

FIBROBLAST ACTIVATION PROTEIN-TARGETED COMPOSITIONS AND METHODS OF USE THEREOF

RELATED APPLICATIONS

This application claims the benefit of U.S. Provisional Application Serial No. 63/627,309, filed January 31, 2024 and U.S. Provisional Application Serial No. 63/529,522, filed July 28, 2023, the entire teachings of which are incorporated herein by reference.

FIELD OF THE INVENTION

The present technology relates to targeted imaging and therapy agents, more particularly compounds useful in the diagnosis and treatment of disease. For example, the compositions described herein may be used as radiopharmaceutical agents, or conjugated to optical dyes or fluorophores, or drug/toxin conjugates, useful for the diagnosis and treatment of cancers and fibrotic disease in tissues.

BACKGROUND OF THE INVENTION

Fibroblast activation protein alpha ("FAP") is a 170 kDa type-II membrane-bound enzyme that demonstrates serine protease activity. A soluble form of FAP is present in blood plasma, lacking the intracellular and transmembranal sequences of the full-length protein. Other common names for FAP include Prolyl Endopeptidase and Surface Expressed Protease (seprase).

FAP is one of several members of the S9B prolyl oligopeptidase subfamily, which includes among other proteins, DPP4, DPP8 and DPP9. Substrates for FAP include neuropeptide Y, peptide YY, substance P, B-type natriuretic peptide, fibroblast growth factor 21 (FGF-21), alpha2 antiplasmin, and denatured collagen I and III.

FAP is actively expressed in tissues that are undergoing wound healing and remodeling, but is otherwise not expressed, or expressed at extremely low levels in healthy, mature tissues. Tumors are localized regions of histological injury to the host, and are active at remodeling local vasculature and endothelium, as well as several other phenomena that serve to hide the tumor from immune surveillance and promote wound healing – a microenvironment that allows the neoplastic cells to proliferate. Accordingly, FAP

expression is correlated with regions of tumorigenic tissues, particularly the tumor stroma and therefore presents an excellent molecular target for the diagnosis and treatment of various cancers. Its expression has been confirmed in numerous cancers, such as pancreatic, liver, gall bladder, neuroblastoma, breast, ovarian, esophageal, kidney, melanoma and many other deadly and aggressive tumors and cancer types. FAP expression is also detected in fibrotic tissues and can also be a marker for a wide spectrum of clinical conditions including systemic fibrotic diseases such as systemic sclerosis (SSc), sclerodermatous graft vs. host disease, and nephrogenic systemic fibrosis, as well as numerous organ-specific disorders including radiation-induced fibrosis and cardiac, pulmonary, liver (such as NAFLD: non-alcoholic fatty liver disease and NASH: non-alcoholic steatohepatitis), and kidney fibrosis.

While several researchers have explored FAP as a cancer target, the development of agents that can serve as diagnostics or even therapeutics have proven only marginally useful, due to their pharmacokinetic limitations. To date FAP has proven to be a difficult target due to the low and limited expression of the target and the limited residence time the prior art compounds display *in vivo*. What is needed are improved FAP-binding agents that demonstrate better binding kinetics and biodistribution, thereby providing the basis for improved FAP-targeted diagnostic and therapeutic agents, i.e., compounds that can accumulate to a greater degree in tumors without unacceptable uptake in normal non-target tissues and organs.

SUMMARY OF THE INVENTION

FAP-targeted therapeutics are emerging as a potential methodology for treating a wide variety of cancers that express FAP in the microenvironment and/or tumor cells themselves. Several FAP-targeted molecules, such as FAPI-04, FAPI-46, FAP-2286, PNT6555 have been studied preclinically and in early clinical trials. FAP-targeted therapies have shown some initial promise in humans; however relatively short tumor retention is a significant limitation. Current FAP-targeted compounds clear relatively rapidly from tumor tissue, limiting the radioactive absorbed dose to the tumor. Efforts have been made to improve tumor retention of FAP-targeted radiotracers with limited success.

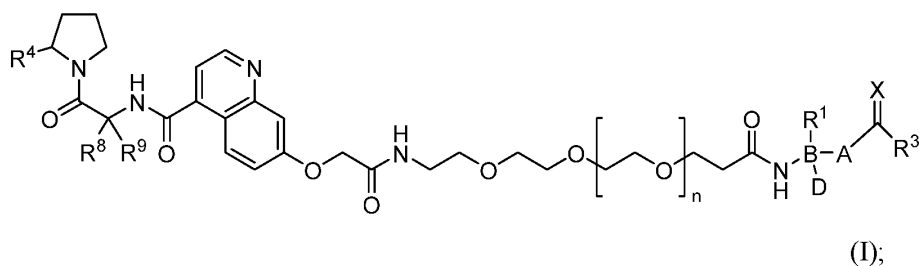
Disclosed herein are a series of novel compounds that bind to the extracellular domain of FAP. Unlike existing compounds, the disclosed compounds exhibit a unique chemical

structure that allows for significantly higher affinity to FAP (see Example 2 & 3), leading to improved tumor retention.

The compounds disclosed herein can be attached to chelating groups for radionuclide binding and are therefore suitable for radioimaging and/or radiotherapy applications, for example the disclosed compounds can be radiolabeled with a positron emitter such as ^{18}F , ^{68}Ga or ^{64}Cu , and used for positron emission tomography (PET) (see Example 4).

Alternatively, the compounds can be radiolabeled with an alpha particle emitter such as ^{225}Ac , a beta particle emitter such as ^{67}Cu or ^{177}Lu , or an Auger electron emitter (e.g. ^{111}In , ^{67}Ga , $^{99\text{m}}\text{Tc}$, $^{195\text{m}}\text{Pt}$, ^{125}I and ^{123}I). The compounds can also be attached to a cytotoxic agent for targeted delivery of the cytotoxic agent to tumors, for example conjugated to gemcitabine or doxycycline, or a venom. Likewise, the compounds can be conjugated to compounds having physiological effects, such as TLR agonists to stimulate the immune response of a recipient. The disclosed compounds have the advantage of surprisingly high affinity to FAP. Many of these compounds also have albumin binding/increased circulatory residence time but it is the potency/affinity to FAP that is believed to result in the significant tumor residence time. The increased circulatory residence time has the effects of increasing target loading while reducing accumulation of the compound in non-target tissues (see Example 6). Accordingly, the favorable binding kinetics of the compounds disclosed herein reduce the “wash-out” effect (i.e., low residence time) seen with prior art FAP-targeted compounds.

One embodiment of the invention is a compound represented by the following structural formula (I):



or a pharmaceutically acceptable salt thereof, wherein:

n is 0 or 1;

A is NH , O , S or CR^6R^7 ;

B comprises or is a branched, unbranched or cyclic aliphatic group of up to 30 carbon atoms optionally interrupted by up to 10 heteroatoms or a peptidyl chain of up to 20 amino acid residues (for example, 3-20 or 3-15 carbon atoms optionally interrupted by up to 6

heteroatoms or up to 5 amino acid residues), wherein B is optionally substituted with 1-5 groups selected from F, Cl, Br, I, =O, OR⁶, OCOR⁶, COOR⁶, CN, =NR⁶, NR⁶R⁷, =S, and SR⁶, provided that B comprises at least 3 atoms in a chain between group D and the group A;

D is selected from the group consisting of OPO₃H₂, PO₃H₂, OSO₃H, SO₃H and COOH or a C₁-C₄ alkyl ester thereof;

X is O or S;

R¹ is a chelating group, an optical dye or fluorophore, a cytotoxic agent, an immune stimulant, or a benzoyl group optionally substituted by one or more groups represented by R⁵;

R³ is C₁-C₈ alkyl or C₁-C₄ aralkyl, wherein:

the alkyl and aryl portions of the aralkyl are each optionally and independently substituted with F, Cl, Br, I, branched, unbranched or cyclic C₁-C₆ aliphatic group, OR⁶, OCOR⁶, COOR⁶, CHO, COR⁶, CH₂OR⁶, NR⁶R⁷, CH₂NR⁶R⁷, SR⁶, =O, =S and =NH;

R⁴ is CN or B(OH)₂;

each R⁵ is independently selected from halo, cyano, halomethyl, N⁺(CH₃)₃W⁻ wherein W⁻ is a pharmaceutically acceptable anion;

R⁶ and R⁷ are independently selected from the group consisting of H or a C₁-C₆ alkyl; and

R⁸ is C₁₋₄alkyl and R⁹ is selected from H and C₁-C₄ alkyl or R⁸ and R⁹ taken together with their intervening carbon atom form a C₃-C₆ cycloalkyl.

Another embodiment of the invention is a pharmaceutical composition comprising: i) a compound disclosed herein or a pharmaceutically acceptable salt thereof; and ii) a pharmaceutically acceptable carrier or diluent. For compounds comprising a chelating group, the chelating group is preferably chelated with a radionuclide.

Another embodiment of the invention is a method of treating a subject with diseased tissue that expresses fibroblast activation protein alpha. The diseased tissue in one aspect can be a cancer or a fibrotic tissue. The method comprises administering an effective amount of the compound disclosed herein or pharmaceutically acceptable salt to the subject. Preferably, the compound used for therapy comprises a cytotoxic agent, such as a chelating group having a radionuclide that emits beta, alpha, Auger or other cytotoxic radiation which can kill the diseased tissue.

Yet another embodiment of the invention is a method of imaging a region in a subject having or suspected of having a cancer or a fibrotic tissue disease which expresses fibroblast activation protein alpha or fibrotic tissue, comprising:

- a. administering to the subject a diagnostically effective amount of a compound disclosed herein or pharmaceutically acceptable salt thereof or a pharmaceutical composition disclosed herein;
- b. exposing a region in the subject to an imaging device, the region suspected of having diseased tissue; and
- c. obtaining an image of diseased tissue in the region.

Preferably, the compound used for imaging comprises a chelating group having a radionuclide that emits gamma-rays or positrons or other detectible radiation. In another aspect, the compound comprises an optical dye or a fluorophore, the emissions of which can be detected.

Yet another embodiment of the invention is a method of imaging tumors. The method comprises:

- a. administering to a subject, a compound disclosed herein, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, in an amount effective to contact and bind to the tumor and/or surrounding tissue;
- b. irradiating the tumor and/or surrounding tissue at a wavelength absorbed by the bound compound;
- c. and detecting a signal from the irradiated bound compound, thereby imaging the tumor and/or surrounding tissue.

Preferably, the compound used for imaging comprises a chelating group having a radionuclide that emits gamma-rays or positrons, or an optical dye or a fluorophore, or other detectible radiation.

Still another embodiment of the invention is a method of treating diseased tissue. The method comprises:

- a. administering to a subject, a compound disclosed herein, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, in an amount effective to contact and bind to the diseased tissue;

- b. using the compound as a fiducial, irradiating the region of the bound compound with one or more doses of external beam radiation, thereby treating the diseased tissue with radiation.

Preferably, the compound used for the fiducial comprises a chelating group having a radionuclide that emits gamma-rays or positrons, or an optical dye or a fluorophore, or other detectible radiation.

Even still another embodiment of the invention is a method of treating diseased tissue. The method comprises:

- a. administering to a subject, a compound disclosed herein, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, in an amount effective to contact and bind to the diseased tissue;
- b. using the compound as a fiducial for guided surgery applications, to resect the region of the diseased tissue thereby excising the diseased tissue.

Preferably, the compound used for the fiducial comprises a chelating group having a radionuclide that emits gamma-rays or positrons, or an optical dye or a fluorophore, or other detectible radiation.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1. Show the organ biodistribution of [Lu-177]RTX-1371R in BALB/C nude mice previously transplanted with U-87/MG cells.

FIG. 2. Show the organ biodistribution of [Lu-177]RTX-1391R in BALB/C nude mice previously transplanted with U-87/MG cells.

FIG. 3. Show the organ biodistribution of [Lu-177]RTX-1392R in BALB/C nude mice previously transplanted with U-87/MG cells.

FIG. 4. shows the organ biodistribution of [Lu-177]RTX-1356R and [Lu-177]RTX-1391R in BALB/C nude mice previously transplanted with U-87/MG cells.

FIG. 5. shows the organ biodistribution of [Lu-177]RTX-1357R and [Lu-177]RTX-1392R in BALB/C nude mice previously transplanted with U-87/MG cells.

FIG. 6. shows the organ biodistribution of [Lu-177]RTX-1359R and [Lu-177]RTX-1371R in BALB/C nude mice previously transplanted with U-87/MG cells.

FIG. 7. shows the organ biodistribution of [Lu-177]RTX-1427R and [Lu-177]RTX-1411R in BALB/C nude mice previously transplanted with U-87/MG cells.

FIG. 8. shows the organ biodistribution of [Lu-177]RTX-1418R in BALB/C nude mice previously transplanted with U-87/MG cells.

FIG. 9. shows the organ biodistribution of [Lu-177]RTX-1386S in BALB/C nude mice previously transplanted with U-87/MG cells.

FIG. 10. shows the organ biodistribution of [ZW800-1]RTX-1384S in female Nu/J or NCr-Foxn1 nude mice previously transplanted with U-87/MG cells.

FIG. 11. shows the organ biodistribution of [ZW800-1]RTX-1384S normalized to muscle in female Nu/J or NCr-Foxn1 nude mice previously transplanted with U-87/MG cells.

FIG. 12. shows the efficacy of [Ac-225]RTX-1359R at various dosages in female Nu/J mice previously transplanted with U-87/MG cells.

FIG. 13. shows the survival percentage of female Nu/J mice previously transplanted with U-87/MG cells after receiving a treatment of [Ac-225]RTX-1359R.

FIG. 14. shows the efficacy of [Ac-225]RTX-1392R at various dosages in female Nu/J mice previously transplanted with U-87/MG cells.

FIG. 15. shows the survival percentage of female Nu/J mice previously transplanted with U-87/MG cells after receiving a treatment of [Ac-225]RTX-1392R.

FIG. 16. shows a comparison of the efficacy between [Ac-225]RTX-1392R and [Ac-225]RTX1411R in female Nu/J mice previously transplanted with U-87/MG cells.

FIG. 17. shows a comparison of the survival percentage of female Nu/J mice previously transplanted with U-87/MG cells after receiving a treatment of [Ac-225]RTX-1392R or [Ac-225]RTX1411R.

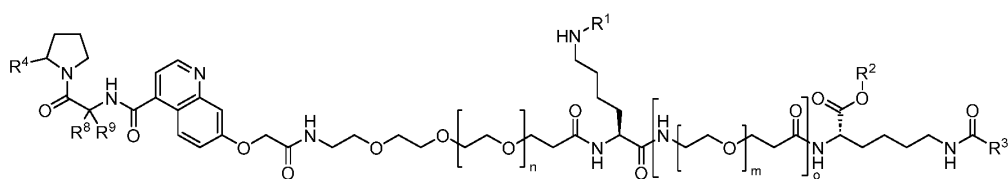
DETAILED DESCRIPTION

Disclosed herein are a series of compounds that bind with high affinity to the extracellular domain of FAP and are capable of delivering a payload to a tissue expressing FAP. Compounds of the invention are described herein below.

A first embodiment of the invention is a compound represented by structural formula (I) or a pharmaceutically acceptable salt thereof, wherein the variables are as described above for structural formula (I).

A second embodiment of the invention is a compound represented by structural formula (I) or a pharmaceutically acceptable salt thereof, wherein B is independently a branched or unbranched aliphatic group of 3 to 20 carbon atoms optionally interrupted by up to 10 heteroatoms or a peptidyl chain of up to 5 amino acid residues, wherein the aliphatic group is optionally substituted with F, Cl, Br, I, =O, OR⁶, OCOR⁶, COOR⁶, CN, =NR⁶, NR⁶R⁷, =S, or SR⁶, and the remainder of the variables are as described above for structural formula (I).

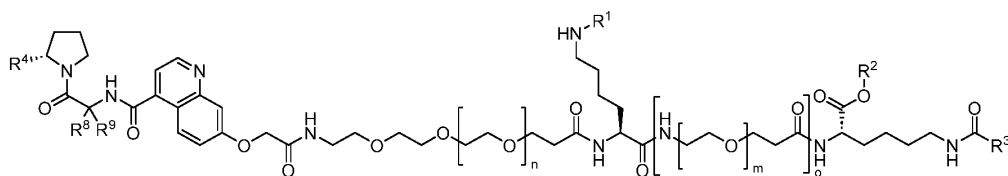
A third embodiment of the invention is compound represented by structural formula (II):



(II);

or a pharmaceutically acceptable salt thereof, wherein m is an integer from 0 to 12; o is 0 or 1; and R² is H or C₁-C₄ alkyl; and the remainder of the variables are as described in the first or second embodiment.

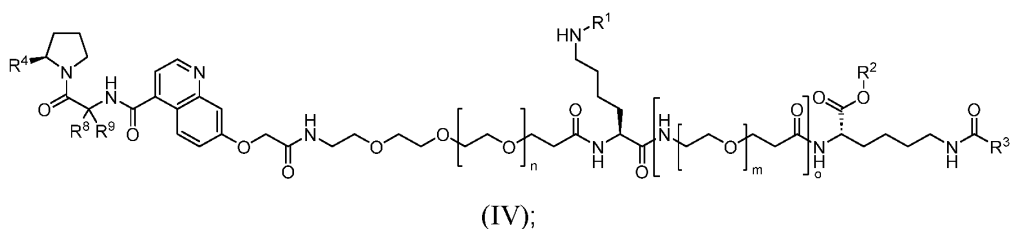
A fourth embodiment of the invention is a compound represented by structural formula (III):



(III);

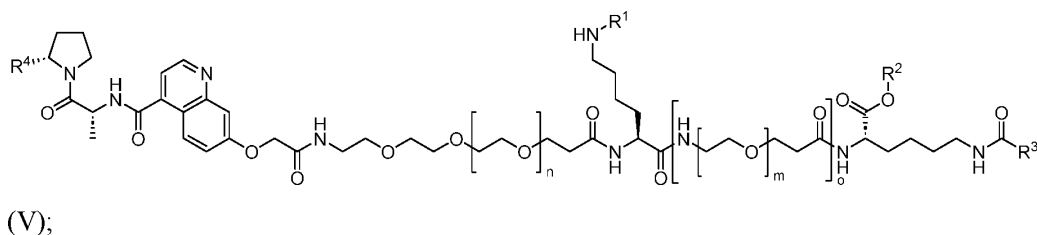
or a pharmaceutically acceptable salt thereof, wherein the variables are as described in the third embodiment.

A fifth embodiment of the invention is a compound represented by structural formula (IV):



or a pharmaceutically acceptable salt thereof; wherein the variables are as described in the third embodiment.

A sixth embodiment of the invention is a compound represented by structural formula (V):



or a pharmaceutically acceptable salt thereof; wherein the variables are as described in the third embodiment.

A seventh embodiment of the invention is a compound represented by structural formula (I), (II), (III), (IV), or (V), or a pharmaceutically acceptable salt thereof, wherein R³ is C₁₋₈alkyl or C₁₋₄aralkyl optionally substituted with F, Cl, Br, I, or C₁₋₄alkyl, and the remainder of the variables are as described in the first, second or third embodiments.

An eighth embodiment of the invention is a compound represented by structural formula (I), (II), (III), (IV), or (V), or a pharmaceutically acceptable salt thereof, wherein R³ is C₁₋₈alkyl or C₁₋₄aralkyl optionally substituted with I or C₁₋₄alkyl, and the remainder of the variables are as described in the first, second, third, or seventh embodiments.

A ninth embodiment of the invention is a compound represented by structural formula (I), (II), (III), (IV), or (V), or a pharmaceutically acceptable salt thereof, wherein R³ is C₁-C₈ alkyl or C₁-C₄ aralkyl optionally substituted with C₁-C₄ alkyl, and the remainder of the variables are as described in the first, second, or third, seventh, or eighth embodiment.

An tenth embodiment of the invention is a compound represented by structural formula (I), (II), (III), (IV), or (V), or a pharmaceutically acceptable salt thereof, wherein R³ methyl, (4-isobutylphenyl)methyl, (4-isobutylphenyl)propyl, (4-iodophenyl)methyl, or (4-iodophenyl) propyl, and the remainder of the variables are as describe in the first, second, third, seventh, or eighth embodiment.

An eleventh embodiment of the invention is a compound represented by structural formula (I), (II), (III), (IV), or (V), or a pharmaceutically acceptable salt thereof, wherein R^3 is methyl, (4-isobutylphenyl)methyl, and (4-isobutylphenyl)propyl, and the remainder of the variables are as described in the first, second, third, seventh, eighth, ninth, or tenth embodiment.

A twelfth embodiment of the invention is a compound represented by structural formula (I), (II), (III), (IV), or (V), or a pharmaceutically acceptable salt thereof, wherein R^8 is methyl and R^9 is H, and the remainder of the variables are as described in the first, second, third, seventh, eighth, ninth, tenth, or eleventh embodiment.

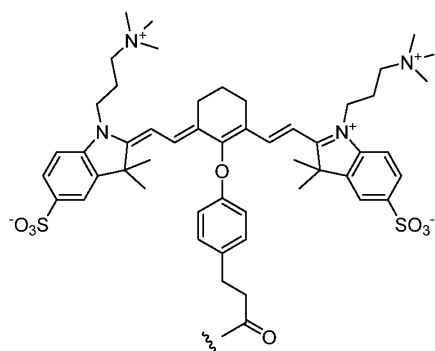
A thirteenth embodiment of the invention is a compound represented by structural formula (II), (III), (IV), or (V), or a pharmaceutically acceptable salt thereof, wherein o is 1 and m is 3 to 12, and the remainder of the variables are as described in the third, seventh, eighth, ninth, tenth, eleventh, or twelfth embodiment.

A fourteenth embodiment of the invention is a compound represented by structural formula (II), (III), (IV), or (V), or a pharmaceutically acceptable salt thereof, wherein m is 8 and the remainder of the variables are as described in the thirteenth embodiment.

An fifteenth embodiment of the invention is a compound represented by structural formula (II), (III), (IV), or (V), or a pharmaceutically acceptable salt thereof, wherein o is 0; and the remainder of the variables are as described for the third, seventh, eighth, ninth, tenth, eleventh, or twelfth embodiment.

A sixteenth embodiment of the invention is a compound represented by structural formula (I), (II), (III), (IV), or (V), or a pharmaceutically acceptable salt thereof, wherein n is 1; and the remainder of the variables are as described for the first, second, third, seventh, eighth, ninth, tenth, eleventh, twelfth, thirteenth, fourteenth, or fifteenth embodiment.

A seventeenth embodiment of the invention is a compound represented by structural formula (I), (II), (III) (IV), or (V), or a pharmaceutically acceptable salt thereof, wherein R^1 is a fluorophore or optical dye; and the remainder of the variables are as described in the first, second, third, seventh, eighth, ninth, tenth, eleventh, twelfth, thirteenth, fourteenth, fifteenth, or sixteenth embodiment. In one aspect, the fluorophore is



; and the optical dye is carbocyanin, indocarbocyanin, oxacarbocyanin, thiocarbocyanin, merocyanin, polymethine, coumarin, rhodamine, xanthene, fluorescein, Borodipyrromethane (BODIPY), VivoTag-680, VivoTag-S750, AlexaFluor dyes (e.g., AlexaFluor660, AlexaFluor680, AlexaFluor700, AlexaFluor750, AlexaFluor790) and DylightFluor dyes.

An eighteenth embodiment of the invention is a compound represented by structural formula (I), (II), (III) (IV), or (V), or a pharmaceutically acceptable salt thereof, wherein R^1 is a chelating group that is the residue of a chelating agent; and the remainder of the variables are as described in the first, second, third, seventh, eighth, ninth, tenth, eleventh twelfth, thirteenth, fourteenth, fifteenth, or sixteenth embodiment. Suitable chelating agents and residues of chelating agents are described below.

A nineteenth embodiment of the invention is a compound represented by structural formula (I), (II), (III), (IV), or (V), or a pharmaceutically acceptable salt thereof, wherein R^1 is a benzoyl group optionally substituted by one or more groups represented by R^5 ; each R^5 is independently selected from halo, cyano, halomethyl, $N^+(CH_3)_3W^-$; and W^- is a pharmaceutically acceptable anion; and the remainder of the variables are as described in the first, second, third, seventh, eighth, ninth, tenth, eleventh, twelfth, thirteenth, fourteenth, fifteenth, or sixteenth embodiment. In one aspect, the halo group represented by R^5 is ^{18}F .

A twentieth embodiment of the invention is a compound represented by structural formula (I), (II), (III), (IV), or (V), or a pharmaceutically acceptable salt thereof, wherein each R^5 is independently selected from fluoro, cyano, trifluoromethyl, $N^+(CH_3)_3W^-$; and the remainder of the variables are as described in the nineteenth embodiment. In one aspect, the fluoro group represented by R^5 is F^{18} .

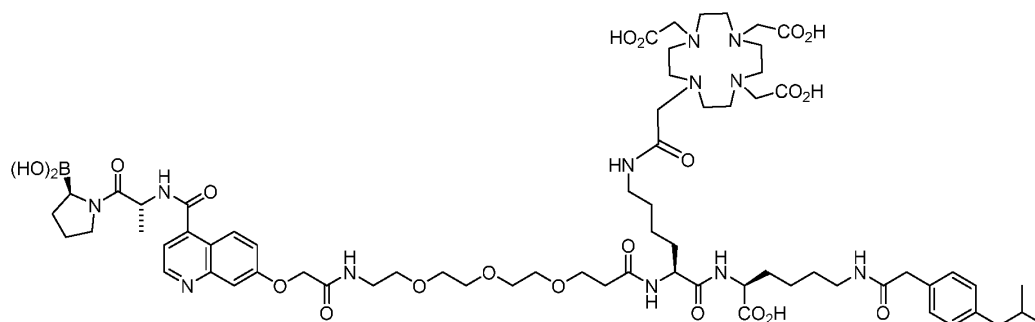
A twenty-first embodiment of the invention is a compound represented by structural formula (I), (II), (III), (IV), or (V), or a pharmaceutically acceptable salt thereof, wherein R^2 is H and R^4 is $B(OH)_2$; and the remainder of the variables are as described in the first, second,

third, seventh, eighth, ninth, tenth, eleventh, twelfth, thirteenth, fourteenth, fifteenth, sixteenth, seventeenth, eighteenth, nineteenth, or twentieth embodiment.

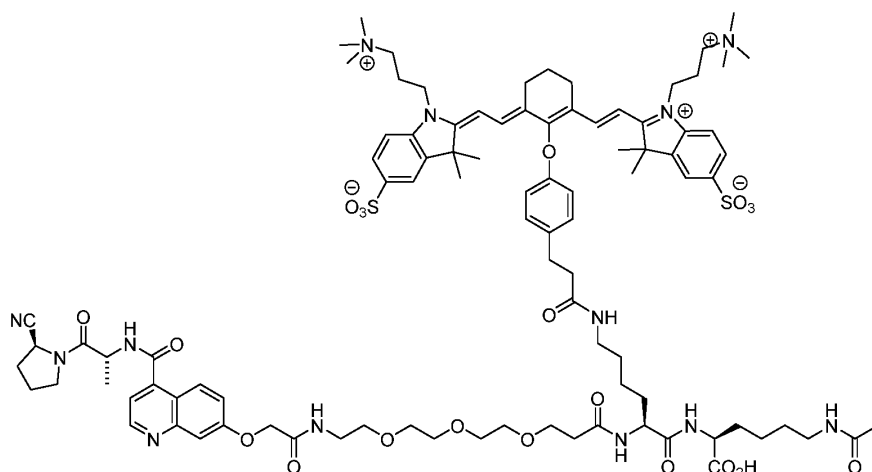
A twenty-second embodiment of the invention is a compound represented by structural formula (I), (II), (III), (IV), or (V), or a pharmaceutically acceptable salt thereof, R^2 is H and R^4 is CN; and the remainder of the variables are as described in the first, second, third, seventh, eighth, ninth, tenth, eleventh, twelfth, thirteenth, fourteenth, fifteenth, sixteenth, seventeenth, eighteenth, nineteenth, or twentieth embodiment.

Also included in the invention are the compounds whose preparation is described in the Exemplification and shown in the Figures, both pharmaceutically acceptable salts thereof and the neutral form. For those compounds comprising a chelating group, chelation with a radionuclide is also included in the invention.

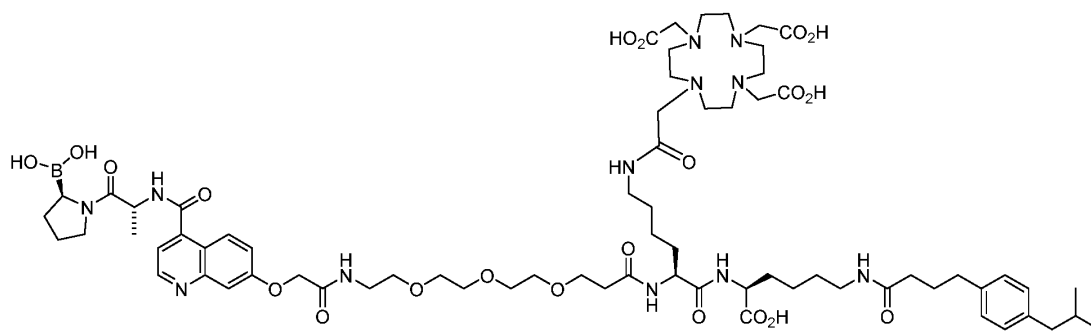
Also included in the invention are the compounds shown below, both pharmaceutically acceptable salts thereof and the neutral form. Chelation with a radionuclide is also included in the invention.



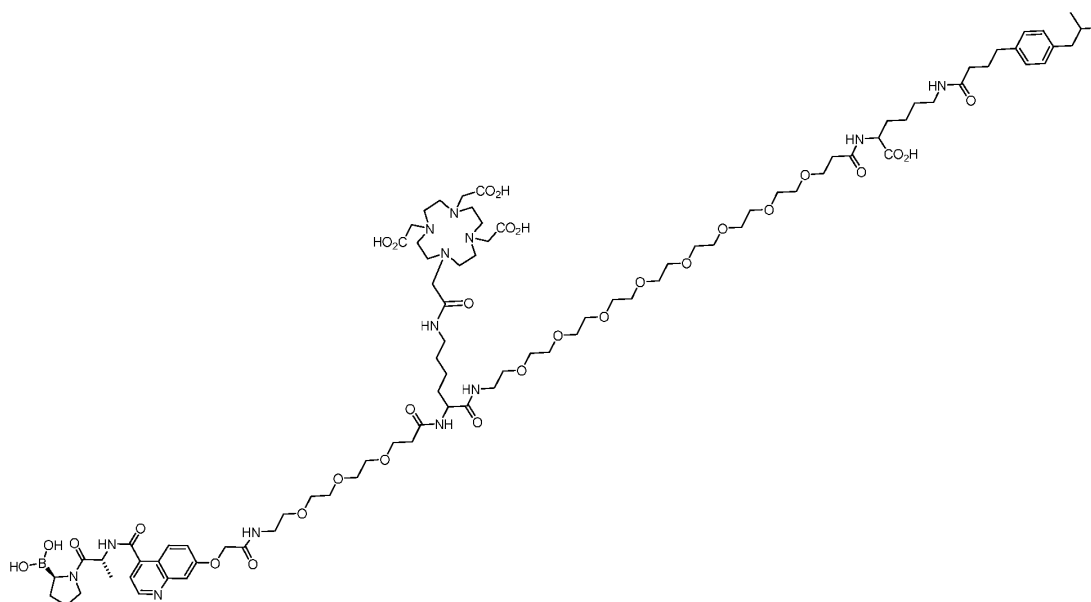
RTX-1371R



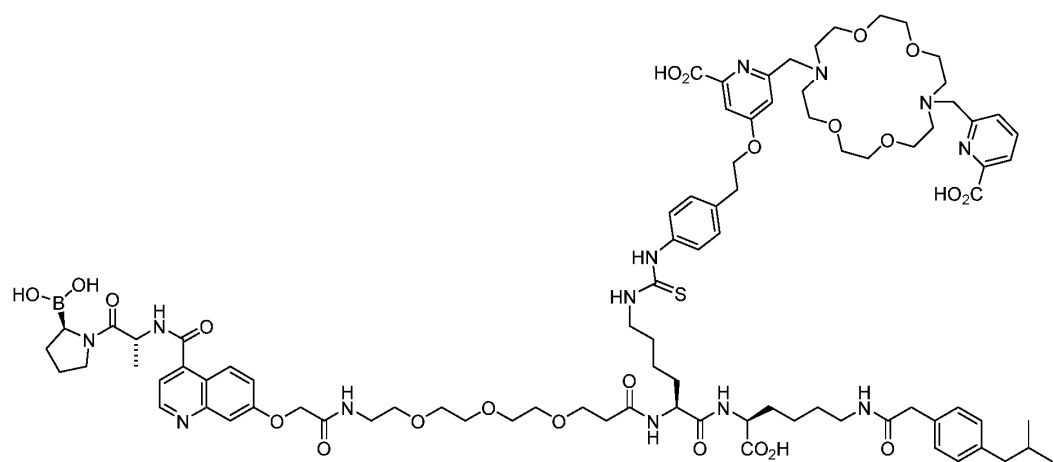
RTX-1384S



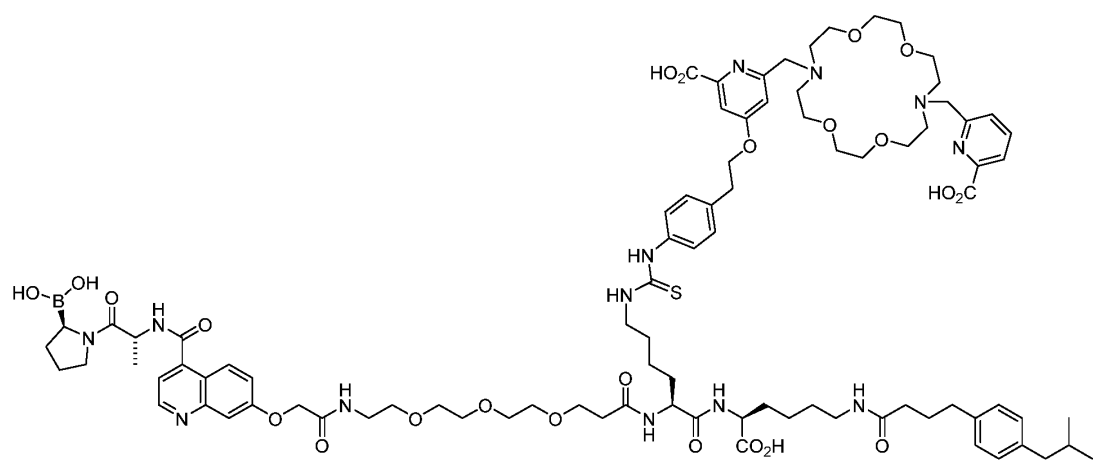
RTX-1391R



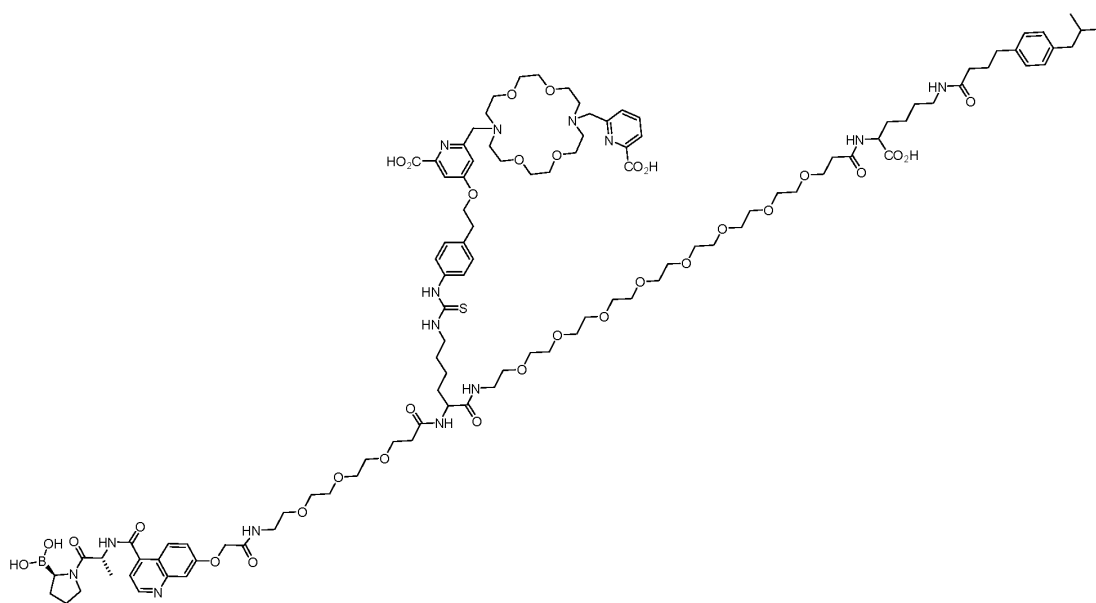
RTX-1392R



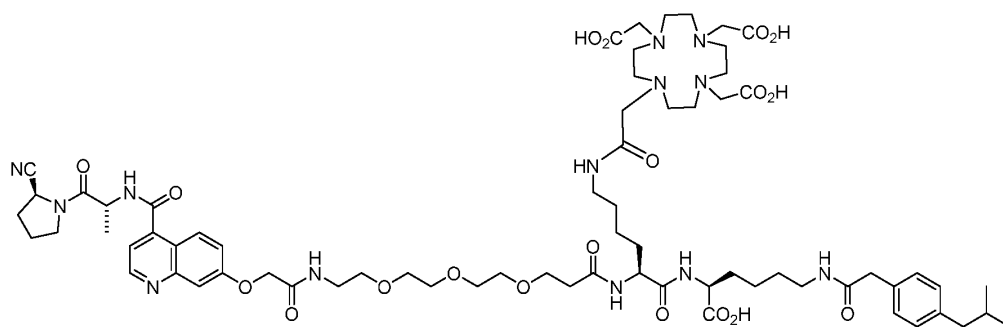
RTX-1400R



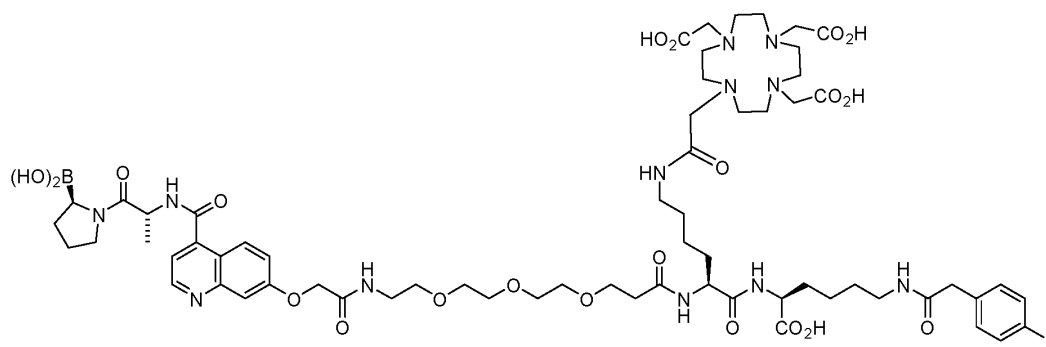
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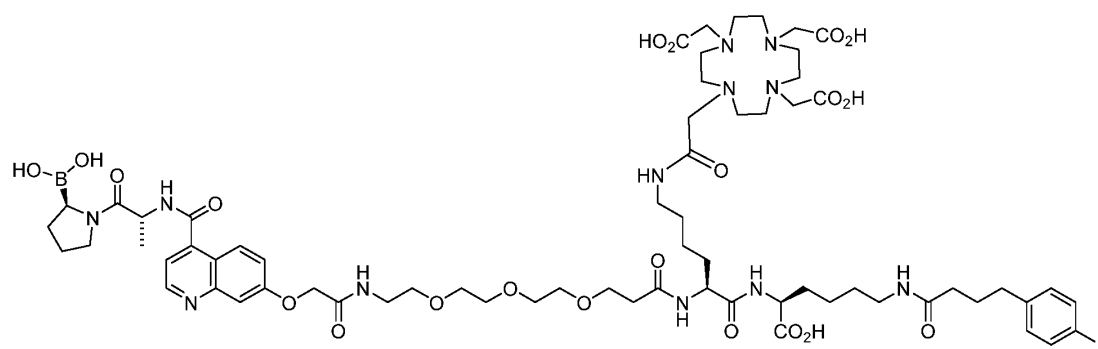
RTX-1402R



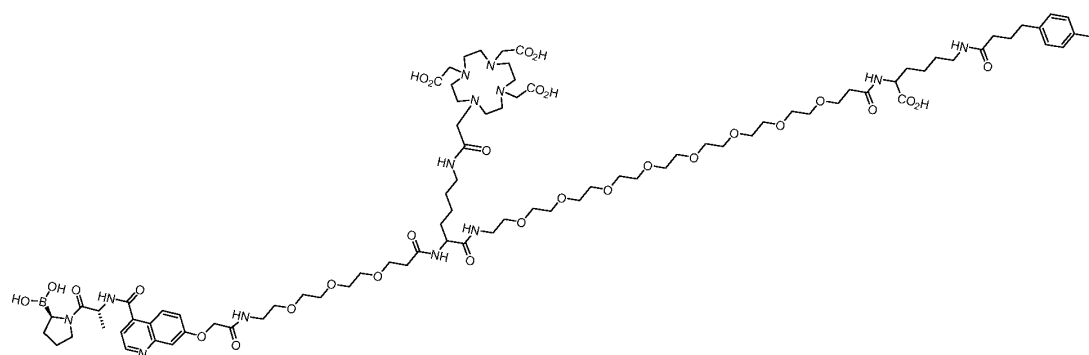
RTX-1407S



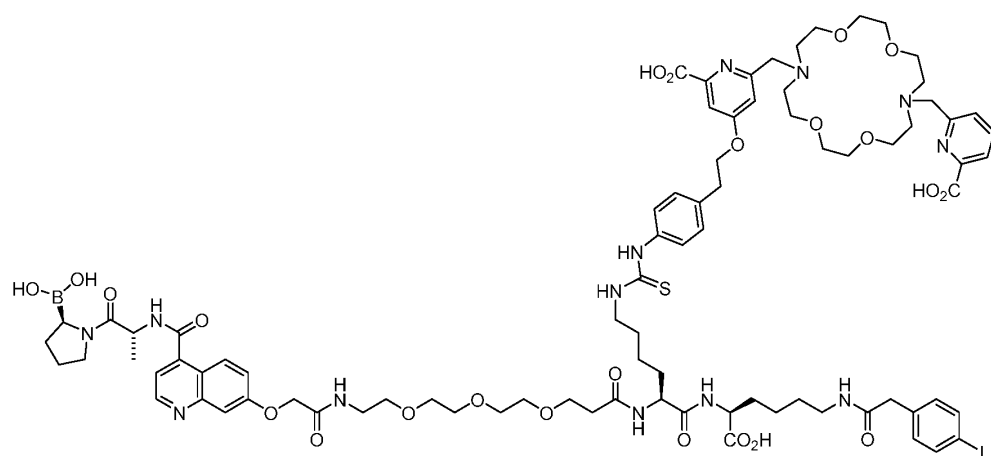
RTX-1409R



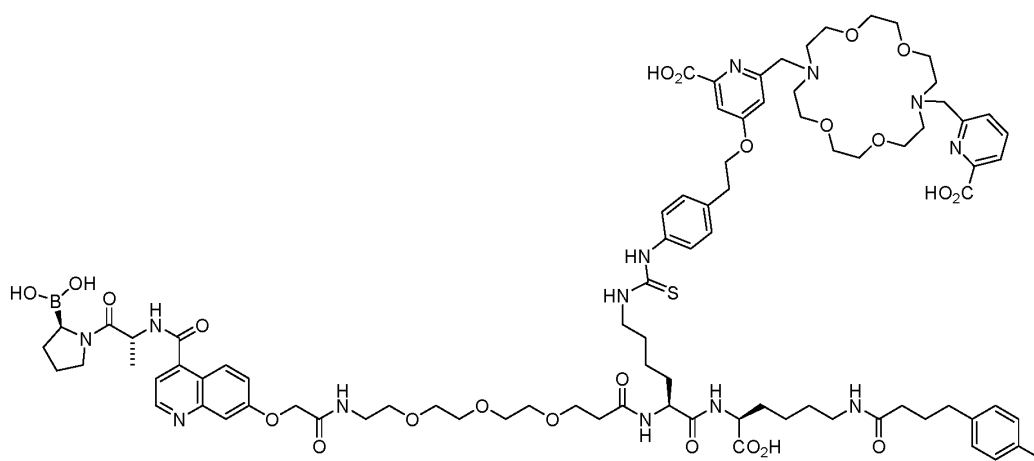
RTX-1410R



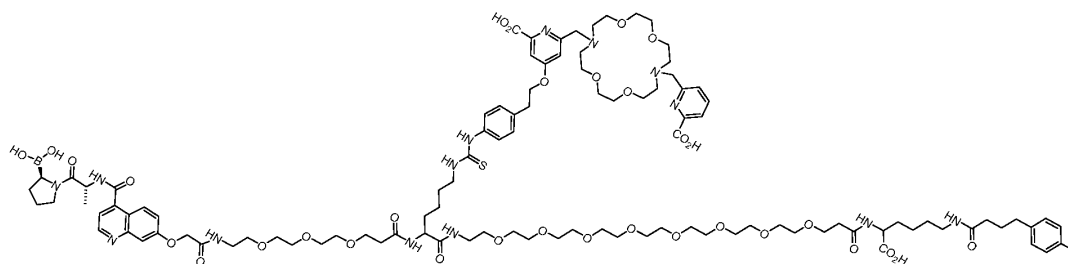
RTX-1411R



RTX-1413R



RTX-1414R



RTX-1415R

The nomenclature in which a compound name is preceded by an isotope indicates that the isotope is chelated to the chelating group of the compound. For example, “[⁶⁸Ga]RTX-1371R” refers to RTX-1371R in which its chelating group is chelated with ⁶⁸Ga. Exemplary compounds of the invention (with their chelating group) include [68Ga]RTX-1371R; [68Ga]RTX-1391R; [68Ga]RTX-1392R; [68Ga]RTX-1411R; [212Pb]RTX-1371R; [212Pb]RTX-1391R; [212Pb]RTX-1392R; [212Pb]RTX-1411R; [90Y]RTX-1371R; [90Y]RTX-1391R; [90Y]RTX-1392R; [90Y]RTX-1411R; [117Sn]RTX-1371R; [117Sn]RTX-1391R; [117Sn]RTX-1392R; [117Sn]RTX-1411R; [186Re]RTX-1371R; [186Re]RTX-1391R; [186Re]RTX-1392R; [186Re]RTX-1411R; [188Re]RTX-1371R;

[188Re]RTX-1391R; [188Re]RTX-1392R; [188Re]RTX-1411R; [169Er]RTX-1371R; [169Er]RTX-1391R; [169Er]RTX-1392R; [169Er]RTX-1411R; [153Sm]RTX-1371R; [153Sm]RTX-1391R; [153Sm]RTX-1392R; [153Sm]RTX-1411R; [223Ra]RTX-1371R; [223Ra]RTX-1391R; [223Ra]RTX-1392R; [223Ra]RTX-1411R; [67Cu]RTX-1371R; [67Cu]RTX-1391R; [67Cu]RTX-1392R; [67Cu]RTX-1411R; [161Tb]RTX-1371R; [161Tb]RTX-1391R; [161Tb]RTX-1392R; [161Tb]RTX-1411R; [213Bi]RTX-1371R; [213Bi]RTX-1391R; [213Bi]RTX-1392R; [213Bi]RTX-1411R; [166Ho]RTX-1371R; [166Ho]RTX-1391R; [166Ho]RTX-1392R; [166Ho]RTX-1411R; [149Tb]RTX-1371R; [149Tb]RTX-1391R; [149Tb]RTX-1392R; [149Tb]RTX-1411R; [47Sc]RTX-1371R; [47Sc]RTX-1391R; [47Sc]RTX-1392R; [47Sc]RTX-1411R; [227Th]RTX-1371R; [227Th]RTX-1391R; [227Th]RTX-1392R; [227Th]RTX-1411R; [177Lu]RTX-1371R; [177Lu]RTX-1391R; [177Lu]RTX-1392R; [177Lu]RTX-1411R; [225Ac]RTX-1371R; [225Ac]RTX-1391R; [225Ac]RTX-1392R; and [225Ac]RTX-1411R.

Compounds with Macropa chelators are commonly chelated with 225Ac.

Accordingly, another compound of the invention (with their chelating group) includes [Ac225]RTX-1400R, [Ac225]RTX-1401R, [Ac225]RTX-1402R, [Ac225]RTX-1407S, [Ac225]RTX-1413R, [Ac225]RTX-1414R, [Ac225]RTX-1415R, [134Ce]RTX-1400R, [134Ce]RTX-1401R, [134Ce]RTX-1402R, [134Ce]RTX-1407S, [134Ce]RTX-1413R, [134Ce]RTX-1414R, and [134Ce]RTX-1415R.

“Aliphatic” means a saturated or unsaturated straight-chain or branched monovalent or bivalent hydrocarbon radical. Unless otherwise specified, an aliphatic group typically has 1 to 10 carbon atoms. “Alkyl” means a saturated aliphatic straight-chain or branched monovalent aliphatic radical. Unless otherwise specified, an alkyl group typically has 1 to 10 carbon atoms (C₁₋₁₀ alkyl), alternatively, 1 to 6 carbon atoms (C₁₋₃ alkyl) (i.e., 1, 2 or 3).

“Cyclic aliphatic” means a saturated or unsaturated, monovalent or bivalent, cyclic hydrocarbon ring radical. Unless otherwise specified, a cyclic aliphatic has 3 to 8 ring carbon atoms (C₃₋₈ cycloalkyl). “Cycloalkyl” means a saturated aliphatic cyclic aliphatic. Unless otherwise specified, a cycloalkyl has 3 to 8 ring carbon atoms.

“Aryl”, alone or part or a larger moiety such as “aralkyl” is carbocyclic aromatic group such as phenyl or naphthyl.

“Aralkyl” refers to an alkyl group substituted with an aryl group. “C₁-C_x aralkyl” refers to an aralkyl group in which the alkyl portion has 1 to x carbon atoms.

Compounds having one or more chiral centers can exist in various stereoisomeric forms, *i.e.*, each chiral center can have an *R* or *S* configuration or can be a mixture of both. Stereoisomers are compounds that differ only in their spatial arrangement. Stereoisomers include all diastereomeric and enantiomeric forms of a compound. Enantiomers are stereoisomers that are non-superimposable mirror images of each other. Diastereomers are stereoisomers having two or more chiral centers that are not identical and are not mirror images of each other.

When the stereochemical configuration at a chiral center in a compound having one or more chiral centers is depicted by its chemical name (*e.g.*, where the configuration is indicated in the chemical name by “*R*” or “*S*”) or structure (*e.g.*, the configuration is indicated by “wedge” bonds), the enrichment of the indicated configuration relative to the opposite configuration is greater than 50%, 60%, 70%, 80%, 90%, 99% or 99.9%. “Enrichment of the indicated configuration relative to the opposite configuration” is a mole percent and is determined by dividing the number of compounds with the indicated stereochemical configuration at the chiral center(s) by the total number of all of the compounds with the same or opposite stereochemical configuration in a mixture.

When a disclosed compound having a chiral center is depicted by a structure without showing a configuration at that chiral center, the structure is meant to encompass the compound with the *S* configuration at that chiral center, the compound with the *R* configuration at that chiral center, or the compound with a mixture of the *R* and *S* configuration at that chiral center. When a disclosed compound having a chiral center is depicted by its chemical name without indicating a configuration at that chiral center with “*S*” or “*R*”, the name is meant to encompass the compound with the *S* configuration at that chiral center, the compound with the *R* configuration at that chiral center or the compound with a mixture of the *R* and *S* configuration at that chiral center.

The FAP-targeted compounds of the present invention are useful imaging agents for diagnostic applications. For example, these can be conjugated to various metals for magnetic resonance imaging applications or conjugated to an optical dye or a fluorophore or other detectable moiety (*i.e.*, dyes, quantum dots, etc.) for histochemistry applications and luminescence imaging applications. Likewise, the compounds can be radiolabeled and used in nuclear medicine applications. Radionuclides that can be used for imaging applications are referred to herein as “imaging radionuclides”. Nonlimiting examples of imaging

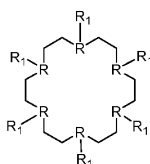
radionuclides include ^{18}F , ^{64}Cu or ^{68}Ga , which are suitable for use in PET imaging applications, and ^{67}Cu or ^{177}Lu , which are typically therapy nucleotides but are also suitable for use in SPECT imaging applications.

The FAP-targeting compounds of the present invention are useful therapy compounds. Such a therapy compound includes a FAP-targeted compound of the invention with a suitable therapeutic moiety. The FAP-targeted compound may be separated from the therapeutic moiety by a covalent linker. The separation between these (on the basis of a contiguous atom count) may be from about 4 atoms to about 100 atoms. Furthermore, by including additional targeting structures on the compound, the pharmacokinetics of the compound can be altered. For example, using a blood-targeting moiety it is possible to increase circulatory residence time, which has the effects of increasing tumor perfusion and loading while reducing accumulation of the radiotherapy compound in non-target tissues. See, for example U.S. Patent 11,285,277 which describes a trifunctional (“Trillium”) compound having a tumor-targeting domain, a blood protein binding domain and a third domain constituting a cytotoxic or cytostatic therapeutic agent. In a currently preferred embodiment, the FAP-binding compounds of the present invention are capable of being adapted to the Trillium scaffold by covalent linkage, to constitute the tumor-targeting domain for such FAP-targeted Trillium agents. Exemplary constructs include drug conjugates having a toxin, a venom, a metabolic poison, or a chemotherapy drug, as well as radiotherapy compounds having an alpha-emitting radionuclide, a beta-emitting radionuclide, an Auger electron emitting radionuclide or one that emits a spectrum of radiation upon decay (including positron emissions, which are also suitable for diagnostic uses).

For radiotherapy, the FAP-targeted compound is conjugated to a chelator, which is selected based on its suitability to hold an appropriate therapeutic radionuclide. A “therapeutic radionuclide” is a radionuclide that can be used for therapeutic purposes, e.g., for treating cancer or fibrotic tissue due to their radioactive emissions, which have cytotoxic effects on targeted tissues (i.e., FAP-expressing cancers and tumor microenvironments, malignancies, and fibrotic cells). Although targeted radiotherapy has been practiced for some time using macrocyclic complexes of radionuclides, the macrocycles currently in use (e.g., DOTA) generally form complexes with many therapeutic radionuclide metals, such as actinium, radium, bismuth, astatine, lutetium, and lead isotopes among others. Instability of many known macrocyclic-containing compounds can result in some dissociation of the radionuclide from the macrocycle, and this results in a lack of selective delivery to the

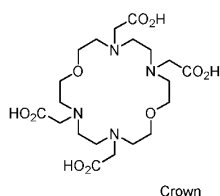
intended targeted tissue, which can also result in toxicity to non-targeted tissue. Alpha-emitting radionuclides such as ^{225}Ac can provide much greater cytotoxic effects, and thus, for therapy are considered substantially more potent than beta-emitting radionuclides. But this toxicity requires a chelator with increased retention of the chelated metal. U.S. Patent 11,279,698 (see also, PCT/US2018/025488, and PCT/US2019/062479) describes a novel chelator (“Macropa”) and its’ use as a chelator for ^{225}Ac , including as a component of a Trillium PK-tuned, targeted radiotherapy agent. The ratio of tumor activity to kidney activity of 1 or greater may persist up to about 36 hours after administration of the radiotherapeutic, and in the case of an ^{225}Ac Trillium-based therapeutic may persist for 72, or even 128 hours, or longer, maximizing the therapeutic effects of the radiation on the target tissues.

Accordingly, an exemplary preferred FAP-targeted Trillium compound will have in its’ third (non-targeting) domain, a chelator. Macropa is the currently preferred chelator for ^{225}Ac -FAP-targeted Trillium compounds. (see also, PCT/CA2021/050226).

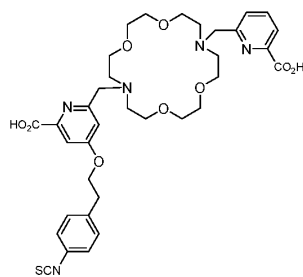
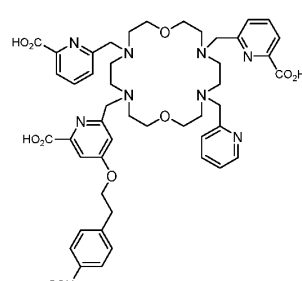
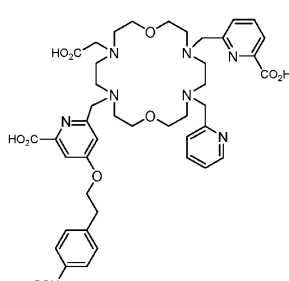
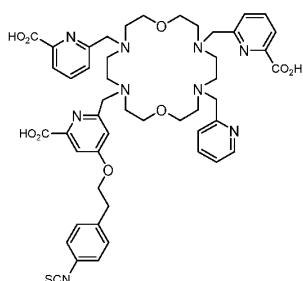
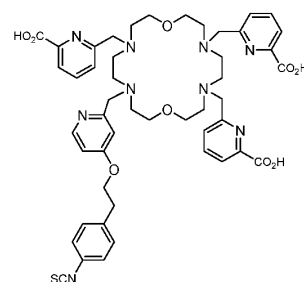
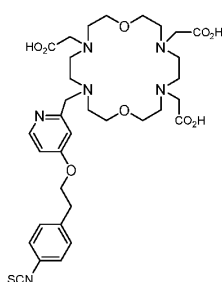


R can be O, N or S

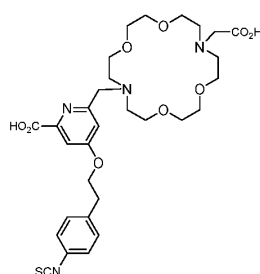
Some examples of R_1 are $-\text{CH}_2\text{CO}_2\text{H}$, alkyl, Targeting compound, 4-(4-isothiocyanatophenethoxy)picolinyl-2-methyl, picolinyl-2-methyl, 2-methyl pyridine, 2-methyl-4-(4-isothiocyanatophenethoxy)pyridine



Crown



Macropa



The radionuclide usable with the compounds disclosed herein depends on the application, radiation type desired and half-life as will be apparent to those of skill in the art. Exemplary radionuclides include: ^{177}Lu , ^{175}Lu , ^{45}Sc , ^{47}Sc , ^{64}Cu , ^{67}Cu , ^{68}Cu , ^{66}Ga , ^{67}Ga , ^{68}Ga , ^{69}Ga , ^{71}Ga , ^{90}Y , ^{89}Y , ^{86}Y , ^{89}Zr , ^{90}Y , $^{99\text{m}}\text{Tc}$, ^{111}In , ^{113}In , ^{115}In , ^{117}Sn , ^{153}Sm , ^{139}La , ^{134}Ce , ^{136}Ce , ^{138}Ce , ^{140}Ce , ^{142}Ce , ^{151}Eu , ^{153}Eu , ^{152}Dy , ^{149}Tb , ^{159}Tb , ^{154}Gd , ^{155}Gd , ^{156}Gd , ^{157}Gd , ^{158}Gd , ^{160}Gd , ^{161}Tb , ^{166}Ho , ^{169}Er , ^{188}Re , ^{186}Re , ^{213}Bi , ^{211}At , ^{217}At , ^{227}Th , ^{226}Th , ^{225}Ac , ^{233}Ra , ^{152}Dy , ^{213}Bi , ^{212}Bi , ^{211}Bi , ^{203}Pb , ^{212}Pb , ^{223}Ra , ^{255}Fm , and uranium-230. The radionuclide of any embodiment herein may be both a therapeutic radionuclide, and a diagnostic radionuclide

depending on its' decay profile. Currently preferred alpha-emitting radionuclides for therapy applications include ^{225}Ac , ^{233}Ra , and ^{212}Pb . Currently preferred beta-emitting radionuclides for therapy applications include ^{177}Lu , ^{90}Y , and ^{67}Cu .

Chelating groups and polyaza polycarboxylic macrocycles useful in the present technology include, and refer to a group that can chelate, bind or otherwise deliver a radionuclide to a therapeutic or diagnostic target. A chelating group is the residue of a chelating agent after the chelating agent reacts with a nucleophilic group in a compound to form a targeted bivalent radio pharmaceutical or radio diagnostic agent that can bind and deliver a radionuclide. In the case of the disclosed compounds, the reactive group is the side chain amine of the lysyl group in the penultimate precursor that reacts with the chelating agent to form the disclosed compounds. Examples of chelating agents include, but are not limited to, a covalently conjugated substituted or unsubstituted member of the following group: 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), p-SCN-Bn-NOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), p-SCN-Bn-DOTA (also known as 2B-DOTA-NCS), PIP-DOTA, diethylenetriaminepentaacetic acid (DTPA), PIP-DTPA, AZEP-DTPA, ethylenediamine tetraacetic acid (EDTA), triethylenetetraamine-N,N',N'',N''',N''''-hexa-acetic acid (TTHA), 7-[2-(bis-carboxymethylamino)-ethyl]-4,10-bis-carboxymethyl-1,4,7,10-tetraaza-cyclododec-1-yl-acetic acid (DEPA), 2,2',2''-(10-(2-(bis(carboxymethyl)amino)-5-(4-isothiocyanatophenyl)pentyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (3p-C-DEPA-NCS), NETA, {4-carboxymethyl-7-[2-(carboxymethylamino)-ethyl]-perhydro-1,4,7-triazonin-1-yl}-acetic acid (NPTA), diacetylpyridinebis(benzoylhydrazone), 1,4,7,10,13,16-hexaazacyclooctadecane N,N',N'',N''',N''''-hexa-acetic acid (HEHA), octadentate terephthalamide ligands, 2,2'-(4-(2-(bis(carboxymethyl)amino)-5-(4-isothiocyanatophenyl)pentyl)-10-(2-(bis(carboxymethyl)amino)ethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid, N,N'-bis[(6-carboxy-2-pyridyl)methyl]-4,13-diaza-18-crown-6 (H2macropa), 6-((16-((6-carboxypyridin-2-yl)methyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecan-7-yl)methyl)-4-isothiocyanatopicolinic acid (macropa-NCS), 6-((16-((6-carboxypyridin-2-yl)methyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecan-7-yl)methyl)-4-isocyanatopicolinic acid (macropa-NCO), 3,9-carboxymethyl-6-(2-methoxy-5-isothiocyanatophenyl)carboxymethyl-3,6,9,15-tetraazabicyclo-[9.3.1]pentadeca-1(15),11,13-triene and 2-[4,7,10-tris(2-amino-2-oxoethyl)-1,4,7,10-tetraazacyclododec-1-yl]acetamide (TCMC or DOTAM). In a currently preferred embodiment, the chelator is the residue of a polyaza polycarboxylic macrocycle,

such as Macropa NCS or NCO-Macropa. In another aspect, the chelator is the residue of a siderophores, In one aspect, ^{225}Ac is the radionuclide for Macropa NCS or NCO-Macropa. In another aspect, the chelating agent is the residue of p-SCN-Bn-DOTA, p-SCN-Bn-NOTA, NOTA or DOTA. In another embodiment, the chelating agent is sarcophagene chelator. In another aspect, the chelating agent is the residue of p-SCN-Bn-DOTA, p-SCN-Bn-NOTA, NOTA or DOTA chelated with ^{68}Ga .

As noted above, complexes of the disclosed compounds or pharmaceutically acceptable salts thereof may contain one or more radionuclides which are suitable for use as radio-imaging agents. Imaging methods include positron emission tomography (PET) or single photon emission computed tomography (SPECT). Accordingly in another aspect, the invention provides for theranostic applications, i.e., methods where a subject with a cancer, a tumor or a fibrotic disease is administered an effective amount of a disclosed compound (or a pharmaceutically acceptable salt thereof) having a chelator, which is complexed to an imaging radionuclide for imaging applications, and administered an effective amount of the compound complexed to a therapeutic radionuclide for treatment. Exemplary cancers which can be imaged and/or treated with the disclosed compounds or a pharmaceutically acceptable salt thereof include pancreatic cancer, liver cancer, gall bladder cancer, neuroblastoma, breast cancer, ovarian cancer, esophageal cancer, kidney cancer, prostate cancer, colorectal cancer, soft tissue sarcoma, bone sarcoma or melanoma.

A "subject" is a mammal in need of medical treatment or diagnosis, preferably a human, but can also be an animal in need of veterinary treatment, e.g., companion animals (e.g., dogs, cats, and the like), farm animals (e.g., cows, sheep, pigs, horses, and the like) and laboratory animals (e.g., rats, mice, guinea pigs, and the like).

The disclosed compounds or pharmaceutically acceptable salts thereof (including chelation with a radionuclide) or pharmaceutical compositions thereof, may be administered orally or via a parenteral route, usually injection or infusion. A "parenteral administration route" means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal and intrasternal injection and infusion.

“Effective amount” of the disclosed compounds or pharmaceutically acceptable salts thereof (including chelation with a radionuclide) means that amount of therapeutic agent alone, or in combination with other therapies, that provides a therapeutic benefit in the treatment or prevention of a disease, that improves overall therapy, reduces or avoids symptoms or causes of disease, or enhances the therapeutic efficacy of or synergies with another therapeutic agent. With respect to imaging, “diagnostically effective amount” refers to the amount which will result in a useful image for diagnosing, e.g., the presence of a tumor. “Effective amount” of the disclosed compounds or pharmaceutically acceptable salt thereof, is determined by the physician on the basis of the patient-specific parameters, such as age, weight, sex, severity of the disease, etc. The dosage is preferably from 0.0001 mg/kg to 100 mg/kg body weight.

Corresponding to the kind of administration, the medicament is suitably formulated, e.g. in the form of solutions or suspensions, simple tablets or dragees, hard or soft gelatine capsules, suppositories, ovules, preparations for injection, which are prepared according to common galenic methods.

When solutions for infusion or injection are used, they are preferably aqueous solutions or suspensions, it being possible to produce them prior to use, e.g. from lyophilized preparations which contain the active substance as such or together with a carrier, such as mannitol, lactose, glucose, albumin and the like. The ready-made solutions are sterilized and, where appropriate, mixed with excipients, e.g. with preservatives, stabilizers, emulsifiers, solubilizers, buffers and/or salts for regulating the osmotic pressure. The sterilization can be obtained by sterile filtration using filters having a small pore size according to which the composition can be lyophilized, where appropriate. Small amounts of antibiotics can also be added to ensure the maintenance of sterility.

According to another aspect, a pharmaceutical composition is provided, which is suitable for *in vivo* imaging and/or radiotherapy of a target tissue. Suitable pharmaceutical compositions may contain a radioimaging agent that has a radionuclide either as an element, (i.e., ^{18}F), or a diagnostic radioactive metal chelate complex (e.g., with ^{64}Cu or ^{68}Ga), or a radiotherapeutic agent which is radioactive metal chelate complex, in an amount sufficient for binding to the target tissue, together with a pharmaceutically acceptable radiological vehicle. The radiological vehicle should be suitable for injection or aspiration, such as human serum albumin; aqueous buffer solutions, e.g., tris(hydromethyl) aminomethane (and its

salts), phosphate, citrate, bicarbonate, etc; sterile water physiological saline; and balanced ionic solutions containing chloride and or dicarbonate salts or normal blood plasma cautions such as calcium potassium, sodium and magnesium.

The concentration of the radiopharmaceutical agent in the radiological vehicle should be sufficient to provide reasonable binding to the target tissue, such as about 4% to 20% ID/gram. For example, when using an aqueous solution, the human dosage can range from about 1.0 to 500 millicuries of activity. The actual dose administered to a patient for imaging or therapeutic purposes, however, is determined by the physician administering treatment. The imaging agent or therapeutic agent should be administered so as to remain in the patient for about 1 hour to 10 days, although both longer and shorter time periods are acceptable. Therefore, convenient ampoules containing 1 to 10 mL of aqueous solution may be prepared.

Imaging may be carried out in the normal manner, for example by injecting a sufficient amount of the imaging composition to provide adequate imaging and then scanning with a suitable imaging or scanning machine, such as a tomograph or gamma camera. In certain embodiments, a method of imaging a region in a patient includes the steps of: (i) administering to a patient a diagnostically effective amount of a compound complexed with a radionuclide; exposing a region of the patient to the scanning device; and (ii) obtaining an image of the region of the patient. Accordingly, the invention provides a method for obtaining an image of a mammalian subject following administration of the compound. Likewise, imaging can be performed after administration of a therapeutic drug or radiotherapy cycle to assess efficacy. Thus, obtaining an image after administration of the radiotherapeutic may occur after about 1 hour, about 4 hours, about 9 hours, about 12 hours, about 16 hours, about 20 hours, about 24 hours, about 36 hours, about 48 hours, about 72 hours, about 96 hours, about one week, about two weeks, about four weeks, or after completion of a cycle of therapeutic treatments. Thus, in some embodiments, a method of imaging tissue such as FAP-expressing tumor tissue is provided including contacting the tissue with a complex synthesized by contacting an imaging radionuclide with a disclosed compound.

According to another aspect, complexes of the disclosed compounds or pharmaceutically acceptable salts thereof may contain one or more radionuclides which are suitable for use as radio-imaging agents in the field of image guided radiation therapy (IGRT). As described in U.S. Patent No. US 10,688,320 B2, IGRT uses images acquired

before a treatment session to guide the application of therapeutic radiation during a treatment session. The concentration of the imaging agent or the therapeutic agent in the radiological vehicle should be sufficient to provide satisfactory imaging. For example, when using an aqueous solution, the dosage is about 1.0 to 100 millicuries. Imaging can be performed to provide a fiducial, for guidance for the target region to receive a calculated radiation fluence from a therapeutic radiation source. Similar uses of the compounds as fiducials can be used in guided surgery applications.

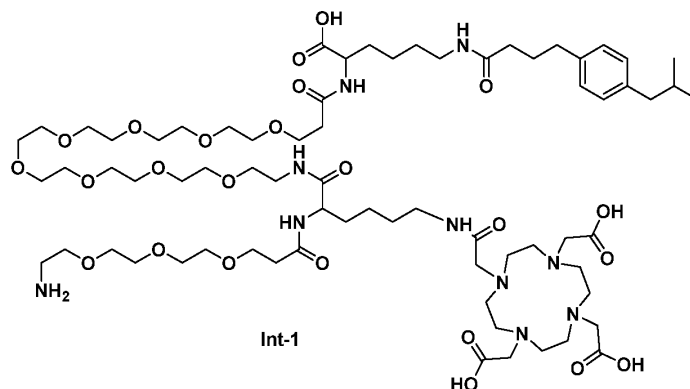
The amount of the compound of the present invention, or a formulation comprising a complex of a metal and a compound or pharmaceutically acceptable salt thereof that is administered to a patient depends on several physiological factors that are routinely used by the physician, including the nature of the procedure to be carried out, the volume of tissue to be targeted for imaging or therapy and the body weight and medical history of the patient to be imaged or treated using the compounds.

The examples herein are provided to illustrate advantages of the present technology and to further assist a person of ordinary skill in the art with preparing or using the compounds of the present technology or salts, pharmaceutical compositions, derivatives, prodrugs, or tautomeric forms thereof. The examples herein are also presented in order to more fully illustrate the preferred aspects of the present technology. The examples should in no way be construed as limiting the scope of the present technology, as defined by the appended claims. The examples can include or incorporate any of the variations, aspects or embodiments of the present technology described above. The variations, aspects or embodiments described above may also further each include or incorporate the variations of any or all other variations, aspects or embodiments of the present technology.

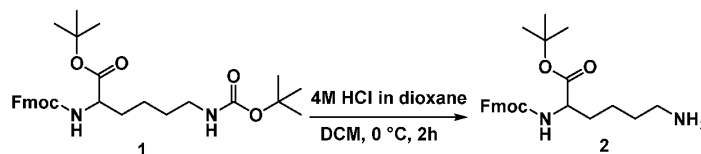
EXEMPLIFICATION

Example 1 – Synthesis of Compounds

Preparation of Int-1:

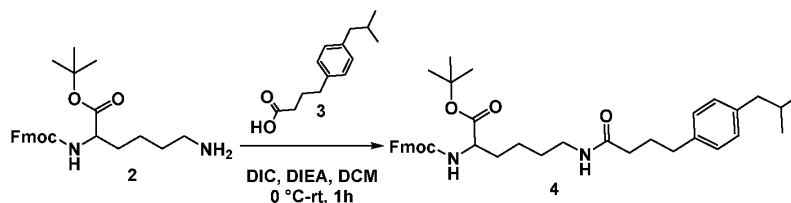


Procedure 1: Synthesis of tert-butyl 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-aminohexanoate (2):



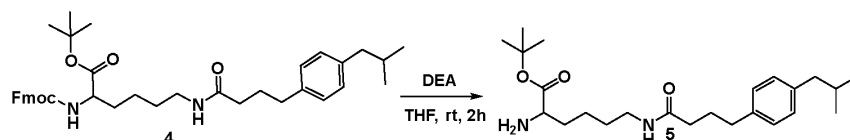
To a solution of tert-butyl 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)hexanoate (2.0 g, 3.81 mmol) in DCM (10 ml), was added, dropwise, HCl in dioxane (4 M, 20 ml) at 0 °C, and the reaction mixture was stirred at 0 °C for 2 h. The solvents were evaporated and the residue was co-evaporated with hexane and dried in vacuo to obtain HCl salt of the product (1.62 g, 100%) as a white solid. The crude product was used for the next step without further purification. LCMS: $\text{C}_{25}\text{H}_{32}\text{N}_2\text{O}_4$; m/z: 424.53, observed m/z = 425.3 $[\text{M}+\text{H}]^+$.

Procedure 2: Synthesis of tert-butyl 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-(4-(4-isobutylphenyl)-butanamido)hexanoate (4):



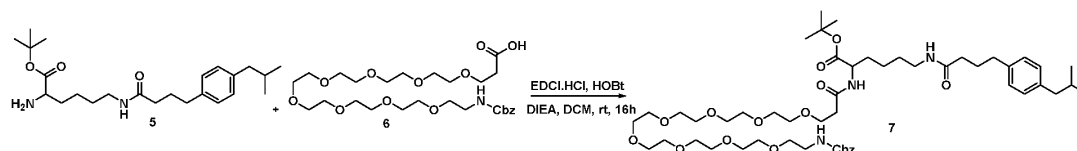
To a chilled solution of 4-(4-isobutylphenyl)butanoic acid (1.0 g, 4.57 mmol) in 10 ml of DCM was added DIC (0.71 ml, 4.47 mmol), dropwise, in an ice bath. The mixture was stirred for 30 minutes and filtered. The filtrate was added to a solution of tert-butyl 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-amino hexanoate (1.62 g, 3.81 mmol) in 5 ml of DCM, and followed by a dropwise addition of DIEA (0.66 ml, 3.81 mmol). The mixture was stirred for another 30 minutes. The solvents were evaporated under reduced pressure, and the crude product was purified by flash chromatography (EtOAc/hexane, eluted at 50% EtOAc in hexane) to afford the product (1.93 g, 82% yield) as a white solid. LCMS: $C_{39}H_{50}N_2O_5$; m/z : 626.82, observed $m/z = 627.4 [M+H]^+$.

Procedure 3: Synthesis of tert-butyl 2-amino-6-(4-(4-isobutylphenyl)butanamido)hexanoate (5):



To a solution of tert-butyl 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-(4-(4-isobutylphenyl)butanamido)-hexanoate (0.95 g, 1.51 mmol) in THF (8 ml) was added diethylamine (8 ml) at room temperature. The reaction mixture was stirred at ambient temperature for 2 hours. The solvent was evaporated, and the residue was washed with hexane (2x) and dried in vacuo to obtain the product (613 mg, 100%). The compound was used as is for the next chemical transformation. LCMS: $C_{24}H_{40}N_2O_3$; m/z : 404.59, observed $m/z = 405.4 [M+H]^+$.

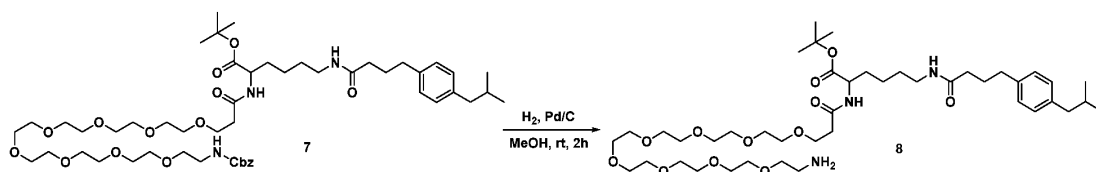
Procedure 4: Synthesis of tert-butyl 33-(4-(4-(4-isobutylphenyl)butanamido)butyl)-3,31-dioxo-1-phenyl-2,7,10,13,16,19,22,25,28-nona-4,32-diazatetracontan-34-oate (7):



To a solution of 3-oxo-1-phenyl-2,7,10,13,16,19,22,25,28-nona-4-azahentriacontan-31-oic acid (872 mg, 1.51 mmol) in 10 ml of DCM was added EDCI.HCl (434 mg, 2.27 mmol) and HOBt (203 mg, 1.51 mmol) and the reaction mixture was stirred at rt for 5 min. Tert-butyl 2-

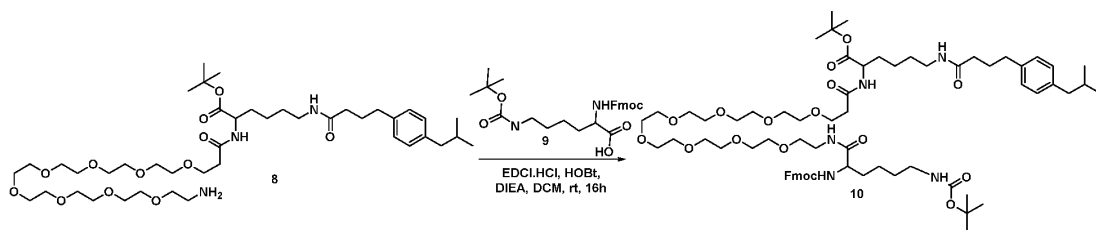
amino-6-(4-(4-isobutylphenyl)butanamido)hexanoate (613 mg, 1.51 mmol) in DCM (10 ml) and DIEA (0.8 ml, 4.53 mmol) were added sequentially and the mixture was stirred at room temperature for 16 hours. The reaction mixture was diluted with 20 ml of DCM and then washed with water. The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by flash chromatography (MeOH/DCM, eluted at 3-4% MeOH in DCM) to afford the product (1.0 g, 69% yield) as a colorless liquid. LCMS: C₅₁H₈₃N₃O₁₄: m/z: 962.22, observed m/z = 962.9 [M+H]⁺.

Procedure 5: Synthesis of tert-butyl 1-amino-29-(4-(4-(4-isobutylphenyl)butanamido)butyl)-27-oxo-3,6,9,12,15,18,21,24-octaoxa-28-azatriacontan-30-oate (8):



Palladium on carbon (10%, 0.2 g, wet, 67%) was suspended in a solution of tert-butyl 33-(4-(4-isobutylphenyl)butanamido)butyl)-3,31-dioxo-1-phenyl-2,7,10,13,16,19,22,25,28-nona-4,32-diazatetratriacontan-34-oate (1.0 g, 1.03 mmol) in MeOH (25 ml). The suspension was stirred for 2 h at room temperature under H₂ at balloon pressure. The mixture was filtered through celite, and the filtrate was evaporated to afford the product (850 mg, 100%) as a white solid. The crude product was used for the next step without further purification. LCMS: C₄₃H₇₇N₃O₁₂: m/z: 828.08, observed m/z = 828.7 [M+H]⁺.

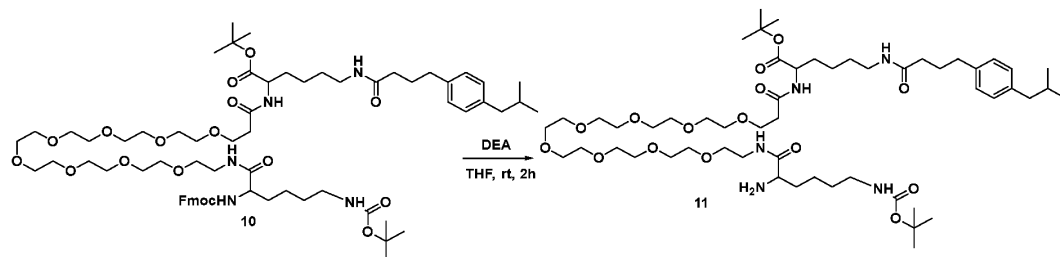
Procedure 6: Synthesis of tert-butyl 10-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-41-(4-(4-isobutylphenyl)butanamido)butyl)-2,2-dimethyl-4,11,39-trioxo-3,15,18,21,24,27,30,33,36-nona-5,12,40-triaza-dotetracontan-42-oate (10):



To a solution of 2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)hexanoic acid (0.48 g, 1.02 mmol) in 10 ml of DCM was added EDCI.HCl (0.29 g, 1.53 mmol) and HOBT (0.14 g, 1.02 mmol) and the reaction mixture was stirred at rt for 5 min. Tert-butyl 1-amino-29-(4-(4-(4-isobutylphenyl)butanamido)butyl)-27-

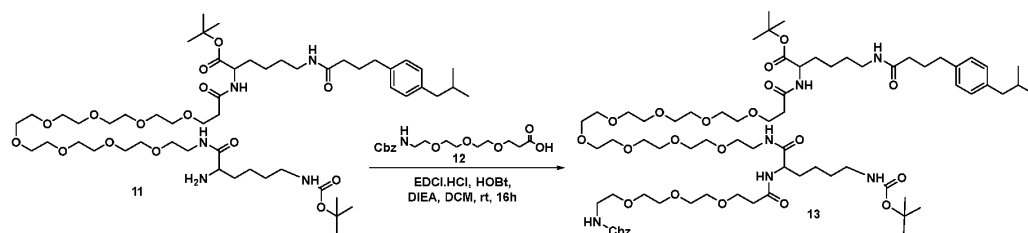
oxo-3,6,9,12,15,18,21,24-octaoxa-28-azatriacontan-30-oate (0.85 g, 1.02 mmol) in DCM (10 ml) and DIEA (0.53 ml, 3.07 mmol) were added sequentially and the mixture was stirred at room temperature for 16 hours. The reaction mixture was diluted with 20 ml of DCM and washed with water. The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by flash chromatography (MeOH/DCM, eluted at 5% MeOH in DCM) to afford the product (0.66 g, 50% yield) as a white solid. LCMS: C₆₉H₁₀₇N₅O₁₇: m/z: 1278.61, observed m/z = 1279.1 [M+H]⁺.

Procedure 7: Synthesis of tert-butyl 10-amino-41-(4-(4-(4-isobutylphenyl)butanamido)butyl)-2,2-dimethyl-4,11,39-trioxo-3,15,18,21,24,27,30,33,36-nonaoxa-5,12,40-triazadotetracontan-42-oate (11):



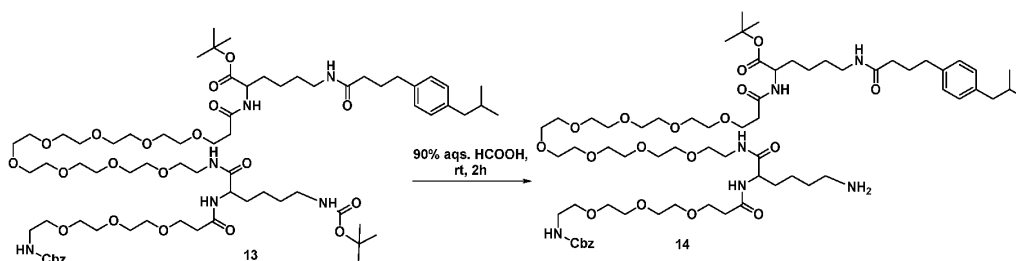
To a solution of tert-butyl 10-(((9H-fluoren-9-yl)methoxy)carbonyl)amino-41-(4-(4-(4-isobutylphenyl)butanamido)butyl)-2,2-dimethyl-4,11,39-trioxo-3,15,18,21,24,27,30,33,36-nonaoxa-5,12,40-triazadotetracontan-42-oate (0.66 g, 0.51 mmol) in THF (7 ml) was added diethylamine (7 ml) at room temperature. The reaction mixture was stirred at ambient temperature for 2 hours, and the solvents were evaporated. The residue was washed with hexane (2x) and dried in vacuo to obtain the product (700 mg, 100%). The compound was used for the next step without further purification. LCMS: C₅₄H₉₇N₅O₁₅: m/z: 1056.37, observed m/z = 1057.1 [M+H]⁺.

Procedure 8: Synthesis of tert-butyl 18-(4-((tert-butoxycarbonyl)amino)butyl)-49-(4-(4-(4-isobutylphenyl)butanamido)butyl)-3,16,19,47-tetraoxo-1-phenyl-2,7,10,13,23,26,29,32,35,38,41,44-dodecaoxa-4,17,20,48-tetraazapentacontan-50-oate (13):



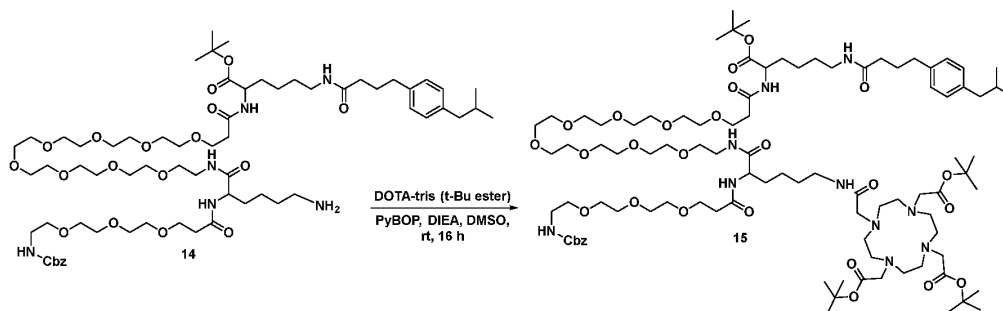
To a solution of 3-oxo-1-phenyl-2,7,10,13-tetraoxa-4-azahexadecan-16-oic acid (0.22 g, 0.62 mmol) in DCM (8 ml) was added EDCI.HCl (0.18 g, 0.93 mmol) and HOBt (0.08 g, 0.62 mmol) and the reaction mixture was stirred at rt for 5 min. Tert-butyl 10-amino-41-(4-(4-(4-isobutylphenyl)-butanamido)butyl)-2,2-dimethyl-4,11,39-trioxo-3,15,18,21,24,27,30,33,36-nona-5,12,40-triazadotetra-contan-42-oate (0.66 g, 0.62 mmol) in DCM (5 ml) and DIEA (0.32 ml, 1.87 mmol) were added sequentially and the mixture was stirred at room temperature for 16 hours. The reaction mixture was diluted with 20 ml of DCM and washed with water. The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by flash chromatography (MeOH/DCM, eluted at 8-10% MeOH in DCM) to afford the product (0.70 g, 50% yield) as a white solid. LCMS: C₇₁H₁₂₀N₆O₂₁: m/z: 1393.74, observed m/z = 1394.7 [M+H]⁺.

Procedure 9: Synthesis of tert-butyl 18-(4-aminobutyl)-49-(4-(4-(4-isobutylphenyl)butanamido)butyl)-3,16,19,47-tetraoxo-1-phenyl-2,7,10,13,23,26,29,32,35,38,41,44-dodecaoxa-4,17,20,48-tetraazapentacontan-50-oate (14):



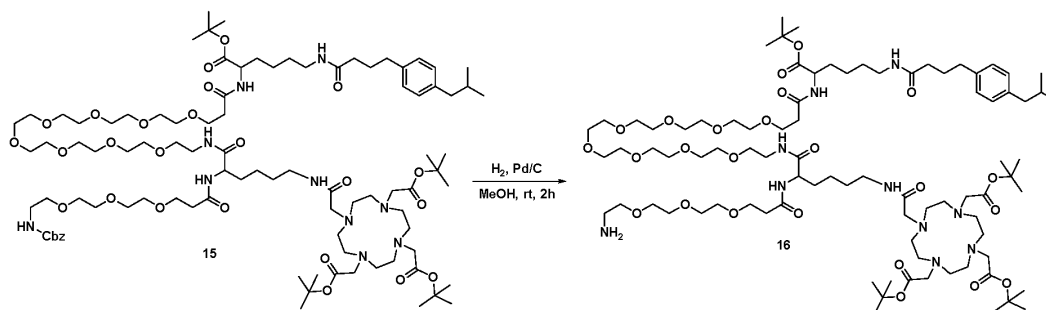
A solution of tert-butyl 18-(4-((tert-butoxycarbonyl)amino)butyl)-49-(4-(4-(4-isobutylphenyl)butanamido)butyl)-3,16,19,47-tetraoxo-1-phenyl-2,7,10,13,23,26,29,32,35,38,41,44-dodecaoxa-4,17,20,48-tetraazapentacontan-50-oate (0.70 g, 1.22 mmol) in 90% aq. formic acid (10 ml) was stirred at ambient temperature for 2 h. Full conversion was detected by LCMS. The solvents were evaporated, and the residue was co-evaporated with ACN (3x) and toluene (3x). The residue was dissolved in a mixture MeOH/water (1:1, 25 ml) and treated with ion-exchange resin AmberLite HPR550 (OH-form) at ambient temperature for 15 minutes. The resin was filtered and washed with MeOH and water. The filtrate was evaporated to obtain target material (free base, 0.62 g, 95% yield) as a colorless liquid. The crude amine was used for the step without further purification. LCMS: C₆₆H₁₁₂N₆O₁₉: m/z: 1293.62, observed m/z = 1294.1 [M+H]⁺.

Procedure 10: Synthesis of tri-tert-butyl 2,2',2''-(10-(39-(tert-butoxycarbonyl)-48-(4-isobutylphenyl)-2,9,37,45-tetraoxo-8-(3-oxo-1-phenyl-2,7,10,13-tetraoxa-4-azahexadecanamido)-13,16,19,22,25,28,31,34-octaoxa-3,10,38,44-tetraazaoctatetracontyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (15):



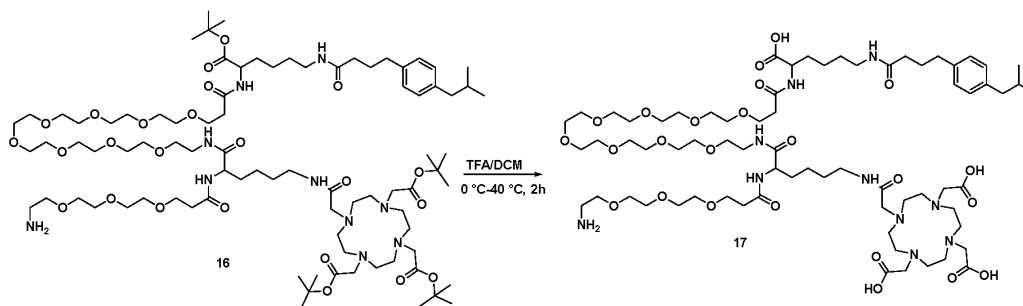
To a solution of tert-butyl 18-(4-aminobutyl)-49-(4-(4-(4-isobutylphenyl)butanamido)butyl)-3,16,19,47-tetraoxo-1-phenyl-2,7,10,13,23,26,29,32,35,38,41,44-dodecaoxa-4,17,20,48-tetraazapentacontan-50-oate (0.62 g, 0.48 mmol), DOTA-tris(t-Bu-ester) (0.27 g, 0.48 mmol) and PyBop (0.30 g, 0.56 mmol) in DMSO (8 ml) was added DIEA (0.20 ml, 1.15 mmol) and the reaction mixture was stirred at ambient temperature for 16 h. Water (20 ml) was added, and the mixture was extracted with EtOAc (30 ml). The separated organic layer was washed with water (7x) and brine (8x) to remove excess PyBOP. The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure obtained target product (820 mg, 93% yield) as colorless liquid. The compound was used for the next step without further purification. LCMS: C₉₄H₁₆₂N₁₀O₂₆: m/z: 1848.34, observed m/z = 1849.7 [M+H]⁺.

Procedure 11: Synthesis of tri-tert-butyl 2,2',2''-(10-(8-(3-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)propanamido)-39-(tert-butoxycarbonyl)-48-(4-isobutylphenyl)-2,9,37,45-tetraoxo-13,16,19,22,25,28,31,34-octaoxa-3,10,38,44-tetraazaoctatetracontyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (16):



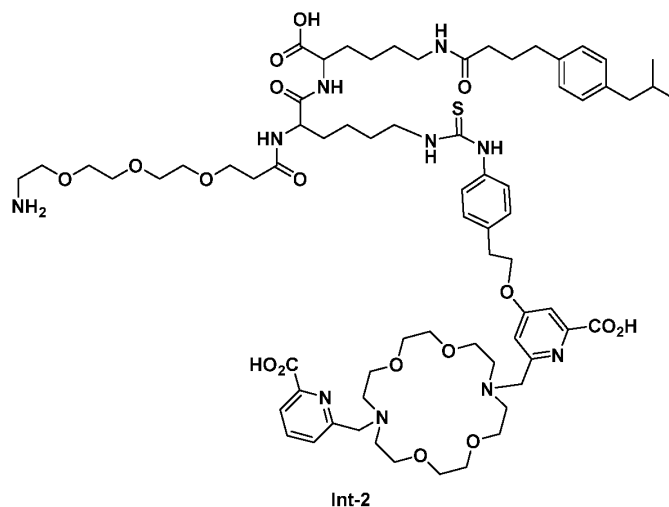
Palladium on carbon (10%, 0.12 g, wet, 67%) was suspended in a solution of tri-tert-butyl 2,2',2''-(10-(39-(tert-butoxycarbonyl)-48-(4-isobutylphenyl)-2,9,37,45-tetraoxo-8-(3-oxo-1-phenyl-2,7,10,13-tetraoxa-4-azahexadecanamido)-13,16,19,22,25,28,31,34-octaoxa-3,10,38,44-tetraazaooctatetracontyl)-1,4,7,10-tetra-azacyclododecane-1,4,7-triyl)triacetate (500 mg, 0.270 mmol) in MeOH (15 ml). The suspension was stirred for 2 h at room temperature under H₂ at balloon pressure. The mixture was filtered through celite, and the filtrate was evaporated to afford target compound (387 mg, 83% yield) as a colorless liquid. The crude product was used for the next step without further purification. LCMS: C₈₆H₁₅₆N₁₀O₂₄: m/z: 1714.21, observed m/z = 1714.7 [M+H]⁺.

Procedure 12: Synthesis of 2,2',2''-(10-(8-(3-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)propanamido)-39-carboxy-48-(4-isobutylphenyl)-2,9,37,45-tetraoxo-13,16,19,22,25,28,31,34-octaoxa-3,10,38,44-tetraazaooctatetracon-tyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (17):

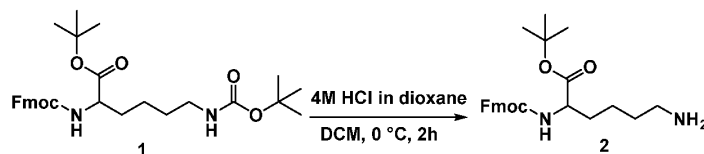


To a 0 °C solution of tri-tert-butyl 2,2',2''-(10-(8-(3-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)propanamido)-39-(tert-butoxycarbonyl)-48-(4-isobutylphenyl)-2,9,37,45-tetraoxo-13,16,19,22,25,28,31,34-octaoxa-3,10,38,44-tetraazaooctatetracontyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (387 mg, 0.225 mmol) in DCM (4 ml), was added TFA (2 ml). The reaction mixture was stirred at 40 °C for 2 h and the solvents were evaporated under reduced pressure. The residue was washed with hexane (2x) and ether (2x) to obtain the product (340 mg, 100%) as a white powder. The crude product was used for the next step without further purification. LCMS: C₇₀H₁₂₄N₁₀O₂₄: m/z: 1489.79, observed m/z = 1490.5 [M+H]⁺.

Preparation of Int 2:

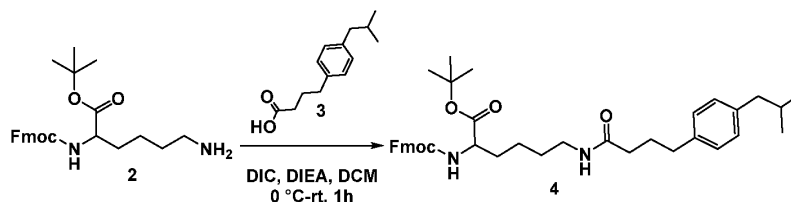


Procedure 13: Synthesis of tert-butyl 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-aminohexanoate (2):



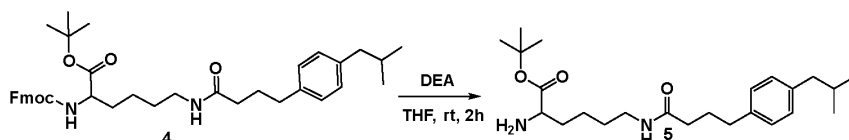
To a solution of tert-butyl 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)hexanoate (2.0 g, 3.81 mmol) in DCM (10 ml), was added, dropwise, HCl in dioxane (4 M, 20 ml) at 0 °C, and the reaction mixture was stirred at 0 °C for 2 h. The solvents were evaporated and the residue was co-evaporated with hexane and dried in vacuo to obtain HCl salt of the product (1.62 g, 100%) as a white solid. The crude product was used for the next step without further purification. LCMS: $C_{25}H_{32}N_2O_4$: m/z: 424.53, observed m/z = 425.3 $[M+H]^+$.

Procedure 14: Synthesis of tert-butyl 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-(4-(4-isobutylphenyl)-butanamido)hexanoate (4):



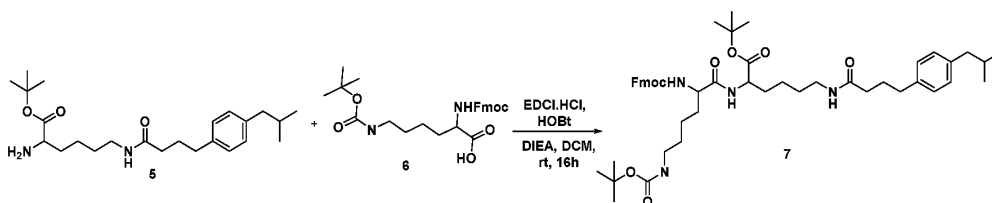
To a chilled solution of 4-(4-isobutylphenyl)butanoic acid (1.0 g, 4.57 mmol) in 10 ml of DCM was added DIC (0.71 ml, 4.47 mmol), dropwise, in an ice bath. The mixture was stirred for 30 minutes and filtered. The filtrate was added to a solution of tert-butyl 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-aminohexanoate (1.62 g, 3.81 mmol) in 5 ml of DCM, and followed by a dropwise addition of DIEA (0.66 ml, 3.81 mmol). The mixture was stirred for another 30 minutes. The solvents were evaporated under reduced pressure, and the crude product was purified by flash chromatography (EtOAc/hexane, eluted at 50% EtOAc in hexane) to afford the product (1.93 g, 82% yield) as a white solid. LCMS: $C_{39}H_{50}N_2O_5$; m/z : 626.82, observed $m/z = 627.4 [M+H]^+$.

Procedure 15: Synthesis of tert-butyl 2-amino-6-(4-(4-isobutylphenyl)butanamido)hexanoate (5):



To a solution of tert-butyl 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-(4-(4-isobutylphenyl)butanamido)-hexanoate (0.95 g, 1.51 mmol) in THF (8 ml) was added diethylamine (8 ml) at room temperature. The reaction mixture was stirred at ambient temperature for 2 hours. The solvent was evaporated, and the residue was washed with hexane (2x) and dried in vacuo to obtain the product (613 mg, 100%). The compound was used as is for the next chemical transformation. LCMS: $C_{24}H_{40}N_2O_3$; m/z : 404.59, observed $m/z = 405.4 [M+H]^+$.

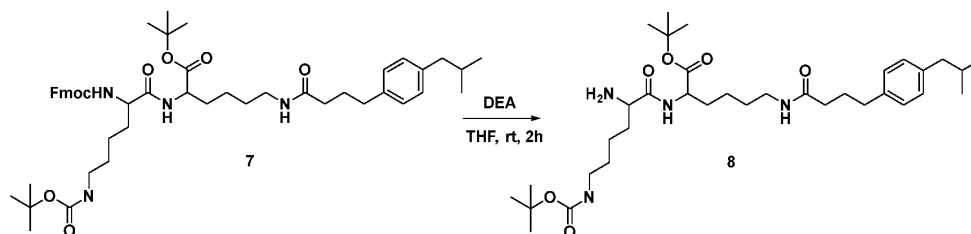
Procedure 16: Synthesis of tert-butyl 2-(2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-((tert-butoxycarbonyl)-amino)hexanamido)-6-(4-(4-isobutylphenyl)butanamido)hexanoate (7):



To a solution of 2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)hexanoic acid (1.50 g, 3.19 mmol) in 15 ml of DCM was added

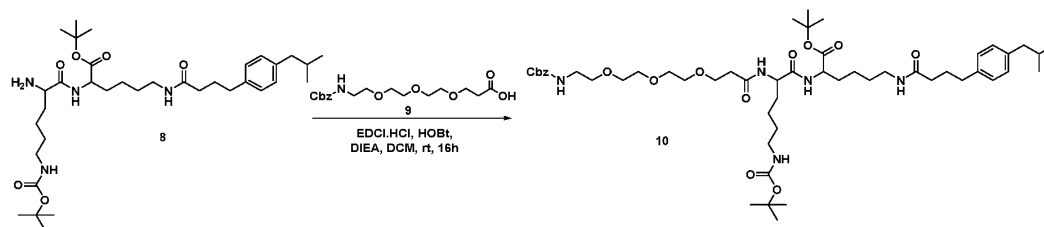
EDCI.HCl (0.92 g, 4.78 mmol) and HOBT (0.43 g, 3.19 mmol) and the reaction mixture was stirred at rt for 5 min. Tert-butyl 2-amino-6-(4-(4-isobutylphenyl)butanamido)hexanoate (1.29 g, 3.19 mmol) in DCM (10 ml) and DIEA (1.66 ml, 9.57 mmol) were added sequentially and the mixture was stirred at room temperature for 16 hours. The reaction mixture was diluted with DCM (20 ml) and washed with water. The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by flash chromatography (EtOAc/hexane, eluted at 75% EtOAc in hexane) to afford the product (1.18 g, 43% yield) as a glassy solid. LCMS: C₁₂H₂₄N₂O₃: m/z: 855.11, observed m/z = 245.4 [M+H]⁺.

Procedure 17: Synthesis of tert-butyl 2-(2-amino-6-((tert-butoxycarbonyl)amino)hexanamido)-6-(4-(4-isobutyl-phenyl)butanamido)hexanoate (8):



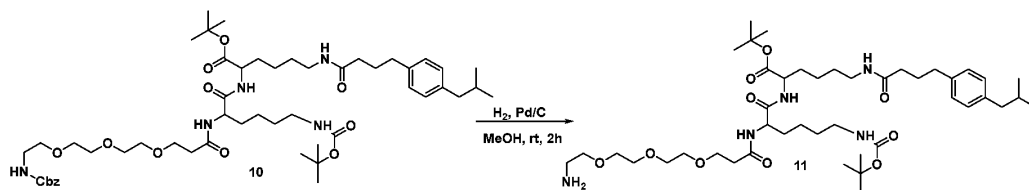
To a solution of tert-butyl 2-(2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-((tert-butoxycarbonyl)amino)hexanamido)-6-(4-(4-isobutylphenyl)butanamido)hexanoate (1.18 g, 1.38 mmol) in THF (10 ml) was added diethylamine (10 ml) at room temperature. The reaction mixture was stirred at ambient temperature for 2 hours. The solvents were evaporated and the residue was washed with hexane (2x) and dried in vacuo to obtain the product (900 mg, 100%). The compound was used for the next step without further purification. LCMS: C₃₅H₆₀N₄O₆: m/z: 632.87, observed m/z = 633.9 [M+H]⁺.

Procedure 18: Synthesis of tert-butyl 18-(4-((tert-butoxycarbonyl)amino)butyl)-21-(4-(4-isobutylphenyl)butan-amido)butyl)-3,16,19-trioxo-1-phenyl-2,7,10,13-tetraoxa-4,17,20-triazadocosan-22-oate (10):



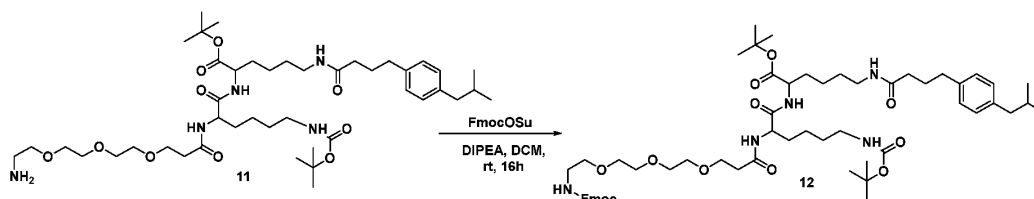
To a solution of 3-oxo-1-phenyl-2,7,10,13-tetraoxa-4-azahexadecan-16-oic acid (0.50 g, 1.42 mmol) in 8 ml of DCM was added EDCI.HCl (0.41 g, 2.13 mmol) and HOBT (0.19 g, 1.42 mmol) and the reaction mixture was stirred at rt for 5 min. Tert-butyl 2-(2-amino-6-((tert-butoxycarbonyl)amino)hexan-amido)-6-(4-(4-isobutylphenyl)butanamido)hexanoate (0.90 g, 1.42 mmol) in DCM (10 ml) and DIEA (0.72 ml, 4.27 mmol) were added sequentially and the mixture was stirred at room temperature for 16 hours. The reaction mixture was diluted with 20 ml of DCM and washed with water. The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure. The crude product was purified by flash chromatography (MeOH/DCM, eluted at 3-4% MeOH in DCM) to afford the product (0.95 g, 69% yield) as a colorless oil. LCMS: C₅₂H₈₃N₅O₁₂: m/z: 970.24, observed m/z = 970.8 [M+H]⁺.

Procedure 19: Synthesis of tert-butyl 1-amino-14-(4-((tert-butoxycarbonyl)amino)butyl)-17-(4-(4-(4-isobutylphenyl)butanamido)butyl)-12,15-dioxo-3,6,9-trioxa-13,16-diazaoctadecan-18-oate (11):



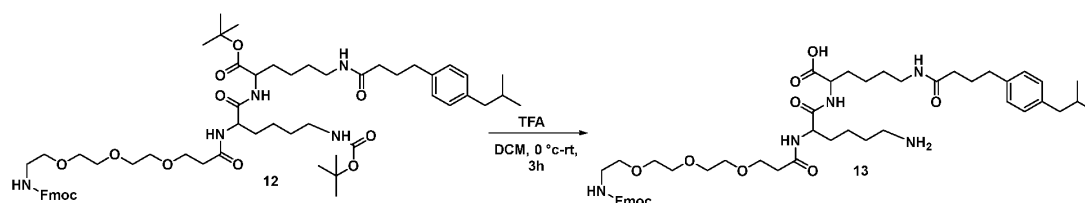
Palladium on carbon (10%, 0.040 g, wet, 67%) was suspended in a solution of tert-butyl 18-(4-((tert-butoxycarbonyl)amino)butyl)-21-(4-(4-(4-isobutylphenyl)butanamido)butyl)-3,16,19-trioxo-1-phenyl-2,7,10,13-tetraoxa-4,17,20-triazadocosan-22-oate (160 mg, 0.165 mmol) in MeOH (5 ml). The suspension was stirred for 2 h at room temperature under H₂ at balloon pressure. The mixture was then filtered through celite, and the filtrate was evaporated to afford the target compound (150 mg, 100%) as a white solid. The crude product was used for the next step without further purification. LCMS: C₄₄H₇₇N₅O₁₀: m/z: 836.11, observed m/z = 836.80 [M+H]⁺.

Procedure 20: Synthesis of tert-butyl 18-(4-((tert-butoxycarbonyl)amino)butyl)-1-(9H-fluoren-9-yl)-21-(4-(4-(4-isobutylphenyl)butanamido)butyl)-3,16,19-trioxo-2,7,10,13-tetraoxa-4,17,20-triazadocosan-22-oate (12):



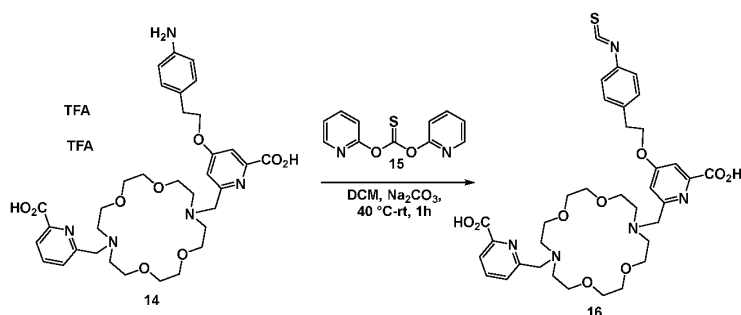
To a rt solution of the tert-butyl 1-amino-14-(4-((tert-butoxycarbonyl)amino)butyl)-17-(4-(4-(4-isobutylphenyl)-butanamido)butyl)-12,15-dioxo-3,6,9-trioxa-13,16-diazaoctadecan-18-oate (150 mg, 0.179 mmol) in DCM (4 ml) were added FmocOSu (73 mg, 0.215 mmol) and DIPEA (0.1 ml, 0.538 mmol) and the reaction was stirred at rt for 16 h. Water was added and the mixture was extracted with DCM. The organic layer was dried over Na₂SO₄, filtered, and the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (EtOAc/hexane, eluted at 50% EtOAc in hexane) to afford the product (145 mg, 77% yield) as a white solid. LCMS: C₅₉H₈₇N₅O₁₂: m/z: 1058.35, observed m/z = 1059.0 [M+H]⁺.

Procedure 21: Synthesis of 18-(4-aminobutyl)-1-(9H-fluoren-9-yl)-21-(4-(4-(4-isobutylphenyl)butanamido)butyl)-3,16,19-trioxo-2,7,10,13-tetraoxa-4,17,20-triazadocosan-22-oic acid (13):



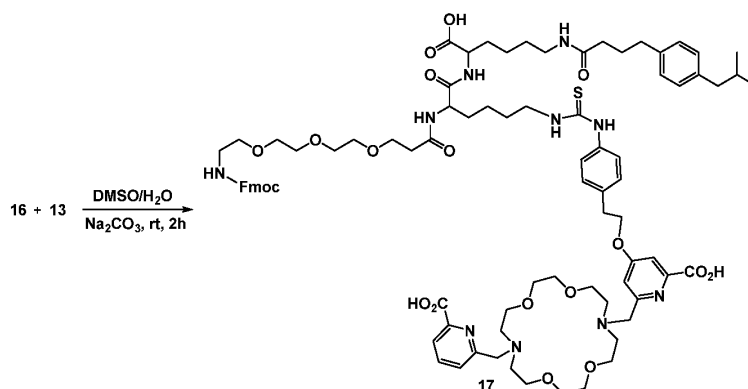
To a 0 °C solution of the tert-butyl 18-(4-((tert-butoxycarbonyl)amino)butyl)-1-(9H-fluoren-9-yl)-21-(4-(4-(4-isobutylphenyl)butanamido)butyl)-3,16,19-trioxo-2,7,10,13-tetraoxa-4,17,20-triazadocosan-22-oate (145 mg, 0.137 mmol) in DCM (3 ml) was added TFA (1 ml) and the reaction mixture was stirred at rt for 3 h. The solvents were evaporated under reduced pressure and the crude product was submitted for HPLC purification. Desired fractions were combined and lyophilized to obtain the product (65 mg, 53%) as an off-white solid. LCMS: C₅₀H₇₁N₅O₁₀: m/z: 902.13, observed m/z = 902.9 [M+H]⁺

Procedure 22: Synthesis of 6-((16-((6-carboxypyridin-2-yl)methyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecan-7-yl)methyl)-4-(4-isothiocyanatophenethoxy)picolinic acid (16):



To a solution of 4-(4-aminophenethoxy)-6-((16-((6-carboxypyridin-2-yl)methyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecan-7-yl)methyl)picolinic acid.2xTFA (120 mg, 0.14 mmol) in DCM (3.5 ml), was added Na₂CO₃ (44 mg, 0.42 mmol). The reaction mixture was stirred at 40 °C for 15 min (until a homogeneous solution was obtained). The reaction mixture was cooled to rt and O,O-di(pyridin-2-yl) carbonothioate (36 mg, 0.15 mmol) in 0.5 ml DCM was added to the reaction mixture. After LCMS analysis indicated consumption of the amine, the solids were removed by filtration and volatiles were removed under reduced pressure obtained the product **16** (100 mg, 100%) as a yellow color oil. LCMS: C₃₅H₄₃N₅O₉S: m/z: 709.81, observed m/z = 710.4 [M+H]⁺.

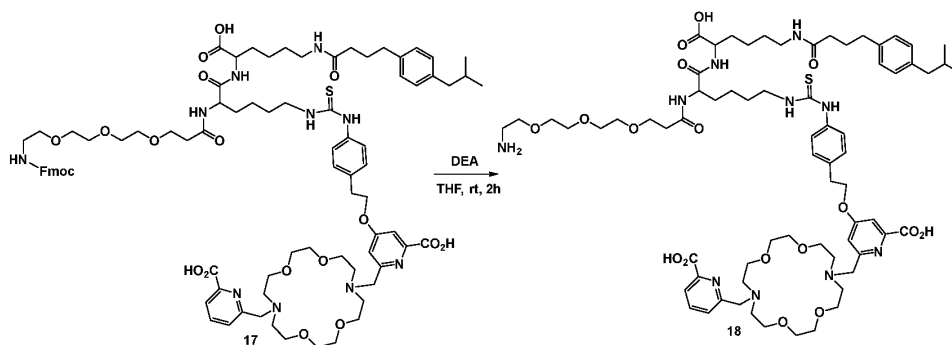
Procedure 23: Synthesis of 4-(4-(3-(18-((1-carboxy-5-(4-(4-isobutylphenyl)butanamido)pentyl)carbamoyl)-1-(9H-fluoren-9-yl)-3,16-dioxo-2,7,10,13-tetraoxa-4,17-diazadocosan-22-yl)thioureido)phenethoxy)-6-((16-((6-carboxypyridin-2-yl)methyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecan-7-yl)methyl)picolinic acid (**17**):



To a solution of 18-(4-aminobutyl)-1-(9H-fluoren-9-yl)-21-(4-(4-(4-isobutylphenyl)butanamido)butyl)-3,16,19-trioxo-2,7,10,13-tetraoxa-4,17,20-triazadocosan-22-oic acid (21 mg, 0.023 mmol) in DMSO: H₂O (0.5 ml:0.5 ml), were added Na₂CO₃ (8 mg, 0.069 mmol) and 6-((16-((6-carboxypyridin-2-yl)methyl)-1,4,10,13-tetraoxa-7,16-

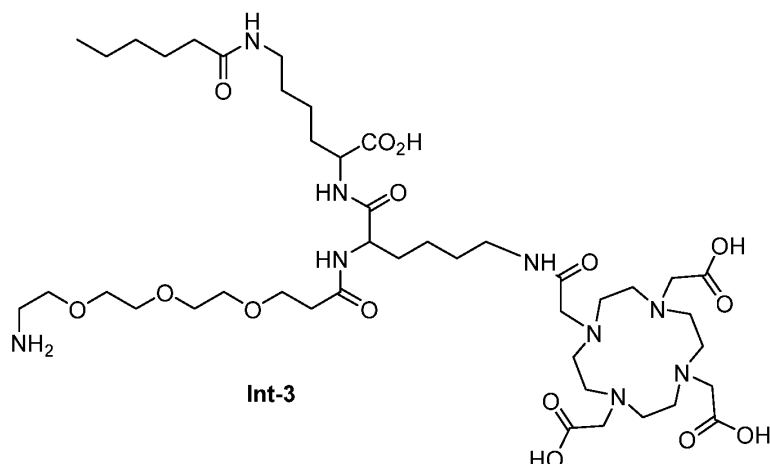
diazacyclooctadecan-7-yl)methyl)-4-(4-isothiocyanatophenethoxy)picolinic acid (20 mg, 0.027 mmol, dissolved in 0.5 ml DMSO), and stirred the reaction mixture at rt for 2 h. After LCMS analysis indicated complete consumption of the amine, the solids were removed by filtration and the crude product was submitted for HPLC purification. Desired fractions were combined and lyophilized to obtain the product (28 mg, 76%) as an off-white solid. LCMS: $C_{85}H_{114}N_{10}O_{19}S$: m/z: 1611.94, observed m/z = 1612.4 $[M+H]^+$.

Procedure 24: Synthesis of 4-(4-(3-(1-amino-14-((1-carboxy-5-(4-(4-isobutylphenyl)butanamido)pentyl)carbamoyl)-12-oxo-3,6,9-trioxa-13-azaoctadecan-18-yl)thioureido)phenethoxy)-6-((16-(((6-carboxypyridin-2-yl)methyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecan-7-yl)methyl)picolinic acid(18):

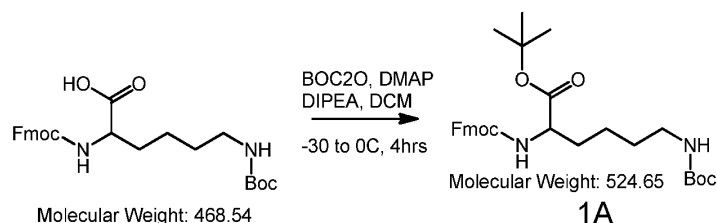


To a rt solution of 4-(4-(3-(18-((1-carboxy-5-(4-(4-isobutylphenyl)butanamido)pentyl)carbamoyl)-1-(9H-fluoren-9-yl)-3,16-dioxo-2,7,10,13-tetraoxa-4,17-diazadocosan-22-yl)thioureido)phenethoxy)-6-((16-(((6-carboxypyridin-2-yl)methyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecan-7-yl)methyl)picolinic acid (28 mg, 0.017 mmol) in THF (0.5 ml) was added diethylamine (0.5 ml). The reaction mixture was stirred at ambient temperature for 2 hours, the solvents were evaporated, and the crude residue was subjected to HPLC purification. Desired fractions were combined and lyophilized to obtain the product (13 mg, 54%) as an off-white solid. LCMS: $C_{70}H_{104}N_{10}O_{17}S$: m/z: 1389.70, observed m/z = 1390.2 $[M+H]^+$.

Synthesis of Int-3



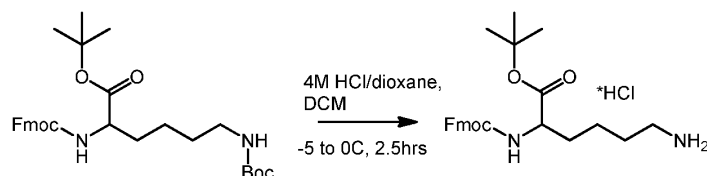
Procedure 25: Preparation of Tert-Butyl-(S)-2-[(S)-2-(3-{2-[2-(2-aminoethoxy)ethoxy]ethoxy} propionylamino)-6-(tert-butoxycarbonylamino)hexanoylamino]-6-(hexanoylamino)hexanoate: Fmoc-L-Lys (Boc)-OtBu,



Fmoc-L-Lys(Boc)-OH (47 g, 100 mmol) was dissolved in DCM (470 ml), and diisopropylethylamine (25 ml) was added at -30 °C. The reaction mixture was stirred at -30 °C for 5min, followed by slow addition of Boc anhydride (30.1 g, 140 mmol) at -30 °C. The reaction mixture was stirred at -30 °C for a further 30 minutes, then N,N-dimethylaminopyridine (1.7g, 14 mmol) was added at -30°C. The reaction mixture was slowly warmed to 0 °C over the course of 2 hours and was stirred at 0°C for another 2 hours at which time LCMS analysis of the reaction mixture showed complete consumption of the starting carboxylic acid). The reaction mixture was cooled to -30 °C and 1M HCl aqueous solution was added slowly to obtain a pH~3 (the temperature of mixture was kept below -20 °C). The resulting biphasic mixture was separated and the organic layer was washed with water (200 ml) and 0.1M NaHCO₃ (200 ml), dried with MgSO₄, filtered and evaporated to dryness. The residue

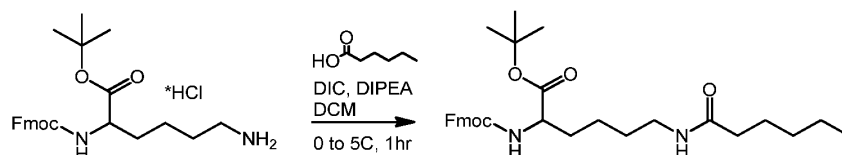
was diluted with 30 ml of ethyl acetate, heated to reflux and diluted with 300 ml of hexane. The mixture was stirred at room temperature for 2 hours and filtered. The precipitate was washed with 100 ml of 20% ethyl acetate in hexane and the residual solvent was removed in vacuo overnight. Yield = 23g (44%).

Procedure 26: Fmoc-L-Lys (Boc)-OtBu



To a solution of Fmoc-L-Lys(Boc)-OtBu (23 g, 44 mmol) in 130 ml DCM was added 4M HCl in dioxane (275 ml) at -5 °C. The mixture was stirred for 2.5 hour at -5 °C to 0 °C (until LCMS showed no starting material). The solvents were evaporated in vacuo without heating, re-evaporated with 150 ml of DCM, and residual solvents were removed under vacuum at room temperature overnight. The product was obtained as a white amorphous solid (22.6 g, 110%).

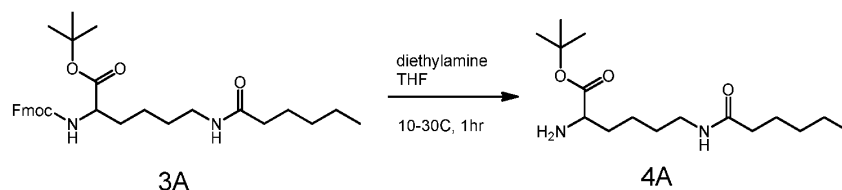
Procedure 27: tert-Butyl-(S)-2-[(9H-fluoren-9-yl)methoxycarbonylamino]-6-(hexanoylamino) hexanoate



Diisopropylcarbodiimide (DIC, 16.7 g, 132 mmol) was added dropwise to a solution of hexanoic acid (30.7 g, 264 mmol) in DCM (120 ml) at 0 °C and the reaction mixture was stirred for 30 minutes at 0 °C. The precipitated solid was removed by filtration. The filtrate was added to a suspension of Fmoc-L-Lys-OtBu hydrochloride (20.3 g, 44 mmol) in DCM (30 ml), followed by the addition of DIPEA (7.7 ml, 1 eq); the temperature was held at 0 °C to 5 °C during the addition. The reaction mixture was stirred for 30 minutes at 5 °C, at which time LCMS showed no starting material remaining. The mixture was diluted with 150 ml of DCM, and the organic layer was washed with 0.5 M HCl solution (100 ml), washed with 0.1 M NaHCO₃ solution (100ml), dried over MgSO₄ and evaporated. The residue was purified by

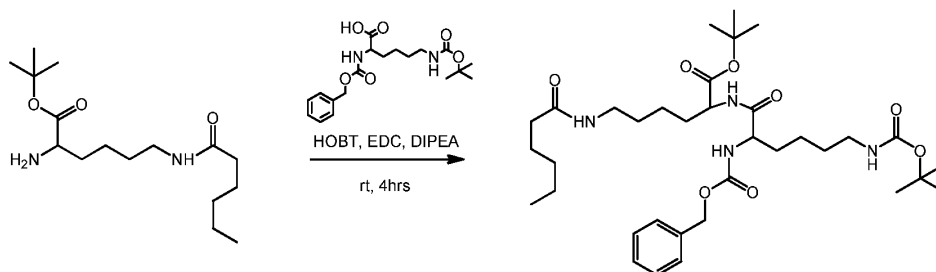
flash chromatography (330 g silica gel column, 0% to 50% ethyl acetate in hexane) to obtain the target compound (16.8 g, 73%) as a white glass.

Procedure 28: tert-Butyl (S)-2-amino-6-(hexanoylamino)hexanoate



To a solution of tert-butyl-(S)-2-[(9H-fluoren-9-yl)methoxycarbonylamino]-6-(hexanoylamino) hexanoate (16 g, 30 mmol) in THF (200 ml) was added diethylamine (92 ml, 900 mmol) at 10 °C. The reaction mixture was stirred for 1 hour and evaporated to dryness under vacuum without heating. The residue was re-evaporated with toluene (100 ml) at 25-30 °C and subjected to silica gel purification (220 g column, 0% to 15% MeOH in DCM) to obtain the product (7.1 g, 79%) as a viscous yellow oil.

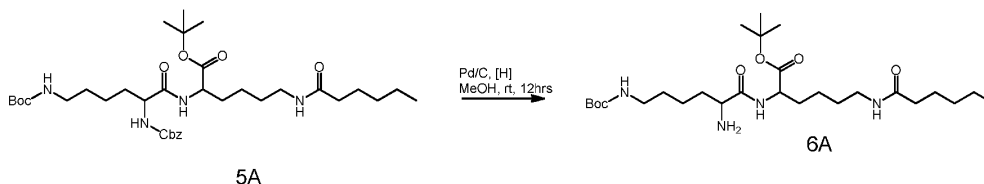
Procedure 29: tert-Butyl-(S)-2-[(S)-2-amino-6-(tert-butoxycarbonylamino)hexanoylamino]-6-(hexanoylamino)hexanoate



To a solution of CBZ-Lys(Boc)-OH (9.7 g, 25.5 mmol) in dry DCM (100 ml), HOBT (3.45 g, 25.5 mmol) was added and the mixture was stirred for 10 min at ambient temperature. A solution of tert-butyl (S)-2-amino-6-(hexanoylamino)hexanoate (6.9 g, 23 mmol) in DCM, DIPEA (12.2 ml) and EDC (5.35 g) were added sequentially. The reaction mixture was stirred at ambient temperature for 4 hours (until LCMS showed tert-butyl (S)-2-amino-6-(hexanoylamino)hexanoate was consumed), diluted with DCM (200 ml), washed with water (150 ml) and saturated NaCl solution (100 ml), and dried over anhydrous MgSO₄. The organic solution was concentrated under reduced pressure and the residue was purified by

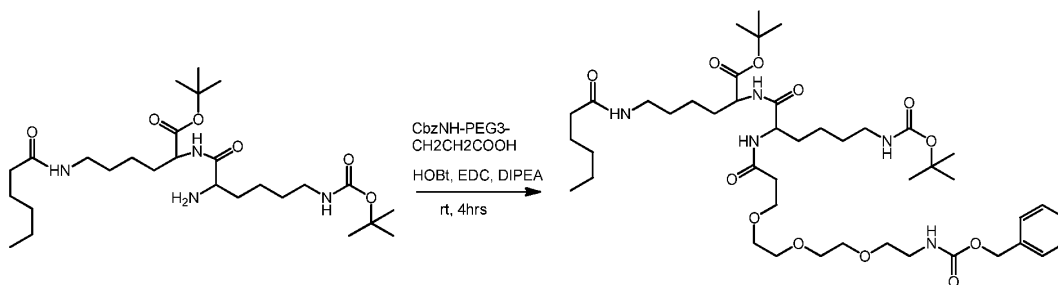
silica gel chromatography (220 g column, 15% to 80% ethyl acetate in hexane) to obtain the product (11.4 g, 75%) as an amorphous yellow solid.

Procedure 30: tert-Butyl-(S)-2-[(S)-2-amino-6-(tert-butoxycarbonylamino)hexanoylamino]-6-(hexanoylamino)hexanoate



To a solution of tert-butyl-(S)-2-[(S)-2-amino-6-(tert-butoxycarbonylamino)hexanoylamino]-6-(hexanoylamino)hexanoate (11 g, 16.6 mmol) in methanol (400 ml) was added 10% palladium (50% wet) on activated carbon (2 g). The suspension was subjected to catalytic hydrogenation (H_2 , 1 atm, overnight) at ambient temperature (after 12 h LCMS indicated complete consumption of starting material). The reaction mixture was filtered through Celite. The filtrate was concentrated under reduced pressure, re-evaporated twice with 200 ml of acetonitrile, and dried for 4 hours under high vacuum to give the product (8.96 g, 100%) as a colorless glass.

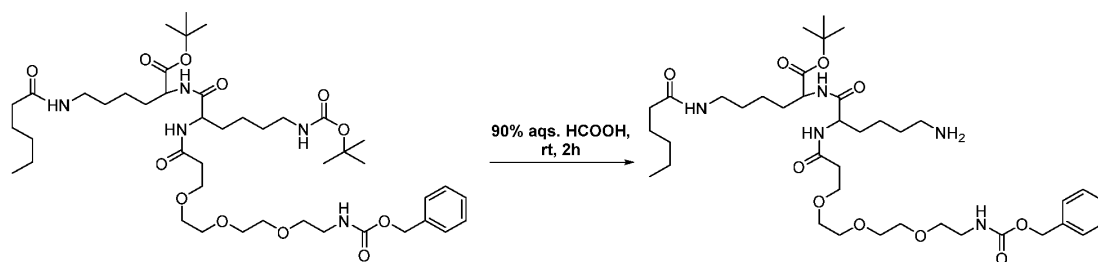
Procedure 31: tert-Butyl-(S)-2-[(S)-2-[3-(2-{2-[2-(benzyloxycarbonylamino)ethoxy]ethoxy}ethoxy)propionylamino]-6-(tert-butoxycarbonylamino)hexanoylamino]-6-(hexanoylamino)hexanoate



To a solution of 3-(2-{2-[2-(benzyloxycarbonylamino)ethoxy]ethoxy}ethoxy)propionic acid (5.8 g, 16.6 mmol) in dry DCM (50 ml), HOBt (2.53 g, 18.7 mmol) was added and the mixture was stirred for 10 min at ambient temperature. A solution of tert-butyl-(S)-2-[(S)-2-amino-6-(tert-butoxycarbonylamino)hexanoylamino]-6-(hexanoylamino)hexanoate (8.9 g, 16.6 mmol) in DCM (50 ml) EDC (5.35 g) and DIPEA (12.2 ml) were added subsequently. The reaction mixture was stirred 4 h (LCMS monitoring), diluted with DCM (200 ml),

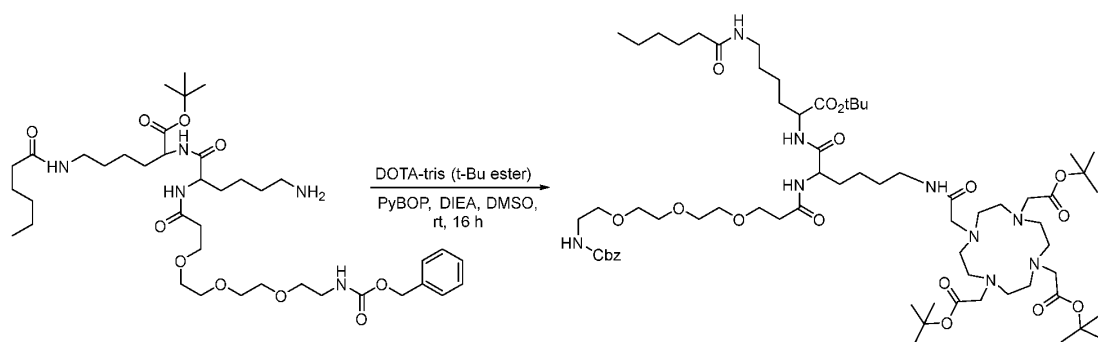
washed with water (150 ml) and saturated NaCl solution (100 ml), and dried over MgSO₄. The organic layer was concentrated under reduced pressure and the residue was purified by silica gel chromatography (220 g column, 0% to 10% MeOH in DCM) to give target product (9.63 g, 67%) as a colorless glass.

Procedure 32: Synthesis of tert-butyl 18-(4-aminobutyl)-49-(4-(4-(4-isobutylphenyl)butanamido)butyl)-3,16,19,47-tetraoxo-1-phenyl-2,7,10,13,23,26,29,32,35,38,41,44-dodecaoxa-4,17,20,48-tetraazapentacontan-50-oate



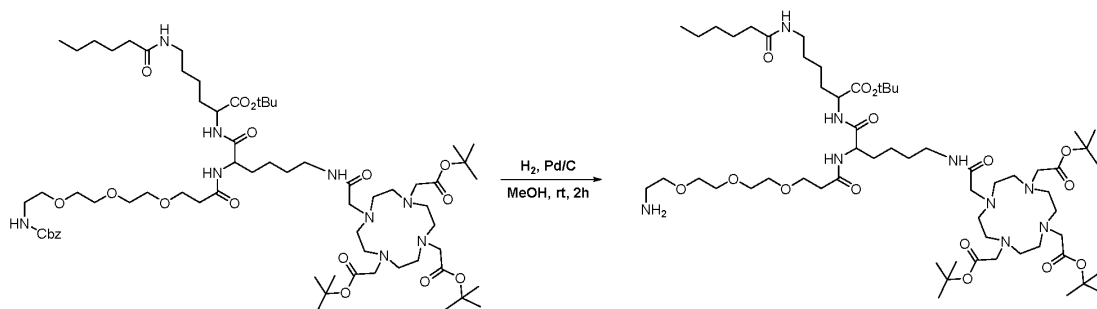
A solution of tert-butyl 18-(4-((tert-butoxycarbonyl)amino)butyl)-49-(4-(4-(4-isobutylphenyl)butanamido)butyl)-3,16,19,47-tetraoxo-1-phenyl-2,7,10,13,23,26,29,32,35,38,41,44-dodecaoxa-4,17,20,48-tetraazapentacontan-50-oate (0.70 g, 1.22 mmol) in 90% aq. formic acid (10 ml) was stirred at ambient temperature for 2 h. Full conversion was detected by LCMS. The solvents were evaporated, and the residue was co-evaporated with ACN (3x) and toluene (3x). The residue was dissolved in a mixture MeOH/water (1:1, 25 ml) and treated with ion-exchange resin AmberLite HPR550 (OH-form) at ambient temperature for 15 minutes. The resin was filtered and washed with MeOH and water. The filtrate was evaporated to obtain target material (free base, 0.62 g, 95% yield) as a colorless liquid. The crude amine was used for the step without further purification. LCMS: C₆₆H₁₁₂N₆O₁₉: m/z: 1293.62, observed m/z = 1294.1 [M+H]⁺.

Procedure 33: Synthesis of tri-tert-butyl 2,2',2''-(10-(39-(tert-butoxycarbonyl)-48-(4-isobutylphenyl)-2,9,37,45-tetraoxo-8-(3-oxo-1-phenyl-2,7,10,13-tetraoxa-4-azahexadecanamido)-13,16,19,22,25,28,31,34-octaoxa-3,10,38,44-tetraaza-octatetracontyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (15):



To a solution of tert-butyl 18-(4-aminobutyl)-49-(4-(4-(4-isobutylphenyl)butanamido)butyl)-3,16,19,47-tetraoxo-1-phenyl-2,7,10,13,23,26,29,32,35,38,41,44-dodecaoxa-4,17,20,48-tetraazapentacontan-50-oate (0.62 g, 0.48 mmol), DOTA-tris(t-Bu-ester) (0.27 g, 0.48 mmol) and PyBop (0.30 g, 0.56 mmol) in DMSO (8 ml) was added DIEA (0.20 ml, 1.15 mmol) and the reaction mixture was stirred at ambient temperature for 16 h. Water (20 ml) was added, and the mixture was extracted with EtOAc (30 ml). The separated organic layer was washed with water (7x) and brine (8x) to remove excess PyBOP. The organic layer was dried over Na₂SO₄ and concentrated under reduced pressure obtained target product (820 mg, 93% yield) as colorless liquid. The compound was used for the nest step without further purification. LCMS: C₉₄H₁₆₂N₁₀O₂₆: m/z: 1848.34, observed m/z = 1849.7 [M+H]⁺.

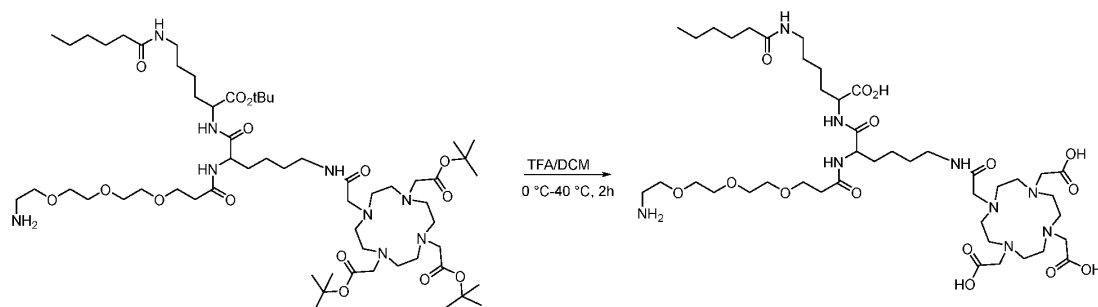
Procedure 34: Synthesis of tri-tert-butyl 2,2',2''-(10-(8-(3-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)propanamido)-39-(tert-butoxycarbonyl)-48-(4-isobutylphenyl)-2,9,37,45-tetraoxo-13,16,19,22,25,28,31,34-octaoxa-3,10,38,44-tetraazaoctatetracontyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (16):



Palladium on carbon (10%, 0.12 g, wet, 67%) was suspended in a solution of tri-tert-butyl 2,2',2''-(10-(39-(tert-butoxycarbonyl)-48-(4-isobutylphenyl)-2,9,37,45-tetraoxo-8-(3-oxo-1-phenyl-2,7,10,13-tetraoxa-4-azahexadecanamido)-13,16,19,22,25,28,31,34-octaoxa-3,10,38,44-tetraazaoctatetracontyl)-1,4,7,10-tetra-azacyclododecane-1,4,7-triyl)triacetate (500 mg, 0.270 mmol) in MeOH (15 ml). The suspension was stirred for 2 h at room

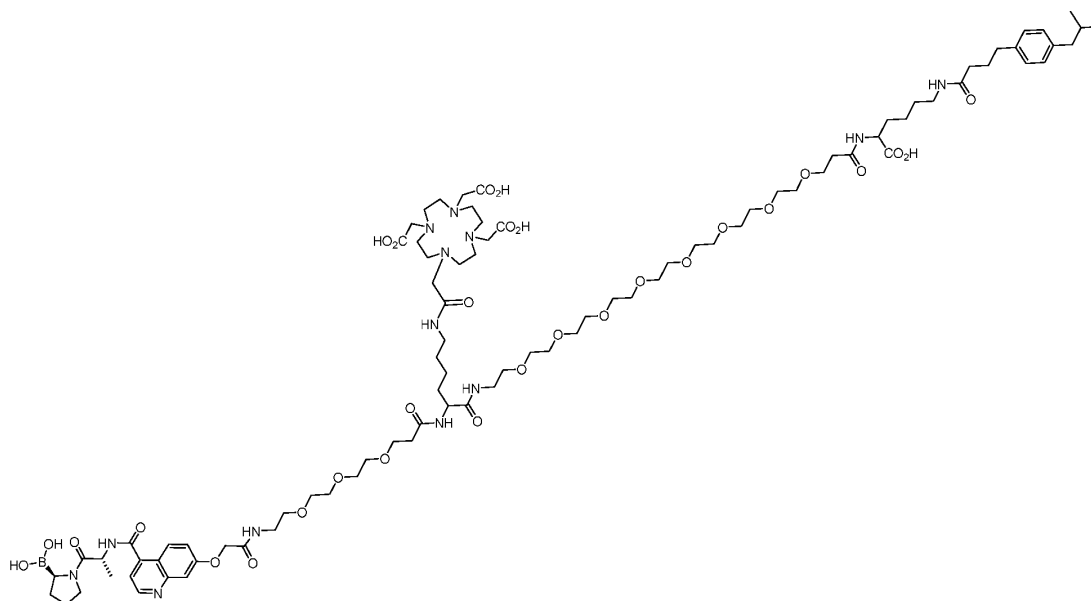
temperature under H₂ at balloon pressure. The mixture was filtered through celite, and the filtrate was evaporated to afford target compound (387 mg, 83% yield) as a colorless liquid. The crude product was used for the next step without further purification. LCMS: C₈₆H₁₅₆N₁₀O₂₄: m/z: 1714.21, observed m/z = 1714.7 [M+H]⁺.

Procedure 35: Synthesis of 2,2',2''-(10-(8-(3-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)propanamido)-39-carboxy-48-(4-isobutylphenyl)-2,9,37,45-tetraoxo-13,16,19,22,25,28,31,34-octaoxa-3,10,38,44-tetraazaocatactetracon-tyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (17):



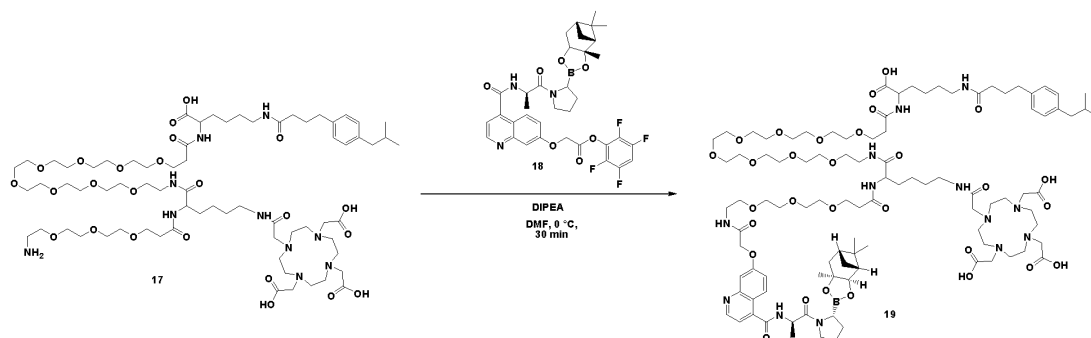
To a 0 °C solution of tri-tert-butyl 2,2',2''-(10-(8-(3-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)propanamido)-39-(tert-butoxycarbonyl)-48-(4-isobutylphenyl)-2,9,37,45-tetraoxo-13,16,19,22,25,28,31,34-octaoxa-3,10,38,44-tetraazaocatactetracon-tyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (387 mg, 0.225 mmol) in DCM (4 ml), was added TFA (2 ml). The reaction mixture was stirred at 40 °C for 2 h and the solvents were evaporated under reduced pressure. The residue was washed with hexane (2x) and ether (2x) to obtain the product (340 mg, 100%) as a white powder. The crude product was used for the next step without further purification. LCMS: C₇₀H₁₂₄N₁₀O₂₄: m/z: 1489.79, observed m/z = 1490.5 [M+H]⁺.

Synthesis of RTX-1392R



Using Int-1 as starting material, the following procedures were used to prepare RTX-1392R:

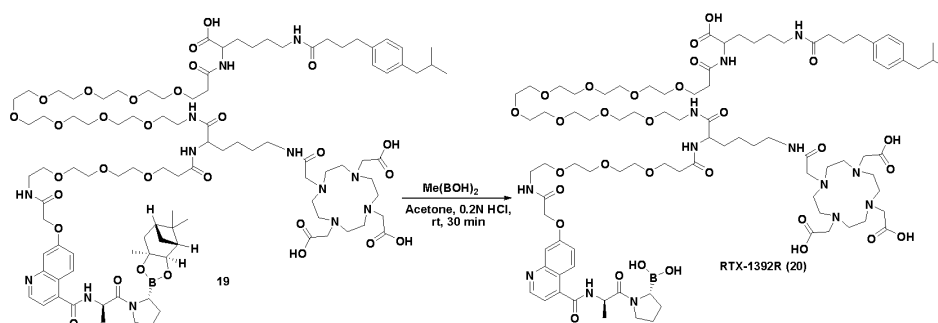
Procedure 36: Synthesis of 2,2',2''-(10-(39-carboxy-48-(4-isobutylphenyl)-2,9,37,45-tetraoxo-8-(2-oxo-1-((4-(((R)-1-oxo-1-((R)-2-((3aR,4R,6R,7aS)-5,5,7a-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)pyrrolidin-1-yl)propan-2-yl)carbamoyl)quinolin-7-yl)oxy)-6,9,12-trioxa-3-azapentadecanami-do)-13,16,19,22,25,28,31,34-octaoxa-3,10,38,44-tetrazaoctatetracontyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (19):



To a 0 °C solution of 2,2',2''-(10-(8-(3-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)propanamido)-39-carboxy-48-(4-isobutylphenyl)-2,9,37,45-tetraoxo-13,16,19,22,25,28,31,34-octaoxa-3,10,38,44-tetrazaoctatetracontyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (200 mg, 0.134 mmol) in DMF (2 ml), was added DIPEA (0.19 ml) and the reaction mixture was stirred at 0 °C for 5 min. 2,3,5,6-Tetrafluorophenyl 2-((4-(((2R)-1-oxo-1-((2R)-2-

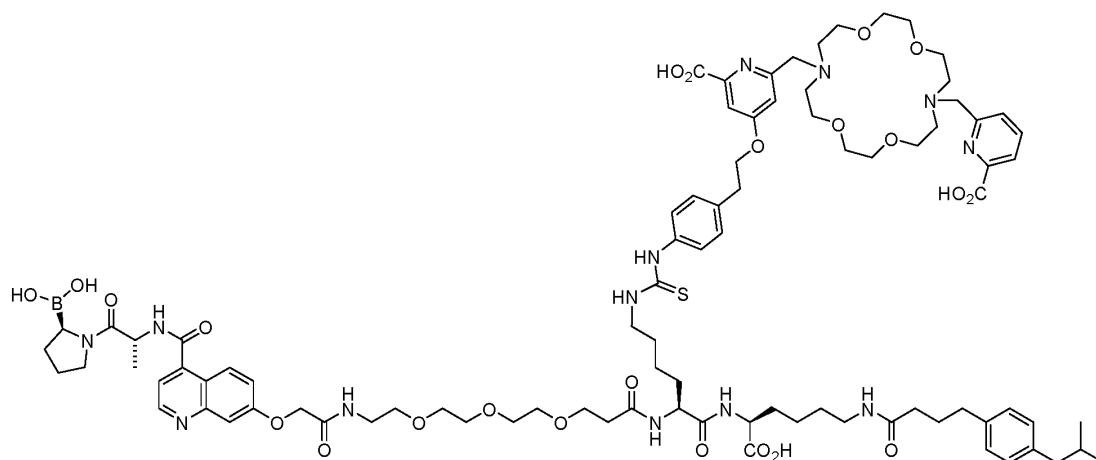
((3aS,4R,6R)-3a,5,5-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)pyrrolidin-1-yl)propan-2-yl)carbamoyl)quinolin-7-yl)oxy)acetate (121 mg, 0.174 mmol) in DMF (1 ml) was added and the reaction mixture was stirred at 0 °C for 0.5 h. After LCMS indicated complete consumption of the amine, the crude product was submitted for HPLC purification. Desired fractions were combined and lyophilized to obtain the product (72 mg, 26%) as an off-white solid. LCMS: C₉₉H₁₅₈BN₁₃O₃₀: m/z: 2021.19, observed m/z = 1011.7 [M/2+H]⁺.

Procedure 37: Synthesis of 2,2',2''-(10-(8-(1-(((R)-1-((R)-2-boronopyrrolidin-1-yl)-1-oxopropan-2-yl)carbamoyl)quinolin-7-yl)oxy)-2-oxo-6,9,12-trioxa-3-azapentadecanamido)-39-carboxy-48-(4-isobutylphenyl)-2,9,37,45-tetraoxo-13,16,19,22,25,28,31,34-octaoxa-3,10,38,44-tetraazaocatetracontyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (RTX-1392R (20)):



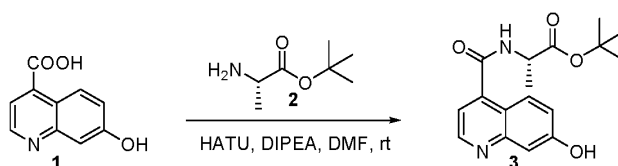
To a rt solution of 2,2',2''-(10-(39-carboxy-48-(4-isobutylphenyl)-2,9,37,45-tetraoxo-8-(2-oxo-1-(((R)-1-oxo-1-((R)-2-((3aR,4R,6R,7aS)-5,5,7a-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)pyrrolidin-1-yl)propan-2-yl)carbamoyl)quinolin-7-yl)oxy)-6,9,12-trioxa-3-azapentadecanamido)-13,16,19,22,25,28,31,34-octaoxa-3,10,38,44-tetraazaocatetracontyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (70 mg, 0.034 mmol) in acetone (1.5 ml) and 0.2N HCl (1.5 ml), was added MeB(OH)₂ (10 mg, 0.173 mmol) and the reaction mixture was stirred at ambient temperature for 30 min. After LCMS indicated complete consumption of the starting material, the crude product was submitted for HPLC purification. Desired fractions were combined and lyophilized to obtain the product (31 mg, 48%) as an off-white solid. LCMS: C₈₉H₁₄₄BN₁₃O₃₀: m/z: 1886.98, observed m/z = 943.9 [M-OH/2]⁺.

Synthesis of RTX-1401R:



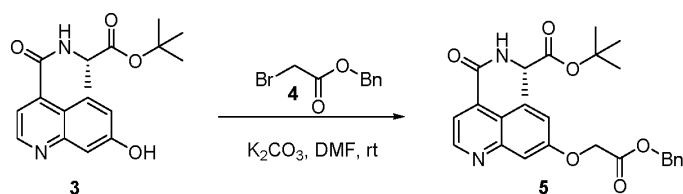
Using Int-2 as starting material, the following procedures were used to prepare RTX-1401R:

Procedure 38: Synthesis of tert-butyl (R)-2-[(7-hydroxy-4-quinolyl)carbonylamino]propionate



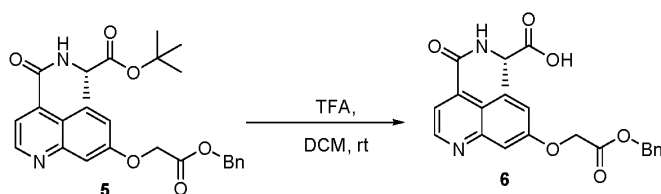
7-Hydroxy-4-quinolinecarboxylic acid 2.0g, (10.5 mmol, 1 eq), (R)-Ala-t-butyl ester hydrochloride (2.0 g, 11.5 mmol, 1.1 eq) and HATU (5.98 g, 15.75 mmol, 1.5 eq) in 40 ml DMF was added DIPEA (4.6 ml, 2.5 eq) at 5 °C and the mixture was stirred at RT for 2h. Upon completion (indicated by LCMS), H₂O was added and the product was extracted with n-butanol. The organic layer was separated, dried over Na₂SO₄ and evaporated under reduced pressure. The resulting residue was purified by flash column (0-10% MeOH in DCM) to afford **3** (2.01g, 61% yield) as an off-white powder. ESI-MS m/z calc.316.351, found 317.0 [M+H]⁺.

Procedure 39: Synthesis of tert-butyl (R)-2-({7-[(benzyloxycarbonyl)methoxy]-4-quinolyl}carbonylamino)propionate



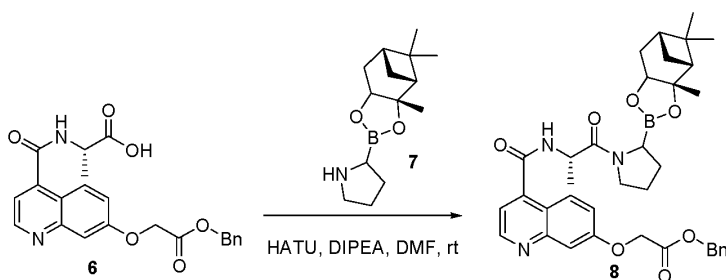
To a suspension of tert-butyl (R)-2-[(7-hydroxy-4-quinolyl)carbonylamino]propionate (2.0 g, 6.4 mmol, 1 eq) and K_2CO_3 (2.7g, 19.2 mmol, 3eq) in DMF was added benzyl bromoacetate (4.4 g, 19.2 mmol, 3 eq) at RT and the mixtures was allowed to stir at 60 °C for 16 h. Upon completion, H_2O was added and the product was extracted with EtOAc. The organic layer was separated, dried over Na_2SO_4 and evaporated under reduced pressure. The resulting residue was purified by flash column chromatography (0-80% ethyl acetate in hexane) to afford **5** (2 g, 67% yield) as an off-white powder. ESI-MS m/z calc.464.509, found 465.1 $[M+H]^+$.

Procedure 40: Synthesis of (R)-2-({ 7-[(benzyloxycarbonyl)methoxy]-4-quinolyl}carbonylamino)-propionic acid



To tert-butyl (R)-2-({ 7-[(benzyloxycarbonyl)methoxy]-4-quinolyl}carbonylamino)propionate (2.0 g, 4.3 mmol) in DCM was added 20 ml TFA/DCM (DCM:TFA, 1:2) at RT and the mixture was stirred for 4h. Upon completion, the solvents were evaporated under reduced pressure to afford **6** (1.75 g, 100%) as an off-white solid which was used for the next step without further purification. ESI-MS m/z calc.408.403, found 409.3 $(M+1)^+$.

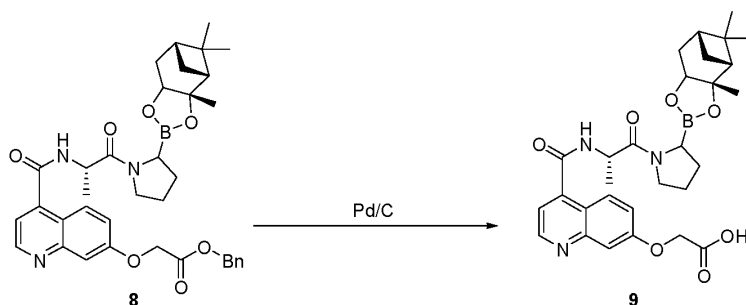
Procedure 41: Synthesis of benzyl(4-{[(R)-2-(2-{(1R,2S,8S)-2,9,9-trimethyl-3,5-dioxo-4-boratricyclo[6.1.1.0^{2,6}]dec-4-yl}-1-pyrrolidinyl)-1-methyl-2-oxoethylamino]carbonyl}-7-quinolyloxy)acetate



To a solution of (R)-2-({ 7-[(benzyloxycarbonyl)methoxy]-4-quinolyl}carbonylamino)propionic acid (1.75 g, 4.3 mmol, 1 eq), (R)-boropro-(+)-pinanediol hydrochloride (1.35 g, 4.73 mmol, 1.1 eq) and HATU (2.45 g, 6.45 mmol, 1.5 eq) in DMF (15 ml) was added DIPEA (1.9 ml, 10.75 mmol, 2.5 eq) at 5 °C. The mixture was stirred at RT for 2 h, at which time completion of reaction was indicated by LCMS. H_2O was added

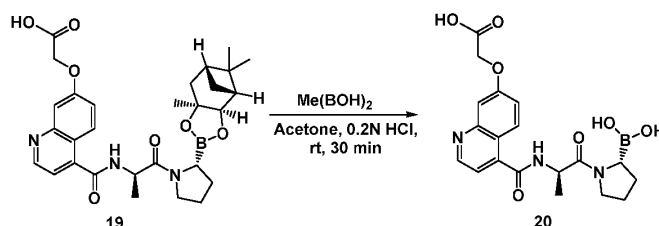
and the product was extracted with EtOAc. The organic layer was separated, dried over Na_2SO_4 and evaporated under reduced pressure. The resulting residue was purified by flash chromatography (0-10% MeOH in DCM) to afford **8** (1.75 g, 64% yield)) as an off-white powder. ESI-MS m/z calc.639.547, found 640.2 $[\text{M}+\text{H}]^+$.

Procedure 42: Synthesis of (4-{[(R)-2-(2-{(1R,2S,8S)-2,9,9-trimethyl-3,5-dioxo-4-boratricyclo[6.1.1.0^{2,6}]dec-4-yl}-1-pyrrolidinyl)-1-methyl-2-oxoethylamino]carbonyl}-7-quinolyloxy)acetic acid



Activated palladium on carbon (5%, 0.4 g) was suspended in a solution of **8** (1.75 g, 2.75 mmol) in MeOH (150 mL). The suspension was stirred for 1 h at room temperature under H_2 atmosphere (40 psi). The mixture was then filtered through celite, and the filtrate was concentrated under reduced pressure to afford 1.45 g of crude product. This material was purified by reverse phase HPLC (30-100% gradient, 0.1% formic acid / ACN) to afford **9** (200 mg, 14% yield) as an off-white solid. ESI-MS m/z calc.549.425, found 550.3 $[\text{M}+\text{H}]^+$.

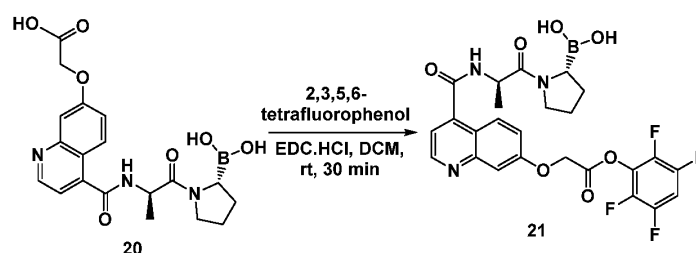
Procedure 43: Synthesis of 2-((4-(((2R)-1-(2-boronopyrrolidin-1-yl)-1-oxopropan-2-yl)carbamoyl)quinolin-7-yl)oxy)acetic acid (20):



To a solution of 2-((4-(((R)-1-oxo-1-((R)-2-((3aR,4R,6R,7aS)-5,5,7a-trimethylhexahydro-4,6-methano-benzo[d][1,3,2]dioxaborol-2-yl)pyrrolidin-1-yl)propan-2-yl)carbamoyl)quinolin-7-yl)oxy)acetic acid (5 mg, 0.009 mmol) in acetone (1.5 ml) and 0.2 N HCl (1.5 ml) mixture was added $\text{MeB}(\text{OH})_2$ (10 mg, 0.173 mmol) at rt and the reaction mixture was stirred at ambient temperature for 30 min. Water was added and the reaction mixture was extracted with DCM.

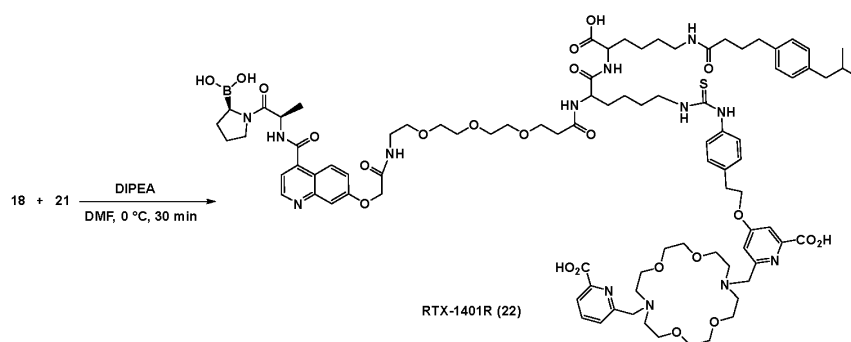
The organic layer was separated, dried over Na₂SO₄, and concentrated under reduced pressure obtained the product (8 mg, 100%) as a colorless oil. The crude product was used for the next step without further purification. LCMS: C₁₉H₂₂BN₃O₇: m/z: 415.20, observed m/z = 416.2 [M+H]⁺.

Procedure 44: Synthesis of ((R)-1-((R)-2-(7-(2-oxo-2-(2,3,5,6-tetrafluorophenoxy)ethoxy)quinoline-4-carboxamido)propanoyl)pyrrolidin-2-yl)boronic acid (21):



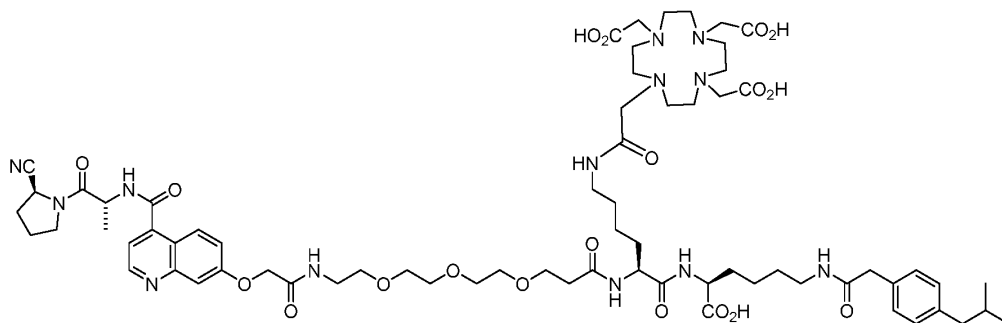
To a solution of 2-((4-(((2R)-1-(2-boronopyrrolidin-1-yl)-1-oxopropan-2-yl)carbamoyl)quinolin-7-yl)-oxy)acetic acid (8 mg, 0.019 mmol) and 2,3,5,6-tetrafluorophenol (5 mg, 0.028 mmol) in 0.5 ml of DCM was added EDC.HCl (5.5 mg, 0.028 mmol), and the reaction mixture was stirred at rt for 0.5h. Water was added to the reaction mixture and extracted with DCM. The organic layer was separated, dried over Na₂SO₄ and concentrated under reduced pressure to obtain the activated ester (10 mg, 100%) as a white powder. The crude product was used for the next step without further purification. LCMS: C₂₅H₂₂BF₄N₃O₇: m/z: 563.26, observed m/z = 563.9 [M+H]⁺.

Procedure 45: Synthesis of 4-(4-(3-(1-((4-(((R)-1-(R)-2-boronopyrrolidin-1-yl)-1-oxopropan-2-yl)carbamoyl)quinolin-7-yl)oxy)-17-((1-carboxy-5-(4-(4-isobutylphenyl)butanamido)pentyl)carbamoyl)-2,15-dioxo-6,9,12-trioxa-3,16-diazahenicosan-21-yl)thioureido)phenethoxy)-6-((16-((6-carboxypyridin-2-yl)methyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecan-7-yl)methyl)picolinic acid (RTX-1401R (22):



To a 0 °C solution of 4-(4-(3-(1-amino-14-((1-carboxy-5-(4-(4-isobutylphenyl)butanamido)pentyl)carbamoyl)-12-oxo-3,6,9-trioxa-13-azaoctadecan-18-yl)thioureido)phenethoxy)-6-((16-((6-carboxypyridin-2-yl)methyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecan-7-yl)methyl)picolinic acid (6 mg, 0.0043 mmol) in DMF (0.3 ml), was added DIPEA (6 uL) and the reaction mixture was stirred at 0 °C for 5 min. ((R)-1-((R)-2-(7-(2-Oxo-2-(2,3,5,6-tetrafluorophenoxy)ethoxy)quinoline-4-carboxamido)propano-yl)pyrrolidin-2-yl)boronic acid (3 mg, 0.0051 mmol) in DMF (0.1 ml) was added and the reaction mixture was stirred at 0 °C for 0.5 h. After LCMS analysis indicated complete consumption of the amine, the crude product was submitted for HPLC purification. Desired fractions were combined and lyophilized to obtain the product (2.5 mg, 32%) as an off-white solid. LCMS: C₈₉H₁₂₄BN₁₃O₂₃S; m/z: 1786.89, observed m/z = 1769.4 [M-OH]⁺.

Synthesis of RTX-1407S:

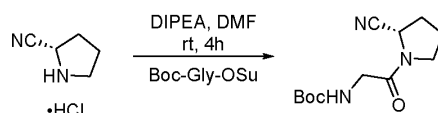


Using Int-3 as starting material, the following procedures were used to prepare RTX-1407S.

Int-3 was prepared with the following changes.

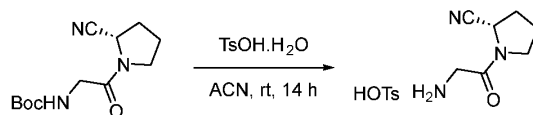
In Procedure 24, 2-(4-isobutylphenyl)acetic acid was used in lieu of hexanoic acid.

Procedure 46: Preparation of [2-((S)-2-cyano-pyrrolidin-1-yl)-2-oxo-ethyl]-carbamic acid tert-butyl ester:



To a solution of (S)-pyrrolidine-2-carbonitrile hydrochloride (1 g, 7.5 mmol) in DCM (35 ml) was added DIPEA (3.9 ml, 22.5 mmol) and Boc-Gly-OSu (2.26 g, 8.3 mmol). The reaction mixture was stirred at ambient temperature for 14 h. The solvents were evaporated and the residue was dissolved in DCM, washed with water, dried over anhydrous Mg_2SO_4 and filtered. The filtrate was concentrated to 20% of the initial volume and loaded onto a CombiFlash™ apparatus and eluted (gradient 0-10% MeOH/DCM). The desired fractions were combined and evaporated to obtain target product (1.1 g, 58%) as an amorphous solid.

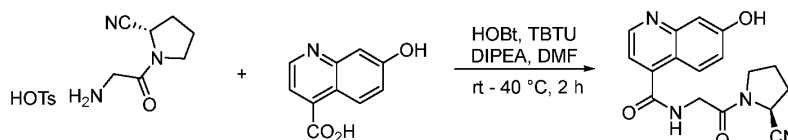
Procedure 47: Preparation of (S)-1-(2-amino-acetyl)-pyrrolidine-2-carbonitrile:



p-Toluenesulfonic acid monohydrate (1.25 g, 6.6 mmol) was added to a stirred solution of (2-((S)-2-cyano-pyrrolidin-1-yl)-2-oxo-ethyl)-carbamic acid tert-butyl ester (1.1 g, 4.4 mmol) in acetonitrile (20 ml) and the mixture was stirred at ambient temperature for 14 h. The solvent was removed at reduced pressure to obtain the tosylate salt of the target product (1.8 g, yield

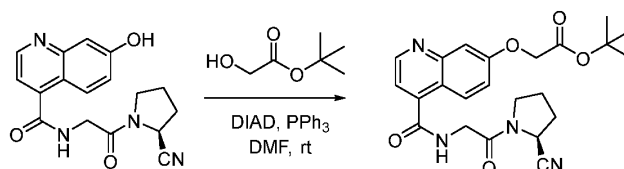
exceeded theoretical). The compound was sufficiently pure to carry through to the next chemical transformation.

Procedure 48: Preparation of (S)-N-(2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)-7-hydroxyquinoline-4-carboxamide:



A solution of 7-hydroxyquinoline-4-carboxylic acid (307 mg, 1.62 mmol), HOBT (221 mg, 1.62 mmol), and TBTU (521 mg, 1.62 mmol) in DMF (15 mL) was stirred at room temperature for 5 min. A solution of (S)-1-(2-amino-acetyl)-pyrrolidine-2-carbonitrile (1.5 tosylate) (1.78 mmol) and DIPEA (0.74 mL, 4.86 mmol) in DMF (5 mL) was added to the activated ester solution and the resulting mixture was stirred at room temperature for 2 h while the reaction progress was monitored by LCMS. Upon full conversion, the reaction mixture was concentrated under reduced pressure, dissolved in a small amount of dichloromethane, and filtered. The filtrate was evaporated, dissolved in DCM, loaded onto a CombiFlash™ silica gel column, and purified (gradient 0-20% MeOH/DCM) to obtain the target product (422 mg, 80% yield) as an off-white solid.

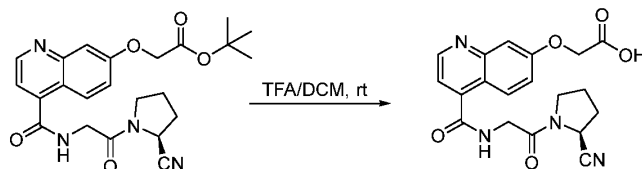
Procedure 49: Preparation of (S)-tert-butyl 2-((4-((2-(2-cyanopyrrolidin-1-yl)-2-oxoethyl)carbamoyl)quinolin-7-yl)oxy)acetate:



A flask containing 7-hydroxyquinoline-4-carboxylic acid [2-((S)-2-cyanopyrrolidin-1-yl)-2-oxo-ethyl]-amide (407 mg, 1.25 mmol), 2-tert-butyl glycolate (249 mg, 1.88 mmol), and triphenylphosphine (395 mg, 1.51 mmol) in DMF (15 mL) was chilled in an ice water bath. Di-isopropyl azodicarboxylate (300 μ L, 1.51 mmol) was added dropwise to the chilled reaction mixture. The ice water bath was removed, and the resulting solution was stirred at room temperature and monitored by LCMS. Upon completion, the solvent was removed under reduced pressure and the residue was dissolved in DCM, loaded onto a CombiFlash™

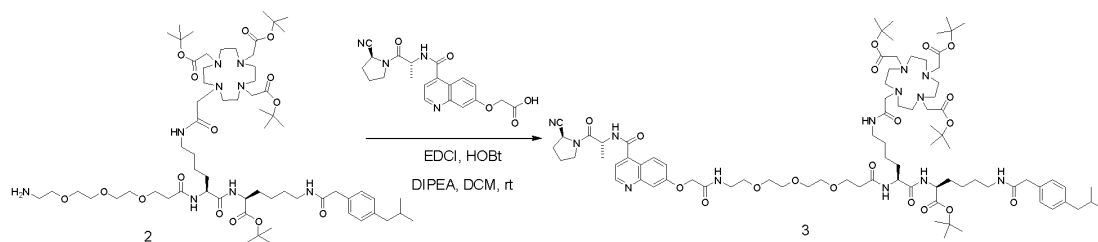
silica gel column (gradient 0-10% MeOH/DCM) and purified to obtain the target product (404 mg, 61% yield) as a glassy solid.

Procedure 50: Preparation of {4-[2-((S)-2-Cyano-pyrrolidin-1-yl)-2-oxo-ethylcarbamoyl]-quinolin-7-yloxy}-acetic acid:



To a solution of {4-[2-((S)-2-cyano-pyrrolidin-1-yl)-2-oxo-ethylcarbamoyl]-quinolin-7-yloxy}-acetic acid tert-butyl ester (141 mg, 0.322 mmol) in DCM (1.5 ml) was added TFA (1.51 ml, 19.64 mmol) dropwise at 0 °C. The temperature of the stirred reaction mixture was allowed to rise to room temperature over the course of 1 h, at which time full conversion was detected by LCMS. The solvent was evaporated at rt and residue was co-evaporated with toluene (x3) at 40 °C. The crude compound, Intermediate 2, was sufficiently pure to use for subsequent transformations, but it was unstable to hydrolysis upon long standing. For this reason, the compound was prepared immediately before use.

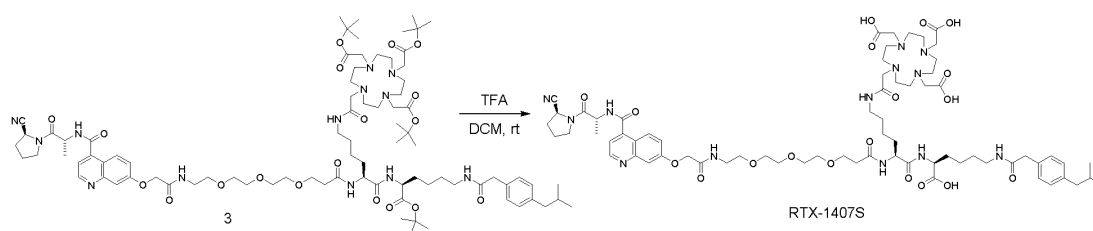
Procedure 51: Synthesis of tert-butyl 2-[2-(3-{2-[2-(2-{2-[4-((R)-2-[(S)-2-cyano-1-pyrrolidinyl]-1-methyl-2-oxoethylamino}carbonyl)-7-quinolyloxy]acetyl-amino}ethoxy)ethoxy]ethoxy}propionyl-amino)-6-{2-[4,7,10-tris(tert-butoxycarbonylmethyl)-1,4,7,10-tetraaza-1-cyclododecyl]-acetyl-amino}hexanoylamino]-6-[2-(p-isobutylphenyl)acetyl-amino]hexanoate (3)



To a solution of tert-butyl 2-[2-(3-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}propionylamino)-6-{2-[4,7,10-tris(tert-butoxycarbonylmethyl)-1,4,7,10-tetraaza-1-cyclododecyl]acetyl-amino}hexano-ylamino]-6-[2-(p-isobutylphenyl)acetyl-amino]hexanoate

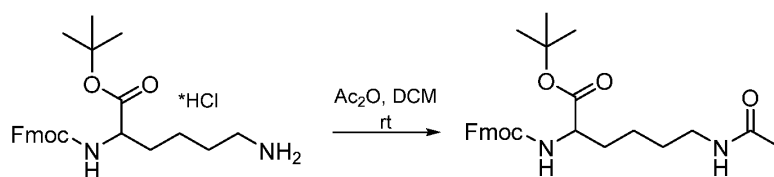
(59 mg, 0.046 mmol, 1 eq) in DCM was added [4-({(R)-2-[(S)-2-cyano-1-pyrrolidinyl]-1-methyl-2-oxoethylamino}carbonyl)-7-quinolyl-oxy]acetic acid (18 mg, 0.046 mmol, 1 eq) and DIPEA (25 μ L, 0.699 mmol, 3 eq), HOBt (6.8 mg, 0.050 mmol, 1.1 eq), and EDCI (7.8 mg, 0.050 mmol, 1.1 eq). The mixture was stirred at rt for 16 h before diluting it with DCM (5 ml) and water (5 ml). The aqueous layer was extracted with DCM (3x5 ml) and the combined organic layers were dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (0-20% MeOH in DCM) to yield the product (62 mg, 82%) as a colorless solid. LCMS: $\text{C}_{85}\text{H}_{133}\text{N}_{13}\text{O}_{19}$; m/z : 1641.04, observed $m/z = 1642.32$ $[\text{M}+\text{H}]^+$.

Procedure 52: Synthesis of 2-[2-(3-{2-[2-(2-{2-[4-({(R)-2-[(S)-2-cyano-1-pyrrolidinyl]-1-methyl-2-oxoethylamino}carbonyl)-7-quinolyloxy]acetyl-amino}ethoxy)ethoxy}ethoxy}propionyl-amino)-6-{2-[4,7,10-tris(carboxymethyl)-1,4,7,10-tetraaza-1-cyclododecyl]acetyl-amino}-hexanoylamino)-6-[2-(p-isobutylphenyl)acetyl-amino]hexanoic acid (RTX 1407S)



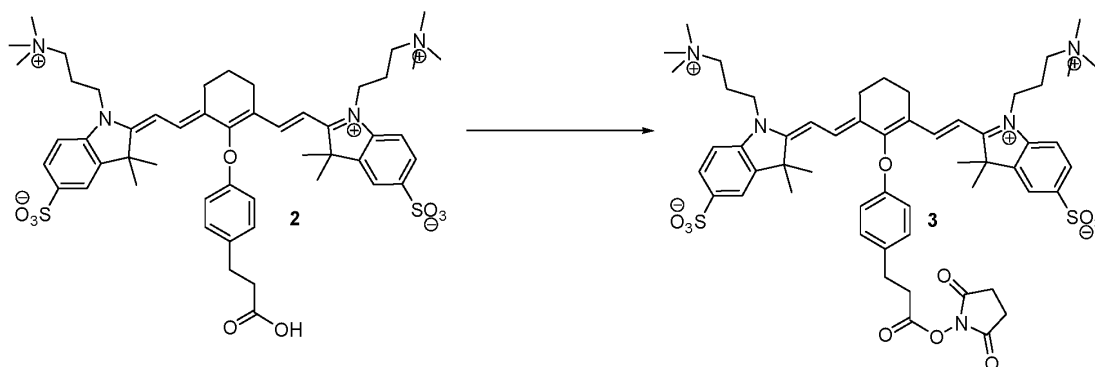
To a solution of tert-butyl 2-[2-(3-{2-[2-(2-{2-[4-({(R)-2-[(S)-2-cyano-1-pyrrolidinyl]-1-methyl-2-oxoethylamino}carbonyl)-7-quinolyloxy]acetyl-amino}ethoxy)ethoxy}ethoxy}propionyl-amino)-6-{2-[4,7,10-tris(tert-butoxycarbonylmethyl)-1,4,7,10-tetraaza-1-cyclododecyl]acetyl-amino}hexanoylamino)-6-[2-(p-isobutylphenyl)acetyl-amino]hexanoate (62 mg, 0.037 mmol, 1 eq) in DCM (0.62 ml) was added TFA (283 μ L, 3.70 mmol). The resulting mixture was stirred for 10 h. The volatiles were removed and the residue was purified by HPLC to yield the product (35.8 mg, 68%) as a colorless solid. LCMS: $\text{C}_{69}\text{H}_{101}\text{N}_{13}\text{O}_{19}$; m/z : 1416.62, observed $m/z = 1417.60$ $[\text{M}+\text{H}]^+$.

Procedure 53: Synthesis of tert-butyl N2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-acetyllysinate



To a stirred solution of 5-(((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-(tert-butoxy)-6-oxohexan-1-aminium chloride (3 g, 4.6 mmol) and DIEA (1.76 ml, 10.12 mmol) in DCM (24 ml) was added acetic anhydride (524 μ l, 5.51 mmol). The mixture was stirred at room temperature for 90 min, at which time LCMS indicated complete conversion. The reaction mixture was diluted with EtOAc, washed with water and brine, dried over anhydrous MgSO_4 and filtered. Evaporation of the volatiles under reduced pressure left the target compound (2.9 g, 91%) as off-white solid.

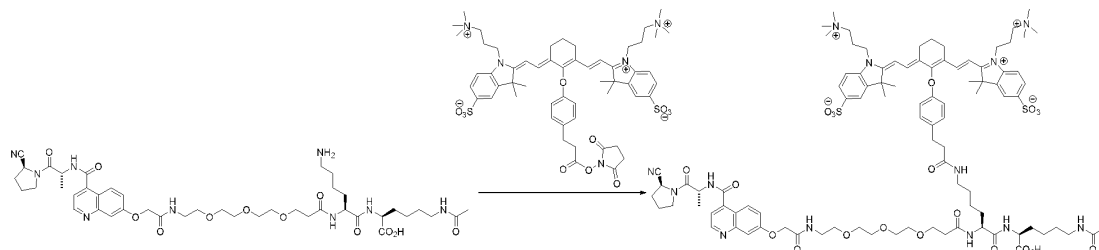
Procedure 54: Synthesis of 2-((E)-2-((E)-3-((E)-2-(3,3-dimethyl-5-sulfonato-1-(3-(trimethylammonio)propyl)indolin-2-ylidene)ethylidene)-2-(4-(3-((2,5-dioxopyrrolidin-1-yl)oxy)-3-oxopropyl)phenoxy)cyclohex-1-en-1-yl)vinyl)-3,3-dimethyl-1-(3-(trimethylammonio)propyl)-3H-indol-1-ium-5-sulfonate:



To a solution of ZW800-1 (100 mg, 0.105 mmol) in anhydrous DMSO (10 ml) was added dipyrrolidino(N-succinimidyl)oxy)carbenium hexafluorophosphate (130 mg, 0.3 mmol) followed by the addition of N,N-diisopropylethylamine (0.2 mL, 1.1 mmol) dropwise at room temperature. The reaction mixture was stirred at ambient temperature for 16 hours, at which time full conversion was detected by LCMS. The mixture was treated with a solution of 1:1:1 ethanol:ethylacetate:acetone (150 mL) and 0.1% trifluoroacetic acid (0.9 mL) and was allowed to mix and sit for 30 min. The solids were filtered and dried under vacuum to obtain 2-((E)-2-((E)-3-((E)-2-(3,3-dimethyl-5-sulfonato-1-(3-(trimethylammonio)propyl)indolin-2-ylidene)ethylidene)-2-(4-(3-((2,5-dioxo-pyrrolidin-1-yl)oxy)-3-oxopropyl)phenoxy)cycl-

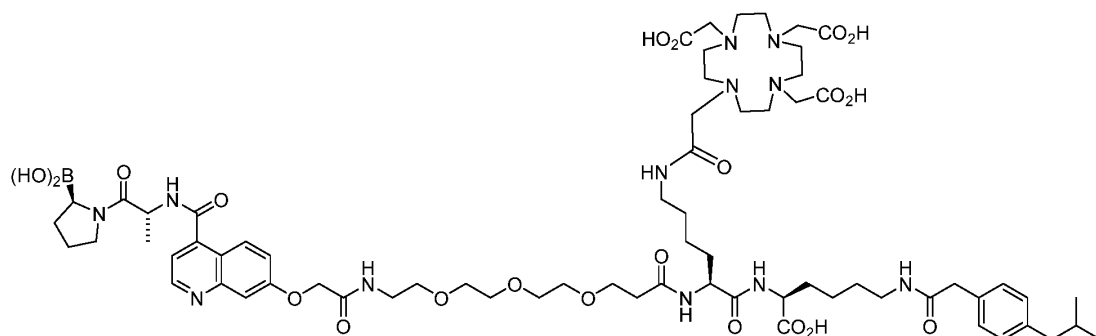
ohex-1-en-1-yl)vinyl)-3,3-dimethyl-1-(3-(trimethylammonio)propyl)-3H-indol-1-ium-5-sulfonate as a green powder (51 mg, 46%) which was used directly for the next step without further purification. LCMS: $C_{46}H_{61}N_9O_{12}$; m/z : 1040.32, observed m/z = 1040.5 $[M]^+$.

Procedure 55:



To the solution of 2-((E)-2-((E)-3-(2-((E)-3,3-dimethyl-5-sulfonato-1-(3-(trimethylammonio)propyl)indolin-2-ylidene)ethylidene)-2-(4-(3-((2,5-dioxopyrrolidin-1-yl)oxy)-3-oxopropyl)phenoxy)cyclohex-1-en-1-yl)vinyl)-3,3-dimethyl-1-(3-(trimethylammonio)propyl)-3H-indol-1-ium-5-sulfonate (0.070 g, 0.067 mmol) in DMSO (1 mL) was added a solution of N6-acetyl-N2-((1-((4-((R)-1-((S)-2-cyanopyrrolidin-1-yl)-1-oxopropan-2-yl)carbamoyl)quinolin-7-yl)oxy)-2-oxo-6,9,12-trioxa-3-azapentadecan-15-oyl)-L-lysyl)-L-lysine (0.040 g, 0.045 mmol) in DMSO (1 mL). N,N-Diisopropylethylamine (0.070 g, 0.067 mmol) was added at room temperature, and the mixture was allowed to stir for 16h. Full conversion was detected by LCMS. After the reaction completion, the volatiles were removed and the residue was purified by HPLC to yield the desired product.

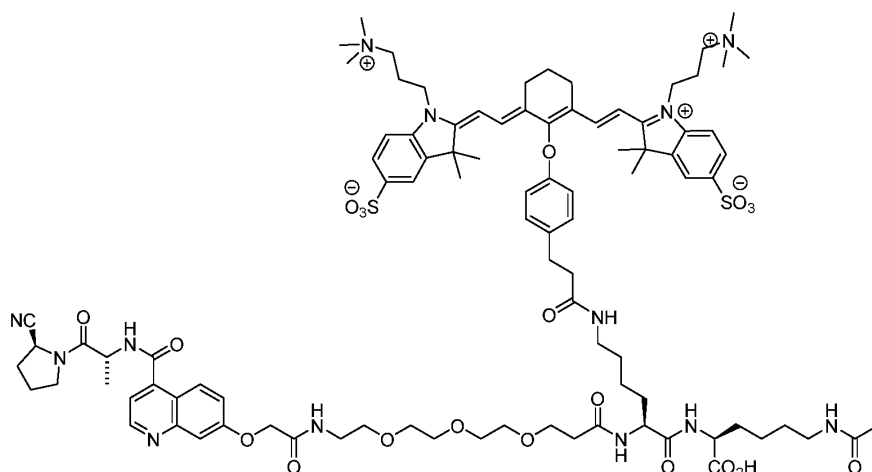
Synthesis of RTX-1371R:



Prepared according procedures analogous to those of RTX-1401R using Int-3 as a starting material. Int-3 was prepared with the following changes:

In Procedure 27, 2-(4-isobutylphenyl)acetic acid was used in lieu of hexanoic acid.

Synthesis of RTX-1384S

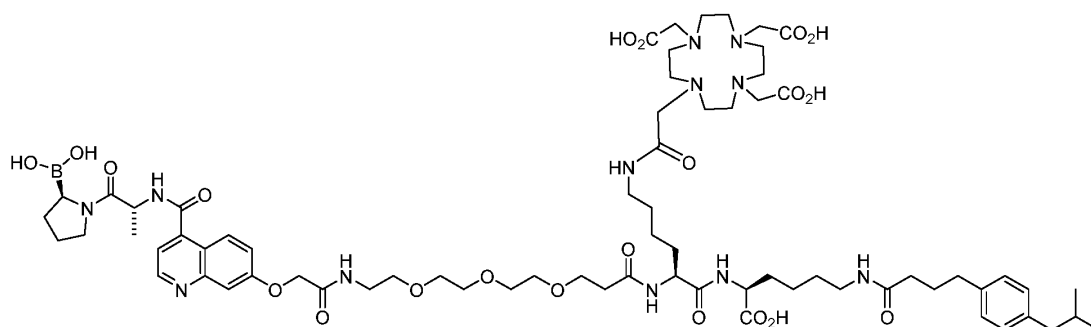


Prepared according procedures analogous to those to prepare Int-2 with the following changes:

Procedure 53 was used in lieu of Procedure 14.

The product of procedure 19 was utilized for the further synthesis of RTX-1384S by subjecting this product to procedures 51 to install the quinoline and 52 to remove protecting groups. This was followed by procedures 54 and 55 to install the fluorophore.

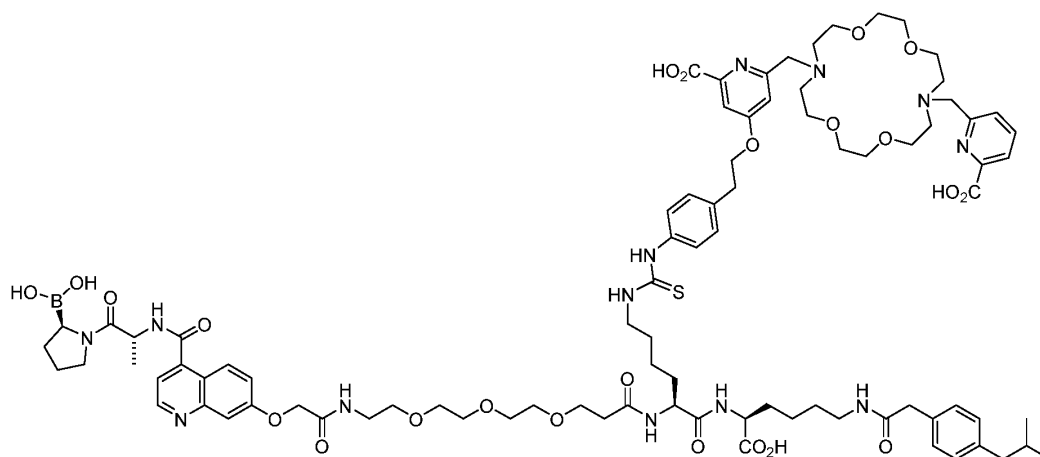
Synthesis of RTX-1391R:



Prepared according to procedures analogous to those of RTX-1401R using Int-3 as starting material. Int-3 was prepared with the following changes:

In Procedure 27, 2-(4-isobutylphenyl)butanoic acid was used in lieu of hexanoic acid.

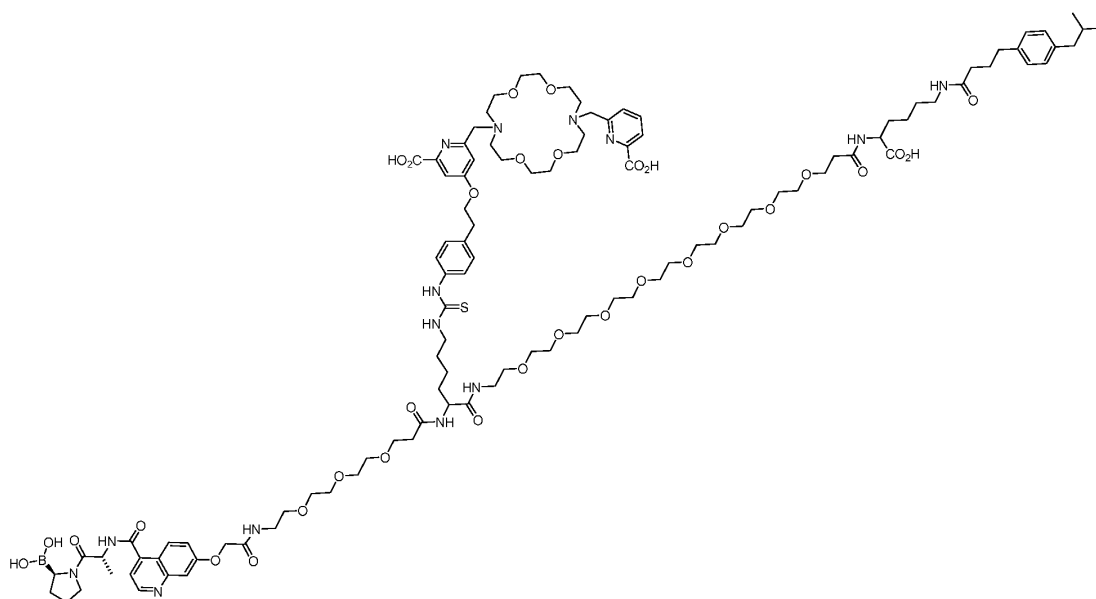
Synthesis of RTX-1400R



Prepared according to procedure analogous to those of RTX-1401R using Int-2 as starting material. Int-2 was prepared with the following changes:

In Procedure 14, 2-(4-isobutylphenyl)acetic acid was used in lieu of 2-(4-isobutylphenyl)butanoic acid.

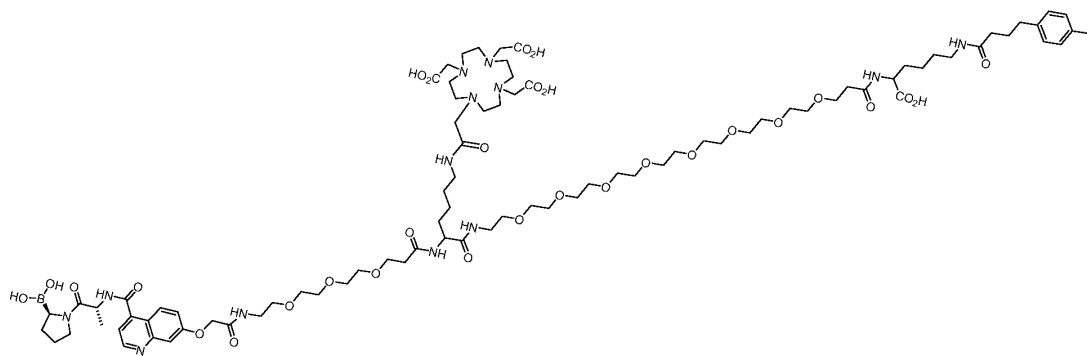
Synthesis of RTX-1402R



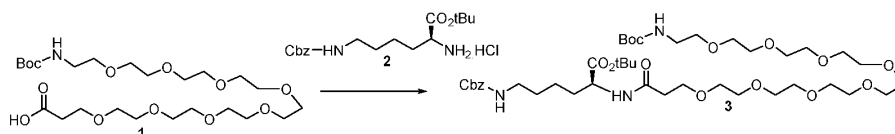
Prepared according to procedures analogous to those of RTX-1392 using Int-1 as starting material. Int-1 was prepared with the following changes:

Procedure 23 was used in lieu of Procedure 10 to install the Macropa chelator.

Synthesis of RTX-1411R

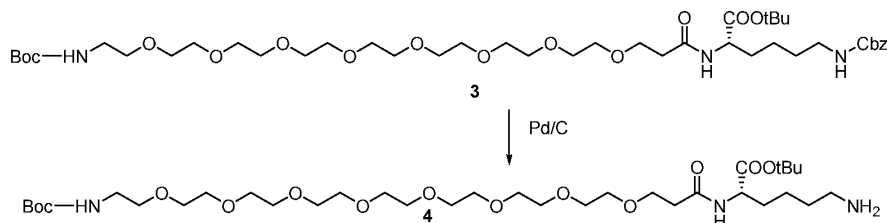


Procedure 56: (tert-butyl N6-((benzyloxy)carbonyl)-N2-(2,2-dimethyl-4-oxo-3,8,11,14,17,20,23, 26,29-nonaoxa-5-azadotriacontan-32-oyl)lysinate) (3):



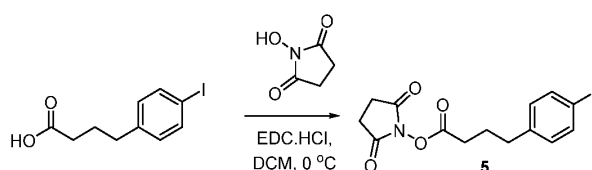
To a mixture of 1 (1 eq), 2 (1.1 eq) and HATU (1.2 eq) in DMF at 0 °C, DIPEA (2.2 eq) was added dropwise. The resulting reaction mixture was stirred at rt for 3 h. Completion of the reaction was monitored by LCMS. The solvent was evaporated to dryness and the resulting crude reaction mixture was purified by Combi Flash using 0-100% EtOAc in hexane (quantitative yield) LCMS: $C_{42}H_{73}N_3O_{15}$: m/z: 859.50, observed m/z = 860.6 $[M+H]^+$.

Procedure 57: tert-butyl (2,2-dimethyl-4-oxo-3,8,11,14,17,20,23,26,29-nonaoxa-5-azadotriacontan-32-oyl)lysinate (4):



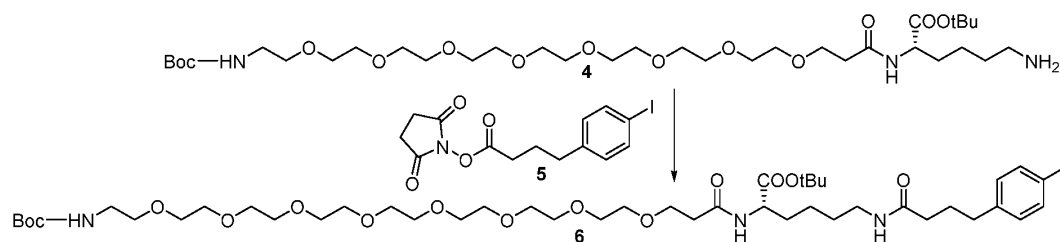
To a solution of **3** (0.5 g) in methanol (10 ml) was added 10% palladium on activated carbon (100 mg). The suspension was subjected to catalytic hydrogenation (H_2 , under balloon pressure) at ambient temperature for 3 h (LCMS control - consumption of starting material). The reaction mixture was filtered through Celite, and the filtrate was concentrated under reduced pressure. The residue was dried under high vacuum to give target product (quant.) as a colorless liquid. LCMS: $C_{34}H_{67}N_3O_{13}$; m/z: 825.92, observed m/z = 826.9 $[M+H]^+$.

Procedure 58: 2,5-dioxopyrrolidin-1-yl 4-(4-iodophenyl)butanoate (**5**):



To a stirred solution of iodophenylbutanoic acid (2.0 g, 1 eq) in DCM (20 ml) was added N-hydroxy succinimide (1.3 eq) at 0 °C. The reaction mixture was stirred for 5 minutes at the same temperature, and EDC.HCl (1.3 eq) was added. The resulting solution was stirred 1 h at rt (reaction progress was monitored by TLC). The reaction mixture was diluted DCM (20 ml), washed with water (50 ml), separated, and the organic layer was dried over Na_2SO_4 and evaporated. The residue was washed with diethyl ether (2 X 20 ml) to obtain compound **5** (2.0 g) as an off-white solid.

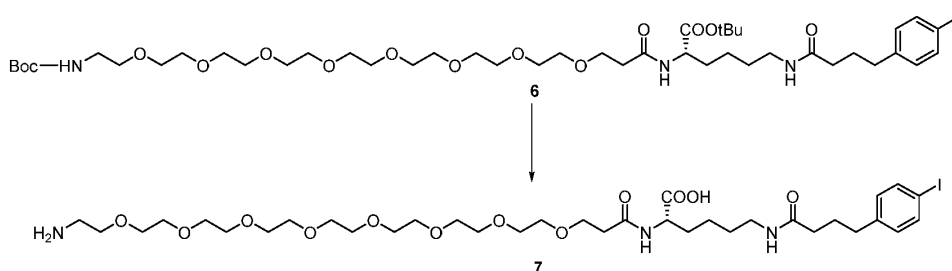
Procedure 59: tert-butyl N2-(2,2-dimethyl-4-oxo-3,8,11,14,17,20,23,26,29-nonaoxa-5-azadotriacontan-32-oyl)-N6-(4-(4-iodophenyl)butanoyl)lysinate (**6**):



To a solution of **4** (2.0 g, 1 eq) in DCM (25 ml) was added 2,5-dioxopyrrolidin-1-yl 4-(4-iodophenyl)butanoate (**5**) (1.0 eq) at room temperature, followed by the addition of DIPEA (1.2 eq). The reaction mixture was stirred for 30 minutes. After completion of the reaction (reaction progress was monitored by LCMS), the solvent was evaporated under reduced pressure and the crude residue was purified by Combi Flash using MeOH: DCM (0 – 20%) to

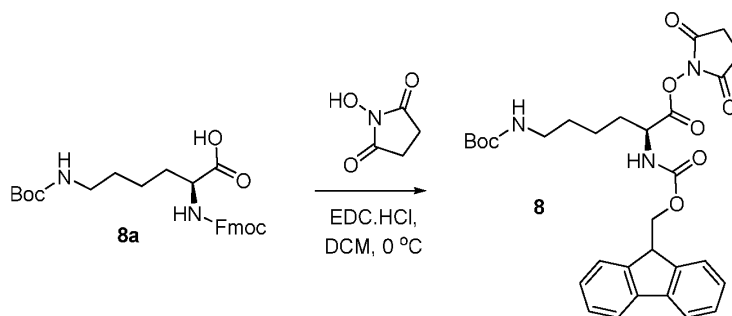
obtain compound **6** as light brown viscous oil (1.8 g). LCMS: $C_{44}H_{76}IN_3O_{14}$: m/z : 997.44, observed (loss of tert-butyl group) $m/z = 471.45 [M/2+H]^+$.

Procedure 60: N2-(1-amino-3,6,9,12,15,18,21,24-octaoxaheptacosan-27-oyl)-N6-(4-(4-iodophenyl)butanoyl)lysine (**7**):



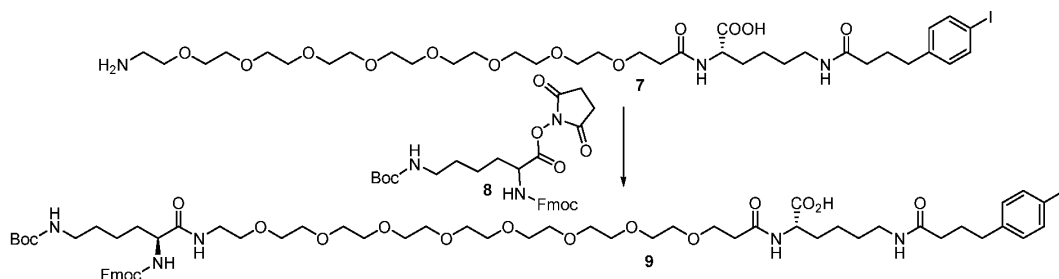
To a stirred solution of **6** (1.8 g, 1 eq) in DCM (10 ml) was added TFA (10 ml) at room temperature and the reaction mixture was stirred for 30 minutes. After completion of the reaction (reaction progress was monitored by LCMS), the volatiles were evaporated under reduced pressure and co-distilled with acetonitrile (3 X 30 ml) and dried under high vacuum for 30 minutes. The crude product (2.0 g) was used without further purification for the next step in the reaction sequence). LCMS: $C_{35}H_{60}IN_3O_{12}$: m/z : 841.32, observed $m/z = 842.32[M+H]^+$.

Procedure 61: 2,5-dioxopyrrolidin-1-ylN2-(((9H-fluoren-9-yl)methoxy)carbonyl)-N6-(tert-butoxycarbonyl)lysinate (**8**):



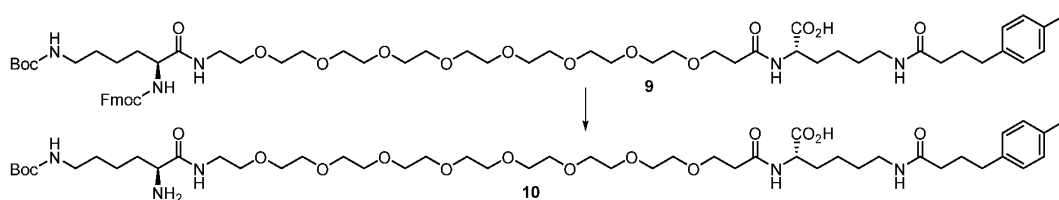
To a stirred solution of **8a** (2.0 g, 1 eq) and NHS (1.3 eq) in DCM (10 ml) was added EDC.HCl at 0 °C and the mixture was stirred at room temperature for 30 minutes. The reaction mixture was diluted with 20 ml of DCM, washed with 50 ml of water, dried over Na_2SO_4 and evaporated. The residue was washed with diethyl ether (2 X 25 ml) to obtain compound **8** (2.0 g) as off-white solid. LCMS: $C_{30}H_{35}N_3O_8$: m/z : 565.24, observed $m/z = 588.19 [M+Na]^+$.

Procedure 62: N2-(10-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-2,2-dimethyl-4,11-dioxo-3, 15,18,21,24,27,30,33,36-nonaoxa-5,12-diazanonatriacontan-39-oyl)-N6-(4-(4-iodophenyl) butanoyl)lysine (9):



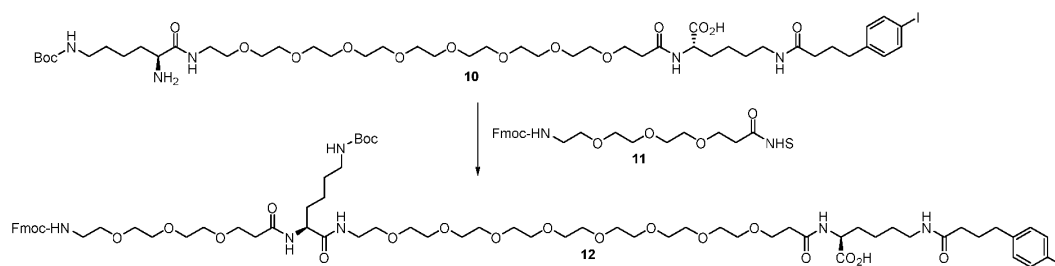
To a solution of 7 (2.0 g, 1 eq) in DMF (10 ml) was added DIPEA (3.0 eq), followed by a solution of 8 (1.0 eq) in DMF at room temperature, and stirred for 30 minutes. After completion of the reaction (reaction progress was monitored by LCMS), the solvent was evaporated and the crude residue was purified by silica gel column chromatography eluting in MeOH:DCM (0 – 40%) to obtain compound 9 as light brown viscous oil (1.7 g). LCMS: $C_{61}H_{90}IN_5O_{17}$: m/z: 1291.54, observed m/z = 647.46[M/2+H]⁺

Procedure 63: N2-(10-amino-2,2-dimethyl-4,11-dioxo-3,15,18,21,24,27,30,33,36-nonaoxa-5,12-diazanonatriacontan-39-oyl)-N6-(4-(4-iodophenyl)butanoyl)lysine (10):



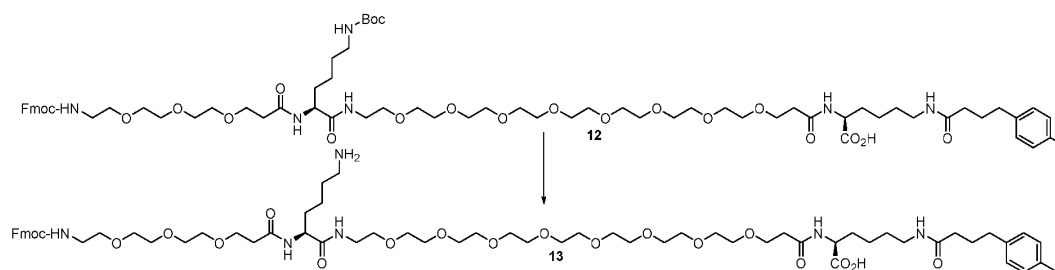
To a stirred solution of 9 (0.4 g, 1 eq) in DCM (4 ml) was added diethyl amine (4 ml) at room temperature and the reaction mixture was stirred for 30 minutes. After completion of the reaction (reaction progress was monitored by LCMS), solvent was evaporated under reduced pressure and co-distilled with acetonitrile (3 X 30 ml) and dried under high vacuum for 30 minutes. The crude product 10 (0.5 g) was used as obtained for the next step without any further purification. LCMS: $C_{46}H_{80}IN_5O_{15}$: m/z: 1069.47, observed m/z = 535.98 [M/2+H]⁺.

Procedure 64: N2-(10-(1-(9H-fluoren-9-yl)-3-oxo-2,7,10,13-tetraoxa-4-azahexadecan-16-amido)-2,2-dimethyl-4,11-dioxo-3,15,18,21,24,27,30,33,36-nonaoxa-5,12-diazanonatriacontan-39-oyl)-N6-(4-(4-iodophenyl)butanoyl)lysine (12):



DIPEA (3.0 eq) was added to a solution of 10 (0.5 g, crude 1 eq) in DMF (5 ml) followed by the addition of 11 (1.0 eq) in DMF (5 ml) at room temperature, and the reaction mixture was stirred for 30 minutes. After completion of the reaction (reaction progress was monitored by LCMS), the solvent was evaporated. The crude residue was purified by silica gel column chromatography (MeOH:DCM, 0 – 30%) to obtain compound 9 as light brown viscous oil (0.7 g, crude). LCMS: $C_{70}H_{107}IN_6O_{21}$: m/z: 1494.65, observed m/z = 499.24 $[M/3+H]^+$, 521.5 $[M/3+Na]^+$.

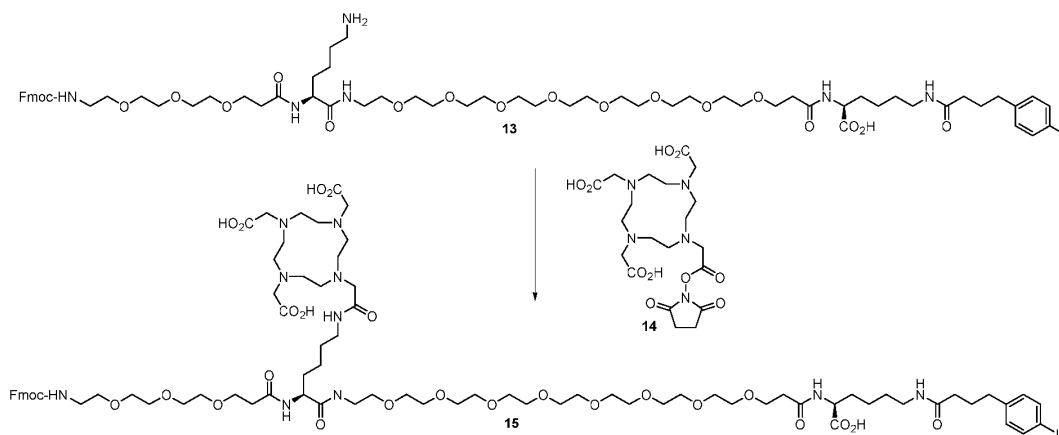
Procedure 65: N2-(30-(1-(9H-fluoren-9-yl)-3-oxo-2,7,10,13-tetraoxa-4-azahexadecan-16-amido)-34-amino-29-oxo-4,7,10,13,16,19,22,25-octaoxa-28-azatetratriacontanoyl)-N6-(4-(4-iodophenyl) butanoyl)lysine (13):



To a stirred solution of 12 (0.7 g, crude 1 eq) in DCM (4 ml) was added TFA (6 ml) at room temperature and the mixture was stirred for 30 minutes. After completion of the reaction (reaction progress was monitored by LCMS), the solvent was evaporated under reduced pressure, co-distilled with acetonitrile (3 X 10 ml), and dried under high vacuum for 30 minutes. The crude product (0.6 g) was used as obtained for the next step without any further purification. LCMS: $C_{65}H_{99}IN_6O_{19}$: m/z: 1394.65, observed m/z = 698.85 $[M/2+H]^+$.

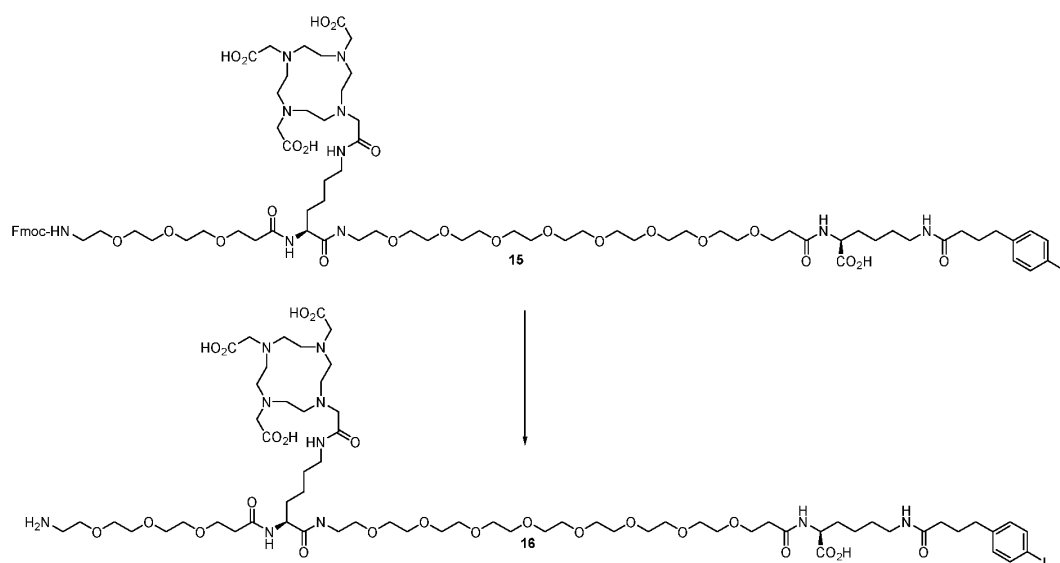
Procedure 66: 2,2',2''-(10-(8-(1-(9H-fluoren-9-yl)-3-oxo-2,7,10,13-tetraoxa-4-azahexadecan-16-amido)-39-carboxy-48-(4-iodophenyl)-2,9,37,45-tetraoxo-13,16,19,22,25,28,31,34-

octaoxa-3,10,38,44-tetraazaooctatetracontyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (15):



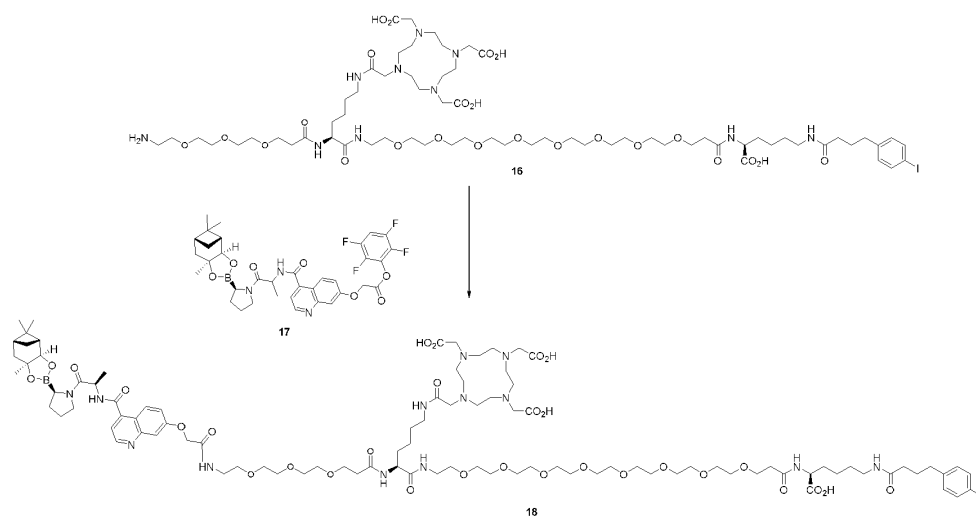
To a stirred solution of solution 13 (0.6 g, crude 1 eq), in DMF (5 ml) was added DIPEA (2 eq) followed by the addition of DOTA NHS ester (14, 1 eq) and the reaction mixture was stirred for 30 minutes. After completion of the reaction (reaction progress was monitored by LCMS), the solvent was evaporated, and the crude residue was used as isolated for the next step without further purification (0.65 g, crude). LCMS: $C_{81}H_{125}IN_{10}O_{26}$: m/z : 1780.78, observed m/z = 892.12 $[M/2+H]^+$.

Procedure 67: 2,2',2''-(10-(8-(3-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)propanamido)-39-carboxy-48-(4-iodophenyl)-2,9,37,45-tetraoxo-13,16,19,22,25,28,31,34-octaoxa-3,10,38,44-tetraazaooctatetracontyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (16):



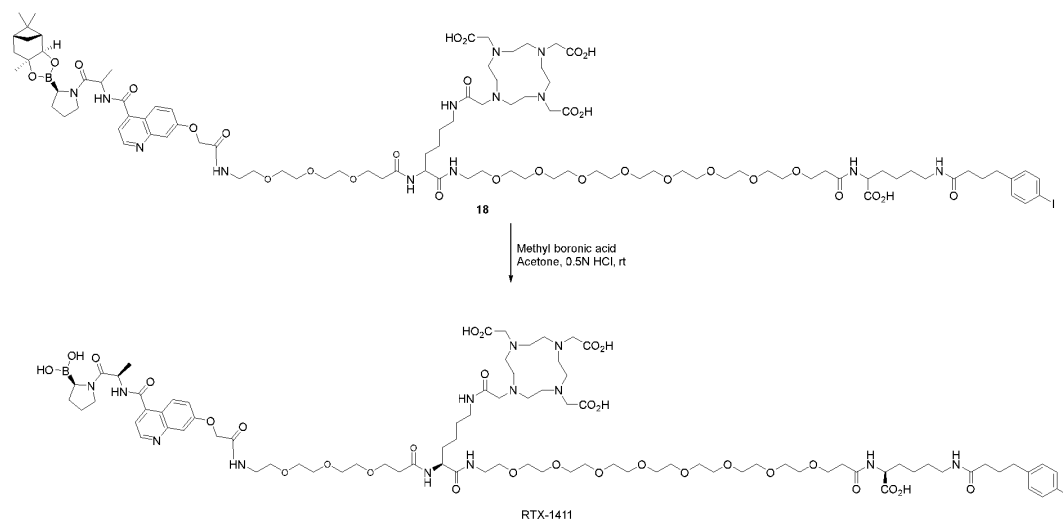
Diethyl amine (5 ml) was added **15** (0.65 g) at room temperature and the reaction mixture was stirred at the same temperature for 30 mins. Upon completion (the reaction progress was monitored by LCMS), the solvent was evaporated and the crude residue was purified by prep HPLC (ACN:H₂O, 5-95%, 40 mins) to give pure compound of **16** as colorless solid (240 mg). LCMS: C₆₆H₁₁₅IN₁₀O₂₄; m/z: 1558.71, observed m/z = 780.46 [M/2+H]⁺.

Procedure 68: 2,2',2''-(10-(39-carboxy-48-(4-iodophenyl)-2,9,37,45-tetraoxo-8-(2-oxo-1-((4-((1-oxo-1-((R)-2-((3aR,4R,6R,7aS)-5,5,7a-trimethylhexahydro-4,6-methanobenzo[d][1,3,2]dioxaborol-2-yl)pyrrolidin-1-yl)propan-2-yl)carbamoyl)quinolin-7-yl)oxy)-6,9,12-trioxa-3-azapentadecan-15-amido)-13,16,19,22,25,28,31,34-octaoxa-3,10,38,44-tetraazaocatetracontyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (**18**):



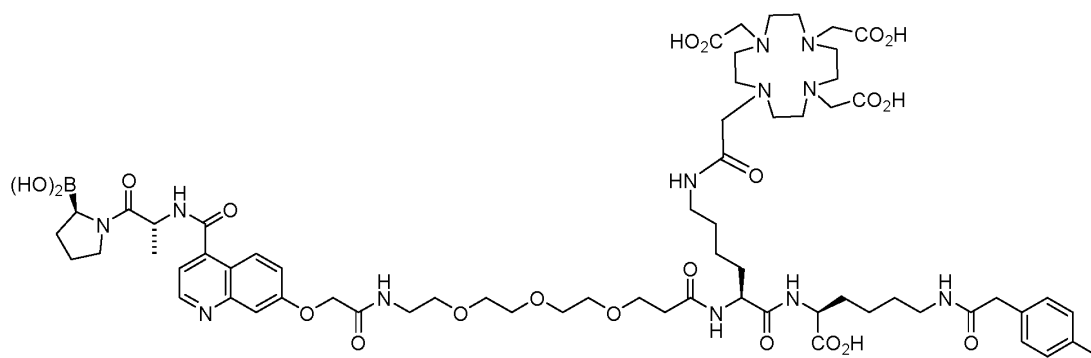
To a stirred solution of 16 (20 mg, 1 eq), in DMF (5 ml) was added DIPEA (2 eq) followed by the addition of compound 17 (1.5 eq) and the reaction mixture was stirred for 30 minutes. Upon completion (the reaction progress was monitored by LCMS), the solvent was evaporated, and the crude residue was purified by prep HPLC (ACN:H₂O, 5-95%, 40 mins) to give pure compound of 18 as white solid (10 mg). LCMS: C₆₆H₁₁₅IN₁₀O₂₄: m/z: 2089.97, observed m/z = 698.31 [M/3+H]⁺.

Procedure 69: 2,2',2''-(10-(8-(1-((4-((1-((R)-2-boronopyrrolidin-1-yl)-1-oxopropan-2-yl) carbamoyl)quinolin-7-yl)oxy)-2-oxo-6,9,12-trioxo-3-azapentadecan-15-amido)-39-carboxy-48-(4-iodophenyl)-2,9,37,45-tetraoxo-13,16,19,22,25,28,31,34-octaoxa-3,10,38,44-tetraazaoctatetracontyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (141 IR):



To a stirred solution of compound 16 (10 mg, 1 eq) in acetone (1 ml) was added 1N HCl (1 ml) followed by methyl boronic acid (5 eq) and the reaction mixture was stirred at room temperature for 12h. After completion of the reaction (monitored by LC-MS), the compound purified by prep HPLC (ACN:H₂O, 5-95%, 40 mins (60% in 30 mins followed by 95% in 40 mins) to give pure compound of as white solid (4 mg). LCMS: C₈₅H₁₃₅BiN₁₃O₃₀: m/z: 1955.86, observed m/z = 647.31 [M-18/3+H]⁺.

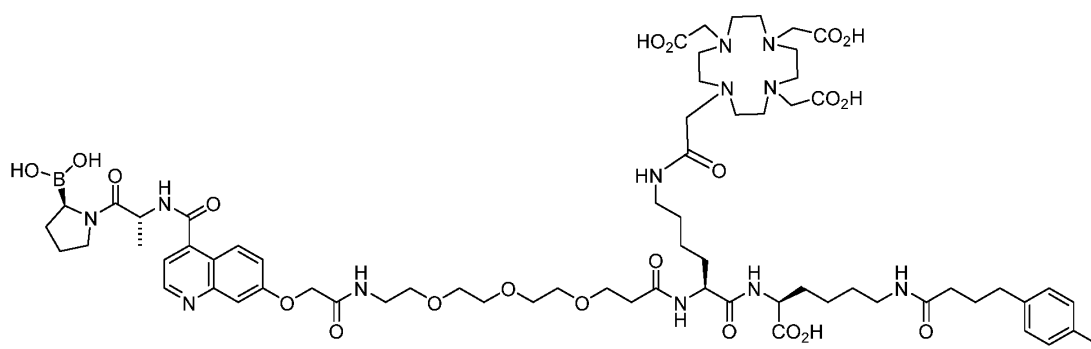
Synthesis of RTX-1409R



Prepared according to procedures analogous to those of RTX-1411R using Boc-L-Lys(H)-OtBu as the starting material with the following changes:

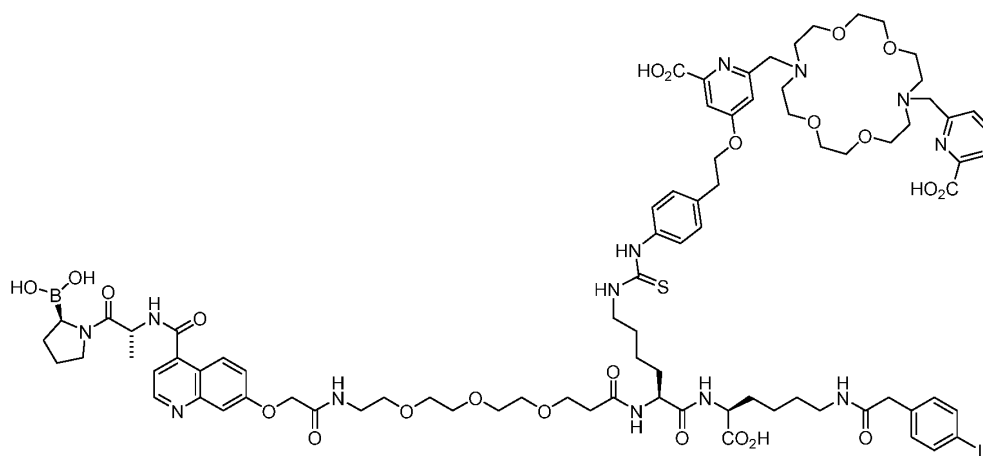
2-(4-iodophenyl)acetic acid was used in lieu of iodophenylbutanoic acid in Procedure 58.

Synthesis of RTX-1410R



Prepared according to procedures analogous to those of RTX-1411R using Boc-L-Lys(H)-OtBu as the starting material.

Synthesis of RTX-1413R

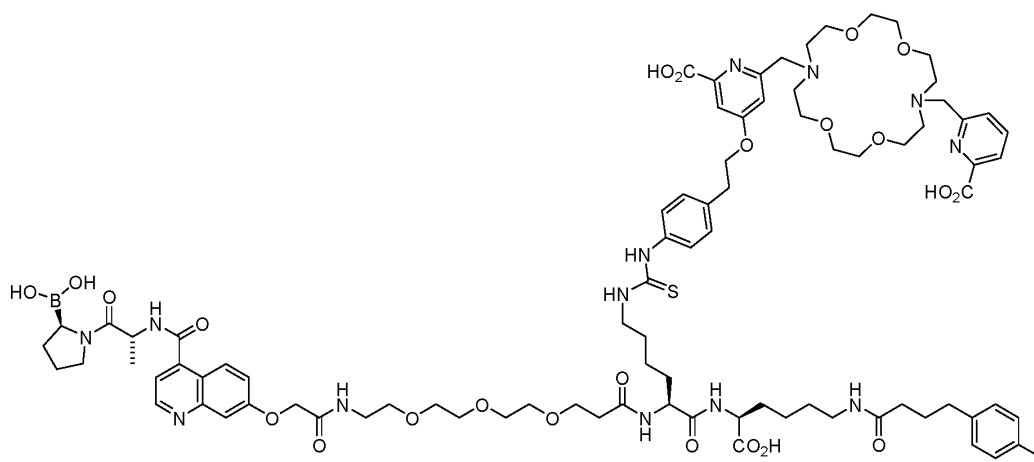


Prepared according to procedures analogous to those of RTX-1411R using Boc-L-Lys(H)-OtBu as the starting material with the following changes:

2-(4-iodophenyl)acetic acid was used in lieu of iodophenylbutanoic acid in Procedure 58.

Macropa chelator was installed using Procedure 23 in lieu of in Procedure 66.

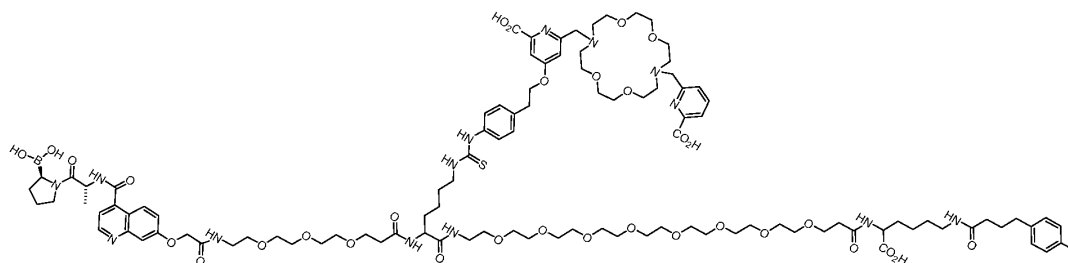
Synthesis of RTX-1414R



Prepared according to procedures analogous to those of RTX-1411R using Boc-L-Lys(H)-OtBu as the starting material with the following changes:

Macropa chelator was installed using Procedure 23 in lieu of in Procedure 66.

Synthesis of RTX-1415R



Prepared according to procedures analogous to those of RTX-1411R with the following changes:

Macropa chelator was installed using Procedure 23 in lieu of in Procedure 66.

Example 2 – Compounds of the Invention Bind to FAP With High Affinity

Protease reactions were assembled in 384 well plates (Greiner) in a total volume of 20 uL as described below.

Recombinant proteins were pre-diluted in assay buffer comprising of 100mM HEPES, pH 7.5, 0.1% BSA, 0.01% Triton X-100, 1mM DTT, and dispensed into 384 well plate (10 uL per well). Test compounds were serially pre-diluted in DMSO and added to the assay wells by acoustic dispensing (Labcyte Echo 550). Control samples (0%-inhibition in the absence of inhibitor, DMSO only) and 100%-inhibition (in the absence of enzyme) were assembled in replicates of four and used to calculate the %-inhibition in the presence of compounds. Concentration of DMSO was equalized to 1% in all samples.

Compounds were pre-incubated with enzymes for 15 minutes. Human FAP was obtained from Enzo, catalogue number BML-SE409-0010. The reactions are initiated by addition of 10 uL of 2x FAM-labeled substrate peptide (FAM-GPRPFNYLAKK-NH₂) prepared in the same assay buffer. Final concentration of enzymes were 0.5 nM. Final concentration of substrate peptides was 1 uM.

The reactions were allowed to proceed at room temperature. Incubation time was three hours for human FAP, 0.5 hours for mouse FAP. After incubation, kinase reactions were quenched by addition of 50 uL of termination buffer: assay buffer supplemented with reference inhibitor at 100 x IC₅₀.

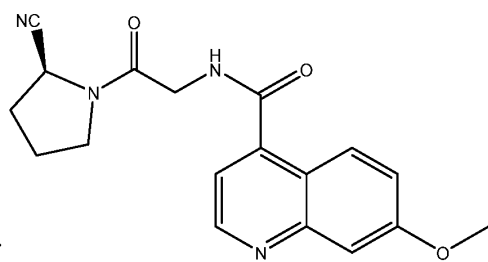
Terminated plates were analyzed using a microfluidic electrophoresis instrument (Caliper LabChip® 3000, Caliper Life Sciences/Perkin Elmer). A change in relative intensity of the peptide substrate and cleaved product was the parameter measured. Activity in each test sample was determined as the product to sum ratio (PSR): $P/(S+P)$, where P is the peak height of the product, and S is the peak height of the substrate. Percent inhibition (P_{inh}) was determined using the following equation: $P_{inh} = (PSR_{0\%inh} - PSR_{compound}) / (PSR_{0\%inh} - PSR_{100\%inh}) * 100$, in which: $PSR_{compound}$ is the product/sum ratio in the presence of compound, $PSR_{0\%inh}$ is the product/sum ratio in the absence of compound and the $PSR_{100\%inh}$ is the product/sum ratio in the absence of the enzyme. To determine IC₅₀ of compounds (50%-inhibition) the %-inh data (P_{inh} versus compound concentration) are fitted by a 4 parameter sigmoid dose-response model using XLfit software (IDBS). These values are

compiled in Table 1 and grouped, where AA represents an $IC_{50} < 250$ pM, A represents an $IC_{50} < 0.1$ nM; B is an IC_{50} from 0.1 to 0.5 nM; C is an IC_{50} from 0.5 to 5.0 nM; D is an IC_{50} from 5.0 to 100 nM; E an $IC_{50} > 100$ nM. Some compounds of the present disclosure afforded IC_{50} values beyond the limit of detection of this assay (*i.e.* below 250 pM).

Table 1. Results for Binding Affinity Studies

Compound	IC_{50} (nM)	MS Calc (Found)	Compound	IC_{50} (nM)	MS Calc (Found)
RTX-1371R	A	1434.75 (701.4)	RTX-1407S	C	1415.73 (1417.6)
RTX-1384S	B	1823.89 (912.7)	RTX-1409R	AA	1504.58 (744)
RTX-1391R	A	1462.78 (715.2)	RTX-1410R	AA	1532.61 (758)
RTX-1392R	A	1886.02 (927.1)	RTX-1411R	AA	
RTX-1400R	A	1757.84 (862.9)	RTX-1413R	AA	
RTX-1401R	A	1785.87 (876.7)	RTX-1414R	AA	
RTX-1402R	A	2209.12	RTX-1415R	AA	

The S stereoisomer with the S configuration at the pyrrolidine are considerably more potent than the corresponding R stereoisomers. The inhibition studies above also revealed that



extending the chain off of the quinolinyl core of which has an LC_{50} of 1.5 nM, significantly improved activity. Specifically, the lengthening the chain piecemeal provided an improvement with each extension, and the addition of the final amino acid (lysine) provided an additional increase in activity.

Greater discrimination was observed between certain compounds of the invention and FAPI-46 in a surface plasmon resonance assay data (FAP-46 was measured to have an

affinity of 255 pM, whereas certain disclosed compounds had an affinity of 20 pM or stronger).

Example 3 – Surface Plasmon Resonance Studies to Investigate the Binding Affinity of Compounds of the Invention

As discussed in Example 2, some compounds of the present invention have binding affinities beyond the limit of detection of the assay (*i.e.* approximately 250 pM). Surface Plasmon Resonance provides a significantly lower limit of detection around 1- 10 pM. Both unmetalated and non-radioactive lanthanum (La) and lutetium (Lu) labeled compounds of the present invention were subjected to surface Plasmon resonance assay to analyze their binding affinity to human, mouse, and rat FAP.

SPR Methods:

The SPR experiments were performed at 37 °C using a Biacore 8K (Cytiva Europe GmbH). Ligand was immobilized by SA-biotin capture (mouse FAP, Biosystems Acro/Cat. No. FAP-M82Q8 and human FAP, Biosystems Acro/Cat. No FAP-H82Q6) on a SA-Chip (Cytiva #BR100531) or by amide coupling (rat FAP, Biosystems Acro/Cat. No. FAP-R5246) on a CM5 chip (Cytiva #29149603) in flow cell 2 for all 8 channels. For immobilization a ligand solution with a concentration of 10-20 microgram/mL was prepared and running buffer HBS-P+ (Cytiva #BR100671) without DMSO was used at a flow rate of 5 microliter/min over the surface of the selected flow cells at a temperature of 25 °C. Flow cell 1 on each of the 8 channels was left blank to serve as a reference surface.

K_D determination for the compounds was performed in single cycle experiments. As running buffer for the experiments HBS-P+ with 1% DMSO was used. A 1:4 dilution series with 6 concentrations (100 nM, 25 nM, 6.25 nM, 1.56125 nM, 0.390625 nM, 0.0976525 nM or 400 nM, 100 nM, 25 nM, 6.25 nM, 1.56125 nM, 0.390625 nM) and 0-value as blank was used. The experiments were performed at a flow rate of 100 microliters per minute with a contact time for each solution of 80 seconds and a final dissociation time of 2500 seconds. Solvent correction was performed at the start and end of the run.

All data was analyzed using the Biacore Insight Evaluation Software (Version 5.0.18.22102 Cytiva 2022). In all channels flow cell 1 was used as reference and solvent correctio was applied to all experiments via the measured calibration curves. Single cycle

data was analyzed by 1:1 binding kinetics model. All measurements were performed as duplicates or triplicates.

The mean dissociation constant (K_D) values, determined from $n = 2$ or 3 replicates, is summarized in Table 2 below. Some of the compounds of the present invention provided binding affinities beyond the limit of detection of this assay (*i.e.* < 1 pM). For the compounds tested, labeling with non-radioactive La or Lu did not significantly alter FAP binding affinity, as assessed within the limitations of the SPR assay.

Table 2. Results for SPR Binding Studies

Compound	Mean K_D (picomolar)		
	Human FAP	Rat FAP	Mouse FAP
FAP1-46	255	n.d.	n.d.
RTX-1359R	< 1	n.d.	< 1
La-RTX-1359R	< 1	n.d.	< 1
Lu-RTX-1359R	< 1	< 1	< 1
La-RTX-1411R	< 1	< 1	< 1
Lu-RTX-1411R	< 1	< 1	< 1
RTX-1392R	1.2	n.d.	< 1
La-RTX-1392R	< 1	< 1	< 1
Lu-RTX-1392R	< 1	< 1	< 1

Example 4 – Radiolabeling of a FAP-targeted compound with ^{18}F direct labelling

^{18}F is received from the manufacturer, is loaded on to a Sep Pak QMA Light Plus cartridge, and eluted into a glass reaction vial using a $\text{Cs}_2\text{CO}_3/\text{K222}$ solution. Eluted ^{18}F is azeotropically dried using acetonitrile (3×1 ml) at 95°C under a stream of N_2 . 2.5mg of the FAP-targeted compound is dissolved in in 0.3 ml DMSO which is then added to the above vial containing the dried ^{18}F . The vial is sealed and heated to 90°C for 15 minutes. The vial is cooled and a solution of 1 N HCl is added to vial, which is then sealed and heated to 95°C for 10 minutes. The reaction mixture is cooled and neutralized and then loaded on to a Semi Prep HPLC column for purification and formulated appropriately for use. Alternatively, a suitable FAP-targeted compound having a chelator can be used for ^{18}F AIF labelling, wherein AlCl_3 stock in acetate buffer (22.5 μL , 45 nmol, 0.9 eq) is added to a ^{18}F solution in sodium acetate (200uL) and the reaction vial is left at room temperature for 5 min. From precursor stock, a FAP-targeted compound solution (50 nmol scale, 12.5 μL) is then added to the above

vial. pH is corrected to about 4.0 by addition of 1% v/v acetic acid in water (15 μ L). Co-solvent, 200 μ L of EtOH, is added and the reaction vial is sealed, then heated at 100°C for 15 mins. The reaction mixture is diluted to 9.5 ml and loaded on to a C18 Sep Pak cartridge. Product is eluted with 300uL acidified EtOH and formulated for use.

Example 5 – Radiolabeling Methods and Results

General procedure: 20 μ g of precursor (unless otherwise noted) are combined with the indicated radioisotopes. The radioisotopes are either eluted from a generator (for ^{68}Ga) or obtained commercially in HCl solutions that were buffered with varying amounts of 3 N NaOAc to obtain a final pH of 4-6. Reactions with a C18 Sep-Pak Lite cartridge as needed for additional purity and/or reformulation for injection. Labeling results are shown in Table 3.

Table 3. Results for representative labeling reactions

Compound	Isotope	Precursor (μ g)	T ($^{\circ}\text{C}$)	Time (min)	Buffer	n	RC Y (%)	RC P (%)	Purification
RTX-1371R	^{68}Ga	20	90	10	3N NaOAc	3	54.0	99.9	C-18 Lite
RTX-1371R	^{177}Lu	5	90	10	3N NaOAc	2	98.0	98.1	None
RTX-1387S	^{68}Ga	20	90	10	3N NaOAc	1	54.3	99.9	C-18 Lite
RTX-1391R	^{68}Ga	20	90	10	3N NaOAc	1	87.2	96.5	None
RTX-1392R	^{68}Ga	20	90	10	3N NaOAc	3	85.5	94.7	None
RTX-1392R	^{177}Lu	5	90	10	3N NaOAc	2	94.2	94.3	None
RTX-1407S	^{68}Ga	20	90	10	3N NaOAc	1	90.3	99.9	None
RTX-1407S	^{177}Lu	5	90	10	3N NaOAc	1	99.5	99.6	None
RTX-1409R	^{68}Ga	20	75	10	3N NaOAc	1	88.3	97.8	C-18 Lite

RTX-1409R	¹⁷⁷ Lu	5	90	15	3N NaOAc	1	98.9	99.0	C-18 Lite
RTX-1410R	⁶⁸ Ga	20	75	10	3N NaOAc	1	89.3	98.9	C-18 Lite
RTX-1410R	¹⁷⁷ Lu	5	90	15	3N NaOAc	1	99.9	100	C-18 Lite
RTX-1411R	⁶⁸ Ga	20	75	10	3N NaOAc	1	88.2	97.7	C-18 Lite
RTX-1411R	¹⁷⁷ Lu	5	90	15	3N NaOAc	1	87.3	96.7	C-18 Lite
RTX-1411R	²²⁵ Ac	50	90	15	1M NH ₄ OA c	1	99.3	99.4	None

Example 6 - Detection of Tumors in Murine Models with Compounds of the Invention

BALB/c nude mice are transplanted with approximately 10^7 U-87 (human glioblastoma) cells and the tumors are developed. A quantity of an ¹⁸F-labeled FAP-targeted agent of the invention is administered to the mice by intravenous injection (e.g., tail vein), which is sacrificed after one hour. The mouse is imaged to evaluate the tumor binding of the ¹⁸F-labeled FAP targeted compound. Biodistribution of the compound is evaluated by analysis of resected tissue samples from each organ.

Example 7 – Biodistribution studies

Female BALB/C nude mice were inoculated subcutaneously on the right shoulder with U87MG cells in 1:1 matrigel:PBS. When the tumors reached a volume of 150-500 mm³, radiolabeled ligand was administered intravenously (IV) via tail vein. At various time points post-injection, mice were humanely euthanized via exsanguination and tissue samples (bladder, blood, urine, bone (femur), heart, lungs, liver, both kidneys, small intestines (including contents), large intestines (including contents), muscle (quadriceps), tumor, and tail) were resected, weighed and counted with a gamma counter. The activity of each collected tissue was measured in units of counts per minute (CPM). Triplicate aliquots of the radiotracer were also assayed in the gamma counter to calculate a factor for converting counts to units of activity (μCi/CPM). Values were decay corrected to the time of injection and

corrected for background radiation. The biodistribution of compounds [177Lu]RTX-1371R, [177Lu]RTX-1391R, [177Lu]RTX-1392R showed localization of the compounds within the tumor and minimal concentrations within other organs (See FIG. 1-3). This biodistribution demonstrated increased selectivity for the tumor over time demonstrating the high affinity for FAP. The incorporation of D-alanine results in significantly greater tumor retention at 72 and 168 hour time points. (See FIG 4-6). Compounds of the present disclosure demonstrate significantly greater tumor retention at late time points (48-168 h post injection) compared to existing compounds [177Lu]PNT5555 ([177Lu]RTX-1418R), and [177Lu]3BP-2286 ([177Lu]RTX-1386S), See FIG 7 and 8.

Example 8 – Cryo-fluorescence tomography (CFT) Experiments

Female Nu/J or NCr-Foxn1 nude mice were inoculated subcutaneously on the right shoulder with U-87MG cells in 1:1 matrigel:PBS. When the tumors reached a volume of at least 150 mm³, 1.5 µg of ligand diluted in sterile PBS was administered intravenously (IV) via tail injection. At various time points post-injection, mice were humanely euthanized via CO₂ inhalation and cervical dislocation and frozen for ten minutes in a hexane bath that had been cooled by dry ice surrounding the metal container full of hexanes for 30 minutes before freezing occurred. Mice were stored at -80°C until they were embedded in a block of frozen OCT compound and cryo-fluorescence tomography (CFT) was used to image the distribution of the ligand in the mice on the Xerra (Emit Imaging). Cuts 35 µm in thickness were sliced off the top of the block by a razor blade followed by fluorescence excitation and then a fluorescence emission reading for 500 ms at appropriate wavelengths for the fluorophore conjugated to the ligand. RGB images were captured after each slice in addition to the fluorescence images. Slices followed by image capture were taken throughout the entire block. Data were processed using the Xerra Recon application (Emit Imaging) and analyzed via Vivoquant (Invivo). A single, spherical ROI that was within the bounds of the organ of interest was drawn for the heart, liver, lungs (one ROI in each), kidneys (one ROI in each), joint (right rear knee), muscle (right rear quad), and brain. Whole body regions of interest (ROIs) were also drawn. 2D ROIs were drawn throughout the tumor and then interpolated to form a 3D ROI that captured the entire tumor volume. Mean fluorescence values for ROIs (arbitrary units) in mice injected at different time points and with different compounds were compared, See FIG. 10. Mean organ ROIs were normalized to whole body or to muscle ROI values by dividing all other organ values by the whole body or muscle values, See FIG. 11.

These normalized mean ROI values were compared between compounds and time points to assess signal to noise ratios.

Example 9 – Efficacy and Tolerability Studies in U-87 Xenografts

Female Nu/J mice were inoculated subcutaneously on the right flank with U-87MG cells in 1:1 matrigel:PBS. When the tumors reached a volume (using the formula $\frac{1}{2} \text{ length}^2 * \text{width}$) between 120 mm³ and 250 mm³, mice were weighed and randomized, based on tumor volume, into treatment groups. One µg of ligand at an [²²⁵Ac] activity of 15, 30 or 60 kilobecquerel (kBq), diluted in sterile PBS, was administered intravenously (IV) via tail injection once for treatment groups with a single administration. Multiple dose groups received 4 biweekly IV tail vein injections (spaced 2-3 days apart) of 15 kBq activity, 1 µg ligand, for a cumulative dose of 60 kBq activity. Body weights and tumor volumes were recorded twice per week. If one mouse had a decrease in body weight greater than 10% of the weight at the start of the study, DietGel® Boost (ClearH₂O) was given to every cage in the study. Tumor volume, body weight and survival were plotted using GraphPad Prism, *See* FIG. 12-17. Measurements continued biweekly for every mouse until they reached one of the following endpoint criteria: a) tumor volume $\geq 2000\text{mm}^3$, b) $\geq 20\%$ drop in body weight from the start of the study c) tumor became ulcerated; at which time the mouse was euthanized via CO₂ inhalation and cervical dislocation.

EQUIVALENTS

While certain embodiments have been illustrated and described a person with ordinary the art, after reading the foregoing specification, can effect changes, substitutions of equivalents and other types of alterations to the compounds of the present technology or salts, pharmaceutical compositions, derivatives, prodrugs, metabolites, tautomers or racemic mixtures thereof as set forth herein. Each aspect and embodiment described above can also have included or incorporated therewith such variations or aspects as disclosed in regard to any or all of the other aspects and embodiments.

The present technology is also not to be limited in terms of the particular aspects described herein, which are intended as single illustrations of individual aspects of the present technology. Many modifications and variations of this present technology can be made

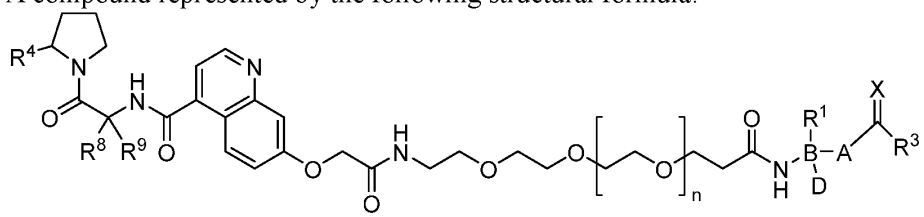
without departing from its spirit and scope, as will be apparent to those skilled in the art. Functionally equivalent methods within the scope of the present technology, in addition to those enumerated herein, will be apparent to those skilled in the art from the foregoing descriptions. Such modifications and variations are intended to fall within the scope of the appended claims. It is to be understood that this present technology is not limited to particular methods, reagents, compounds, compositions, labeled compounds or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting. Thus, it is intended that the specification be considered as exemplary only with the breadth, scope and spirit of the present technology indicated only by the appended claims, definitions therein and any equivalents thereof.

All publications, patents, and other documents referred to in this specification are herein incorporated by reference in its entirety.

CLAIMS

We claim:

1. A compound represented by the following structural formula:



or a pharmaceutically acceptable salt thereof, wherein:

n is 0 or 1;

Z is NH, O, S, CR⁶R⁷, NHCO, CONH, or a 4-7 membered nitrogen containing heterocycle bonded to Y through a ring nitrogen atom of the heterocycle.

A is NH, O, S or CR⁶R⁷;

B comprises a branched, unbranched or cyclic aliphatic group of up to 30 carbon atoms optionally interrupted by up to 10 heteroatoms or a peptidyl chain of up to 20 amino acid residues, wherein B is optionally substituted with 1-5 groups selected from F, Cl, Br, I, =O, OR⁶, OCOR⁶, COOR⁶, CN, =NR⁶, NR⁶R⁷, =S, and SR⁶, provided that B comprises at least 3 atoms in a chain between group D and the group A;

D is selected from the group consisting of OPO₃H₂, PO₃H₂, OSO₃H, SO₃H and COOH or a C₁-C₄ alkyl ester thereof;

X is O or S;

R¹ is a chelating group, an optical dye or fluorophore, a cytotoxic agent, or an immune stimulant, or a benzoyl group optionally substituted by one or more groups represented by R⁵;

R³ is C₁-C₈ alkyl or C₁-C₄ aralkyl, wherein:

the alkyl and aryl portions of the aralkyl are each optionally and independently substituted with F, Cl, Br, I, branched, unbranched or cyclic C₁-C₆ aliphatic group, OR⁶, OCOR⁶, COOR⁶, CHO, COR⁶, CH₂OR⁶, NR⁶R⁷, CH₂NR⁶R⁷, SR⁶, =O, =S and =NH;

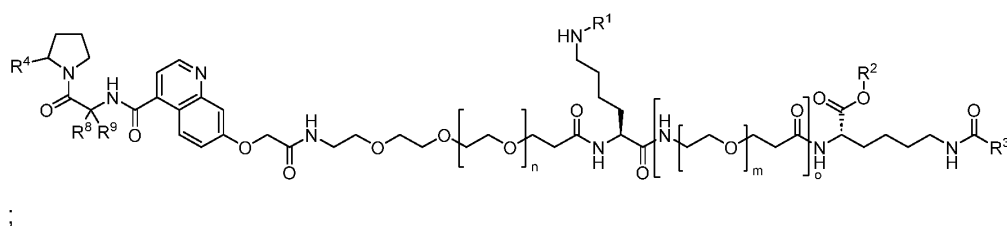
R⁴ is CN or B(OH)₂;

each R⁵ is independently selected from halo, cyano, halomethyl, N⁺(CH₃)₃W⁻ wherein W⁻ is a pharmaceutically acceptable anion;

R^6 and R^7 are independently selected from the group consisting of H or a C_1 - C_6 alkyl; and

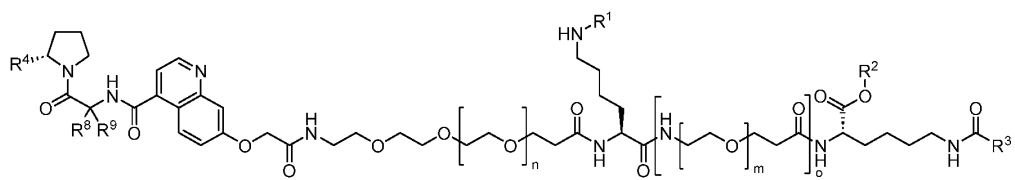
R^8 is C_1 - C_4 alkyl and R^9 is selected from H and C_1 - C_4 alkyl or R^8 and R^9 taken together with their intervening carbon atom form a C_3 - C_6 cycloalkyl.

2. The compound of claim 1, wherein B is independently a branched or unbranched aliphatic group of 3 to 20 carbon atoms optionally interrupted by up to 10 heteroatoms or a peptidyl chain of up to 5 amino acid residues, wherein the aliphatic group is optionally substituted with F, Cl, Br, I, =O, OR^6 , $OCOR^6$, $COOR^6$, CN, =NR⁶, NR⁶R⁷, =S, or SR⁶.
3. The compound of claim 1 or 2 represented by the following structural formula:



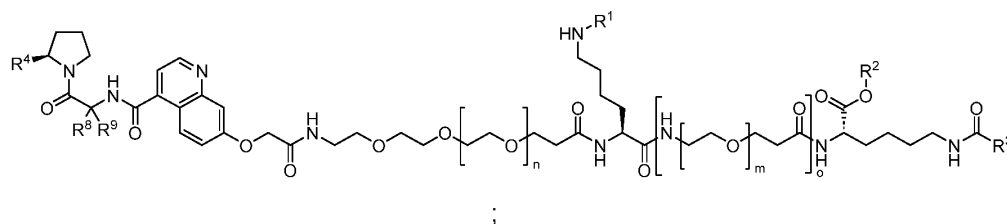
or a pharmaceutically acceptable salt thereof, wherein m is an integer from 0 to 12; o is 0 or 1; and R^2 is H or C_1 - C_4 alkyl.

4. The compound of claim 3 represented by the following structural formula:



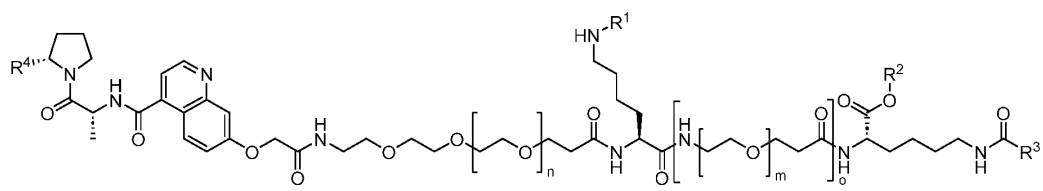
or a pharmaceutically acceptable salt thereof.

5. The compound of claim 3 represented by the following structural formula:



or a pharmaceutically acceptable salt thereof.

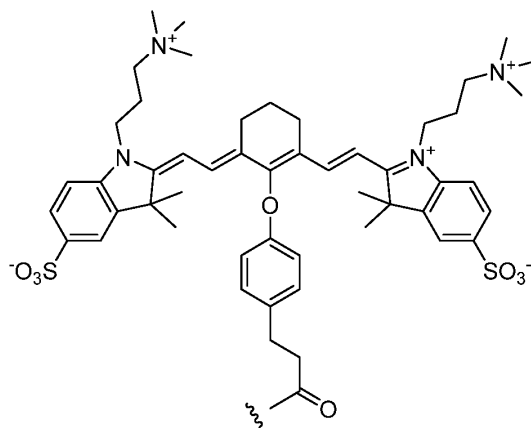
6. The compound of claims 3 or 4 represented by the following structural formula



or a pharmaceutically acceptable salt thereof.

7. The compound of any one of claims 1-6, or a pharmaceutically acceptable salt thereof, wherein R³ is C₁₋₈alkyl or C₁₋₄aralkyl optionally substituted with F, Cl, Br, I, or C₁₋₄alkyl.
8. The compound of any one of claims 1-7, or a pharmaceutically acceptable salt thereof, wherein R³ is C₁₋₈alkyl or C₁₋₄aralkyl optionally substituted with I or C₁₋₄alkyl.
9. The compound of any one of claims 1-8, or a pharmaceutically acceptable salt thereof, wherein R³ is C_{1-C₈} alkyl or C_{1-C₄} aralkyl optionally substituted with C_{1-C₄} alkyl.
10. The compound of any one of claims 1-8, or a pharmaceutically acceptable salt thereof, wherein R³ methyl, (4-isobutylphenyl)methyl, (4-isobutylphenyl)propyl, (4-iodophenyl)methyl, or (4-iodophenyl)propyl.
11. The compound of any one of claims 1-10, or a pharmaceutically acceptable salt thereof, wherein R³ is methyl, (4-isobutylphenyl)methyl, and (4-isobutylphenyl)propyl.
12. The compound of any one of claims 1-11, or a pharmaceutically acceptable salt thereof, wherein R⁸ is methyl and R⁹ is H.
13. The compound of any one of claims 3 to 12, or a pharmaceutically acceptable salt thereof, wherein o is 1 and m is 3 to 12.
14. The compound of claim 13, or a pharmaceutically acceptable salt thereof, wherein m is 8.
15. The compound of any one of claims 3 to 12, or a pharmaceutically acceptable salt thereof, wherein o is 0.
16. The compound of any one of claims 1 to 15, or a pharmaceutically acceptable salt thereof, wherein n is 1.
17. The compound of any one of claims 1 to 16, or a pharmaceutically acceptable salt thereof, wherein R¹ is a fluorophore or an optical dye.

18. The compound of claim 17, or a pharmaceutically acceptable salt thereof, wherein the



fluorophore is ; and the optical dye is selected from the group consisting of: a carbocyanin, indocarbocyanin, oxacarbocyanin, thiocarbocyanin, merocyanin, polymethine, coumarin, rhodamine, xanthene, fluorescein, Borodipyrromethane (BODIPY), VivoTag-680, VivoTag-S750, AlexaFluor dyes (e.g., AlexaFluor660, AlexaFluor680, AlexaFluor700, AlexaFluor750, AlexaFluor790) and DylightFluor dyes.

19. The compound of any one of claims 1 to 16, or a pharmaceutically acceptable salt thereof, wherein R¹ is a chelating group that is the residue of a chelating agent.

20. The compound of claim 19 or a pharmaceutically acceptable salt thereof, wherein the chelating group is the residue of a chelating agent selected from 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), p-SCN-Bn-NOTA, 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA), p-SCN-Bn-DOTA (also known as 2B-DOTA-NCS), PIP-DOTA, diethylenetriaminepentaacetic acid (DTPA), PIP-DTPA, AZEP-DTPA, ethylenediamine tetraacetic acid (EDTA), triethylenetetraamine-N,N',N'',N''',N''''-hexa-acetic acid (TTHA), 7-[2-(bis-carboxymethylamino)-ethyl]-4,10-bis-carboxymethyl-1,4,7,10-tetraaza-cyclododec-1-yl-acetic acid (DEPA), 2,2',2''-(10-(2-(bis(carboxymethyl)amino)-5-(4-isothiocyanatophenyl) pentyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (3p-C-DEPA-NCS), NETA, {4-carboxymethyl-7-[2-(carboxymethylamino)-ethyl]-perhydro-1,4,7-triazonin-1-yl}-acetic acid (NPTA), diacetylpyridinebis(benzoylhydrazine), 1,4,7,10,13,16-hexaazacyclooctadecane N,N',N'',N''',N''''-hexa-acetic acid (HEHA), octadentate terephthalamide ligands, 2,2'-(4-(2-(bis(carboxymethyl)amino)-5-(4-isothiocyanatophenyl)pentyl)-10-(2-(bis(carboxymethyl)amino)ethyl)-1,4,7,10-tetraazacyclododecane-1,7-diyl)diacetic acid, N,N'-bis[(6-carboxy-2-pyridyl)methyl]-4,13-diaza-18-crown-6 (H2macropa), 6-((16-((6-carboxypyridin-2-yl)methyl)-1,4,10,13-tetraoxa-

7,16-diazacyclooctadecan-7-yl)methyl)-4-isocyanatopicolinic acid (macropa-NCO), 6-((16-((6-carboxypyridin-2-yl)methyl)-1,4,10,13-tetraoxa-7,16-diazacyclooctadecan-7-yl)methyl)-4-isothiocyantopicolinic acid (macropa-NCS), 3,9-carboxymethyl-6-(2-methoxy-5-isothiocyantophenyl)carboxymethyl-3,6,9,15-tetraazabicyclo-[9.3.1]pentadeca-1(15),11,13-triene and 2-[4,7,10-tris(2-amino-2-oxoethyl)-1,4,7,10-tetrazacyclododec-1-yl]acetamide (TCMC or DOTAM).

21. The compound of claim 19 or a pharmaceutically acceptable salt thereof, wherein the residue of the chelating agent is the residue of macropa-NCS or macropa-NCO.

22. The compound of claim 19 or a pharmaceutically acceptable salt thereof, wherein the residue of a chelating agent is the residue of p-SCN-Bn-NOTA, p-SCN-Bn-DOTA, NOTA or DOTA,

23. The compound of any one of claims 1-16 or 19, or a pharmaceutically acceptable salt thereof, wherein the chelating group is the residue of a siderophore.

24. The compound of any one of claims 1 to 16, or a pharmaceutically acceptable salt thereof, wherein:

R^1 is a benzoyl group optionally substituted by one or more groups represented by R^5 ;

each R^5 is independently selected from halo, cyano, halomethyl, $N^+(CH_3)_3W^-$; and

W^- is a pharmaceutically acceptable anion.

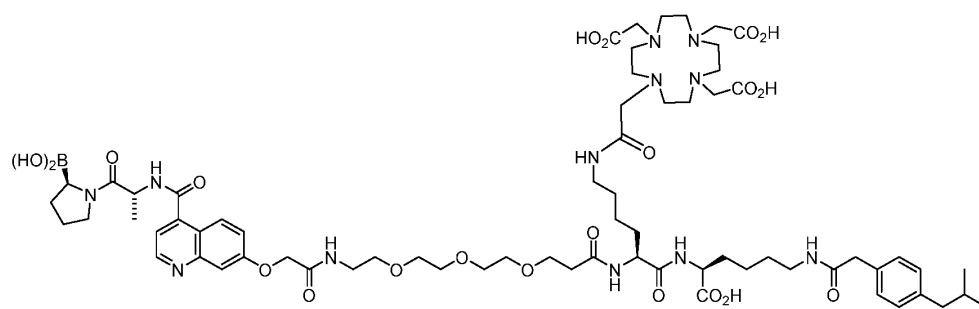
25. The compound of claim 24 or a pharmaceutically acceptable salt thereof, wherein each R^5 is independently selected from fluoro, cyano, trifluoromethyl, $N^+(CH_3)_3W^-$.

26. The compound of claim 24 or 25, or a pharmaceutically acceptable salt thereof, wherein the halo or fluoro group represented by R^5 is ^{18}F .

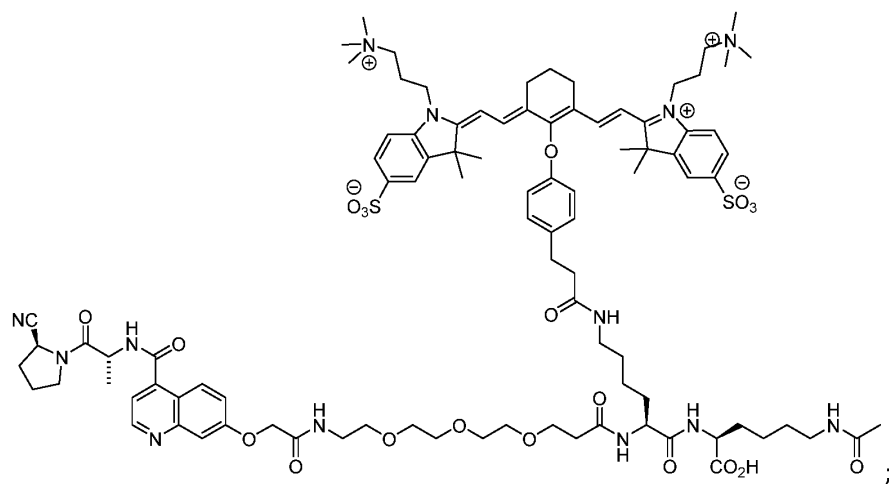
27. The compound of any one of claims 1-26 or a pharmaceutically acceptable salt thereof, wherein R^2 is H and R^4 is $B(OH)_2$.

28. The compound of any one of claims 1-26 or a pharmaceutically acceptable salt thereof, wherein R^2 is H and R^4 is CN.

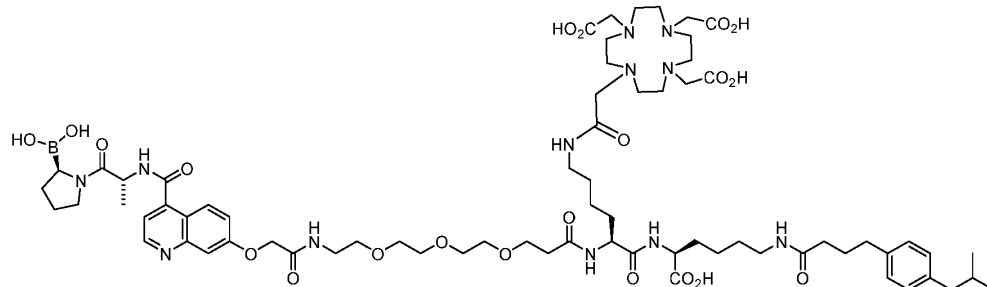
29. The compound of claim 1 or pharmaceutically acceptable salt thereof, represented by a structural formula selected from:



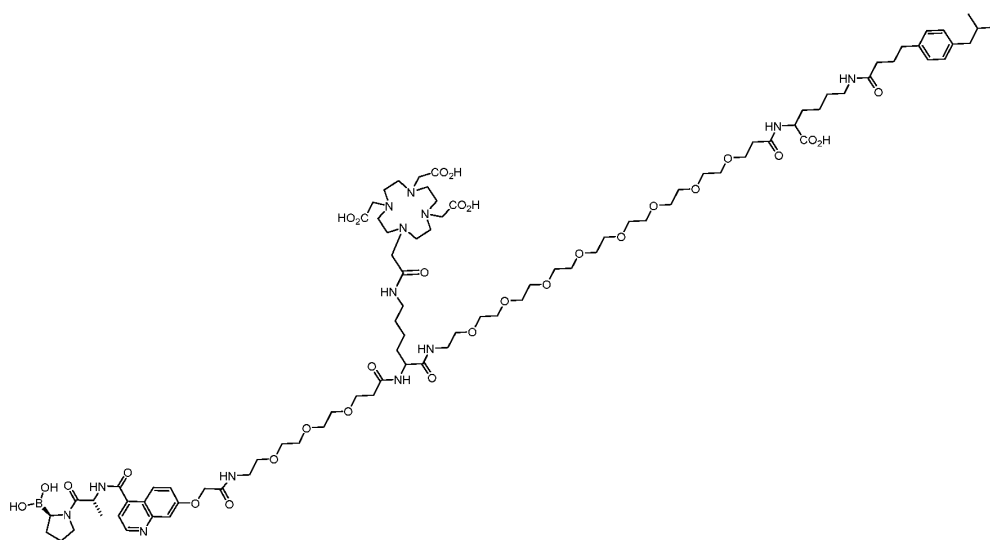
;



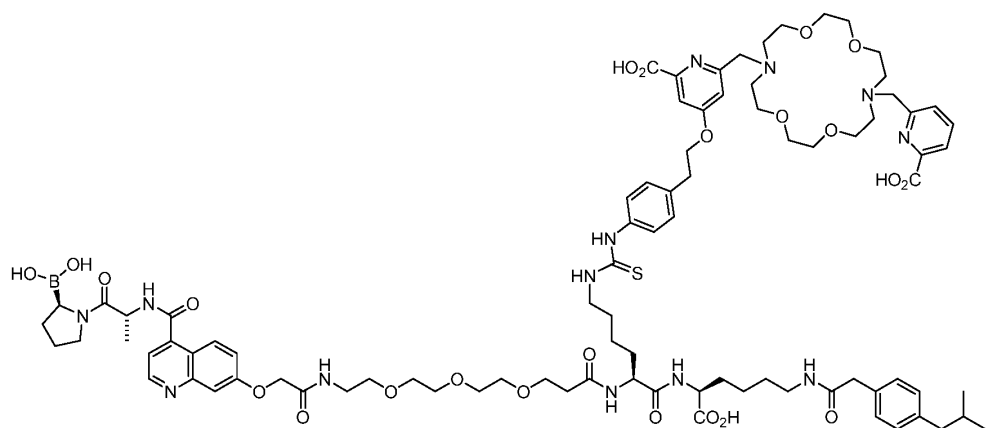
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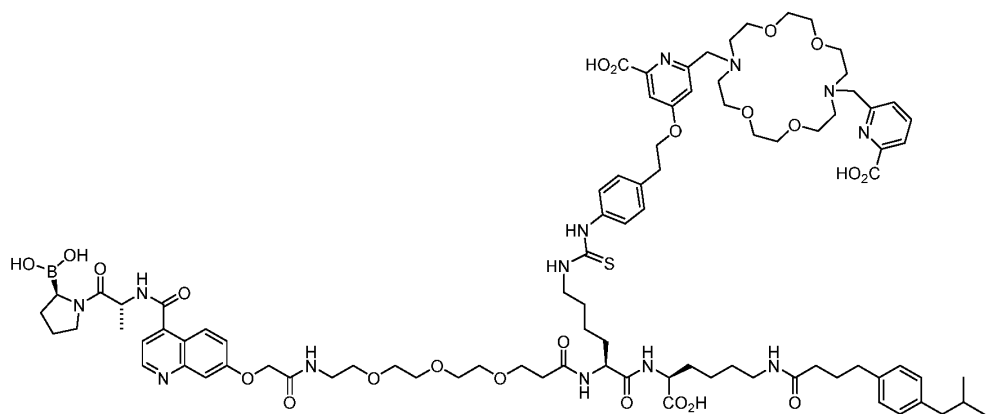
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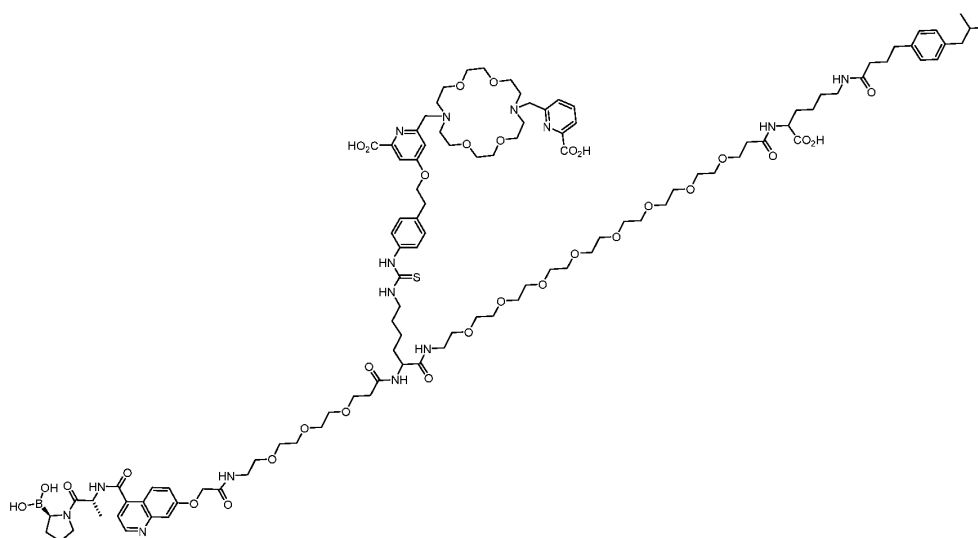
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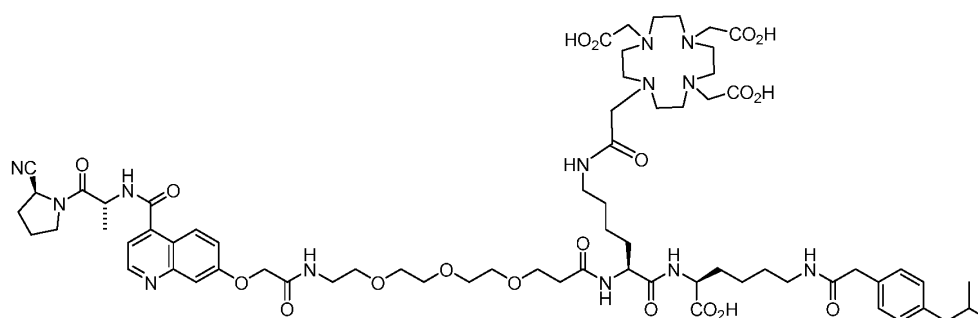
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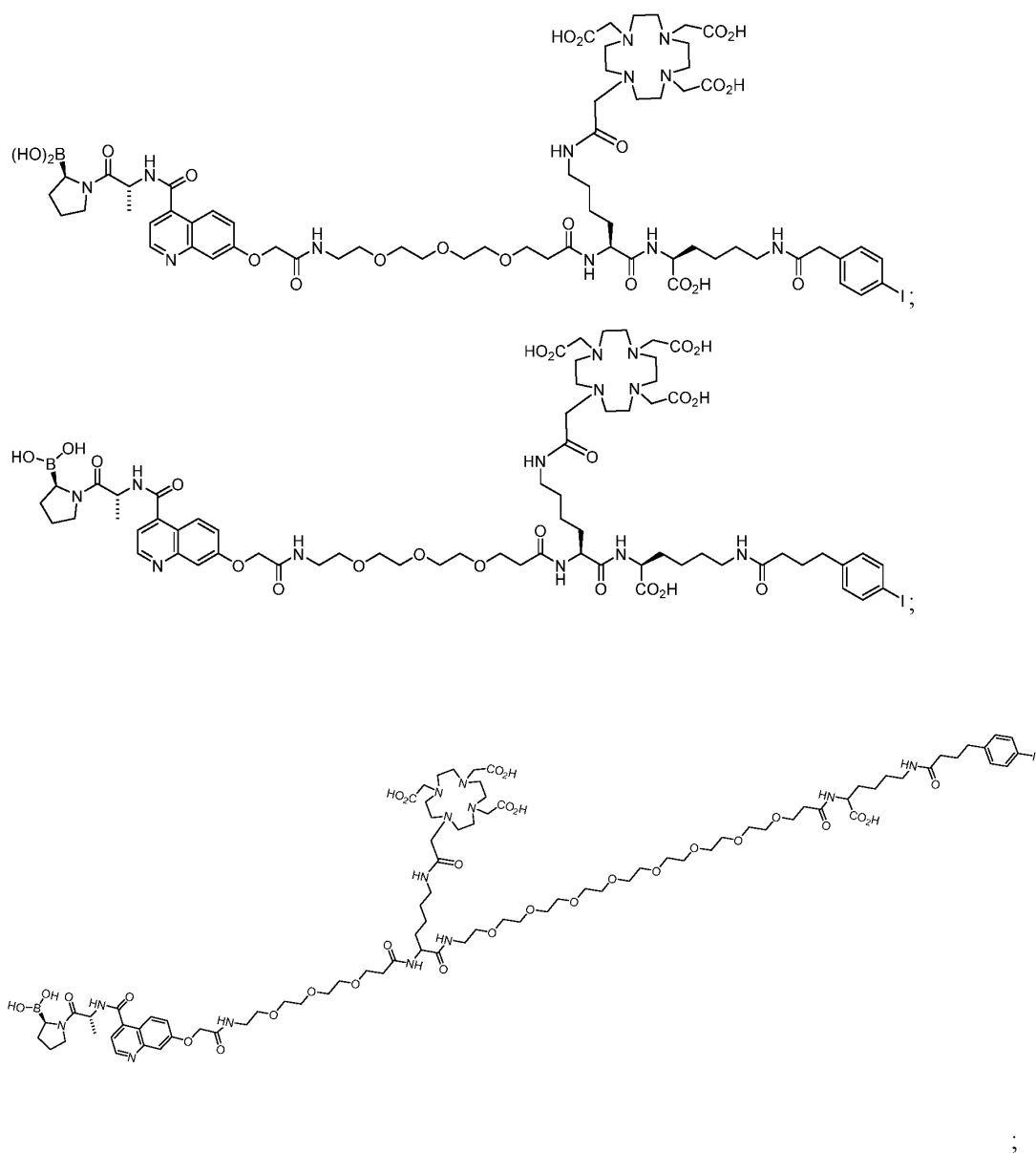
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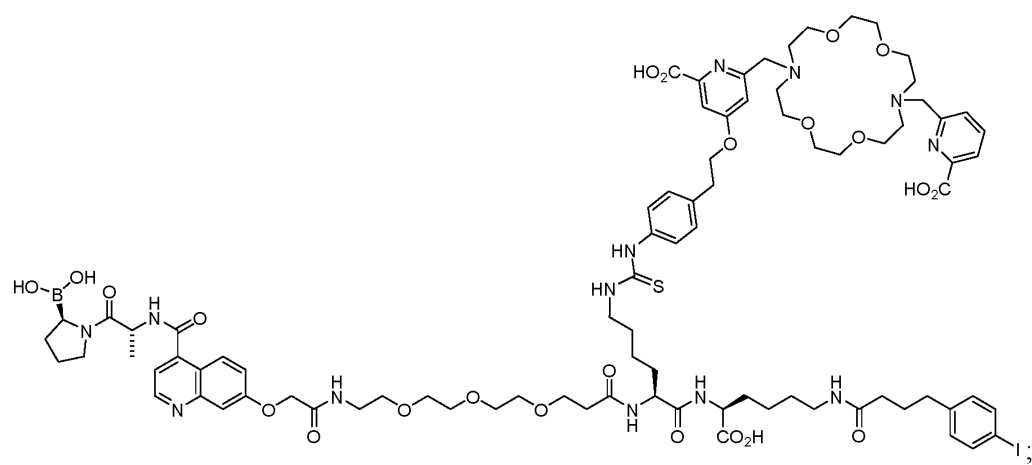
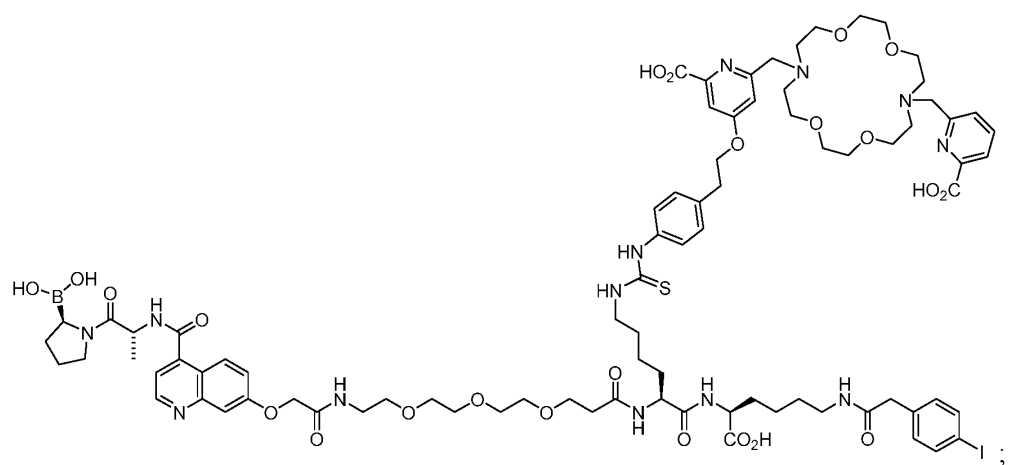


; and

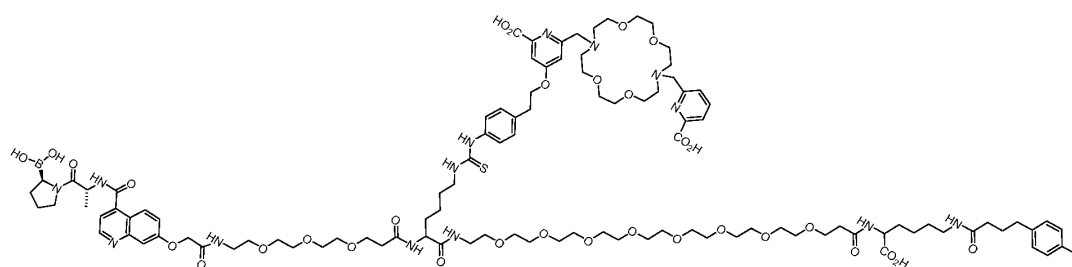


30. The compound of claim 1 or pharmaceutically acceptable salt thereof, represented by a structural formula selected from:





and



31. The compound of any one of claims 1 to 16, 19-23, or 27-30, or a pharmaceutically acceptable salt thereof, wherein the residue of the chelating agent is chelated with a radionuclide.
32. The compound of claim 31 or a pharmaceutically acceptable salt thereof, wherein the radionuclide is selected from ^{177}Lu , ^{175}Lu , ^{45}Sc , ^{64}Cu , ^{67}Cu , ^{68}Cu , ^{66}Ga , ^{67}Ga , ^{68}Ga , ^{69}Ga , ^{71}Ga , ^{90}Y , ^{89}Y , ^{86}Y , ^{89}Zr , ^{90}Y , $^{99\text{m}}\text{Tc}$, ^{111}In , ^{113}In , ^{115}In , ^{139}La , ^{134}Ce , ^{136}Ce , ^{138}Ce , ^{140}Ce , ^{142}Ce , ^{151}Eu , ^{153}Eu , ^{152}Dy , ^{149}Tb , ^{159}Tb , ^{154}Gd , ^{155}Gd , ^{156}Gd , ^{157}Gd , ^{158}Gd , ^{160}Gd , ^{188}Re , ^{186}Re , ^{213}Bi , ^{211}At , ^{217}At , ^{227}Th , ^{226}Th , ^{225}Ac , ^{233}Ra , ^{152}Dy , ^{213}Bi , ^{212}Bi , ^{211}Bi , ^{203}Pb , ^{212}Pb , ^{255}Fm , and uranium-230.
33. The compound of claim 31 or a pharmaceutically acceptable salt thereof, wherein the radionuclide is an alpha-emitting radionuclide such as ^{225}Ac , ^{233}Ra , and ^{212}Pb .
34. The compound of claim 31 or a pharmaceutically acceptable salt thereof, wherein the radionuclide is an Auger electron emitting radionuclide or a beta-emitting radionuclide such as ^{177}Lu , ^{90}Y , and ^{67}Cu .
35. The compound of claim 21 or a pharmaceutically acceptable salt thereof, wherein the residue of macropa-NCS or macropa-NCO is chelated with ^{225}Ac .
36. A pharmaceutical composition comprising:
- the compound of any one of claims 1-35 or a pharmaceutically acceptable salt thereof; and
 - a pharmaceutically acceptable carrier or diluent.
37. A method of treating diseased tissue in a subject, wherein the diseased tissue expresses fibroblast activation protein alpha, comprising administering an effective amount of the compound or pharmaceutically acceptable salt of any one of claims 31-35 or the pharmaceutical composition of claim 36 to the subject and wherein the radionuclide is a therapeutic radionuclide.
38. The method of claim 37, the diseased tissue is a cancer.
39. The method of claim 38, wherein the cancer is pancreatic cancer, liver cancer, gall bladder cancer, neuroblastoma, breast cancer, ovarian cancer, esophageal cancer, kidney cancer, prostate cancer, colorectal cancer, soft tissue sarcoma, bone sarcoma or melanoma.
40. The method of claim 38, wherein the diseased tissue is fibrotic.
41. A method of imaging a region in a subject having or suspected of having diseased tissue which expresses fibroblast activation protein alpha or fibrotic tissue, comprising:
- administering to the subject a diagnostically effective amount of a compound or pharmaceutically acceptable salt thereof of any one of claims 16-19, 25-30 or

- 32-35 or the pharmaceutical composition of claim 36 and wherein the radionuclide is a diagnostic radionuclide;
- b. exposing the region in the subject to an imaging device; and
 - c. obtaining an image of the diseased tissue in the region.
42. The method of claim 41, wherein the region has or is suspected of having diseased tissue that includes a primary cancer or a metastasis of the cancer.
43. The method of claim 41, wherein the region has or is suspected of having diseased tissue that includes fibrotic tissue.
44. A method of imaging tumors, the method comprising:
- a. contacting the tumor and/or surrounding tissue with a compound or pharmaceutically acceptable salt thereof of any one of claims 18-19 in an amount sufficient to bind to the tumor;
 - b. irradiating the tumor and/or surrounding tissue at a wavelength absorbed by the compound;
 - c. and detecting a signal from the compound, thereby imaging the tumor and/or surrounding tissue.
45. A method of treating diseased tissue, comprising:
- a. administering to a subject, a compound of any one of claims 18-19, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, in an amount effective to contact and bind to the diseased tissue;
 - b. using the compound as a fiducial, irradiating the region of the bound compound with one or more doses of external beam radiation, thereby treating the diseased tissue with radiation.
46. The method of claim 45, wherein the compound comprises a chelating group having a radionuclide that emits gamma-rays or positrons, or an optical dye or a fluorophore, or other detectible radiation.
47. A method of treating diseased tissue, comprising: administering to a subject, the compound of any one of claims 18-19, or a pharmaceutically acceptable salt thereof, or a pharmaceutical composition thereof, in an amount effective to contact and bind to the diseased tissue; and using the compound as a fiducial for guided surgery applications, to resect the region of the diseased tissue thereby excising the diseased tissue.
48. The method of claim 47, wherein the compound comprises a chelating group having a radionuclide that emits gamma-rays or positrons, or an optical dye or a fluorophore, or other detectible radiation.

49. The method of claim 37, further comprising administering a chemotherapeutic, immunotherapeutic, or irradiating the diseased tissue with one or more doses of external beam radiation.
50. The method of claim 38, wherein the compound or pharmaceutically acceptable salt thereof is administered to subject in need thereof intravenously, subcutaneously, intramuscularly, topically, or directly into the bladder.