Supramolecular Solubilisation of Hydrophilic Dyes by Using Individual Dendritic Branches

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Abstract: Individual dendritic branches can solubilise hydrophilic dyes in apolar media. The functional group at the focal point of the dendritic branch plays a key role in the dye uptake process. Supramolecular interactions between carboxylic acid and amine groups have been shown to be effective in enabling efficient solubilisation to occur. The necessary complementarity of this interaction is further illustrated by a series of control experiments. The extent of dendritic branching (i.e. dendritic generation) plays a key role in controlling the extent of dye uptake, with higher-generation dendritic branches exhibiting more efficient uptake at lower concentrations. UV/Visible spectroscopic methods have shown that the dendritic branches, in addition to the tuning of the extent of dye uptake, also tune the optical properties of the solubilised dye and this provides further insight into the interactions occurring between the solubilised dye and the individual dendritic branches. Furthermore, it is shown that suitably functionalised dendritic branches can transport hydrophilic dyes through an apolar phase and deliver them continuously into an aqueous medium.

Keywords: aggregation · dendrimers · dyes/pigments · hydrogen bonds · supramolecular chemistry

Introduction

The unique properties of dendritic macromolecules discovered during the last five years have intensified efforts in this area of research.[1] In particular, it has been shown that a dendritic shell can generate a unique microenvironment deep within its branched architecture and modulates optical properties,[2] as well as behaviour such as redox chemistry, molecular recognition and catalysis.[3] The microenvironmental control within dendrimers is analogous to that of enzymes, which control their internal environments in order to achieve optimum efficiency. The preparation of dendrimers, however, requires considerable synthetic input and consequently, supramolecular dendrimer chemistry and self-assembling systems are of increasing interest as a consequence of their ability to short-cut some of this synthesis.[4, 5]

Perhaps the earliest use of dendrimers in supramolecular chemistry was for the solubilisation of dyes in unusual solvent environments. There have been extensive investigations of this type, primarily because dyes are ideal visual probes of solubilisation, but also because they can report on the environment in which they find themselves. In 1991, Newkome and co-workers reported the use of a dendrimer as a unimolecular micelle for guest solubilisation.[6] The surface of this dendrimer possessed charged carboxylate groups, whilst the interior was purely aliphatic in nature. The micellar behaviour of this dendrimer in aqueous solution was established through its ability to encapsulate hydrophobic probes, such as phenol blue. Analogously, Fréchet and co-workers reported a dendritic micelle possessing peripheral carboxylate groups and interior aromatic-ether branching, which was capable of solubilising pyrene in aqueous solution.[7] This "dendritic-micelle" strategy has also been applied in reverse by using dendrimers in which the surface of a polar dendritic interior has been functionalised with a high density of hydrophobic groups.[8] Such dendrimers will solubilise hydrophilic dyes in apolar media. With the periphery of a polar spherical dendrimer functionalised with fluorous groups, the dendrimer was shown to solubilise methyl orange, a hydrophilic dye, in liquid CO_2.[9] In a landmark paper, Meijer and co-workers not only solubilised dyes within a dendritic structure, but also permanently trapped them inside the dendritic superstructure by using bulky, hydrogen-bonding surface groups.[10] Shape-selective liberation of the encapsulated guests could then be achieved by deprotection of the multivalent dendritic surface. There have been a number of recent reports in which the ability of spherical dendrimers to

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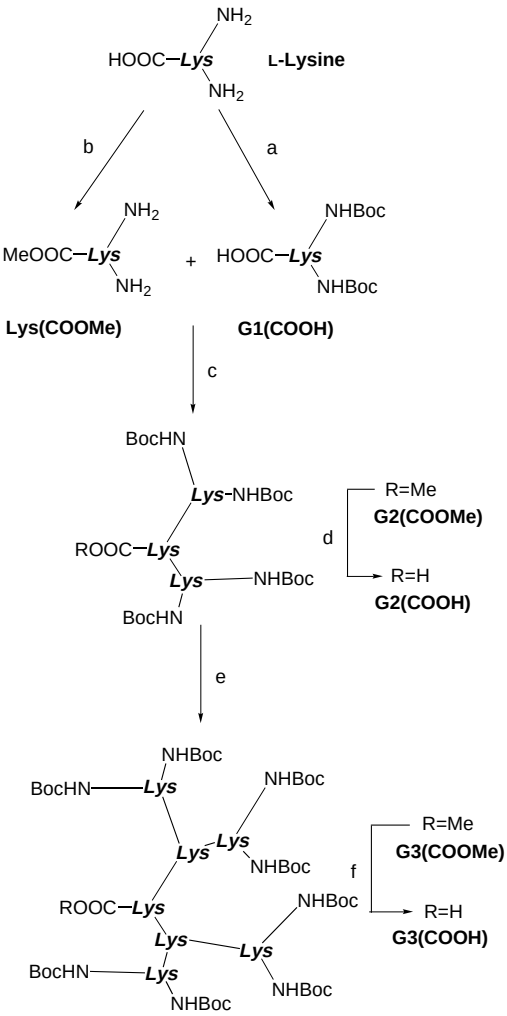
act as unimolecular solubilisation agents has been further explored.[11]

Rather than simply controlling polarity, however, it is also possible to use a more selective approach to the supramolecular encapsulation. There are many examples in which specific supramolecular complexation occurs within the framework of a spherical dendrimer.[4, 12] Particularly noteworthy in this context are the reports of Diederich and co-workers, illustrating that a dendritic cyclophane (dendrophane) generates a unique environment at its selective encapsulated binding site, a feature which was probed by using the λ_{max} value of 6-(*p*-toluidino)naphthalene-2-sulfonate (TNS), which is a fluorescent probe of the dendritic microenvironment.[13] In 1999, Twyman and co-workers reported a water-soluble, polar spherical dendrimer possessing interior amine groups that was capable of solubilising organic acids (such as benzoic acid) in aqueous solution.[14] It was argued that specific carboxylic acid–amine interactions played a key role in the solubilisation process.[15, 16]

At around the same time, we also reported the use of simple interactions between carboxylic acid and amine groups in order to achieve dendritic solubilisation.[17] In our case, however, rather than using spherical dendrimers, we focussed on the use of individual dendritic branches (up to the third generation) to solubilise dyes. Individual dendritic branches have, in fact, previously been used for dye solubilisation, for example, the surfactant poly(ethyleneglycol)-dendrimer block copolymers reported by Chapman and co-workers.[18] These hydraamphiphiles (fourth generation) form aggregates in aqueous solution, solubilising orange OT (1-(*o*-tolylazo)-2-naphthol). The solubilisation process using such hydraamphiphiles, however, is polarity dependent, whereas in our case[17] it appeared that specific supramolecular interactions between carboxylic acid and amine groups were essential for efficient solubilisation to occur.

In this full paper, we significantly expand the scope of our novel methodology for the supramolecular dendritic solubilisation of hydrophilic dyes and illustrate its general applicability to different hydrophilic dyes *and* different dendritic branches. Using a range of carefully chosen control experiments, we report and discuss the main factors which control the process of supramolecular dendritic solubilisation. Furthermore, we also describe the ability of these dendritic branches to act as transport agents delivering hydrophilic dyes through an apolar phase and into an aqueous medium.

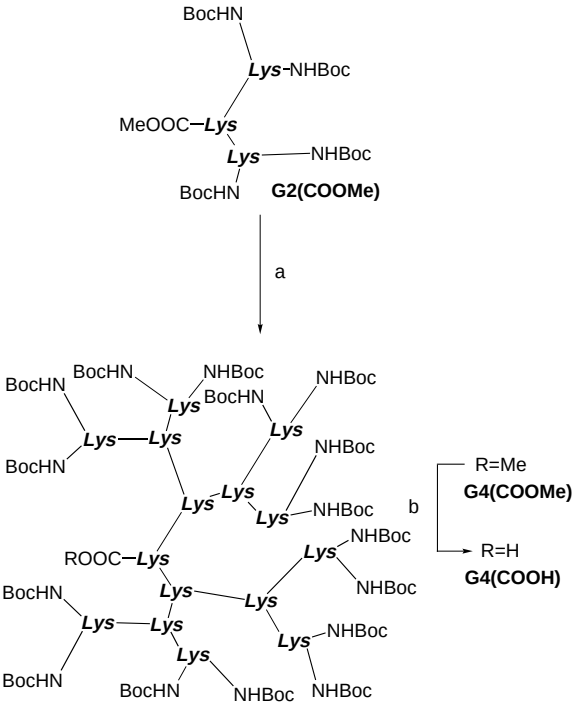


Scheme 1. Synthesis of $\mathbf{G_n(COOH)}$ ($n = 1-3$). a) Boc_2O, NaOH, H_2O, dioxane, 97%; b) 2,2-dimethoxypropanone, MeOH, HCl, 93%; c) DCC, HOBt, Et_3N, EtOAc, 82%; d) NaOH, MeOH, H_2O, 90%; e) **Lys(COOMe)**, DCC, HOBt, Et_3N, EtOAc, 86%; f) NaOH, MeOH, H_2O, 92%.

Results and Discussion

Synthesis of dendritic branches: The relative abilities of two different series of dendritic branches ($\mathbf{G_n(COOH)}$ and $\mathbf{G_n(NH_2)}$) to solubilise hydrophilic dyes in apolar media were investigated. Acidic branches, $\mathbf{G_n(COOH)}$, were based on peptide-coupled L-lysine amino acids and synthesised from generation 1 (**G1(COOH)**) to generation 4 (**G4(COOH)**) (see Schemes 1 and 2 for the optimised convergent synthesis). This type of dendritic branching is well-known in the literature and was first introduced by Denkewalter.[19] Surprisingly, full experimental data for this type of individual dendritic branch based on L-lysine have, to the best of our knowledge, not previously been reported, and consequently are incorporated in the Experimental Section of this paper. A variety of different solution-phase synthetic methods were employed during the course of this project, and their relative advantages and disadvantages were compared for lysine dendrimers for the first time.

Divergent strategy: This proved to be an efficient method for synthesising dendritic branches of all generations. Perhaps surprisingly, it was easy to obtain complete reaction of all available sites on the dendritic surface by coupling with DCC (dicyclohexyl carbodiimide) and HOBt (hydroxybenzotriazole, to suppress racemisation),[20] and column chromatography allowed the isolation of analytically pure material at all generations (**G2(COOH)**: SiO_2 chromatography; **G3(COOH)** and **G4(COOH)**: gel-permeation chromatography). It was necessary to perform this chromatography in order to remove all traces of DCU (dicyclohexylurea) a side product in the coupling reaction. This was a difficult



Scheme 2. Synthesis of $\mathbf{G_n(COOH)}$ ($n=4$) by using a double exponential growth strategy. a) i) CF_3COOH, CH_2Cl_2; ii) NEt_3, EtOAc; iii) **G2-(COOH)**, DCC, HOBt, 81%; b) NaOH, MeOH, H_2O, 90%.

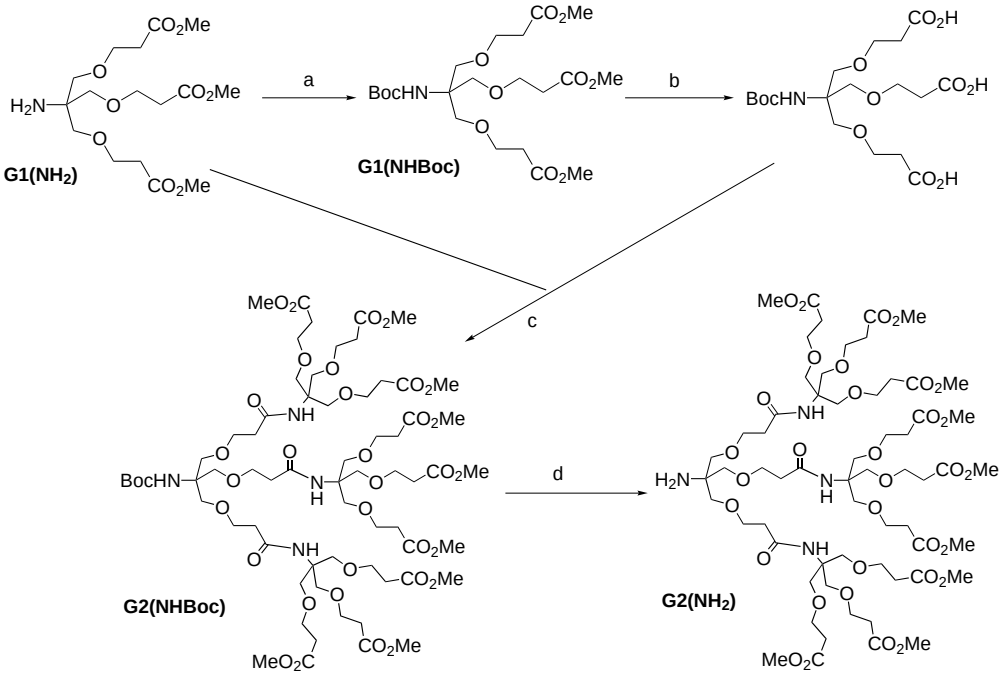
procedure for **G3(COOH)** and **G4(COOH)** (Scheme 2), which form gels in many organic solvents at the concentrations required for chromatography. This necessitated the use of polar solvents (Sephadex LH-20 gel: MeOH, or Biobeads SX-1: 90/10, CH_2Cl_2/MeOH). Attempts to replace DCC with EDCI (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, which generates a readily removed byproduct)[21] led to poor yields.

Convergent strategy: As fewer coupling reactions are performed at each generation for a dendrimer undergoing convergent growth, it was reasoned that problems caused by the DCU side product would be less marked using this approach. This was indeed the case, although some chromatography was still required (Biobeads SX-1: 90/10, CH_2Cl_2/MeOH). Nonetheless, the convergent strategy gives good yields of monodisperse products; this is the first report of the use of the convergent solution-phase approach for the synthesis of lysine based dendritic branches. Convergent synthesis is generally considered to yield the highest purity dendritic products. In order to synthesise fourth-generation dendritic branches in the most efficient way, a *double exponential growth* strategy[22] was used in which two second-generation building blocks were combined. This allows the synthesis of **G4(COOH)** in multigram quantities.

Amino branches, $\mathbf{G_n(NH_2)}$ (Scheme 3), were synthesised by using the methodology first introduced by Newkome and coworkers and further modified by Diederich and co-workers.[23] In this study, we employed different protecting groups compared with the branches used by Diederich and coworkers (Boc, *tert*-butoxycarbonyl group, rather than CBz, benzyloxycarbonyl group), but in all other respects the synthetic procedure was the same. Therefore, the data and syntheses for these compounds are not reproduced here. First- ($\mathbf{G1(NH_2)}$) and second- ($\mathbf{G2(NH_2)}$) generation dendritic branches with an amino group at the focal point were employed in this investigation.

All dendritic branches were fully characterised by using all available techniques, and proven as monodisperse by using electrospray mass spectrometry and analytical gel-permeation chromatography (Shodex gel).

Investigation of dye solubilisation, proflavine hydrochloride (1): compound **1** is a hydrophilic aromatic dye possessing two



Scheme 3. Synthesis of $\mathbf{G_n(NH_2)}$. a) Boc_2O, Et_3N, dioxane, 46%; b) NaOH, H_2O, MeOH, 93%; c) DCC, HOBt, THF, 50%; d) CF_3COOH, CH_2Cl_2, 80%.

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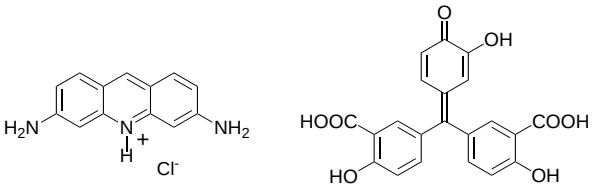
free amino groups (Scheme 4). We postulated that this dye should form $COOH\cdots NH_2$ hydrogen-bond interactions with dendritic branches **G_n(COOH)**, and reasoned that such interactions could manifest themselves in the form of an altered solubility profile for the dye that should reflect to some extent the solubility profile of the dendritic branch to which it was complexed. Furthermore, it was hoped that the optical properties of the dye (e.g. absorption wavelength) would reflect the dendritically encapsulated environment in which it found itself.



1, proflavine hydrochloride **2**, aurin tricarboxylic acid

Scheme 4. Dyes solubilised by individual dendritic branches.

The *solubilisation* of proflavine hydrochloride in CH_2Cl_2 solution was therefore investigated using solid–liquid extraction experiments. These solid–liquid extraction experiments were performed by using an optimised method[24] in which a solution (3 mL, 5 mM) of dendritic branch in CH_2Cl_2 was mixed with solid proflavine hydrochloride (10 mg) and stirred for 18 hours. The resultant solution was then diluted to 50 mL and it (5 mL) was filtered through a pad of Biobeads gel (SX-1) in a pipette, which was washed with additional solvent and made up to 10 mL in a volumetric flask. The degree of dye uptake in this solution was then monitored by using UV/Visible spectroscopy. All uptake experiments were repeated a minimum of three times and average values taken. The reproducibility of these experiments was extremely high.

Each of dendritic branches **G_n(COOH)** exhibited uptake of proflavine hydrochloride in CH_2Cl_2 to some extent, whereas the dye showed no solubility in CH_2Cl_2 with no dendritic additive present (Table 1). Interestingly, as the extent of dendritic branching increases, so does the uptake of the dye; this observation is consistent with a model in which the hydrophilic dye interacts with the carboxylic acid at the focal point of the branch and becomes encapsulated within the branched environment. In this way, the dendritic branching can shield the hydrophilic dye from the apolar solvent, and consequently, more extensive branching gives rise to a greater degree of uptake. For fourth-generation dendritic branch **G4(COOH)**, the degree of uptake was indeed dramatic and gave rise to a bright orange solution.

Table 1. Degree of uptake of proflavine hydrochloride into CH_2Cl_2 by using a variety of different additives [all 5 mM] and λ_{max} [nm] values of the solubilised dye. The degree of uptake is, in each case, quoted relative to that observed with **G1(COOH)** and is calculated from the ratio of intensities of the UV/Vis spectra.

Additive [5 mM]	Degree of solubilisation of proflavine hydrochloride	λ_{max} [nm]
None	0	–
G1(COOH)	1.0	440
G2(COOH)	1.4	441
G3(COOH)	5.3	443
G4(COOH)	97	461
CH_3COOH	0.10	441
$C_{17}H_{35}COOH$	0.21	441
G2(COOMe)	0.56	440
G3(COOMe)	2.4	441
G4(COOMe)	44	441
G2(NH_2)	0.19	broad

A number of *control experiments* were subsequently performed to provide additional evidence for this hypothesis. Simple carboxylic acids, lacking dendritic branching, were used in an attempt to solubilise proflavine hydrochloride by $COOH\cdots NH_2$ interactions alone. Acetic acid (CH_3COOH) and stearic acid ($C_{17}H_{35}COOH$) both showed very poor uptake of the dye (less than **G1(COOH)**, Table 1). This result, coupled with the enhanced effect of dendritic generation described above, indicates that the *dendritic branching* does indeed *play a key role* in the dye solubilisation process.

Dendritic branches in which the available carboxylic acids had been *protected* as ester groups were also investigated. **G2(COOMe)** showed negligible uptake of the dye, especially when compared with **G2(COOH)**. This would indicate that the *carboxylic acid* also *plays a key role* in the solubilisation process. Interestingly, however, **G4(COOMe)** did show significant uptake of proflavine hydrochloride (although still less than half the uptake of **G4(COOH)**). This was unexpected using our simple model, but is perhaps not surprising. **G4(COOMe)** contains 30 amide N–H groups, any of which can donate hydrogen bonds to the amine groups on proflavine hydrochloride. Whilst this process is not as favourable as the formation of $COOH\cdots NH_2$ hydrogen bonds, it will still occur, and given the large number of N–H groups, such secondary interactions probably explain the uptake of some dye by this protected fourth-generation dendritic branch. Nonetheless, dye uptake by **G4(COOMe)** was markedly less than by **G4(COOH)**, and this reinforces the *important role of the carboxylic acid group* in assisting dye solubilisation. This result provides strong evidence for the importance of hydrogen-bond interactions between the carboxylic acid at the focal point of the branch and the solubilised dye molecule. It seems clear from these results that the carboxylic acid group and the dendritic branching *act cooperatively* to solubilise the dye, presumably by the formation of supramolecular interactions within the shielded dendritic environment.

Interestingly, the *optical properties* of the dye also varied as a function of dendritic generation, with a pronounced red-shift of λ_{max} from 441 to 461 nm on progressing from **G1(COOH)** to **G4(COOH)**. This type of bathochromic shift was previously observed by Seel and Vögtle for the encapsulation of proflavine hydrochloride using an acidic endoreceptor.[25] It is known that a bathochromic shift in absorption wavelength for this dye is associated with increased protonation, indeed the λ_{max} values for deprotonated, monoprotonated and diprotonated proflavine in H_2O are approximately 390, 445 and 460 nm, respectively.[26] For **G4(COOH)** it is possible that the degree of proton transfer in the hydrogen bond is greater within the more deeply encapsulated dendritic microenvironment.[27] Interestingly, when solubilised by **G4(COOMe)**, proflavine hydrochloride has an absorption

maximum at 441 nm, whilst as explained above, with **G4(COOH)**, this maximum shifted bathochromically to 461 nm. This indicates that it is not simply the polarity of the extensive dendritic branching which generates a solvatochromic shift in λ_{max}, and illustrates the *key role of the encapsulated carboxylic acid in generating the dendritically induced shift in absorption wavelength*.

The degree of uptake of proflavine hydrochloride by **G3(COOH)** and **G4(COOH)** was also monitored by NMR spectroscopy. The solvent was removed from the volumetric solution by rotary evaporation, the solution was dissolved in CD_3OD, and the relative intensities of the dye and branch signals were integrated. In this way, it was ascertained that when the uptake experiment is performed with **G3(COOH)** approximately 0.1 equivalents of dye are dissolved, whilst uptake with **G4(COOH)** dissolves approximately 1.9 equivalents of dye; this is a very significant extent of solubilisation and a result consistent with the observations from UV/Visible spectroscopy.[28]

In an attempt to observe individual COOH$\cdots$NH$_2$ interactions, IR spectroscopy of the dye solution solubilised by **G3(COOH)** was performed and compared with the dendritic branches alone in CH_2Cl_2. Unfortunately, no major differences in the IR spectra were observed, but this is not surprising as the most important regions associated with C=O, O–H and N–H stretches are obscured as a consequence of the large number of amide groups present within the dendritic branching.

The *effect of the concentration* of individual dendritic branches on the uptake of the dye in CH_2Cl_2 was then investigated.[29] Figure 1 illustrates that, as expected, as the concentration of the branches increases, the concentration of dissolved dye also goes up. However, neither for **G1(COOH)** nor for **G2(COOH)** does much dye dissolve at any concentration (even up to 100 mM). This offers further proof that the solubilisation is not simply caused by nonspecific polarity effects. For **G3(COOH)**, however, large quantities of dye dissolve at concentrations of 10 mM and above, whilst for **G4(COOH)** large quantities of dye dissolve at concentrations of 0.5 mM and above. This clearly indicates the effect of the dendritic branching in facilitating the solubilisation process; this is presumably by providing a more effective encapsulated environment. It is interesting to note that the most commonly used reverse micelles, such as AO^T (Bis(2-ethylhexyl)sulfosuccinate sodium salt) operate at concentrations around 1 mM, but the presence of H_2O or other hydrogen-bonding solvent is essential.[30] The potential of these individual dendritic branches driven by supramolecular interactions (and not dependent on additional solvent) to rival such systems is therefore clear.

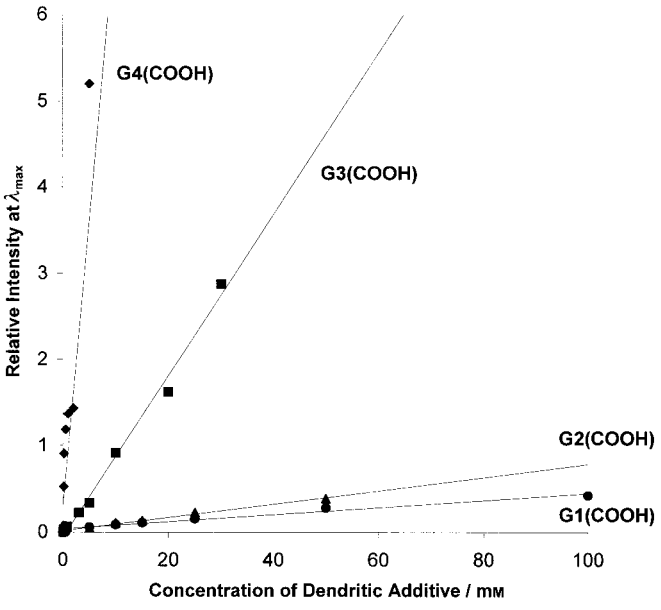


Figure 1. Dependence of proflavine hydrochloride solubilisation (absorbance at λ_{max}) on concentration of dendritic branches **G$_n$(COOH)**.

The ability of **G$_n$(NH$_2$)** dendritic branches to solubilise proflavine hydrochloride was then investigated (Table 1). In this case, the dendritic branches should not be able to form any favourable supramolecular interactions with the dye, and consequently no uptake should be observed. For **G2(NH$_2$)** this was indeed the case: the branch did not solubilise the dye to any significant extent. This agrees with our model: *complementary interactions between COOH and NH$_2$ are crucial to the solubilisation process.*

Investigation of dye solubilisation, aurin tricarboxylic acid (2): In order to demonstrate the generality of this approach to supramolecular dendritic solubilisation, we then considered the solubilisation of a hydrophilic dye which is a polyacid, aurin tricarboxylic acid **2** (Scheme 4). We proposed that dendritic branches **G$_n$(NH$_2$)** would solubilise this dye as a consequence of the formation of complementary COOH$\cdots$NH$_2$ interactions. Uptake experiments were performed as described above (Table 2). Like proflavine hydrochloride, aurin tricarboxylic acid shows little solubility in CH_2Cl_2 in the absence of additives. The use of **G1(NH$_2$)** and **G2(NH$_2$)** as additives, however, increased the degree of uptake, in the case of **G2(NH$_2$)** this effect was dramatic with a bright red solution being obtained. Therefore, as observed previously, dendritic branching enhanced the degree of dye solubilisation. The simple model system, *tert*-butylamine, however, showed little uptake of the dye and this result further illustrated the *key role played by the branching*. Protected dendritic branches, **G1(NHBoc)** and **G2(NHBoc)** also showed much lower dye

Table 2. Degree of uptake of aurin tricarboxylic acid into CH_2Cl_2 by using a variety of different additives [all 5 mM] and λ_{max} [nm] values of the solubilised dye. The degree of uptake is, in each case, quoted relative to that observed with **G1(NH$_2$)**.

Additive [5 mM]	Degree of solubilisation of aurin tricarboxylic acid	λ_{max} [nm]
None	0.06	–
G1(NH$_2$)	1.0	517
G2(NH$_2$)	13.9	528
***t*BuNH$_2$**	0.26	broad
G1(NHBoc)	0.25	broad
G2(NHBoc)	2.0	486
G1(COOH)	0.21	484
G2(COOH)	1.1	484
G3(COOH)	1.3	484

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uptake than their free amine equivalents. This clearly illustrates the *crucial role of the amine group* in interacting with the carboxylic acid groups on the dye. **G2(NHBoc)**, however, did exhibit some uptake of the dye; like **G4(COOMe)**, which shows some solubilisation of proflavine hydrochloride, this effect can be ascribed to weaker secondary hydrogen-bond interactions between amide groups within the branch and the dye.

Once again, the dendritic branching has an effect on the *absorption wavelength* of the dye. In the presence of dendritic branching with an amine group at the focal point, λ_{max} is shifted. Most strikingly, in the presence of **G2(NH_2)** this maximum occurs at 528 nm whilst with **G2(NHBoc)**, the dye has an absorption maximum at just 486 nm, a shift of 42 nm.

The *concentration dependence* of this dendritic solubilisation process was investigated. **G2(NH_2)** showed a high degree of dye uptake at concentrations above 3 mM, whilst even **G1(NH_2)** showed some uptake of the dye at concentrations above 20 mM.

The ability of **G_n(COOH)** to solubilise aurin tricarboxylic acid was then investigated (Table 2). These branches exhibited significantly less dye solubilisation than their amine functionalised counterparts and this observation once again reinforces the important concept of acid–amine complementarity. It is possible that COOH···COOH or COOH···OH interactions can occur between branch and dye in this case and they provide the small amount of solubilisation observed.

Transport experiments: The ability of these dendritic branches to transport a hydrophilic dye through an apolar phase with subsequent delivery into an aqueous medium was then studied. A solid–liquid–liquid transport apparatus was designed (Figure 2) in which a solid hydrophilic dye could

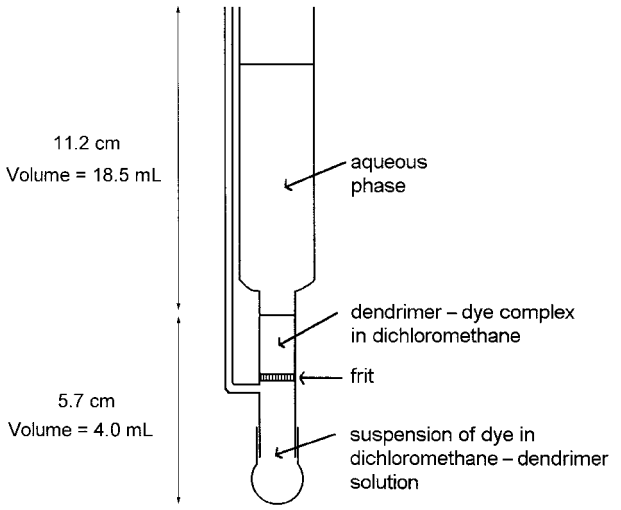

Figure 2. Equipment for solid–liquid–liquid transport experiment.

be dissolved in a stirred solution of dendritic branch in apolar CH_2Cl_2 (0.1 mM), transported in solution through a frit and then delivered into a stirred aqueous phase. This apparatus was modelled on a previous experiment designed by Vögtle and Seel.[25] The rate of transport of the dye into the aqueous phase was monitored by sampling the aqueous solution at different times and measuring the intensity of the UV/Vis absorption band at 440 nm. This experiment was performed by using branches **G_n(COOH)** and proflavine hydrochloride. As illustrated in Figure 3, transport of the dye through the

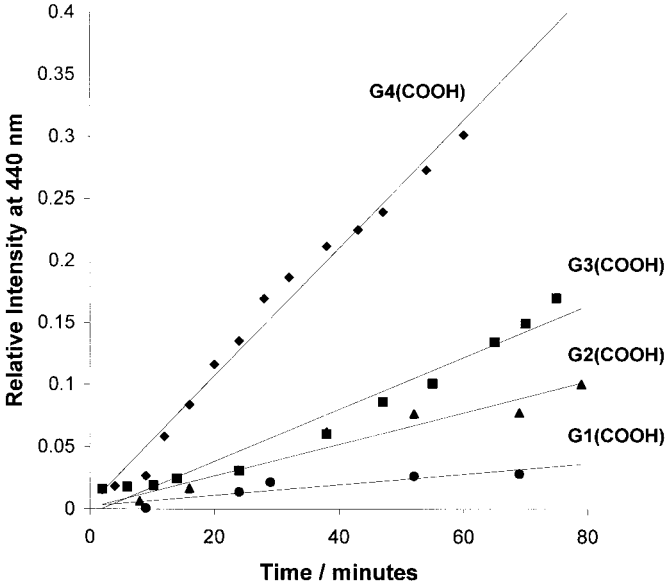

Figure 3. Continuous transport of proflavine hydrochloride through an apolar solvent into an aqueous phase by dendritic branches **G_n(COOH)** as monitored by UV/Vis spectroscopy on the aqueous phase.

apolar phase was indeed observed. Interestingly, transport was quickest with higher-generation dendritic branches (reflecting their greater ability at dye solubilisation). Transport was effective even at very low concentrations of **G4(COOH)** (0.1 mM) and furthermore was a continuous process, with active transport of the dye continuing for at least 24 hours. This proves that the dendritic branches do not become inactivated during the transport experiment and indicates the potential of such dendritic systems to act as efficient transport and delivery vehicles. By using this experimental setup, in 24 hours, 1 mole equivalent of **G4(COOH)** transports 3.1 mole equivalents of proflavine hydrochloride.[31]

Clearly the results from this experiment will reflect a complicated mix of different factors: a) rate of diffusion, b) solubility, c) rate of solubilisation and d) dimensions of the apparatus. Stirring of the organic and aqueous phases was employed to limit the effects of slow diffusion, although the experimental data do show some evidence of a brief lag time before transport is recorded. Separate experiments were performed to determine the rate of solubilisation caused by the dendritic additive. This was achieved by using regular sampling from a stirred solution combined with UV/Vis spectroscopy to analyse the extent of uptake. For **G4(COOH)** the uptake was fast, with the maximum degree of solubilisation being achieved after approximately five minutes. As a consequence of this observation, it is therefore proposed that the degree of solubility of the dye caused by the presence of the dendritic carrier molecule is the key factor in controlling the rate of continuous transport over the period of 24 hours

through the apolar phase. This is in agreement with the observation that the rates of transport for different dendritic branches follow the same pattern as the extent of dye solubilisation in each case.

In any case, the transport results reported in this paper should be considered as illustrative of the continuous transport which *can* be achieved by using this type of dendritic carrier system, rather than absolute measures, as the values determined are dependent on the precise dimensions of the apparatus.

By comparison, protected dendritic branches, as seen from the results for their methyl esters, showed diminished rates of transport of proflavine hydrochloride through the apolar phase. Once again this emphasises the importance of complementary acid–amine interactions, although as before **G4(COOMe)** shows some transport ability, presumably as a consequence of the multiple secondary interactions possible within the cavities of the branches.

Conclusion

These experiments indicate the generality of this supramolecular approach to encapsulation by using individual dendritic branches. The methodology has been extended to higher-generation branches and most importantly, in a predictive manner, to a different dendritic branch and a different template dye. The key principles of the process are now clear. Complementary supramolecular $COOH\cdots NH_2$ hydrogen-bonding interactions play an important role in controlling the aggregation, whilst the degree of dendritic branching plays an important role in providing the encapsulated environment and allowing efficient uptake at low concentrations of dendritic additive. The optical properties of the solubilised dye are modified within the assembly and can be used to read out information about the encapsulated microenvironment. In addition, efficient transport of the hydrophilic dyes through an apolar phase can be mediated by suitably functionalised dendritic branches. The possibilities for encapsulation and modification of function by using this approach would appear to be wide-ranging, and a number of investigations into modified chemical and materials properties are currently in progress.[32]

Experimental Section

Solvents and reagents were used as supplied. Silica column chromatography was carried out by using silica gel provided by Fluorochem Ltd. (35–60 Å). Thin-layer chromatography was performed on commercially available Merck aluminium backed silica plates. Preparative gel-permeation chromatography was carried out by using a glass column (2 m) packed with Biobeads SX-1, supplied by Biorad. Analytical gel-permeation chromatographs were recorded by using a Waters instrument incorporating two Shodex columns in series (KF-802.5 and KF-803) with THF as eluent. Proton and carbon NMR spectra were recorded on either a Jeol EX-270 (^{1}H 270 MHz, ^{13}C 67.9 MHz) or a Bruker AMX-500 (^{1}H 500 MHz, ^{13}C 125 MHz) at 25 °C. Chemical shifts (δ) are quoted in parts per million, referenced to residual solvent. Coupling constant values (J) are given in Hz. DEPT (distortionless enhancement by polarisation transfer) experiments were used to assist in the assignment of ^{13}C NMR spectra. Melting points were measured on an Electrothermal IA 9100 digital melting point apparatus and are uncorrected. Optical rotation was measured as $[\alpha]_D$ on a JASCO DIP-370 digital polarimeter. Positive ion electrospray mass spectra were recorded on a Finnigan LCQ mass spectrometer. Positive ion fast atom bombardment mass spectra were recorded on a Fisons Instruments Autospec mass spectrometer. The isotope distribution observed for mass spectral ions of the larger molecules was consistent with data calculated from isotopic abundances. Infrared spectra were recorded by using an ATI Mattson Research Series 1 FTIR spectrometer. Compounds **Lys(COOMe)**[33] and **G1(COOH)**[34] were prepared according to literature methods.

Compound G2(COOMe): L-Lysine methyl ester dihydrochloride **(Lys-(COOMe)** (0.560 g, 2.4 mmol) was suspended in ethyl acetate (20 mL). Triethylamine (0.506 g, 5.0 mmol) was added, followed by **G1(COOH)** (1.732 g, 5.0 mmol). The mixture was stirred under nitrogen for two minutes and then cooled to 0 °C. Hydroxybenzotriazole (HOBt, 0.681 g, 5.0 mmol) and dicyclohexyl carbodiimide (DCC, 1.032 g, 5.0 mmol) were added simultaneously as a mixture of solids. The reaction mixture was allowed to warm to room temperature and stirred for 24 h. The precipitate was removed by filtration and discarded. The filtrate was then washed with $NaHCO_3$ aq. (satd.), then $NaHSO_4$ aq. (8 g in 50 mL), $NaHCO_3$ aq. (satd.) and then finally H_2O. The solution was dried ($MgSO_4$) and then the solvent was removed by rotary evaporation to produce a white solid. This crude product was purified by column chromatography (silica, CH_2Cl_2:MeOH:NEt_3, 95:5:0.1) to give the product (1.63 g, 1.98 mmol, 82 %).

Colourless solid, m.p. 75–80 °C; $R_f = 0.48$ (CH_2Cl_2/MeOH 90:10); $[\alpha]_D^{293} = -41.9$ ($c = 1.0$ in $CHCl_3$), -20.5 ($c = 1.0$ in CH_3COCH_3); ^{1}H NMR (500 MHz, CD_3COCD_3): $\delta = 7.56$ (brs, 1H; CONH), 7.33 (brs, 1H; CONH), 6.14 (brs, 1H; N*H*Boc), 6.09 (d, $^3J = 7.5$ Hz, 1H; N*H*Boc), 5.92 (brs, 2H; N*H*Boc), 4.36 (brm, 1H; COC*H*(R)NH), 4.23 (brm, 1H; COC*H*(R)NH), 4.05 (brm, 1H; COC*H*(R)NH), 3.68 (s, 3H; CO_2CH_3), 3.06 (m, 6H; CH_2NH), 1.85–1.35 (m, 54H; CH_2 and CH_3); ^{13}C NMR (125 MHz, CD_3COCD_3): $\delta = 173.5$ (*C*O_2Me), 173.3 (CONH × 2), 156.8 (*C*OOC(CH$_3$)$_3$ × 4), 79.5 (O*C*(CH$_3$)$_3$ × 2), 78.4 (O*C*(CH$_3$)$_3$ × 2), 55.6 (CO*C*H(R)NH), 55.1 (CO*C*H(R)NH), 53.0 (CO*C*H(R)NH), 52.3 (CO_2CH_3), 40.9 ($CH_2NH \times 2$), 39.0 (CH_2NH), 33.3, 33.1, 31.9, 30.6, 30.5, 29.8 (All CH_2), 28.8 ($CH_3 \times 6$), 28.7 ($CH_3 \times 6$), 23.8, 23.5, 23.3 (All CH_2); IR (KBr disc): $\tilde{\nu} = 3330$ (m), 3062 (w), 2978 (m), 2934 (m), 2866 (w), 1712 (m), 1694 (s), 1660 (s), 1527 (s), 1456 (w), 1392 (m), 1366 (m), 1250 (m), 1172 cm^{-1} (s); MS (Electrospray) $[M+Na]^+$: $C_{39}H_{72}N_6O_{12}Na$ ($M_r = 840.0$): m/z (%): 840.0 (40), 839.0 (100); HRMS (FAB) ($C_{39}H_{72}N_6O_{12}Na$) $[M+Na]^+$: found 839.5101; calcd 839.5106.

Compound G2(COOH): **G2(COOMe)** (0.500 g, 0.612 mmol) was dissolved in methanol (25 mL). The solution was cooled to 0 °C and then aqueous sodium hydroxide solution (1.8 mL, 1M, 1.8 mmol) was added. The reaction was stirred under nitrogen for 24 hours. The solvent was removed by rotary evaporation, water was added (35 mL) and then the mixture was acidified to pH 3 with aqueous sodium hydrogen sulfate. The product was extracted with ethyl acetate and then washed with water and brine. The resulting solution was dried ($MgSO_4$) and then the solvent was removed by rotary evaporation to dryness. Diethyl ether (20 mL) was added and the solvent was removed from the mixture by rotary evaporation to give product (0.44 g, 0.55 mmol, 90 %).

Colourless solid, m.p. 90–100 °C; $R_f = 0.15$ (CH_2Cl_2/MeOH 90:10); $[\alpha]_D^{293} = -7.6$ ($c = 1.0$ in $CHCl_3$), -14.7 ($c = 1.0$ in CH_3COCH_3), -9.3 ($c = 1.0$ in MeOH); ^{1}H NMR (500 MHz, CD_3COCD_3): $\delta = 7.54$ (brs, 1H; CONH), 7.40 (brs, 1H; CONH), 6.17 (d, $^3J = 7.5$ Hz, 1H; N*H*Boc), 6.13 (d, $^3J = 7.5$ Hz, 1H; N*H*Boc), 5.95 (brs, 2H; N*H*Boc), 4.40 (brm, 1H; COC*H*(R)NH), 4.23 (brm, 1H; COC*H*(R)NH), 4.07 (brm, 1H; COC*H*(R)NH), 3.06 (m, 6H; CH_2NH), 1.91–1.33 (m, 54H; CH_2 and CH_3); ^{13}C NMR (125 MHz, CD_3COCD_3): $\delta = 173.8$ (CO_2H), 173.4 (CONH × 2), 156.9 (*C*OOC(CH$_3$)$_3$ × 4), 79.5 (O*C*(CH$_3$)$_3$ × 2), 78.4 (O*C*(CH$_3$)$_3$ × 2), 55.5 (CO*C*H(R)NH), 55.2 (CO*C*H(R)NH), 52.8 (CO*C*H(R)NH), 40.8 ($CH_2NH \times 2$), 39.0 (CH_2NH), 33.1, 32.0, 30.5, 30.1, 29.7 (All CH_2), 28.8 ($CH_3 \times 6$), 28.7 ($CH_3 \times 6$), 25.7, 23.7, 23.6, 23.2 (All CH_2); IR (KBr disc): $\tilde{\nu} = 3325$ (m), 3062 (w), 2978 (m), 2936 (m), 2868 (w), 1698 (m), 1696 (s), 1663 (s), 1533 (s), 1457 (w), 1405 (m), 1367 (m), 1251 (m), 1172 cm^{-1} (s); MS (Electrospray) $[M+Na]^+$: $C_{38}H_{70}N_6O_{12}Na$ ($M_r = 826.0$): m/z (%): 826.2 (40), 825.1 (100); HRMS (FAB) ($C_{38}H_{70}N_6O_{12}Na$) $[M+Na]^+$: found 825.4955; calcd 825.4949.

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Compound G3(COOMe): L-Lysine methyl ester dihydrochloride (**Lys-(COOMe)**) (0.552 g, 2.37 mmol) was suspended in ethyl acetate (100 mL). Triethylamine (0.499 g, 4.93 mmol) was added, followed by **G2(COOH)** (3.96 g, 4.93 mmol). The mixture was stirred under nitrogen for 2 min and then cooled to 0 °C. Hydroxybenzotriazole (HOBt, 0.671 g, 4.93 mmol) and dicyclohexyl carbodiimide (DCC, 1.017 g, 4.93 mmol) were added simultaneously as a mixture of solids. The reaction mixture was allowed to warm to room temperature and stirred for 60 h. The precipitate (containing the product) was filtered off and washed with ethyl acetate. The product was extracted from this solid with excess CH_2Cl_2 (by using an ultrasonic bath to break up the large solid particles). The yield of crude product was 4.71 g. A small portion of the crude product (1.00 g) was purified by size-exclusion chromatography (Biobeads, CH_2Cl_2/MeOH, 90:10) to give a white solid product with a yield of 0.746 g (0.431 mmol). This extrapolated to an overall yield for the reaction of 86%.

Colourless solid, m.p. 135–140 °C; $R_f = 0.46$ (CH_2Cl_2:MeOH 90:10); $[\alpha]_D^{293} = -28.1$ ($c = 1.0$ in $CHCl_3$), −18.0 ($c = 1.0$ in MeOH); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 4.36$ (m, 2H; COC*H*(R)NH), 4.27 (m, 1H; COC*H*(R)NH), 4.04 (m, 2H; COC*H*(R)NH), 3.97 (m, 2H; COC*H*(R)NH), 3.71 (s, 3H; CO_2CH_3), 3.17 (m, 6H; C*H*$_2$NH), 3.02 (m, 8H; C*H*$_2$NH), 1.88–1.26 (m, 114H; C*H*$_2$ and C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD): $\delta = 175.2$, 175.1, 175.0, 174.2, 174.0, 174.0 (All *C*O$_2$Me, CONH × 6), 158.5 (NH*C*OOC(CH$_3$)$_3$ × 4), 158.1 (NH*C*OOC(CH$_3$)$_3$), 157.9 (NH*C*OOC(CH$_3$)$_3$), 157.8 (NH*C*OOC(CH$_3$)$_3$ × 2), 80.6 (O*C*(CH$_3$)$_3$ × 4), 79.8 (O*C*(CH$_3$)$_3$ × 4), 56.1, 55.9, 54.6, 54.2, 53.6 (All CO*C*H(R)NH × 7), 52.8 (CO_2CH_3), 41.1 (CH_2NH × 4), 40.0 (CH_2NH × 2), 39.9 (CH_2NH), 33.3, 33.0, 32.9, 32.7, 32.6, 32.5, 20.6, 30.0, 29.9, 29.9, 29.7 (All CH_2), 28.9, 28.8, 28.8 (All CH_3), 24.2, 24.1, 24.0 (All CH_2); IR (KBr disc): $\tilde{\nu} = 3326$ (m), 3062 (w), 2975 (m), 2938 (m), 2864 (w), 1696 (s), 1653 (s), 1526 (s), 1457 (w), 1392 (m), 1366 (m), 1275 (m), 1250 (m), 1168 cm^{-1} (s); MS (Electrospray) $[M+Na]^+$: $C_{83}H_{152}N_{14}O_{24}Na$ ($M_r = 1753.2$): *m*/*z* (%): 1754.0 (35), 1753.0 (80), 1752.0 (100).

Compound G3(COOH): Compound **G3(COOMe)** (0.32 g, 0.18 mmol) was dissolved in methanol (25 mL). The solution was cooled to 0 °C, then aqueous sodium hydroxide solution (0.6 mL, 1M, 0.6 mmol) was added. The reaction was stirred under nitrogen for 24 h. TLC showed that the reaction was not complete, so a further aliquot of sodium hydroxide solution (0.6 mL, 1M, 0.6 mmol) was added and stirred for a further 24 h. The solvent was removed on a rotary evaporator, water was added (35 mL) and then the mixture was acidified to pH 3 with aqueous $NaHSO_4$. The product was extracted with ethyl acetate and then washed with water and brine. The resulting solution was dried ($MgSO_4$) and then the solvent was removed by rotary evaporation to dryness. Diethyl ether (20 mL) was added and the solvent was removed from the mixture by rotary evaporation to provide product (0.29 g, 0.17 mmol, 92%).

Colourless solid, m.p. 100–120 °C; $R_f = 0.18$ (CH_2Cl_2:MeOH 90:10); $[\alpha]_D^{293} = -25.7$ ($c = 1.0$ in $CHCl_3$), −17.3 ($c = 1.0$ in MeOH); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 4.37$ (m, 2H; COC*H*(R)NH), 4.28 (m, 1H; COC*H*(R)NH), 4.03 (m, 2H; COC*H*(R)NH), 3.97 (m, 2H; COC*H*(R)NH), 3.18 (m, 6H; C*H*$_2$NH), 3.02 (m, 8H; C*H*$_2$NH), 1.92–1.28 (m, 114H; C*H*$_2$ and C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD): $\delta = 175.3$, 175.1, 175.0, 173.9 (All *C*O$_2$Me, CONH × 6), 158.5 (NH*C*OOC(CH$_3$)$_3$ × 4), 158.0 (NH*C*OOC(CH$_3$)$_3$ × 2), 157.8 (NH*C*OOC(CH$_3$)$_3$ × 2), 80.6 (O*C*(CH$_3$)$_3$ × 4), 79.8 (O*C*(CH$_3$)$_3$ × 4), 56.1, 55.9, 54.5, 54.3, 53.5 (All CO*C*H(R)NH × 7), 41.0 (CH_2NH × 4), 40.0 (CH_2NH × 3), 33.2, 33.0, 32.8, 32.6, 32.2, 30.6, 30.1, 29.9, 29.7 (All CH_2), 28.8, 28.8 (CH_3 × 24), 24.2, 23.7 (CH_2); IR (KBr disc): $\tilde{\nu} = 3339$ (m), 2978 (m), 2933 (m), 2868 (w), 1693 (s), 1660 (s), 1530 (s), 1454 (w), 1391 (w), 1366 (m), 1251 (m), 1173 cm^{-1} (s); MS (Electrospray) $[M+Na]^+$: $C_{82}H_{150}N_{14}O_{24}Na$ ($M_r = 1739.2$): *m*/*z* (%): 1739.9 (40), 1738.9 (85), 1737.8 (100), 1760 [25, $M+2Na-H]^+$.

Compound G4(COOMe): Synthesis using a *double exponential growth strategy*. Compound **G2(COOMe)** (0.339 g, 0.415 mmol) was dissolved in dichloromethane (3 mL). Trifluoroacetic acid (3 mL) was then added and the solution stirred under nitrogen for 30 min. The solvent was removed from the solution by rotary evaporation and the sample was then dried under high vacuum for 1 h. Ethyl acetate (30 mL) was added followed by triethylamine (0.336 g, 3.3 mmol, 2 equiv per NH_3^+) to produce the free amine. Compound **G2(COOH)** (1.43 g, 1.78 mmol, 4.3 equiv) was then added and the solution stirred under nitrogen for two minutes, before being cooled to 0 °C. Hydroxybenzotriazole (HOBt, 0.241 g, 1.78 mmol, 4.3 equiv) and dicyclohexylcarbodiimide (DCC, 0.367 g, 1.78 mmol, 4.3 equiv) were then added simultaneously as a mixture of solids. The reaction mixture was allowed to warm to room temperature and stirred for 90 h. The solvent was removed by rotary evaporation and the mixture purified by size-exclusion chromatography (Biobeads, CH_2Cl_2/MeOH, 90:10) to give the product (1.20 g, 0.337 mmol, 81% with respect to **G2(COOMe)**).

Colourless solid, m.p. 170–172 °C (decomposed); $R_f = 0.32$ (CH_2Cl_2:MeOH 90:10); $[\alpha]_D^{293} = -25.2$ ($c = 1.0$ in $CHCl_3$), −19.0 ($c = 1.0$ in MeOH); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 4.37$ (m, 4H; COC*H*(R)NH), 4.28 (m, 3H; COC*H*(R)NH), 4.02 (m, 8H; COC*H*(R)NH), 3.71 (s, 3H; CO_2CH_3), 3.17 (m, 14H; C*H*$_2$NH), 3.02 (m, 16H; C*H*$_2$NH), 1.86–1.31 (m, 234H; C*H*$_2$ and C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD): $\delta = 175.1$, 173.9 (*C*O$_2$Me, CONH × 12), 158.5 (NH*C*OOC(CH$_3$)$_3$ × 8), 158.0 (NH*C*OOC(CH$_3$)$_3$ × 4), 157.8 (NH*C*OOC(CH$_3$)$_3$ × 4), 80.7, 80.5 (O*C*(CH$_3$)$_3$ × 8), 79.8 (OC(CH$_3$)$_3$ × 8), 56.1, 54.6 (CO*C*H(R)NH × 15), 53.7 (CO_2CH_3), 41.0 (CH_2NH × 8), 40.1 (CH_2NH × 7), 34.7, 33.3, 32.9, 32.7, 32.0, 30.6, 30.0, 29.8 (All CH_2), 28.8 (CH_3), 26.8, 26.0, 24.2, 24.0 (All CH_2); IR (KBr disc): $\tilde{\nu} = 3336$ (m), 3064 (w), 2978 (m), 2932 (m), 2866 (w), 1691 (s), 1653 (s), 1528 (s), 1456 (w), 1392 (w), 1366 (m), 1251 (m), 1173 cm^{-1} (s); MS (Electrospray) $[M+2Na]^{2+}$: $C_{171}H_{312}N_{30}O_{48}Na_2$ ($M_r = 3602.6$, doubly charged ion therefore will appear at 1801.3): *m*/*z* (%): 1802.5 (20), 1801.9 (40), 1801.5 (70), 1801.0 (100), 1800.5 (100), 1800.0 (50).

Compound G4(COOH): Compound **G4(COOMe)** (0.30 g, 0.084 mmol) was dissolved in methanol (15 mL). The solution was cooled to 0 °C, then aqueous sodium hydroxide solution (0.25 mL, 1M, 0.25 mmol) was added. The reaction was stirred under nitrogen for 24 h. TLC showed that the reaction was not complete, so a further aliquot of $NaOH_{aq}$ (0.25 mL, 1M, 0.25 mmol) was added and stirred until TLC indicated the reaction was complete (a further 24 h). The solvent was removed on a rotary evaporator, water was added (25 mL) and the mixture was acidified to pH 3 with aqueous $NaHSO_4$. The solid product was washed with water, dried and then collected into a flask using methanol. The solvent was evaporated. Diethyl ether (20 mL) was added and the solvent was removed from the mixture by rotary evaporation to give product (0.27 g, 0.076 mmol, 90%).

Colourless solid, m.p. 115–125 °C (decomposed); $R_f = 0.26$ (CH_2Cl_2/MeOH 90:10); $[\alpha]_D^{293} = -17.6$ ($c = 1.0$ in $CHCl_3$), −17.2 ($c = 1.0$ in MeOH); ^{1}H NMR (500 MHz, CD_3OD): $\delta = 4.35$ (m, 7H; COC*H*(R)NH), 4.02 (m, 8H; COC*H*(R)NH), 3.17 (m, 14H; C*H*$_2$NH), 3.02 (m, 16H; C*H*$_2$NH), 1.85–1.28 (m, 234H; C*H*$_2$ and C*H*$_3$); ^{13}C NMR (125 MHz, CD_3OD): $\delta =$ 173.8–172.6 (*C*O$_2$Me, CONH × 12), 157.2 (NH*C*OOC(CH$_3$)$_3$ × 8), 156.5 (NH*C*OOC(CH$_3$)$_3$ × 8), 79.2 (O*C*(CH$_3$)$_3$ × 8), 78.5 (OC(CH$_3$)$_3$ × 8), 54.8, 53.2 (CO*C*H(R)NH × 15), 39.7 (CH_2NH × 8), 38.8 (CH_2NH × 7), 33.4–28.5 (CH_2), 27.6 (CH_3), 25.4–22.9 (CH_2); IR (KBr disc): $\tilde{\nu} = 3336$ (m), 3064 (w), 2978 (m), 2932 (m), 2866 (w), 1691 (s), 1653 (s), 1528 (s), 1456 (w), 1392 (w), 1366 (m), 1251 (m), 1173 cm^{-1} (s); MS (Electrospray) $[M+2Na]^{2+}$: $C_{170}H_{310}N_{30}O_{48}Na_2$ ($M_r = 3588.8$, doubly charged ion therefore will appear at 1794.4): *m*/*z* (%): 1795.4 (20), 1794.9 (40), 1794.4 (65), 1794.0 (100), 1793.6 (80), 1793.1 (25); Also observed $[M+3Na-H]^{2+}$: $C_{170}H_{309}N_{30}O_{48}Na_3$ ($M_r = 3610.8$, doubly charged ion therefore will appear at 1805.4): *m*/*z* (%): 1806.4 (20), 1805.9 (42), 1805.4 (73), 1804.9 (100), 1804.5 (90), 1804.1 (35).

Procedure for solid–liquid solubilisation studies: A 5mM (typically) solution of the dendritic branch in the hydrophobic CH_2Cl_2 solvent was prepared. A portion (3 mL) of this solution was stirred with a quantity of solid hydrophilic dye at ambient temperature for 18 h. This solution was then diluted to 50 mL in a volumetric flask (not for aurin tricarboxylic acid studies) and the solution (5 mL) was filtered through a pad of Biobeads gel in a pipette. The gel was washed with additional solvent and the solution made up to 10 mL in a volumetric flask. The solution was then analysed by using UV/Visible spectroscopy to assess the degree of dye uptake.

Procedure for solid–liquid–liquid transport experiments: Solid–liquid–liquid transport experiments were performed by using a purpose built apparatus based on the design of Vögtle and Seel (Figure 2).[25] The dimensions of this apparatus were as shown in Figure 2. Proflavine dye (10 mg) was placed in the flask before the apparatus was assembled. A solution of dendritic branch in CH_2Cl_2 (0.1mM, 6 mL) was then added through the frit. The frit was present to stop the solid dye from reaching the dichloromethane–water interface directly. Water (15 mL) was added to the top of the dichloromethane phase and the phases were gently stirred.

Magnetic followers, in the bottom flask and above the frit, stirred the dichloromethane phase; an overhead stirrer stirred the aqueous phase. The transport of the dye from the solid, through the apolar phase, into the aqueous phase was monitored by UV/Visible spectroscopy. An aliquot (≈3 mL) from the aqueous phase was removed at regular intervals and the UV/Visible spectrum was recorded, before the sample was returned to the apparatus.

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