

Support Information

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1. Decomposition of Dye **1** in the Presence of Bases

Our original attempts of direct alkylation of the imine group of dye **1** in the presence of bases such as LDA, tBuOK, NaOMe, NaHMDS and NaH were fruitless. These reactions were tested using good electrophiles such as propargyl bromide (3-bromopropyne), MeI, or ethyl bromoacetate. In addition, a wide range of temperatures between -78 and 60 °C and different solvents (e.g., THF, DMSO, CH₃CN) were also considered. Almost no conversion was observed with weaker bases such as K₂CO₃. Control experiments in the absence of electrophile revealed a rapid decomposition of **1** caused by the presence of strong bases regardless of the solvent and temperature used. Most of these products did not show any fluorescence under UV-light. In general, the evolution of the reaction mixtures could be clearly monitored by TLC analysis (Figure S1). We hypothesized that a ring-opening cascade reaction initiated by the nucleophilic unprotonated imine could explain the decomposition process (Figure S2). Moreover, the alternative strategy involving the hydrolysis of the imine group and subsequent alkylation (this would give a permanent positive charge to the molecule on the benzimidazole ring) provided also a similar decomposition outcome.

Figure S1. Representative analytical TLC analysis of several reaction mixtures. Labels: **A** = reaction between dye **1** and propargyl bromide using LDA as base in THF at -78 °C \rightarrow 0 °C; **B** = reaction between dye **1** and anhydride **2** at 90 °C as described in the main text; **C** = reaction between dye **1** and acetic anhydride at 90 °C; **D** = co-spot (A+B+C+E); **E** = dye **1**. Eluent: EtOAc/hexanes 4:1 v/v.

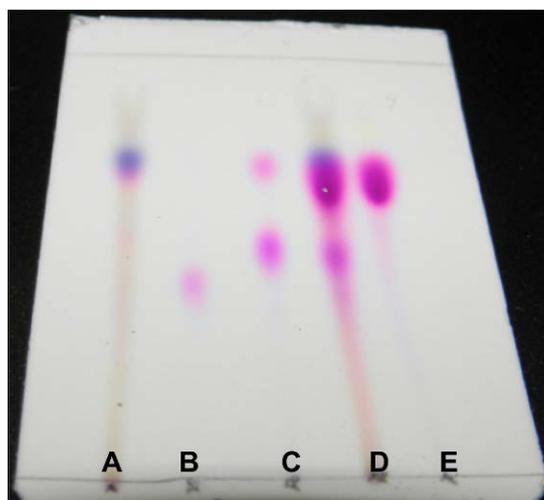
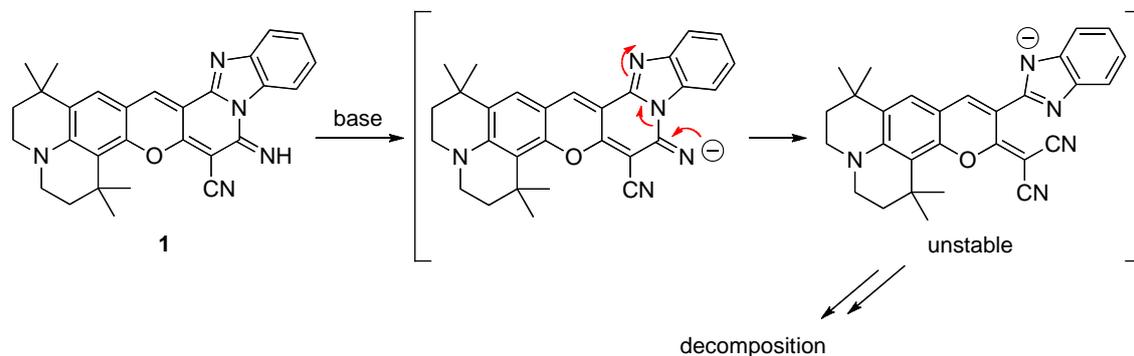


Figure S2. Plausible decomposition path for **1** in the presence of bases via ring-opening cascade reaction initiated by the nucleophilic attack of the unprotonated imine group.



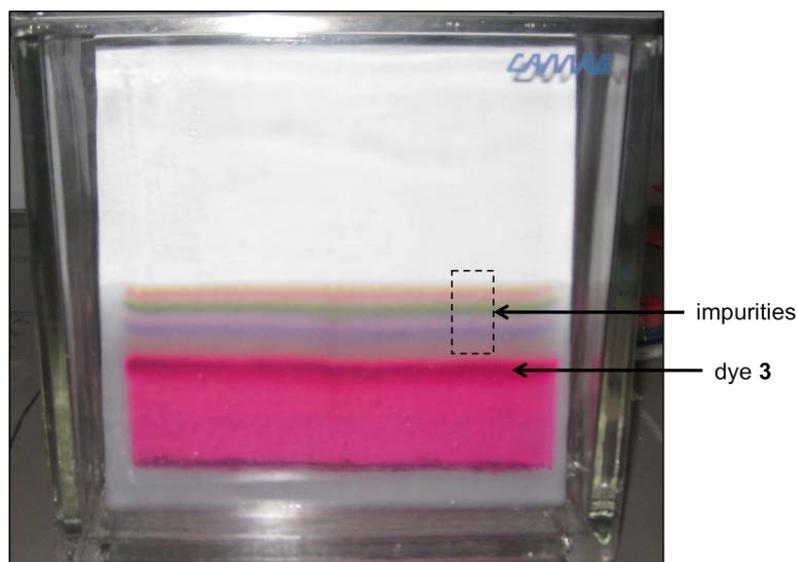
2. Considerations for the Purification of Modified Dye 3

Exhaustive washing of the organic phase with water is recommended for removing most of the anhydride in excess, which makes the column chromatography easier. A large column of *ca.* 45 cm height \times 5 cm diameter is recommended for the preliminary purification of a reaction carried out with *ca.* 20 mg of unmodified dye **1**. The loaded column should be eluted first with a mixture EtOAc/hexanes 1:1 v/v to ensure the removal of unreacted starting dye, remaining anhydride and most of byproducts. Note that most of these products stick considerably to the stationary phase making difficult to obtain the desired compound only after this procedure. Further purification by preparative TLC is necessary to isolate the pure product (Figure S3). The silica gel plate (20 cm \times 20 cm) should be loaded with no more than 4–5 mg of mixture.

The title compound and one of the byproducts have a very similar R_f value. The optimum separation of the two products is achieved by eluting with EtOAc/hexanes 4:1 v/v. To observe this byproduct by analytical TLC during the synthesis are necessary several runs eluting with EtOAc/hexanes 1:1 v/v.

The title compound should be stored in a sealed vial, under nitrogen/argon atmosphere and in the dark.

Figure S3. Typical aspect of a preparative TLC loaded with modified dye **3** (eluting with EtOAc/hexanes 4:1 v/v).



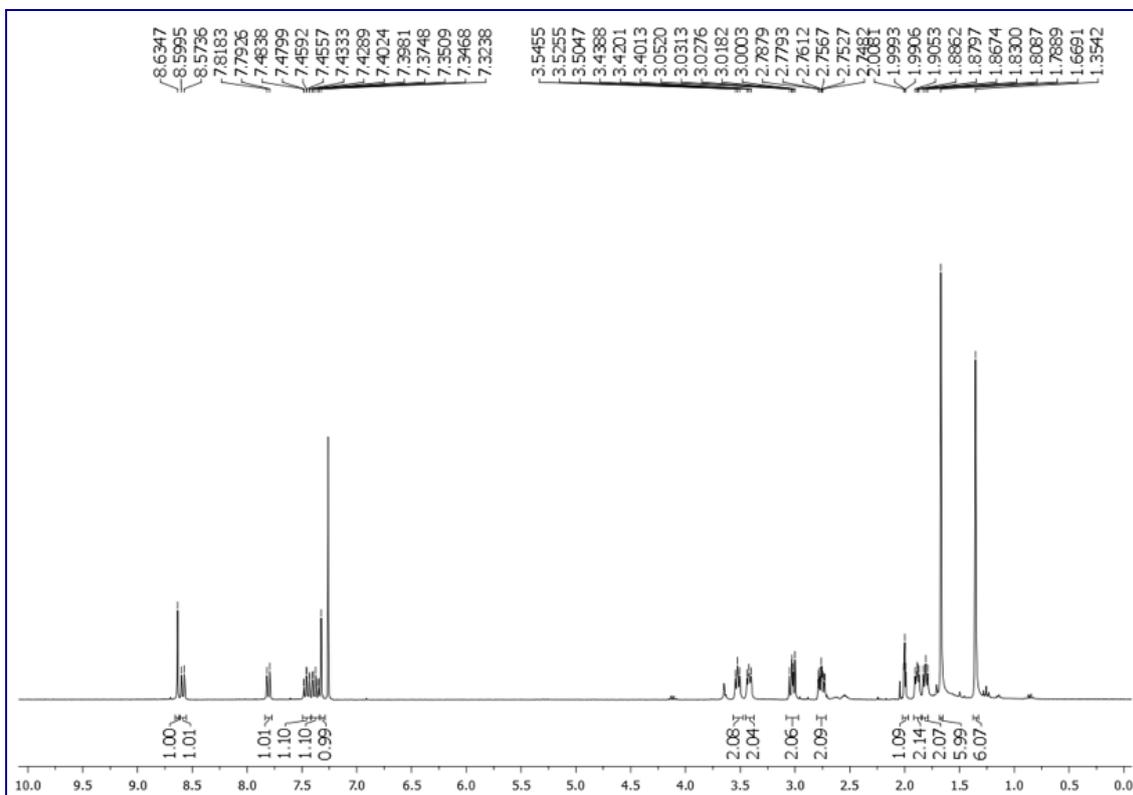
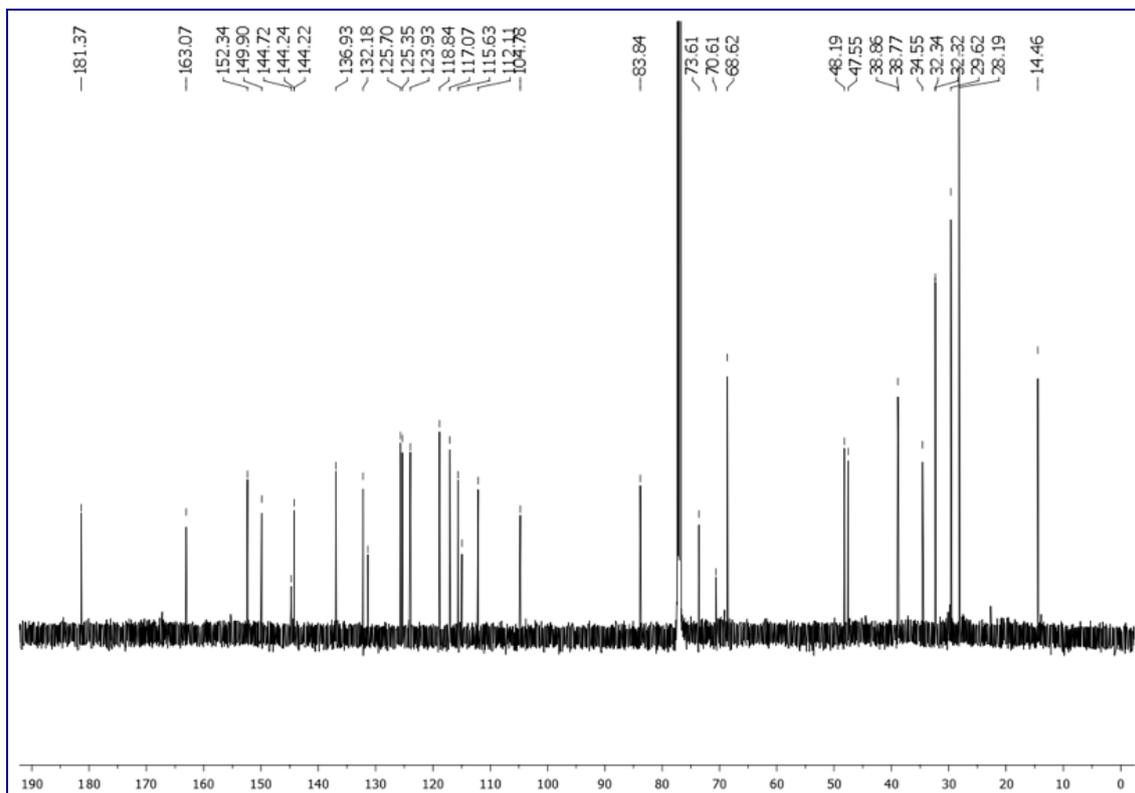
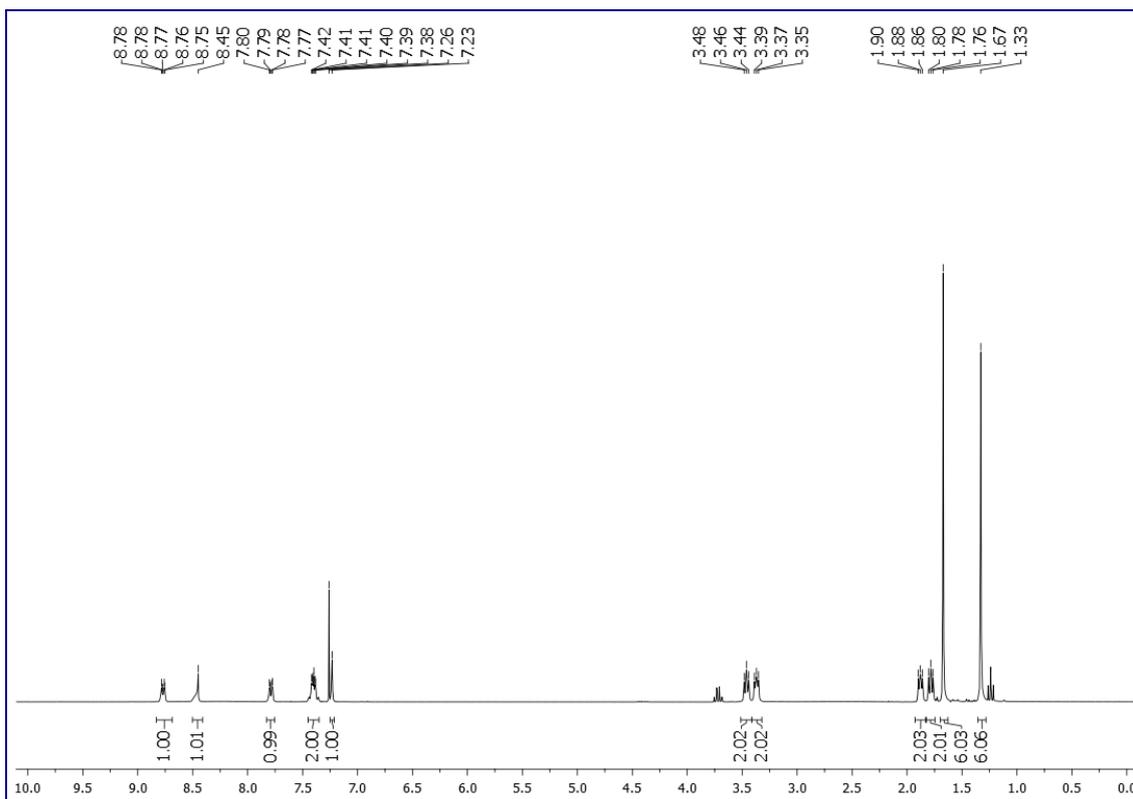
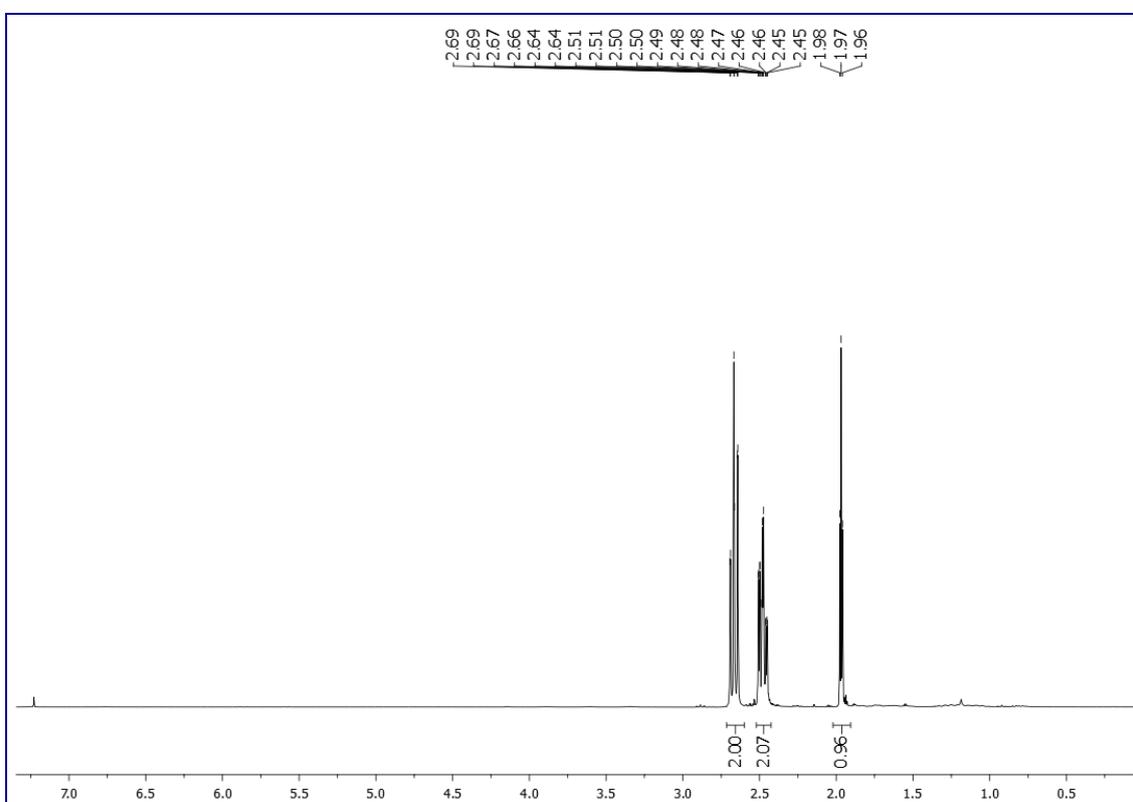
3. ^1H - and ^{13}C -NMR spectroscopyFigure S4. ^1H -NMR spectrum of title compound **3** (300 MHz, CDCl_3).Figure S5. ^{13}C -NMR spectrum of title compound **3** (151 MHz, CDCl_3).

Figure S6. $^1\text{H-NMR}$ spectrum of commercial dye **1** (300 MHz, CDCl_3).**Figure S7.** $^1\text{H-NMR}$ spectrum of pent-4-ynoic anhydride (**2**) (300 MHz, CDCl_3).

4. UV-vis Spectroscopy and Correlation of Absorption with Solvent Polarity

Figure S8. (A–C) Comparative UV-vis spectra of the original dye **1** and derivative **3** in 1,4-dioxane, acetone and DMSO ($c = 3.8 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$). (D) Wavelength of the two characteristic absorption maxima for each case. Abbreviation: sh = shoulder.

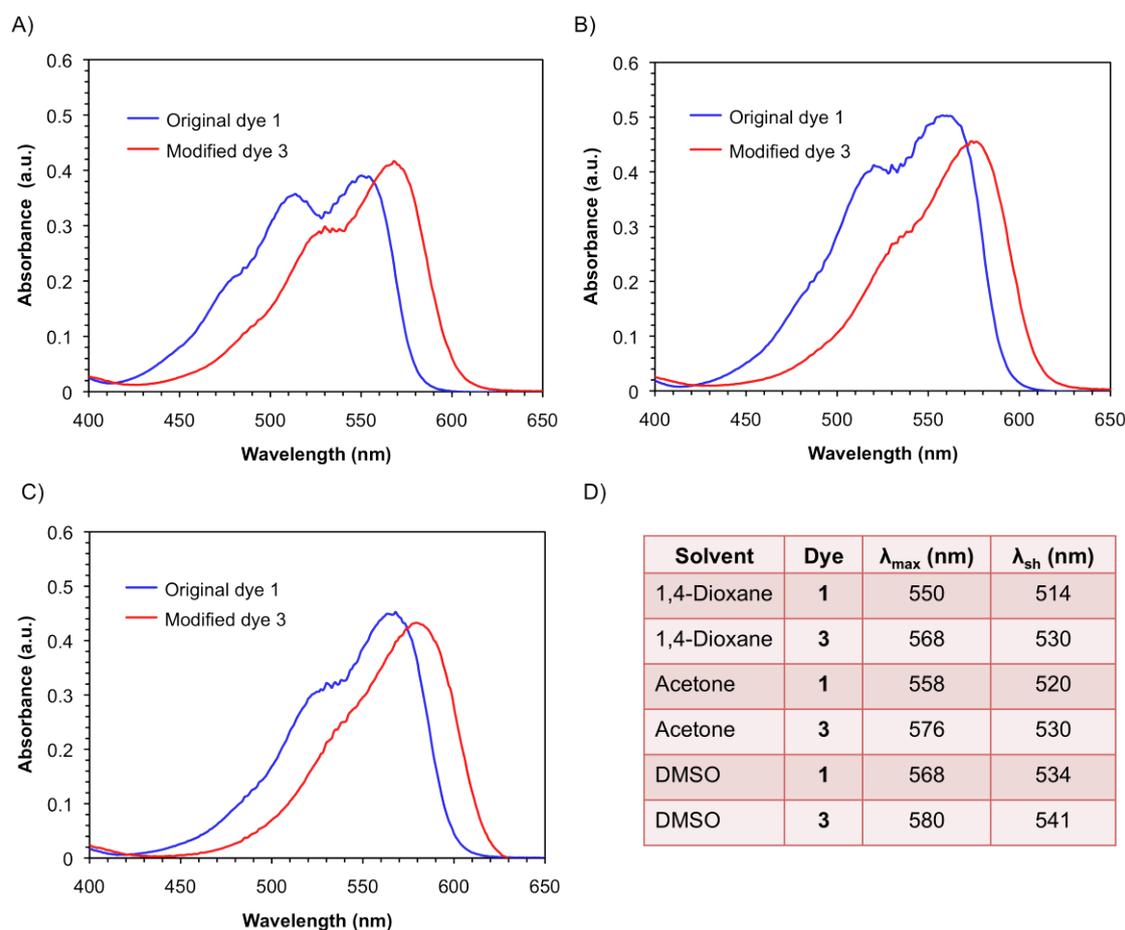


Table S1. Positive solvatochromism showed by modified dye **3** ($c = 3.8 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$). The solvents are sorted by dielectric permittivity (ϵ_{R}) in increasing order.

| Entry | Solvent | ϵ_{R} ^[a] | λ_{max} (nm) | λ_{sh} (nm) ^[b] |
|-------|-------------|--------------------------------------|-----------------------------|---|
| 1 | 1,4-Dioxane | 2.2 | 568 | 534 |
| 2 | EtOAc | 6.0 | 572 | 534 |
| 3 | THF | 7.6 | 573 | 535 |
| 4 | 2-BuOH | 16.6 | 578 | 538 |
| 5 | 1-PrOH | 20.5 | 579 | 540 |
| 6 | Acetone | 20.7 | 576 | 539 |
| 7 | EtOH | 24.6 | 579 | 540 |
| 8 | MeCN | 35.9 | 577 | 538 |
| 9 | DMF | 36.7 | 579 | 538 |
| 10 | DMSO | 46.5 | 580 | 541 |

^[a] Source: Riddich, J.A.; Bunger, W.B.; Sakano, T.K. *Organic Solvents. Physical Properties and Methods of Purification*, 4th ed.; Wiley & Sons: New York, NY, USA, 1986, Volume II. ^[b] Abbreviation: sh = shoulder.

5. Excitation-Emission Fluorescence Spectroscopy

Figure S9. Excitation-emission spectrum of modified dye **3** in EtOH ($c = 1.43 \times 10^{-5} \text{ mol}\cdot\text{L}^{-1}$). Excitation wavelength (λ_{ex}) = 580 nm. A stock solution of rhodamine-6G in EtOH ($c = 8.62 \times 10^{-7} \text{ mol}\cdot\text{L}^{-1}$, $\phi = 0.95$, $\lambda_{\text{ex}} = 530 \text{ nm}$) was used as standard sample for the calculation of the quantum yield according to Parker's law.

