3472 *Chem. Mater.* **2010,** *22,* 3472–3481
DOI:10.1021/cm100599g

CHEMISTRY OF MATERIALS
Article

Selective Fluorescence Sensing of Zinc and Mercury Ions with Hydrophilic 1,2,3-Triazolyl Fluorene Probes

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Received February 26, 2010. Revised Manuscript Received April 15, 2010

The ability to rapidly detect biologically and environmentally significant metal ions such as zinc and mercury is important to study a number of important cellular and environmental processes. Hydrophilic bis(1,2,3-triazolyl)fluorene-based derivatives containing a 1,2,3-triazole-based recognition moiety were synthesized through Click chemistry and characterized by UV−vis absorption, fluorescence emission, and two-photon absorption as new fluorescence sensing probes, selective for Zn^{2+} and Hg^{2+} ions. The UV−vis absorption and fluorescence emission spectra of the complexes exhibited blue-shifted absorption and emission spectra upon chelation to Zn^{2+} and Hg^{2+} ions, resulting in ca. 2-fold enhancement in fluorescence. Fluorometric titration revealed that 1:2 and 1:3 ligand to metal complexes formed with binding constants of 10^8 and 10^{16} for Zn^{2+} and Hg^{2+}, respectively. The two-photon absorption cross sections for the probes and probe-metal ion complexes ranged from 200 to 350 GM at 800 nm. These novel fluorescent compounds may have potential as new metal ion sensors to probe cellular and biological environments.

Introduction

Heavy metal ions can disturb normal biological functions and evoke cellular stress responses.[1] Zinc is the second most abundant heavy metal ion in vivo and is an essential component of many protein scaffolds present in cells.[2,3] It is a major regulatory ion in many channels and receptors, in the metabolism of cells, in neural signal transmission, and in metalloenzyme regulation.[4,5] Zinc also plays an important role in neurophysiology, inducing the formation of β-amyloid that is related to Alzheimer's disease.[6] In high concentration, Zn^{2+} is also a known neurotoxin, and excess zinc in the brain has been implicated in neuropathology. Zinc release also contributes to hypoglycemia-induced neuronal death.[7] Therefore, a zinc-sensing molecule is quite desirable for biological studies and for the diagnosis of certain diseases.[8] Mercury, a nonessential heavy metal, is highly toxic to cells.[9] It is known that mercury is a severe neurotoxic, genotoxic, and immunotoxic reagent in mammals. In plants, mercury can reduce chlorophyll content in leaves.[10] Moreover, mercury can induce aberrations in microtubules, ion channels, and mitochondria.[11] A molecule for sensing heavy metal ions, such as zinc in vivo or mercury in contaminated sources, should exhibit high selectivity for zinc or mercury over other relevant metal ions. Since Zn^{2+} and Hg^{2+} are relatively inert with a closed $3d^{10}$ electronic configuration, fluorometric methods have been employed as a technique of choice to detect both ions for various applications.[12−14]

New fluorescence sensing molecules for metal ion detection should have high affinity, bind rapidly and reversibly, and be nontoxic with high luminescence quantum yield and photostability. Reported zinc ion sensing molecules employ aryl sulfonamides of 8-aminoquinoline,[15] fluorescein platform,[16−18] porphyrin-based,[19] and calix-[4]arene derivatives.[20] Current mercury sensing molecules

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Published on Web 05/04/2010

employ rhodamine-based,[21] fluorescein-based,[22,23] perylene derivatives,[24] calix[4]arenediazacrown ether,[25] and porphyrin-based dyes.[26]

The introduction of multiphoton microscopy has shown tremendous advantages over single-photon excitation since two-photon excitation (2PE) shows greater penetration into cells with reduced photodamage than conventional confocal microscopy.[27] Recently, in this laboratory, we designed fluorescent probes that exhibit high two-photon absorption (2PA) with a fluorenyl chromophore core derivatized to target specific cellular proteins.[28,29] The fluorene derivatives are known for their good photophysical properties, high fluorescence quantum yields, large 2PA cross-sections (δ_{2PA}), and excellent photostability.[30−35] The design of our current metal sensing probes is based on a donor−π-electron-bridge−acceptor (D−π−A) fluorene-based chromophore system. The probe, based on an integrated fluoroionophore,[13] has a 1,2,3-triazole-based recognition moiety. The triazole derivative was synthesized using a Cu(I)-catalyzed Huigsen cycloaddition reaction of an alkyne with an azide,[36,37] commonly referred to as the "Click" reaction,[38] which is now appearing more frequently in the design and synthesis of molecular receptors. Recently, Zhu and co-workers used this approach to synthesize a number of triazolyl compounds.[39] Herein, we report the use of an amino bis-triazolyl chelator as an ionophore, attached to a hydrophilic fluorene-based receptor. Greater specificity in the binding with Zn^{2+} and Hg^{2+} over other metals was observed. Detailed linear and nonlinear photophysical properties were investigated in both organic and aqueous media. The metal to ligand stoichiometry and binding constants were investigated, as was the reversibility of metal complexation. The versatility and synthetic accessibility of the 1,2,3-triazole ligand makes this an especially attractive ion receptor.

Experimental Section

Materials. Organic reagents were obtained from Acros and Aldrich, and used as received. Al^{3+}, Ca^{2+}, Cd^{2+}, Fe^{2+}, Hg^{2+}, Mg^{2+}, Pb^{2+}, Zn^{2+} as perchlorate salts, Co^{2+}, Cu^{2+} as acetate salts, Ba^{2+}, Cu^{+}, Fe^{3+}, Hg^{+}, Li^{+} as chloride salts, and Mn^{2+} nitrate were used to prepare metal ion stock solutions. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Mercury 300 MHz or a Varian 500 MHz spectrometer. High resolution mass spectrometry (HRMS) data was provided by the Mass Spectrometry Laboratory at the University of Florida. Silica gel for chromatography was obtained from Silicycle (particle size of 40−63 μm).

Synthesis. 2-(4-Ethenylphenyl)benzothiazole was synthesized according to the literature.[40] 2-Iodofluorene (**2**),[41] 2-(2-ethoxyethoxy)ethyl 4-methylbenzenesulfonate (**3a**),[42] and pegylated tosylate **3b**[43] were prepared by modifications of literature procedures, and are described in the Supporting Information. Safety was taken during preparation of 1-azidobutane and 1-azido-11-undecanthiol since azides are potentially explosive. Their syntheses were performed by heating at moderate temperatures and by working in small scale with the reaction behind a shield.

2-Iodo-9,9-di-2-(2-ethoxyethoxy)ethylfluorene (***4a***). 2-Iodofluorene (**2**) (1.80 g, 6 mmol) was dissolved in dry DMSO (30 mL) in a 100 mL flask. The solution was degassed with N_2 for 10 min, followed by addition of KI (0.10 g, 0.6 mmol) and KOH (1.00 g, 18 mmol). To this solution was added **3a** (5.40 g, 18 mmol) dropwise over 10 min. The resulting solution was stirred overnight and then poured into water (50 mL) to which CH_2Cl_2 (50 mL) was added. The organic phase was then removed, and the aqueous phase was extracted three times with CH_2Cl_2 (40 mL). The organic phases were combined, washed with water, dried over Na_2SO_4, and then evaporated under reduced pressure to obtain a crude product. Purification was accomplished on a silica gel column using hexane and EtOAc (7:3) as the eluent, resulting in 2-iodo-9,9-di-2-(2-ethoxyethoxy)ethylfluorene (**4a**) (1.90 g, 3.6 mmol) as a white solid with mp of 49−50 °C (60% yd). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.78 (s, Ar−H, 1H), 7.70−7.75 (m, Ar−H, 2H), 7.22−7.25 (m, Ar−H, 2H), 7.10−7.15 (dd, Ar−H, 2H), 3.35−3,43 (m, OCH_2, 8H), 3.18−3.20 (m, OCH_2, 4H), 2.75−2.78 (m, CH_2CH_2O, 4H), 2.38−2.42 (m, CH_3CH_2O, 4H), 1.13−1.18 (m, CH_3, 6H). ^{13}C NMR (500 MHz, $CDCl_3$, δ): 152.89, 150.12, 147.80, 146.0, 137.75, 137.45, 133.63, 123.66, 123.01, 120.05, 119.01, 95.54 (C−I), 70.12, 69.59, 66.75, 66.64, 52.24 (C9), 39.19 (CH_2CH_2O), 15.06 (CH_3). HRMS calcd for $C_{25}H_{33}O_4I$, 524.4383; found 524.1417.

*Fluorene Derivative **4b***. Tosylate **3b** (3.80 g, 6 mmol) was used in a procedure identical to the synthesis of **4a** above. Purification by column chromatography, using silica gel as the stationary phase and CH_2Cl_2 and CH_3OH (95:5) as the eluent, afforded an oily liquid **4b** (40% yd). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.75 (s, Ar−H, 1H), 7.65−7.68 (m, Ar−H, 2H), 7.38−7.42 (m, Ar−H, 2H), 7.32−7.35 (m, Ar−H, 2H), 3.55−3.65 (m, 50H), 3.38−3.42 (s, 6H), 2.65−2.68 (m, CH_2CH_2O, 4H), 2.32−2.36 (m, CH_3-CH_2O, 4H). ^{13}C NMR (500 MHz, $CDCl_3$, δ): 148.33, 145.31,

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137.09, 136.46, 133.33, 129.46, 125.20, 124.48, 120.12, 118.47, 116.94, 89.62, 68.87, 68.49, 68.36, 67.66, 67.61, 67.58, 67.53, 67.50, 63.81, 56.05 (OCH_3), 48.39 (C9), 36.46 (CH_2CH_2O). HRMS calcd $C_{47}H_{77}O_{16}I$ for 1025.0207; found 1024.9921.

2-Iodo-7-nitro-9,9-di-2-(2-ethoxyethoxy)ethylfluorene ***5a***. 2-Iodo-9,9-di-2-(2-ethoxyethoxy)ethylfluorene **4a** (0.53 g, 1 mmol) was dissolved in a mixture of acetic acid (2 mL) and acetic anhydride (2 mL) in a 50 mL flask. To this solution, nitric acid (0.30 g, 0.22 mL, 3 mmol) was added, and the reaction mixture was stirred overnight. The resulting solution was poured into water (50 mL), followed by addition of CH_2Cl_2 (40 mL). The organic phase was removed, and then the aqueous phase was extracted 3 times with CH_2Cl_2 (40 mL). The organic phases were combined, washed with water, and then dried over Na_2SO_4. The filtrate was removed under reduced pressure to obtain a crude product which was further purified on a silica gel column using hexane and EtOAc as the eluent to obtain 2-iodo-7-nitro-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene **5a** (0.30 g, 0.50 mmol) with mp of 55−56 °C as a light yellow solid (50% yd). ^{1}HNMR (500 MHz, $CDCl_3$, δ): 8.15−8.17 (m, Ar−H, 2H), 7.80−7.82 (s, 1H), 7.75 −7.78 (m, Ar−H, 2H), 7.35−7.38 (m, 1H), 3.38−3.42 (m, 4H), 3.28−3.32 (m, 4H), 3.15−3.19 (m, OCH_2, 4H), 2.80−2.88 (dd, CH_2CH_2O, 4H), 2.40−2,48 (dd, CH_3CH_2O, 4H), 1.10−1,15 (m, CH_3, 6H). ^{13}C NMR (500 MHz, $CDCl_3$, δ): 151.30, 148.32, 140.07, 139.46, 136.27, 132.45, 128.01, 127.82, 123.52, 121.65, 119.92, 92.64 (C−I), 70.07, 69.67, 66.89, 66.60, 51.37 (C9), 39.55 (CH_2CH_2O), 15.10 (CH_3). HRMS calcd $C_{25}H_{32}NIO_6$ for 569.4359; found 569.1268.

Compound ***5b***. Compound **4b** (1.20 g, 1 mmol) was used in a procedure identical to the synthesis of **5a** above. Purification by column chromatography, using silica gel as the stationary phase and CH_2Cl_2 and CH_3OH (95:5) as the eluent, produced an oily yellowish liquid **5b** (40% yd). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 8.25−8.30 (m, Ar−H, 2H), 7.75−7.82 (m, Ar−H, 3H), 7.50−7.55 (m, Ar−H, 1H), 7.50 (m, Ar−H, 1H), 3.4--3.75 (m, 72H), 3.35−3.42 (s, 6H), 2.78−2.82 (d, CH_2CH_2O, 4H), 2.38−2.42 (d, CH_3CH_2O, 4H). ^{13}C NMR (500 MHz, $CDCl_3$, δ): 149.85, 147.06, 144.47, 142.85, 134.74, 134.04, 130.05, 120.78, 120.0, 117.15, 116.0, 92.51 (C−I), 74.35, 74.10, 73.84, 69.16, 68.95, 68.75, 68.66, 68.55, 68.45, 68.33, 68.16, 67.79, 67.66, 66.41, 56.10 (OCH_3), 49.22 (C9), 36.23 (CH_2CH_2O). HRMS calcd $C_{47}H_{76}O_{18}NI$ for 1070.0183; found 1070.8651.

2-Nitro-7-styrylbenzothiazolyl-9,9-di-2-(2-ethoxyethoxy)ethylfluorene ***6a***. 2-Iodo-7-nitro-9,9-di-2-(2-ethoxyethoxy)ethylfluorene **5a** (2.00 g, 3.5 mmol) was dissolved in a mixture of DMF (25 mL) and triethylamine (5 mL) in a 50 mL flask. The solution was degassed with N_2 for 10 min, and then palladium acetate (0.04 g, 0.18 mmol), tri(o-tolyl)phosphine (0.10 g, 3.3 mmol), and 2-(4-ethenylphenyl)benzothiazole[41] (1.00 g, 4.2 mmol) were added. The resulting solution was heated at 90 °C for 40 h. At the end of this period, the solution was allowed to cool to room temperature and then poured into water (200 mL) to which CH_2Cl_2 (50 mL) was added. The organic phase was removed. The aqueous phase was extracted thrice with CH_2Cl_2 (40 mL). The organic phases were combined, washed with water, and dried over Na_2SO_4. The filtrate was removed under reduced pressure to obtain a crude product. Purification of the crude product was accomplished on a silica gel column using hexane and ethyl acetate (3:7) as the eluent to obtain 2-nitro-7-styrylbenzothiazolyl-9,9-di-2-(2-ethoxyethoxy)-ethylfluorene **6a** (1.40 g, 2.1 mmol) as yellow solid with mp of 145−147 °C (60% yd). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 8.12−8.15 (dd, Ar−H, 2H), 8.02−8.09 (dd, Ar−H, 3H), 7.83−7.88 (m, Ar−H, 1H), 7.75−7.78 (dd, Ar−H, 2H), 7.65−7.70 (m, Ar−H, 3H), 7.55−7.57 (m, Ar−H, 1H), 7.48−7.52 (m, Ar−H, 1H), 7.35−7.38 (m, Ar−H, 1H), 7.22−7.30 (m, Ar−H, 2H), 3.05−3.38 (m, OCH_2, 12H), 2.75−2.81 (dd, CH_2CH_2O, 4H), 2.38−2.45 (m, CH_3CH_2O, 4H), 1.0−1.04 (m, CH_3, 6H). ^{13}C NMR (500 MHz, $CDCl_3$, δ): 167.95 (C═N), 154.22 (sp^2 C−N), 151.90, 151.80, 147.63, 146.45, 139.66, 138.34, 138.08, 135.75, 133.80, 129.89, 128.90, 128.01, 127.16, 127.05, 126.95, 126.43, 124.85, 123.70, 123.21, 121.65, 121.55, 119.88, 118.94, 70.15, 69.61, 66.86, 66.61, 51.95 (C9), 39.45 (CH_2CH_2), 15.05 (CH_3). HRMS calcd for $C_{40}H_{42}O_6N_2S$, 678.8416; found 678.2827.

Compound ***6b***. Fluorenyl **5b** (2.18 g, 1.75 mmol) was used in a procedure identical to the synthesis of **6a** above. Purification by column chromatography, using silica gel as the stationary phase and CH_2Cl_2 and CH_3OH (95:5) as the eluent, provided an oily liquid **6b** (1.65 g, 1.20 mmol) in 70% yield. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 8.20−8.22 (dd, 2H), 8.02−8.10 (dd, 3H), 7.85−7.90 (m, 1H), 7.70−7.75 (d, 2H), 7.62−7.68 (dd, Ar−H, 3H), 7.55−7.57 (d, Ar−H, 1H), 7.45−7.48 (m, Ar−H, 1H), 7.30−7.35 (m, Ar−H, 1H), 7.22−7.25 (m, Ar−H, 2H), 3.40−3.62 (m, 66H), 3.30−3.38 (m, 8H), 2.70−2.80 (d, CH_2CH_2O, 4H), 2.38−2.41 (m, CH_3CH_2O, 4H). ^{13}C NMR (500 MHz, $CDCl_3$, δ): 167.90, 154.01, 151.7, 151.05, 147.06, 146.10, 140.00, 138.20, 138.10, 135.02, 132.89, 130.02, 129.80, 129.50, 128.07, 127.55, 126.80, 125.99, 125.80, 124.00, 123.80, 123.50, 123.20, 122.00, 120.00, 119.50, 119.00, 71.89, 71.00, 70.76, 70.63, 70.52, 70.32, 70.26, 70.04, 66.80, 59.01 (OCH_3), 51.89 (C9), 39.80 (CH_2CH_2O). HRMS calcd $C_{54}H_{70}O_{14}N_2S$ for 1003.212; found 1002.9711.

2-Amino-7-styrylbenzothiazolyl-9,9-di-2-(2-ethoxyethoxy)ethyl-fluorene ***7a***. 2-Nitro-7-styrylbenzothiazolyl-9,9-di-2-(2-ethoxy-ethoxy)ethylfluorene **6a** (1.20 g, 1.8 mmol) was dissolved in a mixture of EtOH (25 mL) and THF (25 mL) in a 200 mL flask. The resulting solution was degassed with N_2 for 10 min, and 10% palladium on carbon (0.12 g) was then added. The solution was heated to 60 °C under N_2. Hydrazine hydrate (0.50 g) was then added dropwise to the reaction mixture over 10 min. The solution was then refluxed at 70 °C for 20 h. At the end of this period, the solution was allowed to cool to room temperature, filtered using a short silica gel plug, and washed with THF (100 mL). The filtrate was removed under reduced pressure, affording a crude product that was further purified on a silica gel column, using EtOAc and THF (4:1) as eluent, to obtain 2-amino-7-styrylbenzothiazolyl-9,9-di-2-(2-ethoxyethoxy)ethyl-fluorene **7a** (1.00 g, 1.5 mmol) as yellow solid (mp 185−188 °C). IR (neat, cm^{-1}): 3400, 3340, 3230 ($-NH_2$). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.95−8.05 (m, Ar−H, 3H), 7.82−7.85 (d, Ar−H, 1H), 7.58−7.62 (d, Ar−H, 2H), 7.30−7.50 (m, Ar−H, 6H), 7.10−7.18 (m, Ar−H, 2H), 6.65 (s, Ar−H, 1H), 6.56−6.58 (d, Ar−H, 1H), 3.85 (s, NH_2, 2H), 3.25−3.38 (m, OCH_2, 8H), 3.15−3.18 (m, 4H), 2.62−2.78 (m, CH_2CH_2O, 4H), 2.21−2.42 (m, CH_3CH_2O, 4H), 1.02−1.12 (m, CH_3, 6H). ^{13}C NMR ($CDCl_3$, 300 MHz, δ): 167.87 (C═N), 154.15 (sp^2 C−N), 151.70, 148.62, 146.76, 141.86, 140.55, 135.01, 134.65, 132.05, 131.24, 131.04, 128.13, 127.05, 126.87, 126.61, 126.54, 126.05, 123.33, 121.82, 121.10, 121.05, 119.10, 114.35, 110.15, 70.41, 70.00, 67.37, 66.89, 51.06 (C9), 40.40 (CH_2CH_2O), 15.48 (CH_3). HRMS calcd for $C_{40}H_{44}N_2O_4S$, 648.8586; found 648.3016.

Compound ***7b***. Fluorene **6b** (2.40 g, 1.70 mmol) was used in a procedure identical to the synthesis of **7a** above. Purification by column chromatography, using silica gel as the stationary phase and CH_2Cl_2 and CH_3OH (95:5) as the eluent, produced an oily liquid **7b** (2.05 g, 1.50 mmol) in 90% yield. IR (neat, cm^{-1}): 3447, 3355, 3236 ($-NH_2$), 2870, 1602, 1468, 1354, 1293, 1248, 1101 (C−O), 1031, 958, 871, 822, 762. ^{1}H NMR (500 MHz, $CDCl_3$,

δ): 8.10−8.15 (m, Ar−H, 3H), 7.90−7.93 (d, Ar−H, 1H), 7.65−7.68 (d, Ar−H, 2H), 7.40−7.58 (m, Ar−H, 6H), 7.18−7.20 (d, Ar−H, 1H), 6.79 (s, Ar−H, 1H), 6.70 (d, Ar−H, 1H), 3.95−4.02 (s, 2H), 3.50−3.70 (m, OCH_2, 68H), 3.35−3.45 (m, OCH_3,10H), 2.78−2.80 (m, CH_2CH_2O, 4H), 2.30−2,42 (d, CH_3CH_2O, 4H). ^{13}C NMR (500 MHz, $CDCl_3$, δ): 167.79, 154.23, 148.48, 140.37, 135.01, 134.50, 132.27, 131.50, 131.21, 128.02, 127.84, 126.96, 126.50, 126.20, 125.55, 123.85, 121.81, 121.50, 121.15, 121.05, 119.50, 115.48, 114.85, 109.80, 71.92, 70.77, 70.54, 70.46, 70.30, 70.00, 67.04, 59.10 (OCH_3), 50.73 (C9), 40.05 (CH_2CH_2O). HRMS calcd $C_{58}H_{80}O_{14}N_2S$ for 1065.3204; found 1065.6587.

2-N,N-Dipropargylamino-7-styrylbenzothiazolyl-9,9-di-2-(2-ethoxyethoxy)ethylfluorene ***8a***. 2-Amino-7-styrylbenzothiazolyl-9,9-di-2-(2-ethoxyethoxy)ethylfluorene **7a** (0.65 g, 1.0 mmol) was dissolved in DMF (15 mL). The solution was degassed with N_2 for 10 min, followed by addition of K_2CO_3 (0.50 g, 3.6 mmol) and a solution of 80% propargyl bromide (0.50 g, 3.0 mmol) in toluene. The resulting solution was heated under N_2 to 95 °C for 20 h. The solution was then allowed to cool to room temperature and poured into water (50 mL) to which CH_2Cl_2 (40 mL) was added. The organic phase was removed. The aqueous phase was extracted 3 times with CH_2Cl_2 (40 mL). The organic phases were combined, washed with water, and dried over Na_2SO_4. The filtrate was removed under reduced pressure, followed by purification of the crude product on a silica gel column using hexane and EtOAc (3:7) as eluent to obtain 2-*N*,*N*-di(2-ethylacetate)amino-9,9-di-2-(2-ethoxyethoxy)ethylfluorene **8a** (0.61 g, 0.75 mmol) as a yellow solid with mp of 103−105 °C. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 8.02−8.08 (m, Ar−H, 3H), 7,82−7.85 (d, Ar−H, 1H), 7.58−7.62 (m, Ar−H, 3H), 7.40−7.58 (m, Ar−H, 3H), 7.30−7.38 (d, Ar−H, 2H), 7.25 (d, Ar−H, 1H), 7.10−7.12 (d, Ar−H, 1H), 6.52−6.59 (m, Ar−H, 2H), 4.10−4.18 (m, CO_2CH_2, NCH_2, 8H), 3.30−3.38 (m, OCH_2, 8H), 3.10−3.18 (m, 4H), 2.65−2.78 (m, CH_2CH_2O, 4H), 2.22−2.43 (m, CH_3-CH_2O, 4H), 0.98−1.02 (m, CH_3, 6H).^{13}C NMR (500 MHz, $CDCl_3$, δ): 170.86 ($CO_2C_2H_5$), 167.98 (C=N), 154.11 (sp^2 C−N), 150.55, 150.15, 148.35, 148.25, 148.08, 140.47, 140.27, 140.13, 135.01, 134.65, 132.03, 131.25, 131.01, 127.94, 126.67, 126.35, 126.16, 125.87, 125.32, 122.28, 122.18, 121.96, 120.75, 120.45, 118.37, 112.12, 106.42, 70.06, 69.66, 67.05, 66.54, 61.28, 53.83 (NCH_2), 50.89 (C9), 39.92 (CH_2CH_2O), 15.05 (CH_3), 14.27 (CH_3). HRMS calcd for $C_{48}H_{56}O_8N_2S$, 821.039; found 820.3751.

Compound ***8b***. Fluorene **7b** (2.30 g, 1.75 mmol) was used in a procedure identical to the synthesis of **8a** above. Purification by column chromatography, using silica gel as the stationary phase and CH_2Cl_2 and CH_3OH (95:5) as eluent, revealed oily liquid **8b** (1.60 g, 1.10 mmol) in 65% yield. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 8.12−8.15 (m, Ar−H, 3H), 7.92−7.95 (d, 1H), 7.65−7.72 (d, Ar−H, 2H), 7.55−7.58 (m, Ar−H, 3H), 7.50−7.55 (m, Ar−H, 2H), 7.38−7.39 (d, Ar−H, 1H), 7.30−7.32 (d, Ar−H, 1H), 7.20−7.22 (d, Ar−H, 1H), 6.90−6.95 (m, 2H), 4.20 (m, 4H), 3.54−3.65 (m, 70H), 3.37−3.42 (m, 12H), 3.22 (m, 4H), 2.80 (m, CH_2CH_2O, 4H), 2.38−2.42 (m, CH_3CH_2O, 4H). ^{13}C NMR (500 MHz, $CDCl_3$, δ): 167.77, 154.23, 150.35, 149.10, 148.00, 140.95, 140.28, 135.01, 132.33, 132.19, 128.03, 127.84, 127.16, 126.99, 126.87, 125.95, 125.23, 123.76, 121.80, 121.35, 119.62, 119.43, 115.78, 115.61, 110.25, 110.15, 79.50, 73.55, 71.91, 70.77, 70.65, 70.53, 70.45, 70.41, 70.27, 69.99, 58.90 (OCH_3), 50.97 (C9), 40.50 (CH_2CH_2O), 39.2 (C−N). HRMS calcd $C_{60}H_{76}O_{12}N_2S$ for 1049.3266; found 1048.7501.

(44) Ciampi, S.; Böcking, T.; Kilian, K. A.; James, M.; Harper, J. B.; Gooding, J. J. *Langmuir* **2007**, *23*, 9320–9329.

1-Azidobutane[44]. 1-Bromobutane (5.50 g, 40 mmol) was dissolved in DMSO (50 mL) at room temperature in a 200 mL flask to which NaN_3 (3.90 g, 60 mmol) was added. The solution was then heated to 95 °C under nitrogen for 24 h. At the end of this period, the solution was allowed to cool to room temperature. A white precipitate formed. The resulting mixture was transferred into water (100 mL) in a separatory flask to which Et_2O (50 mL) was added. The organic phase was then removed. The aqueous phase was extracted thrice with portion of Et_2O (100 mL). The ether phases were then combined, washed with water, dried with Na_2SO_4, and evaporated under reduced pressure to yield 1-azidobutane (3.00 g, 3.20 mmol) as colorless liquid (80% yield). IR: 2090 cm^{-1} (N_3). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 3.18−3.21 (m, 2H), 1.49−1.55 (m, 2H), 1.30−1.37 (m, 2H), 0.86−0.89 (m, 3H). ^{13}C NMR (500 MHz, $CDCl_3$, δ (ppm)): 39.97 (C−N), 29.82, 18.87, 12.60 (CH_3).

1-Azido-11-undecanol. 1-Bromo-11-undecanol (5.00 g, 20 mmol) was dissolved in acetonitrile (125 mL) in a 500 mL flask. To this solution was added NaN_3 (2.00 g, 30 mmol). The resulting solution was refluxed at 80 °C under nitrogen for 20 h. At the end of this period, the solution was allowed to cool to room temperature, and then filtered and washed with acetonitrile (50 mL). The filtrate was evaporated under reduced pressure to obtain 1-azido-11-undecanol (4.20 g, 19 mmol) as a colorless liquid in 97% yield. The product was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 3.59−3.61 (m, 2H), 3.23−3.25 (m, 2H), 1.53−1.60 (m, 4H), 1.27−1.34 (m, 14H). ^{13}C NMR (500 MHz, $CDCl_3$, δ (ppm): 62.91 (OCH_2), 51.46 (C−N), 32.75, 29.54, 29.44, 29.40, 29.25, 29.12, 28.81, 26.69, 25.73.

1-Azido-11-undecanyl 4-methylbenzenesulfonate. 1-Azido-11-undecanol (4.00 g, 19 mmol) was dissolved in pyridine (10 mL) in a 100 mL flask immersed in an ice bath at 5 °C. To this solution, 4-methylenebenzenesulfonyl chloride (3.50 g, 18 mmol) was added in portions in 20 min. The resulting solution was stirred at 5 °C under a nitrogen atmosphere for 3 h. At the end of this period, a white precipitate formed and the solution was poured into a cold solution of hydrochloric acid (10 mL) in water (50 mL). The resulting solution was transferred into a separatory flask to which of CH_2Cl_2 (40 mL) was added. The organic phase was removed, and the aqueous phase was extracted 3 times with portions of CH_2Cl_2 (100 mL). The organic phases were combined, washed with a solution of 2% $NaHCO_3$ (100 mL), water, and then dried over Na_2SO_4. The filtrate was removed under reduced pressure and in vacuo to obtain 1-azido-11-undecanyl tosylate (6.30 g, 18 mmol) as a colorless oily liquid in 92% yield. The product was pure enough for the next reaction without further purification. ^{1}H NMR (500 MHz, $CDCl_3$, δ): 7.70−7.72 (d, Ar−H, 2H), 7.20−7.28 (d, Ar−H, 2H), 3.93−3.95 (m, 2H), 3.17−3.19 (m, 2H), 2.37−2.50 (s, 3H), 1.44−1.57 (m, 4H), 1.15−1.36 (m, 14H). ^{13}C NMR (500 MHz, $CDCl_3$, δ (ppm)): 144.65, 133.19, 129.73, 127.91, 70.70 (SO_3C), 51.46 (C−N), 29.39, 29.34, 29.10, 28.89, 28.82, 28.79, 26.68, 25.31, 21.68.

1-Azido-11-undecanethiol[52]. 1-Azido-11-undecanyl tosylate (6.00 g, 16 mmol) was dissolved in EtOH (40 mL) in a 250 mL flask. To this solution, thiourea (1.80 g, 24 mmol) was added and the solution was refluxed at 80 °C under N_2 atmosphere for 10 h. At the end of this period, the solution was allowed to cool to room temperature. A solution of 10% NaOH (60 mL) was then added, followed by reflux at 100 °C for 4 h. The solution was allowed to cool to room temperature and then neutralized with 10% HCl. The solution was transferred into a separatory flask

to which CH_2Cl_2 (50 mL) was added. The organic phase was removed, and the aqueous phase was extracted thrice with CH_2Cl_2 (40 mL). The organic phases were combined, washed with water, and then dried over Na_2SO_4. The filtrate was removed under reduced pressure to obtain a white solid. The crude product was purified on a silica gel column using hexane and CH_2Cl_2 (7:3) as the eluent to obtain 1-azido-11-undecanethiol (3.00 g, 20 mmol) as colorless liquid in 80% yd. IR (Perkin-Elmer): 2098 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$, δ): 3.15−3.20 (m, 2H), 2.42−2.62 (m, 2H), 1.47−1.63 (m, 4H), 1.21−1.32 (m, 15H). ^{13}C NMR (500 MHz, $CDCl_3$, δ (ppm): 51.47 (C−N_3), 39.15 (C−S), 34.04, 29.45, 29.20, 29.05, 28.83, 27.72, 28.36, 26.71, 24.66.

*Compound **9a***. 2-N,N-Dipropargylamino-7-styrylbenzothiazolyl-9,9-di-2-(2-ethoxyethoxy)ethylfluorene **8a** (0.36 g, 0.50 mmol) was dissolved into a mixture of dioxane (10 mL) and Et_3N (1 mL) in a 50 mL flask at room temperature. The solution was degassed with nitrogen for 10 min, and CuI (0.10 g) and 1-azidobutane (0.20 g, 2 mmol) were added. The resulting solution was stirred under N_2 for 24 h. The solution was then poured into water (40 mL) to which CH_2Cl_2 (30 mL) was added. The organic phase was removed, and the aqueous phase was extracted thrice with CH_2Cl_2 (40 mL). The organic phases were combined and dried over Na_2SO_4. The filtrate was removed under reduced pressure. The crude product was purified on a silica gel column using EtOAc and THF (8:2) as eluent, resulting in **9a** (0.33 g, 0.36 mmol) as oily liquid in 72% yd. ^1H NMR (500 MHz, $CDCl_3$, δ): 8.05−8.08 (m, Ar−H, 4H), 7.82−7.85 (d, Ar−H, 1H), 7.62−7.65 (m, Ar−H, 2H), 7.40−7.45 (m, Ar−H, 6H), 7.30−7.35 (m, Ar−H, 1H), 7.20−7.22 (m, Ar−H, 1H), 7.05−7.10 (d, Ar−H, 1H), 6.85−6.90 (m, Ar−H, 2H), 4.65−4.70 (s, N−CH_2, 4H), 4.22−4.28 (d, NCCH_2, 4H), 3.30−3.38 (m, OCH_2, 8H), 3.10−3.18 (m, OCH_2, 4H), 2.75−2.78 (m, CH_2-CH_2O, 4H), 2.22−2.30 (d, CH_3CH_2O, 4H), 1.82−1.89 (m, CH_2, 4H), 1.26−1.35 (m, CH_2, 4H), 1.09−1.13 (m, OCCH_3, 6H), 0.91−0.95 (m, CH_3, 6H). ^{13}C NMR (500 MHz, $CDCl_3$, δ (ppm)): 167.80 (C═N), 154.25 (sp^2 C−N), 150.98, 148.37, 145.0, 141.52, 140.77, 135.87, 134.65, 134.25, 132.17, 130.84, 127.98, 126.55, 126.06, 125.78, 123.25, 122.0, 121.75, 121.45, 118.85, 110.37, 108.89, 70.01, 69.81, 66.87, 66.47, 50.15 (C9), 49.85(C−N), 46.0 (C−N), 39.85 (CH_2CH_2O), 32.12, 19.95, 14.89 (CH_3), 13.67 (CH_3). HRMS calcd for $C_{54}H_{66}O_4N_8S$, 923.2266; found 922.4922.

*Fluorene **9b***. **8a** (0.36 g, 0.50 mmol) was used in a procedure identical to the synthesis of **9a** above; however, the reaction was heated at 70 °C for 48 h. Purification by column chromatography, using silica gel as the stationary phase and EtOAc and THF (8:2) as eluent, affording oily liquid **9b** (0.35 g, 0.30 mmol) in 50% yield. ^1H NMR (500 MHz, $CDCl_3$, δ): 8.10−8.15 (m, Ar−H, 4H), 7.90−7.92 (d, Ar−H, 1H), 7.65−7.70 (d, Ar−H, 3H), 7.50−7.58 (m, Ar−H, 6H), 7.35−7.38 (m, Ar−H, 1H), 7.30−7.34 (m, Ar−H, 1H), 7.15−7.18 (d, Ar−H, 1H), 7.05−7.10 (m, Ar−H, 2H), 4.75−4.78 (s, NCH_2, 4H), 4.25−4.28 (m, $NCCH_2$, 4H), 3.39−3.41 (dd, OCH_2, 8H), 3.20−3.28 (m, OCH_2, 4H), 2.75−2.82 (m, 4H), 2.62−2.65 (m, CH_2CH_2O, 4H), 2.30−2.42 (dd, CH_3CH_2O, 4H), 1.82−1.85 (m, CH_2, 4H), 1.50−1.65 (m, CH_2, 10H), 1.20−1.28 (m, CH_2, 24H) 1.10−1.12 (m, CH_3, 6H). ^{13}C NMR (500 MHz, $CDCl_3$, δ (ppm)): 167.85 (C═N), 154.20 (sp^2 C−N), 151.05, 148.27, 145.11, 141.54, 140.15, 135.19, 134.27, 132.18, 130.47, 127.94, 126.70, 126.22, 125.14, 122.87, 122.15, 121.79, 121.05, 119.06, 112.58, 108.26, 70.06, 69.72, 66.83, 66.57, 51.58 (C9), 50.39 (C−N), 46.75 (C−N), 39.13 (CH_2CH_2O), 30.35, 29.48, 29.42, 29.25, 29.15, 29.03, 28.84, 28.51, 26.72, 26.53, 15.09 (CH_3). HRMS calcd for $C_{68}H_{94}O_4N_8S_3$, 1183.7218; found 1183.6347.

*Fluorene **9c***. **8b** (0.70 g, 0.50 mmol) was used in a procedure identical to the synthesis of **8b** above using a Cu(I) complex[45] (0.20 g). Purification by column chromatography, using silica gel as a stationary phase and CH_2Cl_2 and CH_3OH (95:5) as eluent, afforded oily liquid **9c** (0.40 g, 0.20 mmol) in 40% yd. ^1H NMR (500 MHz, $CDCl_3$, δ): 8.10−8.13 (m, Ar−H, 4H), 7.80−7.85 (d, Ar−H, 1H), 7.62−7.64 (d, Ar−H, 2H), 7.42−7.58 (m, Ar−H, 6H), 7.35−7.38 (m, Ar−H, 1H), 7.25−7.28 (m, Ar−H, 1H), 7.18−7.20 (m, Ar−H, 1H), 6.90−6.98 (m, Ar−H, 2H), 4.70−4.75 (s, NCH_2, 4H), 4.32−4.35 (m, $NCCH_2$, 4H), 3.42−3.62 (m, OCH_2, 58H), 3.35−3.42 (m, OCH_2, 10H), 3.20−3.25 (m, OCH_3, OCH_2, 10H), 2.90−2.92 (m, 4H), 2.78−2.81 (m, 4H), 2.62−2.65 (m, CCH_2O, 4H), 2.38−2.42 (m, 4H), 1.80−1.85 (m, CH_2, 4H), 1.48−1.78 (m, CH_2, 10H), 1.20−1.40 (m, CH_2, CH_3, 24H). ^{13}C NMR (500 MHz, $CDCl_3$, δ (ppm)): 167.89 (C═N), 154.25 (sp^2 C−N), 151.11, 148.34, 145.01, 141.11, 140.21, 134.92, 134.62, 132.25, 130.05, 127.79, 127.15, 125.01, 123.15, 122.27, 121.88, 121.03, 119.29, 113.59, 108.47, 72.01, 71.10, 70.92, 70.52, 70.31, 70.11, 69.82, 66.58, 58.85 (OCH_3), 50.78 (C9), 50.18 (C−N), 46.25 (C−N), 39.53 (CH_2CH_2O), 30.41, 30.35, 29.56, 29.40, 29.19, 28.79, 28.56,28.21, 27.76, 27.63, 27.41, 26.55. HRMS calcd $C_{74}H_{106}O_8N_8S_3$ for 1331.8802; found 1332.3254.

Photophysical Characterization. *General Instrumental Information*. UV−vis absorption spectra were obtained using a Hewlett-Packard (Model 8453) diode-array spectrophotometer equipped with 1.0 cm quartz cells. Fluorescence emission spectra were obtained with a Photon Technology International Quantamaster spectrofluorimeter equipped with a 75-W continuous Xe-arc lamp as an excitation light source.

Fluorescence Quantum Yield Determination. The fluorescence quantum yield was determined by comparison of the integrated area of the corrected emission spectrum of the sample with that of a known standard (reference), r, such as 9,10-diphenylanthracene (ϕ = 0.95 in cyclohexane). The quantum yield of the sample was calculated according to eq 1[46]

$$\phi = \phi_r \frac{I}{I_r} \frac{OD_r}{OD} \frac{n^2}{n_r^2} \quad (1)$$

where I and I_r are the corrected integrated fluorescence intensity of the sample and the standard; OD and OD_r are the optical density (absorbance) of the sample and the standard at their respective wavelengths; and n and n_r are the refractive index of the sample and the standard, respectively.

Two-Photon Absorption Cross Section Determination[47]. Two-photon absorption (2PA) (δ, 1 GM = 1×10^{-50} cm^4 s $photon^{-1}$ mol^{-1}) measurements via the fluorescence method were performed with a mode-locked Coherent Mira 900 laser system. The pulse width of the laser was 200 fs with a repetition rate of 76 MHz and 700 mW of average power. The numerical estimation of the 2PA cross-section, δ, was performed by comparison with a known reference,[47,48] and calculated using eq 2

$$\delta = \delta_r \frac{\langle F \rangle n^2 C_r \phi_r P_r^2}{\langle F_r \rangle n_r^2 C \phi P^2} \quad (2)$$

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where r represents the reference; $\langle F \rangle$ is the average integrated fluorescence intensity from the two-photon fluorescence (2PF) spectrum; n is the refractive index of the solvent; C is the concentration; ϕ is the quantum yield, and P is the incident power in the sample.

UV−vis Absorption and Fluorescence Emission Titration Procedure. UV−vis absorption and fluorescence emission titrations were performed in EtOH. Dye solution (3.0 mL) was prepared and the corresponding metal ion stock solution was added. The resulting solution was agitated and the UV−vis absorption and fluorescence emission spectra recorded. Experiments were performed in triplicate for data analysis.

Determination of Stoichiometry Number of the Complex. The basic eq 3 for determination of the ligand−metal complexation is

$$D + nM \rightleftharpoons C \tag{3}$$

where D is the dye molecule (ligand); M is the metal ion, and C is the complex.

Calculation of the Binding Constants. The binding constant K of the metal complex was determined by eq 4, assuming the concentration of free metal is about equal to its total concentration ($[M] \cong [M]_t$),[49−51]

$$\frac{F - F_o}{F_m - F} = \frac{[C]}{[D]} = K[M]^n \tag{4}$$

where F_o, F, and F_m are the corrected fluorescence emission intensity of the complex at initial, interval t, and the final state at which the complex was fully formed upon addition of metal ion, respectively. The binding constant K was determined from the plot of the linear regression of $\log[(F - F_0)/(F_m - F)]$ vs $\log[M]$ in eq 5, derived from eq 4, to obtain the intercept as $\log K$ and the slope as n.

$$\log \frac{F - F_o}{F_m - F} = \log K + n\log[M] \tag{5}$$

Results and Discussion

Synthesis. 1,2,3-Triazolyl fluorene derivatives **9a**−**c** were synthesized from fluorene (Scheme 1). The three new probes produced were based on a D−π−A push−pull system, the donor being the bis(1,2,3-triazolyl)amino, whereas benzothiazole served as an organic accepting moiety. The styryl group increased the conjugation of the chromophore's π-electron system, increasing λ_{max} of linear absorption, emission, and 2PA. Probe **9a** contained a butyl chain attached to the triazole ring, whereas the triazolyl moieties were derivatized at the 2-position with an undecanylthiol substituent in probes **9b** and **9c**. Two long polyethoxy chains were introduced in the fluorenyl 9-position to increase the hydrophilicity of probe **9c**.

The preparation of compound **9a**, involved alkylation of 2-iodofluorene (**2**) with 2-(2-ethoxyethoxy)ethyl tosylate (**3a**) to obtain 2-iodo-9,9-di-2-(2-ethoxy ethoxy)ethylfluorene **4a**. Nitric acid was used to nitrate compound **4a** at room temperature to obtain 2-iodo-7-nitro-9,9-di-2-(2-ethoxyethoxy)ethylfluorene (**5a**). Heck coupling was then employed to prepare **6a** in 60% yield from the reaction of **5a** with 2-(4-ethenylphenyl)benzothiazole.[40] This was followed by reduction using hydrazine with 10% Pd/C in a mixture of EtOH and THF at 70 °C, affording **7a** in 95% yield. Compound **7a** underwent N-alkylation with propargyl bromide in DMF to obtain bis-alkyne **8a** in 75% yield. 1-Azidobutane was synthesized in a 80% yield by reaction of 1-bromobutane with NaN_3 in DMSO at 95 °C for 24 h. Likewise, 1-azido-11-undecanethiol was synthesized in 80% overall yield from 1-bromo-11-undecanol (Scheme 2). The NMR data of 1-azidobutane and 1-azido-11-undecanethiol agreed with values reported in the literature.[52,53]

"Click" reaction of the bis-alkyne **8a** with 1-azidobutane produced **9a** in 72% yield. The ^{1}H NMR of compound **9a** exhibited signals at 4.7, 1.8, 1.3, and 0.9 ppm that were integrated for a total of 18 alkyl protons of the two butyl groups. The ^{13}C NMR spectrum of **9a** also showed signals at 49.9, 46.0, 32.1, 20.0, and 13.7 ppm for amino and alkyl carbons of the butyl substituents on the 1,2,3-triazoles. Compound **9b** was synthesized in 50% yield by a "Click" reaction of **8a** with 1-azido-11-undecanethiol. The ^{1}H NMR spectrum of **9b** had resonances at 8.1, 7.9, 7.7, 7.6, 7.4, 7.4, and 7.1 that were integrated for a total of 18 aromatic protons. In addition, signals were present at 4.7, 2.6, 1.8, 1.5, and 1.2−1.3 ppm for a total of 46 alkyl protons of the undecanyl substituents on the 1,2,3-triazoles. The ^{13}C NMR spectrum of **9b** exhibited resonances at 50.4, 46.8, 30.1, 29.4, 28.4, and 26 ppm for amino and alkyl carbons of the undecanylthiol moieties. Likewise, **9c** was obtained in 40% yield from bis-alkyne **8b**. The ^{1}H NMR spectrum of **9c** also showed signals at 4.7, 2.6, 1.8, 1.5, and 1.2−1.4 ppm that integrated for a total of 46 aliphatic protons of the two undecanylthiol groups. The ^{13}C NMR spectrum of **9c** revealed signals at 50.2, 46.3, 30.4, 29.6, 28.8, 27.8, and 26.6 ppm, corresponding to the aliphatic carbons of undecanyl groups. The HRMS analysis of compounds **9a** and **9b** showed molecular ions at 922.4922 and 1183.6347, consistent with formulas of $C_{54}H_{66}O_4N_8S$ and $C_{68}H_{94}O_4N_8S_3$, respectively.

Linear Photophysical Properties. *Response with Metal Ions*. Several metal ions were evaluated with the new probes by UV−vis absorption and fluorescence emission spectroscopy. The fluorescence response factor (F/F_0) was measured by comparison of the maximum integrated area of the corrected emission spectrum of the metal complex (F) with the uncomplexed dye before addition of metal ion (F_0). The metal ions that were investigated

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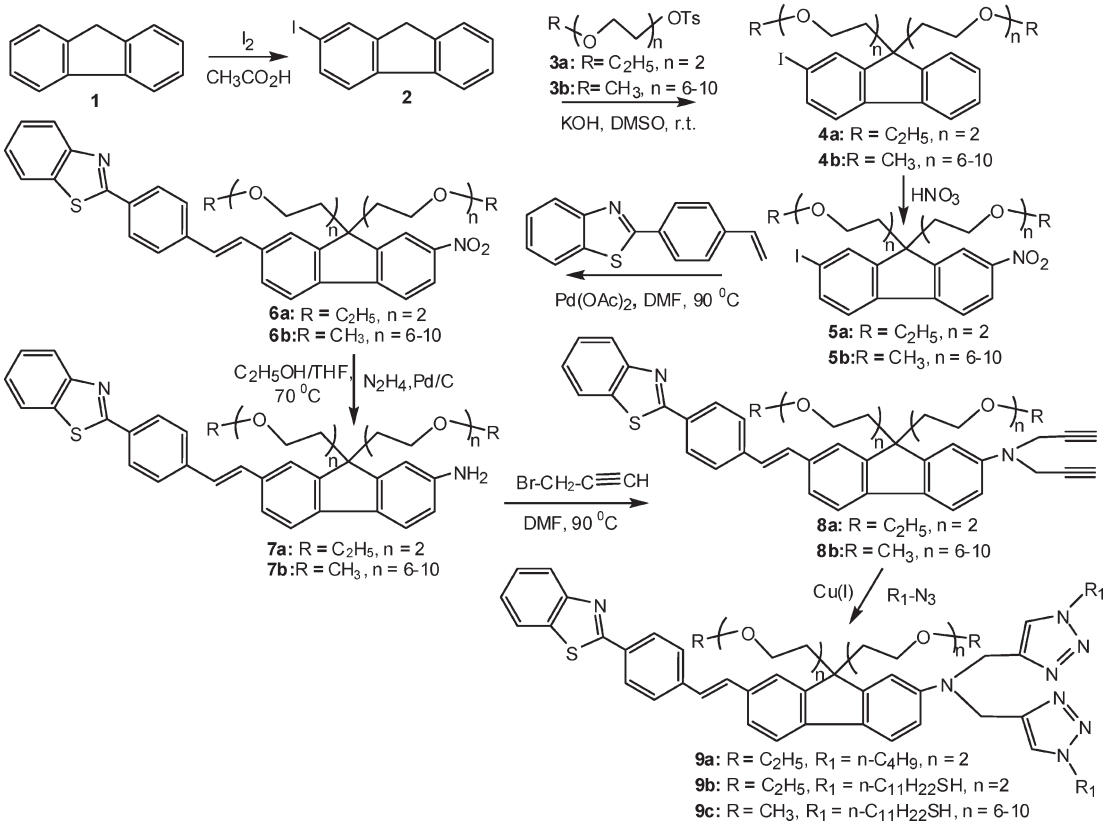
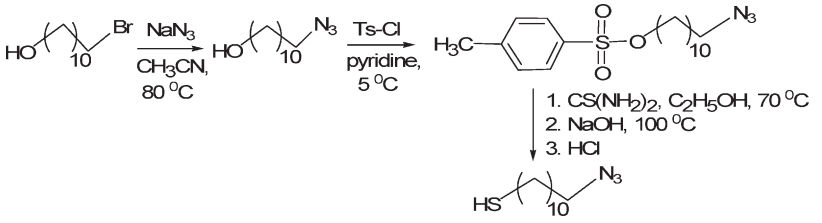
Scheme 1. Synthesis of 1,2,3-Triazolyl Fluorene-Based Derivatives 9a−c



Scheme 2. Synthesis of 1-Azido-11-undecanethiol



can be divided into four groups. Group A consisted of Li^+, Na^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, and Mn^{2+} in which the fluorescence response was unchanged; group B consisted of Al^{3+}, Fe^{2+}, Ni^{2+}, Cu^+, and Cu^{2+}, where a slight decrease in the fluorescence response was observed; group C consisted of Fe^{3+} and Co^{3+} and exhibited quenching or reduction of the fluorescence response, and group D consisted of Zn^{2+}, Hg^{2+}, Cd^{2+}, and Pb^{2+} and displayed an enhanced fluorescence response. The fluorescence response of selected metal ions with probe **9a** is shown in Figure 1; probes **9b** and **9c** exhibited similar responses. The concentration of probe **9a** in ethanol was 4×10^{-6} M while the metal ion concentration was 4×10^{-6} to 10^{-4} M.

The UV−vis absorption and fluorescence emission spectra of complexes of probes **9a−c** with Zn^{2+} (Figures 2 and 3) and Hg^{2+} (Figure 4) displayed enhanced fluorescence responses, accompanied by a blue-shift in wavelength. The UV−vis absorption and fluorescence emission of the complexes of probes **9a−c** with zinc and

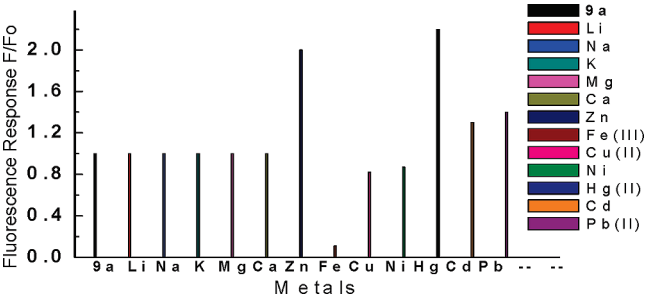


Figure 1. Fluorescence response of probe **9a** at 4×10^{-6} M in ethanol toward various metal ions in concentrations ranging from 1×10^{-6} to 1×10^{-4} M.

mercury were studied in acetonitrile, ethanol, THF, and an aqueous solution of acetonitrile. The UV−vis absorption and fluorescence emission spectra of the complexes of probes **9a−c** with metals were quite similar in organic solvents. As expected, a large Stokes shift was observed in fluorescence emission when upon increase in solvent polarity (Table 1). Titrations with Zn^{2+} and Hg^{2+} for

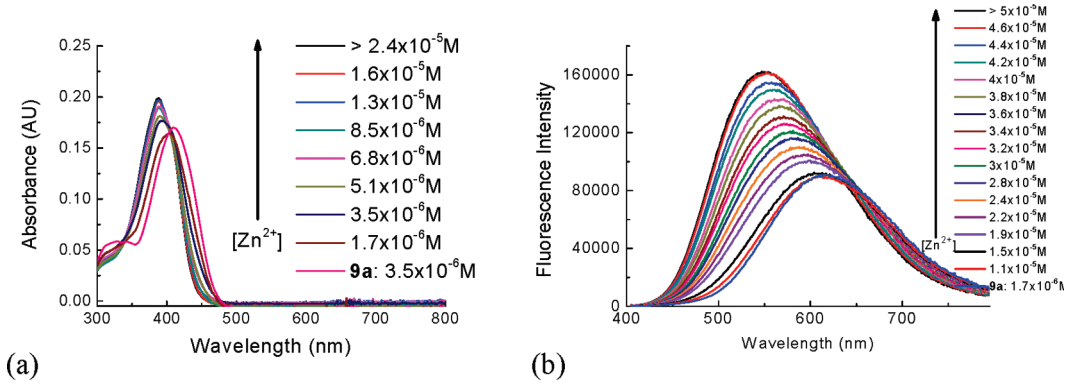


Figure 2. (a) UV−vis absorption spectra of probe **9a** at 3.5×10^{-6} M in ethanol at room temperature as a function of various concentrations of zinc perchlorate (1×10^{-6} to 1×10^{-4} M). (b) Fluorescence emission spectra of probe **9a** at 1.7×10^{-6} M in ethanol excited at 405 nm as a function of zinc(II) concentration.

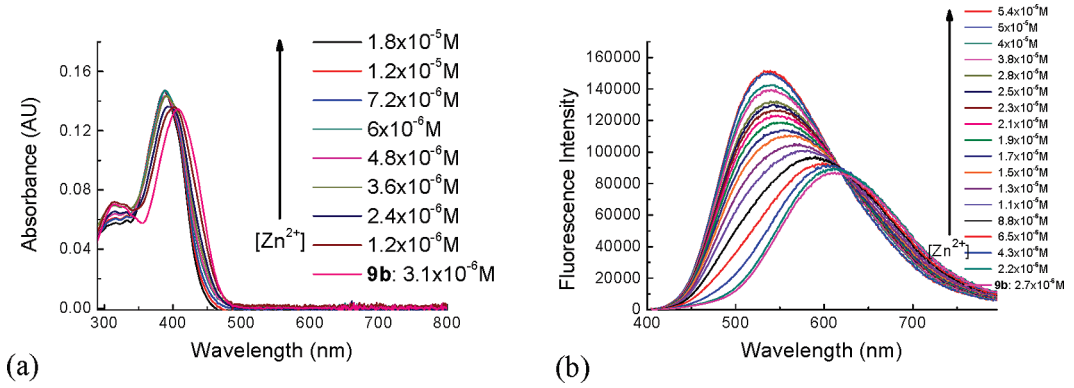


Figure 3. (a) UV−vis absorption spectra of probe **9b** at 3.1×10^{-6} M in ethanol at room temperature as a function of various concentrations of zinc perchlorate (10^{-6} to 10^{-4} M). (b) Fluorescence emission spectra of probe **9a** at 2.7×10^{-6} M in ethanol excited at 405 nm as a function of zinc(II) concentration.

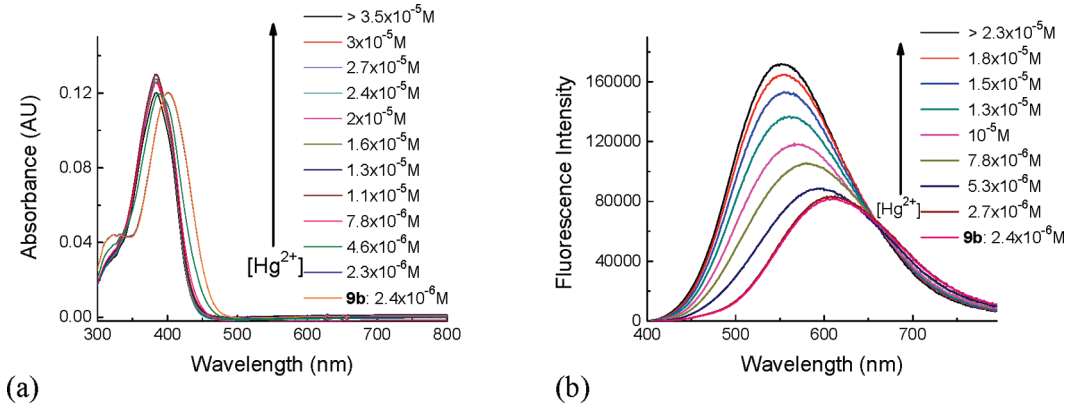


Figure 4. (a) UV−vis absorption spectra of probe **9b** at 2.4×10^{-6} M in ethanol at room temperature as a function of various concentrations of mercuric perchlorate (1×10^{-6} to 1×10^{-4} M). (b) Fluorescence emission spectra of probe **9b** at 2.4×10^{-6} M in ethanol excited at 405 nm as a function of Hg(II) concentration.

probes **9a**−**c** were consistent with complex formation, i.e., a single set of isosbestic points was observed in both their absorption and emission spectra, suggesting a single equilibrium was reached during complexation.

Binding Constants of Metal Complexes. Binding constants, *K*, for **9a**−**c** with Zn^{2+} and Hg^{2+} were calculated from the fluorescence titration curves. The titrations showed that the complexation of **9a**−**c** with Zn^{2+} and Hg^{2+} formed 1:2 and 1:3 ligand to metal ratios, respectively, at equilibrium. These results are comparable to that reported in the literature for the 1:2 complexation of ligand to metal by the work of Videva et al. for a 1:2 zinc-butylcalix[4]arene,[53] Bukhari et al. with a cobalt-quercetin,[54] and Chen et al. with a lithium-anthraquinone cryptand.[55] The values of the binding constant were determined by linear fitting, eq 5, in Figures 5 and 6. A

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Table 1. Linear Photophysical Properties of Probes 9a−c and their metal complexes

	solvent								
	EtOH			THF		ACN		ACN:H_2O 80:20	
compd	ϕ	λ^{ab}_{max}	λ^{em}_{max}	λ^{ab}_{max}	λ^{em}_{max}	λ^{em}_{max}	λ^{ab}_{max}	λ^{ab}_{max}	λ^{em}_{max}
9a	0.32	400	609	401	568	403	616	404	638
9b	0.31	401	612	401	570	402	608	403	641
9c	0.31	403	610	403	569	405	621	404	642
9a + Zn	0.51	385	542	384	521	385	548	400	590
9b + Zn	0.41	386	545	384	511	388	546	399	592
9c + Zn	0.45	384	537	384	510	387	555	399	593
9a + Hg	0.46	382	540	382	517	383	558	396	578
9b + Hg	0.47	383	530	382	519	384	561	395	575
9c + Hg	0.45	382	521	382	520	384	560	394	572

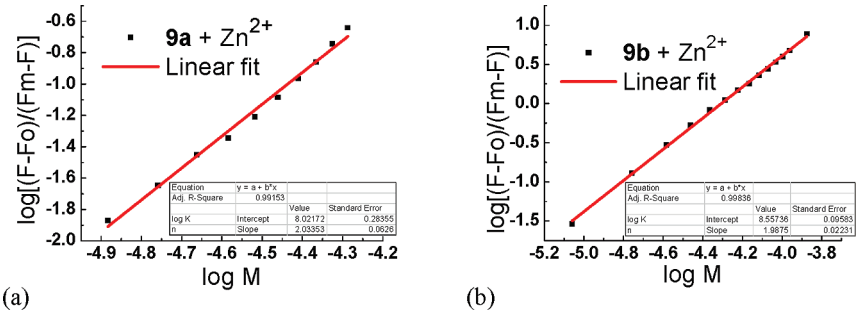

Figure 5. (a) Determination of the binding constant for **9a** with Zn^{2+} in ethanol and (b) **9b** toward Zn^{2+} in ethanol using a linear regression plot of $\log[(F - F_0)/(F_m - F)]$ vs $\log [Zn^{2+}]$.

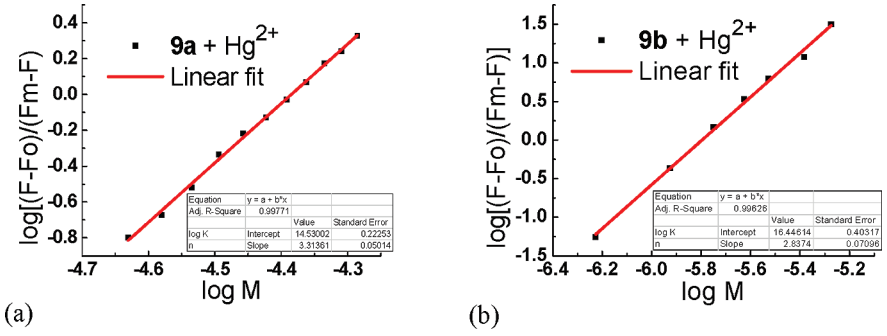

Figure 6. (a) Determination of the binding constant for **9a** with Hg^{2+} in ethanol and (b) **9b** toward Hg^{2+} in ethanol using a linear regression plot of $\log [(F - F_0)/(F_m - F)]$ vs $\log [Hg^{2+}]$.

summary is provided in Table 2. No suitable model for the 1:2 and 1:3 equilibrium with Zn^{2+} and Hg^{2+} could be inferred, but it is not unusual for the metal to interact with other parts of a molecule. An interaction of the softer Hg^{2+} ions with the benzothiazole and thiol moiety of the alkyl chains may explain the higher affinity for Hg^{2+} over Zn^{2+} and the higher metal-to-probe ratio. The distant thiols in the undecanyl chains of probes **9b** and **9c** likely have an impact on the complexation process, because both **9b** and **9c** were shown to be more sensitive in the detection of Hg^{2+}, with log K values 2 orders of magnitude higher than that of **9a**.

Reversibility of Complexation. The complexes of probes **9a**−**c** with metals appeared to form rapidly and were stable for several hours. Strong evidence of reversibility was apparent when EDTA was employed to displace the metal from the complexes, releasing the free probe (Figure 7).

Table 2. Binding Constants of Probes 9a and 9b with Zn^{2+} and Hg^{2+} in Ethanol

	Zn		Hg	
compd	log K	n	log K	n
9a	8.0	2	14.5	3.3
9b	8.9	2	16.4	2.8
9c	9	2.1	16.5	3

Two-Photon Absorption. Two-photon absorption (2PA) cross-sections of probes **9a** and **9b** were determined using the two-photon fluorescence (2PF) measurement technique[47] in ethanol in the absence and presence of metal ions. Figure 8, shows the 2PA spectra of the probes and complexes. For probe **9a**, the 2PA maximum wavelength, $\lambda_{max\ (2PA)}$, was 820 nm. The 2PA cross-sections, δ_{2PA}, for **9a** and **9b**, and their Zn^{2+} and Hg^{2+} complexes can be ascertained from Figure 8. As can be seen, the 2PA cross section values of the complexes were lower than the

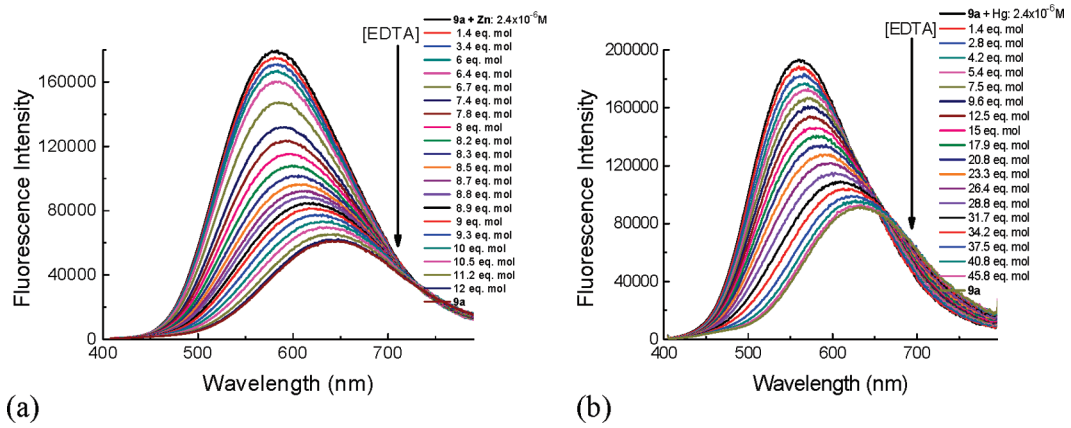

Figure 7. (a) Fluorescence titration of emission of complex **9a** with Zn^{2+} at 2.4×10^{-6} M in ethanol excited at 405 nm as a function of added EDTA. (b) Fluorescence titration of emission of complex **9a** with Hg^{2+} 2.4×10^{-6} M in ethanol excited at 405 nm as a function of added EDTA.

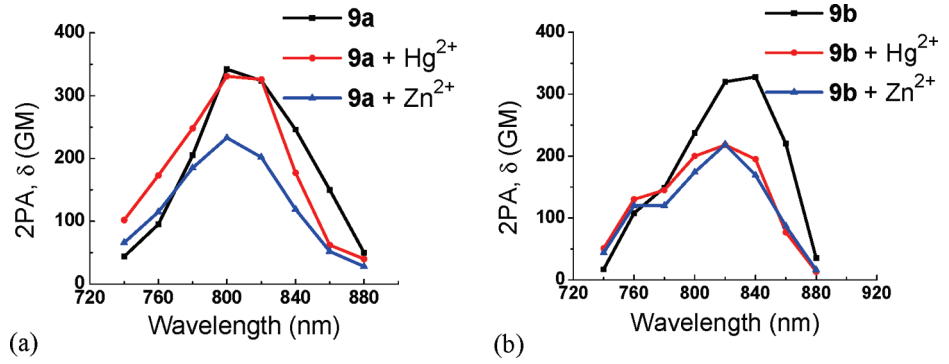

Figure 8. (a) 2PA spectra of **9a** (4×10^{-5} M) and its equilibrium metal ion complexes with Hg^{2+} and Zn^{2+} in ethanol. (b) 2PA spectra of **9b** (4×10^{-5} M) and its equilibrium metal ion complexes with Hg^{2+} and Zn^{2+} in ethanol.

free probes. The 2PA process is a third order nonlinear optical property with a strong dependence on the intramolecular charge transfer (ICT) process.[13] The 2PA cross sections of linear conjugated molecules with a D$-\pi-$A motif strongly depends on the donating ability of the donor moiety. A stronger donor will generally increase charge transfer and enhance the transition dipole moments, contributing to a larger value of δ_{2PA} because of stronger ICT processes. A reduction of intraligand charge-transfer (ILCT) via interaction of a bound cation with the electron-donating amino functionality in **9a** and **9b** may contribute to a reduction in δ_{2PA} of the complexes, consistent with that reported in the literature.[56,57] The 2PA cross-sections at 800 nm, ranging from ca. 200−350 GM are reasonably high, suggesting potential applications of **9a** and **9b** for 2PF metal ion sensing.

Conclusion

Hydrophilic bis(1,2,3-triazolyl)fluorene-based derivatives were prepared and their linear and nonlinear photophysical properties determined. The probes exhibited specific metal ion selectivity, particularly for Zn^{2+} and Hg^{2+}. Complexation of probes **9a**−**c** with metal ions was fast and reversible. The new probes exhibited an approximately 2-fold increase in fluorescence response upon binding to Zn^{2+} and Hg^{2+}. In addition, 1:2 and 1:3 metal to ligand complexes exhibited large binding constant values. This, along with their reasonably large 2PA cross sections at 800 nm and metal ion selective fluorescence emission response positions them as candidates for two-photon based metal ion sensing in biological and environmental applications. Additionally, the synthetic ease to form 1,2,3-triazole ligands via the Cu(I)-catalyzed Huigsen Click reaction, combined with the observed ion selectivity, makes this an especially attractive ion receptor.

Acknowledgment. This work was supported by the National Science Foundation (CHE-0832622) and the National Institutes of Health (1 R15 EB008858-01).

Supporting Information Available: Synthesis of 2-iodofluorene (**2**), **3a**, and **3b**, derivation of eq 4; additional UV−vis absorption and emission spectra of relevant metal complexes of probes **9a**−**c**; and binding constant determination of probe **9c** with metal ions (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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