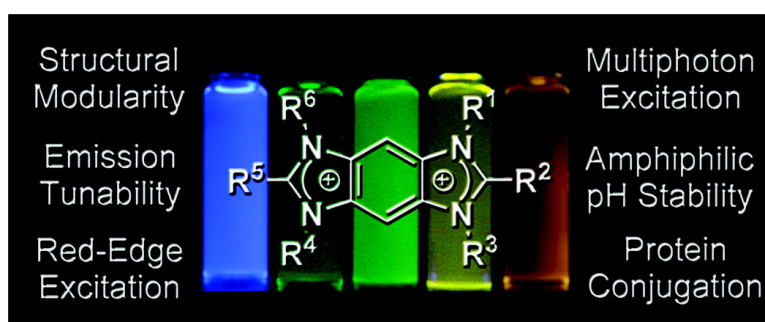Article

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Modular Fluorescent Benzobis(imidazolium) Salts: Syntheses, Photophysical Analyses, and Applications

Andrew J. Boydston, Peter D. Vu, Olga L. Dykhno, Vicki Chang, Alvin R. Wyatt, II, Adam S. Stockett, Eric T. Ritschdorff, Jason B. Shear, and Christopher W. Bielawski*

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Abstract: A series of benzobis(imidazolium) (BBI) salts has been prepared and studied as a new class of versatile fluorescent materials. Using a high yielding, modular synthetic strategy, BBI salts with a range of functionality poised for investigating fundamental and applications-oriented characteristics, including emission wavelength tunability, solvatochromism, red-edge excitation, chemical stability, multiphoton excitation, and protein conjugation, were prepared in overall yields of 40–97%. Through structural variation, the BBIs exhibited λ_{em} ranging between 329 and 561 nm while displaying Φ_fs up to 0.91. In addition, the emission characteristics of these salts were found to exhibit strong solvent dependencies with Stokes shifts ranging from 4570 to 13 793 cm^{-1}, depending on the nature of the BBI core. Although red-edge effects for BBI salts with Br and BF$_4$ counterions were found to be similar, unique characteristics were displayed by an analogue with MeSO$_4$ anions. The stability of an amphiphilic BBI was quantified in aqueous solutions of varying pH, and >85% of the emission intensity was retained after 2 h at pH 3–9. Through multiphoton excitation experiments in aqueous solutions, a BBI salt was found to exhibit three-photon fluorescence action cross sections similar to serotonin. The application of BBI salts as fluorescent protein tags was demonstrated by conjugating bovine serum albumin to a maleimide-functionalized derivative.

Introduction

The discovery and development of novel organic emissive materials is essential for advancement in a multitude of areas of chemical research.[1] As an emerging subclass, fluorescent organic salts are receiving considerable attention due to their unique attributes. They not only exhibit tunable electronic and physical characteristics, including solution-based and solid-state fluorescence, but also offer other advantages ascribed specifically to their charged nature. For example, the incorporation of ionic moieties in emissive salts has been credited with imparting high thermal stabilities, phase tunabilities, water-solubility, chemoselective sensing via electrostatic interactions, and strengthened solid-state intermolecular interactions. These features have already inspired research in a number of areas including fundamental photophysical investigations,[2,3] sensory materials,[4–6]

novel materials for display applications,[7,8] multiphoton excitation (MPE),[9] and nanoscopic fluorescent ionic liquids.[10] Overall, these materials encompass a tremendous breadth of structural diversity, each targeting a specific application or area of study.

To complement these systems, we sought a single, modular platform from which task-specific fluorophores could be obtained rapidly and efficiently. Benzobis(imidazolium) (BBI) salts are one class of such compounds that feature a number of attributes poised for achieving this goal (see Figure 1). The BBI architecture features two imidazolium ring systems annulated to a common arene linker. An important consequence of this arrangement is that the charged moieties are embodied within the system's chromophore, which distinguishes these materials from common types of fluorescent organic salts. In addition, BBIs feature four N-substituents, two C1-substituents, and two

(1) For excellent reviews, see the following: (a) Thomas, S. W., III.; Joly, G. D.; Swager, T. M. *Chem. Rev.* **2007**, *107*, 1339. (b) Finney, N. S *Curr. Opin. Chem. Biol.* **2006**, *10*, 238. (c) Glasbeek, M. Zhang, H. *Chem. Rev.* **2004**, *104*, 1929. (d) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 2nd ed.; Plenum Publishing: New York, 1999.

(2) (a) Mandal, P. K.; Paul, A.; Samanta, A. *J. Photochem. Photobiol., A* **2006**, *182*,113. (b) Samanta, A. *J. Phys. Chem. B* **2006**, *110*, 13704. (c) Das, S.; Frölich, R.; Pramanik, A. *Org. Lett.* **2006**, *8*, 4263. (d) Paul, A.; Mandal, P. K.; Samanta, A. *J. Phys. Chem. B* **2005**, *109*, 9148. (e) Paul, A.; Mandal, P. K.; Samanta, A. *Chem. Phys. Lett.* **2005**, *402*, 375. (f) Mandal, P. K.; Sarkar, M.; Samanta, A. *J. Phys. Chem. A* **2004**, *108*, 9048.

(3) For excellent reviews, see the following: (a) Hu, Z.; Margulis, C. J. *Acc. Chem. Res.* **2007**, *40*, 1097. (b) Demchenko, A. P. *Luminescence* **2002**, *17*, 19.

(4) Wang, S.; Chang, Y.-T. *J. Am. Chem. Soc.* **2006**, *128*, 10380.

(5) Chen, L.; McBranch, D. W.; Wang, H.-L.; Helgeson, R.; Wudl, F.; Whitten, D. G. *Proc. Nat. Acad. Sci. U.S.A.* **1999**, *96*, 12287.

(6) (a) Harrison, B. S.; Ramey, M. B.; Reynolds, J. R.; Schanze, K. S. *J. Am. Chem. Soc.* **2000**, *122*, 8561. (b) Balanda, P. B.; Ramey, M. B.; Reynolds, J. R. *Macromolecules* **1999**, *32*, 3970.

(7) Xu, H.; Meng, R.; Xu, C.; Zhang, J.; He, G.; Cui, Y. *Appl. Phys. Lett.* **2003**, *83*, 1020.

(8) Chondroudis, K.; Mitzi, D. B. *Appl. Phys. Lett.* **2000**, *76*, 58.

(9) (a) Krishna, T. R.; Parent, M.; Wets, M. H. V.; Moreaux, L.; Gmouh, S.; Charpak, S.; Caminade, A.-M.; Majoral, J.-P.; Blanchard-Desce, M. *Angew. Chem.*, *Int. Ed.* **2006**, *45*, 4645. (b) Woo, H. Y.; Liu, B.; Kohler, B.; Korystov, D.; Mikhailovsky, A.; Bazan, G. C. *J. Am. Chem. Soc.* **2005**, *127*, 14721. (c) Woo, H. Y.; Korystov, D.; Mikhailovsky, A.; Nguyen, T.-Q.; Bazan, G. C. *J. Am. Chem. Soc.* **2005**, *127*, 13794. (d) Abbotto, A.; Beverina, L.; Bozio, R.; Facchetti, A.; Ferrante, C.; Pagani, G. A.; Pedron, D.; Signorini, R. *Org. Lett.* **2002**, *4*, 1495.

(10) For an example of an low-T_g, ionic PAMAM dendrimer exhibiting blue photoluminescence, see the following: Huang, J.-F.; Luo, H.; Liang, C.; Sun, I-W.; Baker, G. A.; Dai, S. *J. Am. Chem. Soc.* **2005**, *127*, 12784.
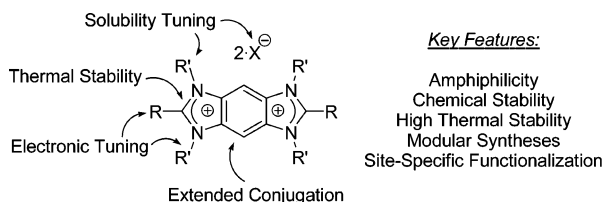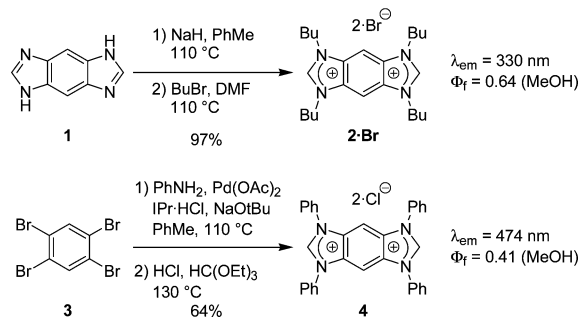
Figure 1. Key features of fluorescent benzobis(imidazolium) salts.

Scheme 1. Synthesis and Photoluminescence Properties of Tetrabutyl BBI Salt **2·Br** and Tetraphenyl BBI Salt **4**

**Scheme 2.** Synthesis of BBI Salts via Anion Metathesis

counterions which can be independently varied. As such, the physical, electronic, and chemical properties exhibited by these materials may be finely tuned. Combined, these features may synergistically open new opportunities for fluorescent organic salts that not only display versatile photoluminescence characteristics but also may be used in a broad array of applications.

Our preliminary interest in annulated bis(imidazolium) salts was as precursors to facially opposed bis(*N*-heterocyclic carbene)s.[11] As such, syntheses were designed to provide predominantly symmetric and exclusively C1-unsubstituted systems (i.e., R = H, Figure 1). Initial efforts focused on two primary variants exemplified in Scheme 1: one featuring four *N*-alkyl groups (e.g., **2·Br**) and the other containing four *N*-aryl groups (e.g., **4**). In general, syntheses of the former were accomplished via 4-fold alkylation of benzobis(imidazole) (**1**) with an appropriate alkyl halide.[11d] The latter were prepared from 1,2,4,5-tetrabromobenzene (**3**) via Pd-catalyzed 4-fold aryl amination[12,13] using 1,3-bis(2,6-diisopropylphenyl)imidazolium chloride (IPr·HCl) as a preligand. Subsequent formylative cyclization of the tetraaminobenzene intermediates (not shown) resulted in BBIs such as **4** in modest to excellent overall yields (39−88%).[11c]

During our initial studies, we observed that systems such as **2·Br** and **4** were highly photoluminescent with λ_{em} of 330 and 474 nm, respectively. In addition, high photoluminescence efficiencies (Φ_f) were observed from each BBI, with $\Phi_f = 0.64$ and 0.41 for **2·Br** and **4**, respectively. These results inspired the design and synthesis of new subclasses of fluorescent, phase-tunable (i.e., ionic liquid and ionic liquid crystal) BBI salts.[14]
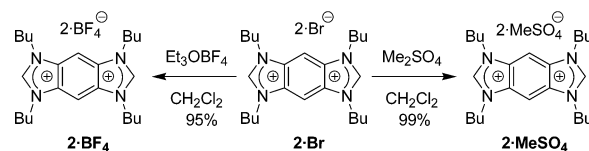
To further investigate the photophysics and applications of fluorescent BBI salts, a systematic series of these materials was prepared and studied. Syntheses and analyses were aimed at understanding how electronic charge delocalizes throughout the BBI chromophores, methods for controlling their emission properties, and incorporation of task-specific functionalities. Herein, we report a series of designer BBI fluorophores and studies involving their absorption and emission characteristics, solvatochromism, red-edge excitation, and chemical stability, as well as applications in MPE and protein conjugation.

Results and Discussion

Syntheses of Benzobis(imidazolium) Salts. To develop comprehensive structure−photophysical property relationships based upon BBI salts, focus was placed on controlling four areas of structural modulation: (1) counterions; (2) regioisomers; (3) *N*-aryl electronics; and (4) *C1*-aryl electronics. Photophysical studies and applications of BBI salts are described in subsequent sections. General syntheses utilized one of four strategies, summarized in Schemes 2−5 below. Overall, syntheses of BBIs **2**, **6**, **9**, **15**, **16**, and **26**−**32** required a maximum of four steps, were chromatography-free, and afforded products in yields ranging from 40 to 97% from commercially available starting materials.

(a) Counterions. A unique feature of organic salts is that, in principle, both their cation and anion constituents may be independently tuned. While the anion has been shown to play a key role in controlling physical properties,[14,15] photophysical anion dependencies have not been fully explored.[16] This is likely due to the fact that, for emissive organic salts, focus is placed primarily on cationic chromophores. However, ion-pairing interactions may greatly influence the photophysical characteristics of an organic salt under specific conditions. For example, red-edge effects (REEs)[2,3] from charged compounds have been attributed to different orientations of associated species.[2,3] Thus, one might expect that the counterion would influence a fluorescent salt's response to red-edge excitation.

To facilitate a comparative study involving counterion effects on photophysical properties of BBIs, compounds with simple, highly symmetric dicationic cores and either Br, BF₄, or MeSO₄ counterions were investigated. Capitalizing on the nucleophilic nature of halides, **2·Br** was treated with either Et₃O·BF₄ or Me₂-SO₄ to produce anion metathesis[17] products **2·BF₄** and **2·MeSO₄**, respectively, as shown in Scheme 2. Subsequent removal of

(11) (a) Khramov, D. M.; Boydston, A. J.; Bielawski, C. W. *Angew. Chem., Int. Ed.* **2006**, *45*, 6186. (b) Boydston, A. J.; Rice, J. D.; Sanderson, M. D.; Dykhno, O. L.; Bielawski, C. W. *Organometallics* **2006**, *25*, 6087. (c) Khramov, D. M.; Boydston, A. J.; Bielawski, C. W. *Org. Lett.* **2006**, *8*, 1831. (d) Boydston, A. J.; Williams, K. A.; Bielawski, C. W. *J. Am. Chem. Soc.* **2005**, *127*, 12496.
(12) For additional reports regarding aryl amination, see the following: (a) Prim, D.; Campagne, J.-M.; Joseph, D.; Andrioletti, B. *Tetrahedron* **2002**, *58*, 2041. (b) Wolfe, J. P.; Wagaw, S.; Marcox, J.-F.; Buchwald, S. L. *Acc. Chem. Res.* **1998**, *31*, 805. (c) Hartwig, J. F. *Angew. Chem., Int. Ed.* **1998**, *37*, 2046.
(13) For another example of 4-fold aryl amination of tetrahaloarenes, see the following: Wenderski, T.; Light, K. M.; Ogrin, D.; Bott, S. G.; Harlan, C. J. *Tetrahedron Lett.* **2004**, *45*, 6851.
(14) Boydston, A. J.; Pecinovsky, C. S.; Chao, S. T.; Bielawski, C. W. *J. Am. Chem. Soc.* **2007**, *129*, 14550.

(15) (a) Kouwer, P. H. J.; Swager, T. M. *J. Am. Chem. Soc.* **2007**, *129*, 14042. (b) López-Martin, I.; Burello, E.; Davey, P. N.; Seddon, K. R.; Rothenberg, G. *Chem. Phys. Chem.* **2007**, *8*, 690. (c) Yoshio, M.; Ichikawa, T.; Shimura, H.; Kagata, T.; Hamasaki, A.; Mukai, T.; Ohno, H.; Kato, T. *Bull. Chem. Soc. Jpn.* **2007**, *80*, 1836. (d) Binnemans, K. *Chem. Rev.* **2005**, *105*, 4148.
(16) Fluorescent anion sensors require separate consideration, see ref 1a and the following: (a) Gunnlaugsson, T.; Glynn, M.; Tocci, G. M.; Kruger, P. E.; Pfeffer, F. M. *Coord. Chem. Rev.* **2006**, *250*, 3094. (b) Badugu, R.; Lakowicz, J. R.; Geddes, C. D. *Curr. Anal. Chem.* **2005**, *1*, 157. (c) Martinez-Máñez, R.; Sancenón, F. *Chem. Rev.* **2003**, *103*, 4419. (d) Sessler, J. L.; Tvermoes, N. A.; Davis, J.; Anzenbacher, P., Jr.; Jursíková, K.; Sato, W.; Siedel, D.; Lynch, V.; Black, C. B.; Try, A.; Andrioletti, B.; Hemmi, G.; Mody, T. D.; Magda, D. J.; Král, V. *Pure Appl. Chem.* **1999**, *71*, 2009.
(17) Vu, P. D.; Boydston, A. J.; Bielawski, C. W. *Green Chem.* **2007**, *9*, 1158.

Scheme 3. Synthesis of Regioisomeric Diphenyl Dimethylbenzobis(imidazolium) Salts

Scheme 4. Synthesis of BBI Salts with Electronically Modified N-Aryl Substituents
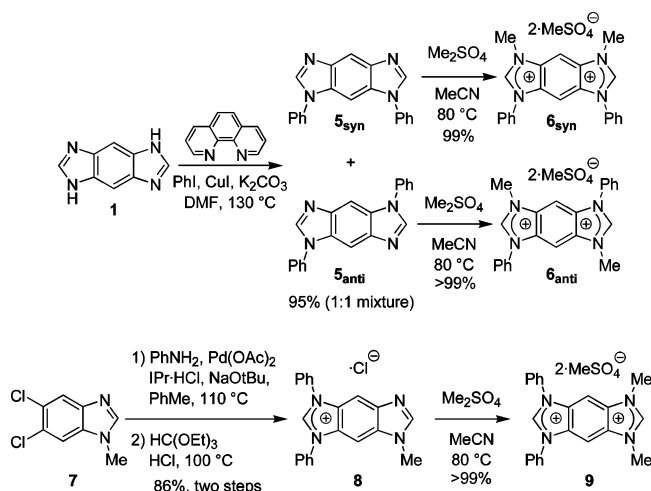
residual, volatile byproducts afforded each of the desired BBIs in excellent isolated yields ($\geq$95%). As will be discussed below, the counterions had little effect on the λ_{em}, provided excitation was performed at the λ_{abs}; however, analysis of these compounds ultimately revealed counterion-dependent REEs in dilute solutions.

(b) Regioisomers. Incorporation of distinct functional groups around the BBI core was expected to provide an additional degree of control over the λ_{em} of the resulting BBIs. Prior to engaging in the synthesis of compounds bearing substituents with different electronic properties, however, we investigated the effects of site-specific N-arylation. Hence, BBI regioisomers bearing two N-phenyl and two N-methyl groups in various substitution patterns were synthesized as shown in Scheme 3. Benzobis(imidazole) **1** underwent Cu-catalyzed cross coupling with PhI to provide a 1:1 mixture of 1,7-diphenyl- (**5**$_{syn}$) and 1,5-diphenylbenzobis(imidazole)s (**5**$_{anti}$) in 95% combined yield.[18,19] Separation of the two isomers was accomplished via recrystallization from hot DMSO.[20] Subsequent alkylation with Me$_2$SO$_4$ afforded BBIs **6**$_{syn}$ and **6**$_{anti}$, respectively.[21] As shown in Scheme 3, the other possible regioisomer (**9**) was obtained from 5,6-dichloro-1-methylbenzimidazole (**7**).[22] Aryl amination with PhNH$_2$, followed by formylative cyclization of the resulting diamine with HC(OEt)$_3$, afforded benzimidazolium chloride **8** in 86% yield. Treatment of **8** with 2.0 equiv of Me$_2$SO$_4$ not only resulted in N-methylation but also effectively removed the Cl anion (as gaseous MeCl), affording the desired bis(methyl sulfate) salt (**9**) in 85% overall yield from **7**. As will be discussed below, BBI **6**$_{syn}$ displayed a longer λ_{em} (447 nm) than **6**$_{anti}$ (442 nm) and **9** (403 nm). Hence, further structural modulation focused on 1,7-diaryl BBIs.

(c) N-Aryl Electronic Effects. As stated previously, the photophysics of BBI salts were found to be dependent on the
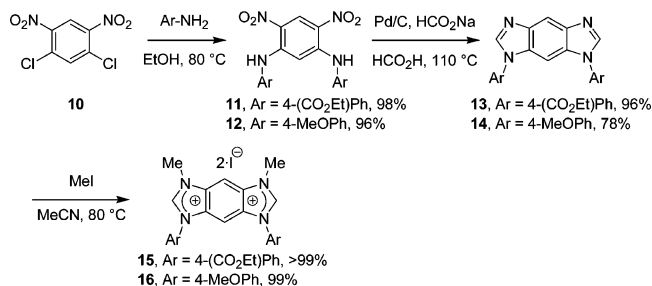
nature of the N-substituent (cf., **2**·Br and **4** in Scheme 1).[14] To further explore the effects of differential N-aryl electronic factors, BBIs bearing electron-donating and withdrawing N-aryl groups were synthesized as shown in Scheme 4. This was accomplished from 2,4-dinitro-1,5-dichlorobenzene (**10**), which underwent S$_N$Ar reactions with benzocaine and p-anisidine to provide dinitroarenes **11** and **12**, respectively. Reduction followed by in situ formylative cyclization afforded the corresponding benzobis(imidazole)s **13** and **14**. Finally, alkylation with MeI afforded the BBI salts **15** and **16** in 94 and 74% overall yield, respectively, from **10**. As will be discussed below, incorporation of electron-rich N-arenes bathochromically shifted the λ_{em} of these compounds by as much as 61 nm (compare: **16**, λ_{em} = 469 nm, versus **15**, λ_{em} = 408 nm, in MeOH). In contrast, electron-poor N-arenes resulted in significant hypsochromic shifts in the λ_{em}.

(d) Differential N- and C1-Substitution Effects. The ability to independently functionalize the N- and C1-positions of the BBI structure provides an additional handle for precisely tuning BBI photophysical properties. To facilitate solubility in common organic solvents (see below), BBIs bearing N-(4-BuPh) groups were targeted. Furthermore, these BBIs provided a platform for independently comparing different C1-substituents while maintaining constant N-groups (i.e., BBIs **26−29**). In addition to solubility enhancement, the N-aryl substituents were also used to modulate the electronic characteristics of the BBI chromophores. Specifically, N-(4-MeOPh) groups were incorporated into C1-substituted BBIs **30** and **31** to ultimately provide longer wavelength λ_{em} than other analogues (see below for a full discussion).

Dinitroarenes **12** (Scheme 4) and **17** (Scheme 5) were each synthesized from S$_N$Ar reactions of 1,5-dichloro-2,4-dinitrobenzene (**10**) with the corresponding aryl amines. Reduction of the nitro groups using Pd/C and hydrazine under acidic conditions afforded the corresponding tetraaminobenzenes **18** and **19**, which were each isolated as their hydrochloride salts in >99% yield.[23] Reaction of these tetraamines with various aroyl chlorides afforded diamide intermediates (structures not shown), which underwent dehydrocyclization in AcOH to give benzobis-(imidazole)s **20−25** in yields ranging from 78 to 99% over the two steps.[24] The aroyl chlorides chosen for study were commercially available and encompassed both electron-withdrawing (e.g., R = 4-CNPh) and -donating functional groups (e.g., R =

(18) In this context, we use the subscripts "syn" and "anti" to indicate the relative positions of the N-phenyl groups about the BBI core.
(19) For an example of this type of coupling using benzimidazole as a substrate, see the following: Harkal, S.; Rataboul, F.; Zapf, A.; Fuhrmann, C.; Riermeier, T.; Monsees, A.; Beller, M. Adv. Synth. Catal. **2004**, 346, 1742.
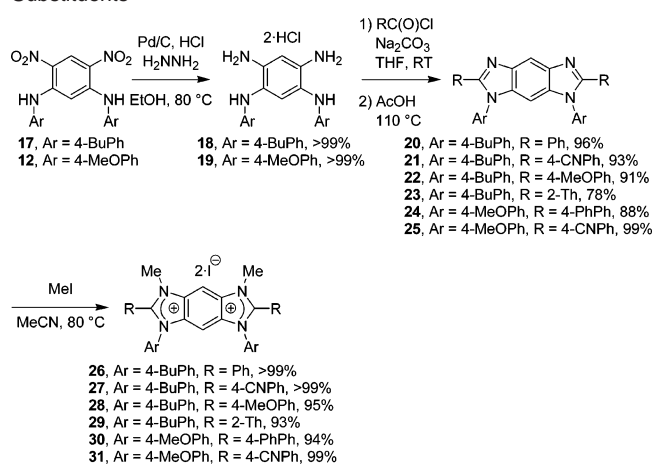(20) Chromatographic separation on silica gel (1:1 EtOAc/Et$_2$O eluent) was also effective. An alternate synthesis of **5**$_{syn}$ has been reported.[14]
(21) An alternate synthesis of BBI **6**$_{syn}$ has been previously reported.[14]
(22) For a synthesis of **7**, see the following: Feitelson, B. N.; Mamalis, P.; Moualim, R. J.; Petrow, V.; Stephenson, O.; Sturgeon, B. J. Chem. Soc. **1952**, 2389.

(23) These compounds were found to decompose gradually upon storage under ambient conditions. Under dry N$_2$, they showed no signs of decomposition for >2 months.
(24) For related synthetic approaches to C1-aryl benzimidazoles, see the following: (a) Xin, Z. et al. J. Med. Chem. **2006**, 49, 4459. (b) Gong, B.; Hong, F.; Kohm, C.; Bonham, L.; Klein, P. Bioorg. Med. Chem. Lett. **2004**, 14, 1455. (c) McKee, R.; Bost, R. J. Am. Chem. Soc. **1947**, 69, 471.

Scheme 5. Synthesis of BBI Salts Bearing *N*- and *C1*-Aryl Substituents
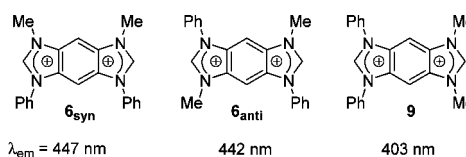

Figure 2. BBI salts used to evaluate the electronic impacts of regioisomeric placement of *N*-aryl and *N*-alkyl substituents.
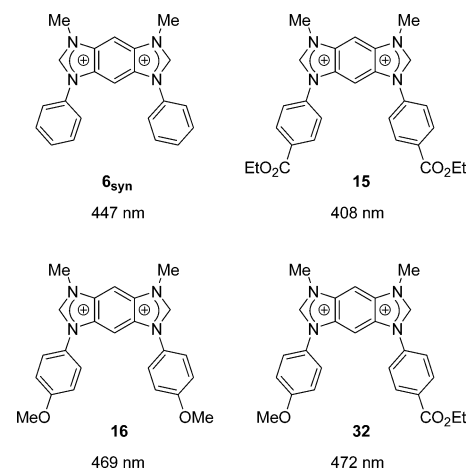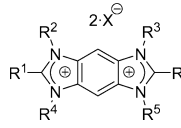

Figure 3. BBI salts used to evaluate *N*-aryl electronic effects on λ_{em}. Values shown are λ_{em} (excitation at the λ_{abs}) in MeOH under ambient conditions.

4-MeOPh, 2-thienyl, and 4-biphenyl). Subsequent alkylation of bis(imidazole)s **20**−**25** with MeI provided a series of highly functionalized BBI salts (**26**−**31**) in overall yields of 67−94% based on **10**. As discussed below, the different electron-donating and -withdrawing capabilities of the C1-substituents lead to λ_{em} tunability over a broad range of wavelengths (393−561 nm). Ultimately, through judicious combinations of N- and C1-aryl groups, we were able to prepare BBI salts with λ_{em} that were bathochromically shifted by up to 227 nm relative to BBI **2**· Br; specific details are discussed in the following sections.

Photophysical Analyses. With a systematic series of BBI salts in hand, attention turned toward studying the electronic and physical characteristics of these materials; a summary of key results is shown in Table 1. Collectively, these BBI salts displayed good Φ_fs and molar absorptivities as well as a broad range of λ_{em}. The following sections summarize our investigations of probing how structural, substituent, and solvent variations influence the photophysical properties of BBI fluorophores. The utilities of BBI salts in a variety of applications are also discussed.

As stated previously, the emission properties of BBI salts are strongly influenced by the nature of its N-substituents. In particular, comparing **2**·Br versus **4** revealed a relatively large $\Delta\lambda_{em}$ (124 nm) by incorporating *N*-phenyl (**4**) versus *N*-alkyl (**2**·Br) groups. Hence, we aimed to better understand the impact of systematically placing *N*-aryl groups at different positions on the BBI core. Specifically, regioisomeric BBIs **6**$_{syn}$, **6**$_{anti}$, and **9** (see Figure 2) were analyzed by UV−vis and fluorescence spectroscopies in methanolic solutions. While each of the regioisomers displayed similar λ_{abs} (345−347 nm), the longest wavelength λ_{em} were obtained when *N*-phenyl substituents were placed on opposite imidazolium rings. In particular, BBIs **6**$_{syn}$ and **6**$_{anti}$ showed λ_{em} at 447 and 442 nm, respectively, whereas BBI **9**, which bears two *N*-phenyl substituents on the same imidazolium ring, exhibited a λ_{em} at 403 nm. Considering BBI **6**$_{syn}$ displayed the longest λ_{em} and was straightforward to synthesize, subsequent efforts focused on studying derivatives of this compound to further tune its emissive properties (see below).[25]

Effects of Incorporating Functional *N*-Aryl Groups. To compare the effects of electronically tuning the *N*-aryl substit-

uents, we compared BBIs **6**$_{syn}$, **15**, and **16** (see Figure 3). Whereas BBI **6**$_{syn}$ showed a λ_{em} at 447 nm, incorporation of electron-withdrawing groups on the *N*-aryl substituents (**15**) resulted in a hypsochromically shifted λ_{em} at 408 nm. In contrast, BBI **16**, which bears electron-donating groups on the *N*-aryl substituents, showed a λ_{em} at 469 nm. Thus, from this series, it was apparent that increased electron density at the N-substituent effectively increased the λ_{em} of BBIs.

To further study electronic communication within the BBI chromophore, and to probe a new avenue for further tuning the λ_{em} exhibited by these materials, we prepared an N-to-N′ "donor−acceptor" BBI (**32**) featuring *N*-(4-MeOPh) and *N*′-(4-EtO₂CPh) groups (see Figure 3 for the structure of this compound). The synthesis (not shown) of **32** began with 1,5-dichloro-2,4-dinitrobenzene (**10**), which was treated in a stepwise fashion with benzocaine (to afford intermediate **33**) followed by *p*-anisidine to provide the respective dinitroarene **34** in 93% yield over the two steps. Following reductive cyclization, the resulting benzobis(imidazole) **35** was alkylated with MeI to provide BBI **32** in 78% overall yield from **10**. Analysis of the emission spectra of this compound revealed a λ_{em} at 472 nm, representing a slight bathochromic shift relative to **16**. Collectively, comparison of various BBI salts used to evaluate *N*-aryl electronic effects (shown in Figure 3) not only revealed an ability to tune the emission characteristics through *N*-aryl variation but also established a means to increase λ_{em}, primarily via incorporation of electron-rich *N*-arenes.

Next, we turned our attention toward evaluating the feasibility of tuning fluorescence properties through C1-substitution. Analysis of BBIs **26**−**31** (see Figure 4) revealed a strong dependence of the λ_{em} on the nature of the C1-substituent. Since C1-substituted BBI fluorophores were essentially unexplored, we initially investigated the effects of incorporating phenyl groups at these positions. Thus, we compared BBI **6**$_{syn}$, which is unsubstituted at C1, with C1-Ph substituted BBI **26**. The λ_{em} of the latter appeared at a considerably longer wavelength (507

(25) For comparison, 1-methyl-3-phenylbenzimidazolium iodide exhibited λ_{abs} at 270 nm and λ_{em} at 392 nm under identical conditions.

Table 1. Photophysical Properties of Benzobis(imidazolium) Salts[a]

BBI	R[1]	R[2]	R[3]	R[4]	R[5]	X	λ_{abs} (nm)[a]	$\log(\epsilon)$[a]	λ_{em} (nm)[a]	Φ_f[b]	yield (%)[c]
2·Br	H	Bu	Bu	Bu	Bu	Br	288	4.29	330	0.64	97
2·BF4	H	Bu	Bu	Bu	Bu	BF4	290	4.25	329	0.55	92
2·MeSO4	H	Bu	Bu	Bu	Bu	MeSO4	289	4.26	331	0.63	96
4	H	Ph	Ph	Ph	Ph	Cl	352	3.94	474	0.41	64
6$_{syn}$	H	Me	Me	Ph	Ph	MeSO4	345	3.31	447	0.91	86
6$_{anti}$	H	Ph	Me	Me	Ph	MeSO4	345	4.05	442	0.38	40
9	H	Ph	Me	Ph	Me	MeSO4	347	3.92	403	0.31	85[d]
15	H	Me	Me	4-(CO2Et)Ph	4-(CO2Et)Ph	I	360	4.13	408	0.02	94
16	H	Me	Me	4-MeOPh	4-MeOPh	I	343	3.93	469	0.05	74
32	H	Me	Me	4-MeOPh	4-(CO2Et)Ph	I	362	3.60	472	0.01	78
26	Ph	Me	Me	4-BuPh	4-BuPh	I	311	4.54	507	0.14	90
27	4-CNPh	Me	Me	4-BuPh	4-BuPh	I	388	4.94	551	0.43	87
28	4-MeOPh	Me	Me	4-BuPh	4-BuPh	I	332	4.51	393	0.55	86
29	2-Th	Me	Me	4-BuPh	4-BuPh	I	349	4.00	400	0.18	67
30	4-PhPh	Me	Me	4-MeOPh	4-MeOPh	I	331	4.61	461	0.05	79
31	4-CNPh	Me	Me	4-MeOPh	4-MeOPh	I	389	4.91	561	0.06	94

[a] Data taken in MeOH under ambient conditions. [b] Quantum efficiencies (Φ_f) were determined relative to *E*-stilbene, anthracene, or 9,10-bis(phenylethynyl)anthracene. [c] Isolated overall yield from commercial material. [d] Isolated overall yield from 5,6-dichloro-1-methylbenzimidazole (**7**).

26, R = Bu, Ar = Ph
27, R = Bu, Ar = 4-CNPh
28, R = Bu, Ar = 4-MeOPh
29, R = Bu, Ar = 2-Th
30, R = MeO, Ar = 4-PhPh
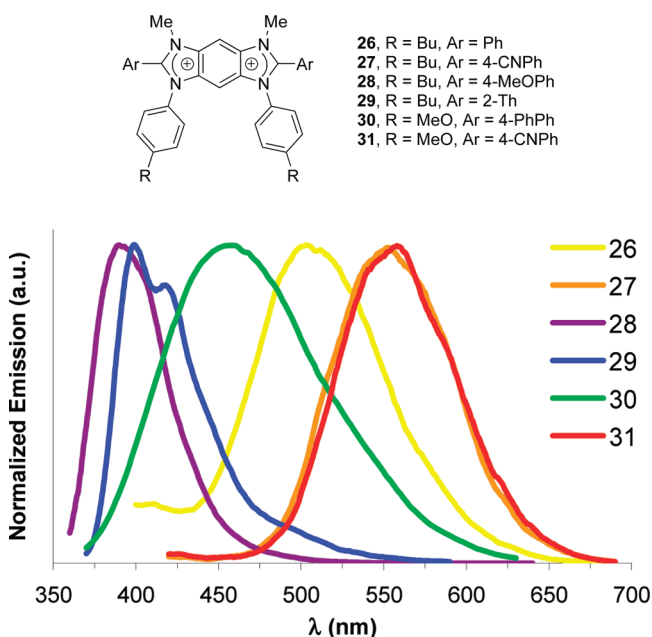**31**, R = MeO, Ar = 4-CNPh

Figure 4. Photoluminescence spectra of *C1*-aryl BBI salts in MeOH, excited at the absorption maximum, under ambient conditions. The concentration is ca. 5 μM; signal intensities have been normalized.
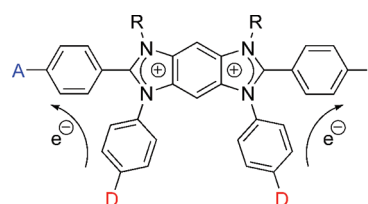

Figure 5. Diagram of the proposed major electronic communication pathways throughout the BBI chromophore: D = electron donor; A = electron-acceptor.

the λ_{em} of these BBIs to appear at wavelengths that were lower than even C1-unsubstituted BBI **6$_{syn}$**.

Electron-withdrawing C1-substituents, on the other hand, increased λ_{em} relative to BBI **26**. For example, installation of 4-CNPh groups at C1 (**27**) resulted in a λ_{em} at 551 nm, which is bathochromically shifted by 44 nm relative to **26**. Overall, it appeared that electronic influences from the *N*- and C1-substituents were complementary in that *increased* electron density at the N-positions and *decreased* electron density at C1 each resulted in longer λ_{em}. Considering these two factors, we envisioned it should be possible to take advantage of a donor–acceptor functionalized BBI featuring electron-donating *N*-aryl groups in combination with electron-withdrawing *C1*-aryl groups to further increase λ_{em}. Such a system was expected to display electronic communication as depicted in Figure 5.

To investigate, we synthesized BBI **31** which features 4-MeOPh groups at the 1- and 7-positions and 4-CNPh groups at C1. The λ_{em} of BBI **31** appeared at 561 nm, which is the longest λ_{em} of all the BBIs described herein. Although the emission spectra of **31** and **27** were very similar (see Figure 4), close examination revealed that the λ_{em} for **31** was slightly bathochromically shifted relative to **27** (λ_{em} = 551 nm). Notably, both BBI **27** and **31** undergo efficient excitation in the visible region, as the absorption cutoff of each appeared at approximately 450 nm.

Solvent Effects on Absorption and Emission Properties. Many classes of molecules exhibit solvent-dependent photo-

nm) than the former (447 nm). Having revealed a strong dependence of the λ_{em} on the C1-substituent, we considered electronic variation at this position as an additional parameter for tuning this property.

Installation of electron-donating C1-aryl groups such as 4-MeOPh (**28**), 2-thienyl (**29**), or 4-biphenyl (**30**) resulted in hypsochromic shifts in the λ_{em} relative to BBI **26**. The magnitudes of these shifts were consistent with the relative electron-donating abilities of the respective C1-arenes (i.e., 4-MeOPh > 2-thienyl > 4-biphenyl). In other words, increased electron density at C1 was consistently accompanied by a decrease in λ_{em}. Interestingly, the effect of increased electron density at C1 in BBIs **28** and **29** was significant enough to cause
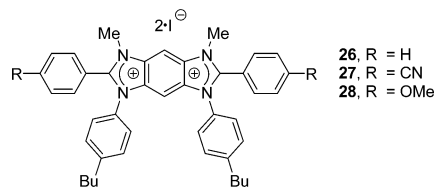
Figure 6. BBI salts used to evaluate photophysical solvent effects as a function of structure.

Table 2. Solvent Effects on the Photophysical Properties [$\lambda_{abs}/\lambda_{em}$ (nm)] of Various BBI Salts[a]

BBI	MeCN	(MeO)$_3$PO	DMF	DMSO
27	312/437	313/439	315/551	315/557
27·BF$_4$	312/446	313/436	315/550	316/561
26	310/420	310/412	312/412	314/438
28	331/392	331/390	332/394	333/395
21	350/436	336/438	358/439	354/442

[a] BBIs **26**−**28** were used as their diiodide salts. Data were taken under ambient conditions. Concentration were ca. 5 μM. Solvent donor numbers and dielectric constants respectively are as follows: MeCN = 14.1, 38.0; (MeO)$_3$PO = 23.0, 20.6; DMF = 26.6, 38.3; DMSO = 29.8, 45.0.[34]

physical properties.[26−28] Commonly, the λ_{abs} and λ_{em} respond to changes in solvent dielectric in a manner consistent with changes in the polarity of the chromophore as it transitions from the ground state to an excited state. Other factors such as solvent reorganization energies, solvent donor and acceptor abilities, and chemical reactions taking place in an excited state can each alter the Stokes shift, and thus the λ_{em}, as the solvent is varied.[29,30] Regardless of the origins, it is important to note that solvent dependency can offer an excellent handle for tuning the photophysical characteristics of a solute.[31]

To probe the effects of different solvents on the photophysical properties of BBI salts, the absorption and emission spectra of BBI **27** (see Figure 6) were analyzed in MeCN, (MeO)$_3$PO, DMF, and DMSO; limited solubilities precluded analysis in less polar solvents (i.e., PhMe, THF, CH$_2$Cl$_2$, dioxane, etc.).[32] For comparison, more electron-rich BBIs **26** and **28**, as well as benzobis(imidazole) **21**, were also investigated (see below for further discussion). As shown in Table 2, the λ_{abs} of **27** remained fairly constant upon increasing solvent polarity. The λ_{em} of this compound, however, was found to be strongly dependent on the nature of the solvent, displaying positive solvatofluorochromism with λ_{em} ranging from 437 to 557 nm.[33]

The Stokes shifts exhibited by BBI **27** ranged from 9168 to 13 793 cm^{-1}, depending on the donor number (DN) of the solvent used.[34] These values are in the range of unusually large Stokes shifts (5000−15 000 cm^{-1}), commonly ascribed to structural reorganization of the fluorophore or excited-state

(26) For excellent reviews, see the following: (a) Reichardt, C. *Green Chem.* **2005**, *7*, 339. (b) Minkin, V. I. *Chem. Rev.* **2004**, *104*, 2751. (c) Reichardt, C. *Chem. Rev.* **1994**, *94*, 2319. (d) Buncel, E.; Rajagopal, S. *Acc. Chem. Rev.* **1990**, *23*, 226.
(27) For a discussion of solvatochromism in quadrupolar fluorophores, see the following: Terenziani, F.; Painelli, A.; Katan, C.; Charlot, M.; Blanchard-Desce, M. *J. Am. Chem. Soc.* **2006**, *128*, 15742.
(28) Reichardt, C. *Solvents and Solvent Effects in Organic Chemistry*, 3rd ed.; Wiley-VCH: Weinheim, Germany, 2003.
(29) Mertz, E. L.; Tikhomirov, V. A.; Krishtalik, L. I. *J. Phys. Chem. A* **1997**, *101*, 3433.
(30) Marcus, R. A. *J. Chem. Phys.* **1963**, *38*, 1858.
(31) Joshi, H. S.; Jamshidi, R.; Tor, Y. *Angew. Chem., Int. Ed.* **1999**, *38*, 2722.
(32) Similarly, BBI **43** was excluded from comparative analyses due to poor solubility. See Experimental Section for details.
(33) To clarify, the terms "solvatochromism" and "solvatofluorochromism" are used to describe solvent-induced changes in λ_{abs} and λ_{em}, respectively.
(34) Gutmann, V. *Coordination Chemistry in Non-Aqueous Solutions*; Springer-Verlag: New York, 1968.

Scheme 6. Proposed Excited-State Reaction Giving Rise to Large Stokes Shifts and Positive Solvatofluorochromism
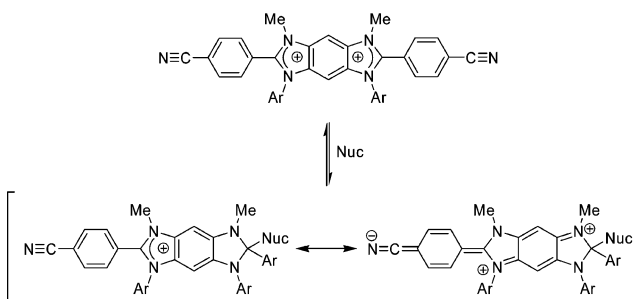
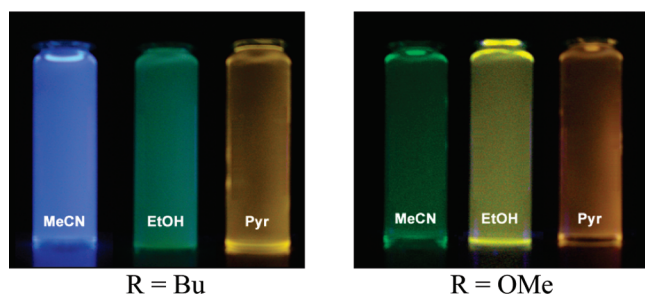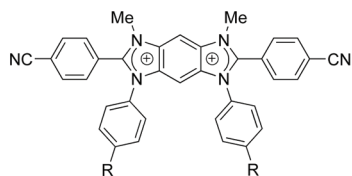**Table 3.** Stokes Shifts (cm^{-1}) of BBIs **26**−**28** and Bis(imidazole) **21** in Different Solvents

BBI	MeCN	(MeO)$_3$PO	DMF	DMSO
27	9168	9170	13597	13793
26	8449	7986	7779	9016
28	4701	4570	4740	4714
21	5636	6931	5154	5624

[a] Stokes shifts, in various solvents, given in cm^{-1} by $10^7(1/\lambda_{em} - 1/\lambda_{abs})$. Solvents are listed in order of increasing donor number (DN).

reactions.[35] For comparison, the Stokes shifts exhibited by BBIs **26** (7779−9016 cm^{-1}) and **28** (4570−4740 cm^{-1}) were smaller than **27**. The relative magnitudes of the Stokes shifts observed for these BBIs were consistent with the trend in λ_{em} and may be rationalized in terms of the relative electron densities on the BBI cores.

As described in Scheme 6, one plausible reorganization pathway may involve nucleophilic attack at one of the electrophilic C1-positions. Such an event would result in a strongly dipolar system capable of extended delocalization, features commonly manifested in large positive solvatofluorochromism.[26,27] The most likely nucleophilic species (Nuc) are either an iodide counterion or a solvent molecule. To exclude the former from consideration, we prepared **27·BF$_4$** from **27** (which contains iodide counteranions) using the anion metathesis method previously described.[17] As shown in Table 2, nearly identical spectroscopic signals were observed in each of the aforementioned solvents suggesting that excited-state structural changes were independent of the anion.

We next considered the solvent as a possible nucleophile in the mechanism described in Scheme 6. As stated above, an interesting relationship was realized after comparing the solvatofluorochromic shifts with solvent DNs, which is a semiquantitative comparison of solvent nucleophilicities,[34] and dielectric constants (κ).[34] The λ_{em} for BBI **27** correlated better with increasing solvent DN than with κ. For example, MeCN and DMF have nearly identical κ (38.0 and 38.3, respectively), but different DN (14.1 and 26.6, respectively). The λ_{em} of **27** appeared at 437 nm in MeCN but was shifted to 551 nm in DMF. This strong dependence on solvent DN, in combination with counterion independence (cf., **27** and **27·BF$_4$**; see above), further supported the proposed excited-state reaction described in Scheme 6.

To further explore the source of the observed solvatofluorochromism, we compared the solvent dependencies of the λ_{em} and Stokes shifts exhibited by BBIs **26** and **28**, as well as neutral benzobis(imidazole) **21**, with the more electron-deficient dicyano

(35) Doroshenko, A. O. *Theor. Exp. Chem.* **2002**, *38*, 135.

R = Bu   R = OMe

Figure 7. Solutions of BBIs **27** (left) and **31** (right) irradiated with a 365 nm (5 W) lamp. Solvents in each series from left to right: MeCN; EtOH; pyridine.

Figure 8. Electronic absorption (solid line), excitation (dashed line, emission intensity monitored at 330 nm), and emission (dotted line, excitation at 290 nm) spectra of **2**·MeSO$_4$ in MeOH under ambient conditions.

Figure 9. Emission spectra of **2**·Br (1 μM) in MeOH under ambient conditions excited at 330 (solid line), 340 (dashed line), and 350 nm (dotted line).

BBI **27** (see Tables 2 and 3). In contrast with **27**, BBI **26** showed only relatively small changes in λ_{em} (412−438 nm) and Stokes shifts (7779−9016 cm^{-1}) upon varying the solvent (Table 3). Similar effects were observed with BBI **28** and benzobis-(imidazole) **21**, which also exhibited relatively narrow ranges of λ_{em} and Stokes shifts. However, more generally, no discernible trends were observed between the λ_{em} or Stokes shifts for **21**, **26**, and **28** and solvent DN. In other words, a positive correlation between λ_{em} (and Stokes shifts) and solvent DN was observed only for a relatively electron deficient BBI (i.e., **27**). The other compounds studied were apparently not sufficiently electrophilic to undergo nucleophilic attack from solvent molecules (Scheme 6). Hence, the dicationic nature of BBI **27**, in combination with the electron-withdrawing C1-substituents, appear to be primarily responsible for the large solvatofluorochromic shifts. In particular, the electrophilic C1-position may be involved in an excited-state reaction that is facilitated by electron-withdrawing C1-substituents but impaired by electron-donating *C1*-arenes.

Since electron-withdrawing C1-substituents appeared to be mandatory for observing solvatofluorochromism, we envisioned tuning solvent effects by modulating the *N*-arenes while maintaining constant *C1*-(4-CNPh) groups. Indeed, as can be seen in Figure 7, marked differences in the photoluminescent properties of BBIs **27** and **31** were observed upon irradiation of these compounds in different solvents. Overall, these results underscore the potential for solvent effects to influence the emission characteristics of BBIs.

Red-Edge Effects. A red-edge effect (REE) refers to shifting of the λ_{em} to longer wavelengths in response to an increase in the excitation wavelength, along the red-edge of the absorption band.[3,36] Multiple polar organic fluorophores have been found to display REEs, phenomena that have found applications in a number of areas.[2a,3] Particularly interesting to our studies were recent reports of REEs from imidazolium-based ionic liquids.[2] The specific cause of the REE in imidazolium species is not entirely understood, but it has been speculated that its origins
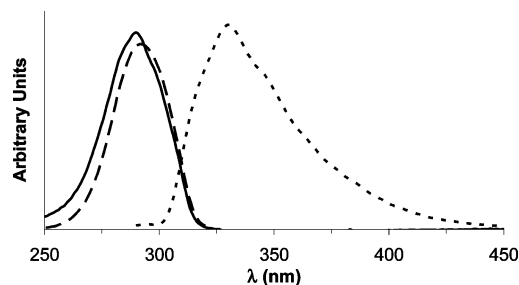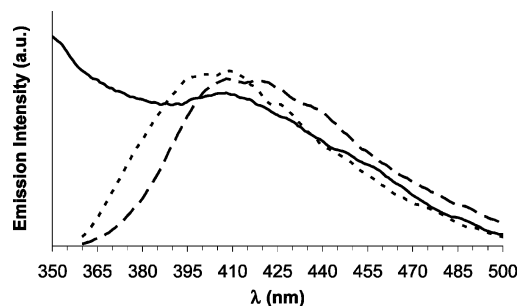
involve different ground-state orientations of associated species.[2,37] Considering the BBI structure comprises two imidazolium moieties, we envisioned REEs may be observed from these materials. In addition, differential ion-pairing interactions of BBIs **2**·Br, **2**·BF$_4$, and **2**·MeSO$_4$ may manifest in counterion-dependent REEs.

Since REEs of BBI-type fluorophores were unexplored, we began our investigations with a simple BBI structure (**2**) that would also be amenable to probing counterion effects. To determine if a REE was observable from BBI solutions, and explore any resulting counterion dependencies, MeOH solutions (ca. 1 μM) of BBIs **2**·Br, **2**·BF$_4$, and **2**·MeSO$_4$ were each excited at various wavelengths along the red-edge of their excitation spectra. As shown in Table 1, the absorption and emission spectra (excitation at the λ_{abs}) for **2** in MeOH were independent of the counterion; a representative set of spectra for **2**·MeSO$_4$ is shown in Figure 8.

Both **2**·Br and **2**·BF$_4$ gave nearly identical spectra upon red-edge excitation; the resulting spectra for **2**·Br are depicted in Figure 9. Excitation at 330 nm resulted in a shoulder protruding at ca. 410 nm, which became the only apparent peak at $\lambda_{ex} \geq$ 340 nm. Notably, a mixture of **2**·Br and **2**·BF$_4$ (1:1 molar ratio) also gave the same response. In contrast, red-edge excitation of **2**·MeSO$_4$ gave bimodal emission spectra that continued to shift bathochromically when excited at 330, 340, and 350 nm (see Figure 10). The difference in fine structure and overall response to red-edge excitation between **2**·MeSO$_4$ and **2**·Br/BF$_4$ strongly suggested that the counterion plays a decisive role in dictating the photophysical properties of **2** under these

(36) (a) Galley, W. C.; Purkey, R. M. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *67*, 1116. (b) Rubinov, A. N.; Tomin, V. I. *Opt. Spektrosk.* **1970**, *29*, 1082. (c) Weber, G.; Shinitzky, M. *Proc. Natl. Acad. Sci. U.S.A.* **1970**, *65*, 823. (d) Weber, G. *Biochem. J.* **1960**, *75*, 335.

(37) In solution, a REE is attributed to excitation of ground-state molecules that are most strongly interacting with solvent molecules; see the following: Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*; Plenum Publishing: New York, 1983.
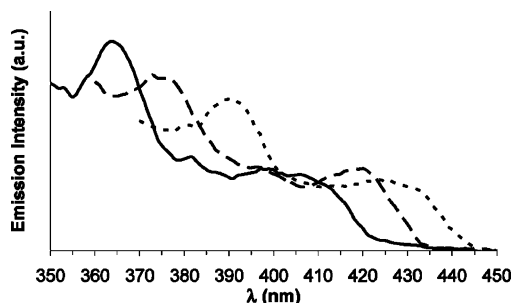
Figure 10. Emission spectra of **2**·MeSO$_4$ (1 μM) in MeOH under ambient conditions excited at 330 (solid line), 340 (dashed line), and 350 nm (dotted line).

conditions. These results demonstrate, for the first time, REEs in extended, highly photoluminescent imidazolium-based chromophores and support the contention that REEs may be influenced by cation−anion interactions.

Multiphoton Excitation. Multiphoton-excited fluorescence of organic chromophores has provided important capabilities for three-dimensionally resolved microscopy,[38,39] simultaneous measurements of multiple fluorophores,[39] and low-background analytical assays.[40] Although two- and three-photon fluorescence action cross-section values are typically small, fluorescence can be promoted with relatively low excitation powers by using high peak-power pulsed sources such as a femtosecond titanium: sapphire (Ti:S) laser. Recently, particular attention has been given to water-soluble organic salt fluorophores for applications in biological membranes, cells, and pH sensing.[9] Since multiphoton excitation (MPE) in BBI chromophores has not been investigated, we probed the ability of this new class of fluorophore to undergo MPE, particularly in aqueous solutions. Thus, multiphoton fluorescence action cross sections of BBI **4** were determined semiquantitatively for excitation between 740 and 860 nm.

To begin investigating MPE of BBI salts, BBI **4** was prepared in unbuffered water at 265 μM. Power studies were then performed at 740, 760, 820, and 860 nm to determine the laser power dependence of multiphoton-excited fluorescence. Logarithmic plots of the fluorescence emission versus incident laser power yielded linear least-square fits whose slopes represent the number of photons required for molecular excitation. At all wavelengths examined herein, BBI **4** was found to be excited primarily via a three-photon mechanism. Specifically, log−log slopes at 740, 780, 820, and 860 nm were found to be 3.00, 3.00, 3.01, and 2.98, respectively.

Three-photon fluorescence action cross sections were determined over this wavelength range using estimates of the spectral functions of the optics and detector, laser pulse width, and beamwaist size.[38] As shown in Figure 11, the cross section of BBI **4** was greatest at the short wavelength limit of these studies. These results show absolute three-photon cross sections similar to those determined for the native biological fluorophore, serotonin,[38] a compound that has been used in a range of microscopy[38,41] and microanalysis applications.[42,43]
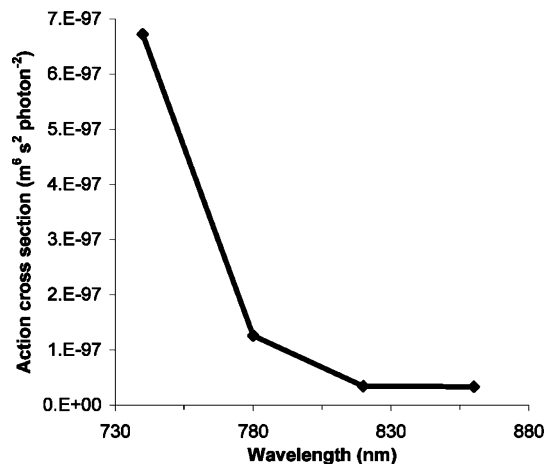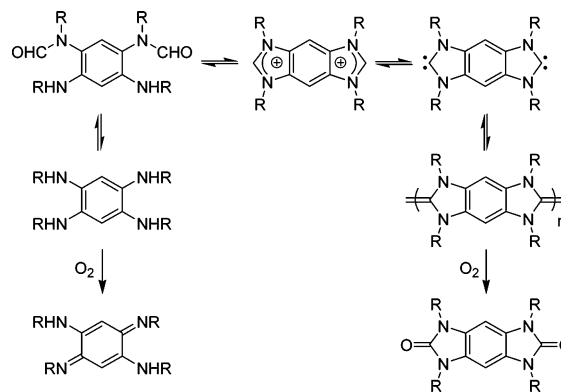

Figure 11. Three-photon fluorescence action cross section spectrum of BBI **4**.

Scheme 7. Possible Reaction Pathways of BBI Salts under Basic Aqueous Conditions

pH Stability. The potential of BBI fluorophores will ultimately depend on their stabilities in basic and acidic media. We have previously demonstrated that BBI salts can be deprotonated[44] under strongly basic conditions to generate bis-(N-heterocyclic carbene)s.[45] When the N-substituents were small, such as 1°-alkyl groups (e.g., **2**·Br), carbene dimerization ensued which resulted in the formation of poly(enetetraamine)s as depicted in Scheme 7. Enetetraamines are well-known to be extremely oxygen sensitive and rapidly convert to ureas upon exposure to air, a fate that would ultimately preclude the use of BBI fluorophores at high pH.[46] Decomposition of imidazolium species under acidic conditions, on the other hand, has not been investigated; hence, a lower pH limit of stability may also exist for this class of molecules. Notably, an alternate decomposition pathway to deprotonation may involve hydrolysis of the BBI to generate a diamide, which upon further hydrolysis and oxidation would result in a diamino−benzoquinonediimine. The uncertainty of the pH tolerance of BBI fluorophores prompted us to explore the pH stability of a representative BBI (**2**·Br) in aqueous solutions; key data are summarized in Table 4.

(38) Maiti, S.; Shear, J. B.; Williams, R. M.; Zipfel, W. R.; Webb, W. W. *Science* **1997**, *275*, 530.
(39) Zipfel, W. R.; Williams, R. M.; Webb, W. W. *Nat. Biotechnol.* **2003**, *21*, 1369.
(40) Okerberg, E.; Shear, J. B. *Anal. Chem.* **2001**, *73*, 1610.

(41) Williams, R. M.; Shear, J. B.; Zipfel, W. R.; Maiti, S.; Webb, W. W. *Biophys. J.* **1999**, *76*, 1835.
(42) Gostkowski, M. L.; Wei, J.; Shear, J. B. *Anal. Biochem.* **1998**, *260*, 244.
(43) Wise, D. D.; Shear, J. B. *J. Chromatogr., A* **2006**, *1111*, 153.
(44) The pK_a of imidazolium salts has been estimated to be 18−22; see the following: Amyes, T. L.; Diver, S. T.; Richard, J. P.; Rivas, F. M.; Toth, K. *J. Am. Chem. Soc.* **2004**, *126*, 4366.
(45) Kamplain, J. W.; Bielawski, C. W. *Chem. Commun.* **2006**, 1727.
(46) (a) Shi, Z.; Thummel, R. P. *J. Org. Chem.* **1995**, *60*, 5935. (b) Winberg, H. E.; Downing, J. R.; Coffman, D. D. *J. Am. Chem. Soc.* **1965**, *87*, 2054.

Table 4. Relative Emission Intensities Observed from BBI **2·**Br at Various Conditions and Durations[a]

	I_x/I_7 at time n			I_n/I_0 at pH X	
pH	0 min	30 min	120 min	30 min	120 min
1	0.09	0.09	0.10	0.94	0.93
3	0.87	0.92	1.00	0.98	0.98
5	0.87	0.89	1.04	0.95	1.02
7	1.00	1.00	1.00	0.93	0.85
9	0.86	0.91	0.98	0.98	0.97
11	0.01	0.01	0.01	0.98	1.03

[a] Emission data collected under ambient conditions. Excitation at 290 nm. I_x = total integrated emission intensity at pH = X and time indicated. I_7 = total integrated emission intensity at pH = 7 and time indicated. I_0 = total integrated emission intensity at time 0 for the pH indicated.

Initial efforts focused on studying BBI **2·**Br in neutral H_2O. Under these conditions, this compound showed absorption and emission characteristics nearly identical with those observed in MeOH. After 30 min in H_2O (pH 7), emission intensity of **2·** Br was at 93% of its initial value (see Table 4). After 2 h, 85% of the emission intensity persisted.

To investigate the emission properties of **2·**Br under basic conditions, a fresh sample was dissolved in aqueous media buffered to pH 9. The emission intensity remained essentially unchanged from that at pH 7 and did not diminish significantly even after 2 h. At pH 11, the photoluminescence disappeared almost instantly and was not returned upon acidification to pH 7. Thus, the upper limit of tolerable pH levels appeared to be approximately 9. Furthermore, the irreversibility of the fluorescence loss suggested that the BBI chromophore was in fact destroyed via hydrolysis or oxidation, as described in Scheme 7.

To elucidate the degradation pathway of BBI **2·**Br under basic aqueous conditions, we performed NMR-scale experiments in D_2O/NaOH (pH = 11). Analysis of the products after 2 h at room temperature revealed complete loss of the C1-proton signals ([1]H: δ = 9.57 in D_2O). In addition, signals attributable to arene protons were found at δ = 8.22, 7.47, 7.42, and 6.76 ppm. These four singlets, which were each upfield of the respective BBI arene signal, likely resulted from isomeric hydrolysis products. Indeed, mass-spectral analysis confirmed products consistent with the bis(formamide) shown in Scheme 7.

Next, we explored the stability of **2·**Br under acidic aqueous conditions. At pH levels of 3 and 5, the emission intensities remained essentially unchanged after 2 h and were similar to the emission intensity observed at pH 7. Upon further acidification (pH 1), however, photoluminescence was lost immediately and was not regained upon neutralization to pH 7. Analysis of the degradation products of **2·**Br in acidic D_2O using NMR spectroscopy revealed upfield shifts of the arene and alkyl signals. In addition, there appeared to be loss of butyl groups relative to arene signals. Mass-spectral analysis confirmed the presence of dibutyl benzobis(imidazole) suggesting that the BBI is subject to dealkylation under strongly acidic conditions. Overall, despite reactive imidazolium moieties, BBI **2·**Br showed excellent stability in aqueous solutions ranging in pH from 3 to 9.

Protein Conjugation. Encouraged by the amphiphilic nature and broad pH stability of the BBI salts, we next addressed the challenge of synthesizing a task-specific BBI designed for protein conjugation. To accomplish this goal, we envisioned

Scheme 8. Synthesis of Task-Specific BBI Salt **36**
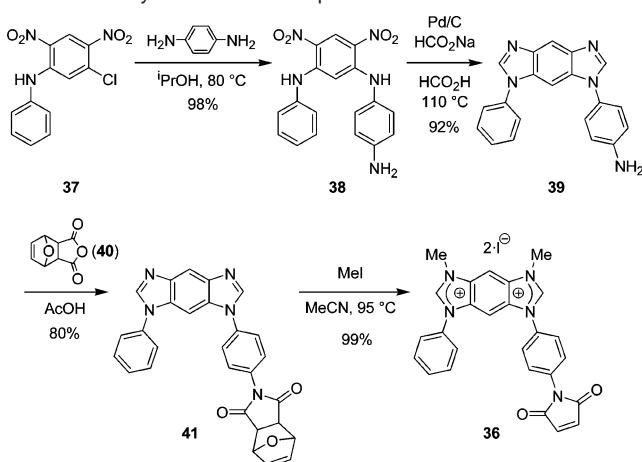
Scheme 9. Conjugation of BBI Salt **36** with MPA
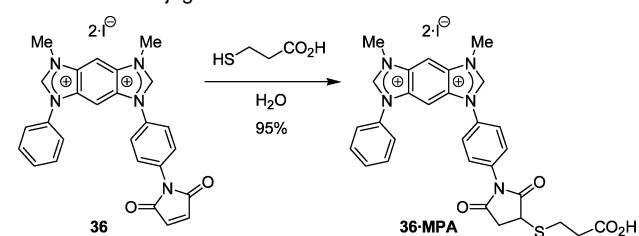
using a BBI fluorophore that featured a pendant substituent predisposed to reacting with specific protein residues. Considering maleimides are commonly used to conjugate with cysteine residues,[47] initial efforts were focused on incorporating this functional group into a BBI. Thus, we targeted **36** (see Scheme 8) which features (1) two *N*-aryl groups to provide emission in the visible region, (2) small *N*-methyl substituents to facilitate solubility in aqueous media, and (3) a pendant maleimide to undergo reactions with thiols.

The synthesis of **36** is shown in Scheme 8 and was based upon the S_NAr/reductive cyclization/alkylation methodology previously described.[14] Starting from dinitroarene **37**, S_NAr reaction with *p*-phenylenediamine provided dinitroarene **38** in 98% yield. Reductive cyclization of **37** at 110 °C gave benzobis-(imidazole) **39** in 92% yield. To install a maleimide functionality, **39** was first treated with anhydride **40**. Condensation of **40** with amine **39** resulted in clean formation of imide **41**, a protected maleimide. Subsequent alkylation of the benzobis-(imidazole) **41**, and concomitant deprotection via a retro Diels−Alder reaction at elevated temperature, provided BBI **36** in 67% overall yield from **37**.

The λ_{abs} and λ_{em} of **36** in MeOH appeared at 346 and 432 nm, respectively. Although the Φ_f was low (0.01), this may be related to the maleimide functionality which has been found to reduce quantum efficiencies in other fluorophores.[47a] To determine if **36** would be a feasible Michael acceptor for protein conjugation, and to explore the photophysical impacts of altering the maleimide moiety, we performed a control experiment using 3-mercaptopropionic acid (MPA) as a model for a nucleophilic thiol residue. Thus, BBI **36** was dissolved in H_2O and treated with an equimolar amount of MPA as shown in Scheme 9. After

(47) (a) Guy, J.; Caron, K.; Dufresne, S.; Michnick, S. W.; Skene, W. G.; Keilor, J. W. *J. Am. Chem. Soc.* **2007**, *129*, 11969. (b) Corrie, J. E. T. *J. Chem. Soc., Perkin Trans.* **1994**, *1*, 2975.
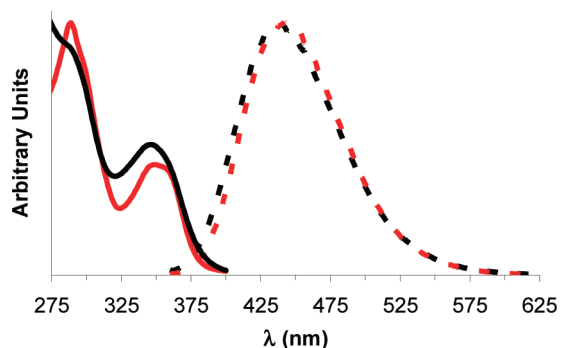
Figure 12. Absorption (solid lines) and emission (dotted lines) of **36**·MPA (red) and **36**·BSA (black) in MeOH under ambient conditions.

4 h at room temperature, the solution was concentrated to provide **36**·MPA in 95% yield. In comparison with free **36**, attachment of the thiol to the maleimide significantly increased the Φ_f of the BBI to 0.34.

The efficacy of **36** in protein conjugation was studied using bovine serum albumin (BSA). BSA was dissolved in H_2O and treated with a slight excess of **36**. After 4 h at room temperature, the conjugate **36**·BSA was subjected to dialysis against distilled H_2O for 48 h. After removal of excess H_2O under vacuum, the conjugate product was isolated in 90% yield and analyzed by NMR, UV−vis, and fluorescence spectroscopy. Confirmation of successful conjugation was evidenced by an absence of maleimide proton signals in the ¹H NMR spectrum (diagnostic signal at $\delta = 7.27$ in DMSO-d_6). In addition, **36**·BSA displayed λ_{abs} and λ_{em} at 287 (additional peak at 345 nm) and 445 nm, which were consistent with those observed from **36** and **36**·MPA (see Figure 12).

Conclusions

We have synthesized and analyzed a series of benzobis-(imidazolium) salts which represent a new class of highly versatile, robust fluorophores. A modular synthetic design facilitated access to fluorophores whose structures were tailored to probe various photophysical and applications-based characteristics, including emission wavelength tunability, solvatochromic effects, red-edge excitation, multiphoton excitation, pH stability, and protein conjugation. Additionally, the syntheses provided each of the fluorophores in few synthetic manipulations and without the need for chromatographic purification.

The fluorophores displayed structure-dependent λ_{em} ranging from 329 to 561 nm in MeOH. Depending on the nature of the fluorogenic core, solvent-dependent λ_{em} were also observed in conjunction with large Stokes shifts. Although the nature of the counterion had no detectable impacts in these studies, investigation of red-edge effects revealed a strong counterion dependency. Specifically, while Br and BF_4 counterions gave similar results, a strikingly different REE was observed when $MeSO_4$ counterions were incorporated. Additionally, preliminary experiments were conducted to investigate the BBI's ability to display multiphoton excited fluorescence. Aqueous solutions of tetraphenyl BBI **4** exhibited three-photon excitation and fluorescence action cross sections similar to those observed from serotonin.

Encouraged by broad emission tunability, ease of functionalization, and good pH stability, we synthesized a task-specific fluorophore predisposed for protein conjugation. A maleimide-

functionalized fluorophore was successfully conjugated with 3-mercaptopropionic acid as a control experiment and was ultimately attached to bovine serum albumin in aqueous media. The absorption and emission spectra obtained after protein conjugation, in comparison with those of the control experiment, confirmed attachment of the fluorophore to the protein.

Collectively, this new class of fluorophores has many desirable attributes that have been disclosed herein. In addition, the ease with which the fluorogenic cores may be functionalized and finely tuned should increase the efficiency with which they may be deployed in a range of applications. These materials are expected to open new opportunities and enable advancements in a range of areas, including fundamental photophysical studies, biological imaging, sensory materials, and electronic display applications.

Experimental Section

General Considerations. ¹H and ¹³C NMR spectra were recorded using a Varian Unity Plus 300 or 400 spectrometer. Chemical shifts (δ) are expressed in ppm downfield from tetramethylsilane using the residual protio solvent as an internal standard (CDCl₃, ¹H, 7.26 ppm, and ¹³C, 77.0 ppm; DMSO-d_6, ¹H, 2.49 ppm, and ¹³C, 39.5 ppm). Coupling constants are expressed in hertz (Hz). HRMS (ESI, CI) were obtained with a VG analytical ZAB2-E instrument. UV−vis spectra were recorded using a Perkin-Elmer Instruments Lambda 35 spectrometer. Emission spectra were recorded using a QuantaMaster Photon Technology International fluorometer. Unless otherwise noted, all reactions were performed under ambient atmosphere. All starting materials and solvents were of reagent quality and used as received from commercial suppliers.

1,3,5,7-Tetrabutylbenzobis(imidazolium) Bis(tetrafluoroborate) (2·BF₄). After suspension of BBI **2**·Br (992 mg, 1.82 mmol) in dry CH_2Cl_2, Et₃O·BF₄ (692 mg, 3.64 mmol) was added in a single portion. The reaction progress was monitored by ¹H NMR spectroscopy, and upon completion (ca. 10 min), the solution was concentrated to provide 969 mg (95% yield) of the desired product as a white powder. ¹H NMR (400 MHz, DMSO-d_6): δ 10.10 (s, 2H), 8.97 (s, 2H), 4.59 (t, $J = 7.2$ Hz, 8H), 2.00−1.93 (m, 8H), 1.42−1.33 (m, 8H), 0.94 (t, $J = 7.2$ Hz, 12H). ¹³C NMR (75 MHz, DMSO-d_6): δ 145.5, 130.2, 98.8, 47.1, 30.2, 19.1, 13.4. HRMS (m/z): calcd for BF₄, 87.0029; found, 87.0026.

1,3,5,7-Tetrabutylbenzobis(imidazolium) Bis(methyl sulfate) (2·MeSO₄). After suspension of BBI **2**·Br (488 mg, 0.90 mmol) in dry CH_2Cl_2, Me₂SO₄ (0.17 mL, 1.80 mmol) was added in a single portion. The reaction progress was monitored by ¹H NMR spectroscopy, and upon completion (ca. 4 h), the solution was concentrated to provide 542 mg (99% yield) of the desired product as a beige powder. ¹H NMR (400 MHz, CDCl₃): δ 9.94 (s, 2H), 8.84 (s, 2H), 4.72, (t, $J = 7.0$ Hz, 8H), 3.76 (s, 6H), 1.96−1.89 (m, 8H), 1.42−1.32 (m, 8H), 0.93 (t, $J = 7.2$ Hz, 12H). ¹³C NMR (100 MHz, CDCl₃ + DMSO-d_6): δ 145.3, 130.3, 99.7, 54.5, 47.8, 31.1, 19.5, 13.4.

1,5-Diphenylbenzobis(imidazole) (5$_{anti}$). Benzobis(imidazole) **1** (500 mg, 3.16 mmol) was dissolved in dry DMF (15 mL) in a screw-cap vial. Under nitrogen atmosphere, PhI (1.42 mL, 12.6 mmol), CuI (60 mg, 0.32 mmol), 1,10-phenanthroline (114 mg, 0.63 mmol), and K₂-CO₃ (1.75 g, 12.6 mmol) were added. The vial was then sealed with a Teflon-lined cap, and the mixture was stirred at 130 °C for 17 h. Afterward the mixture was poured into H_2O (30 mL). Collection of the precipitated solids via vacuum filtration afforded 932 mg (95% yield) as a 1:1 mixture of two regioisomers **5**$_{syn}$ and **5**$_{anti}$. Recrystallization of the mixture from hot DMSO provided 392 mg (40% yield based on **1**) of **5**$_{anti}$. Spectral data for **5**$_{syn}$ were consistent with those previously reported.[14] Data for **5**$_{anti}$: ¹H NMR (300 MHz, CDCl₃): δ 8.20 (s, 2H), 8.00 (s, 2H), 7.63−7.62 (m, 8H), 7.52−7.45 (m, 2H).

^{13}C NMR (75 MHz, CDCl$_3$) δ 143.7, 142.2, 136.6, 131.5, 130.1, 128.0, 123.9, 100.4; HRMS m/z calcd for C$_{20}$H$_{15}$N$_4$ [M + H$^+$] 311.1297, found 311.1299.

1,5-Dimethyl-3,7-diphenylbenzobis(imidazolium) Bis(methyl sulfate) (6$_{anti}$). After diphenylbenzobis(imidazole) 5$_{anti}$ (50 mg, 0.15 mmol) was dissolved in MeCN (1.0 mL) in a screw-cap vial, Me$_2$SO$_4$ (29 μL, 0.31 mmol) was added in a single portion. The vial was then sealed with a Teflon-lined cap and placed in an oil bath at 80 °C for 4 h. The reaction mixture was then cooled and concentrated to afford 84 mg (>99% yield) of the desired product as a beige wax. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.35 (s, 2H), 8.64 (s, 2H), 7.92 (d, J = 7.6 Hz, 4H), 7.85−7.78 (m, 6H), 4.24 (s, 6H), 3.34 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 147.4, 146.3, 132.9, 131.2, 130.9, 130.5, 130.1, 125.7, 99.1, 52.9, 34.0. HRMS (m/z): calcd for C$_{22}$H$_{19}$N$_4$ [M − H$^+$], 339.1610; found, 339.1609.

Benzimidazolium Chloride 8. In a nitrogen-filled drybox, a screw-cap vial was charged with 5,6-dichloro-1-methylbenzimidazole (7) (375 mg, 1.87 mmol) and PhMe (10 mL). To the solution was added Pd-(OAc)$_2$ (4 mg, 0.02 mmol), 1,3-bis(2,6-diisopropylphenyl)imidazolium chloride (16 mg, 0.04 mmol), NaO^{t}Bu (368 mg, 3.83 mmol), and PhNH$_2$ (869 mg, 9.33 mmol). The vial was then sealed with a Teflon-lined cap and placed in an oil bath at 120 °C for 12 h. After cooling of the reaction mixture to ambient temperature, it was poured into a solution of HC(OEt)$_3$ (50 mL) and concentrated HCl (1.0 mL). The resulting mixture was then stirred in an oil bath at 150 °C for 12 h, allowed to cool, and then poured into Et$_2$O (100 mL). The precipitated solids were collected via vacuum filtration, rinsed with Et$_2$O, and redissolved in a saturated solution of Na$_2$CO$_3$ in ^{i}PrOH. After standing for 1 h, the mixture was filtered through Celite and the filtrate was concentrated to provide 523 mg (86% yield) of the desired product as a light brown powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.58 (s, 1H), 8.57 (s, 1H), 8.13 (s, 1H), 8.09 (s, 1H), 8.05−8.02 (m, 4H), 7.83−7.72 (m, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 149.2, 143.2, 143.0, 134.9, 133.5, 133.4, 130.5, 130.4, 128.1, 127.7, 125.6, 125.4, 102.6, 94.6, 31.5. HRMS (m/z): calcd for C$_{21}$H$_{17}$N$_4$ [M$^+$], 325.1453; found, 325.1454.

1,3-Dimethyl-5,7-diphenylbenzobis(imidazolium) Bis(methyl sulfate) (9). After benzimidazolium chloride 8 (50 mg, 0.15 mmol) was dissolved in MeCN (1.0 mL), Me$_2$SO$_4$ (39 mg, 0.31 mmol) was added in a single portion. After stirring the resulting solution at 80 °C for 2 h, it was concentrated to afford 84 mg (>99% yield) of the desired product as a tan solid. ^{1}H NMR (400 MHz, CDCl$_3$): δ 10.81 (s, 1H), 10.04, (s, 1H), 8.60 (s, 2H), 8.04 (d, J = 7.2 Hz, 4H), 7.85−7.78 (m, 6H), 4.18 (s, 6H), 3.35 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 147.4, 146.3, 132.9, 131.2, 130.9, 130.5, 130.1, 125.7, 99.1, 52.9, 34.0. HRMS (m/z): calcd for C$_{22}$H$_{19}$N$_4$ [M − H$^+$], 339.1610; found, 339.1607.

1,5-Bis(4-carboethoxyphenyl)amino-3,4-dinitrobenzene (11). A screw-cap vial was charged with 1,5-dichloro-3,4-dinitrobenzene (10) (413 mg, 1.74 mmol), benzocaine (1.44 g, 8.71 mmol), ^{i}PrOH (15 mL), and a stir bar. The vial was sealed with a Teflon-lined cap, placed in an oil bath at 120 °C, and stirred for 48 h. After the mixture was poured into 5% HCl (50 mL), the precipitated solids were collected via vacuum filtration, rinsed with H$_2$O, and dried under vacuum to provide 1.66 g (98% yield) of the desired product as a yellow powder. ^{1}H NMR (400 MHz, CDCl$_3$): δ 9.92 (br s, 2H), 9.32 (s, 1H), 8.05 (d, J = 8.4 Hz, 4H), 7.25 (d, J = 8.4 Hz, 4H), 6.83 (s, 1H), 4.38 (quart, J = 7.2 Hz, 4H), 1.41 (t, J = 7.2 Hz, 6H). ^{13}C NMR (100 MHz, CDCl$_3$): δ 165.4, 145.6, 141.2, 131.3, 129.2, 128.2, 126.1, 123.0, 96.7, 61.2, 14.3. HRMS (m/z): calcd for C$_{24}$H$_{23}$N$_4$O$_8$ [M + H$^+$], 495.1516; found, 495.1517.

1,5-Bis(4-methoxyphenyl)amino-3,4-dinitrobenzene (12). After 1,5-dichloro-3,4-dinitrobenzene (10) (1.00 g, 4.22 mmol) was dissolved in EtOH (75 mL), p-anisidine (2.08 g, 16.9 mmol) was added in a single portion. The mixture was then placed in an oil bath at 80 °C and stirred for 48 h. The mixture was then poured into H$_2$O (200 mL) which caused solids to precipitate. The solids were collected via vacuum

filtration, rinsed with H$_2$O, and dried under vacuum to provide 1.42 g (96% yield) of the desired product as a red-orange powder. ^{1}H NMR (300 MHz, CDCl$_3$): δ 9.57 (br s, 2H), 9.31 (s, 1H), 7.05 (d, J = 8.9 Hz, 4H), 6.84 (d, J = 8.9 Hz, 4H), 6.18 (s, 1H), 3.80 (s, 3H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 158.2, 1147.7, 129.8, 129.4, 126.7, 125.0, 114.8, 94.9, 55.5. HRMS (m/z): calcd for C$_{20}$H$_{19}$N$_4$O$_6$ [M + H$^+$], 411.1305; found, 411.1301.

1,7-Bis(4-carboethoxyphenyl)benzobis(imidazole) (13). Dinitroarene 11 (400 mg, 0.81 mmol) was dissolved in HC(OEt)$_3$ (10 mL) in a screw-cap vial. To the solution was added HCO$_2$H (88%, 1 mL), HCO$_2$Na (1.65 g, 24.3 mmol), and Pd/C (172 mg, 5 wt %, 0.08 mmol Pd). The vial was then sealed with a Teflon-lined cap, and the mixture was heated in an oil bath at 110 °C for 48 h. Upon cooling of the mixture to ambient temperature, it was filtered through Celite. The filtrate was treated with 5% HCl, stirred for ca. 30 min, and then brought to pH 9 using saturated aqueous Na$_2$CO$_3$. Precipitated solids were subsequently collected via vacuum filtration, rinsed with H$_2$O, and dried under vacuum to provide 352 mg (96% yield) of the desired product as a tan powder. ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.38 (s, 1H), 8.26 (d, J = 8.4 Hz, 4H), 8.21 (s, 2H), 7.63 (s, 1H), 7.61 (d, J = 8.4 Hz, 4H), 4.43 (quart, J = 7.2 Hz, 4H), 1.43 (t, J = 7.2 Hz, 6H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 165.5, 143.1, 141.8, 140.1, 131.8, 131.7, 129.9, 123.3, 111.6, 90.4, 61.4, 14.3. HRMS (m/z): calcd for C$_{26}$H$_{23}$N$_4$O$_4$ [M + H$^+$], 455.1719; found, 455.1716.

1,7-Bis(4-methoxyphenyl)benzobis(imidazole) (14). Dinitroarene 12 (1.00 g, 2.44 mmol) was suspended in HCO$_2$H (88%, 100 mL). To the suspension was added HCO$_2$Na (1.99 mg, 29.2 mmol) and Pd/C (103 mg, 5 wt %, 0.05 mmol Pd). The mixture was heated in an oil bath at 110 °C for 48 h. Upon completion, the cooled reaction mixture was filtered through Celite and the filtrate volume was reduced to ca. 20 mL under vacuum. The solution was then added slowly into a vigorously stirred saturated solution of aqueous Na$_2$CO$_3$ (150 mL). Precipitated solids were collected via vacuum filtration, rinsed with H$_2$O, and dried under vacuum to provide 705 mg (78% yield) of the desired product as a tan powder. ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.31 (s, 1H), 8.08 (s, 2H), 7.41 (d, J = 8.8 Hz, 4H), 7.35 (s, 1H), 7.06 (d, J = 8.8 Hz, 4H), 3.88 (s, 6H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 159.2, 143.6, 141.2, 132.8, 129.3, 125.9, 115.2, 110.5, 89.8, 55.6. HRMS (m/z): calcd for C$_{22}$H$_{19}$N$_4$O$_2$ [M + H$^+$], 371.1508; found, 371.1511.

1,7-Bis(4-carboethoxyphenyl)-3,5-dimethylbenzobis(imidazolium) Diiodide (15). Benzobis(imidazole) 13 (300 mg, 0.66 mmol) was dissolved in MeCN (10 mL) and MeI (1.0 mL) in a screw-cap vial. The vial was then sealed with a Teflon-lined cap, and the mixture was heated at 80 °C for 8 h. Afterward, the mixture was concentrated under vacuum to provide 485 mg (>99% yield) of the desired product as a dark brown powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.48 (s, 2H), 9.12 (s, 1H), 8.27 (d, J = 8.2 Hz, 4H), 8.24 (s, 1H), 8.03 (d, J = 8.2 Hz, 4H), 4.38 (quart, J = 7.2 Hz, 4H), 4.30 (s, 6H), 1.35 (t, J = 7.0 Hz, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 164.7, 147.4, 136.6, 131.6, 131.3, 131.2, 129.9, 125.7, 107.3, 99.9, 98.5, 61.4, 34.4, 14.2. HRMS (m/z): calcd for C$_{28}$H$_{27}$N$_4$O$_4$ [M − H$^+$], 483.2032; found, 483.2033.

1,7-Bis(4-methoxyphenyl)-3,5-dimethylbenzobis(imidazolium) Diiodide (16). Benzobis(imidazole) 14 (500 mg, 1.35 mmol) was dissolved in MeCN (10 mL) and MeI (1.0 mL) in a screw-cap vial. The vial was then sealed with a Teflon-lined cap, and the mixture was heated at 80 °C for 8 h. Upon completion, the mixture was concentrated under vacuum to provide 877 mg (99% yield) of the desired product as a dark brown powder. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.30 (s, 2H), 9.06 (s, 1H), 7.87 (s, 1H), 7.76 (d, J = 8.8 Hz, 4H), 7.25 (d, J = 8.8 Hz, 4H), 4.27 (s, 6H), 3.86 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.7, 147.1, 130.9, 130.6, 127.0, 125.4, 115.5, 99.4, 97.8, 55.8, 34.2. HRMS (m/z): calcd for C$_{24}$H$_{23}$N$_4$O$_2$ [M − H$^+$], 399.1821; found, 399.1817.

1,5-Bis(4-butylphenyl)amino-3,4-dinitrobenzene (17). Following a similar procedure used to prepare 12, 1,5-dichloro-3,4-dinitrobenzene (10) (1.00 g, 4.22 mmol) and 4-butylaniline (2.52 g, 16.88 mmol)

afforded 1.83 g (94% yield) of the desired product as a burnt orange powder. ¹H NMR (400 MHz, CDCl₃): δ 9.68 (br s, 2H), 9.30 (s, 1H), 7.14 (d, $J = 8.2$ Hz, 4H), 7.05 (d, $J = 8.2$ Hz, 4H), 6.48 (s, 1H), 2.58 (t, $J = 7.6$ Hz, 4H), 1.61−1.53 (m, 4H), 1.40−1.31 (m, 4H), 0.95 (t, $J = 7.2$ Hz, 6H). ¹³C NMR (100 MHz, CDCl₃): δ 147.0, 141.5, 134.6, 129.4, 129.3, 125.1, 124.6, 95.1, 35.1, 33.6, 22.2, 13.9. HRMS (*m/z*): calcd for $C_{26}H_{31}N_4O_4$ [M + H⁺], 463.2345; found, 463.2343.

General Procedure for the Reduction of Dinitroarenes 18 and 19. After the dinitroarene (1.0 mmol, 1.0 equiv) was dissolved in EtOH (20 mL) in a 100 mL flask, Pd/C (5 wt %, 0.10 mmol Pd, 0.10 equiv) was added. The flask was then fitted with a H₂O-jacketed condenser, and H₂NNH₂·H₂O (80%, 16 mmol, 16 equiv) was added dropwise followed by 1.2 N HCl (7 mL, 8.0 mmol, 8.0 equiv). The solution was stirred in an oil bath at 80 °C for 4 h. The cooled reaction mixture was then filtered through Celite and concentrated under vacuum to provide the corresponding tetraamines as their dihydrochloride salts. These products were used directly in subsequent steps without further purification. Note: these materials were found to decompose upon prolonged standing under ambient atmosphere and thus were stored in a nitrogen-filled desiccator prior to use.

Tetraaminobenzene Dihydrochloride 18. Yield: 4.20 g (>99%). ¹H NMR (300 MHz, CDCl₃): δ 6.97 (d, $J = 6.6$ Hz, 4H), 6.85 (s, 1H), 6.56 (d, $J = 6.6$ Hz, 4H), 6.26 (s, 1H), 4.86 (br s, 2H), 3.76 (br s, 4H), 2.49 (br t, 4H), 1.59−1.49 (m, 4H), 1.40−1.27 (m, 4H), 0.91 (t, $J = 7.2$ Hz, 6H). ¹³C NMR (75 MHz, CDCl₃): δ 144.8, 142.5, 132.9, 129.1, 126.5, 119.6, 114.0, 102.3, 34.7, 34.0, 22.3, 14.0. HRMS (*m/z*): calcd for $C_{26}H_{35}N_4$ [M + H⁺], 403.2862; found, 403.2858.

Tetraaminobenzene Dihydrochloride 19. Yield: 1.15 g (>99%). ¹H NMR (400 MHz, CDCl₃): δ 6.79 (s, 1H), 6.75 (d, $J = 8.8$ Hz, 4H), 6.59 (d, $J = 8.8$ Hz, 4H), 6.25 (s, 4H), 4.74 (br s, 2H), 3.74 (s, 6H). ¹³C NMR (100 MHz, CDCl₃): δ 152.8, 141.9, 140.8, 125.1, 120.5, 115.5, 114.8, 102.6, 55.7. HRMS (*m/z*): calcd for $C_{20}H_{23}N_4O_2$ [M + H⁺], 351.1821; found, 351.1818.

General Procedure for the Synthesis of *C1*-Aryl Benzobis-(imidazole)s 20−25. Under an atmosphere of dry nitrogen, the corresponding tetraaminobenzene (1.0 mmol, 1.0 equiv) was suspended in THF (10 mL). The respective aroyl chloride (2.0 mmol, 2.0 equiv) was added, followed by Na₂CO₃ (10.0 mmol, 10.0 equiv). After stirring of the solution for 12 h at ambient temperature, it was slowly poured into AcOH (30 mL). The resulting suspension was then heated open-air in an oil bath at 110 °C for 24 h, during which time AcOH was added in portions to maintain the solution volume at ca. 30 mL. Upon completion, the solvent volume was reduced to ca. 10 mL under reduced pressure, and the resulting mixture was slowly poured into a vigorously stirred solution of 10% NaOH containing an equal weight of crushed ice. The resulting solids were collected via vacuum filtration, rinsed with 10% NaOH and then H₂O, and dried under vacuum.

1,7-Bis(4-butylphenyl)-2,6-diphenylbenzobis(imidazole) (20). Yield: 580 mg (96%). ¹H NMR (300 MHz, CDCl₃): δ 8.36 (s, 1H), 7.58 (dd, $J = 6.3, 1.8$ Hz, 4H), 7.37−7.26 (m, 10H), 7.20 (d, $J = 8.7$ Hz, 4H), 6.97 (s, 1H), 2.68 (t, $J = 2.68$ Hz, 4H), 1.70−1.60 (m, 4H), 1.45−1.32 (m, 4H), 0.96 (t, $J = 7.3$ Hz, 6H). ¹³C NMR (75 MHz, CDCl₃): δ 153.1, 143.4, 135.8, 134.8, 130.1, 129.8, 129.4, 129.3, 128.2, 127.3, 109.0, 90.2, 35.3, 33.3, 22.3, 13.9. HRMS (*m/z*): calcd for $C_{40}H_{39}N_4$ [M + H⁺], 575.3175; found, 575.3176.

1,7-Bis(4-butylphenyl)-2,6-bis(4-cyanophenyl)benzobis(imida-zole) (21). Yield: 308 mg (93%). ¹H NMR (300 MHz, CDCl₃): δ 8.37 (s, 1H), 7.69 (d, $J = 8.7$ Hz, 4H), 7.58 (d, $J = 8.7$ Hz, 4H), 7.32 (d, $J = 8.4$ Hz, 4H), 7.19 (d, $J = 8.4$ Hz, 4H), 6.96 (s, 1H), 2.71 (t, $J = 7.7$ Hz, 4H), 1.72−1.61 (m, 4H), 1.46−1.34 (m, 4H), 0.97 (t, $J = 7.2$ Hz, 6H). ¹³C NMR (75 MHz, CDCl₃): δ 151.2, 144.3, 140.8, 136.5, 134.3, 134.0, 132.0, 130.2, 129.7, 127.1, 118.4, 112.8, 109.9, 90.5, 35.3, 33.3, 22.4, 13.9. HRMS (*m/z*): calcd for $C_{42}H_{37}N_6$ [M + H⁺], 625.3080; found, 625.3078.

1,7-Bis(4-butylphenyl)-2,6-bis(4-methoxyphenyl)benzobis(imida-zole) (22). Yield: 287 mg (91%). ¹H NMR (300 MHz, CDCl₃): δ

8.28 (s, 1H), 7.50 (d, $J = 8.1$ Hz, 4H), 7.27 (d, $J = 7.3$ Hz, 4H), 7.19 (d, $J = 7.3$ Hz, 4H), 6.90 (s, 1H), 6.80 (d, $J = 8.1$ Hz, 4H), 3.80 (s, 6H), 2.68 (t, $J = 7.7$ Hz, 4H), 1.70−1.60 (m, 4H), 1.45−1.33 (m, 4H), 0.96 (t, $J = 7.2$ Hz, 6H). ¹³C NMR (75 MHz, CDCl₃): δ 160.3, 152.9, 143.3, 140.5, 135.6, 135.0, 130.8, 129.8, 127.3, 122.7, 113.6, 108.4, 89.9, 55.2, 35.3, 33.3, 22.3, 13.9. HRMS (*m/z*): calcd for $C_{42}H_{43}N_4O_2$ [M + H⁺], 635.3386; found, 635.3390.

1,7-Bis(4-butylphenyl)-2,6-bis(2-thienyl)benzobis(imidazole) (23). Yield: 200 mg (78%). ¹H NMR (300 MHz, CDCl₃): δ 8.25 (s, 1H), 7.37−7.26 (m, 10H), 6.89 (dd appearing as t, $J = 4.5$ Hz, 2H), 6.77 (d, $J = 3.6$ Hz, 2H), 6.59 (s, 1H), 2.73 (t, $J = 7.7$ Hz, 4H), 1.74−1.64 (m, 4H), 1.48−1.35 (m, 4H), 0.97 (t, $J = 7.2$ Hz, 6H). ¹³C NMR (75 MHz, CDCl₃): δ 148.2, 144.6, 140.5, 136.4, 134.0, 132.9, 130.0, 128.25, 128.16, 127.9, 127.4, 108.4, 89.6, 35.4, 33.3, 22.4, 13.9. HRMS (*m/z*): calcd for $C_{36}H_{35}N_4S_2$ [M + H⁺], 587.2303; found, 587.2307.

2,6-Bis(4-biphenyl)-1,7-bis(4-methoxyphenyl)benzobis(imida-zole) (24). Yield: 492 mg (88%). ¹H NMR (400 MHz, CDCl₃): δ 8.36 (s, 1H), 7.67 (d, $J = 8.4$ Hz, 4H), 7.59 (d, $J = 7.2$ Hz, 4H), 7.54 (d, $J = 8.4$ Hz, 4H), 7.45−7.41 (m, 4H), 7.37−7.33 (m, 2H), 7.25 (d, $J = 6.8$ Hz, 4H), 7.01 (d, $J = 8.8$ Hz, 4H), 6.85 (s, 1H), 3.88 (s, 6H). ¹³C NMR (75 MHz, CDCl₃): δ 159.4, 152.9, 141.8, 140.6, 140.1, 136.3, 130.0, 129.7, 129.0, 128.82, 128.78, 127.7, 127.0, 126.8, 115.2, 108.9, 90.0, 55.5. HRMS (*m/z*): calcd for $C_{46}H_{35}N_4O_2$ [M + H⁺], 675.2760; found, 675.2763.

2,6-Bis(4-cyanophenyl)-1,7-bis(4-methoxyphenyl)benzobis(imida-zole) (25). Yield: 469 mg (99%). ¹H NMR (400 MHz, CDCl₃ + DMSO-d_6): δ 8.29 (s, 1H), 7.68 (d, $J = 8.2$ Hz, 4H), 7.55 (d, $J = 8.2$ Hz, 4H), 7.16 (d, $J = 8.4$ Hz, 4H), 6.97 (d, $J = 8.4$ Hz, 4H), 6.80 (s, 1H), 3.84 (s, 6H). ¹³C NMR (75 MHz, CDCl₃ + DMSO-d_6): δ 159.7, 151.1, 140.6, 136.8, 134.2, 131.9, 129.5, 128.9, 128.5, 118.2, 115.4, 112.6, 109.6, 90.3, 55.5. HRMS (*m/z*): calcd for $C_{36}H_{25}N_6O_2$ [M + H⁺], 573.2039; found, 573.2038.

General Procedure for the Alkylation of Benzobis(imidazole)s 20−25 To Provide BBI Salts 26−31. The respective benzobis-(imidazole) (1.0 mmol, 1.0 equiv) was dissolved or suspended in MeCN (10 mL) and MeI (5.0 mmol, 5.0 equiv) in a screw-cap vial. After the vial was sealed with a Teflon-lined cap, the reaction mixture was stirred in an oil bath at 100 °C for 2−10 h. The reaction progress was monitored by No-D ¹H NMR spectroscopy[48] of aliquots taken periodically. Upon completion of the reaction, the reaction mixtures were cooled to ambient temperature and concentrated. Crude materials were then rinsed with cold EtOAc and then dried under vacuum to provide the desired products.

1,7-Bis(4-butylphenyl)-3,5-dimethyl-2,6-diphenylbenzobis(imida-zolium) Diiodide (26). Yield: 149 mg (>99%). ¹H NMR (300 MHz, CDCl₃): δ 8.44 (s, 1H), 7.88 (d, $J = 7.5$ Hz, 4H), 7.65−7.50 (m, 10H), 7.31 (s, 1H), 7.23 (d, $J = 8.4$ Hz, 4H), 4.27 (s, 6H), 1.60−1.50 (m, 4H), 1.35−1.23 (m, 4H), 0.89 (t, $J = 7.2$ Hz, 6H). ¹³C NMR (75 MHz, CDCl₃): δ 153.7, 146.4, 133.1, 132.9, 132.0, 131.4, 130.2, 129.5, 129.3, 127.6, 120.6, 99.9, 98.0, 35.2, 34.9, 32.9, 22.1, 13.8. HRMS (*m/z*): calcd for $C_{42}H_{43}N_4$ [M − H⁺], 603.3488; found, 603.3492.

1,7-Bis(4-butylphenyl)-2,6-bis(4-cyanophenyl)benzobis(imidazo-lium) Diiodide (27). Yield: 255 mg (>99%). ¹H NMR (400 MHz, DMSO-d_6): δ 9.33 (s, 1H), 8.12 (d, $J = 7.8$ Hz, 4H), 8.05 (d, $J = 7.8$ Hz, 4H), 7.49 (d, $J = 8.2$ Hz, 4H), 7.38 (s, 1H), 7.36 (d, $J = 8.2$ Hz, 4H), 4.19 (s, 3H), 2.59 (t, $J = 7.4$ Hz, 4H), 1.52−1.48 (m, 4H), 1.25−1.20 (m, 4H), 0.84 (t, $J = 7.2$ Hz, 6H). ¹³C NMR (75 MHz, CDCl₃ + DMSO-d_6): δ 151.7, 146.5, 132.6, 132.3, 132.1, 131.8, 129.9, 128.7, 127.0, 124.8, 116.7, 116.3, 99.8, 97.3, 34.7, 34.3, 32.4, 21.7, 13.3. HRMS (*m/z*): calcd for $C_{44}H_{41}N_6$ [M − H⁺], 653.3393; found, 653.3398.

1,7-Bis(4-butylphenyl)-2,6-bis(4-cyanophenyl)benzobis(imidazo-lium) Bis(tetrafluoroborate) (27·BF₄). This compound was prepared

(48) Hoye, T. R.; Eklov, B. M.; Ryba, T. D.; Voloshin, M.; Yao, L. J. *Org. Lett.* **2004**, *6*, 953.

analogously to **2·BF₄** from BBI **27** (59 mg, 0.06 mmol) to provide 49 mg (98% yield) of the desired product. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.30 (s, 1H), 8.15 (d, $J = 8.4$ Hz, 4H), 7.99 (d, $J = 8.4$ Hz, 4H), 7.46 (d, $J = 8.1$ Hz, 4H), 7.43 (s, 1H), 7.38 (d, $J = 8.7$ Hz, 4H), 4.20 (s, 6H), 2.62 (t, $J = 7.8$ Hz, 4H), 1.57−1.47 (m, 4H), 1.31−1.19 (m, 4H), 0.86 (t, $J = 7.2$ Hz, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 152.4, 145.8, 132.8, 132.3, 131.9, 131.3, 130.2, 129.5, 127.4, 125.4, 117.6, 115.5, 99.4, 96.8, 34.3, 33.8, 32.5, 21.6, 13.7.

1,7-Bis(4-butylphenyl)-2,6-bis(4-methoxyphenyl)-3,5-dimethyl-benzobis(imidazolium) Diiodide (28). Yield: 425 mg (95%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.22 (s, 1H), 7.73 (d, $J = 8.6$ Hz, 4H), 7.48 (d, $J = 8.2$ Hz, 4H), 7.37 (d, $J = 8.2$ Hz, 4H), 7.15 (s, 1H), 7.13 (d, $J = 8.6$ Hz, 4H), 4.19 (s, 6H), 3.82 (s, 6H), 2.61 (t, $J = 7.6$ Hz, 4H), 1.55−1.48 (m, 4H), 1.26−1.21 (m, 4H), 0.85 (t, $J = 7.2$ Hz, 6H). ^{13}C NMR (400 MHz, DMSO-d_6): δ 162.5, 153.8, 145.4, 133.4, 131.7, 131.1, 130.14, 130.09, 127.5, 114.5, 112.5, 55.7, 34.3, 34.0, 32.5, 21.6, 13.7. HRMS (m/z): calcd for $C_{44}H_{47}N_4O_2$ [M − H$^+$], 663.3699; found, 663.3701.

1,7-Bis(4-butylphenyl)-2,6-bis(2-thienyl)-3,5-dimethylbenzobis-(imidazolium) Diiodide (29). Yield: 276 mg (93%). ^{1}H NMR (400 MHz, CDCl₃): δ 8.48 (s, 1H), 7.80 (d, $J = 3.6$ Hz, 2H), 7.78 (d, $J = 4.8$ Hz, 2H), 7.39 (d, $J = 8.2$ Hz, 4H), 7.31 (d, $J = 8.2$ Hz, 4H), 7.25 (d, $J = 4.8$ Hz, 2H), 7.11 (s, 1H), 4.32 (s, 6H), 2.67 (t, $J = 7.8$ Hz, 4H), 1.64−1.56 (m, 4H), 1.38−1.29 (m, 4H), 0.92 (t, $J = 7.4$ Hz, 6H). ^{13}C NMR (100 MHz, CDCl₃): δ 148.7, 147.1, 137.6, 135.5, 132.8, 132.2, 130.6, 129.5, 128.7, 127.8, 119.0, 98.8, 97.0, 35.3, 34.4, 33.0, 22.2, 13.8. HRMS (m/z): calcd for $C_{38}H_{39}N_4S_2$ [M − H$^+$], 615.2616; found, 615.2612.

2,6-Bis(4-biphenyl)-1,7-bis(4-methoxyphenyl)-3,5-dimethylbenzo-bis(imidazolium) Diiodide (30). Yield: 186 mg (94%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.28 (s, 1H), 7.95 (d, $J = 8.4$ Hz, 4H), 7.90 (d, $J = 8.4$ Hz, 4H), 7.58−7.43 (m, 10H), 7.37 (s, 1H), 7.10 (d, $J = 9.2$ Hz, 4H), 4.24 (s, 6H), 3.76 (s, 6H). ^{13}C NMR (75 MHz, DMSO-d_6): δ 160.6, 153.8, 144.1, 138.0, 132.3, 132.0, 131.2, 129.2, 128.8, 127.0, 126.9, 124.7, 119.8, 115.5, 99.1, 96.4, 55.6, 34.1. HRMS (m/z): calcd for $C_{48}H_{39}N_4O_2$ [M − H$^+$], 703.3073; found, 703.3077.

2,6-Bis(4-cyanophenyl)-1,7-bis(4-methoxyphenyl)-3,5-dimethyl-benzobis(imidazolium) Diiodide (31). Yield: 102 mg (99%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.30 (s, 1H), 8.14 (d, $J = 8.6$ Hz, 4H), 8.04 (d, $J = 8.6$ Hz, 4H), 7.51 (d, $J = 9.0$ Hz, 4H), 7.49 (s, 1H), 7.07 (d, $J = 9.0$ Hz, 4H), 4.18 (s, 6H), 3.76 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 160.7, 152.5, 132.8, 132.4, 131.3, 129.1, 125.5, 124.2, 117.6, 115.5, 115.4, 96.9, 88.0, 55.6, 34.0. HRMS (m/z): calcd for $C_{38}H_{29}N_6O_2$ [M − H$^+$], 601.2352; found, 601.2355.

1-(4-Carboethoxyphenyl)-7-(4-methoxyphenyl)-3,5-dimethylben-zobis(imidazolium) Diiodide (32). This compound was prepared analogously to BBI **15** from benzobis(imidazole) **35** (100 mg, 0.24 mmol) to provide 169 mg (>99% yield) of the desired product as a pale yellow powder. ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.48 (s, 1H), 10.33 (s, 1H), 9.1 (s, 1H), 8.27 (d, $J = 8.7$ Hz, 2H), 8.07 (s, 1H), 8.04 (d, $J = 9.0$ Hz, 2H), 7.80 (d, $J = 9.0$ Hz, 2H), 7.25 (d, $J = 9.0$ Hz, 2H), 4.38 (quart, $J = 6.9$ Hz, 2H), 4.30 (s, 3H), 4.29 (s, 3H), 3.86 (s, 3H), 1.35 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.7, 160.6, 147.2, 136.7, 131.6, 131.3, 131.1, 131.0, 130.6, 129.8, 127.0, 125.8, 125.5, 115.5, 99.7, 98.2, 61.4, 55.8, 34.4, 34.3, 14.1. HRMS (m/z): calcd for $C_{26}H_{25}N_4O_3$ [M − H$^+$], 441.1927; found, 441.1930.

1-(4-Carboethoxyphenyl)amino-5-chloro-2,4-dinitrobenzene (33). A screw-cap vial was charged with 1,5-dichloro-3,4-dinitrobenzene (**10**) (595 mg, 2.51 mmol), benzocaine (829 g, 5.02 mmol), EtOH (10 mL), and a stir bar. The vial was then sealed with a Teflon-lined cap, and the mixture was placed in an oil bath at 50 °C and stirred for 12 h. Upon completion, the mixture was poured into 5% HCl (50 mL). Precipitated solids were collected via vacuum filtration, rinsed with H₂O, and dried under vacuum to provide 875 mg (95% yield) of the desired product as a yellow powder. ^{1}H NMR (400 MHz, CDCl₃): δ

9.90 (br s, 1H), 9.06 (s, 1H), 8.18 (d, $J = 8.8$ Hz, 2H), 7.38 (d, $J = 8.8$ Hz, 2H), 7.32 (s, 1H), 4.41 (quart, $J = 7.2$ Hz, 2H), 1.42 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (100 MHz, CDCl₃): δ 165.4, 144.2, 140.4, 136.7, 135.8, 131.7, 130.3, 129.4, 126.5, 124.0, 118.1, 61.4, 14.3. HRMS (m/z): calcd for $C_{15}H_{13}N_3O_6Cl$ [M + H$^+$], 366.0493; found, 366.0489.

1-(4-Carboethoxyphenyl)amino-2,4-dinitro-5-(4-methoxyphenyl)-aminobenzene (34). After aryl chloride **33** (380 mg, 1.04 mmol) was dissolved in EtOH (10 mL), p-anisidine (256 mg, 2.08 mmol) was added in a single portion. The mixture was then stirred at 80 °C for 24 h. After being cooled to ambient temperature, the mixture was poured into 5% HCl (50 mL). Precipitated solids were collected via vacuum filtration, rinsed with H₂O, and dried under vacuum to provide 462 mg (98% yield) of the desired product as an orange powder. ^{1}H NMR (300 MHz, CDCl₃): δ 9.85 (br s, 1H), 9.65 (br s, 1H), 9.33 (s, 1H), 7.98 (d, $J = 8.7$ Hz, 2H), 7.17 (d, $J = 8.7$ Hz, 2H), 7.12 (d, $J = 8.7$ Hz, 2H), 6.92 (d, $J = 9.0$ Hz, 2H), 6.53 (s, 1H), 4.38 (quart, $J = 7.2$ Hz, 2H), 3.81 (s, 3H), 1.41 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (100 MHz, CDCl₃): δ 165.6, 158.7, 148.1, 145.1, 141.7, 131.1, 129.5, 129.3, 127.6, 127.0, 125.64, 125.55, 122.6, 115.1, 96.1, 61.1, 55.5, 14.3. HRMS (m/z): calcd for $C_{22}H_{21}N_4O_7$ [M + H$^+$], 453.1410; found, 453.1406.

1-(4-Carboethoxyphenyl)-7-(4-methoxyphenyl)benzobis(imida-zole) (35). This compound was prepared analogously to **13** from dinitroarene **34** (354 mg, 0.78 mmol) to provide 272 mg (84% yield) of the desired product as an off-white solid. ^{1}H NMR (300 MHz, CDCl₃): δ 8.33 (s, 1H), 8.24 (d, $J = 8.7$ Hz, 2H), 8.19 (s, 1H), 8.10 (s, 1H), 7.61 (d, $J = 9.0$ Hz, 2H), 7.49 (s, 1H), 7.41 (d, $J = 9.0$ Hz, 2H), 7.08 (d, $J = 9.0$ Hz, 2H), 4.42 (quart, $J = 7.2$ Hz, 2H), 3.89 (s, 3H), 1.42 (t, $J = 7.2$ Hz, 3H). ^{13}C NMR (75 MHz, CDCl₃): δ 165.5, 159.4, 143.9, 142.7, 141.6, 141.5, 140.3, 133.1, 131.6, 131.5, 129.6, 129.1, 125.9, 123.2, 115.3, 111.1, 90.1, 61.4, 55.6, 14.3. HRMS (m/z): calcd for $C_{24}H_{21}N_4O_3$ [M + H$^+$], 413.1614; found, 413.1611.

Benzobis(imidazolium) Diiodide (36). Benzobis(imidazole) **41** (2.00 g, 4.23 mmol), was dissolved in dry MeCN (20 mL) and MeI (5.99 g, 42.3 mmol) in a screw-cap vial. The vial was then sealed with a Teflon-lined cap and placed in an oil bath at 95 °C for 12 h. The solution was then cooled, opened to air, and then stirred in an oil bath at 95 °C until the solvent volume reached ca. 1 mL. The residue was then dried under vacuum to provide 2.88 g (99% yield) of the desired product. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.45 (s, 1H), 10.43 (s, 1H), 9.15 (s, 1H), 8.13 (s, 1H), 8.04 (d, $J = 8$ Hz), 7.93 (d, $J = 8$ Hz, 2H), 7.73 (m, 5H), 7.27 (s, 2H), 4.32 (s, 2H), 4.31 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.6, 147.2, 147.1, 134.9, 133.4, 133.0, 131.8, 131.1, 131.1, 130.7, 130.5, 130.0, 129.9, 128.3, 125.8, 125.3, 99.7, 98.3. HRMS (m/z): calcd for $C_{26}H_{21}IN_5O_2$ [M + I], 562.0740; found, 562.0716.

Conjugate 36·MPA. BBI **36** (130 mg, 0.19 mmol) and 3-mercap-topropionic acid (20 mg, 0.19 mmol) were dissolved in H₂O (2.0 mL) in a screw-cap vial. The mixture was stirred for 12 h at room temperature and then concentrated under vacuum to provide 142 mg (95% yield) of the desired product. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.47 (s, 1H), 10.43 (s, 1H), 9.16 (s, 1H), 8.14 (s, 1H) 8.07 (d, $J = 8$ Hz, 2H), 7.93 (d, $J = 8$ Hz, 2H), 7.71 (m, 5H), 4.31 (s, 3H), 4.30 (s, 3H), 4.25−4.22 (m, 1H) 3.44−3.37 (m, 1H), 3.06−2.89 (m, 2H), 2.76−2.70 (m, 1H), 2.65−2.60 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.8, 174.0, 172.8, 147.3, 147.1, 133.9, 133.0, 132.6, 131.1, 130.7, 130.5, 130.0, 129.8, 128.9, 125.9, 125.2, 99.8, 98.3, 36.2, 34.4, 34.3, 34.1, 26.3. HRMS (m/z): calcd for $C_{29}H_{27}IN_5O_4S$ [M + I], 668.0828; found, 668.0828.

Conjugate 36·BSA. BBI **36** (61 mg, 0.08 mmol) and bovine serum albumin (BSA) (200 mg, 0.003 mmol) were dissolved in distilled H₂O (3 mL). The resulting solution was stirred at 23 °C for 4 h. Upon completion, the solution was transferred to a 6000 Da dialysis membrane and dialyzed against distilled H₂O for 48 h. Afterward, the solution was concentrated under vacuum and analyzed.

1-Chloro-2,4-dinitro-5-(phenylamino)benzene (37). 1,5-Dichloro-2,4-dinitrobenzene (**10**) (10.0 g, 42.2 mmol) and PhNH₂ (7.85 g, 84.4 mmol) were dissolved in ^{i}PrOH (250 mL), and the resulting mixture

was stirred at room temperature for 48 h. The resulting slurry was then poured into H_2O (250 mL), and the solids were collected by vacuum filtration, rinsed with H_2O, and dried under vacuum to provide 11.6 g (94% yield) of the desired product. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.14, (s, 1H), 8.88 (s, 1H), 7.51 (m, 2H), 7.38 (m, 3H), 6.96 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 145.3, 137.3, 134.6, 133.3, 130.0, 127.3, 126.6, 125.8, 117.9. HRMS (*m/z*): calcd for $C_{12}H_9ClN_3O_4$ [M + H]$^+$, 294.0282; found, 294.0283.

1-(4-Aminophenyl)amino-5-(phenyl)amino-2,4-dinitrobenzene (38). In a flask fitted with a H_2O-jacketed condenser, aryl chloride **37** (5.00 g, 17.1 mmol) and *p*-phenylenediamine (3.69 g, 34.1 mmol) were dissolved in *i*PrOH (75 mL). The resulting mixture was stirred in an oil bath at 80 °C for 24 h. Afterward, the mixture was poured into 100 mL of water, which caused solids to precipitate. After collection by vacuum filtration, the solids were rinsed with H_2O and then dried under vacuum to provide 6.10 g (98% yield) of the desired product. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.54 (s, 1H), 9.56 (s, 1H), 9.01 (s, 1H), 7.35– 7.16 (m, 2H), 7.25 (d, *J* = 6.0 Hz, 2H), 7.16 (t, *J* = 8.0 Hz, 2H) 6.89 (d, *J* = 8.0 Hz, 2H), 6.21 (d, *J* = 8.0 Hz, 2H), 6.33 (s, 1H), 5.17 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 147.4, 147.3, 145.6, 137.8, 129.3, 128.5, 126.7, 125.7, 125.3, 124.7, 124.6, 124.3, 114.1, 95.4. HRMS (*m/z*): calcd for $C_{18}H_{16}N_5O_4$ [M + H]$^+$, 366.1202; found, 366.1197.

1-(4-Aminophenyl)-7-(phenyl)benzobis(imidazole) (39). This compound was prepared analogously to benzobis(imidazole) **14** from dinitroarene **38** (5.00 g, 15.4 mmol). Analysis of the crude product mixture by ^{1}H NMR spectroscopy and LC-MS revealed the presence of a 1-(4-formamidophenyl) analogue of the desired product. To effect hydrolysis of the formamide, the crude product was suspended in 10% aqueous H_2SO_4 (100 mL) and heated at 100 °C for 3 h. The cooled solution was then neutralized with K_2CO_3 powder, and the resulting precipitate was collected via vacuum filtration, rinsed with H_2O, and dried under vacuum to provide 4.14 g (92% yield) of the desired product. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.54 (s, 1H), 8.36 (s, 1H), 8.06 (s, 1H) 7.69 (d, *J* = 8.0 Hz, 2H), 7.63–7.59 (m, 2H), 7.47–7.43 (m, 2H), 7.27 (d, *J* = 8.0 Hz, 2H), 6.72 (d, *J* = 8.0 Hz, 2H), 5.42 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 148.7, 144.5, 143.9, 140.9, 140.8, 136.3, 132.2, 131.1, 130.1, 127.6, 125.3, 124.2, 123.6, 114.4, 109.0, 90.0. HRMS (*m/z*): calcd for $C_{20}H_{16}N_5$ [M + H]$^+$, 326.1406; found, 326.1407.

Benzobis(imidazole) 41. Benzobis(imidazole) **39** (3.00 g, 9.23 mmol) and 4,10-dioxatricyclo[5.2.1.0]dec-8-ene-3,5-dione (**40**) (1.53 g, 9.23 mmol) were dissolved in AcOH (150 mL), and the resulting mixture was stirred at ambient temperature for 12 h. Upon completion, the solution was neutralized with K_2CO_3 powder and the resulting precipitate was collected via vacuum filtration, rinsed with H_2O, and dried under vacuum to provide 3.49 g (80% yield) of the desired product. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.64 (s, 1H), 8.59 (s, 1H), 8.13 (s, 1H), 7.87 (d, *J* = 8 Hz, 2H), 7.74 (d, *J* = 8 Hz, 2H), 7.70 (s, 1H), 7.65–7.61 (m, 2H), 7.49–7.44 (m, 3H), 6.62 (s, 2H), 5.27 (s, 2H), 3.12 (s, 2H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 175.7, 172.2, 144.4, 141.23, 141.18, 136.7, 136.2, 136.0, 131.3, 131.0, 130.2, 128.5, 127.7, 124.1, 123.7, 109.5, 90.6, 80.8, 64.8, 47.6. HRMS (*m/z*): calcd for $C_{28}H_{20}N_5O_3$ [M + H]$^+$, 474.1566; found, 474.1560.

2,6-Bis(4-cyanophenyl)-1,7-diphenylbenzobis(imidazole) (42). This compound was prepared analogously to benzobis(imidazole)s **20–25** from 1,5-diamino-2,4-bis(phenylamino)benzene dihydrochloride to provide 500 mg (96% yield) of the desired compound. ^{1}H NMR (400 MHz, CDCl$_3$): δ 8.39 (s, 1H), 7.70 (d, *J* = 8.4 Hz, 4H), 7.59 (d, *J* = 8.4 Hz, 4H), 7.53–7.52 (m, 6H), 7.31–7.29 (m, 4H), 6.94 (s, 1H). ^{13}C NMR (75 MHz, CDCl$_3$): δ 151.2, 140.9, 136.6, 136.5, 134.2, 132.0, 130.4, 129.7, 129.2, 127.4, 118.3, 113.0, 110.1, 90.3. HRMS (*m/z*): calcd for $C_{34}H_{21}N_6$ [M + H]$^+$, 513.1828; found, 513.1822.

2,6-Bis(4-cyanophenyl)-1,7-dimethyl-3,5-diphenylbenzobis(imida-zolium) Diiodide (43). This compound was prepared analogously to BBIs **26–31** from benzobis(imidazole) **42** to provide 255 mg (>99% yield) of the desired compound. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.35 (s, 1H), 8.13 (d, *J* = 8.6 Hz, 4H), 8.06 (d, *J* = 8.6 Hz, 4H), 7.61–7.55 (m, 10H), 7.48 (s, 1H), 4.20 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6): δ 152.3, 132.7, 132.5, 132.0, 131.9, 131.4, 130.5, 127.7, 125.4, 117.6, 115.5, 99.7, 96.9, 54.4, 34.1. HRMS (*m/z*): calcd for $C_{36}H_{25}N_6$ [M − H]$^+$, 541.2141; found, 541.2137. UV–vis and fluorescence data in MeOH: λ_{abs} = 384, log(ϵ) = 4.19, λ_{em} = 547, Φ_f = 0.33.

Multiphoton Excitation. All experiments were performed using a mode-locked Ti:S oscillator (Coherent Mira 900F) pumped by a 532 nm 10 W frequency-doubled neodymium–vanadate (Coherent Verdi) laser. This source produces ca. 150 fs pulses at a repetition rate of 76 MHz. The near-IR output of the Ti:S was attenuated to desired powers using a half-wave plate/polarizing beam splitting pair. After attenuation, the beam was passed through a long-pass dichroic mirror (Chroma Technology Corp., 575DCXR) and directed into the back aperture of a 100× 1.3-NA oil-immersion objective (Fluar, Zeiss). Fluorescence generated at the beam focus within a sample cuvette was collected by the Fluar objective and reflected by the dichroic mirror through a series of filters (5 cm saturated aqueous $CuSO_4$ and two 3-mm thick BG-39 filters) before striking a bialkali photomultiplier tube (PMT; Hamamatsu HC-125) detector. Signal was sent to a photon counter (Stanford Research Systems SR-400), which collected data in 1 s intervals. Data were corrected for background counts from the cuvette and solvent. The collection efficiency of the instrument was estimated from the fluorescence emission spectrum of BBI **4** and measured/reported transmission spectra of the optics, filters, and PMT.

Supporting Information Available: A full list of authors for ref 24a as well as ^{1}H and ^{13}C NMR spectra for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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