COMPOUNDS TARGETING FIBROBLAST-ACTIVATION PROTEIN AND METHODS OF USE THEREOF

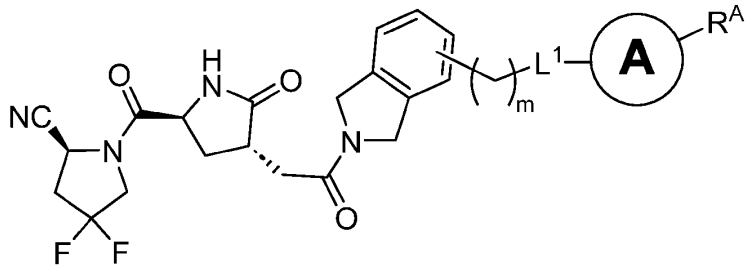
CROSS-REFERENCE

[0001] This application claims priority to U.S. Provisional Application Number 63/551,362 filed February 8, 2024, the contents of which are incorporated herein by reference.

SUMMARY

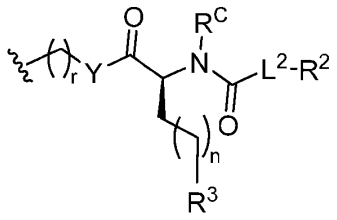
[0002] The present disclosure provides, in certain embodiments, compounds such as complexes that are chelated or covalently bound to therapeutic or diagnostic radionuclides (e.g., a chelated complex represented by Formula (I), e.g., a chelated complex represented by Formula (I-A), Formula (I-B), or Formula (I-C)) and their methods of use in the treatment of a disease (e.g., a disease described herein) and/or imaging. Also described herein, in certain embodiments, are non-chelated compounds of the complexes described herein (e.g., a compound represented by Formula (I), e.g., a compound represented by Formula (I-A), Formula (I-B), or Formula (I-C)).

[0003] In certain embodiments, provided herein is a chelated complex represented by Formula (I):

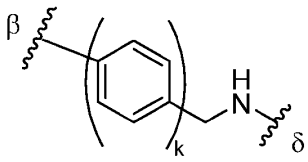


Formula (I)

or a pharmaceutically acceptable salt thereof, wherein: ring A is selected from the group consisting of 3 to 6-membered heterocyclyl, 6-membered heteroaryl, and C_{3-6} cycloalkyl; L^1 is selected from a covalent bond and a linker; R^A is a moiety represented by:

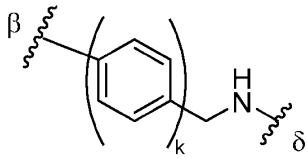


Y is a covalent bond or NR^D; R^C is selected from hydrogen and C_1-C_3 alkyl; R^D is selected from hydrogen and C_1-C_3 alkyl; L^2 is selected from the group consisting of: a covalent bond, and a moiety represented by:



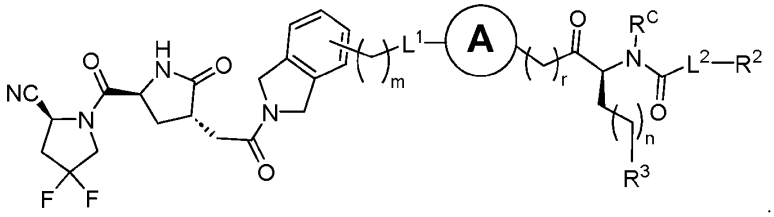
wherein β represents the point of attachment to the carbonyl group and δ represents the point of attachment to R^2; each of R^2 and R^3 is independently selected from an albumin binding ligand and a ligand chelated or covalently linked to a therapeutic radionuclide or a diagnostic radionuclide, wherein at least one of R^2 and R^3 is a therapeutic radionuclide or a diagnostic radionuclide; each of k, m, and r is independently 0 or 1; and n is an integer from 1 to 6; provided that:

when L^1 is a covalent bond, m is 1, L^2 is a moiety represented by:



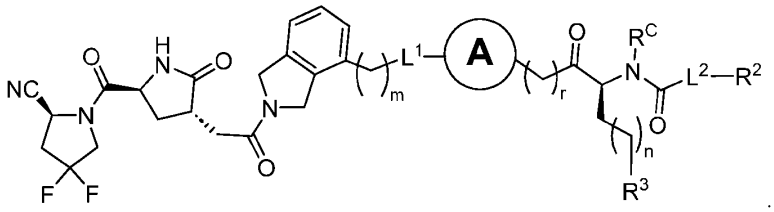
, and A is piperazinyl having a ring nitrogen attached to L^1, then k is 0.

[0004] In some embodiments, the complex is represented by Formula (I-A):



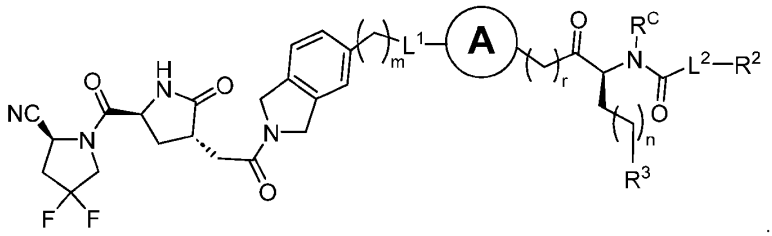
Formula (I-A).

[0005] In some embodiments, the complex is represented by Formula (I-B):



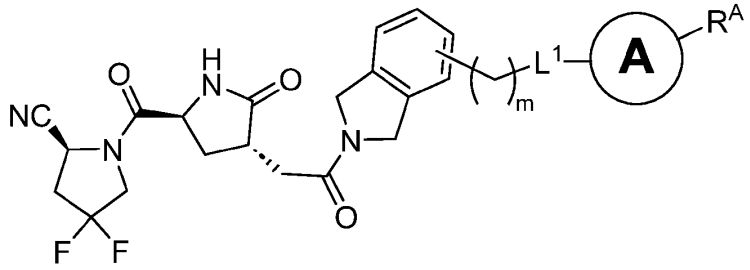
Formula (I-B).

[0006] In some embodiments, the complex is represented by Formula (I-C):



Formula (I-C).

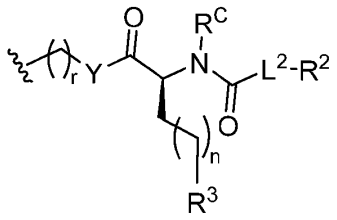
[0007] In certain embodiments, provided herein is a compound represented by Formula (I):



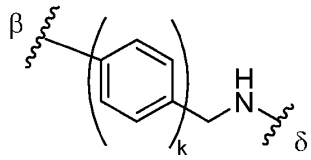
Formula (I)

or a pharmaceutically acceptable salt thereof, wherein:

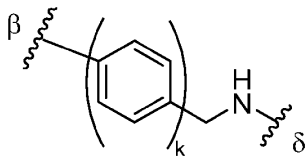
ring A is selected from the group consisting of 3 to 6-membered heterocyclyl, 6-membered heteroaryl, and C_{3-6} cycloalkyl; L^1 is selected from a covalent bond and a linker; R^A is a moiety represented by:



Y is a covalent bond or NR^D; R^C is selected from hydrogen and C_1-C_3 alkyl; R^D is selected from hydrogen and C_1-C_3 alkyl; L^2 is selected from the group consisting of: a covalent bond, and a moiety represented by:

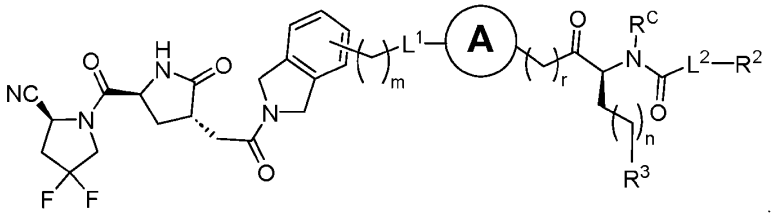


wherein β represents the point of attachment to the carbonyl group and δ represents the point of attachment to R^2; each of R^2 and R^3 is independently selected from an albumin binding ligand and an unchelated ligand capable of chelating to or covalently bonding to a therapeutic radionuclide or a diagnostic radionuclide wherein at least one of R^2 and R^3 is an unchelated ligand capable of chelating to or covalently bonding to a therapeutic radionuclide or a diagnostic radionuclide; each of k, m, and r is independently 0 or 1; and n is an integer from 1 to 6; provided that: when L^1 is a covalent bond, m is 1, L^2 is a moiety represented by:



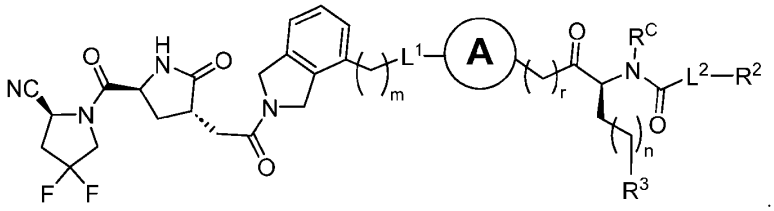
, and A is piperazinyl having a ring nitrogen attached to L^1, then k is 0.

[0008] In some embodiments, the compound is represented by Formula (I-A):



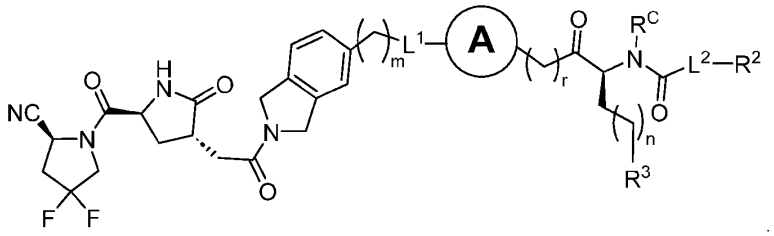
Formula (I-A).

[0009] In some embodiments, the compound is represented by Formula (I-B):



Formula (I-B).

[00010] In some embodiments, the compound is represented by Formula (I-C):



Formula (I-C).

[00011] In certain embodiments, provided herein is a pharmaceutical composition comprising a compound described herein (e.g., a complex represented by Formula (I) or compound represented by Formula (I)), or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

[00012] In certain embodiments, disclosed herein is a method of treating a disease (e.g., a disease described herein) in a patient in need thereof, comprising administering to the patient a therapeutically effective amount of a compound described herein (e.g., a complex represented by Formula (I)).

[00013] Additionally disclosed herein, in certain embodiments, is a method of imaging a cancer-associated fibroblast in a patient in need thereof, comprising administering to the patient an effective amount of a compound described herein (e.g., a complex represented by Formula (I)), and identifying the location of the cancer-associated fibroblast in the patient.

BRIEF DESCRIPTION OF THE DRAWINGS

[00014] **FIG. 1A** depicts a radiochromatogram of ^{177}Lu- Compound 9. **FIG. 1B** depicts a radio-reverse phase HPLC chromatogram of ^{177}Lu-Compound 9.

[00015] **FIG. 2A** depicts a radiochromatogram of ^{177}Lu-Compound 7. **FIG. 2B** depicts an iTLC chromatogram of ^{177}Lu-Compound 7.

[00016] **FIG. 3A** depicts a radiochromatogram of ^{177}Lu- Compound 6. **FIG. 3B** depicts an iTLC chromatogram of ^{177}Lu- Compound 6.

[00017] **FIG. 4A** depicts a radiochromatogram of ^{177}Lu-Compound 5. **FIG. 4B** depicts an iTLC chromatogram of ^{177}Lu- Compound 5.

[00018] **FIG. 5A** depicts a radiochromatogram of ^{177}Lu- Compound 4. **FIG. 5B** depicts an iTLC chromatogram of ^{177}Lu-Compound 4.

[00019] **FIG. 6A** depicts a radio-reverse phase HPLC chromatogram of ^{177}Lu-Compound 13. **FIG. 6B** depicts a radio-reverse phase HPLC chromatogram of ^{177}Lu-Compound 13.

[00020] **FIG. 7** depicts a radio-reverse phase HPLC chromatogram of ^{177}Lu-Compound 18.

[00021] **FIG. 8** depicts a radio-reverse phase HPLC chromatogram of ^{177}Lu-Compound 24.

[00022] **FIG. 9** depicts a radio-reverse phase HPLC chromatogram of ^{177}Lu-Compound 15.

[00023] **FIG. 10** depicts a radio-reverse phase HPLC chromatogram of ^{177}Lu-Compound 23.

[00024] **FIG. 11** depicts a radio-reverse phase HPLC chromatogram of ^{177}Lu-Compound 8.

[00025] **FIG. 12** depicts an iTLC chromatogram of ^{177}Lu-Compound 8.

[00026] **FIG. 13** depicts a radio-reverse phase HPLC chromatogram of ^{177}Lu-Compound 2.

[00027] **FIG. 14** depicts a radio-reverse phase HPLC chromatogram of ^{177}Lu-Compound 10.

[00028] **FIG. 15** depicts a radio-reverse phase HPLC chromatogram of ^{177}Lu-Compound 21.

[00029] **FIG. 16** depicts a radio-reverse phase HPLC chromatogram of ^{177}Lu-Compound 22.

[00030] **FIG. 17** depicts a radio-reverse phase HPLC chromatogram of ^{177}Lu-Compound 17.

[00031] **FIG. 18** depicts a radio-reverse phase HPLC chromatogram of ^{177}Lu-Compound 19.

[00032] **FIG. 19** depicts a radio-reverse phase HPLC chromatogram of ^{177}Lu-Compound 20.

[00033] **FIG. 20** depicts SPECT/CT scans at 3 hours, 24 hours, 48 hours, 96 hours, and 192 hours post injection of ^{177}Lu-Compound 4 using Balb/c mice bearing U87MG tumors.

[00034] **FIG. 21** depicts SPECT/CT scans at 3 hours, 24 hours, 48 hours, 96 hours, and 192 hours post injection of ^{177}Lu-Compound 7 using Balb/c mice bearing U87MG tumors.

[00035] **FIG. 22** depicts SPECT/CT scans at 3 hours, 24 hours, 48 hours, 96 hours, and 192 hours post injection of ^{177}Lu-Compound 8 using Balb/c mice bearing U87MG tumors.

[00036] **FIG. 23** depicts SPECT/CT scans at 3 hours, 24 hours, 48 hours, 96 hours, and 192 hours post injection of ^{177}Lu-Compound 9 using Balb/c mice bearing U87MG tumors.

[00037] **FIG. 24** depicts SPECT/CT scans at 3 hours, 24 hours, 48 hours, 96 hours, and 192 hours post injection of ^{177}Lu-Compound 13 using Balb/c mice bearing U87MG tumors.

[00038] **FIG. 25** depicts SPECT/CT scans at 3 hours, 24 hours, 48 hours, 96 hours, and 192 hours post injection of ^{177}Lu-Compound 15 using Balb/c mice bearing U87MG tumors.

[00039] **FIG. 26** depicts SPECT/CT scans at 3 hours, 24 hours, 48 hours, 96 hours, and 192 hours post injection of ^{177}Lu-Compound 23 using Balb/c mice bearing U87MG tumors.

[00040] **FIG. 27** depicts SPECT/CT scans at 3 hours, 24 hours, 48 hours, 96 hours, and 192 hours post injection of ^{177}Lu-Compound 24 using Balb/c mice bearing U87MG tumors.

DETAILED DESCRIPTION

Definitions

[00041] As used herein the specification, "a" or "an" may mean one or more. As used herein, when used in conjunction with the word "comprising", the words "a" or "an" may mean one or more than one. As used herein "another" may mean at least a second or more. Still further, the terms "having", "including", "containing" and "comprising" are interchangeable and one of skill in the art is cognizant that these terms are open ended terms. Some embodiments of the disclosure may consist of or consist essentially of one or more elements, method steps, and/or methods of the disclosure. It is contemplated that any method, compound, or composition described herein can be implemented with respect to any other method, compound, or composition described herein.

[00042] "About" and "approximately" shall generally mean an acceptable degree of error for the quantity measured given the nature or precision of the measurements. Exemplary degrees of error are within 20 percent (%), typically, within 10%, and more typically, within 5% of a given value or range of values.

[00043] As used herein, "alkyl" refers to a radical of a straight–chain or branched saturated hydrocarbon group, *e.g.*, having 1 to 20 carbon atoms ("$C_{1–20}$ alkyl"). In some embodiments, an alkyl group has 1 to 10 carbon atoms ("$C_{1–10}$ alkyl"). In some embodiments, an alkyl group has 1 to 9 carbon atoms ("$C_{1–9}$ alkyl"). In some embodiments, an alkyl group has 1 to 8 carbon atoms ("$C_{1–8}$ alkyl"). In some embodiments, an alkyl group has 1 to 7 carbon atoms ("$C_{1–7}$ alkyl"). In some embodiments, an alkyl group has 1 to 6 carbon atoms ("$C_{1–6}$ alkyl"). In some embodiments, an alkyl group has 1 to 5 carbon atoms ("$C_{1–5}$ alkyl"). In some embodiments, an alkyl group has 1 to 4 carbon atoms ("$C_{1–4}$ alkyl"). In some embodiments, an alkyl group has 1 to 3 carbon atoms ("$C_{1–3}$ alkyl"). In some embodiments, an alkyl group has 1 to 2 carbon atoms ("C_{1-2} alkyl"). In some embodiments, an alkyl group has 1 carbon atom ("C_1 alkyl"). Examples of $C_{1–6}$ alkyl groups include methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, hexyl, and the like. In some embodiments, "alkyl" is unsubstituted or substituted with one or more substituents as described herein.

[00044] As used herein, "cycloalkyl" refers to a monovalent saturated cyclic, bicyclic, or bridged cyclic (e.g., adamantyl) hydrocarbon group of 3-12, 3-10, 3-8, 4-8, or 4-6 carbons ("C_{3-10}cycloalkyl") derived from a cycloalkane. Exemplary cycloalkyl groups include, but are not limited to, cyclohexanes, cyclopentanes, cyclobutanes and cyclopropanes. A some embodiments, a cycloalkyl group is a monovalent saturated cyclic hydrocarbon group of 3-6 carbons ("C_{3-6} cycloalkyl").

[00045] As used herein, "halogen" refers to -F, -Cl, -Br, or -I.

[00046] As used herein, "heterocyclyl" refers to a radical of a 3– to 10– membered non–aromatic ring system having ring carbon atoms and 1 to 4 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("3–10 membered heterocyclyl"). In some embodiments, in heterocyclyl groups that contain one or more nitrogen atoms, the point of attachment is a carbon or nitrogen atom, as valency permits. In some embodiments, a heterocyclyl group is either be monocyclic ("monocyclic heterocyclyl") or a fused, bridged or spiro ring system such as a bicyclic system ("bicyclic heterocyclyl"), and is saturated or is partially unsaturated. In some embodiments, heterocyclyl bicyclic ring systems include one or more heteroatoms in one or both rings. "Heterocyclyl" also includes ring systems wherein the heterocyclyl ring, as defined above, is fused with one or more cycloalkyl groups wherein the point of attachment is either on the cycloalkyl or heterocyclyl ring, or ring systems wherein the heterocyclyl ring, as defined above, is fused with one or more phenyl or heteroaryl groups, wherein the point of attachment is on the heterocyclyl ring, and in such instances, the number of ring members continue to designate the number of ring members in the heterocyclyl ring system. The terms "heterocycle," "heterocyclyl," "heterocyclyl ring," "heterocyclic group," "heterocyclic moiety," and "heterocyclic radical," are used interchangeably. In some embodiments, a heterocyclyl group is a 3-6 membered non-aromatic ring system having ring carbon atoms and 1-4 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("3 to 6-membered heterocyclyl"). In some embodiments, a heterocyclyl group is a 6 membered non-aromatic ring system having ring carbon atoms and 1-4 ring heteroatoms, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("6-membered heterocyclyl").

[00047] As used herein, "heteroaryl" refers to a radical of a 5–10 membered monocyclic or bicyclic 4n+2 aromatic ring system (e.g., having 6 or 10 electrons shared in a cyclic array) having ring carbon atoms and 1–4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen and

sulfur ("5–10 membered heteroaryl"). In some embodiments, in heteroaryl groups that contain one or more nitrogen atoms, the point of attachment is a carbon or nitrogen atom, as valency permits. In some embodiments, a heteroaryl group is a 6 membered aromatic ring system having ring carbon atoms and 1–4 ring heteroatoms provided in the aromatic ring system, wherein each heteroatom is independently selected from nitrogen, oxygen, and sulfur ("6-membered heteroaryl").

[00048] Exemplary 6–membered heteroaryl groups containing one heteroatom include, without limitation, pyridinyl. Exemplary 6–membered heteroaryl groups containing two heteroatoms include, without limitation, pyridazinyl, pyrimidinyl, and pyrazinyl. Exemplary 6– membered heteroaryl groups containing three or four heteroatoms include, without limitation, triazinyl and tetrazinyl, respectively.

[00049] It will be appreciated that the compounds, as described herein, may be substituted with any number of substituents or functional moieties. In general, the term "substituted" whether preceded by the term "optionally" or not, and substituents contained in formulas of this disclosure, refer to the replacement of hydrogen radicals in a given structure with the radical of a specified substituent. When more than one position in any given structure may be substituted with more than one substituent selected from a specified group, the substituent may be either the same or different at every position.

[00050] Unless otherwise indicated, any heteroatom with unsatisfied valences is assumed to have hydrogen atoms sufficient to satisfy the valences.

[00051] Certain compounds of the present disclosure may exist in particular geometric or stereoisomeric forms. The present disclosure contemplates all such compounds, including cis- and trans-isomers, R- and S-enantiomers, diastereomers, (d)-isomers, (l)-isomers, the racemic mixtures thereof, and other mixtures thereof, as falling within the scope of the disclosure. Additional asymmetric carbon atoms may be present in a substituent such as an alkyl group. All such isomers, as well as mixtures thereof, are intended to be included in this disclosure.

[00052] All stereoisomers of the present compounds (for example, those which may exist due to asymmetric carbons on various substituents), including enantiomeric forms and diastereomeric forms, are contemplated within the scope of this disclosure. Individual stereoisomers of the compounds of the disclosure may, for example, be substantially free of other isomers (*e.g.,* as a pure or substantially pure optical isomer having a specified activity), or may be admixed, for example, as racemates or with all other, or other selected, stereoisomers. The chiral centers of the present disclosure may have the S or R configuration

as defined by the International Union of Pure and Applied Chemistry (IUPAC) 1974 Recommendations. The racemic forms can be resolved by physical methods, such as, for example, fractional crystallization, separation or crystallization of diastereomeric derivatives, or separation by chiral column chromatography. The individual optical isomers can be obtained from the racemates by any suitable method, including without limitation, conventional methods, such as, for example, salt formation with an optically active acid followed by crystallization.

[00053] All configurational isomers of the compounds of the present disclosure are contemplated, either in admixture or in pure or substantially pure form. The definition of compounds of the present disclosure embraces both cis (Z) and trans (E) alkene isomers, as well as cis and trans isomers of cyclic hydrocarbon or heterocyclic rings.

[00054] Isomeric mixtures containing any of a variety of isomer ratios may be utilized in accordance with the present disclosure. For example, where only two isomers are combined, mixtures containing 50:50, 60:40, 70:30, 80:20, 90:10, 95:5, 96:4, 97:3, 98:2, 99:1, or 100:0 isomer ratios are all contemplated by the present disclosure.

[00055] The present disclosure also includes isotopically-labeled compounds, which are identical to the compounds disclosed herein, but for the fact that one or more atoms are replaced by an atom having an atomic mass or mass number different from the atomic mass or mass number usually found in nature. Examples of isotopes that can be incorporated into compounds of the present disclosure include isotopes of hydrogen, carbon, nitrogen, oxygen, phosphorous, sulfur, fluorine, and chlorine, such as ^{2}H, ^{3}H, ^{13}C, ^{11}C, ^{14}C, ^{15}N, ^{18}O, ^{17}O, ^{31}P, ^{32}P, ^{35}S, ^{18}F, and ^{36}Cl, respectively. Compounds of the present disclosure, or a stereoisomer, tautomer, or pharmaceutically acceptable salt or solvate thereof, which contain the aforementioned isotopes and/or other isotopes of other atoms are within the scope of this disclosure.

[00056] As used herein, "pharmaceutically acceptable salt" refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art. For example, Berge *et al.*, describes pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences* (1977) 66:1–19. Pharmaceutically acceptable salts of the compounds of this disclosure include those derived from suitable inorganic and organic acids and bases. Examples of pharmaceutically acceptable, nontoxic acid addition salts are salts of an amino group formed with inorganic acids such as hydrochloric acid,

hydrobromic acid, phosphoric acid, sulfuric acid and perchloric acid or with organic acids such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid or malonic acid or by using other methods used in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2–hydroxy–ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2–naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3–phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, p–toluenesulfonate, undecanoate, valerate salts, and the like. Pharmaceutically acceptable salts derived from appropriate bases include alkali metal, alkaline earth metal, ammonium and $N^+(C_{1-4}alkyl)_4$ salts. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like. Further pharmaceutically acceptable salts include, when appropriate, nontoxic ammonium, quaternary ammonium, and amine cations formed using counterions such as halide, hydroxide, carboxylate, sulfate, phosphate, nitrate, lower alkyl sulfonate, and aryl sulfonate.

[00057] As used herein, "pharmaceutically acceptable excipient" refers to any substance in a pharmaceutical formulation other than an active pharmaceutical ingredient(s). Exemplary pharmaceutical excipients include those that aid the manufacturing process; protect, support or enhance stability; increase bioavailability; or increase patient acceptability. They may also assist in product identification or enhance the overall safety or function of the product during storage or use.

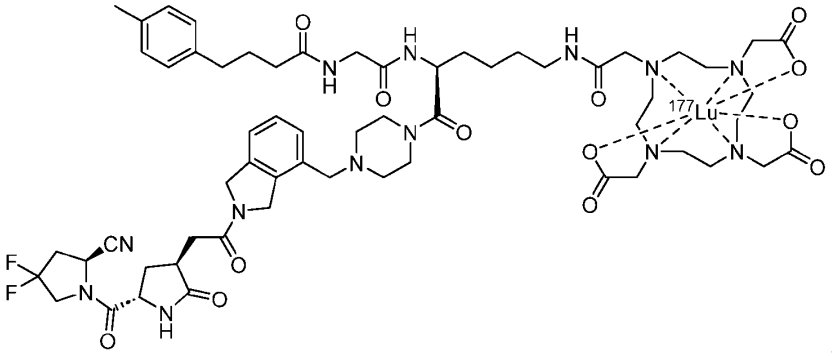
[00058] As used herein, a "subject" to which administration is contemplated includes, but is not limited to, humans (i.e., a male or female of any age group, e.g., a pediatric subject (e.g., infant, child, adolescent) or adult subject (e.g., young adult, middle–aged adult or senior adult)) and/or a non-human animal, e.g., a mammal such as primates (e.g., cynomolgus monkeys, rhesus monkeys), cattle, pigs, horses, sheep, goats, rodents, cats, and/or dogs. In some embodiments, the subject is a human. In some embodiments, the subject is a non-human animal. The terms "human," "patient," "subject," and "individual" are used interchangeably herein. None of these terms require the active supervision of medical personnel.

[00059] Disease, disorder, and condition are used interchangeably herein.

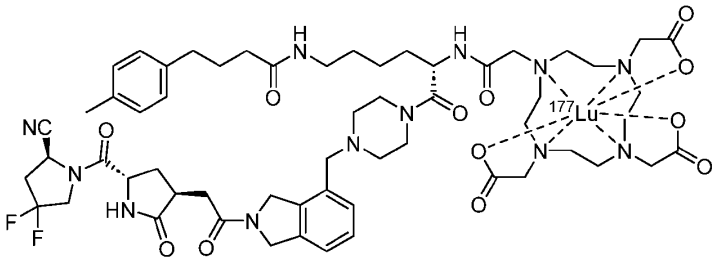
[00060] As used herein, and unless otherwise specified, the terms "treat," "treating" and "treatment" contemplate an action that occurs while a subject is suffering from the specified disease, disorder or condition, which reduces the severity of the disease, disorder or condition, or reverses or slows the progression of the disease, disorder or condition (also "therapeutic treatment").

[00061] In general, the "effective amount" of a compound refers to an amount sufficient to elicit the desired biological response. As will be appreciated by those of ordinary skill in this art, the effective amount of a compound of the disclosure may vary depending on such factors as the desired biological endpoint, the pharmacokinetics of the compound, the disease being treated, the mode of administration, and the age, weight, health, and condition of the subject. A "therapeutically effective amount" of a compound is an amount sufficient to provide a therapeutic benefit in the treatment of a disease, disorder or condition, or to delay or minimize one or more symptoms associated with the disease, disorder or condition. A therapeutically effective amount of a compound means an amount of therapeutic agent, alone or in combination with other therapies, which provides a therapeutic benefit in the treatment of the disease, disorder or condition. The term "therapeutically effective amount" can encompass an amount that improves overall therapy, reduces or avoids symptoms or causes of disease or condition, or enhances the therapeutic efficacy of another therapeutic agent. A "prophylactically effective amount" of a compound is an amount sufficient to prevent a disease, disorder or condition, or one or more symptoms associated with the disease, disorder or condition, or prevent its recurrence. A prophylactically effective amount of a compound means an amount of a therapeutic agent, alone or in combination with other agents, which provides a prophylactic benefit in the prevention of the disease, disorder or condition. The term "prophylactically effective amount" can encompass an amount that improves overall prophylaxis or enhances the prophylactic efficacy of another prophylactic agent. A "prophylactic treatment" contemplates an action that occurs before a subject begins to suffer from the specified disease, disorder or condition.

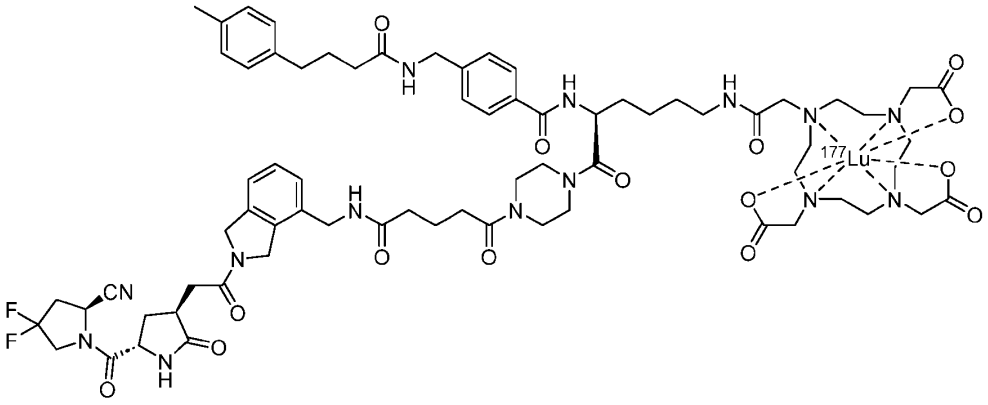
[00062] As used herein, "^{177}Lu-Compound 1" refers to a chelated complex represented by the formula:



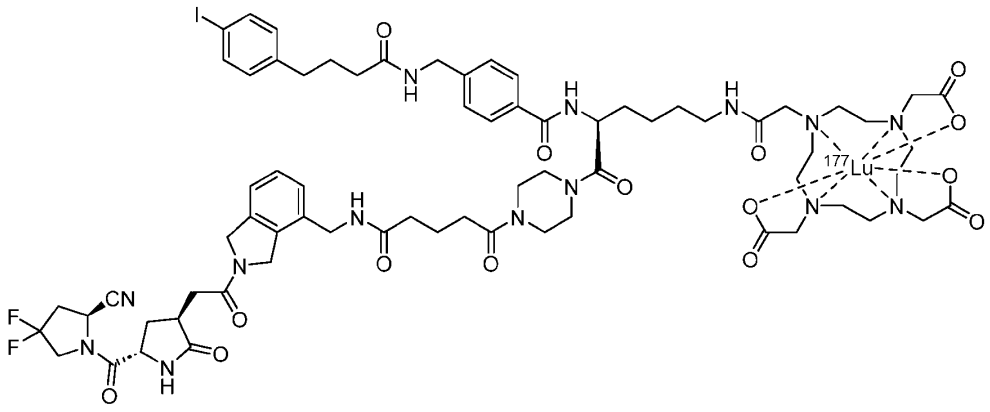
[00063] As used herein, "^{177}Lu-Compound 2" refers to a chelated complex represented by the formula:



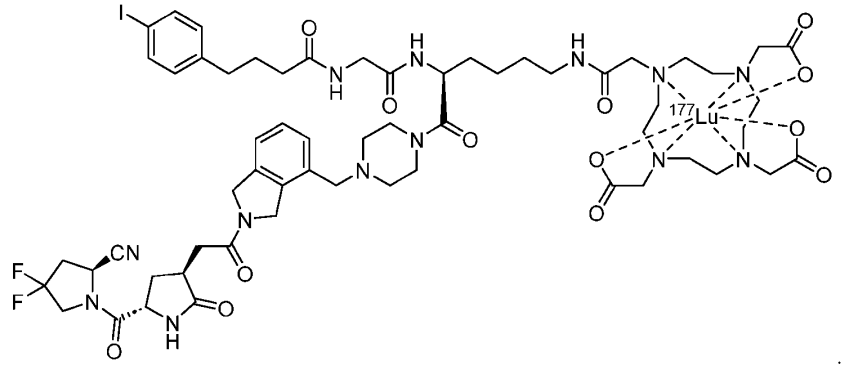
[00064] As used herein, "^{177}Lu-Compound 3" refers to a chelated complex represented by the formula:



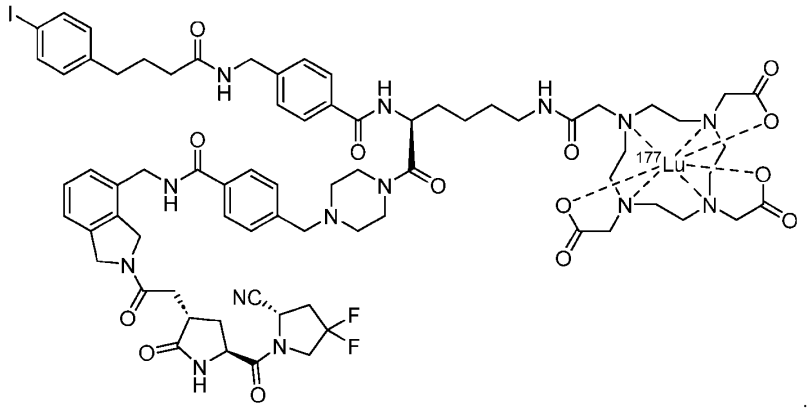
[00065] As used herein, "^{177}Lu-Compound 4" refers to a chelated complex represented by the formula:



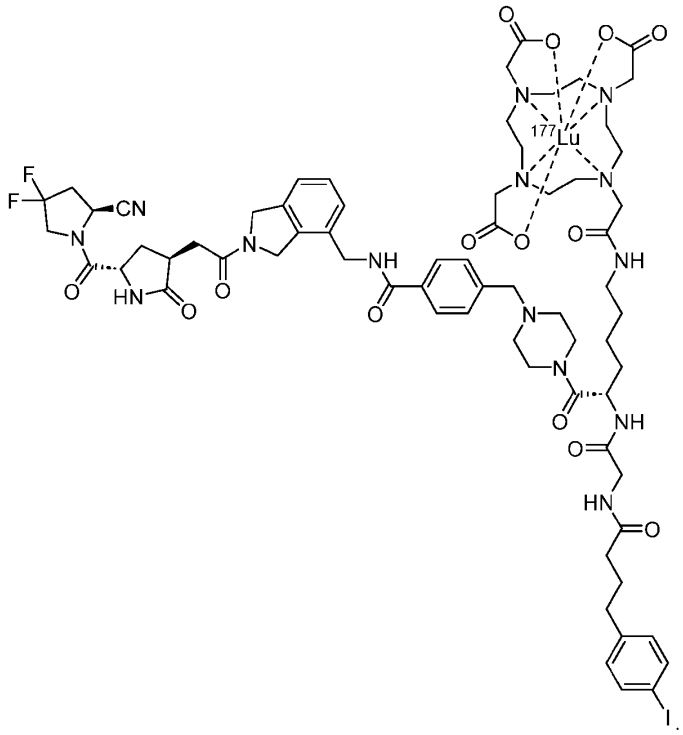
[00066] As used herein, "^{177}Lu-Compound 5" refers to a chelated complex represented by the formula:



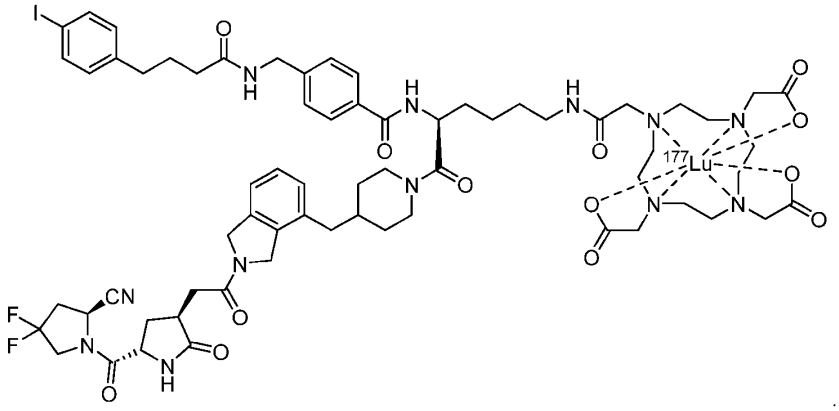
[00067] As used herein, "^{177}Lu-Compound 6" refers to a chelated complex represented by the formula:



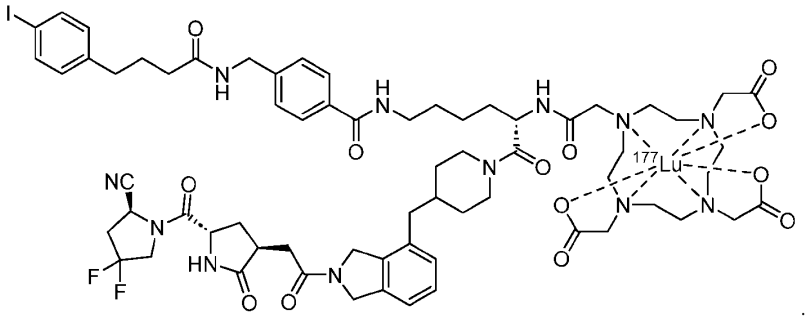
[00068] As used herein, "^{177}Lu-Compound 7" refers to a chelated complex represented by the formula:



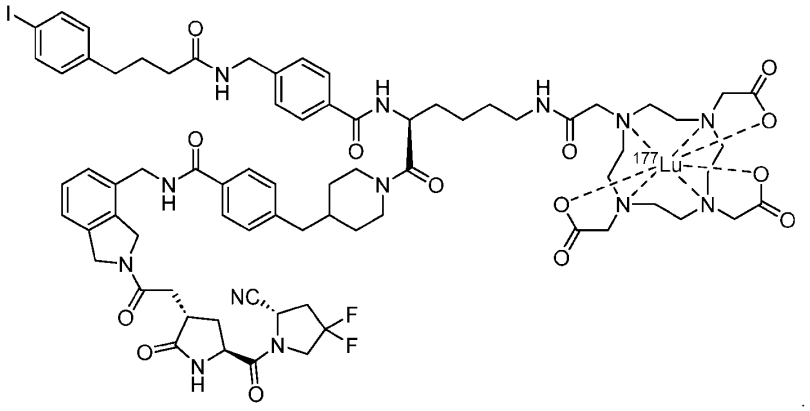
[00069] As used herein, "^{177}Lu-Compound 8" refers to a chelated complex represented by the formula:



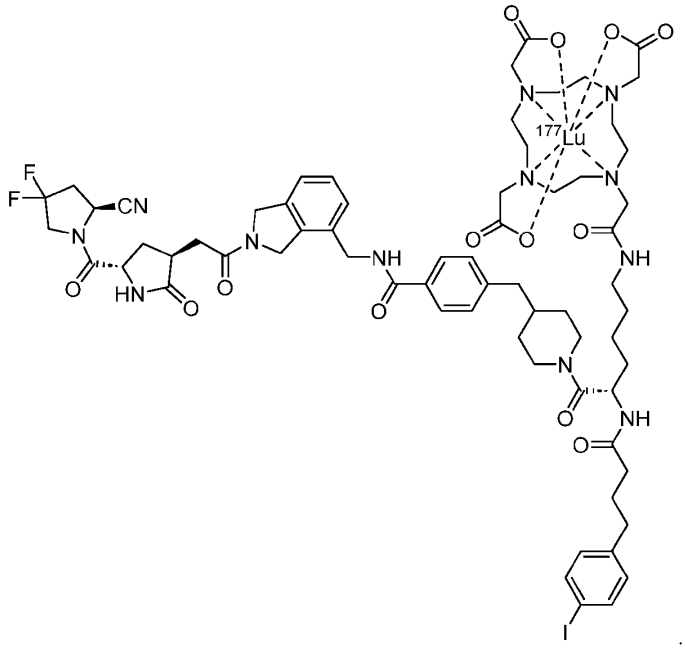
[00070] As used herein, "^{177}Lu-Compound 9" refers to a chelated complex represented by the formula:



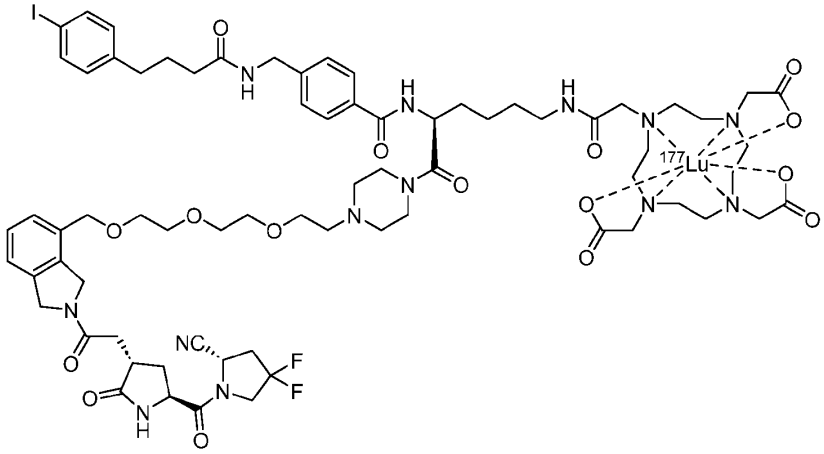
[00071] As used herein, "^{177}Lu-Compound 10" refers to a chelated complex represented by the formula:



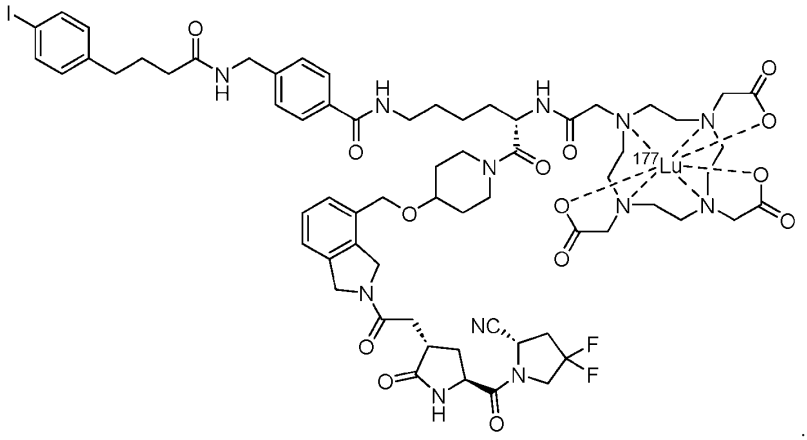
[00072] As used herein, "^{177}Lu-Compound 11" refers to a chelated complex represented by the formula:



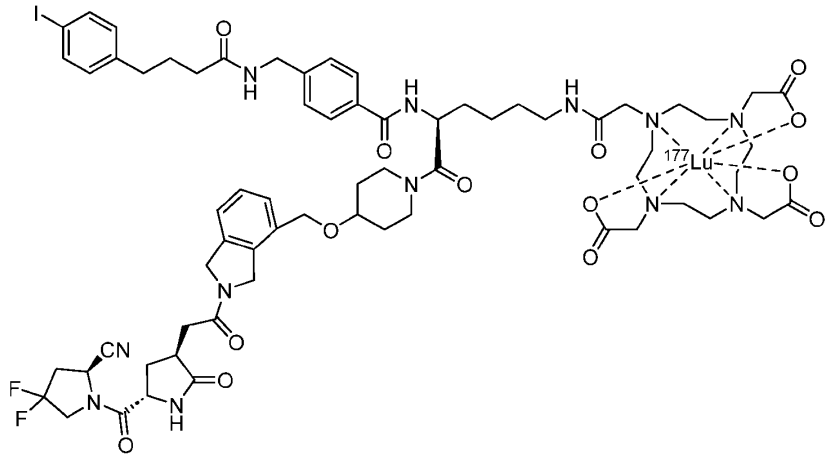
[00073] As used herein, "^{177}Lu-Compound 12" refers to a chelated complex represented by the formula:



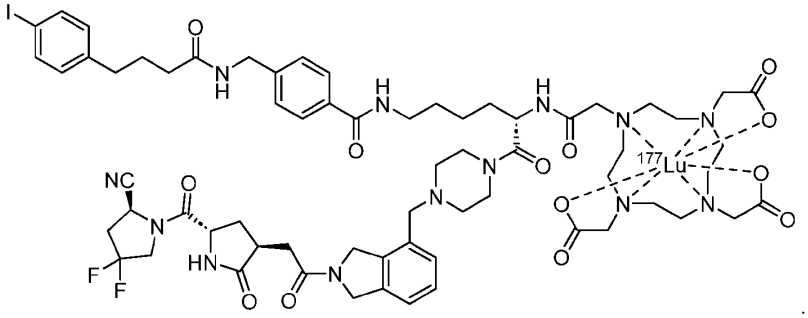
[00074] As used herein, "^{177}Lu-Compound 13" refers to a chelated complex represented by the formula:



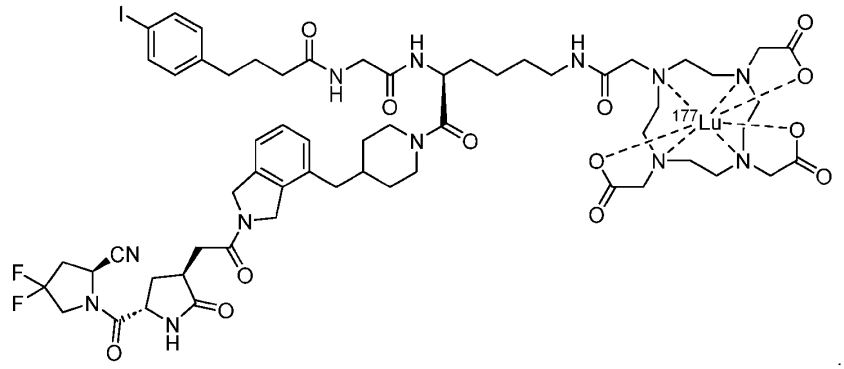
[00075] As used herein, "^{177}Lu-Compound 14" refers to a chelated complex represented by the formula:



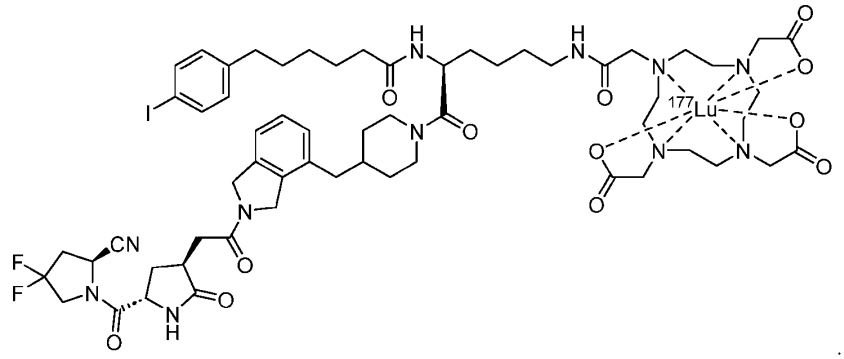
[00076] As used herein, "^{177}Lu-Compound 15" refers to a chelated complex represented by the formula:



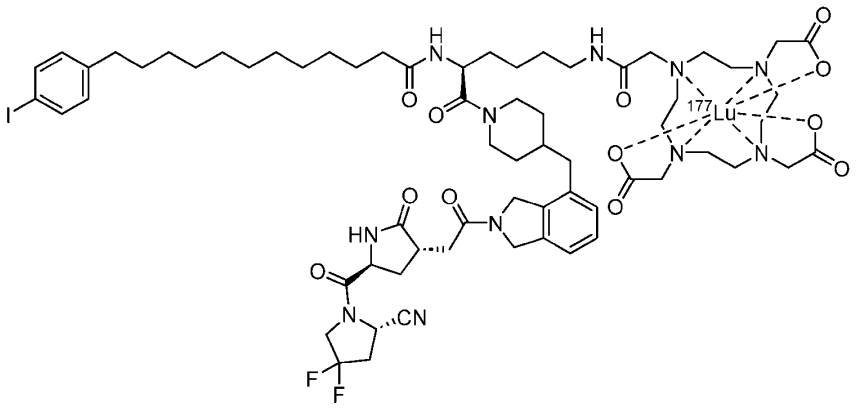
[00077] As used herein, "^{177}Lu-Compound 16" refers to a chelated complex represented by the formula:



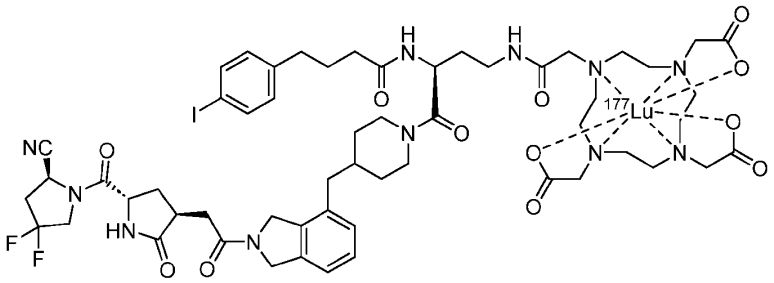
[00078] As used herein, "^{177}Lu-Compound 17" refers to a chelated complex represented by the formula:



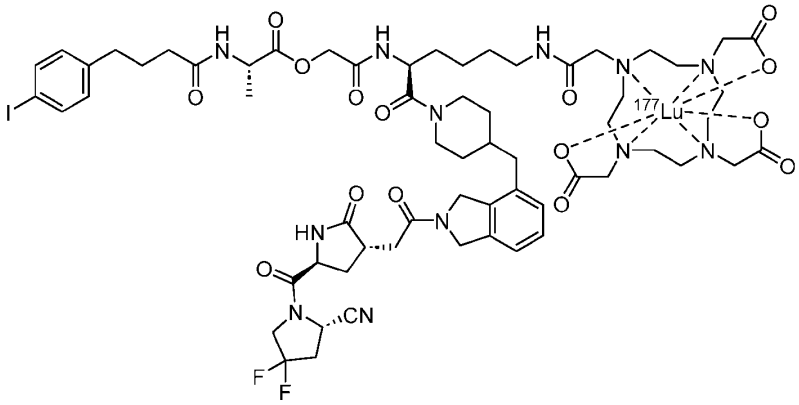
[00079] As used herein, "^{177}Lu-Compound 18" refers to a chelated complex represented by the formula:



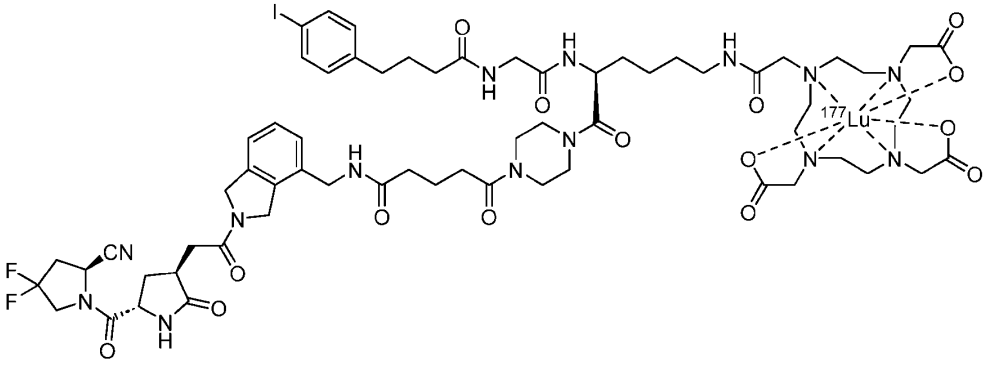
[00080] As used herein, "^{177}Lu-Compound 19" refers to a chelated complex represented by the formula:



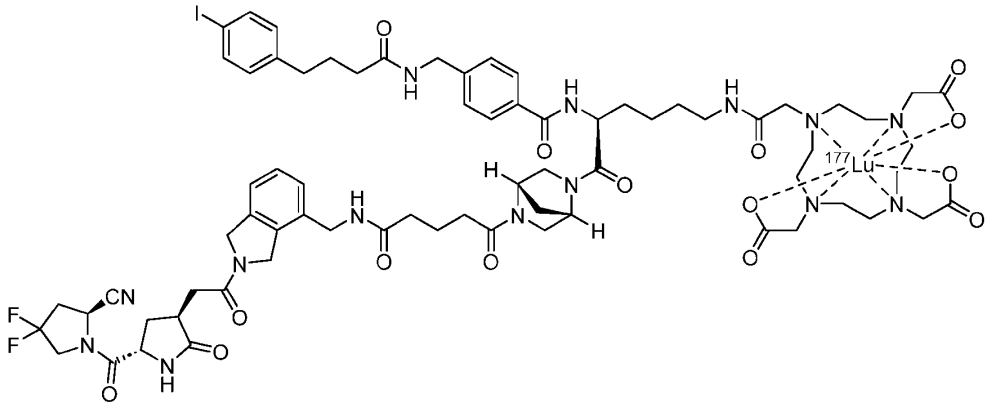
[00081] As used herein, "^{177}Lu-Compound 20" refers to a chelated complex represented by the formula:



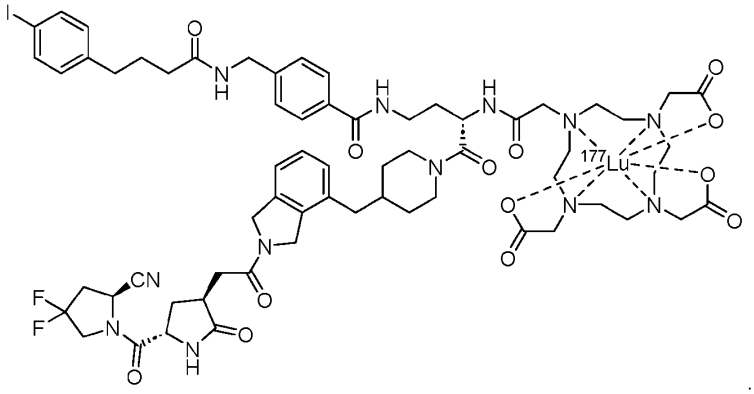
[00082] As used herein, "^{177}Lu-Compound 21" refers to a chelated complex represented by the formula:



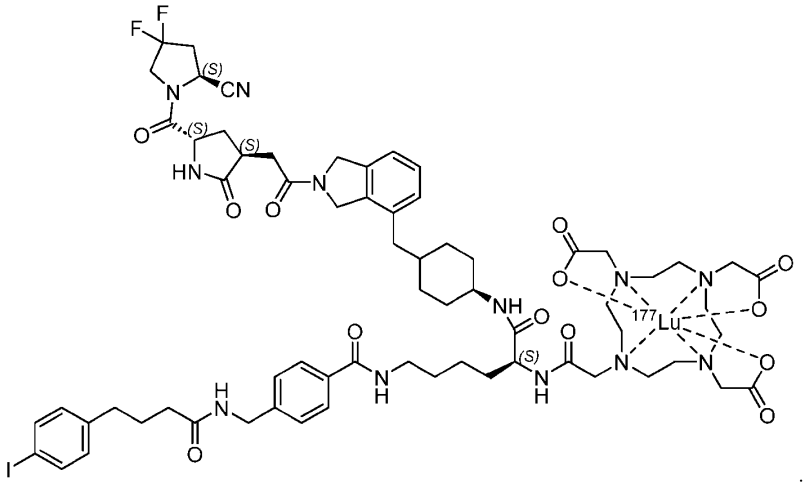
[00083] As used herein, "^{177}Lu-Compound 22" refers to a chelated complex represented by the formula:



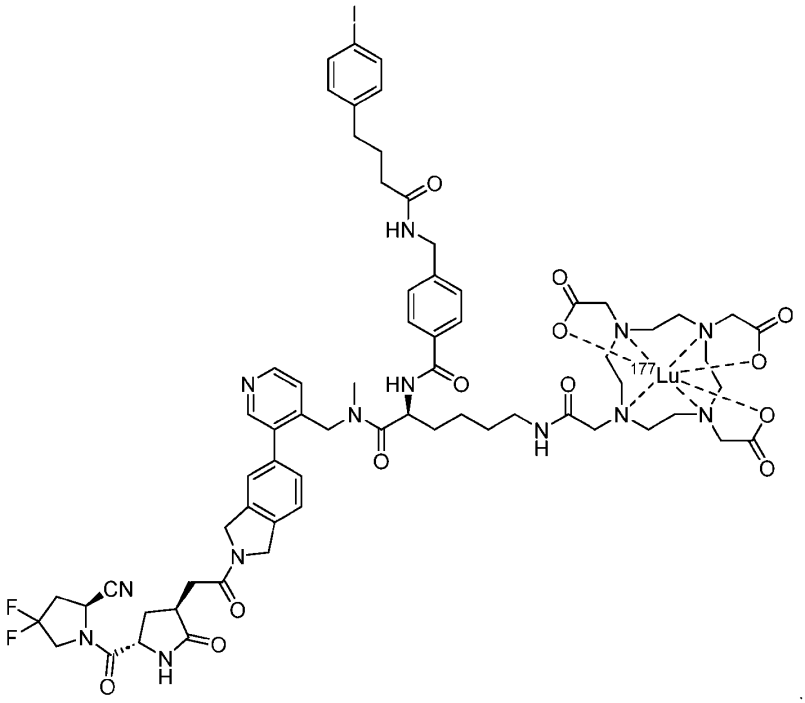
[00084] As used herein, "^{177}Lu-Compound 23" refers to a chelated complex represented by the formula:



[00085] As used herein, "^{177}Lu-Compound 24" refers to a chelated complex represented by the formula:

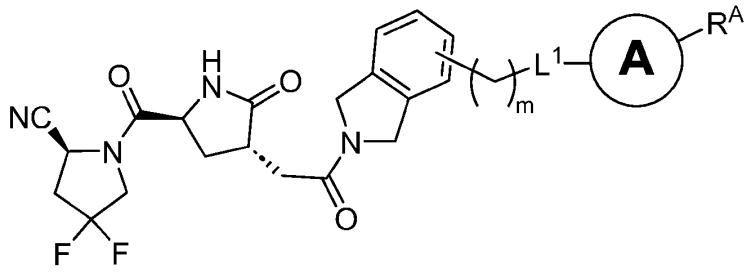


[00086] As used herein, "^{177}Lu-Compound 25" refers to a chelated complex represented by the formula:



Compounds

[00087] In certain embodiments, provided herein is a complex of a compound represented by Formula (I):



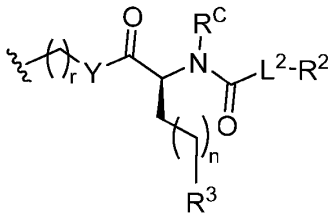
Formula (I)

or a pharmaceutically acceptable salt thereof, wherein:

ring A is selected from the group consisting of 3 to 6-membered heterocyclyl, 6-membered heteroaryl, and C_{3-6} cycloalkyl;

L^1 is selected from a covalent bond and a linker;

R^A is a moiety represented by:



Y is a covalent bond or NR^D;

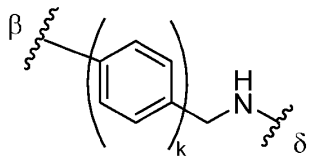
R^C is selected from hydrogen and C_1-C_3 alkyl;

R^D is selected from hydrogen and C_1-C_3 alkyl;

L^2 is selected from the group consisting of:

a covalent bond, and

a moiety represented by:



wherein β represents the point of attachment to the carbonyl group and δ represents the point of attachment to R^2;

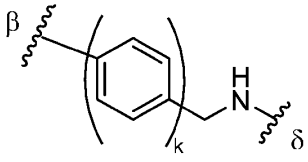
each of R^2 and R^3 is independently selected from an albumin binding ligand and a ligand chelated or covalently linked to a therapeutic radionuclide or a diagnostic radionuclide, wherein at least one of R^2 and R^3 is a therapeutic radionuclide or a diagnostic radionuclide;

each of k, m, and r is independently 0 or 1; and

n is an integer from 1 to 6;

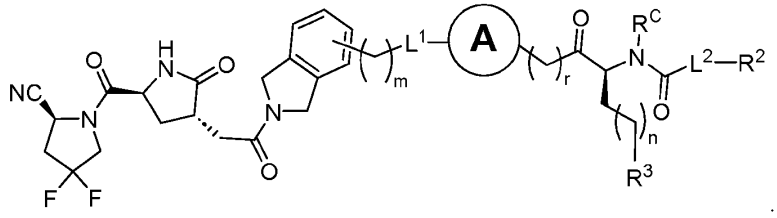
provided that:

when L^1 is a covalent bond, m is 1, L^2 is a moiety represented by:



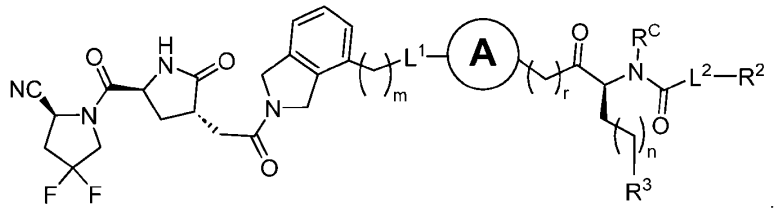
, and A is piperazinyl having a ring nitrogen attached to L^1, then k is 0.

[00088] In some embodiments, the complex of Formula (I) is represented by Formula (I-A):



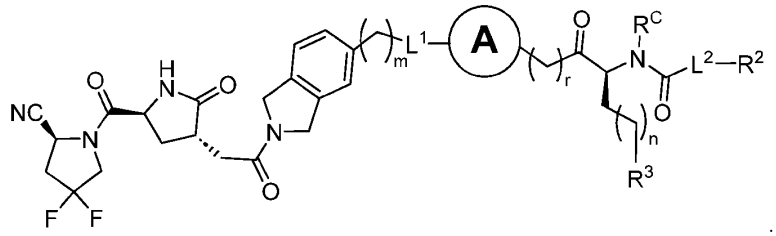
Formula (I-A).

[00089] In some embodiments, the complex of Formula (I) is represented by Formula (I-B):



Formula (I-B).

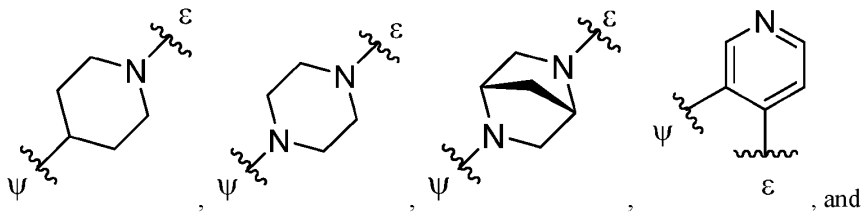
[00090] In some embodiments, the complex of Formula (I) is represented by Formula (I-C):



Formula (I-C).

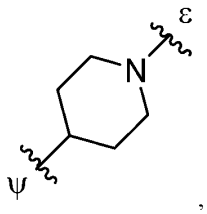
[00091] In some embodiments, ring A is 6-membered heterocyclyl. In some embodiments, ring A is 6-membered heteroaryl. In some embodiments, ring A is cyclohexyl.

[00092] In some embodiments, ring A is selected from the group consisting of:



, wherein each ψ represents the point of attachment to L^1, and each ε represents the point of attachment to R^A.

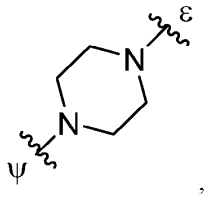
[00093] In some embodiments, ring A is:



,

wherein ψ represents the point of attachment to L^1, and ε represents the point of attachment to R^A.

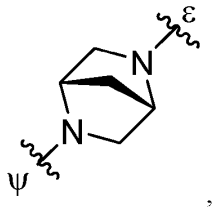
[00094] In some embodiments, ring A is:



,

wherein ψ represents the point of attachment to L^1, and ε represents the point of attachment to R^A.

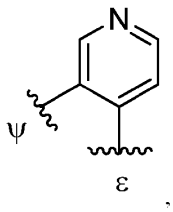
[00095] In some embodiments, ring A is:



,

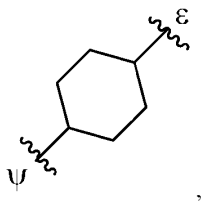
wherein ψ represents the point of attachment to L^1, and ε represents the point of attachment to R^A.

[00096] In some embodiments, ring A is:



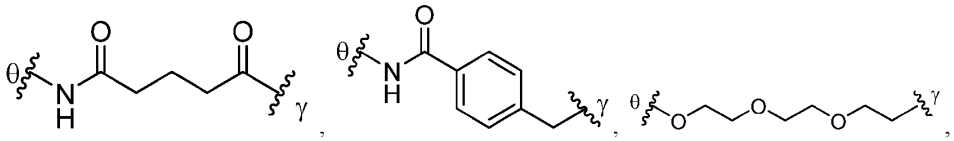
wherein ψ represents the point of attachment to L^1, and ε represents the point of attachment to R^A.

[00097] In some embodiments, ring A is:



wherein ψ represents the point of attachment to L^1, and ε represents the point of attachment to R^A.

[00098] In some embodiments, L^1 is a covalent bond. In some embodiments, L^1 is a linker. In some embodiments, L^1 is a linker selected from the group consisting of:



and -O-,

wherein each θ is the point of attachment to the methylene group and each γ is the point of attachment to ring A.

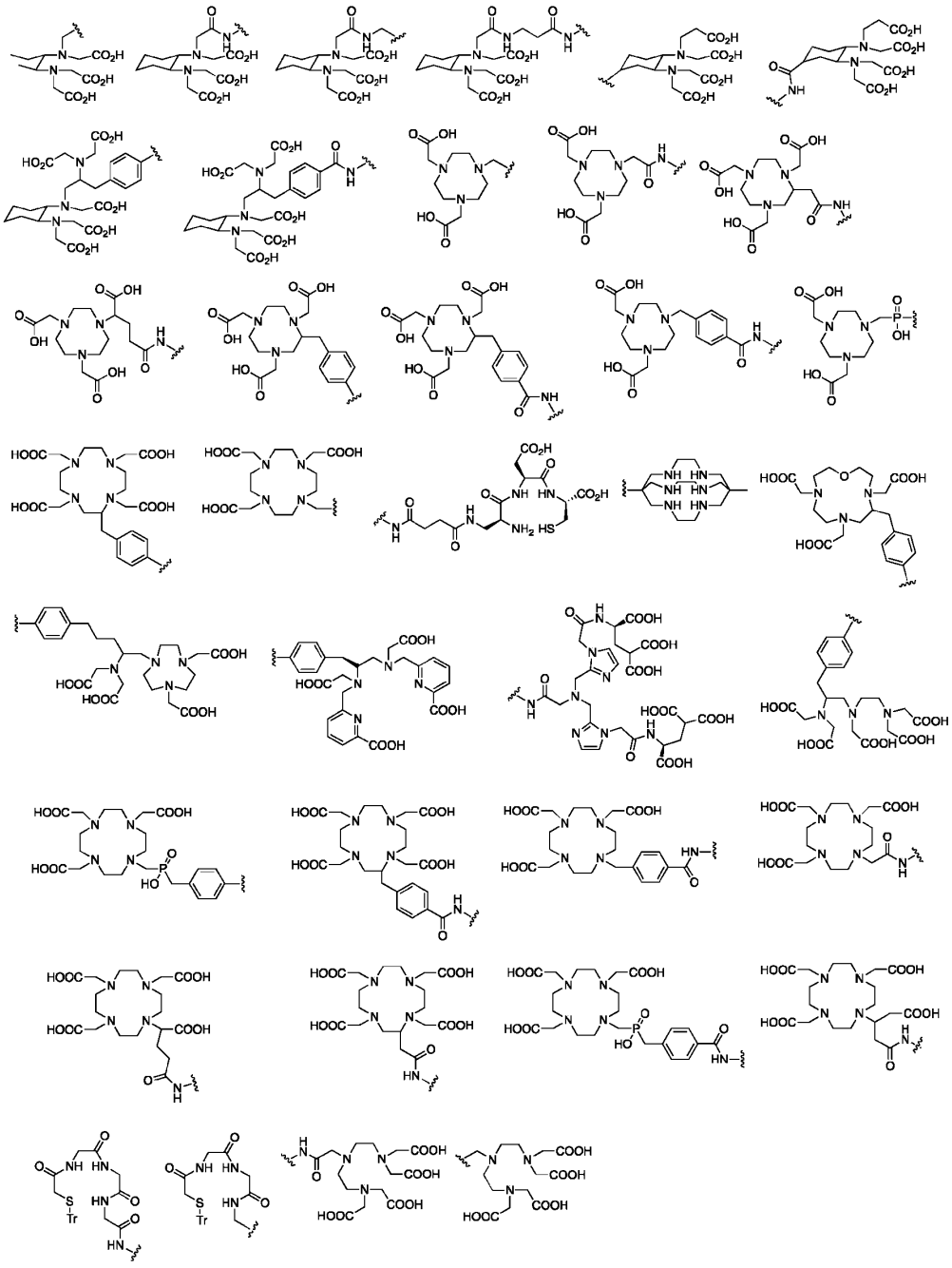
[00099] In some embodiments, m is 0. In some embodiments, m is 1.

[000100] In some embodiments, n is 3.

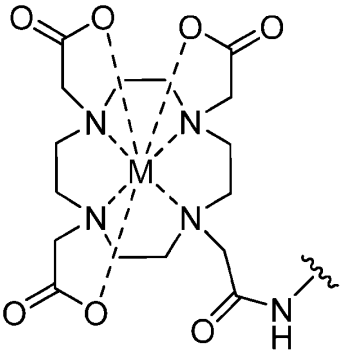
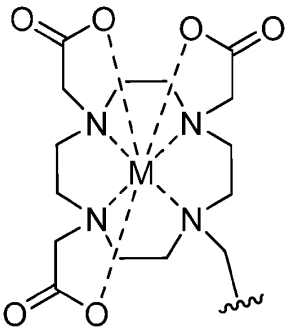
[000101] In some embodiments, r is 0. In some embodiments, r is 1.

[000102] In some embodiments, k is 0. In some embodiments, k is 1.

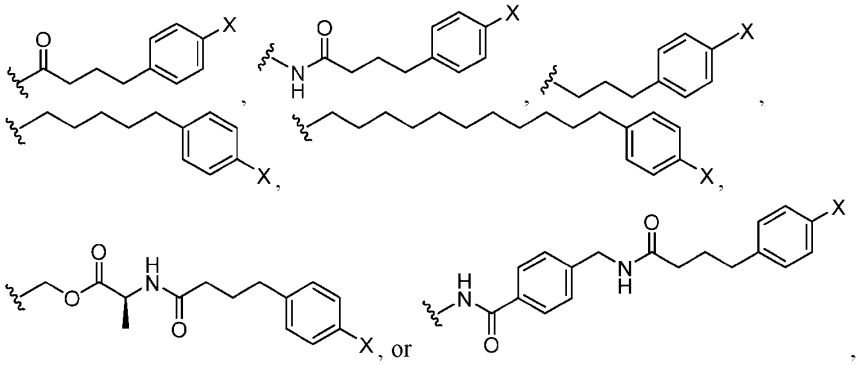
[000103] In some embodiments, one of R^2 and R^3 is a ligand chelated to or covalently linked to a therapeutic radionuclide or a diagnostic radionuclide, wherein the ligand is selected from the group consisting of:



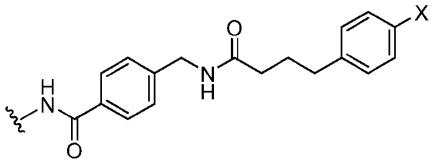
[000104] In some embodiments, one of R^2 and R^3 is a ligand chelated to or covalently linked to a therapeutic radionuclide or a diagnostic radionuclide, wherein the therapeutic radionuclide or the diagnostic radionuclide is represented by:


or

, wherein M is a therapeutic radionuclide or a diagnostic radionuclide.

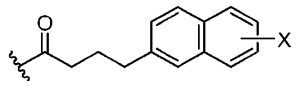
[000105] In some embodiments, one of R^2 and R^3 is an albumin binding ligand represented by:


, wherein X is selected from the group consisting of halogen and C_{1-6} alkyl.

[000106] In some embodiments, one of R^2 and R^3 is an albumin binding ligand represented by:


, wherein X is selected from hydrogen, halogen, and C_{1-6} alkyl.

[000107] In some embodiments, one of R^2 and R^3 is an albumin binding ligand represented by:

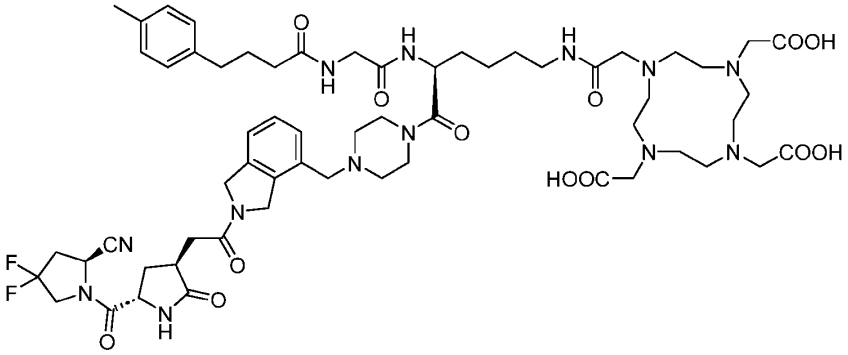


wherein X is selected from the group consisting of hydrogen, halogen and C_{1-6} alkyl.

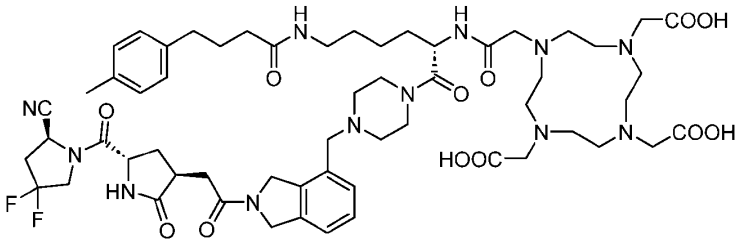
[000108] In some embodiments, R^C is hydrogen.

[000109] In some embodiments, when one of R^2 and R^3 is the albumin binding ligand, the other is the ligand chelated to or covalently linked to a therapeutic radionuclide or a diagnostic radionuclide.

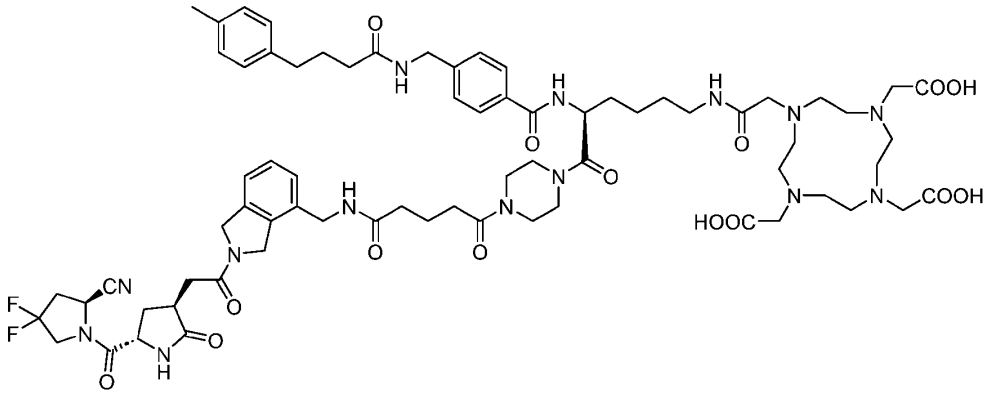
[000110] In certain embodiments, provided herein is a complex of a compound selected from the group consisting of



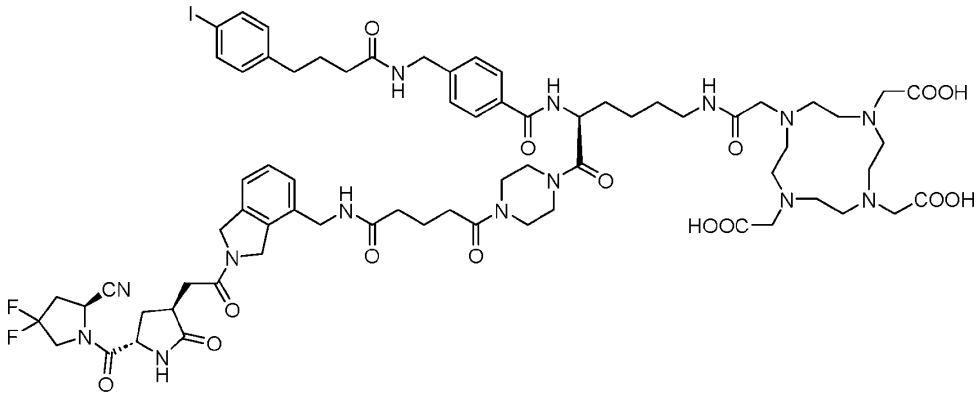
Compound 1,



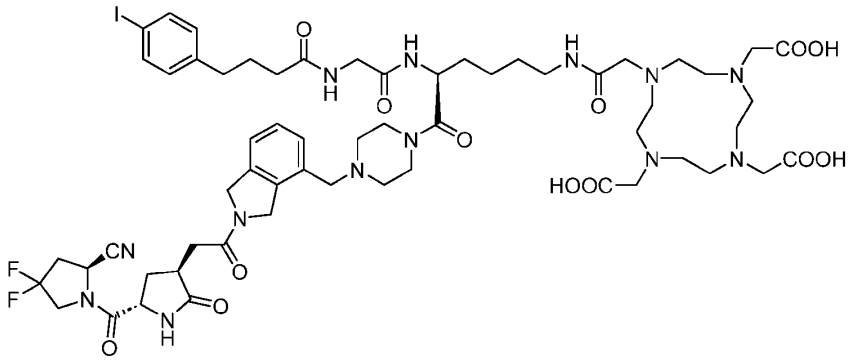
Compound 2,



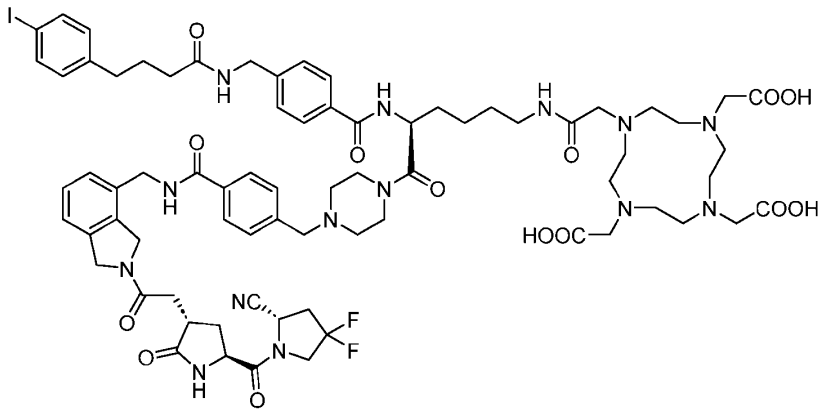
Compound 3,



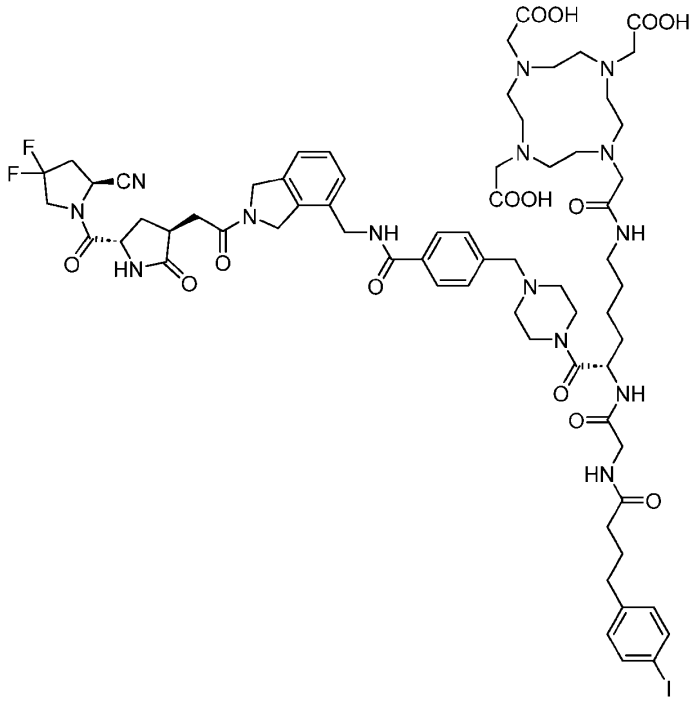
Compound 4,



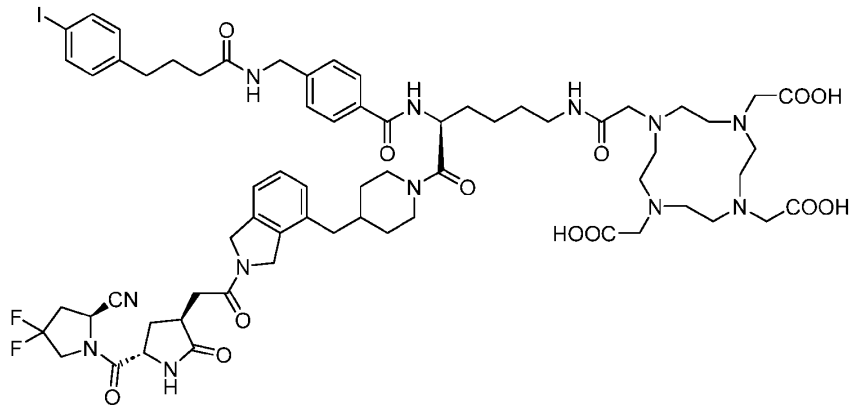
Compound 5,



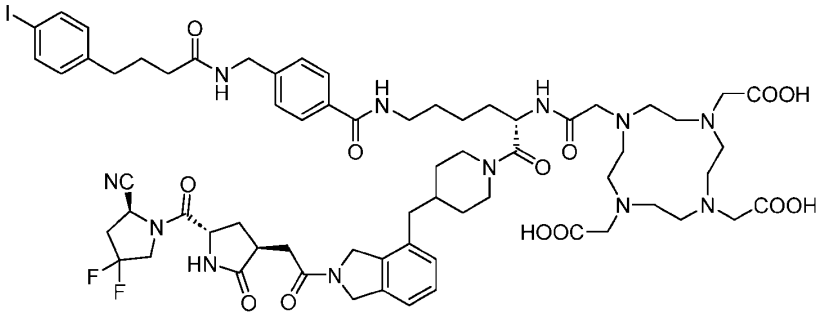
Compound 6,



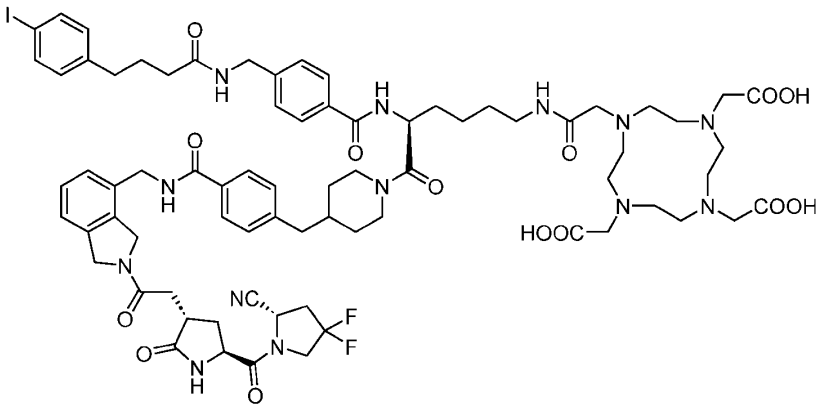
Compound 7,



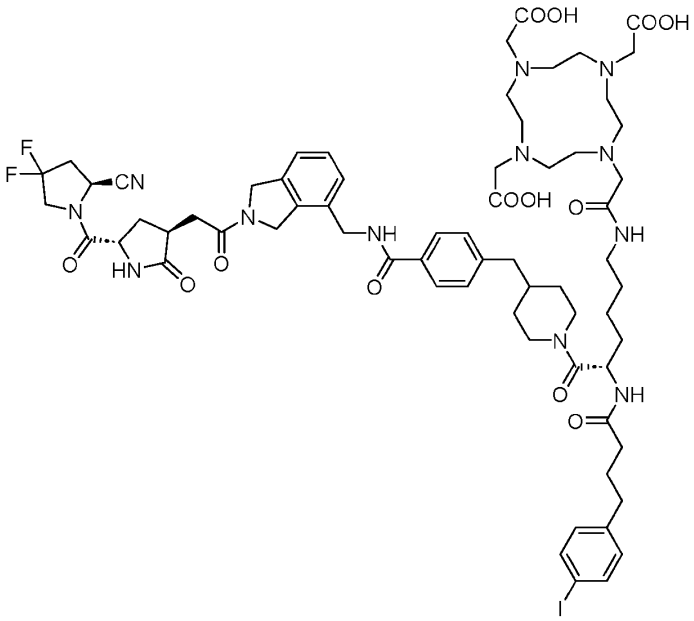
Compound 8,



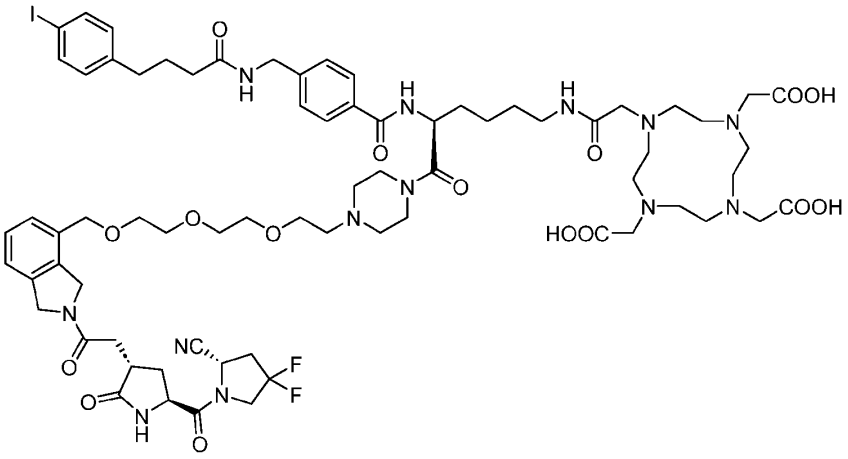
Compound 9,



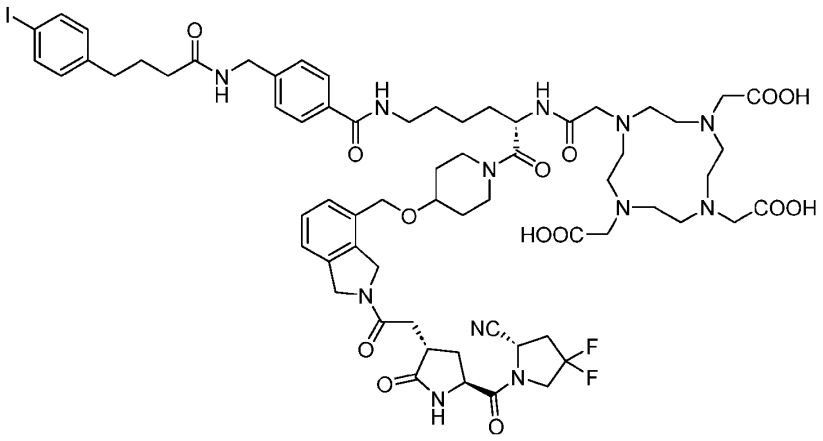
Compound 10,



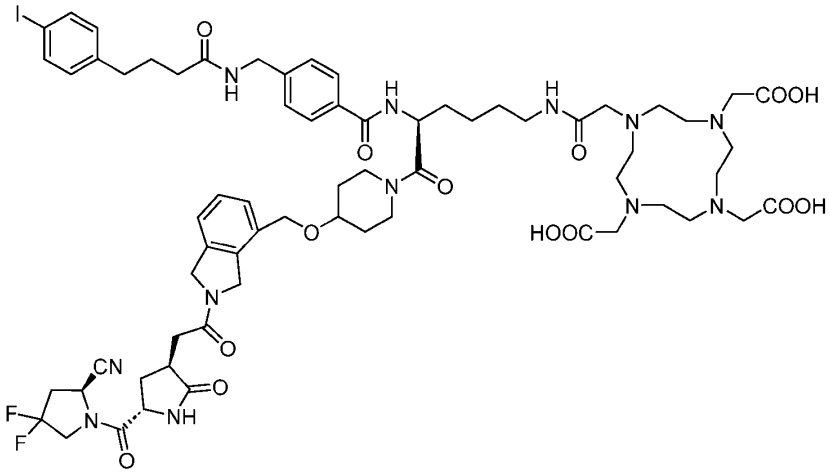
Compound 11,



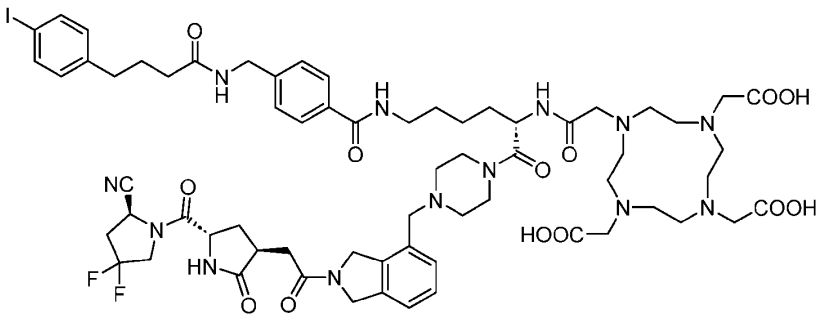
Compound 12,



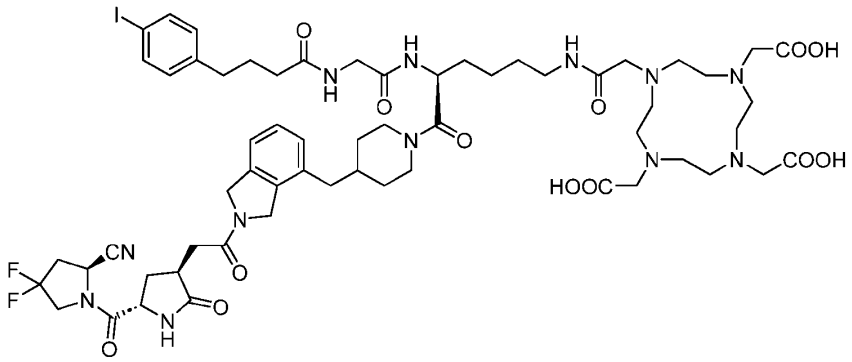
Compound 13,



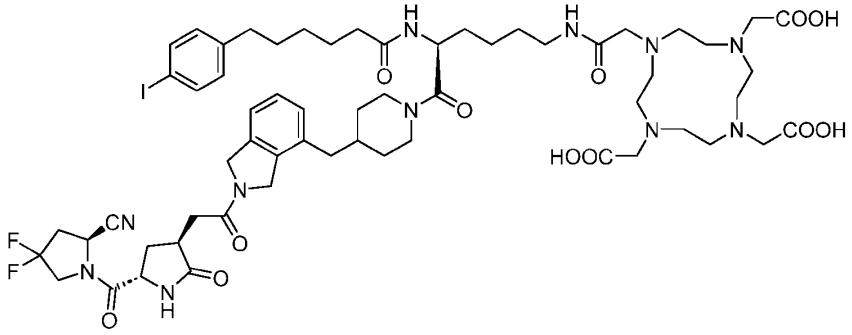
Compound 14,



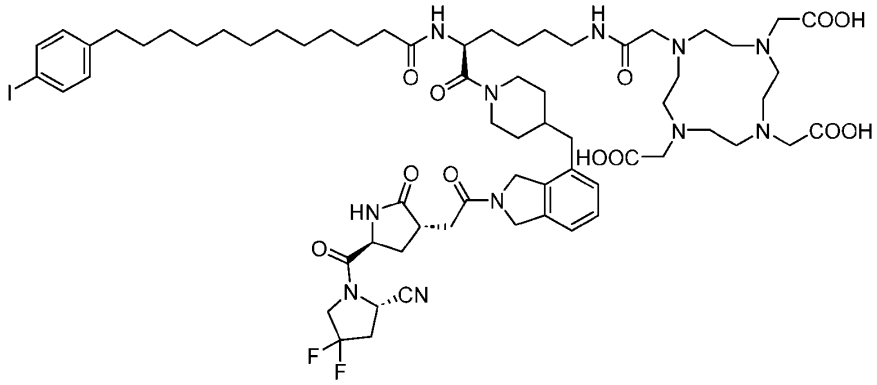
Compound 15,



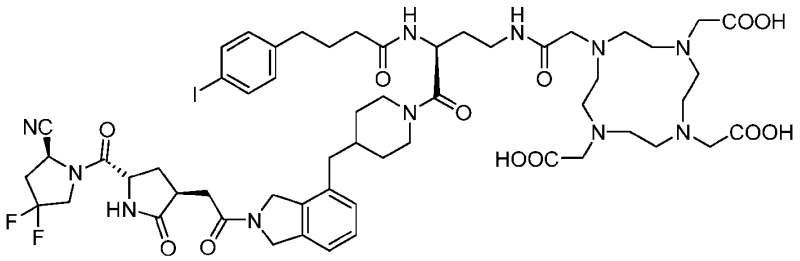
Compound 16,



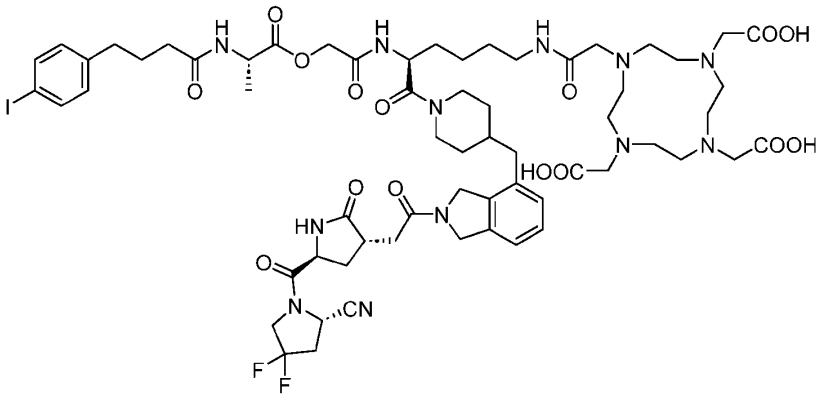
Compound 17,



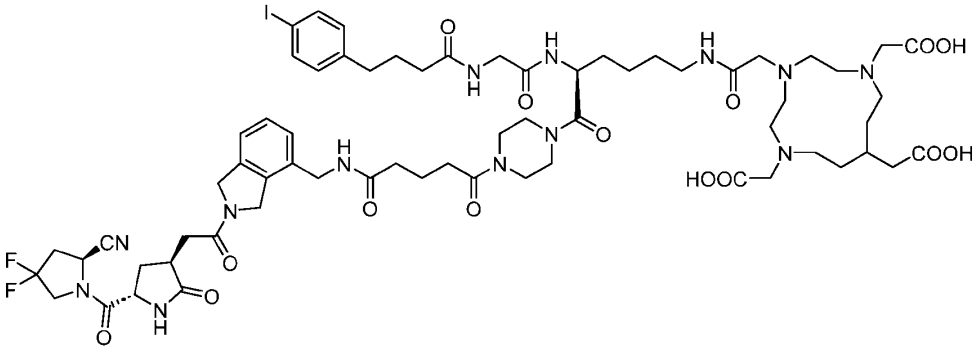
Compound 18,



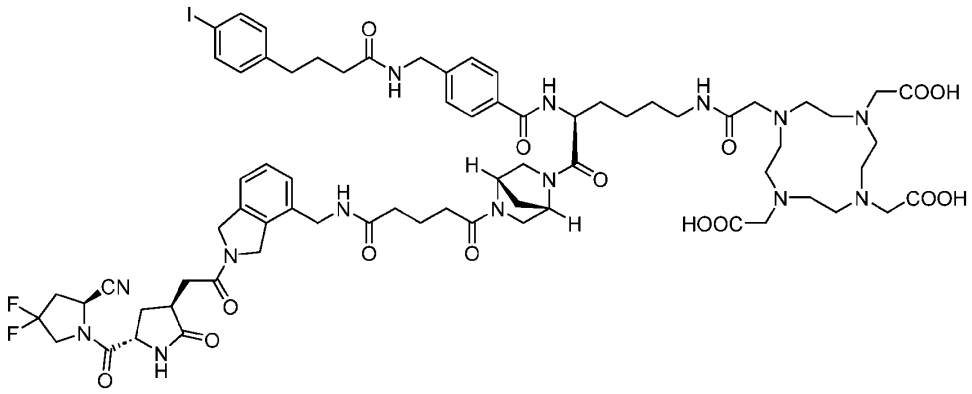
Compound 19,



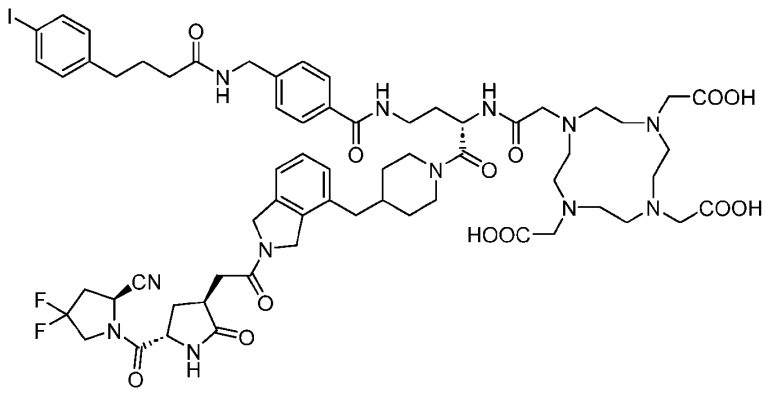
Compound 20,



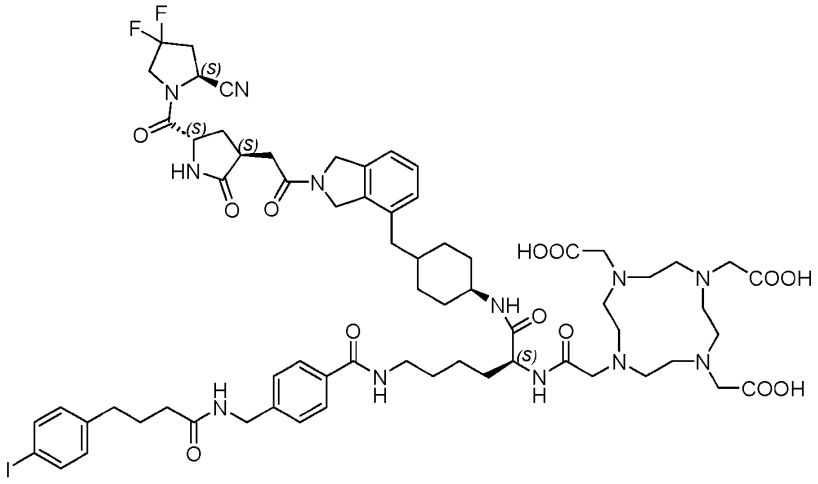
Compound 21,



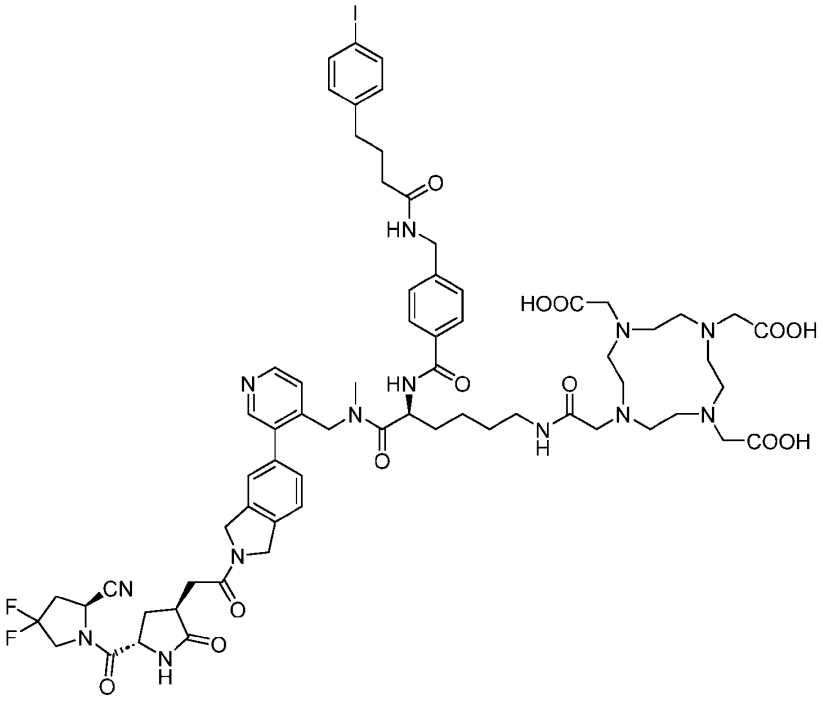
Compound 22,



Compound 23,



Compound 24, and



Compound 25,

or a pharmaceutically acceptable salt thereof; and

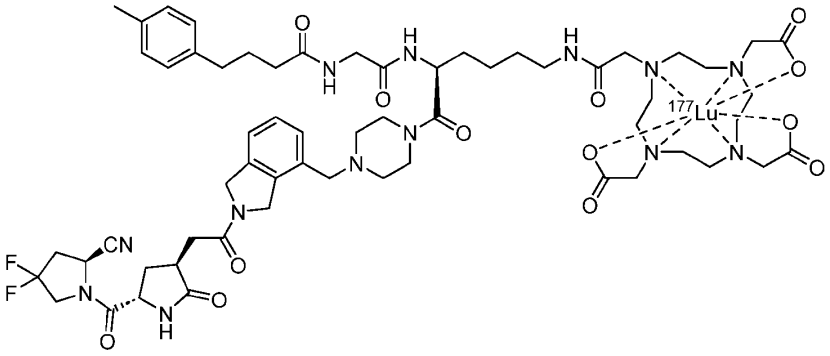
a therapeutic radionuclide or a diagnostic radionuclide.

[000111] In some embodiments, the therapeutic radionuclide is selected from the group consisting of ^{177}Lu, ^{90}Y, ^{155}Tb, ^{161}Tb, ^{225}Ac, ^{67}Cu, ^{223}Ra, ^{186}Re, ^{188}Re, ^{131}I, ^{212}Pb, ^{212}Bi, ^{213}Bi, and ^{211}At. In some embodiments, the therapeutic radionuclide is ^{177}Lu.

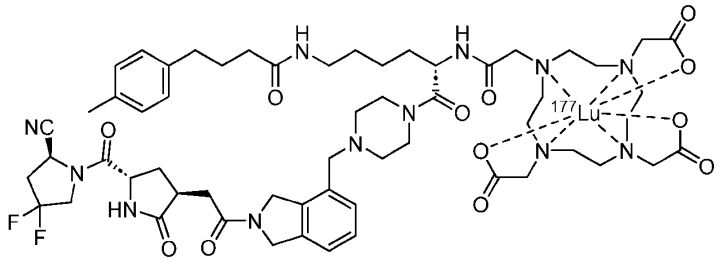
[000112] In some embodiments, the diagnostic radionuclide is selected from the group consisting of ^{99m}Tc, ^{111}In, ^{18}F, ^{66}Ga, ^{67}Ga, ^{68}Ga, ^{124}I, ^{125}I, ^{44}Sc, ^{47}Sc, ^{64}Cu, ^{52}Mn, ^{55}Co, ^{86}Y, ^{89}Zr, ^{149}Tb, ^{152}Tb, and ^{203}Pb. In some embodiments, the diagnostic radionuclide is ^{111}In.

[000113] It is to be understood that radionuclides disclosed herein may have both diagnostic and therapeutic properties (e.g., "theranostic radionuclides"). In some embodiments, compounds of the disclosure (e.g., complexes of compounds disclosed herein chelated or covalently bound to radionuclides) may have both diagnostic and therapeutic properties.

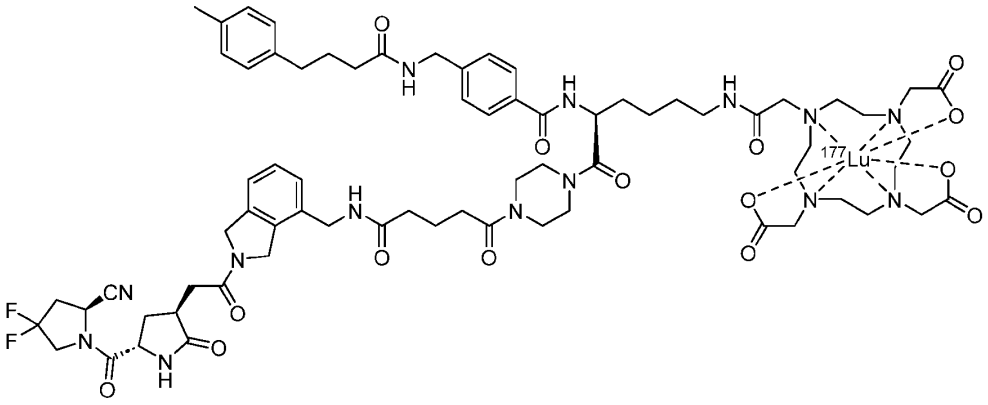
[000114] In certain embodiments, provided herein is a complex selected from the group consisting of



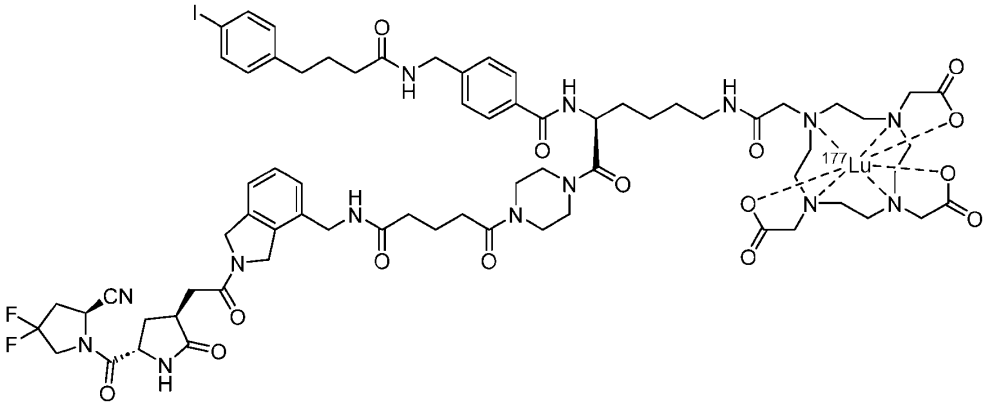
^{177}Lu-Compound 1,



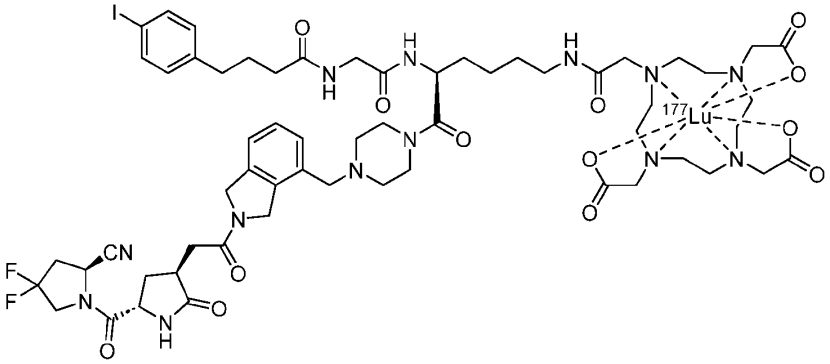
^{177}Lu-Compound 2,



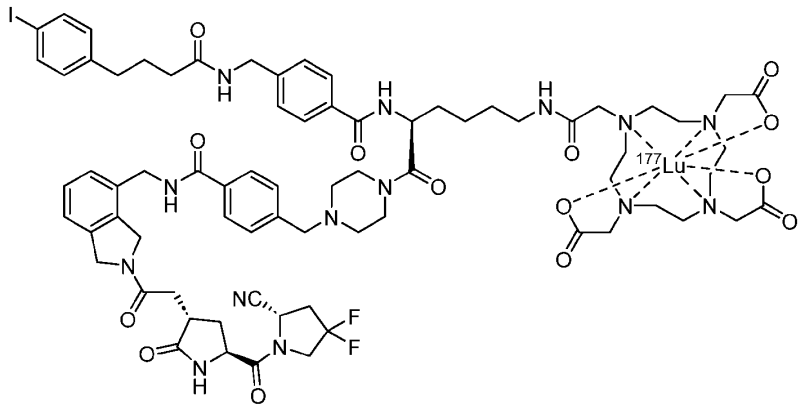
^{177}**Lu-Compound 3**,



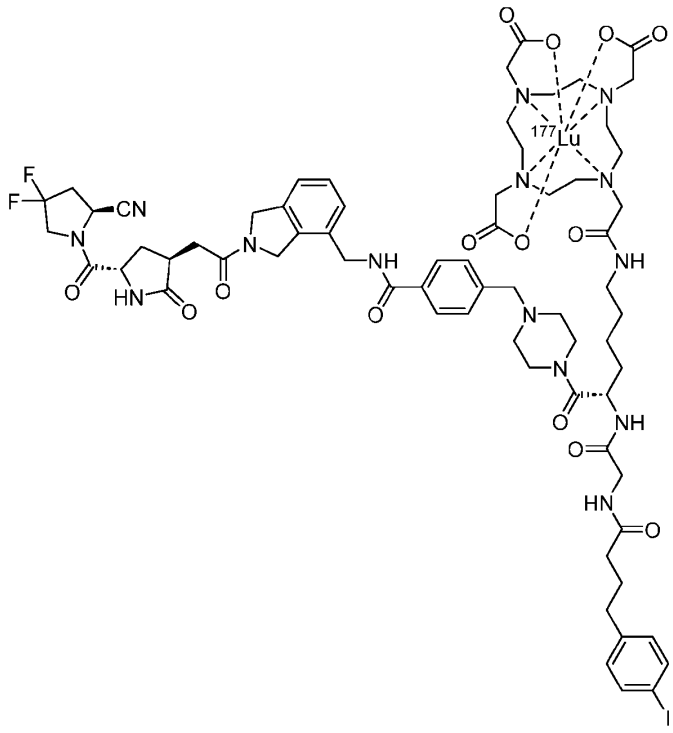
^{177}**Lu-Compound 4**,



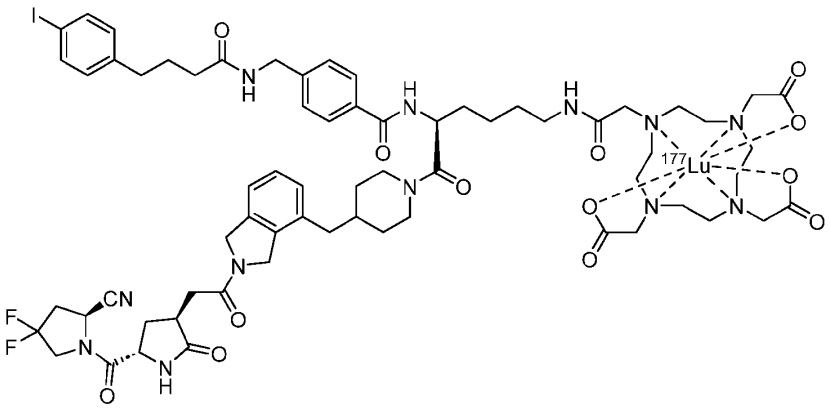
^{177}**Lu-Compound 5**,



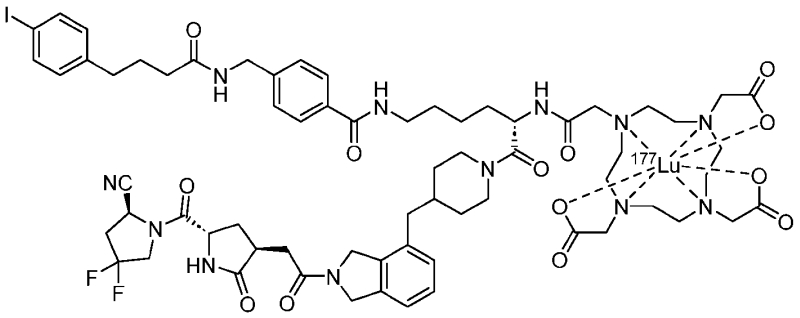
^{177}**Lu-Compound 6**,



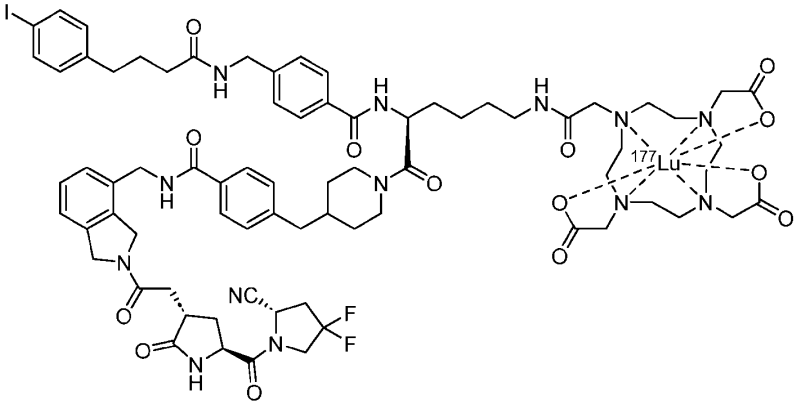
^{177}**Lu-Compound 7**,



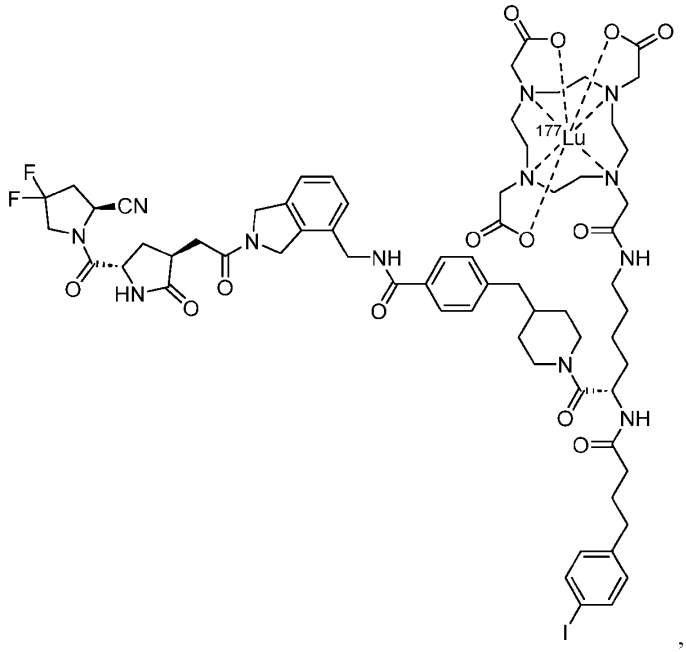
^{177}**Lu-Compound 8**,



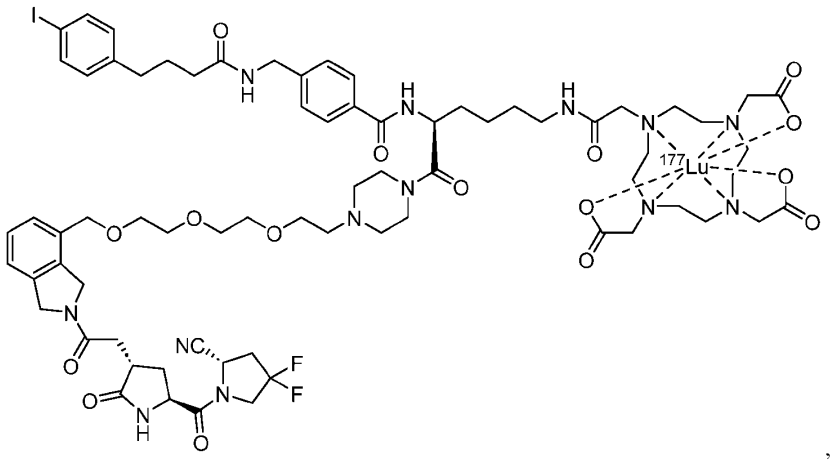
^{177}**Lu-Compound 9**,



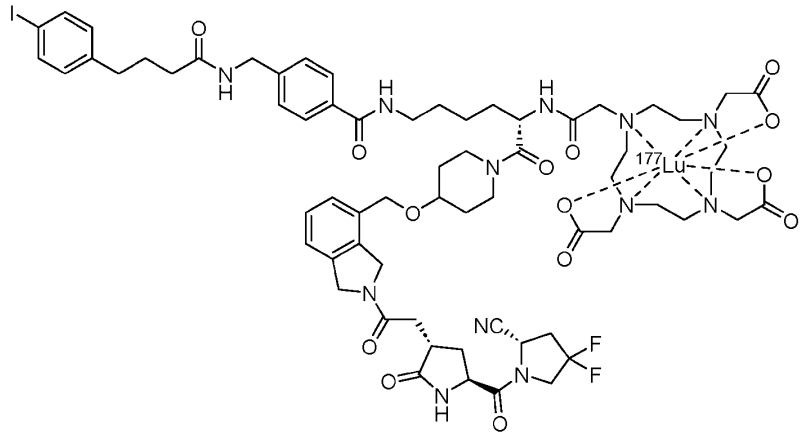
^{177}**Lu-Compound 10**,



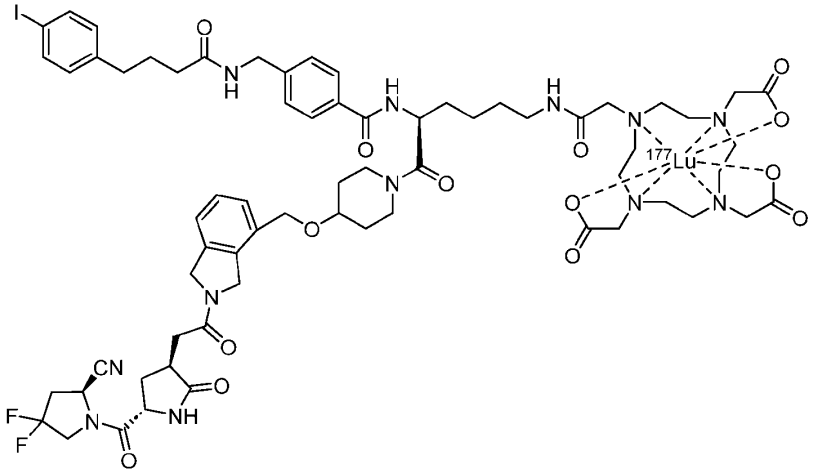
^{177}**Lu-Compound 11**,



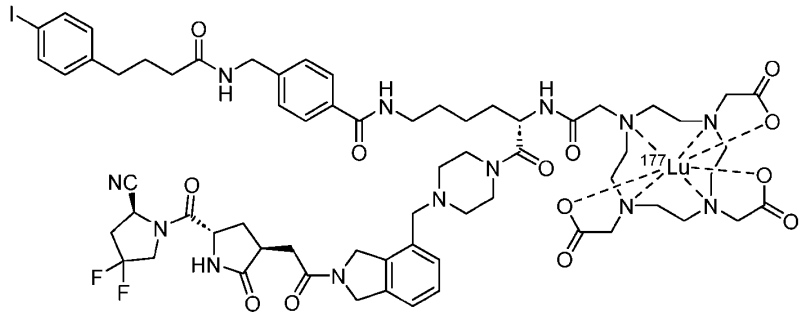
^{177}**Lu-Compound 12**,



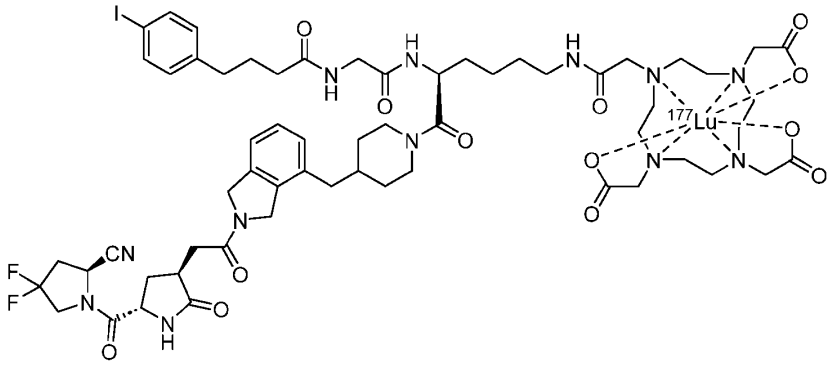
^{177}**Lu-Compound 13**,



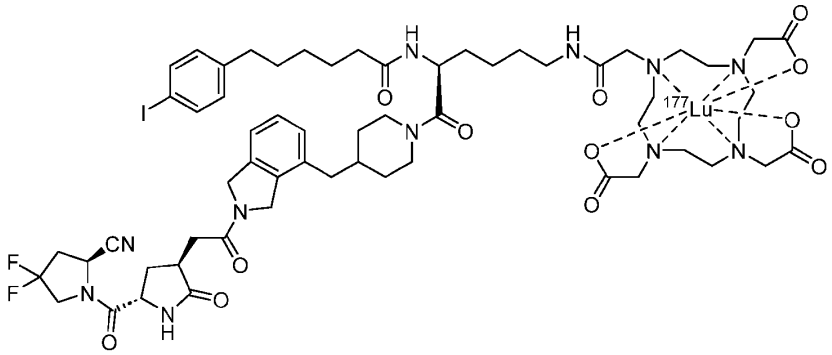
^{177}**Lu-Compound 14**,



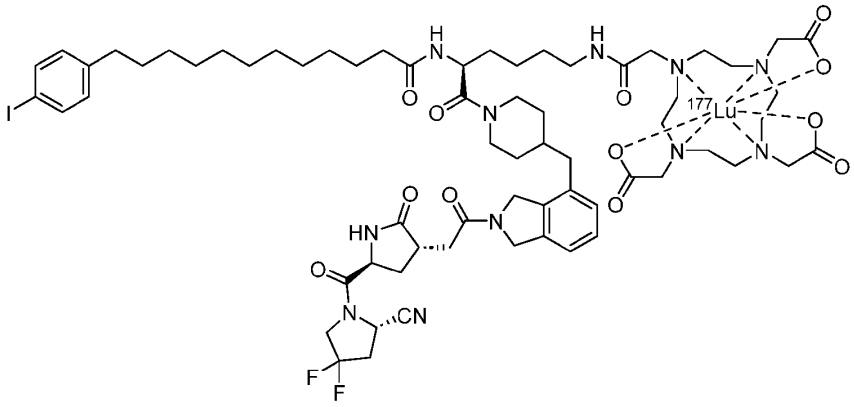
^{177}**Lu-Compound 15**,



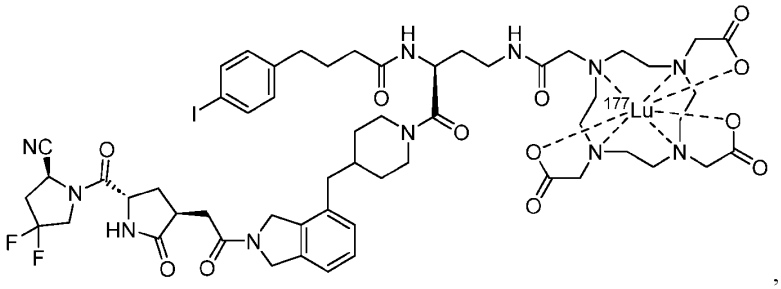
^{177}**Lu-Compound 16**,



^{177}**Lu-Compound 17**,

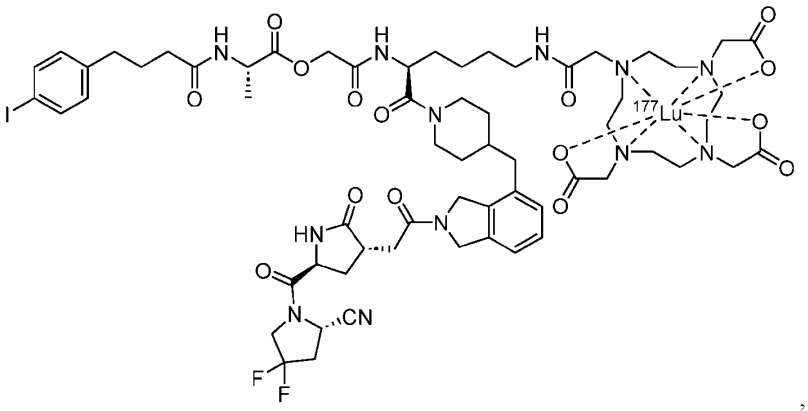


^{177}**Lu-Compound 18**,



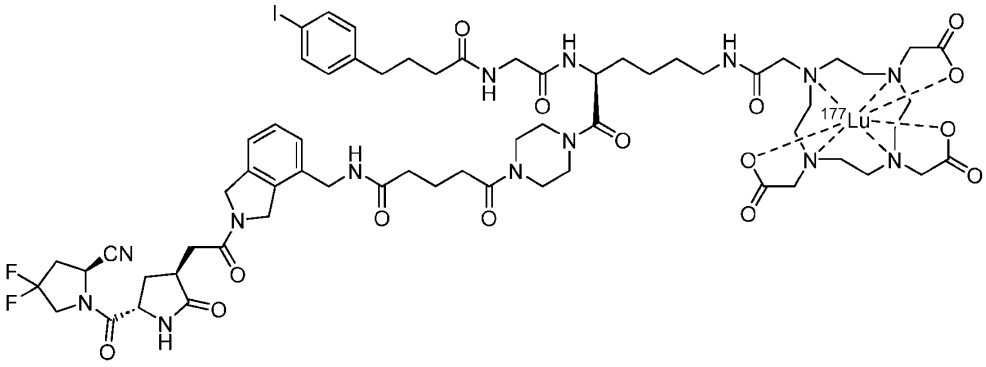
,

^{177}**Lu-Compound 19**,



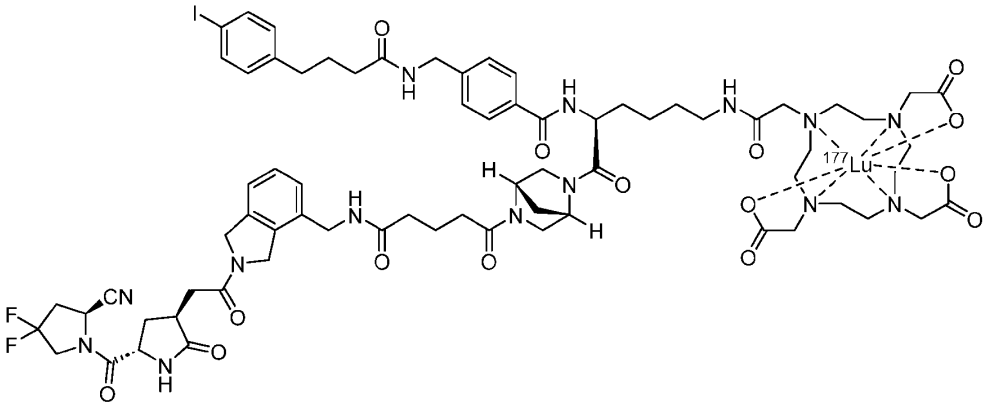
,

^{177}**Lu-Compound 20**,

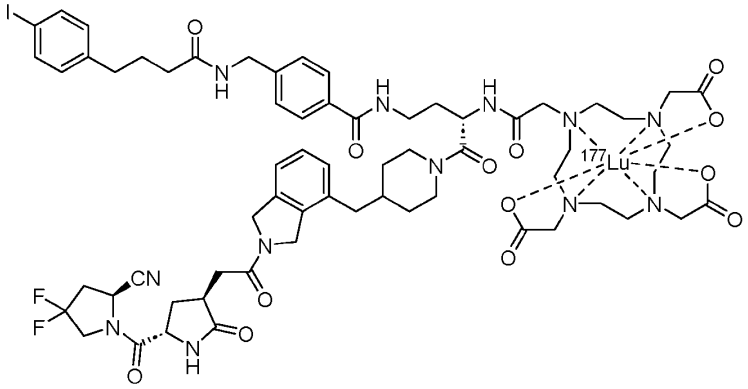


,

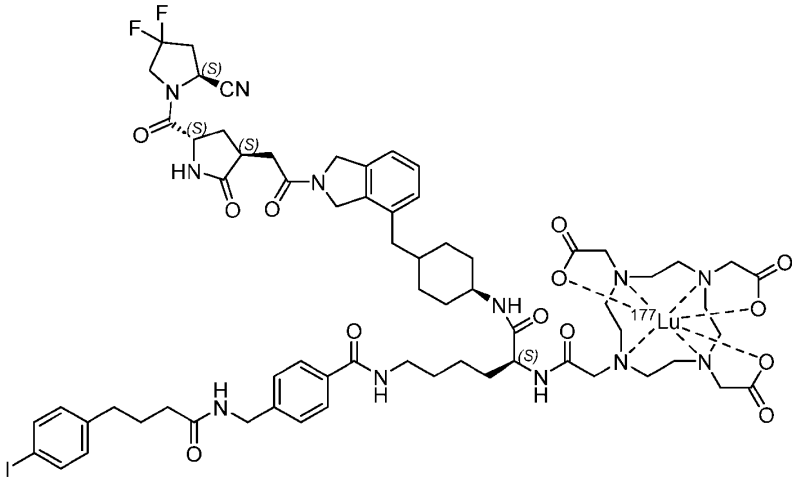
^{177}**Lu-Compound 21**,



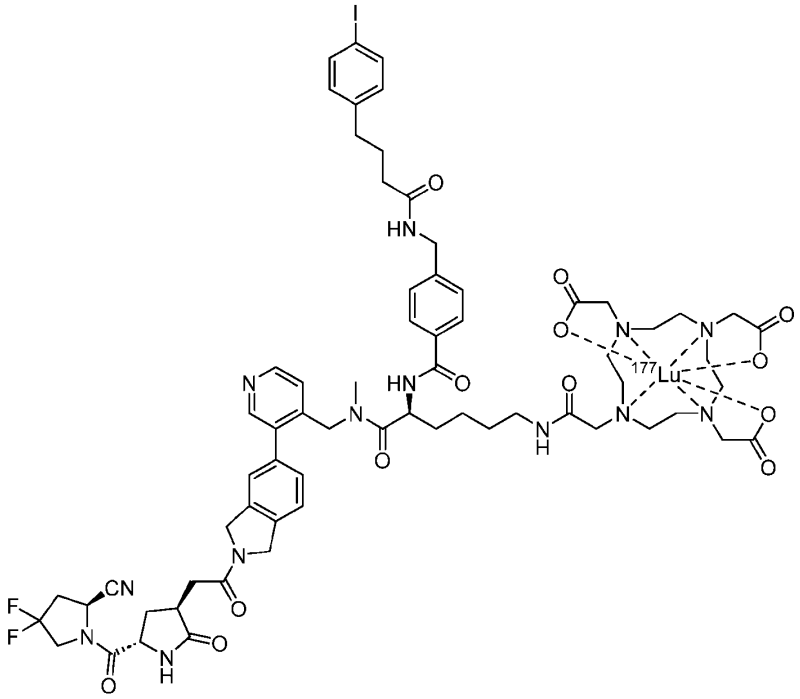
^{177}**Lu-Compound 22**,



^{177}**Lu-Compound 23**,

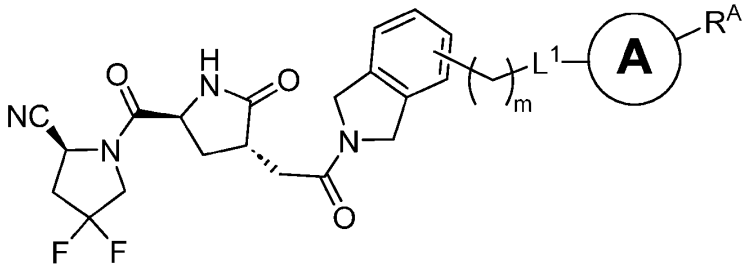


^{177}**Lu-Compound 24**, and



^{177}**Lu-Compound 25**.

[000115] In certain embodiments, provided herein is a compound represented by Formula (I):



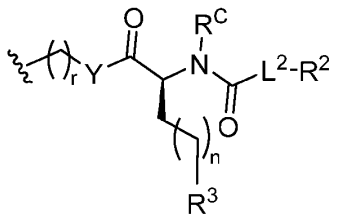
Formula (I)

or a pharmaceutically acceptable salt thereof, wherein:

ring A is selected from the group consisting of 3 to 6-membered heterocyclyl, 6-membered heteroaryl, and C_{3-6} cycloalkyl;

L^1 is selected from a covalent bond and a linker;

R^A is a moiety represented by:



Y is a covalent bond or NR^D;

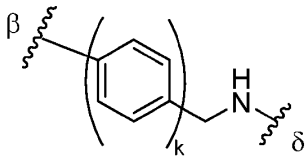
R^C is selected from hydrogen and C_1-C_3 alkyl;

R^D is selected from hydrogen and C_1-C_3 alkyl;

L^2 is selected from the group consisting of:

a covalent bond, and

a moiety represented by:



wherein β represents the point of attachment to the carbonyl group and δ represents the point of attachment to R^2;

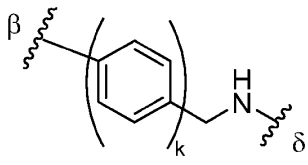
each of R^2 and R^3 is independently selected from an albumin binding ligand and an unchelated ligand capable of chelating to or covalently bonding to a therapeutic radionuclide or a diagnostic radionuclide wherein at least one of R^2 and R^3 is an unchelated ligand capable of chelating to or covalently bonding to therapeutic radionuclide or a diagnostic radionuclide;

each of k, m, and r is independently 0 or 1; and

n is an integer from 1 to 6;

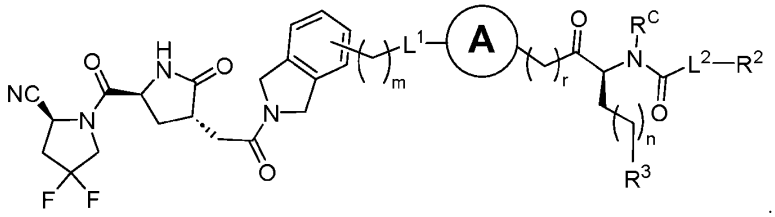
provided that:

when L^1 is a covalent bond, m is 1, L^2 is a moiety represented by:



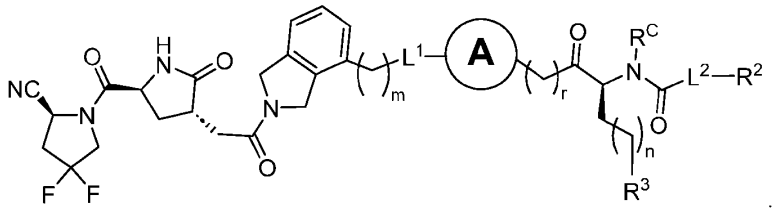
, and A is piperazinyl having a ring nitrogen attached to L^1, then k is 0.

[000116] In some embodiments, the compound of Formula (I) is represented by Formula (I-A):



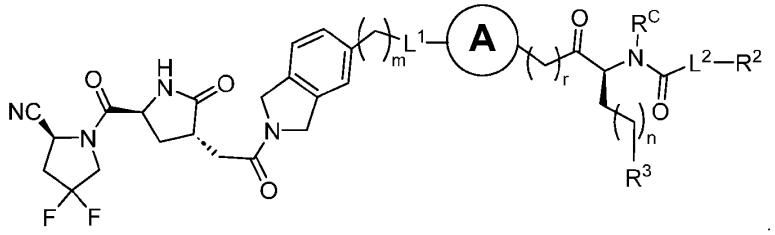
Formula (I-A).

[000117] In some embodiments, the compound of Formula (I) is represented by Formula (I-B):



Formula (I-B).

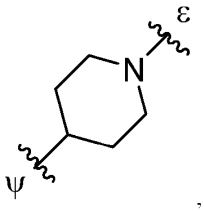
[000118] In some embodiments, the compound of Formula (I) is represented by Formula (I-C):

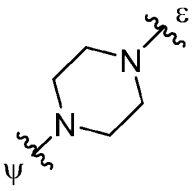


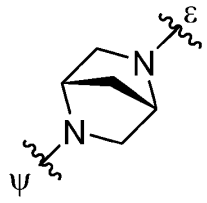
Formula (I-C).

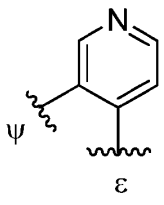
[000119] In some embodiments, ring A is 6-membered heterocyclyl. In some embodiments, ring A is 6-membered heteroaryl. In some embodiments, ring A is cyclohexyl.

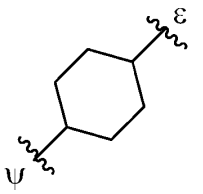
[000120] In some embodiments, wherein ring A is selected from the group consisting of:



,


,


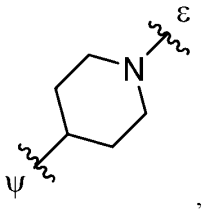
,


, and


,

wherein each ψ represents the point of attachment to L^1, and each ε represents the point of attachment to R^A.

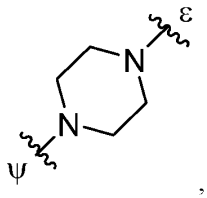
[000121] In some embodiments, ring A is:



,

wherein ψ represents the point of attachment to L^1, and ε represents the point of attachment to R^A.

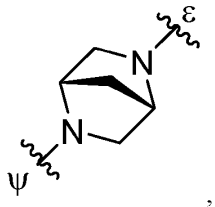
[000122] In some embodiments, ring A is:



,

wherein ψ represents the point of attachment to L^1, and ε represents the point of attachment to R^A.

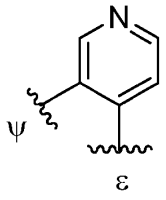
[000123] In some embodiments, ring A is:



,

wherein ψ represents the point of attachment to L^1, and ε represents the point of attachment to R^A.

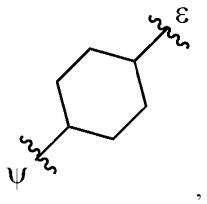
[000124] In some embodiments, ring A is:



,

wherein ψ represents the point of attachment to L^1, and ε represents the point of attachment to R^A.

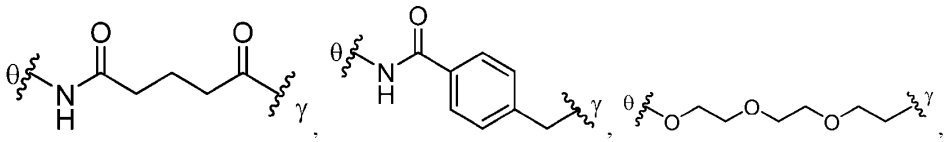
[000125] In some embodiments, ring A is:



,

wherein ψ represents the point of attachment to L^1, and ε represents the point of attachment to R^A.

[000126] In some embodiments, L^1 is a covalent bond. In some embodiments, L^1 is a linker. In some embodiments, L^1 is a linker selected from the group consisting of:



and -O-,

wherein each θ is the point of attachment to the methylene group and each γ is the point of attachment to ring A.

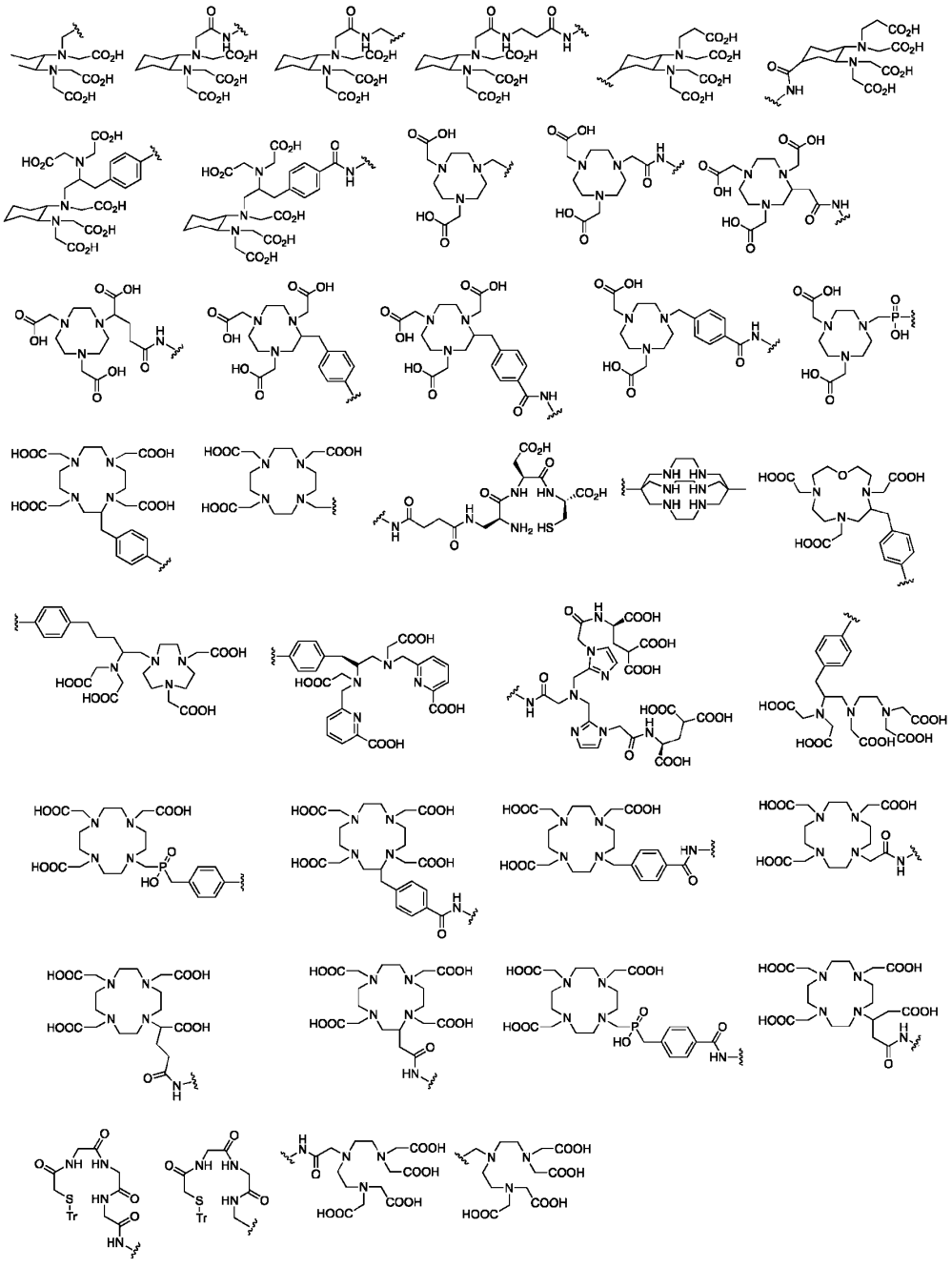
[000127] In some embodiments, m is 0. In some embodiments, m is 1.

[000128] In some embodiments, n is 3.

[000129] In some embodiments, r is 0. In some embodiments, r is 1.

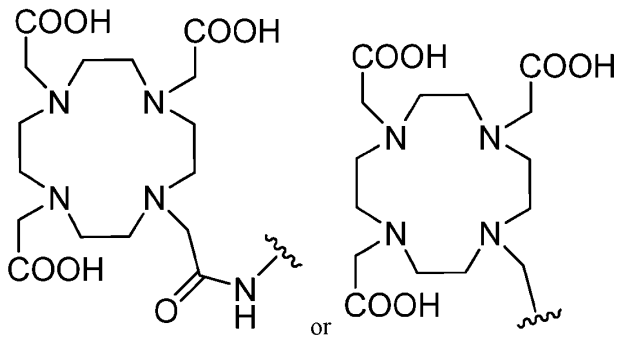
[000130] In some embodiments, k is 0. In some embodiments, k is 1.

[000131] In some embodiments, one of R^2 and R^3 is an unchelated ligand capable of chelating to or covalently binding to a therapeutic radionuclide or a diagnostic radionuclide selected from the group consisting of:



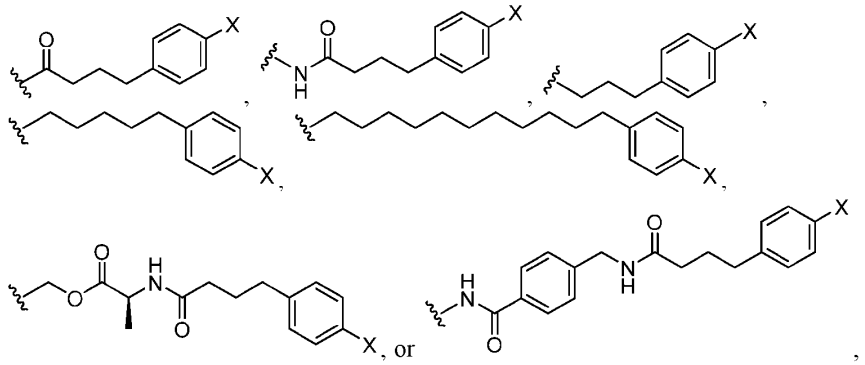
.

[000132] In some embodiments, one of R^2 and R^3 is an unchelated ligand capable of chelating to or covalently binding to a therapeutic radionuclide or a diagnostic radionuclide, represented by:



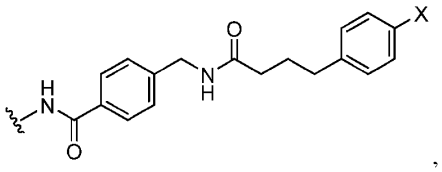
or

[000133] In some embodiments, one of R^2 and R^3 is an albumin binding ligand represented by:



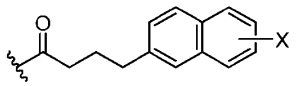
wherein X is selected from the group consisting of halogen and C_{1-6} alkyl.

[000134] In some embodiments, one of R^2 and R^3 is an albumin binding ligand represented by:



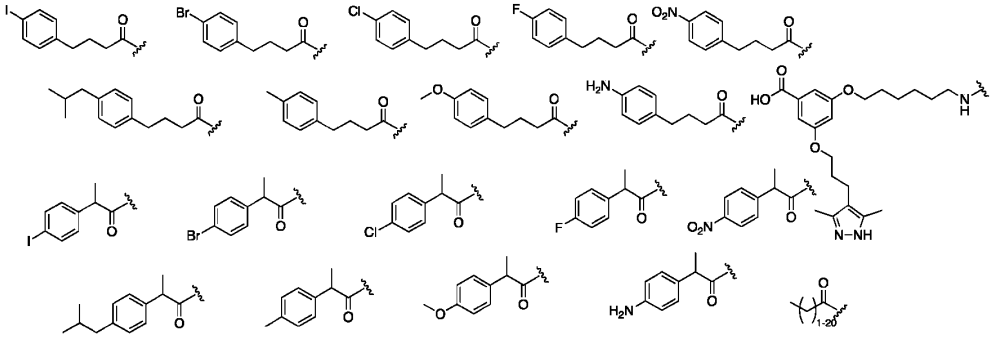
wherein X is selected from hydrogen, halogen, and C_{1-6} alkyl.

[000135] In some embodiments, one of R^2 and R^3 is an albumin binding ligand represented by:



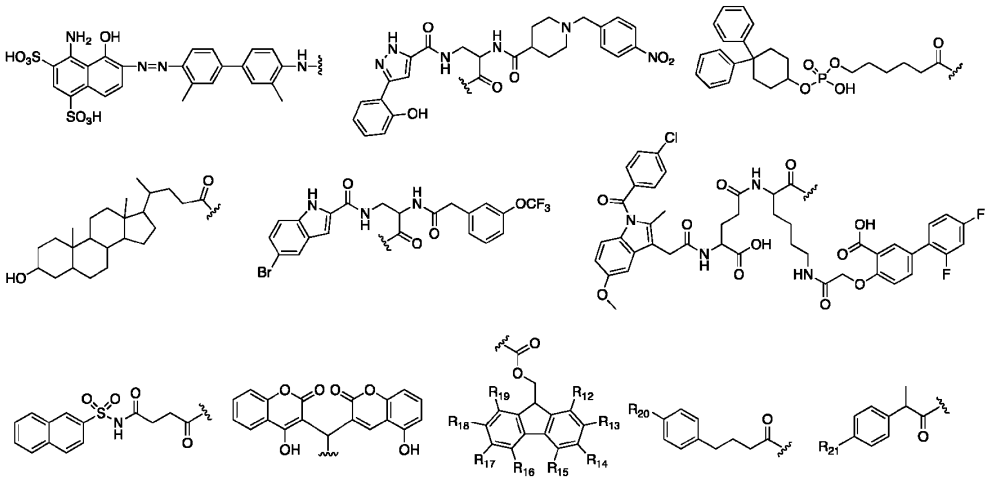
wherein X is selected from the group consisting of hydrogen, halogen and C_{1-6} alkyl.

[000136] In some embodiments, an albumin binding ligand disclosed herein (e.g., an albumin binding ligand of R^2 or R^3) may be selected from the group consisting of



.

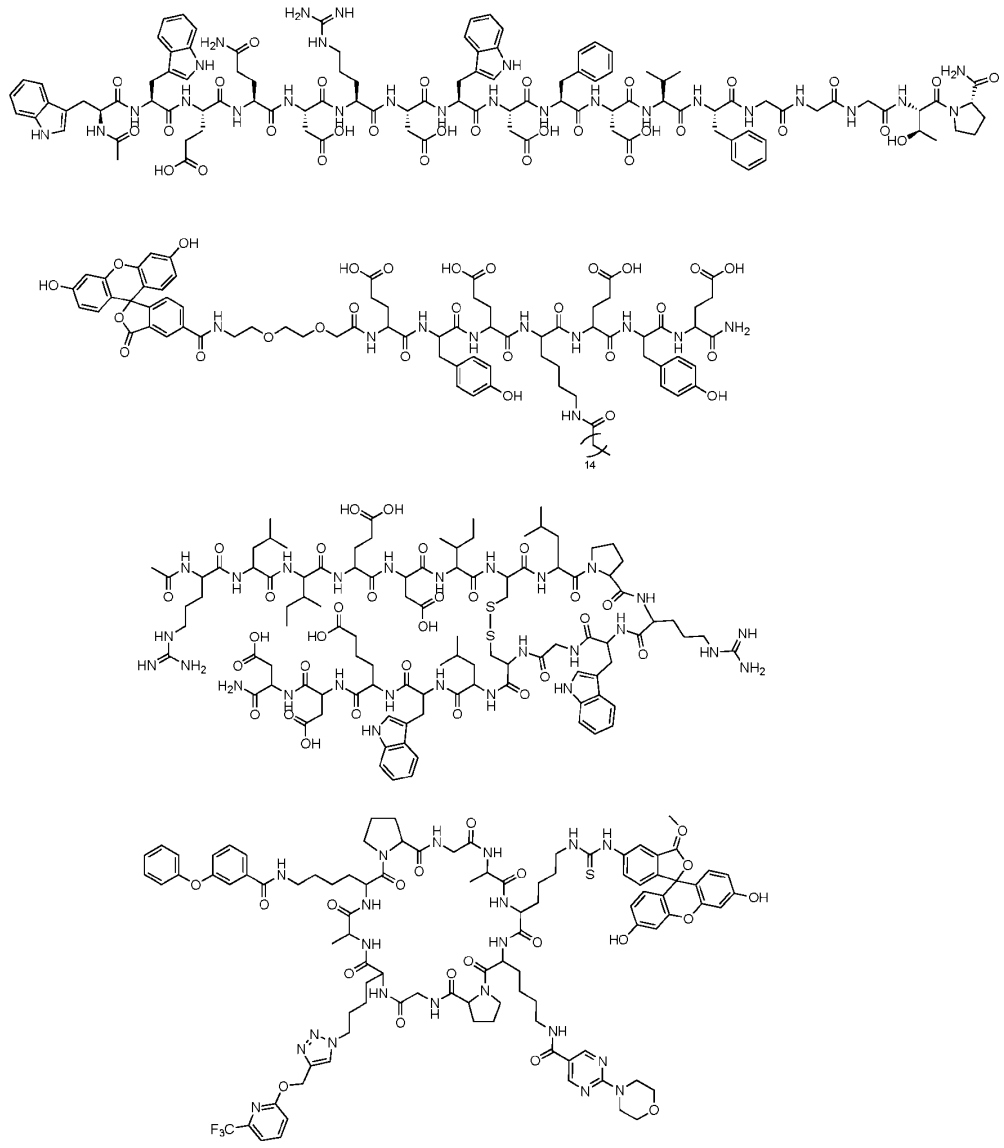
[000137] In some embodiments, an albumin binding ligand disclosed herein (e.g., an albumin binding ligand of R^2 or R^3) may be selected from the group consisting of



,

wherein R_{12-19} are each independently -H, -C_1-C_6 alkyl, -F, -Cl, -Br, -I, -CN, -CHO, -$B(OH)_2$, -C(O)alkyl, -C(O)aryl-, -C=C-C(O)aryl, -C=C-$S(O)_2$aryl, -CO_2H, -SO_3H, -SO_2NH_2, -PO_3H_2, or -SO_2F; R_{20} and R_{21} is independently -H, -C_1-C_6 alkyl, -F, -Cl, -Br, -I, -O-C_{1-6} alkyl, -CN, -CHO, -$B(OH)_2$, -C=C-C(O)aryl, -C=C-$S(O)_2$aryl, -CO_2H, -SO_3H, -SO_2NH_2, -PO_3H_2, -SO_2F, or CF_3.

[000138] In some embodiments, an albumin binding ligand disclosed herein (e.g., an albumin binding ligand of R^2 or R^3) may be an albumin binding peptide selected from the group consisting of



.

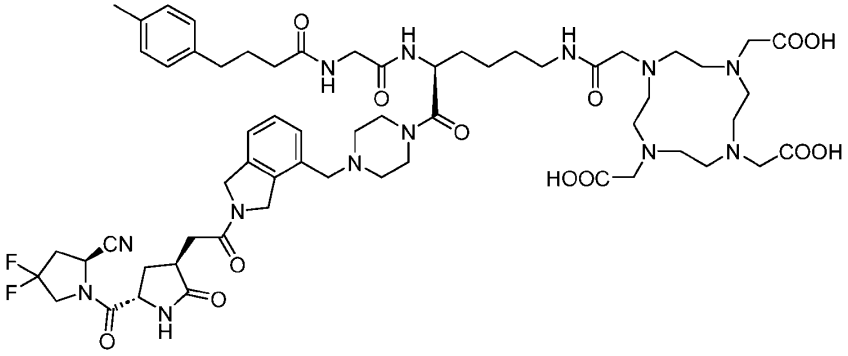
[000139] In some embodiments, an albumin binding ligand disclosed herein (e.g., an albumin binding ligand of R^2 or R^3) may be an albumin binding protein selected from the group consisting of ABD035, ABDCon, DARPins, dsFv CA645, Nanobody, and VNAR (E06).

[000140] In some embodiments, X is iodo.

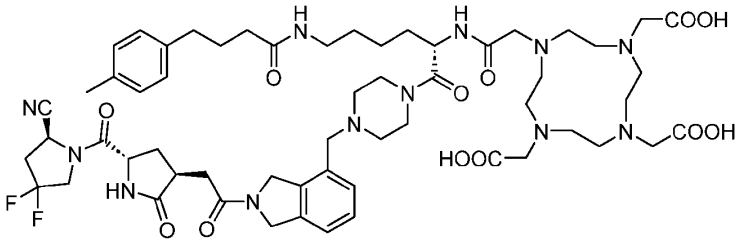
[000141] In some embodiments, R^C is hydrogen.

[000142] In some embodiments, when one of R^2 and R^3 is the albumin binding ligand, the other is the unchelated ligand capable of chelating to or covalently binding to a therapeutic radionuclide or a diagnostic radionuclide.

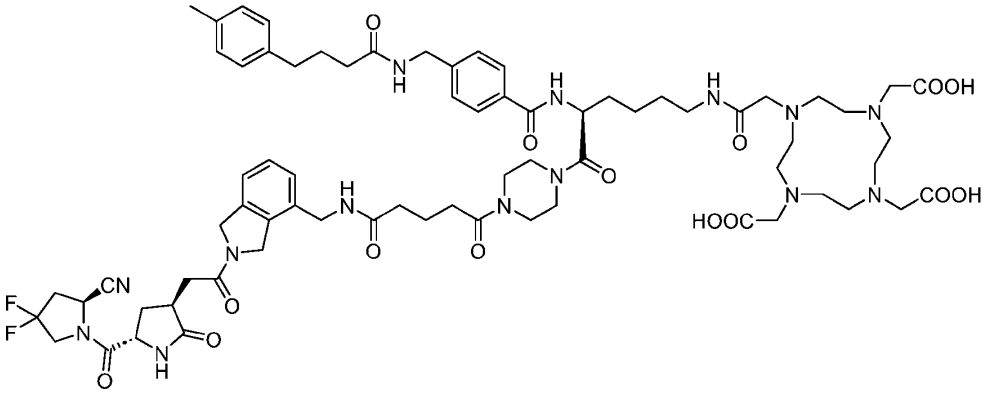
[000143] In certain embodiments, provided herein is a compound selected from the group consisting of



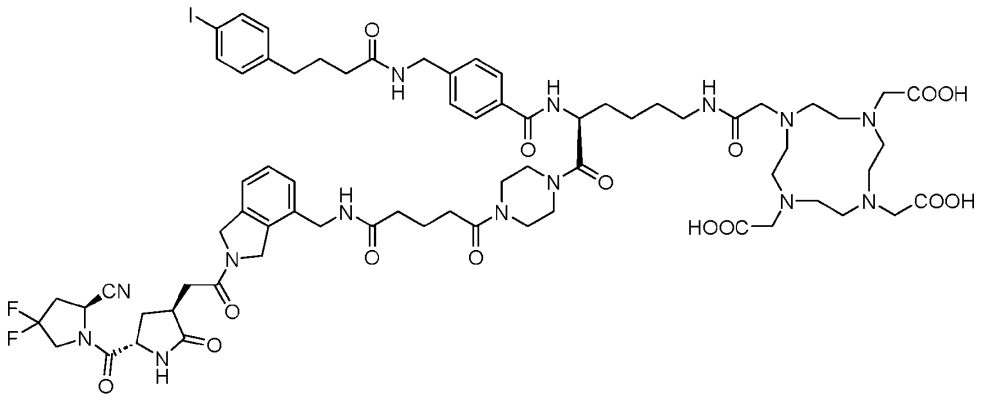
Compound 1,



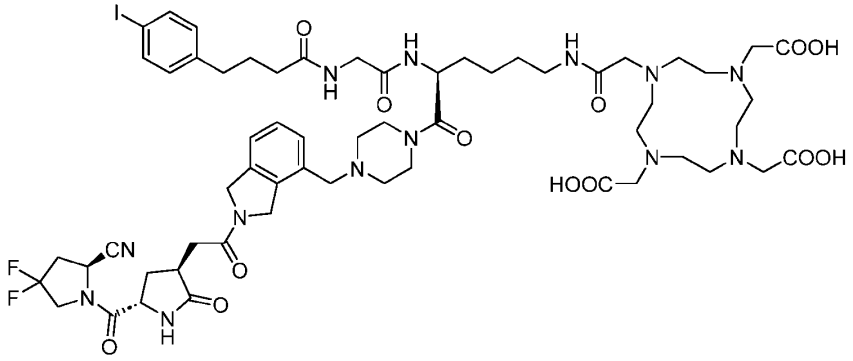
Compound 2,



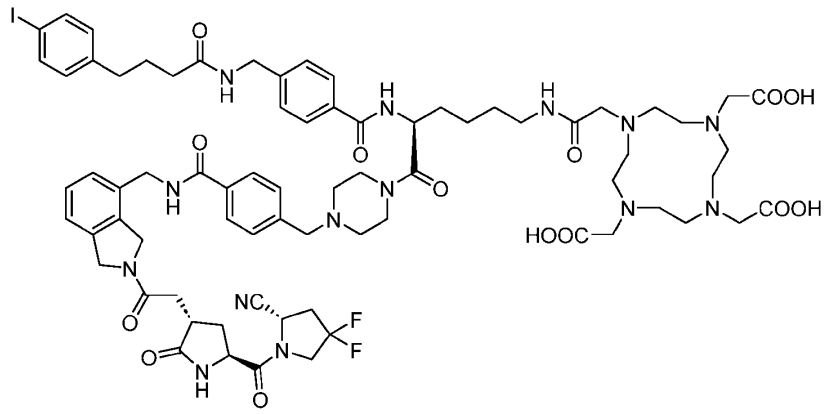
Compound 3,



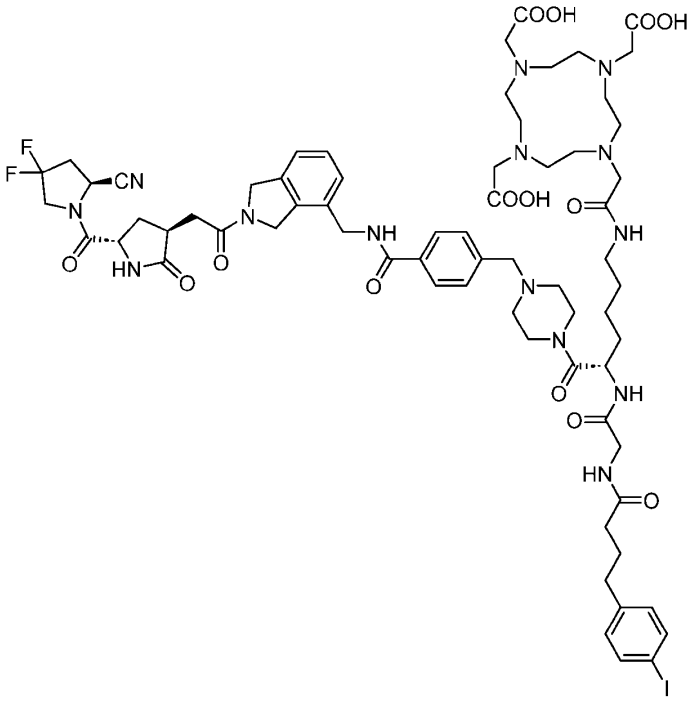
Compound 4,



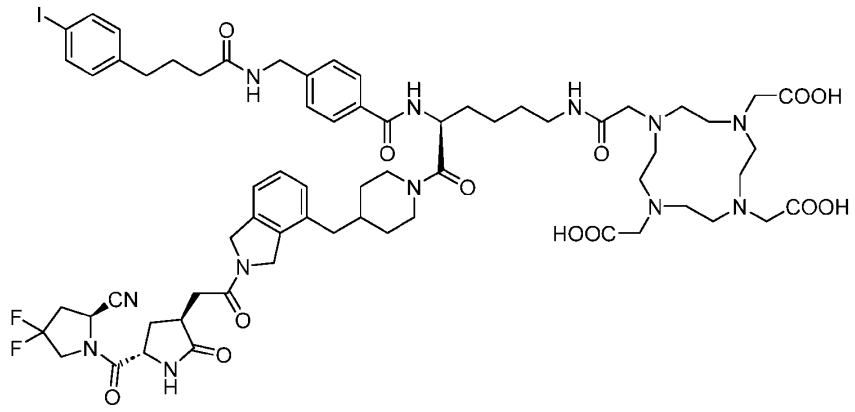
Compound 5,



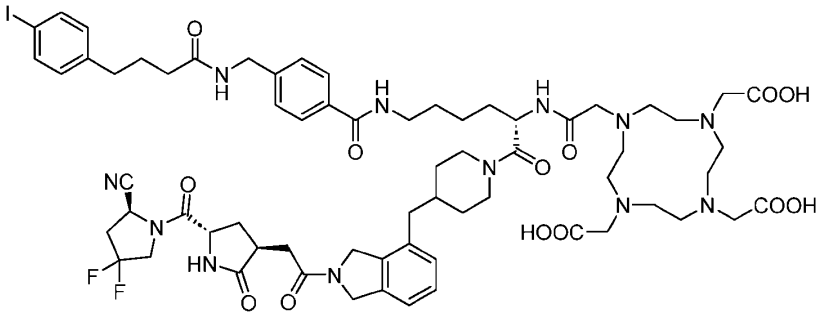
Compound 6,



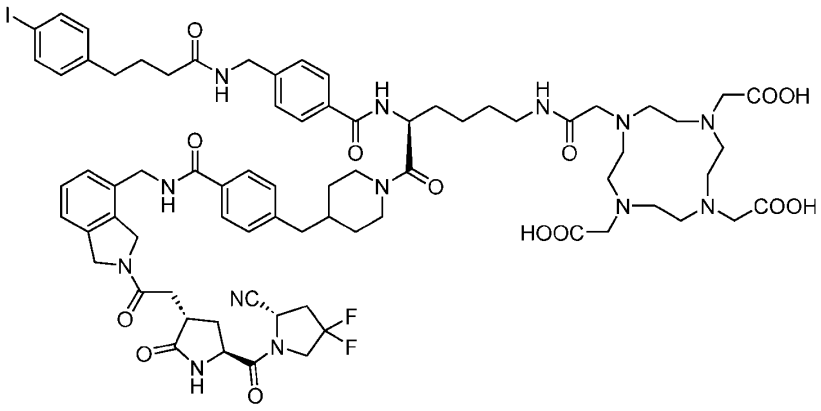
Compound 7,



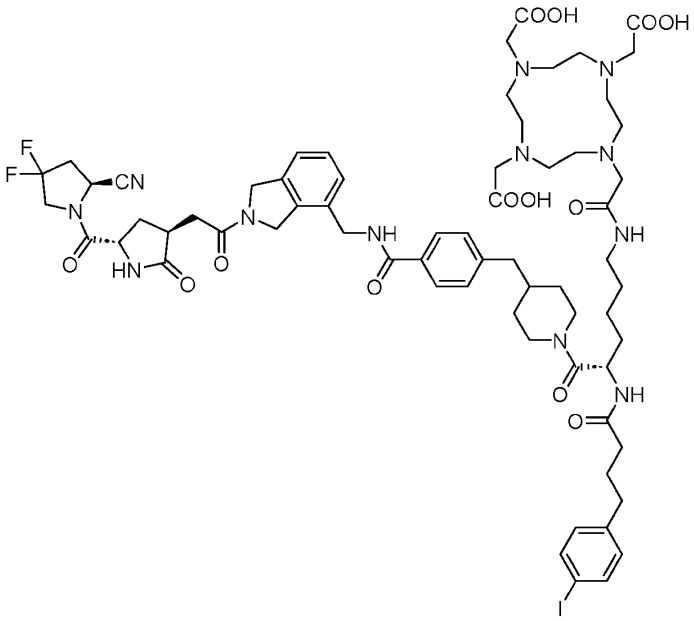
Compound 8,



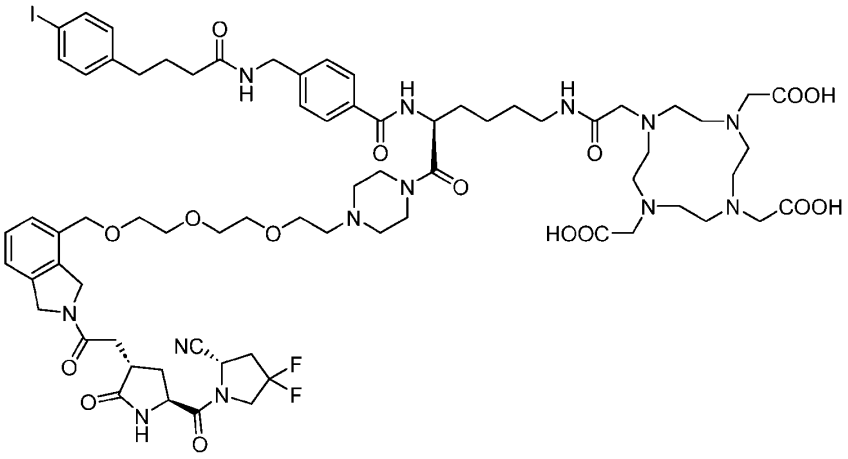
Compound 9,



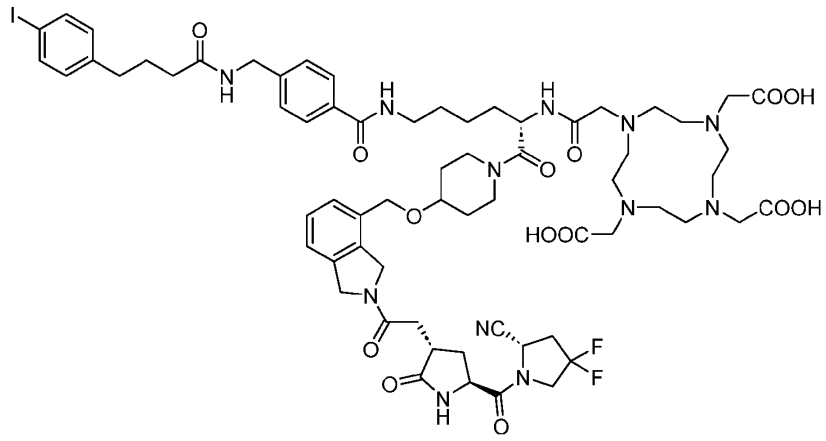
Compound 10,



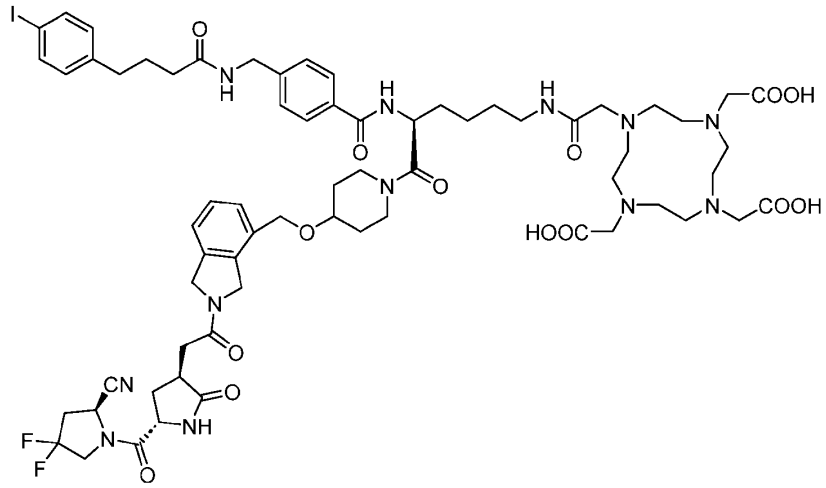
Compound 11,



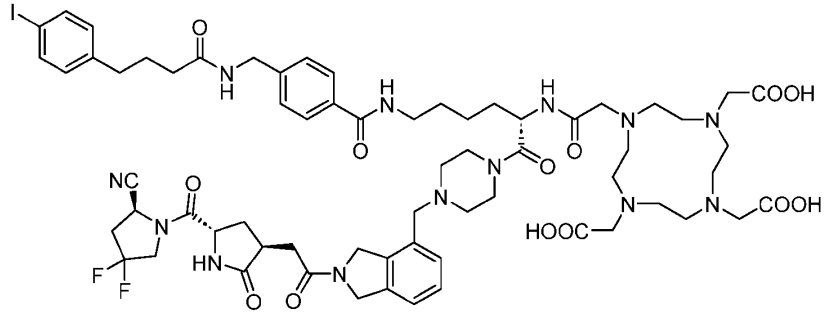
Compound 12,



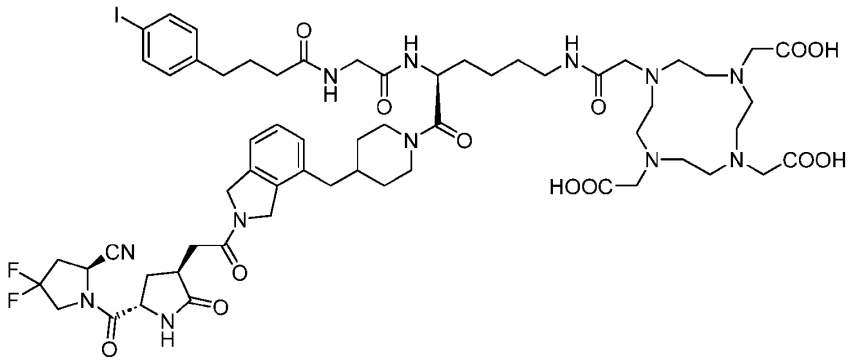
Compound 13,



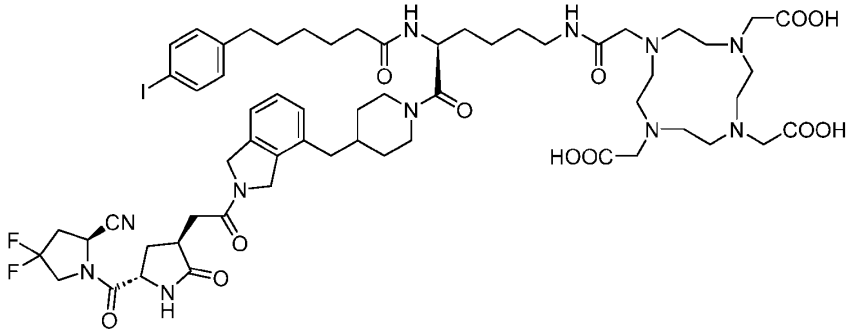
Compound 14,



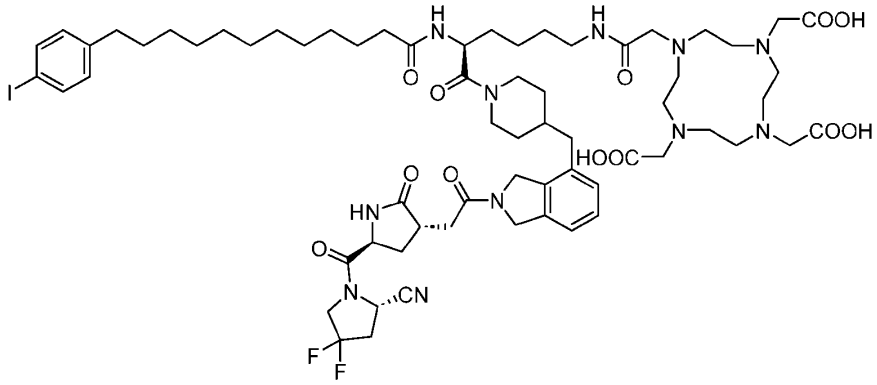
Compound 15,



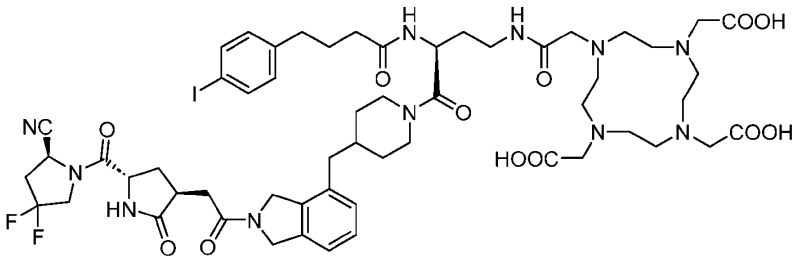
Compound 16,



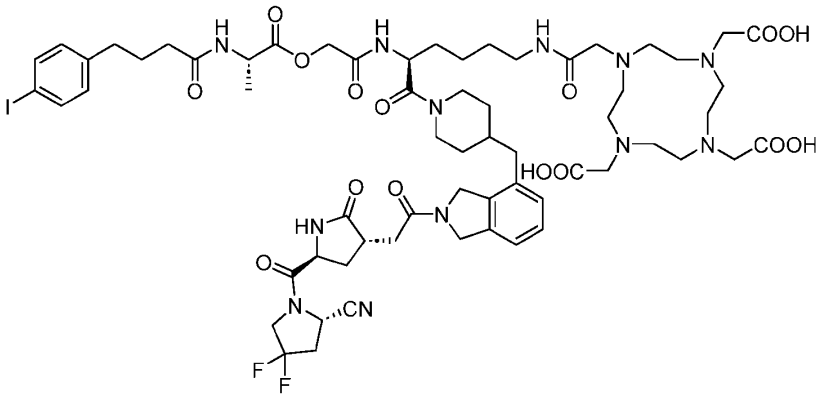
Compound 17,



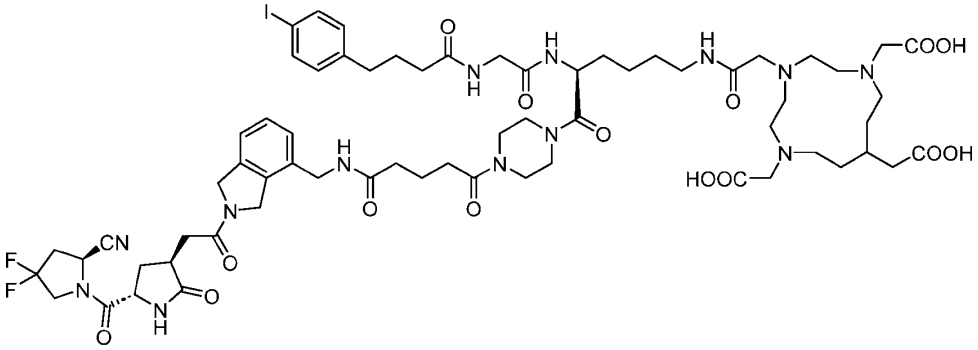
Compound 18,



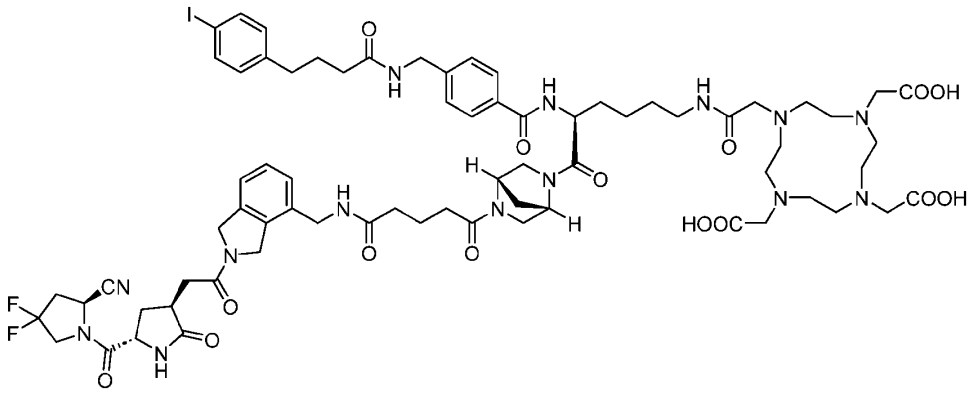
Compound 19,



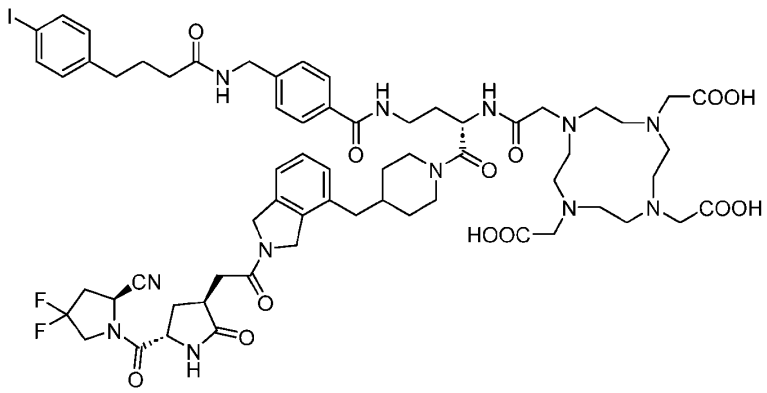
Compound 20,



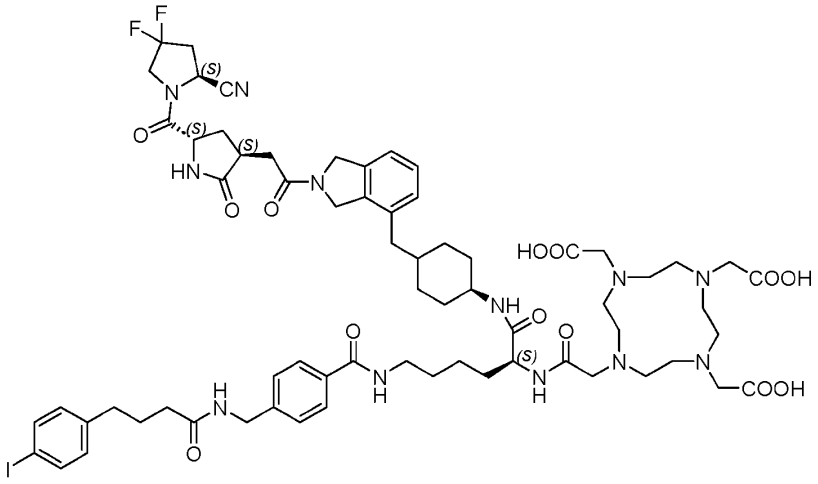
Compound 21,



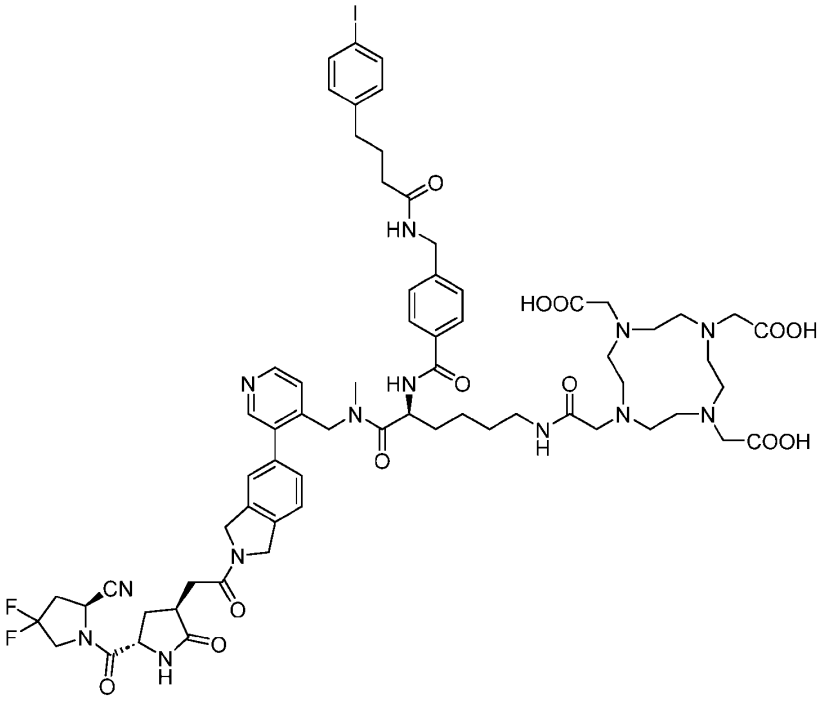
Compound 22,



Compound 23,



Compound 24, and



Compound 25,

or a pharmaceutically acceptable salt thereof.

Pharmaceutical Compositions

[000144] Compounds provided in accordance with the present disclosure, in certain embodiments, are administered in the form of pharmaceutical compositions. This disclosure therefore provides, in certain embodiments, pharmaceutical compositions that comprise, as the active ingredient, one or more of the compounds described, or a pharmaceutically acceptable salt thereof, and one or more pharmaceutically acceptable excipients, including inert solid diluents and fillers, diluents, including sterile aqueous solution and various organic solvents, permeation enhancers, solubilizers and adjuvants. In certain embodiments, the pharmaceutical compositions are administered in combination with other therapeutic agents.

[000145] Provided herein, in certain embodiments, is a pharmaceutical composition, comprising: a) a compound disclosed herein, or a pharmaceutically acceptable salt thereof; and b) a pharmaceutically acceptable excipient.

[000146] In certain embodiments, the pharmaceutical compositions are administered in a single dose by any of the accepted modes of administration of agents having similar utilities, including rectal, buccal, intranasal and transdermal routes, by intra-arterial injection, intravenously, intraperitoneally, parenterally, intramuscularly, subcutaneously, orally, topically, as an inhalant, or via an impregnated or coated device such as a stent, for example, or an artery-inserted cylindrical polymer. In certain embodiments, the pharmaceutical compositions are administered in multiple doses.

[000147] One mode for administration is parenteral, for example by injection. Administration by injection comprises, in some embodiments, aqueous or oil suspensions, or emulsions, with sesame oil, corn oil, cottonseed oil, or peanut oil, as well as elixirs, mannitol, dextrose, or a sterile aqueous solution, and similar pharmaceutical vehicles. Aqueous solutions in saline are also conventionally used for injection. In some embodiments, compositions suitable for injection comprise ethanol, glycerol, propylene glycol, liquid polyethylene glycol, or the like (and suitable mixtures thereof), cyclodextrin derivatives, or vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, or the like.

[000148] Sterile injectable solutions are prepared by incorporating a compound according to the present disclosure in the required amount in the appropriate solvent with various other ingredients as enumerated above, as required, followed by filtered sterilization.

Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[000149] Oral administration is another route for administration of compounds in accordance with the disclosure. Oral administration includes, for example, capsule or enteric coated tablets, or the like. In making the pharmaceutical compositions that include at least one compound described herein, the active ingredient is, in some embodiments, diluted by an excipient and/or enclosed within such a carrier that can be in the form of a capsule, sachet, paper or other container. When the excipient serves as a diluent, it can be in the form of a solid, semi-solid, or liquid material (as above), which acts as a vehicle, carrier or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols (as a solid or in a liquid medium), ointments comprising the active compound, soft and hard gelatin capsules, sterile injectable solutions, or sterile packaged powders.

[000150] The compositions of the disclosure can be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures known in the art. Controlled release drug delivery systems for oral administration include osmotic pump systems and dissolutional systems containing polymer-coated reservoirs or drug-polymer matrix formulations. Another formulation for use in the methods of the present disclosure employs transdermal delivery devices ("patches"). Such transdermal patches may be used to provide continuous or discontinuous infusion of the compounds of the present disclosure in controlled amounts. Such patches may be constructed for continuous, pulsatile, or on demand delivery of pharmaceutical agents.

[000151] The compositions are preferably formulated in a unit dosage form. The term "unit dosage forms" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical excipient (*e.g.*, a tablet, capsule, or ampoule). The compounds are generally administered in a pharmaceutically effective amount. It will be understood, however, that the amount of the compound actually administered usually will be determined by a physician, in the light of the relevant circumstances, including the condition to be treated, the chosen route

of administration, the actual compound administered and its relative activity, the age, weight, and response of the individual patient, the severity of the patient's symptoms, and the like.

[000152] For preparing solid compositions such as tablets, the principal active ingredient is mixed with a pharmaceutical excipient to form a solid preformulation composition containing a homogeneous mixture of a compound of the present disclosure. When referring to these preformulation compositions as homogeneous, it is meant that the active ingredient is dispersed evenly throughout the composition so that the composition may be readily subdivided into equally effective unit dosage forms such as tablets, pills and capsules.

[000153] In certain embodiments, the tablets or pills of the present disclosure are coated or otherwise compounded to provide a dosage form affording the advantage of prolonged action, or to protect from the acid conditions of the stomach. For example, the tablet or pill can comprise an inner dosage and an outer dosage component, the latter being in the form of an envelope over the former. The two components can be separated by an enteric layer that serves to resist disintegration in the stomach and permit the inner component to pass intact into the duodenum or to be delayed in release. A variety of materials can be used for such enteric layers or coatings, such materials including a number of polymeric acids and mixtures of polymeric acids with such materials as shellac, cetyl alcohol, and cellulose acetate.

[000154] Compositions for inhalation or insufflation include solutions and suspensions in pharmaceutically acceptable, aqueous or organic solvents, or mixtures thereof, and powders. In some embodiments, the compositions are administered by the oral or nasal respiratory route for local or systemic effect. Compositions in preferably pharmaceutically acceptable solvents may be nebulized by use of inert gases. Nebulized solutions may be inhaled directly from the nebulizing device or the nebulizing device may be attached to a facemask tent, or intermittent positive pressure breathing machine. Solution, suspension, or powder compositions may be administered, preferably orally or nasally, from devices that deliver the formulation in an appropriate manner.

Methods of Use

[000155] In certain embodiments, provided herein is a method of treating cancer in a patient in need thereof, comprising administering to the patient a therapeutically effective amount of a compound described herein (e.g., a complex of Formula (I) described herein, e.g., a complex of Formula (I-A), Formula (I-B), or Formula (I-C)). In certain embodiments, the cancer is selected from the group consisting of breast cancer, pancreatic cancer,

mesothelioma, esophageal cancer, cholangiocellular carcinoma, head and neck cancer, carcinoma of unknown primary origin, brain metastases, sarcoma, salivary gland carcinoma, lung cancer, prostate cancer, thymus cancer, ovarian cancer, desmoid, chordoma, colorectal cancer, anal carcinoma, neuroendocrine tumors, small intestine cancer, medullary thyroid cancer, cervical cancer, hepatocellular carcinoma, gastric cancer, adenoid cystic cancer, pheochromocytoma, differentiated thyroid cancer, insulinoma, renal cell cancer, gastric cancer, renal cancer, bone metastases, lymph node metastases, lung metastases, pleura, bladder cancer, myeloma, lymphoma, brain cancer, endometrial cancer, testicular cancer, neuroblastoma, astrocytoma, meningioma, rectal cancer, cervical cancer, stomach cancer, skin cancer, thyroid cancer, and/or liver cancer.

[000156] In certain embodiments, provided herein is a method of treating a disease mediated by cancer-associated fibroblasts in a patient in need thereof, comprising administering to the patient a therapeutically effective amount of a compound described herein (e.g., a complex of Formula (I) described herein, e.g., a complex of Formula (I-A), Formula (I-B), or Formula (I-C)). In certain embodiments, the disease is cancer. In certain embodiments, the cancer is selected from the group consisting of breast cancer, pancreatic cancer, mesothelioma, esophageal cancer, cholangiocellular carcinoma, head and neck cancer, carcinoma of unknown primary origin, brain metastases, sarcoma, salivary gland carcinoma, lung cancer, prostate cancer, thymus cancer, ovarian cancer, desmoid, chordoma, colorectal cancer, anal carcinoma, neuroendocrine tumors, small intestine cancer, medullary thyroid cancer, cervical cancer, hepatocellular carcinoma, gastric cancer, adenoid cystic cancer, pheochromocytoma, differentiated thyroid cancer, insulinoma, renal cell cancer, gastric cancer, renal cancer, bone metastases, lymph node metastases, lung metastases, pleura, bladder cancer, myeloma, lymphoma, brain cancer, endometrial cancer, testicular cancer, neuroblastoma, astrocytoma, meningioma, rectal cancer, cervical cancer, stomach cancer, skin cancer, thyroid cancer, and/or liver cancer.

[000157] In certain embodiments, provided herein is a method of imaging a cancer-associated fibroblast in a patient in need thereof, comprising administering to the patient an effective amount of a compound described herein (e.g., a complex of Formula (I) described herein, e.g., a complex of Formula (I-A), Formula (I-B), or Formula (I-C)), and identifying the location of the cancer-associated fibroblast in the patient.

[000158] In certain embodiments, provided herein is a method of imaging activated fibroblasts in a patient with idiopathic pulmonary fibrosis, cirrhosis or liver fibrosis, Crohn's disease, rheumatoid arthritis, ulcerative colitis, sclerosis, cardiac fibrosis, and/or kidney

fibrosis in need thereof, comprising administering to the patient an effective amount of a compound described herein (e.g., a complex of Formula (I) described herein, e.g., a complex of Formula (I-A), Formula (I-B), or Formula (I-C)), or a pharmaceutically acceptable salt thereof, and identifying the location of activated fibroblasts in the patient for noninvasive diagnosis.

Dosage Regimens

[000159] Dosage regimens may be adjusted to provide the optimum desired response. The skilled artisan would appreciate, based upon the disclosure provided herein, that the dose and dosing regimen is adjusted in accordance with methods well-known in the therapeutic arts. That is, the maximum tolerable dose can be readily established, and the effective amount providing a detectable therapeutic benefit to a patient may also be determined, as can the temporal requirements for administering each agent to provide a detectable therapeutic benefit to the patient. Accordingly, while certain dose and administration regimens are exemplified herein, these examples in no way limit the dose and administration regimen that may be provided to a patient in practicing the present disclosure.

[000160] It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated and may include single or multiple doses. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition. For example, doses may be adjusted based on pharmacokinetic or pharmacodynamic parameters, which may include clinical effects such as toxic effects and/or laboratory values. Thus, the present disclosure encompasses intra-patient dose-escalation as determined by the skilled artisan. Determining appropriate dosages and regimens for administration of the chemotherapeutic agent are well-known in the relevant art and would be understood to be encompassed by the skilled artisan once provided the teachings disclosed herein.

[000161] The amount of the compound of the disclosure administered will be dependent on the subject being treated, the severity of the disorder or condition, the rate of administration, the disposition of the compound and the discretion of the prescribing physician. However, an effective dosage is in the range of about 0.001 to about 100 mg per kg body weight per day. In some instances, dosage levels below the lower limit of the

aforesaid range may be more than adequate, while in other cases still larger doses may be employed without causing any harmful side effect, provided that such larger doses are first divided into several small doses for administration throughout the day.

Kits

[000162] In another aspect provided herein is a kit, comprising materials useful for the treatment or prevention of the diseases and disorders described above. In some embodiments, the kit comprises a container comprising a compound described herein (e.g., a complex of Formula (I) or compound of Formula (I), or a pharmaceutically acceptable salt thereof). In some embodiments, the kit further comprises a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, blister pack, etc. The container can be formed from a variety of materials such as glass or plastic. The container can hold a compound described herein (e.g., a complex of Formula (I) or a compound of Formula (I), or a pharmaceutically acceptable salt thereof) or a composition thereof which is effective for treating or preventing the condition and can have a sterile access port (for example, the container can be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is a compound described herein (e.g., a complex of Formula (I) or a compound of Formula (I), or a pharmaceutically acceptable salt thereof) or a pharmaceutically acceptable salt thereof. The label or package insert indicates that the composition is used for treating the condition of choice. The label or package insert can also indicate that the composition can be used to treat other disorders. Alternatively, or additionally, the article of manufacture can further comprise a second container comprising a pharmaceutically acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It can further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[000163] The kit can further comprise directions for the administration of a compound described herein (e.g., a complex of Formula (I) or a compound of Formula (I), or a pharmaceutically acceptable salt thereof) or a pharmaceutically acceptable salt thereof and, if present, the second pharmaceutical formulation. For example, if the kit comprises a first composition comprising a compound described herein (e.g., a complex of Formula (I) or a compound of Formula (I), or a pharmaceutically acceptable salt thereof) or a pharmaceutically acceptable salt thereof and a second pharmaceutical formulation, the kit can

further comprise directions for the simultaneous, sequential or separate administration of the first and second pharmaceutical compositions to a patient in need thereof.

[000164] In another embodiment, the kits are suitable for the delivery of solid oral forms of a compound described herein (e.g., a complex of Formula (I) or a compound of Formula (I), or a pharmaceutically acceptable salt thereof) or a pharmaceutically acceptable salt thereof, such as tablets or capsules. Such a kit preferably includes a number of unit dosages. Such kits can include a card having the dosages oriented in the order of their intended use. An example of such a kit is a blister pack. Blister packs are well known in the packaging industry and are widely used for packaging pharmaceutical unit dosage forms. If desired, a memory aid can be provided, for example in the form of numbers, letters, or other markings or with a calendar insert, designating the days in the treatment schedule in which the dosages can be administered.

[000165] In some embodiments, a kit comprises (a) a first container with a compound described herein (e.g., a complex of Formula (I) or a compound of Formula (I), or a pharmaceutically acceptable salt thereof) or a pharmaceutically acceptable salt thereof contained therein; and optionally (b) a second container with a second pharmaceutical formulation contained therein. Alternatively, or additionally, the kit can further comprise a third container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It can further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

[000166] In some embodiments wherein the kit comprises a compound described herein (e.g., a complex of Formula (I) or a compound of Formula (I), or a pharmaceutically acceptable salt thereof) or a pharmaceutically acceptable salt thereof and a second therapeutic agent, the kit can comprise a container for containing the separate compositions such as a divided bottle or a divided foil packet, however, the separate compositions can also be contained within a single, undivided container. Typically, the kit comprises directions for the administration of the separate components. The kit form is particularly advantageous when the separate components are preferably administered in different dosage forms (*e.g.*, oral and parenteral), are administered at different dosage intervals, or when titration of the individual components of the combination is desired by the prescribing physician.

EXAMPLES

General synthetic methods

[000167] The following methods were used in the exemplified Examples, except where noted otherwise.

[000168] Purification of intermediates and final products was carried out via either normal or reverse phase chromatography. Normal phase chromatography was carried out using prepacked SiO_2 cartridges eluting with either gradients of hexane and ethyl acetate or DCM and MeOH. Reverse phase was carried out using C18 column with UV 215 nm to 254 nm, eluting with gradients of solvents A (water with 0.1%TFA) and Solvent B (Acetonitrile) or solvents A: water and Solvent B: Acetonitrile. Reverse phase preparative HPLC was carried out using GEMINI C18 (250*21.2*)5u, AGILENT C18 (50*50mm)5u, Shim-pack C18 (250*20.0mm) 5u and Luna C18 (250*80mm 10µ) with UV detection at 215& 254 nm.

LC-MS/HPLC Employed in characterization of examples

[000169] LC-MS was performed on Waters (Acquity H Class plus coupled with SQ Detector 2,) column: Acquity BEH C18 (2.1*50*mm1.7µ), Mobile phase A:0.01% Formic acid in water, Mobile phase B: Acetonitrile, Diluent; water: acetonitrile (9:1), UV 215 nm, injection volume:1 µL, Run time: 6.6 min.

[000170] Mass detector Parameters: Ionisation mode: ESI, Capillary voltage: 2.0 kV, Cone voltage: 35v, Desolation temp: 450°C

[000171] HPLC was performed on Waters (e2695 with PDA Detector) column: Inertsil ODS 3V (250X4.6 mm5µ), Mobile phase A:0.1%TFA in water, Mobile phase B: Acetonitrile: Water (90:10), Diluent; water: acetonitrile (9:1), UV 215 nm, injection volume:7 µL, Run time: 25 min

Prep HPLC employed for purification

[000172] Purification was performed using Shimadzu Nexera Prep with FRC-40 fraction collector; using with methods (A-H)

Method-A:

Column	Kinetex C18 (250*21.2mm 5µ)
Mobile phase-A	0.1% TFA in Water
Mobile phase-B	Acetonitrile: water (80:20)
Gradient (T/%B)	0/20,10/40,15/45,20/95
Flow Rate	13 mL/min
UV Detection	215nm & 254nm
Solubility	Acetonitrile: water (1:9)

Method-B:

Column	Agilent C18 (50*50mm 5µ) + Kinetex C18 (250*21.2mm 5µ)
Mobile phase-A	0.1% TFA in Water
Mobile phase-B	Acetonitrile: water (80:20)
Gradient (T/%B)	0/25,12/38,25/38,25.1/98
Flow Rate	9 mL/min
UV Detection	215nm & 254nm
Solubility	Acetonitrile: water (1:9)

Method-C:

Column	Gemini C18 (250*21.2mm 5µ)
Mobile phase-A	0.1% TFA in Water
Mobile phase-B	Acetonitrile: water (80:20)
Gradient (T/%B)	0/10,1/10,15/50,18/50,18.05/98
Flow Rate	14 mL/min
UV Detection	215nm & 254nm
Solubility	Acetonitrile: water (1:9)

Method-D:

Column	XBridge C18 (250*19 mm 5µ)
Mobile phase-A	0.1% TFA in Water
Mobile phase-B	Acetonitrile: water (80:20)
Gradient (T/%B)	0/5,2/5,10/38,20/38,21/96
Flow Rate	13 mL/min
UV Detection	215nm & 254nm
Solubility	Acetonitrile: water(1:9)

Method-E:

Column	Shim-pack C18 (250*20 mm 5µ)
Mobile phase-A	0.1% TFA in Water
Mobile phase-B	Acetonitrile: water (80:20)
Gradient (T/%B)	0/30, 2/30, 20/80, 20.1/98
Flow Rate	14 mL/min
UV Detection	215nm & 254nm
Solubility	Acetonitrile: water (1:9)

Method-F:

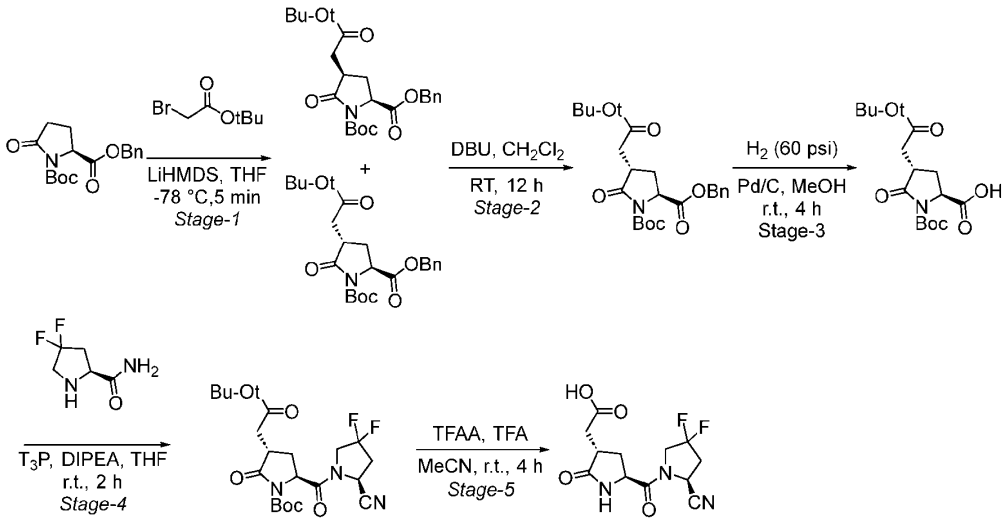
Column name	Luna C18 (250*80 mm 10 µ)
Mobile phase-A	0.1% TFA in Water
Mobile phase-B	0.1% Acetonitrile: Water (80:20)

Method (%B)	0/30, 2/30, 25/60, 26/90, 31/30, 36/30
Flow Rate	180 mL/min
Solubility	Acetonitrile: water (1:9)

NMR Employed in characterization of examples

[000173] ^{1}H NMR spectra were obtained with Brucker spectrometers operating at frequencies as follows: ^{1}H NMR: 400 MHz. Spectra data are reported in the format: Chemical shift (multiplicity, number of hydrogens. Chemical shifts are specified in ppm downfield of a tetramethyl silane internal standard (δ units, TMS=0 ppm) and reference to solvent peaks, which in ^{1}HNMR appear at 2.49 ppm for DMSO-d_6. sample quantity 2 to 3 mg, solvents quantity 0.6 mL

[000174] Example A. Synthesis of 2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetic acid (CDA-5)



[000175] Step 1: To a stirred solution of 2-benzyl 1-(tert-butyl) (2S,4S)-4-(2-(tert-butoxy)-2-oxoethyl)-5-oxopyrrolidine-1,2-dicarboxylate (100 g, 0.313 mol, 1 eq) in THF (1000 mL, 10 Vol), cooled to -78 °C, was added a solution of LiHMDS in THF (1M) drop wise, and the mixture stirred for 1 hour at same temperature, was added a solution of Tert-butyl bromoacetate (50 mL, 0.344 mol, 1.1 eq) in THF (100 mL) dropwise at -78 °C, the reaction mixture continued to stir for 2 hours at same temperature. Upon completion of the reaction, the reaction mixture was quenched saturated aq. ammonium chloride (300 mL) at -

78 °C, allowed to attain ambient temperature and extracted with EtOAc (3×400 mL), the combined organic phases were washed with water (300 mL) and brine (300 mL). Organic phase-separated was dried over anhydrous sodium sulfate, filtered, and dried under vacuum to get crude (135.7 g, yield: 100%).

[000176] Step 2: To a stirred solution of Step-1 crude (135.7 g, 0.313 mol, 1 eq) in DCM (136 mL, 10 Vol), cooled to 0 °C, was added DBU (132 mL, 1.878 mol, 6 eq) dropwise at the same temperature, then allowed to ambient temperature, and the reaction mixture was stirred at ambient temperature for 36 h. The reaction was monitored by TLC (30% EtOAc in Hexanes, R_f = 0.3), TLC indicated conversion of non-polar trans-isomer major product. Upon completion of the reaction, the reaction mixture was quenched with saturated aq. ammonium chloride (150 mL) at ambient temperature and extracted with DCM (2×500 mL), the combined organic phases washed with water (400 mL). The organic phase separated was dried over anhydrous sodium sulfate, filtered and dried under vacuum to get crude (150 g). The crude compound (150 g) was purified by column chromatography using 100-200 silica gel, (Note: Column silica gel pre-eluted with 5% Et_3N in Hexanes before loading crude) [gradient elution with 0-15% EtOAc in Hexanes] to afford 2-benzyl 1-(tert-butyl) (2S,4S)-4-(2-(tert-butoxy)-2-oxoethyl)-5-oxopyrrolidine-1,2-dicarboxylate (50 g, yield: 37%) as an off-white solid.

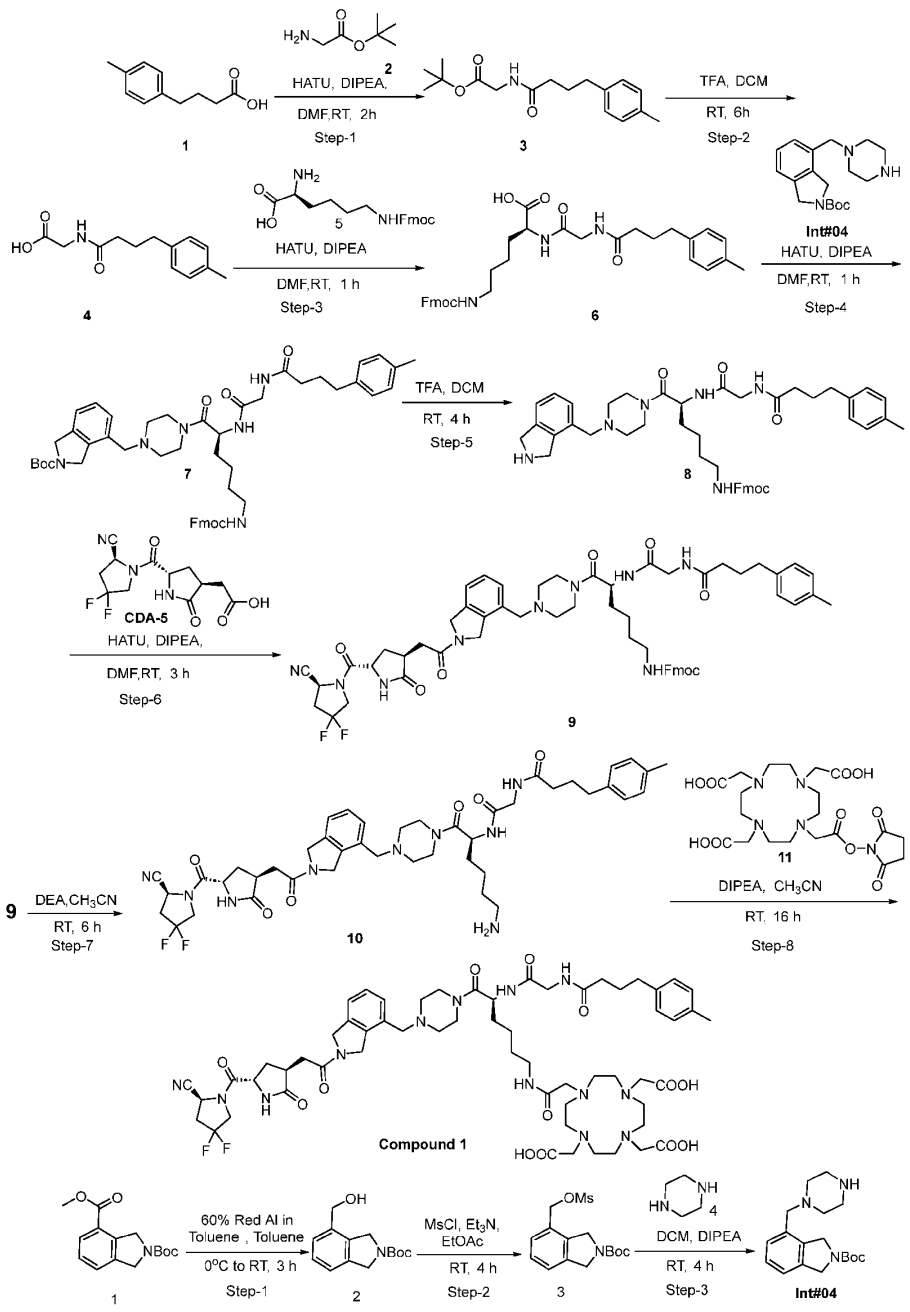
[000177] Step 3: To a stirred solution of 2-Benzyl 1-(tert-butyl) (2*S*,4S)-4-(2-(*tert*-butoxy)-2-oxoethyl)-5-oxopyrrolidine-1,2-dicarboxylate (50 g, 0.1153 mol, 1 eq.,) in methanol (400 mL, 8 Vol), purged with nitrogen for 5 min, was added 10% Pd/C (wet) (5 g, 10 wt %) at ambient temperature, again purged with nitrogen for 5 min. The resulted reaction mixture in the steel bomb was closed tightly and filled with 60 psi H2 gas and was stirred at ambient temperature for 16 h. Upon completion of the reaction, the reaction mixture was filtered on a celite pad, and washed with excess methanol (400 mL). The organic phase separated was concentrated under a vacuum to get crude (50 g). The crude compound (50 g) was triturated with ether (2x200 mL), filtered and dried under vacuum to afford (2*S*,4*S*)-4-(2-(*tert*-butoxy)-2-oxoethyl)-1-(tert-butoxycarbonyl)-5-oxopyrrolidine-2-carboxylic acid (35 g, yield: 88%) as an off-white solid.

[000178] Step 4: To a stirred solution of (2*S*,4*S*)-4-(2-(*tert*-Butoxy)-2-oxoethyl)-1-(*tert*-butoxycarbonyl)-5-oxopyrrolidine-2-carboxylic acid (35 g, 0.102 mol, 1 eq.,) in THF (350 mL, 10 Vol), was added Di-isopropyl ethyl amine (142 mL, 0.82 mol, 8 eq.,), followed by a solution of 50% T3P in EtOAc (194.5 g, 0.62 mol, 6 eq, 389.17 mL) drop wise at ambient temperature, stirred for 10 min. To this was added (*S*)-4,4-difluoropyrrolidine-2-carboxamide

(19.9 g, 0.107 mol, 1.05 eq) at the same temperature, then the reaction mixture was stirred at ambient temperature for 2 h. The reaction was monitored by TLC (40% EtOAc in Hexanes, R_f = 0.3), TLC indicated completion of starting material and formation of intermediate-7. The reaction mixture was further heated to 60 °C and continued to stir for 16 h at the same temperature. Upon completion of the reaction, the reaction mixture was cooled to ambient temperature, diluted with water (400 mL), and extracted with EtOAc (3x400 mL), the combined organic phases were washed with water (400 mL) and brine (400 mL). The organic phase separated was dried over anhydrous sodium sulfate, filtered, and dried under vacuum to get crude (40 g). The crude compound was purified by column chromatography using 60-120 silica gel, 0 to 40% EtOAc in pet ether as an eluent to afford tert-butyl (3S,5S)-3-(2-(tert-butoxy)-2-oxoethyl)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidine-1-carboxylate (28 g, yield: 60%) as an off-white solid.

[000179] Step 5: To a stirred solution of tert-butyl (3*S*,5*S*)-3-(2-(*tert*-butoxy)-2-oxoethyl)-5-((*S*)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidine-1-carboxylate (20 g, 0.1153 mol, 1 eq) in acetonitrile (200 mL, 10 Vol), was added trifluoroacetic anhydride (40 mL, 2 Vol) drop wise at ambient temperature, followed by trifluoroacetic acid (100 mL, 5 Vol) drop wise at the same temperature. The reaction mixture was stirred at ambient temperature for 3 h. Upon completion of the reaction, the reaction mixture was concentrated under a vacuum to get residue. The residue was triturated with ether (100 mL), and the resulted solid was separated and dried under vacuum to get crude material (10.5 g). The crude solid compound was dissolved in water-acetonitrile (2:1) (20 mL) and lyophilized to get an off-white solid compound. The obtained solid was triturated in EtOAc (10 mL), filtered and dried under vacuum to afford 2-((3*S*,5*S*)-5-((*S*)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl) acetic acid (10 g, yield: 75%) as a white solid compound.

[000180] **Example 1. Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-6-oxo-5-(2-(4-(p-tolyl)butanamido)acetamido)hexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 1)**



[000181] Step-1: Synthesis of tert-butyl (4-(p-tolyl)butanoyl)glycinate: To a solution of 4-(p-tolyl)butanoic acid 1 (10 g, 0.056 mol) in DMF (100 mL) at room temperature was added HATU (31.92 g, 0.084 mol), followed by DIPEA (29 mL, 0.16 mol). The mixture was

stirred at room temperature for 30 min, and added tert-butyl glycinate 2 (7.3 g, 0.056 mol). The resultant reaction mixture was stirred at room temperature for 2 h. Upon completion of the reaction, reaction mixture was poured into ice water (500 mL) stirred for 15 min and the aqueous layer was separated and extracted with EtOAc (2* 500 mL). The combined organic layer was washed with ice cold water (250 mL). The organic phase was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to get crude. Crude was purified by silica gel column chromatography (60-120 mesh) using 30% EtOAc in hexane as an eluant. Required fractions were evaporated to afford tert-butyl (4-(p-tolyl)butanoyl)glycinate 3 (7 g, yield: 43%) as an off white solid. MS (ESI) m/z: 292.18 $(M+H)^+$.

[000182] Step-2: Synthesis of (4-(p-tolyl)butanoyl)glycine: TFA (28 mL, 4 Vol) was added to a solution of tert-butyl (4-(p-tolyl)butanoyl)glycinate 3 (7 g, 0.024 mol) in DCM (70 mL, 10 Vol) at 0° C. The resultant reaction mixture was stirred at room temperature for 6 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude. Crude was triturated with diethyl ether (2 * 100 mL) to afford (4-(p-tolyl)butanoyl)glycine 4 (5 g, yield: 89%) as an off white solid. MS (ESI) m/z: 236.03 $(M+H)^+$.

[000183] Step-3: Synthesis of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(p-tolyl)butanoyl)glycyl)-L-lysine: To a solution of (4-(p-tolyl)butanoyl)glycine 4 (6 g, 0.025 mol) in DMF (60 mL, 10 Vol) at room temperature was added DIPEA (8.9 mL, 0.051 mmol) followed by HATU (14.5 g, 0.038 mol). The mixture was stirred at room temperature for 1 h and added tert-butyl N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-L-lysinate 5 (6.5 g, 0.017 mol). Resultant reaction mixture was stirred at room temperature for 1 h. Reaction mixture was diluted with EtOAc (200 mL), cold water (200 mL) and stirred for 15 min. The organic layer was separated, and aqueous layer was further extracted with EtOAc (2 * 150 mL). Combined organic layer was washed with water (150 mL). The separated organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford crude. Crude was triturated with diethyl ether (2 * 100 mL) to afford N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(p-tolyl)butanoyl)glycyl)-L-lysine 6 (5 g, yield: 33%) as an off white solid. MS (ESI) m/z: 585.97 $(M+H)^+$.

[000184] Step-4: tert-butyl 4-((4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(p-tolyl)butanoyl)glycyl)-L-lysyl)piperazin-1-yl)methyl)isoindoline-2-carboxylate: To a solution of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(p-tolyl)butanoyl)glycyl)-L-lysine (4 g, 0.006 mol) in DMF (40 mL, 10 Vol) at room temperature was added HATU (3.9

g, 0.010 mol) followed by DIPEA (2.6 mL, 0.020 mol) and stirred at room temperature for 1 h followed by addition of tert-butyl 4-(piperazin-1-ylmethyl)isoindoline-2-carboxylate Int#04 (2.1 g, 0.006 mol). The resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was diluted with EtOAc (200 mL) and ice-cold water (100 mL) and stirred for 15 min. The aqueous layer was separated and extracted with EtOAc (2 * 250 mL). The combined organic layer was washed with ice cold water (1 * 150 mL), dried over anhydrous sodium sulphate, and concentrated under reduced pressure to get crude. Crude was triturated with n-pentane (2 * 100 mL) to afford tert-butyl 4-((4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(p-tolyl)butanoyl)glycyl)-L-lysyl)piperazin-1-yl)methyl)isoindoline-2-carboxylate 7 (3.2 g) MS (ESI) m/z: 885.45 $(M+H)^+$.

[000185] Step-5: Synthesis of (9H-fluoren-9-yl)methyl (S)-(6-(4-(isoindolin-4-ylmethyl)piperazin-1-yl)-6-oxo-5-(2-(4-(p-tolyl)butanamido)acetamido)hexyl)carbamate: TFA (8 mL, 4 Vol) was added to a solution of tert-butyl 4-((4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(p-tolyl)butanoyl)glycyl)-L-lysyl)piperazin-1-yl)methyl)isoindoline-2-carboxylate 4 (2 g, 0.002 mol) in DCM (20 mL, 10 Vol) at 0° C. The resultant reaction mixture was stirred at room temperature for 4 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude. Crude was triturated with diethyl ether (2 * 100 mL) to afford (9H-fluoren-9-yl)methyl (S)-(6-(4-(isoindolin-4-ylmethyl)piperazin-1-yl)-6-oxo-5-(2-(4-(p-tolyl)butanamido)acetamido)hexyl)carbamate 5 (1.3 g, yield: 76%) as an off white solid. MS (ESI) m/z: 785.4$(M+H)^+$.

[000186] Step-6: Synthesis of (9H-fluoren-9-yl)methyl ((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-6-oxo-5-(2-(4-(p-tolyl)butanamido)acetamido)hexyl)carbamate: To a solution of 2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetic acid CDA-5 (0.75 g, 0.0024 mol) in DMF (7.5 mL, 10 Vol) at room temperature was added DIPEA (0.7 mL, 0.004 mol) followed by HATU (1.4 g, 0.003 mol). The mixture was stirred at room temperature for 1 h and added (9H-fluoren-9-yl)methyl (S)-(6-(4-(isoindolin-4-ylmethyl)piperazin-1-yl)-6-oxo-5-(2-(4-(p-tolyl)butanamido)acetamido)hexyl)carbamate 5 (1.9 g, 0.002 mol). Resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of the reaction, reaction mixture was diluted with EtOAc (150 mL) and ice-cold water (100 mL), stirred for 15 min. Separated aqueous layer was extracted with EtOAc (3 * 100 mL) and combined organic

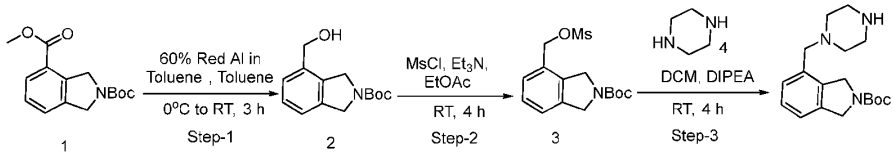
layers was washed with ice cold water (100 mL). Organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to get crude. Crude was triturated with diethyl ether (2 * 100 mL) to afford (9H-fluoren-9-yl)methyl ((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-6-oxo-5-(2-(4-(p-tolyl)butanamido)acetamido)hexyl)carbamate 6 (1.4 g, yield: 53%) as a white solid. MS (ESI) m/z: 1069.78 $(M+H)^+$.

[000187] Step-7: Synthesis of N-(2-(((S)-6-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-1-oxohexan-2-yl)amino)-2-oxoethyl)-4-(p-tolyl)butanamide: Diethyl amine (12.5 mL, 5 Vol) was added to a solution of (9H-fluoren-9-yl)methyl ((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-6-oxo-5-(2-(4-(p-tolyl)butanamido)acetamido)hexyl)carbamate 6 (2.5 g, 0.002 mol) in acetonitrile (20 mL, 8 Vol) at room temperature. The resultant reaction mixture was stirred at room temperature for 6 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to get crude (3.25 g). The crude compound was triturated with cold EtOAc (3 * 50 mL), the resultant solid was dissolved in 10% MeOH in DCM (50 mL), diluted with diethyl ether (50 mL) and the obtained solid was separated to afford N-(2-(((S)-6-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-1-oxohexan-2-yl)amino)-2-oxoethyl)-4-(p-tolyl)butanamide 7 (1.5 g, yield: 75%) as a white solid. MS (ESI) m/z: 846.05 (M+H)+.

[000188] Step-8: Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-6-oxo-5-(2-(4-(p-tolyl)butanamido)acetamido)hexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid: To a solution of N-(2-(((S)-6-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-1-oxohexan-2-yl)amino)-2-oxoethyl)-4-(p-tolyl)butanamide 7 (1.5 g, 0.0017 mmol) in acetonitrile (15 mL, 10 Vol) at room temperature was added DIPEA (2.7 mL, 0.015 mmol) drop wise followed by2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid DOTA NHS Ester (0.89 g, 0.0017 mmol) lot wise. The resultant reaction mixture was stirred at room temperature for 16 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to get residue. The residue was triturated

with EtOAc (2 * 25 mL) filtered. Solid obtained was dissolved in 10% MeOH in DCM (40 mL), and diluted with diethyl ether (40 mL) to regenerate solid. The solid obtained was further triturated with acetonitrile (2 * 50 mL) and filtered. Then the isolated solid was re-dissolved in MeOH (20 mL). The undissolved particles was removed by filtration, and the filtrate was concentrated under reduced pressure to get crude material. The crude material was purified by Prep-HPLC. The required fractions were collected and concentrated under vacuum followed by lyophilized to afford 2,2',2''-(10-(2-(((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-6-oxo-5-(2-(4-(p-tolyl)butanamido)acetamido)hexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (210 mg) as a white solid. MS (ESI) m/z: $(M+H)^+$. ^{1}H-NMR (400 MHz, DMSO-d6): δ 8.5-8.4 (m, 1H), 8.2-8.0 (m, 3H), 7.5-7.4 (m, 3H), 7.1-7.0 (m, 4H), 5.11-5.09 (m, 1H), 5.0-4.5 (m, 7H), 4.6-4.4 (m, 5H), 4.41-4.4 (m, 9H), 4.1-3.7 (m, 5H), 3.4-3.3 (m, 7H), 3.3-3.01 (m, 14H), 3.0-2.7 (m, 4H), 2.5-2.4 (m, 3H), 2.25 (s, 3H), 2.1-2 (m, 2H), 1.8-1.7 (m, 2H), 1.6-1.5 (m, 2H), 1.5-1.4 (m, 3H), 1.3-1.2 (m, 2H)

[000189] Synthesis of tert-butyl 4-(piperazin-1-ylmethyl)isoindoline-2-carboxylate



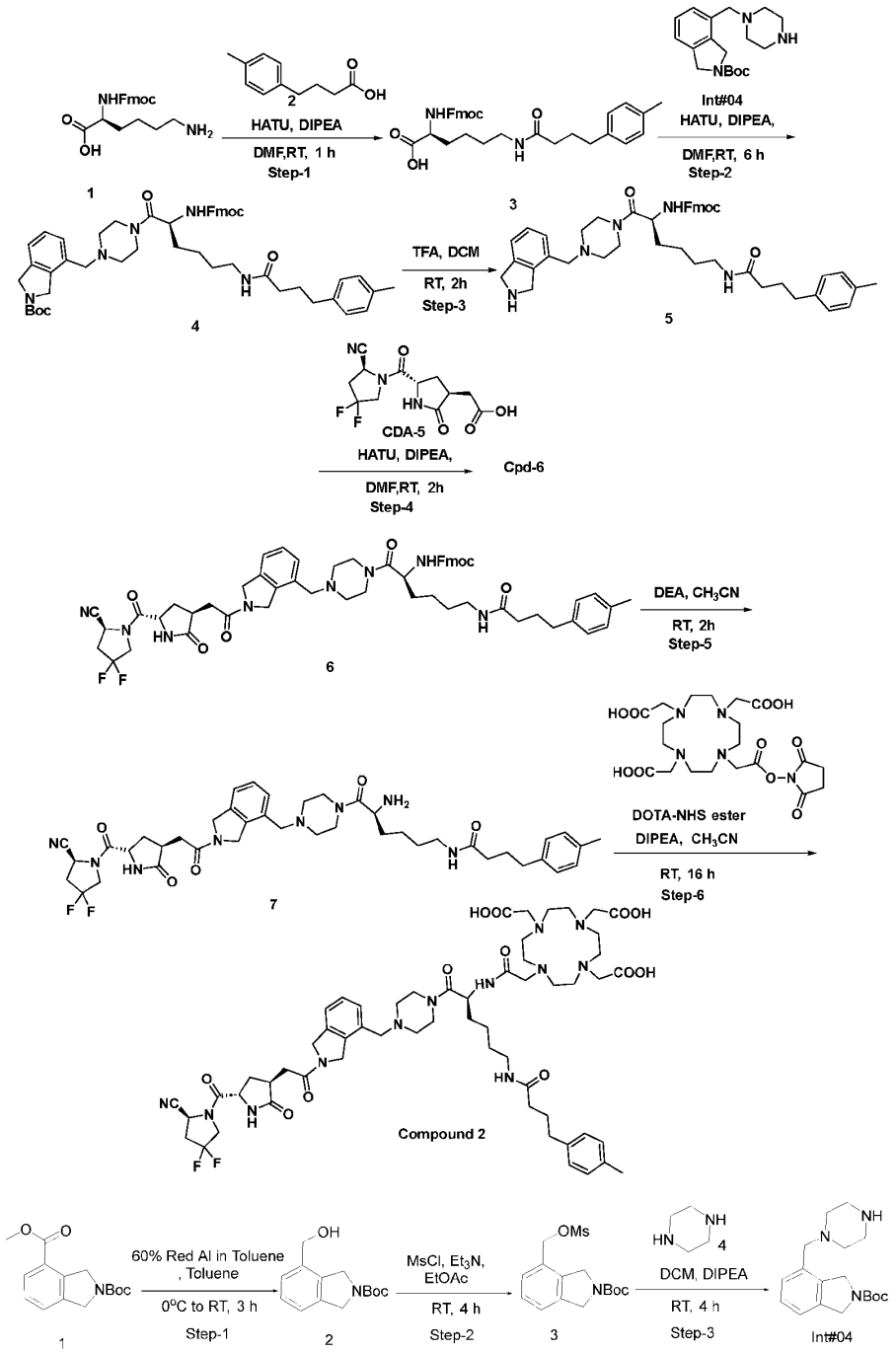
[000190] Step-1: Synthesis of tert-butyl 4-(hydroxymethyl)isoindoline-2-carboxylate: To a solution of 2-benzyl 4-methyl isoindoline-2,4-dicarboxylate 1 (20 g, 0.072 mol) in toluene (200 mL) at 0 °C was added 60 % Red Al in Toluene (31 mL, 0.144 mol). Resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of reaction, the reaction mixture was quenched with 20% KOH solution (100 mL), and compound was extracted in EtOAc (2*200 mL), concentrated under reduced pressure to get crude. The obtained crude was purified by silica gel column chromatography (100-200 mesh), using 40% ethyl acetate in hexane as an eluent. Required fractions were concentrated under reduced pressure to afford tert-butyl 4-(hydroxymethyl)isoindoline-2-carboxylate 2 (15 g, yield: 83%) as an off white solid. MS (ESI) m/z: 250 $[M+H]^+$

[000191] Step-2: tert-butyl 4-(((methylsulfonyl)oxy)methyl)isoindoline-2-carboxylate: To a solution of tert-butyl 4-(hydroxymethyl)isoindoline-2-carboxylate 2 (13.5 g, 0.054 mol)

in EtOAc (135 mL, 10 vol) at 0 °C was added triethyl amine (15.2 mL, 0.108 mol), followed by mesyl chloride (6.2 mL, 0.081 mmol). Resultant reaction mixture was stirred at room temperature for 4 h. Upon completion of the reaction, reaction mixture was diluted with EtOAc (250 mL) and ice-cold water (100 mL), stirred for 15 min. Separated aqueous layer was extracted with EtOAc (3 * 100 mL) and combined organic layers was washed with ice cold water (100 mL). Organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford tert-butyl 4-(((methylsulfonyl)oxy)methyl)isoindoline-2-carboxylate 3 (17.5 g, crude material) as a brown liquid. The crude material was forwarded to the next step without purification.

[000192] Step-3: Synthesis of *tert*-butyl 4-(piperazin-1-ylmethyl)isoindoline-2-carboxylate: To a solution of tert-butyl 4-(((methylsulfonyl)oxy)methyl)isoindoline-2-carboxylate 3 (52 g, 0.159 mol) in DCM (420 mL, 10 Vol) at room temperature was added piperazine 4 (54 g, dissolved in DCM, 0.636 mol) followed by addition of DIPEA (138.2 mL, 0.795 mol). Resultant reaction mixture was stirred at room temperature for 4 h. The reaction mixture was cooled to room temperature and diluted with ice-cold water (300 mL), stirred for 15 min and separated the layers. The aqueous layer was extracted with DCM (2 * 200 mL). Combined organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford crude material. Crude was purified by silica gel column chromatography (100-200 mesh) using 5% MeOH in dichloromethane as an eluent. compound contained fractions were concentrated under reduced pressure to afford *tert*-butyl 4-(piperazin-1-ylmethyl)isoindoline-2-carboxylate (38 g, 76%) as a pale yellow gummy liquid. MS (ESI) m/z: 318.2 $[M+H]^+$, ^{1}H-NMR (400 MHz, DMSO-d6): δ 7.2-7.1 (m, 3H), 4.52-4.51 (m, 4H), 3.5-3.4 (2H), 2.5-2.4 (4H), 2.3-2.2(m, 5H), 1.5 (s, 9H).

[000193] Example 2. Synthesis of 2,2',2''-(10-(2-(((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-1-oxo-6-(4-(p-tolyl)butanamido)hexan-2-yl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 2)



[000194] Step-1: Synthesis of N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(4-(p-tolyl)butanoyl)-L-lysine: To a solution of 4-(p-tolyl) butanoic acid 2 (7 g, 0.039 mol) in DMF (35 mL, 5 Vol) at 0°C, was added DIPEA (10 mL, 0.058 mol) followed by HATU (15 g,

0.039 mol). The mixture was stirred for 30 min and added (((9H-fluoren-9-yl)methoxy)carbonyl)-L-lysine 1 (14.3 g, 0.039 mol). Resultant reaction mixture was stirred at room temperature for 1 h. Reaction mixture was poured into ice water (300 mL) and stirred for 10 min. The solid precipitated was filtered and washed with ice water. The crude obtained was purified by silica gel column chromatography (100-200 mesh) using 70% EtOAc in hexane as an eluant. Required fractions were concentrated under reduced pressure to afford N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(4-(p-tolyl)butanoyl)-L-lysine 3 (9 g, yield: 85%) as a white solid. MS (ESI) m/z: 529.01 $(M+H)^+$.

[000195] Step-2: Synthesis of tert-butyl 4-((4-(N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(4-(p-tolyl)butanoyl)-L-lysyl)piperazin-1-yl)methyl)isoindoline-2-carboxylate: To a solution of N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(4-(p-tolyl)butanoyl)-L-lysine 3 (5 g, 0.0094 mol) in DMF (25 mL, 5 Vol) at 0°C was added DIPEA (3.2 mL, 0.018 mol) followed by HATU (3.57 g, 0.0094 mol) and stirred for 30 min and added tert-butyl 4-(piperazin-1-ylmethyl)isoindoline-2-carboxylate Int#04 (3 g, 0.0094 mol). Resultant reaction mixture was stirred at room temperature for 6 h. Reaction mixture was poured into ice water (100 mL), stirred for 10 min and the precipitated solid was filtered and washed with ice water (50 mL). The solid obtained (6.3 g) was purified by grace reverse phase column chromatography using 50% water in acetonitrile as an eluant. Required fractions were concentrated under reduced pressure to afford tert-butyl 4-((4-(N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(4-(p-tolyl)butanoyl)-L-lysyl)piperazin-1-yl)methyl)isoindoline-2-carboxylate 4 (2.4 g, yield: 19 %) as a white solid. MS (ESI) m/z: 829.52 $(M+H)^+$.

[000196] Step-3: Synthesis of (9H-fluoren-9-yl)methyl (S)-(1-(4-(isoindolin-4-ylmethyl)piperazin-1-yl)-1-oxo-6-(4-(p-tolyl)butanamido)hexan-2-yl)carbamate: TFA (9.8 mL, 4 Vol) was added to a solution of tert-butyl 4-((4-(N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(4-(p-tolyl)butanoyl)-L-lysyl)piperazin-1-yl)methyl)isoindoline-2-carboxylate 4 (2.5 g, 0.003 mol) in DCM (25 mL, 10 vol) at 0 °C. Resultant reaction mixture was stirred at room temperature for 2 h. Reaction mixture was concentrated under reduced pressure to afford crude. Crude was triturated with diethyl ether (30 mL) to afford (9H-fluoren-9-yl)methyl (S)-(1-(4-(isoindolin-4-ylmethyl)piperazin-1-yl)-1-oxo-6-(4-(p-tolyl)butanamido)hexan-2-yl)carbamate 5 (2 g, yield: 49 %) as a pale yellow solid . MS (ESI) m/z: 729.93 $(M+H)^+$.

[000197] Step-4: Synthesis of (9H-fluoren-9-yl)methyl ((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-

yl)methyl)piperazin-1-yl)-1-oxo-6-(4-(p-tolyl)butanamido)hexan-2-yl)carbamate: To a stirred solution of 2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetic acid CDA-5 (1.1 g, 0.0036 mol) in DMF (11 mL, 10 Vol) at 0 °C was added DIPEA (1.87 mL, 0.010 mol) and HATU (1.64 g, 0.0043 mol). The mixture was stirred at room temperature for 10 min and then added (9H-fluoren-9-yl)methyl(S)-(1-(4-(isoindolin-4-ylmethyl)piperazin-1-yl)-1-oxo-6-(4-(p-tolyl)butanamido)hexan-2-yl)carbamate 5 (2.6 g, 0.0036 mol). The resultant reaction mixture was stirred at room temperature for 2 h. After completion of reaction, reaction mixture was diluted with ice-cold water (100 mL) EtOAc (2 * 150 mL), stirred for 15 min and separated the aqueous layer. Aqueous layer was extracted with EtOAc (2 * 150 mL). The combined organic layer was washed with ice cold water (2 * 100 mL). The organic phase was dried over anhydrous sodium sulphate, filtered and concentrated under vacuum to afford (9H-fluoren-9-yl)methyl ((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-1-oxo-6-(4-(p-tolyl)butanamido)hexan-2-yl)carbamate 6 (0.6 g, crude) as a brown colour liquid. MS (ESI) m/z: 1011.70 $(M+H)^+$.

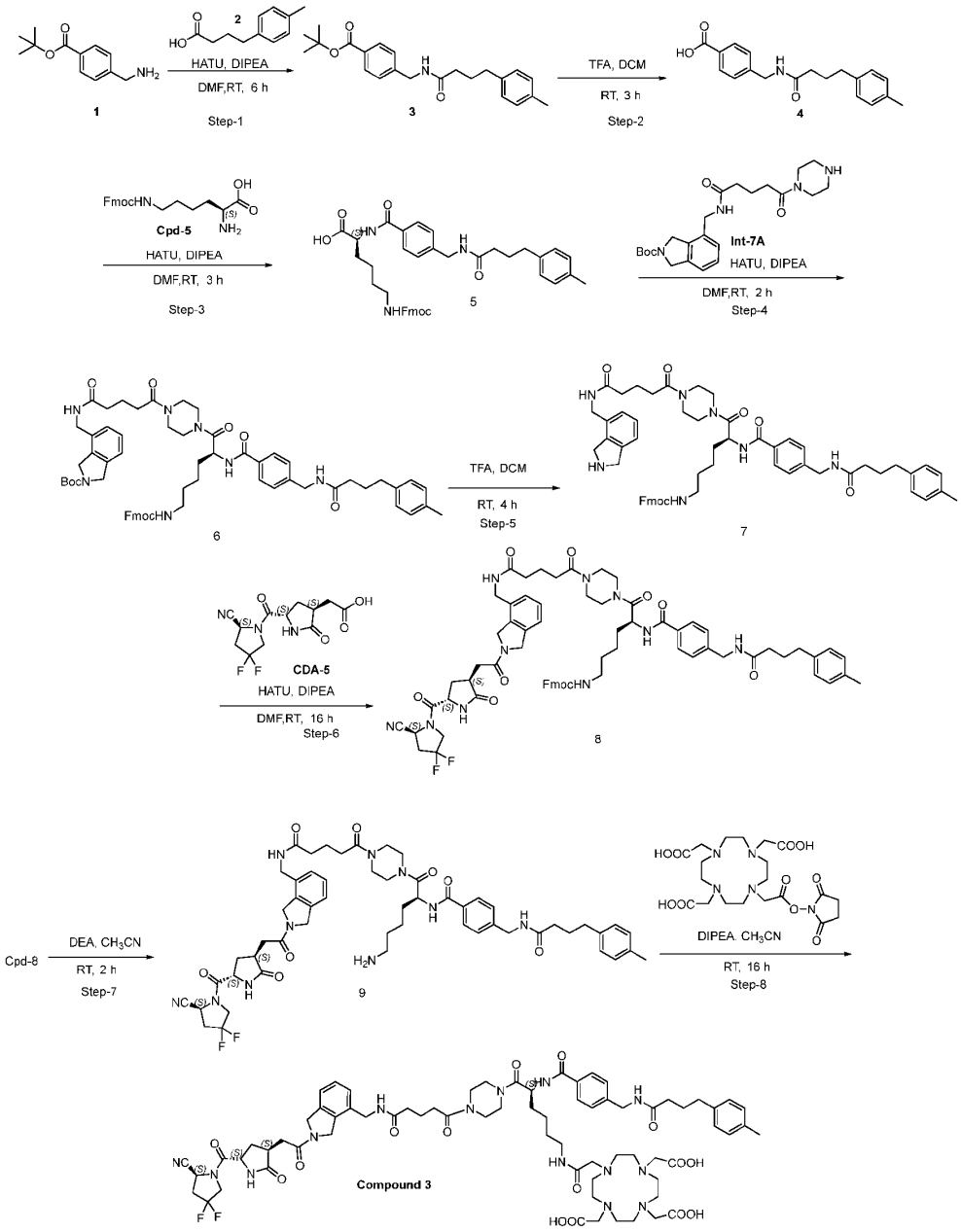
[000198] Step-5: Synthesis of N-((S)-5-amino-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-6-oxohexyl)-4-(p-tolyl)butanamide: Diethyl amine (9 mL, 5 Vol) was added to a solution of (9H-fluoren-9-yl)methyl ((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-1-oxo-6-(4-(p-tolyl)butanamido)hexan-2-yl)carbamate 6 (1.8 g, 0.00178 mol) in acetonitrile (9 mL, 5 Vol) at room temperature . The resultant reaction mixture was stirred at room temperature for 2 h. Reaction mixture was concentrated under reduced pressure to get crude. Crude compound was trituration with cold EtOAc (2 * 25 mL) and filtered. Solid was dissolved in 10% MeOH in DCM (10 mL) and diluted with diethyl ether (50 mL).The regenerated solid was filtered and again dissolved in acetonitrile (30 mL). Undissolved parts were removed by filtration, and the filtrate concentrated under vacuum to afford N-((S)-5-amino-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-6-oxohexyl)-4-(p-tolyl)butanamide 7 (1 g, semi pure) as a pale brown solid . MS (ESI) m/z: 789.82 $(M+H)^+$.

[000199] Step-6: Synthesis of 2,2',2"-(10-(2-(((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-1-oxo-6-(4-(p-tolyl)butanamido)hexan-2-yl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid: To a solution of N-((S)-5-amino-6-

(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-6-oxohexyl)-4-(p-tolyl)butanamide 7 (1 g, 0.0012 mol) in acetonitrile (17 mL, 17 Vol) at room temperature was added and DIPEA (2 mL, 0.011 mmol) followed by 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid DOTA NHS ester (0.90 g, 0.0018 mol). Resultant reaction mixture was stirred at room temperature for 16 h. Reaction mixture was concentrated under reduced pressure to afford crude (1.6 g). Crude material was triturated with EtOAc (2 * 50 mL) and further purified by prep-HPLC. The required fractions were collected and lyophilized to afford 2,2',2''-(10-(2-(((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-1-oxo-6-(4-(p-tolyl)butanamido)hexan-2-yl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (152 mg) as a white solid. MS (ESI) m/z: 1173.8 $(M-H)^-$. ^{1}H-NMR (400 MHz, DMSO-d6): δ 8.8-8.7 (m, 1H), 8.06 (d, J = 9.6 Hz, 1H), 7.9-7.8 (m, 1H), 7.5-7.3 (m, 3H), 7.1-7.0 (m, 4H), 5.11-5.09 (m, 1H), 5.0-4.60 (m, 5H), 4.41-3.7 (m, 17H), 3.6-3.2 (m, 13H), 3.2-3.0 (m, 12H), 2.9-2.7 (m, 4H), 2.32 (s, 3H), 2.2-2.0 (m, 3H), 1.8-1.6 (m, 3H), 1.6-1.5 (m, 1H), 1.4-1.3 (m, 4H)

[000200] The synthesis of tert-butyl 4-(piperazin-1-ylmethyl)isoindoline-2-carboxylate (Int #04) is described in Example 1.

[000201] Example 3. Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-6-oxo-5-(4-((4-(p-tolyl)butanamido)methyl)benzamido) hexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 3)



[000202] Step-1: Synthesis of tert-butyl 4-((4-(4-methylphenyl)butanamido)methyl)benzoate: To a solution of 4-(4-phenyl)butanoic acid 2 (5 g, 28.058 mmol) in DMF (20 mL), at room temperature was added DIPEA (7.34 mL, 42.087 mmol) followed by HATU (10.66 g, 28.058 mmol). The mixture was stirred for 30 min, and

added tert-butyl 4-(aminomethyl)benzoate 1 (5.81 g, 28.058 mmol). Resultant reaction mixture was stirred at room temperature for 6 h. Reaction mixture was poured into ice water (200 mL), and the precipitated solid was filtered and washed with ice water (100 mL). The obtained crude was purified by silica gel column chromatography (100-200 mesh) using 70% EtOAC in hexane as an eluant. Required fractions were concentrated under reduced pressure to afford tert-butyl 4-((4-(4-fluorophenyl)butanamido)methyl)benzoate 3 (7.4 g, yield: 83%) as an off white solid. MS (ESI) m/z: 368 (M+H)+.

[000203] Step-2: Synthesis of 4-((4-(4-methylphenyl)butanamido)methyl)benzoic acid: TFA (30 mL, 4 vol) was added to a solution of 4-((4-(4-methylphenyl)butanamido)methyl)benzoate 3 (7.4 g, 20.136 mmol) in DCM (70 mL, 10 vol), at 0 °C. Resultant reaction mixture was stirred at room temperature for 3 h. Reaction mixture was concentrated under reduced pressure to afford crude. The crude was triturated with diethyl ether (25 mL) and n-pentane (50 mL) to afford 4-((4-(4-methylphenyl)butanamido)methyl)benzoic acid 4 (5.8 g, yield: 92%) as an off white solid. MS (ESI) m/z: 312 $(M+H)^+$. ^{1}H-NMR (400 MHz, DMSO-d6): δ 12.85 (brs, 1H), 8.38 (t, J = 6 Hz, 1H), 7.88 (d, J = 8 Hz, 2H), 7.34 (d, J = 8.4 Hz, 2H), 7.09-7.04 (m, 4H), 4.33 (s, 2H), 2.54-2.49 (m, 2H), 2.26 (s, 3H), 2.18 (t, J = 5 2 Hz, 2H), 1.82-1.80 (m, 2H).

[000204] Step-3: Synthesis of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(p-tolyl)butanamido)methyl)benzoyl)-L-lysine: To a solution of 4-((4-(p-tolyl)butanamido)methyl)benzoic acid 4 (4 g, 12.846 mmol) in DMF (32 mL, 8 Vol) at room temperature was added DIPEA (6.72 mL, 38.535 mmol) followed by HATU (5.85 g, 15.414 mmol). The mixture was stirred for 30 min and added (((9H-fluoren-9-yl)methoxy)carbonyl)-L-lysine 5 (4.73 g, 12.846 mmol). Resultant reaction mixture was stirred at room temperature for 3 h. Reaction mixture was poured into ice water (250 mL), and the precipitated solid was filtered and washed with ice water (100 mL). The resultant material was purified by grace reverse phase column chromatography using 50% H_2O in acetonitrile as an eluant. Required fractions were concentrated under reduced pressure to afford N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(p-tolyl)butanamido)methyl)benzoyl)-L-lysine 5 (3.2 g, yield: 37 %) as an off white solid . MS (ESI) m/z:662.4 $(M+H)^+$.

[000205] Step-4: tert-butyl 4-((5-(4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(p-tolyl)butanamido)methyl)benzoyl)-L-lysyl)piperazin-1-yl)-5-oxopentanamido)methyl)isoindoline-2-carboxylate: To a solution of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(p-tolyl)butanamido)methyl)benzoyl)-L-lysine 5 (3.2 g, 4.835 mmol) in DMF (15 mL, 5 Vol) at 0 °C was added DIPEA (1.18 mL, 6.769 mmol)

followed by HATU (1.83 g, 4.835 mmol). Resultant mixture was stirred at room temperature for 15 min and added tert-butyl 4-((5-oxo-5-(piperazin-1-yl)pentanamido)methyl)isoindoline-2-carboxylate Int-7A (2.08 g, 4.835 mmol) and the reaction mixture was stirred at room temperature for 2 h. Reaction mixture was diluted with EtOAc (200 mL), ice cold water (100 mL) and stirred for 15 minutes. Organic layer was separated and aqueous layer was extracted with EtOAc (3 * 100 mL). The combined organic layers was washed with ice cold water (3 * 50 mL). The organic phase separated was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford tert-butyl 4-((5-(4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(p-tolyl)butanamido)methyl)benzoyl)-L-lysyl)piperazin-1-yl)-5-oxopentanamido)methyl)isoindoline-2-carboxylate 6 (4 g, crude) as a brown colour liquid.

[000206] Step-5: Synthesis of (9H-fluoren-9-yl)methyl (S)-(6-(4-(5-((isoindolin-4-ylmethyl)amino)-5-oxopentanoyl)piperazin-1-yl)-6-oxo-5-(4-((4-(p-tolyl)butanamido)methyl)benzamido)hexyl)carbamate: TFA (16 mL, 4 Vol) was added to a solution of tert-butyl 4-((5-(4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(p-tolyl)butanamido)methyl)benzoyl)-L-lysyl)piperazin-1-yl)-5-oxopentanamido)methyl)isoindoline-2-carboxylate 6 (4 g, 3.723 mmol) in DCM (40 mL, 10 Vol), at 0 °C. Resultant reaction mixture was stirred at room temperature for 4 h. Reaction mixture was concentrated under reduced pressure to get the crude material, which was triturated with diethyl ether (30 mL) to afford (9H-fluoren-9-yl)methyl (S)-(6-(4-(5-((isoindolin-4-ylmethyl)amino)-5-oxopentanoyl)piperazin-1-yl)-6-oxo-5-(4-((4-(p-tolyl)butanamido)methyl)benzamido)hexyl)carbamate 7 (2.7 g, crude) as a brown colour liquid. MS (ESI) m/z: 975.15 $(M+H)^+$.

[000207] Step-6: Synthesis of (9H-fluoren-9-yl)methyl ((S)-6-(4-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-6-oxo-5-(4-((4-(p-tolyl)butanamido)methyl)benzamido)hexyl)carbamate: To a stirred solution of 2-((S)-4,4-difluoro-1-glycylpyrrolidin-2-yl)-N-(3,4-dimethoxybenzyl)-2-hydroxyacetamide (CDA-5) (0.84 g, 2.792 mmol) at 0 °C in DMF (26 mL, 10 Vol), was added DIPEA (0.62 mL, 3.49 mmol) followed by HATU (0.84 g, 2.327 mmol). The resultant mixture was stirred at ambient temperature for 10 min and then added (9H-fluoren-9-yl)methyl (S)-(6-(4-(5-((isoindolin-4-ylmethyl)amino)-5-oxopentanoyl)piperazin-1-yl)-6-oxo-5-(4-((4-(p-tolyl)butanamido)methyl)benzamido)hexyl)carbamate 7 (2.5 g, 2.327 mmol).Resultant reaction mixture was stirred at room temperature for 16 h. Reaction mixture was diluted with

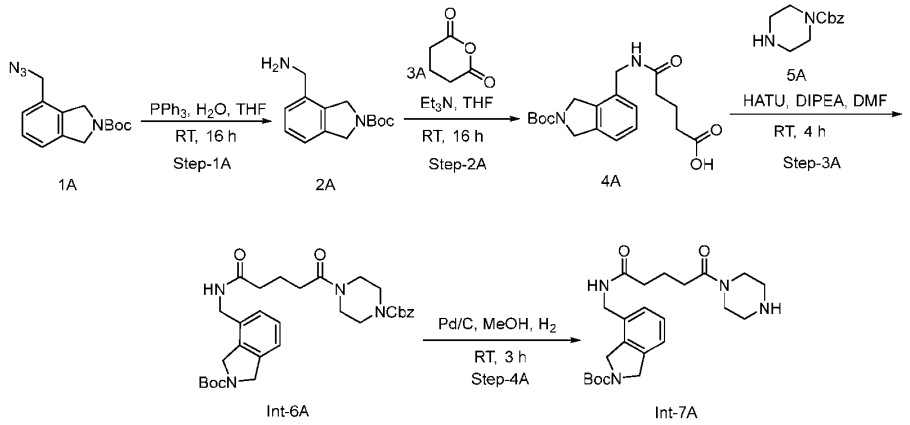
ice-cold water (100 mL) and stirred for 15 min. Organic layer was separated and aqueous layer was extracted with EtOAc (3 * 100 mL). The combined organic layers was washed with water (3 * 50 mL). The organic phase separated was dried over anhydrous sodium sulphate, filtered and concentrated under vacuum to afford 9H-fluoren-9-yl)methyl ((S)-6-(4-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-6-oxo-5-(4-((4-(p-tolyl)butanamido)methyl)benzamido)hexyl)carbamate 8 (2 g, crude) as a pale yellow solid. MS (ESI) m/z: 1258.59 (M+H)+.

[000208] Step-7: Synthesis of N-((S)-6-amino-1-(4-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-1-oxohexan-2-yl)-4-((4-(p-tolyl)butanamido)methyl)benzamide: Diethyl amine was added (10 mL, 5 Vol) to a solution of 9H-fluoren-9-yl)methyl ((S)-6-(4-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-6-oxo-5-(4-((4-(p-tolyl)butanamido)methyl)benzamido)hexyl)carbamate 8 (2 g, 1.590 mmol) at room temperature in acetonitrile (20 mL, 10 Vol), wise. The resultant reaction mixture was stirred at room temperature for 2 h. Reaction mixture was concentrated under vacuum to get crude (1.8 g), which was triturated with cold EtOAc (3 * 20 mL) and filtered Crude was dissolved in 10% MeOH in DCM (10 mL), and diluted with diethyl ether (50 mL). Regenerated solid was again dissolved in acetonitrile (30 mL), and the undissolved particles were removed by filtration. Filtrate was concentrated under vacuum to afford N-((S)-6-amino-1-(4-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-1-oxohexan-2-yl)-4-((4-(p-tolyl)butanamido)methyl)benzamide 9 (1.1 g, crude) as a pale brown Solid. MS (ESI) m/z: 1035.89 $(M+H)^+$.

[000209] Step-8: Synthesis of 2,2',2"-(10-(2-(((S)-6-(4-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-6-oxo-5-(4-((4-(p-tolyl)butanamido)methyl)benzamido)hexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid: To a solution of N-((S)-6-amino-1-(4-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-1-oxohexan-2-yl)-4-((4-(p-tolyl)butanamido)methyl)benzamide 9 (1 g, 0.965 mmol) in acetonitrile (17 mL, 10

Vol) was added DIPEA (1.51 mL, 8.685 mmol) followed by 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid DOTA-NHS ester (0.72 g, 1.447 mmol) at room temperature. Resultant reaction mixture was stirred at room temperature for 16 h. Reaction mixture was concentrated under reduced pressure to get crude which was triturated with EtOAc (2 * 20 mL) and filtered. Crude solid was dissolved in 10% MeOH in DCM (10 mL), and diluted with diethyl ether (40 mL). The regenerated solid was further triturated with acetonitrile (2* 25 mL), and solid was, then the isolated solid was re-dissolved again dissolved in MeOH (10 mL). The undissolved particles was removed by filtration, and the filtrate was concentrated under reduced pressure to get semi-pure compound. The semi-pure material was further purified by Prep-HPLC. The required fractions were collected and concentrated under vacuum followed by lyophilized to afford 2,2',2''-(10-(2-(((S)-6-(4-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-6-oxo-5-(4-((4-(p-tolyl)butanamido)methyl)benzamido)hexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (0.121 g) as a white solid. MS (ESI) m/z: 1421.66 $(M+H)^+$. ^{1}H-NMR (400 MHz, DMSO-d6): δ 12-14 (brs, 2H), 8.57-8.55 (m, 1H), 8.5-8.45 (m, 1H), 8.37 (t, J = 6 Hz, 1H), 8.30-8.28 (m, 1H), 8.04 (s, 1H), 7.83 (d, J = 8 Hz, 2H), 7.61-7.51 (brs, 1H), 7.31 (d, J = 8 Hz, 2H), 7.21-7.11 (m, 1H), 7.1-7.01 (m, 4H), 5.11-5.09 (m, 1H), 4.89-4.60 (m, 4H), 4.70-4.61 (m, 2H), 4.5-4.0 (m, 13H), 3.91-3.82 (m, 2H), 3.71-3.49 (m, 8H), 3.4-3.2 (m, 10H), 3.2-3.0 (m, 10H), 2.5-2.35 (m, 7H), 2.33 (s, 3H), 2.32-2.30 (m, 4H), 1.8-1.6 (m, 6H), 1.5-1.3 (m, 4H)

[000210] Synthesis of tert-butyl 4-((5-oxo-5-(piperazin-1-yl)pentanamido)methyl)isoindoline-2-carboxylate (Int-7A)



[000211] Step-1A: Synthesis of tert-butyl 4-(aminomethyl)isoindoline-2-carboxylate: To a solution of tert-butyl 4-(azidomethyl)isoindoline-2-carboxylate 1A (7 g, 25.54 mmol) in THF (28 mL, 4 vol) and water (14 mL, 2 Vol) at room temperature was added PPh_3 (10 g, 38.27 mmol). Upon completion of the reaction, reaction mixture was diluted with EtOAc (30 mL), ice cold water (30 mL), and stirred for 15 min. Organic layer was separated and the aqueous layer was further extracted with EtOAc (2 * 30 mL). Combined organic layers was washed with ice cold water (50 mL). The separated organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford crude. Crude material was purified by silica gel column chromatography (100-200 mesh) using 50% EtOAC in hexane as an eluant. Compound contained fractions were concentrated under reduced pressure to afford tert-butyl 4-(aminomethyl)isoindoline-2-carboxylate 2A (3.8 g, Yield: 60%) as a brown solid. MS (ESI) m/z: 248.3 $[M+H]^+$

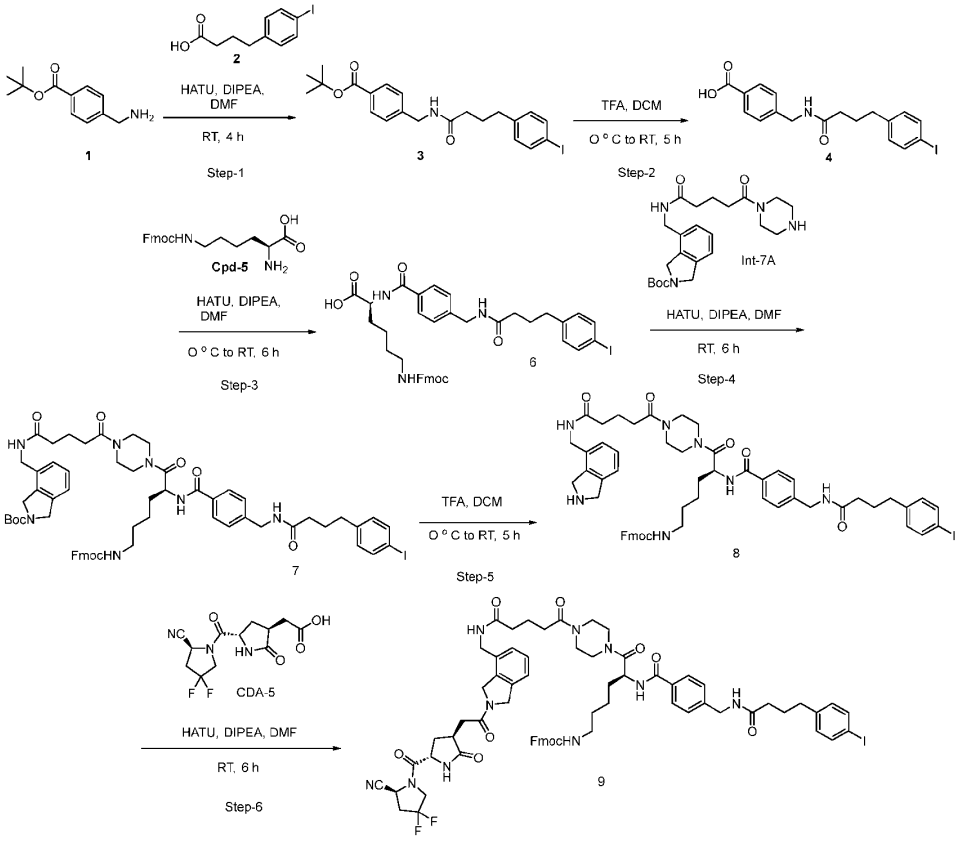
[000212] Step-2A: Synthesis of 5-(((2-(tert-butoxycarbonyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoic acid To a solution of 5-(((2-(tert-butoxycarbonyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoic acid 2A (3.5 g, 14.11 mmol) in THF (52.5mL, 15 Vol) at 0°C, was added Et_3N (2.85 g, 28.21 mmol, 2 eq) followed by dihydro-2H-pyran-2,6(3H)-dione 3A (1.6 g, 14.11 mmol). The resultant reaction mixture was stirred at room temperature for 16 h. Upon completion of the reaction, reaction mixture was diluted with EtOAc (30 mL), ice cold water (30 mL), and stirred for 15 min. Organic layer was separated and aqueous layer was further extracted with EtOAc (2 * 30 mL). Combined organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford 5-(((2-(tert-butoxycarbonyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoic acid 4A (3.2 g, yield: 62%) as a brown solid. MS (ESI) m/z: 362.3 $[M+H]^+$

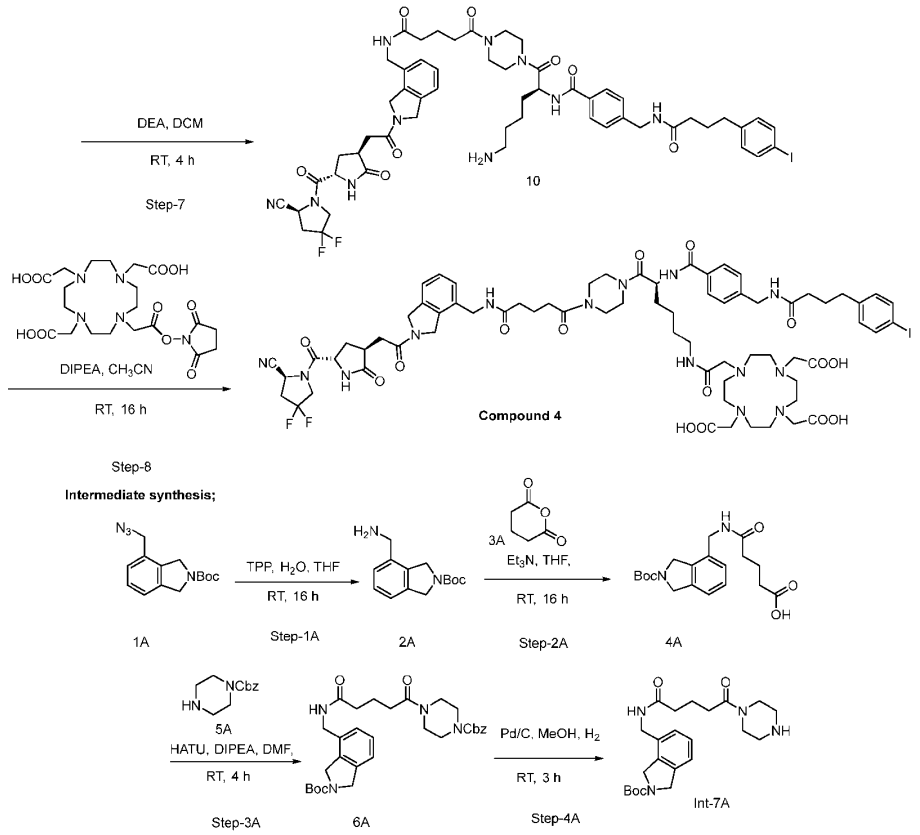
[000213] Step-3A: Synthesis of tert-butyl 4-((5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-5-oxopentanamido)methyl)isoindoline-2-carboxylate: To a solution of 5-(((2-(tert-butoxycarbonyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoic acid 4A (3 g, 8.27 mmol) in DMF (12 mL, 8 Vol) at 0°C, was added DIPEA (2.13 g, 16.5 mmol) followed by benzyl piperazine-1-carboxylate 5A (1.8 g, 8.278 mmol). The resultant reaction mixture was stirred at room temperature for 4 h. Upon completion of the reaction, reaction mixture was diluted with EtOAc (30 mL), ice cold water (30 mL), and stirred for 15 min. Organic layer was separated and aqueous layer was further extracted with EtOAc (2 * 30 mL). Combined organic layers was washed with ice cold water (50 mL). The separated organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to

afford tert-butyl 4-((5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-5-oxopentanamido)methyl)isoindoline-2-carboxylate Int-6A (2.8 g, Yield: 60%) as a brown solid. MS (ESI) m/z: 564.6 $[M+H]^+$

[000214] Step-4A: Synthesis of tert-butyl 4-((5-oxo-5-(piperazin-1-yl)pentanamido)methyl)isoindoline-2-carboxylate: To a solution of tert-butyl 4-((5-(4-((benzyloxy)carbonyl)piperazin-1-yl)-5-oxopentanamido)methyl)isoindoline-2-carboxylate Int-6A (2.8 g, 4.95 mmol) in MeOH (28 mL, 10 Vol) at room temperature, was added Pd/C (900 mg, 30%w/w). Resultant reaction mixture was stirred at room temperature under hydrogen pressure at 30 PSI for 3 h. Upon completion of the reaction, reaction mixture was filtered and filtrate was concentrated under reduced pressure to afford tert-butyl 4-((5-oxo-5-(piperazin-1-yl)pentanamido)methyl)isoindoline-2-carboxylate Int-7A (1.5 g, yield: 70%) as a brown solid. MS (ESI) m/z: 430 $[M+H]^+$

[000215] Example 4. Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 4)


2
HATU, DIPEA, DMF
RT, 4 h
Step-1
1
3
TFA, DCM
0 °C to RT, 5 h
Step-2
4
Cpd-5
HATU, DIPEA, DMF
0 °C to RT, 6 h
Step-3
6
Int-7A
HATU, DIPEA, DMF
RT, 6 h
Step-4
7
TFA, DCM
0 °C to RT, 5 h
Step-5
8
CDA-5
HATU, DIPEA, DMF
RT, 6 h
Step-6
9



[000216] Step-1: Synthesis of tert-butyl 4-((4-(4-iodophenyl)butanamido)methyl)benzsoate: To a solution of 4-(4-iodophenyl)butanoic acid 2 (1.8 g, 6.22 mmol) in DMF (18 mL, 10 Vol) at 0 °C was added DIPEA (2.7 mL, 15.5 mmol) followed by HATU (1.74 g, 6.22 mmol). The Mixture was stirred at room temperature for 30 min, and added tert-butyl 4-(aminomethyl)benzoate 1 (1.2 g, 6.2 mmol). Resultant reaction mixture was stirred at room temperature for 4 h. After completion of reaction, reaction mixture was diluted with EtOAc (200 mL), cold water (100 mL) and stirred for 15 min. The organic layer was separated, and aqueous layer was further extracted with EtOAc (3 * 100 mL). Combined organic layer was washed with water (3 * 50 mL), dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford tert-butyl 4-((4-(4-iodophenyl)butanamido)methyl)benzoate 3 (2.2 g, yield: 74 %) as a brown liquid. MS (ESI) m/z: 480.1 $[M+H]^+$

[000217] Step-2: Synthesis of 4-((4-(4-iodophenyl)butanamido)methyl)benzoic acid: TFA (22 mL, 20.8 mmol) was added to a solution of tert-butyl 4-((4-(4-iodophenyl)butanamido)methyl)benzoate 3 (2.2 g, 4.59 mmol) in DCM (22 mL, 10 Vol) at 0

°C drop wise. Resultant reaction mixture was stirred at room temperature for 5 h. Reaction mixture was concentrated under reduced pressure to afford crude. Crude was triturated with diethyl ether (50 mL) to afford 4-((4-(4-iodophenyl)butanamido)methyl)benzoic acid 4 (1.8 g, yield: 92%) as a brown solid. MS (ESI) m/z: 424.42 $[M+H]^+$

[000218] Step-3: Synthesis of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine: To a solution of 4-((4-(4-iodophenyl)butanamido)methyl)benzoic acid 4 (5 g, 11.8 mmol) in DMF (50 mL, 10 Vol), was added DIPEA (2.4 mL, 14.1 mmol) followed by HATU (4.4 g, 11.8 mmol) at 0 °C. The mixture was stirred at room temperature for 30 min, and added of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-L-lysine 5 (3.9 g, 10.6 mol). The resultant reaction mixture was stirred at room temperature for 30 min. Upon completion of the reaction, the reaction mixture was cool to room temperature, diluted with EtOAc (100 mL) and ice cold water (30 mL), and stirred for 15 min. Organic layer was separated and aqueous layer was extracted with EtOAc (2 * 50 mL). The combined organic layer was washed with ice cold water (50 mL),dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine 6 (3.6 g crude, Yield: 52%) as a white solid. MS (ESI) m/z: 773 [M+H]+

[000219] Step-4: Synthesis of tert-butyl 4-((5-(4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperazin-1-yl)-5-oxopentanamido)methyl)isoindoline-2-carboxylate: To a stirred solution of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine 6 (2 g, 2.58 mmol) in DMF (16 mL, 8 Vol), at 0 °C was added DIPEA (0.82 g, 3.61 mmol) and HATU (983 mg, 2.58 mmol). The mixture was stirred at room temperature for 30 min, and added tert-butyl 4-((5-oxo-5-(piperazin-1-yl)pentanamido)methyl)isoindoline-2-carboxylate Int-7A (1.1 g, 2.58 mmol). The resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was diluted with ice cold water (100 mL), and stirred for 15 min, filtered the solid and triturated with diethyl ether (30 mL) to afford tert-butyl 4-((5-(4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperazin-1-yl)-5-oxopentanamido)methyl)isoindoline-2-carboxylate 7 (2 g, yield: 65%) as a brown solid. MS (ESI) m/z: 1186 $[M+H]^+$

[000220] Step-5: Synthesis of (9H-fluoren-9-yl)methyl (S)-(5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-(4-(5-((isoindolin-4-ylmethyl)amino)-5-oxopentanoyl)piperazin-1-yl)-6-oxohexyl)carbamate: TFA (8.4 mL, 4 vol) was added to a stirred solution of tert-butyl 4-((5-(4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperazin-1-yl)-5-oxopentanamido)methyl)isoindoline-2-carboxylate 7 (2.2 g, 1.85 mmol) in DCM (22 mL, 10 Vol) at 0 °C. The resultant reaction mixture was stirred at room temperature for 60 min. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude. The crude was further triturated with diethyl ether (2 * 50 mL) to afford (9H-fluoren-9-yl)methyl (S)-(5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-(4-(5-((isoindolin-4-ylmethyl)amino)-5-oxopentanoyl)piperazin-1-yl)-6-oxohexyl)carbamate 8 (1.3 g, yield: 64%) as a brown solid. MS (ESI) m/z: 1086 $[M+H]^+$

[000221] Step-6: Synthesis of (9H-fluoren-9-yl)methyl ((S)-6-(4-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)carbamate: To a solution of 2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetic acid (CDA-5) (0.52 g, 1.38 mmol) in DMF (12 mL, 8 Vol) at 0°C, was added DIPEA (0.35 g, 2.76 mmol) followed by HATU (0.52, 1.38 mmol). The mixture was stirred at room temperature for 5 min, followed by addition of (9H-fluoren-9-yl)methyl (S)-(5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-(4-(5-((isoindolin-4-ylmethyl)amino)-5-oxopentanoyl)piperazin-1-yl)-6-oxohexyl)carbamate 8 (1.5 g, 1.38 mmol). The resultant reaction mixture was stirred at room temperature for 60 min. Upon completion of the reaction, reaction mixture was diluted with ice cold water (30 mL), and stirred for 15 min. The solid was filtered and triturated with diethyl ether to afford (9H-fluoren-9-yl)methyl ((S)-6-(4-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)carbamate 9 (1.5 g, yield: 79.3%) as a brown solid. MS (ESI) m/z: 1369 $[M+H]^+$

[000222] Step-7: Synthesis of N-((S)-6-amino-1-(4-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-1-oxohexan-2-yl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide: Diethyl amine (7.2 mL, 4 vol) was added to a stirred solution of (9H-fluoren-9-yl)methyl ((S)-6-(4-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-

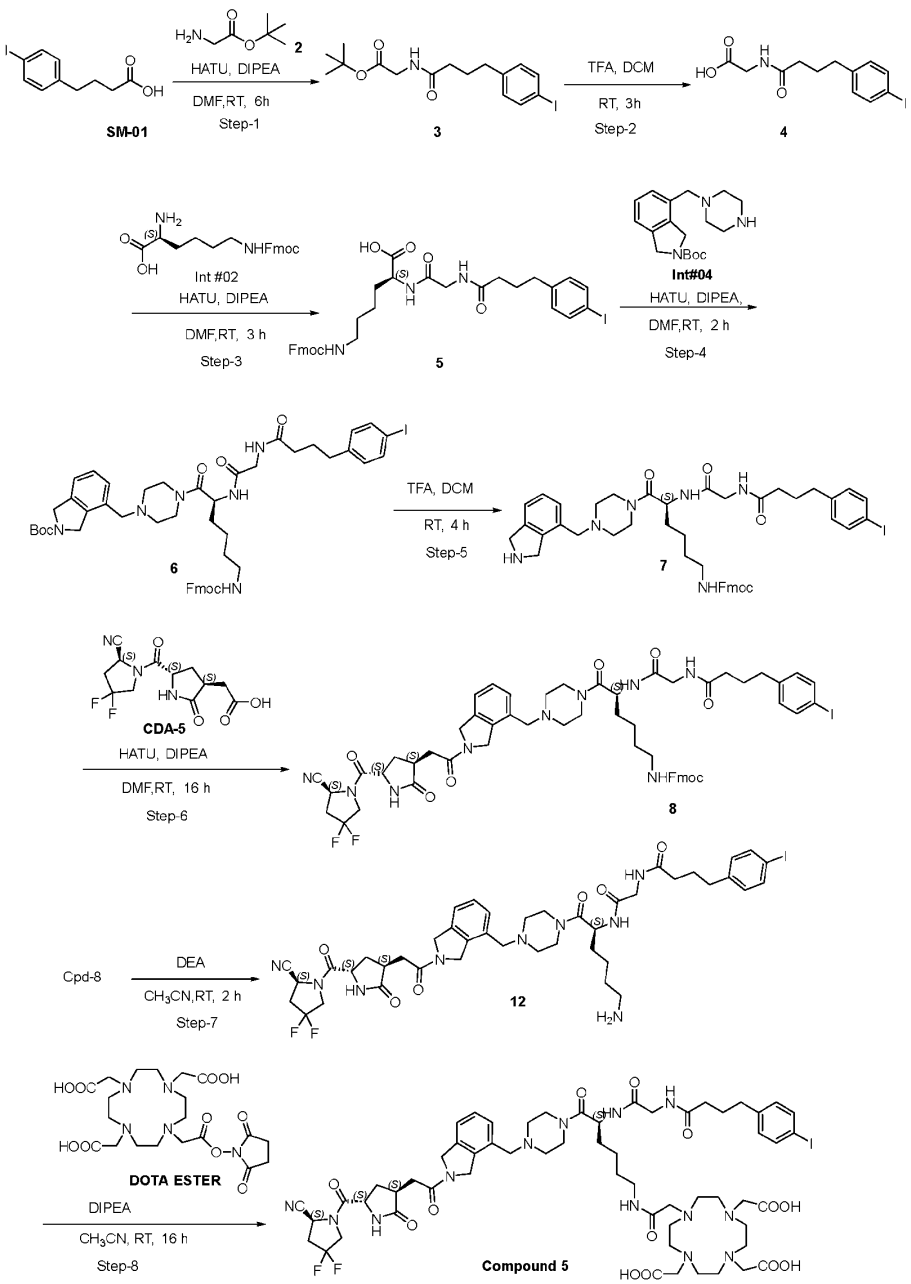
difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)carbamate 9 (1.8 g, 1.314 mmol) in acetonitrile (18 mL, 10 Vol) at room temperature. Resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, the reaction mixture was concentrated under reduced pressure to obtain crude. Crude compound was triturated with cold EtOAc (3*10 mL). The obtained solid was dissolved in 10% MeOH in DCM (20 mL) and precipitated with diethyl ether (30 mL). Resultant solid was filtered, dried under vacuum to afford N-((S)-6-amino-1-(4-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-1-oxohexan-2-yl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 10 (1.2 g, crude) as a pale brown solid. MS (ESI) m/z: 1147 $[M+H]^+$

[000223] Step-8: Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triaceticacid: To a solution of N-((S)-6-amino-1-(4-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-1-oxohexan-2-yl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 10 (1.2 g, 1.04 mmol) in acetonitrile (12 mL, 10 Vol) at room temperature was added DIPEA (1.2 g, 9.43 mmol) followed by 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid DOTA NHS Ester (0.786 g, 1.56 mmol). The resultant reaction mixture was stirred at room temperature for 16 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude (1.6 g). The crude was triturated with EtOAc (30 mL) and further purified by prep HPLC. The required fractions were collected and lyophilized to afford 2,2',2''-(10-(2-(((S)-6-(4-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triaceticacid (0.183 g, Yield: 11.4%) as a white solid. MS (ESI) m/z: 1533.41 $[M+H]^+$ ^{1}H-NMR (400 MHz, DMSO-d6): δ 8.6-8.5 (m, 1H), 8.5-8.45 (m, 1H), 8.40 (t, J = 6 Hz, 1H), 8.2-8.1 (m, 1H), 8.1 (s, 1H), 7.83 (d, J = 8 Hz, 2H), 7.62 (d, J = 8.4 Hz, 2H), 7.3 (d, J = 8 Hz, 2H), 7.3-7.2 (m, 2 H), 7.2-7.1 (m, 1 H), 7.00 (d, J = 8 Hz,

2H), 5.09-5.06 (m, 1H), 4.9-4.8 (m, 2H), 4.7-4.6 (m, 2 H), 4.4-4.0 (m, 13 H), 3.9-3.8 (m, 10 H), 3.7-3.5 (m, 10 H), 3.4-3.3 (m, 8 H), 3.2-3.0 (m, 9 H), 2.9-2.7 (m, 4H), 2.4-2.0 (m, 6H), 1.6-1.9 (m, 4H), 1.5-1.3 (m, 4H)

[000224] The synthesis of tert-butyl 4-((5-oxo-5-(piperazin-1-yl)pentanamido)methyl)isoindoline-2-carboxylate (Int-7A) is described in Example 3.

[000225] Example 5. Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 5)



[000226] Step-1: Synthesis of tert-butyl (4-(4-iodophenyl)butanoyl)glycinate: To a solution of 4-(4-phenyl)butanoic acid SM-01(7 g, 0.02412 mol) in DMF (54 mL) at room temperature was added HATU (13.7 g, 0.036 mol) followed by DIPEA (12.6 mL, 0.07238 mol) and tert-butyl glycinate 2 (3.5 g, 0.024 mol). Resultant reaction mixture was stirred at room temperature for 6 h. Upon completion of the reaction, reaction mixture was cool to 0 °C

and poured into ice water (300 mL). The precipitated solid was filtered and washed with ice water (20 mL) to get crude. Crude was purified by silica gel column chromatography (100-200 mesh) using 70% EtOAC in hexane as an eluant. Compound contained fractions were concentrated under reduced pressure to afford tert-butyl (4-(4-iodophenyl)butanoyl)glycinate 3 (7.2 g, yield: 74%) as an off white solid. MS (ESI) m/z: 403 $(M+H)^+$.

[000227] Step-2: Synthesis of (4-(4-iodophenyl)butanoyl)glycine: TFA (28.8 mL, 4 vol) was added to a solution of tert-butyl (4-(4-iodophenyl)butanoyl)glycinate 3 (7.2 g, 0.017 mol) in DCM (72 mL, 10 vol), at 0 °C. Resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of reaction, the reaction mixture was concentrated under reduced pressure to afford crude. Crude was triturated with diethyl ether (25 mL) and n-pentane (50 mL) to afford (4-(4-iodophenyl)butanoyl)glycine 4 (5.5 g, yield: 88%) as an off white solid. MS (ESI) m/z: 348 $(M+H)^+$.

[000228] Step-3: Synthesis of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysine: To a solution of (4-(4-iodophenyl)butanoyl)glycine 4 (5.5 g, 0.015 mol) in DMF (44 mL, 8 Vol), at room temperature was added HATU (6 g, 0.015 mol) followed by DIPEA (5.5 mL, 0.031 mol) The mixture was stirred for 30 min before adding N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-L-lysine Int #02 (4 g, 0.011 mol). Resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of reaction, the reaction mixture was cool to 0 °C and poured into ice water (50 mL), the precipitated solid was filtered and washed with ice water (50 mL). Crude was purified by mPLC column chromatography using 50 % H_2O in acetonitrile as an eluant. Compound contained fractions were concentrated under reduced pressure to afford N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysine 5 (5 g, yield: 45%) as an off white solid. MS (ESI) m/z: 698.7 $(M+H)^-$.

[000229] Step-4: tert-butyl 4-((4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysyl)piperazin-1-yl)methyl)isoindoline-2-carboxylate: To a solution of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysine 5 (5 g, 0.0071 mol) in DMF (50 mL, 10 Vol) at 0 °C, was added DIPEA (3.7 mL, 0.021 mol) followed by HATU (4 g, 0.010 mol). The mixture was stirred for 30 min before adding tert-butyl 4-((5-oxo-5-(piperazin-1-yl)pentanamido)methyl)isoindoline-2-carboxylate Int#04 (2.2 g, 0.0071 mol). Resultant reaction mixture was stirred at room temperature for 2 h. Upon completion of reaction, the reaction mixture was diluted with EtOAc (100 mL), ice cold water (50 mL) stirred for 15 min. Organic layer was separated and aqueous layer was extracted with EtOAc (3 * 100 mL).

The combined organic layers was washed with ice cold water (3 * 50 mL). The organic phase separated was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford tert-butyl 4-((4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysyl)piperazin-1-yl)methyl)isoindoline-2-carboxylate 6 (5 g, yield: 70%) as a pale yellow solid . MS (ESI) m/z: 997.05 $(M+H)^+$

[000230] Step-5: Synthesis of 9H-fluoren-9-yl)methyl (S)-(5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-(4-(isoindolin-4-ylmethyl)piperazin-1-yl)-6-oxohexyl)carbamate: To a solution afford tert-butyl 4-((4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysyl)piperazin-1-yl)methyl)isoindoline-2-carboxylate 6 (5 g, 0.005 mmol) at 0 °C in DCM (50 mL, 10 Vol), was added TFA (20 mL, 4Vol) drop wise. Resultant reaction mixture was stirred at room temperature for 4 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to get the crude material, which was triturated with diethyl ether (30 mL) to afford 9H-fluoren-9-yl)methyl (S)-(5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-(4-(isoindolin-4-ylmethyl)piperazin-1-yl)-6-oxohexyl)carbamate brown colour solid 7 (3.5 g, crude, yield: 77%) as a MS (ESI) m/z: 897. $(M+H)^+$.

[000231] Step-6: Synthesis of (9H-fluoren-9-yl)methyl ((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-oxohexyl)carbamate: To a solution of 2-((S)-4,4-difluoro-1-glycylpyrrolidin-2-yl)-N-(3,4-dimethoxybenzyl)-2-hydroxyacetamide CDA-5 (0.9 g, 0.0039 mol) in DMF (35 mL, 10 Vol) at 0 °C was added DIPEA (2 mL, 0.011 mol) followed by HATU (2.2 g, 0.005 mol). Resultant mixture was stirred at room temperature for 10 min followed by addition of 9H-fluoren-9-yl)methyl (S)-(5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-(4-(isoindolin-4-ylmethyl)piperazin-1-yl)-6-oxohexyl)carbamate 7 (3.5 g, 0.0039 mol). Resultant reaction mixture was stirred at room temperature for 16 h. Reaction mixture was diluted with ice-cold water (100 mL) and stirred for 15 min. Organic layer was separated and aqueous layer was extracted with EtOAc (3 * 100 mL). The combined organic layers were washed with water (3 * 50 mL). The organic phase separated was dried over anhydrous sodium sulphate, filtered and concentrated under vacuum to afford (9H-fluoren-9-yl)methyl ((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-oxohexyl)carbamate 8 (2.5 g, crude) as a pale yellow solid. MS (ESI) m/z: 1145 $(M+H)^+$.

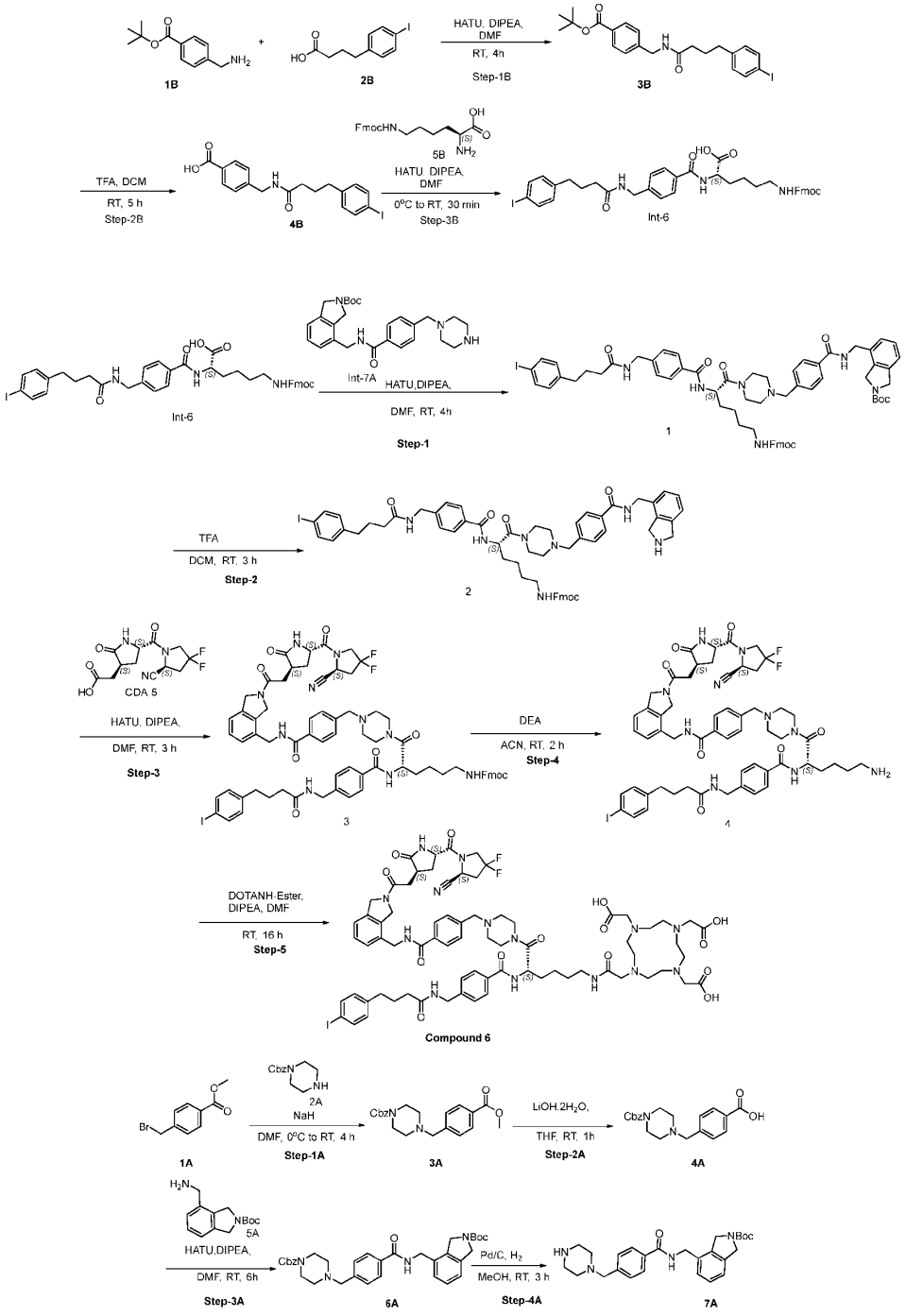
[000232] Step-7: Synthesis of N-(2-(((S)-6-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-1-oxohexan-2-yl)amino)-2-oxoethyl)-4-(4-iodophenyl)butanamide: To a solution of (9H-fluoren-9-yl)methyl ((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-oxohexyl)carbamate 8 (2.5 g, 0.0021 mol) at room temperature in acetonitrile (25 mL, 10 Vol), was added diethyl amine (12.5 mL, 5 Vol) drop wise. The resultant reaction mixture was stirred at room temperature for 2 h. Reaction mixture was concentrated under vacuum to get crude (3.2 g), which was triturated with cold EtOAc (3 * 20 mL) and filtered. Crude was dissolved in 10% MeOH in DCM (10 mL), and diluted with diethyl ether (50 mL). Regenerated solid was again dissolved in acetonitrile (30 mL), and the undissolved particles were removed by filtration. Filtrate was concentrated under vacuum to afford N-(2-(((S)-6-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-1-oxohexan-2-yl)amino)-2-oxoethyl)-4-(4-iodophenyl)butanamide 9 (1.5 g crude, yield: 68%) as a pale brown liquid. MS (ESI) m/z: 959 $(M+H)^+$.

[000233] Step-8: Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid: To a solution of N-(2-(((S)-6-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-1-oxohexan-2-yl)amino)-2-oxoethyl)-4-(4-iodophenyl)butanamide (1.5 g, 0.0015 mmol) in acetonitrile (15 mL, 10 Vol), at room temperature was added DIPEA (2.4 mL, 0.014 mmol) and 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid DOTA NHS Ester (1.1 g, 0.002 mmol) lot wise. The resultant reaction mixture was stirred at room temperature for 16 h. Reaction mixture was concentrated under reduced pressure to get crude which was triturated with EtOAc (2 * 20 mL) and filtered. Crude solid was dissolved in 10% MeOH in DCM (10 mL), and diluted with diethyl ether (40 mL). The regenerated solid was further triturated with acetonitrile (2* 25 mL), and solid was, then the isolated solid was re-dissolved again dissolved in MeOH (10 mL). The undissolved particles was removed by filtration, and the filtrate was concentrated under reduced pressure get semi-pure compound. The semi-pure material was further purified by Prep-HPLC. The required

fractions were collected and concentrated under vacuum followed by lyophilization to afford 2,2',2''-(10-(2-(((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (203 mg) as a white solid. MS (ESI) m/z: 1344.8 $(M+H)^+$. ^{1}H-NMR (400 MHz, DMSO-d6): δ 8.44-8.42 (m, 1H), 8.2- 8.0 (m, 3H), 7.61 (d, J = 8.4 Hz, 2H), 7.51-7.40 (m, 3H), 7.00 (d, J = 8.4 Hz, 2H), 5.12-5.10 (m, 1H), 5.0-4.6 (m, 5H), 4.4-4.1 (m, 10 H), 4.00-3.51 (m, 12 H), 3.51-3.01 (m, 20H), 3.0-2.5 (m, 4 H), 3.1-2.5 (m, 6 H), 2.2-2.0 (m, 2H), 1.8-1.6 (m, 3H), 1.51-1.41 (m, 3H), 1.3-1.2 (m, 2H)

[000234] The synthesis of tert-butyl 4-(piperazin-1-ylmethyl)isoindoline-2-carboxylate (Int#04) is described in Example 1.

[000235] Example 6. Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl) piperazin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 6)


1B
2B
HATU, DIPEA, DMF
RT, 4h
Step-1B
3B
5B
TFA, DCM
RT, 5 h
Step-2B
4B
HATU, DIPEA, DMF
0°C to RT, 30 min
Step-3B
Int-6
Int-7A
HATU,DIPEA,
DMF, RT, 4h
Step-1
1
TFA
DCM, RT, 3 h
Step-2
2
CDA 5
HATU, DIPEA,
DMF, RT, 3 h
Step-3
3
DEA
ACN, RT, 2 h
Step-4
4
DOTANH-Ester,
DIPEA, DMF
RT, 16 h
Step-5
Compound 6
2A
NaH
DMF, 0°C to RT, 4 h
Step-1A
1A
3A
LiOH.2H2O,
THF, RT, 1h
Step-2A
4A
5A
HATU,DIPEA,
DMF, RT, 6h
Step-3A
6A
Pd/C, H2
MeOH, RT, 3 h
Step-4A
7A

[000236] Step-1B: Synthesis of tert-butyl 4-((4-(4-iodophenyl)butanamido)methyl)benzoate 3: To a solution of 4-(4-iodophenyl)butanoic acid 2B (1.8 g, 6.22 mmol) in DMF (18 mL, 10 Vol) at 0 °C, was added DIPEA (2.7 mL, 15.5 mmol) followed by HATU (1.74 g, 6.22 mmol). The mixture was stirred at room temperature for 30 min, and added tert-butyl 4-(aminomethyl)benzoate 1B (1.2 g, 6.2 mmol). Resultant reaction mixture was further stirred at room temperature for 4 h. Reaction mixture was diluted with EtOAc (200 mL), cold water (100 mL) and stirred for 15 min. The organic layer was separated, and aqueous layer was further extracted with EtOAc (3 * 100 mL). Combined organic layers was washed with water (3 * 50 mL), dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford tert-butyl 4-((4-(4-iodophenyl)butanamido)methyl)benzoate 3B (2.2 g, yield: 74 %) as a brown liquid. MS (ESI) m/z: 480.1 $[M+H]^+$

[000237] Step-2B: Synthesis of 4-((4-(4-iodophenyl)butanamido)methyl)benzoic acid: TFA (22 mL, 20.8 mmol) was added to a solution of tert-butyl 4-((4-(4-iodophenyl)butanamido)methyl)benzoate 3B (2.2 g, 4.59 mmol) in DCM (22 mL, 10 Vol) at 0 °C. Resultant reaction mixture was stirred at room temperature for 5 h. Reaction mixture was concentrated under reduced pressure to afford crude. Crude was triturated with diethyl ether (50 mL) to afford 4-((4-(4-iodophenyl)butanamido)methyl)benzoic acid 4B (1.8 g, yield: 92%) as a brown solid. MS (ESI) m/z: 424.42 $[M+H]^+$

[000238] Step-3B: Synthesis of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine: To a solution of 4-((4-(4-iodophenyl)butanamido)methyl)benzoic acid 4B (5 g, 11.8 mmol) in DMF (50 mL, 10 Vol), was added DIPEA (2.4 mL, 14.1 mmol) followed by HATU (4.4 g, 11.8 mmol). The mixture was stirred at room temperature for 30 min and added N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-L-lysine 5B (3.9 g, 10.6 mol). The resultant reaction mixture was stirred at room temperature for 30 min. Upon completion of the reaction, the reaction mixture was diluted with EtOAc (100 mL) and ice-cold water (30 mL), and stirred for 15 min. The organic layer was separated, and aqueous layer was further extracted with EtOAc (3 * 100 mL). Combined organic layers was washed with water (3 * 50 mL), dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine Int-6 (3.6 g crude, Yield: 52%) as a white solid. MS (ESI) m/z: 773 [M+H]+

[000239] Step-1: tert-butyl 4-((4-((4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperazin-1-

yl)methyl)benzamido)methyl)isoindoline-2-carboxylate: To a solution of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine Int-6 (2.6 g, 3.3635 mmol) in DMF (26 mL, 8 Vol) at room temperature, was added DIPEA (0.82 mL, 4.708 mmol) followed by HATU (1.27 g, 3.3635 mmol). The mixture was stirred at room temperature for 30 min and added tert-butyl 4-((4-(piperazin-1-ylmethyl)benzamido)methyl)isoindoline-2-carboxylate Int-7A (1.51 g, 3.3635 mmol). Resultant reaction mixture was stirred at room temperature for 3 h. The reaction mixture was poured into ice water (50 mL) and stirred for 10 min. The solid precipitated was filtered and washed with ice water (50 mL). The crude obtained was purified by reverse phase column chromatography using 60% H_2O in acetonitrile as an eluant. Compound contained fractions were concentrated under reduced pressure to afford tert-butyl4-((4-((4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl) butanamido)methyl)benzoyl)-L-lysyl)piperazin-1-yl)methyl)benzamido)methyl)isoindoline-2-carboxylate 1 (3.4 g, yield: 83 %) as an brown colour solid.MS (ESI) m/z:1207.68 $(M+H)^+$.

[000240] Step-2: Synthesis of (9H-fluoren-9-yl)methyl (S)-(5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-(4-(4-((isoindolin-4-ylmethyl)carbamoyl)benzyl)piperazin-1-yl)-6-oxohexyl)carbamate: TFA (12 mL, 3 vol) was added to a solution of tert-butyl 4-((4-((4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl) piperazin-1-yl)methyl)benzamido)methyl)isoindoline-2-carboxylate 1 (3.9 g, 3.23 mmol) in DCM (13 mL, 10 vol) at 0 °C. Resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of reaction, the reaction mixture was concentrated under reduced pressure to afford crude. Crude was triturated with diethyl ether (25 mL) and n-pentane (50 mL) to afford (9H-fluoren-9-yl)methyl (S)-(5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-(4-(4-((isoindolin-4-ylmethyl)carbamoyl)benzyl)piperazin-1-yl)-6-oxohexyl)carbamate 2 (2.8 g, yield: 78%) as an off white solid. MS (ESI) m/z: 1107.53 $(M+H)^+$.

[000241] Step-3: Synthesis of (9H-fluoren-9-yl)methyl ((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperazin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)carbamate: To a solution of (9H-fluoren-9-yl)methyl (S)-(5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-(4-(4-((isoindolin-4-ylmethyl)carbamoyl)benzyl)piperazin-1-yl)-6-oxohexyl)carbamate 2 (2.5 g, 2.32 mmol) in DMF (26 mL, 10 vol) at 0 °C, was added DIPEA (0.62 mL, 3.25 mmol) followed by HATU (0.84 g, 2.32 mmol). The mixture was stirred at room temperature for 10

min and added 2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetic acid CDA-5 (0.7 g, 2.32 mol). The resultant reaction mixture was stirred at ambient temperature for 3 h. The progress of reaction was monitored by TLC. Upon completion of reaction, the reaction mixture was poured into ice water (50 mL), and the solid precipitated was filtered and washed with ice water (50 mL) to afford crude. The crude was purified by mPLC column chromatography using 50% H_2O in acetonitrile as an eluant. Compound contained fractions were concentrated under reduced pressure to afford (9H-fluoren-9-yl)methyl ((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperazin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)carbamate 3 (2.7 g, crude) as a pale yellow solid. MS (ESI) m/z: 1398.30 $(M+H)^+$.

[000242] Step-4: Synthesis of N-((S)-6-amino-1-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperazin-1-yl)-1-oxohexan-2-yl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide: Diethyl amine (12 mL, 5 Vol) was added to a solution of (9H-fluoren-9-yl)methyl ((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperazin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)carbamate 3 (2.4 g, 1.727 mmol) in acetonitrile (24 mL, 10 Vol) at room temperature. Resultant reaction mixture was stirred at room temperature for 2 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude. Crude compound (4.5 g) was triturated with cold EtOAc (3*10 mL). The obtained solid was dissolved in 10% MeOH in DCM (20 mL) and precipitated with diethyl ether (30 mL). The resultant solid was filtered, dried under vacuum to afford N-((S)-6-amino-1-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperazin-1-yl)-1-oxohexan-2-yl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 4 (1.3 g, crude) as a pale brown solid. MS (ESI) m/z: 1167.54 $(M+H)^+$.

[000243] Step-5: Synthesis of 2,2',2"-(10-(2-(((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperazin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid: To a stirred solution of N-((S)-6-amino-1-(4-

(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-1-oxohexan-2-yl)-4-((4-(p-tolyl)butanamido)methyl)benzamide 4 (1.3 g, 1.3366 mmol) in acetonitrile (17 mL, 10 Vol)at room temperature was added DIPEA (2.1 mL, 12.029 mmol) followed by 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (1 g, 2.0049 mmol). The resultant reaction mixture was stirred at ambient temperature for 16 h. The progress of reaction was monitored by TLC. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude (1.2 g). The crude material was triturated with EtOAc (30 mL) and further purified by prep HPLC. The required fractions were collected and concentrated under vacuum followed by lyophilization to afford 2,2',2''-(10-(2-(((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperazin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (0.142 g) as a white solid. MS (ESI) m/z: 1554.53 $(M+H)^+$. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 9.2-9.1 (m, 1H), 8.59-8.57 (m, 2H), 8.5-8.48 (brs, 1H), 8.41 (t, J = 6 Hz, 1H), 8.04 (s, 1H), 7.99-7.96 (m, 2H), 7.82 (d, J = 8 Hz, 2H), 7.63-7.57 (m, 2H), 7.31 (d, J = 8.4 Hz, 2H), 7.21-7.3 (m, 2H), 7.01 (d, J = 8.4 Hz, 2H), 5.12-5.11 (m, 1H), 4.9-4.5 (m, 8H), 4.5-4.0 (m, 16 H), 3.9-3.8 (m, 3H), 3.8-3.5 (m, 6H), 3.5-3.3 (m, 10 H), 3.2-3.0 (m, 13 H), 2.9-2.6 (m, 4H), 2.5-2.4 (m, 2H), 2.3-2.2 (m, 1H), 2.2-2.1 (m, 2H), 1.7-1.8 (m, 2H), 1.7-1.6 (m, 2H), 1.5-1.4 (m, 2H), 1.3-1.2 (m, 2H)

[000244] Step-1A: Synthesis of benzyl 4-(4-(methoxycarbonyl)benzyl)piperazine-1-carboxylate: To a solution of benzyl piperazine-1-carboxylate 2A (24 g, 109.09 mmol) in DMF (120 mL), at 0 °C was added 60% NaH (8.7 g, 218.18 mmol). The mixture was stirred for 30 min and added methyl 4-(bromomethyl)benzoate 1A (4.1 g, 15.886 mmol). Resultant reaction mixture was stirred at room temperature for 4h. Upon completion of the reaction, reaction mixture was diluted with EtOAc (100 mL), ice cold water (400 mL) and stirred for 15 min. Organic layer was separated and the aqueous layer was further extracted with EtOAc (2 * 150 mL). Combined organic layers was washed with ice cold water (200 mL), dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford crude. The crude was purified by silica gel column chromatography (100-200 mesh) using 60% ethyl acetate in hexane as an eluent. Compound contained fractions were concentrated under reduced pressure to afford benzyl 4-(4-(methoxycarbonyl)benzyl)piperazine-1-carboxylate 3A (30 g, yield: 78%) as an off white solid. MS (ESI) m/z: 369.1 $(M+H)^+$.

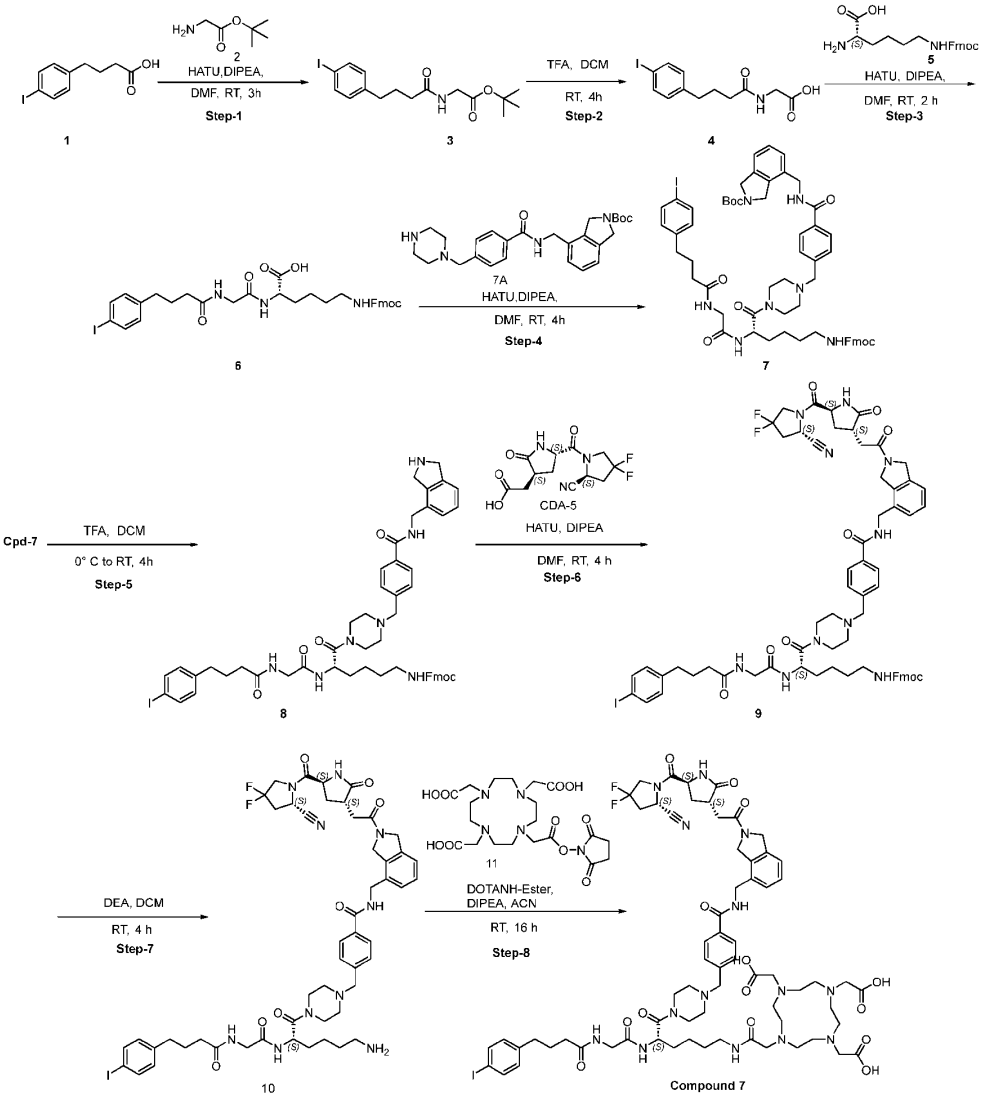
[000245] Step-2A: Synthesis of 4-((4-((benzyloxy)carbonyl)piperazin-1-yl)methyl)benzoic acid: To a solution of benzyl 4-(4-(methoxycarbonyl)benzyl)piperazine-1-carboxylate 3A (15 g, 40.76 mmol) in THF (75 mL, 5 vol) and water (75, 5 vol) at room temperature was added LiOH.H_2O (2.4 g, 101.92 mmol). Resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was diluted ice water (200 mL), acidified with 1N HCl (10 mL) and extracted with 10% MeOH in DCM, organic layer was dried over sodium sulphate and concentrated under vacuum to afford crude. The crude was purified by silica gel column chromatography (100-200 mesh) using 50% EtOAC in hexane as an eluant. Compound contained fractions were concentrated under reduced pressure to afford 4-((4-((benzyloxy)carbonyl)piperazin-1-yl)methyl)benzoic acid 4A (7 g, yield: 50%) as an off white solid. MS (ESI) m/z: 354.4 $(M+H)^+$.

[000246] Step-3A: Synthesis of tert-butyl 4-((4-((4-((benzyloxy)carbonyl)piperazin-1-yl)methyl)benzamido)methyl)isoindoline-2-carboxylate: To a solution of 4-((4-((benzyloxy)carbonyl)piperazin-1-yl)methyl)benzoic acid 4A (7 g, 19.77 mmol) in DMF (10 mL), at room temperature was added DIPEA (4.8 mL, 27.68 mmol) followed by HATU (8.2 g, 21.75 mmol). The mixture was stirred at room temperature for 30 min and added tert-butyl 4-(aminomethyl)isoindoline-2-carboxylate 5A (4.9 g, 19.774 mmol). Resultant reaction mixture was stirred at room temperature for 6h. The progress of the reaction was monitored by TLC. Upon completion of the reaction, mixture was poured into ice cold water (200 mL), the precipitated solid was filtered and washed with ice water (20 mL) to afford crude. The crude was purified by silica gel column chromatography (100-200 mesh) using 40% EtOAC in hexane as an eluant. Compound contained fractions were concentrated under reduced pressure to afford tert-butyl 4-((4-((4-((benzyloxy)carbonyl)piperazin-1-yl)methyl)benzamido)methyl)isoindoline-2-carboxylate 6A (10 g, yield: 63%) as an off white solid. MS (ESI) m/z: 584.75 $(M+H)^+$.

[000247] Step-4A: Synthesis of tert-butyl 4-((4-(piperazin-1-ylmethyl)benzamido)methyl)isoindoline-2-carboxylate: To a solution of tert-butyl 4-((4-((4-((benzyloxy)carbonyl)piperazin-1-yl)methyl)benzamido)methyl)isoindoline-2-carboxylate 6A (3.9 g, 2.48 mmol) in methanol (50 mL, 12 Vol) at room temperature was added 10% Pd/C (1.3 g, 2.487 mmol) under nitrogen atmosphere. The resultant reaction mixture was hydrogenated under 30 *psi* for 3 h. Upon completion of reaction, mixture was filtered through celite pad. The filtrate was concentrated under reduced pressure to afford crude (3.5 g). Crude was purified by reverse phase purification using 50% H_2O in acetonitrile as an eluant. Compound contained fractions were concentrated under reduced pressure to afford tert-butyl

4-((4-(piperazin-1-ylmethyl)benzamido)methyl)isoindoline-2-carboxylate 7A (2 g, yield: 66 %) as an off white solid . MS (ESI) m/z:451.70 $(M+H)^+$.

[000248] Example 7. Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperazin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido) acetamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 7)



[000249] Step-1: Synthesis of tert-butyl (4-(4-iodophenyl)butanoyl)glycinate: To a solution of 4-(4-phenyl)butanoic acid SM-01 (7 g, 0.02412 mol) in DMF (54 mL) at room

temperature was added HATU (13.7 g, 0.036 mol) followed by DIPEA (12.6 mL, 0.07238 mol). The mixture was stirred for 30 min and added tert-butyl glycinate 2 (3.5 g, 0.024 mol). Resultant reaction mixture was stirred at room temperature for 6 h. Upon completion of the reaction, reaction mixture was poured into ice water (300 mL), and the precipitated solid was filtered and washed with ice water (200 mL) to get crude. The crude obtained was purified by silica gel column chromatography (100-200 mesh) using 70% EtOAC in hexane as an eluant. Required fractions were concentrated under reduced pressure to afford tert-butyl (4-(4-iodophenyl)butanoyl)glycinate 3 (7.2 g, yield: 74%) as an off white solid. MS (ESI) m/z: 403 $(M+H)^+$.

[000250] Step-2: Synthesis of (4-(4-iodophenyl)butanoyl)glycine:TFA (28.8 mL, 4 vol) was added to a solution of tert-butyl (4-(4-iodophenyl)butanoyl)glycinate 3 (7.2 g, 0.017 mol) in DCM (72 mL, 10 vol), at 0 °C. Resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of reaction, the reaction mixture was concentrated under reduced pressure to afford crude. Crude was triturated with diethyl ether (50 mL) and n-pentane (50 mL) to afford (4-(4-iodophenyl)butanoyl)glycine 4 (5.5 g, yield: 88%) as an off white solid. MS (ESI) m/z: 348 $(M+H)^+$.

[000251] Step-3: Synthesis of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysine : To a solution of (4-(4-iodophenyl)butanoyl)glycine 4 (6 g, 0.017 mol) in DMF (60 mL) at room temperature, was added HATU (6.5 g, 0.017 mol) followed by DIPEA (4.5 mL, 0.025 mol). The mixture was stirred for 30 min and added N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-L-lysine 5 (5 g, 0.013 mol). Resultant reaction mixture was stirred at room temperature for 2 h. Upon completion of the reaction, reaction mixture was poured into ice water (200 mL) stirred for 15 min, the precipitated solid was filtered and washed with ice water (50 mL). The solid obtained was dissolved in 10% MeOH in DCM (30 mL), and triturated with diethyl ether (60 mL). The regenerated solid was filter to afford N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysine 6 (4.3 g, yield: 35%) as a white solid. MS (ESI) m/z: 698.35 $(M+H)^+$

[000252] Step-4: Synthesis of tert-butyl 4-((4-((4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysyl)piperazin-1-yl)methyl)benzamido)methyl)isoindoline-2-carboxylate: To a solution of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysine 6 (4 g, 0.0057 mol) in DMF (40 mL) at room temperature, was added HATU (2.2 g, 0.0057 mol) followed by DIPEA (2 mL, 0.014 mol). The mixture was stirred for 30 min and added tert-butyl 4-((4-(piperazin-1-ylmethyl)benzamido)methyl)isoindoline-2-carboxylate 7A (2.5 g, 0.0057 mol).

The resultant reaction mixture was stirred at room temperature for 4 h. Upon completion of the reaction, reaction mixture was poured into ice water (150 mL) stirred for 15 min, the precipitated solid was filtered and washed with ice water (50 mL). The solid obtained was dissolved in 10% MeOH in DCM (30 mL), and triturated with diethyl ether (60 mL). The regenerated solid was filter to afford tert-butyl 4-((4-((4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysyl)piperazin-1-yl)methyl)benzamido)methyl)isoindoline-2-carboxylate 7 (3.8 g, yield: 59%) as a white solid. MS (ESI) m/z: 1131.65 $(M+H)^+$

[000253] Step-5: Synthesis of (9H-fluoren-9-yl)methyl (S)-(5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-(4-(4-((isoindolin-4-ylmethyl)carbamoyl)benzyl)piperazin-1-yl)-6-oxohexyl)carbamate : TFA (15.2 mL, 4 Vol) was added to a solution of tert-butyl 4-((4-((4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysyl)piperazin-1-yl)methyl)benzamido)methyl)isoindoline-2-carboxylate 7 (3.8 g, 0.003 mmol) in DCM (38 mL, 10 Vol) at 0° C. The resultant reaction mixture was stirred at room temperature for 4 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude. Crude was triturated with diethyl ether (2 * 50 mL) to afford (9H-fluoren-9-yl)methyl (S)-(5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-(4-(4-((isoindolin-4-ylmethyl)carbamoyl)benzyl)piperazin-1-yl)-6-oxohexyl)carbamate 8 (3 g, yield: 78%) as a white solid. MS (ESI) m/z: 1030.55 $(M+H)^+$.

[000254] Step-6 : Synthesis of (9H-fluoren-9-yl)methyl ((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperazin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-oxohexyl)carbamate: To a solution of 2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetic acid CDA-5 (1.15 g, 0.003 mol) in DMF (30 mL) at room temperature was added HATU (1.49 g, 0.003 mol) followed by DIPEA (1.3 mL, 0.007 mol). The mixture was stirred for 30 min and added (9H-fluoren-9-yl)methyl (S)-(5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-(4-(4-((isoindolin-4-ylmethyl)carbamoyl)benzyl)piperazin-1-yl)-6-oxohexyl)carbamate 8 (3.09 g, 0.003 mol). The resultant reaction mixture was stirred at room temperature for 4 h. Upon completion of the reaction, reaction mixture was poured into ice water (150 mL) stirred for 15, the precipitated solid was filtered and washed with ice water (50 mL). The solid obtained was dissolved in 10% MeOH in DCM (30 mL), and triturated with diethyl ether (60 mL). The regenerated solid was filter to afford (9H-fluoren-9-yl)methyl ((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-

cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperazin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-oxohexyl)carbamate 9 (3 g, yield: 60%) as a white solid. MS (ESI) m/z: 1314.67 $(M+H)^+$

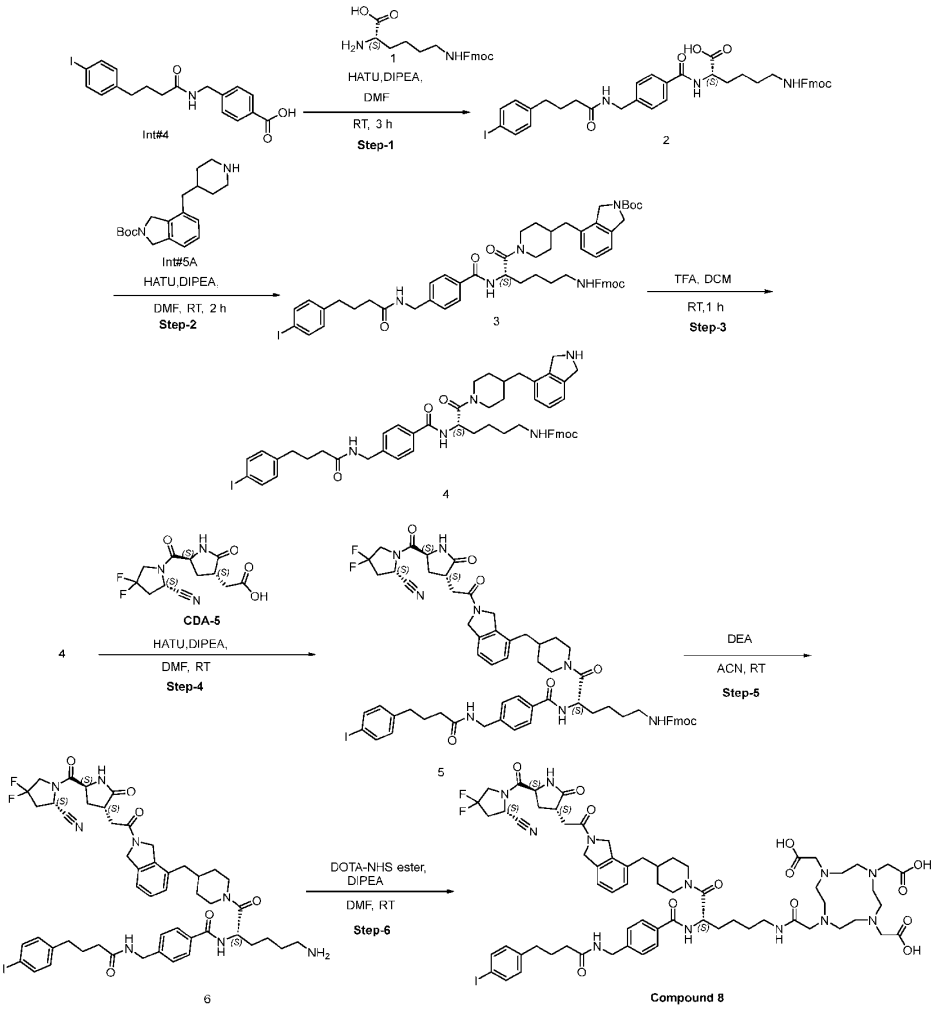
[000255] Step-7: N-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)-4-((4-((4-(4-iodophenyl)butanoyl)glycyl-L-lysyl)piperazin-1-yl)methyl)benzamide: Diethyl amine (13 mL, 5 Vol) was added to a solution of (9H-fluoren-9-yl)methyl ((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperazin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-oxohexyl)carbamate 9 (2.6 g, 0.0019 mol) in acetonitrile (2.5 mL, 10 Vol) at room temperature. Resultant reaction mixture was stirred at room temperature for 4h. Reaction mixture was concentrated under reduced pressure to get crude. Crude compound was triturated with cold EtOAc (3 * 25 mL) and filtered. Solid was dissolved in 10% MeOH in DCM (10 mL) and diluted with diethyl ether (50 mL). The regenerated solid was filtered and again dissolved in acetonitrile (30 mL). Undissolved parts were removed by filtration, and the filtrate concentrated under vacuum to afford N-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)-4-((4-((4-(4-iodophenyl)butanoyl)glycyl-L-lysyl)piperazin-1-yl)methyl)benzamide 10 (1.7 g, yield: 80%) as a white solid. MS (ESI) m/z: 1091.52 $(M+H)^+$.

[000256] Step-8 : Synthesis of 2,2',2"-(10-(2-(((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperazin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid: To a solution of N-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)-4-((4-((4-(4-iodophenyl)butanoyl)glycyl-L-lysyl)piperazin-1-yl)methyl)benzamide 10 (1.7 g, 0.0015 mol) in CH_3CN (8.5 mL, 10 Vol) at room temperature was added DIPEA (2.4 mL, 0.014 mol), followed by 2,2',2"-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid DOTA-NHS Ester (1.1 g, 0.0023 mol). Resultant reaction mixture was stirred at room temperature for 16 h. Reaction mixture was concentrated under reduced pressure to get crude. Crude was triturated with EtOAc (2 * 50 mL). The solid obtained was dissolved in 10% MeOH in DCM (30 mL), and triturated with diethyl ether (60 mL). The solid obtained was trituration with acetonitrile (2 * 50 mL), isolated solid was re-dissolved in MeOH (20 mL), the undissolved parts were removed by filtration, the organic phase collected

was concentrated under reduced pressure to get crude. Purification: The crude material was purified by Prep-HPLC. The required fractions were collected and concentrated under vacuum followed by lyophilization to afford 2,2',2''-(10-(2-(((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperazin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (215 mg) as a white solid. MS (ESI) m/z: 1478.63 $(M-H)^-$. ^{1}H-NMR (400 MHz, DMSO-d6): δ 13.2-12.8 (brs, 1H), 9.14-9.12 (m, 1H), 8.46 (brs, 1H), 8.14-8.11 (m, 2H), 8.06 (brs, 1H), 7.99-7.96 (m, 2H), 7.62-7.57 (m, 4H), 7.31-7.21 (m, 3H), 7.01 (d, J = 8.4 Hz, 2H), 5.11-5.09 (m, 1H), 4.94 (brs, 1H), 4.86 (brs, 1H), 4.75-4.70 (m, 1H), 4.65-4.62 (m, 2H), 4.45 (d, J = 4.8 Hz, 2H), 4.33 (brs, 3H), 4.29-4.10 (m, 5H), 4.14.0 (m, 3H), 3.88 (brs, 2H), 3.6 (d, J = 5.6 Hz, 2H), 3.59-3.55 (m, 4H), 3.37 (brs, 6H), 3.35-3.30 (m, 2H), 3.16-3.09 (m, 12H), 2.92-2.67 (m, 7H), 2.45-2.40 (m, 1H), 2.30-2.25 (m, 1H), 2.12 (t, J = 7.2 Hz, 2H), 1.78-1.76 (m, 2H), 1.65-1.55 (m, 1H), 1.49-1.35 (m, 3H), 1.28-1.20 (m, 2H).

[000257] The synthesis of tert-butyl 4-((4-(piperazin-1-ylmethyl)benzamido)methyl)isoindoline-2-carboxylate (7A) is described in Example 6.

[000258] Example 8. Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 8)



[000259] Step-1: Synthesis of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine (2): To a solution of 4-((4-(4-iodophenyl)butanamido)methyl)benzoic acid (Int#4) (5 g, 11.82 mmol) in DMF (50 mL, 10 Vol) at 0 °C was added DIPEA (4.1 mL, 23.64 mmol) followed by HATU (4.9 g, 11.82 mmol). The mixture was stirred at ambient temperature for 30 min, followed by N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-L-lysine 1 (4.35 g, 11.82 mmol). Resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of the reaction, reaction mixture was diluted with EtOAc (200 mL), cold water (200 mL) and stirred for 15 min. The organic layer was separated, and aqueous layer was further extracted with EtOAc (2 * 150 mL). Combined organic layer was washed with water (150 mL), dried over anhydrous sodium sulphate, filtered and concentrated under reduced to afford crude. The crude was purified by

silica gel column chromatography (100-200 mesh) using 50% ethyl acetate in hexane an eluant. Required fractions were concentrated under reduced pressure to afford N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine 2 (4 g, yield: 44%) as an off white solid. MS (ESI) m/z: 774.26 $(M+H)^+$.

[000260] Step-2: Synthesis of tert-butyl 4-((1-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperidin-4-yl)methyl)isoindoline-2-carboxylate(3): To a solution of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine 2 (5 g, 0.006 mol) in DMF (50 mL, 10 Vol) mixture was stirred at room temperature for 15 min, followed by addition of tert-butyl 4-(piperidin-4-ylmethyl)isoindoline-2-carboxylate (Int#5A) (2.04 g, 0.006 mol) . Resultant reaction mixture was stirred at ambient temperature for 1 h. Upon completion of the reaction, reaction mixture was diluted with EtOAc (100 mL), ice cold water (100mL) and stirred for 15 min. The aqueous layer was further extracted with EtOAc (3 * 100 mL), combined organic layer was washed with ice cold water (2 * 100 mL). The organic phase separated was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to get tert-butyl 4-((1-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperidin-4-yl)methyl)isoindoline-2-carboxylate 3 (4.6 g, yield: 66%) as an off white solid. MS (ESI) m/z:1072.53 $(M+H)^+$.

[000261] Step-3: Synthesis of(9H-fluoren-9-yl)methyl (S)-(5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-(4-(isoindolin-4-ylmethyl)piperidin-1-yl)-6-oxohexyl)carbamate (4): TFA (20 mL, 4 Vol) was added to a solution of tert-butyl 4-((1-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperidin-4-yl)methyl)isoindoline-2-carboxylate 3 (5 g, 0.004 mol) in DCM (50 mL, 5 Vol) at 0 °C. Resultant reaction mixture was stirred at room temperature for 2 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude. The crude was triturated with diethyl ether (30 mL), resulted solid was filtered and dried under vacuum to afford (9H-fluoren-9-yl)methyl (S)-(5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-(4-(isoindolin-4-ylmethyl)piperidin-1-yl)-6-oxohexyl)carbamate 4 (4 g, yield: 88%) as a brown solid. MS (ESI) m/z: 972.28 $(M+H)^+$.

[000262] Step-4: Synthesis of(9H-fluoren-9-yl)methyl ((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-

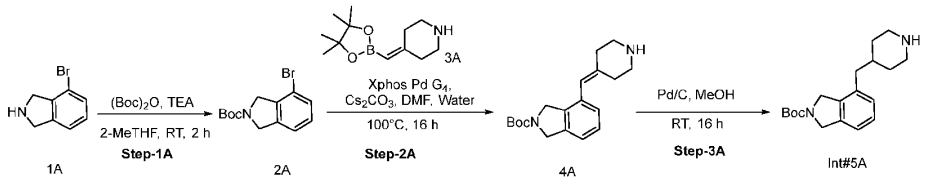
oxohexyl)carbamate (5): To a solution of 2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetic acid CDA-5 (2.41 g, 0.002 mol) in DMF (7.5 mL, 10 Vol) at 0 °C, was added DIPEA (0.91 mL, 0.004 mol) followed by HATU (1.041 g, 0.0027 mol).The mixture was stirred at room temperature for 30 min followed by addition of (9H-fluoren-9-yl)methyl (S)-(5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-(4-(isoindolin-4-ylmethyl)piperidin-1-yl)-6-oxohexyl)carbamate 4 (750 mg, 0.002 mol). The resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was diluted with 10% MeOH in DCM (100 mL) and ice water (50 mL) and stirred for 15 min. The organic layer was separated and the aqueous layer was further extracted with 10% MeOH in DCM (2 *50 mL). The combined organic layer was washed with water (2 * 50 mL), dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford (9H-fluoren-9-yl)methyl ((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)carbamate 5 (2.1 g, yield: 21%) as a brown solid. MS (ESI) m/z:1256.60 $(M+H)^+$. Note: Crude material was forwarded to the next step without purification.

[000263] Step-5: N-((S)-6-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-1-oxohexan-2-yl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide(6): Diethyl amine (9.2 mL, 4 Vol) was added to a solution of (9H-fluoren-9-yl)methyl ((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-5-(4-((4-(4-iodophenyl) butanamido) methyl) benzamido)-6-oxohexyl)carbamate 5 (2.3 g, 0.0018 mol) in acetonitrile (2.3 mL, 10 Vol) at room temperature. The resultant reaction mixture was stirred at room temperature for 2 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude. Crude was triturated with cold EtOAc (50 mL) followed by diethyl ether (50 mL) to afford N-((S)-6-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-1-oxohexan-2-yl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 6 (1.5 g, yield: 79%) as an off white solid. MS (ESI) m/z: 1034.42 $(M+H)^+$.

[000264] Step-6: Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-

yl)methyl)piperidin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid: To a solution of N-((S)-5-amino-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-6-oxohexyl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 6 (850 mg, 0.823 mol) in acetonitrile (8.5 mL, 10 Vol) at room temperature was added DIPEA (1.36 mL, 7.42mol) followed by 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid DOTA NHS ester (618 mg, 1.235 mol). Resultant reaction mixture was stirred at room temperature for 16 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude (780 mg crude). Crude was triturated with EtOAc (20 mL) and further purified by prep HPLC using the below condition. The required fractions were collected and lyophilized to afford 2,2',2''-(10-(2-(((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (174.8 mg, yield: 14.5%) as a white solid. MS (ESI) m/z: 1419.56 $[M+H]^+$. ^{1}H-NMR (400 MHz, DMSO-d6): δ 14-12.5 (brs, 2H), 8.6-8.4 (m, 3H), 8.1 (s, 1H), 7.9-7.8 (m, 2H), 7.62 (d, J = 8.0 Hz, 2H), 7.33-7.28 (m, 2H), 7.02-7.03 (m, 1H), 7.00 (d, J = 8 Hz, 2H), 5.13-5.11 (m, 1H), 4.9-4.8 (m, 3H), 4.7-4.6 (m, 2H), 4.5-4.4 (m, 4H), 4.3-4.0 (m, 5H), 3.8-3.9 (m, 4H), 3.5-3.4 (m, 9 H), 3.4-3.3 (m, 10 H), 3.3-3.0 (m, 12 H), 2.9-2.7 (m, 4H), 2.3-2.2 (m, 3H), 1.9-1.8 (m, 3H), 1.7-1.6 (m, 2H), 1.5-1.4 (m, 4H), 1.3-1.2 (m, 2H).

[000265] Synthesis of tert-butyl 4-(piperidin-4-ylmethyl)isoindoline-2-carboxylate (Int#5A)



[000266] Step-1A: Synthesis of tert-butyl 4-bromoisoindoline-2-carboxylate (2A): To a solution of 4-bromoisoindoline 1A (1 g, 0.005 mol) in 2-MeTHF (10 mL, 10 Vol) at 0 °C was added Et_3N (2.9 mL, 0.02 mol) followed by $(Boc)_2O$ (1.47 mL, 0.0067 mol). The reaction mixture was stirred at room temperature for 2h. Upon completion of the reaction, reaction mixture was diluted with EtOAc (30 mL), water (30 mL) and stirred for 15 min. The organic

layer was separated, and aqueous layer was further extracted with EtOAc (3 * 30 mL). Combined organic layers was washed with water (3 * 30 mL), dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to get crude. Crude was purified by silica gel column chromatography (100-200 mesh) using 5% EtOAc in pet ether as an eluent. Required fraction were concentrated under reduced pressure to afford tert-butyl 4-bromoisoindoline-2-carboxylate 2A (1.14 g, yield: 76%) as a white solid.

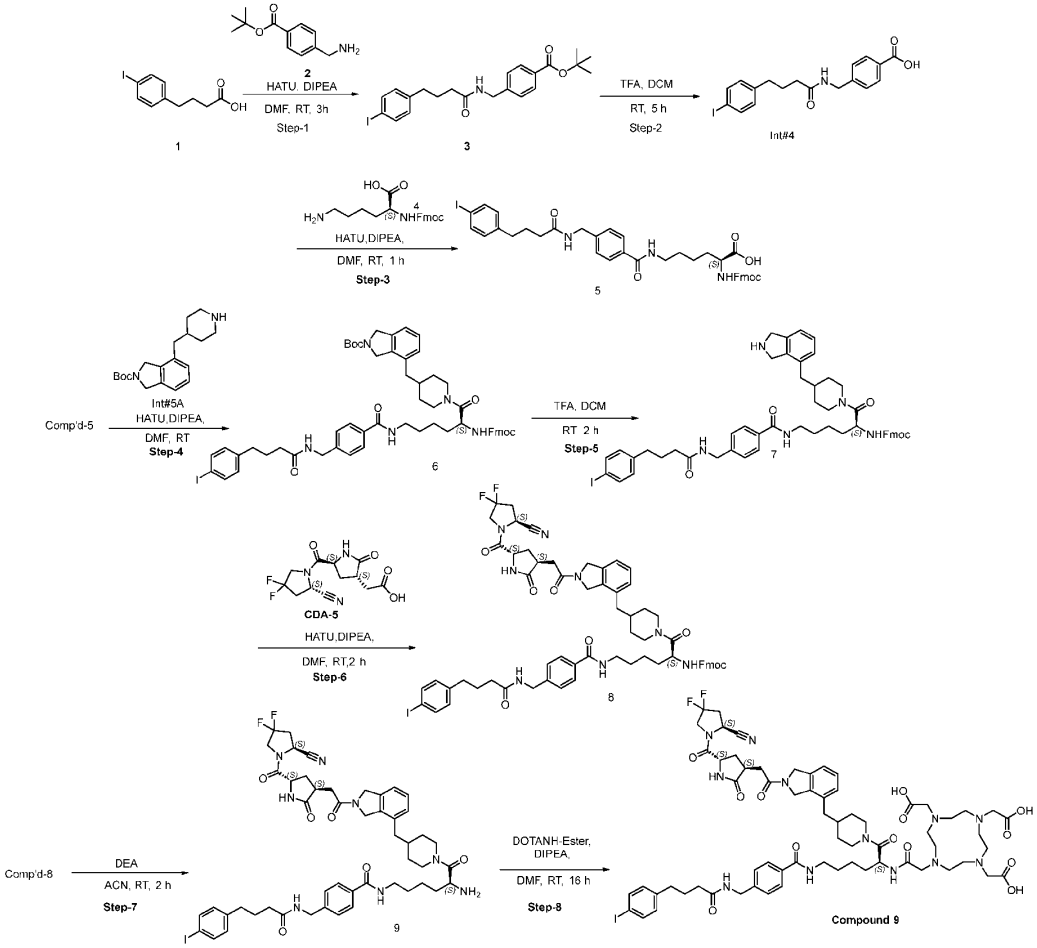
[000267] Step-2A: Synthesis of tert-butyl 4-(piperidin-4-ylidenemethyl)isoindoline-2-carboxylate (4A): To a solution of tert-butyl 4-bromoisoindoline-2-carboxylate 2A (500 mg, 1.67 mol) in DMF:H_2O (9:1, 10 vol) was added 4-((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methylene)piperidine 3A (561.5 mg, 2.51 mmol) followed by Cs_2CO_3 (1.63 g, 5.03 mmol). The mixture was degassed with argon for 15 min followed by addition of XPhos Pd G4 (80 mg, 0.67 mmol), and resultant reaction mixture was stirred at 100 °C for 16 h. Upon completion of the reaction, reaction mixture was filtered through celite bed washed with ethyl acetate (20 mL), filtrate was diluted with water (30 mL). The organic layer was separated, and aqueous layer was further extracted with EtOAc (3 * 30 mL). Combined organic layer was washed with water (3 * 30 mL), dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford crude. Crude was purified by silica gel column chromatography (100-200 mesh) using 10% MeOH in DCM. Required fractions were concentrated under reduced pressure to afford tert-butyl 4-(piperidin-4-ylidenemethyl)isoindoline-2-carboxylate 4A (0.415 g, yield: 79%) as a brown solid. MS (ESI) m/z: 315.1$[M+H]^+$

[000268] Step-3A: Synthesis of tert-butyl 4-(piperidin-4-ylmethyl)isoindoline-2-carboxylate (Int#5A: To a solution of tert-butyl 4-(piperidin-4-ylidenemethyl)isoindoline-2-carboxylate 4A (680 mg, 2.162 mmol) in MeOH (3 mL) was added 10% Pd/C (680 mg, wt/wt) under nitrogen atmosphere. The resultant reaction mixture was stirred at room temperature for 16 h under hydrogen pressure 30 *psi*. Upon completion of the reaction, reaction mixture was filtered through celite bed, washed with MeOH (20 mL), filtrated and concentrated under reduced pressure to afford tert-butyl 4-(piperidin-4-ylmethyl)isoindoline-2-carboxylate (Int#5A) (523 mg, crude) as an off white solid. MS (ESI) m/z: 317.09 $[M+H]^+$

[000269] Step-1B: Synthesis of 4-((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methylene)piperidine (3A): To a solution of tert-butyl 4-((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methylene)piperidine-1-carboxylate 1B (1 g, 0.003 mmol) in DCM (10 mL, 10 vol), was added 4M HCl in 1,4-dioxane (6 mL, 6 vol) at 0 °C . The reaction mixture was stirred at room temperature for 1h. Upon completion of the reaction, reaction mixture

was concentrated under reduced pressure to get crude (1.3 g). Crude was triturated with diethyl ether, resulted solid was filtered and dried under vacuum to afford 4-((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methylene)piperidine 3A (635 mg, yield: 92%) as an off white solid. MS (ESI) m/z: 224 $[M+H]^+$

[000270] Example 9. Synthesis of 2,2',2''-(10-(2-(((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 9)



[000271] Step-1: Synthesis of tert-butyl 4-((4-(4-iodophenyl)butanamido)methyl)benzoate (3): To a solution of 4-(4-iodophenyl)butanoic acid 1 (30 g, 0.103 mol) in DMF (60 mL, 2 Vol) at 0 °C was added DIPEA (27 mL, 0.155 mol)

followed by HATU (39.3 g, 0.103 mol). The mixture was stirred at room temperature for 30 min, and added tert-butyl 4-(aminomethyl)benzoate 2 (21.4 g, 0.103 mol). Resultant reaction mixture was stirred at room temperature for 4 h. Upon completion of the reaction, reaction mixture was diluted with EtOAc (500 mL), cold water (400 mL) and stirred for 15 min. The organic layer was separated, and aqueous layer was further extracted with EtOAc (2 * 400 mL). Combined organic layer was washed with water (400 mL). The separated organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford tert-butyl 4-((4-(4-iodophenyl)butanamido)methyl)benzoate 3 (36.1 g, crude) as a brown liquid. MS (ESI) m/z: 480.1 $[M+H]^+$

[000272] Step-2: Synthesis of 4-((4-(4-iodophenyl)butanamido)methyl)benzoic acid (4): TFA (160 mL, 4 vol) was added to a solution of tert-butyl 4-((4-(4-iodophenyl)butanamido)methyl)benzoate 3 (40 g, 0.083 mol) in DCM (200 mL, 5 Vol) at 0 °C. Resultant reaction mixture was stirred at room temperature for 5 h. Reaction mixture was concentrated under reduced pressure to afford crude. Crude was triturated with diethyl ether (200 mL) to afford 4-((4-(4-iodophenyl)butanamido)methyl)benzoic acid 4 (31 g, yield: 88%) as a brown solid. MS (ESI) m/z: 424.42 $[M+H]^-$

[000273] Step-3: Synthesis of N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine (5): To a solution of 4-((4-(4-iodophenyl)butanamido)methyl)benzoic acid (Int#4) (5 g, 11.82 mmol) in DMF (50 mL, 10 Vol) at 0 °C, was added DIPEA (4.1 mL, 23.64 mmol) followed by HATU (4.49 g, 11.82 mmol). The mixture was stirred at room temperature for 30 min, and added (((9H-fluoren-9-yl)methoxy)carbonyl)-L-lysine 4 (4.77 g, 11.84 mmol). Resultant reaction mixture was stirred at room temperature for 1 h. Reaction mixture was diluted with EtOAc (200 mL), cold water (200 mL) and stirred for 15 min. The organic layer was separated, and aqueous layer was further extracted with EtOAc (2 * 150 mL). Combined organic layer was washed with water (150 mL). The separated organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford crude. Crude was purified by silica gel column chromatography (100-200 mesh) using 50% Ethyl acetate in hexane as an eluant. Required fractions were concentrated under reduced pressure to afford N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine 5 (4 g, yield: 44 %) as a white solid. MS (ESI) m/z:774.3 $(M+H)^+$.

[000274] Step-4: Synthesis of tert-butyl 4-((1-(N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperidin-4-yl)methyl)isoindoline-2-carboxylate (6): To a solution of N2-(((9H-fluoren-

9-yl)methoxy)carbonyl)-N6-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine 5 (170 mg, 0.219 mmol) in DMF (2 mL, 10 Vol) at 0 °C was added DIPEA (0.076 mL, 0.439 mmol) followed by HATU (83.4 mg, 0.219 mmol, 1 eq). The mixture was stirred at room temperature for 15 min and added tert-butyl 4-(piperidin-4-ylmethyl)isoindoline-2-carboxylate (Int#5A) (69.5 mg, 0.219 mmol). Resultant reaction mixture was stirred at room temperature for 2 h. Reaction mixture was diluted with EtOAc (50 mL), ice cold water (50 mL) and stirred for 15 min. The aqueous layer was extracted with EtOAc (2 * 25 mL), combined organic layer was washed with ice cold water (2 * 25 mL). The organic phase separated was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to get tert-butyl 4-((1-(N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperidin-4-yl)methyl)isoindoline-2-carboxylate 6 (120 mg, yield: 51%) as a white solid. MS (ESI) m/z: 1072.4 $(M+H)^+$.

[000275] Step-5: Synthesis of (9H-fluoren-9-yl)methyl (S)-(6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-(4-(isoindolin-4-ylmethyl)piperidin-1-yl)-1-oxohexan-2-yl)carbamate (7): TFA (20 mL, 4 Vol) was added to a solution of 4-((1-(N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperidin-4-yl)methyl)isoindoline-2-carboxylate 6 (5 g, 4.66 mmol) in DCM (50 mL, 10 Vol) at 0 °C. The resultant reaction mixture was stirred at room for 2 h. Reaction mixture was concentrated under reduced pressure to afford crude. Crude was triturated with diethyl ether (30 mL), to afford (9H-fluoren-9-yl)methyl (S)-(6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-(4-(isoindolin-4-ylmethyl)piperidin-1-yl)-1-oxohexan-2-yl)carbamate 7 (4 g, yield: 51%) as a brown solid. MS (ESI) m/z: 972.98 $(M+H)^+$.

[000276] Step-6: Synthesis of (9H-fluoren-9-yl)methyl ((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)carbamate (8): To a solution of CDA-5 (81 mg, 0.269 mmol) in DMF (2.6 mL, 10 Vol) at 0°C was added DIPEA (70 mg, 0.539 mmol) followed by addition of HATU (363 mg, 0.295 mmol). The mixture was stirred at room temperature for 30 min and added (9H-fluoren-9-yl)methyl (S)-(6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-(4-(isoindolin-4-ylmethyl)piperidin-1-yl)-1-oxohexan-2-yl)carbamate 7 (262 mg, 0.269 mmol). The resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was diluted with 10% MeOH in DCM (25 mL) and ice water (20 mL), organic layer was separated and the aqueous layer was extracted with 10% MeOH

in DCM (2 *25 mL). Organic layer separated was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford (9H-fluoren-9-yl)methyl ((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)carbamate 8 (200 mg, yield: 51%) as a brown colour solid. MS (ESI) m/z: $(M+H)^+$. Note: Crude material was forwarded to the next step without purification.

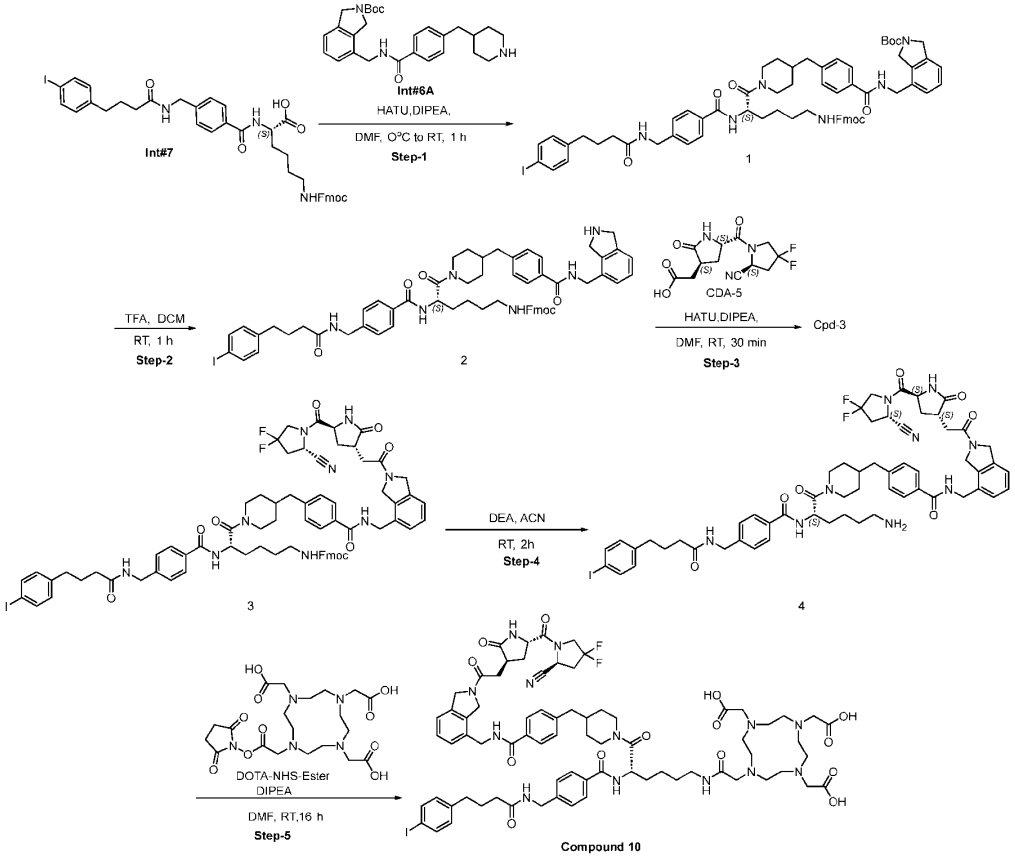
[000277] Step-7: Synthesis of N-((S)-5-amino-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-6-oxohexyl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide: (9) Diethyl amine (0.4 mL, 4 Vol) was added to a solution of (9H-fluoren-9-yl)methyl ((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)carbamate 8 (1 g, 0.796 mmol) in acetonitrile (10 mL, 10 Vol) at room temperature. Resultant reaction mixture was stirred at room temperature for 2 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to get crude. Crude material was triturated with cold EtOAc (10 mL) followed by diethyl ether (10 mL) to afford N-((S)-5-amino-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-6-oxohexyl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 9 (0.66 g, yield: 81%) as a white solid. MS (ESI) m/z: 1033 $(M+H)^+$.

[000278] Step-8: Synthesis of 2,2',2''-(10-(2-(((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid: To a solution of N-((S)-5-amino-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-6-oxohexyl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 9 (720 mg, 0.697 mmol) in acetonitrile (8 mL, 10 Vol), at room temperature, was added DIPEA (1.07 mL, 6.273 mmol) followed by 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid DOTA NHS ester (523 mg, 1.045 mmol). The resultant reaction mixture was stirred at room temperature for 16 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude (430 mg crude). The crude

material was triturated with EtOAc (20 mL) and further purified by prep HPLC. The required fractions were collected and lyophilized to afford 2,2',2"-(10-(2-(((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (120 mg, yield: 12%) as a white solid. MS (ESI) m/z: 1491.5 [M+H]. ^{1}H-NMR (400 MHz, DMSO-d*6*): δ 14-12 (brs, 2H), 8.8-8.7 (Brs, 1H), 8.39-8.36 (m, 2H), 8.06-8.03 (m, 1H), 7.79-7.74 (m, 2H), 7.62 (*dd*, *J* = 2Hz, 8.4 Hz, 2H), 7.31-7.16 (m, 2H), 7.2-7.1 (m, 2 H), 7.1-7.0 (m, 1H), 7.02 (d, *J* = 2.4 Hz, 8 Hz, 2H), 5.12-5.09 (m, 1H), 4.9-4.8 (m, 2H), 4.7-4.6 (m, 3H), 4.4-4.3 (m, 4H), 4.2-4.0 (m, 5H), 3.8-3.7 (m, 2H), 3.7-3.5 (m, 4H), 3.5-3.3 (m, 16 H), 3.3-3.0 (m, 10H), 2.9-2.7 (m, 4H), 2.3-2.2 (m, 1H), 2.2-2.1 (m, 3H), 1.8-1.7 (m, 3H), 1.7-1.6 (m, 2H), 1.5-1.4 (m, 4H), 1.4-1.3 (m, 2H), 1.2-1 (m, 2H)

[000279] The synthesis of tert-butyl 4-(piperidin-4-ylmethyl)isoindoline-2-carboxylate is described in Example 8.

[000280] Example 10. Synthesis of 2,2',2"-(10-(2-(((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperidin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 10)



[000281] Step-1: Synthesis of tert-butyl 4-((4-((1-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperidin-4-yl)methyl)benzamido)methyl)isoindoline-2-carboxylate (1): To a solution of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine (Int#7) (2.8 g, 0.003 mol) in DMF (28 mL, 10 Vol) at 0 °C, was added DIPEA (1.2 mL, 0.007 mol) followed by HATU (1.3 g, 0.003 mol). The mixture was stirred at room temperature for 10 min, followed by addition of tert-butyl 4-((4-(piperidin-4-ylmethyl)benzamido)methyl)isoindoline-2-carboxylate (1.62 g, 0.003 mol). Resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was diluted with ice cold water (100 mL) and stirred for 15 min. Solid precipitated was filtered, washed with ice cold water (2 * 50 mL), hexene (50 mL) and dried under reduced pressure to afford *tert*-butyl4-((4-((1-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-

lysyl)piperidin-4-yl)methyl)benzamido)methyl)isoindoline-2-carboxylate 1 as an off white solid (3 g, Yield 69%). MS (ESI) m/z: 1205.99 $(M+H)^+$.

[000282] Step-2: Synthesis of (9H-fluoren-9-yl)methyl (S)-(5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-(4-(4-((isoindolin-4-ylmethyl)carbamoyl)benzyl)piperidin-1-yl)-6-oxohexyl)carbamate(2): TFA (28 mL, 4 Vol) was added to a solution of tert-butyl4-((4-((1-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperidin-4-yl)methyl)benzamido)methyl)isoindoline-2-carboxylate 1 (7 g, 0.006 mol) in DCM (70 mL, 10 Vol) at 0°C. The reaction mixture was stirred at room temperature for 1h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude. Crude was triturated with diethyl ether (50 mL), and resultant solid was filtered to afford (9H-fluoren-9-yl)methyl (S)-(5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-(4-(4-((isoindolin-4-ylmethyl)carbamoyl)benzyl)piperidin-1-yl)-6-oxohexyl)carbamate 2 (3.5 g, yield: 54%) as a light brown solid. MS (ESI) m/z:1105.13 $(M+H)^+$.

[000283] Step-3: Synthesis of (9H-fluoren-9-yl)methyl ((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperidin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)carbamate (3): To a solution of 2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetic acid CDA-5 (800 mg, 2.65 mol) in DMF (8 mL, 10 Vol) at 0 °C was added DIPEA (0.9 mL, 5.31 mol) followed by HATU (1.11 g, 2.92 mol). The mixture was stirred for 15 min and added (9H-fluoren-9-yl)methyl (S)-(5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-(4-(4-((isoindolin-4-ylmethyl)carbamoyl)benzyl)piperidin-1-yl)-6-oxohexyl)carbamate 2 (2.9 g, 2.65 mol). Upon completion of the reaction, reaction mixture was diluted with EtOAc (30 mL), ice cold water (30 mL), and stirred for 15 min. Organic layer was separated and the aqueous layer was further extracted with EtOAc (2 * 30 mL). Combined organic layer was washed with ice cold water (50 mL), dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford (9H-fluoren-9-yl)methyl ((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperidin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)carbamate 3 (2.5 g, yield: 69%) as a brown solid. MS (ESI) m/z: 1389$(M+H)^+$.

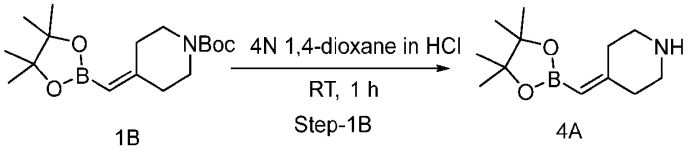
[000284] Step-4: Synthesis of N-((S)-6-amino-1-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperidin-1-yl)-1-oxohexan-2-yl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide(4): Diethyl amine (10.8 mL, 4 Vol) was added to a solution of (9H-fluoren-9-yl)methyl ((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperidin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)carbamate 3 (2.7 g, 0.001 mol) in acetonitrile (27 mL, 10 Vol) at room temperature. Reaction mixture was stirred at room temperature for 2 h. Reaction mixture was concentrated under reduced pressure to afford crude. Crude material was triturated with cold EtOAc followed by diethyl ether (2*50 mL) to afford N-((S)-6-amino-1-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperidin-1-yl)-1-oxohexan-2-yl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 4 (1.57 g, yield: 69%) as an off white solid. MS (ESI) m/z:1166.59 $(M+H)^+$.

[000285] Step-5: Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperidin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic: To a solution of N-((S)-6-amino-1-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperidin-1-yl)-1-oxohexan-2-yl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 4 (1.6 mg, 0.001 mol) in acetonitrile (16 mL, 10 Vol) at room temperature was added DIPEA (2.15 mL, 0.012 mol) followed by 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid DOTA NHS ester (1.03 g, 0.002 mol). Resultant reaction mixture was stirred at room temperature for 16 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude (3.5 g crude). The crude material was triturated with EtOAc (25*2 mL) and further purified by prep HPLC using the below condition. The required fractions were collected and lyophilized to afford 2,2',2''-(10-(2-(((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperidin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic (200 mg, yield: 9.5%) as a white solid. MS (ESI)

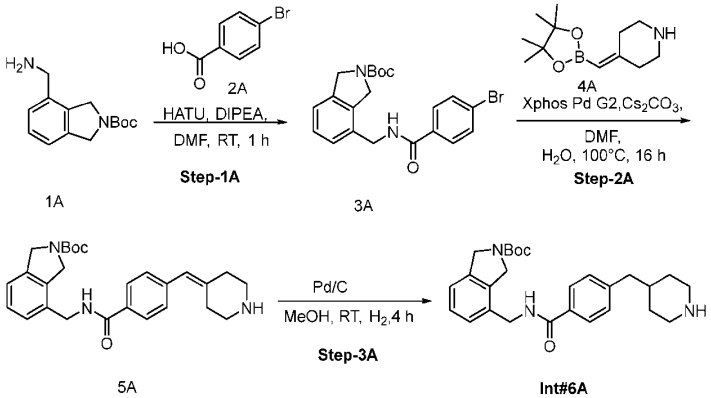
m/z: 155.57 $[M+H]^+$. ^{1}H-NMR (400 MHz, DMSO-d*6*): δ 14-12 (brs, 2H), 9.0-8.9 (m, 1H), 8.5-8.36 (m, 3H), 8.06 (d, *J* = 5.2 Hz, 1H), 7.79-7.6 (m, 2H), 7.62 (*d*, *J* = 8 Hz, 2H), 7.4-7.2 (m, 7H), 7.0 (d, *J* = 8 Hz, 2 H), 5.1-4.9 (m, 1H), 4.9-4.8 (m, 3H), 4.7-4.6 (m, 2H), 4.5-4.4 (m, 2H), 4.4-4.3 (m, 4H), 4.3-4.0 (m, 4H), 4.0-3.8 (m, 4H), 3.7-3.5 (m, 4H), 3.4-3.3 (m, 10 H), 3.3-3.0 (m, 11 H), 2.9-2.6 (m, 6H), 2.4-2.2 (m, 2H), 2.3-2.1 (m, 3H), 1.8-1.7 (m, 3H), 1.7-1.6 (m, 3H), 1.5-1.4 (m, 3H), 1.4-1.3 (m, 2H), 1.2-1 (m, 2H)

[000286] Synthesis of Int#6A

[000287] Synthesis of 4-((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methylene)piperidine (4A)



[000288] Step-1B: To a solution of tert-butyl 4-((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methylene)piperidine-1-carboxylate 1B (15 g, 0.046 mmol) in DCM (150 mL, 10 vol) at 0 °C was added 4M HCl in 1,4-dioxane (30 mL, 2 vol). Resultant reaction mixture was stirred at room temperature for 1h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to get crude. Crude was triturated with diethyl ether to afford 4-((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methylene)piperidine (10 g, yield: 97%) as an off white solid. MS (ESI) m/z: 224 $[M+H]^+$



[000289] Step 1A: Synthesis of tert-butyl 4-((4-bromobenzamido)methyl)isoindoline-2-carboxylate (3A): To a solution of tert-butyl 4-(aminomethyl)isoindoline-2-carboxylate 1A (2.5 g, 0.012 mol) in DMF (25 mL, 10 Vol) at 0 °C, were added DIPEA (4.3 mL, 0.024 mol) followed by HATU (5.1 g, 0.013 mol). The reaction mixture was stirred at room temperature for 10 min, and added 4-bromobenzoic acid 2A (3.08 g, 0.012 mol). Resultant reaction

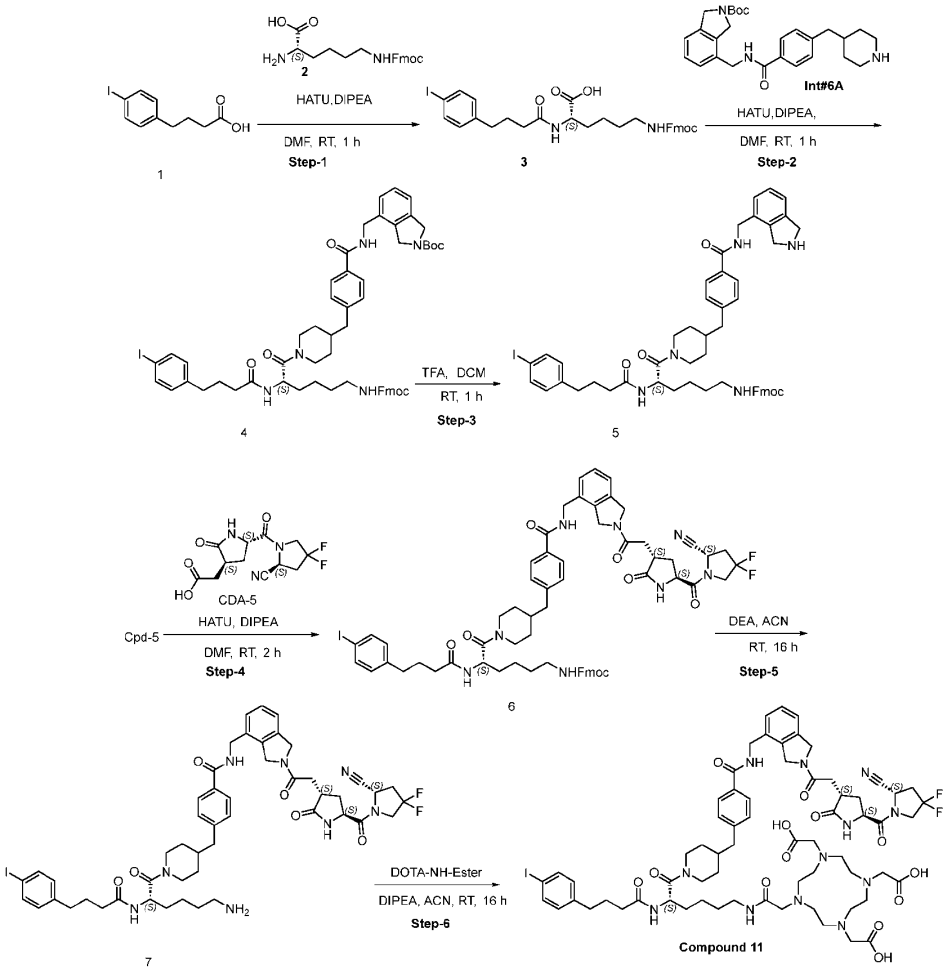
mixture was stirred at room temperature for 1h. Upon completion of the reaction, reaction mixture was diluted with ice cold water (100 mL) and stirred for 15 min, precipitated solid was filtered and washed with ice water (100 mL), hexene (50 mL), dried under reduced pressure to afford tert-butyl 4-((4-bromobenzamido)methyl)isoindoline-2-carboxylate 3A as an off white solid (4 g, Yield 75%). MS (ESI) m/z: 332 $(M+H\text{-}Boc)^+$.

[000290] Step-2A: Synthesis of tert-butyl 4-((4-(piperidin-4-ylidenemethyl)benzamido)methyl)isoindoline-2-carboxylate (5A): To a solution of tert-butyl 4-((4-bromobenzamido)methyl)isoindoline-2-carboxylate 3A (5.2 g, 0.012 mol) in DMF:H_2O (6:1, 10 vol) were added 4-((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methylene)piperidine 4A (4 g, 0.018 mol), Cs_2CO_3 (11.7 g, 0.036 mol) and degassed with argon for 15 min followed by addition of XPhos Pd G4 (948 mg, 0.001 mol). The resultant reaction mixture was stirred at 100°C for 16 h. Upon completion of the reaction, reaction mixture was filtered through celite bed, washed with 10%MeOH/DCM (2*30 mL), filtrate was diluted with water (30 mL). The organic layer was separated, and aqueous layer was further extracted with 10%MeOH/DCM (30 mL). Combined organic layer was washed with water (2 * 30 mL). The separated organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to get crude (4.5 g). Crude was purified by silica gel (100-200 mesh) column chromatography using 10% MeOH in DCM as an eluent, collected fraction were concentrated under reduced pressure to afford tert-butyl 4-((4-(piperidin-4-ylidenemethyl)benzamido)methyl)isoindoline-2-carboxylate 5A (4 g, yield: 74%) as a brown solid. MS (ESI) m/z: 448.65 $[M+H]^+$

[000291] Step-3A: Synthesis of tert-butyl 4-((4-(piperidin-4-ylmethyl)benzamido)methyl)isoindoline-2-carboxylate(Int#6A): To a solution of tert-butyl 4-((4-(piperidin-4-ylidenemethyl)benzamido)methyl)isoindoline-2-carboxylate 5A (4 g, 0.008 mol) in MeOH (40 mL) was added 10% Pd/C (4 g, wt/wt) under nitrogen atmosphere. The resultant reaction mixture was stirred at room temperature for 4 h under hydrogen pressure 30 *psi*. Upon completion of the reaction, reaction mixture was filtered through celite bed, washed with MeOH (20 mL). Filtrate was concentrated under reduced pressure to afford tert-butyl 4-((4-(piperidin-4-ylmethyl)benzamido)methyl)isoindoline-2-carboxylate (Int#6A) (3 g, yield: 75%) as an off white solid. MS (ESI) m/z: 450.50 $[M+H]^+$

[000292] **Example 11. Synthesis of 2,2',2"-(10-(2-(((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperidin-1-yl)-5-(4-(4-iodophenyl)butanamido)-6-**

oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 11)



[000293] Step-1: Synthesis of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-(4-iodophenyl)butanoyl)-L-lysine (3): To a solution of 4-(4-iodophenyl)butanoic acid 1 (5 g, 0.017 mol) in DMF (50 mL, 10 Vol) at 0 °C was added DIPEA (6 mL, 0.034 mol) followed by HATU (7.2 g, 0.018 mol). The mixture was stirred at room temperature for 30 min, and added N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-L-lysine 2 (6.1 g, 0.017 mol). The resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of reaction, reaction mixture was diluted with cold water (50 mL), and the precipitated solid was filtered and dried under vaccuo to afford N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-(4-iodophenyl)butanoyl)-L-lysine 3 (7.1 g, crude) as an off white solid. MS (ESI) m/z: 641.16 $[M+H]^+$

[000294] Step-2: Synthesis of tert-butyl 4-((4-((1-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-(4-iodophenyl)butanoyl)-L-lysyl)piperidin-4-yl)methyl)benzamido)methyl)isoindoline-2-carboxylate (4): To a solution of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-(4-iodophenyl)butanoyl)-L-lysine 3 (2 g, 0.0031 mol) in DMF (20 mL, 10 Vol) at 0 °C was added DIPEA (1 mL, 0.006 mol) followed by HATU (1.3 g, 0.003 mol). The mixture was stirred at room temperature for 30 min, followed by tert-butyl 4-((4-(piperidin-4-ylmethyl)benzamido)methyl)isoindoline-2-carboxylate (Int#6A) (1.4 g, 0.0031 mol). The resultant reaction mixture was further stirred at room temperature for 1 h. Upon completion of reaction, reaction mixture was diluted with cold water (20 mL), precipitated solid was filtered, and dried under vaccuo to afford tert-butyl 4-((4-((1-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-(4-iodophenyl)butanoyl)-L-lysyl)piperidin-4-yl)methyl)benzamido)methyl)isoindoline-2-carboxylate 4 (3 g, crude) as white solid. MS (ESI) m/z: 1072.41 $[M+H]^+$

[000295] Step-3: Synthesis of (9H-fluoren-9-yl)methyl (S)-(5-(4-(4-iodophenyl)butanamido)-6-(4-(4-((isoindolin-4-ylmethyl)carbamoyl)benzyl)piperidin-1-yl)-6-oxohexyl)carbamate (5): TFA (4 mL, 4 vol) was added to a solution of tert-butyl 4-((4-((1-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-(4-iodophenyl)butanoyl)-L-lysyl)piperidin-4-yl)methyl)benzamido)methyl)isoindoline-2-carboxylate 4 (1 g, 9.328 mol) in DCM (5 mL, 5 Vol) at 0 °C. The resultant reaction mixture was stirred at room temperature for 1 h. Reaction mixture was concentrated under reduced pressure to afford crude. Crude was triturated with diethyl ether (20 mL) to afford (9H-fluoren-9-yl)methyl (S)-(5-(4-(4-iodophenyl)butanamido)-6-(4-(4-((isoindolin-4-ylmethyl)carbamoyl)benzyl)piperidin-1-yl)-6-oxohexyl)carbamate 5 (540 mg, crude) as a white solid. MS (ESI) m/z: 972.68 $[M+H]^+$

[000296] Step-4: Synthesis of (9H-fluoren-9-yl)methyl ((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperidin-1-yl)-5-(4-(4-iodophenyl)butanamido)-6-oxohexyl)carbamate (6): To a solution of 2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetic acid CDA-5 (0.155 g 4.93 mol) in DMF (1 mL) at 0 °C, was added DIPEA (0.18 mL, 9.86 mol) followed by HATU (0.19 g, 4.93 mol). The mixture was stirred at room temperature for 30 min, and added (9H-fluoren-9-yl)methyl (S)-(5-(4-(4-iodophenyl)butanamido)-6-(4-(4-((isoindolin-4-ylmethyl)carbamoyl)benzyl)piperidin-1-yl)-6-oxohexyl)carbamate 5 (0.48 g, 4.93 mol). The resultant reaction mixture was stirred at room temperature for 2 h. Upon completion of the

reaction, reaction mixture was diluted with EtOAc (20 mL), ice cold water (10 mL) and stirred for 15 min. The aqueous layer was further extracted with EtOAc (3 * 5 mL). The combined organic layer was washed with ice cold water (2 * 10 mL), dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to get (9H-fluoren-9-yl)methyl ((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperidin-1-yl)-5-(4-(4-iodophenyl)butanamido)-6-oxohexyl)carbamate 6 (0.4 g, yield: 65%) as an off white solid. MS (ESI) m/z: 1255.8 $(M+H)^+$.

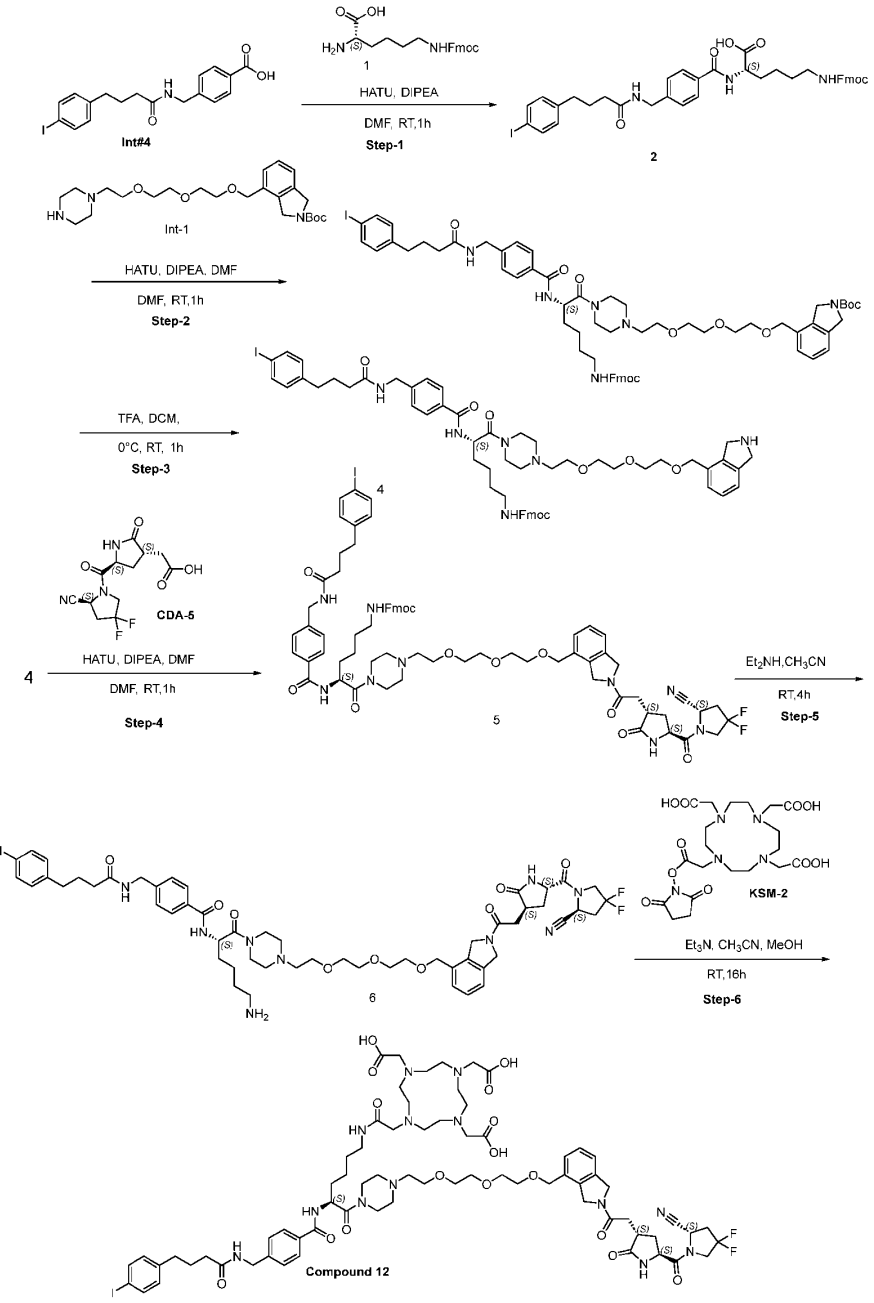
[000297] Step-5: Synthesis of N-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)-4-((1-((4-(4-iodophenyl)butanoyl)-L-lysyl)piperidin-4-yl)methyl)benzamide: Diethyl amine (4 mL, 4 Vol) was added to a solution of (9H-fluoren-9-yl)methyl ((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperidin-1-yl)-5-(4-(4-iodophenyl)butanamido)-6-oxohexyl)carbamate 6 (1 g, 7.96 mol) in acetonitrile (10 mL, 10 Vol) at room temperature. The resultant reaction mixture was stirred at room temperature for 2 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude. The crude was triturated with cold EtOAc (20 mL) to afford N-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)-4-((1-((4-(4-iodophenyl)butanoyl)-L-lysyl)piperidin-4-yl)methyl)benzamide 7 (0.6 g, crude) as an off white solid. MS (ESI) m/z: 1033.6 $(M+H)^+$.

[000298] Step-6: Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperidin-1-yl)-5-(4-(4-iodophenyl)butanamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid: To a solution of N-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)-4-((1-((4-(4-iodophenyl)butanoyl)-L-lysyl)piperidin-4-yl)methyl)benzamide 7 (1.8 g, 0.0017 mol) in acetonitrile (18 mL, 10 Vol) at room temperature was added DIPEA (2.84 mL, 0.015 mol) followed by addition of 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid DOTA-NHS Ester (1.27 g, 0.0025 mol) the resultant reaction mixture was stirred at room temperature for 16 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude (1.2 g crude). The crude was triturated with EtOAc (15 mL) to obtained solid. The solid material was further purified by prep-HPLC

using the below condition. The required fractions were collected and lyophilized to afford 2,2',2''-(10-(2-(((S)-6-(4-(4-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)carbamoyl)benzyl)piperidin-1-yl)-5-(4-(4-iodophenyl)butanamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (195.7 mg, yield: 7.8%) as a white solid. MS (ESI) m/z: 1419.86 [M+H]. ^{1}H-NMR (400 MHz, DMSO-d*6*): δ 14-13 (brs, 2H), 8.97-8.96 (brs, 1H), 8.4-8.5 (m, 1H), 8.1-8.02 (m, 1H), 7.82-7.79 (m, 2H), 7.62 (*t*, *J* = 8 Hz, 2H), 7.6-7.4 (m, 1H), 7.3-7.2 (m, 6H), 7.00 (t, *J* = 8.4 Hz, 2H), 5.09-5.07 (m, 1H), 4.9-4.8 (m, 2H), 4.7-4.6 (m, 3H), 4.4-4.3 (m, 5H), 4.6-4.0 (m, 4H), 3.5-3.2 (m, 12H), 3.2-3.0 (m, 13 H), 3.0-2.6 (m, 6H), 2.4-2.3 (m, 2H), 2.3-2.2 (m, 2H), 2.2-2.1 (m, 3H), 1.8-1.7 (m, 4H), 1.6-1.5 (m, 3H), 1.6-1.5 (m, 4H), 1.3-1.2 (m, 2H), 1.2-1.1 (m, 2H).

[000299] The synthesis of tert-butyl 4-((4-(piperidin-4-ylmethyl)benzamido)methyl)isoindoline-2-carboxylate (Int#6A) is described in Example 10.

[000300] Example 12. Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-(2-(2-(2-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 12)



[000301] Step-1: Synthesis of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine: To a solution of 4-((4-(4-iodophenyl)butanamido)methyl)benzoic acid (Int#4) (5 g, 0.011 mol) in DMF (50 mL) at

room temperature was added DIPEA (4.1 mL, 0.023 mol) followed by HATU (4.94 g, 0.013 mol).Mixture was stirred for 10 min and then added N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-L-lysine (4.35 g, 0.011 mol). Resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was poured into ice water (250 mL) stirred for 15 min and obtained solid was filtered. Solid was dissolved in 10% MeOH in DCM (30 mL), and diluted with diethyl ether (50 mL) to afford N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine 2 (4.6 g, yield: 50%) as a white solid. MS (ESI) m/z: 774.13 $(M+H)^+$.

[000302] Step-2: Synthesis of tert-butyl 4-((2-(2-(2-(4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperazin-1-yl)ethoxy)ethoxy)ethoxy)methyl)isoindoline-2-carboxylate: To a solution of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine 2 (1 g, 1.29 mmol) in DMF (10 mL) at room temperature was added DIPEA (0.4 mL, 2.58 mmol) followed by HATU (540 mg, 1.42 mmol). Mixture was stirred for 10 min followed by addition of tert-butyl 4-((2-(2-(2-(piperazin-1-yl)ethoxy)ethoxy)ethoxy)methyl)isoindoline-2-carboxylate Int-1 (580 mg, 1.29 mmol). The resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was poured into ice water (50 mL) stirred for 15 min and obtained solid was filtered. The solid compound was dissolved in 10% MeOH in DCM (20 mL) and diluted with diethyl ether (50 mL) to afford tert-butyl 4-((2-(2-(2-(4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperazin-1-yl)ethoxy)ethoxy)ethoxy)methyl)isoindoline-2-carboxylate 3 (750 mg, yield: 48%) as an off white solid. MS (ESI) m/z: 1206.09 $(M+H)^+$

[000303] Step-3: Synthesis of (9H-fluoren-9-yl)methyl (S)-(5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-(4-(2-(2-(2-(isoindolin-4-ylmethoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)-6-oxohexyl)carbamate: TFA (4 mL, 4 Vol) was added to a solution of tert-butyl 4-((2-(2-(2-(4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperazin-1-yl)ethoxy)ethoxy)ethoxy)methyl)isoindoline-2-carboxylate 3 (1 g, 0.82 mmol, in DCM (10 mL, 10 Vol) at 0° C. The resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was concentrated under reduced. Resultant crude was triturated with diethyl ether (50 mL), filtered and dried under vacuum to afford (9H-fluoren-9-yl)methyl (S)-(5-(4-((4-(4-

iodophenyl)butanamido)methyl)benzamido)-6-(4-(2-(2-(2-(isoindolin-4-ylmethoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)-6-oxohexyl)carbamate 4 (750 mg, yield: 71%) as a white solid. MS (ESI) m/z: 1106.61 $(M+H)^+$.

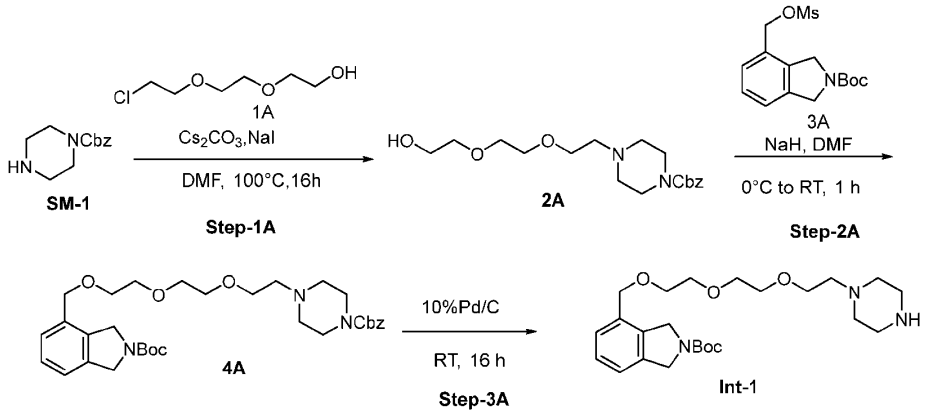
[000304] Step-4: Synthesis of (9H-fluoren-9-yl)methyl ((S)-6-(4-(2-(2-(2-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)carbamate: To a solution of 2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetic acid CDA-5 (245 mg, 0.81 mmol) in DMF (9 mL, 10 Vol) at room temperature was added DIPEA (0.28 mL, 1.62 mmol) followed by HATU (340 mg, 0.89 mol). The reaction mixture was stirred at room temperature for 10 min followed by (9H-fluoren-9-yl)methyl (S)-(5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-(4-(2-(2-(2-(isoindolin-4-ylmethoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)-6-oxohexyl)carbamate 4 (900 mg, 0.81 mmol). The resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was poured into ice water (50 mL) stirred for 15 min and solid obtained was filtered. Solid was dissolved in 10% MeOH in DCM (10 mL) and diluted with diethyl ether (25 mL) to afford (9H-fluoren-9-yl)methyl ((S)-6-(4-(2-(2-(2-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)-5-(4-((4-(4-iodophenyl) butanamido) methyl) benzamido)-6-oxohexyl)carbamate 5 (420 mg, yield: 37%) as a white solid. MS (ESI) m/z: 1389.41 $(M+H)^+$.

[000305] Step-5: Synthesis of N-((S)-6-amino-1-(4-(2-(2-(2-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)-1-oxohexan-2-yl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide: Diethyl amine (3 mL, 5 Vol) was added to a solution of (9H-fluoren-9-yl)methyl ((S)-6-(4-(2-(2-(2-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)carbamate 5 (600 mg, 0.43 mmol) in acetonitrile (3 mL, 10 Vol) at room temperature . The resultant reaction mixture was stirred at room temperature for 4 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to get crude. The crude compound was triturated with EtOAc (3 * 25 mL) and resultant solid was dissolved in 10% MeOH in DCM (25 mL), diluted with diethyl ether (30 mL) to afford N-((S)-6-amino-1-(4-(2-(2-(2-((2-(2-((3S,5S)-5-

((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)-1-oxohexan-2-yl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 6 (320 mg, yield: 63%) as a white solid. MS (ESI) m/z: 1166.47 $(M+H)^+$.

[000306] Step-6: Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-(2-(2-(2-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid: To a solution of N-((S)-6-amino-1-(4-(2-(2-(2-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)-1-oxohexan-2-yl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 6 (300 mg, 0.25 mmol) in CH_3CN (3 mL, 10 Vol) at room temperature was added DIPEA (0.39 mL, 2.31 mmol) followed by lot wise addition of 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid DOTA-NHS Ester (193 mg, 0.38 mmol). The resultant reaction mixture was stirred at room temperature for 16 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to get residue. The residue was trituration with EtOAc (2 * 50 mL), the isolated solid was dissolved in 10% MeOH in DCM (40 mL), diluted with diethyl ether (40 mL) to get crude material. The crude material was purified by Prep-HPLC. The required fractions were collected and concentrated under vacuum followed by lyophilized to afford 2,2',2''-(10-(2-(((S)-6-(4-(2-(2-(2-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)ethoxy)ethoxy)ethyl)piperazin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (56 mg) as a white solid. MS (ESI) m/z: 1550.90 $(M-H)^-$. ^{1}H-NMR (400 MHz, DMSO-d6): δ 8.60 (d, J = 7.6 Hz, 1H), 8.43 - 8.40 (m, 2H), 8.06 (s, 1H), 7.83-7.81 (m, 2H), 7.62 (d, J = 8.0 Hz, 2H), 7.31-7.28 (m, 4H), 7.25-7.22 (m, 1H), 6.98 (d, J = 10.8 Hz, 2H), 5.11-5.09 (m, 1H), 4.85-4.83 (m, 3H), 4.65-4.63 (m, 2H), 4.50- (s, 2H), 4.31-4.30 (m, 3H), 4.22-4.15 (m, 2H), 4.05-4.00 (m, 2H), 3.85-3.80 (m, 2H), 3.75-3.70 (m, 2H), 3.55-3.50 (m, 6H), 3.5-3.4 (m, 10 H), 3.40-3.20 (m, 14H), 3.15-3.10 (m, 13H), 2.90-2.80 (m, 4H), 2.30-2.20 (m, 1H), 2.14 (t, J = 7.6 Hz, 4H), 1.82-1.78 (m, 2H), 1.71-1.65 (m, 2H), 1.45-1.40 (m, 2H)1.35-1.25 (m, 2H).

[000307] Synthesis of Int. 1



[000308] Step-1A: Synthesis of benzyl 4-(2-(2-(2-hydroxyethoxy)ethoxy)ethyl)piperazine-1-carboxylate: To a solution of benzyl piperazine-1-carboxylate SM-1 (5 g, 0.02 mol) in DMF (50 mL, 10 Vol) at 0 °C was added Cs_2CO_3 (11 g, 0.03 mol) followed by NaI (340 mg, 0.002 mol). Resultant mixture was stirred at room temperature for 10 min and added 2-(2-(2-chloroethoxy)ethoxy)ethan-1-ol 1A (5 g, 0.03 mol). Resultant reaction mixture was stirred at 100°C for 16 h. Upon completion of the reaction, reaction mixture was diluted with DCM (250 mL), water (100 mL) and stirred for 15 min. Organic layer was separated and aqueous layer was extracted with DCM (2 * 100 mL), combined organic layer was washed with ice cold water (100 mL), dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to get crude. Crude material was purified by silica gel (60-120 mesh) column chromatography using 5% MeOH in DCM as an eluent. The compound contained fractions were concentrated under reduced pressure to afford benzyl 4-(2-(2-(2-hydroxyethoxy)ethoxy)ethyl)piperazine-1-carboxylate 2A (4.5 g, yield: 56 %) as a liquid. MS (ESI) m/z: 353.42 $(M+H)^+$

[000309] Step-2A: Synthesis of tert-butyl 4-((2-(2-(2-(4-((benzyloxy)carbonyl)piperazin-1-yl)ethoxy)ethoxy)ethoxy)methyl)isoindoline-2-carboxylate: To a solution of benzyl 4-(2-(2-(2-hydroxyethoxy)ethoxy)ethyl)piperazine-1-carboxylate 2A (2 g, 5.68 mmol) in THF (20 mL, 10 Vol) at 0 °C was added NaH (400 mg, 11.36 mmol) and stirred for 15 min followed by addition of tert-butyl 4-(((methylsulfonyl)oxy)methyl)isoindoline-2-carboxylate 3A (2.2 g, 6.81 mmol). Resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was diluted with water (50 mL), and stirred for 15 min. Compound was extracted with DCM (3 * 100 mL) and combined organic layer was washed with ice cold

water (2 * 50 mL). Organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to get crude. The crude was purified by silica gel (60-120 mesh) column chromatography eluted with 2% MeOH in DCM, and compound contained fractions were concentrated under reduced pressure to afford tert-butyl 4-((2-(2-(2-(4-((benzyloxy)carbonyl)piperazin-1-yl)ethoxy)ethoxy)ethoxy)methyl)isoindoline-2-carboxylate 4A (2.5 g) as a gummy liquid. MS (ESI) m/z: 584.51 $(M+H)^+$.

[000310] Step-3A: Synthesis of tert-butyl 4-((2-(2-(2-(piperazin-1-yl)ethoxy)ethoxy)ethoxy)methyl)isoindoline-2-carboxylate: To a solution of tert-butyl 4-((2-(2-(2-(4-((benzyloxy)carbonyl)piperazin-1-yl)ethoxy)ethoxy)ethoxy)methyl)isoindoline-2-carboxylate 4A (1 g, 1.71 mmol) in MeOH (10 mL, 10 Vol) at room temperature was added 10% Pd/C (300 mg). Resultant reaction mixture was stirred at room temperature for 16 h under H_2 gas at 30 *PSI*. Upon completion of the reaction, reaction mixture was filtered through celite bed wash with MeOH (50 mL). The filtrate was concentrated to afford tert-butyl 4-((2-(2-(2-(piperazin-1-yl)ethoxy)ethoxy)ethoxy)methyl)isoindoline-2-carboxylate Int-1 (1.2 g crude) as a liquid. The crude material was taken to the next step without purification. MS (ESI) m/z: 450.26 $(M+H)^+$.

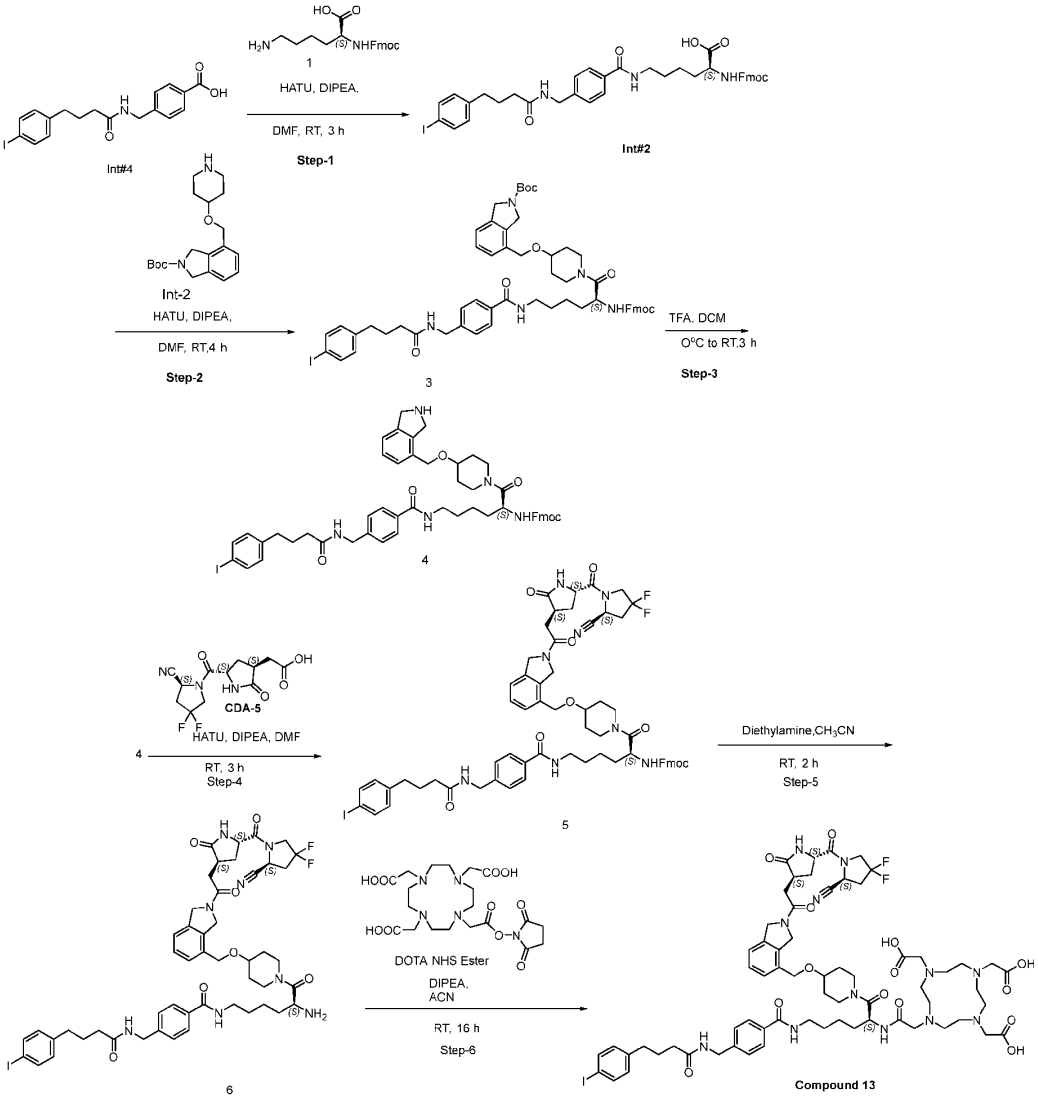
[000311] Synthesis of Int#4:

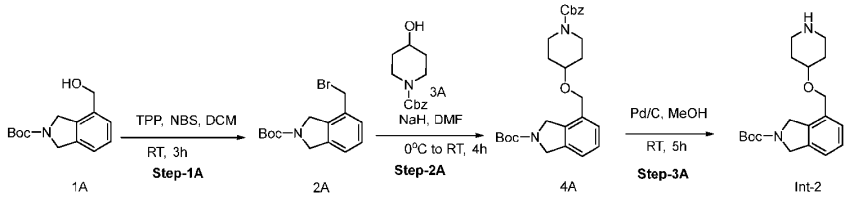
[000312] Step-1: Synthesis of tert-butyl 4-((4-(4-iodophenyl)butanamido)methyl)benzsoate: To a solution of 4-(4-iodophenyl)butanoic acid 2 (1.8 g, 6.22 mmol) in DMF (18 mL, 10 Vol) at 0 °C was added DIPEA (2.7 mL, 15.5 mmol) followed by HATU (1.74 g, 6.22 mmol). Reaction mixture was stirred at room temperature for 30 min, and added tert-butyl 4-(aminomethyl)benzoate 1 (1.2 g, 6.2 mmol). Resultant reaction mixture was stirred at room temperature for 4 h. Reaction mixture was diluted with EtOAc (200 mL), cold water (100 mL) and stirred for 15 min. The organic layer was separated, and aqueous layer was further extracted with EtOAc (3 * 100 mL). Combined organic layers was washed with water (3 * 50 mL). The separated organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford tert-butyl 4-((4-(4-iodophenyl)butanamido)methyl)benzoate 3 (2.2 g, yield: 74 %) as a brown liquid. MS (ESI) m/z: 480.1 $[M+H]^+$

[000313] Step-2: Synthesis of 4-((4-(4-iodophenyl)butanamido)methyl)benzoic acid: TFA (22 mL, 20.8 mmol) was added to a solution of tert-butyl 4-((4-(4-iodophenyl)butanamido)methyl)benzoate 3 (2.2 g, 4.59 mmol) in DCM (22 mL, 10 Vol) at 0 °C. Resultant reaction mixture was stirred at room temperature for 5 h. Reaction mixture was concentrated under reduced pressure to afford crude. Crude was triturated with diethyl ether

(50 mL) to afford 4-((4-(4-iodophenyl)butanamido)methyl)benzoic acid Int#4 (1.8 g, yield: 92%) as a brown solid. MS (ESI) m/z: 424.42 $[M+H]^+$

[000314] Example 13. Synthesis of 2,2',2''-(10-(2-(((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)piperidin-1-yl)-6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 13)





[000315] The synthesis of 4-((4-(4-iodophenyl)butanamido)methyl)benzoic acid (Int#4) is described in Example 9.

[000316] Step-1: Synthesis of N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine: To a solution of 4-((4-(4-iodophenyl)butanamido)methyl)benzoic acid Int#4 (7.5 g, 17.72 mmol) in DMF (56 mL, 8 Vol) at room temperature was added DIPEA (6.18 mL, 35.44 mmol) followed by HATU (8.08 g, 21.2 mmol). The reaction mixture was stirred for 1 h and added (((9H-fluoren-9-yl)methoxy)carbonyl)-L-lysine 1 (6.5 g, 17.72 mmol). The resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of the reaction, reaction mixture was cool to 0 °C and poured into ice water (50 mL).Solid precipitated was filtered and dried under vacuum to afford N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine Int#2 (6.5 g, yield: 47 %) as a white solid . MS (ESI) m/z:774.47 $(M+H)^+$.

[000317] Step-2: Synthesis of tert-butyl 4-(((1-(N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperidin-4-yl)oxy)methyl)isoindoline-2-carboxylate: To a solution of N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine Int#2 (5 g, 6.462 mmol) in DMF (40 mL, 8 Vol) at room temperature was added DIPEA (3.38 mL, 19.386 mmol) followed by HATU (3.68 g, 9.693 mmol).Mixture was stirred for 1 h and added tert-butyl 4-((piperidin-4-yloxy)methyl)isoindoline-2-carboxylate Int-2 (3.21 g, 9.693 mmol). The resultant reaction mixture was stirred at room temperature for 4 h. Upon completion of reaction, the reaction mixture was cool to 0 °C and poured into ice water (50 mL). Precipitated solid was filtered and washed with ice water (50 mL) to afford tert-butyl 4-(((1-(N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperidin-4-yl)oxy)methyl)isoindoline-2-carboxylate 3 (3 g, yield: 43 %) as a brown solid. MS (ESI) m/z: 1088.35 $(M+H)^+$.

[000318] Step-3: Synthesis of (9H-fluoren-9-yl)methyl (S)-(6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-(4-(isoindolin-4-ylmethoxy)piperidin-1-yl)-1-

oxohexan-2-yl)carbamate: TFA (3.3 mL, 3 vol) was added to a solution of tert-butyl 4-((4-((4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperazin-1-yl)methyl)benzamido)methyl)isoindoline-2-carboxylate 3 (3.1 g, 2.849 mmol) in DCM (30 mL, 10 vol) at 0 °C. The resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure. Resultant crude was triturated with diethyl ether (25 mL) and n-pentane (50 mL) to afford (9H-fluoren-9-yl)methyl (S)-(6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-(4-(isoindolin-4-ylmethoxy)piperidin-1-yl)-1-oxohexan-2-yl)carbamate 4 (2.1 g, yield: 75%) as an off white solid. MS (ESI) m/z: 988.45 $(M+H)^+$.

[000319] Step-4: Synthesis of (9H-fluoren-9-yl)methyl ((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)piperidin-1-yl)-6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)carbamate: To a solution of 2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetic acid CDA-5 (0.76 g, 2.53 mmol) in DMF (35 mL, 10 Vol) at 0 °C was added DIPEA (0.62 mL, 3.54 mmol) followed by HATU (0.96 g, 2.53 mmol). Mixture was stirred at room temperature for 10 min followed by addition of (9H-fluoren-9-yl)methyl (S)-(6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-(4-(isoindolin-4-ylmethoxy)piperidin-1-yl)-1-oxohexan-2-yl)carbamate 4 (2.5 g, 2.53 mmol). Resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of reaction, the reaction mixture was cool to 0 °C and poured into ice water (50 mL).Precipitated solid was filtered and washed with ice water (50 mL), dried under vacuum to afford (9H-fluoren-9-yl)methyl ((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)piperidin-1-yl)-6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)carbamate 5 (2.5 g, crude) as a pale yellow solid. MS (ESI) m/z: 1271.2 $(M+H)^+$.

[000320] Step-5: Synthesis of N-((S)-5-amino-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)piperidin-1-yl)-6-oxohexyl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide: Diethyl amine (10 mL, 4 Vol) was added to a solution of (9H-fluoren-9-yl)methyl ((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)piperidin-1-yl)-6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)carbamate 5 (2.5 g, 1.96

mmol) in acetonitrile (25 mL, 10 Vol), at room temperature . The resultant reaction mixture was stirred at room temperature for 2 h. Upon completion of reaction, the reaction mixture was concentrated under vacuum to get crude. The crude compound was triturated with cold EtOAc (2* 10 mL) and acetonitrile (30 mL) to afford N-((S)-5-amino-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)piperidin-1-yl)-6-oxohexyl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 6 (1 g, crude) as a pale brown solid. MS (ESI) m/z: 1049.85 $(M+H)^+$.

[000321] Step-6: Synthesis of 2,2',2''-(10-(2-(((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)piperidin-1-yl)-6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid: To a solution of N-((S)-5-amino-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)piperidin-1-yl)-6-oxohexyl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 6 (1 g, 0.95 mmol) in acetonitrile (20 mL, 20 Vol) at room temperature was added DIPEA (1.4 mL, 8.55 mmol) followed by lot wise addition of 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid 7 (0.716 g, 1.42 mmol). The resultant reaction mixture was stirred at room temperature for 16 h. Upon completion of the reaction, the reaction mixture was concentrated under reduced pressure to get residue. The residue was triturated with EtOAc (2 * 20 mL) and filtered solid was dissolved in 10% MeOH in DCM (10 mL) and diluted with diethyl ether (40 mL) to get crude as pale-yellow solid. Crude material was purified by Prep-HPLC using below condition: The required fractions were collected and concentrated under vacuum followed by lyophilization to afford 2,2',2''-(10-(2-(((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)piperidin-1-yl)-6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (0.142 g) as a white solid. MS (ESI) m/z: 1435.97 $(M+H)^+$. ^{1}H-NMR (400 MHz, DMSO-d6): δ 13.1-12.8 (brs, 1H), 8.9-8.8 (m, 1H), 8.45- 8.41 (m, 1H), 8.2-8.1 (m, 1H), 7.76 (d, J = 7.6 Hz, 2H), 7.62 (d, J = 8 Hz, 2H), 7.30-7.24 (m, 5H), 7.01 (d, J = 8 Hz, 2H), 5.11-5.09 (m, 1H), 4.9-4.8 (m, 2H), 4.8-4.6 (m, 3 H), 4.5 (s, 2 H), 4.4-4.2 (m, 4H), 4.2-4.0 (m, 4H), 3.9-3.7 (m, 5H), 3.7-3.5 (m, 5 H), 3.5-3.3 (m, 12 H), 3.2-3.0 (m, 8H), 2.8-2.7 (m, 4H), 2.5-2.4 (m, 2H), 2.32-2.230 (m, 1H), 2.2-2.1 (m, 3H), 1.9-1.6 (m, 6H), 1.51-1.41 (m, 4H), 1.4-1.3 (m, 2H)

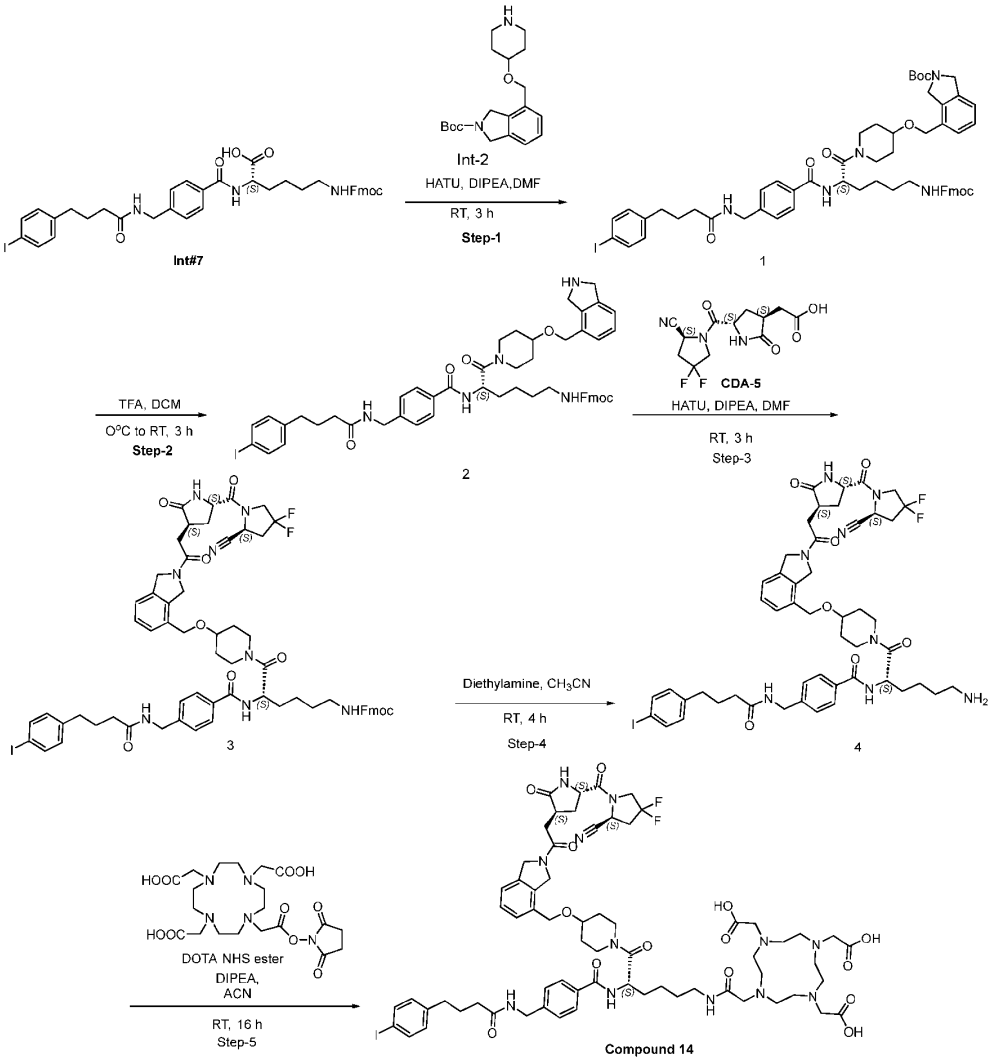
[000322] Step-1A: Synthesis of tert-butyl 4-(bromomethyl)isoindoline-2-carboxylate: To a solution of tert-butyl 4-(hydroxymethyl)isoindoline-2-carboxylate (3 g, 12.0 mmol) in DCM (30 mL) at 0 °C was added PPh_3 (3.1 g, 12.0 mmol). The reaction mixture was stirred for 30 min and added NBS (3.1 g, 17.4 mmol). Resultant reaction mixture was stirred at room temperature for 3h. Upon completion of the reaction, reaction mixture was poured into water (100 mL),and extracted with DCM (2*100 mL), concentrated under reduced pressure to afford, crude material. Crude was purified by silica gel column chromatography (100-200 mesh) using 70% ethyl acetate in hexane as an eluent. Compound contained fractions were concentrated under reduced pressure to afford tert-butyl 4-(bromomethyl)isoindoline-2-carboxylate 2A (2.6 g, yield: 69%) as an off white solid.

[000323] Step-2A: Synthesis of tert-butyl 4-(((1-((benzyloxy)carbonyl)piperidin-4-yl)oxy)methyl)isoindoline-2-carboxylate: To a solution of benzyl 4-hydroxypiperidine-1-carboxylate 3A (7.5 g, 32.05 mmol) in DMF (50 mL, 5 vol) at 0 °C was added 60% NaH (1.8 g, 64.1 mmol). Mixture was stirred at 0 °C for 30 min followed by addition of tert-butyl 4-(bromomethyl)isoindoline-2-carboxylate 2A (10 g, 32.05 mmol). Resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was poured into water (100 mL), and extracted with ethyl acetate (2*100 mL). Organic layer was dried over Na_2SO_4, concentrated under reduced pressure to get crude. Crude was purified by silica gel column chromatography (100-200 mesh) using 60% ethyl acetate in hexane. Compound contained fractions were concentrated under reduced pressure to afford tert-butyl 4-(((1-((benzyloxy)carbonyl)piperidin-4-yl)oxy)methyl)isoindoline-2-carboxylate 4A (6.5 g, yield: 58%) as an off white solid. MS (ESI) m/z: 467 $(M+H)^+$.

[000324] Step-3A: Synthesis of tert-butyl 4-((piperidin-4-yloxy)methyl)isoindoline-2-carboxylate: To a solution of tert-butyl 4-(((1-((benzyloxy)carbonyl)piperidin-4-yl)oxy)methyl)isoindoline-2-carboxylate 4A (10 g, 21.45 mmol) in MeOH (100 mL, 10 vol), at room temperature was added 10% Pd/C (3.3 g), the reaction mixture was stirred at 120 *psi* under hydrogen atmosphere for 5 h. Upon completion of reaction, the reaction mixture was filter and washed with methanol. Filtrate was concentrated under reduced pressure to get crude (7.8 g), which was purified by silica gel column chromatography (100-200 mesh) using 70% ethyl acetate in hexane as an eluent. Compound contained fractions were concentrated under reduced pressure to afford tert-butyl 4-((piperidin-4-yloxy)methyl)isoindoline-2-carboxylate Int-2 (10 g, yield: 63%) as an off white solid. MS (ESI) m/z: 333.11 $(M+H)^+$. MS (ESI) m/z: 312 $(M+H)^+$. ^{1}H-NMR (400 MHz, DMSO-d_6): δ 12.85 (brs, 1H), 8.38 (t, J = 6 Hz,

1H), 7.88 (d, J = 8 Hz, 2H), 7.34 (d, J = 8.4 Hz, 2H), 7.09-7.04 (m, 4H), 4.33 (s, 2H), 2.54-2.49 (m, 2H), 2.26 (s, 3H), 2.18 (t, J = 5 2 Hz, 2H), 1.82-1.80 (m, 2H).

[000325] Example 14. Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl) methoxy)piperidin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 14)



[000326] Step-1: tert-butyl 4-(((1-(N6-((((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperidin-4-yl)oxy)methyl)isoindoline-2-carboxylate: To a solution of N6-((((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-

iodophenyl)butanamido)methyl)benzoyl)-L-lysine Int#7 (3.1 g, 4.010 mmol) in DMF (25 mL, 8 Vol) at room temperature was added DIPEA (0.98 mL, 5.61 mmol) followed by HATU (1.5 g, 4.010 mmol). Mixture was stirred for 1 h followed by addition of tert-butyl 4-((piperidin-4-yloxy)methyl)isoindoline-2-carboxylate Int-2 (1.3 g, 4.010 mmol). Resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of reaction, the reaction mixture was poured into ice water (50 mL),and the precipitated solid was filtered and washed with ice water (50 mL). The crude material was purified by grace reverse phase column chromatography using 50% H_2O in acetonitrile as an eluant. Compound contained fractions were concentrated under reduced pressure to afford tert-butyl 4-(((1-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperidin-4-yl)oxy)methyl)isoindoline-2-carboxylate 3 (2.5 g, yield: 57 %) as a white solid. MS (ESI) m/z:1088.56 $(M+H)^+$.

[000327] Step-2: Synthesis of (9H-fluoren-9-yl)methyl (S)-(5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-(4-(isoindolin-4-ylmethoxy)piperidin-1-yl)-6-oxohexyl)carbamate: TFA (13.5 mL, 3 vol) was added to a solution of tert-butyl 4-(((1-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperidin-4-yl)oxy)methyl)isoindoline-2-carboxylate 3 (4.5 g, 4.135 mmol) in DCM (45 mL, 10 vol) at 0 °C . The resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure. Resultantcrude was triturated with diethyl ether (25 mL) and n-pentane (50 mL) to afford (9H-fluoren-9-yl)methyl (S)-(5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-(4-(isoindolin-4-ylmethoxy)piperidin-1-yl)-6-oxohexyl)carbamate 4 (4 g, yield: 98%) as an off white solid. MS (ESI) m/z: 989.30 $(M+H)^+$.

[000328] Step-3: Synthesis of (9H-fluoren-9-yl)methyl ((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)piperidin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)carbamate: To a solution of 2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetic acid CDA-5 (0.87 g, 2.90 mmol) at 0 °C in DMF (30 mL, 10 Vol) at room temperature was added DIPEA (0.7 mL, 4.06 mmol) followed by HATU (1.1 g, 2.90 mmol). Mixture was stirred at room temperature for 10 min followed by addition of (9H-fluoren-9-yl)methyl (S)-(5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-(4-(isoindolin-4-ylmethoxy)piperidin-1-yl)-6-oxohexyl)carbamate 4 (2.9 g, 2.90 mmol) at room temperature. Resultant reaction mixture

was stirred at room temperature for 3 h. Upon completion of reaction, the reaction mixture was cool to 0 °C and poured into ice water (50 mL), the precipitated solid was filtered and washed with ice water (50 mL). The crude material was purified by mPLC column chromatography using 50% H_2O in acetonitrile as an eluant. Compound contained fractions were concentrated under reduced pressure to afford (9H-fluoren-9-yl)methyl ((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)piperidin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)carbamate 5 (2.5 g, crude) as a pale yellow solid. MS (ESI) m/z: 1271.94 $(M+H)^+$.

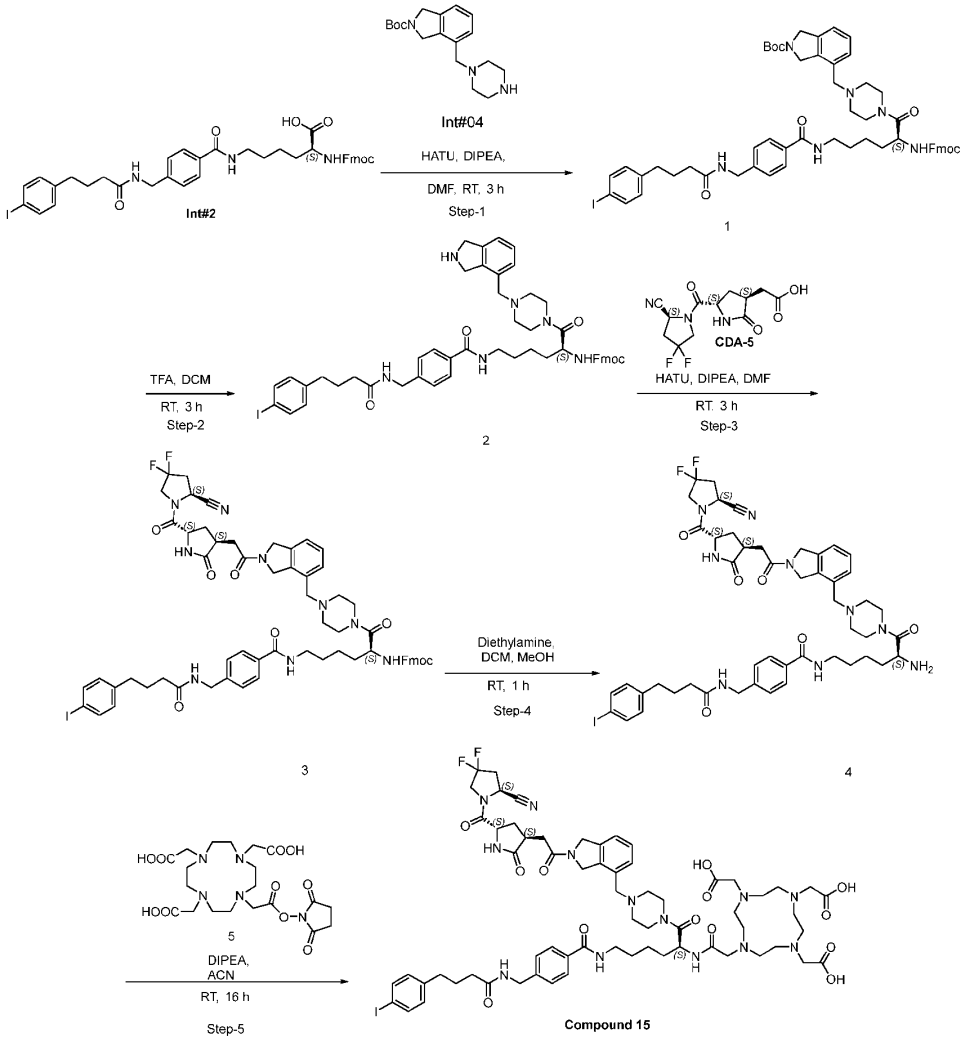
[000329] Step-4: Synthesis of N-((S)-6-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)piperidin-1-yl)-1-oxohexan-2-yl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide: Diethyl amine (8 mL, 4 Vol) was added to a solution of (9H-fluoren-9-yl)methyl ((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)piperidin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)carbamate 5 (2 g, 1.573 mmol) in acetonitrile (20 mL, 10 Vol) at room temperature. The resultant reaction mixture was stirred at room temperature for 4 h. Upon completion of the reaction, reaction mixture was concentrated under vacuum to get crude (1.6 g). The crude compound was triturated with cold EtOAc (3 * 10 mL) and filtered solid was dissolved in 10% MeOH in DCM (10 mL) diluted with diethyl ether (50 mL) and solid obtained was dried under vacuum to afford N-((S)-6-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)piperidin-1-yl)-1-oxohexan-2-yl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 6 (1.3 g, crude) as a pale brown solid. MS (ESI) m/z: 1049.85 $(M+H)^+$.

[000330] Step-5: Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)piperidin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid: To a solution of N-((S)-6-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)piperidin-1-yl)-1-oxohexan-2-yl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 6 (1.1 g, 1.049 mmol) in acetonitrile (20 mL, 20 Vol) at room temperature was added DIPEA (1.7 mL, 9.44 mmol)

followed by lot wise addition of 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid DOTA NHS ester (0.78 g, 1.574 mmol). The resultant reaction mixture was stirred at room temperature for 16 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to get residue. The residue was trituration with EtOAc (2 * 30 mL), the isolated solid was dissolved in 10% MeOH in DCM (10 mL), triturated with diethyl ether (40 mL) to get crude compound. The crude material was purified by Prep-HPLC using below condition. The required fractions were collected and concentrated under vacuum followed by lyophilized to afford 2,2',2''-(10-(2-(((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methoxy)piperidin-1-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (219.8 mg) as a white solid. MS (ESI) m/z: 1436.08 $(M+H)^+$. ^{1}H-NMR (400 MHz, DMSO-d6): δ 13-11 (brs, 2H), 8.56-8.55 (m, 1H), 8.45- 8.41 (m, 1H), 8.4 (t, J = 6 Hz, 1H), 8.05 (s, 1H), 7.83 (d, J = 8.4 Hz, 2H), 7.62 (d, J = 8 Hz, 2H), 7.31-7.25 (m, 5H), 7.01 (d, J = 8 Hz, 2H), 5.11-5.09 (m, 1H), 4.9-4.80 (m, 3H), 4.7-4.6 (m, 2H), 4.6-4.5 (m, 2 H), 4.41-4.3 (m, 3H), 4.21-4.01 (m, 2H), 4.0-3.9 (m, 1H), 3.8-3.7 (m, 3H), 3.7-3.5 (m, 11 H), 3.21-2.9 (m, 19 H), 2.9-2.6 (m, 5H), 2.5-2.4 (m, 2H), 2.14 (t, J = 7.2 Hz, 3H), 1.9-1.6 (m, 5 H), 1.5-1.2 (m, 6 H).

[000331] The synthesis of tert-butyl 4-((piperidin-4-yloxy)methyl)isoindoline-2-carboxylate is described in Example 13.

[000332] Example 15. Synthesis of 2,2',2''-(10-(2-(((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 15)



[000333] Step-1: tert-butyl 4-((4-(N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperazin-1-yl)methyl)isoindoline-2-carboxylate: To a solution of N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine Int#2 (3 g, 3.88 mmol) in DMF (24 mL, 8 Vol) at room temperature was added DIPEA (0.9 mL, 5.43 mmol) followed by HATU (1.4 g, 3.88 mmol). Mixture was stirred at room temperature for 30 min and added tert-butyl 4-(piperazin-1-ylmethyl)isoindoline-2-carboxylate Int#04 (1.3 g, 4.01 mmol). Resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of reaction, the reaction mixture was cool to 0 °C and poured into ice water (50 mL), and the precipitated solid was filtered and washed with ice water (50 mL). Crude was purified by mPLC column

chromatography using 50% H_2O in acetonitrile as an eluant. Compound contained fractions were concentrated under reduced pressure to afford tert-butyl 4-((4-(N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperazin-1-yl)methyl)isoindoline-2-carboxylate 1 (3.6 g, yield: 86 %) as an off white solid. MS (ESI) m/z:1074.36 (M+H)+.

[000334] Step-2: Synthesis of (9H-fluoren-9-yl)methyl (S)-(6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-(4-(isoindolin-4-ylmethyl)piperazin-1-yl)-1-oxohexan-2-yl)carbamate: TFA (10.8 mL, 3 vol) was added to a stirred solution of tert-butyl 4-((4-(N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)piperazin-1-yl)methyl)isoindoline-2-carboxylate 1 (3.6 g, 3.35 mmol) in DCM (36 mL, 10 vol) at 0 °C . The resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of reaction, the reaction mixture was concentrated under reduced pressure to afford crude material. The crude material was triturated with diethyl ether (25 mL) and n-pentane (50 mL) to afford (9H-fluoren-9-yl)methyl (S)-(6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-(4-(isoindolin-4-ylmethyl)piperazin-1-yl)-1-oxohexan-2-yl)carbamate 2 (2.4 g, yield: 88%) as an off white solid. MS (ESI) m/z: 973.68 $(M+H)^+$.

[000335] Step-3: Synthesis of (9H-fluoren-9-yl)methyl ((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)carbamate: To a solution of 2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetic acid CDA-5 (1.3 g, 4.31 mmol) in DMF (42 mL, 10 Vol) at 0 °C was added DIPEA (1 mL, 6.046 mmol) followed by HATU (1.6 g, 4.31 mol). Mixture was stirred at room temperature for 10 min followed by addition of (9H-fluoren-9-yl)methyl (S)-(6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-(4-(isoindolin-4-ylmethyl)piperazin-1-yl)-1-oxohexan-2-yl)carbamate 2 (4.2 g, 4.31 mmol). The resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of reaction, the reaction mixture was cool to 0 °C and poured into ice water (50 mL).Precipitated solid was filtered and washed with ice water (50 mL). Crude was purified by grace reverse phase column chromatography using 50% H_2O in acetonitrile as an eluant. Compound contained fractions were concentrated under reduced pressure to afford (9H-fluoren-9-yl)methyl ((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-6-(4-((4-(4-

iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)carbamate 3 (3.7 g, crude) as a pale yellow solid. MS (ESI) m/z: 1257.04 $(M+H)^+$.

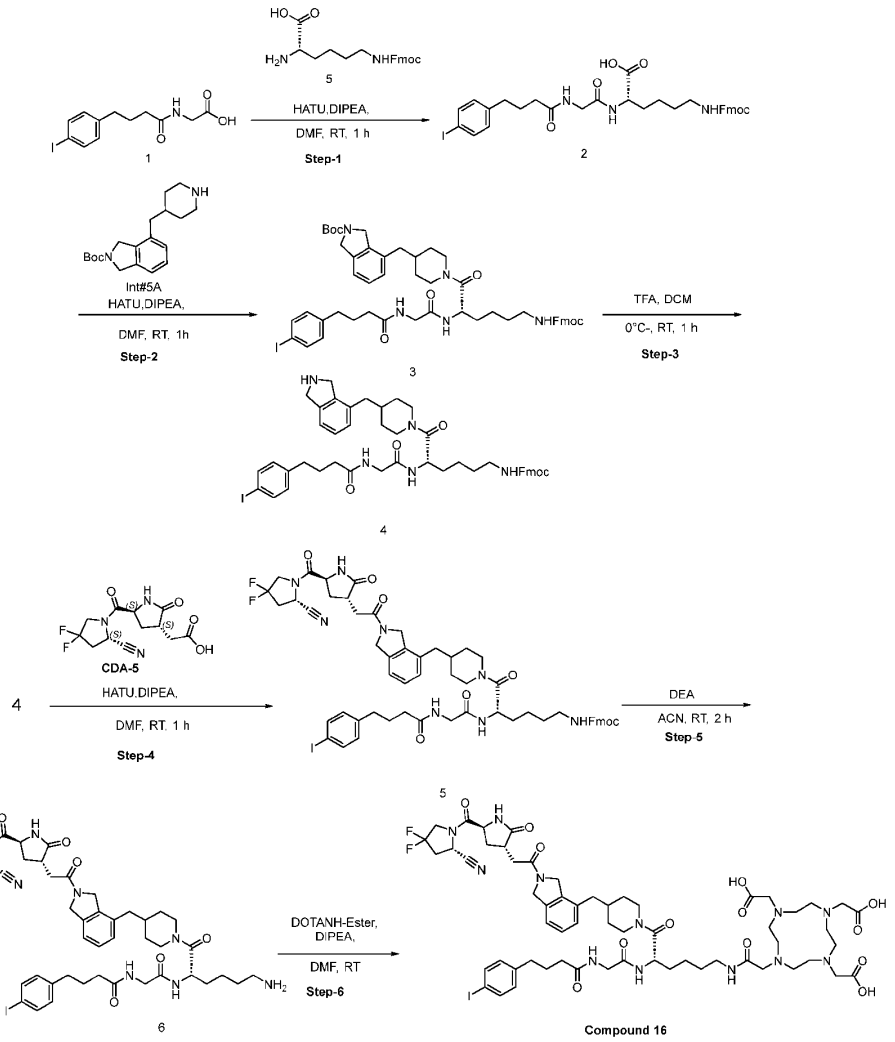
[000336] Step-4: Synthesis of N-((S)-5-amino-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-6-oxohexyl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide: Diethyl amine (14.8 mL, 4 Vol) was added to a solution of (9H-fluoren-9-yl)methyl ((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)carbamate 3 (3.7 g, 2.945 mmol) in acetonitrile (40 mL, 10 Vol) at room temperature. The resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, the reaction mixture was concentrated under reduced pressure. Resultant crude compound was triturated with cold EtOAc (2 * 10 mL) followed by dissolution in 10% MeOH in DCM (10 mL). Diethyl ether (50 mL) was added to the solution to afford N-((S)-5-amino-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-6-oxohexyl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 4 (2 g, crude) as a pale brown solid. MS (ESI) m/z: 1034.74 $(M+H)^+$.

[000337] Step-5: Synthesis of 2,2',2''-(10-(2-(((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid: To a solution of N-((S)-5-amino-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-6-oxohexyl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 4 (2 g, 1.934 mmol) in acetonitrile (20 mL, 20 Vol) at room temperature was added DIPEA (1.7 mL, 9.44 mmol) followed by addition of 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid DOTA-NHS-ester (1.45 g, 2.901 mmol). The resultant reaction mixture was stirred at room temperature for 16 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to get residue. The residue was triturated with EtOAc (2 * 10 mL) to afford solid. Isolated solid was dissolved in 10% MeOH in DCM (10 mL), and added diethyl ether (40 mL) to afford solid. The solid crude material was purified by Prep-HPLC using below condition. The required fractions were collected and concentrated under vacuum followed by lyophilization to afford 2,2',2''-

(10-(2-(((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (212 mg) as a white solid. MS (ESI) m/z: 1421.77 $(M+H)^+$. ^{1}H-NMR (400 MHz, DMSO-d6): δ 13.5-12 (brs, 1H), 8.46-8.39 (m, 2H), 8.07 (d, *J* = 10.8 Hz, 1H), 7.77-7.74 (m, 1 H), 7.62 (d, *J* = 8 Hz, 2H), 7.45-7.42 (brs, 3H), 7.25 (d, *J* = 8 Hz, 2H), 7.01 (d, *J* = 8.4 Hz, 2H), 5.14-5.12 (m, 1H), 4.95-4.90 (m, 2H), 4.80 (m, 1H), 4.7-4.6 (m, 2H), 4.4-4.3 (m, 4H), 4.3-4.0 (m, 10 H), 4.0-3.7 (m, 8H), 3.6-3.5 (m, 2H), 3.5-3.4 (m, 2H), 3.3-3.2 (m, 8H), 3.2-3.0 (m, 10 H), 2.9-2.6 (m, 5H), 2.5-2.4 (m, 2H), 2.3-2.2 (m, 1H), 2.2-2.1 (m, 2H), 1.8-1.7 (m, 2H), 1.7-1.6 (m, 1H), 1.6-1.5 (m, 3H), 1.4-1.2 (m, 2H).

[000338] The synthesis of tert-butyl 4-(piperazin-1-ylmethyl)isoindoline-2-carboxylate (Int#04) is described in Example 1.

[000339] Example 16. Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 16)



[000340] Step-1: Synthesis of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysine: To a solution of (4-(4-iodophenyl)butanoyl)glycine 1 (3 g, 0.0086 mol) in DMF (30 mL, 10 Vol) at room temperature was added DIPEA (2.9 mL, 0.017 mmol) followed by HATU (3.61 g, 0.095 mol). Mixture was for 10 min and then added N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-L-lysine 5 (2.8 g, 0.077 mol). Resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was poured into ice water (100 mL), stirred for 15 min and solid obtained was filtered. Solid was dissolved in 10% MeOH in DCM (30 mL), and diluted with diethyl ether (50 mL) to afford N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-

iodophenyl)butanoyl)glycyl)-L-lysine 2 (4 g, yield: 66%) as an off white solid. MS (ESI) m/z: 698.00 $(M+H)^+$.

[000341] Step-2: Synthesis of tert-butyl 4-((1-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysyl)piperidin-4-yl)methyl)isoindoline-2-carboxylate: To a solution of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysine 2 (1 g, 0.0014 mol) in DMF (10 mL, 10 Vol) at room temperature was added DIPEA (0.49 mL, 0.0028 mmol) followed by HATU (545 mg, 0.0014 mol).Mixture was stirred at room temperature for 10 min and added tert-butyl 4-(piperidin-4-ylmethyl)isoindoline-2-carboxylate Int#5A (408 mg, 0.0012 mol). The resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was poured into ice water (100 mL), stirred for 15 min and solid obtained was filtered. Solid was dissolved in 10% MeOH in DCM (25 mL), and diluted with diethyl ether (50 mL) to afford tert-butyl 4-((1-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysyl)piperidin-4-yl)methyl)isoindoline-2-carboxylate 3 (1.1 g, yield: 78%) as an off white solid. MS (ESI) m/z: 996.23 $(M+H)^+$.

[000342] Step-3: Synthesis of (9H-fluoren-9-yl)methyl (S)-(5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-(4-(isoindolin-4-ylmethyl)piperidin-1-yl)-6-oxohexyl)carbamate: TFA (16 mL, 4 Vol) was added to a solution of tert-butyl 4-((1-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysyl)piperidin-4-yl)methyl)isoindoline-2-carboxylate 3 (4 g, 0.004 mol) in DCM (40 mL, 10 Vol) at 0° C. The resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure. The resultant crude was triturated diethyl ether (50 mL) to afford (9H-fluoren-9-yl)methyl (S)-(5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-(4-(isoindolin-4-ylmethyl)piperidin-1-yl)-6-oxohexyl)carbamate 4 (2.8 g, yield: 80%) as a brown gummy solid. MS (ESI) m/z: 896.54 $(M+H)^+$.

[000343] Step-4: Synthesis of (9H-fluoren-9-yl)methyl ((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-oxohexyl)carbamate: To a solution of 2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetic acid CDA-5 (1 g, 0.003 mol) in DMF (10 mL, 10 Vol) at room temperature was added DIPEA (1.03 mL, 0.006 mol) followed by HATU (1.26 g, 0.003 mmol). Mixture was stirred at room temperature for 10 min followed by addition of

(9H-fluoren-9-yl)methyl (S)-(5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-(4-(isoindolin-4-ylmethyl)piperidin-1-yl)-6-oxohexyl)carbamate 4 (2.68 g, 0.003 mmol). Resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was poured into ice water (10 mL), stirred for 15 min and obtained solid was filtered. The resulted solid was dissolved in 10% MeOH in DCM (25 mL), diluted with diethyl ether (50 mL), obtained compound was filter and dried under vacuum to afford (9H-fluoren-9-yl)methyl ((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-oxohexyl)carbamate 5 (2.5 g, yield: 71%) as an off white solid. MS (ESI) m/z: 1179.74 $(M+H)^+$.

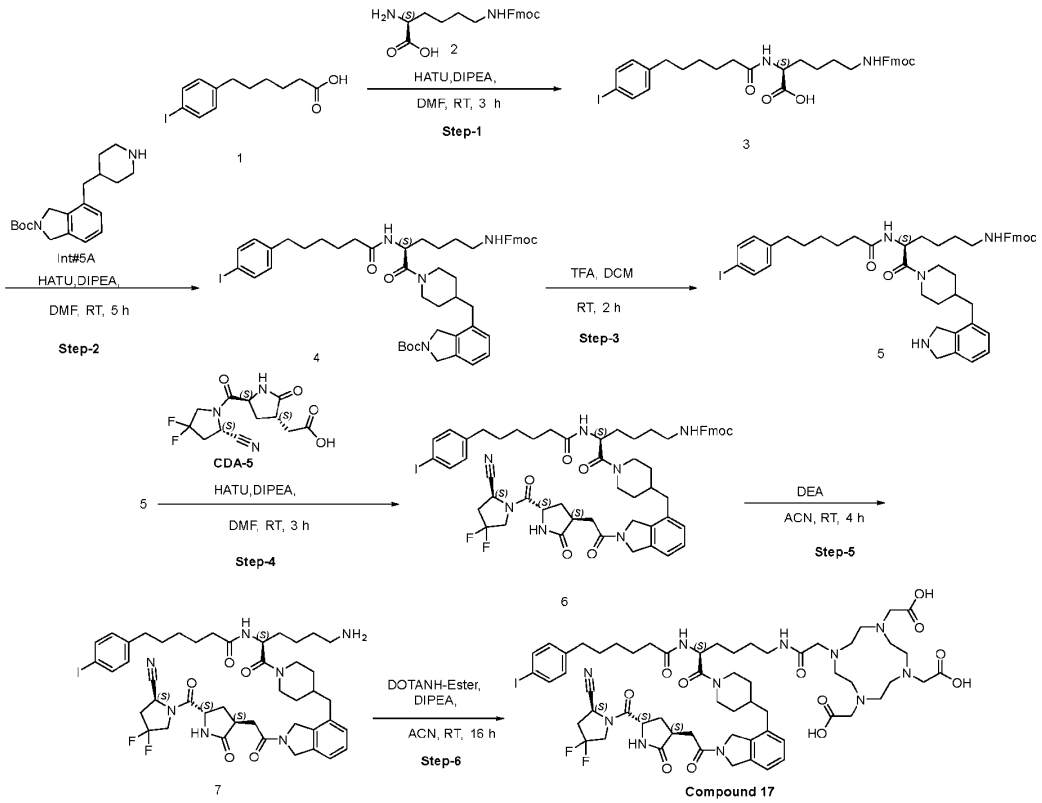
[000344] Step-5: Synthesis of N-(2-(((S)-6-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-1-oxohexan-2-yl)amino)-2-oxoethyl)-4-(4-iodophenyl)butanamide: Diethyl amine (12.5 mL, 5 Vol) was added to a solution of (9H-fluoren-9-yl)methyl ((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-oxohexyl)carbamate 5 (2.5 g, 0.002 mol) in acetonitrile (25 mL, 10 Vol), MeOH (25 mL, 10 Vol) at room temperature. The resultant reaction mixture was stirred at room temperature for 4 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to get crude (3.5 g). The crude compound was triturated with EtOAc (3 * 50 mL), the resultant solid was dissolved in 10% MeOH in DCM (25 mL) and added diethyl ether (50 mL), the obtained solid was filter to afford N-(2-(((S)-6-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-1-oxohexan-2-yl)amino)-2-oxoethyl)-4-(4-iodophenyl)butanamide 6 (1.5 g, yield: 75%) as a white solid. MS (ESI) m/z: 957.27 $(M+H)^+$.

[000345] Step-6: Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid: To a solution of N-(2-(((S)-6-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-1-oxohexan-2-yl)amino)-2-oxoethyl)-4-(4-iodophenyl)butanamide 6 (1 g, 1.04 mmol) in CH_3CN (10 mL, 10 Vol) at room temperature was added DIPEA (1.61 mL, 9.36 mmol) followed by 2,2',2''-(10-(2-((2,5-

dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid DOTANHS-Ester (750 mg, 1.56 mmol). The resultant reaction mixture was stirred at room temperature for 16 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to get residue. The residue was trituration with EtOAc (2 * 25 mL), the isolated solid was dissolved in 10% MeOH in DCM (40 mL), diluted with diethyl ether (40 mL) to get crude. Crude material was purified by Prep-HPLC. The required fractions were collected and concentrated under vacuum followed by lyophilization to afford 2,2',2''-(10-(2-(((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (211 mg) as a white solid. MS (ESI) m/z: 1343.61 $[M+H]^+$ ^{1}H-NMR (400 MHz, DMSO-d6): δ 8.06 (brs, 1H), 8.11-8.06 (m, 2H), 8.03-7.96 (m, 1H), 7.61 (d, J = 8.0 Hz, 1H), 7.25-7.16 (m, 2H), 7.11-7.07 (m, 1H), 7.03-6.98 (m, 2H), 5.12-5.11 (m, 1H), 4.9-4.85 (m, 2H), 4.74-4.60 (m, 3H), 4.48-4.31 (m, 2H), 4.25-4.15 (m, 2H), 3.90-3.80 (m, 2H), 3.75-3.65 (m, 4H), 3.62-3.50 (m, 4H), 3.20-3.00 (m, 19H), 2.90-2.60 (m, 8H), 2.60-2.50 (m, 2H), 2.45-2.40 (m, 2H), 2.30-2.20 (m, 1H), 2.15-2.10 (m, 3H), 1.85-1.75 (m, 3H), 1.65-1.50 (m, 3H), 1.49-1.35 (m, 3H), 1.30-1.20 (m, 2H), 1.15-1.10 (m, 1H), 1.05-0.90 (m, 1H).

[000346] The synthesis of tert-butyl 4-(piperidin-4-ylmethyl)isoindoline-2-carboxylate (Int#5A) is described in Example 8.

[000347] Example 17. Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-5-(6-(4-iodophenyl)hexanamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 17)



[000348] Step-1: Synthesis of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(6-(4-iodophenyl)hexanoyl)-L-lysine (3): To a solution of 6-(4-iodophenyl)hexanoic acid 1 (3 g, 9.42 mmol) in DMF (30 mL, 10 Vol) at 0 °C was added DIPEA (2.4 mL, 14.13 mmol) followed by HATU (3.5 g, 9.42 mmol). The mixture was stirred at room temperature for 15 min, and added N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-L-lysine 2 (3.12 g, 8.47 mmol). The resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of the reaction, reaction mixture was diluted with EtOAc (50 mL), cold water (50 mL) stirred for 15 min. The organic layer was separated, and aqueous layer was further extracted with EtOAc (2 * 30 mL). Combined organic layer was washed with water (30 mL), dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(6-(4-iodophenyl)hexanoyl)-L-lysine 3 (6 g, crude) as a pale yellow liquid. MS (ESI) m/z: 669.5 $[M+H]^+$

[000349] Step-2: Synthesis of tert-butyl 4-((1-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(6-(4-iodophenyl)hexanoyl)-L-lysyl)piperidin-4-yl)methyl)isoindoline-2-carboxylate (4): To a solution of N6-(((9H-fluoren-9-

yl)methoxy)carbonyl)-N2-(6-(4-iodophenyl)hexanoyl)-L-lysine 3 (6 g, 8.97 mmol) in DMF (60 mL, 10 Vol) at 0 °C was added DIPEA (3.12 mL, 17.94 mmol) followed by HATU (3.4 g, 8.97 mmol). The mixture was stirred at room temperature for 15 min, followed by tert-butyl 4-(piperidin-4-ylmethyl)isoindoline-2-carboxylate (Int#5A) (2.8 g, 8.97 mmol). Resultant reaction mixture was stirred at room temperature for 5 h. Upon completion of reaction, reaction mixture was diluted with EtOAc (100 mL), ice cold water (20 mL) and stirred for 15 min. Separated aqueous layer was extracted with EtOAc (2 * 100 mL), combined organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford tert-butyl 4-((1-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(6-(4-iodophenyl)hexanoyl)-L-lysyl)piperidin-4-yl)methyl)isoindoline-2-carboxylate 4 (7 g, yield: 62 %) as a gummy liquid. MS (ESI) m/z: 968 $(M+H)^+$.

[000350] Step-3: Synthesis of (9H-fluoren-9-yl)methyl (S)-(5-(6-(4-iodophenyl)hexanamido)-6-(4-(isoindolin-4-ylmethyl)piperidin-1-yl)-6-oxohexyl)carbamate (4): TFA (28.8 mL, 4 Vol) was added to a solution of tert-butyl 4-((1-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(6-(4-iodophenyl)hexanoyl)-L-lysyl)piperidin-4-yl)methyl)isoindoline-2-carboxylate 4 (7.2 g 7.44 mmol) in DCM (36 mL, 5 Vol) at 0 °C. The resultant reaction mixture was stirred at room temperature for 2 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude, crude was triturated with diethyl ether (30 mL) and filter, dried under vacuum to afford (9H-fluoren-9-yl)methyl (S)-(5-(6-(4-iodophenyl)hexanamido)-6-(4-(isoindolin-4-ylmethyl)piperidin-1-yl)-6-oxohexyl)carbamate 5 (4.1 g, yield: 53%) as a brown solid. MS (ESI) m/z: 867 $(M+H)^+$.

[000351] Step-4: Synthesis of (9H-fluoren-9-yl)methyl ((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-5-(6-(4-iodophenyl)hexanamido)-6-oxohexyl)carbamate (6): To a solution of 2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetic acid CDA-5 (1.32 g, 4.40 mmol) in DMF (35 mL, 10 Vol) at 0 °C was added DIPEA (1.5 ml, 8.80 mmol) followed by HATU (1.84 g, 4.84 mmol). The mixture was stirred for 30 min and added (9H-fluoren-9-yl)methyl-(S)-(5-(6-(4-iodophenyl)hexanamido)-6-(4-(isoindolin-4-ylmethyl)piperidin-1-yl)-6-oxohexyl)carbamate 5 (3.5 g, 4.40 mmol). Resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of reaction, reaction mixture was diluted with 10% MeOH in DCM (50 mL) and ice cold water (25 mL), stirred for 15 min. The organic layer was separated and aqueous layer was extracted with 10% MeOH in DCM (2* 30 mL), combined organic layer was dried over anhydrous sodium

sulphate, filtered and concentrated under reduced pressure to afford (9H-fluoren-9-yl)methyl ((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-5-(6-(4-iodophenyl)hexanamido)-6-oxohexyl)carbamate 6 (3.8 g, yield:58 %) as a brown solid. MS (ESI) m/z:1150 $(M+H)^+$.

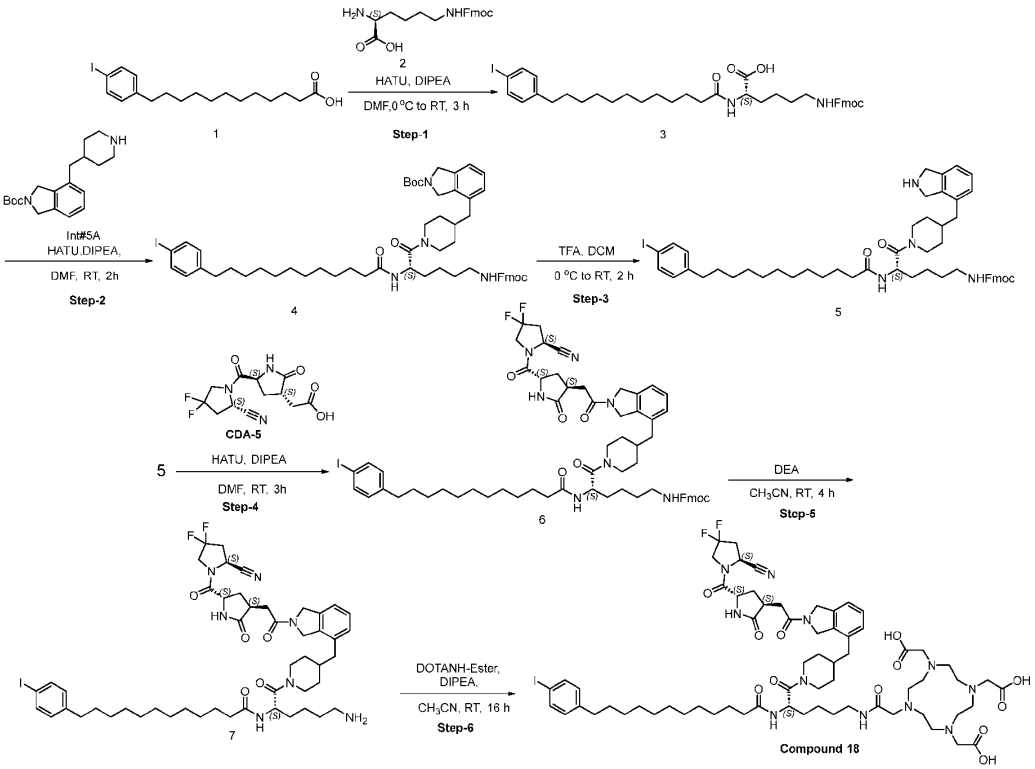
[000352] Step-5: Synthesis of N-((S)-6-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-1-oxohexan-2-yl)-6-(4-iodophenyl)hexanamide (7): Diethyl amine (6 mL, 4 Vol) was added to a solution of (9H-fluoren-9-yl)methyl ((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-5-(6-(4-iodophenyl)hexanamido)-6-oxohexyl)carbamate 6 (1.5 g, 1.305 mmol) in acetonitrile (15 mL, 10 Vol) at room temperature. The resultant reaction mixture was stirred at room temperature for 4 h. Upon completion of reaction, reaction mixture was concentrated under reduced pressure to get crude. The crude was triturated with cold EtOAc (20 mL) followed by diethyl ether (30 mL) to afford N-((S)-6-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-1-oxohexan-2-yl)-6-(4-iodophenyl)hexanamide 7 (0.95 g, yield: 63%) as a brown solid. MS (ESI) m/z: 929 $(M+H)^+$.

[000353] Step-6: Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-5-(6-(4-iodophenyl)hexanamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid : To a solution of N-((S)-6-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-1-oxohexan-2-yl)-6-(4-iodophenyl)hexanamide 7 (950 mg, 1.02 mmol) in acetonitrile (10 mL, 10 Vol) was added DIPEA (1.6 mL, 9.12 mmol) at room temperature followed by 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid DOTA-NHS Ester (769 mg, 1.53 mmol). The resultant reaction mixture was stirred at room temperature for 16 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude. The crude was triturated with EtOAc (15 mL) and further purified by prep HPLC. The required fractions were collected and lyophilized to afford 2,2',2''-(10-(2-(((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-5-(6-(4-iodophenyl)hexanamido)-6-oxohexyl)amino)-2-oxoethyl)-

1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (140 mg, yield: 10%) as a white solid. MS (ESI) m/z: 1313[M-H]$^-$. ^{1}H-NMR (400 MHz, DMSO-d6): 13.20-12.80 (brs, 1H), 8.43 (brs, 1H), 8.06-7.99 (m, 2H), 7.61-7.59 (m, 2H), 7.23-7.18 (m, 2H), 7.10-7.04 (m, 1H), 7.01.6.99 (m, 2H), 5.13-5.10 (m, 1H), 4.85-4.80 (m, 2H), 4.70-4.64 (m, 3H), 4.40-4.30 (m, 2H), 4.25-4.20 (m, 1H), 4.15-4.05 (m, 4H), 3.95-3.85 (m, 6H), 3.60-3.50 (m, 4H), 3.40-3.20 (m, 8H), 3.20-3.00 (m, 12H), 2.90-2.70 (m, 4H), 2.30-2.25 (m, 1H), 2.15-2.05 (m, 3H), 1.90-1.70 (m, 2H), 1.65-1.40 (m, 11H), 1.30-1.25 (m, 4H), 1.15-0.95 (m, 2H).

[000354] The synthesis of tert-butyl 4-(piperidin-4-ylmethyl)isoindoline-2-carboxylate (Int#5A) is described in Example 8.

[000355] Example 18. Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-5-(12-(4-iodophenyl)dodecanamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 18)



[000356] Step-1: Synthesis of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(12-(4-iodophenyl)dodecanoyl)-L-lysine (3): To a solution of 12-(4-iodophenyl)dodecanoic acid 1

(3.5 g, 8.72 mmol) in DMF (35 mL, 10 Vol) at 0 °C, was added DIPEA (2.25 mL, 13.08 mmol) followed by HATU (3.3 g, 8.67 mmol). The mixture was stirred at room temperature for 30 min, and added N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-L-lysine 2 (2.88 g, 7.84 mmol). Resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of the reaction, reaction mixture was diluted with EtOAc (50 mL), cold water (50 mL) and stirred for 15 min. The organic layer was separated, and aqueous layer was further extracted with EtOAc (2 * 50 mL). Combined organic layer was washed with water (50 mL). The separated organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(12-(4-iodophenyl)dodecanoyl)-L-lysine 3 (4.8 g, crude) as a light yellow liquid. MS (ESI) m/z: 753 $[M+H]^+$

[000357] Step-2: Synthesis of tert-butyl 4-((1-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(12-(4-iodophenyl)dodecanoyl)-L-lysyl)piperidin-4-yl)methyl)isoindoline-2-carboxylate (4): To a solution of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(12-(4-iodophenyl)dodecanoyl)-L-lysine 3 (4.8 g, 6.38 mmol) in DMF (48 mL, 10 Vol) at 0 °C, was added DIPEA (2.2 mL, 12.76 mmol) followed by HATU (2.4 g, 6.38 mmol). The mixture was stirred at room temperature for 15 min, and added tert-butyl 4-(piperidin-4-ylmethyl)isoindoline-2-carboxylate (Int#5A) (2 g, 6.38 mmol). Upon completion of the reaction, reaction mixture was diluted with EtOAc (150 mL), ice cold water (100 mL) and stirred for 15 min. The aqueous layer was extracted with EtOAc (3 * 50 mL), combined organic layer was washed with ice cold water (2 * 50 mL). The organic phase separated was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to get tert-butyl 4-((1-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(12-(4-iodophenyl)dodecanoyl)-L-lysyl)piperidin-4-yl)methyl)isoindoline-2-carboxylate 4 (5 g, yield: 74 %) as a light yellow gummy liquid. MS (ESI) m/z: 1052 $(M+H)^+$.

[000358] Step-3: Synthesis of (9H-fluoren-9-yl)methyl (S)-(5-(12-(4-iodophenyl)dodecanamido)-6-(4-(isoindolin-4-ylmethyl)piperidin-1-yl)-6-oxohexyl)carbamate (5): TFA (20 mL, 4 Vol) was added to a solution of tert-butyl 4-((1-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(12-(4-iodophenyl)dodecanoyl)-L-lysyl)piperidin-4-yl)methyl)isoindoline-2-carboxylate 4 (5 g 4.75 mmol) in DCM (20 mL, 5 Vol) at 0 °C. The resultant reaction mixture was stirred at room temperature for 2 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude. Crude was triturated with diethyl ether (30 mL), filtered and dried under vacuum to afford (9H-fluoren-9-yl)methyl (S)-(5-(12-(4-iodophenyl)dodecanamido)-6-(4-

(isoindolin-4-ylmethyl)piperidin-1-yl)-6-oxohexyl)carbamate 5 (3.3 g, yield: 40%) as a brown solid. MS (ESI) m/z: 952 $(M+H)^+$.

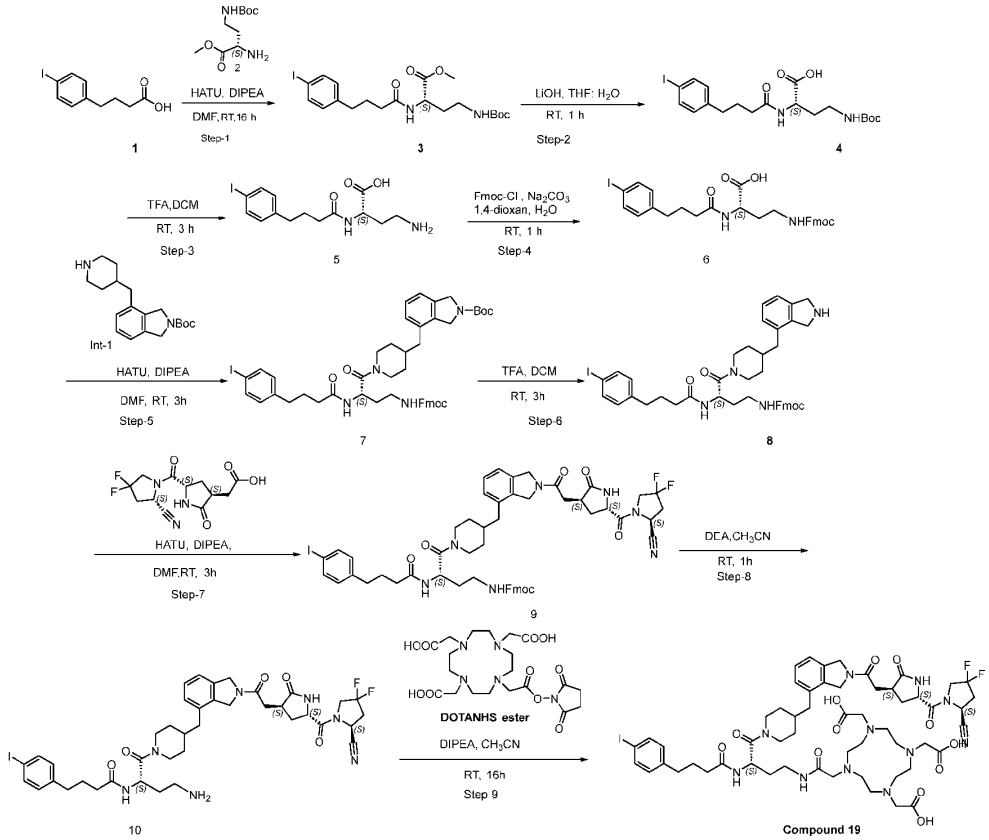
[000359] Step-4: Synthesis of (9H-fluoren-9-yl)methyl ((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-5-(12-(4-iodophenyl)dodecanamido)-6-oxohexyl)carbamate (6): To a solution of CDA-5 (1.04 g, 3.47 mmol) in DMF (33 mL, 10 Vol) at 0 °C, was added DIPEA (1.5 mL, 8.80 mmol) followed by HATU (1.84 g, 4.84 mmol). The mixture was stirred at room temperature for 30 min followed by addition of (9H-fluoren-9-yl)methyl (S)-(5-(12-(4-iodophenyl)dodecanamido)-6-(4-(isoindolin-4-ylmethyl)piperidin-1-yl)-6-oxohexyl)carbamate 5 (3.3 g, 3.47 mmol). The resultant reaction mixture was stirred at room temperature for 3 h. After completion of reaction, reaction mixture was diluted with ice-cold water (100 mL) EtOAc (2 * 150 mL), stirred for 15 min and separated the aqueous layer. Aqueous layer was extracted with EtOAc (2 * 100 mL). The combined organic layer was washed with ice cold water (2 * 100 mL). The organic phase was dried over anhydrous sodium sulphate, filtered and concentrated under vacuum to afford (9H-fluoren-9-yl)methyl ((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-5-(12-(4-iodophenyl)dodecanamido)-6-oxohexyl)carbamate 6 (3 g, yield: 50 %) as a brown colour solid. MS (ESI) m/z: 1234 $(M+H)^+$.

[000360] Step-5: Synthesis of N-((S)-6-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-1-oxohexan-2-yl)-12-(4-iodophenyl)dodecanamide (7): Diethyl amine (12 mL, 4 Vol) was added to a solution of (9H-fluoren-9-yl)methyl ((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-5-(12-(4-iodophenyl)dodecanamido)-6-oxohexyl)carbamate 6 (3 g, 2.43 mmol) in acetonitrile (30 mL, 10 Vol) at room temperature. The resultant reaction mixture was stirred at room temperature for 4 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to get crude. Crude material was triturated with cold EtOAc (50 mL) followed by diethyl ether (50 mL) to afford N-((S)-6-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-1-oxohexan-2-yl)-12-(4-iodophenyl)dodecanamide 7 (1.5 g, yield: 42%) as a brown solid. MS (ESI) m/z: 1013 $(M+H)^+$.

[000361] Step-6: Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-5-(12-(4-iodophenyl)dodecanamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid: To a solution of N-((S)-6-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-1-oxohexan-2-yl)-12-(4-iodophenyl)dodecanamide 7 (1.5 g, 1.07 mmol) in acetonitrile (15 mL, 10 Vol) at room temperature, was added DIPEA (1.6 mL, 9.65 mmol) followed by 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid DOTA NHS ester (804 mg, 1.60 mmol). The resultant reaction mixture was stirred at room temperature for 16 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude (1.5 g crude). The crude material was triturated with EtOAc (20 mL) and further purified by prep-HPLC. The required fractions were collected and lyophilized to afford 2,2',2''-(10-(2-(((S)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-5-(12-(4-iodophenyl)dodecanamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (174 mg, yield: 11%) as a white solid. MS (ESI) m/z: 1399.4 $[M+H]^+$. ^{1}H-NMR (400 MHz, DMSO-d6): δ 8.20 (brs, 1H), 8.05-7.95 (brs, 2H), 7.60 (d, J = 8 Hz, 2H), 7.21-7.16 (m, 3H), 7.09-7.00 (m, 2H), 6.98-6.96 (m, 3H), 5.11-5.10 (m, 1H), 4.8-4.7 (m, 2H), 4.7-4.6 (m, 4H), 4.4-4.3 (m, 2H), 4.3-4.1 (m, 2H), 3.9-3.8 (m, 2H), 3.6-3.5 (m, 6H), 3.3-3.0 (m, 21 H), 2.8-2.7 (m, 6 H), 2.3-2.0 (m, 4H), 1.8-1.7 (m, 1H), 1.4-1.1 (m, 12 H), 1.21 (s, 18H), 1.06 (m, 1H)

[000362] The synthesis of tert-butyl 4-(piperidin-4-ylmethyl)isoindoline-2-carboxylate (Int#5A) is described in Example 8:

[000363] Example 19. Synthesis of 2,2',2''-(10-(2-(((S)-4-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-3-(4-(4-iodophenyl)butanamido)-4-oxobutyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 19)



[000364] Step-1A: Synthesis of tert-butyl 4-(piperidin-4-ylidenemethyl)isoindoline-2-carboxylate: To a solution of tert-butyl 4-bromoisoindoline-2-carboxylate 2A (8.3 g, 0.0278 mol) in DMF:H_2O (9:1, 83 mL, 10 Vol) were added 4-((4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)methylene)piperidine (7.4 g, 0.0334 mol) Cs_2CO_3 (27 g, 0.0835 mol) and degassed with argon for 15 min followed by addition of XPhos Pd-G4 (2.4 g, 0.0024 mmol). The resultant reaction mixture was stirred at 100°C for 16 h. Upon completion of the reaction, reaction mixture was filtered through celite bed and washed with ethyl acetate (50 mL).Filtrate was diluted with water with water (30 mL). The organic layer was separated, and aqueous layer was further extracted with EtOAc (3 * 30 mL). Combined organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to get crude material, the crude material was purified by silica gel column chromatography (100-200 mesh)using 10% MeOH in DCM as an eluent. Compound fractions were concentrated under reduced pressure to afford tert-butyl 4-(piperidin-4-

ylidenemethyl)isoindoline-2-carboxylate 4A (6.5 g, yield: 74%) as a brown solid. MS (ESI) m/z: 315.12 $[M+H]^+$

[000365] Step-2A: Synthesis of tert-butyl 4-(piperidin-4-ylmethyl)isoindoline-2-carboxylate: To a solution of tert-butyl 4-(piperidin-4-ylidenemethyl)isoindoline-2-carboxylate 4A (6.5 g, 22.611 mmol) in MeOH (80 mL) at room temperature was added 10% Pd/C (6.5 g, wt/wt)), the resultant reaction mixture was stirred at room temperature for 16 h. Upon completion of the reaction, reaction mixture was filtered through celite bed and washed with MeOH (20 mL), filtrate was concentrated under reduced pressure to get tert-butyl 4-(piperidin-4-ylmethyl)isoindoline-2-carboxylate int#5A (4.9 g, crude) as an off white solid. MS (ESI) m/z: 317.27 $[M+H]^+$

[000366] Step-1: Synthesis of methyl (S)-4-((tert-butoxycarbonyl)amino)-2-(4-(4-iodophenyl)butanamido)butanoate: To a solution of 4-(4-iodophenyl)butanoic acid 1 (5 g, 17.24 mmol) in DMF (20 mL) at room temperature was added DIPEA (6 mL, 34.48 mmol) followed by HATU (6.5 g, 17.24 mmol). The mixture was stirred for 10 min and added (9H-fluoren-9-yl)methyl methyl (S)-2-amino-4-((tert-butoxycarbonyl)amino)butanoate 2 (4.6 g, 17.24 mmol). Resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of the reaction, reaction mixture was cool to 0 °C and poured into ice water (200 mL), the precipitated solid was filtered and washed with ice water (10 mL) to get crude. Crude was purified by silica gel column chromatography (100-200 mesh) using 70% EtOAC in hexane as an eluant. Required fractions were concentrated under reduced pressure to afford methyl (S)-4-((tert-butoxycarbonyl)amino)-2-(4-(4-iodophenyl)butanamido)butanoate 3 (7.5 g, yield: 86%) as a white solid. MS (ESI) m/z: 505.13 $(M+H)^+$.

[000367] Step-2: (S)-4-((tert-butoxycarbonyl)amino)-2-(4-(4-iodophenyl)butanamido)butanoic acid: To a solution of methyl (S)-4-((tert-butoxycarbonyl)amino)-2-(4-(4-iodophenyl)butanamido)butanoate 3 (7 g, 13.8 mmol) in THF (70 mL, 10 vol) and water (35 mL, 5 vol) at 0 °C was added $LiOH.H_2O$ (1.1 g, 27.7 mmol). Resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to get crude. The crude was poured into ice water (200 ml) adjust the pH ~2 with 1N HCl, and extracted with 10% MeOH in DCM (2*30 mL). Organic layer was dried over sodium sulphate and concentrated under vacuum to get crude. The crude obtained was purified by silica gel column chromatography (100-200 mesh) using 70% EtOAC in hexane as an eluant. Required fractions were concentrated under reduced pressure to afford (S)-4-((tert-butoxycarbonyl)amino)-2-(4-(4-iodophenyl)butanamido)butanoic acid.

MS (ESI) m/z: 491.25 $(M+H)^+$.

[000368] Step-3: Synthesis of (S)-4-amino-2-(4-(4-iodophenyl)butanamido)butanoic acid: TFA (12 mL, 3 vol) was added to a solution of (S)-4-((tert-butoxycarbonyl)amino)-2-(4-(4-iodophenyl)butanamido)butanoic acid (4 g, 8.161 mmol) in DCM (40 mL, 10 vol) at 0 °C. The resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of reaction, the reaction mixture was concentrated under reduced pressure to afford crude material. The crude material was triturated with diethyl ether (25 mL) and n-pentane (50 mL) to afford (S)-4-amino-2-(4-(4-iodophenyl)butanamido)butanoic acid (1.8 g, yield: 56%) as an off white solid. MS (ESI) m/z: 391.14 $(M+H)^+$.

[000369] Step-4: Synthesis of (S)-4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-(4-(4-iodophenyl)butanamido)butanoic acid: To a stirred solution of (S)-4-amino-2-(4-(4-iodophenyl)butanamido)butanoic acid (1.8 g, 4.161 mmol) in 1,4-dioxane (10 mL) and water (10 mL) at 0 °C was added sodium carbonate (968 mg, 9.22 mmol) followed by Fmoc-Osu (1.8 g, 5.23 mmol). The resultant reaction mixture was stirred at room temperature for 1h. Upon completion of the reaction, reaction mixture was cool to room temperature and diluted with EtOAc (100 mL), ice cold water (50 mL), and stirred for 15 min. Organic layer was separated and the aqueous layer was further extracted with EtOAc (2 * 30 mL). Combined organic layer was washed with ice cold water (50 mL). The separated organic layers were dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford crude material. The crude material was purified by silica gel column chromatography (100-200 mesh) using 60 % EtOAC in hexane as an eluant. Compound contained fractions were concentrated under reduced pressure to afford (S)-4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-(4-(4-iodophenyl)butanamido)butanoic acid 6 (1.6 g, yield: 56%) as an off white solid. MS (ESI) m/z: 613.15 $(M+H)^+$.

[000370] Step-5: Synthesis of tert-butyl (S)-4-((1-(4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-(4-(4-iodophenyl)butanamido)butanoyl)piperidin-4-yl)methyl)isoindoline-2-carboxylate: To a solution of (S)-4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-(4-(4-iodophenyl)butanamido)butanoic acid 6 (1.64 g, 2.679 mmol) in DMF (16 mL, 8 Vol) at room temperature was added DIPEA (0.65 mL, 3.75 mmol) followed by HATU (1 g, 2.67 mmol). The mixture was stirred for 1 h and added tert-butyl 4-(piperidin-4-ylmethyl)isoindoline-2-carboxylate Int-1 (847.1 mg, 2.679 mmol). Resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of reaction, the reaction mixture was cool to 0 °C and poured into ice water (50 mL), the precipitated solid was filtered and washed with ice water (50 mL). The crude material was purified by mPLC

column chromatography using 50% H_2O in acetonitrile as an eluant. Compound contained fractions were concentrated under reduced pressure to afford (9H-fluoren-9-yl)methyl ((S)-5-((R)-4-((tert-butoxycarbonyl)amino)-2-(4-(4-iodophenyl)butanamido)butanamido)-6-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-6-oxohexyl)carbamate 7 (2.1 g, yield: 86 %) as an off white solid.

MS (ESI) m/z: 911.56 $(M+H)^+$.

[000371] Step-6: Synthesis of (9H-fluoren-9-yl)methyl (S)-(3-(4-(4-iodophenyl)butanamido)-4-(4-(isoindolin-4-ylmethyl)piperidin-1-yl)-4-oxobutyl)carbamate: TFA (6.5 mL, 3 vol) was added to a solution of tert-butyl (S)-4-((1-(4-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-(4-(4-iodophenyl)butanamido)butanoyl)piperidin-4-yl)methyl)isoindoline-2-carboxylate 7 (2.1 g, 2.306 mmol) in DCM (25 mL, 10 vol) at 0 °C . Resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of reaction, the reaction mixture was concentrated under reduced pressure to afford crude material. The crude was triturated with diethyl ether (25 mL) and n-pentane (50 mL) to afford (9H-fluoren-9-yl)methyl (S)-(3-(4-(4-iodophenyl)butanamido)-4-(4-(isoindolin-4-ylmethyl)piperidin-1-yl)-4-oxobutyl)carbamate 8 (1.7 g, crude) as a brown colour liquid. MS (ESI) m/z: 811.45 $(M+H)^+$.

[000372] Step-7: Synthesis of (9H-fluoren-9-yl)methyl ((S)-4-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-3-(4-(4-iodophenyl)butanamido)-4-oxobutyl)carbamate: To a solution of 2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetic acid CDA-5 (847.1 mg, 2.679 mmol) in DMF (16 mL, 8 Vol) at room temperature was added DIPEA (0.52 mL, 3.005 mmol) followed by HATU (816 mg, 2.147 mmol). The mixture was stirred for 10 min and added (9H-fluoren-9-yl)methyl (S)-(3-(4-(4-iodophenyl)butanamido)-4-(4-(isoindolin-4-ylmethyl)piperidin-1-yl)-4-oxobutyl)carbamate 9 (1.74 g, 2.147 mmol). The resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of reaction, the reaction mixture was cool to 0 °C and poured into ice water (50 mL), and the precipitated solid was filtered and washed with ice water (50 mL). The crude was purified by mPLC column chromatography using 50% H_2O in acetonitrile as an eluant. Compound contained fractions were concentrated under reduced pressure to afford (9H-fluoren-9-yl)methyl ((S)-4-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-3-(4-(4-

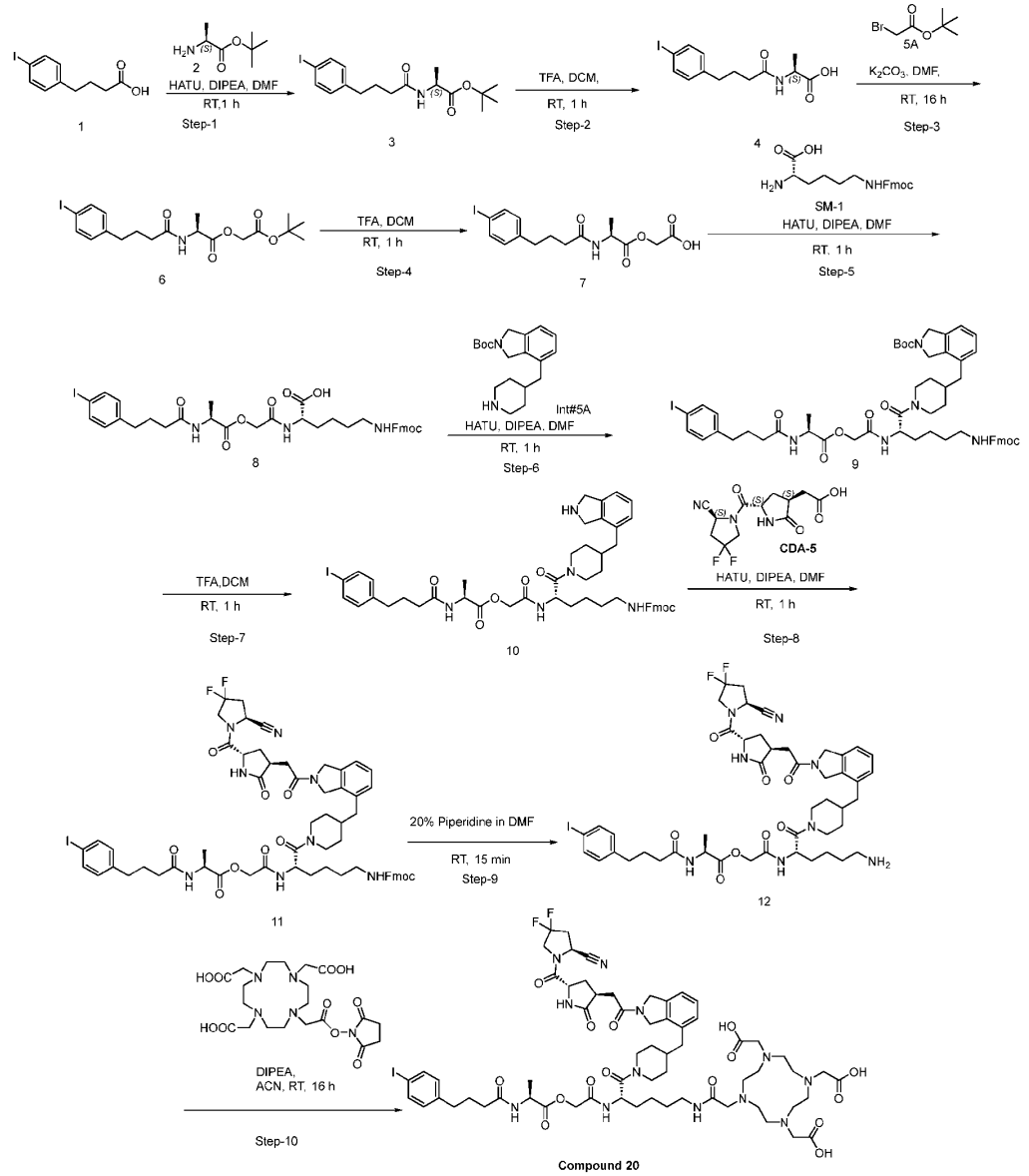
iodophenyl)butanamido)-4-oxobutyl)carbamate 9 (2.1 g, yield: 89 %) as an off white solid. MS (ESI) m/z: 1094.61 $(M+H)^+$.

[000373] Step-8: Synthesis of N-((S)-4-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-1-oxobutan-2-yl)-4-(4-iodophenyl)butanamide: Diethyl amine (8.4 mL, 4 Vol) was added to a solution of (9H-fluoren-9-yl)methyl ((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperazin-1-yl)-6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)carbamate 9 (2.1 g, 1.921 mmol) at room temperature in acetonitrile (40 mL, 10 Vol). The resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, the reaction mixture was concentrated under vacuum to get crude (3.2 g). The crude compound was trituration with cold EtOAc (3 * 50 mL), the resultant solid was dissolved in 10% MeOH in DCM (10 mL), diluted with diethyl ether (50 mL) and the obtained solid filter to afford N-((S)-4-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-1-oxobutan-2-yl)-4-(4-iodophenyl)butanamide 10 (1.4 g, crude) as a pale brown Solid. MS (ESI) m/z: 872.47 $(M+H)^+$.

[000374] Step-9: Synthesis of 2,2',2''-(10-(2-(((S)-4-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-3-(4-(4-iodophenyl)butanamido)-4-oxobutyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid: To a solution of N-((S)-4-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-1-oxobutan-2-yl)-4-(4-iodophenyl)butanamide 10 (1.4 g, 1.6068 mmol) in acetonitrile (20 mL, 20 Vol) at room temperature was added DIPEA (2.52 mL, 14.461 mmol) followed by 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid DOTA-NHS ester (1.2 g, 2.410 mmol) lot wise. Resultant reaction mixture was stirred at room temperature for 16 h. Upon completion of the reaction, the reaction mixture was concentrated under reduced pressure to get residue. The residue was triturated with EtOAc (2 * 30 mL).Solid was dissolved in 10% MeOH in DCM (10 mL), diluted with diethyl ether (40 mL) to regenerate the solid compound. The crude was purified by Prep-HPLC using below condition. The required fractions were collected and concentrated under reduced pressure followed by lyophilization to afford 2,2',2''-(10-(2-(((S)-4-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-

yl)methyl)piperidin-1-yl)-3-(4-(4-iodophenyl)butanamido)-4-oxobutyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (218.7 mg) as a white solid. MS (ESI) m/z: 1257.18 $(M+H)^+$. ^{1}H-NMR (400 MHz, DMSO-d6): 14-12 (brs, 2H), 8.5-8.41 (m, 1H), 8.2- 8.1 (m, 1H), 7.62 (t, J = 8.4 Hz, 2H), 7.21-6.93 (m, 5H), 5.11-5.09 (m, 1H), 4.89-4.70 (m, 3H), 4.7-4.6 (m, 2 H), 4.41-4.01 (m, 8 H), 3.71-3.51 (m, 4H), 3.9-3.6 (m, 6 H), 3.5-3.2 (m, 9 H), 3.3-3.0 (m, 10 H), 2.9-2.6 (m, 4H), 2.33 (m, 2H), 2.32-2.10 (m, 4H), 1.9-1.7 (m, 4H), 1.71-1.51 (m, 3H), 1.3-1.0 (m, 2H).

[000375] Example 20. Synthesis of 2,2',2''-(10-((8S,14S)-8-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidine-1-carbonyl)-19-(4-iodophenyl)-14-methyl-2,10,13,16-tetraoxo-12-oxa-3,9,15-triazanonadecyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 20)



[000376] Step-1: Synthesis of tert-butyl (4-(4-iodophenyl)butanoyl)-L-alaninate (3): To a solution of 4-(4-iodophenyl)butanoic acid 1 (5 g, 0.0172 mol) in DMF (50 mL, 10 Vol) at 0 °C was added DIPEA (6.34 mL, 0.034 mol) followed by HATU (7.2 g, 0.0189 mol). The reaction mixture was stirred at room temperature for 10 min, and added tert-butyl L-alaninate 2 (3.12 g, 0.0172 mol). Resultant reaction mixture was stirred at room temperature for 1h. Upon completion of the reaction, reaction mixture was diluted with ice cold water (100 mL) and extracted with ethyl acetate (3*50). Organic layer was dried over sodium sulphate and

concentrated under reduced pressure to get tert-butyl (4-(4-iodophenyl)butanoyl)-L-alaninat 3 (4 g, yield: 64%) as a pale brown gummy solid. MS (ESI) m/z: 418 $(M+H)^+$.

[000377] Step-2: Synthesis of (4-(4-iodophenyl)butanoyl)-L-alanine (4): TFA (20 mL, 4 Vol) was added to a solution of tert-butyl (4-(4-iodophenyl)butanoyl)-L-alaninat 3 (5 g, 0.011 mol) in DCM (50 mL, 10 Vol) at 0°C. The reaction mixture was stirred at room temperature for 1h. Upon completion of reaction, reaction was concentrated under reduced pressure to afford crude material, the crude material was triturated with diethyl ether (50 mL) and dried under vacuum to afford (4-(4-iodophenyl)butanoyl)-L-alanine 4 (3.5 g, yield: 81%) as a light brown solid. MS (ESI) m/z: 362.17 $(M+H)^+$.

[000378] Step-3: Synthesis of 2-(tert-butoxy)-2-oxoethyl (4-(4-iodophenyl)butanoyl)-L-alaninate(6): To a solution of (4-(4-iodophenyl)butanoyl)-L-alanine 4 (12 g, 0.033 mol) in DMF (120 mL, 10 Vol) at 0°C was added tert-butyl 2-bromoacetate 5A (7.1 g, 0.036 mol) followed by K_2CO_3 (6.88 g, 0.049 mol), KI (0.55 g, 0.003 mol). The resultant reaction mixture was stirred for 16 h at 100°C temperature. Upon completion of the reaction, reaction mixture was quenched with ice cold water stirred for 5 min and solid was filtered, dried under vacuum to afford 2-(tert-butoxy)-2-oxoethyl (4-(4-iodophenyl)butanoyl)-L-alaninate 6 (12 g, yield: 69%) as a brown solid. MS (ESI) m/z: 476 $(M+H)^+$.

[000379] Step-4: Synthesis of 2-(((4-(4-iodophenyl)butanoyl)-L-alanyl)oxy)acetic acid (7): TFA (40 mL, 4 Vol) was added to a solution of 2-(tert-butoxy)-2-oxoethyl (4-(4-iodophenyl)butanoyl)-L-alaninate 6 (10 g, 0.021 mol) in DCM (100 mL, 10 Vol) at 0°C. The reaction mixture was stirred at room temperature for 1h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude material. Crude was triturated with diethyl ether (50 mL) and dried under vacuum to afford 2-(((4-(4-iodophenyl)butanoyl)-L-alanyl)oxy)acetic acid 7 (9 g, yield: 90.9%) as a light brown solid. MS (ESI) m/z:420 $(M+H)^-$.

[000380] Step-5: Synthesis of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(2-(((4-(4-iodophenyl)butanoyl)-L-alanyl)oxy)acetyl)-L-lysine (8): To a solution of 2-(((4-(4-iodophenyl)butanoyl)-L-alanyl)oxy)acetic acid 7 (3 g, 0.007 mol) in DMF (30 mL, 10 Vol) at 0°C was added DIPEA (2.6 mL, 0.0143 mol) and 50%T_3P in EtOAc (2.46 mL, 0.0077 mol). The reaction mixture was stirred for 10 min and added N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-L-lysine SM-1 (2.63 g, 0.007 mol). Resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was diluted with ice cold water (100 mL) and stirred for 15 min to afford solid. The precipitated solid was filtered, dried under vacuum to get crude (2.5 g). Crude was purified by mPLC

column chromatography, eluted with 10% water in acetonitrile, required fraction were concentrated under reduced pressure to afford N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(2-(((4-(4-iodophenyl)butanoyl)-L-alanyl)oxy)acetyl)-L-lysine 8 (750 mg, Yield: 13%). MS (ESI) m/z:770 $(M+H)^+$.

[000381] Step-6: Synthesis of tert-butyl 4-((1-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(2-(((4-(4-iodophenyl)butanoyl)-L-alanyl)oxy)acetyl)-L-lysyl)piperidin-4-yl)methyl)isoindoline-2-carboxylate (9): To a solution of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(2-(((4-(4-iodophenyl)butanoyl)-L-alanyl)oxy)acetyl)-L-lysine 8 (410 mg, 0.533 mol) in DMF (4.1 mL, 10 Vol) at 0 °C was added DIPEA (0.19 mL, 1.066 mol) followed by HATU (222.8 mg, 0.586 mol). The reaction mixture was stirred at room temperature for 5 min and added tert-butyl 4-(piperidin-4-ylmethyl)isoindoline-2-carboxylate (Int#5A) (1.62 g, 0.003 mol). The resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was diluted with ice cold water (100 mL) and stirred for 15 min, the precipitated solid was filtered and washed with ice water (100 mL), hexene (50mL) and dried under vacuum to afford tert-butyl 4-((1-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(2-(((4-(4-iodophenyl)butanoyl)-L-alanyl)oxy)acetyl)-L-lysyl)piperidin-4-yl)methyl)isoindoline-2-carboxylate 9 as an off white solid(350 mg, yield: 62%). MS (ESI) m/z: 1069 $(M+H)^+$.

[000382] Step-7: Synthesis of 2-(((S)-6-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-1-(4-(isoindolin-4-ylmethyl)piperidin-1-yl)-1-oxohexan-2-yl)amino)-2-oxoethyl (4-(4-iodophenyl)butanoyl)-L-alaninate: TFA (2 mL, 4 Vol) was added to a solution of tert-butyl 4-((1-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(2-(((4-(4-iodophenyl)butanoyl)-L-alanyl)oxy)acetyl)-L-lysyl)piperidin-4-yl)methyl)isoindoline-2-carboxylate 9 (500 mg, 0.468 mol) in DCM (5 mL, 10 Vol) at 0°C. The resultant reaction mixture was stirred at room temperature for 1h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude, the crude was triturated with diethyl ether (30 mL) to obtained solid. The solid material was dried under vacuum to afford 2-(((S)-6-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-1-(4-(isoindolin-4-ylmethyl)piperidin-1-yl)-1-oxohexan-2-yl)amino)-2-oxoethyl (4-(4-iodophenyl)butanoyl)-L-alaninate 10 (300 mg, yield: 66%) as a light brown solid. MS (ESI) m/z: 968 $(M+H)^+$.

[000383] Step-8: Synthesis of 2-(((S)-6-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-1-oxohexan-2-yl)amino)-2-oxoethyl (4-(4-iodophenyl)butanoyl)-L-alaninate (11): To a solution of 2-((3S,5S)-5-((S)-2-cyano-4,4-

difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetic acid CDA-5 (155 mg, 0.514 mmol) in DMF (2 mL, 10 Vol) at 0 °C was added DIPEA (0.18 mL, 1.029 mol) followed by HATU (215 mg, 0.566 mol)) . The resultant reaction mixture was stirred at room temperature for 5 min and added 2-(((S)-6-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-1-(4-(isoindolin-4-ylmethyl)piperidin-1-yl)-1-oxohexan-2-yl)amino)-2-oxoethyl (4-(4-iodophenyl)butanoyl)-L-alaninate 10 (500 mg, 0.514 mol). Resultant reaction mixture was stirred at room temperature for 10 min. Upon completion of reaction, reaction mixture was quenched with ice cold water and solid precipitated was filtered under vacuum to afford solid . The solid compound was washed with hexene (20 mL), diethyl ether (20 mL) and dried under vacuum to afford 2-(((S)-6-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-1-oxohexan-2-yl)amino)-2-oxoethyl (4-(4-iodophenyl)butanoyl)-L-alaninate 11 (150 mg, yield: 23%) as a brown solid. MS (ESI) m/z: 1251.76 $(M+H)^+$.

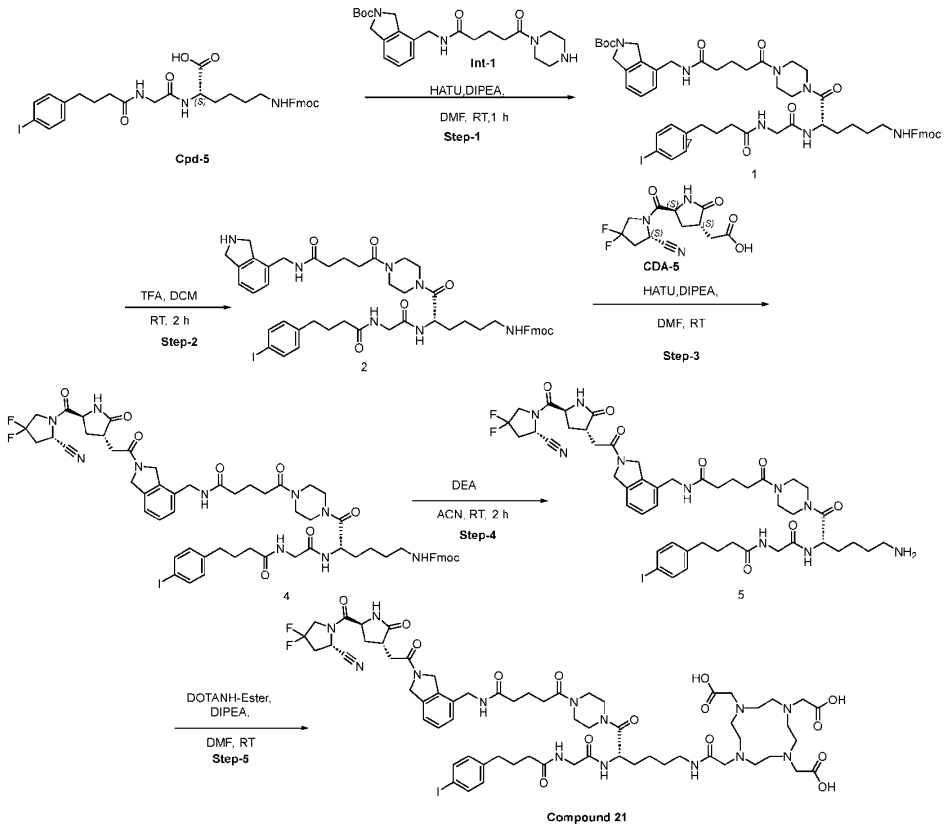
[000384] Step-9: Synthesis of 2-(((S)-6-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-1-oxohexan-2-yl)amino)-2-oxoethyl (4-(4-iodophenyl)butanoyl)-L-alaninate(12): To a solution of 2-(((S)-6-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-1-oxohexan-2-yl)amino)-2-oxoethyl (4-(4-iodophenyl)butanoyl)-L-alaninate 11 (500 mg, 0.04 mol) in acetonitrile (2.5 mL, 10 Vol) at room temperature was added 20% Piperidine in DMF (1.5 mL, 3 Vol). Resultant reaction mixture was stirred at room temperature for 15 min. Upon completion of reaction, reaction mixture was concentrated under reduced pressure to get crude. The crude material was triturated with cold EtOAc followed by diethyl ether (50*2 mL) to afford 2-(((S)-6-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-1-oxohexan-2-yl)amino)-2-oxoethyl (4-(4-iodophenyl)butanoyl)-L-alaninate 12 (250 mg, yield: 62 %) as an off white solid. MS (ESI) m/z:1029.56 $(M+H)^+$.

[000385] Step-10: Synthesis of 2,2',2''-(10-((8S,14S)-8-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidine-1-carbonyl)-19-(4-iodophenyl)-14-methyl-2,10,13,16-tetraoxo-12-oxa-3,9,15-triazanonadecyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid: To a solution of 2-(((S)-6-amino-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-

carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-1-oxohexan-2-yl)amino)-2-oxoethyl (4-(4-iodophenyl)butanoyl)-L-alaninate 12 (600 mg, 0.583 mol) in acetonitrile (6 mL, 10 Vol) at room temperature was added DIPEA (0.96 mL, 5.252 mol) followed by 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid DOTA-NHS-Ester (438 mg, 0.875 mol).Resultant reaction mixture was stirred at room temperature for 16 h. Upon completion of reaction, reaction mixture was concentrated under reduced pressure to afford crude (900 mg crude). The crude was triturated with EtOAc (25*2 mL) to afford solid, further purified by prep HPLC using the below condition. The required fractions were collected and lyophilized to afford 2,2',2''-(10-((8S,14S)-8-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidine-1-carbonyl)-19-(4-iodophenyl)-14-methyl-2,10,13,16-tetraoxo-12-oxa-3,9,15-triazanonadecyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (105.7 mg, yield: 13%) as a white solid. MS (ESI) m/z: 1415.77 $[M+H]^+$. ^{1}H-NMR (400 MHz, DMSO-d6): δ 13.4-12.8 (brs, 2H), 8.5-8.36 (m, 2H), 7.61 (d, *J* = 8 Hz, 1H), 7.31-7.16 (m, 3H), 7.19-7.12 (m, 2 H), 7.00 (d, *J* = 8 Hz, 2H), 5.12-5.09 (m, 1H), 4.9-4.8 (m, 2H), 4.7-4.5 (m, 5H), 4.4-4.3 (m, 4H), 4.2-4.0 (m, 4H), 3.9-3.7 (m, 4H), 3.4-3.3 (m, 12H), 3.3-3.0 (m, 12 H), 2.9-2.7 (m, 6H), 2.3-2.2 (m, 1H), 2.2-2.1 (m, 3H), 1.9-1.7 (m, 4H), 1.7-1.5 (m, 3H), 1.5-1.3 (m, 4H), 1.3-1.2 (m, 7H), 1.2-1 (m, 2H)

[000386] The synthesis of tert-butyl 4-(piperidin-4-ylmethyl)isoindoline-2-carboxylate is described in Example 8.

[000387] Example 21. Synthesis of 2,2',2''-(10-(2-(((S)-6-(4-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 21)



[000388] Step-1: Synthesis of tert-butyl 4-((5-(4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysyl)piperazin-1-yl)-5-oxopentanamido)methyl)isoindoline-2-carboxylate: To a solution of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysine Int#5 (5 g, 0.0071 mmol) in DMF (50 mL) at room temperature was added DIPEA (1.85 mL, 0.010 mol) followed by HATU (2.8 g, 0.0071 mol). The mixture was stirred for 10 min and added tert-butyl 4-((5-oxo-5-(piperazin-1-yl)pentanamido)methyl)isoindoline-2-carboxylate Int-1 (3 g, 0.0071 mol). Resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was poured into ice water (200 mL) stirred for 15 min and resultant solid was filtered. Solid was dissolved in 10% MeOH in DCM (30 mL), diluted with diethyl ether (60 mL) and solid obtained was filtered, dried under vacuum to afford tert-butyl 4-((5-(4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysyl)piperazin-1-yl)-5-oxopentanamido)methyl)isoindoline-2-carboxylate 1 (4 g, yield: 50%) as an off white solid. MS (ESI) m/z: 1110.81 $(M+H)^+$.

[000389] Step-2: Synthesis of (9H-fluoren-9-yl)methyl (S)-(5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-(4-(5-((isoindolin-4-ylmethyl)amino)-5-oxopentanoyl)piperazin-1-yl)-6-oxohexyl)carbamate: TFA (16 mL, 4 vol) was added to a solution tert-butyl 4-((5-(4-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysyl)piperazin-1-yl)-5-oxopentanamido)methyl)isoindoline-2-carboxylate 1 (4 g, 0.003 mol) in DCM (40 mL, 10 Vol) at 0 °C. Resultant reaction mixture was stirred at room temperature for 2 h. Upon completion of reaction, reaction mixture was concentrated under reduced pressure to afford crude. Crude was triturated with diethyl ether (100 mL), filtered and dried under vacuum to afford (9H-fluoren-9-yl)methyl(S)-(5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-(4-(5-((isoindolin-4-ylmethyl)amino)-5-oxopentanoyl)piperazin-1-yl)-6-oxohexyl)carbamate 2 (2.8 g, yield: 77%) as a brown solid. MS (ESI) m/z: 1010.47 $[M+H]^+$

[000390] Step-3: Synthesis of (9H-fluoren-9-yl)methyl ((S)-6-(4-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-oxohexyl)carbamate: To a solution of 2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetic acid CDA-5 (1 g, 0.003 mol) in DMF (10 mL) at room temperature was added DIPEA (1 mL, 0.006 mol) followed by HATU (1.2 g, 0.003 mol). The reaction mixture was stirred for 10 min and added (9H-fluoren-9-yl)methyl (S)-(5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-(4-(5-((isoindolin-4-ylmethyl)amino)-5-oxopentanoyl)piperazin-1-yl)-6-oxohexyl)carbamate 3 (3 g, 0.003 mol). Resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was poured into ice water (200 mL) stirred for 15 min and resulted solid was filtered. The solid was dissolved in 10% MeOH in DCM (30 mL), triturated with diethyl ether (60 mL) and regenerated solid was filtered, dried under vacuum to afford (9H-fluoren-9-yl)methyl ((S)-6-(4-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-oxohexyl)carbamate 4 (2.5 g, yield: 65%) as an off white solid. MS (ESI) m/z: 1293.48 $(M+H)^+$.

[000391] Step-4: Synthesis of N-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)-5-(4-((4-(4-iodophenyl)butanoyl)glycyl-L-lysyl)piperazin-1-yl)-5-oxopentanamide: Diethyl amine (12.5 mL, 5 Vol) was added to a solution of (9H-fluoren-9-yl)methyl ((S)-6-(4-(5-(((2-(2-((3S,5S)-

5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-oxohexyl)carbamate 4 (2.5 g, 0.0019 mol) in acetonitrile (25 mL, 10 Vol) at room temperature. The resultant reaction mixture was stirred at room temperature for 2 h. Upon completion of reaction, reaction mixture was concentrated under reduced pressure to get crude (3.8 g). The crude was triturated with cold EtOAc (3 * 25 mL), resultant solid was dissolved in 10% MeOH in DCM (25 mL), diluted with diethyl ether (30 mL) and the obtained solid was filter and dried under vacuum to afford N-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)-5-(4-((4-(4-iodophenyl)butanoyl)glycyl-L-lysyl)piperazin-1-yl)-5-oxopentanamide 5 (1.5 g, yield: 75%) as a white solid. MS (ESI) m/z: 1072.17 $(M+H)^+$.

[000392] Step-5 : Synthesis of 2,2',2"-(10-(2-(((S)-6-(4-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triaceticacid: To a solution of N-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)-5-(4-((4-(4-iodophenyl)butanoyl)glycyl-L-lysyl)piperazin-1-yl)-5-oxopentanamide 5 (1.5 g, 0.0014 mol) in CH_3CN (15 mL, 10 Vol), MeOH (15 mL, 10 Vol) at room temperature was added DIPEA (2.1 mL, 0.012 mol) followed by 2,2',2"-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid DOTA NHS-ester (1 g, 0.002 mol). The resultant reaction mixture was stirred at room temperature for 16 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to get residue. The residue was trituration with EtOAc (2 * 25 mL), the isolated solid was dissolved in 10% MeOH in DCM (40 mL), diluted with diethyl ether (40 mL) the obtained solid was filtered to get crude. The crude material was purified by Prep-HPLC. The required fractions were collected and concentrated under vacuum followed by lyophilization to afford 2,2',2"-(10-(2-(((S)-6-(4-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)piperazin-1-yl)-5-(2-(4-(4-iodophenyl)butanamido)acetamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triaceticacid (38.7 mg) as a white solid. MS (ESI) m/z: 1455.32 $(M-H)^-$. ^{1}H-NMR (400 MHz, DMSO-d6): δ 13.20-12.80 (brs, 1H), 8.34-8.30 (m, 2H), 8.08-8.05 (m, 3H), 7.62 (d, J = 8.0 Hz, 2H), 7.29-

7.21 (m, 2H), 7.21-7.15 (m, 1H), 7.01 (d, J = 8.0 Hz, 2H), 5.11-5.10 (m, 1H), 4.85 (brs, 2H), 4.66-4.64 (m, 3H), 4.34-4.32 (m, 1H), 4.22-4.20 (m, 2H), 4.15-4.10 (m, 2H), 3.95-3.85 (m, 3H), 3.70-3.65 (m, 2H), 3.60-3.50 (m, 8H), 3.39-3.20 (m, 14H), 3.16-3.00 (m, 10H), 2.90-2.70 (m, 4H), 2.35-2.25 (m, 2H), 2.20-2.10 (m, 5H), 1.80-1.70 (m, 4H), 1.69-1.58 (m, 1H), 1.80-1.70 (m, 4H), 1.69-1.58 (m, 1H), 1.49-1.35 (m, 3H), 1.30-1.20 (m, 2H).

[000393] Synthesis of Cpd-5

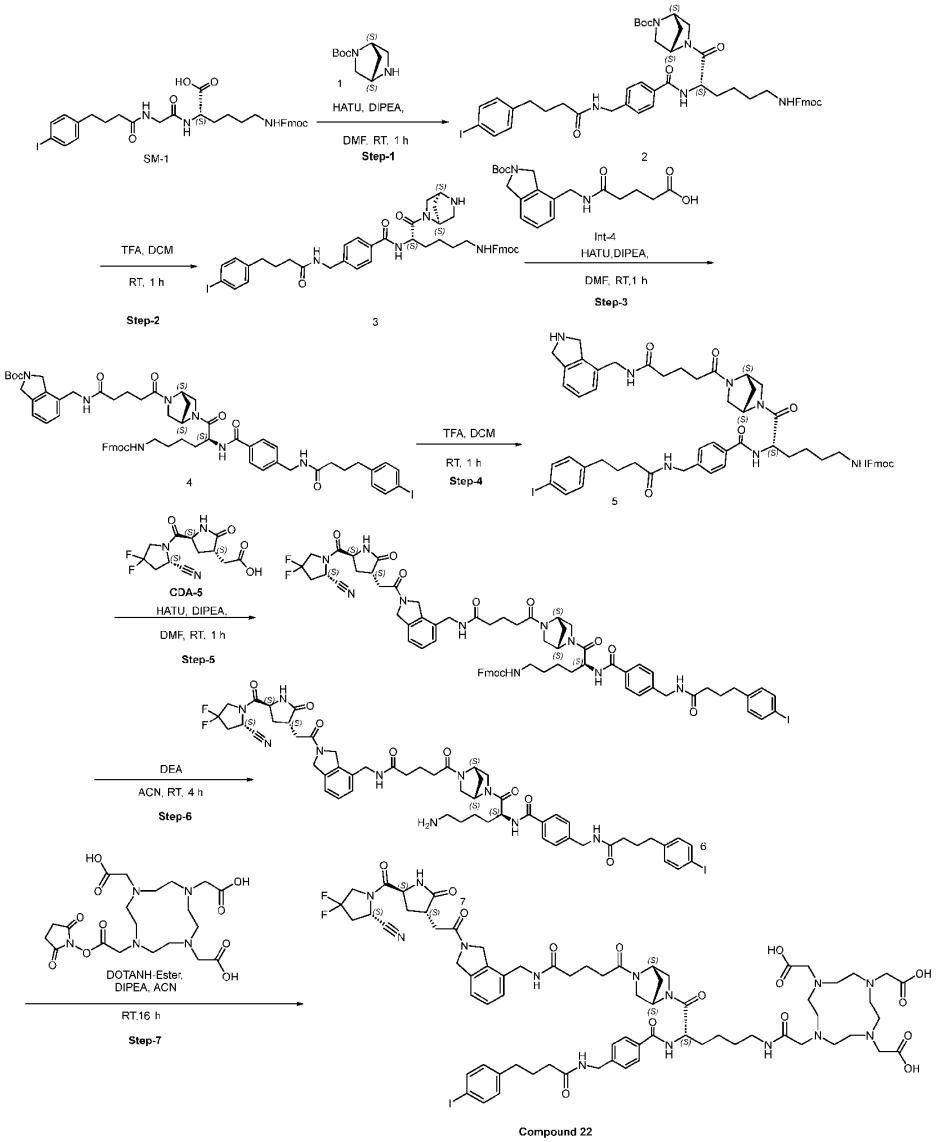
[000394] Step-1: Synthesis of tert-butyl (4-(4-iodophenyl)butanoyl)glycinate: To a solution of 4-(4-iodophenyl)butanoic acid SM-1 (5 g, 0.017 mol) in DMF (50 mL, 10 vol) at room temperature was added DIPEA (6 mL, 0.034 mol) followed by HATU (6.5 g, 0.017 mol). The reaction mixture was stirred for 10 min and added tert-butyl glycinate 1 (2.2 g, 0.01 mol). Resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was poured into ice water (250 mL) and extracted with diethyl ether (2 * 200 mL), organic layer was dried over sodium sulphate, concentrated under reduced pressure to get crude. Crude was dissolved in EtOAc (1 mL) and triturated with hexane (10 mL) to afford tert-butyl (4-(4-iodophenyl)butanoyl)glycinate 2 (4.5 g, yield: 65%) as a white solid.

[000395] Step-2: Synthesis of (4-(4-iodophenyl)butanoyl)glycine: TFA (20 mL, 4 Vol) was added to a solution of tert-butyl (4-(4-iodophenyl)butanoyl)glycinate 2 (5 g, 0.12 mmol) in DCM (50 mL, 10 Vol) at 0° C. The resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude. Crude was dissolved in EtOAc (100 mL) and triturated with hexane (2 * 50 mL) to afford (4-(4-iodophenyl)butanoyl)glycine 3 (3 g, yield: 88%) as an off white solid. MS (ESI) m/z: 347.90 $(M+H)^+$.

[000396] Step-3: Synthesis of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysine: To a solution of (4-(4-iodophenyl)butanoyl)glycine 3 (5 g, 0.014 mol) in DMF (50 mL, 10 Vol) at room temperature was added HATU (5.47 g, 0.014 mol) followed by DIPEA (3.7 mL, 0.021 mol), the mixture was stirred for 10 min, and added N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-L-lysine 4 (4.7 g, 0.012 mol). The reaction mixture was stirred at room temperature for 1 h. Upon completion of reaction, reaction mixture was poured into ice water (50 mL) stirred for 15 min and obtained solid was filtered. The resulted solid was dissolved in 10% MeOH in DCM (30 mL), diluted with diethyl ether (50 mL) obtained solid was filter to afford N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysine -Cpd-5 (4 g, yield: 40%) as an off white solid. MS (ESI) m/z: 698.25 $(M+H)^+$.

[000397] The synthesis of tert-butyl 4-((5-oxo-5-(piperazin-1-yl)pentanamido)methyl)isoindoline-2-carboxylate (Int-1) is described in Example 3.

[000398] **Example 22. Synthesis of 2,2',2''-(10-(2-(((S)-6-((1S,4S)-5-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 22)**



[000399] Step-1: Synthesis of tert-butyl (1S,4S)-5-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate: To a solution of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysine SM-1 (6 g, 0.007 mol) in DMF (60 mL) at room temperature was added DIPEA (1.8 mL, 0.010 mol) followed by HATU (2.94 g, 0.07 mol). The mixture was stirred for 10 min and added tert-butyl (1S,4S)-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate 1 (1.38 g, 0.007 mol). The resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was poured into ice water (200 mL) stirred for 15 min and solid obtained was filtered and dissolved in 10% MeOH in DCM (30 mL), and added diethyl ether (60 mL), obtained solid was filter, dried under vacuum to afford tert-butyl (1S,4S)-5-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate 2 (5.2 g, yield: 78%) as a brown solid. MS (ESI) m/z: 954.34 $(M+H)^+$

[000400] Step-2: Synthesis of (9H-fluoren-9-yl)methyl ((S)-6-((1S,4S)-2,5-diazabicyclo[2.2.1]heptan-2-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)carbamate: TFA (24 mL, 4 Vol) was added to a solution of tert-butyl (1S,4S)-5-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)-2,5-diazabicyclo[2.2.1]heptane-2-carboxylate 2 (6 g, 0.006 mmol) in DCM (60 mL, 10 Vol) at 0° C. The resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of reaction, reaction mixture was concentrated under reduced pressure to afford crude. The crude was triturated with diethyl ether (2 * 50 mL) and obtained solid was filtered to afford (9H-fluoren-9-yl)methyl ((S)-6-((1S,4S)-2,5-diazabicyclo[2.2.1]heptan-2-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)carbamate 3 (4.2 g, yield: 79%) as a brown solid. MS (ESI) m/z: 854.24 $(M+H)^+$.

[000401] Step-3: Synthesis of tert-butyl 4-((5-((1S,4S)-5-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)-5-oxopentanamido)methyl)isoindoline-2-carboxylate: To a solution of 5-(((2-(tert-butoxycarbonyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoic acid Int-4 (2.3 g, 0.006 mol) in DMF (23 mL) at room temperature was added DIPEA (2.0 mL, 0.012 mol) followed by HATU (2.41 g, 0.006 mol). The mixture was stirred at room temperature for 10 min and added (9H-fluoren-9-yl)methyl ((S)-6-((1S,4S)-2,5-diazabicyclo[2.2.1]heptan-2-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-

oxohexyl)carbamate 3 (5.1 g, 0.006 mol). The resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of reaction, reaction mixture was poured into ice water (150 mL) stirred for 15 min and solid obtained was filtered. Solid was dissolved in 10% MeOH in DCM (30 mL), added diethyl ether (60 mL) and regenerated solid was filtered, dried under vacuum to afford tert-butyl 4-((5-((1S,4S)-5-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)-5-oxopentanamido)methyl)isoindoline-2-carboxylate 4 (4 g, yield: 56%) as a brown solid. MS (ESI) m/z: 1198.54 $(M+H)^+$

[000402] Step-4: Synthesis of (9H-fluoren-9-yl)methyl ((S)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-((1S,4S)-5-(5-((isoindolin-4-ylmethyl)amino)-5-oxopentanoyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)-6-oxohexyl)carbamate: TFA (20 mL, 4 Vol) was added to a solution of tert-butyl 4-((5-((1S,4S)-5-(N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)-5-oxopentanamido)methyl)isoindoline-2-carboxylate 4 (5 g, 0.004 mol) in DCM (50 mL, 10 Vol) at 0° C. The resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of reaction, reaction mixture was concentrated under reduced pressure to afford crude. Crude was triturated with diethyl ether (2 * 50mL) and obtained solid was filter to afford (9H-fluoren-9-yl)methyl ((S)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-((1S,4S)-5-(5-((isoindolin-4-ylmethyl)amino)-5-oxopentanoyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)-6-oxohexyl)carbamate 5 (3.5 g, yield: 77%) as a white solid . MS (ESI) m/z: 1099.43 $(M+H)^+$.

[000403] Step-5: Synthesis of (9H-fluoren-9-yl)methyl ((S)-6-((1S,4S)-5-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)carbamate: To a solution of 2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetic acid CDA-5 (1 g, 0.003 mol) in DMF (10 mL) at room temperature was added DIPEA (0.58 mL, 0.006 mol) followed by HATU (1.26 g, 0.003 mol). The mixture was stirred for 10 min and added (9H-fluoren-9-yl)methyl ((S)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-((1S,4S)-5-(5-((isoindolin-4-ylmethyl)amino)-5-oxopentanoyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)-6-oxohexyl)carbamate 5 (3.64 g, 0.003 mol). The resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of reaction, reaction mixture was poured into ice water (150 mL) stirred for 15 min and solid obtained was filtered and again dissolved in 10% MeOH in DCM (30 mL),

diluted with diethyl ether (60 mL) and obtained solid was filtered, dried under vacuum to afford (9H-fluoren-9-yl)methyl ((S)-6-((1S,4S)-5-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)carbamate 6 (2.8 g, yield: 62%) as a brown solid. MS (ESI) m/z: 1381.60 $(M+H)^+$

[000404] Step-6 : Synthesis of N-((S)-6-amino-1-((1S,4S)-5-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)-1-oxohexan-2-yl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide: Diethyl amine (8.5 mL, 5 Vol) was added to a solution of (9H-fluoren-9-yl)methyl ((S)-6-((1S,4S)-5-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)carbamate 6 (1.7 g, 0.001 mol) in acetonitrile (1.7 mL, 10 Vol) at room temperature. The resultant reaction mixture was stirred at room temperature for 4 h. Upon completion of reaction, reaction mixture was concentrated under reduced pressure to get crude. The crude compound was triturated with cold EtOAc (3 * 25 mL), the resultant solid was dissolved in 10% MeOH in DCM (25 mL), diluted with diethyl ether (30 mL) and the obtained solid was filter to afford N-((S)-6-amino-1-((1S,4S)-5-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)-1-oxohexan-2-yl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 7 (1 g, yield: 71%) as a white solid. MS (ESI) m/z: 1160.08 $(M+H)^+$.

[000405] Step-7: Synthesis of 2,2',2''-(10-(2-(((S)-6-((1S,4S)-5-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid: to a stirred solution of N-((S)-6-amino-1-((1S,4S)-5-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)-1-oxohexan-2-yl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 7 (1 g, 0.0008 mol) in CH_3CN (10 mL, 10 Vol) at room temperature was added DIPEA (1.2 mL, 0.0072 mol) and 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic

acid DOTA-NHS-ester (600 mg, 0.0012 mol). Resultant reaction mixture was stirred at room temperature for 16 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to get residue. The residue was trituration with EtOAc (2 * 25 mL), the isolated solid was dissolved in 10% MeOH in DCM (40 mL) and triturated with diethyl ether (40 mL) to get crude material. The crude material was purified by Prep-HPLC. Prep HPLC conditions: The required fractions were collected and concentrated under vacuum followed by lyophilization to afford 2,2',2"-(10-(2-(((S)-6-((1S,4S)-5-(5-(((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoyl)-2,5-diazabicyclo[2.2.1]heptan-2-yl)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (205 mg) as a white solid. MS (ESI) m/z: 1544.06 $(M-H)^-$. ^{1}H-NMR (400 MHz, DMSO-d6): δ 14.1-12.98 (brs, 1H), 8.68-8.64 (m, 1H), 8.55-8.45 (m, 2H), 8.40-8.30 (m, 1H), 8.10 (brs, 1H), 7.85-7.70 (m, 2H), 7.65-7.60 (m, 2H), 7.58-7.45 (brs, 1H), 7.42-7.10 (m, 5H), 7.12-6.98 (m, 2H), 5.15-5.10 (m, 1H), 4.90-4.80 (m, 2H), 4.79-4.60 (m, 5H), 4.35-4.29 (m, 3H), 4.25-4.15 (m, 4H), 4.12-4.05 (m, 4H), 3.70-3.50 (m, 8H), 3.45-3.20 (m, 12H), 3.19-3.00 (m, 10H), 2.85-2.62 (m, 4H), 2.30-2.10 (m, 8H), 1.85-1.65 (m, 8H), 1.55-1.30 (m, 4H).

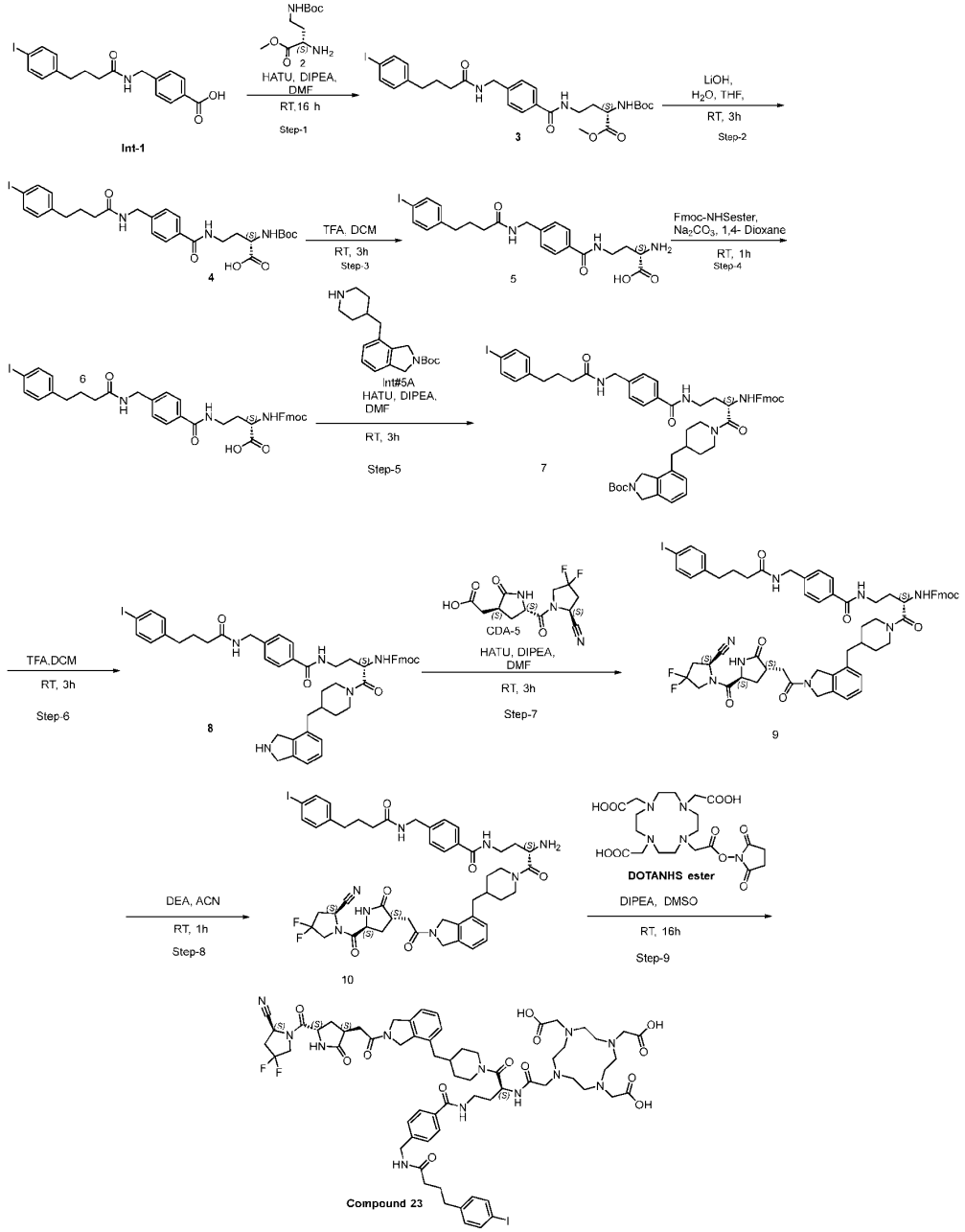
[000406] The synthesis of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-((4-(4-iodophenyl)butanoyl)glycyl)-L-lysine (SM-1) is described in Example 16.

[000407] Synthesis of 5-(((2-(tert-butoxycarbonyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoic acid (Int-4).

[000408] Step-1A: Synthesis of tert-butyl 4-(aminomethyl)isoindoline-2-carboxylate: To a solution of tert-butyl 4-(azidomethyl)isoindoline-2-carboxylate 1A (7 g, 25.54 mmol) in THF (28 mL, 4 vol) and water (14 mL, 2 Vol) at room temperature was added PPh_3 (10 g, 38.27 mmol). The resultant reaction was stirred at room temperature for 16 h. Upon completion of reaction, reaction mixture was diluted with EtOAc (30 mL), ice cold water (30 mL), and stirred for 15 min. Organic layer was separated and aqueous layer was further extracted with EtOAc (2 * 30 mL). Combined organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford crude. Crude was purified by silica gel column chromatography (100-200 mesh) using 50% EtOAc in hexane as an eluant. Compound contained fractions were concentrated under reduced pressure to afford tert-butyl 4-(aminomethyl)isoindoline-2-carboxylate 2A (3.8 g, Yield: 60%) as a brown solid. MS (ESI) m/z: 248.3 $[M+H]^+$

[000409] Step-2A: Synthesis of 5-(((2-(tert-butoxycarbonyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoic acid: To a solution of 5-(((2-(tert-butoxycarbonyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoic acid 2A (3.5 g, 14.11 mmol) in THF (52.5 mL, 15 Vol) at 0°C, was added Et_3N (2.85 g, 28.21 mmol) followed by dihydro-2H-pyran-2,6(3H)-dione 3A (1.6 g, 14.11 mmol). The resultant reaction mixture was stirred at room temperature for 16 h. Upon completion of reaction, reaction mixture was diluted with EtOAc (30 mL), ice cold water (30 mL), and stirred for 15 min. Organic layer was separated and aqueous layer was further extracted with EtOAc (2 * 30 mL), combined organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford 5-(((2-(tert-butoxycarbonyl)isoindolin-4-yl)methyl)amino)-5-oxopentanoic acid 4A (3.2 g, yield: 62%) as a brown solid. MS (ESI) m/z: 362.3 $[M+H]^+$

[000410] Example 23. Synthesis of 2,2',2''-(10-(2-(((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-4-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxobutan-2-yl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 23)



[000411] Step-1: Synthesis of methyl (S)-2-((tert-butoxycarbonyl)amino)-4-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)butanoate: To a solution of 4-((4-(4-iodophenyl)butanamido)methyl)benzoic acid Int-1 (5 g, 11.8 mmol) in DMF (50 mL) at room temperature was added DIPEA (6.1 mL, 35.9 mmol) followed by HATU (4.49 g, 11.8

mmol). The reaction mixture was stirred for 10 min and added methyl (S)-2-amino-4-((tert-butoxycarbonyl)amino)butanoate 2 (3.17 g, 11.8 mmol). The resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of reaction, reaction mixture was diluted with water (200 mL), and the solid precipitated was filtered to get crude. Crude was purified by silica gel column chromatography (100-200 mesh) using 70% EtOAC in hexane as an eluant. Compound contained fractions were concentrated under reduced pressure to afford methyl (S)-2-((tert-butoxycarbonyl)amino)-4-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)butanoate 3 (5.2 g, yield: 69%) as an off white solid. MS (ESI) m/z: 638.28 $(M+H)^+$.

[000412] Step-2: Synthesis of (S)-2-((tert-butoxycarbonyl)amino)-4-(4-((4-(4-iodophenyl) butanamido) methyl) benzamido) butanoic acid: To a solution of methyl (S)-2-((tert-butoxycarbonyl)amino)-4-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)butanoate 3 (5.2 g, 8.161 mmol) in THF (15.6 mL, 3 vol) and water (15, 3 vol) at 0 °C was added $LiOH.H_2O$ (0.65 g, 16.32 mmol). Resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of reaction, reaction mixture was concentrated under vacuum to get crude. Crude was dissolved in water (50 mL) and acidified (pH 2-3) with 1N HCl and extracted with 10% MeOH in DCM, organic layer was dried over sodium sulphate and concentrated under vacuum to get crude . Obtained crude material was purified by silica gel column chromatography (100-200 mesh) using 70% EtOAc in hexane as an eluant. Compound contained fractions were concentrated under reduced pressure to afford (S)-2-((tert-butoxycarbonyl)amino)-4-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)butanoic acid 4 (500 mg). MS (ESI) m/z: 624.21 $(M+H)^+$.

[000413] Step-3: Synthesis of (S)-2-amino-4-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)butanoic acid: TFA (20 mL, 4 vol) was added to a solution of (S)-2-((tert-butoxycarbonyl)amino)-4-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)butanoic acid 4 (5 g, 8.01 mmol) in DCM (50 mL, 10 vol), at 0 °C. The resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of reaction, reaction mixture was concentrated under reduced pressure to afford crude. The crude was triturated with diethyl ether (25 mL) and n-pentane (50 mL) to afford (S)-2-amino-4-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)butanoic acid 5 (2.45 g, yield: 58%) as a white solid. MS (ESI) m/z: 524.12 $(M+H)^+$.

[000414] Step-4: Synthesis of (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)butanoic acid: To a solution of (S)-2-

amino-4-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)butanoic acid 5 (1.8 g, 4.681 mmol) in 1,4 dioxane (10 mL) and water (10 mL) at 0 °C was added sodium carbonate (968 mg, 9.362 mmol), stirred for 5 min, and added Fmoc-Osu (1.8 g, 5.617 mmol). The resultant reaction mixture was stirred at room temperature for 1h. Upon completion of reaction, reaction mixture was diluted with EtOAc (30 mL), ice cold water (30 mL), and stirred for 15 min. Organic layer was separated and the aqueous layer was further extracted with EtOAc (2 * 30 mL). Combined organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford crude. Crude was purified by silica gel column chromatography (100-200 mesh) using 70 % EtOAC in hexane as an eluant. Compound contained fractions were concentrated under reduced pressure to afford (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)butanoic acid 6 (1.7 g, yield: 48%) as an off white solid. MS (ESI) m/z: 746.32 $(M+H)^+$.

[000415] Step-5: Synthesis of tert-butyl (S)-4-((1-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)butanoyl)piperidin-4-yl)methyl)isoindoline-2-carboxylate: To a solution of (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)butanoic acid 6 (1.1 g, 1.475 mmol) in DMF (12 mL, 10 Vol), at room temperature was added DIPEA (0.3 mL, 2.066 mmol) followed by HATU (560 mg, 1.475 mmol). The reaction mixture was stirred for 10 min and added tert-butyl 4-(piperidin-4-ylmethyl)isoindoline-2-carboxylate Int#5A (466 mg, 1.475 mmol). The resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of reaction, reaction mixture was cool to 0 °C and poured into ice water (50 mL),the solid precipitated was filtered and washed with ice water (50 mL). Crude was purified by grace reverse phase column chromatography using 50% H_2O in acetonitrile as an eluant. Compound contained fractions were concentrated under reduced pressure to afford tert-butyl (S)-4-((1-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)butanoyl)piperidin-4-yl)methyl)isoindoline-2-carboxylate 7 (1.4 g, yield: 91 %) as an off white solid. MS (ESI) m/z: 1044.74 $(M+H)^+$.

[000416] Step-6: (9H-fluoren-9-yl)methyl (S)-(4-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-(4-(isoindolin-4-ylmethyl)piperidin-1-yl)-1-oxobutan-2-yl)carbamate: TFA (4.2 mL, 3 vol) was added to a solution of tert-butyl (S)-4-((1-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-4-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)butanoyl)piperidin-4-yl)methyl)isoindoline-2-

carboxylate 7 (1.4 g, 1.340 mmol) in DCM (15 mL, 10 vol), at 0 °C. The resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of reaction, the reaction mixture was concentrated under reduced pressure to afford crude material. The crude material was triturated with diethyl ether (25 mL) and n-pentane (50 mL) to afford (9H-fluoren-9-yl)methyl (S)-(4-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-(4-(isoindolin-4-ylmethyl)piperidin-1-yl)-1-oxobutan-2-yl)carbamate 8 (1.1 g, crude) as a brown colour gummy liquid. MS (ESI) m/z: 944.55 $(M+H)^+$.

[000417] Step-7: Synthesis of (9H-fluoren-9-yl)methyl ((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-4-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxobutan-2-yl)carbamate: To a solution of 2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetic acid CDA-5 (351 mg, 1.166 mmol) in DMF (16 mL, 8 Vol) at room temperature was added DIPEA (0.3 mL, 1.632 mmol) followed by HATU (8.16 mg, 1.166 mmol). The reaction mixture was stirred for 15 min and added (9H-fluoren-9-yl)methyl (S)-(4-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-(4-(isoindolin-4-ylmethyl)piperidin-1-yl)-1-oxobutan-2-yl)carbamate 8 (1.1 g, 1.166 mmol). Resultant reaction mixture was stirred at room temperature for 3 h. Upon completion of reaction, reaction mixture was cool to 0 °C and poured into ice water (50 mL), the solid precipitated was filtered and washed with ice water (50 mL). Crude was purified by grace reverse phase column chromatography using 50% H_2O in acetonitrile as an eluant. Compound contained fractions were concentrated under reduced pressure to afford (9H-fluoren-9-yl)methyl ((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-4-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxobutan-2-yl)carbamate 9 (1.3 g, yield: 90 %) as a white solid. MS (ESI) m/z: 1227.84 $(M+H)^+$.

[000418] Step-8: Synthesis of N-((S)-3-amino-4-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-4-oxobutyl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide: Diethyl amine (5.2 mL, 4 Vol) was added to a solution of (9H-fluoren-9-yl)methyl ((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-4-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxobutan-2-yl)carbamate 9 (1.3 g, 1.059 mmol) in acetonitrile (40 mL, 10 Vol) at room temperature. The resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of reaction, reaction mixture was

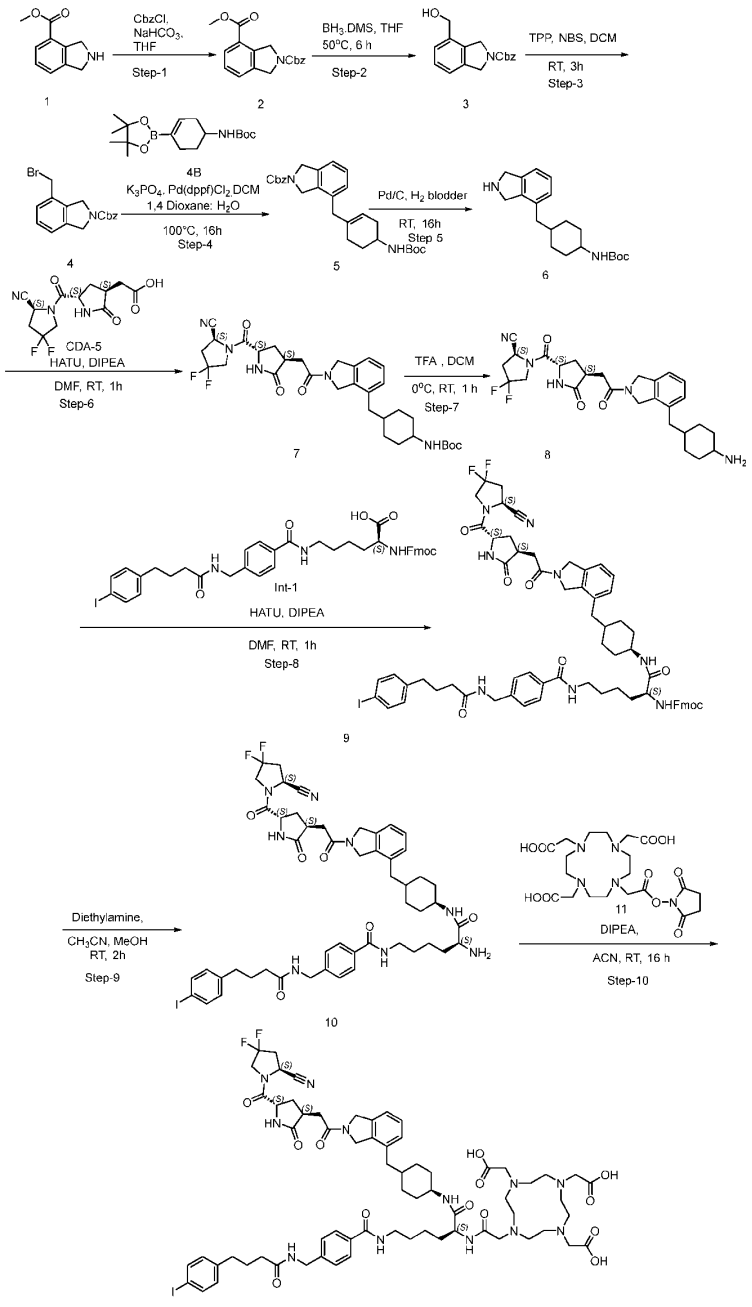
concentrated under vacuum to get crude. The crude was triturated with cold EtOAc (30 mL) and filtered. Filtered solid was dissolved in 10% MeOH in DCM (20 mL) and added diethyl ether (30 mL), regenerated solid was filtered, dried under vacuum to afford N-((S)-3-amino-4-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-4-oxobutyl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 10 (900 mg, crude) as a pale brown solid. MS (ESI) m/z: 1005.57 $(M+H)^+$.

[000419] Step-9: Synthesis of 2,2',2''-(10-(2-(((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-4-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxobutan-2-yl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid: To a solution of N-((S)-3-amino-4-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-4-oxobutyl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 10 (900 mg, 0.896 mmol) in acetonitrile (18 mL, 20 Vol) at room temperature was added DIPEA (1.40 mL, 8.064 mmol) followed by 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid DOTA-NHS ester (673.34 mg, 1.344 mmol) lot wise. Resultant reaction mixture was stirred at room temperature for 16 h, Upon completion of reaction, reaction mixture was concentrated under reduced pressure to get residue. The residue was triturated with EtOAc (2* 30 mL) and acetonitrile (2* 25 mL) to get crude. The crude was purified by Prep-HPLC column chromatography by using below condition. The required fractions were collected and concentrated under vacuum followed by lyophilization to afford 2,2',2''-(10-(2-(((S)-1-(4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)piperidin-1-yl)-4-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxobutan-2-yl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (220 mg) as a white solid. MS (ESI) m/z: 1390.31 $(M+H)^+$. ^{1}H-NMR (400 MHz, DMSO-d6): δ 14-11 (brs, 2H), 8.8-8.6 (m, 1H), 8.5-8.31 (m, 2H), 8.1 (s, 1H), 7.9-7.8 (m, 2H), 7.62 (d, J = 8 Hz, 2H), 7.41-7.3 (m, 2H), 7.21-7.11 (m, 2H), 7.10-7.00 (m, 3H), 5.11-5.09 (m, 1H), 4.99-4.80 (m, 1H), 4.8-4.7 (m, 2 H), 4.71-4.61 (m, 2 H), 4.4-4.3 (m, 4H), 4.3-4.01 (m, 4H), 4.0-3.8 (m, 4H), 3.71-3.5 (m, 8H), 3.4-3.3 (m, 4H), 3.4-3.3 (m, 8H), 3.2-3.0 (m, 11H), 2.9-2.6 (m, 4H), 2.5-2.4 (m, 2H), 2.4-2.3 (m, 1H), 2.2-2.1 (m, 3H), 2.0-1.9 (m, 1H), 1.81-1.61 (m, 3H), 1.3-1.1 (m, 1H)

[000420] The synthesis of tert-butyl 4-(piperidin-4-ylmethyl)isoindoline-2-carboxylate (Int#5A) is described in Example 8.

[000421] The synthesis of 4-((4-(4-iodophenyl)butanamido)methyl)benzoic acid (Int-1) is described in Example 4.

[000422] Example 24. Synthesis of N-((5S)-5-amino-6-((4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl) methyl) cyclohexyl)amino)-6-oxohexyl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide (Compound 24)



[000423] Step-1: To a solution of methyl isoindoline-4-carboxylate 1 (15 g, 0.070 mol) in THF (150 mL) at 0 °C was added $NaHCO_3$ (17 g, 0.211 mol) followed by benzyl chloroformate (19 mL, 0.14 mol). Resultant reaction mixture was stirred at room temperature for 16h, after completion of the reaction, reaction mixture was poured into water (100 mL),

and extracted with ethyl acetate (2*500 mL). The organic layer was separated, dried over sodium sulphate, filtered and concentrated under reduced pressure to afford 2-benzyl 4-methyl isoindoline-2,4-dicarboxylate 2 (13 g) as an off white solid. MS (ESI) m/z: 311.98 $[M+H]^+$

[000424] Step-2: To a solution of 2-benzyl 4-methyl isoindoline-2,4-dicarboxylate 3 (13 g, 0.04 mol) in THF (130 mL) at 0 °C was added BH_3DMS (2M in THF) (13 mL, 0.12 mmol). The resultant reaction mixture was stirred at 50 °C for 6 h. After completion of reaction, reaction mixture was quenched with MeOH (100 mL), concentrated under reduced pressure to get crude. The obtained crude was purified by silica gel column chromatography (100-200 mesh) using 30- 40% ethyl acetate in hexane as an eluent. Compound contained fractions were concentrated under reduced pressure to afford benzyl 4-(hydroxymethyl)isoindoline-2-carboxylate 4 (9 g, yield:75%) as an off white solid.

[000425] Step-3: Synthesis of benzyl 4-(bromomethyl)isoindoline-2-carboxylate : To a solution of benzyl 4-(hydroxymethyl)isoindoline-2-carboxylate 3 (11.5 g, 0.04 mol) in DCM (115 mL) at 0 °C was PPh_3 (21.3 g, 0.08 mol) added followed by NBS (21.6 g, 0.12 mol). The resultant reaction mixture was stirred at room temperature for 3h. After completion of the reaction, reaction mixture was poured in to water (200 mL), obtained solid was filtered and washed with ice water (100 mL) to get crude. Crude material was purified by silica gel column chromatography (100-200 mesh) using 20-30% Ethyl acetate in hexane as an eluent. Compound fractions were concentrated under reduced pressure to afford benzyl 4-(bromomethyl)isoindoline-2-carboxylate 4 (7 g, yield: 50%) as a white solid. MS (ESI) m/z: 346.02 $[M+H]^+$

[000426] Step-4: Synthesis of benzyl 4-((4-((tert-butoxycarbonyl)amino)cyclohex-1-en-1-yl)methyl)isoindoline-2-carboxylate :A 100 mL seal tube, to a solution of benzyl 4-(bromomethyl)isoindoline-2-carboxylate 4 (5 g, 0.015 mol) in 1,4 dioxane : H_2O (9:1, 50 mL, 10 vol) at room temperature was added K_3PO_4 (8.2 g, 0.038 mol) and tert-butyl (4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)cyclohex-3-en-1-yl)carbamate 4B (4.8 g, 0.017 mol). The mixture was purged with argon for 15 min, and added Pd(dppf)Cl_2.DCM (1.2 g, 0.0015 mmol). The resultant reaction mixture was sealed and heated at 100 °C for 16 h. After completion of reaction, reaction mixture was filtered through celite pad and filtrate was concentrated under vacuum and the resultant residue was diluted with water (50 mL), extracted with ethyl acetate (2 * 100 mL). The organic layer was dried over sodium sulphate and concentrated under vacuum to get crude. The crude was purified by silica gel column chromatography (100-200 mesh) using 20% EtOAc in hexane as an eluent. The compound

fractions were concentrated under reduced presser to afford benzyl 4-((4-((tert-butoxycarbonyl)amino)cyclohex-1-en-1-yl)methyl)isoindoline-2-carboxylate 5 (5.2 g, yield: 78%) as a brown solid. MS (ESI) m/z:462.15 $[M+H]^+$

[000427] Step-5: Synthesis of tert-butyl (4-(isoindolin-4-ylmethyl)cyclohexyl)carbamate: 10% Pd/C (3 g) was added to a solution of benzyl 4-((4-((tert-butoxycarbonyl)amino)cyclohex-1-en-1-yl)methyl)isoindoline-2-carboxylate 5 (5.2 g, 0.011 mol) in MeOH (50 mL) . The reaction mixture was stirred at room temperature for 16 h under H_2 gas at 20 *psi*. After completion of the reaction the reaction mixture was filtered through celite pad, the filtrate was concentrated under reduced presser to afford tert-butyl (4-(isoindolin-4-ylmethyl)cyclohexyl)carbamate 6 (3 g, 81%) as a brown liquid. MS (ESI) m/z: 331.3 $(M+H)^+$.

[000428] Step-6: Synthesis of tert-butyl (4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)cyclohexyl)carbamate: To a solution of 2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetic acid CDA-5 (4 g, 0.013 mmol) in DMF (40 mL) at room temperature was added DIPEA (4.47 mL, 0.026 mol) followed by HATU (4.94 g, 0.013 mol). The mixture was stirred for 10 min and added tert-butyl (4-(isoindolin-4-ylmethyl)cyclohexyl)carbamate 6 (4.29 g, 0.013 mol). The resultant reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, reaction mixture was poured into ice water (200 mL) stirred for 15 min and solid obtained was filtered. The solid was dissolved in 10% MeOH in DCM (30 mL), and diluted with diethyl ether (60 mL) and regenerated solid was filtered, dried under vacuum to afford tert-butyl (4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)cyclohexyl)carbamate 7 (3.8 g, yield: 46%) as an off white solid . MS (ESI) m/z: 614.37 $(M+H)^+$.

[000429] Step-7: Synthesis of (S)-1-((2S,4S)-4-(2-(4-((4-aminocyclohexyl)methyl)isoindolin-2-yl)-2-oxoethyl)-5-oxopyrrolidine-2-carbonyl)-4,4-difluoropyrrolidine-2-carbonitrile: TFA (10 mL, 4 vol) was added to a solution of tert-butyl (4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)cyclohexyl)carbamate 7 (2.5 g, 0.004 mol) in DCM (25 mL, 10 Vol) at 0 °C. The resultant reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude. The crude material was diluted with diethyl ether (100 mL) and regenerated solid was filter to afford (S)-1-((2S,4S)-4-(2-(4-((4-aminocyclohexyl)methyl)isoindolin-2-yl)-

2-oxoethyl)-5-oxopyrrolidine-2-carbonyl)-4,4-difluoropyrrolidine-2-carbonitrile 8 (1.5 g, yield: 75%) as a brown solid. MS (ESI) m/z: 514.30 $[M+H]^+$

[000430] Step-8: Synthesis of (9H-fluoren-9-yl)methyl ((2S)-1-((4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)cyclohexyl)amino)-6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)carbamate: To a solution of N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine 8 (4 g, 0.0051 mol) in DMF (40 mL) at room temperature was added DIPEA (1.7 mL, 0.010 mol) followed by HATU (1.9 g, 0.0051 mol). The mixture was stirred for 10 min and added (S)-1-((2S,4S)-4-(2-(4-((4-aminocyclohexyl)methyl)isoindolin-2-yl)-2-oxoethyl)-5-oxopyrrolidine-2-carbonyl)-4,4-difluoropyrrolidine-2-carbonitrile Int-1 (3.1 g, 0.006 mol). The resultant reaction mixture was stirred at room temperature for 1 h. After completion of reaction, reaction mixture was poured into ice water (200 mL), stirred for 15 min and solid obtained was filtered. The solid was dissolved in 10% MeOH in DCM (30 mL), and added diethyl ether (60 mL), regenerated solid was filtered, dried under vacuum to afford (9H-fluoren-9-yl)methyl ((2S)-1-((4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)cyclohexyl)amino)-6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)carbamate 9 (3.8 g, yield: 60%) as an off white solid. MS (ESI) m/z: 1269.65 $(M+H)^+$

[000431] Step-9: Synthesis of N-((5S)-5-amino-6-((4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)cyclohexyl)amino)-6-oxohexyl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide: Diethyl amine (19.5 mL, 5 Vol) was added to a solution of (9H-fluoren-9-yl)methyl ((2S)-1-((4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)cyclohexyl)amino)-6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)carbamate 9 (3.9 g, 0.003 mol) in acetonitrile (31.2 mL, 8 Vol) at room temperature. The resultant reaction mixture was stirred at room temperature for 2 h. After completion of reaction, reaction mixture was concentrated under reduced pressure to get crude. Crude was triturated with cold EtOAc (50 mL), the resultant solid was dissolved in 10% MeOH in DCM (25 mL), and diluted with diethyl ether (30 mL) and regenerated solid was filter to afford N-((5S)-5-amino-6-((4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)cyclohexyl)amino)-6-oxohexyl)-4-((4-(4-

iodophenyl)butanamido)methyl)benzamide 10 (2.5 g, yield: 78%) as a white solid. MS (ESI) m/z: 1047.65 $(M+H)^+$.

[000432] Step-10: Synthesis of 2,2',2''-(10-(2-(((2S)-1-((4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)cyclohexyl)amino)-6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid: To a solution of N-((5S)-5-amino-6-((4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)cyclohexyl)amino)-6-oxohexyl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 10 (1.2 g, 0.0011 mol) in CH_3CN (12 mL, 10 Vol) was added DIPEA (1.77 mL, 0.0103 mol) at room temperature followed by addition of 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid DOTA-NHS-ester (820 mg, 0.0016 mol) as lot wise. The resultant reaction mixture was stirred at room temperature for 16 h. After completion of reaction, reaction mixture was concentrated under reduced pressure to get residue. The residue was triturated with EtOAc (2 * 50 mL) and obtained solid was dissolved in 10% MeOH in DCM (50 mL) diluted with diethyl ether (100 mL), regenerated solid was filtered. The solid was triturated with acetonitrile (30 mL) to get crude. Crude material was purified by Prep-HPLC using below condition. The required fractions were collected and concentrated under vacuum followed by lyophilization to afford 2,2',2''-(10-(2-(((2S)-1-((4-((2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-4-yl)methyl)cyclohexyl)amino)-6-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-1-oxohexan-2-yl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (220 mg,) as a white solid. MS (ESI) m/z: 1431.96 $(M-H)^-$. ^{1}H-NMR (400 MHz, DMSO-d6):δ 13.80-11.50 (brs, 1H), 8.63 (brs, 1H), 8.38 (t, J = 6.0 Hz, 2H), 8.05 (brs, 1H), 7.99-7.90 (m, 1H), 7.74 (d, J = 4.0 Hz, 2H), 7.62 (d, J = 8.0 Hz, 2H), 7.28 (d, J = 7.6, 2H), 7.24-7.23 (m, 1H), 7.17 (t, J = 7.2 Hz, 1H), 7.06 (d, J = 7.2 Hz, 1H), 7.00 (d, J = 8.4 Hz, 2H), 5.15-5.10 (m, 1H), 4.90-4.82 (m, 2H), 4.62 (d, J = 9.2 Hz, 2H), 4.35-4.32 (m, 3H), 4.30-4.20 (m, 2H), 4.10-3.90 (m, 5H), 3.70-3.60 (m, 4 H), 3.40-3.32 (m, 10H), 3.30-3.20 (m, 4H), 3.12-3.00 (m, 10H), 2.90-2.70 (m, 4H), 2.62-2.60 (m, 1H), 2.41-2.40 (m, 2H), 2.30-2.25 (m, 1H), 2.14 (t, J = 7.2 Hz, 3H), 1.81-1.77 (m, 2H), 1.72-1.70 (m, 1H), 1.65-1.40 (m, 7H), 1.32-1.30 (m, 2H), 1.15-1.00 (m, 3H).

[000433] Synthesis of Int-1:

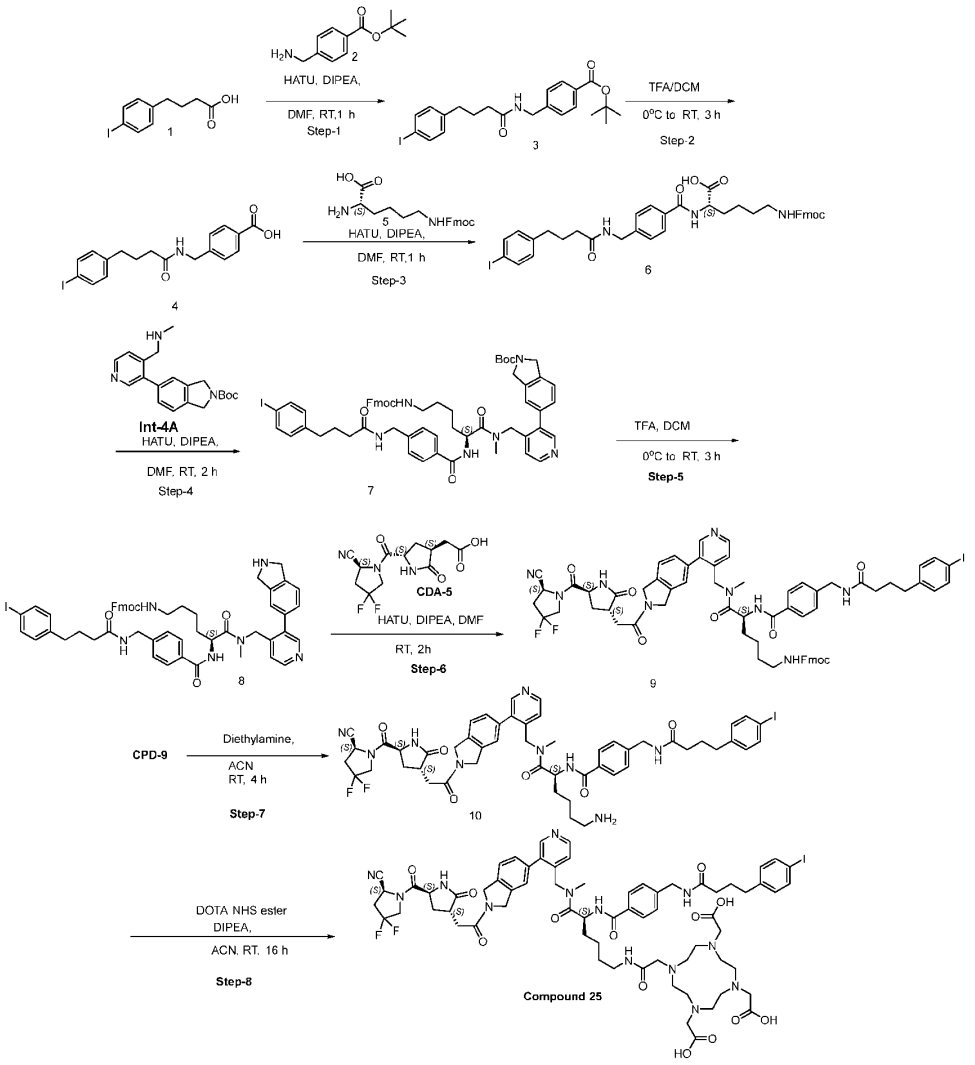
[000434] Step-1: Synthesis of tert-butyl 4-((4-(4-iodophenyl)butanamido)methyl)benzoate (3): To a solution of 4-(4-iodophenyl)butanoic acid 1 (30 g, 0.103 mol) in DMF (60 mL, 2 Vol) at 0 °C was added DIPEA (27 mL, 0.155 mol) followed by HATU (39.3 g, 0.103 mol). The mixture was stirred at room temperature for 30 min, followed by addition of tert-butyl 4-(aminomethyl)benzoate 2 (21.4 g, 0.103 mol). Resultant reaction mixture was stirred at room temperature for 4 h. Upon completion of reaction, reaction mixture was diluted with EtOAc (500 mL), cold water (400 mL) and stirred for 15 min. Organic layer was separated, and aqueous layer was further extracted with EtOAc (2 * 400 mL). Combined organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford tert-butyl 4-((4-(4-iodophenyl)butanamido)methyl)benzoate 3 (36.1 g, crude) as a brown liquid. MS (ESI) m/z: 480.1 $[M+H]^+$

[000435] Step-2: Synthesis of 4-((4-(4-iodophenyl)butanamido)methyl)benzoic acid (4): TFA (160 mL, 4 vol) was added to a solution of tert-butyl 4-((4-(4-iodophenyl)butanamido)methyl)benzoate 3 (40 g, 0.083 mol) in DCM (200 mL, 5 Vol) at 0 °C. Resultant reaction mixture was stirred at room temperature for 5 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude. Crude was triturated with diethyl ether (200 mL) to afford 4-((4-(4-iodophenyl)butanamido)methyl)benzoic acid 4 (31 g, yield: 88%) as a brown solid. MS (ESI) m/z: 424.42 $[M+H]^+$

[000436] Step-3: Synthesis of N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine (5): To a solution of 4-((4-(4-iodophenyl)butanamido)methyl)benzoic acid (Int#4) (5 g, 11.82 mmol) in DMF (50 mL, 10 Vol) at 0 °C, was added DIPEA (4.1 mL, 23.64 mmol) followed by HATU (4.49 g, 11.82 mmol). The mixture was stirred at room temperature for 30 min, and added (((9H-fluoren-9-yl)methoxy)carbonyl)-L-lysine 4 (4.77 g, 11.84 mmol). Resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of reaction, reaction mixture was diluted with EtOAc (200 mL), cold water (200 mL) and stirred for 15 min. The organic layer was separated, and aqueous layer was further extracted with EtOAc (2 * 150 mL). Combined organic layer was dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford crude, resultant crude was purified by silica gel column chromatography (100-200 mesh) using 50% Ethyl acetate in hexane as an eluant. Required fractions were concentrated under reduced pressure to afford N2-(((9H-fluoren-9-

yl)methoxy)carbonyl)-N6-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine 5 (4 g, yield: 44 %) as a white solid. MS (ESI) m/z:774.3 $(M+H)^+$.

[000437] Example 25. Synthesis of 2,2',2''-(10-(2-(((S)-6-(((3-(2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-5-yl)pyridin-4-yl)methyl)(methyl)amino)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (Compound 25)



[000438] Step-1: Synthesis of tert-butyl 4-((4-(4-iodophenyl)butanamido)methyl)benzsoate: To a solution of 4-(4-iodophenyl)butanoic acid 2 (1.8 g, 6.22 mmol) in DMF (18 mL, 10 Vol) at 0 °C was added DIPEA (2.7 mL, 15.5 mmol) followed by HATU (1.74 g, 6.22 mmol). Reaction mixture was stirred at room temperature for 30 min, and added tert-butyl 4-(aminomethyl)benzoate 1 (1.2 g, 6.2 mmol). Resultant reaction mixture was stirred at room temperature for 4 h. Reaction mixture was diluted with EtOAc (200 mL), cold water (100 mL) and stirred for 15 min. The organic layer was separated, and aqueous layer was further extracted with EtOAc (3 * 100 mL). Combined organic layers was washed with water (3 * 50 mL) dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to afford tert-butyl 4-((4-(4-iodophenyl)butanamido)methyl)benzoate 3 (2.2 g, yield: 74 %) as a brown liquid. MS (ESI) m/z: 480.1 $[M+H]^+$

[000439] Step-2: Synthesis of 4-((4-(4-iodophenyl)butanamido)methyl)benzoic acid: TFA (22 mL, 20.8 mmol) was added to a solution of tert-butyl 4-((4-(4-iodophenyl)butanamido)methyl)benzoate 3 (2.2 g, 4.59 mmol) in DCM (22 mL, 10 Vol) at 0 °C drop wise. Resultant reaction mixture was stirred at room temperature for 5 h. Reaction mixture was concentrated under reduced pressure to afford crude. Crude was triturated with diethyl ether (50 mL) to afford 4-((4-(4-iodophenyl)butanamido)methyl)benzoic acid 4 (1.8 g, yield: 92%) as a brown solid. MS (ESI) m/z: 424.42 $[M+H]^+$

[000440] Step-3: Synthesis of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine: To a solution of 4-((4-(4-iodophenyl)butanamido)methyl)benzoic acid 4 (5 g, 0.011 mol) in DMF (50 mL, 10 Vol) at room temperature was added DIPEA (2.8 mL, 0.016 mmol) followed by HATU (4.18 g, 0.011 mol). The reaction mixture was stirred for 10 min followed by addition of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-L-lysine 5 (3.9 g, 0.010 mol). Resultant reaction mixture was stirred at room temperature for 1 h. After completion of the reaction, reaction mixture was poured in to water (200 mL) and stirred for 10 min. Solid obtained was filtered and dried under vacuum to afford N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-iodophenyl)butanamido)methyl)benzoyl)-L-lysine 6 (4 g, yield: 44%) as a white solid. MS (ESI) m/z: 774.50 $(M+H)^+$.

[000441] Step-4: Synthesis of tert-butyl (S)-5-(4-((6-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-(2-(4-((4-(4-iodophenyl)butanamido)methyl)phenyl)-2-oxoethyl)-N-methylhexanamido)methyl)pyridin-3-yl)isoindoline-2-carboxylate: To a solution of N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(4-((4-(4-

Iodophenyl)butanamido)methyl)benzoyl)-L-lysine 6 (1 g, 1.288 mol) in DMF (10 mL, 10 Vol) at room temperature was added DIPEA (0.4 mL, 2.56 mmol) followed by HATU (490 mg, 1.288 mmol), the reaction mixture was stirred for 10 min followed by addition of tert-butyl 5-(4-((methylamino)methyl)pyridin-3-yl)isoindoline-2-carboxylate Int-4A (439 mg, 1.288 mmol). Resultant reaction mixture was stirred at room temperature for 2 h. After completion of the reaction, reaction mixture was poured into ice water (50 mL) stirred for 15 min and obtained solid was filtered. Resulted solid was dissolved in 10% MeOH in DCM (25 mL), diluted with diethyl ether (50 mL) and regenerated solid was filtered to afford tert-butyl (S)-5-(4-((6-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-(2-(4-((4-(4-iodophenyl)butanamido)methyl)phenyl)-2-oxoethyl)-N-methylhexanamido)methyl)pyridin-3-yl)isoindoline-2-carboxylate 7 (1.1 g, yield: 78%) as a white solid. MS (ESI) m/z: 1095.31 $(M+H)^+$.

[000442] Step-5: Synthesis of (9H-fluoren-9-yl)methyl (S)-(5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-(((3-(isoindolin-5-yl)pyridin-4-yl)methyl)(methyl)amino)-6-oxohexyl)carbamate: TFA (4 mL, 4 Vol) was added to a solution of tert-butyl (S)-5-(4-((6-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2-(2-(4-((4-(4-iodophenyl)butanamido)methyl)phenyl)-2-oxoethyl)-N-methylhexanamido)methyl)pyridin-3-yl)isoindoline-2-carboxylate 7 (1 g, 0.91 mmol) in DCM (10 mL, 10 Vol) at 0° C. The resultant reaction mixture was stirred at room temperature for 3 h. After completion of the reaction, reaction mixture was concentrated under reduced pressure to afford crude material. The crude material was dissolved in 10% MeOH in DCM (25 mL), diluted with diethyl ether (50 mL). The regenerated solid was filtered to afford (9H-fluoren-9-yl)methyl (S)-(5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-(((3-(isoindolin-5-yl)pyridin-4-yl)methyl)(methyl)amino)-6-oxohexyl)carbamate 8 (800 mg, yield: 88%) as a brown gummy solid. MS (ESI) m/z: 995.68 $(M+H)^+$.

[000443] Step-6: Synthesis of (9H-fluoren-9-yl)methyl ((S)-6-(((3-(2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-5-yl)pyridin-4-yl)methyl)(methyl)amino)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)carbamate: To a solution of 2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetic acid CDA-5 (600 mg, 1.99 mmol) in DMF (6 mL, 10 Vol) at room temperature was added DIPEA (0.68 mL, 3.98 mmol) followed by HATU (756 mg, 1.99 mmol). The reaction mixture was stirred at room temperature for 10 min followed by addition of (9H-fluoren-9-yl)methyl (S)-

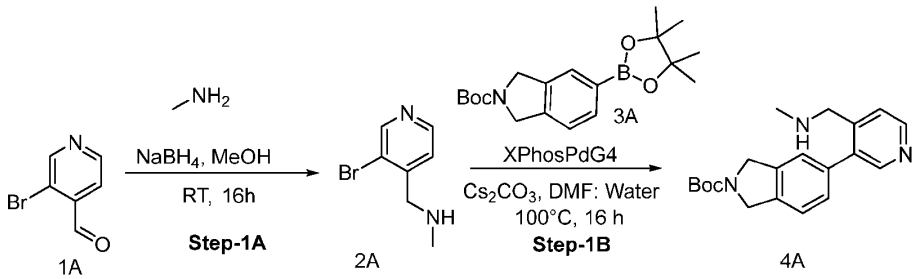
(5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-(((3-(isoindolin-5-yl)pyridin-4-yl)methyl)(methyl)amino)-6-oxohexyl)carbamate 8 (1.9 g, 1.99 mmol). The resultant reaction mixture was stirred at room temperature for 2h. Upon completion of the reaction, reaction mixture was poured into ice water (50 mL) and stirred for 15 min, obtained solid was filtered. Resulted solid was dissolved in 10% MeOH in DCM (25 mL) and diluted with diethyl ether (50 mL) to afford solid. Regenerated solid was filtered, dried under vacuum to afford (9H-fluoren-9-yl)methyl ((S)-6-(((3-(2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-5-yl)pyridin-4-yl)methyl)(methyl)amino)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)carbamate 9 (1.5 g, yield: 66%) as an off white solid. MS (ESI) m/z: 1278.75 $(M+H)^+$.

[000444] Step-7: Synthesis of N-((S)-6-amino-1-(((3-(2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-5-yl)pyridin-4-yl)methyl)(methyl)amino)-1-oxohexan-2-yl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide: Diethyl amine (2.8 mL, 5 Vol) was added to a solution of (9H-fluoren-9-yl)methyl ((S)-6-(((3-(2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-5-yl)pyridin-4-yl)methyl)(methyl)amino)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)carbamate 9 (700 mg, 0.54 mmol) in acetonitrile (3.5 mL, 10 Vol) at room temperature . The resultant reaction mixture was stirred at room temperature for 4 h. After completion of the reaction, reaction mixture was concentrated under reduced pressure to get crude (900 mg). The crude was triturated with cold EtOAc (25 mL), the resultant solid was dissolved in 10% MeOH in DCM (25 mL), diluted with diethyl ether (50 mL). The regenerated solid was filtered and dried under vacuum to afford N-((S)-6-amino-1-(((3-(2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrregeolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-5-yl)pyridin-4-yl)methyl)(methyl)amino)-1-oxohexan-2-yl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 10 (500 mg, yield: 86%) as a white solid. MS (ESI) m/z: 1056.53 $(M+H)^+$.

[000445] Step-8: Synthesis of 2,2',2''-(10-(2-(((S)-6-(((3-(2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-5-yl)pyridin-4-yl)methyl)(methyl)amino)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid: To a solution of N-((S)-6-amino-1-(((3-(2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-5-yl)pyridin-4-yl)methyl)(methyl)amino)-1-oxohexan-2-yl)-4-((4-(4-iodophenyl)butanamido)methyl)benzamide 10 (500 mg, 0.47 mmol)

in CH_3CN (2.5 mL, 10 Vol) at room temperature was added DIPEA (0.73 mL, 4.26 mmol) followed by 2,2',2''-(10-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid DOTA NHS ester (356 mg, 0.71 mmol) lot wise. Resultant reaction mixture was stirred at room temperature for 16 h. Upon completion of the reaction, reaction mixture was concentrated under reduced pressure to get residue. The residue was triturated with EtOAc (2 * 25 mL) and the isolated solid was dissolved in 10% MeOH in DCM (20 mL), diluted with diethyl ether (40 mL) to obtained solid, the solid was filtered to get crude material. The crude material was purified by Prep-HPLC. The required fractions were collected and concentrated under vacuum followed by lyophilization to afford 2,2',2''-(10-(2-(((S)-6-(((3-(2-(2-((3S,5S)-5-((S)-2-cyano-4,4-difluoropyrrolidine-1-carbonyl)-2-oxopyrrolidin-3-yl)acetyl)isoindolin-5-yl)pyridin-4-yl)methyl)(methyl)amino)-5-(4-((4-(4-iodophenyl)butanamido)methyl)benzamido)-6-oxohexyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (200 mg) as a white solid. MS (ESI) m/z: 1440.8 $(M-H)^-$. ^{1}H-NMR (400 MHz, DMSO-d6): δ 14-12 (brs, 1H), 8.6-8.5 (m, 2H), 8.5-8.4 (m, 2H), 8.4-8.3 (m, 2H), 8.1-8.0 (m, 1H), 7.9-7.8 (m, 2H), 7.7-7.60 (m, 3H), 7.51-7.2 (m, 6H), 7.1-7.0 (m, 2H), 5.12-5.0 (m, 1H), 4.9-4.8 (m, 3H), 4.8-4.6 (m, 4H), 4.5-4.4 (m, 1H), 4.4-4.3 (m, 4H), 4.3-4 (m, 5H), 3.9-3.8 (m, 4H), 3.6-3.5 (m, 5H), 3.3-3.2 (10 H), 3.2-3.0 (m, 10 H), 2.9-2.7 (m, 4H), 2.4-2.3 (m, 1H), 2.2-2.1 (m, 3H), 1.9-1.8 (m, 2H), 1.7-1.6 (m, 2H), 1.5 (m, 1H).

[000446] Synthesis of tert-butyl 5-(4-((methylamino)methyl)pyridin-3-yl)isoindoline-2-carboxylate (Int-4a):



[000447] Step-1A: Synthesis of 1-(3-bromopyridin-4-yl)-N-methylmethanamine: In a sealed tube, to a solution of 3-bromoisonicotinaldehyde 1A (5 g, 0.02 mol) in MeOH (50 mL) at room temperature was added CH_3NH_2 (1M in THF, 50 mL, 10 Vol). The reaction mixture was stirred for 2 h and added $NaBH_4$ (5.1 g, 0.13 mol). Resultant reaction mixture was stirred at room temperature for 16 h. After completion of reaction, the reaction mixture was concentrated under reduced pressure, then diluted with cold water (50 mL) and extracted with

ethyl acetate (3*100 mL). The organic layer was dried over sodium sulphate and concentrated under reduced pressure to get crude. Crude material was purified by silica gel column chromatography (60-120 mesh), using 5% MeOH:DCM as an eluent. The compound contained fractions were concentrated under reduced presser to afford 1-(3-bromopyridin-4-yl)-N-methylmethanamine 2A (4 g, 74%) as a yellow liquid MS (ESI) m/z: 203.19 $(M+H)^+$.

[000448] Step-1B: Synthesis of tert-butyl 5-(4-((methylamino)methyl)pyridin-3-yl)isoindoline-2-carboxylate: In a 100 mL seal tube, to a solution of 1-(3-bromopyridin-4-yl)-N-methylmethanamine 2A (2 g, 9.95 mmol) in *N,N*-dimethylformamide : water (20 mL) at room temperature was added Cs_2CO_3 (9.7 g, 29.8 mmol) and tert-butyl 5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoindoline-2-carboxylate 3A (3.4 g, 9.95 mmol). Mixture was purged with argon for 15 min and added Xphos Pd G4 (774 mg, 0.9 mmol). Resultant reaction mixture was stirred at 100 °C for 16 h. After completion of reaction, the mixture was filtered through celite pad, and filtrate was concentrated in vacuo, resultant residue was diluted with water (50 mL), extracted with ethyl acetate (3*100 mL). Organic layer was dried over sodium sulphate and concentrated under vacuum to get crude. Crude was purified by column chromatography (60-120 silica mesh) using 5% MeOH in DCM as an eluent. The compound contained fractions were concentrated under reduced presser to afford tert-butyl 5-(4-((methylamino)methyl)pyridin-3-yl)isoindoline-2-carboxylate 4A (1.2 g, 36%) as a brown liquid. MS (ESI) m/z: 340.21 $(M+H)^+$.

[000449] Synthesis (4-(4-iodophenyl)butanoyl)glycine

[000450] Step-1: Synthesis of tert-butyl (4-(4-iodophenyl)butanoyl)glycinate: To a solution of 4-(4-iodophenyl)butanoic acid SM-1 (5 g, 0.017 mol) in DMF (50 mL, 10 vol) at room temperature was added DIPEA (6 mL, 0.034 mol) followed by HATU (6.5 g, 0.017 mol). The reaction mixture was stirred for 10 min and added tert-butyl glycinate 1 (2.2 g, 0.01 mol). Resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was poured into ice water (250 mL) and extracted with diethyl ether (2 * 200 mL), organic layer was dried over sodium sulphate, concentrated under reduced pressure to get crude. Crude was dissolved in EtOAc (1 mL) and triturated with hexane (10 mL) to afford tert-butyl (4-(4-iodophenyl)butanoyl)glycinate 2 (4.5 g, yield: 65%) as a white solid.

[000451] Step-2: Synthesis of (4-(4-iodophenyl)butanoyl)glycine: TFA (20 mL, 4 Vol) was added to a solution of tert-butyl (4-(4-iodophenyl)butanoyl)glycinate 2 (5 g, 0.12 mmol) in DCM (50 mL, 10 Vol) at 0° C. The resultant reaction mixture was stirred at room temperature for 1 h. Upon completion of the reaction, reaction mixture was concentrated

under reduced pressure to afford crude . The crude was dissolved in EtOAc (100 mL) and triturated with hexane (2 * 50 mL) to afford (4-(4-iodophenyl)butanoyl)glycine (3 g, yield: 88%) as an off white solid. MS (ESI) m/z: 347.90 $(M+H)^+$.

[000452] Example 26. Preparation of ^{177}Lu-chelated compounds

[000453] Exemplary non-chelated/non-complexed compounds of the disclosure were each reconstituted in 1 mL of trace Select water at approximately (1 mg/mL) in a reaction vial, and to reaction vial was added 100 uL of buffer (0.2M NaOAc or 0.1M NH3OAc) pH 5.5-6. To reaction vial was added 3 µg of the exemplary compound, and the mixture was allowed to sit at room temperature for 15 minutes. An appropriate amount of stock [^{177}Lu]$LuCl_3$ solution in 0.01 N HCl (5 µL= 550 µCi) was added to the reaction vial, and the vial was placed on a thermomixer for 30 min. at 55° C, with mixing at 300 rpm. The reaction was monitored with instant thin layer chromatography (iTLC), using 0.1 M ammonium acetate, 50 mM EDTA, pH 5.5-6.0 at 30 min, and the reaction was analyzed with RP-HPLC, spiking vials with 10% 5mM EDTA solution. Table 1 describes the characterization conditions and results. The results demonstrate that most of the test articles radiolabeled with lutetium-177 at a reasonable initial radio-purity, which were then subsequently optimized through formulation and development.

Table 1. Radiolabeling conditions and results for ^{177}Lu-chelated compounds

Compound	**177Lu-Compound 15**	**177Lu-Compound 23**	**177Lu-Compound 13**	**177Lu-Compound 9**	**177Lu-Compound 18**	**177Lu-Compound 24**
Assay Time	11:18	11:19	14:10	14:12	14:05	14:07
Isotope Starting Activity (mCi)	9.5	9.5	9.4	7.0	5.17	5.17
Isotope Volume (µL)	24	24	24	18	12	12
Buffer Volume (µL)	80	80	80	80	60	60
TA Mass (µg)	25	25	25	17	9.8	9.8
TA Volume (µL)	25	25	25	17	9.8	9.8
Additional DMSO	20	20	20	20	30	30

Total Reaction Volume (µL)	149	149	149	135	112.8	112.8
Labeling Efficiency via HPLC (%)	96.1	95.0	92.8	97.7	98	96.3
Specific Activity at Assay Time (µCi/µg)	365	361	349	402	NA	NA
Purified Activity (µCi)	9.1	9.1	9.0	6.8	512	508
Radiochemical concentration (µCi/µL)	30.3	30.3	30	23	4.56	4.70
Radiochemical Purity (%)	96.1	95.0	92.8	97.7	6.51	6.7
Yield (%)	96.1	95.0	92.8	97.7	98	96.3
EOS Time	12:20	12:21	15:15	15:16	98	96.3

[000454] Characterization results for the ^{177}Lu-chelated compounds are further provided in the radio-reverse phase HPLC chromagraphs and the iTLC chromatograms depicted in **FIGS. 1A-19**.

[000455] Example 27. Pharmacokinetic studies of exemplary compounds of the disclosure in male athymic nude mice

[000456] Male athymic nude mice were administered exemplary compounds of the disclosure. Exemplary dose administration details are provided in Table 2 (the same procedures were followed for all tested compounds), and the sample collection details are provided in Table 3.

Table 2. Exemplary dose administration for pharmacokinetic studies

Group	Number of Animals	Compound ID	Vehicle	Dose Level (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Dose Route
1	6 (n=3/cohort)	Compound 7	PBS	1	0.2	5	IV
2	6 (n=3/cohort)	Compound 8	PBS	1	0.2	5	IV
3	6 (n=3/cohort)	Compound 9	PBS	1	0.2	5	IV

IV = intravenous

Table 3. Sample collection details for pharmacokinetic studies

Group Number	Blood Collection Schedule (h)
1 – 3 Cohort A (n=3/group/cohort)	0.0833, 0.5, 3, 12 and 48 hours post dose
1 – 3 Cohort B (n=3/group/cohort)	0.25, 1, 6 and 24 hours post dose
Sample Volume/Tube Size/Additive	~45µL in K_2EDTA tubes

[000457] Results of the pharmacokinetic studies are provided in Table 4.

Table 4. Pharmacokinetic results

Mouse PK	Cmpd 4	Cmpd 7	Cmpd 8	Cmpd 9	Cmpd 13	Cmpd 15	Cmpd 23	Cmpd 24
$AUC_{(0\text{-}48\ hours)}$ (h*ng/mL)	47,406	19,984	62,105	70,531	123,048	72,046	127,471	131,728
t(½) (hours)	8.05	5.03	7.41	6.04	9.47	7.65	9.00	14.2

Mouse PK	Compound 2	Compound 5	Compound 10	Compound 12	Compound 18	Compound 21	Compound 22
$AUC_{(0\text{-}48\ hours)}$ (h*ng/mL)	10,661	57,134	17,139	18,028	11,080	10,150	62,944
t(½) (hours)	1.08	6.11	7.79	7.55	3.19	1.68	7.69

Mouse PK	Compound 1	Compound 3	Compound 6	Compound 17	Compound 19	Compound 20
$AUC_{(0\text{-}48\ hours)}$ (h*ng/mL)	7,549	10,864	22,076	1,428	4,878	48,894
t(½) (hours)	-	3.53	4.53	0.97	7.64	

[000458] **Example 28. Dialysis studies of human plasma protein binding for exemplary compounds of the disclosure**

[000459] Dialysis studies were conducted to investigate the human plasma protein binding of exemplary compounds of the disclosure.

[000460] On the day of the experiment, frozen human plasma was thawed under running cold tap water until the pellet disappeared, and then placed at room temperature for

use. The plasma was centrifuged at 3220 ×g for 5 min to remove the clots. The pH of the resulting plasma was measured and adjusted to 7.4 ± 0.1 using 1% phosphoric acid or 1 M sodium hydroxide, as required.

[000461] Dialysis membrane strips (molecular weight cut off 12-14 KDa) were soaked in ultra pure water at room temperature for approximately 1 hr. After that, each membrane strip that containing two membranes was separated and soaked in ethanol:water (20:80 v:v) for approximately 20 min, after which the membranes were ready for use or were stored in the solution at 2-8°C for up to 1 month. Prior to the experiment, the membranes were rinsed and soaked for 20 min in ultra pure water for use.

[000462] Aliquots of 50 µL loading matrix (2 µM test compound) or control compound (2 µM warfarin) in triplicate were transferred to dialysis sample collection plate, respectively. The samples were matched with opposite blank buffer to obtain a final volume of 100 µL with a volume ratio of matrix:Dialysis Buffer (1:1, v:v) in each well immediately. The stop solution (500 µL of acetonitrile containing tolbutamide at 250 nmol/L and labetalol at 250 nmol/L) was added to these T_0 samples of test compound and control compound. The plate was sealed and shaken at 800 rpm for 10 min. Then these T_0 samples were stored at 2-8°C pending further processing along with other post-dialysis samples.

[000463] An aliquot of 100 µL of the loading matrix containing test compound or control compound was transferred to the donor side of each dialysis well in triplicate and 100 µL of the dialysis buffer (100 mM sodium phosphate and 150 mM NaCl, pH 7.4 ± 0.1) was loaded to the receiver side of the well. Then the plate was rotated at approximately 100 rpm in a humidified incubator with 5% CO_2 at 37±1°C for 4 hours.

[000464] At the end of the dialysis, aliquots of 50 µL samples from the buffer side and matrix side of the dialysis device were taken into new 96-well plates (Sample Collection Plates). An equal volume of opposite blank matrix (buffer or matrix) in each sample was added to reach a final volume of 100 µL with volume ratio of matrix : Dialysis Buffer at 1:1 (v:v) in each well. Stop solution (500 µL) was added to these samples. The mixture was vortexed and centrifuged at 4000 rpm for about 20 minutes. An aliquot of 100 µL of supernatant of all the samples was then removed for LC-MS/MS analysis.

[000465] The %Unbound, %Bound and %Recovery were calculated using the following equations:

$$\%\text{Unbound} = 100 \times F / T$$

$$\%\text{Bound} = 100 - \%\text{Unbound}$$

$$\%\text{Recovery} = 100 \times (F + T) / T_0$$

F = the peak area ratio of compound and internal standard on the buffer side of the membrane after 4 hours of incubation

T = the peak area ratio of compound and internal standard on the plasma side of the membrane after 4 hours of incubation

T_0 = the peak area ratio of compound and internal standard at time zero

[000466] Results of the plasma protein binding studies are provided in Table 5.

Table 5. Plasma protein binding dialysis results

Human PPB	Cmpd 4	Cmpd 7	Cmpd 8	Cmpd 9	Cmpd 13	Cmpd 15	Cmpd 23	Cmpd 24
% Bound	94.34	97.88	98.62	97.95	97.31	97.32	99.07	99.21
% Remaining @ 6 hours	111.00	81.83	89.18	55.02	83.23	91.19	89.16	48.52

Human PPB	Compound 2	Compound 5	Compound 10	Compound 12	Compound 18	Compound 21	Compound 22
% Bound	74.27	92.14	99.75	94.21	>99.99	83.56	91.10
% Remaining @ 6 hours	88.44	113.24	82.28	80.49	84.85	88.74	80.14

Human PPB	Compound 1	Compound 3	Compound 6	Compound 17	Compound 19	Compound 20
% Bound	-	-	-	99.39	97.61	97.20
% Remaining @ 6 hours	-	-	-	96.80	80.02	36.47

[000467] Example 29. Surface plasmon resonance binding to FAPα of exemplary compounds of the disclosure

[000468] Binding of exemplary compounds of the disclosure to human fibroblast activation protein α (FAPα) was investigated by surface plasmon resonance.

[000469] Human FAPα was covalently immobilized on CM5 chip, with an immobilization level 3500 RU. The experiments used 20 mM Tris pH 7.5, 150 mM NaCl, 0.05% Tween 20, and 1% DMSO as buffer. Covalent immobilization via primary amine-groups on CM5 chip was accomplished using EDC/NHS coupling chemistry. 30 µg/ml Human FAPα Proteros construct was injected at pH 4.5 for 120 sec.

[000470] Exemplary compounds of the disclosure were injected at 8 concentrations, 3-fold dilution series, 100 nM top concentration.

[000471] Measurements were made in single-cycle kinetics (SCK) mode, as slow off rate was expected. In SCK, analyte is injected at increasing concentration without dissociation in between cycles, followed by a long dissociation at the end of injection cycle.

[000472] The surface plasmon resonance results are provided in Table 6.

Table 6. Surface plasmon resonance binding assay results

Human SPR	Cmpd 4	Cmpd 7	Cmpd 8	Cmpd 9	Cmpd 13	Cmpd 15	Cmpd 23	Cmpd 24
K_d (pM)	63.5	40.4	57.6	< 1	< 1	< 1	11	4
K_{off} (1/s)	2.1E-4	1.6E-4	8.8E-5	7.0E-08	1.1E-6	5.8E-8	6.2E-5	2.6E-6

Human SPR	Compound 2	Compound 5	Compound 10	Compound 12	Compound 18	Compound 21	Compound 22
K_d (pM)	< 1	30.6	14	20	238	48	11
K_{off} (1/s)	1.1E-9	5.5E-5	1.9E-2	1.2E-4	9.3E-6	1.2E-4	2.2E-2

Human SPR	Compound 1	Compound 3	Compound 6	Compound 17	Compound 19	Compound 20
K_d (pM)	75	331	21.8	10	27	16
K_{off} (1/s)	4.0E-5	2.8E-4	3.2E-5	2.0E-4	9.1E-5	9.6E-5

[000473] Example 30: Mouse xenograft tumor growth inhibition studies of the efficacy of ^{177}Lu-chelated radioligands

[000474] Female athymic nude mice (n = 150) were received from Charles River Laboratories (Hollister, CA). Upon arrival at the laboratory, each animal was inspected upon transfer to caging, and given a unique identifier. The animals were judged to be in good health and began their acclimation period. At study initiation, n = 100 animals were enrolled; the animals were 8 weeks old. All animals were acclimated for a minimum of 48 hours. During acclimation and study periods, the animals were socially housed in polycarbonate Innovive IVC® disposable cages containing sterile absorbent bedding in an environmentally-monitored room. A cage card was affixed to the cage displaying unique barcodes and the permanent animal number. All animals received unique IDs upon arrival tracked by ear punch, ear tag or tail mark using permanent marker. The animals were observed daily for any abnormalities indicative of health problems. Only healthy animals were placed on the study.

The animal housing rooms were integrated with a computerized environmental monitoring system to monitor and control room temperatures and light cycles. Room temperature was maintained at 72° ± 4° F and humidity was ambient. Animal rooms were maintained on 12 hour alternating light and dark cycles. Purina LabDiet® Pico 5053 was made available ad libitum throughout acclimation and the biological phase of the study. Upon arrival, the feed lot number and date were logged with the expiration date. Food was stored in a temperature- and humidity-controlled room with the use date logged on the barrel. Feed was analyzed by the manufacturer for nutritional contents and any contamination that may have adversely affected the health of the animals. DietGel and hydrogel was added to all cages if one mouse in the study lost 10% of initial body weight or more. Water was purchased in pre-filled bottles from Innovive (Aquavive® pre-filled water bottle). It was delivered double-bagged and irradiated. Water was provided ad libitum throughout acclimation and testing periods. There were no known contaminants in the food or water that would have adversely affected the results of this study.

[000475] All mice on study (n = 100) were inoculated with 5×10^6 U87MG cells. Mice were randomized into 10 groups and injected with exemplary ^{177}Lu-chelated compounds of the disclosure to determine the efficacious response. The injected dosages were 500 μCi/nmol and 150 μCi/nmol with vehicle control. All mice were monitored for up to 60 days with body weight and tumor volume measurements taken 3 times weekly. All cells were cultured in the Invicro cell culture suite under sterile conditions. U87MG cells at passage P5 were used for all inoculations. SC inoculations of cells for tumor generation were performed one animal at a time using the skin tent method. Once tumors were observed, caliper measurements and body weights were taken three times weekly. Per IACUC guidelines, maximum tumor volume size was 3000 mm^3. Animals bearing tumors that exceeded this limit were euthanized. Additionally, sponsor requested animals with two consecutive measurements greater than or equal to 1500 mm^3 be euthanized. Individual mice were euthanized prior to study completion if they exhibited any of the following:

- Two consecutive tumor measurements > 1500 mm^3
- A single tumor volume measurement > 3000 mm^3
- A decrease in body weight loss > 20% relative to Day -1 weights

[000476] The animals were dosed on Day 0 with body weight and tumor volume measurements occurring on Day 1. Tumor volumes at time of enrollment (Day 1) for individual animals ranged between 108 - 196 mm^3. Single intravenous (IV) doses were administered using a 1 mL insulin syringe in a dosage volume of 10 mL/kg. The dosing

syringe was assayed in a dose calibrator before and after injection. The activity in the emptied syringe was decay-corrected and subtracted from the loaded syringe to determine the actual activity administered to each subject. For sample preparation, tumors were excised and bifurcated. They were then dried and weighed with one half fixed for 48 hours in 10% NBF and then transferred to 70% EtOH following fixation. The other half was snap frozen in liquid nitrogen and then stored at -80 °C. Both the left and right kidneys were collected, along with the spleen, heart, and bladder. All organs were dried then weighed. They were then fixed for 48 hours in 10% NBF, then transferred to 70% EtOH following fixation. The bone marrow was collected, and bone marrow smears were performed (in triplicates) for determining myeloid:erythroid ratios.

[000477] The efficacy of the test articles was evaluated using tumor growth inhibition (%), which can be defined as stated in Equation 1:

$$TGI\ (\%) = \left[1 - \frac{T_i - T_0}{V_i - V_0}\right] \times 100\%$$

Where, T_i and T_0 = average tumor volume of the treatment group on the given day and when treatment started (i.e. Day -1), respectively
V_i and V_0 = average tumor volume of the vehicle group on the given day and when the treatment started, respectively.

[000478] The efficacy results are provided in Table 7.

Table 7. Efficacy results of *in vivo* studies

U87MG antitumor activity	Study 1			Study 2			
	177Lu Compound 4	177Lu Compound 7	177Lu Compound 9	177Lu Compound 9	177Lu Compound 13	177Lu Compound 15	177Lu Compound 23
Tumor growth inhibition (Day 13)	133%	127%	130%	86%	90%	84%	86%
Tumor growth inhibition (Day 20)	111%	111%	117%	92%	94%	94%	90%
Tumor growth inhibition (Day 27)	102%	103%	108%	93%	87%	88%	81%

[000479] Example 31: *In vivo* radioimaging and *ex vivo* biodistribution quantification in mouse xenograft model treated with ^{177}Lu-chelated radioligands

[000480] Twenty-four (n = 24) female athymic nude mice were received from Charles River Laboratories (Hollister, CA). Upon arrival at the laboratory, each animal was inspected prior to transferring to caging, and given a unique identifier. The animals were judged to be in good health and began their acclimation period. At study origination the animals were 9 weeks old. Acclimation, housing conditions, diet, and euthanasia criteria were the same as for the radiotherapy studies. Tumor cell preparation and inoculation were as described in Example 31.

[000481] Fifteen female athymic nude mice (n = 4 + 1 spare per group) with U87MG tumors on their right shoulder were enrolled into 3 groups, randomized by tumor volume, for the imaging study. On the first day of imaging each animal was weighed, and tumor volume measurements were taken. The tumor volume ranged from 177.8 – 270.1 mm^3 with an average tumor volume of 215.4 ± 8.2 mm^3 at enrollment. Body weight and tumor volume measurements were recorded at every imaging time point. Animals were dosed intravenously (IV) with Lutetium-177 radiolabeled compounds of the disclosure while awake until the first imaging time point, 3 hours post-injection, respectively. Following dose administration, animals underwent 30-minute whole body static SPECT scans at five time points in a 3-bed hotel. CT scans were acquired prior to SPECT scanning at all time points for anatomical reference. Each animal was weighed on the day of dose administration. The animals ranged in weight from 19.6 to 26.6 g with an average weight of 23.3 ± 0.5 g. Single intravenous (IV) doses were administered into awake animals using a 1 mL insulin syringe to provide a dosage volume range of 105 – 110 µL to achieve the target activity of 450 µCi. The dosing syringe was assayed in a dose calibrator before and after injection. The activity in the emptied syringe was decay-corrected and subtracted from the loaded syringe to determine the actual activity administered to each subject.

Table 8. SPECT Acquisition Parameters

SPECT Acquisition Parameters	
System	Nanoscan (Mediso, Inc.)
Scan Range	Whole body
Scan Duration & Time Points	30 min static @ 4, 24, 48, 96, 192hrs
Energy Window	Primary: 208.4 keV Secondary: 112.90 keV Tertiary: 56.10 keV
Number of Projections	180
Time Per Projection	~34 s
Axial Aperture FOV	17 (Apt. #63)

SPECT Reconstruction Parameters	
Regularization	High
Resolution	Low (64)
Iterations	Low (32)
Subset Size	Low (1)
Monte Carlo Quality	Medium
Attenuation Correction	Yes
Scatter Correction	Yes
CT Acquisition Parameters	
Projections	360
Scan Method	Helical
Pitch	1
Xray Energy (kVp)	50 kVp
Exposure Time (ms)	170 ms
Zoom	Maximum FOV
Binning	1:4
Current (μA)	980
Scan Duration	~3 min
CT Reconstruction Parameters	
Voxel Size	0.250 x 0.250 x 0.250 mm
Filter Type	Cosine

[000482] Animals were anesthetized by isoflurane gas for all imaging procedures. Animals were injected while awake and were allowed to move freely between injection and imaging.

- Induction: 4-5% isoflurane 0.5-1 L/min of medical grade O_2 or air
- Maintenance: 1-2% isoflurane 0.5 L/min of medical grade O_2 or air

[000483] Blood was collected after the 3h and 24h imaging time points. Approximately 40 μL of whole blood was collected via survival bleed from two non-sampled animals from each group at each time point for gamma counting analysis to verify the SPECT/CT quantification of the blood pool in the heart. Following the final imaging time point, approximately 100 μL of terminal whole blood was collected along with resected tumor and kidneys from all animals (n = 4 plus 1 spare) for ex vivo gamma counting analysis. Whole blood and kidneys were counted on the Perkin Elmer Wizard 2470 (Table 10) with tumors counted on the Perkin Elmer Wizard 2480 (Table 11) Gamma Counters. Tumors were resected with half placed in 10% Neutral Buffer Formalin (NBF) for 24 hours, then transferred to 70% EtOH and stored at 4 °C. The remaining half was weighed, and gamma counted, separately. Left and right kidneys were harvested, weighed, and gamma counted separately. Additional tissues that were resected included the kidneys, and following the final imaging time point they were placed in 10% NBF for 48 hours and then transferred to 70% EtOH and stored at 4 °C.

Table 9. Wizard 2470 – Gamma Counting Acquisition Parameters

Isotope Settings	
System	Wizard 2470 Gamma Counter
Manufacturer	Perkin Elmer
Isotope	Lu177
Counting Window Information	
Counting Window Type	Dynamic-keV
Low Boundary (keV)	42 keV
High Boundary (keV)	500 keV
Peak Position (keV)	113 keV
Window Coverage	74
Counting Spectrum Type	Many Peaks
Crosstalk Correction	Yes
Acquisition Protocol and Corrections	
Protocol ID	7
Counting Time	60s
Decay correction	Off
Background correction	Off

Table 10. Wizard 2480 – Gamma Counting Acquisition Parameters

Isotope Settings	
System	Wizard 2480 Gamma Counter
Manufacturer	Perkin Elmer
Isotope	Lu177
Counting Window Information	
Counting Window Type	Dynamic-keV
Low Boundary (keV)	42 keV
High Boundary (keV)	500 keV
Peak Position (keV)	113 keV
Window Coverage	74
Counting Spectrum Type	Many Peaks
Crosstalk Correction	Yes
Acquisition Protocol and Corrections	
Protocol ID	7
Counting Time	60s
Decay correction	Off
Background correction	Off

[000484] Reconstructed Images from the NanoScan SPECT/CTTM were generated in units of activity. Namely, the values assigned to the voxels (volume elements) comprising the 3D reconstructed SPECT images were in units of μCi. Reconstructed images were co-registered to one another, resampled to 0.2 mm^3 voxels, and cropped to a uniform size prior to analysis. Regions of interest (ROIs) were defined using VivoQuant software. The kidneys and heart were segmented as fixed volumed phantoms and registered using the CT for anatomical reference. The tumor and tail were manually segmented based on relevant anatomy in the CT. Fixed volume spheres (#) were used to create ROIs for the liver (2). Spheres were placed at appropriate anatomical locations based on CT. The bladder ROI was segmented using thresholding based on the SPECT signal, if no SPECT signal was present a

fixed volume sphere was placed in the bladder using the CT as anatomical reference. Group and individual master spreadsheets were generated which included the volume, activity, and concentration (Activity/Volume) at each time point for each ROI generated. Additionally, plots of activity were generated using Python tools to highlight trends in the data. Outputs of each region were plotted and reported in percent injected dose per gram (%ID/g) and regions which were fully segmented (tumor, tail, whole body) were additionally presented in percent injected dose (%ID). Tail correction was applied to all animals at all time points if any animal exhibited tail uptake greater than 5 %ID. Plots of body weights and tumor volumes measured manually in the laboratory were also created in the same manner as described above. Results were presented in units of percent injected dose (%ID) and percent injected dose per gram (%ID/g). The units were calculated the same way for both *in vivo* and *ex vivo* (gamma counting) results. The definition of these units can be found in the equations below.

- The %ID for each analyzed region from the *in vivo* imaging data or *ex vivo* gamma counted data can be defined as stated in *Equation 2:*

$$\%ID = \frac{Uptake}{Injected\ Dose} * 100$$

Where, Uptake = Radioactivity (µCi) in a particular ROI or gamma counting sample, decay-corrected to the time of injection.
Injected dose= Radioactivity (µCi) injected into the subject.

- The %ID/g for each analyzed region from the *in vivo* imaging data or *ex vivo* gamma counted data can be defined as stated in *Equation 3:*

$$\%\frac{ID}{g} = \frac{\frac{Uptake}{Injected\ Dose} * 100}{ROI\ weight}$$

Where, Uptake = Radioactivity (µCi) in a particular ROI or gamma counting sample, decay-corrected to the time of injection.
Injected dose= Radioactivity (µCi) injected into the subject.
Weight = For *in vivo*, this is the volume of the particular ROI in mL. For *ex vivo* samples, this is the sample weight of the gamma counted tissue in g. Assumption: tissue density of 1 g/mL.

[000485] After the pre-processing steps described above, individual maximum intensity projections (MIPs) were created with VivoQuant software for each subject at each time point and scaled in percent injected dose per gram (%ID/g). The SPECT was windowed at various ranges to highlight different regions of uptake. Images were then stitched together using Python tools to create montages of subject over time and time points over groups. The activity of each collected tissue was measured in units of counts per minute (CPM). Values were decay corrected to the time of injection and corrected for background radiation. A global efficiency factor for Lu-177 was used to convert counts to units of (µCi/CPM). Sample

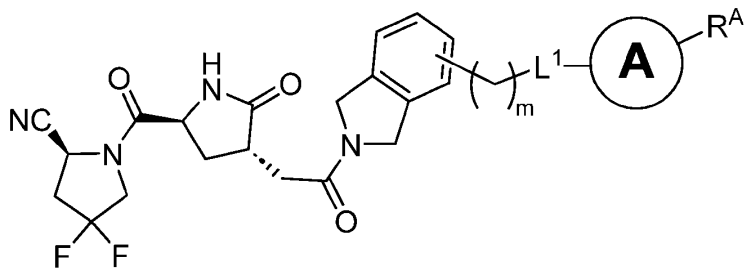
activities were corrected for background radiation and decay-corrected to the injection time. Tissue weights were used to calculate the reported concentration (%ID/g). The SPECT/CT scans are depicted in **FIGS. 20-27**. The activities of the collected tissues are described in Table 11.

Table 11. *Ex vivo* quantifications by gamma counter of selected ^{177}Lu–chelated compounds of the disclosure in U87MG tumor-bearing nude mice 8 days post-injection

Gamma counter %ID/g (Day 8)	177Lu Cmpd 4	177Lu Cmpd 7	177Lu Cmpd 8	177Lu Cmpd 9	177Lu Cmpd 13	177Lu Cmpd 15	177Lu Cmpd 23	177Lu Cmpd 24
Tumor	5.17%	1.23%	4.94%	7.38%	10.24%	9.97%	6.51%	20.49%
Kidneys	1.83%	0.27%	1.61%	1.38%	1.65%	1.43%	1.20%	5.62%
Blood	0.39%	0.00%	0.52%	0.75%	0.70%	0.63%	0.76%	4.69%

CLAIMS

1. A complex represented by Formula (I):



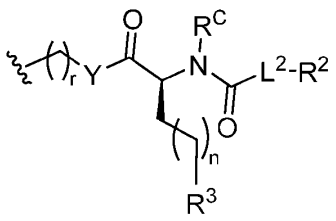
Formula (I)

or a pharmaceutically acceptable salt thereof, wherein:

ring A is selected from the group consisting of 3 to 6-membered heterocyclyl, 6-membered heteroaryl, and C_{3-6} cycloalkyl;

L^1 is selected from a covalent bond and a linker;

R^A is a moiety represented by:



Y is a covalent bond or NR^D;

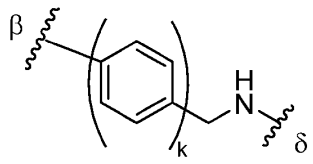
R^C is selected from hydrogen and C_1-C_3 alkyl;

R^D is selected from hydrogen and C_1-C_3 alkyl;

L^2 is selected from the group consisting of:

a covalent bond, and

a moiety represented by:



wherein β represents the point of attachment to the carbonyl group and δ represents the point of attachment to R^2;

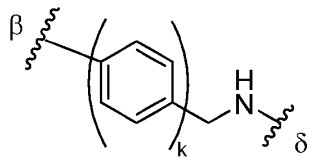
each of R^2 and R^3 is independently selected from an albumin binding ligand and a ligand chelated or covalently linked to a therapeutic radionuclide or a diagnostic radionuclide, wherein at least one of R^2 and R^3 is a therapeutic radionuclide or a diagnostic radionuclide;

each of k, m, and r is independently 0 or 1; and

n is an integer from 1 to 6;

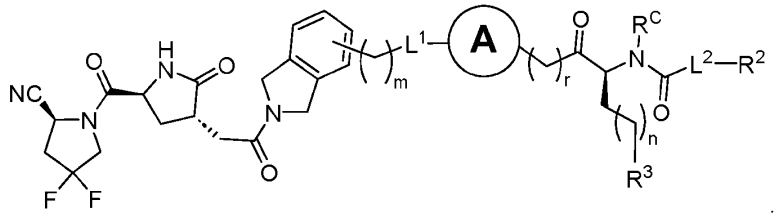
provided that:

when L^1 is a covalent bond, m is 1, L^2 is a moiety represented by:



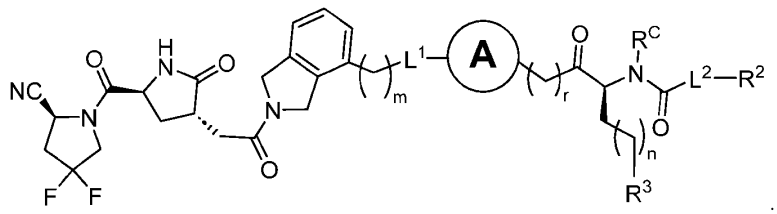
, and A is piperazinyl having a ring nitrogen attached to L^1, then k is 0.

2. The complex of claim 1, wherein the complex of Formula (I) is represented by Formula (I-A):



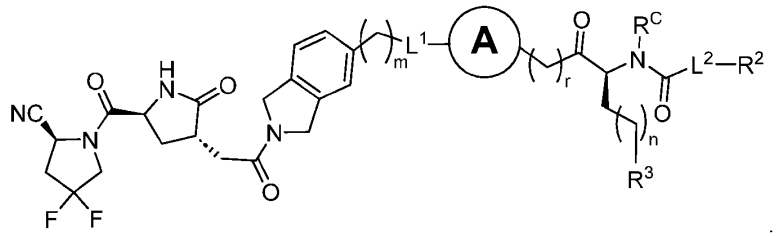
Formula (I-A)

3. The complex of claim 1, wherein the complex of Formula (I) is represented by Formula (I-B):



Formula (I-B)

4. The complex of claim 1, wherein the complex of Formula (I) is represented by Formula (I-C):



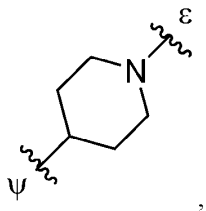
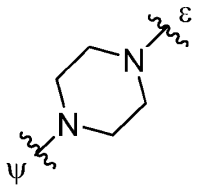
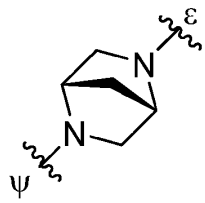
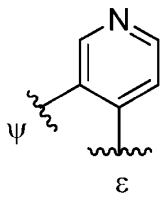
Formula (I-C)

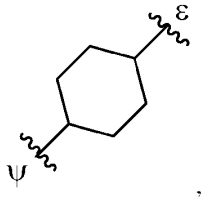
5. The complex of any one of claims 1-4, wherein ring A is 6-membered heterocyclyl.

6. The complex of any one of claims 1-4, wherein ring A is 6-membered heteroaryl.

7. The complex of any one of claims 1-4, wherein ring A is cyclohexyl.

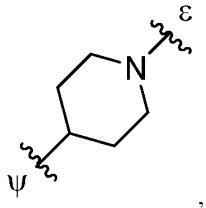
8. The complex of any one of claims 1-4 wherein ring A is selected from the group consisting of:





, and


,

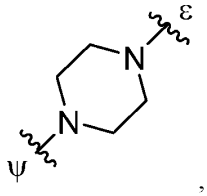
wherein each ψ represents the point of attachment to L^1, and each ε represents the point of attachment to R^A.

9. The complex of any one of claims 1-4 and 8, wherein ring A is:


,

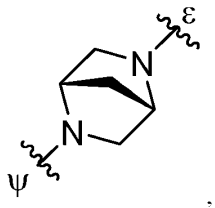
wherein ψ represents the point of attachment to L^1, and ε represents the point of attachment to R^A.

10. The complex of any one of claims 1-4 and 8, wherein ring A is:


,

wherein ψ represents the point of attachment to L^1, and ε represents the point of attachment to R^A.

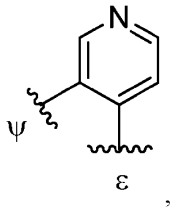
11. The complex of any one of claims 1-4 and 8, wherein ring A is:



,

wherein ψ represents the point of attachment to L^1, and ε represents the point of attachment to R^A.

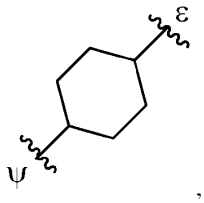
12. The complex of any one of claims 1-4 and 8, wherein ring A is:



,

wherein ψ represents the point of attachment to L^1, and ε represents the point of attachment to R^A.

13. The complex of any one of claims 1-4 and 8, wherein ring A is:



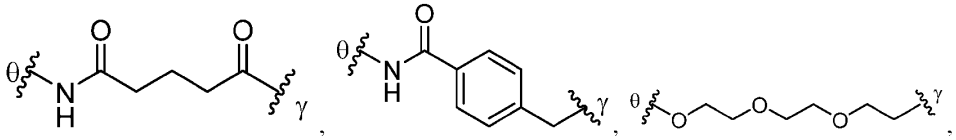
,

wherein ψ represents the point of attachment to L^1, and ε represents the point of attachment to R^A.

14. The complex of any one of claims 1-13, wherein L^1 is a covalent bond.

15. The complex of any one of claims 1-13, wherein L^1 is a linker.

16. The complex of any one of claims 1-13 and 15, wherein L^1 is a linker selected from the group consisting of:



and -O-,

wherein each θ is the point of attachment to the methylene group and each γ is the point of attachment to ring A.

17. The complex of any one of claims 1-16, wherein m is 0.

18. The complex of any one of claims 1-16, wherein m is 1.

19. The complex of any one of claims 1-18, wherein n is 3.

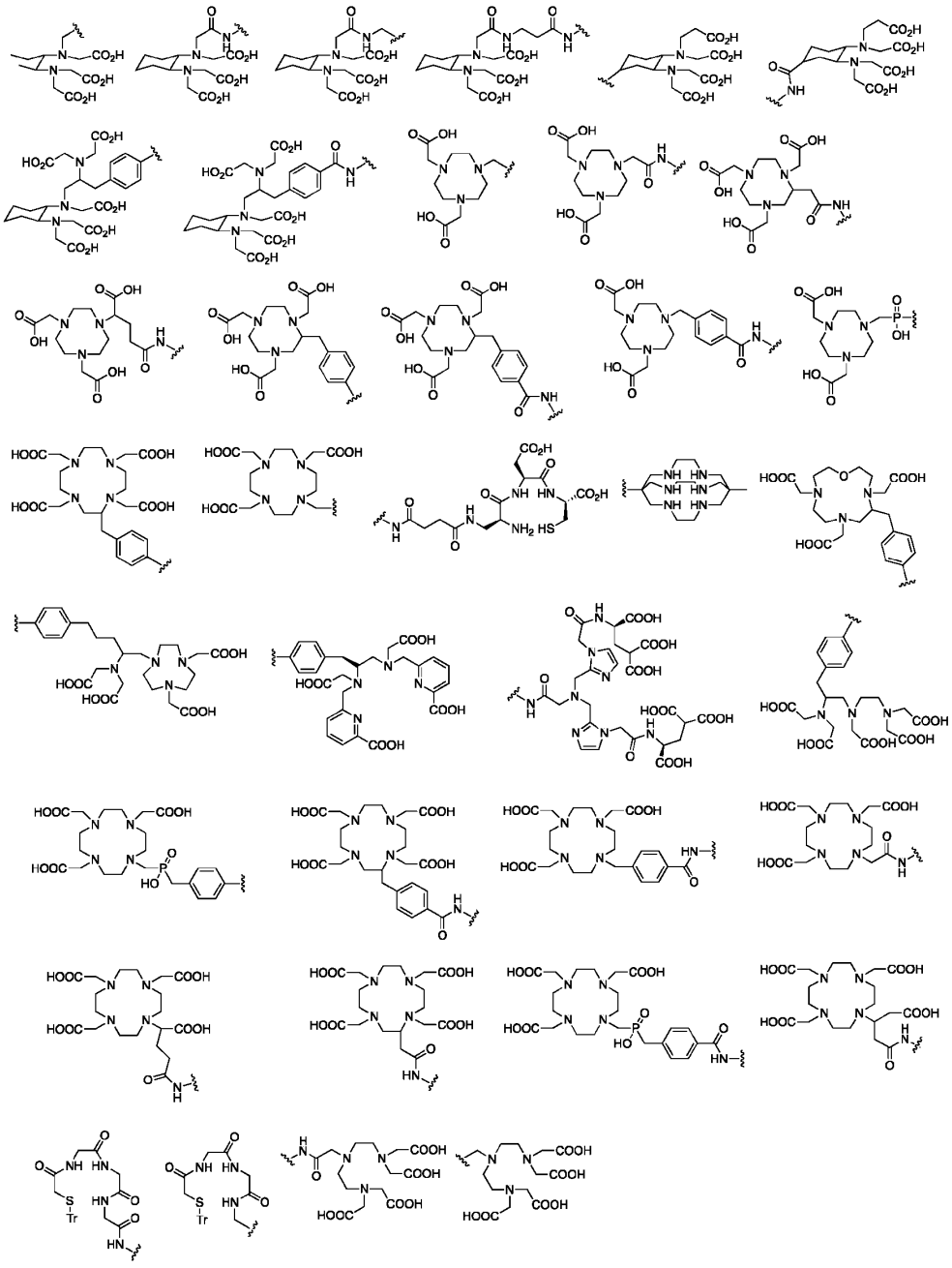
20. The complex of any one of claims 1-19, wherein r is 0.

21. The complex of any one of claims 1-19, wherein r is 1.

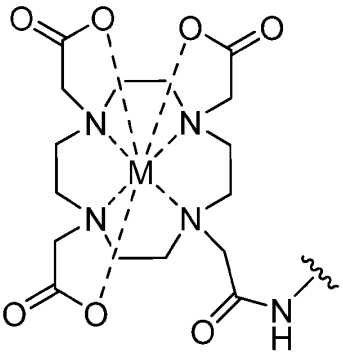
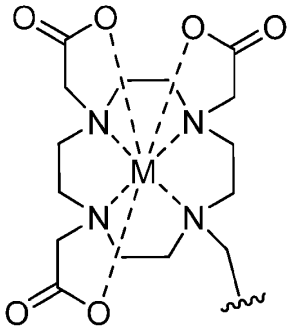
22. The complex of any one of claims 1-21, wherein k is 0.

23. The complex of any one of claims 1-21, wherein k is 1.

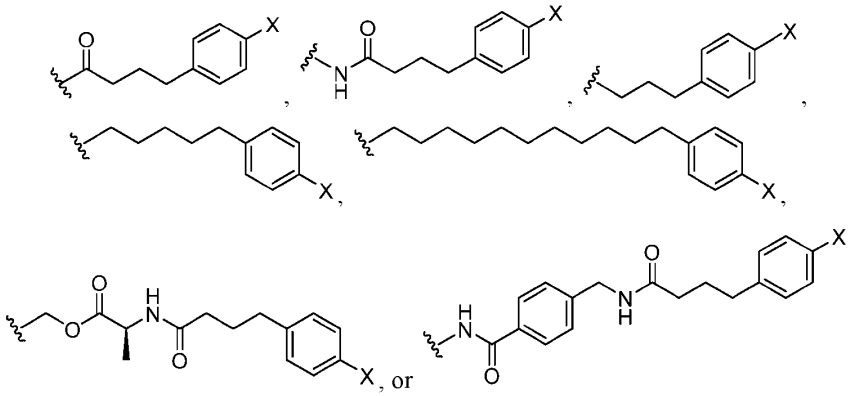
24. The complex of any one of claims 1-21 wherein one of R^2 and R^3 is a ligand chelated to or covalently linked to a therapeutic radionuclide or a diagnostic radionuclide, wherein the ligand is selected from the group consisting of:



25. The complex of any one of claims 1-21 and 24, wherein one of R^2 and R^3 is a ligand chelated to or covalently linked to a therapeutic radionuclide or a diagnostic radionuclide, wherein the therapeutic radionuclide or the diagnostic radionuclide is represented by:

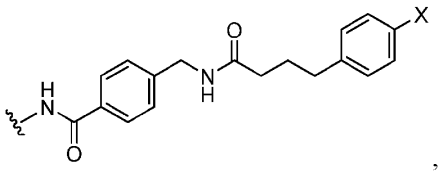

or

, wherein M is a therapeutic radionuclide or a diagnostic radionuclide.

26. The complex of any one of claims 1-21, wherein one of R^2 and R^3 is an albumin binding ligand represented by:


, or

wherein X is selected from the group consisting of halogen and C_{1-6} alkyl.

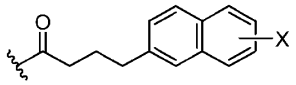
27. The complex of any one of claims 1-21, wherein one of R^2 and R^3 is an albumin binding ligand represented by:



,

wherein X is selected from hydrogen, halogen, and C_{1-6} alkyl.

28. The complex of any one of claims 1-21, wherein one of R^2 and R^3 is an albumin binding ligand represented by:

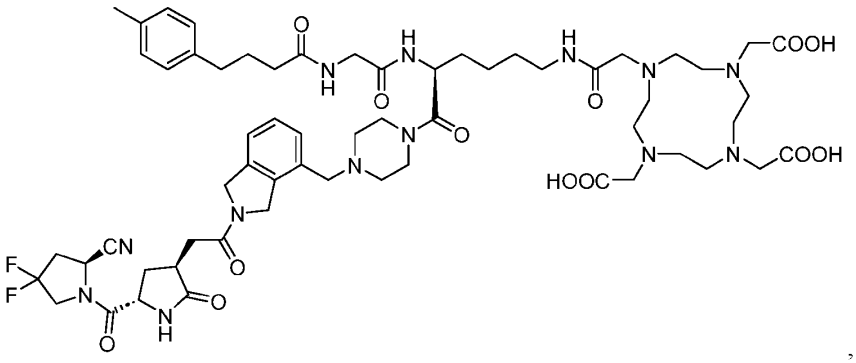


wherein X is selected from the group consisting of hydrogen, halogen and C_{1-6} alkyl.

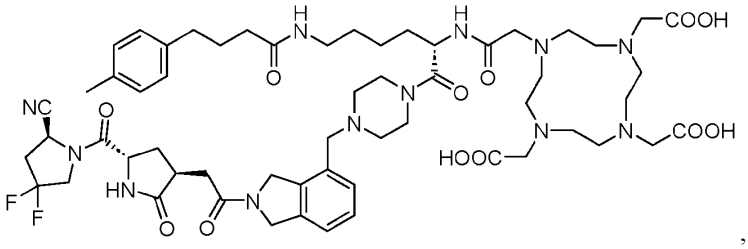
29. The complex of any one of claims 1-28, wherein R^C is hydrogen.

30. The complex of any one of claims 1-29, wherein when one of R^2 and R^3 is the albumin binding ligand, the other is the ligand chelated to or covalently linked to a therapeutic radionuclide or a diagnostic radionuclide.

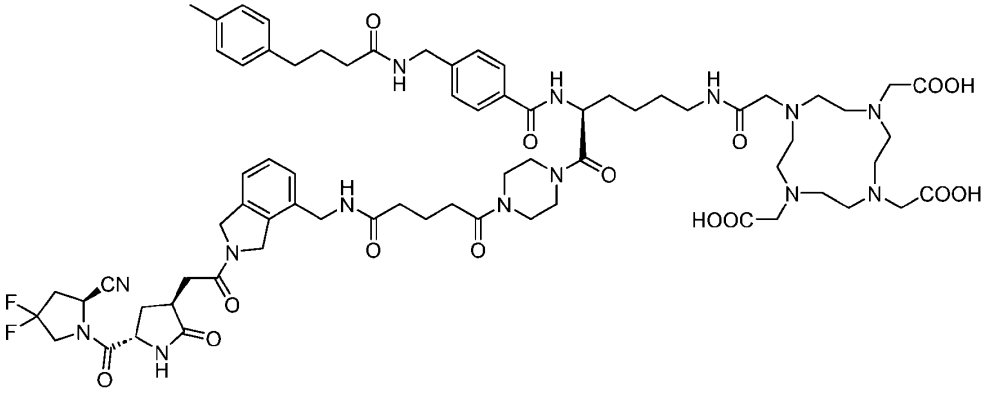
31. A complex of a compound selected from the group consisting of



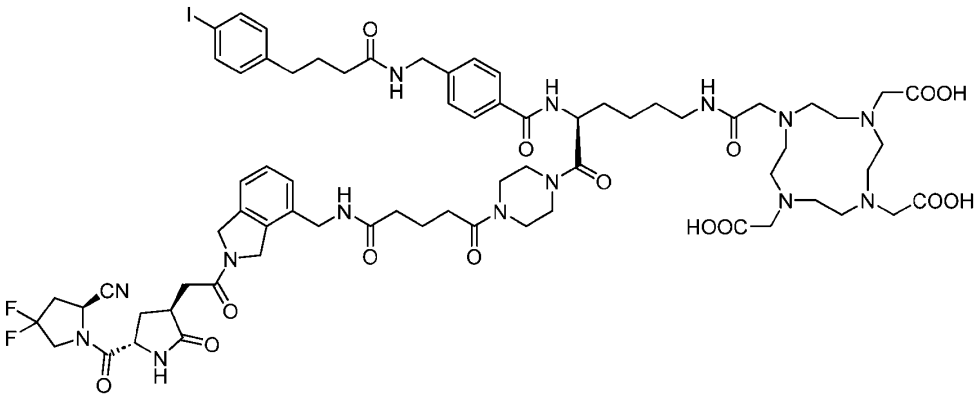
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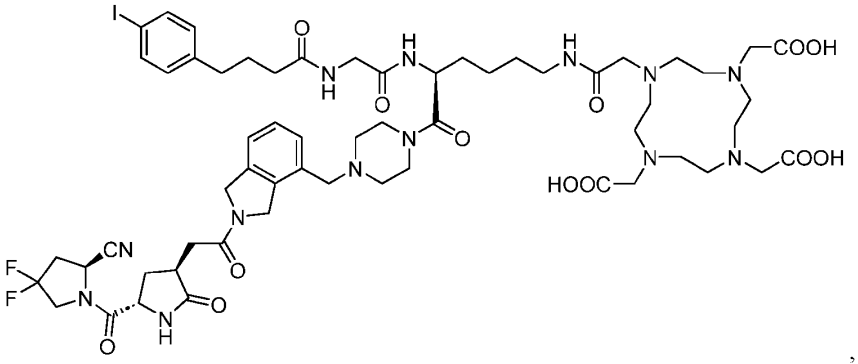
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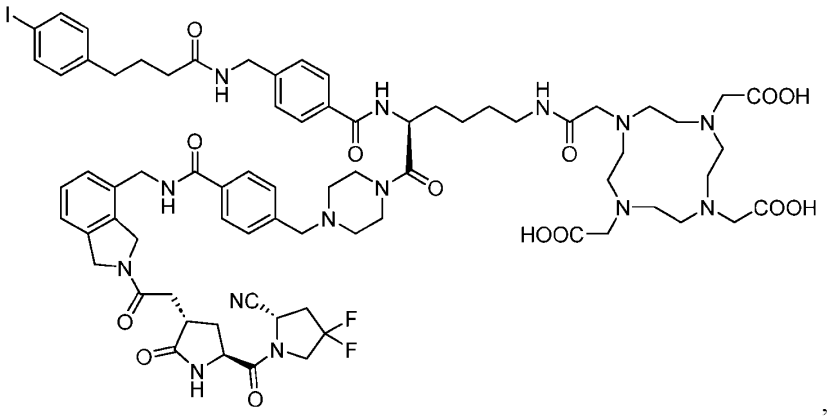
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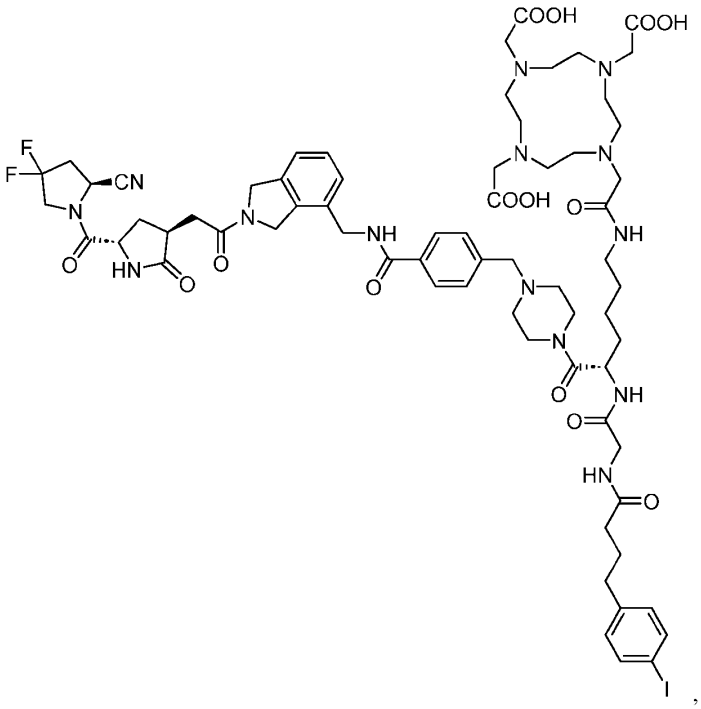
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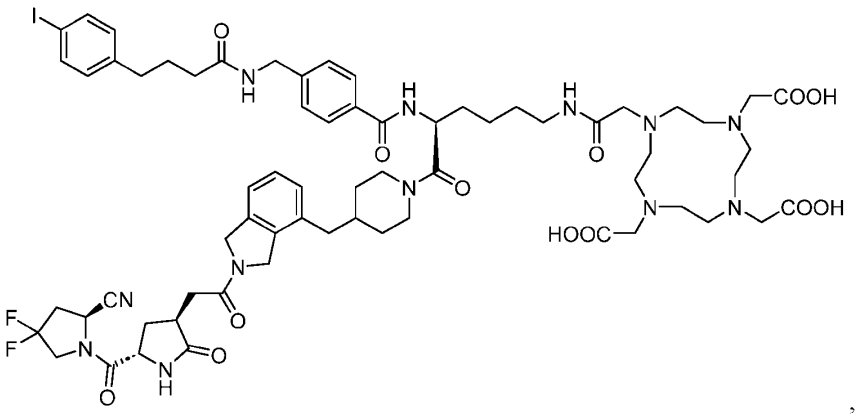
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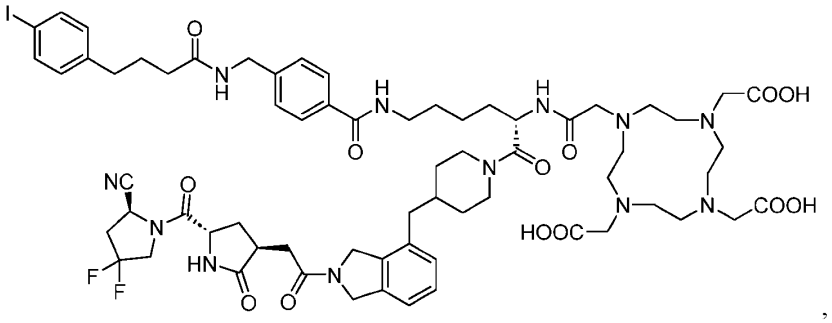
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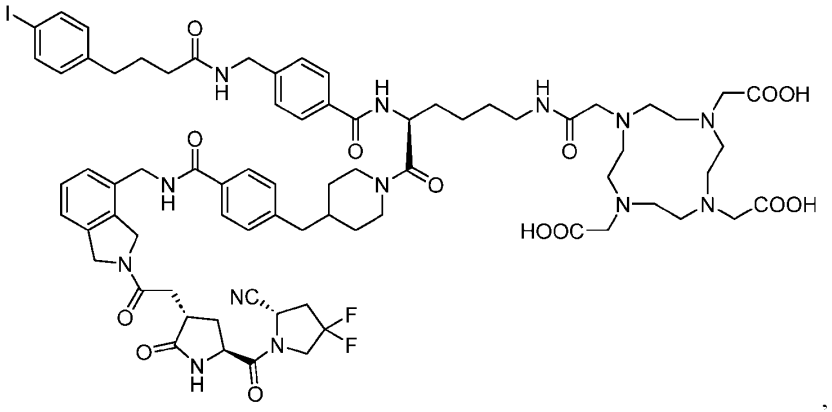
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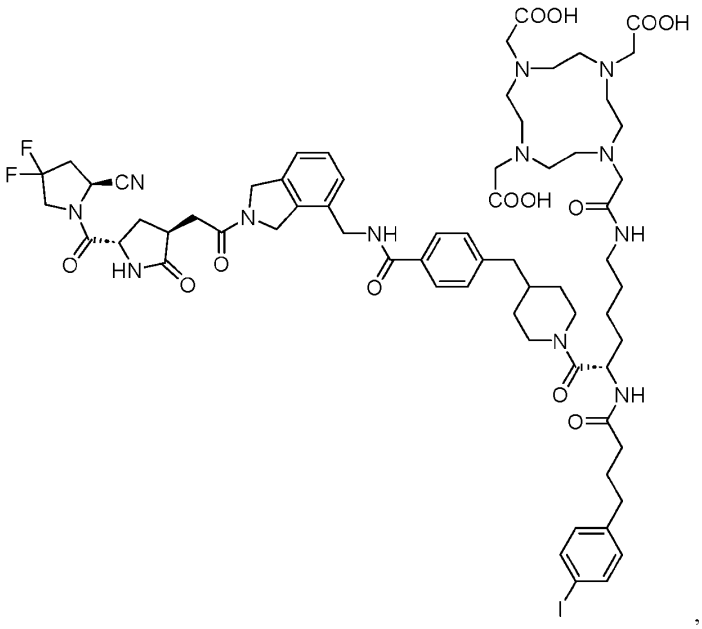
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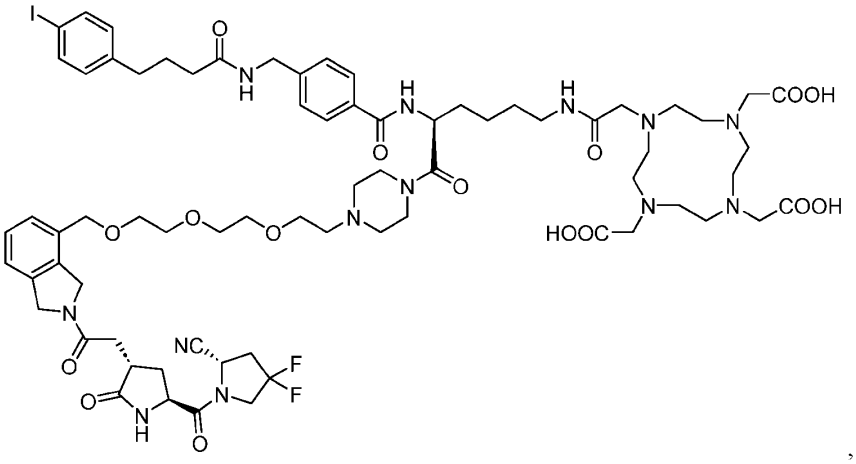
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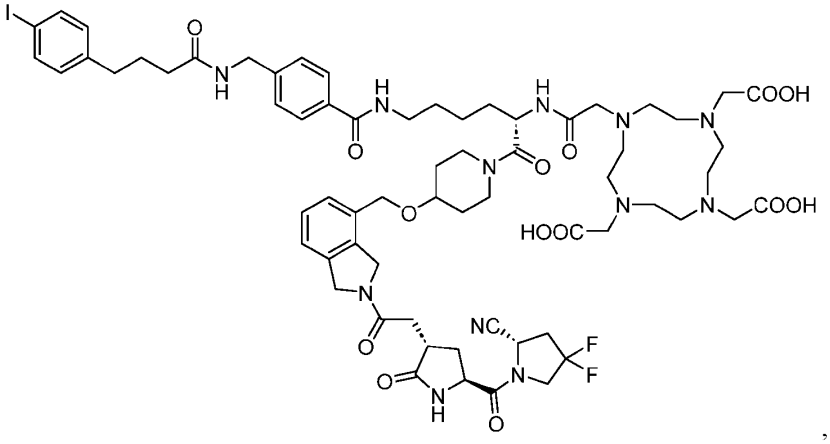
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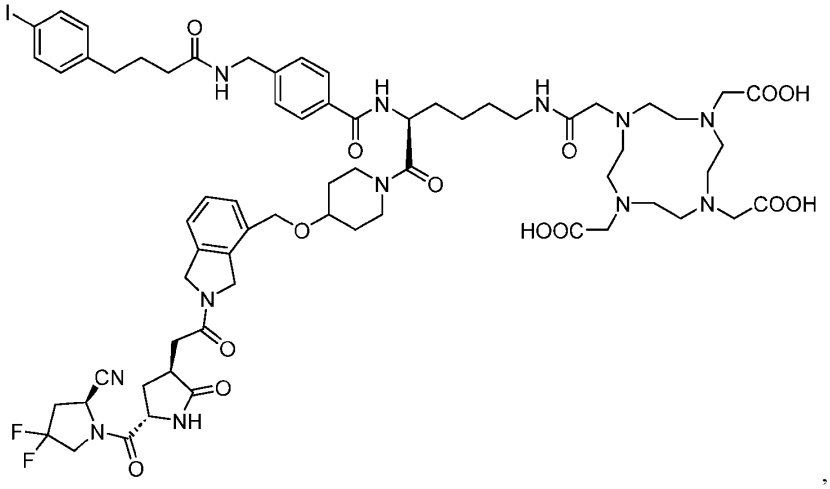
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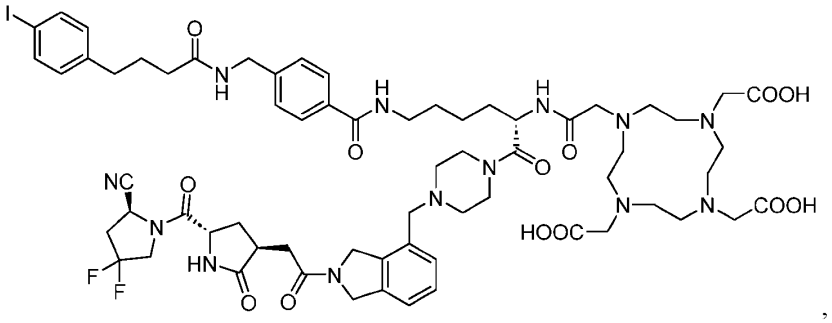
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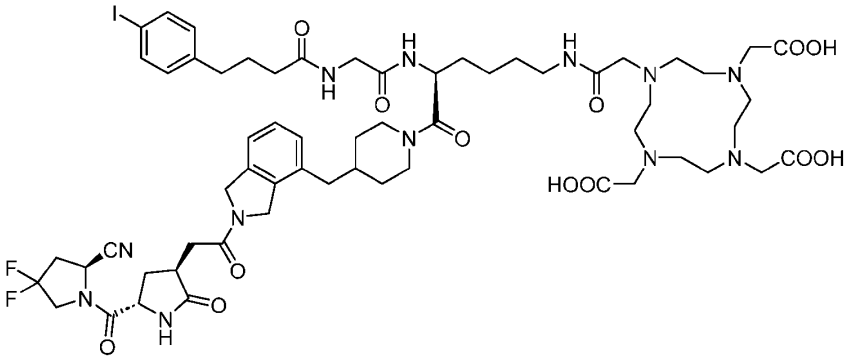
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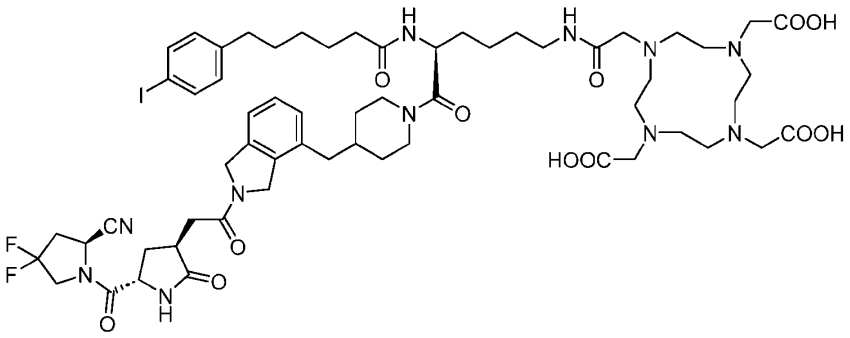
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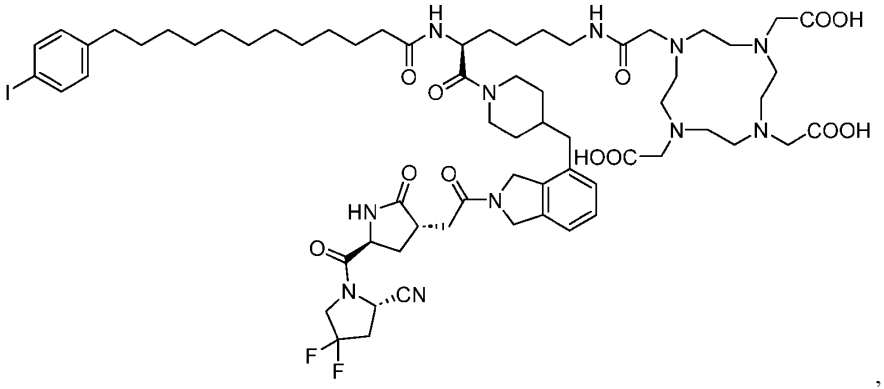
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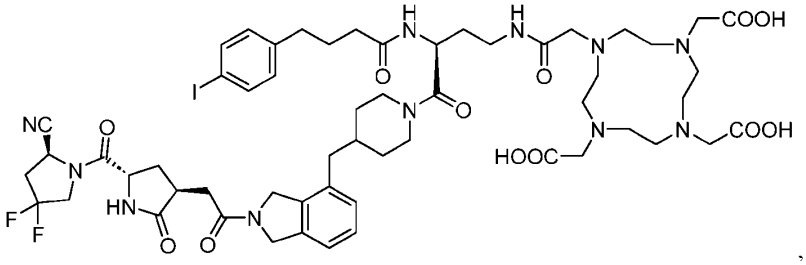
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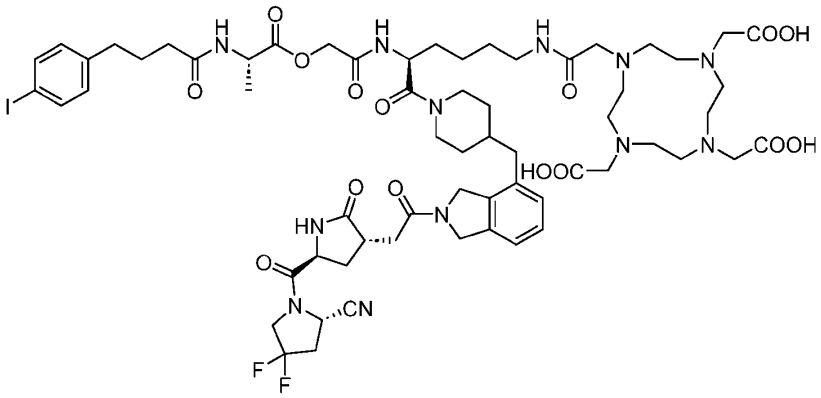
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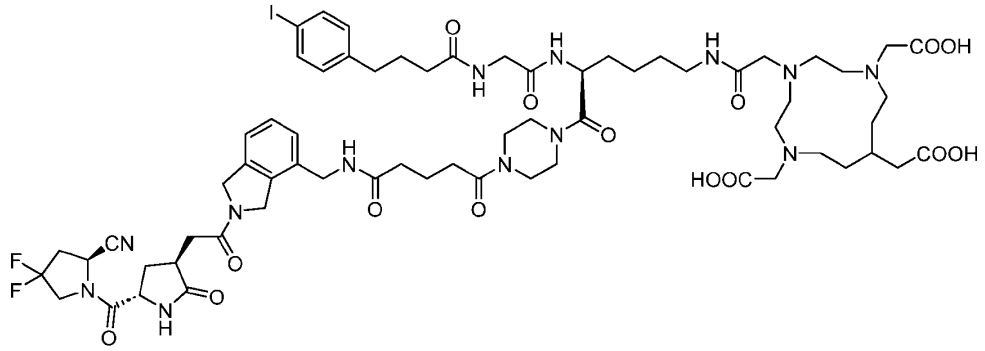
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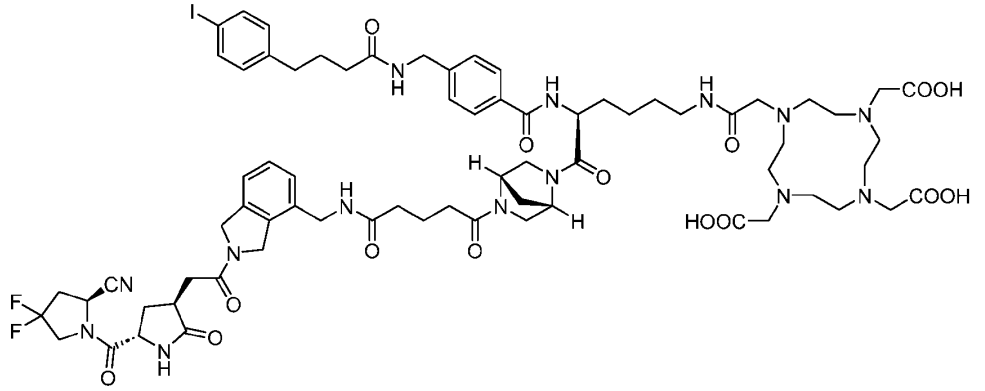
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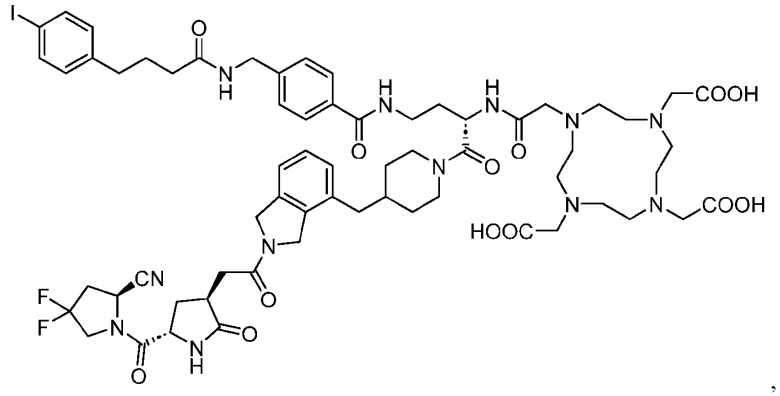
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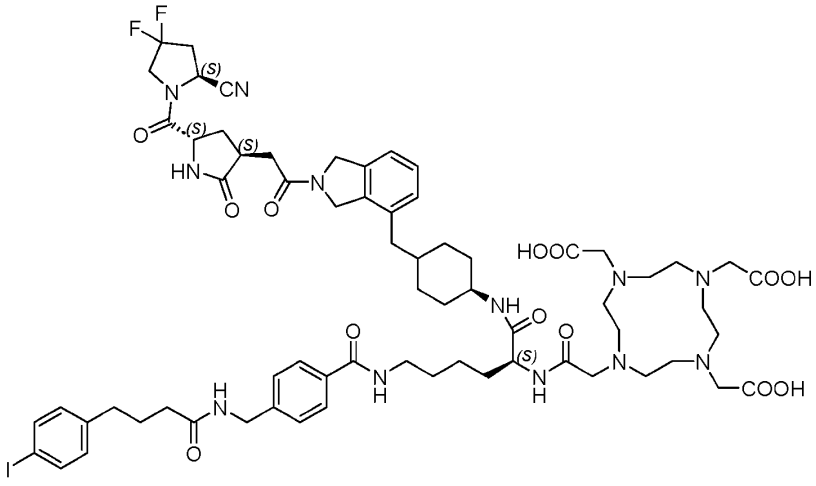
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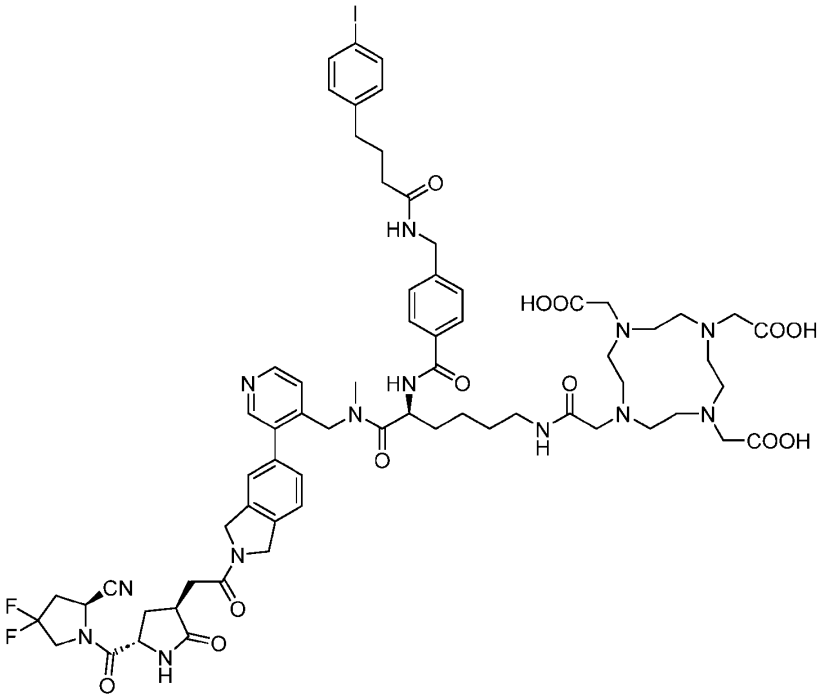
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, and



, or a pharmaceutically acceptable salt thereof; and

a therapeutic radionuclide or a diagnostic radionuclide.

32. The complex of any one of claims 1-31, wherein the therapeutic radionuclide is selected from the group consisting of ^{177}Lu, ^{90}Y, ^{155}Tb, ^{161}Tb, ^{225}Ac, ^{67}Cu, ^{223}Ra, ^{186}Re, ^{188}Re, ^{131}I, ^{212}Pb, ^{212}Bi, ^{213}Bi, and ^{211}At.

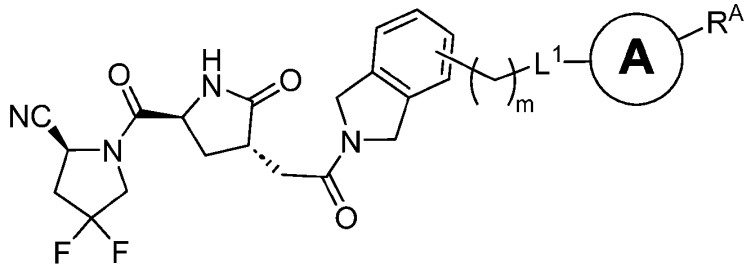
33. The complex of any one of claims 1-32, wherein the therapeutic radionuclide is ^{177}Lu.

34. The complex of any one of claims 1-31, wherein the diagnostic radionuclide is selected from the group consisting of ^{99m}Tc, ^{111}In, ^{18}F, ^{66}Ga, ^{67}Ga, ^{68}Ga, ^{124}I, ^{125}I, ^{44}Sc, ^{47}Sc, ^{64}Cu, ^{52}Mn, ^{55}Co, ^{86}Y, ^{89}Zr, ^{149}Tb, ^{152}Tb, and ^{203}Pb.

35. The complex of any one of claims 1-31 and 34, wherein the diagnostic radionuclide is ^{111}In.

36. A pharmaceutical composition comprising the complex of any one of claims 1-35, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

37. A compound represented by Formula (I):



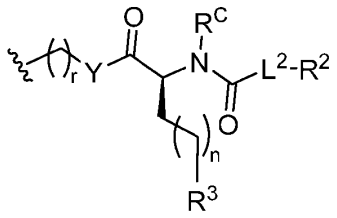
Formula (I)

or a pharmaceutically acceptable salt thereof, wherein:

ring A is selected from the group consisting of 3 to 6-membered heterocyclyl, 6-membered heteroaryl, and C_{3-6} cycloalkyl;

L^1 is selected from a covalent bond and a linker;

R^A is a moiety represented by:



Y is a covalent bond or NR^D;

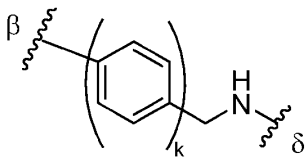
R^C is selected from hydrogen and C_1-C_3 alkyl;

R^D is selected from hydrogen and C_1-C_3 alkyl;

L^2 is selected from the group consisting of:

a covalent bond, and

a moiety represented by:



wherein β represents the point of attachment to the carbonyl group and δ represents the point of attachment to R^2;

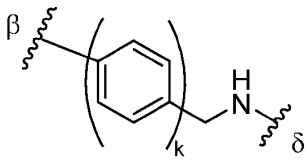
each of R^2 and R^3 is independently selected from an albumin binding ligand and an unchelated ligand capable of chelating to or covalently bonding to a therapeutic radionuclide or a diagnostic radionuclide wherein at least one of R^2 and R^3 is an unchelated ligand capable of chelating to or covalently bonding to therapeutic radionuclide or a diagnostic radionuclide;

each of k, m, and r is independently 0 or 1; and

n is an integer from 1 to 6;

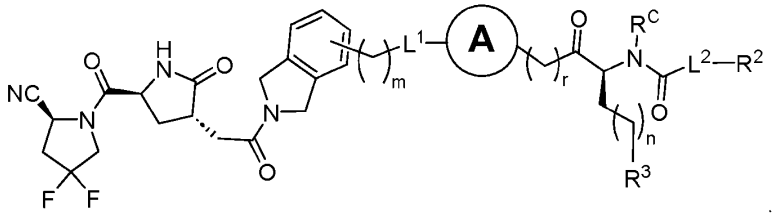
provided that:

when L^1 is a covalent bond, m is 1, L^2 is a moiety represented by:



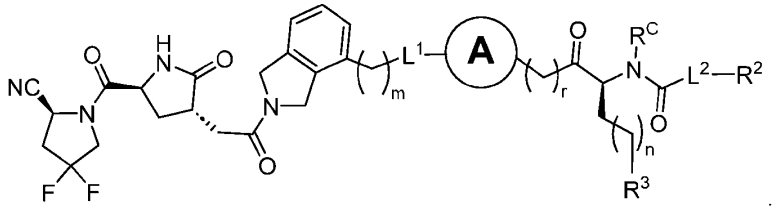
, and A is piperazinyl having a ring nitrogen attached to L^1, then k is 0.

38. The compound of claim 37, wherein the compound of Formula (I) is represented by Formula (I-A):



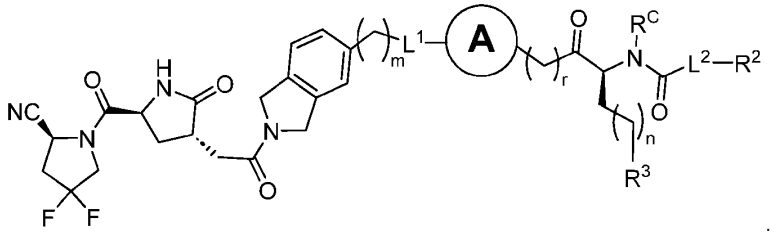
Formula (I-A)

39. The compound of claim 37, wherein the compound of Formula (I) is represented by Formula (I-B):



Formula (I-B)

40. The compound of claim 37, wherein the compound of Formula (I) is represented by Formula (I-C):



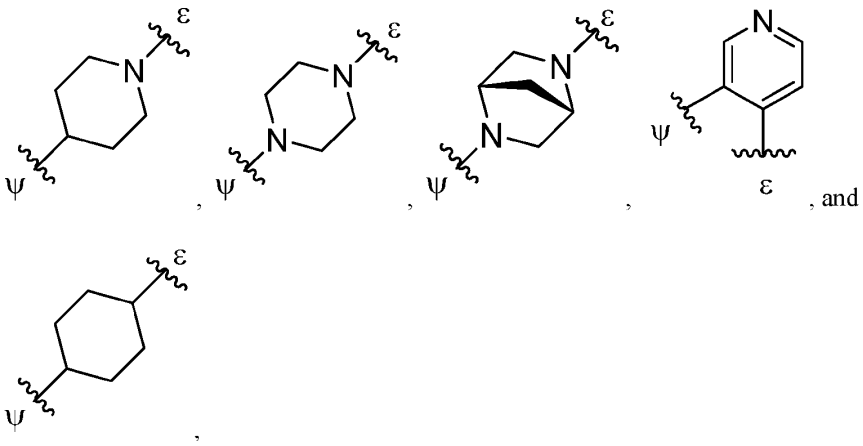
Formula (I-C)

41. The compound of any one of claims 37-40, wherein ring A is 6-membered heterocyclyl.

42. The compound of any one of claims 37-40, wherein ring A is 6-membered heteroaryl.

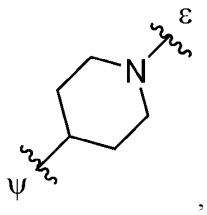
43. The compound of any one of claims 37-40, wherein ring A is cyclohexyl.

44. The compound of any one of claims 37-40, wherein ring A is selected from the group consisting of:



wherein each ψ represents the point of attachment to L^1, and each ε represents the point of attachment to R^A.

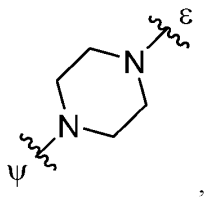
45. The compound of any one of claims 37-40 and 44, wherein ring A is:



,

wherein ψ represents the point of attachment to L^1, and ε represents the point of attachment to R^A.

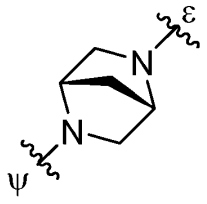
46. The compound of any one of claims 37-40 and 44, wherein ring A is:



,

wherein ψ represents the point of attachment to L^1, and ε represents the point of attachment to R^A.

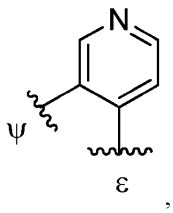
47. The compound of any one of claims 37-40 and 44, wherein ring A is:



,

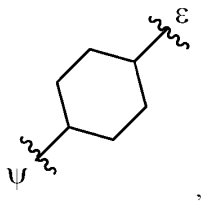
wherein ψ represents the point of attachment to L^1, and ε represents the point of attachment to R^A.

48. The compound of any one of claims 37-40 and 44, wherein ring A is:



wherein ψ represents the point of attachment to L^1, and ε represents the point of attachment to R^A.

49. The compound of any one of claims 37-40 and 44, wherein ring A is:

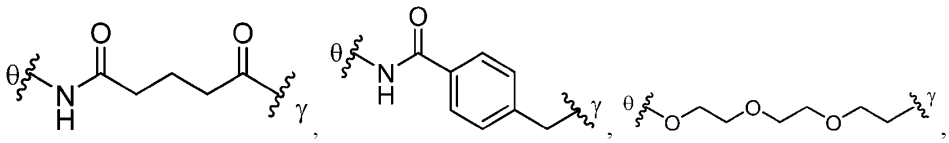


wherein ψ represents the point of attachment to L^1, and ε represents the point of attachment to R^A.

50. The compound of any one of claims 37-49, wherein L^1 is a covalent bond.

51. The compound of any one of claims 37-49, wherein L^1 is a linker.

52. The compound of any one of claims 37-49 and 51, wherein L^1 is a linker selected from the group consisting of:



and -O-,

wherein each θ is the point of attachment to the methylene group and each γ is the point of attachment to ring A.

53. The compound of any one of claims 37-52, wherein m is 0.

54. The compound of any one of claims 37-52, wherein m is 1.

55. The compound of any one of claims 37-54, wherein n is 3.

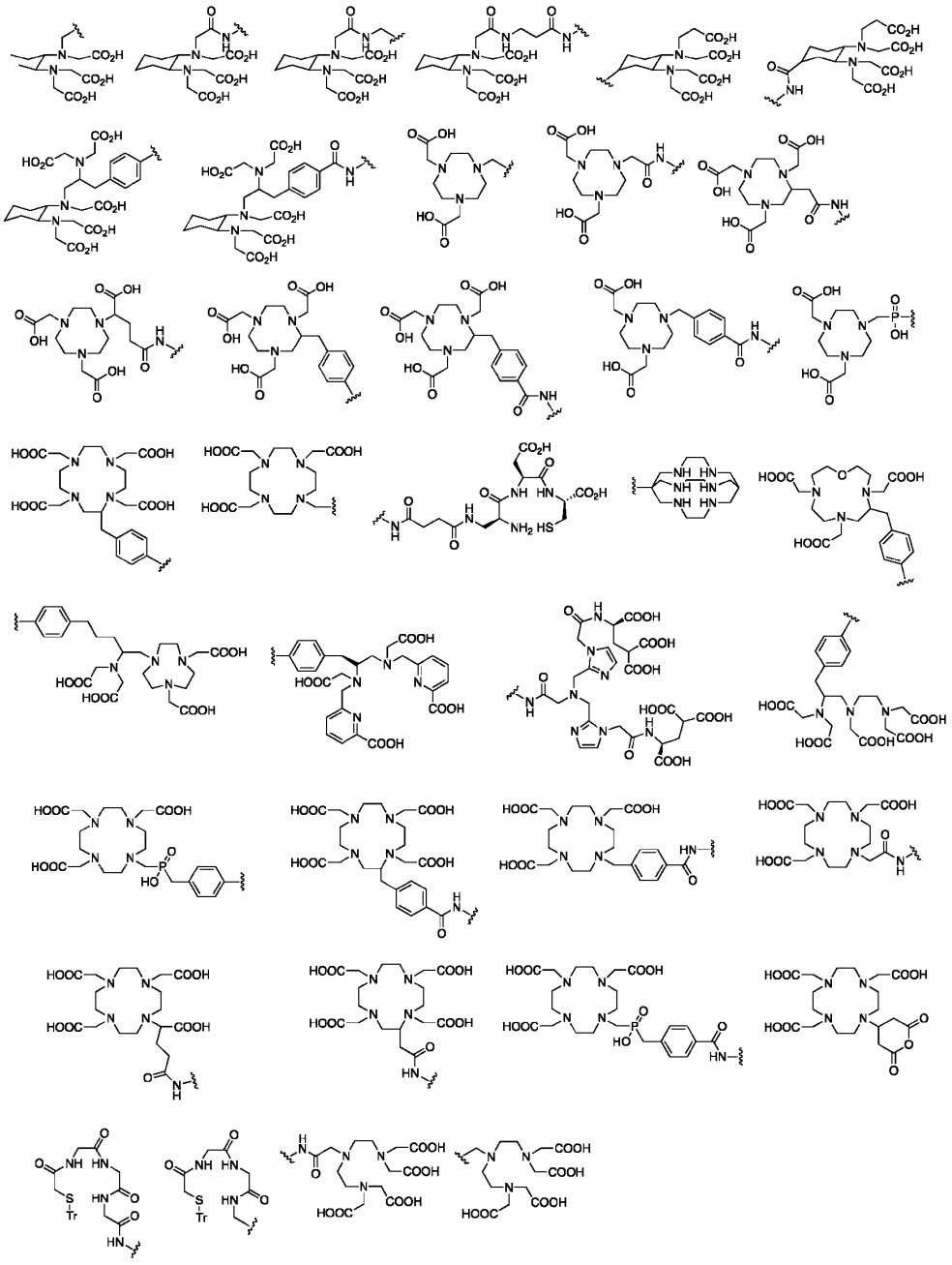
56. The compound of any one of claims 37-55, wherein r is 0.

57. The compound of any one of claims 37-55, wherein r is 1.

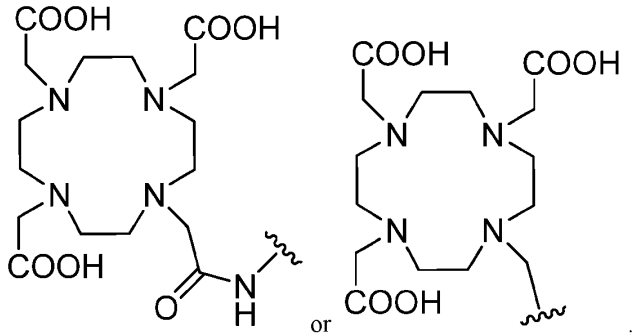
58. The compound of any one of claims 37-57, wherein k is 0.

59. The compound of any one of claims 37-57, wherein k is 1.

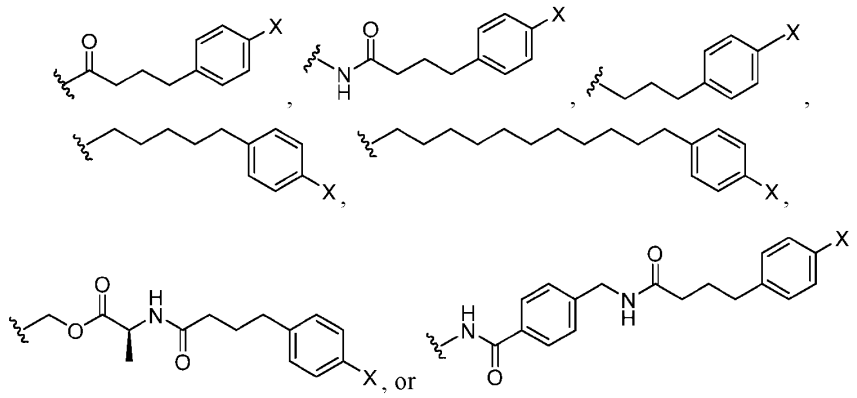
60. The compound of any one of claims 37-59, wherein one of R^2 and R^3 is an unchelated ligand capable of chelating to or covalently binding to a therapeutic radionuclide or a diagnostic radionuclide, selected from the group consisting of:



61. The compound of any one of claims 37-60, wherein one of R^2 and R^3 is an unchelated ligand capable of chelating to or covalently binding to a therapeutic radionuclide or a diagnostic radionuclide, represented by:

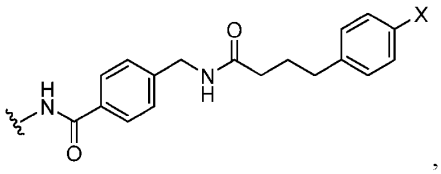


62. The compound of any one of claims 37-60, wherein one of R^2 and R^3 is an albumin binding ligand represented by:



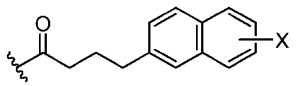
wherein X is selected from the group consisting of halogen and C_{1-6} alkyl.

63. The compound of any one of claims 37-60, wherein one of R^2 and R^3 is an albumin binding ligand represented by:



wherein X is selected from hydrogen, halogen, and C_{1-6} alkyl.

64. The compound of any one of claims 37-61, wherein one of R^2 and R^3 is albumin binding ligand represented by:



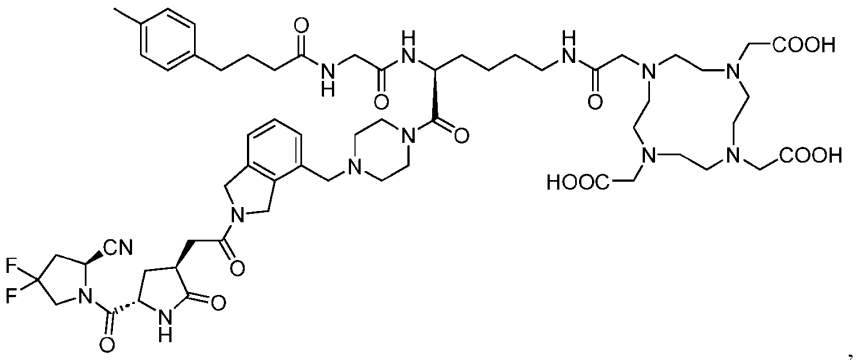
wherein X is selected from the group consisting of hydrogen, halogen and C_{1-6} alkyl.

65. The compound of any one of claims 62-64, wherein X is iodo.

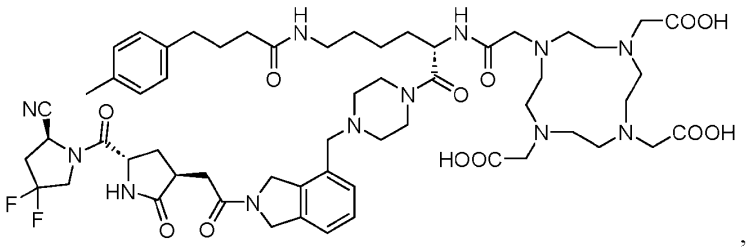
66. The compound of any one of claims 37-65, wherein R^C is hydrogen.

67. The compound of any one of claims 37-66, wherein when one of R^2 and R^3 is the albumin binding ligand, the other is the unchelated ligand capable of chelating to or covalently binding to a therapeutic radionuclide or a diagnostic radionuclide.

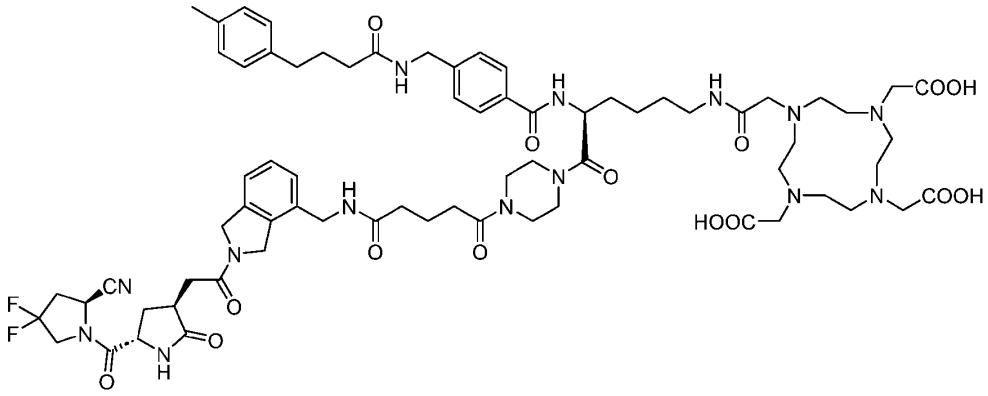
68. A compound selected from the group consisting of



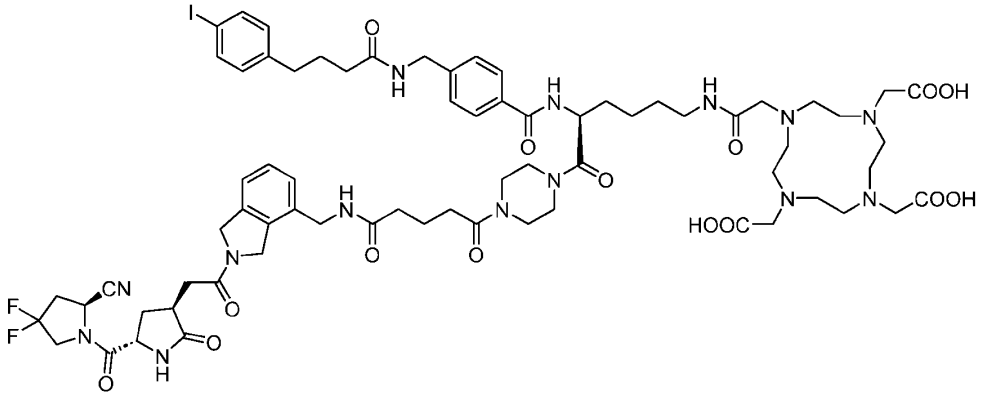
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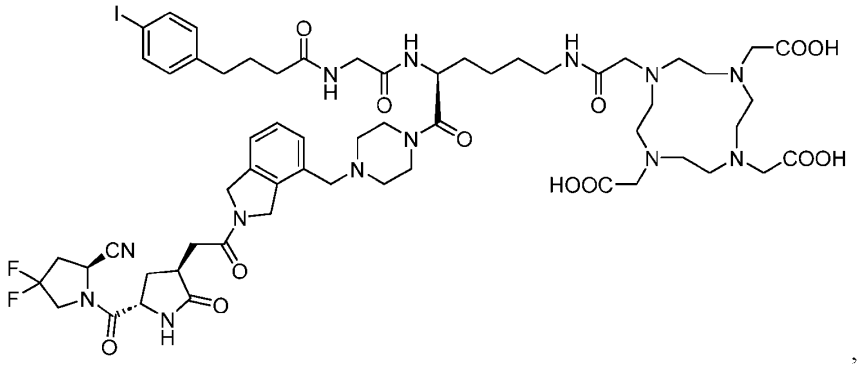
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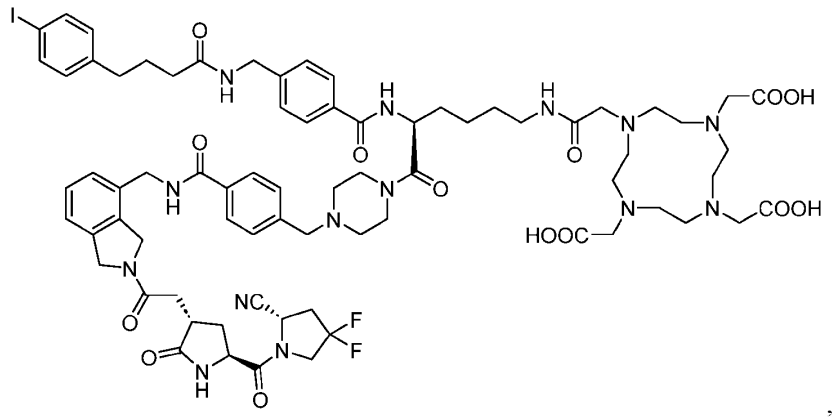
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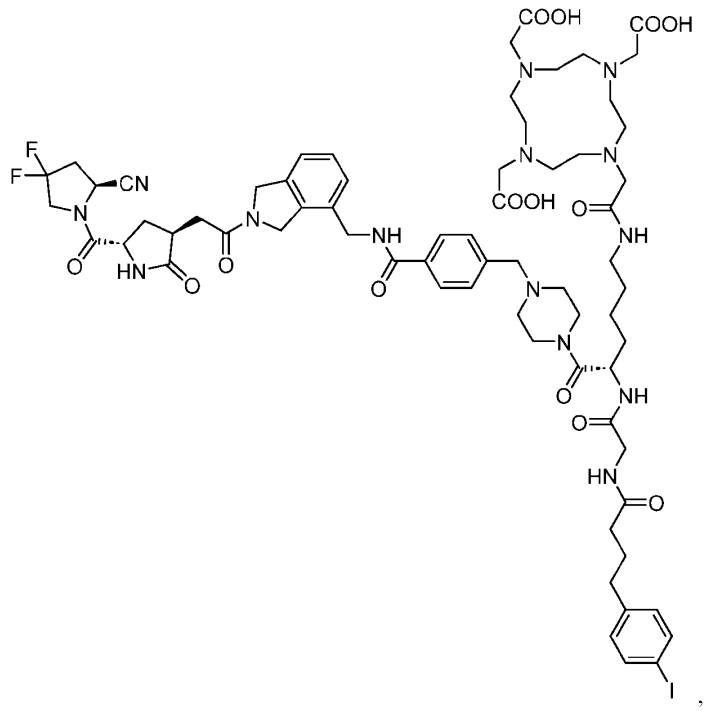


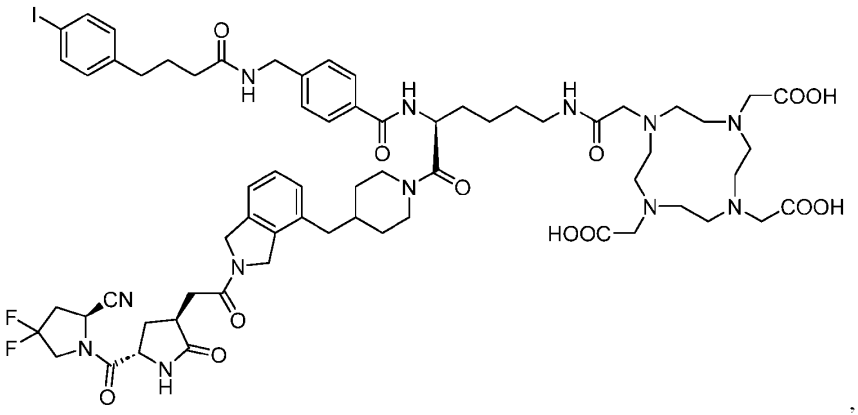
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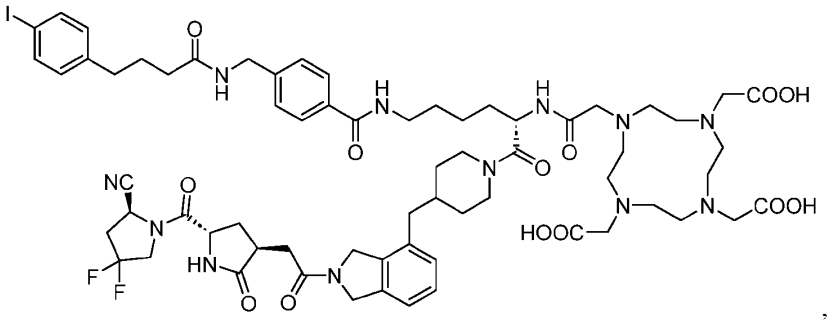
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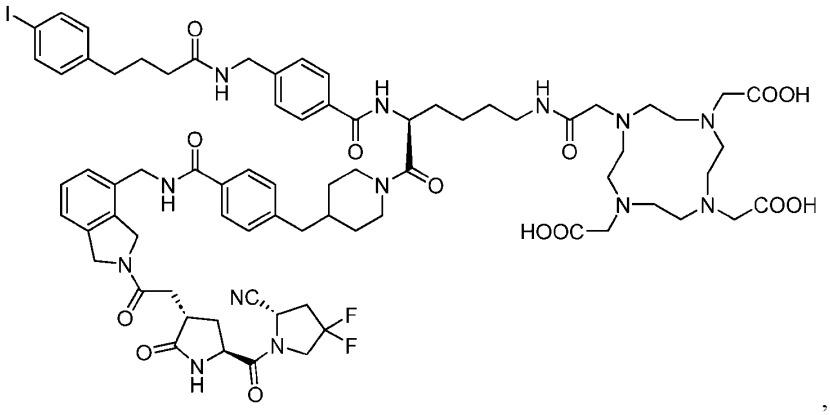




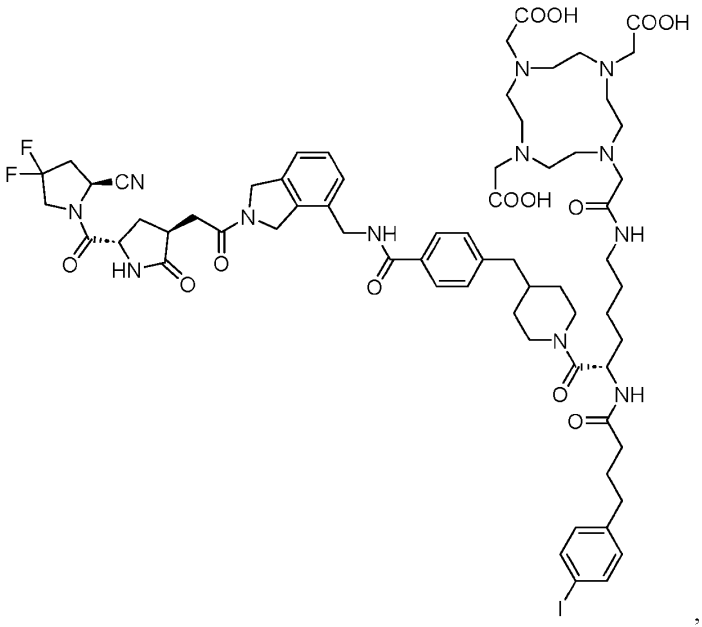
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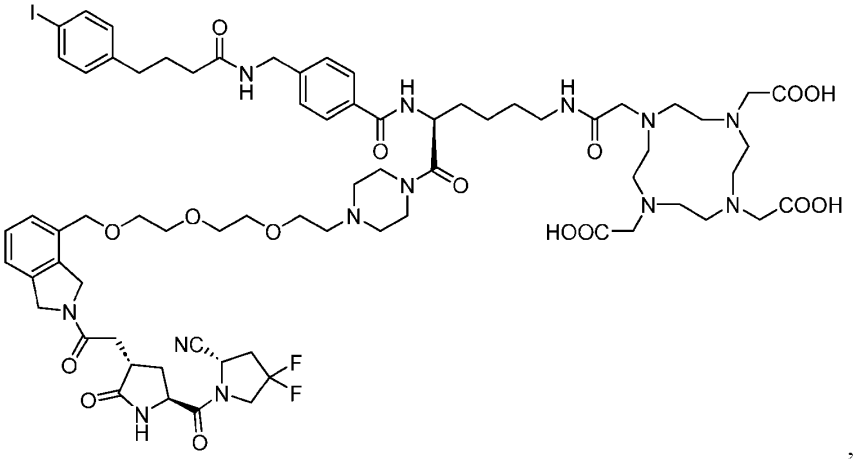
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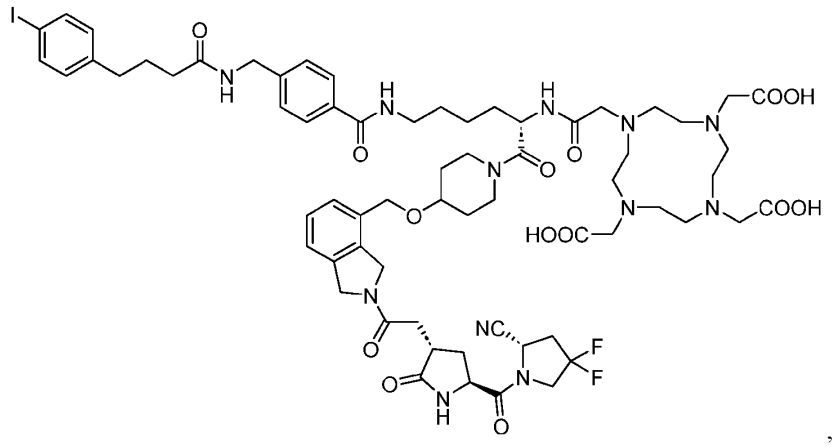
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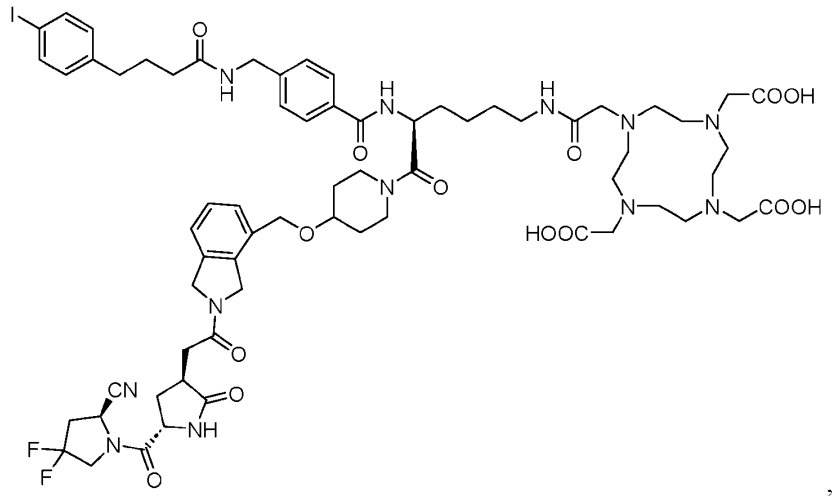
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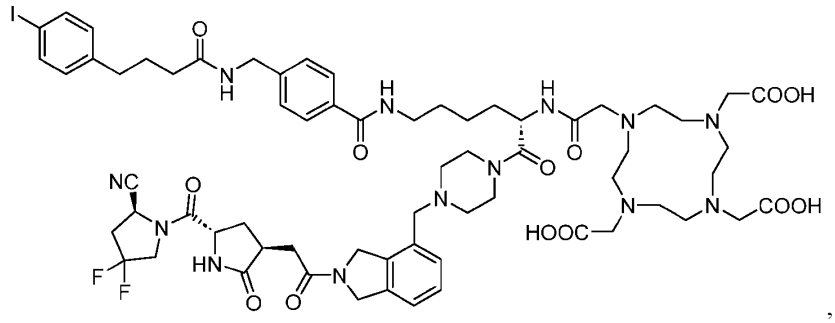
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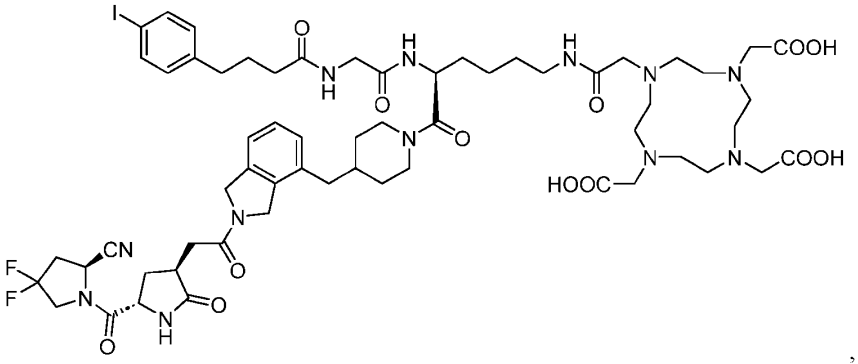
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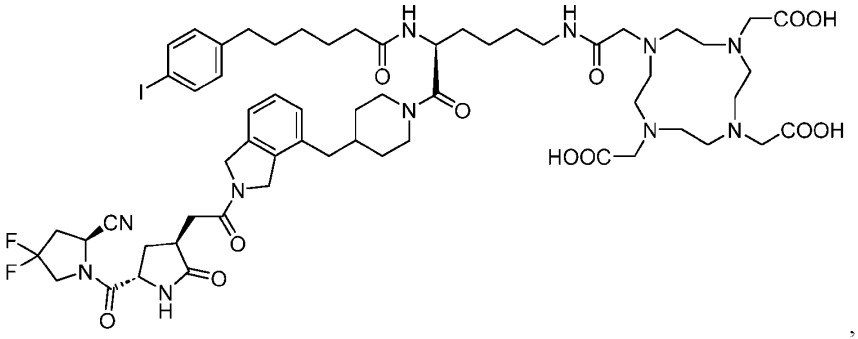
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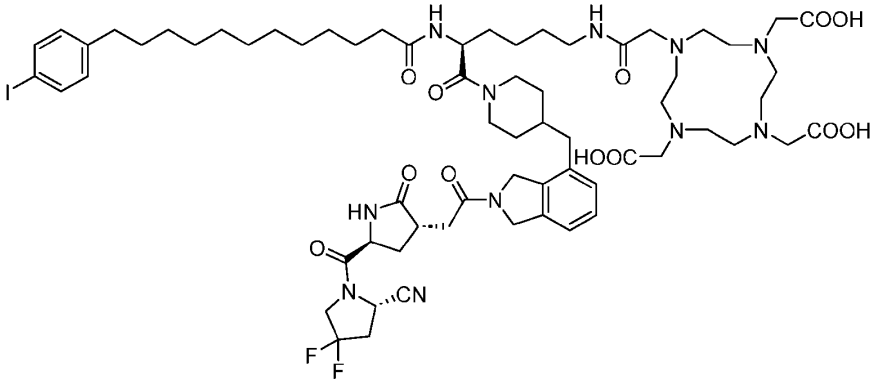
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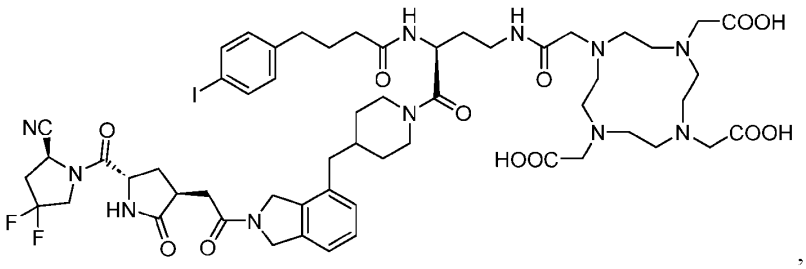
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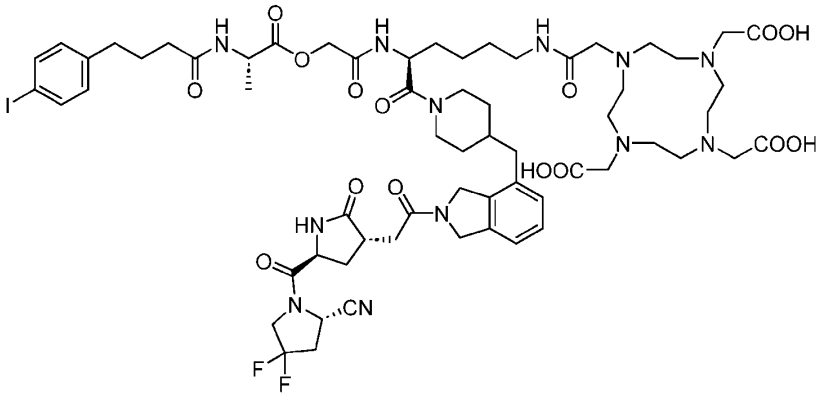
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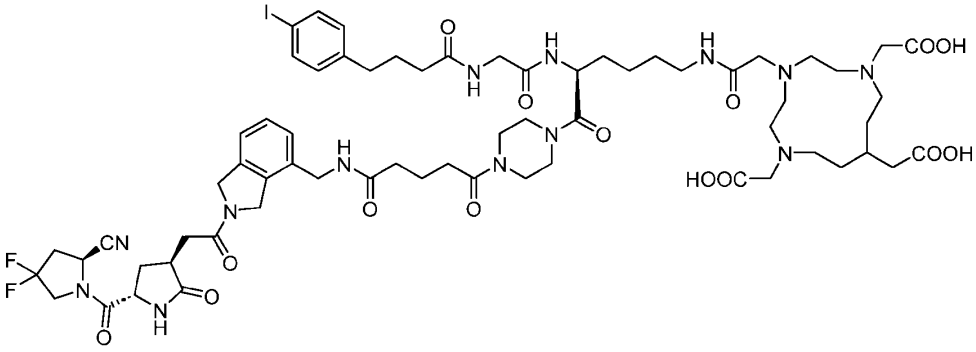
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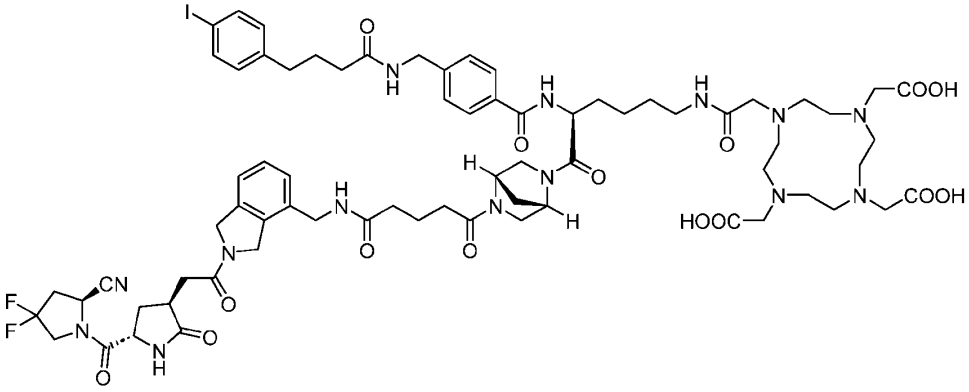
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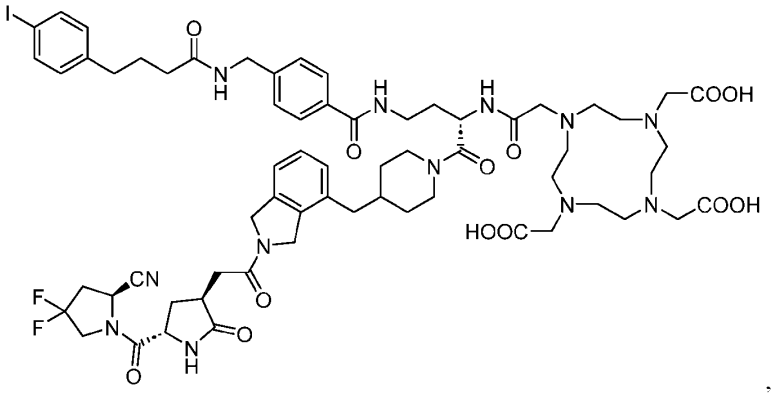
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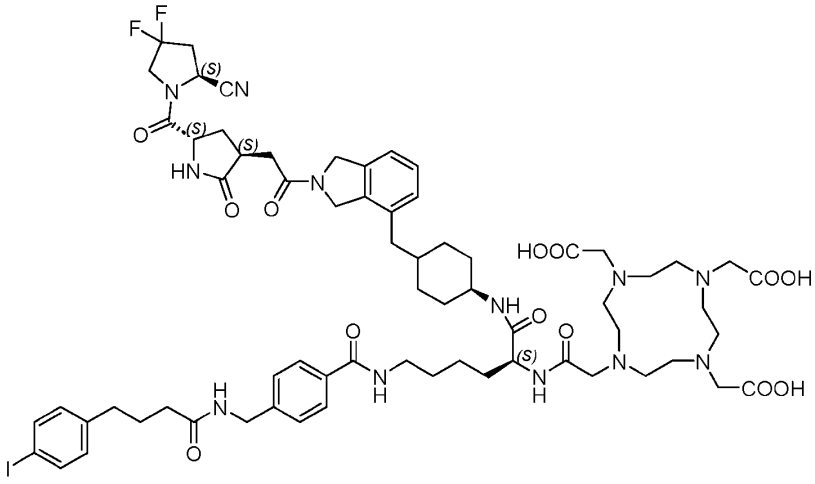
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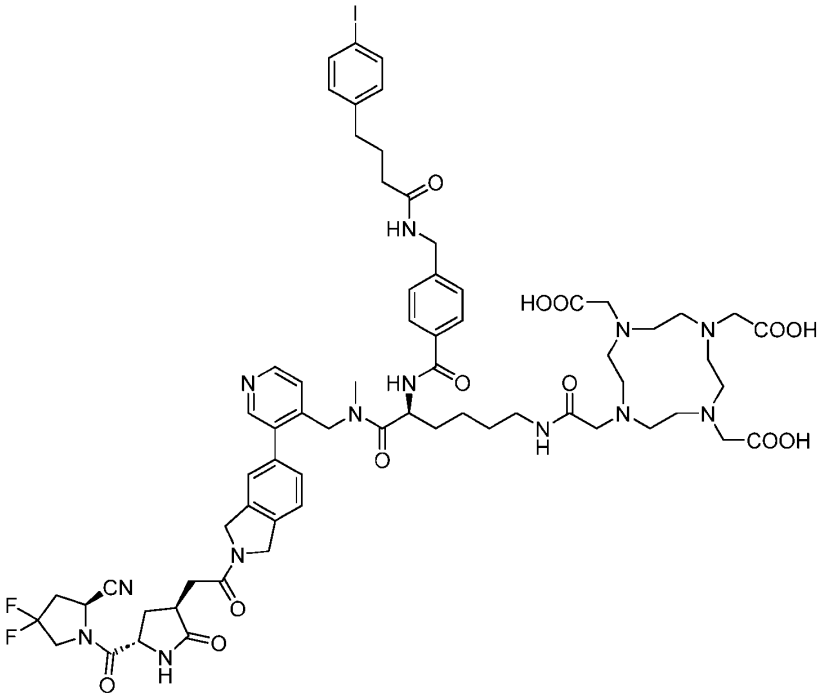
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, or a pharmaceutically acceptable salt thereof.

69. A pharmaceutical composition comprising the compound of any one of claims 37-68, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable excipient.

70. A method of treating cancer in a patient in need thereof, comprising administering to the patient a therapeutically effective amount of the complex of any one of claims 1-36, or a pharmaceutically acceptable salt thereof.

71. The method of claim 70, wherein the cancer is selected from the group consisting of breast cancer, pancreatic cancer, mesothelioma, esophageal cancer, cholangiocellular carcinoma, head and neck cancer, carcinoma of unknown primary origin, brain metastases, sarcoma, salivary gland carcinoma, lung cancer, prostate cancer, thymus cancer, ovarian cancer, desmoid, chordoma, colorectal cancer, anal carcinoma, neuroendocrine tumors, small intestine cancer, medullary thyroid cancer, cervical cancer, hepatocellular carcinoma, gastric cancer, adenoid cystic cancer, pheochromocytoma, differentiated thyroid cancer, insulinoma, renal cell cancer, gastric cancer, renal cancer, bone metastases, lymph node metastases, lung metastases, pleura, bladder cancer, myeloma, lymphoma, brain cancer, endometrial cancer,

testicular cancer, neuroblastoma, astrocytoma, meningioma, rectal cancer, cervical cancer, stomach cancer, skin cancer, thyroid cancer, and/or liver cancer.

72. A method of treating a disease mediated by cancer-associated fibroblasts in a patient in need thereof, comprising administering to the patient a therapeutically effective amount of the complex of any one of claims 1-36, or a pharmaceutically acceptable salt thereof.

73. The method of claim 72, wherein the disease is cancer.

74. The method of claim 73, wherein the cancer is selected from the group consisting of breast cancer, pancreatic cancer, mesothelioma, esophageal cancer, cholangiocellular carcinoma, head and neck cancer, carcinoma of unknown primary origin, brain metastases, sarcoma, salivary gland carcinoma, lung cancer, prostate cancer, thymus cancer, ovarian cancer, desmoid, chordoma, colorectal cancer, anal carcinoma, neuroendocrine tumors, small intestine cancer, medullary thyroid cancer, cervical cancer, hepatocellular carcinoma, gastric cancer, adenoid cystic cancer, pheochromocytoma, differentiated thyroid cancer, insulinoma, renal cell cancer, gastric cancer, renal cancer, bone metastases, lymph node metastases, lung metastases, pleura, bladder cancer, myeloma, lymphoma, brain cancer, endometrial cancer, testicular cancer, neuroblastoma, astrocytoma, meningioma, rectal cancer, cervical cancer, stomach cancer, skin cancer, thyroid cancer, and/or liver cancer.

75. A method of imaging a cancer-associated fibroblast in a patient in need thereof, comprising administering to the patient an effective amount of the complex of any one of claims 1-31 and 34-35, or a pharmaceutically acceptable salt thereof, and identifying the location of the cancer-associated fibroblast in the patient.

76. A method of imaging activated fibroblasts in a patient with idiopathic pulmonary fibrosis, cirrhosis or liver fibrosis, Crohn's disease, rheumatoid arthritis, ulcerative colitis, sclerosis, cardiac fibrosis, and/or kidney fibrosis in need thereof, comprising administering to the patient an effective amount of the chelated complex of any one of claims 1-31 and 34-35, or a pharmaceutically acceptable salt thereof, and identifying the location of activated fibroblasts in the patient for noninvasive diagnosis.