Supplementary Information

To D or not to D

On estimating the microenvironment polarity of biomolecular cavities

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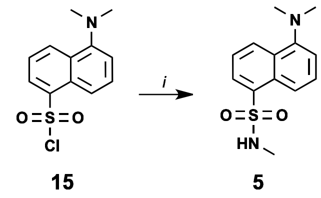
Synthesis general

All materials obtained from commercial suppliers were used without further purification. Anhydrous triethylamine, DiPEA and CH_3CN were obtained from Sigma-Aldrich. Anhydrous DMF was acquired using a two-column purification system (Glasscontour System, Irvine, CA). Reactions were conducted under and argon atmosphere if required. NMR spectra were recorded on a Varian Mercury 400 MHz spectrometer. NMR solvents were purchased from Cambridge Isotope Laboratories (Andover, MA). Mass spectra were recorded at the UCSD Chemistry and Biochemistry Mass Spectrometry Facility, utilizing either a LCQDECA (Finnigan) ESI with a quadrapole ion trap or a MAT900XL (ThermoFinnigan) FAB double focusing mass spectrometer.

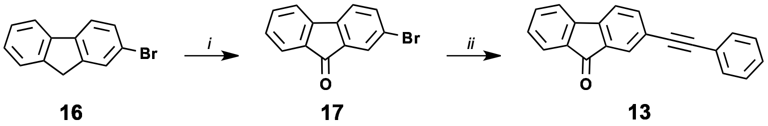
Synthesis of 5-(dimethylamino)-*N*-methylnaphthalene-1-sulfonamide (5)



Scheme 1: Synthesis of 5-dimethylamine-naphthalene-1-N-methyl-sulfonamide. i) Methylamine (2 M in THF, 2 equiv.), DiPEA (2 equiv.), CH_3CN, 0°C to rt, 1 h, 72%.

A solution of dansylchloride (**15**) (0.997 g, 3.696 mmol) and DiPEA (1.27 mL, 0.955 g, 7.392 mmol) in dry CH_3CN (15 mL) is cooled to 0°C. After dropwise addition of CH_3NH_2 (3.70 mL, 7.392 mmol, of a 2.0 M solution in THF) the reaction mixture is stirred for 1 h allowing the temperature to rise to rt. TLC analysis (silica, CH_2Cl_2, R_f = 0.2) indicates full conversion. The crude reaction mixture is concentrated *in vacuo* after which the remaining solid is redisolved in EtOAc (50 mL) and subsequently washed with water (3 × 50 mL) and brine (50 mL). The organic layer is dried over $MgSO_4$ and filtered over a glass filter and concentrated *in vacuo*. The remaining solid is purified by column chromatography (silica, CH_2Cl_2, R_f = 0.2) to obtain 5-(dimethylamino)-*N*-methylnaphthalene-1-sulfonamide (**5**) as a very bright yellow solid with a greenish glow (0.71 g, 2.67 mmol, 72%). ^{1}H-NMR (DMSO, 400 MHz) δ (ppm): 8.45 (1H, d, 8.8 Hz), 8.26 (1H, d, 8.8 Hz), 8.07 (1H, d, *J* = 7.3 Hz), 7.73 (1H, q, *J* = 4.52, N-H), 7.64-7.56 (2 1H, 2 t overlapping, *J* = 7.3 Hz and *J* = 8.4 Hz), 7.25 (1H, d, *J* = 7.3 Hz), 2.81 (6H, s, 2 × CH_3), 2.40 (3H, d, *J* = 4.8 Hz, N-CH_3); ^{13}C-NMR (DMSO, 100 MHz) δ (ppm): 151.4, 134.8, 129.5, 129.14, 129.10, 128.7, 127.9, 123.6, 119.1, 115.2, 45.1, 28.6; FAB-HRMS calc. 264.0927, found: 264.0929.

Synthesis of 2-phenylethynylfluorenone (13)



*Scheme 2: Oxidation of 2-bromofluorene (**16**) to 2-bromofluorenone (**17**) and subsequent coupling to phenylacetylene under Sonogashira conditions gave 2-phenylethynylfluorenone (**13**). i) Bu_4NOH (0.13 equiv), pyridine, rt, 5 days, 52-65%. ii) Phenylacetylene (2 equiv.), $PdCl_2(dppf)$ (5 mol%), CuI (5 mol%), DMF/Et_3N (4:1), 70°C, 2 h, ~100 %.*

A solution of phenylacetylene (0.27 mL, 0.255 g, 2.500 mmol) and Et_3N (0.8 mL) in DMF (3.2 mL) is purged with argon for 15 minutes and cannulated into a roundbottom flask containing 2-bromofluorenone (**17**)[1] (0.324 g, 1.250 mmol), Pd(dppf)Cl_2 (0.0510 g, 0.063 mmol) and CuI (0.012 g, 0.062 mmol) under an argon atmosphere. The reaction mixture is heated to 50°C for 17 h under continuous stirring after which it is concentrated *in vacuo*. The remaining solid is taken up in EtOAc (20 mL) and washed with 0.1 M HCl aq. (3 × 20 mL) and brine (20 mL). The organic layer is dried over $MgSO_4$ and filtered over a glass filter. The *in vacuo* concentrated filtrate is purified by column chromatography (silica, 20% CH_2Cl_2 in hexane, R_f = 0.3) yielding 2-phenylethynylfluorenone (**13**) as a bright yellow solid (0.352 g, 1.256 mmol, ~100%). ^{1}H-NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.81 (1H, dd, J = 1.1 Hz, *J* = 0.7 Hz), 7.68 (1H, d, *J* = 7.3 Hz), 7.65 (1H, dd, *J* = 1.5 Hz, *J* = 7.8 Hz), 7.55-7.49 (5H, m), 7.38-7.35 (3H, m), 7.32 (1H, dt, *J* = 7.1 Hz, *J* = 1.8 Hz); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ (ppm): 193.2 (*C*=O), 144.1, 143.8, 137.7, 135.0, 134.4, 134.3, 131.8, 129.5, 128.7, 128.5, 127.4, 124.6, 124.3, 122.9, 120.7, 120.4, 91.2 (*C*≡CPh), 88.7 (C≡*C*Ph); FAB-HRMS calc. 280.0883, found: 280.0884.

Spectroscopy general

UV-Vis spectra were recorded on a Hewlett Packard 8453 Diode Array Spectrometer. Steady state fluorescence experiments were carried out at room temperature in a 1 mL quartz fluorescence cell with a path length of 1.0 cm (Hellma GmbH & Co KG, Müllheim, Germany) on a Jobin Yvon Horiba FluoroMax-3 luminescence spectrometer. All samples were prepared from a stock solution in DMSO, hence, all samples contain 0.4 *v*% DMSO.

Spectroscopic properties of 5-(dimethylamino)-*N*-methylnaphthalene-1-sulfonamide (**5**)

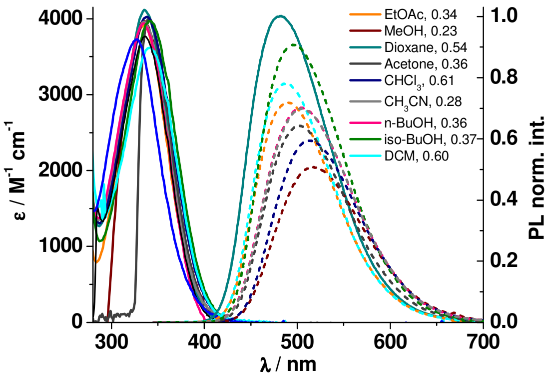


*Figure 1: Absorption (solid lines) and emission curves (dashed lines) of dansylamide **5** in, dioxane (dark cyan), i-BuOH (green), DCM (light blue), EtOAc (Orange), n-BuOH (pink), CH_3CN (gray), acetone (dark gray), $CHCl_3$ (blue), MeOH (dark red). Quantum yields relative to anthracene are reported in the legend. All samples are measured at 1.02 × 10^{-5} M concentration and contain 0.4 v% DMSO to ensure solubility. All emission spectra are recorded after excitation at the long wavelength absorption maximum.*

Tabel 1: Spectral properties of 5-(dimethylamino)-*N*-methylnaphthalene-1-sulfonamide (**5**) and solvent polarity parameters ε, $E_T(30)$ and Δ*f*.

Solvent	**Abs** [nm]	**Em** [nm]	**Stokes shift** [cm^{-1}]	**rel. QY**	**ε** [D]	**$E_T(30)$** [kcal mol^{-1}]	**Δ*f***
EtOAc	335	488.0	9359	0.34	6.05	38.3	0.200
MeOH	335	518.0	10546	0.23	33.52	55.2	0.309
Dioxane	335	478.5	8952	0.54	2.22	36.3	0.022
Acetone	341	502.0	9405	0.36	21.40	42.7	0.285
$CHCl_3$	341	486.0	8749	0.61	4.81	39.2	0.148
CH_3CN	339	512.0	9967	0.28	37.50	45.9	0.305
DCM	340	495.5	9230	0.60	8.90	41.3	0.217
n-BuOH	335	505.0	10049	0.36	17.92	49.6	0.264
iso-BuOH	335	503.5	9990	0.37	17.84	48.9	0.265
Toluene	336	475.0	8709	0.40	2.39	34.8	0.014
Water	328	561.5	12678	--	80.18	--	0.320

The quantum yield is determined relative to anthracene in EtOH (0.27). All $E_T(30)$ are determined experimentally,[2] the orientational polarizability is determined according Lippert[3]-Mataga[4] based on refractive indexes taken from the Sigma-Aldrich website[5] as reported for the pure solvents. The dielectric constants are taken from Wohlfarth.[6]

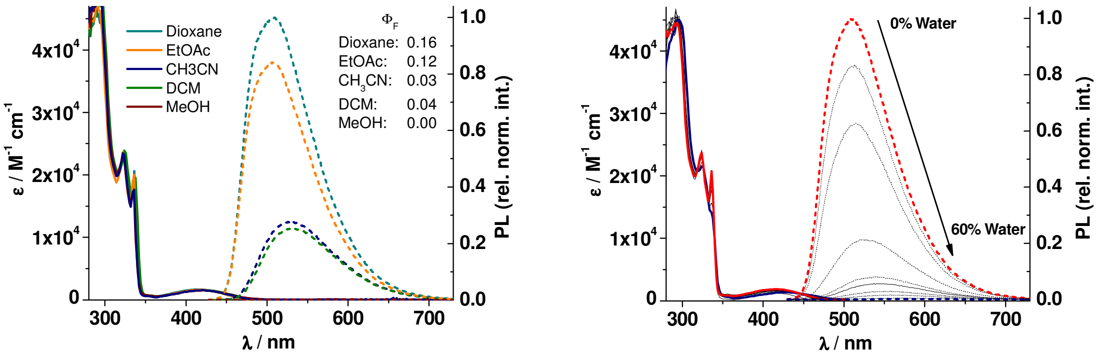
Spectroscopic properties of Prodan (12)

Tabel 2: Spectral properties of Prodan (**12**) and solvent polarity parameters ε, $E_T(30)$ and Δ*f*.

Solvent	Abs [nm]	Em [nm]	Stokes shift [cm^{-1}]	ε [D]	$E_T(30)$ [kcal mol^{-1}]	Δ*f*
cyclohexane	342	401	4302	2.02	30.9	0.001
benzene	355	421	4416	2.28	34.3	0.002
triethylamine	343	406	4523	2.43	32.1	0.102
chlorobenzene	254	430	4992	5.65	36.8	0.143
chloroform	357	440	5284	4.81	39.1	0.185
acetone	350	452	6448	21.40	42.2	0.287
DMF	355	461	6477	38.70	43.2	0.276
acetonitrile	350	462	6926	37.50	45.6	0.304
ethylene glycol	375	515	7249	41.82	56.3	0.274
propylene glycol	370	510	7419	30.00	54.1	0.270
ethanol	360	496	7616	25.29	51.9	0.298
methanol	362	505	8206	33.52	55.4	0.308
water	364	531	8640	80.18	63.1	0.320

Absorption and emission data are taken from Weber and Farris.[7] All $E_T(30)$ are taken from Reichardt,[2] the orientational polarizability is determined according Lippert[8]-Mataga[9] based on refractive indexes taken from the Sigma-Aldrich website[10] as reported for the pure solvents. The dielectric constants are taken from Wohlfarth.[11]

Spectroscopic properties of 2-phenylethynylfluorenone (10)



*Figures 2: left) Absorption spectra of **5** in dioxane (blue line) EtOAc (orange line), CH_3CN (blue line), DCM (green line) and MeOH (red line) and their corresponding PL spectra (dashed lines); right) Absorption spectra (solid lines) and PL spectra (dashed lines) of **5** in various water dioxane mixtures with pure water and pure dioxane depicted in blue and red respectively. All samples are measured at a concentration of 4.71 × 10^{-5} M and contain 0.4 v% DMSO to ensure solubility. PL spectra are recorded after excitation at the long wavelength absorption maximum.*

Table 3: Spectral properties of 2-phenylethynylfluorenone and solvent parmeters ε, $E_T(30)$ and Δf.

Solvent	**Abs** [nm]	**Em** [nm]	**Stokes shift** [cm^{-1}]	**ε** [D]	**$E_T(30)$** [kcal mol^{-1}]	**Δf**
EtOAc	418	505	4121	6.05	38.3	0.200
Dioxane	418	509	4277	2.22	36.3	0.022
CH_3CN	420	528	4870	37.50	45.9	0.305
DCM	422	529	4793	8.90	41.9	0.217
MeOH	420	593	6946	33.52	55.3	0.309
fraction water in dioxane						
0	418	512	4392	2.08	36.4	0.008
6.00E-04	418	513	4430	2.10	36.6	0.009
0.003	416	515	4621	2.16	37.3	0.016
0.015	418	526	4912	2.49	39.9	0.048
0.042	420	540	5291	3.35	43.4	0.106
0.060	416	543	5622	4.00	44.7	0.135
0.120	420	551	5661	6.65	46.8	0.199
0.210	420	556	5824	11.85	48.6	0.243
0.420	424	571	6072	28.06	52.1	0.278
0.600	424	583	6432	44.21	54.9	0.289

$E_T(30)$ values are determined experimentally with Reichardt's dye.[2] The dielectric constants for the pure solvents and the water-dioxane mixtures are taken from ref 6 and 12 respectively. The orientational polarizabilities are calculated according Lippert[3] and Mataga[4] with refractive indexes for the pure solvents and water-dioxane mixtures from ref 5 and 13 respectively and, where necessary, interpolation of the data was performed after curve fitting using Microcal Origin Pro v. 7.5.

References

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