Dendritic biomimicry: microenvironmental effects on tryptophan fluorescence†

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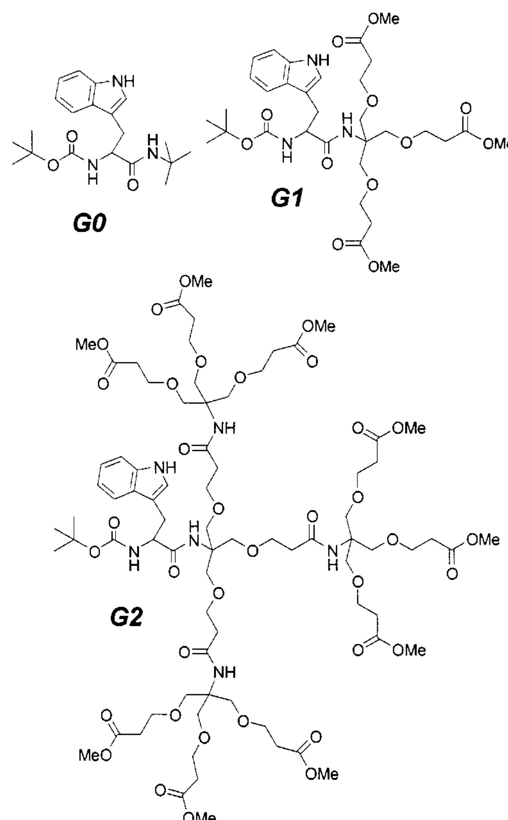
Branched tryptophan derivatives were synthesised and their optical properties investigated in a range of solvents: the steady state fluorescent emission from the indole ring sheds light on both the nature of a hydrogen bonding dendritic microenvironment and the optical behaviour of tryptophan residues in biological systems.

Tryptophan is undoubtedly the most important source of emission in proteins.[1] The wavelength of emission has proven to be very sensitive to the environmental conditions, and has consequently been extensively used as a probe of the local environment within a protein.[2] It has recently been proposed that branched macromolecules (dendrimers)[3] can mimic some aspects of protein behaviour,[4] in particular by creating unique microenvironments within their three dimensional superstructures.[5] Simple, well-understood environmental probes have been used in an attempt to yield a better understanding of this effect of dendritic encapsulation.[6] Here we target the synthesis of dendritic tryptophan derivatives containing a single tryptophan unit buried within the branched environment,[7] the emissive properties of which should respond to the environment generated by the branched shell. The results provide new information about the dendritic microenvironment, and have relevance to understanding the emissive properties of tryptophan in the protein environment, an area of intense research activity.[1]

Tryptophan derivatives **G0**, **G1** and **G2** were synthesised using a convergent coupling strategy in which *N-tert*-butoxycarbonyl-protected L-tryptophan was coupled with Bu^tNH_2 or pre-formed dendritic branches of first or second generation respectively.[8] Coupling was achieved using DCC/HOBt in CH_2Cl_2 with Et_3N as base; typical reaction times were 72 h. Products were purified by silica or gel permeation column chromatography as appropriate.†

The steady state fluorescence emission spectra of these new dendritic amino acids were measured in a range of different solvents.‡ It is well-known that environmental polarity is important in controlling the red shift of tryptophan fluorescence.[9] Firstly, compound **G0** was investigated in order to ascertain the effect of solvent on derivatives with this type of functionalisation—the emission wavelengths are provided in Table 1. Dependent on the solvent, fluorescent emission was observed between 321.5 and 343.5 nm. Attempts to correlate the data with Kamlet and Taft's π^* parameter,[10] a measure of polarity which is divorced from specific interactions such as hydrogen bonds, failed. Analysis of the data indicates that hydrogen bond acceptor solvents (THF, EtOAc and MeCN) were more red shifted than would have been expected on the basis of π^* alone, whilst hydrogen bond acceptor and donor solvents (Pr^iOH and MeOH) were shifted yet further to the red. Therefore, the hydrogen bond donor and acceptor ability of the solvent, as well as its polarity, apparently control the emission wavelength.[11] This is in stark contrast to the solvatochromic probe used by Fréchet and co-workers to investigate a dendritic microenvironment,[5a] the emission wavelength of which correlated well with π^* (polarity) alone.

As expected, therefore, the solvent plays key roles in controlling the fluorescence. The major question, however, was



whether the dendritic shell would modify this behaviour, generating a unique microenvironment for the tryptophan residue. Consequently, the steady state emissive behaviour of **G1** and **G2** was investigated and compared with that of **G0** (Table 1).

The branched shell did indeed have a dramatic and progressive effect on the emission of the tryptophan residue. Fréchet and co-workers found that, in their system, the effect of the dendritic branching correlated with the solvent polarity

Table 1 Emissive wavelengths for **G0**, **G1** and **G2** measured in a range of solvents. All wavelengths are ±0.5 nm

Solvent	π^{*a}	λ_{max}/nm **G0**	**G1**	**G2**	Dendritic effect^b/nm
Non-hydrogen bonding					
Cyclohexane	0.00	321.5	326.5	332	10.5
Benzene	0.59	330	331	333.5	3.5
CH_2Cl_2	0.82	332.5	333.5	335.5	3.0
Hydrogen bond acceptors					
EtOAc	0.55	332	332.5	333.5	1.5
THF	0.58	332.5	333	333	0.5
MeCN	0.75	337.5	337	338	0.5
Hydrogen bond donors and acceptors					
Pr^iOH	0.48	340.5	340	339	−1.5
MeOH	0.60	343.5	343	342.25	−1.25

^{a} Kamlet and Taft's polarity parameter (ref. 10). ^{b} λ_{max}(**G2**) − λ_{max}(**G0**).

† Spectral data for **G0**, **G1** and **G2** are available from the RSC web site, see http://www.rsc.org/suppdata/cc/1999/1915/

parameter π^*, indicating that the effect of the branched shell was to moderate the polarity experienced by the solvatochromic probe. In this case, however, no correlation was observed. Rather, the magnitude of the dendritic effect was primarily dependent on the ability of the solvent to form specific interactions, in particular, hydrogen bonds. Three different types of behaviour were observed:

(i) Non-hydrogen bonding solvents: The most marked effect occurred in primarily non-hydrogen bonding solvents (cyclohexane, benzene, CH_2Cl_2) with the emission wavelength shifting bathochromically on dendritic functionalisation. In cyclohexane, for example, **G0** emitted at 321.5 nm, **G1** at 326.5 nm and **G2** at 332 nm, a remarkable total shift (dendritic effect) of 10.5 nm. This indicates that the branched shell strongly perturbs the local environment of the tryptophan residue.[5,6a] Furthermore, whilst for **G0** there was a large difference in emission wavelength between apolar cyclohexane and more polar CH_2Cl_2 (11 nm), this solvent-generated difference was much smaller for branched analogue **G2** (3.5 nm). In other words, attachment of the branched shell strongly reduces the effect of solvent polarity.

(ii) Hydrogen bond acceptor solvents: In such solvents (EtOAc, THF, MeCN), the dendritic shell had a much smaller effect on the emission wavelength (dendritic effects of between 0.5 and 1.5 nm), even though some of these solvents have polarities comparable to, or lower than, CH_2Cl_2. This provides strong supporting evidence that polarity is not the only important factor in controlling tryptophan fluorescence.

(iii) Hydrogen bond acceptor and donor solvents: In such solvents (MeOH, Pr^iOH) the dendritic effect is once again of smaller magnitude, but this time in the opposite direction, with the emission maximum shifted hypsochromically by around 1.5 nm after dendritic functionalisation.

It is therefore clear that the dendritic effect is much smaller in competitive solvents than in non-competitive solvents. This indicates that specific tryptophan solvation effects, such as hydrogen bonding, play a crucial role. It is proposed that, in non-competitive solvents, an intramolecular *hydrogen bond* is formed between the dendritic shell (C=O) and the N–H group of the tryptophan residue at the focal point. Such a hydrogen bond would act as a tether, holding the polar peptidic branched shell in close proximity to the tryptophan subunit, modulating the polarity of its local environment, and hence perturbing its fluorescence spectrum much more strongly.[12] In competitive solvents, the solvent is itself able to interact with the tryptophan residue, and the branched shell consequently has a much smaller effect.

Further evidence for the dendritic creation of a hydrogen bonding interaction in non-competitive solvents is provided by the downfield perturbation of the ^1H NMR shift (in $CDCl_3$) of the N–H proton in the indole ring. This resonance shifts from δ 8.10 for **G0** to δ 8.60 for **G1** to δ 9.45 for **G2**.

Such hydrogen bonding microenvironments have been previously postulated as being important in controlling the behaviour of dendritic receptors.[13] This report clearly illustrates the dramatic effect that such hydrogen bond interactions can have inside branched superstructures—in clear analogy with the importance of hydrogen bonds in controlling the structure and behaviour of enzymes.

In summary, dendritically modified tryptophans have been synthesised and their optical properties investigated. A remarkable dendritic effect[5] on emission wavelengths was observed. It is proposed that in non-competitive solvents, there is a *hydrogen bonding microenvironment*[13] for the encapsulated tryptophan residue, leading to enhanced dendritic effects. In addition to providing clear characterisation of this type of dendritic microenvironment, these results emphasise the potential importance of microenvironmental hydrogen bonds in controlling the fluorescence of tryptophan residues in the apolar interior of enzymes. This is significant given the wide use of tryptophan emission as a probe of protein structure.[1] Further work using these, and more deeply encapsulated, dendritic tryptophan derivatives to probe kinetic aspects of fluorescence quenching is planned. Other biologically relevant fragments will also be encapsulated in order to ascertain additional microenvironmental effects on function, and generate new, easily tuneable forms of molecular behaviour.

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Notes and references

‡ Emission spectra measured after excitation at 290 nm. [Tryptophan derivative] = 1×10^{-4} M, except from **G1** and **G2** in cyclohexane which exhibited limited solubility, [**G1**] = 5×10^{-5} M, [**G2**] = 2×10^{-5} M.

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