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Synthesis and properties of novel dendrimers for molecular delivery

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ABSTRACT
The primary aim of this investigation was to synthesize novel water-soluble dendrimer materials which would be able to encapsulate a model non-polar drug (a water-insoluble dye) at a low generation number. Fairly long-chain alkyl carboxylate branching units were used to help encapsulate the non-polar guest molecules efficiently at a low generation number. 3,5-dihydroxybenzyl alcohol was used as a building block, and ethyl 6-bromohexanoate as a branching unit; two different core centers were used, namely biphenyl and terphenyl, in order to assess the effects of the different structures on the dendrimers' ability to encapsulate nonpolar molecules. The structures and relevant physico-chemical properties of these new molecules were investigated using surface tension, molar conductivity, dye encapsulation and the normal spectrometric techniques.
Keywords: *Dendrimer, oil red O, dye encapsulation, hyperbranched polymer, CMC.*

1. INTRODUCTION
Hyperbranched polymers, and most notably dendrimers, constitute a class of macromolecules whose distinctive properties such as their degree of branching, multi-valency and defined molecular shape can make them suitable as platforms for molecular encapsulation and applications including drug delivery [1]. Dendrimers can offer two different interfacial environments [2], namely their interior and their outer surfaces. The outer surface determines molecular features such as solubility, and is where the terminal groups are located, providing an interface with the surrounding medium. When a hydrophilic substituent is introduced onto this surface (the periphery) a dendrimer becomes more hydrophilic, and potentially water-soluble. The interior of the dendrimer can provide a non-polar environment suitable to incorporate hydrophobic molecules.

It is known that having a hydrophobic interior and a hydrophilic surface can make such dendrimers good delivery vehicles for drugs with poor aqueous solubility, and some of the important ongoing work in this area is reviewed in references [3-7]. The present paper reports the synthesis and properties of some novel, low-cost dendritic materials with the potential for use as drug-delivery vectors.

2. EXPERIMENTAL SECTION
Abbreviations used in this work:

DCM= dichloromethane; THF = tetrahydrofuran. The letter G is used for the generation number, and C for the number of phenyl groups in the core (2C for a biphenyl and 3C for a terphenyl core).

2.1. Materials and methods.
All the synthetic chemicals used in this research were purchased from Sigma Aldrich Ltd. (UK) or Lancaster Ltd., and were used as supplied unless stated otherwise. All solvents used during the reaction, work-up and purification procedures were supplied by the Fisher Chemical Co. Electrical conductivity measurements [8] were made using a conductivity meter (Wooden Precision Apparatus Limited, CMD 830 WPA), calibrated by a standard aqueous KCl solution.

Solution surface tensions were measured using the De Nouy ring method [9,10], due to its simplicity and accuracy. In the dye encapsulation experiment, Oil Red O dye was used; it has an absorption maximum at around 518nm and has an empirical formula of $C_{26}H_{24}N_4O$; the structure of this compound is shown in Figure 1. Absorbance measurements to determine the solubility profiles were made on a Kontron Uvikon 940 UV-visible spectrophotometer. Particle sizes of the nanoparticulate products in aqueous media were determined by means of a Malvern Instruments Zetasizer, model 3000HS.

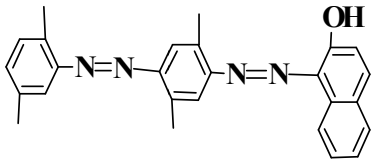
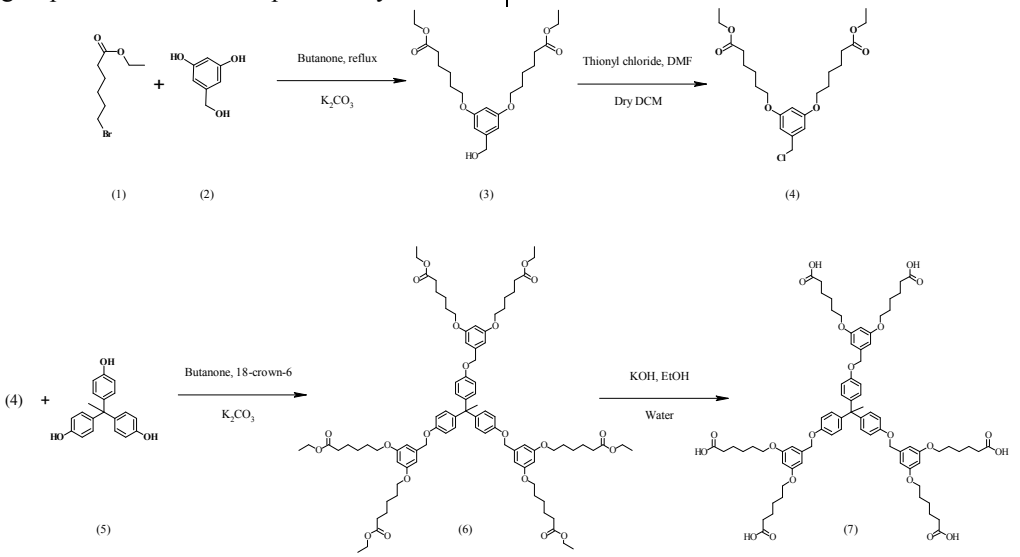


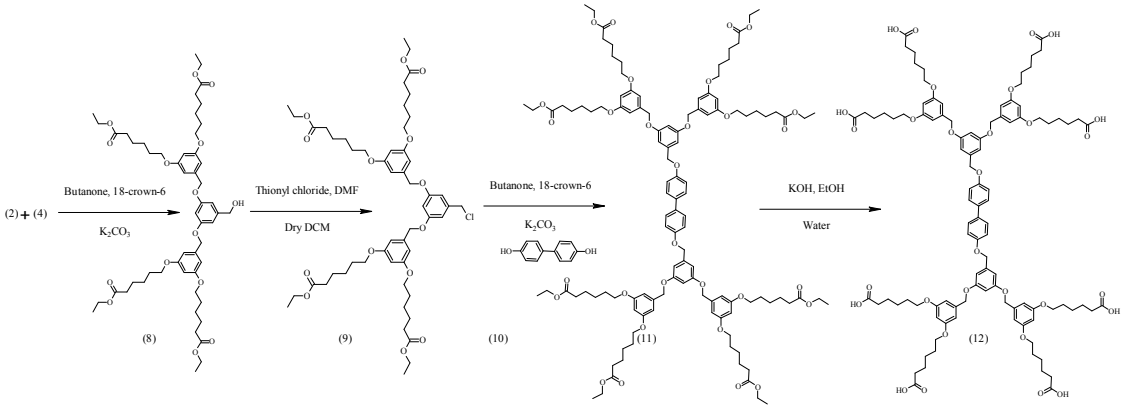
Figure 1. Structure of Oil Red O dye.

2.2. Synthesis of compound 7, $(HOOC)_6$-G1-3C (Scheme 1).
The starting material chosen to produce the chain ends with potentially hydrophilic functional groups was ethyl 6-bromohexanoate **(1).** To help provide a hydrophobic interior containing electron-rich aromatic rings, it was decided to employ 3,5-dihydroxybenzyl alcohol as a building block. The generation growth step was started by reacting two equivalents of **1** with 3,5-dihydroxybenzyl alcohol (**2**). This reaction produced the first-generation alcohol $(EtO_2C)_2$-G1-OH (**3**) with an ethyl ester chain-end. Activation of the hydroxymethyl group at the focal point of **3** was achieved by reaction with thionyl chloride and a catalytic amount of DMF in the presence of dry DCM, which gave the

corresponding chloride $(EtO_2C)_2$-G1-Cl (**4**). The next stage was to react three equivalents of **4** with 1,1,1-tris(4-hydroxyphenyl)ethane (**5**). The resultant dendritic polyether macromolecule $(EtO_2C)_6$-G1-3C (**6**) was isolated, and transformation of the terminal ethyl ester groups of **6** into the desired carboxylate groups was then accomplished by alkaline hydrolysis [11]. Reaction of **6** with a large excess of potassium hydroxide was found to proceed satisfactorily in the mixed solvent system of tetrahydrofuran/water/ethanol. Acidification of the reaction mixture by a well-known literature method [12] gave the carboxylate-terminated molecule **7,** $(HOOC)_6$-G1-3C.



Scheme 1. Synthesis of compound 7, $(HOOC)_6$-G1-3C.



Scheme 2. Synthesis of compound 12, (HOOC)8-G2-2C.

2.2.1.Spectroscopic characterisation of compound 7 ^{1}H (CDCl$_3$), ppm).

2.2 (t, 12H, C***H*****$_2$**COOH)$_6$, J=7.4Hz); 1.41 (overlapped peaks), 12H; (C***H*****$_2$**CH$_2$)$_6$, 1.6 (overlapped peaks, 24H (C***H*****$_2$**CH$_2$)12); 4.05 (t, 12H, (C***H*****$_2$**OAr)$_6$, J=6.4 Hz); 6.3 (d, C***H***, 4-ArH); 6.26 (t, 6H, C***H***, 2,6-ArH); 5.20 (s, ArC***H*****$_2$**OAr); 6.70 (d, 6H, C***H***, 3,5-Ar in core); 7.1 (d, 6H, C***H***, 2,6-ArH in core); 12.35 (s, 6H, O***H***), 2.28 (q, 3H, C***H*****$_3$** core). ^{13}C NMR (ppm):177.21 (**C**OOH, carbonyl); 36 (**C**H$_2$COOH); 25 (**C**H$_2$CH$_2$COOH); 25.5 (**C**H$_2$CH$_2$CH$_2$COH); 29.3 (**C**H$_2$CH$_2$OAr); 68.82 (**C**H$_2$OAr); 99.5 (**C**H, 4 Ar); 159 (**C**O, 3,5-Ar); 102 (**C**H, 2,6-ArC); 142 (**C**, 4-Ar); 71.4 (**C**CH$_2$OAr); 158.0 (**C**, 4-Ar in core); 129.2 (**C**H, 2,6-Ar in core); 114.8 (**C**H, 3,5-ArC in core); 135 (**C**, 1-Ar in core); 12.8 (**C**H$_3$, attached to core); 48 (C-CH$_3$ in core).IR ν max (KBr, cm^{-1}):2950, 2972 (R-CH$_3$); 2916, 2936 (R'-CH$_2$R''); 3010, 3097 (*p*-di-subst.); 3010, 3079 (1,3,5-tri-subst.); 2900, 3100 (C-C-COOH); 1210, 1310 (Ph-O-C, ester); 1710 (C=O)

High-resolution mass spectrometry:Found mono-isotopic mass = 1356.6478; Calculated mono-isotopic mass = 1356.6444.

2.3. Synthesis of compound 12, (HOOC)$_8$-G2-2C (Scheme 2).

Synthesis of the second-generation dendron was achieved by reacting two equivalents of (4) with one equivalent of 3,5-dihydroxybenzyl alcohol (**2**) in the presence of potassium carbonate, 18-crown 6 and butanone, heated under reflux to give $(EtO_2C)_4$-G2-OH (**8**) with four ethyl ester chain-ends. Activation of the hydroxymethyl group, the focal point of **8**, by reaction with thionyl chloride and several drops of DMF as a proton scavenger, proceeded to give the corresponding chloride $(EtO_2C)_4$-G2Cl (**9**). The next stage was to use the biphenyl moiety of (4,4'-dihydroxybiphenyl) as a core unit (**10**). Two equivalents of **9** were reacted with one equivalent of biphenol core in the presence of potassium carbonate, 18-crown-6 and butanone to give $(EtO_2)_8$-G2-2C (**11**) with eight ester chain-ends. The transformation of the terminal ethyl ester groups of **11** into the desired carboxylate

groups was accomplished by alkaline hydrolysis. Reaction of **11** with a large excess of potassium hydroxide proceeded satisfactorily in a mixed solvent system of tetrahydrofuran/water/ethanol. Acidification gave the carboxyl-terminated macromolecule $(HOOC)_8$-G2-2C **12.**

2.3.1. Spectroscopic characterisation of compound 12.

^{1}H (CDCl$_3$, ppm):2.2 (t, 16H, (C***H***$_2$COOH)$_8$, J=7.2Hz); 1.46 (overlapped peaks, 16H, (C***H***$_2$CH$_2$)4; 1.66 (overlapped peaks, 32H, (C***H***$_2$CH$_2$CH$_2$)$_8$); 3.89 (t, 16H, (C***H***$_2$OAr)$_4$, J=6.2 Hz); 6.30 (C***H***, 6H,4-Ar)$_6$; 6.26 (t,12 H, C***H***, 2,6-Ar); 5.2 (s,12H,ArC***H***$_2$OAr)$_4$; 6.78 (d,4H,C***H***, in biphenyl core); 7.32 (d, 4H, C***H*** in biphenyl core)$_4$); 12.4 (d, 8H, COO***H***)$_8$.

^{13}C NMR (ppm):34.32 (**C**H$_2$COOR); 24.8 (**C**H$_2$CH$_2$COOR)$_8$; 25.07 (**C**H$_2$CH$_2$CH$_2$COOR); 28.99 (**C**H$_2$CH$_2$OAr); 65.9 (**C**H$_2$OAr); 99.5 (**C**H, 1-Ar); 99.3 (**C**H, 4-Ar); 158 (**C**OR, 3,5-Ar); 102.5 (**C**H, 2.6-Ar); 142.2 (**C**CH$_2$OAr); 71.4 (Ar**C**H$_2$OAr); 161.5 (**C**, 3,5-Ar inner ring); 103.5 (**C**, 2,6-Ar in inner ring); 143 (**C**, 1-Ar in inner ring); 159.5 (**C**, 4 in biphenyl); 129 (**C**, 2,6 in biphenyl); 14.8 (**C**, 3,5 in biphenyl core); 173.2 (**C**, in carbonyl).

IR ν max (KBr,cm^{-1}): Broad peak 3200, 3600 (COOH), shoulder in region about 2800-3000 (acid characteristic), 1707 (C=O), 1596.68 (C=C), 1456.54 (C-H), 1374.56, 1295.62 (CO, aryl ether), 1162(O-C=C), 824 (C-H bend).

High-resolution mass spectrometry: Calculated monoisotopic mass: 1830.8334; Found monoisotopic mass: 1830.8342.

3. RESULTS SECTION

3.1. Solubility data for compounds 7 and 12.

Figure 2 shows the pH profiles (UV absorbance data) for compounds **7** and **12**. The maximum solubility of both compounds were achieved at pH 7.4, and their solubility and molar absorptivity at that pH are shown in Table 1.

Table 1. Absorptivity and solubility of dendrimers 7 and 12 at pH 7.4.

Compound name	Absorptivity /dm^{-3}.mol^{-1}.cm^{-1}	Solubility /mol.dm^{-3}
$(HOOC)_6$-G1-3C (**7**)	5.04	0.140
$(HOOC)_8$-G2-2C (**12**)	30.63	0.05

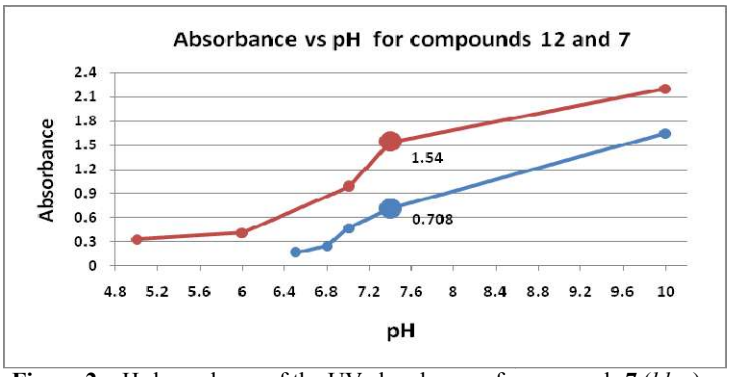


Figure 2. pH-dependence of the UV absorbance of compounds **7** (*blue*) and **12** (*red*) at λ=278nm.

3.2. CMC results and micellarproperties in aqueous media.

The critical micelle concentrations (CMC) of the products were measured by three different methods in order to determine whether they were forming micelles. It should be recalled that the dye used in this experiment for encapsulation was the water-insoluble Oil Red O dye, which is known to have a moderate solubility in ethanol [13]. Studies of surface tension, molar conductivity and dye encapsulation for compounds **7** and **12** revealed that both compounds had a CMC, which indicates that many units of these molecules come together to form micellar structures rather than forming unimolecular micelles.

This was supported by simple molecular modelling using Quantum CAChe software (Fujitsu Ltd.), together with Zetasizer measurements, which indicated that the micellar structures for these two substances were much larger than the average size of the individual dendrimer molecules. The CMC value found by dye encapsulation was greater than those obtained from measurements of surface tension and molecular conductivity; this seems reasonable, due to the likely expansion of the micelles after they encapsulate a dye.

Table 2. Average CMC values found by two different methods for compounds 7 and 12.

CMC Method	$(HOOC)_6$-G1-3C (7) /mol.dm^{-3}	$(HOOC)_8$-G2-2C(12) /mol.dm^{-3}
Surface tension	1.1 x10^{-4}	1.5 x 10^{-4}
Molar conductivity	1.4 x 10^{-4}	1.1 x 10^{-4}
Average CMC	1.25 x 10^{-4}	1.3 x 10^{-4}

Table 3.CMC values by dye-encapsulation for compounds 7 and 12.

CMC Method	$(HOOC)_6$-G1-3C (7) /mol.dm^{-3}	$(HOOC)_8$-G2-2C (12) /mol.dm^{-3}
Dye encapsulation	7.15 x 10^{-4}	7.75 x 10^{-4}

Table 4. Sizes of compounds 7 & 12 from modelling and from Zetasizer.

Dendrimer molecule	Size of molecule modelled in flat conformation/nm	Size of micelle found by Zetasizer/nm	Polydispersity Index
$(HOOC)_6$-G1-3C, (**7**)	5006	136	1.52
$(HOOC)_8$-G2-2C, (**12**)	6146	1247	1.12

The CMC value found by dye encapsulation was greater than those obtained from measurements of surface tension and molecular conductivity; this seems reasonable, due to the likely expansion of the micelles after they encapsulate a dye.

Compounds **7** and **12** both had good solubility in water at pH 7.4 and they were able to encapsulate the water-insoluble dye and improve its solubility. In order to have more information about the properties of these macromolecules it is useful to compare their CMC values to those previously reported for well-known surfactants. (The CMCs of the compounds in Table 5 are average values obtained by several methods such as surface tension and molar conductivity).

Table 5. Relative molar masses and CMC for dendritic macromolecules and two well-known surfactants.

Surfactant or Dendrimer	CMC found / mol.dm^{-3}	Molar mass/Da
Pluronic P105	4.3 x 10^{-4} [14]	6500
Hexadecylpyridinium bromide	7.3 x 10^{-4} [15]	384
$(HOOC)_6$-G1-3C	3.3 x 10^{-4}	1357
$(HOOC)_8$-G2-2C	3.6 x 10^{-4}	1832

Pluronic co-polymers (also known as Synperonic P105 or Poloxamer P105) have been used extensively in a variety of pharmaceutical applications including the delivery of low molecular mass drugs and polypeptides [16]. The Pluronic (P105) tri-block copolymer used for comparison here was composed of polyethylene oxide (POE) and polypropylene oxide (PPO) segments. It had a relative molar mass of 6500 [17]. The other surfactant used, hexadecylpyridinium bromide, a cationic surfactant comprising a pyridinium head and a hexadecyl tail. Pluronic P105 has the highest molar mass and hexadecylpyridinium bromide the lowest. The CMCs of our dendritic macromolecules lie in between these two, which could be due to their compact structure. Pluronic P105 had the highest CMC due to its straight-chain conformation.

4. CONCLUSIONS

It has been widely documented that the solubility of hydrophobic compounds in water can be enhanced by the addition of surfactant molecules at a concentration above their CMC [18], by an encapsulation process. Investigation of compounds **7** and **12** in the present work has revealed that they encapsulate the water-insoluble ORO dye and greatly improve its solubility. Further modification of the structure of these compounds may enhance their potential to be used as molecular delivery agents.

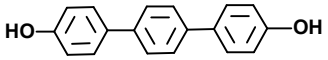


Figure 3. Precursor for terphenyl core.

In future, synthesis of next-generation dendrimers will be attempted, although it is likely that as the generations increase, the synthesis will become more difficult. The physical and chemical properties of the new dendritic compounds (such as solubility, formation of micelles and dye uptake) will be investigated. A feature of the continuing investigations is to make bespoke cores such as linear terphenyls which could be expected to produce larger dendrimers with bigger cavities.

To increase the potential size and encapsulation capacity of the dendrimer, it is planned to increase the length of the dendrons by reacting our current dendrons with spacers; one spacer type is based on *para*-substituted phenyl systems to effectively lengthen the "trunk" section of the dendrimer tree.

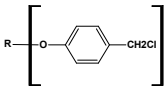


Figure 4. An example of a linking spacer.

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5. ACKNOWLEDGEMENTS

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