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Novel sulphur-rich BODIPY systems that enable stepwise fluorescent O-atom turn-on and H_2O_2 neuronal system probing†‡

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Bis-arylsulfide BODIPY systems were prepared and studied for multiple O-atom sensing (at 522 nm); 2- and 3-atom loading was optimal (50-fold, "turn on"). Neuronal studies showed greater H_2O_2 sensitivity than 2′,7′-dichlorofluorescein diacetate. The novel 1,3,6-trimethyl BODIPY formed as a biproduct under Lindsey conditions.

Reactive oxygen species (ROS) by their name and nature are extremely unstable. High levels can cause biological damage implicated in cancer, neurodegeneration, diabetes, *etc*. Molecular level effects such as extensive lipid peroxidation, protein oxidation, and mitochondrial DNA mutation arise from excess biological hydrogen peroxide (H_2O_2), superoxide (O_2^-), hydroxyl ($^{\bullet}OH$), singlet oxygen, and lipid hydroperoxide concentrations. Selective and sensitive detection and quantification of such species by luminescent molecules may allow for a better understanding of the intricacies of biological processes. The rational synthesis of well-defined systems paves the way for novel synthetic probes with improved and varying functions.[1–5] Oxidative transformations in synthetic systems have involved boronate, quinone, olefin, reduced moieties, and lanthanide-based systems, and the photomechanisms of PET, FRET, phosphorescence *etc*.[1]

BODIPY (4,4-difluoro-4-bora-3a, 4a-diaza-*s*-indacene) species are resilient, easily derivatizable and have been prepared for a host of optical applications.[2] They are commonly synthesized by the straight-forward Lindsey method.[4] Sulphide- or thienyl-functionalized BODIPY platforms for fluorescent ROS detection are few.[5] Direct *chemical* sulphide oxidation accesses valence states 4 and 6. The oxidation of Met in proteins might access, *e.g*., neurodegenerative disease pathways. Dyes with *multiple* inputting are also important targets.[3] Herein, we report a full study of novel and fully characterized 4-tolyl sulphide-containing thienyl BODIPY species that allow for ROS sensing.

Synthetically, 3-thiophene carboxaldehyde was selected as a starting aldehyde to enable sequential asymmetric sulphide incorporation. First, *N*-bromo succinimide (NBS) effected dibromination of the thiophene carboxaldehyde (DMF). Arylsulphide intermediates 2,5-bis(4-tolylsulfide)- (**1**) and 5-bromo-2-(4-tolylsulfide)-3-thiophene carboxaldehyde (**1a**) were prepared efficiently by an adapted CuI/BtH-catalyzed coupling of 2,5-dibromothiophene carboxaldehyde with *p*-toluenethiol (see ESI†).[6] The BODIPY derivatives **2** and **2a** were then conveniently prepared *via* the Lindsey method (Scheme 1);[3] authentication was made by ^{1}H, ^{13}C, and ^{11}B NMR spectroscopy, and high-resolution ES-mass spectrometry (ESI†). UV-PL data of **2** (Φ_F = 0.01), and **2a** (Φ_F = 0.06) were obtained (Fig. 1 and ESI†). Formal incorporation of thiol groups greatly limits BODIPY fluorescence; compound **2**, because of its lower Φ_F value ("turned off"), was used further in potential ROS "turn on" assays.

Compound **2** was assayed by ROS-type species to determine the extent of sulphur oxidation (Fig. 2) as determined by fluorescence changes (Fig. 3). *m*-CPBA (1.0×10^{-4} M) in MeCN : H_2O (70 : 30 by vol) with **2** (1.0×10^{-6} M) gave a "turn-on" response (2 h: 11-*fold*; 15 h: 50-*fold*) at 522 nm (Fig. 1 and ESI†). Interestingly, **2** was unresponsive with H_2O_2, NaOCl, KO_2, $^{\bullet}OH$, $^{\bullet}O^tBu$, and ^{t}BuOOH under analogous conditions. Interestingly, this reactivity pattern is different

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† Electronic supplementary information (ESI) available: Experimental details, in-line spectral data, reproductions of NMR spectra, HR MS spectra, Crystallographic data, structural bond lengths and angles, *etc*. CCDC 860722, 869054 and 869055. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c2cc33076h

‡ Crystallographic Data: Compound **7** (CCDC 860722): $C_{31}H_{29}BF_2N_2O_3S_3$, F.W.: 622.55. Monoclinic, *C*2/*c*, *Z* = 8, *a* = 37.899(9) Å, *b* = 7.743(2) Å, *c* = 25.029(7) Å, *β* = 122.015(11)°, *V* = 6227(3) Å$_3$, *T* = 296(2)K, No. reflections = 28247, No. independent reflections = 7373 [*R*(int) = 0.1082], R_1 = 0.0845, wR_2 = 0.2158, μ/mm^{-1} = 0.285, Largest diff. peak and hole = 0.793 and 0.553 e−/$^{-3}$. Compound **8** (CCDC 869055): $C_{31}H_{29}BF_2N_2O_4S_3$, F.W. = 638.55, monoclinic, *C*2/*c*, *Z* = 8, *a* = 38.241(9) Å, *b* = 7.6428(18) Å. *c* = 25.192(7) Å, *β* = 121.40(2)°, *V* = 6285(3) Å3, *T* = 296(2)K. No. of reflections = 27 214, No. of independent reflections = 4422 [*R*(int) = 0.0713], R_1 = 0.0508, wR_2 = 0.1466, μ/mm^{-1} = 0.286, Largest diff. peak and hole = 0.331 and −0.264 e−/$^{-3}$. Compound **9** (CCDC 869054): $C_{12}H_{13}B1F_2N_2$, F.W. = 234.05, Monoclinic, $P2_1/c$, *Z* = 4, *a* = 6.350(3) Å, *b* = 13.032(7) Å. *c* = 14.253(7) Å, *β* = 94.75(2)°, *V* = 1175.4(10) Å^3, *T* = 296(2)K. No of reflections = 16 333, No. of independent reflections = 4133 [R(int) = 0.0857], R_1 = 0.0992, wR_2 = 0.2003, μ/mm^{-1} = 0.100, Largest diff. peak and hole = 0.287 and −0.178 e−/$^{-3}$.

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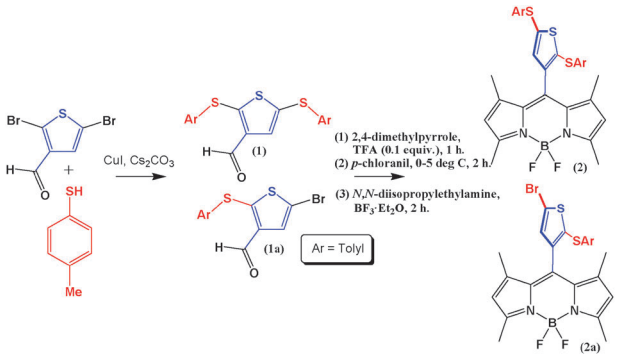


Scheme 1 Synthesis of the mono- and dithiol-derivatized aldehydes (**1** and **1a**) from 3-thiophene carboxaldehyde, and BODIPY compounds **2** and **2a** (ESI†).

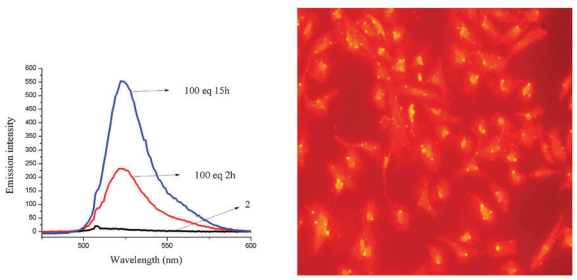


Fig. 1 (*left*) Cuvette emission spectra of compound **2** (1×10^{-6} M) and "turn-on" response (50-fold, 522 nm) with *m*-CPBA (100 eq) in acetonitrile/water (70 : 30) after 2 and 15 h ($\lambda_{exc} = \lambda_{abs} = 506$ nm) (ESI†). (*right*) Fluorescence microscopy images of cultured neuroblastoma for **2** at 100 μmol with H_2O_2 after incubation (2 h, 37 °C; $\lambda_{exc} = 543$ nm, $\lambda_{em} = 592$ nm) (see ESI†).

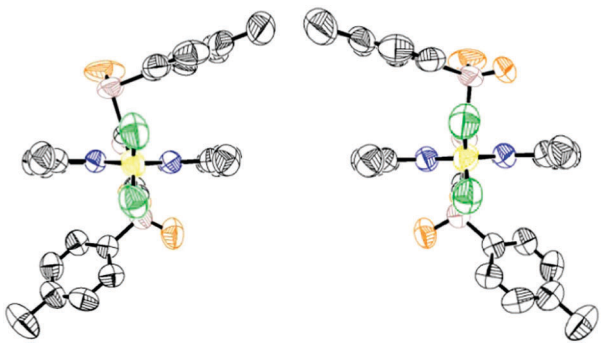


Fig. 2 BODIPY crystal structures of (*left*) the *trio*xidized **7**, an arylsulfoxide–arylsulfone and (*right*) the *tetra*oxidized **8**, a bis-arylsulfone (CCDC #'s: 860722 & 869055, respectively). Hydrogen atoms are omitted for clarity, percentage of thermal ellipsoids = 30%.

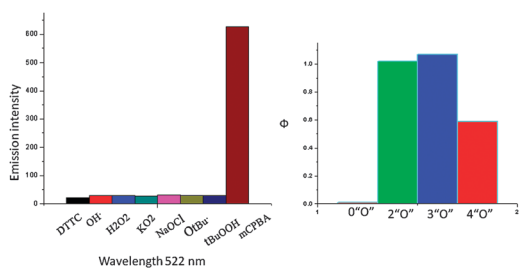


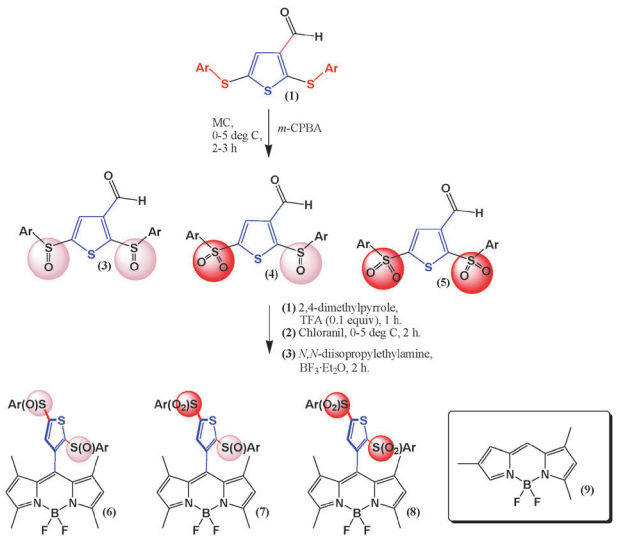
Fig. 3 (*left*) Selectivity of **2** for *m*-CPBA, and (*right*) comparison of Φ_F values for compounds **2**, **6**, **7**, and **8** (522 nm).

from that reported for the related 2-SMe-substitution, selective for NaOCl.[5]

Next, cellular fluorescence microscopy studies with SH-SY5Y neuroblastoma were carried out on **2** and the commercial 2′,7′-dichlorofluorescein diacetate (DCFDA) for comparison (Fig. 1 and ESI†); **2** compartmentalized in the cytosol and was found to be sensitive to endogenous H_2O_2, in fact moreso than the fluorescein species was (30 min).[7] Various conditions were measured (ESI†). Furthermore, compound **2** was found non-neurotoxic at 100 μM, as determined by LDH assaying (ESI†). The calculated *c* log *P* value for **2** (2.75) indicates promise for possible blood-brain barrier penetrability.

Next, to consider how the emission signal and molecular geometry are attenuated upon stepwise [O]-atom incorporation, we synthesised, isolated and characterized compounds **6–8**, the discrete proposed BODIPY products of oxidation (Scheme 2, Fig. 2, ESI†), Aldehyde **1** was oxidized (*m*-CPBA) and its products were isolated and characterized for convenient BODIPY (**6–8**) preparation (ESI†). ^{1}H NMR spectra show inequivalent 1,3,5,7-position methyls and 2,6-position methines for **6** and **7** (ESI†). These compounds are also highly fluorescent as predicted ($\Phi_F = \sim 1.00$). To confirm that **6–8** are genuine products of *m*-CPBA oxidation, **2** was treated separately with excess *m*-CPBA (CH_2Cl_2, 0 °C); ^{1}H NMR spectral analysis revealed three major products assigned to **6–8**.

Structural data was also obtained and can be brought into line with spectroscopic data. Crystal structures of **7** and **8** confirm the expected BODIPY species with the trioxidized arylsulfoxide–arylsulfone and tetraoxidized bis-arylsulfone moieties, respectively (Fig. 2). The crystal structure of **7** reveals one sulfoxide and one sulfone (Fig. 2). In contrast, that for compound **8** reveals two sulfones and is C_s-symmetric by ^{1}H NMR spectroscopy, suggesting rapid arylsulfone motion on the NMR time-scale (RT, $CDCl_3$). The final oxidation site is at the proximal sulfide. The Φ_F value of **8** (~0.59) is 40% lower than that of **7**. From the emission data, importantly, the



Scheme 2 Preparation and characterization of BODIPY oxidation products of **2** with *p*-chloranil, and the simple BODIPY by-product species **9**.

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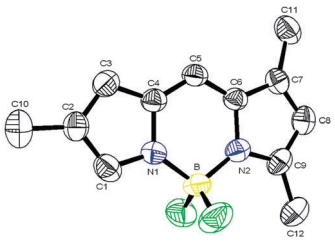

Fig. 4 Crystal structure of **9** (CCDC #: 869054). Hydrogen atoms are omitted for clarity, percentage of thermal ellipsoids = 30%.

first-oxygenation of the proximal sulphur allows for a ~100-fold increase in Φ_F value; upon oxidation, the entire 8-aryl moiety gains steric hindrance, rotational inhibition allows the fluorophore to possess higher fluorescence.[3,8] The sulfone in **8**, involves a combined electronic and steric attenuation of fluorescence (ESI†); structurally, the fourth [O] must direct itself toward the BODIPY fluorophore (Fig. 2). Red shifting for the series (**6** < **7** < **8**: λ_{ABS}: 509 < 512 < 515; λ_{EM}: 523 < 524 < 527) suggests a growing heteroatomic influence of the thienyl donor moiety to the BODIPY acceptor. Oxidation at the distal sulfide imparts a subtle photophysical effect not underscored here. Compound **2** was also assayed with potentially interfering metal cations (50 equiv); no fluorescence perturbations were found, except for minor quenching effect for Cu^{2+} (−20%) and Hg^{2+} (−15%). For Hg^{2+}, very slight bathochromic shifting for **6** (3 nm) and **7** (4 nm), and hypsochromic shifting for **8** (2 nm) was found in MeCN (ESI†). *c* log *P* values were calculated and found to decrease: **6**: 1.62 > **7**: 1.06 > **8**: 0.498, suggesting worsening BBB penetrability with more O-loading.

Surprisingly, the reaction of **5** with 2,4-dimethyl pyrrole gave the novel and highly fluorescent 1,3,6-trimethyl-4,4-difluoroboradiazaindacene (Φ_F = 0.98) as a side product in 9.9% yield (R_f 0.70, CH_2Cl_2). High resolution-MS confirmed a low MW molecule (257.1038 (cal.), 257.1033 (exp.)). The structure by solution ^{1}H NMR spectroscopy reveals four aromatic singlets (δ 7.46, 7.06, 6.68, 6.09) and three aliphatic singlets (δ 2.54, 2.23, 2.10). The asymmetric nature of **9**, where two methyls exist on one side and a single methyl is present on the opposite side of the molecule, is supported structurally as well (Fig. 4). Interestingly, in a deviation from the known Lindsey reaction, the formation of **9** requires **5**, but not TFA. Remarkably, no product formation was detected in the presence of **4**, suggesting the subtlety of proximal sulfone/sulfoxide sterics in product formation in which the 4-methyl carbon of the first 2,4-dimethylpyrrole bonds to the ring 5-carbon of the second 2,4-dimethylpyrrole *via* oxidation/condensation.

Herein, we report the preparation and characterization of various closely-related sulphur-containing BODIPY species for ROS sensing. Stepwise chemical *S*-oxidations effected by *m*-CPBA or H_2O_2 can greatly enhance molecular fluorescence. Specifically, oxygen atom loading gives respective sulfoxide/sulfone groups: enhancement is found with 3[O]-atom loading (**7**); a diminished capacity is found for 4[O]-atom loading (**8**). The greatest emission changes are seen when the sulfide that is proximal to the chromophore is oxidized; cuvette optical data show selective "turn-on" behavior (50-fold) with *m*-CPBA (over H_2O_2, NaOCl, KO_2, $^{\bullet}$OH, $^{\bullet}$O^{t}Bu, and ^{t}BuOOH) in 30 + % water. Neuroblastoma cellular trials with **2** show responses for H_2O_2. Structural data support the asymmetric findings gleaned from NMR spectral data. Geometric impositions give rise to increased molecular fluorescence to a point. Lastly, bis-arylsulfone aldehyde **5** facilitates the unexpected formation of a simple, asymmetric trimethyl BODIPY species (1,3,6-trimethyl-4,4-difluoroboradiazaindacene) (Φ_F = ~1.00). We hope to fine-tune the concept of stepwise oxidation for neurodegenerative disease research through future studies.

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