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Synthesis and Photophysical Properties of a Series of Cyclopenta[*b*]naphthalene Solvatochromic Fluorophores

Erica Benedetti, Laura S. Kocsis, and Kay M. Brummond*

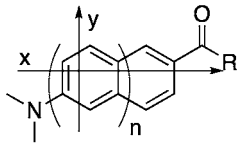
Department of Chemistry, University of Pittsburgh, Pittsburgh, Pennsylvania 15260, United States

Supporting Information

ABSTRACT: The synthesis and photophysical properties of a series of naphthalene-containing solvatochromic fluorophores are described within. These novel fluorophores are prepared using a microwave-assisted dehydrogenative Diels–Alder reaction of styrene, followed by a palladium-catalyzed cross coupling reaction to install an electron donating amine group. The new fluorophores are structurally related to Prodan. Photophysical properties of the new fluorophores were studied and intriguing solvatochromic behavior was observed. For most of these fluorophores, high quantum yields (60–99%) were observed in methylene chloride in addition to large Stokes shifts (95–226 nm) in this same solvent. As the solvent polarity increased, so did the observed Stokes shift with one derivative displaying a Stokes shift of ∼300 nm in ethanol. All fluorophore emission maxima, and nearly all absorption maxima were significantly red-shifted when compared to Prodan. Shifting the absorption and emission maxima of a fluorophore into the visible region increases its utility in biological applications. Moreover, the cyclopentane portion of the fluorophore structure provides an attachment point for biomolecules that will minimize disruptions of the photophysical properties.

Fluorescent-based tools are widely used to monitor environments of biological events.[1] Fluorescence is used for a variety of reasons, including the nondestructive nature of these measurements. Of the fluorescent probes available, small organic fluorophores are gaining in popularity due to rapid response times for monitoring real-time events with excellent spatial resolution.[2] In addition, their relatively small size minimizes disruption of the environment being studied. The widespread use of these probes is enabled by the commercial availability of hundreds of fluorescent dyes.[3] However, there are many elements to consider when choosing the optimal fluorescent tool, including quantum yield, absorption and emission wavelengths, photo- and chemical stability, and Stokes shift to name just a few. Each of the commercially available dyes comes with a list of advantages and compromises that must be weighed for any application. Thus, new small molecule-based fluorescent probes are continually being developed to encompass all the desired elements while minimizing compromises.[4]

A common structural feature in many fluorophores is a conjugated π-system functionalized with both an electron-withdrawing and an electron-donating group. For many of these fluorescent compounds, the π-system is composed of a naphthalene moiety. The synthesis of these fluorescent chromophores usually begins with commercially available naphthalene derivatives or, alternatively, new naphthalene-based dyes are developed by modifying an existing fluorophore. An excellent example of the latter protocol involves Prodan (**1a**), a compound whose fluorescent emission wavelength and quantum yield are unusually dependent upon solvent polarity (Figure 1). For example, in cyclohexane, the fluorescent



1a, R = CH_2CH_3, n = 1
1b, R = $(CH_2)_{10}CH_3$, n = 1
1c, R = $CHCH_2$, n = 1
1d, R = CH_2Br, n = 1
1e, R = $CH_2C(CO_2H)NHFmoc$, n = 1
1f, R = CH_2CH_3, n = 2

Figure 1. Prodan and Its Derivatives.

emission of Prodan is 410 nm and in water it is 534 nm, a bathochromic shift of 124 nm.[5] Environmentally sensitive dyes are of special interest as probes, and Prodan and its analogues are considered to be state of the art for application in biological systems.[6] Structural variants of Prodan include the lipophilic Laurdan (**1b**), the thiol reactive Acrylodan (**1c**) and Badan (**1d**), and the amino acid-containing Aladan (**1e**).[7] The latter dye is important for monitoring protein interactions and dynamics.[8] In addition, a spectrally red-shifted compound, Anthradan (**1f**), has been prepared that incorporates an anthracene ring between the electron donating and electron withdrawing groups resulting in an emission spectra in hexanes of 483 nm and of 604 nm in methanol.[9] Recently, a fluorene analogue of Prodan was prepared that showed excellent brightness when compared to Prodan.[10] In addition, Prodan has been conjugated to four nucleosides to aid in the structure determination of DNA (structures of the latter two examples are not shown).[11]

For each of these Prodan derivatives, the donor-acceptor substituents are located along the *x*-axis (longer axis) of the naphthalene π-system, and the amino group can be characterized as exonuclear and sterically unhindered. For the anthracene analogue, even though the emission wavelength was significantly red-shifted, the quantum efficiency was lower. The synthesis of fluorophores possessing more desirable properties could be significantly enhanced by more efficient and versatile methods for the construction of naphthalene derivatives.[12]

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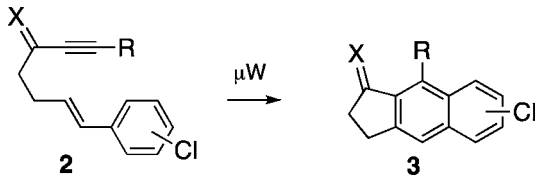
In another paper, we described the synthesis of a series of structurally novel naphthalene compounds using a microwave-assisted dehydrogenative Diels–Alder reaction of styrene (Scheme 1).[13] One of these cycloadducts was functionalized

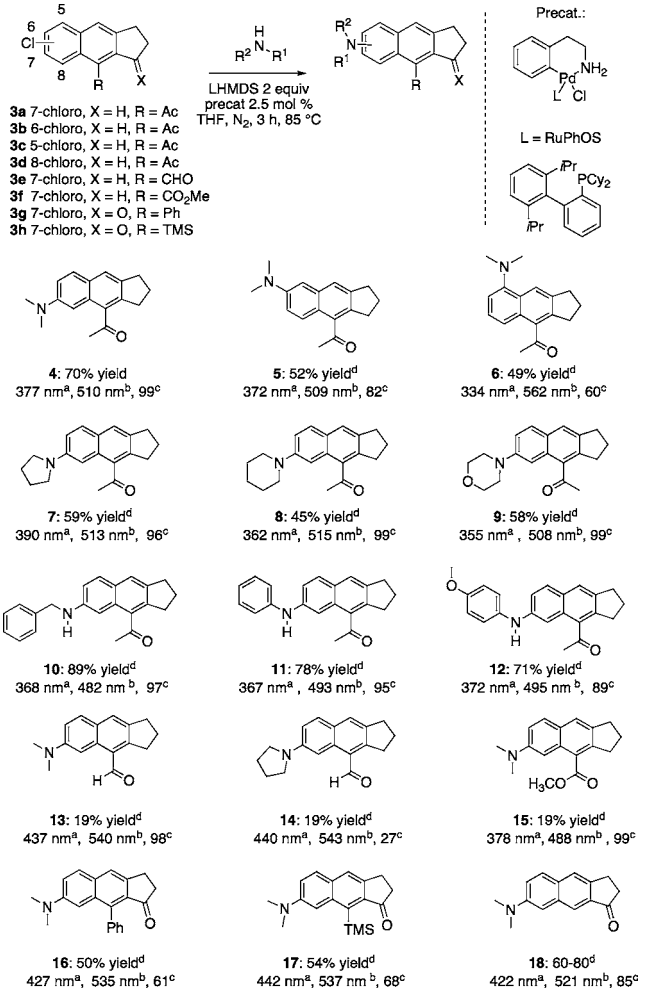
Scheme 1. Dehydrogenative Diels–Alder Reaction



with acetyl and dimethylamino groups to give compound **4**. This compound showed very interesting optical properties when compared to Prodan in that the absorption and emission maxima in methylene chloride (CH_2Cl_2) were significantly red-shifted by 22 and 70 nm, respectively. Moreover, this compound was highly fluorescent with a quantum yield of 99%! Thus, simply changing the positions of the electron-donating and electron-withdrawing groups on the naphthalene ring resulted in a photophysical behavior previously only available by adding an additional ring to the π-system, as in both Anthradan (**1e**) and a fluorene analogue of Prodan.[9] However, unlike these Prodan derivatives, compound **4** is relatively small, thereby limiting potential disruption of the system under study, while displaying a high quantum yield. Furthermore, the bathochromic shift of the absorption of compound **4** (22 nm) into the visible region allows for excitation with visible light, compared to Prodan which absorbs in the UV range limiting its application to biology. These interesting properties prompted us to prepare a number of structurally related compounds in search of fluorescent dyes with superior photophysical properties. Moreover, the versatility of our synthetic approach using the dehydrogenative Diels–Alder reaction enables a systematic study and rationally designed fluorophores with desirable properties for biological applications.

With an eye toward the preparation of a series of aminonaphthalene derivatives, *ortho*-, *meta*-, and *para*-chlorinated styrenyl Diels–Alder precursors **2** were subjected to the dehydrogenative Diels–Alder reaction to afford the corresponding chlorinated cyclopenta[*b*]naphthalene derivatives **3** (Scheme 1). Next, cross-coupling reactions were examined for the introduction of electron-donating amino groups via the chloro group of the naphthalene derivatives **3**. For this process, a palladium-catalyzed amination of aryl chlorides emerged as a valuable tool for producing the fluorophore structure; the advantages to carrying a chloro group through a synthetic sequence instead of the more reactive bromo or iodo groups cannot be overstated.[14,15] For the first generation of fluorescent compounds, dimethylamine and chloronaphthalenes **3a–d** were synthesized and reacted with dimethylamine to produce Prodan-like fluorophores so that photophysical properties of these first generation fluorophores could be directly compared to that of Prodan (Scheme 2). The coupling reaction of **3a** and **3c** using a commercially available RuPhos precatalyst (2.5 mol %) and lithium hexamethyldisilazide (LHMDS) in tetrahydrofuran (THF) afforded the corresponding *N*,*N*-dimethylamine substituted cyclopenta[*b*]naphthalenes **4** and **6** in 70% and 49% yield, respectively.[16] Different palladium catalysts ($Pd(OAc)_2$) and bases (K_3PO_4, Cs_2CO_3) were also screened,

Scheme 2. Pd-Catalyzed Cross-Coupling Reaction and Photophysical Properties of Functionalized Naphthalenes



[a]Absorption maximum in CH_2Cl_2. [b]Emission maximum in CH_2Cl_2. [c]Fluorescence quantum yield vs Prodan in DMSO (91%). [d]Yield optimizations were not performed on this substrate.

but all resulted in lower yields of the coupling products. Attempts were not made to further optimize the reaction conditions. Each amine compound in Scheme 2 was obtained from the corresponding chloronaphthalene using these same reaction conditions. Interestingly, a mixture of **3b** and **3d** gave two products, amine **5** in 52% yield along with the dehalogenated cyclopenta[*b*]naphthalene. It is hypothesized that this latter compound arises from a palladium-catalyzed dehalogenation reaction of **3d** facilitated by the close proximity of the methyl ketone. The photophysical properties of these first generation fluorophores, including the solvatochromic properties were examined (below). For our second generation of fluorophores, a number of amines were coupled with **3a**. Secondary cyclic amines such as pyrrolidine, piperidine, and morpholine gave the corresponding tertiary amines **7**, **8**, and **9** in 59%, 45%, and 58% yield, respectively. Primary amines such as benzylamine, aniline, and *para*-methoxyaniline were also successfully coupled with **3a** to afford **10**, **11**, and **12** in 89%, 78%, and 71% yield, respectively. Compounds **13**, **14**, and **15** were prepared in a similar manner from aldehyde **3e** and ester **3f**. Finally, ketones **16** and **17** were prepared from the corresponding cyclopentanones **3g** and **3h**. Desilylation of silyl

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Table 1. Spectroscopic Properties of Fluorophores 4−6 in Comparison to Prodan

		Prodan			4			5			6		
Entry	Solvent	λ_{abs}[a] nm	λ_{em}[b] nm	ϕ_f %	λ_{abs}[a] nm	λ_{em}[b] nm	ϕ_f[c] %	λ_{abs}[a] nm	λ_{em}[b] nm	ϕ_f[c] %	λ_{abs}[a] nm	λ_{em}[b] nm	ϕ_f[c] %
1	*n*-Hexane	340	389	2.0	/	/	/	/	/	/	/	/	/
2	Cyclohexane	/	/	/	373	466	45	363	445	8	314	480	28
3	Toluene	346	416	56	376	490	62	368	481	25	322	520	47
4	1,4-Dioxane	346	422	75	376	495	46	370	495	38	319	534	44
5	THF	348	430	78	377	497	75	370	505	34	330	543	39
6	CH_2Cl_2	355	440	98	377	510	99	372	509	82	334	562	60
7	$CHCl_3$	/	/	/	377	516	77	373	511	41	334	562	40
8	Acetonitrile	350	455	80	375	529	99	370	545	18	316	590	23
9	DMSO	357	462	91	377	536	85	374	558	37	334	598	48
10	EtOH	362	485	71	377	578	5	374	605	3	334	634	1

[a]Absorption maximum. [b]Emission maximum. [c]Fluorescence quantum yield vs Prodan in DMSO (91%), excitation at 334 nm (10^{-5} M).

naphthalene **17** with tetra-*n*-butyl ammonium fluoride afforded **18**.

With a series of novel fluorophore compounds in hand, absorption and emission maxima along with quantum yields were measured in CH_2Cl_2 (Scheme 2). Notable trends for this series of compounds were observed. For example, compound **6** containing a 1,5-substituted cyclopenta[*b*]naphthalene moiety absorbed light at a much shorter wavelength (334 nm) and fluoresced at a much longer wavelength (562 nm) than either the 1,7- or 1,6-disubstituted compounds **4** or **5** (absorption and emission maxima for both are ∼375 and ∼510 nm). Interestingly, the absorption and emission maxima along with the quantum yield of compounds **5** and **6** are almost identical to previously reported, and structurally related, analogues of Prodan lacking the five-membered ring.[17] Thus, it can be concluded that the cyclopentane ring has little effect on the photophysical properties of these new fluorophores. The tertiary cyclic amino ketone series of compounds **7**, **8**, and **9** showed a range of absorption maxima (355−390 nm), while the emission maxima remained relatively constant (508−515 nm). For example, the absorption of the pyrrolidine-substituted naphthalene **7** was red-shifted by 13 nm when compared to the dimethylamine derivative **4**. This subtle change in structure affords a compound that may be more amenable to biological studies because the absorption maximum is in the visible region. The emission spectra of the secondary amines **10**−**12** were significantly blue-shifted (482−495 nm) when compared to the tertiary amines, but there were not significant differences in absorption maxima between each of the secondary amines.

Substitution of the ketone group with an aldehyde or ester significantly affects the photophysical properties. For example, the absorption maxima of aldehydes **13** and **14** are red-shifted by 50−60 nm when compared to ketones **4** and **7**. However, the pyrrolidine group of aldehyde **14** had almost no effect on the absorption maximum when compared to aldehyde **13**. The emission maximum for both aldehydes **13** and **14** were red-shifted by 30 nm when compared to ketones **4** and **7**. Substitution of the ketone group with an ester group results in a blue shift in the emission maximum of 22 nm (see compounds **4** and **15** for a comparison).

A series of compounds with a carbonyl in the five membered ring was synthesized and displayed a significant red shift in the absorption and emission maxima comparable to that observed for aldehydes **13** and **14**. For example, fluorophores **16**, **17**, and **18** have absorption maxima at 427, 442, and 422 nm, respectively, with respective emission maxima of 535, 537, and 521 nm. The effect that the silyl group has on the absorption is in agreement with the bathochromic shift that was previously reported for other monosilyl substituted naphthalene derivatives, only more pronounced (20 nm vs 9 nm).[18] Finally, the quantum yields for all fluorophores in Scheme 2 were extremely high (82−99%), with four exceptions, compounds **6**, **14**, **16**, and **17**.

Finally, solvatochromic properties for the first generation fluorophores **4**−**6** were measured in a number of solvents varying in polarity (Table 1 and Scheme 2). Several important findings emerged from these measurements, the first being that for all three fluorophores, the emission maxima are significantly red-shifted as the solvent polarity increases. For example, the emission maxima for compounds **4**, **5**, and **6** are 466, 445, and 480 nm in cyclohexane and 578, 605, and 634 nm in ethanol, respectively. The range of emission and absorption wavelengths for these fluorophores along with the large Stokes shift of 300 nm for compound **6** are potentially valuable for multiplexing experiments.[19] Moreover, the solvatochromic emission spectra are significantly red-shifted when compared to Prodan, which emits at 389 nm in hexanes and 485 nm in ethanol. Red-shifted fluorescent emissions are important for biological applications where background fluorescence can limit the magnitude of the fluorescent change for fluorophores emitting and absorbing at shorter wavelengths.[15] A brief examination of the photobleaching properties was performed on compounds **4**−**6** in CH_2Cl_2. All three fluorophores were found to have photodegradation curves similar to that of Prodan (see Supporting Information).

In conclusion, ready access to a series of environmentally sensitive fluorescent compounds has been accomplished by using a dehydrogenative Diels−Alder cycloaddition reaction followed by a palladium-catalyzed cross-coupling reaction. It is anticipated that these compounds or other readily prepared fluorophores will prove useful as fluorescent markers in

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biological systems. Moreover, the ease with which the compounds can be prepared, and our ability to incorporate a diversity of electron-withdrawing and electron-donating groups into the synthetic route, will aid in a deeper understanding of the photophysical properties of not only this class of fluorophores, but also others, thus, contributing to the arsenal of rationally designed fluorescent probes. While some chemical modifications of the Prodan scaffold have been studied, systematic construction of a library of Prodan derivatives has not been reported, in part, due to synthetic challenges. Our approach provides ready access to a variety of Prodan-like fluorophores. Furthermore, it is concluded that the fused five-membered ring does not affect the photophysical properties of naphthalene-containing compounds **4**–**15**. The fused ring provides a point of attachment for biomolecules or water solublizing groups with the ultimate goal of developing new probes that can be used more effectively for imaging in living cells and whole organisms. Finally, the red-shift of the absorption and emission maxima of the cyclopentanone-containing compounds is intriguing and supports the premise that this class of compounds emit from a planar intramolecular charge transfer (PICT) excited state.[20] Studies are ongoing to delineate the effect these subtle structural changes have on the photophysical properties and to design improved fluorophores for application to biological systems.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures and characterization data for all new compounds along with fluorescent emission spectra for compounds **4**–**18**; photodegradation decay curves for compounds **4**–**6** and solvatochromic spectra for compounds for **4**–**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

kbrummon@pitt.edu

Notes

The authors declare no competing financial interest.

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