Angewandte Communications

Fluorescence Hot Paper

International Edition: DOI: 10.1002/anie.201502365
German Edition: DOI: 10.1002/ange.201502365

Single Benzene Green Fluorophore: Solid-State Emissive, Water-Soluble, and Solvent- and pH-Independent Fluorescence with Large Stokes Shifts**

*Teruo Beppu, Kosuke Tomiguchi, Akito Masuhara, Yong-Jin Pu, and Hiroshi Katagiri**

Abstract: *Benzene is the simplest aromatic hydrocarbon with a six-membered ring. It is one of the most basic structural units for the construction of π conjugated systems, which are widely used as fluorescent dyes and other luminescent materials for imaging applications and displays because of their enhanced spectroscopic signal. Presented herein is 2,5-bis(methylsulfonyl)-1,4-diaminobenzene as a novel architecture for green fluorophores, established based on an effective push–pull system supported by intramolecular hydrogen bonding. This compound demonstrates high fluorescence emission and photostability and is solid-state emissive, water-soluble, and solvent- and pH-independent with quantum yields of $\Phi = 0.67$ and Stokes shift of 140 nm (in water). This architecture is a significant departure from conventional extended π-conjugated systems based on a flat and rigid molecular design and provides a minimum requirement for green fluorophores comprising a single benzene ring.*

Novel designs for fundamental fluorescent scaffolds have been attracting the attention of chemists because of their potential applications in fluorescent bioimaging[1] and as light-emitting layers in organic light-emitting diodes.[2] There are two general strategies for the construction of organic fluorescent scaffolds; one is an extended π-conjugated system based on a flat and rigid framework[3] and the other is a push–pull system based on a donor-π-conjugated-acceptor structure.[4] The extended π-conjugated system leads to low solubility in many solvents, and the ease of intermolecular stacking at high concentrations leads to subsequent quenching and lowered photosensitivity through intermolecular energy-transfer processes. Conversely, the push–pull system is constructed by relatively small π-conjugated systems and is widely used as a fundamental scaffold in organic dyes and optoelectronic materials. As desirable features for fluorescent molecules used in bioimaging, solid-state emission properties help to prevent self-quenching,[5] and in general, a large Stokes shift helps to avoid the reabsorption of emitted photons, which allows higher contrast in fluorescence imaging. There are sometimes disadvantages for basic scaffolds of organic fluorophores. For example, cyanine dyes show aggregation and photobleaching,[6] fluorescein is pH sensitive,[7] and BODIPY has a small Stokes shift.[8] Therefore, many researchers are intent on solving these problems through synthetic modification.[9]

Herein, we report a new class of sulfonylaniline-based fluorophores, 2,5-bis(methylsulfonyl)-1,4-diaminobenzene (BMeS-*p*-A), as robust fluorescent scaffolds through a push–pull system; the fluorophores are solid-state emissive, water-soluble, environmentally insensitive, and display large Stokes shift performances.

Our approach to install fluorescence at a longer wavelength relied on our previous concept of a push–pull system between amino and sulfonyl moieties supported by intramolecular hydrogen bonding, which showed good photostability and efficient fluorescence in the blue range (about 400 nm) in both solution and solid state.[10] This observation prompted us to explore more effective combinations of amino and sulfonyl moieties demonstrating longer wavelengths and smaller sizes. The 1,4-diaminobenzene unit is one of the strongest donating groups with a high HOMO and is used as a component in conductive polymers;[11] 1,4-bis(alkenyl)-2,5-dipiperidinobenzenes have been reported as visible fluorophores;[12] 2,5-bis(phenylsulfonyl)-1,4-diaminobenzene has been reported as a yellow crystalline compound.[13]

BMeS-*p*-A was prepared according to Scheme 1. Commercially available compound **1** was methylated at sulfur atoms to give sulfide **2**. The amino groups were then protected with acetyl groups, the sulfur atoms were oxidized with *m*-CPBA, and the acetyl groups were removed by deprotection in basic conditions to afford the desired BMeS-*p*-A in 62 % overall yield over four steps. In addition to the ease of synthesis, the oxidation and deprotection processes were conducted at ambient conditions in a one-pot procedure, and all products could be isolated without column chromatography; synthetic intermediate compounds **2** and **3** were isolated by filtration, and the target molecule BMeS-*p*-A was purified by recrystallization (see the synthesis in the Supporting Information). Owing to the high yields and simple work-up procedures, BMeS-*p*-A was conveniently prepared on a gram scale.

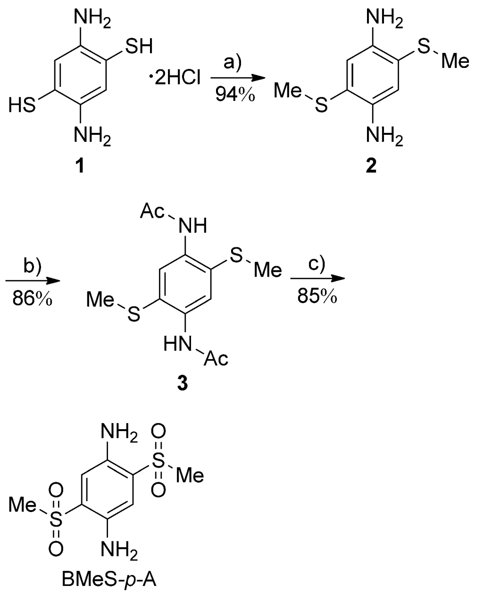
BMeS-*p*-A is soluble in water and various organic solvents and exhibits green fluorescence with high quantum yields

---

[*] T. Beppu, K. Tomiguchi, Prof. Dr. A. Masuhara, Prof. Dr. Y.-J. Pu, Prof. Dr. H. Katagiri
Graduate School of Science and Engineering
Yamagata University
4-3-16 Jonan, Yonezawa, Yamagata 992-8510 (Japan)
E-mail: kgri7078@yz.yamagata-u.ac.jp

[**] We thank Mr. R. Satake (Yamagata University) for technical support in quantum yield measurements. This study was partially supported by Izumi Science and Technology Foundation (grant number H26-J-031 to H.K.).

Supporting information for this article, including ^{1}H and ^{13}C NMR spectroscopic data, high-resolution mass spectrometry data for all new compounds, X-ray crystallographic data and crystallographic files, powder XRD data, UV/Vis and fluorescence spectra, MO calculation details, and photostability measurements, is available on the WWW under http://dx.doi.org/10.1002/anie.201502365.



Scheme 1. Synthetic route toward BMeS-*p*-A. Conditions and reagents: a) sodium (4.1 equiv), iodemethane (2.1 equiv), methanol, RT, 5 h. b) Triethylamine (10 equiv), acetic anhydride (10 equiv), dichloromethane, RT, one day. c) 1. *m*-Chloroperbenzoic acid (10 equiv), dichloromethane, RT, two days. Sodium hydroxide aqueous solution (2. 6 м), tetrahydrofuran, reflux, one day.

Table 1: Absorption and emission properties of BMeS-*p*-A in selected solvents.[a]

Solvent	λ^{max}_{abs} [nm]	λ^{max}_{em} [nm][b]	ε^{max} [$\mathrm{M}^{-1}\,\mathrm{cm}^{-1}$]	Φ[c]
THF	384	490	4170	0.51
MeOH	385	505	3990	0.47
DMSO	394	509	4360	0.70
water	377	517	3820	0.67
powder	380[d]	477	–	0.69[e]

[a] UV/Vis spectra recorded at 1.5×10^{-4} м; fluorescence spectra recorded at 5.0×10^{-6} м. [b] Excitation at $\lambda = 350$ nm. [c] Determined relative to 9,10-diphenylanthracene in cyclohexane ($\Phi = 0.95$). [d] Reflection spectra in an integrating sphere. [e] Determined in an integrating sphere.

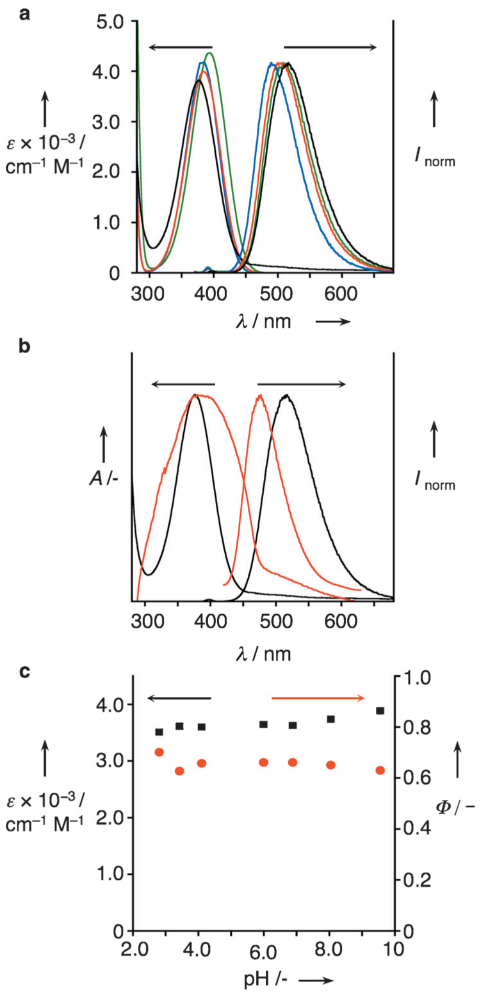


Figure 1. a) Absorption and fluorescence spectra of BMeS-*p*-A in tetrahydrofuran (blue), methanol (red), dimethylsulfoxide (green), and water (black). b) Absorption and fluorescence spectra of BMeS-*p*-A in water (black) and solid state (crystalline powder, red). Solid-state absorption spectrum was measured using the reflection method in an integrating sphere with a lamp switching wavelength of 330 nm. c) Plot of molar extinction coefficient (black square) and fluorescence quantum yields (red circle) of BMeS-p-A in phosphate buffers (pH 2.8–9.6).

(Table 1). Notably, large Stokes shifts up to 140 nm were observed. Of particular interest, a 140 nm shift was observed in water, which is six times larger than that in fluorescein. In addition, fluorescent properties such as λ^{max}_{abs}, λ^{max}_{em}, ε, and Φ showed comparatively small dependence on the solvent. Moreover, the pH dependence of the fluorescence intensity was quite small (Figure 1), which is quite different from that of fluorescein (see absorption and fluorescence spectra in the Supporting Information). The pH sensitivity is generally a disadvantage because the fluorescence intensity drastically decreases below pH 6; thus, the experimental conditions need to be maintained between pH 6 and 10.[7] To avoid this, Tokyo Green was developed by replacing the ionic carboxylic acid of fluorescein with a non-ionic methyl group.[9j] BMeS-*p*-A is non-ionic and composed of a single benzene platform, which most likely contributes to the pH independence of the fluorescence intensity.

The X-ray single-crystal structure of BMeS-*p*-A was dominated by strong intramolecular and intermolecular hydrogen bonding between amino and sulfonyl groups, which remarkably adopt Etter's empirical hydrogen bonding rules for organic compounds.[14] Six-membered ring intramolecular hydrogen bonding prompts both methylsulfonyl groups to adopt a syn configuration (Figure 2 a); intermolecular hydrogen bonding supports ladder structures along the *a*–*c* plane (Figure 2 b). These structural features prevented intermolecular π-stacking between benzene units, allowing the generation of solid-state emission (Figure 2 c). The powder X-ray pattern of the sample used for fluorescent measurements was in agreement with the simulation from the single-crystal X-ray diffraction (see the powder X-ray diffraction pattern in the Supporting Information).

Density functional theory (DFT) calculations in water at the PCM/B3LYP/6-31G(d) level were performed to inves-

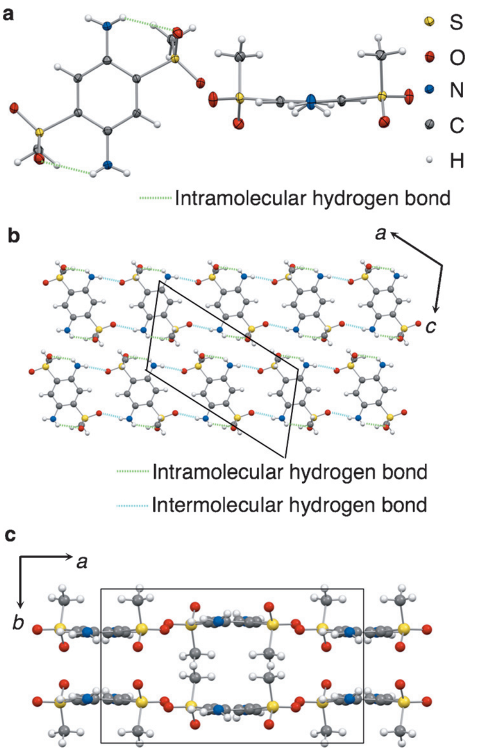


Figure 2. Crystal structure of BMeS-*p*-A. a) Thermal ellipsoid drawing of side and top views at 50% probability level. b) Partial view with the indication of intramolecular and intermolecular hydrogen bonding. c) Packing structure along the *c* axis.

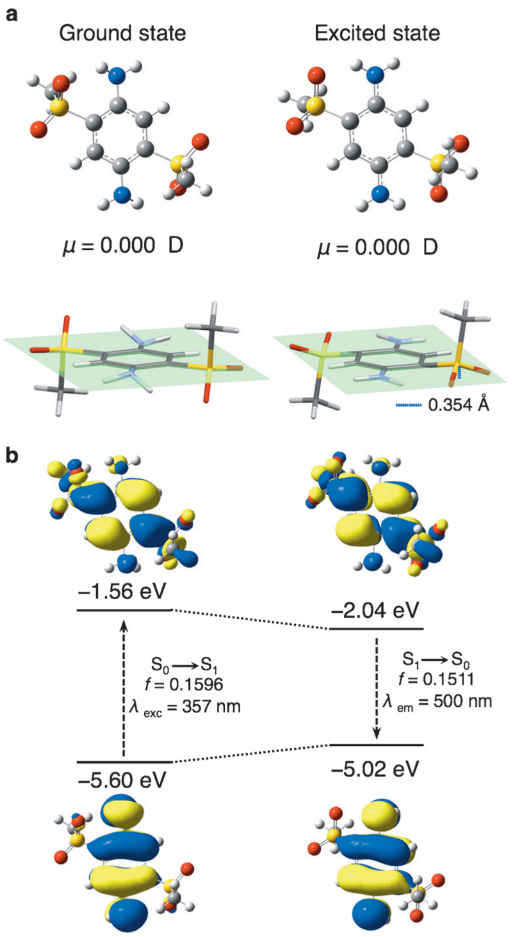


Figure 3. MO calculation of BMeS-*p*-A. a) Geometry of BMeS-*p*-A in the ground and excited states. b) Rationalization for the UV/Vis absorption, emission, and large Stokes shift of BMeS-*p*-A; the geometry relaxation and the frontier molecular orbitals involved in the vertical excitation (i.e., UV/Vis absorption, left column) and emission (right column) of BMeS-*p*-A. The vertical-excitation-related calculations are based on the optimized geometry of the ground state (S_0). The emission-related calculations were based on the optimized geometry of the excited state (S_1). Water was used as the solvent for calculations (polarizable continuum model) at the B3LYP/6-31G(d) level.

tigate the optical properties of BMeS-*p*-A. These results were in good agreement with the absorption and emission spectra (Figure 3).[9g] The anti conformer was the most stable, as determined from the conformation search. The optimized structures in both the ground state (DFT) and excited state (time-dependent DFT) exhibited HOMOs that were located at the central 1,4-diaminobenzene moieties and LUMOs that were distributed over the entire molecule via the sulfonyl groups. The resulting HOMO–LUMO asymmetry indicated that an effective push–pull system was established for the BMeS-*p*-A framework. Moreover, the ^{1}H NMR spectrum of BMeS-*p*-A showed that the proton signal assigned to NH_2 resonated at 5.45 ppm, shifting downfield from that of sulfide precursor **2**, which resonated at 4.38 ppm. These results suggest that the sulfonyl groups withdraw electron density from nitrogen and its adjacent protons. The six-membered ring intramolecular hydrogen bonds are also predominantly controlled by the push–pull system, promoting the downfield shift of NH_2 protons in the ^{1}H NMR spectra. As a notable feature of the optimized structures, structural differences between the ground and excited states were observed. In the excited state, the sulfonyl groups were 0.327 Å above the benzene plane and not in line with the aromatic plane (Figure 3). In addition, the amino groups in the excited state clearly had sp^2 geometry, whereas the ground state had sp^3 geometry. Moreover, the C-N distance in the exited state was shorter than that in the ground state. These structural properties indicate that the ground state has a neutral, planar structure, and the excited state has a zwitterionic, non-planar structure. Generally, the asymmetric push–pull system exhibits significantly different dipole moments between the ground and excited states, which gives strong solvatochromic shifts of the absorption and emission bands.[15] Conversely, BMeS-*p*-A has a symmetrical push–pull system, and the difference in dipole moments between the ground and excited state was almost zero (see MO calculations in the Supporting Information), which is most likely a reason for the solvent independence of the optical properties of BMeS-*p*-A. Furthermore, the seemingly small structural transformation from the planar geometry to non-planar geometry of the sulfonyl groups from the ground state to excited state prompted a large difference in BMeS-*p*-A because of its small molecular size, which is reasonable and clearly explains the large Stokes shift of BMeS-*p*-A.

Finally, we demonstrated the photostability of BMeS-*p*-A in water (Figure 4). BMeS-*p*-A showed higher stability than

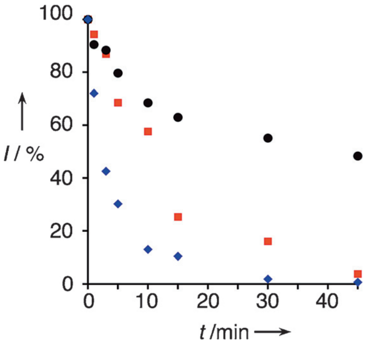


Figure 4. Plot of decreased fluorescence percentage versus irradiation time for BMeS-*p*-A (black circle), fluorescein (red square), and BODIPY (blue rhombus) obtained using a 150 W xenon lamp in water for BMeS-*p*-A, 0.1 м aqueous sodium hydroxide for fluorescein, and 10% (*v*/*v*) DMSO in water for BODIPY. BODIPY: [[(3,5-dimethyl-1*H*-pyrrol-2-yl)(3,5-dimethyl-2*H*-pyrrol-2-ylidene)methyl]methane](difluoroborane).

ordinary green fluorescent dyes such as fluorescein and BODIPY. The high stability of BMeS-*p*-A allowed a convenient, long-term storage of a stock solution in water, showing great potential for long-term time-lapse imaging.

In conclusion, a minimum architecture for green fluorophores has been successfully designed and synthesized. This study demonstrates the unparalleled fluorescence emission and photostability with solid-state emissive, water-soluble, solvent- and pH-independent, and large Stokes shift performances. We believe that this scaffold, which is a significant departure from conventional extended π-conjugated systems, may provide a means to construct a wide variety of fluorescent materials, as well as reignite interest in the fundamental chemistry of substituted benzenes.

Keywords: fluorescence · hydrogen bonds · solid-state emission · stokes shifts · water solubility

How to cite: *Angew. Chem. Int. Ed.* **2015**, *54*, 7332–7335
Angew. Chem. **2015**, *127*, 7440–7443

---

[1] a) I. Johnson, M. T. Z. Spence, *The Molecular Probes Handbook*: *A Guide to Fluorescent Probes and Labeling Technologies*, *11th ed.*, Life Technologies Corporation, Grand Island, NY, **2010**; b) L. D. Lavis, R. T. Raines, *ACS Chem. Biol.* **2008**, *3*, 142–145; c) H. Kobayashi, M. Ogawa, R. Alford, P. L. Choyke, Y. Urano, *Chem. Rev.* **2010**, *110*, 2620–2640; d) X. Li, X. Gao, W. Shi, H. Ma, *Chem. Rev.* **2014**, *114*, 590–659.

[2] K. Müllen, U. Scherf, *Organic Light Emitting Devices*, *Synthesis Properties and Applications*, Wiley-VCH, Weinheim, **2005**.

[3] a) R. E. Martin, F. Diederich, *Angew. Chem. Int. Ed.* **1999**, *38*, 1350–1377; *Angew. Chem.* **1999**, *111*, 1440–1469; b) M. D. Watson, A. Fechtenkötter, K. Müllen, *Chem. Rev.* **2001**, *101*, 1267–1300.

[4] a) R. Gompper, H. U. Wagner, *Angew. Chem. Int. Ed. Engl.* **1988**, *27*, 1437–1455; *Angew. Chem.* **1988**, *100*, 1492–1511; b) H. Meier, *Angew. Chem. Int. Ed.* **2005**, *44*, 2482–2506; *Angew. Chem.* **2005**, *117*, 2536–2561; c) S. J. Lord, H.-L. D. Lee, R. Samuel, R. Weber, N. Liu, N. R. Conley, M. A. Thompson, R. J. Twieg, W. E. Moerner, *J. Phys. Chem. B* **2010**, *114*, 14157–14167.

[5] a) M. Shimizu, T. Hiyama, *Chem. Asian J.* **2010**, *5*, 1516–1531; b) Y. Hong, J. W. Y. Lam, B. Z. Tang, *Chem. Soc. Rev.* **2011**, *40*, 5361–5388; c) D. Ding, K. Li, B. Liu, B. Z. Tang, *Acc. Chem. Res.* **2013**, *46*, 2441–2453.

[6] A. Mishra, R. K. Behera, P. K. Behera, B. K. Mishra, G. B. Behera, *Chem. Rev.* **2000**, *100*, 1973–2011.

[7] R. Sjöback, J. Nygren, M. Kubista, *Spectrochim. Acta Part A* **1995**, *51*, L7–L21.

[8] A. Loudet, K. Burgess, *Chem. Rev.* **2007**, *107*, 4891–4932.

[9] For comprehensive reviews, see: a) M. S. T. Gonçalves, *Chem. Rev.* **2009**, *109*, 190–212; b) L. D. Lavis, R. T. Raines, *ACS Chem. Biol.* **2014**, *9*, 855–866; for cyanine dyes, see: c) R. B. Mujumdar, L. A. Ernst, S. R. Mujumdar, C. J. Lewis, A. S. Waggoner, *Bioconjugate Chem.* **1993**, *4*, 105–111; d) B. R. Renikuntla, H. C. Rose, J. Eldo, A. S. Waggoner, B. A. Armitage, *Org. lett.* **2004**, *6*, 909–912; e) A. Toutchkine, D. V. Nguyen, K. M. Hahn, *Org. lett.* **2007**, *9*, 2775–2777; for BODIPY, see: f) M. Bröring, R. Krüger, S. Link, C. Kleeberg, S. Köhler, X. Xie, B. Ventura, L. Flmigni, *Chem. Eur. J.* **2008**, *14*, 2976–2983; g) Y. Chen, J. Zhao, H. Guo, L. Xie, *J. Org. Chem.* **2012**, *77*, 2192–2206; for fluorescein, see: h) N. Panchuk-Voloshina, R. P. Haugland, J. Bishop-Stewart, M. K. Bhalgat, P. J. Millard, F. Mao, W.-Y. Leung, R. P. Haugland, *J. Histochem. Cytochem.* **1999**, *47*, 1179–1188; i) W. C. Sun, K. R. Gee, D. H. Klaubert, R. P. Haugland, *J. Org. Chem.* **1997**, *62*, 6469–6475; j) Y. Urano, M. Kamiya, K. Kanda, T. Ueno, K. Hirose, T. Nagano, *J. Am. Chem. Soc.* **2005**, *127*, 4888–4894; k) L. F. Mottram, S. Boonyarattanakalin, R. E. Kovel, B. R. Peterson, *Org. Lett.* **2006**, *8*, 581–584.

[10] T. Beppu, S. Kawata, N. Aizawa, Y. J. Pu, Y. Abe, Y. Ohba, H. Katagiri, *ChemPlusChem* **2014**, *79*, 536–545.

[11] a) J. D. Stenger-Smith, P. Zarras, L. H. Merwin, S. E. Shaheen, B. Kippelen, N. Peyghambarian, *Macromolecules* **1998**, *31*, 7566–7569; b) T. Q. Nguyen, R. Y. Yee, B. J. Schwartz, *J. Photochem. Photobiol. A* **2001**, *144*, 21–30.

[12] M. Shimizu, Y. Takeda, M. Higashi, T. Hiyama, *Angew. Chem. Int. Ed.* **2009**, *48*, 3653–3656; *Angew. Chem.* **2009**, *121*, 3707–3710.

[13] R. Adams, M. D. Nair, *J. Am. Chem. Soc.* **1956**, *78*, 5932–5938.

[14] Etter's empirical hydrogen bonding rules; "(1) All good proton donors and acceptors are used in hydrogen bonding: (2) Six-membered-ring intramolecular hydrogen bonds form in preference to intermolecular hydrogen bonds: (3) The best proton donors and acceptors remaining after intramolecular hydrogen-bond formation form intermolecular hydrogen bonds to one another." a) M. C. Etter, *Acc. Chem. Res.* **1990**, *23*, 120–126; b) M. C. Etter, *J. Phys. Chem.* **1991**, *95*, 4601–4610; c) J. Bernstein, R. E. Davis, L. Shimoni, N. L. Chang, *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 1555–1573; *Angew. Chem.* **1995**, *107*, 1689–1708.

[15] a) C. Reichardt, *Chem. Rev.* **1994**, *94*, 2319–2358; b) Z. R. Grabowski, K. Rotkiewicz, W. Rettig, *Chem. Rev.* **2003**, *103*, 3899–4031; c) F. A. Chipem, A. Mishra, G. Krishnamoorthy, *Phys. Chem. Chem. Phys.* **2012**, *14*, 8775–8790; d) G. Weber, F. J. Farris, *Biochemistry* **1979**, *18*, 3075–3078; e) G. F. Mes, B. Jong, H. J. Ramesdonk, J. W. Verhoeven, J. M. Warman, M. P. Haas, L. E. W. Horsmanvandendool, *J. Am. Chem. Soc.* **1984**, *106*, 6524–6528; f) F. Vollmer, W. Rettig, E. Birckner, *J. Fluoresc.* **1994**, *4*, 65–69; g) B. C. Lobo, C. J. Abelt, *J. Phys. Chem. A* **2003**, *107*, 10938–10943.

Received: March 13, 2015