purification of the resultant product (for example by reverse phase liquid chromatography or reverse osmosis).

[0008] There is a need for an efficient method of producing AMG 416, or a pharmaceutically acceptable salt thereof (e.g., AMG 416 HCl), and particularly one appropriate for commercial scale manufacturing.

SUMMARY

[0009] In view of the above-described problems, it is an objective of the disclosure to provide a method for preparing AMG 416, or a pharmaceutically acceptable salt thereof, among other things.

[0010] In one embodiment, provided is a method for preparing AMG 416, the method comprising: providing a resin-bound peptide having a structure selected from the group consisting of Fmoc-D-Cys(Trt)-D-Ala-D-Arg(Pbf)-D-Arg(Pbf)-D-Arg(Pbf)-D-Ala-D-Arg(Pbf)-[Resin] (SEQ ID NO:2) and Ac-D-Cys(Trt)-D-Ala-D-Arg(Pbf)-D-Arg(Pbf)-D-Arg(Pbf)-D-Ala-D-Arg(Pbf)-[Resin] (SEQ ID NO:3); cleaving the peptide from the solid support; and activating the side chain of the D-Cys residue of the cleaved peptide.

[0011] In one or more embodiments related to the embodiment disclosed in [0010], the cleaving and the activating steps occur in the same vessel.

[0012] In one or more further embodiments, the resin-bound peptide is contacted with a solution comprising water, trifluoroacetic acid, triisopropylsilane and dipyridyldisulfide.

[0013] In a first aspect, provided is a method for preparing AMG 416 or a pharmaceutically acceptable salt thereof, the method comprising: contacting a peptide having a structure of Ac-D-Cys(SPy)-D-Ala-D-Arg-D-Arg-D-Arg-D-Ala-D-Arg-NH_2 (SEQ ID NO:4)with L-Cys to produce a conjugated product of formula Ac-c(C)arrrar-NH_2 (SEQ ID NO: 1), or a pharmaceutically acceptable salt thereof.

[0013a] In a second aspect, provided is a method for preparing AMG 416 or a pharmaceutically acceptable salt thereof: the method comprising purifying by HPLC a peptide having the structure Ac-D-Cys(SPy)-D-Ala-D-Arg-D-Arg-D-Arg-D-Ala-D-Arg-NH_2 (SEQ ID NO:4) in a solution of TFA; performing solvent exchange by azeotropic distillation on the purified peptide; and contacting the purified peptide with L-Cys to produce a conjugated product of formula Ac-c(C)arrrar-NH_2 (SEQ ID NO: 1) .

[0013b] In another embodiment, provided is a method for synthesizing D-Arg(Pbf)-OH comprising: converting Boc-D-Arg-OH to Boc-D-Arg(Pbf)-OH by mixing the Boc-D-Arg-OH with PbfCl, NaOH and NaI.

[0014] In some embodiments related to the first aspect, the peptide is contacted with an aqueous solution comprising L-Cys and trifluoroacetic acid.

[0015] In some further embodiment related to the first aspect, the method further comprises lyophilizing the conjugated product.

[0016] In yet some further embodiments, the method of the first aspect further comprises contacting the conjugated product with an aqueous solution comprising isopropyl alcohol (IPA) and hydrochloric acid (HCl), thereby producing a precipitate comprising AMG 416 HCl.

[0017] In yet one or more further embodiments related to the first aspect, the method further comprises purifying the precipitate by high performance liquid chromatography (HPLC).

[0018] In yet another embodiment provided is a method for preparing AMG 416, the method comprising: providing a resin-bound peptide having a structure selected from the group consisting of Fmoc-D-Cys(Trt)-D-Ala-D-Arg(Pbf)-D-Arg(Pbf)-D-Arg(Pbf)-D-Ala-D-Arg(Pbf)-[Resin] (SEQ ID NO:2) and Ac-D-Cys(Trt)-D-Ala-D-Arg(Pbf)-D-Arg(Pbf)-D-Arg(Pbf)-D-Ala-D-Arg(Pbf)-[Resin] (SEQ ID NO:3); cleaving the peptide from the solid support, i.e., to provide an unsupported peptide, and activating the side chain of the D-Cys residue of the unsupported peptide to generate an AMG 416 SPy intermediate (where SPy is 2-pyridinesulfenyl or S-Pyr), dissolving the AMG 416 SPy intermediate in an aqueous 0.1% TFA (trifluoroacetic acid solution), and purifying the AMG 416 SPy derivative by HPLC.

What is claimed is:

1. A method for preparing etelcalcetide (AMG 416) or a pharmaceutically acceptable salt thereof, the method comprising:

contacting a peptide having a structure of Ac-D-Cys(SPy)-D-Ala-D-Arg-D-Arg-D-Arg-D-Ala-D-Arg-NH_2 (SEQ ID NO:4)

with L-Cys to produce a conjugated product of formula Ac-c(C)arrrar-NH_2 (SEQ ID NO: 1), or a pharmaceutically acceptable salt thereof.

2. The method of claim 1, wherein said contacting comprises dissolving the peptide in an aqueous solution comprising L-Cys and trifluoroacetic acid (TFA).

3. The method of claim 1, further comprising lyophilizing the conjugated product.

4. The method of claim 1, further comprising contacting the conjugated product with an aqueous solution comprising isopropyl alcohol (IPA) and HCl, thereby producing a precipitate comprising AMG 416 HCl .

5. The method of claim 4, further comprising purifying the precipitate by HPLC.

6. The method of claim 2, wherein said contacting occurs at room temperature for about 15 minutes.

7. The method of claim 3, further comprising contacting the conjugated product with an aqueous solution comprising IPA and HCl, thereby producing a precipitate comprising AMG 416 HCl.

8. The method of claim 7, further comprising washing the precipitate with isopropyl alcohol.

9. The method of claim 8, further comprising dissolving the isopropyl alcohol washed precipitate in water to produce a dissolved precipitate.

10. The method of claim 9, further comprising concentrating the dissolved precipitate via distillation.

11. The method of claim 1, wherein the method further comprises deriving the peptide having structure Ac-D-Cys(SPy)-D-Ala-D-Arg-D-Arg-D-Arg-D-Ala-D-Arg-NH_2 (SEQ ID NO:4) from a resin-bound peptide having a structure of Ac-D-Cys(Trt)-D-Ala-D-Arg(Pbf)-D-Arg(Pbf)-D-Arg(Pbf)-D-Ala-D-Arg(Pbf)-[Resin] (SEQ ID NO:3).

12. The method of claim 11, wherein the deriving comprises contacting the resin-bound peptide with a solution comprising water, trifluoroacetic acid, triisopropylsilane and dipyridyldisulfide.

13. The method of claim 1, wherein the peptide comprises a TFA salt of Ac-D-Cys(SPy)-D-Ala-D-Arg-D-Arg-D-Arg-D-Ala-D-Arg-NH_2 (SEQ ID NO:4).

14. A method for preparing etelcalcetide (AMG 416) or a pharmaceutically acceptable salt thereof, the method comprising:

purifying by HPLC a peptide having the structure Ac-D-Cys(SPy)-D-Ala-D-Arg-D-Arg-D-Arg-D-Ala-D-Arg-NH_2 (SEQ ID NO:4) in a solution of TFA;

performing solvent exchange by azeotropic distillation on the purified peptide; and

contacting the purified peptide with L-Cys to produce a conjugated product of formula Ac-c(C)arrrar-NH_2 (SEQ ID NO: 1).

15. The method of claim 14, wherein L-Cys is in a solution of water and IPA.

16. The method of claim 15, further comprising contacting the conjugated product with an aqueous solution comprising IPA and HCl, thereby producing a precipitate comprising AMG 416 HCl.

17. The method of claim 16, further comprising lyophilizing the precipitate.