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(54) **Title**: PSMA INHIBITORS

(57) **Abstract**: Compounds as defined herein are provided which are useful in (1) diagnostic methods for detecting and/or identifying cells presenting PSMA; and (2) methods for preparing the compounds.

PSMA INHIBITORS

BACKGROUND OF THE INVENTION

FIELD OF THE INVENTION

[0001] The present invention relates to small molecules having high affinity and specificity to prostrate-specific membrane antigen (PSMA), methods for making the molecules, and their use for diagnostic purposes.

SUMMARY OF THE RELATED ART

[0002] Prostate-specific membrane antigen (PSMA) is uniquely overexpressed on the surface of prostate cancer cells as well as in the neovasculature of a variety of solid tumors. As a result, PSMA has attracted attention as a clinical biomarker for detection and management of prostate cancer. Generally, these approaches utilize an antibody specifically targeted at PSMA to direct imaging or therapeutic agents. For example, ProstaScint (Cytogen, Philadelphia, PA), which has been approved by the FDA for the detection and imaging of prostate cancer, utilizes an antibody to deliver a chelated radioisotope (Indium-111). However, it is now recognized that the ProstaScint technology is limited to the detection of dead cells and therefore its clinical relevance is questionable.

[0003] The success of cancer diagnosis and therapy using antibodies is limited by challenges such as immunogenicity and poor vascular permeability. In addition, large antibodies bound to cell-surface targets present a barrier for subsequent binding of additional antibodies at neighboring cell-surface sites resulting in a decreased cell-surface labeling.

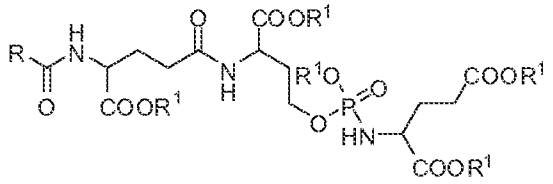
[0004] In addition to serving as a cell-surface target for antibodies delivering diagnostic or therapeutic agents, a largely overlooked and unique property of PSMA is its enzymatic activity. That is, PSMA is capable of recognizing and processing molecules as small as dipeptides. Despite the existence of this property, it has been largely unexplored in terms of the development of novel diagnostic and therapeutic strategies. There are a few recent examples in the literature that have described results in detecting prostate cancer cells using labeled small-molecule inhibitors of PSMA.

[0005] Certain phosphoramidate PSMA inhibitors have been described in U.S. Patent Application Publication No. US-2007-0219165-A1. And one ^{18}F-labeled PMSA inhibitor is disclosed in Lapi, S.E., et al., *J. Nucl. Med.* **2009**, *50*(12), 2042. Although the stereochemical configuration of PSMA inhibitors plays a role in binding of PSMA inhibitors, binding has been observed for stereoisomers of the same inhibitor (Wu, L. Y., et al., *Bioorg Med Chem* 15 *(2007)* 7434–7443).

SUMMARY OF THE INVENTION

[0006] Provided herein are diagnostic compounds and methods for PSMA presenting cells, such as prostate cancer, that capitalize on the potency and specific affinity of small-molecule inhibitors. The diagnostic agents can be used to monitor and stratify patients for treatment with appropriate therapeutic agents.

[0007] In one aspect, the disclosure provides compounds that are in the form of formula (I),



(I)

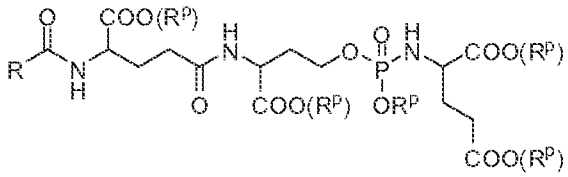
[0008] or the form of a pharmaceutically acceptable salt thereof, wherein

[0009] R is phenyl or pyridyl, each substituted with either one [^{18}F]-Fluoro group or one [^{19}F]-Fluoro group and optionally substituted with a second group selected from the group consisting of chloro and cyano; and

[0010] each R^1 is independently hydrogen or a protecting group.

[0011] In another aspect the present disclosure provides pharmaceutical compositions comprising a compound of the preceding aspect and a pharmaceutically acceptable carrier.

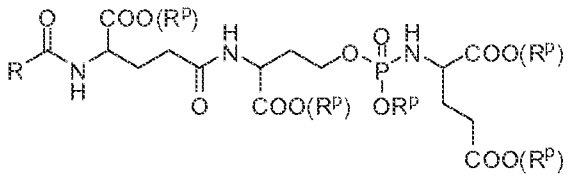
[0012] In another aspect, the disclosure provides methods for preparing a compound comprising deprotecting a compound of the formula (I'),



(I')

wherein R is phenyl or pyridyl, each substituted with either one [^{18}F]-Fluoro group or one [^{19}F]-Fluoro group and optionally substituted with a second group selected from the group consisting of chloro and cyano; and each R^P is a protecting group (e.g., t-butyl or benzyl); under conditions suitable for removing each of the R^P groups.

[0013] In another aspect, the disclosure provides compounds of formula (II),



(II)

[0014] wherein R is phenyl or pyridyl, each substituted with one leaving group and optionally substituted with a second group selected from the group consisting of chloro and cyano; and each R^p is a protecting group (e.g., t-butyl or benzyl).

[0015] In another aspect, the disclosure provides methods for preparing a compound (e.g., a compound of formula (I)) comprising contacting a compound of the formula (II), with a fluoride or radiofluoride source.

[0016] In another aspect the present disclosure provides methods for detecting and/or identifying cells presenting PSMA comprising contacting a cell suspected of presenting PSMA with a compound or a composition of the preceding aspects, wherein R^1 is hydrogen.

[0017] In another aspect the present disclosure provides methods for imaging one or more prostate cancer cells in a patient comprising administering to the patient a compound or a pharmaceutical composition of either of the preceding aspects, wherein R^1 is hydrogen. In certain embodiments, the methods herein are useful for imaging of diseases associated with elevated expression of Prostate Specific Membrane Antigen (PSMA).

[0018] In another aspect the present disclosure provides for the use of compounds of general formula I, wherein R^1 is hydrogen, for conducting biological assays and chromatographic identification. In certain embodiments, the compounds of formula (I) contain a [^{18}F]-Fluoro group. In certain other embodiments, the compounds of formula (I) contain a [^{19}F]-Fluoro group.

[0019] In another aspect the present disclosure provides for the use of compounds of formula (I) that contain a [^{19}F]-Fluoro group and wherein R^1 is hydrogen as references and/or measurement agents.

[0020] In another aspect the present disclosure provides for the use compounds of formula (I) , wherein R^1 is hydrogen, for the manufacture of an imaging tracer or radiopharmaceutical agent for imaging diseases associated with altered expression of Prostate Specific Membrane Antigen PSMA. In certain embodiments, altered expression of Prostate Specific Membrane Antigen PSMA refers to elevated expression of Prostate Specific Membrane Antigen PSMA.

[0021] In another aspect the present disclosure provides methods for imaging or diagnosing of diseases associated with elevated expression of Prostate Specific Membrane Antigen PSMA comprising, administering to a mammal an effective amount of a compound of formula (I), wherein R^1 is hydrogen; obtaining images of the mammal; and assessing the images.

[0022] The foregoing merely summarizes certain aspects of the present invention and is not intended to be limiting. A more expansive and complete description of the various aspects and embodiments of the present invention is provided below. All patents, patent applications, and publications are hereby incorporated by reference in their entirety with the caveat that the present disclosure shall supersede or take precedence over that of prior patents, patent applications, and publications incorporated herein by reference in the event of any conflict or inconsistency.

BRIEF DESCRIPTION OF THE DRAWINGS

[0023] **Figure 1** shows PET quantification results for [^{18}F]-SFB-CTT54 in LNCaP bearing nude mice Top: micro-PETCT (10283, 101210, M3, 8,1 MBq, 50-70 min p.i.110325); Bottom: PET quantification results.

[0024] **Figure 2** shows [^{18}F]-SFB-hCTT54 in LNCaP bearing nude mice. Top: micro-PETCT (10284, 101209, M3, 8.6 MBq, 50-70 min p.i.); Bottom: PET quantification results.

[0025] **Figure 3** shows [^{18}F]-SFB-CTT54 in LNCaP bearing nude mice. Top: micro-PETCT (11001, 110325, M3, 4,2 MBq, 50-70 min p.i.); Bottom: PET quantification results.

[0026] **Figure 4** shows [^{18}F]-SFN-hCTT54 in LNCaP bearing nude mice. Top: micro-PETCT (11003, 110228, M2 NMRI, 8.8 MBq, 50-70 min p.i..); Bottom: PET quantification results.

[0027] **Figure 5** shows [^{18}F]-SClFN-hCTT54 in LNCaP bearing nude mice. Top: micro-PETCT (11004, 110317, M3, [^{18}F]-SClFN-hCTT54, 7.4 MBq, 50-70 min p.i.); Bottom: PET quantification results

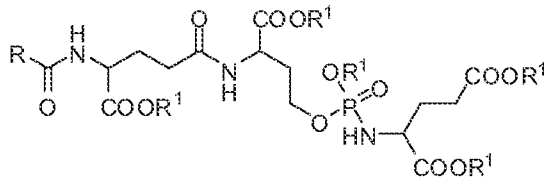
[0028] **Figure 6** shows [^{18}F]-SClFN-hCTT54 in 22RV1 bearing nude mice. Top: micro-PETCT (11168, 110624, M1, 8.2 MBq, 50-70 min p.i.); Bottom: PET quantification results

[0029] **Figure 7** shows [^{18}F]-SFN-hCTT54 in 22RV1 bearing nude mice. Top: micro-PETCT (11215, 110901, M2, 6.6 MBq, 50-70 min p.i.); Bottom: PET quantification results.

[0030] **Figure 8** shows [^{18}F]-SClFN-hCTT54 in 786-O bearing nude mice. Top: micro-PETCT (11084, 110509, M7, 7.2 MBq, 50-70 min p.i.); Bottom: PET quantification results.

DETAILED DESCRIPTION OF THE INVENTION

[0031] In one aspect, the disclosure provides compounds that are in the form of formula (I),



(I)

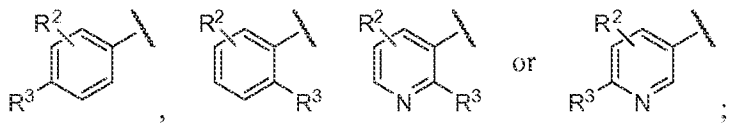
[0032] or the form of a pharmaceutically acceptable salt thereof, wherein

[0033] R is phenyl or pyridyl, each substituted with either one [^{18}F]-fluoro group or one [^{19}F]-fluoro group and optionally substituted with a second group selected from the group consisting of chloro and cyano; and

[0034] each R^1 is independently hydrogen or a protecting group.

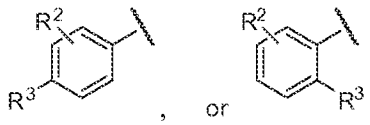
[0035] A "protecting group" as used herein is substituted benzyl, t-butyl ester, allyl ester, alkyl esters (e.g., methyl, ethyl), fluorenylmethoxycarbonyl groups, and carboxylic and phosphorus acid protecting groups described in Greene's Protective Groups in Organic Synthesis, 4th Edition (the relevant parts of which are incorporated by reference). Substituted benzyl groups include, but are not limited to, triphenylmethyl (trityl), diphenylmethyl, o-nitrobenzyl, 2,4,6-trimethylbenzyl, p-bromobenzyl, p-nitrobenzyl, p-methoxybenzyl (PMB), 2,6-dimethoxybenzyl, 4-(methylsulfinyl)benzyl, 4-sulfobenzyl, 4-azidomethoxybenzyl, and piperonyl, and benzyl protecting groups for carboxylic and phosphorus acids disclosed in Greene's Protective Groups in Organic Synthesis (the relevant parts of which are incorporated by reference).

[0036] In an embodiment of formula (I), R is



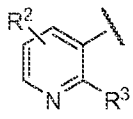
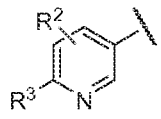
wherein R^3 is -F or -^{18}F; and R^2 is chloro or cyano.

[0037] In an embodiment of formula (I), R is

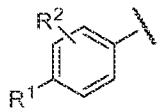


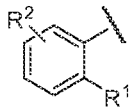
wherein R^3 is -F or -^{18}F; and R^2 is chloro or cyano.

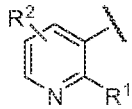
[0038] In an embodiment of formula (I), R is

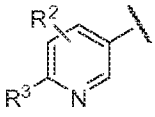
 or  ;

wherein R^3 is -F or -^{18}F; and R^2 is chloro or cyano.

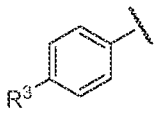
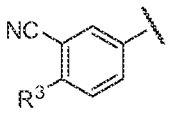
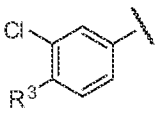
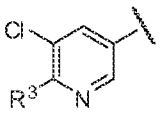
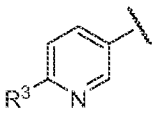
[0039] In an embodiment of formula (I), R is  , wherein R^3 is -F or -^{18}F; and R^2 is chloro or cyano.

[0040] In an embodiment of formula (I), R is  , wherein R^3 is -F or -^{18}F; and R^2 is chloro or cyano.

[0041] In an embodiment of formula (I), R is  , wherein R^3 is -F or -^{18}F; and R^2 is chloro or cyano.

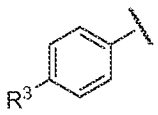
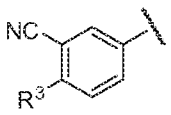
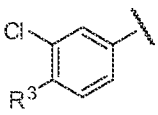
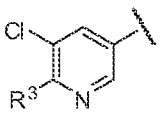
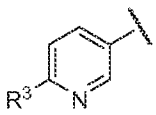
[0042] In an embodiment of formula (I), R is  , wherein R^3 is -F or -^{18}F; and R^2 is chloro or cyano.

[0043] In an embodiment of formula (I), R is

 ,  ,  ,  , or  ;

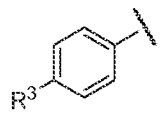
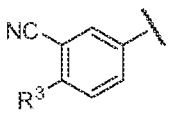
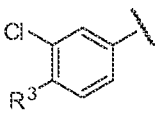
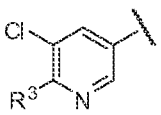
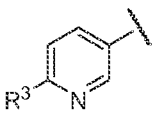
wherein R^3 is -F or -^{18}F.

[0044] In an embodiment of formula (I), R is

 ,  ,  ,  , or  ;

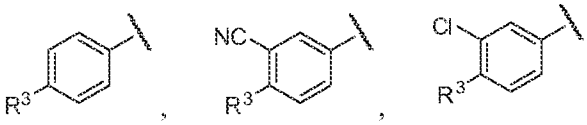
wherein R^3 is -F.

[0045] In an embodiment of formula (I), R is

 ,  ,  ,  , or  ;

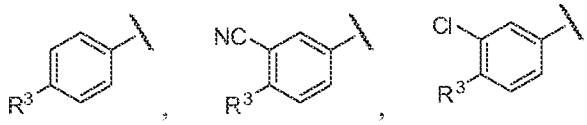
wherein R^3 is -^{18}F.

[0046] In an embodiment of formula (I), R is



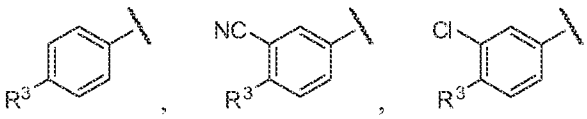
wherein $R^3$is -F or -^{18}F.

[0047] In an embodiment of formula (I), R is



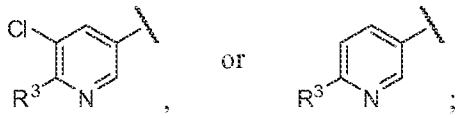
wherein R^3 is -F.

[0048] In an embodiment of formula (I), R is



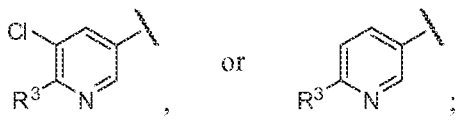
wherein R^3 is -^{18}F.

[0049] In an embodiment of formula (I), R is



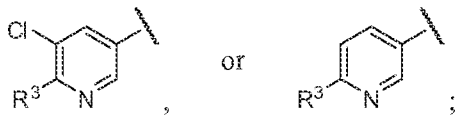
wherein R^3 is -F or -^{18}F.

[0050] In an embodiment of formula (I), R is

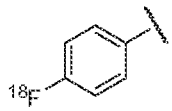


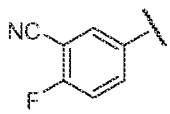
wherein R^3 is -F.

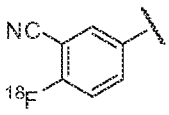
[0051] In an embodiment of formula (I), R is

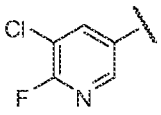


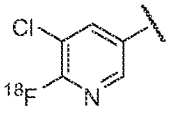
wherein R^3 is -^{18}F.

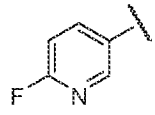
[0052] In another embodiment of formula (I), R is  .

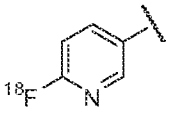
[0053] In another embodiment of formula (I), R is  .

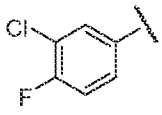
[0054] In another embodiment of formula (I), R is  .

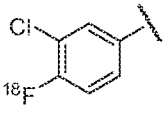
[0055] In another embodiment of formula (I), R is  ..

[0056] In another embodiment of formula (I), R is  .

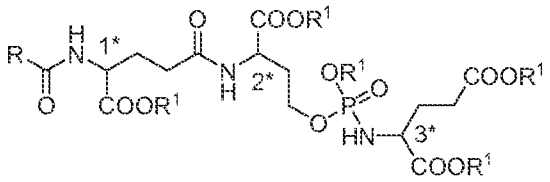
[0057] In another embodiment of formula (I), R is  .

[0058] In another embodiment of formula (I), R is  .

[0059] In another embodiment of formula (I), R is  .

[0060] In another embodiment of formula (I), R is  .

[0061] Compounds of structural formula (I) have three chiral centers. Accordingly, in another aspect of the invention, the invention comprises compounds of formula (I) of the formula (I*),



(I*)

[0062] and pharmaceutically acceptable salts thereof, wherein R and R^1 are defined according to any one of the embodiments described above for formulae (I), and one, two, or three of the chiral centers 1*, 2*, and 3* is enantiomerically enriched (defined herein as having >50% R or S stereochemistry) or enantiomerically pure (defined herein as having greater than 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% R or S stereochemistry).

[0063] In structure (I*), 1*, 2*, and 3* are chiral centers that are independently racemic (rac) or in the S or R stereoconfiguration. Thus, compounds according to this aspect include those with the following combinations of stereoconfigurations, and mixtures thereof:

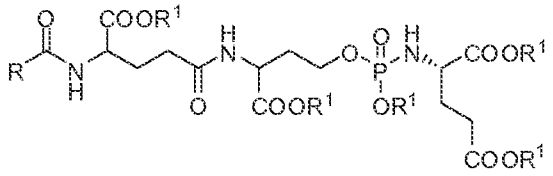
1*	2*	3*
S	S	S
S	S	R
S	R	S
R	S	S
S	R	R

1*	2*	3*
R	S	R
R	R	S
R	R	R
rac	S	S
rac	S	R

1*	2*	3*
rac	R	S
rac	R	R
S	rac	S
S	rac	R
R	rac	S

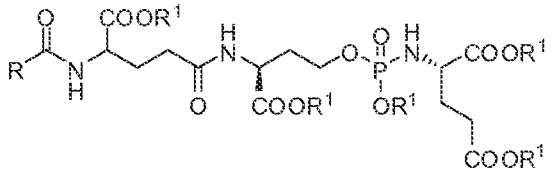
1*	2*	3*
R	rac	R
S	S	rac
S	R	rac
R	S	rac
R	R	rac

[0064] In an embodiment of any of the preceding embodiments, the compound can be of the formula (Ia),



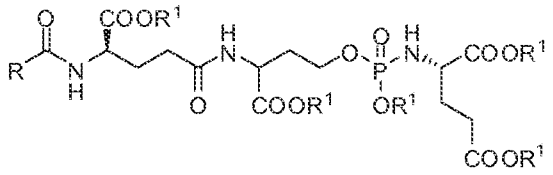
(Ia).

[0065] In an embodiment of any of the preceding embodiments, the compound can be of the formula (Ib),



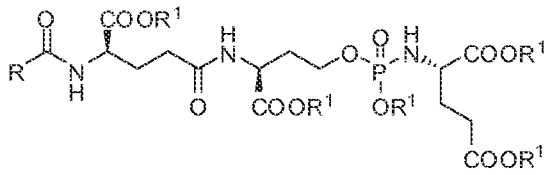
(Ib).

[0066] In an embodiment of any of the preceding embodiments, the compound can be of the formula (Ic),



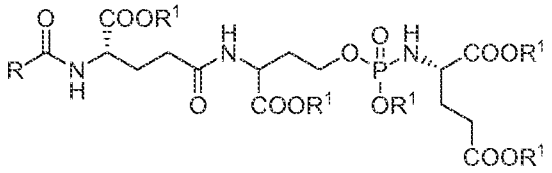
(Ic).

[0067] In an embodiment of any of the preceding embodiments, the compound can be of the formula (Id),



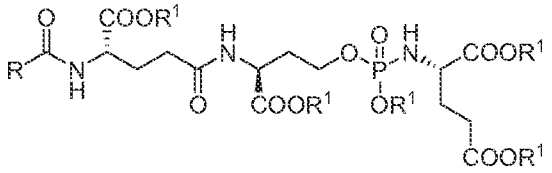
(Id).

[0068] In an embodiment of any of the preceding embodiments, the compound can be of the formula (Ie),



(Ie).

[0069] In an embodiment of any of the preceding embodiments, the compound can be of the formula (If),



(If).

[0070] In an embodiment of any of the preceding embodiments, R^1 is hydrogen.

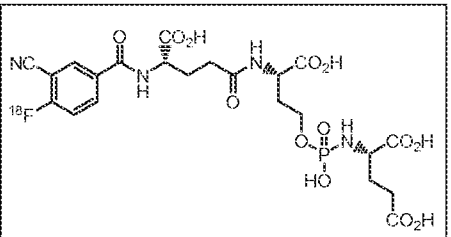
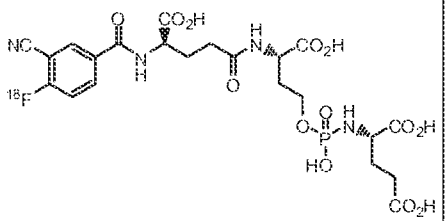
[0071] In an embodiment of any of the preceding embodiments, R^1 is a protecting group.

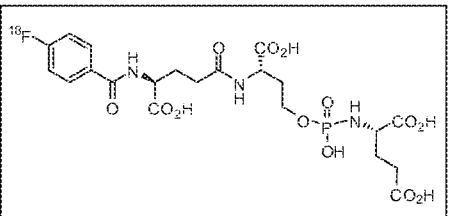
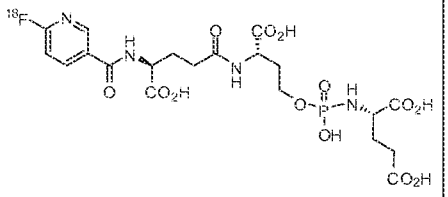
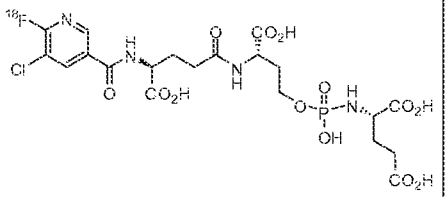
[0072] In an embodiment of any of the preceding embodiments, R^1 is t-butyl or benzyl.

[0073] In an embodiment of any of the preceding embodiments, R^1 is t-butyl.

[0074] In an embodiment of any of the preceding embodiments, R^1 is benzyl.

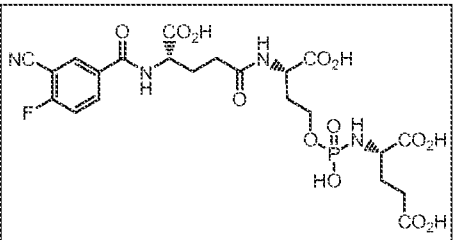
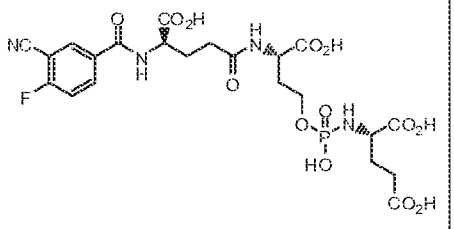
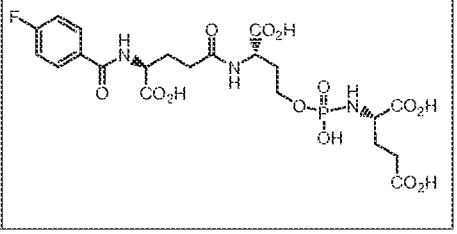
[0075] In another embodiment, the compound of formula (I) is

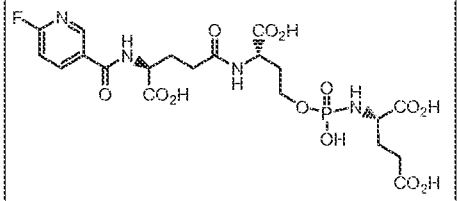
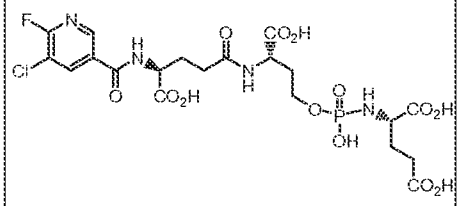
[^{18}F]CTT1143 [^{18}F]F,CNBz-hCTT54;	(*N*-(3-cyano-4-[^{18}F]fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-dicarboxypropyl]amino}(hydroxy)phosphoryl]-L-homoserine	
	(*N*-(3-cyano-4-[^{18}F]fluorobenzoyl)-D-γ-glutamyl)-*O*-[{[(1S)-1,3-dicarboxypropyl]amino}(hydroxy)phosphoryl]-L-homoserine	

[^{18}F]SFB-hCTT54;	(*N*-(4-[^{18}F]fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-dicarboxypropyl]amino}(hydroxy)phosphoryl]-L-homoserine	
[^{18}F]-SFN-hCTT54;	(*N*-(6-[^{18}F]fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-dicarboxypropyl]amino}(hydroxy)phosphoryl]-L-homoserine	
[^{18}F]-SClFN-hCTT-54;	(*N*-(5-chloro-6-[^{18}F]fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-dicarboxypropyl]amino}(hydroxy)phosphoryl]-L-homoserine	

and a pharmaceutically acceptable salts thereof.

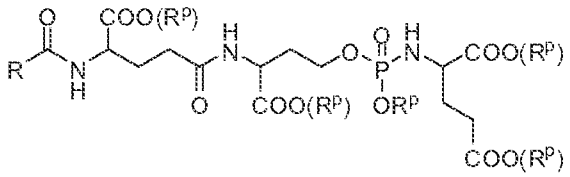
[0076] In another aspect, the disclosure provides compounds of formula (I) that are

CTT1143 = F,CNBz-hCTT54;	(*N*-(3-cyano-4-fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-dicarboxypropyl]amino}(hydroxy)phosphoryl]-L-homoserine	
	(*N*-(3-cyano-4-fluorobenzoyl)-D-γ-glutamyl)-*O*-[{[(1S)-1,3-dicarboxypropyl]amino}(hydroxy)phosphoryl]-L-homoserine	
SFB-hCTT54;	(*N*-(4-fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-dicarboxypropyl]amino}(hydroxy)phosphoryl]-L-homoserine	

SFN-hCTT54;	(*N*-(6-fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-dicarboxypropyl]amino}(hydroxy)phosphoryl]-L-homoserine	
SClFN-hCTT-54;	(*N*-(5-chloro-6-fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-dicarboxypropyl]amino}(hydroxy)phosphoryl]-L-homoserine	

and a pharmaceutically acceptable salts thereof.

[0077] The preceding compounds, where R^1 is hydrogen, can be prepared by a method comprising deprotecting a compound of the formula (I'),



(I')

[0078] wherein R is phenyl or pyridyl, each substituted with either one [^{18}F]-Fluoro group or one [^{19}F]-Fluoro group and optionally substituted with a second group selected from the group consisting of chloro and cyano; and each R^p is a protecting group (e.g., t-butyl or benzyl); under conditions suitable for removing each of the R^P groups.

[0079] As would be clear to one skilled in the art, removal of the R^p groups in the preceding results in the formation of the corresponding compound wherein R^p is hydrogen, or a salt thereof (e.g., a compound of formula (I) where R^1 is hydrogen).

[0080] When R^p is a t-butyl group, the method can be maintained under anhydrous conditions to prevent degradation of the compounds as the phosphoramidate moiety is known to be unstable in aqueous acidic media. In various embodiment, each of the following deprotection conditions can be utilized for removal of t-butyl groups:

i) Contacting the compound with an acid selected from the groups consisting of, trifluoroacetic acid, hydrochloric acid, formic acid, glacial acetic acid, chloroacetic acid, and mixtures thereof;

ii) Contacting the compound with an acid (selected as in (i)) in a solvent selected from the group consisting of diethyl ether, ethyl acetate, dioxane, 1,2-dichloroethane, dichloromethane, t-butanol, glyme, methyl t-butylether, tetrahydrofuran, and mixtures thereof;

iii) Contacting the compound with a neat acid;

iv) Contacting the compound any of the preceding with the addition of scavengers, such as, but not limited to triethylsilane (TES);

v) Contacting the compound as in any of the preceding at a temperatures between room temperature (e.g., 25 °C) and 180 °C;

vi) Contacting the compound as in any of the preceding with microwave heating;

vii) Contacting the compound with a base such as, but not limited to, NaOH;

viii) Contacting the compound as in any of the preceding, where the reaction is allowed to proceed for a period of time between about 15 seconds and 15 minutes;

ix) Contacting the compound with trimethylsilyl iodide (TMS-I, may be formed *in situ* from trimethylsilyl chloride and sodium iodide),

x) Contacting the compound with trimethylsilyl triflate (TMSOTf) and triethylamine (TEA);

xi) Contacting the compound with quinoline at elevated temperatures, *e.g.*, greater than 150 °C, such as, 180°C;

xii) Contacting the compound with LiI in ethyl acetate;

[0081] In certain embodiments, the conditions include contacting the compound with formic acid. In certain other embodiments, the conditions include contacting the compound with neat formic acid.

[0082] In certain embodiments, the conditions include contacting the compound with formic acid at a temperature between about room temperature (e.g., 25 °C) and 100°C. In

certain embodiments, the conditions include contacting the compound with formic acid at a temperature between about room temperature (e.g., 25 °C) and 75°C. In certain embodiments, the conditions include contacting the compound with formic acid at a temperature between about 35 °C and 75°C. In certain embodiments, the conditions include contacting the compound with formic acid at a temperature between about 40 °C and 60°C. In certain embodiments, the conditions include contacting the compound with formic acid at a temperature between about 45 °C and 55 °C.

[0083] In certain embodiments, the conditions include contacting the compound with neat formic acid at a temperature between about room temperature (e.g., 25 °C) and 100°C. In certain embodiments, the conditions include contacting the compound with neat formic acid at a temperature between about room temperature (e.g., 25 °C) and 75°C. In certain embodiments, the conditions include contacting the compound with neat formic acid at a temperature between about 35 °C and 75°C. In certain embodiments, the conditions include contacting the compound with neat formic acid at a temperature between about 40 °C and 60°C. In certain embodiments, the conditions include contacting the compound with neat formic acid at a temperature between about 45 °C and 55 °C.

[0084] In any of the preceding embodiments using formic acid or neat formic acid, the compound can be heated at a desired temperature (e.g., between about 45 °C and 55 °C) for a period of time between about 15 seconds and 15 minutes. In certain embodiment, the heating is for between about 15 seconds and 10 minutes; or 15 seconds and 8 minutes; or 1 minute and 8 minutes; or 2 minutes and 8 minutes; or 3 minutes and 8 minutes; or 4 minutes and 6 minutes; or about 5 minutes. Following the termination of the desired time period for heating the compound, any solvents and/or acids can be removed from the reaction mixture by methods familiar to those skilled in the art, such as *in vacuo* removal or by purging the reaction mixture with an inert gas, such as Ar, He, or N_2.

[0085] In certain embodiments, the conditions include contacting the compound with trifluoroacetic acid. In certain other embodiments, the conditions include contacting the compound with trifluoroacetic acid in a solvent. In certain embodiments, the solvent is 1,2-dichloroethane.

[0086] In certain embodiments, the conditions include contacting the compound with trifluoroacetic acid and a scavenger, such as triethylsilane. In certain other embodiments, the conditions include contacting the compound with trifluoroacetic acid and triethylsilane in a solvent. In certain embodiments, the solvent is 1,2-dichloroethane.

[0087] In certain embodiments, the conditions include contacting the compound with trifluoroacetic acid and triethylsilane in 1,2-dichloroethane at a temperature between about room temperature (e.g., 25 °C) and 150°C. In certain embodiments, the conditions include contacting the compound with trifluoroacetic acid and triethylsilane in 1,2-dichloroethane at a temperature between about 50 °C and 150°C. In certain embodiments, the conditions include contacting the compound with trifluoroacetic acid and triethylsilane in 1,2-dichloroethane at a temperature between about 75 °C and 125°C. In certain embodiments, the conditions include contacting the compound with trifluoroacetic acid and triethylsilane in 1,2-dichloroethane at a temperature between about 90 °C and 110°C.

[0088] In any of the preceding embodiments using trifluoroacetic acid and optionally triethylsilane, the compound can be heated at a desired temperature (e.g., between about 90 °C and 10 °C) for a period of time between about 15 seconds and 15 minutes. In certain embodiment, the heating is for between about 1 minute and 15 minutes; or about 1 minute and 12 minutes; or 5 minute and 15 minutes; or 5 minutes and 12 minutes; or 7 minutes and 12 minutes; or 9 minutes and 11 minutes; or about 10 minutes. Following the termination of the desired time period for heating the compound, any solvents and/or acids can be removed from the reaction mixture by methods familiar to those skilled in the art, such as *in vacuo* removal or by purging the reaction mixture with an inert gas, such as Ar or N_2.

[0089] In certain embodiments, each R^p is an optionally substituted benzyl group. In certain other embodiments, each R^p is a benzyl group. In other embodiments, each R^p is a substituted benzyl group.

[0090] When R^p is an optionally substituted benzyl group (e.g., unsubstituted benzyl), suitable deprotection conditions include, but are not limited to, hydrogenolysis conditions (e.g., H_2 and Pd/C) or catalytic hydrogen transfer using ammonium formate and Pd/C. Other hydrogenation catalysts may be used as are familiar to those skilled in the art.

[0091] In certain embodiments, alternative hydrogen sources may be used including, but not limited to ammonium formate, sodium formate, or formic acid with triethylamine. In certain embodiments, the hydrogen source is ammonium formate.

[0092] The hydrogenation may be undertake in a suitable solvent, selected from, but not limited to, ethanol, tetrahydrofuran, water, or phosphate buffered saline, or a mixture thereof.

[0093] For example, in certain embodiments, the deprotection can be setup in a cartridge where the Pd/C catalyst is loaded in a layer or distributed in inert material, then, the halogenated or radiolabeled sample (e.g., containing -F or -^{18}F) dissolved in a solvent (such

as ethanol), is further dissolved in ammonium formate and flushed through the cartridge to yield deprotected material without the need for further purification.

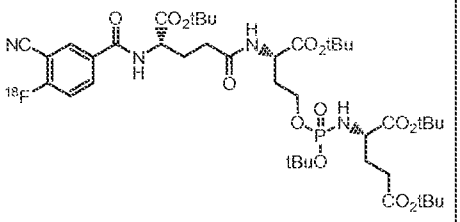
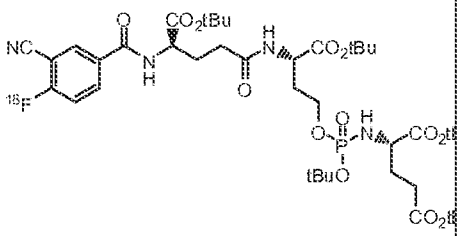
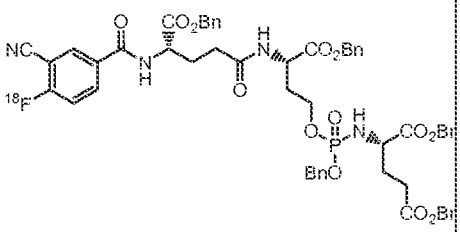
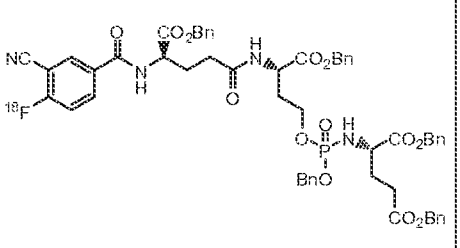
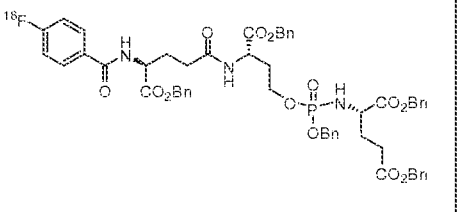
[0094] In any of the preceding embodiments using Pd/C as a catalyst for deprotection, 5 – 10 wt% Pd/C can be used. In certain embodiments, 10 wt% Pd/C is used. About 0.01 to about 0.40 molar equivalents of Pd/C to the compound being deprotected can be used. In certain embodiments, about 0.01 to about 0.30 molar equivalents are used. In other embodiments, about 0.01 to about 0.20 molar equivalents; or 0.01 to about 0.10 molar equivalents; about 0.05 to about 0.40 molar equivalents; or about 0.05 to about 0.30 molar equivalents; or about 0.05 to about 0.20 0.01 to about 0.2 molar equivalents; or about 0.05 to about 0.10 molar equivalents; or about 0.075 to about 0.40 molar equivalents; or about 0.075 to about 0.30; or about 0.075 to about 0.20 molar equivalents; or about 0.075 to about 0.10 molar equivalents are used.

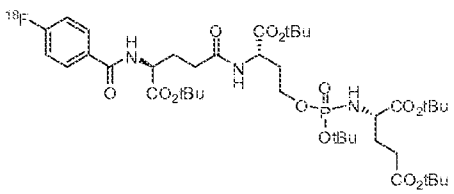
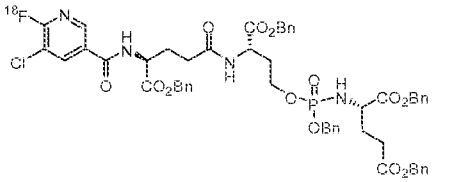
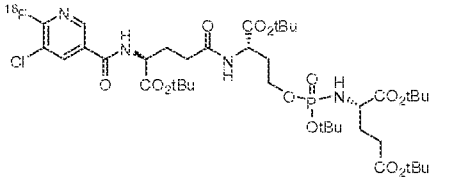
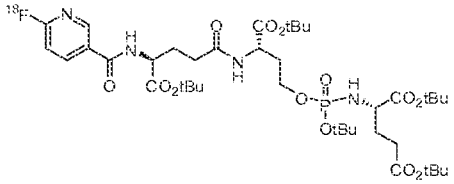
[0095] Further, in any of the preceding embodiments using Pd/C as a catalyst for deprotection, less than about 20 % of the ^{18}F label is removed from the compound during the deprotection step. That is, in going removing the benzyl groups, the yield of the reaction step is greater than about 80 %.

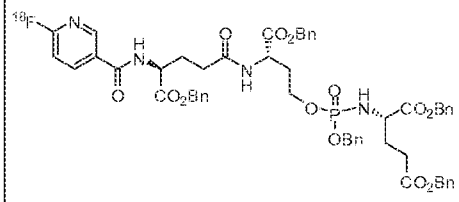
[0096] In other embodiments, less than about 10 % of the ^{18}F label is removed from the compound during the deprotection step (greater than about 90 % yield). In other embodiments, less than about 5 % of the ^{18}F label is removed from the compound during the deprotection step (greater than about 95 % yield). In other embodiments, less than about 3 % of the ^{18}F label is removed from the compound during the deprotection step (greater than about 97 % yield). In other embodiments, less than about 2 % of the ^{18}F label is removed from the compound during the deprotection step (greater than about 98% yield). In other embodiments, less than about 1 % of the ^{18}F label is removed from the compound during the deprotection step (greater than about 99 % yield). In other embodiments, essentially none of the ^{18}F label is removed from the compound during the deprotection step (essentially quantitative yield).

[0097] In other embodiments, the deprotection can be completed in less than about 30 minutes. For example, the deprotection step can be completed in less than about 20 minutes, ot about 15 minutes, or about 10 minutes. In yet other embodiments, the deprotection can be completed in between about 1 minute and about 30 minutes; or about 1 minute and about 20 minutes; or about 1 minute and about 15 minutes; or about 1 minute and about 10 minutes; or about 5 minutes and about 30 minutes; or about 5 minutes and about 20 minutes; or about 5 minutes and about 15 minutes; or about 5 minutes and about 10 minutes.

[0098] In particular embodiments, the compound is

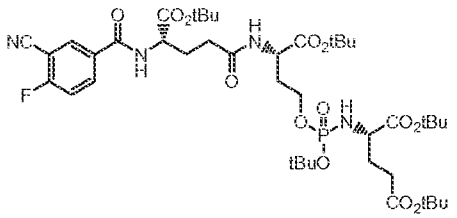
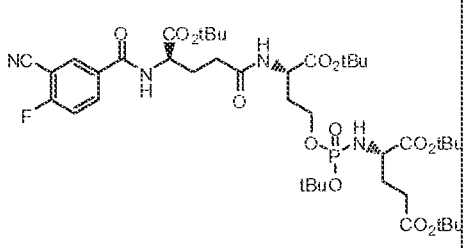
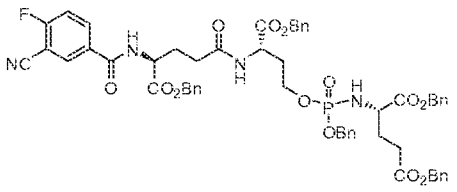
[^{18}F]CTT1138= [^{18}F]F,CNBz-hCTT54(5tBu);	*t*-butyl (*O*-*t*-butyl-*N*-(3-cyano-4-[^{18}F]fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine	
	t-butyl (*O*-*t*-butyl-*N*-(3-cyano-4-[^{18}F]fluorobenzoyl)-D-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine	
[^{18}F]F,CNBz-hCTT54(5Bn);	benzyl (*O*-benzyl-*N*-(3-cyano-4-[^{18}F]fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine	
	benzyl (*O*-benzyl-*N*-(3-cyano-4-[^{18}F]fluorobenzoyl)-D-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine	
[^{18}F]SFB-hCTT54(5Bn);	benzyl (*O*-benzyl-*N*-(4-[^{18}F]fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine	

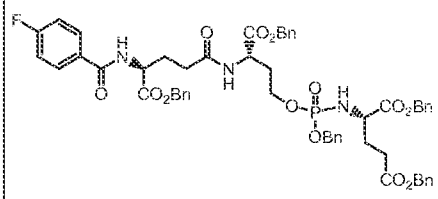
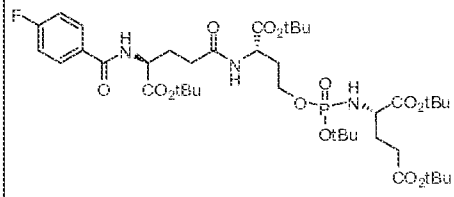
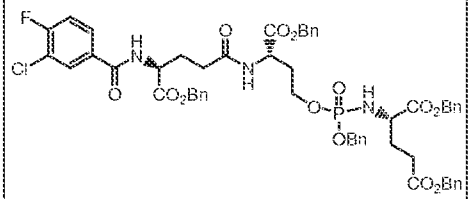
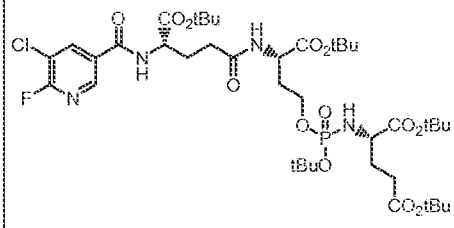
[^{18}F]SFB-hCTT54(5tBu);	*t*-butyl (*O*-*t*-butyl-*N*-(4-[^{18}F]fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine	
[^{18}F]FClPy-hCTT54(5Bn);	benzyl (*O*-benzyl-*N*-(5-chloro-6-[^{18}F]fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine	
[^{18}F]FClPy-hCTT54(5tBu);	*t*-butyl (*O*-*t*-butyl-*N*-(5-chloro-6-[^{18}F]fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine	
[^{18}F]SFN-hCTT54(5tBu);	*t*-butyl (*O*-*t*-butyl-*N*-(6-[^{18}F]fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine	

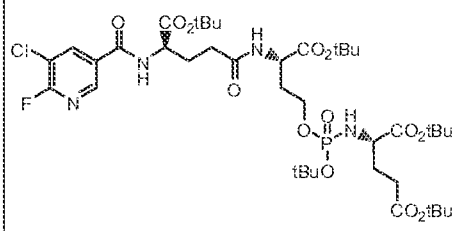
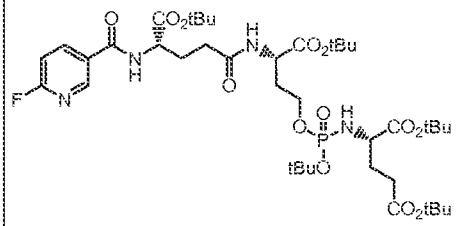
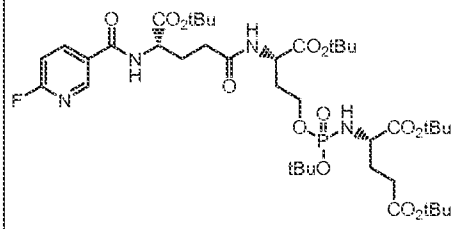
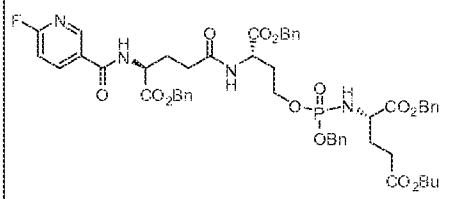
[^{18}F]SFN-hCTT54(5Bn);	benzyl (*O*-benzyl-*N*-(6-[^{18}F]fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine	

and pharmaceutically acceptable salts thereof.

[0099] In particular other embodiments, the compound is

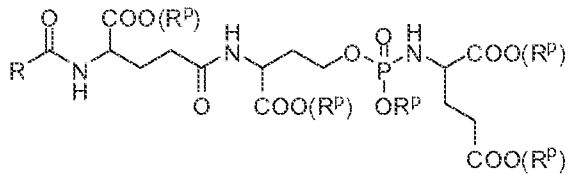
CTT1138 = F,CNBz-hCTT54(5tBu);	*t*-butyl (*O*-*t*-butyl-*N*-(3-cyano-4-fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine	
	t-butyl (*O*-*t*-butyl-*N*-(3-cyano-4-fluorobenzoyl)-D-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine	
F,CNBz-hCTT54(5Bn);	benzyl (*O*-benzyl-*N*-(3-cyano-4-fluorobenzoyl) -L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine	

SFB-hCTT54(5Bn);	benzyl (*O*-benzyl-*N*-(4-fluorobenzoyl) -L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine	
SFB-hCTT54(5tBu);	*t*-butyl (*O*-*t*-butyl-*N*-(4-fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine	
FClPy-hCTT54(5Bn);	benzyl (*O*-benzyl-*N*-(5-chloro-6-fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine	
FClPy-hCTT54(5tBu);	*t*-butyl (*O*-*t*-butyl-*N*-(5-chloro-6-fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine	

	t-butyl (*O*-*t*-butyl-*N*-(5-chloro-6-fluoro-pyrid-3-yl)carbonyl-D-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine	
SFN-hCTT54(5tBu);	*t*-butyl (*O*-*t*-butyl-*N*-(6-fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine	
	t-butyl (*O*-*t*-butyl-*N*-(6-fluoro-pyrid-3-yl)carbonyl-D-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine	
SFN-hCTT54(5Bn);	benzyl (*O*-benzyl-*N*-(6-fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine	

and a pharmaceutically acceptable salts thereof.

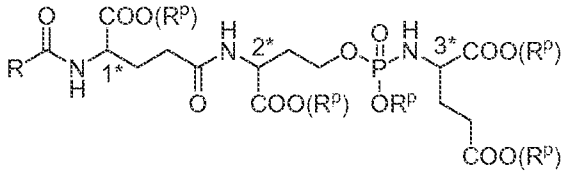
[0100] In another aspect are provided compounds of formula (II),



(II)

[0101] wherein R is phenyl or pyridyl, each substituted with one leaving group and optionally substituted with a second group selected from the group consisting of chloro and cyano; and each R^P is a protecting group (e.g., t-butyl or benzyl).

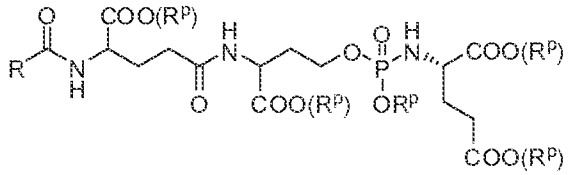
[0102] In particular embodiments of the compounds of formula (II), the compound is of the formula (II*):



(II*)

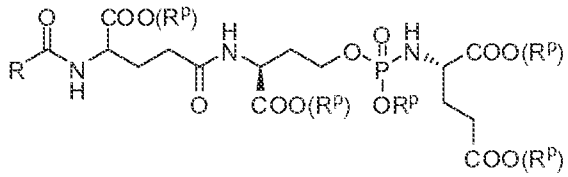
and a pharmaceutically acceptable salt thereof, wherein R and R^P are as defined according to any one of the embodiments described above for formula (II), and the stereoconfiguration of 1*, 2*, and 3* are as defined above for compounds of formula (I*).

[0103] In one embodiment, the compound of formula (II) can be of the formula,



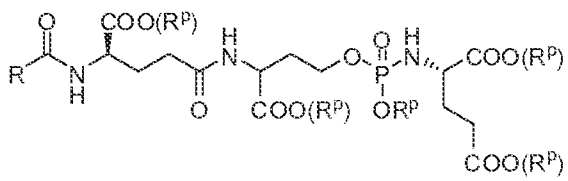
(IIa).

[0104] In another embodiment, the compound of formula (II) can be of the formula,



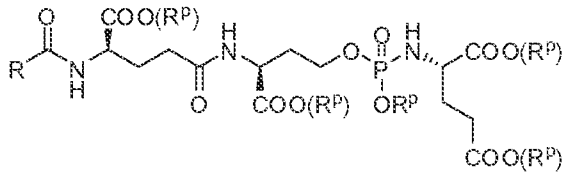
(IIb).

[0105] In another embodiment, the compound of formula (II) can be of the formula,



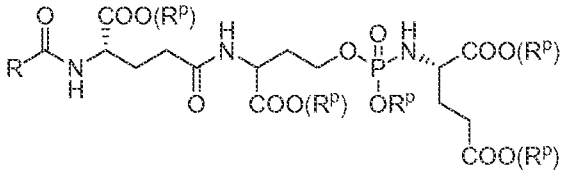
(IIc).

[0106] In another embodiment, the compound of formula (II) can be of the formula,



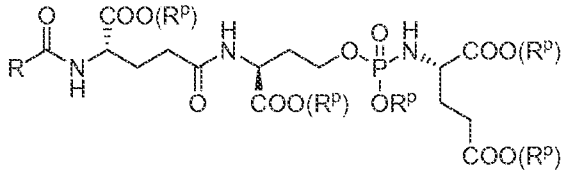
(IId).

[0107] In another embodiment, the compound of formula (II) can be of the formula,



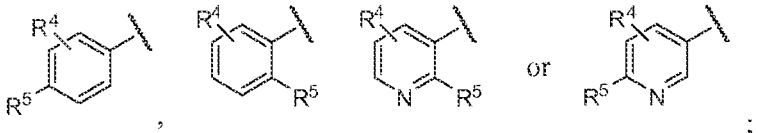
(IIe).

[0108] In another embodiment, the compound of formula (II) can be of the formula,



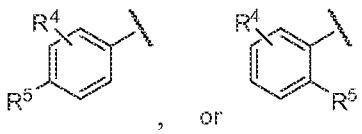
(IIf).

[0109] In an embodiment of formulas (II) and (IIa-d), R is

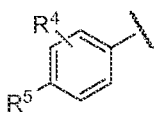


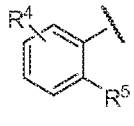
wherein R^5 is a leaving group; and R^4 is chloro or cyano.

[0110] In an embodiment of formulas (II) and (IIa-d), R is

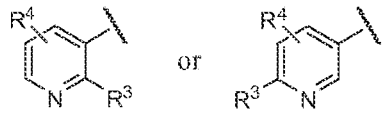


wherein R^5 is a leaving group; and R^4 is chloro or cyano.

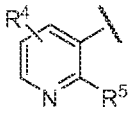
[0111] In an embodiment of formulas (II) and (IIa-d), R is  wherein R^5 is a leaving group; and R^4 is chloro or cyano.

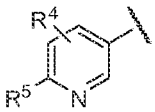
[0112] In an embodiment of formulas (II) and (IIa-d), R is  , wherein R^5 is a leaving group; and R^4 is chloro or cyano.

[0113] In an embodiment of formulas (II) and (IIa-d), R is

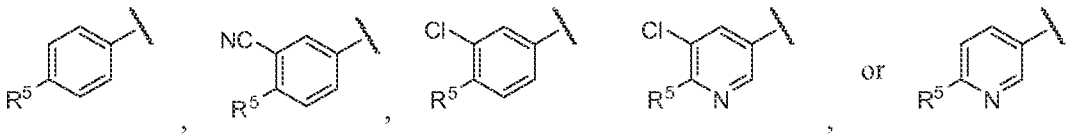


wherein R^5 is a leaving group; and R^4 is chloro or cyano.

[0114] In an embodiment of formulas (II) and (IIa-d), R is  , wherein R^5 is a leaving group; and R^4 is chloro or cyano.

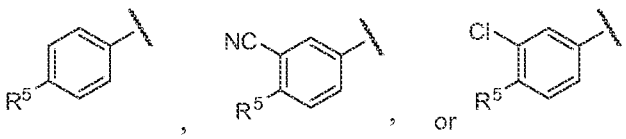
[0115] In an embodiment of formulas (II) and (IIa-d), R is  , wherein R^5 is a leaving group; and R^4 is chloro or cyano.

[0116] In an embodiment of formulas (II) and (IIa-d), R is



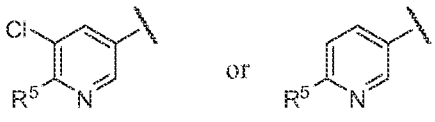
wherein R^5 is a leaving group.

[0117] In an embodiment of formulas (II) and (IIa-d), R is



wherein R^5 is a leaving group.

[0118] In an embodiment of formulas (II) and (IIa-d), R is



wherein R^5 is a leaving group.

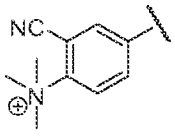
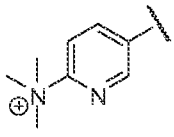
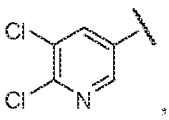
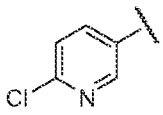
[0119] As used herein, a "leaving group" is a chemical entity that is capable of being displaced from a phenyl or pyridyl ring under S_NAr conditions are as familiar to those skilled

in the art. For example, see March, J., *Advanced Organic Chemistry*, 4th Ed. (1992), at pages 642-644, which are hereby incorporated by reference in their entirety.

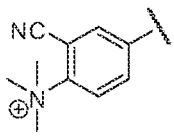
[0120] In any of the preceding embodiments, the leaving group is nitro, trimethylammonium, trimethylstannyl, benzotriazol-1-yloxy, chloro, bromo, iodo, C_1-C_{10}alkylsulfonate, C_1-C_{10}haloalkylsulfonate, or phenylsulfonate, wherein the phenyl is optionally substituted with 1, 2, or 3 groups which are each independently halogen or C_1-C_4 alkyl (e.g., besylate, tosylate, mesylate $(CH_3S(O)_2O^-)$,, triflate $(CF_3S(O)_2O^-)$, nonaflate $(CF_3CF_2CF_2CF_2S(O)_2O^-)$, 2,4,6-trimethylbenzenesulfonate, or 2,4,6-triisopropylbenzenesulfonate).

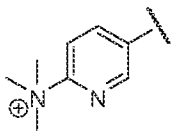
[0121] In an embodiment of any of the preceding embodiments of formulas (II) and (IIa-d), R^5 is trimethylammonium, and A- is a monovalent anion.

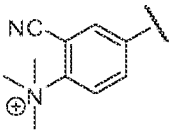
[0122] In an embodiment of formulas (II) and (IIa-d), R is

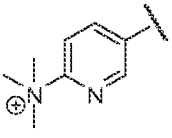

A⁻,

A⁻,

, or

;

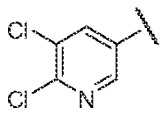
wherein A^- is a monovalent anion.

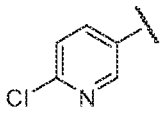
[0123] In an embodiment of formulas (II) and (IIa-d), R is  wherein A^- is an monovalent anion.

[0124] In an embodiment of formulas (II) and (IIa-d), R is  wherein A^- is an monovalent anion.

[0125] In an embodiment of formulas (II) and (IIa-d), R is  wherein A^- is triflate.

[0126] In an embodiment of formulas (II) and (IIa-d), R is  wherein A^- is triflate.

[0127] In an embodiment of formulas (II) and (IIa-d), R is  .

[0128] In an embodiment of formulas (II) and (IIa-d), R is  .

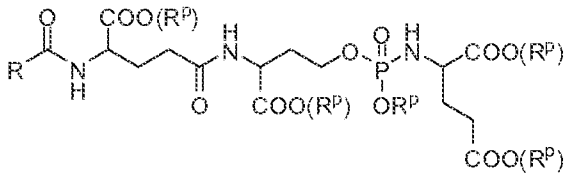
[0129] In an embodiment of any of the preceding embodiments, R^P is t-butyl or benzyl.

[0130] In an embodiment of any of the preceding embodiments, R^P is t-butyl.

[0131] In an embodiment of any of the preceding embodiments, R^P is benzyl.

[0132] In any of the preceding embodiments, the monovalent anion is chloride, bromide, iodide, hydrogen sulfate, formate, trifluoromethanesulfonate (i.e., triflate), toluenesulfonate, methanesulfonate, methyl sulfonate, nitrate, benzoate, or acetate. In certain embodiments, the monovalent anion is iodide, trifluoromethanesulfonate (i.e., triflate), methyl sulfonate, or acetate. In certain embodiments, the monovalent anion is trifluoromethanesulfonate (i.e., triflate).

[0133] In another aspect, the fluorinated compounds (e.g., of formula (I)) can be prepared according to methods comprising contacting a compound of the formula (II), as defined in any of the preceding embodiments of formula (II) with a fluoride or radiofluoride source. For example, the compounds can be prepared according to a method comprising contacting a compound of the formula,



wherein

R is phenyl or pyridyl, each substituted with one leaving group and optionally substituted with a second group selected from the group consisting of chloro and cyano; and each R^P is a protecting group (e.g., t-butyl or benzyl);

with a fluoride or radiofluoride source.

[0134] In one embodiment, the radiofluoride source is $Na^{18}F$, $K^{18}F$, $Cs^{18}F$, tetra(C_1-C_6)alkylammonium^{18}F fluoride, or tetra(C_1-C_6)alkylphosphonium ^{18}F fluoride. In another embodiment, the fluoride source is NaF, KF, CsF, tetra(C_1-C_6)alkylammonium fluoride, or tetra(C_1-C_6)alkylphosphonium fluoride.

[0135] In other embodiments, a base may be used in combination with the fluoride or radiofluoride source. Suitable bases include, but are not limited to, potassium carbonate,

potassium bicarbonate, potassium oxalate, potassium sulfonates, potassium tert-alkoxylates, cesium carbonate, cesium bicarbonate, tetrabutylammonium hydroxide (TBAOH), tetrabutylammonium bicarbonate ($TBAHCO_3$), and tetrabutylammonium mesylate (TBAOMs).

[0136] To increase the reactivity of the fluoride, a phase transfer catalyst such as an aminopolyether or crown ether, for example, 4,7,13,16,21,24 hexaoxa-1,10-diazabicyclo[8,8,8]hexacosane (Kryptofix 2.2.2; K222) may be added and the reaction performed in a non protic solvent.

[0137] The treatment with fluoride or radiofluoride anion can be effected in the presence of a suitable organic solvent such as acetonitrile, dimethylformamide, dimethylacetamide, dimethyl sulfoxide, tetrahydrofuran, dioxane, 1,2 dimethoxyethane, ethanol, methanol, iso-propanol, n-butanol, t-butanol, amyl alcohol, sulfolane, N-methylpyrrolidone, toluene, benzene, dichlorobenzenes, dichloromethane, xylenes, or mixtures thereof, at a non-extreme temperature, for example, 15 °C to 180 °C, preferably at ambient to elevated temperatures, such as 20 °C to 150 °C; or 20 °C to 120 °C; or 20 °C to 100 °C; 20 °C to 70 °C. The reaction solution can be heated using microwave irradiation for about 1 minute to about 1 hour; for example, about 5 to 15 minutes.

[0138] In one embodiment, the base used in combination with the fluoride or radiofluoride source is cesium carbonate or tetrabutylammonium bicarbonate. In one embodiment, the base used in combination with the fluoride or radiofluoride source is cesium carbonate. In one embodiment, the base used in combination with the fluoride or radiofluoride source is tetrabutylammonium bicarbonate.

[0139] In one embodiment, the base used in combination with the fluoride or radiofluoride source is cesium carbonate or tetrabutylammonium bicarbonate at a temperature between about 50 and 70°C. In one embodiment, the base used in combination with the fluoride or radiofluoride source is cesium carbonate at a temperature between about 50 and 70°C. In one embodiment, the base used in combination with the fluoride or radiofluoride source is tetrabutylammonium bicarbonate at a temperature between about 50 and 70°C.

[0140] In another embodiment, the base used in combination with the fluoride or radiofluoride source is tetrabutylammonium hydroxide. In another embodiment, the base used in combination with the fluoride or radiofluoride source is tetrabutylammonium hydroxide at a temperature between about 90 °C and 110 °C (e.g., 100°C). In another embodiment, the base used in combination with the fluoride or radiofluoride source is tetrabutylammonium

hydroxide at a temperature between about 90 °C and 110 °C (e.g., 100°C), where the temperature is maintained for about 5 minutes to about 15 minutes (e.g., about 10 min.).

[0141] Following the reaction, excess fluoride or radiofluoride anion may optionally be removed from the solution of the fluoride-labeled or radiofluoride-labeled compound by any suitable means, for example by distillation, chromatography such as by silica gel and C-18 reversed phase chromatography, or alternatively by ion-exchange chromatography or solid phase absorbents, for example by anionic exchange resin or a quaternary alkylated amino resin.

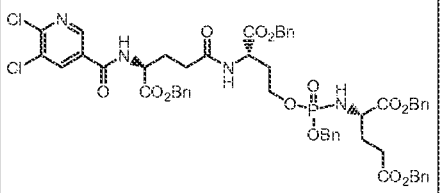
[0142] An anionic exchange resin is a resin containing a cation group, typically amino groups that are protonated to give ammonium salt or quaternary alkylated amino groups, which attract and retain anions present in the solution surrounding the said resin.

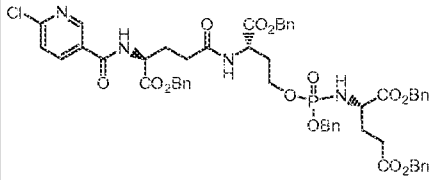
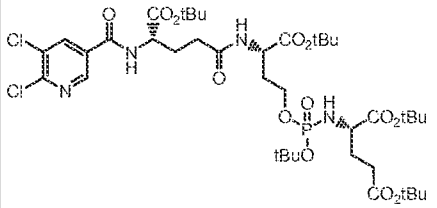
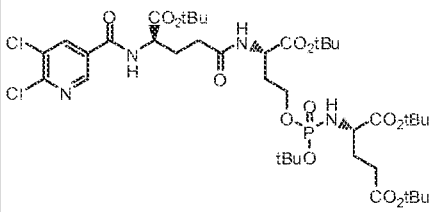
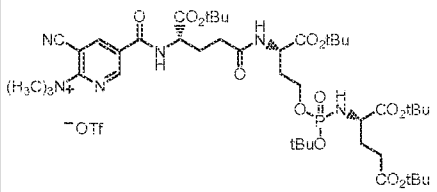
[0143] A resin is organic polymer or functionalized silica that is insoluble in most organic solvents, aqueous solutions and mixtures thereof.

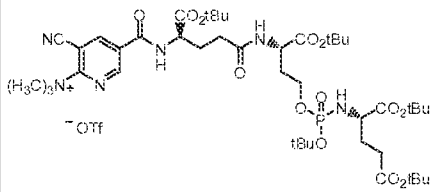
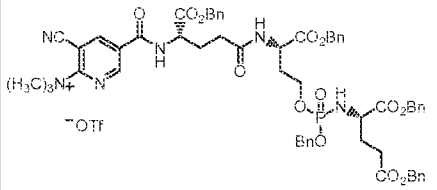
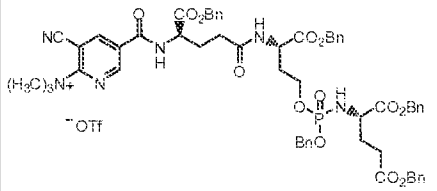
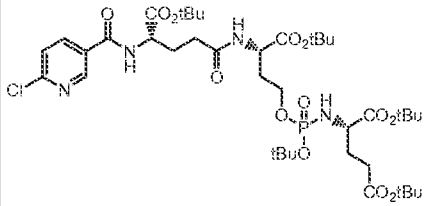
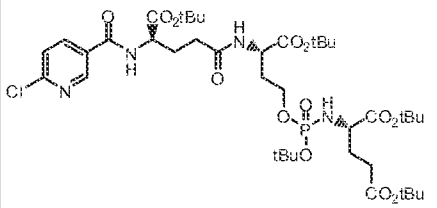
[0144] A quaternary alkylated amino resin is a resin that it functionalized with one or more amino groups and these amino groups are substituted independently with three alkyl or alkylaryl groups or mixture thereof to give an ammonium salt ($N^+R^1R^2R^3R^4$) where are R^1 is the resin R^2, R^3 and R^4 can be methyl, ethyl, propyl, butyl, benzyl, ethylphenyl.

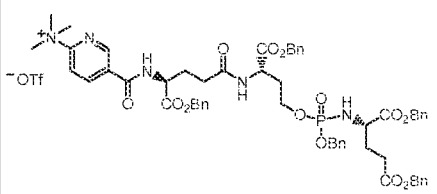
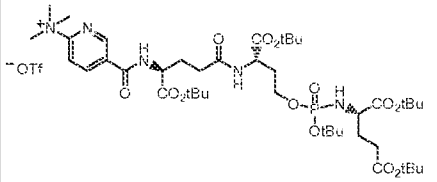
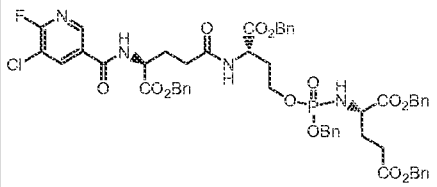
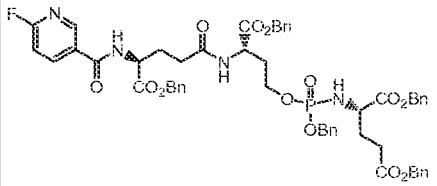
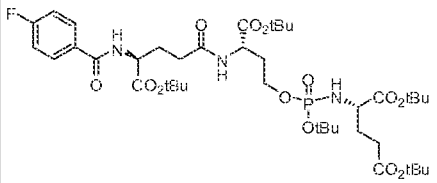
[0145] For example, a resin or solid, that allows trapping of ^{18}F fluoride may be used, such as a QMA or PS-30 cartridge. In other examples, chromatography over SepPak™ cartridges (Waters Corp., Milford, MA; e.g., C_{18} Silica, Florisil™, or Alumina A, B, N chemistries) may be used as are familiar to those skilled in the art. Suitable ion-exchange resins include BIO-RAD AG 1-X8 or Waters QMA and suitable solid phase absorbents include alumina.

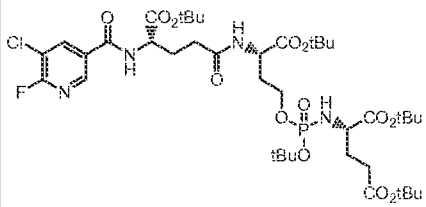
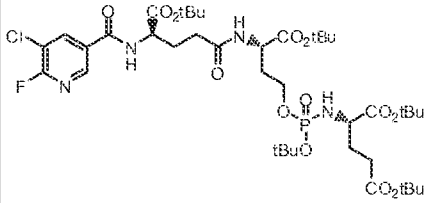
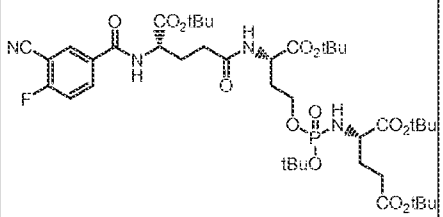
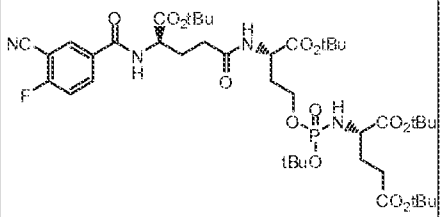
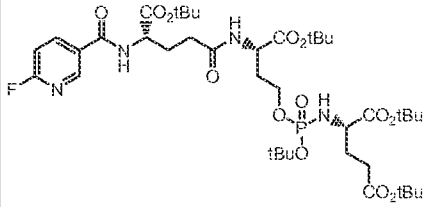
[0146] In another aspect, the disclosure provides compounds that are

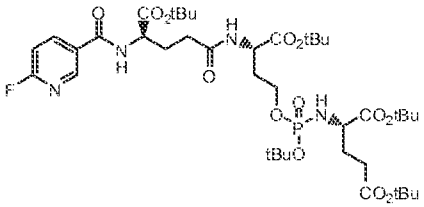
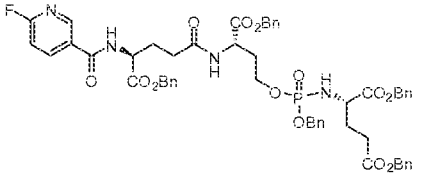
diClPy-hCTT54(5Bn);	benzyl (*O*-benzyl-*N*-(5,6-dichloro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine	

SClN-hCTT54(5Bn);	benzyl (*O*-benzyl-*N*-(6-chloro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine	
diClPy-hCTT54(5tBu);	*t*-butyl (*O*-*t*-butyl-*N*-(5,6-dichloro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine	
	t-butyl (*O*-*t*-butyl-*N*-(5,6-dichloro-pyrid-3-yl)carbonyl-D-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine	
CN,TMABz-hCTT-54(5tBu) Triflate CTT1136;	*t*-butyl (*O*-*t*-butyl-*N*-(3-cyano-4-(trimethylammonio)benzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine triflate	

	t-butyl (*O*-*t*-butyl-*N*-(3-cyano-4-(trimethylammonio)benzoyl)-D-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine triflate	
TMA,CNBz-hCTT-54(5Bn);	benzyl (*O*-benzyl-*N*-(3-cyano-4-(trimethylammonio)benzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine triflate	
	benzyl (*O*-benzyl-*N*-(3-cyano-4-(trimethylammonio)benzoyl)-D-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine triflate	
SCIN-hCTT54(5tBu);	*t*-butyl (*O*-*t*-butyl-*N*-(6-chloro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine	
	t-butyl (*O*-*t*-butyl-*N*-(6-chloro-pyrid-3-yl)carbonyl-D-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine	

	benzyl (*O*-benzyl-*N*-(6-(trimethylammonio)-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine triflate	
	t-butyl (*O*-*t*-butyl-*N*-(6-(trimethyammonio)-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine triflate	
FClPy-hCTT54(5Bn);	benzyl (*O*-benzyl-*N*-(5-chloro-6-fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine	
SFB-hCTT54(5Bn);	benzyl (*O*-benzyl-*N*-(4-fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzyloxy)phosphoryl]-L-homoserine	
SFB-hCTT54(5tBu);	*t*-butyl (*O*-*t*-butyl-*N*-(4-fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine	

FClPy-hCTT54(5tBu);	*t*-butyl (*O*-*t*-butyl-*N*-(5-chloro-6-fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine	
	t-butyl (*O*-*t*-butyl-*N*-(5-chloro-6-fluoro-pyrid-3-yl)carbonyl-D-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine	
CN,FBz-hCTT54(5tBu);	*t*-butyl (*O*-*t*-butyl-*N*-(3-cyano-4-fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine	
	t-butyl (*O*-*t*-butyl-*N*-(3-cyano-4-fluorobenzoyl)-D-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine	
SFN-hCTT54(5tBu);	*t*-butyl (*O*-*t*-butyl-*N*-(6-fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine	

	t-butyl (*O*-*t*-butyl-*N*-(6-fluoro-pyrid-3-yl)carbonyl-D-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine	
SFN-hCTT54(5Bn);	benzyl (*O*-benzyl-*N*-(6-fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine	

and a pharmaceutically acceptable salts thereof.

Advantages of Direct ^{18}F Labeling of PSMA Inhibitors.

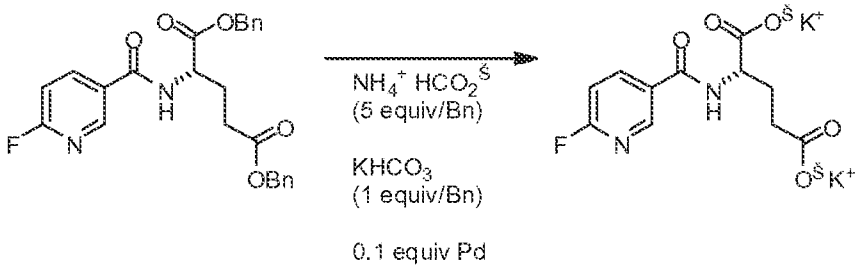
[0147] The compounds described in the examples herein comprise a radiolabeled pendant group connected to the parent structure (PMSA inhibitor or fragment thereof) via an amide bond. Although examples of structures of such pendant groups alone (not attached through an amide) can be found in the literature as substrates for fluoride substitution (^{18}F or ^{19}F), few examples have an amide bond on the pendant group. The reactivity of F with pendant groups alone or without an amide substituent on the pendant group does not allow one to predict if the same results would be obtained when the amide group is present on the pendant groups. In fact, we have found that in some cases, the literature precedent for F reaction with a pendant group alone did not correlate to our results when that pendant group was attached through an amide bond to a model peptide mimic.

[0148] Protecting groups on the PSMA inhibitor, such as benzyl and t-butyl groups, can be later removed after the incorporation of the radiolabel (^{18}F) on a pendant group. Furthermore, once the radiolabel has been incorporated into a pendant group attached to a PSMA inhibitor precursor, a final deprotection step can remove all the protecting groups on the PSMA inhibitor in a single step (e.g., t-butyl or benzyl esters).

[0149] For maximal utility as a labeled probe for PET, (1) the deprotection reaction is preferably rapid, e.g., occurring within a fraction of the half-life of the radionuclide on the pendant group (e.g., $t_{1/2} \approx 110$ min. for ^{18}F); and (2) the conditions of deprotection should not result in the loss of the radiolabel on the pendant group.

[0150] The compounds herein can be deprotected, for example, using catalytic hydrogen transfer. Conventional hydrogenolysis with H_2 gas and Pd/C is known to result in dehalogenation on aromatic rings. In fact we have observed this with pendant groups substituted with fluoride on model compounds. However, we have found conditions for catalytic hydrogen transfer in which defluorination is minimized and the reaction is complete within 20 min, and as little as 6 min without the loss of fluoride.

[0151] Defluorination can be minimized and/or avoided by controlling the amount of catalyst (Pd/C) used in the deprotection step. In model experiments,



[0152] with the fluoro-nicotinamide derivative of glutamate dibenzyl ester we found that with 0.1 molar equivalents of Pd (using 10% Pd/C) the benzyl groups were deprotected within 10 minutes and no defluorination was observed. However, using 0.4 equivalents of Pd, the deprotection of the benzyl esters was complete within 3 minutes but 10% defluorination was observed.

[0153] Such optimized yields allows for the use of less of each starting material (i.e., the PSMA inhibitor having a leaving group) and the ^{18}F anion source, while still providing a final radiolabeled product in high yield.

[0154] By utilizing the methods described herein the time and chemical and chromatographic steps involved after labeling can be shortened by a step as compared to methods using the fluorine-18 labeled N-succinimidyl benzoate (SFB) as described in Lapi, S.E., et al., *J. Nucl. Med.* **2009**, *50*(12), 2042.

Imaging Methods

[0155] In another aspect, the present disclosure provides methods for detecting and/or identifying cells presenting PSMA comprising contacting a cell suspected of presenting PSMA with a compound as discussed above, or a composition comprising the compound.

[0156] In one embodiment, the methods are suitable for imaging studies of PSMA inhibitors, for example, by studying competitive binding of non-radiolabeled inhibitors.

[0157] In still another embodiment, the methods are suitable for imaging of cancer, tumor or neoplasm. In a further embodiment, the cancer is selected from eye or ocular cancer, rectal

cancer, colon cancer, cervical cancer, prostate cancer, breast cancer and bladder cancer, oral cancer, benign and malignant tumors, stomach cancer, liver cancer, pancreatic cancer, lung cancer, corpus uteri, ovary cancer, prostate cancer, testicular cancer, renal cancer, brain cancer (e.g., gliomas), throat cancer, skin melanoma, acute lymphocytic leukemia, acute myelogenous leukemia, Ewing's Sarcoma, Kaposi's Sarcoma, basal cell carcinoma and squamous cell carcinoma, small cell lung cancer, choriocarcinoma, rhabdomyosarcoma, angiosarcoma, hemangioendothelioma, Wilms Tumor, neuroblastoma, mouth/pharynx cancer, esophageal cancer, larynx cancer, lymphoma, neurofibromatosis, tuberous sclerosis, hemangiomas, and lymphangiogenesis.

[0158] The methods are suitable for imaging any physiological process or feature in which PSMA is involved. Typically, imaging methods are suitable for identification of areas of tissues or targets which express high concentrations of PSMA. Typical applications include imaging glutamateric neurotransmission, presynaptic glutamatergic neurotransmission, malignant tumors or cancers that express PSMA, prostate cancer (including metastasized prostate cancer), and angiogenesis. Essentially all solid tumors express PSMA in the neovasculture. Therefore, present methods can be used to image nearly all solid tumors including lung, renal cell, glioblastoma, pancreas, bladder, sarcoma, melanoma, breast, colon, germ cell, pheochromocytoma, esophageal and stomach. Also, certain benign lesions and tissues including endometrium, schwannoma and Barrett's esophagus can be imaged according to the present methods.

[0159] In certain embodiments, the radiolabeled compound is detected by positron emission tomography (PET).

[0160] In certain other embodiments, the radiolabeled compound is detected by positron emission tomography - computed tomography (PET/CT).

[0161] In one embodiment, the subject of the methods may be a human, rat, mouse, cat, dog, horse, sheep, cow, monkey, avian, or amphibian. In another embodiment, the cell is in vivo or in vitro. In certain embodiments, the cells being images or detected are *in vivo*.

[0162] Typical subjects to which compounds described herein may be administered will be mammals, particularly primates, especially humans. For veterinary applications, a wide variety of subjects will be suitable, e.g. livestock such as cattle, sheep, goats, cows, swine and the like; poultry such as chickens, ducks, geese, turkeys, and the like; and domesticated animals particularly pets such as dogs and cats. For diagnostic or research applications, a wide variety of mammals will be suitable subjects including rodents (e.g. mice, rats, hamsters), rabbits, primates, and swine such as inbred pigs and the like. Additionally, for in

vitro applications, such as in vitro diagnostic and research applications, body fluids and cell samples of the above subjects will be suitable for use such as mammalian, particularly primate such as human, blood, urine or tissue samples, or blood urine or tissue samples of the animals mentioned for veterinary applications.

[0163] In certain embodiments, a kit can be provided that contains from about 1 to about 30 mCi of the radionuclide-labeled imaging agent described above, in combination with a pharmaceutically acceptable carrier. The imaging agent and carrier may be provided in solution or in lyophilized form. When the imaging agent and carrier of the kit are in lyophilized form, the kit may optionally contain a sterile and physiologically acceptable reconstitution medium such as water, saline, buffered saline, and the like. The kit may provide a compound, as discussed above, in solution or in lyophilized form, and these kit components may optionally contain stabilizers such as NaCl, silicate, phosphate buffers, ascorbic acid, gentisic acid, and the like. Additional stabilization of kit components may be provided in this embodiment, for example, by providing the reducing agent in an oxidation-resistant form. Determination and optimization of such stabilizers and stabilization methods are well within the level of skill in the art.

[0164] In certain embodiments, a kit provides a non-radiolabeled precursor to be combined with a radiolabeled reagent on-site, such as Na[^{18}F] or K[^{18}F].

[0165] The radiolabeled compounds herein (i.e., imaging agents) may be used in accordance with the methods described herein by one of skill in the art. Images can be generated by virtue of differences in the spatial distribution of the imaging agents which accumulate at a site when contacted with PSMA. The spatial distribution may be measured using any means suitable for the particular label, for example, a PET apparatus. The extent of accumulation of the imaging agent may be quantified using known methods for quantifying radioactive emissions. A particularly useful imaging approach employs more than one imaging agent to perform simultaneous studies.

[0166] In general, a detectably effective amount of the imaging agent is administered to a subject. As used herein, "a detectably effective amount" of an imaging agent is an amount sufficient to yield an acceptable image using equipment which is available for clinical use. A detectably effective amount of an imaging agent may be administered in more than one injection. The detectably effective amount of the imaging agent can vary according to factors such as the degree of susceptibility of the individual, the age, sex, and weight of the individual, idiosyncratic responses of the individual, and the dosimetry. Detectably effective

amounts of the imaging agent can also vary according to instrument and film-related factors. Optimization of such factors is well within the level of skill in the art.

[0167] The amount of imaging agent used for diagnostic purposes and the duration of the imaging study will depend upon the radionuclide used to label the agent, the body mass of the patient, the nature and severity of the condition being treated, the nature of therapeutic treatments which the patient has undergone, and on the idiosyncratic responses of the patient. Ultimately, the attending physician will decide the amount of imaging agent to administer to each individual patient and the duration of the imaging study. In certain embodiments, a safe and sufficient amount of the compounds herein can be in the range of from about 0.01 mg to about 200 mg per dose.

Definitions

[0168] The compounds herein described may have one or more charged atoms. For example, the compounds may be zwitterionic, but may be neutral overall. Other embodiments may have one or more charged groups, depending on the pH and other factors. In these embodiments, the compound may be associated with a suitable counter-ion. It is well known in the art how to prepare salts or exchange counter-ions. Generally, such salts can be prepared by reacting free acid forms of these compounds with a stoichiometric amount of the appropriate base (such as Na, Ca, Mg, or K hydroxide, carbonate, bicarbonate, or the like), or by reacting free base forms of these compounds with a stoichiometric amount of the appropriate acid. Such reactions are typically carried out in water or in an organic solvent, or in a mixture of the two. Counter-ions may be changed, for example, by ion-exchange techniques such as ion-exchange chromatography. All zwitterions, salts and counter-ions are intended, unless the counter-ion or salt is specifically indicated. In certain embodiments, the salt or counter-ion may be pharmaceutically acceptable, for administration to a subject. Pharmaceutically acceptable salts are discussed later.

[0169] The term "alkyl" as used herein, means a straight or branched chain hydrocarbon containing from 1 to 10 carbon atoms, unless otherwise specified. Representative examples of alkyl include, but are not limited to, methyl, ethyl, n-propyl, iso-propyl, n-butyl, sec-butyl, iso-butyl, tert-butyl, n-pentyl, isopentyl, neopentyl, n-hexyl, 3-methylhexyl, 2,2-dimethylpentyl, 2,3-dimethylpentyl, n-heptyl, n-octyl, n-nonyl, and n-decyl.

[0170] The term "haloalkyl" as used herein, means an alkyl group, as defined herein, substituted with one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, or 9) halogen groups (i.e., F, Cl, Br, and/or I). Examples of haloalkyl groups include, but are not limited to fluoromethyl,

difluoromethyl, trifluoromethyl, chloromethyl, dichloromethyl, trichloromethyl, and n-nonafluorobutyl.

[0171] As used herein, the term "cell" is meant to refer to a cell that is *in vitro*, ex vivo or *in vivo*. In some embodiments, an ex vivo cell can be part of a tissue sample excised from an organism such as a mammal. In some embodiments, an *in vitro* cell can be a cell in a cell culture. In some embodiments, an *in vivo* cell is a cell living in an organism such as a mammal.

[0172] As used herein, the term "contacting" refers to the bringing together of indicated moieties in an *in vitro* system or an *in vivo* system. For example, "contacting" PSMA with a compound includes the administration of a compound described herein to an individual or patient, such as a human, as well as, for example, introducing a compound into a sample containing a cellular or purified preparation containing PSMA.

[0173] As used herein, the phrase "pharmaceutically acceptable salt" refers to both pharmaceutically acceptable acid and base addition salts and solvates. Such pharmaceutically acceptable salts include salts of acids such as hydrochloric, phosphoric, hydrobromic, sulfuric, sulfinic, formic, trifluoromethanesulfonic (i.e., triflic), toluenesulfonic, methanesulfonic, methyl sulfonate, nitric, benzoic, citric, tartaric, maleic, hydroiodic, alkanoic such as acetic, HOOC-$(CH_2)_n$-COOH where n is 0-4, and the like. "Pharmaceutically acceptable salts" also include, for example, salts formed by the quaternization (e.g., alkylation) of a suitable site in the compound itself, such as methylation of a dimethylamine to form a trimethylammonium group. In such cases, the counterion can be, for example, but not limited to, chloride, phosphate, hydrogen phosphate, bromide, sulfate, hydrogen sulfate, sulfinate, formate, trifluoromethanesulfonate (i.e., triflate), toluenesulfonate, methanesulfonate, methyl sulfonate, nitrate, benzoate, citrate, tartarate, maleate, iodide, acetate, and the like. Non-toxic pharmaceutical base addition salts include salts of bases such as sodium, potassium, calcium, ammonium, and the like. In certain embodiments, the pharmaceutically acceptable salt is a potassium salt. Those skilled in the art will recognize a wide variety of non-toxic pharmaceutically acceptable addition salts.

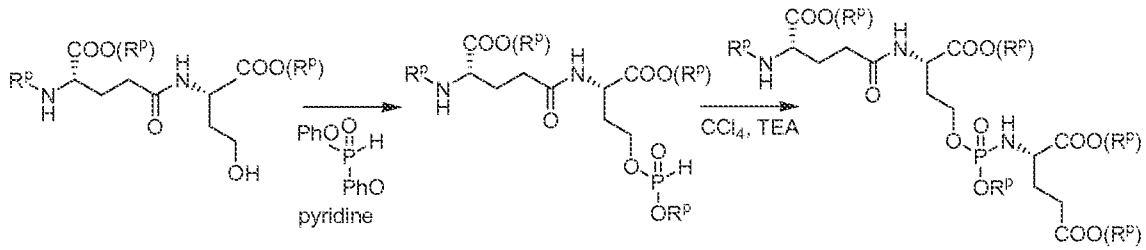
[0174] Pharmaceutical compositions suitable for parenteral administration, such as, for example, by intraarticular (in the joints), intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening

agents, stabilizers, and preservatives. Compositions can be administered, for example, by intravenous infusion, orally, topically, intraperitoneally, intravesically or intrathecally.

[0175] In certain embodiments, a "pharmaceutically acceptable carrier" refers to a biocompatible solution, having due regard to sterility, p[Eta], isotonicity, stability, and the like and can include any and all solvents, diluents (including sterile saline, Sodium Chloride Injection, Ringer's Injection, Dextrose Injection, Dextrose and Sodium Chloride Injection, Lactated Ringer's Injection and other aqueous buffer solutions), dispersion media, coatings, antibacterial and antifungal agents, isotonic agents, and the like. The pharmaceutically acceptable carrier may also contain stabilizers, preservatives, antioxidants, or other additives, which are well known to one of skill in the art, or other vehicle as known in the art.

EXAMPLES

Example 1 *Synthesis of radiolabeling precursor.*

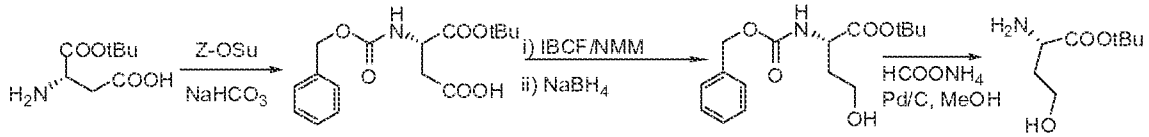


[0176] *Synthesis of Z-Asp-OtBu.* To a solution of H-Asp-OtBu (15 g, 79.3 mmol) and $NaHCO_3$ (19 g, 3eq) in water (300 mL) chilled in an ice bath was added Z-OSu (19.8 g, 1 eq) in acetone (50 mL). The mixture was allowed to stir for 5 hours, followed by washing with ether (30 mL x 3). The aqueous solution was chilled in an ice bath and acidified with 3 N aq. HCl to pH 3-4, and extracted with ethyl acetate (50 mL x 4) four times. The organic phases were combined and washed with brine (25 mL x 2), dried over $MgSO_4$. The solvent was evaporated and the residue was dried in vacuo overnight. Yield: 25 g, 98% Purity (TLC, hexane/ethyl acetate, 1/1, ninhydrin): one spot.

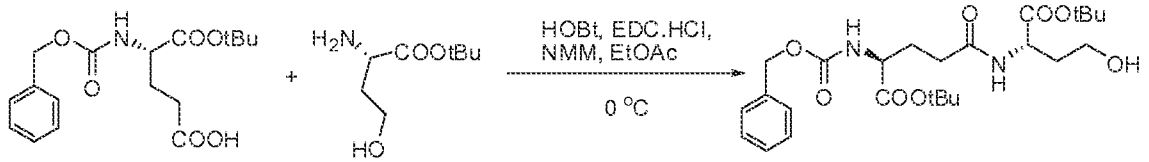
[0177] *Synthesis of Z-HSer-OtBu.* To a solution of Z-Asp-OtBu (10 g, 31 mmol) and NMM (N-methylmorpholine, 3.4 mL, 1 eq) in dry THF (50 mL) chilled in acetonitrile/dry ice bath (-20°C) was added IBCF (isobutyl chloroformate, 4.0 mL, 1eq) solution in dry THF during 10 min. The mixture was stirred for 30 min at between this temperature and at 0 °C. The solid was filtered off and the filter cake was washed with dry THF (10 mL). The washing was combined with the filtrate.

[0178] In a large beaker (1 L), water (100 mL) and $NaBH_4$ (2.4 g, 2 eq.) were added and chilled in an ice bath. While vigorous stirring, the anhydride filtrate was added in portions to keep the temperature below 15 °C. The mixture was allowed to stir for 2-3 hours. TLC monitored the completion of the reduction. The reaction was then quenched by 1N HCl. The solution was extracted with ethyl acetate three times (50 mL x 3). The organic phases were combined and washed with 1N HCl (50 mL) once and 10% aq. $NaHCO_3$ (30 mL x 3), and brine (30 mL) once, dried over Na_2SO_4. The filtrate was concentrated to dryness, and purified by column chromatography (hexane/ethyl acetate, 1/1). Yield: ~7.5g, 78%.

[0179] *Synthesis of H-HSer-OtBu.* Z-HSer-OtBu (7 g, 22 mmol), $HCOONH_4$ (4.3 g, 3 eq), 10% Pd/C (5 weight ratio%) were added into methanol (50 mL) in a round bottom flask. The mixture was allowed to stir for 3 hours. TLC indicated the completion of the reaction. The catalyst was filtered off via Celite®. The solvent was evaporated to dryness. The residue was dissolved in ethyl acetate and the solid was filtered off. The filtrate was concentrated for direct use. Yield: >90%.

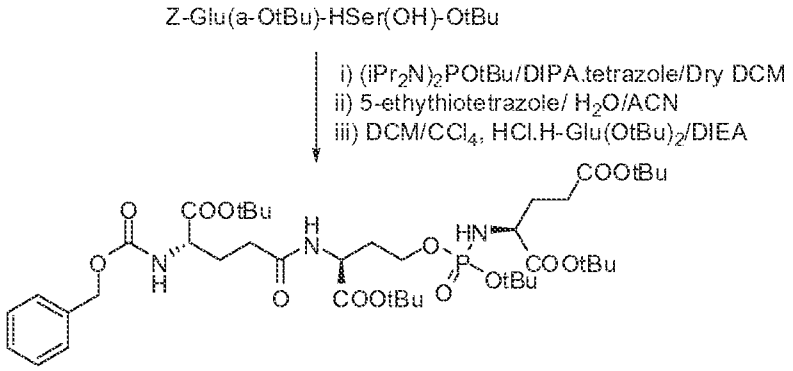


[0180] *Synthesis of Z-Glu(HSer-OtBu)-OtBu.* Z-Glu-OtBu (5 g, 15 mmol), H-HSer-OtBu (3g, 17mmol), 1-hydroxybenzotriazole hydrate (HOBt)(2.9 g, 1.1 eq), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide) hydrochloride (EDC·HCl) (4.2 g, 1.3 eq) were mixed in ethyl acetate (200 mL). The mixture was chilled in an ice bath, followed by addition of N-methylmorpholine (NMM)(5.6 mL, 3 eq) while stirring. The mixture was allowed to stir for 2 hours, followed by washing with 1N HCl (30 mL x 3), 10% $NaHCO_3$ (30 mL x 3) and brine (30mL x 2), drying over Na_2SO_4. The solvent was evaporated to dryness to give the dipeptide. Yield: 5.5 g, 74%; Purity (TLC, hexane/ethyl acetate, 1/1, ninhydrin): >95%.



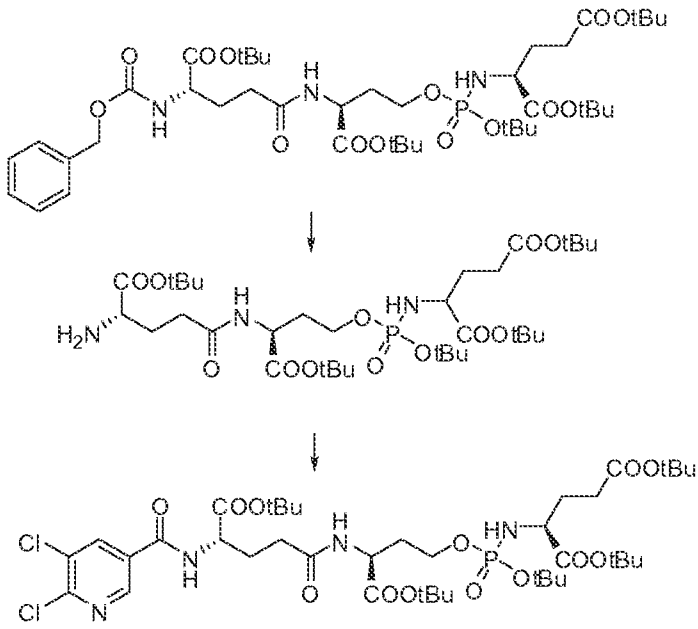
[0181] *Synthesis of pendant group-coupled precursor.* The solution of dipeptide (5 g, 10 mmol) and N,N-diisopropyl ammonium tetrazolide (1.2 equiv.) in dry CH_2Cl_2 was chilled in an ice bath followed by addition of $(iPr_2N)_2P(OtBu)$ (1.2 equiv.) in portions. The mixture was allowed to stir for 2 hours. The solvent was evaporated *in vacuo*. The residue was dissolved

in acetonitrile (25 mL) and chilled in an ice bath. A solution of 5-ethylthio-1H-tetrazole (4 equiv.) in a mixture of water (4 mL) and acetonitrile (20 mL) was added and the reaction mixture was stirred for 20 min at 0 °C, then an additional 1 hour at room temperature. The solvent was evaporated *in vacuo* and the residue was partitioned with ethyl acetate (25 mL)/1 N $HCl_{(aq)}$ (25 mL). The organic phase was collected and washed twice with 10% $NaHCO_{3(aq)}$ (30mL), and twice with brine (30 mL). After drying over Na_2SO_4, the solvent was removed *in vacuo*. The resulting peptide, and HCl.H-Glu(OtBu)$_2$ (4 g, 1.4 eq), CCl_4 (4 mL) and dichloromethane (10 mL) and diisopropylethylamine (2 eq) were mixed and stirred overnight. The solvent was evaporated and the residue was purified by column chromatography (Elute: hexane/ethyl acetate, 1/1). Yield: 5.4 g, 62% Purity (TLC, ethyl acetate/hexane, 2/1): one spot.



[0182] *Synthesis of radiolabeling precursor.* The starting peptide (5 g), $HCOONH_4$ (1.4 g, 4 eq) and 10% Pd/C (0.5 g, 5-10% in weight ratio) were mixed in methanol (40 mL) at room temperature for 2 hours. TLC indicated the completion of the reaction. The catalyst was filtered off through Celite® and the solvent was evaporated to dryness. The residue was dissolved in dichloromethane and the insoluble solid was filtered off and the filter cake was washed with dichloromethane (20 mL) once. The washing and the filtrate were combined and evaporated to dryness to yield a white powder.

[0183] The white powder (1 g) and diisopropylethylamine (DIEA) (1 eq) were dissolved in N,N-dimethylformamide (DMF) (5 mL). A pendant N-hydroxysuccinimide (NHS) ester was added to the solution and the mixture was allowed to stir overnight. The residue was dissolved in ethyl acetate (20 mL) and washed with 1N HCl (10 mL x 3) and 5% $NaHCO_3$ (10 mL x 3) and brine (10 mL x 2), dried over Na_2SO_4. The solution was evaporated and purified by column chromatography (hexane/ethyl acetate, 2/1 to 1/1, or dichloromethane/methanol, 10/1). Yield: 550mg, 45% Purity (TLC, ethyl acetate/hexane, 2/1): one spot.



Example 2 Preparation of [^{18}F]SFClN-hCTT54 (Precursor = diClPy-hCTT54(5tBu))

[0184] **HPLC Column:**

ACE 3µ C18 50 * 4,6 mm (Agilent 7)\

ACE S/No: N/A

ACE 5 C18 250*10mm (Agilent 3)

ACE 121-2510 S/No: A44243

ZIC-Hilic, 100 * 4.6 mm 5 µm 200 Å (Agilent 5)

SeQuant; Serial No. 934340; Sorbent Lot# S080813

[0185] **Eluents:**

A: 1mM K_2HPO_4 in water (Agilent 7)

B: Acetonitrile

A: H_2O + 0.1% trifluoroacetic acid (Agilent 3)

B: Acetonitrile + 0.1% trifluoroacetic acid

A: 0.1M ammonium formate pH 3.2 (Agilent 5)

B: Acetonitrile

[0186] **Gradient Flow:**

ZIC HILIC (agil.5) 2 mL/min		ACE 3µ (agil.7) 2mL/min.	
00:00	70/80%B	00:00	05%B
05:00	70/80%B	07:00	95%B
06:00	10%B	07:10	100%B
08:00	10%B	08:80	100%B

09:00	70/80%B	09:00	05%B
11:00	70/80%B	12:00	05%B

prep ACE (Knauer 7) 4 mL/min

isocratic 80%B

[0187] A QMA SepPak (SepPak QMA light Waters PN: WAT023525) was preconditioned with 5 mL 0.5 M K_2CO_3, washed with 10 mL water, and air pushed through to dry. 600 µL of F-18 (Na[^{18}F] (E&Z EURO-PET Berlin GmbHz)) was passed through a QMA and air pushed through to dry. The F-18 from the QMA was eluted with 600 µL $TBAHCO_3$ mixture and put into a vial (8µL $TBAHCO_3$ (w=40% in water) in 600µL acetonitrile/H_2O 1:1)[1742MBq].

[0188] This vial was heated under gentle N_2 stream for 10 min at 140°C (heating block temperature) and 1 mL acetonitrile was added to the vial. The vial was then heated under gentle N_2 stream for 5 min at 140 °C (heating block temperature) and to cooled at room temperature over 5 min. in a lead pig (Vial 1, 1511MBq=97.16% decay corrected).

[0189] Precursor (2 mg) in DMSO (300 µL) was added to the dried [^{18}F]fluoride complex and the vial was incubated for 10 min. at 70 °C. The reaction mixture was transferred into 4 mL water, the vial washed with 500 µL acetonitrile and this was transferred into the same aqueous solution (884.5 MBq) and this solution was purified by preparative HPLC, collecting the main peak: (370.3 MBq).

[0190] The collected fraction was diluted with 25 mL water, passed through a C18 light (SepPak C18 light (Waters), preconditioned with 5 mL Ethanol and 10 mL water), the SPE was washed with 5 mL water, and then eluted with 1 mL acetonitrile. The acetonitrile eluent was passed the solution through a dry SPE (255.4 MBq).

[0191] *Deprotection* (trifluoroacetic acid/1,2-dichloroethane):

[0192] 300 µL of the acetonitrile solution was concentrated under a gentle N_2-stream for 10 min. at 60 °C. 100 µL of Trifluoroacetic acid, 100 µL 1,2-dichloroethane, and 50 µL triethylsilane were added, and the reaction mixture heated for 10 min. at 100 °C. After heating, the solution was concentrated under gentle N_2-stream for 10 min. at 80 °C and redissolved in 250 µL PBS.

[0193] *Deprotection* (Formic acid, RT) :

[0194] 300 µL of the acetonitrile solution was concentrated under a gentle N_2-stream for 5 min. at room temperature and for 10 min at 60 °C. 250 µL formic acid was added and the solution maintained for 5 min. at room temperature. The solution was concentrated under gentle N_2-stream for 10 min at 80 °C and redissolved in 250 µL PBS.

[0195] *Deprotection* (Formic acid, 50 °C):

[0196] 300 µL of the acetonitrile solution was concentrated under a gentle N_2-stream for 5 min. at 50 °C and 10 min. at 60 °C. 250 µL Formic acid was added and the solution maintained for 5min. at 50°C. The solution was concentrated under gentle N_2-stream for 10 min at 80°C and redissolved in 250 µL PBS.

Example 3 Preparation of [^{18}F]SFClN-hCTT54 (Precursor = diClPy-Bz-hCTT54(5tBu))

[0197] **HPLC Column:**

ACE 3µ C18 50 * 4.6 mm (Agilent 7)

ACE S/No: N/A

ACE 5 C18 250*10mm (Agilent 3)

ACE 121-2510 S/No: A44243

ZIC-Hilic, 100 * 4.6mm 5 µm 200 Å (Agilent 5)

SeQuant; Serial No. 934340; Sorbent Lot# S080813

Zorbax Bonus RP, 5µm 9.4mm*250mm (Agilent 3)

S/N: USAYB01016

[0198] **Eluents:**

A: 1 mM K_2HPO_4 in water (Agilent 7)

B: Acetonitrile

A: H_2O + 0.1 % trifluoroacetic acid (Agilent 3)

B: Acetonitrile + 0.1 % trifluoroacetic acid

A: 0.1 M ammonium formate pH 3.2 (Agilent 5)

B: Acetonitrile

[0199] Gradient Flow:

ZIC HILIC (agil.5) 2 mL/min		ACE 3µ (agil.7) 2 mL/min	
00:00	70/80%B	00:00	05%B
05:00	70/80%B	07:00	95%B
06:00	10%B	07:10	100%B
08:00	10%B	08:80	100%B
09:00	70/80%B	09:00	05%B
11:00	70/80%B	12:00	05%B
prep ACE (Agilent 3) 4 mL/min		prep Zorbax (Agilent 3) 3 mL/min	

isocratic 80%B	00:00	05%B
	02:00	05%B
	22:00	50%B

[0200] A QMA SepPak (SepPak QMA light Waters PN: WAT023525) was preconditioned with 5 mL 0.5M K_2CO_3, washed with 10 mL water, and air pushed through to dry. 600 µL of F-18 (Na[^{18}F] (E&Z EURO-PET Berlin GmbHz)) was passed through a QMA and air pushed through to dry. The F-18 from the QMA was eluted with 600 µL $TBAHCO_3$ mixture and put into a Vial (8 µL $TBAHCO_3$ (w=40% in water) in 600 µL acetonitrile/H_2O 1:1)[3537MBq].

[0201] This vial was heated under gentle N_2 stream for 10 min at 140°C (heating block temperature) and 1 mL acetonitrile was added to the vial. The vial was then heated under gentle N_2 stream for 5 min at 140 °C (heating block temperature) and to cooled at room temperature over 5 min. in a lead pig

[0202] Precursor (2 mg) in DMSO (300 µL) was added to the dried [^{18}F]fluoride complex and the vial was incubated for 10 min. at 70 °C. The reaction mixture was transferred into 4 mL water, the vial washed with 500 µL acetonitrile and this was transferred into the same aqueous solution (1657 MBq) and this solution was purified by preparative HPLC, collecting the main peak: (815.7MBq). The collected fraction was diluted with 25 mL water and passed through the C18 light (SepPak C18 light (Waters); preconditioned with 5 mL Ethanol and 10 mL water). The SPE was washed with 5 mL water, eluted with 1 mL acetonitrile, and the eluent passed through a Dry SPE (714.8MBq).

Deprotection (trifluoroacetic acid/1,2-dichloroethane):

[0203] 500 µL of the acetonitrile solution was concentrated under gentle N_2-stream for 10 min. at 60 °C. 100 µL trifluoroacetic acid, 100 µL 1,2-dichloroethane and 50 µL triethylsilane were added and the reaction maintained for 10 min at 100°C. The reaction solution was concentrated under gentle N_2-stream for 10 min, at 80°C, and redissolved in 250 µL PBS.

[0204] The PBS solution was diluted with 4 mL PBS and purified by prep HPLC, collecting the main peak (62.25 MBq). The collected fraction was diluted with 15 mL 0.02M K_2CO_3 and loaded on QMA (preconditioned with 5 mL Methanol and 10 mL 0.02M K_2CO_3), the QMA washed with 2 mL water (load: 19.42 MBq, wash: 1.035 MBq, QMA: 32.84 MBq) and product eluted with 0.3 mL of 0.5 M NaCl into 0.2 mL PBS buffer (32.24 MBq).

Deprotection (formic acid):

[0205] 1000 µL of the acetonitrile solution was concentrated under gentle N_2-stream for 10 min. at 60 °C. 250 µL formic acid was added and the reaction maintained for 5 min. at 50°C. The mixture was concentrated under gentle N_2-stream for 10 min. at 80 °C and redissolved in 250µL PBS.

[0206] The PBS solution was diluted with 4 mL PBS and purified by prep HPLC, collecting the main peak (54.50 MBq). diluted the fraction with 15 mL 0.02 M K_2CO_3 and loaded on QMA (preconditioned with 5 mL methanol and 10 mL 0.02 M K_2CO_3) washed with 2 mL water (load: 19.83 MBq, wash: 0.85 MBq) and product eluted with 0.3 mL 0.5M NaCl into 0.2 mL PBS buffer (32.04 MBq).

Example 4 Preparation of [^{18}F]SFClN-hCTT54 (Precursor = diClPy-Bz-hCTT54(5tBu))

[0207] **HPLC Column:**

ACE 3µ C18 50 * 4.6 mm (Agilent 7)

ACE S/No: N/A

ACE 5 C18 250*10 mm (Agilent 3)

ACE 121-2510 S/No: A44243

ZIC-Hilic, 100 * 4.6 mm 5 µm 200 Å (Agilent 5)

SeQuant; Serial No. 934340; Sorbent Lot# S080813

Zorbax Bonus RP, 5 µm 9.4 mm*250 mm (Agilent 3)

S/N: USAYB01016

[0208] **Eluents:**

A: 1 mM K_2HPO_4 in water (Agilent 7)

B: Acetonitrile

A: H_2O + 0.1 % trifluoroacetic acid (Agilent 3)

B: Acetonitrile + 0.1 % trifluoroacetic acid

A: 0.1 M ammoniumformate pH 3.2 (Agilent 5)

B: Acetonitrile

[0209] **Gradient Flow:**

ZIC HILIC (agil.5) 2 mL/min		ACE 3µ (agil.7) 2 mL/min	
00:00	70/80%B	00:00	05%B
05:00	70/80%B	07:00	95%B
06:00	10%B	07:10	100%B
08:00	10%B	08:80	100%B
09:00	70/80%B	09:00	05%B

11:00	70/80%B	12:00	05%B

prep ACE (Agilent 3) 4ml/min		prep	Zorbax	(Agilent 3)	3ml/min
isocratic 80%B		00:00	10%B		
		20:00	50%B		

[0210] A QMA SepPak (SepPak QMA light Waters PN: WAT023525) was preconditioned with 5 mL 0.5M K_2CO_3, washed with 10 mL water, and air pushed through to dry. 600 µL of F-18 (Na[^{18}F] (E&Z EURO-PET Berlin GmbHz)) was passed through a QMA and air pushed through to dry. The F-18 from the QMA was eluted with 600 µL $TBAHCO_3$ mixture and put into a Vial (8 µL $TBAHCO_3$ (w=40% in water) in 600 µL acetonitrile/H_2O 1:1)[3743MBq].

[0211] This vial was heated under gentle N_2 stream for 10 min at 140°C (heating block temperature) and 1 mL acetonitrile was added to the vial. The vial was then heated under gentle N_2 stream for 5 min at 140 °C (heating block temperature) and to cooled at room temperature over 5 min. in a lead pig (3259 MBq).

[0212] Precursor (2 mg) in DMSO (300 µL) was added to the dried [^{18}F]fluoride complex and the vial was incubated for 10 min. at 70 °C. The reaction mixture was transferred into 4 mL water, the vial washed with 500 µL acetonitrile and this was transferred into the same aqueous solution (2121 MBq) and this solution was purified by preparative HPLC, collecting the main peak: (568.6 MBq). The collected fraction was diluted with 25 mL water and passed through the C18 light (SepPak C18 light (Waters); preconditioned with 5 mL Ethanol and 10 mL water). The SPE was washed with 5 mL water, eluted with 1 mL acetonitrile, and the eluent passed through a Dry SPE (498.2 MBq).

Deprotection (formic acid):

[0213] 1000 µL of the acetonitrile solution was concentrated under a gentle N_2-stream for 10 min. at 60 °C (237.9 MBq). 250 µL formic acid was added and the reaction maintained for 5 min. at 50 °C. The mixture was concentrated under gentle N_2-stream for 10 min. at 80 °C, and redissolved in 250 µL PBS. The PBS solution was diluted with 4 mL PBS and purified by prep HPLC (211.4 MBq), collecting the main peak (89.16 MBq). The collected fraction was diluted with 15 mL 0.02 M K_2CO_3 and loaded on QMA (preconditioned with 5 mL methanol and 10 mL 0.02 M K_2CO_3), the QMA was washed with 2 mL water and the produce was eluted with 0.3 mL of 0.5M NaCl into 0.2 mL PBS buffer (49.84 MBq).

Example 5 Preparation of [^{18}F]SClFN-hCTT54 (precursor=diClPy-hCTT54(5tBu))

[0214] HPLC Column:

ACE 3µ C18 50 * 4.6 mm (Agilent 7)

ACE S/No: N/A

ACE 5 C18 HL 250*10 mm (Knauer 3)

ACE 121-2510 S/No: A44243

ZIC-Hilic, 100 * 4.6 mm 5 µm 200 Å (Agilent 5)

SeQuant; Serial No. 934340; Sorbent

Zorbax Bonus RP, 5 µm 9.4 mm*250 mm (Knauer 3)

S/N: USAYB01016

[0215] Eluents:

A: 1 mM K_2HPO_4 in water (Agilent 7)

B: Acetonitrile

A: water (Knauer 3)

B: Acetonitrile

A: H_2O + 0.1 % trifluoroacetic acid (Agilent 3)

B: Acetonitrile + 0.1 % trifluoroacetic acid

A: 0.1 M ammonium formate pH 3.2 (Agilent 5)

B: Acetonitrile

[0216] Gradient Flow:

ZIC HILIC (agil.5) 2 mL/min		ACE 3µ (agil.7) 2 mL/min	
00:00	75%B	00:00	05%B
05:00	75%B	07:00	95%B
06:00	10%B	07:10	100%B
08:00	10%B	08:80	100%B
09:00	75%B	09:00	05%B
11:00	75%B	12:00	05%B

prep ACE (Knauer 3) 4ml/min		prep Zorbax (Knauer 3) 4 mL/min	
isocratic 80%B		00:00	05%B
		02:00	05%B
		22:00	50%B

[0217] A QMA SepPak (SepPak QMA light Waters PN: WAT023525) was preconditioned with 5 mL 0.5M K_2CO_3, washed with 10 mL water, and air pushed through to

dry. 600 µL of F-18 (Na[^{18}F] (E&Z EURO-PET Berlin GmbHz)) was passed through a QMA and air pushed through to dry. The F-18 from the QMA was eluted with 600 µL $TBAHCO_3$ mixture and put into a Vial (8 µL $TBAHCO_3$ (w=40% in water) in 600 µL acetonitrile/H_2O 1:1)[4158 MBq].

[0218] This vial was heated under gentle N_2 stream for 10 min at 140°C (heating block temperature) and 1 mL acetonitrile was added to the vial. The vial was then heated under gentle N_2 stream for 5 min at 140 °C (heating block temperature) and to cooled at room temperature over 5 min. in a lead pig (3613MBq =97.94% decay corrected).

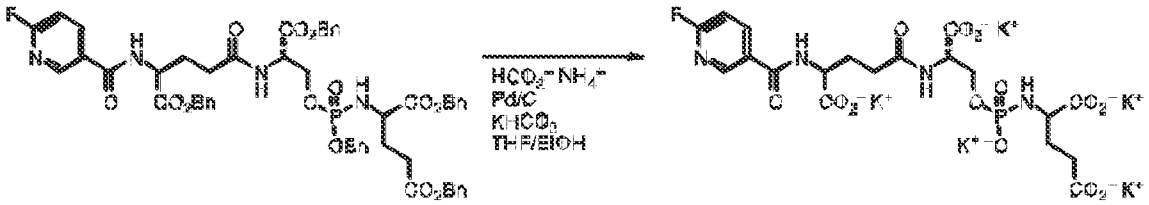
[0219] Precursor (2 mg) in DMSO (300 µL) was added to the dried [^{18}F]fluoride complex and the vial was incubated for 10 min. at 70 °C. The reaction mixture was transferred into 4 mL water, the vial washed with 500 µL acetonitrile and this was transferred into the same aqueous solution (2347 MBq) and this solution was purified by preparative HPLC, collecting the main peak: (304.3 MBq). The collected fraction was diluted with 25 mL water and passed through the C18 light (SepPak C18 light (Waters); preconditioned with 5 mL Ethanol and 10 mL water). The SPE was washed with 5 mL water, eluted with 1 mL acetonitrile, and the eluent passed through a Dry SPE (258.2 MBq).

[0220] *Deprotection* (trifluoroacetic acid/1,2-dichloroethane):

[0221] The ethanol solution was concentrated under gentle N_2-stream for 10 min. at 60°C. 100 µL trifluoroacetic acid, 100 µL 1,2-dichloroethane, and 50 µL triethylsilane were added and the solution maintained for 10 min at 100°C. The solution was concentrated under gentle N_2-stream for 10 min. at 80°C, and redissolved in 250µL PBS. The PBS solution was diluted with 4 mL PBS and purified by prep HPLC, collecting the main peak (60.83 MBq). The collected fraction was diluted the fraction with 15 mL 0.02 M K_2CO_3 and loaded on QMA (preconditioned with 5 mL methanol and 10 mL 0.02 M K_2CO_3). The QMA was washed with 2 mL water (QMA: 41.11 MBq) and product eluted with 0.3 mL of 0.5M NaCl into 0.2 mL PBS buffer (38.52 MBq).

Example 6 Benzyl deprotection

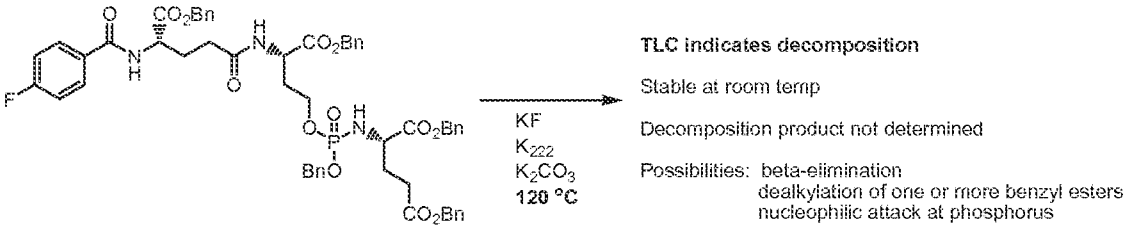
[0222] The following procedure represents a typical deprotection protocol for benzyl protected precursors.



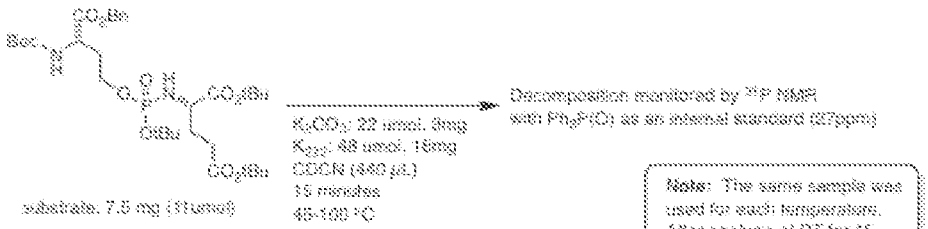
[0223] The starting material (8.9 mg, 0.00875 mmol) was dissolved in a mixture of tetrahydrofuran (2 drops) and ethanol (400 µL). To the reaction vessel was added a 200 µL (0.00256 mmol Pd) of a suspension of Pd/C in ethanol (10.9 mg 10 % Pd/C in 800 µL ethanol), 20 µL (0.044 mmol) of an aqueous $KHCO_3$ solution (2.17 M; 54.3 mg/250 µL), and a solution of ammonium formate (31 mg/200 µL water, 0.49 mmol). The reaction was stirred open (no cap or septa) at room temperature for 20 min. Consumption of starting material was monitored by TLC. The reaction mixture was then filtered though a 0.2 µm PTFE Whatman disc and the filter was washed with 10 mL of an ethanol:water (9:1 vol:vol ratio) solution. The filtrate and wash solutions were combined and the solvents were removed *in vacuo*.

Example 7 Labeling Stability Studies

[0224] The stability of the hCTT54 core was examined at conditions typical for 18F labeling. Elevated temperatures and basic conditions lead to decomposition of benzyl protected scaffold, presumably through the loss of a benzyl protecting group or cleavage of a phosphorus-homoserine oxygen bond.



[0225] To increase the scaffold stability under radiolabeling conditions, the tBu protecting group was examined. Due to the acidic pH stability noted above, the tBu protecting group was considered a viable protecting group. It was noted that the hCTT54 scaffold was stable when treated to labeling conditions with tBu protecting groups suggesting a steric block to the decomposition mechanism at work with the benzyl protecting groups.

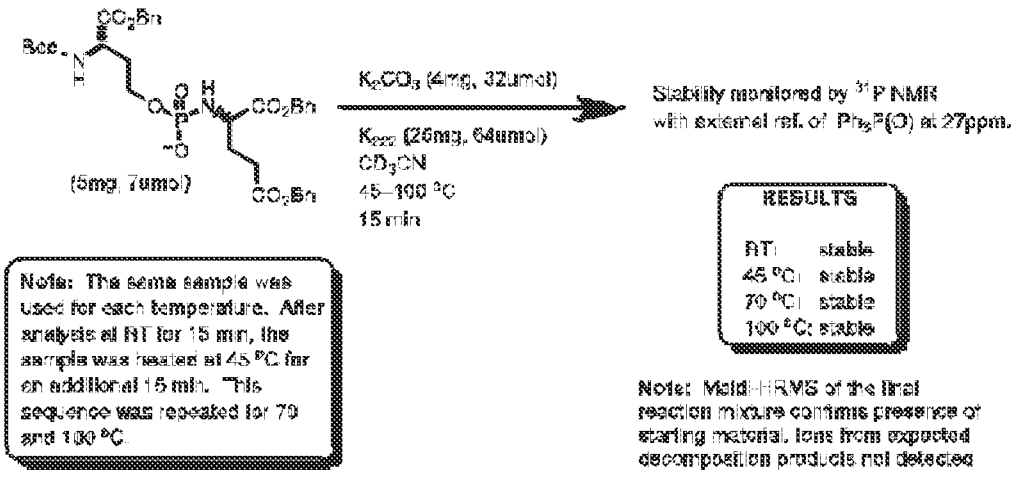


RESULTS		
	^{31}P NMR peaks	Stable
RT	2.99 & 2.87	Yes
45 °C	2.86 & 2.84	Yes
70 °C	2.84 & 2.82	Yes
100 °C	2.81 & 0.13	Some decomposition was observed

TLC (C18 and silica) showed the substrate was present and no major decomposition products observed.

[0226] ^{31}P NMR data: The two resonances reported represent two stereoisomers, unresolved at phosphorus.

[0227] In order to determine if the phosphorus was a problematic center in the radiolabeling conditions with the benzyl protecting groups, the following compound was prepared to block nucleophilic attack on the phosphorus center by having it deprotected. It was noted that this model compound was stable to radiolabeling conditions supporting the hypothesis that the phosphorus center was sensitive to the radiolabeling conditions, which could be circumvented using sterically bulky protecting groups (tBu) or by imparting a negative charge on the phosphorus center through deprotection prior to radiolabeling conditions.



Biological Example 1 ***In Vitro*** **Studies – Potency and Reversibility of Binding to PSMA**

[0228] The IC_{50} for CTT-54 was 12 nM using PSMA isolated from LNCaP cells as a membrane fraction. This was consistent with the IC_{50} value of 14 nM that was obtained using purified PSMA. Methods for IC_{50} determinations with purified PSMA or PSMA from the cell membrane fraction of LNCaP cells are described below.

[0229] *IC_{50} determination.* Inhibition studies were performed as described previously with only minor modifications (1-3). Working solutions of the substrate N-[4-(phenylazo)-benzoyl]-glutamyl-γ-glutamic acid (PABGgG) and inhibitor were made in TRIS buffer (50 mM, pH 7.4). Working solutions of purified PSMA or PSMA from the cell membrane fraction of LNCaP cells were diluted in TRIS buffer (50 mM, pH 7.4 containing 1% Triton X-100) to provide from 15% to 20% conversion of substrate to product in the absence of inhibitor. A typical incubation mixture (final volume 250 µL) was prepared by the addition of either 25 µL of an inhibitor solution or 25 µL TRIS buffer (50 mM, pH 7.4) to 175 µL TRIS buffer (50 mM, pH 7.4 containing 1% Triton X-100) in a test tube. PABGgG (25 µL, 10 µM) was added to the above solution. The enzymatic reaction was initiated by the addition of 25 µL of the PSMA working solution. In all cases, the final concentration of PABGgG was 1 µM while the enzyme was incubated with five serially diluted inhibitor concentrations providing a range of inhibition from 10% to 90%. The reaction was allowed to proceed for 15 min with constant shaking at 37°C and was terminated by the addition of 25 µL methanolic trifluoroacetic acid (2% trifluoroacetic acid by volume in methanol) followed by vortexing. The quenched incubation mixture was quickly buffered by the addition of 25 µl K_2HPO_4 (0.1 M), vortexed, and centrifuged (10 min at 7.000g). An 85 µL aliquot of the resulting supernatant was subsequently quantified by HPLC as previously described (see Anderson et al. Substrate specificity of prostate-specific membrane antigen. Bioorg Med Chem. 2007;15(21):6678-86). IC_{50} values were calculated using KaleidaGraph 3.6 (Synergy Software).

Biological Example 2 **PET imaging studies in tumor-bearing rodents**

[0230] The goal of the study was to demonstrate proof of concept for the novel homoserine class of ^{18}F labeled phosphoramidate molecules *in vivo*. For this purpose, three compounds of the homoserine class (SFB-hCTT54, SFN-hCTT54, and SCLFN-hCTT54) were tested in nude mice bearing human tumor xenografts (LNCaP, 22RV1) and compared to

SFB-CTT54. All compounds tested were produced by indirect radiolabeling procedures described above.

[0231] **Methods**. All animal experiments were performed in compliance with the current version of the German law concerning animal protection and welfare. PET imaging and biodistribution experiments were performed with tumor bearing mice. Male Balb/c nude mice (Taconic, Ry, Denmark or Janvier, Le Genest-Saint-Isle, France) were used for subcutaneous inoculation of human xenografts. Female NMRI nu/nu mice were obtained from Taconic, Ry, Denmark and used for intracardiac injection of 786-O-luc tumor cells.

[0232] *LNCaP.* Mice (BALB/c nu/nu, provided by Taconic or Janvier, male, 7-8 weeks), were inoculated ~4 weeks before the PET study by s.c. injection of 1x107 LNCaP cells (human prostate cancer) in a volume of 100µL Matrigel into the right shoulder and allowed to grow for approximately four weeks. 2-4 days before inoculation of the tumor cells, a testosterone pellet (Innovative Research of America, Cat.# NA-151) was implanted.

[0233] *22RV1.* Mice (NMRI nu/nu, provided by Taconic, male, ~30g), were inoculated ~3 weeks before the PET study by s.c. injection of $5x10^6$ 22Rv1 cells (human prostate cancer) in a volume of 100µL Matrigel into the right shoulder.

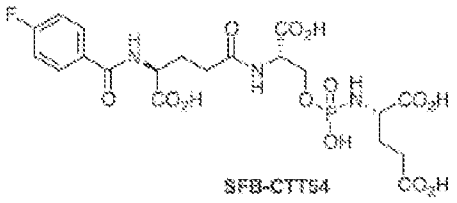
[0234] *786-O-luc.* Mice (NMRI nu/nu, provided by Taconic, male, ~30g), were inoculated ~3 weeks before the PET study by intracardiac injection of $1x10^5$ 786-O-luc cells (human kidney cancer) in a volume of 100µL PBS (for details refer to Strube et al. Clin Exp Metastasis. 2010 May;27(5):319-30).

[0235] Tumor carrying animals were injected intravenously, without anesthesia, into the tail vein with 7-10 MBq of different PET tracers in 100-200 µL PBS buffer. Immediately prior to the initiation of the micro PET measurement, the animals were anesthetized with isoflurane. PET/CT imaging studies were performed using the Inveon small animal PET/CT scanner (Siemens, Knoxville, TN). PET data were acquired from 50 - 70 min post injection. After completion of data acquisition, the animals were sacrificed, and tissues of interest were collected and weighed. The amount of radioactivity was determined with a gamma-counter to calculate uptake as the percentage injected dose per gram of tissue (%ID/g) (biodistribution data, see Table 2).

[0236] In addition, PET/CT images were analyzed by defining regions of interest (ROI) and quantifiying tracer uptake (%ID/g, mean averaged over time) by the instrument software.

[0237] **Results.** A summary of microPET/CT studies is provided in **Table 1**. Exemplified images from the studies and a summary of biodistribution data are provided in **Table 2** and Figures 1-8, respectively.

[0238] To allow for comparison of several candidate tracers with SFB-CTT54:



,

it was decided to restrict the initial experimental series to single time point (50-70 min p.i.), static PET analyses. Therefore, all experiments with novel PET tracers were initially carried out in LNCaP bearing nude mice at 50-70 min post injection (p.i.) for which good imaging quality for SFB-CTT54 had been obtained previously.

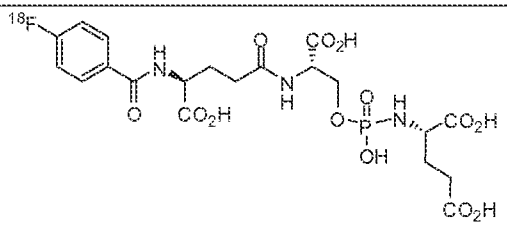
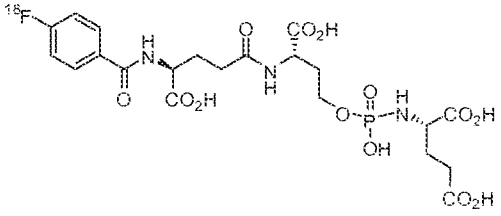
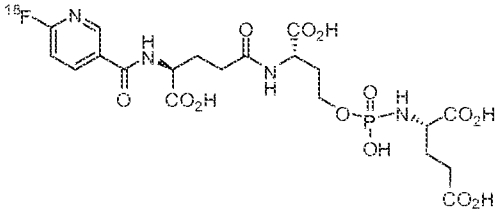
[0239] In a first series of experiments (10283, 10284, 10285), SFB-CTT54 was compared to SFB-hCTT54 and SClFN-hCTT54. The image quality obtained with SFB-CTT54 (10283) in LNCaP bearing animals was comparable to previous experiments (*see*. Lapi et al. 2009). LNCaP tumors were well visualized whereas significant tracer uptake into other organs was only recorded for kidney and bladder (**Figure 1**). SFB-hCTT54 (**Figure 2**) and SFN-hCTT54 (data not shown) were tested in 10284 and 10285, respectively, and resulted in image quality comparable to SFB-CTT54 (**Figure 2, Figure 3**). However, quantitative comparison was not possible because separation of the UV inactive precursor from the final radiopreparation had not been assessed. Since the precursor has high affinity for PSMA, large precursor quantities in the final radiopreparation may have resulted in blocking of tracer binding. In all subsequent experiments (except 11215) precursor separation was confirmed by Corona detection methodology.

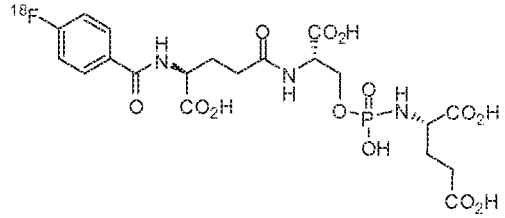
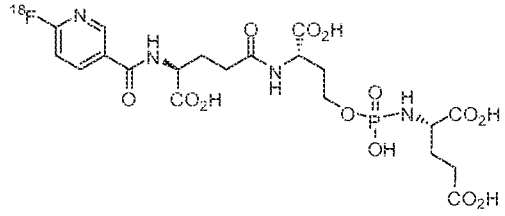
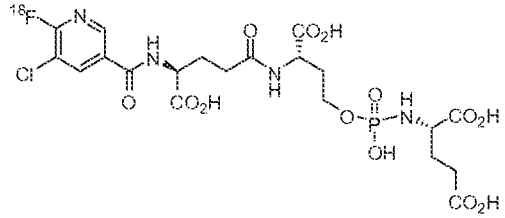
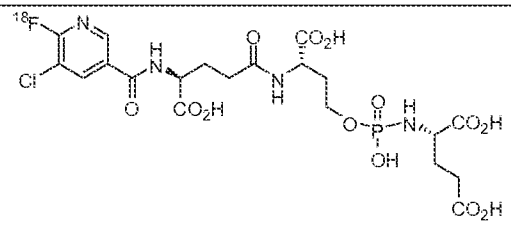
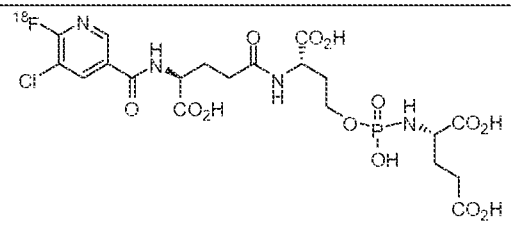
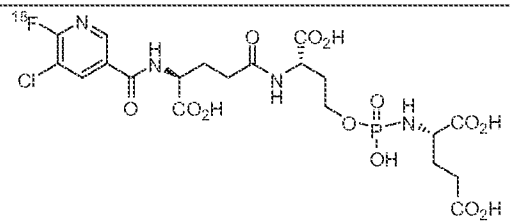
[0240] Findings from the first series of experiments were reproduced in a second series of experiments (11001, 11003, 11004, 11109), whereby SFN-hCTT54 and SClFN-hCTT54, respectively, were compared to SFB-CTT54. Tumor uptake of the novel PET tracers SFN-hCTT54 and SClFN-hCTT54 was comparable to SFB-CTT54. However, SClFN-hCTT54 and in particular SFN-hCTT54 showed lower background uptake and consequently higher tumor to blood ratios, overall resulting in improved imaging quality of the novel tracers. The most promising results in this series were obtained with SFN-hCTT54 which showed good tumor uptake (mean 2.8 ± 1.0 %ID/g), excellent tumor to blood ratios (mean 52.9 ± 15.5), and low uptake into bone (mean 0.1 ± 0.090 %ID/g) pointing to little defluorination and high stability of the compound.

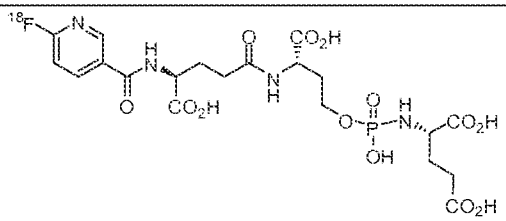
[0241] SFN-hCTT54 and SClFN-hCTT54 were tested in 22RV1 xenografts (11168, 11215). PSMA expression in 22RV1 xenografts was reported to be considerably lower compared to LNCaP xenografts (Regino et al. Curr Radiopharm. 2009 Jan;2(1):9-17). Nevertheless, imaging of 22RV1 tumors was possible with both compounds, although, as expected, tumor uptake values were lower compared to LNCaP xenografts.

[0242] An exploratory experiment was performed in 786-O xenografts to assess the specificity of SClFN-hCTT54 in bone lesions. 786-O cells originated from a renal cell carcinoma and show no expression of PSMA based on mRNA and protein level. Upon intracardiac injection of 786-O cells osteolytic bone metastases form with high penetrance. As expected, low uptake of SClFN-hCTT54 was found which might result from residual fluoride in the radiopreparation and/or defluorination of the compound (see **Figure 8**).

[0243] Table 1: Summary of microPET/CT studies in tumor bearing nude mice.

Study #	**Compound**	**Rad. Yield [%] d.c.**	**Rad. Purity [%]**	**Residual fluoride [%]**	**Tumor (# animals)**
10283	 SFB-CTT54	26.8	98.2	1.8	LNCaP (3)
10284	 SFB-hCTT54	19.2	98.7	1.3	LNCaP (3)
10285	 SFN-hCTT54	2.2	100	0	LNCaP (1)

Study #	Compound	Rad. Yield [%] d.c.	Rad. Purity [%]	Residual fluoride [%]	Tumor (# animals)
11001	 SFB-CTT54	2.46 1.2 1.2	86.5 100 96.6	0 0 0	LNCaP (3)
11003	 SFN-hCTT54	4.3 2.8	100 100	0 0	LNCaP (3)
11004	 SClFN-nCTT54	1.1	100	0	LNCaP (3)
11084	 SClFN-nCTT54	1.5	100	0	786-O (2)
11109	 SClFN-nCTT54	2.5	100	0	LNCaP (4)
11168	 SClFN-nCTT54	2	100	0	22RV1 (3)

Study #	Compound	Rad. Yield [%] d.c.	Rad. Purity [%]	Residual fluoride [%]	Tumor (# animals)
11215	 SFN-hCTT54	1.1	100	0	22RV1 (3)

[0244] Table 2: Biodistribution data (mouse strain Balb/c nu; T/B: tumor to blood ratio)

Study #	PET Tracer	Animal	Kidney	Liver	Femur	Tumor	Blood	T/B
10283	SFB-CTT54	1	7.90	0.55	0.40	3.19	0.40	7.98
		2	5.80	0.50	0.67	3.74	0.32	11.69
		3	9.40	0.50	0.97	3.27	0.35	9.34
10284	SFB-hCTT54	1	5.50	0.42	1.24	1.85	0.26	7.12
		2	3.70	0.40	0.06	2.17	0.21	10.33
		3	4.00	0.50	0.08	1.97	0.31	6.35
10285	SFN-hCTT54	1	*	*	*	*	*	*
11001	SFB-CTT54	1	7.20	0.88	0.22	2.33	0.84	2.77
		2	4.40	0.50	0.34	5.24	0.43	12.19
		3	9.20	0.80	1.31	5.04	0.59	8.54
11003	SFN-hCTT54	1	15.40	0.20	0.20	3.86	0.10	38.60
		2	*	*	*	*	*	*
		3	*	*	*	*	*	*
11004	SCIFN-nCTT54	1	17.40	0.30	0.50	4.12	0.14	29.43
		2	*	*	*	*	*	*
		3	*	*	*	*	*	*
11084	SCIFN-nCTT54	1	15.49	*	*	*	0.28	*
		2	21.92	*	*	*	0.07	*
11109	SCIFN-nCTT54	1	9.40	0.10	0.55	2.08	0.10	20.80
		2	4.30	0.10	0.37	1.60	0.11	14.55
		3	5.00	0.09	0.42	1.96	0.09	21.78
		4	3.80	0.10	0.48	1.68	0.11	15.27

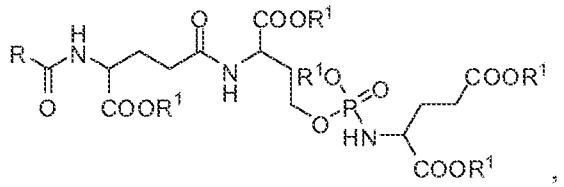
[00100] Table 3: Biodistribution data (mouse strain NMRI nu; T/B: tumor to blood ratio)

Study #	PET Tracer	Animal	Kidney	Liver	Femur	Tumor	Blood	T/B
11003	SFN-hCTT54	1	5.50	0.11	0.17	1.94	0.04	50.79
		2	5.00	0.10	0.03	2.64	0.04	69.29
		3	*	*	*	*	*	*
11168	SCIFN-nCTT54	1	15.20	0.10	2.80	2.23	0.12	18.58
		2	12.60	0.10	0.60	2.19	0.13	16.85
		3	12.80	0.10	0.50	1.42	0.06	23.67

Study #	PET Tracer	Animal	Kidney	Liver	Femur	Tumor	Blood	T/B
11215	SFN-hCTT54	1	9.80	0.30	0.10	1.42	0.10	1.420
		2	8.60	0.20	0.20	1.09	0.08	13.63
		3	6.80	0.20	0.10	0.93	0.22	4.23

We claim:

1. A compound that is in the form of formula (I),



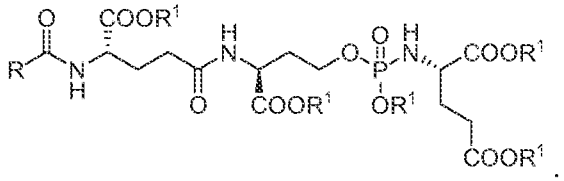
(I)

or the form of a pharmaceutically acceptable salt thereof, wherein

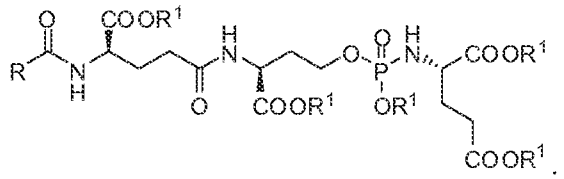
R is phenyl or pyridyl, each substituted with either one [^{18}F]-fluoro group or one [^{19}F]-fluoro group and optionally substituted with a second group selected from the group consisting of chloro and cyano; and

each R^1 is independently hydrogen or a protecting group.

2. The compound of claim 1 of structure



3. The compound of claim 1 of structure



4. The compound of claim 1 that is

(*N*-(3-cyano-4-[^{18}F]fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-dicarboxypropyl]amino}(hydroxy)phosphoryl]-L-homoserine;

(*N*-(4-[^{18}F]fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-dicarboxypropyl]amino}(hydroxy)phosphoryl]-L-homoserine;

(*N*-(6-[^{18}F]fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-dicarboxypropyl]amino}(hydroxy)phosphoryl]-L-homoserine;

(*N*-(5-chloro-6-[^{18}F]fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-dicarboxypropyl]amino}(hydroxy)phosphoryl]-L-homoserine;

or a pharmaceutically acceptable salt thereof.

5. The compound of claim 1 that is

t-butyl (*O*-*t*-butyl-*N*-(3-cyano-4-[^{18}F]fluorobenzoyl)-L-γ-glutamyl)-O-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine;

benzyl (*O*-benzyl-*N*-(3-cyano-4-[^{18}F]fluorobenzoyl)-L-γ-glutamyl)-O-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine;

benzyl (*O*-benzyl-*N*-(4-[^{18}F]fluorobenzoyl)-L-γ-glutamyl)-O-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine;

t-butyl (*O*-*t*-butyl-*N*-(4-[^{18}F]fluorobenzoyl)-L-γ-glutamyl)-O-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine;

benzyl (*O*-benzyl-*N*-(5-chloro-6-[^{18}F]fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-O-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine;

t-butyl (*O*-*t*-butyl-*N*-(5-chloro-6-[^{18}F]fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-O-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine;

t-butyl (*O*-*t*-butyl-*N*-(6-[^{18}F]fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-O-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine;

benzyl (*O*-benzyl-*N*-(6-[^{18}F]fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-O-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine;

or a pharmaceutically acceptable salt thereof.

6. The compound of claim 1 that is

(*N*-(3-cyano-4-fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-dicarboxypropyl]amino}(hydroxy)phosphoryl]-L-homoserine;

(*N*-(4-fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-dicarboxypropyl]amino}(hydroxy)phosphoryl]-L-homoserine;

(*N*-(6-fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-dicarboxypropyl]amino}(hydroxy)phosphoryl]-L-homoserine;

(*N*-(5-chloro-6-fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-dicarboxypropyl]amino}(hydroxy)phosphoryl]-L-homoserine;

or a pharmaceutically acceptable salt thereof.

7. The compound of claim 1 that is

t-butyl (*O*-*t*-butyl-*N*-(3-cyano-4-fluorobenzoyl)-L-γ-glutamyl)-O-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine;

benzyl (*O*-benzyl-*N*-(3-cyano-4-fluorobenzoyl) -L-γ-glutamyl)-O-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine;

benzyl (*O*-benzyl-*N*-(4-fluorobenzoyl) -L-γ-glutamyl)-O-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine;

t-butyl (*O*-*t*-butyl-*N*-(4-fluorobenzoyl)-L-γ-glutamyl)-O-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine;

benzyl (*O*-benzyl-*N*-(5-chloro-6-fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-O-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine;

t-butyl (*O*-*t*-butyl-*N*-(5-chloro-6-fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-O-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine;

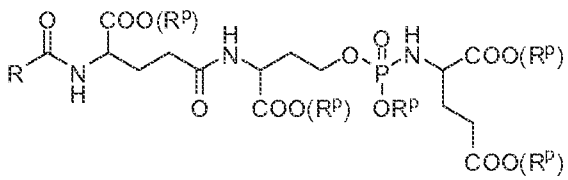
t-butyl (*O*-*t*-butyl-*N*-(6-fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-O-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine;

benzyl (*O*-benzyl-*N*-(6-fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-O-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine;

or a pharmaceutically acceptable salt thereof.

8. A composition comprising a compound of claim 1, wherein R^1 is hydrogen, or a compound of claim 2, together with a pharmaceutically acceptable carrier, excipient, and/or diluent.

9. A compound of formula (II),



(II)

wherein

R is phenyl or pyridyl, each substituted with one leaving group and optionally substituted with a second group selected from the group consisting of chloro and cyano;

and each R^P is a protecting group (e.g., t-butyl or benzyl).

10. The compound of claim 9 that is

benzyl (*O*-benzyl-*N*-(5,6-dichloro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-O-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine;

benzyl (*O*-benzyl-*N*-(6-chloro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-O-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine;

t-butyl (*O*-*t*-butyl-*N*-(5,6-dichloro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-O-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine;

t-butyl (O-*t*-butyl-N-(3-cyano-4-(trimethylammonio)benzoyl) -L-γ-glutamyl)-O-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine triflate;

benzyl (*O*-benzyl-*N*-(3-cyano-4-(trimethylammonio)benzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine triflate;

t-butyl (*O*-*t*-butyl-*N*-(6-chloro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine;

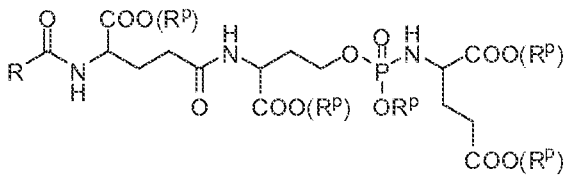
benzyl (*O*-benzyl-*N*-(6-(trimethylammonio)-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine triflate;

t-butyl (*O*-*t*-butyl-*N*-(6-(trimethyammonio)-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine triflate;

or a pharmaceutically acceptable salt thereof.

11. A method for preparing a compound comprising

deprotecting a compound of the formula,



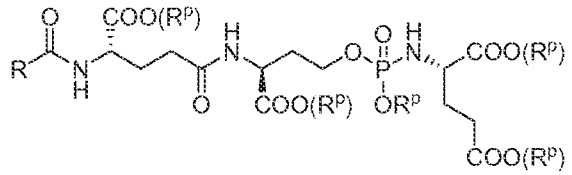
wherein

R is phenyl or pyridyl, each substituted with either one [^{18}F]-Fluoro group or one [^{19}F]-Fluoro group and optionally substituted with a second group selected from the group consisting of chloro and cyano; and

each R^P is a protecting group (e.g., t-butyl or benzyl);

under conditions suitable for removing each of the R^P groups.

12. The method of claim 11, wherein the compound is of the formula,



13. The method of claim 11, wherein the compound is

t-butyl (*O*-*t*-butyl-*N*-(3-cyano-4-[^{18}F]fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine;

benzyl (*O*-benzyl-*N*-(3-cyano-4-[^{18}F]fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine;

benzyl (*O*-benzyl-*N*-(4-[^{18}F]fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine;

t-butyl (*O*-*t*-butyl-*N*-(4-[^{18}F]fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine;

benzyl (*O*-benzyl-*N*-(5-chloro-6-[^{18}F]fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine;

t-butyl (*O*-*t*-butyl-*N*-(5-chloro-6-[^{18}F]fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine;

t-butyl (*O*-*t*-butyl-*N*-(6-[^{18}F]fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine;

benzyl (*O*-benzyl-*N*-(6-[^{18}F]fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine;

t-butyl (*O*-t-butyl-*N*-(3-cyano-4-fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine;

benzyl (*O*-benzyl-*N*-(3-cyano-4-fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine;

benzyl (*O*-benzyl-*N*-(4-fluorobenzoyl) -L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine;

t-butyl (*O*-t-butyl-*N*-(4-fluorobenzoyl)-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine;

benzyl (*O*-benzyl-*N*-(5-chloro-6-fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-

[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine;

t-butyl (*O*-*t*-butyl-*N*-(5-chloro-6-fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(t-butoxycarbonyl)propyl]amino}(t-butoxy)phosphoryl]-L-homoserine;

t-butyl (*O*-t-butyl-*N*-(6-fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(*t*-butoxycarbonyl)propyl]amino}(*t*-butoxy)phosphoryl]-L-homoserine;

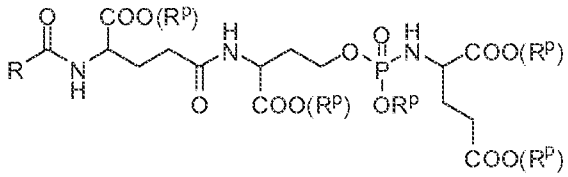
benzyl (*O*-benzyl-*N*-(6-fluoro-pyrid-3-yl)carbonyl-L-γ-glutamyl)-*O*-[{[(1S)-1,3-di(benzoxycarbonyl)propyl]amino}(benzoxy)phosphoryl]-L-homoserine;

or a pharmaceutically acceptable salt thereof.

14. The method of claim 9, wherein R^P is t-butyl and the conditions comprise contacting the compound with trifluoroacetic acid or formic acid.

15. A method for preparing a compound comprising

contacting a compound of the formula,



wherein

R is phenyl or pyridyl, each substituted with one leaving group and optionally substituted with a second group selected from the group consisting of chloro and cyano; and each R^P is a protecting group (e.g., t-butyl or benzyl);

with a fluoride or radiofluoride source.

16. A method for detecting and/or identifying cells presenting PSMA comprising contacting a cell suspected of presenting PSMA with a compound of claim 1, wherein R^1 is hydrogen, or a composition of claim 6.

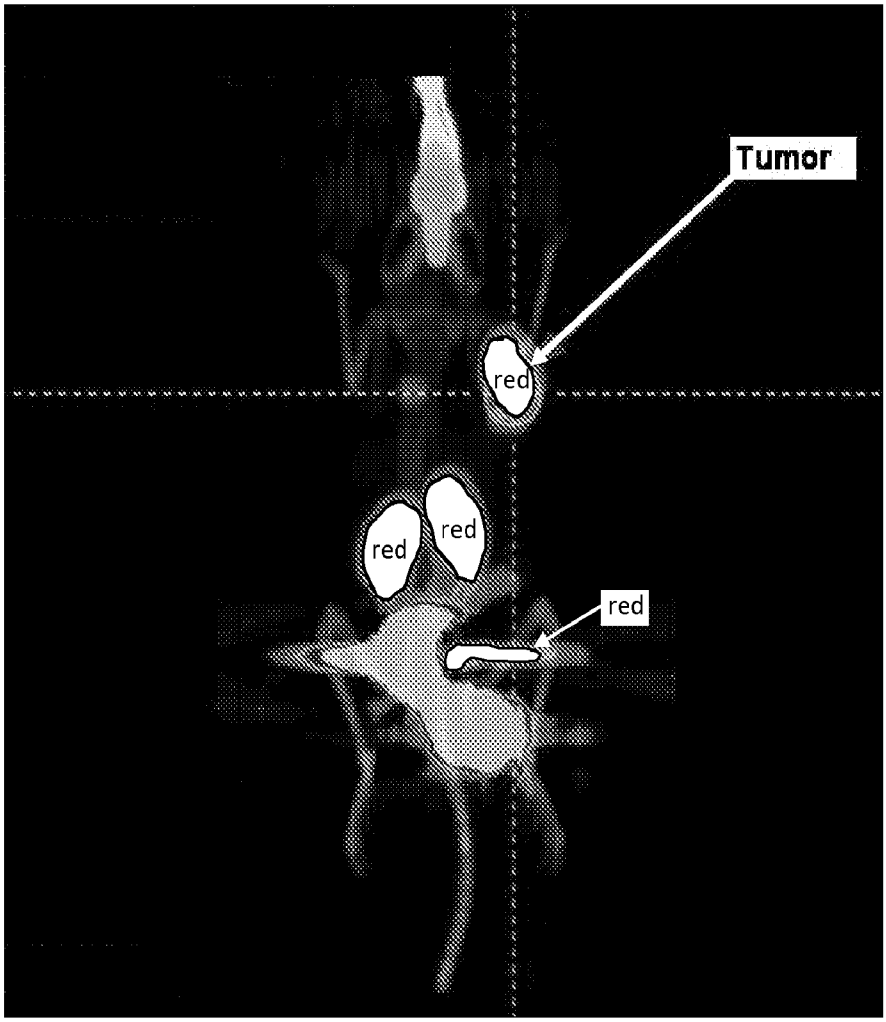
17. The method of claim 16, further comprising detecting the radiolabeled cells.

18. The method of claim 17, wherein the detecting is by positron emission tomography (PET).

19. The method of claim 17, wherein the detecting is by positron emission tomography - computed tomography (PET/CT).

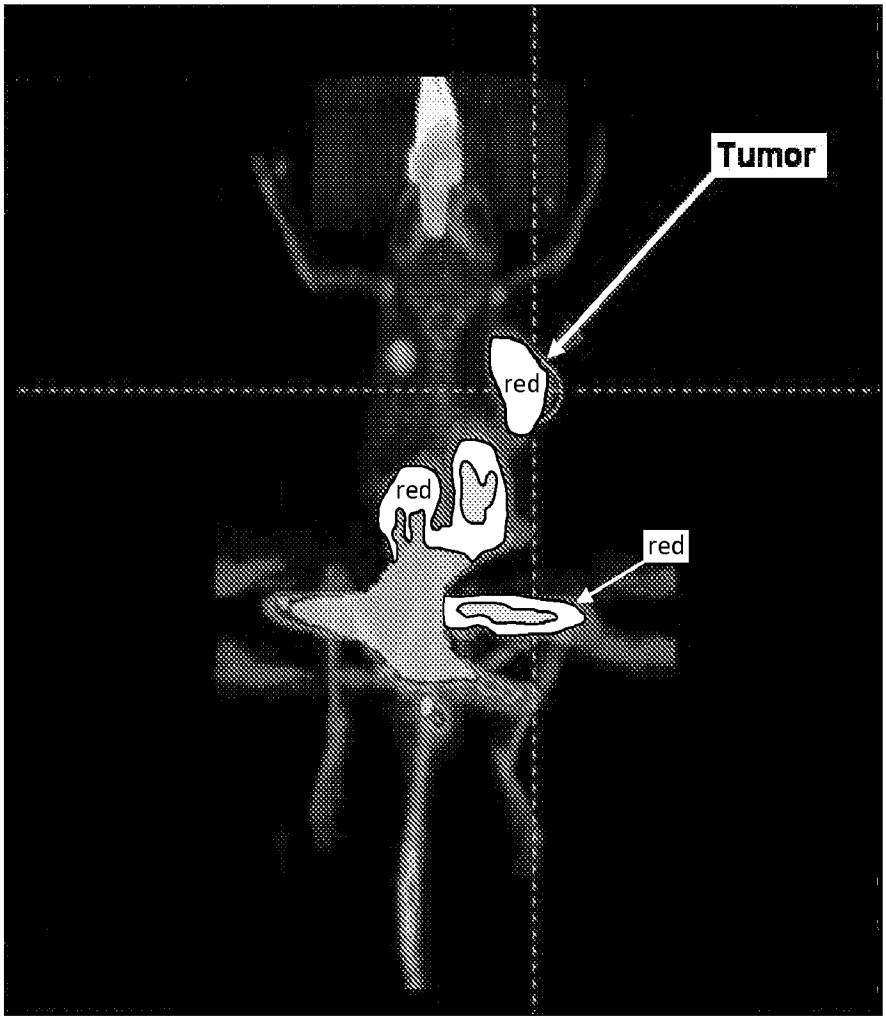
20. The method of any one of claims 16-19, wherein the cell is *in vivo*.

21. A method for imaging or diagnosing of a disease associated with elevated expression of Prostate Specific Membrane Antigen (PSMA) comprising,
administering to a mammal an effective amount of a compound claim 1, wherein R^1 is hydrogen;
obtaining images of the mammal; and
assessing the images.



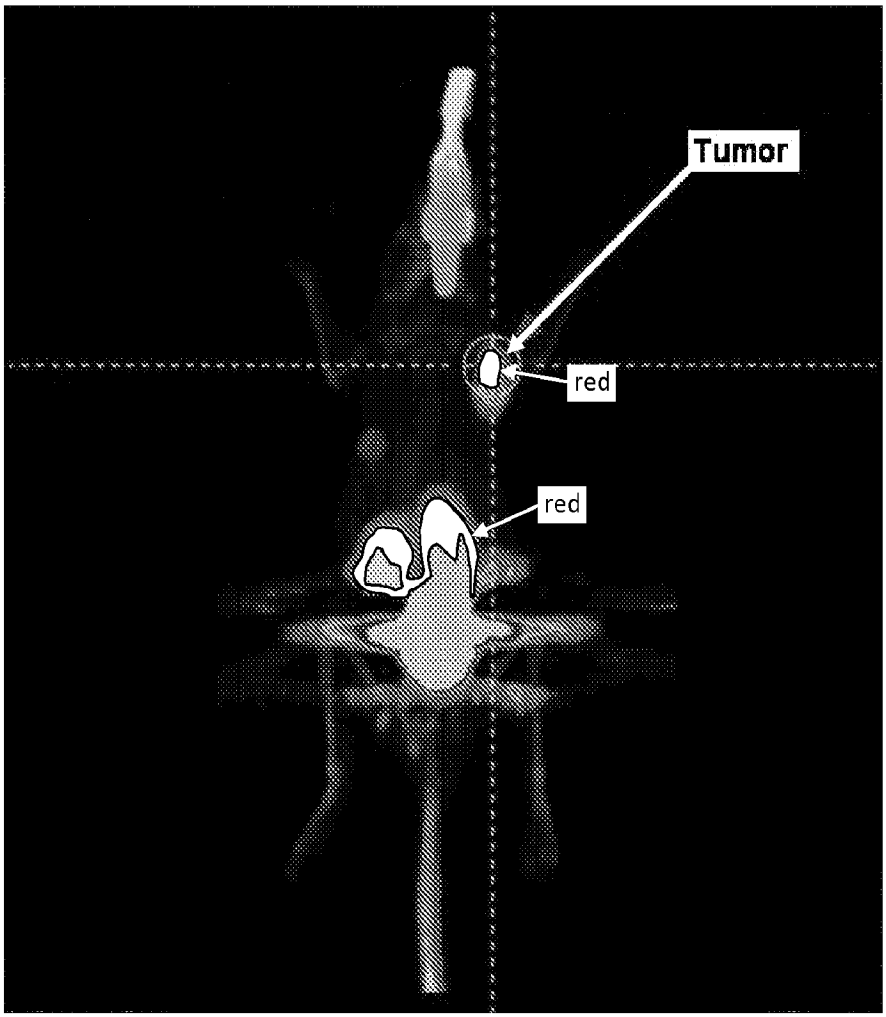
KM10283	PET Quantification %ID/g		
[18F]-SFB-CTT54	Mouse 1	Mouse 2	Mouse 3
BAY1065322	50-70 min	50-70 min	50-70 min
tumor	2,8	2,1	2,2
background	0,3	0,27	0,255
shoulder joint	0,58	0,36	0,42
kidney right	3,7	3,3	3
liver (part)	0,5	0,56	0,42

Figure 1



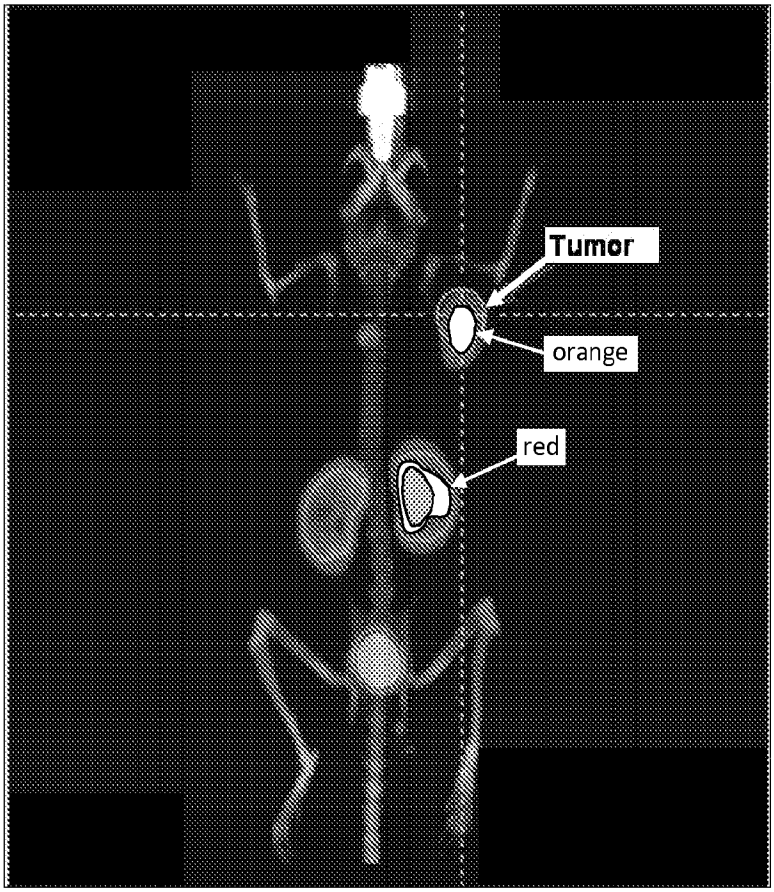
KM10284	PET Quantification %ID/g		
[18F]-SFB-hCTT54	Mouse 1	Mouse 2	Mouse 3
BAY1126798	50-70 min	50-70 min	50-70 min
tumor	1,9	1,5	1,5
background	0,14	0,08	0,17
shoulder joint	0,38	0,32	0,41
kidney right	2,9	1,9	2,2
liver (part)	0,41	0,32	0,397

Figure 2



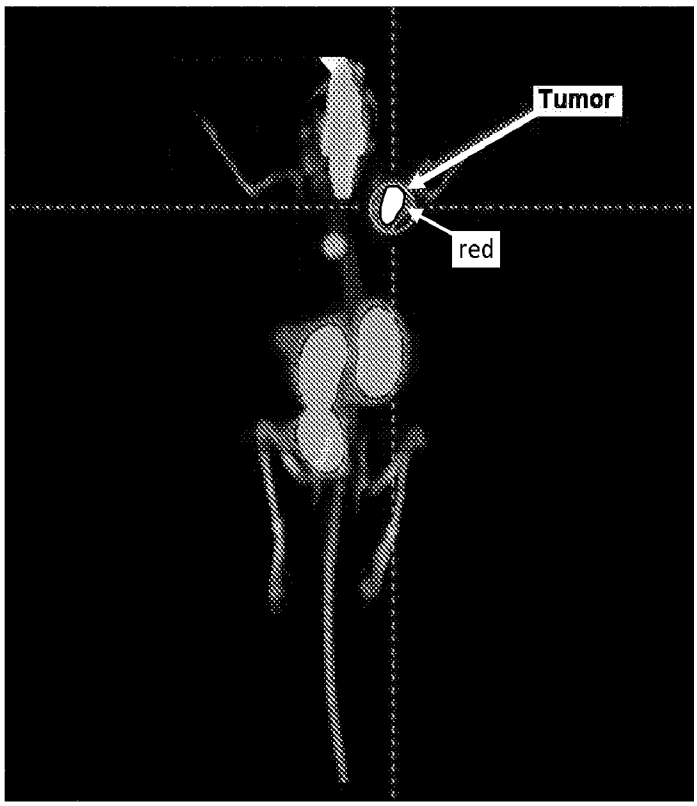
KM11001	PET Quantification %ID/g		
[18F]-SFB-CTT54	Mouse 1	Mouse 2	Mouse 3
BAY1065322	50-70 min	50-70 min	50-70 min
tumor	1,8	1,8	2,2
background	0,39	0,2	0,42
kidney right	4,2	1,8	4,7
bladder w.artifacts	50	15,7	128,4
pellet	0,79	0,46	0,55

Figure 3



KM11003 NMRI	PET Quantification %ID/g		
[18F]-SFN-hCTT54	Mouse 1	Mouse 2	Mouse 3
BAY1129022	50-70 min	50-70 min	50-70 min
tumor	1,9	1,3	0,94
kidney left	2,0	1,7	1,9
testost. pellet	0,2	0,1	0,16
KM11003 Balb/c	PET Quantification %ID/g		
[18F]-SFN-hCTT54	Mouse 1	Mouse 2	Mouse 3*
BAY1129022	50-70 min	50-70 min	50-70 min
tumor	2,2	1,5	9,4
background	0,3	0,18	1,4
kidney right	11,3	7,4	37,9
bladder w. artifacts	279,7	102,4	498,8
testost.pellet	1,3	0,63	3,2

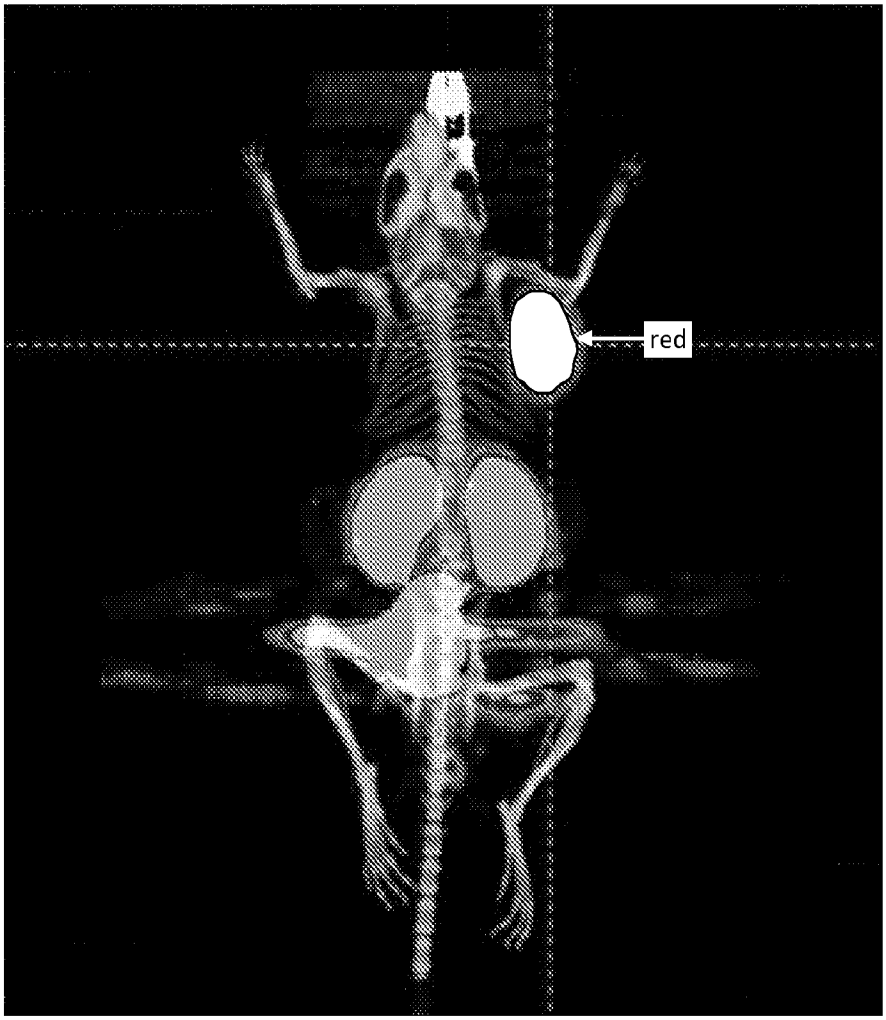
Figure 4



KM11004	PET Quantification %ID/g		
[18F]-SCIFN-hCTT54	Mouse 1	Mouse 2	Mouse 3
BAY1149526	50-70 min	50-70 min	50-70 min
tumor	2,4	0,9	2,7
background	0,2	0,1	0,3
kidney right	21,8	11,0	18,2
bladder w. artifacts	135,8	15,6	26,8
shoulder joint	nd	0,3	0,7
testost. pellet	0,7	0,7	0,5

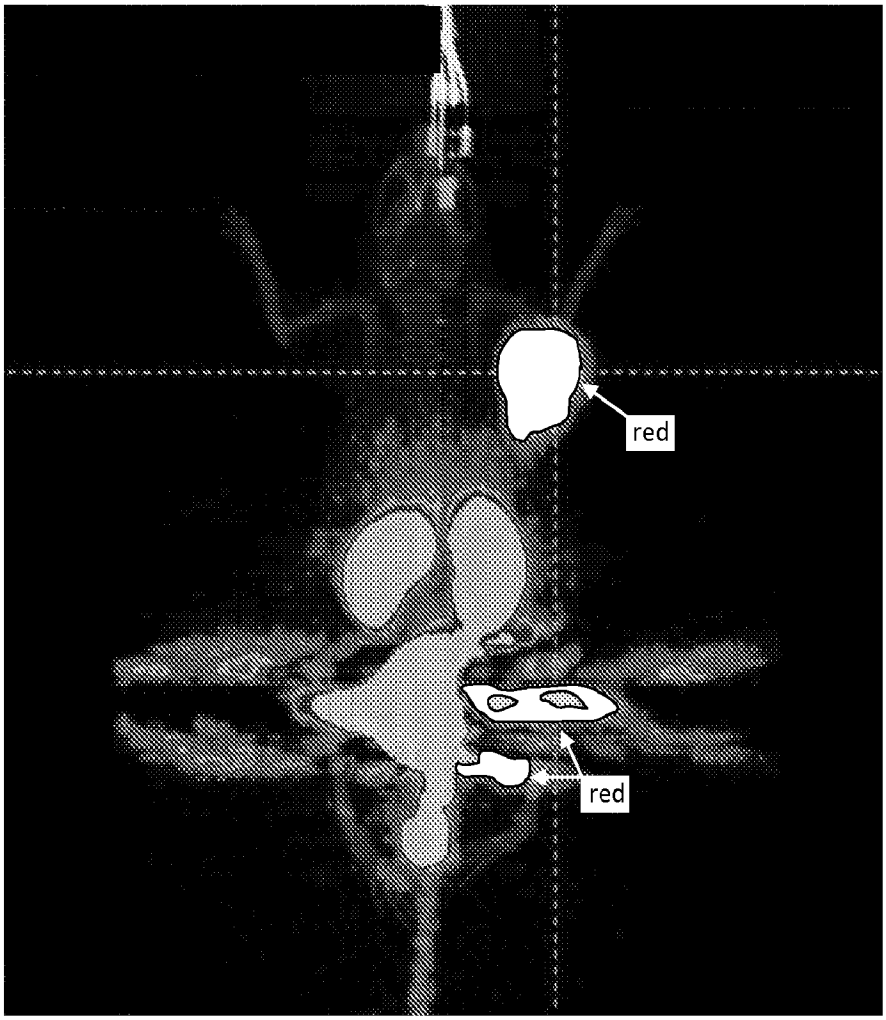
KM11009	PET Quantification %ID/g			
[18F]-SCIFN-hCTT54	M1	M2	M3	M4
BAY1149526	50-70 min	50-70 min	50-70 min	50-70 min
tumor	0,8	0,7	0,4	0,5
background	0,1	0,1	0,1	0,1
kidney right	2,9	1,9	2,2	1,6
testost. Pellet	0,3	0,2	0,3	0,2
shoulder joint	0,3	0,2	0,2	0,3
bladder	18,8	20,0	19,3	20,2

Figure 5



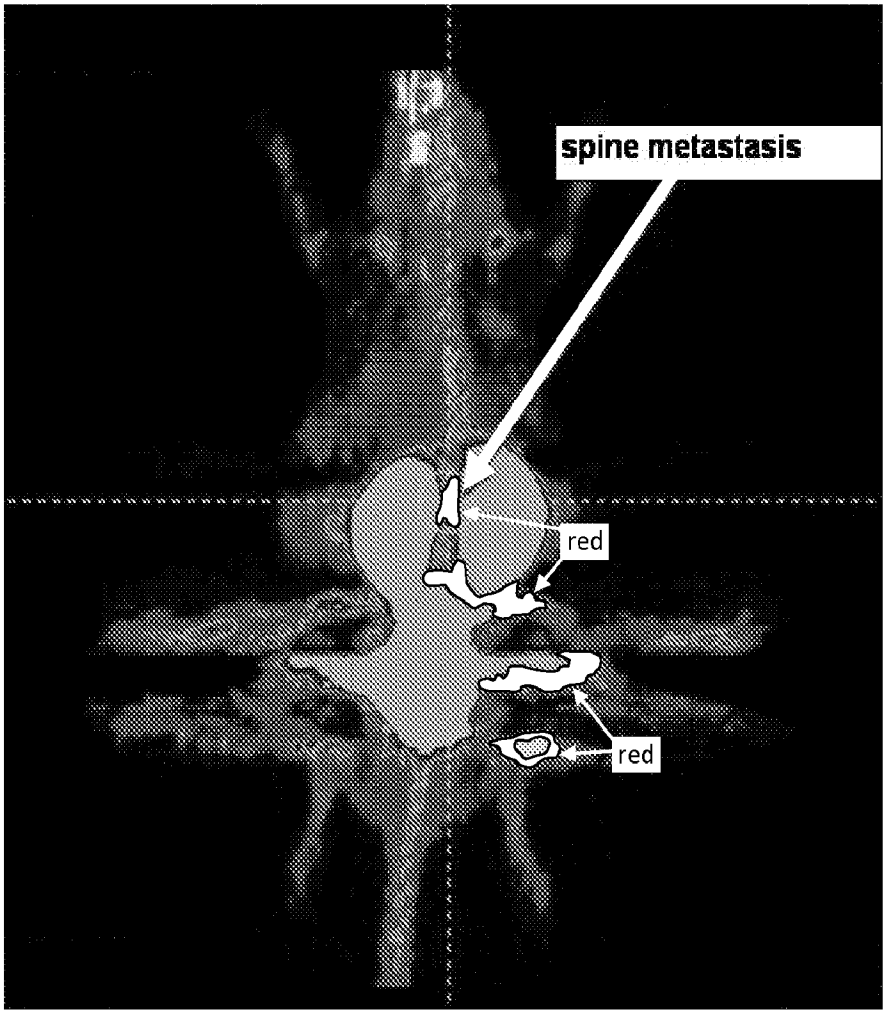
KM11168	PET Quantification %ID/g		
[18F]-SCIFN-hCTT54	Mouse 1	Mouse 2	Mouse 3
BAY1149526	50-70 min	50-70 min	50-70 min
tumor	1,8	1,6	1,2
background	0,3	0,2	0,1
kidney right	12,8	16,3	10,1
shoulder joint	0,9	0,7	0,6

Figure 6



KM11215 [18F]-SFN-hCTT54 BAY1129022	PET Quantification %ID/g		
	Mouse 1	Mouse 2	Mouse 3
	50-70 min	50-70 min	50-70 min
tumor	0,8	0,9	0,8
background	0,1	0,1	0,1
kidney right	6,6	8,5	4,7
liver (part)	0,3	0,3	0,3
bladder (artefacts)	215,3	215,2	264,0

Figure 7



KM11084	PET Quantification %ID/g	
[18F]-SCIFN-hCTT54	Mouse 1	Mouse 2
BAY1149526	50-70 min	0-60 min
spine metastasis	0,9	1,1
shoulder joint R	0,8	1,0
shoulder joint L	0,7	1,0
Background	0,2	nd
blood	nd	0,4
kidney right	12,4	8,7

Figure 8

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/041353

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07F9/58 C07F9/24 A61P13/08 A61K31/664 A61K31/675
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07F A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	S. E. LAPI ET AL: "Assessment of an 18F-Labeled Phosphoramidate Peptidomimetic as a New Prostate-Specific Membrane Antigen-Targeted Imaging Agent for Prostate Cancer", THE JOURNAL OF NUCLEAR MEDICINE, vol. 50, no. 12, 1 December 2009 (2009-12-01), pages 2042-2048, XP055034294, ISSN: 0161-5505, DOI: 10.2967/jnumed.109.066589 figure 3 compound (3)	1-21

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 June 2013	27/06/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Eberhard, Michael

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/041353

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P	WO 2012/174136 A1 (CANCER TARGETED TECHNOLOGY LLC [US]; BERKMAN CLIFFORD [US]; LANGTON-WE) 20 December 2012 (2012-12-20) paragraphs [0027], [0029], [0030] compound (B)	1-21
Y,P	WO 2012/064914 A2 (UNIV WASHINGTON STATE RES FDN [US]; UNIV CALIFORNIA [US]; BERKMAN CLIF) 18 May 2012 (2012-05-18) paragraph [0148] example 1 claim 19	1-21

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2013/041353

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012174136 A1	20-12-2012	NONE	
WO 2012064914 A2	18-05-2012	NONE	