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Authors

Kocsis, Laura S
Elbel, Kristyna M
Hardigree, Billie A
et al.

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Cyclopenta[*b*]naphthalene cyanoacrylate dyes: synthesis and evaluation as fluorescent molecular rotors†

Laura S. Kocsis,[a] Kristyna M. Elbel,[b] Billie A. Hardigree,[c] Kay M. Brummond,*[a] Mark A. Haidekker*[c] and Emmanuel A. Theodorakis*[b]

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We describe the design, synthesis and fluorescent profile of a family of environment-sensitive dyes in which a dimethylamino (donor) group is conjugated to a cyanoacrylate (acceptor) unit *via* a cyclopenta[*b*]-naphthalene ring system. This assembly satisfies the typical D–π-A motif of a fluorescent molecular rotor and exhibits solvatochromic and viscosity-sensitive fluorescence emission. The central naphthalene ring system of these dyes was synthesized *via* a novel intramolecular dehydrogenative dehydro-Diels–Alder (IDDDA) reaction that permits incorporation of the donor and acceptor groups in variable positions around the aromatic core. A bathochromic shift of excitation and emission peaks was observed with increasing solvent polarity but the dyes exhibited a complex emission pattern with a second red emission band when dissolved in nonpolar solvents. Consistent with other known molecular rotors, the emission intensity increased with increasing viscosity. Interestingly, closer spatial proximity between the donor and the acceptor groups led to decreased viscosity sensitivity combined with an increased quantum yield. This observation indicates that structural hindrance of intramolecular rotation dominates when the donor and acceptor groups are in close proximity. The examined compounds give insight into how excited state intramolecular rotation can be influenced by both the solvent and the chemical structure.

Introduction

The study of events in chemistry, biology, and materials science requires the development of sophisticated sensory devices that can accurately measure changes that occur in their environment.[1] For instance, questions related to cell function, tissue organization and organ activities in normal and diseased states cannot be answered without the use of specifically designed sensors.[2] A subclass of these sensors can provide dynamic information of their surrounding stimuli by accordingly modifying their fluorescence emission due to solvatochromism.[3] Often referred to as fluorescent molecular rotors (FMRs), these environment-sensitive dyes[4] can be used to measure fluctuations in properties of their microenvironment (*e.g.* pH, viscosity, polarity, voltage and presence of specific analytes) in real time and with high spatial-temporal resolution.[5,6]

Typically, the FMR motif consists of an electron donor group that is in conjugation with an electron acceptor group (D–π-A motif).[7] In this configuration, the probe responds to photoexcitation with an intramolecular charge transfer from the donor to acceptor. Environmental factors (*e.g.* solvent polarity) affect the energy levels of either the ground or excited states and shift the fluorescence emission wavelength accordingly. Moreover, relaxation from the excited state can occur *via* fluorescence emission and/or *via* mechanical de-excitation (*e.g.* rotation across the σ-bonds). Thus, the fluorescence quantum yield of FMRs depends on the rigidity, *i.e.* viscosity, of their surrounding environment.[8] Specifically, if the intramolecular rotation around the σ-bonds between donor and acceptor becomes hindered, *e.g.* within a viscous or rigid environment, the quantum yield of fluorescence intensity increases. Thus, photoexcitation of an FMR in the solid state leads mainly to fluorescence emission with a quantum yield close to unity, since the σ-bonds of the molecule cannot rotate. On the other hand, photoexcitation in a fluid environment (*e.g.* low viscosity or high free volume) leads to both fluorescence and non-fluorescence relaxation processes, the ratio of which is related to the fluidity of the medium.[9]

[a]*Department of Chemistry, University of Pittsburgh, 219 Parkman Avenue, Pittsburgh, PA 15260, USA. E-mail: kbrummond@pitt.edu*
[b]*Department of Chemistry & Biochemistry, University of California, San Diego, 9500 Gilman Drive MC: 0358, La Jolla, CA 92093-0358, USA. E-mail: etheodor@ucsd.edu; Fax: +1-858-822-0386; Tel: +1-858-822-0456*
[c]*College of Engineering, University of Georgia, 597 D. W. Brooks Drive, Athens, GA 30602, USA. E-mail: mhaidekk@uga.edu*
† Electronic supplementary information (ESI) available: ^{1}H and ^{13}C NMR spectra for all compounds. See DOI: 10.1039/c4ob02563f

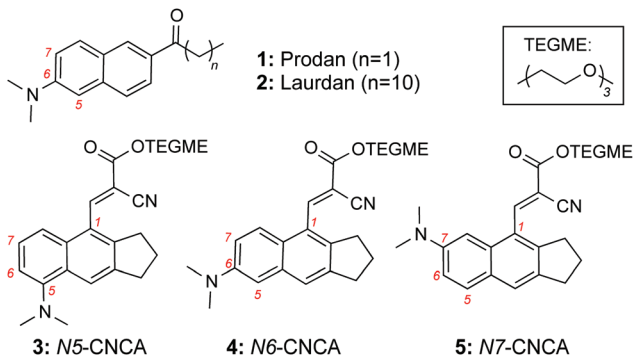


Fig. 1 Chemical structures of Prodan (**1**), Laurdan (**2**), and cyclopenta[*b*]-naphthalene cyanoacrylate (CNCA) dyes (**3–5**).

The fluorescent dye Prodan (6-propionyl-2-dimethylamino-naphthalene, **1**) is one of the best-known FMRs as it exhibits a strong bathochromic shift of its emission wavelength in polar solvents.[10] Both Prodan and its more lipophilic analogue Laurdan (6-dodecanoyl-2-dimethylaminonaphthalene, **2**) are frequently used to measure changes in viscosity and polarity in phospholipid bilayers and cell membranes.[11] Various structural modifications of Prodan that maintain its fluorophoric motif but have altered substitutions at the alkyl side chain, *e.g.* acrylodan,[12] badan[13] and aladan,[14] have been evaluated as FMRs. In addition, replacement of the naphthalene motif by an anthracene[15] and a fluorene[16] ring system has produced red-shifted dyes suitable for various biological applications. Inspired by this data, we sought to apply a novel intramolecular dehydrogenative dehydro-Diels–Alder (IDDDA) reaction[17] for the synthesis of a new family of functionalized naphthalenes, referred to here as CNCA dyes, and evaluate them as FMRs. Importantly, this key reaction allows the incorporation of the donor–acceptor pair at different positions of the naphthalene ring thereby permitting a methodical evaluation of structure–photophysical property relationships.[17a,18] Herein, we present the synthesis, fluorescent profile and viscosity/polarity sensitivity of these probes (Fig. 1).

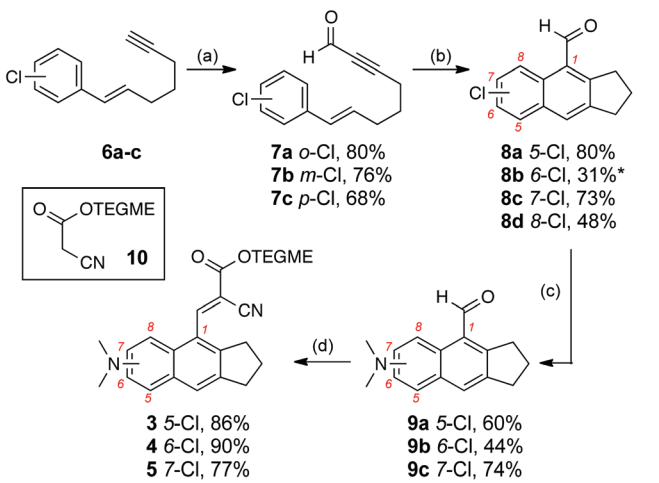
Results and discussion

Design criteria of CNCA probes

Our recently developed IDDDA reaction of styrenyl derivatives provides efficient and *de novo* access to a variably functionalized cyclopenta[*b*]naphthalene scaffold. We envisioned that further substitution of this scaffold with an amine (donor group) and a cyanoacrylate (acceptor group) would create the desired D–π-A fluorophore, referred to here as the cyclopenta-[*b*]naphthalene cyanoacrylate (CNCA) motif. In accordance with previous studies,[19] we anticipated that this motif would exhibit a viscosity-sensitive emission behavior. To increase solubility of the lipophilic motif in polar solvents, we decided to attach at the acceptor unit a triethylene glycol monomethyl ether (TEGME) group at the acceptor unit.[20] The synthesized CNCA dyes were designed to test the effect of the relative position of the dimethylamino and the cyanoacrylate groups on solvatochromism and viscosity sensitivity.

Synthesis of CNCA probes

The CNCA dyes **3–5** were synthesized *via* a four-step protocol beginning from enyne substrates **6a–c** (Scheme 1). A pyridinium chlorochromate oxidation of commercially available 5-hexyn-1-ol, followed by a Horner–Wadsworth–Emmons reaction of the resulting aldehyde with *n*-butyllithium and chlorobenzylphosphonate produced the enyne precursors **6a–c**, as previously reported.[17a] Formylation of the lithium acetylide of **6a–c** by addition of *N*,*N*-dimethylformamide formed aldehydes **7a–c** in 68–80% yield. Naphthalene derivatives **8a–c** were then generated by a microwave-assisted IDDDA reaction of **7a–c** at 180 °C for 1 h (73–80% yield).[17a] The IDDDA reaction of **7b** resulted in formation of 8-chloro-naphthalene derivative **8d** in addition to **8b** in a 79% combined yield (1 : 1.5 **8b** : **8d**). Conversion of chloro-substituted naphthalenes **8a–c** to the amine derivatives **9a–c** was conducted *via* a palladium-catalyzed Buchwald–Hartwig cross-coupling reaction employing RuPhos palladacycle, cesium carbonate, and dimethylamine (44–74% yield).[18] Attempts to perform a Buchwald–Hartwig cross-coupling reaction[21] on the sterically hindered naphthalene **8d** were unsuccessful. Subsequent Knoevenagel condensation reactions of **9a–c** utilizing cyanoester **10**[20] and catalytic 1,8-diazabicycloundec-7-ene (DBU) provided CNCA dyes **3–5** in 77–90% yield. This condensation is known to produce the thermodynamically stable *trans* alkenes, in which the ester group and the aromatic ring reside at the opposite face of the newly formed double bond. Nonetheless, upon purification of **3–5** we observed partial double bond isomerization (*trans*/*cis* ratio:



Scheme 1 *Reagents and conditions*: (a) **6a–c** (1.0 equiv.), *n*-butyllithium (1.0 equiv.), DMF (2.0 equiv.), THF, −78 °C to rt; (b) **7a–c**, *o*-dichlorobenzene, μW, 180 °C, 1 h; (c) RuPhos palladacycle (3 mol%), cesium carbonate (2.0 equiv.), **8a–c** (1.0 equiv.), dimethylamine (2.0 M in THF, 3.0 equiv.), THF, 85 °C, 2.5–5 h; (d) **9a–c** (1.0 equiv.), **10** (0.8 equiv.), DBU (0.010 equiv.), THF, 50 °C, 3 h. *Formed as a mixture with 48% of 8-Cl substituted product **8d**.

2.2/1) that likely occurred due to the acidic nature of the silica gel. In fact, treating the resulting mixture of isomers with catalytic DBU for 1 h at 50 °C reformed exclusively the *trans*-isomer. To avoid this isomerization, we pretreated the silica gel with 10% triethylamine/hexanes prior to purification of the crude substrates and were then able to isolate exclusively the desired *trans* isomer. It is also worth mentioning that a small amount of **9** (between 5–10%) was also recovered during purification of compounds **3–5**. This retro-Knoevenagel reaction is likely a result of hydrolysis due to water retained by the silica gel.

Solvatochromic behavior of CNCA probes

The solvatochromic behavior of compounds **3–5** was studied in solvents of various polarities (Table 1). A condensed overview of the excitation and emission maxima of **3–5** with their associated approximate peak intensity values (given in 10^6 photon counts per second, cps) is provided in Table 1.

In general, compound **3**, containing a 1,5-substituted cyclopenta[*b*]naphthalene moiety, absorbed light at shorter wavelengths (320–395 nm) in most solvents used as compared to **4** (292–382 nm) and **5** (280–436 nm). Compound **5**, containing a 1,7-substituted cyclopenta[*b*]naphthalene moiety, has the strongest fluorescence intensity in all solvents. The CNCA dyes exhibited multiple emission peaks, and the solvent polarity influenced the balance between the two peaks. In **3** and **4**, the emission from the red band (emission peak near λ = 610 nm) is best visible in toluene and less prominent than that of **5** (λ = 522 nm). A bathochromic shift of both peak excitation and peak emission with increasing solvent polarity can most clearly be seen in compound **3**. In addition, **5** (and to a lesser extend **3** and **4**) can be excited at 280 nm. Most likely, this is a direct excitation of the naphthalene core since naphthalene itself is fluorescent.[23]

Viscosity-dependent behavior

The emission spectra of **3**, **4**, and **5** in different mixtures of methanol, ethylene glycol, and glycerol were obtained by excitation at the optimum wavelength and are shown in Fig. 2. Compound **5** appeared to emit fluorescence only in one broad band that showed almost no variability with the solvent. On the other hand, compounds **3** and **4** exhibited two distinct emission peaks. The emission intensity of **3** and **4** increased with solvents of increased viscosity. Only the longer-wavelength emission peak of **3** exhibited a noticeable hypsochromic shift in solvents with higher glycerol content.

When a power-law relationship between viscosity and quantum yield (and therefore also intensity) exists (eqn (1)), data points of intensity, drawn over viscosity in a double-logarithmic scale, should lie on a straight line, and the slope of the line is the exponent x in eqn (1).

$$\phi F = \phi_0 \cdot \left(\frac{\eta}{\sigma}\right)^x \quad (1)$$

The log–log plots of peak intensity over viscosity are shown in Fig. 3. Straight lines were fitted by linear regression. In the case of **5**, the line did not have a slope significantly different from zero. Conversely, the slopes of the intensity peaks of **4** were 0.12 and 0.14, respectively. For **3**, we found slopes of 0.1 and 0.37, all of them significantly different from zero ($P < 0.005$). Relative intensities (*i.e.*, the *y*-intercept of the slope, which is the extrapolated value at a hypothetical viscosity of 1 mPa s) were 54 700 cps (red-shifted emission band) and 234 000 cps (blue-shifted emission band) for compound **3**, 247 000 (blue-shifted emission band) and 209 000 cps (red-shifted emission band) for compound **4**, and 750 000 cps for the single emission band of compound **5**.

Fluorescent molecular rotors are generally considered to have two emission bands: one from the planar state, and one

Table 1 Dominant fluorescence peaks of CNCA dyes **3**, **4** and **5** in solvents of different polarity (polarity is given relative to water)[22]

Solvent	Compound **3**			Compound **4**			Compound **5**		
(Polarity)[22]	Excitation λ (nm)	Emission λ (nm)	Peak Intensity ($\times 10^6$)	Excitation λ (nm)	Emission λ (nm)	Peak Intensity ($\times 10^6$)	Excitation λ (nm)	Emission λ (nm)	Peak Intensity ($\times 10^6$)
Toluene	395	616	0.8	363	603	0.42	425	522	2.7
(0.1)	333	414	0.25	306	325	3.0	363	413	2.5
							302	413	1.4
DMSO	335	425	0.6	365	430	1.7	436	562	2.5
(0.44)	280	360	0.95	340	369	1.4	364	430	3.6
							280	430	2.5
Acetonitrile	348	434	0.2	382	425	0.8	425	548	2.5
(0.46)				314	425	0.3	368	427	2.2
							280	427	1.4
Methanol	322	439	0.65	360	427	0.85	416	500	0.6
(0.76)				315	433	0.95	365	422	2.3
							280	422	1.4
Water/methanol	320	446	0.2	343	433	0.4	384	475	1.1
(0.88)				292	433	0.3	356	432	1.05
							295	432	0.7

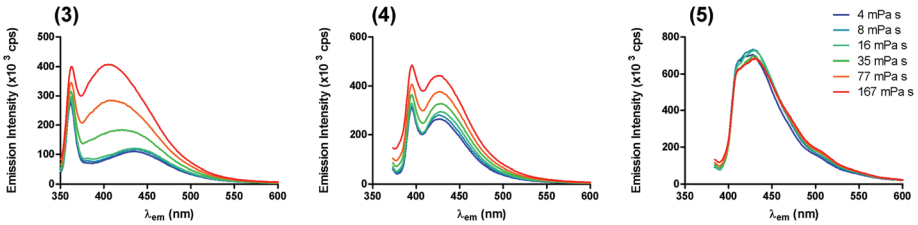


Fig. 2 Emission spectra of **3**, **4**, and **5** in alcohol mixtures of different viscosity. While **5** shows no major change in the different alcohol mixtures, **4** and notably **3** exhibit increased peak emission from the red-shifted band in alcohols with higher viscosity.

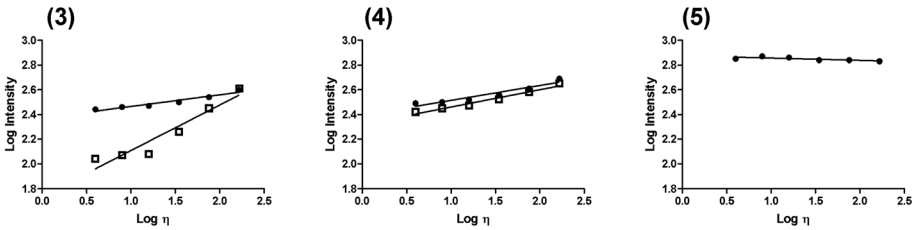


Fig. 3 Peak emission intensity plotted over solvent viscosity in a double-logarithmic scale. Compounds **3** and **4** have significantly positive slopes, and the slope can be used as an indication of the sensitivity towards viscosity. The slope of **5** is not significantly different from zero. Circles indicate the blue-shifted band (and the single emission of **5**) and open squares indicate the red-shifted band.

from the twisted intramolecular charge transfer (TICT) state. A good example is DMABN (dimethylaminobenzonitrile), whose photophysical properties are well-explored.[24] In the ground state, the energy of the twisted conformation is higher than that of the planar state. In the first excited state, we find the exact opposite. Therefore, excitation takes place preferentially from the planar state, and emission from the TICT state, unless rotation is hindered in some way. A reduction of the intramolecular rotation rate, for example due to viscous microfriction, leads to dual-band emission with increasing intensity emitted from the planar state (blue-shifted band). It is also possible that the energy gap between excited and ground states in the twisted conformation is so low that no photon emission occurs. Those molecular rotors, of which a representative example is DCVJ (9-dicyano-vinyl julolidine), do not have any red-shifted emission. Their quantum yield depends on emission from the planar state, which increases with increased microfriction. In the case of **3** and **4**, we observe a dual emission. Increase of solvent viscosity leads to a sharp increase of the red-shifted band (most pronounced in compound **3**) whereas the blue-shifted band increases less strongly. One possible explanation is a reversal of the energetically-preferred conformations, as found, for example, in BODIPY-related dyes.[25] Since both emission peaks of **3** and **4** increase with increasing viscosity, it is also possible that a third energy level exists which is comparatively weak and shows an extreme Stokes shift. This is consistent with the observation of emission in the 600 nm range in toluene. If this interpretation is correct, the dual emission occurs from a planar or almost-planar state, and twisted-state emission is invisible due to its low energy gap.

It is interesting to correlate the structural and photophysical differences between compounds **3**, **4** and **5**. CNCA dye **5**, containing a 1,7-substituted cyclopenta[*b*]naphthalene moiety, was found to have the strongest fluorescence emission and was the least viscosity sensitive among all compounds tested. On the other hand, compound **3**, containing a 1,5-substituted cyclopenta[*b*]naphthalene moiety, was shown to be the least fluorescent but most viscosity sensitive, while compound **4**, containing a 1,6-substituted cyclopenta[*b*]naphthalene moiety, had intermediate values of fluorescence intensity and viscosity sensitivity. The inverse relationship between fluorescence emission and viscosity sensitivity is a general characteristic of FMRs and has been previously observed for related naphthalene-based fluorophores.[26] It is likely that placement of the donor (D) and acceptor (A) groups in close proximity (*e.g.* in compound **5**) decreases their rotational ability and thus increases the energy level of the TICT state (Fig. 4). This effect translates into fluorescence deexcitation without significant passage through the environment-sensitive TICT state. Thus, compound **5** becomes more fluorescent but less sensitive to solvent viscosity.

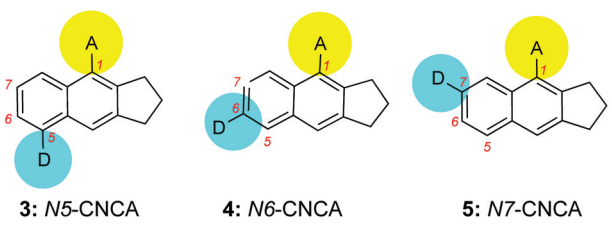


Fig. 4 Comparison of the relative position of donor (D, Me_2N) and acceptor (A, CH═C(CN)CO_2TEGME) substituents in CNCA dyes. Placing the D and A groups in close proximity decreases their rotational ability thereby allowing emission from the planar excited state.

Conclusion

The intramolecular dehydrogenative dehydro-Diels–Alder reaction (IDDDA) of chlorinated styrenes allowed access to cyclopenta[*b*]naphthalene carbaldehydes containing a chlorine atom in various positions of the aromatic motif. Conversion of the aryl chloride to the corresponding dimethylamino group (donor) under Buchwald–Hartwig cross-coupling conditions followed by Knoevenagel condensation of the carbonyl group with a cyanoacrylate motif (acceptor) produced a family of fluorescent dyes containing the D–π-A array. Fluorescence emission of these dyes was found to depend on the solvent polarity and viscosity, a behavior that is typical for molecular rotors. Moreover, placing the donor and acceptor groups in close proximity along the π-ring structure (*i.e.* CNCA dye **5**) was found to influence viscosity sensitivity. In this case, absence of viscosity sensitivity combined with a high quantum yield points to the inability of the molecule to perform intramolecular twisting. The pattern of viscosity sensitivity in CNCA dyes **3** and **4** alludes to the existence of a twisted ground state with nonradiative emission from a planar excited state. These observations help to understand the influence of chemical structure on viscosity sensitivity of fluorescent molecular rotors.

Experimental

General notes

All reagents were procured from commercial sources and were used as received. Tetrahydrofuran (THF) was dried by passing through alumina using the Sol-Tek ST-002 solvent purification system. All microwave-mediated reactions were conducted using either a Biotage Initiator Exp or Anton Paar Monowave 300 microwave synthesizer. The microwave parameters were set to variable power, constant temperature, and a fixed hold time. Thin-layer chromatography (TLC) analyses were performed on Silicycle SiliaPlate G silica gel glass plates (60F-254, 0.25 mm thickness) and visualized under UV light. Purification of substrates by flash column chromatography was performed with silica gel (40–63 μm particle size, 60 Å pore size) purchased from Sorbent Technologies. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 300, 400, or 500 MHz spectrometers. Spectra were referenced to residual chloroform (7.27 ppm, ^{1}H, 77.16 ppm, ^{13}C). Chemical shifts are reported in ppm, and multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), p (pentet), m (multiplet), and b (broad). Coupling constants, *J*, are reported in hertz (Hz). Infrared (IR) spectra were obtained using a Nicolet Avatar E.S.P. 360 FT-IR. Electron spray ionization (ES) spectra were recorded on a Waters Q-TOF Ultima API, Micromass UK Limited high resolution mass spectrometer. Substrates **6a–c** were previously synthesized.[17a]

General procedure A for the synthesis of formyl enynes 7. To a flame-dried two-neck round-bottomed flask equipped with an argon inlet adapter, a septum, and a stir bar was added enyne **6a–c** (1.0 equiv.) and THF (0.4 M) *via* syringe. The solution was cooled at −78 °C (bath temperature) in a dry ice/acetone bath, and *n*-butyllithium (1.0 equiv. of a 1.6 M solution in hexanes) was added dropwise *via* syringe. The reaction was stirred at −78 °C for 45 min, and anhydrous *N*,*N*-dimethylformamide (DMF, 2.0 equiv.) was added dropwise *via* syringe. The reaction mixture was stirred at −78 °C for 30 min, and was then warmed to rt and stirred for 2 h. The reaction mixture was added to a cold solution of ethyl acetate and 10% KH_2PO_4, and stirred for 30 min. The aqueous layer was separated and extracted with ethyl acetate (1×). The organic layer was then washed with brine, dried over magnesium sulfate, gravity filtered, and concentrated under reduced pressure. The crude product was purified by flash silica gel column chromatography.

*(E)-8-(2-Chlorophenyl)oct-7-en-2-ynal (**7a**).* Follows general procedure A: enyne **6a** (0.500 g, 2.44 mmol), THF (6.5 mL), *n*-butyllithium (1.52 mL, 2.44 mmol), DMF (0.38 mL, 4.88 mmol), ethyl acetate (8 mL), 10% KH_2PO_4 (15 mL). The reaction mixture turned orange upon addition of *n*-butyllithium and yellow upon addition of DMF. The crude product was purified by flash silica gel column chromatography (3.0 cm column, 5% ethyl acetate/hexanes) to yield the title compound as a light yellow oil (0.455 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.19 (s, 1H), 7.48 (d, J = 7.8, 1.2 Hz, 1H), 7.34 (d, J = 7.8, 1.2 Hz, 1H), 7.22–7.13 (m, 2H), 6.81 (d, J = 15.6 Hz, 1H), 6.15 (dt, J = 15.6, 7.2 Hz, 1H), 2.49 (t, J = 7.2 Hz, 2H), 2.39 (q, J = 7.2 Hz, 2H), 1.82 (p, J = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.9, 135.4, 132.5, 131.7, 129.5, 128.1, 127.5, 126.7, 126.6, 98.4, 82.0, 32.0, 26.2, 18.4; IR (thin film) 3307, 3066, 2938, 2860, 2740, 2278, 2200, 1667, 796, 751 cm^{-1}; LRMS (TOF MSMS ES+) *m*/*z* (%) 233 (100), 214 (35), 205 (20); HRMS (TOF MS ES+) calculated for $C_{14}H_{14}OCl$ $(M + H)^+$ 233.0733, found 233.0726; TLC (UV, $KMnO_4$ stain) R_f = 0.3 (10% ethyl acetate/hexanes).

*(E)-8-(3-Chlorophenyl)oct-7-en-2-ynal (**7b**).* Follows general procedure A: enyne **6b** (0.495 g, 2.42 mmol), THF (6.5 mL), *n*-butyllithium (1.51 mL, 2.42 mmol), DMF (0.37 mL, 4.84 mmol), ethyl acetate (8 mL), 10% KH_2PO_4 (15 mL). The reaction mixture turned yellow upon addition of *n*-butyllithium and lighter upon addition of DMF. The crude product was purified by flash silica gel column chromatography (3.0 cm column, 5% ethyl acetate/hexanes) to yield the title compound as a light yellow oil (0.426 g, 76%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.19 (t, J = 0.9 Hz, 1H), 7.33 (t, J = 1.8 Hz, 1H), 7.23–7.16 (m, 3H), 6.38 (d, J = 15.9 Hz, 1H), 6.18 (dt, J = 15.9, 7.2 Hz, 1H), 2.47 (t, J = 7.2 Hz, 2H), 2.35 (dq, J = 7.2, 1.2 Hz, 2H), 1.79 (p, J = 7.2 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.1, 139.1, 134.3, 130.3, 129.9, 129.7, 127.0, 125.8, 124.2, 98.4, 81.9, 31.7, 26.9, 18.4; IR (thin film) 3072, 3023, 2938, 2860, 2733, 2277, 2200, 1667, 1592, 1476, 776 cm^{-1}; LRMS (TOF MSMS ES+) *m*/*z* (%) 233 (100), 215 (59), 205 (86), 198 (12), 197 (29), 179 (16); HRMS (TOF MS ES+) calculated for $C_{14}H_{14}OCl$ $(M + H)^+$ 233.0733, found 233.0735; TLC (UV, $KMnO_4$ stain) R_f = 0.4 (10% ethyl acetate/hexanes).

*(E)-8-(4-Chlorophenyl)oct-7-en-2-ynal (**7c**).* Follows general procedure A: enyne **6c** (0.500 g, 2.44 mmol), THF (6.5 mL),

n-butyllithium (1.52 mL, 2.44 mmol), DMF (0.38 mL, 4.88 mmol), ethyl acetate (8 mL), 10% KH_2PO_4 (15 mL). The reaction mixture turned light yellow upon addition of DMF. The crude product was purified by flash silica gel column chromatography (3.0 cm column, 5% ethyl acetate/hexanes) to yield the title compound as a light yellow oil (0.389 g, 68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.19 (s, 1H), 7.27 (s, 4H), 6.39 (d, J = 15.9 Hz, 1H), 6.15 (dt, J = 15.9, 6.9 Hz, 1H), 2.48 (t, J = 6.9 Hz, 2H), 2.35 (q, J = 6.9 Hz, 2H), 1.80 (p, J = 6.9 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 177.0, 135.8, 132.5, 129.9, 129.4, 128.5 (2C), 127.1 (2C), 98.4, 81.9, 31.7, 26.9, 18.4; IR (thin film) 3026, 2936, 2857, 2729, 2200, 1665, 1489 cm^{-1}; LRMS (TOF MSMS ES+) *m*/*z* (%) 233 (100), 232 (92), 215 (35); HRMS (TOF MS ES+) calculated for $C_{14}H_{13}OCl$ $(M)^+$ 232.0655, found 232.0632; TLC (UV, $KMnO_4$ stain) R_f = 0.3 (10% ethyl acetate/hexanes).

General procedure B for the synthesis of naphthalene carbaldehydes 8. To a microwave irradiation vial equipped with a stir bar was added enyne **7a–c** in *o*-dichlorobenzene (0.06–0.09 M). The reaction mixture was irradiated with stirring at 180 °C for 60 min. During this time, the reaction mixture turned golden in color. The reaction mixture was then purified directly by flash silica gel column chromatography.

*8-Chloro-2,3-dihydro-1H-cyclopenta[b]naphthalene-4-carbaldehyde (**8a**).* Follows general procedure B: aldehyde **7a** (0.279 g, 1.20 mmol), *o*-dichlorobenzene (20 mL). The reaction mixture was purified directly by flash silica gel column chromatography (2.5 cm column, 0–10% ethyl acetate/hexanes) to yield the title compound as a white solid (0.222 g, 80%). MP: 92–96 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.8 (s, 1H), 9.02 (d, J = 8.7 Hz, 1H), 8.41 (s, 1H), 7.58 (d, J = 7.5 Hz, 1H), 7.47 (t, J = 7.5 Hz, 1H), 3.47 (t, J = 7.5 Hz, 2H), 3.13 (dt, J = 7.5, 1.2 Hz, 2H), 2.25 (p, J = 7.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.1, 152.5, 144.9, 132.0, 131.7, 130.5, 127.5, 126.3, 125.7, 125.5, 123.4, 32.1, 31.6, 25.6; IR (thin film) 3080, 2968, 2929, 2868, 2766, 1677, 1615, 1596, 1490 cm^{-1}; LRMS (TOF MSMS ES+) *m*/*z* (%) 231 (42), 203 (100), 195 (30), 175 (44), 168 (50), 167 (20); HRMS (TOF MS ES+) calculated for $C_{14}H_{12}OCl$ $(M + H)^+$ 231.0577, found 231.0581; TLC (UV, $KMnO_4$ stain) R_f = 0.3 (10% ethyl acetate/hexanes).

*7-Chloro-2,3-dihydro-1H-cyclopenta[b]naphthalene-4-carbaldehyde (**8b**).* Follows general procedure B: aldehyde **7b** (0.122 g, 0.52 mmol), *o*-dichlorobenzene (7 mL). The reaction mixture was first concentrated under high vacuum, and then the crude product was purified by flash silica gel column chromatography (3.0 cm column, 5% ethyl acetate/hexanes) to yield the title compound as a white solid (0.037 g, 31%). Substrate **8b** was formed as a 1 : 1.5 mixture with **8d** in a total yield of 79%. MP: 66–67 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.8 (s, 1H), 9.07 (d, J = 9.1 Hz, 1H), 7.80 (s, 1H), 7.77 (d, J = 2.1 Hz, 1H), 7.51 (dd, J = 9.1, 2.1 Hz, 1H), 3.45 (t, J = 7.5 Hz, 2H), 3.08 (dt, J = 7.5, 1.2 Hz, 2H), 2.24 (p, J = 7.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.1, 152.4, 144.8, 134.1, 131.7, 128.4 (2C), 128.3, 126.6, 126.2, 125.5, 31.8, 31.3, 25.6; IR (thin film) 2956, 2868, 2843, 2756, 1682, 1621, 1596, 1489 cm^{-1}; LRMS (TOF MSMS ES+) *m*/*z* (%) 230 (10), 229 (63), 212 (40), 201 (48), 175 (100), 168 (71); HRMS (TOF MS ES+) calculated for $C_{14}H_{12}OCl$ $(M + H)^+$ 231.0577, found 231.0568; TLC (UV, $KMnO_4$ stain) R_f = 0.4 (10% ethyl acetate/hexanes).

*6-Chloro-2,3-dihydro-1H-cyclopenta[b]naphthalene-4-carbaldehyde (**8c**).* Follows general procedure B: aldehyde **7c** (0.425 g, 1.83 mmol), *o*-dichlorobenzene (20 mL). The reaction mixture was first concentrated under high vacuum, and then the crude product was purified by flash silica gel column chromatography (3.0 cm column, 5% ethyl acetate/hexanes) to yield the title compound as a white solid (0.308 g, 73%). MP: 93–96 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.7 (s, 1H), 9.18 (s, 1H), 7.87 (s, 1H), 7.72 (d, J = 8.7 Hz, 1H), 7.44 (dd, J = 8.7, 1.8 Hz, 1H), 3.47 (t, J = 7.5 Hz, 2H), 3.05 (dq, J = 7.5, 2.7 Hz, 2H), 2.24 (p, J = 7.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.8, 153.3, 143.8, 134.1, 131.4, 130.5, 129.2 (2C), 126.7, 124.5, 123.8, 31.6, 31.2, 25.5; IR (thin film) 3096, 2958, 2913, 2868, 2753, 1675 cm^{-1}; LRMS (TOF MSMS ES+) *m*/*z* (%) 231 (100), 230 (75), 203 (45), 195 (32); HRMS (TOF MS ES+) calculated for $C_{14}H_{11}OCl$ $(M)^+$ 230.0498, found 230.0471; TLC (UV, $KMnO_4$ stain) R_f = 0.3 (10% ethyl acetate/hexanes).

*5-Chloro-2,3-dihydro-1H-cyclopenta[b]naphthalene-4-carbaldehyde (**8d**).* Formed as a mixture with **8b**. White solid (0.058 g, 48%); MP: 84–86 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.2 (s, 1H), 7.77 (s, 1H), 7.73 (dd, J = 7.5, 1.2 Hz, 1H), 7.44 (dd, J = 7.5, 1.2 Hz, 1H), 7.36 (t, J = 7.5 Hz, 1H), 3.19 (t, J = 7.5 Hz, 2H), 3.06 (dt, J = 7.5, 1.2 Hz, 2H), 2.14 (p, J = 7.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 194.3, 147.0, 144.6, 135.1, 129.8, 129.7, 128.3, 128.1, 127.7, 126.5, 125.6, 33.1, 32.3, 25.6; IR (thin film) 2959, 2927, 2853, 2751, 1682 cm^{-1}; LRMS (TOF MSMS ES+) *m*/*z* (%) 203 (100), 175 (25), 168 (60); HRMS (TOF MS ES+) calculated for $C_{14}H_{12}OCl$ $(M + H)^+$ 231.0577, found 231.0564; TLC (UV, $KMnO_4$ stain) R_f = 0.5 (10% ethyl acetate/hexanes).

General procedure C for the synthesis of aminonaphthalenes 9. To an oven-dried microwave irradiation vial equipped with a stir bar was added RuPhos palladacycle (3 mol%), cesium carbonate (2.0–2.2 equiv.), and naphthalene **8a–c** (1.0 equiv.). The vial was capped and evacuated and refilled with argon (3×) by piercing the septum of the cap with a small gauge needle connected to an argon manifold. THF (0.5 M) was added *via* syringe with stirring, followed by dimethylamine (3.0–3.3 equiv. of a 2.0 M solution in THF). The argon inlet was removed and the reaction mixture was lowered into a preheated 85 °C oil bath and stirred until complete by TLC. The reaction mixture was then cooled to rt and quenched with aqueous saturated ammonium chloride. The aqueous layer was separated and extracted with ethyl acetate (3×). The combined organic layers were washed with brine, dried over magnesium sulfate, gravity filtered, and concentrated under reduced pressure. The crude product was then purified by flash silica gel column chromatography.

*8-(Dimethylamino)-2,3-dihydro-1H-cyclopenta[b]naphthalene-4-carbaldehyde (**9a**).* Follows general procedure C: 0.5–2 mL vial, RuPhos palladacycle (0.004 g, 0.0055 mmol), cesium carbonate (0.143 g, 0.44 mmol), naphthalene **8a** (0.050 g, 0.22 mmol), THF (0.43 mL), dimethylamine (0.33 mL, 0.66 mmol). The reaction mixture was heated for 4.5 h and turned from tan to orange in color over time. The crude product was purified by

flash silica gel column chromatography (2.0 cm column, 5% ethyl acetate/hexanes) to yield the title compound as a yellow solid (0.032 g, 60%). MP: 59–61 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.8 (s, 1H), 8.72 (d, J = 8.4 Hz, 1H), 8.40 (s, 1H), 7.49 (t, J = 8.4 Hz, 1H), 7.13 (d, J = 7.2 Hz, 1H), 3.45 (t, J = 7.5 Hz, 2H), 3.10 (dt, J = 7.5, 1.2 Hz, 2H), 2.88 (s, 6H), 2.22 (p, J = 7.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.6, 151.3, 151.1, 142.8, 132.1, 128.8, 127.7, 126.0, 125.6, 119.0, 114.5, 45.5 (2C), 32.2, 31.8, 25.7; IR (thin film) 3583, 3072, 2942, 2859, 2828, 2783, 1680, 1616, 1593 cm^{-1}; LRMS (TOF MSMS ES+) m/z (%) 240 (65), 225 (100), 211 (78), 197 (86); HRMS (TOF MS ES+) calculated for $C_{16}H_{18}NO$ $(M + H)^+$ 240.1388, found 240.1372; TLC (UV, $KMnO_4$ stain) R_f = 0.3 (10% ethyl acetate/hexanes).

*7-(Dimethylamino)-2,3-dihydro-1H-cyclopenta[b]naphthalene-4-carbaldehyde (**9b**).* Follows general procedure C: 0.5–2 mL vial, RuPhos palladacycle (0.005 g, 0.0063 mmol), cesium carbonate (0.164 g, 0.50 mmol), naphthalene **8b** (0.052 g, 0.23 mmol), THF (0.50 mL), dimethylamine (0.38 mL, 0.75 mmol). The reaction mixture was heated for 3 h and turned from burnt orange to fluorescent yellow in color over time. The crude product was purified by flash silica gel column chromatography (1.75 cm column, 5% ethyl acetate/hexanes) to yield the title compound as a yellow solid (0.024 g, 44%). MP: 122–125 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.8 (s, 1H), 8.95 (d, J = 9.3 Hz, 1H), 7.73 (s, 1H), 7.26–7.21 (m, 1H), 6.88 (s, 1H), 3.40 (t, J = 7.5 Hz, 2H), 3.05–3.00 (m, 8H), 2.19 (p, J = 7.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 192.8, 148.4, 148.2, 143.7, 135.1, 128.1, 125.5, 125.2, 123.2, 117.6, 106.8, 40.6 (2C), 31.9, 31.2, 25.8; IR (thin film) 2953, 2921, 2843, 2802, 2745, 1668, 1618, 1605, 1510 cm^{-1}; LRMS (TOF MSMS ES+) m/z (%) 239 (25), 238 (75), 225 (100), 210 (45), 197 (35); HRMS (TOF MS ES+) calculated for $C_{16}H_{18}NO$ $(M + H)^+$ 240.1388, found 240.1399; TLC (UV, $KMnO_4$ stain) R_f = 0.1 (10% ethyl acetate/hexanes).

*6-(Dimethylamino)-2,3-dihydro-1H-cyclopenta[b]naphthalene-4-carbaldehyde (**9c**).* Follows general procedure C: 2–5 mL vial, RuPhos palladacycle (0.017 g, 0.023 mmol), cesium carbonate (0.590 g, 1.81 mmol), naphthalene **8c** (0.200 g, 0.87 mmol), THF (1.8 mL), dimethylamine (1.37 mL, 2.73 mmol). The reaction mixture was heated for 2.5 h and turned from tan to orange in color over time. The crude product was purified by flash silica gel column chromatography (2.5 cm column, 5% ethyl acetate/hexanes) to yield the title compound as an orange solid (0.154 g, 74%). MP: 132–133 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 10.7 (s, 1H), 8.43 (d, J = 2.4 Hz, 1H), 7.74 (s, 1H), 7.64 (d, J = 9.1 Hz, 1H), 7.12 (dd, J = 9.1, 2.4 Hz, 1H), 3.43 (t, J = 7.5 Hz, 2H), 3.11 (s, 6H), 3.01 (t, J = 7.5 Hz, 2H), 2.19 (p, J = 7.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 192.5, 153.4, 150.5, 138.5, 132.2, 129.8, 129.0, 126.6, 123.6, 115.2, 103.6, 40.6 (2C), 31.6, 31.5, 25.7; IR (thin film) 3068, 2952, 2847, 2794, 2751, 1665, 1612, 1511, 1159 cm^{-1}; LRMS (TOF MSMS ES+) m/z (%) 240 (80), 239 (100), 226 (59), 211 (60); HRMS (TOF MS ES+) calculated for $C_{16}H_{17}NO$ $(M)^+$ 239.1310, found 239.1289; TLC (UV, $KMnO_4$ stain) R_f = 0.5 (25% ethyl acetate/hexanes).

General procedure D for the synthesis of CNCA dyes 3–5. To stirring solution of aldehyde **9a–c** (1.0 equiv.) and **10** (0.8 equiv.) in THF was added DBU (0.01–0.03 equiv.) under argon. The reaction mixture was heated at 50 °C for 2–3 h then cooled to rt and concentrated under reduced pressure. The residue was purified by flash silica gel column chromatography to give CNCA dyes as colored oils.

*N5-CNCA (**3**).* Follows general procedure D: **9a** (0.020 g, 0.084 mmol), **10** (0.015 g, 0.067 mmol), THF (0.33 mL), and DBU (0.001 mmol). The reaction mixture was heated at 50 °C for 2 h. The residue was purified by flash silica gel column chromatography (0–20% ethyl acetate/hexanes) to yield the title compound as an orange oil (0.026 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.96 (s, 1H), 8.23 (s, 1H), 7.41–7.39 (m, 2H), 7.11 (dd, J = 4.0, 1.2 Hz, 1H), 4.53–4.51 (m, 2H), 3.87–3.85 (m, 2H), 3.76–3.74 (m, 2H), 3.71–3.66 (m, 4H), 3.56–3.54 (m, 2H), 3.36 (s, 3H), 3.14–3.09 (m, 4H), 2.88 (s, 6H), 2.20–2.14 (m, 2H); ^{13}C (100 MHz, $CDCl_3$) δ 162.0, 156.8, 151.5, 144.6, 143.0, 131.7, 128.3, 126.8, 124.8, 122.3, 118.7, 115.0, 114.6, 109.6, 72.0, 71.0, 70.8, 70.7, 68.8, 66.2, 59.0, 45.5 (2C), 33.4, 33.3, 29.8 (grease), 26.7; HRMS (ESI) calculated for $C_{26}H_{33}N_2O_5$ $(M + H)^+$ 453.2384, found 453.2387; TLC (UV) R_f = 0.3 (40% ethyl acetate/hexanes).

*N6-CNCA (**4**).* Follows general procedure D: **9b** (0.0070 g, 0.030 mmol), **10** (0.0054 g, 0.023 mmol), THF (0.20 mL), and DBU (0.001 mmol). The reaction mixture was heated at 50 °C for 2 h. The residue was purified by flash silica gel column chromatography (0–40% ethyl acetate/hexanes) to yield the title compound as an orange oil (0.0094 g, 90%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.92 (s, 1H), 7.61–7.58 (m, 2H), 7.15 (dd, J = 12.0, 4.0 Hz, 1H), 6.89 (d, J = 4.0 Hz, 1H), 4.52–4.50 (m, 2H), 3.87–3.85 (m, 2H), 3.75–3.74 (m, 2H), 3.71–3.66 (m, 5H), 3.56–3.54 (m, 2H), 3.37 (s, 3H), 3.05 (m, 9H), 2.17–2.10 (m, 2H); ^{13}C (100 MHz, $CDCl_3$) δ 162.2, 156.7, 148.6, 143.9, 141.2, 134.5, 125.0, 124.8, 124.3, 123.0, 116.2, 115.0, 109.1, 107.3, 72.1, 71.0, 70.8, 70.7, 68.8, 65.9, 59.2, 40.9 (2C), 33.1, 33.0, 29.8 (grease), 26.7; HRMS (ESI) calculated for $C_{26}H_{33}N_2O_5$ $(M + H)^+$ 453.2384, found 453.2381; TLC (UV) R_f = 0.2 (40% ethyl acetate/hexanes).

*N7-CNCA (**5**).* Follows general procedure D: **9c** (0.020 g, 0.084 mmol), **10** (0.015 g, 0.067 mmol), THF (0.33 mL), and DBU (0.001 mmol). The reaction mixture was heated at 50 °C for 3 h. The residue was purified by flash silica gel column chromatography (0–60% ethyl acetate/hexanes) to yield the title compound as a red oil (0.023 g, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 7.67–7.64 (m, 2H), 7.11 (dd, J = 8.0, 4.0 Hz, 1H), 6.63 (bs, 1H), 4.52–4.50 (m, 2H), 3.88–3.85 (m, 2H), 3.76–3.74 (m, 2H), 3.70–3.66 (m, 4H), 3.56–3.54 (m, 2H), 3.36 (s, 3H), 3.10–3.03 (m, 10H), 2.17–2.10 (m, 2H); ^{13}C (100 MHz, $CDCl_3$) δ 162.5, 156.4, 148.9, 145.9, 139.2, 132.0, 129.4, 126.3, 125.8, 122.7, 115.6, 115.3, 108.0, 103.2, 72.1, 71.0, 70.8, 70.7, 68.8, 65.9, 59.2, 40.9 (2C), 33.8, 32.7, 29.8 (grease), 26.7; HRMS (ESI) calculated for $C_{26}H_{33}N_2O_5$ $(M + H)^+$ 453.2384, found 453.2386; TLC (UV) R_f = 0.34 (50% ethyl acetate/hexanes).

Table 2 Mixture ratios and resulting viscosity of the alcohol gradient

Target viscosity (mPa s)	Pre-stained ethylene glycol	Unstained ethylene glycol	Methanol	Glycerol
4	1 mL	2 mL	2 mL	—
8	1 mL	3 mL	1 mL	—
16	1 mL	4 mL	—	—
35	1 mL	3 mL	—	1 mL
77	1 mL	2 mL	—	2 mL
167	1 mL	1 mL	—	3 mL

Fluorescence spectroscopy

Dye stock solutions were prepared by dissolving each dye in methanol at a concentration of 5 mM and were kept at 4 °C until used. Final dye solutions were prepared by pipetting 10 μL of the stock solution into 5 mL of the solvent under vigorous stirring for a resulting concentration of 10 μM. Solutions of the compounds in toluene, acetonitrile, DMSO, methanol, and a 50% by volume mix of water and methanol were analyzed. Viscosity gradients were composed of a mixture of methanol, ethylene glycol, and glycerol at different concentrations as reported elsewhere.[27] Specifically, mixtures of methanol, ethylene glycol, and glycerol were prepared by prestaining 10 mL of ethylene glycol with 10 μL dye stock solution. One mL of prestained ethylene glycol was then mixed with either methanol or glycerol and unstained ethylene glycol to afford solutions of different viscosity and constant dye concentration at a total volume of 5 mL as detailed in Table 2. Viscosity values for the individual solvents were taken from the labels of the stock bottles or from the pertinent literature[28] as 0.5 mPa s for methanol, 16 mPa s for ethylene glycol, and 864 mPa s for glycerol, all at 22 °C. The viscosity η_{mix} of the mixtures was calculated with eqn (2)

$$\ln \eta_{mix} = \sum_i w_i \ln \eta_i \quad (2)$$

where w_i is the volume fraction and η_i the viscosity of the i-th solvent. Solvent polarity values for methanol of 0.762, for ethylene glycol of 0.790, and for glycerol of 0.812 were used.[22] For comparison, the relative polarity of toluene is 0.099, of acetonitrile 0.460, and of DMSO 0.444.

Spectroscopy was performed using a Fluoromax-3 fluorophotometer (Jobin-Yvon, Edison, NJ) with a temperature-controlled four-sample holder (Turret-400, Quantum Northwest, Liberty Lake, WA) set to 22.0 °C. Disposable methacrylate cuvettes were used for the viscosity gradients, and glass cuvettes for all other solvents. Unless otherwise stated, emission scans were performed from the optimum excitation wavelength.

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