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The Synthesis of Water Soluble Dendrimers, and their Application as Possible Drug Delivery Systems.

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Abstract: The synthesis of two water soluble dendrimers is described. The formation of water soluble inclusion complexes with a variety of small, *hydrophobic* guest molecules is also described. Moreover, when these guest molecules are drug moieties, then the resulting drug/dendrimer complexes can be considered ideal candidates for use as novel drug delivery systems.

The therapeutic effectiveness of any drug is often diminished by its inability to gain access to the site of action in an appropriate dose. This is often due to the poor solubility of the drug in the body's aqueous environment. Medicinal chemists initially attempt to address this problem by synthesizing a water soluble derivative of the drug moiety: unfortunately, even small structural changes can often reduce the efficacy of the drug. Another method of aiding solublisation is to encapsulate the drug within the hydrophobic domain of a colloidal or surfactant based system (i.e. emulsions, liquid crystals or micelles).[1] However, the unstable dynamics of these systems, as well as their sensitivity to other functionality and *pH*, can lead to uncontrolled and premature release of the bound drug moieties, once again rendering this approach ineffectual. Ideally a *static* or *covalent* micellar system is desirable.

Micellar, or surfactant based delivery systems, in effect *dissolve* drugs within their hydrophobic interior, *water solubility* is then provided by the hydrophilic exterior of the micellar or surfactant based assembly. It is possible to incorporate these properties within a *dendrimeric* structure and therefore overcome the problems associated with traditional surfactant based delivery systems.[2] Dendrimers are spherical, hyperbranched macromolecules possessing a large number of terminal groups.[3] When these terminal groups are either charged or polar, then these novel molecules can indeed be considered *static, covalent micelles.*[4] The synthesis of two water soluble dendrimers, **1** and **2**, was achieved following a modified Tomalia PAMAM synthesis.[5] A methanolic solution of ethylene diamine was treated with four

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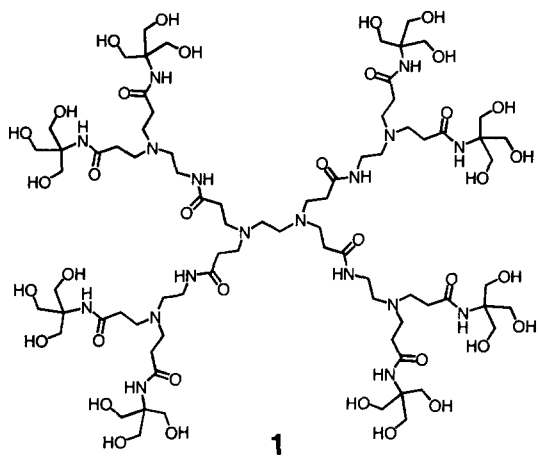

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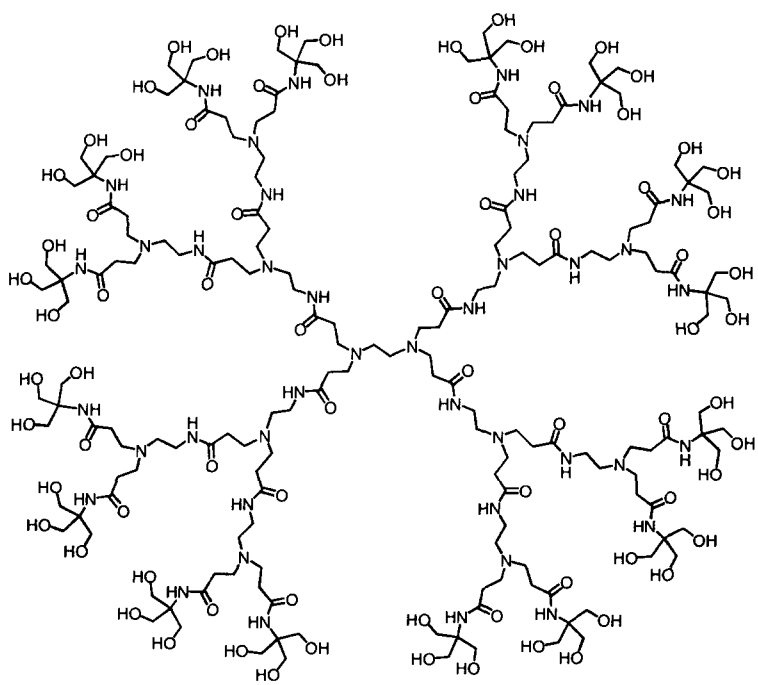
molar equivalents of methyl acrylate to give the desired half generation ester terminated dendron (G=0.5) in 99% yield. Subsequent reaction of this half generation dendrimer with a twenty fold excess of ethylene diamine gave the first generation amine terminated dendrimer (G=1.0) in 98% yield. Repetition of these reactions yields the larger ester terminated dendrimers G=1.5 and G=2.5, containing eight and sixteen ester terminal groups respectively. Treatment of these dendrimers with tris(hydroxymethyl) amino methane (TRIS) in the presence of potassium carbonate yields the final dendrimers **1** and **2,** in 78 and 54% respectively. [6] As expected both dendrimers proved to be extremely water soluble, so much so they were found to be completely miscible with water in all proportions. Benzoic acid is essentially insoluble in water at neutral *pH* (2.9 mg/ml),[7] and is therefore an ideal substrate for an initial study into the dendrimer's solubilising and complex forming ability. The most successful procedure for complex formation first involved the dissolution of both substances in methanol, thus ensuring that the crystal lattice of both substrates were completely disrupted. The methanol was then removed under vacuum giving a dendrimer/benzoic acid co-precipitate.[8]



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On addition of phosphate buffered water (*pH* 7), an aqueous soluble benzoic acid/dendrimer complex was observed.[9] The pH of this benzoic acid/dendrimer complex solution remained at pH 7.0.[10] Arbitrary dendrimer concentrations ranging from 0.25M to 2.5M were initially chosen for the dendrimer/benzoic acid complex's. These complexes remained stable at neutral *pH,* even after storage for several weeks. At this stage only 1:1 complex's were being assembled, even so, we had increased the solubility of benzoic acid to 305 mg/ml *via* complex formation with dendrimer 1.[9] Measurement of the complex's UV spectrum confirmed both the presence and concentration of benzoic acid. In an attempt to prove further inclusion a comparison was made of the UV spectra of benzoic acid before and after complexation. Unfortunately no change in the spectra could be detected, similarly, no change in the ^{1}H NMR spectra could be measured. Attempts to form inclusion complexes with other aqueous insoluble substrates and dendrimer **1** were also attempted (again at arbitrary concentrations between 0.25M and 2.5M), the solubility [9] of the resulting complexes is displayed in **Table 1**. These substrates were chosen because of their antifungal or antibacterial properties. This would enable the use of microcalorimetric techniques to evaluate the drug delivery potential of these drug/dendrimer complexes.[11] The preliminary results of the calorimetric experiments clearly demonstrated that the bound substrates could be released upon contact with the target organism [12] (more detailed results concerning drug delivery will be reported elsewhere). Once again no spectroscopic changes could be observed for these drug dendrimer complexes.

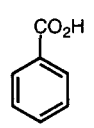
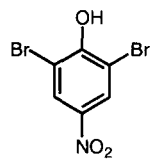
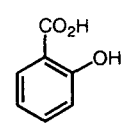
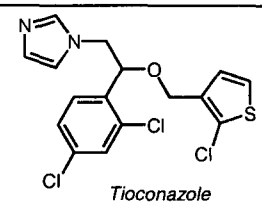
Substrate				 *Tioconazole*
Solubility [9]	Infinitely soluble complex	Infinitely soluble complex	Infinitely soluble complex	No complex formed

Table 1.

Unfortunately all drug/dendrimer complexes were unstable to acid, and the bound substrate began to precipitate after only 10 minutes at *pH* 2. This indicates that the nitrogens within the dendrimer may be important with regard to binding, for as they are protonated (at *pH* 2), the ability of the dendrimer to bind its guest is lost. Additionally, tioconazole and other small *non-polar* molecules could not be retained within the dendrimer, again, suggesting an interaction between the dendrimer and the polar regions of the *acidic* guest molecules. More work is required in order to determine the exact nature of this binding, but it seems likely that an interaction between the acidic portion of the guests (drug moieties) and the basic tertiary nitrogens of the dendrimeric hosts is involved. An initial attempt was made to determine the maximum benzoic acid loading of these dendrimers. As before a co-precipitate was formed, and on this occasion an excess of

benzoic acid (100 equivalents) and 1 equivalent of either dendrimer **1** or **2** was used. Buffered water, pH 7, was then added (giving a 0.5M solution of dendrimer), and the resulting suspensions left to settle overnight, the excess benzoic acid was removed *via* filtration and the UV spectra recorded. From the Beer-Lambert plots a maximum loading of around 22 for dendrimer **1** and 46 benzoic acids for dendrimer **2** were determined. This demonstrates that as more space becomes available within the host molecule, then an increased number of guest molecules can be retained. However, these results still fail to give us any useful information concerning the exact mode and mechanism of binding.

In conclusion, we have developed a convenient route to highly water soluble dendrimers using a modified PAMAM synthesis. Additionally, we have demonstrated that these water soluble dendrimers are capable of binding, and solubilising, small *acidic* hydrophobic molecules. By virtue of the complex's infinite water solubility, the solubility of the bound hydrophobic guests can also be considered infinite.[9] Additionally, when these bound guests are drug molecules the resulting complexes can be considered as potential drug delivery systems. Further work is going on in our laboratory to determine the exact nature of the host/guest binding, as well as to develop these dendrimeric hosts as novel drug delivery vehicles.

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References and Notes

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6. All new compounds gave satisfactory spectral data. *Experimental for dendrimer 1.* A DMSO solution (15ml) of ester terminated dendrimer, G=1.5 (2.0g, 1.67mmol) [5] trs-(hydroxymethyl) aminomethane (1.8g, 15.0mmol) and potassium carbonate (2.1g, 15.0mmol) was stirred for 70 hours at 50°C. The solution was then filtered, and the solvent removed under vacuum to give a thick honey coloured oil. The compound was then purified by precipitation with acetone from the minimum amount of water. The white solid was collected and dried in a vacuum oven. Yield 78%. Analytical reverse phase HPLC, using methanol as eluent, indicated a single compound, retention time = 204 sec (1ml/min). ν_{max} cm^{-1} 3320 (b), 1667 and 1642. δ C ppm (67.5MHz, D_2O); 34.4, 34.7, 38.4, 48.1, 50.0, 51.1, 53.5, 62.6, 63.6, 174.8, 176.0. δ H ppm (270 MHz, D_2O); 3.83 (48H, s, C***H***$_2$OH), 2.47-2.93 (68H, series of broad multiplets, remaining C***H***$_2$'s). *m/z*FAB 1917$[M+H]^+$and 1939$[M+Na]^+$, $C_{78}H_{152}N_{18}O_{36}$ requires 1916.
7. *AnalaR Standards for Laboratory Chemicals,* 8th Edition.
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9. Due to the dendrimer and dendrimer/substrate complexes being *miscible with water in all proportions*, the concept of concentration needs to be carefully considered. In large volumes of water the dendrimer/substrate complex is indeed dissolved within the water, nevertheless, in small aqueous volumes, it is now the water that is dissolved within the dendrimer/substrate complex. As a result the subsequent dendrimer complexes should therefore be considered *infinitely water soluble.*
10. The pKa of dendrimer **1** was measured to be 9.0. Although the amine groups are in principle capable of deprotonating the buffer/benzoic acid, *measurements confirmed that the pH of the buffer/complex solution remained at pH 7.0.*
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