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Electrochemiluminescence properties of a carboxy functionalised BODIPY

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ABSTRACT

A novel electrochemiluminescent reagent, 2,6-Diethyl-1,3,5,7-tetramethyl-8-[(2-fluorophenyl)-6-methoxy-1,5-naphthyridine-3-carboxy]-4,4′-difluoroboradiazaindacene, BODIPY-COOH, capable of direct conjugation to a wide range of biomolecules has been developed. BODIPY-COOH shows a high fluorescence quantum yield, $\phi = 0.67 \pm 0.03$, which significantly exceeds the range of 0.33 to 0.41 previously reported for structurally similar dyes. In the presence of persulfate, the dye generates significant electrochemiluminescence, ECL. Significantly, at high concentrations, quenching of the ECL is observed due to dimerisation.

1. Introduction

Electrochemiluminescence, ECL, is a process where electrochemically generated intermediates undergo a highly exergonic reaction to produce an electronically excited state that then goes on to emit light [1,2]. There is an ongoing need for high efficiency ECL reagents that can be coupled to biomolecules that emit at different wavelengths so as to enable high sensitivity, multiplexed bioassays. BODIPY, 4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene, dyes exhibit a very high quantum yield in optically driven emission and find wide application as laser dyes [3] and as fluorescent labels for proteins and other biological materials in biochemistry and molecular biology, surgical treatments and photodynamic therapies [4]. Significantly, the spectroscopic properties of these dyes depend strongly on the location and nature of the substituents, *i.e.* small structural changes can result in major differences in their fluorescence emission wavelengths and their optical properties can be sensitive to environmental factors like pH and polarity making them useful for binding assays [5,6]. However, typical BODIPYs emit in the green-yellow region of the spectrum which often overlaps the autofluorescence of biomaterials. Moreover, their Stokes shift is typically small making it challenging to resolve the fluorescence from the excitation wavelength and trivial quenching can occur even at micromolar concentrations. Therefore, there is significant interest in extending the conjugation or introducing donor/acceptor groups so as to tune the emission wavelength and increase the Stokes Shift. For example, Bröring and co-workers, have been successful in developing BODIPY compounds that exhibit an 80 nm Stokes shift [7]. However, the impact of the Stokes shift and self-absorbance on the electrochemiluminescence and optically driven emission intensities ought to be distinctly different. For example, ECL is generated from the oxidised or reduced form of the ECL reagent often in the presence of a co-reactant. Therefore, unlike an optically driven experiment, the emitting species is created within the diffusion layer close to the electrode surface and is surrounded by oxidised/reduced forms of the luminophore whose absorption spectrum is very different from the ground state of the emitting species. Surprisingly, this issue has not been addressed previously.

In this contribution, we report on the electrochemical, photophysical and electrochemiluminescent properties of 2,6-Diethyl-1,3,5,7-tetramethyl-8-[(2-fluorophenyl)-6-methoxy-1,5-naphthyridine-3-carboxy]-4,4′-difluoroboradiazaindacene, BODIPY-COOH. This BODIPY-COOH dye is especially attractive for biological assays as it contains a carboxyl terminal which can be easily conjugated to biomolecules. Moreover, we report on the effect of substitution of the quantum yield, ϕ, since ϕ for substituted BODIPYs is typically between 0.33 and 0.41 compared to unsubstituted derivatives which have quantum yields of the order of 0.85 [8].

2. Experimental

2.1. Materials and methods

Fluorescent lifetimes were measured on a Fluotime 100 machine with a 450 nm laser excitation. ECL and cyclic voltammetry, CV, experiments were performed as previously reported [2]. All potentials are quoted versus a silver wire quasi reference electrode and all measurements were performed at 22 ± 2 °C. All compounds were characterised using ^{1}H and ^{13}C NMR.

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2.2. Synthesis

*2.2.1. 1,3,5,7-tetramethyl-8-[(2-fluorophenyl)-6-methoxy-1,5-naphthyridine-3-ethyl carboxylate]-4,4′-difluoroboradiazaindacene (**2**)*

Ethyl 4-(2-fluoro-4-formylphenyl)-6-methoxy-1,5-naphthyridine-3-carboxylate (**1**) (0.5 mmol), 3-ethyl-2,4-dimethyl-1*H*-pyrrole (1.1 equiv) and TFA (cat) were dissolved in N_2-purged DCM (35 mL) and stirred for 4 h under N_2 atmosphere until the aldehyde was fully consumed (TLC). Tetrachlorobenzoquinone (1 equiv) in N_2-purged DCM (10 mL) was then added and stirred for 30 min. Et_3N (1.31 mL) and $BF_3 \cdot OEt_2$ (1.31 mL) were then added and the reaction stirred at room temperature overnight. The crude mixture was then washed with water (2×50 mL), dried over $MgSO_4$ and evaporated to dryness. The residue was purified on silica gel (20% EtOAc:Hex). (163 mg, 58%) as an bright red powder, R_f 0.2 (hexane/EtOAc 8:2). Found: C: 64.7%; H: 5.9%; N: 9.5%. $C_{31}H_{28}BF_3N_4O_3$ requires C: 65.0%; H: 4.9%; N: 9.8%); λ_{max} (ε [$M^{-1}\,cm^{-1}$]) = 501 nm (79,000 ± 1800); λ_{em} = 520 nm (λ_{ex} = 499 nm), Φ_f = 0.61 (±0.04), τ_f = 4.1 ns.

*2.2.2. 2,6-Diethyl-1,3,5,7-tetramethyl-8-[(2-fluorophenyl)-6-methoxy-1,5-naphthyridine-3-carboxy]-4,4′-difluoroboradiazaindacene (**3**)*

1,3,5,7-tetramethyl-8-[(2-fluorophenyl)-6-methoxy-1,5-naphthyridine-3-ethyl carboxylate]-4,4′-difluoroboradiazaindacene (**2**) (475 mg, 0.74 mmol) was added to DCM (18 mL) with stirring. Crushed NaOH pellets (126.5 mg, 2.96 mmol) were dissolved in MeOH (2 mL) and added to the reaction mixture which was stirred overnight at room temperature. The solvents were then removed under *vacuo* to yield a dark solid which was then dissolved in H_2O (15 mL). Acidification using 10% HCl resulted in precipitation of the crude product. This was purified on silica gel (100% EtOAc) which yielded **3** (91 mg, 20%) as a bright red solid, R_f 0.1 (EtOAc). Found: C: 65.8%; H: 5.3%; N: 9.6%. $C_{33}H_{32}BF_3N_4O_3$ requires C: 66.0%; H: 5.4%; N: 9.3%); λ_{max} (ε [$M^{-1}\,cm^{-1}$]) = 526 nm (65,000 ± 1500); λ_{em} = 548 nm (λ_{ex} = 524 nm), Φ_f = 0.67 (±0.03), τ_f = 6.0 ns.

3. Results and discussion

3.1. Synthesis of BODIPY-COOH

Here, a standard Knoevenagel reaction of formyl naphthyridine **1** with 3-ethyl-2,4-dimethylpyrrole was employed and the crude product purified on silica gel by slow elution of the fluorescent spot using 20% EtOAc:hexane to yield **2**. The ester group was then hydrolysed by treating a DCM/MeOH (9:1) solution of **2** with NaOH (4 equiv) at ca. 20 °C for 12 h [9]. This was followed by the removal of the solvent *in vacuo*, dissolution in H_2O, acidification and filtration to yield the crude product. Purification on silica gel yielded the bright red powder (27%).

3.2. Electrochemistry

The inset of Fig. 1 shows the BODIPY-COOH structure whilst the main figure shows the cyclic voltammogram of a 0.5 mM solution of the dye dissolved in tetrabutylammonium-tetrafluoroborate, TBATBF in MeCN at a 1.5 mm radius platinum electrode where the scan rate is 0.06 V s^{-1}. This figure reveals two closely spaced oxidation processes at peak potentials, E_P, of +1.38 V and +1.61 V. The first oxidation is shifted by +0.17 V relative to the unsubstituted parent indicating that the electron density is lower in BODIPY-COOH [10]. Aromatic substitution of the parent fluorophore in the 8 position is known to induce additional oxidation processes [11,12].

A chemically irreversible reduction peak is observed at −0.28 V which shifts in a negative potential direction as the scan rate is increased. The behaviour contrasts with the parent fluorophore that exhibits a reversible reduction at −1.4 V *vs.* SCE in MeCN [8]. The less negative reduction potential observed for BODIPY-COOH again indicates that the electron density is lower relative to the parent [10]. The peak currents vary linearly with square root of scan rate and a diffusion coefficient of $1.2 \pm 0.01 \times 10^{-6}\,cm^2\,s^{-1}$ was obtained which is consistent with other reports [13].

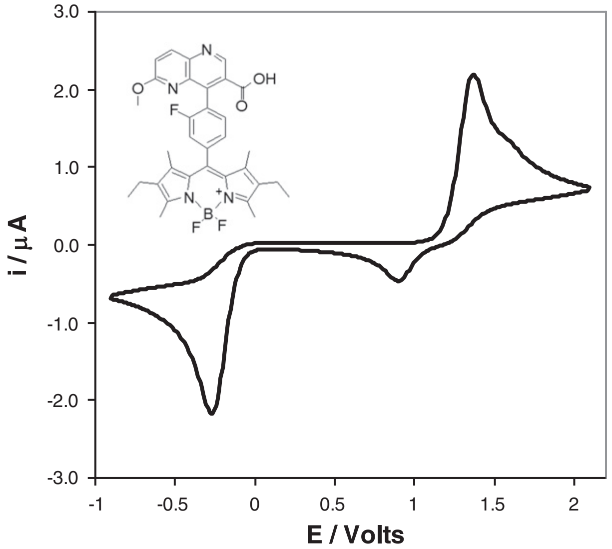

Fig. 1. Background corrected cyclic voltammogram of 0.5 mM BODIPY-COOH dissolved in acetonitrile with 0.1 M TBATBF as supporting electrolyte. The working electrode is a 3 mm platinum working electrode and the scan rate is 0.06 V s^{-1}. The inset shows the BODIPY-COOH structure.

3.3. Electrochemiluminescence using $[S_2O_8]^{2-}$ as Co-reactant

One important approach to generating electrochemiluminescence is the annihilation reaction between the oxidised and reduced forms of the ECL reagent. Significantly, no ECL is observed when the potential at the working electrode was stepped between +1.8 V and −0.4 V where oxidised and reduced forms were produced within the diffusion layer. Fluorescence spectroscopy at 77 K reveals that the wavelength of maximum emission, λ_{max} is 533 nm indicating that 2.32 eV is required to directly populate the single excited state. Using the first oxidation and first reduction wave from the cyclic voltammogram, the total free energy available from annihilation is approximately 1.5 eV. Thus, this system is energy insufficient to create the singlet state directly using the annihilation approach. The triplet energy of structurally related BODIPY dyes is approximately 1.6 eV [14]. Thus, creation of the triplet state is also energy deficient [10] and electrochemiluminescence is not observed by the annihilation approach.

Under these circumstances, one might reasonably expect electrochemiluminescence in the presence of a co-reactant such as persulfate. For example, as shown in Eq. (1), similar to the $[Ru(bpy)_3]^{2+}$–$[S_2O_8]^{2-}$ system [15], the strong oxidant, $SO_4^{-\bullet}$, could lead to the creation of an electronically excited state leading to ECL.

$$\begin{aligned} &S_2O_8{}^{2-} + e \rightarrow SO_4{}^{2-} + SO_4{}^{-\cdot} \\ &\text{BODIPY} + e \rightarrow \text{BODIPY}^{-\cdot} \\ &\text{BODIPY}^{-\cdot} + SO_4{}^{-\cdot} \rightarrow \text{BODIPY}^{*} + SO_4{}^{2-} \\ &\text{BODIPY}^{*} \rightarrow \text{BODIPY} + h\upsilon \end{aligned} \qquad (1)$$

In sharp contrast to the behaviour observed under annihilation conditions, Fig. 2 shows that an intense electrochemiluminescence is observed at negative potentials in the presence of 1 mM $[S_2O_8]^{2-}$. To avoid any issues, e.g., oxidative de-carboxylation of the BODIPY-COOH, the initial potential was 0.000 V and the potential was scanned