or crystalline salt of a compound herein can have, for example, a chemical purity ranging from about 90% to 100%. The chemical purity can be, for example, about 92% to 100%, about 94% to 100%, about 96% to 100%, about 98% to 100%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%. The percentage can be, for example, based on the total weight of the compound, its salt, crystalline compound, or its salt. The percentage can be, for example, arrived at using HPLC. The percentage can be arrived at, for example, using NMR, for example proton NMR. The chemical purity can be arrived at, for example, using elemental analysis.

Enantiomeric Excess

[0062] Herein, unless otherwise indicated, any compound, salt thereof, crystalline compound, or crystalline salt of a compound, can have an enantiomeric excess. The enantiomeric excess can be, for example, from about 80% to 100%, from about 85% to 100%, from about 90% to 100%, from about 95% to 100%, from about 96% to 100%, from about 97% to 100%, from about 98% to 100%, from about 99% to 100%, about 95%, about 96%, about 97%, about 97.2%, about 98%, about 99%, or 100%. The enantiomeric excess can be, for example, greater than 95%, greater than 96%, greater than 97%, greater than 98%, or greater than 99%. Herein, unless otherwise indicated, enantiomeric excess can be calculated, for example, by the formula: enantiomeric excess (ee) = ((P-S)/(P+S))x100%, where P and S represent the moles, respectively, of the predominant and subdominant enantiomer produced or present in a sample. For example, if the more moles of the (R) enantiomer are produced at than moles of the (S) enantiomer, moles of (R) enantiomer are designated as R, and moles of the (S) enantiomer are designated as S, then the enantiomeric excess formula becomes: ee (%) = ((R-S)/(R+S))x100%. Herein, unless otherwise indicated, the amount (*e.g.*, moles) or enantiomer produced can be determined, for example, by chiral HPLC, by chiral GC, or via a chiral NMR shift reagent using NMR spectroscopy.

Optical Purity

[0063] Herein, unless otherwise indicated, any compound, its salt, crystalline compound, or crystalline salt of a compound, can have an optical purity. The optical purity can be,

for example, from about 80% to 100%, from about 85% to 100%, from about 90% to 100%, from about 95% to 100%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100%. Herein, unless otherwise indicated, optical purity can be calculated using the formula: optical purity (%) = ([α]observed/[α]maximal)*100%, where [α]observed is the specific rotation of the sample, and [α]maximal is the specific rotation of the pure enantiomer. Herein, unless otherwise indicated, specific rotation can be defined as the observed angle of optical rotation, α, when plane-polarized light is passed through a sample with a path length of 1 decimeter and a sample concentration of 1 gram per 1 millilitre. The specific rotation can be obtained, for example, at 20 °C and at a wavelength of light of 589 nanometers (*e.g.*, the sodium D line). Herein, unless otherwise indicated, the specific rotation can be obtained, for example, with a polarimeter. Herein, unless otherwise indicated, the solvent the sample is dissolved in can be any suitable solvent or solvent combination, for example, ethanol, methanol, chloroform, dichloromethane, carbon tetrachloride, water, DMSO, N,N-DMF, diethyl ether, tetrahydrofuran, hexane, pentane, acetone, or any combination thereof.

Diastereomeric Excess

[0064] Herein, unless otherwise indicated, the compounds, salts, crystalline compounds, or crystalline salts of compounds herein can be diastereomers. When this is so, the compounds, crystalline compounds, or crystalline salts of compounds herein can have a diastereomeric excess of, for example, from about 80% to 100%, from about 85% to 100%, from about 90% to 100%, from about 95% to 100%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100%. Herein, unless otherwise indicated, the diastereomeric excess, for example, in a mixture of two diastereomers, can be calculated, for example, by the formula: diastereomeric excess de % = ((D1-D2)/(D1+D2))*100%, wherein D1 represents, for example, the mole or percent weight of a first and most abundant diastereomer, and D2 represents , for example, the mole or percent weight of a second and least abundant diastereomer, where mole percent is used consistently (*e.g.*, alone) in the calculation, or where percent weight is used consistently (*e.g.*, alone) in the calculation.

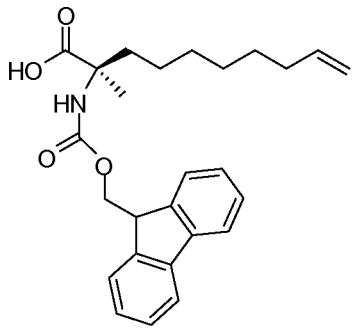
Converted Enantiomeric Excess or Optical Purity

[0065] Unless otherwise indicated, any compound, salt thereof, crystalline compound, or crystalline salt thereof, herein, that is a diastereomer, can be converted to an enantiomer or enantiomeric mixture having one stereocenter (*e.g.*, * in Formula (I))

by, for example, removal of a nitrogen protecting group (*e.g.*, removal of the nitrogen protecting group R_2 in the crystalline compound of Formula (I) or its crystalline salt that, together with the stereocenter *, creates a diastereomer), and the resulting enantiomer or enantiomeric mixture can then have its enantiomeric excess or optical purity determined as described herein. The resulting enantiomeric excess or optical purity, in these circumstances, is termed a converted enantiomeric excess or converted optical purity. The converted enantiomeric excess can be, for example, from about 80% to 100%, from about 85% to 100%, from about 90% to 100%, from about 95% to100%, from about 96% to 100%, from about 97% to 100%, from about 98% to 100%, from about 99% to 100%, about 95%, about 96%, about 97%, about 97.2%, about 98%, about 99%, or 100%. The converted enantiomeric excess can be, for example, greater than 95%, greater than 96%, greater than 97%, greater than 98%, or greater than 99%. The converted optical purity can be, for example, from about 80% to 100%, from about 85% to 100%, from about 90% to 100%, from about 95% to 100%, about 95%, about 96%, about 97%, about 98%, about 99%, or 100%. Thus, any optionally crystalline diastereomer or its optionally crystalline salt herein, unless otherwise indicated, can have a converted enantiomeric excess or converted optical purity.

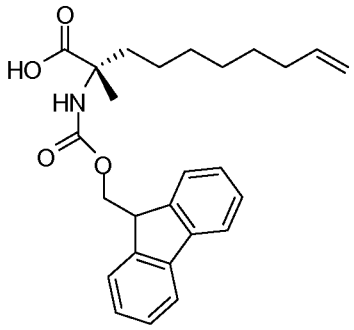
Specifically Exemplified Crystalline Compounds and Crystalline Salts Thereof

[0066] Herein, unless otherwise indicated, the crystalline compound of Formula (I) or its crystalline salt can be a compound of Formula (IIa) or its crystalline salt:



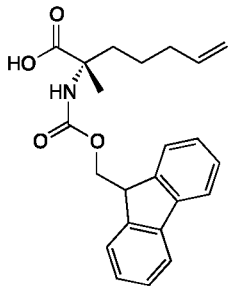
(IIa).

[0067] Herein, unless otherwise indicated, the crystalline compound of Formula (I) or its crystalline salt can be a compound of Formula (IIb) or its crystalline salt:



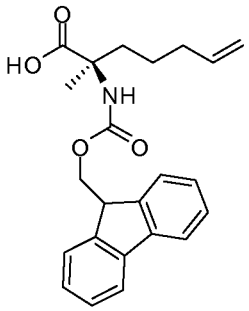
(IIb).

[0068] Herein, unless otherwise indicated, the crystalline compound of Formula (I) or its crystalline salt can be a compound of Formula (IIIa) or its crystalline salt:



(IIIa).

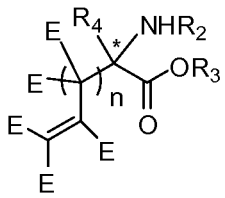
[0069] Herein, unless otherwise indicated, the crystalline compound of Formula (I) or its crystalline salt can be a compound of Formula (IIIb) or its crystalline salt:



(IIIb).

Deuterated and Halogenated Compounds and Their Salts

[0070] Also provided herein, unless otherwise indicated, are optionally crystalline compounds and their optionally crystalline salts of Formula (IV):



Formula **(IV)** ,

wherein R_2, R_3, n, and * are the same as in the crystalline compound or its crystalline salt of Formula (I), each E is independently selected from the group consisting of deuterium and halogen, and R_4 is selected from the group consisting of C_1-C_3 alkyl, C_1-C_3 deuteroalkyl and C_1-C_3 haloalkyl.

Deuterium

[0071] Herein, unless otherwise indicated, for any deuterated: compound, its salt, crystalline compound, or its crystalline salt; greater than 90%, greater than 92%, greater than 94%, greater than 96%, or greater than 98%, of the deuterated: compound, its salt, crystalline compound, or its crystalline salt; has a deuterium atom at each position designated as deuterium (D) in the deuterated: compound, its salt, the crystalline compound, or its crystalline salt.

Methods of Making

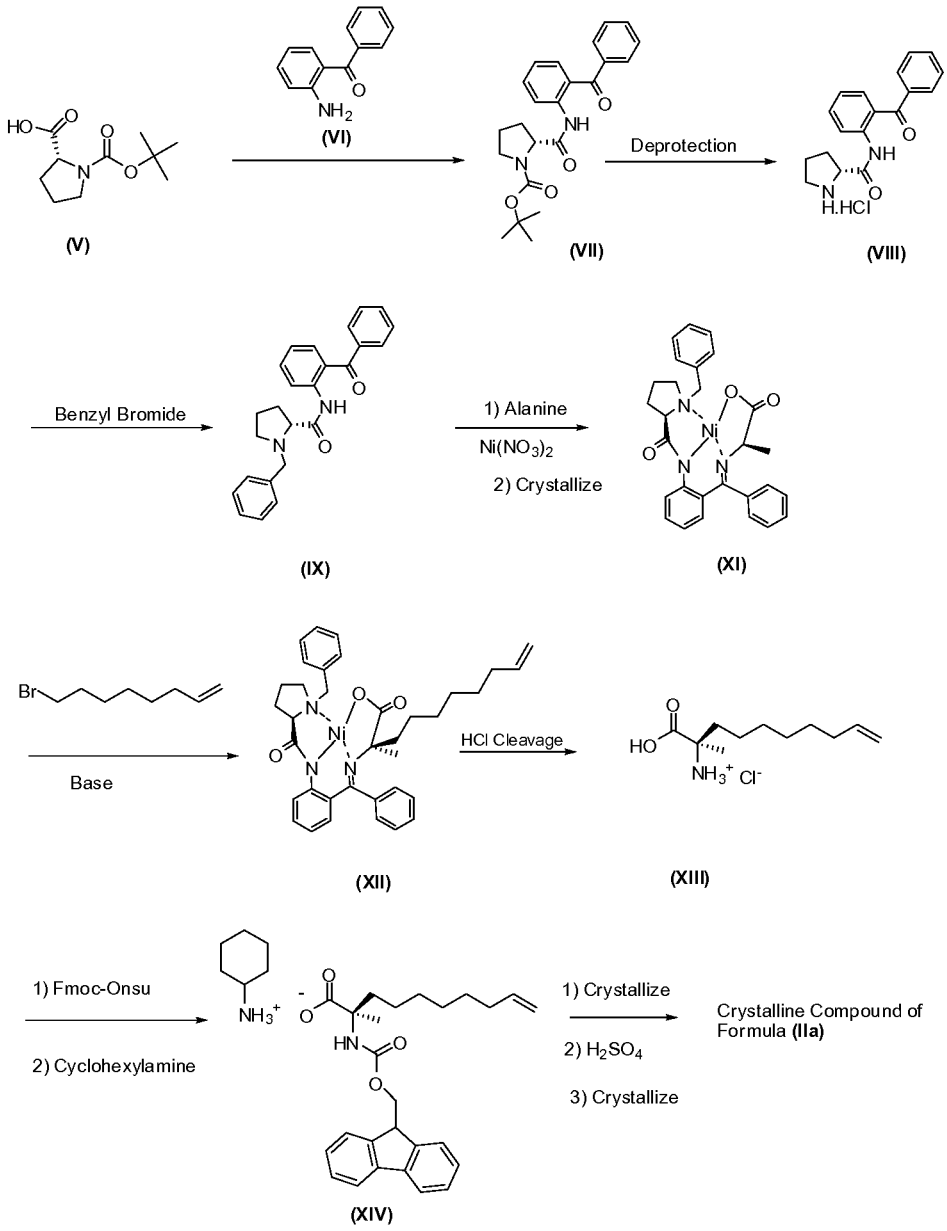
[0072] The compounds and their salts herein can be advantageously made by methods disclosed herein that result in at least one of the following advantages:

- the compounds or their salts that are produced are crystalline;
- the compounds and their salts (both of which can be crystalline) are advantageously produced in high yield;
- the compounds and their salts (both of which can be crystalline) are advantageously produced in high chemical purities;
- the compounds and their salts (both of which can be crystalline) are advantageously produced in high enantiomeric excess, optical purity, diastereomeric excess, high converted enantiomeric excess, or high converted optical purity; or
- the compounds and their salts (both of which can be crystalline) are produced without chromatographic purification (*e.g.*, without chromatography).

[0073] Unless otherwise indicated, the compounds, their salts, crystalline compounds, and their crystalline salts, herein can be produced using for example exemplary Scheme I (with modifications that would be readily apparent to a skilled artisan). Scheme I depicts formation of the crystalline N-Fmoc-(R)-α-methyl-α-aminodec-9-enoic acid (*i.e.*, the crystalline compound of Formula (IIa)). Sequence I starts with Boc-D-proline (*i.e.*, the compound of Formula (V)). It is understood that by starting with Boc-L-proline, compounds with the opposite stereochemistry of the compound of Formula (IIa) can be produced (*e.g.*, the compound of Formula (IIb) can be produced). It is also understood that the stereochemisty of the amino acid used to

form the metal complex (*e.g.*, alanine used to form the metal complex of Formula (XI) in Scheme I) is not dispositive of the stereochemistry in the resulting crystalline compound (e.g., of Formula (IIa)) or its crystalline salt.

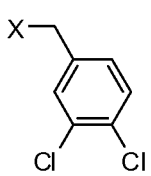
Scheme I



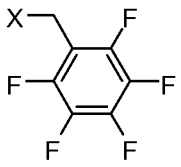
[0074] In Scheme I, Boc-D-proline (Compound of Formula (V)) is first reacted with 2-aminobenzophenone (compound of Formula (VI)) to form the compound of Formula (VII). Next, the compound of Formula (VII) is deprotected to form the HCl salt of the compound of Formula (VIII). A skilled artisan would readily understand that the synthetic scheme contemplates use of acids other than HCl, including organic acids

and inorganic acids, for example, nitric acid, phosphoric acid, sulfuric acid, boric acid, hydrofloric acid, hydrobromic acid, and perchloric acid.

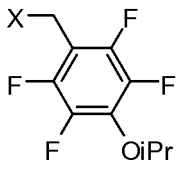
[0075] The salt of the compound of Formula (VIII) is next reacted with benzyl bromide, and for example, a base, to form the compound of Formula (IX). A skilled artisan would readily understand that substituted benzyl halides could be employed in place of benzyl bromide. For example, the following benzyl halides, where X = Cl, Br, or I, could be employed:



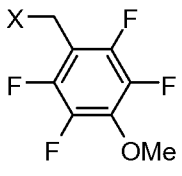
(X_a)



(X_b)

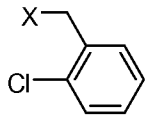


(X_c)

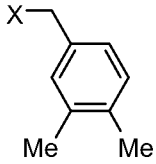


(X_d)

Representative benzyl halides are found in Belokon, Y. N., *et al.*, "Halo-substituted (S)-N-(2- benzoylphenyl)-1-benzylpyrolidine-2-carboxamides as new chiral auxiliaries for the asymmetric synthesis of (S)-α-amino acids,"*Russian Chemical Bulletin, International Edition, 51(8)*: 1593-1599 (2002). Further and different benzyl halides could also be employed:



(X_e)



(X_f)

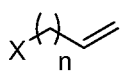
These representative benzyl halides are found in Saghiyan, A. S., *et al.*, "New chiral NiII complexes of Schiff's bases of glycine and alanine for efficient asymmetric synthesis of α-amino acids," *Tedrahedron: Asymmetry 17*: 455-467 (2006).

[0076] Next, the compound of Formula (IX) is reacted with L-alanine and $Ni(NO_3)_2$ to form the metal complex of Formula (XI). The skilled artisan would understand that other amino acids other than alanine could be employed in Scheme I. For example, glycine; 2-aminobutanoic acid, 2-aminopentanoic acid, and valine, for example in their D or L forms, could be employed. The $Ni(NO_3)_2$ can be a hydrate, for example, a hexahydrate. The reaction can be run in an alcoholic solvent, for example, methanol. The reaction can be run at an elevated temperature, for example, from

about 40 °C to about 60 °C. The reaction can be run in the presence of a base, for example, a hydroxide, for example an inorganic hydroxide, for example, potassium hydroxide. Other hydroxides are contemplated, including sodium hydroxide, cesium hydroxide, lithium hydroxide, magnesium hydroxide, and ammonium hydroxide.

[0077] To increase purity of the final product from Scheme I, the metal complex of Formula (XI) can be crystallized one or more times from one or more solvents, for example a cyclic ether and a non-cyclic ether. In one embodiment, the solvent is tetrahydrofuran and methyl tert-butyl ether. In some cases the ratio of the cyclic ether to the non-cyclic ether is at most 0.5:10, 1.0:10, 1.5:10, 2.0:10, 2.5:10, 3.0:10, 3.5:10, 4.0:10, 4.5:10 or 5:10. In other cases the ratio of the cyclic ether to the non-cyclic ether is at least 0.5:10, 1.0:10, 1.5:10, 2.0:10, 2.5:10, 3.0:10, 3.5:10, 4.0:10, 4.5:10 or 5:10. For example, some cases the metal complex of Formula (XI) is crystallized from a mixture of tetrahydrofuran and methyl tert-butyl ether in ratio of at most 0.5:10, 1.0:10, 1.5:10, 2.0:10, 2.5:10, 3.0:10, 3.5:10, 4.0:10, 4.5:10 or 5:10. In other cases the ratio of and tetrahydrofuran and methyl tert-butyl ether is at least 0.5:10, 1.0:10, 1.5:10, 2.0:10, 2.5:10, 3.0:10, 3.5:10, 4.0:10, 4.5:10 or 5:10. In some cases the ratio of tetrahydrofuran and methyl tert-butyl ether is 1.5:10. The metal complex of Formula (XI) may also be crystallized with esters, for example with ethyl acetate or isopropyl acetate. The product or crystallized product of Formula (IX) can alternatively or additionally be crystallized or recrystallized from a solvent, for example an alcohol, for example isopropyl alcohol. Other alcohols are contemplated, including methanol, ethanol, n-propanol, a butanol, n-butanol, iso-butanol, sec-butanol, and tert-butanol.

[0078] The metal complex of Formula (XI) is then alkylated with 8-bromooct-1-ene to form the alkylated metal complex of Formula (XII). The skilled artisan would understand that other alkylating agents, including other halo alkyl olefins, could be used in place of 8-bromooct-1-ene. For example, alkylating agents of the Formula (XV) could be used:



(XV) ,

wherein X is Cl, Br, or I, and n is an integer from 1 to 20. For example, n can be from 3 to 11, from 3 to 6, or 3 or 6. Some or all of the hydrogen atoms present in the compound of Formula (XV) can be replaced with deuterium atoms or halogen atoms. The alkylation can be performed in one or more solvents, for example a polar aprotic solvent, for

example N, N-dimethyl formamide (DMF). The alkylation can be performed, for example, at a temperature of less than 20 °C, for example, from less than 20 °C to 5 °C, from less than 20 °C to 10 °C, or at about 10 °C. The skilled artisan would also understand that when glycine is used to form the metal complex, two alkylations could be performed one after the other. For example, the first alkylation could be performed using a C_1-C_3 alkane with a leaving group such as a halogen (*e.g.*, methyl bromide, ethyl bromide, n-propyl bromide), or a C_1-C_3 deuteroalkane with a leaving group such as a halogen (*e.g.*, CD_3Br, CD_3CD_2Br, $CD_3CD_2CD_2Br$), or a C_1-C_3 haloalkane with a leaving group such as a more reactive halogen than the other halogens in the haloalkane (*e.g.*, CF_3Br, CF_3CF_2Br, $CF_3CF_2CF_2Br$). Then, the second alkylation could be performed using the alkylating agent of Formula (XV). The order of the first and second alkylations can be reversed.

[0079] Purification of Formula (XII) may be achieved by crystallization one or more times from one or more solvents including cyclic and non-cyclic ethers, esters, hexanes and heptanes. For example crystallization may be achieved using a combination of ethyl acetate and hexanes, ethyl acetate and heptanes, isopropyl acetate and hexanes, isopropyl acetate and heptanes, methyl tertiary-butyl ether and hexanes, methyl tertiary-butyl ether and heptanes or isopropyl acetate and methyl tertiary-butyl ether.

[0080] The metal complex of Formula (XII) is then cleaved with an acid, for example HCl, using one or more solvents, for example an ether, for example a cyclic ether, for example tetrahydrofuran, to form the amino acid HCl salt of Formula (XIII). The skilled artisan would understand that other acids in addition to HCl are contemplated, for example organic or inorganic acids, for example, nitric acid, phosphoric acid, sulfuric acid, boric acid, hydrofloric acid, hydrobromic acid, or perchloric acid. The salt of Formula (XIII) may be further purified by crystallization one or more times with one or more solvents. The solvent may be any suitable solvent including tetrahydrofuran, methyl tertiary-butyl ether, ethyl acetate, isopropyl acetate, ethanol, methanol, isopropanol, acetonitrile, or a combination thereof. In one embodiment, the solvent is acetonitrile.

[0081] The amino acid salt of Formula (XIII) is then nitrogen protected with a nitrogen protecting group, in this case an Fmoc group, and the cyclohexylamine addition salt of the protected amino acid is formed, yielding the protected amino acid cyclohexylamine salt of Formula (XIV). Formation of the salt of Formula (XIV) can be achieved in any suitable solvent including acetonitrile, methyl tertiary-butyl ether, tetrahydrofuran or a combination thereof. In one embodiment, the solvent is methyl

tertiary-butyl ether. A skilled artisan would understand that other amines, for example other cyclic amines, for example cyclopropylamine, cyclobutyl amine, cyclopentylamine, cycloheptylamine, and cyclooctylamine, are contemplated. One of skill in the art would also readily understand that other nitrogen protecting groups are contemplated, for example the nitrogen protecting groups for R_2 in the crystalline compound of Formula (I) or its crystalline salt herein.

[0082] The protected amino acid cyclohexylamine salt of Formula (XIV) can then be crystallized from one or more ethers, for example, two ethers, for example a cyclic ether and a non cyclic ether, for example tetrahydrofuran and methyl tert-butyl ether.

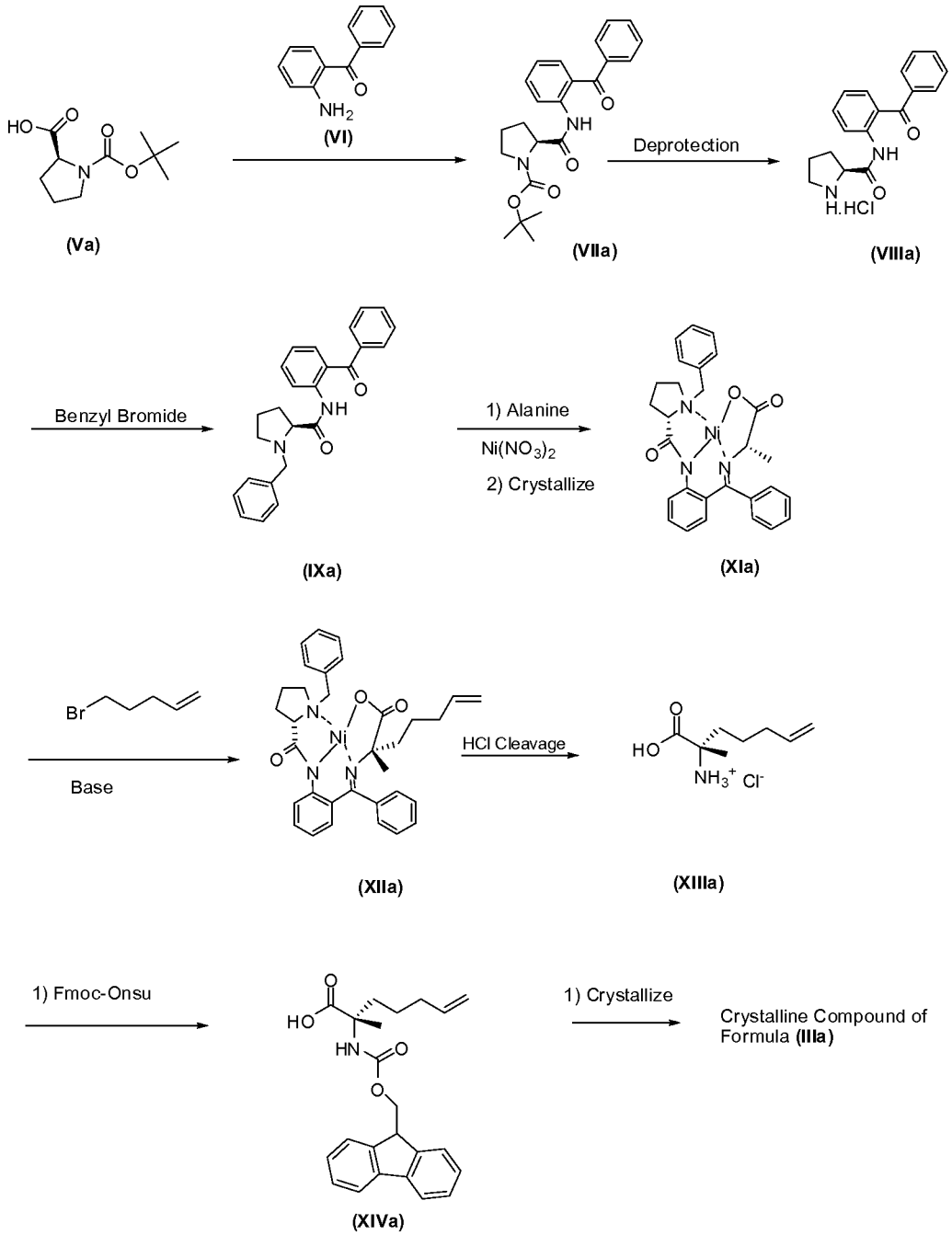
[0083] The crystallized amino acid cyclohexylamine salt of Formula (XIV) is then treated with sulfuric acid, and subsequently crystallized to form the crystalline compound of Formula (IIa). The skilled artisan would understand that acids other than sulfuric acid are contemplated, for example organic or inorganic acids, for example, nitric acid, phosphoric acid, sulfuric acid, boric acid, hydrofloric acid, hydrobromic acid, or perchloric acid. The crystallization can be performed using one or more solvents, for example two solvents, for example an alkane and haloalkane, for example hexanes and chloroform. In some cases the ratio of the alkane to the haloalkane is at least 6:1, 5:1, 4:1, 3:1, 2:1, or 1:10. In some cases the ratio of the alkane to the haloalkane is at most 6:1, 5:1, 4:1, 3:1, 2:1, or 1:10. For example, the crystalline compound of Formula (IIa) may be obtained by crystallization from a mixture of hexanes and chloroform in the ratio of at least 6:1, 5:1, 4:1, 3:1, 2:1, or 1:1. The crystalised IIa may also obtained by crystallization from a mixture of hexanes and chloroform in the ratio of at most 6:1, 5:1, 4:1, 3:1, 2:1, or 1:1. In some cases the ratio of hexanes and chloroform is 3:1.

[0084] The crystallization can be performed at a temperature ranging from, for example, about -5 ^{o}C to about -20 ^{o}C, about -10 ^{o}C to about -20 ^{o}C, or about -15 ^{o}C to -20 ^{o}C.

[0085] The skilled artisan would understand, for example, that the crystalline compound of Formula (IIa) could be further activated or protected at its carboxylic acid function with, for example, a protecting or activating group R_3 of the crystalline compound of Formula (I) or its crystalline salt. Unless otherwise indicated, the compounds, their salts, crystalline compounds, and their crystalline salts, herein can be produced using exemplary Scheme II (with modifications that would be readily apparent to a skilled artisan). Scheme II depicts formation of the crystalline N-Fmoc-(S)-α-methyl-α-aminohept-6-enoic acid (*i.e.*, the crystalline compound of Formula (IIIa)). Sequence

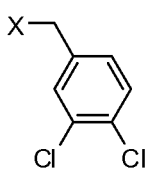
II starts with Boc-L-Proline (*i.e.*, the compound of Formula (Va)). It is understood that by starting with Boc-D-proline, compounds with the opposite stereochemistry of the compound of Formula (IIIa) can be produced (*e.g.*, the compound of Formula (IIIb) can be produced). It is also understood that the stereochemisty of the amino acid used to form the metal complex, and whose alpha carbon atom is subsequently alkylated by the haloolefin (*e.g.*, alanine in Formula (XIa)) is not dispositive of the stereochemistry in the resulting crystalline compound (*e.g.*, of Formula (IIIa)) or its crystalline salt.

Scheme II

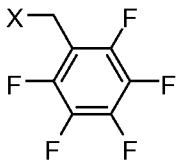


[0086] In Scheme II, Boc-L-proline (Compound of Formula (Va)) is first reacted with 2-aminobenzophenone (compound of Formula (VI)) to form the compound of Formula (VIIa). Next, the compound of Formula (VIIa) is deprotected to form the HCl salt of the compound of Formula (VIIIa). A skilled artisan would readily understand that the synthetic scheme contemplates use of acids other than HCl, including organic acids and inorganic acids, for example, nitric acid, phosphoric acid, sulfuric acid, boric acid, hydrofloric acid, hydrobromic acid, and perchloric acid.

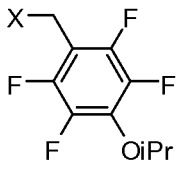
[0087] The salt of the compound of Formula (VIIIa) is next reacted with benzyl bromide, and for example a base, to form the compound of Formula (IXa). A skilled artisan would readily understand that substituted benzyl halides could be employed in place of benzyl bromide. For example, the following benzyl halides, where X = Cl, Br, or I, could be employed:



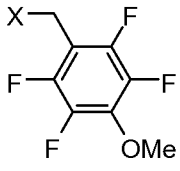
(X_a)



(X_b)

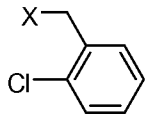


(X_c)

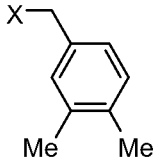


(X_d)

[0088] Representative benzyl halides are found in Belokon, Y. N., *et al.*, "Halo-substituted (S)-N-(2 benzoylphenyl)-1-benzylpyrolidine-2-carboxamides as new chiral auxiliaries for the asymmetric synthesis of (S)-α-amino acids,"*Russian Chemical Bulletin, International Edition, 51(8)*: 1593-1599 (2002). Further and different benzyl halides could also be employed:



(X_e)



(X_f)

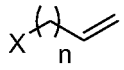
[0089] These representative benzyl halides are found in Saghiyan, A. S., *et al.*, "New chiral NiII complexes of Schiff's bases of glycine and alanine for efficient asymmetric synthesis of α-amino acids," *Tedrahedron: Asymmetry 17*: 455-467 (2006).

[0090] Next, the compound of Formula (IXa) is reacted with L-alanine and $Ni(NO_3)_2$ to form the metal complex of Formula (XIa). The skilled artisan would understand that other

amino acids other than alanine could be employed in Scheme II. For example, glycine; 2-aminobutanoic acid, 2-aminopentanoic acid, and valine could be employed, for example in their D or L forms. The $Ni(NO_3)_2$ can be a hydrate, for example, a hexahydrate. The reaction can be run in an alcoholic solvent, for example, methanol. The reaction can be run at an elevated temperature, for example, from about 40 °C to about 60 °C. The reaction can be run in the presence of a base, for example, a hydroxide, for example an inorganic hydroxide, for example, potassium hydroxide. Other hydroxides are contemplated, including sodium hydroxide, lithium hydroxide, cesium hydroxide, and magnesium hydroxide.

[0091] To increase purity of the final product from Scheme II, the metal complex of Formula (XIa) can be crystallized one or more times from one or more solvents, for example a cyclic ether and a non-cyclic ether, for example tetrahydrofuran and methyl tert-butyl ether. In some cases the ratio of the cyclic ether to the non-cyclic ether is at most 0.5:10, 1.0:10, 1.5:10, 2.0:10, 2.5:10, 3.0:10, 3.5:10, 4.0:10, 4.5:10 or 5:10. In other cases the ratio of the cyclic ether to the non-cyclic ether is at least 0.5:10, 1.0:10, 1.5:10, 2.0:10, 2.5:10, 3.0:10, 3.5:10, 4.0:10, 4.5:10 or 5:10. For example, some cases the metal complex of Formula (XIa) is crystallized from a mixture of tetrahydrofuran and methyl tert-butyl ether in ratio of at most 0.5:10, 1.0:10, 1.5:10, 2.0:10, 2.5:10, 3.0:10, 3.5:10, 4.0:10, 4.5:10 or 5:10. In other cases the ratio of and tetrahydrofuran and methyl tert-butyl ether is at least 0.5:10, 1.0:10, 1.5:10, 2.0:10, 2.5:10, 3.0:10, 3.5:10, 4.0:10, 4.5:10 or 5:10. In some cases the ratio of tetrahydrofuran and methyl tert-butyl ether is 1.5:10. The product or crystallized product of Formula (IXa) can be crystallized or recrystallized from a solvent, for example an alcohol, for example isopropyl alcohol. Other alcohols are contemplated, including methanol, ethanol, n-propanol, a butanol, n-butanol, iso-butanol, sec-butanol, and t-butanol. Other solvents suitable for crystallization or recrystallization of Formula (XIa) include esters, for example ethyl acetate or isopropyl acetate.

[0092] The metal complex of Formula (XIa) is then alkylated with 5-bromopent-1-ene to form the alkylated metal complex of Formula (XIIa). The skilled artisan would understand that other alkylating agents, including other halo alkyl olefins, could be used in place of 5-bromopent-1-ene. For example, alkylating agents of the Formula (XV) could be used:



(XV) ,

wherein X is Cl, Br, or I, and n is an integer from 1 to 20. For example, n can be from 3 to 11, from 3 to 6, or 3 or 6. Some or all of the hydrogen atoms present in the compound of Formula (XV) can be replaced with deuterium atoms or halogen atoms. The alkylation can be performed in one or more solvents, for example a polar aprotic solvent, for example N, N-dimethyl formamide (DMF). The alkylation can be performed, for example, at a temperature of less than 20 °C, for example, from less than 20 °C to 5 °C, from less than 20 °C to 10 °C, or at about 10 °C. The skilled artisan would also understand that when glycine is used to form the metal complex, two alkylations could be performed one after the other. For example, the first alkylation could be performed using a C_1-C_3 alkane with a leaving group such as a halogen (*e.g.*, methyl bromide, ethyl bromide, n-propyl bromide), or a C_1-C_3 deuteroalkane with a leaving group such as a halogen (*e.g.*, CD_3Br, CD_3CD_2Br, $CD_3CD_2CD_2Br$), or a C_1-C_3 haloalkane with a leaving group such as a more reactive halogen than the other halogens in the haloalkane (*e.g.*, CF_3Br, CF_3CF_2Br, $CF_3CF_2CF_2Br$). Then, the second alkylation could be performed using the alkylating agent of Formula (XV). The order of the first and second alkylations can be reversed.

[0093] Purification of Formula (XIIa) may be achieved by crystallization one or more times from one or more solvents including cyclic and non-cyclic ethers, esters, hexanes and heptanes. For example crystallization may be achieved by using a combination of ethyl acetate and hexanes, ethyl acetate and heptanes, isopropyl acetate and hexanes, isopropyl acetate and heptanes, methyl tertiary-butyl ether and hexanes, methyl tertiary-butyl ether and heptanes or isopropyl acetate and methyl tertiary-butyl ether.

[0094] The metal complex of Formula (XIIa) is then cleaved with an acid, for example HCl, using one or more solvents, for example an ether, for example a cyclic ether, for example tetrahydrofuran, to form the amino acid HCl salt of Formula (XIIIa). The skilled artisan would understand that other acids in addition to HCl are contemplated, for example organic or inorganic acids, for example, nitric acid, phosphoric acid, sulfuric acid, boric acid, hydrofloric acid, hydrobromic acid, or perchloric acid.

[0095] The salt of Formula (XIIIa) may be further purified by crystallization one or more times with one or more solvents. The solvent may be any suitable solvent including

tetrahydrofuran, methyl tertiary-butyl ether, ethyl acetate, isopropyl acetate, ethanol, methanol, isopropanol, acetonitrile, or a combination thereof. In one embodiment, the solvent is acetonitrile.

[0096] The amino acid salt of Formula (XIIIa) is then nitrogen protected with a nitrogen protecting group, in this case an Fmoc group, yielding the protected amino acid of Formula (XIVa). In some embodiments, the compound of Formula (XIVa) is taken on to the crystallization step as is. In other embodiments, the compound of Formula (XIVa) is converted to a salt prior to crystallization. Formation of the salt of Formula (XIVa) may be achieved in any suitable solvent including acetonitrile, methyl tertiary-butyl ether, tetrahydrofuran or a combination thereof. One of skill in the art would also readily understand that other nitrogen protecting groups are contemplated, for example the nitrogen protecting groups for R_2 in the crystalline compound of Formula (I) or its crystalline salt herein. For example, a protected amino acid cyclohexylamine salt of Formula (XIVa) can then be crystallized from one or more ethers, for example, two ethers, for example a cyclic ether and a non cyclic ether, for example tetrahydrofuran and methyl tert-butyl ether.

[0097] The protected amino acid cyclohexylamine salt of Formula (XIVa) can then be crystallized to form the crystalline compound of Formula (IIIa).

[0098] The crystallization can be performed using one or more solvents, for example two solvents, for example an alkane and haloalkane, for example hexanes and chloroform. In some cases the ratio of the alkane to the haloalkane is at least 6:1, 5:1, 4:1, 3:1, 2:1, or 1:10. In some cases the ratio of the alkane to the haloalkane is at most 6:1, 5:1, 4:1, 3:1, 2:1, or 1:10. For example, the crystalline compound of Formula (IIIa) may be obtained by crystallization from a mixture of hexanes and chloroform in the ratio of at least 6:1, 5:1, 4:1, 3:1, 2:1, or 1:1. The crystalised IIIa may also obtained by crystallization from a mixture of hexanes and chloroform in the ratio of at most 6:1, 5:1, 4:1, 3:1, 2:1, or 1:1. In some cases the ratio of hexanes and chloroform is 2:1.

[0099] The crystallization can be performed at a temperature ranging from, for example, about -5 °C to about -20 °C, about -10 °C to about -20 °C, or about -15 °C to -20 °C. Herein, unless otherwise indicated, any compound or its salt may be crystalline. Herein, unless otherwise indicated, any compound or its salt may be crystalline at a temperature, for example, of about 0 °C or less, about -5 °C or less, about - 10 °C or less, about -15 °C or less, about -20 °C or less, about -5 °C, about -6 °C, about -7 °C, about -8 °C, about -9 °C, about -10 °C, about -11 °C, about -12 °C, about -13 °C, about

-14 °C, about -15 °C, about -16 °C, about -17 °C, about -18 °C, about -19 °C, or about -20 °C.

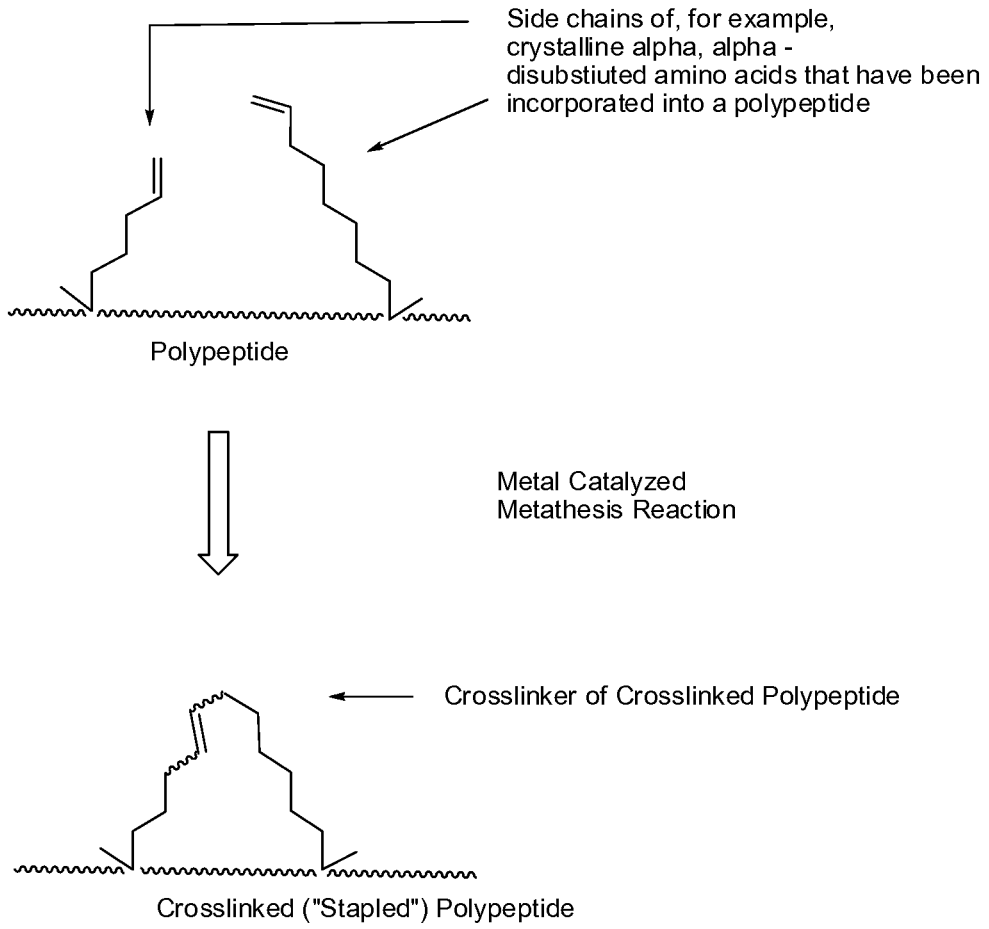
[00100] The skilled artisan would understand, for example, that the crystalline compound of Formula (IIIa) could be further activated or protected at its carboxylic acid function with, for example, a protecting or activating group R_3 of the crystalline compound of Formula (I) or its crystalline salt.

Stapled and Stitched Polypeptides

[00101] The crystalline compounds and their crystalline salts of Formula (I), including the crystalline compounds and their crystalline salts of Formulae (IIa), (IIb), (IIIa) and (IIIb), as well as the optionally crystalline compounds and their optionally crystalline salts of Formula (IV), can be used to synthesize peptides, polypeptides, and crosslinked polypeptides that are useful for treating and preventing diseases.

[00102] The crosslinked polypeptides can contain secondary structures such as a helix, for example, an alpha helix. The crosslinker can stablize the secondary structures relative to an otherwise identical but uncrosslinked polypeptide. And the crosslinker can be formed by, for example, joining the terminal alkene side chains of, for example, two crystalline alkene α, α-disubstituted amino acids or their crystalline salts herein that are incorporated into a polypeptide through, for example, a metal catalyzed olefin metathesis reaction (*e.g.*, forming a stapled peptide). This process is depicted in Scheme III, below:

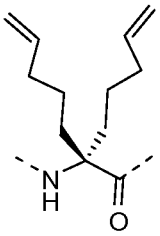
Scheme III



[00103] Examples of stapled polypeptides are found, *inter alia*, for example, in International Application No. PCT/US2004/038403.

[00104] The crystalline compounds and their crystalline salts of Formula (I), including the crystalline compounds and their crystalline salts of Formulae (IIa), (IIb), (IIIa) and (IIIb), as well as the optionally crystalline compounds and their optionally crystalline salts of Formula (IV), can be used to synthesize peptides, polypeptides, and stitched polypeptides that are useful for treating and preventing diseases.

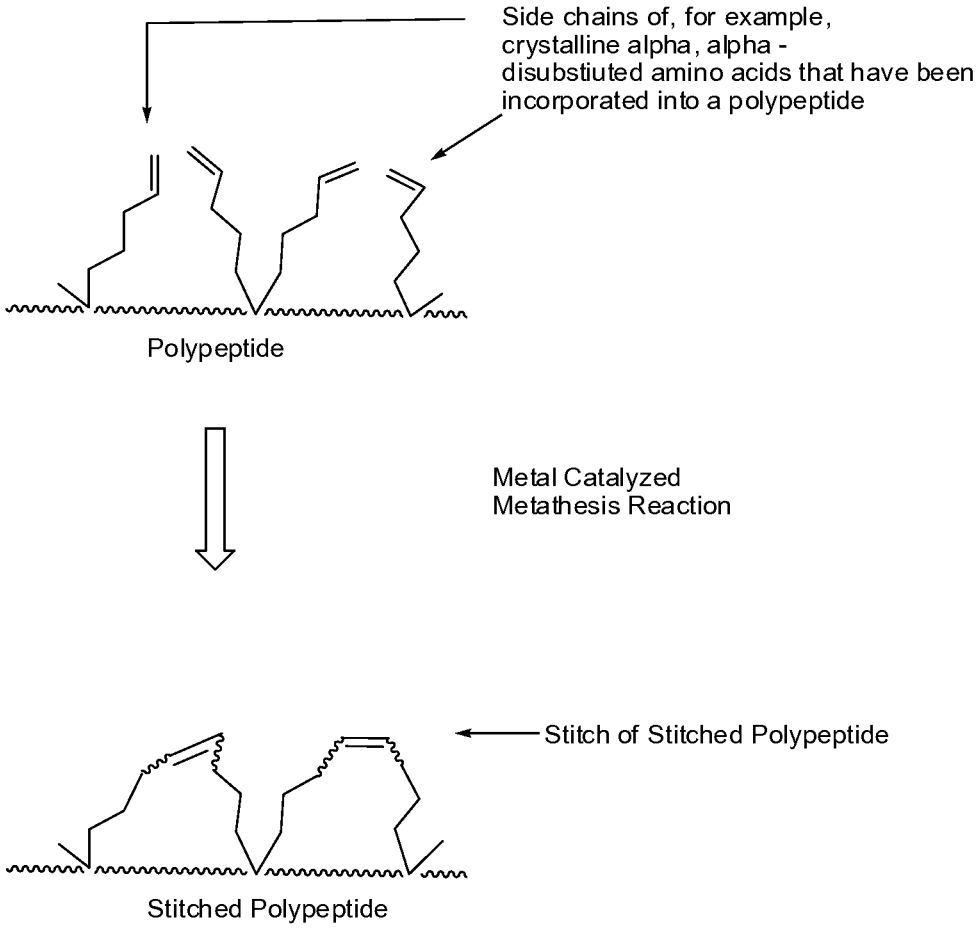
[00105] For example, two of the crystalline compounds and their crystalline salts of Formula (I), can be incorporated into a polypeptide backbone along with an α, α-disbustituted amino acid having terminal olefins on each of its side chains, for example the compound of Formula (XVI):



(XVI) ,

as shown in scheme IV. Metal catalyzed metathesis reaction of the olefins yields a stitched peptide.

Scheme IV



[00106] Examples of stitched polypeptides are found, for example, in International Application Publication No. WO2008/121767.

[00107] Methods to effect formation of peptidomimetic macrocycles which are known in the art can be employed. For example, the preparation of peptidomimetic macrocycles are described in Schafmeister *et al., J. Am. Chem. Soc.* 122:5891-5892 (2000); Schafmeister & Verdine, *J. Am. Chem. Soc.* 122:5891 (2005); Walensky *et al.*,

Science 305:1466-1470 (2004); U.S. Patent No. 7,192,713 and International Pat. App. Pub. No. WO 2008/121767.

[00108] Herein, unless otherwise indicated, the term "peptide synthesis" encompasses coupling of two or more amino acids with the aid of a coupling reagent. Peptide synthesis may be performed in" liquid" or "solution" phase where the coupling of the amino acids is performed in a solvent system. Peptide synthesis may also, or alternatively, be performed on "solid phase" where an amino acid is attached to a polymeric or solid support by a covalent bond at the N- or C-terminus of an amino acid. Peptides can be made, for example, by chemical synthesis methods, such as those described in Fields *et al.*, Chapter 3 in Synthetic Peptides: *A User's Guide*, ed. Grant, W. H. Freeman & Co., New York, N. Y., 1992, p. 77,; and Goodman, M., *et al.*, Houben-Weyl Methods in Organic Chemistry: Synthesis of Peptides and Peptidomimetics, *Thieme Publishers*, *Volumes 1-5*, (1994). For example, peptides can be synthesized using automated Merrifield techniques of solid phase synthesis with the amino groups of the amino acids employed in the synthesis protected, for example by *t*-Boc or Fmoc protecting groups. An automated peptide synthesizer (*e.g.*, Applied Biosystems (Foster City, CA), Model 430A, 431, or 433) can be employed in making peptides.

[00109] Herein unless otherwise indicated, peptidomimetic precursors and peptidomimetic macrocycles and their salts described herein can be produced using solid phase peptide synthesis (SPPS), where for example, a C-terminal amino acid is attached to a cross-linked polystyrene resin *via* an acid or base labile bond with a linker. The resin can be, for example, insoluble in the solvents used for synthesis, making it relatively simple and fast to wash away excess reagents and by-products. The N-terminus of each amino acid added to the growing peptide chain can be protected, for example, with an Fmoc group, which is stable in acid, but removable by base. Side chain functional groups can be protected, as necessary or desirable, for example, with base stable, acid labile groups.

[00110] Herein, unless otherwise indicated, the peptidomimetic precursors can be made, for example, in a high-throughput, combinatorial fashion using, for example, a high-throughput polychannel combinatorial synthesizer (*e.g.*, Thuramed TETRAS multichannel peptide synthesizer from CreoSalus, Louisville, KY or Model Apex 396 multichannel peptide synthesizer from AAPPTEC, Inc., Louisville, KY).

[00111] Herein, unless otherwise indicated, solution peptide synthesis can be performed in a manner wherein reagents are fully or partially dissolved in, for example, an appropriate solvent, for example, a polar aprotic solvent. In a representative case employing, for example, a solid crystalline N-terminally protected olefinic amino acid with a removable protecting group (*e.g.*, t-Butyloxycarbonyl, Benzyloxycarbonyl, Fluorenylmethoxycarbonyl) and a C-protected amino acid with a selectively removable ester (*e.g.*, methyl, benzyl, t-butyl), the amino acids can be fully or partially dissolved in a solvent and an activating agent is added to accomplish peptide bond formation between the amino acids. Solution peptide synthesis can also utilize first formation of active esters of N-protected olefinic amino acids (*e.g.*, N-hydroxysuccinamide, p-nitrophenyl, 2, 4, 6-trichlorophenyl, pentafluorophenyl) and then subsequent reaction of the activated amino acid with an unprotected or C-protected amino acid. The active esters of olefinic amino acids can be prepared, for example, by reacting a solid N-protected olefinic amino acid with an appropriate alcohol with help of the condensing agent (*e.g.*, dicyclohexylcarbodiiimide). These same procedures can also be used, for example, when one or both of the amino acids to be reacted are part of, and incorporated into, respectively, for example, one or two peptides.

[00112] Formation of C-terminally protected olefinic amino acids can easily be facilitated by reacting dry solid olefinic amino acid(s) with an appropriate alcohol (*e.g.*, methyl, ethyl, benzyl) under, for example, anhydrous conditions. Formation of a peptide where olefinic amino acid is located in the C-terminal position can accomplished, for example, in the similar way. Solution methods of peptide preparation can be easily adapted to process scale. The starting materials and reagents used herein in preparing any compound herein and as above-and-below disclosed, unless otherwise indicated, for example, can be available from commercial sources such as Aldrich, Sigma or Bachem, or can be prepared by methods known to those skilled in the art following procedures set forth, for example, in references such as: Fieser and Fieser's Reagents for Org. Syn. Vol. 1-17, Organic Reactions Vol. 1-40, March's Advanced Organic Synthesis, Larock's Comprehensive Organic Transformations, Bodansky and Bodansky's The Practice of Peptide Synthesis, Greene's Protective Groups in Organic Synthesis, Wei, Q., *et al.*, *Tetrahedron 56*: 2577-2582 (2000), Belokon, Y. N., *et al.*, *Tetrahedron: Asymmetry 9*: 4249-4252 (1998), Belokon, Y., *Pure & App. Chem. 64(12)*: 1917-1924 (1992), Ueki, H., *et al.*, *J. Org. Chem.* 68: 7104-7107 (2003).

[00113] These schemes herein are illustrative of some methods by which compounds herein and their salts (which can be crystalline) can be synthesized, and various modifications to these schemes can be made and will be suggested to one skilled in the art having referred to this disclosure.

[00114] The starting materials and intermediates of the reactions of any embodiment herein, herein and as-above disclosed, unless otherwise indicated, may be isolated and purified if desired using conventional techniques, including, but not limited to filtration, distillation, crystallization, chromatogram, flash chromotography, HPLC, MPLC, Chromatotron®, ion exchange chromatography, crystallization with Mosher acids or Mosher esters, and the like. Such materials may be characterized using conventional means, including physical constructs and spectral data, for example proton NMR, carbon NMR, IR spectroscopy, polarimetry, atomic absorption, elemental analysis, UV spectroscopy, FTIR spectroscopy, and the like. In any embodiment here and as-above described, unless otherwise indicated, chromatography can be excluded in making any of the compounds or their salts.

[00115] Unless specified to the contrary, the reactions described herein can take place at, for example, from about 0.001 to about 100 atmospheres (atm), for example, about 0.001 atm, about 0.01 atm, about 0.1 atm, about 1 atm, about 2 atm, about 3 atm, about 4 atm, about 5 atm, about 10 atm, about 20 atm, about 50 atm, or about 100 atm.

[00116] Reactions in any embodiment herein, unless otherwise indicated, can be run, unless otherwise specified, for example, open to the atmosphere, or under an inert gas atmosphere such as, for example, nitrogen or argon.

[00117] Reactions in any embodiment herein, unless otherwise indicated, can be run, unless otherwise specified, for example, at temperatures from about -78°C to about 150 °C, for example from about -78 °C, about -50 °C, about -20 °C, about 0 °C, about 10 °C, about 20 °C, about 23 °C, about 25 °C, about 27 °C, about 30 °C, about 40 °C, about 50 °C, about 100 °C, about 125 °C, about 150 °C, at about ambient temperature, or at about room temperature.

[00118] Reactions herein, unless otherwise indicated, can have a yield, unless otherwise explicitly stated, based on the theoretical yield, for example, ranging from about 1% to about 99%. The yield can be, for example, about 99%, about 98%, about 97%, about 96%, about 95%, about 90%, about 85%, about 80%, about 75%, about 70%, about 65%, about 60%, about 55%, about 50%, about 45%, about 40%, about 35%, about 30%, about 25%, about 20%, about 15%, about 10%, or about 5%.

[00119] Reactions herein, unless otherwise indicated, can be run, unless otherwise specified, for example, for a time ranging from about 0.1 to about 96 hours, *e.g.*, for about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours, about 48 hours, about 72 hours, or about 96 hours,

Selective Uses of Crosslinked Peptidomimetic Macrocycles (Stitched and Stapled Peptides)

[00120] Crosslinked peptidomimetic macrocycles (stitched or stapled peptides), made with for example at least one of the crystalline compounds and their crystalline salts of Formula (I), including the crystalline compounds and their salts of Formulae (IIa), (IIb), (IIIa) and (IIIb), as well as the optionally crystalline compounds and their optionally crystalline salts of Formula (IV), can be used to treat or prevent diseases.

For example, the crosslinked peptidomimetic macrocycles (stitched or stapled peptides) can be used to treat or prevent cancers. Selected examples of cancers include, for example, fibrosarcoma, myosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, gastric cancer, esophageal cancer, rectal cancer, pancreatic cancer, ovarian cancer, prostate cancer, uterine cancer, cancer of the head and neck, skin cancer, brain cancer, squamous cell carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinoma, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular cancer, small cell lung carcinoma, non-small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, retinoblastoma, leukemia, lymphoma, or Kaposi sarcoma.

[00121] Diseases which can be treated by stitched or stapled peptides can be found, for example, in International Application No. PCT/US2004/038403 ("the '403

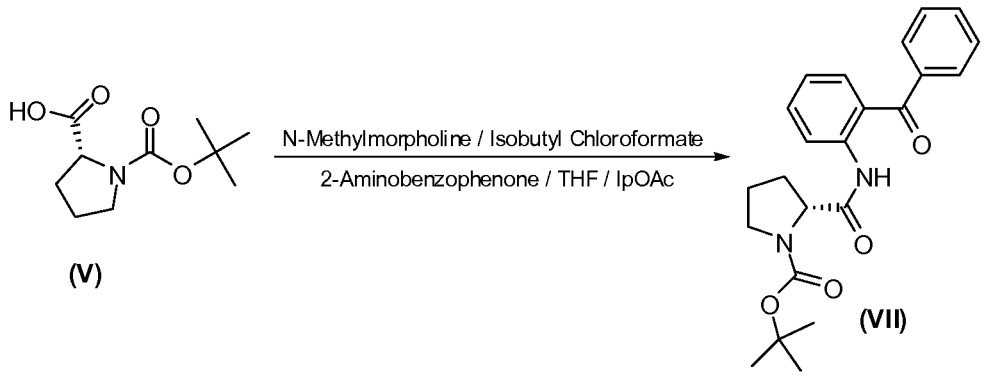
application") and International Application Publication No. WO2008/121767 ("the '767 publication').

[00122] While inventive embodiments have been shown and described herein, such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the inventive disclosure herein. The following Examples are illustrative and should not be construed as limiting.

Examples

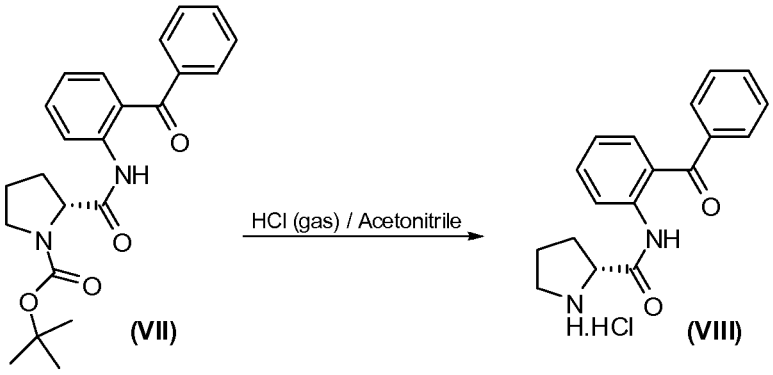
Example 1: Preparation of crystalline N-Fmoc-(R)-α-methyl-α-aminodec-9-enoic acid

Example 1a: Preparation of (R)-2-[N-(N'-Boc-prolyl)amino]benzophenone



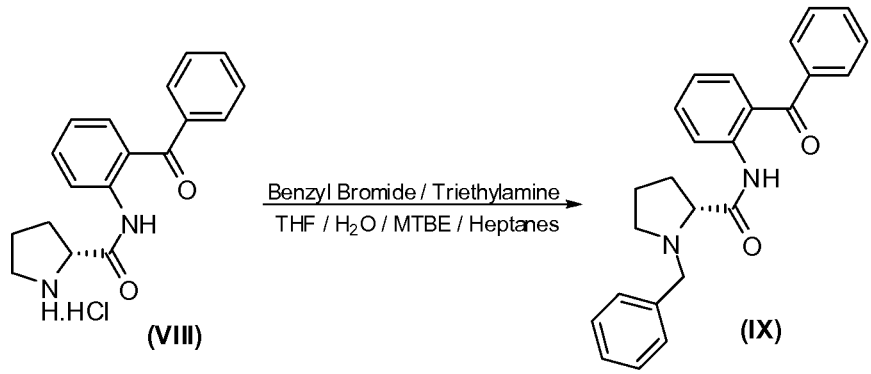
[00123] Tetrahydrofuran and 9.6 kg (1.0 equivs.) of Boc-D-proline (V) were added to a reactor and cooled to -5 °C. 5.3 kg (1.15 equivs.) of N-methylmorpholine were charged followed by a slow addition of 6.1 kg (1.0 equivs.) of isobutyl chloroformate in tetrahydrofuran while maintaining the internal temperature at < 5 °C. The mixture was allowed to agitate at 20 – 25 °C for 45 - 60 minutes and then was analyzed by TLC for completion. A solution of 8.2 kg (0.9 equvs.) of 2-aminobenzophenone / tetrahydrofuran was charged and the mixture was allowed to agitate at 20 – 25° C until the reaction is deemed complete. The mixture was concentrated to ½ volume and isopropyl acetate was charged. The organic product layer was then washed with a 5% sodium bicarbonate solution, water was charged, then the pH was adjusted to 2.0 – 2.5 with 25% sulfuric acid. The layers were split and the organic product layer was washed again with water. The organic product solution was then concentrated and crystallized from isopropyl acetate and washed with methyl tert-butyl ether. Product (VII) was isolated and dried under heat and vacuum. Yield: 12 kg, 66.7 %.

Example 1b: Preparation of D-Proline-2-Aminobenzophenone amide



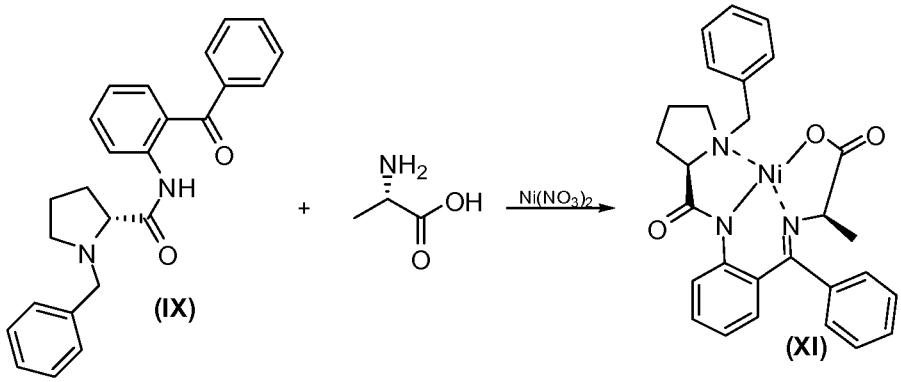
[00124] 12.0 kg (1.0 equivs.) of Boc-D-proline-2-aminobenzophenone (VII) amide was dissolved into acetonitrile. 2.2 kg (2.0 equivs.) of hydrogen chloride gas was then charged / bubbled into the solution. The resulting mixture was then allowed to agitate at 20 – 25 °C until the reaction was complete. Methyl *tert*-butyl ether was added and the solid product was isolated out of the reaction solution and washed with additional methyl *tert*-butyl ether. The product (VIII) was dried under heat and vacuum. Yield: 9.1 kg, 100%.

Example 1c: Preparation of (R)-2-[N-(N'-benzylprolyl)amino]benzophenone (D-BPB)



[00125] 9.1 kg (1.0 equivs.) of D-proline-2-aminobenzophenone amide · HCl (VIII) was dissolved into tetrahydrofuran and water. 8.1 kg (2.4 equivs.) of triethylamine was then charged, followed by a slow addition of 7.9 kg (1.4 equivs.) of benzyl bromide. The mixture was then allowed to agitate at 20 – 25 °C until the reaction was complete. Methyl *tert*-butyl ether and water were added and the resulting solution was pH adjusted to 2.0 – 2.5 with a 1N hydrochloric acid solution. The mixture was concentrated to remove all the tetrahydrofuran. The product slurry was then isolated and washed with methyl *tert*-butyl ether. The product (IX) was dried under heat and vacuumYield: 10.5 kg, 82.7%.

Example 1d: Preparation of (R)-Ala-Ni-BPB



[00126] 10.5 kg (1.0 equivs.) of D-BPB (IX) , 14.1 kg (1.78 equivs.) nickel (II) nitrate hexahydrate, 4.9 kg (2.0 equivs) of L-alanine, and methanol were charged to a reactor. The mixture was heated to 40 °C and a solution of 12.2 kg (8.0 equivs.) of potassium hydroxide / methanol was slowly added while maintaining the internal temperature of < 50°C. The reaction mixture was then heated up to 60 °C and allowed to agitate at temperature until the reaction was complete. The mixture was then cooled to 20 – 25 °C and 8.2 kg (5.0 equivs.) of acetic acid was slowly charged while maintaining an internal temperature of < 35 °C. The reaction solution was concentrated to a solid. Tetrahydrofuran and isopropyl acetate were then added to dissolve the solid(s) and the organic product layer was washed 2x with water. The solution was then concentrated again and material was subsequently crystallized out of tetrahydrofuran and methyl *tert*-butyl ether. The product was isolated, rinsed with additional methyl *tert*-butyl ether and analyzed for purity. To improve purity the product (XI) was recrystallized out of isopropyl alcohol and then isolated, and dried under heat and vacuum. Yield: 6.8 kg, 48.6%.

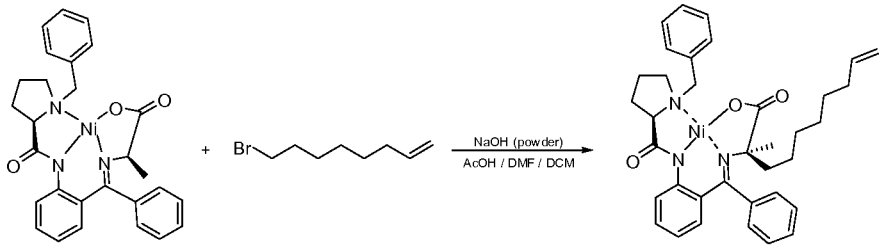
Recrystallization procedure

[00127] THF was added to the crude product (15 mL per 10 g of starting material (D-BPB)) and the resulting mixture was heated to 50 °C. The mixture was maintained at 50 °C for 1 h, then methyl tertiary-butyl ether was added (50 mL per 10 g of starting material (D-BPB)). The mixture was maintained at 50 °C for additional 1 h after which it was cooled to 35 °C. The mixture was filtered and the resulting solid was washed with methyl tertiary-butyl ether (20 mL per 10 g of starting material (D-BPB)) to obtain the crystalline product XI.

Alternate recrystallization procedure

[00128] Isopropyl acetate was added to the crude product (40 mL per 4 g of starting material (D-BPB)) and the resulting mixture was maintained at room temperature for 30 min. The mixture was then filtered to obtain the crystalline product XI.

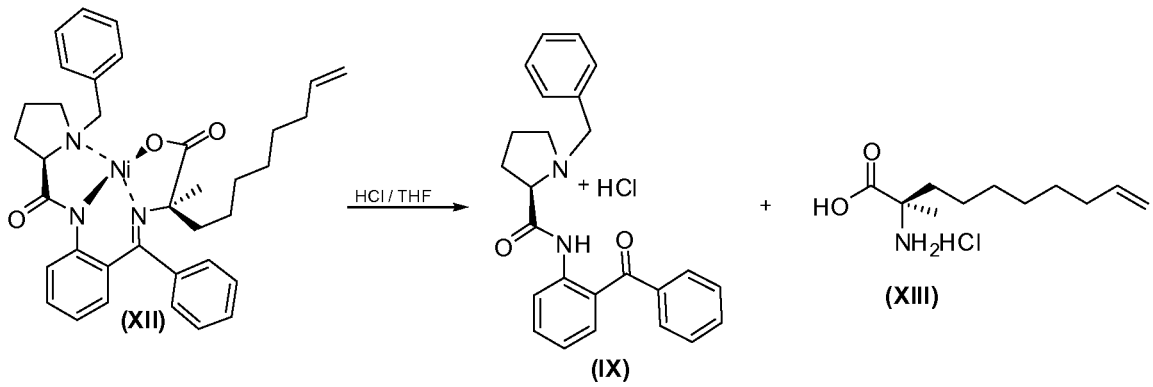
Example 1e: Preparation of R8-Ni-BPB



(XI) (XII)

[00129] 6.8 kg (1.0 equivs.) of (R)-Ala-Ni-BPB (XI) was charged to a reactor and dissolved up into dimethylformamide and cooled to 10 °C. 1.4 kg (2.5 equivs.) of sodium hydroxide (powder) was then charged to the same reactor and the mixture was sparged with nitrogen and agitated until a solution formed at 10 °C. 5.2 kg (2.0 equivs.) of 8-bromo-1-octene was charged to the reactor while maintaining an internal temperature of < 20 °C. The mixture was then allowed to agitate at 20 – 25 °C until the reaction was complete. Once the reaction was complete, the solution was cooled to 10 °C and 0.5 kg (0.6 equivs.) of acetic acid was charged maintaining the internal temperature < 25 °C. Water was then charged followed by methyl *tert*-butyl ether and the organic layer was washed. The organic layer was then washed 2 more times with water and then concentrated. The product oil was then co-stripped with methylene chloride and dissolved up in additional methylene chloride. The product (XII) solution was taken on into the next processing step.

Example 1f: Preparation of (R)-2-Amino-2-methyl-dec-9-enoic acid



(XII) (IX) (XIII)

[00130] The R8-Ni-BPB (XII) / DCM solution was charged to a 50-L chem-glass reactor and stripped to an oil.Tetrahydrofuran was then added and the mixture was agitated at 20 – 25 °C until a solution formed. 7.8 kg (5.0 equvs.) of 32% hydrochloric acid was charged slowly while maintaining an internal temperature of < 30 °C. The mixture was then allowed to agitate for 6 – 8 hours at ambient temperature. The mixture was then concentrated to remove tetrahydrofuran to yield a slurry. Additional water was added and the slurry was agitated at ambient temperature for 1 – 2 hours. The solid BPB salts were isolated by filtration and rinsed with additional water followed by methyl *tert*-butyl ether. The product filtrates were then re-charged to the reactor yielding a tri-phased solution. The lower-most layer was split from the upper two layers. The combined two organic layers were then washed 3x with water and concentrated to an oil. Acetonitrile was then added and the mixture was warmed to 70 °C for 30 minutes. The mixture was then cooled to 25 – 30 °C and the solid product was isolated. The solid filter-cake was washed with acetonitrile and methyl *tert*-butyl ether, then analyzed for purity. The product was then re-slurried out of additional acetonitrile and washed with acetonitrile and methyl *tert*-butyl ether. The material (XIII) was isolated and dried under heat and vacuum. Yield: 1.55 kg, 48 %.

Recrystallization procedure

[00131] Acetonitrile (23 mL per 10 g of starting material (oil of (R)-Ala-Ni-BPB (XI))) was added to the crude product and the resulting mixture was heated to 70 °C for 30 min after which it was cooled to 20 °C. The mixture was filtered and the resulting solid

was washed with acetonitrile (5 mL) and methyl tertiary-butyl ether (8.5 mL) to obtain the crystalline product XIII.

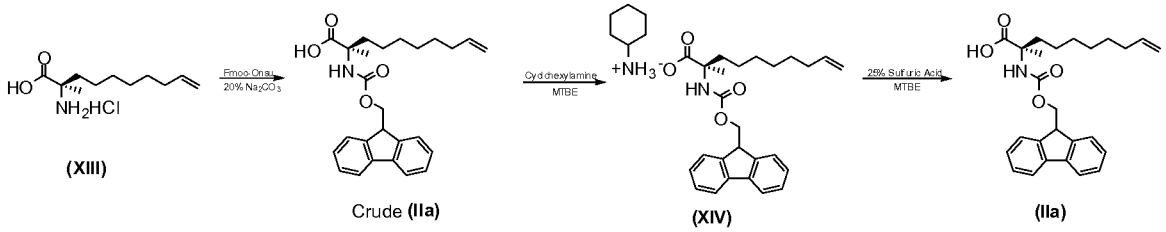
Alternate recrystallization procedure to prepare XIII -I

[00132] Acetonitrile (30 mL per 10 g of starting material (oil of (R)-Ala-Ni-BPB (XI))) was added to the crude product and the resulting mixture was heated at 60 °C for 30 min followed by cooling to 30 °C. The mixture was then filtered and washed with 5 mL acetonitrile to obtain the crystalline product XIII.

Alternate recrystallization procedure to prepare XIII -II

[00133] Acetonitrile (23 mL per 10 g of starting material (oil of (R)-Ala-Ni-BPB (XI))) was added to the crude product and the resulting mixture was heated at 40 °C for 30 min followed by cooling to room temperature. The mixture was then filtered and washed twice with 5 mL acetonitrile to obtain the crystalline product XIII.

Example 1g: Preparation of Crystalline N-Fmoc-(R)-α-methyl-α-aminodec-9-enoic acid



[00134] 1.55 kg (1.0 equiv.) of 2-amino-2-methyl-dec-9-enoic acid· HCl (XIII) was suspended in water and polished filtered to remove trace amounts of D-BPB · HCl from the solution. Methyl *tert*-butyl ether was added and the aqueous product layer was extracted once with methyl *tert*-butyl ether. The aqueous product layer was re-charged and tetrahydrofuran was added. A 20% aqueous sodium carbonate solution (2.75 equiv.) was charged to the mixture followed by Fmoc-OSu (0.89 equiv.). The mixture was allowed to react at 20 – 25 °C while maintaining the pH between 8.5 – 9.0 with additional amounts of the 20% sodium carbonate solution until the reaction was complete. The mixture was pH adjusted down to pH 2.0 – 2.5 with conc. hydrochloric acid. Tetrahydrofuran was distilled off and methyl *tert*-butyl ether was charged. The layers were separated and the organic layer was washed 3 more times with additional

water. The organic layer was then concentrated under vacuum and co-stripped with methyl *tert*-butyl ether. The resulting crude oil was re-dissolved in methyl *tert*-butyl ether and cyclohexylamine (1.10 equiv.) was added slowly to obtain a pH range of 8.5 – 9.0. The slurry was agitated at ambient temperature (20 – 25 °C) for 3 hours and the solid product salt (XIV) was isolated by filtration. The solids were rinsed twice with additional methyl *tert*-butyl ether and the solid wetcake was recharged to a clean reactor. The wetcake was recrystallized from tetrahydrofuran and methyl *tert*-butyl ether to improve the purity. The solid salt was suspended in methyl *tert*-butyl ether and water and the pH adjusted to 2.0 – 2.5 with 25% sulfuric acid. The organic product layer was washed with water until all of the cyclohexylamine was removed. The organic product layer was concentrated and co-stripped with hexanes to a loose oil. The product (IIa) was then crystallized out of chloroform and hexanes and dried at < 0°C under a 1.0 cfm nitrogen sweep. Yield: 1.12 kg, 41.5 %

Recrystallization procedure

[00135] Methyl tertiary-butyl ether (800 mL per 36 g of starting material XIII) was added to the crude product and the pH of the resulting mixture was adjusted to 8-9 using CHA at 20 °C. The mixture was mixed at 20 °C and after 1h crystals started forming. Additional methyl tertiary-butyl ether was added (200 mL) and the resulting slurry was mixed for 18 h. The mixture was filtered and the resulting solid was washed with twice methyl tertiary-butyl ether (200 mL and 8.5 mL) to obtain the crystalline product XIII. The product was analyzed for chiral purity, and if the results were less than 95% Fmoc-R8 vs. Fmoc-S8 then crystallization was performed to upgrade the chiral purity by dissolving dry FmocR/S (50 g) in THF (50 mL). Once FmocR/S was dissolved, methyl tertiary-butyl ether was added (900 mL) and the mixture was mixed at 20 °C for 18 h. The mixture was then filtered and washed twice with methyl tertiary-butyl ether (100 mL each). The chiral purity of the resulting crystalline product XIV was about 97.8%

Alternate recrystallization procedure for XIV-I

[00136] Methyl tertiary-butyl ether (1500 mL per 47 g of starting material XIII) was added to the crude product and the pH of the resulting mixture was adjusted to 8-9 using CHA at 20 °C. The mixture was mixed at this temperature for 3 h after which it was filtered and the resulting solid was washed with methyl tertiary-butyl ether (250 mL).

Alternate recrystallization procedure for XIV-II

[00137] Methyl tertiary-butyl ether (400 mL per 20 g of starting material XIII) was added to the crude product and the pH of the resulting mixture was adjusted to 8-9 using CHA at 20 °C. Additional 200 mL methyl tertiary-butyl ether was added and the mixture was mixed at this temperature for 2 h after which it was filtered and the resulting solid was washed with methyl tertiary-butyl ether (10 mL).

Alternate recrystallization procedure for XIV -III

[00138] Methyl tertiary-butyl ether (50 mL per 4 g of starting material XIII) was added to the crude product and the pH of the resulting mixture was adjusted to 8-9 using CHA at 20 °C. The mixture was mixed at this temperature for 45 min after which it was filtered and the resulting solid was washed with methyl tertiary-butyl ether (10 mL).

Recrystallization procedure for IIa

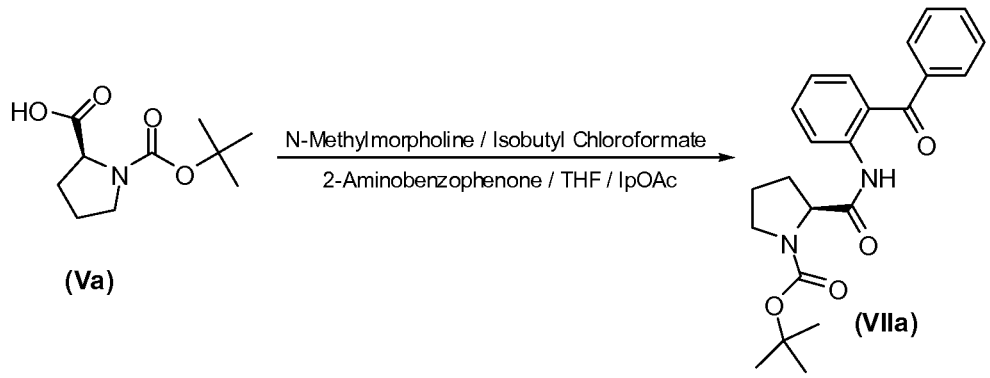
[00139] Chloroform (70 mL) was added to the crude product (25 g) and the resulting mixture was cooled to 0 °C. Hexanes (210 mL) were then slowly added so as to maintain the temperature at 0 °C. The mixture was further maintained at this temperature for 1h after which it was filtered cooled and the resulting solid was dried under vacuum at 0 °C.

Alternate recrystallization procedure for IIa -I

[00140] Chloroform (2200 m L) was added to the crude product (1100 g). Hexanes (6600 L) were then added slowly and the resulting mixture was cooled to less than 0 °C. The mixture was further mixed at temperature below 0 °C for 1h after which it was filtered at less than 0 °C and the resulting solid was dried under vacuum at temperature below 0 °C.

Example 2: Preparation of crystalline N-Fmoc-(S)-α-methyl-α-aminohept-6-enoic acid

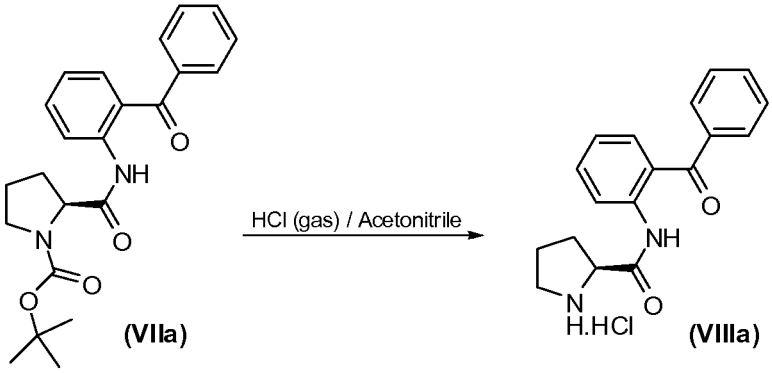
Example 2a: Preparation of (S)-2-[N-(N'-Boc-prolyl)amino]benzophenone



(Va) (VIIa)

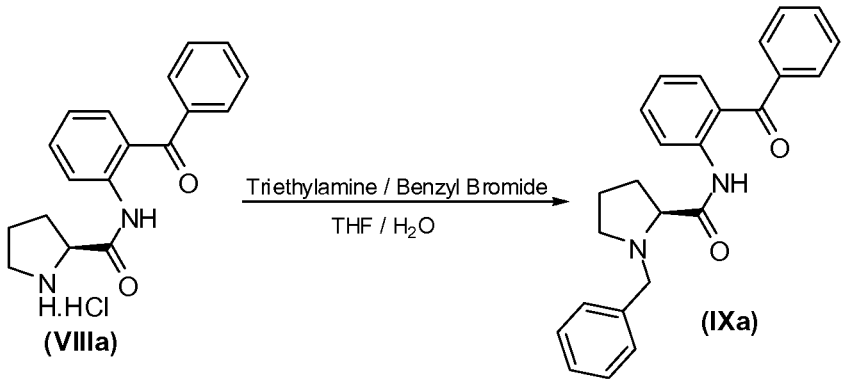
[00141] Tetrahydrofuran and 7.5 kg (1.0 equivs.) of Boc-L-proline (Va) were added to a reactor and the resulting solution was cooled to -5 °C. 4.2 kg (1.05 equivs.) of N-methylmorpholine were charged, followed by slow addition of 5.3 kg (1.0 equivs.) of isobutyl chloroformate in tetrahydrofuran while maintaining an internal temperature of < 5 °C. The mixture was allowed to agitate at 20 – 25 °C for 45 - 60 minutes and then was analyzed by TLC for completion. A solution of 6.2 kg (0.9 equvs.) of 2-aminobenzophenone / tetrahydrofuran was charged and the mixture was allowed to agitate at 20 – 25 °C until the reaction was shown to be complete by TLC. The mixture was concentrated to ½ volume and isopropyl acetate was charged. The organic product layer was then washed with a 5% sodium bicarbonate solution, water was charged, and then pH adjusted to 2.0 – 2.5 with 25% sulfuric acid. Layers were split and the organic product layer was washed again with water. The organic product solution/layer was then concentrated and crystallized from isopropyl acetate and washed with methyl *tert*-butyl ether. Product (VIIa) was then isolated and dried under heat and vacuum. Yield: 9.3 kg, 75 %.

Example 2b: Preparation of L-Proline-2-Aminobenzophenone amide



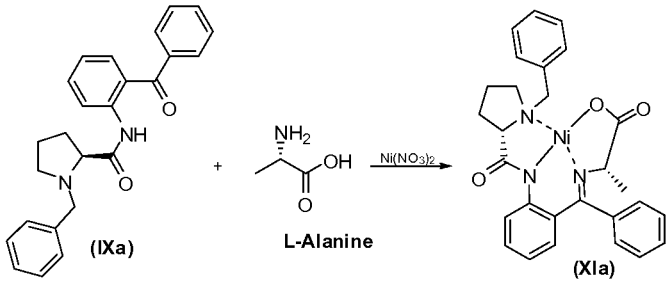
[00142] 9.4 kg (1.0 equivs.) of Boc-L-proline-2-aminobenzophenone amide (VIIa) was dissolved into acetonitrile. 1.7 kg (2.0 equivs.) of hydrogen chloride gas were then charged / bubbled into the solution. This mixture was allowed to agitate at 20 – 25 °C until the reaction was demonstrated to be complete by TLC. Methyl *tert*-butyl ether was added and a solid product was isolated out of the reaction solution and washed with additional methyl *tert*-butyl ether. The product (VIIIa) was dried under heat and vacuum. Yield: 7.0 kg, 100 %.

Example 2c: Preparation of (S)-2-[N-(N'-benzylprolyl)amino]benzophenone (L-BPB)



[00143] 7.1 kg (1.0 equivs.) of L-proline- 2-aminobenzophenone amide · HCl (VIIIa) was dissolved into tetrahydrofuran and water. 5.8 kg (2.4 equivs.) of triethylamine were then charged followed by a slow addition of 5.9 kg (1.4 equivs.) of benzyl bromide. The mixture was then allowed to agitate at 20 – 25 °C until the reaction was complete. Methyl *tert*-butyl ether and water were added and the solution pH was adjusted to 2.0 – 2.5 with a 1N hydrochloric acid solution. The mixture was concentrated to remove all the tetrahydrofuran. The product slurry was then isolated and washed with methyl *tert*-butyl ether. The product (IXa) was dried under heat and vacuum. Yield 7.7 kg, 84.0 %.

Example 2d: Preparation of (S)-Ala-Ni-BPB



[00144] 7.9 kg (1.0 equivs.) of L-BPB (IXa), 12.1 kg (1.78 equivs.) nickel (II) nitrate hexahydrate, 3.7 kg (2.0 equivs) of L-alanine, and methanol were charged to a reactor. The mixture was heated to 40 °C and a solution of 8.2 kg (8.0 equivs.) of potassium hydroxide / methanol was slowly added while maintaining the internal temperature at < 50 °C. The reaction mixture was then heated up to 60 °C and allowed to agitate at temperature until the reaction was complete. The mixture was subsequently cooled to 20 – 25 °C and 8.9 kg (5.0 equivs.) of acetic acid was slowly charged while maintaining the internal temperature at < 35 °C. The reaction solution was then concentrated to a solid. Tetrahydrofuran and isopropyl acetate were added to dissolve the solids and the organic product layer was washed twice with water. The solution was concentrated again and material crystallized out of tetrahydrofuran and methyl *tert*-butyl ether. The product was isolated, rinsed with additional methyl *tert*-butyl ether and analyzed for purity. To improve purity the product (XIa) was recrystallized out of isopropyl alcohol and then isolated, and dried under heat and vacuum. Yield: 6.0 kg, 56.0 %.

Recrystallization procedure for XIa

[00145] Methyl tertiary-butyl ether (550 mL per 50 g of starting material L-BPB) was added to the crude product (S)-Ala-Ni-BPB and the slurry was then heated to 50 °C before cooling it to 20 °C. The mixture was mixed at 20 °C for 16 h. The mixture was filtered and the resulting solid was washed with methyl tertiary-butyl ether (100 mL) to obtain the crystalline product XIa.

Alternate recrystallization procedure for XIa -I

[00146] Methyl tertiary-butyl ether (600 mL per 50 g of starting material L-BPB) was added to the crude product (S)-Ala-Ni-BPB and the slurry was then heated to 50-60 °C and maintained at this temperature for 1 h. The mixture was then filtered at 35 °C and washed with methyl tertiary-butyl ether (100 mL) to obtain the crystalline product XIa.

Alternate recrystallization procedure for XIa -II

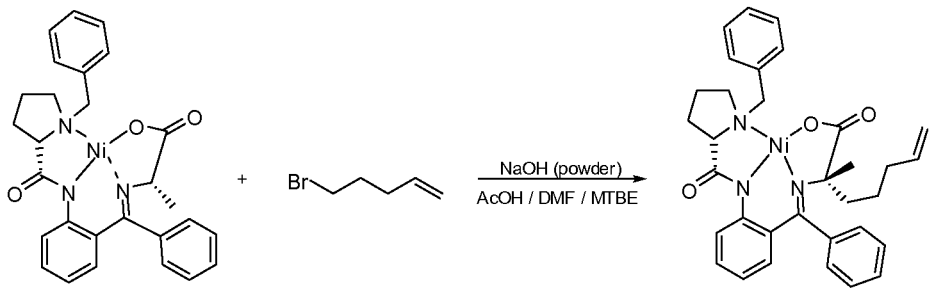
[00147] Methyl tertiary-butyl ether (500 mL per 50 g of starting material L-BPB) was added to the crude product (S)-Ala-Ni-BPB and the slurry was then heated to 45-50 °C and maintained at this temperature for 1 h. The mixture was then filtered at 35 °C and washed with methyl tertiary-butyl ether (100 mL) to obtain the crystalline product XIa.

Alternate recrystallization procedure-III

[00148] Methyl tertiary-butyl ether (2000 mL per 280 g of starting material L-BPB) was added to the crude product (S)-Ala-Ni-BPB and the slurry was then heated to 45-50 °C and maintained at this temperature for 30 min. The mixture was then cooled to 20 °C and mixed at this temperature for 8 h. The resulting solid was then filtered and washed with methyl tertiary-butyl ether (100 mL).

[00149] (S)-Ala-Ni-BPB (300 g) was recrystallized by dissolving in THF (450 mL). The mixture was heated to 50 °C for 1 h followed by the addition of methyl tertiary-butyl ether (1500 mL) at 50 °C. The resulting mixture was mixed at this temperature for additional 1h. The slurry was then cooled to 20 °C and mixed at 20 °C for 1 h. The resulting solid was then filtered and washed with methyl tertiary-butyl ether (300 mL) to obtain the crystalline product XIa.

Example 2e: Preparation of S5-Ni-BPB



(XIa) (XIIa)

[00150] 5.8 kg (1.0 equivs.) of (S)-Ala-Ni-BPB (Xia) was charged to a reactor and dissolved up into dimethylformamide and cooled to 10 °C. 1.2 kg (2.5 equivs.) of sodium hydroxide (powder) was then charged to the same reactor and the mixture was sparged with nitrogen and agitated until a solution formed at 10 °C. 3.3 kg (2.0 equivs.) of 5-bromo-1-pentene was then charged to the reactor maintaining the internal temperature of < 20°C. The mixture was then allowed to agitate at 20 – 25 °C until the reaction was complete. Once the reaction was complete, the solution was cooled to 10 °C and 0.4 kg (1.5 equivs.) of acetic acid was charged maintaining an internal temperature of < 25 °C. Water was then charged, followed by methyl *tert*-butyl ether, and the organic layer was washed. The organic layer was then washed 2 more times with water and then concentrated. The product (XIIa) was crystallized out of isopropyl acetate, isolated and dried under heat and vacuum. Yield: 2.2 kg, 32.4 %.

Recrystallization procedure

[00151] Isopropyl acetate (200 mL per 12.5 g of starting material XIa) was added to the crude product S5-Ni-BPB and the mixture was mixed at 20 °C for 30 min then hexanes (500 mL) were added. The mixture was further mixed for 30 min following which it was filtered to obtain the crystalline product XIIa.

Alternate recrystallization procedure-I

[00152] Isopropyl acetate (80 mL per 39 g of starting material XIa) was added to the crude product S5-Ni-BPB and the mixture was mixed at 20 °C for 2 h. The mixture was filtered and washed with isopropyl acetate (35 mL). The filtrate and the washed were combined and heptanes (170 mL) were added. The resulting slurry was mixed for 1 h, then filtered and washed with heptanes (360 mL) to obtain the crystalline product XIIa.

Alternate recrystallization procedure-II

[00153] Isopropyl acetate (1000 mL per 205 g of starting material XIa) was added to the crude product S5-Ni-BPB and the mixture was dissolved at 70-80 °C. The solution was cooled to 20 °C and the mixture was mixed at this temperature for 1 h during which no crystallization was observed. The mixture was filtered over celite and the solvent was removed under vacuum at 40 °C. Methyl tertiary-butyl ether (1000 mL) was added and the mixture was heated to 60 °C then cooled to 20 °C and mixed for 24 h. The solid was filtered and washed with methyl tertiary-butyl ether (300 mL) and to obtain the crystalline product XIIa.

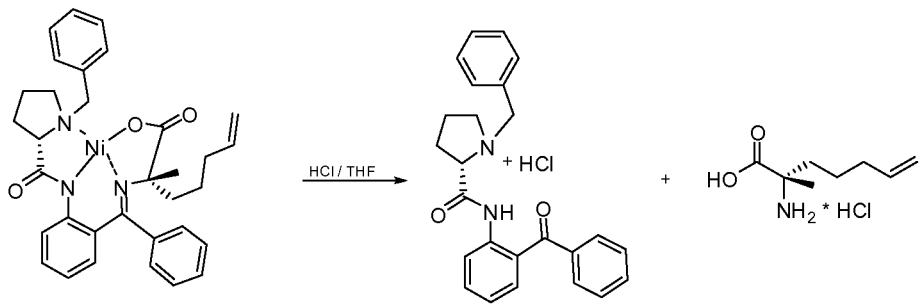
Alternate recrystallization procedure-III

[00154] Ethyl acetate (100 mL per 12.5 g of starting material XIa) was added to the crude product S5-Ni-BPB and the mixture was mixed at 20 °C for 30 min. hexanes (500 mL) were added and the resulting slurry was mixed for further 30 min after which it was filtered to obtain the crystalline product XIIa.

Alternate recrystallization procedure-IV

[00155] Methyl tertiary-butyl ether (100 mL per 12.5 g of starting material XIa) was added to the crude product S5-Ni-BPB and the mixture was heated to 45-50 °C. Heptanes (400 mL) were added 45-50 °C. The resulting slurry was cooled to 20 °C and filtered to obtain the crystalline product XIIa.

Example 2f: Preparation of (S)-2-Amino-2-methyl-hept-6-enoic acid



(XIIa) (IXa) (XIIIa)

[00156] 2.2 kg (1.0 equivs.) of S5-Ni-BPB (XIIa) was charged to a 15-L chem-glassreactor. Tetrahydrofuran was added and the mixture agitated at 20 – 25 °C until a solution formed. 1.8 kg (4.5 equvs.) of 32% hydrochloric acid was charged slowly while maintaining an internal temperature of < 30 °C. The mixture was then allowed to agitate for 6 – 8 hours at ambient temperature. The mixture was concentrated to remove tetrahydrofuran to yield a slurry. Additional water was added and the slurry was agitated at ambient temperature for 1 – 2 hours. The solid BPB salts were isolated by filtration and rinsed with additional water followed by methyl *tert*-butyl ether. The product filtrates were then re-charged to the reactor yielding a tri-phased solution. The lower-most layer was split from the upper two layers. The combined two organic layers were then washed 3x with water and concentrated to an oil. Acetonitrile was added and the mixture was warmed to 70 °C for 30 minutes. The mixture was then cooled to 25 – 30 °C and the solid product was isolated. The solid filter-cake was washed with acetonitrile and methyl *tert*-butyl ether, then analyzed for chemical purity. The product was then re-slurried out of additional acetonitrile and washed with acetonitrile and methyl *tert*-butyl ether. The material (XIIIa) was isolated and dried under heat and vacuum. Yield: 0.585 kg, 80.0 %

Recrystallization procedure for XIIIa

[00157] Acetonitrile (100 mL per 20 g of starting material S5-Ni-BPB (XIIa)) was added to the crude product and the mixture was mixed at 20 °C for 1 h. The mixture was then filtered and washed with acetonitrile (40 mL) to obtain the crystalline product XIIIa.

Alternate recrystallization procedure for XIIIa-I

[00158] Acetonitrile (500 mL per 185 g of starting material XIIa) was added to the crude product S5-Ni-BPB and the slurry was dissolved at 45-50 °C. The solvent was removed under vacuum at 45-50 °C, 500 mL acetonitrile was added and the resulting mixture was heated to 45-50 °C. The mixture was then cooled to 35 °C, filtered and washed with acetonitrile (50 mL) to obtain the crystalline product XIIIa.

Alternate recrystallization procedure for XIIIa -II

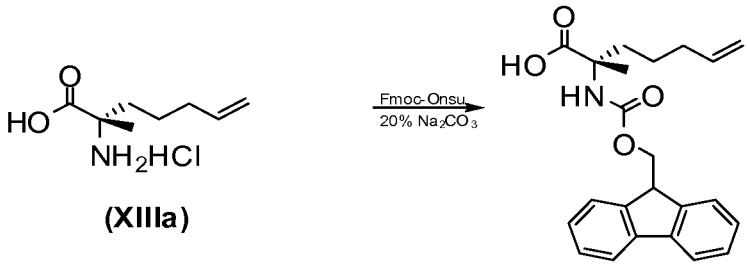
[00159] Acetonitrile (270 mL per 35 g of starting material XIIa) was added and the slurry was heated to 45-50 °C. The mixture was then cooled to 20 °C and mixed at this

temperature for 2 h. The mixture was then filtered and washed with acetonitrile (50 mL) and methyl tertiary-butyl ether (50 mL) to obtain the crystalline product XIIIa.

Alternate recrystallization procedure for XIIIa -III

[00160] Isopropyl acetate (60 mL per 15 g of XIIIa) was added and the mixture was heated to 70 °C. Acetonitrile (180 mL) was added and the resulting mixture was cooled to 20 °C. The mixture was filtered and the resulting solid was washed with acetonitrile (50 mL) to obtain the crystalline product XIIIa.

Example 2g: Preparation of N-Fmoc-(S)-α-methyl-α-aminohept-6-enoic acid



(XIIIa) **(IIIa)**

[00161] 0.585 kg (1.0 equiv.) of 2-amino-2-methyl-hept-6-enoic acid ·HCl (XIIIa) was suspended in water and polished filtered to remove trace amounts of L-BPB · HCl from the solution. Methyl *tert*-butyl ether was added and the aqueous product layer extracted once with methyl *tert*-butyl ether. The aqueous product layer was re-charged and tetrahydrofuran was added. An aqueous 20% sodium carbonate solution (2.75 equiv.) was charged to the mixture, followed by Fmoc-Onsu (0.95 equiv.). The mixture was allowed to react at 20 – 25 °C, while maintaining the pH between 8.5 – 9.0 with additional amounts of the 20% sodium carbonate solution until the reaction was complete. The mixture was pH adjusted down to pH 2.0 – 2.5 with conc. hydrochloric acid. Tetrahydrofuran was distilled off and methyl *tert*-butyl ether is charged. The layers were separated and the organic layer was washed 3 more times with additional water. The organic layer was then concentrated under vacuum and co-stripped with methyl *tert*-butyl ether. The organic product layer was concentrated and co-stripped with hexanes to a loose oil. The product (IIIa) was then crystallized out of

chloroform and hexanes and dried at < 0°C under a 1.0 cfm nitrogen sweep. Yield: 0.831 kg, 76.0 %.

Recrystallization procedure for IIIa

[00162] Chloroform (30 mL per 9 g of starting material XIIIa) was added to the crude product. Hexanes (100 mL) were added and the mixture was cooled to 0 °C. The resulting solid was filtered at 0 °C and washed with cold hexanes to obtain the crystalline product IIIa.

Recrystallization procedure for cyclohexylamine salt of IIIa

[00163] Acetonitrile (300 mL per 19.04 g of starting material XIIIa) was added to the crude product and the pH was adjusted to 8-9 using cyclohexylamine at 20 °C. The resulting mixture was mixed at 20 °C for 2 h and then filtered and washed with acetonitrile (50 mL) to obtain the crystalline cyclohexylamine salt of IIIa.

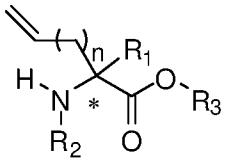
Alternate recrystallization procedure for cyclohexylamine salt of IIIa-I

[00164] Methyl tertiary-butyl ether (200 mL per 5 g of starting material XIIIa) was added to the crude product and the pH was adjusted to 8-9 using cyclohexylamine at 20 °C. The resulting mixture was mixed at 20 °C for 1 h and then filtered and washed with methyl tertiary-butyl ether (50 mL) to obtain the crystalline cyclohexylamine salt of IIIa.

CLAIMS

WHAT IS CLAIMED IS:

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



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 9-Fluorenylmethoxycarbonyl (Fmoc); and

R_3 is –H; wherein the salt is a cyclo alkyl amine salt.

2. The crystalline salt of claim 1, wherein R_1 is C_1-C_3 alkyl.

3. The crystalline salt of any preceding claim, wherein R_1 is methyl.

4. The crystalline salt of any preceding claim, wherein n is an integer from 3 to 11.

5. The crystalline salt of any preceding claim, wherein n is selected from the group consisting of: 3 and 6.

6. The crystalline salt of any of claims 1-5, wherein the stereocenter * is (S).

7. The crystalline salt of any of claims 1-5, wherein the stereocenter * is (R).

8. The crystalline salt of any of claims 1-7, having a chemical purity ranging from about 90% to 100%.

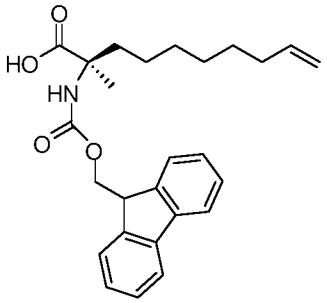
9. The crystalline salt of any of claims 1-7, having an optical purity ranging from about 90% to 100%.

10. The crystalline salt of any of claims 1-7, having an optical purity ranging from about 95% to 100%.

11. The crystalline salt of any of claims 1-7, having an enantiomeric excess ranging from about 90% to 100%.

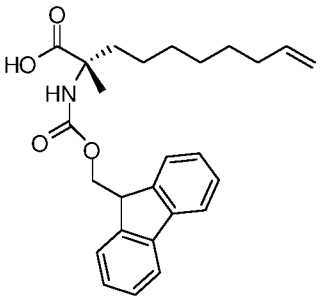
12. The crystalline salt of any of claims 1-7, having an enantiomeric excess ranging from about 95% to 100%.

13. The crystalline salt of claim 1, having the Formula (IIa):



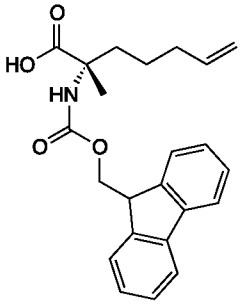
Formula (IIa).

14. The crystalline salt of claim 1, having the Formula (IIb):



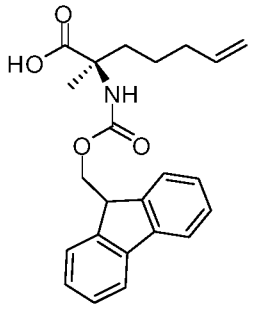
Formula (IIb).

15. The crystalline salt of claim 1, having the Formula (IIIa):



Formula (IIIa).

16. The crystalline salt of claim 1, having the Formula (IIIb):

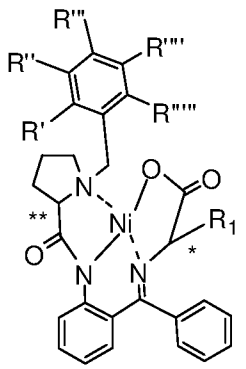


Formula (IIIb).

17. The crystalline salt of any one of claims 13-16, which has an enantiomeric excess of about 95% to 100%.

18. A method of preparing the 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)



Formula (XIb)

from one or more solvents ** is a stereocenter independent of the stereocenter *, 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;

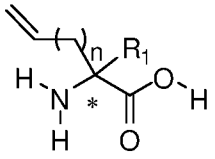
F, F, OiPr, F, F;

F, F, OMe, F, F;

Cl, H, H, H, H; or

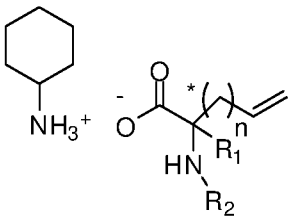
H, H, Me, Me, H;

2) precipitating a compound of Formula (Ia) as a precipitated HCl salt:



Formula (1a);

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



Formula (XIVb); and

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

19. The method of claim 18, comprising performing steps 2, 3, and 4.

20. The method of claim 18, comprising steps 2 and 4.

21. The method of claim 18, wherein crystallization of the metal complex of Formula (XIb) in step 1 is conducted with methyl tertiary-butyl ether.

22. The method of claim 18, wherein crystallization of the metal complex of Formula (XIb) in step 1 is conducted with tetrahydrofuran and methyl tertiary-butyl ether.

23. The method of claim 18, wherein crystallization of the metal complex of Formula (XIb) in step 1 is conducted with isopropyl acetate.

24. The method of claim 18, wherein the precipitated HCl salt in step 2 is further crystallized with acetonitrile.

25. The method of claim 18, wherein the precipitated HCl salt in step 2 is further crystallized with acetonitrile and methyl tertiary-butyl ether.

26. The method of claim 18, wherein the precipitated HCl salt in step 2 is further crystallized with isopropyl acetate.

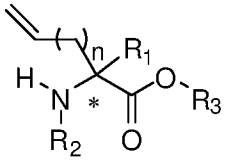
27. The method of claim 18, wherein step 3 is conducted in methyl tertiary-butyl ether.

28. The method of claim 18, wherein step 3 is conducted in acetonitrile.

29. The method of claim 18, wherein step 4 comprises crystallizing a cyclohexyl amine salt of Formula (I) with tetrahydrofuran and methyl tertiary-butyl ether.

30. A method of making a polypeptide, comprising making the polypeptide with the crystalline salt of any of claims 1-17.

31. A crystalline composition comprising a crystalline compound of Formula (I):



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 9-Fluorenylmethoxycarbonyl (Fmoc); and

R_3 is –H; wherein the crystalline composition further comprises an alkane and an halo alkane.

32. The crystalline salt of claim 1, wherein the cyclo alkyl amine is cyclopropylamine, cyclobutylamine, cycopentylamine, cyclohexylamine, cycloheptylamine, or cyclooctylamine.

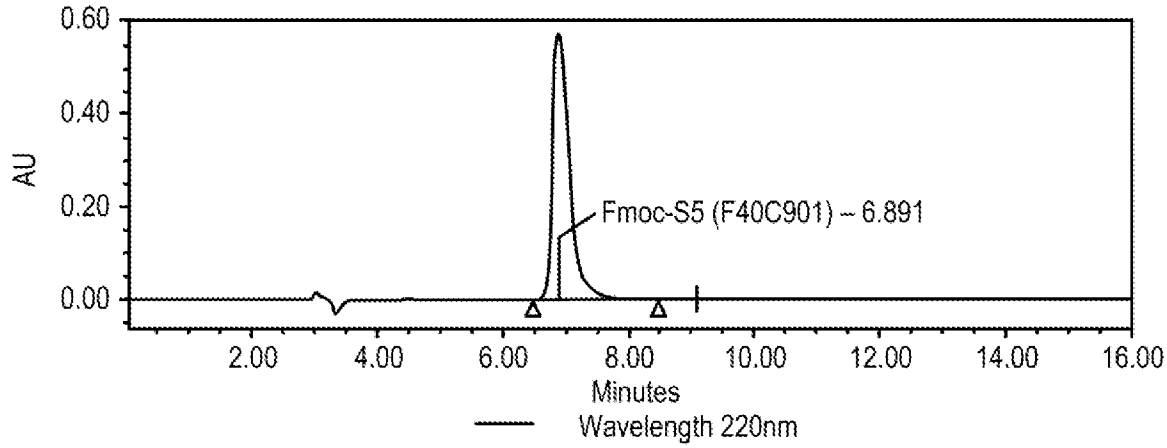
33. The crystalline salt of claim 1, wherein the cyclo alkyl amine is cyclohexylamine.

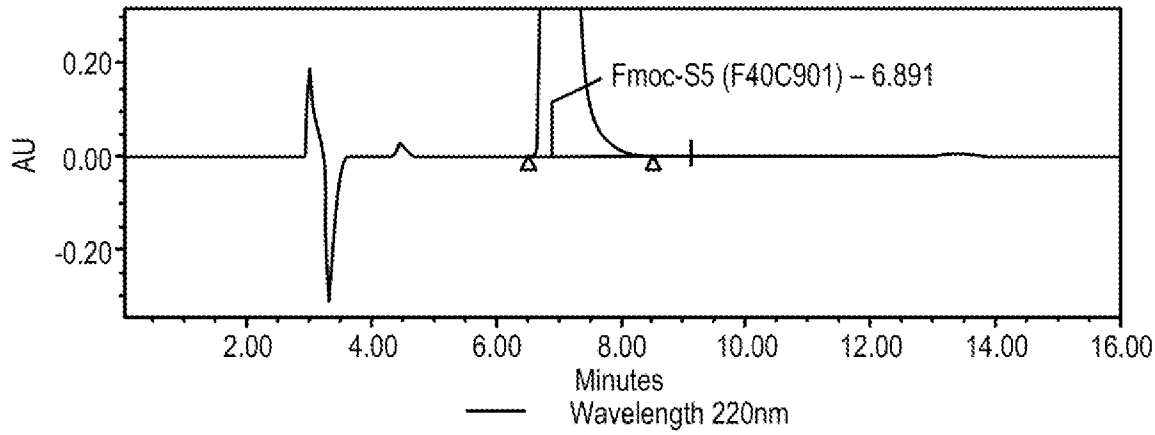
34. The crystalline composition of claim 31, wherein the alkane is hexane.

35. The crystalline composition of claim 31, wherein the haloalkane is chloroform.

Column Used: Chiralpak AD-H QC#167
Vial: 73 Injection: 1
Injection Volume: 5.00 ul
Sample Concentration: 48.0mg/25mL DS
Additional Sample Information: Finished Product

Solvent A: 90%Hex.anes/10%IPA/0.1%TFA
Solvent B: 90%Hex.anes/10%IPA
Solvent C: IPA
Solvent C: EtOH





Results processed by 220nm

	Peak Name	Retention Time	Peak Area	% Peak Area
1	Fmoc-55 (F40C901)	6.891	9858806	100.00

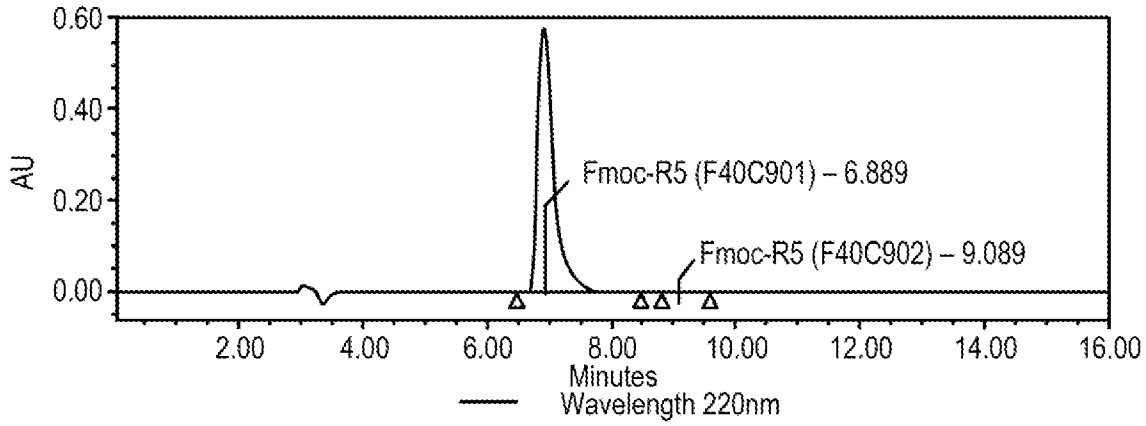
	Peak Name	Retention Time	Peak Area	% Peak Area
2	Fmoc-55 (F40C902)	9.100		

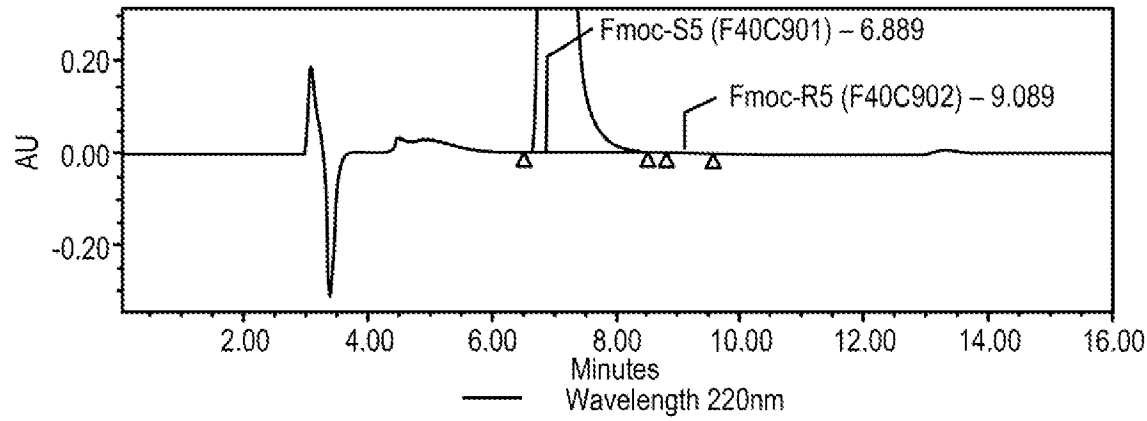
User Name: System Current Date: 12/19/2011 2:19:54 PM 1 of 1

FIG. 1

Column Used: Chiralpak AD-H QC#167
Vial: 74 Injection: 1
Injection Volume: 5.00 ul
Sample Concentration: 48.0mg/25mL DS + 0.5% R5
Additional Sample Information: Sample+0.5% R5

Solvent A: 90%Hex.anes/10%IPA/0.1%TFA
Solvent B: 90%Hex.anes/10%IPA
Solvent C: IPA
Solvent C: EtOH





Results processed by 220nm

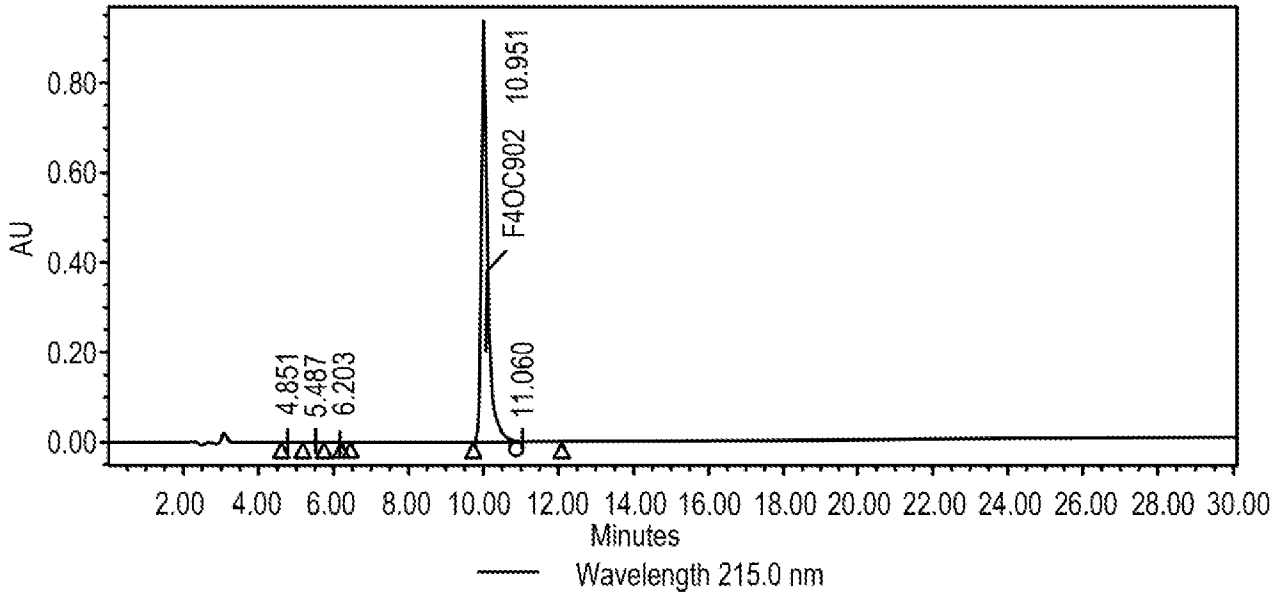
	Peak Name	Retention Time	Peak Area	% Peak Area
1	Fmoc-55 (F40C901)	6.889	9847318	99.83

	Peak Name	Retention Time	Peak Area	% Peak Area
2	Fmoc-55 (F40C902)	9.089	16328	0.17

User Name: System Current Date: 12/19/2011 2:41:41 PM 1 of 1

FIG. 2

Acq Method Set: F40C902_60to80in25min
Column Used: Waters Spherisorb ODS2#131
Date Acquired: 12/20/2011 1:53:06 PM
Vial: 35 Injection: 1
Processing Method F40C902_60to80in25min
Injection Volume: 5.00 ul
Date Processed: 12/20/2011 3:21:49 PM
Sample Concentration: 26.1mg/25mL DS
Additional Sample Information: Sample



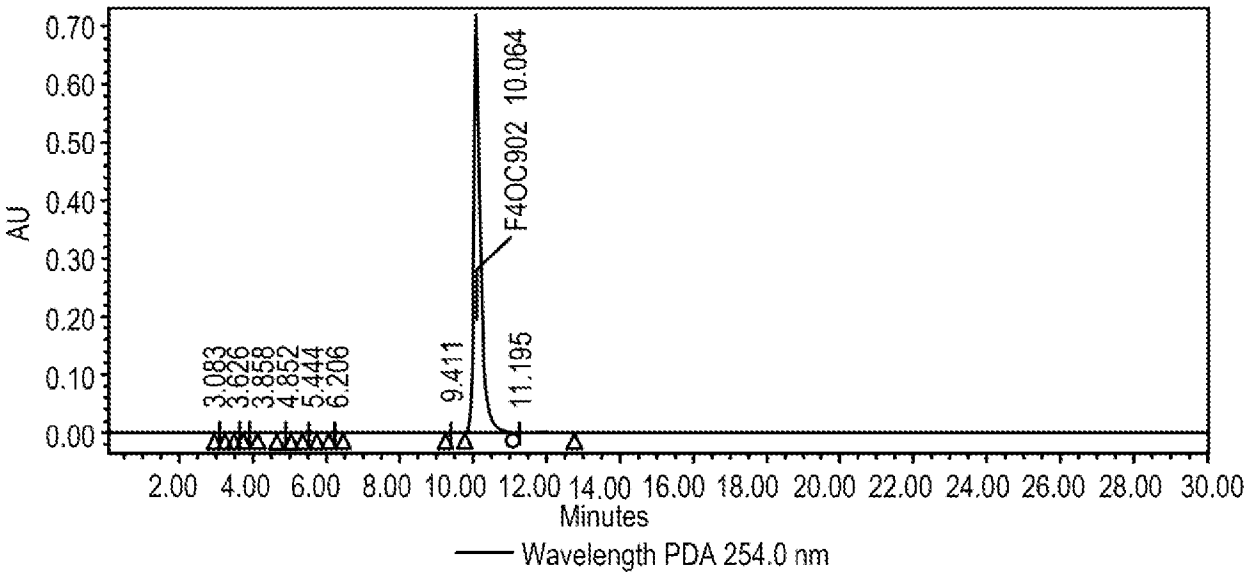
Data from Chromatogram 215nm

	Peak Name	Retention Time	Height (μV)	% Height	Peak Area	% Peak Area
1		4.851	3099	0.33	24138	0.20
2		5.487	1580	0.17	21810	0.18
3		6.203	6569	0.71	62741	9.52
4	F40C902	10.054	925370	98.56	11863328	98.49
5		11.060	2192	0.23	73331	0.61

FIG. 3

Column Used: Waters Spherisorb ODS2#131
Vial: 35 Injection: 1
Injection Volume: 5.00 ul
Date Acquired: 12/20/2011 1:53:06 PM
Processing Method F40C902_60to80in25min
Date Processed: 12/20/2011 3:19:31 PM

Sample Concentration: 26.1mg/25mL DS
Additional Sample Information: Sample

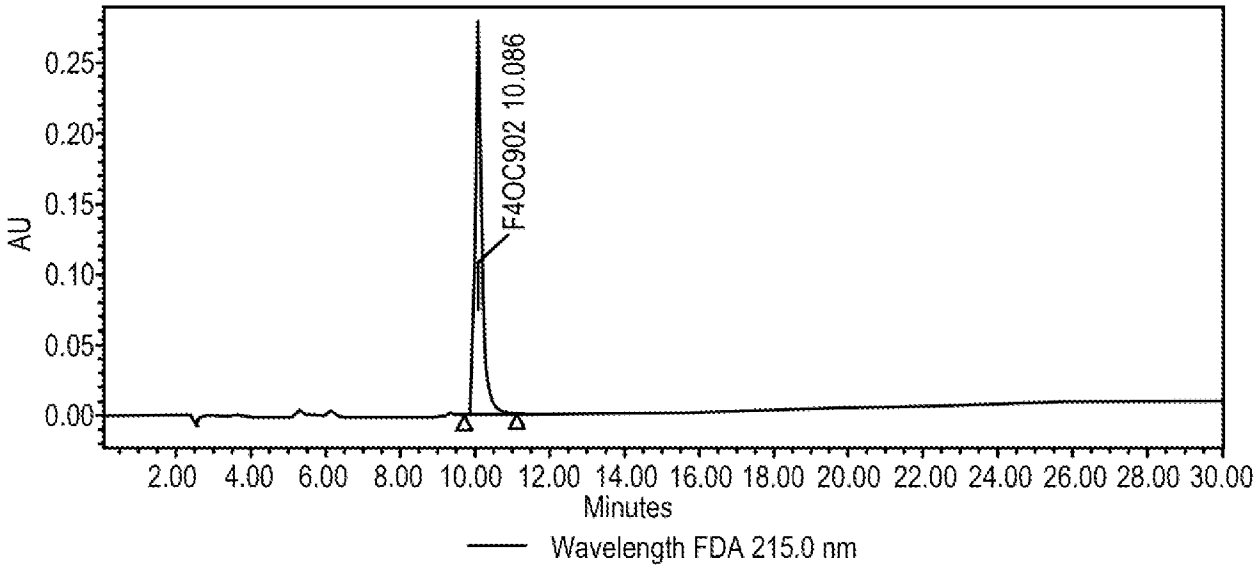


Data from Chromatogram 254nm

	Peak Name	Retention Time	Height (µV)	% Height	Peak Area	% Peak Area
1		3.083	914	0.13	5380	0.06
2		3.626	379	0.05	2548	0.03
3		3.858	612	0.09	7057	0.08
4		4.852	2303	0.32	17314	0.19
5		5.484	967	0.14	8412	0.09
6		6.206	2206	0.31	20714	0.23
7		9.411	1420	0.20	14463	0.16
8	F40C902	10.054	698574	98.50	8845126	98.36
9		11.195	1818	0.26	71822	0.80

FIG. 4

Column Used: Waters Spherisorb ODS2 #131
Vial: 36 Injection: 1
Injection Volume: 5.00 ul
Date Acquired: 12/20/2011 2:44:05 PM
Processing Method F40C902_60to80in25min
Date Processed: 12/20/2011 3:24:26 PM
Sample Concentration: 0.5mg/25mL DS
Additional Sample Information: Customer Control



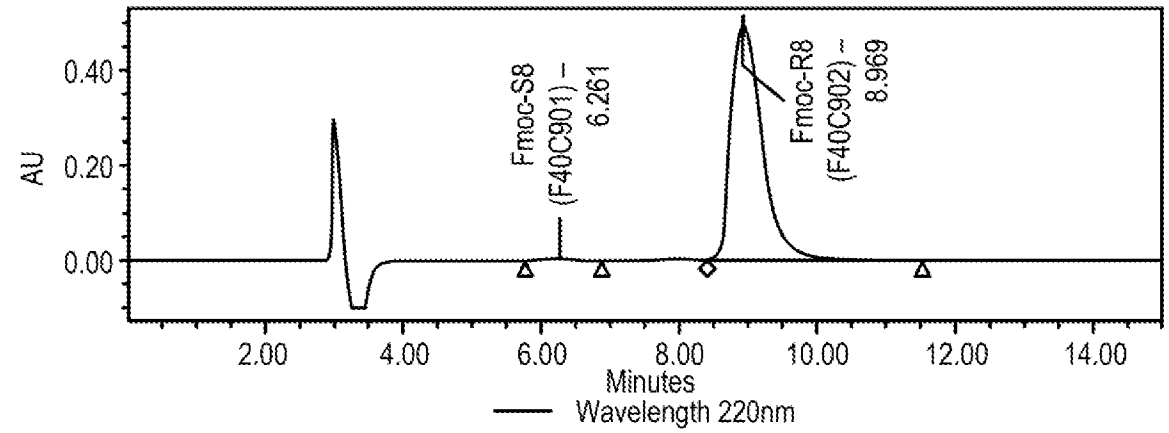
Data from Chromatogram 215nm

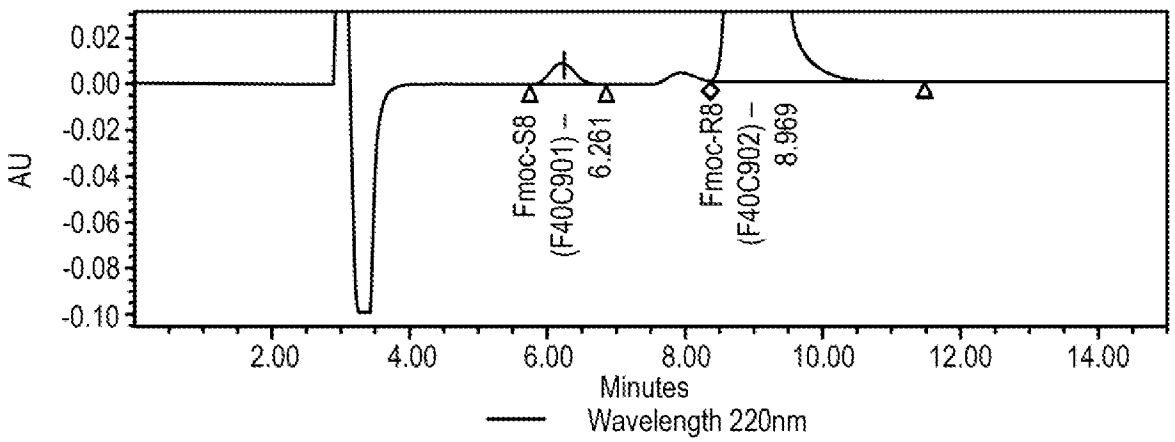
	Peak Name	Retention Time	Height (µV)	% Height	Peak Area	% Peak Area
1	F40C902	10.086	273598	100.00	3562385	100.05

FIG. 5

Column Used: CHiralpak AD-H QC#167
Vial: 86 Injection: 1
Injection Volume: 20.00 ul
Sample Concentration: 25.5mg (N2 Dried)/25mL IPA
Additional Sample Information:
N-Fmoc-(R)-a-Me-a-Aminodec-9-Enoic Acid; Sample; 100% MP-A; 1.0mL/min; 25 C; 730 psi;

Solvent A: 90%Hexanes/10%IPA/0.1%TFA
Solvent B: 90%Hexanes/10%IPA
Solvent C: IPA
Solvent D: EtOH





Results processed by 220nm

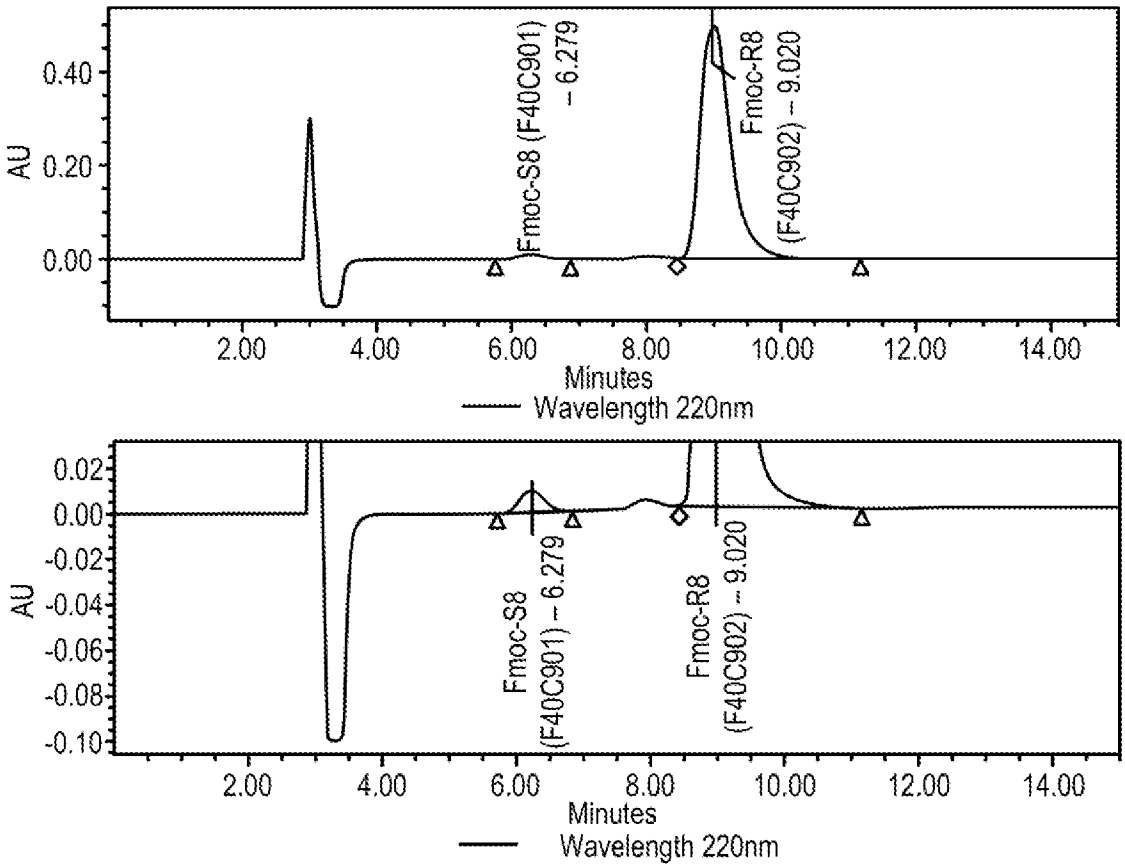
	Peak Name	Retention Time	Peak Area	% Peak Area
1	Fmoc-S8 (F40C901)	6.261	215326	1.37

	Peak Name	Retention Time	Peak Area	% Peak Area
2	Fmoc-S8 (F40C902)	8.969	15530958	98.63

FIG. 6

Column Used: CHiralpak AD-H QC#167
Vial: 87 Injection: 1
Injection Volume: 20.00 ul
Sample Concentration: 50uL S8 RSTD/10mL Sx Soln.
Additional Sample Information:
N-Fmoc-(R)-a-Me-a-Aminodec-9-Enoic Acid; Sample; 100% MP-A; 1.0mL/min; 25 C; 730 psi;

Date Processed: 11/17/2011 7:38:20 AM
Solvent A: 90%Hexanes/10%IPA/0.1%TFA
Solvent B: 90%Hexanes/10%IPA
Solvent C: IPA
Solvent D: EtOH



Results processed by 220nm

	Peak Name	Retention Time	Peak Area	% Peak Area
1	Fmoc-S8 (F40C901)	6.279	236343	1.48
2	Fmoc-S8 (F40C902)	9.020	15717883	98.52

User Name: System Current Date: 11/17/2011 7:38:49 PM 1 of 1

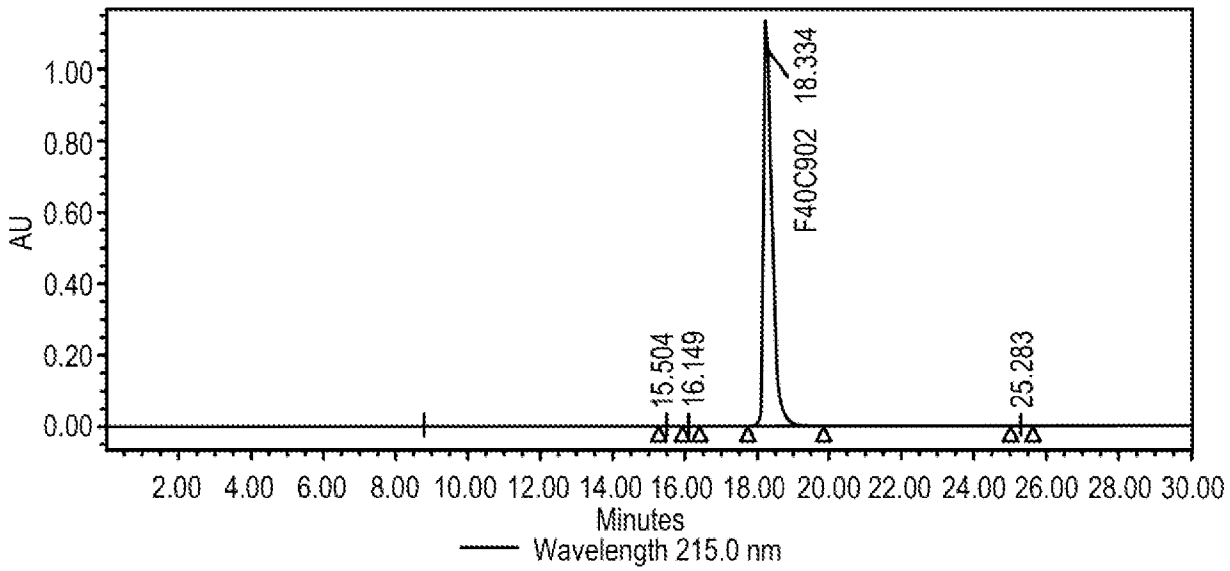
FIG. 7

Column Used: Waters Spherisorb ODS2 #131
Vial: 46 Injection: 1
Injection Volume: 5.00 ul

Date Processed: 11/16/2011 7:19:45 PM
Processing Method F40C902_60to80in25min
Date Processed: 11/17/2011 4:04:32 PM

Sample Concentration: 14.6mg/10mL DS

Additional Sample Information: N-Fmoc-(R)-a-Me-a-Aminodec-9-enoic Acid; FP;

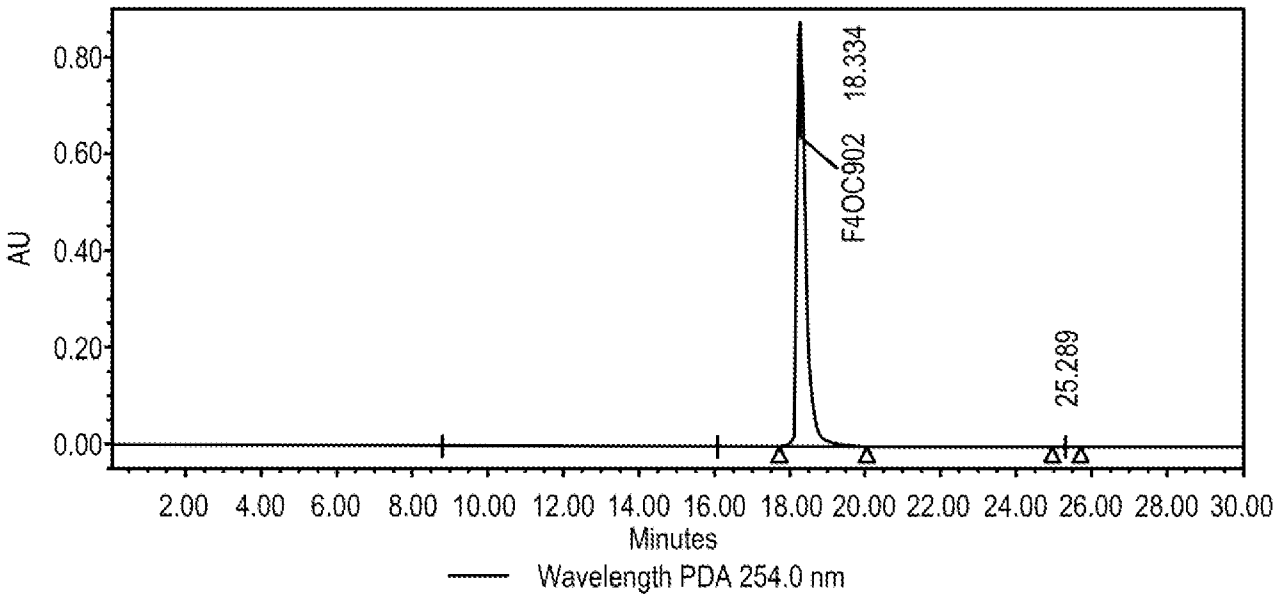


	Peak Name	Retention Time	Height (µV)	% Height	Peak Area	% Peak Area
1	Fmoc-OnSu	8.800				
2		15.504	494	0.04	9432	0.05
3	40C902	16.100				
4		16.149	1013	0.09	13695	0.08
5	F40C902	18.334	1106791	99.81	17679588	99.82
6		25.283	552	0.05	8025	0.05

FIG. 8

Column Used: Waters Spherisorb ODS2 #131
Vial: 46 Injection: 1
Injection Volume: 5.00 ul
Date Acquired: 11/16/2011 7:19:45 PM
Processing Method F40C902_60to80in25min
Date Processed: 11/17/2011 6:11:21 PM

Sample Concentration: 14.6mg/10mL DS
Additional Sample Information:
N-Fmoc-(R)-a-Me-a-Aminodec-9-enoic Acid; FP;



Data from Chromatogram 254nm

	Peak Name	Retention Time	Height (µV)	% Height	Peak Area	% Peak Area
1	Fmoc-OnSu	8.800				
2	40C902	16.100				
3	F40C902	18.334	856815	99.96	13404558	99.95
4		25.289	382	0.04	7053	0.05

FIG. 9