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# Synthesis and Properties of $BF_2$-3,3′-Dimethyldiarylazadipyrromethene Near-Infrared Fluorophores

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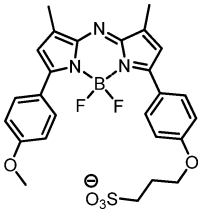
## ABSTRACT



**The first synthesis of both organic and aqueous soluble $BF_2$ chelated 3,3′-dimethyl-5,5′-diarylazadipyrromethenes has been achieved. The fluorophores are emissive in organic and aqueous solvents with high quantum yields in the key biological near-infrared (NIR) spectral region of 675−700 nm. Following efficient cellular uptake from aqueous media the fluorophore can be readily visualized with confocal microscopy.**

The ability to visualize *in vitro* and *in vivo* using molecular near-infrared (NIR) fluorescence is a rapidly evolving research field. Opportunities exist for the development of new fluorescent platforms with strong absorption and emissions in the low energy spectral regions (650−800 nm) that most readily pass through biological tissues. Potential biomedical uses for such techniques range from clinical diagnosis of disease states to real time imaging for intraoperative guided surgery.[1] Our recent interest in this field stems from our development of boron chelates of tetraarylazadipyrromethenes **3** (Scheme 1).[2] These fluorophores exhibit NIR absorption and emissions and have high fluorescence quantum yields (0.3−0.4) and excellent photostability.[3] Their synthesis derives from tetraarylazadipyrromethenes **2** which were first reported in 1943 as unexpected, deep blue, colored products obtained from treatment of diaryl-$\gamma$-nitro ketones **1** with ammonium formate (Scheme 1).[4] In more recent times we have developed new and optimized routes to these compounds and specifically explored their boron chelated derivatives as NIR fluorophores **3** (Scheme 1).[5] Their advantageous photophysical properties have encouraged investigations for potential applications such as fluorochromes,[6] sensors/energy transfer cassettes,[7] and donor/acceptor conjugates for solar cells.[8] Despite their growing importance, the 3,3′,5,5′-tetraaryl substituted derivatives

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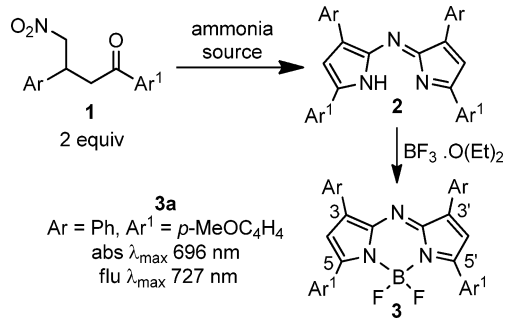
10.1021/ol401434c 

**Scheme 1.** Synthesis of 3,3′,5,5′-Tetraarylazadipyrromethenes




have been the only substitution pattern explored to date. For some specific uses it would be beneficial to reduce the lipophilicity of this fluorophore class, though this would need to be achieved without loss of their valuable photophysical properties. One approach to achieving this would be removal of the aryl rings from the 3 and 3′ positions of **3** and would, in effect, reduce the molecular weight of the fluorophore by approximately one-third (Scheme 1). An examination of the literature reveals that the synthesis of 5,5′-diaryl substrates **4** has been independently reported three times via two different routes (Scheme 2).[9a−c] It has also been reported that attempts to produce **4** by a similar reaction used to produce **2** from the reaction of 4-nitro-1-arylbutanone **5** with ammonium acetate failed to generate **4** (Scheme 2).[4b,9a]

The first claimed synthesis was by Knott in 1947 in which the reaction of 3-benzoylpropionitrile **7** (Ar = Ph) with hydroxylamine hydrochloride gave a dark blue colored chromophore which was assigned the structure **4** (Scheme 2).[9a] Due to the era of this report, the structural assignment was only based upon compound color and in analogy with the earlier publication[4] describing the synthesis of tetraaryl derivatives **2**. It was also shown that other ammonium sources such as ammonium acetate failed to give the product assigned as **4**. This synthesis was later reproduced by Boyer et al. in 1993 though no characterization data was reported for the product other than an absorbance $\lambda_{max}$ of 595 nm and, tellingly, attempts to convert this material into a $BF_2$ chelate using $BF_3 \cdot OEt_2$ and TEA failed, giving only "intractable mixtures".[9b] In a 1992 report Bird et al. described access to **4** (Ar = Ph) by the reaction of succinonitrile with phenylmagnesium bromide followed by heating of the resulting crude extract with ammonium chloride.[9c] Unfortunately again, the only analytical data provided to confirm the assigned structure as **4** was an absorption maximum of 595 nm. Our insight gained from a mechanistic investigation into the synthesis of the tetraaryl derivatives **2**[5b] and the lack of characterization data raised doubts about these results, so repeat syntheses of **4** from **6** and **7** were attempted.

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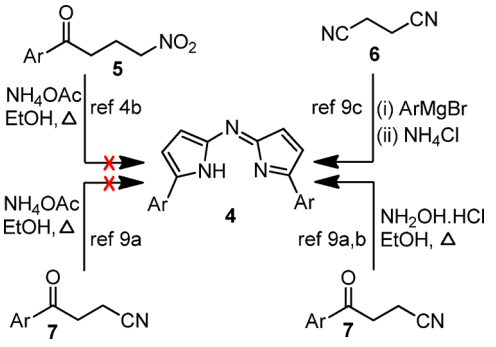
**Scheme 2.** Previous Attempted Syntheses of 5,5′-Diarylazadipyrromethene **4**




In our hands we were unable to obtain sufficient quantities of the dark blue chromophore from the route utilizing Grignard addition to succinonitrile,[9c] so we turned our attention to the reaction of 3-benzoylpropionitrile **7** (Ar = Ph) with hydroxylamine hydrochloride.[9a] Following the heating of **7** with $NH_2OH \cdot HCl$ in EtOH for 2 h, a dark blue colored precipitate was obtained. The precipitate was isolated and washed to remove excess hydroxylamine. The resultant solid was bench stable but decomposed in a matter of hours as a solution in THF, MeCN, acetone, or MeOH. The absorption spectrum taken in $CHCl_3$ corresponded with the previously reported $\lambda_{max}$ value of 595 nm (Figure S1 (Supporting Information, SI)).[9b,c] But the $^1H$ NMR did not contain the expected signals for four $\beta$-pyrrole protons and the $^{13}C$ NMR showed 16 signals where 8 would be predicted for structure **4** (SI). Additionally, ES-MS analysis gave an $[M+H]^+$ value of 314.1304 corresponding to a molecular formula of $C_{20}H_{15}N_3O$ indicating that the synthesis of **4** remains unsubstantiated (SI).

Due to the issues concerning **4** the 3,3′-dimethyl-5,5′-diaryl substituted derivative **8** was chosen as a more suitable alternative target structure that would still achieve our goal of providing access to derivatives of this fluorophore class with lower lipophilicity (Figure 1).

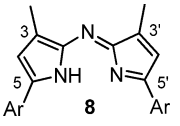
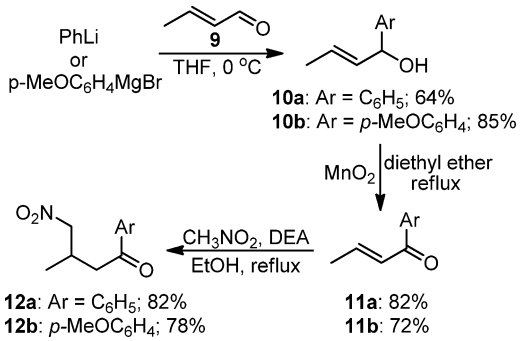


**Figure 1.** 3,3′-Dimethyl-5,5′-diarylazadipyrromethene.

The route selected was via the 3-methyl-4-nitro-1-arylbutan-1-ones **12**, which could be accessed in three steps from crotonaldehyde **9** (Scheme 3). 1,2-Addition to crotonaldehyde with phenyllithium and *p*-methoxyphenylmagnesium bromide gave the allylic alcohols **10a**,**b**, and subsequent oxidation with $MnO_2$ generated the α,β-unsaturated ketones **11a**,**b**.

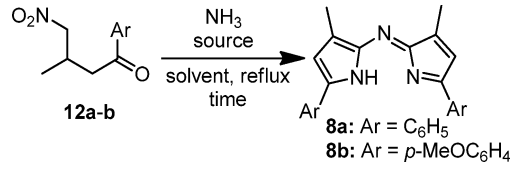
Conjugate addition of nitromethane to **11a**,**b** in EtOH with diethylamine (DEA) gave the nitro-ketones **12a**,**b** in

**Scheme 3.** Synthesis of 3-Methyl-4-nitro-1-arylbutan-1-ones




good yields (Scheme 3). Substrate **12b** was selected to optimize reaction conditions for its transformation into azadipyrromethene **8b** (Table 1). Screening of ammonia sources showed that heating **8b** in EtOH with $NH_2OH \cdot HCl$, $NH_4Cl$, $(NH_4)_2SO_4$, and $(NH_4)_2CO_3$ all failed to give any product formation (entries 1–4). In contrast, ammonium formate and ammonium acetate both gave the desired product **8b** in 15 and 17% yield respectively after 10 h of reflux (entries 5, 6). A further examination of reaction times and solvents (EtOH, MeOH, *i*PrOH, BuOH) identified reflux in MeOH for 10 h as optimal conditions providing a 28% yield of **8b** (entries 8–11). Applying these conditions to **12a** gave the corresponding product **8a** in a 19% yield (entries 12, 13). NMR and HRMS analyses for **8a**,**b** were consistent with these structures, and revealingly, the absorption spectrum of **8a** ($\lambda_{max}$ 561 nm) differed significantly from the compound previously claimed to be structure **4** (Figure S1 (SI)).

**Table 1.** Investigation of 3,3′-Dimethyldiarylazadipyrromethene Formation

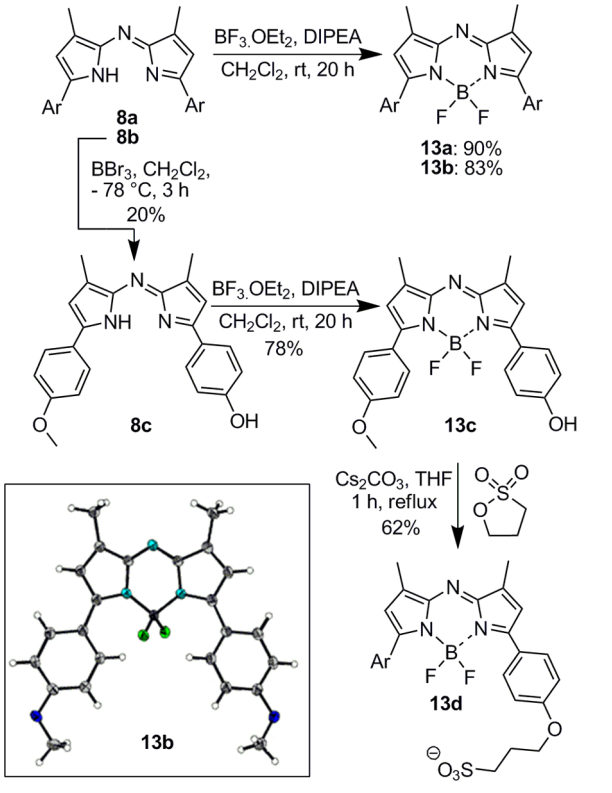



| entry | sub-strate | $NH_3$ source | solvent | time (h) | 8 | yield (%) |
|---|---|---|---|---|---|---|
| 1 | **12b** | $NH_2OH$ | EtOH | 20 | **8b** | 0 |
| 2 | **12b** | $NH_4Cl$ | EtOH | 20 | **8b** | 0 |
| 3 | **12b** | $(NH_4)_2SO_4$ | EtOH | 20 | **8b** | 0 |
| 4 | **12b** | $(NH_4)_2CO_3$ | EtOH | 20 | **8b** | 0 |
| 5 | **12b** | $HCOONH_4$ | EtOH | 10 | **8b** | 15 |
| 6 | **12b** | $NH_4OAc$ | EtOH | 10 | **8b** | 17 |
| 7 | **12b** | $NH_4OAc$ | EtOH | 20 | **8b** | 10 |
| 8 | **12b** | $NH_4OAc$ | EtOH | 6 | **8b** | 26 |
| 9 | **12b** | $NH_4OAc$ | MeOH | 10 | **8b** | 28 |
| 10 | **12b** | $NH_4OAc$ | *i*PrOH | 4 | **8b** | 21 |
| 11 | **12b** | $NH_4OAc$ | BuOH | 1 | **8b** | 22 |
| 12 | **12a** | $NH_4OAc$ | EtOH | 6 | **8a** | 19 |
| 13 | **12a** | $NH_4OAc$ | MeOH | 10 | **8a** | 19 |

Conversion of **8a**,**b** to their corresponding $BF_2$ chelates **13a**,**b** was achieved in excellent yields by their treatment with $BF_3$ etherate and *N*,*N*-diiopropylethylamine (DIPEA) in $CH_2Cl_2$ (Scheme 4). This boron chelation restricts rotation about the central bridging C–N–C bonds which limits excited state quenching and provided the first diaryl substituted azadipyrromethene fluorophores. To expand the aryl substituent pattern the bismethoxy substituted **8b** was monodemethylated with $BBr_3$ giving **8c** which upon chelation provided the monophenol-monomethoxyphenyl substituted derivative **13c** (Scheme 4).

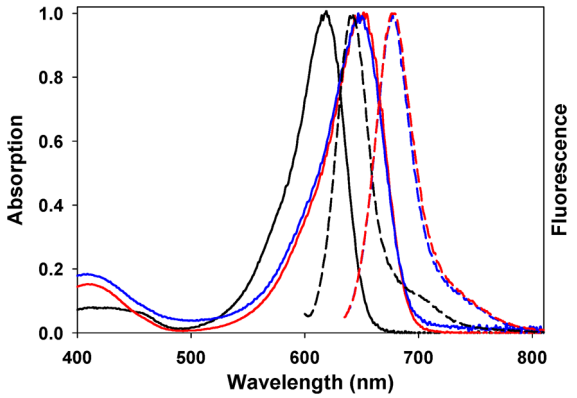
The structure of **13b** was confirmed with X-ray analysis of a single crystal grown by slow evaporation of a dichloromethane solution (Scheme 4, inset). Analysis of the crystal structure of **13b** confirmed the overall conjugated nature of the fluorophore with a planar 5–6–5 fused ring system. The methoxy-substituted aryl rings had torsion angles of 23.9(3)° and 34.2(3)° with the pyrrole rings, indicating a strong electronic interaction with the central fluorophore unit which would impact upon absorption and emission wavelengths.

**Scheme 4.** Synthesis of NIR Fluorophores **13a**–**d** (Inset: X-ray crystal structure of **13b** with thermal ellipsoids drawn at 50% probability level)




Using $CHCl_3$ as a representative solvent the absorption spectra of **13a**–**c** showed sharp bands (fwhm between 35 and 43 nm) between 618 and 651 nm (Table 2). The aryl *p*-OMe substituents of **13b** had a pronounced bathochromic shift of over 30 nm when compared to the unsubstituted phenyl derivative **13a** (entries 1, 2). Emission maxima

**Table 2.** UV−vis and Fluorescence Properties of **13a**−**c**[a]




| entry | 13 | $\lambda_{max}$ abs (nm) | fwhm (nm)[b] | ext. coeff. ($M^{-1}$ $cm^{-1}$) | $\lambda_{max}$ flu (nm) | $\Phi_{flu}$[c] |
|---|---|---|---|---|---|---|
| 1 | **a** | 618 | 35 | 64 000 | 642 | 0.41 |
| 2 | **b** | 648 | 39 | 81 000 | 678 | 0.44 |
| 3 | **c** | 651 | 39 | 81 000 | 679 | 0.45 |

[a] **13a** (black), **13b** (blue), **13c** (red). In $CHCl_3$. [b] Full width at half-maximum height. [c] Compound **3a** (Ar = Ph, Ar′ = *p*-$MeOC_6H_4$; $\Phi$ = 0.36) used as a standard.[3]

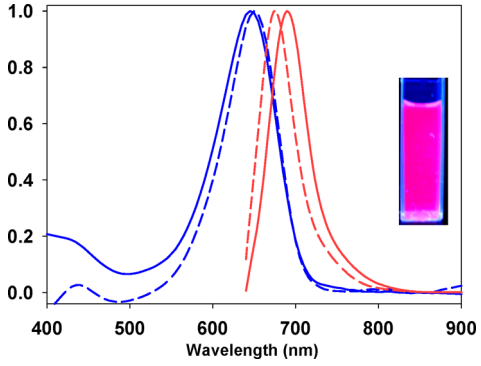



**Figure 2.** Absorbance (blue) and fluorescence (red) spectra of **13d** in PBS at pH 7.2 (solid lines) ($\lambda_{max}$ abs 651 nm, em 691 nm) and DMEM (dashed lines) ($\lambda_{max}$ abs 654 nm, em 676 nm).

for **13b** and **c** were at 678 and 679 nm with high quantum yields of 0.44 and 0.45 respectively (Table 2, entries 2, 3). Examination of abs and flu properties in various organic solvents showed little solvent effects (Table S1, (SI)). To complete the first phase of our work on this new substitution pattern **13c** was converted into the water solubilized **13d** by its reaction with 1,3-propanesultone (Scheme 4).

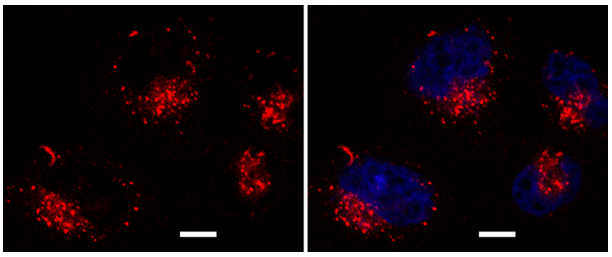


**Figure 3.** Left: Confocal imaging of **13d** in HeLa cells after 1 h of incubation. Right: Overlay with cell nuclei stained blue fluorescent with DAPI. Scale bar = 10 $\mu$m.

Absorption and emission spectra of **13d** taken in phosphate buffered saline (PBS) were encouraging with minor broadening observed in the UV−visible spectrum ($\lambda_{max}$ 651 nm) and an emission maximum at 691 nm (Figure 2). Incubation of **13d** with HeLa cells in Dulbecco's modified eagle's medium (DMEM) for 60 min and confocal imaging showed efficient internalization of the fluorophore (Figure 3). Confocal Z-stack imaging of individual cellular focal planes showed localization of **13d** restricted to the cytosol (Figure S2 (SI)).

In conclusion, we have described the first synthesis of $BF_2$ chelated 3,3′-dimethyl-5,5′-diarylazadipyrromethenes which exhibit excellent NIR photophysical properties in organic and aqueous solutions. These results are encouraging indications that they could be adapted for *in vitro* and *in vivo* NIR-fluorescence imaging. Development of routes to further substitution patterns is ongoing. Literature confusion with respect to claimed syntheses of 5,5′-diarylazadipyrromethenes has been clarified.

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**Supporting Information Available.** All experimental procedures, NMR spectra, X-ray data, and confocal Z-stack images. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare the following competing financial interest(s): A patent application has been filed by DOS on azadipyrromethene based NIR fluorophores (PCT/EP2010/065991).