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SYNTHESIS AND PROPERTIES OF NOVEL DENDRIMER MATERIALS

THESIS

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DECLARATION

This thesis entitled "Synthesis and properties of novel dendrimer material" is based upon work conducted by the author in the School of Pharmacy and Chemistry at Kingston University London between Month Year and Month Year. All of the work described herein is original unless otherwise acknowledged in the text or by references. None of the work has been submitted for another degree in this or any other universities.

Abdollah Ataei

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Many thanks to my all friend for giving me the joy and happiness, mainly for their great patience throughout this period.

My sincere gratitude goes to my family for their constant encouragement and emotional support all the way throughout these years.

Abstract:

The field of drug delivery is developing rapidly and is gaining the attention of scientists and the pharmaceutical industry. Serious challenges in designing drug delivery systems are that many new drug entities such as cancer drugs are water-insoluble.

Hyperbranched polymers, most notably dendrimers, are a class of macromolecules whose unique properties such as their degree of branching, multi-valency and defined molecular shape make them suitable as new scaffolds for drug delivery. Dendrimers can offer two different environments which are very important in drug delivery, namely the interior and the outer surface. The outer surface represents .molecular features such as solubility where the terminal groups are located, and its interface with the surrounding medium. When a hydrophilic substituent is introduced into the surface (the periphery) the dendrimer becomes hydrophilic and water-soluble. The interior of these water soluble dendrimers gives a hydrophobic environment to the incorporated organic molecule.

Having a hydrophobic interior and a hydrophilic surface makes dendrimers potentially very good drug delivery vehicles.

The primary aim of this investigation was to synthesise a suitable water soluble dendritic material which was able to encapsulate the non-polar drugs (water insoluble dye) in a lower generation; the idea was that the large branching unit could the encapsulate drugs at lower generation.

The selection of building blocks for the preparation of dendritic material was very important. In this project, the monomer selected to build the dendritic macromolecule was 3,5-dihydroxybenzyl alcohol, and initially ethyl 6-bromohexanoate as a branching unit. The cores used were 4,4-dihydroxybiphenyl and 1,1,1-tris (4-hydroxy-phenyl) ethane; in the later stage of this project, physical and chemical properties of dendrimers such as solubility at different pH, maximum solubility at pH 7.4, Absorptivity of the molecules, and finding their CMC using dye encapsulation, surface tension, and molar conductivity were investigated.

The first part of this investigation was the synthesis of dendritic macromolecules achieved by attaching first and second generation dendrons to 4,4-dihydroxybiphenyl and 1,1,1-tris(4-hydroxyphenyl) ethane, and then hydrolysis prior to measurement of their physical-chemical properties.

Some of the products had properties that could improve the solubility of a water insoluble dye by encapsulation. Their critical micelle concentration was found by surface tension measurement and was verified by two other analytical techniques (molar conductivity and dye encapsulation).

Study of physico-chemical results and molecular modelling of these compounds revealed that they have a potential to be used in drug delivery systems, although further modification of structure would improve their properties for this purpose.

LIST OF ABBREVIATION

b.p.	boiling point
Conc	concentrated
DMF	N,N-dimethyl foramide
EA	elemental analysis
Et	ethyl
h	hours
m.p.	melting point
Me	methyl
min	minutes
MS	mass spectrometry
NMR	nuclear magnetic resonance
Ph	phenyl
THF	tetrahydrofuran
TLC	thin-layer chromatography
PPI	poly(propylene imine
UV	ultra-violet
PAMAM	poly(amidoamine)
C	concentration (mol dm^{-3})
cm	centimetre
DCM	dichloromethane

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CHAPTER 1: INTRODUCTION

GENERAL INTRODUCTION

This chapter is intended to introduce the reader to several areas of the chemistry and physical science of dendrimers which are described in this thesis. The first part of this chapter is concerned with macromolecules. A brief historical background of macromolecules, their properties and their differences from dendrimers, dendrimer structures, the various classes and categories of dendrimers will be described.

In the last few years synthesis of dendritic structures and the investigation of their properties have been the subject of major research effort worldwide. This chapter presents a brief introduction to different potential applications of dendrimers in pharmaceutics and industry, in particular in the field of drug delivery. The amphiphilic properties and micelle formation of drug carrier macromolecules and methods of measuring CMC are described.

The second part deals with common synthetic procedures used to construct dendrimers, their historical background and the advantages and disadvantages of production methods via well-known synthetic routes.

The third part of this chapter will look at physical and chemical properties of dendrimers, in particular the amphiphilic properties and micelle formation of water-soluble dendrimers, methods of measuring CMC via surface tension and the ability of dendrimers to encapsulate host or drug molecules. The final section will describe the aims and objectives of this project

MACROMOLECULES

1.1. INTRODUCTION

Hyper-branched polymers, most notably dendrimers, are a class of macromolecules; hence it is important first to discuss the context of macromolecules in general and then specifically for the class of compound in which the macromolecule is highly branched. Macromolecules have large molecular masses, but their bulk densities are comparable with those of small molecules of similar composition. For an un-branched macromolecule, most of the conformations are asymmetric and relatively open, although if the macromolecule has a high density of branches that appear in a regular pattern, more compact conformations with a closer approach to spherical symmetry would be possible, which gives rise to molecules called dendrimers.

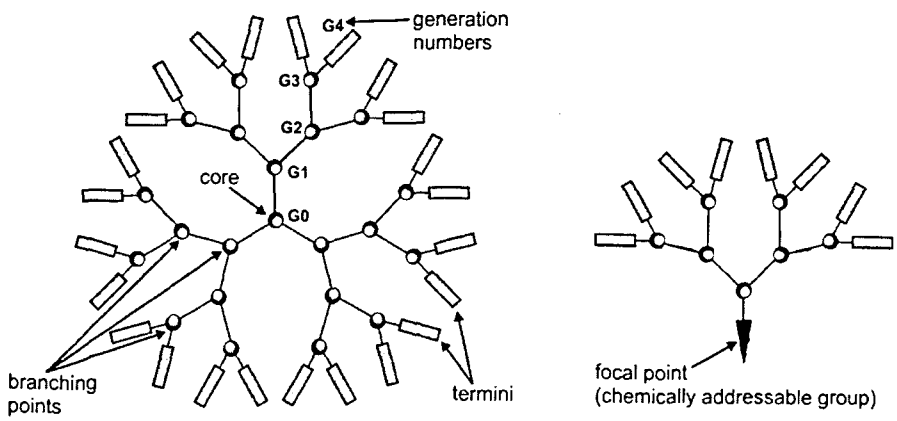


Figure 1.1: Dendron and dendrimer reported by Müllen and coworkers in Chem.-Eur. J., 2002, 3858-3864.

1.2. HISTORICAL BACKGROUND

From a historical perspective, progress from classically prepared long chain linear polymers to highly branched polymers and finally iteratively constructed macromolecules, commonly known as dendrimers or cascade macromolecules is considered to have occurred in three stages.

The first period started roughly from the late 1860's to the early 1940's, when macromolecules were considered as responsible for the insoluble and intractable materials formed in polymerisation processes. All the synthetic controls, mechanical separation and physical characterisation were primitive in comparison with current standards. The need for new synthetic resources to meet the increased demand for materials was driven by World War II to replace natural materials such as latex, with synthetic or un-natural substitutes.

From the early 1940's to the late 1970's, branched structures were considered primarily from a theoretical viewpoint with initial attempts at preparation via classical, or single-pot, polymerisation of functionally differentiated monomers, as noted by Flory et. al [1]. The current stage of research started in the late 1970's and early 1980's in which successful progress toward macromolecule assembly based on iterative methodology became the corner-stone of dendritic chemistry; by then analytical procedures were well enough developed to establish the purity and structure of

dendrimers. It was during this period (1978) that Vögtle. et. al (1978) reported the first example of an iterative synthetic procedure leading to a well-defined branched structure [2,3]. In the early 1980's Denkewalter [4] patented the synthesis of L-lysine-based dendrimers. Interestingly features of these dendritic polymers include a 1→2 asymmetric branching pattern and the incorporation of multiple chiral centres at each tier.[4]. The first dendritic structures that were investigated and received widespread attention were Tomalia's PAMAM (polyamidoamine) dendrimers and Newkome's 'arborol' system [5]. PAMAM dendrimers were synthesised by the divergent method (in which they are constructed from the core outwards) using ammonia initiator core reagents. Tomalia [5] reported the first preparation of an entire series of dendrimers possessing trigonal, 1→2N-based, branching centres. It is interesting to note that in 1985 Bidd and Whitting [6] described an iterative-based synthesis of a series of pure, linear alkyl hydrocarbons possessing 102, 150, 198, 264 and 390 carbon atoms; their methodology involved repetitive coupling and cycling acetal hydrolysis reactions. In 1993, based on the original work of Vögtle, polypropylene imines (PPI) dendrimers were created from the core to the periphery by Meijer and co-workers[7] , Meijer and co-workers functionalised the PPI family at the terminal with second order nonlinear optical chromospheres,[8], e.g., 4-(N,N-dimethyl) phenylcarboxamide end groups prepared by reaction with the corresponding acyl chloride. Employing the hyper-Rayleigh scattering technique to study hyper-polarisabilities, dendritic solution structure and symmetry were found to

be flexible at lower generations, and, on average, sphere-like. At higher generations, however, the dendrimers were found to be even more spherical and to become more rigid.

Today, these PPI dendrimers are synthesised in large quantities and are commercially available. In 1990 the first convergent (i.e. built from outside in) preparation of dendrimers resulted in poly (arylether) architecture, as reported by Fréchet and Hawker [9]. Their innovative use of a pivotal phenoxide-based, benzylic bromide displacement sequence has since led to many creative and novel macromolecular assemblies. During the same year (1990), Miller and Neenan [10] published their efforts with respect to the convergent preparation of the first series of aromatic-based, all-hydrocarbon dendrimers.

1.3. DENDRIMER STRUCTURE

The name dendrimer is derived from Greek words 'dendron' meaning tree and 'meros' meaning part. Dendrimers are highly branched three dimensional macromolecules with highly controlled structures, and theoretically all the molecule should have a single molecular weight. At higher generations, they have a tendency to adopt a globular shape. These highly branched polymers consist of multi-functional core unit where a monomeric unit is attached. Branching units are attached to the core and the terminal group.[11]

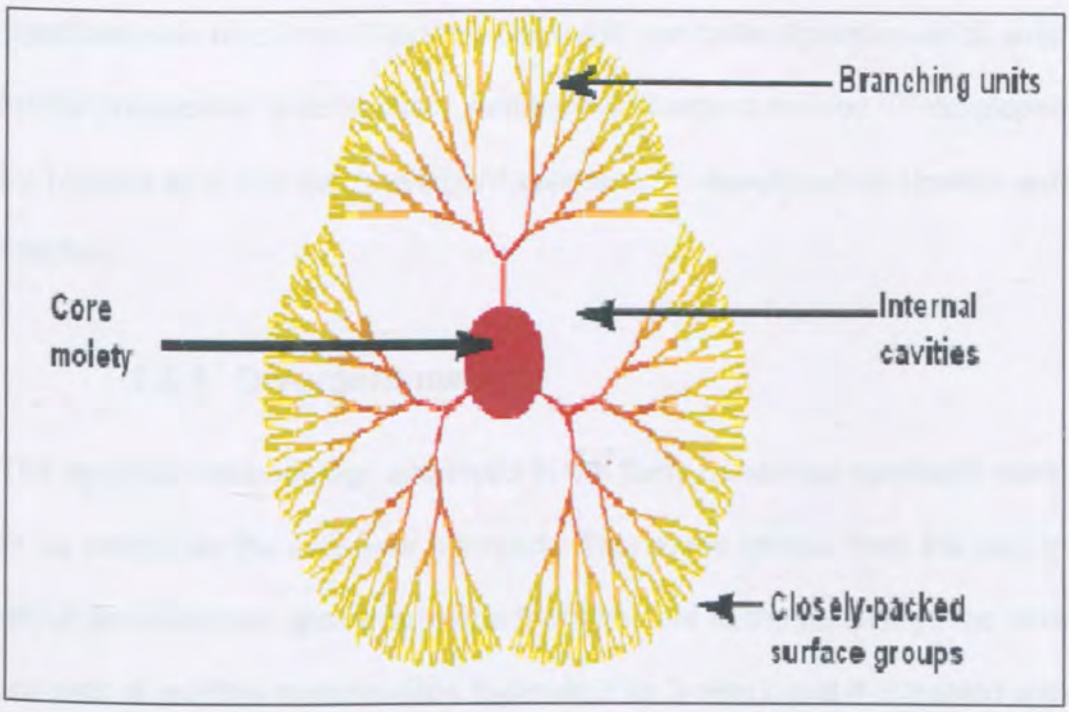


Figure 1.2: Dendrimer Structure, Benly, A. Accessed on 22.05.08:www.bitspace.com/bendy/dendrimer,1997

In this example the dendrimer has a functionality of three, with one reactive site attached to the core and the other making up a branching unit (G_1=Generation one). The branching unit can then react with a further monomer to produce G_2 (generation two) and a molecule with four end groups (terminal groups).

1.4. CONSTRUCTION OF DENDRIMERS

Generally synthesis of dendrimers involves the repeating of a two-step reaction sequence which comprises a generation growth step and an activation growth step. In order to obtain dendrimers without structure defects, both of these reactions must be clean and occur in high yield with no

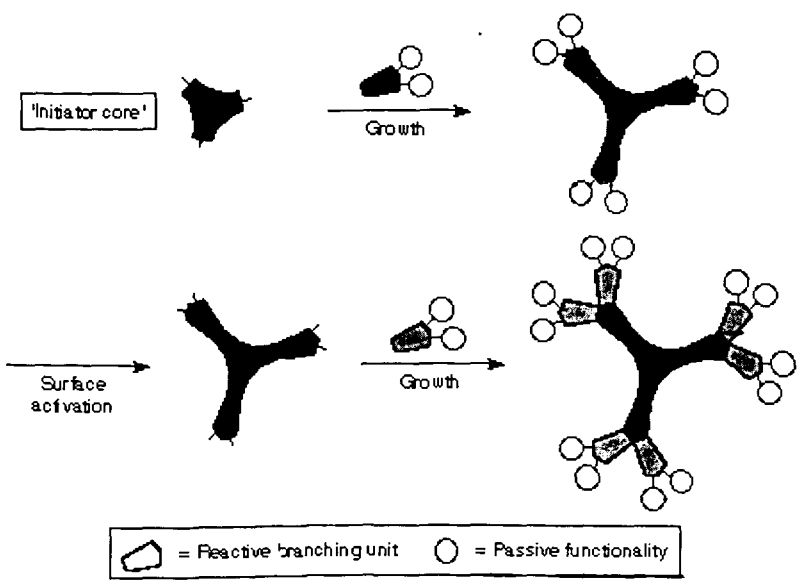
significant side reactions. There are two major synthetic strategies which exist for the preparation of dendrimers, namely the divergent method [12] developed by Tomalia et al and the convergent approach [13] developed by Hawker and Fréchet.

1.4.1. Divergent method

The synthetic methodology employed in the early dendrimer synthesis came to be known as the divergent approach. This name comes from the way in which the dendrimer grows outwards from the core to the periphery. The core consists of multiple reaction sites (typically 2 or 3 sites), and it is treated with an excess of the first monomer reacting with all the core reaction sites. The monomer also has reactive groups that are ready to react. An excess of a second monomer is reacted with the half generation (core and monomer), giving rise to the first generation. Continuation of this iterative reaction leads to second and third generations, but it is only in the fourth generation where the dendrimer becomes a highly branched sphere. Above the fifth generation steric overcrowding can occur, and may prevent complete reaction of the molecules, and it also damages the shape of the uniform structure of dendrimers[14,15,16]. A key feature of the divergent method is the exponentially increasing number of reactions that are required for the attachment of each subsequent layer or generation. Branching is dependent on monomer valency (this includes the cores since they are a special class of building block); therefore a core possessing one reactive moiety, such as a primary amine, is divalent and will accommodate two monomers assuming a neutral tri-

substituted amine product; branching therefore proceeds in a 1→2 manner. With three monomers, the resultant product is an ammonium salt, in which branching proceeds in a 1→3 fashion. Perhaps one of the most well known divergent procedures is the synthesis of PAMAM.

Diagram of divergent synthesis:



Scheme 1.1: Benly, A., Accessed on 20.04.08:www.bitspace.com/bendy/dendrimer,1997

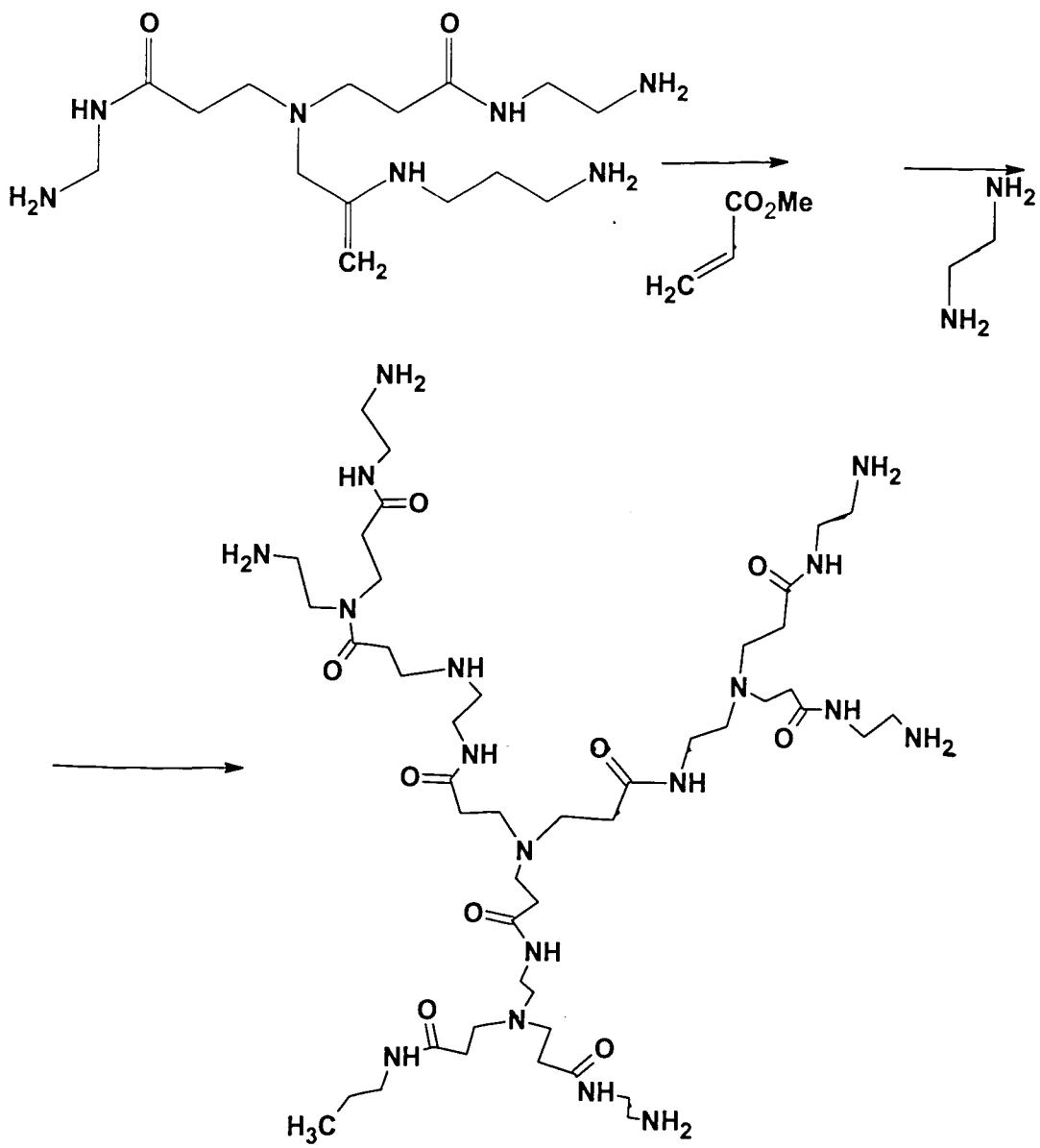
1.4.1.2. Early procedures

In 1978 Vögtle and co-workers [17] in Bonn reported the first preparation, separation, and mass spectrometric characterisation of simple fractal-like structures by an iterative methodology; this is defined as cascade synthesis, comprising reaction sequences which can be carried out repeatedly. In the period of the time between 1979 and 1981, Denkewalter et al[18] reported in three patents the first divergent preparation of a dendritic polypeptide utilising the protected amino acid N,N'-bis(tert-butoxycarbonyl)-L-lysine as the

monomeric building block. In 1985, Newkome et al and Tomalia et al [19,20] published different divergent routes to 1→3C-branching arborols and 1→N-branching dendrimer respectively. These works describe the construction of poly-functional molecules that possessed multiple branching centres and offered spectral characterisation supporting the structural assignment. There is another subtle but important difference between these two divergent processes: in the initial Vögtle-Tomalia routes, branching occurred at the surface substituent(s), whereas the Denkewalter- Newkome approaches involved a single surface attachment and the monomer possessed a 2- or 3-branching centre. This latter examples can be either divergent (with simple branched monomers) or convergent (with complex monomer addition) processes, (The general construction of both is shown below in schemes I.2 and I.3 [21, 22].

General construction

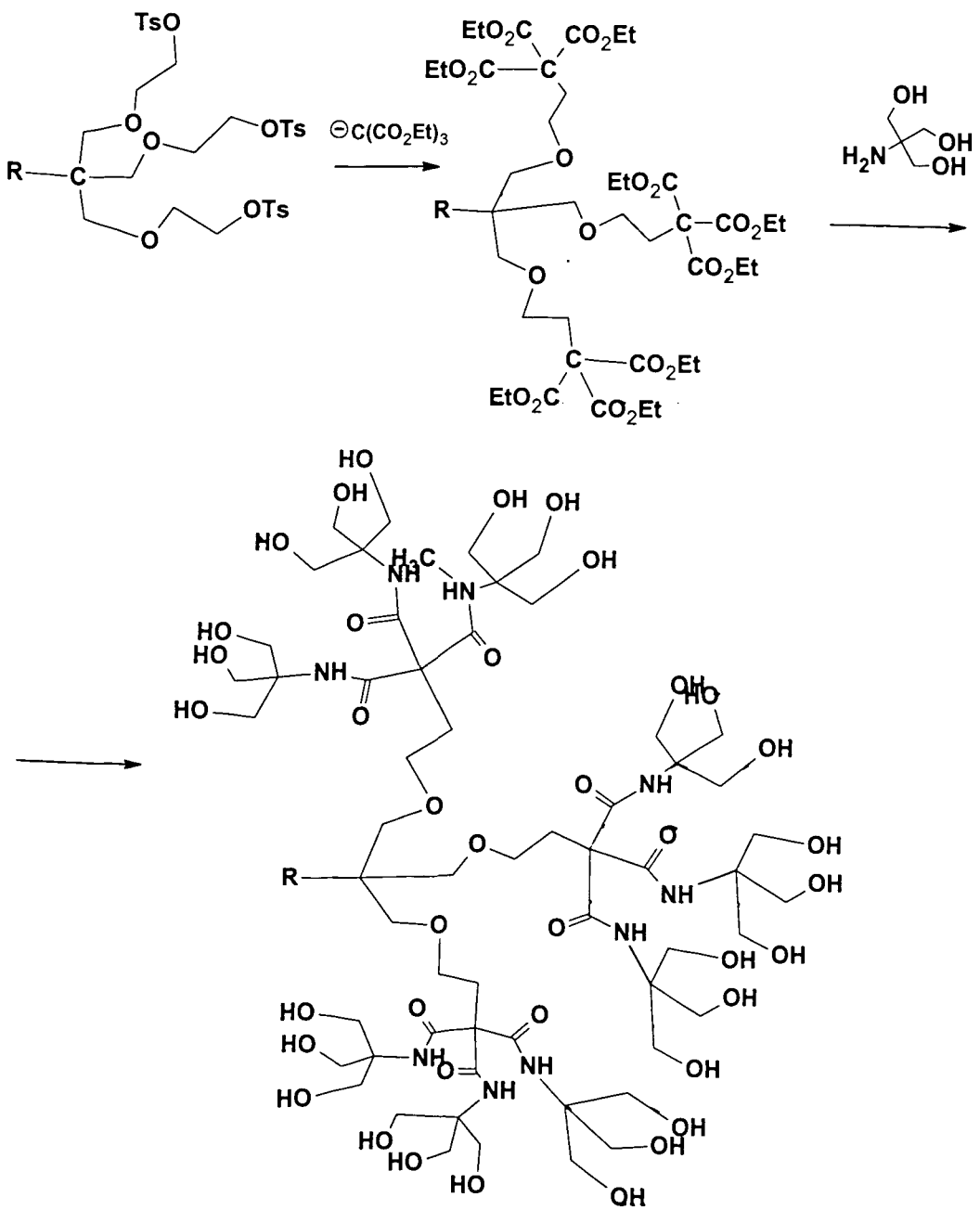
1→ 2N- Branching (Tomalia)



Scheme 1.2: Tomalia et al. (1→ 2N – branching)

General construction

1 ⟶ 3-C-Branched (Newkome)



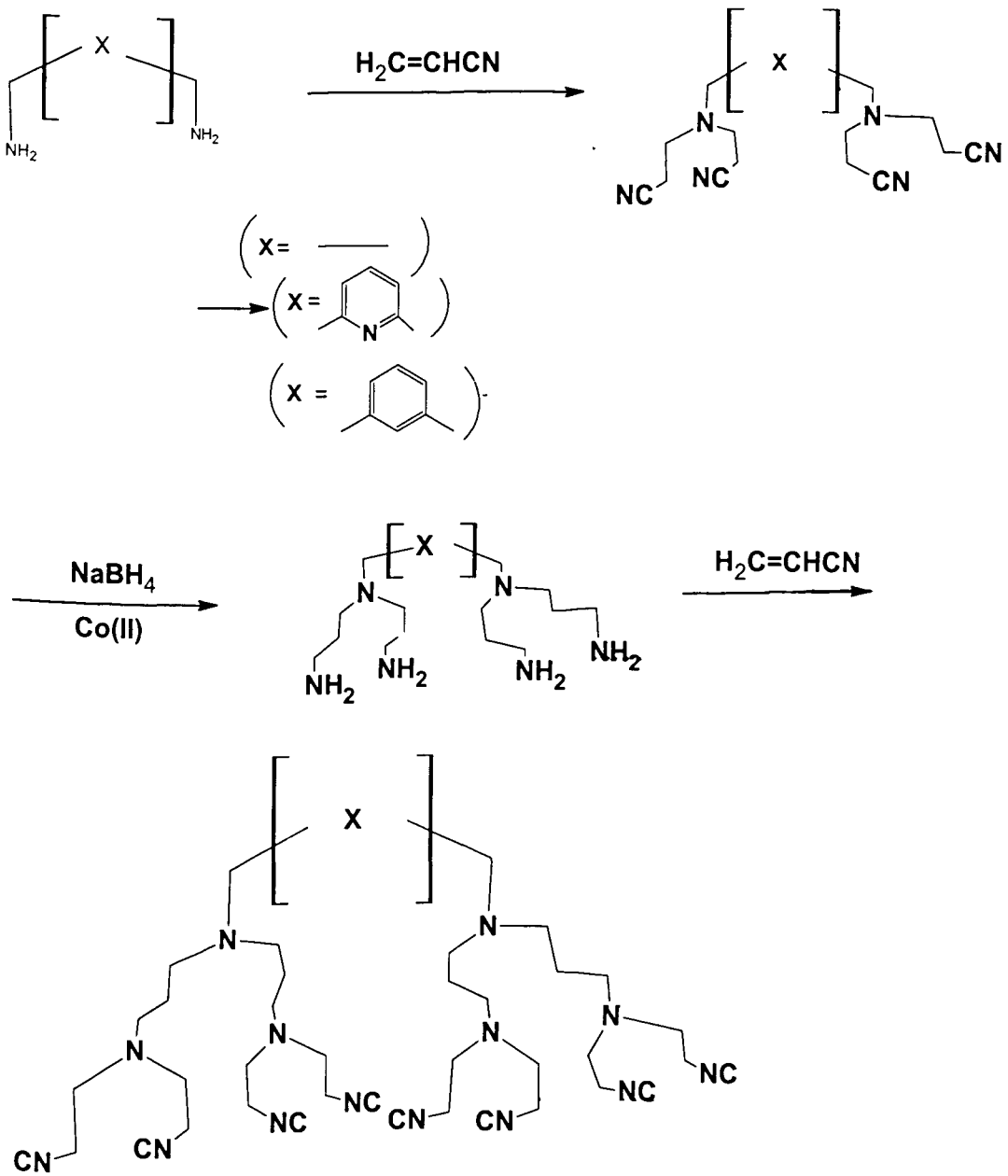
Scheme 1.3: G.R.Newkome, C. N. Moorefield, F., 2001, P 55 Dendrimers and Dendrons

1→ 2 Branched systems.

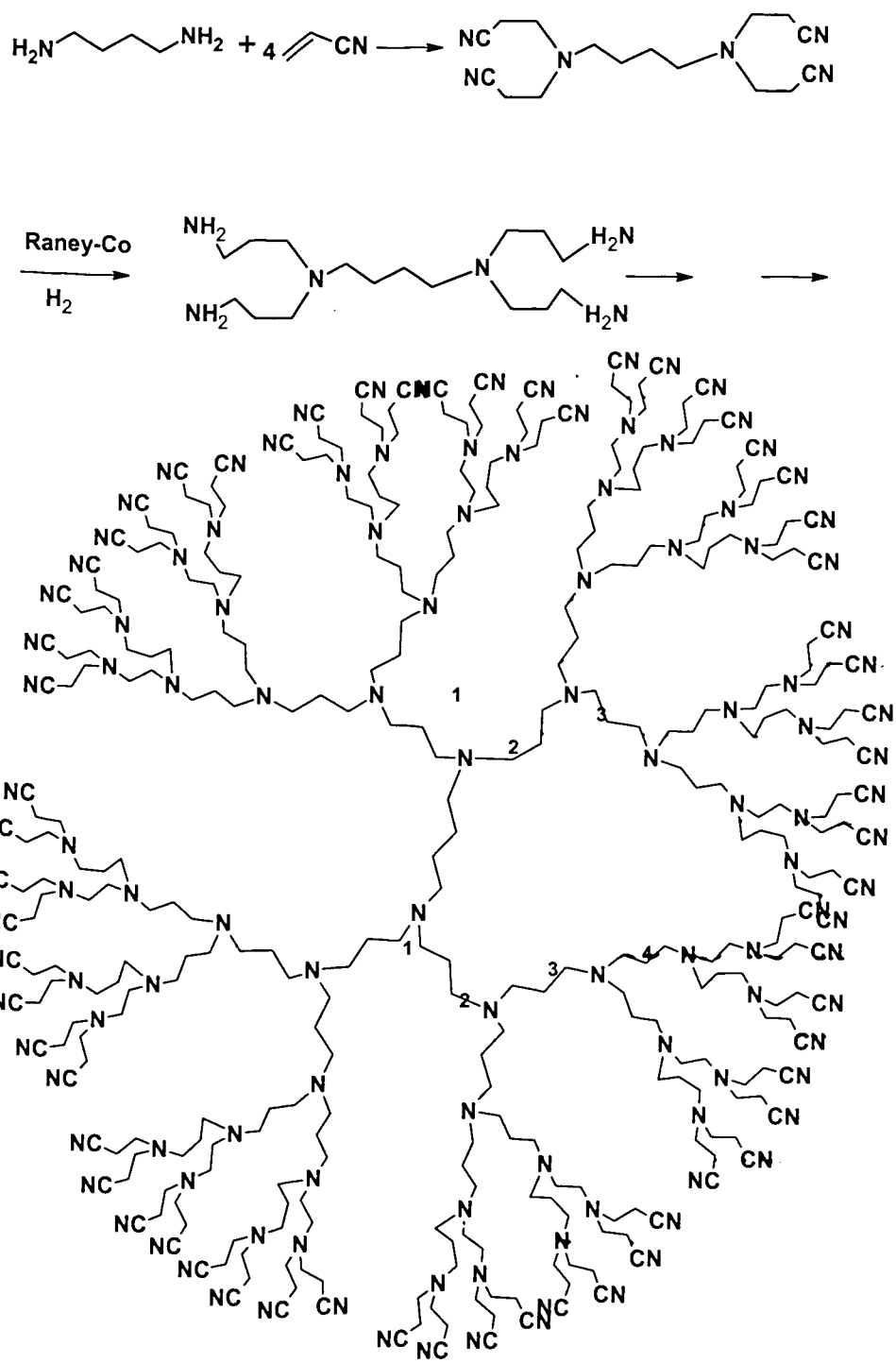
1→ 2N-Branched dendrimers and their connectivity:

In their quest for large molecular cavities capable of binding molecules or ionic guests, Vögtle et al [23] synthesised nanocyclic polyaza compounds by a repetitive stepwise process. The use of two-directional cores (Scheme I.4), e.g., ethylenediamine, 2,6-bis(amino-methyl)pyridine, or 1,3-bis(amino-methyl) benzene, in conjunction with acrylonitrile afforded tetranitriles, which were reduced with borohydride under Co(II)-catalysis to produce hexamines. These terminal groups were subsequently reacted with excess acrylonitrile to generate the octanitriles; although the process was terminated at this stage, a foundation for dendrimer construction was established. Worner and Mulhaupt [24] improved the Vögtle procedure by modifications that do not require the use of excess reagents and complicated purifications. They first improved the nitrile reduction step by the use of Raney nickel [25] at ambient temperature (8 bar H_2) in the presence of trace of NaOH in EtOH. Then they found that when the cyanoethylation was conducted in MeOH, it occurred without the generation of monosubstituted side products. By application of these synthetic modifications [26] the original route could be extended to the 5th generation nitrile, with excellent overall yields for most steps. Meijer and de Brabander-van den Berg [26,27] also reported (in back-to-back manuscripts with Worner and Mulhaupt [28])procedures for the large scale preparation of poly (propylene imine) dendrimers (herein denoted as PPIs also known as POPAM) using a sequence of reactions analogous to those originally

employed.[28] Thus, repetitive addition of a primary amine to two equivalents of acrylonitrile, followed by catalytic (Raney (cobalt)) hydrogenation, afforded the desired polyamine-terminated macromolecules. Scheme 1.4 and 1.5 show dendrimers derived by divergent procedures using 1→2 branching.

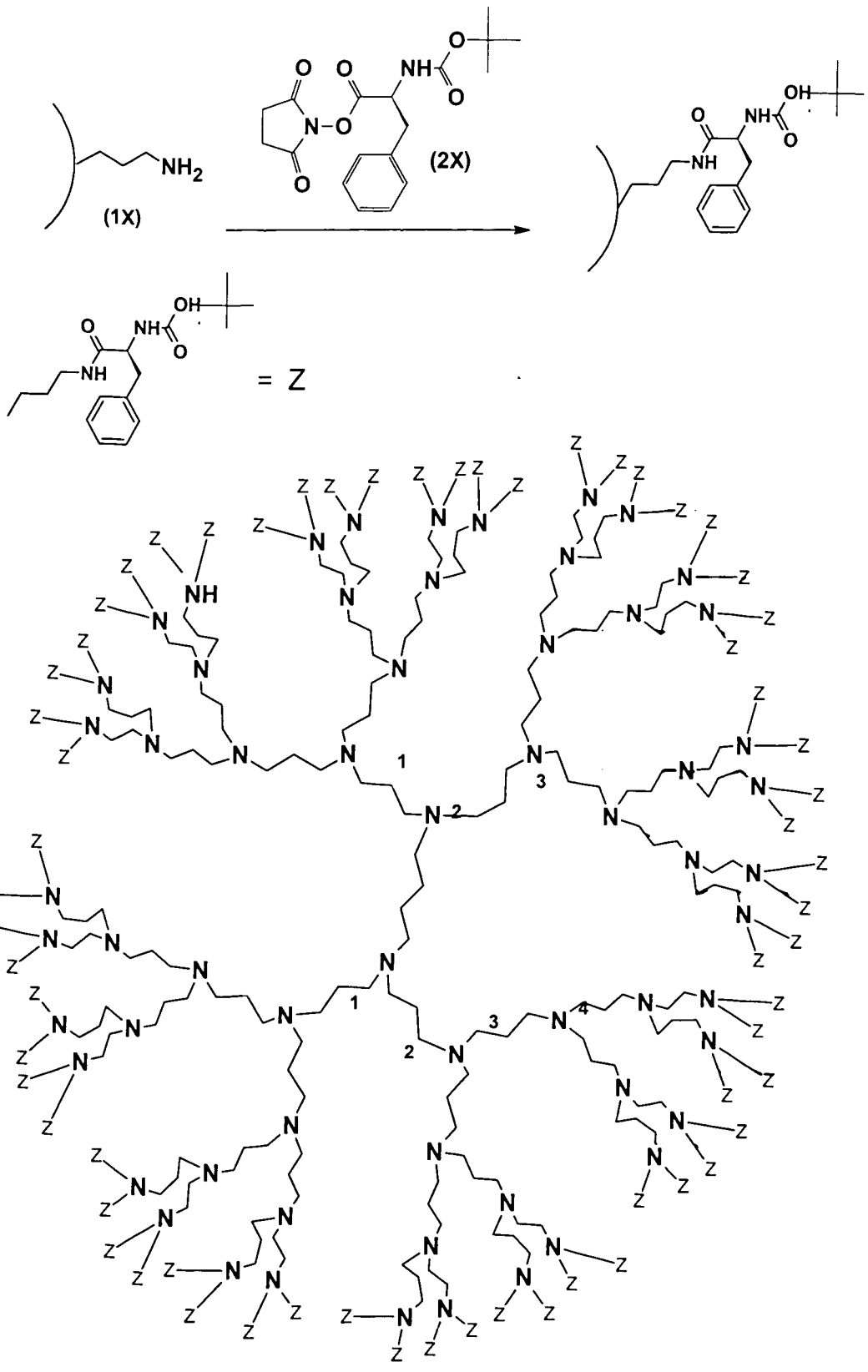


Scheme 1.4: Vögtle repetitive N-alkylation and reduction route for the construction of polyamine and polynitriles [28]



Scheme 1.5: Procedure for the large-scale synthesis of poly(propylene imine)dendrimers(PPI)[28].

Meijer et al.[29] utilised the 5th generation polyamine, (denoted as letter X) generated by reduction of the corresponding nitrile, for the construction of host dendritic ' boxes' that allowed the steric entrapment of guest molecules. The concept of "trapping topologically by shell molecules" was considered theoretically by Maciejewski [30] and was reported by Denkewalter et al [31], for their lysine based dendrimers, which were classified as "non-draining" spheres. Meijer's dendritic boxes with molecular weights up to 24,000 amu, were prepared [32] by surface modification of X with an activated chiral ester, for example the N-hydroxysuccinimide ester of a t-Boc-protected amino acid,(phenylanaline shown; 2X) as shown in Scheme I.6. The concept of a dendritic box was expanded [33, 34] by the introduction of other surface amino acid groups, e.g. L-alanine, L-t-Bu-serine, L-Tyr-cystein, and L-t-Bu-aspartic ester. Additional studies concerning the chirality of encapsulated guests [35], the encapsulation of Rose Bengal [36,37] and of triplet radical pairs[38] have been carried out; an excellent review of the host-guest ramifications of dendrimers has also appeared.[39] Goddard et al[40] have described a molecular dynamics analysis of the encapsulation of Rose Bengal within the dendritic box.[41,42,43] Cavallo and Fraternali [44] also performed a molecular dynamics study detailing such properties as H-bonding, solvent- accessible surface areas, and excluded volumes for the first five generations of these N-t-Boc-L-phenylalanine- terminated frameworks.


(1X)
(2X)
= Z

Scheme 1.6: Poly(propylene imine)s terminally functionalized with t-Boc-protected phenylaniline units

Verlhac et al [45] prepared branched per-fluorinated alkanes by reaction of the tosylated, linear monomer with either polyamine or similar molecules, respectively, under basic conditions (K_2CO_3, CHCN). These small dendritic molecules were subsequently employed as perfluorinated Cu(I) ligands for the catalytic, intermolecular cyclisation of alkenyl tri-chloromethyl esters under "fluoro biphasic" conditions [46] which allowed for ease of work-up and catalyst recovery. See **fig 1.3**

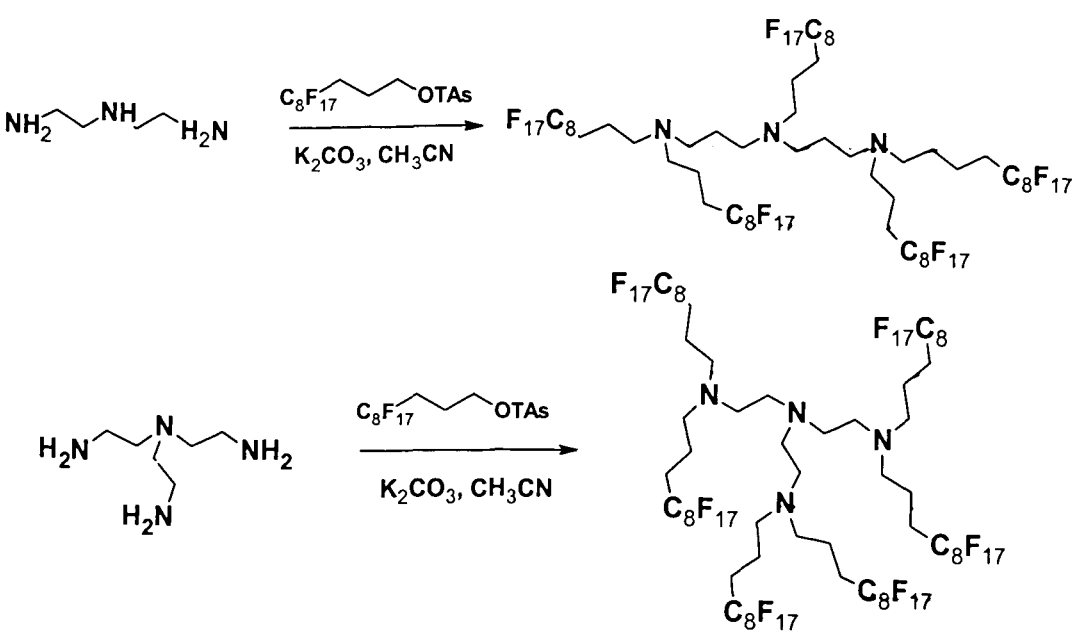


Figure 1.3: 1 → 2 Branched systems - Synthetic route to per fluorinated N-dendrimers [46]

Vögtle, Balzani, De Cola et al [47, 48] customised the surface of PPIs, through to the 5th generation, with dansyl groups. The products were studied with

regard to their protonation, absorption, and photo-physical properties, as well as intra-dendrimer quenching and sensitising processes. The 4th generation of these coated PPIs was shown [49] to exhibit a strong fluorescence, which was quenched when a Co^{2+} ion was N-coordinated within its interior; a Co^{2+} concentration of 4.6 X 10^{-7} M resulted in a 5% decrease in fluorescence intensity, revealing the potential to fine-tune this effect.

Pan and Ford [50,51] reported the creation of an amphiphilic architecture (figure 1.4) possessing hydrophobic and hydrophilic terminal chains. These materials, prepared as water-soluble catalysts, were accessed in five steps from the 32-PPI. Terminal modification with octanoyl chloride, reduction ($LiAlH_4$) of the resulting amide to give the terminal secondary amine, subsequent reaction of the latter with the triethylene glycol acid chloride, and further reduction, gave the polyamine precursor of the methyl iodide treated poly-ammonium catalyst [52]. Tnese cationic chloride dendrimers act as host for guests, such as Reichardt's dye and pyrene, in an aqueous environment and enhance (500X over water alone) the rate of decarboxylation of 6-nitrobenzisoxazole-3-carboxylic acid. Water soluble, oligo (ethyleneoxy)-terminated PPIs, based on a 3,4,5–(PEG) benzoate moiety, have been shown to act as unimolecular micelles [53]. Coating of the PPIs with porphyrin groups has been accomplished [54,55] and the products were employed in a time resolved fluorescence study to examine the dynamics of electronic energy transfer. **Fig I.4** below shows dendrimers derived by divergent procedures using 1→ branching motifs

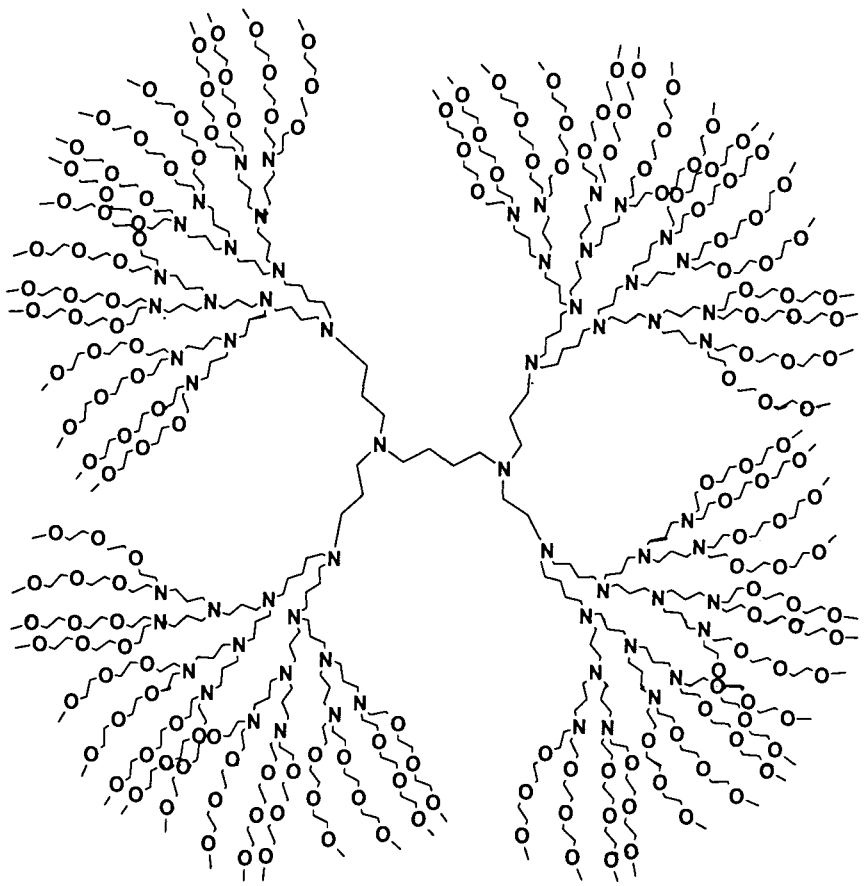
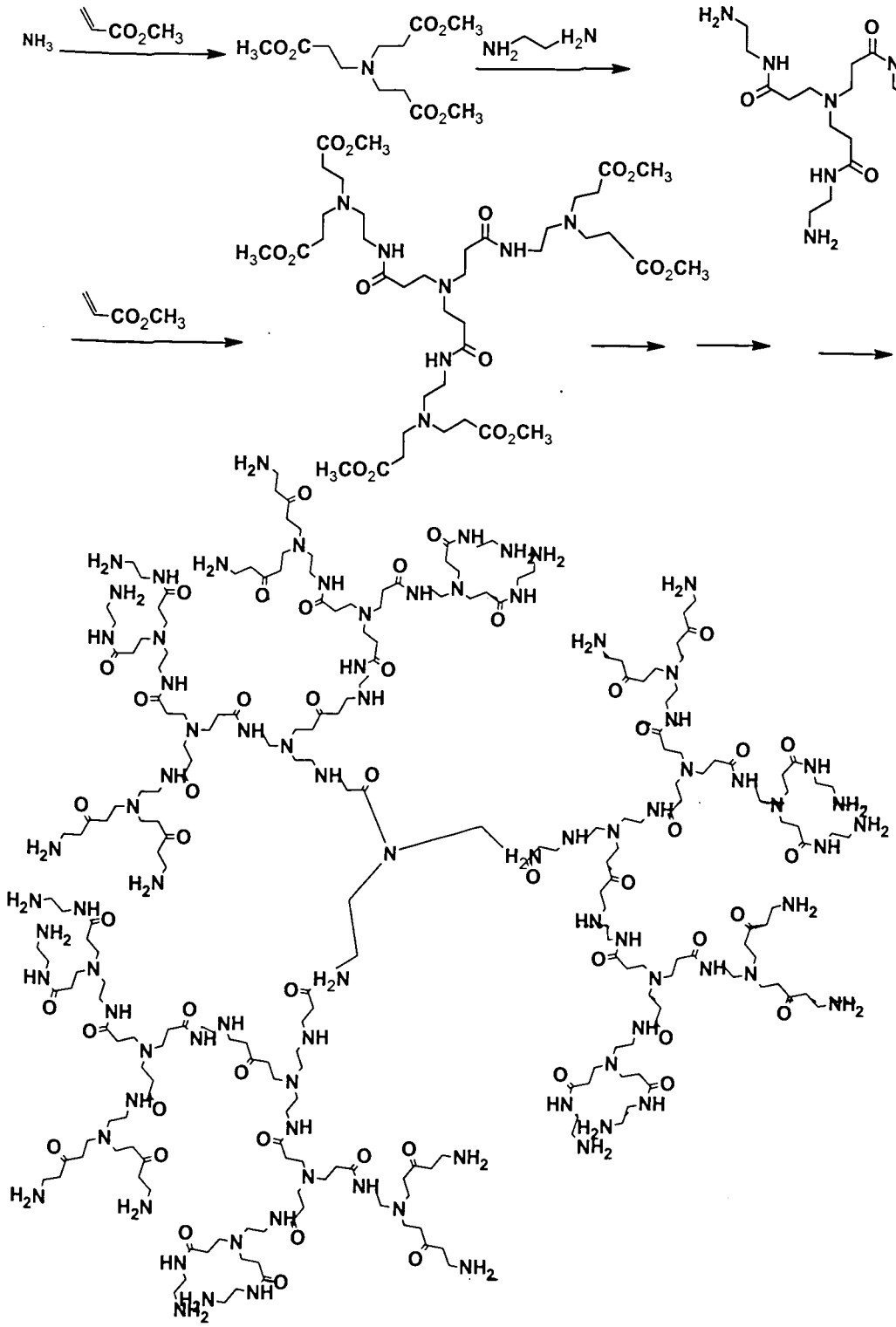


Figure 1.4: Pan and Ford's amphiphilic dendrimers for use as catalysts in aqueous media [50]

In 1985 and 1986, Tomalia et al. described [56] the preparation of polyamidoamine (denoted herein simply as PAMAMs), which were generated from a three-directional core (e.g., ammonia) and possessed a three-directional N-branching centre as well as amide connectivity (Scheme I.7). Each generation was synthesized by exhaustive Michael-type addition of methyl acrylate to amines (e.g., for an ammonia core) to generate a β-amino acid ester, followed by amidation with excess ethylenediamine to produce a new branched polyamine. The generation procedure was repeated to create the higher generations.

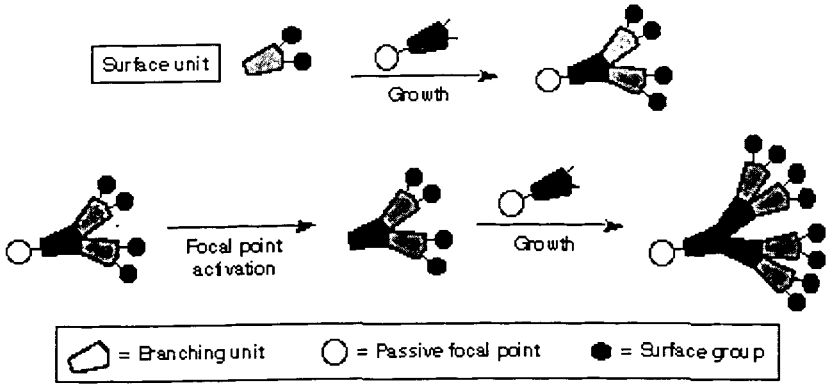


Scheme 1.7: Procedure for the preparation of PAMAM polymer [56]

There are so many different variations of connectivity in divergent synthesis such as 1→2N-branched, aryl connectivity - 1→2N-branched, Si-connectivity, 1→2N-branched, N-&amide–connectivity - 1→2aryl-branched, ester connectivity- 1→2C-branched, Ether and Sulfonates Connectivity.

1.4.2. Convergent procedure

The convergent method was developed in response to some of the weaknesses of divergent synthesis [57]. In convergent synthesis, dendrimer growth begins at the chain end and proceeds inwards through successive addition of the growing dendritic molecules to a single monomer unit. When the growing wedges are large enough, several can be attached to a suitable core to give a complete dendrimer. The advantage of convergent synthesis over divergent synthesis is that for convergent growth only two simultaneous reactions are required for any generation-adding step. Another advantage of convergent synthesis is that purification becomes less problematic than in the divergent case. Convergent synthesis makes it easier to yield the desired dendrimers and to have complete control over all molecular design parameters. There are some disadvantages of this synthesis; for example the number of steps to build up a large structure is not reduced compared with the divergent approach, yet a great deal more starting material is required. It also suffers from low yields in the synthesis of large structures.



Scheme 1.8: Diagram of convergent synthesis, Benly, A., Accessed on 2008:www.bitspace.com/bendy/dendrimer,1997

1.4.2.2. Early procedure

In 1990, the first and now the best-known convergent dendritic synthesis based on a combination of 3,5-dihydroxybenzyl alcohol branching ethereal bond connections, was reported by Hawker and Fréchet [58]. Two simple synthetic transformations were used [59]

i) Selective alkylations of a phenolic hydroxyl group.

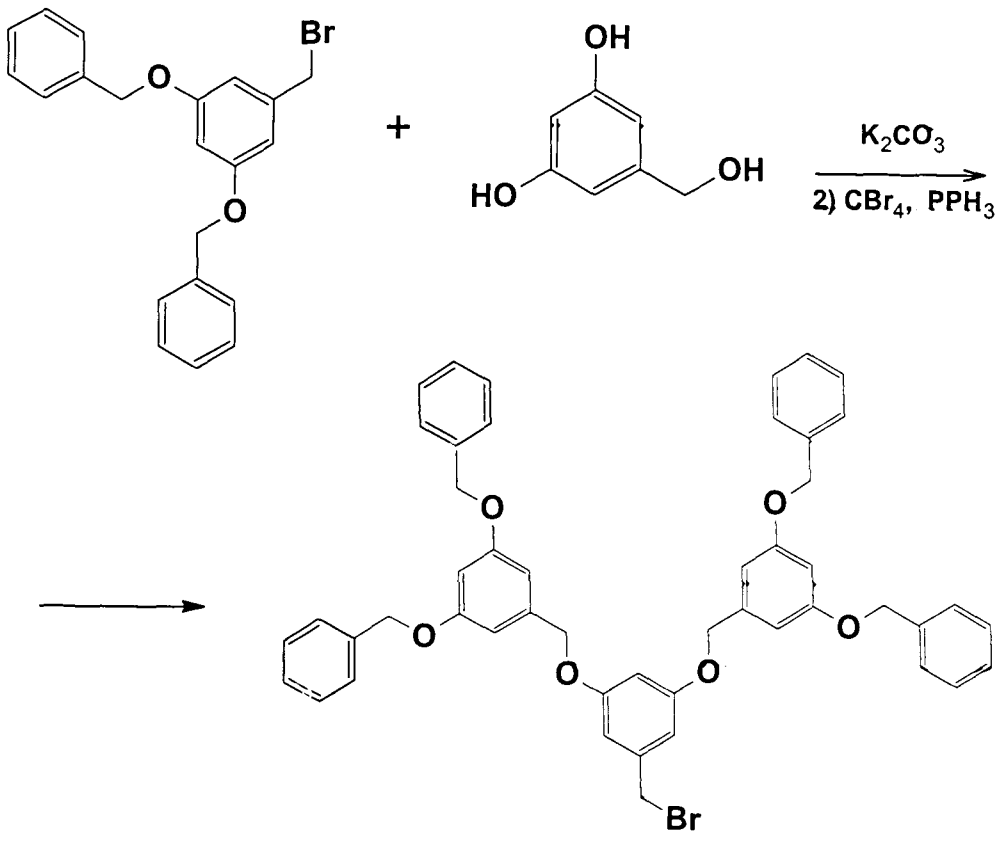
ii) Conversion of benzyl alcohol to benzyl bromide

Thus, addition of two equivalents, of the previously reported benzylic bromide to the initial monomer yielded the hepta-phenyl ethereal wedge, which was converted (CBr_4, PPh_3) to the corresponding bromide to generate a reactive focal moiety.[60] This sequence was successfully repeated to prepared dendritic wedges through to the 6th generation.

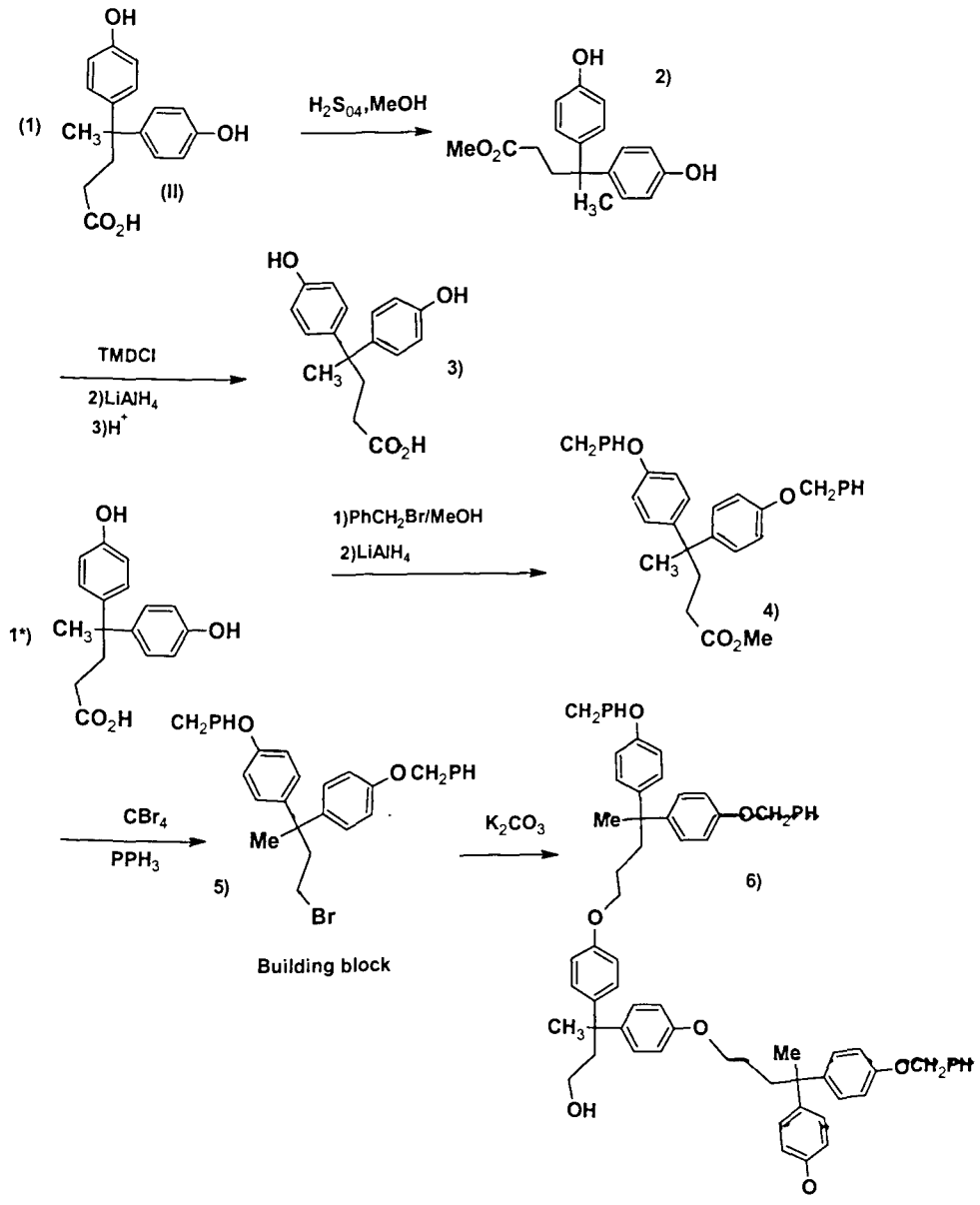
Scheme I.9 shows original convergent methodology of Fréchet et al.

Tyler and Hanson [61] developed a synthesis of poly-aryl ether whereby monomer connectivity was based on the total phenolate displacement of benzylic bromide in a protocol that is essentially a reversed Fréchet method. There are many convergent procedures to make a dendrimer, for example

1→2C branched, ether connectivity, described by Woolley, Hawker, and Fréchet [62,65], involving a double-stage convergent growth approach that led to dendrimers comprised of different monomers at different generations (Scheme 1.10). The branches or wedges of the potential dendrimers, were convergently prepared, using 4,4-bis(4`-hydroxyphenyl) pentanol(III), which was obtained from the corresponding pentanoic acid (II) by four simple steps.



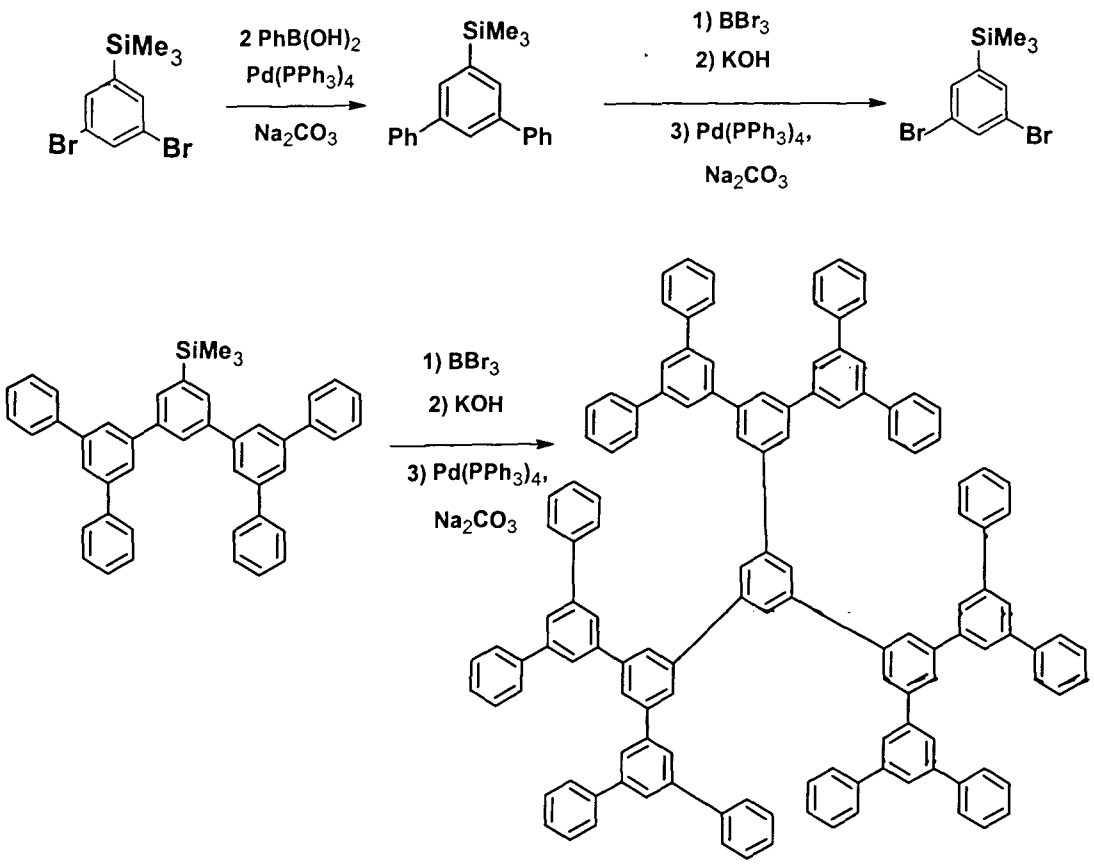
Scheme 1.9: Convergent synthesis 1→2 Aryl-Branched, Ether Connectivity Original convergent methodology of Fréchet et al [61]



Scheme 1.10: Double- stage convergent growth [62]

At about the same time as Hawker and Fréchet's initial publication, [63-64] Miller and Neenan reported [65] the synthesis of monodisperse molecular spheres based on a 1,3,5-trisubstituted benzene. The completely aromatic hydrocarbon was prepared using 1,3-dibromo-5-(trimethylsilyl)benzene as the key monomer. Two equivalents of phenylboronic acid were catalytically [$Pd(PPh_3)_4$] coupled to the dibromobenzene yielding terphenylsilane, the silyl

group of which was subsequently transformed to a boronic acid moiety and further coupled to monomer. The resultant heptaphenyl trimethylsilyl wedge was boron added and attached to the three-directional core to generate their hydrocarbon, termed a 12-Cascade : benzene[3-1,5]:(1,3,5-phenylene)^2 :benzene.



Scheme 1.11: Employment of the Suzuki coupling leads to the construction of all-aromatic dendrimers[67]

There are many variations of synthesis in convergent mode such as 1→2 C-branched, ester connectivity – 1→2C-branched, ether & imides connectivity – 1→2 aryl-branched, ether connectivity.

Selective protection of terminal monomers has allowed access to water-soluble PEG conjugates that have potential as dendrimer–based drug carriers

[68] Attached model drugs include cholesterol, phenylalanine, and tryptophan moieties. Cell-targetable cationic amphiphilics capable of gene delivery and possessing glycosyl residues have also been reported [69,70]

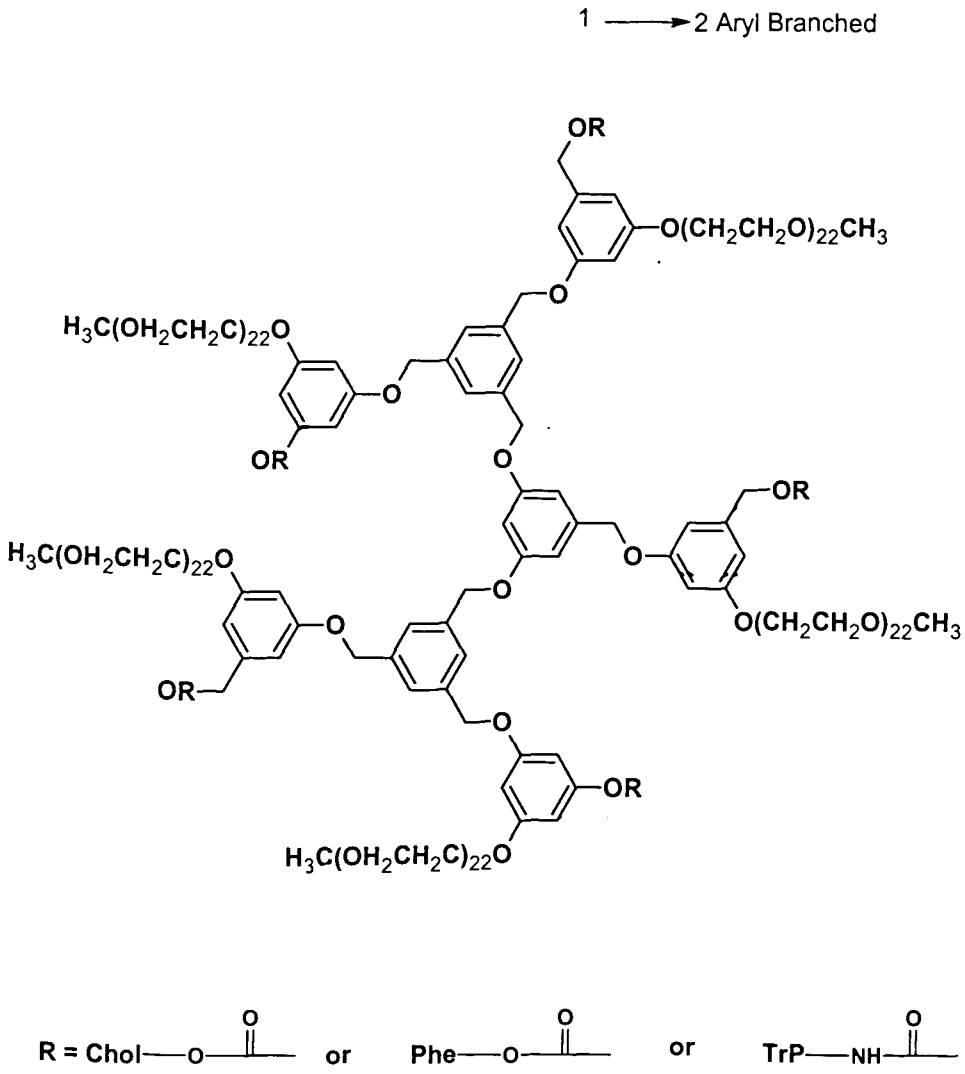
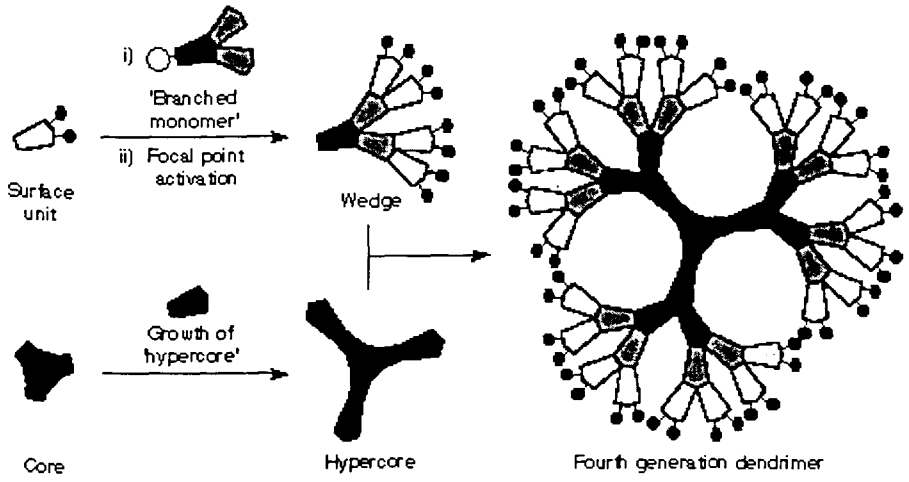


Figure 1.5: Star-Like conjugates for potential drug carriers [68]

1.4.3. Hyper-cores and Branched Monomers

This kind of synthesis is a direct, one–step poly-condensation of ABX monomers where X ≥ 2. In the early 1990's, after Fréchet and his group established convergent synthesis, they focused on ways to accelerate the dendrimer synthesis. The outcome of this research was a demonstration of

`hyper-cores` and `branched monomers`. This method involve the pre-assembly of oligomeric species which can be linked together to give dendrimers in fewer steps or higher yields.[71,72]

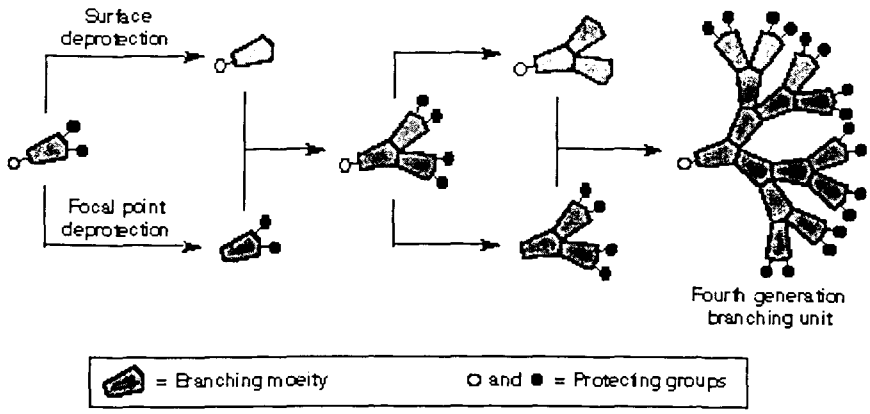


Scheme 1.12: Diagram for Hyper-Cores and Branched Monomers, F. Benly ,A. , www.bitspace.com/bendy/dendrimer,1997(Accessed on 20.03.08)

1.4.4. Double Exponential and Mixed Growth

The first use of a versatile building block in this way was performed by Shinkai although the full potential of this idea was not realized at that time[73]. Double exponential growth, similar to a rapid growth technique for linear polymers, involves an AB2 monomer with orthogonal protecting groups for the A and B functionalities. This approach allows the preparation of monomers for both convergent and divergent growth from a single starting material. These two products are reacted together to give an orthogonally protected trimer, which may be used to repeat the growth process again.

Double exponential – type methodology has also been employed by Fréchet, Sharpless, Roy, and Schluter (as shown in I.12).



Scheme 1.13: Diagram for Double Exponential and Mixed Growth, Benly, A.(ref), www.bitspace.com/bendy/dendrimer,1997 . (Accessed on 16.02.08)

1.5. PHYSICAL AND CHEMICAL PROPERTIES OF DENDRIMERS

Most of the physical and chemical properties of dendrimer such as solubility and viscosity are affected by the terminal groups in the dendrimer. Because of their molecular architecture, dendrimers show some significantly improved physical and chemical properties when compared to traditional linear polymers. In solution, a linear chain polymer exists as a flexible coil; in contrast dendrimers form a tightly-packed ball. This has a great impact on their rheological properties. Dendrimer solutions have significantly lower viscosity than linear polymers [74]. When the molecular mass of dendrimer increases their intrinsic viscosity goes through a maximum at the fourth generation and then begins to decline; [75] such behaviour is unlike that of linear polymers. For classical polymers the intrinsic viscosity increases continuously with molecular mass; this effect is believed to be a consequence of the globular shapes of high generation dendrimers leaving them unable to tangle with one another. Mourey et al [76,77] evaluated the viscosities of poly

(benzyl ether) dendrimers [78,79] by means of size-exclusion chromatography coupled with differential viscometry. For the 0-6th generations, their intrinsic viscosity was found to pass through a maximum at the 3rd generation, whereas the refractive index was found to pass through a minimum at the 2nd generation. The results support Lescanec and Multhkumar's[79] theoretical model of dendrimers, which indicates higher internal density relative to surface density, but are at variance with the de Gennes and Hervet model[80] which suggest greater surface congestion.

The melt viscosity behaviour for the family of dendrimers generated from 3,5-dihydroxybenzyl alcohol has been investigated [80,81,and 82]. The presence of many chain ends is responsible for their high solubility and for high reactivity. Dendrimer solubility is strongly influenced by the nature of the surface groups. Dendrimers terminated in hydrophilic groups are soluble in polar solvents (in our case carboxylate end groups) and those terminated with hydrophobic end groups are soluble in non polar solvents. In general, evaluation of the melt viscosity revealed a profile with no critical molecular weight for molecules as large as 85,000 amu. At high molecular weights, branching and surface congestion in this family prevent appreciable intermolecular entanglement, affording " ball-bearing – like" macromolecules. The zero shear melt viscosity has been measured for a variety of these poly(benzyl ether) dendrimers [83]. For higher generation materials, a direct correlation with molecular mass was observed, while lower generations exhibited a "stronger" dependence. The viscosity was also found to scale with molecular mass for mono-and tri-dendrons rather than with generation number

1.5.1. Monodispersity

Step-wise synthetic processes enable at least theoretically the production of dendrimers with defined surface functionality. Monodispersity is one of the most important differences between dendrimers and polymers. The classical polymerisation process which results in linear polymers is usually random in nature and produces molecules of different sizes, whereas size and molecular mass of dendrimers can be specifically controlled during synthesis

1.5.2. Poly-valency

The structure of a dendrimer permits functionalisation fairly easily, allowing multiple functionalities to be added [84,85]. Poly-valency of dendrimers produces multiple interactions with biological receptor sites, for example in the design of antiviral therapeutic agents [86]. Different ligands can be coupled to dendrimers for use as transfection reagents, e.g., ligands recognising only the surface of a certain cell types combined with ligands that facilitate the escape from the endosome.

1.5.3. Biocompatibility and Low toxicity

Some dendrimer systems display low toxicity levels with dendrimers carrying anionic groups being less toxic than those carrying cationic groups. Dendrimers commonly also show negligible or very low immunogenic response when injected or used topically. These properties make them highly suitable for drug delivery and bio-labelling. High biocompatibility is crucial both for preventing toxic reactions and for seeking biodegradability options [87].

1.5.4. Loading capacity

The internal cavities of dendritic structures can be used to carry and/ or store a wide range of metals and organic or inorganic molecules by encapsulation and absorption. The appropriate type (and degree) of functionalisation will result in the desired loading capacity [88]. This property makes dendrimers very suitable as drug delivery vehicles and also appropriate for building electro-optic magnetic devices. The possibility of transporting materials makes dendrimers attractive potential carriers of bio-substrates or as additives for special materials.

1.5.5. Defined architecture, size and shape control

Dendrimers branch out in a highly predictable fashion to form amplified three-dimensional structures with highly ordered architectures. This property is relevant for applications such as protein modelling or catalysis. Size control is also important in therapeutic applications, as different molecular sizes exhibit different pharmacokinetics.

1.6. APPLICATIONS OF DENDRIMERS

There are now more than fifty families of dendrimers, each with unique properties, as the surface and core can be tailored to different type of applications, many potential application dendrimers are based on their unique properties of molecular uniformity, multi-functional surface and presence of internal cavities[89]. These specific properties make dendrimers suitable for a variety of uses including biomedical, electronic and industrial applications.

Dendrimers can be used in many areas of bioscience including drug delivery, immunology, the development of vaccines, antimicrobials and ant i- virals[90]. Recent progress has been made in the application of biocompatible and biodegradable dendrimers to cancer treatment, including their use for delivery of potent anticancer drugs such as cis-platin and doxorubicin. Some dendrimer backbones, especially those designed to be water soluble and biocompatible, such as poly-amidoamine (PAMAM)[91] dendrimers prepared using divergent synthesis, are widely used in biology (although the surface amine group of this dendrimer must be modified to neutral or anionic moieties to avoid toxicity in the liver [92].

fig 1.6 shows the structure of a commercially available PAMAM dendrimer.

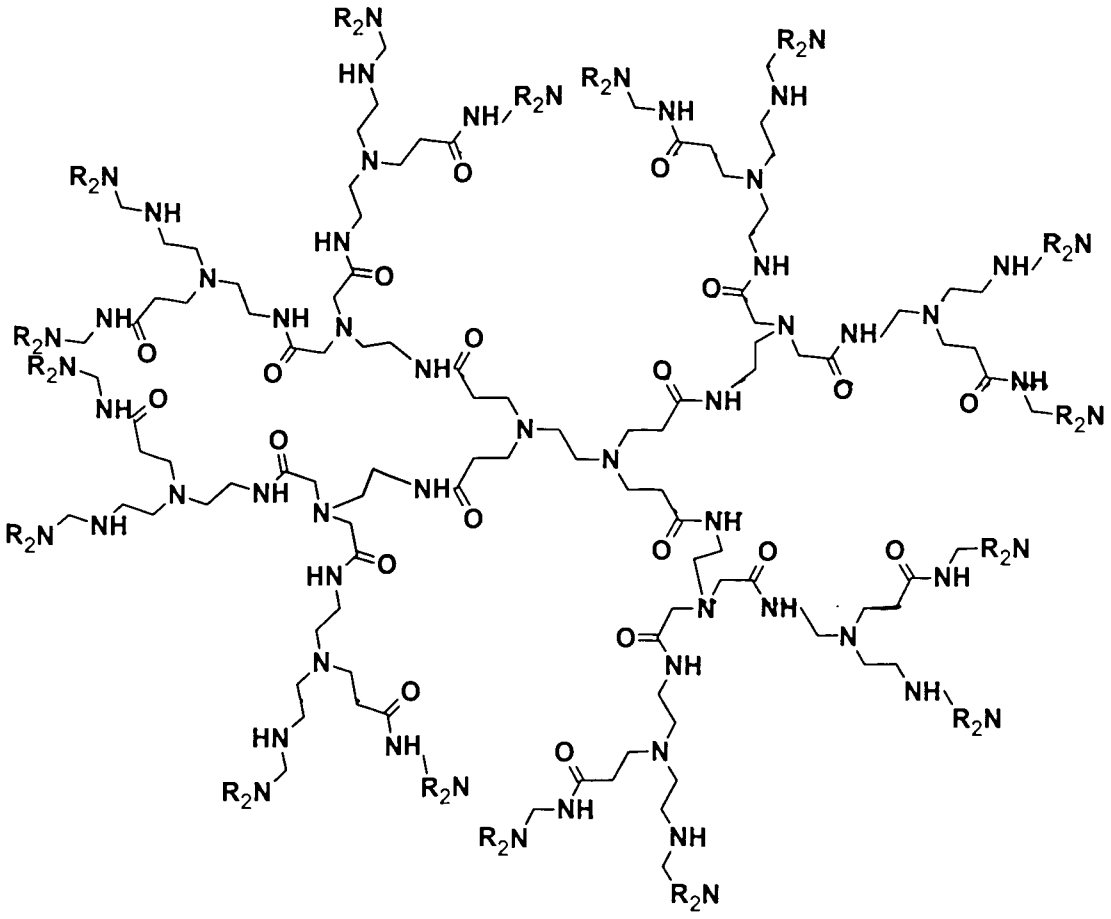


Figure 1.6: Commercially available PAMAM dendrimer [91]

Some of the industrial uses of dendrimers can be mentioned as, i) in vitro diagnostics, ii) light harvesting antennae, iii) contrast agents, IV) catalysts and as a drug carriers. This project is focused on using dendritic materials as drug carriers; therefore the other uses of dendritic material as mentioned above will only be explained briefly.

Dendritic materials can be used for *in vitro* diagnostic methods. Proteins present in a blood sample bind to immunoglobulin which is fixed by dendrimers to a sheet of glass. The result can be used to detect heart muscle damage. This method significantly reduces the waiting time for the blood test results to about 8 minutes [93]. It is possible to create dendrimers which can act as extremely efficient light harvesting antenna. Absorbing dyes are placed at the periphery of the dendrimer which transfer the energy of light to other chromospheres located in the core. The absorption of the whole molecule is particularly broad because the peripheral chromospheres cover a wide wavelength range. The energy transfer process converts this broad absorption into the narrow emission of the central dye. The light harvesting ability increases with generation due to the increase in the number of peripheral chromophores [94] **fig 1.7**

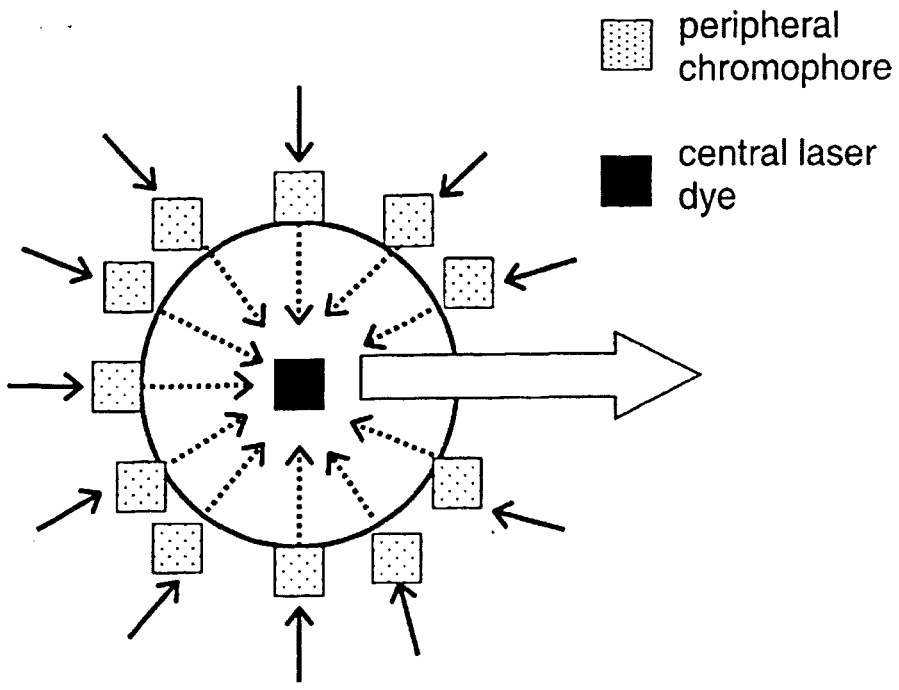


Figure 1.7: Light harvesting properties of dendrimers. Antenna function : [95,96,97,98]

Dendrimers have been tested in preclinical studies as contrast agents for magnetic resonance imaging (MRI)[99,100]. MRI is a diagnostic method producing anatomical images of organs and blood vessels by placing the patient in a generated, defined, inhomogeneous magnetic field resulting in the nuclear resonance signal of water which is assigned to its place of origin and converted into pictures. Addition of contrast agents (paramagnetic metal cations) improve the sensitivity and specificity of the method[101]. The gadolinium salt of di-ethylenetriaminepentaaceticacid (DTPA) is used clinically but it diffuses to extravenous areas due to its low molecular mass. Dendrimers, due to their properties are highly suited for use as image contrast media [102,103]. Several groups have prepared dendrimers containing gadolinium ion chelated on the surface [103-104]. The use of dendrimers in medical imaging rest mainly on the fact that they have a multiplicity of reactive chain ends. This allows a large number of contrast agents to be introduced on

to a single molecule in a controllable manner, thereby enhancing the imaging sensitivity.

Dendrimers also can be used as catalysts, using dendritic material as a catalyst is one of the most promising applications in dendrimer research. Dendrimer have nanoscopic cavities that act as micro-environments for molecular reactions [105] the cavities provide nano-scale reactor sites for catalysis. There are two possible catalytic sites being investigated, one at the core and the other at the surface. Many attempts have been made in using dendrimers to enhance reaction rate and reaction activity. There is a macro-polarity around the core, thereby influencing its molecular recognition and catalytic properties [106]. Dendrimers are appealing molecules with well-defined structures in the nanometre dimension. The figure below shows two dendritic catalyst in which the catalyst is located at the core (figure I.8), and at the periphery (figure I.9) of the dendrimer.

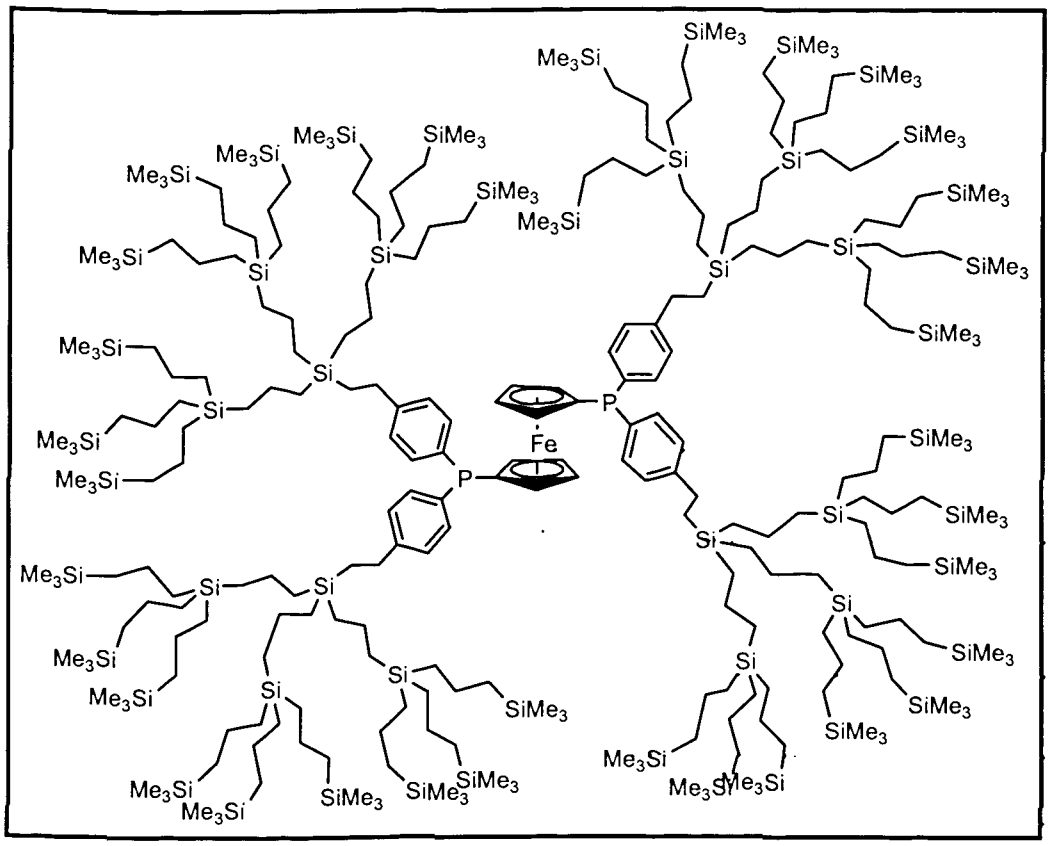


Figure 1.8: A dendritic catalyst in which the catalyst is located at the core[208]

Perhaps the most important properties of dendrimers are their ability to be used as drug carriers. The concepts of molecular shape and well- defined structure, size mono-dispersity and controllable surface functionalities of dendrimers make them excellent candidates for evaluation as drug carriers. As early as 1982, Maciejewski [107] proposed the possibility of constructing a dendritic "core-shell molecule" to topologically entrap small molecules.

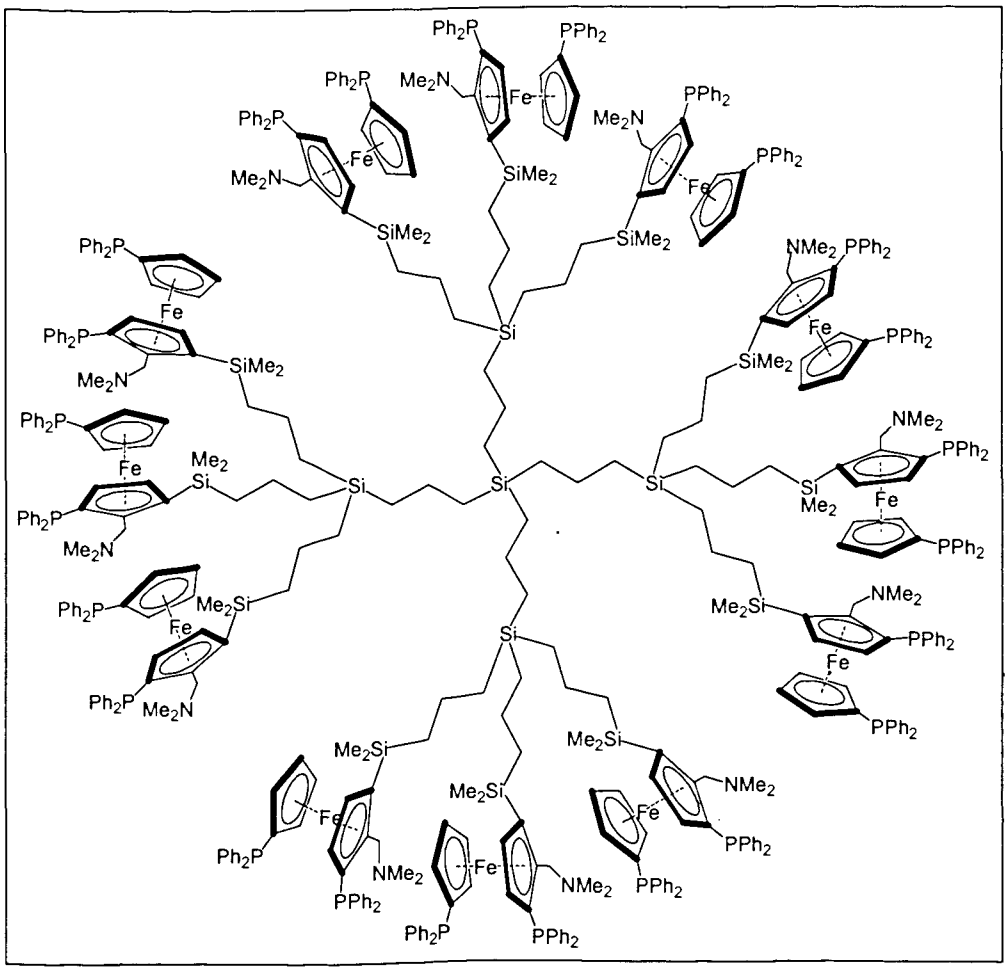


Figure 1.9: A dendritic catalyst in which the catalyst is located at periphery[209]

Generally drugs can be carried by a dendrimer in two main ways.

1.) Drug molecules can be covalently attached onto surface or other functionalities to afford dendrimer-drug conjugates.(exo-receptors) [108]

e.g. Gene therapy is a branch of medicine that aims to correct genetic defects by transferring active genes into damaged cells[109].

2) The internal cavity of an appropriately designed dendritic structure could be used for the entrapment of drugs with the possibility of subsequent controlled release (endo-receptors) [110]

e.g. Fréchet and coworkers have developed novel dendritic unimolecular micelles as drug delivery vehicles[111,112] based on a core of hydrophobic

Fréchet type dendrimer able to interact with hydrophobic drugs, in conjunction with a shell of hydrophilic PEG chain [113].

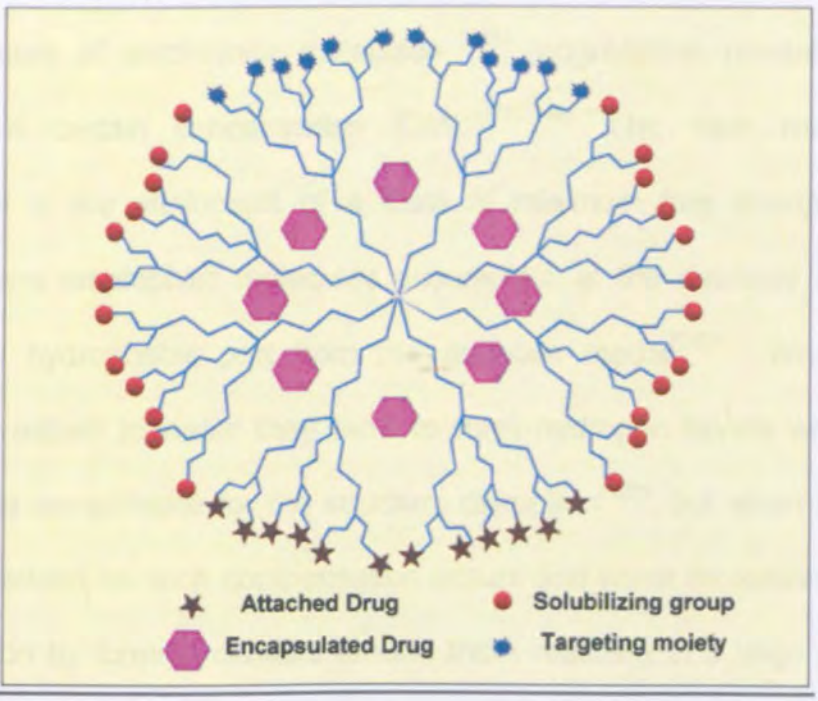


Figure 1.10: possible covalent attachment for drugs, encapsulating of drugs, solubilising moieties [114]

1.7. AMPHIPHILIC COMPOUNDS

The existence of hydrophilic (water-liking) and hydrophobic (water-hating) moieties in one molecule is known as amphiphilic and compounds having such a characters are referred as amphiphilic molecules. Water soluble dendrimers have such properties (hydrophilic terminal groups and hydrophobic core). This dual structure of amphiphilic molecules gives the unique characteristic of having surface activity at different interfaces. Micellisation is one of the most important consequences of such an activity [115]

1.8. MICELLISATION PROCESS

Micellisation is the process of forming micelles, which are aggregates of a unique number of amphiphilic molecules [116] (aggregation number) which occurs at a certain concentration (CMC)[117,118]. The main reason for micellisation is the attainment of a state of minimum free energy at low concentrations amphiphilic molecules accumulate at the interface so as to remove the hydrophobic part from the aqueous media[119]. When polar groups are added to water they tend to form hydrogen bonds with water molecules to compensate for the structure disruption[120], but when nonpolar groups are added no such compensation occurs and water molecules tend to resist solution by forming clusters around them resulting in a large negative entropy change, to overcome this change and maintain a state of minimum free energy these non-polar moieties tend to withdraw from water either by self -associating into micelles or by orienting themselves at the interface[121] fig 1.11.

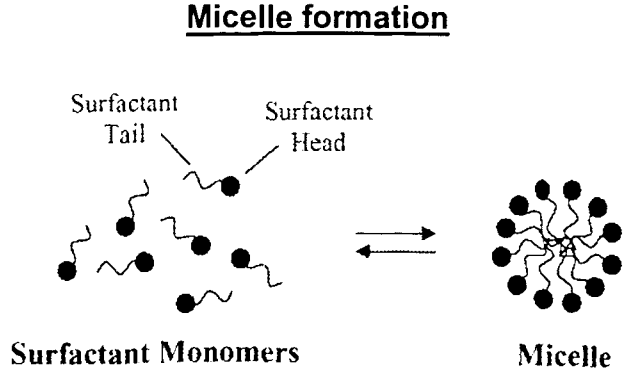


Figure 1.11: Alton 2002

When the surface of an apolar dendrimer contains charged functional groups, its gross structure resembles that of a micelle [122] with a completely hydrophobic alkane interior. Fréchet and coworkers have developed novel dendritic unimolecular micelles as drug delivery vehicles [123] based on a hydrophobic core. Fréchet type dendrimers are able to interact with hydrophobic drugs, in conjunction with a shell of hydrophilic PEG chain [124]. Meijer and co-workers [125] reported the synthesis of a series of inverted unimolecular dendritic micelles by reacting the amino end groups of a poly(propylene imine) dendrimer with aliphatic acid chlorides. The resultant dendritic macromolecules have a hydrophobic shell and a hydrophilic interior. The binding of the hydrophilic molecules such as rose Bengal dye in the dendritic interior occurs in hexane solution, whereas the dye is released by adding toluene but not water. In contrast to normal micelles, these inverted unimolecular micelles make it possible to solubilize polar molecules in non-polar solvents through the shell separation created by the dendritic framework [126]

1.8.2. The structure and types of micelle

There are two types of micelles, ionic and non-ionic micelles. Ionic micelles have the hydrophobic part of a surfactant as their core and surrounding that core is a compact layer containing the hydrophilic groups and their bound counter ions (Stern layer) [127]. The outer surface of this layer is referred to as the shear surface of the micelle, (e.g. water soluble dendrimer). The Stern layer and the core of the micelle together are referred to as the kinetic micelle [128]. The counter ions required to neutralize the kinetic micelle occupy another

diffuse layer surrounding the Stern layer called the Gouy-Chapman electrical double layer [129]. Non-ionic micelles usually have a larger size than their ionic counterparts and as a result of their large size they tend to be asymmetrical.[130] Non–ionic micelles have a hydrophobic core, and surrounding this core are shells of oxyethylene chains; this layer is usually referred to as the palisade layer. Figure I.13 shows a schematic representation of (a) ionic and (b) non ionic micelles [131]. Micelles could also be divided into two different groups according to their molecular weights: low–molecular weight surfactant micelles and polymeric micelles Polymeric micelles[132,133,134] have been found to be advantageous over low molecular weight micelles in term of drug loading, adverse effects, stability, and targeting of tumours. There are different shapes of micelles and the structure of the amphiphilic molecule and the environment control the shape of the micelles.[135,135] **Fig 1.12**

PROPERTIES of the IDEAL MICELLE SYSTEM

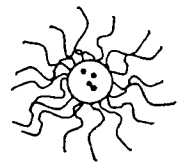
The Micelle Core	*The Micelle as a Whole*	*The Micelle Corona*
 Cargo Space		 Stabilizing Interface
•**high loading** capacity •**controlled release** profile high degree of **compatibility** between the drug and core - forming block	• suitable **size** between 10 - 100nm • **high** degree of **stability** low CMC • **slow** rate of **disassembly**	• **efficient steric barrier** high surface density of poly(ethylene oxide) corona shell of sufficient thickness • effective **coverage of core**

Figure 1.12: Properties of ideal micelle systems

Perhaps one of the most important properties of micelle delivery systems is their stability, where some micelles tend to break down into their monomeric units, and release their encapsulated drug component when put in a dilute system such as blood.

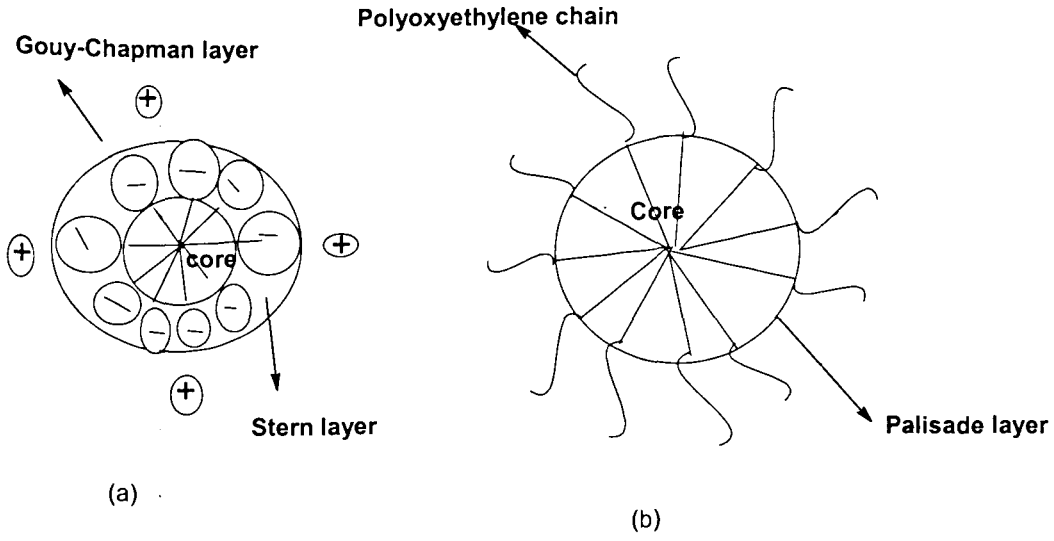


Figure 1.13: A schematic presentation of (a) ionic and (b) non-ionic micelles.(Aulton 2002).

1.8.3. Unimolecular micelles

Dendrimers consisting of an apolar core and polar shell have been referred to as "unimolecular micelles".[136] Unlike conventional micelles, however dendritic structure is independent of dendrimer concentration [137]. The first such structure was an arborol reported by Newkome et al [138] in 1985. The same group also synthesised a symmetrical, four-directional saturated hydrocarbon cascade polymer containing 36 carboxylic acid moieties with a neopentyl core [139]. It was shown that lipophilic probes were located within the lipophilic infrastructure of the dendritic structures and it was concluded

that the polymers exist as single molecules capable of molecular inclusion and therefore act as unimolecular micelles [140].

Figure 1.14 shows the structure of water soluble dendritic unimolecular micelles.

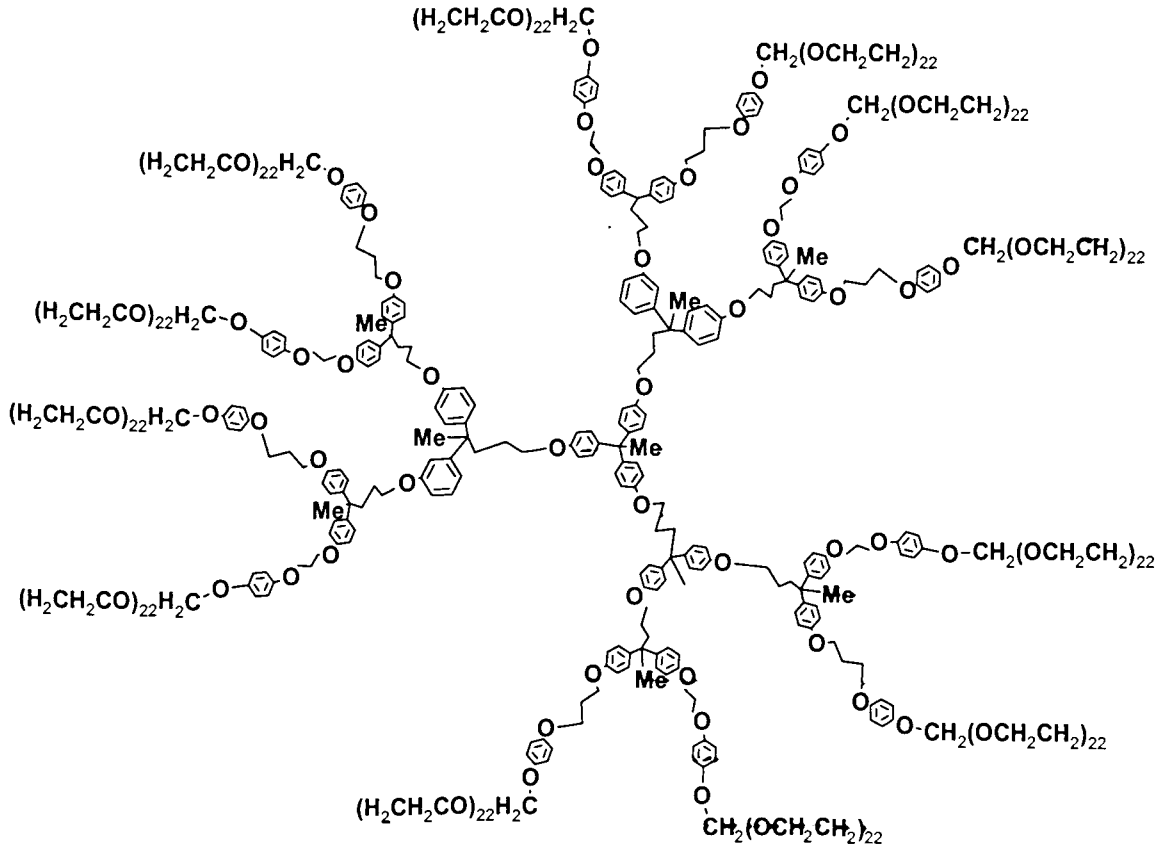


Figure 1.14: Unimolecular micelle based on 4-4'-bis(4'-hydroxyphenyl) pentanol building block and surface shell of polyethylene glycol (PEG) chain [138]

1.8.4. Applications of micelles

Several drug delivery systems have been developed to enhance drug bioavailability and to prevent harmful side-effect of drugs[141], such as lipoproteins, liposomes, natural and synthetic polymers[142,143] and micelles. What distinguishes micelles from other colloidal particles is the fact that

monomers and micelles are in dynamic equilibrium and that is why they are also referred to as association colloids.[144,145]

1.8.5. Advantages of micelles in drug delivery

1) They are easily prepared and can be produced in large amounts [146]

2) Their shape and size can be changed to suit their purpose

3) They can be used in targeted drug delivery [147,148]

4) They minimise the side effects of drugs [149]

5) They increase the drugs stability inside the micelle, and enhance its permeability.[150]

6) The connectivity feature of polymeric micelles is also of importance for the enhancement of drug bioavailability and targeting [151]

7) Their size permits them to accumulate in leaky vascular regions

8) The longevity feature of polymeric micelles is important due to the enhancement of drug bioavailability and targeting [152]

1.8.6. Critical micelle concentration (CMC)

In chemistry, the critical micelle concentration (CMC) is defined as the concentration of surfactant above which micelles are spontaneously formed. At this concentration the surface area between two liquids is fully loaded with surfactants and there is no room for additional ones. Any further addition of surfactants will lead to the formation of micelles [152,153]

Figure 1.15 shows the possible formation of micelles in water.

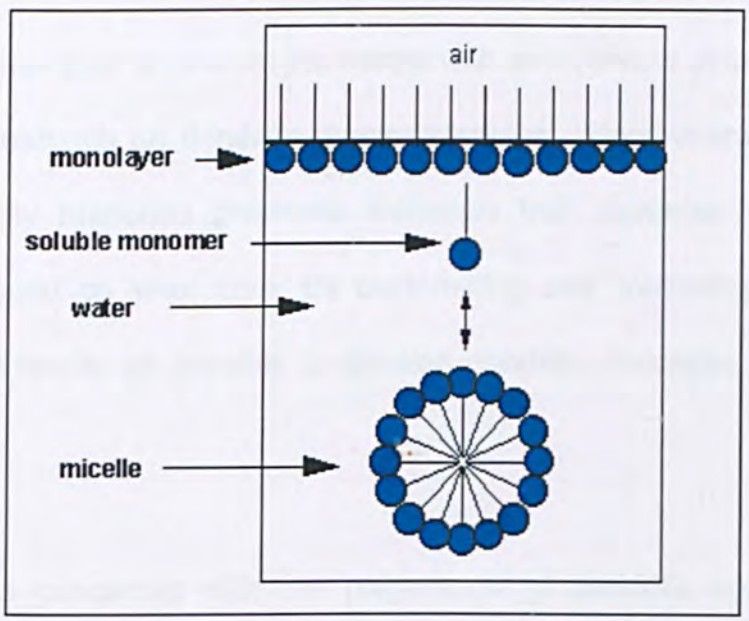


Figure 1.15: Micelle formation in water [152]

Many properties of surfactant solutions (in our case hydrolysed dendrimer), if plotted against the concentration, appear to change at a different rate above and below the CMC region [154]. By extrapolating the loci of such properties above and below this range until they intersect, a value may be obtained known as CMC. Due to many physico-chemical quantities that might be affected during the formation of micelles, a large number of methods for determining the critical micelles concentration therefore exists. In this project two main methods have been used which were dye encapsulation, and the tensiometry method. [155]

1.9. AIMS AND OBJECTIVES OF PROJECT

This chapter has tried to present the reader with an overview of several areas of scientific research on dendritic macromolecules. Dendrimers are a new class of highly branched polymeric materials that comprise a series of branches around an inner core. By customising and controlling dendrimer architecture it would be possible to develop dendritic structures suitable for drug delivery.

This thesis is concerned with the preparation of dendritic poly(arylether) macromolecules based on a 3,5-dihydroxybenzyl alcohol building block and having carboxylate chain-ends groups using convergent synthesis method. The idea having a hydrophobic centre capable of encapsulating water insoluble drugs, combined with a surface that is water soluble and having lipidic properties similar to cell membranes. The aim is to facilate merging of the dendrimer with the cell, the outer surface should also have the potential to be modified. During this project two different types of core will be used, namely 4-4'-dihydroxybiphenyl" or 1,1,1-tris(4-hydroxyphenyl)ethane "tri phenyl".

The primary aim of this investigation is to synthesise hydrophilic dendrimers of lower generation using ethyl 6-bromohexanoate as a branching unit and 3, 5-dihydroxybenzyl alcohol as a building block. Carboxylate as the terminal moiety in the later stage will be hydrolysed to improve the solubility of compound.

The first part of chapter 2 describes some synthetic routes to make triphenyl and biphenyl dendrimers and then the hydrolysis of these dendrimers. The second part of this chapter deals with an investigation of their physico-chemical properties by different analytical methods.

The first part of chapter 3 of this thesis describes all the spectrometric results obtained from synthesis of these dendritic macromolecules, such as nuclear magnetic resonance, infra red and mass spectrometry, whereas the second part shows all the physico-chemical results such as solubility at different pH, surface tension, molar conductivity and dye encapsulation. The results were analysed by these methods in order to provide a good understanding of the nature of these materials.

The results from chapter 3 are then summarised in chapter 4, and some general conclusions are drawn about the investigations presented in this project and the possible future work.

CHAPTER 2: EXPERIMENTAL

GENERAL INTRODUCTION

This chapter is concerned with the synthesis, characterisation and physical measurement of dendritic poly-arylether macromolecules based on electron-rich 3,5-dihydroxybenzyl alcohol building blocks prepared by the convergent growth approach. The experimental section in this project is divided in to two main parts. Part 1 which describes the synthetic methodology including all the characterisation of molecules synthesised (^{1}H, ^{13}C NMR, IR, and MS). Purity of these molecules synthesised was determined either by TLC or elemental analysis (EA). In part 2 the physico-chemical properties of the macromolecules made in this project were investigated; this included pH profile, solubility testing, critical micelle concentration (found by surface tension, dye encapsulation and conductivity), maximum solubility at (pH 7.4), and uv-visible absorptivity.

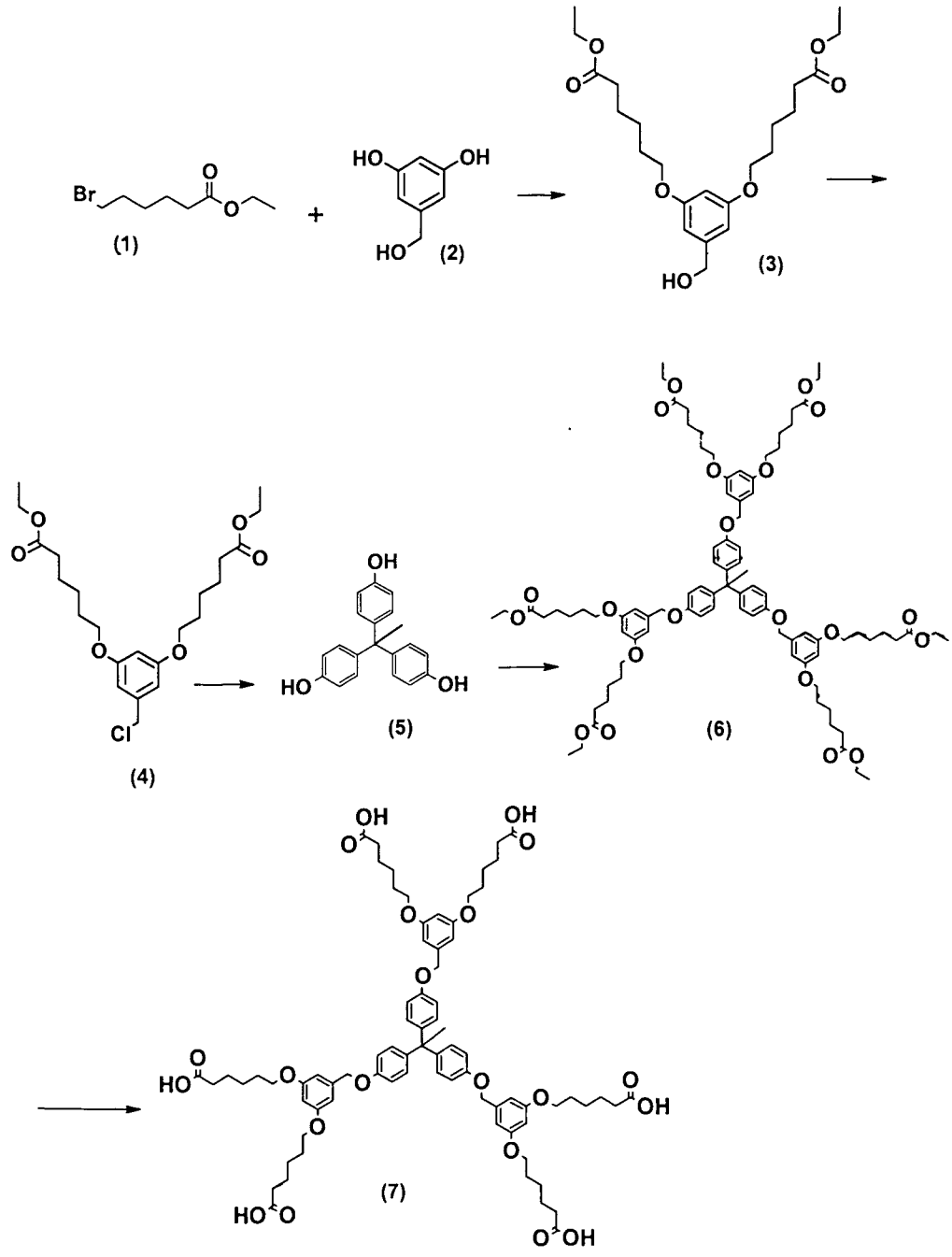
During this investigation letter G used for number of generation, and C for the number of phenyl groups in the core for example C2 for biphenyl and C3 for triphenyl core.

The synthesis of dendritic material in this chapter is described in five different stages. The first stage is the formation of first generation dendrimer, using the tri-phenyl core and the subsequent hydrolysis of the resultant molecules which have their physico-chemical properties investigated as discussed in the second part of this chapter, In the second and third stages, the attachment of the first and second generation dendrons to a different kind of core (bi-phenyl) is described and then the resultant products were hydrolysed, using alkaline conditions. The last stage describes the formation of a different type of

dendrite, and the attachment of a spacer to the first dendrite in order to alter the shape of dendritic macromolecules.

The starting material which became the chain ends that contained potentially hydrophilic functional groups was chosen to be ethyl 6-bromo-hexanoate **(1).** To provide an interior containing electron-rich aromatic rings, it was decided to employ 3,5-dihydroxybenzyl alcohol as the building block and a two-step generation growth process, consisting of activation growth step by chlorination and a generation growth step,(see scheme 1). The generation growth step starts by attaching two molecules of **1** which will become the terminal groups, to the monomer unit 3, 5-dihydroxybenzyl alcohol **(2).** In this reaction attachment occurred readily in the presence of potassium carbonate and butanone when heated under reflux, which gave 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** occurred by reaction with thionyl chloride and a catalytic amount of DMF in the presence of dry DCM. This proceeded smoothly to give the corresponding chloride $(EtO_2C)_2$-G1-Cl **(4).** The next stage was reacting three molecules of **4** with 1,1,1-tris (4-hydroxyphenyl) ethane **(5)** in the presence of 18-crown6 and potassium carbonate in butanone under reflux. The dendritic poly ether macromolecule $(EtO_2C)_6$-G1-3C **(6)** was isolated then the transformation of terminal ethyl ester groups of **6** in to the desired carboxylate groups was accomplished by alkaline hydrolysis. 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 this reaction mixture gave the carboxylate-terminated molecule $(HOOC)_6$-G1-3C **(7).**

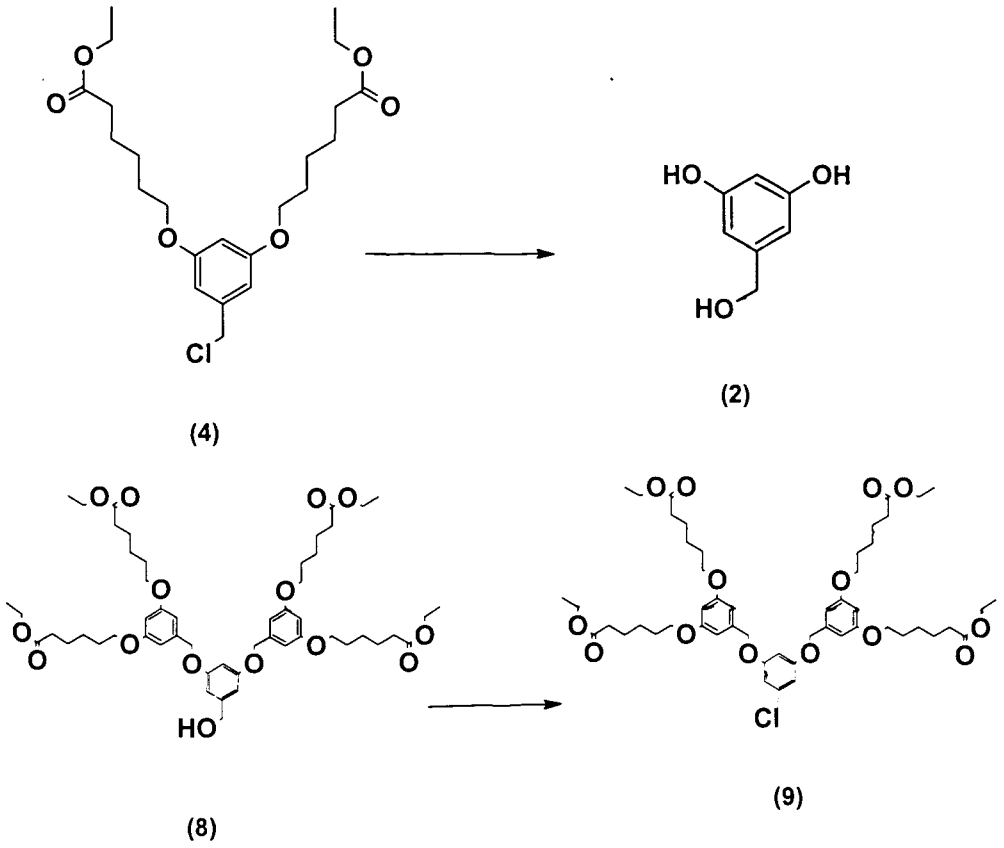
Scheme (1)



In the next stage, (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 at 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, several drops of DMF and a proton scavenger, proceeded to give the corresponding chloride $(EtO_2C)_4$-G2-Cl **(9)**. See scheme 2

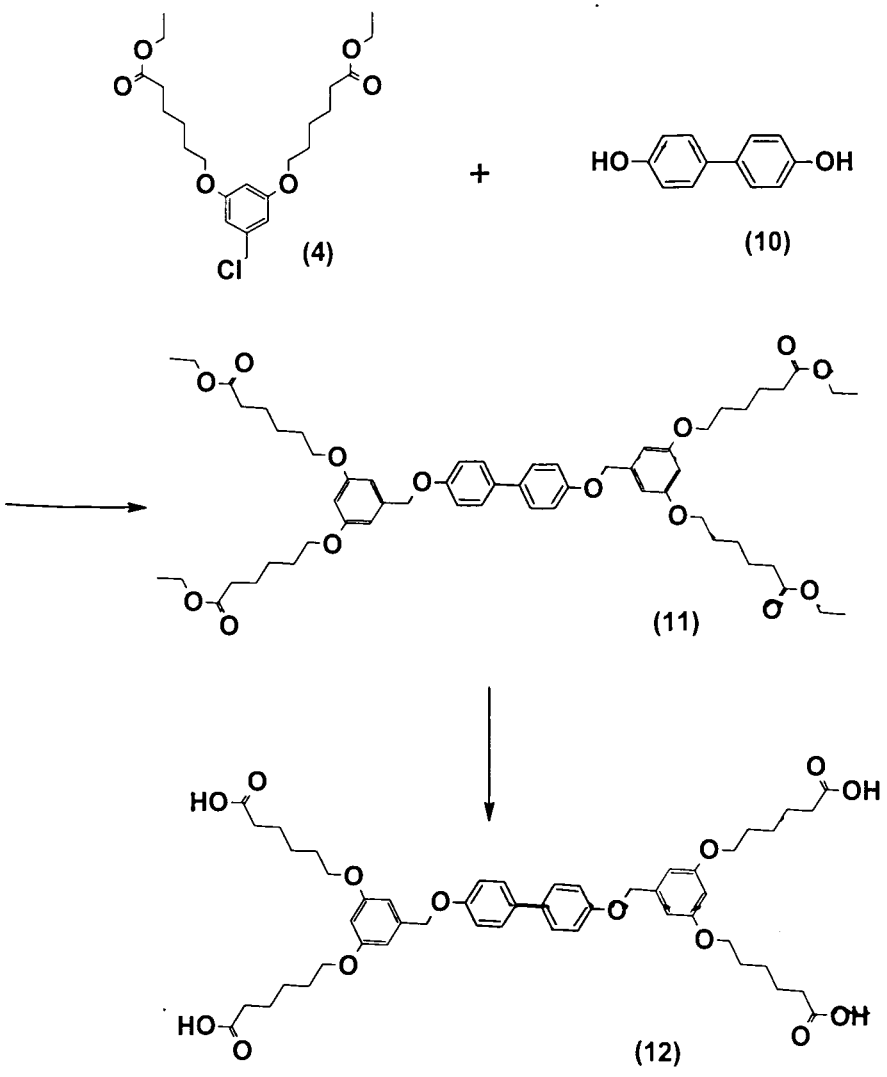
Scheme 2



Reaction of three equivalents of **9** with one equivalent of triphenyl core **5** was tried but due to the steric hindrance between the carbon chains thr product didn'r form; therefore it was decided to use a biphenyl core of (4,4'-dihydroxybiphenyl), instead of the three phenyl core 1,1,1-tris(4-hydroxy-phenyl) ethane. The biphenyl core **(10)** in this case was used because the dendrons can spread in two opposite directions, therefore reaction of two molecules of **4** with one molecule of **(10)** in the presence of potassium

carbonate, 18-crown6, and butanone under reflux gave $(EtO_2)_4$-G1-2C.**(11).** Transformation of the methyl ester end groups in **11** into the desired carboxylate groups was accomplished by alkaline hydrolysis. Reaction of **11** with a large excess of potassium hydroxide was found to proceed satisfactorily. Acidification of reaction mixture gave the carboxylate–terminated molecule $(HOOC)_4$-G1-2C .**(12). (see scheme 3).**

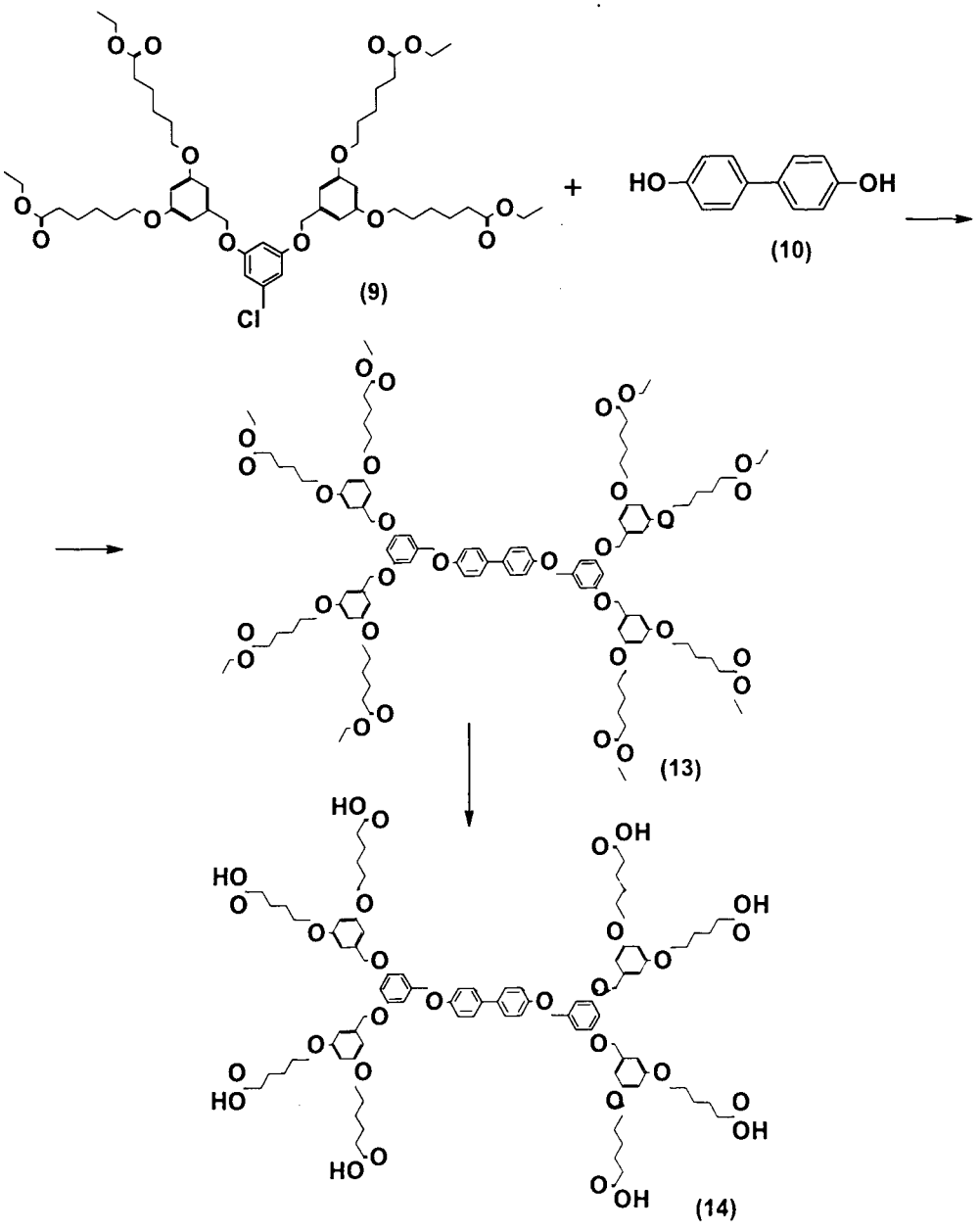
Scheme 3:



In the first stage two equivalents of **9** was reacted with one equivqlent of biphenyl core, 4',4-dihydroxybiphenyl in the presence of potassium carbonate, 18-crown6 and butanone to give $(EtO_2)_8$-G2-2C **(13)** with eight ester chain

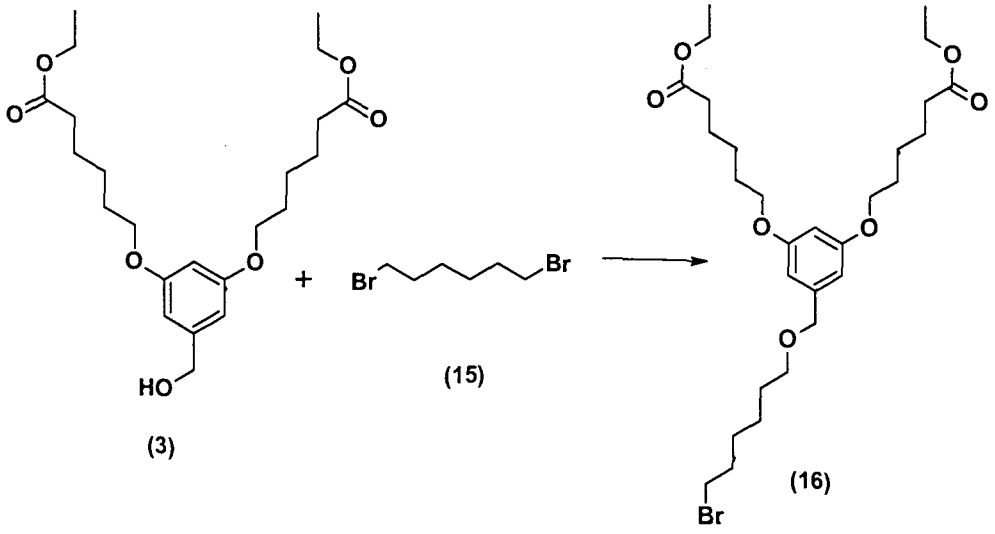
ends. The transformation of the terminal ethyl ester groups of **13** into the desired carboxylate groups was accomplished by alkaline hydrolysis. Reaction of **13** with large excess of potassium hydroxide proceeded satisfactorily in a mixed solvent system of tetrahydrofuran/water/ethanol. Acidification of this gave the carboxy-terminated macromolecule $(HOOC)_8$-G2-2C **(14)**. See scheme 4

Scheme (4)



In the fifth synthesis, it was decided to use a cross-linker or spacer that would make the dendrons bigger and then react them with a different kind of core; for this reason 1,6-dibromohexane **(15)** was chosen to be reacted with dendrite, by reacting 4 equivalents of 1,6-dibromohexane with one equivalent of dendrite **3** in the presence of potassium-*tert*-butoxide, 18-crown-6 and dry diethylether to produce compound **(16),(see scheme 5.1)**

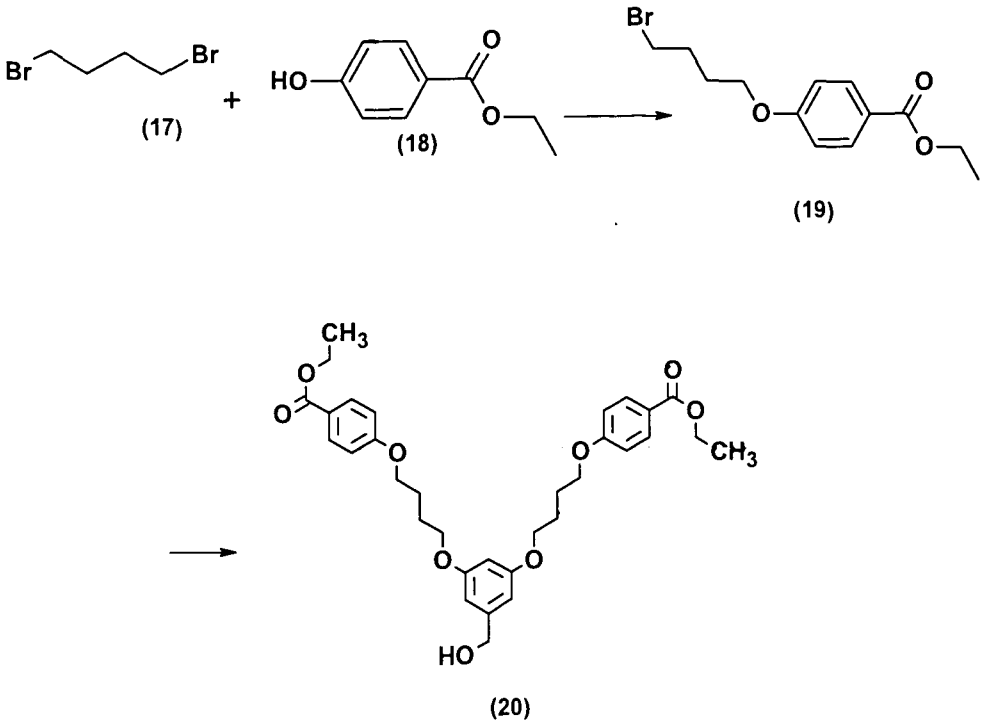
Scheme 5.1:



but this reaction gave significantly lower yield than was expected less than 10%; therefore it was decided to make a different branching unit with carboxylate chain ends by reacting one equivalent of 1,4–dibromobutane **(17)** with one equivalent of ethyl 4-hydroxybenzoate **(18)** in the presence of potassium carbonate and butanone in reflux conditions to produce different kind of branching monomer **(19)** with bromine at its focal point, which by reacting two equivalent of **19** with monomer unit **2** (3,5-dihydroxybenzyl

alcohol) in the presence of potassium carbonate , 18-crown 6 and butanone produced a second type of dendrite **(20) , see scheme 5.2**

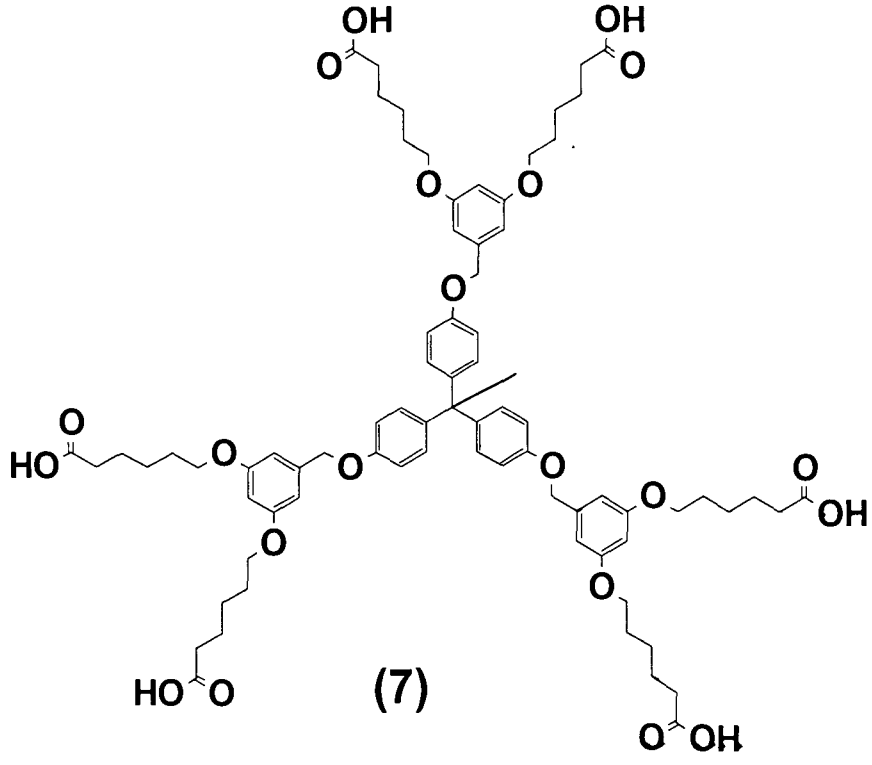
Scheme (5.2)



In part 2, physico-chemical properties of macromolecules **7**, **12** and **14** were measured; these included solubility tests, pH profile, amphiphilic properties of dendrimers, UV/Visible absorptivity, maximum absorbance at pH 7.4 and finding the CMC by surface tension, dye encapsulation, molar conductivity measurements and overall percentage yield.

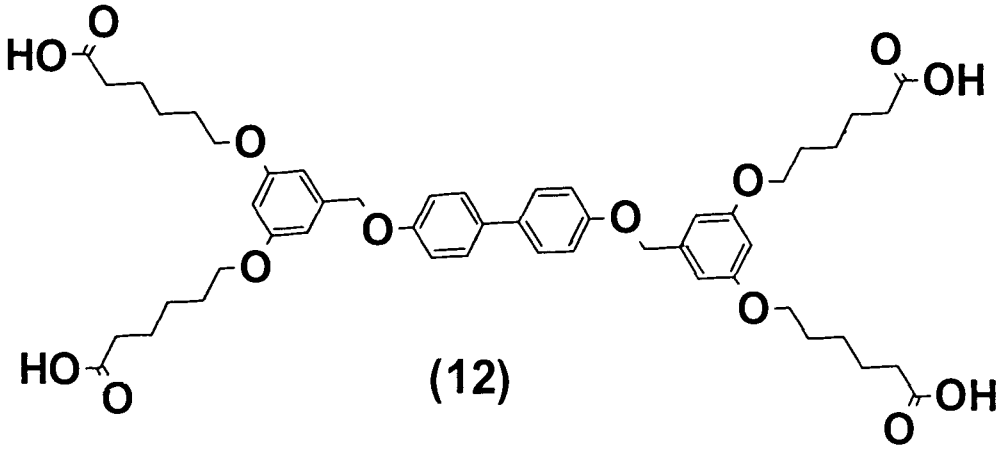
Structure of dednrimers whose physico-chemical properties were analysed

(7) = $(COOH)_6$-G1-2C



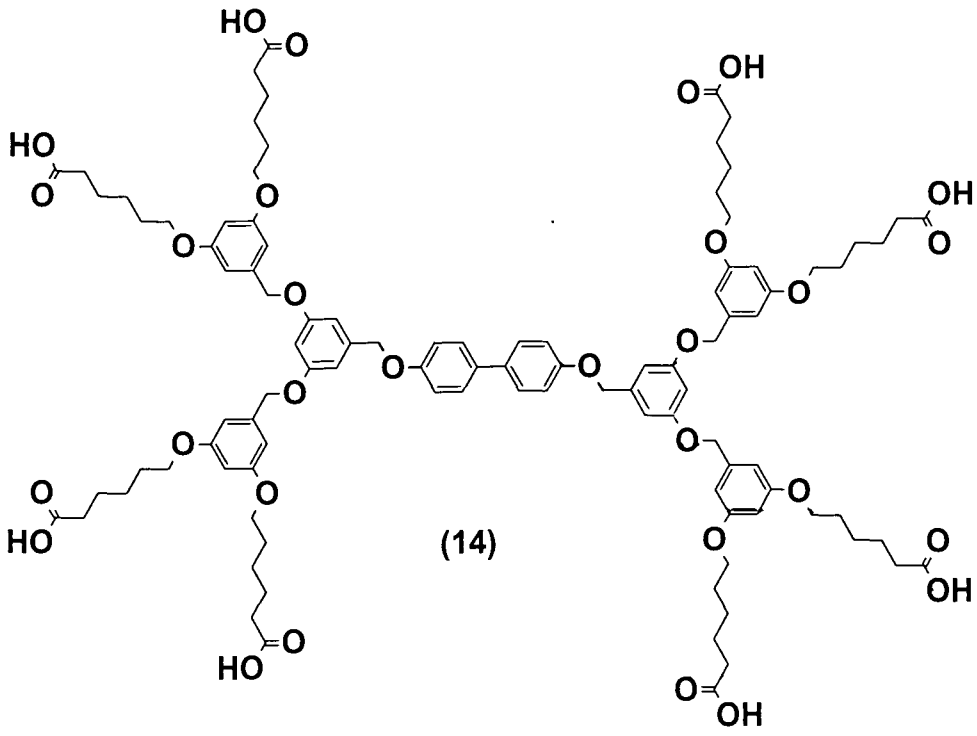
(7)

(12)= $(COOH)_4$-G1-2C



(12)

(14) = $(COOH)_8$-G2-2C



(14)

2.1. GENERAL PROCEDURES AND INSTRUMENTATION

2.1.1. Chemicals and solvents

All chemicals used during the course of this research were purchased from Sigma Aldrich or Lancaster and were used as supplied, unless stated otherwise. All solvents used during reaction, work-up and purification procedures were commercially available.

2.1.2. Chromatography Techniques

Analytical thin-layer chromatography (TLC) was carried out on silica gel of pore diameter 60Å with a fluorescent indicator. The TLC plate was then visualized by UV light (254nm). Flash chromatography was performed on silica gel with 0.46 g/ml with 35 micrometre -70 micrometre pores

2.1.3. Spectroscopy Techniques

Infra–red spectra were recorded using a Perkin–Elmer (PARAGON 1000), spectrometer. Proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C N.M.R) spectra were measured using an Eclipse + 400EXC NMR spectrometer

Mass spectra were determined using a 1200 L Quadrupole mass spectrometer, Unless otherwise stated, the mass spectra were recorded under electron impact (EI) conditions with a source temperature of 250 °C and an electron energy of 70 eV. High Resolution mass spectrometry was measured on a Water's L.C.T using TOF high resolution mode.

Ultra-violet/visible spectra were recorded using a Kontron Uvikon 940 UV/Vis spectrophotometer.

2.1.4. Purity of Compounds Synthesized

Purity of the compounds synthesized was assessed either by elemental analysis (EA) or by TLC. Elemental analyses were carried out by MEDAC LTD, Zetasizer, (Malvern Instrument).

2.1.5. Physico – chemical characteristic techniques

2.1.5.1. Electrical conductivity

Electrical conductivity is a measure of a material's ability to conduct an electric current [156,157]. The electrical conductivity measurements were made using a conductivity meter (Wooden Precision Apparatus Limited, CMD 830 WPA). The cell constant was determined by measuring the conductance of potassium chloride (0.1M) using a conductance meter; the conductivity of KCl (0.1M) was taken to be 1.131 (CMD830 WPA: from laboratory data book).

The formula from which the electrical conductivity was calculated is shown below

Equation (1)

$$Kcell = \left[\frac{k(0.1.MKcell)}{G(0.1.MKcell)} \right]$$

where K_{cell} = cell constant

k= conductivity of (0.1 M KCl)

G = Conductance of (0.1 MKCl), measured from the conductance meter.

The conductivity for each concentration of the surfactant is then calculated using the following equation:

Equation (2) $$k = K_{cell} (G-G_0)$$

where

k= conductivity of the surfactant:

K_{cell}= cell constant:

G= conductance of surfactant

G_0= Conductance of the distilled water

The molar conductivity for each concentration of the surfactant is then calculated using the following equation:

Equation (3) $$A = (k/C)$$

where

A= molar conductivity: (Scm^{-1} mol^{-1})

K=conductivity: (Scm^{-1})

C=surfactant concentration

The molar conductivity was plotted against the surfactant concentration, and the point where a change in slope is observed is the critical micelle concentration [158]

2.1.5.2. Surface tension

Surface tension is caused by the attraction between the molecules of a liquid due to various intermolecular forces. In the bulk of the liquid each molecule is pulled equally in all directions by neighbouring liquid molecules, resulting in a

net force of zero. At the surface of liquid, the molecules are pulled inwards by other molecules deeper inside the liquid but they are not attracted as intensely by the molecules in the neighbouring medium (be it vacuum, air or another liquid). Therefore all the molecules at the surface are subject to an inward force of molecular attraction which can be balanced only by the resistance of the liquid to compression. Thus the liquid squeezes itself together until it has the lowest surface area possible. This property is what makes the liquid surface act like an elastic membrane; the surface tension is measured in Newton per metre $N.m^{-1}$. Several methods can be used to measure surface tension such as Wilhelmy plate method, drop weight and drop volume methods, capillary rise method [159,160], and ring method. In this thesis the De Nouy's ring method [161,162] was used due to its simplicity and accuracy, which can be high as ± 0.25% .This method measures the force required to detach a platinum ring from a surface.[163,164]

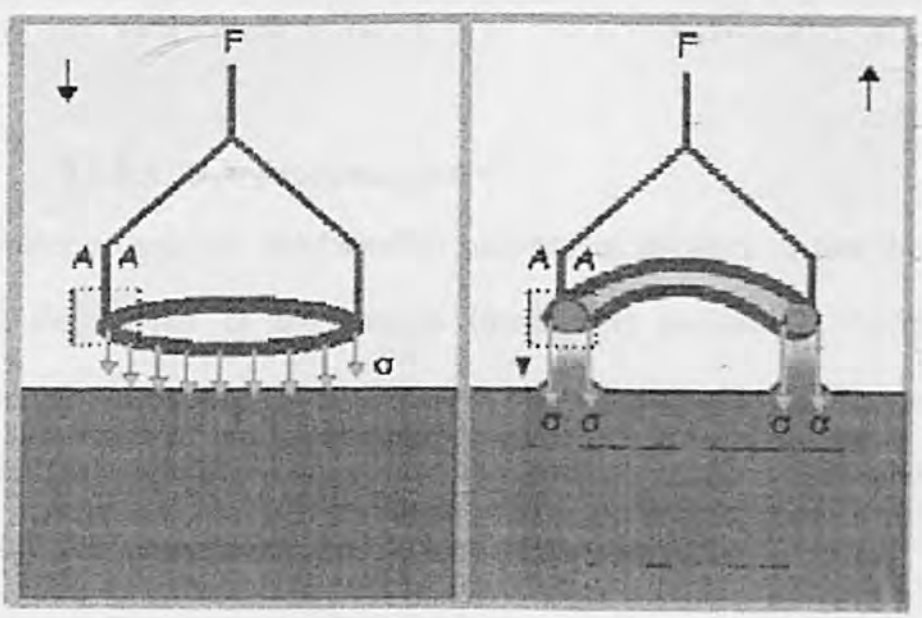


Figure 2.1: Schematic representation of the detachment of the platinum ring from a surface [165]

Surface tension measurements were taken using a SIGMA 703 De Nuoy tensiometer. (KSV INSTRUMENT) [166.167]

The surfactant concentration is related to the original concentration by the equation:

Equation (4) $$C = C^0 (V / V^0 + V)$$

Where

C = Surfactant concentration.

C^0 = original concentration

V= volume of solution added to water

V^0 = original volume of the distilled water

The surface tension obtained from the tensiometer was plotted against the surface concentration C, the point where a change in slope is observed is the CMC

2.1.5.3. Dye encapsulation

This method uses the solubilisation property of micelles to find the CMC, where the interior of the micelle (dendrimer) provides a hydrophobic environment to accommodate the non polar compound (dye). The constant absorbance reading of a water insoluble compound within the micelles will therefore give indication on the formation of micelles. [168,169].

Oil red dye is a water insoluble dye, which has moderate solubility in ethanol. This dye has an absorption at around 518 nm, and has the empirical formula $C_{26}H_{24}N_4O$. [170]

The structure of this compound is shown in fig 2.2

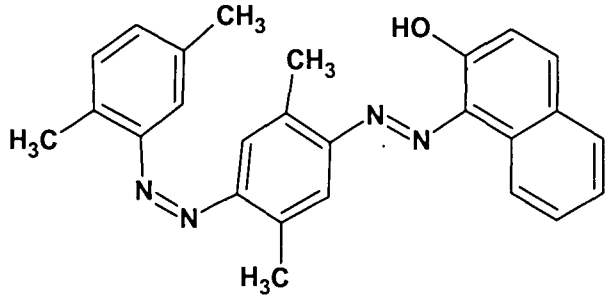
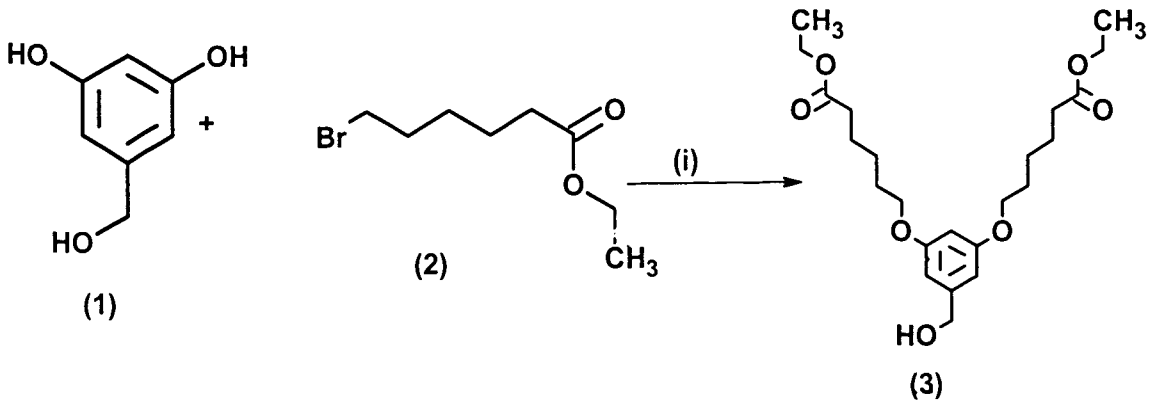


Figure 2.2: Structure of the Oil red dye [168]

2.2. PART (1)

2.2.1. Synthesis of first dendritic alcohol $(EtO_2C)_2$-G1-OH,(3).



(i) Anhydrous potassium carbonate,
Butanone

Preparation

To a 250cm^3 round bottomed flask equipped with a condenser and nitrogen gas bubbler, were added 150cm^3 butanone, 3,5-dihydroxybenzyl alcohol (5g , 0.0357mol), ethyl-6-bromohexanoate (13.3cm^3, 0.0714mol), and K_2CO_3 (22.7g, 0.164mol). The solution was refluxed with constant stirring under nitrogen for 72 h. The presence of a product was confirmed by TLC (hexane: ethyl acetate 1:5). The solution was filtered and washed with distilled water (3 x 10cm^3), extracted with DCM (3 x 10cm^3); the extract was dried over $MgSO_4$ and the solvent evaporated. The product was purified by column chromatography (SiO_2) using hexane /ethyl acetate (1:5) as the eluent. The product was a very light yellowish oil. (4g, 93%)

^{1}H ($CDCl_3$,δ ppm):

1.18 (t, 6H, $(CH_3CH_2)_2$, J=7.2Hz), 1.42-1.60 (overlapped peaks, 4H, $(CH_2CH_2)_2$, 1.2-1.8 (overlapped peaks, 4H, $(CH_2CH_2CH_2)_2$, 2.26 (t, 4H, $(CH_2COOR)_2$, J=7.2 Hz), 3.80 (t, 4H, $(CH_2OAr)_2$, J=6.4 Hz), 4.06 (q, 4H, $(CH_2CH_3)_2$, J= 7.2 Hz), 4.6 (d, 2H, CH_2OH), 3.5 (t, 1H, O*H*), 6.25 (d, 2H, 2,6-Ar*H*), 6.3 (3,1H, 4-Ar*H*).

^{13}C NMR (ppm):

14.31 (**C**H_3CH_2), 34 (**C**H_2COOR), 25.08 (**C**H_2CH_2COOR), 25.4 (**C**$H_2CH_2CH_2COOR$), 29.5 (**C**H_2CH_2OAr), 68.5 (**C**H_2OAr), 100 (**C**H, 4-Ar), 158.4 (**C**OR, 3, 5-Ar), 104.2 (**C**H, 2, 6-Ar), 140.2 (**C**, position 1-Ar), 68.65 (**C**H_2OH), 173.2 (**C**O, carbonyl), 61.4 (**C**H_2CH_3)

MS: (m/z):424

ν_{max} (KBr) IR:

3400- 2950 (OH, methyl alcohol), 2940, 2870 (CH, alkenes), 1732,1712 (C=O, ester), 1598 (C=C), 1454 (CH, aromatic), 1374,1322,1296 (CO, aryl ether), 1162 (O-C=C), 836 (C-H bend) cm^{-1}

Elemental analysis:%

Calculated: C (66.79), H (8.14)

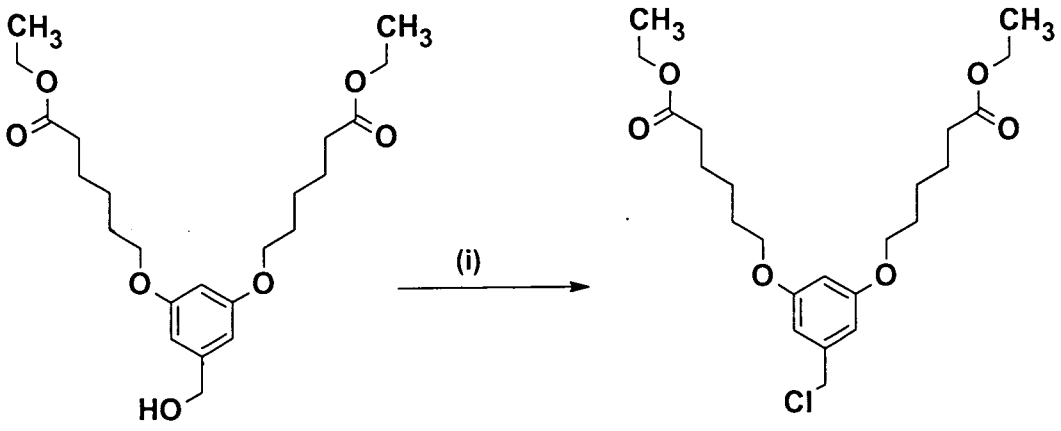
Found: C (66.51),H (8.60)

High resolution mass spectrometry:

Mono-isotopic ion: 424.2461

Found: 424.2468

2.2.2. Synthetic procedure for the conversion of the dendritic alcohol into the corresponding chloride $(EtO_2C)_2$-G1-Cl ,(4).



(3) (4)

i) **DMF, dry DCM , Thionyl chloride**

Preparation

A mixture of dry DCM ($100cm^3$), dendrite **3** (10g , 0.024mol), and a catalytic amount of DMF was added to a two–necked $150cm^3$ flask, and thionyl chloride ($2.3cm^3$) dissolved in $10cm^3$ dry DCM was added to the mixture with stirring at room temperature. Approximately 30 minutes after the addition of $SOCl_2$ the reaction was complete (TLC, 5:1 hexane: ethyl acetate). Water was then added drop-wise to destroy excess $SOCl_2$. The solution was washed with water ($30\ cm^3$) and extracted with DCM ($3 \times 10cm^3$), the extract was dried over $MgSO_4$, and the solvent removed under reduced pressure. The product was purified by flash chromatography, eluting with hexane: ethyl acetate (5:1) to give a light yellowish oil. (6.9g, 65%)

^{1}H ($CDCl_3$,δ ppm):

1.2(t, 6H, $(CH_3CH_2)_2$, J=7.2Hz), 1.41 (overlapped peaks, 4H, $(CH_2CH_2)_2$, 1.60 (overlapped peaks, 8H, $(CH_2CH_2CH_2)_2$, 2.26 (t, 4H, $CH_2COOR)_2$, J=7.4Hz), 3.8 (t, 4H, $(CH_2OAr)_2$, J=6.42 Hz), 4.06 (q, 4H, $(CH_2OCOR)_2$, J= 7.2 Hz), 4.53 (s, 2H, CH_2Cl), 6.28 (d, 2H, 2,6-Ar*H*), 6.48 (t, H, 4-Ar*H*)

^{13}C NMR (ppm):

14.31 (CH_3CH_2), 33.9 (CH_2COOR), 24.8 (CH_2CH_2COOR), 25.08 ($CH_2CH_2CH_2COOR$), 29.3 (CH_2CH_2OAr), 68.1 (CH_2OAr), 102 (**C**H, 4-Ar), 160 (**C**OR, 3,5-Ar), 104 (**C**H, 2,6-Ar), 139 (CCH_2Cl, 1-Ar), 45.9 (CH_2Cl), 173 (**C**, carbonyl), 61.3 (CH_2CH_3)

MS:

MS: m/z : 442, 444 (M+)

ν_{max} **(KBr)**

2940, 2870 (CH, alkane), 1732, 1712 (C=O, ester), 1598 (C=C), 1454 (C-H aromatic), 1374,1322 ,1296 (CO Aryl ether), 1162 (O-C=C), 832 (C-H bend), 716 (C-Cl) cm^{-1}

Elemental analysis:%

Calculated: C (62.36), H (7.96)

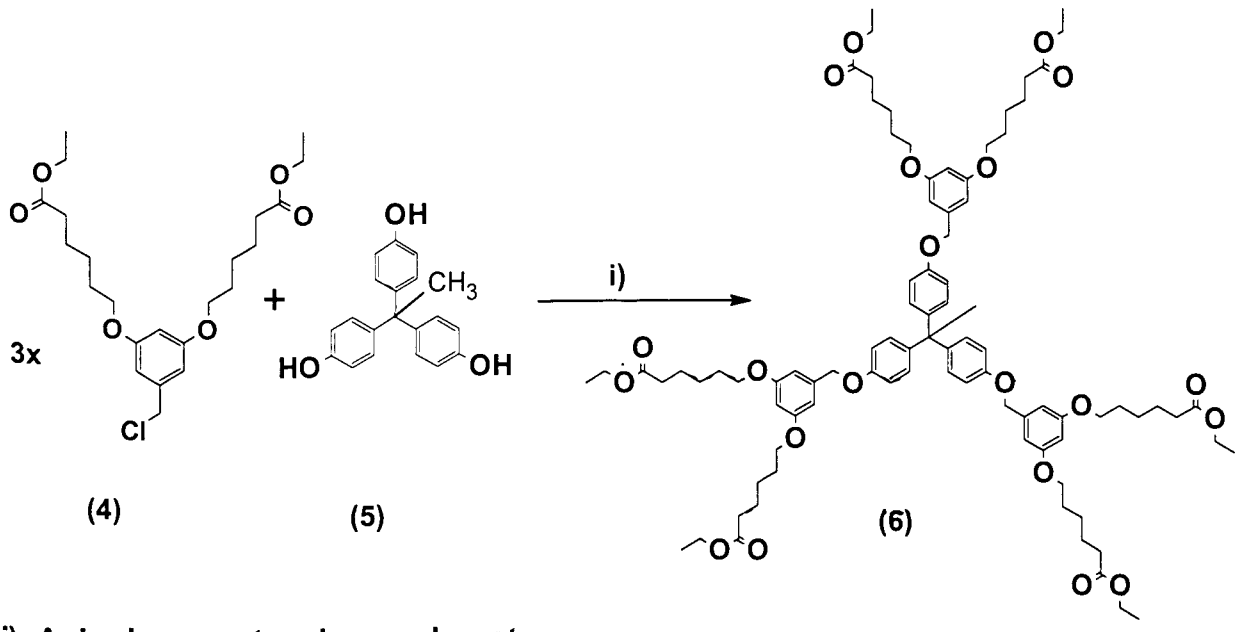
Found: C (62.42), H (8.1)

High resolution mass spectrometry:

Calculated mono-isotopic mass: 442.2120

Found mass = 442.2106

2.2.3. Synthesis of compound $(EtO_2C)_6$-G1-3C ,(6). with 1,1,1-Tris(4-Hydroxyphenyl) Ethane.



i) Anhydrous potassium carbonate
18-Crown 6, butanone

Preparation:

A mixture of chlorinated dendrite **4** (2.10g, 4.75 x 10^{-3}mol), 1,1,1-tris(4-hydroxyphenyl) ethane **5** (0.48g, 1.58 x 10^{-3}mol), 18-crown 6 (0.25g, 9.4 x10^{-4} mol), potassium carbonate (3.0g, 0.0119mol) in butanone (150cm^3) were refluxed under nitrogen with stirring for 72h. The solution was then filtered, and the product was washed with water (30cm^3) and extracted with DCM (3 x 10cm^3); the extract was dried over $MgSO_4$ and the solvent evaporated. The product was purified with flash chromatography (SiO_2) eluting with ether/petroleum ether (60-80 ^{0}C) (6:4). The product was a light beige oil (1.4g, 60%).

^{1}H ($CDCl_3$,δ ppm):

1.18 (t,18H, $(CH_3CH_2)_6$, J=7.2), 1.38-1.75 (overlapped peaks, 12H, $(CH_2CH_2)_6$, 1.4-1.8 (overlapped peaks, 24H, $(CH_2CH_2CH_2)_6$), 2.1 (s, 3H, CH_3), 2.26 (t, 12H, $(CH_2COOR)_6$), J=7.2), 3.86 (t, 12H, $(CH_2OAr)_6$, J=6.4), 4.06 (q, 12H, $(CH_2CH_3)_6$, J=7.41), 4.88 (s, CH_2 not coupling, 6H), 6.45 (t, 3H, C**H** , 4-ArH)$_3$, 6.30 (d, 6H, C'**H**, 2,6-ArH)$_3$, 5.20 (s, 6H, ArCH_2OAr)$_3$, 6.78 (d, 6H, 2,6-Ar***H*** in core)$_3$, 6.92 (d, 6H, 3,5-Ar***H*** in core)

^{13}C NMR (ppm):

14.1 (**C**H_3CH_2), 16.2 (**C**H_2CH_3), 173.1 (**C**=O, Carbonyl), 34.9 (**C**H_2COOR), 25 (**C**H_2CH_2COOR), 25.5 (**C**$H_2CH_2CH_2$COOR), 29.3 (**C**H_2CH_2OAr)$_6$, 68.82 (**C**H_2OAr), 159 (**C**-O, 3,5-Ar), 99.5 (**C**H, 4-Ar), 102.5 (**C**H, 2,6-Ar), 142.4 (**C**CH_2OAr, 1-Ar), 71.5 (Ar**C**H_2OAr),114.5 (**C**H, 3,5-Ar in core), 129.2 (**C**H 2,6-Ar in core), 158.1(**C**, 4-Ar in core)$_3$, 135 (**C**,1-Ar), 48.5 (**C**CH_3, in core), 12.8(**C**H_3, in core)

ν_{max} (KBr)

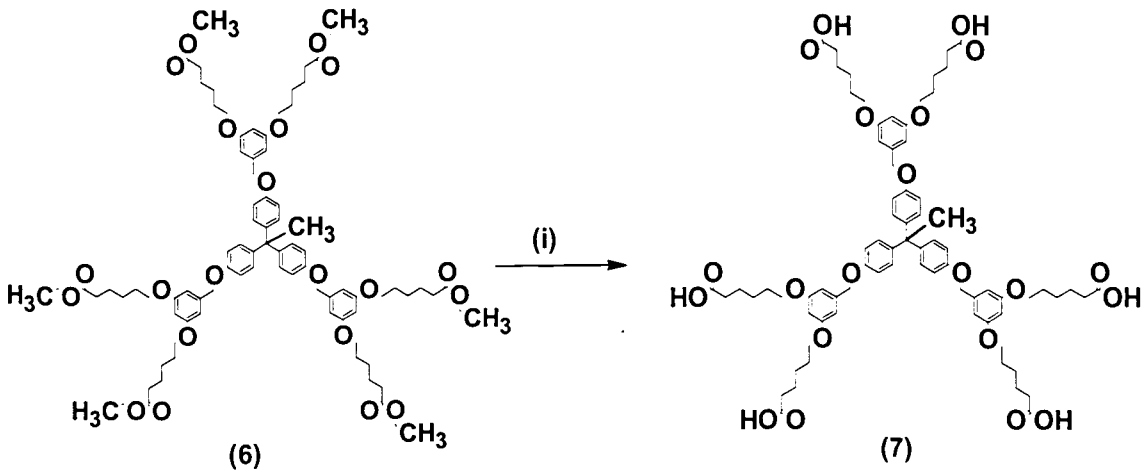
2950, 2970 (R-CH_3 , Alkane), 2915,2936 (R'-$(CH_2)_4$-R''),1598 (C=C), 1545 (CH, aromatic), 1735-1750 (C=O, ester), 1210.1310 (C=O-C), 1374,1322.1296 (CO, aryl ether), 1162 (O-C=C)

High resolution mass spectrometry:

Calculated mono isotopic mass = 1524.8322

Found mono isotopic mass = 1524.8337

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



i) **Anhydrous potassium carbonate**

18-Crown6 , Butanone

Preparation:

Compound **6** (0.96g, 7.15 x 10^{-4}mol) was dissolved in 20cm^3 solvent (ethanol 10cm^3, THF 10cm^3), and transferred to a 100cm^3 quick-fit flask equipped with condenser and nitrogen bubbler. Potassium hydroxide (1g, 0.0171mol) dissolved in distilled water (20cm^3) was added to the flask with constant stirring under reflux. After 4 hours the reaction was complete (TLC, ether: hexane, 4:6).The product was cooled, poured into distilled (100cm^3) water, acidified with HCl, extracted with DCM (3 x 10cm^3), dried over $MgSO_4$, and evaporated under reduced pressure. The product was dark beige dense oil (0.7g, 72%).

^{1}H ($CDCl_3$,δ ppm):

2.2(t, 12H, C***H2***$COOH)_6$, J=7.4Hz), 1.41(overlapped peaks, 12H, ($C\boldsymbol{H_2}CH_2)_6$, 1.60 (overlapped peaks, 24H ($C\boldsymbol{H_2}CH_2)_{12}$, 4.05 (t, 12H, ($C\boldsymbol{H_2}OAr)_6$, J=6.4 Hz), 6.3 (d, C***H***, 4-ArH), 6.26 (t, 6H, C***H***, 2,6-ArH), 5.20 (s, 6H, Ar$C\boldsymbol{H_2}$OAr), 6.70 (d, 6H, 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***H3*** core,)

^{13}C NMR (ppm):

177.2.1 (**C**OOH, Carbonyl), 36 (**C**H_2COOH), 25 (**C**H_2CH_2COOH), 25.5 (**C**$H_2CH_2CH_2$COH), 29.3 (**C**H_2CH_2OAr), 68.82 (**C**H_2OAr), 99.5 (**C**H, 4 Ar), 159 (**C**O, 3,5-ArC), 102 (**C**H, 2,6-ArC), 142.4 (**C**, 4-Ar), 71.4 (**C**CH_2OAr), 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)

ν_{max} (KBr)

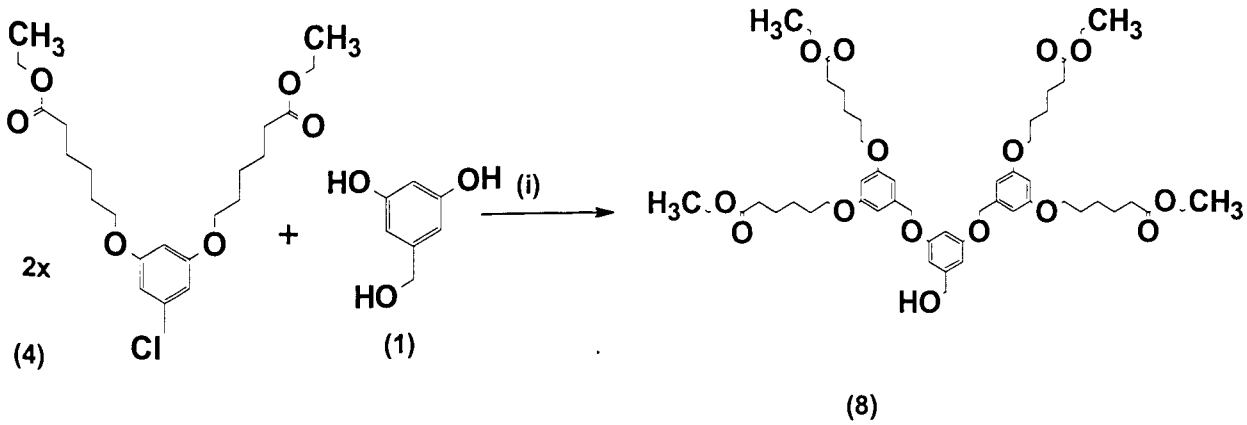
2950, 2972 (R-CH_3), 2916,2936 (R'-CH_2R''), 3010,3079 (P-Di-subst), 3010,3079 (1,3,5 tri-subst), 2900,3100 (C-C-COOH), 1210,1310 (Ph-O-C, Ester), 1710 (C=O) cm^{-1}

High resolution mass spectrometry:

Found mono isotopic mass: 1356.6478

Calculated mono-isotopic mass: 1356.6444

2.2.5. Synthesis of compound $(EtO_2C)_4$-G2-OH,(8).



i) Anhydrous potassium carbonate, 18-crown 6

Butanone

Preparation

To a 250cm^3 round-bottomed flask was added butanone (150cm^3), the chlorinated dendrite **4** (6g, 0.014mol), 3,5-di-hydroxybenzyl alcohol (1g , 7 x 10^{-3}mol), K_2CO_3 (4.14g , 0.03mol), and 18 crown-6 (0.4g , 0.0014mol); the resultant solution was vigorously stirred under reflux for 4 days. The solution was filtered and washed with water (30cm^3) and extracted with DCM (3 x 10cm^3), dried over $MgSO_4$ and evaporated under reduced pressure to give the product, which was then purified using flash chromatography (SiO_2), eluting by diethyl ether: petroleum ether (8:2 , v/v). The product was dense beige oil (4g, 60%).

^{1}H NMR (ppm):

1.25 (t, 12H, (C***H***$_3$CH$_2$)$_4$ J=7.14Hz), 1.46 (over-lapped peaks, 8H, (C***H***$_2$CH$_2$)$_4$, 1.66 (overlapped peaks, 16H, (C***H***$_2$CH$_2$)$_8$, 2.3 (t, 8H, (C***H***$_2$COOR)$_4$ J=7.4Hz), 3.89 (t, 8H, (C***H***$_2$OAr)$_4$ J=6.2 Hz), 4.06 (q, 8H, (C***H***$_2$CH$_3$)$_4$ J=7.2 Hz), 4.6 (d, 2H, C***H***$_2$OH), 5.20 (s, 4H, (Ar-***CH***$_2$O-Ar)$_2$, 5.29 (d, 1H, O***H***), 6.35 (t, 3H, 4-Ar***H***), 6.46 (d, 6H, 2,6-Ar***H***)$_6$

^{13}C NMR (ppm):

14.34 (**C**H$_3$CH$_2$), 34 (**C**H$_2$COOR), 25.07 (**C**H$_2$CH$_2$COOR), 25.4 (**C**H$_2$CH$_2$CH$_2$COOR), 29.5 (**C**H$_2$CH$_2$OAr), 65.9 (**C**H$_2$OAr), 99.5 (**C**, 4-Ar), 158.2 (**C**OR, 3,5-Ar), 102.8 (**C**H, 2,6-Ar), 142.4 (**C**, 1-Ar), 71.4 (C**C**H$_2$OAr), 100.4 (**C**, 4-Ar), 161.8 (**C**OR, 3,5-Ar in core), 104.9 (**C**H, 2,6-Ar),139.8 (**C**,1-Ar in core), 68 (**C**H$_2$OH),173.1 (**C**, carbonyl), 61.3 (**C**H$_2$CH$_3$)

ν_{max} (KBr)

340 , 3200 (R-CH$_2$-OH), 2936,2916 (R'-(CH$_2$)4-O), 2900 , 3100 (CCOOH),1310 ,1210 (Ph-O-C), 2940,2870 (CH, alkenes), 1732 , 1712 (C=O, ester), 1598 (C=C), 1454 (CH, aromatic), 1374 ,1322 ,1296 (CO, aryl ether), 1162 (O-C=C), 836 (C-H bend) cm^{-1}

Elemental analysis:%

Calculated: C (66.79), H (8.04)

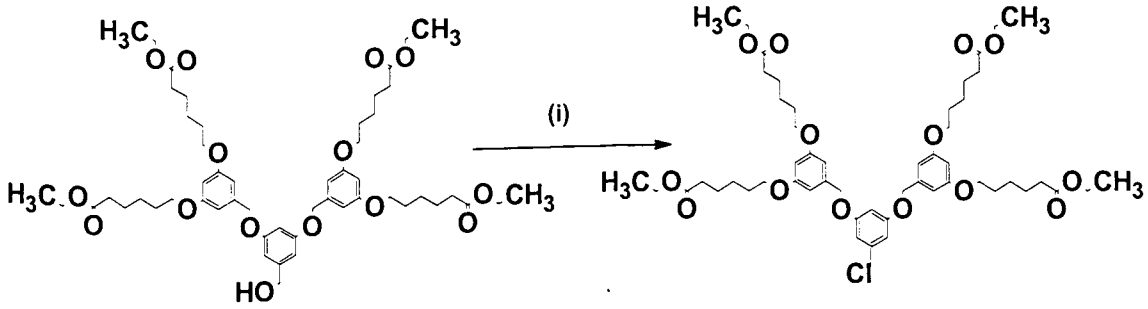
Found: C (66.60), H (8.44)

High resolution mass spectrometry:

Calculated mono isotopic mass: 952.5184

Found mass: 952.5179

2.2.6. Conversion of the dendron (with methyl alcohol) to corresponding chloride $(EtO_2C)_4$-G2-Cl,(9).



(8) (9)

(i) Thionyl chloride, dry DCM

Catalytic amount of DMF

Preparation:

A mixture of dry DCM ($100cm^3$), dendron **8** (2.20g , 2.35×10^{-3}mol), and a catalytic amount of DMF were added to a two–necked $150cm^3$ flask, then thionyl chloride ($0.24cm^3$, 3.3×10^{-3}mol) dissolved in dry DCM ($10cm^3$) was added drop wise over a period of 20 minutes to the mixture with stirring at room temperature. 30 minutes after the addition of $SOCl_2$ the reaction was complete (TLC, 5:1 Hexane: ethyl-acetate). Water ($100cm^3$) was added drop-wise to destroy the excess $SOCl_2$. The resultant solution was washed with water ($30\ cm^3$) and extracted with DCM ($3 \times 10cm^3$), dried over $MgSO_4$, and distilled under reduced pressure to remove the solvent. The product was purified using flash chromatography (SiO_2) eluting with hexane: ethyl acetate (5:1, v/v). The product was a beige semi-solid oil (1.8g,78%).

^{1}H NMR (ppm):

1.3 (t, 12H, $\boldsymbol{CH_3}CH_2)_4$, J=7.2Hz), 1.46 (overlapped peaks, 8H, $(\boldsymbol{CH_2}CH_2)_4$, 1.66 (overlapped peaks, 16H, $(\boldsymbol{CH_2}CH_2CH_2)_8$, 2.3 (t, 8H, $\boldsymbol{CH_2}COOR)_4$, J=7.4Hz), 3.89 (t, 8H, $(\boldsymbol{CH_2}OAr)_4$ J=6.22 Hz), 4.06 (q, 8H, $(\boldsymbol{CH_2}CH_3)_4$, J= 7.2 Hz), 4.65 (s, 2H, $\boldsymbol{CH_2}Cl$), 4.9(s,2H,Ar-$\mathbf{CH_2}$O-Ar), 6.30 (*d*, 2H, 4-**ArH**)$_2$, 6.26 (t, 4H, 2,6-***ArH***), 6.32 (t, 2H, 2,6-***ArH*** in lower ring), 6.37 (t, 1H, 4-***ArH***),

^{13}C NMR (ppm):

14.24 ($\mathbf{C}H_3CH_2)_4$, 34 ($\mathbf{C}H_2COOR)_4$, 25.07 ($\mathbf{C}H_2CH_2COOR)_4$, 25.4 ($\mathbf{C}H_2CH_2CH_2COOR)_4$, 29.5 ($\mathbf{C}H_2CH_2OAr)_4$, 65.9 ($\mathbf{C}H_2OAr)_4$, 99.5 (**C**H, 4-Ar)$_3$, 100 (**C**H, 4 in lower ring), 141.2 (**C**-$CH_2OAr)_2$, 139(**C**CH_2Cl), 173(**C,** carbonyl), 158.4 (**C**OR, 3,5-Ar)$_4$, 161 (**C**OCH_2Ar, 3,5-Ar in lower ring)$_2$, 102.5 (**C**H, 2,6-Ar)$_4$, 105 (**C**H, 2,6-Ar in lower ring)$_2$, 140 (**C**CH_2Cl, 1-Ar), 47 ($\mathbf{C}H_2Cl$)

ν_{max} (KBr)

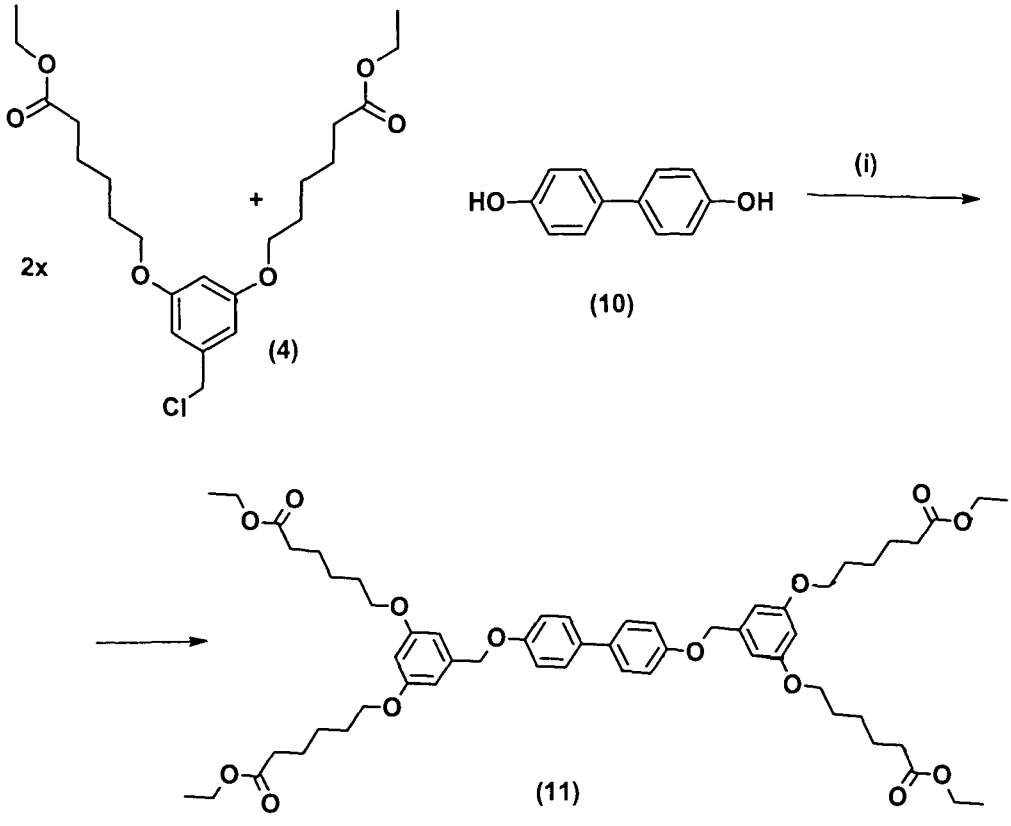
2972 , 2916 (R-CH_3), 2940,2870 (CH, alkane), 2936 , 2916 (R$(CH_2)_4$-O), 1750 , 1725 (C=O), 1732,1712 (C=O, ester), 1598 (C=C), 1454 (C-H aromatic), 1374,1322,1296 (CO aryl ether), 1310,1210 (Ph-O-C, ether), 1162 (O-C=C), 832 (C-H bend), 716 (C-Cl), aryl ether) cm^{-1}

High resolution mass spectrometry:

Calculated mono isotopic mass (M+H):971.4923

Found mono-isotopic mass: 971.4971

2.2.7. Synthesis of the first generation biphenyl dendrimer $(EtO_2C)_4$-G1-2C,(11).



(i) Anhydrous potassium carbonate

18- crown 6, butanone

Preparation:

To a 150cm^3 round bottomed flask equipped with a condenser and nitrogen bubbler, was added butanone (100cm^3), the chlorinated dendrite **4** (6g, 0.0136mol), 4,4'-dihydroxybiphenyl (1.3g, 6.78 x 10^{-3}mol), 18-crown 6 (0.7g , 2.72 x 10^{-3} mol), and potassium carbonate (4.5g , 0.033mol). The solution was refluxed with constant stirring under nitrogen for 72 hours. Once the formation of product was confirmed by TLC (hexane: ethyl acetate, 8:2), the

solution was filtered, washed with water (30 cm^3) and extracted by DCM (3 x 10cm^3), dried over $MgSO_4$, and evaporated under reduced pressure. The product was further purified using silica gel, eluting with hexane: ethyl acetate, 8:2, the product was a beige liquid (8.8g , 65%).

^{1}H ($CDCl_3$,δ ppm):

1.2(t, 12H, (C***H***$_3$CH_2)$_2$, J=7.2Hz), 1.41 (overlapped peaks, 8H, (C***H***$_2$CH_2)$_2$, 1.60 (overlapped peaks, 16H, (C***H***$_2$CH_2CH_2), 2.26 (t, 8H, C***H***$_2$COOR)$_4$, J=7.4Hz), 3.8(t, 8H, (C***H***$_2$OAr)$_4$, J=6.41 Hz), 4.06 (q, 8H, (C***H***$_2$OCOR)$_4$, J= 7.2 Hz), 5.25 (s, 4H, (ArC***H***$_2$OAr)$_2$, 6.35 (d, 2H, 4-Ar***H***)$_2$, 6.26 (t, 4H, (2,6-Ar***H***)$_2$, J=2.38 Hz), 6.95 (d, 4H, (2,6-Ar***H*** in biphenyl)$_4$, 7.8 (d, 4H, (3,5-Ar***H*** in biphenyl core)$_4$

^{13}C NMR (ppm):

14.2 (**C**H_3CH_2), 33.9 (**C**H_2COOR), 24.6 (**C**H_2CH_2COOR), 25.06 (**C**H_2CH_2CH_2COOR), 29.3 (**C**H_2CH_2OAr), 68.4 (**C**H_2OAr), 102 (**C**H, 4-ArH), 159 (**C**OR, 3,5-Ar), 104 (**C**H, 2,6-ArH), 142 (**C**CH_2OAr, 1-ArH), 71.5 (ArCH_2OAr), 173 (**C**, carbonyl), 61.2 (**C**H_2CH_3), 113.6 (**C**, 2,6-Ar biphenyl core), 160 (C, 1-Ar biphenyl core), 130 (C, 3,5-Ar biphenyl core), 129 (**C**, 4-Ar biphenyl core)

ν_{max} (KBr):

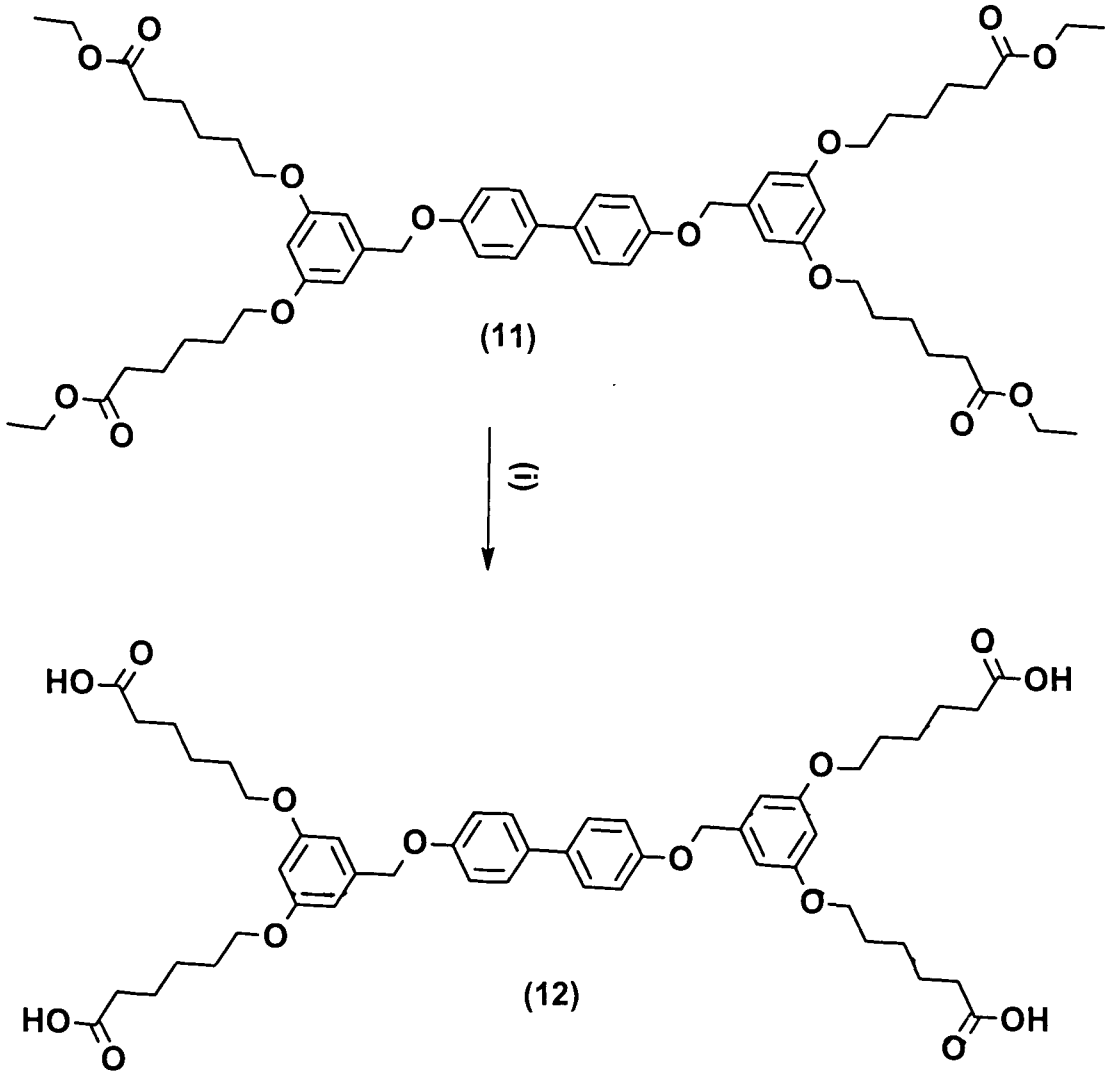
29539 , 2869 (R-CH_3), 1732.94 (C=O of ester), 1596 (C=C), 1456 (C-H), 1374 , 1295. (CO, aryl ether), 1162 (O-C=C), 824 (C-H bend) cm^{-1}

High resolution mass spectrometry:

Calculated mono-isotopic mass: 998.5361

Found mono-isotopic mass: 998.5339

2.2.8. Hydrolysis of first generation biphenyl dendrimer: $(HOOC)_4$-G2-2C,(12).



(i) Potassium hydroxide , Ethanol, THF

Distilled water, H_3O^+

Preparation:

To a 150cm^3 two necked flask equipped with a condenser and nitrogen bubbler, the bi-phenyl dendrimer **11** (4.7g, 4.68 x 10^{-3} mol) dissolved in 20cm^3 (ethanol, THF 10:10 v/v), KOH (4g, 0.075mol) dissolved in distilled water (20cm^3) were added with constant stirring at 45 ^{0}C temperature. After 4 hours under reflux, the reaction was found to be complete (TLC: hexane: ether 6:4).

The product was cooled and then added to distilled water ($100cm^3$), concentarated HCl was added with constant stirring, until the solution was acidic; it was then washed with water ($30cm^3$) and extracted using DCM (3 x $10cm^3$), dried over $MgSO_4$,and put under reduced pressure, to remove the solvent. The product was a beige semi-solid (2.7g, 65%).

^{1}H ($CDCl_3$,δ ppm):

12.4 (s, 4H, COO***H***), (over-lapped peaks, 8H, (C***H***$_2$CH$_2$)$_2$, 1.60 (overlapped peaks, 16H, (C***H***$_2$CH$_2$CH$_2$), 2.26 (t, 8H, C***H***$_2$COOR)$_2$, J=7.4Hz), 3.8 (t, 8H, (C***H***$_2$OAr)$_2$, J=6.42 Hz), 5.20 (d, 4H, ArC***H***$_2$OAr), 6.25 (d, 4H, 2, 4-Ar***H***), 6.30 (d, 2H, 4-Ar***H***), 6.84(d, 4H, 2,6-Ar***H*** in core), 7.65 (d, 4H, 3,5-Ar***H*** in core),

^{13}C NMR (ppm):

34.9 (**C**H$_2$COOH), 24.6 (**C**H$_2$CH$_2$COOH), 25.06 (**C**H$_2$CH$_2$CH$_2$COOH), 29.3 (**C**H$_2$CH$_2$OAr), 68.4 (**C**H$_2$OAr), 102 (**C**H, position 2,6-Ar), 159 (**CH**, 1 Ar), 158.5(**C**OR, 3,5-Ar) 99.6 (**C**H, 4-Ar), 142 (**C**CH$_2$OAr, 1-Ar), 143.4 (**C**H$_2$OAr), 173 (**C**, carbonyl), 128 (**C**, 2,6-Ar, biphenyl core), 116 (**C**, 3,5-Ar, biphenyl core), 159 (**C**, 4-Ar, biphenyl core),126 (**C**, 1-Ar)

ν_{max} (KBr)

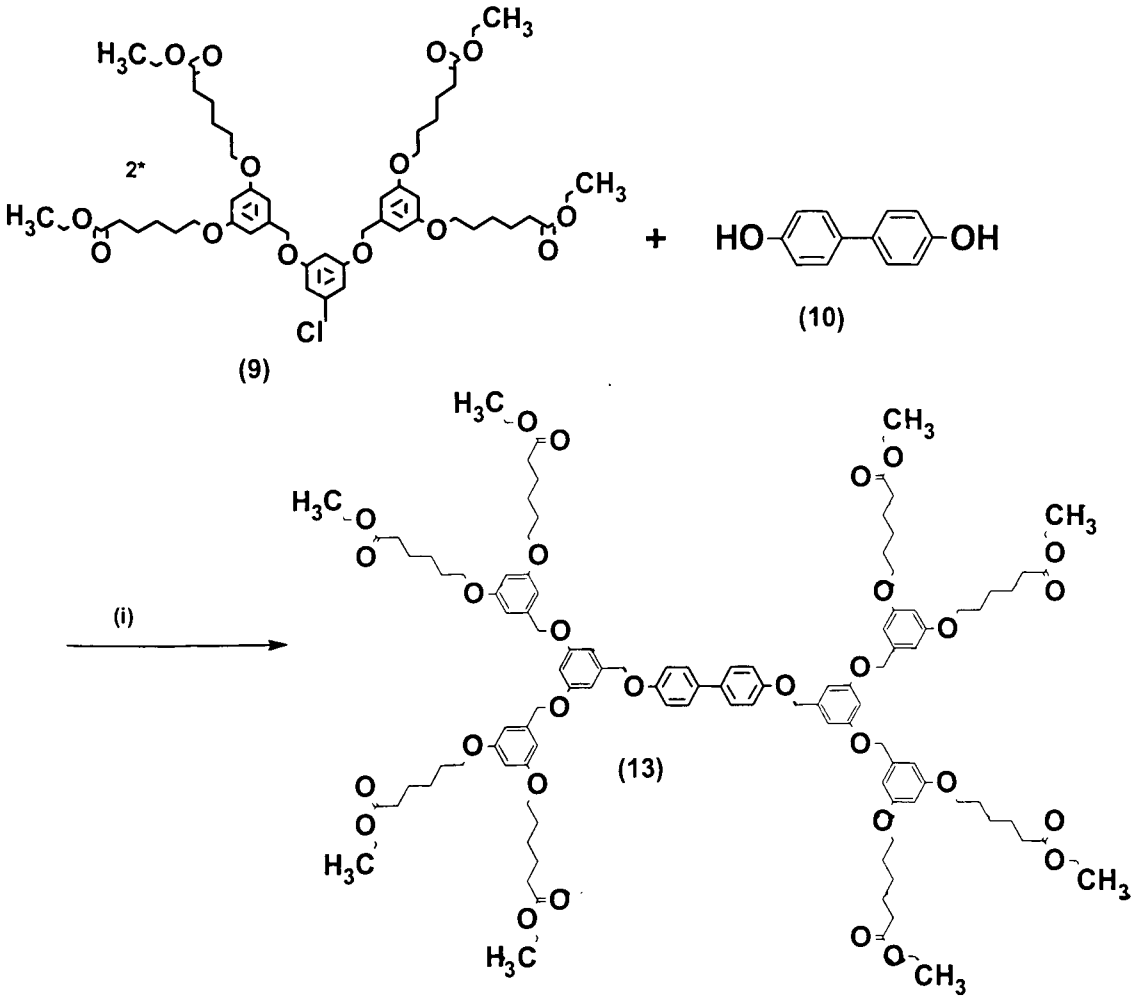
29539 , 2869 (R-CH$_3$), 1705 (-COOR of ester), 1596.68 (C=C), 1456.54 (C-H), 1374.56,1295.62 (CO, aryl ether), 1162 (O-C=C), 824 (C-H bend) cm^{-1}

High resolution mass spectrometry:

Calculated mono-isotopic mass: 886.4139

Found mono-isotopic mass: 886.4100

2.2.9. Synthesis of second generation biphenyl dendrimer $(EtO_2C)_8$-G2-2C,(13).



(i) Anhydrous potassium carbonate, Butanone

18-crown6

Preparation:

To a 250cm^3 round bottomed flask fitted with a condenser and nitrogen bubbler, were added butanone (150cm^3), the chlorinated dendron **9** (2g , 2.1 x 10^{-3}mol), K_2CO_3 (0.4g , 0.362mol), 4,4'–di-hydroxybiphenyl (0.2g, 1.05x10^{-3}mol), and 18-crown6 (0.1g , 2.1 x 10^{-4}mol). The resultant solution was

refluxed with constant stirring under nitrogen for four days, then washed with water (30 cm^3) and extracted with DCM (3 x 10cm^3), dried over $MgSO_4$, and evaporated under reduced pressure. The product was then purified with flash chromatography (SiO_2) eluting by hexane: ether (4:6); to give beige oil (1.4g, 65%).

^{1}H NMR (ppm):

1.25 (t, 24H, $(CH_3CH_2)_8$, J=7.2Hz), 1.46 (overlapped peaks,16H,$(CH_2CH_2)_8$, 1.66 (overlapped peaks, 32H, $(CH_2CH_2CH_2)_{16}$, 2.3 (t, 16H, $CH_2COOR)_8$, J=7.4Hz), 3.89 (t,16H, $(CH_2OAr)_8$, J=6.2 Hz), 4.06 (q, 16H, $(CH_2CH_3)_8$, J= 7.2 Hz), 6.30 (t, 6H, 4-ArH)$_6$, 6.25 (t,12H, 3,5-ArH)$_{12}$, 5.20 (s, 12H, $(ArCH_2OAr)_6$), 6.85 (d, 4H, CH, 3,5-Biphenyl core)$_4$, 7.60 (d, 4H, CH, 2,6-Biphenyl core)$_4$

^{13}C NMR (ppm):

14.34 (CH_3CH_2), 34.32 (CH_2COOR), 24.8 $(CH_2CH_2COOR)_8$, 25.07 ($CH_2CH_2CH_2COOR$), 28.99 (CH_2CH_2OAr), 65.9 (CH_2OAr), 60.35 (CH_2CH_3), 99.5 (CH, 4-Ar), 99.3 (C',H, 4-Ar),158 (COR, 3,5-Ar), 102.5 (CH, 2,6-Ar), 142.2 (C' ,1-Ar), 71.5 ($ArCH_2OAr$), 72 (CCH_2OBiphenyl), 159.5 (C, 4-Biphenyl), 114.8 (C, 3,5-Biphenyl core), 129 (C, 2,6-Biphenyl), 128.5 (C, 1-Biphenyl), 173.2 (C, Carbonyl)

ν_{max} (KBr)

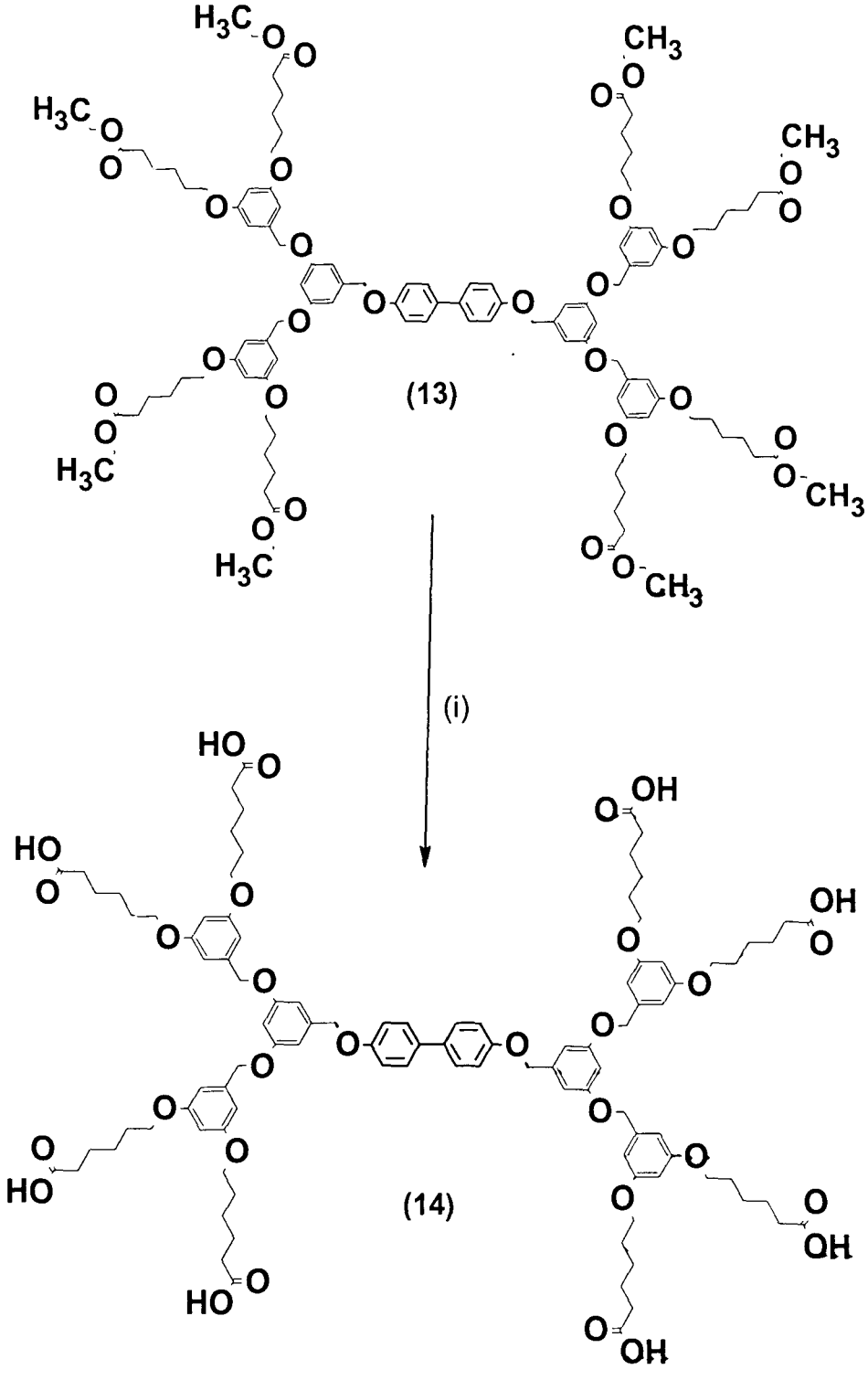
29539 , 2869 ($R-CH_3$), 1732 (C=O), 1596.68 (C=C), 1456.54 (C-H), 1374.56 ,1295.62 (CO, aryl ether), 1162 (O-C=C), 824 (C-H bend) cm^{-1}

High resolution mass spectrometry:

Theoretical isotopic mass: 2055.0837

Found isotopic mass: 2055.0800

2.2.10. Hydrolysis of biphenyl dendrimer (13) to: $(HOOC)_8$-G2-2C),(14).



(i) Potassium hydroxide, Ethanol-THF, H_2O, H_3O^+

Preparation:

To a 150cm^3 two necked flask equipped with a condenser and nitrogen bubbler, was added biphenyl dendrimer **13** (1.88g, 9.18 x 10^{-4}mol) dissolved in 20cm^3 (ethanol : THF , 1:1), KOH (2g , 0.04mol) dissolved in distilled water (20cm^3) with constant stirring at room temperature, After 4 hours the reaction was complete (TLC: hexane: ether 6:4). The mixture was then cooled and added to distilled water (120cm^3). Concentrated HCl was added with constant stirring, until the solution was acidic. It was then extracted using DCM (3 x 10cm^3), dried over $MgSO_4$, and put under reduced pressure, to remove the solvent. The product was a dense beige semi-solid (1.1g, 65%).

^{1}H NMR (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, 12H, C***H2***, 2,6-Ar), 5.20 (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 in lower ring), 103.5(C , 2,6-Ar in lower ring), 143 (C , 1-Ar in lower ring), 159.5 (**C** , 4 in Biphenyl), 129 (**C** ,2,6 in Biphenyl), 114.8 (**C**, 3,5 in Biphenyl core), 173.2 (**C**, Carbonyl)

ν_{max} **(KBr)**

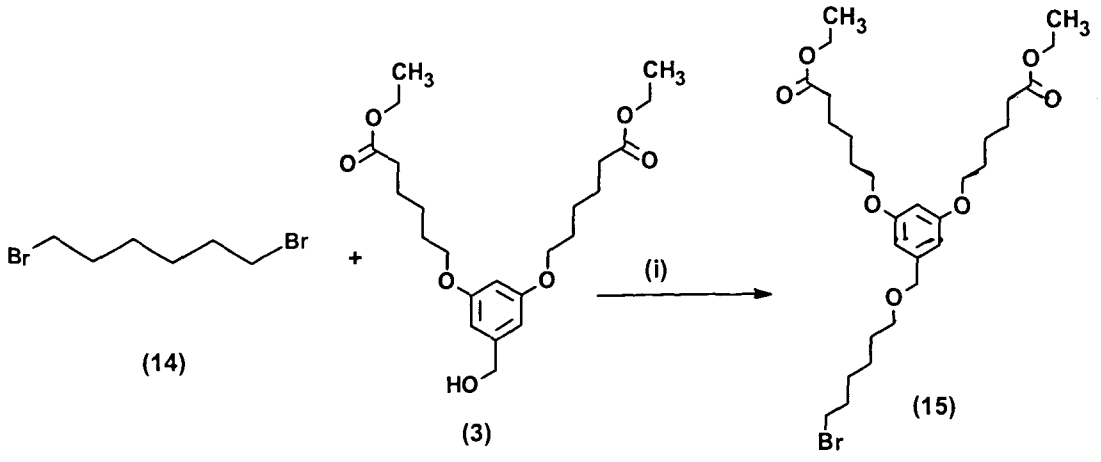
Broad peak 3200, 3600 (COOH), shoulder peak at region about 2800-3000(acid characteristic), 1707 (C=O), 1596.68 (C=C), 1456.54 (C-H), 1374.56, 1295.62 (CO, aryl ether), 1162 (O-C=C), 824 (C-H bend) cm^{-1}

High resolution mass spectrometry:

Calculated isotopic mass: 1830.8334

Found isotopic mass: 1830.8342

2.2.11. Preparation of 6-[3-(6-bromo-hexyloxymethyl)-5-(5-ethoxycarbonyl-pentyloxy)-phenoxy-hexanoic acid ethyl ester. (15).



(i) Dry diethyl ether, 18-crown 6

Anhydrous potassium tert-butoxide

Preparation:

To a $250cm^3$ round bottomed flask equipped with a guard tube, were added diethyl ether ($150cm^3$) , the first dendrite **3** (9g , 0.0212mol), potassium *tert*-butoxide (9.43g, 0.0848mol), and 18-crown 6 (1.2g , 4.24×10^{-3}mol) and the

mixture was stirred for 15 minutes. 1,6-dibromobutane (22.3cm^3, 0.0848mol) was poured into the mixture and it was stirred for 48h at room temperature, then it was washed with water (30 cm^3), filtered, extracted using diethyl ether (3 x 10cm^3), dried over $MgSO_4$, and evaporated under reduced pressure to give the product, which was purified by flash chromatography (SiO_2) eluting by hexane : ethyl acetate (20:1). The product was a very light yellowish oil (0.7g, 6%).

^{1}H NMR (ppm):

1..30 (t, 6H, $(CH_3CH_2)_2$, J=7.4Hz), 1.29-1.71 (overlapped peaks, 12H, $(CH_2CH_2CH_2)_2$, 1.5-1.8(overlapped peaks, 8H, $(CH_2CH_2)_6$, 2.58 (t, 4H, $CH_2COOR)_2$, J=7.4Hz), 3.32(t, 2H, (CH_2OR), J=6.77 Hz), 3.4 (t, 2H,CH_2Br, J=6.59), 3.58 (t, 4H, $CH_2OAr)_2$, J=6.41), 3.35 (d,2H, CH_2OCH_2Ar),4.1 (4H, CH_2CH_3, J=7.41)$_2$, 4.65 (s, 2H, Ar CH_2OR), 6.27 (t, 2H,C*H*, 2,6-Ar*H*)$_2$ 6.38 (1H, d, C*H* , 1-Ar*H*).

^{13}C NMR (ppm):

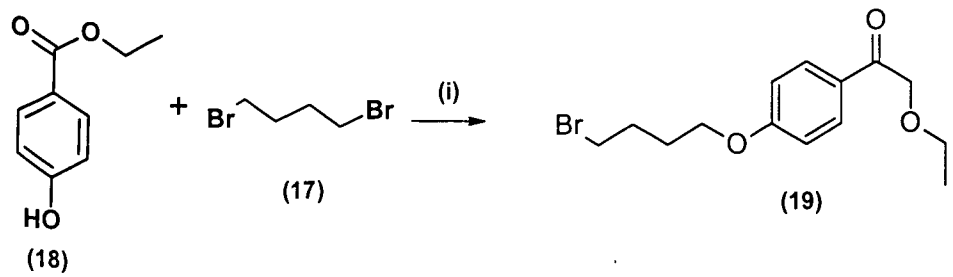
14.31 (CH_3CH_2), 33.9 (CH_2COOR), 25 (CH_2CH_2COOR), 25.3 ($CH_2CH_2CH_2COOR$), 29.3 (CH_2CH_2OAr), 61.6(CH_2CH_3), 68.8 (CH_2OAr), 99.7 (CH, 1-Ar), 157.9 (CH_2, 2,6-Ar), 103.1(CH, 3,5-Ar), 138.8 (**C,** position 4-Ar), 75.5 ($ArCH_2OR$), 72(CH_2OCH_2Ar), 30 ($CH_2CH_2OCH_2Ar$),24.9 ($CH_2CH_2CH_2OCH_2Ar$), 33.7 (CH_2Br), 32.6 (CH_2CH_2Br), 173.1(**C**, carbonyl)

MS: m/z :586- 588 m$^+$

ν_{max} (KBr)

2935.35 , 2959.23 , 2857.32 (RCH_3), 1733.10 C=O,(ester), 1596.01(C=C), 1216.85 (C-C=O-O), 727.61 (CBr), cm^{-1}

2.2.12. Preparation of 4-(4-bromo-butoxy)-benzoic acid ethyl ester, (18).



(I) Anhydrous Potassium carbonate, 18-crown 6

Butanone

Preparation:

To a 150cm^3 round bottomed flask equipped with a condenser and nitrogen bubbler, were added 100cm^3 dry diethyl ether , ethyl 4-hydroxybenzoate (5g, 0.03mol), 18 crown-6 (1.6g , 6 x 10^{-3}mol), and potassium tert-butoxide (8.44g , 0.0752 mol), under constant stirring. After 15 min 1,4-dibromobutane (8.9cm^3 , 0.75mol) was added to the flask, and stirring continued for 48 hours, TLC (hexane: ethyl acetate 9:1), the product was purified by flash chromatography, eluting with hexane : ethyl acetate 9:1, then using Kugelrohr to remove excess 1,4-dibromobutane. The product was dense light yellowish oil (4.9g, 55%).

^{1}H NMR (ppm):

1.35(t, 3H, C***H***$_3$CH$_2$, J=7.2Hz), 1.9-2.1(overlapped peaks, 4H, C***H***$_2$), 3.4 (t, 2H, C***H***$_2$Br, J=6.3) , (4.06, t, 2H, C***H2***OAr, J=6.4), 4.3 (q, 2H, C***H***$_2$CH$_3$), 7.0 (d,2H, C***H***, 3,5-Ar), 7.8 (d, 2H,C***H***, 2,6-Ar),

^{13}C NMR (ppm):

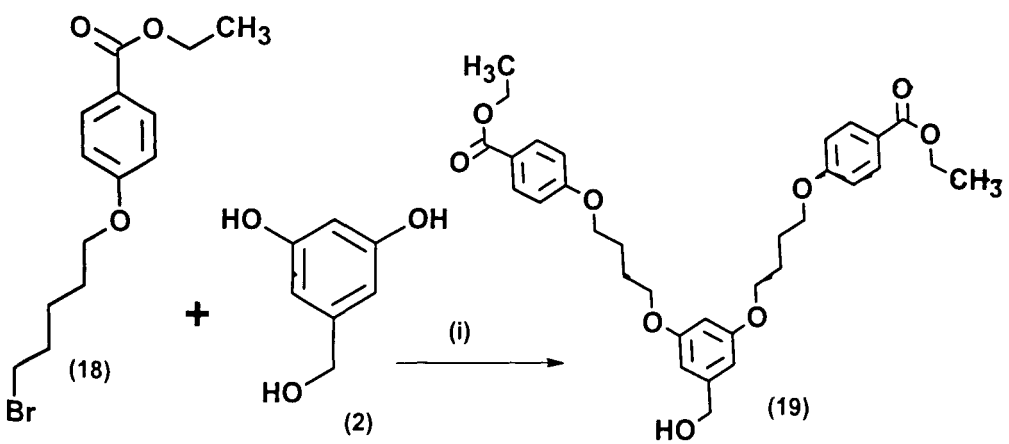
14.2 (**C**H$_3$CH$_2$), 60 (**C**H$_2$CH3), 166 (**C**, Carbonyl), 121.8 (**C**, 4-Ar), 130.5 (**C**, 2, 6- Ar), 114.2 (**C**H, 3, 5-Ar), 161.8 (**C**, position 4-Ar), 67 (**C**H$_2$OAr), 28.6 (**C**H$_2$CH$_2$OAr), 28.9 (**C**H$_2$CH$_2$Br), 32.6 (**C**H$_2$Br), 165.8 (**C**, Carbonyl)

MS: m/z: 302-304 M^+

ν_{max} **(KBr)**

2972, 2952 (R-CH$_3$), 2936, 2916 (AlkanesR'-CH$_2$-R''), 2936, 2916 (R' (CH$_2$)$_4$O), 1750, 1735 (ester, R-CO-OR, C=O), 1740, 1715 (ester, Ph -CO-O-OR, C=O), cm^{-1}

2.2.13. Diethyl-4,4'-(5-(hydroxymethyl)-1,3-phenylene)bis(oxy)bis(butane-4,1-diyl-1))bis(oxy)dibenzoate,(19).



(i) Anhydrous potassium carbonate

Butanone, 18-crown- 6

Preparation

To a 150cm^3 round bottomed flask equipped with a condenser and nitrogen bubbler, were added butanone (100cm^3), 3,5-dihydroxybenzyl alcohol (2g , 0.014mol), 4-hydroxyethylbenzoate (4.3g , 0.014), and potassium carbonate (4.6g , 0.034). The resultant solution was refluxed with constant stirring under nitrogen for 48 hours. After the presence of product had been confirmed by TLC (hexane: ethyl acetate – 9.5, 0.5), the solution was filtered, washed with water (30 cm^3), extracted using DCM (3 x 10cm^3), dried over $MgSO_4$, and put under reduced pressure to evaporate the solvent. The product was purified by flash chromatography (SiO_2) using hexane/ethyl acetate (9.5:0.5) as the eluent. The product was very light yellowish oil (5.5g, 65%).

^{1}H NMR (ppm):

1.3(t, 6H, (CH_3CH_2)$_2$, J=7.14Hz), 1.89-1.92 (overlapped peaks, 8H,(CH_2)$_4$), 4.3(q, 4H,CH_2CH_3 ,J=7.116) , 7.90 (d, 2H, CH, 2,6-Ar, J=6.42), 7.05 (d, 2H, CH, 3,5- Ar), 4.2 (t, 4H, CH_2OAr, J =7.4, higher ring), 4.2 (t, 4H, CH_2OAr, J=7.4 in lower ring), 6.30 (s, 1H, 1-Ar, in lower ring), 6.26 (d, 2H, CH, 3,5-Ar), 4.80 (s, 2H, CH_2OH), 5.38 (s, 1H, OH)

^{13}C NMR (ppm):

14.2 (CH_3CH_2), 60.8 (CH_2CH_3), 166 (C, Carbonyl), 121.8 (C,para Ar), 130.5 (C,2,6 Ar), 114.2 (CH, 3,5 Ar), 161.8 (C, position 4, Ar), 68.5(CH_2O, higher ring), 68.5 (CH_2O, lower ring), 28.6 (CH_2CH_2OAr, higher ring) 28.6 (CH_2CH_2OAr, in lower ring), 68.5 (CH_2OAr), 99.5 (CH, Para Ar), 102.6 (COR, 2,6 Ar), 142.4 (CCH_2OH, 4 Ar). 68.6(CH_2OH)

MS: m/z : 580

ν_{max} **(KBr)** :

3450 , 3200 (OH), 2972 , 2952 (R-CH_3), 2936 , 2916 (AlkanesR'-CH_2-R''), 2936 , 2916 (R'$(CH_2)_4$O) , 1710 (ester, R-CO-OR, C=O), 1606 (carbonyl),1260 (OH), 1510.71 (C=C aromatic) cm^{-1}

2.3. PART B:

Physico-chemical properties of the dendrimer: $(HOOC)_6$-G1-3C,(7).

2.3.1. Solubility testing:

The solubility of each dendrimer was determined in buffer solutions at various pHs. The various buffer solutions were prepared by literature methods (**Table 3.1).**

2.3.2. Preparation of Buffer Solutions

The recipe for preparing buffers at different pH values were taken from the following site < www.Liv.ac.uk/buffercalc.html> in APPENDIX 1

2.3.3. Procedure:

0.1g of each hydrolysed dendrimer, e.g. $(HOOC)_6$-G1-3C,**(7).** was added in $50cm^3$ of each buffer solution, and the resultant solutions were then stirred for 2 hours, filtered and their absorbance were measured at the λ =276nm (Stock solution 1), $50cm^3$ of stock solution 1 was taken and put in-to a $100cm^3$ flask

and made up to the mark with appropriate buffer (stock solution 2) and the absorbance was measured.

2.3.4. Absorptivity calculation of $(HOOC)_6$-G1-3C,(7).

In order to find the maximum solubility of a dendrimer in pH 7.4, one must first find the absorptivity which is the gradient of a calibration graph of absorbance against concentration. In the first step, a saturated solution of hydrolysed dendrimer, (e.g. compound 7) was made in 50 ml of buffer at pH 7.4; then in the next step a series of solutions of concentration between 0.02 to 0.12M were made. Using the absorbance of 0.1g compound 7 in buffer 7.4 (1.43), the dilution factor for each concentration can be calculated. For example the dilution factor for solution of 0.02 M was calculated as → 1.43 / 0.02 = 7.15. As every cuvette has a $3.5cm^3$ capacity on dividing the capacity of cuvette by the dilution factor the amount of hydrolyzed dendrimer from stock solution (2) can be found; then in the next stage the remained of the cuvette was filled with buffer solution (pH=7.4). Other concentrations were calculated in the same way. Once the absorbance at each concentration was calculated, plotting absorbance against concentration gave a straight line of which the gradient was the Absorptivity (Table 3.2, 3.3 and fig 3.14)

2.3.5. CMC by Dye encapsulation,(7).

The first step in this procedure was to make a non-polar dye solution with a specific concentration, for example 5mg/ml; in order to make this, 75mg of dye powder was dissolved in $15cm^3$; of chloroform and dissolved thoroughly,

then by the mean of a micro-pipette 300μL of dye solution was added to 13 stoppered tubes one at a time and swirled gently for 2-3 minutes in the fume hood until the chloroform had evaporated. At this stage using burettes, different amounts of surfactant (hydrolyzed dendrimer) from $1cm^3$ to $10cm^3$ was added to each tube, the tubes were shaken for 2 minutes to disperse the powder, the sample tubes were placed in an oven set at 40 ^0C for 20 minutes or until the surfactant solutions showed a gradation in colour due to dye uptake. All the samples were taken out of the oven and allowed to equilibrate at room temperature. Using a clean membrane filter and plastic syringe for each set of solutions starting with the most dilute, undissolved dye was filtered off and then transferred directly into the cuvette. Finally the absorption of each solution at 518nm was measured. Each measurement was repeated three times and then their average and standard deviations were calculated. A graph was obtained by plotting the absorbance at 518nm of dye against concentration (mg/ml) which was used to find the CMC. All the measurement are shown in table 3.4 and fig 3.15

2.3.6. CMC by surface tension for $(HOOC)_6$-G1-3C,(7).

All the glassware in this experiment was cleaned thoroughly, rinsed with distilled water and acetone and dried in a drying cabinet. $25cm^3$ of distilled water was pipetted into a $100cm^3$ beaker. By means of a burette, 1ml of the main stock solution (hydrolyzed dendrimer dissolved at pH 7.4) was added and the surface tension was noted each time the ring was detached. A total volume of $50cm^3$ of the main stock solution was used. The platinum ring was

heated to redness between each trial. Each measurement was repeated three times (table 3.5) the plot of surface tension against concentration shows the critical micelles concentration (fig 3.16).

2.3.7. CMC by molar conductivity for $(HOOC)_6$-G1-3C,(7).

All glassware was cleaned thoroughly, rinsed with distilled water and acetone, and dried in a drying cabinet. $25cm^3$ of distilled water was pipetted into a $100cm^3$ beaker and its conductance measured. By the means of a burette $2cm^3$ of the main surfactant stock solution was added and the conductance noted after each addition. The conductance of a 0.100 M KCl solution was also measured to find the cell constant value. Each experiment was repeated three times, the results shown in table 3.6 – 3.7. The plot of molar conductivity against concentration gives the critical micelle concentration (**fig 3.17**) at the point of change of slope.

2.3.8. pH profile for $(HOOC)_8$-G2-2C,(14).

The procedure was the same as in 2.3.3 $(HOOC)_6$-G1-3C. (**Table : 3.8**)

2.3.9. Surface tension for $(HOOC)_8$-G2-2C ,(14).

The procedure was the same as in 2.3.6. $(HOOC)_6$-G1-3C. (**Table 3.11 and fig 3.19**). For $(COOH)_8$-G2-2C; 0.1 g of hydrolyzed biphenyl dendrimer (Rmm = 1382.07 g/mol) was dissolved completely in $67cm^3$ of buffer at pH

7.4; therefore the concentration of hydrolyzed biphenyl dendrimer was calculated as $1.08x10^{-3}$ M.

2.3.10. Absorptivity measurement for $(HOO_2C)_8$-G2-OH,(14).

The procedure was the same as in 2.3.4. $(HOOC)_6$-G1-3C (**Table 3.9 – 3.10 and fig 3.18**)

2.3.11. CMC by molar conductivity for $(HOOC)_8$-G2-2C,(14).

The procedure was the same as in 2.3.7 (HOOC)6–G1-3C

To find the concentration of surfactant the formula $C_1 \times V_1 = C_2 \times V_2$ can be used

Concentration of surfactant in the burette: $8.146x10^{-4}$ M

8.146×10^{-4} M x 0.001dm^3 = C_2 x 0.026dm^3 → C= $3.13x10^{-5}$ (**table 13,12 and fig 3.20**)

2.3.12. CMC by Dye encapsulation $(HOOC)_8$-G2-2C ,(14).

The procedure was the same as in 2.3.5 for compound 7. (HOOC)6–G1-3C

All the measurements are shown in the **table 3.13 and fig 3.21**

2.3.13. pH Profile for: $(HOOC)_4$-G1-2C,(12).

Procedure was the same as 2.33, $(HOOC)_6$-G1-3C. **Table 3.15**

2.3.14. Absorptivity calculation for $(HOOC)_4$-G1-2C,(12).

The procedure was the same as 2.3.4, $(HOOC)_6$-G1-3C,(7). **Table 3.16, 3.17 and fig 3.22**

2.3.15. Surface tension procedure for $(HOOC)_4$-G1-2C,(12).

The procedure was the same as 2.3.6.

Concentration of hydrolyzed dendrimer $(HOOC)_4$-G1-2C $=1.123 \times 10^{-3}$ M

$C_1 \times V_2 = C_2 \times V_2 \rightarrow 1.123 \times 10^{-3} M \times 0.001 dm^3 = C_2 \times 0.026 dm^3 \rightarrow C = 4.15 \times 10^{-5}$. **Table 3.18 and fig 3.23**

2.3.16. CMC by molar conductivity for $(HOOC)_4$-G1-2C,(12).

The procedure was the same as in 2.3.7

Table 3.19, 3.20 and fig 3.24

CHAPTER 3: RESULTS AND DISCUSSION

GENERAL INTRODUCTION

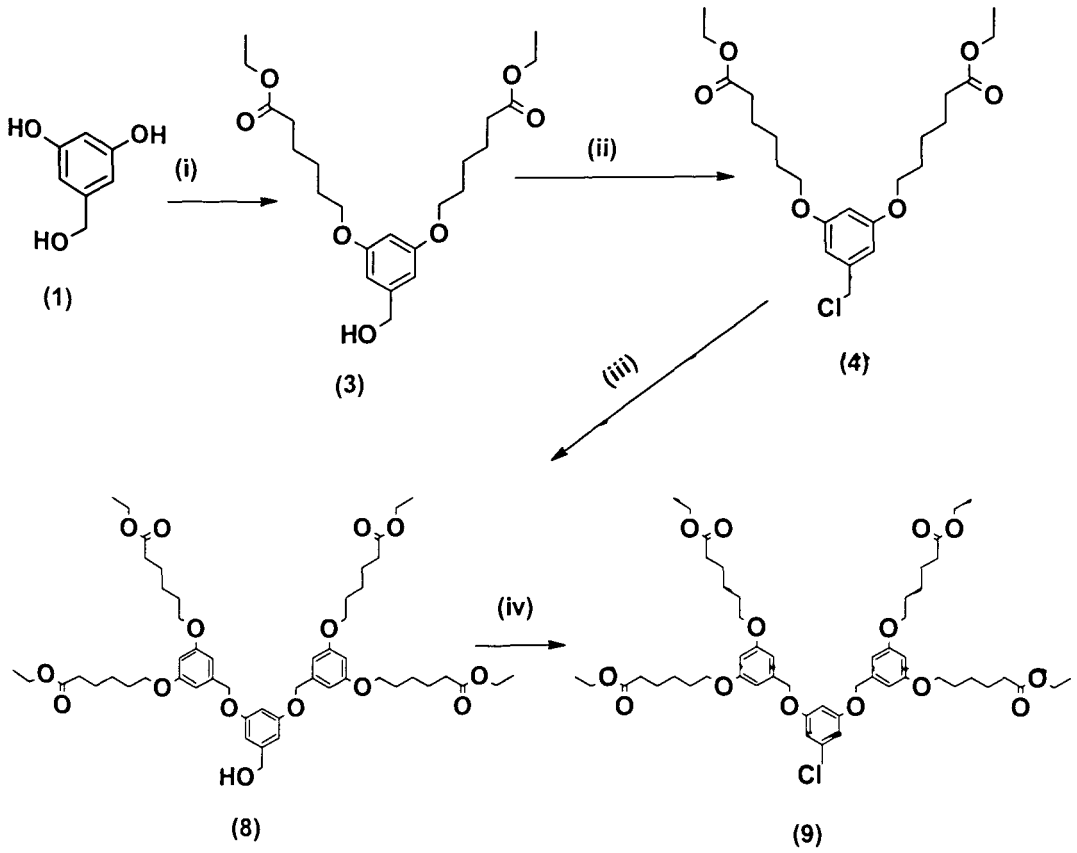
The previous chapter of this thesis has been concerned with the design and synthesis of dendritic macromolecules based on 3,5-dihydroxybenzyl alcohol as a building block and ethyl 6-bromohexanoate as branching unit. This chapter describes the synthesis of these dendritic macromolecules, and then the investigation of the physico-chemical properties of the hydrolysed dendritic macromolecules.

The first part of this chapter will only be concerned with the characterization of dendritic macromolecules synthesized. Their structure was determined as outlined in the experimental chapter, by using MS, IR and NMR spectroscopic techniques.

The synthetic part is described in five different stages. The first stage is the formation of first and second generation dendrons, the second stage describes the formation of the first generation dendrimer, using a tri-phenyl core and the subsequent hydrolysis of the resultant molecules which had their physico-chemical properties investigated as discussed in the second part of this chapter. In the third and fourth stages, the attachment of the first and second generation dendrons to a different kind of core (bi-phenyl) is described and then the resultant products were hydrolysed, using alkaline conditions. The last stage describes the synthesis of a different type of dendrite, and the attachment of a spacer to the first dendrite in order to alter the shape of dendritic macromolecules. The second part of this chapter will deal with the physico-chemical properties of the hydrolysed dendritic macromolecules. The results for solubility, absorptivity and formation of micelles using methods

such as surface tension, dye encapsulation and molar conductivity method will be discussed

3.1. Synthesis of the first and second generation dendrite and dendrons.



i) Anhydrous K_2CO_3, ethyl 6-bromohexanoate, butanone

ii) Thionyl chloride, DMF, dry ~~diethyl ether~~

iii) Anhydrous K_2CO_3, chlorinated dendrite, butanone, 18 crown-6

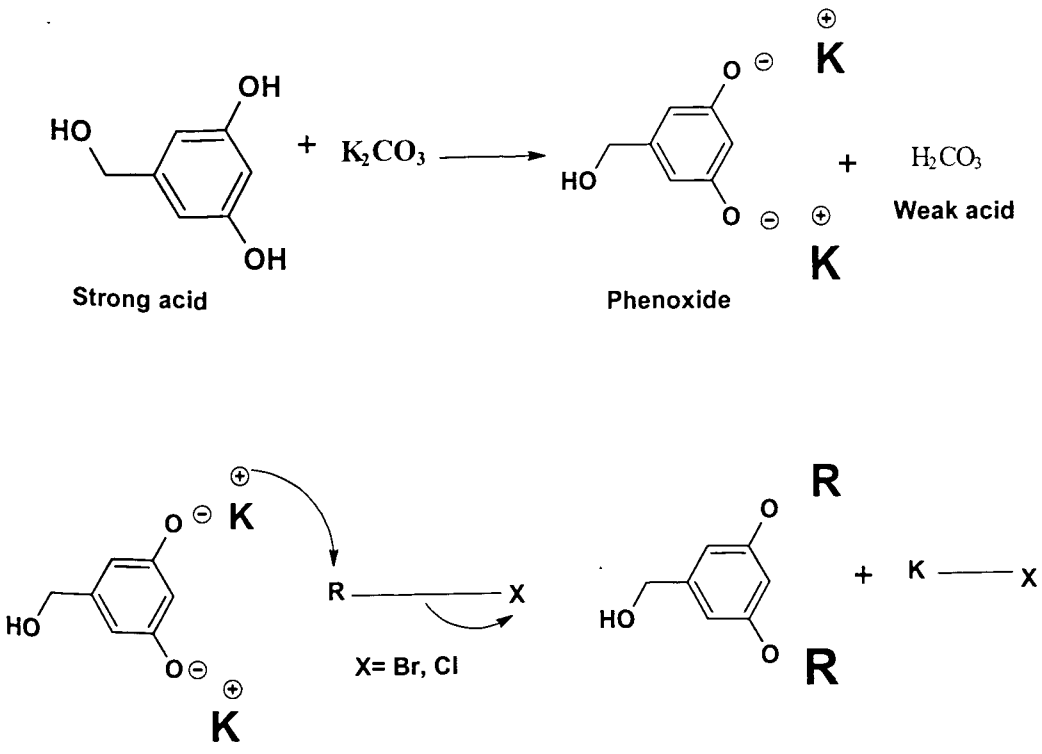
iv) Thionyl chloride, several drops of DMF, dry diethyl ether

The primary aim of this project was to synthesis amphiphilic dendritic macromolecules and then to investigate the possibility of encapsulating drug molecules within their branches. This offers the potential for dendritic

macromolecules to interact with labile or poorly-soluble drugs or dyes. The synthesis started with the reaction of ethyl 6-bromohexanoate with 3,5-dihydroxybenzyl alcohol, using a convergent growth approach. This reaction is a Fréchet type synthesis [171-172]. The reaction sequence in this project involved a generation growth step (formation of first dendrite with methylol group at its focal point) and an activation growth step which was the conversion of benzyl alcohol to benzyl chloride. Using the strategy given in scheme (3.1, III.1) initially two moles of ethyl 6-bromohexanoate were reacted with one mole of 3,5-dihydroxybenzyl alcohol; the reaction is typical example of a Williamson ether synthesis, [173]. The reaction proceeded at an excellent rate and gave a yield of 93%. The Williamson synthesis, discovered in 1850, is still considered the best general method for the preparation of unsymmetrical or symmetrical ethers [174]. It involves the nucleophilic substitution of alkoxide or phenoxide ion for halide ion. The reaction mechanism is shown below in scheme 3.1

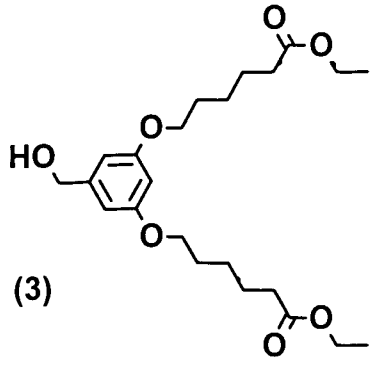
The alkoxide ion reacts with the substrate in an S_N2 reaction, with resulting formation of ether. The substrate must have a good leaving group. Typical substrates are alkyl halides, alkyl sulfonates, and dialkyl sulphates, i.e., [175,176]

Living groups = -Br, -I, $-OSO_2R''$ or $-OSO_2OR'$



Scheme 3.1: Williamson reaction [175]

3.2. Formation of first dendrite: (EtO_2C)-G1-OH,(3).



In this reaction butanone was used as a solvent as its boiling point (80-90 ^{0}C) and polarity were suitable for the reaction. During the experiment, samples were taken every five hours for TLC testing using hexane: ethyl acetate (1: 5). The presence of one single new spot after 48 hours was a good indication of the completion of this reaction. The percentage yield was 93% which

confirmed the good yield and purification produced a good quality sample product. Mass spectrometry, IR, and NMR results provide evidence for the structure of the dendrite. For instance, the proton NMR (fig 3.1) a doublet peak at 4.53 ppm (due to CH_2OH), a peak at 3.8 ppm (due to CH_2OAr) and two peaks at 6.28 ppm and 6.48 ppm (due to ArH) are good indications of the structure of the dendrite; all other peaks at lower chemical shifts in ^1H spectrum are due to the CH_2's and CH_3 in the carbon chain which are similar for both the reactant and product. By comparison of the proton NMR of the reactant and product, the degree of completion of reaction can be monitored.

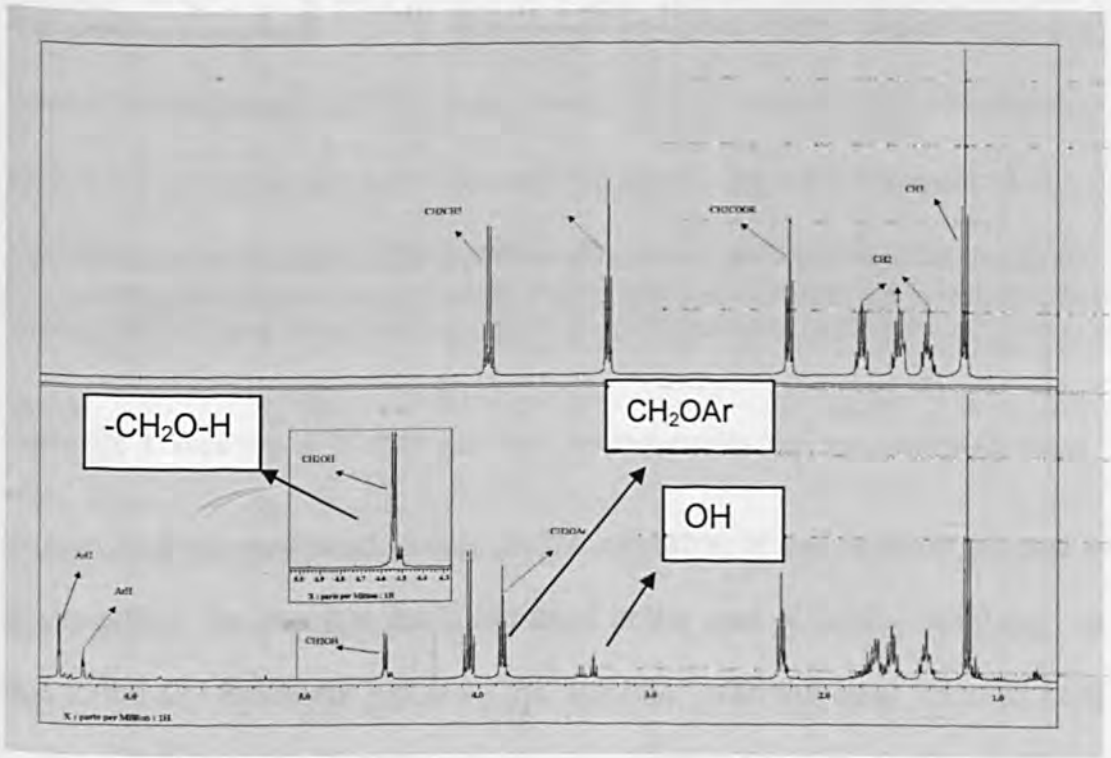


Figure 3.1: Comparison of the proton NMR spectra of ethyl 6-bromohexanoate (top) and dendrite (EtO_2C)-G1-OH),(3).

Carbon NMR (fig 3.2) was also useful to prove the formation of product, for example the peak at 140.2 ppm (C, 1-Ar), 100 ppm (C, 4-Ar), and 158.4 ppm (C, COR 3,5-Ar) positions are all indicate the completion of reaction. By

comparison of the carbon NMR of reactant and resultant product, information regarding the completion and formation of product was obtained.

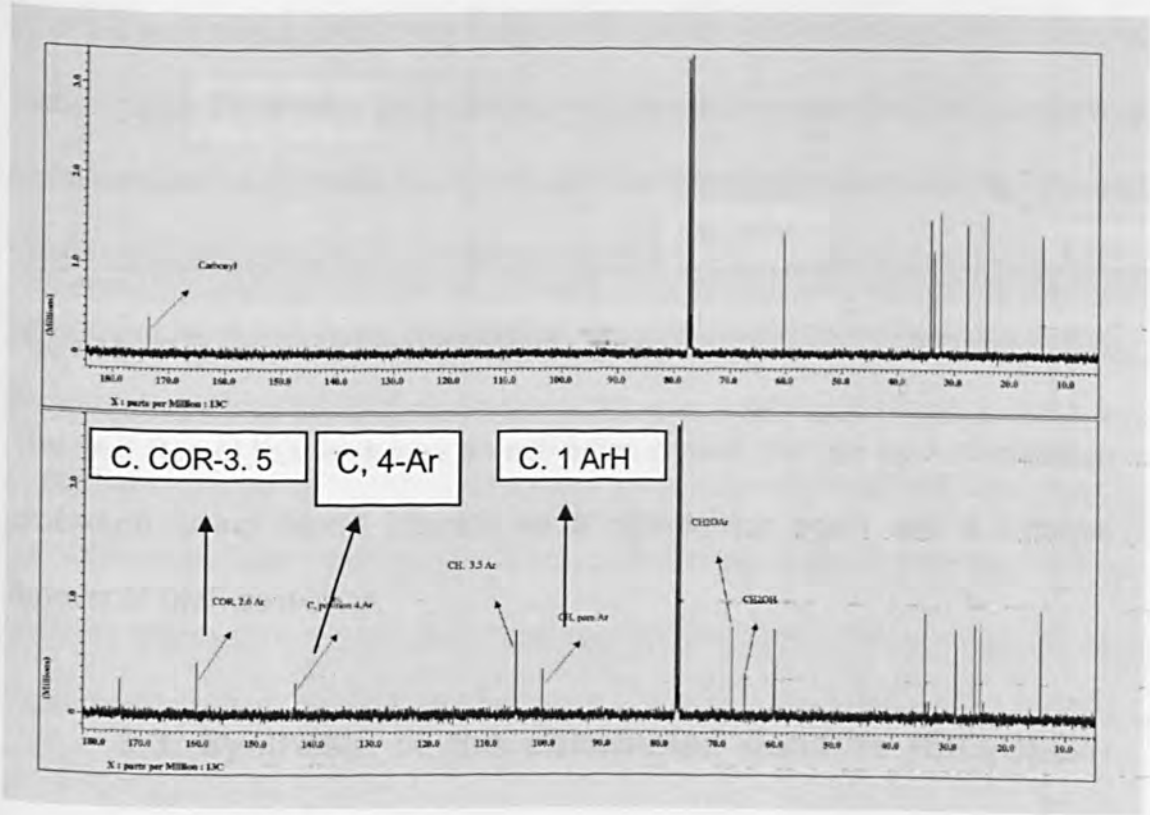


Figure 3.2: Comparison of ^{13}C NMR spectra of reactant sample (top) and dendrite (3) below

IR spectrometry was used to confirm the formation of the required product in this reaction; for example the broad band in the area of 3600 – 3200 cm^{-1} is due to the OH functional group on the dendrite; also the ether band at 1162 cm^{-1}, and carbonyl at 1732 cm^{-1} all indicate the formation of the dendrite (compound 3).

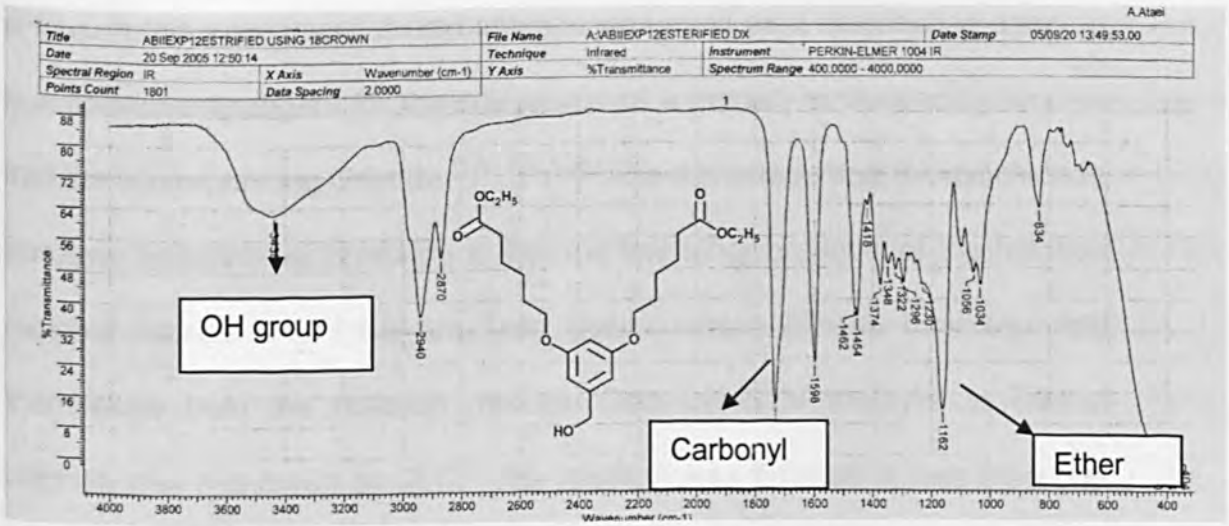
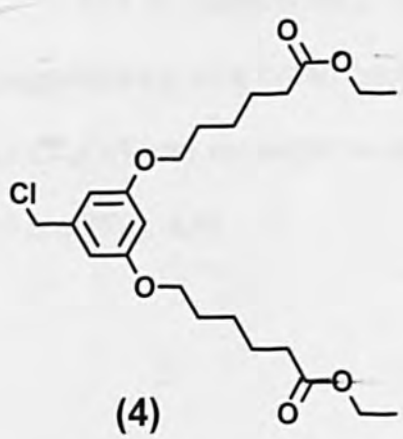


Figure 3.3: IR spectrum of dendrite (EtO2C)-G1-OH,(3).

The next step of this work was an activation-growth process by a chlorination procedure, using thionyl chloride as a chlorinating agent and a catalytic amount of DMF as a base.

3.3. Synthesis of the chlorinated dendrite (EtO_2C)-G1-Cl,(4).



(4)

Conversion of the dendritic alcohol to the dendritic chloride can be achieved by chlorination using either thionyl chloride ($SOCl_2$) or phosphorus trichloride

(PCl_3). In this experiment thionyl chloride was used as a chlorinating agent; it is a convenient reagent for the conversion of a primary or secondary alcohol into the corresponding chloride [177, 178,179]. The advantage that thionyl chloride has over phosphorus trichloride is that the two other products of the reaction (sulphur dioxide and HCl) are both gases, which means they separate themselves from the reaction mixture. Conversion of methylol to methyl chloride was monitored by TLC. The reaction was finished in less than one hour at room temperature; for purification, flash chromatography was used, and the yield was 65%. One of the most important factors that affect the percentage of yield in this reaction is that all the solvent and glassware must be completely dried before the reaction proceeds; otherwise the percentage yield is reduced. It is also important that thionyl chloride is added to the reaction mixture over a period of 20-30 minutes, as this increases the percentage yield of product.

Conversion of methylol to methyl chloride and completion of reaction was monitored by ^{1}H, ^{13}C and IR spectrometry. For example in the IR spectrum, there was the disappearance of a broad band in the area of 3600-3200 cm^{-1} which was due to CH_2OH and appearance of small sharp peak in the finger-print region at 716 cm^{-1}(**Fig. 3.4**).

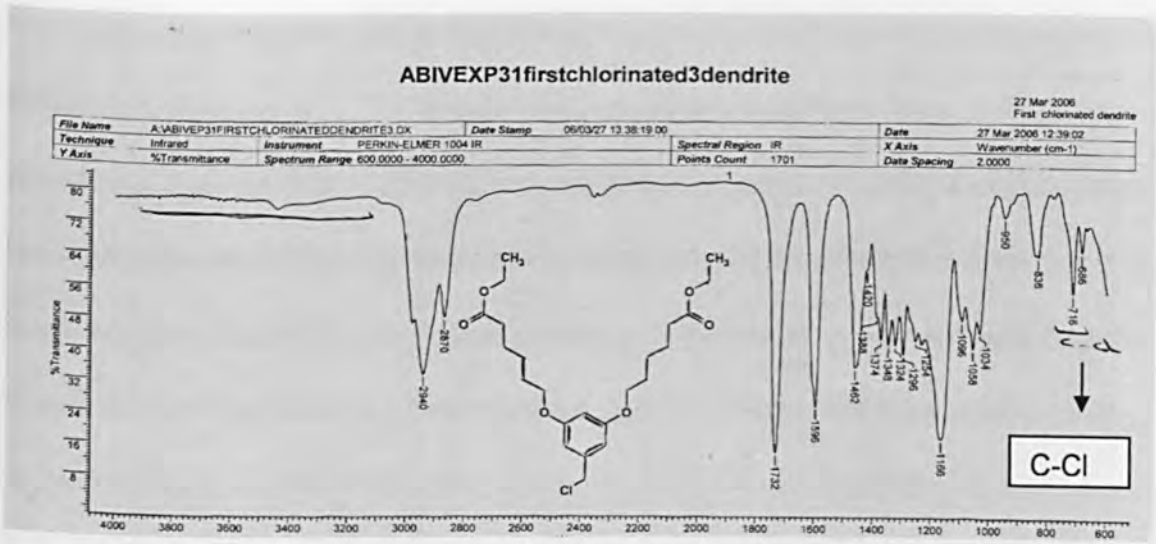


Figure 3.4: IR spectrum of chlorinated dendrite ,(4).

By comparing the proton NMR of the dendrite **(3)** to the chlorinated dendrite **(4)** one can clearly see the different resonances for methyl alcohol and methyl chloride. The proton peak for the methylol group (CH_2OH) can be seen as an easily distinguishable doublet shifted about 0.2 ppm downfield in relation to the methyl chloride (CH_2Cl). The remaining peaks in the proton NMR of the chlorinated dendrite (compound 4) are similar to the non-chlorinated (compound 3) dendrite with methyl alcohol as its focal point. Fig 3.5 shows the proton and carbon NMR of the chlorinated dendrite **(4)**.

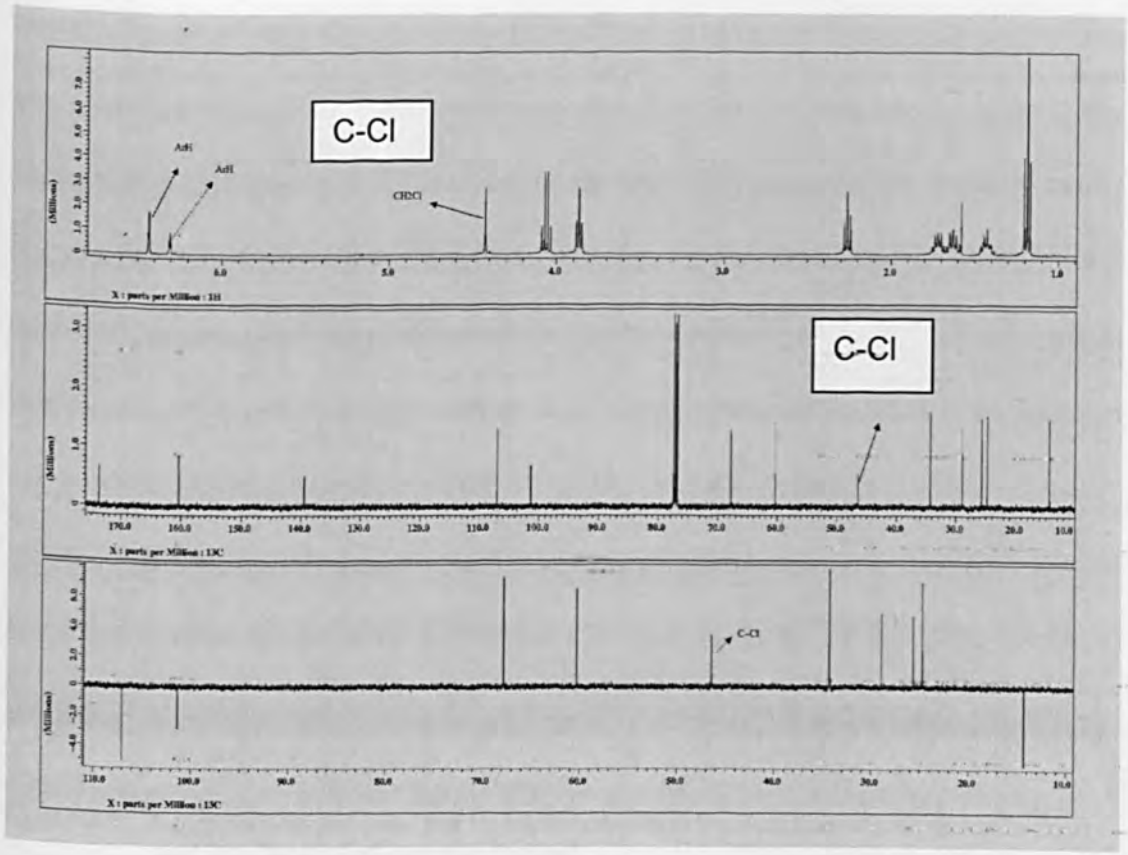


Figure 3.5: The ^{1}H and ^{13}C NMR spectra of chlorinated dendrite ,(4).

The disappearance of the peak at 65.5 ppm in the carbon NMR (due to CH_2OH) and appearance of the peak at 45.5 ppm (due to the C-Cl), are good indications of the successful chlorination process. Elemental analysis and high resolution mass spectrometry also confirmed the structure of chlorinated dendrite (compound 4).

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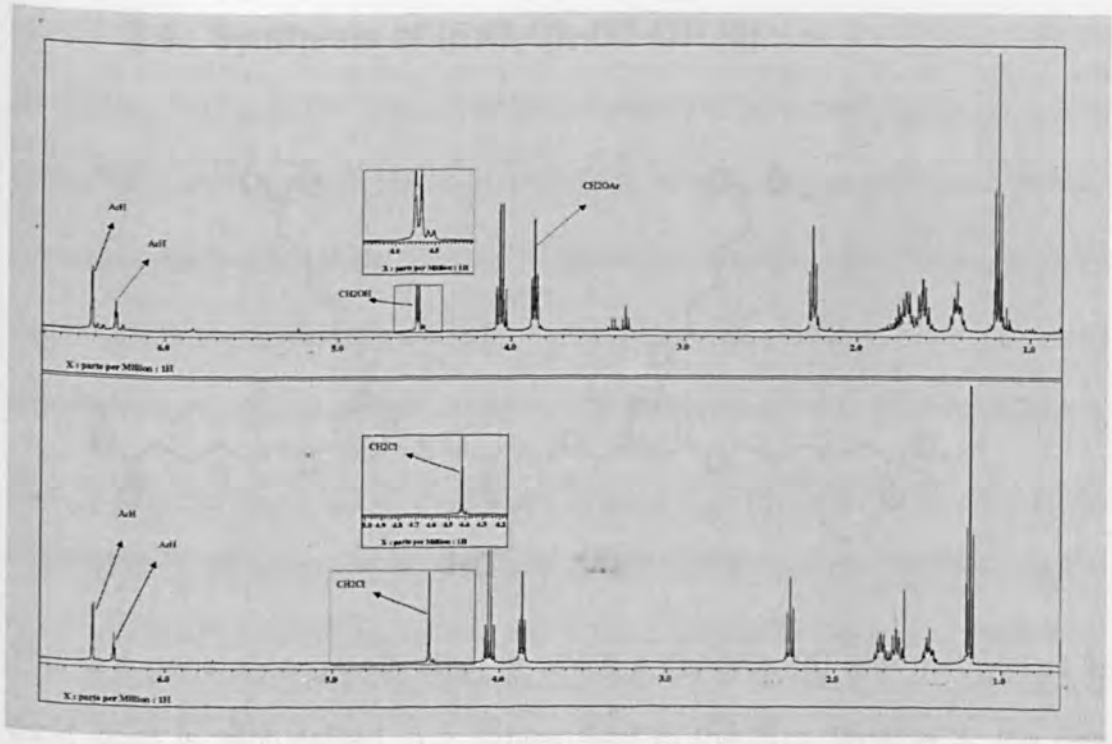
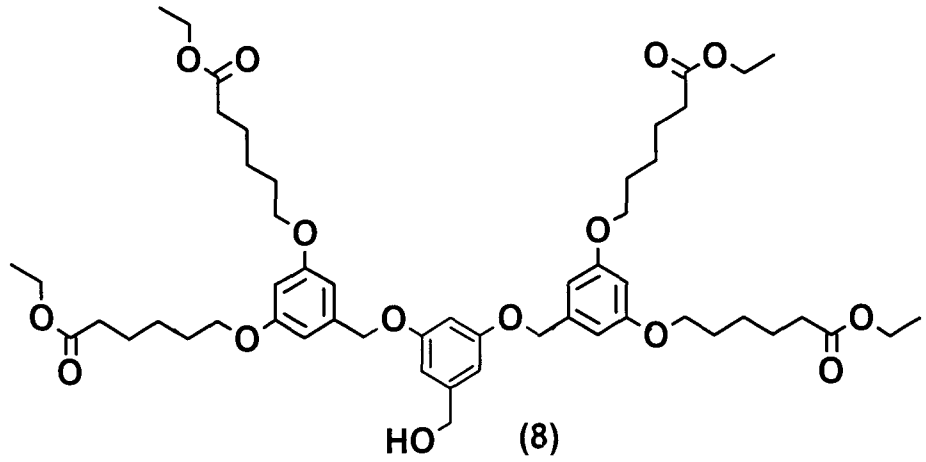


Figure 3.6: A comparison between the ^{1}H NMR spectra of the non chlorinated dendrite (top) and the chlorinated dendrite (below)

All the proton NMR peaks for the dendrite 3 and chlorinated dendrite 4 are the same, with the exception of CH_2Cl and CH_2OH; the CH_2OH peak is a doublet shifted about 0.2 ppm downfield from the CH_2Cl which is a singlet. In the next stage the synthesis of a second generation dendron 8 was carried out using a strategy similar to the one in scheme (3.1) by reacting two equivalents of chlorinated dendrite 4 with one equivalent of 3,5-dihydroxybenzyl alcohol which again involved a Williamson synthesis.

3.4. Synthesis of $(EtO_2C)_4$-G2-OH,(8).

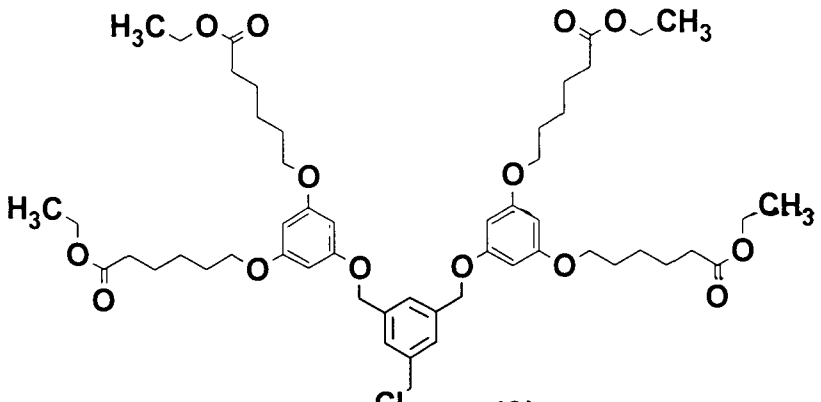


(8)

This is a generation-growth step, in which a dendron (8) with methylol at its focal point is synthesised in a similar way to the first dendrite 3; the only difference in this synthesis was the use of 18-crown6 [180]. 18-crown6 is a complexing agent and it can solvate alkali, and alkali earth metal ions, thereby increasing the strength of the base in the reaction and improving the percentage yield [181]. The percentage yield of reaction was 60% on average. It should be noted that as the generation growth step increases, the formation of higher generation dendrons should be more difficult, because of steric hindrance between the carbon chains. Further purification improved the quality of the sample product. IR, NMR and mass spectrometry results provided evidence for the structure of this dendron. For example in the IR spectrum the appearance of a broad band in 3600-3200 cm^{-1} due to the OH in the methyl alcohol of the dendron and disappearance of the C-Cl peak in the finger print area of the chlorinated dendrite is a good indication of the of the dendron formation.

Proton and carbon NMR results also provide evidence for the structure of the dendron, for example in the proton NMR the single peak at 5.20 ppm due to $ArCH_2OAr$, and formation of a doublet instead of a singlet peak at about 4.7 ppm in proton NMR are good indications of the desired structure. In the carbon NMR a peak at about 68 (due to the OH group) is a good indication of completion of reaction. Once sample 8 was purified, it was chlorinated to become activated for the next reaction step. The chlorination procedure in this reaction was similar to the synthesis of compound 4.

3.5. Synthesis of the first chlorinated dendron $(EtO_2C)_4$-G2-Cl,(9).



(9)

This is an activation-growth step. The chlorination of second-generation dendron with thionyl chloride in the presence of several drops of DMF was completed over a longer period of time (2 hours). The main problem associated with this reaction was decomposition of the chlorinated dendron over a period of time, and in order to overcome this, an excess of Et_3N or other similar organic base was used to retard the decomposition. That didn't completely suppress the problem; therefore it was decided to use a stronger

proton scavenger such as 2,6-di-*tert*-butyl-4-methyl pyridine or 2,6-di-*tert*butylpyridine [182,183], and no decomposition was observed after this. Further purification using flash chromatography removed the impurity shown in the base-line of the TLC.

It should be noted that bromination of the benzylic alcohol moiety of dendritic wedges with CBr_4/PPh_3 was tried [184,185] but the problem associated with this method was the formation of triphenylphosphine oxide by-product; also the reaction was considerably slower and required an excess of the reagent, so it was decided to use a chlorination process rather than bromination [186]. The result was much better due to the short reaction time, low temperature (room temperature) and ease of manipulation. The yield for this reaction was 78%. NMR, IR, and high resolution mass spectrometry confirmed the structure of this product. Proton and carbon NMR with the exception of the appearance and disappearance of CH_2Cl and CH_2OH were all the same as the reactants.

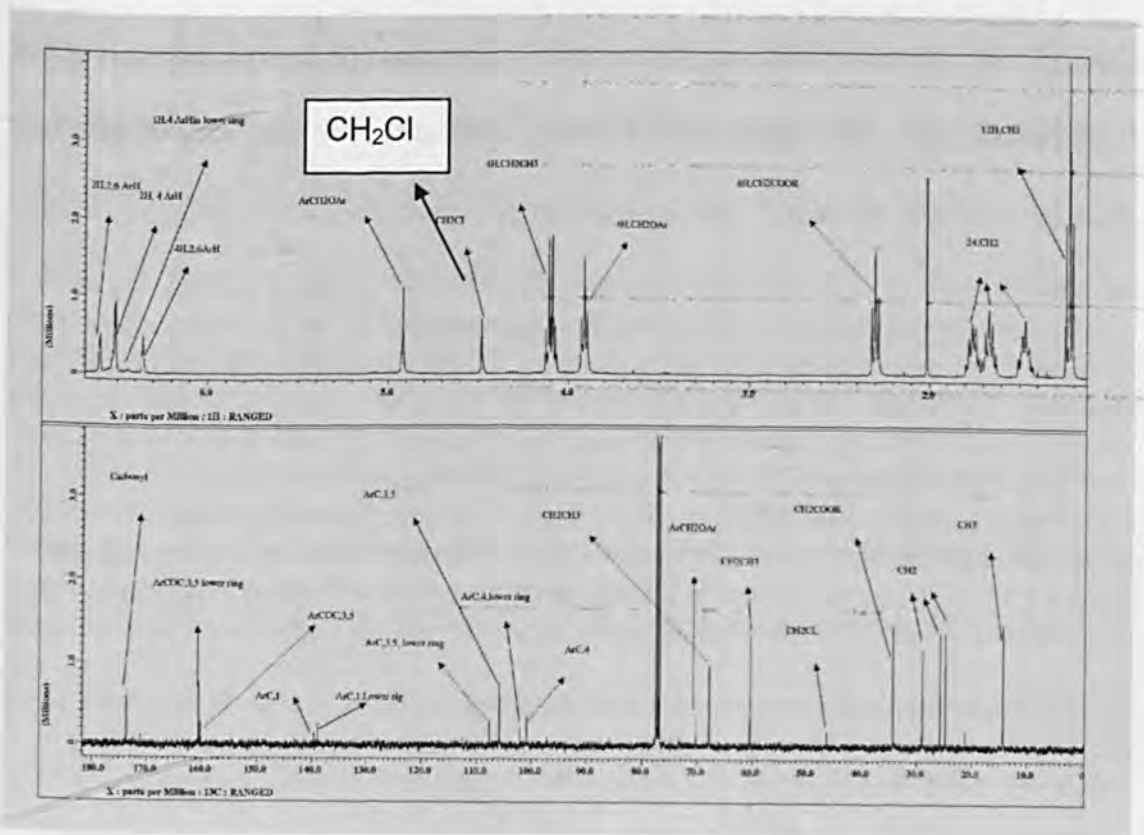


Figure 3.7: Proton and carbon NMR spectrum of chlorinated dendron $(EtO_2C)_4$-G2-Cl,(9).

The IR spectra give some evidence to support the formation of the chlorinated dendron; for example the disappearance of the broad band at 3600-3200 cm^{-1} and appearance of a peak at 716 cm^{-1} (due to C-Cl) are good indications of a successful method. Mass spectrometry also confirmed the molecular weight of desired molecule.

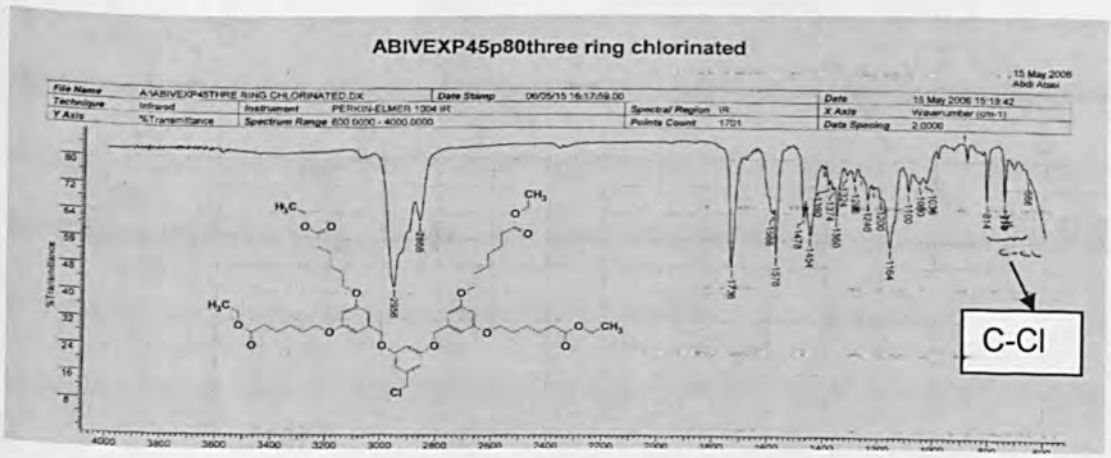
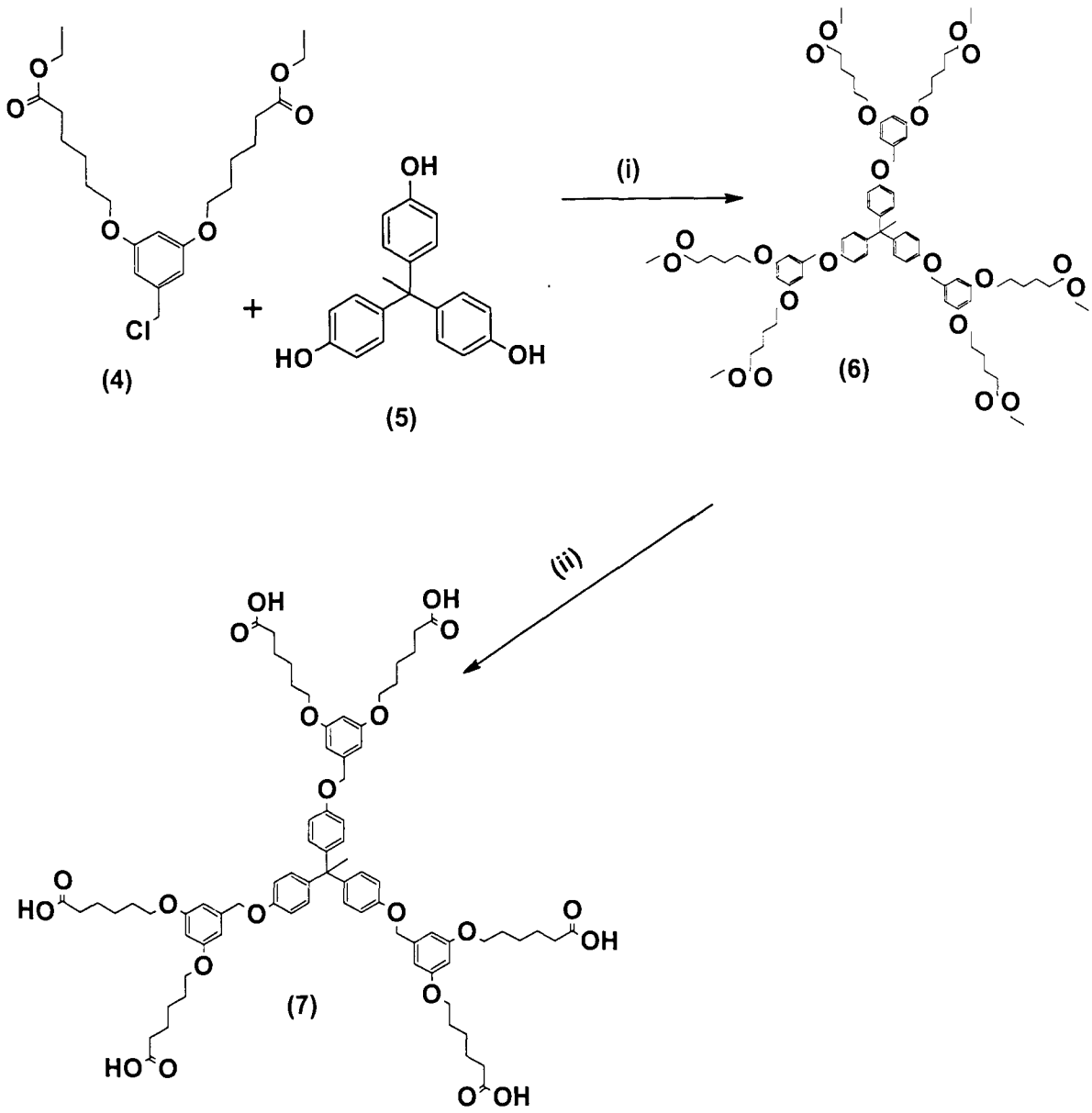


Figure 3.8: IR spectrum of $(EtO_2C)_4$-G2-Cl,(9).

In the next scheme (**3.6**) reaction of chlorinated dendrite to the tri-phenyl core and hydrolysis of the resultant product by alkali will be described.

3.6. Synthesis of first triphenyl dendrimer.

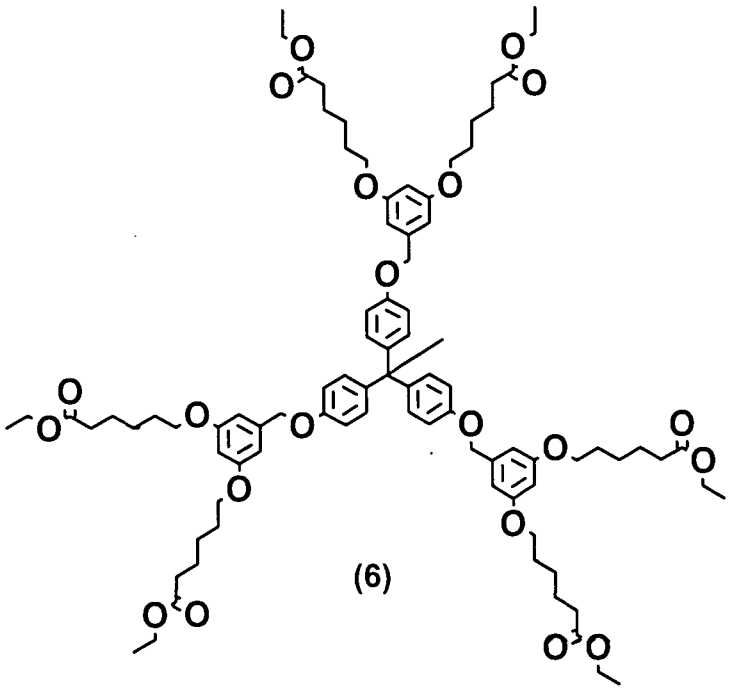


i) **Anhydrous K_2CO_3, 18-crown6, butanone**

ii) **KOH, (Ethanol-THF), H_2O**

Using the strategy given in scheme (3.6), three equivalents of chlorinated dendrite (4) were reacted with one equivalent of (5), to form molecule 6.

3.7. Synthesis of first dendrimer $(EtO_2C)_6$-G1-3C,(6).



(6)

During the experiment samples were taken every four hours for TLC testing, but because of the structure of this dendritic macromolecule the TLC spot wasn't clear and showed a smear rather than a single spot; the addition of a few drops of acetic acid to the solvent produced a better separation and showed clearer spots. By comparing the TLC movement of reactant and product, the completion of reaction was monitored.

The change in spectral changes occurring upon attachment of benzyl chloride to the polyfunctional core was also used to monitor the process and completion of reaction. The spectra of the resulting product indicated that the attachment of the chlorinated dendrite was successful. For example, the disappearing C-Cl peak at 45.5 ppm in the carbon NMR and appearance of peak at 5.20 ppm in proton NMR (due to $ArCH_2OAr$) again are good indications of the completion of the reaction; also the peak at 2.30 ppm in

proton NMR (due to CH_3 in the poly functional core) is good evidence to confirm the formation of product molecule. The IR spectrum was also used to monitor and provide evidence for the structure of molecule; for instance disappearing the peak at 716 cm^{-1} due to the C-Cl bond is a good indication of consuming benzyl chloride and formation of the product. Mass spectrometry also confirmed the formation of desired molecule. The yield for this reaction was 60% after purification by flash chromatography. The synthesis and purification become more difficult as the extent of branching increased.

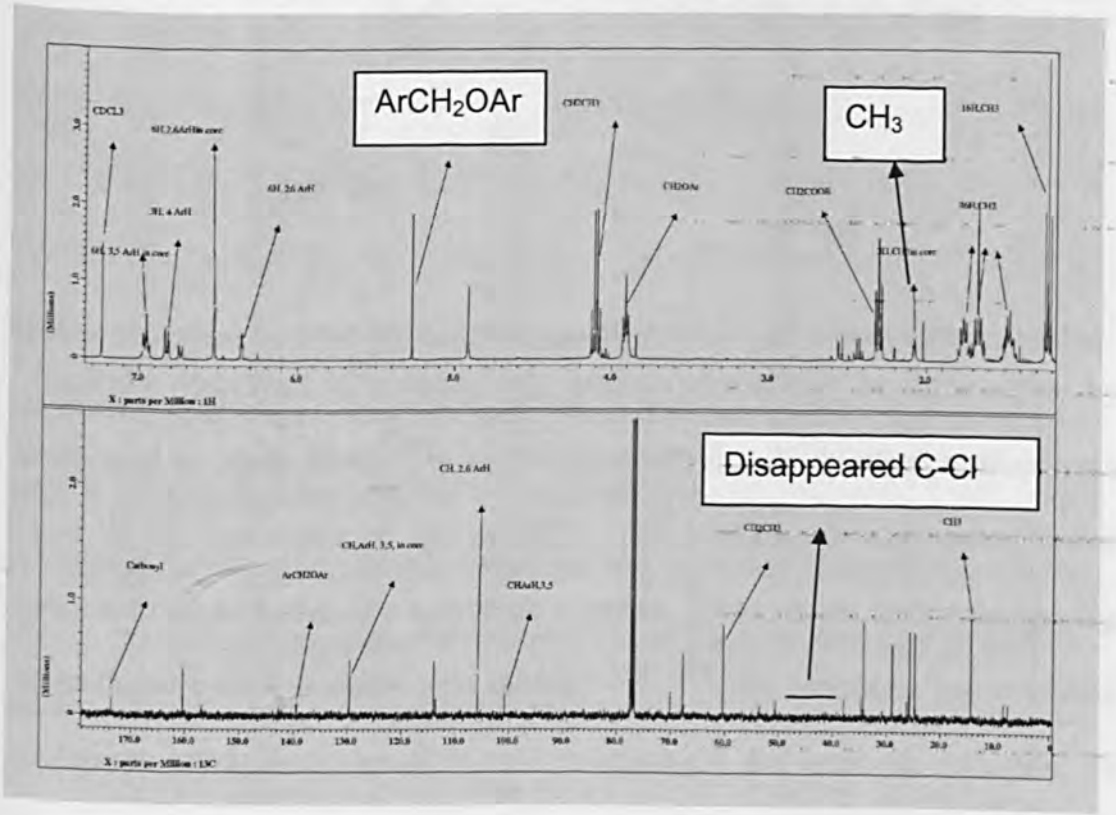
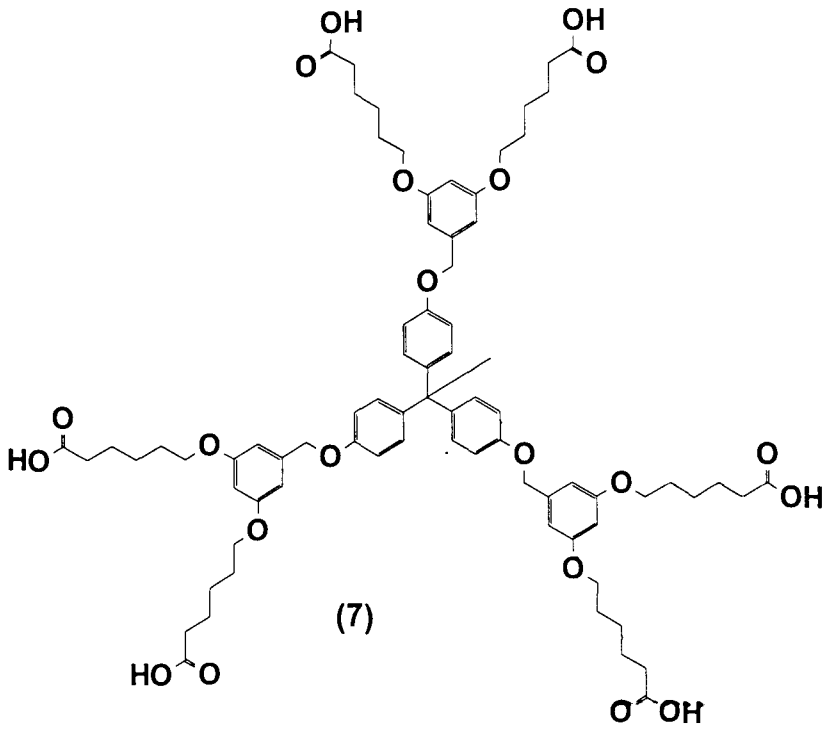


Figure 3.9: NMR spectrum of $(EtO_2C)_6$-G1-3C,(6).

In the next step dendritic macromolecule 6 was hydrolysed to compound 7 using an alkaline hydrolysis method.

3.8. Synthesis of $(HOOC)_6$-G1-3C,(7).



(7)

Typically hydrolysis is reaction with water, but this can be done either by dilute acid or dilute alkali [187]; in this experiment a dilute alkali method was used for the hydrolysis of compound 6. This compound was heated under reflux with dilute potassium hydroxide solution. Using dilute alkali has two big advantages over the dilute acid method [188, 189], the reactions are one-way processes rather than reversible, and the products are easier to separate. In the reaction equation below, X represents the un-reacted part of molecule 6.

$$X\text{-}\ COOC_2H_5 + KOH \rightarrow X\text{-}COOK + C_2H_5OH$$

In this reaction the product was easy to separate, provided an excess of KOH solution is used. Addition of a strong acid like dilute hydrochloric acid to the solution produces the carboxylic acid. During the experiment, samples were taken every 10 minutes for TLC testing; ideally putting a spot from compound

6 and one from compound 7 that the completion of reaction can be monitored. Once the spot remained at the base line (with the same eluent) this was a good indication of completion of the reactions.

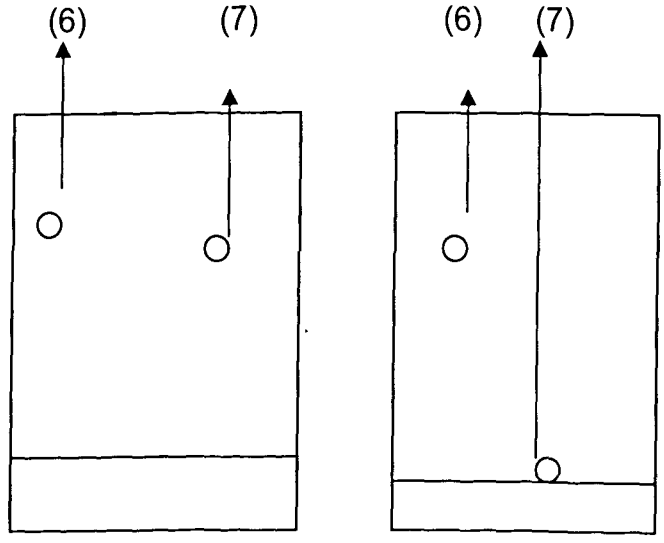


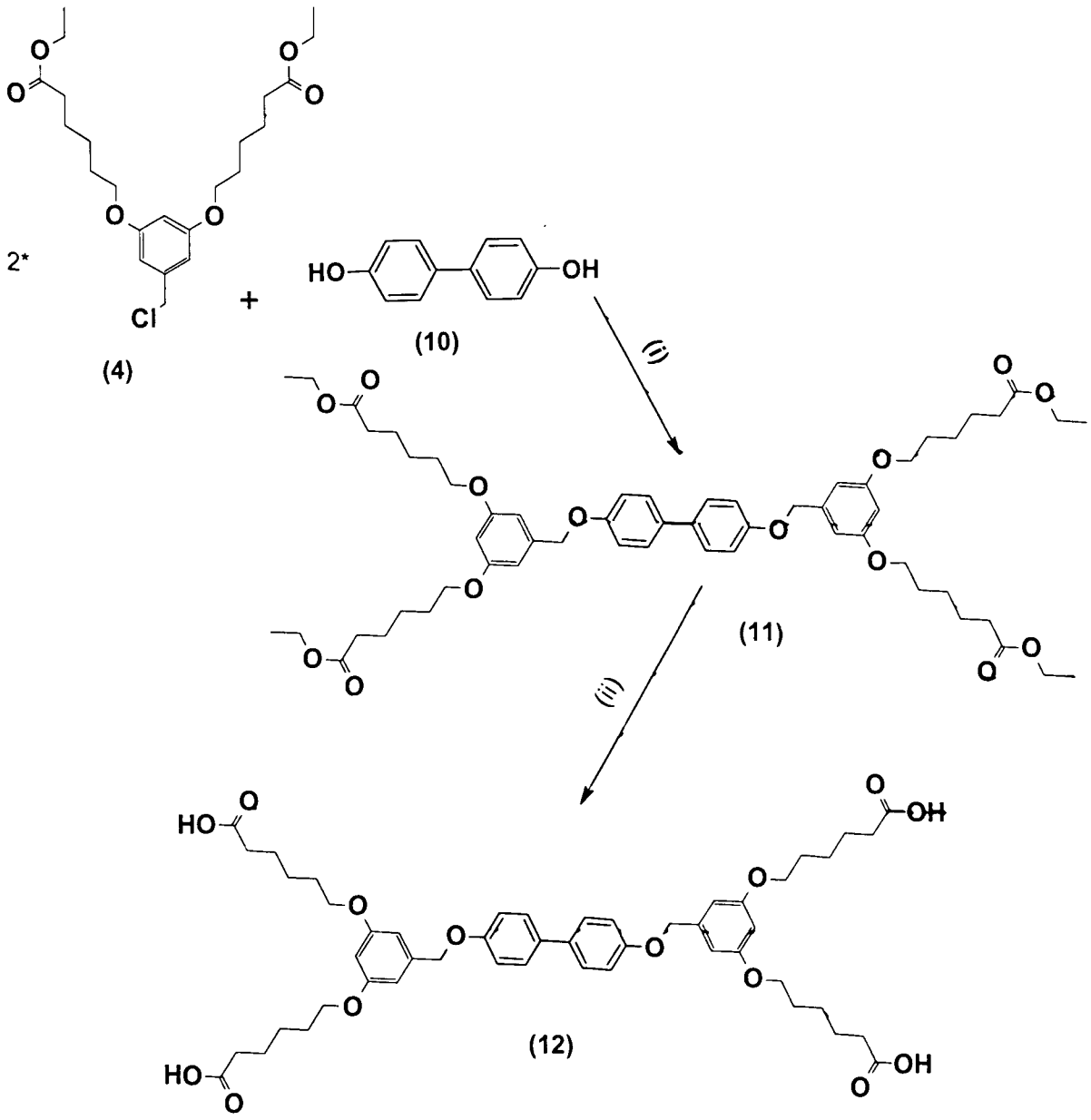
Figure 3.10: TLC plate for monitoring conversion of 6 →7

In proton NMR the disappearance of the quartet at 4.3 ppm (due to CH_2CH_3), and the appearance of a singlet peak at around 12.2 ppm (due to OH in carboxylic acid) and disappearance of the CH_3 peak at 1.8 ppm, were good indications of formation of hydrolysed dendrimer 7. This was also used to monitor the conversion of ethanoate to carboxylic acid. The IR spectrum also gave good evidence of the structure of molecule 7, for example the special shapes of the broad bands at 3500-3000 cm^{-1} and 2800-2350 cm^{-1} which are characteristic of carboxylic acid, as well as the shifting of the carbonyl peak from 1720 cm^{-1} in molecule 6 to 1705 cm^{-1} in compound 7.

The yield in this reaction was 72%. It is important that extraction of the product from water was repeated several times in order to collect as much product as possible.

Reaction of second generation dendron 9 with a tri-phenyl core was attempted but was not successful, which could be due to steric hindrance between carbon chains which prevents formation of the product; therefore it was decided to attach a chlorinated dendrite 4 and dendron 9 to a bi-phenyl core, because the branching spreads in two different directions and reduces the steric hindrance.

3.9. Synthesis of first generation bi-phenyl dendrimers, (12). Scheme 3.7

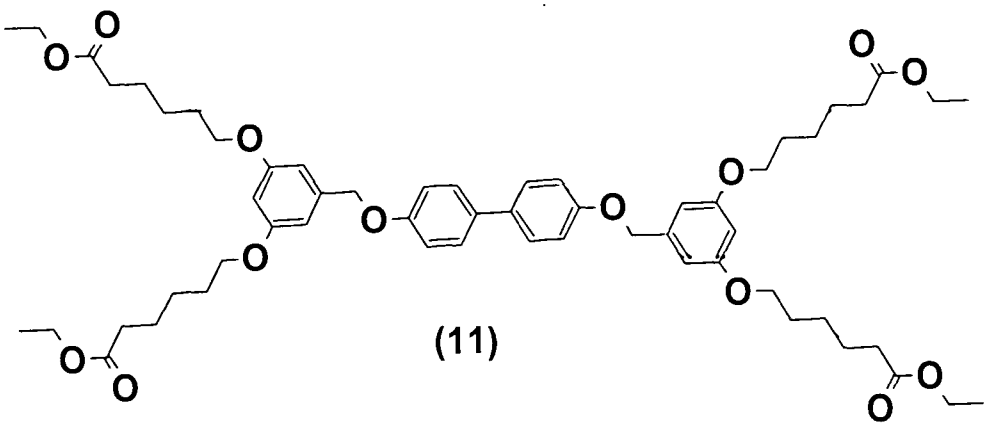


i) Anhydrous K_2CO_3, 18-Crown6, butanone

ii) KOH, (Ethanol-THF) , H_2O

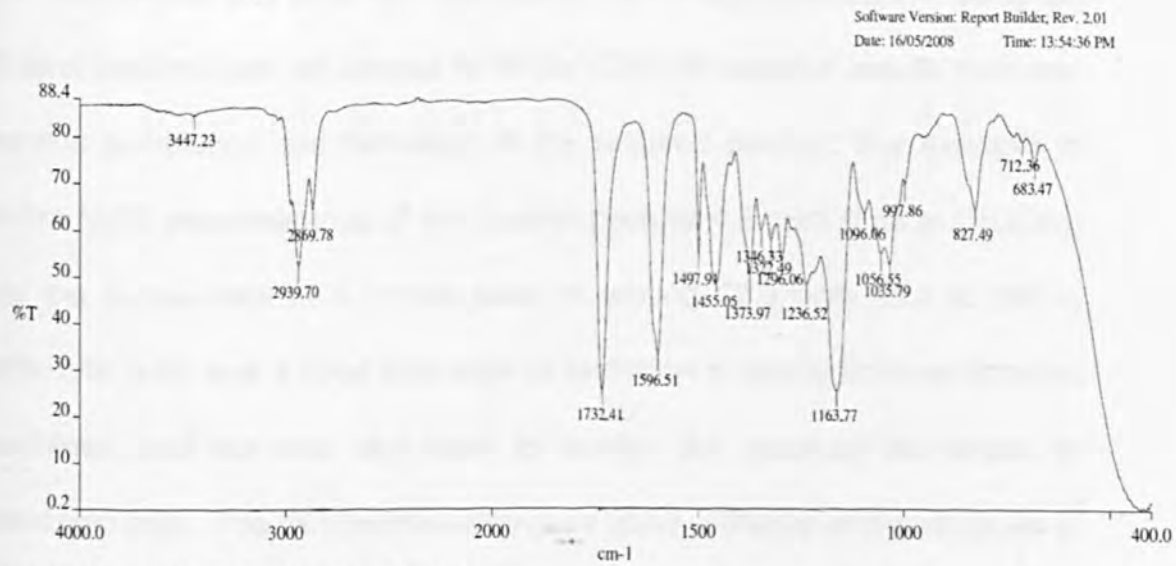
Using the strategy given in scheme (3.7) the first generation bi-phenyl dendrimer was synthesised by reaction of two equivalents of chlorinated dendrite (4) with one equivalent of bi-phenyl core (10), followed by hydrolysis to the carboxylic acid.

3.10. Synthesis of $(EtO_2C)_4$-G1-2C,(11).



(11)

The spectra confirmed the structure of the product. For example in the proton NMR, the appearance of a peak at around 5.20 ppm (due to $ArCH_2OAr$) and two doublet peaks at around 6.85 ppm and 7.65 ppm (due to biphenyl core) are good indicators of the formation of the biphenyl dendrimer product. In the carbon NMR the peak at 14.6 ppm (due to $ArCH_2OAr$) and disappearance of the peak at 45.5 ppm (due to C-Cl band) confirmed the completion of the reaction. NMR, high resolution mass spectrometry and IR all provided evidence for the structure of this molecule (as mentioned in the experimental part).

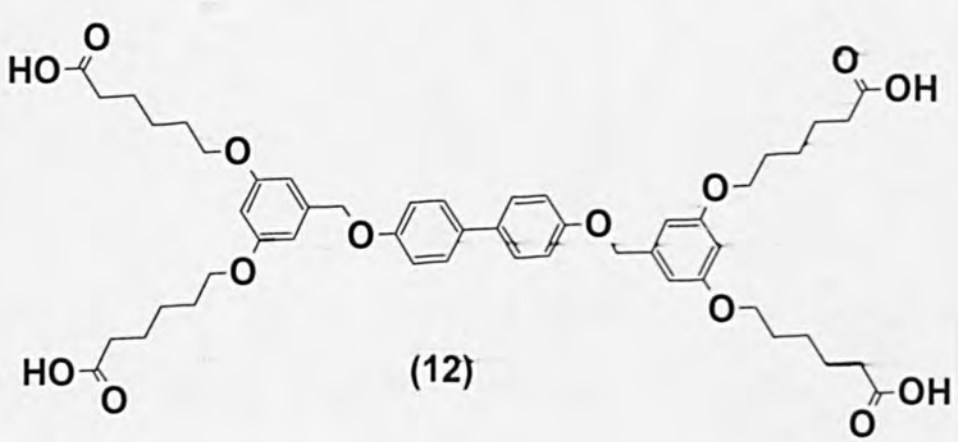


IR spectrum of $(EtO_2C)_4$-G1-2C,(11).

For instance in the IR spectrum, disappearance of the C-Cl band in the finger-print region provided good evidence for completion of the reaction. The reaction proceeded at an excellent rate and gave a yield of 65%.

The next stage of this work was the hydrolysis of sample 11 using the strategy given in section (3.8) by alkaline hydrolysis.

3.11. Synthesis of $(COOH)_4$-G1-2C,(12).

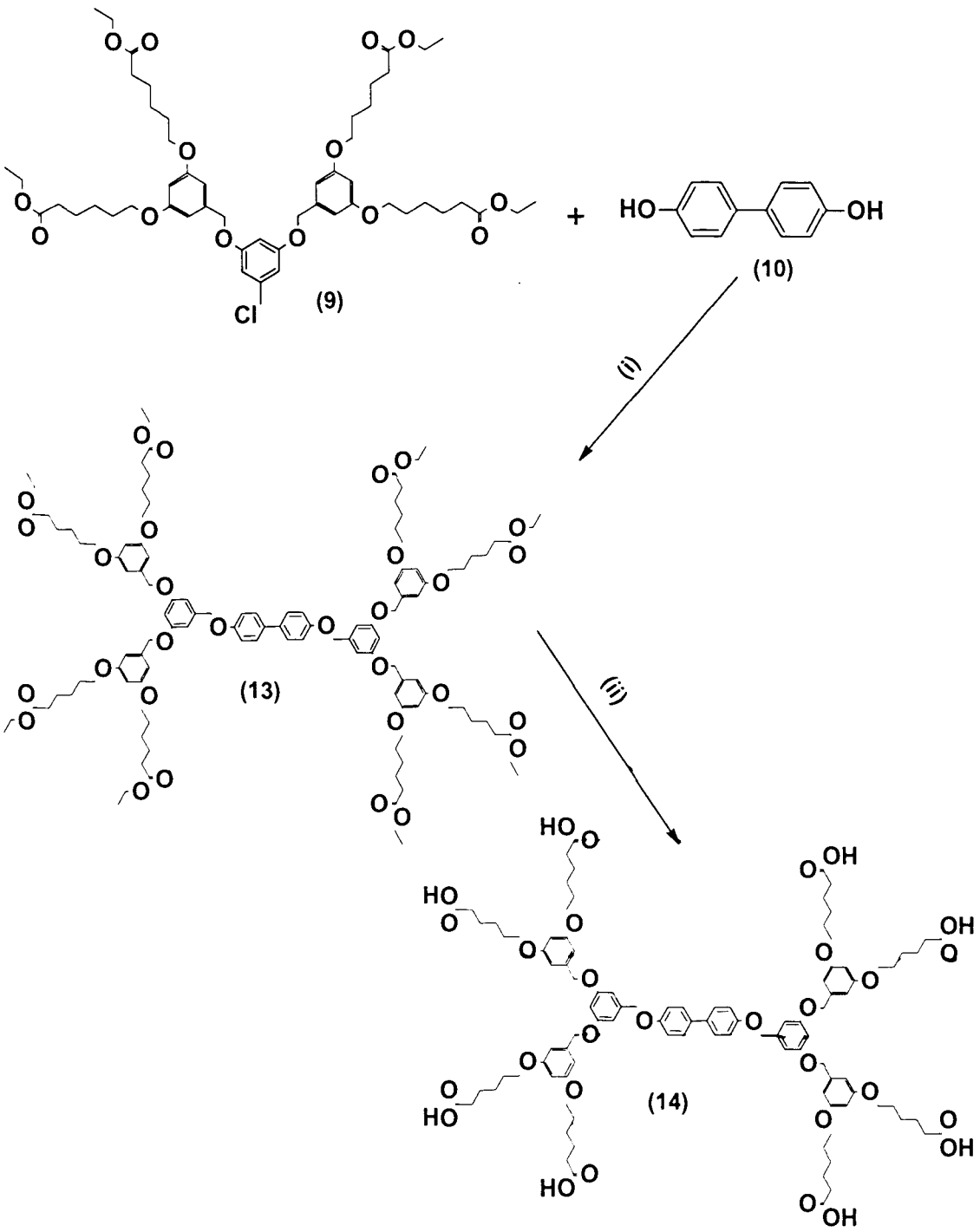


(12)

The hydrolysis of this ester was identical to that in experiment 2.2.4, using the alkaline method, with an excess of dilute KOH. All spectral results indicated reaction completion and formation of the required product. For example in proton NMR disappearance of the quartet peak at 4.4 ppm (due to CH_2CH_3) and the appearance of a singlet peak at around 12.3 ppm (due to OH in carboxylic acid) was a good indication of formation of the hydrolysed biphenyl dendrimer, and this was also used to monitor the complete conversion to carboxylic acid. The IR spectrum also gave good evidence of the structure of molecule **12**, for example the broad band at 3450-3100 cm^{-1} and 2800-2350 cm^{-1} was characteristic of carboxylic acid, together with the shifting of the carbonyl peak from 1720 cm^{-1} in molecule 11 to 1710 cm^{-1} in **12**. The reaction proceeded at an excellent rate and gave a yield of 65%.

The next step of this work was the synthesis of a second generation bi-phenyl dendrimer.

3.12. Synthesis of second generation bi-phenyl dendrimer. Scheme 3.8

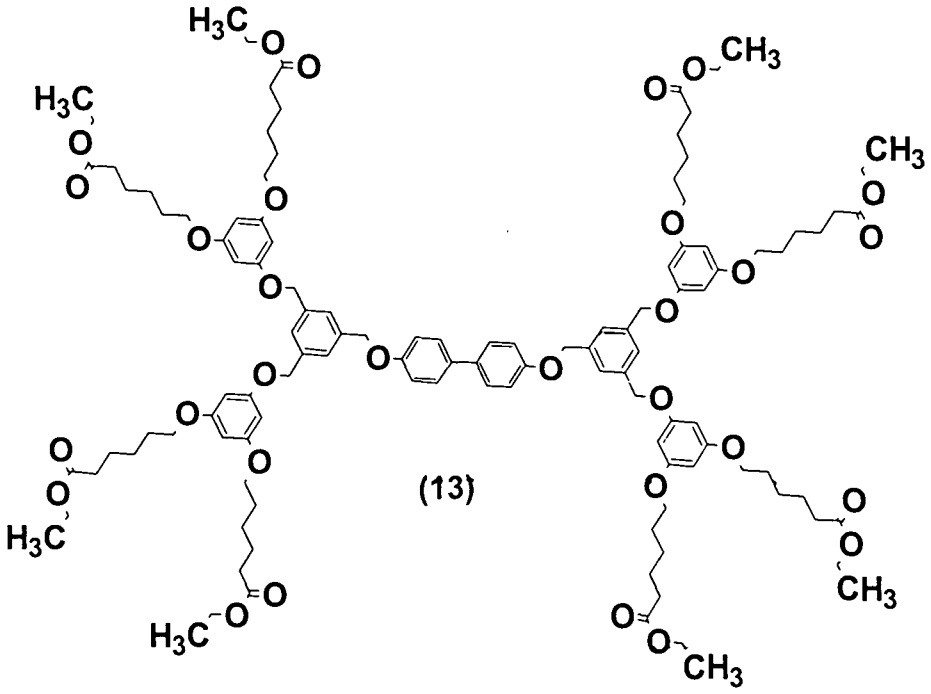


i) Anhydrous K_2CO_3, 18-Crown6, butanone

ii) KOH, (Ethanol, THF), H_2O

Using the synthetic strategy given in scheme (3.8), synthesis of the second generation bi-phenyl dendrimer proceeded successfully.

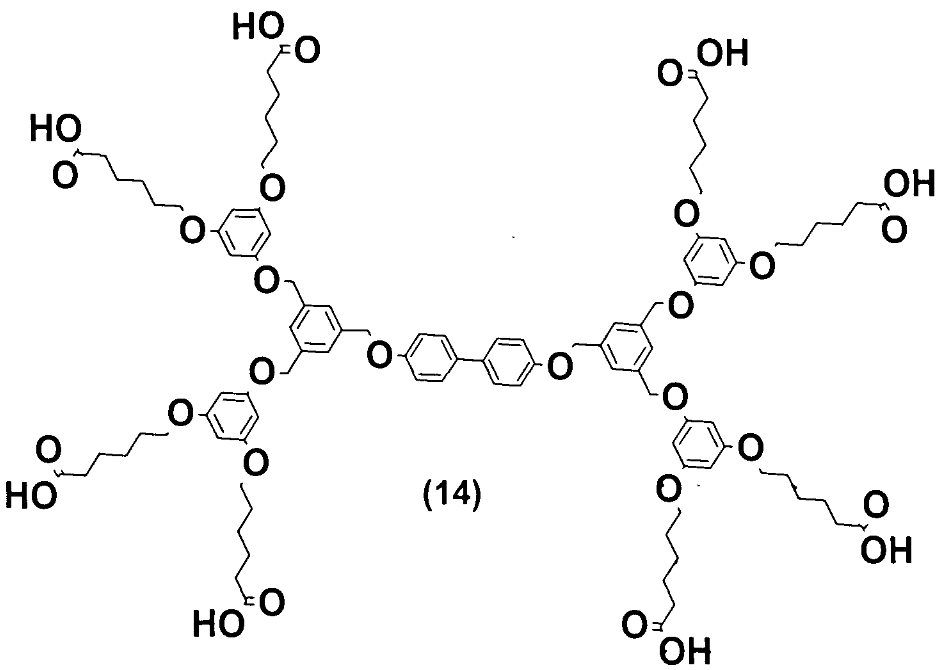
3.13. Synthesis of $(EtO_2C)_8$ -G2-2C,(13).



All the spectra of the resulting product indicated that the attachment of the chlorinated dendron to bi-phenyl core had been successful. Proton and carbon NMR of the second generation biphenyl were more or less the same as those of the first generation. The only major difficulty in the preparation of the second generation biphenyl dendrimer (13) was separation and purification. This was probably because of the existence of several large carbon chains in the molecule; hence purification became much more difficult, but was successfully achieved. Using NMR, IR and high resolution mass spectrometry provided evidence for the structure. The reaction proceeded at an excellent rate and gave a yield of 65%.

Transformation of methyl ester end groups in 13 into the desired carboxylate groups was accomplished by alkaline hydrolysis.

3.14. Synthesis of $(HOOC)_8$-G2-2C,(14).



(14)

The hydrolysis procedure was similar to that for compound 7 using the alkaline method.

Disappearance of the quartet peak at around 4.2 ppm (due to CH_3 coupling with CH_2), absence of triplet peak at around 1.4 (due to CH_3), and formation of a doublet peak (due to OH of carboxylic acid) at around 12 ppm were good indications of hydrolysis to biphenyl dendrimer (14). IR of the hydrolysed biphenyl dendrimer showed a broad band in the area of 3500-2900 cm^{-1} characteristic of carboxylate OH and this together with shifting of the carbonyl peak toward higher wavenumber, are good indications of success.

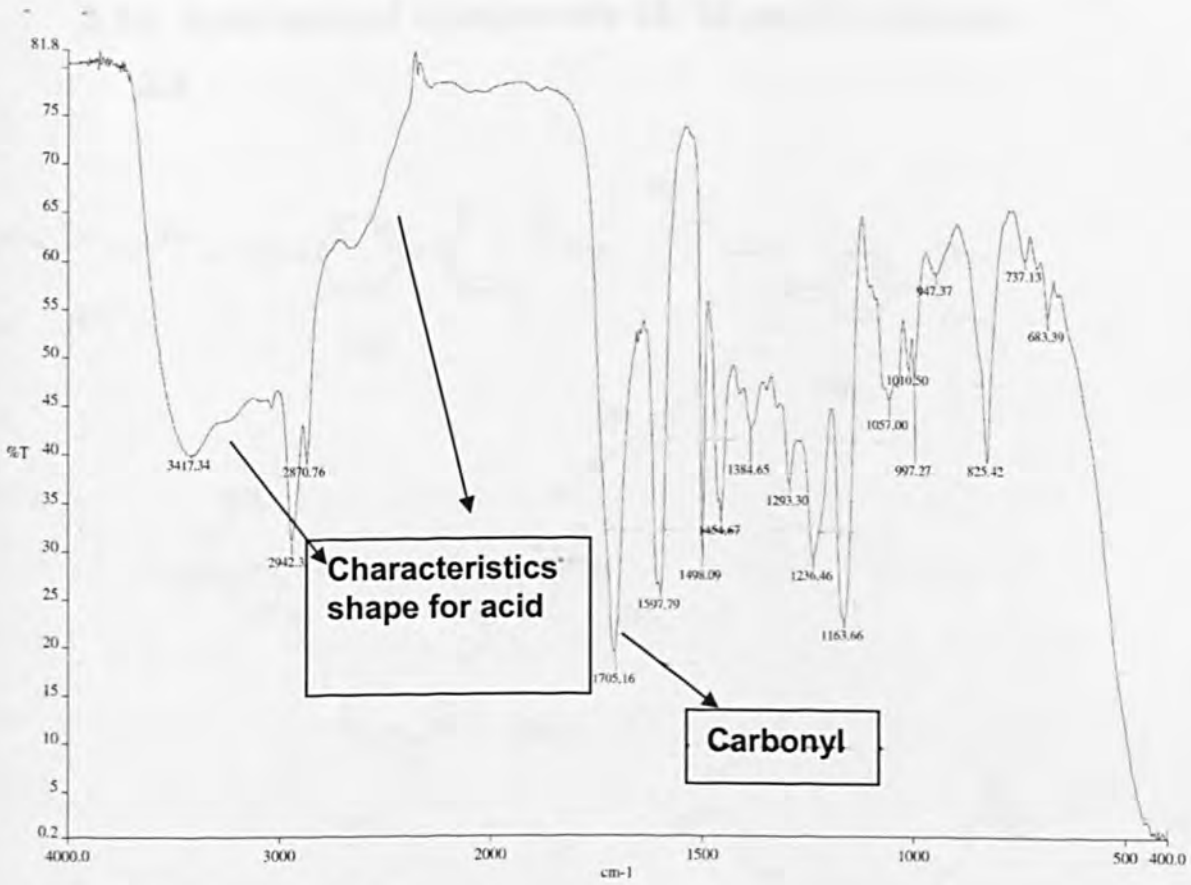
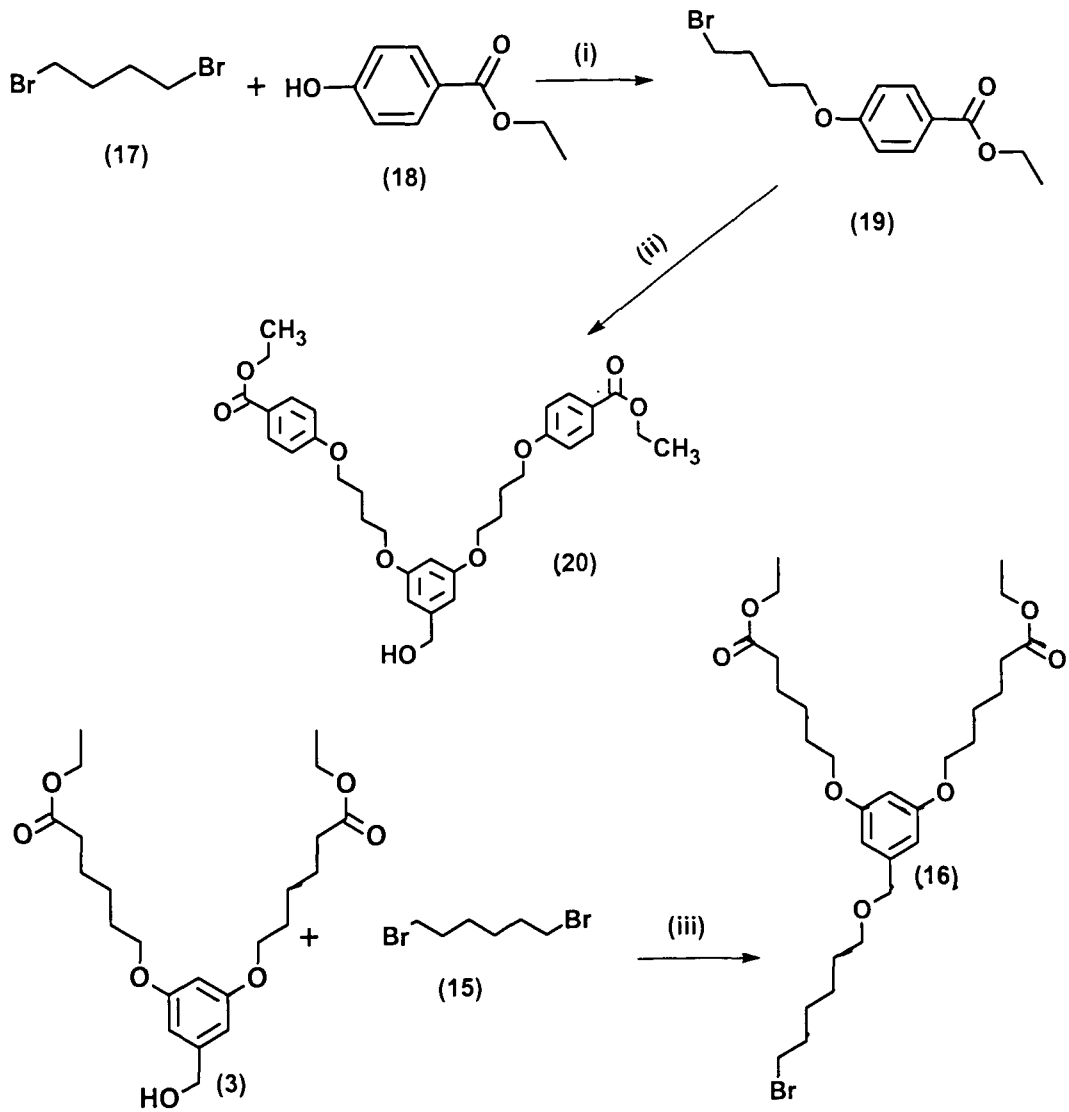


Figure 3.11: IR spectrum $(COOH)_8$-G2-2C,(14).

Figure 3.12 shows the IR spectrum of hydrolysed biphenyl dendrimer **14**. The main difference between biphenyl dendrimer **13** and the hydrolysed biphenyl dendrimer **14** is the formation of a broad band in the area of 2800-2200 cm^{-1} which is characteristic of the acid. The other obvious difference between these two IR spectra is the position of the carbonyl peak; in the case of the hydrolysed biphenyl this peak is shifted to a lower wave-number. The yield of the reaction was 65%.

In this stage different types and shapes of dendrite were synthesised. With the aim of enlarging the cavity size within the dendrimer by alkyl spacer group within the structure.

3.15. Synthesis of compounds 16, 19 and 20. Scheme 3.9



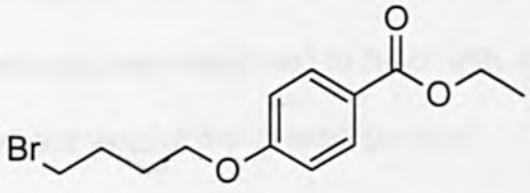
i) **Anhydrous K_2CO_3, 18-Crown6, butanone**

ii) **Anhydrous K_2CO_3, 18-Crown6, butanone**

iii) **anhydrous dry diethyl ether, potassium *tert*-butoxide, 1,6-dibromohexane**

Using the strategy given in scheme (3.9) a new branching type was synthesised.(19).

3.16. Synthesis of 4-(4-Bromo-butoxy)-benzoic acid ethyl ester,(19).



(19)

The reaction proceeded very well under reflux conditions and gave 55% yield. During the reaction, samples were taken every 4 hours for TLC testing. This showed two spots, which indicated that further purification by flash chromatography was required. The spectra of the resulting products indicated that the synthesis of 1,4-dibromobutane with ethyl 4-hydroxybenzoate had been successful.

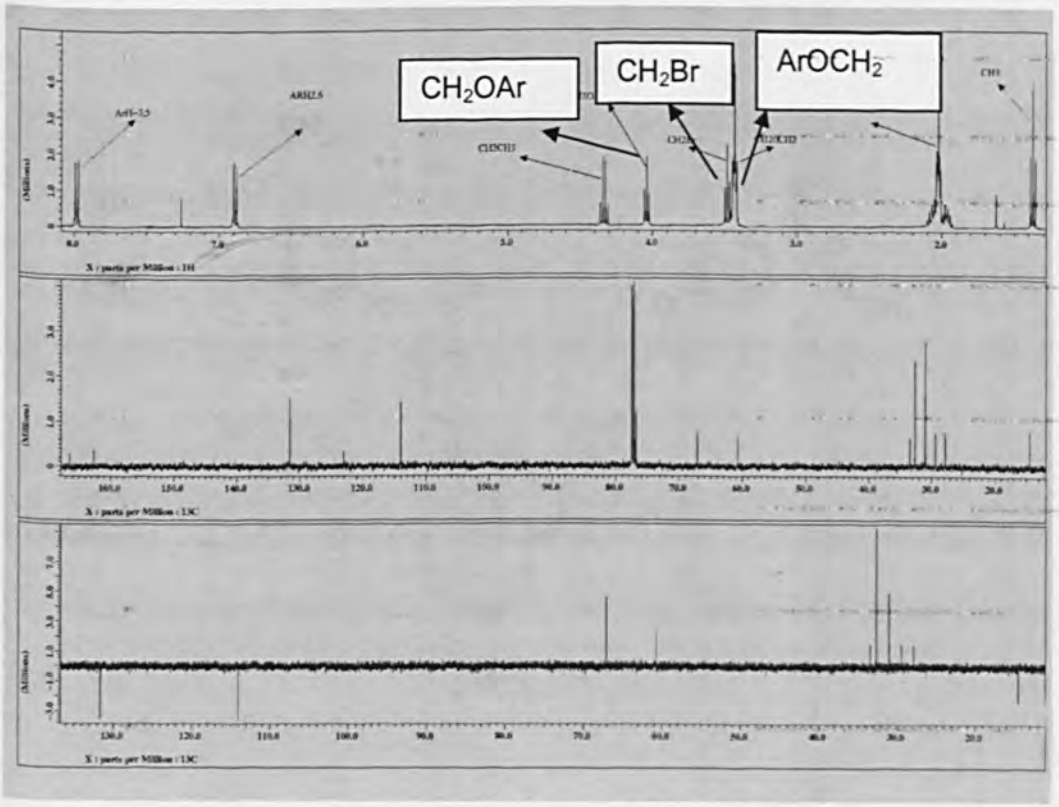
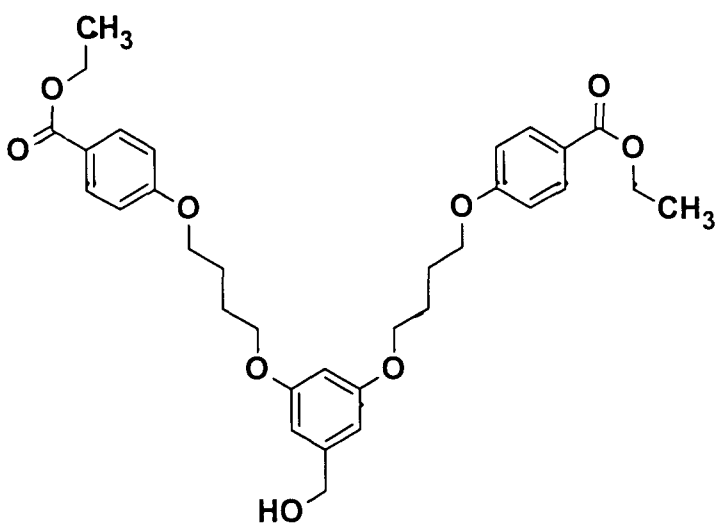


Figure 3.12: Proton and carbon NMR of 4-(4-bromo-butoxy)-benzoic acid ethyl ester ,(19).

In the proton NMR spectrum figure 3.13, the triplet peak 3.3 ppm(due to CH_2Br) and the triplet peak at 2.95ppm (due to CH_2OAr), clearly indicates the formation of the required molecule. In this reaction, it should be noted that an excess of 1,4-dibromobutane was used to react with ethyl 4-hydroxybenzoate in order to improve the yield of the desired product.

The next stage of this work was the reaction of two equivalents of this new branching monomer to the electron-rich interior of 3,5-dihydroxybenzyl alcohol, to form a new type of dendrite (20).

3.16.2. Synthesis of Diethyl4,4'-(5-(hydroxymethyl)-1,3-phenylene)bis(oxy)bis(butane-4,1-diyl-1))bis(oxy)dibenzoate, (20).



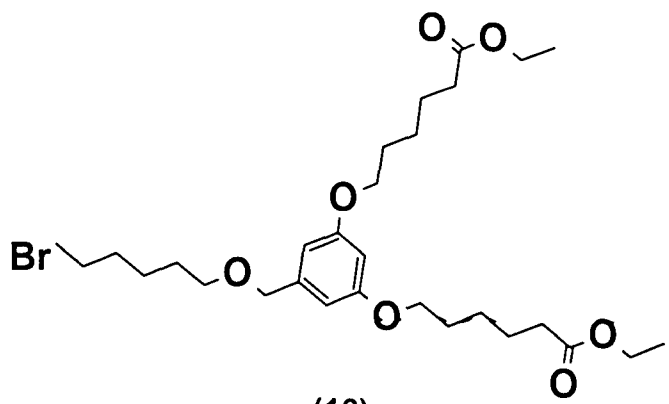
(20)

This reaction is a generation-growth step. Samples were taken every 5 hours for TLC testing, which showed two different spots, so purification by flash

chromatography was required. All the spectra of the resulting products indicated completion of the reaction. The reaction to produce (20) gave a good yield of 65% after purification by flash chromatography. Proton and carbon NMR provided evidence for the formation of this molecule. IR spectrometry showed a broad band in the region of 3100-3500 cm^{-1} which was due to OH.

In the next step, it was decided to synthesise another type of dendrite by reaction between 1,6-dibromohexane and compound (3), to form 6-[3-(5-ethoxycarbonyl-pentyloxy)-5-heptyloxymethyl-phenoxyy]-hexanoic acid ethyl ester. (20). The initial aim was to react the first dendrite with a six-carbon spacer and then the tri-phenyl core, in order to change the space of the dendritic cavities.

3.17. Synthesis of 6-[3-(6-bromo-hexyloxymethyl)-5-(5-ethoxycarbonyl-pentyloxy)-phenoxy]- hexanoic acid ethyl ester,(16).



(16)

The yield of this reaction was very low (4%); this could be due to the acidic hydrogen in the dendrite attached to the carbon adjacent to the carbonyl

which would neutralize the base, but even increasing the amount of base didn't improve the yield, NMR, IR and mass spectrometry provided evidence for the structure of the expected product. Formation of CH_2Br and CH_2OCH_2 in the area of 3.4 ppm - 3.5 ppm clearly indicates the formation of the required molecule, and the remaining peaks were very similar to those of the simple dendrite. TLC showed two spots but after purification by flash chromatography, a single spot was obtained. Mass spectrometry identified the expected molecular ion, and the IR spectrum showed the absence of broad band (due to OH) which was a good indication of complete reaction.

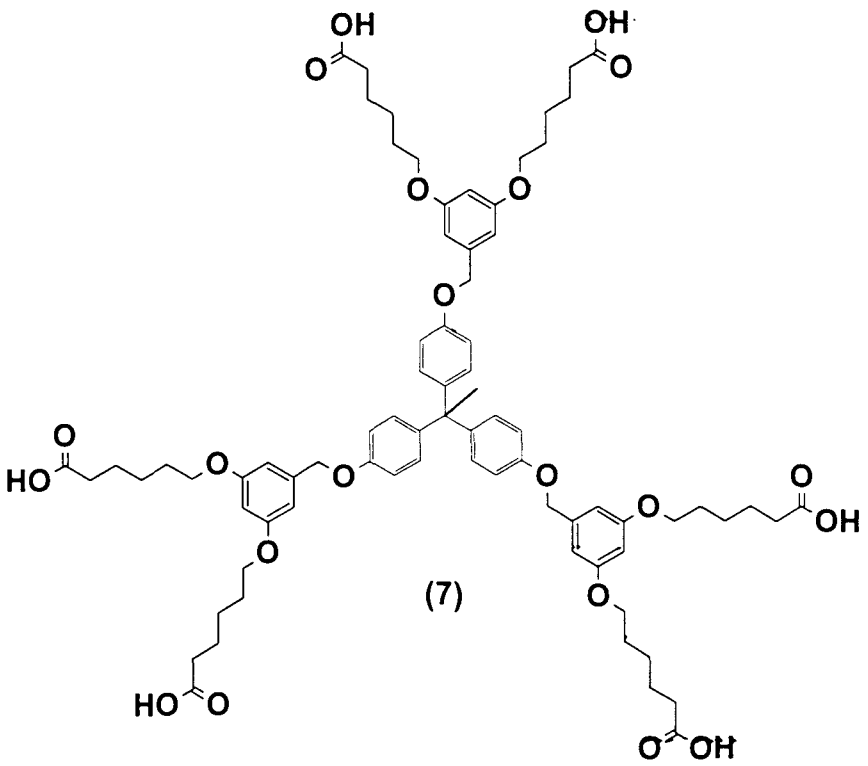
In the next section of this chapter physico-chemical properties of the dendritic macromolecules synthesised will be reported.

Unfortunately time didn't allow me to further develope this synthetic route to produce new dendrimers with larger cavities.

3.18. Physico-chemical properties (B)

In this section physico-chemical properties of compounds 7, 12 and 14 were investigated. One of the most important properties of any dendritic macromolecules used as drug carriers is their solubility, therefore the solubilities of these compounds in buffers at different pH were investigated.

3.18.1. $(COOH)_6$-G1-3C,(7).



(7)

3.18.1.1. pH Profile for $(HOOC)_6$-G1-3C,(7).

Table 3.1 represents the absorbance of $(HOOC)_6$-G1-3C at different pH in stock solution 1 (the mother solution), and in the diluted solution, stock solution 2

pH	Absorbance (Stock solution 1) $7.37*10^{-4}$ M	Absorbance (Stock solution 2) $1.47*10^{-3}$ M
10	3.55	1.65
7.4	1.43	0.708
7	1.02	0.466
6.8	0.511	0.250
6.5	0.307	0.160

Table 3.1: pH profile for $(HOOC)_6$-G1-3C

Table 3.1 shows the absorbance of compound 7 at two different concentrations at different pH. As expected, increasing the pH of the buffer increased the solubility of the dendritic macromolecule.

In order to determine the maximum solubility of these compounds in one cm^3 of particular buffer eg. Compound 7 at pH 7.4, the first parameter to be found is the absorptivity. This was done by using a saturated solution of compound 7 in a buffer at pH 7.4 and using a series of dilutions to make up a calibration curve, by plotting absorbance against concentration. The slope of this graph is the absorptivity. Having absorptivity, cell length and absorbance, the maximum concentration of this compound at pH 7.4 can be found.

3.18.1.2. Absorbance of $(HOOC)_6$-G1-3C,(7). At λ= 278nm

Conc.(M)	Dilution factor	Volume of stock solution(ml)	Volume of buffer(pH=7.4)ml
0.02	7.15	0.49	3.03
0.04	3.58	0.98	2.52
0.06	2.38	1.47	2.03
0.08	1.79	1.96	1.54
0.1	1.43	2.45	1.05
0.12	1.19	2.94	0.56

Table 3.2: Absorptivity calculated for $(HOOC)_8$-G1-3C. (7).

Absorbance and Concentration of $(HOOC)_6$-G1-3C,(7). At λ=278nm

Abs(1)	Abs(2)	Abs(3)	Ave	± SD n = 3	Conc.(M)
0.112	0.119	0.116	0.116	0.004	0.02
0.204	0.209	0.202	0.208	0.004	0.04
0.321	0.311	0.309	0.314	0.006	0.06
0.404	0.394	0.4	0.399	0.005	0.08
0.498	0.487	0.492	0.492	0.006	0.1
0.588	0.604	0.608	0.608	0.011	0.12

Table 3.3: UV-Visible absorbance of $(HOOC)_6$-G1-3C,(7).

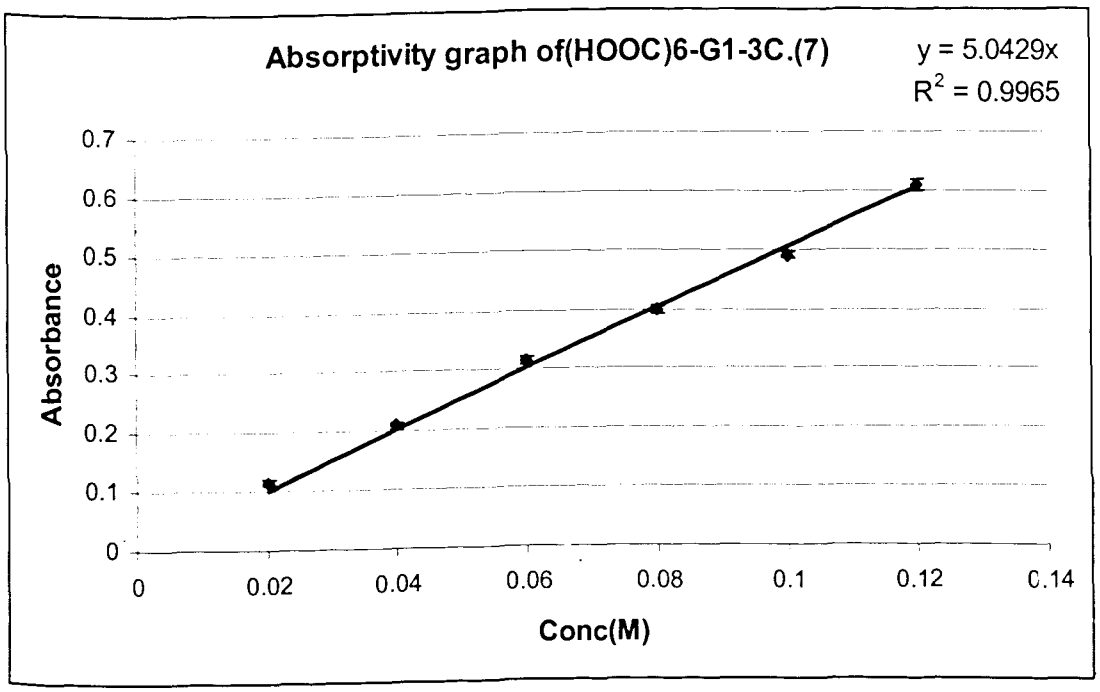


Figure 3.13: Absorptivity graph for $(HOOC)_6$-G1-3C,(7).

From the graph (compound 7) above slope of the line is calculated as absorptivity

$$\varepsilon = 5.04\ M^{-1}cm^{-1}$$

Having absorptivity, cell length and absorbance, maximum concentration of compound 7 at pH 7.4 can be calculated using Beer lambert low. This was calculated as follow.

Equation 5 → $A = \varepsilon.C.L.$

A = observed absorbance at pH 7.4 from stuck solution 2 = 0.708

C = concentration (5 $M^{-1}cm^{-1}$)

L = cell path length (1cm)

The calculation is therefore as follows:

$C = A/ \varepsilon \times L$

$C= 0.708 / 5.04 \times 1 \rightarrow C = 0.140\ M$

Therfore the maximum solubility of compound 7 in buffer at pH 7.4 is 0.140 M

As these macromolecules have amphiphilic properties, they were expected to form micelles and to show a critical micelle concentration (CMC). There is a relatively small range of concentrations separating the limit below which virtually no micelles are detected and the limit above which virtually all additional dendritic macromolecules form micelles. Many properties of surfactant solutions, if plotted against the concentration, appear to change at a different rate above and below this range. By extrapolating the loci of such properties above and below this range until they intersect, the CMC can be found. There are many methods that can be used to determine the value of the CMC. In this part, the dye encapsulation method was used [190]. The method uses the solubilisation properties of micelles, where the interior of the micelles provides a hydrophobic environment for the dye (a non-polar compound) to be accommodated. The absorbance value of the dye within the micelles will therefore give an indication of the formation of micelles [191,192].

3.18.1.3. CMC for $(HOOC)_6$-G1-3C dye encapsulation,(7).

Abs(1)	Abs(2)	Abs(3)	Ave	±SD N = 3	Conc.(M)X10^{-4})
0.1	0.08	0.09	0.09	0.01	7.06
0.16	0.18	0.14	0.16	0.02	7.10
0.18	0.2	0.22	0.2	0.02	7.13
0.4	0.5	0.3	0.4	0.1	7.15
0.5	0.52	0.46	0.49	0.03	7.17
0.5	0.47	0.54	0.5	0.0	7.19
0.52	0.51	0.49	0.51	0.02	7.20

Table 3.4: Data for calculation of the CMC by use of dye encapsulation for $(COOH)_6$-G1-C. (7).

0.1 g of compound 7 dissolved in 100 cm^3 of buffer at pH7.4→

Concentration of $(HOOC)_6$-G1-3C = 7.37 x 10^{-4} M

Using equation 6, new concentration can be found. All other concentrations were calculated in the same way.

Equation (6) $C_1 \times V_1 = C_2 \times V_2$

It should be remembered that only sample tube 7 to 14 shows the absorbance for dye

C_1= 7.37 x 10^{-4} M

V_1 = 0.007 dm^3

C_2 = total volume (300μ = 0.0003 + 0.007) = 0.01 dm^3

e.g. $C_1 \times V_1 = C_2 \times V_2$ → 7.37 x 10^{-4} x 0.007 = 0.01 x C_2 → C_2 = 5.159 x 10^{-4}M

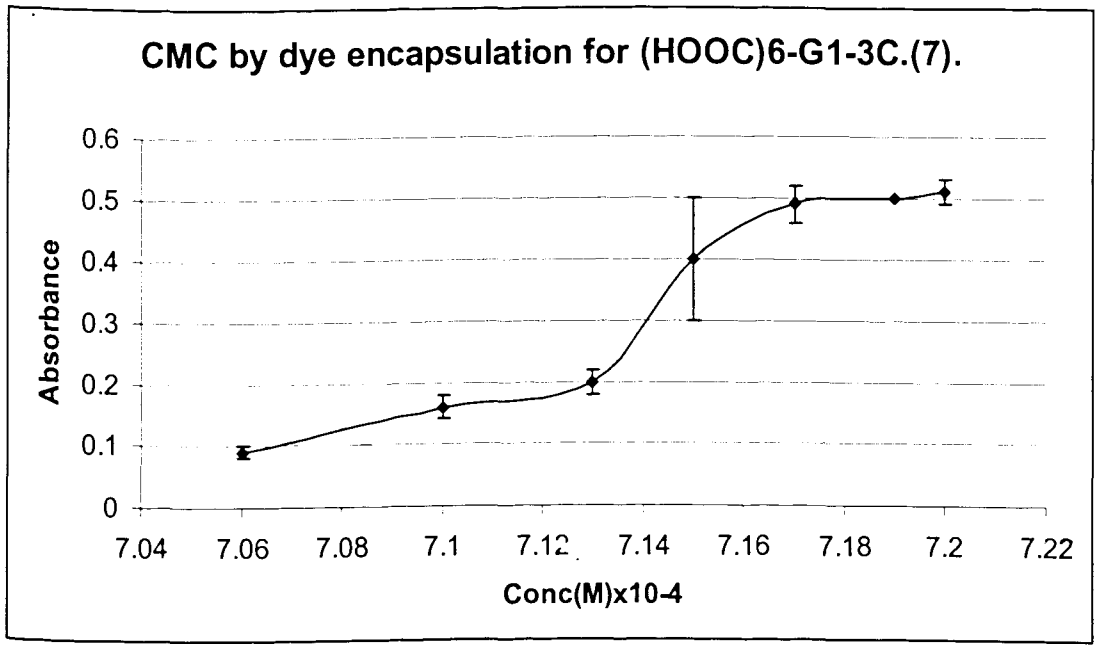


Figure 3.14: Dye encapsulation graph for $(HOOC)_6$-G1-3C,(7).

According to the graph above, the CMC occurred at around 7.15×10^{-4} M. In the dye encapsulation experiment, as the concentration of biphenyl dendrimer was increased, an increase in the colour intensity was observed. It should be noted that the dye used in this experiment was not soluble in water,

and the results shown here prove that the bi-phenyl compound improved the solubility of the dye by encapsulating it.

In the next experiment, the surface tension method was used to find the critical micelle concentration in order to verify the result found for CMC by dye encapsulation method.

CMC by surface tension calculation:

The concentration of the solution was found using equation 4

Equation (4)

$$C = C^0 (V / V^0 + V)$$

in which

C= Surfactant concentration

C^0 = Original concentration

V= Volume of solution added to water

V^0 = Original volume of distilled water

e.g. for the concentration of compound 7. $(COOH)_6$-G1-3C= 7.37×10^{-4} M

C= $7.37 \times 10^{-4} \times (0.001 / 0.0010 + 0.25) = 2.84 \times 10^{-5}$ M

3.18.1.4. CMC for $(HOOC)_6$-G1-3C by surface tension,(7).

Volume(HD)	1st (mNm^{-1})	2nd (mNm^{-1})	3rd (mNm^{-1})	Ave (mNm^{-1})	±SD n =3	Conc.(M)
1	69.5	69	69.4	69.3	0.26	2.84×10^{-5}
2	65.6	66	66.8	66.13	0.61	5.46×10^{-5}
4	61.4	61.3	61.2	61.3	0.08	1.02×10^{-4}
6	60.9	60.8	60.5	60.7	0.17	1.43×10^{-4}
8	59.8	60.2	60	60	0.16	1.78×10^{-4}
10	60.4	60.6	60.2	60.4	0.2	2.11×10^{-4}
12	60.8	60.4	60.1	60.3	0.35	2.39×10^{-4}
14	59.8	59.8	59.6	59.7	0.12	2.65×10^{-4}
16	59.4	60.2	60.1	59.9	0.44	2.89×10^{-4}
18	60.4	60.1	60.2	60.2	0.15	3.09×10^{-4}
20	61	61	60.2	60.7	0.46	3.28×10^{-4}

Table 3.5: Measurements for surface tension of $(HOOC)_6$-G1-3C,(7).

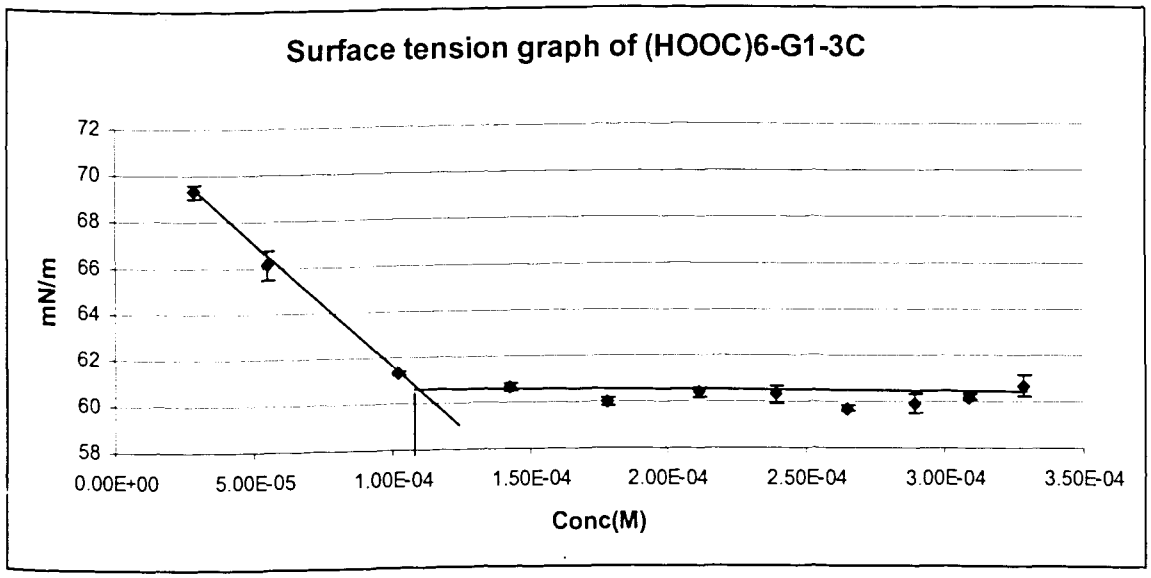


Figure 3.15: The surface tension of $(HOOC)_6$-G1-3C,(7). showing CMC at the intersection

According to the graph above the critical micelle concentration occurs at a region about 1.1 x 10^{-4} M

In the next part, molar conductivity methods were used to verify the result found by surface tension, using equation 1,2and 3

Concentration of surfactant is (C) = 7.37x 10^{-4} M

Equation 1) $$Kcell = \left[\frac{k(0.1MKCl\ )}{G(0.1MKCl)} \right]$$

Equation 2) $$k = K_{cell}\,(G - G_0)$$

Equation 3) $$A = (k/C)$$

3.18.1.5. CMC for $(HOOC)_6$-G1-3C by Molar conductivity,(7).

G1 (mS)	G2 (mS)	G3 (mS)	G^0 (mS)	Kcell cm^{-1}	K1 ($mScm^{-1}$)	K2 ($mScm^{-1}$)	k3 ($mScm^{-1}$)
1.3	1.32	1.31	0.4	1.26	1.13	1.16	1.13
2.3	2.35	2.3	0.4	1.26	2.5	2.4	2.4
3.6	3.5	3.4	0.4	1.26	4.03	3.91	3.8
4.2	4.4	4.3	0.4	1.26	4.79	5.04	4.91
4.9	5.1	5.1	0.4	1.26	5.67	5.92	5.92
5.5	5.7	5.4	0.4	1.26	6.43	6.68	6.3
6.1	6.3	6.3	0.4	1.26	7.18	7.43	7.43
6.7	6.9	6.8	0.4	1.26	7.94	8.2	8.06
7.2	7.3	7.2	0.4	1.26	8.57	8.7	8.57
7.6	7.7	7.6	0.4	1.26	9.07	9.2	9.07

Table 3.6: Conductance data and calculation molar conductivity for $(COOH)_6$-G1-3C,(7).

A1 $mScm^{-1}M^{-1}$	A2 $mScm^{-1}M^{-1}$	A3 $mScm^{-1}M^{-1}$	A(Ave) $mScm^{-1}M^{-1}$	±SD N = 3	Conc.(M)
103.5	106.2	103.5	104.40	1.56	5.46×10^{-5}
122.55	118	118	119.52	2.63	1.02×10^{-4}
141	136.7	133	136.90	4.00	1.43×10^{-4}
133.8	140.8	137.2	137.27	3.50	1.79×10^{-4}
134.4	140.3	140.3	138.33	3.41	2.11×10^{-4}
134.5	139.7	132	135.40	3.93	2.39×10^{-4}
135.5	140.2	140.2	138.63	2.71	2.65×10^{-4}
130.4	142.4	140	137.60	6.35	2.88×10^{-4}
138.7	140.8	138.7	139.40	1.21	3.09×10^{-4}
138.7	140.7	138.7	139.37	1.15	3.27×10^{-4}

Table 3.7: Data for the Molar conductivity for $(HOOC)_6$-G1-3,(7).

Molar conductivity graph for $(HOOC)_6$-G1-3C,(7).

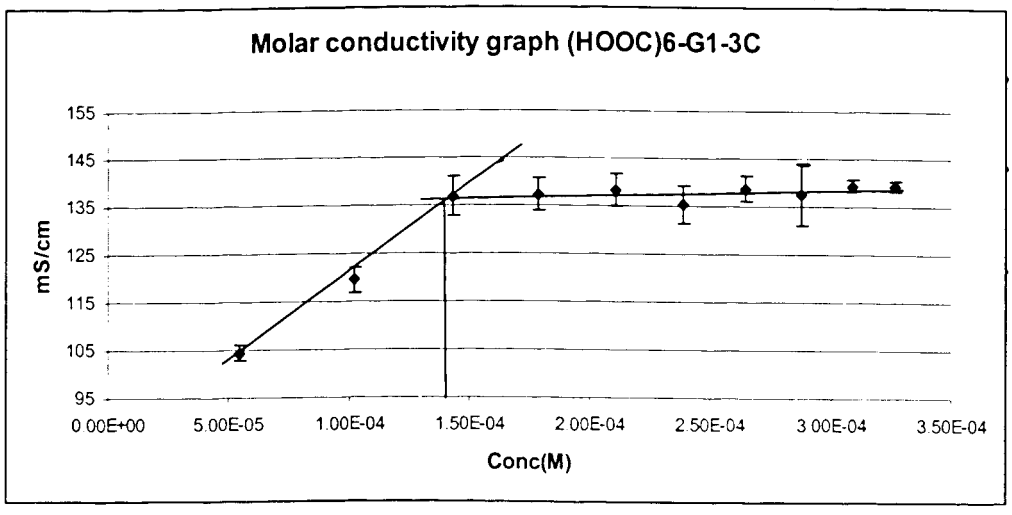
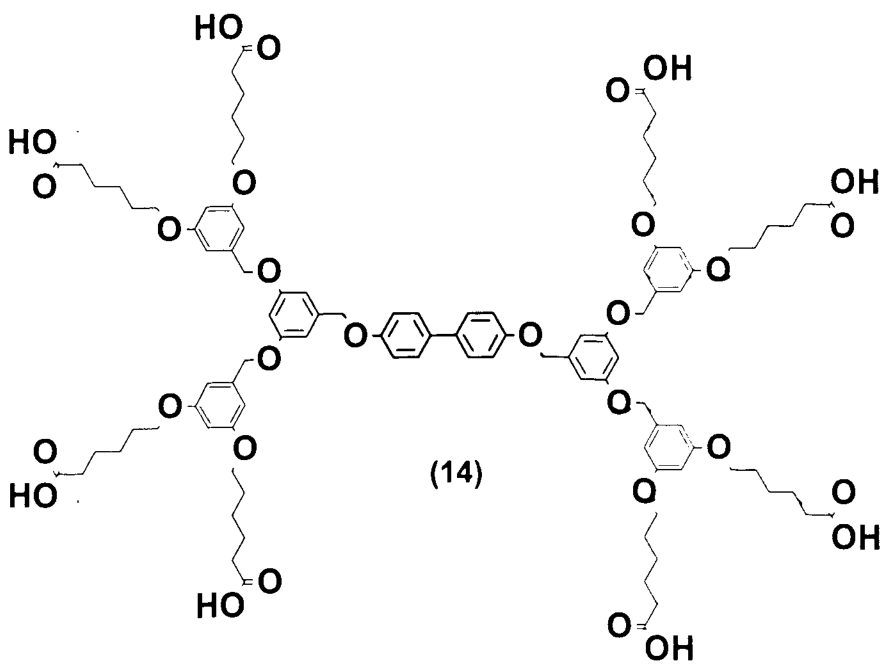


Figure 3.16. The molar conductivity of $(COOH)_6$-G1-3C, showing the CMC at the intersection point,(7).

The CMC from the graph above found to be 1.4 x 10^{-4} $mol^{-1}dm^{-3}$

Below the *CMC*, the addition of surfactant to an aqueous solution causes an increase in the number of charge carriers, an increase in the conductivity. Above the *CMC*, further addition of surfactant increases the micelle concentration while the monomer concentration remains approximately constant (at the *CMC* level). Since a micelle is much larger than a surfactant monomer it diffuses more slowly through solution and so is a less efficient charge carrier. A plot of conductivity against surfactant concentration is, thus expected to show a break at the *CMC*.

3.19. $(HOOC)_8$-G2-2C,(14).



(14)

3.19.1. pH Profile of biphenyl dendrimer $(HOOC)_8$-G2-2C ,(14).

pH	Absorbance (stock solution 1) $5.46*10^{-4}$ M	Absorbance (stock solution 2) $1.09*10^{-3}$ M
10	3.4	2.2
7.4	3.45	1.54
7	2.024	0.98
6	1.01	0.408
5	0.742	0.331

Table 3.8: pH Profile for $(HOOC)_8$-G2-2C,(14).

As the pH increases the absorbance increases, which means the compound become more soluble at higher pH, probably due to the nature of the carboxylic acid. As the carboxylic acid is a weak acid, it would have better dissociation in high pH than low pH.

3.19.2. Absorbance of $(HOOC)_8$-G2-2C.(14). at λ = 278nm

Dilution Factor	Hydrolysed dendrimer(ml)	Buffer(pH:7.4), (ml)	Conc .(M)
7.7	0.45	3.1	0.02
3.85	0.91	2.59	0.04
2.56	1.36	2.13	0.06
1.93	1.82	1.68	0.08

Table 3.9: Dilution factor calculation for $(HOOC)_8$-G2-2C,(14).

Absorbance and concentration of $(HOOC)_8$-G_2-2C,(14). at λ =278 nm

Abs(1)	Abs(2)	Abs(3)	Average(A)	±SD N = 3	Conc.(M)
0.57	0.62	0.58	0.58	0.04	0.02
1.24	1.25	1.27	1.25	0.02	0.04
1.79	1.86	1.80	1.82	0.04	0.06
2.42	2.49	2.46	2.46	0.04	0.08

Table 3.10: Data for absorptivity measurements $(HOOC)_8$-G2-2C,(14).

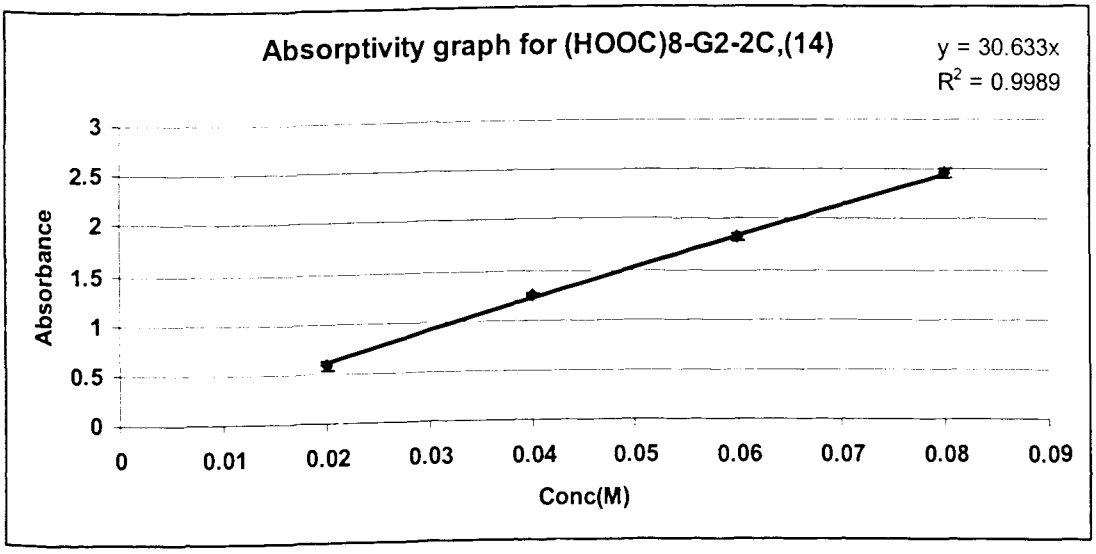


Figure 3.17: Absorptivity graph for $(HOOC)_8$-G2-2C,(14).

Absorptivity calculation:

Absorptivity of compound 14 is the slope of the graph above, shown as below.

$\varepsilon = 30.6\ M^{-1}cm^{-1}$

Having the absorptivity, the maximum solubility of compound 14 at pH 7.4 can be calculated (using equation 1)

$A = \varepsilon . C . L$ in which C = concentration, L= length of cell, ε = Absorptivity

A= 1.54

L = 1cm

$\varepsilon = 30.6\ M^{-1}cm^{-1}$

Therefore the maximum concentration of compound 14 at pH 7.4 was calculated as shown below

C = I.54 / 3.06 X 1 → C = 0.050 M

This is maximum solubility of compound 14 in pH 7.4

Surface tension calculation for compound 14:

All the surface tension calculations and measurements were done according to equation 4→ $C = C^0 (V / V^0 + V)$

Concentration of $(HOOC)_8$-G2-2C compound..(14). = 8.146×10^{-4} M.

3.19.3. CMC of $(HOOC)_8$-G2-2C by surface tension,(14).

Volume	1st (mNm^{-1})	2nd (mNm^{-1})	3rd (mNm^{-1})	Average (mNm^{-1})	±SD n = 3	Conc.(M)
1	66.4	66	66.2	66.2	0.2	4.15×10^{-5}
2	62.04	62	62.4	62.15	0.2	8.00×10^{-5}
4	56.8	56.2	56	56.33	0.42	1.49×10^{-4}
6	53	53.2	52.9	53.03	0.15	2.09×10^{-4}
8	52	51.8	52	51.93	0.12	2.62×10^{-4}
10	51.8	51.6	51.6	51.67	0.12	3.09×10^{-4}
12	51.6	51.2	51.7	51.5	0.27	3.50×10^{-4}
14	51.4	51.6	51.3	51.43	0.15	3.88×10^{-4}
16	51	51.4	51.2	51.2	0.2	4.21×10^{-4}
18	52	52.06	52	52.02	0.03	4.52×10^{-4}

Table 3.11: Surface tension data for $(HOOC)_8$-G2-2C,(14).

Surface tension graph of $(HOOC)_8$-G2-2C,(14).

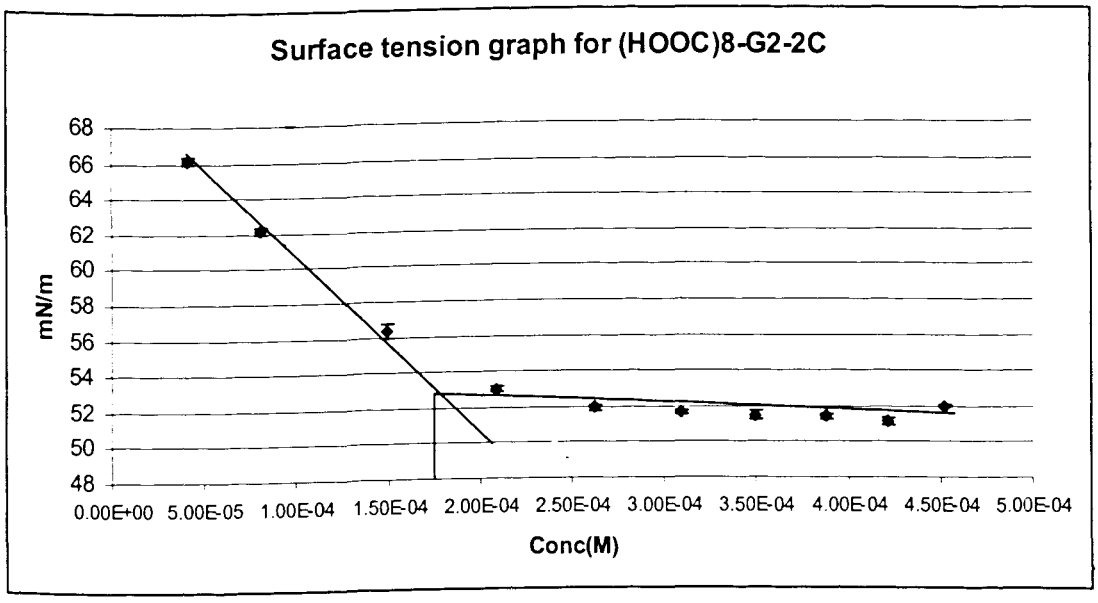


Figure 3.18: MC by surface tension graph for $(HOOC)_8$-G2-2C,(14).

According to the surface tension graph above the critical micelles concentration occurs at region about 1.5×10^{-4} M ($mol\,dm^{-3}$)

In the next step the molar conductivity of $(HOOC)_8$-G2-2C compound 14 was investigated to verify the result found by the surface tension method.

Concentration of surfactant in the burette: 8.146×10^{-4} M

Using equation 6 → $C_1 \times V_1 = C_2 \times V_2$

Other concentrations were calculated as follow:

e.g.→8.146×10^{-4} M x0.001 dm^3 = C_2 x 0.026 dm^3 → C= 3.13×10^{-5} $mol^{-1}dm^{-3}$

K= conductivity of (0.1 M KCl) from CRC hand book of physical &chemical contents = 1.131 Sm^{-1}

G = conductance of (0.1 MKCl), measured from the conductance meter=0.9 S

Using equation 1 → $$Kcell = \left[\frac{k(0.1MKCl\ )}{G(0.1MKCl)}\right]$$

K_{cell} (cell constant)= 1.26 m^{-1}

The conductivity for each concentration of the surfactant is then calculated

using the following equation 2 $$k = K_{cell}\,(G - G_0)$$

where

k= conductivity of surfactant

K_{cell}= cell constant

G= conductance of surfactant measured via a conductance meter = 0.9

G_0= Conductance of the distilled water → measured via a conductance meter= 0.4

The molar conductivity for each concentration of the surfactant is then calculated using the following equation 3

$$A = (k/C)$$

In which C= surfactant concentration

A= molar conductivity of surfactant = (k/C)

The remaining molar conductivities for each surfactant concentration were calculated in the same way as shown in the tables below. (Conductivity of distilled water = 0.4)

3.19.4. CMC for $(HOOC)_8$-G2-2C by Molar conductivity,(14).

G1 (mS)	G2 (mS)	G3 (mS)	G_0 mS	Kcell (cm^{-1})	k_1 ($mScm^{-1}$)	K_2 ($mScm^{-1}$)	K_3 ($mScm^{-1}$)
1.3	1.25	1.3	0.4	1.26	1.134	1.1	1.134
2.4	2.6	2.5	0.4	1.26	2.52	2.77	2.65
3.3	3.4	3.3	0.4	1.26	3.65	3.78	3.65
4.2	4.3	4.4	0.4	1.26	4.79	4.95	5.04
4.9	4.8	5	0.4	1.26	5.67	5.54	5.79
5.5	5.4	5.5	0.4	1.26	6.42	6.3	6.42
6	6.2	5.95	0.4	1.26	7.1	7.31	7
6.3	6.2	6.35	0.4	1.26	7.34	7.31	7.5
7	7.1	6.98	0.4	1.26	8.32	8.44	8.42
7.3	7.2	7.35	0.4	1.26	8.7	8.6	8.8

Table 3.12: Conductance data of molar conductivities for $(HOOC)_8$-G1-2C ,(14).

A_1 $mScm^{-1}M^{-1}$	A_2 $mScm^{-1}M^{-1}$	A_3 $mScm^{-1}M^{-1}$	Average A $mScm^{-1}M^{-1}$	± SD n = 3	Conc.(M)
94.2	91.2	94.02	93.14	1.68	6.03×10^{-5}
112.5	123.7	118.3	118.17	5.60	1.12×10^{-4}
115.5	119.6	115.5	116.87	2.37	1.58×10^{-4}
118.7	125	127.3	123.67	4.45	1.98×10^{-4}
121.7	119	124.2	121.63	2.60	2.33×10^{-4}
121.6	119.32	121.5	120.81	1.29	2.64×10^{-4}
121.6	125.2	120	122.27	2.66	2.92×10^{-4}

Table 3.13: Conductance data for molar conductivity of compound,(14).

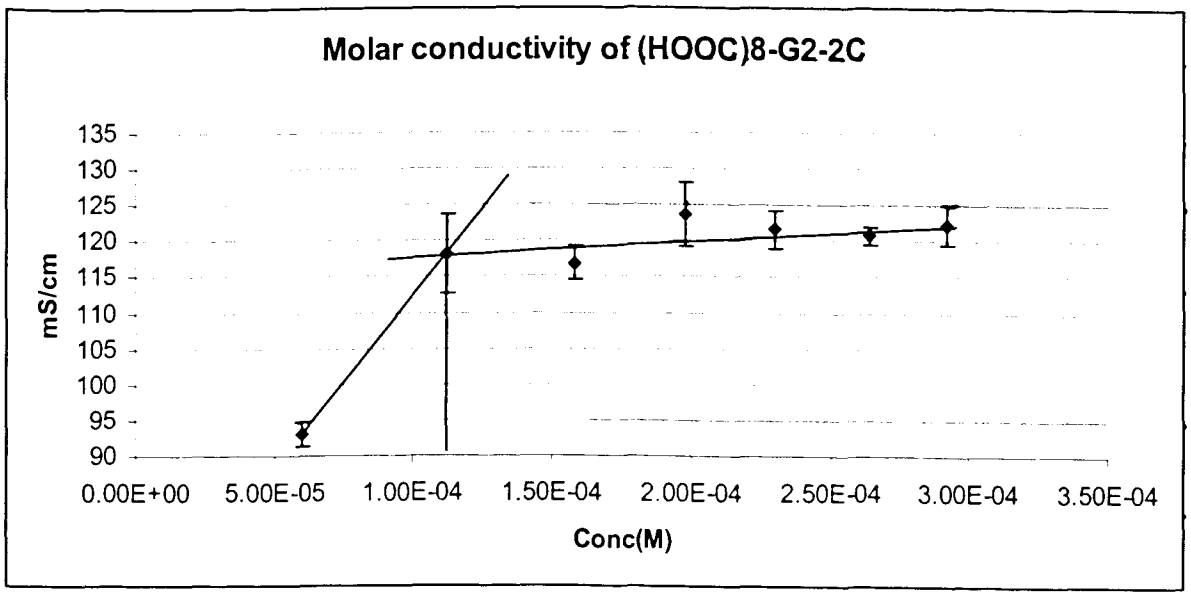


Figure 3.19: CMC by molar conductivity graph for $(HOOC)_8$-G2-2C,(14).

Molar conductivity is a very convenient way of quantifying conductivity because it highlights the properties of the electrolyte. For instance, doubling the concentration of an electrolyte solution would be expected to double the number of ions and thus to double the conductivity. In this case the molar conductivity would be unchanged. In most cases, however, the molar conductivity actually decreases with increasing concentration owing to the influence of concentration on interactions between electrolyte ions or on an ionic dissociation process.

The molar conductivity of an electrolyte depends upon the extent to which the electrolyte dissociates into ions. Strong electrolytes (e.g. KCl) are almost completely onised, whilst weak electrolytes (e.g. CH3COOH) are ionised to only a small extent. Dilution of an electrolyte solution increases the extent of dissociation.

The plot of molar conductivity against surfactant concentration can be used to found CMC.

3.19.5. CMC of $(HOOC)_8$-G2-2C by dye encapsulation,(14).

Volume(ml)	Abs(1)	Abs(2)	Abs(3)	Average	±SD N = 3	Conc.(M) x 10^{-4}
3	0.1	0.06	0.08	0.08	0.02	7.41
4	0.12	0.14	0.13	0.13	0.01	7.58
5	0.24	0.26	0.22	0.24	0.02	7.69
6	0.5	0.53	0.51	0.51	0.02	7.75
7	0.6	0.62	0.64	0.62	0.02	7.81
8	0.72	0.7	0.69	0.70	0.02	7.85
9	0.74	0.72	0.76	0.74	0.02	7.85
10	0.75	0.77	0.78	0.77	0.02	7.91

Table 3.14: Dye encapsulation measurement for $(HOOC)_8$-G2-2C,(14).

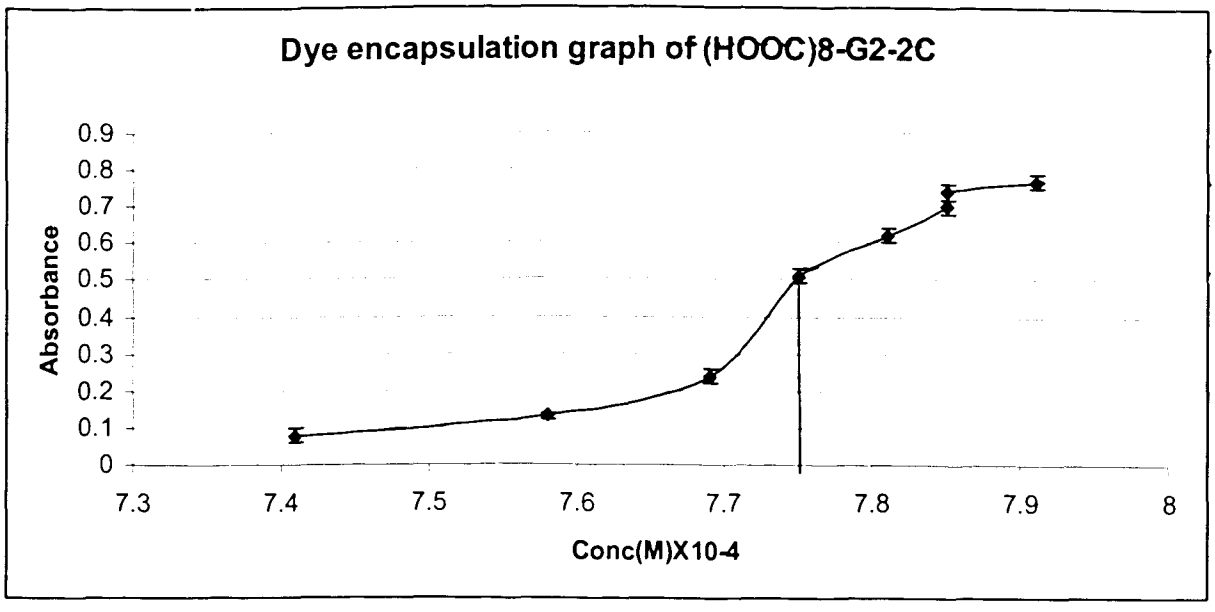
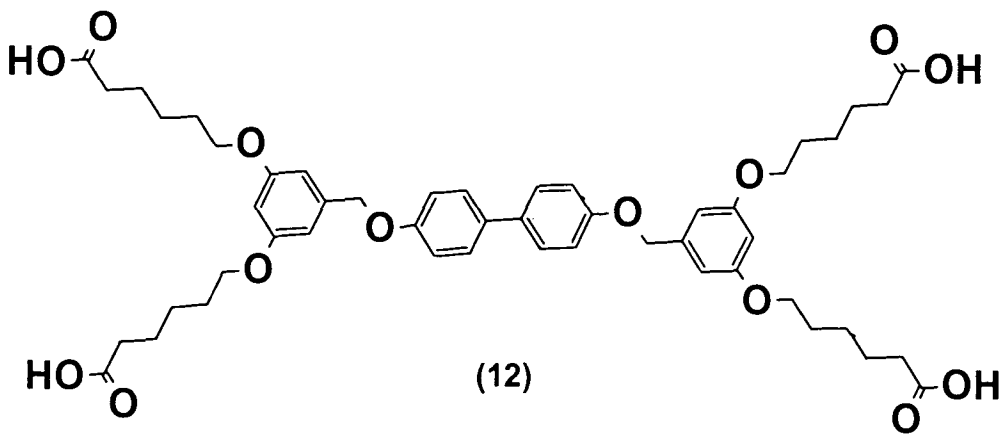


Figure 3.20: CMC by dye encapsulation graph OF $(HOOC)_8$-G2-2C,(14).

Critical micelle concentration in the dye encapsulation graph above occurs at around 7.75×10^{-4} M.

3.20. $(HOOC)_4$-G1-2C,(12).



(12)

3.20.1. pH Profile for $(HOOC)_4$-G1-2C,(12).

pH	Absorbance(First solution,(1) 1.13×10^{-3} M	Absorbance(Second solution,(2) 2.25×10^{-3} M
6.5	2.08	0.905
6.8	2.71	1.51
7	3.05	1.81
7.4	3.103	2.85
10	3.2	3.1

Table 3.15: pH Profile for $(HOOC)_4$-G1-2C,(12).

3.20.2. Absorbance of $(HOOC)_4$-G1-2C,(12). at λ = 274 nm

Conc.(Mol)	Dilution factor	Volume of stock solution(ml)	Volume of buffer(pH=7.4) (ml)
0.2	14.25	0.25	3.25
0.4	7.13	0.5	3.0
0.6	4.75	0.74	2.74
0.8	3.6	1	2.5

Table 3.16: Preparation of solutions for absorptivity measurements for $(HOOC)_4$-G1-2C.

Absorbance and Concentration of $(HOOC)_4$-G1-2C,(12), at λ = 274 nm

Abs(1)	Abs(2)	Abs(3)	Average	±SD n =3	Conc.(M)
0.55	0.52	0.47	0.51	0.04	0.02
1.38	1.45	1.42	1.41	0.04	0.04
2.1	1.98	2.05	2.10	0.06	0.06
2.85	2.65	2.78	2.76	0.10	0.08

Table 3.17: UV-visible absorbance of $(HOOC)_4$-G1-2C solution at 278 nm.

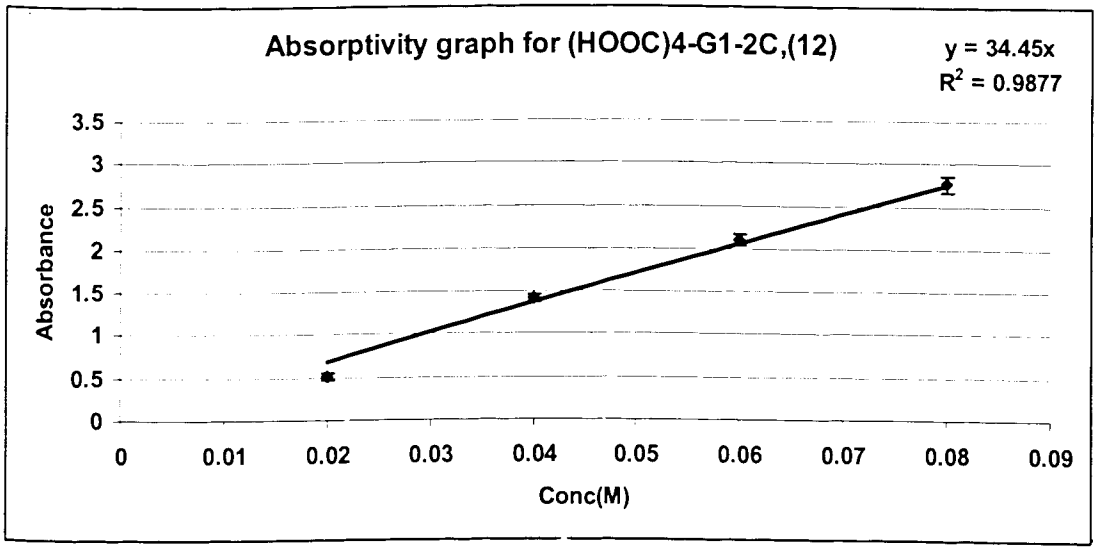


Figure 3.21: Absorptivity graph for $(COOH)_4$-G1-2C,(12).

The slope of the graph above (**fig 3.22**) is absorptivity. As shown below

$\varepsilon = 34.45\ M^{-1}cm^{-1}$ $C = A/\ \varepsilon \times L$ $C = 1.51 / 34.45\ M^{-1}\ cm^{-1} \times 1cm = 0.044\ M$

This is the maximum solubility of compound 12 in pH 7.4

In the next experiment, the surface tension was method used to find critical micelles concentration in order to verify the result found for CMC by dye encapsulation method.

CMC by surface tension calculation:

Concentration of solution was found using equation 4

Equation (4)

$$C = C^0 (V/ V^0 + V)$$

Mass = 0.1 g $R_{mm} = 887.018$ g/mol → $n = 1.13X\ 10^{-3} / 0.1dm^3$ →Using eq (4). New concentration can be calculated $C = 4.33 \times 10^{-5}\ M$

Remaining calculations were done in the same way:

3.20.3. Surface tension for $(HOOC)_4$-G1-2C,(12).

Volume added	1st ($mN.m^{-1}$)	2nd ($mN.m^{-1}$)	3rd ($mN.m^{-1}$)	Ave ($mN.m^{-1}$)	SD	Conc.(M)
25ml of water	72.3	72.5	73	72.60	0.36	--------
1	68.9	68.6	68.5	68.67	0.21	4.33×10^{-5}
2	66.6	66.8	66.9	66.77	0.15	9.41×10^{-5}
3	63.8	63	62.4	63.07	0.70	1.36×10^{-4}
4	61.9	61.2	62	61.70	0.44	1.75×10^{-4}
5	59.8	59.6	60.2	59.87	0.31	2.12×10^{-4}
6	56	55.4	56.2	55.87	0.42	2.46×10^{-4}
8	54.6	54	54.3	54.30	0.30	2.78×10^{-4}
10	51	52.8	52.2	52.00	0.92	3.63×10^{-4}
12	52.6	51	51.9	51.83	0.80	4.12×10^{-4}
14	51.8	52.4	51.2	51.80	0.60	4.56×10^{-4}
16	53	51.9	53.2	52.70	0.70	4.95×10^{-4}
18	52.4	51.8	53.2	52.47	0.70	5.32×10^{-4}
20	51.6	52.3	52.1	52.00	0.36	5.64×10^{-4}
22	53.2	54	53.6	53.60	0.40	5.94×10^{-4}
24	51.2	51.8	51.4	51.47	0.31	6.22×10^{-4}
26	52.2	52.9	52.4	52.50	0.36	6.47×10^{-4}
28	53.4	53.6	53.4	53.47	0.12	6.70×10^{-4}
32	51.8	51.2	51.8	51.60	0.35	7.13×10^{-4}
38	52.3	52	52.6	52.30	0.30	7.66×10^{-4}
42	53.6	53.1	54.1	53.60	0.50	7.96×10^{-4}
50	51.9	52.6	51.8	52.10	0.44	8.47×10^{-4}

Table 3.18: Surface tension data vs concentration for $(COOH)_4$-G1-2C,(12).

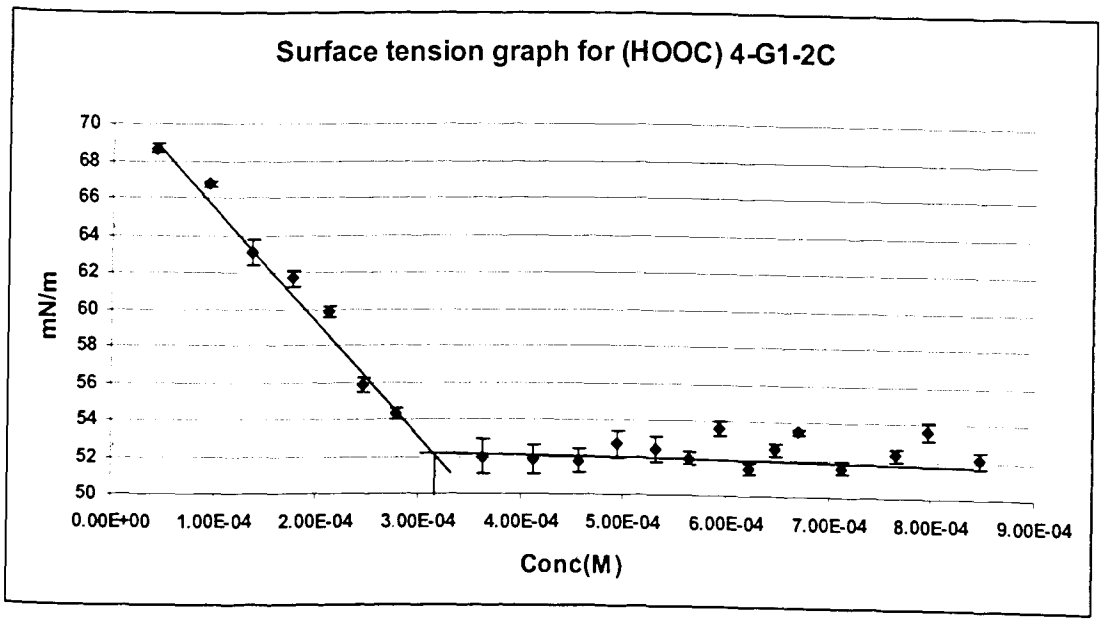


Figure 3.22: CMC by surface tension graph for $(COOH)_4$-G1-2C,(12)

According to the surface tension graph above the critical micelle concentration occurs at around 3.2×10^{-4} M.

3.20.4. Molar conductivity for $(HOOC)_4$-G1-2C,(12).

Concentration of compound 12 in buffer at pH 7.4 is 1.123×10^{-3} M.

C = $(HOOC)_4$-G1-2C is 1.123×10^{-3} M.

To find the molar conductivity, equations 1, 2, 3 and 5 were used.

G1 mS	G2 mS	G3 mS	G0 mS	K_{cell} $mScm^{-1}$	K1 $mScm^{-1}$	K2 $mScm^{-1}$	K3 $mScm^{-1}$
1.02	1	1.04	0.4	1.26	0.78	0.76	0.81
1.8	1.7	1.8	0.4	1.26	1.76	1.64	1.76
2.8	2.6	2.64	0.4	1.26	3.02	2.77	2.82
3.9	3.6	3.4	0.4	1.26	4.41	4.03	3.78
4.6	4.2	4.8	0.4	1.26	5.29	4.79	5.54
5.2	4.9	5.4	0.4	1.26	6.05	5.67	6.30
5.9	5.6	5.4	0.4	1.26	6.93	6.55	6.30
6.2	5.9	6.4	0.4	1.26	7.31	6.93	7.56
6.8	6.6	6.9	0.4	1.26	8.06	7.81	8.19
7.02	7.04	7.06	0.4	1.26	8.34	8.37	8.37
7.3	7.5	7.2	0.4	1.26	8.69	8.94	8.57
8	8.2	8.3	0.4	1.26	9.58	9.83	9.95

Table 3.19: Conductance data of molar conductivity for $(COOH)_4$-G1-2C,(12).

In which

Where K_{cell} = cell constant

k= conductivity of surfactant

k= conductivity of surfactant

G = Conductance of (0.1 M KCl), measured from the conductance meter

A1 $mScm^{-1}M^{-1}$	A2 $mScm^{-1}M^{-1}$	A3 $mScm^{-1}M^{-1}$	Average (A)	±SD n= 3	Conc.(M)
42.81	41.71	44.46	42.99	1.38	9.11×10^{-5}
56.77	52.90	56.70	55.46	2.21	1.55×10^{-4}
69.59	63.82	64.98	66.13	3.05	2.17×10^{-4}
78.47	71.74	67.26	72.49	5.64	2.81×10^{-4}
82.43	74.58	86.36	81.12	6.00	3.21×10^{-4}
83.08	77.88	86.54	82.50	4.36	3.64×10^{-4}
85.98	81.29	78.16	81.81	3.94	4.03×10^{-4}
83.42	86.30	86.30	85.34	1.66	4.38×10^{-4}
85.79	87.13	87.13	86.68	0.77	4.70×10^{-4}
83.57	78.26	83.87	81.90	3.16	4.99×10^{-4}
82.60	81.46	81.46	81.84	0.66	5.26×10^{-4}
85.35	88.72	88.72	87.60	1.95	5.61×10^{-4}

Table 3.20: Conductance measurement and calculation for $(COOH)_4$-G1-2C,(12).

$A = (K / C)$

In which

A = molar conductivity

K = conductivity

C = concentration

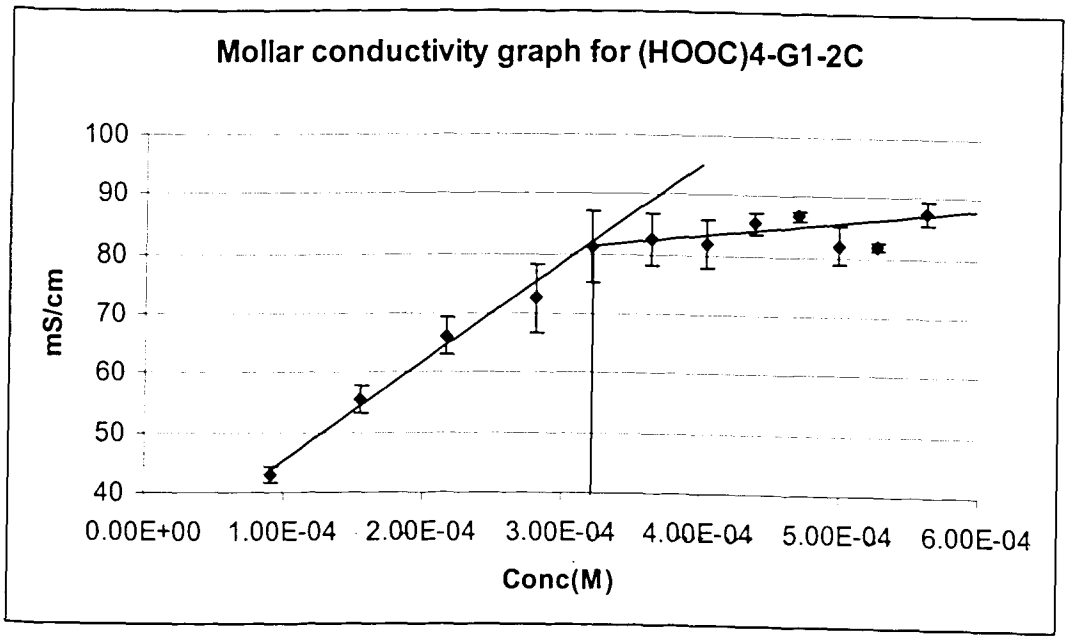


Figure 3.23: CMC by molar conductivity graph for $(COOH)_4$-G1-2C,(12).

According to the graph of molar conductivity above, the CMC occurs around 3.3×10^{-4} M

3.21. Solubility properties of compounds 7, 12 and 14

The solubility of dendritic macromolecules synthesis in this project was investigated in buffer solutions at different pH. Many factors affect the solubility of dendritic macromolecules

i) Polar compounds dissolve in polar solvents and non-polar in non-polar solvents

ii) Temperature: an increase in temperature decreases solubility in exothermic and increases the solubility in endothermic systems

iii) The molecular size and structure (branching of a compound). Generally compounds with a high molecular weight tend to have a lower solubility than those of lower molecular weights in the same homologous series.

iv) Type of bonding in compound

Table 3.21 represents the effect of bonding type on the solubility of a compound in water

Solubilisation refers to the amount of a substance that can be dissolved in a given solvent under specified conditions. Micelles are capable of increasing the solubility of most organics in water or buffer solution at pH 7.4. The mechanism by which the solubilisation occurs is the incorporation of organic molecules into the micelles. The interior of micelles provides a hydrophobic environment for the apolar compound to be accommodated and solubilised; a completely water-soluble drug can be adsorbed within the hydrophilic core of the micelle.

3.21.1. Type of bonding in compound

Bonding type	Solubility in water
Ionic	most soluble
Metallic	Insoluble – unless they react with water
Polar covalent	Soluble if it has H bonds
Non – polar covalent	Most insoluble – some slightly soluble
Covalent lattice	Insoluble

Table 3.21: The effect of bonding type on the solubility of a compound in water. [205]

The macromolecules made in this project had hydrophobic cores and hydrophilic chain ends; therefore it is expected that as the number of hydrolysed chain ends increases, the solubility of dendritic macromolecules will increase. In comparison, the second generation bi-phenyl dendrimer had higher solubility than the other two which was in good agreement with our prediction. **Table 3.22** represent the absorbances and maximum solubility of compound 7, 12 and 14 in buffer at pH at 7.4. As expected, increasing the number of hydrophilic functional groups in the structure increases the solubility.

3.21.2. Absorptivity and solubility of dendritic macromolecules at pH 7.4

Compound name	Absorptivity ($M^{-1}cm^{-1}$)	Solubility at pH :7.4 (M)
$(HOOC)_6$-G1-3C,(**7**).	5.04	0.14
$(HOOC)_4$-G1-2C,(**12**).	34.45	0.041
$(HOOC)_8$-G2-2C,(**14**).	30.63	0.05

Table 3.22: The solubility and absorptivity of dendritic macromolecules synthesised

3.21.3. Average CMC found by three different methods for dendritic macromolecules.

$(HOC)_6$-G1-3C Compund,(7).	$(HOC)_4$-G1-2C Compound,(12)	$(HOC)_8$-G2-2C Compound,(14).	$(HOC)_8$-G2-2C Compound,(14).
Surface tension	1.1×10^{-4}	3.2×10^{-4}	1.5×10^{-4}
Molar conductivity	1.4×10^{-4}	3.3×10^{-4}	1.1×10^{-4}
Average CMC	1.25×10^{-4}	3.25×10^{-4}	1.3×10^{-4}

Table 3.23: CMC found by two different methods for (compound 7 and14) and their averages

CMC	$(HOC)_6$-G1-3C Compund,(7).	$(HOC)_4$-G1-2C Compound,(12)	$(HOC)_8$-G2-2C Compound,(14).
Dye encapsulation	7.15×10^{-4}	Not measureable	7.75×10^{-4}

Table 3.24: CMC found by Dye encapsulation for compound 7 and 14

The CMC's found by surface tension and molar conductivity for compound 7 and 14 is in reasonably good agreement. As expected, the average CMC for $(COOH)_8$-G2-2C. (14) is bigger than those for the remaining two dendritic macromolecule, because of size and bulky structure. $(COOH)_4$-G1-2C didn't show any CMC by dye encapsulation; this could be predicted because it is already known that the size of the dendritic macromolecule is relevant to its three dimensional shape [193]; for example lower generation dendrimers tend to have open and amorphous structures, whereas higher generations can adopt spherical conformations capable of incorporating drug molecules [194] . The $(COOH)_8$-G2-2C and $(COOH)_6$-G1-3C dendritic macromolecules are amphiphilic and they are predicted to have hydrophobic cores and hydrophilic shells. Generally dendritic macromolecules consisting of an apolar core and polar shell are referred to as "unimolecular micelles" and unlike conventional micelles the dendritic structure is independent of dendrimer concentration [195,196]. The two dendritic macromolecules in this project might be predicted to have that sort of micelle property, although these structures are partial dendrimers and they are in a lower generation. Having a critical micelle concentration indicates that they don't form unimolecular micelles, rather those different segments could gather together and form micelles. The CMCs

found by surface tension and molar conductivity in this project were very similar but the CMC found by dye encapsulation method is slightly higher, which could be due to dye molecules might having affected the CMC value.

Another parameter that can be mentioned about these dendritic macromolecules is that they resemble weak bolaamphiphiles [197,198] and they have a weak hydrophilic group such as COO^- in their chain end. Bolaamphiphiles are molecules with two hydrophilic heads connected by one or two hydrophobic chains, and in comparison to uni-polar surfactants they have a smaller size and higher CMC [199]. In order to have more information about the properties of these macromolecules it was necessary to compare their CMC to those of other well known surfactants.

3.21.4. CMC of different type of surfactant

Surfactant	Literature CMC	Molecular weight
Hexadecylpyridinium Bromide	0.73×10^{-3}	384 Da
Pluronic P105	4.3×10^{-4}	6500 Da
$(COOH)_8$-G2-2C,(**14**).	3.6×10^{-4}	1832 Da
$(COOH)_6$-G1-3C,(**7**).	3.3×10^{-4}	1357 Da
$(COOH)_4$-G1-2C ,(**12**).	3.3×10^{-4}	887 Da

Table 3.25: Relation between molecular weight and CMC for dendritic macromolecules and two well known surfactants

Pluronics have been used extensively in a variety of pharmaceuticals including delivery of low molecular mass drugs and polypeptides [200]. There are many kind of pluronics; the one used in this project for comparison is Pluronic (P105 also known as either Synperonic P105, or Poloxamer P105). This tri-block copolymer is composed of polyethylene oxide (POE) and polypropylene oxide (PPO) segments. The approximate formula of the copolymer used is $PEO_{37}PPO_{56}PEO_{37}$, it has an average molecular weight of 6,500 and a melting point of 35 ^{0}C. [201] .

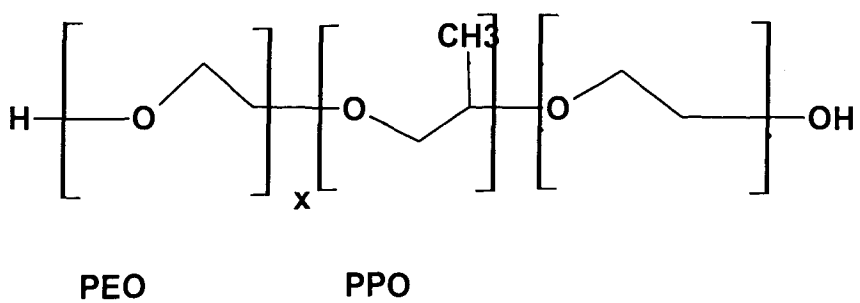


Figure 3.24: . The structure of Pluronic co-polymers from Sigma Aldrich

Hexadecylpyridinium bromide is a cationic surfactant, containing a pyridinium bromide derivative and hexadecyl alkyl chain.

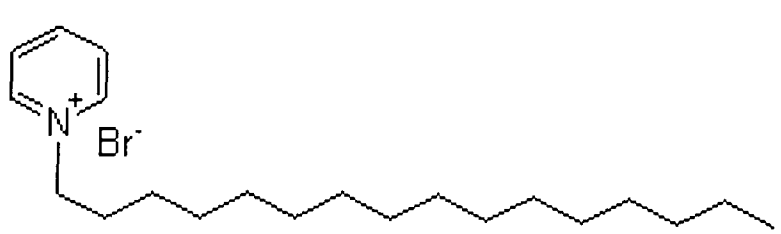


Figure 3.25: The structure of Hexadecylpyridinium bromide [202]

Pluronic (P105) has the highest molecular weight, and hexadecylpyridinium bromide the lowest. CMCs of our dendritic macromolecules are in between these two, which could be due to the bulky structure. Pluronic P105 has the highest CMC due to its straight chain conformation. By comparison, our dendritic macromolecules are intermediate in structure.

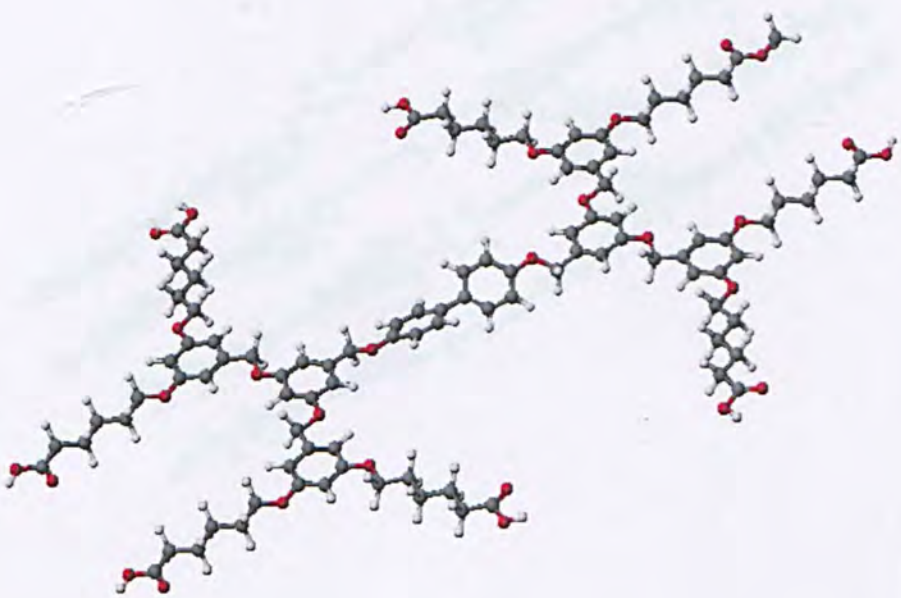
The actual internal geometry of dendritic macromolecules is likely to be determined by the type of molecule used to form the branches (nature of hydrophobic chain; also factors such as the nature of the hydrophilic end moiety, and size of the dendritic macromolecule will also be relevant to the three dimensional shape. Dendritic macromolecules synthesised in this project are partial dendrimers. In most similar synthetic procedures to make dendritic macromolecules for drug delivery, the number of carbons in the branching unit is 2 or maximum 3, and the reason is that a shorter carbon chain reduces the hydrophobicity and the same time increases the hydrophilicity of macromolecules; this improves the solubility, which is crucial in drug delivery. In this project it was decided to use six carbons in the branching unit which is attached to the 3,5-di-hydroxybenzyl alcohol, and the idea was the large number of carbon atoms in the branching unit (hydrophobic segment) might cover and hold the organic drugs with a dendrimer of lower generation number. It has been already known that an increase in the hydrocarbon chain length in dendritic macromolecules with identical polar groups, results in a decrease in the critical micelle concentration and an increase in the molecular size[203,204], so it was expected that in comparison with similar synthetic procedures our dendritic macromolecules should have

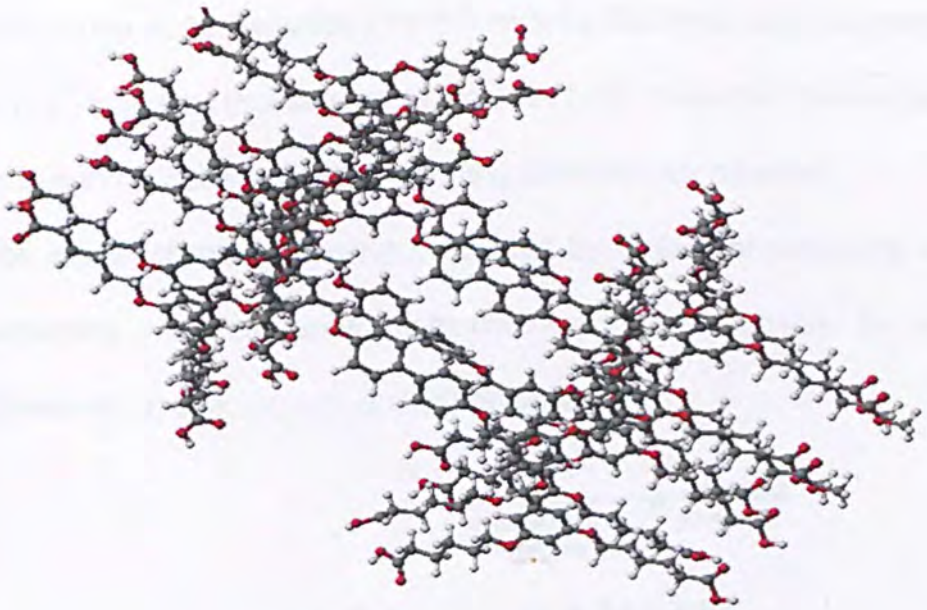
lower CMCs and bigger size. Another factor that can be mentioned here is the nature and size of the hydrophilic groups; usually a larger hydrophilic group results in an increase in the CMC; the dendritic macromolecules synthesised in this project has carboxylate end groups which has average size.

Overall percentage yield for compound 7,14 nd 12 are 72.5%, 65% and 72% respectively.

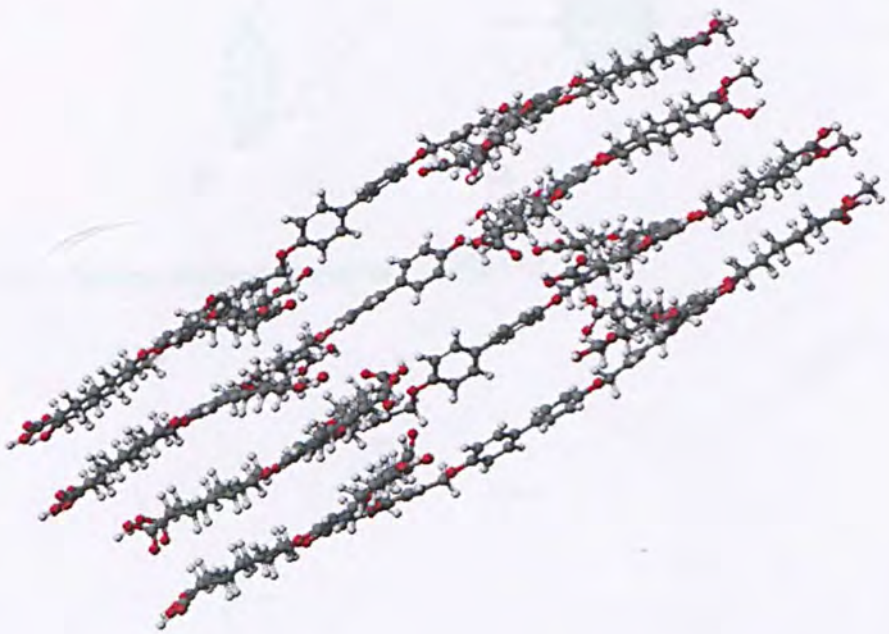
Molecular modelling for $(HOOC)_6$-G1-3C,(7). and $(HOOC)_8$-G1-2C,(14) was done using Quantum CAChe software..In the first step a MM3 calculation was done, and then from the calculation of the most stable conformer (lowest energy), using the PM3 program. It should be noted that all compounds below were modelled as being solvated in water

3.21.5. Molecular modelling for Second generation biphenyl dendrimer $(HOOC)_8$-G2-2C,(14).





Molecular modelling for layers of hydrolysed second generation biphenyl dendrimer:



,

The average size of compound 14 visually in the flat form is about 6146nm or 6146000 pm , comparing it with the average size of this compound in micellar

form found by he Zetasizer (1247.3 nm= 1247000pm, with poly-dispersity of 0.12), indicates that several molecules of this molecule come together and form each micelle rather than forming unimolecular micelles.

The shape of the compound calculated by molecular modelling here is one possibility, micelles can form in different kinds of shape*: for example* (a) spherical, (b) disk, (c) rod, and (d) reversed.

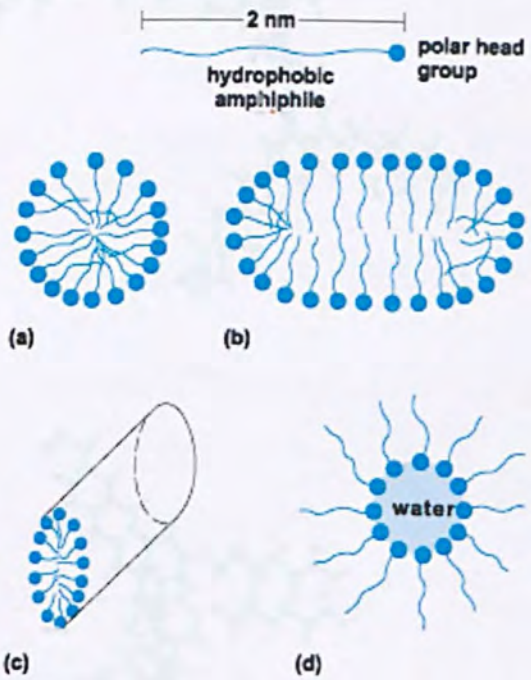
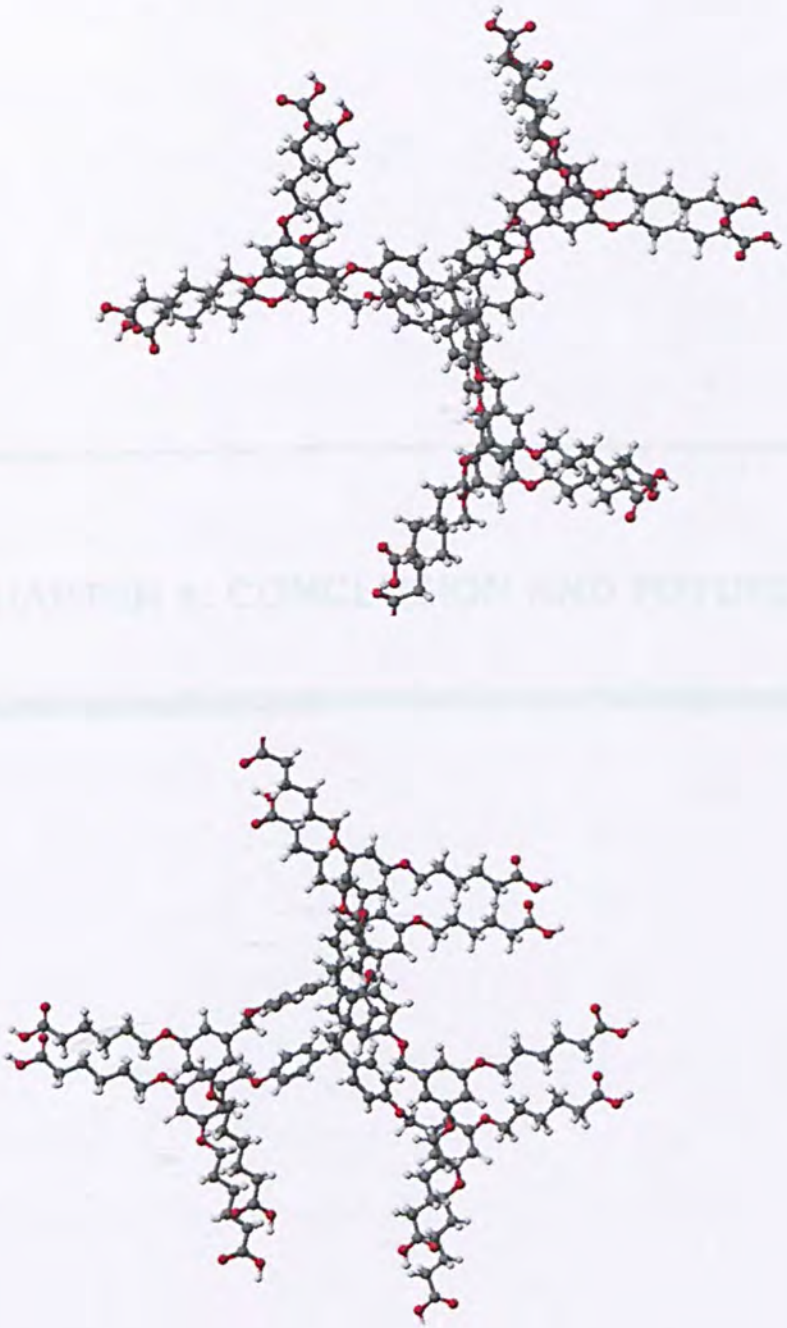


Figure 3.26: Possible different shapes of micelles [206]

***Molecular modelling for $(HOOC)_6$-G1-3C,(7)* Solvated in water.**



The average size of compound 7 visually in the flat shape is about 5006nm = 5006000pm, compare it to the average size of this compound in micellar form found ny Zetasizer (136.6nm = 136600pm with polydispersity 0.52) indicates that many units of this molecule come together and form micelles rather than forming,unimolecular,micelles.

CHAPTER 4: CONCLUSION AND FUTURE WORK

The work presented in this thesis was the synthesis of carboxylated-terminated polyether dendritic macromolecules. These were based on an electron-rich 3,5-dihydroxybenzyl alcohol building block, and were prepared by the convergent method approach.

In this project first and second generation dendrons were attached to the tri and biphenyl cores and their surfaces were modified to give a hydrophilic carboxylic acid surface groups, the physical and chemical properties of these molecules was then investigated.

Dendritic macromolecules were synthesised in this project as follows:

1) $(HOOC)_4$-G1-2C (Compound 12)
2) $(HOOC)_8$-G2-2C (Compound 14)
3) $(HOOC)_6$-G1-3C (compound 7)

It has been widely documented that the solubility of hydrophobic compounds in water can be dramatically enhanced by the addition of the surfactant molecules at a concentration above the CMC [207] by an encapsulation process.

Investigation of dye encapsulation by compounds 12, 14, and 7 revealed that only compounds 14 and 7 could encapsulate a water-insoluble dye; this could be due to the number of carboxylate groups in the structures of these molecules (molecular modelling of compound 14 and 7, p180-183) in which the branching units spread in opposite directions and they were able to

encapsulate the dye molecules. The other thing that can be mentioned here is that compound 14 had the highest CMC compared with the other two compounds; this could be expected because of the structure and molecular weight of compound 14.

Studies of dye encapsulation, molar conductivity and surface tension revealed that compounds 12, 14 and 7 all showed a CMC, which indicates that they did not form unimolecular micelle, where only one macromolecule is the micelle. This is confirmed by molecular modelling using Quantum CAChe, together by Zetasizer measurements which indicated that the micelle structure was much larger than the size of individual dendrimer molecules.

The dendrimers have good solubility in water at pH 7.4 and compound 7 and 14 are able to encapsulate a water-insoluble dye and improve its solubility. This indicates that by further modification of the structure of these compounds, they may have the potential to be used as drug delivery agents.

The formation of micelle can be measured by many physical parameters, and the measurement of properties such as surface tension, dye encapsulation and molar conductivity techniques give a good indication of the onset of micelle formation.

Much of the investigation performed in this research could be developed further. The author suggests the following main ideas:

The synthesis of higher generation dendrons should be attempted, and the physical and chemical properties of new dendrimers (such as solubility, formation of micelles and dye uptake) investigated.

It is apparent that as the generations increase synthesis becomes more difficult. An approach to be further investigated could be to make bespoke cores such as linear terphenyls (synthesised by Suzuki coupling reactions) which can be designed to produce a larger dendrimer with bigger cavaties. A simple example could be to replace the current biphenyl core with the terphenyl core which should increase cavity size (fig 3.28).

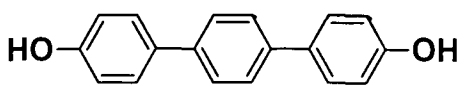


Figure 3.27: Figure of terphenyl core

It may be nessecary to increase the length of the outer carboxylic acid chains length to close the cavity. Essentially a series of large cores could be pepared using Suzukii coupling techniques, an example of such a core could be (fig 3.29).

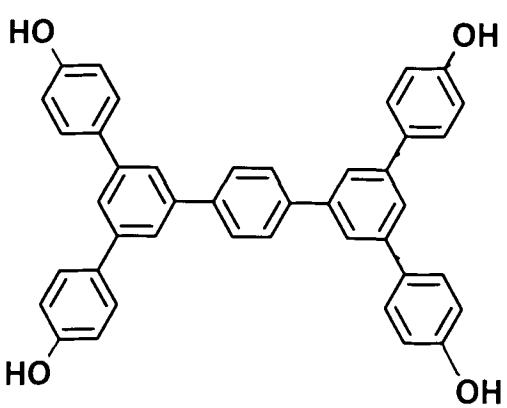
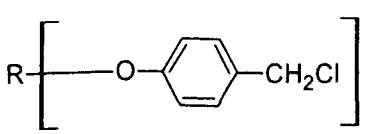


Figure 3.28: Figure of new kind of core

This would allow our current second generation dendrons to be attached and produce a larger dendrimer with larger cavities.

The aim would be to produce a range of bespoke cores which could be developed with the aid of molecular modelling to have variable size cavities designed to encapsulate different insoluble drugs of different sizes. As the size of the dendrimers increase they may form animalcular micelles.

To increase the size and capacity of the dendrimer, it should also be possible to increase the length of dendrons by reacting our current dendrons with spacers, some work has started on this, but another possibility would be to use para-substituted phenyl systems to effectively lengthen the "trunk" section of our dendrimer tree. An example shown in figure 3.20 would give more space and increase the dendrimer size and cavity size (R stands for our dendron).



Extra length = Link spacer

Figure 3.29: Figure of link spacer

This "pick and mix" process of using bespoke cores combined with variable spacer length and variable outer dendrons could make it possible to design bespoke dendrimers tailored to specific drugs. Obviously these systems would need to be evaluated by using cell cultures and with other water insoluble molecules such as pyrene that can be done by using fluorescent measurements.

Another approach to using these dendrimer systems would be to combine the dendrons with an electro-active polypyrrole backbone, via a flexible spacer of variable length. The idea being to investigate the potential of the system to take up insoluble drugs and release them, controlled by electromagnetic fields. An example of such a polymer is fig 3.31

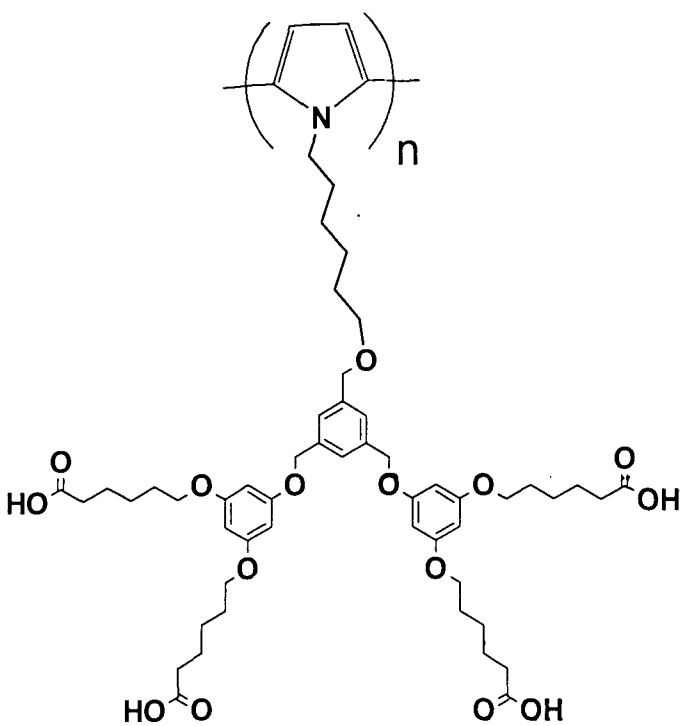


Figure 3.30: Figure of possible attachment of polypyrrole to the dendron

It could be anticipated that varying the length of the spacer between the polypyrrole backbone and the dendron would vary the cavity size to encapsulate a drug or other materials. These systems may also have potential for use in drug sensing. It would be interesting to also investigate the effect of the dendrons on the properties of the electroactive polymers, particularly if the dendrons were designed to exhibit liquid crystalline properties. They could also contain polar groups that can respond to electric of magnetic fields.

One of the reasons for having carboxylic acid terminal groups on our dendrons is their potential to be combined with amino acids and peptides. This should make possible to combine our dendrons with cell penetrating peptides [211], so as to facilate entry into the target cells to deliver drugs or potential gene therapy molecules. For example one dendron may have an outer shell combined with appropriate cell peneterating peptides. This would make up part of the dendrimer and facilate entry into appropriate target cells.

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APPENDICES

BES Buffer

BES has pKa of 7.1 at 25 ^{0}C and it is usefull fro a pH range of 6.4 and 7.8

The chemical structure and molecular formula of this compound is cshown below:

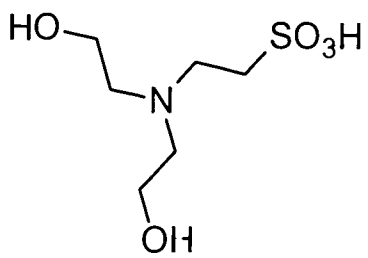

Figure 3.31: The structure of BES buffer

Synonym:

N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid, N,N-Bis(2-hydroxyethyl)taurine.

Molecular Formula : $C_6H_{15}NO_5S$

Molecular Weight : 213.25

BES sodium salt

It has pKa of 7.1 and is usefull for a Ph range between 6.4 and 7.8. The chemical structure, and molecular formuls of this compound is shown below.

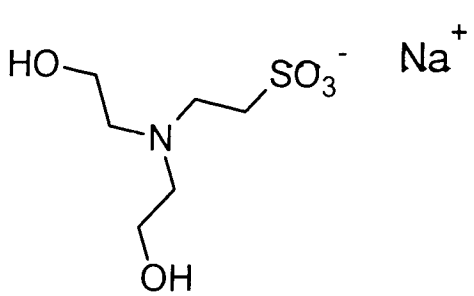

Figure 3.32: The structure of the BES sodium salt

Synonym

N,N-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid sodium salt

Molecular Formula : $C_6H_{14}NNaO_5S$

Molecular Weight : 235.23

Buffer 6.5

To make 1000 ml of 0.01 M BES (pKa=7.26) Buffer, pH= 6.5,

Prepared at 20°C, used at 37 °C

Recipe:

Dissolve 0.0073 mol of acid component

Dissolve 0.0026 mol of basic component

Add 8.859 g NaCl.

Make up to 1000 ml with pure water

Buffer 6.8

To make 1000ml of 0.01 M (pKa =.26) Buffer, pH =**6.8**

Prepared at 20 ^{0}C, used at 22 ^{0}C

Recipe:

Dissolved 0.007 mol of acid component

Dissolved 0.0029mol of basic component

Add 8.844 g NaCl

Make up to 100 ml ml with pure water.

Buffer 7

To make 1000ml of 0.01 M BES(pKa = 7.26) Buffer, Ph =**7**

Prepared at 20 ^{0}C , used at 22 ^{0}CC

Recipe:

Dissolve 0.006 mol of acid component

Dissolve 0.0039 mol of basic component

Add 8.783 g NaCl

Make up to 1000ml with pure water

Buffer 7.4

To make 1000 ml of 0.01 M BES (pKa = 7.26) Buffer, pH= **7.4**

Prepared at 20 ^{0}C, used at 37 ^{0}C

Recipe:

Dissolved 0.0025 mol of acid component

Dissolved 0.0074 mol of basic component

Add .58 g NaCl

Make up to 100 ml with pure water

Buffer 10

Burrate Buffer Solution pH 10: Dissolve 24.64g of boric acid in 900 ml of distilled water. Adjust the Ph using a 400 g/l solution of sodium hydroxide. Dilute to 1000 ml with distilled water.