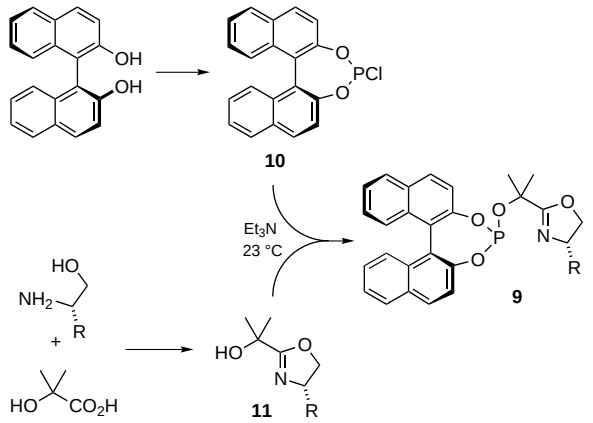


Scheme 4. Synthesis of ligands **9**. The preparation of compounds **10**[10] and **11**[11] is described elsewhere.

palladium catalyst. By using a new type of chiral P,N ligand, practically useful enantio- and regioselectivities have been obtained in the reaction with 1- and 3-aryl-2-propenyl acetates.[8] Although at present the range of substrates that afford good selectivities is limited to aryl-substituted derivatives, the concepts used for the design of ligands **9** may serve as guidelines for the development of new catalysts for different classes of substrates. In addition, these ligands have other possible applications in asymmetric catalysis.[9]

*Experimental Section*

**9a**: All reactions were performed under argon in degassed solvents. A solution of **10**[10] (1.1 g, 3.2 mmol) in toluene (10 mL) was added dropwise to $Et_3N$ (2.6 g, 25.7 mmol) in toluene (20 mL) at $-78^\circ C$. This solution was stirred for 5 min at $-78^\circ C$ before a solution of **11**[11] (0.6 g, 3.2 mmol) in toluene (5 mL) was added quickly. The solution was allowed to gradually warm up to room temperature and stirred for 12 h. The white precipitate was removed by filtration. Evaporation of the solvent afforded a yellow oil, which was purified by column chromatography (silica gel, $3.0 \times 20$ cm; *n*-hexane/EtOAc 4/1, $R_f = 0.4$) to afford (*S*,*S*)-**9a** (890 mg, 56%) as an amorphous solid. $[\alpha]_{589} = +269$ ($CHCl_3$, $c = 3.1$, 23°C); $^1$H NMR (200 MHz, $CDCl_3$): $\delta = 0.96$ (s, 9H; *t*Bu), 1.65 (s, 3H; Me), 1.75 (s, 3H; Me), 3.95–4.00 (m, 1H; HCN), 4.20–4.35 (m, 2H; $CH_2O$), 7.22–7.52 (m, 8H; arom. CH), 7.89–7.96 (m, 4H; arom. CH); $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta = 25.8$ (*t*Bu), 28.1 (d, $J = 7.9$ Hz; Me), 28.2 (d, $J = 5.7$ Hz; Me), 33.9 (*t*Bu), 69.7 ($CH_2O$), 75.7 (CHN), 77.2 (C), 121.9/122.4 (arom. CH), 123.2 (arom. C), 124.5 (d, $J =$ 3.1 Hz; arom. C), 124.6/124.8/125.8/126.0/126.9/127.0/128.1/128.2/129.3/130.0 (arom. CH), 131.1/131.4/132.7/132.8 (arom. C), 147.9 (d, $J = 2.3$ Hz; arom. C), 148.0 (d, $J = 3.7$ Hz; arom. C), 168.3 (C=N); $^{31}$P NMR (100 MHz, $CDCl_3$): $\delta = 151.5$.

Catalytic reactions (Tables 1 and 2) were carried out according to the literature procedure.[12] Purification and analytical data of the products are described elsewhere.[3b, 4].

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## Synthesis of a New Class of Solvent-Sensitive Fluorescent Labels

James J. La Clair*

Charge transfer (CT) labels such as 5-(dimethylamino)-naphthalene-1-sulfonyl (dansyl) chloride have been used extensively for the detection, characterization, and localization of carbohydrates, phospholipids, proteins, oligonucleotides, and numerous other synthetic and natural substances.[1] These materials typically experience shifts in their UV/Vis absorption and/or fluorescence bands depending on the nature of their solvent shells.[2] This effect together with modifications of fluorescence lifetimes, extent of intersystem crossing, and fluorescence quantum yield have encouraged

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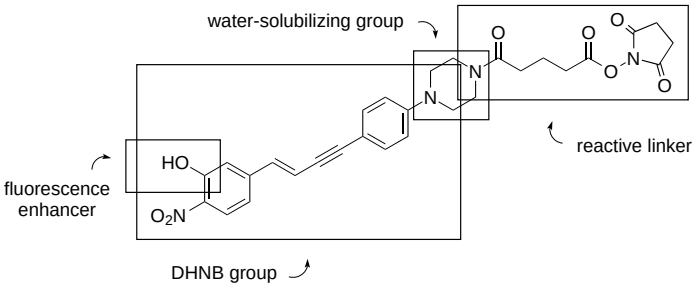
the use of such compounds as practical sensors for monitoring interactions between biologically relevant macromolecules.[3] Because of the extent of its aromaticity, the dansyl group absorbs light between $\lambda = 190$ and 400 nm, limiting its excitation primarily to the ultraviolet region.[4] We describe here the synthesis of a new class of intramolecular charge-transfer labels that absorbs both visible and ultraviolet light, displays dramatic solvent sensitivity, and can be detected at the single-molecule level.

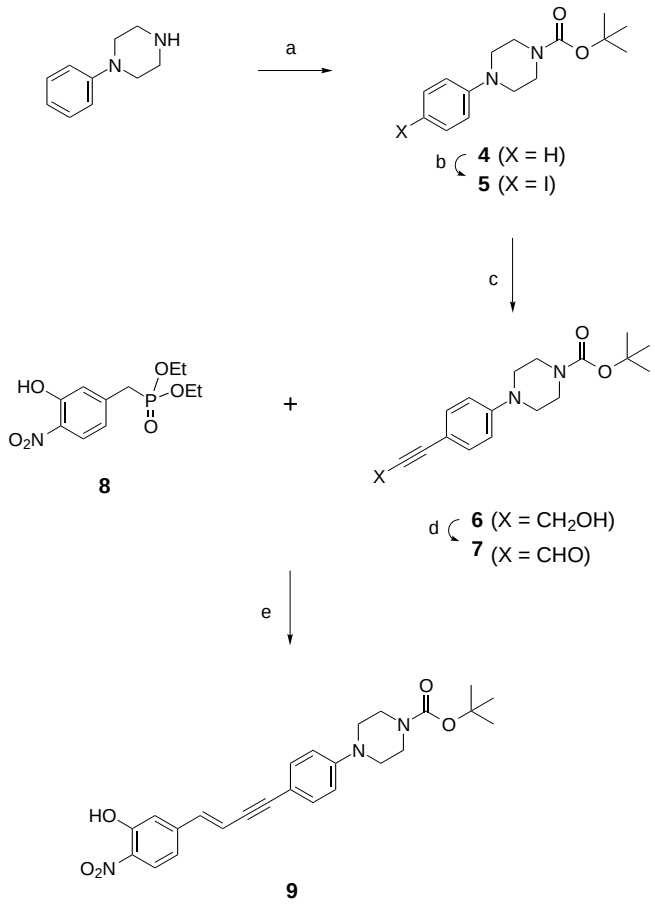
Recent studies have shown that fluorescence from 1-[4-(*N,N*-dimethylamino)phenyl]-4-(3-hydroxy-4-nitrophenyl)-3-(*E*)-buten-1-yne (DHNB) is dramatically affected by the nature of its solvent shell, whereas its UV/Vis absorption is altered only minimally.[5] For example, DHNB dissolved in methanol absorbs light with a maximum at 421 nm ($\varepsilon = 50\,800\ cm^{-1}M^{-1}$) and fluoresces at 528 nm with a quantum efficiency of 0.000017. However, in a nonpolar aprotic solvent such as *n*-hepane the absorption maximum undergoes a bathochromic shift to 435 nm ($\varepsilon = 47\,400\ cm^{-1}M^{-1}$), and the fluorescence quantum yield is enhanced by approximately 1000-fold to 0.0188 and 0.0205 at 593 and 570 nm, respectively. In an effort to devise new systems for monitoring the interactions of single molecules, we employed DHNB to selectively detect single aggregates of a complex consisting of a carbohydrate and a protein.[5] This communication describes the synthesis of a family of DHNB derivatives that can be used to label a wide variety of biologically significant molecules.

Scheme 1 depicts the current design, which mimics the functionality in the DHNB group. A hydroxy group adjacent




Scheme 1. Design of a new fluorescence label.

to the nitro group enhances the fluorescence quantum yield and serves as a site for the attachment of an additional functionality.[6] The dimethylamino group of DHNB was then replaced with a piperazine unit, which not only enhances the water solubility of the dye but also provides a handle for further linkage. *N*-Phenylpiperazine was chosen as the starting material for this synthesis because it is commercially available and inexpensive (ca. 1 DM per gram). Synthesis of the model compound begins with the halogenation of protected piperazine **4**[7] with iodine in a biphasic mixture of aqueous sodium bicarbonate and dichloromethane (Scheme 2). After one recrystallization, iodide **5** was coupled with the aid of transition metal catalysis to the C terminus of propargyl alcohol. The resulting alcohol **6** was then oxidized to aldehyde **7** with manganese dioxide, and subsequently




Scheme 2. a) Di-*tert*-butyl dicarbonate, $Et_3N$, DMAP, $CH_2Cl_2$, 0 °C → RT, 94 %; b) 1. iodine, $NaHCO_3$, $CH_2Cl_2$, $H_2O$, 12–15 °C, 10 min; 2. RT, 30 min, 90 %; c) propargyl alcohol, $[PdCl_2(PPh_3)_2]$, CuI, $Et_3N$, THF, RT, 18 h, 85 %; d) $MnO_2$, $CH_2Cl_2$, RT, 6 h, 98 %; e) 1. addition of NaHMDS in THF to **8** in DMF, 0 °C → RT, 1 h; 2. addition of **7** in THF, −20 °C → RT, 8 h, 62 %.

condensed with the dianion of phosphate **8**[6] to produce **9** (Table 1). The overall yield of this process ranged from 41 to 45 % on a one-gram scale. Deprotection of piperazine **9** with sulfuric acid in wet tetrahydrofuran provided a junction for attachment of a wide variety of reactive linkages. For example, the amine-reactive label SENSI-OH (**1**) was prepared by allowing the deprotected piperazine to react with glutaric anhydride and then converting the terminal carboxylic acid group into an *N*-hydroxysuccinimide ester functionality with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC; Scheme 3). The phenol function can also be used as a site for linkage, as shown in SENSI (**2**). Attachment to this site offers the advantage that the bond can be broken hydrolytically. Synthesis of **2** was achieved by converting the *t*boc-protected derivative **9** into **10**[8] (Table 1, *t*boc = *tert*-butoxycarbonyl), which in turn reacted with *N*-hydroxysuccinimidyl glutaryl chloride to provide **2**. Other systems, such as the one-carbon-linked SENSIm (**3**), were prepared by the same route. These three derivatives readily reacted with bovine serum albumin (BSA) under standard conditions. Addition of four equivalents of **1** per equivalent of BSA provided conjugates containing approximately 2.1 molecules of dye per molecule of BSA. The efficiency of the two phenol-

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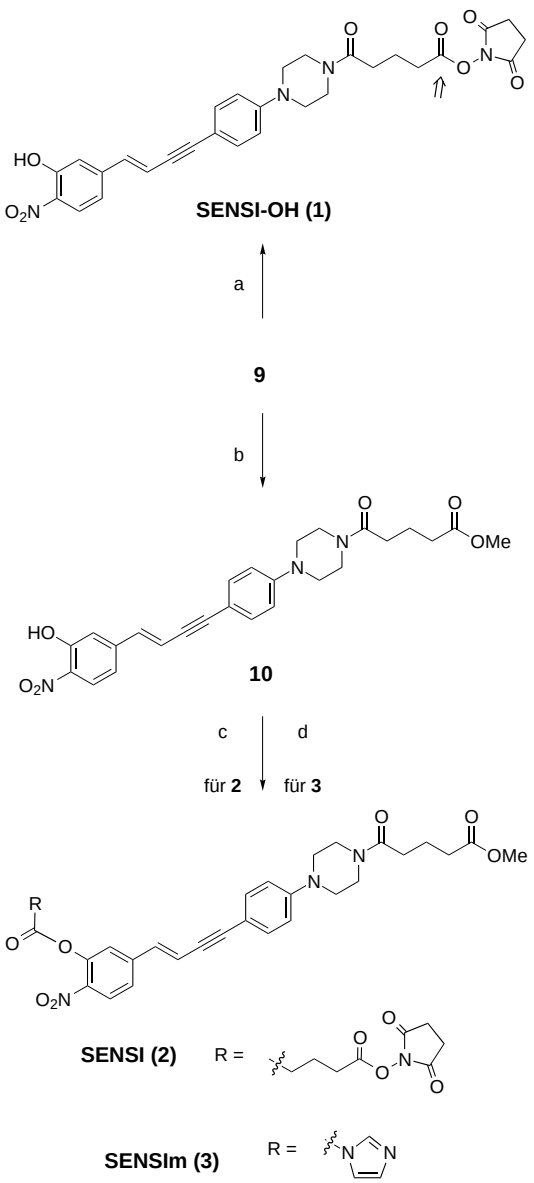


Scheme 3. a) 1. $H_2SO_4$, $H_2O$, THF, RT; 2. glutaric anhydride, THF, RT, 2 h; 3. *N*-hydroxysuccinimide, EDC, THF, RT, 12 h, 81%; b) 1. $H_2SO_4$, $H_2O$, THF, RT; 2. glutaric anhydride, THF, RT, 2 h; 3. EDC, MeOH, THF, RT, 4 h, 77%; c) *N*-hydroxysuccinimidyl glutaryl chloride, DMAP, THF, RT, 12 h, 64%; d) *N,N*-carbonyldimidazole (2.5 equiv), DMAP (0.05 equiv), THF, RT, 14 h, 72%.

linked derivatives **2** and **3** was much lower (1.2 and 0.8 fluorescent units per molecule of BSA, respectively). This is probably a result of hydrolysis of the phenolic ester linkage during coupling. The conjugates of BSA with **2** and **3** were stable between pH 5.9 and 8.1, where only minimal dye release was visible during dialysis.[9] However, considerable hydrolysis occurred outside this region, often leaving only small amounts of label intact.

The spectroscopic properties of the new materials resemble those of DHNB. For instance, the UV/Vis absorption maximum of **10** was red-shifted by 33 nm upon changing the solvent from *n*-heptane to methanol (Table 2). The extinction coefficient was largest in aromatic solvents such as benzene and toluene, and decreased with increasing polarity. The intensity of the B band was between 14 and 63% greater than that of the K band in most solvents other than DMF.[10] The fluorescence band of **10** shifted by 111 nm and was 120 times more intense when methanol was replaced by *n*-heptane. Enhancement of fluorescence intensity was only one-eighth that observed with the parent DHNB. This may be due to the fact that the piperazine ring has a greater tendency to conjugate with the aryl π system than the dimethylamino group, which restricts its freedom to rotate and form additional fluorescence states.[11] As indicated in Table 3, single

Table 1. Selected physical data for **1**–**3**, **5**–**7**, **9**, and **10**.

**1** ($C_{29}H_{28}N_4O_8$, 560.56): $R_f = 0.41$ (ethyl acetate/toluene/methanol 10/1/1); m.p. 223.1–225.6 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 10.6$ (s, 1H), 8.04 (d, $J = 8.8$ Hz, 1H), 7.38 (d, $J = 8.7$ Hz, 2H), 7.09 (d, $J = 1.6$ Hz, 1H), 7.01 (dd, $J = 1.6$, 8.8 Hz, 1H), 6.87 (d, $J = 16.1$ Hz, 1H), 6.83 (d, $J = 8.7$ Hz, 2H), 6.54 (d, $J = 16.1$ Hz, 1H), 3.73 (dd, $J = 4.8$, 4.9 Hz, 2H), 3.63 (dd, $J = 4.8$, 4.9 Hz, 2H), 3.24 (m, 4H), 2.83 (s, 4H), 2.74 (t, $J = 6.7$ Hz, 2H), 2.52 (t, $J = 7.2$ Hz, 2H), 2.10 (dddd, $J = 6.7$, 6.7, 7.2, 7.2 Hz, 2H); $^{13}$C NMR (63 MHz, $CDCl_3$/[$D_6$]DMSO, 2/1): $\delta = 18.9$, 24.4, 29.0, 29.8, 43.5, 46.5, 46.8, 113.1, 113.9, 115.5, 116.3, 124.4, 131.6, 135.9, 149.2, 168.3

**2** ($C_{35}H_{36}N_4O_{11}$, 688.69): $R_f = 0.62$ (ethyl acetate/toluene/methanol 10/1/1); m.p. 161.1–162.9 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 8.08$ (d, $J = 8.5$ Hz, 1H), 7.37 (d, $J = 8.7$ Hz, 2H), 7.35 (dd, $J = 1.1$, 8.5 Hz, 1H), 7.20 (d, $J = 1.1$ Hz, 1H), 6.90 (d, $J = 16.1$ Hz, 1H), 6.83 (d, $J = 8.7$ Hz, 2H), 6.51 (d, $J = 16.1$ Hz, 2H), 3.75 (t, $J = 4.9$ Hz, 2H), 3.65 (s, 3H), 3.63 (t, $J = 4.9$ Hz, 2H), 3.23 (m, 4H), 2.83 (s, 4H), 2.80 (t, $J = 7.1$ Hz, 2H), 2.42 (t, $J = 7.1$ Hz, 4H), 2.20 (quint, $J = 7.1$ Hz, 2H), 1.96 (quint, $J = 7.1$ Hz, 2H), 1.23 (t, $J = 7.1$ Hz, 2H); $^{13}$C NMR (53 MHz, $CDCl_3$): $\delta = 19.4$, 20.3, 25.6, 29.8, 32.1, 32.4, 33.0, 41.1, 45.0, 48.0, 48.4, 51.6, 87.1, 113.1, 114.4, 115.3, 122.0, 123.8, 126.5, 133.0, 136.3, 140.1, 143.7, 144.5, 168.0, 169.0, 170.3, 173.7

**3** ($C_{30}H_{29}N_5O_7$, 571.59): $R_f = 0.12$ (ethyl acetate/*n*-hexane 1/1); m.p. 192.5–193.1 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 8.04$ (d, $J = 8.8$ Hz, 1H), 7.67 (s, 1H), 7.38 (d, $J = 8.6$ Hz, 2H), 7.10 (s, 2H), 7.01 (d, $J = 8.8$ Hz, 1H), 6.88 (d, $J = 16.0$ Hz, 1H), 6.84 (d, $J = 8.7$ Hz, 2H), 6.54 (t, $J = 16.0$ Hz, 1H), 3.74 (m, 2H), 3.66 (s, 3H), 3.63 (m, 2H), 3.24 (m, 4H), 2.42 (t, $J = 7.1$ Hz, 2H), 1.97 (quint, $J = 7.1$ Hz, 2H), 0.85 (t, $J = 7.1$ Hz, 2H)

**5** ($C_{15}H_{21}IN_2O_2$, 388.25): $R_f = 0.85$ (ethyl acetate/*n*-hexane 1/1); m.p. 153.1–153.8 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 7.50$ (d, $J = 8.7$ Hz, 2H), 6.65 (d, $J = 8.7$ Hz, 2H), 3.54 (dd, $J = 5.2$, 5.2 Hz, 2H), 3.07 (dd, $J = 5.2$, 5.2 Hz, 2H), 1.45 (s, 9H); $^{13}$C NMR (63 MHz, $CDCl_3$): $\delta = 28.4$, 48.9, 118.5, 137.8, 150.8, 154.6

**6** ($C_{18}H_{24}N_2O_3$, 316.40): $R_f = 0.52$ (ethyl acetate/hexane 1/1); m.p. 158.2–159.1 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 7.30$ (d, $J = 8.8$ Hz, 2H), 6.77 (d, $J = 8.9$ Hz, 2H), 4.44 (s, 2H), 3.53 (dd, $J = 5.2$, 5.2 Hz, 4H), 3.13 (dd, $J = 5.2$, 5.2 Hz, 4H), 1.45 (s, 9H); $^{13}$C NMR (63 MHz, $CDCl_3$): $\delta = 28.4$, 48.4, 51.6, 80.0, 85.9, 113.1, 115.5, 132.8, 150.8

**7** ($C_{18}H_{22}N_2O_3$, 314.38): $R_f = 0.74$ (ethyl acetate/*n*-hexane 1/1); m.p. 103.5–104.7 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 9.34$ (s, 1H), 7.48 (d, $J = 8.9$ Hz, 2H), 6.81 (d, $J = 8.9$ Hz, 2H), 3.55 (dd, $J = 5.5$, 5.5 Hz, 4H), 3.13 (dd, $J = 5.5$, 5.5 Hz, 4H), 1.46 (s, 9H); $^{13}$C NMR (63 MHz, $CDCl_3$): $\delta = 28.4$, 47.2, 80.2, 89.4, 98.1, 108.2, 114.5, 135.3, 152.5, 154.6, 176.6

**9** ($C_{25}H_{27}N_3O_5$, 449.20): $R_f = 0.83$ (ethyl acetate/*n*-hexane 1/1); m.p. 214.3–216.0 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 10.7$ (s, 1H), 8.03 (d, $J = 8.8$ Hz, 1H), 7.37 (d, $J = 8.8$ Hz, 2H), 7.07 (d, $J = 1.5$ Hz, 1H), 7.00 (dd, $J = 1.5$, 8.8 Hz, 1H), 6.87 (d, $J = 16.0$ Hz, 1H), 6.83 (d, $J = 8.9$ Hz, 2H), 6.53 (d, $J = 16.0$ Hz, 2H), 3.55 (dd, $J = 5.2$, 5.2 Hz, 4H), 3.20 (dd, $J = 5.2$, 5.2 Hz, 4H), 1.46 (s, 9H); $^{13}$C NMR (63 MHz, [$D_6$]DMSO): $\delta = 28.4$, 48.1, 87.2, 114.8, 115.3, 116.6, 117.7, 125.6, 133.0, 136.9, 145.9, 151.1, 154.2

**10** ($C_{30}H_{29}N_5O_7$, 571.59): $R_f = 0.13$ (ethyl acetate/*n*-hexane 1/1); m.p. 166.3–168.0 °C; $^1$H NMR (300 MHz, $CDCl_3$): $\delta = 10.6$ (s, 1H), 8.02 (d, $J = 8.8$ Hz, 1H), 7.36 (d, $J = 8.8$ Hz, 2H), 7.07 (d, $J = 1.5$ Hz, 1H), 6.89 (dd, $J = 1.5$, 8.8 Hz, 1H), 6.86 (d, $J = 16.0$ Hz, 1H), 6.81 (d, $J = 8.9$ Hz, 2H), 6.51 (d, $J = 16.0$ Hz, 2H), 3.74 (t, $J = 1.4$ Hz, 2H), 3.65 (s, 3H), 3.62 (t, $J = 1.4$ Hz, 2H), 3.22 (m, 4H), 2.40 (dt, $J = 1.4$, 6.6 Hz, 2H), 1.95 (quint, $J = 7.2$ Hz, 2H), 1.22 (t, $J = 7.2$ Hz, 2H)

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Table 2. UV/Vis absorption maxima ($\lambda_{A,max}$), range, extinction coefficients ($\varepsilon$), fluorescence maxima ($\lambda_{F,max}$), range, and quantum yields ($\Phi_F$) for **10** in anhydrous solvents.[a]

| Solvent | $E_T$[b] | $\lambda_{A,max}$[c] [nm] | $\varepsilon$ [$cm^{-1}M^{-1}$] | $\lambda_A$ range[d] [nm] | $\lambda_{F,max}$ [nm] | $\Phi_F$[e] | $\lambda_F$ range[d] [nm] |
|---|---|---|---|---|---|---|---|
| *n*-heptane | 30.9 | 425 | 72500 | 190 – 505 | 595 | 0.082 | 575 – 740 |
| | | 305 | 61800 | | 579 | 0.079 | |
| toluene | 33.9 | 422 | 71700 | 190 – 495 | 601 | 0.041 | 510 – 730 |
| | | 314 | 62800 | | | | |
| benzene | 34.5 | 420 | 119300 | 190 – 495 | 606 | 0.057 | 520 – 730 |
| | | 318 | 93700 | | | | |
| THF | 37.4 | 412 | 65200 | 190 – 510 | 596 | 0.0067 | 450 – 760 |
| | | 306 | 47500 | | | | |
| ethyl acetate | 38.1 | 410 | 85100 | 190 – 495 | 624 | 0.0086 | 440 – 725 |
| | | 310 | 60500 | | | | |
| $CHCl_3$ | 39.1 | 422 | 59200 | 190 – 505 | 666 | 0.0061 | 460 – 780 |
| | | 316 | 46900 | | | | |
| $CH_2Cl_2$ | 41.4 | 422 | 69400 | 190 – 505 | 657 | 0.0051 | 450 – 670 |
| | | 316 | 47200 | | | | |
| acetone | 42.2 | 410 | 67400 | 190 – 495 | 468 | 0.0053 | 430 – 565 |
| DMF | 43.8 | 468 | 32900 | 190 – 515 | 473 | 0.0039 | 405 – 510 |
| | | 364 | 73200 | | | | |
| DMSO | 45.0 | 390 | 44900 | 190 – 510 | 480 | 0.0021 | 425 – 545 |
| | | 310 | 34700 | | | | |
| acetonitrile | 46.0 | 398 | 45300 | 190 – 495 | 473 | 0.0014 | 430 – 585 |
| | | 316 | 34100 | | | | |
| *n*-butanol | 50.2 | 406 | 45700 | 190 – 490 | 586 | 0.0019 | 432 – 705 |
| | | 316 | 28200 | | | | |
| ethanol | 51.9 | 394 | 63900 | 190 – 490 | 482 | 0.0016 | 425 – 690 |
| | | 318 | 40800 | | | | |
| methanol | 55.5 | 392 | 46700 | 190 – 485 | 490 | 0.00068 | 425 – 545 |
| | | 318 | 28500 | | | | |

[a] The reported data have been averaged over several iterations with deviations of less than 5%. Unavoidable traces of moisture undoubtedly decreased the intensity of these values. Precautions were taken to minimize contact with moisture, and all solvents were either purchased dry or distilled. [b] $E_T$ is a measure of solvent polarity. See ref. [2a]. [c] The K band is defined as the lower and the B band as the higher wavelength band. [d] The range is defined as the region within which the signal intensity exceeded 5% of the maximum absorption or fluorescence. [e] Quantum yields were standardized against the value 0.70 for rhodamine B in ethanol.

Table 3. Single-molecule detection of **10**.[a]

| Solvent | Detection limit [nM][b] | Extent of decomposition after 5 min [%] |
|---|---|---|
| *n*-heptane | 0.015 | 1 |
| THF | 12 | 4 |
| $CHCl_3$ | 0.95 | 3 |
| acetone | 14 | 4 |
| DMF | 17 | 3 |
| acetonitrile | 89 | 2 |
| ethanol | 128 | 2 |
| methanol | 205 | 5 |
| water | 625 | 4 |

[a] Excitation with an argon ion laser at 457 nm (0.5 mW). The fluorescence was observed through a filter with a cutoff at 545 nm. [b] The detection limit is the concentration at which transients from single molecules were no longer resolved from the baseline.

molecules derived from these dyes were detectable at submicromolar concentrations in polar solvents (e.g., THF, chloroform, acetone, acetonitrile, DMF, ethanol, methanol, and water) with the aid of a confocal fluorescence correlation spectrometer.[12] Single molecules were detected at far lower concentrations in nonpolar solvents such as *n*-heptane, providing a gain in response of approximately 40000 for selective detection of single molecules. Investigations into the use of these markers as a probe for interactions between molecules and lipophilic surfaces are currently in progress.

## *Experimental Section*

**5**: Di-*tert*-butyl dicarbonate (4.5 g, 20.6 mmol) was added in small portions over 15 min to a mixture of *N*-phenylpiperazine (3.0 mL, 19.6 mmol), triethylamine (8.2 mL, 58.9 mmol), and 4-dimethylaminopyridine (DMAP; 86 mg) in anhydrous $CH_2Cl_2$ (100 mL) at 0 °C. Ten minutes after addition was complete the mixture was warmed to room temperature (RT). After 1.5 h it was diluted with $CH_2Cl_2$ (200 mL) and water (100 mL), and extracted. The aqueous phase was further extracted with $CH_2Cl_2$ (2 × 100 mL). The combined organic extracts were washed with saturated aqueous NaCl, dried over $Na_2SO_4$, and concentrated to yield 4.87 g (94%) of **4**. Iodine (5.0 g, 19.7 mmol) was added over 45 min to a mixture of **4** (5.46 g, 20.8 mmol) and $NaHCO_3$ (2.62 g, 31.2 mmol) in $CH_2Cl_2$ (80 mL) and water (60 mL), which was kept between 12 and 15 °C. After addition was complete the mixture was warmed to room temperature, and this temperature was maintained for 30 min. The mixture was then diluted with $CH_2Cl_2$ (500 mL) and water (100 mL), and the organic phase was collected and washed consecutively with water (50 mL), a solution ofL sodium thiosulfate (100 m), water (2 × 100 mL), and a saturated solution of aqueous NaCl (100 mL). The crude product was dried over $Na_2SO_4$, concentrated, and recrystallized from *n*-hexane/THF (10/1) to yield 6.95 g (90%) of **5**.

**9**: A mixture of **5** (1.17 g, 3.02 mmol), $[PdCl_2(PPh_3)_2]$ (21.2 mg, 0.032 mmol), and CuI (5.7 mg, 0.032 mmol) in THF (2 mL) and triethylamine (5 mL) was degassed by rapid bubbling of dry argon through it for 15 min. 2-Propyn-1-ol (0.193 mL, 3.32 mmol) was then added to the vigorously stirred mixture. Three hours later a second batch of $[PdCl_2(PPh_3)_2]$ and CuI was added. The reaction was complete within 18 h. Strict maintenance of an argon atmosphere was crucial to the yield of this step. The crude solution was filtered through silica gel (30 g) with methanol/ethyl acetate (5/95), and the eluate was concentrated. Flash chromatography ($SiO_2$, ethyl acetate/hexane 1/3) led to pure **6** (815.2 mg,

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85%). Activated $MnO_2$ (0.507 g, 5.83 mmol) was added to **6** (615.0 mg, 1.94 mol) in $CH_2Cl_2$ (18 mL) at RT. After 6 h the reaction mixture was purified directly by flash chromatography ($SiO_2$, ethyl acetate/hexane 1/3), yielding 602.1 mg (98%) of **7**. Sodium bis(trimethylsilyl)amide (4.79 mL, 1.0 M in THF, 4.79 mmol) was added to a solution of phosphonate **8** (621 mg, 2.15 mmol) in DMF (6.0 mL) at 0 °C. The solution was warmed to RT after 30 min at 0 °C and kept at RT for 20 min, after which it was again cooled to −20 °C. A solution of aldehyde **7** (376.5 mg, 1.19 mmol) in THF (10 mL) was added by syringe. After 8 h at room temperature water (5 mL) was added, the pH adjusted to 7 with 5% aqueous HCl, and the mixture further diluted with a saturated solution of aqueous NaCl (10 mL). The crude product was obtained by extraction with THF/$CH_2Cl_2$ (1/9, 3 × 40 mL), drying over $Na_2SO_4$, and concentration. Flash chromatography ($SiO_2$, $CHCl_3$/hexane 1/2) and recrystallization from *n*-heptane/THF (10/1) yielded 331.4 mg (62%) of **9**.

**1**: Concentrated sulfuric acid (130 μL) was added to **9** (82.1 mg, 0.178 mmol) in THF/$H_2O$ (1/9, 9 mL) at 0 °C. After 20 min a saturated solution of aqueous sodium bicarbonate was added until a pH of 6.5 was reached, followed by a saturated solution of aqueous NaCl (15 mL). The crude product was obtained by extraction with THF/$CH_2Cl_2$ (1/9, 3 × 60 mL), drying over $Na_2SO_4$, and concentration. The residue was treated with glutaric anhydride (26.4 mg, 0.231 mmol) and DMAP (≈3 mg) in anhydrous THF (8 mL). After 2 h at room temperature *N*-hydroxysuccinimide (40.9 mg, 0.356 mmol) was added followed by EDC (119.4 g, 0.623 mmol). Twelve hours later water (10 mL) was added, and the pH adjusted to 6.5 with dilute Hcl. The mixture was extracted with THF/$CH_2Cl_2$ (1/9, 3 × 80 mL), dried over $Na_2SO_4$, and evaporated. Recrystallization from *n*-heptane/THF (2.5/1) provided 80.7 mg (81%) of pure **1**.

**2**: Concentrated sulfuric acid (80 μL) was added to **9** (49.5 g, 0.107 mmol) in THF/$H_2O$ (1/9, 5 mL) at 0 °C. After 20 min a saturated solution of aqueous sodium bicarbonate was introduced until the pH reached 7.0, followed by a saturated solution of aqueous NaCl (10 mL). The crude product was obtained by extraction with THF/$CH_2Cl_2$ (1/9; 3 × 40 mL), drying over $Na_2SO_4$, and concentration. The residue was immediately dissolved in anhydrous THF (5 mL) and treated with glutaric anhydride (14.6 mg, 0.128 mmol) and DMAP (≈2 mg). After 2 h at room temperature anhydrous methanol (0.2 mL) was added followed by EDC (61.7 mg, 0.321 mmol). Esterification was complete after 4 h (TLC). Water (10 mL) was then added, and the product isolated by adjustment of the pH to 6.5 with dilute HCl followed by extraction with THF/$CH_2Cl_2$ (1/9, 3 × 40 mL), drying with $Na_2SO_4$, and rotary evaporation. Recrystallization from *n*-hexane/THF (3/1) provided 39.2 mg (77%) of pure **10**. *N*-Hydroxysuccinimidyl glutaryl chloride (81.1 mg, 0.327 mmol) was added to **10** (41.9 mg, 0.0953 mmol) and DMAP (ca. 1 mg) in anhydrous THF (5 mL). After 12 h at room temperature water was added (10 mL), and the product isolated by adjustment of the pH to 6.5 with dilute HCl followed by extraction with THF/$CH_2Cl_2$ (1/9, 3 × 30 mL). The extract was dried over $Na_2SO_4$ and concentrated by rotary evaporation. Recrystallization from a mixture of *n*-heptane/THF (3/1) led to 40.9 mg (64%) of **2**.

Coupling with BSA: Bovine serum albumin (BSA) used in these studies was purchased from Sigma, product A7030. A solution of **1** (1.2 mg, 2.14 μmol) in DMF (50 μL) was added in five portions to a solution of BSA (35.9 mg, 0.54 μmol) in a phosphate-buffered solution of NaCl (PBS, 5 mL; 0.15 mM NaCl, 8.1 mM $Na_2HPO_4$, 1.5 mM $NaH_2PO_4$). The mixture was allowed to react to completion for 8 h at room temperature, diluted with water (5 mL), dialyzed extensively against water (Spectra/por MWCO 12000–14000), and lyophilized to dryness. An absorption spectrum was recorded from 2–3 mg of this material, which was dissolved in 500 μL of PBS. The approximate number of fluorophores per protein molecule was then calculated based on the known absorption of **1** in PBS and the concentration of labeled BSA.

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