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(54) Title: DYES, COMPOSITIONS AND RELATED METHODS OF USE

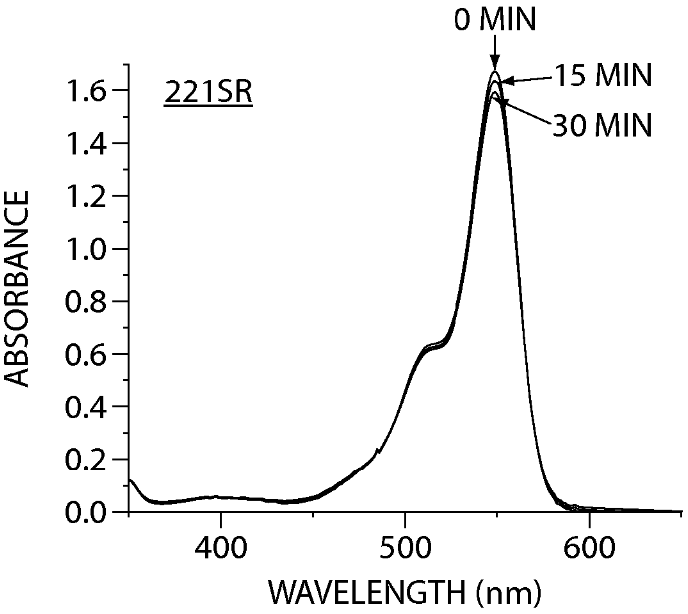

Fig. 1

(57) Abstract: Donor-acceptor dyes, compositions, and related methods of use are described herein.

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DYES, COMPOSITIONS AND RELATED METHODS OF USE

RELATED APPLICATIONS

This application claims priority under 35 U.S.C. §119 to U.S. Provisional Application No. 61/112,363 filed November 7, 2008, the entire contents of which is incorporated by reference.

FIELD OF THE INVENTION

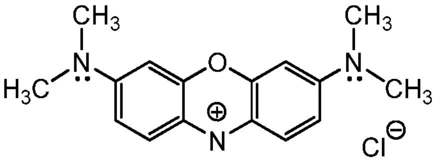
The invention relates to donor-acceptor dyes, compositions, and related methods of use.

BACKGROUND OF INVENTION

Donor-acceptor dyes are well known and of considerable commercial importance. These dyes can be characterized by the presence of one or more electron donating auxochrome groups, which typically contain a heteroatom having a non-bonded pair of electrons such as O, N or S. These electron donating groups are directly connected via a sigma and a partial pi-bond to a simple or complex electron accepting moiety, most often a ring system (Griffiths, J. *Colour and Constitution of Organic Molecules*, London, Academic Press, 1976, Chapters 6 & 7). In the absence of a donor group the acceptor moiety is typically colorless, or at most light yellow, and vice versa.

The donor-acceptor (DA) family is made up of a large number of different classes of dyes having a wide variety of properties (Griffiths, J. *Colour and Constitution of Organic Molecules*, London, Academic Press, 1976, Chapters 7 & 9). Many DA dyes are non-fluorescent and are used for coloring materials; examples include the anthraquinones, triarylmethanes and aminoazo dyes. Some DA dyes are photosensitizers such as the phenothiazine dye Methylene Blue and are used in photomedicine and in photopolymerization systems. Another important class of DA dyes includes those that fluoresce when illuminated with light of the appropriate wavelength; examples include but are not limited to rhodamines, oxazines and squaraines. Fluorescent dyes can be useful in situations where a small amount of dye must give an intense signal, such as in biological systems where it is desirable to perturb the system as little as possible.

Donor-acceptor dyes can be composed of one donor and one acceptor, such as the laser dye DCM; two donors and one acceptor, such as the fluorescent dye Rhodamine B; or three donors and one acceptor, such as the cationic colorant Crystal Violet. An exemplary "two donor-one acceptor" dye is Capri Blue:



Capri Blue

In the above structure the dimethylamino groups constitute the two electron donors, each of which is directly attached via a sigma bond and a partial pi bond to a xanthene ring system, which constitutes the complex electron acceptor moiety. During the absorption of a photon, electron density flows from the two amino moieties to the xanthene ring to give an excited state species. Conversely, relaxation of the excited state, either by the generation of heat (internal conversion) or light (fluorescence) corresponds to the reverse process.

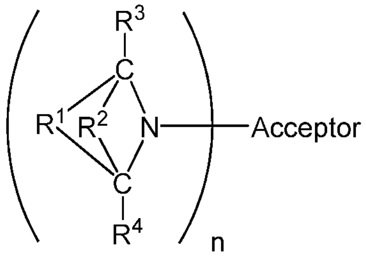
Many organic dyes are unstable to prolonged exposure to light. In general, the photodegradation of such dyes leads to complex mixtures of products (Kuznetsova *et al.*, in Z. Yoshida and T. Kitao (Eds.), *Chemistry of Functional Dyes,* MITA press, Tokyo, 1989, p. 183-185). When the electron donating moiety is an amino group substituted with alkyl substituents, photooxidative degradation usually leads to stepwise dealkylation reactions until all of the alkyl groups have been removed from the amino groups. This type of degradation can be undesirable for one or more of the following reasons: (1) it can cause a significant blue shift in the dye's color; (2) the resulting NH groups can be acidic and reactive in alkaline environments; and (3) the resulting NH groups can react with reactive bioconjugation groups and therefore preclude their use if degradation occurs during storage but before bioconjugation. An additional property of dyes with dialkylamino donors that can be undesirable is their propensity to induce decreasing fluorescence quantum yields as the temperature of the environment rises.

It is highly desirable to develop novel fluorescent dyes that have improved characteristics, such as simultaneous high photostability and high fluorescent quantum yields. Such dyes will be particularly beneficial where little dye is present, such as in single molecule detection experiments, as the combination of these two properties allows the maximum number of photons to be observed from each single chromophore.

SUMMARY OF INVENTION

The inventors have discovered novel dyes, compositions comprising the dyes, and methods of using the dyes.

In one aspect, the invention features a donor-acceptor dye of formula (I),



Formula (I)

wherein n is 1, 2 or 3;

each R^1 and R^2 is independently C_{2-4} alkylenyl, C_{2-4} heteroalkylenyl, C_{2-4} alkenylenyl, arylenyl, or heteroarylenyl; each of which is optionally substituted with 1-4 R^5;

each R^3 and R^4 is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, or $-C(=O)OR^c$; each of which is optionally substituted with 1-4 R^6;

each R^5 and R^6 is independently oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e_2$, $-SR^f$ or $-SO_2R^g$;

each R^a is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino or halo;

each R^b is independently hydrogen, hydroxy, alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, or alkylamino;

each R^c is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, or alkylamino;

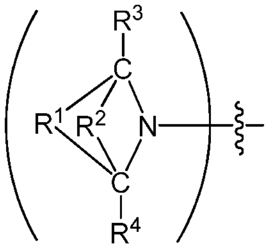
each R^d is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, or alkylamino;

each R^e is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, or alkylamino;

each R^f is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl alkylhydroxy, haloalkyl, or alkylamino;

each R^g is independently hydrogen, hydroxy, halo, alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl alkylhydroxy, haloalkyl, alkylamino or halo;

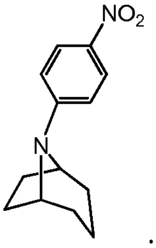
wherein



is a donor; and

the acceptor is a moiety that can accept electron density from the donor moiety under the influence of light of the appropriate wavelength.

In some embodiments, the compound of formula (I) is not



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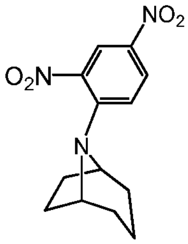
In some embodiments, the compound of formula (I) is not



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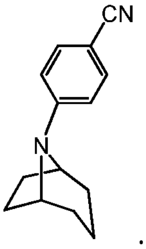
In some embodiments, the compound of formula (I) is not



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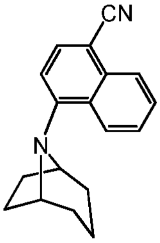
In some embodiments, the compound of formula (I) is not



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In some embodiments, the compound is a closed shell compound. In some embodiments, acceptor moiety comprises at least ten contiguous pi-bonded carbon, nitrogen, oxygen and sulfur atoms (e.g., at least ten, at least twelve, at least thirteen, or at least fourteen).

In some embodiments, the compound is a fluorescent compound. In some embodiments, the compound is a zwitterion. In some embodiments, the compound is a salt.

In some embodiments, R^1 is C_{2-4} alkylenyl, C_{2-4} heteroalkylenyl, C_{2-4} alkenylenyl, arylenyl, or heteroarylenyl; each of which is optionally substituted with 1-4 R^5. In some embodiments, R^1 is C_{2-4} alkylenyl, such as C_{2-4} alkylenyl substituted with 1-4 R^5. In some embodiments, R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e_2$, $-SR^f$ or $-SO_2R^g$. In some embodiments, R^1 is C_{2-4} alkylenyl substituted with 1 R^5 (e.g., wherein R^5 is oxo, halo, $-NO_2$, -CN, -

$C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$). In some embodiments, R^5 is $-OR^d$ or oxo.

In some embodiments, R^1 is C_{2-4} heteroalkylenyl, such as C_{2-4} heteroalkylenyl substituted with 1-4 R^5. In some embodiments, R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$. In some embodiments, R^1 is heteroalkylenyl substituted with 1 R^5 (e.g., wherein R^5 is oxo, halo, $-NO_2$, -CN, -$C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.)

In some embodiments, R^1 is alkenylenyl, such as alkenylenyl substituted with 1-4 R^5. In some embodiments, R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, -$C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$. In some embodiments, R^1 is alkenylenyl substituted with 1 R^5 (e.g., wherein R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, -$C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.)

In some embodiments, R^1 is arylenyl, such as arylenyl substituted with 1-4 R^5. In some embodiments, R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, -$C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$. In some embodiments, R^1 is arylenyl substituted with 1 R^5 (e.g. wherein R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, -$C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.)

In some embodiments, R^1 is unsubstituted.

In some embodiments, R^2 is C_{2-4} alkylenyl, C_{2-4} heteroalkylenyl, C_{2-4} alkenylenyl, arylenyl, or heteroarylenyl; each of which is optionally substituted with 1-4 R^5. In some embodiments, R^2 is alkylenyl, such as alkylenyl substituted with 1-4 R^5. In some embodiments, R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, -$C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, or $-SR^f$ or $-SO_2R^g$. In some embodiments, R^2 is alkylenyl substituted with 1 R^5 (e.g., wherein R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, -$C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.) In some embodiments, R^5 is $-OR^d$ or oxo.

In some embodiments, R^2 is C_{2-4} heteroalkylenyl, such as C_{2-4} heteroalkylenyl substituted with 1-4 R^5. In some embodiments, R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$. In some embodiments, R^2 is heteroalkylenyl substituted with 1 R^5 (e.g., wherein R^5 is oxo, halo, $-NO_2$, -CN, -$C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.)

In some embodiments, R^2 is alkenylenyl, such as alkenylenyl substituted with 1-4 R^5. In some embodiments, R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$. In some embodiments, R^2 is alkenylenyl substituted with 1 R^5 (e.g., wherein R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.)

In some embodiments, R^2 is arylenyl, such as arylenyl substituted with 1-4 R^5. In some embodiments, R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$. In some embodiments, R^2 is arylenyl substituted with 1 R^5 (e.g. wherein R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.)

In some embodiments, R^2 is unsubstituted.

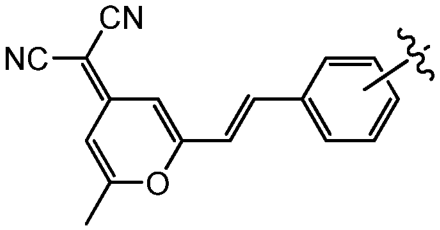
In some embodiments, R^3 is hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, or $-C(=O)OR^c$; each of which is optionally substituted with 1-4 R^6. In some embodiments, R^3 is $-C(=O)OR^c$, alkyl, or alkyl substituted with 1-4 R^6 (e.g., wherein R^6 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.)

In some embodiments, R^4 is hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, or $-C(=O)OR^c$; each of which is optionally substituted with 1-4 R^6. In some embodiments, R^4 is $-C(=O)OR^c$, alkyl, or alkyl substituted with 1-4 R^6 (e.g., wherein R^6 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.)

In some embodiments, the donor is selected from 7-azabicyclo[2.2.1]heptyl, 7-azabicyclo[2.2.1]heptenyl, 7-azabicyclo[2.2.1]heptadienyl, 8-azabicyclo[3.2.1]octyl, 8-azabicyclo[3.2.1]octenyl, 8-azabicyclo[3.2.1]octadienyl, 9-azabicyclo[3.3.1]nonyl, 9-azabicyclo[3.3.1]nonenyl, 9-azabicyclo[3.3.1]nonadienyl, 7-aza-[b]-benzobicyclo[2.2.1]heptyl, and 7-aza-[b,e]-dibenzobicyclo[2.2.1]heptyl.

In some embodiments, n is 1, e.g., the dye may have one donor moiety.

In some embodiments, the acceptor is



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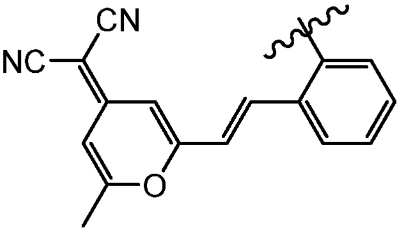

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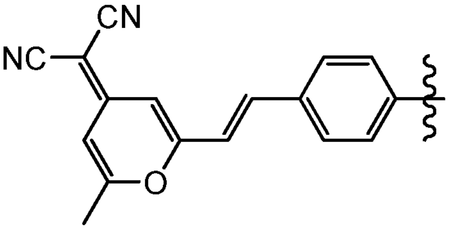

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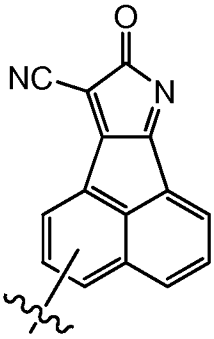
In some embodiments, the acceptor is



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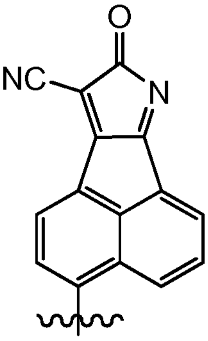

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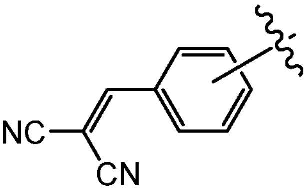
In some embodiments, the acceptor is



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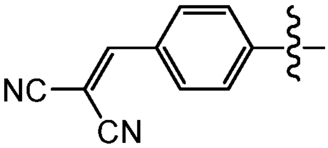

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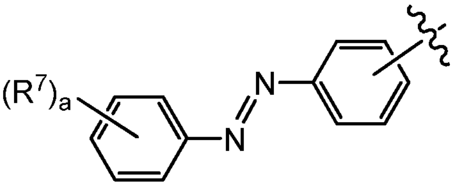
In some embodiments, the acceptor is



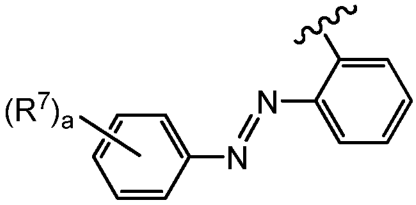
wherein:

a is 0, 1, 2, 3, 4 or 5;

each R^7 is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e_2$, $-SR^f$ or $-SO_2R^g$, each of which is optionally substituted with 1-4 R^8; and

each R^8 is independently halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e_2$, $-SR^f$ or $-SO_2R^g$.

In some embodiments, the acceptor is



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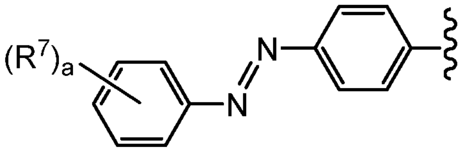

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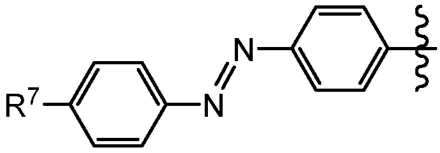
In some embodiments, a is 1.

In some embodiments, the acceptor is



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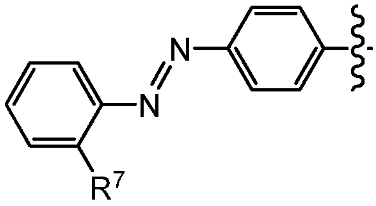

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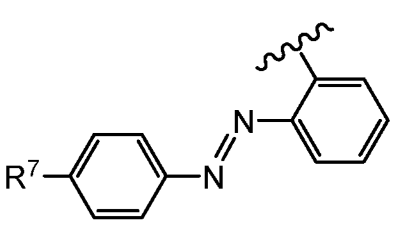

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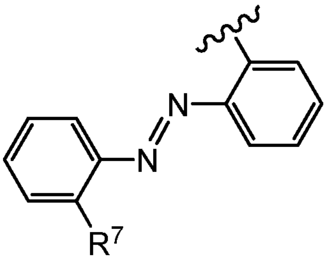

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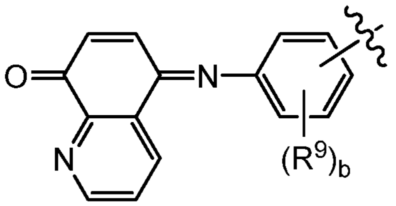
In some embodiments, the acceptor is



wherein:

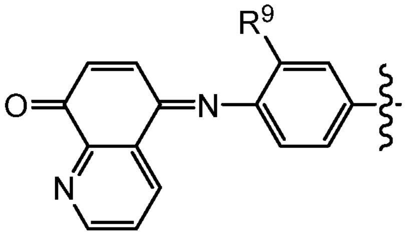
b is 0, 1, 2, 3 or 4;

each R^9 is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e_2$, $-SR^f$ or $-SO_2R^g$, each of which is optionally substituted with 1-4 R^{10}; and

each R^{10} is independently halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e_2$, $-SR^f$ or $-SO_2R^g$.

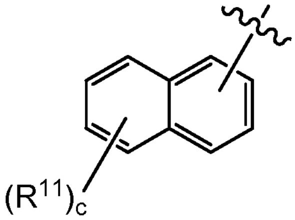
In some embodiments, b is 1. In some embodiments, R^9 is $-OR^d$ (e.g., wherein R^d is alkyl.)

In some embodiments, the acceptor is



.

In some embodiments, the acceptor is



wherein:

c is 1, 2 or 3;

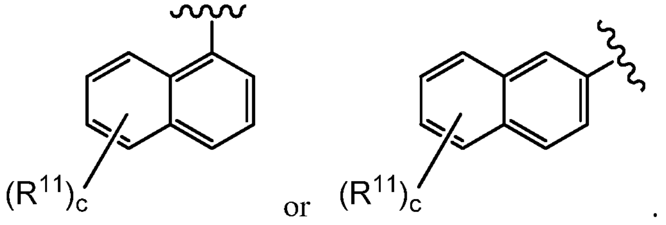
each R^{11} is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, -

C(=O)OR^c, -OR^d, -NR$^e_2$, -SR^f or -SO$_2$R^g, each of which is optionally substituted with 1-4 R^{12}; and

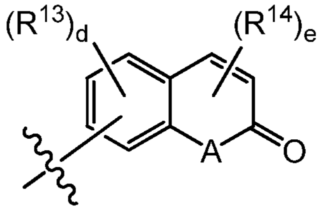
each R^{12} is independently halo, -NO$_2$, -CN, -C(=O)R^a, -C(=O)N(R^b)$_2$, -C(=O)OR^c, -OR^d, -NR$^e_2$, -SR^f or -SO$_2$R^g.

In some embodiments, c is 1. In some embodiments, R^{11} is -C(=O)R^a (e.g., wherein R^a is alkenyl). In some embodiments, R^{11} is -SO$_2$R^g (e.g., wherein R^g is hydroxyl or halo.

In some embodiments, the acceptor is



In some embodiments, the acceptor is:



wherein:

d is 0, 1 or 2;

e is 0, 1 or 2;

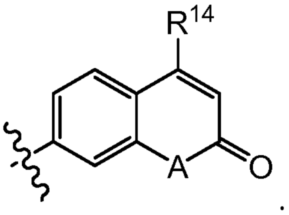
A is O or NH;

each R^{13} and R^{14} is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, halo, -NO$_2$, -CN, -C(=O)R^a, -C(=O)N(R^b)$_2$, -C(=O)OR^c, -OR^d, -NR$^e_2$, -SR^f or -SO$_2$R^g, each of which is optionally substituted with 1-4 R^{15}; and

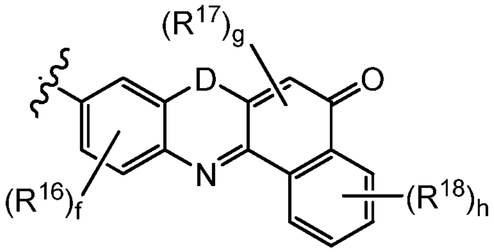
each R^{15} is independently halo, -NO$_2$, -CN, -C(=O)R^a, -C(=O)N(R^b)$_2$, -C(=O)OR^c, -OR^d, -NR$^e_2$, -SR^f or -SO$_2$R^g.

In some embodiments, R^{13} is not positioned ortho to the donor moiety. In some embodiments, A is O or NH. In some embodiments, e is 1. In some embodiments, R^{14} is alkyl.

In some embodiments, the acceptor is



In some embodiments, the acceptor is



wherein:

f is 0, 1, 2 or 3;

g is 0 or 1;

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

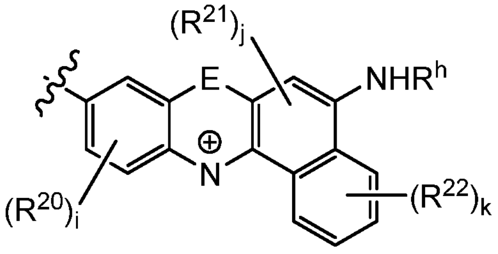
D is O, S or Se;

each R^{16}, R^{17} and R^{18} is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, arylenyl, heteroarylenyl, halo, -C(=O)R^a, -C(=O)N$(R^b)_2$, -C(=O)OR^c, -OR^d, -NR^e_2 , -SR^f or -SO_2R^g, each of which is optionally substituted with 1-4 R^{19}; and

each R^{19} is independently halo, -NO_2, -CN, -C(=O)R^a, -C(=O)N$(R^b)_2$, -C(=O)OR^c, -OR^d, -NR^e_2 , -SR^f or -SO_2R^g.

In some embodiments, R^{16} is not positioned ortho to the donor moiety. In some embodiments, D is O. In some embodiments, h is 1. In some embodiments, R^{18} is -OR^d (e.g., wherein R^d is alkyl.)

In some embodiments, the acceptor is



wherein:

i is 0, 1 or 2;

j is 0, or 1;

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

E is O, S or Se;

each R^{20}, R^{21} and R^{22} is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, halo, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$, each of which is optionally substituted with 1-4 R^{23};

each R^{23} is independently halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$;

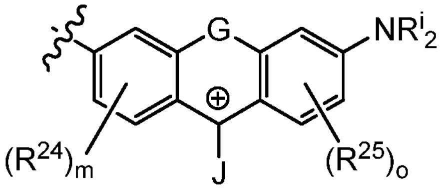
R^h is hydrogen or alkyl;

wherein at least one of R^{20}, R^{21}, R^{22} or R^{23} comprises a negatively charged moiety, or the dye further comprises a negatively charged counterion Z^-; and

Z^- is an anion such as halide, acetate, tosylate, azide, tetrafluoroborate, tetraphenylborate, hexafluorophosphate, phosphate, sulfate, perchlorate, trifluoromethanesulfonate or hexafluoroantimonate.

In some embodiments, E is O. In some embodiments, R^{20} is not positioned ortho to the donor moiety. In some embodiments, j is 1. In some embodiments, R^{21} is halo. In some embodiments, k is 1. In some embodiments, R^{22} is halo. In some embodiments, R^h is hydrogen or alkyl.

In some embodiments, the acceptor is



wherein:

m is 0, 1 or 2;

o is 0, 1 or 2;

G is O, S, $CR^{26}R^{27}$, $SiR^{28}R^{29}$, or NR^{30};

J is hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, alkylhydroxy, haloalkyl, alkylamino, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, each of which is optionally substituted with 1-6 R^{31};

each R^{31} and R^{32} is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e_2$, $-SR^f$ or $-SO_2R^g$, each of which is optionally substituted with 1-4 R^{32};

each R^{31} and R^{32} is independently halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e_2$, or $-SR^f$ or $-SO_2R^g$;

each R^{26}, R^{27}, R^{28}, R^{29}, and R^{30} is independently hydrogen, alkyl, alkenyl or alkynyl;

each R^i is independently hydrogen or alkyl;

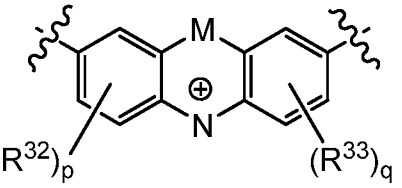
wherein at least one of R^{24}, R^{25}, R^{31} or R^{32} comprises a negatively charged moiety, or the dye further comprises a negatively charged counterion Z^-; and

Z^- is an anion such as halide, acetate, tosylate, azide, tetrafluoroborate, tetraphenylborate, hexafluorophosphate, phosphate, sulfate, perchlorate, trifluoromethanesulfonate or hexafluoroantimonate.

In some embodiments, R^{24} and R^{25} are not positioned ortho to the donor moieties. In some embodiments, G is O or NR^{30}. In some embodiments, J is hydrogen, aryl optionally substituted with 1 R^{31}, $-C(=O)OR^c$, $-SO_2R^g$, alkyl optionally substituted with 1-3 R^{31} (e.g., wherein R^{31} is halo), or -CN. In some embodiments, R^i is hydrogen or alkyl.

In some embodiments, n is 2, e.g., the dye may have two donor moieties.

In some embodiments, the acceptor is



wherein:

p is 0, 1 or 2;

q is 0, 1 or 2;

M is O, S, $CR^{34}R^{35}$, $SiR^{36}R^{37}$, or NR^{38};

each R^{32} and R^{33} is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, halo, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$, each of which is optionally substituted with 1-4 R^{39};

each R^{39} is independently halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$;

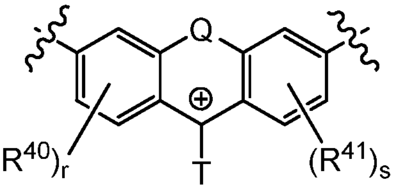
each R^{34}, R^{35}, R^{36}, R^{37} and R^{38} is independently hydrogen, alkyl, alkenyl or alkynyl;

wherein at least one of R^{32}, R^{33} or R^{39} comprises a negatively charged moiety, or the dye further comprises a negatively charged counterion Z^-; and

Z^- is an anion such as halide, acetate, tosylate, azide, tetrafluoroborate, tetraphenylborate, hexafluorophosphate, phosphate, sulfate, perchlorate, trifluoromethanesulfonate or hexafluoroantimonate.

In some embodiments, wherein R^{32} and R^{33} are not positioned ortho to the donor moieties. In some embodiments, M is O or S.

In some embodiments, the acceptor is:



wherein:

r is 0, 1 or 2;

s is 0, 1 or 2;

Q is O, S, $CR^{42}R^{43}$, $SiR^{44}R^{45}$, or NR^{46};

T is hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, alkylhydroxy, haloalkyl, alkylamino, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, each of which is optionally substituted with 1-6 R^{47};

each R^{40} and R^{41} is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$, each of which is optionally substituted with 1-4 R^{48};

each R^{47} and R^{48} is independently halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, or $-SR^f$ or $-SO_2R^g$;

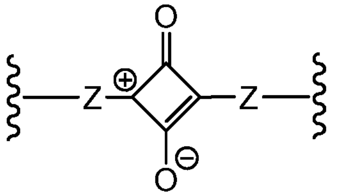
each R^{42}, R^{43}, R^{44}, R^{45}, and R^{46} is independently hydrogen, alkyl, alkenyl or alkynyl;

wherein at least one of R^{40}, R^{41}, R^{47} or R^{48} comprises a negatively charged moiety, or the dye further comprises a negatively charged counterion Z^-; and

Z^- is an anion such as halide, acetate, tosylate, azide, tetrafluoroborate, tetraphenylborate, hexafluorophosphate, phosphate, sulfate, perchlorate, trifluoromethanesulfonate or hexafluoroantimonate.

In some embodiments, R^{40} and R^{41} are not positioned ortho to the donor moieties. In some embodiments, Q is O or NR^{37}. In some embodiments, T is hydrogen, aryl optionally substituted with 1 R^{47} (e.g., wherein R^{47} is $-C(=O)OR^c$ or $-SO_2R^g$), alkyl optionally substituted with 1-3 R^{47} (e.g., wherein R^{47} is halo), or -CN.

In some embodiments, the acceptor is



wherein:

each Z is independently 5- or 6-membered aryl or heteroaryl, optionally substituted with 1-4 R^{49};

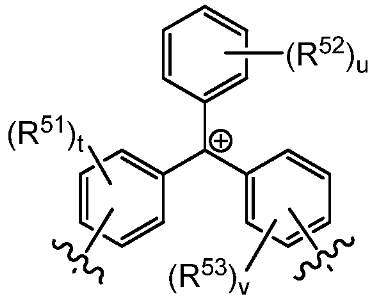
each R^{49} is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, -

C(=O)OR^c, -OR^d, -NR$^e_2$, -SR^f or -SO$_2$R^g, each of which is optionally substituted with 1-4 R^{50}; and

each R^{50} is independently halo, -NO$_2$, -CN, -C(=O)R^a, -C(=O)N(R^b)$_2$, -C(=O)OR^c, -OR^d, -NR$^e_2$, -SR^f or -SO$_2$R^g.

In some embodiments, Z is phenyl or thienyl.

In some embodiments, the acceptor is



wherein:

t is 0, 1, 2, 3, 4 or 5;

u is 0, 1, 2, 3, 4 or 5;

v is 0, 1, 2, 3, 4 or 5;

each R^{51}, R^{52} and R^{53} is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, halo, -NO$_2$, -CN, -C(=O)R^a, -C(=O)N(R^b)$_2$, -C(=O)OR^c, -OR^d, -NR$^e_2$, -SR^f or -SO$_2$R^g, each of which is optionally substituted with 1-4 R^{54};

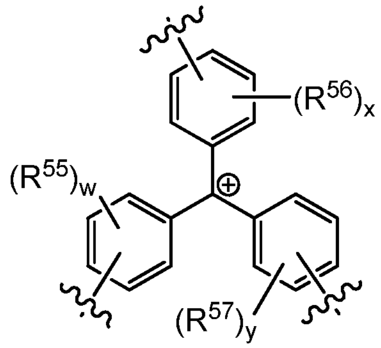
each R^{54} is independently halo, -NO$_2$, -CN, -C(=O)R^a, -C(=O)N(R^b)$_2$, -C(=O)OR^c, -OR^d, -NR$^e_2$, -SR^f or -SO$_2$R^g;

wherein at least one of R^{51}, R^{52}, R^{53} or R^{54} comprises a negatively charged moiety, or the dye further comprises a negatively charged counterion Z$^-$; and

Z$^-$ is an anion such as halide, acetate, tosylate, azide, tetrafluoroborate, tetraphenylborate, hexafluorophosphate, phosphate, sulfate, perchlorate, trifluoromethanesulfonate or hexafluoroantimonate.

In some embodiments, n is 3, e.g., the dye may have three donor moieties.

In some embodiments, the acceptor is:



wherein:

w is 0, 1, 2 or 3;

x is 0, 1, 2 or 3;

y is 0, 1, 2 or 3;

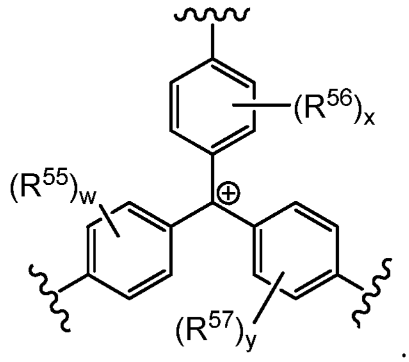
each $R^{55,}$ R^{56} and R^{57} is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e_2$, $-SR^f$ or $-SO_2R^g$, each of which is optionally substituted with 1-4 R^{58};

each R^{58} is independently halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e_2$, $-SR^f$ or $-SO_2R^g$;

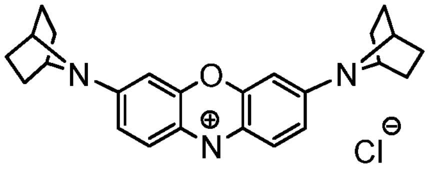
wherein at least one of R^{55}, R^{56}, R^{57} or R^{58} comprises a negatively charged moiety, or the dye further comprises a negatively charged counterion Z^-; and

Z^- is an anion such as halide, acetate, tosylate, azide, tetrafluoroborate, tetraphenylborate, hexafluorophosphate, phosphate, sulfate, perchlorate, trifluoromethanesulfonate or hexafluoroantimonate.

In some embodiments, the acceptor is:



In some embodiments, the dye is selected from: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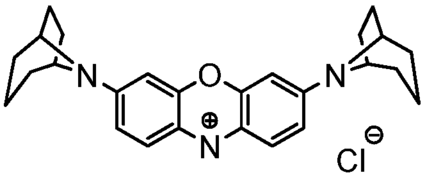
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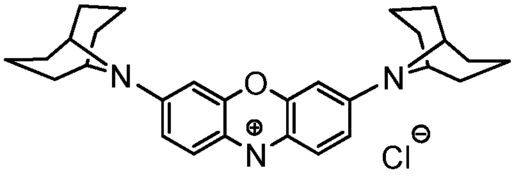
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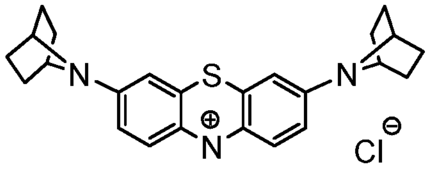
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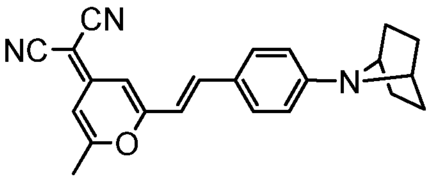
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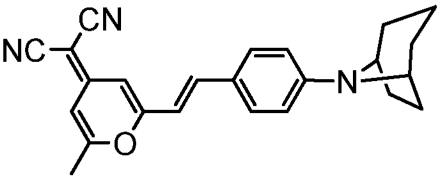
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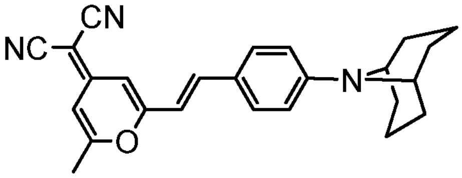
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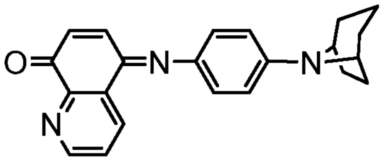
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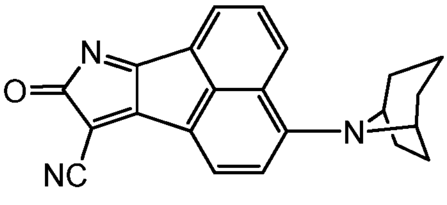
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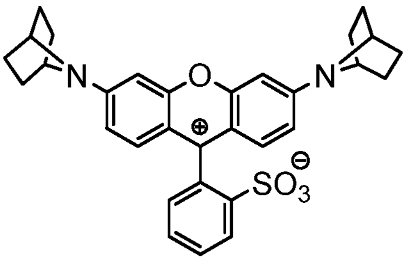
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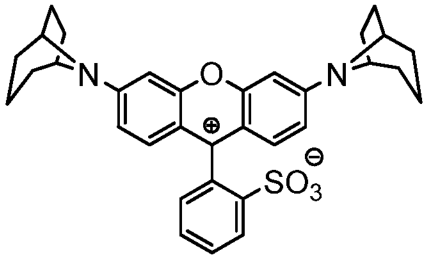
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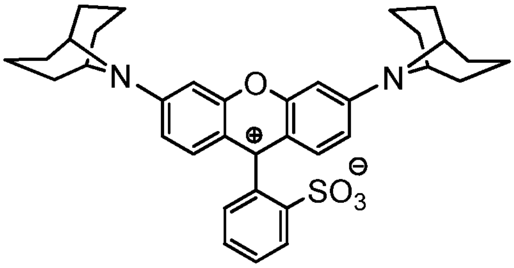
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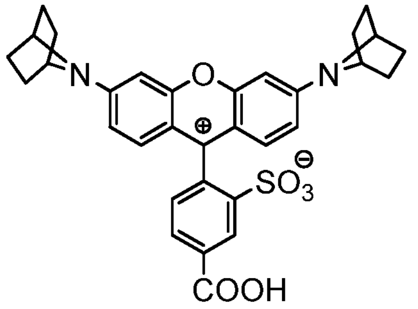

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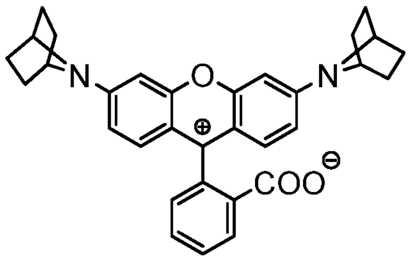

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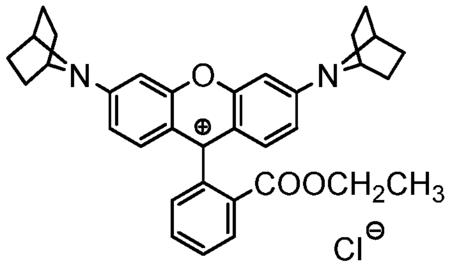

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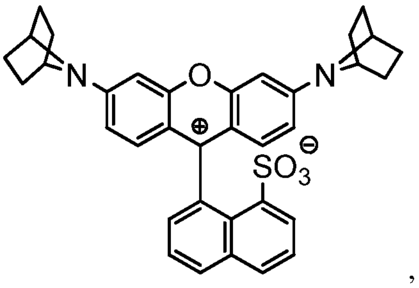

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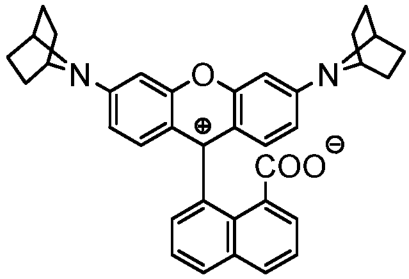

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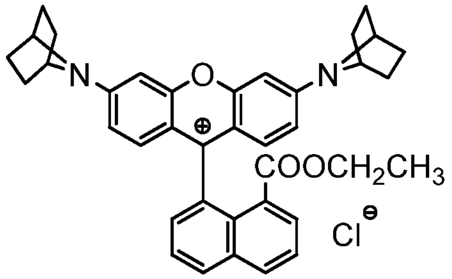

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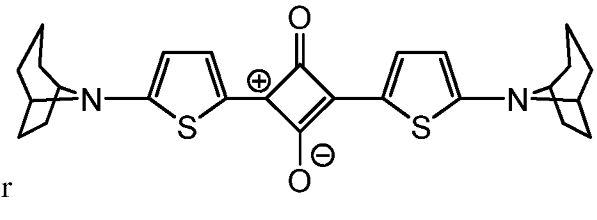

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In some embodiments, the dye has a photostability of at least 1.5 times that of a reference dye when subjected to irradiation, as measured by a decrease in optical density (e.g., wherein the reference dye is a dye having an amino, alkylamino or dialkylamino donor moiety).

In some embodiments, the dye further comprises a reactive moiety. In some embodiments, the dye further comprises a linker and a reactive moiety.

In some embodiments, the dye has the following formula (II):

$$\left[\text{Formula (I)}\right]\!-\!L_z-X$$

(II)

wherein:

L is a linker; and

z is an integer between 0 and 20;

X is a reactive moiety.

In some embodiments, the linker and reactive moiety are attached to the acceptor. In some embodiments, the linker and reactive moiety are attached to the donor. In some embodiments, the reactive moiety is selected from the group consisting of Michael acceptor (e.g., an α,β-unsaturated carbonyl moiety), carboxylic acid or an activated derivative thereof (e.g., a succinimidyl-containing group), maleimido-containing group, isothiocyanate, sulfonic acid or activated derivative thereof (e.g., a sulfonyl chloride), aldehyde, ketone, carbonyl azide, iodoacetamide, alkyne and azide.

In some embodiments, the reactive moiety is directly attached to the dye. In some embodiments, the reactive moiety is attached to the dye via a linker. In some embodiments, the linker is selected from the group consisting of:

$–(C_1\text{-}C_4 \text{ alkylenyl})_{a'}–$

$–[(C_1\text{-}C_4 \text{ alkylenyl})\text{-}O]_{a'}–$

$–NH\text{-}(C_1\text{-}C_4 \text{ alkylenyl})_{a'}\text{-}NH–$

$–NH\text{-}(C_1\text{-}C_4 \text{ alkylenyl})_{a'}\text{-}CO–$

$–NH\text{-}(C_1\text{-}C_4 \text{ alkylenyl})_{a'}\text{-}COO–$

$–NH\text{-}(C_1\text{-}C_4 \text{ alkylenyl})_{a'}\text{-}SO_2NH–$

$–CO\text{-}(C_1\text{-}C_4 \text{ alkylenyl})_{a'}\text{-}CO–$

$–CO\text{-}(C_1\text{-}C_4 \text{ alkylenyl})_{a'}\text{-}COO–$

$–CO\text{-}(C_1\text{-}C_4 \text{ alkylenyl})_{a'}\text{-}SO_2NH–$

$–COO\text{-}(C_1\text{-}C_4 \text{ alkylenyl})_{a'}\text{-}COO–$

$–COO\text{-}(C_1\text{-}C_4 \text{ alkylenyl})_{a'}\text{-}SO_2NH–$

$–SO_2NH\text{-}(C_1\text{-}C_4 \text{ alkylenyl})_{a'}\text{-}SO_2NH–$

$–NH-[(C_1-C_4\ \text{alkylenyl})-O]_{a'}-NH–$

$–NH-[(C_1-C_4\ \text{alkylenyl})-O]_{a'}-CO–$

$–NH-[(C_1-C_4\ \text{alkylenyl})-O]_{a'}-COO–$

$–NH-[(C_1-C_4\ \text{alkylenyl})-O]_{a'}-SO_2NH–$

$–CO-[(C_1-C_4\ \text{alkylenyl})-O]_{a'}-CO–$

$–CO-[(C_1-C_4\ \text{alkylenyl})-O]_{a'}-COO–$

$–CO-[(C_1-C_4\ \text{alkylenyl})-O]_{a'}-SO_2NH–$

$–COO-[(C_1-C_4\ \text{alkylenyl})-O]_{a'}-COO–$

$–COO-[(C_1-C_4\ \text{alkylenyl})-O]_{a'}-SO_2NH–$

$–SO_2NH-[(C_1-C_4\ \text{alkylenyl})-O]_{a'}-SO_2NH–$

and combinations thereof, wherein:

a’ is an integer between 1 and 20;

the linker as drawn may be positioned between the donor and acceptor in either direction; and

the alkyl chains may have varying degrees of unsaturation.

In some embodiments, the linker is $–(CH_2)_{a'}–$, $–(CH_2-CH_2-O)_{a'}–$, or $–NH-CH_2-CH_2-O-CH_2-CH_2-O-CH_2-COO–$.

In some embodiments, the dye is conjugated to a substrate. In some embodiments, the dye is conjugated to a substrate via a covalent bond. In some embodiments, the dye is conjugated to a substrate via a linker and a reactive moiety.

In some embodiments, the dye has the following formula (III):

$$\left[\text{Formula (II)}\right]_{b'}\text{—substrate}$$

(III)

wherein b’ is 1, 2 or 3; and

the reactive moiety in the dye of formula (II) is the residual product of the reaction between the reactive moiety and the substrate.

In some embodiments, the substrate is a biomolecule (e.g., an amino acid, a polypeptide, a nucleic acid, an antibody, or an antigen), a pharmaceutical agent, a metabolite, a diagnostic agent, a controlled substance, a toxin, biotin, a polymer, or a textile (e.g., an article of clothing). In some embodiments, the substrate has been previously labeled with another dye.

In one aspect, the invention features a composition comprising a dye of formula (I) and an additional component.

In some embodiments, the component is a solvent. In some embodiments, the component is a substrate. In come embodiments, the substrate is a biomolecule (e.g., an amino acid, a polypeptide, a nucleic acid, an antibody, or an antigen), a pharmaceutical agent, a metabolite, a diagnostic agent, a controlled substance, a toxin, biotin, a polymer, or a textile (e.g., an article of clothing). In some embodiments, the substrate has been previously labeled with another dye.

In some embodiments, the component is a reagent (e.g., an acid, a base, a reducing agent, or a coupling agent). In some embodiments, the component is a textile (e.g., an article of clothing).

In some embodiments, the composition comprises a plurality of components. In some embodiments, the composition features a dye of formula (II).

In one aspect, the invention features a kit comprising a dye of formula (I).

In some embodiments, the kit further comprises a container (e.g., a vial). In some embodiments, the kit further comprises instructions for use of the dye. In some embodiments, the kit further comprises a substrate. In some embodiments, the kit further comprises a reference standard. In some embodiments, the kit comprises a dye of formula (II).

In one aspect, the invention features a method of labeling a substrate, the method comprising mixing a dye of formula (I) with the substrate under conditions sufficient to label the substrate, thereby labeling the substrate.

In some embodiments, the dye is of formula (II). In some embodiments, the substrate is a biomolecule (e.g., an amino acid, a polypeptide, a nucleic acid, an antibody, or an antigen), a pharmaceutical agent, a metabolite, a diagnostic agent, a controlled substance, a toxin, biotin, a polymer, or a textile (e.g., an article of clothing). In some embodiments, the substrate has been previously labeled with another dye.

In some embodiments, the dye is of formula (III), the biomolecule of formula (III) is an antigen, and the substrate is an antibody. In some embodiments, the dye is of formula (III), the biomolecule of formula (III) is an antibody, and the substrate is an antigen.

In some embodiments, the method further comprises evaluating the labeled substrate. In some embodiments, the amount of labeled substrate is evaluated qualitatively. In some embodiments, the amount of labeled substrate is evaluated quantitatively. In some embodiments, the amount of labeled substrate is evaluated in comparison to a control or reference standard.

In some embodiments, the substrate is labeled in an imagewise manner.

In one aspect, the invention features a method of staining an object with a dye of formula (I), the method comprising contacting the dye with the object, thereby staining the object.

In some embodiments, the object is a cell or group of cells. In some embodiments, the object is a polymer. In some embodiments, the object is a textile (e.g., an article of clothing).

The dyes described herein can have one or more beneficial properties, including properties that are improved over one or more dyes known in the art. Exemplary properties described herein may include one or more of the following: (1) colors and extinction coefficients comparable to or better than those dyes known in the art; (2) increased resistance to photooxidative dealkylation; (3) fluorescence quantum yields that are as good or better than the best known members in their class, regardless of the substitution pattern or rigidity of the electron donor moieties; (4) fluorescence quantum yields that are unaffected or little affected by temperature; (5) are as readily synthesized as are dyes known in the art; and (6) improved solubilities over those known in the art.

BRIEF DESCRIPTION OF DRAWINGS

The accompanying drawings are not intended to be drawn to scale. In the drawings, each identical or nearly identical component that is illustrated in various figures is represented by a like numeral. For purposes of clarity, not every component may be labeled in every drawing. In the drawings:

FIG. 1 is the absorption spectrum of dye 221SR as a function of time of illumination by a 1.7W, 514 nm CW argon laser in anaerobic trifluoroethanol. The structure of the dye is given in Example 20.

FIG. 2 is the absorption spectrum of dye 331SR as a function of time of illumination by a 1.7W, 514 nm CW argon laser in anaerobic trifluoroethanol. The structure of the dye is given in Example 20.

FIG. 3 is the absorption spectrum of control dye TMSR as a function of time of illumination by a 1.7W, 514 nm CW argon laser in anaerobic trifluoroethanol. The structure of the dye is given in Example 20.

FIG. 4 shows the absorption spectra of sulfonaphthylrhodamine dyes 221SNR and control TMSNR as a function of time of illumination by a 1.7W, 514 nm CW argon laser in anaerobic trifluoroethanol. The structures of the dyes are given in Example 24.

FIG. 5 is a plot of the optical density at wavelength of maximum absorption vs. time of illumination by a 1W bank of green LEDs for dyes 221RE, 221SR, 221SNR of this invention and control dyes TMRE and TMSR. The structures of the dyes are given in Example 25.

DEFINITIONS

The term “alkyl” refers to a hydrocarbon chain that may be a straight chain or branched chain, containing the indicated number of carbon atoms. For example, C_1-C_{12} alkyl indicates that the group may have from 1 to 12 (inclusive) carbon atoms in it. The term “arylalkyl” refers to an alkyl moiety in which an alkyl hydrogen atom is replaced by an aryl group. Examples of “arylalkyl” include benzyl, 2-phenylethyl, 3-phenylpropyl, 9-fluorenyl, benzhydryl, and trityl groups. The term “alkylhydroxy” refers to an alkyl moiety in which an alkyl hydrogen atom is replaced by a hydroxyl group. The term “haloalkyl” refers to an alkyl in which one or more hydrogen atoms are replaced by halo, and includes alkyl moieties in which all hydrogens have been replaced by halo (e.g., perfluoroalkyl). The term “alkylamino” refers to an -NH(alkyl) or -N(alkyl)$_2$ radical. The term “alkylenyl” refers to a divalent alkyl, e.g., -CH_2-, -CH_2CH_2-, and -$CH_2CH_2CH_2$-.

The term “heteroalkyl” refers to an alkyl moiety in which one or more of the -CH_2- groups has been replaced with a heteroatom such as O, S or NH. The term “heteroalkylenyl” refers to a divalent heteroalkyl, e.g., -CH_2-O-CH_2-, -CH_2-CH_2-NH-CH_2-, and -CH_2-CH_2-S-CH_2-CH_2-.

The term “alkenyl” refers to a straight or branched hydrocarbon chain containing 2-12 carbon atoms and having one or more double bonds. Examples of alkenyl groups include, but are not limited to, allyl, propenyl, 2-butenyl, 3-hexenyl and 3-octenyl groups. One of the double bond carbons may optionally be the point of attachment of the alkenyl substituent. The term “alkenylenyl” refers to a divalent alkenyl, e.g., -CH=CH-, -CH=CH-CH_2-, and -CH=CH-CH=CH-.

The term “alkynyl” refers to a straight or branched hydrocarbon chain containing 2-12 carbon atoms and characterized in having one or more triple bonds. Examples of alkynyl groups include, but are not limited to, ethynyl, propargyl, and 3-hexynyl. One of the triple bond carbons may optionally be the point of attachment of the alkynyl substituent.

The term “aryl” refers to an aromatic monocyclic, bicyclic, or tricyclic hydrocarbon ring system, wherein any ring atom capable of substitution can be substituted (e.g., by one or more substituents). Examples of aryl moieties include, but are not limited to, phenyl, naphthyl, and anthracenyl. The term “arylenyl” refers to a divalent aryl, e.g., -C_6H_4-.

The term “heteroaryl” refers to an aromatic 5-8 membered monocyclic, 8-12 membered bicyclic, or 11-14 membered tricyclic ring system having 1-3 heteroatoms if monocyclic, 1-6 heteroatoms if bicyclic, or 1-9 heteroatoms if tricyclic, said heteroatoms selected from O, N, or S (e.g., carbon atoms and 1-3, 1-6, or 1-9 heteroatoms of N, O, or S if monocyclic, bicyclic, or tricyclic, respectively). Any ring atom can be substituted (e.g., by one or more substituents). The term “heteroarylenyl” refers to a divalent heteroaryl, e.g., -C_5H_3N-.

The term “halo” or “halogen” refers to any radical of fluorine, chlorine, bromine or iodine.

The term “oxo” refers to an oxygen atom, =O, that is double-bonded to the atom to which it is attached; it forms a carbonyl when attached to carbon, an N-oxide when attached to nitrogen, and a sulfoxide or sulfone when attached to sulfur.

The term “substituents” refers to a group “substituted” on an alkyl, alkylenyl, alkenyl, alkenylenyl, alkynyl, aryl, arylenyl, heteroaryl or heteroarylenyl group at any atom of that group. Any atom can be substituted. Suitable substituents include, without limitation, alkyl (e.g., C1, C2, C3, C4, C5, C6, C7, C8, C9, C10, C11, C12 straight or branched chain alkyl), haloalkyl (e.g., perfluoroalkyl such as CF_3), aryl, heteroaryl, arylalkyl, alkenyl, alkynyl, alkoxy, haloalkoxy (e.g., perfluoroalkoxy such as OCF_3), halo, hydroxy, carboxy, carboxylate, cyano, nitro, amino, alkyl amino, SO_3H, sulfate, phosphate, methylenedioxy (-O-CH_2-O- wherein oxygens are attached to vicinal atoms), ethylenedioxy, oxo, thioxo (e.g., C=S), imino (alkyl, aryl, arylalkyl), $S(O)_n$alkyl (where n is 0-2), $S(O)_n$ aryl (where n is 0-2), $S(O)_n$ heteroaryl (where n is 0-2), $S(O)_n$ heterocyclyl (where n is 0-2), amine (mono-, di-, alkyl, cycloalkyl, arylalkyl, heteroarylalkyl, aryl, heteroaryl, and combinations thereof), ester (alkyl, arylalkyl, heteroarylalkyl, aryl, heteroaryl), amide (mono-, di-, alkyl, arylalkyl, heteroarylalkyl, aryl, heteroaryl, and combinations thereof), sulfonamide (mono-, di-, alkyl, arylalkyl, heteroarylalkyl, and combinations thereof). In one aspect, the substituents on a group are independently any one single, or any subset of the aforementioned substituents. In another aspect, a substituent may itself be substituted with any one of the above substituents.

As used herein, the term “partial pi-bond” refers to a bond in which there is direct p-orbital overlap between the two atoms but the resulting pi bond does not necessarily contain two electrons as is the case in an isolated carbon-carbon double bond.

As used herein, the term “donor” refers to a moiety that readily releases electrons to an acceptor.

As used herein, the term "acceptor" refers to a moiety that accepts the electron density from the donor moiety.

As used herein, the term "complex acceptor" refers to a moiety that accepts electron density from the donor moiety and can provide extended conjugation.

As used herein, "donor-acceptor dye" (abbreviated DA dye) refers to a dye comprising a donor and an acceptor as defined above, wherein the donor is directly connected to the acceptor via a sigma and partial pi-pond as defined above.

As used herein, the term "closed-shell molecule" refers to a molecule in which all of the ground state electrons are paired.

As used herein, "optical density" (abbreviated OD) refers to a measure of the amount of light transmitted through a solution of an organic molecule using a spectrophotometer.

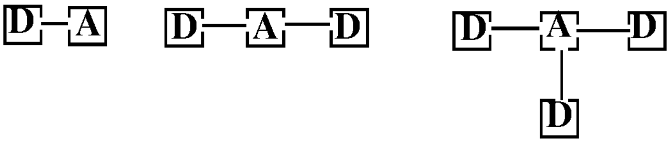
As used herein, "photostability" refers to the % change in OD of a solution of the dye under the influence of irradiation.

DETAILED DESCRIPTION

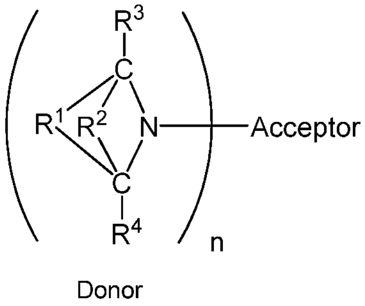
Dyes

Described herein are donor-acceptor dyes, including those described generically and specifically herein. In general, the dyes described herein are closed shell compounds. Included herein are dyes that are zwitterions or salts. In some embodiments, the dye has a photostability of at least 1.5 times that of a reference dye (e.g., a reference dye having an alkylamino or dialkylamino donor moiety) when subjected to irradiation, as measured by a decrease in optical density.

The dyes described herein can have one or more donor moieties (e.g., 1, 2, or 3 donor moieties) attached to an acceptor moiety. The dyes may be represented by the generic structures below, in which D represents a donor and A is an acceptor. In the cases in which multiple donors are employed, the donors may be same or different.

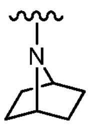


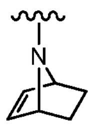
The donor moieties of the dyes herein generally include one or more apex-N-substituted azabicycloalkanes and alkenes, for example, as shown in formula (I) below.

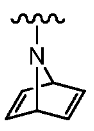


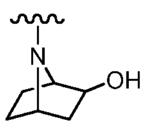
Formula (I)

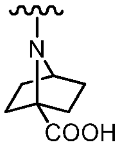
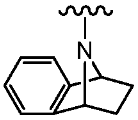
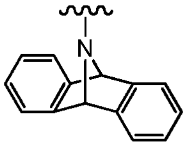
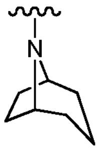
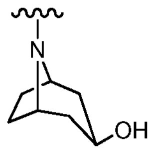
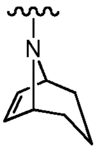
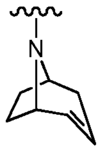
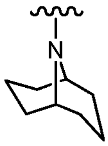
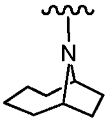
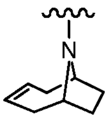
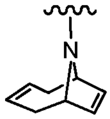
In formula (I), R^1 and R^2 of the donor moiety represent two branches of an azabicyclic ring system. In general, R^1 and R^2 are each independently carbon chains (e.g., C_{2-4} alkylenyl, C_{2-4} heteroalkylenyl, C_{2-4} alkenylenyl) or a cyclic moiety (e.g., arylenyl or heteroarylenyl). Each of R^1 and R^2 can be independently substituted or unsubstituted, for example, as described herein. In certain embodiments, one or both branches are alkylenyl. In certain embodiments, one or both branches are alkenylenyl. In certain embodiments, one or both branches are aryl, e.g., phenyl. R^1 and R^2 may be the same or different. R^1 and R^2 can in some instances be substituted with one or more substituents. Exemplary substituents include alkyl groups, oxo groups, alcohols and carboxylic acids. In addition to substituents on R^1 and R^2, in formula (I) the bridgehead carbons may also be substituted, as provided above. For example, each of R^3 and R^4 can independently be hydrogen, or a substituent such as alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, -CN, -C(=O)R^a, -C(=O)N(R^b)$_2$, or -C(=O)OR^c. Each of R^3 and R^4 can be independently further substituted. Exemplary donor moieties include the following:



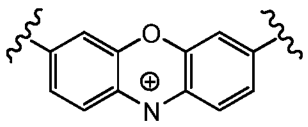
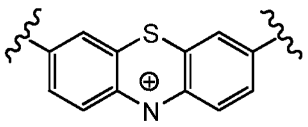
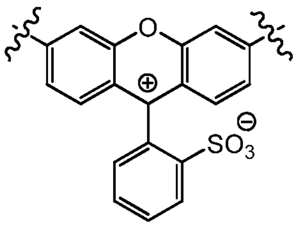
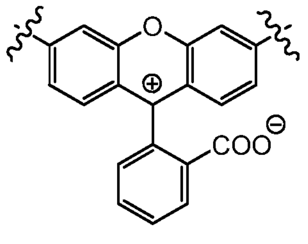
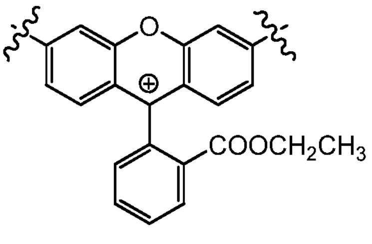
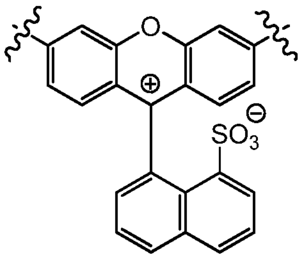


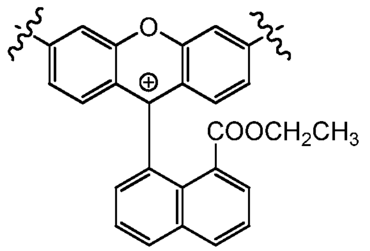


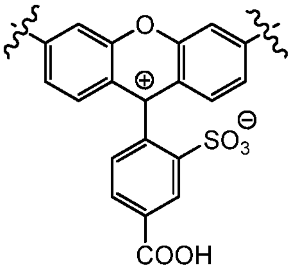


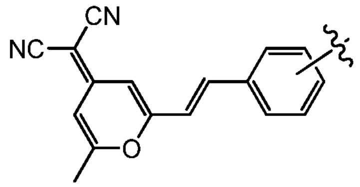












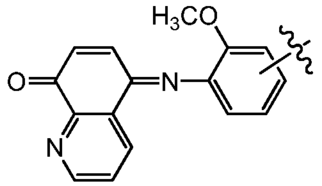
As described herein, an acceptor moiety is a moiety that can accept electron density from the donor moiety under the influence of light of the appropriate wavelength. Exemplary acceptor moieties include at least ten contiguous pi-bonded carbon, nitrogen, oxygen and sulfur atoms (e.g., at least 10, 11, 12, 13, 14, 15, 16, 17, or 18). As shown in formula (I), a dye can include one or more donor moieties such as those described herein. As shown in formula (I), the acceptor moiety can include an aromatic moiety. In certain embodiments, the acceptor moiety is a phenoxazine, phenothiazine, xanthene, coumarin, naphthalene, aryl azo compound, triarylmethane or squaraine. Exemplary acceptor moieties include the following:

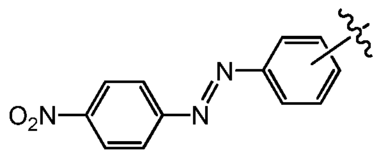







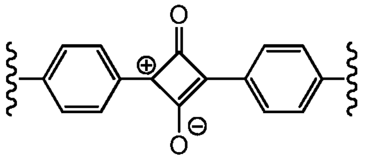


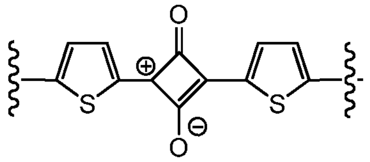


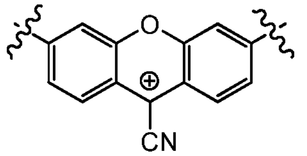


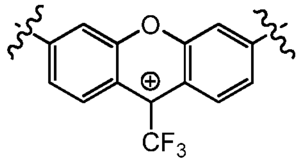


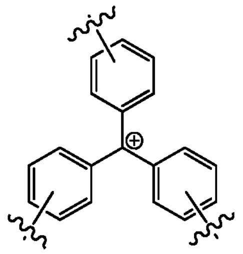


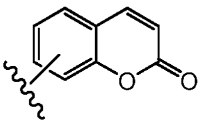


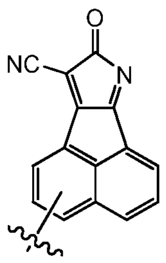












Exemplary dyes include those described in the examples.

In some instances, a dye described herein may be zwitterionic. In some instances, a dye described herein may be a salt. For example, a dye described herein may have a positive charge on the acceptor moiety, and thus also include a negatively charged counterion such as halide, acetate, tosylate, azide, tetrafluoroborate, tetraphenylborate, phosphorus hexafluoride, phosphate, sulfate, perchlorate, trifluoromethanesulfonate or hexafluoroantimonate.

In some instances, a dye described herein includes a substituent, for example, on a donor or acceptor moiety (e.g., the acceptor moiety), which allows the dye to form a bond with a substrate. For example, a dye described herein can include a reactive moiety, which allows the dye to form a bond such as a covalent, hydrogen, ionic or electrostatic bond with a substrate. The reactive moiety can be directly attached to the dye, for example, through a covalent bond, or can be attached to the dye through a linker (e.g., a linker including a carbon chain). Exemplary dyes that include a reactive moiety are provided in Formula (II) below, in which L is a linker, z is an integer between 0 and 20, and X is a reactive moiety.

$$\left[\text{Formula (I)}\right]\!\!-\!L_z\!-\!X$$

(II)

The reactive moiety can be any group which can react with a substrate. Exemplary reactive moieties include Michael acceptors, carboxylic acids or an activated derivative thereof, maleimido-containing groups, isothiocyanates, sulfonic acids or activated derivatives thereof, aldehydes, ketones, carbonyl azides, iodoacetamides, alkynes and azides.

The linker moiety can be any group that attaches the reactive moiety to the dye. Exemplary linker moieties include: –(C_1-C_4 alkylenyl)$_{a'}$–, –[(C_1-C_4 alkylenyl)-O]$_{a'}$–, –NH-(C_1-C_4 alkylenyl)$_{a'}$-NH–, –NH-(C_1-C_4 alkylenyl)$_{a'}$-CO–, –NH-(C_1-C_4 alkylenyl)$_{a'}$-COO–, –NH-(C_1-C_4 alkylenyl)$_{a'}$-SO_2NH–, –CO-(C_1-C_4 alkylenyl)$_{a'}$-CO–, –CO-(C_1-C_4 alkylenyl)$_{a'}$-COO–, –CO-(C_1-C_4 alkylenyl)$_{a'}$-SO_2NH–, –COO-(C_1-C_4 alkylenyl)$_{a'}$-COO–, –COO-(C_1-C_4 alkylenyl)$_{a'}$-SO_2NH–, –SO_2NH-(C_1-C_4 alkylenyl)$_{a'}$-SO_2NH–, –NH-[(C_1-C_4 alkylenyl)-O]$_{a'}$-NH–, –NH-[(C_1-C_4 alkylenyl)-O]$_{a'}$-CO–, –NH-[(C_1-C_4 alkylenyl)-O]$_{a'}$-COO–, –NH-[(C_1-C_4 alkylenyl)-O]$_{a'}$-SO_2NH–, –CO-[(C_1-C_4 alkylenyl)-O]$_{a'}$-CO–, –CO-[(C_1-C_4 alkylenyl)-O]$_{a'}$-COO–, –CO-[(C_1-C_4 alkylenyl)-O]$_{a'}$-SO_2NH–, –COO-[(C_1-C_4 alkylenyl)-O]$_{a'}$-COO–, –COO-[(C_1-C_4 alkylenyl)-O]$_{a'}$-SO_2NH–, –SO_2NH-[(C_1-C_4 alkylenyl)-O]$_{a'}$-SO_2NH– and combinations thereof, where a' is an integer between 1 and 20. The linker moieties can attach the reactive moiety to the dye in either direction from that drawn above.

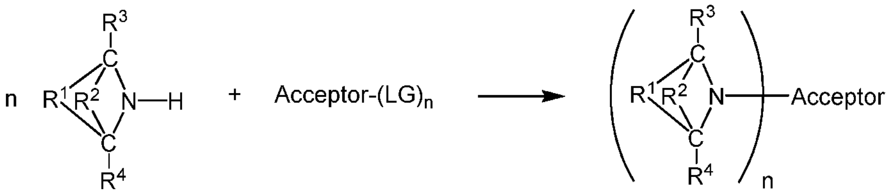
In some embodiments, a dye described herein is conjugated to a substrate (e.g., a biomolecule such as an antibody, antigen or nucleic acid), or an organic compound (e.g., a pharmaceutical compound, a metabolite, a controlled substance, or a toxin). The substrate can be conjugated directly to the dye or can be conjugated through a reactive moiety, for example, a reactive moiety described above. As described above, the reactive moiety can be attached to the dye directly (e.g., through a covalent bond) or through a linker such as a linker described herein. Exemplary dye conjugates are provided in formula (III) below, where b' is 1, 2 or 3.

$$\left[\text{Formula (II)}\right]_{b'}\text{—substrate}$$

(III)

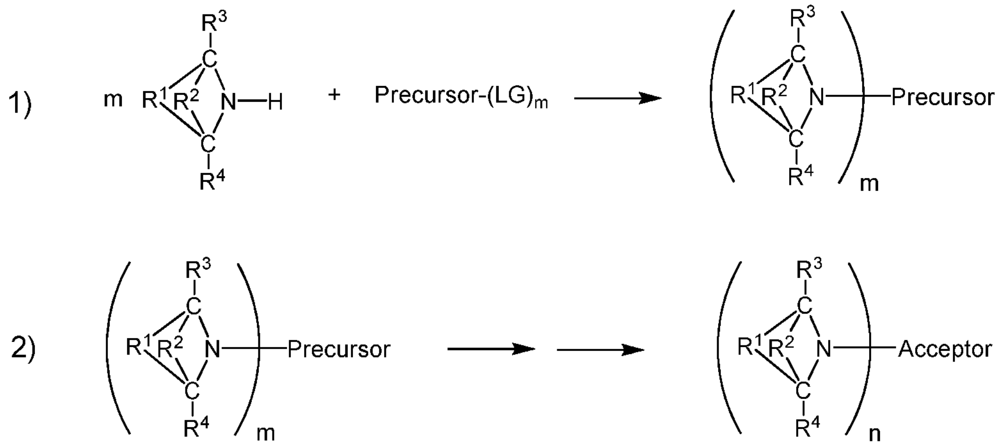
Synthesis of dyes

Described herein are methods for synthesizing the dyes described herein. Methods for synthesizing the dyes vary depending on the specific acceptor moiety to be used. In certain embodiments, the dye is synthesized by reaction of the acceptor bearing an appropriate leaving group with the donor amine, e.g., apex-N-substituted azabicyclic amine, according to the following scheme:



In the above scheme, LG is a leaving group and n is 1, 2 or 3. In preferred embodiments, the acceptor is an aromatic moiety and the leaving group is a halogen such as fluorine, chlorine or bromine, and the reaction proceeds via nucleophilic aromatic substitution. If the acceptor is not commercially available with the appropriate halogen substituents, it may be synthesized by methods known to those skilled in the art, such as chlorination with phosphorus pentachloride.

In other embodiments, the appropriate azabicyclic amine is reacted with an aromatic molecule that is not the acceptor itself but rather a precursor to the desired acceptor, according to the following scheme:



In the above scheme, m is 1 or 2, LG is a leaving group and n is 1, 2 or 3. In certain embodiments, in reaction 1 the precursor is an aromatic moiety and the leaving group is a halogen such as fluorine, chlorine or bromine, and the reaction proceeds via nucleophilic aromatic substitution. In certain embodiments, reaction 2 comprises acid-catalyzed condensation, as described in Example 2 below.

Many of the donor moieties, e.g., apex-N-substituted azabicyclic amines, are commercially available. Those that are not commercially available may be synthesized using methods that will be known to those of ordinary skill in the art.

As can be appreciated by the skilled artisan, methods of synthesizing the compounds of the formulae herein will be evident to those of ordinary skill in the art. Synthetic chemistry transformations useful in synthesizing the compounds described herein are known in the art and include, for example, those such as described in R. Larock, *Comprehensive Organic Transformations*, VCH Publishers (1989); L. Fieser and M. Fieser, *Fieser and Fieser's Reagents for Organic Synthesis*, John Wiley and Sons (1994); and L. Paquette, ed., *Encyclopedia of Reagents for Organic Synthesis*, John Wiley and Sons (1995), and subsequent editions thereof.

Methods of use

Described herein are methods of using the dyes and compositions described herein. Exemplary methods include methods of labeling a substrate with a dye described herein. The substrate may be a biomolecule such as a polypeptide or nucleic acid, or an organic molecule such as a pharmaceutical agent, metabolite, controlled substance or toxin.

The dyes described herein, e.g., dyes of formulas (I), (II) and (III), can be used in a variety of methods, for example, where the labeling of a substrate is desirable. Exemplary methods and techniques appropriate for the dyes described herein include: fluorescence microscopy, Förster resonance energy transfer (FRET), fluorescent immunoassays (FIA), flow cytometry, fluorescence-activated cell sorting (FACS), single-molecule fluorescence spectroscopy, DNA sequencing, fluorescence in-situ hybridization (FISH), quantitative real-time polymerase chain reaction (qPCR), DNA microarrays, dye lasers, stains, photodynamic therapy (PDT), inkjet applications and clothing dyes.

Fluorescence microscopy

The dyes described herein, e.g., the dyes of formulas (I), (II) and (III), could be used in fluorescence microscopy. In this technique, the specimen, such as a cell or group of cells containing a substrate labeled with a dye described herein, is illuminated with light of a specific wavelength which is absorbed by the dye, causing it to emit a longer wavelength of light (of a different color than the absorbed light). The illumination light may be separated from the much weaker emitted fluorescence through the use of an emission filter. This technique makes it possible to identify cells and sub-microscopic cellular components with a high degree of specificity.

The photostability of the dyes described herein (e.g., a fluorescent dye) can provide an advantage to researchers using fluorescence microscopy. For example, in some instances, a dye described herein can allow the use of a lower concentration of the dye and also provide for longer time periods of illumination.

Förster resonance energy transfer

The dyes described herein, e.g., the dyes of formulas (I), (II) and (III), could be used in Förster resonance energy transfer (FRET), also known as fluorescence resonance energy transfer. FRET results from a distance-dependent interaction between the electronic excited states of two molecules in which excitation is transferred from a donor fluorophore to an acceptor fluorophore without emission of a photon. The process of energy transfer results in a reduction (quenching) of fluorescence intensity and excited state lifetime of the donor fluorophore and, where the acceptor is a fluorophore, can produce an increase in the emission intensity of the acceptor fluorophore.

FRET is a useful tool to quantify molecular dynamics in biophysics and biochemistry, such as protein-protein interactions, protein-DNA interactions, and protein conformational changes. For monitoring the complex formation between two molecules, one of them is labeled with a donor fluorophore and the other with an acceptor fluorophore, and these fluorophore-labeled molecules are mixed. When they are dissociated, the donor fluorophore emission is detected upon the donor fluorophore excitation. On the other hand, when the donor fluorophore and acceptor fluorophore are in proximity (1-10 nm) due to the interaction of the two molecules, the acceptor fluorophore emission is predominantly observed because of the intermolecular FRET from the donor fluorophore to the acceptor fluorophore.

Fluorescent Immunoassays

The dyes described herein, e.g., the dyes of formulas (I), (II) and (III), could be used in a Fluorescent Immunoassay, abbreviated FIA. This is a biochemical technique used mainly in immunology to detect the presence of an analyte, such as an antibody or an antigen, in a sample. In this technique, an unknown amount of antigen is affixed to a surface, and then a specific antibody may be washed over the surface so that it can bind to the antigen. The antigen or the antibody may be labeled with a dye described herein, allowing it to be detected upon exposing the sample to light of the appropriate wavelength. The amount of antigen in the sample can be inferred through the magnitude of the fluorescence, and can be compared to a standard curve generated with known amounts of antigen.

Flow cytometry

The dyes described herein, e.g., the dyes of formulas (I), (II) and (III), could be used in flow cytometry, which is a technique for counting, examining, and sorting microscopic particles suspended in a stream of fluid. It allows simultaneous multiparametric analysis of the physical and/or chemical characteristics of single cells flowing through an optical and/or electronic detection apparatus. A beam of light of a single wavelength, often supplied by a laser, is directed onto a hydro-dynamically focused stream of fluid. A number of detectors are aimed at the point where the stream passes through the light beam and one or more fluorescent detectors. Each suspended particle passing through the beam scatters the light in some way, and fluorescent dyes found in the particle or attached to the particle may be excited into emitting light at a longer wavelength than the light source. This combination of scattered and fluorescent light is picked up by the detectors, and by analyzing fluctuations in brightness at each detector (one for each fluorescent emission peak) it is then possible to derive various types of information about the physical and chemical structure of each individual particle.

Fluorescence-activated cell sorting

The dyes described herein, e.g., the dyes of formulas (I), (II) and (III), could be used in fluorescence-activated cell sorting (FACS), which is a specialized type of flow cytometry. It provides a method for sorting a heterogeneous mixture of biological cells into two or more containers, one cell at a time, based upon the specific light scattering and fluorescent characteristics of each cell. It is a useful scientific instrument as it provides fast, objective and quantitative recording of fluorescent signals from individual cells as well as physical separation of cells of particular interest.

Single-molecule fluorescence

The dyes described herein, e.g., the dyes of formulas (I), (II) and (III), could be used in single-molecule fluorescence experiments. Single-molecule fluorescence has emerged as a useful tool for probing various processes which cannot be fully understood on the bulk level, using the fluorescence of a molecule to record information pertaining to its environment, structure, and position. The technique affords the ability to obtain information otherwise not available due to ensemble averaging of a bulk material. A dye described herein may provide simultaneous high photostability and fluorescence quantum yields that are useful in single-molecule experiments.

DNA sequencing

The dyes described herein, e.g., the dyes of formulas (I), (II) and (III), could be used in DNA sequencing. DNA sequencing is a biological method for determining the order of the nucleotide bases, adenine, guanine, cytosine, and thymine, in a DNA oligonucleotide. Determining DNA sequences is useful in basic research studying fundamental biological processes, as well as in applied fields such as diagnostic or forensic research. The advent of DNA sequencing has significantly accelerated biological research and discovery. The rapid speed of sequencing attained with modern DNA sequencing technology has been instrumental in the large-scale sequencing of the human genome, as well as the sequencing of many animal, plant, and microbial genomes.

DNA sequencing is often accomplished using 'dye-terminator sequencing', which allows the sequencing to be performed in a single reaction. The reaction requires a DNA template, a DNA primer, a DNA polymerase, the four deoxynucleotides, and four fluorescently-labeled dideoxynucleotides. Each of the four dideoxynucleotides is labeled with a different fluorescent dye, each fluorescing at a different wavelength. These serve as chain-terminating nucleotides, lacking the 3'-OH group required for the formation of a new phosphodiester bond. Incorporation of a dideoxynucleotide into the elongating DNA strand therefore terminates DNA strand extension, resulting in various DNA fragments of varying length. These DNA fragments are separated by size by gel electrophoresis, and the DNA sequence can be read by measuring the fluorescence of each of the separated DNA fragments.

Fluorescence in-situ hybridization

The dyes described herein, e.g., the dyes of formulas (I), (II) and (III), could be used in fluorescence in situ hybridization (FISH). FISH is a cytogenetic technique that can be used to detect and localize the presence or absence of specific DNA sequences on chromosomes. It uses fluorescently-labeled oligonucleotide probes that bind to only those parts of the chromosome with which they show a high degree of sequence similarity. Fluorescence microscopy can then be used to determine where the fluorescent probe has bound to the chromosomes. FISH is often used for finding specific features in DNA for use in genetic counseling, medicine, and species identification. FISH can also be used to detect and localize specific mRNAs within tissue samples. In this context, it can help define the spatial-temporal patterns of gene expression within cells and tissues.

Quantitative real-time polymerase chain reaction

The dyes described herein, e.g., the dyes of formulas (I), (II) and (III), could be used in quantitative real time polymerase chain reaction (qPCR). In molecular biology, qPCR is a laboratory technique based on the polymerase chain reaction, which is used to amplify and simultaneously quantify a targeted DNA molecule. It enables both detection and quantification (as absolute number of copies or relative amount when normalized to DNA input or additional normalizing genes) of a specific sequence in a DNA sample. The procedure follows the general principle of polymerase chain reaction. The amplified DNA is generally quantified as it accumulates in the reaction in real time after each amplification cycle. Two common methods of quantification are the use of fluorescent dyes that intercalate with double-stranded DNA, and the use of fluorescently-labeled DNA oligonucleotide probes that fluoresce when hybridized with a complementary DNA.

DNA Microarrays

The dyes described herein, e.g., the dyes of formulas (I), (II) and (III), could be used in DNA microarrays. A DNA microarray is a multiplex technology used in molecular biology and in medicine. It includes an arrayed series of thousands of microscopic spots of DNA oligonucleotides, each containing picomoles of a specific DNA sequence. This can be a short section of a gene or other DNA element, which is used as a probe hybridize a cDNA or cRNA sample (called a target) under high-stringency conditions. Probe-target hybridization is usually detected and quantified by fluorescence-based detection of fluorophore-labeled targets to determine relative abundance of nucleic acid sequences in the target. DNA microarrays can be used to measure changes in expression levels or to detect single nucleotide polymorphisms (SNPs).

Laser Dyes

The dyes described herein, e.g., the dyes of formulas (I), (II) and (III), could be used as laser dyes. A dye laser is a laser that uses an organic dye as the lasing medium, usually as a liquid solution. Compared to gases and most solid state lasing media, a dye can usually be used for a much wider range of wavelengths. The dye solution is typically circulated from a large reservoir and through a thin dye jet that serves as the gain medium, so that the dye molecules are used only for a short time within the pump and laser beam and have a long time to recover before they are used again.

Stains

The dyes described herein, e.g., the dyes of formulas (I), (II) and (III), could be used as stains. Staining is an auxiliary technique used in microscopy to enhance contrast in the microscopic image. In biochemistry it involves adding a class-specific (DNA, proteins, lipids, carbohydrates) dye to a substrate to qualify or quantify the presence of a specific compound. Stains and dyes are frequently used in biology and medicine to highlight structures in biological tissues for viewing, often with the aid of different microscopes. Stains may be used to define and examine bulk tissues (highlighting, for example, muscle fibers or connective tissue), cell populations (classifying different blood cells, for instance), or organelles within individual cells. Staining is not limited to biological materials, it can also be used to study the morphology of other materials for example the lamellar structures of semicrystalline polymers or the domain structures of block copolymers.

Photodynamic Therapy

The dyes described herein, e.g., the dyes of formulas (I), (II) and (III), could be used in photodynamic therapy (PDT). In PDT a targeted biomolecule, pathogen or diseased tissue is selectively stained with a dye that is relatively nontoxic in the dark. The surrounding healthy tissue contains relatively little dye. Upon illumination with light of the appropriate wavelength the PDT dye either becomes, or generates, a toxin which can inactivate the targeted substrate. Often the toxin that is generated by the PDT agent is a reactive oxygen species such as singlet oxygen, superoxide or hydrogen peroxide. Killing of the biomolecule, pathogen or diseased tissue only occurs where light and dye are simultaneously present. Thus the cleansing organs, such as the liver, spleen and kidneys, which remove dye from the blood and consequently can contain considerable quantities of dye, are not damaged because they reside in dark parts of the animal and the dye therefore does not become activated.

Inkjet applications

The dyes described herein, e.g., the dyes of formulas (I), (II) and (III), could be used in inkjet applications. Inkjet printers are important in numerous applications in the generation of documents as well as photographs. The dye is dissolved in an appropriate solvent (usually aqueous with one or more cosolvents) and applied via inkjet to a substrate in an imagewise manner. Each individual pixel of ink is applied via one or more ink nozzles.

Clothing dyes

The dyes described herein, e.g., the dyes of formulas (I), (II) and (III), could be used as clothing dyes. Imparting a specific color to a garment is typically accomplished in one of two ways. In the first procedure, a material web is produced from color-dyed threads. Each thread is pulled through a dyeing bath in a circulating fashion within a line dyeing machine. A second procedure involves dyeing of the competed garments in drum dyeing machines. Digital printing methods such as inkjet printing (see above) are becoming increasingly important for printing of textiles as well.

Compositions

A dye described herein may be part of a composition. Exemplary compositions include a dye of formula (I), (II) or (III) and an additional component such as a solvent, substrate or reagent.

The compositions described herein may include a substrate that is to be labeled with a dye described herein. Exemplary substrates include those described herein, for example, a component of interest in an assay such as a biological assay. In some embodiments, the substrate may be a biomolecule such as a polypeptide or nucleic acid. In some embodiments, the substrate may be an organic molecule described herein such as a pharmaceutical agent or an analyte.

The compositions described herein may include a solvent. The solvent may be water, buffer, or an organic solvent such as DMSO. In certain embodiments, the composition may include multiple solvents, for example in the case that the dye and the substrate are not soluble in the same solvent. In one embodiment, the substrate is a polypeptide dissolved in a buffer and the dye is dissolved in an organic solvent such as DMSO, and the two are admixed in order to label the substrate.

The compositions described herein may include a reagent. For example, a composition can include a reagent which can facilitate the reaction with one or more components in the composition, such as a reaction between a dye and a substrate. In some embodiments, the reagent is an acid, base or reducing agent. In some embodiments, the reagent is a coupling agent such as a carbodiimide, which would facilitate labeling of a substrate with a dye described herein. In some instances, a composition described herein is subjected to energy, for example, energy sufficient to cause the dye to fluoresce.

The compositions described herein can be used in the methods described herein. Exemplary methods include methods of labeling a substrate with a dye described herein.

Kits

A dye described herein can be provided in a kit. The kit includes (a) a dye described herein, e.g., a composition that includes a dye described herein, and, optionally (b) a reference standard, and (c) informational material. The informational material can be descriptive, instructional, marketing or other material that relates to the use of a dye described herein for the methods described herein.

The informational material of the kits is not limited in its form. In one embodiment, the informational material can include information about production of the dye, molecular weight of the dye, concentration, date of expiration, batch or production site information, and so forth. In one embodiment, the informational material relates to methods for labeling a substrate with the dye.

In one embodiment, the informational material can include instructions to label a substrate with a dye described herein. In another embodiment, the informational material can include instructions to label the reference standard provided in the kit.

The informational material of the kits is not limited in its form. In many cases, the informational material, e.g., instructions, is provided in printed matter, e.g., a printed text, drawing, and/or photograph, e.g., a label or printed sheet. However, the informational material can also be provided in other formats, such as Braille, computer readable material, video recording, or audio recording. In another embodiment, the informational material of the kit is contact information, e.g., a physical address, email address, website, or telephone number, where a user of the kit can obtain substantive information about a dye described herein and/or its use in the methods described herein. Of course, the informational material can also be provided in any combination of formats.

A dye described herein can be provided in any form, e.g., solution, dried or lyophilized form. It is preferred that a dye described herein be substantially pure.

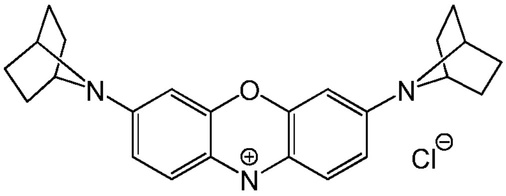
When a dye described herein is provided in a liquid solution, the liquid solution may be an aqueous solution or an organic solution. When a dye described herein is provided as a dried form, reconstitution generally is by the addition of a suitable solvent. The solvent, e.g., water, buffer or DMSO, can optionally be provided in the kit.

The kit can include one or more containers for the composition containing a dye described herein. In some embodiments, the kit contains separate containers, dividers or compartments for the composition and informational material. For example, the composition can be contained in a bottle or vial, and the informational material can be contained in a plastic sleeve or packet. In other embodiments, the separate elements of the kit are contained within a single, undivided container. For example, the composition is contained in a bottle or vial that has attached thereto the informational material in the form of a label. In some embodiments, the kit includes a plurality (e.g., a pack) of individual containers, each containing one or more samples of a dye described herein. For example, the kit includes a plurality of ampules, vials or bottles, each containing a sample of a dye described herein. The containers of the kits can be air tight, waterproof (e.g., impermeable to changes in moisture or evaporation), and/or light-tight.

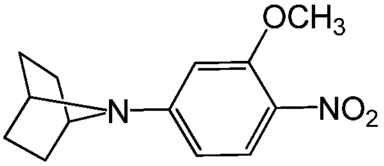
EXAMPLES

Example 1

Preparation of 3,7-bis(7'-azabicylco[2.2.1]heptyl)oxazinium chloride having the formula:



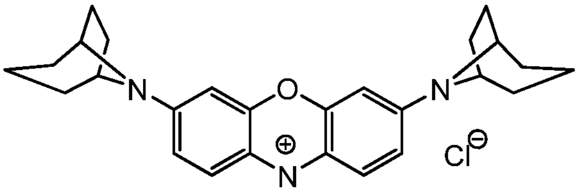
(a) A mixture of 0.5 g (5.15 mmol) of 7-azabicyclo[2.2.1]cycloheptane and 0.68 g (4 mmol) of 3-fluoro-6-nitroanisole and 0.6 g of potassium carbonate were heated in 5 mL of dry DMSO at 100 °C for 16 hrs. The solution was poured into water and a yellow solid precipitated. The solid was washed with water, air dried and purified by column chromatography using silica gel and eluting with 10% acetone in methylene chloride to give 0.85 g of a pure sample of the desired aniline. Structure and purity were established by high resolution (400 MHz) NMR spectroscopy and shown to be of the formula:



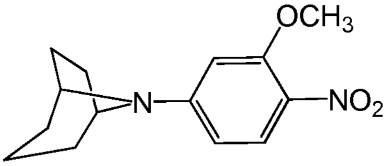
(b) A 125 mg sample of the compound prepared in step (a) was placed in 4 mL of aqueous 48% HI and heated on a steam bath 7 hrs. The colorless solution was poured onto an excess of solid potassium carbonate and the resulting mixture extracted three times with methylene chloride. The methylene chloride extracts were washed with 1N aqueous HCl and twice with brine too ensure the counter ion of the dye was chloride. After drying this solution over sodium sulfate, the solution was poured onto 50 g of silica gel and placed in a crystallizing dish which was then exposed to ambient conditions in a laboratory hood. The silica gel rapidly turned a dark blue color. After standing overnight, a portion of the colored silica gel was place on top of a silica gel column. Elution with a gradient of methanol/methylene chloride (3-7% methanol) gave 65 mg of the desired dye which NMR showed contained several contaminants. The compound was again subjected to column chromatography to afford a pure sample of the desired oxazine dye which was identified by mass spectroscopy, NMR and a characteristic blue color.

Example 2

Preparation of 3,7-bis(8'-aza[3.2.1]bicyclooctyl)oxazinium chloride having the formula:



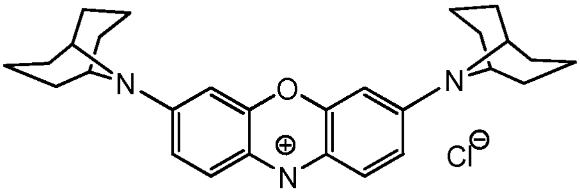
(a) A mixture of 1.2 g (11 mmol) of 8-azabicyclo[3.2.1]cyclooctane and 0.85 g (5 mmol) of 3-fluoro-6-nitroanisole and 0.7 g of potassium carbonate were heated in 5 mL of dye DMSO at 100 °C for 16 hrs. The solution was poured into water and a yellow solid precipitated. The solid was washed with water, air dried and purified by column chromatography using silica gel and eluting with 10% acetone in methylene chloride to give 1.2 g of a pure sample of the desired aniline. Structure and purity were established by high resolution (400 MHz) NMR spectroscopy and shown to be of the formula:



(b) A 120 mg sample of the compound prepared in step (a) was placed in 5 mL of aqueous 48% HI and heated on a steam bath 6 hrs. The colorless solution was poured onto an excess of solid potassium carbonate and the resulting mixture extracted three times with methylene chloride. The methylene chloride extracts were washed with 1N aqueous HCl and twice with brine too ensure the counter ion of the dye was chloride. After drying this solution over sodium sulfate, the solution was poured onto 4.5 g of silica gel and placed in a crystallizing dish which was then exposed to ambient conditions in a laboratory hood. The silica gel rapidly turned a dark blue color. After standing overnight, one third of the colored silica gel was place on top of a silica gel column. Elution with acetone/methylene chloride was followed by elution with a gradient of methanol/methylene chloride (3-6% methanol) to give the desired dye which NMR showed contained several contaminants. The compound was again subjected to column chromatography using the same conditions as used previously to afford a pure sample of the desired oxazine dye which was identified by mass spectroscopy, NMR spectroscopy and a characteristic blue color.

Example 3

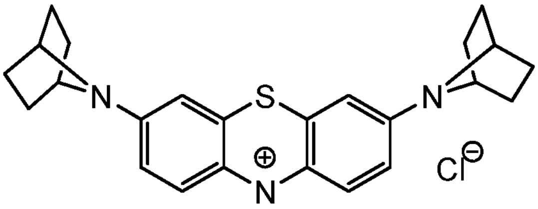
Preparation of 3,7-bis(9'-azabicyclo[3.3.1]nonyl)oxazinium chloride having the formula:



This compound was prepared using substantially the same method as used in Examples 1 and 2 with the exception that the amine used in step (a) was the hydrochloride salt of 9-azabicyclo[3.3.1]nonane.

Example 4

Preparation of the compound having the formula:

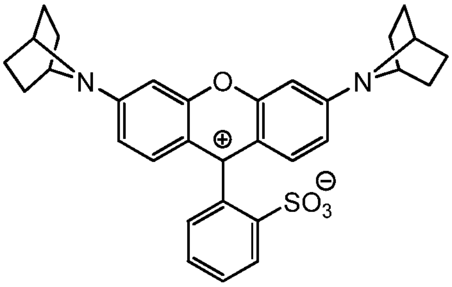


Phenothiazine (1.541 g) was taken up in 75 mL ether. To this solution was added dropwise 0.8 mL Br_2 in 11 mL glacial acetic acid over 5 minutes. A precipitate rapidly formed which was isolated by filtration. The solid was washed with ether until the washings were colorless and air dried. Yield was 3.040 g (90.1%). A 1.881 g sample of this phenothiazinium bromide was placed in a 100 mL flask and diluted with 20 mL methanol. 1.677 g 7-azabicyclo[2.2.1]heptane in 10 mL methanol was added dropwise. The reaction was stirred at room temperature overnight. UV/vis spectrum the following morning showed a strong peak at 650 nm. TLC (10% CH_3OH/CH_2Cl_2) showed cyan product mixed with a variety of contaminants. The solvent was evaporated, the reaction mixture was dissolved in CH_2Cl_2, and the solution was loaded on a silica gel column and eluted with a CH_2Cl_2 – 5% CH_3OH/CH_2Cl_2 solvent gradient. Fractions containing product were combined to yield 1.00 g solid that appeared pure by TLC. However, NMR analysis indicated that the product was contaminated with several impurities.

A pure sample of the desired compound was obtained by preparative TLC and had a wavelength absorption maximum at 652 nm in ethanol.

Example 5

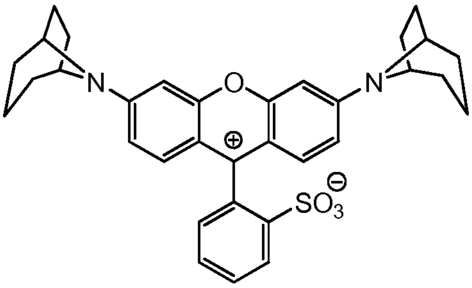
Preparation of a compound having the formula:



A 0.820 g sample of 7-azabicyclo[2.2.1]heptane and 0.855 g 3,6-dichlorosulfofluoran were heated overnight at reflux temperature in ethanol. The UV/vis spectrum of the solution showed a strong peak at 546 nm. TLC (10% CH_3OH/CH_2Cl_2) showed desired product plus impurities which included an amine-fluoran 1:1 side-product, which ran as an orange spot just below the desired product. To this solution was added .5 g of piperazine and heating was continued until no 1: 1 adduct remained and a new magenta spot that remained near the origin of the TLC plate had been formed. The magenta reaction mixture was dissolved in CH_2Cl_2 and loaded onto a silica gel column, which was eluted with a CH_2Cl_2 – 5% CH_3OH/CH_2Cl_2 solvent gradient to give a pure sample of the desired dye. The purity of the dye was determined by HPLC and high field NMR spectroscopy. The structure was further verified by high resolution mass spectroscopy and by absorption spectroscopy. The dye had an absorption maximum at 548 and extinction coefficient = 112, 000 L/mol-cm.

Example 6

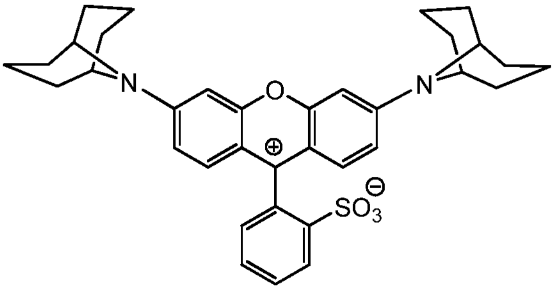
Preparation of a compound having the formula:



This compound was prepared using substantially the same method used in Example 5 except that 8-azabicyclo[3.2.1]cyclooctane was used as the secondary amine. Purity was established using HPLC, TLC and NMR spectroscopy. The structure was further verified by high resolution mass spectroscopy.

Example 7

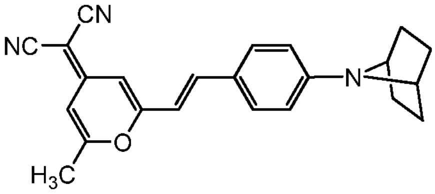
Preparation of a compound having the formula:



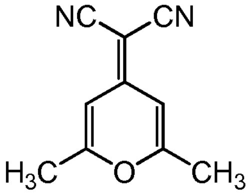
This compound was prepared using substantially the same method used in Example 5 except that 9-azabicyclo[3.3.1]nonane was the secondary amine used. Purity was established using HPLC, TLC and NMR spectroscopy. The structure was further verified by high resolution mass spectroscopy.

Example 8

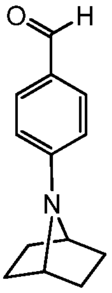
Preparation of a compound having the formula:



(a) A 10.00 g sample of 2,6-dimethyl-γ-pyrone was dissolved in 40 mL of acetic anhydride. To this solution was added 5.3 mL of malononitrile and the mixture was heated in an oil bath for 90 min at reflux temperature. Using a distillation apparatus, the volume was reduced by one half. The brown solution was added to 600 mL warm H_2O, resulting in a dark brown crystalline solid, which was collected by vacuum filtration to yield 7.901 g (57%) (NMR ($CDCl_3$) δ 6.57 (s, 2, pyrone), 2.32 (s, 6, CH_3.)) of a compound having the formula:.



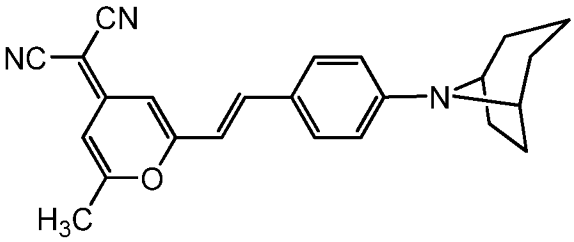
(b) A 0.521 g sample of 7-azabicyclo[2.2.1]heptane , 0.5 mL 4-fluorobenzaldehyde, 0.741 g K_2CO_3, and 8 mL dimethylformamide were placed in a 25 mL round bottom equipped with a water-cooled condenser. The reaction apparatus was placed under argon and the flask heated in an oil bath to 124°C overnight. TLC (eluted with CH_2Cl_2) indicated that not all of the 4-fluorobenzaldehyde was consumed. Heating at 152°C for five more hours did not further the progress of the reaction. The reaction mixture was diluted with 75 mL H_2O and extracted with 3 x 70 mL ethyl acetate. The organic layers were combined, dried over $MgSO_4$, and concentrated to give an orange-brown oil. The oil was chromatographed on a silica gel column eluted with 40% CH_2Cl_2 in hexanes. The desired product came off the column as a yellow band. The product was concentrated to give 1.30 g of a yellow oil (40%). NMR ($CDCl_3$) δ 9.75 (s, 1, C<u>H</u>O), 8.02 (s, 1.5, DMF), 7.70 (d, 2, aromatic), 6.90 (d, 2, aromatic), 5.30 (s, 1.75, CH_2Cl_2), 4.32 (s, 2, bridgehead $\underline{H}$s), 2.98 (s, 4.5, DMF), 2.82 (s, 4.5, DMF), 1.82 (d, 4, C2$\underline{H}$s), 1.50 (d, 4, C2$\underline{H}$s.). The product had the formula:



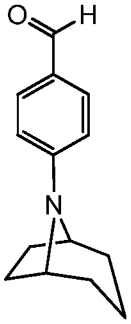
(c) A 0.378 g sample of the product of step (b), 7-azabicyclo[2.2.1]heptane-para-benzaldehyde, was taken up in a solution of 0.143 g KOH pellets (85% KOH) in 5 mL CH_3OH. To this solution was added 0.378 g dicyano-pyran, the product of step (a), and the reaction mixture was stirred overnight at 24°C. It was then heated to reflux for 1/2 hour. TLC eluted with 1% CH_3OH/CH_2Cl_2 showed fluorescent orange-red dye. The mixture was diluted with 80 mL H_2O and extracted with 3 x 75 mL CH_2Cl_2. Organic layers were then dried over $MgSO_4$, concentrated, and chromatographed on a silica gel column and eluted with 1% CH_3OH/CH_2Cl_2. Product-containing fractions showed evidence of aldehyde contamination by NMR analysis. The material was again subjected to column chromatography using neutral alumina as solid phase and eluting with 40% hexanes/CH_2Cl_2 to yield 94 mg of dye. The dye was dissolved in 5 mL CH_2Cl_2, and 15 mL hexanes were added to the mixture. The CH_2Cl_2 was then gently evaporated via rotary evaporation, and the hexane was concentrated to 10 mL to give a pure sample of the desired dye as red crystals. Yield was 11 mg. NMR ($CDCl_3$) δ 7.41-7.33 (2d, 3, aromatic and alkenyl), 6.87 (d, 2, aromatic), 6.61 (s, 1), 6.53-6.47 (2d, 2), 4.22 (s, 2, bridgehead $\underline{H}$s), 2.39 (s, 3, $C\underline{H}_3$), 1.81 (d, 4, C2$\underline{H}$s), 1.55 (s, 4, unknown), 1.47 (d, 4, C2$\underline{H}$s.) Dye absorption maximum was 435 nm; fluorescence emission maximum was 620 nm in methanol. Fluorescence lifetime in methanol was 1.24 nsec at RT.

Example 9

Preparation of a compound having the formula:



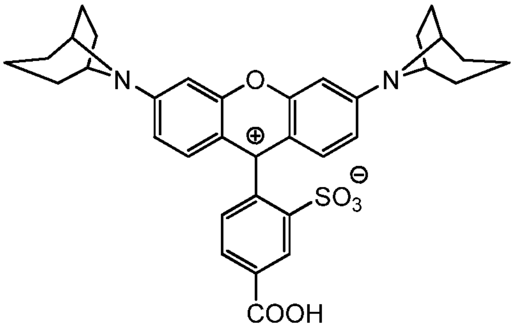
This compound was made using substantially the same method as used in Example 8 with the exception that the aldehyde used in step (c) was a compound of formula:



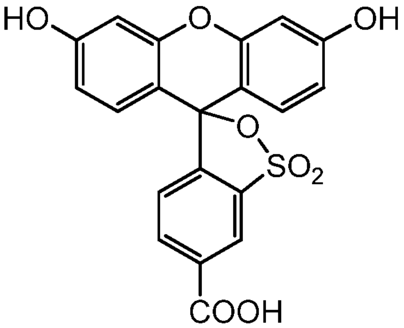
The desired compound was prepared in 60% yield (0.75 g). NMR ($CDCl_3$) δ 9.72 (s, 1, C<u>H</u>O), 7.75 (d, 2, aromatic), 6.78 (d, 2, aromatic), 4.32 (s, 2, bridgehead <u>H</u>s), 2.12 (m, 2, C2<u>H</u>s), 1.87 (m, 4, C2<u>H</u>s), 1.50 (m, 1, C2<u>H</u>s), 1.39 (d, 2, C2<u>H</u>s), 1.22 (d, 1, C2<u>H</u>s.) The absorption maximum of the dye in methanol was 462 nm and the fluorescence maximum in the same solvent was 615 nm. The dye has a fluorescence lifetime of 1.33 nsec in methanol at RT.

Example 10

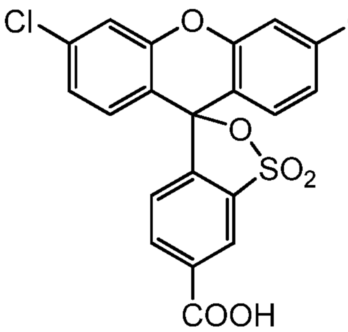
Preparation of the compound having the formula:



(a) A 28 g (123mmol) sample of the monosodium salt of sulfoterephthalic acid was heated at reflux temperature for 48 hr in 150 mL of thionyl chloride under an argon atmosphere. The solution was filtered to remove solid sodium chloride and the solvent was removed under vacuum to give a colorless oil. This oil was stirred with cold water briefly and then heated on a steam bath for 2 hr. The water was removed under heat and vacuum to give a white solid in 89% yield. This material was reacted with 27 g (200 mmol) of resorcinol in 200 mL of methanesulfonic acid at 90 °C for 24 hr. The mixture was cooled, poured into ice water. The resulting solid was collected by filtration, was washed with water and air dried to give a compound of the formula:



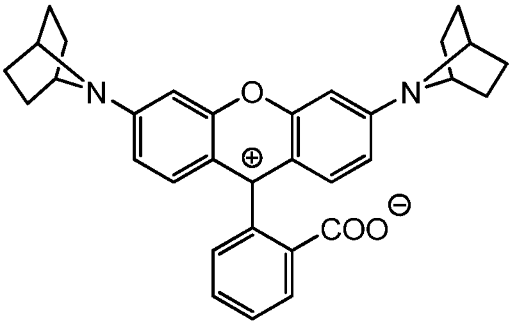
(b) One equivalent of the compound prepared in (a) was placed in acetonitrile. To this mixture was added 12 equivalents of phosphorus pentachloride. The mixture was heated at reflux temperature for 2 hr whereupon all of the sulfofluorescein dye had dissolved. The solution was cooled and poured into ice-water to give a yellow precipitate of formula:



(c) A sample of the dichlorosulfofluoran prepared in step (b) was treated with excess 8-azabicyclo[3.2.1]octane in DMSO and heated on steam bath for 30 min. The resulting magenta solution was poured into water and extracted with methylene chloride. The organic solution of dye was dried over sodium sulfate and applied to a silica gel coated glass plate. The plate was developed with a solvent mixture of methanol-methylene chloride. The isolated magenta dye was determined to be pure by TLC. The targeted sulforhodamine-4'carboxylic acid dye has an absorption maximum at 568 nm in methanol, an emission maximum at 587 nm and a fluorescence lifetime of 3.5 nsec at room temperature in the same solvent.

Example 11

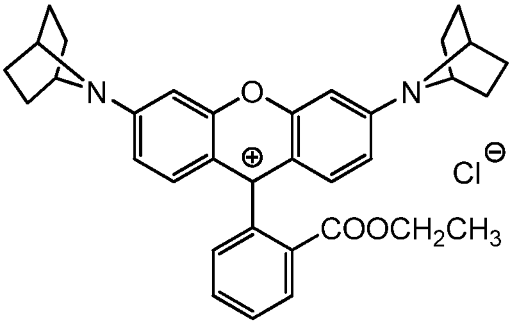
Preparation of a compound of the formula:



(a) A 0.37 g (1 mmol) sample of 3,6-dichlorofluoran was mixed with 0.4 g (4 mmol) of 7-azabicyclo[2.2.1]heptane, 0.5 g of anhydrous zinc chloride and 0.15 g of zinc oxide in a high pressure reactor and heated to 190 °C for 12 hr. Upon cooling to room temperature, the contents of the bomb were extracted with methylene chloride to give a red solution. The sample was subjected to purification using flash chromatography with silica gel solid phase and 5% methanol/methylene chloride as eluting solvent. TLC analysis indicated the isolated dye was not pure. The sample was further purified by preparative TLC to give 0.2 g of pure product (40% yield). In methanol the dye has an absorption maximum at 547 nm, an emission maximum at 572 nm and a fluorescence lifetime of 4.02 nsec.

Example 12

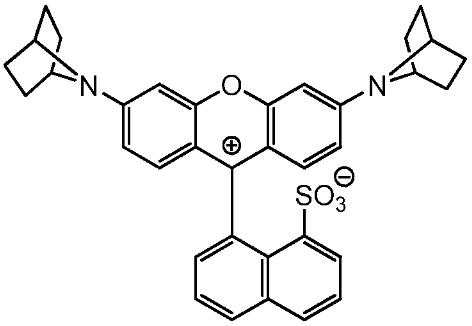
Preparation of a compound of the formula:



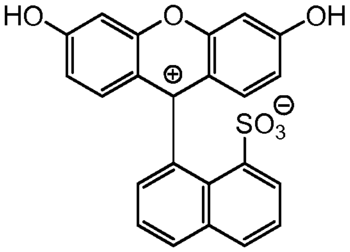
(a) Anhydrous HCl gas was bubbled through a solution consisting of 40 mg (0.08 mmol) of the compound prepared in Example 11 and 15 mL of anhydrous ethanol for 30 min. The resulting red solution was heated at reflux temperature for 6 hr. The solvent was removed using a rotary-evaporator under vacuum to leave a red/magenta solid. The dye was purified using flash chromatography with silica gel as solid phase and 10% methanol/methylene chloride as eluent. The desired dye was further purified using preparative TLC using silica gel coated plates and dichloromethane/acetone (2:1) as eluent. The structure of the dye was verified using NMR analysis which indicated a small amount of impurity remained in the sample. Absorption maximum was at 549 nm, emission maximum was at 573 nm and fluorescent lifetime was measured to be 4.05 nsec in methanol at room temperature.

Example 13

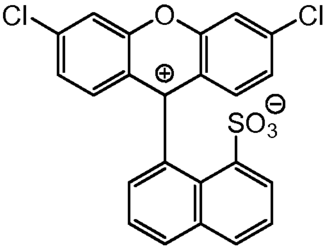
Preparation of a compound of the formula:



(a) A 0.6 g (2.56 mmol) sample of 1 –sulfo-8-naphthoic anhydride, made using the method described in the journal Youji Huaxue (1995) 15, 433-440, was heated to 140 °C for 5 hr with 1 g (10.3 mmol) of resorcinol. The melt was cooled to room temperature and extracted into aqueous 1N sodium hydroxide. The solution had a bright fluorescein-like color. The solution was neutralized with 6N HCl to give a yellow/brown precipitate which was washed with water and dried in the air to give a compound of formula:



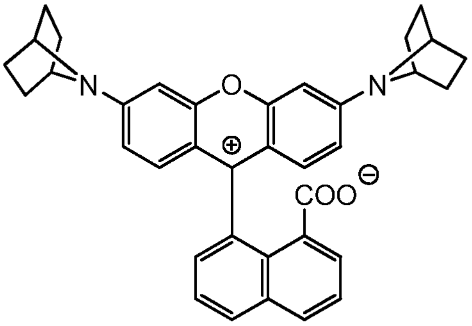
(b) A 0.1 g (0.24 mmol) sample of the compound made in (a) was reacted with 0.6 g (2.9 mmol) of phosphorus pentachloride in 3 mL of dry DMF at a temperature of 87 °C for 2 hr. The solution was cooled to room temperature and poured into ice water to give a yellow/brown solid which was dried in the air. NMR analysis indicated the product was of the formula:



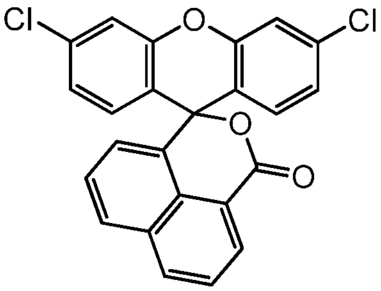
(c) A mixture of 23 mg (0.05 mmol) of the dichloro compound prepared in step (b) was heated on a steam bath with 200 mg (2.0 mmol) of 7-azabicyclo[2.2.1]heptane in 5 mL of DMSO for 1 hr. Cold water (40 mL) was added to the cooled red mixture. The aqueous mixture was extracted with 50 mL of methylene chloride and the organic layer dried over sodium sulfate. The targeted dye was purified by flash chromatography using silica gel as solid phase and 5% methanol /methylene chloride as solvent to give 18 mg of pure dye (63%). The dye has an absorption maximum at 550 nm, an emission maximum at 575 nm and a fluorescence lifetime of 4.59 nsec in methanol at room temperature.

Example 14

Preparation of a compound of the formula:



(a) A 0.15 g (0.39 mmol) sample of 3',6'-dihydroxyspiro[1H,3H-naphtho[1,8-cd]pyran-1,9'-[9H]xanthen]-3-one, made according to the method described in the Journal of General Chemistry of the USSR (1959) 29, 241-3 (English version 2207-2209), was heated at reflux temperature in 6 mL of thionyl chloride for 3 hr. The solution was added to ice water and a solid formed. The solid was isolated by filtration, washed with 1N sodium hydroxide, then with water and air dried to give 0.14 grams of an impure sample of the compound of the formula:

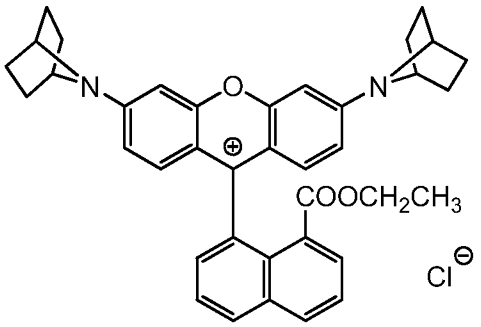


This material was used without further purification.

(b) In a 50 mL high pressure vessel was placed 0.42g (1 mmol) of the dichloro compound prepared in step (a), 0.45 g of anhydrous zinc chloride and 0.15 g of anhydrous zinc oxide. The mixture was heated to 180 °C for 6 hr. The mixture was cooled to room temperature and the contents of the vessel taken up in methylene chloride. The mixture was purified using flash chromatography with silica gel solid phase and 10% acetone/methylene chloride as eluent to give 10 mg of pure dye having an absorption maximum at 555 nm, an emission maximum at 582 nm and a fluorescence lifetime of 4.18 nsec in methanol at room temperature.

Example 15

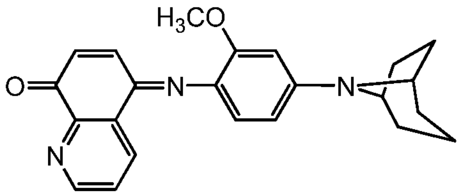
Preparation of a compound of the formula:



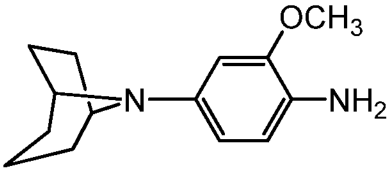
The dye was prepared using the same method used in Example 12 with the exception that the starting dye was the compound prepared in Example 14. The targeted dye has an absorption maximum at 558 nm, an emission maximum at 582 nm and a fluorescence lifetime of 4.22 nsec in methanol at room temperature.

Example 16

Preparation of a compound of the formula:



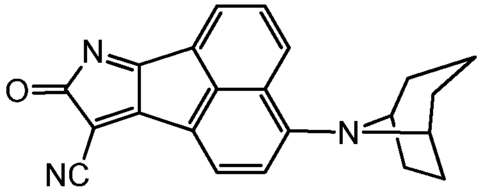
(a) The nitro compound prepared in step (a) of Example 2 was reduced in a hydrogen atmosphere at 40 psi using 10% Pd/C catalyst in ethanol. The solution was filtered through Celite to remove the catalyst. Removal of the solvent on a rotary evaporator gave a compound of the formula:



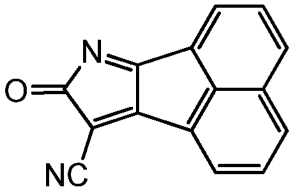
(b) The phenylenediamine prepared in step (a) was treated with an excess of 8-hydroxyquinoline and ammonium persulfate in water at room temperature. After approximately 5 min the blue solution was extracted with methylene chloride. The organic layer was concentrated and the targeted dye was purified by preparative TLC. The desired dye has an absorption maximum at 654 nm in methylene chloride. Addition of $CuBr_2$ to a methylene chloride solution of the title dye gave a copper(II) chelate compound having an absorption maximum at 714 nm. Similarly, the nickel(II) chelate of the title dye was observed to have an absorption maximum at 735 nm.

Example 17

Preparation of a company having the formula:



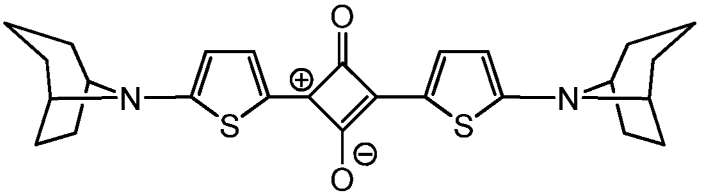
A sample of the compound having the formula:



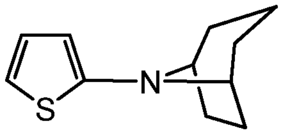
which was synthesized using the method described in Chemical Communications of the Royal Society of Chemistry (2005) 239-241, was placed in acetonitrile and treated with an equivalent amount of 8-azabicyclo[3.2.1]octane at room temperature. A purple color formed rapidly. The solvent was evaporated and the residue purified by preparative TLC using silica gel as solid phase and acetone/methylene chloride as eluent to give the desired dye. The dye has an absorption maximum at 594 nm, an emission maximum at 610 nm and a fluorescence lifetime of 0.5nsec in methylene chloride at room temperature.

Example 18

Preparation of the compound having the formula:



(a) A 0.3 g sample of 2-mercaptothiophene was heated at reflux temperature with 0.4 g of 8-azabicyclo[3.2.1]octane in toluene for 3 hr. The solvent was removed and the residue subjected to flash chromatography using silica gel as solid phase and hexane/ethyl acetate as eluent to give a colorless oil. NMR analysis indicated some impurities were present in the compound of the formula:

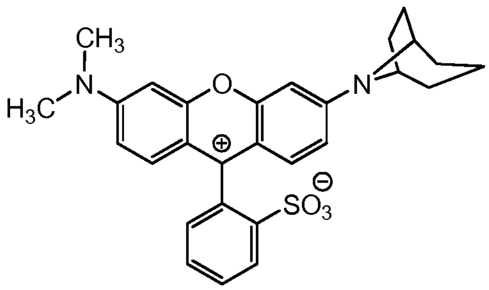


This compound was used without further purification.

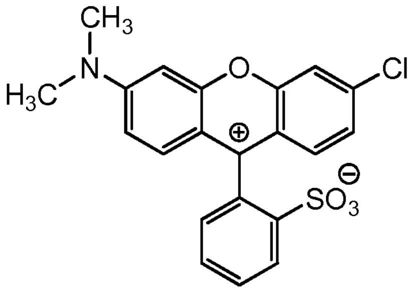
(b) An excess of the compound prepared in step (a) was dissolved in a mixture of toluene and n-butanol and treated with a small amount of squaric acid. The solution was heated at reflux temperature for 5 min. The resulting blue solution was evaporated to dryness and the residue purified by preparative TLC. The targeted dye has an absorption maximum at 671 nm, an emission maximum at 682 nm and a fluorescence lifetime of 0.36 nsec in acetone at room temperature.

Example 19

Preparation of the compound of the formula:



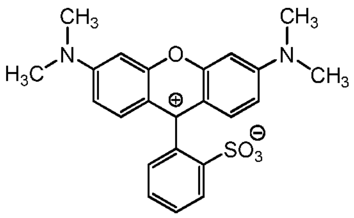
(a) One equivalent of 3,6-dichlorosulfofluoran and one equivalent of dimethylamine were heated in ethanol at reflux temperature for 1 hr. The resulting orange solution was cooled and the solvent remover. TLC analysis indicated that small amounts of starting material and the magenta dye tetramethylsulforhodamine contaminated the desired 1:1 adduct. The sample was subjected to column chromatography using silica gel and 5-7% methanol/methylene chloride as eluent. The desired dye is an orange solid having an absorption maximum at 490 nm and a less intense band at 530 nm in ethanol, an emission maximum at 572 nm with a shoulder at 604 nm and a fluorescence lifetime of 0.08 nsec. Unlike highly fluorescent 2:1 adducts, this 1:1 adduct does not appear to be fluorescent to the eye when in solution. This material had the formula:



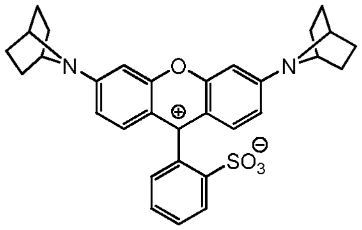
(b) A sample of the compound prepared in step (a) was treated with excess 8-azabicyclo[3.2.1]octane in DMSO and heated on a steam bath for 1 hr during which time the solution turned from orange to magenta. The mixture was treated with water and the dye extracted into methylene chloride. The dye was purified by TLC using silica gel as solid phase and methanol/methylene chloride as eluent. In methanol the targeted dye has an absorption maximum at 558 nm, an emission maximum at 576 nm and a fluorescence lifetime of 3.72 nsec.

Example 20

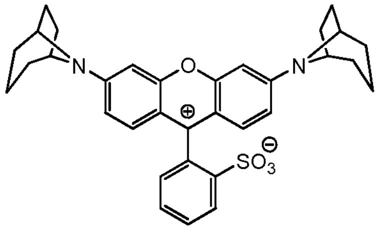
Compounds of the following formulae:



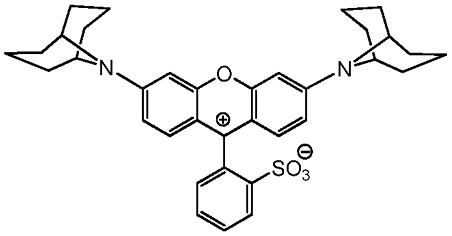
TMSR (control)



221SR



321SR



331SR

were dissolved in methanol and their photophysical properties measured at 20°C and 60°C. Table 1 contains the absorption maximum, fluorescence maximum and the fluorescence quantum yields, Φ, at both temperatures.

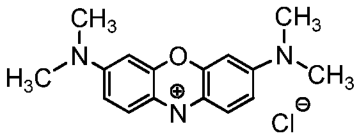
Table 1

Dye	**λ_{max} abs**	**λ_{max} fl**	**Φ 20°C**	**Φ 60°C**
TMSR (control)	550 nm	568 nm	0.65	0.38
221SR	548 nm	567 nm	0.95	0.95
321SR	565 nm	585 nm	0.89	0.78
331SR	571 nm	592 nm	0.47	0.33

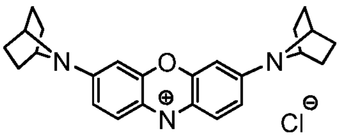
Two of the new dyes of this invention, 221SR and 321SR, have improved fluorescence quantum yields as compared to the control TMSR. Moreover, all of the new dyes are less adversely affected by a temperature increase from 20 to 60 °C.

Example 21

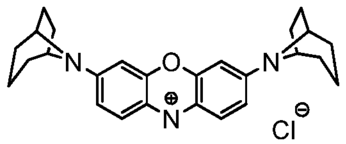
Compounds of the formulae including control fluorophores Oxazine 1 and JulOx:



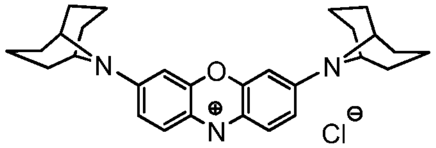
Oxazine 1 (control)



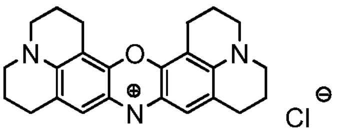
221Ox



321Ox



331Ox



JulOx (control)

were dissolved in ethanol and their photophysical properties measured at 20°C and 60°C. Table 2 contains the absorption maximum, fluorescence maximum and the fluorescence quantum yields, Φ, at both temperatures.

Table 2

Dye	**λ_{max} abs**	**λ_{max} fl**	**Φ 20°C**	**Φ 60°C**
Oxazine 1 (control)	647 nm	693 nm	0.11	0.02
221Ox	636 nm	650 nm	0.69	0.66
321Ox	658 nm	675 nm	0.45	0.39
331Ox	666 nm	685 nm	0.07	0.03
JulOx (control)	667 nm	690 nm	0.37	0.36

These data demonstrate that 221Ox has a significantly higher fluorescent quantum yield than control dyes Oxazine 1 and JulOx whereas 321Ox has an emission efficiency that is equivalent to the JulOx control in spite of being an untethered donor as compared to the tethered JulOx control.

Example 22

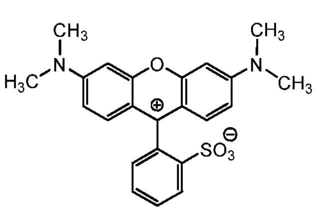
The compounds 221Ox, 321Ox and JulOx having formulae depicted in Example 21 were dissolved in methanol and two drops of each were placed in parallel columns on a silica gel TLC plate. One column was protected from exposure to light by a foil cover while the other was exposed to ambient light provided by fluorescent room lighting at bench top level. Control dye JulOx was deposited at two levels, one relatively dilute and one more concentrated. After 63 hours of exposure to room light the spots of JulOx, which were originally cyan in color, had turned into a greenish color whereas the 321Ox and 221Ox appeared to retain their original color. The optical density of the spots in the red spectral region, the region these dyes have maximum absorption, was measured using a Macbeth TD-504 Densitometer and compared to the corresponding spots that had been protected from the light. The following changes in optical density were observed:

Dye	**OD_{Dark}**	**OD_{Light}**	**% Decrease in OD**
JulOx dilute (control)	0.60	0.10	83
JulOx concentrated (control)	1.13	0.29	74
321Oxazine	0.65	0.36	45
221Oxazine	0.39	0.30	23

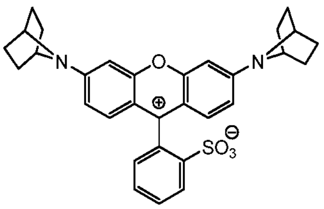
Thus the two dyes of this invention are considerably more stable to light-induced degradation under the conditions of the experiment than is the known laser dye JulOx.

Example 23

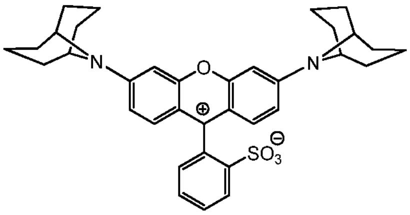
Compounds of the formulae:



TMSR (control)



221SR

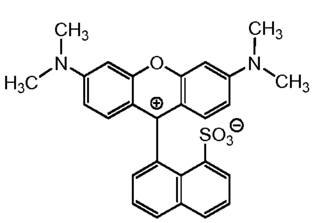


331SR

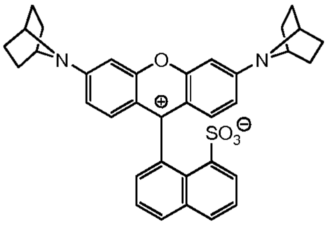
were separately dissolved in trifluoroethanol containing 1% aqueous saturated potassium carbonate and placed in a 1 cm square cuvette. The optical density at 514 nm was adjusted so that all samples had the same absorbance. Argon was bubbled through the solution for 10 min. and then the solution was kept under an argon atmosphere for the remainder of the experiment. A 1.7 W beam of 514 nm light from a CW laser was directed to the surface of each cuvette and the optical density was measured as a function of time. The results of this experiment are shown in Figures 1, 2 and 3 and demonstrate that the two dyes of the present invention are significantly more resistant to light-induced degradation than the control dye TMSR.

Example 24

Compounds of the formulae:



TMSNR (control)

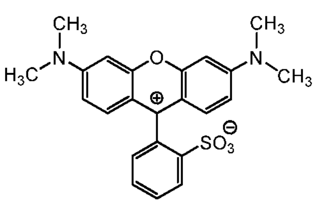


221SNR

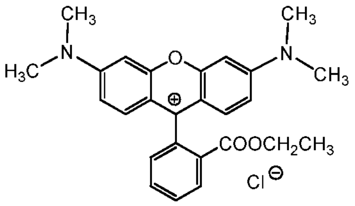
were subjected to the same photolysis conditions described in Example 23. As shown in Figure 4, the dye having the donor group of the present invention is significantly more stable to laser-induced photodegradation.

Example 25

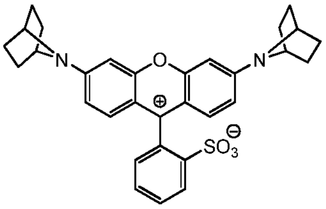
Compounds of the formulae:



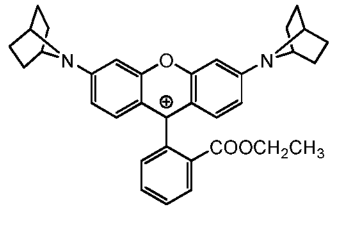
TMSR (control)



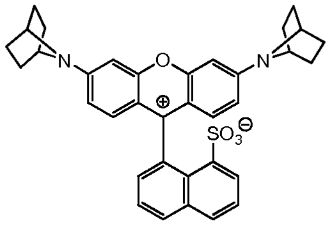
TMRE (control)



221SR



221RE



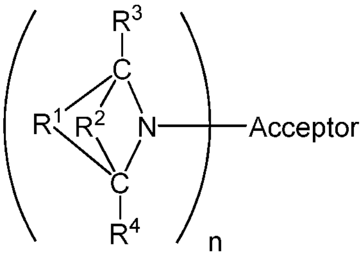
221SNR

were dissolved in trifluoroethanol containing 1% saturated aqueous potassium carbonate and the optical density was adjusted to about 1.5 for each sample. The solutions were placed under argon as in Example D. Each sample was placed in front of a high intensity green emitting bank of LED's and the optical density was measured as a function of time. The results of this experiment are shown in Figure 5 and demonstrate that the dyes containing the donors of the present invention have superior resistance to green light-induced degradation as compared to the two tetramethylrhodamine control compounds. Because the visible absorption bands of TMSR and 221SR are nearly identical a comparison of these rates of photodegradation is especially noteworthy.

Having thus described several aspects of at least one embodiment of this invention, it is to be appreciated various alterations, modifications, and improvements will readily occur to those skilled in the art. Such alterations, modifications, and improvements are intended to be part of this disclosure, and are intended to be within the spirit and scope of the invention. Accordingly, the foregoing description and drawings are by way of example only.

WHAT IS CLAIMED IS:

1. A donor-acceptor dye of formula (I),



Formula (I)

wherein n is 1, 2 or 3;

each R^1 and R^2 is independently C_{2-4} alkylenyl, C_{2-4} heteroalkylenyl, C_{2-4} alkenylenyl, arylenyl, or heteroarylenyl; each of which is optionally substituted with 1-4 R^5;

each R^3 and R^4 is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, -CN, -C(=O)R^a, -C(=O)N(R^b)$_2$, or -C(=O)OR^c; each of which is optionally substituted with 1-4 R^6;

each R^5 and R^6 is independently oxo, halo, -NO_2, -CN, -C(=O)R^a, -C(=O)N(R^b)$_2$, -C(=O)OR^c, -OR^d, -NR^e_2 , -SR^f or -SO_2R^g;

each R^a is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino or halo;

each R^b is independently hydrogen, hydroxy, alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, or alkylamino;

each R^c is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, or alkylamino;

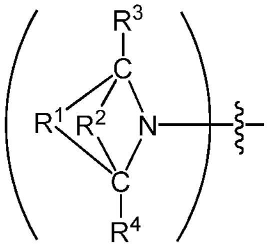
each R^d is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, or alkylamino;

each R^e is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, or alkylamino;

each R^f is independently hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl alkylhydroxy, haloalkyl, or alkylamino;

each R^g is independently hydrogen, hydroxy, halo, alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl alkylhydroxy, haloalkyl, alkylamino or halo;

wherein



is a donor; and

the acceptor is a moiety that can accept electron density from the donor moiety under the influence of light of the appropriate wavelength.

2. The dye of claim 1, wherein the compound is a closed shell compound.

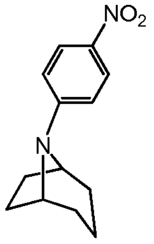
3. The dye of claim 1, wherein the acceptor moiety comprises at least ten contiguous pi-bonded carbon, nitrogen, oxygen and sulfur atoms.

4. The dye of claim 3, wherein the acceptor moiety comprises at least twelve contiguous pi-bonded carbon, nitrogen, oxygen and sulfur atoms.

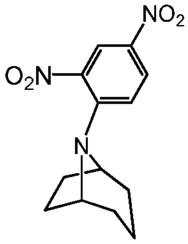
5. The dye of claim 3, wherein the acceptor moiety comprises at least thirteen contiguous pi-bonded carbon, nitrogen, oxygen and sulfur atoms.

6. The dye of claim 3, wherein the acceptor moiety comprises at least fourteen contiguous pi-bonded carbon, nitrogen, oxygen and sulfur atoms.

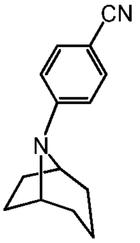
7. The dye of claim 1, wherein the dye does not have the following formula:



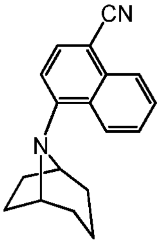
8. The dye of claim 1, wherein the dye does not have the following formula:



9. The dye of claim 1, wherein the dye does not have the following formula:



10. The dye of claim 1, wherein the dye does not have the following formula:



11. The dye of claim 1, wherein the compound is a fluorescent compound.

12. The dye of claim 1, wherein the compound is a zwitterion.

13. The dye of claim 1, wherein the compound is a salt.

14. The dye of claim 1, wherein R^1 is C_{2-4} alkylenyl, C_{2-4} heteroalkylenyl, C_{2-4} alkenylenyl, arylenyl, or heteroarylenyl; each of which is optionally substituted with 1-4 R^5.

15. The dye of claim 14, wherein R^1 is $C_{2\text{-}4}$ alkylenyl.

16. The dye of claim 14, wherein R^1 is $C_{2\text{-}4}$ alkylenyl substituted with 1-4 R^5.

17. The dye of claim 16, wherein R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.

18. The dye of claim 16, wherein R^1 is $C_{2\text{-}4}$ alkylenyl substituted with 1 R^5.

19. The dye of claim 18, wherein R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.

20. The dye of claim 19, wherein R^5 is $-OR^d$.

21. The dye of claim 19, wherein R^5 is oxo.

22. The dye of claim 14, wherein R^1 is $C_{2\text{-}4}$ heteroalkylenyl.

23. The dye of claim 14, wherein R^1 is $C_{2\text{-}4}$ heteroalkylenyl substituted with 1-4 R^5.

24. The dye of claim 23, wherein R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.

25. The dye of claim 23, wherein R^1 is $C_{2\text{-}4}$ heteroalkylenyl substituted with 1 R^5.

26. The dye of claim 25, wherein R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.

27. The dye of claim 14, wherein R^1 is $C_{2\text{-}4}$ alkenylenyl.

28. The dye of claim 14, wherein R^1 is C_{2-4} alkenylenyl substituted with 1-4 R^5.

29. The dye of claim 28, wherein R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.

30. The dye of claim 28, wherein R^1 is C_{2-4} alkenylenyl substituted with 1 R^5.

31. The dye of claim 30, wherein R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.

32. The dye of claim 14, wherein R^1 is arylenyl.

33. The dye of claim 14, wherein R^1 is arylenyl substituted with 1-4 R^5.

34. The dye of claim 33, wherein R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.

35. The dye of claim 33, wherein R^1 is arylenyl substituted with 1 R^5.

36. The dye of claim 35, wherein R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.

37. The dye of claim 14, wherein R^1 is unsubstituted.

38. The dye of claim 1, wherein R^2 is C_{2-4} alkylenyl, C_{2-4} heteroalkylenyl, C_{2-4} alkenylenyl, arylenyl, or heteroarylenyl, each of which is optionally substituted with 1-4 R^5.

39. The dye of claim 38, wherein R^2 is C_{2-4} alkylenyl.

40. The dye of claim 38, wherein R^2 is C_{2-4} alkylenyl substituted with 1-4 R^5.

41. The dye of claim 40, wherein R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, -$C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, or $-SR^f$ or $-SO_2R^g$.

42. The dye of claim 40, wherein R^2 is C_{2-4} alkylenyl substituted with 1 R^5.

43. The dye of claim 42, wherein R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, -$C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.

44. The dye of claim 43, wherein R^5 is $-OR^d$.

45. The dye of claim 43, wherein R^5 is oxo.

46. The dye of claim 38, wherein R^2 is C_{2-4} heteroalkylenyl.

47. The dye of claim 38, wherein R^2 is C_{2-4} heteroalkylenyl substituted with 1-4 R^5.

48. The dye of claim 47, wherein R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, -$C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.

49. The dye of claim 47, wherein R^2 is C_{2-4} heteroalkylenyl substituted with 1 R^5.

50. The dye of claim 49, wherein R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, -$C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.

51. The dye of claim 38, wherein R^2 is C_{2-4} alkenylenyl.

52. The dye of claim 38, wherein R^2 is C_{2-4} alkenylenyl substituted with 1-4 R^5.

53. The dye of claim 52, wherein R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.

54. The dye of claim 52, wherein R^2 is $C_{2\text{-}4}$ alkenylenyl substituted with 1 R^5.

55. The dye of claim 54, wherein R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.

56. The dye of claim 38, wherein R^2 is arylenyl.

57. The dye of claim 38, wherein R^2 is arylenyl substituted with 1-4 R^5.

58. The dye of claim 57, wherein R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.

59. The dye of claim 57, wherein R^2 is arylenyl substituted with 1 R^5.

60. The dye of claim 59, wherein R^5 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.

61. The dye of claim 38, wherein R^2 is unsubstituted.

62. The dye of claim 1, wherein R^3 is hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, or $-C(=O)OR^c$; each of which is optionally substituted with 1-4 R^6.

63. The dye of claim 62, wherein R^3 is $-C(=O)OR^c$.

64. The dye of claim 62, wherein R^3 is alkyl.

65. The dye of claim 62, wherein R^3 is alkyl substituted with 1-4 R^6.

66. The dye of claim 65, wherein R^6 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.

67. The dye of claim 1, wherein R^4 is hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, or $-C(=O)OR^c$; each of which is optionally substituted with 1-4 R^6.

68. The dye of claim 67, wherein R^4 is $-C(=O)OR^c$.

69. The dye of claim 67, wherein R^4 is alkyl.

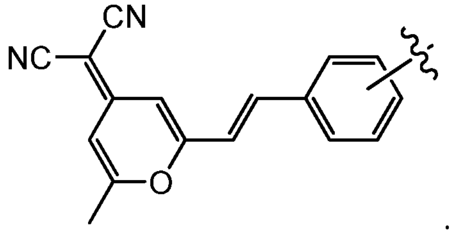
70. The dye of claim 67, wherein R^4 is alkyl substituted with 1-4 R^6.

71. The dye of claim 70, wherein R^6 is oxo, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.

72. The dye of claim 1, wherein the donor is selected from 7-azabicyclo[2.2.1]heptyl, 7-azabicyclo[2.2.1]heptenyl, 7-azabicyclo[2.2.1]heptadienyl, 8-azabicyclo[3.2.1]octyl, 8-azabicyclo[3.2.1]octenyl, 8-azabicyclo[3.2.1]octadienyl, 9-azabicyclo[3.3.1]nonyl, 9-azabicyclo[3.3.1]nonenyl, 9-azabicyclo[3.3.1]nonadienyl, 7-aza-[b]-benzobicyclo[2.2.1]heptyl, and 7-aza-[b,e]-dibenzobicyclo[2.2.1]heptyl.

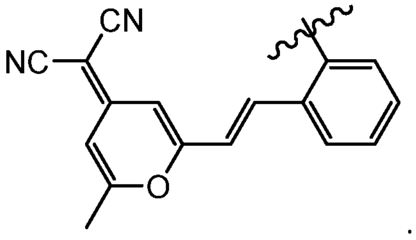
73. The dye of claim 1, wherein n is 1.

74. The dye of claim 73, wherein the acceptor is:

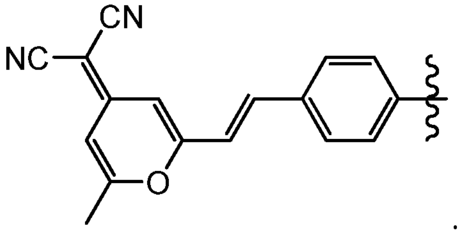


.

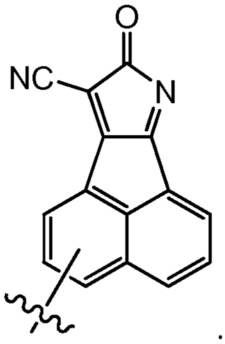
75. The dye of claim 74, wherein the acceptor is:



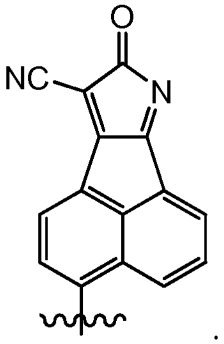
76. The dye of claim 74, wherein the acceptor is:



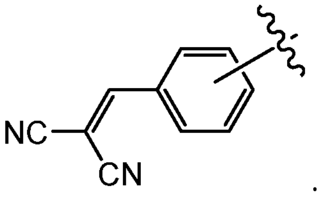
77. The dye of claim 73, wherein the acceptor is:



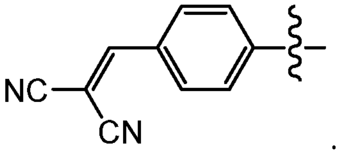
78. The dye of claim 77, wherein the acceptor is:



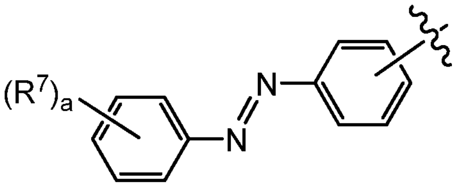
79. The dye of claim 73, wherein the acceptor is:



80. The dye of claim 79, wherein the acceptor is:



81. The dye of claim 73, wherein the acceptor is:



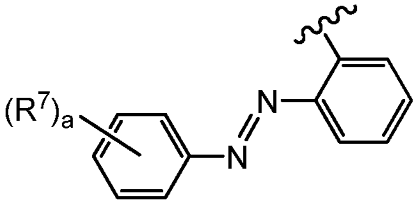
wherein:

a is 0, 1, 2, 3, 4 or 5;

each R^7 is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$, each of which is optionally substituted with 1-4 R^8; and

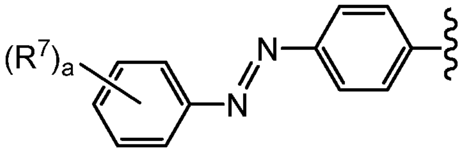
each R^8 is independently halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.

82. The dye of claim 81, wherein the acceptor is:



.

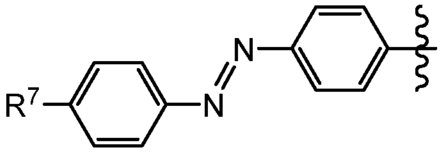
83. The dye of claim 81, wherein the acceptor is:



.

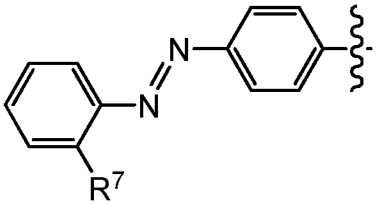
84. The dye of claim 81, wherein a is 1.

85. The dye of claim 84, wherein the acceptor is:



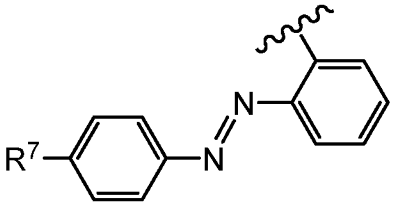
.

86. The dye of claim 84, wherein the acceptor is:



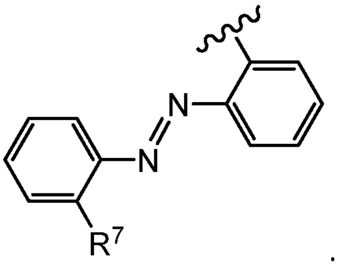
.

87. The dye of claim 84, wherein the acceptor is:

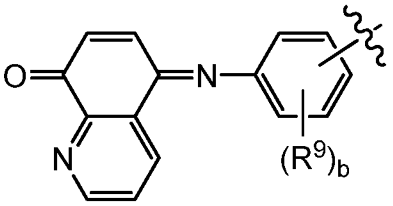


.

88. The dye of claim 84, wherein the acceptor is:



89. The dye of claim 73, wherein the acceptor is:



wherein:

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

each R^9 is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e_2$, $-SR^f$ or $-SO_2R^g$, each of which is optionally substituted with 1-4 R^{10}; and

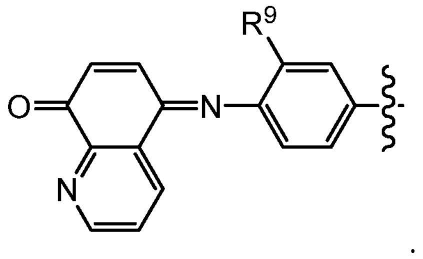
each R^{10} is independently halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e_2$, $-SR^f$ or $-SO_2R^g$.

90. The dye of claim 89, wherein b is 1.

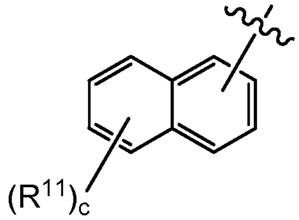
91. The dye of claim 90, wherein R^9 is $-OR^d$.

92. The dye of claim 91, wherein R^d is alkyl.

93. The dye of claim 90, wherein the acceptor is:



94. The dye of claim 73, wherein the acceptor is:



wherein:

c is 1, 2 or 3;

each R^{11} is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$, each of which is optionally substituted with 1-4 R^{12}; and

each R^{12} is independently halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.

95. The dye of claim 94, wherein c is 1.

96. The dye of claim 95, wherein R^{11} is $-C(=O)R^a$.

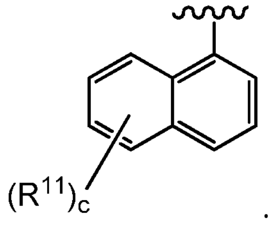
97. The dye of claim 96, wherein R^a is alkenyl.

98. The dye of claim 95, wherein R^{11} is $-SO_2R^g$.

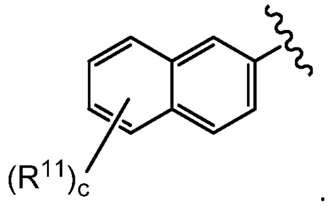
99. The dye of claim 98, wherein R^g is hydroxyl.

100. The dye of claim 98, wherein R^g is halo.

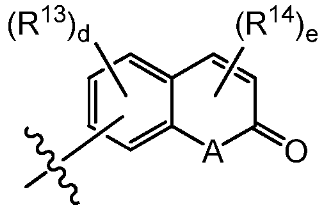
101. The dye of claim 94, wherein the acceptor is:



102. The dye of claim 94, wherein the acceptor is:



103. The dye of claim 73, wherein the acceptor is:



wherein:

d is 0, 1 or 2;

e is 0, 1 or 2;

A is O or NH;

each R^{13} and R^{14} is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e_2$, $-SR^f$ or $-SO_2R^g$, each of which is optionally substituted with 1-4 R^{15}; and

each R^{15} is independently halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e_2$, $-SR^f$ or $-SO_2R^g$.

104. The dye of claim 103, wherein R^{13} is not positioned ortho to the donor moiety.

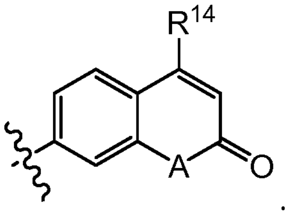
105. The dye of claim 103, wherein A is O.

106. The dye of claim 103, wherein A is NH.

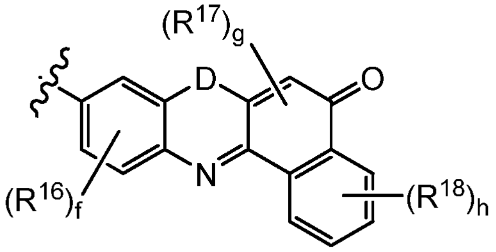
107. The dye of claim 103, wherein e is 1.

108. The dye of claim 107, wherein R^{14} is alkyl.

109. The dye of claim 107, wherein the acceptor is:



110. The dye of claim 73, wherein the acceptor is:



wherein:

f is 0, 1, 2 or 3;

g is 0 or 1;

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

D is O, S or Se;

each R^{16}, R^{17} and R^{18} is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, arylenyl, heteroarylenyl, halo, -C(=O)R^a, -C(=O)N$(R^b)_2$, -C(=O)OR^c, -OR^d, -NR^e_2 , -SR^f or -SO_2R^g, each of which is optionally substituted with 1-4 R^{19}; and each R^{19} is independently halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e_2$, $-SR^f$ or $-SO_2R^g$.

111. The dye of claim 110, wherein R^{16} is not positioned ortho to the donor moiety.

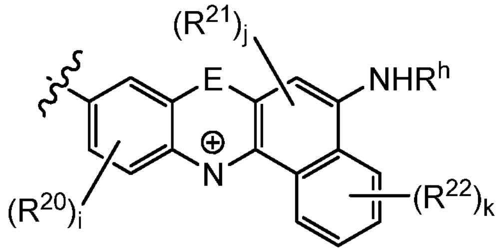
112. The dye of claim 110, wherein D is O.

113. The dye of claim 110, wherein h is 1.

114. The dye of claim 113, wherein R^{18} is $-OR^d$.

115. The dye of claim 114, wherein R^d is alkyl.

116. The dye of claim 73, wherein the acceptor is:



wherein:

i is 0, 1 or 2;

j is 0, or 1;

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

E is O, S or Se;

each R^{20}, R^{21} and R^{22} is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, halo, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e_2$, $-SR^f$ or $-SO_2R^g$, each of which is optionally substituted with 1-4 R^{23};

each R^{23} is independently halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e_2$, $-SR^f$ or $-SO_2R^g$;

R^h is hydrogen or alkyl;

wherein at least one of R^{20}, R^{21}, R^{22} or R^{23} comprises a negatively charged moiety, or the dye further comprises a negatively charged counterion Z^-; and

Z^- is an anion such as halide, acetate, tosylate, azide, tetrafluoroborate, tetraphenylborate, hexafluorophosphate, phosphate, sulfate, perchlorate, trifluoromethanesulfonate or hexafluoroantimonate.

117. The dye of claim 116, wherein R^{20} is not positioned ortho to the donor moiety.

118. The dye of claim 116, wherein E is O.

119. The dye of claim 116, wherein j is 1.

120. The dye of claim 119, wherein R^{21} is halo.

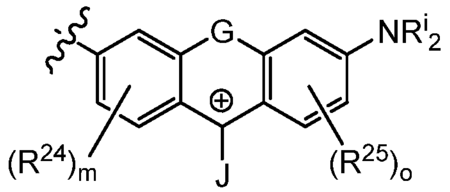
121. The dye of claim 116, wherein k is 1.

122. The dye of claim 121, wherein R^{22} is halo.

123. The dye of claim 116, wherein R^h is hydrogen.

124. The dye of claim 116, wherein R^h is alkyl.

125. The dye of claim 73, wherein the acceptor is:



wherein:

m is 0, 1 or 2;

o is 0, 1 or 2;

G is O, S, $CR^{26}R^{27}$, $SiR^{28}R^{29}$, or NR^{30};

J is hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, alkylhydroxy, haloalkyl, alkylamino, -CN, -C(=O)R^a, -C(=O)N(R^b)$_2$, -C(=O)OR^c, each of which is optionally substituted with 1-6 R^{31};

each R^{31} and R^{32} is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, halo, -NO_2, -CN, -C(=O)R^a, -C(=O)N(R^b)$_2$, -C(=O)OR^c, -OR^d, -NR^e_2 , -SR^f or -SO_2R^g, each of which is optionally substituted with 1-4 R^{32};

each R^{31} and R^{32} is independently halo, -NO_2, -CN, -C(=O)R^a, -C(=O)N(R^b)$_2$, -C(=O)OR^c, -OR^d, -NR^e_2 , or -SR^f or -SO_2R^g;

each R^{26}, R^{27}, R^{28}, R^{29}, and R^{30} is independently hydrogen, alkyl, alkenyl or alkynyl;

each R^i is independently hydrogen or alkyl;

wherein at least one of R^{24}, R^{25}, R^{31} or R^{32} comprises a negatively charged moiety, or the dye further comprises a negatively charged counterion Z^-; and

Z^- is an anion such as halide, acetate, tosylate, azide, tetrafluoroborate, tetraphenylborate, hexafluorophosphate, phosphate, sulfate, perchlorate, trifluoromethanesulfonate or hexafluoroantimonate.

126. The dye of claim 125, wherein R^{24} and R^{25} are not positioned ortho to the donor moieties.

127. The dye of claim 125, wherein G is O.

128. The dye of claim 125, wherein G is NR^{30}.

129. The dye of claim 125, wherein J is hydrogen.

130. The dye of claim 125, wherein J is aryl optionally substituted with 1 R^{31}.

131. The dye of claim 130, wherein R^{31} is -C(=O)OR^c.

132. The dye of claim 130, wherein R^{31} is $-SO_2R^g$.

133. The dye of claim 125, wherein J is alkyl optionally substituted with 1-3 R^{31}.

134. The dye of claim 133, wherein R^{31} is halo.

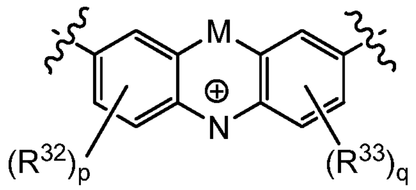
135. The dye of claim 125, wherein J is -CN.

136. The dye of claim 125, wherein R^i is hydrogen.

137. The dye of claim 125, wherein R^i is alkyl.

138. The dye of claim 1, wherein n is 2.

139. The dye of claim 138, wherein the acceptor is:



wherein:

p is 0, 1 or 2;

q is 0, 1 or 2;

M is O, S, $CR^{34}R^{35}$, $SiR^{36}R^{37}$, or NR^{38};

each R^{32} and R^{33} is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, halo, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e_2$, $-SR^f$ or $-SO_2R^g$, each of which is optionally substituted with 1-4 R^{39};

each R^{39} is independently halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e_2$, $-SR^f$ or $-SO_2R^g$;

each R^{34}, R^{35}, R^{36}, R^{37} and R^{38} is independently hydrogen, alkyl, alkenyl or alkynyl;

wherein at least one of R^{32}, R^{33} or R^{39} comprises a negatively charged moiety, or the dye further comprises a negatively charged counterion Z^-; and

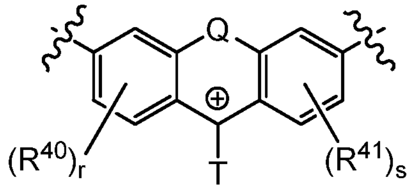
Z^- is an anion such as halide, acetate, tosylate, azide, tetrafluoroborate, tetraphenylborate, hexafluorophosphate, phosphate, sulfate, perchlorate, trifluoromethanesulfonate or hexafluoroantimonate.

140. The dye of claim 139, wherein R^{32} and R^{33} are not positioned ortho to the donor moieties.

141. The dye of claim 139, wherein M is O.

142. The dye of claim 139, wherein M is S.

143. The dye of claim 138, wherein the acceptor is:



wherein:

r is 0, 1 or 2;

s is 0, 1 or 2;

Q is O, S, $CR^{42}R^{43}$, $SiR^{44}R^{45}$, or NR^{46};

T is hydrogen, alkyl, alkenyl, alkynyl, aryl, heteroaryl, alkylhydroxy, haloalkyl, alkylamino, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, each of which is optionally substituted with 1-6 R^{47};

each R^{40} and R^{41} is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e_2$, $-SR^f$ or $-SO_2R^g$, each of which is optionally substituted with 1-4 R^{48};

each R^{47} and R^{48} is independently halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e_2$, or $-SR^f$ or $-SO_2R^g$;

each R^{42}, R^{43}, R^{44}, R^{45}, and R^{46} is independently hydrogen, alkyl, alkenyl or alkynyl;

wherein at least one of R^{40}, R^{41}, R^{47} or R^{48} comprises a negatively charged moiety, or the dye further comprises a negatively charged counterion Z^-; and

Z^- is an anion such as halide, acetate, tosylate, azide, tetrafluoroborate, tetraphenylborate, hexafluorophosphate, phosphate, sulfate, perchlorate, trifluoromethanesulfonate or hexafluoroantimonate.

144. The dye of claim 143, wherein R^{40} and R^{41} are not positioned ortho to the donor moieties.

145. The dye of claim 143, wherein Q is O.

146. The dye of claim 143, wherein Q is NR^{37}.

147. The dye of claim 143, wherein T is hydrogen.

148. The dye of claim 143, wherein T is aryl optionally substituted with 1 R^{47}.

149. The dye of claim 148, wherein R^{47} is $-C(=O)OR^c$.

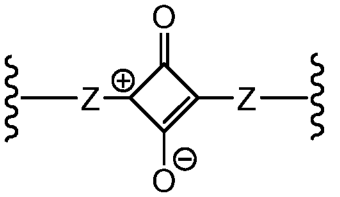
150. The dye of claim 148, wherein R^{47} is $-SO_2R^g$.

151. The dye of claim 143, wherein T is alkyl optionally substituted with 1-3 R^{47}.

152. The dye of claim 151, wherein R^{47} is halo.

153. The dye of claim 143, wherein T is -CN.

154. The dye of claim 138, wherein the acceptor is:



wherein:

each Z is independently 5- or 6-membered aryl or heteroaryl, optionally substituted with 1-4 R^{49};

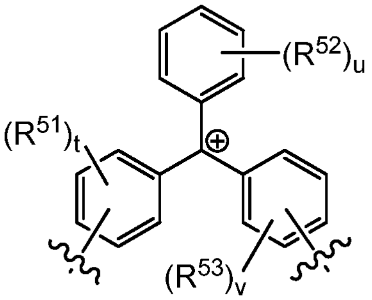
each R^{49} is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$, each of which is optionally substituted with 1-4 R^{50}; and

each R^{50} is independently halo, $-NO_2$, -CN, $-C(=O)R^a$, $-C(=O)N(R^b)_2$, $-C(=O)OR^c$, $-OR^d$, $-NR^e{}_2$, $-SR^f$ or $-SO_2R^g$.

155. The dye of claim 154, wherein Z is phenyl.

156. The dye of claim 154, wherein Z is thienyl.

157. The dye of claim 138, wherein the acceptor is:



wherein:

t is 0, 1, 2, 3, 4 or 5;

u is 0, 1, 2, 3, 4 or 5;

v is 0, 1, 2, 3, 4 or 5;

each R^{51}, R^{52} and R^{53} is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, halo, $-NO_2$, -CN, $-C(=O)R^a$, -

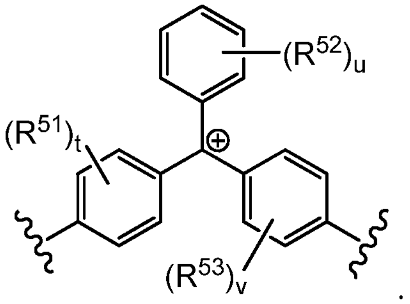
C(=O)N(R^b)$_2$, -C(=O)OR^c, -OR^d, -NR^e_2 , -SR^f or -SO$_2$$R^g$, each of which is optionally substituted with 1-4 R^{54};

each R^{54} is independently halo, -NO$_2$, -CN, -C(=O)R^a, -C(=O)N(R^b)$_2$, -C(=O)OR^c, -OR^d, -NR^e_2 , -SR^f or -SO$_2$$R^g$;

wherein at least one of R^{51}, R^{52}, R^{53} or R^{54} comprises a negatively charged moiety, or the dye further comprises a negatively charged counterion Z^-; and

Z^- is an anion such as halide, acetate, tosylate, azide, tetrafluoroborate, tetraphenylborate, hexafluorophosphate, phosphate, sulfate, perchlorate, trifluoromethanesulfonate or hexafluoroantimonate.

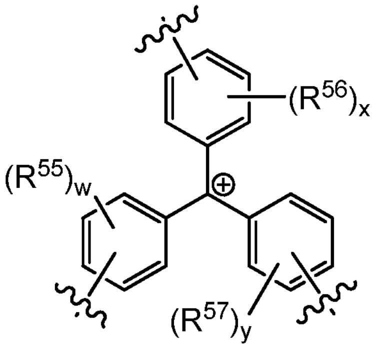
158. The dye of claim 157, wherein the acceptor is:



.

159. The dye of claim 1, wherein n is 3.

160. The dye of claim 159, wherein the acceptor is:



wherein:

w is 0, 1, 2 or 3;

x is 0, 1, 2 or 3;

y is 0, 1, 2 or 3;

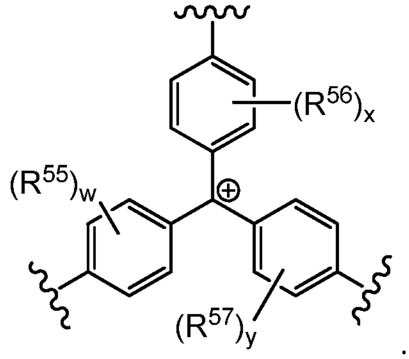
each R^{55}, R^{56} and R^{57} is independently alkyl, alkenyl, alkynyl, aryl, heteroaryl, arylalkyl, alkylhydroxy, haloalkyl, alkylamino, halo, -NO_2, -CN, -C(=O)R^a, -C(=O)N(R^b)$_2$, -C(=O)OR^c, -OR^d, -NR^e_2 , -SR^f or -SO_2R^g, each of which is optionally substituted with 1-4 R^{58};

each R^{58} is independently halo, -NO_2, -CN, -C(=O)R^a, -C(=O)N(R^b)$_2$, -C(=O)OR^c, -OR^d, -NR^e_2 , -SR^f or -SO_2R^g;

wherein at least one of R^{55}, R^{56}, R^{57} or R^{58} comprises a negatively charged moiety, or the dye further comprises a negatively charged counterion Z^-; and

Z^- is an anion such as halide, acetate, tosylate, azide, tetrafluoroborate, tetraphenylborate, hexafluorophosphate, phosphate, sulfate, perchlorate, trifluoromethanesulfonate or hexafluoroantimonate.

161. The dye of claim 160, wherein the acceptor is:



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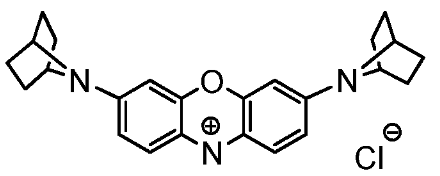
162. The dye of claim 1, wherein the dye is selected from:



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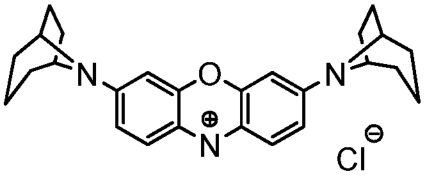

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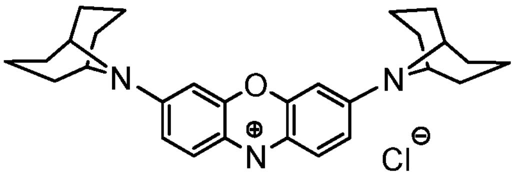

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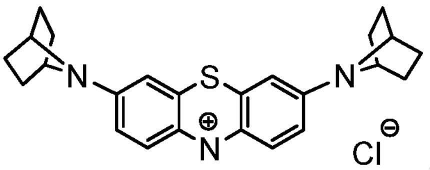

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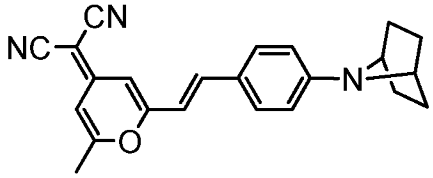

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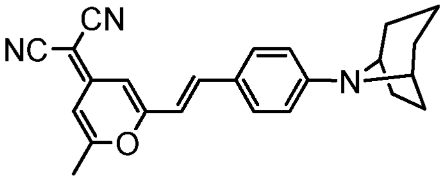

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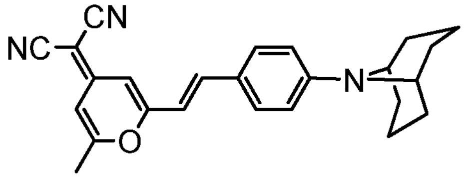

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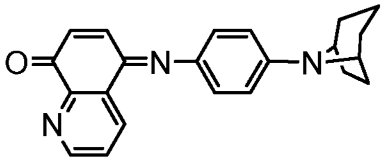

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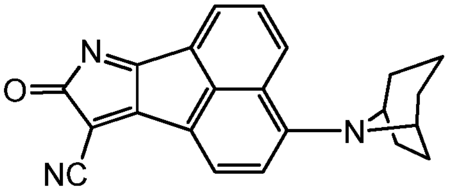

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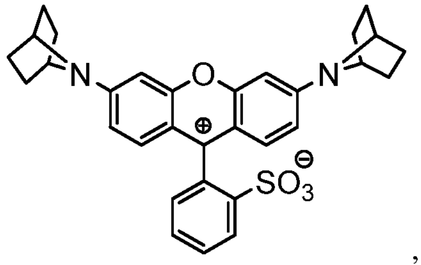

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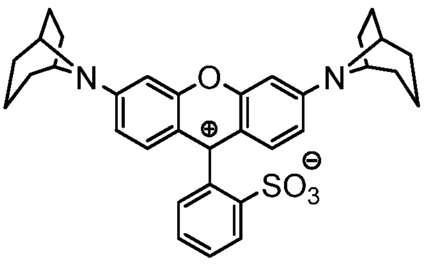

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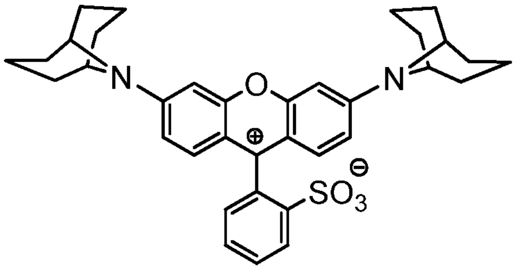

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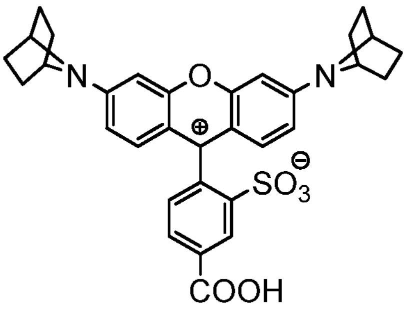

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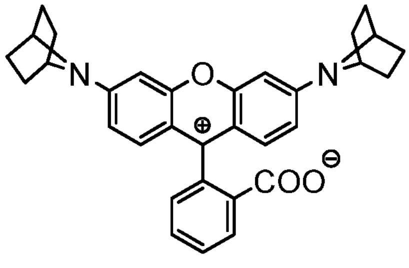

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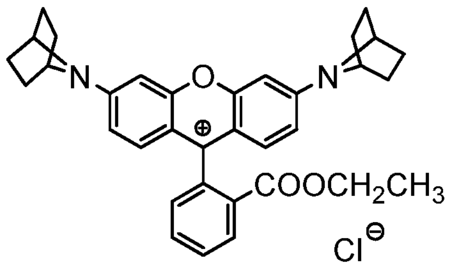

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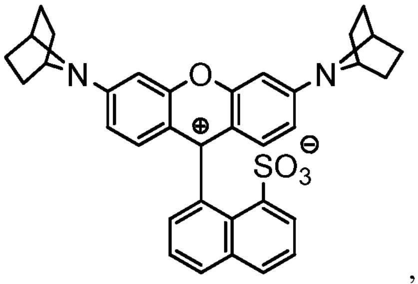

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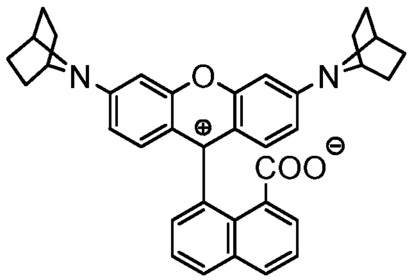

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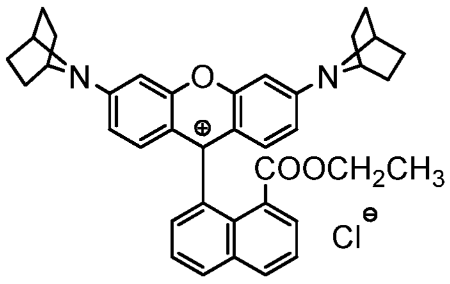

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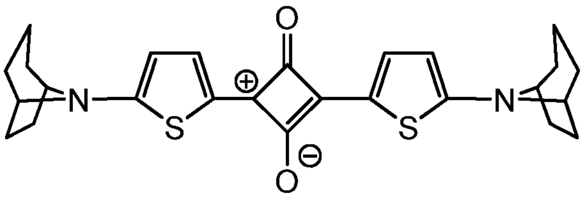
or



.

163. The dye of claim 1, wherein the dye has a photostability of at least 1.5 times that of a reference dye when subjected to irradiation, as measured by a decrease in optical density.

164. The dye of claim 163, wherein the reference dye is a dye having an amino, alkylamino or dialkylamino donor moiety.

165. The dye of claim 1, further comprising a reactive moiety.

166. The dye of claim 1, further comprising a linker and a reactive moiety.

167. The dye of claim 1, having the following formula (II):

$$\left[\text{Formula (I)}\right]\!-L_z-X$$

(II)

wherein:

L is a linker; and

z is an integer between 0 and 20;

X is a reactive moiety.

168. The dye of claim 167, wherein the linker and reactive moiety are attached to the acceptor.

169. The dye of claim 167, wherein the linker and reactive moiety are attached to the donor.

170. The dye of claim 167, wherein the reactive moiety is selected from the group consisting of Michael acceptor, carboxylic acid or an activated derivative thereof, maleimido-containing group, isothiocyanate, sulfonic acid or activated derivative thereof, aldehyde, ketone, carbonyl azide, iodoacetamide, alkyne and azide.

171. The dye of claim 170, wherein the reactive moiety is a succinimidyl-containing group.

172. The dye of claim 170, wherein the reactive moiety is a sulfonyl chloride.

173. The dye of claim 170, wherein the Michael acceptor is an α,β-unsaturated carbonyl moiety.

174. The dye of claim 170, wherein the reactive moiety is a maleimido-containing group.

175. The dye of claim 167, wherein the reactive moiety is directly attached to the dye.

176. The dye of claim 167, wherein the reactive moiety is attached to the dye via a linker.

177. The dye of claim 167, wherein the linker is selected from the group consisting of:

$–(C_1\text{-}C_4\text{ alkylenyl})_{a'}–$
$–[(C_1\text{-}C_4\text{ alkylenyl})\text{-O}]_{a'}–$
$–\text{NH-}(C_1\text{-}C_4\text{ alkylenyl})_{a'}\text{-NH}–$
$–\text{NH-}(C_1\text{-}C_4\text{ alkylenyl})_{a'}\text{-CO}–$
$–\text{NH-}(C_1\text{-}C_4\text{ alkylenyl})_{a'}\text{-COO}–$
$–\text{NH-}(C_1\text{-}C_4\text{ alkylenyl})_{a'}\text{-SO}_2\text{NH}–$
$–\text{CO-}(C_1\text{-}C_4\text{ alkylenyl})_{a'}\text{-CO}–$
$–\text{CO-}(C_1\text{-}C_4\text{ alkylenyl})_{a'}\text{-COO}–$
$–\text{CO-}(C_1\text{-}C_4\text{ alkylenyl})_{a'}\text{-SO}_2\text{NH}–$
$–\text{COO-}(C_1\text{-}C_4\text{ alkylenyl})_{a'}\text{-COO}–$
$–\text{COO-}(C_1\text{-}C_4\text{ alkylenyl})_{a'}\text{-SO}_2\text{NH}–$
$–\text{SO}_2\text{NH-}(C_1\text{-}C_4\text{ alkylenyl})_{a'}\text{-SO}_2\text{NH}–$
$–\text{NH-}[(C_1\text{-}C_4\text{ alkylenyl})\text{-O}]_{a'}\text{-NH}–$
$–\text{NH-}[(C_1\text{-}C_4\text{ alkylenyl})\text{-O}]_{a'}\text{-CO}–$
$–\text{NH-}[(C_1\text{-}C_4\text{ alkylenyl})\text{-O}]_{a'}\text{-COO}–$
$–\text{NH-}[(C_1\text{-}C_4\text{ alkylenyl})\text{-O}]_{a'}\text{-SO}_2\text{NH}–$
$–\text{CO-}[(C_1\text{-}C_4\text{ alkylenyl})\text{-O}]_{a'}\text{-CO}–$
$–\text{CO-}[(C_1\text{-}C_4\text{ alkylenyl})\text{-O}]_{a'}\text{-COO}–$
$–\text{CO-}[(C_1\text{-}C_4\text{ alkylenyl})\text{-O}]_{a'}\text{-SO}_2\text{NH}–$
$–\text{COO-}[(C_1\text{-}C_4\text{ alkylenyl})\text{-O}]_{a'}\text{-COO}–$
$–\text{COO-}[(C_1\text{-}C_4\text{ alkylenyl})\text{-O}]_{a'}\text{-SO}_2\text{NH}–$
$–\text{SO}_2\text{NH-}[(C_1\text{-}C_4\text{ alkylenyl})\text{-O}]_{a'}\text{-SO}_2\text{NH}–$

and combinations thereof, wherein:

a’ is an integer between 1 and 20;

the linker as drawn may be positioned between the donor and acceptor in either direction; and

the alkyl chains may have varying degrees of unsaturation.

178. The dye of claim 177, wherein the linker is $-(CH_2)_{a'}-$.

179. The dye of claim 177, wherein the linker is $-(CH_2\text{-}CH_2\text{-}O)_{a'}-$.

180. The dye of claim 177, wherein the linker is $-NH\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH_2\text{-}CH_2\text{-}O\text{-}CH_2\text{-}COO-$.

181. The dye of claim 167, wherein the dye is conjugated to a substrate.

182. The dye of claim 167, wherein the dye is conjugated to a substrate via a covalent bond.

183. The dye of claim 167, wherein the dye is conjugated to a substrate via a linker and a reactive moiety.

184. The dye of claim 167, having the following formula (III):

$$\left[\text{Formula (II)}\right]_{b'}\!-\text{substrate}$$

(III)

wherein b’ is 1, 2 or 3; and

the reactive moiety in the dye of formula (II) is the residual product of the reaction between the reactive moiety and the substrate.

185. The dye of claim 184, wherein the substrate is a biomolecule.

186. The dye of claim 185, wherein the biomolecule is an amino acid.

187. The dye of claim 185, wherein the biomolecule is a polypeptide.

188. The dye of claim 185, wherein the biomolecule is a nucleic acid.

189. The dye of claim 185, wherein the biomolecule is an antibody.

190. The dye of claim 185, wherein the biomolecule is an antigen.

191. The dye of claim 184, wherein the substrate is a pharmaceutical agent.

192. The dye of claim 184, wherein the substrate is a metabolite.

193. The dye of claim 184, wherein the substrate is a diagnostic agent.

194. The dye of claim 184, wherein the substrate is a controlled substance.

195. The dye of claim 184, wherein the substrate is a toxin.

196. The dye of claim 184, wherein the substrate is biotin.

197. The dye of claim 184, wherein the substrate is a polymer.

198. The dye of claim 184, wherein the substrate is a textile.

199. The dye of claim 198, wherein the textile is an article of clothing.

200. The dye of claim 184, wherein the substrate has been previously labeled with another dye.

201. A composition comprising a dye of formula (I), claim 1, and an additional component.

202. The composition of claim 201, wherein the component is a solvent.

203. The composition of claim 201, wherein the component is a substrate.

204. The composition of claim 203, wherein the substrate is a biomolecule.

205. The composition of claim 204, wherein the biomolecule is an amino acid.

206. The composition of claim 204, wherein the biomolecule is a polypeptide.

207. The composition of claim 204, wherein the biomolecule is a nucleic acid.

208. The composition of claim 204, wherein the biomolecule is an antibody.

209. The composition of claim 204, wherein the biomolecule is an antigen.

210. The composition of claim 203, wherein the substrate is a pharmaceutical agent.

211. The composition of claim 203, wherein the substrate is a metabolite.

212. The composition of claim 203, wherein the substrate is a diagnostic agent.

213. The composition of claim 203, wherein the substrate is a controlled substance.

214. The composition of claim 203, wherein the substrate is a toxin.

215. The composition of claim 203, wherein the substrate is biotin.

216. The composition of claim 203, wherein the substrate is a polymer.

217. The composition of claim 203, wherein the substrate is a textile.

218. The composition of claim 217, wherein the textile is an article of clothing.

219. The composition of claim 203, wherein the substrate has been previously labeled with another dye.

220. The composition of claim 201, wherein the component is a reagent.

221. The composition of claim 220, wherein the reagent is an acid.

222. The composition of claim 220, wherein the reagent is a base.

223. The composition of claim 220, wherein the reagent is a reducing agent.

224. The composition of claim 220, wherein the reagent is a coupling agent.

225. The composition of claim 201, wherein the composition comprises a plurality of components.

226. The composition of claim 201, wherein the dye is of formula (II), claim 167.

227. A kit comprising a dye of formula (I), claim 1.

228. The kit of claim 227, further comprising a container.

229. The kit of claim 228, wherein the container is a vial.

230. The kit of claim 227, comprising instructions for use of the dye.

231. The kit of claim 227, further comprising a substrate.

232. The kit of claim 227, further comprising a reference standard.

233. The kit of claim 227, wherein the dye is of formula (II), claim 167.

234. A method of labeling a substrate, the method comprising mixing a dye of formula (I) with the substrate under conditions sufficient to label the substrate, thereby labeling the substrate.

235. The method of claim 234, wherein the dye is of formula (II), claim 167.

236. The method of claim 234, wherein the substrate is a biomolecule.

237. The method of claim 236, wherein the biomolecule is an amino acid.

238. The method of claim 236, wherein the biomolecule is a polypeptide.

239. The method of claim 236, wherein the biomolecule is a nucleic acid.

240. The method of claim 236, wherein the biomolecule is an antibody.

241. The method of claim 236, wherein the biomolecule is an antigen.

242. The method of claim 234, wherein the substrate is a pharmaceutical agent.

243. The method of claim 234, wherein the substrate is a metabolite.

244. The method of claim 234, wherein the substrate is a diagnostic agent.

245. The method of claim 234, wherein the substrate is a controlled substance.

246. The method of claim 234, wherein the substrate is a toxin.

247. The method of claim 234, wherein the substrate is biotin.

248. The method of claim 234, wherein the substrate is a polymer.

249. The method of claim 234, wherein the substrate is a textile.

250. The method of claim 249, wherein the textile is an article of clothing.

251. The method of claim 234, wherein the dye is of formula (III), the biomolecule of formula (III) is an antigen, and the substrate is an antibody.

252. The method of claim 234, wherein the dye is of formula (III), the biomolecule of formula (III) is an antibody, and the substrate is an antigen.

253. The method of claim 234, further comprising evaluating the labeled substrate.

254. The method of claim 253, wherein the amount of labeled substrate is evaluated qualitatively.

255. The method of claim 253, wherein the amount of labeled substrate is evaluated quantitatively.

256. The method of claim 253, wherein the amount of labeled substrate is evaluated in comparison to a control or reference standard.

257. The method of claim 234, wherein the substrate is labeled in an imagewise manner.

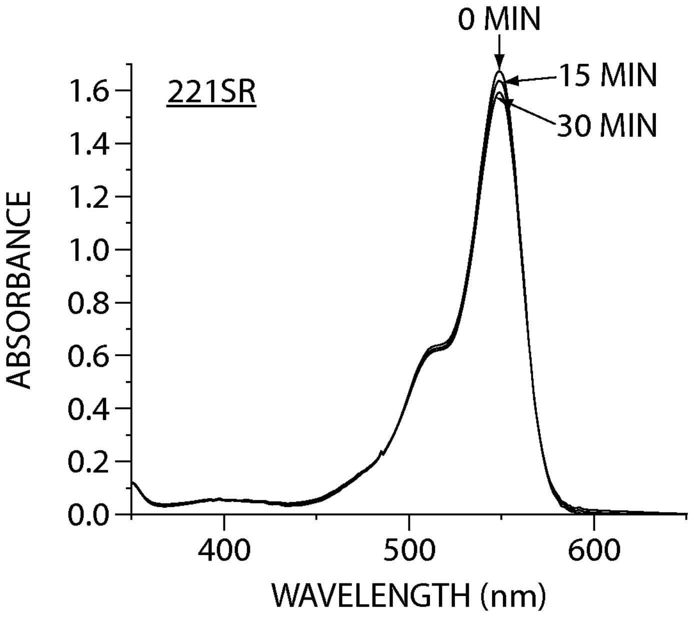
258. A method of staining an object with a dye of formula (I), the method comprising contacting the dye with the object, thereby staining the object.

259. The method of claim 258, wherein the object is a cell or group of cells.

260. The method of claim 258, wherein the object is a polymer.

261. The method of claim 258, wherein the object is a textile.

262. The method of claim 258, wherein the textile is an article of clothing.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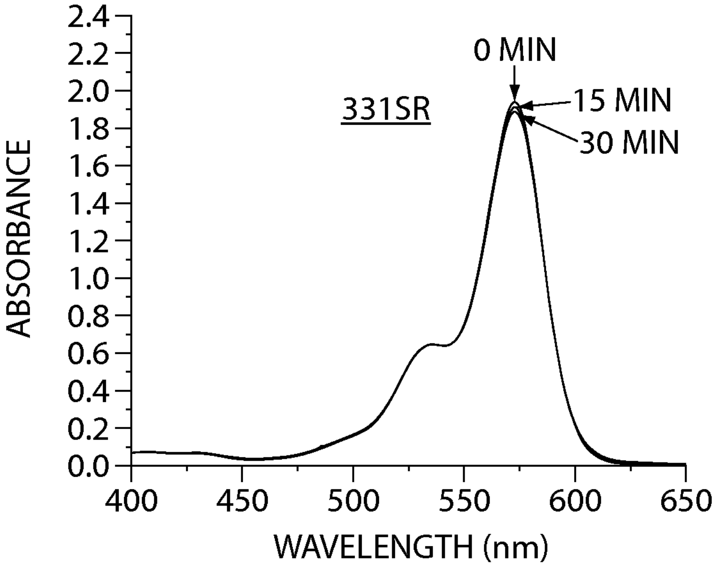
30 MIN
221SR
ABSORBANCE
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
400
500
600
WAVELENGTH (nm)

Fig. 1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331SR
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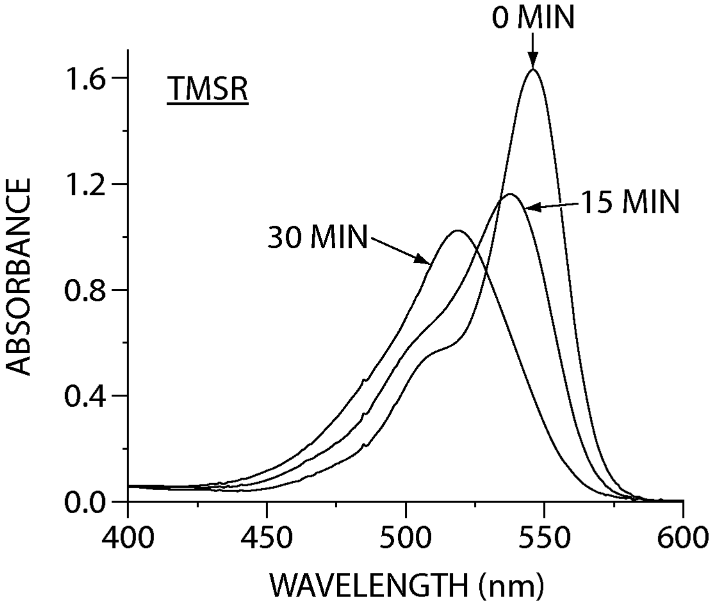
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
400
450
500
550
600
650
WAVELENGTH (nm)

Fig. 2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TMSR
15 MIN
30 MIN
ABSORBANCE
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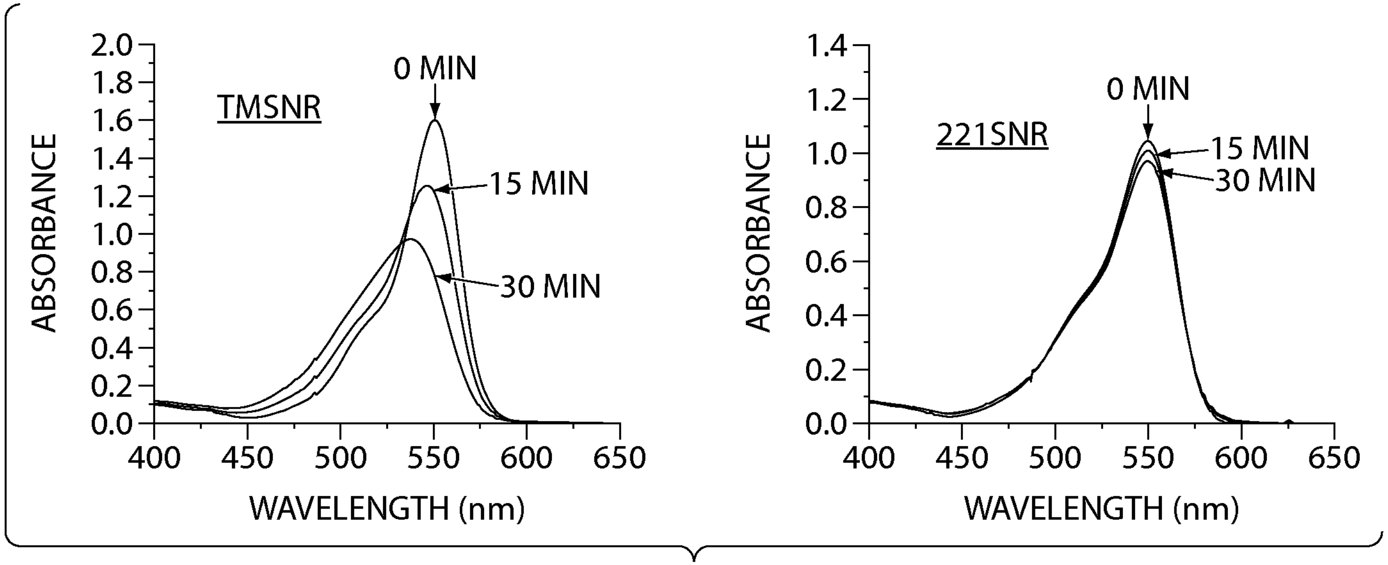
1.2
0.8
0.4
0.0
400
450
500
550
600
WAVELENGTH (nm)

Fig. 3


TMSNR
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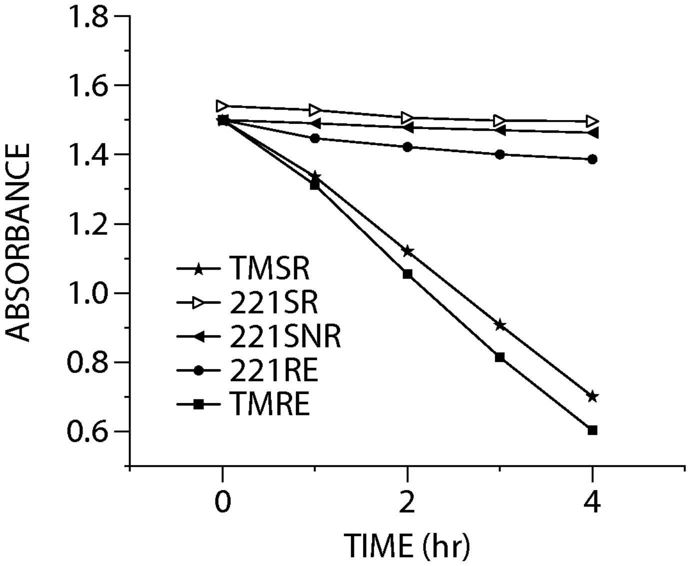
15 MIN
30 MIN
ABSORBANCE
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
400
450
500
550
600
650
WAVELENGTH (nm)
221SNR
0 MIN
15 MIN
30 MIN
ABSORBANCE
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
400
450
500
550
600
650
WAVELENGTH (nm)

Fig. 4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1.6
1.4
1.2
1.0
0.8
0.6
ABSORBANCE
0
2
4
TIME (hr)
★ TMSR
▷ 221SR
◀ 221SNR
● 221RE
■ TMRE

Fig. 5