Supramolecular dendritic solubilisation of a hydrophilic dye and tuning of its optical properties

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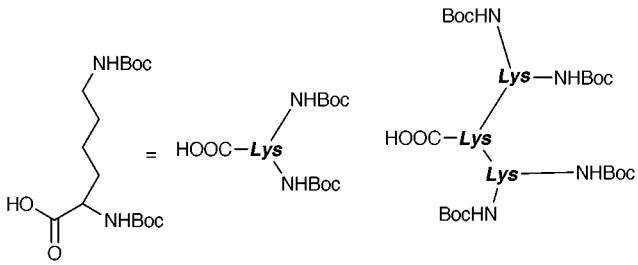
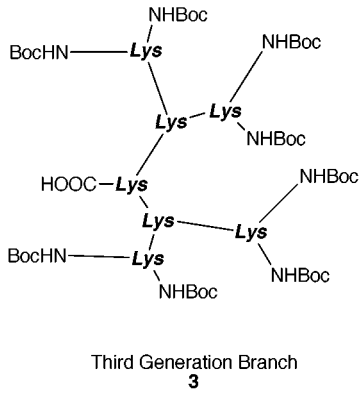
Dendritic peptides with a carboxylic acid at the focal point of the branched structure specifically solubilise a polybasic aromatic dye, the microenvironment and optical properties of which are controlled by the size (generation) of the branched peptide.

The development and investigation of the unique properties of functional dendrimers is a research area of intense current activity.[1] The preparation of dendrimers, however, requires considerable synthetic input and consequently, supramolecular dendrimer chemistry and self-assembling systems are of increasing interest.[2,3] There have been extensive investigations of the use of spherical dendrimers for the supramolecular solubilisation of dyes,[4] which are ideal optical probes of the branched environment. It has also been shown that a dendritic shell can generate a unique microenvironment deep within its branched architecture, modulating optical properties,[5] as well as behaviour such as redox chemistry, molecular recognition and catalysis.[6] Here a new approach to the supramolecular dendritic solubilisation of a hydrophilic dye is reported. Rather than using spherical dendrimers,[4] individual dendritic branches with a functional group at the focal point are used for the supramolecular encapsulation process. Furthermore, the encapsulated dye experiences a unique microenvironment,[5] with its optical properties being dendritically tuned by the extent of the branching assembled around it.

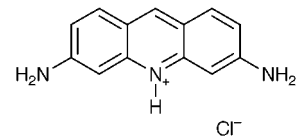
Dendritic branches **1**–**3** were constructed in a solution phase approach, starting from protected L-lysine building blocks.[7] All dendritic branches were fully characterised and monodisperse as proven by electrospray mass spectrometry and analytical gel permeation chromatography (Shodex gel). It was proposed that these dendritic branches containing a free carboxylic acid unit at the focal point should interact with molecules containing basic amine sites through the formation of a hydrogen bonded (acid–base) complex with potential proton transfer.[8] A hydrophilic dye containing amine groups, proflavine **4**, was of great interest as a putative guest. It is intensely coloured and possesses interesting luminescence properties, which assist in monitoring the uptake of the dye by the dendritic branch. Furthermore, its optical behaviour is dependent on the microenvironment in which it is located and this allows the effect of supramolecular dendritic encapsulation to be quantified.[5]



First Generation Branch **1** Second Generation Branch **2**



Third Generation Branch **3**



Proflavine hydrochloride **4**

Solid-liquid extraction (solubilisation) experiments were performed using 2 ml of a solution of dendritic branch in CH_2Cl_2 (13.0 mM) and solid proflavine **4** (20 mg). The mixture was stirred at ambient temperature for 24 h, filtered through a pad of Biobeads gel in a pipette, which was washed with additional solvent, and the resultant solution made up to 5 ml in a volumetric flask. This solution was then analysed using UV-visible and fluorescence spectroscopy to assess the degree of dye uptake.

Each of dendritic branches **1**–**3** solubilised a small but significant quantity of the solid hydrophilic proflavine dye **4** into the apolar CH_2Cl_2 solution, as was clear from UV-visible spectroscopy (Table 1), with the dye molecule exhibiting an absorption maximum around 450 nm. In CH_2Cl_2 without dendritic branch present, however, no uptake was observed and the solution remained colourless. The effect of other additives (13.0 mM) was also investigated. AcOH led to no significant uptake of the dye (a simple carboxylic acid is not effective), whilst the methyl ester of **2** also exhibited no uptake (the dendritic branch without a carboxylic acid group at the focal point is not effective). Stearic acid ($C_{17}H_{35}CO_2H$) also did not solubilise the dye (a long unbranched hydrophobic chain is not effective). It therefore seems clear that the carboxylic acid group and the dendritic branching act cooperatively, solubilis-

Table 1 UV-Visible and fluorescence spectroscopic data after proflavine uptake by the dendritic branches, **1**–**3**

	UV-Visible data		Fluorescence data	
Dendritic branch	Peak maximum/nm	Relative absorption intensity	Emission peak maximum/nm	Relative emission intensity
1	446	1.0	505	1.0
2	448	2.25	503	2.10
3	450	13.7	500.5	7.85

ing proflavine **4** into apolar CH_2Cl_2, presumably *via* the formation of supramolecular interactions between acid and amine groups within the dendritic environment.†

Furthermore, the optical properties of proflavine differ in a progressive manner: as the branched molecule becomes larger, increasing quantities of the dye are solubilised into CH_2Cl_2. This effect is particularly marked between second and third generation branches. This would be consistent with a model in which the hydrophilic dye becomes encapsulated within a branched environment, shielding it from bulk solvent and enhancing its solubility in the hydrophobic solvent phase.[4c–f]

In addition, the UV-visible absorption maximum of proflavine is dependent on the size of the dendritic branch. For branch **1**, this maximum is at 446 nm, whilst for branch **2** it absorbs at 448 nm and for branch **3** it has shifted to 450 nm. These results agree with binding studies performed by Seel and Vögtle on proflavine using an acidic endoreceptor in which the absorption maximum of proflavine shifted bathochromically on encapsulation.[9] The absorption maximum of proflavine is dependent on the protonation state of the molecule, with more highly protonated proflavine absorbing at longer wavelength.[10] It is possible that as the size of the dendritic branch increases, the degree of proton transfer from carboxylic acid to amine accompanying dendritic encapsulation increases. This would agree with theoretical studies,[11] which indicate that carboxylic acid–amine interactions are favoured in a low dielectric (*i.e.* CH_2Cl_2 solvent) whilst carboxylate–ammonium interactions (with proton transfer) are only favoured in a higher dielectric (*i.e.* that generated by the larger polypeptide dendritic branches).

Fluorescence investigations provided further insight (Table 1). On excitation at 450 nm, a proflavine emission peak was observed at 505 (**1**), 503 (**2**) and 500.5 nm (**3**). Interestingly, the relative intensities of these emissions increase less markedly than the intensities of the associated UV-visible absorptions. This would indicate that for the larger dendritic branches there is a greater degree of fluorescence quenching. This once again illustrates the dendritic microenvironment experienced by proflavine.

These results clearly show for the first time that, like their spherical dendrimer counterparts,[4c–f] individual dendritic branches with suitable functionalisation at the focal point can non-covalently solubilise a hydrophilic dye. Furthermore, the optical properties of the encapsulated dye indicate that it experiences a unique microenvironment[5a] as a consequence of supramolecular encapsulation.[5b,9] This has implications for the future design of functional branched systems, as some of the time-consuming covalent synthesis may be avoided. Investigations in this laboratory are currently targeting the synthesis of well-defined aggregates.[3] In addition, dendritic control of a wide variety of molecular function, and the application of assembled branched architectures to the problems of drug encapsulation and delivery,[12] are of considerable interest.

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Notes and references

† The complex still has low solubility, which has thus far prevented an accurate analysis of its stoichiometry, but studies are in progress with other hydrophilic dyes and in different solvents to ascertain how many branches interact with the dye, and hence fully characterise these dendritic aggregates.

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