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Hydrogen-Bonding-Induced Fluorescence: Water-Soluble and Polarity-Independent Solvatochromic Fluorophores

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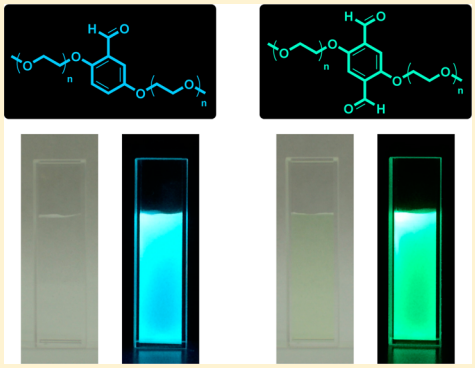
Supporting Information

ABSTRACT: Fluorophores with emission wavelengths that shift depending on their hydrogen-bonding microenvironment in water would be fascinating tools for the study of biological events. Herein we describe the design and synthesis of a series of water-soluble solvatochromic fluorophores, 2,5-bis(oligoethylene glycol)oxybenzaldehydes (**8**−**11**) and 2,5-bis(oligoethylene glycol)oxy-1,4-dibenzaldehydes (**14**−**17**), based on a push−pull strategy. Unlike typical examples in this class of fluorophores, the fluorescence properties of these compounds are independent of solvent polarity and become fluorescent upon intermolecular hydrogen-bonding, exhibiting high quantum yields (up to ϕ = 0.55) and large Stokes shifts (up to 134 nm). Furthermore, their emission wavelengths change depending on their hydrogen-bonding environment. The described fluorophores provide a starting point for unprecedented applications in the fields of chemical biology and medicinal chemistry.



INTRODUCTION

Biological events are primarily regulated by the hydrogen bonding of nucleic acids, proteins, and carbohydrates in aqueous environments. Under such conditions, both the hydrogen-bond donor and acceptor are surrounded by water molecules that mask their donating and accepting properties, yet biomolecules can find and interact with their correct partner in a highly specific manner to induce the corresponding response. However, it remains challenging for chemists to accurately predict hydrogen bonding.[1] The number and positions of hydrogen-bond donors in nucleic acids and proteins can be fine-tuned by replacing the *O*- and/or *N*-hydrogen atom with a methyl group, and such substitutions are common in current chemical biology and medicinal chemistry research.[2] Fluorophores whose emission wavelengths shift depending on their hydrogen-bonding microenvironment in water would provide fascinating tools for the study of biological events.

The design and synthesis of novel fluorophores is in high demand, as they have numerous potential applications in organic light-emitting diodes, chemosensors, and biosensors.[3] The extension of π-conjugated systems over flat and rigid frameworks has provided various unique fluorophores, and a push−pull strategy has also proven to be a promising approach for the construction of small and useful water-soluble variants. Recently, a creative design based on the push−pull strategy enabled the synthesis of a fluorophore containing a single benzene ring that emits bright greenish fluorescence in water with a high quantum yield and large Stokes shift.[4] We have been developing a series of anodic cycloadditions involving intermolecular carbon−carbon bond formation that is facilitated by a lithium perchlorate/nitromethane electrolyte solution.[5] We previously found that dihydrobenzofuran derivatives containing a carbonyl substituent on the aromatic ring emit bright bluish fluorescence in chloroform ($CHCl_3$).[6] This photophysical property results from the push−pull system generated during the reaction, where electron-donating alkoxy substituents and an electron-withdrawing carbonyl substituent are connected through a benzene ring. We also found that dihydrobenzofuran derivatives exhibit solvatochromic properties and that their emission wavelengths change depending on the polarity of the solvent.[7] Herein we describe the design and synthesis of a series of water-soluble solvatochromic fluorophores with high quantum yields and large Stokes shifts, whose emission wavelengths change depending on their hydrogen-bonding environment.

RESULTS AND DISCUSSION

We began by investigating the photophysical properties of benzaldehydes as models (Table 1). Although 2,5-dimethoxybenzaldehyde (DMBA, **1**) emits weakly in $CHCl_3$, it shows bright fluorescence in methanol (MeOH). The corresponding dihydrobenzofuran derivative emits strongly both in $CHCl_3$ and MeOH, suggesting that a rigid dihydrofuran framework is necessary for strong fluorescence in $CHCl_3$ but is unnecessary

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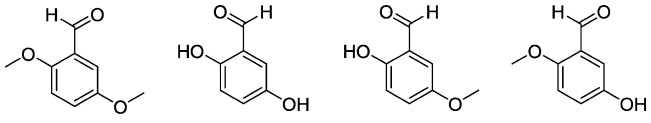
Table 1. Photophysical Data of Benzaldehydes (1−4)



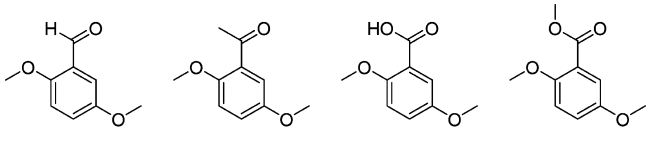
1 2 3 4

compound	solvent	λ^{max}_{abs} [nm][a]	λ^{max}_{em} [nm][a]	ϕ[b]
1	$CHCl_3$	352	427	0.01
1	MeOH	352	466	0.22
2	$CHCl_3$	362	-	<0.01
2	MeOH	364	-	<0.01
3	$CHCl_3$	364	-	<0.01
3	MeOH	359	471	0.03
4	$CHCl_3$	349	433	0.01
4	MeOH	355	489	0.02

[a]Measured at 1.0×10^{-4} M. [b]Absolute quantum yield measured in an integrating sphere.

in MeOH. We also observed that 2,5-dihydroxybenzaldehyde (DHBA, **2**) does not exhibit fluorescence in either $CHCl_3$ or MeOH, clearly indicating that alkylation of the phenol significantly impacts the photophysical properties. The respective monoalkylations impart slight fluorescence to the molecules: 2-hydroxy-5-methoxybenzaldehyde (**3**) and 5-hydroxy-2-methoxybenzaldehyde (**4**) show modest fluorescence in MeOH. Replacement of the aldehyde with ketone (**5**) resulted in a significant decrease in quantum yield while retaining similar absorption and emission wavelengths (Table 2). Although carboxylic acid (**6**) and methyl ester (**7**) have

Table 2. Photophysical Data of 2,5-Dimethoxybenzenes (1, 5−7)



1 5 6 7

compound	solvent	λ^{max}_{abs} [nm][a]	λ^{max}_{em} [nm][a]	ϕ[b]
1	MeOH	352	466	0.22
5	MeOH	334	449	0.10
6	MeOH	314	388	0.24
7	MeOH	318	391	0.64

[a]Measured at 1.0×10^{-4} M. [b]Absolute quantum yield measured in an integrating sphere.

comparable and significantly better quantum yields, respectively, compared to DMBA (**1**), both exhibit large blue shifts in their absorption and emission wavelengths.

There are numerous examples of solvatochromic fluorophores whose emission wavelengths change depending upon the polarity of their environment.[8] In contrast, DMBA (**1**) is poorly fluorescent both in slightly polar and highly polar solvents, including dioxane, tetrahydrofuran (THF), acetone, acetonitrile (MeCN), *N*,*N*-dimethylformamide (DMF), hexamethylphosphoric triamide (HMPA), and dimethyl sulfoxide (DMSO) but shows bright fluorescence in several alcohols (Figure 1; see Table S1 for photophysical data). These results indicate that bright emission by DMBA (**1**) requires a hydrogen-bond donor and is independent of polarity. Furthermore, we found that DHBA (**2**) is fluorescent in

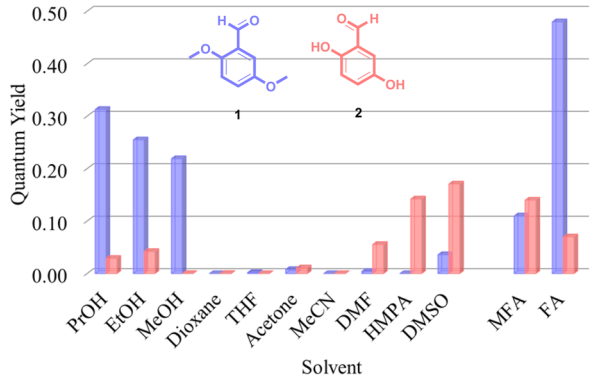
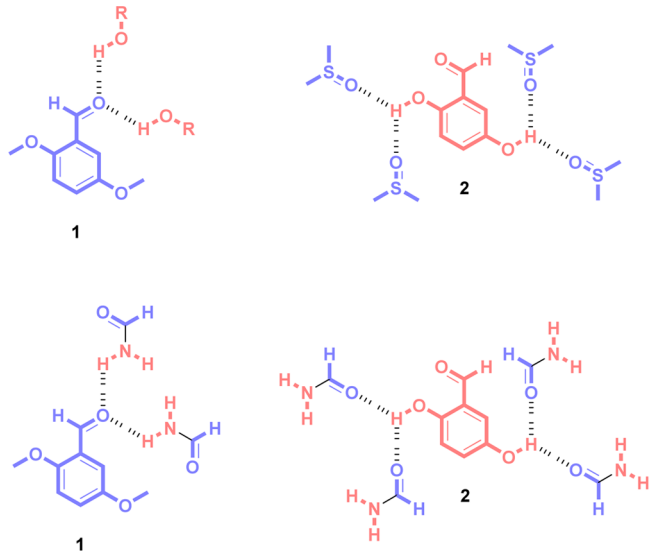


Figure 1. Quantum yields of DMBA (**1**) and DHBA (**2**) in various polar solvents. MFA: *N*-methylformamide, FA: formamide.

DMF, HMPA, and DMSO (Figure 1; see Table S2 for photophysical data). These observations suggest that the hydrogen-bond donor forms an intermolecular hydrogen bond with the aldehyde as the hydrogen-bond acceptor, increasing the electron-withdrawing nature of the hydrogen-bond acceptor. An aprotic hydrogen-bond acceptor forms an intermolecular hydrogen bond with the phenols as hydrogen-bond donors, increasing the electron-donating nature of the donor. This promotes the push−pull system through an aromatic ring (Scheme 1). We therefore tested *N*-methyl-

Scheme 1. Possible Mechanism for Intermolecular Hydrogen-Bonding-Induced Fluorescence of DMBA (1) and DHBA (2) in Several Solvents



formamide (MFA) and formamide (FA) as solvents, because both have hydrogen-bond donor and acceptor groups. To our delight, DMBA (**1**) and DHBA (**2**) were fluorescent in MFA and FA (Figure 1; see Tables S1 and S2 for photophysical data).

The above photophysical study demonstrated that the emission wavelength for DMBA (**1**) changes depending on the nature of the hydrogen-bond donor, and that this shift is not simply due to the acidity of the environment, because acetic acid and trifluoroacetic acid (TFA) quench the fluorescence of DMBA (**1**). Rather, it is likely that acid induces nucleophilic addition to the aldehyde, breaking its electron-withdrawing

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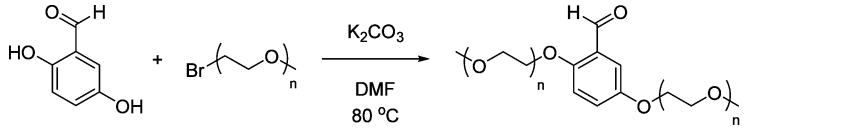
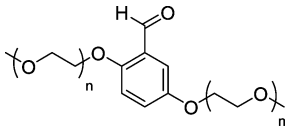
Scheme 2. Preparation of bis(OEG)BAs (8−11)



2 **8** (n = 1, 68%), **9** (n = 2, 65%), **10** (n = 3, 77%), **11** (n = 4, 76%)

nature. To investigate the photophysical properties of compounds related to DMBA (**1**) in water and various alcohols, we prepared a series of 2,5-bis(oligoethylene glycol)-oxybenzaldehydes (bis(OEG)BAs, **8−11**) from DHBA (**2**) in one step (Scheme 2). The oligoethylene glycol is a desirable moiety in fluorophores because it increases the solubility and decreases the crystallinity of the compound without exhibiting significant reactivity. As expected, diethylene and longer glycols imparted water solubility to the molecules. The photophysical properties of DMBA (**1**) and bis(OEG)BAs (**8−11**) in MeOH were comparable (Table 3; see Tables S3−S5 for photophysical data of bis(OEG)BAs (**8−10**)).

Table 3. Photophysical Data of 2,5-Bis(tetraethylene glycol)oxybenzaldehyde (11) in Various Alcohols and Amides



11 (n = 4)

solvent	λ^{max}_{abs}[nm][a]	λ^{max}_{em}[nm][a]	ϕ^b
water	351	481	0.11
MeOH	349	467	0.22
EtOH	349	460	0.25
n-PrOH	349	459	0.27
s-PrOH	349	455	0.24
n-BuOH	349	458	0.32
s-BuOH	350	452	0.29
t-BuOH	350	444	0.19
DMF	350	425	0.02
MFA	349	458	0.10
FA	352	464	0.43

[a]Measured at 1.0×10^{-4} M. [b]Absolute quantum yield measured in an integrating sphere. *N*-methylformamide; FA: formamide.

Bis(OEG)BAs (**8−11**) are soluble in a range of solvents, and in water and various alcohols they exhibited bright bluish fluorescence (445−485 nm) with high quantum yields (up to ϕ = 0.32) and large Stokes shifts (up to 134 nm) (Figure 2; see Table 3 for photophysical data). Primary alcohols caused red shifts, while secondary and tertiary alcohols caused blue shifts. The emission wavelength could be fine-tuned by mixing alcohols, and especially by adding water (Figure 3; see Figure S1 for fluorescence spectra of aqueous *s*-PrOH). These observations were substantiated by studying the fluorescence emission of bis(OEG)BAs (**8−11**) in amides; significant differences were observed among DMF, MFA, and FA (Figure S2). Such differences in the emission wavelengths were still observable even when the amides were mixed with 50% water, suggesting that bis(OEG)BAs (**8−11**) could discriminate organic hydrogen-bond donors from water (Figure 4: see

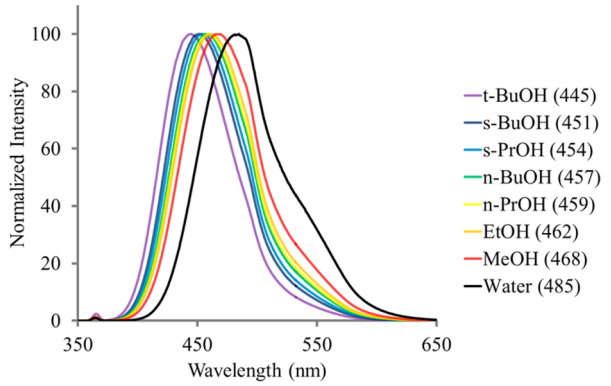


Figure 2. Normalized fluorescence spectra of 2,5-bis(tetraethylene glycol)oxybenzaldehyde (**11**) in various alcohols (1.0×10^{-4} M) upon excitation at 365 nm (λ_{em} are in parentheses).

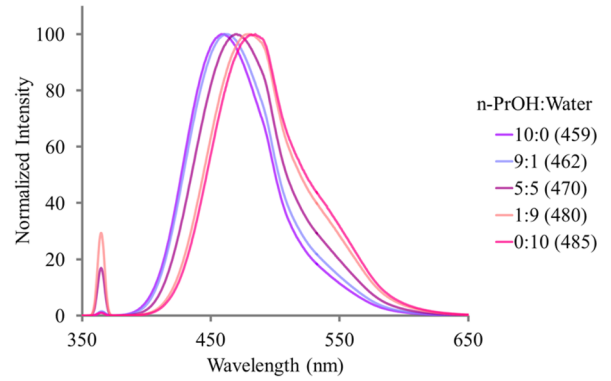


Figure 3. Normalized fluorescence spectra of 2,5-bis(tetraethylene glycol)oxybenzaldehyde (**11**) in aqueous *n*-PrOH (1.0×10^{-4} M) upon excitation at 365 nm (λ_{em} are in parentheses).

Figures S3−S5 for fluorescence spectra of 10% and 90% aqueous amides).

Installation of a second aldehyde on the aromatic ring would be a powerful strategy to enhance the push−pull system, causing a red shift in the emission wavelength. Therefore, a series of 2,5-bis(OEG)oxy-1,4-dibenzaldehydes (bis(OEG)-DBAs, **14−17**) was prepared from 2,5-dimethoxy-1,4-dibenzaldehyde (DMDBA, **12**) in two steps (Scheme 3). Diethylene and longer glycols were required to impart useful water solubility to the molecules. To our satisfaction, the emission wavelengths of bis(OEG)DBAs (**14−17**) in water were red-shifted by 10 nm to provide bright greenish fluorescence (Figure 5), and these compounds exhibited much higher quantum yields (ϕ = 0.55) than those of bis(OEG)BAs (**8−11**), in addition to large Stokes shifts (100 nm), adding new members to the toolbox of water-soluble single-benzene greenish fluorophores (Table 4; see Tables S6−S8 for photophysical data of bis(OEG)DBAs (**14−16**)).

The dependence of the emission wavelengths of bis(OEG)-DBAs (**14−17**) on the nature of the hydrogen-bond donor was

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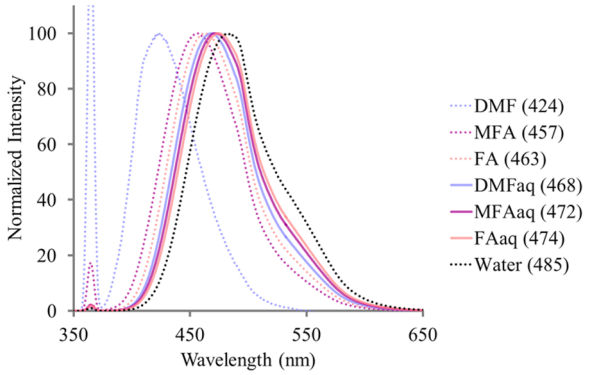


Figure 4. Normalized fluorescence spectra of 2,5-bis(tetraethylene glycol)oxybenzaldehyde (**11**) in 50% aqueous amides (1.0×10^{-4} M) upon excitation at 365 nm (λ_{em} are in parentheses). *N*-methylformamide; FA: formamide.

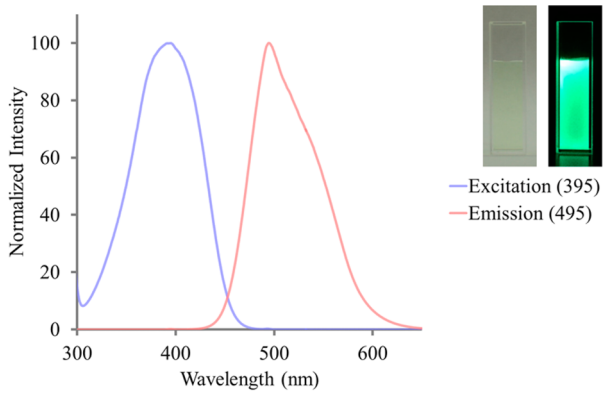
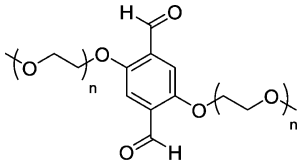


Figure 5. Normalized excitation and emission spectra of 2,5-bis(tetraethylene glycol)oxy-1,4-dibenzaldehyde (**17**) in water (1.0×10^{-4} M) (λ_{max} are in parentheses).

the opposite of that of bis(OEG)BAs (**8**–**11**), with primary alcohols causing blue shifts, and secondary and tertiary alcohols causing red shifts (Figure S6). We confirmed that such differences in the emission wavelengths were still observable in 50% aqueous PrOH and various amides, suggesting that bis(OEG)DBAs (**14**–**17**) could discriminate organic hydrogen-bond donors from water (Figures S7 and S8). Furthermore, we confirmed that 2,5-dihydroxy-1,4-dibenzaldehyde (DHDBA, **13**) fluoresces in DMF, HMPA, and DMSO, supporting the possible mechanism described above for intermolecular hydrogen-bond-induced fluorescence emission (Table S9).

Density functional theory (DFT) calculations were carried out at the B3LYP/6-31G(d) level to gain further insights into the photophysical properties of DMBA (**1**) (Figure 6; see Tables S10 and S11 for Cartesian coordinates), DHBA (**2**) (Figure S9; see Table S12 for Cartesian coordinates), DMDBA (**12**) (Figure 7; see Tables S13, S14 for Cartesian coordinates), and DHDBA (**13**) (Figure S10; see Table S15 for Cartesian coordinates). Intramolecular hydrogen bonding between the phenol as a hydrogen-bond donor and the aldehyde as a hydrogen-bond acceptor apparently brings these groups in close proximity in the most stable conformations of DHBA (**2**) and DHDBA (**13**), whereas steric repulsion explains the most stable conformations of DMBA (**1**) and DMDBA (**12**) because intramolecular hydrogen-bonding is no longer possible. Although the predicted absorption wavelengths are in good agreement with the experimental data, the emission wavelengths are only moderately well predicted, as is the case for both molecules. Moderate prediction of the emission wavelength is likely due to intermolecular hydrogen bonding, which is not considered in the calculations, raising the HOMOs and/or lowering the LUMOs in the excited states to narrow the gaps, thereby contributing to red shifts of the emission wavelengths.

Table 4. Photophysical Data of 2,5-Bis(tetraethylene glycol)oxy-1,4-dibenzaldehydes (17) in Various Alcohols and Amides



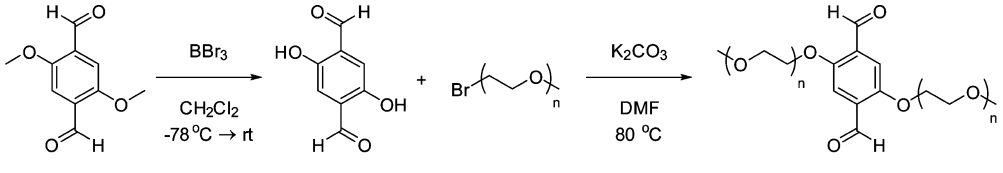
17 (n = 4)

solvent	λ^{max}_{abs} [nm][a]	λ^{max}_{em} [nm][a]	ϕ^b
water	394	495	0.55
MeOH	353	467	0.22
EtOH	364	466	0.18
n-PrOH	365	472	0.14
s-PrOH	390	481	0.11
n-BuOH	375	479	0.09
s-BuOH	392	480	0.10
t-BuOH	393	479	0.12
DMF	392	465	0.04
MFA	350	451	0.05
FA	354	456	0.31

[a]Measured at 1.0×10^{-4} M. [b]Absolute quantum yield measured in an integrating sphere. *N*-methylformamide; FA: formamide.

Finally, we tested the ability of bis(tetraethylene glycol)-oxybenzaldehyde (**11**) and 2,5-bis(tetraethylene glycol)oxy-1,4-dibenzaldehyde (**17**) to discriminate hydrogen-bonding microenvironments using silica gel and octadecylsilyl (ODS) silica gel as simple models (Figure 8; see Figure S10 for photographs with 2,5-bis(tetraethylene glycol)oxy-1,4-dibenzaldehyde (**17**)). Silica gel exhibited brighter fluorescence than expected in the presence of the fluorophore, while almost no fluorescence was observed for the ODS silica gel. Notably, crystalline avidin also

Scheme 3. Preparation of Bis(OEG)DBAs (14–17)



12 **13**, 81% **14** (n = 1, 64%), **15** (n = 2, 61%), **16** (n = 3, 74%), **17** (n = 4, 73%)

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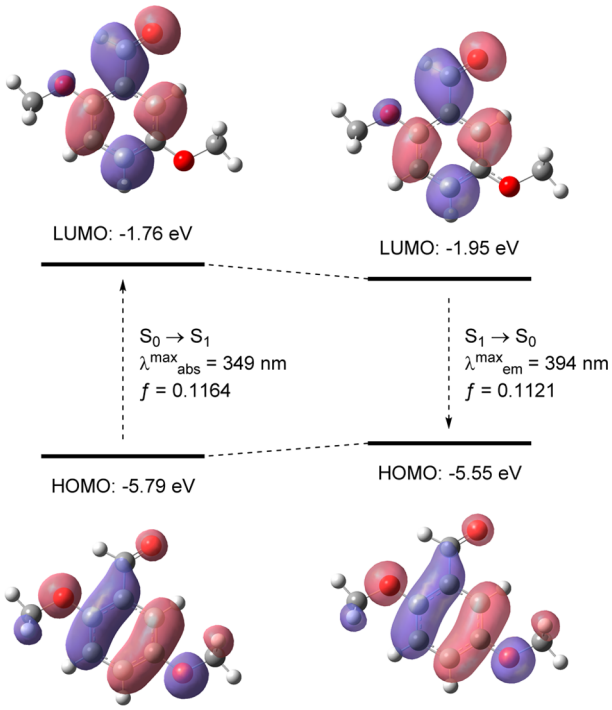

Figure 6. Frontier molecular orbitals involved in UV−vis absorption (left) and fluorescence emission (right) of DMBA (**1**).

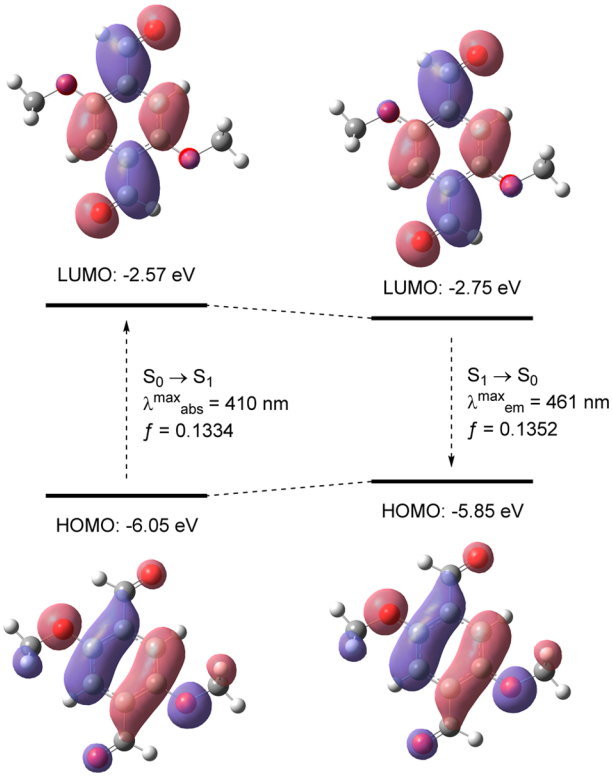

Figure 7. Frontier molecular orbitals involved in UV−vis absorption (left) and fluorescence emission (right) of DMDBA (**12**).

effectively induced fluorescence emission, suggesting that the fluorophore could discriminate hydrogen-bonding microenvironments of biomolecules (Figure 9; see Figure S11 for photographs with 2,5-bis(tetraethylene glycol)oxy-1,4-dibenzaldehyde (**17**)).

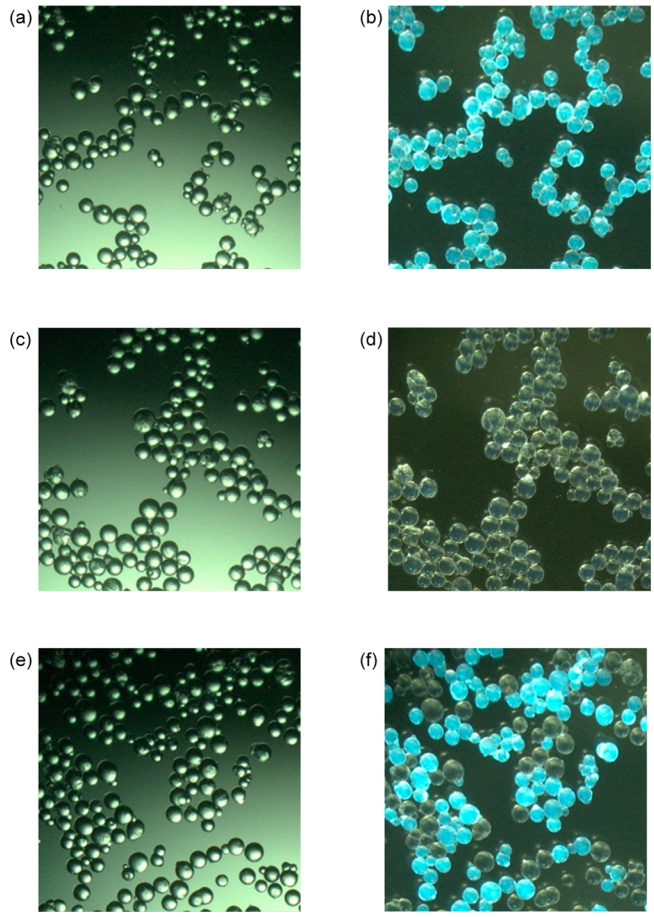

Figure 8. Photographs of (a, b) silica gel, (c, d) ODS silica gels, and (e, f) silica gel + ODS silica gel with 2,5-bis(tetraethylene glycol)oxybenzaldehyde (**11**) under visible light (left) and UV light (340−380 nm, right).

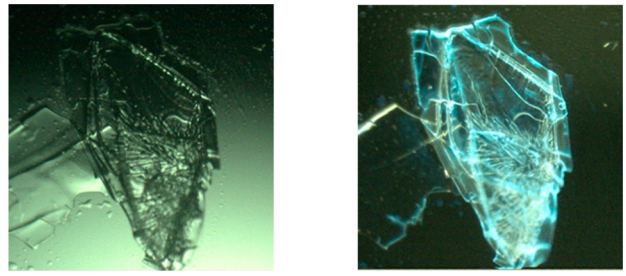

Figure 9. Photographs of crystalline avidin with 2,5-bis(tetraethylene glycol)oxybenzaldehyde (**11**) under visible light (left) and UV light (340−380 nm, right).

CONCLUSIONS

We have designed and synthesized a series of water-soluble and polarity-independent solvatochromic fluorophores, bis(OEG)-BAs (**8**−**11**) and bis(OEG)DBAs (**14**−**17**), based on a push−pull strategy. The fluorophores exhibit high quantum yields and large Stokes shifts due to intermolecular hydrogen-bonding; furthermore, their emission wavelengths change depending on their environment, and they can discriminate organic hydrogen-bond donors from water. It should be noted that such differences in the emission wavelengths are also observed when these compounds are dissolved in various aqueous alcohols and amides, further demonstrating that the fluorophores could

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discriminate organic hydrogen-bond donors from water. In addition, bis(OEG)DBAs (**14**–**17**) emit bright greenish fluorescence in water, thus adding new members to the toolbox of water-soluble, single-benzene greenish fluorophores. We believe that the fluorophores described here will find unprecedented applications in the fields of chemical biology and medicinal chemistry.

EXPERIMENTAL SECTION

General Remarks. All reagents and solvents were purchased from commercial sources and used without further purification. Reactions were monitored by thin-layer chromatography (TLC) carried out on silica gel plates, with detection by UV absorption (254 nm) and by heating the plates after dipping them in a solution of 12 molybdo(VI) phosphoric acid n-hydrate in 95% ethanol. Silica gel (particle size 40–50 μm) was used for column chromatography. ^{1}H NMR spectra were collected on a 500 MHz NMR spectrometer using the deuterated solvent as an internal deuterium reference. Chemical shift data are given in δ units calibrated with residual protic solvent. The multiplicity of a signal is indicated as follows: s, singlet; d, doublet; t, triplet; q, quartet; quin, quintet; m, multiplet. ^{13}C NMR spectra were collected on a 125 MHz spectrometer with proton decoupling using the deuterated solvent as an internal carbon reference. Chemical shift data are given in δ units calibrated with residual solvent. High resolution mass spectra (HRMS) were collected on electrospray ionization (ESI)-time-of-flight (TOF) spectrometer.

Synthesis and Characterization Data. *2,5-Dimethoxybenzaldehyde (**1**).* Pale yellow crystals. Purchased from a commercial source. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.45 (1H, s), 7.34 (1H, d, J = 3.4 Hz), 7.14 (1H, dd, J = 9.1, 3.4 Hz), 6.96 (1H, d, J = 9.1 Hz), 3.90 (3H, m), 3.81 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 189.7, 156.8, 153.7, 125.0, 123.6, 113.5, 110.5, 56.3, 55.9.

*2,5-Dihydroxybenzaldehyde (**2**).* Yellow crystals. Purchased from a commercial source. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.61 (1H, s), 9.83 (1H, s), 7.08 (1H, dd, J = 8.9, 2.9 Hz), 7.01 (1H, d, J = 2.9 Hz), 6.91 (1H, d, J = 8.9 Hz), 4.79 (1H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 196.3, 156.0, 148.6, 125.7, 120.4, 118.9, 118.2.

*2-Hydroxy-5-methoxybenzaldehyde (**3**).* Yellow oil. Purchased from a commercial source. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.66 (1H, s), 9.87 (1H, s), 7.15 (1H, dd, J = 9.1, 2.8 Hz), 7.01 (1H, d, J = 2.8 Hz), 6.94 (1H, d, J = 9.1 Hz), 3.82 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 196.2, 156.0, 152.7, 125.3, 120.1, 118.7, 115.1, 55.9.

*5-Hydroxy-2-methoxybenzaldehyde (**4**),*[9] To a solution of 2,5-dihydroxybenzaldehyde (1.38 g, 10.0 mmol) and iodomethane (684 μL, 11.0 mmol) in DMF (20 mL) stirred at 0 °C was added K_2CO_3 (4.56 g, 33.0 mmol). The resulting reaction mixture was stirred at 0 °C to r.t. overnight, diluted with water (200 mL), and extracted with hot EtOAc (40 mL × 3). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. Silica gel column chromatography (hexane/EtOAc = 2/1) gave the titled compound in 54% yield (819 mg, 5.38 mmol) as yellow crystals. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.42 (1H, s), 7.30 (1H, d, J = 2.9 Hz), 7.10 (1H, dd, J = 9.2, 2.9 Hz), 6.91 Hz (1H, d, J = 9.2 Hz), 4.92 (1H, s), 3.89 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 190.5, 156.8, 150.0, 125.0, 124.0, 113.8, 113.5, 56.3; HRMS $[M + H]^+$ calculated for $C_8H_9O_3$ 153.0552, found 153.0546.

*2′,5′-Dimethoxyacetophenone (**5**).* Pale yellow oil. Purchased from commercial source. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.30 (1H, d, J = 3.1 Hz), 7.04 (1H, dd, J = 9.2, 3.1 Hz), 6.92 (1H, d, J = 9.2 Hz), 3.88 (3H, m), 3.80 (3H, s), 2.62 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 199.2, 153.5, 153.3, 128.1, 120.2, 113.8, 113.1, 55.9, 55.6, 31.8.

*2,5-Dimethoxybenzoic Acid (**6**).* White crystals. Purchased from commercial source. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.99 (1H, s), 7.69 (1H, d, J = 3.4 Hz), 7.13 (1H, dd, J = 9.2, 3.4 Hz), 7.01 (1H, d, J = 9.2 Hz), 4.05 (3H, s), 3.83 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 165.4, 154.5, 152.4, 122.2, 118.2, 116.5, 113.4, 57.4, 56.0.

*Methyl 2,5-Dimethoxybenzoate (**7**).*[10] To a solution of 2,5-dimethoxybenzoic acid (**6**) (951 mg, 5.00 mmol) in MeOH (40 mL) stirred at r.t. was added H_2SO_4 (500 μL). The resulting reaction mixture was stirred at r.t. overnight, diluted with saturated $NaHCO_3$ aq (100 mL), and extracted with EtOAc (40 mL × 3). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. Silica gel column chromatography (hexane/EtOAc = 2/1) gave the titled compound in 81% yield (793 mg, 4.04 mmol) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 (1H, d, J = 3.3 Hz), 7.03 (1H, dd, J = 9.2, 3.3 Hz), 6.93 (1H, d, J = 9.2 Hz), 3.90 (3H, m), 3.87 (3H, s), 3.80 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 166.5, 153.4, 153.0, 120.4, 119.5, 115.9, 113.8, 56.7, 55.8, 52.1; HRMS $[M + Na]^+$ calculated for $C_{10}H_{12}O_4Na$ 219.0633, found 219.0649.

*2,5-Bis(monoethylene glycol)oxybenzaldehyde (**8**).* To a solution of 2,5-dihydroxybenzaldehyde (**2**) (276 mg, 2.00 mmol) and 2-bromoethyl methyl ether (571 μL, 6.00 mmol) in DMF (10 mL) stirred at r.t. was added K_2CO_3 (1.66 g, 12.0 mmol). The resulting reaction mixture was stirred at 80 °C overnight, diluted with water (100 mL), and extracted with hot EtOAc (40 mL × 5). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. Silica gel column chromatography (hexane/EtOAc = 1/1) gave the titled compound in 68% yield (346 mg, 1.36 mmol) as white crystals. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.48 (1H, s), 7.34 (1H, d, J = 3.1 Hz), 7.18 (1H, dd, J = 9.1, 3.1 Hz), 6.96 (1H, d, J = 9.1 Hz), 4.20 (2H, m), 4.12 (2H, m), 3.78 (2H, m), 3.74 (2H, m), 3.45 (3H, s), 3.45 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 189.7, 156.4, 153.2, 125.5, 124.5, 115.1, 111.0, 71.1, 69.1, 68.0, 59.5, 59.4; HRMS $[M + Na]^+$ calculated for $C_{13}H_{18}O_5Na$ 277.1052, found 277.1069.

*2,5-Bis(diethylene glycol)oxybenzaldehyde (**9**).* Pale yellow oil. To a solution of 2,5-dihydroxybenzaldehyde (**2**) (276 mg, 2.00 mmol) and 1-bromo-2-(2-methoxyethoxy)ethane (797 μL, 6.00 mmol) in DMF (10 mL) stirred at r.t. was added K_2CO_3 (1.66 g, 12.0 mmol). The resulting reaction mixture was stirred at 80 °C overnight, diluted with water (100 mL), and extracted with hot EtOAc (40 mL × 5). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. Silica gel column chromatography (EtOAc) gave the titled compound in 65% yield (442 mg, 1.29 mmol) as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.47 (1H, s), 7.33 (1H, d, J = 3.2 Hz), 7.15 (1H, dd, J = 9.1, 3.2 Hz), 6.94 (1H, d, J = 9.1 Hz), 4.22 (2H, m), 4.13 (2H, m), 3.89 (2H, m), 3.85 (2H, m), 3.71 (4H, m), 3.58 (4H, m), 3.39 (6H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 189.7, 156.2, 153.1, 125.5, 124.2, 115.0, 111.2, 72.0, 71.0, 70.8, 69.8, 69.8, 69.0, 68.1, 59.2; HRMS $[M + Na]^+$ calculated for $C_{17}H_{26}O_7Na$ 365.1576, found 365.1560.

*2,5-Bis(triethylene glycol)oxybenzaldehyde (**10**).* To a solution of 2,5-dihydroxybenzaldehyde (**2**) (276 mg, 2.00 mmol) and diethylene glycol 2-bromoethyl methyl ether (1.04 mL, 6.00 mmol) in DMF (10 mL) stirred at r.t. was added K_2CO_3 (1.66 g, 12.0 mmol). The resulting reaction mixture was stirred at 80 °C overnight, diluted with water (100 mL), and extracted with hot EtOAc (40 mL × 5). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. Silica gel column chromatography (EtOAc/MeOH = 40/1) gave the titled compound in 77% yield (665 mg, 1.54 mmol) as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.47 (1H, s), 7.32 (1H, d, J = 3.0 Hz), 7.15 (1H, dd, J = 9.2, 3.2 Hz), 6.95 (1H, d, J = 9.2 Hz), 4.21 (2H, m), 4.12 (2H, m), 3.88 (2H, m), 3.84 (2H, m), 3.73 (4H, m), 3.67 (8H, m), 3.55 (4H, m), 3.38 (3H, s), 3.38 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 189.7, 156.2, 153.2, 125.5, 124.2, 115.1, 111.2, 72.0, 71.0, 70.9, 70.7, 70.7, 69.8, 69.7, 69.1, 68.2, 59.1; HRMS $[M + Na]^+$ calculated for $C_{21}H_{34}O_9Na$ 453.2101, found 453.2097.

*2,5-Bis(tetraethylene glycol)oxybenzaldehyde (**11**).* To a solution of 2,5-dihydroxybenzaldehyde (**2**) (276 mg, 2.00 mmol) and triethylene glycol 2-bromoethyl methyl ether (1.27 mL, 6.00 mmol) in DMF (10 mL) stirred at r.t. was added K_2CO_3 (1.66 g, 12.0 mmol). The resulting reaction mixture was stirred at 80 °C overnight, diluted with water (100 mL), and extracted with hot EtOAc (40 mL × 5). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. Silica gel column chromatography (EtOAc/MeOH = 20/1) gave the titled compound in 76% yield (788 mg, 1.52 mmol) as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.46 (1H, s), 7.32 (1H, d, J = 3.4 Hz), 7.15 (1H, dd, J = 9.2, 3.4 Hz), 6.95 (1H, d,

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J = 9.2 Hz), 4.21 (2H, m), 4.12 (2H, m), 3.88 (2H, m), 3.84 (2H, m), 3.72 (4H, m), 3.66 (16H, m), 3.55 (4H, m), 3.38 (3H, s), 3.37 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 189.6, 156.2, 153.1, 125.4, 124.1, 115.0, 111.1, 71.9, 70.9, 70.8, 70.6, 70.5, 69.7, 69.6, 69.0, 68.1, 59.1; HRMS $[M + Na]^+$ calcd for $C_{25}H_{42}O_{11}Na$ 541.2625, found 541.2604.

2,5-Dimethoxy-1,4-dibenzaldehyde (12). Yellow crystals. Purchased from commercial source. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.22 (2H, s), 9.96 (2H, s), 7.24 (2H, s); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 189.4, 155.9, 129.3, 111.1, 56.4.

2,5-Dihydroxy-1,4-dibenzaldehyde (13). To a solution of 2,5-dimethoxy-1,4-dibenzaldehyde (**12**) (971 mg, 5.00 mmol) in CH_2Cl_2 (20 mL) stirred at −78 °C was added BBr_3 (1.0 M in CH_2Cl_2, 20 mL). The resulting reaction mixture was stirred at −78 °C to r.t. overnight and diluted with water (40 mL), and the organic layer was separated. The aqueous layer was extracted with hot EtOAc (40 mL × 5), and the combined organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. Recrystallization from boiling EtOAc gave the titled compound in 81% yield (669 mg, 4.03 mmol) as an orange crystal. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.22 (2H, s), 9.96 (2H, s), 7.24 (2H, s); ^{13}C NMR (125 MHz, $(CD_3)_2SO$) δ 190.2, 152.8, 127.6, 115.0; HRMS $[M + H]^+$ calcd for $C_8H_7O_4$ 167.0344, found 167.0353.

2,5-Bis(monoethylene glycol)oxy-1,4-dibenzaldehyde (14). To a solution of 2,5-dihydroxy-1,4-dibenzaldehyde (**13**) (332 mg, 2.00 mmol) and 2-bromoethyl methyl ether (571 μL, 6.00 mmol) in DMF (10 mL) stirred at r.t. was added K_2CO_3 (1.66 g, 12.0 mmol). The resulting reaction mixture was stirred at 80 °C overnight, diluted with water (100 mL), and extracted with hot EtOAc (40 mL × 5). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. Silica gel column chromatography (hexane/EtOAc = 1/1) gave the titled compound in 64% yield (359 mg, 1.27 mmol) as yellow crystals. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.53 (2H, s), 7.46 (2H, s), 4.26 (4H, m), 3.79 (4H, m), 3.45 (6H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 189.4, 155.3, 129.6, 112.2, 70.9, 68.9, 59.4; HRMS $[M + Na]^+$ calcd for $C_{14}H_{18}O_6Na$ 305.1001, found 305.1008.

2,5-Bis(diethylene glycol)oxy-1,4-dibenzaldehyde (15).[11] To a solution of 2,5-dihydroxy-1,4-dibenzaldehyde (**13**) (332 mg, 2.00 mmol) and 1-bromo-2-(2-methoxyethoxy)ethane (797 μL, 6.00 mmol) in DMF (10 mL) stirred at r.t. was added K_2CO_3 (1.66 g, 12.0 mmol). The resulting reaction mixture was stirred at 80 °C overnight, diluted with water (100 mL), and extracted with hot EtOAc (40 mL × 5). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. Silica gel column chromatography (EtOAc) gave the titled compound in 61% yield (452 mg, 1.22 mmol) as a yellow crystal. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.53 (2H, s), 7.46 (2H, s), 4.28 (4H, m), 3.90 (4H, m), 3.71 (4H, m), 3.57 (4H, m), 3.39 (6H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 189.4, 155.3, 129.6, 112.2, 72.1, 71.0, 69.7, 69.0, 59.3; HRMS $[M + Na]^+$ calcd for $C_{18}H_{26}O_8Na$ 393.1526, found 393.1525.

2,5-Bis(triethylene glycol)oxy-1,4-dibenzaldehyde (16).[12] To a solution of 2,5-dihydroxy-1,4-dibenzaldehyde (**13**) (332 mg, 2.00 mmol) and diethylene glycol 2-bromoethyl methyl ether (1.04 mL, 6.00 mmol) in DMF (10 mL) stirred at r.t. was added K_2CO_3 (1.66 g, 12.0 mmol). The resulting reaction mixture was stirred at 80 °C overnight, diluted with water (100 mL), and extracted with hot EtOAc (40 mL × 5). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. Silica gel column chromatography (EtOAc/MeOH = 40/1) gave the titled compound in 74% yield (665 mg, 1.54 mmol) as a pale yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.52 (2H, s), 7.46 (2H, s), 4.27 (4H, m), 3.90 (4H, m), 3.73 (4H, m), 3.66 (8H, m), 3.55 (4H, m), 3.38 (6H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 189.4, 155.3, 129.6, 112.3, 72.1, 71.1, 70.8, 70.8, 69.6, 69.0, 59.2; HRMS $[M + Na]^+$ calcd for $C_{22}H_{34}O_{10}Na$ 481.2050, found 481.2074.

2,5-Bis(tetraethylene glycol)oxy-1,4-dibenzaldehyde (17),[11b,13] *9.* To a solution of 2,5-dihydroxy-1,4-dibenzaldehyde (**13**) (332 mg, 2.00 mmol) and triethylene glycol 2-bromoethyl methyl ether (1.27 mL, 6.00 mmol) in DMF (10 mL) stirred at r.t. was added K_2CO_3 (1.66 g, 12.0 mmol). The resulting reaction mixture was stirred at 80 °C overnight, diluted with water (100 mL), and extracted with hot EtOAc (40 mL × 5). The combined organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo. Silica gel column chromatography (EtOAc/MeOH = 20/1) gave the titled compound in 73% yield (794 mg, 1.45 mmol) as a yellow oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.52 (2H, s), 7.46 (2H, s), 4.27 (4H, m), 3.90 (4H, m), 3.72 (4H, m), 3.66 (16H, m), 3.55 (4H, m), 3.37 (6H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 189.4, 155.3, 129.6, 112.2, 72.0, 71.0, 70.7, 70.7, 70.6, 69.6, 69.0, 59.1; HRMS $[M + Na]^+$ calcd for $C_{26}H_{42}O_{12}Na$ 569.2574, found 569.2579.

Theoretical Calculations. Structure optimizations of all stationary points and frequency analyses of 2,5-dimethoxybenzaldehyde (**1**) and 2,5-dimethoxy-1,4-dibenzaldehyde (**12**) were carried out at the RB3LYP level of (time-dependent (TD)) density functional theory (DFT) with the 6-31G(d) basis set in water (PCM model). Structure optimizations of all stationary points and frequency analyses of 2,5-dihydroxybenzaldehyde (**2**) and 2,5-dihydroxy-1,4-dibenzaldehyde (**13**) were carried out at the RB3LYP level of DFT with the 6-31G(d) basis set in DMSO (PCM model). No imaginary frequency was observed for all compounds. Single point energies of 2,5-dimethoxybenzaldehyde (**1**) and 2,5-dimethoxy-1,4-dibenzaldehyde (**12**) were then carried out for the optimized structures at the RB3LYP level of TD-DFT with the 6-31G(d) basis set in water (PCM model).

Imaging Studies. To a solution of bis(tetraethylene glycol)-oxybenzaldehyde (**11**) (1.0 mM) or 2,5-bis(tetraethylene glycol)oxy-1,4-dibenzaldehyde (**17**) (1.0 mM) in EtOAc (1 mL) was dispersed silica gel (40−50 μm, 100 mg), octadecylsilyl silica gel (40−50 μm, 100 mg), or crystalline avidin (10 mg). Droplets of the dispersion were observed without a filter after evaporation under visible light and UV light.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b01991

Additional tables and figures, and copies of ^{1}H and ^{13}C NMR (PDF)

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Notes

The authors declare no competing financial interest.

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