ASYMMETRIC RHODAMINE DYE AND USE THEREOF IN BIOLOGICAL ASSAYS

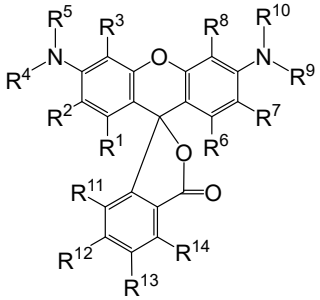
1. BACKGROUND

[001] Using fluorescent rhodamine dyes as detection labels has found widespread use in molecular biology, cell biology and molecular genetics. For example, using fluorescently-labeled oligonucleotides is now widespread in a variety of different assays, including polynucleotide sequencing, fluorescence in situ hybridization (FISH), hybridization assays on nucleic acid arrays, fluorescence polarization studies, and nucleic acid amplification assays, including polymerase chain amplification assays carried out with fluorescent probes and/or primers.

[002] A variety of multiplex assay systems have been described utilizing fluorescent dyes. For example, rhodamine dyes have been described for use in multiplex assay systems, such as those described in WO 2012/067901 for use in human identification assays (HID). Unfortunately, the spectral characteristics of existing dye sets including rhodamine dyes has limited the ability to develop robust and sensitive assay systems using greater than 6-dyes in combination. To enable such higher-plex systems, there is a need for the development of new rhodamine dyes having spectral properties uniquely suited to the creation of such alternative multiplex dye sets.

2. SUMMARY

[003] Fluorescent compounds are described that can be used to label synethetic oligonucleotides. In one example, the compound has the formula (I)



[004] wherein R^1, R^2, R^3, R^6, R^7, R^8, R^{11}, R^{12}, R^{13}, and R^{14}, when taken alone, are each independently of one another selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, $-R^b$, or $-(CH_2)_n-R^b$; or

alternatively, R^1 and R^2 and/or R^6 and R^7 are taken together with the carbon atoms to which they are bonded to form an optionally substituted benzo group;

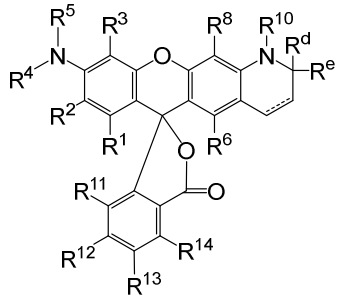
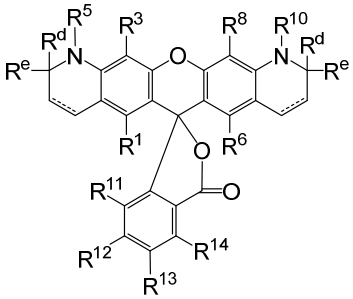
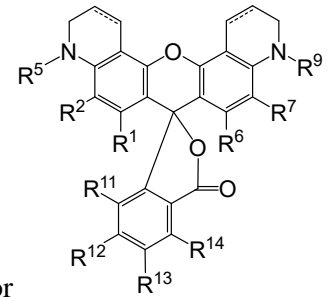
[005] R^4, when taken alone, is selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl; or R^4 and one of R^2 or R^3 are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group;

[006] R^5 is H or a protecting group;

[007] R^9, when taken alone, is selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl; or R^7 and R^9 are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group;

[008] R^{10} is H or protecting group; or R^8 and R^{10} are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group;

[009] at least one of R^7 and R^9 or R^8 and R^{10} are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group, and optionally, R^4 and one of R^2 or R^3 are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group with the proviso that compound is not of the formula


, 
or 
;

[010] each R^a is independently selected from lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, $-CX_3$ and 6-20 membered heteroarylalkyl;

[011] each R^b is independently selected from -X, -OH, $-OR^a$, -SH, $-SR^a$ $-NH_2$, $-NHR^a$, $-NR^cR^c$, $-N^+R^cR^cR^c$, perhalo lower alkyl, trihalomethyl, trifluoromethyl, $-P(O)(OH)_2$, $-P(O)(OR^a)_2$, $P(O)(OH)(OR^a)$, $-OP(O)(OH)_2$, $-OP(O)(OR^a)_2$, $-OP(O)(OR^a)(OH)$, $-S(O)_2OH$,

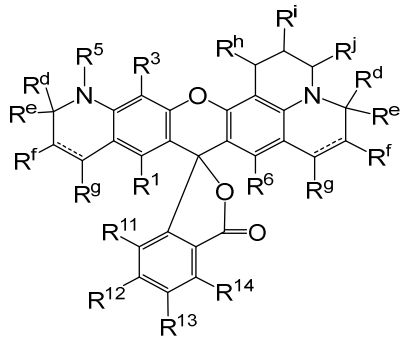
$-S(O)_2R^a$, -C(O)H, $-C(O)R^a$, -C(S)X, $-C(O)OR^a$, -C(O)OH, $-C(O)NH_2$, $-C(O)NHR^a$, $-C(O)NR^cR^c$, $-C(S)NH_2$, $-C(O)NHR^a$, $-C(O)NR^cR^c$, $-C(NH)NH_2$, $-C(NH)NHR^a$, and $-C(NH)NR^cR^c$,

[012] each R^c is independently an R^a, or, alternatively, two R^c bonded to the same nitrogen atom may be taken together with that nitrogen atom to form a 5- to 8-membered saturated or unsaturated ring that may optionally include one or more of the same or different ring heteroatoms, which are typically selected from O, N and S;

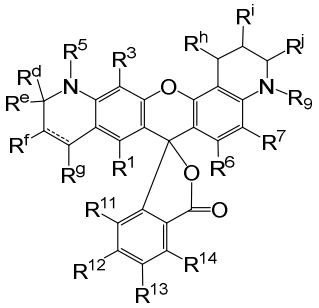
[013] each R^d and R^e, when taken alone, is independently selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, $-R^b$, or $-(CH_2)_n-R^b$; and

[014] n is an integer ranging from 1 to 10.

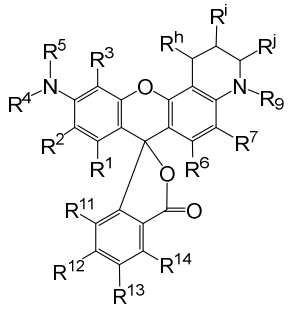
[014a] In one aspect, the present disclosure refers to a compound of the formula (II.1), (II.2), (II.3) or (II.4):



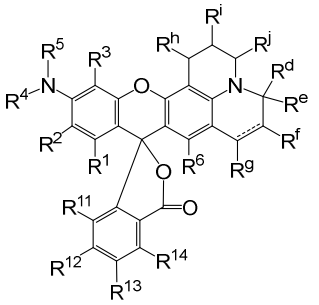
(II.1)



(II.2)



(II.3)



(II.4)

wherein

R^1, R^2, R^3, R^6, R^7, R^{12} and R^{13} when taken alone, are each independently of one another selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, $-R^b$, or $-(CH2)_n-R^b$; or alternatively, R^1 and R^2 and/or R^6 and R^7 are taken together with the carbon atoms to which they are bonded to form an optionally substituted benzo group;

each of R^{11} and R^{14} are halo;

R^4, when taken alone, is selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl; or R^4 and one of R^2 or R^3 are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group;

R^5 is H or a protecting group;

R^9, when taken alone, is selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl; or R^7 and R^9 are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group;

optionally, R^4 and one of R^2 or R^3 are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group;

each R^a is independently selected from lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, $-CX_3$ and 6-20 membered heteroarylalkyl;

each R^b is independently selected from -X, -OH, $-OR^a$, -SH, $-SR^a$ $-NH_2$, $-NHR^a$, $-NR^cR^c$, $-N^+R^cR^cR^c$, perhalo lower alkyl, trihalomethyl, trifluoromethyl, $-P(O)(OH)_2$, $-P(O)(OR^a)_2$, $P(O)(OH)(OR^a)$, $-OP(O)(OH)_2$, $-OP(O)(OR^a)_2$, $-OP(O)(OR^a)(OH)$, $-S(O)_2OH$, $-S(O)_2R^a$, -C(O)H, $-C(O)R^a$, -C(S)X, $-C(O)OR^a$, -C(O)OH, $-C(O)NH_2$, $-C(O)NHR^a$, $-C(O)NR^cR^c$, $-C(S)NH_2$, $-C(O)NHR^a$, $-C(O)NR^cR^c$, $-C(NH)NH_2$, $-C(NH)NHR^a$, and $-C(NH)NR^cR^c$;

each R^c is independently an R^a, or, alternatively, two R^c bonded to the same nitrogen atom may be taken together with that nitrogen atom to form a 5- to 8-membered saturated or unsaturated ring that may optionally include one or more of the same or different ring heteroatoms selected from O, N, and S;

each R^d and R^e, when taken alone, is independently selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, $-R^b$, or $-(CH_2)_n-R^b$;

X is halo;

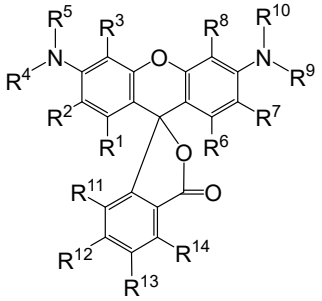
n is an integer ranging from 1 to 10; and

each of R^f, R^g, R^h, R^i, and R^j, when taken alone, are each, independently of one another, selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, $-R^b$, or $-(CH2)_n-R^b$.

[015] In another example, the present disclosure describes oligonucleotide comprising a label moiety produced by reacting an oligonucleotide attached to a solid support with a reagent have a structure of formula:

LM-L-PEP

[016] wherein PEP is a phosphate ester precursor group, L is an optional linker linking the label moiety to the PEP group, and LM comprises an N-protected NH-rhodamine moiety of the formula (I)



[017] wherein R^1, R^2, R^3, R^6, R^7, R^8, R^{11}, R^{12}, R^{13}, and R^{14}, when taken alone, are each independently of one another selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, $-R^b$, or $-(CH2)_n-R^b$; or alternatively, R^1 and R^2 and/or R^6 and R^7 are taken together with the carbon atoms to which they are bonded to form an optionally substituted benzo group; and one of R^2, R^3, R^7, R^8, R^{12}, or R^{13} comprises a group of the formula —Y—, wherein Y is selected from the group consisting of -C(O)-, $-S(O)_2-$, -S- and -NH-;

[018] R^4, when taken alone, is selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl; or R^4 and one of R^2 or R^3 are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted

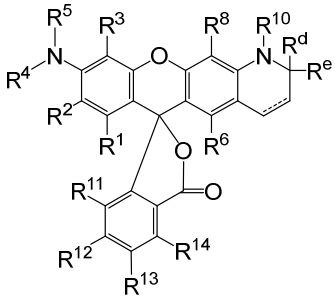
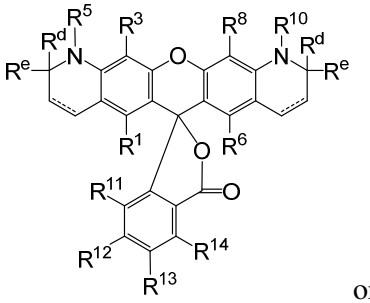
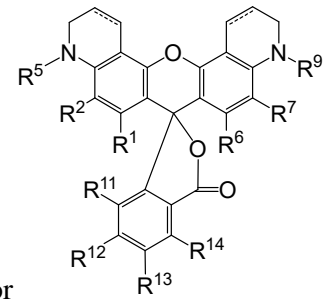
heterocycloalkenyl group, or an optionally substituted heteroaryl group;

[019] R^5 is H or a protecting group;

[020] R^9, when taken alone, is selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl; or R^7 and R^9 are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group;

[021] R^{10} is H or protecting group; or R^8 and R^{10} are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group;

[022] at least one of R^7 and R^9 or R^8 and R^{10} are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group, and optionally, R^4 and one of R^2 or R^3 are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group, with the proviso that compound is not of the formula


, 
or 
;

[023] each R^a is independently selected from lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, $-CX_3$ and 6-20 membered heteroarylalkyl;

[024] each R^b is independently selected from -X, -OH, $-OR^a$, -SH, $-SR^a$ $-NH_2$, $-NHR^a$, $-NR^cR^c$, $-N^+R^cR^cR^c$, perhalo lower alkyl, trihalomethyl, trifluoromethyl, $-P(O)(OH)_2$, $-P(O)(OR^a)_2$, $P(O)(OH)(OR^a)$, $-OP(O)(OH)_2$, $-OP(O)(OR^a)_2$, $-OP(O)(OR^a)(OH)$, $-S(O)_2OH$, $-S(O)_2R^a$, -C(O)H, $-C(O)R^a$, -C(S)X, $-C(O)OR^a$, -C(O)OH, $-C(O)NH_2$, $-C(O)NHR^a$, $-C(O)NR^cR^c$, $-C(S)NH_2$, $-C(O)NHR^a$, $-C(O)NR^cR^c$, $-C(NH)NH_2$, $-C(NH)NHR^a$, and $-C(NH)NR^cR^c$;

[025] each R^c is independently an R^a, or, alternatively, two R^c bonded to the same nitrogen atom may be taken together with that nitrogen atom to form a 5- to 8-membered saturated or

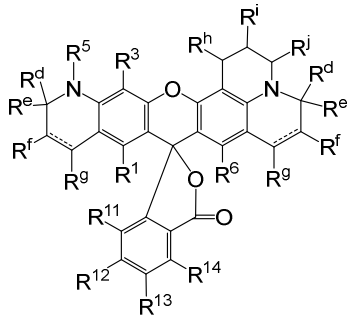
unsaturated ring that may optionally include one or more of the same or different ring heteroatoms, which are typically selected from O, N and S;

[026] each R^d and R^e, when taken alone, is independently selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, $-R^b$, or $-(CH2)_n-R^b$; and

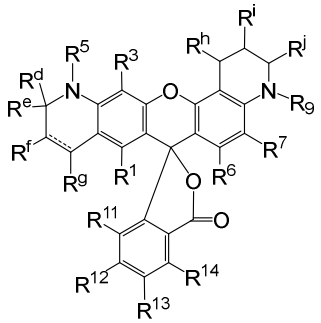
[027] n is an integer ranging from 1 to 10.

[027a] In another aspect, the present disclosure refers to an oligonucleotide comprising a label moiety produced by reacting an oligonucleotide attached to a solid support with a reagent have a structure of formula: LM-L-PEP

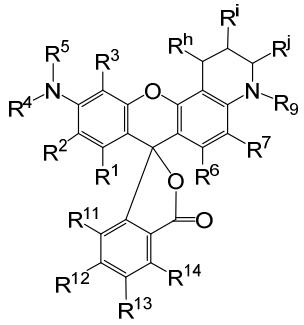
wherein PEP is a phosphate ester precursor group, L is an optional linker linking the label moiety to the PEP group, and LM comprises an N-protected NH-rhodamine moiety of the formula (II.1), (II.2), (II.3) or (II.4):



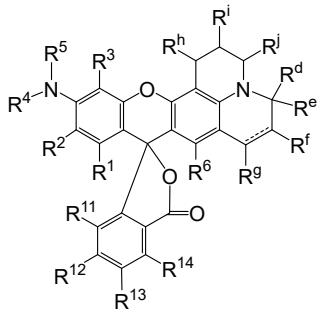
(II.1)



(II.2)



(II.3)



(II.4)

wherein

R^1, R^2, R^3, R^6, R^7, R^{12}, and R^{13}, when taken alone, are each independently of one another selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, $-R^b$, or $-(CH2)_n-R^b$; or alternatively, R^1 and R^2 and/or R^6 and R^7 are taken together with the carbon atoms to which they are bonded to form an optionally substituted benzo group; and one of R^2, R^3, R^7, R^8, R^{12}, or R^{13} comprises a group of the formula —Y—, wherein Y is selected from the group consisting of -C(O)-, $-S(O)_2-$, -S- and -NH-;

each of R11 and R^{14} are halo;

R^4, when taken alone, is selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl; or R^4 and one of R^2 or R^3 are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group;

R^5 is H or a protecting group;

R^9, when taken alone, is selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl; or R^7 and R^9 are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group;

optionally, R^4 and one of R^2 or R^3 are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group;

each R^a is independently selected from lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, $-CX_3$ and 6-20 membered heteroarylalkyl;

each R^b is independently selected from X, -OH, -OR^a -SH, -SR^a -NH_2, -NHR^a -NR^cR^c, -$N^+R^cR^cR^c$, perhalo lower alkyl, trihalomethyl, trifluoromethyl, -$P(O)(OH)_2$, -$P(O)(OR^a)_2$, $P(O)(OH)(OR^a)$, -$OP(O)(OH)_2$, -$OP(O)(OR^a)_2$, -$OP(O)(OR^a)(OH)$, -$S(O)_2OH$, -$S(O)_2R^a$, -C(O)H, -$C(O)R^a$, -C(S)X, -$C(O)OR^a$, -C(O)OH, -$C(O)NH_2$, -$C(O)NHR^a$, -$C(O)NR^cR^c$, -$C(S)NH_2$, -$C(O)NHR^a$, -$C(O)NR^cR^c$, -$C(NH)NH_2$, -$C(NH)NHR^a$, and -$C(NH)NR^cR^c$;

each R^c is independently an R^a, or, alternatively, two R^c bonded to the same nitrogen atom may be taken together with that nitrogen atom to form a 5- to 8-membered saturated or unsaturated ring that may optionally include one or more of the same or different ring heteroatoms selected from O, N, and S;

each R^d and R^e, when taken alone, is independently selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, -R^b, or -$(CH_2)_n$-R^b;

X is halo;

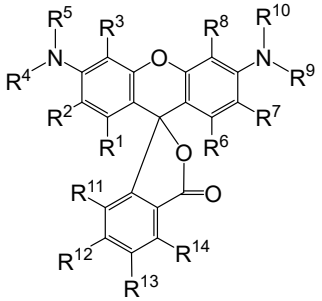
n is an integer ranging from 1 to 10; and

each of R^f, R^g, R^h, R^i, and R^j, when taken alone, are each, independently of one another, selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, -R^b, or -$(CH_2)_n$-R^b.

[028] In another example, a reagent useful for labeling an oligonucleotide, which is a compound according to the structural formula:

LM-L-PEP

[029] wherein LM represents a label moiety that comprises an N-protected NH-rhodamine moiety, PEP is a phosphate ester precursor group which comprises a phosphoramidite group or an H-phosphonate group, and L is an optional linker linking the label moiety to the phosphate ester precursor group, in which the N-protected NH-rhodamine moiety of the structure (I)



[030] wherein R^1, R^2, R^3, R^6, R^7, R^8, R^{11}, R^{12}, R^{13}, and R^{14}, when taken alone, are each independently of one another selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, -R^b, or -$(CH2)_n$-R^b, R^5 is a protecting group, each of R^4, R^9, and R^{10}, when taken alone, is independently hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, -$P(O)(OH)_2$, -$P(O)(OR^a)_2$, $P(O)(OH)(OR^a)$, -$OP(O)(OH)_2$, -$OP(O)(OR^a)_2$, -$OP(O)(OR^a)(OH)$, -$S(O)_2OH$, -$S(O)_2R^a$, -C(O)H, -$C(O)R^a$, -C(S)X, -$C(O)OR^a$, -C(O)OH, -$C(O)NH_2$, -$C(O)NHR^a$, -$C(O)NR^cR^c$, -$C(S)NH_2$, -$C(O)NHR^a$, -$C(O)NR^cR^c$, -$C(NH)NH_2$, -$C(NH)NHR^a$, and -$C(NH)NR^cR^c$, where X is a halo, alternatively, R^1 and R^2 and/or R^6 and R^7 are taken together with the carbon atoms to which they are bonded to form an optionally substituted benzo group, and/or R^7 and R^9 and/or R^8 and R^{10} and/or R^4 and one of R^2 or R^3 are taken together with the carbon atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group;

[031] wherein n is an integer ranging from 1 to 10;

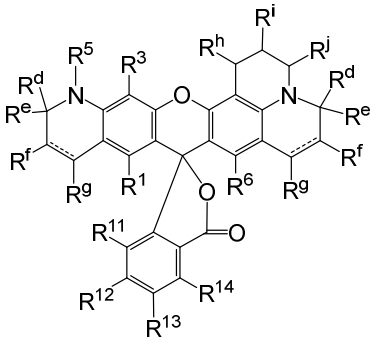
[032] wherein R^b is independently selected from -X, -OH, -OR^a, -SH, -SR^a -NH_2, -NHR^a, -NR^cR^c, -$N^+R^cR^cR^c$, perhalo lower alkyl, trihalomethyl, trifluoromethyl, -$P(O)(OH)_2$, -$P(O)(OR^a)_2$, $P(O)(OH)(OR^a)$, -$OP(O)(OH)_2$, -$OP(O)(OR^a)_2$, -$OP(O)(OR^a)(OH)$, -$S(O)_2OH$, -$S(O)_2R^a$, -C(O)H, -$C(O)R^a$, -C(S)X, -$C(O)OR^a$, -C(O)OH, -$C(O)NH_2$, -$C(O)NHR^a$, -$C(O)NR^cR^c$, -$C(S)NH_2$, -$C(O)NHR^a$, -$C(O)NR^cR^c$, -$C(NH)NH_2$, -$C(NH)NHR^a$, and -$C(NH)NR^cR^c$, each R^a is, independently of the others, selected from lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, -CX_3 and 6-20 membered heteroarylalkyl, and each R^c is, independently of the others, an R^a, or, alternatively, two R^c bonded to the same nitrogen atom may be taken together with that nitrogen atom to form a 5- to 8-membered saturated or unsaturated ring that may optionally include one or more of the same or different ring heteroatoms, which are typically selected from O, N and S; and

[033] with the proviso that at least one of R^2, R^3, R^7, R^8, R^{12},or R^{13} of the compound of formula LM-L-PEP comprises a group of the formula —Y—, wherein Y is selected from the group consisting of —C(O)—, —$S(O)_2$—, —S— and —NH—.

[033a] In yet another aspect, the present disclosure refers to a reagent useful for labeling an oligonucleotide, which is a compound according to the structural formula:

LM-L-PEP (XX)

wherein LM represents a label moiety that comprises an N-protected NH-rhodamine moiety, PEP is a phosphate ester precursor group which comprises a phosphoramidite group or an H-phosphonate group, and L is an optional linker linking the label moiety to the phosphate ester precursor group, in which the N-protected NH-rhodamine moiety has formula



wherein

R^1, R^3, R^6, R^{12} and R^{13}, when taken alone, are each independently of one another selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, -R^b, or -$(CH2)_n$-R^b; and one of R^3, R^{12}, or R^{13} comprises a group of the formula —Y—, wherein Y is selected from the group consisting of -C(O)-, -$S(O)_2$-, -S- and -NH-;

R^{11} and R^{14} are each chloro;

R^5 is H or a protecting group;

each R^a is independently selected from lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, -CX_3 and 6-20 membered heteroarylalkyl;

each R^b is independently selected from X, -OH, -OR^a -SH, -SR^a -NH_2, -NHR^a -NR^cR^c, -$N^+R^cR^cR^c$, perhalo lower alkyl, trihalomethyl, trifluoromethyl, -$P(O)(OH)_2$, -$P(O)(OR^a)_2$, $P(O)(OH)(OR^a)$, -$OP(O)(OH)_2$, -$OP(O)(OR^a)_2$, -$OP(O)(OR^a)(OH)$, -$S(O)_2OH$, -$S(O)_2R^a$, -C(O)H, -$C(O)R^a$, -C(S)X, -$C(O)OR^a$, -C(O)OH, -$C(O)NH_2$, -$C(O)NHR^a$, -$C(O)NR^cR^c$, -$C(S)NH_2$, -$C(O)NHR^a$, -$C(O)NR^cR^c$, -$C(NH)NH_2$, -$C(NH)NHR^a$, and -$C(NH)NR^cR^c$;

each R^c is independently an R^a, or, alternatively, two R^c bonded to the same nitrogen atom may be taken together with that nitrogen atom to form a 5- to 8-membered saturated or unsaturated ring that may optionally include one or more of the same or different ring heteroatoms selected from O, N, and S;

each R^d and R^e, when taken alone, is independently selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, -R^b, or -$(CH_2)_n$-R^b;

X is halo; and

n is an integer ranging from 1 to 10; and

each of R^f, R^g, R^h, R^i, and R^j, when taken alone, are each, independently of one another, selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, $-R^b$, or $-(CH_2)_n-R^bR'$ is selected from $R^{3'}$ and hydrogen.

[034] In another example, a method comprises:

[035] co-amplifying a nucleic acid sample with a plurality of amplification primer pairs to form a plurality of amplifications products, wherein at least one of each of the primer pairs comprises a labeled nucleotide having a structural formula LM-L-PEP wherein each of the amplification products comprises a different genetic loci.

[035a] In still another aspect, the present disclosure refers to a method for the simultaneous amplification and analysis of a plurality of genetic loci comprising:

amplifying a nucleic acid sample with a plurality of amplification primer pairs to form a plurality of amplifications products, wherein at least one of each of the primer pairs comprises a labeled oligonucleotide as disclosed herein wherein each of the amplification products comprises a different genetic loci;

preferably wherein the labeled oligonucleotide as disclosed herein is used to label at least three primer pairs, more preferably wherein the labeled oligonucleotide as disclosed herein is used to label from three to eight primer pairs; or

preferably wherein the nucleic acid sample is isolated from whole blood, a tissue biopsy, lymph, bone, bone marrow, tooth, skin, for example skin cells contained in fingerprints, bone, tooth, amniotic fluid containing placental cells, and amniotic fluid containing fetal cells, hair, skin, semen, anal secretions, feces, urine, vaginal secretions, perspiration, saliva, buccal swabs, various environmental samples (for example, agricultural, water, and soil), research samples generally, purified samples generally, and lysed cells.

[035b] In yet another aspect, the present disclosure refers to a kit comprising oligonucleotide primers for co-amplifying a set of genetic loci of at least one nucleic acid sample to be analyzed; wherein the set of loci can be co-amplified; wherein at least one of the primers comprises a labeled oligonucleotide as disclosed herein wherein the primers are in one or more containers; preferably wherein all of the oligonucleotide primers in the kit are in one container;

preferably further comprising at least one of:

reagents for at least one multiplex amplification reaction; and

a container having at least one size standard; or

preferably wherein at least one of the oligonucleotide primers comprises a labeled nucleotide wherein the labeled nucleotide has different spectral properties from the labeled oligonucleotide as disclosed herein.

3. BRIEF DESCRIPTION OF THE FIGURES

[036] FIG. 1 provides exemplary linkers that can be used to link the various different moieties comprising the reagents described herein to one another;

[037] FIG. 2 provides exemplary embodiments of non-nucleosidic synthesis reagents that do not include synthesis handles;

[038] FIG. 3 provides exemplary embodiments of nucleosidic synthesis reagents that do not include synthesis handles;

[039] FIG. 4 provides exemplary embodiments of non-nucleosidic synthesis reagents that include a synthesis handle;

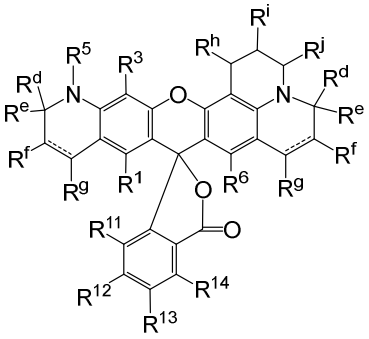
[040] FIG. 5 provides exemplary embodiments of nucleosidic synthesis reagents that include synthesis handles;

[041] FIG. 6 provides exemplary embodiments of non-nucleosidic solid support reagents;

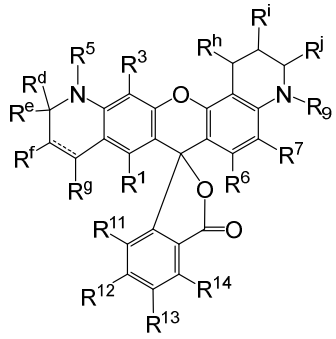
[042] FIG. 7 provides exemplary embodiments of nucleosidic solid support reagents;

What is Claimed is:

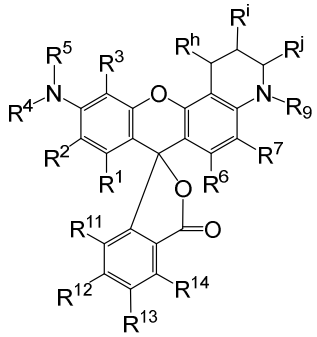
1. A compound of the formula (II.1), (II.2), (II.3) or (II.4):



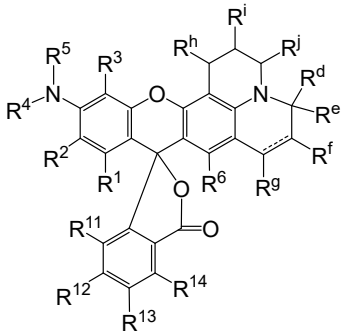
(II.1)



(II.2)



(II.3)



(II.4)

wherein

R^1, R^2, R^3, R^6, R^7, R^{12} and R^{13} when taken alone, are each independently of one another selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, $-R^b$, or $-(CH2)_n-R^b$; or alternatively, R^1 and R^2 and/or R^6 and R^7 are taken together with the carbon atoms to which they are bonded to form an optionally substituted benzo group;

each of R^{11} and R^{14} are halo;

R^4, when taken alone, is selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl; or R^4 and one of R^2 or R^3 are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group;

R^5 is H or a protecting group;

R^9, when taken alone, is selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl; or R^7 and R^9 are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group;

optionally, R^4 and one of R^2 or R^3 are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group;

each R^a is independently selected from lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, $-CX_3$ and 6-20 membered heteroarylalkyl;

each R^b is independently selected from -X, -OH, $-OR^a$, -SH, $-SR^a$ $-NH_2$, $-NHR^a$, $-NR^cR^c$, $-N^+R^cR^cR^c$, perhalo lower alkyl, trihalomethyl, trifluoromethyl, $-P(O)(OH)_2$, $-P(O)(OR^a)_2$, $P(O)(OH)(OR^a)$, $-OP(O)(OH)_2$, $-OP(O)(OR^a)_2$, $-OP(O)(OR^a)(OH)$, $-S(O)_2OH$, $-S(O)_2R^a$, -C(O)H, $-C(O)R^a$, -C(S)X, $-C(O)OR^a$, -C(O)OH, $-C(O)NH_2$, $-C(O)NHR^a$, $-C(O)NR^cR^c$, $-C(S)NH_2$, $-C(O)NHR^a$, $-C(O)NR^cR^c$, $-C(NH)NH_2$, $-C(NH)NHR^a$, and $-C(NH)NR^cR^c$;

each R^c is independently an R^a, or, alternatively, two R^c bonded to the same nitrogen atom may be taken together with that nitrogen atom to form a 5- to 8-membered saturated or

unsaturated ring that may optionally include one or more of the same or different ring heteroatoms selected from O, N, and S;

each R^d and R^e, when taken alone, is independently selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, $-R^b$, or $-(CH_2)_n-R^b$;

X is halo;

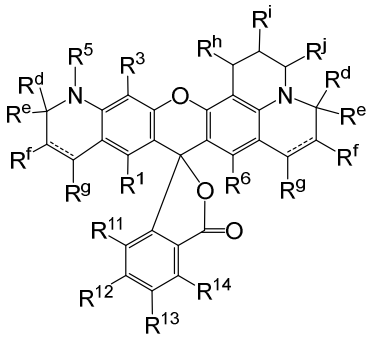
n is an integer ranging from 1 to 10; and

each of R^f, R^g, R^h, R^i, and R^j, when taken alone, are each, independently of one another, selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, $-R^b$, or $-(CH2)_n-R^b$.

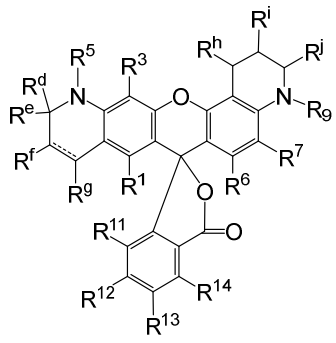
2. An oligonucleotide comprising a label moiety produced by reacting an oligonucleotide attached to a solid support with a reagent have a structure of formula:

LM-L-PEP

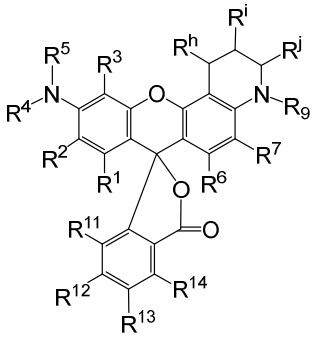
wherein PEP is a phosphate ester precursor group, L is an optional linker linking the label moiety to the PEP group, and LM comprises an N-protected NH-rhodamine moiety of the formula (II.1), (II.2), (II.3) or (II.4):



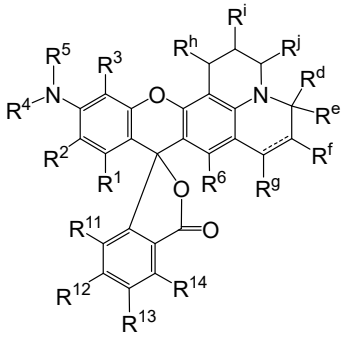
(II.1)



(II.2)



(II.3)



(II.4)

wherein

R^1, R^2, R^3, R^6, R^7, R^{12}, and R^{13}, when taken alone, are each independently of one another selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, $-R^b$, or $-(CH2)_n-R^b$; or alternatively, R^1 and R^2 and/or R^6 and R^7 are taken together with the carbon atoms to which they are bonded to form an optionally substituted benzo group; and one of R^2, R^3, R^7, R^8, R^{12}, or R^{13} comprises a group of the formula —Y—, wherein Y is selected from the group consisting of -C(O)-, $-S(O)_2-$, -S- and -NH-;

each of R11 and R^{14} are halo;

R^4, when taken alone, is selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl; or R^4 and one of R^2 or R^3 are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group;

R^5 is H or a protecting group;

R^9, when taken alone, is selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl; or R^7 and R^9 are

taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group;

optionally, R^4 and one of R^2 or R^3 are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group;

each R^a is independently selected from lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, $-CX_3$ and 6-20 membered heteroarylalkyl;

each R^b is independently selected from X, -OH, $-OR^a$ -SH, $-SR^a$ $-NH_2$, $-NHR^a$ $-NR^cR^c$, $-N^+R^cR^cR^c$, perhalo lower alkyl, trihalomethyl, trifluoromethyl, $-P(O)(OH)_2$, $-P(O)(OR^a)_2$, $P(O)(OH)(OR^a)$, $-OP(O)(OH)_2$, $-OP(O)(OR^a)_2$, $-OP(O)(OR^a)(OH)$, $-S(O)_2OH$, $-S(O)_2R^a$, -C(O)H, $-C(O)R^a$, -C(S)X, $-C(O)OR^a$, -C(O)OH, $-C(O)NH_2$, $-C(O)NHR^a$, $-C(O)NR^cR^c$, $-C(S)NH_2$, $-C(O)NHR^a$, $-C(O)NR^cR^c$, $-C(NH)NH_2$, $-C(NH)NHR^a$, and $-C(NH)NR^cR^c$;

each R^c is independently an R^a, or, alternatively, two R^c bonded to the same nitrogen atom may be taken together with that nitrogen atom to form a 5- to 8-membered saturated or unsaturated ring that may optionally include one or more of the same or different ring heteroatoms selected from O, N, and S;

each R^d and R^e, when taken alone, is independently selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, $-R^b$, or $-(CH_2)_n-R^b$;

X is halo;

n is an integer ranging from 1 to 10; and

each of R^f, R^g, R^h, R^i, and R^j, when taken alone, are each, independently of one another, selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, $-R^b$, or $-(CH_2)_n-R^b$.

3. The oligonucleotide of claim 2, wherein the spirolactone ring of the N-protected NH-rhodamine moiety is in open, acid form and the amine groups are not protected.

4. The oligonucleotide of claim 2 or 3, wherein the label moiety further comprises a donor moiety for the N-protected NH-rhodamine moiety.

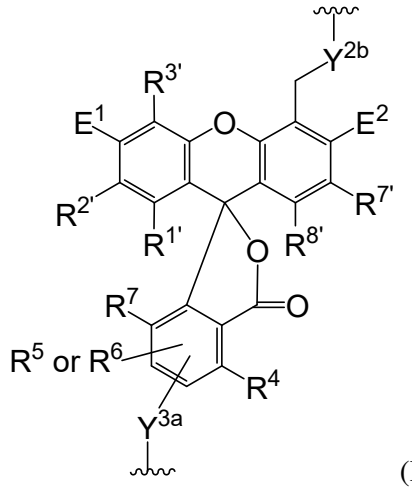
5. The oligonucleotide of claim 4, wherein the donor moiety comprises an N-protected NH-rhodamine moiety or an O-protected fluorescein moiety.

6. The oligonucleotide of claim 5, wherein the label moiety has structural formula (VI):

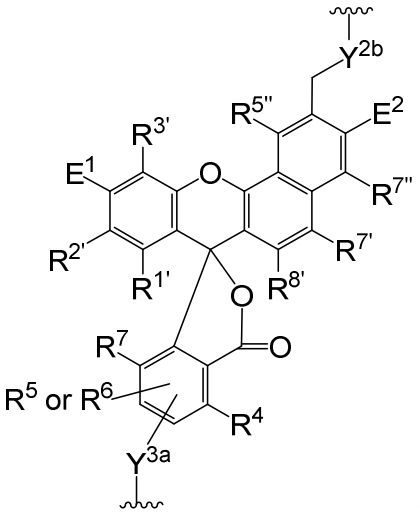
$$A\text{-}Z^1\text{-}Sp\text{-}Z^2\text{-}D \qquad (VI)$$

wherein A represents the N-protected NH-rhodamine moiety, D represents the donor moiety, Z^1 and Z^2, which may be the same or different, represent portions of linkages provided by linking moieties comprising a functional group F^z, and Sp represents a spacing moiety.

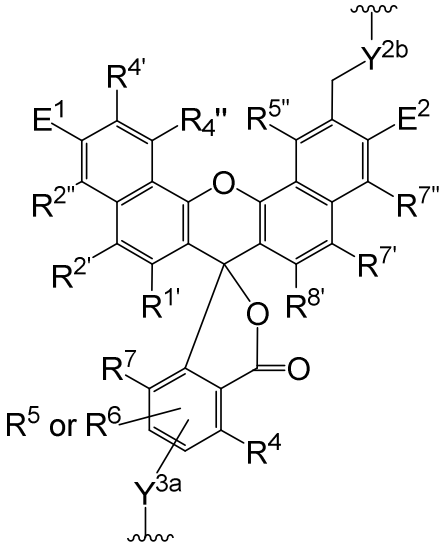
7. The oligonucleotide of claim 6, wherein A is the N-protected NH-rhodamine moiety, and D is selected from the group consisting of moieties having structural formulae D.1, D.2, D.3, D.4, D.5, D.6, D.7, D.8, D.9, D.10, D.11 and D.12:



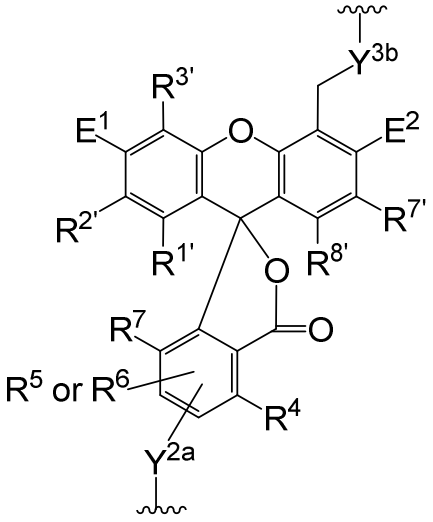
(D.1)



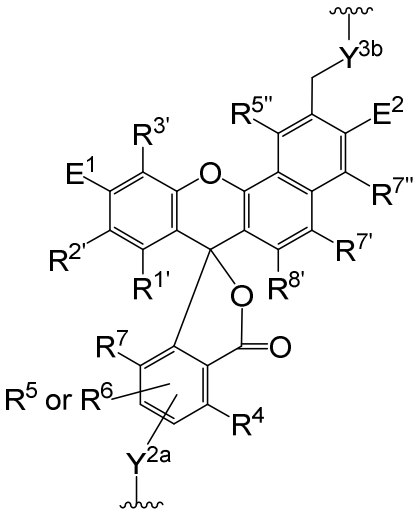
(D.2)



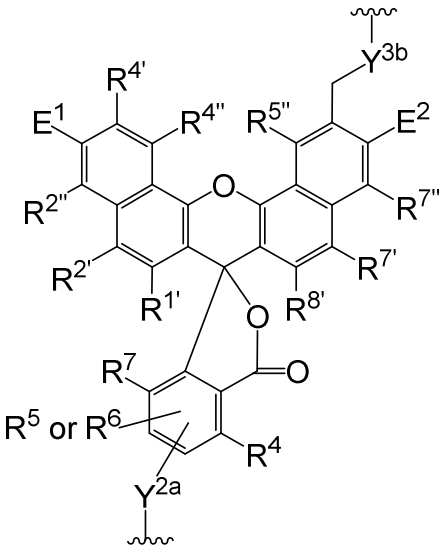
(D.3)



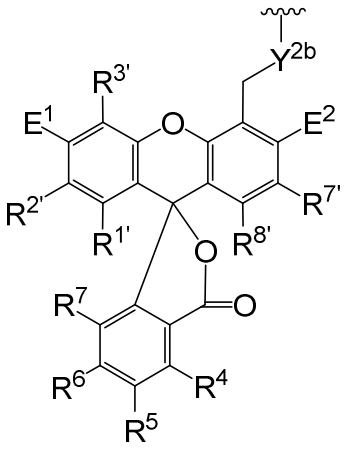
(D.4)


Y3b
R5''
E2
R3'
E1
O
R7''
R2'
R7'
R1'
O
R8'
R7
O
R5 or R6
R4
Y2a

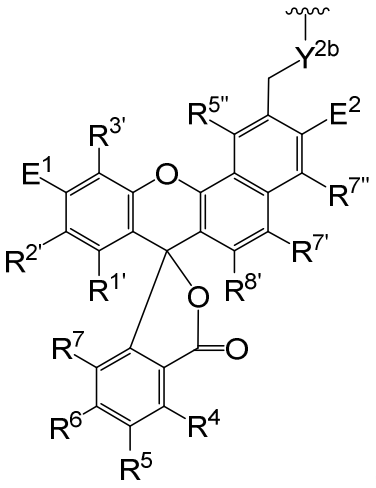
(D.5)


Y3b
R4'
E1
R4''
R5''
E2
O
R2''
R7''
R2'
R7'
R1'
O
R8'
R7
O
R5 or R6
R4
Y2a

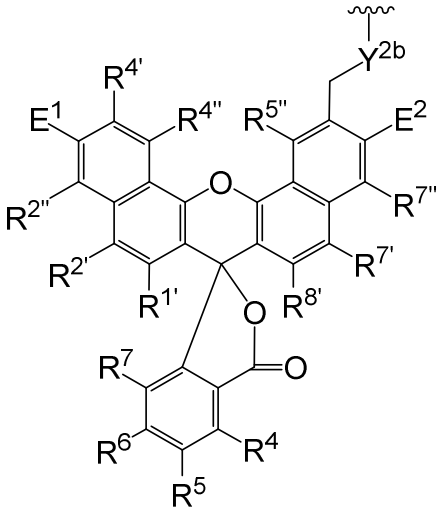
(D.6)


Y2b
R3'
E1
O
E2
R2'
R7'
R1'
O
R8'
R7
O
R6
R4
R5

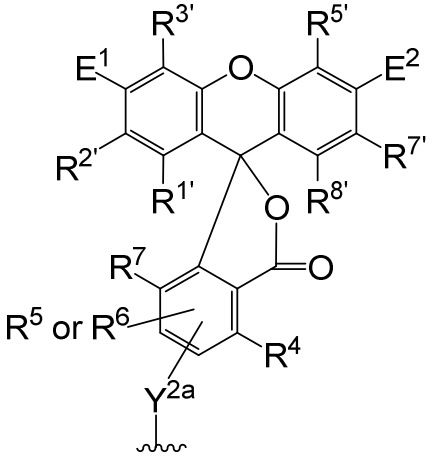
(D.7)



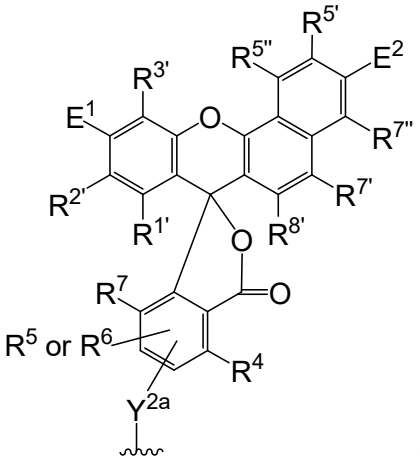
(D.8)



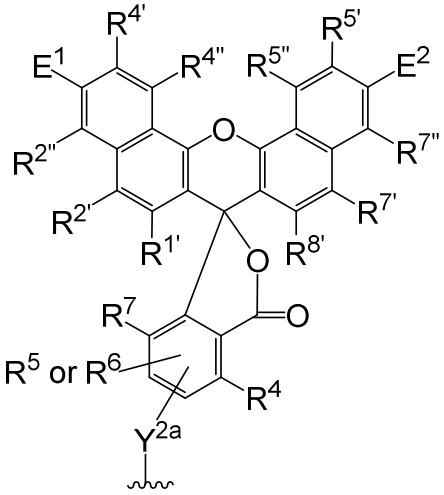
(D.9)



(D.10)



(D.11)



(D.12)

wherein, in each of D.1 – D.12:

each of $R^{1'}$, $R^{2'}$, $R^{2''}$, $R^{4'}$, $R^{4''}$, $R^{5'}$, $R^{5''}$, $R^{7'}$, $R^{7''}$, and $R^{8'}$, when taken alone, is independently selected from the group consisting of hydrogen, a lower alkyl, a (C6-C14) aryl, a (C7-C20) arylalkyl, a 5-14 membered heteroaryl, a 6-20 membered heteroarylalkyl, $-R^b$ and $—(CH_2)_x—R^b$, wherein x is an integer having the value between 1 and 10 and R^b is selected from the group consisting of -X, -OH, $-OR^a$ -SH, $-SR^a$ $-NH_2$, $-NHR^a$ $-NR^cR^c$, $-N^+R^cR^cR^c$, perhalo lower alkyl, trihalomethyl, trifluoromethyl, $-P(O)(OH)_2$, $-P(O)(OR^a)_2$, $P(O)(OH)(OR^a)$, $-OP(O)(OH)_2$, $-OP(O)(OR^a)_2$, $-OP(O)(OR^a)(OH)$, $-S(O)_2OH$, $-S(O)_2R^a$, -C(O)H, $-C(O)R^a$, -C(S)X, $-C(O)OR^a$, -C(O)OH, $-C(O)NH_2$, $-C(O)NHR^a$, $-C(O)NR^cR^c$, $-C(S)NH_2$, $-C(O)NHR^a$, $-C(O)NR^cR^c$, $-C(NH)NH_2$, $-C(NH)NHR^a$, and $-C(NH)NR^cR^c$, wherein X is halo, each R^a is independently selected from the group consisting of a lower alkyl, a (C6-C14) aryl, a (C7-C20) arylalkyl, a 5-14 membered heteroaryl and a 6-20 membered heteroarylalkyl, and each R^c is independently an R^a, or, alternatively, two R^c bonded to the same nitrogen atom may be taken together with that nitrogen atom to form a 5- to 8-membered

saturated or unsaturated ring that may optionally include one or more of the same or different ring heteroatoms selected from the group consisting of O, N, and S;

or, alternatively, $R^{1'}$ and $R^{2'}$ or $R^{7'}$ and $R^{8'}$ are taken together with the carbon atoms to which they are bonded to form an optionally substituted (C6-C14) aryl bridge and/or $R^{4'}$ and $R^{4''}$ and/or $R^{5'}$ and $R^{5''}$are taken together with the carbon atoms to which they are bonded to form a benzo group; and

R^4, R^5, R^6, and R^7 are each, independently of one another, selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 6-14 membered heteroaryl, 7-20 membered heteroarylalkyl, —R^b and —$(CH_2)_x$—R^b;

E^1 is selected from the group consisting of —NHR^9, —NR^9R^{10} and —OR^{9b};

E^2 is selected from the group consisting of —NHR^9, —NR^9R^{10} and —OR^{9b};

R^9, when taken alone, is selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl; or R^7 and R^9 are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group;

R^{10} is H or protecting group; or R^8 and R^{10} are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group;

R^{9b} is R^9;

each of Y^{1a}, Y^{1b}, Y^{2a}, Y^{2b}, Y^{3a} and Y^{3b} is independently selected from the group consisting of —O—, —S—, —NH—, —C(O—) and —$S(O)_2$,

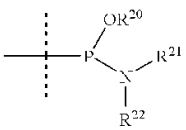
with the proviso that when each of E^1 and E^2 is —OR^{9b}, then $R^{1'}$ and $R^{2'}$ and/or $R^{7'}$ and $R^{8'}$ are may only be taken together with the carbon atoms to which they are bound to form an optionally substituted (C6-C14) aryl bridge.

8. The oligonucleotide of claim 2, wherein the PEP group comprises a phosphoramidite group and an H-phosphonate group.

9. The oligonucleotide of claim 8, wherein:

(i) the phosphate ester precursor group comprises a phosphoramidite of the formula (P.1):

(P.1)



wherein:

R^{20} is selected from a linear, branched or cyclic saturated or unsaturated alkyl containing from 1 to 10 carbon atoms, 2-cyanoethyl, an aryl containing from 6 to 10 ring carbon atoms and an arylalkyl containing from 6 to 10 ring carbon atoms and from 1 to 10 alkylene carbon atoms; and

R^{21} and R^{22} are each, independently of one another, selected from a linear, branched or cyclic, saturated or unsaturated alkyl containing from 1 to 10 carbon atoms, an aryl containing from 6 to 10 ring carbon atoms and an arylalkyl containing from 6 to 10 ring carbon atoms and from 1 to 10 alkylene carbon atoms, or, alternatively, $R^{21}$and R^{22} are taken together with the nitrogen atom to which they are bonded to form a saturated or unsaturated ring that contains from 5 to 6 ring atoms, one or two of which, in addition to the illustrated nitrogen atom, can be heteroatom selected from O, N and S; or

(ii) R^{20} is beta-cyanoethyl and R^{21} and R^{22} are each isopropyl, preferably wherein the synthesis handle has the formula —OR^k, where R^k is an acid-labile protecting group, preferably wherein the acid-labile protecting group is selected from the group consisting of triphenylmethyl (trityl), 4-monomethoxytrityl, 4,4′-dimethoxytrityl, 4,4′,4″-trimethoxytrityl, bis (p-anisyl)phenylmethyl, naphthyldiphenylmethyl, p-(p′-bromophenacyloxy)phenyldiphenylmethyl, 9-anthryl, 9-(9-phenyl)xanthenyl and 9-(9-phenyl-10-oxo)anthryl.

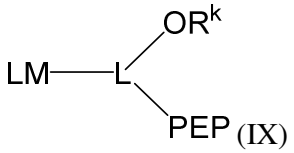
10. The oligonucleotide of claim 2, wherein:

(i) the reagent is a compound according to structural formula (VIII):

$$R^kO\text{-}L\text{-}LM\text{-}L\text{-}PEP \qquad (VIII)$$

wherein R^k represents an acid-labile protecting group, each L represents, independently of the other, an optional linker, LM represents the label moiety and PEP represents the phosphate ester precursor group;

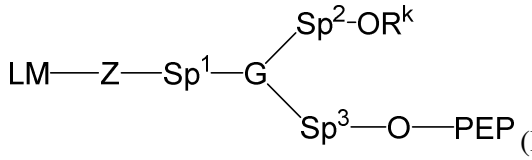
(ii) the reagent is a compound according to structural formula (IX):



(IX)

wherein R^k represents an acid-labile protecting group;

(iii) the reagent is a compound according to structural formula (IX.1):



(IX.1)

wherein -Z- represents a portion of a linkage contributed by a functional group F^z, Sp^1, Sp^2 and Sp^3, which can be the same or different, each represent spacing moieties, and G represents CH, N, or a group comprising an arylene, phenylene, heteroarylene, lower cycloalkylene, cyclohexylene, and/or a lower cycloheteroalkylene, preferably wherein Sp^1, Sp^2 and Sp^3 are each, independently of one another, selected from an alkylene chain containing from 1 to 10 carbon atoms, $-(CH_2)_a-[(Ar)_b-(CH_2)_a]_c-$, $-(CH_2)_a-[C\equiv C-(CH_2)_a]_c-$, $-(CH_2)_a-[C\equiv C-(Ar)_b]_c-(CH_2)_a-$, $-(CH_2)_d-NH-C(O)-[(CH_2)_a-(Ar)-(CH_2)_a-C(O)-NH]_c-(CH_2)_d-$ and $-[CH_2(CH_2)_eO]_f-CH_2(CH_2)-$, where each a represents, independently of the others, an integer ranging from 0 to 4;

each b represents, independently of the others, an integer ranging from 1 to 2;

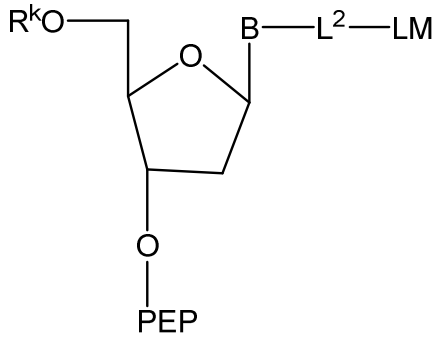
each c represents, independently of the others, an integer ranging from 1 to 5;

each d represents, independently of the others, an integer ranging from 1 to 10;

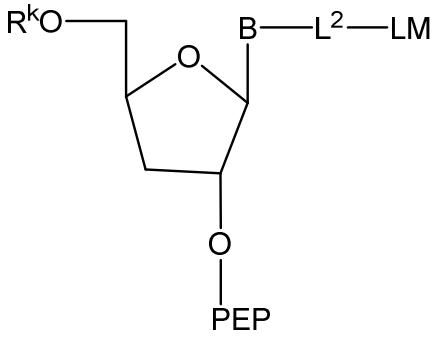
each e represents, independently of the others, an integer ranging from 1 to 4;

each f represents, independently of the others, an integer ranging from 1 to 10; and Ar represents, independently of the others, an optionally substituted monocyclic or polycyclic cycloalkylene, cycloheteroalkylene, arylene or heteroarylene group; or

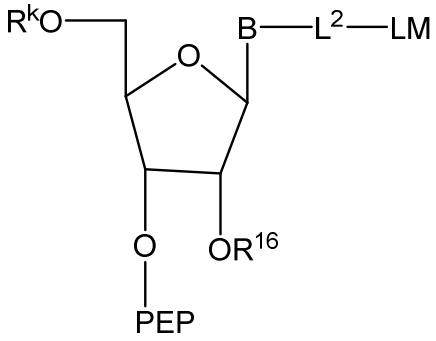
(iv) the reagent is a compound according to structural formula (IX.2), (IX.3), (IX.4) or (IX.5):



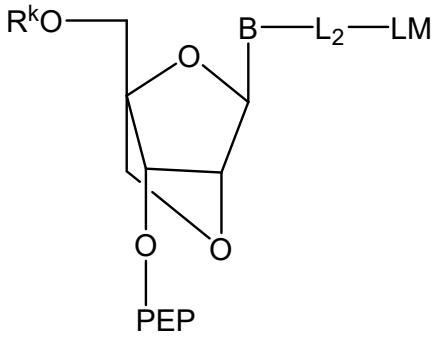
(IX.2)



(IX.3)



(IX.4)



(IX.5)

wherein B represents a suitably protected nucleobase, L^2 represents a linker linking label moiety LM to nucleobase B and, in structure (IX.4), R^{16} represents a protecting group; and the nucleobase is selected from adenine, 7-deazaguanine, guanine, 7-deazaguanine, cytosine, uracil, thymine, inosine, xanthene and hypoxanthene, preferably wherein B of the reagent is selected from A^{iBu}, A^{Pac}, C^{Ac}, $G^{iPr\text{-}Pac}$, T and U, or

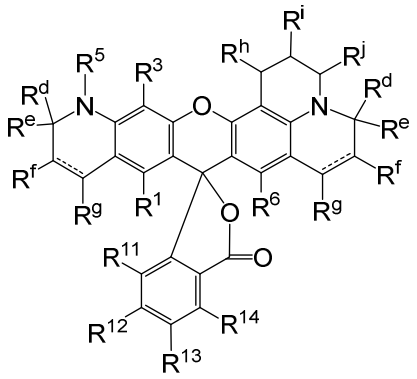
wherein L^2 of the reagent is selected from —C≡C—CH_2—NH—, —C≡C—C(O)—, —CH═CH—NH—, —CH═CH—C(O)—, —C≡C—CH_2—NH—C(O)—$(CH_2)_{1\text{-}6}$—NH—, —CH═CH—C(O)—NH—$(CH_2)_{1\text{-}6}$—NH—C(O)—, —C═CH—CH_2—O—CH_2CH_2—[O—CH_2CH_2]$_{0\text{-}6}$—NH—, —C≡C—C≡C—CH_2—O—CH_2CH_2—[O—CH_2CH_2]$_{0\text{-}6}$—NH—, —C≡C—$(Ar)_{1\text{-}2}$—C≡C—CH_2—O—CH_2CH_2—[O—CH_2CH_2]$_{0\text{-}6}$—NH—, —C≡C—$(Ar)_{1\text{-}2}$—O—CH_2CH_2—[O—CH_2CH_2]$_{0\text{-}6}$—NH— and —C≡C—$(Ar)_{1\text{-}2}$—O—CH_2CH_2—[O—CH_2CH_2]$_{0\text{-}6}$—NH—, where Ar represents, independently of the others, an optionally

substituted monocyclic or polycyclic cycloalkylene, cycloheteroalkynene, arylene or heteroarylene group.

11. A reagent useful for labeling an oligonucleotide, which is a compound according to the structural formula:

LM-L-PEP (XX)

wherein LM represents a label moiety that comprises an N-protected NH-rhodamine moiety, PEP is a phosphate ester precursor group which comprises a phosphoramidite group or an H-phosphonate group, and L is an optional linker linking the label moiety to the phosphate ester precursor group, in which the N-protected NH-rhodamine moiety has formula



wherein

R^1, R^3, R^6, R^{12} and R^{13}, when taken alone, are each independently of one another selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, $-R^b$, or $-(CH2)_n-R^b$; and one of R^3, R^{12}, or R^{13} comprises a group of the formula —Y—, wherein Y is selected from the group consisting of -C(O)-, $-S(O)_2-$, -S- and -NH-;

R^{11} and R^{14} are each chloro;

R^5 is H or a protecting group;

each R^a is independently selected from lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, $-CX_3$ and 6-20 membered heteroarylalkyl;

each R^b is independently selected from X, -OH, $-OR^a$ -SH, $-SR^a$ $-NH_2$, $-NHR^a$ $-NR^cR^c$, $-N^+R^cR^cR^c$, perhalo lower alkyl, trihalomethyl, trifluoromethyl, $-P(O)(OH)_2$,

$-P(O)(OR^a)_2$, $P(O)(OH)(OR^a)$, $-OP(O)(OH)_2$, $-OP(O)(OR^a)_2$, $-OP(O)(OR^a)(OH)$, $-S(O)_2OH$, $-S(O)_2R^a$, -C(O)H, $-C(O)R^a$, -C(S)X, $-C(O)OR^a$, -C(O)OH, $-C(O)NH_2$, $-C(O)NHR^a$, $-C(O)NR^cR^c$, $-C(S)NH_2$, $-C(O)NHR^a$, $-C(O)NR^cR^c$, $-C(NH)NH_2$, $-C(NH)NHR^a$, and $-C(NH)NR^cR^c$;

each R^c is independently an R^a, or, alternatively, two R^c bonded to the same nitrogen atom may be taken together with that nitrogen atom to form a 5- to 8-membered saturated or unsaturated ring that may optionally include one or more of the same or different ring heteroatoms selected from O, N, and S;

each R^d and R^e, when taken alone, is independently selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, $-R^b$, or $-(CH_2)_n-R^b$;

X is halo; and

n is an integer ranging from 1 to 10; and

each of R^f, R^g, R^h, R^i, and R^j, when taken alone, are each, independently of one another, selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl, $-R^b$, or $-(CH_2)_n-R^bR'$ is selected from $R^{3'}$ and hydrogen.

12. The reagent of claim 11, wherein the spiro-lactone ring is in open form and the amine groups are not protected.

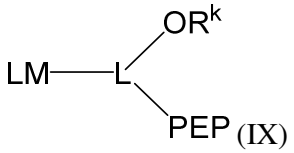
13. The reagent of claim 11 or 12, further comprising a suitably protected synthesis handle, wherein:

(i) the reagent has the formula (VIII):

$$R^kO\text{-}L\text{-}LM\text{-}L\text{-}PEP \qquad (VIII)$$

wherein R^k represents an acid-labile protecting group, each L represents, independently of the other, an optional linker, LM represents the label moiety and PEP represents the phosphate ester precursor group;

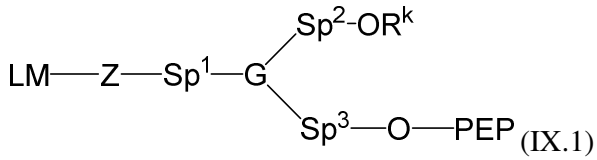
(ii) the reagent is a compound according to structural formula (IX):



(IX)

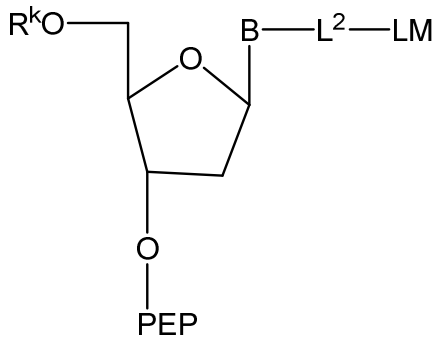
wherein R^k represents an acid-labile protecting group;

(iii) the reagent is a compound according to structural formula (IX.1):

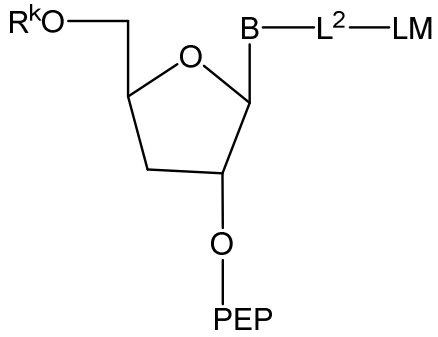


(IX.1)

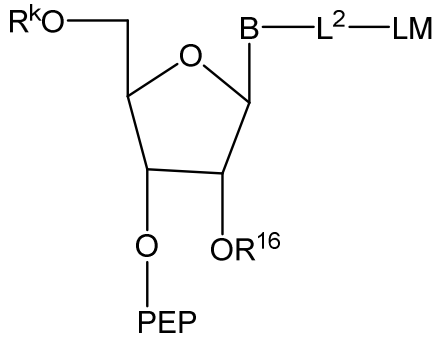
wherein -Z- represents a portion of a linkage contributed by a functional group F^z, Sp^1, Sp^2 and Sp^3, which can be the same or different, each represent spacing moieties, and G represents CH, N, or a group comprising an arylene, phenylene, heteroarylene, lower cycloalkylene, cyclohexylene, and/or a lower cycloheteroalkylene, preferably wherein Sp^1, Sp^2 and Sp^3 are each, independently of one another, selected from an alkylene chain containing from 1 to 10 carbon atoms, $—(CH_2)_a—[(Ar)_b—(CH_2)_a]_c—$, $—(CH_2)_a—[C{\equiv}C—(CH_2)_a]_c—$, $—(CH_2)_a—[C{\equiv}C—(Ar)_b]_c—(CH_2)_a—$, $—(CH_2)_d—NH—C(O)—[(CH_2)_a—(Ar)—(CH_2)_a—C(O)—NH]_c—(CH_2)_d—$ and $—[CH_2(CH_2)_eO]_f—CH_2(CH_2)—$, where each a represents, independently of the others, an integer ranging from 0 to 4;
each b represents, independently of the others, an integer ranging from 1 to 2;
each c represents, independently of the others, an integer ranging from 1 to 5;
each d represents, independently of the others, an integer ranging from 1 to 10;
each e represents, independently of the others, an integer ranging from 1 to 4;
each f represents, independently of the others, an integer ranging from 1 to 10; and Ar represents, independently of the others, an optionally substituted monocyclic or polycyclic cycloalkylene, cycloheteroalkylene, arylene or heteroarylene group; or
(iv) the reagent is a compound according to structural formula (IX.2), (IX.3), (IX.4) or (IX.5):



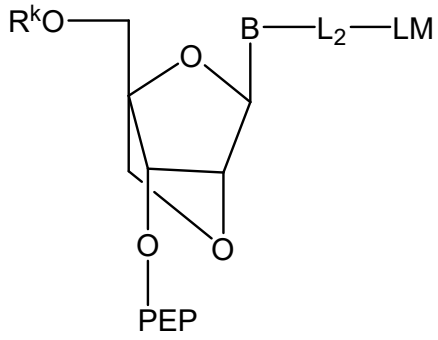
(IX.2)



(IX.3)



(IX.4)



(IX.5)

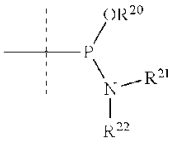
wherein B represents a suitably protected nucleobase, L^2 represents a linker linking label moiety LM to nucleobase B and, and in structure (IX.4), R^{16} represents a protecting group.

14. The reagent of claim 11 or 12, wherein the phosphate ester precursor group comprises a phosphoramidite group and an H-phosphonate group.

15. The reagent of claim 14, wherein

(i) the phosphate ester precursor group comprises a phosphoramidite of the formula (P.1):

(P.1)

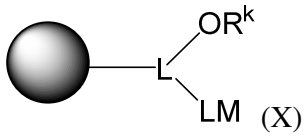


wherein:

R^{20} is selected from a linear, branched or cyclic saturated or unsaturated alkyl containing from 1 to 10 carbon atoms, 2-cyanoethyl, an aryl containing from 6 to 10 ring carbon atoms and an arylalkyl containing from 6 to 10 ring carbon atoms and from 1 to 10 alkylene carbon atoms; and

R^{21} and R^{22} are each, independently of one another, selected from a linear, branched or cyclic, saturated or unsaturated alkyl containing from 1 to 10 carbon atoms, an aryl containing from 6 to 10 ring carbon atoms and an arylalkyl containing from 6 to 10 ring carbon atoms and from 1 to 10 aklylene carbon atoms, or, alternatively, R^{21} and R^{22} are taken together with the nitrogen atom to which they are bonded to form a saturated or unsaturated ring that contains from 5 to 6 ring atoms, one or two of which, in addition to the illustrated nitrogen atom, can be heteroatom selected from O, N and S, preferably wherein R^{20} is beta-cyanoethyl and R^{21} and R^{22} are each isopropyl;

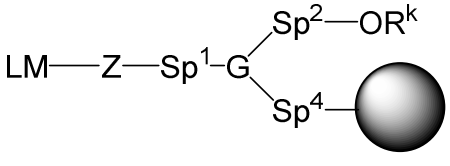
(ii) the reagent is a compound according to structural formula (X):



(X)

wherein LM represents the label moiety, L represents an optional selectively cleavable linker and R^k represents an acid-labile protecting group; or

(iii) the reagent is a compound according to structural formula (X.1):



(X.1)

wherein Z, G, Sp^1 and Sp^2 are as previously defined in claim 13 and Sp^4 represents a selectively cleavable spacing moiety.

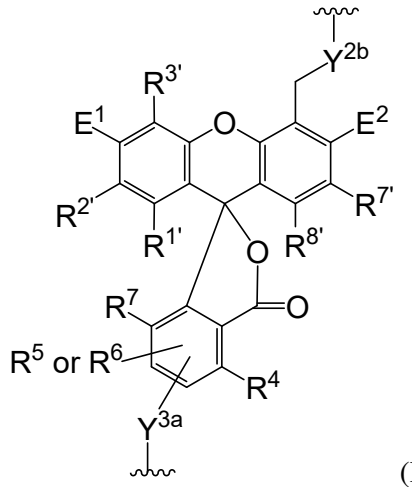
16. The reagent of any one of claims 11-15, wherein the label moiety further comprises a donor moiety for the N-protected NH-rhodamine moiety and wherein the donor moiety comprises an N-protected NH-rhodamine moiety or an O-protected fluorescein moiety.

17. The reagent of claim 16, wherein the 2′-, 2″-, 4′-, 5′-, 7′-, 7″-, 5- or 6-position of the donor moiety is linked to R^3, R^{12}, or R^{13} of the N-protected NH-rhodamine moiety, and the label moiety comprises structural formula (VI):

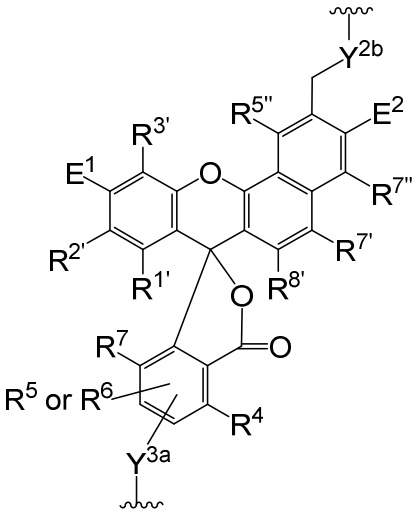
$A\text{-}Z^1\text{-}Sp\text{-}Z^2\text{-}D$ (VI)

wherein A represents the N-protected NH-rhodamine moiety, D represents the donor moiety, Z^1 and Z^2, which may be the same or different, represent portions of linkages provided by linking moieties comprising a functional group F^z, and Sp represents a spacing moiety.

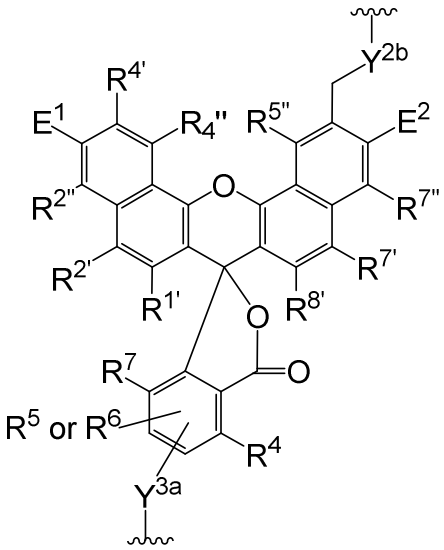
18. The reagent of claim 17, wherein A is the N-protected NH-rhodamine moiety and D is selected from structural formulae D.1, D.2, D.3, D.4, D.5, D.6, D.7, D.8, D.9, D.10, D.11 and D.12;



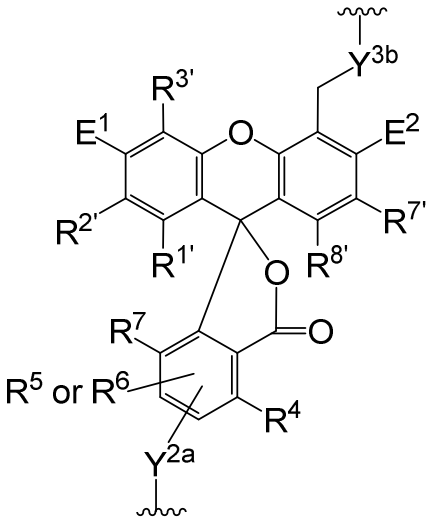
(D.1)


Y2b
R5''
E2
R3'
E1
O
R7''
R2'
R7'
R1'
O
R8'
R7
O
R5 or R6
R4
Y3a

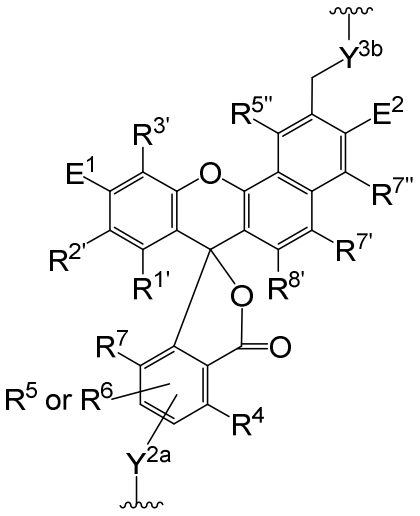
(D.2)


Y2b
R4'
E1
R4''
R5''
E2
O
R2''
R7''
R2'
R7'
R1'
O
R8'
R7
O
R5 or R6
R4
Y3a

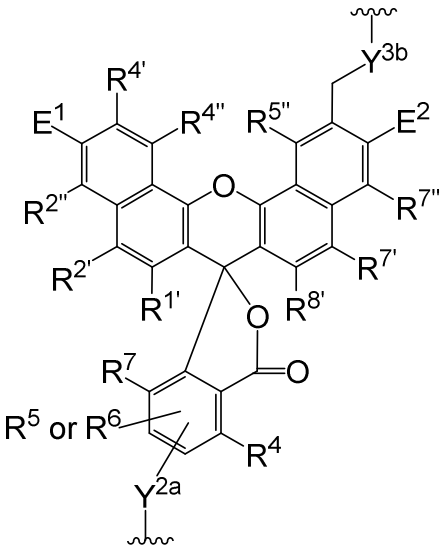
(D.3)


Y3b
R3'
E1
O
E2
R2'
R7'
R1'
O
R8'
R7
O
R5 or R6
R4
Y2a

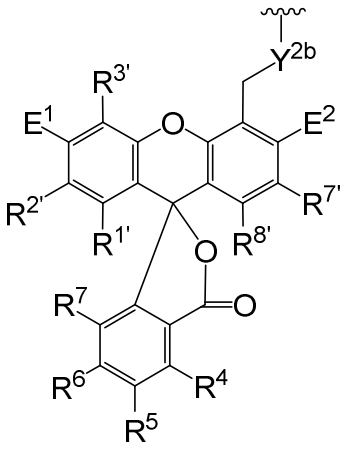
(D.4)


Y3b
R5''
E2
R3'
E1
O
R7''
R2'
R7'
R1'
O
R8'
R7
O
R5 or R6
R4
Y2a

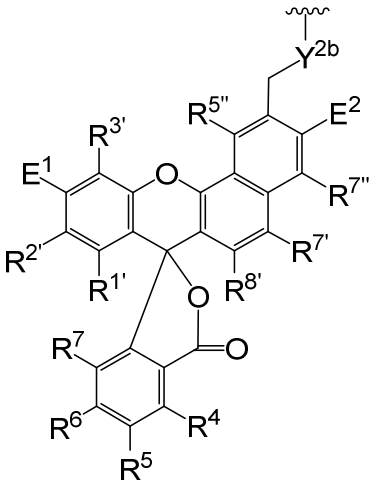
(D.5)


Y3b
R4'
E1
R4''
R5''
E2
O
R2''
R7''
R2'
R7'
R1'
O
R8'
R7
O
R5 or R6
R4
Y2a

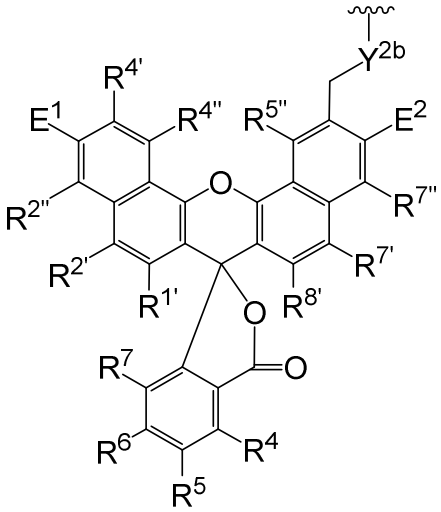
(D.6)


Y2b
R3'
E1
O
E2
R2'
R7'
R1'
O
R8'
R7
O
R6
R4
R5

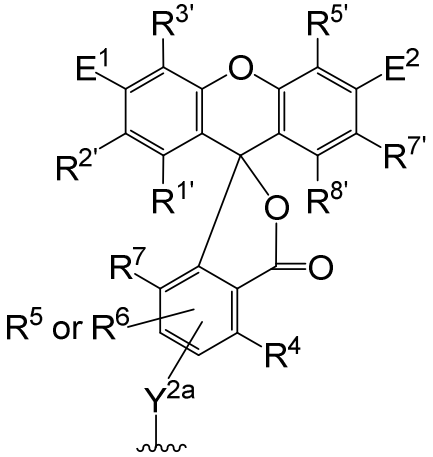
(D.7)


Y2b
R5''
E2
R3'
E1
O
R7''
R2'
R7'
R1'
O
R8'
R7
O
R6
R4
R5

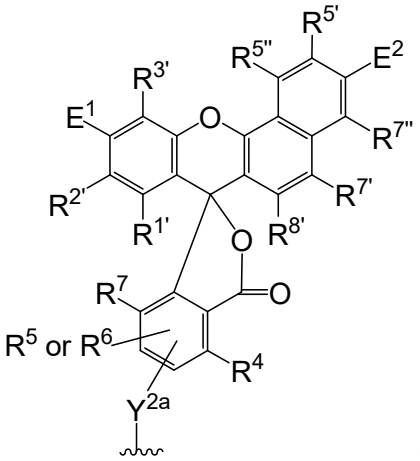
(D.8)


Y2b
R4'
E1
R4''
R5''
E2
O
R2''
R7''
R2'
R7'
R1'
O
R8'
R7
O
R6
R4
R5

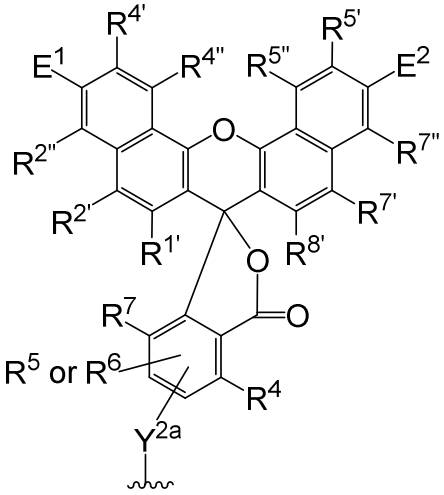
(D.9)


R3'
R5'
E1
O
E2
R2'
R7'
R1'
O
R8'
R7
O
R5 or R6
R4
Y2a

(D.10)



(D.11)



(D.12)

wherein, in each of D.1 – D.12:

each of $R^{1'}$, $R^{2'}$, $R^{2''}$, $R^{4'}$, $R^{4''}$, $R^{5'}$, $R^{5''}$, $R^{7'}$, $R^{7''}$, and $R^{8'}$, when taken alone, is independently selected from the group consisting of hydrogen, a lower alkyl, a (C6-C14) aryl, a (C7-C20) arylalkyl, a 5-14 membered heteroaryl, a 6-20 membered heteroarylalkyl, $-R^b$ and $—(CH_2)_x—R^b$, wherein x is an integer having the value between 1 and 10 and R^b is selected from the group consisting of -X, -OH, $-OR^a$ -SH, $-SR^a$ $-NH_2$, $-NHR^a$ $-NR^cR^c$, $-N^+R^cR^cR^c$, perhalo lower alkyl, trihalomethyl, trifluoromethyl, $-P(O)(OH)_2$, $-P(O)(OR^a)_2$, $P(O)(OH)(OR^a)$, $-OP(O)(OH)_2$, $-OP(O)(OR^a)_2$, $-OP(O)(OR^a)(OH)$, $-S(O)_2OH$, $-S(O)_2R^a$, -C(O)H, $-C(O)R^a$, -C(S)X, $-C(O)OR^a$, -C(O)OH, $-C(O)NH_2$, $-C(O)NHR^a$, $-C(O)NR^cR^c$, $-C(S)NH_2$, $-C(O)NHR^a$, $-C(O)NR^cR^c$, $-C(NH)NH_2$, $-C(NH)NHR^a$, and $-C(NH)NR^cR^c$, wherein X is halo, each R^a is independently selected from the group consisting of a lower alkyl, a (C6-C14) aryl, a (C7-C20) arylalkyl, a 5-14 membered heteroaryl and a 6-20 membered heteroarylalkyl, and each R^c is independently an R^a, or, alternatively, two R^c bonded to the same nitrogen atom may be taken together with that nitrogen atom to form a

5- to 8-membered saturated or unsaturated ring that may optionally include one or more of the same or different ring heteroatoms selected from the group consisting of O, N, and S;

or, alternatively, $R^{1'}$ and $R^{2'}$ or $R^{7'}$ and $R^{8'}$ are taken together with the carbon atoms to which they are bonded to form an optionally substituted (C6-C14) aryl bridge and/or $R^{4'}$ and $R^{4''}$ and/or $R^{5'}$ and $R^{5''}$ are taken together with the carbon atoms to which they are bonded to form a benzo group; and

R^4, R^5, R^6, and R^7 are each, independently of one another, selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 6-14 membered heteroaryl, 7-20 membered heteroarylalkyl, —R^b and —$(CH_2)_x$—R^b;

E^1 is selected from the group consisting of —NHR^9, —NR^9R^{10} and —OR^{9b};

E^2 is selected from the group consisting of —NHR^9, —NR^9R^{10} and —OR^{9b};

R^9, when taken alone, is selected from hydrogen, lower alkyl, (C6-C14) aryl, (C7-C20) arylalkyl, 5-14 membered heteroaryl, 6-20 membered heteroarylalkyl; or R^7 and R^9 are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group;

R^{10} is H or protecting group; or R^8 and R^{10} are taken together with the atoms to which they are bonded to form an optionally substituted heterocycloalkyl group, an optionally substituted heterocycloalkenyl group, or an optionally substituted heteroaryl group;

R^{9b} is R^9;

each of Y^{1a}, Y^{1b}, Y^{2a}, Y^{2b}, Y^{3a} and Y^{3b} is independently selected from the group consisting of —O—, —S—, —NH—, —C(O)—and —$S(O)_2$,

with the proviso that when each of E^1 and E^2 is —OR^{9b}, then $R^{1'}$ and $R^{2'}$ and/or $R^{7'}$ and $R^{8'}$ are may only be taken together with the carbon atoms to which they are bound to form an optionally substituted (C6-C14) aryl bridge.

19. A method for the simultaneous amplification and analysis of a plurality of genetic loci comprising:

amplifying a nucleic acid sample with a plurality of amplification primer pairs to form a plurality of amplifications products, wherein at least one of each of the primer pairs comprises a labeled oligonucleotide of claim 2 wherein each of the amplification products comprises a different genetic loci;

preferably wherein the labeled oligonucleotide of claim 2 is used to label at least three primer pairs, more preferably wherein the labeled oligonucleotide of claim 2 is used to label from three to eight primer pairs; or

preferably wherein the nucleic acid sample is isolated from whole blood, a tissue biopsy, lymph, bone, bone marrow, tooth, skin, for example skin cells contained in fingerprints, bone, tooth, amniotic fluid containing placental cells, and amniotic fluid containing fetal cells, hair, skin, semen, anal secretions, feces, urine, vaginal secretions, perspiration, saliva, buccal swabs, various environmental samples (for example, agricultural, water, and soil), research samples generally, purified samples generally, and lysed cells.

20. A kit comprising oligonucleotide primers for co-amplifying a set of genetic loci of at least one nucleic acid sample to be analyzed; wherein the set of loci can be co-amplified; wherein at least one of the primers comprises a labeled oligonucleotide of claim 2 wherein the primers are in one or more containers; preferably wherein all of the oligonucleotide primers in the kit are in one container;

preferably further comprising at least one of:
reagents for at least one multiplex amplification reaction; and
a container having at least one size standard; or

preferably wherein at least one of the oligonucleotide primers comprises a labeled nucleotide wherein the labeled nucleotide has different spectral properties from the labeled oligonucleotide of claim 2.