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(54) Title: ALPHA-CELL SELECTIVE PROBE FOR EX-VIVO TWO PHOTON IMAGING OF ALPHA CELLS IN INTACT PANCREATIC ISLETS

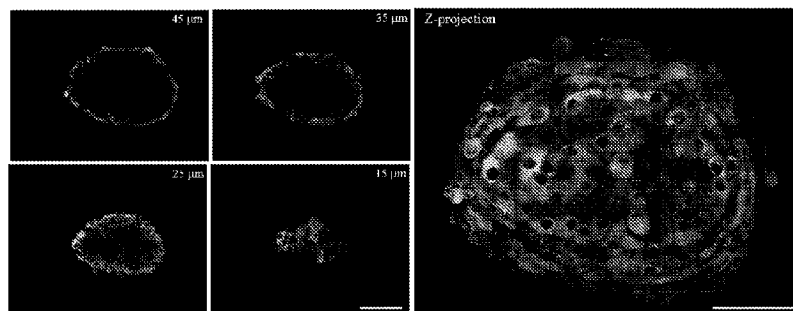
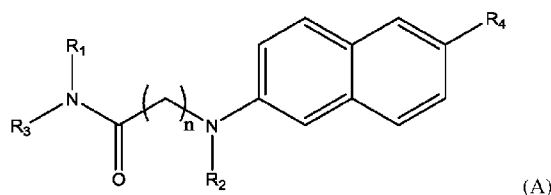


FIG. 3

(57) Abstract: The present invention relates to a composition represented by structural formula (A) [Formula should be entered here] or a salt thereof, as well as a method of visualizing a target cell, the method including (a) contacting a population of the target cell with the composition to form an incubation media, (b) incubating the incubation media for a period of time sufficient to stain the target cells, and (c) visualizing the stained target cells with two-photon microscopy to visualize the target cell.

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ALPHA-CELL SELECTIVE PROBE FOR EX-VIVO TWO PHOTON IMAGING OF ALPHA CELLS IN INTACT PANCREATIC ISLETS

RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 62/103,920, filed on January 15, 2015. The entire teaching of the above application is incorporated herein by reference.

BACKGROUND OF THE INVENTION

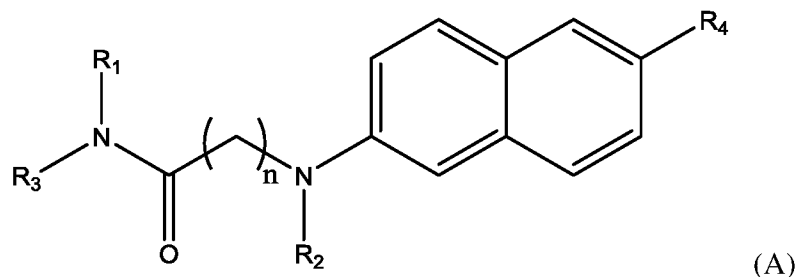
[0002] Pancreatic alpha cells secrete glucagon in response to low blood glucose level. Glucagon counter regulates the hypoglycemic effect of insulin by increasing glycogenolysis and activating hepatic gluconeogenesis. Studies in last decade show that human islet contains more alpha cells (~ 40%) than the well-studied rodent counterpart (~15%); moreover, the unique association of alpha cells with other endocrine cells in human islets has attracted more interest to study pancreatic alpha cell for understanding their function and influence on diabetes. The distribution and function of islet cells has been mostly achieved with either immunostaining or transgenic models. However, small molecule fluorescent probes offer significant advantage over these classic techniques such as, less tedious (does not require transfection unlike its protein counterpart), cheaper, easy and first staining. Two-photon (TP) imaging required two longer wavelength laser light for the excitation of the fluorophore because of longer wavelength TP microscope has deeper achievable penetration depth, no autofluorescence, and less photo bleaching; owing to these advantages, TP microscopy became a preferred technique for live deep

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tissue imaging. There is a strong demand for the development of novel two-photon fluorescent probes.

SUMMARY OF THE INVENTION

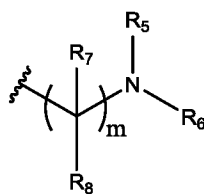
[0003] In a first aspect, the invention is a composition represented by structural formula (A)



or a salt thereof, wherein:

R₁ is (C₁-C₂₀)alkyl, (C₂-C₂₀)alkenyl, (C₂-C₂₀)alkynyl, (C₂-C₂₀)alkoxy, (C₂-C₂₀)alkylamino or (C₃-C₁₀)cycloalkyl, each of which is optionally substituted with one or more groups independently selected from (C₆-C₁₂)aryl, (5-12 atom)heteroaryl, (5-12 atom)heterocycle, -P(O)((C₆-C₁₈)aryl)₂, fused polycyclic carbocyclyl or carbamyl, further optionally substituted with one or more groups selected from halo, NO₂, oxo, (C₁-C₆)alkyl or (C₁-C₆)alkoxy;

R₂ is hydrogen, (C₁-C₆)alkyl, (C₂-C₁₀)alkenyl, (C₆-C₁₈)arylalkyl, cyclohexyl, (C₆-C₁₂)aryl or (5-12 atom)heteroaryl;



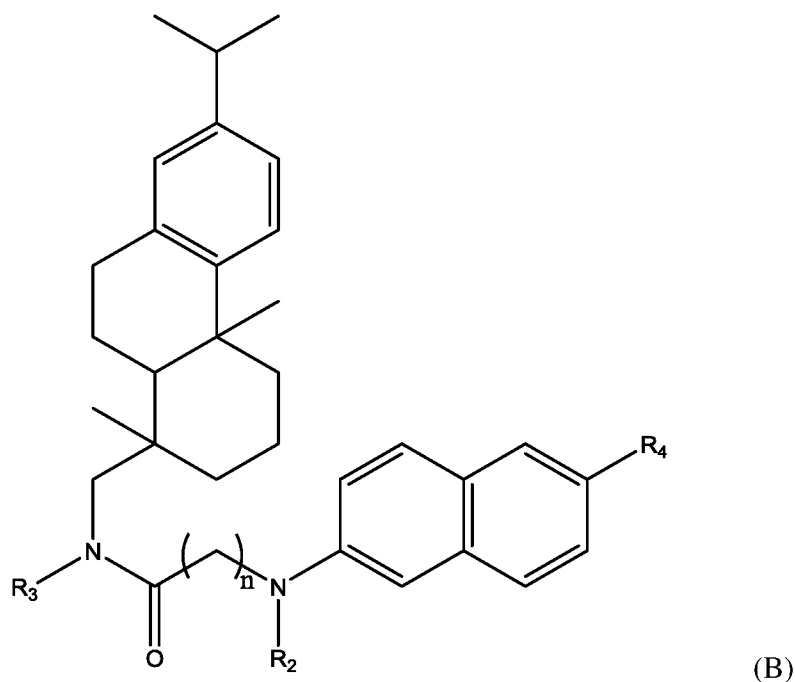
R₃ is hydrogen or , wherein R₅ and R₆ are independently hydrogen, (C₁-C₂₀)alkyl, (C₆-C₁₈)arylalkyl, cyclohexyl, (C₆-C₁₂)aryl or (5-12 atom)heteroaryl, or R₅ and R₆ taken together form a (3-6 atom)heterocycle, R₇ and R₈ are independently hydrogen or (C₁-C₆)alkyl, and m is 1 to 20;

R₄ is -C(O)R₉, -C(O)OR₉, -C(O)OH, -C(O)N(R₉)₂, -S(O)₂N(R₉)₂, -S(O)₂R₉, -NO₂, 2-benzothiazole or -C≡N, wherein R₉ is independently (C₁-C₆)alkyl, (C₂-C₁₀)alkenyl, (C₆-C₁₈)arylalkyl, cyclohexyl, (C₆-C₁₂)aryl or (5-12 atom)heteroaryl; and

n is 0 to 4.

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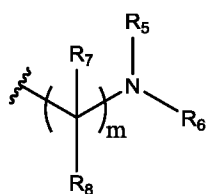
[0004] In a first example embodiment of the first aspect, the composition is represented by structural formula (B)

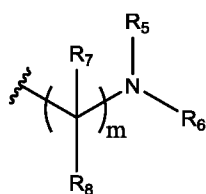


or salt thereof,

wherein:

R₂ is hydrogen, (C₁-C₆)alkyl, (C₂-C₁₀)alkenyl, (C₆-C₁₈)arylalkyl, cyclohexyl, (C₆-C₁₂)aryl or (5-12 atom)heteroaryl;



R₃ is hydrogen or , wherein R₅ and R₆ are independently hydrogen, (C₁-C₂₀)alkyl, (C₆-C₁₈)arylalkyl, cyclohexyl, (C₆-C₁₂)aryl or (5-12 atom)heteroaryl, or R₅ and R₆ taken together form a (3-6 atom)heterocycle, R₇ and R₈ are independently hydrogen or (C₁-C₆)alkyl, and m is 1 to 20;

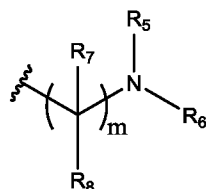
R₄ is -C(O)R₉, -C(O)OR₉, -C(O)OH, -C(O)N(R₉)₂, -S(O)₂N(R₉)₂, -S(O)₂R₉, -NO₂, 2-benzothiazole or -C≡N, wherein R₉ is independently (C₁-C₆)alkyl, (C₂-C₁₀)alkenyl, (C₆-C₁₈)arylalkyl, cyclohexyl, (C₆-C₁₂)aryl or (5-12 atom)heteroaryl; and

n is 0 to 4.

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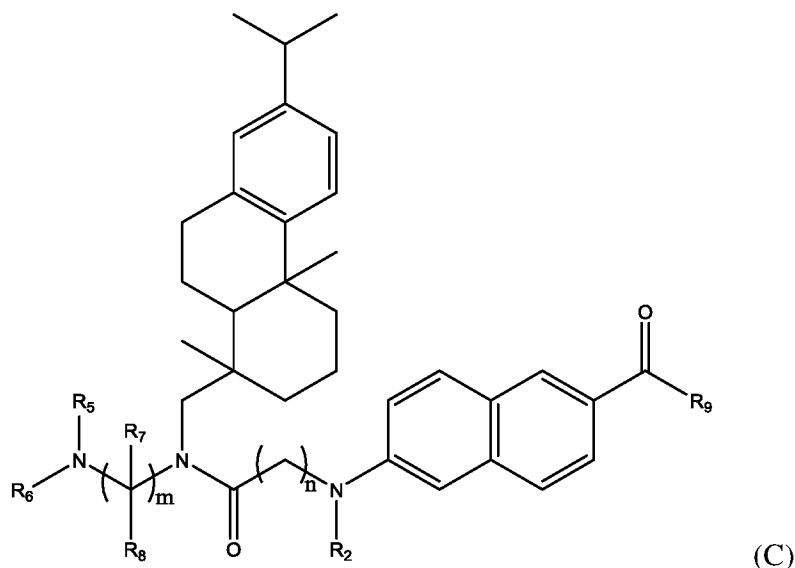
[0005] In a second example embodiment of the first aspect, R_4 of formula (B) is $-C(O)R_9$, $-C(O)OR_9$, $-C(O)N(R_9)_2$, or $-C\equiv N$, wherein R_9 is independently (C_1-C_6) alkyl, (C_2-C_{10}) alkenyl, (C_6-C_{18}) arylalkyl, cyclohexyl, (C_6-C_{12}) aryl or (5-12 atom)heteroaryl.

[0006] In a third example embodiment of the first aspect, R_3 of formula (B) is



, wherein R_5 and R_6 are independently hydrogen, (C_1-C_{20}) alkyl, (C_6-C_{18}) arylalkyl, cyclohexyl, (C_6-C_{12}) aryl or (5-12 atom)heteroaryl, or R_5 and R_6 taken together form a (3-6 atom)heterocycle, R_7 and R_8 are independently hydrogen or (C_1-C_6) alkyl, and m is 1 to 20.

[0007] In a fourth example embodiment of the first aspect, the composition is represented by structural formula (C)



or salt thereof,

wherein:

R_2 is hydrogen, (C_1-C_6) alkyl, (C_2-C_{10}) alkenyl, (C_6-C_{18}) arylalkyl, cyclohexyl, (C_6-C_{12}) aryl or (5-12 atom)heteroaryl;

R_5 and R_6 are independently hydrogen, (C_1-C_{20}) alkyl, (C_6-C_{18}) arylalkyl, cyclohexyl, (C_6-C_{12}) aryl or (5-12 atom)heteroaryl, or R_5 and R_6 taken together form a (3-6 atom)heterocycle;

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R₇ and R₈ are independently hydrogen or (C₁-C₆)alkyl;

R₉ is (C₁-C₆)alkyl, (C₂-C₁₀)alkenyl, (C₆-C₁₈)arylalkyl, cyclohexyl, (C₆-C₁₂)aryl or (5-12 atom)heteroaryl;

m is 2 to 6; and

n is 0 to 4.

[0008] In a fifth example embodiment of the first aspect, R₂ of formula (C) is (C₁-C₆)alkyl.

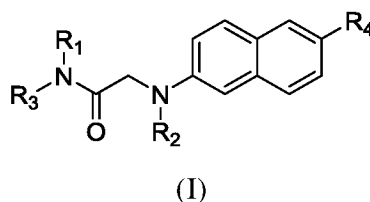
[0009] In a sixth example embodiment of the first aspect, R₅ and R₆ of formula (C) are independently hydrogen or (C₁-C₂₀)alkyl.

[0010] In a seventh example embodiment of the first aspect, R₉ of formula (C) is (C₁-C₆)alkyl.

[0011] In an eighth example embodiment of the first aspect, m of formula (C) is 2 or 3.

[0012] In a ninth example embodiment of the first aspect, n of formula (C) is 1 or 2.

[0013] In a tenth example embodiment of the first aspect, the composition is represented by structural formula (I)



or a salt thereof, wherein:

R₁ is (C₁-C₂₀)alkyl, (C₂-C₂₀)alkenyl, (C₂-C₂₀)alkynyl, (C₂-C₂₀)alkoxy, (C₂-C₂₀)alkylamino or (C₃-C₁₀)cycloalkyl, each of which is optionally substituted with one or more groups independently selected from (C₆-C₁₂)aryl, (5-12 atom)heteroaryl, (5-12 atom)heterocycle, -P(O)((C₆-C₁₈)aryl)₂, fused polycyclic carbocyclyl or carbamyl, further optionally substituted with one or more groups selected from halo, NO₂, oxo, (C₁-C₆)alkyl or (C₁-C₆)alkoxy,

R₂ is (C₁-C₆)alkyl,

R₃ is (C₁-C₂₀)alkylamino, and

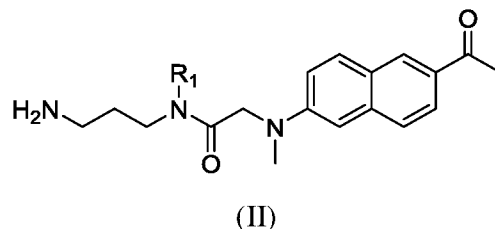
R₄ is acyl.

[0014] In an eleventh example embodiment of the first aspect, R₂ of formula (I) is (C₁-C₃)alkyl.

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[0015] In a twelfth example embodiment of the first aspect, R_3 of formula (I) is (C_1-C_{10}) alkylamino.

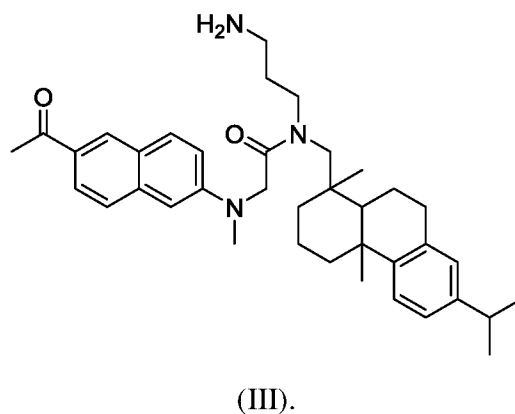
[0016] In a thirteenth example embodiment of the first aspect, the composition is represented by structural formula (II):



wherein:

R_1 is (C_1-C_{20}) alkyl, (C_2-C_{20}) alkenyl, (C_2-C_{20}) alkynyl, (C_2-C_{20}) alkoxy, (C_2-C_{20}) alkylamino or (C_3-C_{10}) cycloalkyl, each of which is optionally substituted with one or more groups independently selected from (C_6-C_{12}) aryl, (5-12 atom) heteroaryl, (5-12 atom) heterocycle, -P(O)((C_6-C_{18})aryl)₂, fused polycyclic carbocyclyl or carbamyl, further optionally substituted with one or more groups selected from halo, NO₂, oxo, (C_1-C_6) alkyl or (C_1-C_6) alkoxy.

[0017] In a fourteenth example embodiment of the first aspect, the composition is represented by structural formula (III) or salt thereof:



[0018] In a second aspect, the invention is a method of visualizing a target cell, the method comprising (a) contacting a population of the target cell with a composition of the first aspect to form an incubation media; (b) incubating the incubation media of step (a) for a period of time sufficient to stain the target cells; and (c) visualizing the stained target cells of step (b) with two-

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photon microscopy to visualize the target cell. In an embodiment of the second aspect, a fluorescence signal produced by the stained target cells allows for the visualizing to take place.

[0019] In an example embodiment of the second aspect, the target cell is a pancreatic islet cell. In another example embodiment of the second aspect, the pancreatic islet cell is an alpha cell.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] FIGs. 1A-C illustrate primary screening and TP- α discovery. (A) The work flow of TPG library primary screening for seeking pancreatic cell selective probe. (B) Structure of TP- α (TPG-456). (C) Representative fluorescence (TP- α ; emission window 430-530 nm) and bright-field images of cell panel stained with 500 nM TP- α , showing bright fluorescence signal from alpha cells.

[0021] FIG. 2 illustrates whole islet staining with TP- α . Isolated mice pancreatic islets were stained with 500 nM TP- α for 1 hr at 37°C, followed by media washing and image acquisition in fresh media. For control 0.1% of DMSO was used. Two-photon images taken with 40X water immersion objective using Leica TCS SP5X MP, Ex:750 nm femto sec laser light, Em: 430-530nm. Scale bar 20 μ m.

[0022] FIG. 3 illustrates two-photon live islet 3D imaging. Individual two-photon optical sections at 10 μ m depth interval of pancreatic islet labeled with 500 nM TP- α incubated at 37°C for 1h. Z-stack projection of whole islet. Two-photon images taken with 40X objective using Leica TCS SP5X MP, Ex:750 nm femto sec laser light, Em: 430-530nm. Scale bar 20 μ m.

[0023] FIGs. 4A-B illustrates spectroscopic information of TP- α . (A) One photon absorption and emission curve of TP- α measured in PBS buffer with 0.1% DMSO. (B) Two-photon absorption cross section of TP- α measured in ethanol.

[0024] FIGs. 5A-C illustrate *in vitro* fluorescence responses of TP- α . (A) Fluorescence spectra of TP- α (10 μ M) without glucagon and upon incubation with different concentration of glucagon (from 10 μ M to 200 μ M) in 10 mM phosphate buffer (1 % DMSO, pH 7.4) under excitation of 370 nm light. Insert is the structure of TP- α . (B) Fractional saturation curve of glucagon with TP- α . Various concentration of glucagon were incubated with 6 μ M TP- α for 30 min on ice in 10 mM phosphate buffer (pH 7.3, 1 % DMSO), followed by measurement of

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fluorescent emission. Experimental $K_D = 65.44 \mu\text{M}$. (C) Selectivity of **TP- α** ($10 \mu\text{M}$) for glucagon in comparison with insulin, other proteins and small molecule analytes (H_2O_2 30 % solution in water). Insert show the selective increase in fluorescent emission of **TP- α** with glucagon in comparison to other analytes. Values are represented as mean and error bars are standard deviation ($n = 3$).

DETAILED DESCRIPTION OF THE INVENTION

[0025] A description of example embodiments of the invention follows.

[0026] Herein is reported the synthesis of a combinatorial two-photon fluorescence dye library (TPG: Two-Photon Green) containing 80 compounds. Primary screening of library compounds against the selected pancreatic cell lines leads to the discovery of an alpha cell imaging probe (TP- α). Further study of this probe demonstrates that TP- α can suitably be used for live alpha cell two-photon imaging of whole pancreatic islets. Moreover, using TP- α staining, bright alpha cell population from the endocrine islet cell mixture can successfully be sorted out.

[0027] Herein are also described probes utilizing “push-pull type” naphthalene-based fluorophores. As Singha et al. (5) describes, donor–acceptor (D–A) type dipolar fluorophores (or “push-pull type” dipolar fluorophores) have been widely used in molecular probes and biological tags owing to their highly emissive nature. Examples of such dipolar dyes include acedan (2-acetyl-6-(dimethylamino)naphthalene), naphthalimide (4-amino-1,8-naphthalimide), coumarin (7-aminocoumarin), benzocoumarin, NBD ((4-nitro-2,1,3-benzoxadiazol-7-yl)amine), dansyl (5-amino-naphthalene-1-sulfonyl), rhodol, Nile red and blue. These dyes mostly contain a dialkylamino group as the electron-donor group and an electron-withdrawing moiety (acetyl, nitro, cyanide, dicyanovinyl, *etc.*), both conjugated to an aromatic system in such a way that the donor and acceptor are electronically conjugated. Such dipolar dyes generate intramolecular charge transfer (ICT) excited states upon irradiation with light, which bestows the dipolar dyes with environment-sensitive photophysical properties: typically, they emit at longer wavelengths as the polarity of the medium increases.

[0028] These types of push-pull naphthalene-based fluorophores are used herein as two-photon imaging probes for whole pancreatic islets since the dipolar dyes are two-photon

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excitable fluorophores. Among the known dipolar dyes, acedan and its analogues constitute an important class of two-photon excitable fluorophores for bioimaging of tissues, as they are small in size, can be readily modified, and show good two-photon absorption properties. Thus, a push-pull type naphthalene-based system like acedan is of great interest and can incorporate any number of electron withdrawing groups in place of acedan's methyl-ketone group while maintaining the ability to fluoresce.

Definitions

[0029] The term “salt,” as used herein, refers to anionic and cationic salts. Examples of anionic salts include the acetate, benzenesulfonate, benzoate, bicarbonate, bitartrate, bromide, calcium edetate, camsylate, carbonate, chloride, citrate, dihydrochloride, edetate, edisylate, estolate, esylate, fumarate, glyceptate, gluconate, glutamate, glycolylarsanilate, hexylresorcinate, hydrobromide, hydrochloride, hydroxynaphthoate, iodide, isethionate, lactate, lactobionate, malate, maleate, mandelate, mesylate, methylsulfate, mucate, napsylate, nitrate, pamoate, pantothenate, phosphate/diphosphate, polygalacturonate, salicylate, stearate, subacetate, succinate, sulfate, tannate, tartrate, teoclate, tosylate, and triethiodide salts. Examples of cationic salts include alkali metal salts (e.g., sodium and potassium), alkaline earth metal salts (e.g., calcium and magnesium), aluminum salts and ammonium salts, as well as salts made from physiologically acceptable organic bases such as trimethylamine, triethylamine, morpholine, pyridine, piperidine, picoline, dicyclohexylamine, N,N'-dibenzylethylenediamine, 2-hydroxyethylamine, bis-(2-hydroxyethyl)amine, tri-(2-hydroxyethyl)amine, procaine, dibenzylpiperidine, dehydroabietylamine, N,N'-bisdehydroabietylamine, glucamine, N-methylglucamine, collidine, quinine, quinoline, and basic amino acids such as lysine and arginine.

[0030] The term “alkyl,” as used herein, refers to both a saturated aliphatic branched or straight-chain monovalent hydrocarbon radical having the specified number of carbon atoms. Thus, “(C₁-C₆) alkyl” means a radical having from 1-6 carbon atoms in a linear or branched arrangement. Examples of “(C₁-C₆) alkyl” include, for example, *n*-propyl, *i*-propyl, *n*-butyl, *i*-butyl, *sec*-butyl, *t*-butyl, *n*-pentyl, *n*-hexyl, 2-methylbutyl, 2-methylpentyl, 2-ethylbutyl, 3-methylpentyl, and 4-methylpentyl. Alkyl can be optionally substituted with halogen, -OH, oxo,

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(C₁-C₆)alkyl, (C₁-C₆)alkoxy, (C₁-C₆) alkoxy(C₁-C₄)alkyl, aryl, heteroaryl, heterocyclyl, cycloalkyl, carbocyclyl, nitro, cyano, amino, acylamino, carbamyl, or -P(O)((C₆-C₁₈)aryl)₂.

[0031] The term “cycloalkyl,” as used herein, refers to saturated aliphatic cyclic hydrocarbon ring. Thus, “(C₃-C₈) cycloalkyl” means (3-8 membered) saturated aliphatic cyclic hydrocarbon ring. (C₃-C₈) cycloalkyl includes, but is not limited to, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, or cyclooctyl. Cycloalkyl can be optionally substituted in the same manner as alkyl, described above.

[0032] The term “carbocyclyl,” as used herein, refers to a cyclic group with only ring carbon atoms. “Carbocyclyl” includes 3-12-membered saturated or unsaturated aliphatic cyclic hydrocarbon rings or 6-12-membered aryl rings. Carbocyclyls are saturated or unsaturated aliphatic cyclic hydrocarbon rings or aromatic hydrocarbon rings having the specified number of carbon atoms. Carbocyclyls include cycloalkyl, cycloalkenyl, cycloalkynyl and aryl. Carbocyclyl can be a monocyclic or fused bicyclic or polycyclic ring system, optionally substituted in the same manner as alkyl or aryl, described herein.

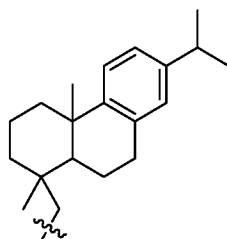
[0033] The term “bicyclic ring system,” as used herein, refers to ring systems having two rings with at least one ring atom in common. Bicyclic ring systems include fused, bridged and spiro ring systems. The two rings can both be aliphatic (*e.g.*, cycloalkyl, cycloalkene, cycloalkyne, or heterocycloalkyl), both be aromatic (*e.g.*, aryl or heteroaryl), or a combination thereof. The bicyclic ring systems can optionally contain 1 to 5 heteroatoms in the ring structure wherein each heteroatom is independently selected from O, N or S. When the heteroatom is N, it can be substituted with H, alkyl, cycloalkyl, alkylene-cycloalkyl, heterocycloalkyl, alkylene-heterocycloalkyl, aryl, alkylene-aryl, heteroaryl, alkylene-heteroaryl, each of which can be optionally substituted with one or more halogen, =O, hydroxy, alkoxy, haloalkyl, alkyl, etc. When the heteroatom is S, it can be optionally mono- or di-oxygenated (*e.g.*, -S(O)- or -S(O)₂-).

[0034] The term “fused bicyclic ring system,” as used herein, refers to ring systems having two rings which have two adjacent ring atoms in common. The two rings can both be aliphatic (*e.g.*, cycloalkyl, cycloalkene, cycloalkyne, or heterocycloalkyl), both be aromatic (*e.g.*, aryl or heteroaryl), or a combination thereof. For example, the first ring can be cycloalkyl or heterocycloalkyl, and the second ring can be a cycloalkyl, cycloalkene, cycloalkyne, aryl, heteroaryl or a heterocycloalkyl. For example, the second ring can be a (C₃-C₆)cycloalkyl, such

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as cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl. Alternatively, the second ring can be an aryl ring (*e.g.*, phenyl). Examples of fused bicyclic ring systems include, but are not limited to, 6,7,8,9-tetrahydro-5H-benzo[7]annulene, 2,3-dihydro-1H-indene, octahydro-1H-indene, tetrahydronaphthalene, decahydronaphthalene, indoline, isoindoline, 2,3-dihydro-1H-benzo[d]imidazole, 2,3-dihydrobenzo[d]oxazole, 2,3-dihydrobenzo[d]thiazole, octahydrobenzo[d]oxazole, octahydro-1H-benzo[d]imidazole, octahydrobenzo[d]thiazole, octahydrocyclopenta[c]pyrrole, 3-azabicyclo[3.1.0]hexane, 3-azabicyclo[3.2.0]heptane, 5,6,7,8-tetrahydroquinoline and 5,6,7,8-tetrahydroisoquinoline, and 2,3,4,5-tetrahydrobenzo[b]oxepine.

[0035] The term “Polycyclic ring system,” as used herein, refers to ring systems having more than two rings (*e.g.*, three rings resulting in a tricyclic ring system) and adjacent rings have at least one ring atom in common. Polycyclic ring systems include fused, bridged and spiro ring systems. A fused polycyclic ring system has at least two rings that have two adjacent ring atoms in common. A spiro polycyclic ring system has at least two rings that have only one ring atom in common. A bridged polycyclic ring system has at least two rings that have three or more adjacent ring atoms in common. Examples of polycyclic ring systems include, but are not limited to, tricyclo[3.3.1.0^{3,7}]nonane (noradamantane), tricyclo[3.3.1.1^{3,7}]decane (adamantane) and 2,3-dihydro-1H-phenalene. An example of a fused polycyclic carbocyclic ring includes, for example:



wherein  represents a point of attachment between two atoms.

[0036] The term “amino,” as used herein, refers to a primary (-NH₂), secondary (-NHR_x), or tertiary (-NR_xR_y) group, wherein R_x and R_y is any alkyl, aryl, heterocyclyl, cycloalkyl or alkenylene, each optionally and independently substituted with one or more substituents described herein. The R_x and R_y substituents may be taken together to form a “ring,” wherein the “ring,” as used herein, is cyclic amino groups such as piperidine and pyrrolidine, and may include heteroatoms such as in morpholine. The terms “alkylamino,” “alkenylamino,” or

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“alkynylamino” as used herein, refer to an alkyl group, an alkenyl group, or an alkynyl group, as defined herein, substituted with an amino group.

[0037] The term “acyl,” as used herein refers to the groups H-C(O)-, alkyl-C(O)-, alkenyl-C(O)-, alkynyl-C(O)-, cycloalkyl-C(O)-, aryl-C(O), heteroaryl-C(O)-, and heterocyclyl-C(O)-, wherein alkyl, alkenyl, alkynyl, cycloalkyl, aryl, heteroaryl, and heterocyclyl are as defined herein.

[0038] The term “acyloxy,” as used herein, refers to the groups alkyl-C(O)O-, alkenyl-C(O)O-, alkynyl-C(O)O-, aryl-C(O)O-, cycloalkyl-C(O)O-, heteroaryl-C(O)O- and heterocyclyl-C(O)O-, wherein alkyl, alkenyl, alkynyl, aryl, cycloalkyl, heteroaryl and heterocyclyl are as defined herein.

[0039] The term “carbamyl,” as used herein, refers to the group -NHC(O)OR_w, wherein R_w is H, alkyl, alkenyl, alkynyl, aryl, cycloalkyl, heteroaryl, or heterocyclyl as defined herein.

[0040] The term “alkenyl,” as used herein, refers to a straight-chain or branched alkyl group having one or more carbon-carbon double bonds. Thus, “(C₂-C₆) alkenyl” means a radical having 2-6 carbon atoms in a linear or branched arrangement having one or more double bonds. Examples of alkenyl groups include, but are not limited to, ethenyl, propenyl, butenyl, pentenyl, hexenyl, butadienyl, pentadienyl, hexadienyl groups, and the like. The one or more carbon-carbon double bonds can be internal (such as in 2-butene) or terminal (such as in 1-butene).

[0041] The term “alkynyl,” as used herein, refers to a straight-chain or branched alkyl group having one or more carbon-carbon triple bonds. Thus, “(C₂-C₆) alkynyl” means a radical having 2-6 carbon atoms in a linear or branched arrangement having one or more triple bonds. Examples of alkynyl groups include, but are not limited to, ethynyl, propynyl, butynyl, pentynyl, and the like. The one or more carbon-carbon triple bonds can be internal (such as in 2-butyne) or terminal (such as in 1-butyne).

[0042] The term “alkoxy,” as used herein, refers to an “alkyl-O-” group, wherein alkyl is defined above. Examples of alkoxy group include methoxy or ethoxy groups.

[0043] The terms “halogen” or “halo,” as used herein, refer to fluorine, chlorine, bromine or iodine.

[0044] The term “aryl,” as used herein, refers to an aromatic monocyclic or polycyclic (e.g. bicyclic or tricyclic) carbocyclic ring system. Thus, “(C₆-C₁₈) aryl” is a 6-18 membered

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monocyclic or polycyclic system. Aryl systems include optionally substituted groups such as phenyl, biphenyl, naphthyl, phenanthryl, anthracenyl, pyrenyl, fluoranthyl or fluorenyl. An aryl can be optionally substituted. Examples of suitable substituents on an aryl include halogen, hydroxyl, (C₁-C₁₂) alkyl, (C₂-C₆) alkenyl, (C₂-C₆) alkynyl, (C₁-C₆) haloalkyl, (C₁-C₃) alkylamino, (C₁-C₃) dialkylamino (C₁-C₆) alkoxy, (C₆-C₁₈) aryloxy, (C₆-C₁₈) arylamino, (C₆-C₁₈) aryl, (C₆-C₁₈) haloaryl, (5-12 atom) heteroaryl, -NO₂, -CN, and oxo.

[0045] In some embodiments, a (C₆-C₁₈) aryl is phenyl, indenyl, naphthyl, azulenyl, heptalenyl, biphenyl, indacenyl, acenaphthylenyl, fluorenyl, phenalenyl, phenanthrenyl, anthracenyl, cyclopentacyclooctenyl or benzocyclooctenyl. In some embodiments, a (C₆-C₁₈) aryl is phenyl, naphthalene, anthracene, 1H-phenalene, tetracene, and pentacene.

[0046] The term “heteroaryl,” as used herein, refers to aromatic groups containing one or more atoms that are a heteroatom (O, S, or N). Thus, “(5-12 atom)heteroaryl” is a 5-12 membered monocyclic or polycyclic aromatic group wherein one or more of the 5 to 12 members are O, S or N. A heteroaryl group can be monocyclic or polycyclic, *e.g.*, a monocyclic heteroaryl ring fused to one or more carbocyclic aromatic groups or other monocyclic heteroaryl groups. The heteroaryl groups of this invention can also include ring systems substituted with one or more oxo moieties. Examples of heteroaryl groups include, but are not limited to, pyridinyl, pyridazinyl, imidazolyl, pyrimidinyl, pyrazolyl, triazolyl, pyrazinyl, quinolyl, isoquinolyl, tetrazolyl, furyl, thienyl, isoxazolyl, thiazolyl, oxazolyl, isothiazolyl, pyrrolyl, quinolinyl, isoquinolinyl, indolyl, benzimidazolyl, benzofuranyl, cinnolyl, indazolyl, indoliziny, phthalazinyl, pyridazinyl, triazinyl, isoindolyl, purinyl, oxadiazolyl, thiazolyl, thiadiazolyl, furazanyl, benzofurazanyl, benzothiophenyl, benzotriazolyl, benzothiazolyl, benzoxazolyl, quinazolinyl, quinoxalinyl, naphthyridinyl, dihydroquinolyl, tetrahydroquinolyl, dihydroisoquinolyl, tetrahydroisoquinolyl, benzofuryl, furopyridinyl, pyrolopyrimidinyl, and azaindolyl.

[0047] In other embodiments, a 5-20-membered heteroaryl group is pyridyl, 1-oxo-pyridyl, furanyl, benzo[1,3]dioxolyl, benzo[1,4]dioxinyl, thienyl, pyrrolyl, oxazolyl, imidazolyl, thiazolyl, a isoxazolyl, quinolinyl, pyrazolyl, isothiazolyl, pyridazinyl, pyrimidinyl, pyrazinyl, a triazinyl, triazolyl, thiadiazolyl, isoquinolinyl, indazolyl, benzoxazolyl, benzofuryl, indoliziny, imidazopyridyl, tetrazolyl, benzimidazolyl, benzothiazolyl, benzothiadiazolyl, benzoxadiazolyl,

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indolyl, tetrahydroindolyl, azaindolyl, imidazopyridyl, quinazolinyl, purinyl, pyrrolo[2,3]pyrimidinyl, pyrazolo[3,4]pyrimidinyl, imidazo[1,2-a]pyridyl, benzothienyl.

[0048] The term “haloalkyl,” as used herein, includes an alkyl substituted with one or more F, Cl, Br, or I, wherein alkyl is defined above.

[0049] The term “haloaryl,” as used herein, includes an aryl substituted with one or more F, Cl, Br, or I, wherein aryl is defined above.

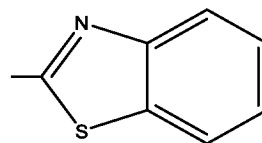
[0050] The term “hetero,” as used herein refers to the replacement of at least one carbon atom member in a ring system with at least one heteroatom selected from N, S or O. “Hetero” also refers to the replacement of at least one carbon atom member in an acyclic system. A hetero ring system or a hetero acyclic system may have 1, 2, or 3 carbon atom members replaced by a heteroatom.

[0051] The terms “heterocyclyl” or “heterocyclic” or “heterocycle,” as used herein, refer to a saturated or unsaturated group having a single ring or multiple condensed rings, from 1 to 10 carbon atoms and from 1 to 4 heteroatoms selected from nitrogen, sulfur or oxygen. Thus, “(5-12 atom)heterocycle” is a 5-12 membered saturated or unsaturated group having a single ring or multiple condensed rings wherein 1 to 4 of the 5 to 12 members are O, S or N. Similarly, “(3-6 atom)heterocycle” is a 3-6 membered saturated or unsaturated group having a single ring or multiple condensed rings wherein 1 to 4 of the 3 to 6 members are O, S or N. In fused ring systems, one or more of the rings can be aryl or heteroaryl, provided that the point of attachment is at the heterocyclyl. Heterocyclyl can be unsubstituted or substituted in accordance with cycloalkyl.

[0052]  represents a point of attachment between two atoms.

[0053] The term “oxo,” as used herein, refers to =O. When an oxo group is a substituent on a carbon atom, they form a carbonyl group (C(O)).

[0054] The term “nitro,” as used herein, refers to -NO₂.



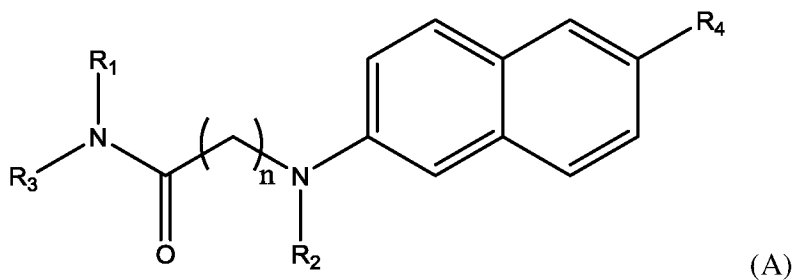
[0055] The term “2-benzothiazole,” as used herein, refers to

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[0056] As shown in Scheme 1, the general synthetic strategy of TPG acid (two-photon green library key intermediate) involves five steps starting from 6-methoxy-2-acetylnaphthalene (A). Compound A undergoes acid mediated demethylation to give compound B in quantitative yield (1). Sodium metabisulfite mediated substitution reaction was performed on B with methylamine, by using water as solvent and heating in microwave at 150 °C for 4 hr to give compound C. Next methyl bromoacetate was reacted to 6-methylamino-2-acetylnaphthalene to give the ester D. Finally D was hydrolyzed by using 2 N KOH, a standard base catalyzed ester hydrolysis method to obtain corresponding TPG acid (2). After synthesizing TPG acid, our previous reported robust solid phase methodology (3) was applied to synthesize an 80 compound TPG library (Scheme 1 and 2, Chart 1).

[0057] Without wishing to be bound by theory, it is believed that the polycyclic system of the TPG library compounds is responsible for the observed (below discussed) selectivity, thus the remainder of the linking groups and the fluorescent components of the probe compounds should be able to be modified without dramatically effecting selectivity and/or fluorescence.

[0058] The 80 compound TPG library described below is one example of a TPG library of probes of the present invention. Numerous other TPG libraries can be synthesized to produce probes of the present invention, for instance those having structural formula (A)

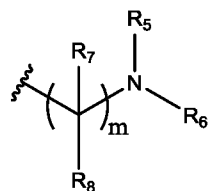


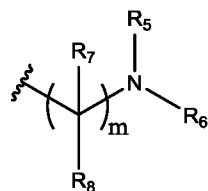
or a salt thereof, wherein:

R₁ is (C₁-C₂₀)alkyl, (C₂-C₂₀)alkenyl, (C₂-C₂₀)alkynyl, (C₂-C₂₀)alkoxy, (C₂-C₂₀)alkylamino or (C₃-C₁₀)cycloalkyl, each of which is optionally substituted with one or more groups independently selected from (C₆-C₁₂)aryl, (5-12 atom)heteroaryl, (5-12 atom)heterocycle, -P(O)((C₆-C₁₈)aryl)₂, fused polycyclic carbocyclyl or carbamyl, further optionally substituted with one or more groups selected from halo, NO₂, oxo, (C₁-C₆)alkyl or (C₁-C₆)alkoxy;

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R₂ is hydrogen, (C₁-C₆)alkyl, (C₂-C₁₀)alkenyl, (C₆-C₁₈)arylalkyl, cyclohexyl, (C₆-C₁₂)aryl or (5-12 atom)heteroaryl;

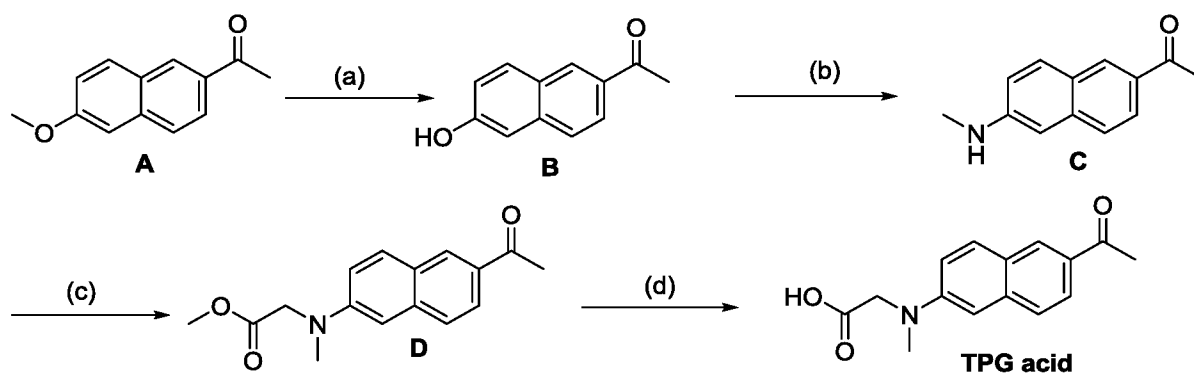


R₃ is hydrogen or , wherein R₅ and R₆ are independently hydrogen, (C₁-C₂₀)alkyl, (C₆-C₁₈)arylalkyl, cyclohexyl, (C₆-C₁₂)aryl or (5-12 atom)heteroaryl, or R₅ and R₆ taken together form a (3-6 atom)heterocycle, R₇ and R₈ are independently hydrogen or (C₁-C₆)alkyl, and m is 1 to 20;

R₄ is -C(O)R₉, -C(O)OR₉, -C(O)OH, -C(O)N(R₉)₂, -S(O)₂N(R₉)₂, -S(O)₂R₉, -NO₂, 2-benzothiazole or -C≡N, wherein R₉ is independently (C₁-C₆)alkyl, (C₂-C₁₀)alkenyl, (C₆-C₁₈)arylalkyl, cyclohexyl, (C₆-C₁₂)aryl or (5-12 atom)heteroaryl; and n is 0 to 4.

[0059] Synthetic scheme of TPG acid: Reagent and conditions: (a) Conc. HCl, TEA, 90°C, 2 h. (b) Methylamine, Na₂S₂O₅, H₂O, MW, 150°C, 2 h. (c) Methylbromoacetate, Na₂HPO₄, NaI, Acetonitrile, Microwave, 140°C, 2 h. (d) KOH, EtOH, Stirring, RT, 5 h.

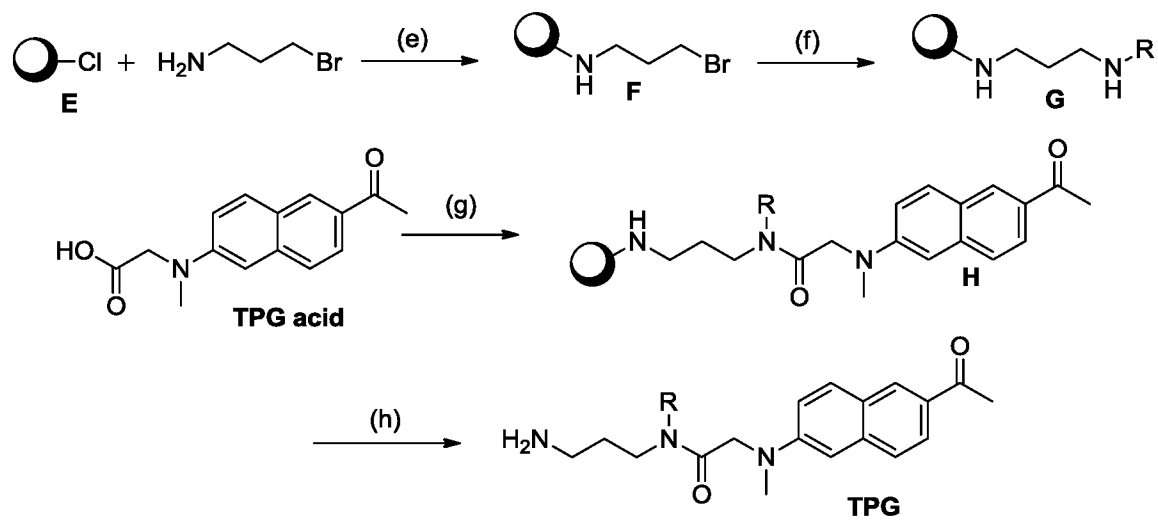
Scheme 1



[0060] Solid phase synthesis of TPG library compounds. Reagents and conditions: (e) DIEA, THF, RT, 12 h. (f) DIEA, RNH₂ (amine building blocks), NMP, 70°C, 12 h. (g) HBTU, HOBt, DIEA, RT, 24 h. (h) 0.5% TFA in DCM, RT, 15 min.

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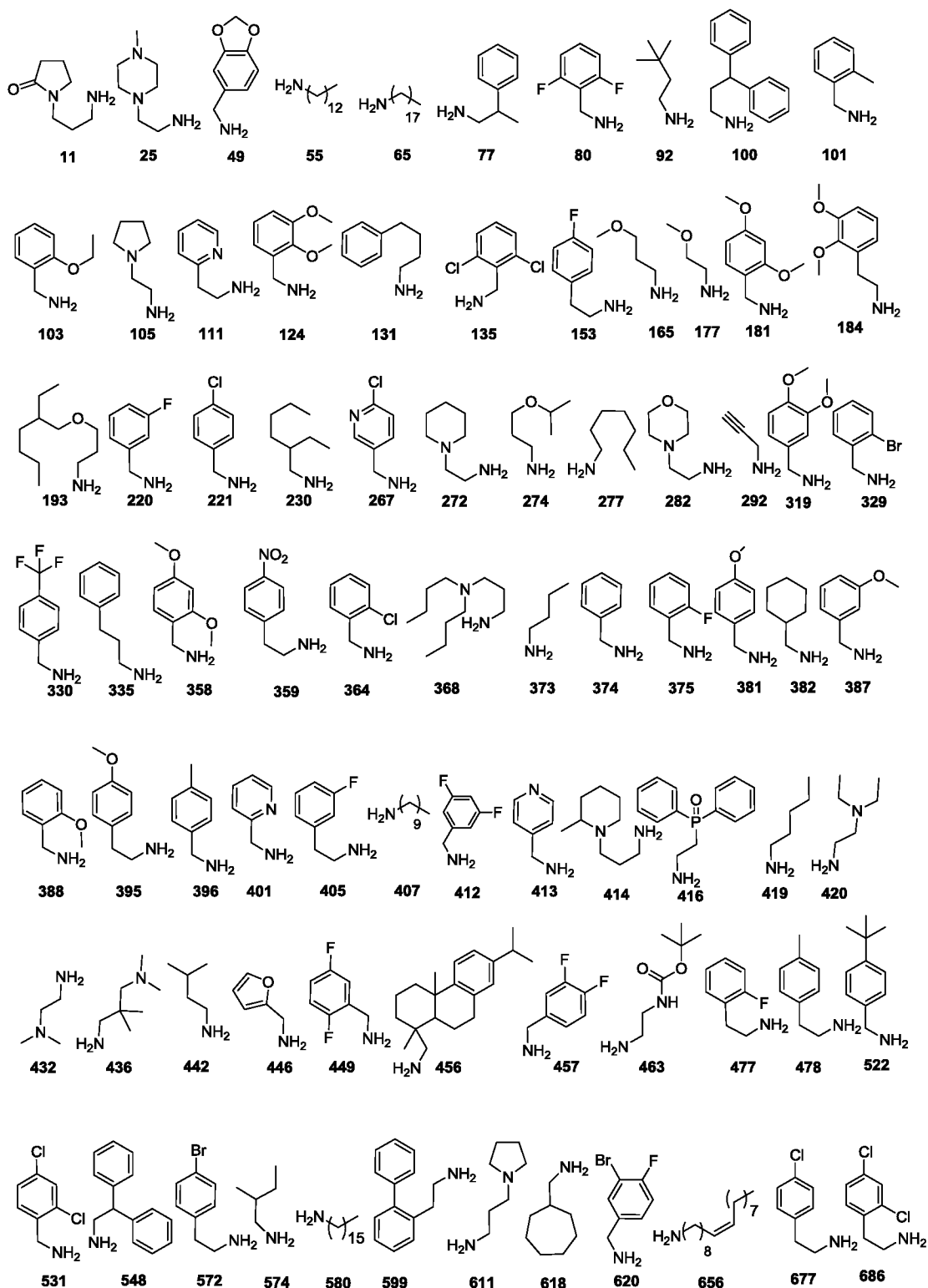
Scheme 2



[0061] Chart 1 illustrates amine building blocks used in the synthesis (R-NH_2 in Scheme 2).

Chart 1

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[0062] To discover the selective probe for pancreatic two-photon imaging application, an 80-membered TPG library was screened against three different cell lines; glucagon producing alpha TC1, insulin producing Beta TC6 and exocrine Acinar cells (FIG. 1A). From the primary screening three TPG compounds were chosen as alpha cells selective hits. Utilizing these three compounds, secondary and tertiary screening was performed and consequently it is confirmed that TPG-456 (FIG. 1B) shows best performance and was thus named TP- α (FIG. 1C).

[0063] Next, it was examined if TP- α can selectively stains alpha cells from the mice pancreatic endocrine tissue. When the cultured dissociated pancreatic islet cells was stained with TP- α , only 14% of the whole islet cells were brightly stained. This subpopulation number matches with the known alpha cell population in mice pancreatic islets which is ~13.5%. Primary islet cells staining with TP- α . One week cultured dissociated islet cells from mice stained with 500 nM TP- α for 1 hr. Fluorescence images analysis showed selective bright staining of 14%, cell subpopulation (based on 3 independent set of experiment), also the brighter cells are ~ 11 fold brighter than the dim population. Two-photon images taken with 63X objective using Leica TCS SP5X MP, Ex:750 nm femto sec laser light, Em: 430-530nm.

[0064] To confirm the cell types of this brightly stained population, immunostaining was performed with anti-glucagon antibody (stains alpha cells), and the result demonstrates the TP- α positive cells are glucagon producing alpha cells. Isolated Pancreatic islet from mice cultured over one week labeled with TP- α , 500 nM for 1hr; followed by immunostaining with Glucagon antibody, and Alexa 546 secondary antibody. Ex: 750nm (MP) Em: 450-520 nm (TP- α) and Ex: 550 nm, Em: 570-620 nm (Alexa 546). Images taken by Leica TCS SP5X with MP.

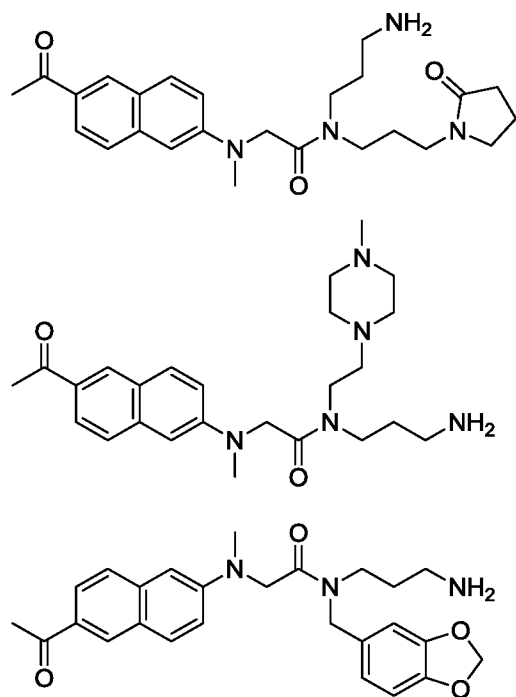
[0065] Finally, two photon imaging for visualizing the natural distribution of alpha cell in the intact pancreatic islets was desired. Handpicked fresh mouse pancreatic islets stained with 500 nM TP- α at 37°C for 1 hour and imaged with two-photon microscope. The two-photon optical sections of live islet at various depths show the distribution of alpha cells at the mantle of the islets. The bright fluorescence signal of TP- α shows three dimensional distributions of alpha cells in live intact islet with distinct cellular resolution. This is the first demonstration where selective alpha cell imaging in live pancreatic islet is achieved with two-photon fluorescent probe (3). Overall, TP- α provides a convenient approach for selective alpha cell staining, and live pancreatic two-photon imaging for alpha cell distribution and survival in real time. TP- α

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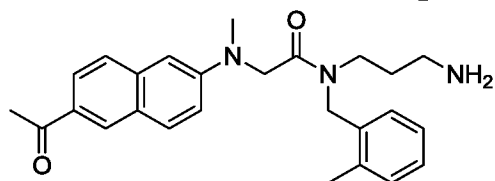
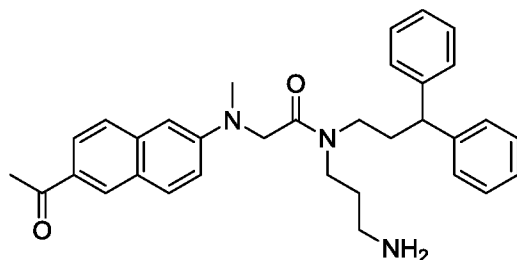
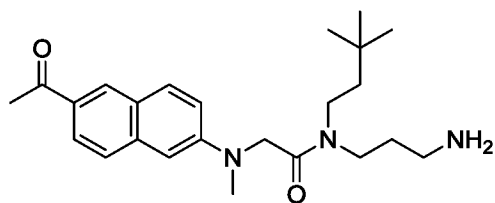
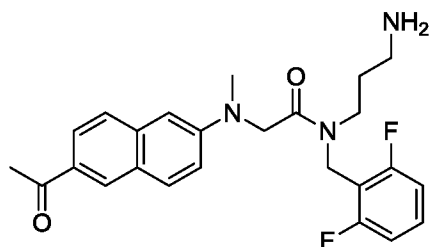
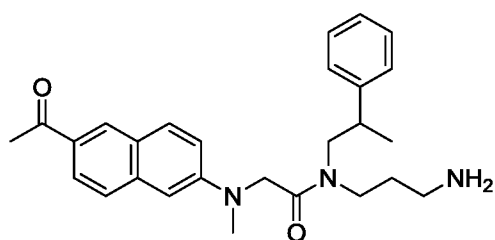
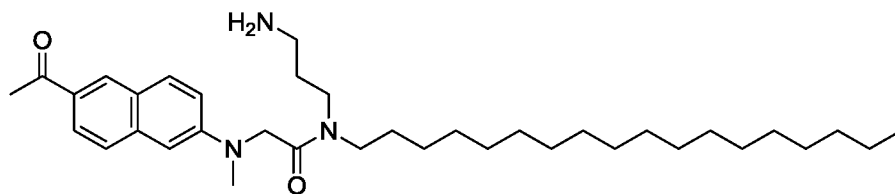
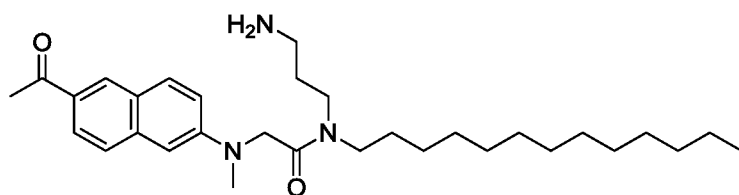
might be a useful tool in diabetic research for understanding and study alpha cell survival and distribution in transplanted islet studies.

[0066] Since TP- α selectively stains glucagon producing alpha cells in comparison with insulin producing beta cells of pancreatic islets, the fluorescence response of TP- α was evaluated with glucagon and insulin first. TP- α not only has a concentration dependent fluorescence response with glucagon (FIG. 5A), but also has more than 8 fold fluorescence intensity with glucagon in comparison with insulin. The fractional saturation curve of TP- α (6 μ M) with glucagon revealed a dissociation constant of 65.44 μ M (FIG. 5B). The fluorescence response of TP- α was also evaluated with pancreatic signaling molecules like acetylcholine, dopamine and found no response with them. Moreover the in vitro study with 23 other biological analytes revealed excellent glucagon selectivity of TP- α (FIG. 5C).

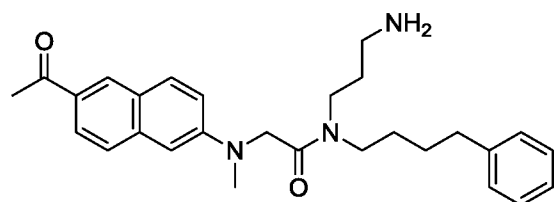
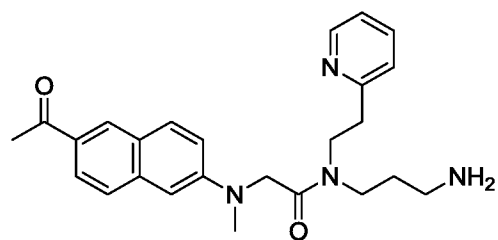
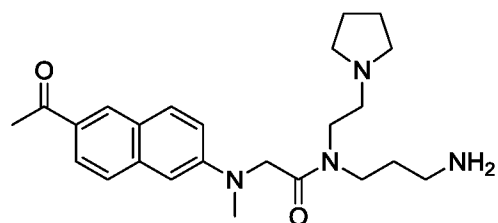
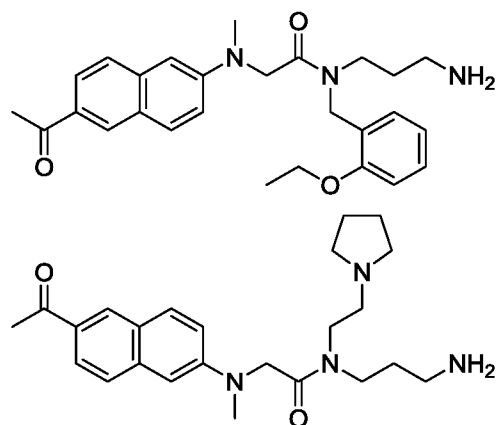
[0067] Additional compounds of the invention include, for example:



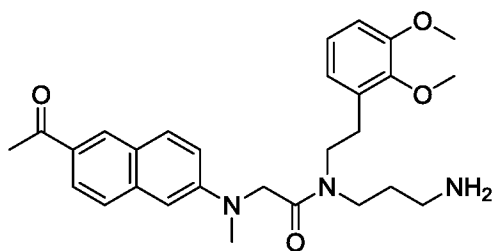
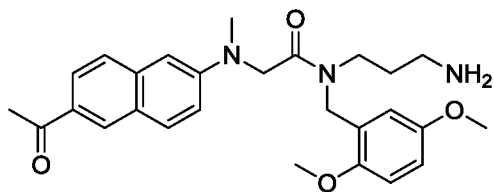
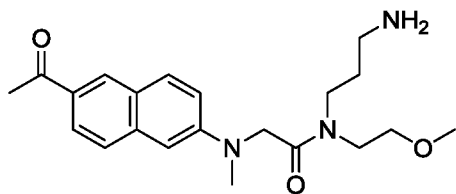
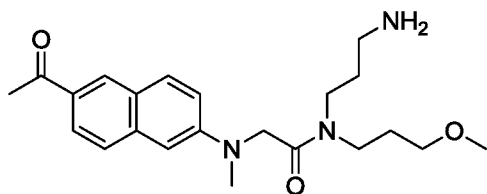
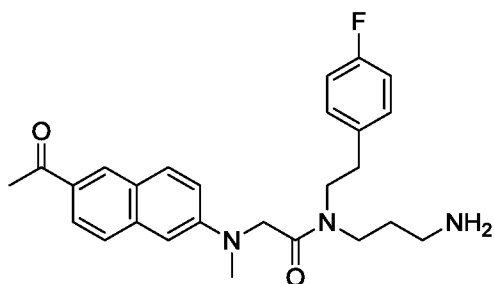
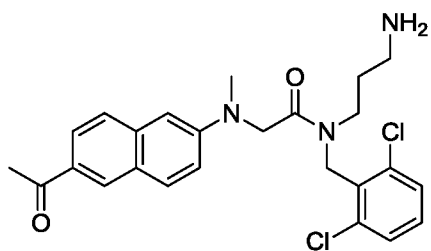
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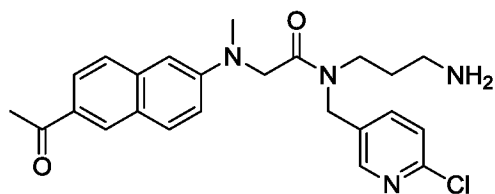
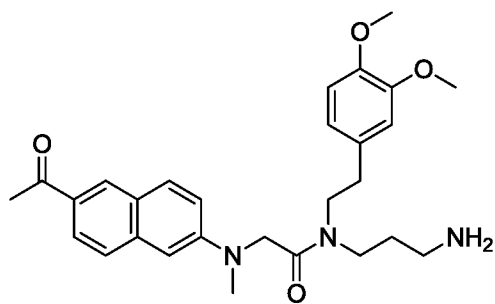
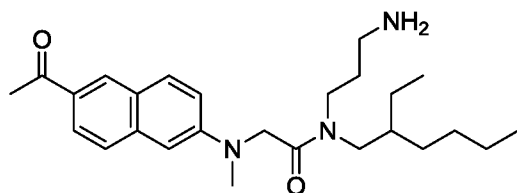
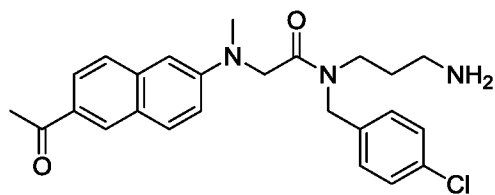
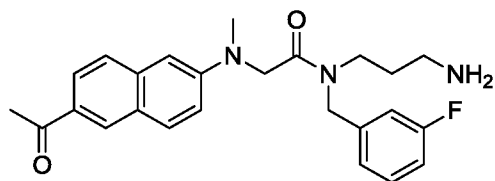
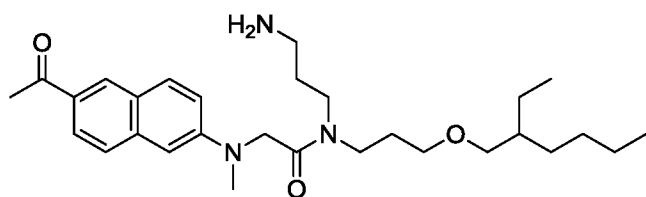
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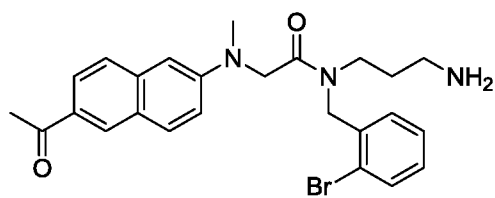
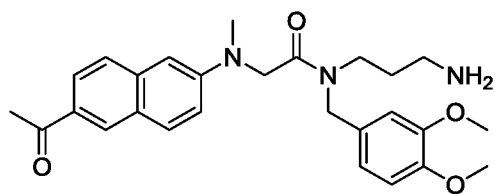
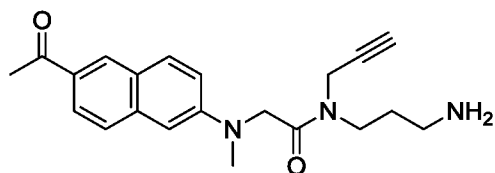
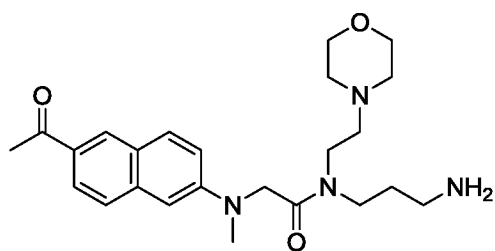
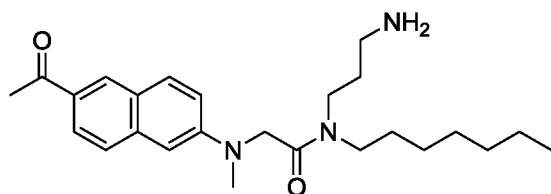
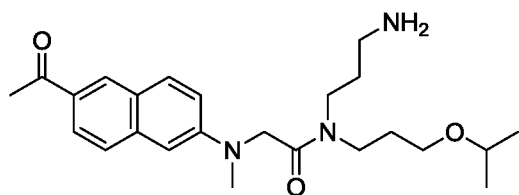
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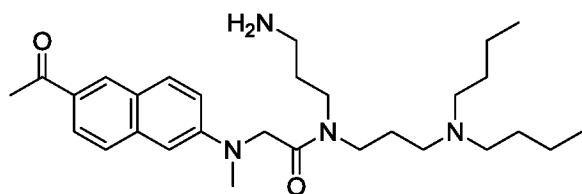
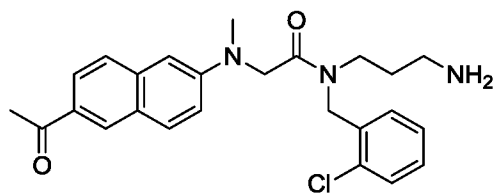
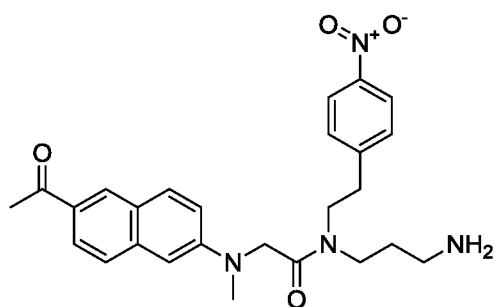
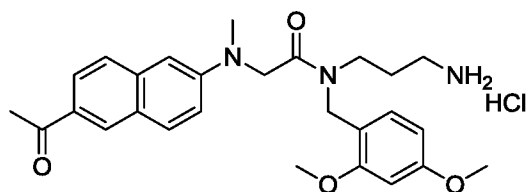
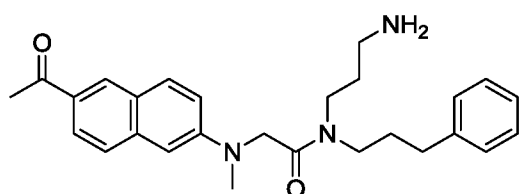
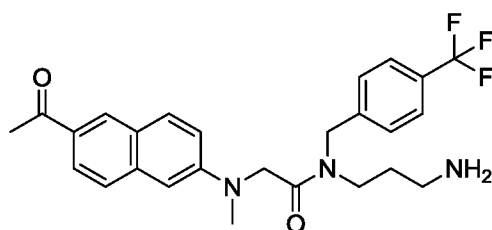
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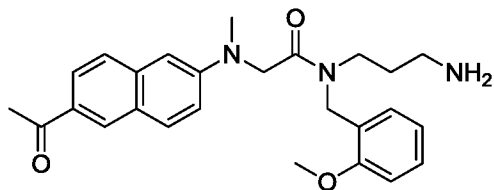
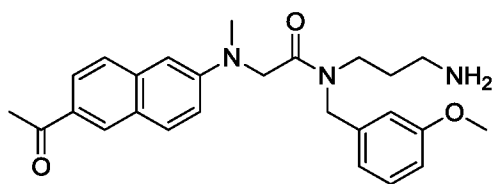
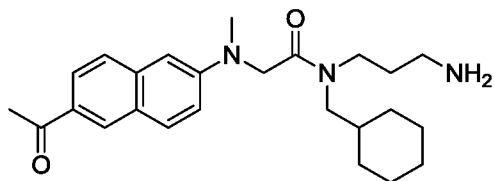
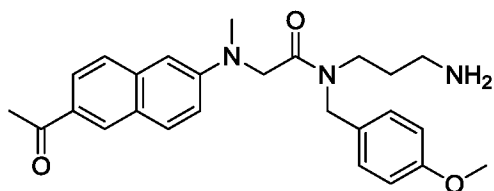
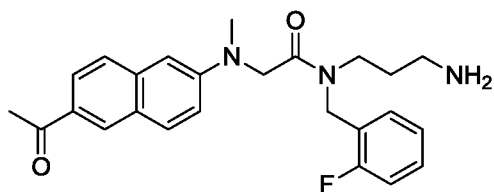
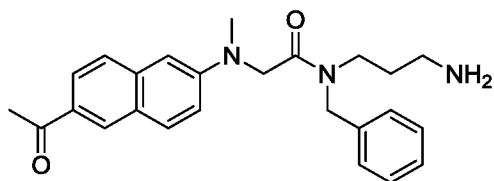
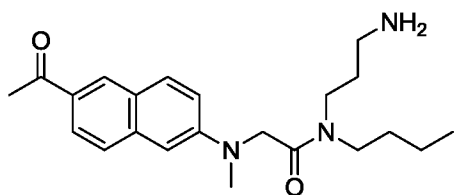
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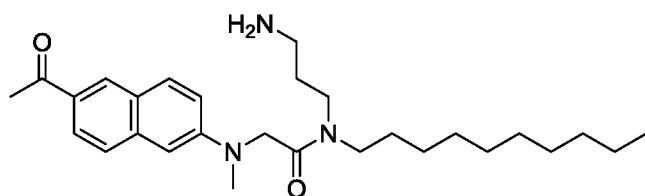
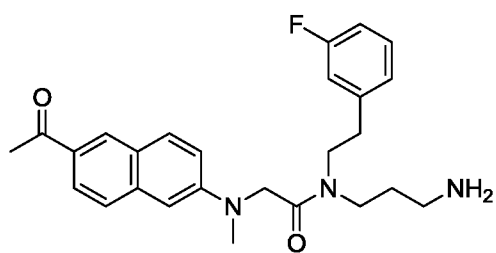
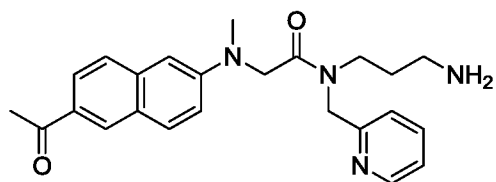
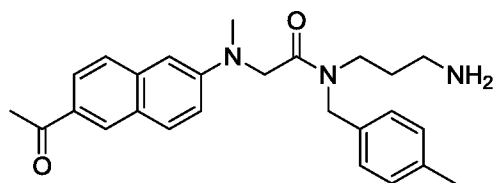
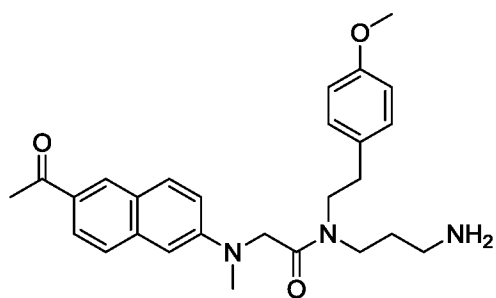
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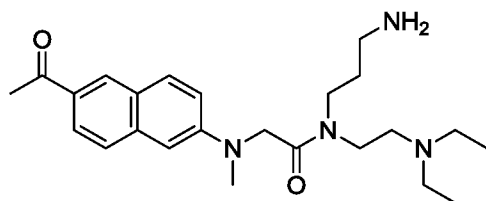
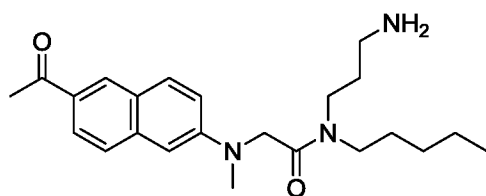
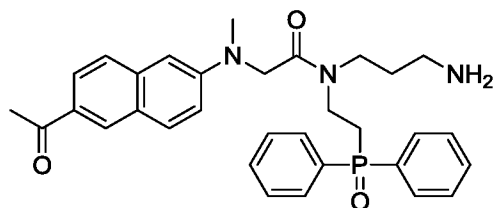
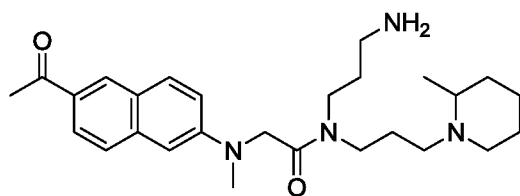
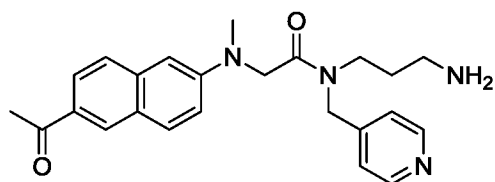
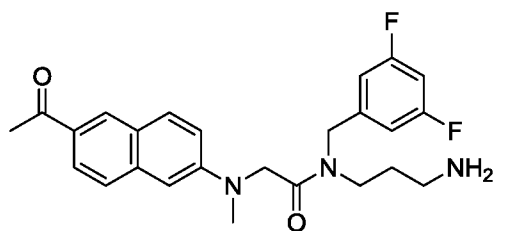
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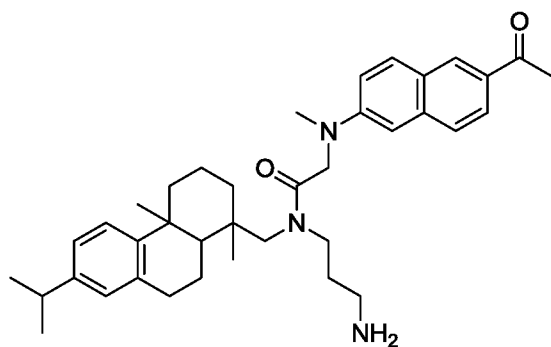
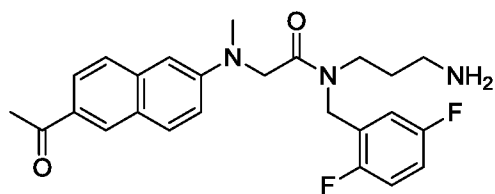
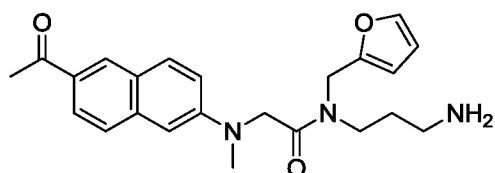
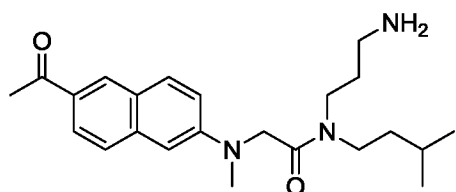
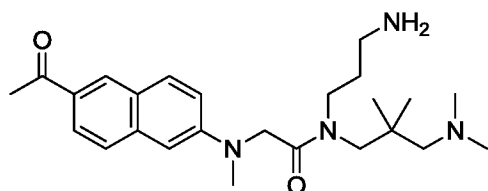
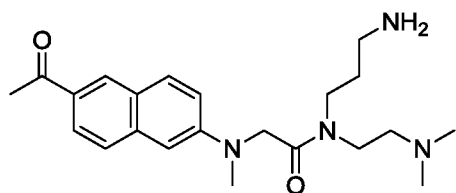
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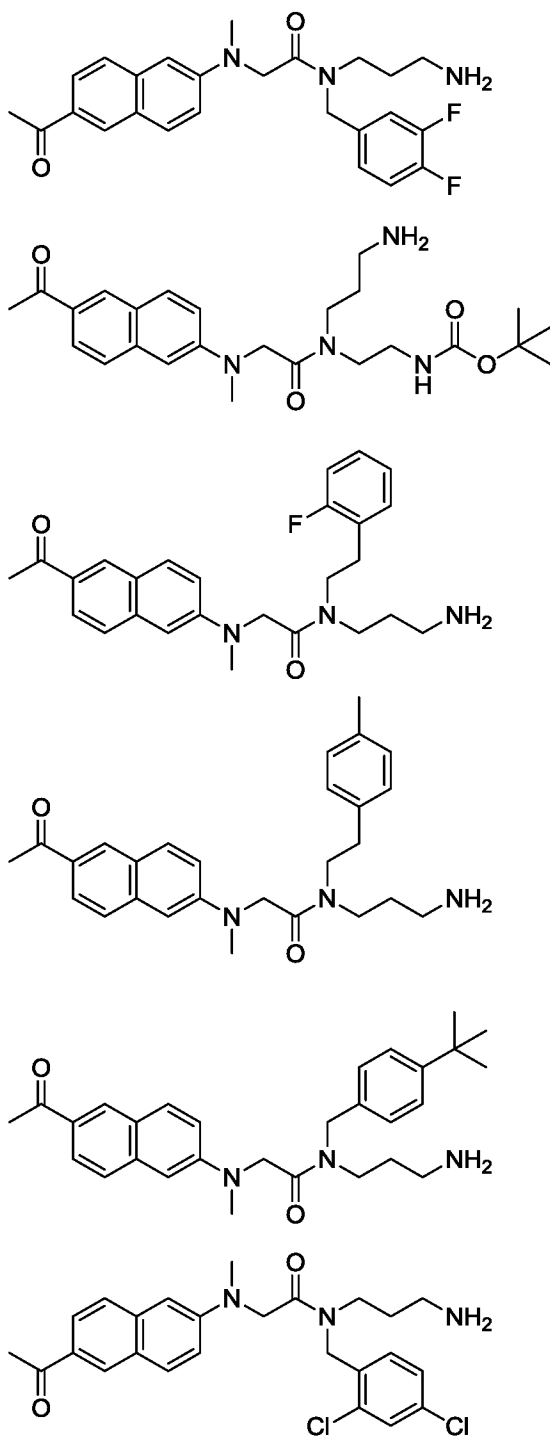
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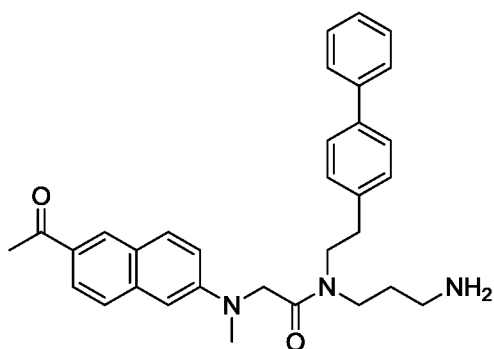
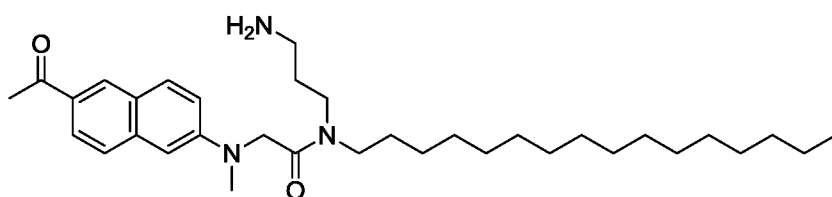
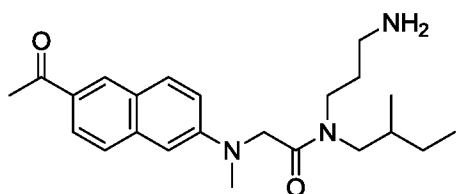
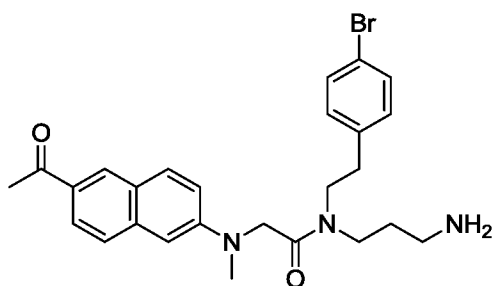
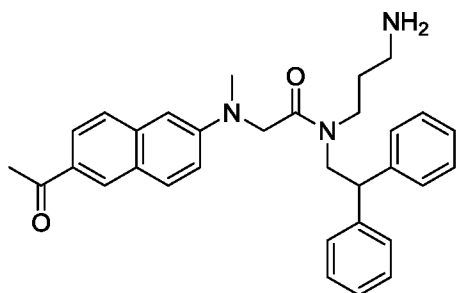
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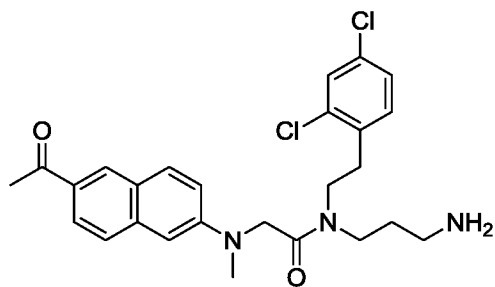
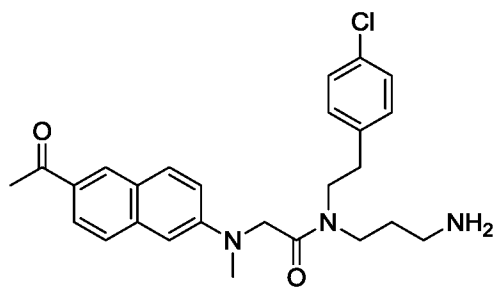
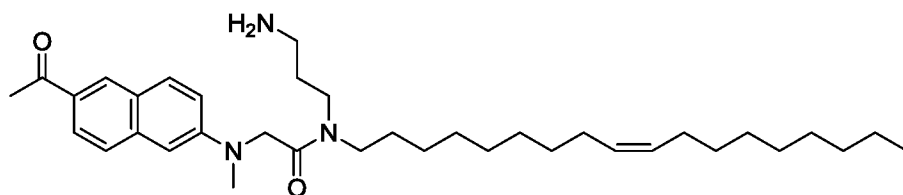
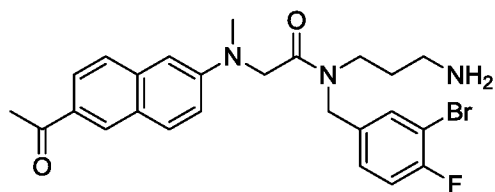
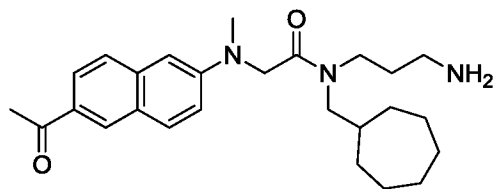
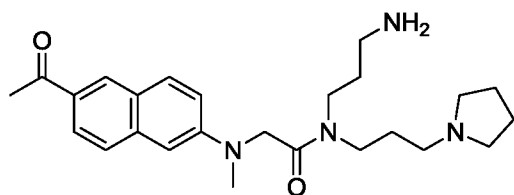
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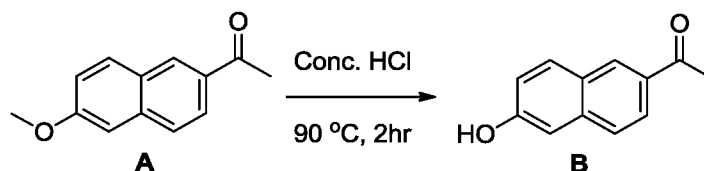
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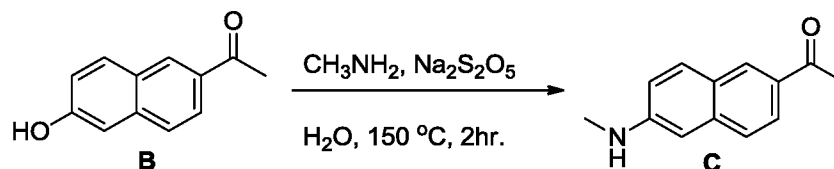
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Exemplification

[0068] Synthesis and characterization of TPG acid intermediates.



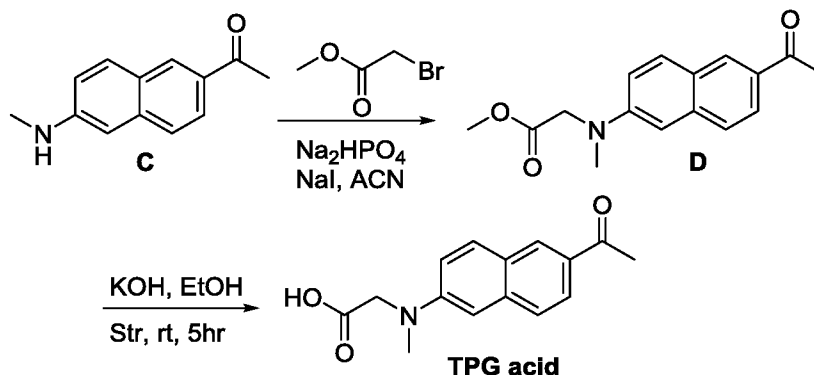
[0069] Synthesis of **B**. To a suspension of 1-(6-methoxy-2-naphthyl)-1-ethanone, **A** (10.0 g, 50 mmol) in 80 mL of HCl ($d=1.18$) in presence of CH_2Cl_2 (2 mL), 15 drops of (~ 0.75 mL) of triethylamine in a 100 mL three-neck round bottom flask equipped with a reflux condenser and an isobaric dropping funnel. The stirred mixture was heated to boil and reflux at 90°C for 2 hr. The hot solution was filtered through a mineral wool plug to remove the oily residue. The solid, after cooling, was filtered out through a glass frit. Then the solid was dissolved in 20 mL of ethyl acetate, washed with brine, dried over with anhydrous magnesium sulfate, and the solvent was removed under reduced pressure to give a crude demethylated product. The solid was dissolved in a base solution, and the diluted HCl was dropped in till white deposit appeared entirely. The solid was filtered out to afford compound **B** as yellow solid (8.09 g, 81%). ^1H NMR (CD_3OD , 500 MHz), δ (ppm) = 8.44 (s, 1H), 7.89 (dd, $J=8.5, 4.0$ Hz, 2H), 7.67 (d, $J=8.5$ Hz, 1H), 7.15 (d, $J=8.5$ Hz, 2H), 4.88 (br s, 1H), 3.02 (s, 3H); ^{13}C NMR (CD_3OD , 500 MHz), δ (ppm) = 200.58, 159.52, 139.36, 133.13, 132.73, 132.06, 128.69, 127.70, 125.21, 120.51, 110.11, 26.63.



[0070] Synthesis of **C**. To a suspension of 2-hydroxy-6-acetylnaphthalene, **B** (1.0 g, 5.37 mmol), $\text{Na}_2\text{S}_2\text{O}_5$ (2 g, 10.74 mmol) with 30 mL H_2O , MeNH_2 (0.8 g, 27 mmol) was added. The mixture was stirred at 170°C for 4 h in the microwave. The product was collected by filtration, washed with ice cold water, and purified by crystallization from $\text{CDCl}_3/\text{EtOH}$ to afford compound **C** as pale yellow crystals (0.67 g, 67%). The reaction was repeated 6 times to scale up **C**. ^1H NMR (CDCl_3 , 500 MHz); δ (ppm) = 8.28 (s, 1H), 7.92 (dd, $J=8.5, 3.0$ Hz, 1H), 7.71

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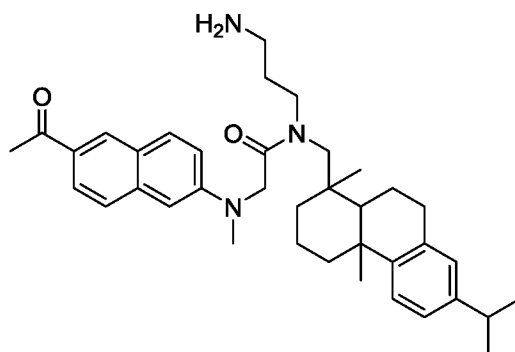
(d, $J = 8.5$ Hz, 1H), 7.63 (d, $J = 8.5$ Hz, 1H), 6.93 (dd, $J = 8.5$ Hz, 2H), 6.7 (s, 1H), 2.94 (s, 3H), 2.65 (s, 3H); ^{13}C NMR (CDCl_3 , 500 MHz); δ (ppm) = 198.14, 148.90, 138.15, 131.01, 130.88, 130.55, 126.19, 124.88, 118.62, 103.77, 30.70, 26.52.



[0071] Synthesis of **TPG acid**. A mixture of **C** (3.0 g, 8.8 mmol), methyl bromoacetate (2.0 g, 13 mmol), Na_2HPO_4 (1.9 g, 13 mmol), and NaI (0.5 g, 3.5 mmol) in MeCN (50 mL) was refluxed under N_2 for 18 h. The product was extracted with ethyl acetate, washed with brine, and purified by crystallization from EtOH to obtained a light yellow powder; yield 2.5 g (70%) ; A mixture of this intermediate (2.0 g, 4.9 mmol) and KOH (0.70 g, 12 mmol) in EtOH (50 mL) was stirred for 5 h. The resultant solution was diluted with ice-water (100 mL) and concentrated HCL (aq) was added slowly at $< 5^\circ\text{C}$ until pH 3 was reached. The resulting precipitate was collected, washed with distilled water, and purified by crystallization from chloroform/petroleum ether to afford dark yellow solid (1.5 g, 79%). ^1H NMR TPG acid (CD_3OD , 500 MHz), δ (ppm) = 8.34 (s, 1H), 7.82 (dd, $J = 9, 3$ Hz, 1H), 7.61 (d, $J = 9.0$ Hz, 1H), 7.15 (d, $J = 9.0$ Hz, 1H), 6.89 (s, 1H), 4.22 (s, 2H), 3.14 (14 (s, 3H), 2.60 (s, 3H); ^{13}C NMR TPG acid (CD_3OD , 500 MHz), δ (ppm) = 200.42, 174.35, 150.94, 139.32, 132.02, 131.97, 131.92, 127.46, 125.19, 117.11, 106.57, 54.85, 39.82, 26.45.

[0072] Characterization of TP- α (TPG-456) compound

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[0073] ^1H NMR **TPG-M456** (300 MHz, MeOH), δ = 8.33 (dd, J =18, 1.8 Hz, 1H), 7.84 (dd, J = 10.5, 1.8 Hz, 1H), 7.80 (s, 1H), 7.56 (dd, J =8.4, 1.2 Hz, 1H), 7.05 (dd, J =9, 2.1 Hz, 1H), 6.95 (m, 1H), 6.85 (d, J =1.5 Hz, 1H), 6.74 (d, J =1.5 Hz, 1H), 4.44 (s, 2H), 3.16 (s, 3H), 3.08 (s, 5H), 2.64 (s, 3H), 2.11 (m, 4H), 1.26 (s, 6H), 1.17 (m, 14H), 1.11 (s, 3H), 0.966 (s, 3H). ^{13}C NMR **TPG-M456** (75 MHz, MeOH), δ = 198.22, 170.60, 161.26, 160.81, 149.03, 146.33, 145.63, 144.95, 133.15, 129.82, 125.57, 125.19, 124.43, 122.86, 117.86, 114.97, 114.00, 104.52, 59.18, 39.95, 39.22, 38.97, 37.24, 36.95, 36.76, 36.47, 36.19, 35.24, 34.54, 32.64, 29.09, 28.60, 28.41, 24.48, 23.47, 22.45, 18.49, 16.09. ESI-MS m/z (M+H) calc'd: 582.40, found 582.20.

Measurement of two-photon absorption cross sections

[0074] The 2PA spectra of library compounds were determined over a broad spectral region by the typical two-photon induced fluorescence (2PF) method relative to Rhodamine B in methanol. A PTI QuantaMaster spectrofluorimeter and femtosecond Ti: Sapphire laser (Mira 900F, 220 fs pulse width, 76 MHz repetition rate, tuning range 740–840 nm, Coherent, USA) were used. Two-photon fluorescence measurements were performed in 10 mm fluorometric quartz cuvettes at $\sim 1 \times 10^{-5}$ M in Methanol, and Rhodamine B as reference at $\sim 1 \times 10^{-5}$ M in Methanol. The experimental fluorescence excitation and detection conditions were conducted with negligible reabsorption processes which can effect 2PA measurements. The two-photon absorption cross section of the probes was calculated at each wavelength according to **equation 1**:

Equation 1 represents two-photon absorption cross section measurement

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$$\delta_{(sample)} = \delta_{(ref)} \frac{\Phi_{(ref)} I_{(sample)} C_{(ref)} n_{(sample)}^2 P_{(ref)}^2}{\Phi_{(sample)} I_{(ref)} C_{(sample)} n_{(ref)}^2 P_{(sample)}^2}$$

where I is the integrated fluorescence intensity, C is the concentration, n is the refractive index, Φ is the quantum yield, and P is the incident power on the sample, subscript 'ref' stands for reference samples, 'sample' stands for samples. The uncertainty in the measured cross sections was about 15%.

Table 1

TP-α Spectral information	
Absorption	370 nm
Emission	510 nm
Quantum yield	0.69
Two-photon absorption cross section	107 GM

Table 2

UC	M ⁺ (cal)	M ⁺ 1(exp.)	Abs(nm)	Em(nm)	QY	Purity (%)
TPG-11	438.26	439.26	363	460	0.49	98
TPG-25	439.29	440.29	361	460	0.42	93
TPG-49	447.22	448.22	361	461	0.48	85
TPG-55	495.38	496.38	361	461	0.37	91
TPG-65	565.46	566.46	355	460	0.44	85
TPG-77	431.26	432.26	367	460	0.60	98
TPG-80	439.21	440.21	363	460	0.49	98
TPG-92	397.27	398.27	359	460	0.38	98
TPG-100	507.29	508.29	360	460	0.52	97
TPG-101	417.24	418.24	361	462	0.48	99
TPG-103	447.25	448.25	363	460	0.43	95
TPG-105	410.27	411.27	362	460	0.50	97
TPG-111	418.24	419.24	363	462	0.29	95
TPG-124	463.25	464.25	359	461	0.39	97

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TPG-131	445.27	446.27	363	460	0.23	98
TPG-135	471.15	472.15	358	460	0.53	96
TPG-153	471.21	472.21	364	461	0.44	97
TPG-165	385.24	386.24	352	464	0.30	98
TPG-177	371.22	372.22	369	461	0.31	98
TPG-181	463.25	464.25	361	460	0.65	99
TPG-184	477.26	478.26	358	460	0.78	97
TPG-193	483.35	484.35	361	463	0.35	92
TPG-220	421.22	422.22	362	460	0.63	96
TPG-221	437.19	438.19	360	460	0.36	97
TPG-230	425.30	426.30	361	460	0.59	95
TPG-266	477.26	478.26	368	460	0.59	95
TPG-267	438.18	439.18	360	461	0.42	91
TPG-274	413.27	414.27	366	460	0.29	99
TPG-277	411.29	412.29	369	462	0.32	97
TPG-282	426.26	427.26	363	460	0.38	91
TPG-292	351.19	352.19	359	455	0.66	81
TPG-319	463.25	464.25	359	461	0.59	96
TPG-329	517.11	518.11	353	458	0.42	89
TPG-330	471.21	472.21	360	460	0.68	95
TPG-335	431.26	432.26	369	460	0.34	97
TPG-358	499.22	500.22	362	460	0.48	98
TPG-359	498.20	499.20	376	450	0.17	92
TPG-364	437.19	438.19	361	462	0.54	97
TPG-368	482.36	483.36	361	460	0.36	93
TPG-373	369.24	370.24	363	460	0.41	97
TPG-374	403.23	404.23	358	460	0.52	98
TPG-375	421.22	422.22	363	462	0.44	94
TPG-381	433.24	434.24	369	460	0.68	95
TPG-382	409.27	410.27	368	460	0.45	92
TPG-387	433.24	434.24	360	460	0.40	97
TPG-388	433.24	434.24	361	462	0.43	97
TPG-395	447.25	448.25	367	461	0.62	98
TPG-396	417.24	418.24	359	461	0.32	98
TPG-401	404.22	405.22	360	462	0.42	91
TPG-405	435.23	436.23	358	461	0.35	92
TPG-407	453.34	454.34	361	460	0.56	96
TPG-412	439.21	440.21	359	460	0.72	98
TPG-413	404.22	405.22	361	461	0.95	92
TPG-414	452.32	453.32	365	460	0.39	93
TPG-416	541.25	542.25	371	460	0.43	85

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TPG-419	383.26	384.26	369	464	0.39	93
TPG-420	412.28	413.28	377	455	0.56	85
TPG-432	384.25	385.25	363	460	0.59	86
TPG-436	426.30	427.30	363	460	0.34	81
TPG-442	383.26	384.26	361	460	0.65	96
TPG-446	393.21	394.21	364	460	0.42	98
TPG-449	439.21	440.21	363	460	0.47	97
TPG-456	581.40	582.40	357	464	0.69	75
TPG-457	439.21	440.21	360	460	0.73	95
TPG-463	456.27	457.27	374	460	0.44	96
TPG-477	435.23	436.23	367	460	0.53	98
TPG-478	431.26	432.26	362	460	0.71	97
TPG-522	459.29	460.29	358	460	0.66	98
TPG-531	471.15	472.15	363	460	0.47	97
TPG-548	493.27	494.27	361	460	0.56	97
TPG-572	495.15	496.15	362	460	0.76	98
TPG-574	383.26	384.26	362	460	0.81	99
TPG-580	537.43	538.43	362	462	0.81	97
TPG-599	493.27	494.27	363	461	0.69	98
TPG-611	424.28	425.28	357	462	0.94	91
TPG-618	423.29	424.29	365	460	0.78	96
TPG-620	499.13	500.13	358	459	0.91	93
TPG-656	563.45	564.45	363	460	0.65	95
TPG-677	451.20	452.20	377	460	0.58	99
TPG-686	485.16	486.16	361	460	0.69	98

*1 Quantum yields were measured in DMSO, using Coumarin 1 as a standard (ϕ : 0.59, in DMSO). *2 Purities were determined according to UV absorption at 350 nm. ESI-MS positive spectra, HPLC conditions: A: H₂O-HCOOH: 99.9:0.1. B: CH₃CN-HCOOH: 99.9:0.1; gradient 100% A to 95% B (6 min), isocratic 95% B (6-8.2min), gradient 95% B to 100% A (8.2-9 min), isocratic 100% A (9-10 min). Reversephase Phenomenex C18 Luna column (4.6 x 50 mm²) 3.5 μ m, flow rate: 1.0 mL/min.

Cell culture

[0075] Insulin producing beta TC-6, glucagon producing alpha TC1 (Clone9), and exocrine acinar 266-6 cells were obtained from the American Type Culture Collection (ATCC) and were maintained according to ATCC protocols. For Beta and acinar cells cultured using Dulbecco's Modified Eagle's Medium (DMEM) with 4500 mg/L D-glucose with 10% FBS and 1%

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penicillin-streptomycin (GIBCO, Life Technologies, Carlsbad, CA, United States). Alpha TC1 cells were cultured in DMEM with 1000 mg/L D-glucose with 15% heat inactivated FBS, 2.0 g/L D-glucose, 15 mM HEPES, 0.1 mM non-essential amino acids (GIBCO, Life Technologies, Carlsbad, CA, United States).

Primary screening

[0076] A primary screening platform was established with the alpha TC 1, beta TC 6 and acinar cells. All three cell lines were seeded onto 384xwell plates in duplicate and cultured overnight before use. For screening all cells were incubated with 1 μ M each TPG library (80) compounds for 30 min to 1 hour. Wells were washed with fresh media before image taking, to reduce the background. To discover the hit candidates in order to develop two-photo imaging probe, we used one photon automated imaging system ImageXpress MicroTM with 10X objectives.

One-photon and Two-photon imaging

[0077] All images were carried out using Leica TCS SP5X MP (Leica Microsystems Ltd.) with 405 nm laser light for one-photon imaging and 750 nm (femto sec pulsed laser) for two-photon imaging. Images were taken using 10x dry, 40x dry, and 100x oil objectives. For two-photon imaging the probes was excited by titanium-sapphire laser light set at wavelength 750 nm and an output power of 2710 mW. To obtain the probe signal the internal photomultiplier tube was set at 430 to 530 nm. Image analysis and intensity measurements were carried out by Leica Application Suite Advanced Fluorescence LAS AF.

Islet isolation and imaging

[0078] Pancreatic islets were isolated from 10-15 week old mouse pancreas by collagenase P digestion and islet peaking methods in accordance with the animal handling regulations of our institution. Briefly, fresh pancreas was cut into small pieces and digested with 0.4% collagenase P (Roche, Indianapolis, IN, USA) solution in Hank's Balanced Salt Solution (HBSS; Invitrogen, Carlsbad, CA, USA) with 0.2% Bovine Serum Albumin for 13 minutes at 37°C on a shaker, followed by neutralization with 0.2% BSA HBSS buffer. Manual peaking of intact islets was

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carried out using Zeiss Stemi DV4 stereomicroscope. Islets were maintained in DMEM with 4500 mg/L glucose + 10% FBS and 1% penicillin-streptomycin (GIBCO, Life Technologies, Carlsbad, CA, United States). Freshly isolated islets were maintained in high glucose MDME for 24 hour before intact islet two photon imaging. 10-20 islets were stained with 500 nM TP- α for 1 hour at 37°C, after incubation islets were transferred to fresh media for two-photon image acquisition.

[0079] For dissociation 50-100 islets were incubated with 0.25% Trypsin-EDTA (1X), phenol red (GIBCO) at 37°C for 2 mins followed by trituration with pipette tips. Finally, the dissociated islet cells were transferred to the 4500 mg/L D-glucose DMEM with 10% FBS and 1% penicillin-streptomycin (GIBCO) for culture. Dissociated cells were cultured for 1 week, with media change every 2-3 days. For image acquisition the cells were stained with 500 nM TP- α for 1 hour at 37°C, after incubation islets were transferred to fresh media before two-photon image acquisition.

Immunohistochemistry

[0080] Primary cells and islets were fixed in 4% PFA and permeabilized with 0.1% Triton-X 100 (BDH chemical). Fixed Cells were identified by secretory markers at the respective dilutions: alpha cell – Glucagon (Sigma-Aldrich 3050 Spruce St. St. Louis) 1:2000, beta cell-insulin (Dako, SPD Scientific, Pvt. Ltd. Singapore) 1:500. For secondary antibody staining, Cy5® Goat Anti-Mouse IgG (Invitrogen, Molecular Probes Inc, PO Box 22010 Eugene, OR) 1:500, and Alexa Fluor® 488 Goat Anti-Guinea Pig IgG (Invitrogen) 1:200 were used. Images were scored using intensity analysis software MetaXpress® and confirmed by image-based visual analysis for the selected hit compounds.

[0081]

REFERENCES

1. Li Jia R.; Zhang G. J., Chem. Crystallogr., 2005, 35, 10, 789.
2. Hwan M. K.; Hyo J. C.; Soon Y. J.; Young G. K.; Won H. P. Seung J. J.; Chul H. K. Taiha J.; Bong R. C.; ChemBioChem., 2007, 8, 553.
3. Das, R. K.; Samanta, A.; Ha, H. H.; Chang, Y. T. RSC Adv., 2011, 1, 573.

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4. Jeffrey D. C.; Stacey B. D.; Kathryn L. C.; Runpei W.; Craig S. N. Biol Proced Online., 2009, 11, 3.
5. Singha, S., et al., Chem. Sci., 2015, 6, 4335.

[0082] The teachings of all patents, published applications and references cited herein are incorporated by reference in their entirety.

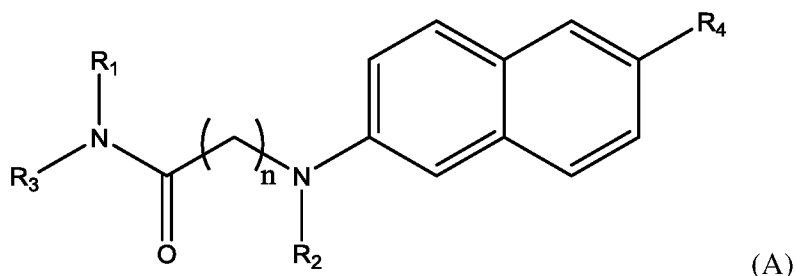
[0083] While this invention has been particularly shown and described with references to example embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

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CLAIMS

What is claimed is:

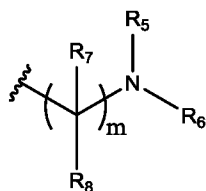
1. A composition represented by structural formula (A):

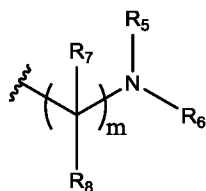


or a salt thereof, wherein:

R₁ is (C₁-C₂₀)alkyl, (C₂-C₂₀)alkenyl, (C₂-C₂₀)alkynyl, (C₂-C₂₀)alkoxy, (C₂-C₂₀)alkylamino or (C₃-C₁₀)cycloalkyl, each of which is optionally substituted with one or more groups independently selected from (C₆-C₁₂)aryl, (5-12 atom)heteroaryl, (5-12 atom)heterocycle, -P(O)((C₆-C₁₈)aryl)₂, fused polycyclic carbocyclyl or carbamyl, further optionally substituted with one or more groups selected from halo, NO₂, oxo, (C₁-C₆)alkyl or (C₁-C₆)alkoxy;

R₂ is hydrogen, (C₁-C₆)alkyl, (C₂-C₁₀)alkenyl, (C₆-C₁₈)arylalkyl, cyclohexyl, (C₆-C₁₂)aryl or (5-12 atom)heteroaryl;



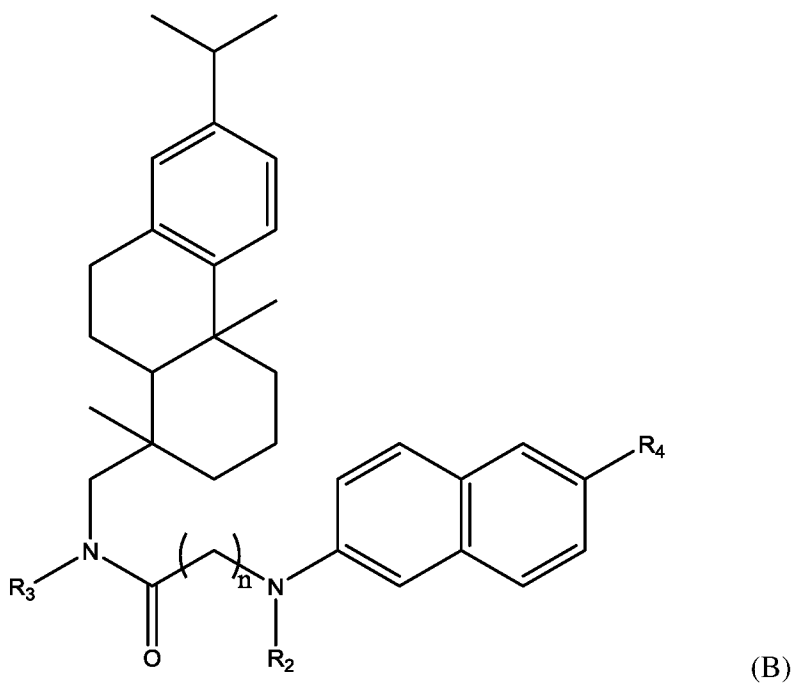
R₃ is hydrogen or , wherein R₅ and R₆ are independently hydrogen, (C₁-C₂₀)alkyl, (C₆-C₁₈)arylalkyl, cyclohexyl, (C₆-C₁₂)aryl or (5-12 atom)heteroaryl, or R₅ and R₆ taken together form a (3-6 atom)heterocycle, R₇ and R₈ are independently hydrogen or (C₁-C₆)alkyl, and m is 1 to 20;

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R_4 is $-C(O)R_9$, $-C(O)OR_9$, $-C(O)OH$, $-C(O)N(R_9)_2$, $-S(O)_2N(R_9)_2$, $-S(O)_2R_9$, $-NO_2$, 2-benzothiazole or $-C\equiv N$, wherein R_9 is independently (C_1-C_6) alkyl, (C_2-C_{10}) alkenyl, (C_6-C_{18}) arylalkyl, cyclohexyl, (C_6-C_{12}) aryl or (5-12 atom)heteroaryl; and

n is 0 to 4.

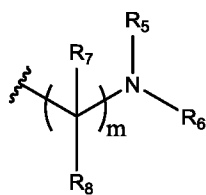
2. The composition of Claim 1, wherein the composition is represented by structural formula (B):

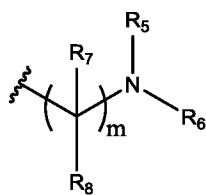


wherein:

R_2 is hydrogen, (C_1-C_6) alkyl, (C_2-C_{10}) alkenyl, (C_6-C_{18}) arylalkyl, cyclohexyl, (C_6-C_{12}) aryl or (5-12 atom)heteroaryl;

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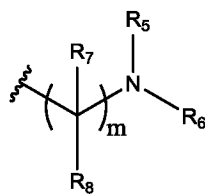


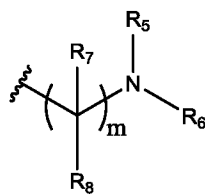
R_3 is hydrogen or , wherein R_5 and R_6 are independently hydrogen, (C₁-C₂₀)alkyl, (C₆-C₁₈)arylalkyl, cyclohexyl, (C₆-C₁₂)aryl or (5-12 atom)heteroaryl, or R_5 and R_6 taken together form a (3-6 atom)heterocycle, R_7 and R_8 are independently hydrogen or (C₁-C₆)alkyl, and m is 1 to 20;

R_4 is $-C(O)R_9$, $-C(O)OR_9$, $-C(O)OH$, $-C(O)N(R_9)_2$, $-S(O)_2N(R_9)_2$, $-S(O)_2R_9$, $-NO_2$, 2-benzothiazole or $-C\equiv N$, wherein R_9 is independently (C₁-C₆)alkyl, (C₂-C₁₀)alkenyl, (C₆-C₁₈)arylalkyl, cyclohexyl, (C₆-C₁₂)aryl or (5-12 atom)heteroaryl; and

n is 0 to 4.

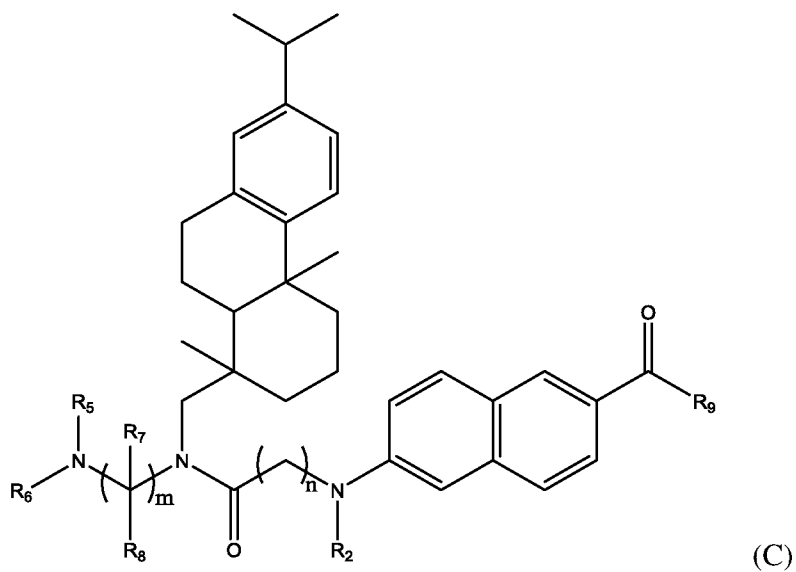
3. The composition of Claim 2, wherein R_4 is $-C(O)R_9$, $-C(O)OR_9$, $-C(O)N(R_9)_2$, or $-C\equiv N$, wherein R_9 is independently (C₁-C₆)alkyl, (C₂-C₁₀)alkenyl, (C₆-C₁₈)arylalkyl, cyclohexyl, (C₆-C₁₂)aryl or (5-12 atom)heteroaryl.



4. The composition of Claim 3, wherein R_3 is , wherein R_5 and R_6 are independently hydrogen, (C₁-C₂₀)alkyl, (C₆-C₁₈)arylalkyl, cyclohexyl, (C₆-C₁₂)aryl or (5-12 atom)heteroaryl, or R_5 and R_6 taken together form a (3-6 atom)heterocycle, R_7 and R_8 are independently hydrogen or (C₁-C₆)alkyl, and m is 1 to 20.

5. The composition of Claim 4, wherein the composition is represented by structural formula (C):

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wherein:

R_2 is hydrogen, (C_1-C_6) alkyl, (C_2-C_{10}) alkenyl, (C_6-C_{18}) arylalkyl, cyclohexyl, (C_6-C_{12}) aryl or $(5-12 \text{ atom})$ heteroaryl;

R_5 and R_6 are independently hydrogen, (C_1-C_{20}) alkyl, (C_6-C_{18}) arylalkyl, cyclohexyl, (C_6-C_{12}) aryl or $(5-12 \text{ atom})$ heteroaryl, or R_5 and R_6 taken together form a $(3-6 \text{ atom})$ heterocycle;

R_7 and R_8 are independently hydrogen or (C_1-C_6) alkyl;

R_9 is (C_1-C_6) alkyl, (C_2-C_{10}) alkenyl, (C_6-C_{18}) arylalkyl, cyclohexyl, (C_6-C_{12}) aryl or $(5-12 \text{ atom})$ heteroaryl;

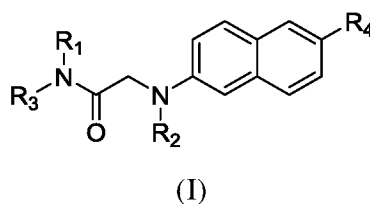
m is 2 to 6; and

n is 0 to 4.

6. The composition of Claim 5, wherein R_2 is (C_1-C_6) alkyl.

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7. The composition of Claim 6, wherein R_5 and R_6 are independently hydrogen or (C_1-C_{20}) alkyl.
8. The composition of Claim 7, wherein R_9 is (C_1-C_6) alkyl.
9. The composition of Claim 8, wherein m is 2 or 3.
10. The composition of Claim 9, wherein n is 1 or 2.
11. The composition of Claim 1, wherein the composition is represented by structural formula (I):



or a salt thereof, wherein:

R_1 is (C_1-C_{20}) alkyl, (C_2-C_{20}) alkenyl, (C_2-C_{20}) alkynyl, (C_2-C_{20}) alkoxy, (C_2-C_{20}) alkylamino or (C_3-C_{10}) cycloalkyl, each of which is optionally substituted with one or more groups independently selected from (C_6-C_{12}) aryl, (5-12 atom) heteroaryl, (5-12 atom) heterocycle, $-P(O)((C_6-C_{18})aryl)_2$, fused polycyclic carbocyclyl or carbamyl, further optionally substituted with one or more groups selected from halo, NO_2 , oxo, (C_1-C_6) alkyl or (C_1-C_6) alkoxy,

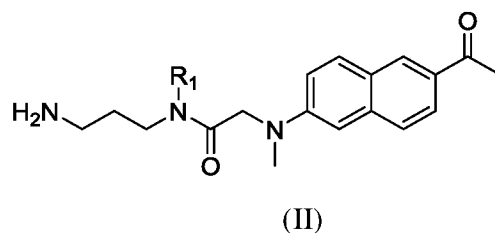
R_2 is (C_1-C_6) alkyl,

R_3 is (C_1-C_{20}) alkylamino, and

R_4 is acyl.

12. The composition of Claim 11, wherein R_2 is (C_1-C_3) alkyl.
13. The composition of Claim 11, wherein R_3 is (C_1-C_{10}) alkylamino.
14. The composition of Claim 11, wherein the composition is represented by structural formula (II):

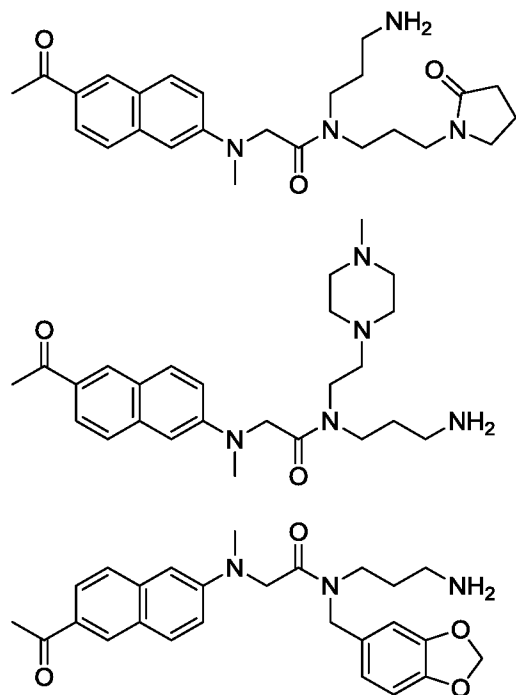
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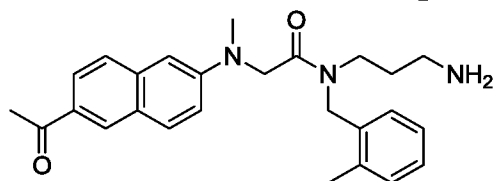
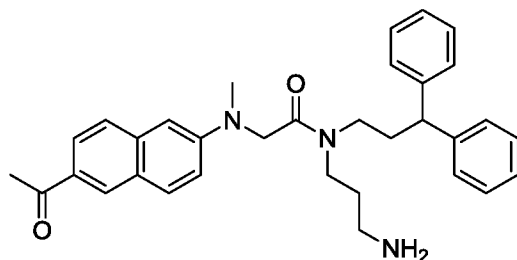
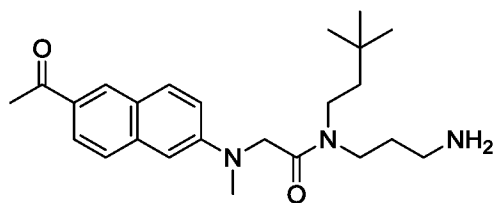
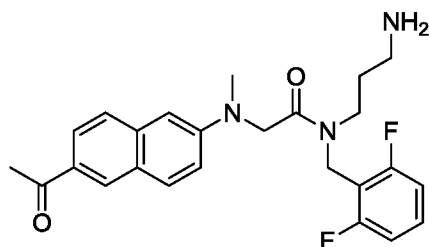
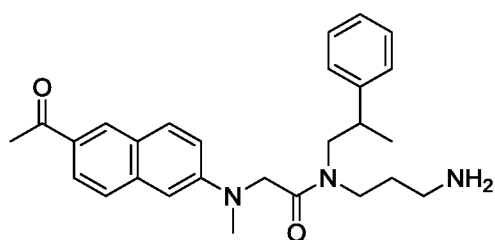
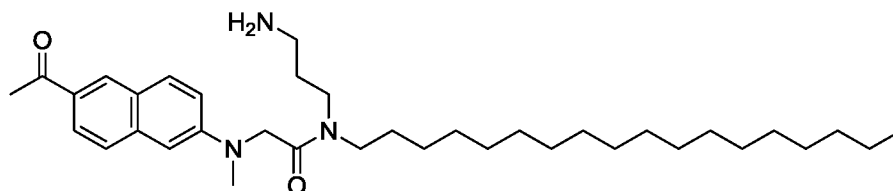
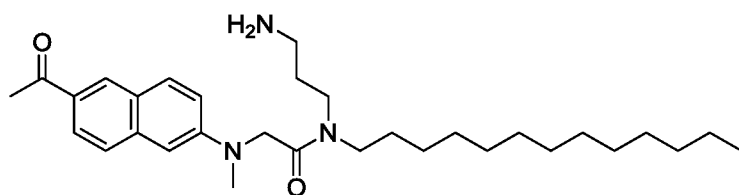
wherein:

R₁ is (C₁-C₂₀)alkyl, (C₂-C₂₀)alkenyl, (C₂-C₂₀)alkynyl, (C₂-C₂₀)alkoxy, (C₂-C₂₀)alkylamino or (C₃-C₁₀)cycloalkyl, each of which is optionally substituted with one or more groups independently selected from (C₆-C₁₂)aryl, (5-12 atom) heteroaryl, (5-12 atom) heterocycle, -P(O)((C₆-C₁₈)aryl)₂, fused polycyclic carbocyclyl or carbamyl, further optionally substituted with one or more groups selected from halo, NO₂, oxo, (C₁-C₆)alkyl or (C₁-C₆)alkoxy.

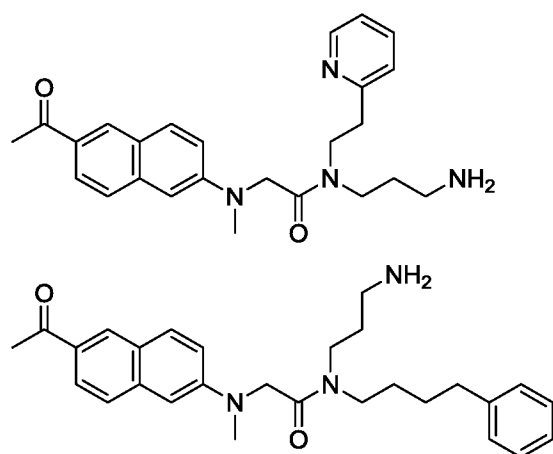
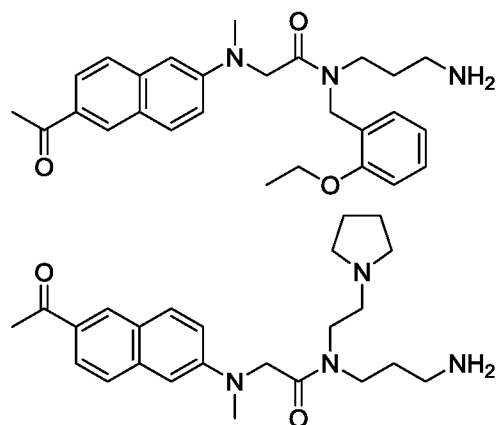
15. The composition of Claim 11, wherein the composition is represented by the structural formula:



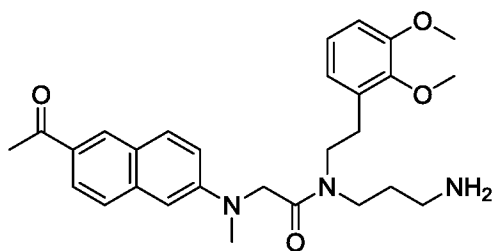
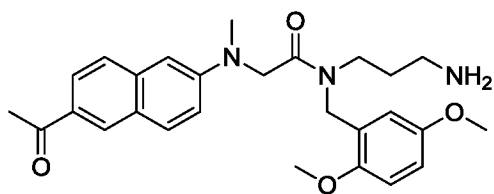
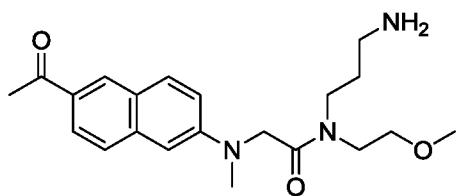
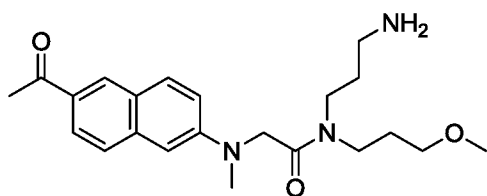
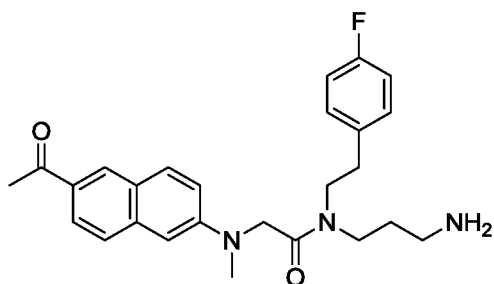
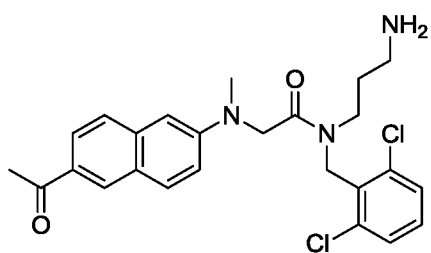
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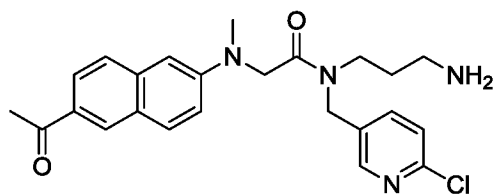
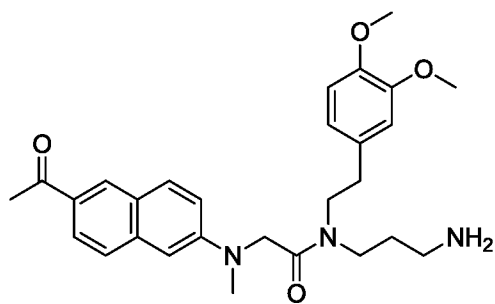
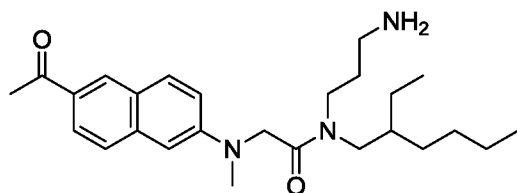
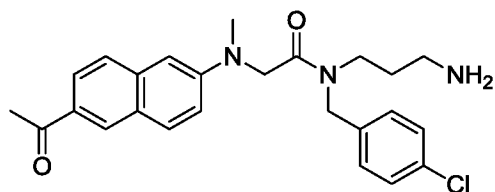
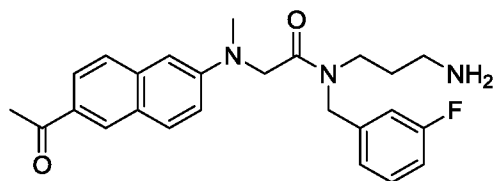
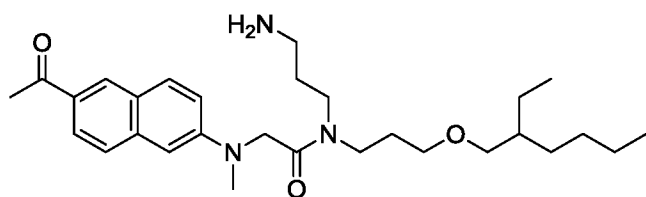
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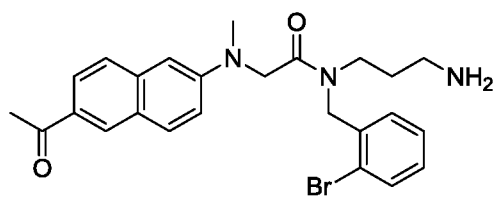
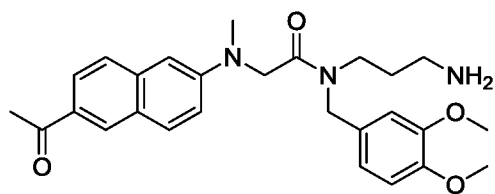
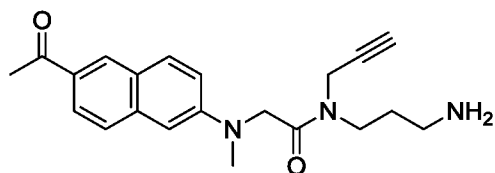
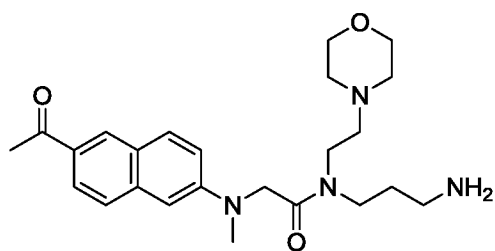
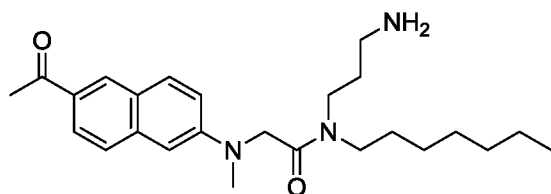
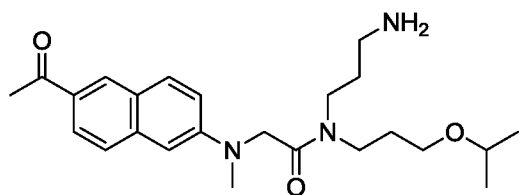
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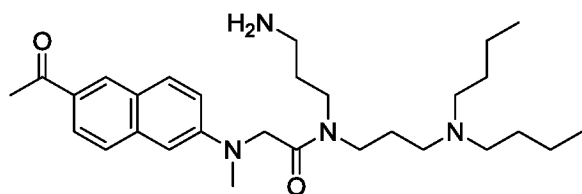
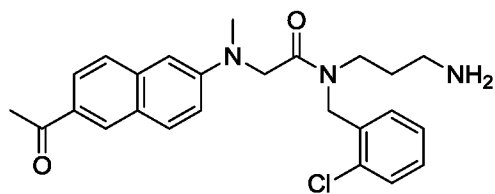
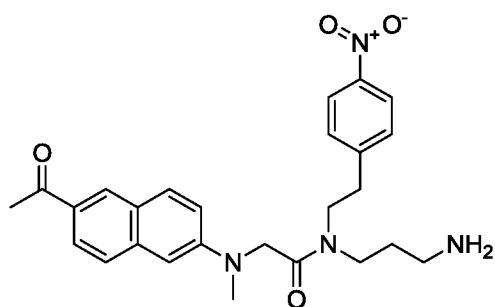
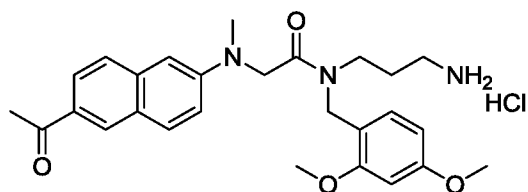
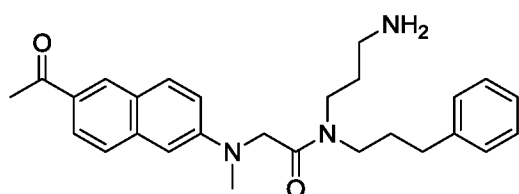
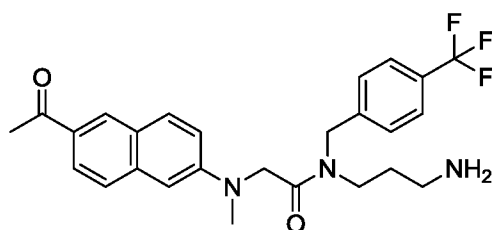
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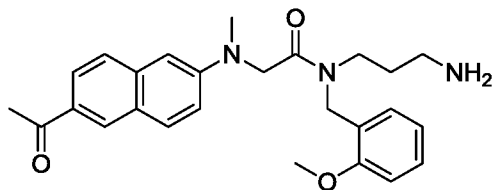
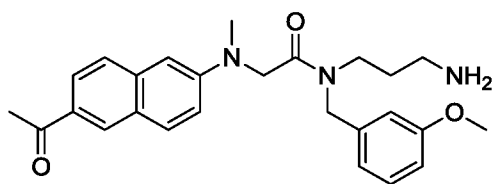
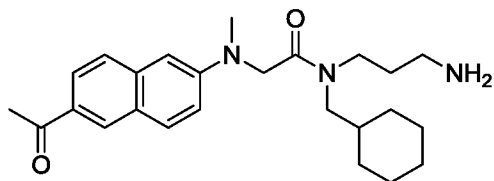
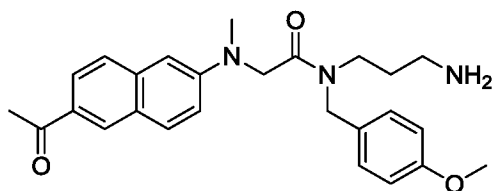
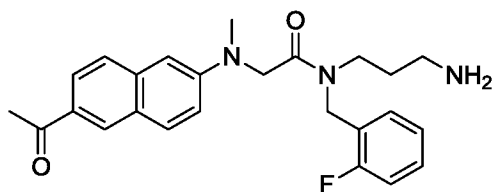
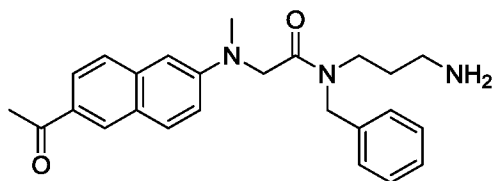
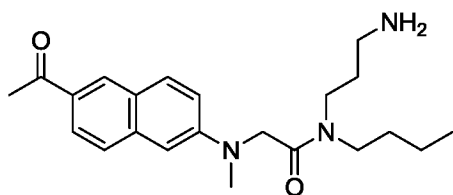
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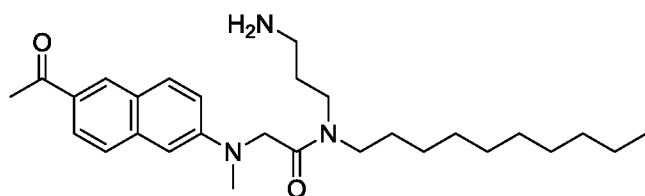
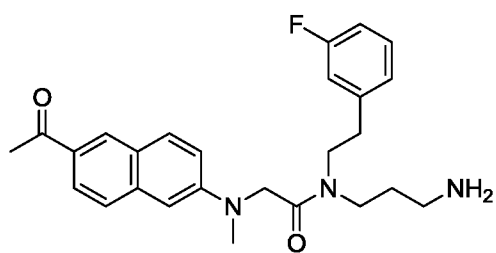
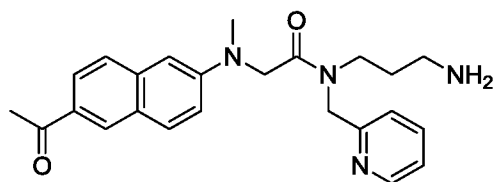
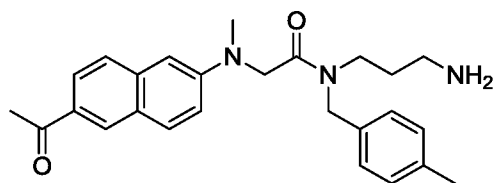
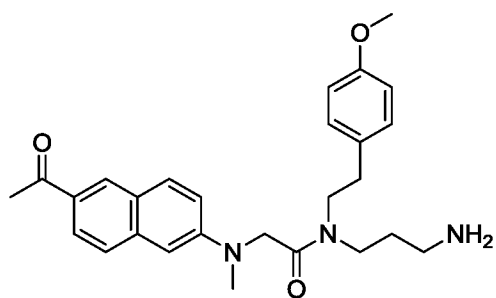
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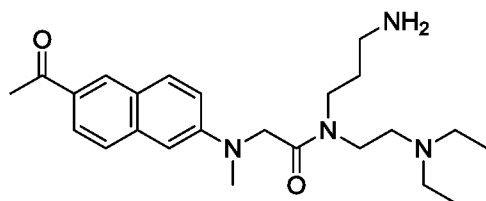
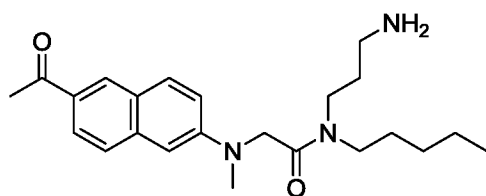
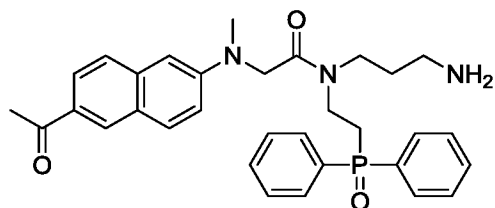
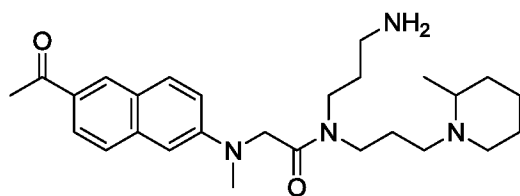
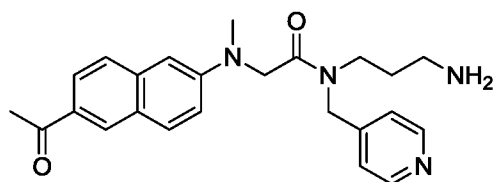
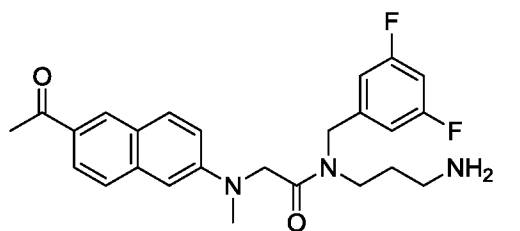
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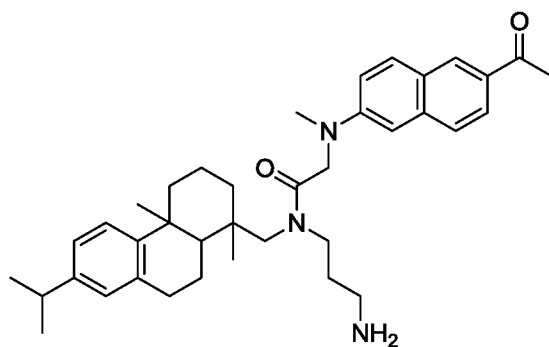
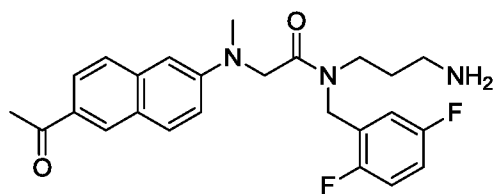
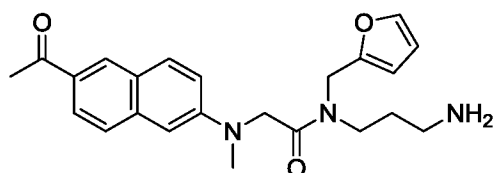
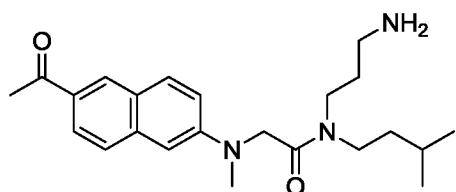
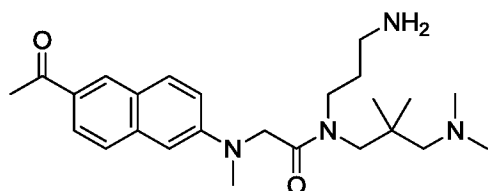
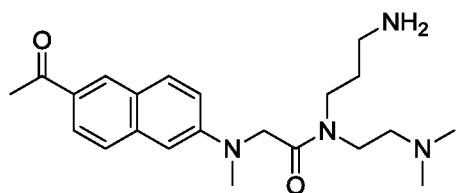
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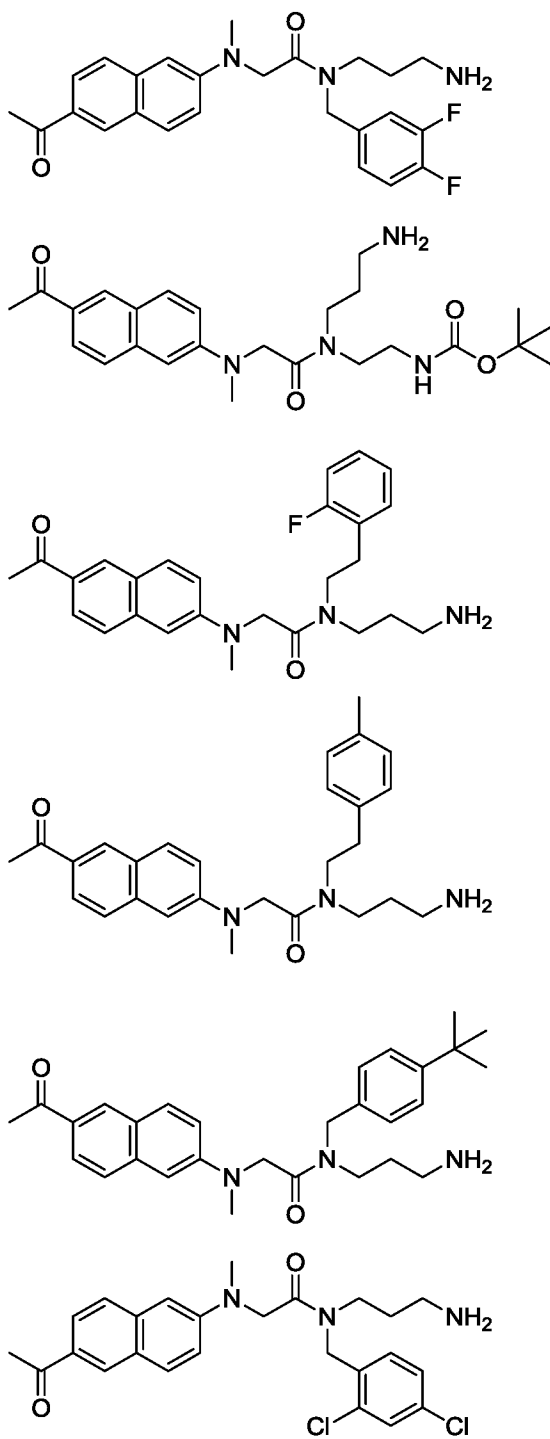
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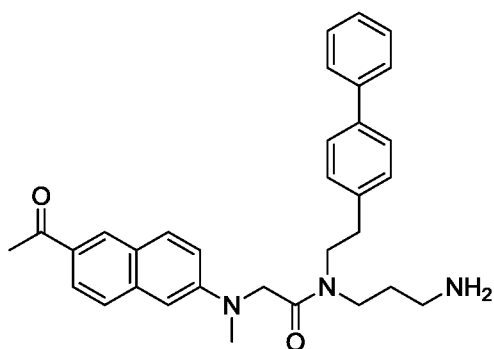
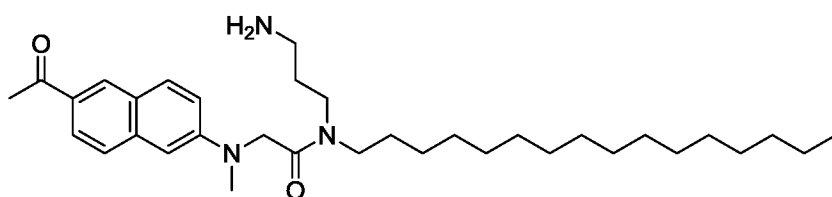
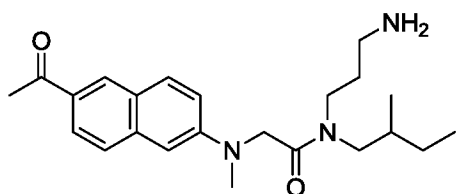
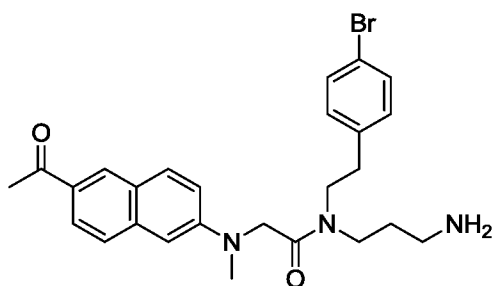
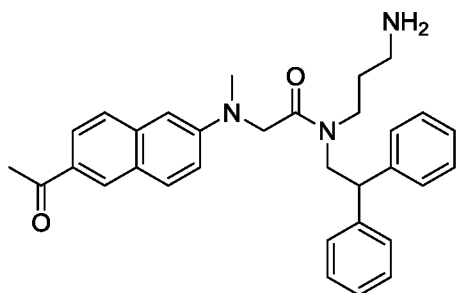
- 58 -



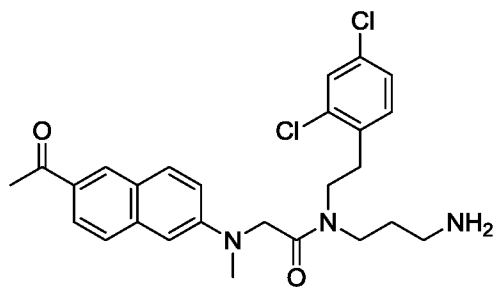
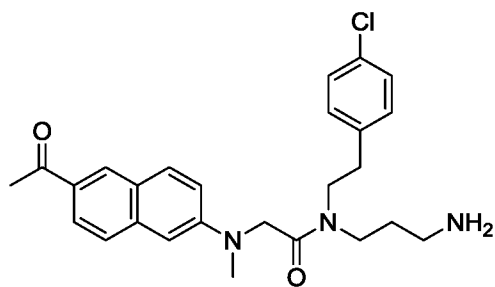
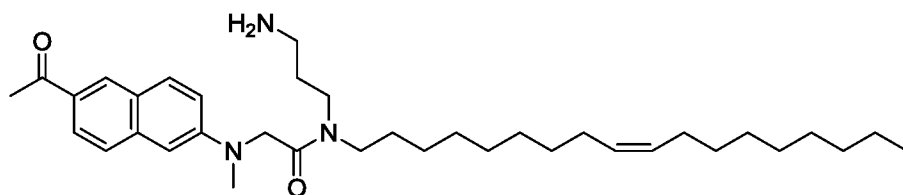
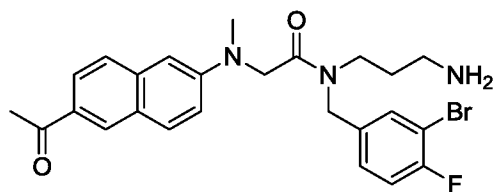
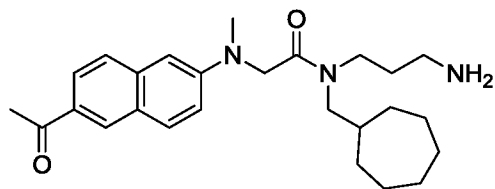
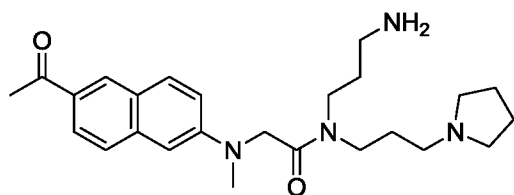
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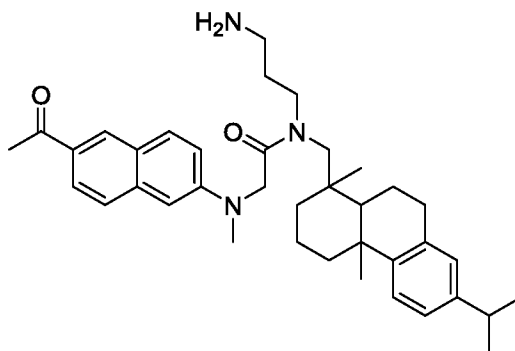


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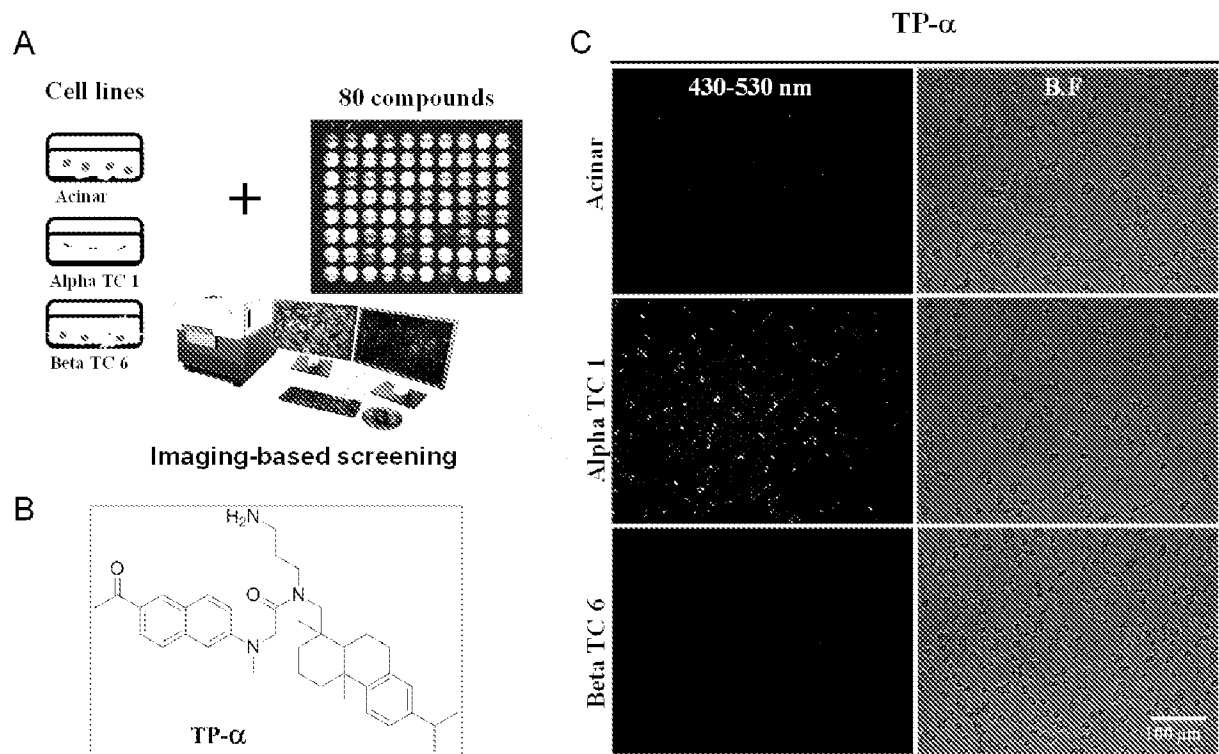
- 62 -

16. The composition of Claim 11, wherein the composition is represented by structural formula (III):



(III).

17. A method of visualizing a target cell, the method comprising:
- (a) contacting a population of the target cell with a composition of any one of Claims 1-16 to form an incubation media;
 - (b) incubating the incubation media of step (a) for a period of time sufficient to stain the target cells; and
 - (c) visualizing the stained target cells of step (b) with two-photon microscopy to visualize the target cell.
18. The method of Claim 17, wherein the target cell is a pancreatic islet cell.
19. The method of Claim 18, wherein the pancreatic islet cell is an alpha cell.



FIGs. 1A-C

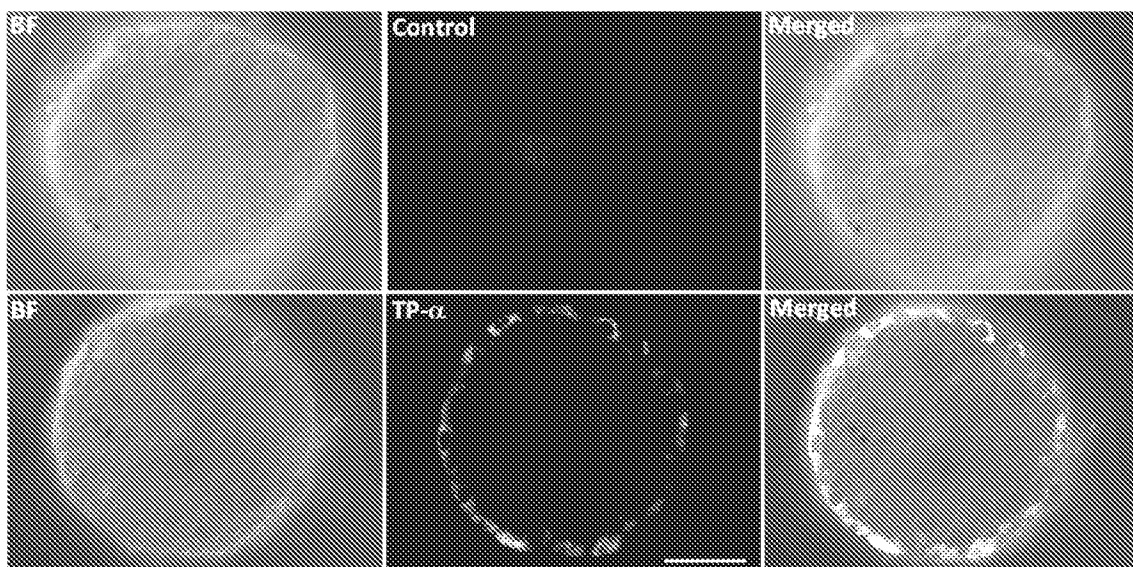


FIG. 2

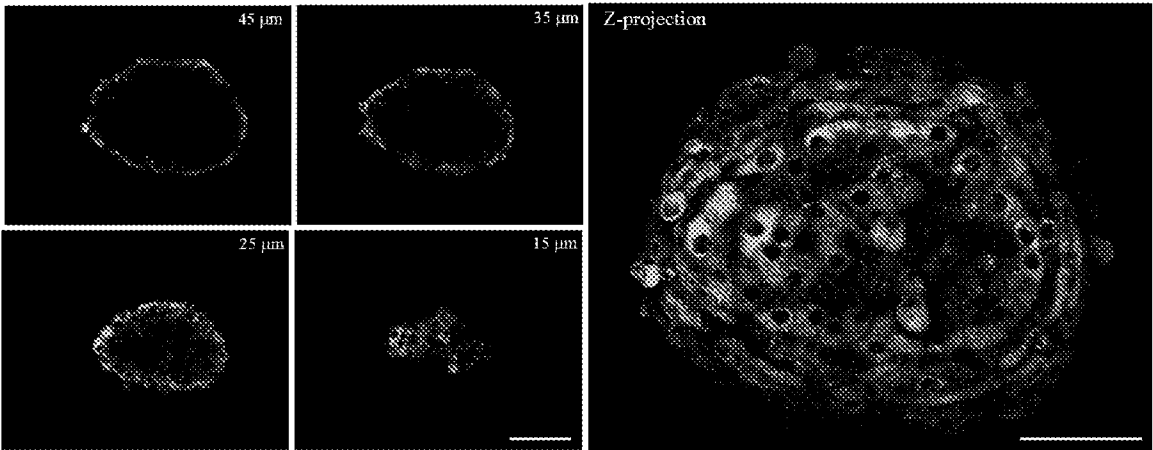
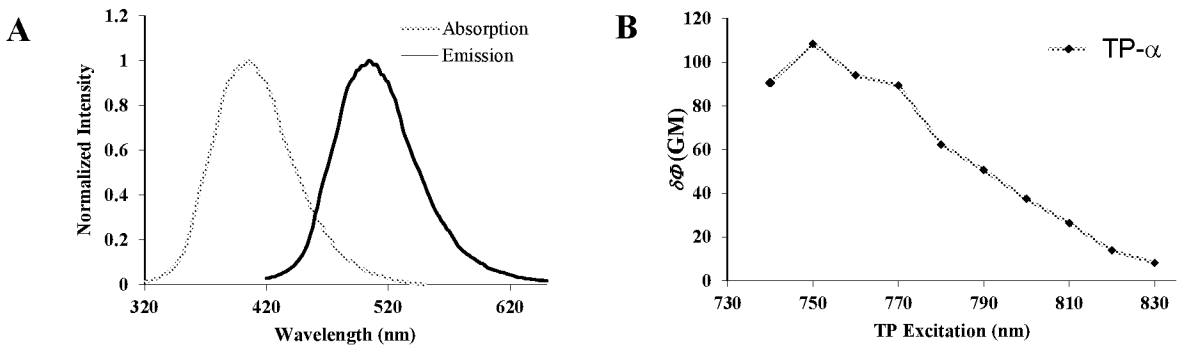
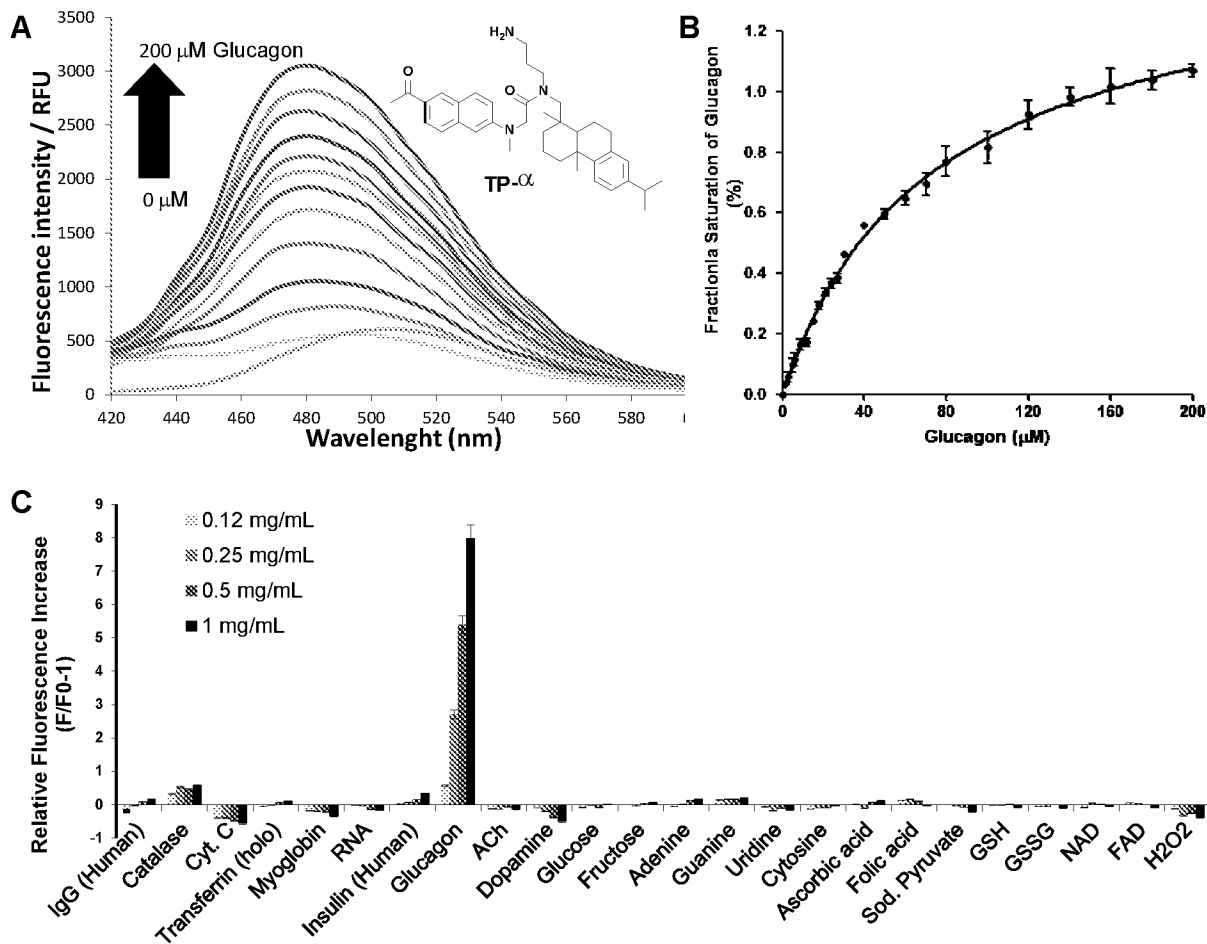


FIG. 3



FIGs. 4A-B



FIGs. 5A-C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG2016/050016

A. CLASSIFICATION OF SUBJECT MATTER

C07C 237/10 (2006.01) C07C 231/02 (2006.01) C07D 317/58 (2006.01) C07D 295/04 (2006.01) C07D 241/04 (2006.01)
C07D 213/60 (2006.01) C07D 213/24 (2006.01) C07D 207/27 (2006.01) C07D 207/06 (2006.01) G01N 33/52 (2006.01)

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)

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)

STN: Chemical Abstract Registry and CAPLUS. Substructure search based on compounds of formula (A).

Patentscope and Espacenet: Applicant and Inventor name search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier application or patent but published on or after the international filing date	"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
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
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Date of the actual completion of the international search 22 February 2016		Date of mailing of the international search report 22 February 2016	
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au		Authorised officer Ben Harris AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +612 6283 2272	

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/SG2016/050016
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/0155837 A1 (CHO) 18 June 2009 see Formula (4) para [0040]; Example 3 para [0060]; para [0041] and [0079]-[0082]	1 and 17
X	US 2001/0049374 A1 (STEELE et al.) 06 December 2001 see Example 69A, 70A, 71A and 75A para [1006]-[1007], [1012]-[1013], [1018]-[1019] and [1041]-[1042]	1
X	US 4008326 A (CALLAHAN et al.) 15 February 1977 see col. 17 Example 7, product of reacting 6-nitro-2-naphthylisocyanate	1

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INTERNATIONAL SEARCH REPORT Information on patent family members		International application No. PCT/SG2016/050016	
This Annex lists known patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.			
Patent Document/s Cited in Search Report		Patent Family Member/s	
Publication Number	Publication Date	Publication Number	Publication Date
US 2009/0155837 A1	18 June 2009	US 2009155837 A1	18 Jun 2009
		US 7955815 B2	07 Jun 2011
		KR 20090066123 A	23 Jun 2009
		KR 100924999 B1	04 Nov 2009
		KR 20100000044 A	06 Jan 2010
		KR 100956410 B1	06 May 2010
US 2001/0049374 A1	06 December 2001	US 2001049374 A1	06 Dec 2001
		US 6258822 B1	10 Jul 2001
		US 6284796 B1	04 Sep 2001
		US 6504031 B1	07 Jan 2003
US 4008326 A	15 February 1977	US 4008326 A	15 Feb 1977
		DE 2460780 A1	10 Jul 1975
		FR 2255902 A1	25 Jul 1975
		FR 2255902 B1	28 Jul 1978
		GB 1498629 A	25 Jan 1978
		JP S5095276 A	29 Jul 1975
		NL 7416238 A	30 Jun 1975
End of Annex			
Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001. Form PCT/ISA/210 (Family Annex)(July 2009)			