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ALKYLAMINO BODIPY DYES AS SELECTIVE FLUORESCENT PROBES FOR PROTEINS AND MOUSE EMBRYONIC STEM CELLS

RELATED APPLICATION(S)

- 5 This application claims the benefit of U.S. Provisional Application No. 61/497,145, filed on June 15, 2011. The entire teachings of the above application(s) are incorporated herein by reference.

BACKGROUND OF THE INVENTION

- 10 The development of small molecule sensors for the selective detection and imaging of proteins and certain cell types has great implications for biomedical and basic research. In particular, small molecule fluorophores have received significant attention as sensors in flow cytometry and fluorescence microscopy. Identification of small molecule fluorophores able to selectively discriminate one protein from a
15 complex mixture is a challenge in this field. Similarly, the development of a sensor capable of selectively staining certain cell types is highly desirable in cell-imaging research.

- BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-s-indacene) has been widely used as a fluorophore due to its high photostability and extinction coefficient, high
20 quantum yield and narrow excitation/emission bandwidth.¹⁻³ In order to develop fluorescent sensors and probes,⁴⁻⁷ several chemical reactions have been adapted to the BODIPY scaffold. Mono- and difunctionalized BODIPY derivatives can be prepared by solution-phase chemistry using nucleophilic and oxidative nucleophilic hydrogen substitutions,⁸⁻¹⁰ Knoevenagel condensations,^{11,12} Liebeskind-Srogl
25 reactions,^{13,14} and palladium-catalyzed couplings¹⁵⁻¹⁸ amongst others. However, solution-phase syntheses of BODIPY dyes often encompass tedious purification steps with typically low recovery yields. This limitation may have little impact on the synthesis of individual compounds, but it seriously hampers the combinatorial derivatization of the BODIPY scaffold,¹⁹⁻²¹ requiring labor intensive purification

processes. Solid-phase methodologies have been successfully applied to the diversification of fluorescent scaffolds that involve challenging purification steps, but the adaptability of the BODIPY structure to solid-phase chemistry has been questioned due to its lability under both basic and acidic conditions.²²

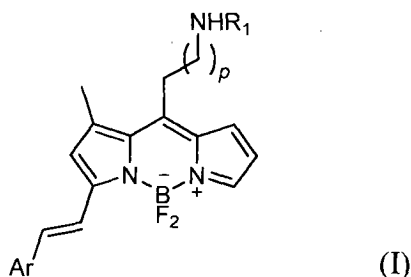
- 5 Due to the current limitations associated with solid-phase synthesis of BODIPY scaffolds, and due to their potential to operate as selective sensors in protein and cell detection and imaging, it is highly desirable to develop a combinatorial solid-phase synthesis of the BODIPY scaffold.

SUMMARY OF THE INVENTION

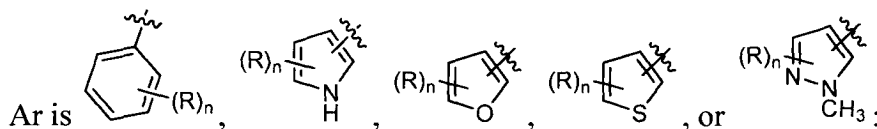
- 10 New methodologies for solid-phase synthesis of alkylamino BODIPY dyes are described herein, and include the development of selective fluorescent probes for proteins (i.e., immunoglobulins) and cell-types (i.e. mouse embryonic stem cells), and their use in target identification approaches by conjugation to chemical or photoreactive tags. These can be used as ratiometric sensors.

- 15 The present invention discloses the first solid-phase synthesis of an alkylamino BODIPY library in high purities after minimum purification steps. The alkylamino BODIPY dyes were further derivatized with chemical and photoreactive tags and the resulting compounds were assayed in two screening platforms: 1) in vitro spectral analysis against diverse purified proteins; 2) high-throughput
20 fluorescence microscopy against a panel of different cell types. These two screenings led to the discovery of an alkylamino BODIPY immunoglobulin fluorescent sensor (Ig Orange, formula (II)), and to an acetylated alkylamino BODIPY dye (CDy9, formula (IV)) which proved to selectively stain mouse embryonic stem cells (mESC) with an excellent potential for cell imaging and flow
25 cytometry applications.

 In one embodiment of the invention, an alkylamino BODIPY dye is disclosed having the structure of formula (I).



wherein:



5 each R is independently selected from H, OH, halogen, nitro, amino, (C₁-C₆)alkyl, (C=O)(C₀-C₆)alkyl, (C=O)O(C₀-C₆)alkyl, NH(C=O)(C₀-C₆)alkyl, OCF₃, CH=CH(C₀-C₆)alkyl aryl, O(C₁-C₆)alkenyl, (C₀-C₆)alkylCH=O, O(C₁-C₆)alkyl, S(C₁-C₆)alkyl, S(C₁-C₆)haloalkyl, O(C₁-C₆)haloalkyl, OCHF₂,
 10 O(C₀-C₆)alkylamino, (C₆-C₁₀)aryl, (C₅-C₁₀)heteroaryl, (C₅-C₁₀)heterocyclyl, (C₁-C₆)alkyl aryl, O(C₀-C₆)alkyl aryl, O(CH₂)_mCHCH₂ or 1-5-membered polycyclyl, wherein each 1-5-membered polycycle optionally and independently contains 1-2 ring heteroatoms selected from oxygen, nitrogen and sulfur;

15 n is 1, 2, 3 or 4;
 m is 0, 1, 2, 3 or 4;
 p is 1, 2, 3, 4, or 5;

wherein, when n is 2 or greater, the R substituents may be optionally taken together to form a fused polycyclic aromatic ring system;

20 and wherein each R is optionally substituted with 1-4 R₂ substituents independently selected from:

(C₁-C₆)alkyl, halo(C₀-C₆)alkyl, hydroxy(C₀-C₆)alkyl, (C₆-C₁₀)aryl, halo (C₆-C₁₀)aryl, hydroxy(C₆-C₁₀)aryl, O(C₁-C₆)alkyl, O(C₁-C₆)haloalkyl, (C₃-C₈)cycloalkyl, halo(C₆-C₁₀)aryl(C₁-C₆)alkoxy, halogen, amino, (C₁-C₆)alkoxy(C₆-C₁₀)aryl(C₁-C₆)alkoxy, nitro, hydroxy, halogen, (C₀-C₆)alkyl(C₆-C₁₀)aryl(C₀-C₆)alkoxy, (C₅-C₁₀)heterocycle, OCF₃, amino, O(C₁-

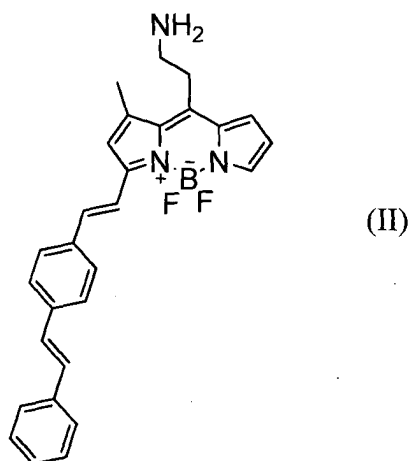
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C₆)alkylamino, (C₆-C₁₀)aryl(C₂-C₆)alkenyl, (C₂-C₆)alkenyl(C₁-C₆)alkoxy, (C₁-C₆)sulfoxy, or (C=O)O(C₀-C₆)alkyl; and

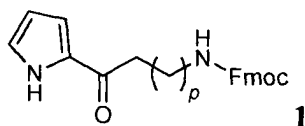
R₁ is H, C=O(C₀-C₆)haloalkyl, C=O(C₀-C₆)alkyl, C=O(C₀-C₆)alkylene or C=O(C₆-C₁₀)aryl;

5 or a pharmaceutically acceptable salt thereof.

In one embodiment of the invention, R in formula (I) is CH=CH(C₀-C₆)alkyl aryl, and n is 1. In a preferred embodiment of the invention, R is (CH=CH)phenyl. In another embodiment of the invention, each R₂ is independently selected from the group comprising H, halogen, (C₁-C₆)haloalkyl, or (C₁-C₆)alkyl. In a more preferred
10 embodiment of the invention, an alkylamino BODIPY dye is disclosed, having the formula (II).



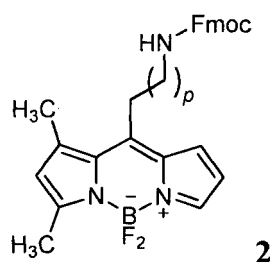
Also disclosed herein is a method for a solid phase synthesis of alkylamino
15 BODIPY dyes having the structure of formula (I), and pharmaceutically acceptable salts thereof. In one aspect of the invention, this method comprises the steps of a) reacting an Fmoc-protected amino acid and pyrrole to form a 2-ketopyrrole of formula 1;



20 wherein *p* is an integer from 1 to 5;

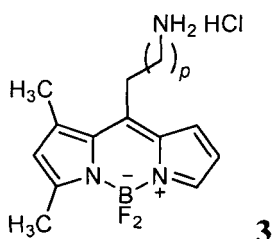
b) condensing the 2-ketopyrrole of formula 1 with 2,4-dimethylpyrrole and an activating agent with *in situ* addition of BF₃•OEt₂ to obtain compound 2;

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wherein p is an integer from 1 to 5;

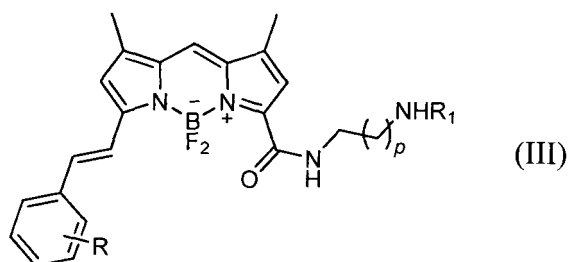
c) reacting compound **2** with a base; d) quenching the reaction mixture of step c) with aqueous HCl to produce derivative **3**;



wherein p is an integer from 1 to 5;

e) loading the derivative of formula **3** on chlorotriptyl chloride-polystyrene (CTC-PS) resin, so that the activated C₃-methyl group of the derivative is modified in a solid-phase adapted Knoevenagel-type reaction; and f) extracting the compounds of formula (I) from step e) by a solid-phase extraction.

In another embodiment of the invention, an alkylamino BODIPY dye having the structure of formula (III) is disclosed.



wherein:

each R is independently selected from H, OH, halogen, nitro, amino, (C₁-C₆)alkyl, (C=O)(C₀-C₆)alkyl, NH(C=O)(C₀-C₆)alkyl, OCF₃, CH=CH(C₀-C₆)alkylaryl, OC₁-C₆ alkenyl, (C₀-C₆)alkylCH=O, O(C₁-C₆)alkyl, S(C₁-C₆)alkyl, S(C₁-C₆)haloalkyl, O(C₁-C₆)haloalkyl, OCHF₂, O(C₀-C₆)alkylamino, (C₆-C₁₀)aryl, (C₅-C₁₀)heteroaryl, (C₅-C₁₀)heterocyclyl, (C₁-C₆)alkyl aryl, O(C₀-C₆)alkyl aryl, O(CH₂)_mCHCH₂ or 1-5 member

polycyclyl, wherein each 1-5 member polycycle optionally and independently contains 1-2 ring heteroatoms selected from oxygen, nitrogen and sulfur;

n is 1, 2, 3 or 4;

m is 0, 1, 2, 3 or 4;

5 p is 1, 2, 3, 4, or 5;

wherein, when n is 2 or greater, the R substituents may be optionally taken together to form a fused polycyclic aromatic ring system;

and wherein each R is optionally substituted with 1-4 R_2 substituents independently selected from:

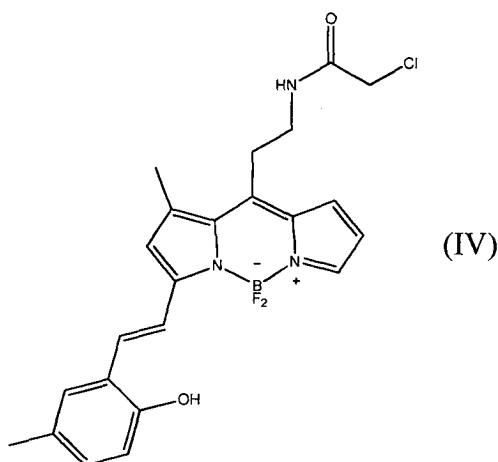
10 (C₁-C₆)alkyl, halo(C₀-C₆)alkyl, hydroxy(C₀-C₆)alkyl, (C₆-C₁₀)aryl, halo(C₆-C₁₀)aryl, hydroxy(C₆-C₁₀)aryl, O(C₁-C₆)alkyl, O(C₁-C₆)haloalkyl, (C₃-C₈)cycloalkyl, halo(C₆-C₁₀)aryl(C₁-C₆)alkoxy, halogen, amino, (C₁-C₆)alkoxy(C₆-C₁₀)aryl(C₁-C₆)alkoxy, nitro, hydroxy, halogen, (C₀-C₆)alkyl(C₆-C₁₀)aryl(C₀-C₆)alkoxy, (C₅-C₁₀)heterocycle, OCF₃, amino, O(C₁-C₆)alkylamino, (C₆-C₁₀)aryl(C₂-C₆)alkenyl, (C₂-C₆)alkenyl(C₁-C₆)alkoxy, (C₁-C₆)sulfoxy, (C=O)O(C₀-C₆)alkyl or N(CH₃)(C₁-C₆)OH;

R_1 is H, C=O(C₀-C₆)haloalkyl, C=O(C₀-C₆)alkyl, C=O(C₀-C₆)alkylene or C=O(C₆-C₁₀)aryl;

or a pharmaceutically acceptable salt thereof.

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In another aspect of the invention, the alkylamino BODIPY dye is represented by formula (IV):



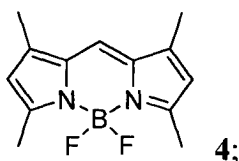
Also described herein are methods of use for the alkylamino BODIPY compounds of the present invention. In one aspect, alkylamino BODIPY dyes

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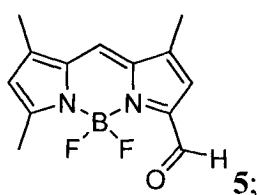
having the structure of formula (I) are used as fluorescent sensors for the identification, detections, and fluorescence imaging of cells, as well as for cytometry applications. A method for protein detection by fluorescence analysis utilizing these compounds is presently disclosed. Similar to compounds of formula (I), compounds of formula (III) may be used for the identification, detections, and fluorescence imaging of cells, and cytometry applications. In one aspect of the invention, the compounds may be used to image mouse embryonic stem cells (mESC). Furthermore, compounds of formula (III) are also utilized in the detection of proteins by fluorescence analysis.

A method for staining cells utilizing a compound of formula (I) or (III) is also disclosed herein. In a particular embodiment of the invention, mouse embryonic stem cells (mESC) are stained.

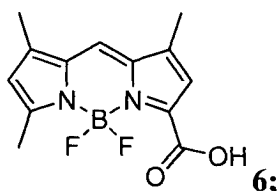
The present invention also discloses a method for the solid phase synthesis of compounds of Formula (III), and pharmaceutically acceptable salts thereof. In one aspect of the invention, this method comprises the steps of a) reacting 3,5-dimethylpyrrole-2-carboxaldehyde with 2,4-dimethylpyrrole to prepare tetramethylBODIPY **4**;



b) treating **4** with an oxidant to obtain compound **5**;



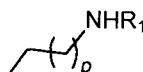
c) reacting compound **5** with a second oxidant to form derivative **6**

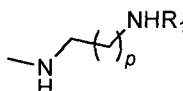


d) coupling **6** with alkylenediamine bound to chlorotriyl chloride-polystyrene (CTC-PS) resin, such that the activated C₃-methyl group of the derivative is modified in a solid-phase adapted Knoevenagel-type reaction; and

e) extracting the compound of Formula (III) from step d) by a solid-phase extraction.

Also disclosed herein are compounds in which the alkylamino moiety

5  on the BODIPY dyes of formula (I) or the alkylamino group

 of formula (III) may be further modified by conjugation to chemically reactive or photoreactive tags.

The alkylamino BODIPY dyes of the invention are fabricated by the first reported solid phase synthesis. This method begins with simple starting materials,
10 and generates the products in high yields after minimum purification, a significant departure from solution phase syntheses which typically require tedious purification and result in low material recovery. This synthetic route is highly tunable, allowing for the rapid generation of a library of alkylamino BODIPY dyes, a task which would have been restricted by previously reported syntheses. Furthermore, this scaffold is
15 easily modified by the addition of chemically reactive or photoreactive tags, emphasizing its utility as a sensor in detection and imaging of proteins and cells. Compounds of the present invention are used as selective fluorescent turn-on probes for protein detection. Furthermore, certain compounds of the present invention are selective for staining mouse embryonic stem cells (mESC) over other differentiated
20 cells types, which holds great utility for imaging, characterization, and isolation. This method is particularly advantageous over established methods because it is non-destructive and requires no genetically modified cells.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing will be apparent from the following more particular
25 description of example embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating embodiments of the present invention.

FIGs. 1a and 1b show the chemical structure of Ig Orange (BDM-69) and its fluorescence response against different proteins and peptides (20 mM HEPES buffer, pH 7.4; excitation wavelength: 510 nm, emission wavelength: 590 nm), and the fluorescence spectra of Ig Orange (BDM-69) upon increasing concentrations of human IgG (0, 1, 2, 4, 8, 17, 33 and 67 μ M) in 20 mM HEPES buffer pH 7.4; excitation wavelength: 530 nm.

FIG. 2 shows the fluorescence response of Ig Orange upon incubation with different concentrations of human IgM, IgA, and IgG.

FIG. 3 shows the fluorescence response of Ig Orange against known concentrations of IgG in immunoglobulin-depleted human serum. Values are represented as means ($n=3$) and error bars as standard deviations. Dotted grey lines indicated a 95% reliability interval. Regression coefficient (R^2) = 0.99.

FIG. 4 shows the SDS-PAGE gel and Western blotting of human non-modified and biotinylated IgG. At left, samples were solved by SDS-PAGE and stained with Coomassie Blue, and at right, samples were solved by Western blotting and immunoblots were incubated with HRP-conjugated streptavidin.

FIG. 5 shows comparative fluorescence increase of Ig Orange after incubation with different concentrations of non-modified and biotinylated human IgG. Values are represented as means ($n=3$) and error bars as standard deviations.

FIGs. 6a and 6b show binding sensorgrams of Ig Orange (0.07, 0.21, 0.62, 1.86, 5.57 and 16.70 μ M) upon injection across an immobilized immunoglobulin chip, which were fit using the equilibrium responses of a steady-state model. The inset of FIG. 6a shows a fitting to an isotherm determined a K_D value of 71.7 ± 0.6 μ M. FIG. 6b shows the determination of the region of human IgG for the binding to Ig Orange (BDM-69) by fluorescence increase of Ig Orange upon incubation with F_{ab} and F_c fragments of human IgG₁ and Job plot analysis. Values are represented as means ($n=3$) and error bars as standard deviations.

FIGs. 7a and 7b show the fluorescence response of Ig Orange (BDM-69) and BD-69 vs. human IgG, in which a 15-fold higher fluorescence increase was observed in the case of Ig Orange indicating the relevant contribution of the aminoethyl group in the interaction with human IgG, and also show binding sensorgrams of the aminoethyl-free compound (BD-69) upon injection across an immobilized

immunoglobulin chip. Different concentrations of BD-69 were injected across a control surface and the immobilized surface serially for Ig Orange. The inset displays a zoom image of the sensorgrams.

FIG. 8 shows the fluorescence intensities of Ig Orange after incubation with polyclonal human IgG and monoclonal human IgG₁. Values are represented as a means (n=3) and error bars as standard deviations.

FIG. 9 shows a fluorescence-based fractional saturation curve of Ig Orange. Values were represented as means (n=3) of the fluorescence fold increase after incubation with the protein, and adjusted to a binding saturation curve (GraphPad Prism 5.0) to estimate a dissociation constant ($K_d = 14.1 \pm 0.9 \mu\text{M}$).

FIGs. 10a and 10b show mouse embryonic stem cell staining and flow cytometry by CDy9. In FIG. 10a, mESC were selectively stained by CDy9 at 500nM for 1 h (right) compared to mouse embryonic fibroblasts (MEF, left), and in FIG. 10b, CDy9 was analyzed by flow cytometry. Cells were loaded after 1h incubation at 500nM. Magnification 10X, fluorescent images (TRITC/TXRed filters for CDy9, DAPI for nuclear staining).

FIGs. 11a and 11b show selective staining of CDy9 in mESC against a wide panel of differentiated cell types. In FIG. 11a, differentiated mESC were no longer stained by CDy9 (500nM, 1 h) upon differentiation, while in FIG. 11b CDy9 selectively stained only mESC and not any differentiated cell lineages such as mouse neuro stem cell (NS5), NS5-derived astrocytes, primary mouse neurons, mouse mixed glial cells, mouse fibroblast (3T3), mouse fibroblast-adipocytes (3T3-L1), mouse alpha TC-1 cells, mouse beta TC-6 cells and mouse primary liver cells.

FIG. 12 shows a CDy9 wash-out experiment.

FIG. 13 shows CDy9 isolation of stem cells from a mixture of MEF and mESC.

FIG. 14 shows the isolation of potential pluripotent stem cells from mouse fat pads using CDy9.

DETAILED DESCRIPTION OF THE INVENTION

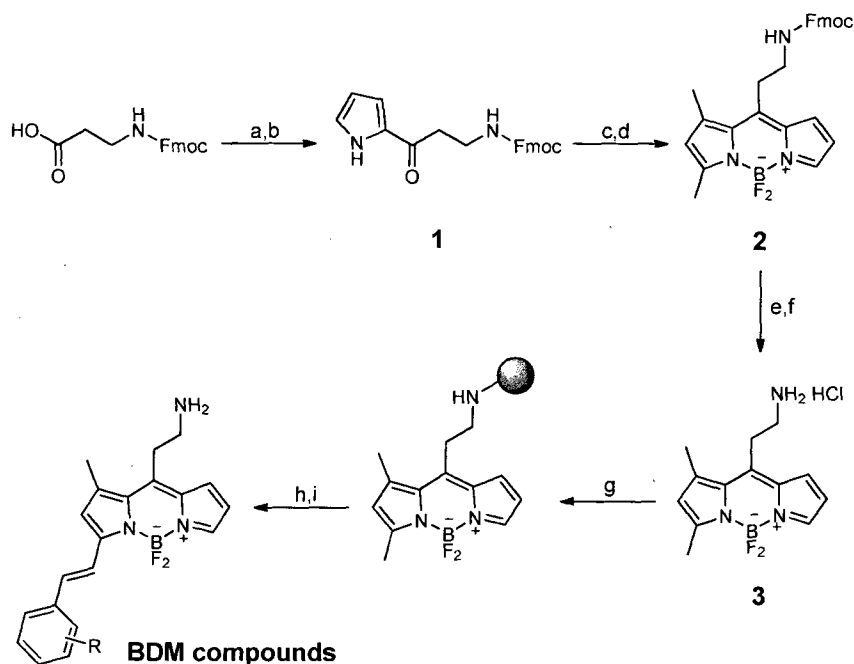
A description of example embodiments of the invention follows.

In this invention, we disclose the first solid-phase synthesis of an alkylamino BODIPY library. The synthesis was designed to include a solid-supported

aminoalkyl BODIPY-scaffold as a common intermediate, which would be efficiently functionalized *via* a solid phase-adapted Knoevenagel reaction in order to generate a library of alkylamino BODIPY dyes. The alkylamino BODIPY dyes of the invention demonstrate diverse utility as sensors for detection and imaging applications in biomedical and basic research. Due to its high photostability and extinction coefficient, as well as its narrow excitation and emission bandwidth, the BODIPY scaffold is an effective fluorophore for use as a sensor with protein samples. Furthermore, these alkylamino BODIPY scaffolds hold potential for use in selective staining of certain lines of stem cells.

Synthesis of an alkylamino BODIPY library.

Scheme 1.



Scheme 1: Representative synthesis of the alkylamino BODIPY scaffold **3** and its derivatization in solid-phase to render substituted aminoethyl BODIPY compounds. Reagents and conditions: a) 2,2'-dipyridyldisulfide, dry tetrahydrofuran, room temperature, 20 h; b) pyrrole, CH_3MgBr , dry tetrahydrofuran, -78°C , 1 h; c) 2,4-dimethylpyrrole, POCl_3 , CH_2Cl_2 , 35°C , 20 h; d) $\text{BF}_3\cdot\text{OEt}_2$, diisopropylethylamine, CH_2Cl_2 , room temperature, 4 h; e) 1,8-Diazabicyclo[5.4.0]undec-7-ene, CH_2Cl_2 , room temperature, 1 h; f) diluted HCl (aq); g) chlorotriptyl chloride-polystyrene (CTC-PS) resin, diisopropylethylamine, N-methylpyrrolidone, room temperature, 20 h; h) RCHO , pyrrolidine, AcOH , dimethylsulfoxide-acetonitrile (1:1), 85°C , 5-10 min; i) trifluoroacetic acid- CH_2Cl_2 (0.5:99.5), room temperature, 2×10 min.

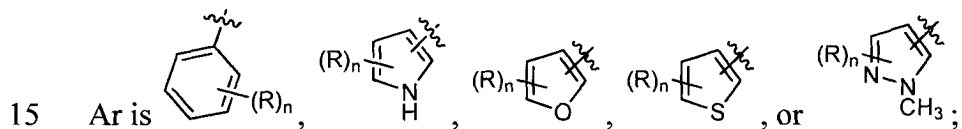
Scheme 1 shows a representative synthesis of an alkylamino-containing BODIPY scaffold **3**, its binding to a solid support resin, and its solid-supported derivitization to a substituted alkylamino-BODIPY dye. The starting material in the invention is an N-Fmoc-alkylamino acid, where Fmoc is 9-fluorenylmethyloxycarbonyl. In some embodiments of the invention, the starting material can be N-Fmoc-7-aminoheptanoic acid, N-Fmoc-6-aminohexanoic acid, N-Fmoc-5-amino-pentanoic acid, N-Fmoc-4-aminobutanoic acid, or N-Fmoc- β -alanine. In a preferred embodiment of the invention, and as is shown in the representative synthesis of an alkylamino-containing BODIPY scaffold **3** in Scheme 1, the starting material for this transformation is N-Fmoc- β -alanine. In steps a-b, N-Fmoc- β -alanine is reacted with 2,2'-dipyridyldisulfide followed by reaction with pyrrole at about -78°C to generate compound **1**. In one aspect of the invention, the preceding transformations take place in an organic solvent selected from a list comprising toluene, tetrahydrofuran, diethyl ether, and dichloromethane, and in a preferred embodiment, the preceding transformation is carried out in tetrahydrofuran.

In steps c-d, the amide formed in the preceding reaction, of which **1** is representative, is then reacted with 2,4-dimethylpyrrole and an activating agent at about 35°C in an organic solvent. In some embodiments of the invention, the activating reagent is trifluoroacetic acid or POCl_3 . In preferred embodiment of the invention, the activating agent is POCl_3 . In certain embodiments of the invention, the organic solvent is a chlorinated organic solvent such as dichloroethane, dichloromethane, or chloroform, and in a more preferred embodiment of the invention, the solvent is dichloromethane. Then, $\text{BF}_3\cdot\text{OEt}_2$ is added *in situ* and a BODIPY derivative is generated, as is represented by **2** in Scheme 1. Next, in step e,

the protecting group is removed from nitrogen by addition of a base. In one aspect of the invention, this is accomplished by the addition of piperidine, solid-supported piperidine or DBU (1,8-Diazabicycloundec-7-ene), and in a preferred embodiment, the deprotection results from the addition of DBU (1,8-Diazabicycloundec-7-ene).

- 5 An acid salt of the amine is then generated in step f, which occurs by addition of an aqueous solution of an acid. In some embodiments of the invention, the acid can be hydrochloric acid, hydrobromic acid, hypochlorous acid, phosphoric acid, or acetic acid, while in preferred embodiments of the invention, the acid salt of the amine is formed from the addition of hydrochloric acid (HCl).

- 10 In step g, the amine salt is loaded onto a solid support resin. In a preferred embodiment, the salt is loaded onto a chlorotriyl chloride polystyrene (CTC-PS) resin. Next, in step h of Scheme 1, the activated C₃-methyl group of the BODIPY structure could be modified in a solid-phase adapted Knoevenagel-type reaction²⁴ by reaction with an aldehyde of the formula ArCHO, wherein:



- each R is independently selected from H, OH, halogen, nitro, amino, (C₁-C₆)alkyl, (C=O)(C₀-C₆)alkyl, (C=O)O(C₀-C₆)alkyl, NH(C=O)(C₀-C₆)alkyl, OCF₃, CH=CH(C₀-C₆)alkyl aryl, O(C₁-C₆)alkenyl, (C₀-C₆)alkylCH=O, O(C₁-C₆)alkyl, S(C₁-C₆)alkyl, S(C₁-C₆)haloalkyl, O(C₁-C₆)haloalkyl, OCHF₂,
20 O(C₀-C₆)alkylamino, (C₆-C₁₀)aryl, (C₅-C₁₀)heteroaryl, (C₅-C₁₀)heterocyclyl, (C₁-C₆)alkyl aryl, O(C₀-C₆)alkyl aryl, O(CH₂)_mCHCH₂ or 1-5membered polycyclyl, wherein each 1-5-membered polycycle optionally and independently contains 1-2 ring heteroatoms selected from oxygen, nitrogen and sulfur;

- n is 1, 2, 3 or 4;

- 25 m is 0, 1, 2, 3 or 4;

wherein, when n is 2 or greater, the R substituents may be optionally taken together to form a fused polycyclic aromatic ring system;

and wherein each R is optionally substituted with 1-4 R₂ substituents independently selected from:

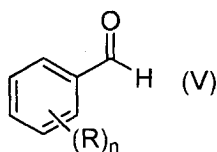
- 30 (C₁-C₆)alkyl, halo(C₀-C₆)alkyl, hydroxy(C₀-C₆)alkyl, (C₆-C₁₀)aryl, halo (C₆-C₁₀)aryl, hydroxy(C₆-C₁₀)aryl, O(C₁-C₆)alkyl, O(C₁-C₆)haloalkyl, (C₃-

C₈)cycloalkyl, halo(C₆-C₁₀)aryl(C₁-C₆)alkoxy, halogen, amino, (C₁-C₆)alkoxy(C₆-C₁₀)aryl(C₁-C₆)alkoxy, nitro, hydroxy, halogen, (C₀-C₆)alkyl(C₆-C₁₀)aryl(C₀-C₆)alkoxy, (C₅-C₁₀)heterocycle, OCF₃, amino, O(C₁-C₆)alkylamino, (C₆-C₁₀)aryl(C₂-C₆)alkenyl, (C₂-C₆)alkenyl(C₁-C₆)alkoxy, (C₁-C₆)sulfoxy, (C=O)O(C₀-C₆)alkyl or
 5 N(CH₃)(C₁-C₆)OH.

The cleavage conditions of step i are provided by about 0.5% trifluoroacetic acid in dichloromethane. A solid-phase extraction renders aminoethyl styryl-BODIPY compounds (BDM) with purities averaging about 91%. Example structures of BDM compounds and the characterization data is found in Table 1.

10 In another aspect of the invention, the incorporation of alkylamino moieties in the BODIPY core has been also accomplished in other positions of the BODIPY structure. This is demonstrated by Scheme 2, which shows a representative pathway for the synthesis of BDMD compounds. The synthesis of BDMD compounds begins by reacting 3,5-dimethylpyrrole-2-carboxaldehyde with 2,4-dimethylpyrrole to
 15 prepare tetramethylBODIPY **4**. Compound **4** is treated with an oxidant in step a to generate compound **5**. In one aspect of the invention, the oxidant is selected from chloranil or DDQ (2,3-Dichloro-5,6-dicyano-1,4-benzoquinone). In a preferred embodiment of the invention, the oxidant is DDQ (2,3-Dichloro-5,6-dicyano-1,4-benzoquinone). Step b converts aldehyde **5** to carboxylic acid **6** by reacting with
 20 additional oxidant. In some preferred aspects of the invention, the oxidant to achieve this transformation is NaClO₂ with 2-methylbut-2-ene and NaH₂PO₄, or else potassium permanganate (KMnO₄).

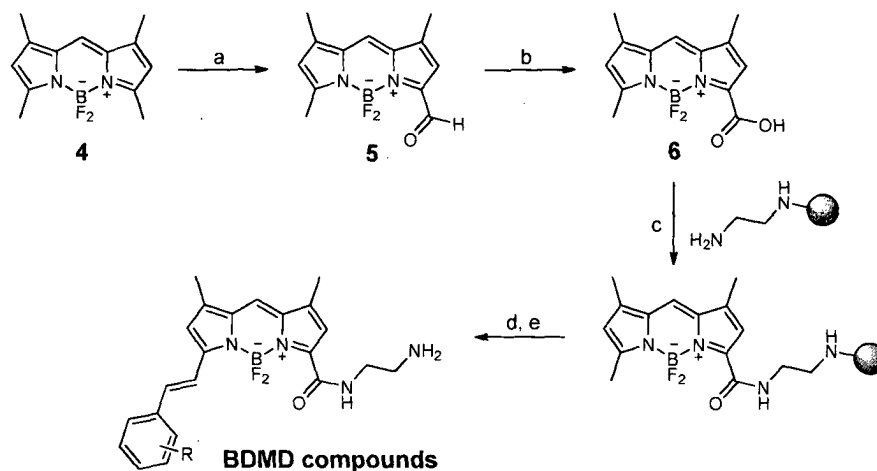
In step c, carboxylic acid **6** is loaded onto a solid-supported resin containing an alkylenediamine linker. In a preferred embodiment of the invention, the acid is
 25 reacted with ethylenediamine-chlorotriyl chloride polystyrene (ethylenediamine-CTC-PS) resin. Analogous to the BDM compounds, in step d, the activated C₃-methyl group of the BODIPY structure could be modified in a solid-phase adapted Knoevenagel-type reaction²⁴ by reaction with an aldehyde of the formula (V).



- Wherein: each R is independently selected from H, OH, halogen, nitro, amino, (C₁-C₆)alkyl, (C=O)(C₀-C₆)alkyl, (C=O)O(C₀-C₆)alkyl, NH(C=O)(C₀-C₆)alkyl, OCF₃, CH=CH(C₀-C₆)alkyl aryl, O(C₁-C₆)alkenyl, (C₀-C₆)alkylCH=O, O(C₁-C₆)alkyl, S(C₁-C₆)alkyl, S(C₁-C₆)haloalkyl, O(C₁-C₆)haloalkyl, OCHF₂,
- 5 O(C₀-C₆)alkylamino, (C₆-C₁₀)aryl, (C₅-C₁₀)heteroaryl, (C₅-C₁₀)heterocyclyl, (C₁-C₆)alkyl aryl, O(C₀-C₆)alkyl aryl, O(CH₂)_mCHCH₂ or 1-5membered polycyclyl, wherein each 1-5-membered polycycle optionally and independently contains 1-2 ring heteroatoms selected from oxygen, nitrogen and sulfur;
- n is 1, 2, 3 or 4;
- 10 m is 0, 1, 2, 3 or 4;
- wherein, when n is 2 or greater, the R' substituents may be optionally taken together to form a fused polycyclic aromatic ring system;
- and wherein each R is optionally substituted with 1-4 R₂ substituents independently selected from:
- 15 (C₁-C₆)alkyl, halo(C₀-C₆)alkyl, hydroxy(C₀-C₆)alkyl, (C₆-C₁₀)aryl, halo (C₆-C₁₀)aryl, hydroxy(C₆-C₁₀)aryl, O(C₁-C₆)alkyl, O(C₁-C₆)haloalkyl, (C₃-C₈)cycloalkyl, halo(C₆-C₁₀)aryl(C₁-C₆)alkoxy, halogen, amino, (C₁-C₆)alkoxy(C₆-C₁₀)aryl(C₁-C₆)alkoxy, nitro, hydroxy, halogen, (C₀-C₆)alkyl(C₆-C₁₀)aryl(C₀-C₆)alkoxy, (C₅-C₁₀)heterocycle, OCF₃, amino, O(C₁-C₆)alkylamino, (C₆-C₁₀)aryl(C₂-
- 20 C₆)alkenyl, (C₂-C₆)alkenyl(C₁-C₆)alkoxy, (C₁-C₆)sulfoxy, (C=O)O(C₀-C₆)alkyl or N(CH₃)(C₁-C₆)OH;

The cleavage conditions of step e are provided by about 0.5% trifluoroacetic acid in dichloromethane.

Scheme 2.



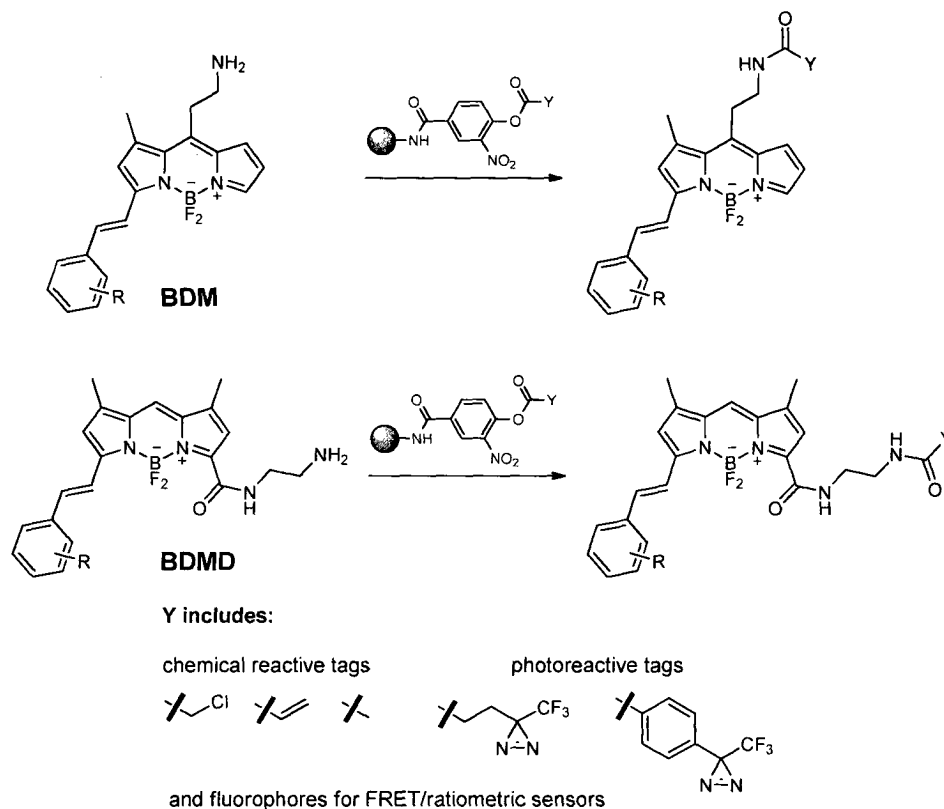
Scheme 2: Representative synthesis of the BODIPY scaffold **6** and its derivatization on solid-phase to render alkylamino BODIPY dyes (BDMD compounds) with different linker position. Reagents and conditions: a) 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone, dry tetrahydrofuran, room temperature, 4 h; b) potassium permanganate, tetrahydrofuran; c) ethylenediamine-CTC-PS resin, diisopropylethylamine, N-methylpyrrolidone, r.t., 20 h; d) RCHO, pyrrolidine, AcOH, DMSO-ACN (1:1), 85 °C, 5-10 min; e) TFA-DCM (0.5:99.5), r.t., 2 × 10 min.

5

The alkylamino linkers in both BDM and BDMD compounds of the present invention are particularly advantageous because they may be easily modified with chemically reactive or photoreactive tags. In some aspects of the invention, chemically reactive and photoreactive tags can be used for target identification studies, which is a key step and one of the bottlenecks of drug discovery and target validation programs. As depicted in Scheme 3, the BDM and BDMD compounds of the invention may undergo chemical modification of alkylamino moiety. In some aspects of the invention, this chemical modification is a purification-free, solid-phase approach that involves reaction of the amine groups with solid-supported reactive esters. In some aspects of the invention, the chemically reactive tag is in the form of an acetyl group, a chloroacetyl group, or an acryloyl group. In another aspect of the invention, the photoreactive tag is an alkyl- or aryl(trifluoromethyl)diazirine. In yet another aspect of the invention, any of the chemically reactive or photoreactive tags are utilized for the construction of FRET and/or ratiometric sensors.

15

Scheme 3.



Scheme 3: Derivatization of BDM and BDMD compounds using a reactive ester support strategy. BDM and BDMD compounds may incorporate chemically reactive tags, photoreactive tags, or react with other acetylating reagents.

5

Use of BDM Compounds.

BDM compounds of the present invention show diverse spectroscopic properties (λ_{abs} : from about 536 to about 622 nm, λ_{em} : from about 561 to about 685 nm), with an average quantum yield around 0.3 that asserts their potential as fluorescent turn-on probes for proteins. In addition to acting as fluorescent sensors, the BDM compounds of the present invention are also particularly suitable for the imaging and staining of certain cell lines.

The discrimination of proteins in complex mixtures remains a challenging issue in sensor development. With the aim of discovering novel BODIPY-based fluorescent turn-on protein probes, the fluorescence properties of the whole library were examined against various proteins and peptides. A number of BDM

15

compounds exhibit a turn-on effect upon incubation with different proteins/peptides, with BDM-69 demonstrating the best selectivity profile among the whole library (FIG. 1a). BDM-69 exhibits a remarkable 75-fold turn-on fluorescence increase in the presence of human IgG (FIG. 1b). On the basis of this selectivity pattern and
5 fluorescence color, we named BDM-69 as Ig Orange.

The following experiments were aimed at determining the dissociation constant of the interaction between Ig Orange and human IgG. Surface Plasmon Resonance (SPR) has been extensively used to measure the binding constants between small molecules and biopolymers, 25 and we modified human IgG with
10 biotin so that it could be immobilized on a neutravidin-coated sensor chip. Upon immobilization of the biotinylated protein on a neutravidin-coated sensor chip CM7, we performed Ig Orange titrations in duplicate with increasing concentrations of the small molecule. The resulting binding sensorgrams were fit using the equilibrium responses (steady-state model) with a K_D value of $71.7 \pm 0.6 \mu\text{M}$ (FIG. 6a).
15 Additional SPR experiments confirmed that both aminoethyl and stilbene moieties of Ig Orange were necessary for the interaction with the immunoglobulin, since the aminoethyl-free derivative of Ig Orange did not show any significant binding to human IgG (FIG. 7).

In order to identify the region of human IgG to which Ig Orange binds, we
20 evaluated the fluorescence response of Ig Orange in the presence of human polyclonal IgG and monoclonal IgG₁, the most abundant subtype. Ig Orange underwent a similar turn-on effect in both cases, indicating that the binding took place at the backbone structure of the immunoglobulin rather than at the antigen-binding site. In view of this result, we analyzed the fluorescence increase after
25 incubation with the separate F_{ab} and F_c fragments of the human monoclonal IgG₁. As shown in FIG. 6b, Ig Orange exhibited a preferred binding to the F_{ab} region of the immunoglobulin when compared to the F_c fragment. To confirm this observation and determine the stoichiometry of the complex between Ig Orange and the full immunoglobulin, we analyzed the interaction by means of a Job plot. A maximum
30 fluorescence increase was detected for a 2:1 Ig Orange:immunoglobulin ratio, which supported our observation that the preferred binding region of Ig Orange corresponded to the F_{ab} fragments of the immunoglobulin (FIG. 7b). In conclusion,

the analysis of the fluorescence response of Ig Orange with the two F_{ab} and F_c fragments of an immunoglobulin pointed out that Ig Orange preferably bound to the F_{ab} region of the backbone structure of the immunoglobulin, which is consistent with the 2:1 stoichiometry for the Ig Orange:immunoglobulin complex determined by Job plot analysis. With this first adaptation of the BODIPY scaffold to a solid-phase approach we accelerated the development of alkylamino BODIPY-based fluorescent probes, being Ig Orange the first immunoglobulin fluorescent sensor.

Alkylamino BODIPY probes may also be used to selectively stain mESC (mouse embryonic stem cells). To determine which BODIPY compounds of the invention are preferred probes, we assayed the staining properties of BDM compounds and their corresponding chloroacetylated derivatives in a high-throughput cell imaging-based screening. CDy9 was identified as the BODIPY compound that stained mESC and not MEF (mouse embryonic fibroblast) in the most selective manner (FIG. 10a). Furthermore, flow cytometry assays proved that CDy9 could be used for the isolation and characterization of mESC (FIG. 10b). In addition to its excellent potential for the imaging, isolation and characterization of mESC, CDy9 overcomes some limitations of the conventionally stem cell research tools for mESC identification such as antibodies or GFP/luciferase-based mESC reporters by providing a protocol that is non-destructive, simple and does not require genetically-modified cells.

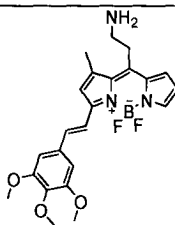
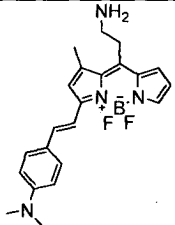
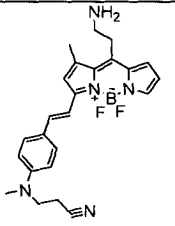
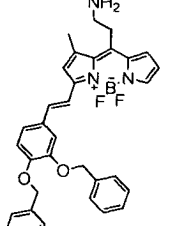
After identifying the potential of CDy9 for mESC staining, we further evaluated its selectivity against a wide panel of different cell lines. Fluorescent mESC probes must ideally turn-on only in the presence of mESC and not fluoresce whenever mESC are differentiated into other lineages. As shown in FIG. 11a, CDy9 can selectively stain mESC and not those cells which undergo a differentiation process. In addition, we proved that CDy9 does not stain any of the three germ layers (ectoderm, mesoderm and endoderm) in which mESC can differentiate to. CDy9 showed no staining in a number of cell lines from the different germ layers (FIG. 11b, ectoderm: mouse neuro stem cell (NS5), NS5-derived astrocytes, primary mouse neurons and mouse mixed glial cells; mesoderm: mouse fibroblasts (3T3 and C2C12) and mouse fibroblast-adipocytes (3T3-L1); endoderm: mouse alpha TC-1 cells, mouse beta TC-6 cells and mouse primary liver cells), which demonstrates its

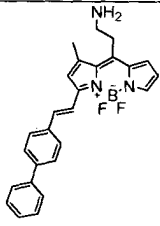
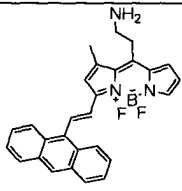
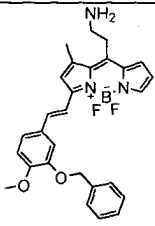
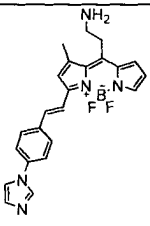
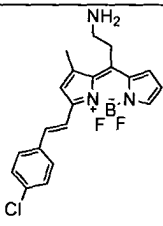
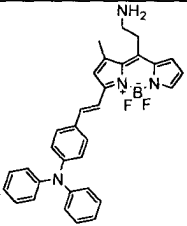
general application as a fluorescent probe for the imaging, isolation and characterization of mESC.

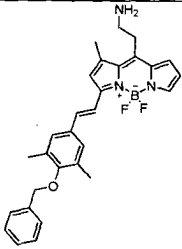
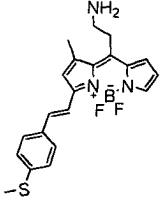
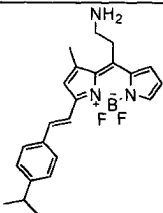
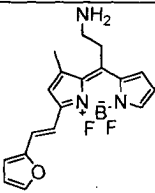
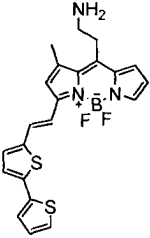
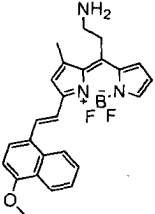
The alkylamino BODIPY dyes of the invention show particular utility as selective fluorescent sensors for use with proteins and certain cell lines.

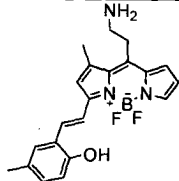
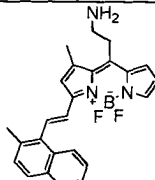
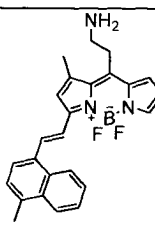
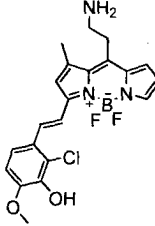
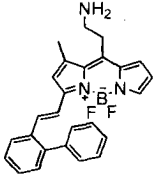
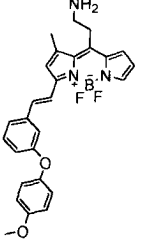
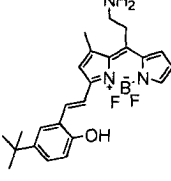
- 5 Furthermore, they selectively stain mESC over MEF, which holds great potential for biomedical research applications.

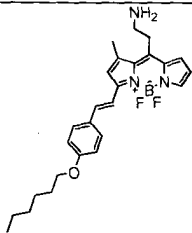
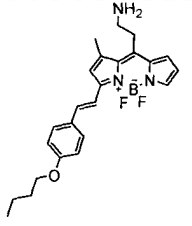
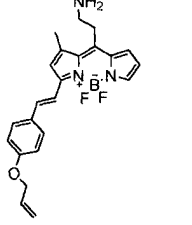
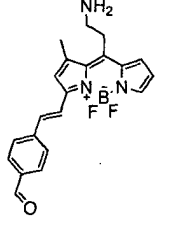
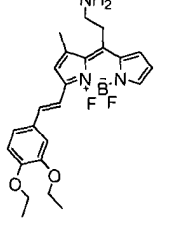
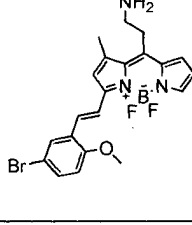
Table 1. Chemical structures and characterization data for the BDM library.

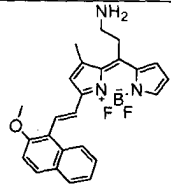
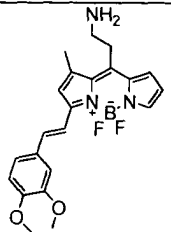
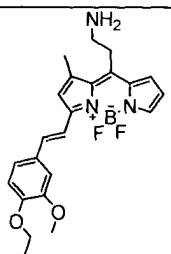
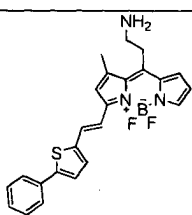
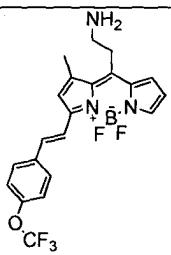
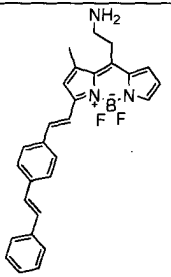
Code	Structure	Mcalc.	Mexp.	tR (min)	purity	λ_{max} (ABS)	λ_{max} (EM)	ϕ	mg
BDM-1		441.3	422.0	3.74	87%	561	586	0.21	5.1
BDM-2		394.3	375.0	3.96	88%	607	685	0.01	5.8
BDM-3		433.3	414.0	4.02	87%	600	683	0.05	5.9
BDM-4		563.4	543.9	5.48	95%	566	590	0.42	6.1

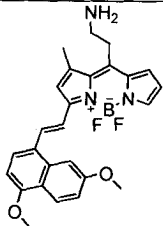
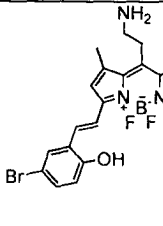
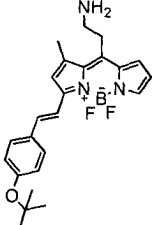
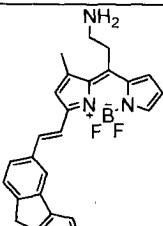
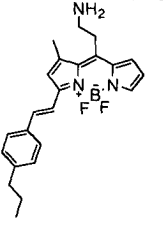
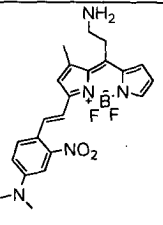
BDM-5		427.3	408.0	5.16	96%	561	575	0.50	7.7
BDM-6		451.3	432.0	4.97	89%	536	682	0.12	9.0
BDM-7		487.3	467.9	4.68	89%	566	591	0.37	6.6
BDM-8		417.3	418.0	2.31	95%	561	575	0.34	3.9
BDM-9		365.3	346.0	4.21	88%	554	567	0.33	3.5
BDM-12		385.3	365.9	4.46	90%	556	567	0.32	6.2
BDM-14		518.3	498.9	6.18	96%	593	624	0.02	7.8

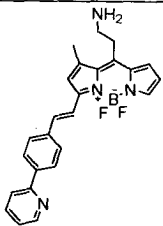
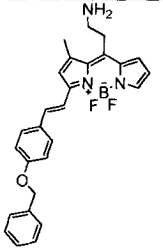
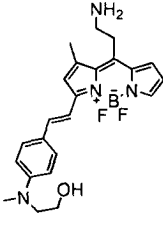
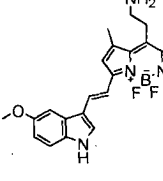
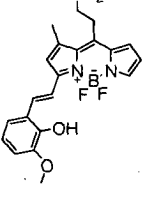
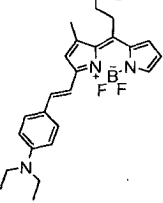
BDM-16		485.4	466.0	5.46	94%	561	574	0.14	4.9
BDM-18		397.3	378.0	4.61	100%	567	588	0.33	6.7
BDM-19		393.3	374.0	4.91	90%	555	569	0.71	8.0
BDM-22		341.3	322.0	3.61	85%	567	584	0.14	5.8
BDM-30		439.3	419.9	4.99	86%	596	634	0.11	4.4
BDM-32		431.3	412.0	4.62	91%	576	624	0.25	5.6

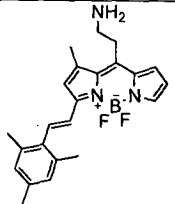
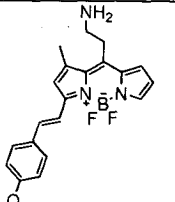
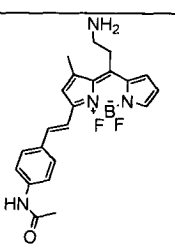
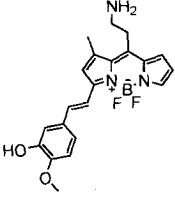
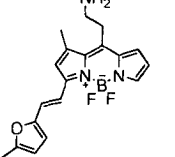
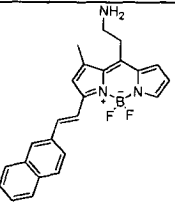
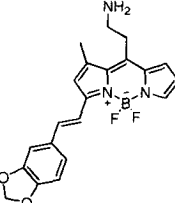
BDM-33		381.3	362.0	3.69	88%	564	580	0.14	9.7
BDM-34		415.3	396.0	4.98	92%	547	581	0.65	7.4
BDM-36		415.3	396.0	5.02	94%	568	595	0.23	6.9
BDM-38		432.3	411.9	3.71	87%	565	579	0.06	9.8
BDM-40		427.3	408.0	4.94	88%	554	567	0.53	5.6
BDM-42		473.3	454.0	5.06	93%	554	563	0.25	4.3
BDM-43		423.3	404.0	4.55	97%	564	579	0.21	4.5

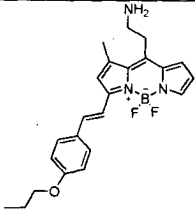
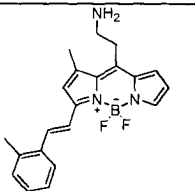
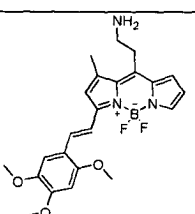
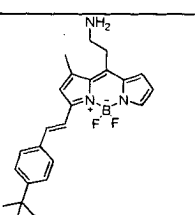
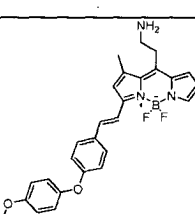
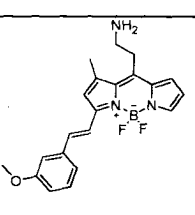
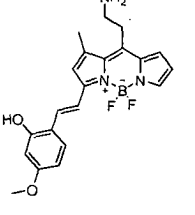
BDM-45		451.3	432.0	5.47	93%	564	583	0.74	4.5
BDM-46		423.3	404.0	5.30	94%	564	583	0.53	6.0
BDM-48		407.3	388.0	4.46	98%	563	583	0.83	4.0
BDM-49		379.2	360.0	3.76	92%	562	572	0.24	5.7
BDM-53		439.3	420.0	4.07	87%	568	600	0.46	5.9
BDM-54		460.3	439.9 441.9	4.39	88%	559	573	0.52	6.2

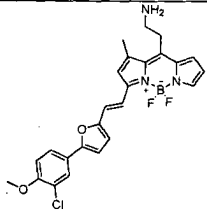
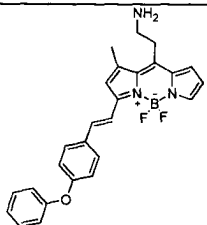
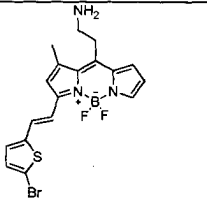
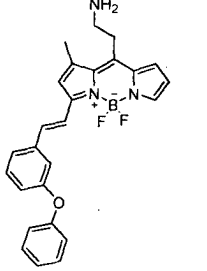
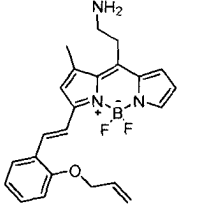
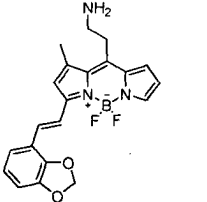
BDM-61		431.3	411.9	4.73	91%	576	610	0.22	8.7
BDM-62		411.3	392.0	3.58	89%	567	594	0.43	5.2
BDM-63		425.3	406.0	4.01	94%	568	597	0.54	6.5
BDM-65		433.3	413.9	5.21	84%	589	615	0.15	7.2
BDM-68		435.3	415.9	4.90	87%	551	561	0.63	5.5
BDM-69		453.3	434.2	5.01	94%	574	594	0.32	8.6

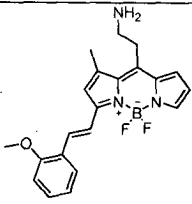
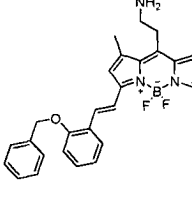
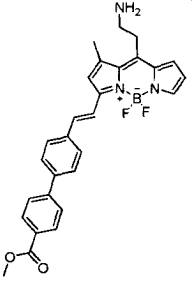
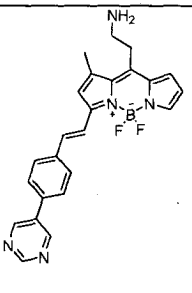
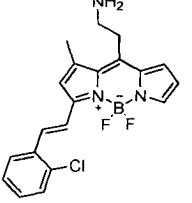
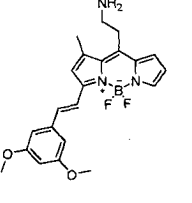
BDM-70		461.3	442.0	4.82	93%	582	642	0.13	7.1
BDM-73		446.3	425.8 427.8	3.89	84%	561	575	0.28	4.4
BDM-75		423.3	404.0	4.64	88%	560	585	0.53	4.4
BDM-76		439.3	420.0	5.20	84%	572	588	0.25	4.0
BDM-77		393.3	374.0	5.03	84%	557	569	0.54	5.2
BDM-78		439.3	420.0	4.55	87%	591	n.d	0.01	5.0

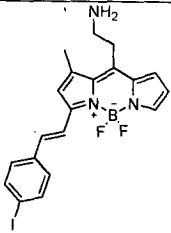
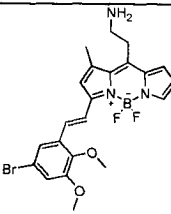
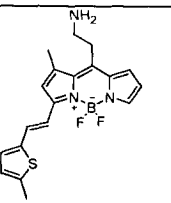
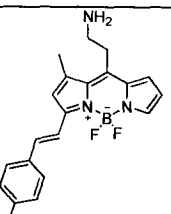
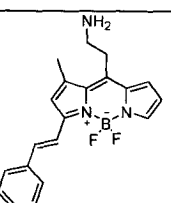
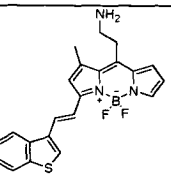
BDM-88		428.3	409.0 429.0	3.57	95%	565	576	0.21	4.9
BDM-90		457.3	438.0	5.18	97%	565	582	0.26	7.3
BDM-91		424.3	405.0	3.59	95%	614	699	0.02	7.0
BDM-93		420.3	401.0	3.95	91%	592	653	0.06	4.0
BDM-97		397.3	378.0	3.68	89%	561	572	0.04	6.8
BDM-107		422.3	403.0	4.22	97%	617	681	0.05	4.7

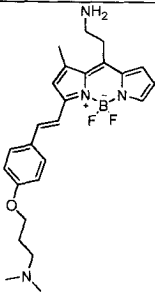
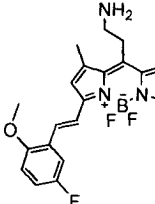
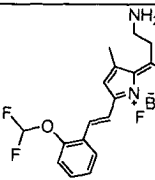
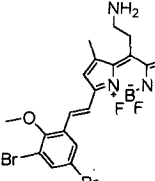
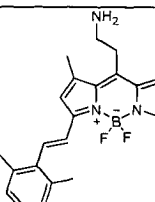
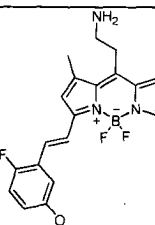
BDM-108		393.3	374.1	4.86	85%	549	573	0.35	6.2
BDM-110		381.3	362.0	4.11	91%	563	583	0.85	3.5
BDM-111		408.3	389.0	3.11	100%	566	589	0.79	4.7
BDM-132		397.3	378.0	3.36	100%	568	594	0.05	4.5
BDM-135		355.3	336.0	3.98	93%	577	601	0.10	4.4
BDM-137		401.3	382.0	4.84	87%	563	575	0.28	7.1
BDM-139		395.2	376.0	3.94	92%	564	584	0.55	3.7

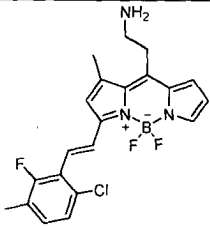
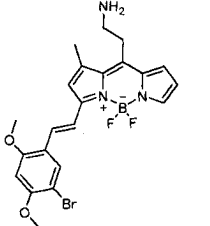
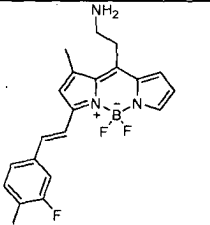
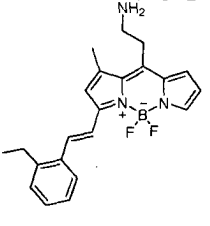
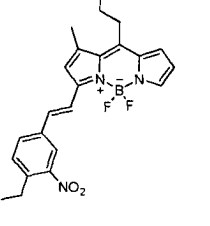
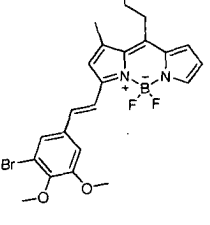
BDM-140		409.3	390.0	4.97	93%	564	585	0.57	5.6
BDM-143		365.2	346.0	4.51	84%	554	566	0.42	6.1
BDM-144		441.3	422.0	3.67	94%	578	612	0.05	5.4
BDM-177		407.3	388.0	5.03	96%	556	567	0.78	4.1
BDM-178		473.2	454.0	5.06	96%	561	577	0.37	5.0
BDM-186		381.2	362.0	4.15	97%	553	564	0.97	5.4
BDM-192		397.2	378.0	3.75	86%	574	605	0.20	4.0

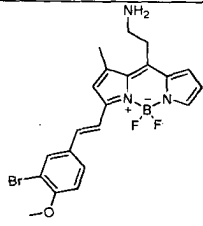
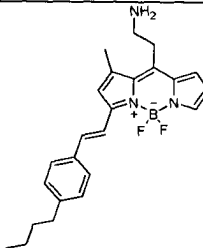
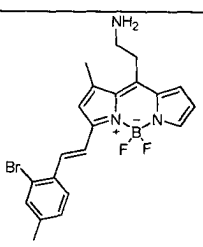
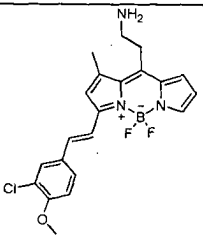
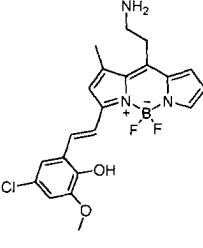
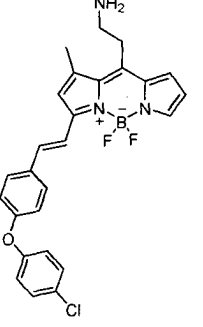
BDM-195		481.7	461.9	5.18	100%	600	663	0.03	6.5
BDM-101		443.3	423.9	5.23	97%	559	576	0.66	5.3
BDM-100		436.1	415.8 417.8	4.49	86%	571	582	0.43	5.0
BDM-20		443.3	424.0	5.09	94%	554	564	0.47	4.4
BDM-202		407.3	388.0	4.64	85%	559	575	0.52	4.1
BDM-231		395.2	376.0	3.94	93%	558	568	0.17	5.9

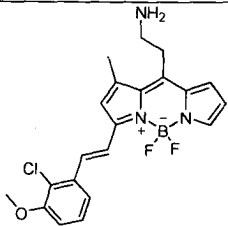
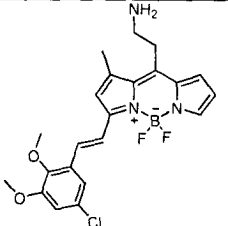
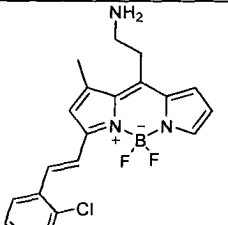
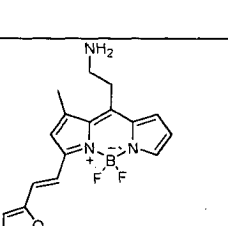
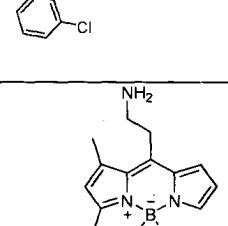
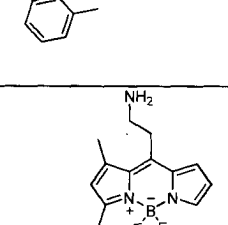
BDM-198		381.2	362.0	4.08	95%	559	574	0.59	5.2
BDM-218		457.3	438.0	5.18	100%	559	574	0.37	5.8
BDM-179		485.3	466.0	5.09	96%	566	581	0.26	5.5
BDM-199		429.3	410.0	3.79	94%	562	574	0.18	7.6
BDM-207		385.7	366.0	4.47	88%	553	566	0.24	3.9
BDM-209		411.3	392.0	4.09	93%	555	567	0.38	4.1

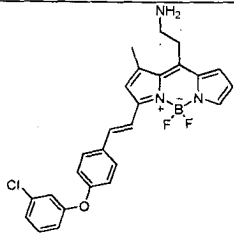
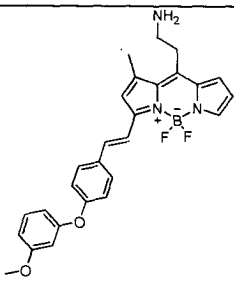
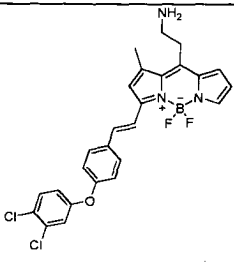
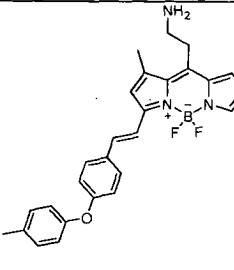
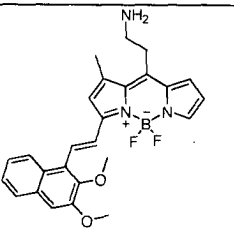
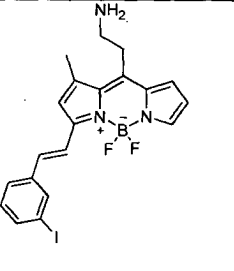
BDM-237		477.1	457.8	4.69	97%	558	572	0.35	5.2
BDM-228		490.2	469.8 471.9	4.48	92%	557	567	0.24	8.8
BDM-236		371.3	352.0	4.29	90%	574	594	0.22	4.9
BDM-208		365.2	346.1	4.54	100%	558	572	0.27	3.8
BDM-27		351.2	332.0	4.01	87%	553	566	0.39	4.2
BDM-37		407.3	387.9	4.81	84%	566	588	0.15	4.5

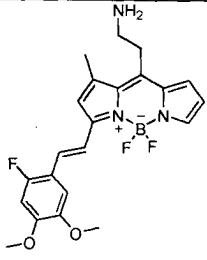
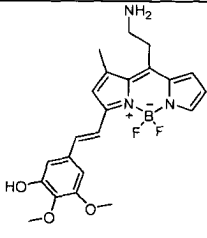
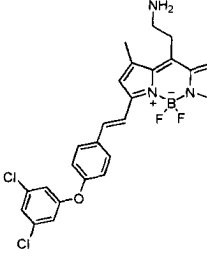
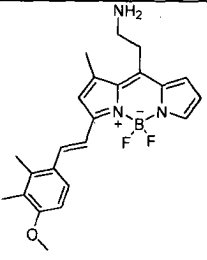
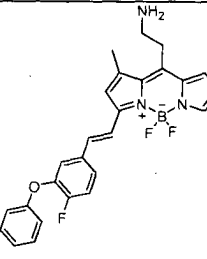
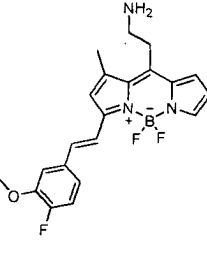
BDM-163		452.4	453.0	2.78	88%	566	586	0.41	5.6
BDM-239		399.2	380.0	4.42	100%	559	572	0.28	5.5
BDM-240		417.2	397.9	4.48	87%	552	565	0.23	5.5
BDM-241		539.0	517.8	4.88	84%	560	576	0.32	6.5
BDM-243		379.3	360.0	4.72	89%	543	565	0.87	3.0
BDM-245		399.2	380.0	4.32	87%	555	563	0.32	4.4

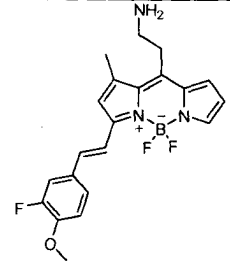
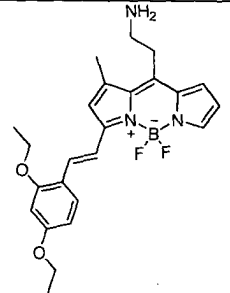
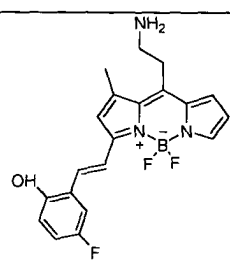
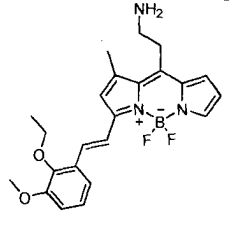
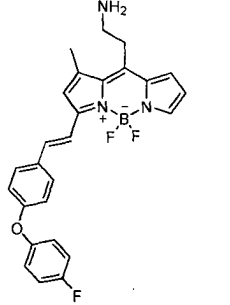
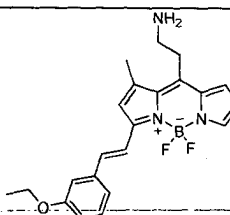
BDM-246		417.7	397.9	4.93	100%	554	566	0.17	5.8
BDM-247		490.2	469.9 471.9	4.48 4.67	90%	571	599	0.54	5.6
BDM-249		383.2	364.0	4.72	100%	557	568	0.24	4.8
BDM-251		379.3	360.0	4.84	100%	556	572	0.22	5.9
BDM-252		424.3	405.0	4.86	91%	556	569	0.08	4.6
BDM-259		490.2	469.8 471.8	4.70	87%	559	573	0.35	5.0

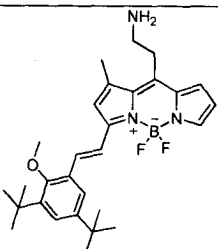
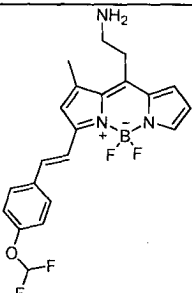
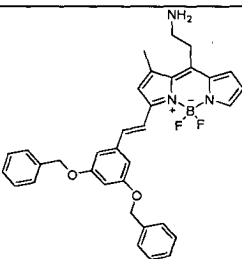
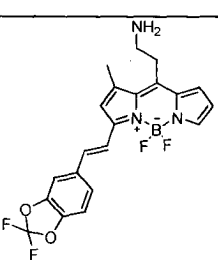
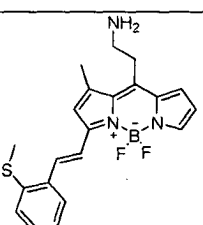
BDM-260		460.1	439.8 441.8	4.55	93%	564	582	0.05	4.0
BDM-263		407.3	388.0	5.69	85%	559	571	0.26	3.9
BDM-267		444.1	423.9 425.9	4.96	92%	558	573	0.21	4.4
BDM-274		415.7	395.9	4.48	93%	562	577	0.46	3.9
BDM-275		431.7	411.9	4.30	85%	562	n.d	0.05	5.9
BDM-277		477.7	457.9	5.71	87%	559	574	0.59	4.9

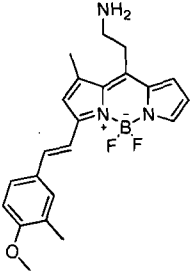
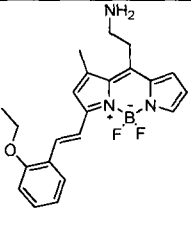
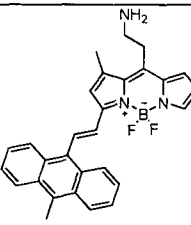
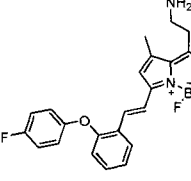
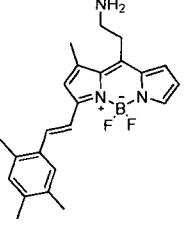
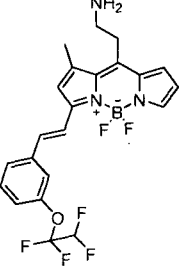
BDM-281		415.7	395.9	4.13	92%	554	567	0.28	5.1
BDM-282		445.7	425.9	4.74	88%	558	565	0.19	4.5
BDM-283		401.7	381.9	3.68	100%	556	567	0.15	4.3
BDM-284		451.7	431.9	5.23	84%	594	622	0.03	4.6
BDM-285		399.7	379.9	4.74	86%	544	561	0.51	6.0
BDM-289		399.7	380.0	4.97	84%	559	570	0.26	4.9

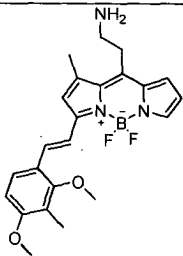
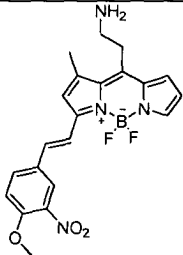
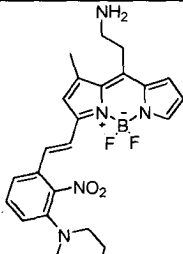
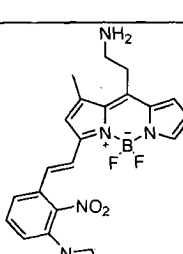
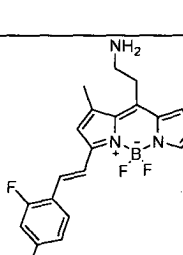
BDM-290		477.7	457.9	5.61	85%	558	574	0.36	4.5
BDM-298		473.3	454.0	5.30	89%	559	568	0.32	6.8
BDM-299		512.2	491.8 493.8	5.89	91%	555	563	0.27	7.1
BDM-300		457.3	438.0	5.65	89%	562	579	0.44	5.3
BDM-301		461.3	442.0	4.76	100%	563	586	0.41	5.6
BDM-303		477.1	457.8	4.92	100%	555	566	0.31	5.6

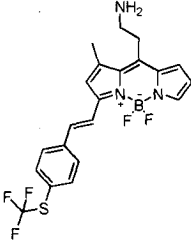
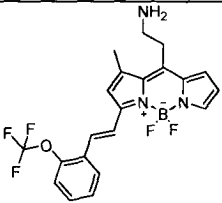
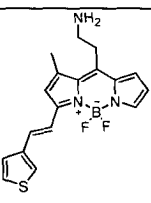
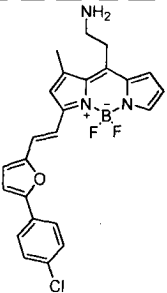
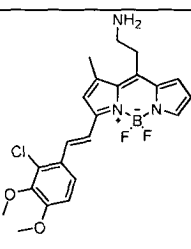
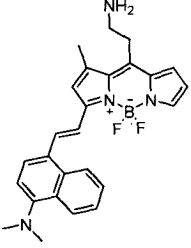
BDM-305		429.2	410.0	3.99	86%	567	587	0.25	8.1
BDM-307		427.3	408.0	3.50	84%	564	580	0.07	4.0
BDM-308		512.2	491.8	6.15	86%	556	567	0.19	4.9
BDM-310		409.3	390.0	4.90	84%	566	600	0.42	6.5
BDM-311		461.3	441.9	5.28	84%	556	565	0.21	5.1
BDM-312		399.2	380.0	4.36	83%	557	568	0.18	3.0

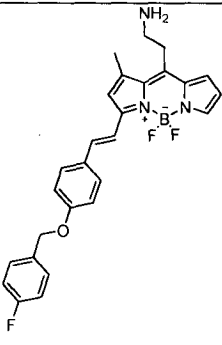
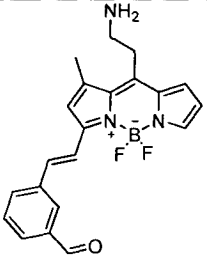
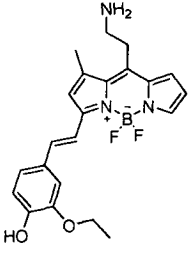
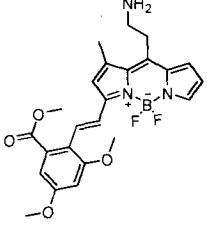
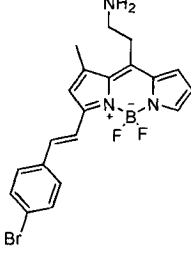
BDM-314		399.2	380.0	4.26	100%	556	570	0.10	4.7
BDM-316		439.3	420.0	4.81	87%, 4.81	572	610	0.46	5.9
BDM-318		385.2	366.0	3.83	85%, 3.83	563	576	0.11	7.4
BDM-320		425.3	406.0	4.46	84%	558	569	0.21	4.3
BDM-322		461.3	441.9	5.37	85%	559	577	0.15	5.5
BDM-323		395.3	376.0	4.61	87%	556	570	0.44	5.0

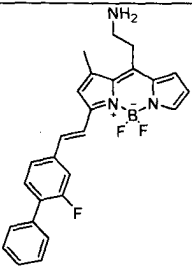
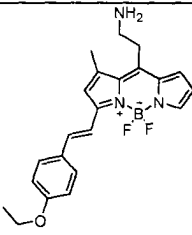
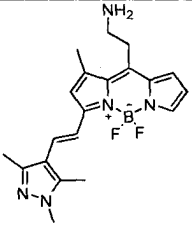
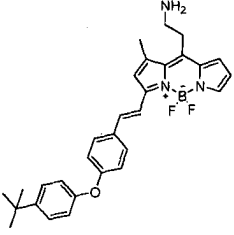
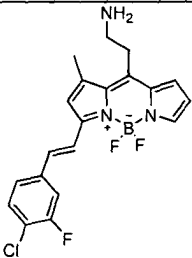
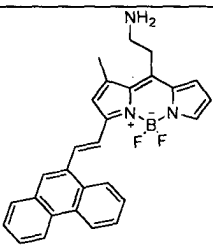
BDM-325		493.4	474.0	6.59	85%	557	573	0.47	5.3
BDM-326		417.2	398.0	4.40	91%	554	565	0.71	5.9
BDM-331		563.4	543.9	6.05	84%	554	566	0.65	9.3
BDM-332		431.2	411.9	4.83	91%	552	562	0.61	5.3
BDM-333		397.3	378.0	4.51	95%	557	575	0.30	5.6

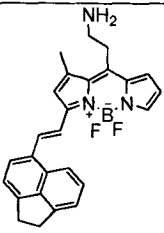
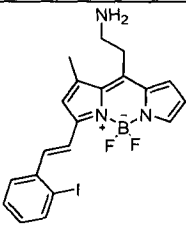
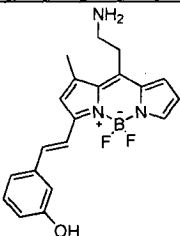
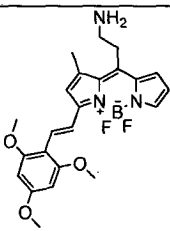
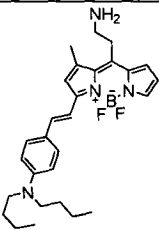
BDM-335		395.3	376.0	4.57	95%	565	591	0.78	6.2
BDM-336		395.3	376.0	4.53	91%	560	577	0.58	3.4
BDM-340		465.3	445.9	5.43 5.57	96%	534	541	0.11	5.0
BDM-341		461.3	441.9	5.09	90%	556	570	0.37	12.4
BDM-343		393.3	374.0	5.08	91%	559	577	0.28	9.6
BDM-345		467.2	447.9	4.92	88%	551	564	0.22	7.4

BDM-347		425.3	406.0	4.67	96%	566	591	0.57	5.3
BDM-350		426.2	406.9	3.93	98%	556	571	0.14	5.9
BDM-356		479.3	460.0	5.16	92%	573	682	0.03	4.2
BDM-357		465.3	446.0	4.72	90%	585	687	0.04	5.0
BDM-359		399.2	380.0	4.33	100%	559	574	0.83	6.6

BDM-363		451.3	431.8	4.98	96%	553	564	0.51	6.1
BDM-365		435.2	415.9	4.77	96%	549	563	0.24	5.2
BDM-369		357.2	337.9	3.96	93%	554	570	0.30	3.6
BDM-371		451.7	431.9	5.35	90%	590	630	0.03	4.8
BDM-372		445.7	425.9	4.34	87%	561	578	0.55	8.5
BDM-377		444.3	445.0	4.76	98%	586	n.d	0.04	6.9

BDM-378		475.3	456.0	5.14	94%	563	585	0.41	3.8
BDM-391		379.2	360.0	3.86	91%	554	563	0.31	5.3
BDM-397		411.2	392.0	3.67	92%	570	614	0.10	4.9
BDM-401		469.3	449.9	4.28	100%	565	590	0.85	5.6
BDM-409		430.1	409.9 411.8	4.93	85%	558	570	0.12	4.0

BDM-425		445.3	425.9	5.27	89%	562	576	0.12	4.4
BDM-429		395.3	376.0	4.49	100%	564	586	0.23	4.6
BDM-430		383.3	364.0	3.09	100%	565	592	0.99	3.3
BDM-435		499.4	480.0	6.14	94%	555	565	0.39	4.9
BDM-437		403.6	383.9	4.62	95%	555	566	0.21	4.1
BDM-440		451.3	432.0	5.42	93%	563	589	0.37	6.1

BDM-89		427.3	408.0	5.15	85%	576	612	0.30	6.1
BDM-384		477.1	457.8	4.60	92%	551	565	0.32	3.1
BDM-105		367.2	348.0	3.48	93%	556	567	0.18	4.4
BDM-82		441.3	422.0	4.36	87%	579	621	0.40	6.6
BDM-17		478.4	459.0 479.0	6.17	89%	622	695	0.03	4.0

HPLC conditions: A: H₂O-HCOOH: 99.9:0.1. B: ACN-HCOOH: 99.9:0.1; gradient 5% B to 95% B (10 min), isocratic 95% B (2 min). Reverse-phase Agilent C₁₈ Zorbax column (2.1 x 30 mm²) 3.5 m, flow rate: 1 mL/min. Purity determined by integration of the absorbance peaks at 350 nm. ESI (+) m/z signals mostly correspond to the [M-F] fragmentation.

BODIPY has been widely used as a fluorophore due to its high photostability and extinction coefficient, high quantum yield and narrow excitation/emission

bandwidth. However, solution-phase syntheses of BODIPY dyes often encompass tedious purification steps with typically low recovery yields, which seriously hampered the combinatorial derivatization of the BODIPY scaffold. With this first adaptation of the BODIPY scaffold to a solid-phase approach we accelerated the development of alkylamino BODIPY-based fluorescent probes. Alkylamino moieties can enhance protein/cell selectivity on BODIPY dyes, as demonstrated with the discovery of Ig Orange or CDy9, and can easily be modified with chemical or photoreactive tags for target identification studies. In addition, CDy9 showed an excellent potential for the imaging, isolation and characterization of mESC and it overcomes some limitations of the currently used-techniques (e.g. antibodies or genetically-modified cells) with a non-destructive, simple and cheap method for selectively staining mESC.

Alkylamino BODIPY tags can easily be modified with chemical or photoreactive tags (Scheme 3) for target identification studies, which is a key step and one of the bottlenecks of drug discovery and target validation programs. For the particular case of CDy9, this fluorescent probe showed an excellent staining selectivity on mESC when compared to a wide range of differentiated cell types, and thus could be used as a good research tool for imaging, isolation and characterization of mESC. Advantages of CDy9 over the conventional stem cell research tools involve a non-destructive and simple staining protocol -unlike antibodies of alkaline-phosphatase assays and do not require genetically-modified cells unlike GFP or luciferase-based mESC reporters.

DEFINITIONS

“Alkyl” means 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. “(C₁-C₆)alkyl” includes methyl, ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, pentyl, and hexyl.

“Alkylene” means a saturated aliphatic straight-chain divalent hydrocarbon radical having the specified number of carbon atoms. Thus, “(C₁-C₆)alkylene” means a divalent saturated aliphatic radical having from 1- 6 carbon atoms in a linear

arrangement. “(C₁-C₆)alkylene” includes methylene, ethylene, propylene, butylene, pentylene and hexylene.

“Heterocycle” means a saturated or partially unsaturated (4-7 membered) monocyclic heterocyclic ring containing one nitrogen atom and optionally 1
5 additional heteroatom independently selected from N, O, or S. When one heteroatom is S, it can be optionally mono- or di-oxygenated (*i.e.*, -S(O)- or -S(O)₂-). Examples of monocyclic heterocycle include, but are not limited to, azetidine, pyrrolidine, piperidine, piperazine, hexahydropyrimidine, tetrahydrofuran, tetrahydropyran, morpholine, thiomorpholine, thiomorpholine 1,1-dioxide, tetrahydro-2H-1,2-
10 thiazine, tetrahydro-2H-1,2-thiazine 1,1-dioxide, isothiazolidine, or isothiazolidine 1,1-dioxide.

“Cycloalkyl” means a saturated aliphatic cyclic hydrocarbon ring. Thus, “C₃-C₇ cycloalkyl” means (3-7 membered) saturated aliphatic cyclic hydrocarbon ring. C₃-C₇ cycloalkyl includes, but is not limited to cyclopropyl, cyclobutyl, cyclopentyl,
15 cyclohexyl and cycloheptyl.

The term “alkoxy” means -O-alkyl; “hydroxyalkyl” means alkyl substituted with hydroxy; “aryl alkyl” means alkyl substituted with an aryl group; “alkoxyalkyl” mean alkyl substituted with an alkoxy group; “alkylamine” means amine substituted with an alkyl group; “cycloalkylalkyl” means alkyl substituted with cycloalkyl;
20 “dialkylamine” means amine substituted with two alkyl groups; “alkylcarbonyl” means -C(O)-A*, wherein A* is alkyl; “alkoxycarbonyl” means -C(O)-OA*, wherein A* is alkyl; and where alkyl is as defined above and OA* includes methoxy, ethoxy, propoxy, butoxy, pentoxy and hexoxy.

“Cycloalkoxy” means a cycloalkyl-O- group wherein the cycloalkyl is as
25 defined above. Exemplary (C₃-C₇)cycloalkyloxy groups include cyclopropoxy, cyclobutoxy, cyclopentoxy, cyclohexoxy and cycloheptoxy.

Haloalkyl and halocycloalkyl include mono, poly, and perhaloalkyl groups where each halogen is independently selected from fluorine, chlorine, and bromine.

“Hetero” refers to the replacement of at least one carbon atom member in a
30 ring system with at least one heteroatom selected from N, S, and O. A hetero ring system may have 1 or 2 carbon atom members replaced by a heteroatom.

“Halogen” and “halo” are interchangeably used herein and each refers to fluorine, chlorine, bromine, or iodine.

“Cyano” means $-C\equiv N$.

“Nitro” means $-NO_2$.

5 “Fmoc” is 9-Fluorenylmethyloxycarbonyl.

As used herein, an amino group may be a primary ($-NH_2$), secondary ($-NHR_x$), or tertiary ($-NR_xR_y$), wherein R_x and R_y may be any of the optionally substituted alkyls described above.

10 The terms “haloalkyl” and “haloalkoxy” mean alkyl or alkoxy, as the case may be, substituted with one or more halogen atoms. The term “halogen” means F, Cl, Br or I. Preferably the halogen in a haloalkyl or haloalkoxy is F.

The term “acyl group” means $-C(O)B^*$, wherein B^* is an optionally substituted alkyl group or aryl group (e.g., optionally substituted phenyl).

15 An “alkylene group” is represented by $-[CH_2]_z-$, wherein z is a positive integer, preferably from one to eight, more preferably from one to four.

An “alkenylene group” is an alkylene in which at least a pair of adjacent methylenes are replaced with $-CH=CH-$.

20 The term “(C₆-C₁₀)aryl” used alone or as part of a larger moiety as in “arylalkyl”, “arylalkoxy”, or “aryloxyalkyl”, means carbocyclic aromatic rings. The term “carbocyclic aromatic group” may be used interchangeably with the terms “aryl”, “aryl ring”, “carbocyclic aromatic ring”, “aryl group” and “carbocyclic aromatic group”. An aryl group typically has 6-14 ring atoms. A “substituted aryl group” is substituted at any one or more substitutable ring atom. The term “C₆₋₁₄ aryl” as used herein means a monocyclic, bicyclic or tricyclic carbocyclic ring system containing from 6 to 14 carbon atoms and includes phenyl, naphthyl, anthracenyl, 1,2-dihydronaphthyl, 1,2,3,4-tetrahydronaphthyl, fluorenyl, indanyl, indenyl and the like.

25 The term “heteroaryl”, “heteroaromatic”, “heteroaryl ring”, “heteroaryl group” and “heteroaromatic group”, used alone or as part of a larger moiety as in “heteroarylalkyl” or “heteroarylalkoxy”, refers to aromatic ring groups having five to fourteen ring atoms selected from carbon and at least one (typically 1 - 4, more typically 1 or 2) heteroatoms (e.g., oxygen, nitrogen or sulfur). They include

monocyclic rings and polycyclic rings in which a monocyclic heteroaromatic ring is fused to one or more other carbocyclic aromatic or heteroaromatic rings. The term "5-14 membered heteroaryl" as used herein means a monocyclic, bicyclic or tricyclic ring system containing one or two aromatic rings and from 5, to 14 atoms of which, unless otherwise specified, one, two, three, four or five are heteroatoms independently selected from N, NH, N(C₁₋₆alkyl), O and S.

The term "2-4 member polycyclyl" is a cyclic compound with 2-4 hydrocarbon loop or ring structures (*e.g.*, benzene rings). The term generally includes all polycyclic aromatic compounds, including the polycyclic aromatic hydrocarbons, the heterocyclic aromatic compounds containing sulfur, nitrogen, oxygen, or another non-carbon atoms, and substituted derivatives of these.

The term "Alkenyl" means a straight or branched hydrocarbon radical having a specified number of carbon atoms and includes at least one double bond. The (C₆-C₁₀)aryl(C₂-C₆)alkenyl group connects to the remainder of the molecule through the (C₂-C₆)alkenyl portion of (C₆-C₁₀)aryl(C₂-C₆)alkenyl.

Another embodiment of the present invention is a pharmaceutical composition comprising one or more pharmaceutically acceptable carrier and/or diluent and a compound disclosed herein or a pharmaceutically acceptable salt thereof.

"Pharmaceutically acceptable carrier" and "pharmaceutically acceptable diluent" means non-therapeutic components that are of sufficient purity and quality for use in the formulation of a composition of the invention that, when appropriately administered to an animal or human, typically do not produce an adverse reaction, and that are used as a vehicle for a drug substance (i.e. a compound of the present invention).

Pharmaceutically acceptable salts of the compounds of the present invention are also included. For example, an acid salt of a compound of the present invention containing an amine or other basic group can be obtained by reacting the compound with a suitable organic or inorganic acid, resulting in pharmaceutically acceptable anionic salt forms. 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, 5 stearate, subacetate, succinate, sulfate, tannate, tartrate, teoclate, tosylate, and triethiodide salts.

Salts of the compounds of the present invention containing a carboxylic acid or other acidic functional group can be prepared by reacting with a suitable base. Such a pharmaceutically acceptable salt may be made with a base which affords a 10 pharmaceutically acceptable cation, which includes alkali metal salts (especially sodium and potassium), alkaline earth metal salts (especially 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'- 15 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.

"FRET sensor" means a sensor utilized in measuring Foerster resonance 20 energy transfer, which is a mechanism describing energy transfer between two chromophores.

"Surface plasmon resonance" is the property of the resonant, collective oscillation of valence electrons in a solid stimulated by incident light. Resonance is reached when the frequency of the light photons matches the frequency of the surface 25 electrons.

A "ratiometric sensor" is a voltage-based sensor measuring fluorescence, wherein the voltage output from the sensor is proportional to the voltage input.

"Flow cytometry" is a laser-based method used for counting and sorting cells, as well as detecting biomarkers in a biological sample.

EXAMPLES

The following examples are provided to illustrate one or more preferred embodiments of the invention, but are not limited embodiments thereof. Numerous variations can be made to the following examples that lie within the scope of the invention.

Example 1: Synthesis of the alkylamino BODIPY scaffold 3 and its derivitization in solid-phase to render BDM compounds.

Using relatively inexpensive starting materials (Fmoc- β -alanine and pyrrole), the 2-ketopyrrole **1** was prepared following slightly modified reported methods.²³

(9H-fluoren-9-yl)methyl 3-oxo-3-(1H-pyrrol-2-yl)propylcarbamate (1). Fmoc- β -Ala-OH (2.5 g, 8.0 mmol) was dissolved in dry THF. 2,2'-dipyridyl disulfide (2.7 g, 12.0 mmol) and PPh₃ (4.2 g, 16.0 mmol) were added, and the mixture was stirred under N₂ atmosphere at r.t for 24 h. In a separate flask, methylmagnesium bromide (12 mL of a 3.0 M solution in THF, 36.0 mmol) was added dropwise at -78 °C to a solution of pyrrole (3.3 mL, 48.0 mmol) in dry THF (50 mL). The mixture was stirred at -78 °C for 30 min and at -20 °C for another 30 min. Then the thioester crude mixture (after 24 h reaction) was added drop wise at -78 °C, and the whole solution was stirred at -78 °C for 30 min and another 30 min at r.t. The reaction was quenched with a saturated solution of NH₄Cl. After dilution with diethyl ether, the organic layer was washed with water (3 × 100 mL) and brine (100 mL), dried over anhydrous sodium sulfate and evaporated under vacuum. Column chromatography (elution with hexane-ethyl acetate 1:1) rendered 2.53 g of **1** as a white solid (yield: 88%). ESI *m/z* (C₂₂H₂₀N₂O₃), calc: 359.2; found (M+Na⁺): 382.9. ¹H-NMR (300 MHz, CDCl₃): 9.68 (bs, 1H), 7.75 (d, 2H, *J* = 7.3Hz), 7.57 (d, 2H, *J* = 7.8Hz), 7.38 (t, 2H, *J* = 7.6Hz), 7.28 (t, 2H, *J* = 7.3Hz), 7.04 (bs, 1H), 6.94 (bs, 1H), 6.28 (d, 1H, *J* = 2.9Hz), 5.48 (bt, 1H, *J* = 5.9Hz), 4.38 (d, 2H, *J* = 7.0Hz), 4.20 (t, 1H, *J* = 7.0Hz), 3.61 (dt, 2H, *J* = 5.9Hz, 6.1Hz), 3.06 (t, 2H, *J* = 5.9Hz). ¹³C-NMR (75 MHz, CDCl₃): 189.0, 156.4, 143.9, 141.3, 131.7, 127.6, 127.0, 125.1, 124.9, 119.9, 116.8, 110.9, 66.7, 47.2, 37.6, 36.3.

Further condensation with 2,4-dimethylpyrrole and POCl₃, and in situ addition of the BF₃·OEt₂ yielded the BODIPY derivative **2**.

10-(2-(((9H-fluoren-9-yl)methoxy)carbonylamino)ethyl)-5,5-difluoro-1,3-dimethyl-5H-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide (2). 1 (1.5 g, 4.2 mmol) and 2,4-dimethylpyrrole (692 μ L, 6.7 mmol) were dissolved in CH_2Cl_2 at 0 °C. After 10 min stirring, the mixture was treated drop wise with POCl_3 (1.3 g, 8.4 mmol), and the resulting solution was stirred at 0 °C for 1 h, and at 35 °C for 20 h. Afterwards, DIEA (2.9 mL, 16.8 mmol) and $\text{BF}_3 \cdot \text{OEt}_2$ (2.3 mL, 16.8 mmol) were added and the crude mixture was stirred for 4 h at r.t. Column chromatography (elution with hexane-ethyl acetate 4:1) rendered 1.2 g of **2** as a reddish solid (yield: 57%). ESI m/z ($\text{C}_{28}\text{H}_{26}\text{BF}_2\text{N}_3\text{O}_2$), calc: 485.2; found (M-F): 466.3. ^1H -NMR (300 MHz, CDCl_3): 7.77 (d, 2H, $J = 7.6$ Hz), 7.61 (bs, 1H), 7.56 (d, 2H, $J = 7.0$ Hz), 7.41 (t, 2H, $J = 7.6$ Hz), 7.31 (t, 2H, $J = 7.3$ Hz), 7.11 (d, 1H, $J = 3.5$ Hz), 6.42 (d, 1H, $J = 3.2$ Hz), 6.17 (bs, 1H), 5.10 (bt, 1H, $J = 6.2$ Hz), 4.43 (d, 2H, $J = 6.7$ Hz), 4.19 (t, 1H, $J = 6.7$ Hz), 3.50 (dt, 2H, $J = 6.7$ Hz, 7.2 Hz), 3.18 (t, 2H, $J = 7.0$ Hz), 2.58 (s, 3H), 2.46 (s, 3H). ^{13}C -NMR (75 MHz, CDCl_3): 161.2, 156.3, 145.2, 145.1, 143.7, 142.3, 141.3, 138.2, 134.0, 127.7, 127.0, 125.0, 124.0, 123.8, 120.0, 116.1, 66.8, 47.2, 42.8, 30.1, 16.2, 15.0. ^{19}F -NMR (282 MHz, CDCl_3): -70.31, -70.53 (dd, $J = 31\text{Hz}$, $J = 62\text{Hz}$, BF_2).

The treatment of **2** with DBU followed by quenching with aqueous HCl afforded **3** as a hydrochloride salt.

10-(2-aminoethyl)-5,5-difluoro-1,3-dimethyl-5H-dipyrrolo[1,2-c:1',2'-f][1,3,2]diazaborinin-4-ium-5-uide hydrochloride (3). A solution of **2** (1.0 g, 2.06 mmol) in CH_2Cl_2 was treated with DBU every 15 min for a total of 4 times ($4 \times 73 \mu\text{L}$, 4×0.51 mmol) and a total reaction time of 1 h. Afterwards, the reaction was quenched with a aqueous solution of 0.5 N HCl and stirred for 15 min at r.t. The resulting red solid was filtered off, and thoroughly washed with diethyl ether to render 350 mg of **3** as the hydrochloride salt (yield: 65%). ESI m/z ($\text{C}_{13}\text{H}_{16}\text{BF}_2\text{N}_3$), calc: 263.1; found (M-F): 244.1. ^1H -NMR (300 MHz, $\text{DMSO}-d_6$): 8.51 (bs, 2H), 7.65 (s, 1H), 7.63 (s, 1H), 6.52 (s, 1H), 6.46 (s, 1H), 3.37 (m, 2H), 3.16 (s, 3H), 3.03 (m, 2H), 2.50 (s, 3H). ^{13}C -NMR (75 MHz, $\text{DMSO}-d_6$): 161.6, 146.3, 141.0, 138.0, 133.5, 133.3, 124.7, 124.0, 116.1, 48.5, 47.8, 15.9, 14.6. ^{19}F -NMR (282 MHz, $\text{DMSO}-d_6$): -66.97, -67.19 (dd, $J = 29$ Hz, $J = 61$ Hz, BF_2).

This product was loaded on CTC-PS resin so that the activated C₃-methyl group of the BODIPY structure could be modified in a solid-phase adapted Knoevenagel-type reaction.²⁴

Synthesis of the BDM library. A solution of **3** (350 mg, 1.3 mmol) and DIEA (1.1 mL, 6.5 mmol) was dissolved in *N*-methylpyrrolidone (NMP) and added to 2-chlorotrityl chloride polystyrene resin (loading: 1.2 mmol/g, 3.3 g, 3.9 mmol). The reaction was shaken at r.t. for 16 h, after which the resin was capped with MeOH (0.8 mL/g resin) for 4 h, and finally filtered off and washed with NMP, DMF and DCM (× 4 each solvent). For every compound, 50 mg of the loaded resin (corresponding to 0.06 mmol of **3**) were re-suspended in 2 mL DMSO-ACN (1:1), and treated with pyrrolidine (75 µL, 0.9 mmol), AcOH (54 µL, 0.9 mmol) and the corresponding aldehydes (0.9 mmol). The resulting suspension was heated at 85 °C for 5 min, cooled down to r.t., filtered off, and washed with DMF and DCM (× 4 each solvent). Afterward, the resins were treated with a solution of TFA-DCM (0.5:99.5) (2 × 10 min), and the resulting filtrates were combined and evaporated under pressure. **BDM** products were isolated as the free-amine compounds after silica-based SPE elution with DCM-MeOH (98:2) containing 1% NH₃ concentration in MeOH (full characterization data on Table 1). The procedure rendered aminoethyl styryl-BODIPY compounds (BDM) with purities averaging 91%. **Ig Orange** (**BDM-69**): ¹H-NMR (300 MHz, CDCl₃): 7.57-7.61-7.69 (m, 8H), 7.39 (d, 4H, *J* = 7.6 Hz), 7.17 (d, 4H, *J* = 8.8 Hz), 6.80 (s, 1H), 6.50 (dd, 1H, *J* = 2.1 Hz, *J* = 4.0 Hz), 3.18 (d, 2H, *J* = 6.4 Hz), 3.12 (d, 2H, *J* = 6.4 Hz), 2.54 (s, 3H), 1.63 (bs, 2H). ¹³C-NMR (75 MHz, CDCl₃): 157.0, 143.9, 140.6, 139.2, 138.9, 137.8, 136.9, 135.0, 134.4, 129.8, 128.6, 128.2, 127.8, 127.8, 126.9, 126.5, 123.4, 122.1, 119.7, 118.0, 116.0, 77.2, 43.4, 31.8, 29.5, 16.2. ¹⁹F-NMR (282 MHz, CDCl₃): -65.82, -66.03 (dd, *J* = 30 Hz, *J* = 59 Hz, BF₂).

Example 2: Quantum Yield Measurements.

Quantum yields were calculated by measuring the integrated emission area of the fluorescent spectra, and referring them to the area measured for Rhodamine B in EtOH after excitation at 510 nm ($\Phi_{\text{Rho-B}} = 0.70$). Quantum yields for the **BDM** products were then calculated using equation below, where *F* represents the area of fluorescent emission, *n* is reflective index of the solvent, and *Abs* is absorbance at

excitation wavelength selected for standards and samples. Emission was integrated from 540 nm to 800 nm.

$$\Phi_{flu}^{sample} = \Phi_{fl}^{reference} \left(\frac{F^{sample}}{F^{reference}} \right) \left(\frac{\eta^{sample}}{\eta^{reference}} \right) \left(\frac{Abs^{reference}}{Abs^{sample}} \right)$$

Example 3: Screening the BDM library.

- 5 Fluorescence intensities were measured using a Spectra Max Gemini XSF plate reader in a 384-well plate. **BDM** compounds were dissolved to a final concentration of 10 μ M (20 mM HEPES buffer, pH 7.4, containing 1% DMSO) and incubated with different proteins and peptides at different serial concentrations in 20 mM HEPES buffer (pH 7.4) (FIG. 1a). The excitation wavelength was set at 510
- 10 nm, and the emission spectra were recorded from 560 to 700 nm. Fluorescence fold increase ratios were determined by referring the maximum fluorescence intensity emission of **BDM** compounds in the presence of the screened proteins and peptides to the maximum fluorescence intensity emission of **BDM** compounds in 20 mM HEPES buffer (pH 7.4) (FIG. 1b).
- 15 **Extended Screening of IgG Orange (BDM-69).** **IgG Orange** was dissolved to a final concentration of 10 μ M (PBS buffer, pH 7.3, containing 1% DMSO) and incubated with human IgG, IgA and IgM at different concentrations in PBS buffer (pH 7.3) (FIG. 2). The excitation wavelength was set at 530 nm, and fluorescence fold increase ratios were determined by referring the maximum fluorescence
- 20 intensity emission (590 nm) of **Ig Orange** in the presence of immunoglobulins to the maximum fluorescence intensity emission of **Ig Orange** in buffer.

Example 4: Ig Orange response against IgG in human serum samples.

- Known amounts of human IgG covering the whole physiological range (3.3, 6.2, 9.4 and 12.5 mg/mL) were added to immunoglobulin(G, A, M)-depleted human serum
- 25 (Sigma Aldrich). The resulting samples were diluted 10 times in PBS buffer (pH 7.3) to reach a final volume of 100 μ L and **Ig Orange** (1 μ L, 1 mM) was added (final **Ig Orange** concentration: 10 μ M). Fluorescence intensities of the samples were recorded on a SpectraMax M2 plate reader (exc: 530 nm; emission: 590 nm) (FIG. 3). The experimental data was fitted to a linear regression using Graphpad
- 30 Prism 5.0 software.

Example 5: Surface Plasmon Resonance and Job Plot Analysis.

Human IgG (5.0 mg, 0.033 μmol) and biotin-OSu (0.11 mg, 0.33 μmol) were dissolved in 0.1 M NaHCO_3 (pH 8.5) and shaken for 1 h at 25 °C. Excess of biotinylating reagent was removed by centrifugation with Microcon 30K filters (3 rounds at 14,000 rpm for 20 min at 4 °C). The purified biotinylated IgG was resuspended in PBS, and characterized by SDS-PAGE and Western blotting with HRP conjugated streptavidin (FIG. 4). The fluorescence response of Ig Orange to IgG and biotinylated IgG was compared (FIG. 5). The concentration of biotinylated IgG before fluorescence and SPR experiments was determined by Bradford assay.

SPR measurements were performed on a T-200 instrument (Biacore AB, GE Healthcare) equipped with Series S sensor chip CM7 (Biacore AB, GE Healthcare). All experiments were performed at 25 °C. Briefly, the carboxymethylated sensor surface was activated for 7 min with 1:1 of 0.2 M EDC (*N*-ethyl-*N*-[3-(diethylamino)propyl] carbodiimide) and 50 mM NHS (*N*-hydroxysuccinimide), then human IgG was coupled to 12,000 RU and finally the surface was deactivated for 7 min with 1 M ethanolamine HCl (pH 8.5). The immobilized immunoglobulin was probed with one positive- and one negative-binding fragment diluted into the running buffer (10 mM sodium phosphate buffer, 150 mM NaCl, 1% DMSO, pH 7.4) to ascertain its functionality. **Ig Orange** (0.07, 0.21, 0.62, 1.86, 5.57 and 16.7 μM , in duplicates) was injected across a control surface and the immobilized surface serially. Sensorgrams obtained were DMSO calibrated and buffer- and reference-subtracted (FIG. 6a). Responses at equilibrium were plotted against analyte concentration and fit to a simple isotherm to obtain the affinity constant using Scrubber 2 software (BioLogics Inc. Australia). **Job Plot Analysis: Ig Orange** (final concentrations: 0, 1.6, 3.2, 4.8, 6.4, 8.0, 9.6, 11.2, 12.8, 14.4, 16 μM) and human IgG (final concentrations: 16, 14.4, 12.8, 11.2, 9.6, 8.0, 6.4, 4.8, 3.2, 1.6, 0 μM) were incubated in PBS buffer (pH 7.3) containing 1% DMSO, and the fluorescence intensities of the different solutions were recorded on a SpectraMax M2 plate reader (excitation: 530 nm; emission: 590 nm). The Job plot is represented as [fluorescence fold increase $\times$ ratio **Ig Orange**] vs. ratio **Ig Orange**, with values as means ($n=3$) and error bars as standard deviations (FIG. 6b).

Example 6: Structure-fluorescence relationships of Ig Orange and additional SPR Experiments.

Ig Orange (BDM-69) (10 μ M) and **BD-69** (10 μ M) were incubated with different concentrations of human IgG in 20 mM HEPES buffer (pH 7.4) and the fluorescence intensities were recorded on a SpectraMax M2 plate reader (excitation: 530 nm; emission: 590 nm), as shown in FIG. 7a. Values are represented as means
5 (n=3) of the fluorescence fold increase after incubation with the protein. FIG. 7b shows a binding sensorgram of the aminoethyl-free compound (BD-69).

Example 7: Binding of Ig Orange to polyclonal IgG and monoclonal IgG₁.

Ig Orange (10 μ M) was incubated with different concentrations of IgG from human serum (polyclonal IgG) and a monoclonal human IgG₁ in 20 mM HEPES
10 buffer (pH 7.4). Fluorescence measurements were recorded on a SpectraMax M2 plate reader (excitation: 530 nm; emission: 590 nm) (FIG. 8).

Example 8: Fractional saturation curve experiments of Ig Orange.

Ig Orange (10 μ M) was incubated with different concentrations of human IgG in 20 mM HEPES buffer (pH 7.4) and the fluorescence intensities were recorded
15 on a SpectraMax M2 plate reader (excitation: 530 nm; emission: 590 nm) (FIG. 9).

Example 9: CDy9 shows specificity to mESC versus MEF.

The specificity of CDy9 to mESC was determined by high throughput screening. MEF was used as the negative control as these cells are often used as feeder cells for co-culture with mESC/ iPSC. 1 μ M of CDy9 was added to the mESC
20 and MEF separately. 1X Hoechst was added to the cells to determine the nuclei staining. After 1 hour incubation, the staining pattern was visualized by image acquisition using the ImageXpress machine followed by image analysis using the MetaXpress software. To further validate and confirm the specificity of CDy9 to mESC, an intensity based analysis such as flow cytometry was carried out. Unstained
25 mESC and MEF were used as control cells. As demonstrated by FIG. 10, CDy9 stains more specifically to mESC than MEF.

Example 10: Cell panel screening.

Cell panel screening was performed to further confirm the specificity of CDy9. In this experiment, many different cell types from different lineages
30 (endoderm, ectoderm, mesoderm) were tested together with mESC. After culturing the different cell types, 1 μ M of CDy9 was added to the cells with 1hour incubation.

1X Hoechst was added to the cells to determine nuclei staining. The cell staining pattern was then visualized by image acquisition using the ImageXpress machine with image analysis using the Metaxpress program. It was determined that CDy9 was highly specific to mESC when compared to all the other cell types.

5 *Example 11: CD9 Wash-out Experiment.*

To investigate the properties of CDy9-stained mESC, a washing experiment was designed to examine survival of CDy9. 1 uM CDy9 was added to mESC. After incubation, the cells were washed with media, PBS, methanol, and 4% PFA, respectively. Utilizing the Ti microscope, 10X images were acquired. As
10 demonstrated by FIG. 12, CDy9 was not washed out of the sample under any of the washing conditions.

Example 12: CDy9 Isolation of Stem Cells from a mixture of MEF and mESC.

We further examined the specificity of CDy9 when used in conjunction with a co-culture of mESC and MEF cells. 1uM of CDy9 was added to these cells. As
15 depicted in FIG. 13, after incubation, the cells were rinsed with PBS (3X) and prepared for FACs. During FACs analysis 10% of the brightest population and 10% of the dimmest population were sorted and collected. These cells were then seeded onto gelatin coated plates and grown for 4-5 days until mESC-like colonies started to appear. The number of colonies formed was counted and the graph was plotted after
20 5 days. It was noticed that the bright population formed at least 2 times more colonies than the dim population indicating that CDy9 stained mESC specifically. Immunohistochemistry was carried out on the colonies formed from the bright population. Primary antibody used was SSEA1 and secondary antibody used was AlexaFluor 488 goat anti mouse IgM.

25 *Example 13: Isolation of Potential Pluripotent Stem Cells from Mouse Fat Pads using CDy9.*

CDy9 was also utilized in the isolation of potential pluripotent stem cells from mouse fat pads using CDy9, depicted in FIG. 14. Mouse epididymal fat pads were collected and isolated into single cells using collagenase type 1. 1uM of CDy9
30 was added to them and incubated for 1 hour before FACs. 10% of the dimmest and 10% of the brightest population were collected and seeded onto mitomycin treated feeder cells. After 14 days, 35 colonies were observed in the bright population

compared to the dim population. 1uM of CDy9 was added per well and 4X images of the entire wells were acquired. Right hand shows enlarged images of selected colonies. Immunohistochemistry was performed on the colonies. Primary antibody used was Oct4 while secondary antibody used was Cy5 goat anti-rabbit IgG (H+L).

5

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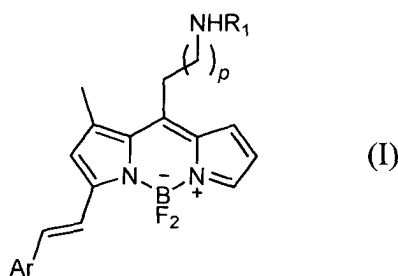
The teachings of all patents, published applications and references cited
20 herein are incorporated by reference in their entirety.

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
25 departing from the scope of the invention encompassed by the appended claims.

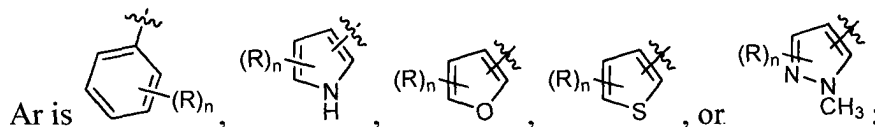
CLAIMS

What is claimed is:

1. A compound represented by structural Formula (I) or pharmaceutically acceptable salts thereof:



wherein:



each R is independently selected from H, OH, halogen, nitro, amino, (C₁-C₆)alkyl, (C=O)(C₀-C₆)alkyl, (C=O)O(C₀-C₆)alkyl, NH(C=O)(C₀-C₆)alkyl, OCF₃, CH=CH(C₀-C₆)alkyl aryl, O(C₁-C₆)alkenyl, (C₀-C₆)alkylCH=O, O(C₁-C₆)alkyl, S(C₁-C₆)alkyl, S(C₁-C₆)haloalkyl, O(C₁-C₆)haloalkyl, OCHF₂, O(C₀-C₆)alkylamino, (C₆-C₁₀)aryl, (C₅-C₁₀)heteroaryl, (C₅-C₁₀)heterocyclyl, (C₁-C₆)alkyl aryl, O(C₀-C₆)alkyl aryl, O(CH₂)_mCHCH₂ or 1-5-membered polycyclyl, wherein each 1-5-membered polycycle optionally and independently contains 1-2 ring heteroatoms selected from oxygen, nitrogen and sulfur;

n is 1, 2, 3 or 4;

m is 0, 1, 2, 3 or 4;

p is 1, 2, 3, 4, or 5;

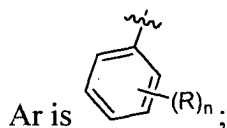
wherein, when n is 2 or greater, the R substituents may be optionally taken together to form a fused polycyclic aromatic ring system;

and wherein each R is optionally substituted with 1-4 R₂ substituents independently selected from:

(C₁-C₆)alkyl, halo(C₀-C₆)alkyl, hydroxy(C₀-C₆)alkyl, (C₆-C₁₀)aryl, halo
 (C₆-C₁₀)aryl, hydroxy(C₆-C₁₀)aryl, O(C₁-C₆)alkyl, O(C₁-C₆)haloalkyl, (C₃-
 C₈)cycloalkyl, halo(C₆-C₁₀)aryl(C₁-C₆)alkoxy, halogen, amino, (C₁-
 C₆)alkoxy(C₆-C₁₀)aryl(C₁-C₆)alkoxy, nitro, hydroxy, halogen, (C₀-
 5 C₆)alkyl(C₆-C₁₀)aryl(C₀-C₆)alkoxy, (C₅-C₁₀)heterocycle, OCF₃, amino, O(C₁-
 C₆)alkylamino, (C₆-C₁₀)aryl(C₂-C₆)alkenyl, (C₂-C₆)alkenyl(C₁-C₆)alkoxy,
 (C₁-C₆)sulfoxy, (C=O)O(C₀-C₆)alkyl or N(CH₃)(C₁-C₆)OH;
 R₁ is H, C=O(C₀-C₆)haloalkyl, C=O(C₀-C₆)alkyl, C=O(C₀-C₆)alkylene or
 C=O(C₆-C₁₀)aryl.

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2. The compound of Claim 1, wherein:



R is CH=CH(C₀-C₆)alkyl aryl; and

n is 1.

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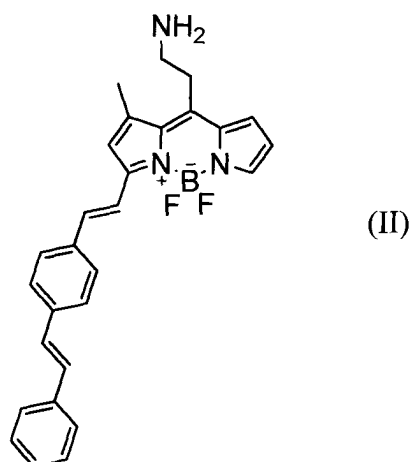
3. The compound of Claim 2, wherein:

R is (CH=CH)phenyl.

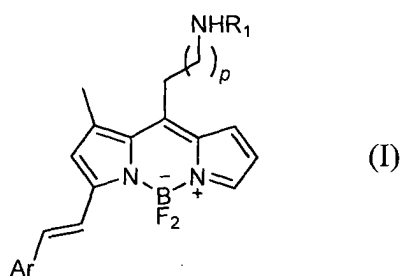
4. The compound of Claim 3, wherein:

20 each R₂ is independently H, halogen, (C₁-C₆)haloalkyl or (C₁-C₆)alkyl .

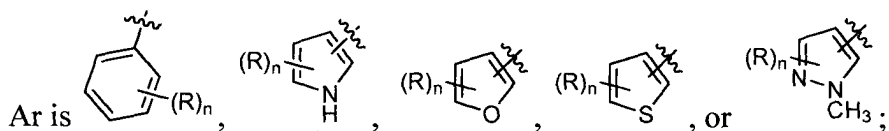
5. The compound of claim 4, wherein the compound is represented by structural
 Formula (II):



6. A method for a solid phase synthesis of a compound of structural Formula (I) or pharmaceutically acceptable salts thereof:



wherein:



each R is independently selected from H, OH, halogen, nitro, amino, (C₁-C₆)alkyl, (C=O)(C₀-C₆)alkyl, (C=O)O(C₀-C₆)alkyl, NH(C=O)(C₀-C₆)alkyl, OCF₃, CH=CH(C₀-C₆)alkylaryl, O(C₁-C₆)alkenyl, (C₀-C₆)alkylCH=O, O(C₁-C₆)alkyl, S(C₁-C₆)alkyl, S(C₁-C₆)haloalkyl, O(C₁-C₆)haloalkyl, OCHF₂, O(C₀-C₆)alkylamino, (C₆-C₁₀)aryl, (C₅-C₁₀)heteroaryl, (C₅-C₁₀)heterocyclyl, (C₁-C₆)alkyl aryl, O(C₀-C₆)alkyl aryl, O(CH₂)_mCHCH₂ or 1-5-membered polycyclyl, wherein each 1-5-membered polycycle optionally and independently contains 1-2 ring heteroatoms selected from oxygen, nitrogen and sulfur;

n is 1, 2, 3 or 4;

m is 0, 1, 2, 3 or 4;

p is 1, 2, 3, 4, or 5;

wherein, when n is 2 or greater, the R substituents may be optionally taken together to form a fused polycyclic aromatic ring system;

and wherein each R is optionally substituted with 1-4 R_2 substituents

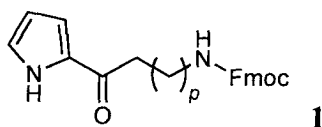
5 independently selected from:

(C_1 - C_6)alkyl, halo(C_0 - C_6)alkyl, hydroxy(C_0 - C_6)alkyl, (C_6 - C_{10})aryl, halo(C_6 - C_{10})aryl, hydroxy(C_6 - C_{10})aryl, O(C_1 - C_6)alkyl, O(C_1 - C_6)haloalkyl, (C_3 - C_8)cycloalkyl, halo(C_6 - C_{10})aryl(C_1 - C_6)alkoxy, halogen, amino, (C_1 - C_6)alkoxy(C_6 - C_{10})aryl(C_1 - C_6)alkoxy, nitro, hydroxy, halogen, (C_0 - C_6)alkyl(C_6 - C_{10})aryl(C_0 - C_6)alkoxy, (C_5 - C_{10})heterocycle, OCF₃, amino, O(C_1 - C_6)alkylamino, (C_6 - C_{10})aryl(C_2 - C_6)alkenyl, (C_2 - C_6)alkenyl(C_1 - C_6)alkoxy, (C_1 - C_6)sulfoxy, (C=O)O(C_0 - C_6)alkyl or N(CH₃)(C_1 - C_6)OH;
 R_1 is H, C=O(C_0 - C_6)haloalkyl, C=O(C_0 - C_6)alkyl, C=O(C_0 - C_6)alkylene or C=O(C_6 - C_{10})aryl;

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comprising the steps of:

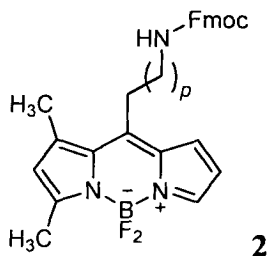
a) reacting an Fmoc-protected amino acid and pyrrole to prepare 2-ketopyrrole (a derivative **1**):



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wherein p is an integer from 1 to 5;

b) condensing derivative **1** with 2,4-dimethylpyrrole and an activating agent, and adding BF₃·OEt₂ *in situ* to obtain derivative **2**:

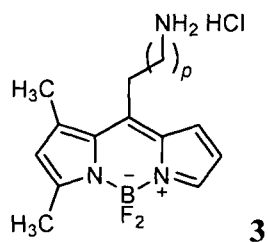


wherein p is an integer from 1 to 5;

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c) reacting derivative **2** with a base;

d) quenching the reaction mixture of step c) with an aqueous HCl to produce derivative **3**:

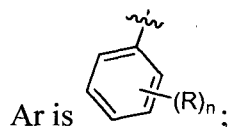


wherein p is an integer from 1 to 5;

e) loading derivative **3** on CTC-PS resin, so that the activated C₃-methyl group of the derivative is modified in a solid-phase adapted Knoevenagel-type reaction; and

f) extracting the compound of Formula I from step e) by a solid-phase extraction.

7. The method of Claim 6, wherein:



R is CH=CH(C₀-C₆)alkyl aryl; and
n is 1.

8. The method of Claim 7, wherein:

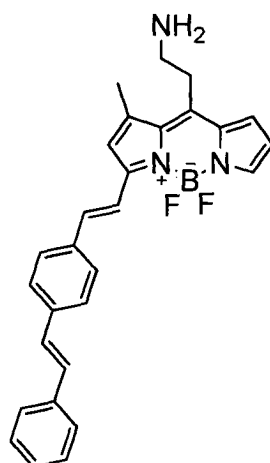
R is (CH=CH)phenyl.

9. The method of Claim 8, wherein:

each R₂ is independently H, halogen, (C₁-C₆)haloalkyl or (C₁-C₆)alkyl .

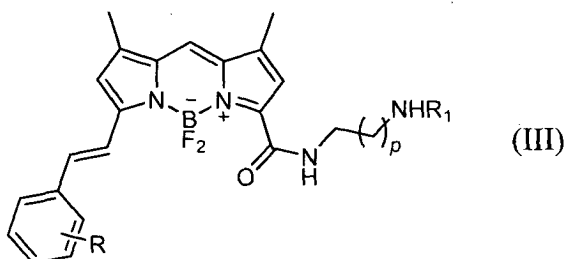
10. The method of Claim 9, wherein the compound is represented by structural Formula (II):

- 66 -



(II).

11. Use of the compound of Claim 1 as a fluorescent sensor.
- 5 12. A method for detection of proteins by fluorescence analysis comprising using a compound of Claim 1.
13. A compound represented by structural Formula (III) or pharmaceutically acceptable salts thereof:



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wherein:

- each R is independently selected from H, OH, halogen, nitro, amino, (C₁-C₆)alkyl, (C=O)(C₀-C₆)alkyl, NH(C=O)(C₀-C₆)alkyl, OCF₃, CH=CH(C₀-C₆)alkylaryl, OC₁-C₆ alkenyl, (C₀-C₆)alkylCH=O, O(C₁-C₆)alkyl, S(C₁-C₆)alkyl, S(C₁-C₆)haloalkyl, O(C₁-C₆)haloalkyl, OCHF₂, O(C₀-C₆)alkylamino, (C₆-C₁₀)aryl, (C₅-C₁₀)heteroaryl, (C₅-C₁₀)heterocyclyl, (C₁-C₆)alkyl aryl, O(C₀-C₆)alkyl aryl, O(CH₂)_mCHCH₂ or 1-5 member polycyclyl, wherein each 1-5 member polycycle optionally and independently contains 1-2 ring heteroatoms selected from oxygen, nitrogen and sulfur;
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n is 1, 2, 3 or 4;

m is 0, 1, 2, 3 or 4;

p is 1, 2, 3, 4, or 5;

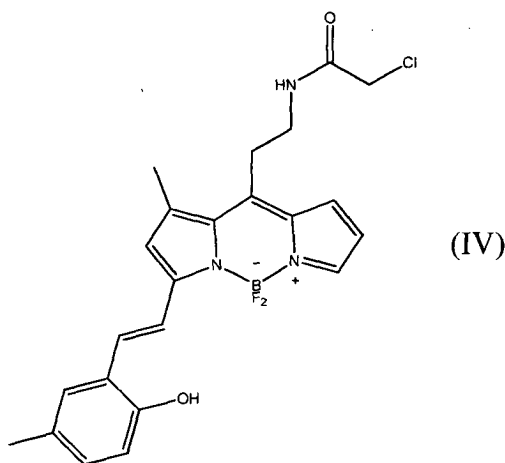
wherein, when n is 2 or greater, the R substituents may be optionally taken together to form a fused polycyclic aromatic ring system;

and wherein each R is optionally substituted with 1-4 R₂ substituents independently selected from:

(C₁-C₆)alkyl, halo(C₀-C₆)alkyl, hydroxy(C₀-C₆)alkyl, (C₆-C₁₀)aryl, halo(C₆-C₁₀)aryl, hydroxy(C₆-C₁₀)aryl, O(C₁-C₆)alkyl, O(C₁-C₆)haloalkyl, (C₃-C₈)cycloalkyl, halo(C₆-C₁₀)aryl(C₁-C₆)alkoxy, halogen, amino, (C₁-C₆)alkoxy(C₆-C₁₀)aryl(C₁-C₆)alkoxy, nitro, hydroxy, halogen, (C₀-C₆)alkyl(C₆-C₁₀)aryl(C₀-C₆)alkoxy, (C₅-C₁₀)heterocycle, OCF₃, amino, O(C₁-C₆)alkylamino, (C₆-C₁₀)aryl(C₂-C₆)alkenyl, (C₂-C₆)alkenyl(C₁-C₆)alkoxy, (C₁-C₆)sulfoxy, (C=O)O(C₀-C₆)alkyl or N(CH₃)(C₁-C₆)OH;

R₁ is H, C=O(C₀-C₆)haloalkyl, C=O(C₀-C₆)alkyl, C=O(C₀-C₆)alkylene or C=O(C₆-C₁₀)aryl;

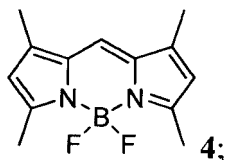
14. The compound of Claim 1, wherein the compound is represented by the structural Formula (IV)



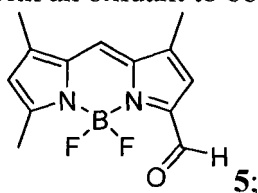
15. Use of compound of Claim 1 for cell imaging and cytometry applications.

- ~~16. Use of compound of Claim 13 for cell imaging and cytometry applications.~~

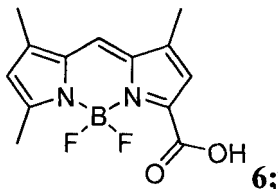
17. A method for staining cells comprising using a compound of Claim 1.
18. A method for the identification, detection, or fluorescence imaging of cells comprising using a compound of Claim 1 or of Claim 13.
19. The method of Claim 16, wherein the cells are mouse embryonic stem cells (mESC).
20. The method of Claim 17, wherein the cells are mouse embryonic stem cells (mESC).
21. A method for a solid phase synthesis of a compound of structural Formula (III), comprising the steps of:
- a) reacting 3,5-dimethylpyrrole-2-carboxaldehyde with 2,4-dimethylpyrrole to prepare tetramethylBODIPY (derivative 4):



- b) reacting derivative 4 with an oxidant to obtain derivative 5:

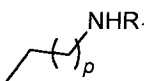


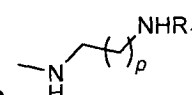
- c) reacting derivative 5 with a second oxidant to form derivative 6;



- d) coupling 6 with alkylendiamine bound to chlorotriyl chloride-polystyrene (CTC-PS) resin, such that the activated C₃-methyl group of the derivative is modified in a solid-phase adapted Knoevenagel-type reaction;
- and

e) extracting the compound of Formula (III) from step d) by a solid-phase extraction.

22. The compound of Claim 1, wherein the group  is further modified with chemically reactive or photoreactive tags.
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23. The compound of Claim 13, wherein the group  is further modified with chemically reactive or photoreactive tags.

- 10 24. A method for detection of proteins by fluorescence analysis comprising using a compound of Claim 13.