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(54) Title: FLUORESCENT PROBES

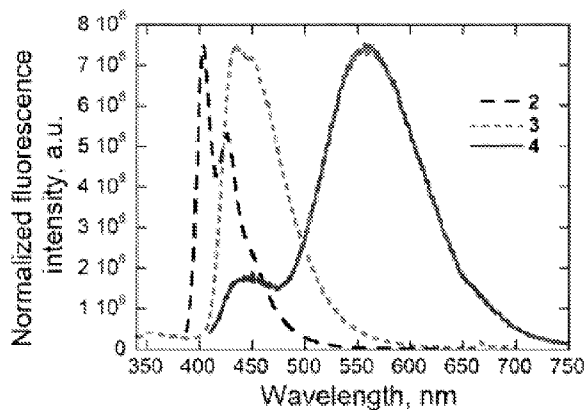


FIG. 15B

(57) Abstract: Described herein are luminescent sila- and germafluorenes. Also described herein are methods of making and using sila- and germafluorenes. The position and the type of substituent impact the absorption and emission properties in solution and in the solid-state and subsequently influence the role of sila- and germafluorenes as biosensors.

FLUORESCENT PROBES

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application No. 63/511,403 filed June 30, 2023, the contents of which are incorporated herein by reference in their entirety.

FIELD OF DISCLOSURE

[0002] Described herein are luminescent sila- and germafluorenes. The position and the type of substituent impact the absorption and emission properties in solution and in the solid-state and subsequently influence the role of sila- and germafluorenes as biosensors.

BACKGROUND OF THE DISCLOSURE

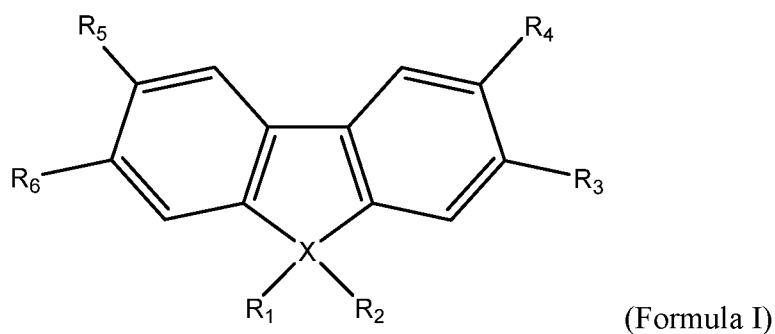
[0003] Compounds with desirable fluorescent properties have the potential for a wide range of applications. Highly efficient polymer light emitting diodes (PLEDs) and organic light emitting diodes (OLEDs) that have high current, power, and external quantum efficiency have been of recent interest for solid state applications. In the solution realm, there is ongoing demand for well-behaved compounds to serve as fluorescent probes of biological behavior. This can be as probes of specific molecular interactions in vitro as well as probes of cellular structures and behavior via confocal microscopy. Those with intramolecular charge transfer (ICT) behavior are sensitive to their environment, or more specifically, are solvatochromic.

[0004] In addition, biological membranes are dynamic in their composition and properties, and local differences participate in cellular processes in a profound way and have been linked to apoptosis, viral entry, and cellular stress. There is a continuing demand for dyes that are water soluble, sensitive (as gauged by extinction coefficient and quantum yield), and that have excellent photostability and favorable excitation and emission wavelengths, that is, those that do not interfere with common chromophores in cells or other dyes when used with them.

[0005] Thus, there is a need in the art for improved fluorescent probes.

BRIEF DESCRIPTION OF THE DISCLOSURE

[0006] In one aspect, disclosed herein is a compound of Formula I,



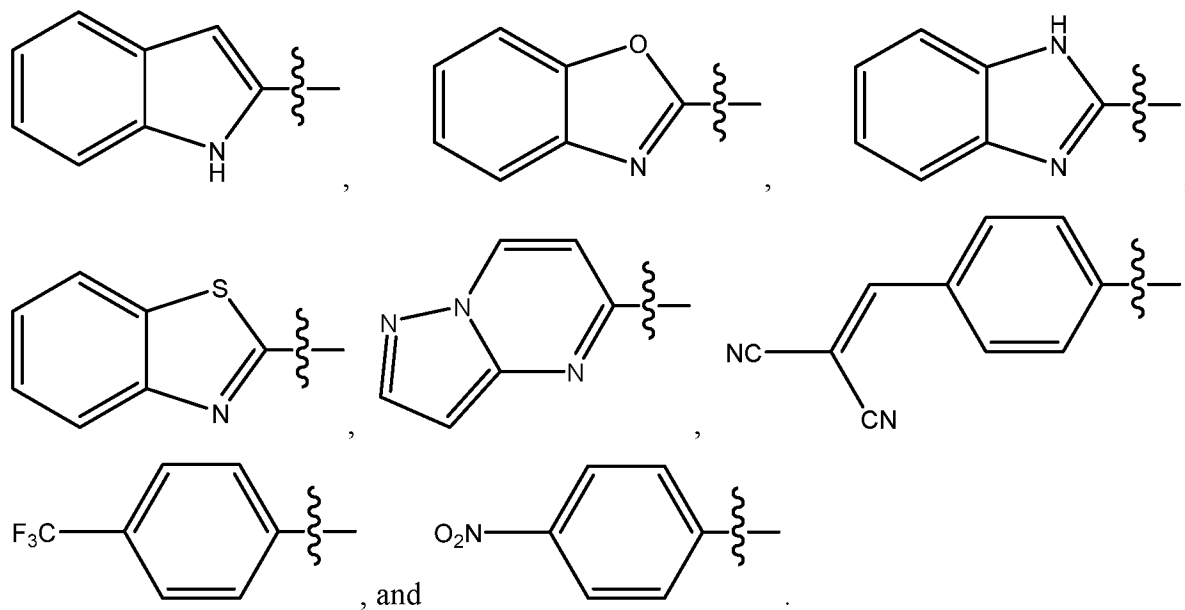
wherein X is Si or Ge;

wherein R₁ and R₂ are each independently selected from the group consisting of hydrogen, alkyl, and aryl;

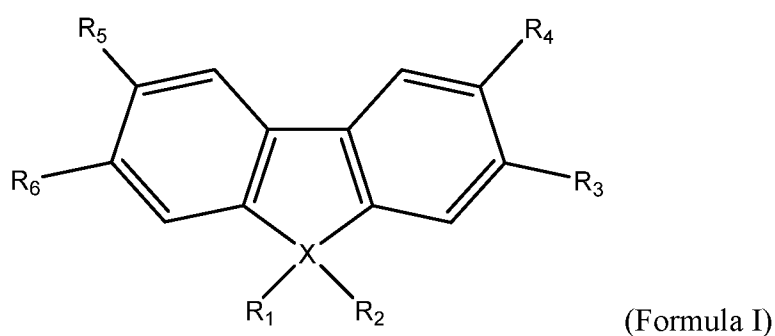
wherein R₃, R₄, R₅, and R₆ are each independently selected from the group consisting of hydrogen, alkoxy, Y, and $\text{---}\overset{\text{S}}{\underset{\text{S}}{\text{C}}}\text{---Y}$, with the proviso that at least two of R₃, R₄, R₅, and

R₆ are selected from the group consisting of Y and $\text{---}\overset{\text{S}}{\underset{\text{S}}{\text{C}}}\text{---Y}$; and

wherein Y is selected from the group consisting of



[0007] In another aspect, disclosed herein is a method of producing a compound of Formula I,



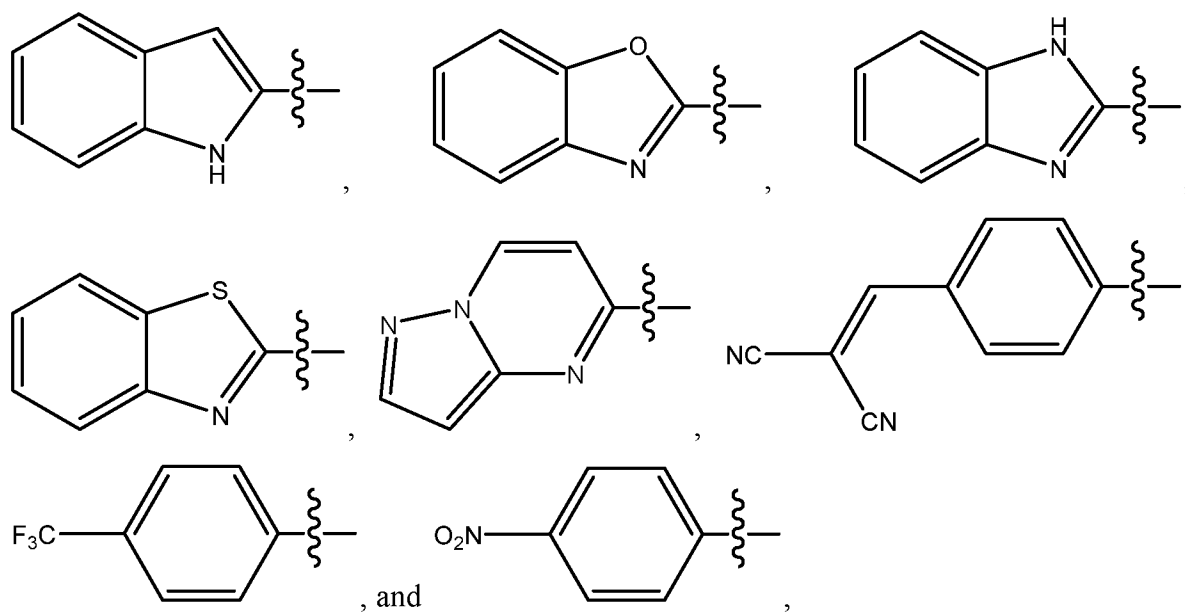
wherein X is Si or Ge;

wherein R_1 and R_2 are each independently selected from the group consisting of hydrogen, alkyl, and aryl;

wherein R_3 , R_4 , R_5 , and R_6 are each independently selected from the group consisting of hydrogen, alkoxy, Y, and $\text{—}\frac{\text{z}}{\text{z}}\equiv\text{Y}$, with the proviso that at least two of R_3 , R_4 , R_5 , and

R_6 are selected from the group consisting of Y and $\text{—}\frac{\text{z}}{\text{z}}\equiv\text{Y}$; and

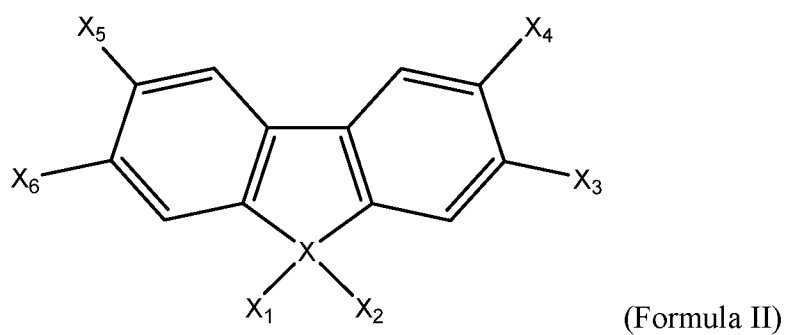
wherein Y is selected from the group consisting of



the method comprising:

forming a mixture comprising

a compound of Formula II,



wherein X is Si or Ge;

wherein X_1 and X_2 are each independently selected from the group consisting of hydrogen, alkyl, and aryl; and

wherein X_3 , X_4 , X_5 , and X_6 are each independently selected from the group consisting of hydrogen, alkoxy, and halogen with the proviso that at least two of X_3 , X_4 , X_5 , and X_6 are halogen;

a compound of Formula III,

R-Y (Formula III)

wherein R is selected from the group consisting of hydrogen, alkynyl, and borono;

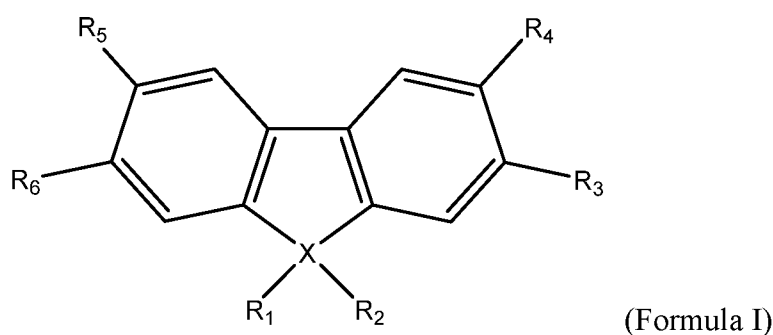
a noble metal catalyst;

a base; and

optionally a co-catalyst comprising a metal; and

reacting the mixture.

[0008] In another aspect, disclosed herein is a method of using a compound of Formula I,



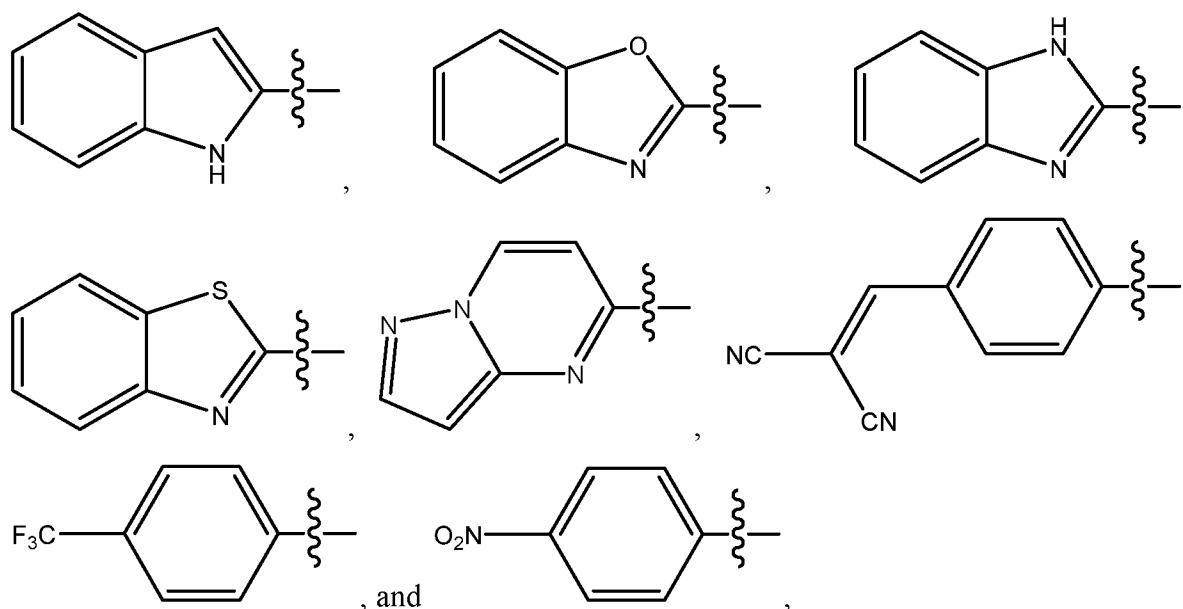
wherein X is Si or Ge;

wherein R₁ and R₂ are each independently selected from the group consisting of hydrogen, alkyl, and aryl;

wherein R₃, R₄, R₅, and R₆ are each independently selected from the group consisting of hydrogen, alkoxy, Y, and $\text{--}\overset{\text{S}}{\underset{\text{Z}}{\text{C}}}\equiv\text{Y}$, with the proviso that at least two of R₃, R₄, R₅, and

R₆ are selected from the group consisting of Y and $\text{--}\overset{\text{S}}{\underset{\text{Z}}{\text{C}}}\equiv\text{Y}$; and

wherein Y is selected from the group consisting of



the method comprising using the compound of Formula I for a purpose selected from the group consisting of measuring luminescence, measuring fluorescence, solvatochromic probing, imaging lipid domains, imaging live cells, providing fluorescence in a polymer light emitting diode, providing fluorescence in an organic light emitting diode, and combinations thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] The file of this patent contains at least one drawing/photograph executed in color. Copies of this patent with color drawing(s)/photograph(s) will be provided by the Office upon request and payment of the necessary fee.

[0010] Figure 1 is a diagram depicting ordered and disordered membrane domains in accordance with the present disclosure.

[0011] Figure 2 is a diagram depicting basic designs of suitable probes in accordance with the present disclosure.

[0012] Figure 3 is a fluorescent image in accordance with the present disclosure. The fluorescent image shows a giant unilamellar vesicle (GUV) showing Lo and Ld domains.

[0013] Figure 4 is a fluorescent image in accordance with the present disclosure. The fluorescent image shows that Compound 1 of Example 1 colocalizes in yeast with Nile Red. Nile Red is shown in the top left, the fluorescence of Compound 1 of Example 1 is shown in the bottom left, transmitted light is shown in the top right, and yellow indicates colocalization in the bottom right. 15 mM probe, 63x magnification.

[0014] Figure 5 depicts a metallafluorene (MF) with different labeled substituents in accordance with the present disclosure.

[0015] Figure 6 shows the HOMO (left) and LUMO (right) for ground state 5 of Compound 5 of Example 1 in accordance with the present disclosure.

[0016] Figure 7 depicts emission of Compound 14 of Example 1 in various organic solvents in accordance with the present disclosure.

[0017] Figure 8 depicts a Lippert-Mataga plot for Compound 14 of Example 1 in accordance with the present disclosure. The Lippert-Mataga plot quantifies solvatochromism. The slope (17,000) is comparable to that of the commercial lipid probe Nile Red (14,000).

[0018] Figure 9 is a photograph illustrating color changes in emission for Compound 14 of Example 1 as a function of solvent in accordance with the present disclosure. The depicted solvents are, from left to right: cyclohexane, toluene, dioxane, chloroform, diethyl ether, diethyl acetate, dichloromethane, dimethyl sulfoxide, acetone, ethanol, acetonitrile, methanol, and water.

[0019] Figure 10 depicts that the emission of Compound 14 of Example 1 in accordance with the present disclosure is sensitive to viscosity in a water-methanol system.

[0020] Figure 11 depicts that Compound 14 of Example 1 in accordance with the present disclosure can be used to stain yeast cells and it co-localizes with the lipid probe Nile Red. Top left: Nile Red; Bottom left: 14; Top right, transmitted light. Bottom right: superposition of Nile Red and compound 14.

[0021] Figure 12 depicts solid state fluorescence (left to right) of Compounds 2, 3 and 4 of Example 2 in accordance with the present disclosure.

[0022] Figure 13 depicts the crystal structure of Compound 2 of Example 2 showing the displacement ellipsoid drawn at 50 % probability level in accordance with the present disclosure. Atomic labels for the asymmetric component alone are shown here. The hydrogen atoms are omitted for clarity.

[0023] Figure 14 depicts a part of the crystal structure of 2 of Example 2 showing the molecular offset stacking via $\pi\cdots\pi$ and C–H $\cdots\pi$ interactions in accordance with the present disclosure, where π_1 =Cg(C1/C2/C3/C4/C5/C6), π_2 = Cg(C9/C10/C11/C12/C13/C14) and π_3 = Cg(C17/C18/C19/C20/C21/C22). The hydrogen atoms not involved in the intermolecular interactions are omitted for clarity.

[0024] Figure 15A depicts absorbance spectra for Compounds 2-4 of Example 2 in dichloromethane in accordance with the present disclosure.

[0025] Figure 15B depicts normalized fluorescence spectra for Compounds 2-4 of Example 2 in dichloromethane in accordance with the present disclosure. The excitation wavelength is at the respective absorbance maximum.

[0026] Figure 15C depicts solid state emission spectra of Compounds 2-4 of Example 2 in dichloromethane in accordance with the present disclosure, following excitation at the respective absorbance maximum.

[0027] Figure 16 depicts the molecular structure of Compound 5 of Example 2 in accordance with the present disclosure.

[0028] Figure 17 depicts the molecular structure of the parent compound 5 of Example 2 showing the displacement ellipsoid drawn at 50% probability level in accordance with the present disclosure. The hydrogen atoms are omitted for clarity.

[0029] Figure 19 depicts part of the crystal structure of Compound 5 of Example 2 in accordance with the present disclosure showing the formation of C–H $\cdots$ Br and C–H $\cdots\pi$ interactions, which constitute the molecular packing in the crystalline solid, where π_1 =

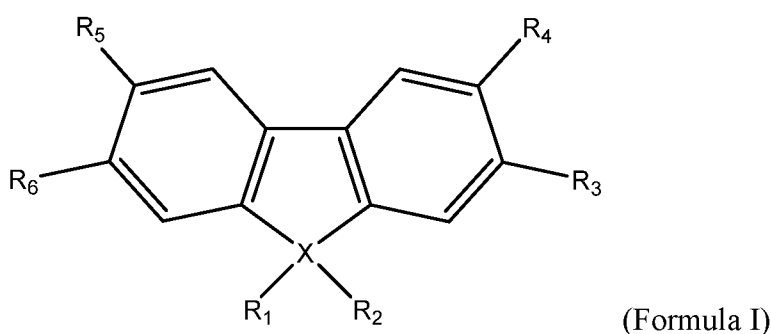
Cg(C1/C2/C3/C4/C5/C6). The hydrogen atoms not involved in the intermolecular interactions are omitted for clarity.

[0030] Figure 21 depicts HOMO (middle)-LUMO (top) orbital diagrams and electrostatic potential diagrams for Compounds 2-4 of Example 2 in accordance with the present disclosure in the ground state in DMF.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0031] Described herein are luminescent sila- and germafluorenes. The position and the type of substituent impact the absorption and emission properties in solution and in the solid-state and for the role of these compounds as biosensors.

[0032] The luminescent sila- and germafluorenes compounds of Formula I:

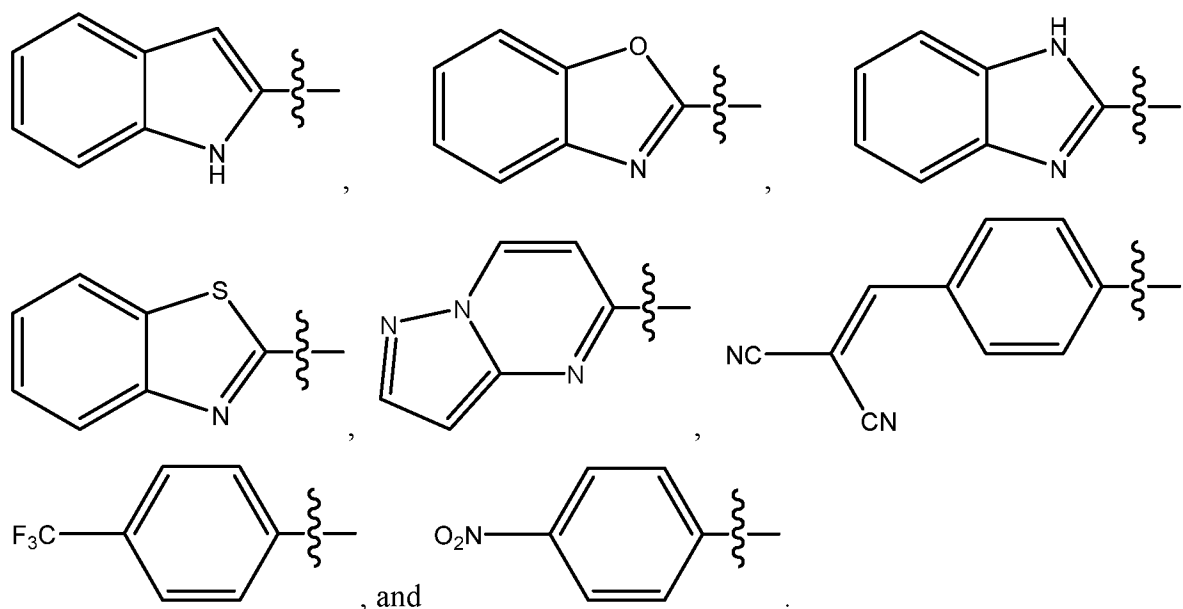


wherein X is Si or Ge;

wherein R₁ and R₂ are each independently selected from the group consisting of hydrogen, alkyl, and aryl;

wherein R₃, R₄, R₅, and R₆ are each independently selected from the group consisting of hydrogen, alkoxy, Y, and $\frac{\xi}{\xi} \equiv \text{Y}$, with the proviso that at least two of R₃, R₄, R₅, and R₆ are selected from the group consisting of Y and $\frac{\xi}{\xi} \equiv \text{Y}$; and

wherein Y is selected from the group consisting of



[0033] In some embodiments, the luminescent sila- and germafluorenes contain alkynyl(aryl) substituents at either the 2,7- or the 3,6- positions. In some embodiments, the luminescent sila- and germafluorenes contain alkynyl(aryl) substituents at the 2,7- positions. In some embodiments, the luminescent sila- and germafluorenes contain alkynyl(aryl) substituents at the 3,6- positions.

[0034] In some embodiments, the luminescent sila- and germafluorenes contain aryl substituents at either the 2,7- or the 3,6- positions. In some embodiments, the luminescent sila- and germafluorenes contain aryl substituents at the 2,7- positions. In some embodiments, the luminescent sila- and germafluorenes contain aryl substituents at the 3,6- positions.

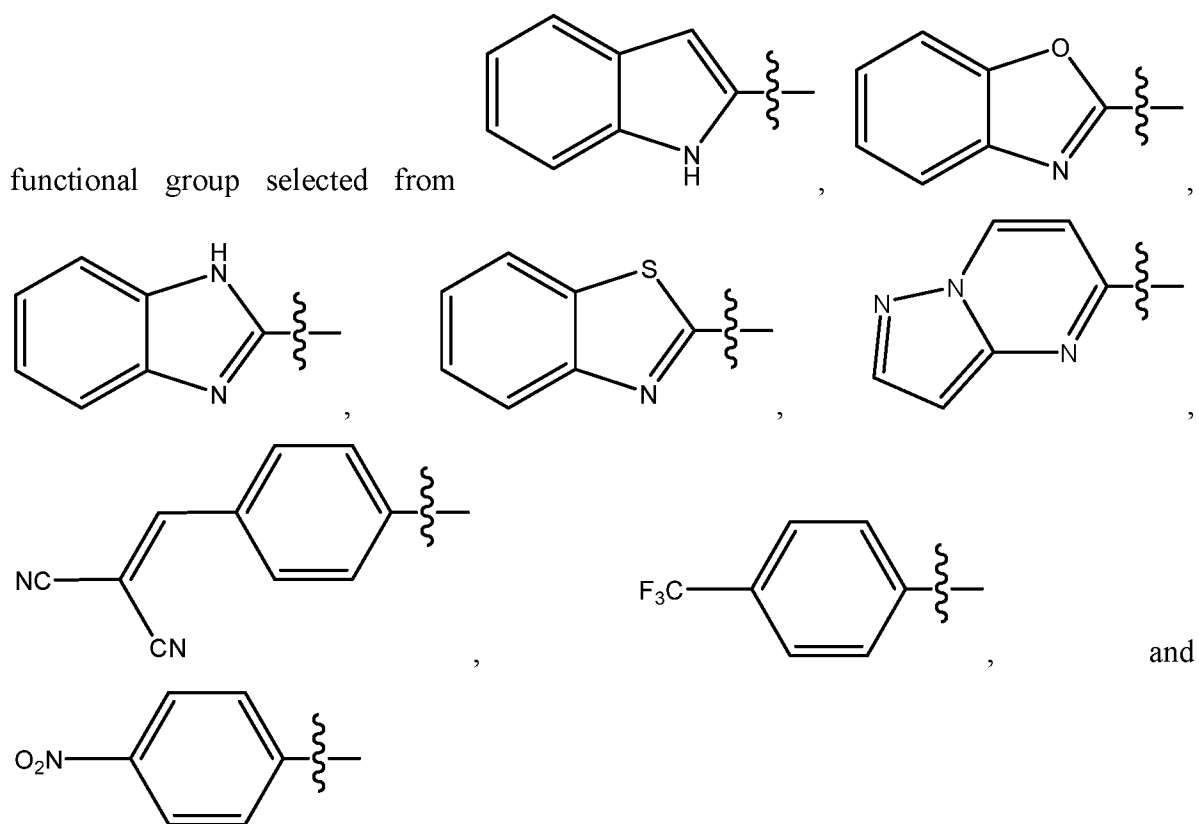
[0035] In some embodiments, the luminescent sila- and germafluorenes contain alkoxy substituents at the 2,7- positions. In some embodiments, the luminescent sila- and germafluorenes contain alkoxy substituents at the 3,6- positions.

[0036] In some embodiments, when the luminescent sila- and germafluorenes contain alkynyl(aryl) substituents at the 2,7- positions, the luminescent sila- and germafluorenes contain alkoxy substituents at the 3,6- positions. In some embodiments, when the luminescent sila- and germafluorenes contain alkynyl(aryl) substituents at the 3,6-

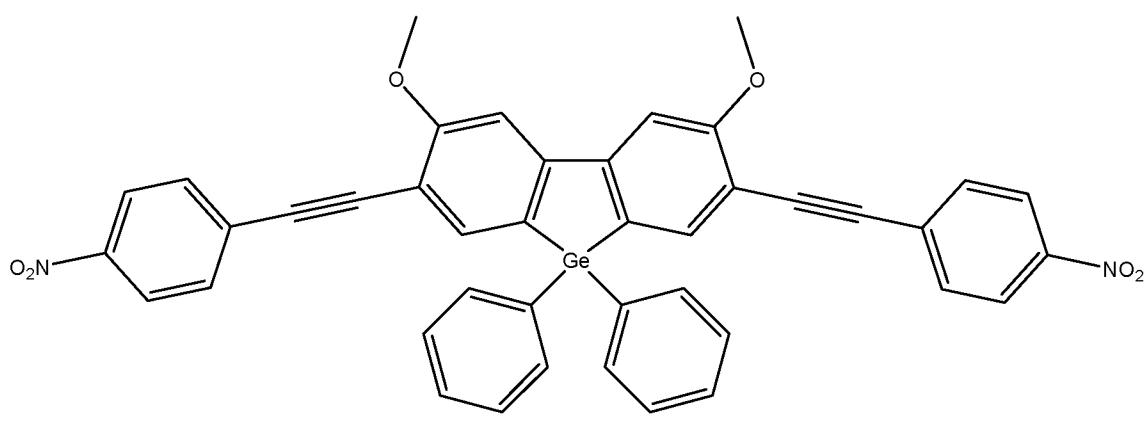
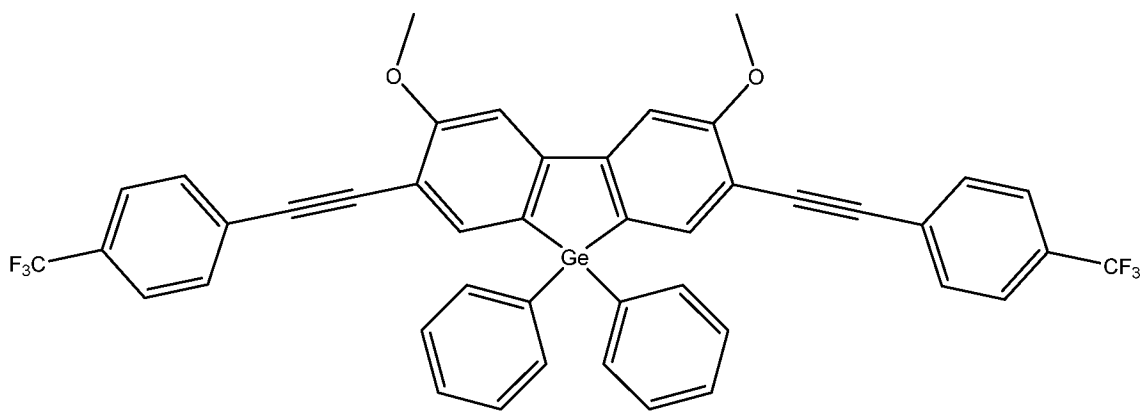
positions, the luminescent sila- and germafluorenes contain alkoxy substituents at the 2,7-positions.

[0037] In some embodiments, when the luminescent sila- and germafluorenes contain aryl substituents at the 2,7-positions, the luminescent sila- and germafluorenes contain alkoxy substituents at the 3,6-positions. In some embodiments, when the luminescent sila- and germafluorenes contain aryl substituents at the 3,6-positions, the luminescent sila- and germafluorenes contain alkoxy substituents at the 2,7-positions.

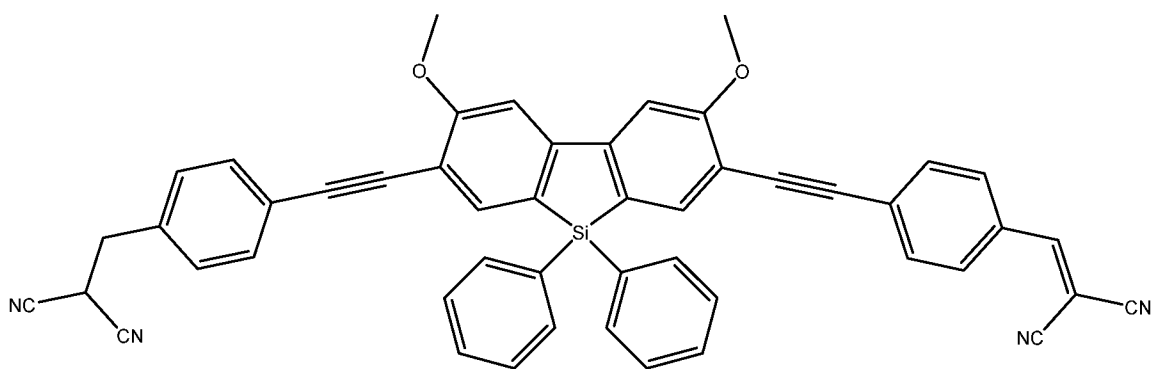
[0038] In some embodiments, the luminescent sila- and germafluorenes contain at least one alkynyl(aryl) substituent at either the 2,7- or the 3,6-positions comprising a



[0039] In some embodiments, the luminescent sila- and germafluorenes are selected from



and



[0040] In some embodiments, the luminescent sila- and germafluorenes contain at least one aryl substituent bound to the silicon or the germanium atom. In some embodiments, the luminescent sila- and germafluorenes contain two aryl substituents bound to the silicon or the germanium atom. In some embodiments, the luminescent sila- and germafluorenes contain two phenyl substituents bound to the silicon or the germanium atom.

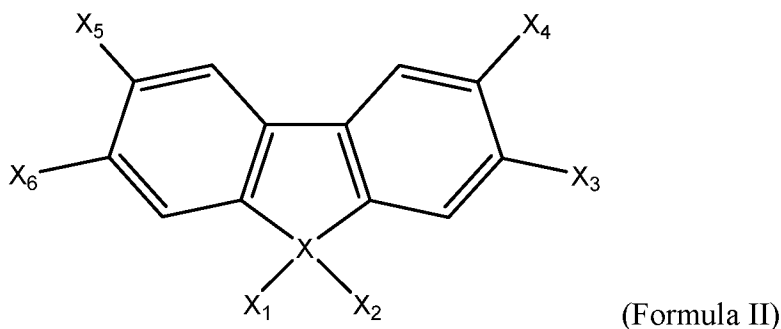
[0041] In some embodiments, a plurality of individual luminescent sila- and germafluorenes are bonded together by their substituents. In some embodiments, a plurality of individual luminescent sila- and germafluorenes are bonded together by their substituents that are identical. In some embodiments, a plurality of individual luminescent sila- and germafluorenes are bonded together by their substituents that are different.

[0042] In some embodiments, the luminescent sila- and germafluorene is solvatochromic.

[0043] In some embodiments, the luminescent sila- and germafluorenes are produced according to a method comprising a reaction selected from an organometallic reaction, a metalation reaction, a coupling reaction, a boron-based coupling reaction, a Suzuki coupling reaction, a Sonogashira coupling reaction, a Negishi coupling reaction, a Heck coupling reaction, a Stille coupling reaction, a Kumada coupling reaction, a Schiff base reaction, and combinations thereof. In some embodiments, the luminescent sila- and germafluorenes are produced according to a method comprising a Sonogashira coupling reaction.

[0044] In some embodiments, the luminescent sila- and germafluorenes are produced according to a method comprising a Sonogashira coupling reaction of

a compound of Formula II,



wherein X is Si or Ge;

wherein X₁ and X₂ are each independently selected from the group consisting of hydrogen, alkyl, and aryl; and

wherein X_3 , X_4 , X_5 , and X_6 are each independently selected from the group consisting of hydrogen, alkoxy, and halogen with the proviso that at least two of X_3 , X_4 , X_5 , and X_6 are halogen; and

a compound of Formula III,

$R-Y$ (Formula III)

wherein R is selected from the group consisting of hydrogen, alkynyl, and borono.

[0045] In some embodiments, the luminescent sila- and germafluorenes are produced according to a method comprising a Sonogashira coupling reaction that utilizes a mixture comprising a noble metal catalyst, a base, and optionally a metal catalyst. In some embodiments, the noble metal catalyst is selected from palladium, $Pd(PPh_3)_4$, $[Pd(PPh_3)_2Cl_2]$, palladium complexes comprising phosphine ligands, gold, silver, ruthenium, rhodium, osmium, iridium, platinum, and combinations thereof. In some embodiments, the base is selected from amines, secondary amines, piperidine, morpholine, diisopropylamine, and combinations thereof. In some embodiments, the metal catalyst is selected from copper, silver, nickel, gold, palladium, dendrimeric palladium complexes, nitrogen ligands, N-heterocyclic carbene palladium complexes, and combinations thereof.

[0046] In some embodiments the luminescent sila- and germafluorenes are produced according to a method comprising heating a reaction mixture to a temperature in the range of about 60 °C to about 100 °C. In some embodiments the luminescent sila- and germafluorenes are produced according to a method comprising heating a reaction mixture to a temperature in the range of about 70 °C to about 90 °C. In some embodiments the luminescent sila- and germafluorenes are produced according to a method comprising heating a reaction mixture to a temperature of about 80 °C.

[0047] In some embodiments, the luminescent sila- and germafluorenes are used for a purpose selected from the group consisting of measuring luminescence, measuring fluorescence, solvatochromic probing, imaging lipid domains, imaging live cells, providing

fluorescence in a polymer light emitting diode, providing fluorescence in an organic light emitting diode, and combinations thereof.

EXAMPLES

[0048] Example 1. Chimeric Environment Sensitive Fluorescent Probes.

[0049] Background.

[0050] Biological membranes are dynamic in their composition and properties, and local differences participate in cellular processes in a profound way and have been linked to apoptosis, viral entry, and cellular stress. It is now understood that there are ordered (Lo) regions of membranes, which are tightly packed and composed of saturated lipids and cholesterol. Other regions containing (more) unsaturated lipids are more fluid and more disordered (Ld) (**Figure 1**). These differences result in detectable differences in polarity and viscosity.

[0051] Fluorescence spectroscopy is an ideal tool for observing these differences. This technique relies on the development of suitable probes. As summarized in **Figure 2**, there are two basic designs: 1, lipid conjugates in which a probe is attached to a lipid, most commonly NBD and BODIPY. 2, inherently lipophilic molecules that are either dyes linked to L(ong) C(hain) H(ydrocarbons) (LCH), of which c-laurdan and Dil/DiO are examples; or inherently planar, extensively conjugated and hydrophobic in character (P(olycyclic) A(romatic) H(ydrocarbons)), of which naphthopyrene is an example. Still others combine features LCH and PAH, of which NR12S and c-laurdan are examples.

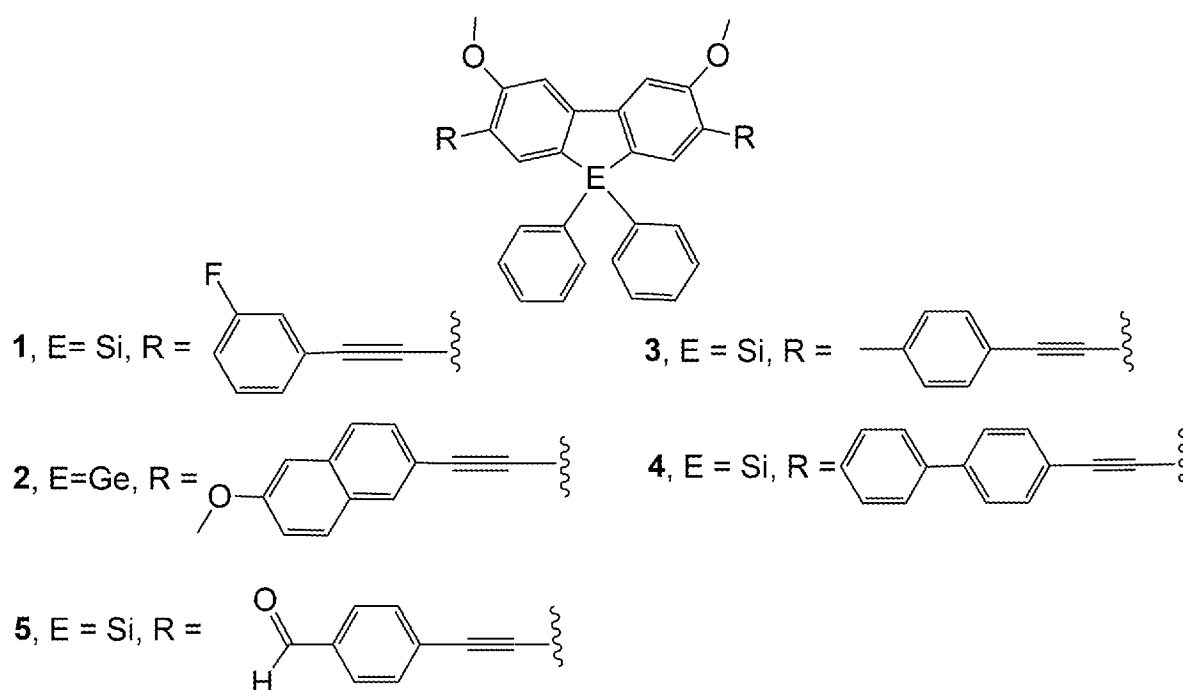
[0052] Solvatochromic probes, which respond to differences in polarity and/or viscosity by emitting light of different wavelengths as a function of molecular environment, are especially advantageous for imaging lipid domains. **Figure 3** illustrates how Nile Red can be used to detect lipid phases in giant unilamellar vesicles (GUVs).

[0053] While there are a number of dyes on the market, there is a continuing demand for dyes that are water soluble; sensitive (as gauged by extinction coefficient and quantum yield), and have excellent photostability and favorable excitation and emission wavelengths, that is, those that do not interfere with common chromophores in cells or other

dyes when used with them. When this is coupled with the rapidly expanding research area, it is no surprise that a call for more probes to meet expanding needs is prominently articulated.

[0054] Metallafluorenes.

[0055] Metallafluorenes are Group 14-centered aromatic polycyclic compounds (**Scheme 1**) that exhibit impressive luminescent properties that are tunable via the 2,7 substituent.



Scheme 1. Small Metallafluorene Library.

[0056] With this small library, it has already been demonstrated that these compounds are soluble and luminescent in aqueous solution at biologically relevant concentrations. They interact with membrane-mimicking surfactants and small unilamellar vesicles, as evidenced by impressive luminescence enhancements and quantum yield. Lastly, they can enter cells and localize to lipid droplets in yeast cells with good photostability.

[0057] Metallafluorenes show promise as being more desirable solvatochromic dyes than Nile Red. It has been shown:

[0058] 1. While Nile Red and MF's have similar quantum yields, MFs have higher extinction coefficients, often over $50,000 \text{ M}^{-1}\text{cm}^{-1}$ relative to $38,000 \text{ M}^{-1}\text{cm}^{-1}$ for Nile Red. This contributes to increased sensitivity.

[0059] 2. They have good photostability. Nile Red has limited photostability.

[0060] 3. They result in more punctate *in vivo* images than Nile Red (**Figure 4**).

[0061] 4. A MF with a benzaldehyde substituent (**5**) is clearly solvatochromic (**Figure 5**).

[0062] Design and preparation of Chimeric Metallafluorenes.

[0063] DFT Calculations Guide Design of New Class of Chimeric Solvatochromic MFs.

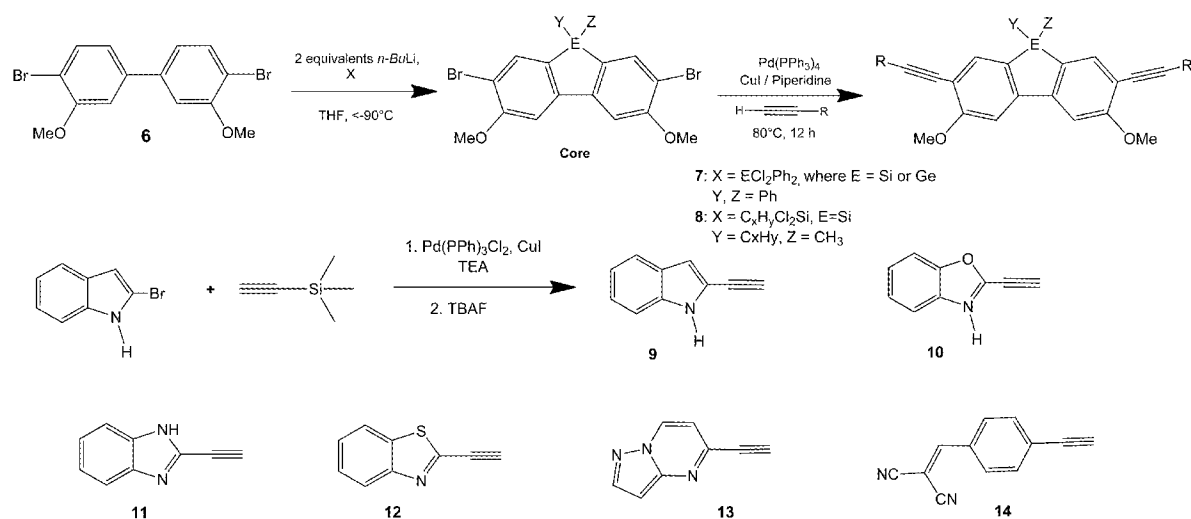
[0064] In Density Functional Theory (DFT) calculations using Spartan software with a density functional B3LYP-6-31G*, the solvatochromic **5** has a high dipole moment and a small HOMO-LUMO gap (**Table 1**). A map of HOMO (highest unoccupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) for **5** clearly shows that upon excitation, electrons move from the MF core into the 2,7 substituents, indicating a charge transfer mechanism (ICT) characteristic of many successful fluorescent lipid probes (**Figure 6**).

[0065] These features could indicate strong potential for solvatochromic behavior and could serve as a guide in the design of a new class of MFs.

[0066] Due to their amenable photophysical properties and synthetically advantageous scaffolding, the MFs represent a unique opportunity to add meaningfully to the current repertoire of lipophilic probes. New chimeric MFs that combine features of known 2,7 substituted MFs with known lipophilic probes have been designed to create hybrid molecules that combines the tunability, high absorbance, solvatochromism, high quantum yield (i.e. sensitivity) of an MF with advantageous features of known fluorescent probes. **Scheme 2** describes the preparation of solvatochromic metallafluorenes. To generate the same core structure as in **1-4**, **6** is reacted with 2 eq. of n-BuLi and ECl_2Ph_2

(where E is either Si or Ge) to yield **7**, which when reacted with (**9-14**) yields (**15Si**, **15Ge**, **17Si**, **17Ge**, **19Si**, **19Ge**, **21Si**, **21Ge**, **23Si**, **23Ge**, **25Si**, **25Ge**).

[0067] To add an acyl chain to each of the silafluorenes, 4,4'-dibromo-2,2'-diiodo-5,5'-dimethoxy-1,1'-biphenyl mixed with dodecylmethyldichlorosilane in the presence of *n*-butyl lithium and tetrahydrofuran as a solvent will yield an alkyl silafluorene core (**8**) which can be used form MFs with a long chain extending from the core (**16Si**, **18Si**, **20Si**, **22Si**, **24Si**, **26Si**).



Scheme 2. Synthesis of 2,7-disubstituted metallafuorenes.

[0068] **Table 1** summarizes the properties of new chimeric solvatochromic metallafuorenes. These compounds exhibit the highest dipole moments observed in the above MFs and their corresponding HOMO-LUMO maps show classic ICT behavior similar to that shown in **Figure 6**. These features are consistent with a high degree of solvatochromism and increased likelihood that these designs will result in higher performance fluorescent probes.

[0069] Table 1. Summary of Metallafuorenes and Properties^a.

Compound	Comment	Dipole moment
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1	Si			Phenyl fluorine	3.4
2	Ge			Methoxy-naphthyl	6.9
3	Si			Tolyl	2.1
4	Si			Biphenyl	3.1
5	Si			benzaldehyde	8.8
	X	Y	R		
15Si, 15Ge	ECl ₂ Ph ₂ , where E = Si or Ge	Ph	9	indole	4.3/6.9 ^b
16Si	C _x H _y Cl ₂ Si	C _x H _y	9	indole	6.5
17Si, 17Ge	ECl ₂ Ph ₂ , where E = Si or Ge	Ph	10	benzoxazole	15/16
18Si	C _x H _y Cl ₂ Si	C _x H _y	10	benzoxazole	-
19Si, 19Ge	ECl ₂ Ph ₂ , where E = Si or Ge	Ph	11	benzimidazole	13
20Si	C _x H _y Cl ₂ Si	C _x H _y	11	benzimidazole	-
21Si, 21Ge	ECl ₂ Ph ₂ , where E = Si or Ge	Ph	12	thiazole	6.4
22Si	C _x H _y Cl ₂ Si	C _x H _y	12	thiazole	-
23Si, 23Ge	ECl ₂ Ph ₂ , where E = Si or Ge	Ph	13	pyrazolo-pyrimidine	8.4
24Si	C _x H _y Cl ₂ Si	C _x H _y	13	pyrazolo-pyrimidine	-
25Si, 25Ge	ECl ₂ Ph ₂ , where E =	Ph	14	malononitrile	5.4

Si or Ge

26Si	$C_xH_yCl_2Si$	C_xH_y	14	malononitrile	-
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^aComputed using Spartan in the ground state under polar conditions (DMF). ^bSi/Ge.

[0070] A 2,7-disubstituted MF can be prepared with the indole group of the fluorescent probes DiD (**15Si**, **15Ge**, **16Si**) and DiO (**17Si**, **17Ge**, **18Si**). A brominated indole, the sidechain of the fluorescent amino acid tryptophan, will be modified to form an ethynyl group at the C2 position (**9**) as per **Scheme 2**; **10** is a commercially available ethynyl benzoxazole. MFs with the remaining R groups (**12-14**, to make **21Si**, **21Ge**, **22Si**, **23Si**, **23Ge**, **24Si**, **25Si**, **25Ge**, **26Si**) also have high computed dipole moments and HOMO-LUMO maps that show ICT behavior. Thus, there is a high probability that they will be solvatochromic. These latter R groups (**12-14**) can be easily prepared.

[0071] The benzylidene malononitrile substituent (**14**) is common to molecular rotors that are specifically sensitive to viscosity. In viscous environments, rotation about bonds is slowed and quantum yield increased. In this way, they can report on the viscosity of microenvironments in lipid structures, which are correlated with Lo and Ld. **25Si**, which has already been synthesized, shows promising characteristics as lipid structure probes such as red-shifted excitation and emission maxima relative to compounds **1-4**. While compounds **1-4** are a yellow powder in the solid state with bright blue emission, **25Si** is red in the solid state with a solvatochromic emission from orange to pale yellow.

[0072] Compound **14** has been demonstrated to be solvatochromic (Figures 7-9). It has also been demonstrated that the emission of Compound **14** is sensitive to viscosity in a water-methanol system (Figure 10). Finally, Compound **14** has been used to stain yeast cells and co localizes with the lipid probe Nile Red (Figure 11).

[0073] Thus, the compounds of this example are useful as chimeric environment sensitive fluorescent probes.

[0074] Example 2. Tuning Emission of Luminescent 2-7 Disubstituted Sila-and Germafluorenes with -(trifluoromethyl)phenyl, -(malononitrile)phenyl, and -nitrobenzene substituents.

[0075] Introduction.

[0076] Compounds with desirable fluorescent properties have the potential for a wide range of applications. Highly efficient polymer light emitting diodes (PLEDs) and organic light emitting diodes (OLEDs) that have high current, power, and external quantum efficiency have been of recent interest for solid state applications.

In the solution realm, there is ongoing demand for well-behaved compounds to serve as fluorescent probes of biological behavior. This can be as probes of specific molecular interactions in vitro as well as probes of cellular structures and behavior via confocal microscopy. Those with push-pull, intramolecular charge transfer (ICT) behavior are sensitive to environment (more specifically, solvatochromic) and therefore of particular interest. The magnitudes of HOMO-LUMO gaps and evidence of intramolecular charge transfer characteristics can be correlated with promising probe behavior. Due to the demand to find molecules that balance a complex mixture of desirable properties such as emission wavelength range, sensitivity and photostability, there is ongoing demand for well-behaved compounds to serve as fluorescent probes.

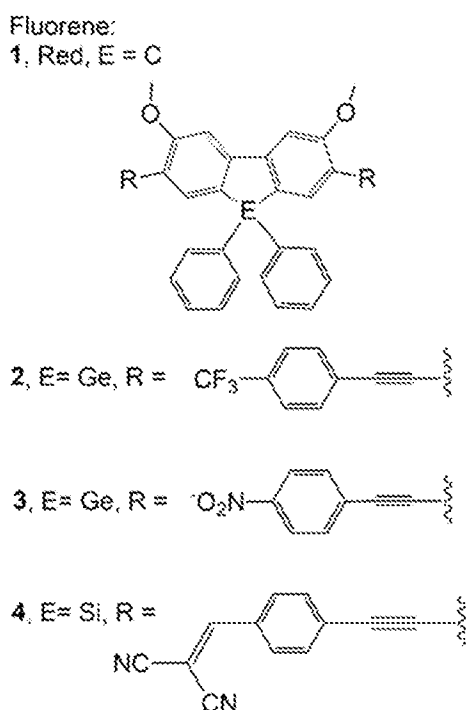
[0077] Group 14 metalloles, which include Si-, Ge-, or Sn-based conjugated molecules with a fluorene core (metallafluorenes (or MFs), **Scheme 3**), offer desirable emission properties both in solid state and in solution. The extended conjugation afforded by these structures provide the potential for push-pull behavior.

[0078] Indeed, fluorenes, sila- and germafluorenes have been applied in OLEDs of varying wavelengths and colors. Since they are structurally related to well-known biological dyes such as prodan and Nile Red, fluorenes, and metallafluorenes are being explored for their potential as probes of membranes and their interactions, including live cell and two photon imaging. The use of fluorophores in stimulated emission depletion spectroscopy (STED) and applications in imaging has been recently reviewed. Here there is also ongoing interest in developing probes that are suitable for this technique.

[0079] With an eye toward the development of useful new compounds, two basic structural themes have emerged in the recent MF literature: one is incorporating another bridge at the MF core. Arsenic bridged sila- and germafluorene with high phosphorescence

capability have been recently reported. Another theme is new substitutions at either the 2,7- or 3,6-positions. Hybrid spirocyclic germafluorene-germoles have been prepared in which the substituents are used to tune emission behavior. Dithiene silole and germoles with dithienylborane substituents have been used to make macrocycles.

[0080] Another structural variation is linker type between the polycyclic core and substituents, which is either a conjugated alkene or the more rigid alkyne to serve as a bridge to more extended structures. Recently, silafluorenes containing 2,7- or 3,6-divinylpyridinium substituents with small HOMO LUMO gaps have been prepared and applied as two-photon probes for live cell imaging. More recently, strongly electron accepting tetracyanobutadiene and tetracyanoquinodimethane moieties were incorporated at the 2,7-position between the aryl conjugated groups to tune the HOMO-LUMO gap.



Scheme 3.

[0081] 2,7-disubstitution via an alkynyl linkage offers more rigidity and provides facile emission wavelength tunability via a wide array of commercially available building blocks. This was demonstrated earlier by preparation of a small library of these compounds via Sonagashira coupling. More complexity has been explored with dithienogermoles that

self assemble into helices. This was followed by more recent reports, which also includes Ge centered compounds. More recently, a large library of 2,7-alkynyl disubstituted silafluorenes with dimethylthio groups at the 3,6-position were reported. These compounds have good photostability and are solvatochromic.

[0082] Recently investigation of the potential of these compounds for solution applications has begun. A small library of MFs has significant sensitivity to a variety of surfactants, exhibiting high emission fold enhancements and high quantum yields, both of which indicate possible uses in wastewater treatment. These results illustrate their potential as probes of biological membranes and in confocal microscopy. In the most recent exploration, it is shown that this same library interacts with small unilamellar vesicles, has excellent photostability, and can be applied in cellular imaging.

[0083] Here the design, preparation, and characterization of unique 2,7-disubstituted metallafluorenes, which show distinctly different λ_{max} that are conferred by the corresponding substituent, is reported. In the design of these molecules, DFT calculations are used to predict push-pull properties. The preparation of additional germafluorenes brings additional breadth to this emerging class of compounds.

[0084] Results and Discussion.

[0085] Synthesis and Characterization.

[0086] Good push-pull compounds have small HOMO LUMO energy gaps and both electron donating and accepting groups separated by extended conjugation. A small library of 2,7-disubstituted metallafluorenes demonstrated HOMO-LUMO gaps of 3.22-3.41 eV. These data provide a baseline of these characteristics and a basis upon which new metallafluorenes can be designed and prepared.

[0087] It is hypothesized that 2,7-substituents that are more electron withdrawing, such as electron withdrawing 2,7 substituents extending from a core with dimethoxy groups, could exhibit desirable push-pull emission characteristics. To that end, commercially available alkynyl building blocks have been surveyed for potentially desirable substituents (**Scheme 3**). A Ge-centered MF with a trifluoromethyl group (**2**) adds to electron

withdrawing nature of the phenyl substituent and is structurally related to a previously prepared silole. Compound **3**, which contains a nitrobenzene group at the 2,7-position, introduces the potential for applications that involve sensitivity to pH. Finally, **4**, which features a malononitrile phenyl substituent at the 2,7-positions, should provide red-shifted UV-Vis and fluorescence spectra relative to the current library of metallafluorenes.

[0088] DFT calculations were conducted on 2-4, and the below table summarizes the above parameters. The HOMO-LUMO gap for 2 is similar to what has been observed with previous MFs. In contrast, the HOMO-LUMO gap for 3 and 4 is narrower.

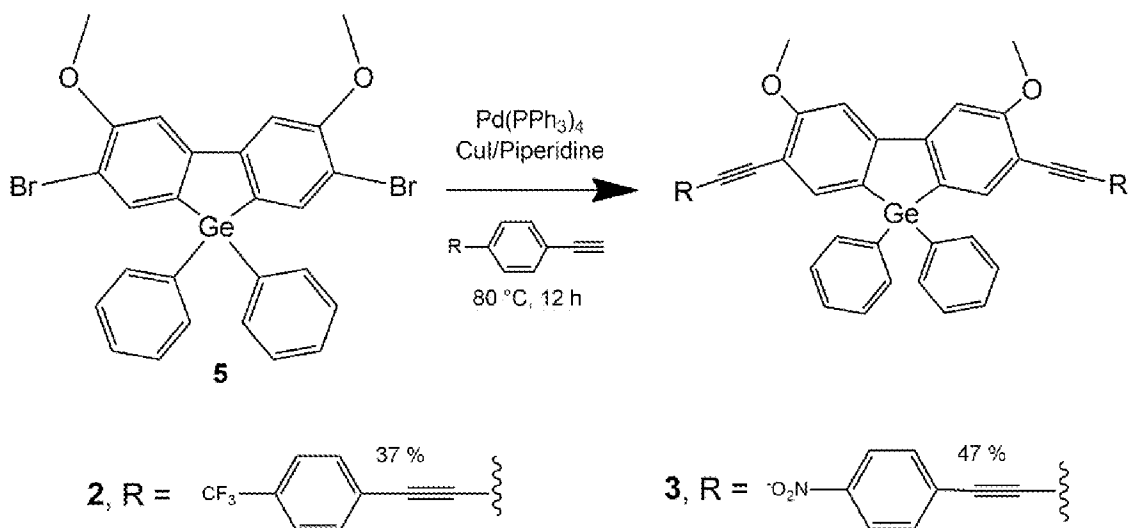
[0089] Theoretical ground state energies (eV) of metallafluorenes.^a

Compound	HOMO	LUMO	HOMO-LUMO Gap
2	-5.51	-2.14	3.37
3	-5.51	-2.99	2.52
4	-6.11	-4.52	1.59

^ain DMF.

[0090] The HOMO-LUMO orbital diagrams for these compounds are shown in **Figure 19**, which shows movement of electrons located on the fluorene core out towards the substituents. This is most modest for 2 but quite dramatic for 3 and 4. The electrostatic potential diagrams show significantly more electron withdrawing to the phenyl substituents at the 2,7-position (red) for 3 and 4 relative to 2.

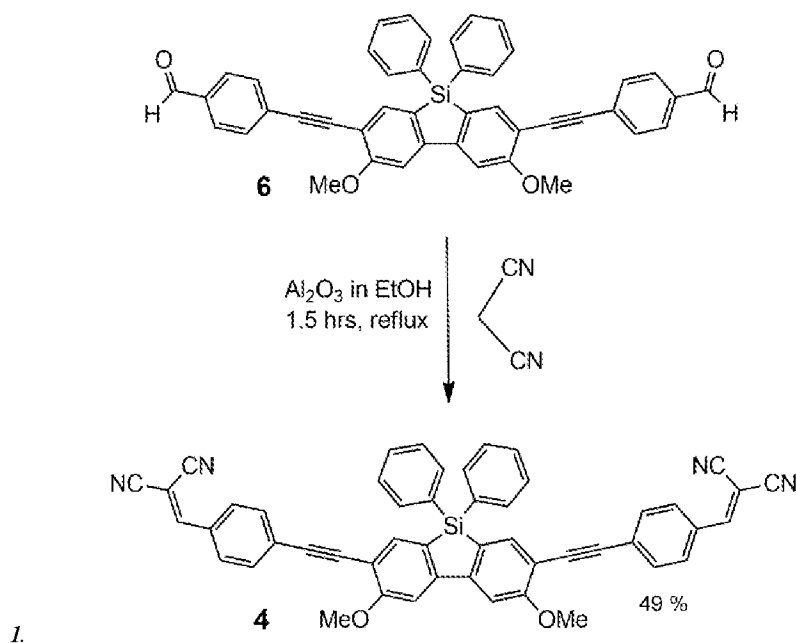
[0091] The weakly fluorescent core silole or germole (2,7-dibromo-3,6-dimethoxy-9,9-diphenyl- sila- or germafluorene) was prepared in good yield following previously published methods. The synthetic route for modification at the 2,7-position is shown in **Scheme 4**. Using commercially available alkynyl(aryl) precursors, **2** and **3** were synthesized via a palladium-catalyzed Sonagashira cross-coupling reaction.



Scheme 4. Synthesis of 2 and 3.

[0092] Both **2** and **3** were characterized by ^1H and ^{13}C $\{^1\text{H}\}$ NMR spectroscopy. IR for both showed no alkynyl proton-carbon stretch, consistent with successful coupling. Both are pale yellow in color and under UV irradiation demonstrate weak fluorescence in the solid state (**Figure 12**). Compound **2** was obtained in 37% yield and gave a correct elemental analysis. Compound **3** was obtained in 49% yield. Despite multiple attempts using elemental analysis, mass spectrometry and scanning electron microscope with energy dispersive composition (elemental) analysis (SEM EDA), neither molecular formula nor mass could be obtained for **3** that corresponds to the NMR and IR spectra, but the compound shows baseline purity via NMR spectroscopy.

[0093] The synthesis of **4** was performed using a Knoevenagel condensation (**Scheme 5**), with a yield of 49 %. The absence of the $\text{C}=\text{O}$ stretch in the IR and aldehyde proton in the ^1H NMR spectrum are consistent with a complete reaction of **6**, and the product shows a nitrile stretch in the IR spectrum at 1577 cm^{-1} and a methine proton in the ^1H NMR spectrum at 7.93 ppm. The compound is a red-orange color and also fluoresces in the solid state (**Figure 12**). Despite multiple approaches, **4** was also resistant to elemental and mass spectrometric analysis that matched clean NMR and IR spectra.



2. **Scheme 5.** Synthesis of **4**, 2,2'-((((2,8-dimethoxy-5,5-diphenyl-5H-dibenzo[b,d]silole-3,7-diyl)bis(ethyne-2,1-diyl))bis(4,1-phenylene))bis(methanylylidene))dimalononitrile.

[0094] X-Ray Crystal Structures.

[0095] Single crystals of **2** and **5** were grown by the slow solvent diffusion method. Compounds **2** and **5** were dissolved in dichloromethane and allowed to diffuse with pentane solvent in a 2:1 ratio. Good diffraction quality crystals of clear yellow color were obtained for **2** and **5** after a few days. Despite the application of multiple crystallization techniques, **3** and **4** did not produce crystals of sufficient quality for X-ray analysis. Intensity data collection and refinement procedure is given in the experimental section. The structure refinement details of compounds **2** and **5** are summarized in **Table 2**.

[0096] Compound **2** crystallized in a monoclinic spacegroup C2/c with half a molecule in the asymmetric unit (Ge-atom lies on the special position) whose corresponding equivalent component has been generated through the symmetry operation ($10x, y, \frac{1}{2}-z$). The molecular structure of **2** is shown in **Figure 13**, and refinement details are summarized in **Table 2**. The germafluorene molecular structure is almost planar except for the phenyl rings that are coordinated to the central Ge atom.

[0097] Table 2. Crystal Structure Refinement Details of **2**.

Parameters		2
Empirical formula		C ₄₄ H ₂₈ F ₆ GeO ₂
Formula weight		775.25
Temperature (K)		100
Wavelength (Å)		0.71073
Crystal system, space group		Monoclinic, C2/c
Unit cell dimensions	a (Å)	25.6908(5)
	b (Å)	11.1338(2)
	c (Å)	13.6378(3)
	α (°)	90
	β (°)	114.1320(10)
	γ (°)	90
Volume (Å ³)		3559.99(13)
Z, Calculated density (Mg/m ³)		4, 1.446
Absorption coefficient (mm ⁻¹)		0.930
F(000)		1576.0
Crystal size (mm)		0.1 × 0.1 × 0.07
Theta range for data collection (°)		2.02 to 27.11
Limiting indices		−32 ≤ h ≤ 32, −14 ≤ k ≤ 14, −16 ≤ l ≤ 17
Reflections collected / unique		39303 / 3940 [R(int) = 0.0178]

Data / restraints / parameters	3940 / 52 / 260
Goodness-of-fit on F^2	1.076
Final R indices [$I > 2\sigma(I)$]	R1 = 0.0266, wR2 = 0.0684
R indices (all data)	R1 = 0.0296, wR2 = 0.0704
Largest diff. peak and hole ($\text{e}\text{\AA}^{-3}$)	0.33 and -0.27

[0098] The substituted phenyl planes deviate significantly from the central molecular moiety showing a dihedral angle of $71.63(2)^\circ$. The geometry around the central Ge atom is tetrahedral, with this atom coordinated to four aromatic carbon atoms. In the molecular structure, the endocyclic C–Ge–C bond angle is observed to be $89.00(9)^\circ$, which is significantly smaller than the other exocyclic C–Ge–C angles which range from $106.71(9)^\circ$ to $116.02(6)^\circ$, respectively. Interestingly, the exocyclic angle formed between the two phenyl moieties is observed to be smaller compared to all other exocyclic angles (e.g., $\text{C17–Ge1–C17}^* = 106.71(9)^\circ$). However, the variation in the endocyclic angle is not the same as for all other previously reported germafluorene molecular structures. This indicates that the substituted phenyl moieties are flexible to move, which may be attributed to the nature of the substitutions at the 2,7-positions of the germafluorene molecular core. In the molecular structure, both the endocyclic and exocyclic Ge–C bond lengths are similar, with an observed Ge1–C1 value of $1.9382(14) \text{ \AA}$ and a Ge1–C17 value of $1.9402(15) \text{ \AA}$, respectively. These values are in agreement with the previously reported structures. The terminal trifluoromethyl phenyl rings (PhF_3) moieties experiences a slight twist around the conjugated triple bond $\text{C1}\equiv\text{C8}$ with a deviation of $6.67(9)^\circ$ with respect to the core germafluorene molecule.

[0099] In the three-dimensional crystal structure, the adjacent molecules are stacked parallelly along the crystallographic $[2\ 0\ 1]$ direction through $\pi\cdots\pi$ ($\pi_1\cdots\pi_2 = 4.014 \text{ \AA}$) and C–H $\cdots\pi$ ($\text{C16}\cdots\pi_3 = 3.615 \text{ \AA}$ and $\text{H16}\cdots\pi_3 = 2.700 \text{ \AA}$) interactions, respectively, in an offset manner (**Figure 14**). The adjacent $[2\ 0\ 1]$ stacking of **2** molecules are further interlinked through a weak C–H $\cdots$ F interaction such as $\text{C13–H13}\cdots\text{F2}$ (where $\text{C13}\cdots\text{F2} =$

3.453 Å, H...F = 2.603 Å and angle C-H...F = 149.21°), which constitute the molecular packing in the crystalline solid.

[0100] From the above crystallographic discussion, compounds **2** and **5** can be compared, that is, when the Br substitution in **5** is replaced by 4-trifluoromethyl -phenyl ethynyl moiety in **2**. It is observed that the Ge-C bond lengths in **2** are comparatively shorter than the Ge-C bond lengths observed in **5**. Similarly, the exocyclic C-Ge-C bond angles in **5** experience more deviation when compared to the exocyclic C-Ge-C bond angles observed in **2**. Interestingly, the C-C bond lengths of the aromatic carbon atom at 2,7-positions of the molecule **2** are observed to be C-C = 1.376(4) Å to 1.400(4) Å, which is shorter when compared to the C-C bond lengths of **5** (C-C = 1.400(2) Å to 1.405(2) Å). Moreover, the C-C-C bond angle at the 2,7-position is observed to be 121.4(3)° and 121.52(3)° in the molecular structure **5**, which is slightly elongated when compared to C-C-C bond angle observed in the molecular structure **2** (118.96(14)°). These deviations in the molecular structures are attributed to the steric effect caused by the nature of substitution at the 2,7-positions of the core molecule.

[0101] Spectral Behavior.

[0102] Absorbance spectra of compounds **2-4** in dichloromethane appear in **Figure 15A**. Spectral data for **5** in DCM has been previously reported, and this compound luminesces far more weakly than **2-4**. This observation, combined with the maps of HOMO and LUMO for **2-4**, are consistent with intramolecular charge transfer behavior afforded by 2,7-disubstitution.

[0103] The spectral features of **2** are typical of those of 2,7-disubstituted MFs reported earlier, with a major peak between 350 and 400 nm. However, **4** (solid trace) has a significantly red shifted peak (442 nm) relative to this position. The absorbance spectrum of **3** (dotted trace) is very distinct in its fine structure and blue shifted features. Compound **2** absorbs most strongly in this small group. The ϵ values for **3** and **4** (**Table 3**) are modest for this class of compounds, with **2** absorbing most strongly. Collectively, this demonstrates how 2,7-disubstitution can dramatically alter absorbance spectral properties to a degree that is uncommon in similar structures.

[0104] Table 3. UV-Visible and Spectra Data for Metallafluorenes 2-4.

Compound	λ_{max} Abs nm	ϵ $\text{M}^{-1} \text{cm}^{-1}$	λ_{max} Fluor nm	Φ_f
2	236, 376	44,100 ^b	403, 426	0.94 ± 0.006
3	250, 333	21,800 ^c	434	0.04 ± 0.02
4	337, 442	25,800 ^d	443, 557	0.25 ± 0.04

All measurements on MFs were performed in CH_2Cl_2 . ^bAt 376 nM. ^cAt 333 nM. ^dAt 442 nM.

[0105] Emission spectra for **2-4** in dichloromethane appear in **Figure 15B** with intensities normalized for easy comparison. Compound **2** (dashed trace) has an emission spectrum similar to those of previously published MFs with the dominant peak at 426 nm. Emission spectra of **3** (dotted trace) and **4** (solid trace) are significantly red shifted relative to this position, with major peaks at 434 and 557 nm, respectively. Interestingly, the intensity ratio of the peaks at 440 and 557 nm in the spectrum of **4** varies with the age of the sample. This suggests excimers, formed by the association of excited and unexcited molecules.

[0106] Solid state emission spectra of **2-4** following excitation at the respective absorbance maximum in dichloromethane appear in **Figure 15C**. Under UV irradiation, all the compounds demonstrate emission. Solid state emission spectra are red shifted but otherwise similar to the solution emission spectra. This further demonstrates the ability of the 2,7-substituents to tune the emission behavior.

[0107] As summarized in **Table 3**, the quantum yield of **2** in dichloromethane is high, comparable to those published previously for other 2,7-disubstituted silafluorenes. That of **3** is very small in dichloromethane. Due to the instability of the monomer form of **4** in dichloromethane, quantum yield measurements with **4** were performed on samples in predominantly the excimer form. Quantum yields follow the trend $\mathbf{2} > \mathbf{4} > \mathbf{3}$, with the red shifted compounds displaying lower values, which is expected of red shifted dyes.

[0108] Experimental.

[0109] General Procedures.

[0110] Reactions were carried out under argon atmosphere using standard Schlenk techniques with solvents dried and purified by standard methods. Chloroform, dichloromethane, pentane, ethanol, and hexane were purchased from Millipore Sigma and used as received. The compounds palladium(II)bis(triphenylphosphine) dichloride, tetrakis(triphenylphosphine)palladium(0), ethynyl- α,α,α -trifluorotoluene, and 1-ethynyl-4-nitrobenzene were purchased from Millipore Sigma, and malononitrile was purchased from TCI America. All were used without further purification.

[0111] All NMR spectra were collected on an Agilent 600 MHz (^1H recorded at 600 MHz, ^{13}C at 151 MHz), Bruker ARX 500 (^1H recorded at 500 MHz, ^{13}C at 126 MHz) or on a Bruker Avance 300 MHz (^1H recorded at 300 MHz and ^{13}C at 75 MHz) at ambient temperature unless otherwise noted. Chloroform- d was purchased from Cambridge Isotopes, Inc. Melting point determinations were obtained on a Mel-Temp melting point apparatus and are uncorrected. UV-vis spectra were measured on a Shimadzu UV-1800 spectrometer. Emission spectra were measured on a Fluorolog 3 fluorimeter (Horiba) using the absorbance λ_{max} as the excitation wavelength unless otherwise specified. For solid state emission spectra, a dichloromethane solution was deposited on a quartz slide and dried. The slide was positioned in the fluorimeter sample holder at 5cdan angle that optimized exposure to both excitation and emission paths. Mass spectral data (ESI) were collected on a Bruker Maxis Plus (maXis HD) quadrupole time-of-flight mass spectrometer or on a QTOF Orbitrap ESI instrument at Washington University. Elemental analyses were conducted by Atlantic Microlab, Inc., Norcross, GA. Elemental analysis was also conducted on a Thermofisher Scientific Apreo 2 HV scanning electron microscope with energy dispersive (elemental) analysis capability (SEM EDA).

[0112] Quantum yields (Φ_f) were measured using the comparative (relative) method. Absorbances were kept below 0.1 to reduce inner filter effects. The same slit widths were used on both instruments (1.0 nm). Refractive indices of organic solvents were obtained from the CRC Handbook, 87th edition. Metallafluorene quantum yields of **2**, **3**,

and **4** were measured using coumarin 102 (ethanol, 350 nm), coumarin 343 (ethanol, 300 nm) and 1-aminonaphthalene (cyclohexane, 400 nm) as standards, respectively.

[0113] Quantum yields were calculated using **Eq. 1**:

$$\Phi_{f,x} = \Phi_{f,s} \left(\frac{m_x}{m_s} \right) \left(\frac{\eta_x}{\eta_s} \right)^2 \quad \text{Eq. 1}$$

where Φ_f is the quantum yield (x, unknown fluorophore; s, reference standard), m is the slope of the integrated fluorescence emission intensity as a function of absorbance, and η is the refractive index of the solvent, the quantum yield of metallafluorenes were calculated. These measurements were performed at least twice times and the results averaged.

[0114] The weakly fluorescent core silole or germole (2,7-dibromo-3,6-dimethoxy-9,9-diphenyl- sila- or germafluorene) **5** was prepared in good yield as previously described.

[0115] *Preparation of 2,8-dimethoxy-5,5-diphenyl-3,7-bis((4-(trifluoromethyl)phenyl)ethynyl)-5H-dibenzo[b,d]germole 2.*

[0116] 2,7-Dibromo-3,6-dimethoxy-9,9-diphenylgermafluorene (**5**, 0.32 g, 0.54 mmol), tetrakis(triphenylphosphine)palladium(0) (0.062 g, 0.054 mmol), and copper (I) iodide (0.021 g, 0.11 mmol) were added to a flask and purged under argon. Then degassed distilled piperidine (6.2 mL, 63 mmol), followed by degassed 4-ethynyltrifluorotoluene (0.26 mL, 1.6 mmol) were added by syringe. The reaction mixture was stirred at 80 °C overnight. After cooling to room temperature, the crude product was filtered through a silica plug. The crude product was further purified by chromatography (silica gel, hexane /ethyl acetate 8/1) to yield the product **2** as a yellow solid (148 mmol, 0.155 g, 37 %). mpt 307 °C, decomp 412 °C.

[0117] ¹H NMR (500 MHz, CDCl₃): δ 7.87 (s, 2H, aromatic), 7.65 (d, J = 8.2 Hz, 4H, aromatic), 7.61 – 7.57 (m, 9H, aromatic), 7.44 – 7.36 (m, 9H, aromatic), 4.10 (s, 6H). ¹³C{¹H} NMR (126 MHz, CDCl₃): δ 162.4, 148.9, 138.7, 134.8, 134.5, 131.9, 130.5, 130.0, 129.9 (q, J = 32.5 Hz), 128.8, 127.5, 125.4 (dd, J = 7.4, 3.6 Hz), 123.0, 112.6, 104.8, (93.6, 88.8, alkyne) 56.2 (OCH₃). ¹⁹F{¹H} NMR (282 MHz, CDCl₃): δ 62.8. Elemental analysis

calculated for $C_{44}H_{28}F_6GeO_2$: C, 68.16; H, 3.64; F, 14.70; Ge, 9.37; O, 4.13. Found: C, 67.70; H, 3.71; F, 14.85.

[0118] *General Procedure for Preparation of 2,8-dimethoxy-3,7-bis((4-nitrophenyl)ethynyl)-5,5-diphenyl-5H-dibenzo[b,d]germole. 3*

[0119] Compound 3 was synthesized using a Sonagashira coupling reaction. 2,7-Dibromo-3,6-dimethoxy-9,9-diphenylgermafluorene (5, 0.27 g, 0.45 mmol), 1-ethynyl 4-nitrobenzene (1.3 mmol, 0.19 g), palladium(II)bis(triphenylphosphine) dichloride ($Pd(PPh_3)_2Cl_2$, 3.1 mg, 4.4 μ mol), and copper (I) iodide (18 mg, 0.94 mmol) were added to 5.2 mL (53 mmol) of piperidine and stirred continuously overnight at 80 °C. Product was purified by silica gel chromatography in 75% dichloromethane/25% hexane at 47 % yield (0.153 g, 0.21 mmol).

[0120] 1H NMR (300 MHz, $CDCl_3$): δ 8.25-8.19 (d, J = 9 Hz, 2H, aromatic), 7.83 (s, 2H, aromatic), 7.71-7.68 (d, J = 9 Hz, 4H, aromatic), 7.56-7.52 (d, J = 12 Hz, 2H, aromatic), 7.44-7.35 (m, 10H, aromatic), 7.25 (s, 2H, aromatic), 4.0 (s, 6H). The $^{13}C\{^1H\}$ NMR (75.4 MHz, $CDCl_3$): δ 158.2, 147.8, 138.4, 135.2, 134.6, 131.9, 129.2, 113.3. IR (neat, cm^{-1}): 3051 (w), 2996 (w), 2960 (w), 2842 (w), 1574 (m). The melting point is 255 °C.

[0121] *General Procedure for Preparation of 2,2'-((((2,8-dimethoxy-5,5-diphenyl-5H-dibenzo[b,d]silole-3,7-diyl)bis(ethyne-2,1-diyl))bis(4,1-phenylene))bis(methanylylidene))dimalononitrile. 4*

[0122] 4 was prepared by refluxing 0.84 mmol (0.55 g) of 6 and 0.92 mmol (0.061 g) of malononitrile under argon in the presence of aluminum hydroxide (0.2 mmol, 0.016 g) in 5 mL of dichloromethane overnight. The product was then purified using silica gel chromatography with a mobile phase of 1:1 dichloromethane and ethyl acetate in 49 % yield (0.307 g, 0.41 mmol).

[0123] 1H NMR (300 MHz, $DMSO-d_6$): δ 8.55 (s, 2H, aromatic), 8.07-7.98 (dd, J = 7 Hz, 4H, aromatic), 7.93 (s, 2H, aromatic), 7.78-7.65 (dd, J = 9 Hz, 6H, aromatic), 7.48-7.40 (m, J = 14 Hz, 10H, aromatic), 4.09 (s, 6H). $^{13}C\{^1H\}$ NMR (151 MHz, $DMSO-D_6$): δ

162.9, 160.0, 135.1, 134.8, 131.9, 130.8, 128.5, 128.1, 127.5, 118.5, 114.2, 113.2, 93.8 (alkyne), 56.4 (OCH₃). IR (neat, cm⁻¹): 2849 (w), 2192 (w), 2120 (w), 1577 (m). The melting point is 308 °C (decomp).

[0124] *X-ray Structure Determinations of 2 and 5.*

[0125] X-Ray intensity data collection of **2** and **5** were performed using Bruker D8 Venture Photon II Diffractometer equipped with MoK α ($\lambda=0.71073\text{\AA}$) microfocus X-ray source. Suitable crystal of size 0.1 x 0.1 x 0.07 mm was chosen for data collection experiment. Data collection, data reduction and absorption correction were carried out using APEX3, SAINT-plus and SADABS program. The crystal structures were solved by direct methods and refined by the method of least squares on F² using SHELX-2018 program.

[0126] In the molecular structure of **2**, the carbon atoms C18 and C19 associated with the phenyl moiety experiences a positional disorder with refined site occupancies of 0.616(11) and 0.384(11) respectively. All hydrogen atoms associated with the C-atoms are optimized to a riding atom model with a C–H distance of 0.95 Å (for aromatic C–H), $U_{\text{iso}}(\text{H}) = 1.2 U_{\text{eq}}(\text{C})$ and 0.98 Å (for methyl C–H), $U_{\text{iso}}(\text{H}) = 1.5 U_{\text{eq}}(\text{C})$ respectively. The molecular structure refinement details are summarized in **Table 4**. The molecular graphics were prepared using *OLEX2* and *mercury* software. The final molecular structure for **2** was deposited in Cambridge Crystallographic Data Centre, and the data can be accessed via CCDC ID 2264641.

[0127] The molecular structure of **5** is shown in **Figure 16**. The compound crystallized in monoclinic spacegroup P2₁/c with four molecules in the unit-cell is shown in **Figure 17**. The data collection and refinement procedures are same as for **2** and the structure refinement details are summarized in **Tables 4**. The finalized molecular structures for **2** and **5** are deposited in Cambridge Crystallographic Data Centre (ID: CCDC 2264641 and 2268554, respectively).

[0128] The germafluorene molecular structure is almost planar except the phenyl rings which are coordinated to the central Ge atom, the substituted phenyl planes deviate significantly from the central molecular moiety with a calculated dihedral angles of 78.05(12)° and 54.79(12)° respectively, the calculated dihedral angle between the phenyl

planes was found to be $73.66(16)^\circ$. The central Ge atom forms a distorted tetrahedral geometry coordinated to four aromatic carbon atoms. In the molecular structure, the endocyclic C–Ge–C bond angle is observed to be $88.46(12)^\circ$ [C1–Ge1–C12] which is significantly smaller compared to other exocyclic C–Ge–C angles which ranges from $107.46(12)^\circ$ to $118.48(12)^\circ$ respectively. Interestingly the exocyclic angle formed between the two phenyl moieties such as C15–Ge1–C21 = $107.46(12)^\circ$ is smaller relative to all other exocyclic angles. In the molecular structure both the endocyclic and exocyclic bond Ge–C bond lengths are observed to be Ge1–C1=1.946(3) Å, Ge1–C12=1.941(3) Å and Ge1–C15=1.946(3) Å, Ge1–C21=1.949(3) respectively. In the crystal structure, the molecular packing is mainly stabilized by the formation of weak C–H...Br interactions such as C14–H14C...Br1 (where C14...Br1 = 4.056 Å, H14...Br1 = 3.079 Å and angle C14–H14C...Br1 = 175.13°) and C–H... π interaction such as C13–H13A... π 1 (where C13... π 1 = 3.647 Å, H13A... π 1 = 2.804 Å and angle C13–H13A... π 1 = 144.61°), respectively (**Figure 18**), no significant strong contact is observed between the molecules.

[0129] Table 4: Crystal Structure Refinement Details for **5** and **2**.

Parameters	5	2
Empirical formula	C ₂₆ H ₂₀ O ₂ Br ₂ Ge	C ₄₄ H ₂₈ O ₂ F ₆ GeO ₂
Formula weight	596.83	775.25
Temperature (K)	100	100
Wavelength (Å)	0.71073	0.71073
Crystal system, space group	Monoclinic, P2 ₁ /c	Monoclinic, C2/c
Unit cell dimensions		
a (Å)	9.2247(4)	25.6908(5)
b (Å)	22.1755(7)	11.1338(2)
c (Å)	11.3947(4)	13.6378(3)
α (°)	90	90
β (°)	98.456(2)	114.1320(10)
γ (°)	90	90
Volume (Å ³)	2305.59(15)	3559.99(13)
Z, Calculated density (Mg/m ³)	4, 1.719	4, 1.446
Absorption coefficient (mm ⁻¹)	4.816	0.930
F(000)	1176.0	1576.0
Crystal size (mm)	0.05 × 0.03 × 0.02	0.1 × 0.1 × 0.07
Theta range for data collection (°)	2.025 to 27.114	2.02 to 27.11
Limiting indices	-11 ≤ h ≤ 11, -28 ≤ k ≤ 28,	-32 ≤ h ≤ 32, -14 ≤ k ≤ 14,

Reflections collected / independent	-14 ≤ 1 ≤ 14 54772 / 5085 [R(int) = 0.0178]	-16 ≤ 1 ≤ 17 39303 / 3940 [R(int) = 0.0178]
Data / restraints / parameters	5085 / 0 / 282	3940 / 52 / 260
Goodness-of-fit on F ²	1.012	1.076
Final R indices [I > 2σ(I)]	R1 = 0.0308, wR2 = 0.0503	R1 = 0.0266, wR2 = 0.0684
R indices (all data)	R1 = 0.0520, wR2 = 0.0572	R1 = 0.0296, wR2 = 0.0704
Largest diff. peak and hole (eÅ ⁻³)	0.49 and -0.51	0.33 and -0.27

[0130] Summary.

[0131] 2,7-disubstitution and DFT guided design have been used to tune emission wavelengths for three new fluorescent 2,7-alkynyl(aryl)-3,6-dimethoxy-9,9-diphenyl-sila- and germafluorenes have been synthesized. Two germafluorenes with trifluoromethyl phenyl and nitrobenzene substituents were prepared using a Pd-catalyzed Sonogashira coupling reaction to incorporate alkynyl(aryl) groups at the 2,7-positions of the ring. Another new silafluorene was prepared by modifying a benzaldehyde-substituted silafluorene to incorporate a malononitrile group. These compounds were characterized utilizing multinuclear NMR, UV-Vis, and fluorescence spectroscopic techniques. The germafluorene with the trifluoromethyl phenyl substituent was also characterized via elemental analysis and X-ray crystallography. These metallafluorenes, which are yellow or red-orange crystals in the solid state, showed a wide range of quantum yields and spectroscopic features, with the malononitrile-containing MF exhibiting significantly red-shifted emission relative to other MFs. These data demonstrate the value in applying DFT to MF design and the dramatic impact the 2,7 substituent has on the optical properties of this class of compounds.

[0132] Although specific features and applications of various embodiments of the disclosure may be shown in some drawings and not in others, this is for convenience only. In accordance with the principles of the disclosure, any feature illustrated herein may be referenced and/or claimed in combination with any feature.

[0133] In the specification and the claims, reference is made to a number of terms, which shall be defined to have the following meanings. The singular forms “a,” “an,” and

“the” include plural references unless the context clearly dictates otherwise. The terms “comprising,” “including,” and “having” are intended to be inclusive and mean that there may be additional elements other than the listed elements. “Optional” or “optionally” means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where the event occurs and instances where it does not.

[0134] Approximating language, as used herein throughout the specification and claims, may be applied to modify any quantitative representation that could permissibly vary without resulting in a change in the basic function to which it is related. Accordingly, a value modified by a term or terms, such as “about,” “approximately,” and “substantially,” are not to be limited to the precise value specified. In at least some instances, the approximating language may correspond to the precision of an instrument for measuring the value. Here and throughout the specification and claims, range limitations may be combined and/or interchanged; such ranges are identified and include all the sub-ranges contained therein unless context or language indicates otherwise.

[0135] As used herein, “aryl” refers to a radical of a monocyclic or poly cyclic (e.g., bicyclic or tricyclic) $4n+2$ aromatic ring system (e.g., having 6, 10, or 14 electrons shared in a cyclic array) having 6-14 ring carbon atoms and zero heteroatoms provided in the aromatic ring system (“C6-14 aryl”). In some embodiments, an aryl group has 6 ring carbon atoms (“C6 aryl”; e.g., phenyl). In some embodiments, an aryl group has 10 ring carbon atoms (“C10 aryl”; e.g., naphthyl such as 1-naphthyl and 2-naphthyl). In some embodiments, an aryl group has 14 ring carbon atoms (“C14 aryl”; e.g., anthracyl).

[0136] As used herein, “heteroaryl” refers to aryl groups that contain at least one heteroatom (such as nitrogen, oxygen, silicon, phosphorous, boron, sulfur, or a halogen atom).

[0137] As used herein, “alkyl” refers to a radical of a straight-chain or branched saturated hydrocarbon group having from, in some embodiments, 1 to 4 carbon atoms (“C1-4 alkyl”), and in other embodiments 1 to 22 carbon atoms (“C1-22 alkyl”). In some embodiments, an alkyl group has 1 to 3 carbon atoms (“C1-3 alkyl”). In some embodiments, an alkyl group has 1 to 2 carbon atoms (“C1-2 alkyl”). In some embodiments, an alkyl

group has 1 carbon atom ("C1 alkyl"). In some embodiments, an alkyl group has 2 to 4 carbon atom ("C2-4 alkyl"). In yet other embodiments, an alkyl group has 1 to 21 carbon atoms ("C1-21 alkyl"), 1 to 20 carbon atoms ("C1-20 alkyl"), 1 to 15 carbon atoms ("C1-15 alkyl"), 1 to 10 carbon atoms ("C1-10 alkyl"), etc. Examples of such alkyl groups include methyl (C1), ethyl (C2), n-propyl (C3), isopropyl (C3), n-butyl (C4), tert-butyl (C4), sec-butyl (C4), iso-butyl (C4), pentyl (C5), and the like.

[0138] As used herein, "alkynyl" or "alkyne" refers to a radical of a straight- chain or branched hydrocarbon group having from 2 to 4 carbon atoms and one or more carbon-carbon triple bonds ("C2-10 alkynyl"). In some embodiments, an alkynyl group has 2 to 3 carbon atoms ("C2-3 alkynyl"). In some embodiments, an alkynyl group has 2 carbon atoms ("C2 alkynyl"). The one or more carbon-carbon triple bonds can be internal (such as in 2-butyne) or terminal (such as in 1-butyne). Examples of C2-4 alkynyl groups include, without limitation, ethynyl (C2), 1-propynyl (C3), 2-propynyl (C3), 1- butynyl (C4), 2-butyne (C4), and the like.

[0139] Alkyl, alkynyl, and aryl groups, as defined herein, are substituted or non-substituted, also referred to herein as "optionally substituted". In general, the term "substituted", whether preceded by the term "optionally" or not, means that at least one hydrogen present on a group (e.g., a carbon or nitrogen atom) is replaced with a permissible substituent, e.g., a substituent which upon substitution results in a stable compound, e.g., a compound which does not spontaneously undergo transformation such as by rearrangement, cyclization, elimination, or other reaction. Unless otherwise indicated, a "substituted" group has a substituent at one or more substitutable positions of the group, and when more than one position in any given structure is substituted, the substituent is either the same or different at each position. The term "substituted" is contemplated to include substitution with all permissible substituents of organic compounds, any of the substituents described herein that result in the formation of a stable compound. The present disclosure contemplates any and all such combinations in order to arrive at a stable compound. For purposes of this disclosure, heteroatoms such as nitrogen may have hydrogen substituents and/or any suitable substituent as described herein which satisfy the valencies of the heteroatoms and results in the formation of a stable moiety.

[0140] As used herein, “alkoxy” refers to a radical of an oxygen atom bonded to an alkyl group. In some embodiments, the alkyl group has 1-6 carbon atoms. In some embodiments, the alkyl group has 1-5 carbon atoms. In some embodiments, the alkyl group has 1-4 carbon atoms. In some embodiments, the alkyl group has 1-3 carbon atoms. In some embodiments, the alkyl group has 1-2 carbon atoms. In some embodiments, the alkyl group has 1 carbon atom.

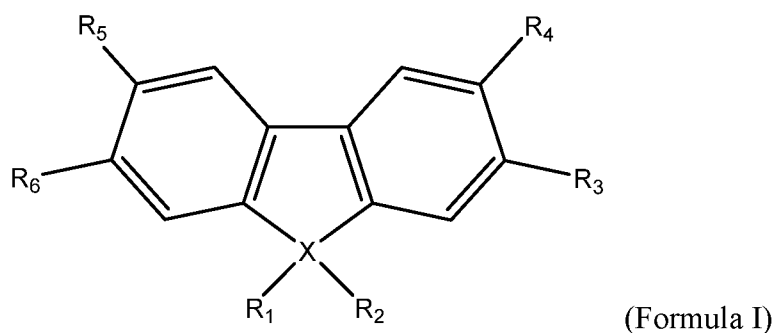
[0141] As used herein, “borono” refers to a radical of a boron atom bonded to at least one oxygen atom. In some embodiments, the borono group comprises an aryl group. In some embodiments, the borono group is selected from Ar(B)(OH)_2 , Ph(B)(OH)_2 . In some embodiments, the borono group is Ph(B)(OH)_2 .

[0142] As used herein, “halogen” refers to a radical of a halogen atom. In some embodiments, the halogen atom is selected from fluorine, chlorine, bromine, and iodine.

[0143] As used herein, a reference to a number on its own (e.g., “5”), refers the compound number of the present disclosure (e.g., “Compound 5”). The definitions for these compounds are found within the examples in which they are defined.

WHAT IS CLAIMED IS:

1. A compound of Formula I,

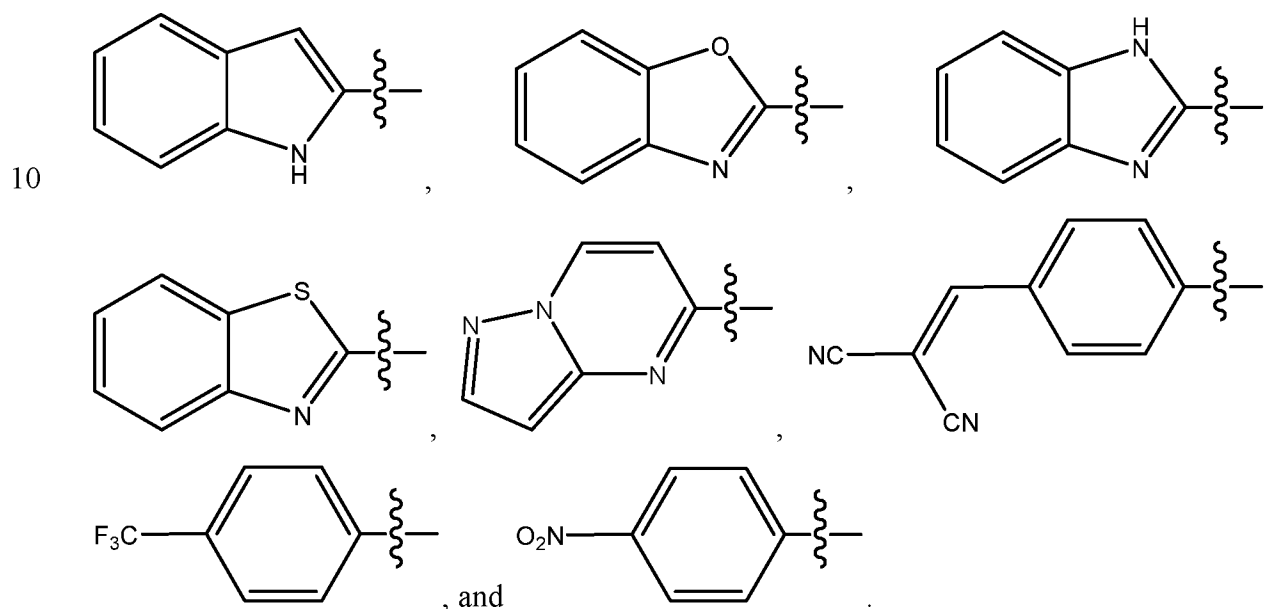


wherein X is Si or Ge;

wherein R₁ and R₂ are each independently selected from the group consisting of hydrogen,
5 alkyl, and aryl;

wherein R₃, R₄, R₅, and R₆ are each independently selected from the group consisting of
hydrogen, alkoxy, Y, and $\text{---}\text{C}\equiv\text{C---Y}$, with the proviso that at least two of R₃, R₄, R₅, and
R₆ are selected from the group consisting of Y and $\text{---}\text{C}\equiv\text{C---Y}$; and

wherein Y is selected from the group consisting of



2. The compound according to claim 1, wherein R_4 and R_5 are selected from the group consisting of Y and $\frac{\xi}{\xi} \equiv Y$ or R_3 and R_6 are selected from the group consisting of Y and $\frac{\xi}{\xi} \equiv Y$.

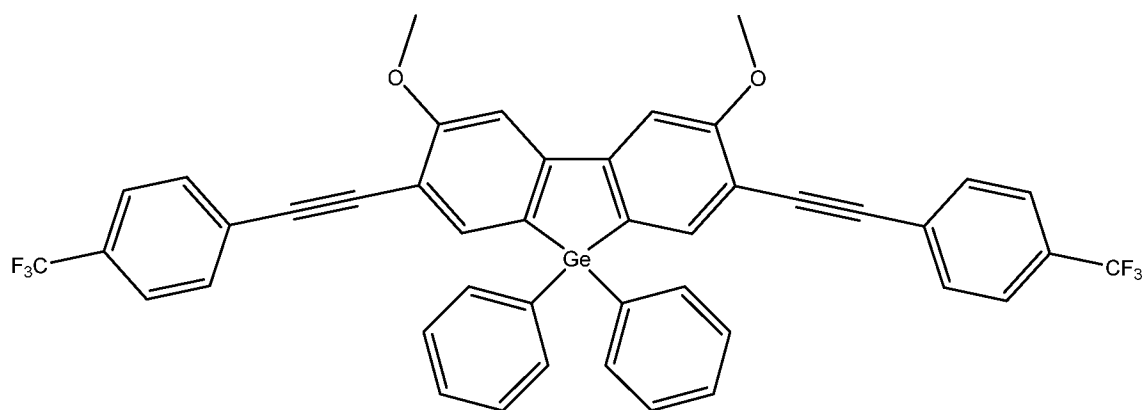
3. The compound according to claim 1, wherein R_4 and R_5 are $\frac{\xi}{\xi} \equiv Y$ or R_3 and R_6 are $\frac{\xi}{\xi} \equiv Y$.

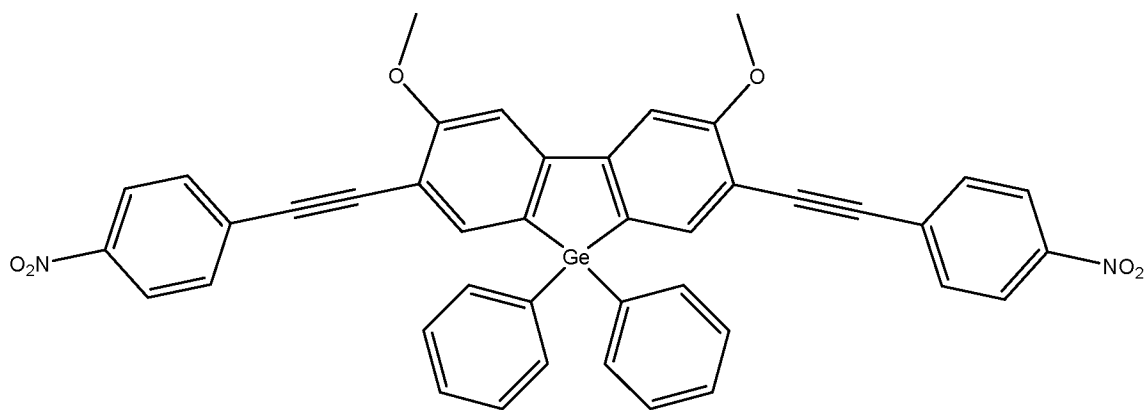
4. The compound according to claim 1, wherein R_3 and R_6 are $\frac{\xi}{\xi} \equiv Y$.

5. The compound according to claim 1, wherein X is Si.

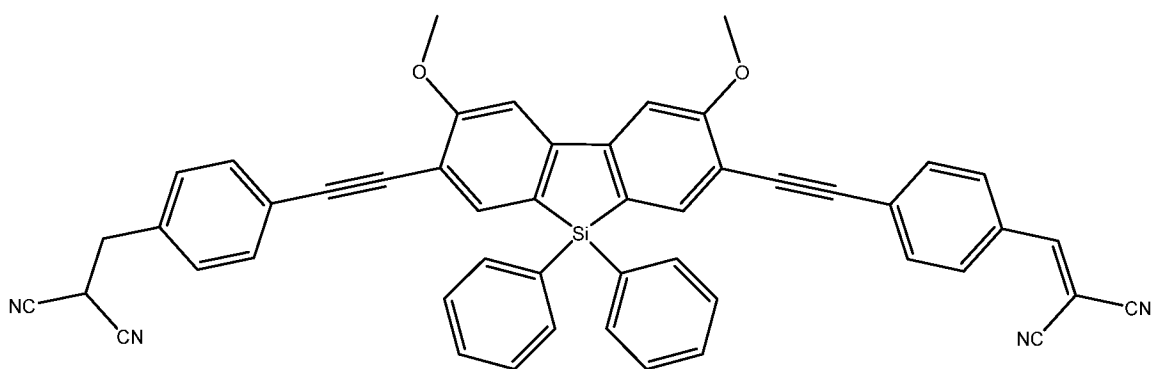
6. The compound according to claim 1, wherein X is Ge.

7. The compound according to claim 1, wherein the compound is selected from the group consisting of



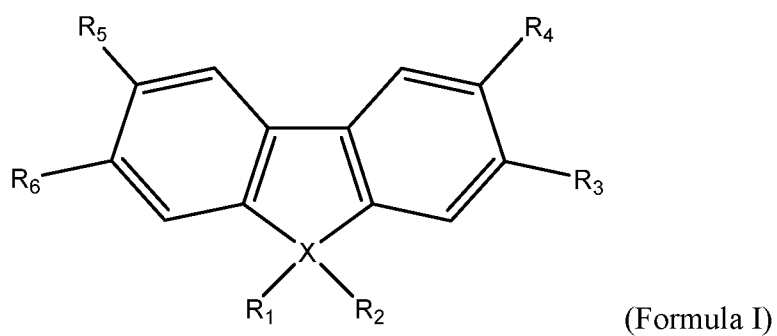


and



8. The compound according to claim 1, wherein the compound is solvatochromic.

9. A method of producing a compound of Formula I,

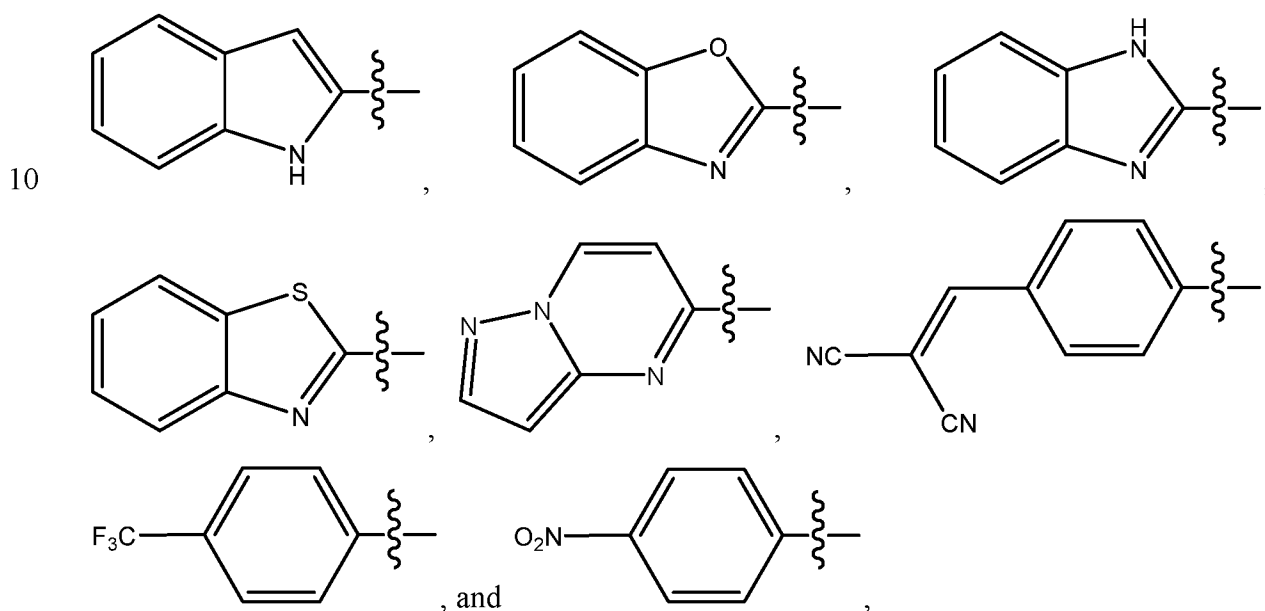


wherein X is Si or Ge;

wherein R₁ and R₂ are each independently selected from the group consisting of hydrogen,
alkyl, and aryl;

wherein R_3 , R_4 , R_5 , and R_6 are each independently selected from the group consisting of hydrogen, alkoxy, Y, and $\xi \equiv Y$, with the proviso that at least two of R_3 , R_4 , R_5 , and R_6 are selected from the group consisting of Y and $\xi \equiv Y$; and

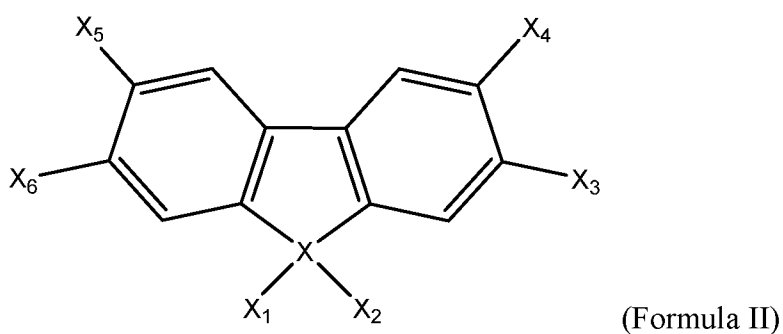
wherein Y is selected from the group consisting of



the method comprising:

forming a mixture comprising

15 a compound of Formula II,



wherein X is Si or Ge;

wherein X_1 and X_2 are each independently selected from the group consisting of hydrogen, alkyl, and aryl; and

20 wherein X_3 , X_4 , X_5 , and X_6 are each independently selected from the group consisting of hydrogen, alkoxy, and halogen with the proviso that at least two of X_3 , X_4 , X_5 , and X_6 are halogen;

a compound of Formula III,

$R-Y$ (Formula III)

25 wherein R is selected from the group consisting of hydrogen, alkynyl, and borono;

a noble metal catalyst;

a base; and

optionally a co-catalyst comprising a metal; and

30 reacting the mixture.

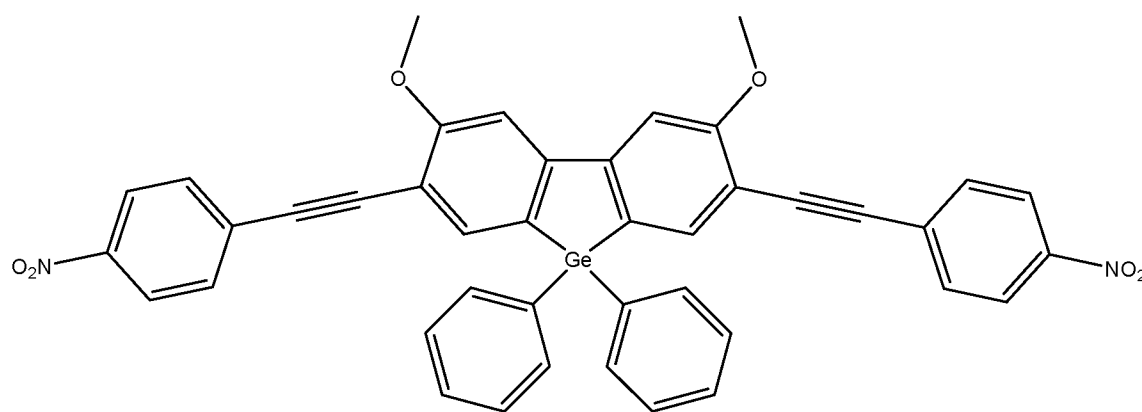
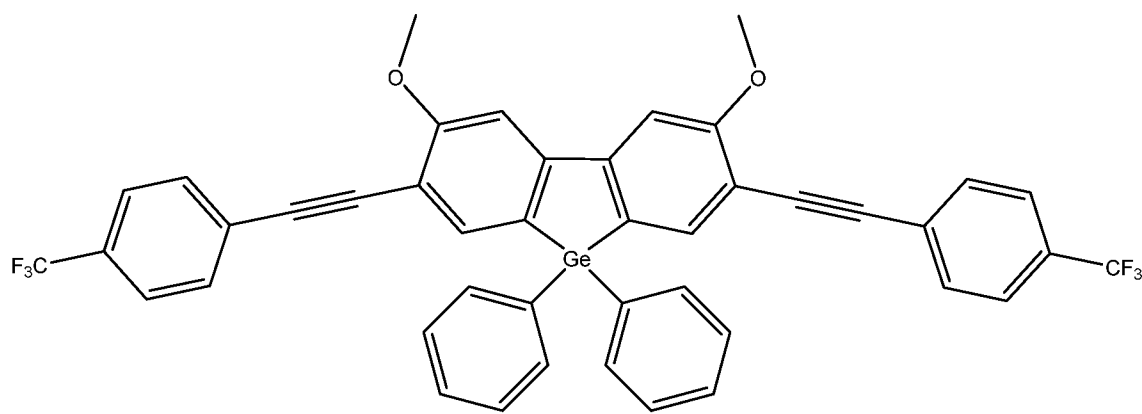
10. The method according to claim 9, wherein X_4 and X_5 are selected from the group consisting of halogen or X_3 and X_6 are selected from the group consisting of halogen.

11. The method according to claim 9, wherein R_4 and R_5 are selected from the group consisting of Y and $\frac{\xi}{\xi} \equiv Y$ or R_3 and R_6 are selected from the group consisting of Y and $\frac{\xi}{\xi} \equiv Y$.

12. The method according to claim 9, wherein R_4 and R_5 are $\frac{\xi}{\xi} \equiv Y$ or R_3 and R_6 are $\frac{\xi}{\xi} \equiv Y$.

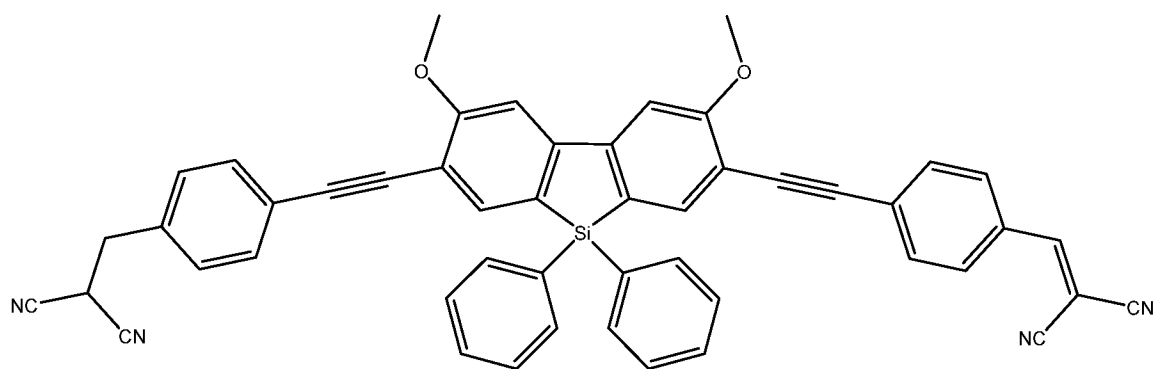
13. The method according to claim 9, wherein R_3 and R_6 are $\frac{\xi}{\xi} \equiv Y$.

14. The method according to claim 9, wherein the compound of Formula I is selected from the group consisting of

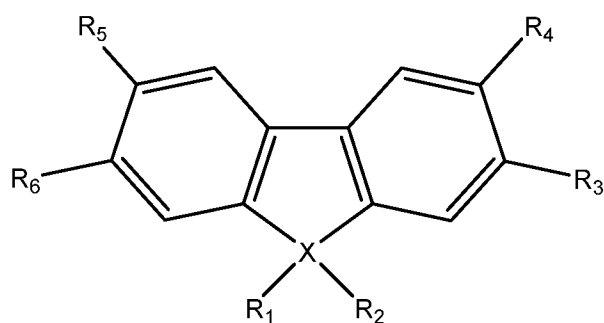


5

and



15. A method of using a compound of Formula I,



(Formula I)

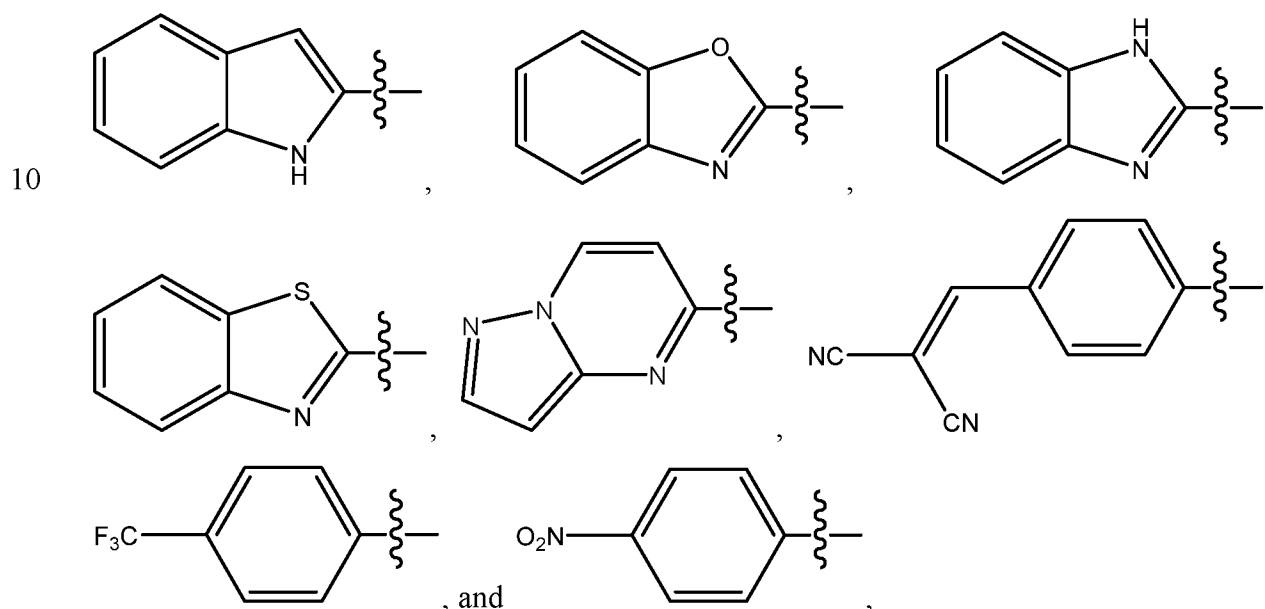
wherein X is Si or Ge;

wherein R₁ and R₂ are each independently selected from the group consisting of hydrogen,
5 alkyl, and aryl;

wherein R₃, R₄, R₅, and R₆ are each independently selected from the group consisting of
hydrogen, alkoxy, Y, and $\text{---}\text{Y}$, with the proviso that at least two of R₃, R₄, R₅, and

R₆ are selected from the group consisting of Y and $\text{---}\text{Y}$; and

wherein Y is selected from the group consisting of



the method comprising using the compound of Formula I for a purpose selected from the
group consisting of measuring luminescence, measuring fluorescence, solvatochromic
15 probing, imaging lipid domains, imaging live cells, providing fluorescence in a polymer

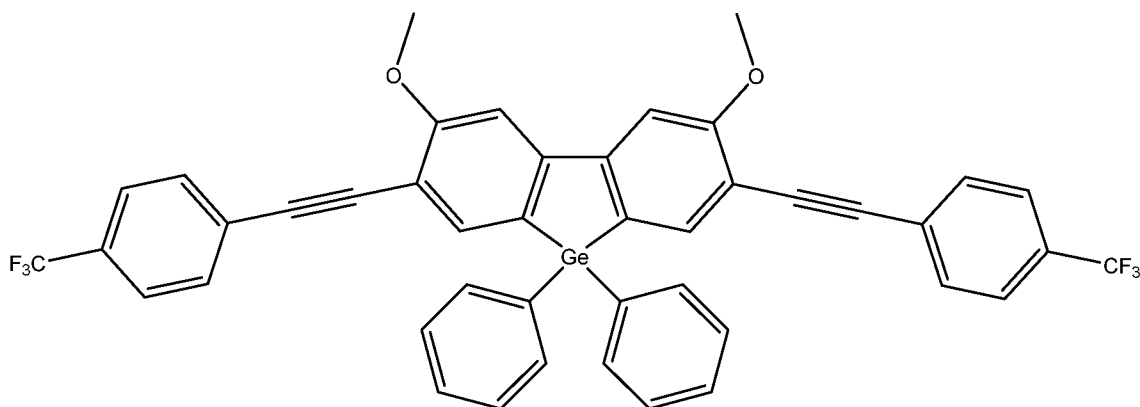
light emitting diode, providing fluorescence in an organic light emitting diode, and combinations thereof.

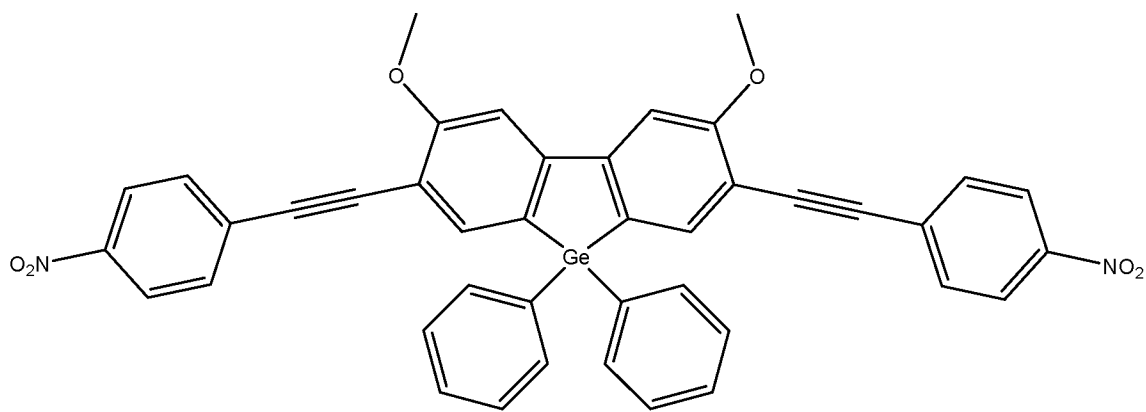
16. The method according to claim 15, wherein R_4 and R_5 are selected from the group consisting of Y and $\frac{\xi}{\xi} \equiv Y$ or R_3 and R_6 are selected from the group consisting of Y and $\frac{\xi}{\xi} \equiv Y$.

17. The method according to claim 15, wherein R_4 and R_5 are $\frac{\xi}{\xi} \equiv Y$ or R_3 and R_6 are $\frac{\xi}{\xi} \equiv Y$.

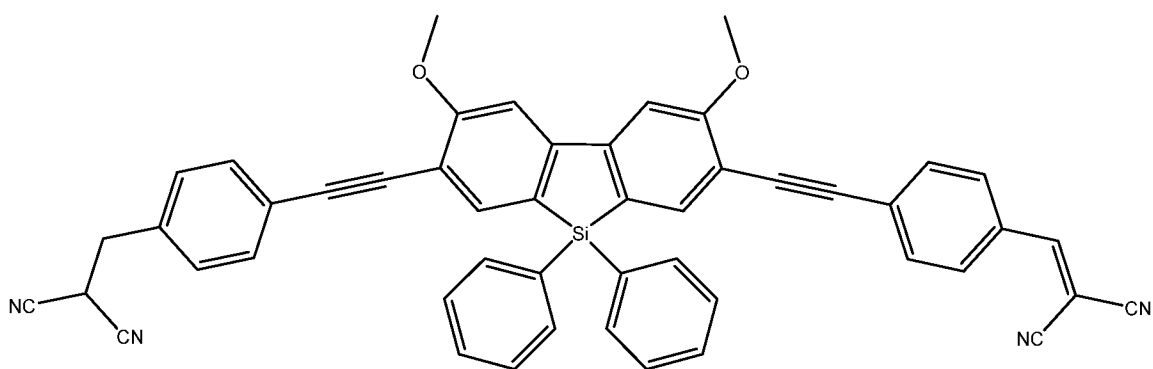
18. The method according to claim 15, wherein R_3 and R_6 are $\frac{\xi}{\xi} \equiv Y$.

19. The method according to claim 15, wherein the compound of Formula I is selected from the group consisting of





and



20. The method according to claim 15, wherein the compound of Formula I is solvatochromic.

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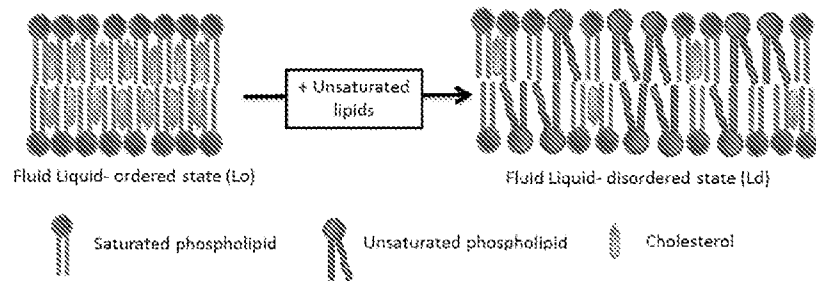


FIG. 1

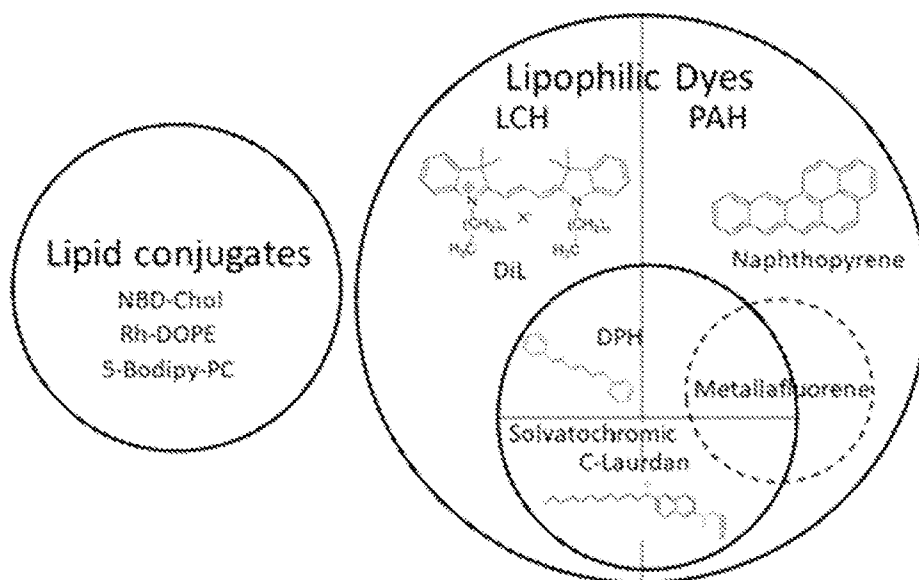


FIG. 2

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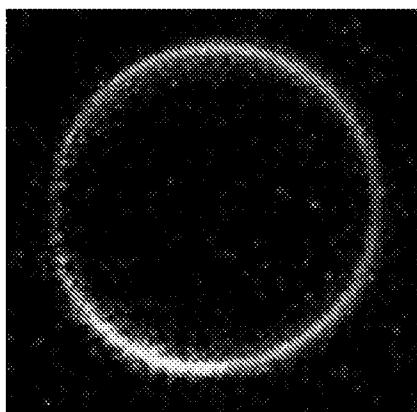


FIG. 3

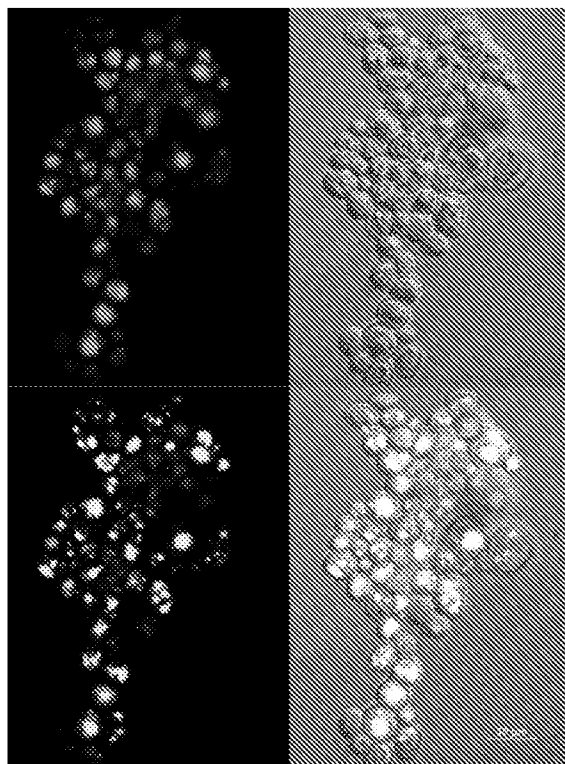


FIG. 4

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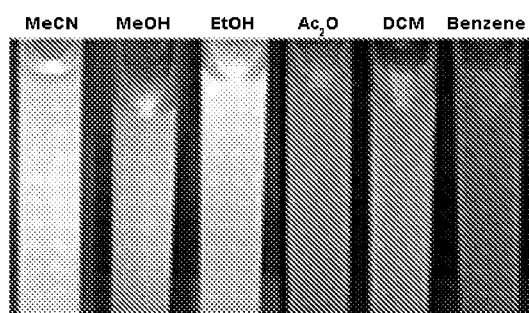


FIG. 5

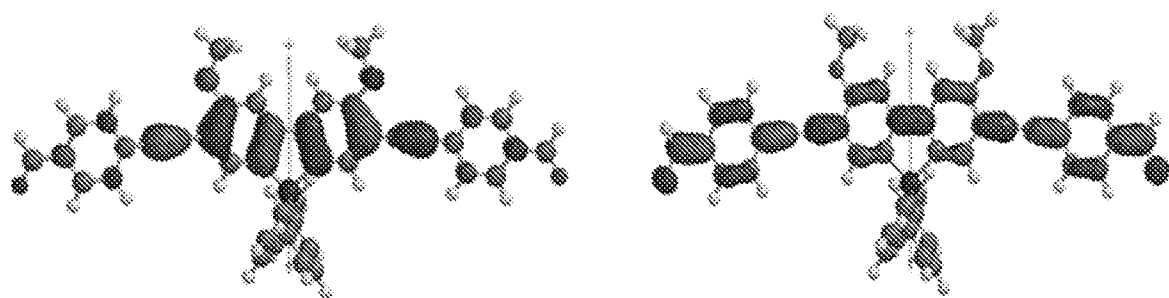


FIG. 6

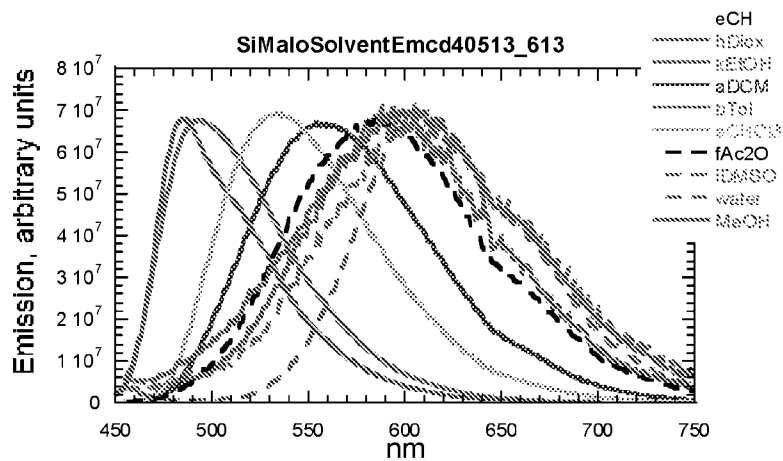


FIG. 7

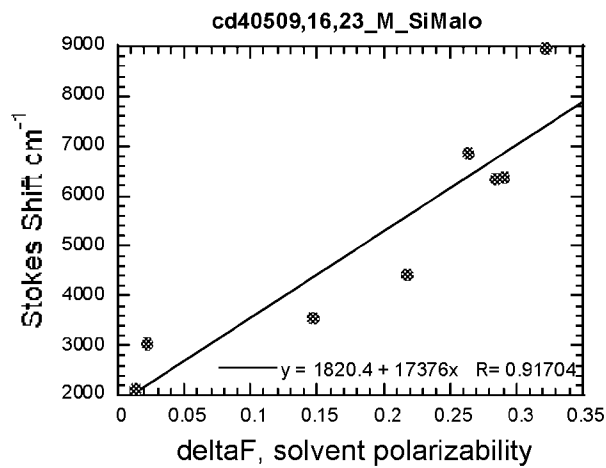


FIG. 8



FIG. 9

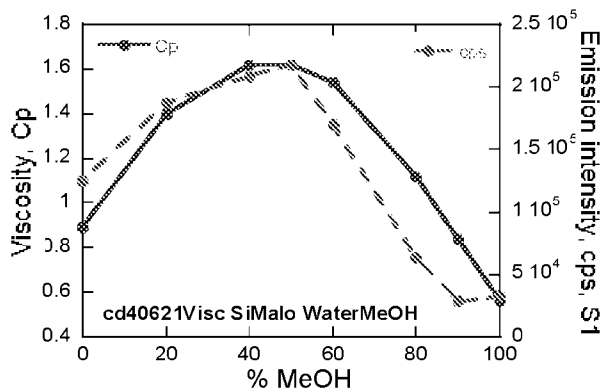


FIG. 10

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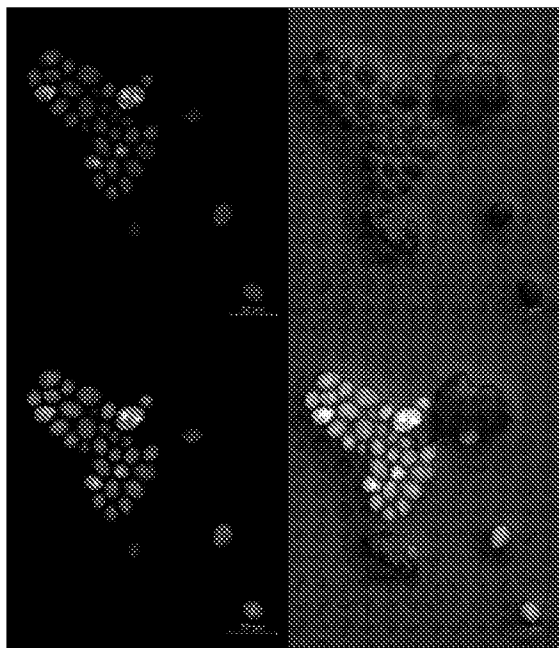


FIG. 11

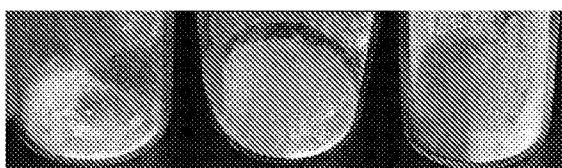


FIG. 12

FIG. 14

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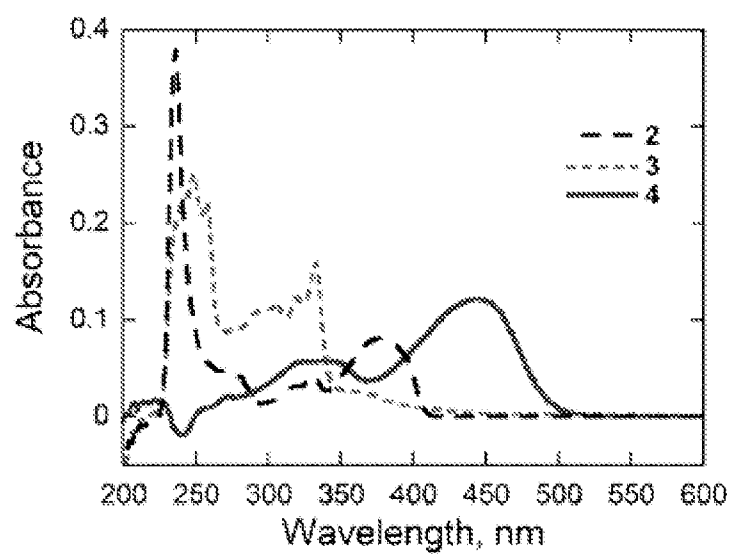


FIG. 15A

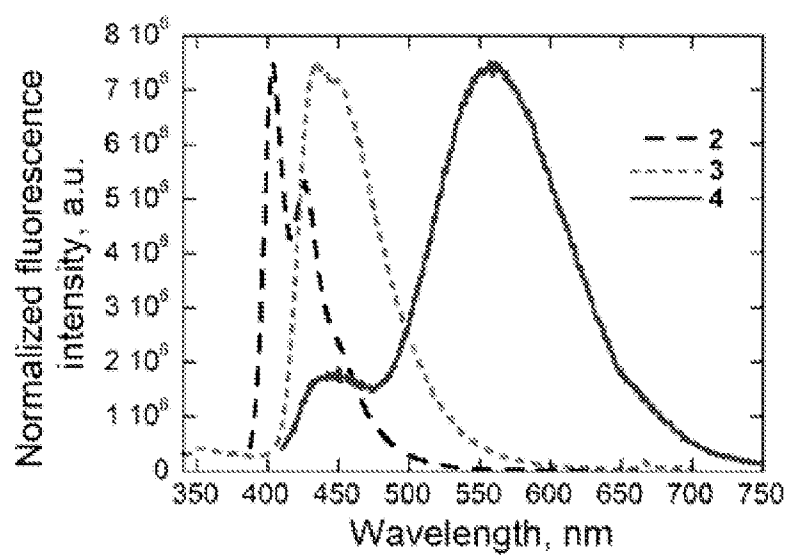


FIG. 15B

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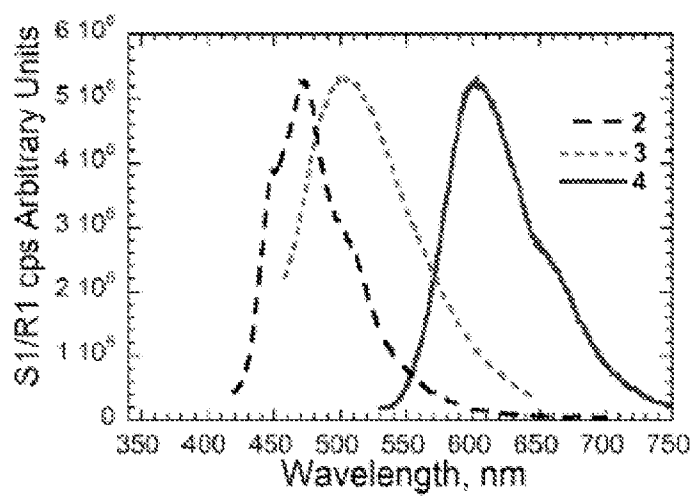


FIG. 15C

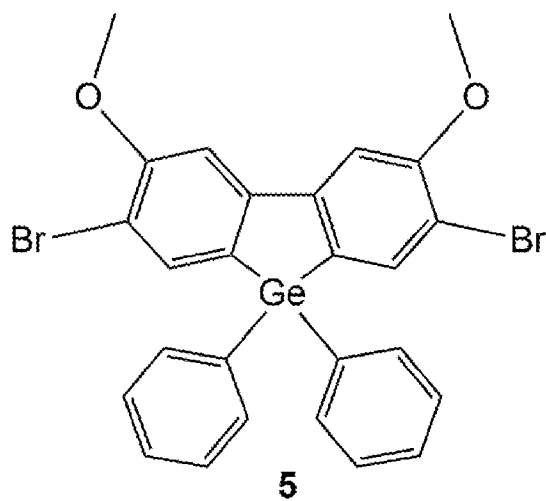


FIG. 16

FIG. 18

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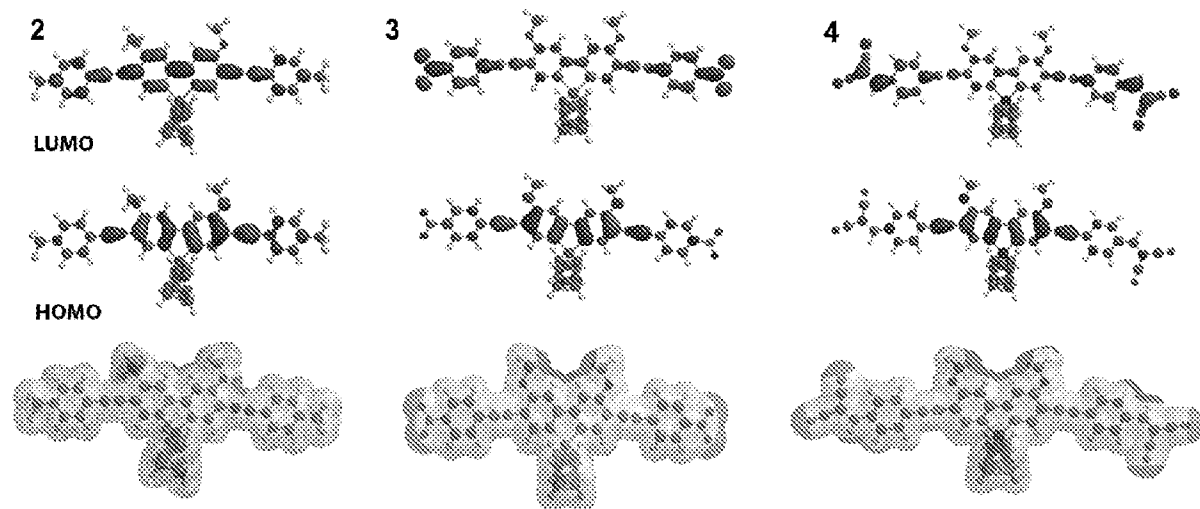


FIG. 19