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Matrix Screening of Substituted *N*-Aryl-1,8-naphthalimides Reveals New Dual Fluorescent Dyes and Unusually Bright Pyridine Derivatives

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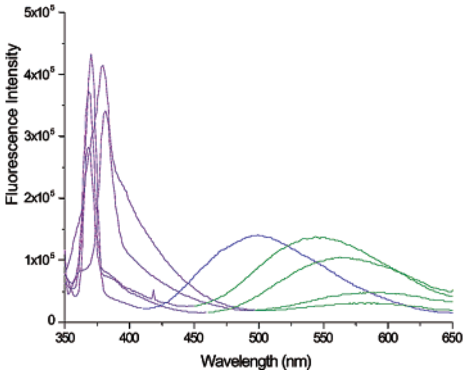

A 3 × 14 matrix of substituted *N*-aryl-1,8-naphthalimides was synthesized for the evaluation and discovery of dual fluorescence. Because of their unique photophysical properties, these dual fluorescent systems represent an exception to the widely studied TICT (Twisted Internal Charge Transfer) fluorescent dyes or tautomeric benzofluorescein class of two-color dyes. The matrix library was designed to investigate the effects of heterocycles, particularly π-excessive and π-deficient systems. Of the 42 compounds surveyed, five displayed well-resolved two-color emission in solvents as nonpolar as hexane. Based on the observed trends in fluorescence $\lambda_{\max}$ and quantum yield, a new model is proposed that predicts LW and SW emission for these systems. In addition, this model provides potential design features for the synthesis of new dual fluorescent species.

Introduction

While the term "atom economy" has been used to describe systems that maximize the efficiency of chemical reactions in organic processes,[1] another application of this phrase may be considered for molecules that display more than one useful photophysical process. With regard to applications such as molecular probes, the phenomena of two-color emission holds distinct advantages over other optical features such as two-photon absorption where the availability of required laser lines and/or the speed of wavelength switching can be restrictive.[2,3] Dyes featuring two emission wavelengths are preferred in methods such as flow cytometry and confocal microscopy where researchers not only observe intracellular analytes but also quantify them via ratiometric calibration.[4] Optical switches, OLED, logic gates, and molecular beacons represent additional research applications for such unique luminescence.[5]

Generally, dual fluorescent molecules can be categorized as either tautomeric systems such as the benzofluorescein class of compounds or TICT systems based

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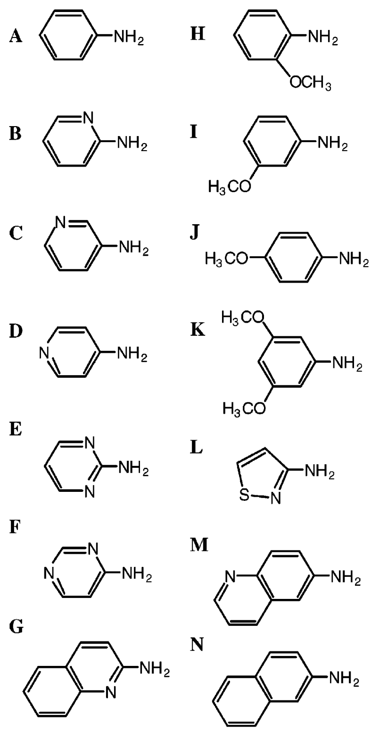
on 4-(*N*,*N*-dimethylamino)benzonitrile (DMABN) and its analogues.[6,7] Molecular Probes' SNARF displays particularly good separation of its two emission bands and has been widely used as a ratiometric probe for intracellular pH.[8] Recently, two-color emission band separation has been improved by exploring the initial and phototautomer states of the 3-hydroxyflavone (3HF) family of compounds.[9] Conversely, dyes which operate under the TICT mechanism tend to undergo fluorescence quenching in protic solvents. While these systems have been utilized as molecular probes for a number of analytes, solvent conditions remain limited to polar aprotic solvents such as acetonitrile in order to promote the longer wavelength emission.[10]

N-Arylnaphthalimdes, specifically 1,2-, 2,3- ,and 1,8-naphthalimides, appear to be a class of fluorescent dyes unlike either category and represent one of the few exceptions to the well-established model of TICT compounds.[11] By comparison, the photophysics of *N*-arylnaphthalimides more closely resemble the excited state properties of azulene where emission from its low-lying S2 state is typically observed.[12] Two emissive states (S1 and S2) capable of yielding both short wavelength (SW) and long wavelength (LW) fluorescence are thought to be present in the excited state of *N*-arylnaphthalimides. Whereas the geometry of the SW state is similar to the canted geometry of the ground state, a coplanar conformation, which results from interannular twisting in the excited state, appears to be responsible for the LW emission. Because of this conformational dependence on LW emission, electronic and steric factors intrinsic to the *N*-arene play a significant role in their fluorescence.

Our interest in these compounds stems from their successful use as a ratiometric probe for carbohydrates in aqueous conditions.[13] Because naphthalimides are inherently more polar than polycyclic aromatic hydrocarbons such as pyrene, additional functional groups enhance their water solubility. Since these compounds can potentially display discrete dual fluorescence in either protic or aprotic solvents, our attention focused on the mechanism responsible for this unique property. Reports by Berces et al. on *N*-phenyl-2,3-naphthalimides highlighted the role of solvent and rotational relaxation by examining several naphthalimides with *N*-aryl groups substituted at ortho, meta, and para positions.[10] Their findings revealed the importance of steric effects on the two emission bands and provided a general model that describes the influence that electron-withdrawing or electron-releasing groups have on SW and LW emission.[14] Because certain *N*-phenyl-1,8-naphthalimides were found to exhibit dual emission when substituted with methoxy at the naphthalene ring,[15] we prepared a matrix library that focuses on this synthetically less versatile component. In addition to probing the effects of substituents, heterocyles were also included to investigate how their more subtle π-excessive and π-deficient properties (as discussed by Marks and Ratner[16]) affect the emission wavelengths of these compounds.

TABLE 1. Chart Showing R^1 Substituent Groups on the Naphthalimide Ring Along with R^2 π-Deficient Substrates a−g and π-Excessive Substrates h−n

R^1	R^2
H Br OMe	

To observe the effects that electron-withdrawing groups have on the naphthalene ring component, we chose commercially available 4-bromo-1,8-naphthalic anhydride as a starting material that could be readily converted to an electron-releasing system such as 4-methoxynaphthalic anhydride. Although the bromo group is a well-known fluorescence quencher, these compounds provide

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SCHEME 1

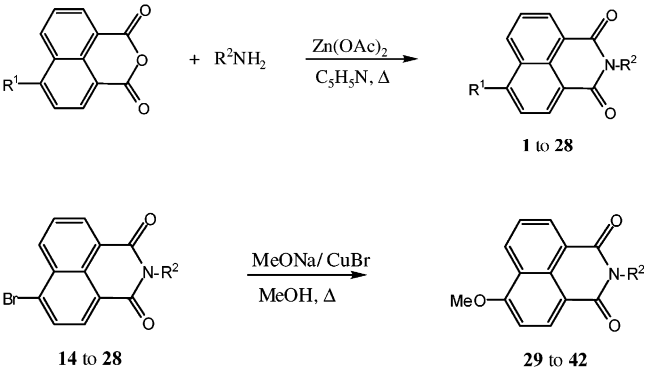

TABLE 2. Matrix Compilation of Substituent Groups for 1,8-Naphthalimide and *N*-Aryl Groups

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
R¹= H	1	2	3	4	5	6	7	8	9	10	11	12	13	14
R¹= Br	15	16	17	18	19	20	21	22	23	24	25	26	27	28
R¹= OMe	29	30	31	32	33	34	35	36	37	38	39	40	41	42

intermediates for an expedient synthetic route via nucleophilic substitution to methoxy-substituted compounds. While other electron-withdrawing groups were considered such as the commercially available 4-nitronaphthalic anhydride, this compound was omitted from our study since reduction of nitro to an electron-releasing amino substituent creates an additional variable of pH sensitivity.

Table 1 lists the 3-substituent groups used for the R1 components along with the π-deficient groups (**a**−**g**) and π-excessive groups (**h**−**n**). The three aminopyridines were selected to investigate the effects of heteroatom position on these heterocyclic systems as well as to screen for their notable fluorescence-quenching properties on the 4-methoxynaphthalimide platform. While similar comparisons can be made for compounds **e** and **f**, these heterocyclics were also chosen for their destabilizing properties on the aromatic system relative to homocylic phenyl groups. Based on theoretical investigations involving hyperpolarization of NLO chromophores, this destabilization has been predicted to enhance the internal charge-transfer properties of excited-state systems.[16] Aminoarene compounds **h** and **j** were selected for comparison with results from a previous report[15] and coupled to 4-methoxynaphthalimide since these compounds were previously found to exhibit dual emission. Finally, the sulfur component in aminothiazole (***l***) was selected for its lower rotational barrier relative to six-membered rings as well as its potential enhancement of internal charge transfer relative to homocyclic aromatic rings. Scheme 1 shows the synthetic route used to generate our matrix of compounds **1**−**28** and **29**−**42**. Table 2 provides the 3 × 14 matrix of compounds that was generated from the reactant elements naphthalic anhydride, substituted naphthalic imides, and aminoarenes.

Results

Figure 1 provides a graphical summary of the trends in quantum yield.[17] As shown in the figure, pyridine derivatives, when coupled to 4-methoxynaphthalene (**30**−**32**), display considerably higher quantum yields (up to 0.69) than their parent naphthalimide analogues (**2**−**4**). Such bright fluorescence for pyridine is typically observed only when methoxy groups are attached directly to the ring.[18] Within the 42-member matrix, 5 compounds displayed well-resolved SW and LW emission bands. Three of these two-color systems originated from dyes with a 4-bromo substituent on the naphthalene ring. Figure 2a−e shows the five spectra for these dual fluorescent compounds.[19,20] In all cases, these systems incorporated a π-excessive arene as the *N*-aryl group.

TABLE 3. Summary of Fluorescence Wavelengths and Quantum Yields[a]

entry	fluorescence λ (nm)	ϕ_F (10^{-3})	entry	fluorescence λ (nm)	ϕ_F (10^{-3})
1	377	0.1	**22**	377	0.8
2	378	4.2	**23**	376	0.7
3	378	2.1	**24**	379	0.3
4	378	12		591	
5	380	15	**25**	378	1.1
6	378	13	**26**	392	2.5
7	375	12	**27**	377	2.4
8	374	15		424	1.3
9	374	10	**28**	377	0.1
10	374	2.5		561	
	569	0.2	**29**	433	889
11	365	0.5	**30**	433	673
12	380	2.4	**31**	433	481
			32	435	694
13	363	0.1	**33**	424	591
	431	0.1	**34**	434	653
14	374	7	**35**	434	880
15	377	1	**36**	432	261
16	390	4.1	**37**	432	253
17	391	2.5	**38**	432	984
18	392	5.6	**39**	431	299
19	391	5.7	**40**	434	479
20	391	4.4	**41**	434	251
21	376	1.5	**42**	427	101

[a] Fluorescence quantum yields relative to quinine sulfate in water. Errors are on the order of ±5% for quantum yields larger than 0.1 and on the order of ±10% for all others. Acetonitrile used as solvent for this data set. Concentrations varied from 1.0×10^{-5} to 1.0×10^{-7} M. Excitation wavelengths used range between 330 and 360 nm.

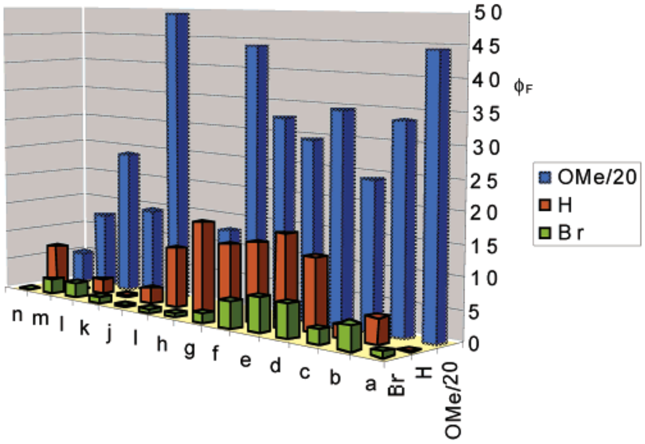

FIGURE 1. Bar graph of fluorescence quantum yield for compounds **1**−**42**.

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(17) No special efforts were made to purge oxygen from fluorescence samples.

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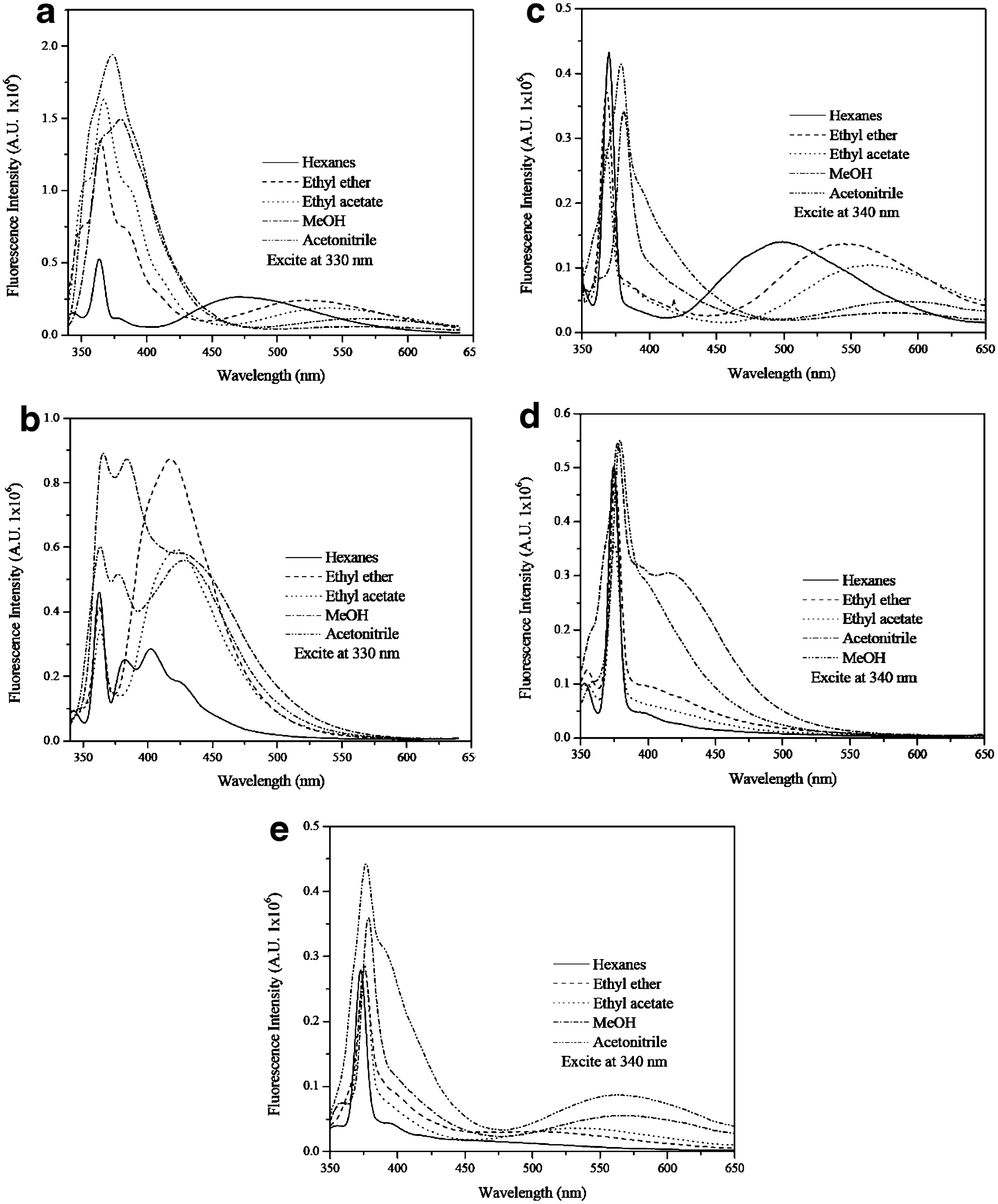

FIGURE 2. (a) Fluorescence spectrum of *N*-(4′-methoxyphenyl)-1,8-naphthalimide (**10**) at λ_{ex} 330 nm. (b) Fluorescence spectrum of *N*-(6′-quinolyl)-1,8-naphthalimide (**13**) at λ_{ex} 330 nm. (c) Fluorescence spectrum of 4-bromo-*N*-(4′-methoxyphenyl)-1,8-naphthalimide (**24**) at λ_{ex} 340 nm. (d) Fluorescence spectrum of 4-bromo-*N*-(6′-quinolyl)-1,8-naphthalimide (**27**) at λ_{ex} 340 nm. (e) Fluorescence spectrum of 4-bromo-*N*-(2′-naphthyl)-1,8-naphthalimide (**28**) at λ_{ex} 340 nm.

Figure 2a shows the weakest intensity for the LW emission within the series of dual fluorescent systems whereas spectrum 2b displays the most intense LW band centered at 475 nm. Compound **13** (Figure 2b) utilizes a 6-quinoline group as the *N*-aryl component; however, the intensity of this band is variable with the highest intensity in diethyl ether. Compound **24** (shown in Figure 2c) appears to give the best separation between bands with the LW emission band (centered at 500 nm) relative to its SW counterpart at 380 nm. Of particular interest,

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(19) In the event that exciplex interaction was possibly responsible for the LW emission, a concentration gradient experiment was conducted to examine concentration effects on emission wavelength. The study confirmed that no LW emission resulted from higher fluorophore concentrations.

(20) All fluorescence measurements were conducted at room temperature.

the LW band occurs in nonpolar hexane, an observation that is typically found for this class of compounds but rarely occurs for TICT dependent dyes. In certain solvent systems, **24** displays an enhanced LW band relative to its SW emission. Compounds **27** and **28** (Figure 2d,e) have similar 4-bromo substitution as well as 10π *N*-arenes coupled to the imide; however, the naphthalene group appears to provide an increased Stokes shift of the LW band.

In general, trends in the emission wavelengths indicated that bromo-substituted naphthalimdes coupled to π-excessive arenes promoted two-color emission. Methoxy substituents on the naphthalimide ring influenced the emission toward a single SW emission along with high quantum yield. In agreement with those findings of Berces, such high quantum yields were not observed when methoxy groups are located on the *N*-aryl component. The parent 1,8-naphthalimide displayed LW emission when coupled to any of the methoxy-substituted anilines and in certain cases dual fluorescence (Figure 2a) if coupled to *p*-methoxyaniline. Because of steric hindrance and therefore prevention of a planar internal charge transfer excited state proposed with these systems, anilines substituted by methoxy at the 2- or 3-position displayed only SW emission. When methoxy groups were located on both the *N*-aryl component and the naphthalimide, only SW emission was observed. Unlike the results reported by Berces et al., compounds **38** and **40** did not display two-color emission under our experimental conditions.[21] Based on our criteria for dual fluorescence, none of the 4-methoxynaphthalimide systems were found to exhibit two well-resolved emission bands. In addition to methoxy-substituted *N*-aryl groups, other π-excessive arenes displayed similar spectroscopic features.

These findings along with quantum yield trends suggest that the substitution on the naphthalimide ring plays the dominant role in SW versus LW fluorescence as well as its relative brightness. Additional evidence is found in the remarkably high quantum yields obtained for pyridine derivatives **30**−**32**. In the case of brominated naphthalimides, the electron-withdrawing properties of this group influenced the fluorescence toward SW emission. This spectral feature occurred regardless of whether a π-excessive or π-deficient group was coupled as the *N*-arene.

Based on these results, we propose a model that summarizes the fluorescence behavior for 1,8-naphthalimides substituted at the naphthalene ring and builds on the earlier model for 2,3-naphthalimides. Because both 1,8- and 2,3-naphthalimdes share a similar point group, C_2 (or C_{2v} for planar conformation), the spectroscopic features of these two systems are expected to be similar. In this modified diagram, the energy levels of the LW and SW excited states are depicted to be sensitive to the substitution on the aryl ring and naphthalene ring, respectively. As shown in Figure 3, the energy level diagram indicates that the substituted 1,8-naphthalimide moeity affects the SW excited state whereas the *N*-aryl group influences the LW excited-state energy level. To illustrate the spectral bias of the naphthalimide component over the *N*-aryl group, the arrows which correspond to the effects of substituents located on the naphthalene ring have been augmented relative to arrows which represent aryl-substituent effects. This modification to the diagram allows one to predict the fluorescence outcome for a given set of substituent groups. For example, if both x and y substituent groups are present and electron releasing, the single emission wavelength is expected to be SW. In this case the SW emission is governed primarily through the x substituent. Fluorescence emission from compounds **29**−**42** appears to follow this trend. If on the other hand, x is hydrogen, and y is π-excessive, the compound should display LW emission or, as in the case of compounds **10** and **13**, both LW and SW.[22] Finally, when the weakly electron-withdrawing properties of the bromine-substituted systems are coupled to π-excessive groups, a mixture of SW and LW excited states occurs and allows for dual fluorescence from compounds **24**, **27**, and **28**.

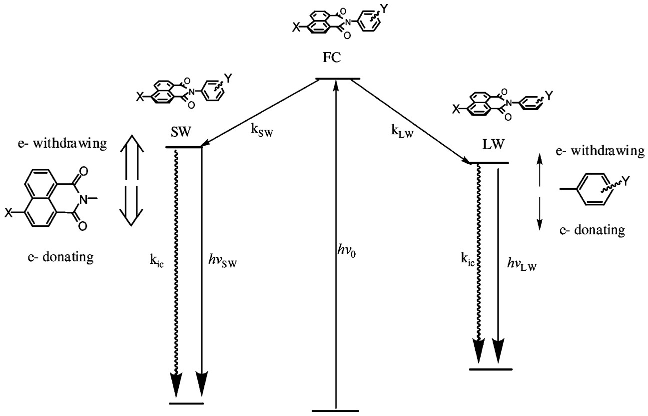

FIGURE 3. Modified energy level diagram depicting the Franck−Condon state (FC) undergoing vibrational relaxation to SW or LW emission.[23] Structures representing the FC state and the SW state show similar structure whereas the LW state indicates the excited state with extended conjugation (ESEC).

These findings portend future designs of dual fluorescent species based on the naphthalimide platform. As mentioned earlier, 1,8- and 2,3-naphthalimides possess a common C_2 symmetry that allows cross comparisons in their spectroscopic features with certain limitations regarding stronger steric interactions for the 1,8- relative to 2,3-systems. Moreover, results from these matrix elements suggest that systems which incorporate electron-attracting groups on the naphthalimide component and electron-releasing groups located at the para-position of the *N*-aryl component should display dual fluorescence. Therefore, alternative electron-attracting groups on the naphthalimide component which are less quenching than bromine (such as cyano, fluoro, or carboxyl ester) represent suitable candidates for two-color emission along with

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(21) Similar to ref 15, we observed absorption maxima at 340, 353, and 369 nm; however, excitation at these wavelengths did not promote LW emission.

(22) Although compounds **13** and **27** have emission bands at 431 and 424 nm, respectively labeled as LW, this wavelength is considered to be the LW emission relative to the shorter wavelength emission that occurs respectively at 373 and 377 nm.

(23) As pointed out in ref 14, certain components of the SW emission originate from the partial repopulation of SW by the LW excited state. According to the diagram from Wintgens et al., an arrow between these two states is depicted which indicates that these two states are kinetically connected.

potentially higher quantum yield. Based on symmetry arguments, this strategy should apply equally well to *N*-aryl-2,3-naphthalimides. Although these fluorescent measurements were not conducted in water due to the limited solubility for this set of dyes, our earlier work with boronic acid functionalized systems indicates that polar groups appended to the aryl component should allow for water-soluble applications.[13]

In summary, a 3 × 14 matrix of *N*-aryl-1,8-naphthalimdes was synthesized to assay for the relatively rare photophysical event of two-color emission. The library was constructed in order to reveal both electron releasing and withdrawing effects on naphthalimide in combination with π-excessive/π-deficient arenes. Four new two-color fluorescent compounds were identified in addition to one reported from a previous study.[14] Fluorescence results obtained from this matrix suggest a modified energy level diagram, which indicates that substituent groups on both aromatic systems can be used to account for the trends in SW vs LW emission. Compounds which utilize pyridine as the *N*-aryl group and also display remarkably high quantum yields provide strong evidence that the naphthalene ring governs their photophysics. Our model for dual substitution of *N*-aryl-1,8-naphthalimides builds on an earlier model proposed for the photophysical properties of monosubstituted *N*-aryl-2,3-naphthalimides. The observed trends in fluorescence lend support to the excited state with the extended conjugation (ESEC) model[11] and allow one to design systems which are biased to either SW or LW or potentially both SW and LW emissive compounds.

Experimental Section

Synthesis for 1 to 14. 1,8-Naphthalenedicarboxylic anhydride (0.30 mmol), arylamine (0.25 mmol), 4 Å molecular sieves, 30 mg of $Zn(OAc)_2$, and 25 mL of pyridine were placed in a 50-mL round-bottom flask, equipped with a Dean–Stark receiver and condenser. The reaction mixture was allowed to reflux for 8 h. Pyridine was removed by distillation and replaced with acetone. The acetone solution was concentrated with use of a rotary evaporator and the crude solid was purified by using preparative layer chromatography (PLC). The reaction afforded white powder.

Synthesis for 15 to 28. 4-Bromo-1,8-naphthalenedicarboxylic anhydride (0.30 mmol), arylamine (0.25 mmol), 4 Å molecular sieves, 30 mg of $Zn(OAc)_2$, and 25 mL of pyridine were placed in a 50-mL round-bottom flask, equipped with a Dean–Stark receiver and condenser. The reaction mixture was allowed to reflux for 8 h. Pyridine was removed by distillation and replaced with acetone. The acetone solution was concentrated with use of a rotary evaporator and the crude solid was purified with silica gel PLC. The reaction afforded white powder.

Synthesis for 4–29 to 4–42. 4-Bromo-1,8-naphthalimide derivatives (compounds **4–15** to **4–28**) (0.20 mmol), 15 mg of CuBr, and a 10:1 stoichiometric ratio of sodium methoxide in methanol were stirred and refluxed for 6 h. Methanol was removed by distillation. Crude product was purified by silica gel PLC. The reaction afforded a tan or yellow powder.

Optical Measurements. For all UV–vis and fluorescence measurements solvent blanks were run for each respective solvent system and subtracted from the recorded sample absorption or emission spectrum.

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Supporting Information Available: UV–vis spectra, NMR, IR, and elemental analyses of the products are included in this section. This material is available free of charge via the Internet at http://pubs.acs.org.

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