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B,O-Chelated Azadipyrromethenes as Near-IR Probes

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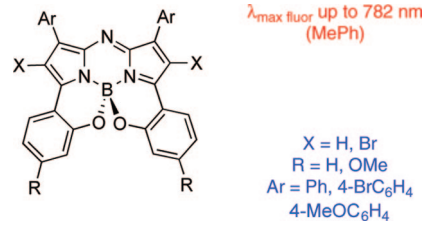
ABSTRACT



Complementary synthetic routes to a new class of near-IR fluorophores are described. These allow facile access (four synthetic steps) to the core fluorophore and substituted derivatives with emissions between 740 and 780 nm in good quantum yields.

Surprisingly few fluorescent probes emit in the near-IR region with high quantum yields.[1,2] Such compounds are valuable for intracellular imaging because autofluorescence in cells tends to obscure emissions at wavelengths below approximately 550 nm, but this factor becomes less of an issue at longer wavelengths. Probes that emit in the 750−900 nm region are, therefore, relatively easy to visualize *in vivo*.[3] Cyanine dyes are currently the most widely used probes for this wavelength region, but their quantum yields and photostabilities are suboptimal.[4] Thus there is need for new fluorescent probes that emit efficiently above 750 nm.

Dyes based on the 5-6-5 fused BODIPY ring system **A** are popular in biotechnology because they tend to have relatively sharp fluorescence emission characteristics, and high quantum yields.[5] However, the emission wavelengths of most BODIPY dyes are between 530 and 630 nm, and this is suboptimal for intracellular or tissue imaging. Previous work by Burgess et al.,[6−8] and others,[9,10] successfully explored a modification strategy for shifting BODIPY-fluorescence emissions to the red (Figure 1). Adaptation of the core fluorophore scaffold by intramolecular B−O ring formation to produce the 6,6-5-6-5-6,6 ring system **B** gave an ca. 65 nm bathochromatic shift (compare **C** and **D**),[11] but the fluorescence emission wavelengths were less than 700 nm.[12]

O'Shea and co-workers have demonstrated that BF_2 chelated tetraaryl-substituted azadipyrromethenes **E** fluoresce

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(1) Sun, C.; Yang, J.; Li, L.; Wu, X.; Liu, Y.; Liu, S. *J. Chromatogr., B* **2004**, 173–90.
(2) Kiyose, K.; Kojima, H.; Nagano, T. *Chem.−Asian J.* **2008**, *50*, 6–515.
(3) Frangioni, J. V. *Curr. Opin. Chem. Biol.* **2003**, 626–34.
(4) Benson, R. C.; Kues, H. A. *J. Chem. Eng. Data* **1977**, 379–83.
(5) Boyer, J. H.; Haag, A. M.; Sathyamoorthi, G.; Soong, M. L.; Thangaraj, K.; Pavlopoulos, T. G. *Heteroat. Chem.* **1993**, 39–49.
(6) Chen, J.; Burghart, A.; Derecskei-Kovacs, A.; Burgess, K. *J. Org. Chem.* **2000**, 2900–2906.
(7) Thivierge, C.; Bandichhor, R.; Burgess, K. *Org. Lett.* **2007**, 2135–2138.
(8) Loudet, A.; Burgess, K. *Chem. Rev.* **2007**, 4891–4932.
(9) Umezawa, K.; Nakamura, Y.; Makino, H.; Citterio, D.; Suzuki, K. *J. Am. Chem. Soc.* **2008**, 1550–1551.
(10) Ulrich, G.; Ziessel, R.; Harriman, A. *Angew. Chem., Int. Ed.* **2008**, 1184–1201.
(11) Kim, H.; Burghart, A.; Welch, M. B.; Reibenspies, J.; Burgess, K. *Chem. Commun.* **1999**, 1889–90.
(12) Burghart, A.; Kim, H.; Welch, M. B.; Thoresen, L. H.; Reibenspies, J.; Burgess, K.; Bergstroem, F.; Johansson, L. B. A. *J. Org. Chem.* **1999**, 7813–7819.

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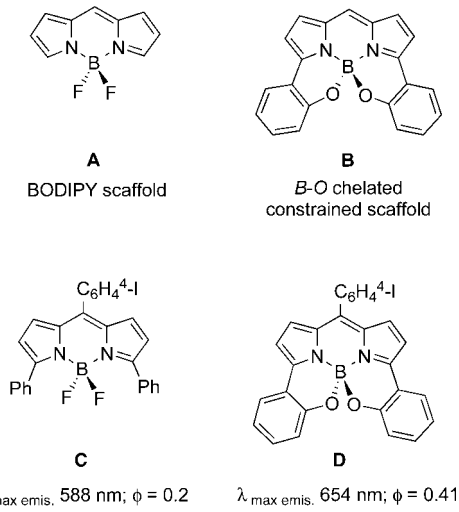


Figure 1. Comparison of fluorescence emission wavelength maxima and quantum yields (in $CHCl_3$) for BODIPY class (**A**) and a B−O ring-extended class (**B**).

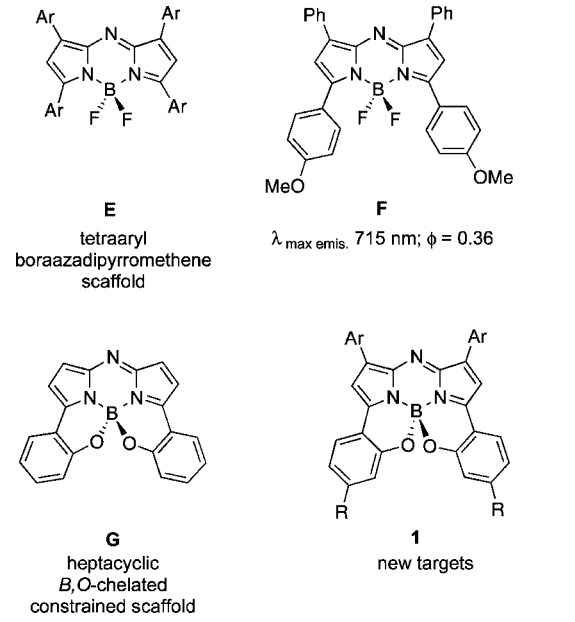
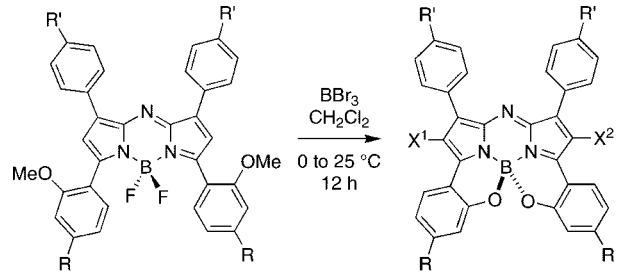


Figure 2. Representative fluorescence emission wavelength maximum and quantum yield (in $CHCl_3$) for BF_2-chelated tetraarylazadipyrromethenes (**E**) and a new B−O ring extended class (**G**).

with wavelength maxima between 670 and 720 nm with para-oriented electron donating groups being particularly effective in enhancing the red-shifted fluorescence, e.g. **F** (Figure 2).[13−19] This is close to the 750−900 nm region at which light permeation through tissue is most effective. Subsequently, it was shown that derivatives with extended aromatic groups do emit in the near-IR region.[20] The premise of this paper is that it would be advantagous to "hybridize" the structures **B** and **E** to shift their emissions beyond 750 nm. On the basis of the structures shown in Figure 1 it was therefore logical to introduce an intramolecular *B*,*O*-chelate on a tetraaryl boraazadipyrromethene scaffold, e.g., **G**. The studies described here feature compounds **1** of this type; it emerges that they can emit in this region.

Scheme 1 shows one approach to the synthesis of the target class **1** from the precursors **2a**−**d** which can be synthesized in four steps from commercially available materials.[21] A solid state X-ray structure determination of **2a** illustrated that both 2-$MeOC_6H_4$ rings lie out of the plane of the central chromophore with torsion angles of 42.7° and 61.4° (Supporting Information). Compounds **1** were obtained from the corresponding **2** by treatment with boron tribromide in dichloromethane. We anticipated that demethylation of the

Scheme 1. Synthesis of Near-IR Fluorophores **1**



	a	b	c	d	e	f
R =	H	H	OMe	NMe_2	H	H
R' =	H	Br	Br	Br	Br	Br
X^1 =	H	H	H	H	Br	Br
X^2 =	H	H	H	H	H	Br
yield (%) =	62	36	n/a	n/a	41	21

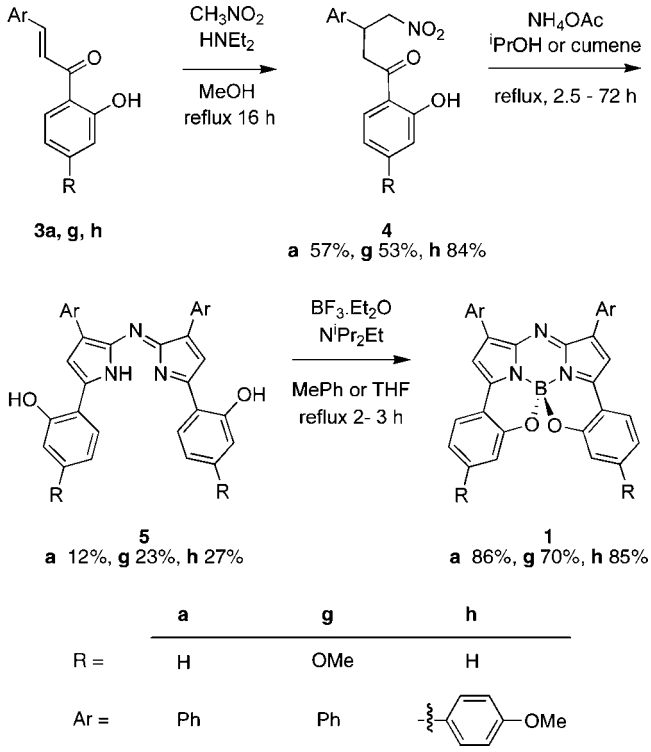
methoxy groups with BBr_3 to the corresponding bisphenols would lead to spontaneous cyclization giving the target materials **1**. This proved correct for substrates **1a** and **1b** with products isolated in 61% and 36% yields, respectively, following chromatography. Furthermore, in addition to **1a** and **1b**, compounds corresponding to bromination of the pyrrole ring, i.e., **1e**−**f**, were also observed. This is somewhat surprising since this did not occur for the synthesis of the **B** class, but it is also potentially useful if the compounds are

(13) Killoran, J.; Allen, L.; Gallagher, J. F.; Gallagher, W. M.; O'Shea, D. F. *Chem. Commun.* **2002**, 1862–1863.
(14) Gorman, A.; Killoran, J.; O'Shea, C.; Kenna, T.; Gallagher, W. M.; O'Shea, D. F. *J. Am. Chem. Soc.* **2004**, 10619–31.
(15) McDonnell, S. O.; Hall, M. J.; Allen, L. T.; Byrne, A.; Gallagher, W. M.; O'Shea, D. F. *J. Am. Chem. Soc.* **2005**, 16360–16361.
(16) Killoran, J.; O'Shea, D. F. *Chem. Commun.* **2006**, 1503–1505.
(17) Hall, M. J.; Allen, L. T.; O'Shea, D. F. *Org. Biomol. Chem.* **2006**, 776–780.
(18) O'Shea, D.; Killoran, J.; Gallagher, W. Compounds useful as photodynamic therapeutic agents. U.S. Patent. 7220732, 20030324, 2003.
(19) Killoran, J.; McDonnell, S. O.; Gallagher, J. F.; O'Shea, D. F. *New J. Chem.* **2008**, 483–489.
(20) Zhao, W.; Carreira, E. M. *Angew. Chem., Int. Ed.* **2005**, 1677–9.
(21) Loudet, A.; Bandichhor, R.; Wu, L.; Burgess, K. *Tetrahedron* **2008**, 3642–3654.

to be modified further.[6] Systems **1c** and **1d** were not air-stable, and were not isolated.

Prior experience with BF_2-chelated tetraarylazadipyrromethene derivatives such as **F** indicated that electron-releasing R-groups might red-shift the fluorescence maxima of the *B*,*O*-chelated compounds **1**. An alternative route, which avoids the aryl demethylation conditions used in Scheme 1, was therefore developed to allow inclusion of 4-methoxy groups (Scheme 2). This required conjugate addition of

Scheme 2. Four-Step Synthesis to Near-IR Fluorophores **1**



nitromethane to the chalcones **3a**,**g**,**h**. Heating of **4a**,**g**,**h** with an ammonium source gave phenolic-substituted azadipyrromethenes **5a**,**g**,**h**. Fluorophores **1a**,**g**,**h** were obtained in a one-pot BF_2 chelation and intramolecular phenolic oxygen−fluorine displacement thereby generating the structurally constraining benzo{1,3,2}oxazaborinine rings. Presumably this synthetic route could be modified to include functional groups in place of the *p*-methoxy position of **1g**,**h** for bioconjugation.

Single-crystal X-ray structures for **1h** and **1f** were obtained (Figure 3 and Supporting Information). The benzo-fused 6,6-5-6-5-6,6 ring structure was shown to be tetrahedral around the boron atom. Consequently the molecule is chiral just as for the other *B*,*O*-chelates **B**.

Figure 4 highlights the dramatic spectroscopic contrast between the constrained derivative **1a** and its unconstrained precursor **2a**. Restrictions caused by the B−O bonds in **1a** gave bathochromic shifts in both the absorption (86 nm) and emission maxima (58 nm) and over a 7-fold increase in the quantum yield from 0.07 to 0.51 (Figure 4, Table 1, entries

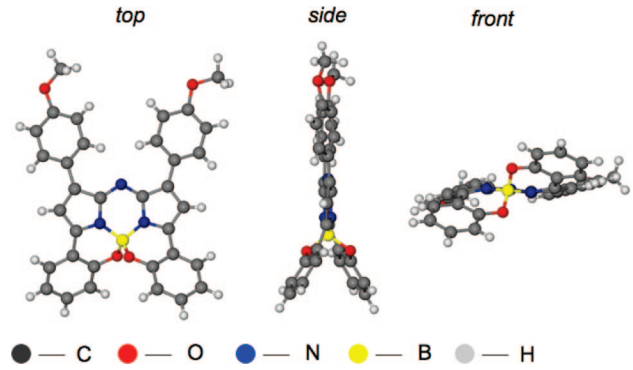


Figure 3. X-ray crystal structure of **1h**. Selected bond lengths B−O = 1.471(2) and 1.476(2) and B−N = 1.513(2) and 1.514(2); bond angles O−B−O = 108.0(1)° and N−B−N = 103.9(1)°.

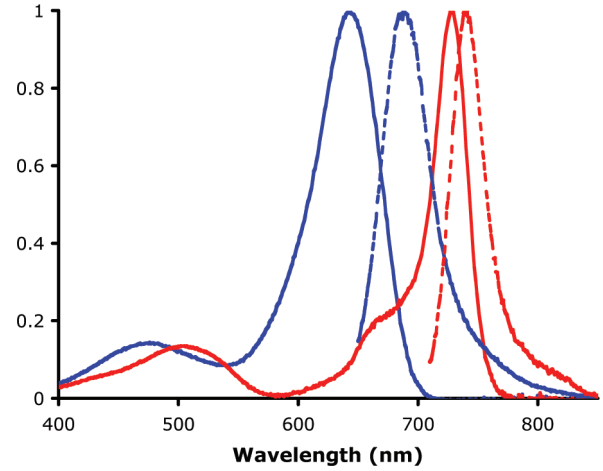


Figure 4. Normalized absorbance (solid line) and emission (dashed line) for **2a** (blue) and **1a** (red) in $CHCl_3$.

Table 1. Photophysical Properties of **1** in Toluene

entry		λ_{max} (nm)	λ_f (nm)	fwhm (nm)[a]	Φ_f[b]
1	**2a**	642	688	43	0.07[10]
2	**1a**	728	746	36	0.51
3	**1b**	745	760	36	0.46
4	**1e**	747	769	38	0.35
5	**1f**	755	776	36	0.36
6	**1g**	765	782	40	0.18[c]
7	**1h**	728	742	36	0.17[c]

[a] Full width at half-maximum height. [b] Measured in 1% pyridinein toluene. ZnPtc (Φ_f 0.30) was used as standard. [c] Measured in $CHCl_3$.

1 and 2). These trends were observed for all derivatives of **1** with additional hypso- and bathochromatic shifts due to the further influence of the aryl and pyrrole substituents.

Introduction of bromine (**1b**) and methoxy (**1h**) aryl-substituents resulted in a 14 nm bathochromic and 4 nm hypsochromic shift for fluorescence maxima, respectively, when compared to **1a** (entries 3 and 7). The longest

absorbance/emission wavelength maxima (765/782 nm) were observed for derivative **1g** containing electron donating methoxy substituents on the benzo{1,3,2}oxazaborinine rings (entry 6). This pronounced red-shift for fluorescence maximum of 94 nm when compared to **2a** and 36 nm from **1a** places it into the near-IR spectral region (Figure 5). Interestingly, the tetrabromo derivative **1f** also had red-shifted absorption and emission profiles and the substituent did not result in a pronounced quenching of the fluorescence (entry 5).[14]

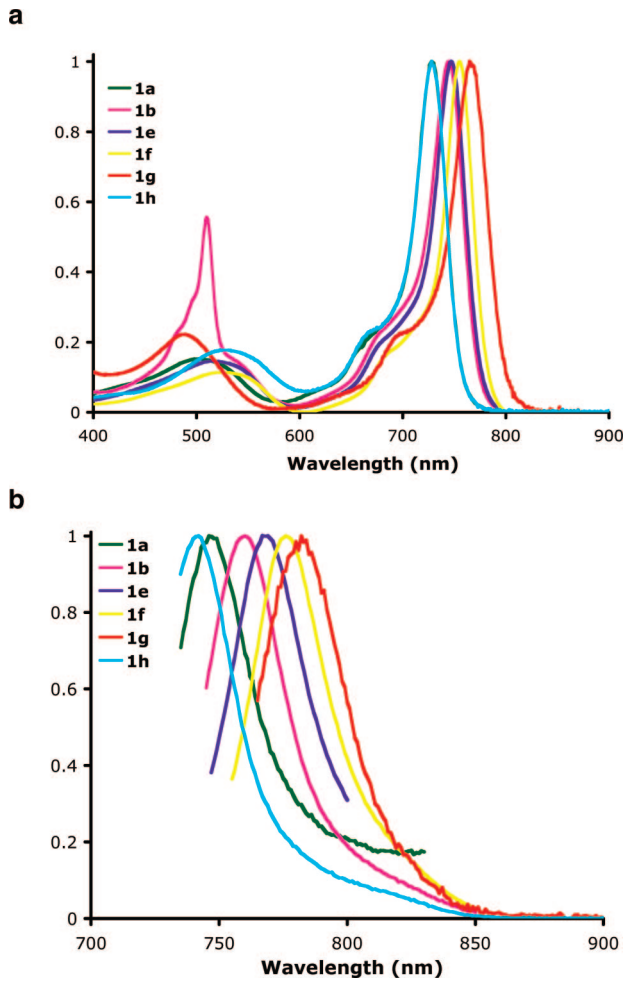


Figure 5. Normalized absorbance (**a**) and (**b**) emission spectra (excited at the respective λ_{max}) of **1a**,**b**,**e**,**f**,**g**,**h** in toluene

In addition the absorbance spectra showed only a small sensitivity to solvent polarity. For example, the $\lambda_{max\ abs}$ of **1g** in DMF is only red-shifted by 5 nm when compared to 761 nm in cyclohexane (Table 2). Inspection of fluorescence maxima for **1g** and **1h** revealed a small blue shift trend in polar solvents (Table 3 and Supporting Information). Large Stokes shift values of 34−56 nm in various solvents were recorded for **2a**; however, this trend did not translate to the conformationally restricted fluorophores **1a**,**g** (9 and 19 nm, Table 3).

Table 2. Effects of the Solvent Polarity on the Absorption.

	$\lambda_{max\ abs}$ (fwhm)[a]/nm				
	$CHCl_3$	THF	dioxane	cyclohexane	DMF
2a	642 (67)	643 (68)	636 (67)	640 (62)	643 (71)
1a	725 (33)	724 (34)	724 (34)	724 (31)	725 (36)
1g	764 (43)	764 (47)	764 (42)	761 (37)	766 (47)

[a] Full width at half-maximum height.

Table 3. Effects of the Solvent Polarity on the Fluorescence

	$\lambda_{max\ abs}$ (Stokes shift)/nm				
	$CHCl_3$	THF	dioxane	cyclohexane	DMF
2a	686 (44)	682 (39)	681 (45)	674 (34)	696 (56)
1a	737 (12)	738 (14)	738 (14)	733 (9)	738 (13)
1g	778 (14)	782 (18)	778 (14)	771 (10)	785 (19)

In conclusion, two efficient synthetic routes to a new class of near-IR fluorophore **1** have been accomplished. The highly favorable emission wavelengths and quantum yields are strongly indicative of future applications in biotechnology. The ease at which key functional groups (halogen and ether) can be included on the core fluorophore indicates that future structural manipulation would be possible to allow adaption for specific use as *in vitro* and noninvasive *in vivo* imaging agents.

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Supporting Information Available: Experimental procedures and characterization data for the new compounds reported. This material is available free of charge via the Internet at http://pubs.acs.org.

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