The Design and Synthesis of Dendrons and Dendronized Polymers for Applications in the Pulp & Paper Industry

by

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Abstract

Dendrimers and dendronized polymers are unique macromolecules with novel characteristics. Dendrons, which share similar properties to dendrimers, are constructed in a layer-by-layer fashion and ending when the desired branching state is achieved. They are synthesized in a very controlled fashion leading to their monodispersity and high surface group density. Dendronized polymers retain some of the properties of dendrons and assume a cylindrical shape. An unexplored area in which dendrons and dendronized polymers can find applications is in the pulp and paper industry, especially in inhibiting biofilm growth. This thesis reports an efficient synthetic methodology to dendrons and dendronized polymers. The dendrons were constructed first from a tetrafunctional core, in which one arm is protected to be used later to couple the dendron to a polymer. We used a combination of copper(I) catalyzed alkyne-azide cycloadditon and the Steglich esterification to synthesize dendrons and dendronized polymers. Utilizing a divergent approach, and linking an alkyne core group to an azide building block, a process to develop a dendron was initiated. It was repeated to construct dendrons of generations 0-2, which were subsequently linked to a linear polymer to create dendronized polymers. These dendrons and dendronized polymers were also functionalized with terminal phosphonate groups which impart water solubility and enhance their biological activity. These macromolecules were subsequently evaluated for their biofilm activity, and they were found to be effective in biofilm inhibition.

Résumé

Les dendrimères et les polymères dendronisés sont des macromolécules uniques possédant des caractéristiques originales. Les propriétés des dendrons sont semblables à celles des dendrimères et ils sont synthétisés couche par couche et se termine lorsque le degré de branchement désiré est atteint. La synthèse de ces molécules est très contrôlée, ce qui mène à leur monodispersité et une densité élevée des groupes de surface. Les polymères dendronisés conservent certaines propriétés des dendrons et adoptent une forme cylindrique. L'industrie des pâtes et papiers, en particulier l'inhibition de la croissance de biofilm, est un domaine d'application inexploré pour les dendrons et les polymères dendronisés. Cette thèse présente une méthodologie de synthèse efficace de dendrons et de polymères dendronisés. La synthèse des dendrons débute d'un noyau tétrafonctionnel dans lequel l'un des quatre bras est protégé pour être utilisé ultérieurement pour coupler le dendron à un polymère. Nous avons utilisé une combinaison de la cycloaddition de Huisgen et de l'estérification de Steglich pour synthétiser les dendrons et les polymères dendronisés. Procédant par l'approche divergente, les groupes alcynes du noyau du dendrons furent liés à des unités d'élongation possédant un groupe azoture. Ce processus fut répété pour construire les dendrons de génération 0 à 2 qui ont ensuite été liés à un polymère linéaire pour créer des polymères dendronisés. Ces dendrons et polymères dendronisés furent ensuite fonctionnalisés avec des groupes phosphonates terminaux. Ceci a pour effet d'augmenter la solubilité dans l'eau et l'activité biologique. Par la suite,

ces macromolécules ont été évaluées pour leur activité biofilm et ce fut déterminé qu'ils inhibent la croissance des biofilms.

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Figure 3.4 Effect of biofilm dendrons of various concentrations on a 4 day old biofilm 83

List of Abbreviations

CuAAC	Copper (I) Catalyzed Alkyne-Azide Cycloaddition
DCM	Dichloromethane
DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic Acid
E. coli B	Escherichia coli B
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDTA	Ethylenediaminetetraacetic Acid
EtOAc	Ethyl Acetate
MALDI-TOF	Matrix Assisted Laser Desorption Ionization – Time of Flight
MS	Mass Spectrometry
MeOH	Methanol
MWCO	Molecular Weight Cutoff
NMR	Nuclear Magnetic Resonance
PAMAM	Poly (amido amine)
PBS	Phosphate-buffered Saline
PPI	Poly(propylene imine)
PTSA	Para-toluenesulfonic Acid
TBDPS	Tert-butylchlorodiphenyl Silane
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TSB	Trypticase Soy Broth

Chapter 1: Introduction

1.1– Dendrimers: Structural Features

Dendrimers are unique macromolecules with novel characteristics. They are different from linear polymers due to their inherent globular shape and monodispersity.[1] A dendrimer inherits these properties from its synthetic elaboration.[2] Generally it is constructed in a layer-by-layer fashion and ending when the desired branching is achieved.[3] Thus dendrimers are synthesized in a very controlled fashion leading to their monodispersity.[4,5] As each layer is added to increase the generation, the number of branching groups is also increased, leading to a hyperbranched macromolecule.[6] This introduction is focused on dendrimers and dendronized polymers, however other macromolecules such as dendrons which form the subject of this thesis, are similar in structure to dendrimers, and possess the same properties as dendrimers. In a dendron, there are unreacted sites at the core, thus making it asymmetrical and not completely globular as is the case with a dendrimer.

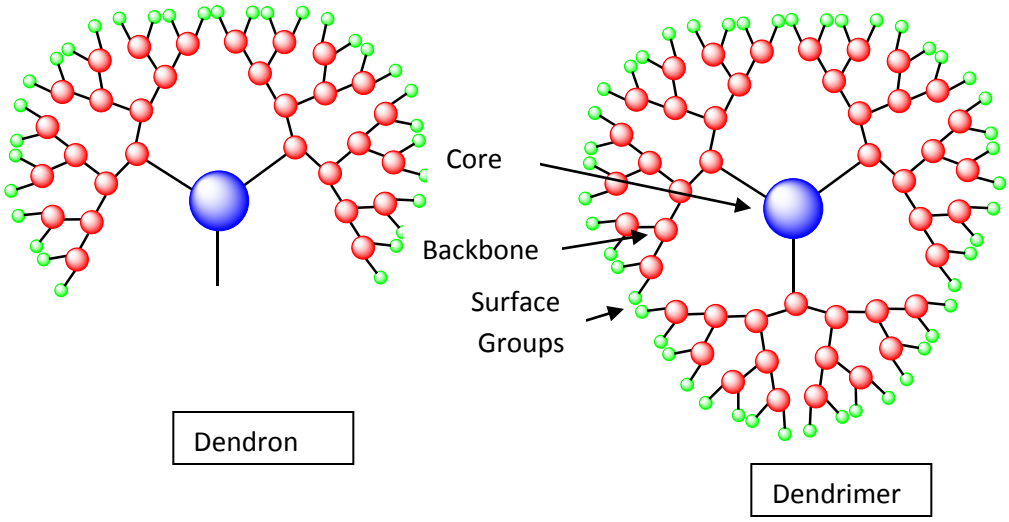


Figure 1.1 – General Dendrimer and Dendron Structure.

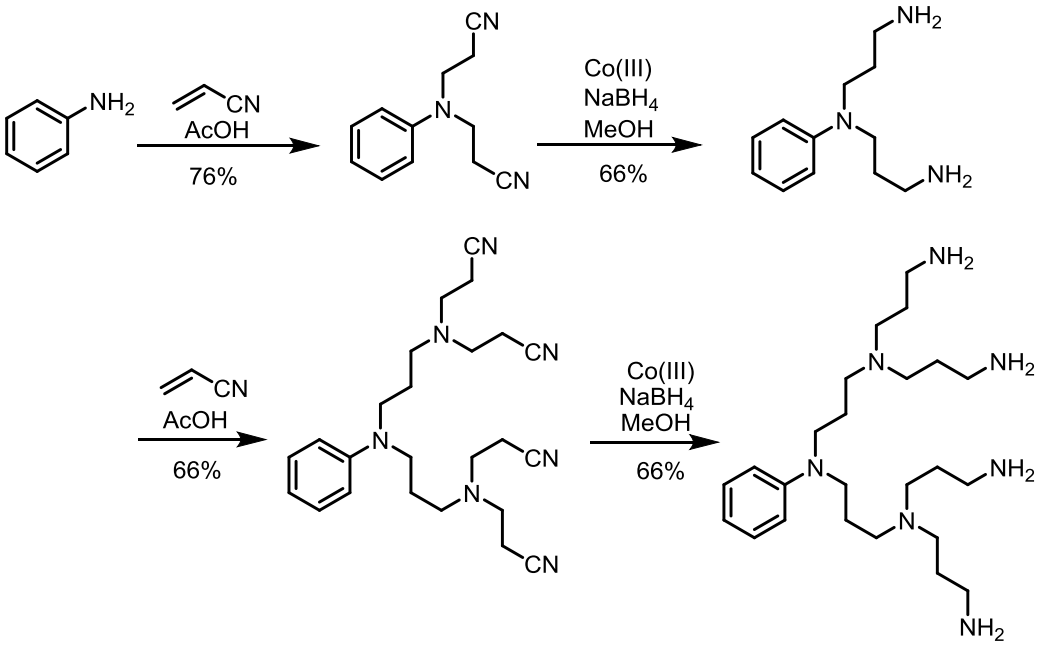
A dendrimer is made up of three parts: the core, branching units, and surface groups.[7] Each of these contributes to the overall function of the dendrimer.[8] The generation number dictates how many surface groups will be present per generation, as well as sets the shape for the given dendrimer. The branching units allow for increased surface groups due to its hyperbranching nature. The branching units can also participate in encapsulating molecules, through hydrogen bonds or other intermolecular forces, within the dendrimer.[9] The surface groups are present on the exterior of the dendrimer, and therefore the properties of molecules attached on the surface will be represented the strongest.

Many other factors can also affect the dendrimer structure, including the rigidity of the core and branching units, as well as the size of internal cavities.[10] The core and the branching units control such properties through varying their size and the amount of branching. For example, using a small core with many branches in combination with a small backbone gives rise to four branches per unit. Such a dendrimer would have very small internal cavities and thus would be

unable to encapsulate larger molecules.[11] The control of rigidity is based on the structure of the core and the branching units; however it is not the size and density of branching, but the type of units used that determines rigidity.[12] A rigid dendrimer could contain benzyl groups and acetylenes to restrict bond rotation, whereas a flexible dendrimer would try to avoid these groups.

1.2– Synthesis of Dendrimers-Inception to Current Methods

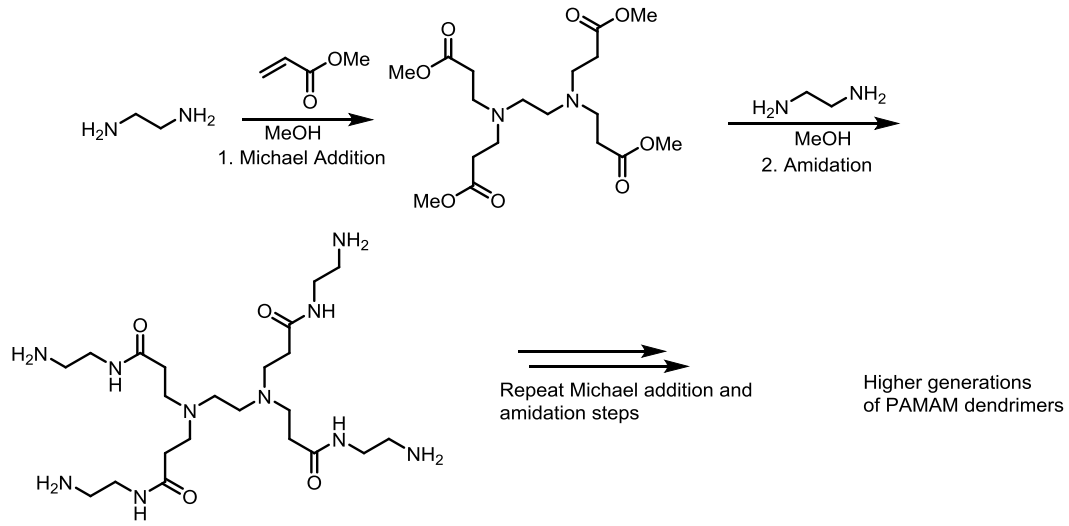
Dendrimers were first discovered in the late 1970's by Fritz Vögtle and his group, at the time however they went by the name cascade molecules.[13] Vögtle and his group worked with monoamines, diamines, and diaza-monocylic rings. These molecules would later be developed into very commonly used poly(propylene imine) (PPI) dendrimers.[13]



Scheme 1.1 – Micheal Addition Based on Vögtle's Cascade Molecules.[13]

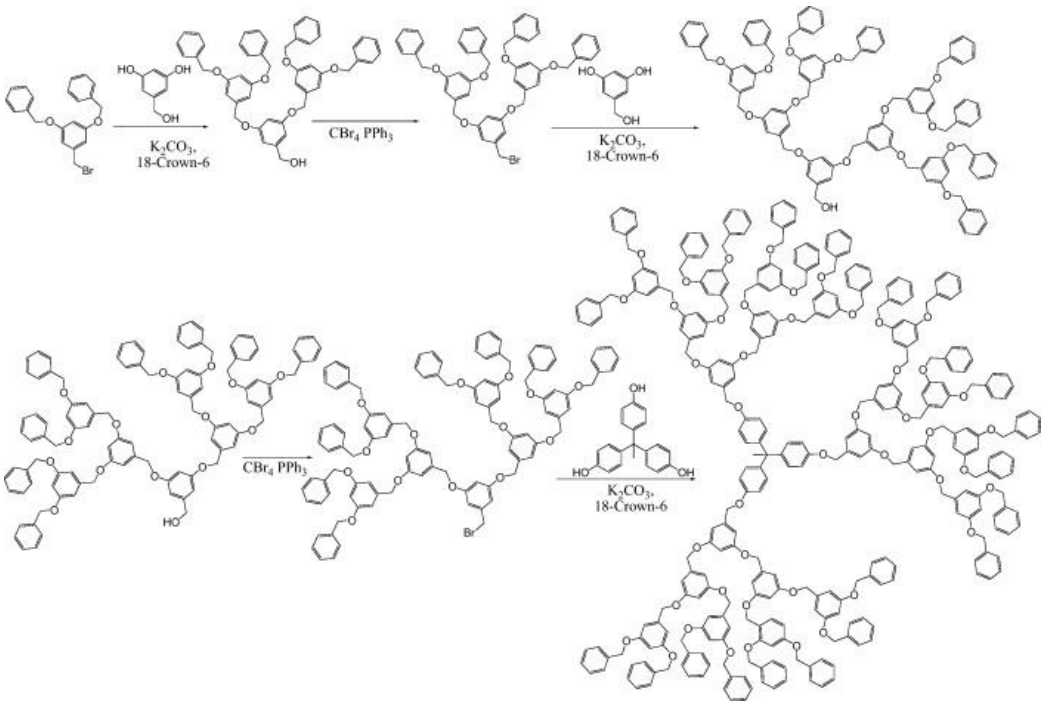
In 1985, Tomalia et al. first coined the term dendrimer, as it represents the Greek word for tree, which aptly describes dendrimers.[14] Tomalia also introduced

divergent synthesis of dendrimers, one of the two main methods for synthesizing dendrimers. Divergent synthesis is the strategy used when, starting with a core molecule, layers are added sequentially, creating a hyperbranching effect, and ending the synthesis with the addition of surface groups. Tomalia and his group also contributed to the field of dendrimers by introducing a well known and well studied dendrimer, the poly(amido amine) dendrimer, also known as the PAMAM dendrimer.[15]



Scheme 1.2 – Tomalia's Divergent Synthesis of a PAMAM Dendrimer.

Another large step taken in dendrimer chemistry was the introduction of the convergent synthetic methodology. In 1990 Fréchet and Hawker et al. developed a new synthetic procedure in which the core is not present at the early stages of synthesis.[16] Instead, dendrons are built up from the building block to the desired dendron length. The last step of the dendrimer synthesis is to attach multiple dendrons to a core moiety. Fréchet and Hawker demonstrated this synthesis with their poly(aryl ether) dendrimers.



Scheme 1.3 – Fréchet and Hawker convergent synthesis of poly(aryl ether) dendrimer. Reprinted with permission from reference [16]. Copyright 1990 American Chemical Society.

Both convergent and divergent syntheses have their advantages and disadvantages, and both are still being used today to prepare dendrimers. Divergent synthesis, with the addition of layers attached to a core, can have some steric hindrance at the surface at higher generations, leading to incomplete functionalization and lack of monodispersity. To avoid this problem, larger (more importantly longer) branching units that are flexible are used which can greatly reduce or eliminate the steric hindrance at the surface of the dendrimer. Divergent synthesis has the advantage of using a wide variety of core molecules. Since the core is the starting material, steric hindrance is rarely a problem. Convergent synthesis suffers from a different steric problem from divergent synthesis. In convergent synthesis the last step in creating the dendrimer is to attach the

branching units and surface groups to a core moiety. In larger dendrimers, it is plain to see that attaching multiple branches to a small core will inevitably have difficulty, as the crowding around the core functional groups will be large. To combat this core attachment issue, a larger core can be utilized to mitigate the steric hindrance issue. It should be noted that convergent synthesis allows for simpler surface group manipulation as the branching groups are not bound to a core molecule.

Dendrimers, up to this point, were all synthesized with the idea in mind that the surface groups can be modified to create the desired functionality in the dendrimer. It was not until 1993, when Inoue et al. synthesized a core functionalized dendrimer, did that change.[17] Inoue used a porphyrin core and a poly(aryl ether) branching unit, in order to create a unique dendrimer. Inoue's goal was to create a large amount of isolation and sterics around the porphyrin core in order to establish the desired biological function. While many methods to isolate a porphyrin core had been established, none had yet used dendrimers, resulting in the first core functionalized dendrimer. After this work, more core functionalized dendrimers were synthesized by other groups, including alterations to Inoues work. Diederich et al. used Inoue's idea for porphyrin core functionalized dendrimers, and used a zinc-porphyrin core, and terminated it with carboxylic acids, creating an entirely different surface functionality.[18]

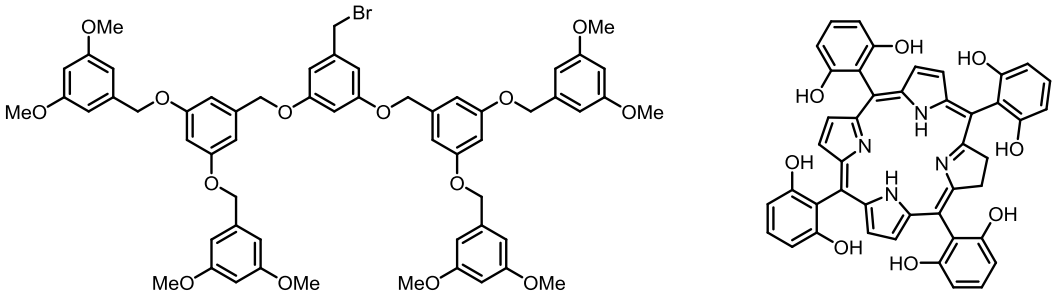


Figure 1.2 – Inoue Core functionalized Dendrimer Core and Branching Unit.

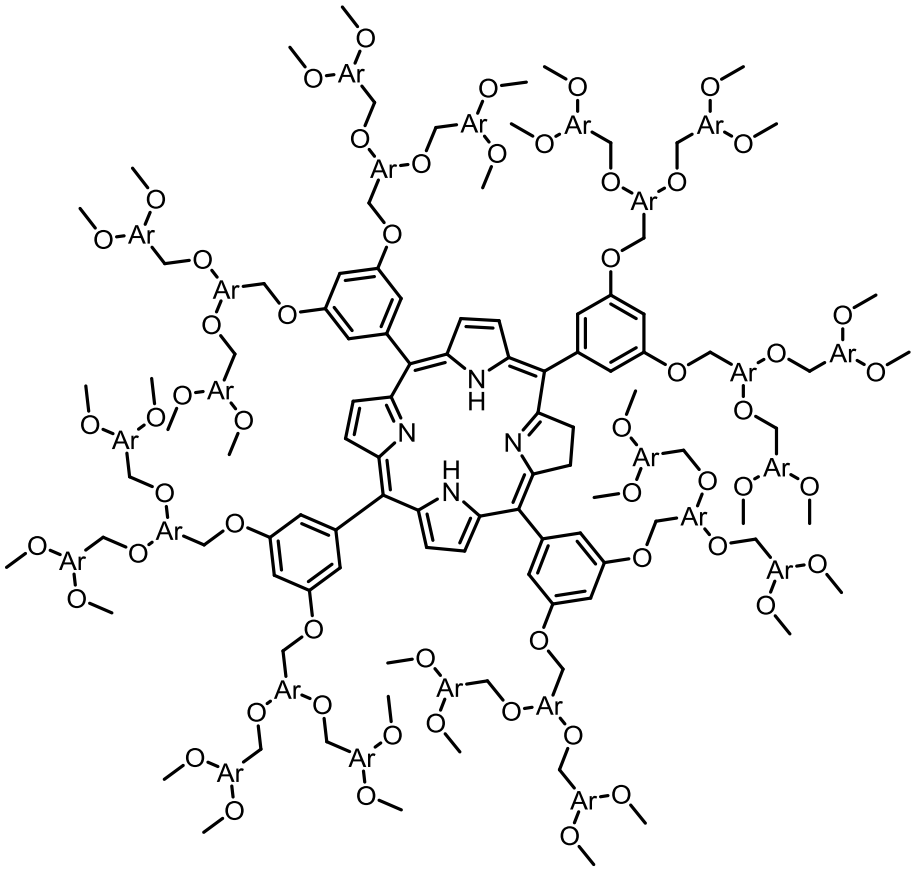


Figure 1.3 – Inoue Core functionalized Dendrimer.

The dendrimer field in the 1990's had gained a multitude of techniques from which the synthesis of large dendrimers could be carried out with increasing variety. From convergent to divergent, poly(amido amine) to poly(aryl ether), a wide array of dendrimers had been synthesized. These dendrimers however all have one type of surface groups present, meaning the dendrimers generally have

monofunctionality on the surface. As dendrimers branched out into various applications, multifunctionality was a desired trait.[19] For instance a dendrimer that could have both an imaging and sensing group on its surface would be extremely useful in biology. In addition, a dendrimer that has therapeutic capabilities, as well as being able to deliver the drug directly to a target would be highly desirable. These dendrimers would later become known as multifunctional dendrimers.

Fréchet et al. would again be a frontrunner in dendrimer ingenuity, being one of the first to publish a multifunctional dendrimer in 1999.[19]

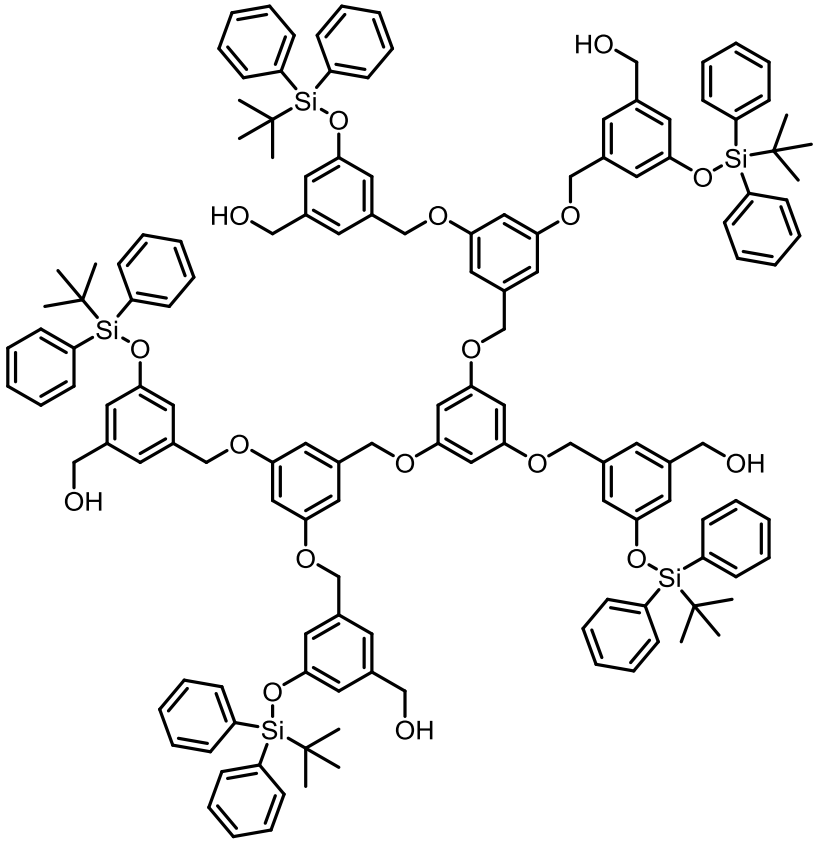
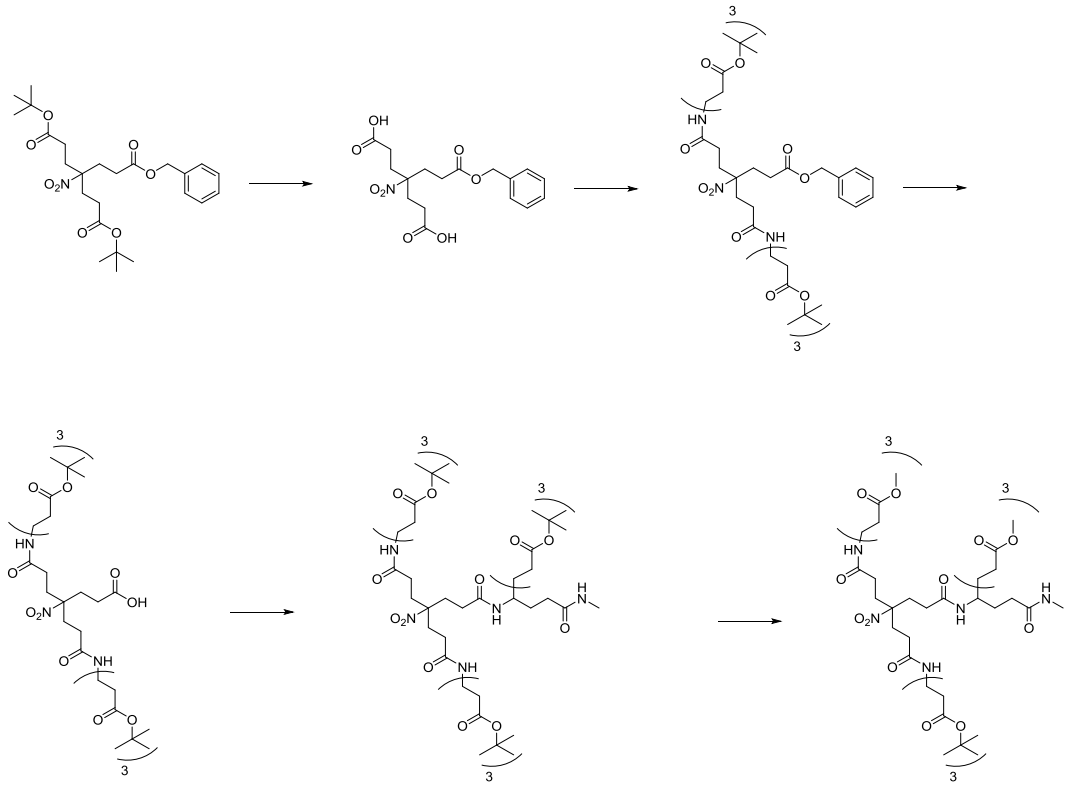


Figure 1.4 – Fréchet's Multifunctional Dendrimer.

Fréchet et al. used a unique structure that involved the selective addition of tert-butylchlorodiphenyl silane (TBDPS) to a single hydroxyl group on a benzyl moiety, leaving behind an additional hydroxyl group. This selectivity, when done on a scale as large as a dendrimer leads to multifunctionality. In Fréchet's case the final dendrimer (Figure 1.4) results in a bulky silane group as well as a free hydroxyl group, both on the surface of the dendrimer and both have substantially different properties. The free hydroxyl groups Fréchet provides on his dendrimer could easily be coupled to many other functionalities; leading to a new set of functions on a similar dendrimer architecture. In this way one can imagine combining multiple functionalities rather easily as long as the mechanism is in place to create two or more different reactive sites on the surface of the dendrimer.

With the addition of multifunctionality came the necessity for dendrimer chemists to explore protecting and deprotecting groups. Having reactive groups that can be activated selectively allows for a more complex dendrimer system. Multi-branch systems can be created, in which one branch of a dendrimer can be totally different from the other; leading to end groups having different functionalities on the same dendrimer. One such example, by Newkome et al., uses several protection and deprotection of esters and amides to selectively build each branch of the dendrimer.[20] Using a benzyl group, and a tertiary butyl group, Newkome could selectively deprotect each ester, and furthermore, once each ester was deprotected, individual branching could occur.



Scheme 1.4 – Newkomes Multifunctional Dendrimer.

With the desired increase in complexity for dendrimers comes the increase in difficulty for synthetically producing them. Protecting groups add a significant amount of time and resources to the complete synthesis of a dendrimer. Not only in synthesis but planning a dendrimer with so many reactive sites can be difficult, accompanied by the already difficult task of creating a monodisperse macromolecule. This difficulty is what led dendrimer chemists to seek out more efficient methods. Eventually click chemistry was incorporated into dendrimer synthesis.

Sharpless et al. developed a method that would greatly aid the development of dendrimers.[21] Since dendrimers are made in a layer-by-layer fashion, there is an inherent need for each reaction to be both high yielding and relatively simple. Without these two properties dendrimer synthesis would be an arduous task that

would result in very low yields. Click chemistry is a reaction that involves the coupling of two reagents with atom economy, high yields, and no side products. There are currently three click type reactions available: i) copper(I) catalyzed alkyne azide reaction, ii) Diels-Alder reaction, and iii) the thiol-ene coupling.[22]

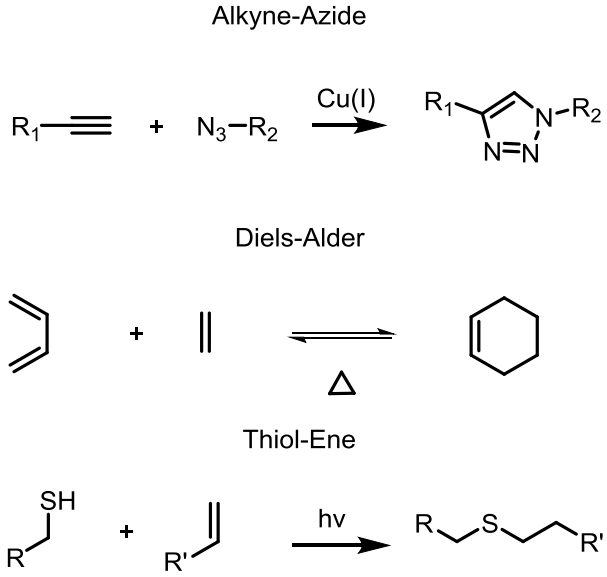


Figure 1.5 – Click Reaction: A General Scheme.[23]

Using a copper(I) catalyst, an alkyne group can be coupled to an azide resulting in a triazole ring, with no side products. Another advantage of the copper(I) alkyne azide coupling is the regioselective nature of the reaction. While the copper(I) alkyne azide coupling was developed in 2001, the first dendrimer synthesis to utilize it was not until 2004 by Hawker et al.[24]

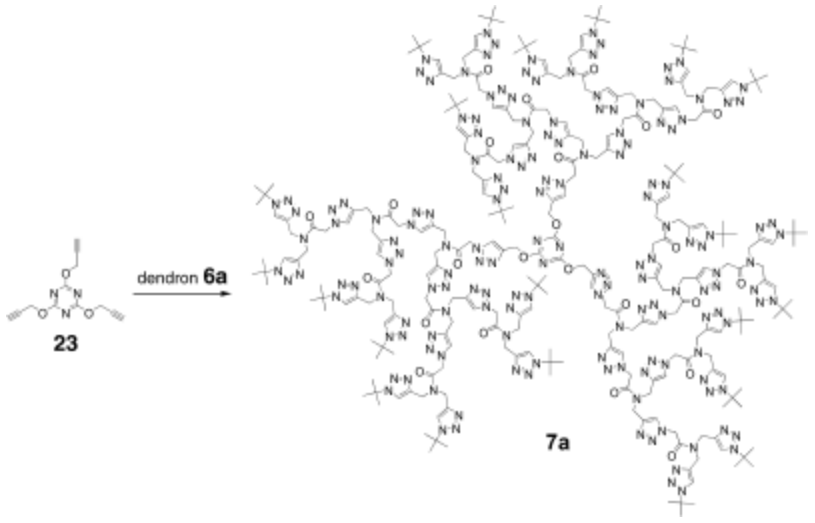
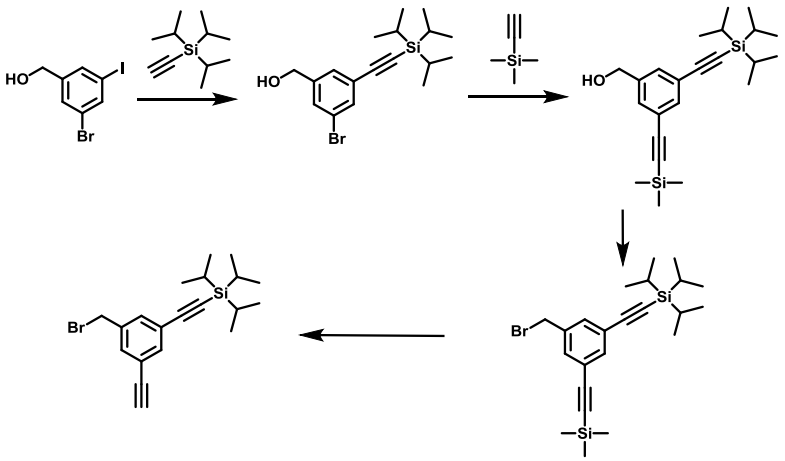
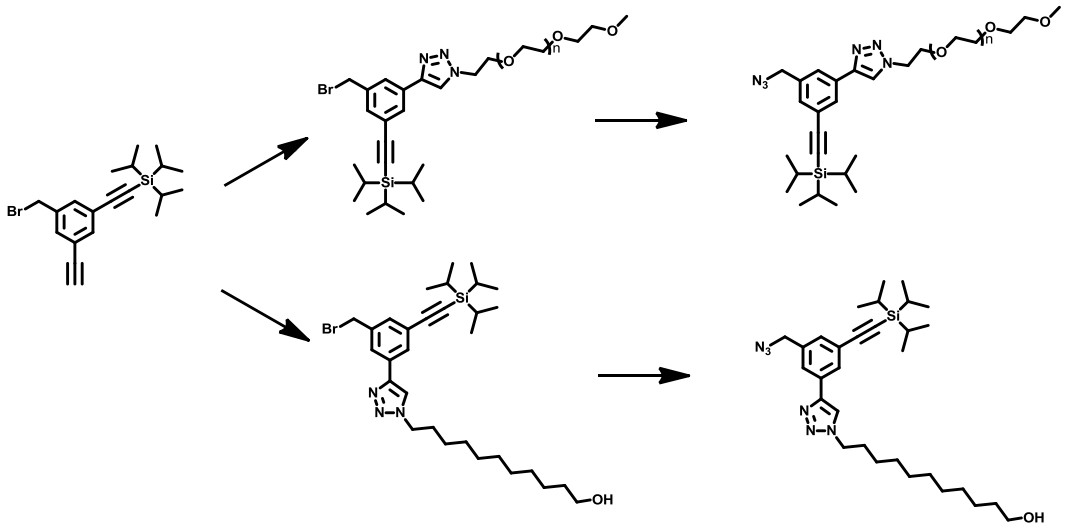


Figure 1.6 – Hawkers Click Dendrimer. Reprinted with permission from reference [24]. Copyright 2004 Angewandte Chemie, International Edition.

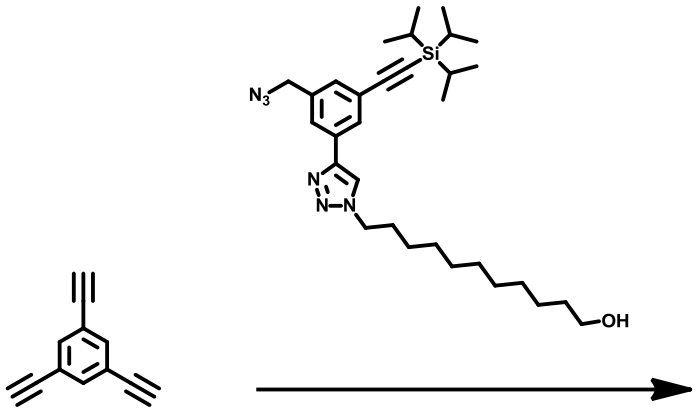
The field of dendrimer chemistry has come a long way from its inception over 30 years ago. With many new tools at the chemist's disposal, very complex and diversified dendrimers can be successfully synthesized. Various combinations of dendrimer structures and functionalities can be experimented with. A recent example from Kakkar's group shows exactly how far dendrimer chemistry has come.[25] They demonstrated as to how using click chemistry, in combination with protecting groups, can be an efficient strategy to synthesize a trifunctional dendrimer.



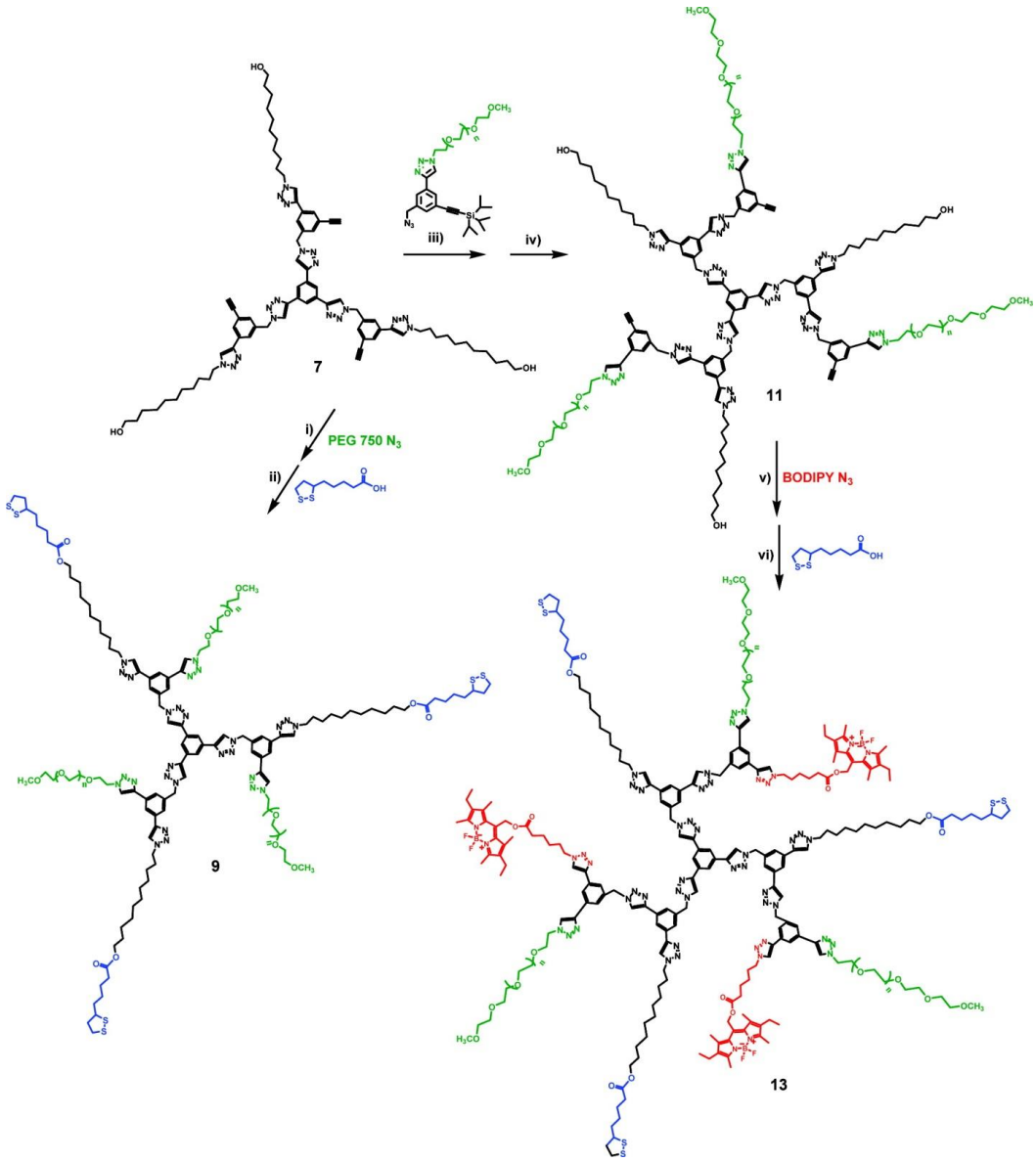
Scheme 1.5a – Synthetic Scheme for Kakkar's Building Blocks.[25]



Scheme 1.5b – Synthetic Scheme for Kakkar's Functionalization.[25]



Scheme 1.5c – Synthetic Scheme for Kakkar's Core Attachment.[25]



Scheme 1.5d – Synthetic Scheme for Kakkar's Dendrimer Combining Drug Delivery, Drug Solubility, and Imaging. Reprinted with permission from reference [25]. Copyright 2011 American Chemical Society.

The key to this dendrimer was its unique core, 3-bromo-5-iodobenylalcohol. This core has an iodo and a bromo group, which while similar, can be reacted separately much like the trimethylsilyl and triisopropyl are used to protect the alkyne groups. Since the alkynes can be deprotected one at a time, two separate

click reactions can occur on the same molecule, leading to two unique branches. The hydroxyl group on the core can be reacted in two steps to form an azide, which then can be clicked once again to have a unique branch. Using this method the Kakkar group was capable of synthesizing a trifunctional dendrimer with surface groups intended for biological applications. These functional groups included lipoic acid to act as a drug, a Bodipy dye for imaging, and polyethylene glycol to aid in solubility. This dendrimer is thus capable of delivering a drug in aqueous conditions, all while being able to image the entire process.

1.3– Applications of Dendrimers

Dendrimers have been explored for a variety of applications due to their inherent structure. Since the surface groups impart most of the functionality, a dendrimer can sometimes be kept exactly the same throughout. In this way monofunctional dendrimers can be rapidly synthesized. One such example is an organometallic Ni(II) polysilane dendrimer developed by van Koten et al.[26] This dendrimer utilizes multiple diaminoarylnickel (II) complexes on its surface. This allows van Koten's dendrimer to catalyze the Kharasch addition of poly(haloalkanes) to alkenes.

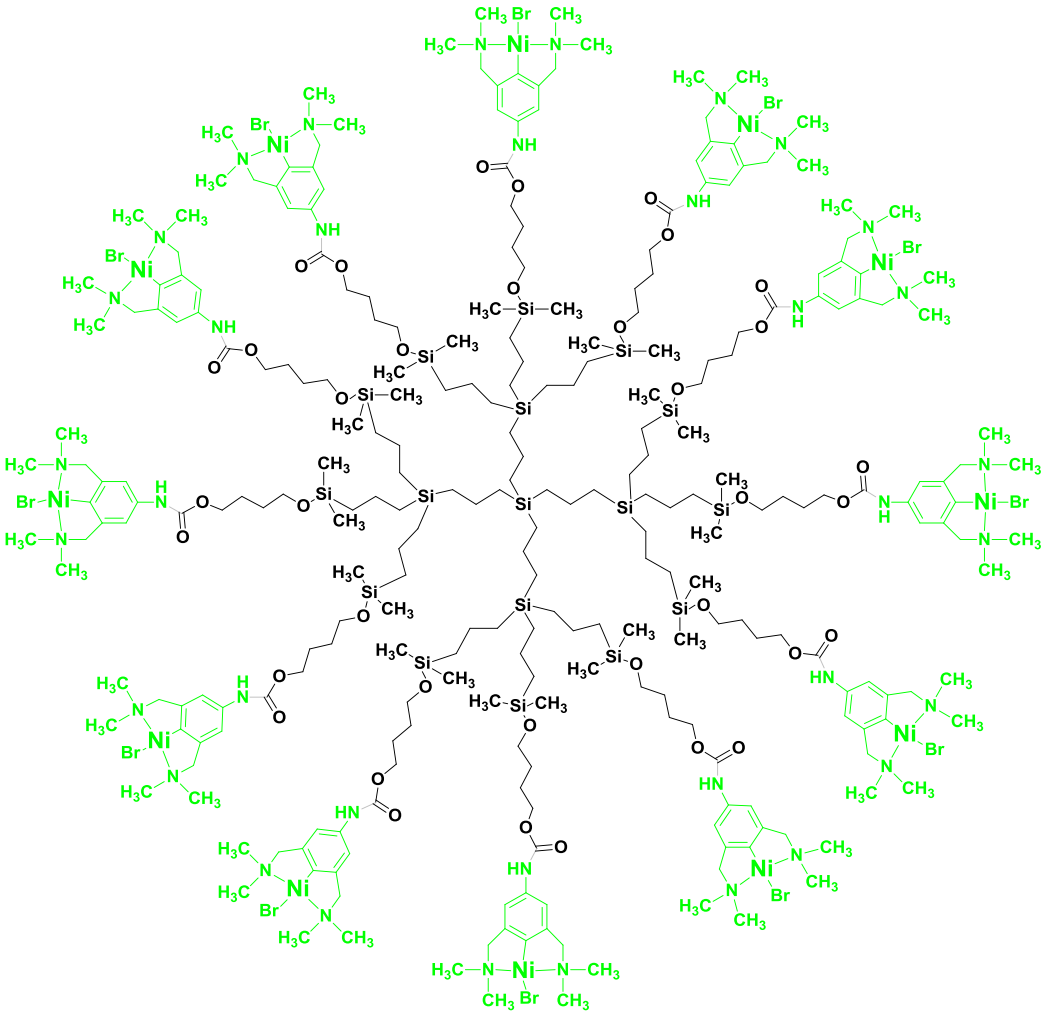


Figure 1.7 – van Koten's Catalytic Dendrimer. Reprinted with permission from reference [26]. Copyright 1994 Nature.

A dendrimer also has the capability to encapsulate molecules, and thus can be used as a carrier.[27] A clear use for this would be in small molecule delivery, where the dendrimer can encapsulate molecules of interest, and the surface groups of the dendrimer can be used to aid in the delivery through biocompatibility, targeting, or imaging.[28] Fréchet et al. developed one such dendrimer.[19] The concept was that the dendrimer could encapsulate a dye, and since the dye fluoresced they could

monitor the release of the dye from the dendrimer over time. On the surface of this dendrimer was polyethylene glycol to enhance the solubility of the dendrimer in aqueous media.

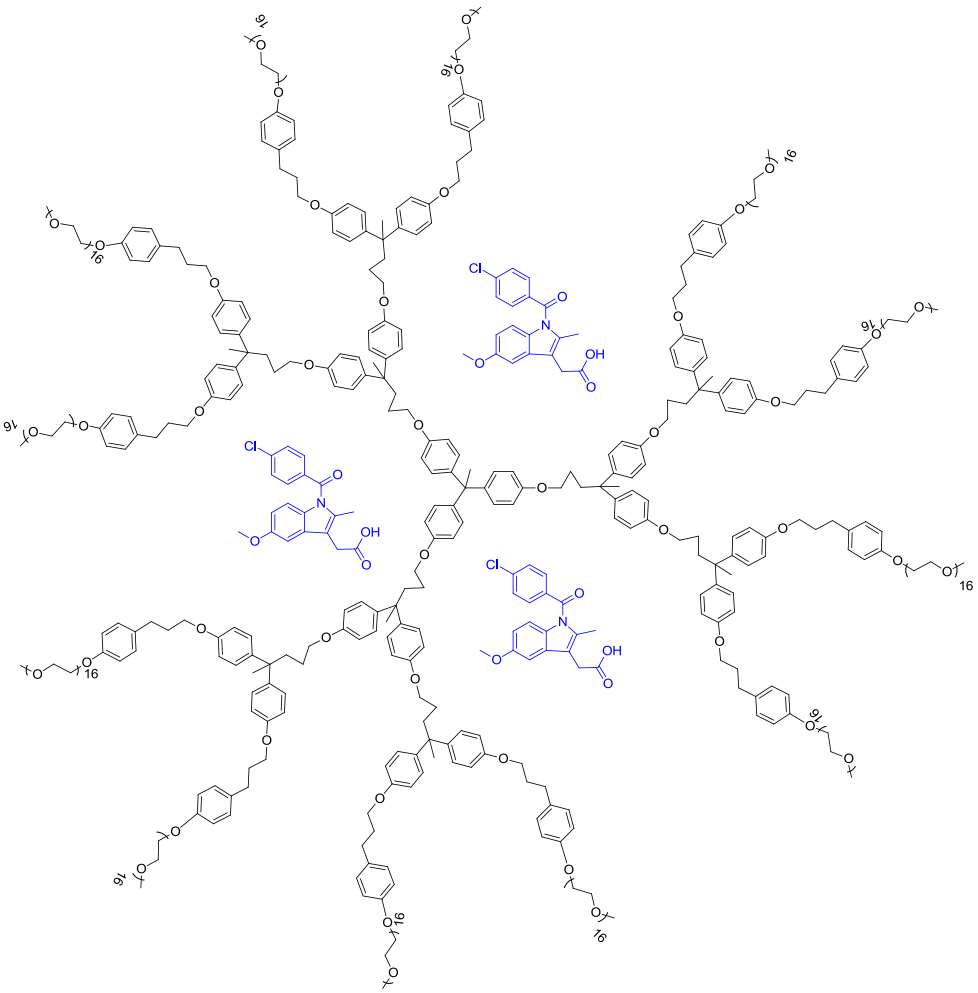


Figure 1.8 – Fréchet's Dye Encapsulated Dendrimer.

As noted earlier, encapsulation is one way for a dendrimer to achieve multifunctionality. A very common method is to have two or more agents present on the surface of a dendrimer, either by having an even distribution across the surface, or by tethering two dendrimers with different functionalities through their core. In this way many dual functions can be achieved. One such example by

Hawker et al. is using a tethering technique, where a dye is on the surface of one half of the dendrimer, and the other half of the dendrimer is a mannose binding unit.[29] The mannose binding unit causes the dendrimer to attach to mannose and thus acts as a sensing unit, where the dye can fluoresce and therefore acts as an imaging agent. The combination of these two functionalities on a single unit allows for the detection of mannose by simple means.

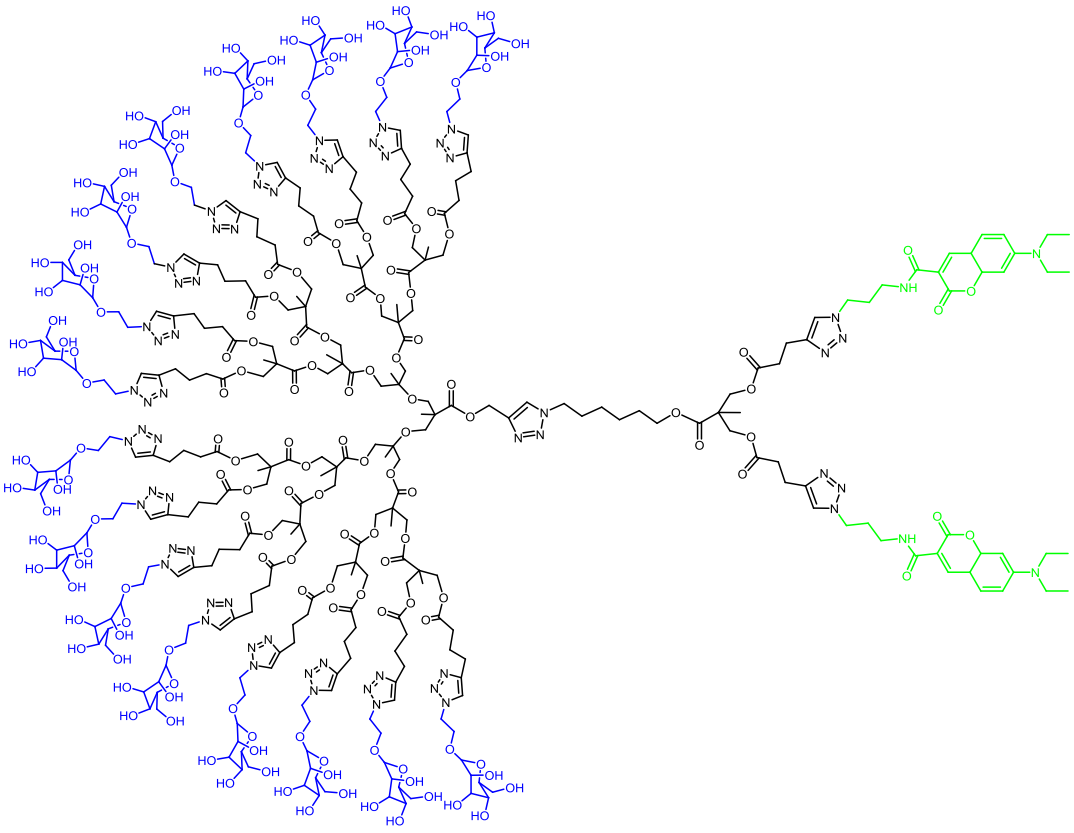


Figure 1.9 – Hawker's Sensing and Imaging Dendrimer.

Hawker, Fréchet, and van Koten dendrimers are only the 'tip of the iceberg' when it comes to possible applications for dendrimers. Theoretically, dendrimers can incorporate any and all functionalities on their surface and can combine multiple functionalities within a single molecule. Other functional

groups include redox reactive sites,[30,31] anti-adhesive,[32] cationic amine,[33] and anionic phosphorus groups. Since so many functional groups are possible and so easily exchangeable, the architecture of the dendrimer and how it affects the functionality of the dendrimer are of great interest. One such example of a unique dendrimer based architecture is the dendritic polymer, or dendronized polymer.

1.4– Dendronized Polymers

Dendronized polymers are a unique combination of a dendron with a linear polymer.[34] These two macromolecules each impart some of their characteristics to the overall structure of dendronized polymers. For example, dendronized polymers are still hyperbranched, resulting in a high surface area but are no longer monodisperse due to being attached to a polydisperse polymer. An interesting consequence of this is that dendronized polymers are not globular like dendrimers, but rather of a cylindrical shape with the diameter dictated by the dendrimer and the length dictated by the polymer.[35] Polymers themselves can have many repeating units and in some ways can be thought of as having a large surface area, but the ability of polymers to coil with itself causes them to lose most of this surface area.[36] This self coiling property of polymers is negated by attaching dendrons, by creating bulk around the polymer itself and turning what would act like a string into more of a thick rope.[37]

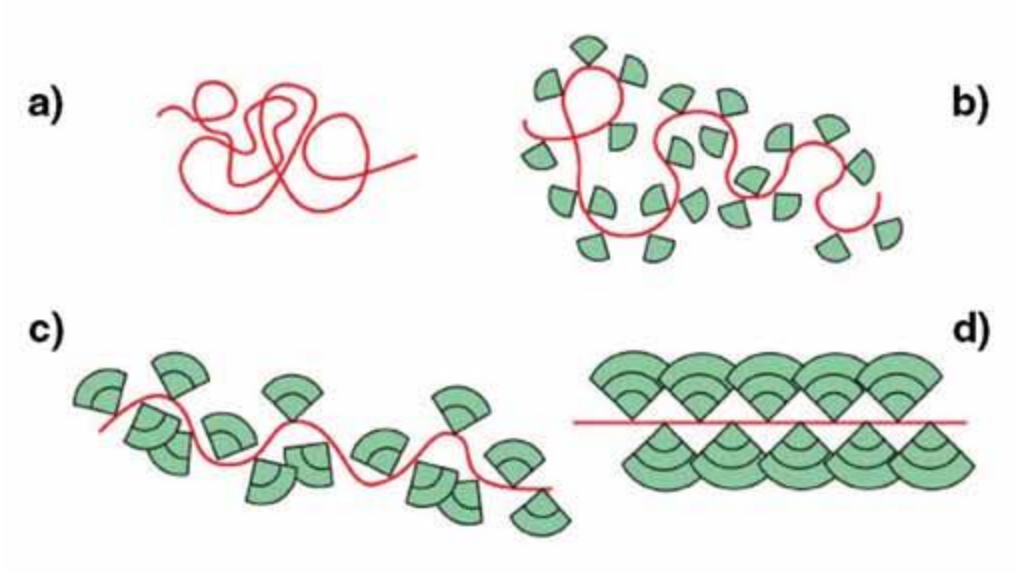


Figure 1.10 –Dendronized Polymers Effect on Self Coiling. Reprinted with permission from reference [35]. Copyright 2003 American Chemical Society.

To successfully synthesize dendronized polymers, three techniques have been developed. These are the 1) graft-to method, 2) the graft from method, and the 3) macromonomer method.[38]

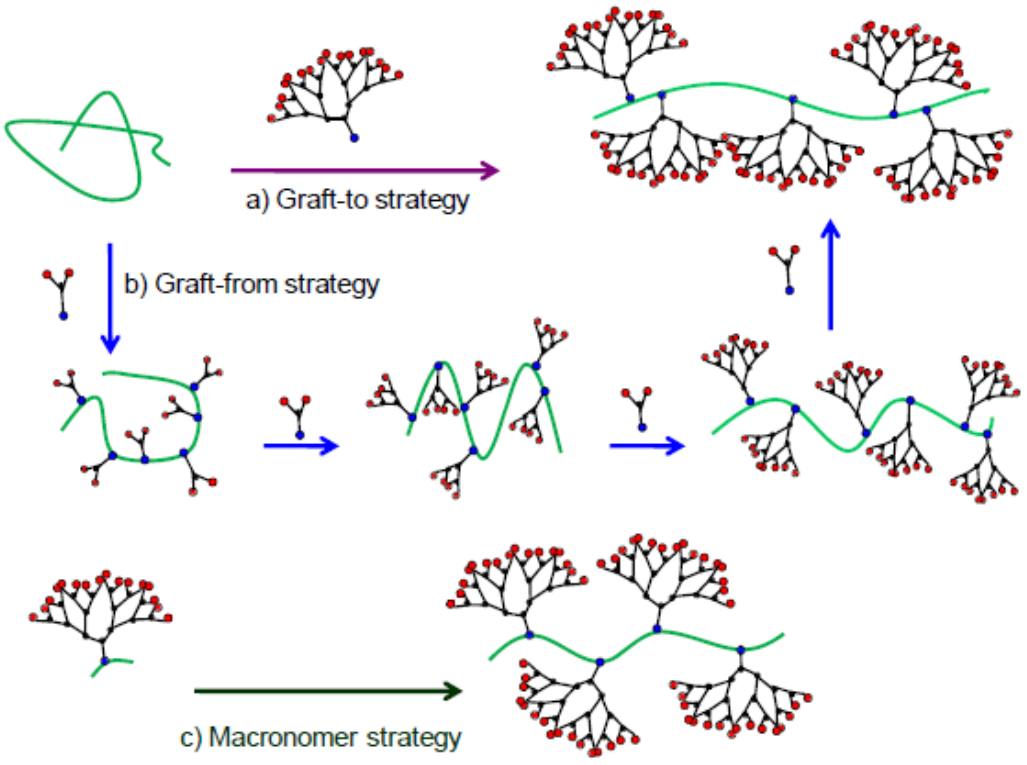


Figure 1.11 –Dendronized Polymers Effect on Self Coiling. Reprinted with permission from reference [36]. Copyright 2012 Polymer.

The graft-to method involves building up a dendrimer to its desired generation, but leaving an arm at the core of the dendrimer protected. Once the dendrimer is built up, the core arm can be deprotected and directly coupled to a polymer. One example of this is Schüll's et al. dendronized polymer.[39]

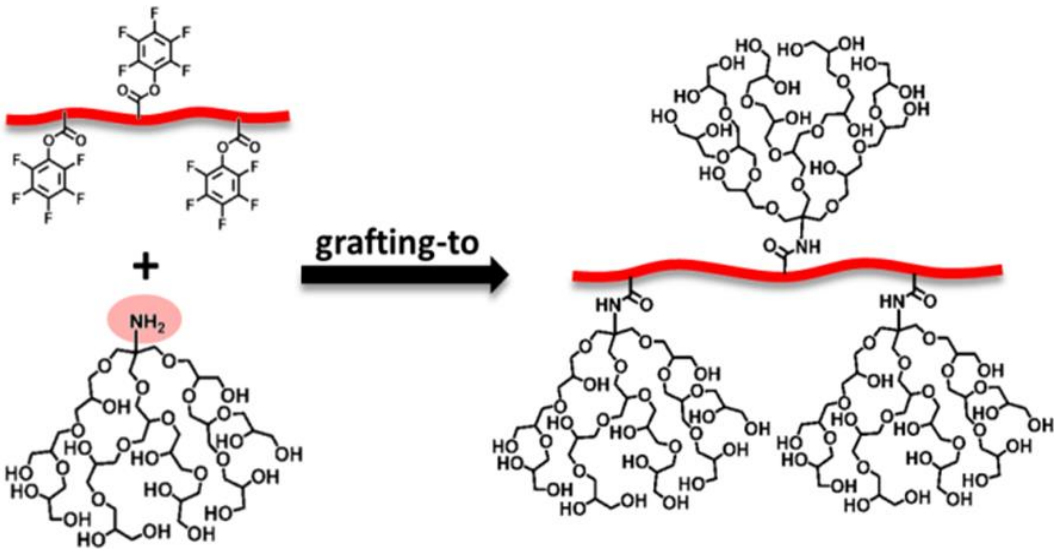
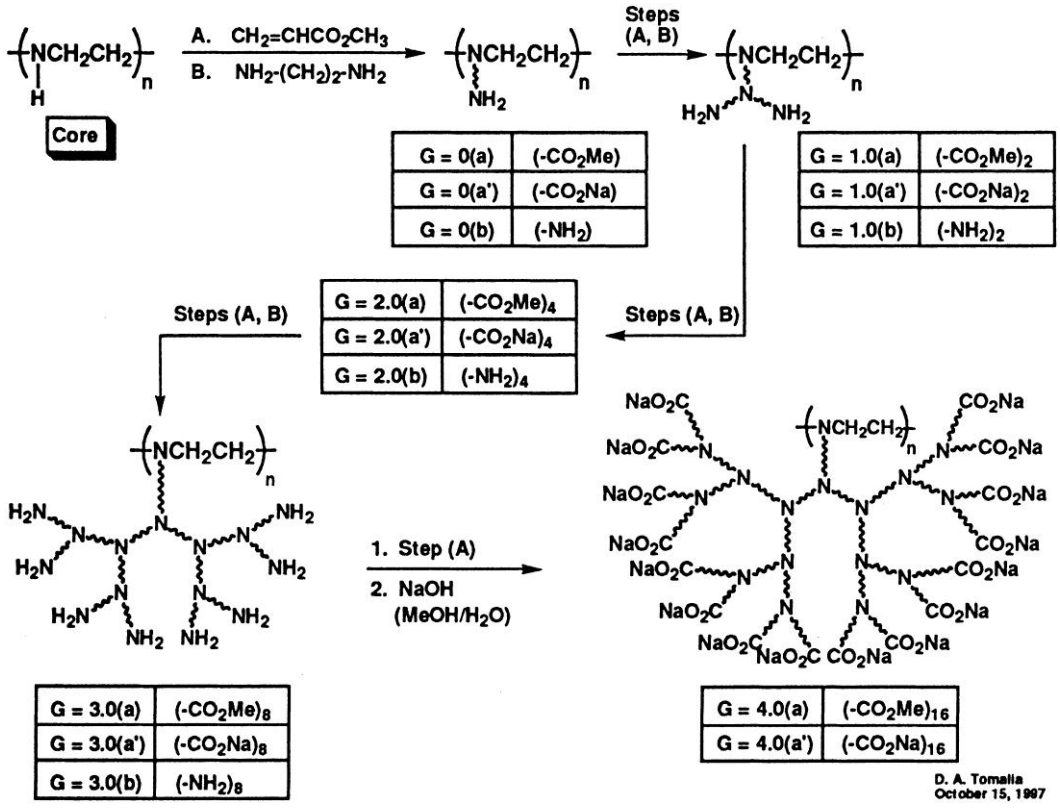


Figure 1.12 –Schüll Graft-to Strategy Reprinted with permission from reference [39]. Copyright 2012 American Chemical Society.

Schüll's strategy to synthesize dendronized polymers is unique in that the grafting reaction process can be monitored. The polymer used contains a unique pentafluorophenol group which is easily identified using fluorine NMR. By using fluorine NMR there is only one moiety which can give rise to a signal, making it relatively easy to see the reaction in progress. When the pentafluorophenol group is attached to the polymer with its ester bond, the chemical shift of the fluorine atoms are greatly shifted compared to the free pentafluorophenol group. Knowing this, Schüll could see if the ester bond was broken and therefore an amide bond could replace it.

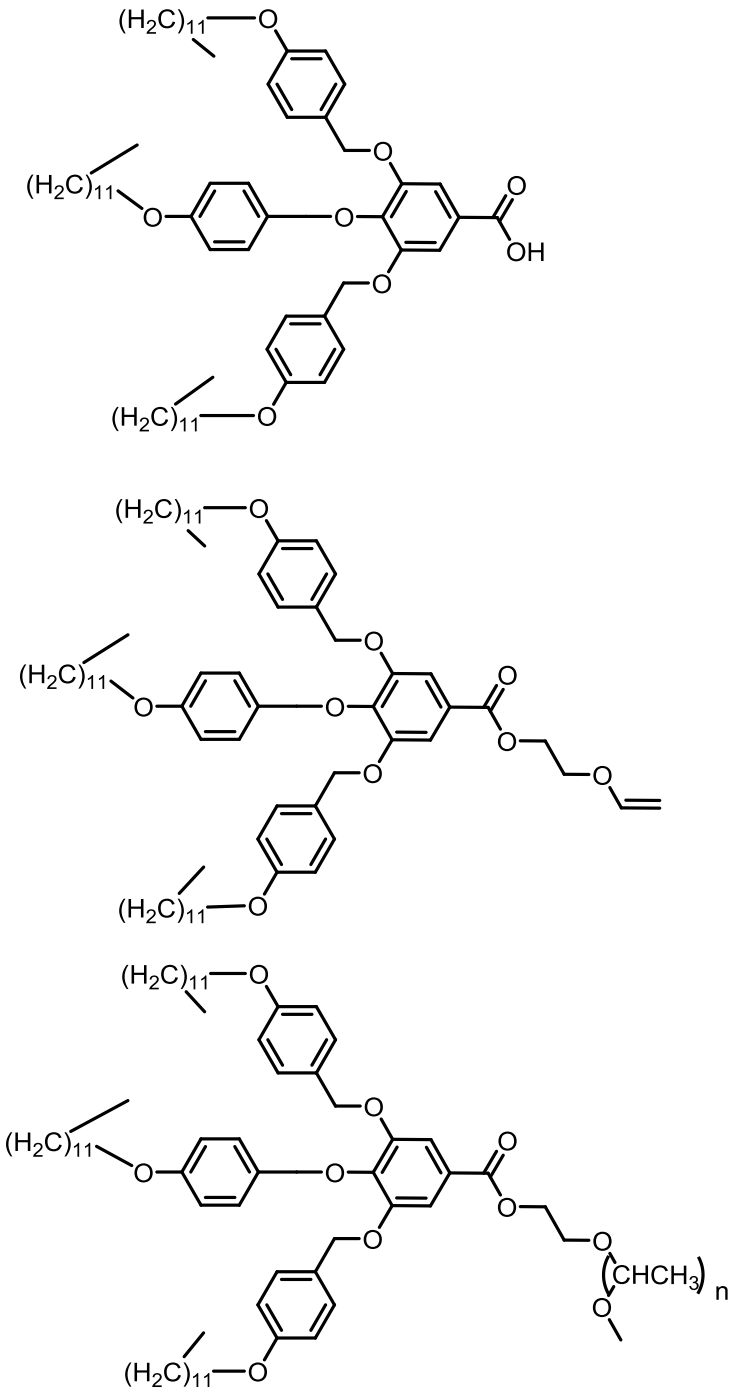
Creating a dendronized polymer by the graft-from method requires a specialized synthetic scheme. Several organic reactions are used to create a dendrimer, but the reactions are all taking place on a polymer core unit. This can cause synthetic difficulties in solubilising and purification of products.[38] One example of a graft-from approach comes from Tomalia and Yin et al., who used a

poly (ethyleneimine) dendrimer to create a dendronized polymer.[40] Using an imine core repeat unit, Tomalia was able to attach an amine group to the polymer, starting the dendronizing process. From there a simple repetition of the first steps produces a dendronized polymer of higher generations. At each generational step, it was possible to add a different reactive group, in this case an ester or acid, to functionalize the dendronized polymer. During each step, however, the potential for polydispersity is high, due to the large amount of reactive sites; therefore reactions are left for long periods of time, making the synthesis much more laborious.



Scheme 1.6 – Tomalia and Yin's Synthesis of Dendronized Polymers via Graft-from Reprinted with permission from reference [40]. Copyright 1998 American Chemical Society.

The third and final approach to synthesizing dendronized polymers involves polymerizing an already formed dendrimer. Called the macromonomer approach, this method's biggest advantage is that the amount of dendron attachment is quantitative, since no alteration is done to this part of the molecule.[36] Its disadvantage is that the steric demands on the polymerization reaction are extremely large, and therefore it is a very delicate reaction that requires forethought. Despite this disadvantage, macromonomer based dendronized polymers are the most intensely studied.[38] Virtually any method used to polymerize a monomer unit can be done to the same effect on a dendron unit, including cationic, anionic, coordination, free-radical, and ring opening metathesis. Percec et al. developed a novel cationic polymerization method to develop dendronized polymers.[41] Percec's goal was to try to create a structure that resembles the tobacco mosaic virus, but his method shows us how simple the macromonomer approach can be.[41] After building up a dendron to sufficient generation, an acid group remains at the core. Using this core acid group, a suitable polymerization group is attached, in this case a poly ethylene glycol. The last step is to carry out the cationic polymerization, which due to the amount of steric bulk, requires extra consideration.



Scheme 1.7 –Macromonomer Approach to Develop Dendronized Polymers.

One can synthesize a variety of dendronized polymers using various reactions available at our disposal. These unique macromolecules are a relatively new field of study in which much interest lies. With their unique structure, dendronized

polymers have the potential to offer solutions to many real world problems. Some areas of intense research include catalysis,[42] ion channel mimics,[43] DNA compactization,[44] optoelectronics,[45,46] and other areas of biosciences.

1.5– Biofilms

Bacteria are incredibly old microorganisms that are prevalent in almost every facet of this planet.[47] Bacteria are so numerous and widespread that there are more bacteria on earth than all plants and animals combined by biomass.[48] These bacteria play an important role in many ecosystems, and can contribute to human wellbeing. Some strains of bacteria, however, are detrimental to human development. Whether it is by growing in ventilation systems, medical equipment, in our food, or even in our bodies, bacteria have the capability to seriously threaten our wellbeing. Bacteria can sometimes be dealt with by simple means like proper cooking, but bacterial infections in the body can be tricky to deal with. The most common method to treat a bacterial infection is with the use of antibiotics. The first real drug developed for use as an antibiotic was penicillin, discovered by Alexander Fleming.[49] Since then many new drugs have been developed and there is widespread use of antibiotics over much of the world. Recently, however, a new development has given cause for concern. Antibiotic resistant bacteria are becoming more prevalent, and with so few new drugs in the pipeline this presents a real problem.[50] Part of the reason for this rise in drug resistant bacteria is due to small populations of bacteria surviving the antibiotics, and this can be attributed in part to biofilms.

Biofilms are an extra-cellular matrix produced by bacteria. The bacteria use this matrix to adhere to a surface. The matrix is made up of polymeric material including polysaccharides, proteins, and DNA.[51] The biofilm formed gives the bacteria numerous advantages over free bacteria including resistance to antibiotics and detergents, a mechanism for growth and dispersal of bacteria, a means for bacteria to communicate, and a method for facilitating nutrients and removal of waste.[52] Biofilms are capable of providing antibiotic resistance by shielding the bacteria and therefore not allowing the antibiotics to penetrate the biofilm layer.[53] Another possible mechanism lies with how a biofilm is layered. The outer layer of a biofilm allows for rapid growth and is more susceptible to antibiotics, whereas the core of the biofilm has mostly dormant bacteria in anaerobic areas where antibiotics are less effective.[54] These dormant bacteria are also for all intensive purposes immune to antibiotics that target replicating bacteria, as there is very little activity present.[55]

The ability of the biofilm to facilitate the growth and dispersal of bacteria is inherent in the way a biofilm is developed. A biofilm is formed over a series of steps, the first of which planktonic bacteria (not biofilm bacteria) adhere to a surface. At this stage bacteria are very vulnerable and it is not until the next stage, when the biofilm first starts development, that some protection is formed. Once the biofilm has been established, it begins to grow in a mushroom like shape until it reaches a particular size. At this point the biofilm breaks in certain areas allowing bacteria to disperse and start the cycle again, thus allowing the biofilm to spread.[52]

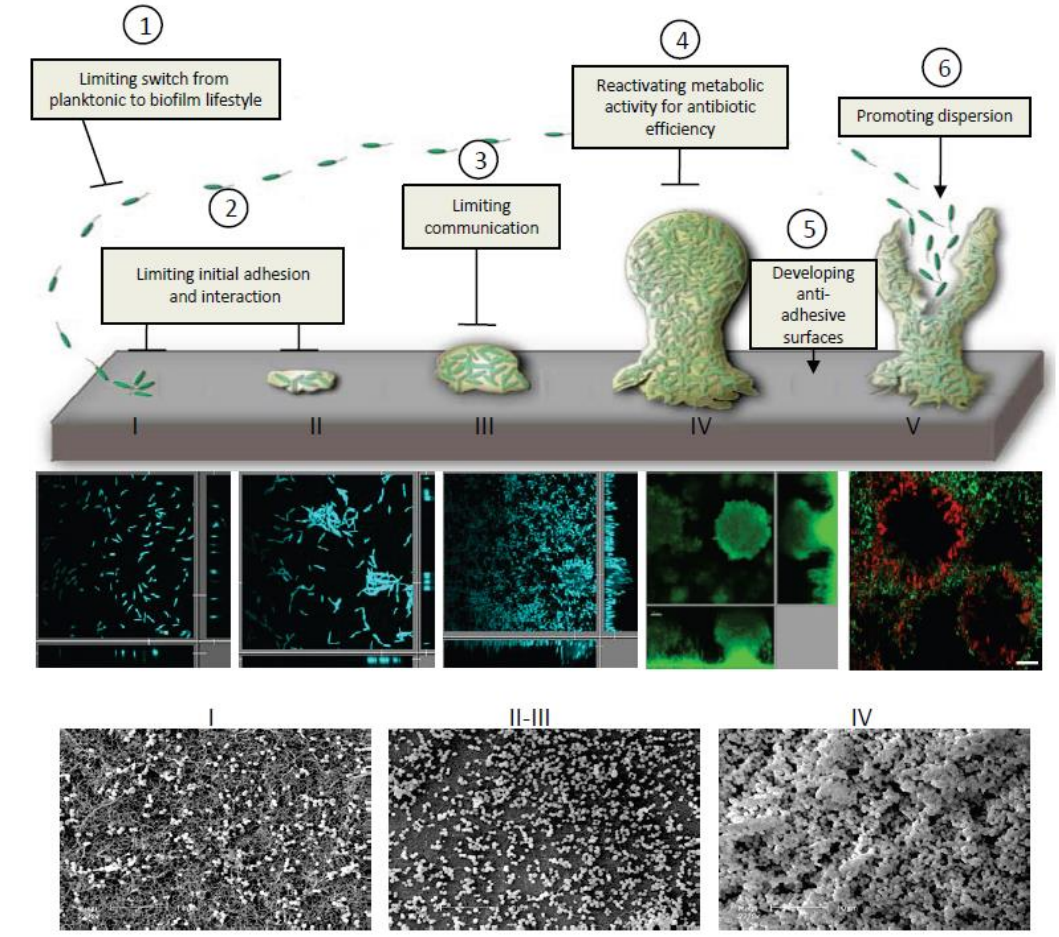


Figure 1.13 – Development Cycle of a Biofilm on a Surface Reprinted with permission from reference [52]. Copyright 2011 Annals of Intensive Care.

Some work has been done to develop an effective method for biofilm inhibition in recent years. Though a relatively new field, some mechanisms have been proposed. Shetye et al. recently found a class of polyolderivatized hydrocarbons that do no inhibit bacterial growth but do inhibit biofilm growth.[56] Another study by Sasaki et al. shows that anionic polymer units have potential as biofilm inhibitors.[57] Despite this work, biofilm inhibition is still a relatively young field and a need for a commercial effective biofilm inhibition drug is apparent.

1.6– Goals

The goals of this thesis include the development of a synthetic pathway to water soluble dendrons and dendronized polymers which is both efficient and simple, and examine their potential as biofilm inhibitors. The synthetic methodology should be versatile in which acetylene terminated dendrons of multiple generations could be generated, and further functionalized with any desired moiety using click chemistry. We chose phosphonate terminal groups for introducing water solubility, as well as enhancing their efficacy as biofilm inhibitors. These dendrons and dendronized polymers will be characterized using a variety of techniques, including NMR (^{1}H & ^{13}C, as well as ^{31}P) and MALDI-TOF and ESI MS, and subsequently their potential in inhibiting biofilm growth will be explored.

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Chapter 2: Design & Synthesis

2.1 – Introduction

Dendrimers and dendronized polymers are an intriguing class of macromolecules, in part due to their wide range of possible applications.[1-5] Dendrimers can utilize their globular structure to have the surface groups dictate most of their solubility and functionality.[6] Similarly, dendronized polymers utilize their cylindrical shape to have the surface groups impart most of their solubility and functionality.[7] These macromolecules have been explored for a variety of applications including catalysis,[8], drug carrier,[9] imaging,[10] and sensing.[11]

Water soluble dendrimers and dendronized polymers have been used for various applications, and are highly desired macromolecules due to the fact that water is used in many systems most importantly in the human body.[12-15] Water is used for many manufacturing processes including the pulp and paper industry, and in many cases these applications require water solubility. In the pulp and paper industry water soluble dendrimers have already been investigated for their efficacy as flocculants,[16] retention aids,[17,18] strengthening agents,[19] and as anti-scalants.[20] Since dendrimers and dendronized polymers are capable of a wide range of applications, it is no surprise that dendrimers and dendronized polymers can be used for several different functions within a single field of study.

The ability of dendrimers and dendronized polymers to have a customizable solubility greatly enhances their potential applications.[6,21,22] Attaching a hydrophobic or hydrophilic unit at the periphery of the dendrimer thus determines the general solubility of the dendrimer.[23] The solubility of

dendronized polymers can also be tailored as the dendrons are built around a polymer. The functionality of the dendronized polymer once again relies on the surface groups attached to the dendron.[24] In the synthetic pathway which will be discussed here, the solubility of the dendrons and dendronized polymers is tailored such that their solubility is very high in water.

Water soluble dendrimers and dendronized polymers are not always made up of hydrophilic units. Often times the core and branching units are hydrophobic in nature, and the water solubility of these dendrimers comes exclusively from the surface groups.[25] In this case the dendrimers and dendronized polymers are known as unicellular micelles, as the inside of the dendrimer and dendronized polymers are shielded from the water molecules.[25] These unicellular micelles are ideal for drug encapsulation of hydrophobic drug molecules. Many drugs are unable to bind to their designated site as they have poor solubility and biocompatibility.[26] This problem can be solved by encapsulating the drug in a dendrimer that is water soluble and using the dendrimer as the delivery method. Other biomedical applications of water soluble dendrimers include contrasting agents for medical devices,[27-29] tissue engineering,[30-32] and gene transfection,[33-35] and as antimicrobial agents.[36-38]

In order to synthesize complex dendrons or dendronized polymers that are water soluble, an efficient methodology needs to be implemented. The versatility of these dendrons and dendronized polymers must be such that the surface groups can be customizable to allow the incorporation of many different functionalities. To accomplish this feat we employed two important reactions. The first is the

copper(I) catalysed alkyne azide coupling reaction.[39] This reaction allows for a variety of units to be clicked on to a dendron in an efficient manner, and in this way the surface groups can be added to the dendron to impart water solubility. The second important reaction is the Steglich esterification reaction.[40] This reaction allows for two units to be attached in a similar manner as click chemistry, but requires different functional groups present. Therefore one can alternate between the copper(I) catalysed alkyne azide coupling reaction, and the Steglich esterification reaction to build up the dendron, and then use the copper(I) catalysed alkyne azide coupling reaction to functionalize the dendron. Lastly, one can use the Steglich esterification reaction to couple the dendron to a polymer, thus creating a functionalized dendronized polymer.

The alkyne azide coupling reaction, developed by Huisgen, has been around for over 50 years.[41] This reaction differs from the copper(I) catalysed alkyne azide coupling reaction in that there is no regioselectivity in the positioning of the resulting triazole ring, and the reaction requires high temperature to complete. For this reason, this reaction was not used nearly as prevalently as it is today. It wasn't until Sharpless et al. developed a way to control the reaction with the addition of copper(I) that the alkyne azide coupling reaction realized its potential.[42] With the catalyst in place the alkyne azide coupling reaction yields exclusively the 1, 4 regioisomer of the triazole ring (Figure 2.1). The 1, 5 regioisomer of the triazole ring no longer forms. Because this reaction involves the coupling of two reagents with high atom economy, high

yields, and no side products, the term click chemistry was coined by Sharpless,[43] as the two reagents are added together like Lego.

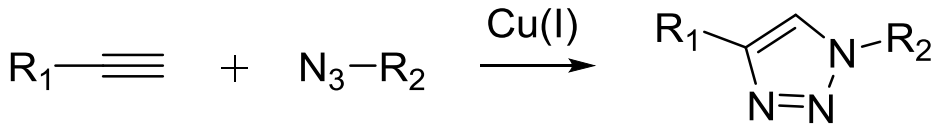


Figure 2.1 – Copper(I) catalysed alkyne azide coupling reaction.

The Steglich esterification reaction creates an ester bond, with a large number of substituents being accepted on both the acid and hydroxyl end allowing for a wide variety of reactions to occur.[44] This can be attributed to the use of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide to act as a cross linker and form an intermediate called *O*-acylisourea. This intermediate can readily be replaced by a nucleophile, allowing the hydroxyl group to replace the *O*-acylisourea. During the Steglich esterification 4-dimethylaminopyridine is also added to act as a nucleophilic catalyst to aid in the ester formation.[44]

The synthesis of dendrons and dendronized polymers reported here requires the use of both the copper(I) catalysed alkyne azide coupling reaction and the Steglich esterification reaction to attach a hyperbranched and globular dendron to a linear polymer. To reach this goal a tetrafunctional core was selected, which allows for one functionality of the dendron to be blocked and still have a trifunctional core thus leading to a high number of surface groups. In order to achieve hyperbranching and increase the dendron in size 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoic acid (1) (Scheme 2.1) was used. This building block contains an acid unit that can be esterified to a molecule with an azide attached. It also contains two hydroxyl units which act as the branching

points, thereby doubling the amount of end groups present per increase in generation. This generation increase can be done by clicking the azide on the core building block with a terminal alkyne group (Fig. 2.2).

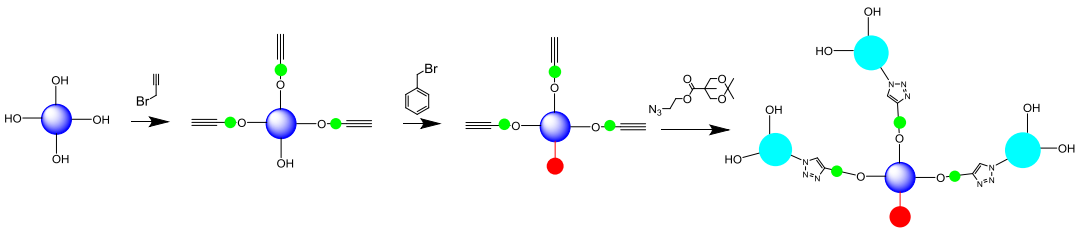


Figure 2.2 – Build up of a Dendron: General Synthetic Strategy.

The sequence of reactions can be repeated on the dendron to achieve the desired generation size, after which, the terminal alkyne groups can be clicked to a desired functional group. The functional unit, a phosphonate azide explored in this thesis, would be able to confer onto the dendron, water solubility as well as the capability to inhibit biofilm growth.[45] This phosphonate azide also plays a role as an antiscalant where it can inhibit scale formation by precipitation threshold inhibition, dispersion, or crystal distortion/modification.[20,46] Lastly, the core molecule can be deprotected to allow for a Steglich esterification reaction with a poly acrylic acid unit, to create a functionalized dendronized polymer (Fig.2.3).

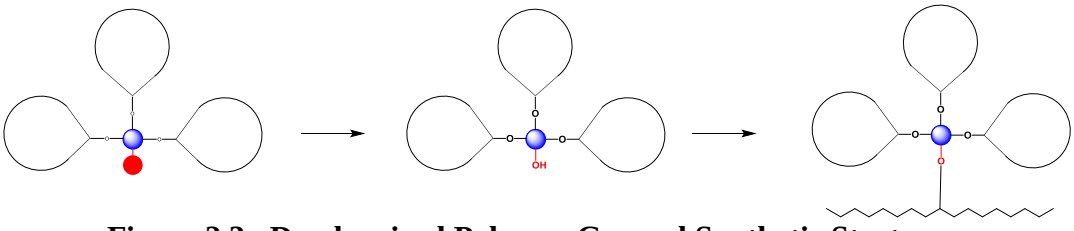
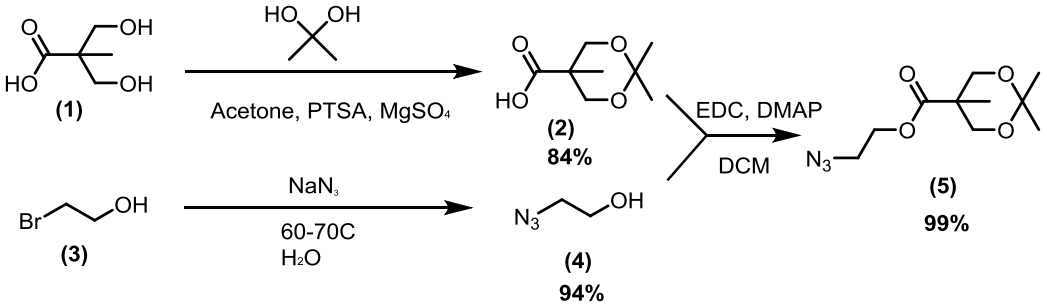


Figure 2.3 –Dendronized Polymer General Synthetic Strategy.

2.2 - Results & Discussion

The design of the dendrons as well as the dendronized polymers was such that their syntheses as well as functionalization were reliant on click chemistry.

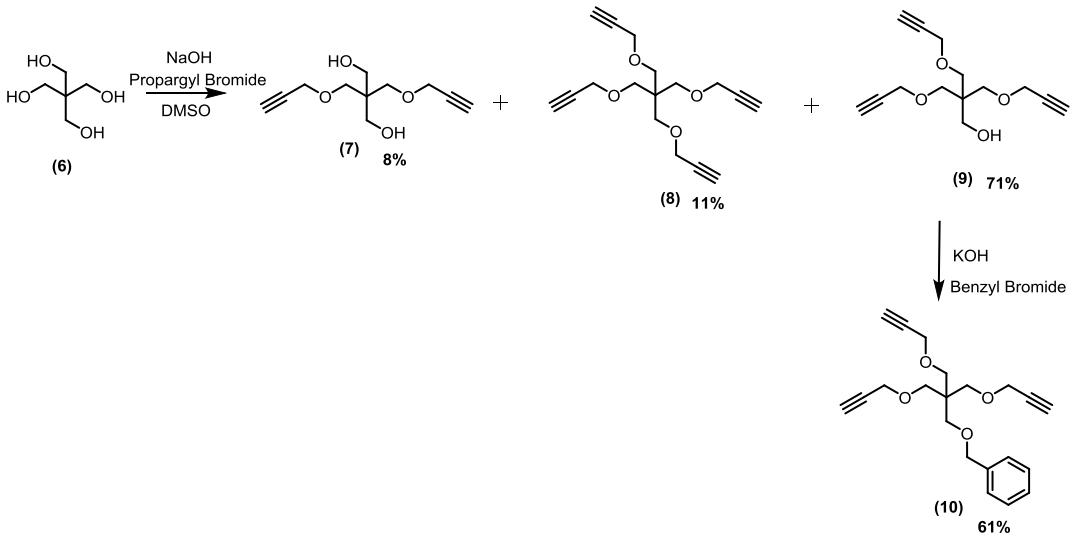
As such, the building block and functionalization groups needed to incorporate either an azide or alkyne moiety, in this case the azide, and the core of the dendron must incorporate the complimentary functional group, the alkyne. The building block chosen, 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoic acid **(5)**, was used as the azide (Scheme **2.1**). To modify the building block a bromoethanol group **(3)** was converted to azidoethanol **(4)** with sodium azide and purified by extraction. At the same time the 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoic acid **(1)** needed to be protected to prevent reactions occurring at these sites. To purify the product in this step a simple neutralization and filtration was carried out. Once both, compound **(2)** and azidoethanol, had been prepared, the two were coupled via an esterification reaction with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and 4-dimethylaminopyridine (DMAP). The product was then purified by column chromatography.



Scheme 2.1 – Synthesis of Building Blocks; PTSA= Para Toluene Sulfonic Acid.

Pentaerythritol **(6)** was chosen as the core, and the first step of the synthesis was to have the other functional groups required for a click reaction, for example the alkyne, on the core. To do this, pentaerythritol was coupled to

propargyl bromide via an etherification, the bromine acts as a leaving group in this S_N2 reaction. Since there are four reactive sites on pentaerythritol, one would expect all these to react given enough propargyl bromide, however, this is not what was observed. During the synthesis three products were formed, the di, tri, and tetra propargylated pentaerythritol. After several modifications of the reaction it seemed that solvent, reaction time, heat, and most importantly the rate of addition of propargyl bromide affects the ratio of the product(s) formed. In our attempts to synthesize the trifunctional core **(9)**, both the difunctional core **(7)** and tetrafunctional core **(8)** were formed in the reaction mixture, however the primary product was the trifunctional core. To purify this reaction column chromatography was used resulting in each product being extracted individually (Scheme **2.2**). After **(9)** is successfully obtained, another etherification was done to protect the remaining hydroxyl group with benzyl ether. Benzyl ether was chosen because of its robust nature, as this protecting group needed to survive a number of reactions and a variety of reaction conditions. The benzyl group is removed through hydrogenation at a later step, which is selective for the core deprotection. Benzyl bromide was used to protect the hydroxyl group in a similar manner to the reaction of hydroxyl groups with propargyl bromide. The resulting product was once again purified by column chromatography. The protected product **(10)** is the core of the dendron which is now ready to undergo the click reaction.



Scheme 2.2 – Synthesis of Core

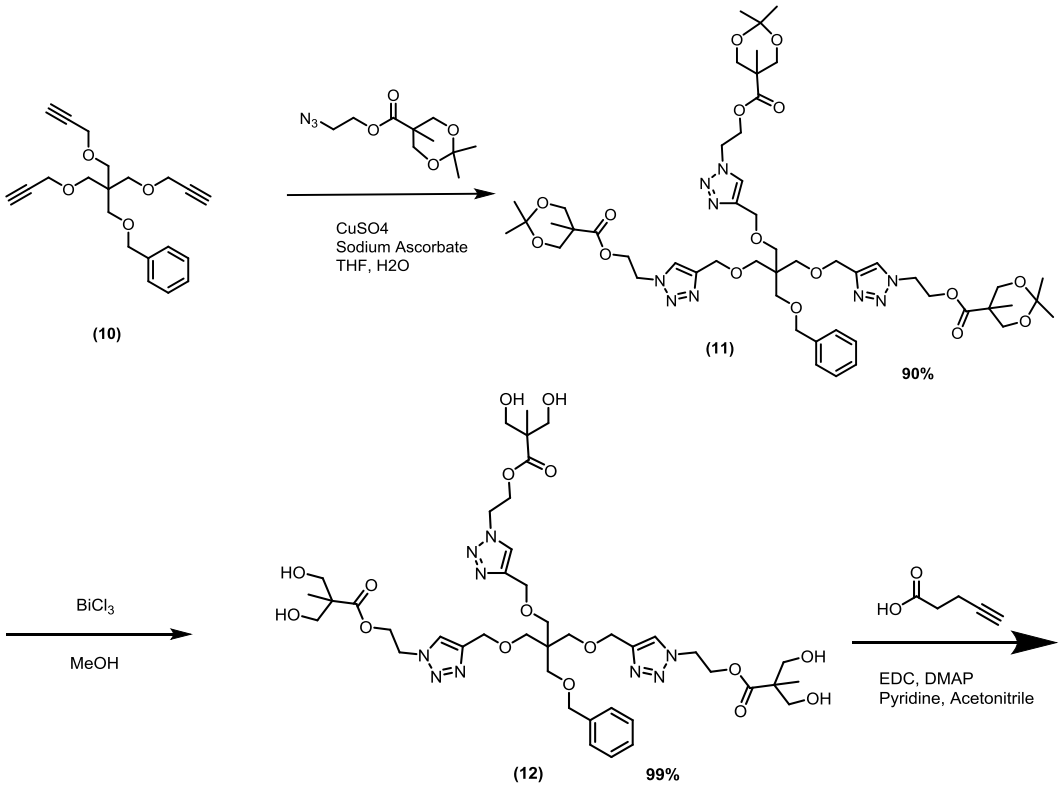
It is important to note that benzyl ether was not the only protecting group investigated or even the only protecting group used. Many other groups were considered, including methoxy methyl ether, t-butyl ether, and a benzyl ester, but not used due to i) not being robust enough to last the synthetic scheme, ii) being so robust that the removal could destroy the dendron, or iii) containing a reactive group that is repeated in the dendron and therefore could have poor selectivity. Protecting groups that were tried but ultimately failed include triisopropylsilyl ether, and trimethylsilyl ether. Both of these compounds, unfortunately, were removed while undergoing the deprotection of the acetonides on the surface of the dendron, and the removal of the trimethylsilyl ether and triisopropylsilyl ether also removed the acetonides, thus making these protecting groups not viable candidates.

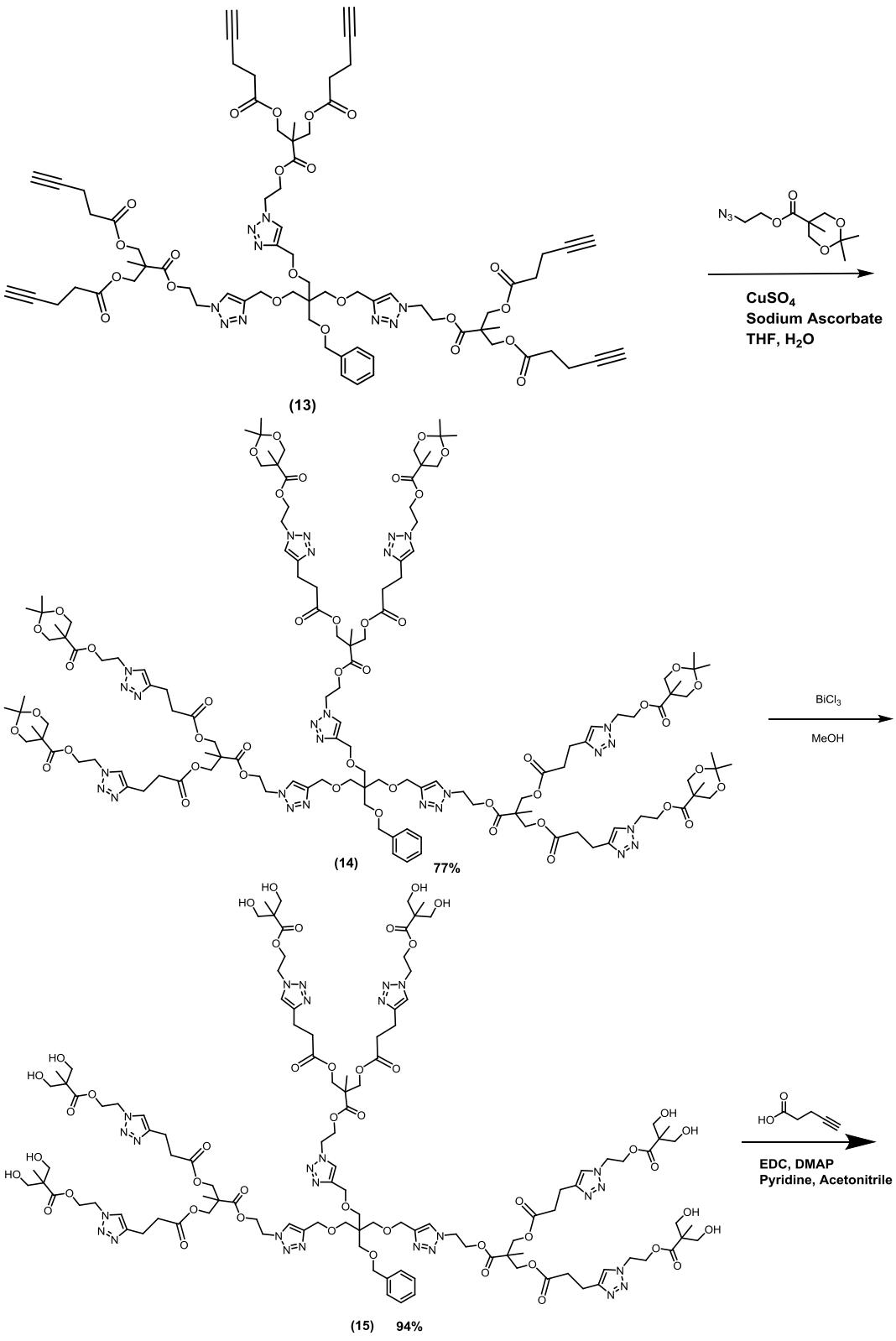
The click reaction was then carried out with the azide functionalized building block and the protected trifunctional core (Scheme **2.3**). We used

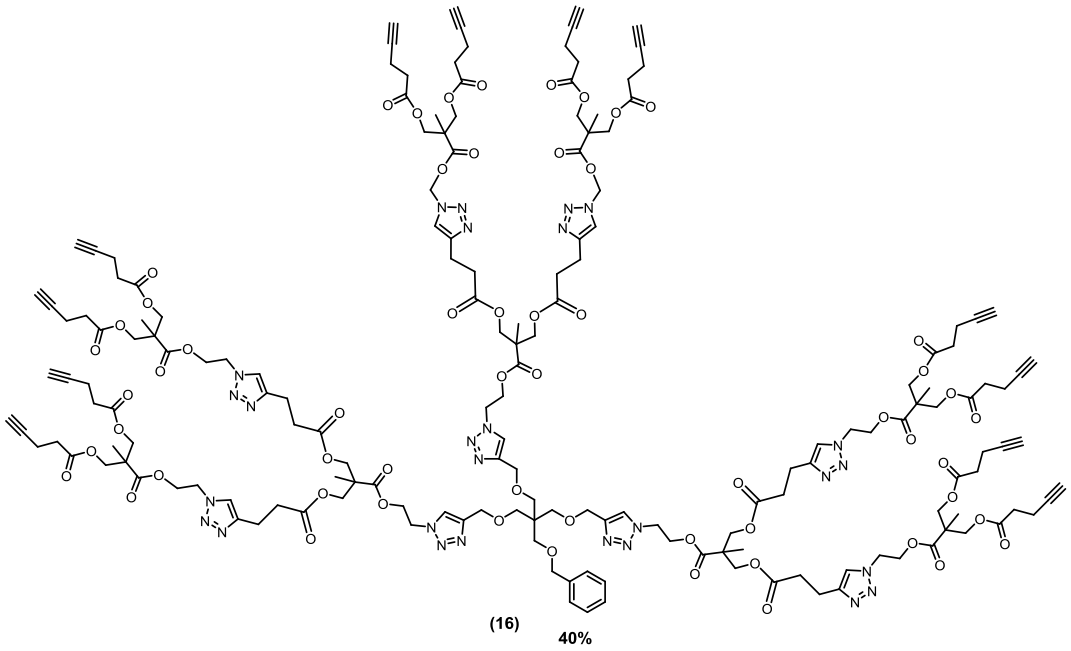
copper(I) catalysed alkyne azide coupling reaction to yield generation 1 **(11)** dendron with six protected hydroxyl units at the periphery. This reaction required removal of copper by ethylenediaminetetraacetic acid (EDTA) washes, as well as column chromatography to remove the excess building block. Once generation 1 dendron **(11)** was synthesized the hydroxyl groups of the dendron could be deprotected. Using a dowex cationic resin the protecting groups on the surface were removed as acetone and the cationic resin filtered off. Evaporation of acetone resulted in deprotected generation 1 dendron **(12)**. This method was found to be initially successful, however, the reliability of it on higher scale reactions was not sufficient for dendrimer chemistry, so a different method was used. It included the addition of a bismuth salt ($BiCl_3$) to the dendron. After the reaction was complete the bismuth salts could be filtered off, and the protecting groups came off as acetone once again. Once the deprotected dendron **(12)** was synthesized the dendron needed to be propargylated to do another click reaction. Unfortunately all attempts to do an etherification with propargyl bromide were unsuccessful. Instead, a Steglich esterification reaction with EDC and DMAP was used with 4-pentynoic acid to produce the activated generation 1 dendron **(13)** and once again the product was purified by column chromatography.

During many attempts to carry out the Steglich esterification reaction, a number of modifications were included. Initially deprotected dendron **(12)** would solubilise in dichloromethane and the reaction would proceed, however with reduced yield. It was later found that **(12)** was insoluble in dichloromethane, however, with the addition of some methanol, **(12)** became soluble. Since the

previous reaction involves methanol, the residual methanol aided in dissolving the deprotected dendron **(12)** in dichloromethane. The reason this went unnoticed was that despite attempts to remove methanol from the reaction mixture in vacuo, the dendron is capable of encapsulating some methanol. Lastly, the use of methanol as a solvent in the Steglich esterification reaction is not a good choice, due to its hydroxyl group competing with those on the dendron in the coupling reaction.




CuSO4
Sodium Ascorbate
THF, H2O
(13)
BiCl3
MeOH
(14)
77%
EDC, DMAP
Pyridine, Acetonitrile
(15)
94%



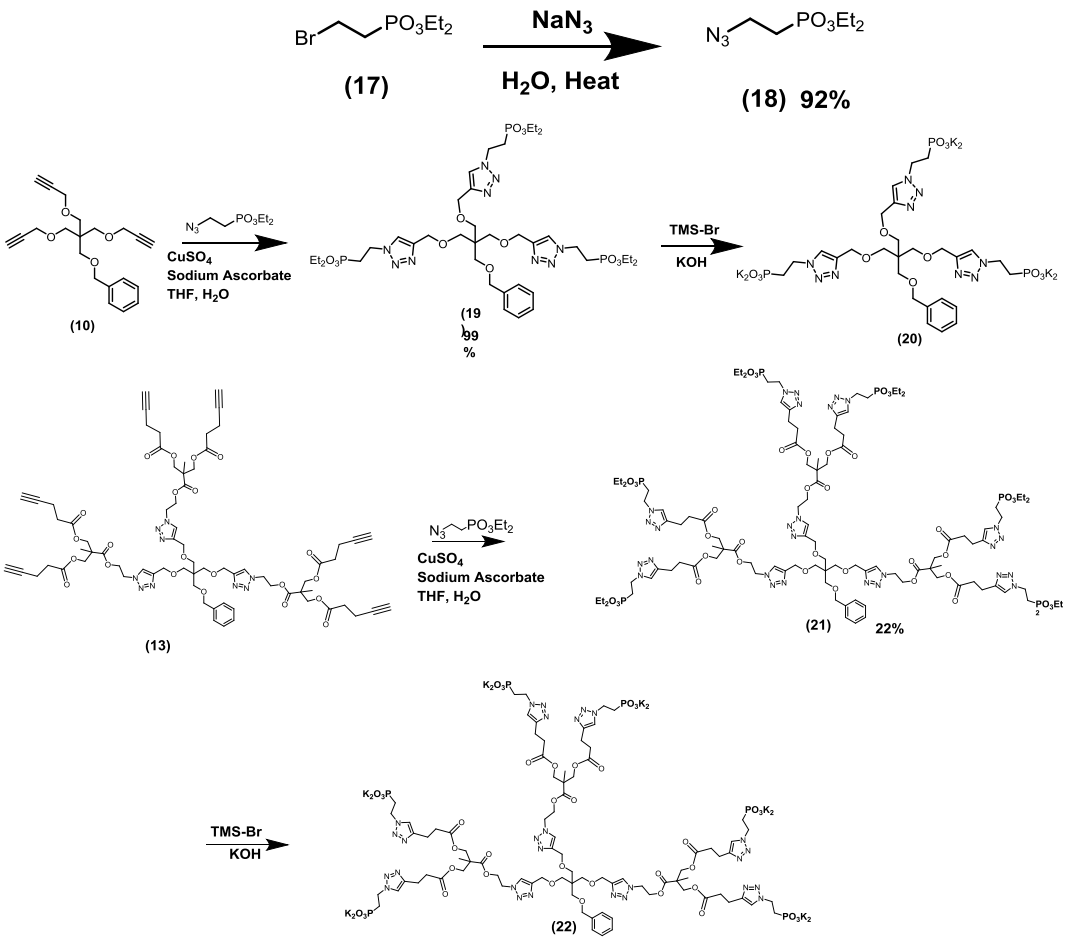
Scheme 2.3 – Buildup of Dendrons.

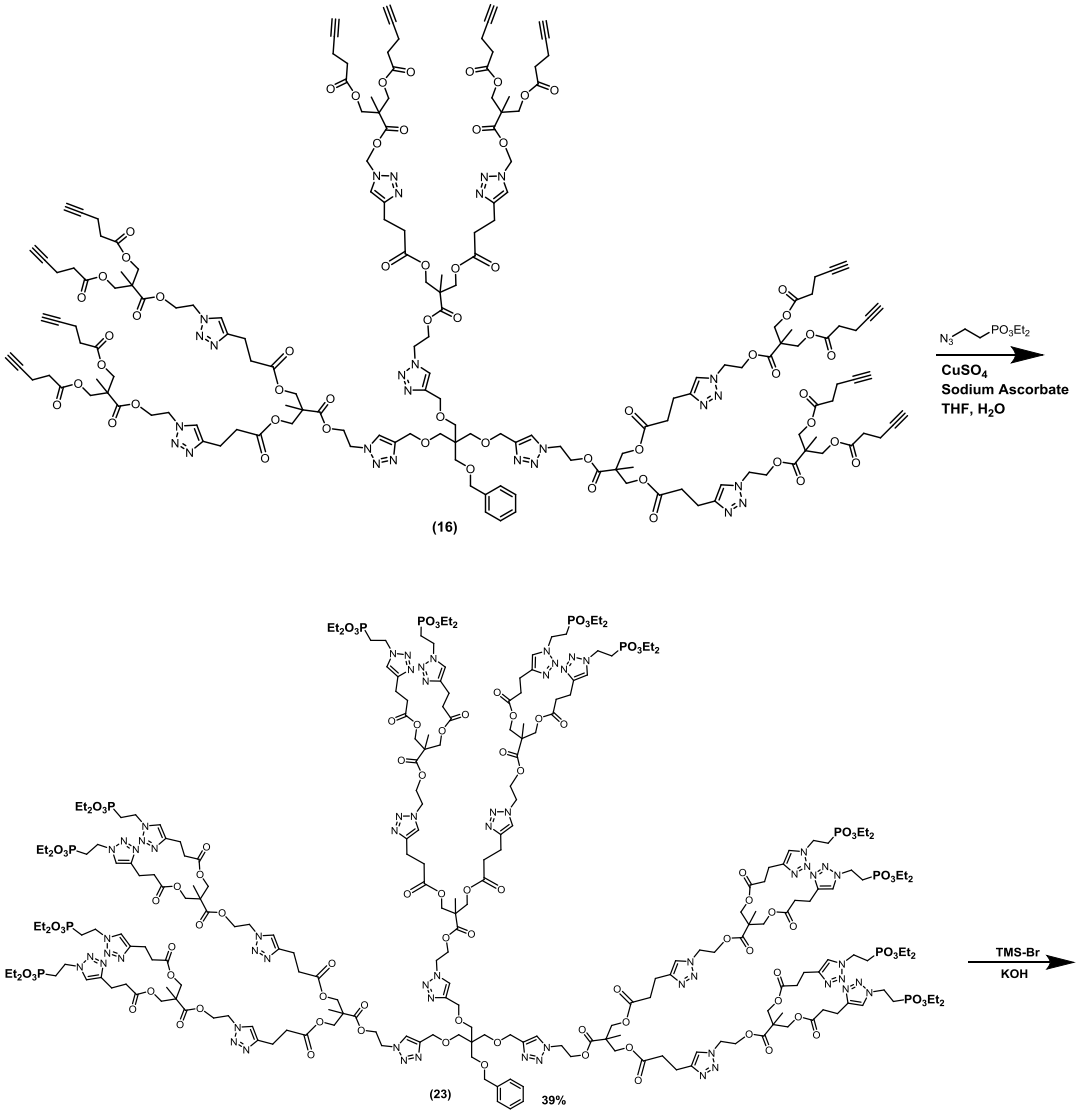
Another click reaction was then carried out with the activated dendron **(13)** and the azide functionalized building block. Using the copper(I) catalysed alkyne azide coupling the two reagents were linked to form generation two dendron **(14)** with 12 protected hydroxyl groups. This reaction once again required removal of residual copper using ethylenediaminetetraacetic acid (EDTA) washes as well as column chromatography to remove the excess building block, just as with generation one dendron. As the generation size increased, the column chromatography became less useful as there was a concern that the dendron would get stuck on the column. As a replacement tool, generally dialysis was conducted to remove small molecule impurities. With the synthesis of generation 2 dendron **(14)** complete, the protecting groups on the surface hydroxyls needed to be removed using bismuth salts ($BiCl_3$). Once again the salts were removed by filtration and the protecting group came off as acetone which

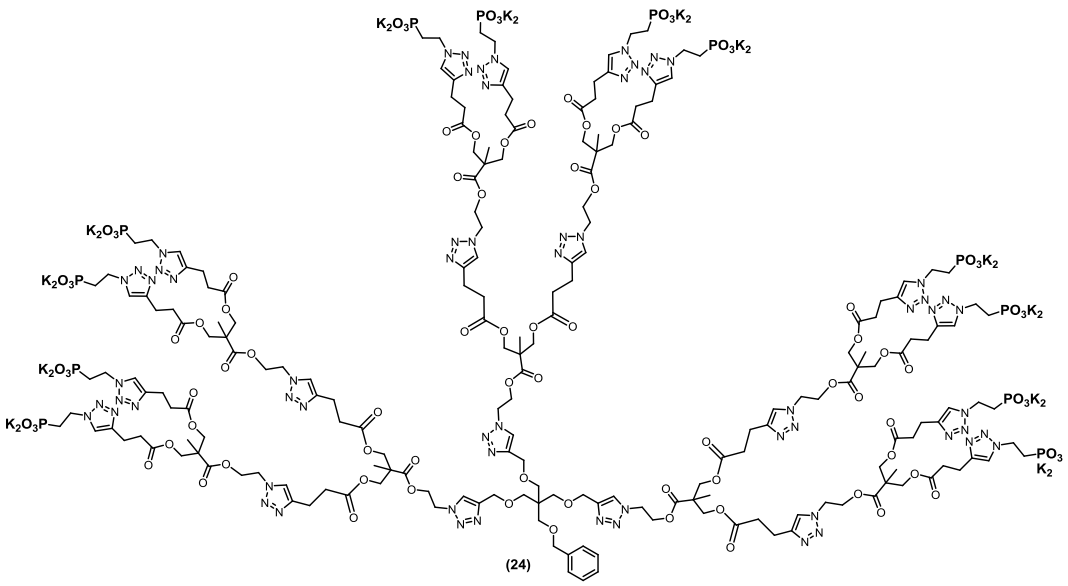
was evaporated in vacuo, resulting in deprotected generation two dendron **(15)**. To do another click reaction the surface groups needed to be propargylated, so compound **(15)** again underwent a Steglich esterification reaction with EDC, DMAP, and 4-pentynoic acid to produce activated generation two dendron **(16)**. The product **(16)** was purified by dialysis with a molecular weight cut off of 1000 daltons. The series of click, deprotection, and esterification described above can be repeated to synthesize a dendron of theoretically any generation.

Once the acetylene terminated dendrons (protected core **(10)** were synthesized, activated generation one dendron **(13)**, activated generation two dendron **(16)**) could now be functionalized by a click reaction with a phosphonate azide group at the periphery (Scheme **2.4**). To synthesize the phosphonate azide **(18)** from commercially available phosphonate bromide **(17)**, azidation with sodium azide was used, similar to the synthesis of azidoethanol[47]. This phosphonate azide was then purified by an extraction. Protected core **(10)**, activated generation one dendron **(13)**, activated generation two dendron **(16)** all followed a similar click reaction where the azidophosponate was added via a copper(I) catalysed alkyne azide coupling reaction to yield generation zero phosphonate **(19)**, generation 1 phosphonate **(21)**, and generation 2 phosphonate **(23)** respectively. Each reaction was first passed through EDTA to remove the copper salts, and generation zero phosphonate **(19)**, generation 1 phosphonate **(21)**, and generation 2 phosphonate **(23)** were purified by precipitating the dendron with ether. The phosphorus units attached to the dendron at this point are protected with ethyl groups. To deprotect them the dendron was first reacted with

bromotrimethylsilane, after which all volatile components were removed. The dendron was then allowed to react with a 1M KOH solution resulting in the phosphorus units being potassiated resulting in **(20)**, **(22)**, and **(24)**. In this form **(20)**, **(22)**, and **(24)** are water soluble.




N3 PO3Et2
CuSO4
Sodium Ascorbate
THF, H2O
(16)
Et2O3P
PO3Et2
TMS-Br
KOH
(23)
39%



Scheme 2.4 – Dendron Functionalization.

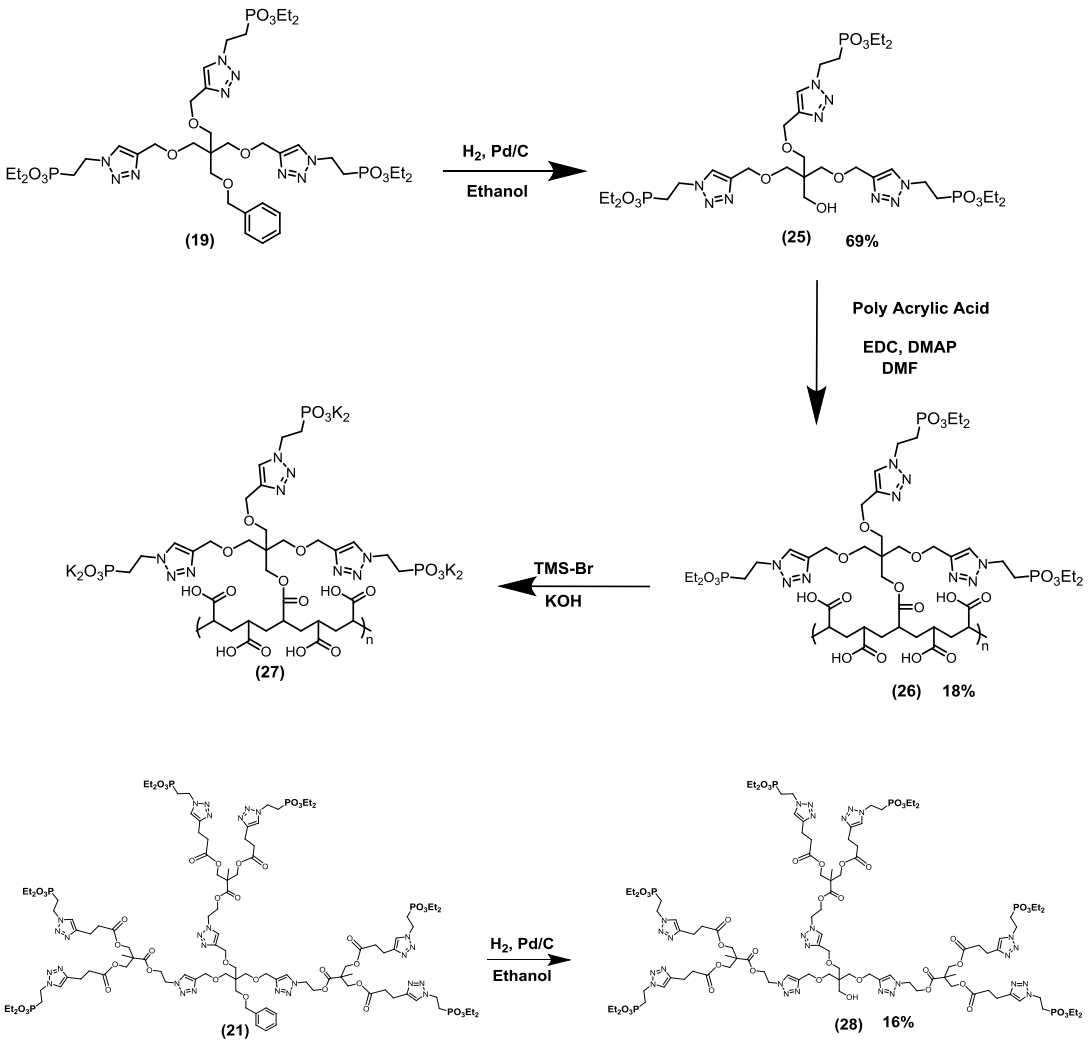
Upon completion of the synthesis of generation 0 phosphonate **(19)**, generation 1 phosphonate **(21)**, and generation 2 phosphonate **(23)**, their core was deprotected. There were four points in the designed synthesis in which the benzyl group could have been removed from the dendron, but it was found that after phosphonation was the best time to do this. The others were found to be inappropriate for three reasons: 1) to remove the benzyl ether when the dendron had alkyne groups was not attempted as we knew that the alkyne groups had the potential to be hydrogenated under these conditions[48]; 2) When the dendron had terminal hydroxyl groups it made no sense to attempt to remove them, as the core and surface would have similar functionalities, and thus a specific reaction afterwards would be difficult; and 3) lastly deprotection of the benzyl ether after a click reaction when the hydroxyls were still protected was attempted. The hydrogenation reaction did successfully remove the benzyl ether, but some of the end groups were also deprotected, and though attempts were made to stop the

reaction before the end groups were removed, it was found that the reactions occur simultaneously, but at differing rates. Thus, benzyl ether was removed at the phophonated stage of the dendron with no deprotection of the surface groups.

To remove benzyl ether, hydrogenation was carried out in a bomb flask filled with the dendron, palladium and charcoal. The flask was filled with 4 atmospheres of hydrogen gas. After the reaction was complete, the dendron was filtered to remove palladium and charcoal, and the protecting group came off as toluene which was evaporated over time resulting in **(25)**, **(28)**, and **(31)**. An important point is that the generation 0 dendron does not need to be protected or deprotected for the synthesis of **(25)**.With the benzyl ether removed, the dendron is ready to be coupled to a polymer. The polymer used was poly(acrylic acid) with a molecular weight of 2000 Daltons. This polymer was chosen for its acid groups which allow it to undergo a Steglich esterification reaction with EDC, and DMAP, and the dendron's free hydroxyl. The resulting product of this coupling was then purified by dialysis with a molecular weight cut off of 1000 Daltons. The purification resulted in dendronized polymer **(26)**. Generation 1 and 2 dendronization synthesis is in progress.

Upon dendronization the product was no longer monodisperse as the polymer used was polydisperse and the amount of dendrons attached to each polymer was not controlled. MS gave peaks for masses of 1, 2, 3, 4, and 5 dendrons attached to the polymer. The last step in this synthesis was to deprotect the end groups of the dendronized polymer to allow water solubility and functionality. The ethyl groups of the phosphorus units were removed from each

dendronized polymer by the addition of bromotrimethylsilane. After the reaction was complete, the bromotrimethylsilane was removed, and 1M KOH was added to form the potassiated dendronized polymers **(27)** which is water soluble.



Scheme 2.5 – Dendronized Polymer Formation and Functionalization.

After purification, the products were characterized using a variety of techniques including ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, and MS. ^{1}H NMR was

extremely useful for dendrons, because most reactions involved a significant change in some protons environment. For example, a click reaction results in a distinctive peak at 7.4 ppm for the triazole hydrogen. This was then followed up with ^{13}C NMR as well as mass spectrometry to further quantify the product. ^{13}C NMR proved useful for dendrons as well. For example, when deprotecting the hydroxyl groups of the dendron, distinctive carbon peaks are removed, allowing for accurate identification. When using mass spectrometry often MALDI-TOF (matrix assisted laser desorption ionization time of flight) was used to insure that the dendrons could be ionized and detected. Lastly, once the dendrons or dendronized polymers were phosphonated, the resulting product could be characterized by ^{31}P NMR. The peak position for ^{31}P NMR when the phosphorus was protected is at 25 ppm compared to the deprotected form at 17 ppm allowing for easy identification.

The three resulting products from the synthetic scheme **(20)**, **(22)**, and **(27)**, were then tested for their effectiveness as a biofilm inhibitor. The results are described in chapter 3.

2.3 – Conclusions

In conclusion, a combination of Sharpless' copper(I) catalysed alkyne azide coupling and Steglich esterification reaction can be efficiently used to synthesize dendrons with a protected core. In addition, the copper(I) catalysed alkyne azide coupling reaction was extremely versatile in both building a dendron, and subsequent functionalization. The series of reactions carried out successfully led to the synthesis of dendrons with multiple surface groups, as well

as the resulting dendronized polymers. Further elaboration to couple different generations of dendrons to linear polymers, and varying their percent functionalization, is currently being pursued. The dendrons and dendronized polymers with phosphonate surface groups have the potential to inhibit biofilm formation. The potential of these functionalized dendrons and dendronized polymers for biofilm applications is explored in Chapter 3.

2.4 – Experimental

Synthesis of Building Blocks: The following building blocks were synthesized using an elaboration and modification of the procedures described in references 49 **(2)**, 47 **(4)**, and 50 **(5)**.

Synthesis of 2,2,5-trimethyl-1,3-dioxane-5-carboxylic acid **(2)**

Para-toluenesulfonic acid (0.598 g, 0.00314 mol) was added to a stirred solution of 2,2-bis(hydroxymethyl)propanoic acid **(1)** (8.43 g, 0.0628 mol) in acetone (34 mL), under nitrogen, in a 250 mL round bottom flask. 2,2-dimethoxypropane (9.81 g, 0.0942 mol) and magnesium sulfate (0.756 g, 0.00628 mol) were then added to the flask. The reaction mixture was left stirring under nitrogen for 2 days. The crude mixture was filtered, extracted with DCM, dried with $MgSO_4$ and the solvent evaporated to yield the product as a white powder (9.14 g, 0.0524 mol, 84% yield). ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.195 (s, -CO-C-C$\underline{H}_3$. 3H), 1.395(s, -O-C-C$\underline{H}_3$, 3H), 1.428 (s, -O-C-C$\underline{H}_3$, 3H), 3.670 (t, -O-C$\underline{H}_2$-C-CO-, 2H), 4.180 (d, -O-C$\underline{H}_2$-C-CO-, 2H) ppm. ^{13}C {^{1}HNMR (300MHz, $CDCl_3$): δ = 18.42 (-CO-C-$\underline{C}H_3$), 22.03 (-O-C-$\underline{C}H_3$), 25.07 (-O-C-$\underline{C}H_3$), 41.71 (-CO-$\underline{C}$-), 65.81 (-O-$\underline{C}H_2$-C-), 98.28 (-O-$\underline{C}$-$(CH_3)_2$), 180.15 (-$\underline{C}$O-) ppm.

Synthesis of Azidoethanol **(4)**

A mixture of bromoethanol **(3)** (10.5 g, 0.0840 mol) and sodium azide (20.0 g, 0.307 mol) in water (50 mL) was left stirring overnight at 65°C. The reaction mixture was then extracted with DCM, the organic layer was isolated and dried with magnesium sulfate. The solvent was then evaporated to yield the product as a yellow oil (6.59 g, 0.0756 mol, 94% yield). ^{1}H NMR (400MHz, $CDCl_3$): δ = 3.46 (t, 2H), 3.79 (q, 2H) ppm. $^{13}C\{^{1}H\}$ NMR (300MHz, $CDCl_3$): δ = 53.6 (N_3-$\underline{C}H_2$-CH_2-), 61.6 (N_3-CH_2-$\underline{C}H_2$-) ppm.

Synthesis of 2-azidoethyl 2,2,5-trimethyl-1,3-dioxane-5-carboxylate **(5)**

A solution of azidoethanol **(4)** (1.70 g, 0.0195 mol), 2,2,5-trimethyl-1,3-dioxane-5-carboxylic acid **(2)** (5.09 g, 0.0292 mol) and 4-dimethylaminopyridine (DMAP) (1.18 g, 0.0966 mol) in anhydrous DCM (23 mL) was left stirring, under nitrogen, for 5 minutes. 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (3.90 g, 0.0203 mol) was added to the reaction mixture, which was then left stirring under nitrogen, at room temperature, overnight. The precipitate was filtered off, extracted in DCM, dried with $MgSO_4$ and the solvent was evaporated to yield a residue that was purified by column chromatography (1:7 EtOAc:Hexane) to yield the product as a white solid (4.71 g, 0.0194 mol, 99.4% yield). ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.21 (s, -CO-C-C$\underline{H}_3$, 3H), 1.39 (s, -O-C-C$\underline{H}_3$, 3H), 1.44 (s, -O-C-C$\underline{H}_3$, 3H), 3.49 (t, N_3-CH_2-CH_2-, 2H), 3.68 (d, -O-C$\underline{H}_2$-C-CO-, 2H), 4.21 (d, -O-C$\underline{H}_2$-C-CO-, 2H), 4.33 (t, N_3-CH_2-CH_2-, 2H) ppm. $^{13}C\{^{1}H\}$ NMR (300MHz, $CDCl_3$): δ = 18.5 (-CO-C-$\underline{C}H_3$), 22.3 (-O-C-$\underline{C}H_3$),

24.9 (-O-C-$\underline{C}H_3$), 42.0 (-C-CH_3), 49.8 (N_3-$\underline{C}H_2$-CH_2-), 63.6 (N_3-CH_2-$\underline{C}H_2$-), 65.9 (-O-$\underline{C}H_2$-C-), 98.1 (-$\underline{C}$-CH_3), 174.0 (-$\underline{C}$O-) ppm.

Synthesis of Core

Synthesis of **(9)**

DMSO (11.25 mL) was added by syringe to a round bottom flask containing a solution of pentaerythritol **(6)** (1.50 g, 0.0110 mol) and NaOH (2.40 g, 0.060 mol) in water (6 mL), with stirring for 30 minutes. A solution of propargyl bromide (8.93 g, 0.0751 mol) in toluene (80%) was then added drop wise to the reaction mixture over 30 minutes. The reaction was left stirring overnight. Water (100 mL) was added to the mixture, which was then extracted with diethyl ether (3x50 mL). The organic layers were isolated, combined, and washed with water (3x50 mL) and brine (3x50 mL). The organic layer was isolated and dried with sodium sulfate. The solvent was removed to yield an orange oil which was purified by column chromatography (1:1 Hexane: ether) to give the product as an orange oil (1.95 g, 0.00780 mol, 71% yield). ^{1}H NMR (400MHz, $CDCl_3$): δ = 2.40 (t, C$\underline{H}$-C-CH_2-, 3H), 3.48 (s, C-C$\underline{H_2}$-OH, 2H) 3.59 (s, C-C$\underline{H_2}$-O-,6H), 4.09 (d, CH-C-C$\underline{H_2}$-, 6H) ppm. ^{13}C{^{1}H} NMR (300MHz, $CDCl_3$) δ = 44.6 ($\underline{C}$-CH_2-O-), 58.7 (CH-C-$\underline{C}H_2$), 65.0 (C-$\underline{C}H_2$-OH), 70.1 (C-$\underline{C}H_2$-O-), 74.5 ($\underline{C}$H-C-CH_2), 79.6 (CH-$\underline{C}$-CH_2) ppm. MALDI-MS: m/z =273.00 [M+Na^+]

During the synthesis of **(9)**, two similar molecules where two of the four hydroxide groups were reacted with propargyl bromide, and where all of the four hydroxyl groups were reacted with propargyl bromide, **(7)** and **(8)** were also obtained respectively. This synthesis was achieved by the same method outlined

above with the exception that the column conditions were changed, where **(8)** comes out in 3:1 hexane: ether mixture, and **(7)** comes out in pure ether. Thus for the same amount of pentaerythritol, of propargyl bromide and of NaOH were used.

(7) : ^{1}H NMR (400MHz, $CDCl_3$): δ = 2.42 (t, C<u>H</u>-C-C<u>H$_2$</u>-, 2H), 3.56 (s, C-C<u>H$_2$</u>-O-,4H), 3.65 (d, C-C<u>H$_2$</u>-OH, 4H), 4.12 (d, CH-C-C<u>H$_2$</u>-, 4H) ppm. ^{13}C{^{1}H} NMR (300MHz, $CDCl_3$): δ = 44.6 (<u>C</u>-CH$_2$-O-), 58.7 (CH-C-<u>C</u>H$_2$), 65.0 (C-<u>C</u>H$_2$-OH), 70.1 (C-<u>C</u>H$_2$-O-), 74.5 (<u>C</u>H-C-CH$_2$), 79.6 (CH-<u>C</u>-CH$_2$) ppm.

(8) : ^{1}H NMR (400MHz, $CDCl_3$): δ = 2.42 (t, C<u>H</u>-C-C<u>H$_2$</u>-, 4H), 3.56 (s, C-C<u>H$_2$</u>-O-,8H), 4.12 (d, CH-C-C<u>H$_2$</u>-, 8H) ppm. ^{13}C{^{1}H} NMR (300MHz, $CDCl_3$): δ = 44.6 (<u>C</u>-CH$_2$-O-), 58.7 (CH-C-<u>C</u>H$_2$), 70.1 (C-<u>C</u>H$_2$-O-), 74.5 (<u>C</u>H-C-CH$_2$), 79.6 (CH-<u>C</u>-CH$_2$) ppm.

<u>Synthesis of **(10)**</u>

In a round bottom flask, **(9)** (2.75 g, 0.0111 mol), benzyl bromide (5.50 g, 0.0321 mol), and KOH (1.20 g, 0.0178 mol) were added. The reaction was stirred for 3 days to yield a orange oil which was purified by column chromatography (9:1 Hexane: Ethyl Acetate) to give the product as a yellow oil (2.17 g, 0.00639 mol, 61% yield). ^{1}H NMR (400MHz, $CDCl_3$): δ = 2.40 (t, C<u>H</u>-C-CH$_2$-, 3H), 3.48 (s, C-C<u>H$_2$</u>-O-C-, 2H) 3.59 (s, C-C<u>H$_2$</u>-O-,6H), 4.09 (d, CH-C-C<u>H$_2$</u>-, 6H), 4.51 (s, CH$_2$-O-C<u>H$_2$</u>-C, 2H), 7.34 (m, C-C<u>H</u>-C<u>H</u>-C<u>H</u>-C<u>H</u>-C<u>H</u>-,5H) ppm. ^{13}C{^{1}H} NMR (300MHz, $CDCl_3$) δ = 44.6 (<u>C</u>-CH$_2$-O-), 58.7 (CH-C-<u>C</u>H$_2$), 69.1 (C-<u>C</u>H$_2$-O-CH$_2$-C), 69.2 (C-<u>C</u>H$_2$-O-CH$_2$-C-CH), 73.2 (C-<u>C</u>H$_2$-O-CH$_2$-C-C), 74.1 (<u>C</u>H-C-CH$_2$),

80.7 (CH-C-CH_2), 127.3 (C-CH-CH-), 127.6 (C-CH-CH-CH), 128.2 (C-CH-CH), 138.8 (C-CH-CH) ppm. MALDI-MS: m/z =363.15582 [M+Na^+]

Synthesis of Dendron

Synthesis of **(11)**

A solution of $CuSO_4 \cdot 5H_2O$ (0.702 g, 0.00282 mol) in water (7.5 mL) was added to a round bottom flask containing a stirred solution of **(10)** (1.53 g, 0.00449 mol) and **(5)** (4.84 g, 0.0199 mol) in THF (30 mL). Sodium ascorbate (1.06 g, 0.00535 mol) was then added and the mixture was allowed to react overnight. The product was purified by column chromatography (100%, Ethyl Acetate, followed by 10% methanol) to yield a yellow oil (4.48 g, 0.00418 mol, 93% yield). ^{1}H NMR (400MHz, CD_3OD): δ = 1.00 (s, -CO-C-CH_3, 9H), 1.22 (s,-O-C-(CH_3)$_2$, 9H), 1.39 (s,-O-C-(CH_3)$_2$, 9H), 3.30 (s, C-CH_2-O-C-, 2H) 3.44 (s, C-CH_2-O-CH_2, 6H), 3.63(dd, CO-C-CH_2-O-, 6H), 4.07(dd, CO-C-CH_2-O-, 6H), 4.31 (s, -O-CH_2-C-N-, 6H), 4.41 (s, CH_2-O-CH_2-C, 2H) 4.47(t, -N-CH_2-CH_2-O-, 6H), 4.72 (t, -N-CH_2-CH_2-O-, 6H), 7.14 (m, C-CH-CH-CH-CH-CH-,5H), 7.60 (s, -C-CH-N-, 1H) ppm. ^{13}C{^{1}H} NMR (300MHz, CD_3OD): δ = 17.19 (-C-CH_3), 20.52 (-O-C-(CH_3)$_2$), 24.93 (-O-C-(CH_3)$_2$), 41.78 (-C-C-CH_3), 45.11 (-C-CH_2-O-), 48.90 (-N-CH_2-CH_2-O-), 62.63 (-N-CH_2-CH_2-O-), 64.06 (-CH_2-O-CH_2-C-N-), 65.47 (-C-CH_2-O-C-), 68.65 (-C-CH_2-O-CH_2-C-N-),69.2 (C-CH_2-O-CH_2-C-CH), 73.2 (C-CH_2-O-CH_2-C-C), 97.97 (O-C-(CH_3)$_2$), 124.07 (-C-CH-N-), 127.3 (C-CH-CH-), 127.6 (C-CH-CH-CH), 128.2 (C-CH-CH), 138.8 (C-CH-CH) 145.00 (-C-N-), 173.80 (-C-O-CH_2-) ppm. MALDI-MS: m/z =1092.46 [M+Na^+]

Synthesis of **(12)**

Using Dowex Cationic Resin:

Dowex Cationic Resin (1.5 g) was added to a solution of **(11)** (3.40 g, 0.00318 mol) in CH_3OH (45 mL). The mixture was left stirring overnight. The resin was then filtered off and the solvent evaporated to yield **(12)** as an orange oil (2.00 g, 0.00210 mol, 66%yield). Unfortunately, this reaction was not consistent enough, and another method was subsequently used.

Using $BiCl_3$:

$BiCl_3$ (0.026 g, 0.0000825 mol) and 5 drops of H_2O were added to a solution of **(11)** (0.25 g, 0.000234 mol) in acetonitrile (5 mL). The reaction mixture was left stirring for 24 hours at 40°C. The reaction was stopped, and $BiCl_3$ was filtered off, and the resulting solvent evaporated to yield a pale yellow oil (0.220 g, 0.000231 mol, 99% yield). ^{1}H NMR (400MHz, CD_3OD): δ = 1.08 (s, -CO-C-C$\underline{H}_3$, 9H), 3.44 (s, C-C$\underline{H}_2$-O-CH_2-C-CH, 2H), 3.49 (s, C-C$\underline{H}_2$-O-, 6H), 3.61 (q, C-C$\underline{H}_2$-OH, 12H), 4.41 (s, CH_2-O-C$\underline{H}_2$-C, 2H), 4.51 (t, -N-CH_2-C$\underline{H}_2$-O-, 6H), 4.55 (s, -O-C$\underline{H}_2$-C-N-, 6H), 4.70 (t, -N-C$\underline{H}_2$-CH_2-O-, 6H), 7.14 (m, C-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-,5H), 8.04 (s, -C-C$\underline{H}$-N-, 3H) ppm. ^{13}C{^{1}H} NMR (300MHz, CD_3OD): δ = 17.40 (-C-$\underline{C}H_3$), 46.53 ($\underline{C}$-CH_2-O-), 50.42 (-$\underline{C}$-CH_3), 51.78 (-N-$\underline{C}H_2$-CH_2-O-), 63.86 (-N-CH_2-$\underline{C}H_2$-O-), 65.40 (-O-$\underline{C}H_2$-C-N-), 65.85 (-C-$\underline{C}H_2$-O-),69.2 (C-$\underline{C}H_2$-O-CH_2-C-CH), 70.09 (C-$\underline{C}H_2$-O-), 73.2 (C-$\underline{C}H_2$-O-CH_2-C-C),125.77 (-C-$\underline{C}$H-N-),127.3 (C-$\underline{C}$H-CH-), 127.6 (C-CH-CH-$\underline{C}$H), 128.2 (C-CH-$\underline{C}$H), 138.8 ($\underline{C}$-CH-CH) 146.35 (-O-CH_2-$\underline{C}$-N-), 176.06 (-C-$\underline{C}$O-O-) ppm. MALDI-MS: m/z = 972.39 [M+Na^+].

Synthesis of **(13)**

DMAP (0.436 g, 0.00357 mol) was added to a stirred mixture of **(12)** (1.11 g, 0.00117 mol) and 4-pentynoic acid (1.06 g, 0.0108 mol) in anhydrous acetonitrile (27 mL), under nitrogen, in a 50 mL round bottom flask. EDC (1.36 g, 0.00708 mol) and pyridine (13 mL) were added to the flask. The reaction mixture was left stirring, under nitrogen, overnight. The crude mixture was filtered and the solvent evaporated. The product was purified by column chromatography (100% EtOAc) to yield the product as a yellow oil (1.30 g, 0.000907 mol, 75% yield). ^{1}H NMR (400MHz, CD_3OD): δ = 1.19(s, 9H, -C-C$\underline{H}_3$), 2.26 (t, 6H, -C-C$\underline{H}$), 2.46-2.53(m, 24H, -O-C-C$\underline{H}_2$-C$\underline{H}_2$-C-),3.44 (s, C-C$\underline{H}_2$-O-CH_2-C-CH, 2H), 3.49(s, 6H, -C-C$\underline{H}_2$-O-CH_2-), 4.18(s, 12H, -C-(C$\underline{H}_2$-O-C-)$_2$), 4.41 (s, CH_2-O-C$\underline{H}_2$-C, 2H), 4.51 (t, -N-CH_2-C$\underline{H}_2$-O-, 6H), 4.55 (s, -O-C$\underline{H}_2$-C-N-, 6H),4.67(t, 6H, -C-CH-N-C$\underline{H}_2$-),7.14 (m, C-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-,5H), 8.01 (s, 3H, -N-C$\underline{H}$-) ppm. ^{13}C{^{1}H} NMR (300MHz, CD_3OD): δ = 15.10 (-$\underline{C}H_2$-C-CH), 18.20 (-C-$\underline{C}H_3$), 34.31 (-$\underline{C}H_2$-CH_2-C-CH), 46.57(-$\underline{C}$-CH_2-O-CH_2-C-N-), 47.70(-$\underline{C}$-CH_3), 50.22(-N-$\underline{C}H_2$-CH_2-O-), 64.51(-N-CH_2-$\underline{C}H_2$-O-), 65.59 (-C-CH_2-O-$\underline{C}H_2$-C-N-), 66.61 (-C-$\underline{C}H_2$-O-C-), 69.2 (C-$\underline{C}H_2$-O-CH_2-C-CH), 70.19 (-C-$\underline{C}H_2$-O-CH_2-C-N-), 70.55 (-C-$\underline{C}$H), 73.2 (C-$\underline{C}H_2$-O-CH_2-C-C), 83.54 (-$\underline{C}$-CH), 125.49 (-C-$\underline{C}$H-N-),127.3 (C-$\underline{C}$H-CH-), 127.6 (C-CH-CH-$\underline{C}$H), 128.2 (C-CH-$\underline{C}$H), 146.51 (-$\underline{C}$-CH-N-), 172.84 (-O-$\underline{C}$-CH_2-), 173.72 (O-$\underline{C}$-C-) ppm. MALDI-MS: m/z = 1452.586 [M+Na^+].

Synthesis of **(14)**

A solution of $CuSO_4 \cdot 5H_2O$ (0.287 g, 0.00115 mol) in water (10 mL) was added to a round bottom flask containing a stirred solution of **(13)** (0.990 g,

0.000692 mol) and **(5)** (1.11 g, 0.00456 mol) in THF (30 mL). Sodium ascorbate (0.133 g, 0.00671 mol) was added and the mixture was allowed to react overnight. The product mixture was evaporated, and then extracted to remove the copper. It was dried with $MgSO_4$ and the solvent evaporated. The resulting oil was then purified by column chromatography (100%, Ethyl Acetate, followed by 10% methanol) to yield a yellow oil (1.55 g, 0.000535 mol, 77% yield). ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.02 (s, 18H, CH_3-C-O-CH_2-C-C$\underline{H}_3$), 1.10 (s, 9H, C-C-O-CH_2-C-C$\underline{H}_3$), 1.28(s, 18H, C$\underline{H}_3$-C-O-CH_2-C-CH_3), 1.39 (s, 18H, C$\underline{H}_3$-C-O-CH_2-C-CH_3), 2.68 (t, 12H, -O-C-CH_2-C$\underline{H}_2$-), 2.94 (t, 12H, -O-C-C$\underline{H}_2$-CH_2-), 3.37 (s, C-C$\underline{H}_2$-O-CH_2-C-CH, 2H),3.43 (s, 6H, -C-C$\underline{H}_2$-O-CH_2-C-N-), 3.62 (dd, 12H, -C-C$\underline{H}_2$-O-C-CH_2), 3.04 (dd, 12H, -C-C$\underline{H}_2$-O-C-CH_2), 4.09 (s, 12H, -C-C$\underline{H}_2$-O-C-CH_3), 4.41 (s, CH_2-O-C$\underline{H}_2$-C, 2H), 4.50 (m, 24H, -N-C$\underline{H}_2$-C$\underline{H}_2$-O-C-C-CH_2-O-C-CH_3), 4.68 (m, 18H, -O-C$\underline{H}_2$-C-CH-N-C$\underline{H}_2$-C$\underline{H}_2$-),7.14 (m, C-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-,5H), 7.56 (s, 3H, -O-CH_2-C-C$\underline{H}$-N-), 7.70 (s, 6H, -C-CH_2-CH_2-C-C$\underline{H}$-N-) ppm. $^{13}C\{^{1}H\}$ NMR (300MHz, CD_3OD): δ = 18.10 (-CH_2-C-O-CH_2-C-$\underline{C}H_3$), 18.68 (CH_3-C-O-CH_2-C-$\underline{C}H_3$), 21.85 (-O-C-$\underline{C}H_3$), 26.49 (-O-C-$\underline{C}H_3$), 34.24 (-O-C-CH_2-$\underline{C}H_2$-C-N-), 43.24 (CH_3-C-O-CH_2-$\underline{C}$-CH_3), 46.57 (-$\underline{C}$-CH_2-O-CH_2-C-N-), 47.67 (-O-C-$\underline{C}H_2$-CH_2-C-N-), 50.03 (-O-CH_2-C-CH-N-$\underline{C}H_2$-CH_2-), 50.17 (-CH_2-C-O-CH_2-$\underline{C}$-), 50.27 (-C-CH_2-CH_2-C-CH-N-$\underline{C}H_2$-), 64.15 (-C-CH_2-CH_2-C-CH-N-CH_2-$\underline{C}H_2$-), 64.48 (-O-CH_2-C-CH-N-CH_2-$\underline{C}H_2$-), 65.59 (-C-CH_2-O-$\underline{C}H_2$-C-N-), 66.51 (-CH_2-C-O-$\underline{C}H_2$-CH_3), 67.04 (CH_3-C-O-$\underline{C}H_2$-C-CH_3), 69.2 (C-$\underline{C}H_2$-O-CH_2-C-CH), 70.22 (-C-$\underline{C}H_2$-O-CH_2-C-N-),73.2 (C-$\underline{C}H_2$-O-CH_2-C-C), 99.44 (-O-$\underline{C}$-CH_3), 124.20 (-C-CH_2-CH_2-C-$\underline{C}$H-N-), 125.51 (-C-CH_2-O-CH_2-C-$\underline{C}$H-),127.3 (C-

CH-CH-), 127.6 (C-CH-CH-CH), 128.2 (C-CH-CH), 146.45 (-C-CH_2-O-CH_2-C-CH-), 147.69 (-C-CH_2-CH_2-C-CH-N-), 173.37 (-O-C-C-CH_2-O-C-CH_3), 173.68 (-C-C-CH_2-O-C-CH_2-), 175.31 (-O-C-CH_2-CH_2-C-) ppm. MALDI-MS: m/z = 1455.17489[M+ $2H^+$].

Synthesis of **(15)**

$BiCl_3$ (0.347 g, 0.00110 mol) and 25 drops of water were added to a stirred solution of **(14)** (1.55 g, 0.000535 mol) in methanol (25 mL). The reaction mixture was left stirring for 24 hours at 40°C under nitrogen. The product mixture was evaporated, dissolved in methanol, and filtered to remove the bismuth salts. The solvent was evaporated to yield a brown oil (1.33 g, 0.000500 mol, 94% yield). ^{1}H NMR (400MHz, CD_3OD): δ = 1.10 (s, 27H, -C-CH_3), 2.69 (t, 12H, -C-CH_2-CH_2-C-N-), 2.95 (t, 12H, -C-CH_2-CH_2-C-N-),3.37 (s, C-CH_2-O-CH_2-C-CH, 2H), 3.43 (s, 6H, -C-CH_2-O-CH_2-C-N-), 3.60 (q, 24H, -C-CH_2-OH), 4.10 (s, 12H, -C-CH_2-O-C-),4.41 (s, CH_2-O-CH_2-C, 2H), 4.48 (m, 24H, -N-CH_2-CH_2-O-C-C-CH_2-OH), 4.66 (m, 12H, -O-CH_2-C-CH-N-CH_2-CH_2-O-), 4.71 (s, 6H, -O-CH_2-C-CH-N-CH_2-CH_2-O-),7.14 (m, C-CH-CH-CH-CH-CH-,5H), 7.86 (s, 6H, -CH_2-CH_2-C-CH-N-), 8.03 (s, 3H, -O-CH_2-C-CH-N-) ppm. ^{13}C{^{1}H} NMR (300MHz, CD_3OD): δ = 17.41 (CH_3-C-CH_2-OH), 18.08 (-C-O-CH_2-C-CH_3), 34.18 (-O-C-CH_2-CH_2-C-N-), 46.53 (-C-CH_2-O-CH_2-), 47.65 (-O-C-CH_2-CH_2-C-N-), 49.99 (-O-CH_2-C-CH-N-CH_2-CH_2-), 50.17 (-CH_2-C-O-CH_2-C-), 50.34 (-C-CH_2-CH_2-C-CH-N-CH_2-), 51.79 (-C-CH_2-OH), 63.91 (-C-CH_2-CH_2-C-CH-N-CH_2-CH_2-), 64.45 (-O-CH_2-C-CH-N-CH_2-CH_2-), 65.51 (-C-CH_2-O-CH_2-C-N-), 65.86 (-C-CH_2-OH), 66.51 (-CH_2-C-O-CH_2-CH_3), 69.2 (C-CH_2-O-CH_2-C-

CH), 70.13 (-C-$\underline{C}H_2$-O-CH_2-C-N-), 124.35 (-C-CH_2-CH_2-C-$\underline{C}$H-N-),73.2 (C-$\underline{C}H_2$-O-CH_2-C-C), 125.61 (-C-CH_2-O-CH_2-C-$\underline{C}$H-),127.3 (C-$\underline{C}$H-CH-), 127.6 (C-CH-CH-$\underline{C}$H), 128.2 (C-CH-$\underline{C}$H), 146.37 (-C-CH_2-O-CH_2-$\underline{C}$-CH-), 147.58 (-C-CH_2-CH_2-$\underline{C}$-CH-N-), 173.47 (-O-$\underline{C}$-C-CH_2-O-C-CH_3), 173.70 (-$\underline{C}$-C-CH_2-O-C-CH_2-), 176.08 (-O-$\underline{C}$-CH_2-CH_2-C-) ppm. MALDI-MS: m/z = 1325.0802[M+ $2H^+$].

Synthesis of **(16)**

DMAP (0.86 g, 0.0070 mol) was added to a stirred mixture of **(15)** (1.03 g, 0.000387 mol) in anhydrous DMF (25 mL), under nitrogen. 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (2.36 g, 0.0123 mol), pyridine (12 mL) and 4-pentynoic acid (0.683 g, 0.00696 mol) were then added to the flask. The reaction mixture was left stirring, under nitrogen, overnight. The crude product mixture was washed with brine, dissolved in DCM, and precipitated with ether to yield the product as a brown oil (0.560 g, 0.000156 mol, 40% yield). ^{1}H NMR (400MHz, CD_3OD): δ = 1.10 (s, 27H, -C-C$\underline{H}_3$), 2.26 (t, 12H, -C-C$\underline{H}$), 2.46-2.53(m, 48H, -O-C-C$\underline{H}_2$-C$\underline{H}_2$-C-), 2.69 (t, 12H, -C-C$\underline{H}_2$-CH_2-C-N-), 2.95 (t, 12H, -C-CH_2-C$\underline{H}_2$-C-N-),3.37 (s, C-C$\underline{H}_2$-O-CH_2-C-CH, 2H), 3.43 (s, 6H, -C-C$\underline{H}_2$-O-CH_2-C-N-), 3.60 (q, 24H, -C-C$\underline{H}_2$-OH), 4.10 (s, 12H, -C-C$\underline{H}_2$-O-C-),4.41 (s, CH_2-O-C$\underline{H}_2$-C, 2H), 4.48 (m, 24H, -N-C$\underline{H}_2$-C$\underline{H}_2$-O-C-C-CH_2-OH), 4.66 (m, 12H, -O-C$\underline{H}_2$-C-CH-N-CH_2-C$\underline{H}_2$-O-), 4.71 (s, 6H, -O-CH_2-C-CH-N-C$\underline{H}_2$-CH_2-O-),7.14 (m, C-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-,5H), 7.86 (s, 6H, -CH_2-CH_2-C-C$\underline{H}$-N-), 8.03 (s, 3H, -O-CH_2-C-C$\underline{H}$-N-) ppm. $^{13}C\{^1H\}$ NMR (300MHz, CD_3OD): δ = 15.10 (-$\underline{C}H_2$-C-CH), 17.41 ($\underline{C}H_3$-C-CH_2-OH), 18.08 (-C-O-CH_2-C-$\underline{C}H_3$), 34.18 (-O-C-CH_2-$\underline{C}H_2$-C-N-), 34.31 (-$\underline{C}H_2$-CH_2-C-CH), 46.53 (-$\underline{C}$-CH_2-O-CH_2-), 47.65 (-O-C-$\underline{C}H_2$-

CH_2-C-N-), 49.99 (-O-CH_2-C-CH-N-$\underline{C}H_2$-CH_2-), 50.17 (-CH_2-C-O-CH_2-$\underline{C}$-), 50.34 (-C-CH_2-CH_2-C-CH-N-$\underline{C}H_2$-), 51.79 (-$\underline{C}$-CH_2-OH), 63.91 (-C-CH_2-CH_2-C-CH-N-CH_2-$\underline{C}H_2$-), 64.45 (-O-CH_2-C-CH-N-CH_2-$\underline{C}H_2$-), 65.51 (-C-CH_2-O-$\underline{C}H_2$-C-N-), 65.86 (-C-$\underline{C}H_2$-OH), 66.51 (-CH_2-C-O-$\underline{C}H_2$-CH_3), 69.2 (C-$\underline{C}H_2$-O-CH_2-C-CH), 70.13 (-C-$\underline{C}H_2$-O-CH_2-C-N-), 70.55 (-C-$\underline{C}$H), 83.54 (-$\underline{C}$-CH), 124.35 (-C-CH_2-CH_2-C-$\underline{C}$H-N-),73.2 (C-$\underline{C}H_2$-O-CH_2-C-C), 125.61 (-C-CH_2-O-CH_2-C-$\underline{C}$H-),127.3 (C-$\underline{C}$H-CH-), 127.6 (C-CH-CH-$\underline{C}$H), 128.2 (C-CH-$\underline{C}$H), 146.37 (-C-CH_2-O-CH_2-$\underline{C}$-CH-), 147.58 (-C-CH_2-CH_2-$\underline{C}$-CH-N-), 172.84 (-O-$\underline{C}$-CH_2-), 173.47 (-O-$\underline{C}$-C-CH_2-O-C-CH_3), 173.70 (-$\underline{C}$-C-CH_2-O-C-CH_2-), 176.08 (-O-$\underline{C}$-CH_2-CH_2-C-) ppm. MALDI-MS: m/z = 1812.025[M+ $2H^+$].

Synthesis of Dendron Functionalization

Synthesis of Azidophosponate **(18)**

Sodium azide (10.6 g, 163 mmol) was added to a solution of 2-bromoethylphosphonate **(17)** (10.4 g, 42.4 mmol) in water (50 mL) and the reaction mixture was stirred for 24 hours at 65°C. An extraction was performed with dichloromethane, the organic phase was dried with $MgSO_4$, filtered and the residue obtained was a yellow oil (8.03 g, 0.0387 mol, 92% yield). ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.18 (t, P-CH_2-C$\underline{H}_3$, 6H), 1.91 (m, P-CH_2-C$\underline{H}_2$, 2H), 3.38 (m, P-C$\underline{H}_2$-CH_2, 2H), 3.97 (m, P-C$\underline{H}_2$-CH_3, 4H) ppm. ^{13}C{^{1}H} NMR (300MHz, $CDCl_3$) δ = 16.27 (d, P-CH_2-$\underline{C}H_3$), 25.8 (d, P-$\underline{C}H_2$-CH_3), 45.2 (d, P-$\underline{C}H_2$-CH_2-), 61.7 (d, P-CH_2-$\underline{C}H_2$-) ppm. ^{31}P{^{1}H} NMR (200MHz, $CDCl_3$) δ = 26.65 (-P-Et) ppm.

Synthesis of **(19)**

A solution of $CuSO_4 \cdot 5H_2O$ (0.23 g, 0.00092 mol) in water (3.5 mL) was added to a round bottom flask containing a stirred solution of **(10)** (0.497 g, 0.00146 mol) and azidophosphonate (1.10 g, 0.00451 mol) in THF (13 mL). Sodium ascorbate (0.348 g, 0.00175 mol) was then added and the mixture was allowed to react overnight. The product was purified by column chromatography (100% Dichloromethane, followed by 10% methanol) to yield an orange oil (1.411 g, 0.001467 mol, 99% yield). ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.19 (t, P-CH_2-C$\underline{H}_3$, 18H), 2.311 (m, P-C$\underline{H}_2$-CH_2, 6H), 3.48 (s, C-C$\underline{H}_2$-O-C-, 2H) 3.59 (s, C-C$\underline{H}_2$-O-,6H), 3.99 (m, P-C$\underline{H}_2$,-CH_3, 12H), 4.30 (s, CH_2-O-C$\underline{H}_2$-C, 2H), 4.51 (s, CH-C-C$\underline{H}_2$- , 6H), 4.55 (s, P-CH_2-C$\underline{H}_2$, 6H), 7.34 (m, C-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-,5H), 7.60 (s, -C-C$\underline{H}$-N-, 1H) ppm. ^{13}C{^{1}H} NMR (300MHz, $CDCl_3$) δ = 16.32 ($\underline{C}H_3$-CH_2-P, d), 27.01 (CH_2-$\underline{C}H_2$-P, d), 44.6 ($\underline{C}$-CH_2-O-), 45.25 (CH_2-$\underline{C}H_2$-P, d), 58.7 (CH-C-$\underline{C}H_2$), 62.01 (CH_3-$\underline{C}H_2$-P, d), 69.1 (C-$\underline{C}H_2$-O-CH_2-C), 69.2 (C-$\underline{C}H_2$-O-CH_2-C-CH), 73.2 (C-$\underline{C}H_2$-O-CH_2-C-C), 74.1 ($\underline{C}$H-C-CH_2), 80.7 (CH-$\underline{C}$-CH_2), 124.07 (-C-$\underline{C}$H-N-), 127.3 (C-$\underline{C}$H-CH-), 127.6 (C-CH-CH-$\underline{C}$H), 128.2 (C-CH-$\underline{C}$H), 138.8 ($\underline{C}$-CH-CH), 145.00 (-$\underline{C}$-N-), ppm. ^{31}P{^{1}H} NMR (200MHz, $CDCl_3$) δ = 25.60 (-P-Et) ppm. MALDI-MS: m/z = 962.404 [$M+H^+$].

Synthesis of **(20)**

In a round bottom flask, **(19)** (0.250 g, 0.000260 mol) and bromotrimethylsilane (0.524 g, 0.00333 mol) were stirred in DCM (5 mL) under nitrogen for 48 hours. Afterwards, all volatile units were removed under vacuum. DCM (5 mL) and 1M KOH were then added and the mixture was stirred for 2 hours. Finally, the DCM was removed by vacuum and the pH was adjusted to 9

with HCl, and once again all volatile molecules were removed under vacuum to produce a solid brown sticky product (0.497 g, 0.000487 mol). The yield was well over 100% due to water being present, which, due to the dendritic nature of the molecule, was difficult to remove. ^{1}H NMR (400MHz, D_2O): δ = 2.311 (m, P-C$\underline{H_2}$-CH_2, 6H), 3.48 (s, C-C$\underline{H_2}$-O-C-, 2H) 3.59 (s, C-C$\underline{H_2}$-O-,6H), 4.30 (s, CH_2-O-C$\underline{H_2}$-C, 2H), 4.51 (s, CH-C-C$\underline{H_2}$- , 6H), 4.55 (s, P-CH_2-C$\underline{H_2}$, 6H), 7.34 (m, C-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-,5H), 7.60 (s, -C-C$\underline{H}$-N-, 1H) ppm. ^{13}C{^{1}H} NMR (300MHz, D_2O) δ = 27.01 (CH_2-$\underline{C}H_2$-P, d), 44.6 ($\underline{C}$-CH_2-O-), 45.25 (CH_2-$\underline{C}H_2$-P, d), 58.7 (CH-C-$\underline{C}H_2$), 69.1 (C-$\underline{C}H_2$-O-CH_2-C), 69.2 (C-$\underline{C}H_2$-O-CH_2-C-CH), 73.2 (C-$\underline{C}H_2$-O-CH_2-C-C), 74.1 ($\underline{C}$H-C-CH_2), 80.7 (CH-$\underline{C}$-CH_2), 124.07 (-C-$\underline{C}$H-N-), 127.3 (C-$\underline{C}$H-CH-), 127.6 (C-CH-CH-$\underline{C}$H), 128.2 (C-CH-$\underline{C}$H), 138.8 ($\underline{C}$-CH-CH), 145.00 (-$\underline{C}$-N-), ppm. ^{31}P{^{1}H} NMR (200MHz, D_2O) δ = 17.41 (-P-K^+) ppm. MALDI-MS: m/z = 894.3416 [M+Na^+].

Synthesis of **(21)**

A solution of $CuSO_4 \cdot 5H_2O$ (0.50 g, 0.00185 mol) in water (10 mL) was added to a round bottom flask containing a stirred solution of **(13)** (0.849 g, 0.000594 mol) and azidophosphonate (1.10 g, 0.00451 mol) in THF (25 mL). Sodium ascorbate (0.702 g, 0.00354 mol) was then added and the mixture was allowed to react overnight under nitrogen. The product was purified by column chromatography (100%, Dichloromethane, followed by 10% methanol), then precipitated with ether, to yield an orange oil (0.336 g, 0.000130 mol, 22% yield). ^{1}H NMR (400MHz, CD_3OD): δ = 1.19(s, 9H, -C-C$\underline{H_3}$), 1.29 (t, P-CH_2-C$\underline{H_3}$, 36H), 2.311 (m, P-C$\underline{H_2}$-CH_2, 6H), 2.69(s, 12H, -O-C-CH_2-C$\underline{H_2}$-C-), 2.93(s, 12H, -

O-C-C$\underline{H_2}$-CH_2-C-),3.44 (s, C-C$\underline{H_2}$-O-CH_2-C-CH, 2H), 3.49(s, 6H, -C-C$\underline{H_2}$-O-CH_2-), 3.99 (m, P-C$\underline{H_2}$,-CH_3, 24H), 4.00(s, 12H, -C-(C$\underline{H_2}$-O-C-)$_2$), 4.41 (s, CH_2-O-C$\underline{H_2}$-C, 2H), 4.51 (t, -N-CH_2-C$\underline{H_2}$-O-, 6H), 4.55 (s, P-CH_2-C$\underline{H_2}$, 12H), 4.55 (s, -O-C$\underline{H_2}$-C-N-, 6H),4.67(t, 6H, -C-CH-N-C$\underline{H_2}$-),7.14 (m, C-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-,5H), 7.31 (s, 6H, -N-C$\underline{H}$-), 8.01 (s, 3H, -N-C$\underline{H}$-) ppm. $^{13}C\{^1H\}$ NMR (300MHz, CD_3OD): δ = 16.32 (C$\underline{H_3}$-CH_2-P, d), 17.10 (-C$\underline{H_2}$-C-CH), 20.20 (-C-C$\underline{H_3}$), 27.01 (CH_2-C$\underline{H_2}$-P, d), 34.31 (-C$\underline{H_2}$-CH_2-C-CH), 45.25 (CH_2-C$\underline{H_2}$-P, d), 46.57(-C-CH_2-O-CH_2-C-N-), 47.70(-C-CH_3), 50.22(-N-C$\underline{H_2}$-CH_2-O-), 62.01 (CH_3-C$\underline{H_2}$-P, d), 64.51(-N-CH_2-C$\underline{H_2}$-O-), 65.59 (-C-CH_2-O-C$\underline{H_2}$-C-N-), 66.61 (-C-C$\underline{H_2}$-O-C-), 69.2 (C-C$\underline{H_2}$-O-CH_2-C-CH), 70.19 (-C-C$\underline{H_2}$-O-CH_2-C-N-), 70.55 (-C-C$\underline{H}$), 73.2 (C-C$\underline{H_2}$-O-CH_2-C-C), 83.54 (-C-CH), 125.49 (-C-C$\underline{H}$-N-),127.3 (C-C$\underline{H}$-CH-), 127.6 (C-CH-CH-C$\underline{H}$), 128.2 (C-CH-C$\underline{H}$), 146.51 (-C-CH-N-), 172.84 (-O-C-CH_2-), 173.72 (O-C-C-) ppm. $^{31}P\{^1H\}$ NMR (200MHz, $CDCl_3$) δ = 25.66 (-P-Et) ppm. MALDI-MS: m/z = 2694.913 [$M+Na^+$].

Synthesis of **(22)**

In a round bottom flask, **(21)** (0.0240 g, 0.00000927 mol) and bromotrimethylsilane (0.060 g, 0.000381 mol) were stirred in DCM (5 mL) under nitrogen for 48 hours. Afterwards, all volatile compounds were removed under vacuum. DCM (5 mL) and 1M KOH were added, and the mixture was stirred for 2 hours. Finally, the DCM was removed by vacuum and the pH was adjusted to 9 with HCl, and once again all volatile compounds were removed under vacuum to produce a solid brown sticky product (0.0319 g, 0.0000118 mol) The yield was well over 100% due to water being present, which, due to the dendritic nature of

the molecule, was difficult to remove. ^{1}H NMR (400MHz, D_2O): δ = 1.19(s, 9H, -C-C$\underline{H}_3$), 2.311 (m, P-C$\underline{H}_2$-CH_2, 6H), 2.69(s, 12H, -O-C-CH_2-C$\underline{H}_2$-C-), 2.93(s, 12H, -O-C-C$\underline{H}_2$-CH_2-C-),3.44 (s, C-C$\underline{H}_2$-O-CH_2-C-CH, 2H), 3.49(s, 6H, -C-C$\underline{H}_2$-O-CH_2-), 4.00(s, 12H, -C-(C$\underline{H}_2$-O-C-)$_2$), 4.41 (s, CH_2-O-C$\underline{H}_2$-C, 2H), 4.51 (t, -N-CH_2-C$\underline{H}_2$-O-, 6H), 4.55 (s, P-CH_2-C$\underline{H}_2$, 12H), 4.55 (s, -O-C$\underline{H}_2$-C-N-, 6H),4.67(t, 6H, -C-CH-N-C$\underline{H}_2$-),7.14 (m, C-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-,5H), 7.31 (s, 6H, -N-C$\underline{H}$-), 8.01 (s, 3H, -N-C$\underline{H}$-) ppm. ^{31}P{^{1}H} NMR (200MHz, D_2O) δ = 18.21 (-P-K) ppm. Carbon and MS difficult due to salt content.

Synthesis of **(23)**

A solution of $CuSO_4 \cdot 5H_2O$ (0.33 g, 0.00122 mol) in water (7 mL) was added to a round bottom flask containing a stirred solution of **(16)** (0.560 g, 0.000156 mol) and azidophosphonate (0.501 g, 0.00242 mol) in THF (17 mL). Sodium ascorbate (0.462 g, 0.00233 mol) was added and the mixture was allowed to react overnight under nitrogen. The product was then washed with brine and EDTA, and finally precipitated from DCM in, to yield an brown oil (0.367 g, 0.0000602 mol, 39% yield). ^{1}H NMR (400MHz, CD_3OD): δ = 1.10 (s, 27H, -C-C$\underline{H}_3$), 1.29 (t, P-CH_2-C$\underline{H}_3$, 72H), 2.311 (m, P-C$\underline{H}_2$-CH_2, 12H), 2.69 (t, 24H, -C-C$\underline{H}_2$-CH_2-C-N-), 2.95 (t, 24H, -C-CH_2-C$\underline{H}_2$-C-N-),3.37 (s, C-C$\underline{H}_2$-O-CH_2-C-CH, 2H), 3.43 (s, 6H, -C-C$\underline{H}_2$-O-CH_2-C-N-), 3.60 (q, 24H, -C-C$\underline{H}_2$-OH), 3.99 (m, P-C$\underline{H}_2$,-CH_3, 48H), 4.10 (s, 12H, -C-C$\underline{H}_2$-O-C-),4.41 (s, CH_2-O-C$\underline{H}_2$-C, 2H), 4.48 (m, 24H, -N-C$\underline{H}_2$-C$\underline{H}_2$-O-C-C-CH_2-OH), 4.55 (s, P-CH_2-C$\underline{H}_2$, 24H), 4.66 (m, 12H, -O-C$\underline{H}_2$-C-CH-N-CH_2-C$\underline{H}_2$-O-), 4.71 (s, 6H, -O-CH_2-C-CH-N-C$\underline{H}_2$-CH_2-O-),7.14 (m, C-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-,5H), 7.31 (s, 12H, -N-C$\underline{H}$-), 7.86 (s, 6H, -CH_2-

CH_2-C-C<u>H</u>-N-), 8.03 (s, 3H, -O-CH_2-C-C<u>H</u>-N-) ppm. $^{13}C\{^1H\}$ NMR (300MHz, CD_3OD): δ = 15.10 (-<u>C</u>H_2-C-CH), 16.32 (<u>C</u>H_3-CH_2-P, d), 17.41 (<u>C</u>H_3-C-CH_2-OH), 18.08 (-C-O-CH_2-C-<u>C</u>H_3), 27.01 (CH_2-<u>C</u>H_2-P, d), 34.18 (-O-C-CH_2-<u>C</u>H_2-C-N-), 34.31 (-<u>C</u>H_2-CH_2-C-CH), 45.25 (CH_2-<u>C</u>H_2-P, d), 46.53 (-<u>C</u>-CH_2-O-CH_2-), 47.65 (-O-C-<u>C</u>H_2-CH_2-C-N-), 49.99 (-O-CH_2-C-CH-N-<u>C</u>H_2-CH_2-), 50.17 (-CH_2-C-O-CH_2-<u>C</u>-), 50.34 (-C-CH_2-CH_2-C-CH-N-<u>C</u>H_2-), 51.79 (-<u>C</u>-CH_2-OH), 62.01 (CH_3-<u>C</u>H_2-P, d), 63.91 (-C-CH_2-CH_2-C-CH-N-CH_2-<u>C</u>H_2-), 64.45 (-O-CH_2-C-CH-N-CH_2-<u>C</u>H_2-), 65.51 (-C-CH_2-O-<u>C</u>H_2-C-N-), 65.86 (-C-<u>C</u>H_2-OH), 66.51 (-CH_2-C-O-<u>C</u>H_2-CH_3), 69.2 (C-<u>C</u>H_2-O-CH_2-C-CH), 70.13 (-C-<u>C</u>H_2-O-CH_2-C-N-), 70.55 (-C-<u>C</u>H), 83.54 (-<u>C</u>-CH), 124.35 (-C-CH_2-CH_2-C-<u>C</u>H-N-),73.2 (C-<u>C</u>H_2-O-CH_2-C-C), 125.61 (-C-CH_2-O-CH_2-C-<u>C</u>H-),127.3 (C-<u>C</u>H-CH-), 127.6 (C-CH-CH-<u>C</u>H), 128.2 (C-CH-<u>C</u>H), 146.37 (-C-CH_2-O-CH_2-<u>C</u>-CH-), 147.58 (-C-CH_2-CH_2-<u>C</u>-CH-N-), 172.84 (-O-<u>C</u>-CH_2-), 172.99 (-O-<u>C</u>-CH_2-), 173.47 (-O-<u>C</u>-C-CH_2-O-C-CH_3), 173.70 (-<u>C</u>-C-CH_2-O-C-CH_2-), 176.08 (-O-<u>C</u>-CH_2-CH_2-C-) ppm. $^{31}P\{^1H\}$ NMR (200MHz, $CDCl_3$) δ = 25.56 (-P-Et) ppm. MALDI-MS: m/z = 2032.127[M+ $3H^+$].

<u>Synthesis of **(24)**</u>

In a round bottom flask, **(23)** (0.090 g, 0.0000148 mol) and bromotrimethylsilane (0.25 g, 0.00159 mol) were stirred in DCM (10 mL) under nitrogen for 48 hours. Afterwards, all volatile compounds were removed under vacuum. DCM (5 mL), and 1M KOH was added and the mixture was stirred for 2 hours. Finally, DCM was removed in vacuo and the pH was adjusted to 9 with HCl, and once again all volatile compounds were removed under vacuum to

produce a solid brown sticky product (0.578 g, 0.0000913 mol). The yield was well over 100% due to water being present, which, due to the dendritic nature of the molecule, was difficult to remove. ^{1}H NMR (400MHz, CD_3OD): δ = 1.10 (s, 27H, -C-C$\underline{H}_3$), 2.311 (m, P-C$\underline{H}_2$-CH_2, 12H), 2.69 (t, 24H, -C-C$\underline{H}_2$-CH_2-C-N-), 2.95 (t, 24H, -C-CH_2-C$\underline{H}_2$-C-N-),3.37 (s, C-C$\underline{H}_2$-O-CH_2-C-CH, 2H), 3.43 (s, 6H, -C-C$\underline{H}_2$-O-CH_2-C-N-), 3.60 (q, 24H, -C-C$\underline{H}_2$-OH), 4.10 (s, 12H, -C-C$\underline{H}_2$-O-C-),4.41 (s, CH_2-O-C$\underline{H}_2$-C, 2H), 4.48 (m, 24H, -N-C$\underline{H}_2$-C$\underline{H}_2$-O-C-C-CH_2-OH), 4.55 (s, P-CH_2-C$\underline{H}_2$, 24H), 4.66 (m, 12H, -O-C$\underline{H}_2$-C-CH-N-CH_2-C$\underline{H}_2$-O-), 4.71 (s, 6H, -O-CH_2-C-CH-N-C$\underline{H}_2$-CH_2-O-),7.14 (m, C-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-,5H), 7.31 (s, 12H, -N-C$\underline{H}$-), 7.86 (s, 6H, -CH_2-CH_2-C-C$\underline{H}$-N-), 8.03 (s, 3H, -O-CH_2-C-C$\underline{H}$-N-) ppm. MALDI-MS: m/z = 6123.828[$M+Na^+$].

Synthesis of Dendronized Polymers and Functionalization

Synthesis of **(25)**

A solution of $CuSO_4 \cdot 5H_2O$ (0.435 g, 0.00175 mol) in water (6.6 mL) was added to a round bottom flask containing a stirred solution of **(9)** (0.941 g, 0.00376 mol) and azidophosphonate (2.57 g, 0.0124 mol) in THF (24.2 mL). Sodium ascorbate (0.659 g, 0.00332 mol) was then added and the mixture was allowed to react overnight. The product was purified by column chromatography (100%, Dichloromethane, followed by 10% methanol) to yield an orange oil (2.25 g, 0.00258 mol, 69% yield). ^{1}H NMR (400MHz, $CDCl_3$): δ = 1.19 (t, P-CH_2-C$\underline{H}_3$, 18H), 2.311 (m, P-C$\underline{H}_2$-CH_2, 6H), 3.48 (s, C-C$\underline{H}_2$-O-C-, 2H) 3.59 (s, C-C$\underline{H}_2$-O-,6H), 3.99 (m, P-C$\underline{H}_2$,-CH_3, 12H), 4.51 (s, CH-C-C$\underline{H}_2$- , 6H), 4.55 (s, P-CH_2-C$\underline{H}_2$,

6H), 7.60 (s, -C-C<u>H</u>-N-, 1H) ppm. $^{31}P\{^{1}H\}$ NMR (200MHz, $CDCl_3$) δ = 25.60 (-P-Et) ppm.

Synthesis of **(26)**

A mixture of **(25)** (0.250 g, 0.000287 mol), poly(acrylic acid) [2000MW] (0.106 g, 0.0000531 mol) and DMAP (0.133 g, 0.0109 mol) were added to a round bottom flask under nitrogen. DMF (10 mL) was added, and the reaction was left to stir for 10 minutes. EDC was added last and the mixture was allowed to stir for 24 hours. Afterwards the mixture was precipitated with ether to remove the DMF, and then the product was purified by dialysis (MWCO = 1000Da, methanol) to yield a brown solid (0.0633 g, 18%yield). ^{1}H NMR (400MHz, CD_3OD): δ = 1.19 (t, P-CH_2-C<u>H</u>$_3$, 18H), 1.83 (m, Polymer, 15H), 2.311 (m, P-C<u>H</u>$_2$-CH_2, 6H), 2.71 (m, Polymer, 20H), 3.48 (s, C-C<u>H</u>$_2$-O-C-, 2H) 3.59 (s, C-C<u>H</u>$_2$-O-,6H), 3.99 (m, P-C<u>H</u>$_2$,-CH_3, 12H), 4.30 (s, CH_2-O-C<u>H</u>$_2$-C, 2H), 4.51 (s, CH-C-C<u>H</u>$_2$- , 6H), 4.55 (s, P-CH_2-C<u>H</u>$_2$, 6H), 7.60 (s, -C-C<u>H</u>-N-, 1H) ppm. $^{13}C\{^{1}H\}$ NMR (300MHz, CD_3OD) δ = 16.32 (<u>C</u>H_3-CH_2-P, d), 25.88 (Polymer), 27.01 (CH_2-<u>C</u>H_2-P, d), 42.1 (Polymer), 44.6 (<u>C</u>-CH_2-O-), 45.25 (CH_2-<u>C</u>H_2-P, d), 58.7 (CH-C-<u>C</u>H_2), 62.01 (CH_3-<u>C</u>H_2-P, d), 69.2 (C-<u>C</u>H_2-O-CH_2-C-CH), 73.2 (C-<u>C</u>H_2-O-CH_2-C-C), 74.1 (<u>C</u>H-C-CH_2), 80.7 (CH-<u>C</u>-CH_2), 124.07 (-C-<u>C</u>H-N), 145.00 (-<u>C</u>-N-), ppm. $^{31}P\{^{1}H\}$ NMR (200MHz, CD_3OD) δ = 27.14 (-P-Et) ppm.

Synthesis of **(27)**

In a round bottom flask, **(26)** (0.0300 g, 0.0000107 mol) and bromotrimethylsilane (0.0464 g, 0.000295 mol) were mixed in MeOH (5 mL) and stirred under nitrogen for 48 hours. Afterwards, all volatile units were removed

under vacuum. MeOH (5 mL) and 1M KOH was added and the mixture was stirred for 2 hours. Finally, MeOH was removed by vacuum and the pH was adjusted to 9 with HCl, and once again all volatile units were removed under vacuum to produce a solid brown sticky product (0.278 g, 0.000102 mol, <100%yield) *Yield over 100% due to water which, due to the dendritic nature of the molecule, was difficult to remove. ^{1}H NMR (400MHz, D_2O): δ = 1.83 (m, Polymer, 15H), 2.311 (m, P-C$\underline{H_2}$-CH_2, 6H), 2.71 (m, Polymer, 20H), 3.48 (s, C-C$\underline{H_2}$-O-C-, 2H) 3.59 (s, C-C$\underline{H_2}$-O-,6H), 4.30 (s, CH_2-O-C$\underline{H_2}$-C, 2H), 4.51 (s, CH-C-C$\underline{H_2}$- , 6H), 4.55 (s, P-CH_2-C$\underline{H_2}$, 6H), 7.34 (m, C-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-C$\underline{H}$-,5H), 7.60 (s, -C-C$\underline{H}$-N-, 1H) ppm. ^{13}C{^{1}H} NMR (300MHz, D_2O) δ = 25.88 (Polymer), 27.01 (CH_2-$\underline{C}H_2$-P, d), 42.1 (Polymer), 44.6 ($\underline{C}$-CH_2-O-), 45.25 (CH_2-$\underline{C}H_2$-P, d), 58.7 (CH-C-$\underline{C}H_2$), 69.1 (C-$\underline{C}H_2$-O-CH_2-C), 69.2 (C-$\underline{C}H_2$-O-CH_2-C-CH), 73.2 (C-$\underline{C}H_2$-O-CH_2-C-C), 74.1 ($\underline{C}$H-C-CH_2), 80.7 (CH-$\underline{C}$-CH_2), 124.07 (-C-$\underline{C}$H-N-), 127.3 (C-$\underline{C}$H-CH-), 127.6 (C-CH-CH-$\underline{C}$H), 128.2 (C-CH-$\underline{C}$H), 138.8 ($\underline{C}$-CH-CH), 145.00 (-$\underline{C}$-N-), ppm. ^{31}P {^{1}H} NMR (200MHz, D_2O) δ = 18.32 (-P-K^+) ppm. *Many of these peaks are difficult to see in ^{1}H NMR and ^{13}C {^{1}H} NMR due to the presence of water.

Synthesis of **(28)**

In a bomb flask, **(21)** (0.300 g, 0.000116 mol) and 5% Pd/C [50% water] (0.300g) were added with ethanol (8 mL). To this flask pressurized hydrogen was added up to 4 atm. The reaction was then left stirring for 24 hours. To remove the Pd/C the mixture was filtered several times, and the solvent was evaporated to yield an orange oil (0.045 g, 0.0000174 mol, 16%yield) ^{1}H NMR (400MHz,

$CDCl_3$): δ = 1.19(s, 9H, -C-C$\underline{H_3}$), 1.29 (t, P-CH_2-C$\underline{H_3}$, 36H), 2.311 (m, P-C$\underline{H_2}$-CH_2, 6H), 2.69(s, 12H, -O-C-CH_2-C$\underline{H_2}$-C-), 2.93(s, 12H, -O-C-C$\underline{H_2}$-CH_2-C-),3.44 (s, C-C$\underline{H_2}$-O-CH_2-C-CH, 2H), 3.49(s, 6H, -C-C$\underline{H_2}$-O-CH_2-), 3.99 (m, P-C$\underline{H_2}$,-CH_3, 24H), 4.00(s, 12H, -C-(C$\underline{H_2}$-O-C-)$_2$, 4.51 (t, -N-CH_2-C$\underline{H_2}$-O-, 6H), 4.55 (s, P-CH_2-C$\underline{H_2}$, 12H), 4.55 (s, -O-C$\underline{H_2}$-C-N-, 6H),4.67(t, 6H, -C-CH-N-C$\underline{H_2}$-), 7.31 (s, 6H, -N-C$\underline{H}$-), 7.74 (s, 3H, -N-C$\underline{H}$-) ppm. $^{13}C\{^1H\}$ NMR (300MHz, $CDCl_3$): δ = 16.32 (C$\underline{H_3}$-CH_2-P, d), 17.10 (-C$\underline{H_2}$-C-CH), 20.20 (-C-C$\underline{H_3}$), 27.01 (CH_2-C$\underline{H_2}$-P, d), 34.31 (-C$\underline{H_2}$-CH_2-C-CH), 45.25 (CH_2-C$\underline{H_2}$-P, d), 46.57(-C-CH_2-O-CH_2-C-N-), 47.70(-C-CH_3), 50.22(-N-C$\underline{H_2}$-CH_2-O-), 62.01 (CH_3-C$\underline{H_2}$-P, d), 64.51(-N-CH_2-C$\underline{H_2}$-O-), 65.59 (-C-CH_2-O-C$\underline{H_2}$-C-N-), 66.61 (-C-C$\underline{H_2}$-O-C-), 70.19 (-C-C$\underline{H_2}$-O-CH_2-C-N-), 70.55 (-C-C$\underline{H}$), 73.2 (C-C$\underline{H_2}$-O-CH_2-C-C), 83.54 (-C-CH), 125.49 (-C-C$\underline{H}$-N-), 146.51 (-C-CH-N-), 172.84 (-O-C-CH_2-), 173.72 (O-C-C-) ppm. $^{31}P\ \{^1H\}$ NMR (200MHz, $CDCl_3$) δ = 25.68 (-P-Et), 18.32 (-P-K^+) ppm. MALDI-MS: m/z = 2604.993 [M+Na^+].

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Chapter 3: An Evaluation of Phosphonate Terminated Dendrons and Dendronized Polymers for Inhibition of Biofilm Formation

3.1 – Introduction

Bacterial biofilms are highly structured, three dimensional communities of bacteria encased in an extra-cellular polymeric matrix. This matrix is comprised of a mixture of biomolecules secreted by bacteria, and is used to protect the cells against environmental threats and, in many cases, keep them bound to biotic and abiotic substrates.[1] Bacteria play a crucial role in many ecosystems, however some bacteria are quite harmful to human health.[2] Furthermore, biofilms can lead to economic loss in industrial settings due to corrosion, blockage of pipes and heat exchangers and contamination of final product. Bacterial infections are tackled with antibiotics in medical settings and with a variety of chemical and physical techniques in industrial settings, none of which are 100% effective towards bacteria in the form of a biofilm.[3] Biofilms have a heightened resistance towards antibiotics and other biocides, requiring higher doses which could adversely affect patients health or in the case of biocides in industrial settings, increase the cost of treatment and potentially have higher environmental impact.[4] Moreover, a portion of cells in the biofilm, known as persister cells, usually survive even high concentrations of biocides.[5] A huge body of research is focused on ways to prevent and/or disrupt biofilms. Shetye et al. reported that polyolderivatized

hydrocarbons prevented biofilm formation,[6] and Sasaki et al. reported that anionic polymers could inhibit the formation of biofilms.[7]

As discussed in chapters 1 and 2, there is a wide range of applications that dendrimers can be employed for, in a large part due to their ease of customization and high number of surface groups.[8-11] One of the goals of this project was to determine whether phosphonate terminated dendrons and dendronized polymers were effective for prevention and/ or dispersal of bacterial biofilms. We examined the potential of dendrons and dendronized polymers synthesized in chapter 2 in biofilm inhibition (generation 0 dendron **(20)**, generation 1 dendron **(22)**, and dendronized generation 0 polymer **(27)** are shown in Figure 3.1).

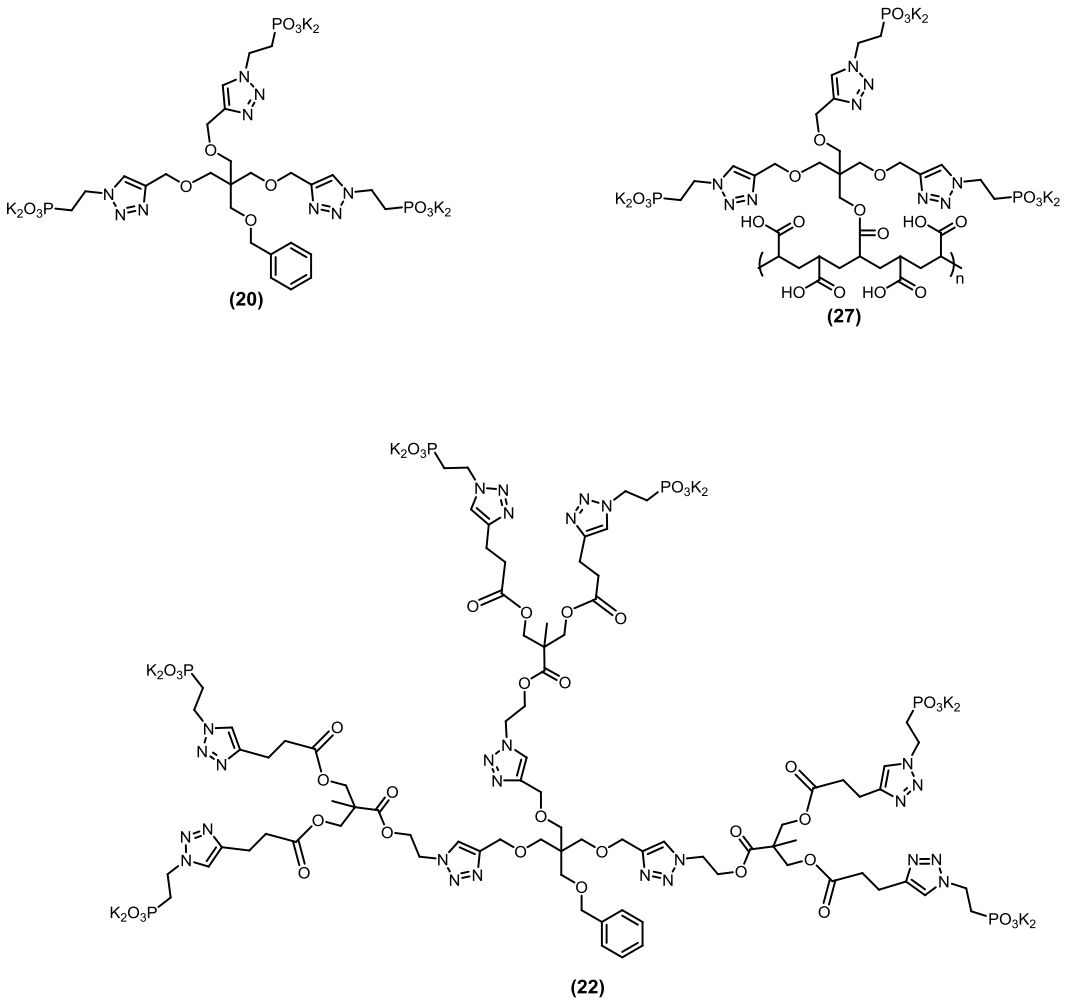

Figure 3.1–Dendrons and dendronized polymers tested for biofilm activity: generation 0 dendron (20), generation 1 dendron (22), and dendronized generation 0 polymer (27).

3.2 – Results and Discussion

To determine whether the dendrons were capable of inhibiting bacterial growth, bacteria were mixed with the dendrons at different concentrations and their growth was monitored for 24 hours. It is necessary to determine whether the dendrons were capable of inhibiting bacterial growth. If our dendrons do inhibit

bacterial growth, then the effect our dendrons have on the formation of biofilms could be as a result of this inhibition, as opposed to a direct effect of the dendrons on the inhibition of biofilm growth. Figure 3.2 shows the growth of bacteria for 24 hours measured by optical density at 600nm in the presence of generation 0 dendron **(20)**, generation 1 dendron **(22)**, and dendronized generation 0 polymer **(27)** at various concentrations. Our preliminary results on **(20)**, **(22)**, and **(27)** show that these macromolecules have no significant effect on the growth of the bacteria, as the rate of growth for the first 24 hours is unchanged compared to the control. This is not a negative result, as inhibiting biofilms and inhibiting bacteria are not necessarily the same process. As well, if our dendrons did inhibit bacterial growth, then any influence it has on the formation of biofilms will be a secondary effect of the dendron treatment. Thus the result of our dendrons having no effect on bacterial growth allows us to compare the effect of our dendrons on biofilm formation as a direct effect.

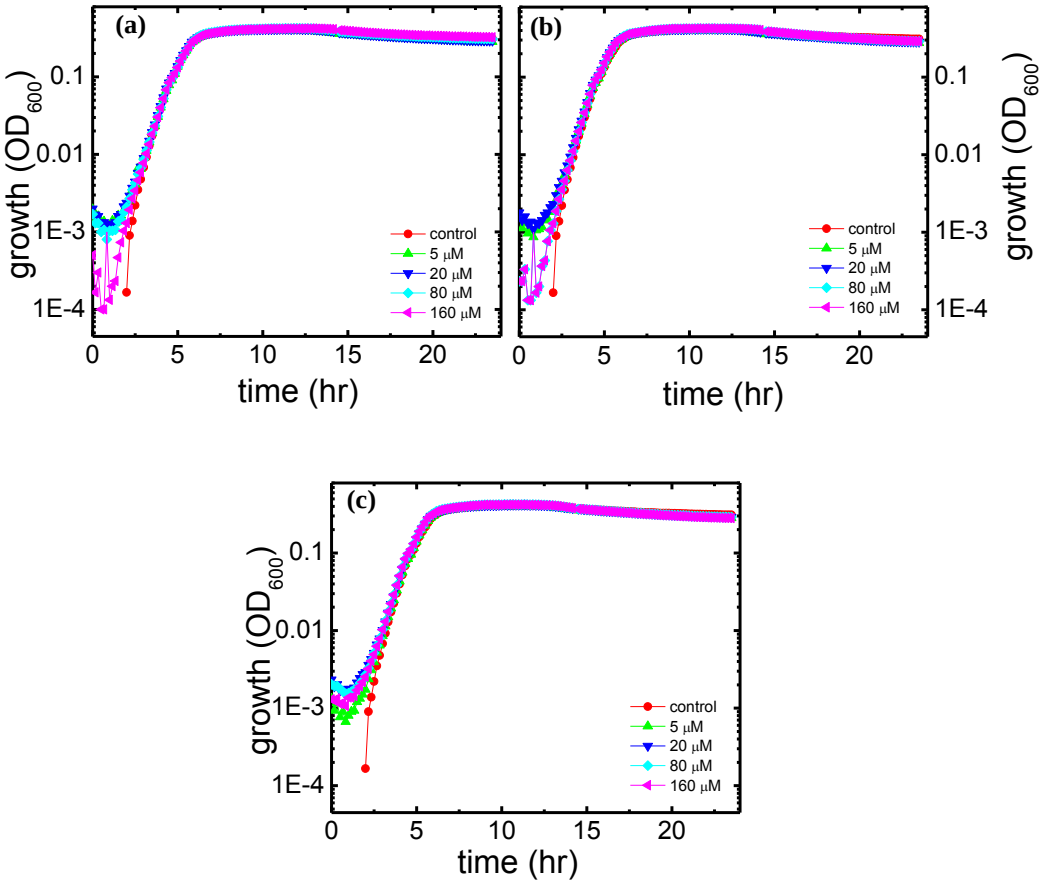

Figure 3.2–Growth curve for *E. coli* inoculated with generation 0 dendron (20), generation 1 dendron (22), and dendronized generation 0 polymer (27).

Since it was determined that the dendrons tested did not inhibit bacterial growth, tests were made to see whether the dendrons affected the ability of bacterial cells to form biofilms. For this purpose, bacterial biofilms were pre-treated and post-treated with the dendrons as explained in the methods section. The first test will determine whether the dendrons are capable of inhibiting the

growth of biofilm, while the second test will determine whether the dendrons have an effect on already grown biofilms.

Figure 3.3 shows the effect that the dendrons had on biofilm growth at various concentrations. At 5μM, generation 0 dendron **(20)** has no effect on biofilm growth, while dendronized generation 0 polymer **(27)** and generation 1 dendron **(22)** have only a minor effect neither of which is statistically significant. As the concentration was increased, however, the reduction in biofilm relative to the control is greatly increased. For generation 0 dendron **(20)** there is about half as much biofilm compared to the control though not statistically significant. Dendronized generation 0 polymer **(27)** and generation 1 dendron **(22)** performed much better, both having reached the detection limit for biofilms at 160μM. Generation 1 dendron **(22)** also had a very pronounced effect on biofilm prevention even at concentrations as low as 20μM (5.41E-3% dendron) where the biofilm level was 10% of the control. Dendronized generation 0 polymer **(27)** and generation 1 dendron **(22)** both had a significant effect on biofilm inhibition at 20, 80, and 160μM concentrations.

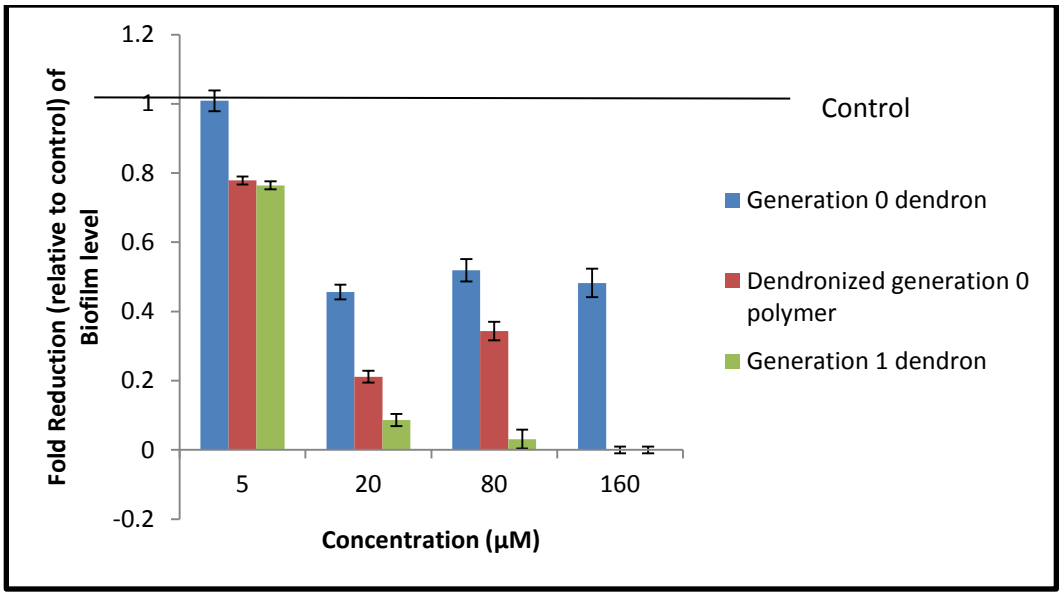

Figure 3.3–Growth of biofilm with dendrons at various concentrations (biofilm prevention).

Figure 3.4 shows the effect of dendrons on 4 day old biofilms. Interestingly, at low concentrations the amount of biofilm present had increased dramatically compared to the control for all dendrons. When the concentration was increased to 80µM the amount of biofilm was similar to that of the control. Lastly when the concentration was increased further to 160µM the amount of biofilm was lower than the control. None of these trials significantly reduced biofilm levels. All three dendrons performed similarly in this experiment, suggesting that there is similar mechanism for dendrons and dendronized polymers interacting with biofilms. This experiment was repeated a second time, and the amount of biofilm present at lower concentrations did not increase significantly. As well, generation 0 dendron **(20)** at 20µM had reduced the amount of biofilm significantly. This effect was also observed for dendronized generation 0 polymer **(27)** at 80µM.

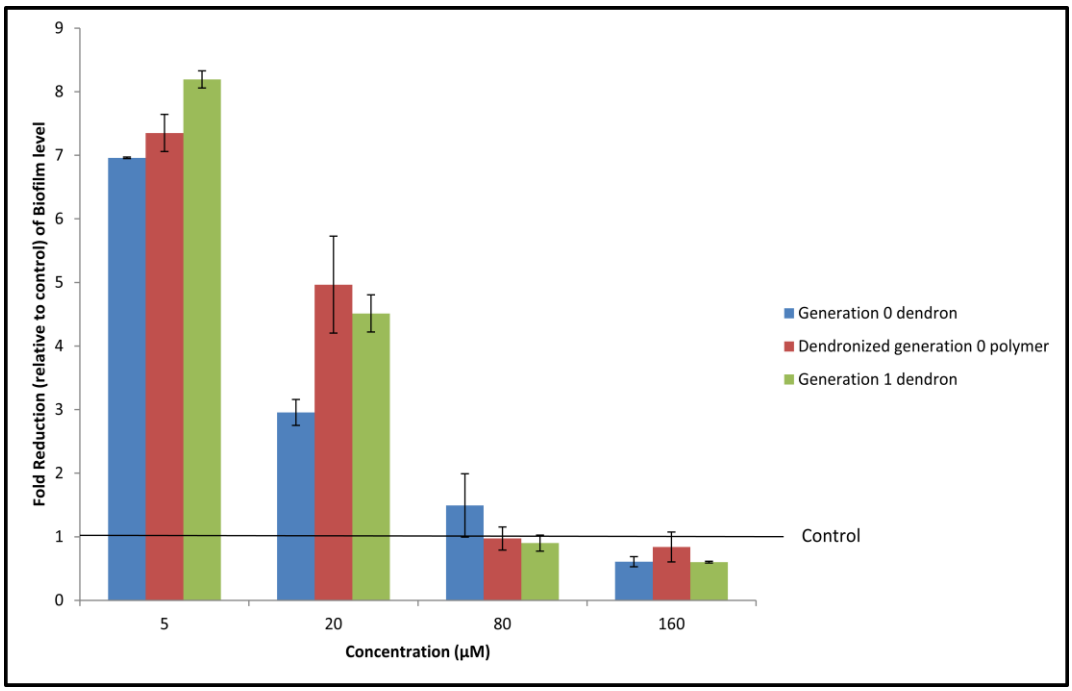

Figure 3.4–Effect of biofilm dendrons of various concentrations on a 4 day old biofilm.

3.3 – Conclusions

In conclusion, the dendrons and dendronized polymers were shown to have an effect on *E. coli* biofilms. The preliminary results indicate that the dendrons and dendronized polymers are much more effective at inhibiting biofilm growth (pre-treatment), as opposed to a post-treatment solution. The results also suggest that at higher concentrations (up to 160uM) these macromolecules are more effective at inhibiting biofilm growth. Lastly, the results indicate that the generation 1 dendron was the most effective, followed by the dendronized polymer, with the generation zero dendron being the least effective. Further

studies are under way to determine the efficacy of higher generation dendrons and dendronized polymers.

3.4 –Experimental

Culture of bacteria

The strain used for this biofilm study was *Escherichia coli* B. To initiate the bacterial culture, an inoculum from a frozen glycerol stock of *E. coli* B was streaked onto a trypticase soy agar plate and incubated overnight at 37 °C. A single colony from the plate was inoculated into 10 mL of trypticase soy broth (TSB), and incubated overnight (37 °C, 120 rpm), from which a 200 μL aliquot was diluted 1:100 in fresh TSB and grown to an OD_{600} of 0.2–0.3.

Bacterial growth curves

The bacterial strain was grown overnight in TSB (37 °C, 120 rpm) from a single colony picked from an agar plate of no more than three days old. This culture was diluted 1:100 in TSB and 100 μLs of it loaded into the wells of an untreated 96-well polystyrene flat-bottomed microtiter plate (Costar; Corning Inc, Corning, NY, USA. Dendrons were added (1:1) to each well. Four dendron concentrations were used (5, 20, 80, 160 uM). Each treatment was applied to 3 wells in each row. Bacteria in control wells were mixed with 0.95% saline instead of dendrons. Bacterial growth in the wells was monitored for 24 hours by measuring the OD_{600} value using a Tecan microplate reader.

An in vitro model for biofilm development

A microtiter plate assay[12] was used to grow and study the biofilms. *E. coli* B was grown overnight in TSB (37 °C, 120 rpm) from a single colony picked from an agar plate of no more than three days old. This culture was diluted 1:100 in TSB and loaded into the wells of an untreated 96-well polystyrene flat-bottomed microtiter plate (Costar; Corning Inc, Corning, NY, USA). The plates were then incubated at 37 °C under static conditions. The biofilms were pretreated or post-treated with dendrons (see below). Firstly, the planktonic bacteria in the wells were transferred to a fresh microtiter plate and their OD_{600} value was measured. The wells were then washed three times with phosphate-buffered saline (PBS) and stained with 1% crystal violet for quantification of the total attached biomass. The OD_{570} value of the anhydrous ethanol subsequently used to dissolve the crystal violet was used as a quantitative measure of the biofilm. The experiments described below were all performed in duplicate.

Inhibition of biofilm formation using dendron (pre-treatment)

To investigate the ability of the dendrons to inhibit the formation of biofilms, the dendrons were added to the wells of the multiwell plate simultaneously with the bacterial inoculums at t = 0. Each dendron treatment was repeated in duplicate. Dendron buffer (0.95 %, pH 7) was added to the control wells (n = 3). Four dendron concentrations were used (5, 20, 80, 160 uM). Each treatment was applied to 3 wells in each row. The biofilm was allowed to develop in the presence of the dendron for a specified period of time (96 hours), after which the biofilm was quantified as detailed above.

Challenging mature biofilms (post-treatment)

The efficacy of the dendrons at eradicating a mature biofilm was investigated by challenging a biofilm grown for 96 hours. The biofilm was inoculated as described above and allowed to develop for 96 hours, after which the planktonic bacteria were removed, the wells were washed (x3) with PBS and the dendrons were added to the wells; three wells were used for each treatment. The biofilm was challenged with the dendron for a specified period of time (24 hours), after which the biofilm level was quantified.

Statistical analysis

All assays were repeated a minimum of two times with triplicates for each sample in each experiment. Results are reported as means ± 95% confidence intervals. The significance of the difference between levels of biofilm was analyzed using the Student's t-test, (Statistica 8.0, Stat Soft. Inc., San Jose, CA) and p-values < 0.05 were considered significant.

3.5 – References

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Chapter 4: Conclusions

4.1 - Summary and Conclusions

Dendrimers and dendronized polymers are unique macromolecules with novel characteristics, and have been used for a variety of applications. An area that has been largely unexplored and in which dendrimers and dendronized polymers can play a significant role is in the pulp and paper industry. In order to examine their potential in biofilm inhibition, it is essential that two parameters are met by these macromolecules; they must be water soluble, and the dendrons must have a biofilm active moiety attached at the periphery. Both of these goals can be achieved by introducing a phosphonate group at the periphery.

The goals of this project included the development of a synthetic pathway to dendrons and dendronized polymers which are both efficient and versatile, as well as examine their potential in biofilm inhibition. A combination of Sharpless' copper(I) catalysed alkyne azide coupling reaction and Steglich esterification reactions were efficiently used to synthesize dendrons with a protected core. The versatility of these reactions was demonstrated by the synthesis, as well as the functionalization of the dendrons and dendronized polymers. The series of reactions carried out successfully led to the synthesis of dendrons with multiple surface groups, as well as their functionalization to linear polymers to yield dendronized polymers. We chose phosphonate terminal groups for introducing water solubility, as well as enhancing their efficacy as biofilm inhibitors.

The dendrons and dendronized polymers were shown to have an effect on *E. coli* biofilms. The results suggested that at higher concentrations all the dendrons and dendronized polymers tested have a significant effect on biofilm formation, and that larger macromolecules are effective at disrupting biofilms.

The results presented in this thesis highlight the importance of intelligent design choice in selection of both the core molecule as well as protecting groups in building dendrons. The core molecule in this dendron was required in order to create the asymmetry necessary for attachment to a polymer. The protecting groups showcase how reactive sites can be selectively utilized to synthesize an asymmetric dendron. We also demonstrate that "click" chemistry is a useful tool for the synthesis of dendrons and dendronized polymers, as well as their post functionalization. This thesis also highlights the graft-to method as an efficient route to synthesize dendronized polymers.

4.2 – Future Outlook

Synthesis of dendrons from generation zero to two was successful, and trials were done on biofilms to understand their biofilm activity. Another application that the pulp and paper industry is interested in is in developing antiscalants. The phosphonate terminated dendrons and dendronized polymers could have antiscalant properties, and tests to determine their efficacy in this regard would be essential to determine the structure property relationship of dendrons and dendronized polymers as antiscalants.

While studies were done on biofilms to determine the efficacy of dendrons and dendronized polymers at inhibiting biofilm formation, they were only tested on three molecules, and at four different concentrations. Three more dendrons, generation 1 dendronized polymer, generation 2 dendron, and generation 2 dendronized polymer, should be tested to determine if they can inhibit biofilm formation, and how they compare to the other molecules. Additionally, these macromolecules could be tested at many more concentrations, and against many other strains of bacteria, to see the full scope these dendrons and dendronized polymers for biofilm inhibition.

Lastly, the synthetic scheme proposed here incorporates only one type of dendron to a polymer using the graft-to method. It would be interesting to see how other dendron types affect the ability of the dendrons to interact with biofilms. As well, many more polymers exist that can readily be coupled to dendrons. These polymers can impart their own properties, which may as well interact with the biofilm formation. For example, poly(vinyl alcohol) could couple to dendrons, and has very different properties compared to poly(acrylic acid). During the synthesis of the core of the dendron, a two armed core was made. This core could be built up in a similar way to the current synthesis shown here, but upon dendronization would have two free hydroxyl reactive sites. It would be interesting to see what effect it would have on its properties if this dendron could polymerize.