Published in final edited form as:

Org Biomol Chem. 2015 August 14; 13(30): 8271–8276. doi:10.1039/c5ob01104c.

Oligoethylene Glycol-substituted Aza-BODIPY Dyes As Red Emitting ER-Probes

Anyanee Kamkaew¹, Sopida Thavornpradit^{1,2}, Thamon Puangsamlee^{1,2}, Dongyue Xin¹, Nantanit Wanichacheva², and Kevin Burgess^{1,3}

¹Department of Chemistry, Texas A & M University, Box 30012, College Station, TX 77842, USA

²Department of Chemistry, Faculty of Science, Silpakorn University, Nakorn Pathom, 73000, Thailand ³Department of Pharmacology, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia

Abstract

This study features aza-BODIPY (BF₂-chelated azadipyrrromethene) dyes with two aromatic substituents linked by oligoethylene glycol fragments to increase hydrophilicity of aza-BODIPY for applications in intracellular imaging. To prepare these, two chalcones were attached α,ω onto oligoethylene glycol fragments, then reacted with nitromethane anion. Conjugate addition products from this reaction were then subjected to typical conditions for synthesis of aza-BODIPY dyes (NH₄OAc, *n*-BuOH, 120 °C); formation of boracycles in this reaction was concomitant with creation of macrocycles containing the oligoethylene glycol fragments. Similar dyes with *acyclic* oligoethylene glycol substituents in the same position were used to compare the efficiencies of the intra- and inter-molecular aza-BODIPY forming reactions, and the characteristics of the products. All the fluors with oligoethylene glycol fragments, *ie* cyclic or acyclic, localized in the endoplasmic reticulum of a fibroblast cell line (WEHI-13VAR), the human pancreatic cancer cell line (PANC-1, rough ER predominates) and human liver cancer cell line (HepG2, smooth ER prevalent). These fluors are potentially useful for near IR ($\lambda_{\text{max emis}}$ at 730 nm) ER staining probes.

Corresponding Author: burgess@tamu.edu.

Electronic Supplementary Information (ESI) available: synthesis of oligoethylene glycol-strapped aza-BODIPY, relative yield experiments, fluorescence spectroscopy of fluors and metal complexes, structural optimization by DFT calculations and cartesian coordinates, live cell imaging of fluors.

Notes

The authors declare no competing financial interests.

RESULTS AND DISCUSSION

Syntheses Of The Strapped Fluors 1 and Controls 2

Scheme 1 describes syntheses of the strapped systems **1**. Reaction of the chalcone **3** with the ethylene glycol containing fragments shown afforded the α,ω -functionalized systems **4**. These Michael electrophiles were reacted with the enolate of nitromethane, and the conjugate addition products **5** formed were condensed with ammonium acetate to form the azadipyrromethenes, then complexation of those with boron trifluoride gave the target molecules **1**.

The non-macrocylic control molecules **2** were prepared via a route similar to Scheme 1, but featuring ω -methyl oligoethylene glycol derivatives (Scheme S1 and S2). Isolated yields for compounds **2a** and **2b** were 83 and 74 %, respectively; these are *significantly higher* than those shown in Scheme 1 for **1a** and **1b**. A HPLC study was performed to confirm that this observation was not an artifact of the isolation procedure. Thus purified samples of the intermediate conjugate addition products **5b** (strapped) and **6b** (acyclic control) were mixed in a 1:2 ratio with excess NH_4OAc then heated to 120 °C for 24 h. HPLC was used to compare the relative intensities of the corresponding azadipyrromethenes with purified authentic samples of those (Figure S1 and S2); the acyclic azadipyrromethene was the dominant product indicating that the two strap lengths examined were detrimental to the yield.

Physiochemical Characteristics Of The Strapped Fluors **1** and The Acyclic Controls **2**

Acetonitrile dissolved all the compounds, as did dichloromethane except **1a** which had limited solubility in that solvent. None of the products **1** or **2** were significantly soluble in pure water, water with approximately 0.1 % Triton X, or aqueous sodium chloride at various concentrations.

The fact that **1** and **2** were *not* soluble well in aqueous media made it difficult to determine how they might complex metal salts. When **1** and **2** (at micromolar concentrations) were dissolved in acetonitrile or dichloromethane and treated with any of a variety of metal salts (including NaCl, KCl, LiCl, ZnCl₂, CaCl₂, CuCl₂, MgCl₂, HgCl₂, CoCl₂; at ~33 μM, see supporting) then there was no significant effect on the fluorescence wavelengths of the fluors. Micromolar concentrations of Cu(ClO₄)₂ diminished the fluorescence of compounds **1** but this is unlikely to be due to strong complexation because the fluorescence of the non-macrocyclic controls **2** were similarly decreased.

Even aside from solubility issues, the experiments described above seem to indicate that the strapped aza-BODIPY dyes **1a** and **1b** are *not* good chelating agents. Consequently, some calculations were performed to try to understand this behavior. Thus the molecules were first minimized via molecular mechanics in a continuous dielectric of 80, then further optimized

using density functional theory {B3LYP density functional, and 6-31G(d') basis set}. This routine was first applied to 15-crown-5 then Na^+ •15-crown-5 as a control. Without sodium the crown converged to irregular conformations, but the expected complex *was* formed with the metal (see supporting). Application of this strategy to **1a** and **1b** led to the calculated energy minimized conformations shown in Figure 1. Sodium cation complexation appears to be possible for both compounds. Predictably, there is less space in the cavity of **1a** and, perhaps less obvious, the constraints caused by the aryl groups in **1a** do not allow so much movement in the ethylene glycol-derived strap. Together these phenomena mean that if the sodium were sequestered by **1a** then the metal would be drawn towards the aza-BODIPY nitrogen. For **1b** however, four of the oxygen atoms in the strap can orientate to complex the Na^+ atom, but the fifth oxygen is unsuitably aligned.

All compounds in the series **1** and **2** have very similar absorption and fluorescence maxima. Moreover, the quantum yields of all the compounds were similar (in the 0.26 – 0.29 range), hence *restriction of the 1,7-aryl substituents with an ethylene glycol linker did not increase the quantum yields of 1 over 2*. We were somewhat surprised by this observation, so checked it by making solutions of all the compounds with almost identical absorbance at the λ_{max} wavelength (~688 nm) and measuring their fluorescence intensities (Figure 2a). In the event, these solutions had almost the same fluorescence intensities, consistent with their having almost identical quantum yields as asserted above.

Figure 2b shows UV absorbance and fluorescence spectra for *equimolar* solutions of the four compounds **1** and **2**, where fluor **1b** is significantly more brilliant than the others. The quantum yields of all the compounds are almost the same (see above) so the relative brilliance of **1b** can be attributed to its higher extinction coefficient (Table 1).

Behaviour Of Fluors **1** and **2** As Near-IR ER-stains

Localization of fluors **1** and **2** in WEHI-13VAR fibroblast cells was investigated first. Incubation with 10 μM of each sample for 3 h, for all the fluors, showed the fluorescence localized in the ER, and co-localized with a blue ER-tracker dye (Figure 3 for **1b** and S10 for all the other probes). That blue standard probe is sold by Life Technologies who also market a BODIPY based ER-tracker that emits around 615 nm (<https://www.lifetechnologies.com/order/catalog/product/E34250>).¹⁰ All four aza-BODIPY dyes in this study localize in the same organelle as that ER-tracker, and it is potentially useful that they emit at a complementary $\lambda_{\text{max emis}}$ (ca 730 nm) that could be observed simultaneously.

Incubation of probes **1** and **2** with two other cell lines was investigated to explore the generality of their affinity for the ER. PANC-1 human pancreatic cancer cells are known to have a prevalent rough ER,¹¹ whereas smooth ER predominates for human liver cancer HepG2 cells.^{12,13} Figure 4 shows both these cell lines were stained just as the fibroblast cells by illustrative probe 1b (the other three probes gave similar images, Figure S11), except there was one difference. The smooth ER of the HepG2 liver cells appeared as just punctates, just as others have observed for the smooth ER.¹²

All the probes **1** and **2** were tested in all three cell types (the fibroblasts, pancreatic cancer, and liver cancer cells) for colocalization with LysoTracker Green, and with MitoTracker

Green, but these did not colocalize. Moreover, there was no significant accumulation of the probes in the cell membranes.

CONCLUSION

Oligoethylene glycol substituents on the aza-BODIPY probes **1** and **2** probably increase their hydrophobicity enough to overcome non-selective accumulation in all hydrophobic membranes, and promote accumulation in the rough and smooth ER. This observation is different to those made by X. Chen *et al.*⁴ for (positively charged) ammonium salts of aza-BODIPY dyes that stained cell membranes, the cytoplasm, and the ER. It is also different to the observations of Perez-Inestrosa's triethylene glycol substituted aza-BODIPY dyes that are *neutral* like the ones reported here, but that localize in the cytoplasm of HeLa cells.⁹ Overall, these comparisons between different aza-BODIPY dyes reflect their potential to be directed to different organelles via relatively small changes to the periphery of the dye core. Probes **1** and **2** are complementary to ER stains that fluoresce in the blue and green region,^{11–14} and they should facilitate clear observations of blue- and green-labeled molecules in the ER without significant cross-talk.

In this work the oligoethylene glycol substituents used contained four or less repeats. This was not enough to render the fluors significantly water-soluble, and the size and shape of the *crown-like* cavities was not conducive to complexation of metals, especially relative to conventional crown ethers (*cf* stability constant of Na⁺•15-crown-5 is *ca.* 6.2).¹⁵ However, since the organelle staining properties of fluorophores tend to be governed by lipophilicity and charge characteristics, we predict variations on organelle-staining properties could be induced by varying the length of the ethylene glycol substituents, and conceivably by their acyclic/cyclic characteristics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Financial support for this project was provided by the National Institutes of Health (GM087981), and the Robert A. Welch Foundation (A-1121) and the High Impact Research (HIR (UM.C/625/1/HIR/MOHE/MED/17 & UM.C/625/1/HIR/MOHE/MED/33) from the Ministry of Higher Education, Malaysia and partial support from Grant RSA 5680034 from the Thailand Research Fund (TRF) and Faculty of Science, Silpakorn University, Thailand. TAMU/LBMS-Applications Laboratory provided mass spectrometric support. The NMR instrumentation at Texas A&M University was supported by a grant from the National Science Foundation (DBI-9970232) and the Texas A&M University System. The Olympus FV1000 confocal microscope acquisition was supported by the Office of the Vice President for Research at Texas A&M University.

References

1. Ziessel R, Ulrich G, Harriman A. *New J Chem.* 2007; 31:496–501.
2. Loudet, A.; Burgess, K. *Handbook of Porphyrin Science: With Applications to Chemistry, Physics, Materials Science, Engineering, Biology and Medicine.* Kadish, K.; Smith, K.; Guillard, R., editors. World Scientific; 2010. p. 203
3. Csende F, Miklos F, Stajer G. *Curr Org Chem.* 2012; 16:1005–1050.

4. Zhang XX, Wang Z, Yue X, Ma Y, Kiesewetter DO, Chen X. *Mol Pharmaceutics*. 2013; 10:1910–1917.
5. Murtagh J, Frimannsson DO, O'Shea DF. *Org Lett*. 2009; 11:5386–5389. [PubMed: 19883098]
6. Tasior M, Murtagh J, Frimannsson DO, McDonnell SO, O'Shea DF. *Org Biomol Chem*. 2010; 8:522–525. [PubMed: 20090964]
7. Hall MJ, Allen LT, O'Shea DF. *Org Biomol Chem*. 2006; 4:776–780. [PubMed: 16493459]
8. Hall MJ, McDonnell SO, Killoran J, O'Shea DF. *J Org Chem*. 2005; 70:5571–5578. [PubMed: 15989339]
9. Collado D, Vida Y, Najera F, Perez-Inestrosa E. *RSC Adv*. 2014; 4:2306–2309.
10. Cole L, Davies D, Hyde GJ, Ashford AE. *J Microsc*. 2000; 197:239–248. [PubMed: 10692127]
11. Chen XQ, Sans MD, Strahler JR, Karnovsky A, Ernst SA, Michailidis G, Andrews PC, Williams JA. *J Proteome Res*. 2010; 9:885–896. [PubMed: 19954227]
12. Zuber C, Cormier JH, Guhl B, Santimaria R, Hebert DN, Roth J. *Proc Natl Acad Sci*. 2007; 104:4407–4412. [PubMed: 17360537]
13. Galteau MM, Antoine B, Reggio H, Embo J. 1985; 4:2793–2800. [PubMed: 4065094]
14. Arai S, Lee SC, Zhai D, Suzuki M, Chang YT. *Sci Rep*. 2014; 4:6701. [PubMed: 25330751]
15. Dishong DM, Gokel GW. *Journal of Organic Chemistry*. 1982; 47:147–148.

Figure 1. Optimized minimum energy conformations of: **a**, **1a** and $\text{Na}^+\bullet\text{1a}$; and, **b**, **1b** and $\text{Na}^+\bullet\text{1b}$. DFT B3LYP/6-31G(d') throughout.

Figure 2.

a Quantum yields of all four compounds **1** and **2** are very similar; however, **b** the strapped system **1b** is more brilliant because it has a higher molar extinction coefficient. CH_2Cl_2 solutions throughout.

Figure 3. Images of WEHI-13VAR cells. **a** Under a bright-field (DIC image); **b** with ER tracker; **c** with fluor 1b; and, **d** overlay of images **a** – **c**.

Figure 4. Parallel images for staining PANC-1 (left) and HepG2 cells (right) with: **a, b** ER tracker; **c, d** with fluor 1b. **e, f** Shows the overlay of images for each cell type.

Scheme 1.
Syntheses of the strapped fluors **1**.

Table 1

Electronic spectra of compounds **1** and **2** in dichloromethane (9.2×10^{-6} M): salient characteristics.

Compound	$\lambda_{\text{max abs}}$ (nm)	ϵ_{max} ($10^4 \text{ M}^{-1}\text{cm}^{-1}$)	$\lambda_{\text{max emis}}$ (nm) ¹	Φ_{fluor}^2
1a	688	2.55	725	0.26 ± 0.003
1b	686	8.22	725	0.29 ± 0.001
2a	690	2.51	725	0.29 ± 0.004
2b	688	5.75	725	0.28 ± 0.004

¹Excited at 650 nm.

²Zn-phthalocyanine is a standard ($\Phi_{\text{fluor}} = 0.30$).