# Fluorescent diaza crown ether sensitive to complexation, conformation and microenvironment

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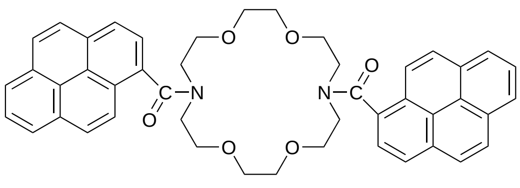
**A newly synthesized diaza crown ether, BPDC, demonstrates sensitivity to solvent polarity, microviscosity and metal complexation with spectroscopic changes in monomer and excimer fluorescence.**

In order to address the sensing challenges presented by complex, real-world matrices, we are designing new molecules tailored with fluorescent sidearms sensitive to the complexation, molecular conformation and microenvironment of the sensing molecule. Specifically, our interest is in the development of a metal sensing probe operable in ship bilge water, a matrix that contains heavy-metal ions in a complex and variable environment of surfactants, oils, and other contaminants. To study the nature of metal complexation in these environments, we synthesized a new, sensor molecule, *N*,*N*-bis(1-pyrenecarbonyl)-7,16-diaza 18-crown-6 (BPDC), designed to signal the sensitive complexation of heavy-metal ions, as well as respond spectroscopically to changes in its microenvironment. While bis-pyrene linked probes have been previously used for monitoring microviscosity,[1] metal ion concentration,[2] and organics,[3] this communication describes the response to all three of these variables together in a single probe.



BPDC

The vibronic fine structure of pyrene fluorescence is very sensitive to solvent polarity changes with changes in the ratio of $I_1/I_3$.[4] According to their protic or aprotic nature, solvents were classified into two, linear polarity scales by BPDC. Comparison of the $I_1/I_3$ ratios and Dimroth's $E_\mathrm{T}$ values are shown in Fig. 1. The $E_\mathrm{T}$ scale is an excellent measure of solvent polarity generated from the intramolecular charge-transfer band of Reichardt's dye.[5]

The ability of BPDC to form an intramolecular excimer provides additional insight into the conformation and microviscosity experienced by this sensor molecule. The intensity of excimer fluorescence, $I_\mathrm{e}$, is correlated with the probability that an excited pyrene moiety can rotate to a position of close, parallel molecular contact with an unexcited pyrene neighbour prior to its own electronic, excited singlet state decay (as monomer fluorescence, $\mathrm{I}_\mathrm{monomer}$).[6] This probability is dependent on both (*i*) the conformation of the molecule in the solvent, a feature that is strongly linked to the solvent's relative permittivity $\varepsilon$, and (*ii*) the viscosity, $\gamma$, of the medium. A plot of the relative monomer emission, $I_\mathrm{m}$ [$I_\mathrm{m} = I_\mathrm{monomer}/(I_\mathrm{monomer}{}^{+}I_\mathrm{e})$], from BPDC *vs.* the product $\varepsilon\gamma$ for various protic and aprotic solvents is shown in Fig. 2. With the exception of *n*-hexane and *n*-heptane, the possibility of intermolecular excimer formation was precluded by sequential dilution of BPDC until no changes were evident in $I_\mathrm{m}$.

Examining the aprotic solvents in Fig. 2, as the solvent changes from *n*-heptane to $CHCl_3$ [(*a*)–(*f*)] there is only a minor change in the viscosity. This indicates that it is the increase in relative permittivity which is primarily responsible for the increase in monomer emission. BPDC has very low solubility in solvents of low relative permittivity, *n*-hexane and *n*-heptane, owing to poor solvation of the crown ether backbone. Aggregation of BPDC could explain the smaller $I_\mathrm{m}$ values apparent in these solvents. As the relative permittivity of the solvent increases, the solvent sheath formed about the crown ether backbone forces apart the two pyrene moieties. For solvents with $\varepsilon > 7$, both the crown ether backbone and pyrene linkages are effectively solvated and BPDC adopts a similar conformation with $I_\mathrm{m}$ *ca.* 0.7.

For protic solvents, as the solvents progress from methanol to pentanol [Fig. 2(*i*)–(*m*)], the increase in viscosity causes the

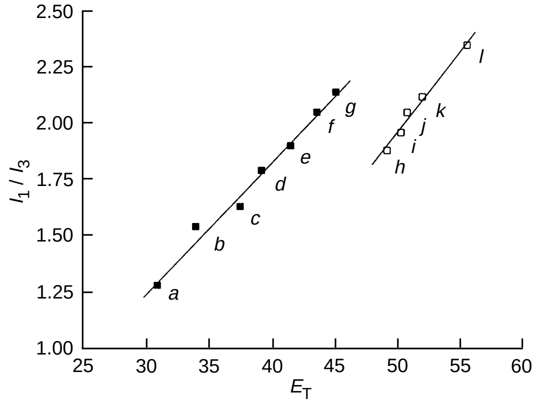


**Fig. 1** Plot of the intensity ratio $I_1/I_3$ from the emission of BPDC *vs.* Dimroth's $E_\mathrm{T}$ values in various aprotic (■) and protic (□) solvents. Solvent: (*a*) *n*-hexane, (*b*) toluene, (*c*) THF, (*d*) $CHCl_3$, (*e*) $CH_2Cl_2$, (*f*) DMF, (*g*) $Me_2SO$, (*h*) *n*-pentanol, (*i*) *n*-butanol, (*j*) *n*-propanol, (*k*) ethanol, (*l*) methanol; [BPDC] = 2.9 μM.

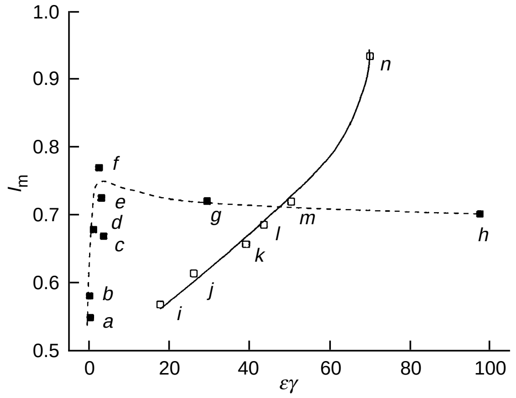


**Fig. 2** Plot of the relative monomer emission, $I_\mathrm{m}$, from BPDC *vs.* the product of the relative permittivity ($\varepsilon$) and the viscosity ($\gamma$). Aprotic solvents (■): (*a*) *n*-heptane (*b*) *n*-hexane, (*c*) $CH_2Cl_2$, (*d*) toluene, (*e*) THF, (*f*) $CHCl_3$, (*g*) DMF, (*h*) $Me_2SO$; protic solvents (□): (*i*) methanol, (*j*) ethanol, (*k*) *n*-propanol, (*l*) *n*-butanol, (*m*) *n*-pentanol, (*n*) water; [BPDC] = 2.9 μM.

monomer emission to increase owing to a reduction in mobility of the pyrene appendages as well as an improvement in solvation of the pyrene groups. Water, because of its extremely high relative permittivity and hydrogen-bonding ability, leads to the highest monomer emission for BPDC. This result is in contrast to the majority of bis-pyrene molecules studied previously, which exhibit significant intramolecular excimer formation in water due to the hydrophobic interaction of the two pyrene appendages.[7,8] For BPDC, water is apparently so effective at stabilizing the crown ether backbone that the pyrene moieties are effectively isolated from one another due to hydrophobic repulsion.

In order to monitor the influence of metal complexation on the conformation of BPDC the $I_1/I_3$ ratio and $I_m$ parameters were additionally studied. From Fig. 3 it is evident that as [Cu$^{II}$] increases, there is a strong quenching of the emission due to a paramagnetic effect of the complexed metal ion.[9] It is interesting to note from the inset chart that both $I_1/I_3$ and $I_m$ decrease linearly with the increase in [Cu$^{II}$]. Fig. 2 indicates that the two pyrene moieties of BPDC are preferentially oriented to one side of the crown ether backbone when dissolved in ethanol. As expected, complexation of a Cu$^{II}$ metal ion does not compete for binding on this side of the compound, but, instead, binds to the exposed, crown ether side. Distortion of the diaza crown ether binding sites for binding of the metal ion results in the two pyrene moieties being brought in closer proximity to each other, increasing intramolecular excimer formation. Similarly, the decrease seen in $I_1/I_3$ is indicative of the more non-polar environment encountered by the molecule due to the enhanced association of the two pyrene appendages on one side of the diaza crown ether.

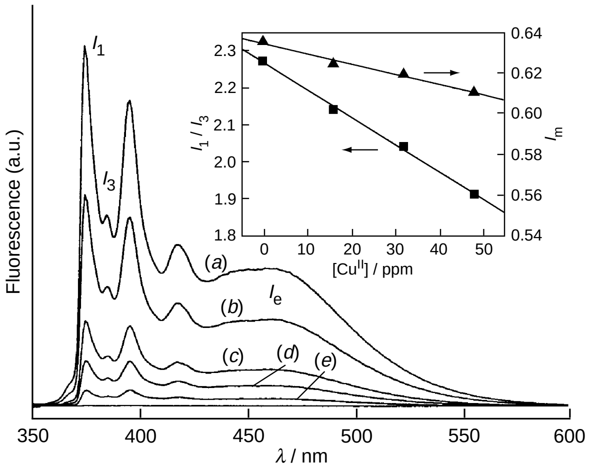


**Fig. 3** Fluorescence spectra recorded for BPDC in ethanol at different $CuCl_2$ concentrations. The inset chart graphs the change in $I_1/I_3$ and $I_m$ with the addition of Cu$^{II}$ metal ions in solution. [$CuCl_2$] in ppm = (*a*) 0, (*b*) 16, (*c*) 32, (*d*) 48, (*e*) 64, (*f*) 128; [BPDC] = 2.9 μM.

In conclusion, the present study demonstrates that the introduction of bis-pyrene appendages to a metal complexing agent can be an important tool for understanding the complexation, conformation and microenvironment of the sensing molecule.

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## Footnotes

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