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Dual Fluorescent *N*-Aryl-2,3-naphthalimides: Applications in Ratiometric DNA Detection and White Organic Light-Emitting Devices

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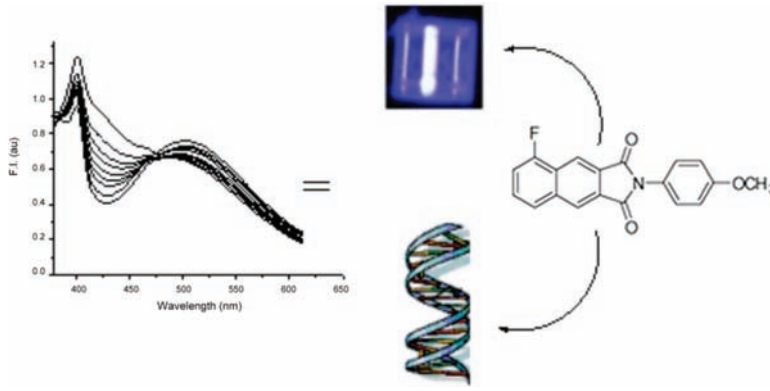
ABSTRACT



A ten element matrix of 5- and 6-substituted-(2,3)-naphthalimides was prepared for the appropriate placement of substituents necessary to promote dual fluorescence (DF). As prescribed by our balanced seesaw photophysical model this matrix yielded nine new DF dyes out of a possible ten compounds. From this set of nine DF dyes, 4-fluoronaphthalic amide (37) was selected as a probe for ratiometric detection of DNA and demonstration of panchromatic emission.

Compared to the intensiometric response of common single-band fluorescent probes, the two-color response of dual fluorescent (DF) dyes[1] imparts key advantages. The ratiometric response of certain DF dyes is independent of probe concentration, reduces errors due to photobleaching, uneven loading of probe, and/or fluctuation in excitation intensity.[2] Such features are keenly sought in cellular studies where the local concentrations of the dyes cannot be controlled. The inherent nature of excited states makes the prediction of fluorescence features a difficult endeavor and in most cases, optimal fluorescence properties are discovered by screening large synthetic libraries.[3]

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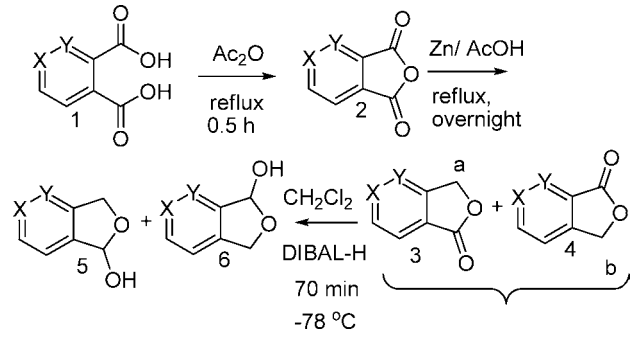
Among the various fluorophore core structures, the naphthalimide class of dyes hold particular interest because of their exceptional brightness and potential for two-color emission.[4] Considering the dramatically different photophysics between the $C_{10}H_8$ isomers azulene and naphthalene,[5] a key hypothesis we sought to examine with our so-called "seesaw model" invokes the use of symmetry as a predictive tool in generating new DF dyes.[5b] Specifically, we sought to compare fluorescence properties of *N*-aryl-2,3-naphthalic anhydride (2,3-NI) systems relative to isomeric 1,8-NI (which the model was originally based upon). The 5-membered imide ring of 2,3-NI imposes a different steric environment and rotational dynamics than the 6-membered imide ring of 1,8-NI. Therefore, to test our excited state model on the basis of the point group (C_{2v}) common to both isomers of NI, we designed a ten element marix of novel 5- and 6-substituted-(2,3)-naphthalimides.

To generate 2,3-naphthalic anhydrides, the inherent anhydride functionality requires an electron-deficient dienophile such as maleic anhydride or dialkyl maleate and therefore restricts electron-deficient dienes from participating.[6] On the basis of the general synthetic approach for these systems with maleic anhydride as the *de facto* dienophile, syntheses involving only electron-rich or neutral dienes have been reported, thereby severely limiting their synthetic scope.[7,8] To further complicate matters, our "seesaw" model requires electron-withdrawing groups at the naphthalic ring to effect DF.

For the appropriate placement of substituents at the 5- and 6-positions of 2,3-naphthalic anhydride, we report a synthetic strategy where the pivotal step involves the use of 1-hydroxyphthalans. The general route to these various hemiacetals is outlined below in Scheme 1. From a practical perspective, our efforts focused on developing a general route to substituted 2,3-naphthalic anhydrides from common substituted phthalic acids. In this procedure, analogues of phthalic dicarboxylic acid were dehydrated in acetic anhydride to give the corresponding anhydrides. Reduction of these phthalic anhydrides with zinc and acetic acid yielded two isomers, α and β (80:20, respectively).

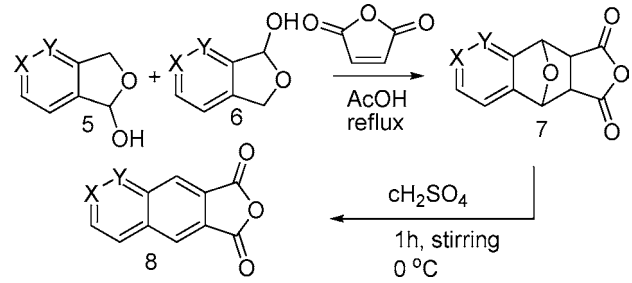
Reduction occurred preferentially at the β-carbonyl function to give the corresponding phthalides as the major product in 3- and 4-substituted phthalic anhydrides, which was confirmed by ^{1}H NMR and TLC analysis (Table 1 in the Supporting Information). In the case of 3-nitrophthalic anhydride (compound **2** in the Supporting Information), the carbonyl group of 3-nitrophthalic anhydride was reduced selectively with $NaBH_4$ at −23 °C, to yield 7-nitrophthalide as the major product. In the case of 3-substituted phthalic anhydride, reduction occurred at the β-carbonyl position and the selective reduction could be due to the formation of chelates.[9] These chelates were formed by complexation of an appropriate substituent (i.e., NO_2, F, N) with zinc. The resulting phthalides were reduced with DIBAL-H in dichloromethane to give 1-hydroxyphthalans.

Scheme 1. Synthesis of Electron Deficient 1-Hydroxyphthalans



In Scheme 2, 1-hydroxyphthalans were used for these unprecedented Diels−Alder reactions. The key step is Diels−Alder addition of in situ generated isobenzofurans by heating of 1-hydroxyphthalans in AcOH with maleic anhydride for 12 h. Vacuum evaporation of the solvent afforded a mixture of *exo* and *endo* isomers in essentially quantitative yield (Table 2 in the Supporting Information). At refluxing temperature, the *exo* product was highly favored and at low temperature *exo* and *endo* products were formed in equal amounts.

Scheme 2. Synthesis of 5- and 6-Substituted-2,3-NI



On the basis of these observations, the *exo* isomer is assigned as the thermodynamic product and the *endo* product as the kinetically favored product. Recognizing this isomer assignment contradicts Alder's endo rule, the *exo* isomer was confirmed by ^{1}H−^{1}H NMR coupling. Similar results were

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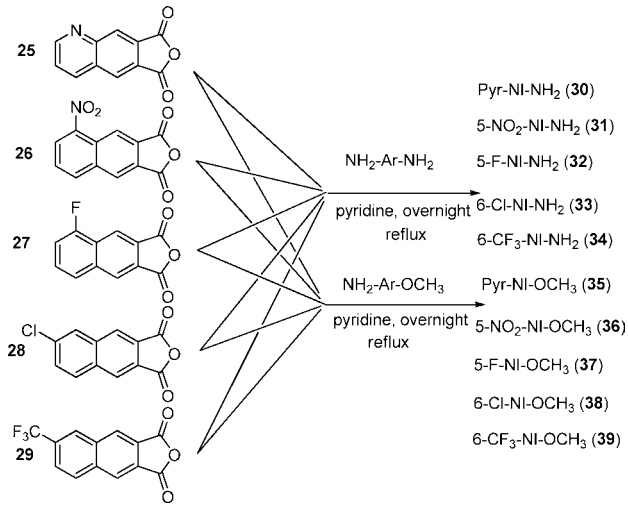
reported by Miller et al.[10] The methine protons at the sites of cycloaddition allowed for the differentiation between *exo* and *endo* products. Specifically, the aliphatic methyl protons in the *exo* isomer displayed two singlets rather than two multiplets.

Scheme 3. Synthetic Scheme for 5 × 2 Matrix Elements of the Ten Compounds and SW (Table 1) Emission



In Scheme 3, **25**, **26**, **27**, **28**, and **29** anhydrides were coupled with electron-releasing aminoarenes that have either 4-methoxy or 4-amino groups. The synthesis of each element of the matrix involved a 1:1 ratio of the **25**, **26**, **27**, **28**, and **29** naphthalimide with the respective aminoarenes in a minimum solvent of pyridine.

Among the pyridine derivatives, compound **30** displayed two-color emission in a number of solvents whereas **35** displayed DF only in water. Both nitro derivatives, **31** and **36**, displayed dual emission in different solvents. For trifluoromethyl derivatives **34** and **39**, compound **39** displayed only a single wavelength emission. Compounds **37** and **33** are shown in Figure 1, whereas the remaining compounds are shown in the Supporting Information, Figure 5.

Table 1. Summary of Fluorescence Wavelengths and Φ_F[a]

entry	excitation (nm)	emission (nm)	molar absorptivity ($L \cdot mol^{-1} \cdot cm^{-1}$)	$10^{-3}\Phi_F$
30	372	435, 527	2×10^5	425, 18
31	373	442, 472	1×10^4	5.1, 6
32	361	415, 495	4.1×10^4	11.6, 4.3
33	365	411, 501	1.1×10^4	27.3, 10
34	360	391, 416	1.4×10^4	20, 21
35	372	369, 432	1×10^4	132, 54
36	375	444, 533	1×10^4	7.0, 182
37	361	407, 546	2.5×10^4	7.9, 4.3
38	360	390, 435	1.2×10^4	20.2, 10
39	370	425	2.5×10^4	20.1

[a] Fluorescence quantum yields relative to quinine sulfate in water. Errors are on the order of ±5% for quantum yields. Value for this data set were obtained from dicholormethane.

DNA Interaction Studies. Out of ten compounds, we selected a fluoro derivative (**37**) with a methoxy group for human DNA detection studies. Because of the interesting behavior that the 5-fluoro naphthalic amide derivative displayed such as well-separated two-color emission it was selected as a ratiometric probe for DNA detection. To the best of our knowlegdge, a dye featuring dual fluorescence has not been reported for ratiometric DNA detection. Figure 2 shows the "OFF-ON" switching behavior at 428 and 506 nm on addition of DNA (0−250 μM) to a solution of **37** provides a two-color emission conducive to ratiometric analysis. To the best of our knowledge, a molecular probe with dual fluorescence has not been reported for ratiometric DNA detection. This probe has an isoemissive point at 475 nm. The relative fluorescence intensities (I_{428}/I_{506}) at the maxima of **37** are plotted against the concentration of DNA from 1 to 250 μM on gradual addition (Figure 1 in the Supporting Information). The ratiometric response in emis-

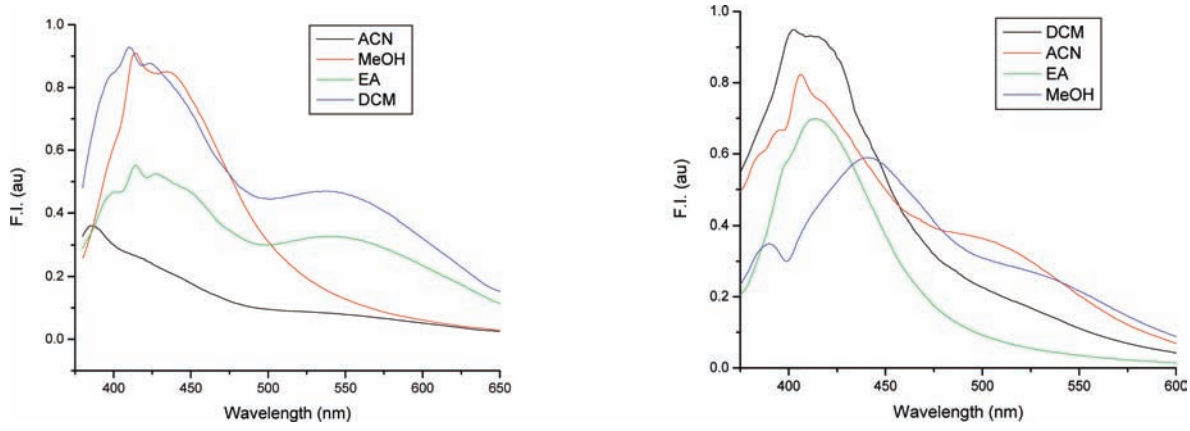


Figure 1. DF spectra of **37** and **33** (1×10^{-5}) in the solvents indicated. Fluorescence spectra are normalized to the maximum of each data set. Excitation wavelengths are shown in Table 1.

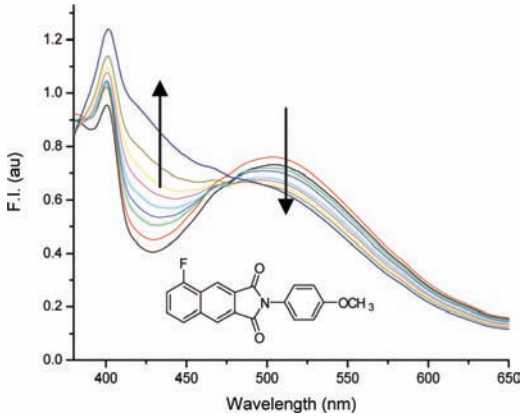


Figure 2. A 1×10^{-6} M **37** sample with 10−250 μM DNA. Ratiometric response showing the emission band at 425 nm increasing upon addition of DNA; the emission band at 525 nm decreasing upon addition of DNA.

sion followed a linear trend. As a control experiment, compound **37** was also tested with helical polypeptide α-polybenzyl[γ]glutamate (PBLG). In this case **37** gave no ratiometric response (supp. Info. Figure 3). So, potentially this compound could be used as a ratiometric probe for DNA detection. Here, we used the following single stranded DNA sequence: TCC TGT GGA GAA GTC TGC CGT TAC TG.

New Lighting Sources. Organic light emitting diodes (OLEDs) have gained attention as one of the most appealing solutions for low energy consumption in solid-state lighting because they display bright colors, reproducibility, and stability.[11] To date, very few small molecule systems have been reported to display white-light emission.[2,12] 4′-Amino-*N*-phenyl-(5-fluoro)-2,3-naphthalic imide (**37**) shows white light emission where two emission bands have similar fluorescence intensity (excitation at 360 nm). Upon excitation at wavelengths less than 360 nm, such as 340 nm, the short emission wavelength is more intense and the perceived light is blue (Figure 3). Finally, for excitation at wavelengths greater than 360 nm, such as 380 nm, the long wavelength emission is more intense, and the emitting light is green.

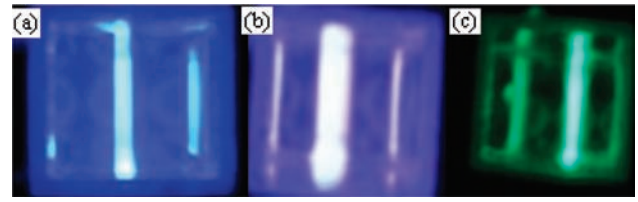


Figure 3. Photographs of **37** at 1×10^{-6} M in 0.1% DMSO (5 mM of phosphate buffer) (a) excitation at 340 nm, (b) excitation at 360 nm (c), and excitation at 380 nm.

On the basis of the present dearth of substituted 2,3-naphthalic anhydrides, this synthetic strategy offers several of these interesting chromophores in a relatively economical procedure. The high-yielding general route can be used to synthesize electron-poor or electron-rich substituted 2,3-naphthalimides from commercially available substituted phthalic acids. As proof of principle, this predictive model yielded nine (DF) dyes from a small synthetic matrix of ten compounds. Our hypothesis that 2,3-NI dyes would exhibit similar photophysics to 1,8-NI on the basis of symmetry proved to be a correct one with the added advantage that the 5-ring carboximide/6-ring *N*-aryl juncture permits broader fluorescence emission than the more restrictive 6-ring carboximide/6-ring *N*-aryl juncture of 1,8 NI. In addition these dyes exceeded our goals prescribed earlier such as water solubility, reduced chromophore size, and molecular weight, as well as possessing a synthetic path that allows synthetic diversity of substituents within the 2,3-NI framework. Immediate applications of these new dyes have been shown in detection of DNA, as well as new white-light fluorophores.

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Supporting Information Available: Synthetic and titration experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

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