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New Water Soluble Terphenylene Diethynylene Fluorophores

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Abstract Inspired by our earlier works on sensors from dendritic phenyleneethynylenes, two new star-shaped water-soluble fluorophores containing terphenylene diethynylene units and anionic carboxylate peripheries are successfully synthesized. The convergent synthesis relies on Sonogashira cross-coupling reactions between *tris*-(4-ethynylphenyl)amine and the iodophenyleneethynylene branches. All of the compounds are characterized by ^1H, ^{13}C NMR, and mass spectrometry. In aqueous solution, the less polar fluorophore **1** shows lower quantum yield than **2** (18 vs 33 %) as a result of hydrophobic induced aggregation. One of these anionic water-soluble fluorophores exhibits a selective fluorescence quenching by Fe^{3+} ion in phosphate buffer pH 8.

Keywords Phenyleneethynylene · Sonogashira ·
Fluorescence · Quenching · Ferric ion

Introduction

Linear conjugated polyelectrolytes have widely been applied for metal ion detection based on its efficient singlet exciton migration within the polymer chain allowing efficient long-range Förster resonance energy transfer (FRET) between the polymers and a quencher [1–8]. Despite offering a potentially greater sensitivity, the exact number of repeating fluorophores in linear conjugated polymers cannot be precisely controlled and their random conformations are unpredictable. Their large molecular weight distributions are responsible for inconsistent quenching efficiency, which reflects the low reproducibility of the detection. As parts of our research program on designs and syntheses of novel signal transducers for fluorescence sensors, we have demonstrated synthesis and applications of a polyanionic diphenylene ethynylene fluorophore as a selective fluorescence sensor for mercuric ion [9]. With a stepwise synthetic approach, we also synthesized variously charged fluorophores and demonstrated a sensor array for protein discrimination [10]. Most recently, we reported the use of a polycationic fluorophore as an energy donor for fluorescein-labeled peptide nucleic acid [11]. Although all of those dendritic fluorophores could serve as fluorescent signal transducers, their quantum efficiencies were relatively low which may result from hydrophobicity-induced π-π stacking in aqueous media. For the application as FRET donor, fluorophores which have an emission spectrum overlapping with absorption of the FRET acceptor is more desirable. In this paper, we report a new design of polyanionic dendritic terphenylene diethynylenes **1**–**2** (Fig. 1). We hypothesized that the extended conjugation from additional phenylene ethynylene units could result in compounds which have emission maxima at longer wavelengths compared to their diphenylene analogues (**3C-**). More importantly, this should give us an opportunity to incorporate some sterically hindered spacer units to prevent the undesirable molecular π-stacking.

Experimental

Chemicals and Instruments

All chemicals were purchased from commercial sources and used without purification. All ^1H and ^{13}C NMR spectra were obtained on a Varian Mercury NMR spectrometer, which operated at 400 MHz for ^1H and 100 MHz for ^{13}C nuclei (Varian Company, CA, USA). Mass spectra were recorded on a Microflex MALDI-TOF mass spectrometer (Bruker

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Fig. 1 Structures of **3C**- and Terphenylene Diethynylene Fluorophores **1**–**2**

3C-

1: R = -CH₂CH₂CH₂CH₃
2: R = -CH₂CH₂CH₂OH
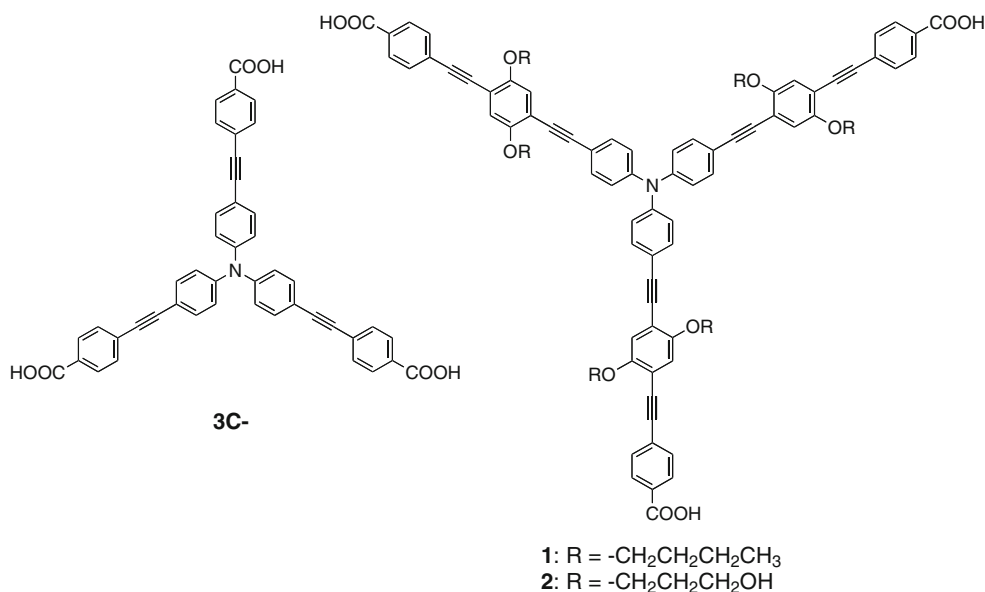
Daltonics) using doubly recrystallized α-cyano-4-hydroxy cinnamic acid (CCA) as a matrix. Absorption spectra were measured by a Varian Cary 50 UV–vis spectrophoto-meter. Fluorescence spectra were obtained from a Varian Cary Eclipse spectrofluorometer.

Synthesis

1,4-Dibutoxybenzene (3a) [13]

To a mixture of 10 mL distilled DMF and hydroquinone (13.4 g, 0.12 mol) was added KOH (28 g, 0.5 mol) with violent stirring for half an hour under nitrogen. Then, *n*-BuBr (44.7 mL, 0.42 mol) was added dropwise to the mixture. After the reaction proceeded for 24 h, the mixture was poured into 100 mL of distilled water. Light yellow solid was filtered and dried under vacuum to afford the product in 17.07 g (64 %). ^{1}H NMR (400 MHz, CDCl$_3$ δ 6.81 (4H, s), 3.91 (4H, t, *J*= 6.5 Hz), 1.80−1.68 (4H, m), 1.55−1.41 (4H, m), 0.97 (6H, t, *J*= 7.3 Hz). ^{13}C NMR (100 MHz, CDCl$_3$) δ ppm 153.2, 115.4, 68.3, 31.5, 19.2, 13.8.

3,3'-(1,4-Phenylenebis(oxy))dipropan-1-ol (3b)

A mixture of hydroquinone (1.0 g, 9.08 mmol), K$_2$CO$_3$ (0.45 g, 3.26 mmol), and 3-chloro-1-propanol (2.28 mL, 0.027 mol) in distilled MeCN (10 mL) was refluxed overnight under nitrogen atmosphere. The reaction was allowed to cool to room temperature and partitioned in CH$_2$Cl$_2$ (50 mL) and water (50 mL). The aqueous layer was separated and extracted with CH$_2$Cl$_2$ (2×50 mL). The organic fractions were collected, dried over Na$_2$SO$_4$, filtered and concentrated under reduced pressure to give a white solid residue. The crude product was crystallized

from dichloromethane to give a white crystalline solid (1.57 g, 76 %). ^{1}H NMR (400 MHz, CDCl$_3$ δ ppm 6.84 (4H, s), 4.08 (4H, t, *J*= 5.8 Hz), 3.86 (4H, t, *J*= 5.8 Hz), 2.03 (4H, quint, *J*= 5.8 Hz). ^{13}C NMR (100 MHz, CDCl$_3$) δ ppm 153.4, 115.8, 66.9, 60.9, 32.4.

3,3'-(1,4-Phenylenebis(oxy))bis(propane-3,1-diyl) diacetate (3c)

To a solution of **3b** (1.0 g, 4.42 mmol) and a catalytic amount of DMAP in 1:1 mixture of CH$_2$Cl$_2$ and pyridine (40 mL) was slowly added AcCl (1.6 mL, 0.022 mol). The mixture was stirred under N$_2$ at room temperature overnight. The solvent was removed, and the resulting slurry was re-dissolved in EtOAc and neutralized with 1 N NH$_4$Cl. A typical extractive work-up afforded product as light yellow powder (1.28 g, 94 %). ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 6.81 (4H, s), 4.24 (4 H, t, *J*= 6.1 Hz), 3.98 (4 H, t, *J*= 6.1 Hz), 2.08 (4 H, m), 2.04 (6H, s). ^{13}C NMR (100 MHz, CDCl$_3$) δ ppm 171.1, 153.1, 115.5, 64.9, 61.4, 28.7, 20.9.

1,4-Dibutoxy-2,5-diiodobenzene (4a)

To a cooled solution (< 15 °C) of ICl (1.31 mL, 0.026 mol) in MeOH (20 mL) was added **3a** (1.33 g, 5.9 mmol) the mixture was allowed to warm, then heated under refluxing conditions for 4 h. A typical extractive work-up afforded **4a** as a pale orange solid (2.08 g, 73 %). ^{1}H NMR (400 MHz, CDCl$_3$ δ ppm 7.17 (2H, s), 3.93 (4H, t, *J*= 6.3 Hz), 1.85−1.73 (4H, m), 1.60−1.47 (4 H, m), 0.98 (6H, t, *J*= 7.3 Hz). ^{13}C NMR (100 MHz, CDCl$_3$) δ ppm 152.8, 122.7, 86.3, 70.0, 31.2, 19.3, 13.9.
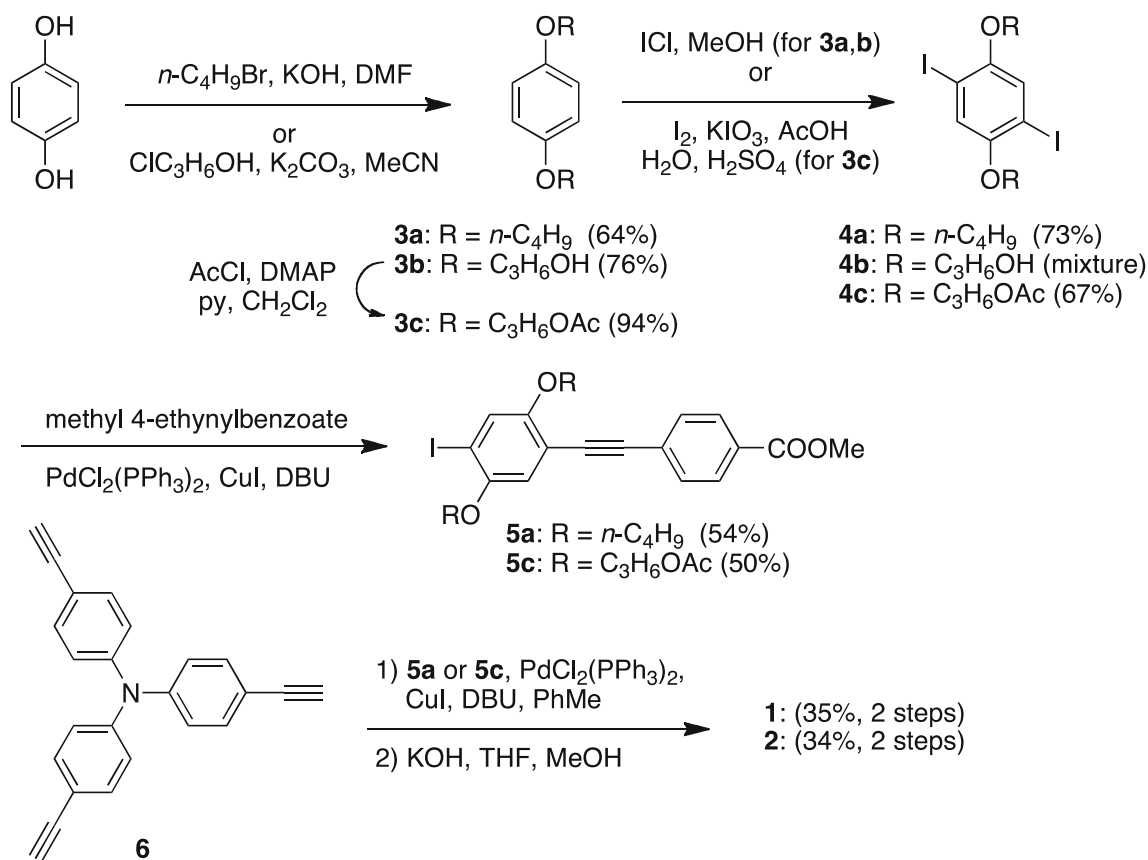
Scheme 1 Synthesis of fluorophore **1**–**2**

*3,3'-(2,5-Diiodo-1,4-phenylene)bis(oxy)bis
(propane-3,1-diyl) diacetate (4c)*

To a solution of I$_2$ (1.03 g, 4.06 mmol) and KIO$_3$ (0.41 g, 1.94 mmol) in glacial acetic acid (9 mL), H$_2$SO$_4$ (0.6 mL) and DI water (6 mL) was added **3c** (1.2 g, 3.87 mmol). The mixture was heated at about 60 °C for 12 h. A typical extractive work-up followed by flash chromatography using hexanes: EtOAc (3:1) as the eluent afforded **4c** as a yellow powder (1.45 g, 67 %). ^{1}H NMR (400 MHz, CDCl$_3$ δ ppm 7.18 (2H, s), 4.32 (4H, t, *J*= 6.1 Hz), 4.02 (4H, t, *J*= 6.1 Hz), 2.18−2.09 (4H, m), 2.07 (6H, s). ^{13}C NMR (100 MHz, CDCl$_3$) δ ppm 171.1, 152.7, 122.8, 86.3, 66.7, 61.2, 28.6, 21.1.

Methyl 4-((2,5-dibutoxy-4-iodophenyl)ethynyl) benzoate (5a)

DBU (0.2 mL, 1.38 mmol) was slowly added to a mixture of **4a** (1.18 g, 2.5 mmol), PdCl$_2$(PPh$_3$)$_2$ (17.5 mg, 0.025 mmol) and CuI (9.5 mg, 0.05 mmol) in toluene (30 mL). Then, a solution of methyl 4-ethynylbenzoate (0.2 g, 1.25 mmol) in toluene (2 mL) was added in small portions. The mixture was stirred at room temperature for 12 h. A typical extractive work-up followed by flash chromatography using gradient solvents from pure hexanes to CH$_2$Cl$_2$/hexanes (1:1) as the eluent afforded **5a** as a yellow solid (0.34 g, 54 % yield). ^{1}H

NMR (400 MHz, CDCl$_3$) δ ppm 8.02 (2H, d, *J*= 8.3 Hz), 7.57 (2H, d, *J*= 8.3 Hz), 7.32 (H, s), 6.91 (1H, s), 3.99 (4H, m), 3.93 (3H, s), 1.85−1.78 (4H, m), 1.60−1.52 (4H, m), 1.00 (6H, m). ^{13}C NMR (100 MHz, CDCl$_3$ δ ppm 166.5, 154.5, 151.9, 131.4, 129.5, 128.1, 123.9, 115.9, 113.0, 93.4, 88.7, 88.4, 69.8, 52.2, 31.3, 19.3, 13.8.

*3,3'-(2-Iodo-5-((4-(methoxycarbonyl)phenyl)
ethynyl)-1,4-phenylene)bis(oxy)bis(propane-3,1-diyl)
diacetate (5c)*

DBU (0.05 mL, 0.34 mmol) was slowly added to a mixture of **4c** (0.37 g, 0.66 mmol), PdCl$_2$(PPh$_3$)$_2$ (4.4 mg, 6.24 μmol) and CuI (2.4 mg, 12.5 μmol) in toluene (10 mL). Then, a

Table 1 Photophysical Properties of **1** and **2** in 50 mM Phosphate Buffer (pH 8.0)

Compound	Absorption		Emission	
	λ$_{max}$ (nm)	Log ε (M^{-1} cm^{-1})	λ$_{max}$ (nm)	Φ$_F$[a]
3C-	374	3.77	454	0.097
1	396	4.66	490	0.18
2	398	4.65	508	0.33

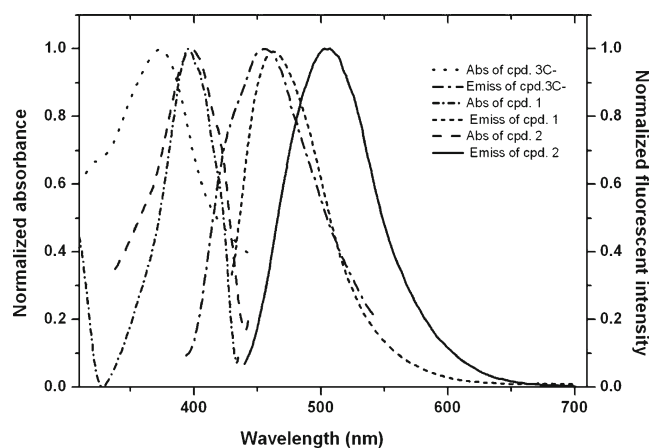[a] Quinine sulfate in 0.1 M H$_2$SO$_4$ (Φ=0.54) was the reference

Fig. 2 Normalized Absorption and Emission Spectra of 3C-, 1, and 2 in 50 mM Phosphate Buffer (pH 8.0)

solution of methyl 4-ethynylbenzoate (0.05 g, 0.31 mmol) was added in small portions. The mixture was stirred at room temperature for 12 h. A typical extractive work-up followed by flash chromatography using gradient solvents from pure hexane to CH_2Cl_2/hexane (1:3) as the eluent afforded 5c as a white solid (91.8 mg, 50 % yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 8.02 (2H, d, J= 8.2 Hz), 7.58 (2H, d, J= 8.2 Hz), 7.33 (1H, s), 6.93 (1H, s), 4.34 (4H, m), 4.08 (4H, m), 3.93 (3H, s), 2.20−2.13 (4H, m), 2.07 (6H, s). ^{13}C NMR (100 MHz, CDCl$_3$) δ ppm 171.1, 166.6, 154.2, 151.8, 131.5, 129.6, 127.9, 123.9, 116.0, 113.2, 93.7, 88.2, 88.1, 66.4, 61.3, 52.3, 28.7, 21.0.

Tris(4-ethynylphenyl)amine (6) [12]

To a stirred solution of tris(4-iodophenyl)amine (2.0 g, 3.21 mmol), PdCl$_2$(PPh$_3$)$_2$ (11.3 mg, 0.016 mmol) and CuI (6.0 mg, 0.032 mmol) in toluene (30 mL) was slowly added DBU (2.16 mL, 14.45 mmol). Then, trimethylsilyl acetylene (2.03 mL, 14.45 mmol) was added and the mixture was stirred at room temperature for 24 h. A typical extractive work-up followed by flash chromatography provided a yellow solid, which was mixed with K$_2$CO$_3$ (0.86 g, 6.19 mmol), CH$_2$Cl$_2$ (30 mL) and

methanol (30 mL). The mixture was stirred at room temperature for 12 h. A typical extractive work-up followed by flash chromatography afforded brown-yellow solid (0.56 g, 86 % yield). ^{1}H NMR (400 MHz, CDCl$_3$) δ ppm 7.38 (6H, d, J= 8.5 Hz), 7.01 (6H, d, J= 8.5 Hz), 3.06 (3H, s). ^{13}C NMR (100 MHz, CDCl$_3$) δ ppm 146.9, 133.2, 123.8, 116.7, 83.3, 82.4.

4,4',4"-(4,4',4"-(4,4',4"-nitrilotris(benzene-4,1-diyl)tris (ethyne-2,1-diyl))tris(2,5-dibutoxybenzene-4,1-diyl)) tris(ethyne-2,1-diyl)tribenzoic acid (1)

DBU (0.1 mL, 0.63 mmol) was slowly added to a mixture of 5a (0.28 g, 0.55 mmol), PdCl$_2$(PPh$_3$)$_2$ (4.4 mg, 6.3 µmol) and CuI (0.6 mg, 3.2 µmol) in toluene (5 mL). Then, solution of 6 (0.05 g, 0.16 mmol) was added and the mixture was stirred for 12 h. A typical extractive work-up followed by flash chromatography afforded a yellow solid. A mixture of this product (100 mg, 0.069 mmol), saturated KOH aqueous solution (0.1 mL) in THF (5 mL), methanol (5 mL) and water (3 mL) was refluxed with stirring for overnight. Volatile solvents were evaporated and the residue dissolved in water (15 mL). Ice was added to the aqueous layer which is acidified and the mixture was stored in a refrigerator. The suspension was centrifuged to afford 1 as a yellow solid (96.9 mg, 35 % yield). ^{1}H NMR (400 MHz, DMSO) δ ppm 7.98 (6H, d, J= 7.8 Hz), 7.61 (6H, d, J= 7.8 Hz), 7.47 (6H, d, J= 7.7 Hz), 7.19 (3H, s), 7.16 (3H, s), 7.12 (6H, d, J= 7.7 Hz), 4.05 (12H, s), 1.68−1.63 (12H, m), 1.46−1.41 (12H, m), 0.95 (18H, t, J= 7.3 Hz). ^{13}C NMR (100 MHz, DMSO) δ ppm 166.6, 153.3, 146.2, 132.7,130.4, 129.5, 126.8, 124.1, 117.3, 116.4, 113.9, 112.3, 94.9, 93.8, 88.9, 85.9, 68.6, 30.7, 18.7, 13.6. MS(MALDI-TOF) Calcd for C$_{93}$H$_{87}$NO$_{12}$: 1410.69 Found: 1410.56.

4,4',4"-(4,4',4"-(4,4',4"-nitrilotris(benzene-4,1-diyl) tris(ethyne-2,1-diyl))tris(2,5-bis(3-hydroxypropoxy) benzene-4,1-diyl))tris(ethyne-2,1-diyl)tribenzoic acid (2)

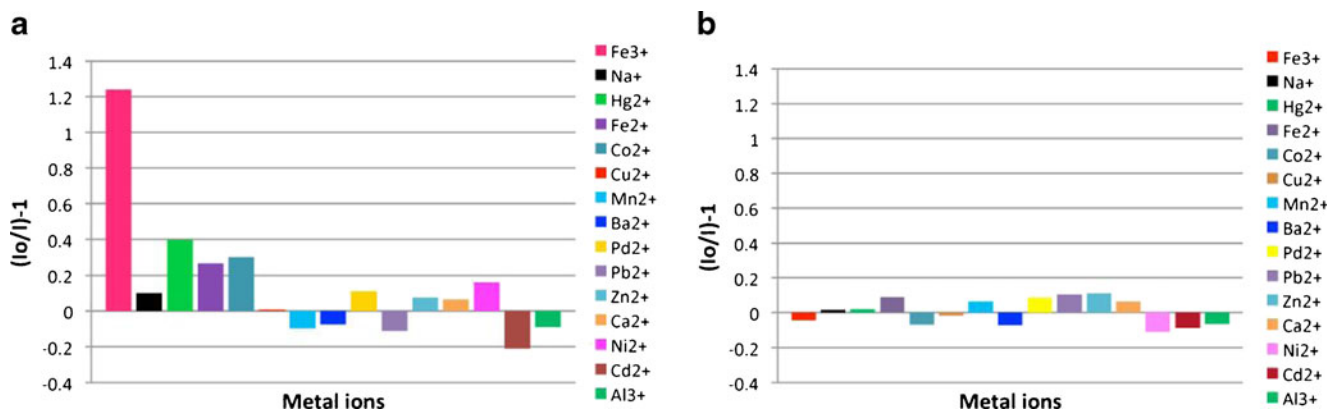This compound was prepared from 5c using the procedure reported for 1. The product was obtained as a yellow solid

Fig. 3 Fluorescence Responses of 1 (a) and 2 (b) (4 µM) to Various Metal Ions (400 µM) in Phosphate Buffer (50 mM, pH 8.0)

(27.1 mg, 34 % yield). ^{1}H NMR (400 MHz, DMSO) δ ppm 8.03 (6H, d, J= 7.9 Hz), 7.67 (6H, d, J= 7.9 Hz), 7.55 (6H, d, J= 8.0 Hz), 7.27 (3H, s), 7.24 (3H, s), 7.14 (6H, d, J= 8.0 Hz), 4.69−4.54 (6H, m), 4.17 (12H, t, J= 6.5 Hz,), 3.69 (12H, t, J= 5.44 Hz), 1.94 (12H, m). ^{13}C NMR (100 MHz, DMSO) δ ppm 166.6, 153.2, 146.3, 132.7, 130.4, 129.5, 126.7, 124.0, 117.3, 116.6, 113.9, 112.3, 94.9, 93.8, 88.7, 85.8, 65.9, 57.2, 32.1. MS(MALDI-TOF) Calcd for $C_{93}H_{87}NO_{12}$: 1422.52 Found: 1423.58.

Results and Discussion

Synthesis of Terphenyleneethynylene Fluorophores 1–2

Our target water-soluble terphenylene diethynylene fluorophores were synthesized by mean of Sonogashira coupling. Prior to that key step, the molecular branches were synthesized by O-alkylation of hydroquinone (Scheme 1). The di-n-butoxy benzene (3a) was successfully iodinated by a reaction with ICl [14], but the same reaction with 3b gave rise to an inseparable mixture of the mono- and diiodo compounds. We then decided to acetylate the hydroxyl group and found that 3c smoothly underwent the diiodination upon treatment with I_2 and KIO_3. A Sonogashira coupling of 4a and 4c with methyl 4-ethynylbenzoate gave rise to 5a and 5c in moderate yields. Fluorophore 1 and 2 by were then assembled via a series of Sonogashira couplings. First, the tris(4-iodophenyl)amine [9] was coupled with trimethylsilyl acetylene, followed by TMS deprotection. A sequential reaction with the dendritic arms (5a or 5c) followed by base-hydrolysis led to fluorophore (1 or 2) in fair yields.

Photophysical Properties of Fluorophores 1–2

The photophysical properties of 1 and 2 in 50 mM phosphate buffer (pH 8.0) were investigated and the results are summarized in Table 1. Both fluorophores have a maximum absorption wavelength around 396–398 nm with an equal molar extinction coefficient, which indicated that the two compounds possessed a similar conjugated system. For the fluorescent spectra, it was found that the maximum emission wavelength of 2 appeared at a longer wavelength (508 nm) compared to that of 1 (490 nm). It seems likely that the more hydrophobic fluorophore 1 is less stabilized in aqueous media upon excitation, while the excited 2 can be well stabilized and a prolonged geometrical relaxation can cause a wider Stoke's shift. The quantum efficiency of 2 (0.33) was also considerably higher than that of 1 (0.18). These results indicate that hydrophobicity in 1 could lead to fluorescence self-quenching. In comparison with our previously reported 3C⁻, these new fluorophores possess more extended conjugation, which results in the absorption and emission maxima at longer wavelengths (Fig. 2).

Metal-sensing Application

For a preliminary screening of applications as fluorescence sensors, we investigated the fluorogenic responses of 1 and 2 in the presence of metal ions such as Fe^{3+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Cd^{2+}, Hg^{2+}, Mn^{2+}, Ba^{2+}, Pd^{2+}, Pb^{2+}, Zn^{2+}, Ca^{2+} and Al^{3+}. It was found that 1 exhibit a selective fluorescence quenching by Fe^{3+} ion without showing significant signal changes by Fe^{2+} or other metal ions (Fig. 3). This selectivity renders an application of the fluorophore as a sensor for trace analysis and diagnosis of iron-related diseases. Although, Lohani and Lee [15] reported the effect of absorbance of Fe^{3+} as an interference effect of the excitation of fluorophore with an excitation wavelength in the UV range, this may not be the case for fluorophore 1 and 2 as both compounds exhibit similar absorption properties but only 1 showed a selectivity toward Fe^{3+}. We proposed that the quenching mechanism should involve a formation of complex between Fe^{3+} ion and the carboxylate groups. This complexation may lead to an aggregation of fluorophore, which would become much less water-soluble than the non-aggregate fluorophore for the case of 1.

Conclusion

Two new terphenylene diethynylene fluorophores containing negatively charged peripheral groups were successfully synthesized. Both compounds displayed high fluorescent quantum yields in aqueous media, which was rationalized by steric-induced deaggregation. The greater quantum efficiency of 2 may result from the hydrophilic –OH groups facilitating the solubility. Fluorophore 1 exhibited a selective fluorescent quenching by Fe^{3+}. The improvements on sensitivity as well as the exploration of further applications for these fluorophores are currently undertaken.

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