

Model systems for flavoenzyme activity: intramolecular self-assembly of a flavin derivative *via* hydrogen bonding and aromatic interactions†

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We have synthesised a flavin derivative incorporating functionalities that promote intramolecular self-assembly *via* hydrogen bonding and aromatic interactions.

Flavoenzymes are proteins that catalyse a variety of biological processes such as redox transformations, signal transduction and electron-transfer.¹ Non-covalent interactions (*e.g.* hydrogen bonding and π -stacking) between the flavin cofactor and the apoenzyme have been shown to modulate the redox properties of the flavin unit by over 500 mV.² Although the flavin co-factor is usually non-covalently bonded to the apoenzyme, in some flavoenzymes the flavin unit is covalently linked to the polypeptide. In these systems, the flavin unit is usually attached through the C8 position of the flavin to the cysteine, tyrosine or histidine moieties of the peptide backbone.³ The flavin cofactor of triethylamine dehydrogenase is covalently attached to the apoenzyme through a C6 thioether link.⁴

In our continuing studies to develop model systems for flavoenzymes, and in particular mimics for flavoenzymes where the cofactor is covalently bound to the apoenzyme, we wanted to develop a model system to study the combined effects of intramolecular aromatic⁵ and hydrogen bonding interactions have on the physical properties of the flavin unit. Compound **1** was designed to allow us to explore these dual effects as the diamidopyridine moiety (DAP) is known to form complementary hydrogen bonds with the imide unit of the flavin,^{6,7} and the electron rich naphthalene (NAP) unit should participate in aromatic interactions with the electron deficient flavin core (Fig. 1).⁸ Molecular modeling studies clearly predicted the formation of an intramolecular self-assembled structure through hydrogen bonding and aromatic interactions (see ESI†). Compound **2** should allow us to explore the role of intramolecular aromatic interactions between the flavin

moiety and NAP in the absence of hydrogen bonds, whereas compounds **3** and **4** were used as controls in NMR, UV-vis and fluorescence spectroscopy experiments. Compound **3** will allow us to explore the physical properties in the absence of supramolecular interactions, whereas compound **4** will allow us to investigate the role intramolecular hydrogen bonding interactions have in modulating flavin properties.

The synthesis of compounds **1–4** is reported in the ESI.† The solution properties of flavin derivatives **1–4** were characterised by ¹H NMR, 2D NOESY, UV-vis and fluorescence spectroscopies. In all cases, non-polar solvents (*e.g.* chloroform and dichloromethane) were used for these experiments to more accurately mimic the polarity of the microenvironment surrounding the flavin moiety of a typical flavoenzyme.⁶ ¹H NMR spectroscopy of flavin **1** revealed upfield shifts for the flavin and NAP moieties and downfield shifts of the flavin imide N–H and two amide N–H groups attached to the DAP moiety (compared to controls **3** and **5**) (Fig. 2). The upfield shifts of the aromatic signals are consistent with the expected ring current effects for face-to-face stacking between the flavin and NAP units.⁸ The downfield shifts for the N–H moieties are in line with hydrogen bonding interactions between the flavin and DAP moieties.^{6,7}

The ¹H NMR spectra of flavin **1** recorded over 1.0×10^{-2} M to 5.3×10^{-4} M in CDCl₃ displayed a limited concentration dependence. Therefore, it is unlikely that supramolecular

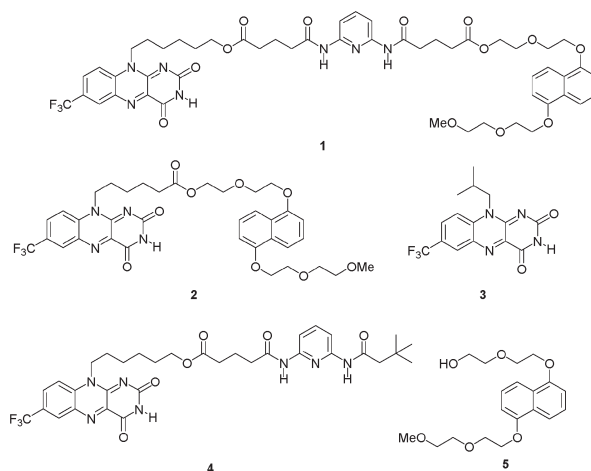


Fig. 1 Structures of derivatives 1–5.

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† Electronic supplementary information (ESI) available: Full synthetic details for the preparation of **1–5** and characterization of their physical properties. See DOI: 10.1039/b809762c

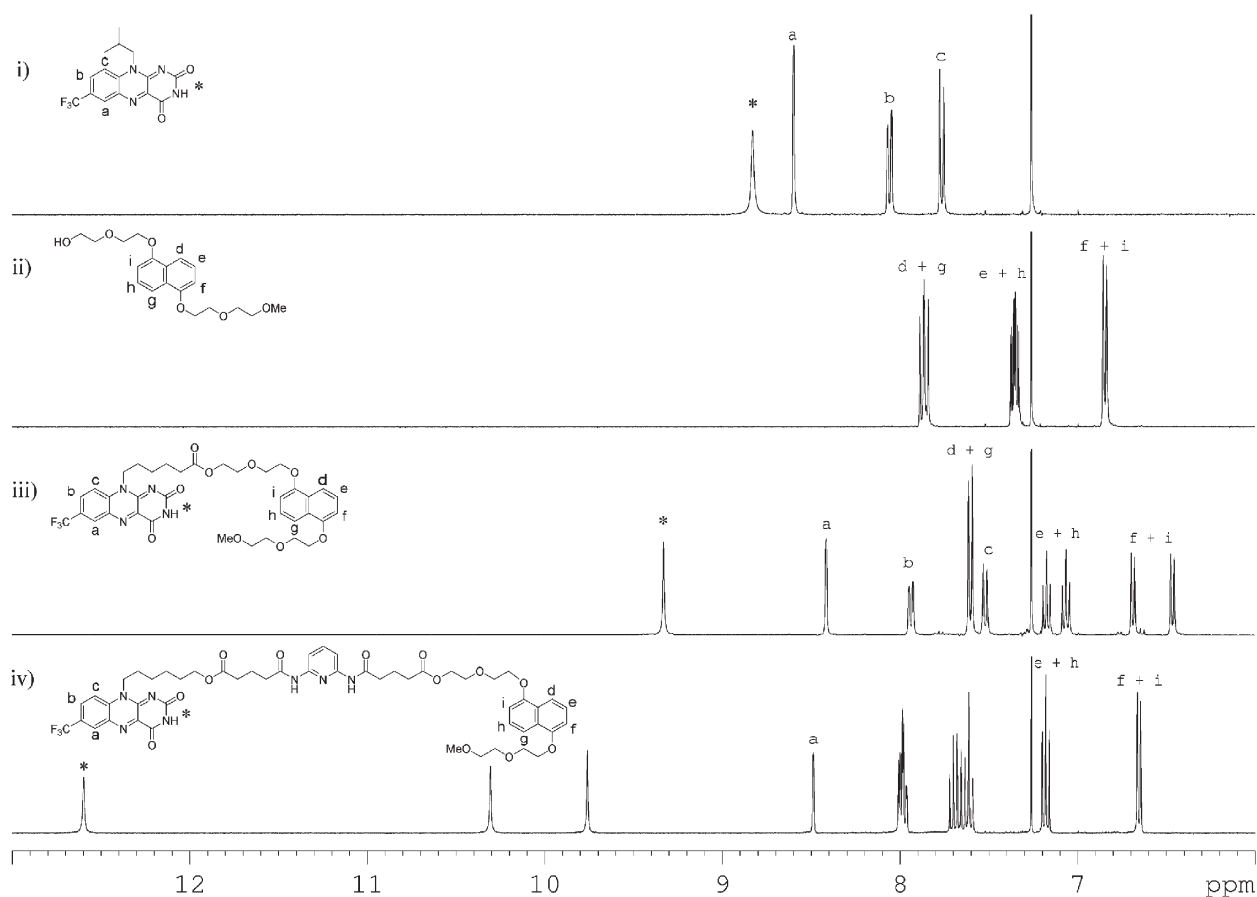


Fig. 2 Partial ^1H NMR spectra of compounds **3**, **5**, **2** and **1** recorded at ~ 31 mM in CDCl_3 .

oligomers are formed to any great extent, and intramolecular interactions are likely to predominate under the conditions examined. Further evidence of intramolecular aromatic interactions in compound **1** were obtained from the NOESY spectrum, which shows weak intensity long range couplings between the aromatic flavin hydrogens and the aromatic NAP hydrogens (Fig. 3). Cross-peaks between the flavin unit and the ethylene glycol “arms” of the naphthalene unit were also observed, indicating that the “arms” are wrapped around the flavin unit.

The ^1H NMR spectrum of flavin **2** recorded in CDCl_3 shows upfield shifts in the signals arising from the NAP unit compared to the signals obtained for the same protons of **5** (Fig. 2). A similar upfield shift was also observed for the aromatic flavin protons compared to control compound **3**.

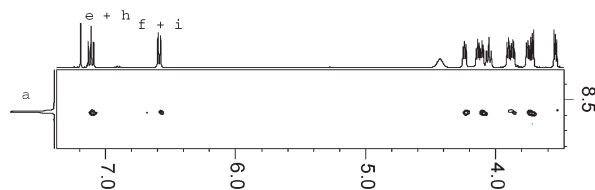


Fig. 3 Partial NOESY spectrum of **1** showing cross peaks between flavin proton *a* (*y*-axis) and naphthalene protons and ethylene glycol “arms” (*x*-axis). Recorded in CDCl_3 using a mixing time of 450 ms.

Therefore, in accordance with the data obtained for derivative **1**, face-to-face aromatic stacking interactions appear to occur between the flavin and NAP moieties. Interestingly, we also observed a downfield shift (~ 0.5 ppm) of the flavin imide hydrogen compared to compound **3**. For compound **4**, the imide of the flavin moiety appeared at a more downfield position compared to control **3**, indicating the formation of hydrogen bonds to the DAP moiety. The ^1H NMR spectra of

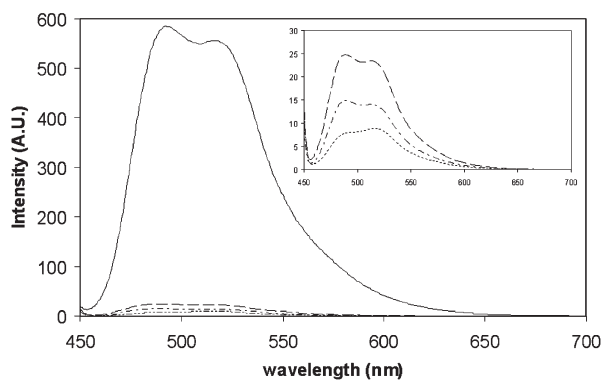


Fig. 4 Fluorescence spectra of compounds **3** (—), **4** (---), **2** (-·-·) and **1** (····) recorded in chloroform ($\sim 4 \times 10^{-6}$ M). Inset shows an expansion of the fluorescence spectra of derivatives **4**, **2** and **1**.

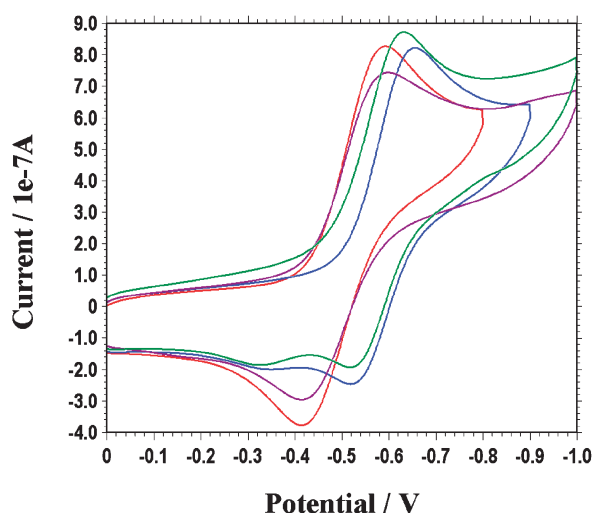


Fig. 5 Cyclic voltammetry of compounds **1** (—), **2** (—), **3** (—) and **4** (—) recorded in dichloromethane ($\sim 1 \times 10^{-4}$ M). Scan rate = 100 mV s^{-1} .

flavins **2** and **4** recorded over 3.6×10^{-2} M to 1.8×10^{-3} M in CDCl_3 displayed limited concentration dependence, suggesting that intramolecular interactions are likely to predominate under the conditions examined.

When compounds **1** or **2** were dissolved in non-polar solvents such as chloroform or toluene red–orange solutions were obtained for these derivatives, whereas derivatives **3** and **4** displayed the standard yellow colour typical of flavin derivatives dissolved in these solvents (see ESI†). When more polar solvents were used (e.g. DMF, DMSO) the colour of derivatives **1** and **2** changed to yellow. Thus the data are consistent with the more polar solvents disrupting aromatic interactions between the naphthalene and flavin moieties of derivatives **1** and **2**, and thus suggest that donor–acceptor interactions are responsible for the complexation processes.^{7b} Due to the complex nature of the UV-vis spectra recorded in chloroform, it was not possible to determine whether charge-transfer bands could be observed. The fluorescence of the flavin nucleus is very sensitive to supramolecular interactions.⁹ The fluorescence spectra of flavins **1–4** recorded in chloroform show dramatic fluorescence quenching for derivatives **1**, **2** and **4** (with respect to flavin **3**), further suggesting that the functionality attached to the N(10) of the flavin unit are participating in supramolecular interactions (Fig. 4).

We have investigated the solution electrochemistry of derivatives **1–4** using cyclic voltammetry (CV) in CH_2Cl_2 (Fig. 5). Derivative **3** afforded CV data in accordance with flavin derivatives recorded in this solvent.¹⁰ CV data for compound **2** showed that the addition of a NAP moiety had very limited effect on the electrochemical properties of the flavin unit. However, the addition of DAP units to the N(10) side chain, as in derivatives **1** and **4**, resulted in a near identical shift of

around 80 mV towards a more positive potential. Thus the electrochemical data indicate that intramolecular complementary hydrogen bonding interactions appear to have a more profound role in stabilizing the flavin radical anion state than intramolecular aromatic interactions.¹¹

In conclusion, we have developed a new flavin model system that has allowed us to probe the combined role intramolecular hydrogen bonding and aromatic interactions have in modulating the physical properties of the flavin moiety. Aromatic interactions appear to play a more important role in perturbing the optical and fluorescence properties, whereas hydrogen bonding appears to play a more important role in tuning the electrochemical properties of the flavin. We are currently using this motif to tune the properties of synthetic flavoenzymes,¹² and to develop new self-assembling polymeric systems. Our endeavours in these areas will be reported in due course.

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