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 a first aspect, 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 first aspect, 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 second aspect, provided is a method for preparing AMG 416, the method comprising: providing 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); and contacting the peptide with L-Cys to produce a conjugated product.

[0013a] In another aspect, provided is a method for preparing AMG 416: the method comprising providing 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; purifying the peptide by HPLC; performing solvent exchange by azeotropic distillation; and contacting the peptide with L-Cys to produce a conjugated product.

[0013b] In another aspect, 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 second aspect, the peptide is contacted with an aqueous solution comprising L-Cys and trifluoroacetic acid.

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

[0016] In yet some further embodiments, the method of the second 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 second aspect, the method further comprises purifying the precipitate by high performance liquid chromatography (HPLC).

[0018] In yet ~~a third aspect~~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.

[0019] In some embodiments related to the method disclosed in paragraph [0018]~~third aspect~~, the method further comprises azeotropic distillation of the AMG 416 SPy intermediate, to thereby effect a solvent exchange to produce a solution of the AMG 416 SPy in the new solvent, e.g., water and isopropyl alcohol.

[0020] In yet some additional embodiments related to the method disclosed in paragraph [0018]~~third aspect~~, the method further comprises contacting the isopropyl alcohol – water solution of the AMG 416 SPy, in some embodiments in the form of its trifluoroacetate salt, with an aqueous solution comprising L-Cys.

[0021] In ~~a fourth aspect~~another embodiment, provided is a method for preparing H-D-Arg(Pbf)-OH, i.e., a suitable starting material for certain of the synthetic methods provided herein.

[0022] In some embodiments related to the method disclosed in paragraph [0021]~~fourth aspect~~, the method comprises converting Boc-D-Arg-OH to Boc-D-Arg(Pbf)-OH in the presence of NaI (sodium iodide). In yet some further embodiments, the sodium iodide is present at a concentration of about 5% mol.

[0023] In some additional embodiments related to the method disclosed in paragraph [0021]~~fourth aspect~~, the method further comprises converting Boc-D-Arg(Pbf)-OH to D-Arg(Pbf)-OH, and crystallizing the D-Arg(Pbf)-OH in an IPA/water solvent system.

[0024] Additional embodiments of the methods described herein will be apparent from the following description, examples, and claims. As can be appreciated from the foregoing and following description, each and every feature described herein, and each and every combination of two or more of such features, is included within the scope of the present disclosure provided that the features included in such a combination are not mutually inconsistent. In addition, any feature or combination of features may be specifically excluded from any embodiment of the present invention

BRIEF DESCRIPTION OF THE DRAWINGS

[0025] FIG. 1 shows the chemical structure of AMG 416 (Ac-D-Cys(L-Cys-OH)-D-Ala-D-Arg-D-Arg-D-Arg-D-Ala-D-Arg-NH_2) (SEQ ID NO:1).

[0026] FIG. 2 shows the chemical structure of Rink Amide AM resin 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).

[0027] FIG. 3 shows a reaction scheme in which the SPy intermediate product (Ac-D-Cys(SPy)-D-Ala-D-Arg-D-Arg-D-Arg-D-Ala-D-Arg-NH_2) (SEQ ID NO:4) is formed from the peptidyl-resin (Ac-D-Cys(Trt)-D-Ala-D-Arg(Pbf)-D-Arg(Pbf)-D-Arg(Pbf)-D-Ala-D-Arg(Pbf)-NH-Resin) (SEQ ID NO:3).

[0028] FIG. 4 shows a reaction scheme in which a TFA salt of AMG 416 is formed from the SPy intermediate ($AA^{1\text{-}7(SPy)}$).

[0029] FIG. 5 shows a reaction scheme in which the HCl salt of AMG 416 is formed from the TFA salt of AMG 416.

[0030] FIG. 6 shows a reaction scheme in which Boc-D-Arg(Pbf)-OH is formed from Boc-D-Arg-OH.

[0031] FIG. 7 shows a reaction scheme in which D-Arg(Pbf)-OH is formed from Boc-D-Arg(Pbf)-OH.