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A New Spirobifluorene-Bridged Bipolar System for a Nitric Oxide Turn-On Fluorescent Probe

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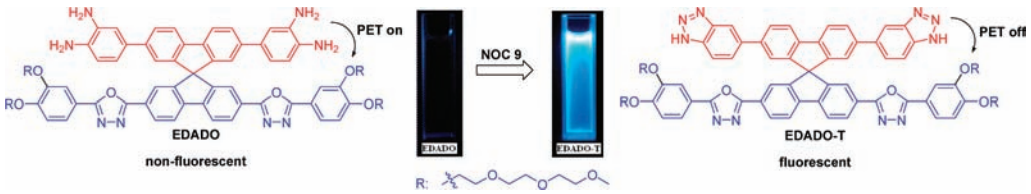
ABSTRACT



A new spirobifluorene-bridged bipolar molecule (EDADO) as a nitric oxide (NO) turn-on fluorescent probe was designed and synthesized. The fluorescence of EDADO is strongly quenched by photoinduced electron transfer (PET) from the electron-donating *o*-phenylenediamine-containing biphenyl branch to the orthogonally arranged electron-accepting 1,3,4-oxadiazole-containing conjugated oligoaryl system. Upon reacting with NO, EDADO is converted to EDADO-T, which exhibits strong fluorescence due to the suppression of PET.

Nitric oxide (NO) is an important biomolecule in a variety of physiological and biological processes, and has a critical role as a signal transmitter in the cardiovascular system, the central and peripheral nervous systems, and the immune system.[1] The pivotal biological role of NO has triggered studies of molecular probes responsive to it. A number of methodologies[2] for detecting NO have been developed, including electrochemical,[3] electron paramagnetic resonance,[4] chemiluminescence,[5] and fluorescence-based[6,7] techniques. Among these methods, fluorimetry holds promise because of its high sensitivity, high selectivity, real-time detection, and simplicity. Several fluorescent probes for NO have been reported to date. They can be divided into two principal categories: (1) organic molecule-based sensors, most notably, *o*-diaminofluoresceins and related molecules, which can detect intracellular NO through the modulation of photoinduced electron transfer (PET) of the probes;[6] and (2) transition metal-based probes, which have also been used for NO sensing via fluorophore displacement.[7]

We previously developed a series of spirobifluorene-bridged bipolar systems that exhibit ultrafast and highly efficient PET behavior.[8] With effective use of such spirobifluorene-bridged bipolar systems, a number of applications, such as electrogenerated chemiluminescence devices,[9]

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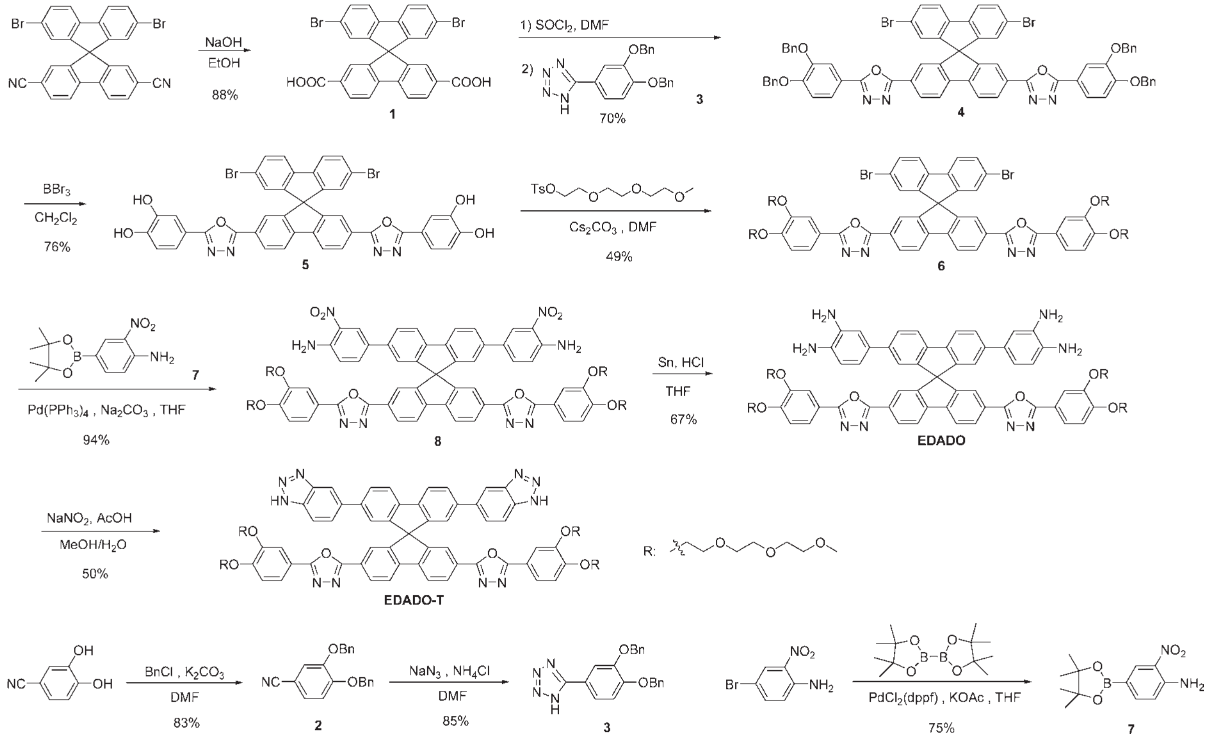
Scheme 1. Synthesis of Spiro-Bridged Bipolar Molecule **EDADO** and Related Intermediates



ultraviolet photodetectors,[10] and phosphorescent organic light-emitting diodes,[11] have been successfully demonstrated. Herein, we report the design and synthesis of a new spirobifluorene-bridged bipolar molecule (**EDADO**, Scheme 1) as a NO-sensitive fluorescent probe. Spirobifluorene appears to be a suitable fluorophore core for highly sensitive fluorescent probes because spirobifluorene-based molecules generally have high extinction coefficients and photoluminescence (PL) quantum yields.[12] The nature of each individual chromophore orthogonally separated by a rigid *σ*-spacer in spirobifluorene-based molecules can be independently tailored. In our design of **EDADO**, the modification involves introduction of *o*-phenylenediamine, which reacts with high selectivity toward NO, to serve as the electron donor and the NO receptor.[6] The 1,3,4-oxadiazole-containing conjugated branch acts as the electron acceptor counterpart. With proper positioning (spiro-configuration) of these two branches, the nonfluorescent **EDADO** ascribed to the strong PET from the *o*-phenylenediamine moiety to the 1,3,4-oxadiazole-containing conjugated oligoaryl moiety can be converted into a highly fluorescent **EDADO-T** by reacting with NO^+ or N_2O_3 under aerobic conditions. Such a change involves transformation of *o*-phenylenediamine to benzotriazole, rendering the resulting chromophore with different electronic character for suppressing the PET. The incorporation of four triethyleneglycol monomethyl ether side chains increases the water solubility of **EDADO**, which may enhance its compatibility with potential biological applications.

The synthetic route for **EDADO** is depicted in Scheme 1. 2,7-Dibromo-2′,7′-dicyano-9,9′-spirobifluorene[8] was hydrolyzed under basic conditions to yield the dicarboxylic acid derivative **1** in 88% yield.[13] The acid groups of **1** were converted to acid chlorides and subsequently reacted with a tetrazole derivative **3** in toluene to give the 1,3,4-oxadiazole-containing derivative **4** in 70% yield.[8,14] Compound **3** was prepared by 1,3-dipolar cycloaddition of sodium azide and the nitrile compound **2**, which was obtained by the benzylation of 3,4-dihydroxybenzonitrile with benzyl chloride. Removal of the benzyl groups in molecule **4** with BBr_3 in CH_2Cl_2 resulted in compound **5**, which was then subjected to nucleophilic substitution with triethylene glycol monomethyl ether tosylate[15] in dimethylformamide (DMF) to afford molecule **6** in 49% yield. Suzuki–Miyaura cross-coupling of the dibromo compound **6** with

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the boronic ester derivative **7**, which was synthesized by Pd-catalyzed borylation of 4-bromo-2-nitroaniline with bis(pinacolato)diboron, produced **8** in 94% yield. Finally, the nitro groups of **8** were reduced with tin powder to generate **EDADO** in 67% yield.[16] For comparison, the benzotriazole-containing **EDADO-T** was synthesized by treating **EDADO** with $NaNO_2$ under acidic conditions (50% yield).[17]

In addtion, model compounds **DAS**, containing the *o*-phenylenediamine chromophore, **DAS-T**, bearing the benzotriazole chromophore, and **EDO**, containing the 1,3,4-oxadiazole chromophore, were also synthesized (Scheme 2). The Pd-catalyzed Suzuki coupling reaction of 4-bromo-2-nitroaniline with 2,7-bis(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-9,9′-spirobifluorene produced intermediate **9** in 50% yield. The reduction of the nitro groups of **9** gave rise to **DAS**, which was subsequently reacted with $NaNO_2$ under acidic conditions to give **DAS-T** in 47% yield. The bromo and benzyl groups of compound **4** could be simultaneously removed by Pd/C-catalyzed hydrogenation to produce compound **10** in 89% yield, which underwent nucleophilic substitution with triethyleneglycol monomethyl ether tosylate in DMF to afford **EDO** in 58% yield.

Scheme 2. Synthesis of the Model Compounds **DAS**, **DAS-T**, and **EDO**

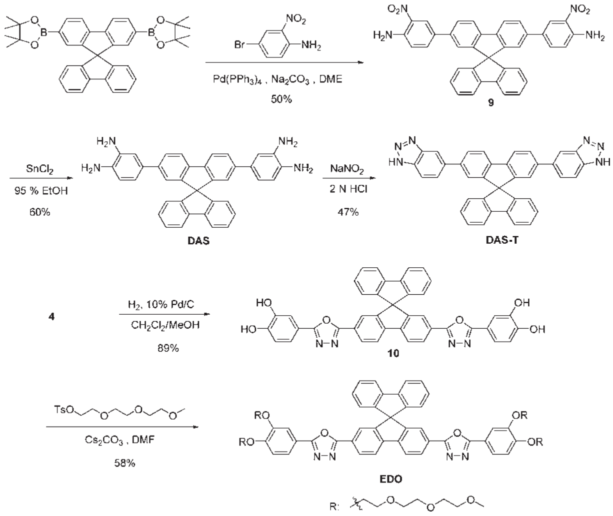

Figure 1 depicts the photophysical behavior of model compounds **DAS** and **EDO**, which are composed of individual donor and acceptor chromophore respectively, as compared to those of **EDADO** in MeCN solution. The photophysical data are summarized in Table 1. **DAS** containing *o*-phenylenediamine donor and **EDO** bearing 1,3,4-oxadiazole acceptor display fluorescence emission maxima centered at 500 and 445 nm, respectively. Due to the rigid nature of the spirobifluorene core, **DAS** and **EDO** exhibit remarkable photoluminescence quantum yields of 0.72 and 0.83, respectively. In contrast, the bipolar probe **EDADO** comprising both donor and acceptor chromophores into a spiro-configuration exhibits weakly detectable fluorescence ($\Phi_F < 0.003$). This result suggests that efficient intramolecular PET occurs in **EDADO**, leading to a charge-separated excited state, which is typically nonfluorescent.

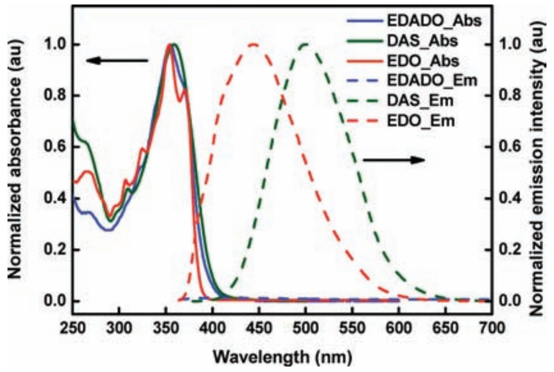

Figure 1. Absorption (solid lines) and emission (broken lines) spectra of **EDADO** (blue), **DAS** (green), and **EDO** (red) in MeCN solution.

Table 1. Photophysical Parameters of **DAS**, **DAS-T**, **EDO**, **EDADO**, and **EDADO-T** in MeCN Solution

compd	λ_{abs} /nm	ε/M^{-1} cm^{-1}	λ_{em}/nm[a]	Φ_F[b]
DAS	359	49 589	500	0.72
DAS-T	336	40 691	388	0.69
EDO	354	62 803	445	0.83
EDADO	355 (354)[c]	74 784[c]	– (–)[c]	0.003 (0.002)[c]
EDADO-T	339 (339)[c]	75 360[c]	443 (461)[c]	0.60 (0.12)[c]

[a] Excited at the absorption maxima. [b] Determined by using a calibrated integrating sphere system. [c] Measured in MeCN/H_2O (1:1, v/v) solution.

To shed light on the emission origin of **EDADO-T**, which is the expected product upon treating **EDADO** with nitric oxide. A comparison study on the photophysical behavior of compounds **DAS-T**, **EDO**, and **EDADO-T** in MeCN was conducted (Figure 2). In sharp contrast to **EDADO**, a strong emission band peaked at 443 nm is observed for **EDADO-T** with a high Φ_F of 0.60, indicating that the PET is efficiently blocked due to the weak electron-donating ability of the benzotriazole branch in **EDADO-T**.[6] Apparently, the emission spectrum of **EDADO-T** superimposes to that of the 1,3,4-oxadiazole-containing model compound **EDO**. Therefore, the emission of **EDADO-T** can be unambiguously assigned from the 1,3,4-oxadiazole-containing branch. It is also noteworthy that the emission spectrum of **DAS-T** partially overlaps with the absorption spectrum of **EDO**. This result indicates that the benzotriazole-containing chromophore (present in **DAS-T**) of **EDADO-T** could potentially serve

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as an energy donor for fluorescence resonance energy transfer (FRET) to the energy acceptor, the 1,3,4-oxadiazole-containing chromophore (present in **EDO**). The reaction of NO with *o*-phenylenediamine apparently not only suppresses PET in **EDADO** but also produces a new chromophore that may additionally contribute to the final fluorescence of **EDADO-T** (Figure 3).

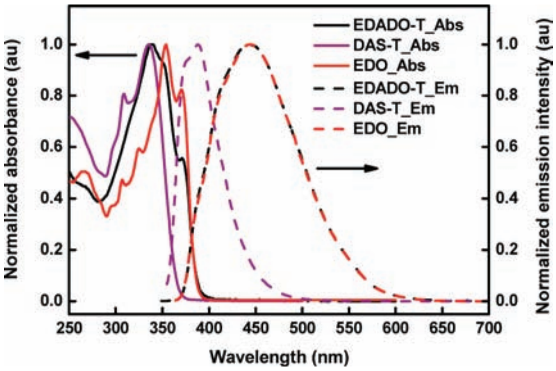

Figure 2. Absorption (solid lines) and emission (broken lines) spectra of **EDADO-T** (black), **DAS-T** (purple), and **EDO** (red) in MeCN solution.

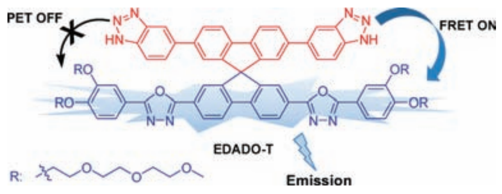

Figure 3. Proposed emission mechanism of **EDADO-T**.

Fluorescence titration experiments were conducted by treating **EDADO** solution [6×10^{-5} M in H_2O/CH_3CN (1:1, v/v) at pH 7.0] with various concentrations of the NO precursor, NOC-9 (2.7 min half-life for releasing NO at 25 °C),[18] and then measuring the fluorescence after 30 min. These were done to evaluate the performance of **EDADO** as a practical probe for NO in the aqueous solution. **EDADO** showed almost no fluorescence in the absence of NOC-9, but the **EDADO** solution was converted to a highly fluorescent mixture upon its addition of NOC-9 (Figure 4).[19] The emission intensity increased incrementally with the loading amount of NOC-9. These results indicate that the emission of this resulting mixture is from **EDADO-T**, as verified by comparing with the emission spectrum of presynthesized **EDADO-T** (Figure S1, Supporting Informaton (SI)). In addition, the formation of **EDADO-T** by mixing **EDADO** and NOC-9 was further confirmed by electrospray ionization mass spectroscopy (ESI-MS) of the resulting mixture, in which a molecular ion (*m*/*z* 1487.62) corresponding to [**EDADO-T**+H]$^+$ was detected (Figure S2, in SI) .

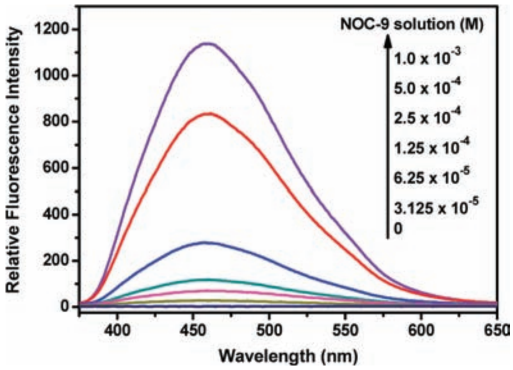

Figure 4. Fluorescence spectra (excitation at 339 nm) of **EDADO** (6×10^{-5} M) in the presence of NOC-9 in MeCN/H_2O (1:1, v/v) at pH 7.0.

In summary, we have synthesized a spiro-configured bipolar molecule (**EDADO**) as a new turn-on fluorescent probe for NO. The success of the designed molecule was primarily due to the judicious selection of the *o*-phenylenediamine moiety as the electron-donating group as well as the NO receptor, which is orthogonally bridged to the highly fluorescent and electron-accepting 1,3,4-oxadiazole-containing oligoaryl via a saturated σ-spacer. Upon treatment of **EDADO** with NO (generated either from $NaNO_2$ in acidic conditions or from NOC-9), the nonfluorescent **EDADO**, due to efficient PET, was converted to the highly fluorescent **EDADO-T**. Comparisons of the photophysical characteristics of **DAS-T** and **EDO** with those of **EDADO-T** indicate that the PL chromophore of **EDADO-T** originated from the 1,3,4-oxadiazole-containing biphenyl branch. The results also indicate the participation of the newly formed benzotriazole-bearing chromophore in intramolecular FRET. These results can provide new ideas for the molecular design of potential molecular probes for NO.

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Supporting Information Available. Synthesis, characterization, copies of ^1H and ^{13}C NMR spectra of new compounds, and ESI-MS spectrum of the fluorescence titration experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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