(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 November 2012 (22.11.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/159072 A4

(51) International Patent Classification:
G01N 33/68 (2006.01) *C07D 473/32* (2006.01)
G01N 33/58 (2006.01) *C07D 407/04* (2006.01)
G01N 33/52 (2006.01)

(21) International Application Number:
PCT/US2012/038678

(22) International Filing Date:
18 May 2012 (18.05.2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/487,461 18 May 2011 (18.05.2011) US

(71) Applicants *(for all designated States except US)*: **CAYMAN CHEMICAL COMPANY, INCORPORATED** [US/US]; 1180 E. Ellsworth Road, Ann Arbor, MI 48108 (US). **THE REGENTS OF THE UNIVERSITY OF MICHIGAN** [US/US]; 1600 Huron Parkway, 2nd Floor, Ann Arbor, MI 48109-2590 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **BARRETT, Stephen, Douglas** [US/US]; 2828 Fenton Road, Hartland, MI 48353 (US). **BOCHAR, Daniel, Austin** [US/US]; 3334 Tiger Lily Drive, Ann Arbor, MI 48103 (US). **BLAZER, Levi, Lynn** [US/US]; 200 Briarcrest Drive, #114, Ann Arbor, MI 48104 (US). **CISKE, Fred, Lawrence** [US/US]; 7375 Ridge Line Circle, Dexter, MI 48130 (US). **ENDRES, Gregory, William** [US/US]; 386 Huntington Drive, Saline, MI 48176 (US). **JOHNSON, Jeffrey, Keith** [US/US]; 5050 Dexter Ann Arbor Road, Ann Arbor, MI 48103 (US). **KEYES, Gregory, Scott** [CA/US]; 8324 Redwood Trail, Dexter, MI 48130 (US). **SIDHU, Ranjinder, Singh** [CA/US]; 1698 Abigail Way, Ann Arbor, MI 48103 (US). **TRIEVEL, Raymond, C.** [US/US]; 4653 Nutmeg Drive, Ypsilanti, MI 48197 (US). **COLLINS, Margaret, Lynn** [US/US]; 42151 Trent Drive, Canton, MI 48188 (US).

(74) Agents: **HAYS, Steven, W.** et al.; Howard & Howard Attorneys PLLC, 450 West Fourth Street, Royal Oak, MI 48067 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *with amended claims and statement (Art. 19(1))*

(88) Date of publication of the international search report:
11 July 2013

Date of publication of the amended claims and statement: 24 October 2013

(54) Title: FLUORESCENT MOLECULAR PROBES FOR USE IN ASSAYS THAT MEASURE TEST COMPOUND COMPETITIVE BINDING WITH SAM-UTILIZING PROTEINS

(57) Abstract: Assay methods may generally comprise forming homogeneous assay mixtures comprising target SAM-utilizing protein, fluorescent detection analyte, and test compound, incubating, and measuring FP or TR-FRET signal emitted in order to determine a measure of test compound-SAM-utilizing protein binding. Assay mixtures comprise a SAM-utilizing protein, and a fluorescent detection analyte that binds with the SAM-utilizing protein in the absence of test compound. Assay mixtures may further comprise a test compound. Assay mixture embodiments may generate FP or TR-FRET signal properties that are a function of the inherent binding interactions of both the test compound and the detection analyte with the SAM- utilizing protein. Fluorescent detection analytes comprise a fluorophore moiety, a covalent linker moiety, and a SAM-utilizing protein ligand moiety and could be utilized in FP or TR-FRET assays to measure test compound binding.

WO 2012/159072 A4

AMENDED CLAIMS
received by the International Bureau on 24 May 2013 (24.05.2013)

WHAT IS CLAIMED IS:

1. A method for identifying compounds that bind to SAM-utilizing proteins comprising:

(a) measuring a fluorescence signal or property of an irradiated assay mixture, wherein the assay mixture comprises:

(1) a fluorescent detection analyte comprising:

(i) a fluorophore moiety;

(ii) a SAM-utilizing protein ligand moiety; and

(iii) a linker moiety that covalently links the fluorophore moiety with the SAM-utilizing protein ligand moiety;

(2) a SAM-utilizing protein or a SAM-utilizing protein labeled with a donor fluorophore or an acceptor fluorophore; and

(3) a test compound;

wherein the level of binding of the test compound to the SAM-utilizing protein or to the SAM-utilizing protein labeled with the donor fluorophore or the acceptor fluorophore is determined from the measured fluorescence signal or property.

2. The method of Claim 1, wherein the SAM-utilizing protein ligand moiety (ii) comprises a nucleoside-type moiety.

3. The method of Claim 2, wherein the nucleoside-type moiety is selected from the group consisting of a sinefungin moiety, a sulfur-based moiety, and a nitrogen-based moiety.

4. The method of Claim 1, wherein the fluorophore moiety (i) comprises a fluorophore having an emission maximum within the wavelength range of 400-750 nm and wherein the SAM-utilizing protein ligand moiety (ii) comprises a sinefungin moiety.

5. The method of Claim 4, wherein the linker moiety that covalently links the fluorophore moiety with the SAM-utilizing protein ligand moiety (iii) comprises a linker moiety that covalently links the fluorophore moiety with the sinefungin moiety through an atom on the sinefungin moiety selected from the group consisting of a ribose 2'-hydroxy oxygen atom and a ribose 3'-hydroxy oxygen atom.

6. The method of Claim 1, wherein the SAM-utilizing protein ligand moiety (ii) comprises a sinefungin moiety, and wherein the linker moiety that covalently links the fluorophore moiety with the sinefungin moiety (iii) comprises a linker moiety that covalently links the fluorophore moiety with the sinefungin moiety through an atom on the base ring or base ring replacement portion of the sinefungin moiety.

7. A method for identifying compounds that bind to SET domain-containing lysine methyltransferase enzymes comprising:

(a) measuring a fluorescence signal or property of an irradiated assay mixture, wherein the assay mixture comprises:

(1) a fluorescent detection analyte comprising:

(i) a fluorophore moiety;

(ii) a sinefungin moiety; and

(iii) a linker moiety that covalently links the fluorophore moiety with the sinefungin moiety through an atom on the sinefungin moiety selected from the group consisting of a ribose 2'-hydroxy oxygen atom, a ribose 3'-hydroxy oxygen atom, and an adenine 2-carbon atom;

(2) a SET domain-containing lysine methyltransferase enzyme or a SET domain-containing lysine methyltransferase enzyme labeled with a donor fluorophore or an acceptor fluorophore; and

(3) a test compound;

wherein the level of binding of the test compound to the SET domain-containing lysine methyltransferase enzyme or to the SET domain-containing lysine methyltransferase enzyme labeled with the donor fluorophore or the acceptor fluorophore is determined from the measured fluorescence signal or property.

8. The method of Claim 7, wherein the SET domain-containing lysine methyltransferase enzyme (2) is selected from the group consisting of SET7/9, GLP, MLL, and G9a.

9. The method of Claim 8, wherein the detection analyte (1) is selected from the group consisting of Sinefungin Probe 1A, Sinefungin Probe 1B, Sinefungin Probe 2A, Sinefungin Probe 3 and Sinefungin Probe 4.

10. A method for identifying compounds that bind to lysine methyltransferase enzyme SET7/9 comprising:

(a) measuring a fluorescence signal or property of an irradiated assay mixture, wherein the assay mixture comprises:

(1) a fluorescent detection analyte comprising:

(i) a fluorophore moiety;

(ii) a sinefungin moiety; and

(iii) a linker moiety that covalently links the fluorophore moiety with the sinefungin moiety through an atom on the sinefungin moiety selected from the group consisting of a ribose 2'-hydroxy oxygen atom and a ribose 3'-hydroxy oxygen atom and the adenine 2-carbon atom;

(2) a lysine methyltransferase enzyme SET7/9 or a lysine methyltransferase enzyme SET7/9 labeled with a donor fluorophore or an acceptor fluorophore; and

(3) a test compound;

wherein the level of binding of the test compound to the lysine methyltransferase enzyme SET7/9 or to the lysine methyltransferase enzyme SET7/9 labeled with the donor fluorophore or the acceptor fluorophore is determined from the measured fluorescence signal or property.

11. The method of Claim 10, wherein the fluorescent detection analyte (1) is selected from the group consisting of Sinefungin Probe 1A, Sinefungin Probe 1B, Sinefungin Probe 2A, Sinefungin Probe 3 and Sinefungin Probe 4.

12. A method for identifying compounds that bind to lysine methyltransferase enzyme G9a comprising:

(a) measuring a fluorescence signal or property of an irradiated assay mixture, wherein the assay mixture comprises:

(1) a fluorescent detection analyte comprising:

(i) a fluorophore moiety;

(ii) a sinefungin moiety; and

(iii) a linker moiety that covalently links the fluorophore moiety with the sinefungin moiety through an atom on the sinefungin moiety selected from the group consisting of a ribose 2'-hydroxy oxygen atom and a ribose 3'-hydroxy oxygen atom;

(2) a lysine methyltransferase enzyme G9a or a lysine methyltransferase enzyme G9a labeled with a donor fluorophore or an acceptor fluorophore; and

(3) a test compound;

wherein the level of binding of the test compound to the lysine methyltransferase enzyme G9a or to the lysine methyltransferase enzyme G9a labeled with the donor fluorophore or the acceptor fluorophore is determined from the measured fluorescence signal or property.

13. The method of Claim 12, wherein the fluorescent detection analyte (1) is selected from the group consisting of Sinefungin Probe 1A, Sinefungin Probe 1B, Sinefungin Probe 2A, Sinefungin Probe 3 and Sinefungin Probe 4.

14. A method for identifying compounds that bind to lysine methyltransferase enzyme GLP comprising:

(a) measuring a fluorescence signal or property of an irradiated assay mixture, wherein the assay mixture comprises:

(1) a fluorescent detection analyte comprising:

(i) a fluorophore moiety;

(ii) a sinefungin moiety; and

(iii) a linker moiety that covalently links the fluorophore moiety with the sinefungin moiety through an atom on the sinefungin moiety selected from the group consisting of a ribose 2'-hydroxy oxygen atom and a ribose 3'-hydroxy oxygen atom;

(2) a lysine methyltransferase enzyme GLP or a lysine methyltransferase enzyme GLP labeled with a donor fluorophore or an acceptor fluorophore; and

(3) a test compound;

wherein the level of binding of the test compound to the lysine methyltransferase enzyme GLP or to the lysine methyltransferase enzyme GLP

labeled with the donor fluorophore or the acceptor fluorophore is determined from the measured fluorescence signal or property.

15. The method of Claim 14, wherein the fluorescent detection analyte (1) is selected from the group consisting of Sinefungin Probe 1A, Sinefungin Probe 1B, Sinefungin Probe 2A, Sinefungin Probe 3 and Sinefungin Probe 4.

16. A method for identifying compounds that bind to lysine methyltransferase enzyme MLL comprising:

(a) measuring a fluorescence signal or property of an irradiated assay mixture, wherein the assay mixture comprises:

(1) a fluorescent detection analyte comprising:

(i) a fluorophore moiety;

(ii) a sinefungin moiety; and

(iii) a linker moiety that covalently links the fluorophore moiety with the sinefungin moiety through an atom on the sinefungin moiety selected from the group consisting of a ribose 2'-hydroxy oxygen atom and a ribose 3'-hydroxy oxygen atom;

(2) a lysine methyltransferase enzyme MLL or a lysine methyltransferase enzyme MLL labeled with a donor fluorophore or an acceptor fluorophore; and

(3) a test compound;

wherein the level of binding of the test compound to the lysine methyltransferase enzyme MLL or to the lysine methyltransferase enzyme MLL labeled with the donor fluorophore or the acceptor fluorophore is determined from the measured fluorescence signal or property.

17. The method of Claim 16, wherein the fluorescent detection analyte (1) is selected from the group consisting of Sinefungin Probe 1A, Sinefungin Probe 1B, Sinefungin Probe 2A, Sinefungin Probe 3 and Sinefungin Probe 4.

18. A method for identifying compounds that bind to SET domain-containing lysine methyltransferase enzymes comprising:

(a) forming an assay mixture comprising:

(1) a fluorescent detection analyte comprising

(i) a fluorophore moiety;

(ii) an *S*-substituted-5'-thioadenosine moiety; and

(iii) a linker moiety that covalently links the fluorophore moiety with the *S*-substituted-5'-thioadenosine moiety;

(2) a SET domain-containing lysine methyltransferase enzyme or a SET domain-containing lysine methyltransferase enzyme labeled with a donor fluorophore; and

(3) a test compound;

wherein the level of binding of the test compound to the SET domain-containing lysine methyltransferase enzyme or to the SET domain-containing lysine methyltransferase enzyme labeled with the donor fluorophore or the acceptor fluorophore is determined from the measured fluorescence signal or property.

19. A method for identifying compounds that bind to the SET domain-containing lysine methyltransferase enzyme PRDM9 comprising:

(a) forming an assay mixture comprising:

(1) a fluorescent detection analyte comprising

(i) a fluorophore moiety;

(ii) an *S*-substituted-5'-thioadenosine moiety; and

(iii) a linker moiety that covalently links the fluorophore moiety with the *S*-substituted-5'-thioadenosine moiety;

(2) the SET domain-containing lysine methyltransferase enzyme PRDM9 or a SET domain-containing lysine methyltransferase enzyme PRDM9 labeled with a donor fluorophore; and

(3) a test compound;

wherein the level of binding of the test compound to the SET domain-containing lysine methyltransferase enzyme PRDM9 or to the SET domain-containing lysine methyltransferase enzyme PRDM9 labeled with the donor fluorophore or the acceptor fluorophore is determined from the measured fluorescence signal or property.

20. The method of Claim 19, wherein the fluorescent detection analyte (1) comprises Thioadenosine Probe 1.

21. A method for identifying compounds that bind to the arginine methyltransferase enzymes comprising:

(a) forming an assay mixture comprising:

(1) a fluorescent detection analyte comprising

(i) a fluorophore moiety;

(ii) an *S*-substituted-5'-thioadenosine moiety; and

(iii) a linker moiety that covalently links the fluorophore moiety with the *S*-substituted-5'-thioadenosine moiety;

(2) a protein arginine methyltransferase (PRMT) enzyme or PRMT enzyme labeled with a donor fluorophore; and

(3) a test compound;

wherein the level of binding of the test compound to the PRMT enzyme or to the PRMT enzyme labeled with the donor fluorophore or the acceptor fluorophore is determined from the measured fluorescence signal or property.

22. The method of Claim 21, wherein the PRMT enzyme is selected from the group consisting of PRMT1 and PRMT4.

23. The method of Claim 21, wherein the fluorescent detection analyte (1) comprises Thioadenosine Probe 1.

24. An assay mixture for identifying compounds that bind to a SAM-utilizing protein comprising:

(a) a detection analyte comprising:

(1) a fluorophore moiety;

(2) a SAM-utilizing protein ligand moiety; and

(3) a linker moiety that covalently links the fluorophore moiety with the SAM-utilizing protein ligand moiety;

(b) a SAM-utilizing protein or a SAM-utilizing protein labeled with a donor fluorophore or an acceptor fluorophore; and

(c) a test compound.

25. The assay mixture of Claim 24, wherein the SAM-utilizing protein ligand moiety comprises a nucleoside-type moiety.

26. The assay mixture of Claim 25, wherein the nucleoside-type moiety is selected from the group consisting of a sinefungin moiety, a sulfur-based moiety, and a nitrogen-based moiety.

27. The assay mixture of Claim 24, wherein the SAM-utilizing protein ligand moiety (2) comprises a sinefungin moiety and wherein the linker moiety that covalently links the fluorophore moiety with the sinefungin moiety (3) comprises a linker moiety that covalently links the fluorophore moiety with the sinefungin moiety through an atom on the sinefungin moiety selected from the group consisting of a ribose 2'-hydroxy oxygen atom and a ribose 3'-hydroxy oxygen atom.

28. The assay mixture of Claim 24, wherein the SAM-utilizing protein ligand moiety (2) comprises a sinefungin moiety and wherein the linker moiety that covalently links the fluorophore moiety with the sinefungin or sinefungin analog moiety (3) comprises a linker moiety that covalently links the fluorophore moiety with the sinefungin moiety through an atom on a base ring or a base ring replacement portion of the sinefungin moiety.

29. An assay mixture for identifying compounds that bind to a SAM-utilizing methyltransferase enzyme comprising:

(a) a detection analyte comprising:

(1) a fluorophore moiety;

(2) a sinefungin moiety; and

(3) a linker moiety that covalently links the fluorophore moiety with the sinefungin moiety;

(b) a SAM-utilizing methyltransferase enzyme or a SAM-utilizing methyltransferase enzyme labeled with a donor fluorophore or an acceptor fluorophore; and

(c) a test compound.

30. An assay mixture for identifying compounds that bind to a SAM-utilizing methyltransferase enzyme comprising:

(b) a detection analyte comprising:

(1) a fluorophore moiety;

(2) a sinefungin moiety; and

(3) a linker moiety that covalently links the fluorophore moiety with the sinefungin moiety through an atom on the sinefungin moiety selected from the group consisting of a ribose 2'-hydroxy oxygen atom, a ribose 3'-hydroxy oxygen atom, and an adenine 2-carbon atom;

(c) a SAM-utilizing methyltransferase enzyme or a SAM-utilizing methyltransferase enzyme labeled with a donor fluorophore or an acceptor fluorophore; and

(d) a test compound.

31. An assay mixture for identifying compounds that bind to a SET domain-containing methyltransferase enzyme comprising:

(a) a detection analyte comprising:

(1) a fluorophore moiety;

(2) a sinefungin moiety; and

(3) a linker moiety that covalently links the fluorophore moiety with the sinefungin moiety through an atom on the sinefungin moiety selected from the group consisting of a ribose 2'-hydroxy oxygen atom, a 3'-hydroxy oxygen atom, and an adenine 2-carbon atom;

(b) a SET domain-containing lysine methyltransferase enzyme or a SET domain-containing lysine methyltransferase enzyme labeled with a donor fluorophore or an acceptor fluorophore; and

(c) a test compound.

32. The assay mixture of Claim 31, wherein the SET domain-containing lysine methyltransferase enzyme (b) is selected from the group consisting of SET7/9, MLL, GLP and G9a.

33. The assay mixture of Claim 32, wherein the detection analyte (a) is selected from the group consisting of Sinefungin Probe 1A, Sinefungin Probe 1B, Sinefungin Probe 2A, Sinefungin Probe 3 and Sinefungin Probe 4.

34. An assay mixture for identifying compounds that bind to a SET domain-containing methyltransferase enzyme comprising:

(a) a detection analyte selected from the group consisting of Sinefungin Probe 1A, Sinefungin Probe 1B, Sinefungin Probe 2A, Sinefungin Probe 3 and Sinefungin Probe 4;

(b) a SET domain-containing lysine methyltransferase enzyme or a SET domain-containing lysine methyltransferase enzyme labeled with a donor fluorophore or an acceptor fluorophore, the SET domain-containing lysine methyltransferase enzyme selected from the group consisting of SET7/9, MLL, GLP and G9a; and

(c) a test compound.

35. An assay mixture for identifying compounds that bind to lysine methyltransferase enzyme SET7/9 comprising:

(a) a detection analyte selected from the group consisting of Sinefungin Probe 1A, Sinefungin Probe 1B, Sinefungin Probe 2A, Sinefungin Probe 3 and Sinefungin Probe 4;

(b) SET7/9 or SET7/9 labeled with a donor fluorophore or an acceptor fluorophore; and

(c) a test compound.

36. An assay mixture for identifying compounds that bind to lysine methyltransferase enzyme G9a comprising:

(a) a detection analyte selected from the group consisting of Sinefungin Probe 1A, Sinefungin Probe 1B, Sinefungin Probe 2A, Sinefungin Probe 3 and Sinefungin Probe 4;

(b) G9a or G9a labeled with a donor fluorophore or an acceptor fluorophore; and

(c) a test compound.

37. An assay mixture for identifying compounds that bind to lysine methyltransferase enzyme GLP comprising:

(a) a detection analyte selected from the group consisting of Sinefungin Probe 1A, Sinefungin Probe 1B, Sinefungin Probe 2A, Sinefungin Probe 3 and Sinefungin Probe 4;

(b) GLP or GLP labeled with a donor fluorophore or an acceptor fluorophore; and

(c) a test compound.

38. An assay mixture for identifying compounds that bind to lysine methyltransferase enzyme MLL comprising:

(a) a detection analyte selected from the group consisting of Sinefungin Probe 1A, Sinefungin Probe 1B, Sinefungin Probe 2A, Sinefungin Probe 3 and Sinefungin Probe 4;

(b) MLL or MLL labeled with a donor fluorophore or an acceptor fluorophore; and

(c) a test compound.

39. An assay mixture for identifying compounds that bind to SET domain-containing lysine methyltransferase enzymes comprising:

(a) a detection analyte comprising:

(1) a fluorophore moiety;

(2) an *S*-substituted-5'-thioadenosine moiety; and

(3) a linker moiety that covalently links the fluorophore moiety with the *S*-substituted-5'-thioadenosine moiety;

(b) a SET domain-containing lysine methyltransferase enzyme or a SET domain-containing lysine methyltransferase enzyme labeled with a donor fluorophore; and

(c) a test compound.

40. An assay mixture for identifying compounds that bind to SET domain-containing lysine methyltransferase enzyme PRDM9 comprising:

(a) a detection analyte comprising:

(1) a fluorophore moiety;

(2) an *S*-substituted-5'-thioadenosine moiety; and

(3) a linker moiety that covalently links the fluorophore moiety with the *S*-substituted-5'-thioadenosine moiety;

(b) the SET domain-containing lysine methyltransferase enzyme PRDM9 or PRDM9 labeled with a donor fluorophore; and

(c) a test compound.

41. The assay mixture of Claim 40, wherein the detection analyte (a) comprises Thioadenosine Probe 1.

42. An assay mixture for identifying compounds that bind to arginine methyltransferase enzymes comprising:

(a) a detection analyte comprising:

(1) a fluorophore moiety;

(2) an *S*-substituted-5'-thioadenosine moiety; and

(3) a linker moiety that covalently links the fluorophore moiety with the *S*-substituted-5'-thioadenosine moiety;

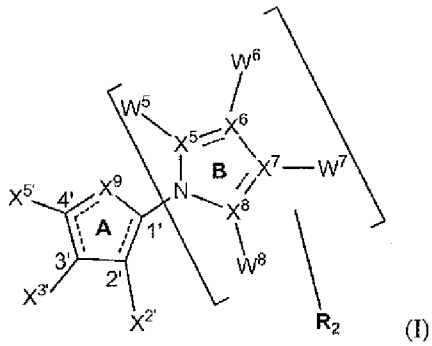
(b) an arginine methyltransferase enzyme or an arginine methyltransferase enzyme labeled with a donor fluorophore; and

(c) a test compound.

43. The assay mixture of Claim 42, wherein the arginine methyltransferase enzyme is selected from the group consisting of PRMT1 and PRMT4.

44. The assay mixture of Claim 42, wherein the detection analyte (a) comprises Thioadenosine Probe 1.

45. A fluorescent detection analyte having the structure of Formula (I):



(I)

wherein the ----- between two atoms in either the **A** ring or the **B** ring represents the bond involving the two atoms is either a single or a double bond, and it may only represent a double bond between the 4'-carbon and X^9 when X^9 is CH;

wherein X^9 is O, NR^7, S, CH (allowed when its bond to the 4'-carbon is a carbon-carbon double bond), or CH_2 (allowed when its bond to the 4'-carbon is a carbon-carbon single bond);

wherein R^7 is hydrogen or methyl;

wherein each of X^5, X^6, X^7, and X^8 is independently a carbon or a nitrogen;

wherein no more than three of X^5, X^6, X^7, and X^8 is nitrogen;

wherein when any X^5, X^6, X^7, or X^8 is nitrogen, the associated W^5, W^6, W^7, and W^8, respectively, is not present;

wherein when X^5 is carbon, W^5 may be hydrogen, methyl, amino, or chloro;

wherein when X^6 is carbon, W^6 may be hydrogen, methyl, amino, acetyl, carboxy, carboxamide, or hydroxy;

wherein when X^7 or X^8 is carbon, W^7 or W^8, respectively, may independently be hydrogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-6} cycloalkyl, (C_{3-6} cycloalkyl)methyl, phenyl, benzyl, five- or six-membered heterocyclyl, five- or six-membered heteroaryl, cyano, amino, acetyl, carboxy, hydroxy or $CONR^8R^9$;

wherein each of R^8 and R^9 is independently hydrogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-6} cycloalkyl, (C_{3-6} cycloalkyl)methyl, phenyl, benzyl, five- or six-membered heterocyclyl, five- or six-membered heteroaryl, or OR^{10}, or together with the nitrogen atom form a pyrrolidine, piperidine, morpholine, or pyrazine ring;

wherein R^{10} is hydrogen, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-6} cycloalkyl, (C_{3-6} cycloalkyl)methyl, phenyl, benzyl, five- or six-membered heterocyclyl, or five- or six-membered heteroaryl;

or wherein W^7 or W^8 together form a five- or six-membered aryl, carbocyclic, heterocyclic, or heteroaryl ring fused with the **B** ring;

wherein $X^{2'}$ is hydrogen, hydroxy, or $OR^{2'}$;

wherein $X^{3'}$ is hydrogen, hydroxy, or $OR^{3'}$;

wherein $X^{5'}$ is $C(=X^4)X^{10}R$;

wherein X^4 is O or H_2;

wherein X^{10} is $C(H)NR^{11}R^{12}$, NR^1, or S;

wherein R^{11} and R^{12} each is independently hydrogen, C_{1-4} alkyl, C_{2-3} alkenyl, C_{2-3} alkynyl, C_{3-6} cycloalkyl, phenyl, benzyl, or acetyl, or together with the nitrogen atom form an aziridine, azetidine, pyrrolidine, piperidine, morpholine, or pyrazine ring;

wherein R and R^1 each is independently hydrogen, C_{1-8} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-6} cycloalkyl, C_{6-10} aryl, five-to-ten-membered heteroaryl, five- to ten-membered heterocyclyl, C_{1-8} acyl, or [(S)-2-aminobutanoic acid]-4-yl,

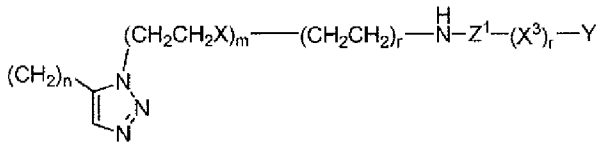
wherein any alkyl, alkenyl, alkynyl, cycloalkyl, heterocyclyl, aryl or heteroaryl ring is optionally substituted with one or more of fluoro, chloro, bromo, iodo, C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, methoxy, ethoxy, trifluoromethoxy, trifluoromethyl, hydroxy, thiomethyl, cyano, NR^8R^9, $-N(H)C(=O)X^{11}$, acetyl, carboxy, carboxy(C_{1-4} alkyl), or $CONR^8R^9$;

wherein X^{11} is C_{1-6} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-6} cycloalkyl, (C_{3-6} cycloalkyl)methyl, phenyl, benzyl, OR^{10}, or NR^8R^9;

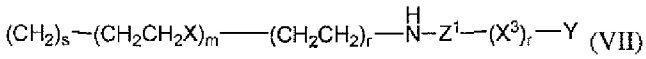
wherein one of R^2, $R^{2'}$ (if it exists), and $R^{3'}$ (if it exists), comprises a linker component, wherein the linker component comprises a linker moiety bonded to a fluorophore moiety, and the other existing of R^2, $R^{2'}$, and $R^{3'}$ are hydrogen, and wherein R^2, when not hydrogen, substitutes a hydrogen atom of the **B** ring or a ring fused to the **B** ring or a functional group covalently bound to the **B** ring or a ring fused to the **B** ring;

wherein the linker moiety comprises a structure as illustrated in Formula (VI) or Formula (VII):

AMENDED SHEET (ARTICLE 19)

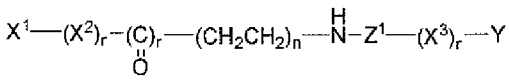


(VI)



(VII)

wherein when R^2 comprises the linker component, the linker moiety may alternatively comprise a structure as illustrated in Formula (VIII):



(VIII)

wherein the $(CH_2)_n$ group of Formula (IV) or the $(CH_2)_s$ group of Formula (V) comprises the site of covalent attachment at one of R^2, $R^{2'}$, and $R^{3'}$ of Formulas (I), (II), and (III);

wherein X is CH_2 or O;

wherein X^1 is N-H, N-CH_3, O, or S;

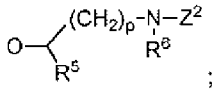
wherein X^2 is N-H, N-CH_3, or, when r is 0 and X^1 is N-H or N-Me, X^2 may alternatively be O;

wherein X^3 is NH or O;

wherein Z^1 is a carbonyl, thiocarbonyl, or sulfonyl group;

AMENDED SHEET (ARTICLE 19)

wherein Y is a covalent bond that binds the linker moiety to the fluorophore moiety, or $(CH_2)_n$ wherein the last CH_2 group in the chain (when n is not 0) is farthest from Z^1 is covalently bound to the fluorophore moiety, or $(CH_2)_n$-N(H)-Z^2, or is of the chemical structure:



;

wherein the oxygen atom end is covalently bound to Z^1;

wherein each R^5 and R^6 is independently H, methyl, or together are $(CH_2)_q$;

wherein q is 1, 2, or 3;

wherein p is 1 or 2; and

wherein Z^2 is a carbonyl or thiocarbonyl group covalently bound by its carbon atom to the fluorophore moiety, or a sulfonyl group covalently bound by sulfur atom to the fluorophore moiety;

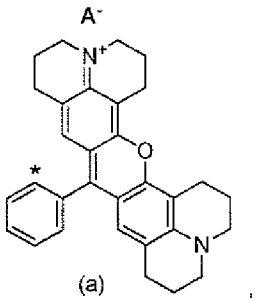
wherein each n is independently 0, 1, 2, 3, 4, or 5;

wherein s is 1, 2, or 3;

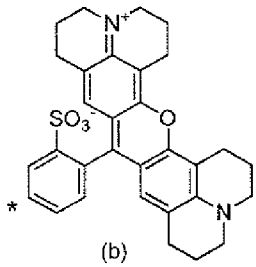
wherein m is 1, 2, or 3;

wherein each r is independently 0 or 1;

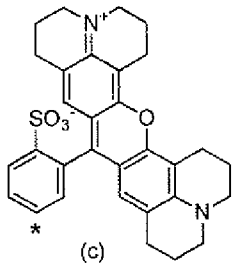
wherein the fluorophore moiety is a structure selected from the group of chemical structures consisting of:



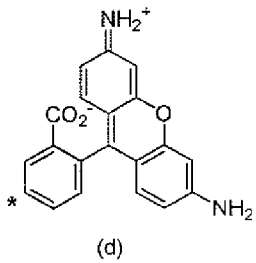
(a),



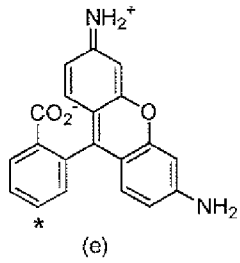
(b),



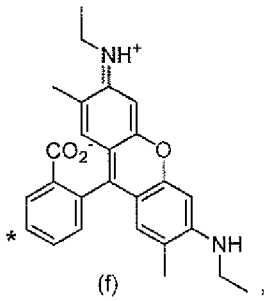
(c),



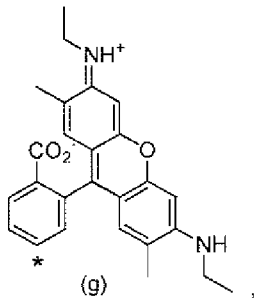
(d),



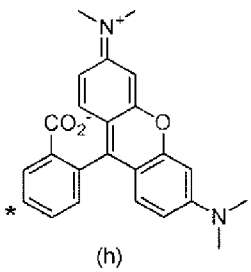
(e),



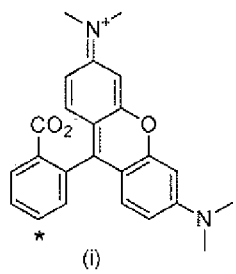
(f),



(g),

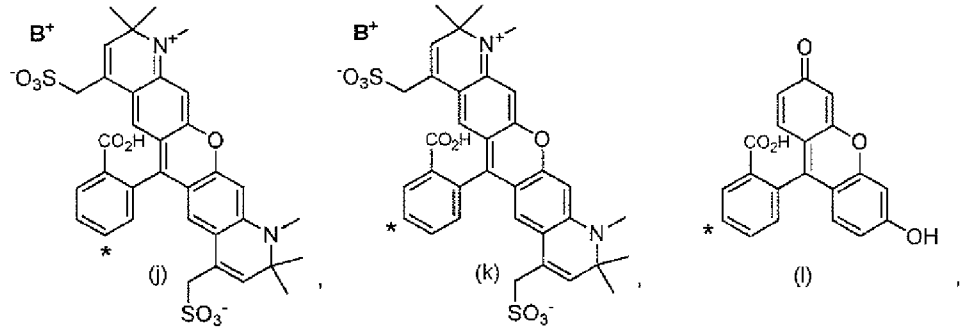


(h),

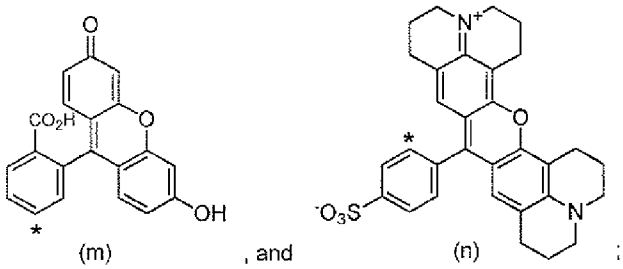


(i),

AMENDED SHEET (ARTICLE 19)



;



, and ;

wherein * represents the position at which the linker moiety is covalently bound to the fluorophore moiety;

wherein A^- is a PF_6^-, trifluoroacetate, acetate, or halide anion;

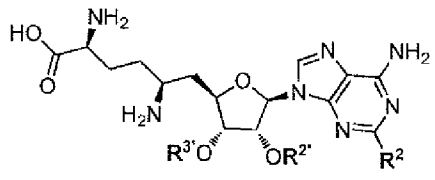
wherein B^+ is a sodium, potassium, cesium, ammonium, or $^+N(R^4)_4$ cation; and

wherein each R^4 is independently H or C_{1-4} alkyl.

46. A fluorescent detection analyte according to Claim 45, wherein the fluorophore moiety comprises a mixture of chemical structure (b) and (c), a mixture of chemical structure (b) and (n), a mixture of chemical structure (d) and (e), a mixture of chemical structure (f) and (g), a mixture of chemical structure (h) and (i), or a mixture of chemical structure (j) and (k).

AMENDED SHEET (ARTICLE 19)

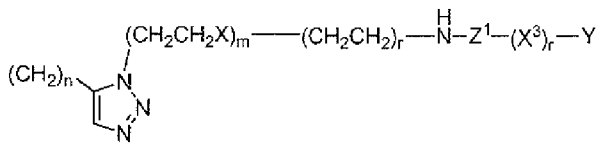
47. A fluorescent detection analyte having the structure of Formula (II):



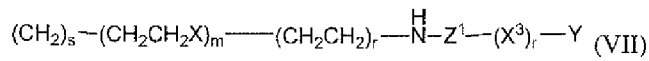
(II)

wherein any two of R^2, $R^{2'}$, and $R^{3'}$ comprises hydrogen and the third comprises a linker component, wherein the linker component comprises a linker moiety bonded to a fluorophore moiety;

wherein the linker moiety comprises a structure as illustrated in Formula (VI) or Formula (VII):

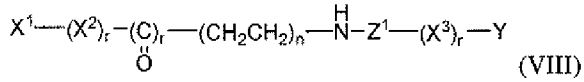


(VI)



(VII)

wherein when R^2 comprises the linker component, the linker moiety may alternatively comprise a structure as illustrated in Formula (VIII):



(VIII)

wherein the $(CH_2)_n$ group of Formula (IV) or the $(CH_2)_s$ group of Formula (V) comprises the site of covalent attachment at one of R^2, $R^{2'}$, and $R^{3'}$ of Formulas (I), (II), and (III);

wherein X is CH_2 or O;

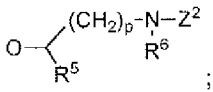
wherein X^1 is N-H, N-CH_3, O, or S;

wherein X^2 is N-H, N-CH_3, or, when r is 0 and X^1 is N-H or N-Me, X^2 may alternatively be O;

wherein X^3 is NH or O;

wherein Z^1 is a carbonyl, thiocarbonyl, or sulfonyl group;

wherein Y is a covalent bond that binds the linker moiety to the fluorophore moiety, or $(CH_2)_n$ wherein the last CH_2 group in the chain (when n is not 0) is farthest from Z^1 is covalently bound to the fluorophore moiety, or $(CH_2)_n$-N(H)-Z^2, or is of the chemical structure:



;

wherein the oxygen atom end is covalently bound to Z^1;

wherein each R^5 and R^6 is independently H, methyl, or together are $(CH_2)_q$;

wherein q is 1, 2, or 3;

wherein p is 1 or 2; and

wherein Z^2 is a carbonyl or thiocarbonyl group covalently bound by its carbon atom to the fluorophore moiety, or a sulfonyl group covalently bound by sulfur atom to the fluorophore moiety;

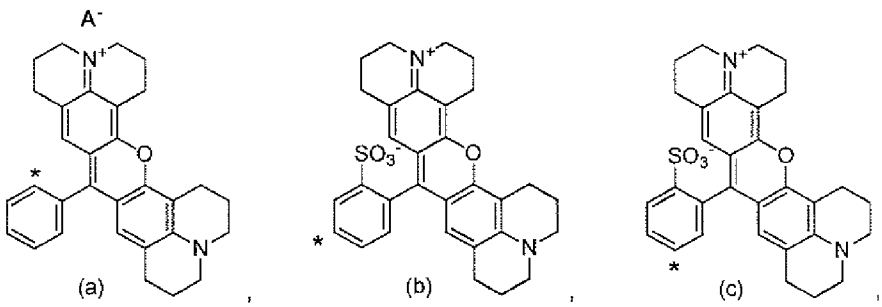
wherein each n is independently 0, 1, 2, 3, 4, or 5;

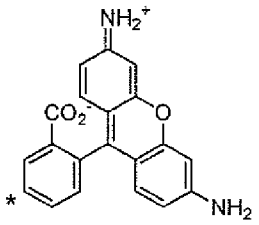
wherein s is 1, 2, or 3;

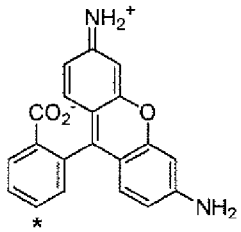
wherein m is 1, 2, or 3;

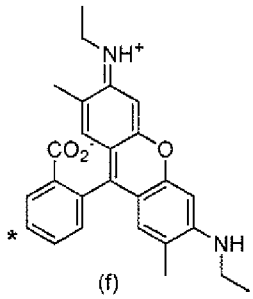
wherein each r is independently 0 or 1;

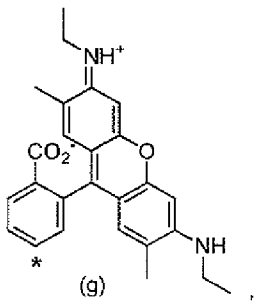
wherein the fluorophore moiety is a structure selected from the group of chemical structures consisting of:

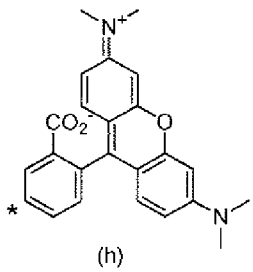


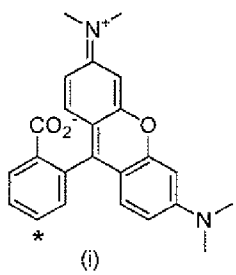

(d),

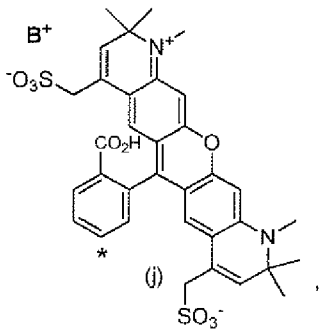

(e),

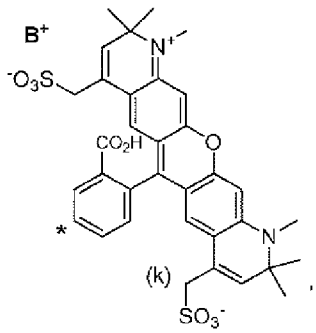

(f),

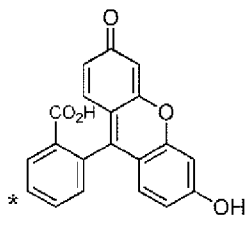

(g),


(h),


(i),

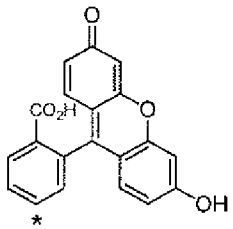

(j),


(k),


(l)

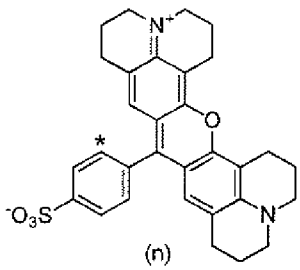
;

AMENDED SHEET (ARTICLE 19)



(m)

, and



(n)

;

wherein * represents the position at which the linker moiety is covalently bound to the fluorophore moiety;

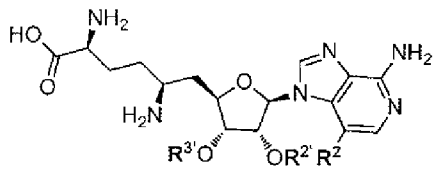
wherein A^- is a PF_6^-, trifluoroacetate, acetate, or halide anion;

wherein B^+ is a sodium, potassium, cesium, ammonium, or $^+N(R^4)_4$ cation; and

wherein each R^4 is independently H or C_{1-4} alkyl.

48. A fluorescent detection analyte according to Claim 47, wherein the fluorophore moiety comprises a mixture of chemical structure (b) and (c), a mixture of chemical structure (b) and (n), a mixture of chemical structure (d) and (e), a mixture of chemical structure (f) and (g), a mixture of chemical structure (h) and (i), or a mixture of chemical structure (j) and (k).

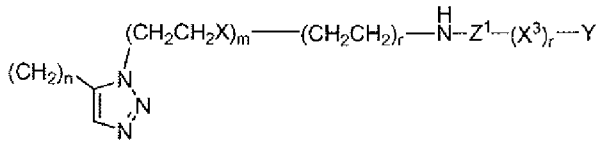
49. A fluorescent detection analyte having the structure of Formula (III):



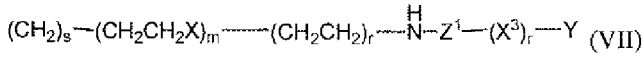
(III)

wherein any two of R^2, $R^{2'}$, and $R^{3'}$ comprises hydrogen and the third comprises a linker component, wherein the linker component comprises a linker moiety bonded to a fluorophore moiety;

wherein the linker moiety comprises a structure as illustrated in Formula (VI) or Formula (VII):

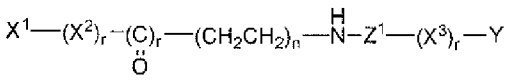


(VI)



(VII)

wherein when R^2 comprises the linker component, the linker moiety may alternatively comprise a structure as illustrated in Formula (VIII):



(VIII)

wherein the $(CH_2)_n$ group of Formula (IV) or the $(CH_2)_s$ group of Formula (V) comprises the site of covalent attachment at one of R^2, $R^{2'}$, and $R^{3'}$ of Formulas (I), (II), and (III);

wherein X is CH_2 or O;

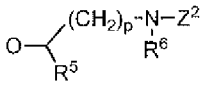
wherein X^1 is N-H, N-CH_3, O, or S;

wherein X^2 is N-H, N-CH_3, or, when r is 0 and X^1 is N-H or N-Me, X^2 may alternatively be O;

wherein X^3 is NH or O;

wherein Z^1 is a carbonyl, thiocarbonyl, or sulfonyl group;

wherein Y is a covalent bond that binds the linker moiety to the fluorophore moiety, or $(CH_2)_n$ wherein the last CH_2 group in the chain (when n is not 0) is farthest from Z^1 is covalently bound to the fluorophore moiety, or $(CH_2)_n$-N(H)-Z^2, or is of the chemical structure:



;

wherein the oxygen atom end is covalently bound to Z^1;

wherein each R^5 and R^6 is independently H, methyl, or together are $(CH_2)_q$;

wherein q is 1, 2, or 3;

wherein p is 1 or 2; and

wherein Z^2 is a carbonyl or thiocarbonyl group covalently bound by its carbon atom to the fluorophore moiety, or a sulfonyl group covalently bound by sulfur atom to the fluorophore moiety;

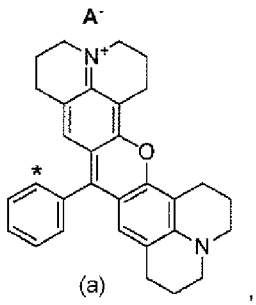
wherein each n is independently 0, 1, 2, 3, 4, or 5;

wherein s is 1, 2, or 3;

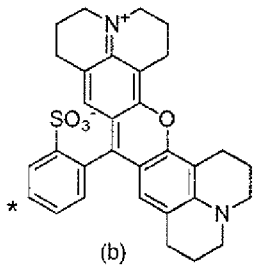
wherein m is 1, 2, or 3;

wherein each r is independently 0 or 1;

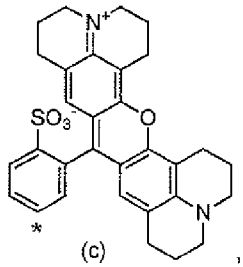
wherein the fluorophore moiety is a structure selected from the group of chemical structures consisting of:



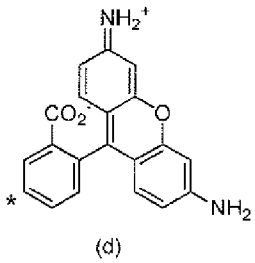
(a),



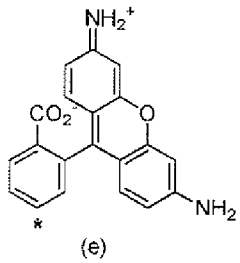
(b),



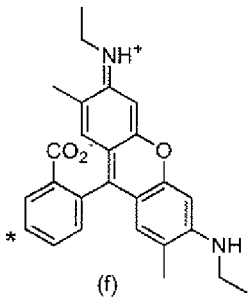
(c),



(d),

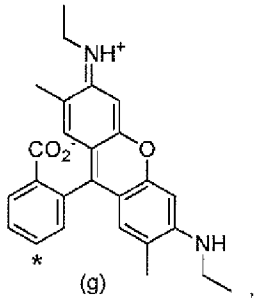


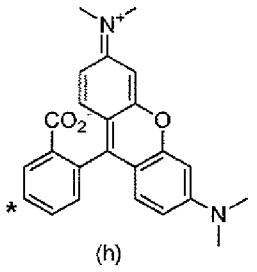
(e),

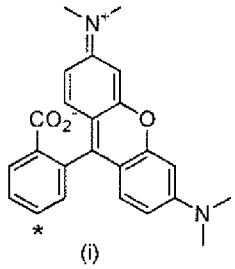


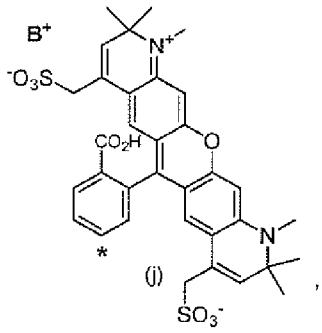
(f),

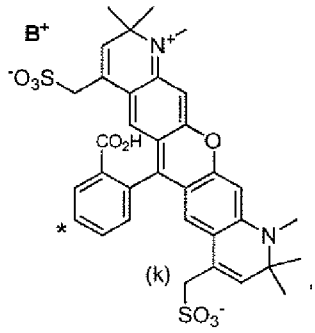
AMENDED SHEET (ARTICLE 19)

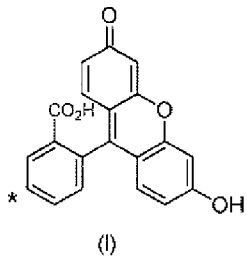

(g),

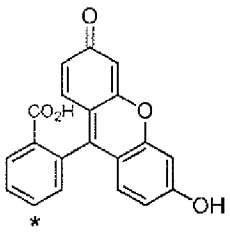

(h),

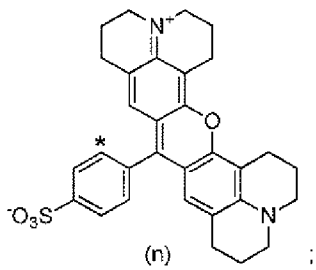

(i),


(j),


(k),


(l),
;


(m), and


(n);

wherein * represents the position at which the linker moiety is covalently bound to the fluorophore moiety;

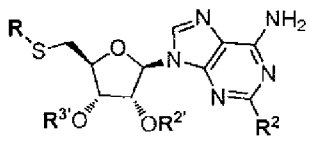
wherein A^- is a PF_6^-, trifluoroacetate, acetate, or halide anion;

wherein B^+ is a sodium, potassium, cesium, ammonium, or $^+N(R^4)_4$ cation; and

wherein each R^4 is independently H or C_{1-4} alkyl.

50. A fluorescent detection analyte according to Claim 49, wherein the fluorophore moiety comprises a mixture of chemical structure (b) and (c), a mixture of chemical structure (b) and (n), a mixture of chemical structure (d) and (e), a mixture of chemical structure (f) and (g), a mixture of chemical structure (h) and (i), or a mixture of chemical structure (j) and (k).

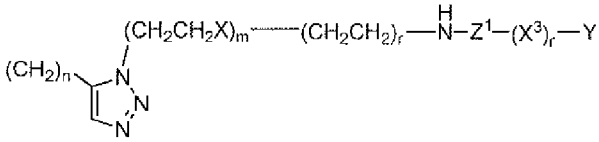
51. A fluorescent detection analyte having the structure of Formula (IV):



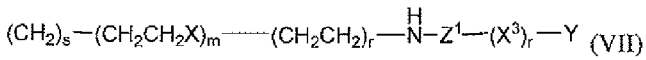
(IV)

wherein any two of R^2, $R^{2'}$, and $R^{3'}$ comprises hydrogen and the third comprises a linker component, wherein the linker component comprises a linker moiety bonded to a fluorophore moiety and wherein R is hydrogen, C_{1-8} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-6} cycloalkyl, C_{6-10} aryl, five-to-ten-membered heteroaryl, five- to ten-membered heterocyclyl, C_{1-8} acyl, or [(S)-2-aminobutanoic acid]-4-yl;

wherein the linker moiety comprises a structure as illustrated in Formula (VI) or Formula (VII):

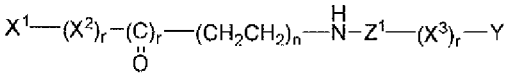


(VI)



(VII)

wherein when R^2 comprises the linker component, the linker moiety may alternatively comprise a structure as illustrated in Formula (VIII):



(VIII)

wherein the $(CH_2)_n$ group of Formula (IV) or the $(CH_2)_s$ group of Formula (V) comprises the site of covalent attachment at one of R^2, $R^{2'}$, and $R^{3'}$ of Formulas (I), (II), and (III);

wherein X is CH_2 or O;

wherein X^1 is N-H, N-CH_3, O, or S;

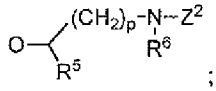
wherein X^2 is N-H, N-CH_3, or, when r is 0 and X^1 is N-H or N-Me, X^2 may alternatively be O;

wherein X^3 is NH or O;

wherein Z^1 is a carbonyl, thiocarbonyl, or sulfonyl group;

wherein Y is a covalent bond that binds the linker moiety to the fluorophore moiety, or $(CH_2)_n$ wherein the last CH_2 group in the chain (when n is not 0) is farthest from Z^1 is covalently bound to the

fluorophore moiety, or $(CH_2)_n$-N(H)-Z^2, or is of the chemical structure:



;

wherein the oxygen atom end is covalently bound to Z^1;

wherein each R^5 and R^6 is independently H, methyl, or together are $(CH_2)_q$;

wherein q is 1, 2, or 3;

wherein p is 1 or 2; and

wherein Z^2 is a carbonyl or thiocarbonyl group covalently bound by its carbon atom to the fluorophore moiety, or a sulfonyl group covalently bound by sulfur atom to the fluorophore moiety;

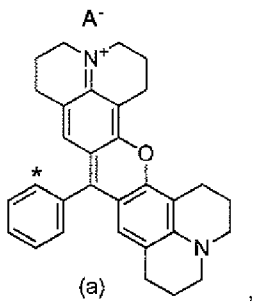
wherein each n is independently 0, 1, 2, 3, 4, or 5;

wherein s is 1, 2, or 3;

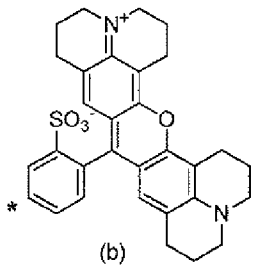
wherein m is 1, 2, or 3;

wherein each r is independently 0 or 1;

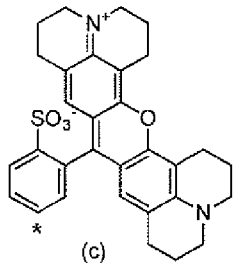
wherein the fluorophore moiety is a structure selected from the group of chemical structures consisting of:


A-
N+
O
N
*

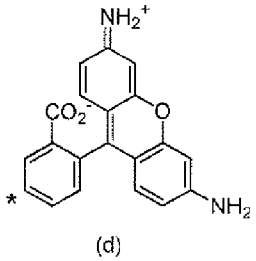
(a),


N+
SO3-
O
N
*

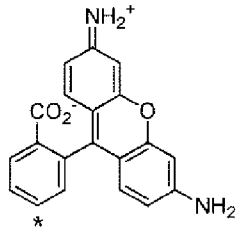
(b),


N+
SO3-
O
N
*

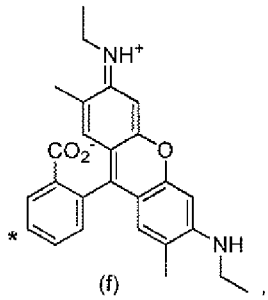
(c),


NH2+
CO2-
O
NH2
*

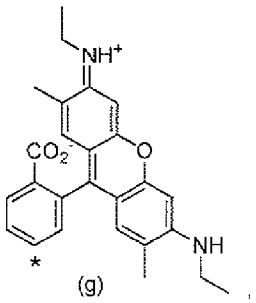
(d),


NH2+
CO2-
O
NH2
*

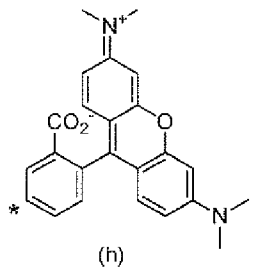
(e),


NH+
CO2-
O
NH
*

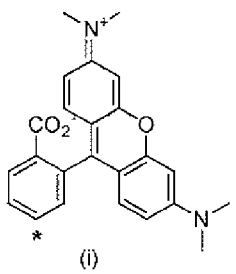
(f),


NH+
CO2-
O
NH
*

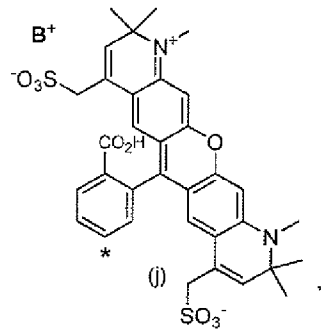
(g),


N+
CO2-
O
N
*

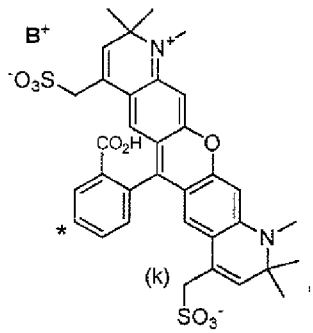
(h),


N+
CO2-
O
N
*

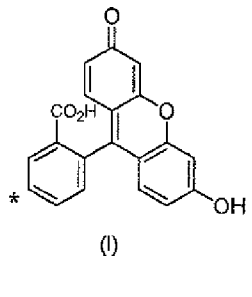
(i),



(j),

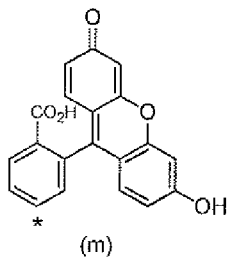


(k),

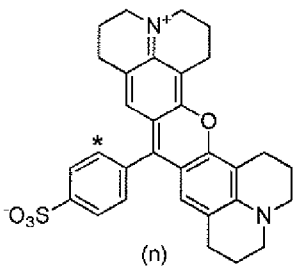


(l),

;



(m), and



(n);

wherein * represents the position at which the linker moiety is covalently bound to the fluorophore moiety;

wherein A^- is a PF_6^-, trifluoroacetate, acetate, or halide anion;

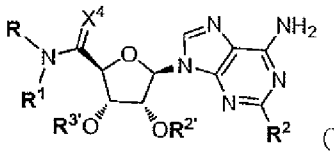
wherein B^+ is a sodium, potassium, cesium, ammonium, or $^+N(R^4)_4$ cation; and

wherein each R^4 is independently H or C_{1-4} alkyl.

52. A fluorescent detection analyte according to Claim 51, wherein the fluorophore moiety comprises a mixture of chemical structure (b) and (c), a mixture of chemical structure (b) and (n), a mixture of chemical structure (d) and (e), a mixture of chemical structure (f) and (g), a mixture of chemical structure (h) and (i), or a mixture of chemical structure (j) and (k).

AMENDED SHEET (ARTICLE 19)

53. A fluorescent detection analyte having the structure of Formula (V):



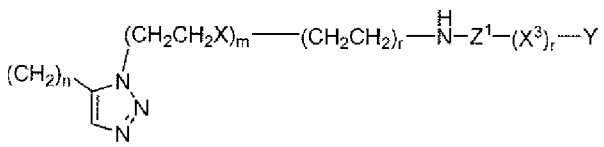
(V)

wherein any two of R^2, $R^{2'}$, and $R^{3'}$ comprises hydrogen and the third comprises a linker component, wherein the linker component comprises a linker moiety bonded to a fluorophore moiety;

wherein R and R^1 are each independently hydrogen, C_{1-8} alkyl, C_{2-6} alkenyl, C_{2-6} alkynyl, C_{3-6} cycloalkyl, C_{6-10} aryl, five-to-ten-membered heteroaryl, five- to ten-membered heterocyclyl, C_{1-8} acyl, or [(S)-2-aminobutanoic acid]-4-yl;

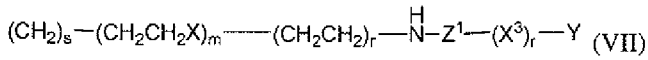
wherein X^4 is O or H_2;

wherein the linker moiety comprises a structure as illustrated in Formula (VI) or Formula (VII):



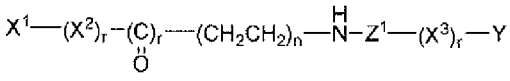
(VI)

AMENDED SHEET (ARTICLE 19)



(VII)

wherein when R^2 comprises the linker component, the linker moiety may alternatively comprise a structure as illustrated in Formula (VIII):



(VIII)

wherein the $(CH_2)_n$ group of Formula (IV) or the $(CH_2)_s$ group of Formula (V) comprises the site of covalent attachment at one of R^2, $R^{2'}$, and R^3 of Formulas (I), (II), and (III);

wherein X is CH_2 or O;

wherein X^1 is N-H, N-CH_3, O, or S;

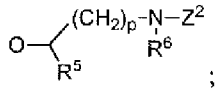
wherein X^2 is N-H, N-CH_3, or, when r is 0 and X^1 is N-H or N-Me, X^2 may alternatively be O;

wherein X^3 is NH or O;

wherein Z^1 is a carbonyl, thiocarbonyl, or sulfonyl group;

wherein Y is a covalent bond that binds the linker moiety to the fluorophore moiety, or $(CH_2)_n$ wherein the last CH_2 group in the chain (when n is not 0) is farthest from Z^1 is covalently bound to the

fluorophore moiety, or $(CH_2)_n$-N(H)-Z^2, or is of the chemical structure:



;

wherein the oxygen atom end is covalently bound to Z^1;

wherein each R^5 and R^6 is independently H, methyl, or together are $(CH_2)_q$;

wherein q is 1, 2, or 3;

wherein p is 1 or 2; and

wherein Z^2 is a carbonyl or thiocarbonyl group covalently bound by its carbon atom to the fluorophore moiety, or a sulfonyl group covalently bound by sulfur atom to the fluorophore moiety;

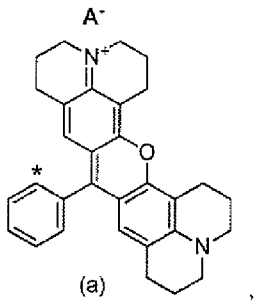
wherein each n is independently 0, 1, 2, 3, 4, or 5;

wherein s is 1, 2, or 3;

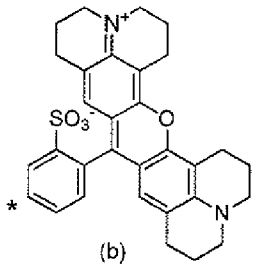
wherein m is 1, 2, or 3;

wherein each r is independently 0 or 1;

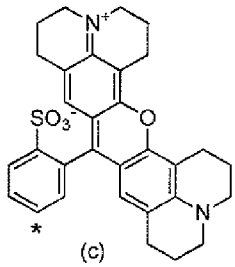
wherein the fluorophore moiety is a structure selected from the group of chemical structures consisting of:



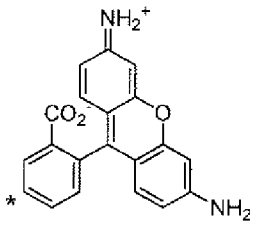
(a),



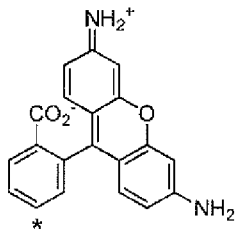
(b),



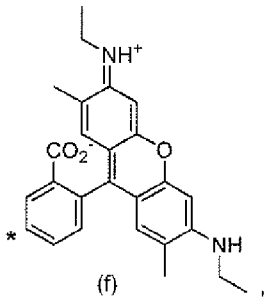
(c),



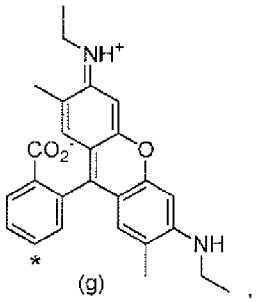
(d),



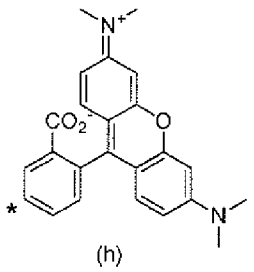
(e),



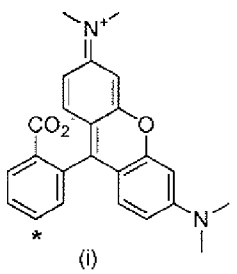
(f),



(g),

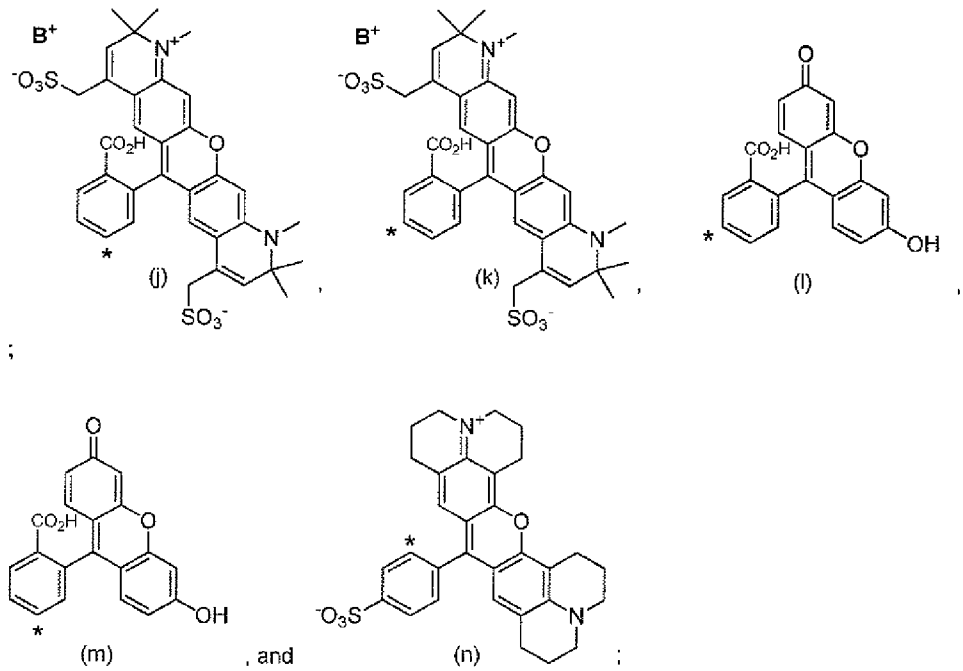


(h),



(i),

AMENDED SHEET (ARTICLE 19)



wherein * represents the position at which the linker moiety is covalently bound to the fluorophore moiety;

wherein A^- is a PF_6^-, trifluoroacetate, acetate, or halide anion;

wherein B^+ is a sodium, potassium, cesium, ammonium, or $^+N(R^4)_4$ cation; and

wherein each R^4 is independently H or C_{1-4} alkyl.

54. A fluorescent detection analyte according to Claim 53, wherein the fluorophore moiety comprises a mixture of chemical structure (b) and (c), a mixture of chemical structure (b) and (n), a mixture of chemical structure (d) and (e), a mixture of chemical structure (f) and (g), a mixture of chemical structure (h) and (i), or a mixture of chemical structure (j) and (k).

AMENDED SHEET (ARTICLE 19)

55. A fluorescent detection analyte selected from the group consisting of Sinefungin Probe 1A, Sinefungin Probe 1B, Sinefungin Probe 2A, Sinefungin Probe 2B, Sinefungin Probe 3, Sinefungin Probe 4, Sinefungin Probe 5, Sinefungin Probe 6, Sinefungin Probe 7, Sinefungin Probe 8, Sinefungin Probe 9, Sinefungin Probe 10, Sinefungin Probe 11, Sinfungin Probe 12, and Sinefungin Probe 13; wherein any A^- is PF_6^-, halide, acetate, or trifluoroacetate, or a mixture thereof.

56. A fluorescent detection analyte selected from the group consisting of Thioadenosine Probe 1 and Thioadenosine Probe 2.

57. A fluorescent detection analyte selected from the group consisting of Aza-adenosine Probe 1, Aza-adenosine Probe 3, Aza-adenosine Probe 5, Aza-adenosine Probe 6, Aza-adenosine Probe 7, Aza-adenosine Probe 8, Aza-adenosine Probe 9, Aza-adenosine Probe 10, and Aza-adenosine Probe 11.

58. A fluorescent detection analyte selected from the group consisting of Aza-adenosine Probe 2, Aza-adenosine Probe 4, Aza-adenosine Probe 12, Aza-adenosine Probe 13, Aza-adenosine Probe 14, Aza-adenosine Probe 15, Aza-adenosine Probe 16, Aza-adenosine Probe 17, Aza-adenosine Probe 18, Aza-adenosine Probe 19, Aza-adenosine Probe 20, and Aza-adenosine Probe 21.

STATEMENT UNDER ARTICLE 19(1)

Claim 40 has been amended to clarify to correct an inadvertent typographical error. Support for this amendment is found throughout the specification as filed.

Item VIII of Written Opinion:

The Written Opinion notes that claim 40 includes a vague and imprecise statement with respect to the preamble including the phrase "Another exemplary embodiment may be directed to an assay mixture." Applicants note that this inclusion of this phrase was an inadvertent typographical error, and have amended claim 40 herein to replace this phrase with the term "An" such that claim 40 is consistent with the remainder with similar independent claims including, for instance, claims 39 and 42. Applicants respectfully submit that claim 40 therefore meets the requirements of PCT Article 6 with respect to clarity.

The Applicant respectfully submits that the claimed invention, as amended, is novel and involves an inventive step. Further and favorable reconsideration of the subject application is hereby requested.