# Dendritic supramolecular assemblies for drug delivery†

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**Dendritic supramolecular assemblies were formed in water with Reichardt's dye or the anticancer drug 10-hydroxycamptothecin and the dendritic macromolecule, ([G4]-PGLSA-OH)$_2$-PEG$_{3400}$.**

Dendrimer-based supramolecular assemblies are of wide-spread interest for applications such as catalysis, micro-reaction vessels, and drug delivery, and range from large extended assemblies to small molecular host–guest complexes.[1–7] Several small molecules including rose bengal,[8] acetylsalicylic acid,[9] pyrene,[10,11] phenol blue,[12] and Reichardt's dye[13] have been successfully encapsulated within poly(aryl ether), poly(propylene imine), or poly(amido amine) dendrimers. We are interested in dendritic macromolecules that are composed of biocompatible monomer units as materials for medical applications.[14–18] Recently, we reported the encapsulation characteristics of a *g*eneration four *p*oly(*g*lycerol-*s*uccinic *a*cid) (**[G4]-PGLSA**-OH) dendrimer.[18] To further elucidate the supramolecular assemblies formed between a dendritic macromolecule and a small molecule guest in water, we investigated the assembly formed between Reichardt's dye and a triblock macromolecule composed of glycerol, succinic acid, and a polyethylene glycol core ((**[G4]-PGLSA**-OH)$_2$-PEG$_{3400}$, Fig. 1). In addition, we report the *in vitro* cytotoxicity against human colon cancer cells of the functional supramolecular assembly created by encapsulation of the anticancer drug 10-hydroxycamptothecin.

This architectural copolymer was synthesized using a divergent approach by successive esterification and hydrogenolysis reactions with succinic acid mono-(2-phenyl-[1,3]-dioxane-5-yl) on a PEG diol core of 3400 Mw.[17] These amphiphilic macromolecules possess a relatively hydrophobic glycerol-succinate dendritic-wedge and a hydrophilic PEG core, with critical aggregation concentrations that decrease from $10^{-3}$M with increasing generation number. Reichardt's dye (**1**; Fig. 2) was encapsulated in the dendritic macromolecule as a model compound for hydrophobic small molecule guests (aqueous solubility 2 μM). It has been used previously to study micelle/solution interfaces, phospholipid bilayers, and microemulsions.[19] Reichardt's dye was successfully encapsulated within the (**[G4]-PGLSA**-OH)$_2$-PEG$_{3400}$ macromolecule, but not within the first generation, ((**[G1]-PGLSA**-OH)$_2$-PEG$_{3400}$) or the PEG$_{3400}$ macromolecule alone. In order to encapsulate Reichardt's dye, it was dissolved in $CH_3OH$ along with the dendritic macromolecule at a 1:1 molar ratio. Water was subsequently added to the solution, which was stirred for an additional hour. Finally, the $CH_3OH$ was removed *via* evaporation over several hours to afford the host–guest dendritic assembly in water. The solution turned red over time and the final $\lambda_{max}$ for the entrapped Reichardt's dye was 504 nm.

To gain further insight into the nature of the encapsulated Reichardt's dye–dendrimer interactions, we performed a series of one and two-dimensional NMR experiments. The $^1$H NMR spectrum of the (**[G4]-PGLSA**-OH)$_2$-PEG$_{3400}$ encapsulated dye in $D_2O$ reveals significant chemical shifts, lineshape changes, and substantial line broadening of the aromatic dye protons compared to free Reichardt's dye in water. For example, the singlet resonances from rings I and II of the free dye at 8.39 and 6.73 ppm shift downfield to 8.50 and 7.02 ppm, respectively, when encapsulated within (**[G4]-PGLSA**-OH)$_2$-PEG$_{3400}$. Similar chemical shifts and line broadening were observed with Reichardt's dye encapsulated within the **[G4]-PGLSA**-OH dendrimer.[18] The $^1$H NMR spin–lattice relaxation time constants, $T_1$, for the protons of ring I and ring II for free Reichardt's dye in $CD_3OD$ decrease from 1.81 and 1.62 $s^{-1}$ to 0.63 and 0.64 $s^{-1}$ when encapsulated within the (**[G4]-PGLSA**-OH)$_2$-PEG$_{3400}$ macromolecule in $D_2O$. Spin–spin relaxation time measurements revealed that the $T_2$ of these singlets also decreased from 1.28 and 1.44 $s^{-1}$ for the free dye in $CD_3OD$ to 0.017 and 0.019 $s^{-1}$, respectively, when encapsulated within the dendrimer in $D_2O$. The decrease in $T_2$, which affords the considerable increase in linewidths, indicates significant changes in molecular motion of the encapsulated dye compared to free dye.

$^1$H-NOESY spectra were recorded to explore the molecular interactions between the (**[G4]-PGLSA**-OH)$_2$-PEG$_{3400}$ dendrimer and Reichardt's dye in this supramolecular assembly (Fig. 3). Strong intermolecular NOEs between the aromatic protons of Reichardt's dye and the methylenes of succinic acid and the methines and methylenes of glycerol were observed, demonstrating close range dipolar interactions. However, no NOE crosspeaks were observed between the aromatic protons of Reichardt's dye and the large number of methylene protons of the PEG core.

To investigate the drug delivery potential of this dendritic supramolecular assembly, the anticancer drug 10-hydroxycamptothecin (10HCPT) (**2**; Fig. 2),[20,21] was encapsulated. 10HCPT is a promising anticancer drug, but possesses poor aqueous solubility (6 μM) and subsequently limited therapeutic efficacy, like many other drugs. The encapsulation procedure for 10HCPT was identical to that for Reichardt's dye. The concentration of the encapsulated 10HCPT, determined by UV-Vis spectroscopy, was

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† Electronic supplementary information (ESI) available: detailed experimental information for the synthesis of the (**[G4]-PGLSA**-OH)$_2$-PEG$_{3400}$ dendrimer, encapsulation procedure, and cell studies. See http://dx.doi.org/10.1039/b502411k

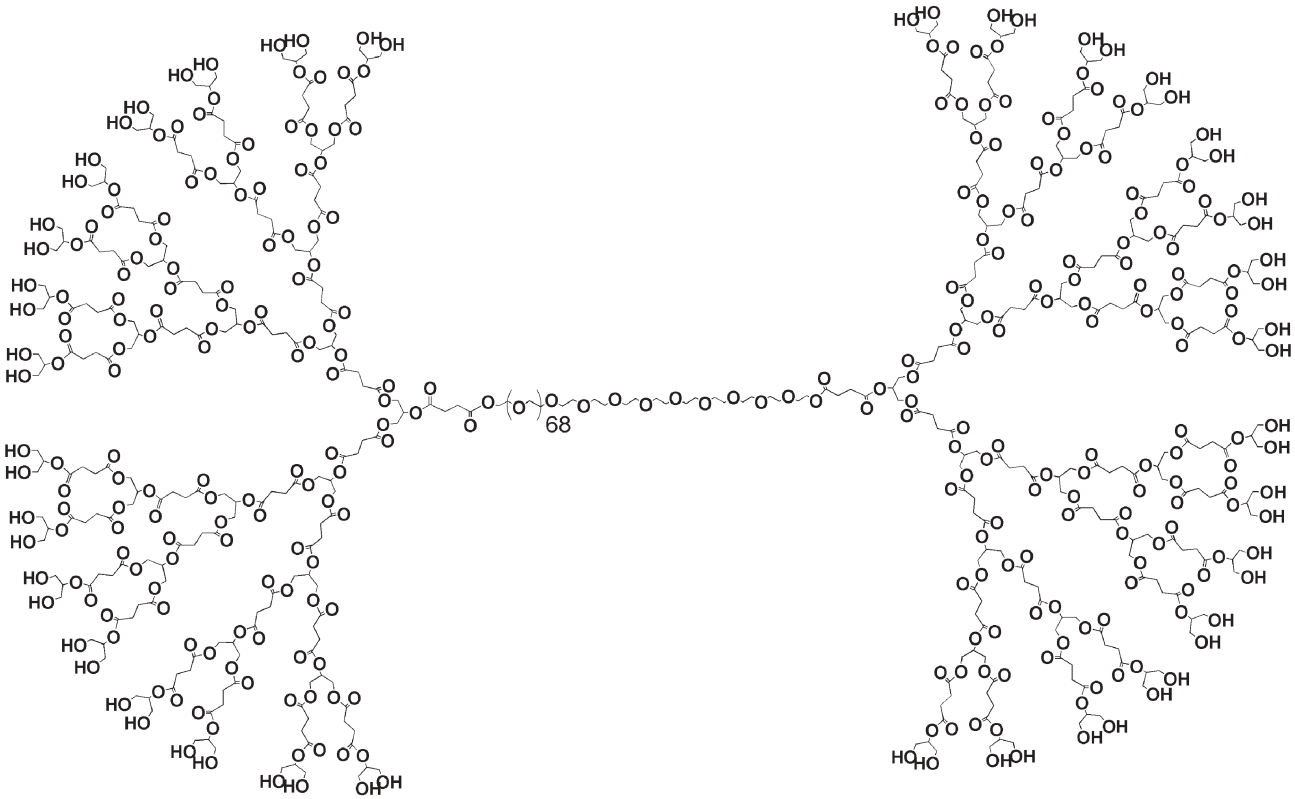

**Fig. 1** Chemical structure of the (**[G4]-PGLSA**-OH)$_2$-PEG$_{3400}$ dendrimer.

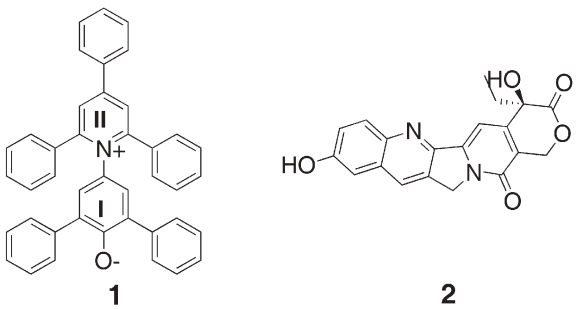

**Fig. 2** Chemical structure of Reichardt's dye (**1**; 2,6-diphenyl-4-(2,4,6-triphenylpyridinio)phenolate) and 10-hydroxycamptothecin (**2**; 10HCPT).

120 μM; a 20-fold increase over the water solubility of 10HCPT (6 μM).

The activity of the encapsulated 10HCPT as an anticancer agent was evaluated using a sulforhodamine B cytotoxicity assay.[18] Varying concentrations of (**[G4]-PGLSA**-OH)$_2$-PEG$_{3400}$, 10HCPT, and (**[G4]-PGLSA**-OH)$_2$-PEG$_{3400}$ 10HCPT assembly were incubated for 0.5 or 2 hours with HT-29 human colon cancer cells (2000 cells per well). Under these experimental conditions, no cytotoxic effects were observed with the (**[G4]-PGLSA**-OH)$_2$-PEG$_{3400}$ dendrimer, whereas cell viability was significantly reduced in the presence of both the free and the encapsulated 10HCPT (Fig. 4). 10HCPT is cytotoxic in its lactone-ring closed form. At a concentration of 2 μM, similar activity was observed for both free and encapsulated 10HCPT. The highest concentration tested of encapsulated 10HCPT (20 μM) showed substantial cytotoxicity with less than 5% of the cells remaining viable. Irinotecan, a camptothecin prodrug with greater aqueous solubility, is used to treat colon cancer, even though the side effects of severe to life-threatening diarrhoea exists in up to 40% of patients.[22] Thus, it is used clinically in combination with strong anti-diarrhoetics.

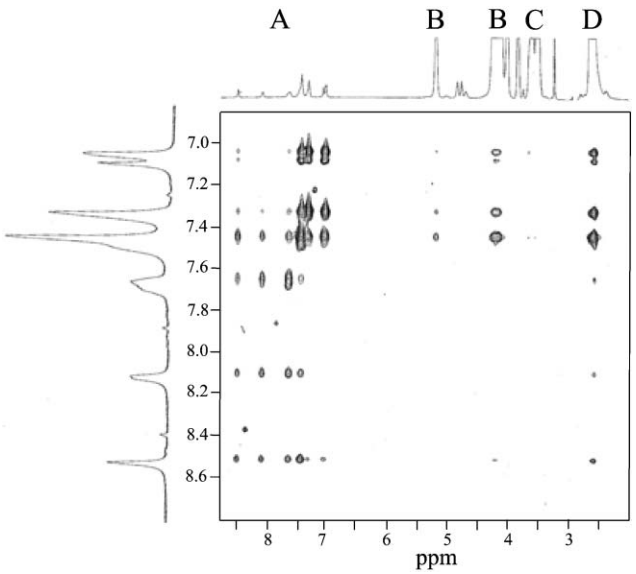


**Fig. 3** $^1$H-NMR NOESY spectrum of the (**[G4]-PGLSA**-OH)$_2$-PEG$_{3400}$ encapsulated Reichardt's dye in $D_2O$. Where A are the aromatic protons of Reichardt's dye, B are the glycerol methine and methylene protons, C are the PEG methylene protons, and D are the succinic acid methylene protons.

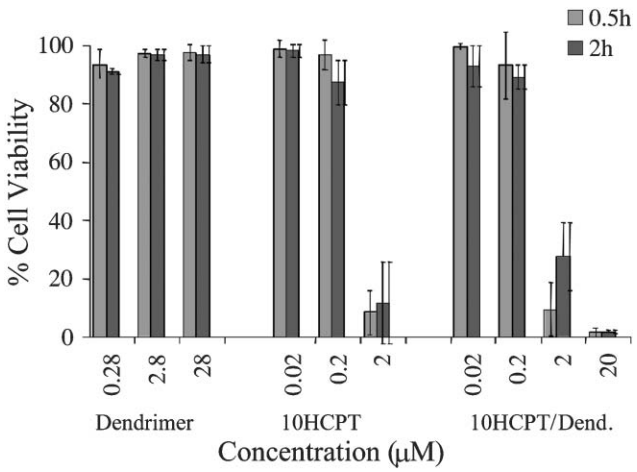


**Fig. 4** Cytotoxicity assay with human colon cancer cells (HT-29, 2000 cells per well, $n = 3$) incubated with the (**[G4]-PGLSA**-OH)$_2$-PEG$_{3400}$ 10HCPT supramolecular assembly.

Four of the requirements for an ideal drug delivery system are: 1) higher effective drug concentrations for smaller delivery volumes, 2) retained potent cytotoxicity of the drug after formulation, 3) targeted or localized delivery, and 4) biocompatible carriers or delivery vehicles. These results provide further motivation for the synthesis and evaluation of new dendritic polymers for drug delivery applications.[23–26] The unique chemical and physical characteristics of dendritic macromolecules, coupled with the control of composition and functionality provide opportunities to synthesize tailored materials for specific medical applications.‡

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## Notes and references

‡ Detailed experimental data for the synthesis of the (**[G4]-PGLSA**-OH)$_2$-PEG$_{3400}$ dendrimer, encapsulation procedure, and cytotoxicity cell studies are available in the supporting information†.

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