The Design and Synthesis of Dendrimers for Applications in the Pulp & Paper Industry

by

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Master of Science

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I would also like to thank Dr. Theo van de Ven and his entire group for their help in bringing the pulp and paper side of my project to fruition. Without their invaluable assistance the bactericidal testing of my dendrimers would have been impossible. Dr. van de Ven has always been a gracious and generous supervisor, and I genuinely appreciated the yearly parties thrown at his charming home.

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Dendrimers find applications in many diverse fields including biology, catalysis, the pulp and paper industry, etc. The distinctive properties of dendrimers leading to their wide applicability include tailorability and high surface group density. In this thesis the design and synthesis of water soluble dendrimers for potential applications as anti-scalants and bactericides, two important areas within the pulp and paper industry, are explored. The dendrimers were constructed on a tetrafunctional core which allows us to obtain a higher number of end groups at a lower generation than in the bi- and trifunctional cores generally employed. We utilized “click” chemistry, or more specifically the copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) reaction, for both dendrimer synthesis and post-functionalization. The implementation of this methodology required the selection of appropriate acetylene- and azide-terminated building blocks. For this purpose we chose i) an acetylene-terminated molecule having a carboxylic acid end group; and ii) an azide-terminated building block with a diol group at its other end. Using a divergent synthetic methodology in which the azide unit was first linked to the tetrafunctional acetylene core, followed by an esterification with the carboxylic acid group of the acetylene-terminated building block, we constructed acetylene-terminated dendrimers of generations 1-3. Generations 0-2 with terminal acetylene groups were subsequently functionalized with cationic amino groups for an evaluation of their behaviour as bactericides, and phosphonate groups for potential application as anti-scalants. The cationic amino-functionalized dendrimers, \( \text{G0-NH}_3^+ \), \( \text{G1-NH}_3^+ \)
and G2-NH$_3^+$, as well as one of the OH-terminated dendrimers, G1, were then evaluated for their bactericidal efficacy. The generation one dendrimer, G1-NH$_3^+$, was found to be the most effective bactericide. Additionally it was determined to be more potent than several previously studied dendrimers. The deprotection of the phosphonate-terminated dendrimers from their ethyl esters to the corresponding alcohols was found to be cumbersome, and prevented their evaluation as anti-scalants.
Résumé

Les dendrimères sont utilisés dans plusieurs domaines scientifiques incluant la biologie, la catalyse, l’industrie papetière, etc. Les aspects uniques des dendrimères qui permettent cette application diverse sont leur haute densité de groupements en périphérique et la capacité de les adapter au besoin. Cette thèse abordera les sujets de l’élaboration et de la synthèse de nouveau dendrimères qui sont solubles à l’eau et qui pourraient être utilisés pour l’inhibition de l’entartrage et comme bactéricide, deux applications indispensables dans l’industrie papetière. Les dendrimères présentés dans ce manuscript ont été construits à partir un noyau tetra-fonctionnel, ce qui permet d’obtenir un plus grand nombre de groupes en périphérique qu’avec les noyaux bi-fonctionnels et tri-fonctionnels qui sont employés habituellement. La chimie « click », soit la réaction de cycloaddition entre un alcyne et un azoture, catalysée par le Cu(I), a été utilisée pour synthétiser les dendrimères. Pour exécuter cette démarche, le choix d’alcynes et d’azotures appropriés a été essentiel. A cet effet, nous avions choisi i) une molécule contenant un terminus acétylénique et un acide carboxylique à son autre extrémité ; ii) une molécule comprenant un azoture à une extrémité et un diol à l’autre extrémité. A l’aide d’une méthode divergente où l’azoture est d’abord lié au noyau acétylénique tetra-fonctionnel, suivi d’une estérisation avec le groupement acide carboxylique du bloc moléculaire i), nous avons construit des dendrimère de génération 1 - 3 incorporant des acétylènes terminaux. D’autres dendrimères de générations 0 - 2 possédant des acétylènes terminaux ont été munis d’amines cationiques afin d’évaluer leur potentiel comme bactéricide. De
même, ces dendrimères ont été aussi munis d’acides phosphoniques afin d’évaluer leur capacité d’inhibition de l’entartrage. L’efficacité comme bactéricide de ces dendrimères fonctionnalisés d’amines cationiques, \textbf{G0-NH}_3^+, \textbf{G1-NH}_3^+ et \textbf{G2-NH}_3^+, ainsi qu’un des dendrimères terminant en OH, \textbf{G1}, a été ensuite évaluée. Le dendrimère de génération un: \textbf{G1-NH}_3^+ a été le meilleur à cet égard. De plus il est le plus puissant que plusieurs autres dendrimères testés dans le passé. La déprotection des dendrimères avec des acides phosphoniques en surface pour les transformer en alcools n’a pas eu lieu, alors l’évaluation de leur efficacité en inhibition d’entartrage n’a pas été réalisé.
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<th>Abbreviation</th>
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<tr>
<td>AMPs</td>
<td>Antimicrobial peptides</td>
</tr>
<tr>
<td>bis-MPA</td>
<td>2,2-Bis(hydroxymethyl)propanoic acid</td>
</tr>
<tr>
<td>BOC₂O</td>
<td>Di-tert-butyl dicarbonate</td>
</tr>
<tr>
<td>cat.</td>
<td>Catalytic</td>
</tr>
<tr>
<td>cmc</td>
<td>Critical micelle concentration</td>
</tr>
<tr>
<td>CuAAC</td>
<td>Copper(I) catalyzed alkyne-azide cycloaddition</td>
</tr>
<tr>
<td>DA</td>
<td>Diels-Alder</td>
</tr>
<tr>
<td>DCC</td>
<td>N,N'-dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DHBA</td>
<td>3,5-Dihydroxybenzyl alcohol</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-Dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulfoxide</td>
</tr>
<tr>
<td>E. coli</td>
<td><em>Escherichia coli</em></td>
</tr>
<tr>
<td>EC₅₀</td>
<td>Half maximum effective concentration</td>
</tr>
<tr>
<td>EDA</td>
<td>Ethylenediamine</td>
</tr>
<tr>
<td>EDC</td>
<td>1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride</td>
</tr>
<tr>
<td>E. faecalis</td>
<td><em>Enterococcus faecalis</em></td>
</tr>
<tr>
<td>ESI-MS</td>
<td>Electrospray ionization mass spectrometry</td>
</tr>
<tr>
<td>Gn</td>
<td>Dendrimer generation number</td>
</tr>
<tr>
<td>HOBT</td>
<td>Hydroxybenzotriazole</td>
</tr>
<tr>
<td>IC₅₀</td>
<td>Half maximum inhibitory concentration</td>
</tr>
<tr>
<td>K. oxytoca</td>
<td><em>Klebsiella oxytoca</em></td>
</tr>
<tr>
<td>LL-37</td>
<td>An antimicrobial peptide found in ocular surface epithelia</td>
</tr>
<tr>
<td>MALDI-TOF MS</td>
<td>Matrix assisted laser desorption/ionization mass spectrometry</td>
</tr>
<tr>
<td>MBC</td>
<td>Minimum bactericidal concentration</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>MHB</td>
<td>Mueller Hinton broth</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum inhibitory concentration</td>
</tr>
<tr>
<td>M. luteus</td>
<td>Micrococcus luteus</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MsCl</td>
<td>Methane sulfonyl chloride</td>
</tr>
<tr>
<td>MWCO</td>
<td>Molecular weight cutoff</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>No.</td>
<td>Number</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>PA</td>
<td>Pseudomonas aeruginosa</td>
</tr>
<tr>
<td>PAMAM</td>
<td>Poly(amido amine)</td>
</tr>
<tr>
<td>PEG</td>
<td>Poly(ethylene glycol)</td>
</tr>
<tr>
<td>PPI</td>
<td>Poly(propylene imine)</td>
</tr>
<tr>
<td>PTSA</td>
<td>Para-toluenesulfonic acid</td>
</tr>
<tr>
<td>P. vulgaris</td>
<td>Proteus vulgaris</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature (≈25°C)</td>
</tr>
<tr>
<td>SA</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>S. suis</td>
<td>Streptococcus suis</td>
</tr>
<tr>
<td>TBDPS</td>
<td>Tert-butylchlorodiphenyl silane</td>
</tr>
<tr>
<td>TEC</td>
<td>Thiol-ene coupling</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TIPS</td>
<td>Triisopropylsilyl</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>Trimethylsilyl</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra-violet</td>
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Contribution of Authors

“The Faculty of Graduate Studies and Research at McGill University allows, as an alternative to the traditional thesis format, a manuscript-based thesis, where the candidate has the option of including, as part of the thesis, the text of one or more papers submitted, or to be submitted, for publication, or the clearly-duplicated text (not the reprints) of one or more published papers. The thesis must be more than a collection of manuscripts. All components must be integrated into a cohesive unit with a logical progression from one chapter to the next. In order to ensure that the thesis has continuity, connecting texts that provide logical bridges preceding and following each manuscript are mandatory.”

“when co-authored papers are included in a thesis, the candidate must be the primary author (the author who has made the most substantial contribution) for all papers included in the thesis. In addition, the candidate is required to make an explicit statement in the thesis as to who contributed to such work and to what extent.”

The review of dendrimer bactericidies reproduced in part in Chapter 1 (Sections 1.3.3.2-9), is a review article written by the author and Dr. Annie Castonguay, a former post-doctoral fellow in the groups of Dr. Ashok Kakkar and Dr. Theo van de Ven. The citation is as follows:

Dr. Castonguay performed the original literature search, while the work of reading the articles and assessing their suitability for inclusion was split evenly between the author and Dr. Castonguay. The author wrote the entirety of Sections 1.3.3.2 & 1.3.3.4-7 and the majority of section 1.3.3.3 (the portion on PAMAM glycodendrimers was written by Dr. Castonguay). Dr. Castonguay wrote sections 1.3.3.8 & 9. All four authors contributed to the final writing, revision and editing of the manuscript.

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Chapter 1: Introduction

1.1 – Dendrimers: An Overview

Dendrimers are hyperbranched, monodisperse macromolecules containing well-defined structural features.\textsuperscript{1-3} The monodispersity and structural regularity of dendrimers arises from the controlled, layer-by-layer fashion by which they are synthesized.\textsuperscript{4-6} In general, each additional layer doubles the number of surface groups and increases the generation number of the dendrimer.\textsuperscript{7} At the centre of every dendrimer is its core, radiating outwards are the backbone units and finally the surface groups at the periphery (Figure 1.1).\textsuperscript{8} Each of these units contributes to the final structure of the dendrimer, and thus its properties and applications.

![Figure 1.1 - Structural Features of a Dendrimer](image)

The surface groups interact primarily with the outside environment and are therefore one of the most important units for determining the final properties of the dendrimer. This is not to say, however, that the contributions of the core or backbone are negligible by any means. For instance, the number of reactive ends,
or arms, of the core molecule will have a large effect on the density of surface
groups presented at each generation.\textsuperscript{9} A fourth generation dendrimer with a
tetrafunctional core has 64 surface groups, while a corresponding dendrimer with
a trifunctional core has only 48.\textsuperscript{10} The flexibility of both the core and backbone
units also has a large effect on the conformation of the structure.\textsuperscript{11} Rigid multiply
bonded units lead to a larger more open structure, while more flexible core and
backbone units correspond to more compact, globular dendrimers as the flexible
arms fold back on themselves.\textsuperscript{12} The flexibility of the dendrimer backbone and
core units also has an effect on the development of internal cavities within the
structure. Internal cavities are formed when the dendrimer arms fold back on
themselves forming a contained internal environment that can be used to
encapsulate small molecules of interest.\textsuperscript{13} If the backbone and core units are more
flexible, internal cavities will form at lower generations as there is less of an
energetic barrier to the dendrons rotating and folding back on themselves.\textsuperscript{12}

\textbf{1.2 - Synthesis: A Historical Perspective}

The synthesis of dendrimers dates back to the late 1970’s with Vögtle \textit{et al.}’s cascade molecules.\textsuperscript{14} Although not yet called as such, these hyperbranched
macromolecules are the first published examples of dendrimers. Containing
either an aromatic or aliphatic core and a poly(propylene imine) (PPI) backbone,
the cascade molecules developed by Vögtle’s group led to one of the most popular
types of dendrimers in use today. Currently known as PPI dendrimers, they are
commercially available under the name Astramol\textsuperscript{TM} (DSM, Sigma-Aldrich) and
have been extensively studied for a variety of applications.\textsuperscript{13,15,16} The synthesis of
PPI dendrimers can be achieved divergently starting with a Michael addition of acrylonitrile to a primary diamine, the resulting terminal nitrile groups are then catalytically reduced to amines and the cycle can be repeated to form subsequent generations (Scheme 1.1.a). The next major developments in dendrimer chemistry came in 1985 with the cascade arboreal systems prepared by Newkome’s laboratory, in parallel with Tomalia et al.’s development of the divergent synthetic methodology.

Dendrimers can be prepared by two main synthetic routes, divergently (Scheme 1.1) or convergently (Scheme 1.2). The divergent method, introduced in 1985 by Tomalia et al., is an inside-out synthesis starting from the core and ending with the attachment of the surface groups. Tomalia’s group is also credited with coining the term dendrimer, as well as inventing what is widely regarded as the most studied type of dendrimer backbone, poly(amido amine)
(PAMAM). PAMAM dendrimers are also commercially available (Dendritech, Sigma-Aldrich), and can be synthesized divergently starting from a primary amino-terminated core such as ethylenediamine (EDA). A Michael addition of methyl acrylate to the terminal amines followed by amidation of the resulting terminal ester groups using EDA regenerates the terminal amine groups and increases the generation number of the structure (Scheme 1.1.b).\textsuperscript{10} The sequence can then be repeated to yield subsequent generations. The convergent synthetic methodology was introduced in 1990 by Hawker and Fréchet.\textsuperscript{18} In a convergent synthesis individual dendrons are first prepared in a layer-by-layer fashion from the outside-in, finally two or more of them are attached to a suitable core as a final step (Scheme 1.2). Both divergent and convergent syntheses have advantages and disadvantages. With divergent synthesis higher generations can become very crowded at the periphery leading to incomplete functionalization and thus structural defects, increasing the polydispersity of the macromolecules. In a

\textbf{Scheme 1.2 - Convergent Synthesis of Fréchet et al.’s Poly(aryl ether) Dendrimers}
convergent synthesis steric hindrance can make it challenging to attach very large dendrons to a comparatively small core molecule. In addition to the convergent methodology, Fréchet’s laboratory is credited with the development of the poly(aryl ether) backbone. Synthesized convergently starting from 3,5-dihydroxybenzyl alcohol (DHBA), poly(aryl ether) dendrimers are constructed through a repeating sequence of etherifications and brominations. Once individual dendrons have been prepared they can be attached to any suitable core molecule to yield the desired dendrimer (Scheme 1.2). As discussed above, for most dendrimers the surface groups provide the bulk of the functionality of the structure. There is one class of dendrimers, however, known as core-functionalized, where the core of the macromolecule determines the majority of its properties. The first published example of a core-functionalized dendrimer was in 1993 by Inoue et al. Possessing a porphyrin core and a poly(aryl ether) backbone, the structure was designed in order to shield the porphyrin core from the outside environment, necessary for the macromolecule to be able to mimic some biological functions. Further work by Diederich’s group employed a poly(ether-amide) backbone and a zinc porphyrin core. Diedrich et al.’s porphyrin dendrimers were synthesized in a divergent manner through an

![Scheme 1.3 - Divergent Synthesis of Diederich et al.’s Core-Functionalized Zinc Porphyrin Dendrimers](image-url)
alternating sequence of peptide couplings to obtain a methyl ester-terminated structure followed by hydrolysis to generate the corresponding carboxylic acid (Scheme 1.3). Although the majority of dendrimers are organic in nature, there are numerous examples of dendrimers containing heteroatoms and/or metal centres. Groundbreaking work in the field of phosphorous dendrimers was accomplished by Majoral’s group in 1994 when they synthesized the first neutral phosphorous-containing dendrimer (Scheme 1.4). The dendrimers contained a pentavalent phosphorous core and branching points, with the fourth generation macromolecule presenting 48 surface groups. The synthesis was achieved through a repeating sequence of two reactions: the first creating a phosphorous-oxygen bond and leaving an aldehyde-terminated structure, and the second to generate an imine bond and leaving a chloro-terminated structure (Scheme 1.4).

Scheme 1.4 - Divergent Synthesis of Majoral et al.'s Neutral Phosphorous-Based Dendrimers

All of the dendrimers discussed so far have only one type of active surface group. For some applications, especially within the medical field, it is desirable to be able to attach more than one type of moiety to the periphery. For example, a dendrimer that delivers a drug molecule while simultaneously providing aqueous solubility, and a group which allows the conjugate to be imaged in vivo would be
highly desirable. Dendrimers that possess more than one type of surface group are known as multifunctional. One of the earliest published examples of a multifunctional dendrimer comes from Fréchet’s group in 1999. Consisting of a poly(aryl ether) backbone, the multifunctionality of the dendrimer was introduced by a selective protection of one of the alcohol groups of methyl 3,5-dihydroxybenzoate with tert-butylchlorodiphenyl silane (TBDPS). The remaining unprotected phenolic alcohol could then be used to attach the molecule to a selected core leaving a free benzylic alcohol and a protected phenolic alcohol that can be selectively coupled to either a poly(ethylene glycol) (PEG) chain, or a drug molecule (Scheme 1.5). Selective protection/deprotection steps are usually necessary in order to create multifunctional dendrimers. A great deal of care must be taken in order to design appropriate building blocks with multiple compatible functionalities. The introduction of click chemistry in 2001 by Sharpless et al. The introduction of click chemistry in 2001 by Sharpless et al. The introduction of click chemistry in 2001 by Sharpless et al. The introduction of click chemistry in 2001 by Sharpless et al.
revolutionized the field of dendrimer synthesis. Since dendrimer synthesis is by definition a cascade process, it is necessary to employ high yielding reactions that work reliably in the presence of multiple diverse functionalities. Click chemistry is ideally suited for this purpose. Click reactions are a class of reaction wherein two units are coupled together with quantitative yields and little to no side products. There are three reactions currently known as click reactions: the first and most commonly employed is the copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC, Scheme 1.6.a), the second is the Diels-Alder cycloaddition (DA, Scheme 1.6.b) and finally the thiol-ene coupling reaction (TEC, Scheme 1.6.c). The synthesis of the first dendrimer prepared using click chemistry was achieved in 2004, using the CuAAC reaction.\(^\text{25}\) A variety of different triazole based structures were generated under collaboration between Hawker, Fréchet, and Sharpless among others. The dendrimers were prepared in a convergent fashion by first clicking together an azide and a diyne with a chloro focal point and then azidifying the chloro group. The sequence was repeated until the desired generation was reached and the resulting dendrons were then clicked onto a polyacetylene core. As mentioned above, click chemistry is extensively used in the preparation of dendrimers. One recent example from Kakkar’s group employed the CuAAC click reaction to synthesize trifunctional dendrimers for
Scheme 1.7 - Synthesis of Kakkar et al.'s Trifunctional Dendrimer for Drug Delivery (α-Lipoic Acid), Increased Drug Solubility (PEG) and Imaging (Fluorescent Dye)
applications in biology. The synthesis depended on the construction of a core with orthogonal functionalities. The synthesis began by exploiting the differing reactivity of the halogen groups of 3-bromo-5-iodobenzylalcohol. Sonogashira coupling reactions were performed selectively at the three and five positions through the use of two different silyl-protected acetylenes. First the iodo group was replaced with triisopropylsilylacetylene, followed by the substitution of the bromo group by trimethylsilylacetylene (Scheme 1.7). The two different protected acetylenes can then be selectively deprotected in order to allow their functionalization with different azide molecules. The hydroxide is then converted to a bromo group, which can later be transformed into an azide. The trimethylsilyl (TMS) group is removed first, leaving a free acetylene that can be coupled to a long chain alcohol functionalized with an azide group, or a PEG chain, also functionalized with an azide group. After the long chain alcohol-functionalized molecules bromo focal point is converted to an azide it can be clicked onto a poly(acetylene) core. The macromolecule’s triisopropylsilyl (TIPS) groups can then be removed, and the resulting free acetylenes can be used to attach the PEG-functionalized molecule with an azide focal point and a TIPS-protected acetylene. The remaining TIPS groups are then removed and the resulting free acetylene used to attach a fluorescent dye with an azide focal point. Finally the long chain alcohol groups can be coupled to the carboxylic acid group of a drug molecule, α-lipoic acid. The resulting trifunctional dendrimer is able to deliver a drug (α-lipoic acid), while simultaneously increasing its aqueous solubility (PEG) and allowing its imaging (fluorescent dye).
1.3 – Applications

1.3.1 – Traditional Applications

The wide range of applications found by dendrimers is largely a consequence of their structural versatility and multivalency. Since the dendritic structure can be tailored for a desired application, the architecture of these macromolecules offers a unique and structurally well-defined way to arrange relevant moieties. Their multifunctionality and high density of surface groups mean that a large concentration of a desired group can be presented in a single unit. One of the biggest applications of dendrimers is in drug delivery.\textsuperscript{13,15,27,28} Many drugs do not make it to the market because of their poor water solubility and bioavailability. Dendrimers offer the potential to aid in the solubilisation of drug molecules,\textsuperscript{22,29,30} as well as to target specific organs or cells,\textsuperscript{31-33} it is even possible to attach a fluorescent molecule or an MRI contrast agent in order to allow imaging of the nanocomposite\textsuperscript{23,26,34,35}. Dendrimers can function as drug delivery vehicles in two main ways: by encapsulation or by covalent attachment. As discussed above, higher generation dendrimers often contain well-defined internal cavities that can be used to encapsulate smaller molecules of interest, including drugs. Drug molecules can also be covalently attached to the surface groups of the dendrimer through a selectively cleavable bond.\textsuperscript{36} Most of the drugs that have been associated with dendrimers are anti-cancer, anti-inflammatory or anti-microbial in nature.\textsuperscript{13} The structure presented below (Figure 1.2) is a generation two poly(aryl ether) dendrimer functionalized with hydrophilic PEG chains.\textsuperscript{22} The hydrophobic nature of the backbone combined with the hydrophilic
nature of the end groups makes this structure what is known as a unimolecular micelle. Unlike micelles formed from multiple polymer chains, unimolecular micelles do not need to reach a critical micelle concentration (cmc) in order to form. This is a great advantage for drug delivery applications as once the nanocomposite is administered to a patient its concentration in the blood stream always falls far below the cmc. The unimolecular micelle presented below (Figure 1.2) was used to encapsulate indomethacin, an anti-inflammatory drug.

Figure 1.2 - Fréchet et al.'s Dendritic Unimolecular Micelle with Encapsulated Indomethacin Dye
Fréchet’s group demonstrated the generation three dendrimers ability to load 11% w/w of the drug and then allow its slow release over about 30h.

Dendrimers are also widely employed in catalysis.\textsuperscript{37-41} The structure of the dendrimer can be designed such that it itself functions as a catalyst, or they can be employed as supports to encapsulate and stabilize other catalytically active agents. The first example of a dendritic catalyst originated in van Koten’s group in 1994 (Figure 1.3).\textsuperscript{42} Consisting of a polysilane backbone and functionalized with

![Organometallic Ni(II) Polysilane Dendrimer for Catalysis](image-url)
diaminoaryl nickel(II) pincer complexes, the dendrimer was able to catalyze the Kharasch addition of poly(haloalkanes) to the corresponding alkene. The advantage of anchoring a homogeneous organometallic catalyst to a dendrimer backbone lies in combining the high selectivity and benign reaction conditions of homogeneous catalysis with the durability and facile isolation of heterogeneous catalysis. When tested, van Koten et al.’s dendrimer showed comparable catalytic activity to the corresponding discrete organometallic complex. Dendrimers are

Figure 1.4 - Vögtle et al.’s PPI Dendrimer with 32 Peripheral Fluorescent Dansyl Units
also employed to aid in the preparation and stabilization of metal nanoparticles.\(^{43-47}\) The structural versatility of dendrimers means that almost any moiety desired to complex specific metal ions can be incorporated into the backbone and/or surface groups. The contained internal environment provided by the internal cavities of higher generation dendrimers also provides an ideal location in which to grow monodisperse nanoparticles. Seminal work in this field was carried out in the mid-1990’s when several groups simultaneously realized the potential of the

**Figure 1.5 – Astruc et al.’s Polysilane/Triazole Dendrimer with 81 Peripheral Redox Active Ferrocene Units**
amine groups of PAMAM dendrimers to complex various metal ions.\textsuperscript{45,46} Dendrimers have also been used as fluorescent sensors in order to detect the complexing of metal ions.\textsuperscript{48-50} Work by Vögtle’s group in this area centered on attaching fluorescent dansyl units to the periphery of PPI dendrimers (Figure 1.4).\textsuperscript{48} The fluorescence of the dansyl units was quenched when the interior tertiary amines of the macromolecule complexed with Co(II) ions. In the case of the generation five dansyl-functionalized dendrimer, the complexation of a single Co(II) ion was adequate to quench the fluorescence of all 64 peripheral dansyl units. This represents a significant increase in sensitivity as opposed to a corresponding monofunctional dansyl unit. Dendrimers have also found use as redox\textsuperscript{51-54} sensors. Studies done by Astruc \textit{et al.} led to the synthesis of a dendrimer with a polysilane/triazole backbone and ferrocenyl surface groups (Figure 1.5).\textsuperscript{55} The triazole groups were able to complex with metal cations and oxo anions which affected the redox potential of the peripheral ferrocene units and could be monitored by cyclic voltammetry. Dendrimers have also found use in a wide range of optoelectronic\textsuperscript{56,57} applications including non-linear optics, stimulated emission and laser emission.\textsuperscript{58} Dendrimers are particularly interesting within the field of non-linear optics due to the precise control they provide over the localization of any desired moiety within their structure. Yokoyama’s group prepared generation four poly(azobenzene) dendrons (Figure 1.6) and measured their first-order molecular hyperpolarizability.\textsuperscript{59} The dendrons were found to be more than 20 times more molecularly hyperpolarizable than the corresponding azobenzene monomer.
1.3.2 – Dendrimers in Pulp and Paper

A new and exciting area where dendrimers have found applications is in the pulp and paper industry. As discussed above, the well-defined globular structure of dendrimers and their resulting high density of surface groups makes them attractive for use in various applications, and those within the pulp and paper industry are no different. Within the field of pulp and paper, dendrimers
have been explored for use as retention aids,\textsuperscript{60,61} strengthening agents,\textsuperscript{62} flocculants\textsuperscript{63} and anti-scalants\textsuperscript{64}. A retention aid is a species added to pulp during the production of paper in order to increase its ability to retain other species. These other species are pulp fines, as well as various polymers and inorganic materials which have been added to the pulp slurry in order to improve a variety of properties of the paper, including drainage and wet and dry strength, as well as to decrease cost. Without retention aids many of these important species would be lost to the waste water. Strengthening agents simply improve the mechanical strength of the paper, while flocculants cause the agglomeration of fines and fillers allowing their incorporation in a forming sheet.\textsuperscript{65} Anti-scalants prevent the build-up of low solubility inorganic salts, known as scale, during the paper-making process. Any industry which extensively employs water, such as the pulp and paper and water treatment industries, suffers from scale build-up, also known as fouling.\textsuperscript{66} Scale consists of low solubility inorganic salts such as CaSO$_4$, CaCO$_3$, Na$_2$CO$_3$, Na$_2$SO$_4$, CaF$_2$ and BaSO$_4$.\textsuperscript{67,68} Generally, scale is formed when the temperature of the aqueous solution decreases, or when the concentration of inorganic material increases (due either to an increase in salt content, or a decrease in water content).\textsuperscript{69} Anti-scalants are typically small molecules or polymers functionalized with phosphonate groups,\textsuperscript{64} which function by three main mechanisms: precipitation threshold inhibition, dispersion or crystal distortion/modification. In the precipitation threshold inhibition mechanism the anti-scalant forms a complex with the scale ions, thus increasing their solubility. The dispersion mechanism prevents further scale aggregation by electrostatic
repulsion caused when the anti-scalant adsorbs on top of the forming scale crystals. In the crystal distortion/modification mechanism the anti-scalant adsorbs on top of the previously formed scale crystals and causes defects during further crystal growth, weakening its structure.\(^7\)

1.3.3 – Dendrimers as Bactericides

1.3.3.1 - Overview

As discussed above, the unique globular structure of dendrimers means that they have a very high density of surface groups, making them suitable for a wide range of applications where a high local concentration of a particular functionality is desired. One such application is in the control of bacterial growth. Although this is a problem relevant to the pulp and paper industry, dendrimers have not yet been applied as bactericides in this field. While preliminary studies on several different structures do exist,\(^7\)\(^1\)-\(^9\)\(^1\) the bactericidal properties of dendrimers have not yet been extensively explored, making the elucidation of structure-property relationships challenging.

1.3.3.2 - Introduction

Bacteria are one of the most ubiquitous forms of life on earth; there are more bacterial cells in the human colon than human cells in the entire body.\(^9\)\(^2\) Although these microorganisms perform many necessary functions such as participating in the carbon, nitrogen and phosphorous cycles and aiding in digestion, their ability to grow in almost any location, from inside steam vents and glaciers to nuclear power plants, means that they often grow in locations where their presence is undesirable to humans.\(^9\)\(^3\)-\(^9\)\(^5\) For instance, bacterial growth in industrial machinery, on medical implants and inside wounds has serious medical
and financial consequences.\textsuperscript{93} This renders the use of bactericides, substances selectively toxic to bacteria, an absolute necessity.\textsuperscript{94} The demand for new bactericidal agents is continuously growing because of the increasing bacterial resistance caused by the overuse of numerous antimicrobials in various fields, as well as the need to have a better control on the build-up of biofilms.\textsuperscript{93} Dendrimers are a relatively new entry to macromolecules, and possess unique properties which have led to applications in a wide-range of areas including biology.\textsuperscript{1,7,8,13,28,35,37,38,96-99} Some of the important properties of dendrimers include a hyperbranched monodisperse architecture where higher generations may contain well-defined internal cavities that can be used to encapsulate small molecules of interest.\textsuperscript{1,7,8,13,28,35,37,38,96-99} Their monodispersity, in contrast to traditional polymers, arises from the controlled, layer-by-layer fashion in which they are synthesized. Compared to polymers, dendrimers have a globular structure, and consequently a higher density of surface groups.\textsuperscript{1,7,8,13,28,35,37,38,96-99} A pictorial representation of a dendrimer’s structure is shown above (Figure 1.1). Key structural features include the core unit, the backbone, the internal cavities and the surface groups. One exciting consequence of this structure is that each distinct unit in it can be varied independently, affecting the overall properties of the dendrimer. The synthetic versatility and high density of surface groups offered by the dendritic structure are some of the factors which make these monodisperse macromolecules attractive for use as bactericides. Compared to various small molecules and polymers which have proven to be active bactericides, the use of dendrimers offers the potential to deliver a high dosage of antimicrobial agents.
using a single scaffold.\textsuperscript{74,81,84,100,101}

1.3.3.3 - Different Modes of Action

Bacteria can be classified into two main categories depending upon the structure of their cell walls: Gram-negative and Gram-positive.\textsuperscript{95} The differences in the structure of the cell wall as well as the presence of an outer membrane in Gram-negative bacteria lead to large differences in the susceptibility of Gram-negative and Gram-positive bacteria to various antimicrobial agents (Figure 1.7.a).\textsuperscript{95} As with antibiotics,\textsuperscript{92,93} some dendrimers are more effective against Gram-negative bacteria,\textsuperscript{72} while others perform better against Gram-positive.\textsuperscript{100,101} The mechanism by which dendrimer bactericides kill or inhibit bacteria is strongly dependent upon the type of their surface groups (Figure 1.7.b).\textsuperscript{73,74,81,83,84,100-104} For instance, dendrimers bearing sugar-type moieties at their periphery (oligosaccharides), referred to as “glycodendrimers”, can mimic the glycoproteins and/or glycolipids which are found at the cell’s surface, and interact with the lectins found at the periphery of the bacteria.\textsuperscript{73} These carbohydrate-protein interactions make the glycodendrimers good anti-adhesive molecules.\textsuperscript{102,103} Since the surface of the bacterial cell walls as well as their

![Figure 1.7 - a) Comparison Between the Cell Walls of Gram-positive and Gram-negative Bacteria; b) Specific Interactions of Respective Dendrimer Surface Groups with Bacterial Membranes](image)
plasma membranes are negatively charged, many bactericides are cationic in nature which maximizes the attractive forces between the two species. Dendrimers bearing charged end groups are believed to function by first penetrating the cell wall and then interacting with the plasma membrane, in order to increase its permeability. The specific interaction with the membrane is dependent upon the precise charged moiety which is employed (Figure 1.7.b), but the result is the same in all cases: leakage of intracellular materials such as potassium ions and nucleic acids, leading to eventual cell death if the bactericide concentration is high enough. It is noteworthy that there are two main factors affecting the strength of bactericidal activity of dendrimers of the same type (similar backbone and surface groups): 

1. The number of surface groups containing bactericidal moieties; and
2. The ability of the dendrimer to cross the cell wall and arrive at the plasma membrane, its site of action (biopermeability of the dendrimer). Obviously these two factors are at odds with one another as generation number increases, often leading to complex outcomes.

1.3.3.4 - Bactericidal Efficiency Quantification

Bactericides can show both bactericidal and bacteriostatic effects, depending upon the type of bacteria (Gram-negative or Gram-positive) and the concentration of bactericide employed. In general, bacteriostasis is caused by an inhibition of metabolic processes, while bactericidal effects are observed as a result of permanent damage to vital cell structures. Two relevant parameters measuring the effectiveness of a bactericide are the minimum inhibitory concentration (MIC), and the minimum bactericidal concentration (MBC). The
MIC is defined as “the lowest concentration of an antimicrobial that will inhibit
the visible growth of a microorganism after overnight incubation”\textsuperscript{,109} while the
MBC, which for a given species is always larger than the MIC, is defined as “the
lowest concentration of an antimicrobial that will prevent the growth of an
organism after sub-culture onto an antibiotic-free media”.\textsuperscript{109} Two related
parameters, the IC\textsubscript{50} (half maximum inhibitory concentration)\textsuperscript{104} and EC\textsubscript{50} (half
maximum effective concentration),\textsuperscript{72} measure the concentrations which inhibit
50\% of the growth and kill 50\% of the bacteria, respectively. Many standard
methods are used to evaluate the efficiency of dendrimers as bactericides (dilution
broth method, the disk diffusion method, etc).\textsuperscript{72,83,104,110} The same parameters
reported using any of these methods can be directly compared.

1.3.3.5 - PAMAM Dendrimers

PAMAM dendrimers are among the most studied dendritic architectures,
and one of the few that are commercially available (Figure 1.8).\textsuperscript{10} These have

![Structure of an Amino Terminated Third Generation PAMAM Dendrimer and Examples of Sugar-type End Groups Used to Prepare Anti-adhesive Glycodendrimers](image)
been extensively employed to deliver active agents encapsulated in their cavities.\textsuperscript{1,7,8,13,28,35,37,38,79,86,96-99,111} It was discovered, by Cai and co-workers\textsuperscript{72} in 2007, that amine-terminated PAMAM dendrimers are themselves effective antibacterial agents. The generation five amine-terminated PAMAM dendrimer was found to be active against both Gram-negative (\textit{Pseudomonas aeruginosa}, \textit{PA}) and Gram-positive (\textit{Staphylococcus aureus}, \textit{SA}) pathogens. The authors mentioned that the dendrimer is even more effective against the \textit{PA} pathogen (EC\textsubscript{50} = 1.5 ± 0.1 mg/L) than \textit{LL-37} (EC\textsubscript{50} = 1.9-2.8 mg/L ± 1.3), an antimicrobial peptide found in ocular surface epithelia,\textsuperscript{112,113} although they did not consider the high error interval reported for the \textit{LL-37} EC\textsubscript{50} value. This PAMAM dendrimer is only moderately active against Gram-positive bacteria (EC\textsubscript{50} = 20.8 mg/L ± 3.4). Partial coating of the dendrimer with PEG chains was shown to greatly reduce its toxicity to human corneal epithelial cells without significantly reducing its bactericidal activity against \textit{PA}. Some reduction in activity was observed for \textit{SA} bacteria (> 50 mg/L), indicating that this type of dendrimer has increased difficulty in crossing the thick crosslinked peptidoglycan layer present in these bacteria. Nevertheless, it clearly demonstrated that PEG-coated PAMAM dendrimers could be potential candidates to act as antibacterial agents incorporated in contact lenses to combat pseudomonal keratitis. In 2009, further studies by Cai and coworkers\textsuperscript{110} allowed the comparison of the bactericidal activity of the fifth generation amino terminated PAMAM dendrimer previously used, with a third generation analogue, against the same pathogens. The MICs (against \textit{PA} and \textit{SA}) for both dendrimers (G3 : 6.3 mg/L, G5 : 12.5 mg/L) are
comparable to LL-37 (1.3-12.5 mg/L), and are within the wide range of the fluoroquinolone antibiotics (0.047-128 mg/L). Interestingly, the smaller third generation dendrimer structure was found to be more potent (0.92 μM, 31 NH₂) than its fifth generation analogue (0.47 μM, 110 NH₂), considering the estimated number of free amino groups present at their periphery. Again, partial PEGylation of the dendrimer surface groups decreased the bactericidal activity against both pathogens, especially for SA bacteria, but also decreased the cytotoxicity towards human corneal epithelial cells. It is noteworthy that slight PEGylation (6%) of the third generation dendrimer was necessary to retain its high bactericidal activity against PA and to significantly reduce its cytotoxicity. In 2010, Kannan and co-workers investigated the antibacterial activity of fourth generation amino-terminated PAMAM dendrimers against the Gram-negative bacteria Escherichia coli (E. coli), and compared the results with a fourth generation hydroxide- and a third-and-a-half generation carboxylic acid-terminated dendrimers. While the amino-terminated structure was the most effective bactericide (IC₅₀ G4-NH₂: 3.8 mg/L, IC₅₀ G4-OH: 5.4 mg/L, IC₅₀ G4-COOH: 22.0 mg/L), it was also the most cytotoxic to human cervical epithelial cells. In vivo experiments using a guinea pig model showed that the hydroxide-terminated structure was effective at treating chorioamnionitis, a condition caused by E. coli. The group also demonstrated that the hydroxyl-terminated dendrimer brings major structural changes to the outer membrane of the bacteria, while the amine-terminated one modifies both the inner and outer membranes (Figure 1.7). They proposed a possible bactericidal mode of action for each of the dendrimers studied (Figure
The same group also reported the suitability of a fourth generation dendrimer in pregnant women without affecting the fetus. Numerous groups have also reported the preparation of PAMAM glycodendrimers which act as anti-adhesive molecules against the Gram-negative bacteria *E. coli* or the Gram-positive bacteria *Streptococcus suis* (*S. Suis*), by linking mannosides, galabiosides and other sugar derivatives at their periphery (Figure 1.8). For instance, in 2004, Pieters and co-workers prepared a first generation PAMAM dendrimer containing disaccharide galabiose units, and it was found to be active against *S. Suis* bacteria, displaying an MIC of 0.3 nM. This 3000-fold (375-fold per galabiose unit) increase in potency was claimed as the first example of a sub-nanomolar MIC for carbohydrate inhibition of bacterial adhesion.

1.3.3.6 - PPI Dendrimers

Although the moieties present at the periphery of the dendrimers are believed to be responsible for their bactericidal activity, altering their backbone can also lead to very interesting results. Cooper and co-workers have carried out intriguing studies in the area of bactericidal PPI dendrimers. In 1999, they determined that amine-terminated PPI dendrimers of the third generation do not show bactericidal effect against Gram-negative bacteria (*E. coli*). In contrast, the third generation amino-terminated PAMAM dendrimer was found to display a great potential against the Gram-negative bacteria *PA* (*vide supra*, MIC : 0.92 μM). It is noteworthy that conclusions about a potential *backbone effect* cannot be drawn from these studies since *i*) both species have not been tested against the same pathogen (*E. coli* vs *PA*) and that *ii*) a third generation PPI dendrimer is
smaller in size and has half as many end groups at its periphery than a PAMAM dendrimer of the third generation. In order to increase the bactericidal potential of the third generation PPI dendrimer, the authors reacted the terminal amine groups with 2-chloroethylisocyanate followed by dimethyldodecylamine (Figure 1.9), leading to a dendrimer decorated with 16 quaternary ammonium groups. They evaluated its bactericidal potential against the Gram-negative bacteria *E. coli* (EC$_{50}$ : 12 mg/L, 1.8 μM) and found that the dendrimer was much more potent than the small molecule n-dodecyltrimethylammonium chloride (EC$_{50}$ : 2000 mg/L, 7579 μM) alone. Interestingly, when tested against the Gram-positive bacteria *S. A*, although very efficient (MIC : 1 mg/L, MBC : 10 mg/L), the results were found to be similar to the ones previously reported for the small molecule n-dodecyltrimethylammonium chloride.$^{116}$ In 2000, the same group$^{81}$ demonstrated that, for PPI dendrimers functionalized with n-dodecyltrimethylammonium chloride, the increase in dendrimer surface charge density is a predominant factor (over permeability), from the third to the fifth dendrimer generation. The bactericidal potential of the G1-G5 series was examined against the Gram-negative bacteria *E. coli*, and the order reported was G5 > G4 > G1 > G2 > G3.
The authors compared the results obtained for the third generation dendrimer with the analogous hyperbranched polymers, and found that the polymers were somewhat less bactericidal. The globular structure of dendrimers can better balance the two opposing trends of the ability to penetrate the cell wall (size) and to interact with the plasma membrane (number of surface groups). The group also studied the impact of the counter-ion, as well as the alkyl chain length of the quaternary ammonium group on the bactericidal activity, and found that Br\(^-\) is a more potent counterion than Cl\(^-\), while end groups having a chain of 10 carbons gave best results. It is important to note that the amino-terminated PPI dendrimers of higher generations (>G4) should be tested as bactericides prior to the quaternization of their end groups, in order to allow a comparison with other dendrimer scaffolds, such as PAMAM.

1.3.3.7 - Carbosilane Dendrimers

Carbosilane dendrimers are much more hydrophobic than either PAMAM or PPI backbones, which inhibits analysis of their antimicrobial properties, due to

![Carbosilane Dendrimers Investigated by Ortega and Co-workers](image-url)
their low water solubility. Quaternary ammonium-functionalized carbosilane dendrimers, however, were shown to be effective bactericides. In 2008, Gomez, Mata and co-workers\textsuperscript{101} tested the first and the second generation of the quaternary ammonium-terminated dendrimers presented in Figure 1.10 (R = A) against Gram-negative (\textit{E. coli}) and Gram-positive (\textit{SA}) bacteria. Both dendrimers were found to be over two orders of magnitude more potent bactericides than their monofunctional counterpart HO-(C\textsubscript{6}H\textsubscript{4})-3-NMe\textsubscript{3}\textsuperscript{+}I (MIC : 512 mg/L), and were more effective against \textit{SA} (MIC G1 : 1 mg/L, MIC G2 : 8 mg/L) than \textit{E. coli} (MIC G1 : 4 mg/L, MIC G2 : 64 mg/L), especially in the case of G2. The first generation was more effective than the second, indicating the importance of biopermeability over surface group density for this system. The bulkier size of the second generation dendrimer might be an explanation for its less potent bactericidal activity, but its lower solubility in water prevents drawing firm conclusions. In order to overcome the water solubility issue, the same group prepared carbosilane dendrimers of the first, second and third generation, functionalized with quaternary ammonium groups linked to the backbone through a second quaternary ammonium group (Figure 1.10, R = B).\textsuperscript{100} This type of end-group effectively doubles the charge density of the structure while not significantly increasing its size. They then tested their bactericidal activity against the same strains of bacteria, \textit{E. coli} and \textit{SA}, and noted some interesting trends. In all cases, as previously observed, all the dendrimers were more effective against \textit{SA} than \textit{E. coli}, however for both bacteria, the activity seemed to reach a plateau at the second generation (MIC Gram-negative : 1.70 \textmu M, MIC Gram-positive :
0.85 μM), with the third generation showing almost identical results (MIC Gram-negative : 1.65 μM, MIC Gram-positive : 0.82 μM). The first generation dendrimer was more potent than higher generations tested in the case of the Gram-positive bacteria (MIC : 0.46 μM), but less potent in the case of the Gram-negative one (MIC : 3.65 μM), indicating that for this particular system, a smaller dendrimer is required to be able to cross the thick, crosslinked peptidoglycan layer present in Gram-positive bacteria. When the bactericidal activity of these dendrimers was compared to their monofunctional counterpart, Et₃Si(CH₂)₃N⁺(Me)(Et)(CH₂)₂N⁺Me₃I⁻₂ (MIC : >115 μM), their unique structure was found to be very beneficial (MICs : 0.46-3.65 μM). Moreover, when compared to the activity of the antibiotic penicillin G potassium, the dendrimers were two orders of magnitude more potent against E. coli (MIC : 1.65-3.65 μM vs 766.0 μM) while slightly less potent against SA (MIC : 0.46-0.85 μM vs 0.09 μM). The fact that the carbosilane dendrimer backbone is much more hydrophobic than the other backbones discussed so far (PAMAM and PPI, vide supra) must be taken into account when attempting to compare results. As previously mentioned, there is a fine balance between the two main factors governing the bactericidal activity of dendrimers, biopermeability and surface group density. However, the biopermeability is believed to not only depend on the size and the molecular weight of the dendrimers but also on the lipophilicity of their backbone and end groups. For instance, the authors investigated the bactericidal activity of a second generation dendrimer functionalized with another type of peripheral group where the counterion was replaced by a Cl⁻, and a methyl group was replaced by
hydrogen on each of the cationic nitrogen atoms (Figure 1.10, R = C). The activity against both pathogens was lower (MIC : 42.49 μM), and it is believed that the presence of N-H bonds may be responsible for the higher hydrophilicity of the dendrimer, leading to a decrease in its biopermeability.

1.3.3.8 - Polypeptide Dendrimers

Some linear peptides are known to adopt conformations which induce bacterial membrane recognition. They are referred to as antimicrobial peptides (AMPs) and usually kill bacteria by modifying their membrane permeability.\textsuperscript{117,118} Most of the AMPs display amphipathic arrangements: their charged basic amino acid units (B) and lipophilic side chains (H) forming various patterns of clusters, which are believed to be responsible for their high affinity towards bacterial membranes. Considering the numerous steps required for the preparation of linear peptides, it is clear that the orthogonal synthesis of dendrimers can offer a great advantage. Consequently, there has been a wide interest in preparing peptide dendrimers in order to compare their bactericidal activity with linear peptides,\textsuperscript{119} and a brief summary of achievements in this area is presented here. Tam and co-workers\textsuperscript{120} have done pioneering work in this area, and in 2002, they reported the synthesis of various peptide dendrimers based on a lysine core. They studied the bactericidal activity of lysine dendrimers of generation 0, 1 and 2, bearing linear tetra- and octapeptides as end groups (R4 and R8, Figure 1.11). The R4 peptide is composed of a \textit{BHHB} microbial surface recognition motif, as the one found in protegrins and tachyplesins, effective AMPs composed of 16-17 residues. The R8
peptide consists of the same petidic chain and an additional R4 unit (Figure 1.11).
Each terminal lysine moiety was coupled to both linear peptides through their amino groups. The study was performed against Gram-negative (E. coli, PA, P. Vulgaris and K. oxytoca) and Gram-positive (SA, M. Luteus and E. faecalis) bacteria. The results indicated that the dendrimers of first and second generation bearing the tetra- and the octapeptide end groups have similar bactericidal activity, displaying MICs lower than 1 μM. The first generation dendrimer with sixteen tetrapeptide units at its periphery showed a comparable activity to protegrins and tachyplesins, as well as to linear repeating tetrapeptides. Urbanczyk-Lipkowska reported a library of a combination of different amino acids to form small dendrimers. Although the bactericidal activity against E. coli or SA strains was not significantly different, the results showed that the topographical location of B or H amino acid units has an influence on the bactericidal activity, but since the differences are not drastic, dendrimers can adopt the proper conformation to interact with the bacteria. Nevertheless, the MICs displayed by all these dendrimers, which are in the range of 16-1327 μM, were higher than the one reported for indolicidin, a 14 unit linear peptide (1.5 μM). Poly(lysine) dendrimers bearing sugar-type end groups at their periphery
have also been reported and are believed to act as anti-adhesive molecules to prevent bacterial infection. An interesting example is the one reported by Roy and co-workers\textsuperscript{122} where dendrimers decorated with mannose units were demonstrated to be efficient candidates to prevent an infection by \textit{E. coli}. IC\textsubscript{50} values found for the inhibition activity of dendrimers bearing 2 to 16 mannose units were in the nM unit range, and the distance between the mannose units was found to be an important factor to consider for the molecules to be efficient \textit{E. coli} anti-adhesives.

Among other dendritic backbones that have shown bactericidal activity include anionic amphiphiles,\textsuperscript{123} aromatic polyethers\textsuperscript{124,125} and poly(propyleneoxide) amine\textsuperscript{126} based structures.

\textbf{1.3.3.9 - Summary and Outlook}

Currently, dendrimers constitute one of the highly explored macromolecules for applications in a wide variety of areas including biology. The synthetic elaboration that has been achieved in numerous laboratories, and their multivalent nature makes these hyperbranched and monodisperse nanomaterials, attractive candidates as bactericides. It is becoming quite apparent that the antibacterial activity of small molecules is enhanced when they are conjugated at the periphery of dendrimers. This conclusion is stated by almost all of the studies reported in the literature. An advantageous consequence of this would be that antimicrobial dendrimers could be less likely to induce bacterial resistance than standard antibiotics, and could help achieve a better control on the build-up of biofilms.
The two main factors influencing the bactericidal activity of dendrimers are the number of active surface groups and the biopermeability of the system; the latter being favoured by small, low molecular weight and lipophilic dendrimers (surface groups or backbone). These two major variables are at odds with one another as generation number increases. The field of bactericidal dendrimers is relatively new, having emerged about a decade ago, and expectedly, up to now, no real structure-activity relationships have been established. This is not only due to the slow emergence of the examples in the literature, but also to the disparity of the results obtained. For instance, there are very few studies where the dendrimer generation effect has been investigated, or where two different types of backbones bearing the same number and type of end groups have been compared. Moreover, the interpretation of the results is also quite a difficult task, since many dendrimers are not generally tested against the same pathogens. We expect that a detailed examination of dendrimer structures, and bringing more variation to their architecture, would really stimulate this field. A better understanding of different parameters governing the bactericidal activity of dendrimers could make them the macromolecules of choice for action against bacteria, and for biology in general.

1.4 - Goals

The goals of this project were to develop a versatile synthetic methodology to dendrimers for applications as bactericides and anti-scalants. This required the design and synthesis of acetylene-terminated dendrimers of generations 0-4, which could be subsequently functionalized with either cationic amino or phosphonate end groups, for bactericidal and anti-scaling activity,
respectively, through the use of click chemistry. These surface groups are expected to impart the dendrimers with increased water solubility, as well as the relevant applications in the pulp and paper industry. The dendrimers were characterized using NMR (\(^1\text{H}\) and \(^{13}\text{C}\}\{\(^1\text{H}\}\}) and MALDI-TOF MS. The potential of these dendrimers for applications in the pulp and paper industry, specifically as bactericides and anti-scalants, was then explored.

1.5 - References

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Chapter 2: Design and Synthesis

2.1 - Introduction

Due to their globular structure, dendrimers present a variety of intriguing properties, one of which is tailorable solubility in a variety of solvents, including water.¹ Some dendrimer backbones are more water soluble than others, obviously this is related to the hydrophilicity of the backbone units used to prepare them. There is also a dendritic effect at play; with higher generation dendrimers generally being less soluble than lower ones.² The type of surface groups employed will also have a large effect on the aqueous solubility, as they are the part of the dendrimer which interacts the most with the outside environment.³ Some dendrimer backbones known to be water soluble without surface modification are: poly(propylene imine) (PPI), poly(lysine) and poly(nucleic acid) based structures.⁴ Dendrimers with more hydrophobic backbones, such as those based on aromatic structures, can be made water soluble by attaching hydrophilic surface groups.⁵ The use of poly(ethylene glycol) (PEG) chains is common, especially when the structure is intended for biomedical applications since PEG is not only water soluble, but also biocompatible.⁶ Dendrimers with hydrophobic backbones and hydrophilic surface groups are known as unimolecular micelles and can be used to aid in the solubilisation and delivery of hydrophobic drug molecules, in addition to other applications.⁷ Drug delivery and solubilisation are very important applications for water soluble dendrimers. Most therapeutic agents are hydrophobic in nature, greatly limiting their solubility in aqueous media such as blood.⁶ Forty percent of new drug molecules do not make it to the
market because of their poor water solubility and thus poor bioavailability.\textsuperscript{8} As a consequence of their monodispersity and structural versatility, as well as the contained internal environment they provide, water soluble dendrimers are ideally suited for applications in drug delivery and solubilisation.\textsuperscript{9} Virtually all dendrimers intended for biomedical applications must be water soluble. Since water is the solvent of life,\textsuperscript{10} the reasons behind this are self-evident. In addition to drug delivery and solubilisation, water soluble dendrimers have been employed as anti-microbial agents,\textsuperscript{11-13} MRI\textsuperscript{14,15} and X-ray\textsuperscript{16} contrast agents, for gene transfection,\textsuperscript{17-19} and in tissue engineering.\textsuperscript{20-22} Outside of the medical field, water soluble dendrimers have been employed within the pulp and paper industry as anti-scalants,\textsuperscript{23} strengthening agents\textsuperscript{24}, flocculants\textsuperscript{25} and retention aids.\textsuperscript{26,27} These dendrimers have also made their mark in metal nanoparticle synthesis and stabilization,\textsuperscript{28,29} catalysis\textsuperscript{30,31} and optoelectronics.\textsuperscript{32}

One of the challenges in the design of water soluble dendrimers is the accessibility to a versatile synthetic methodology which allows their construction and post-functionalization with a variety of desired functional groups. We report here a simple and versatile synthetic route to acetylene-terminated dendrimers which can be functionalized with a variety of surface groups through the use of the copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) reaction. Once clicked onto the dendrimers these surface groups, including cationic amino and phosphonate moieties, impart the dendrimers with water solubility as well as bactericidal and anti-scalant properties. The core molecule that we selected is tetrafunctional, which means that these dendrimers will have a higher density of
surface groups at a lower generation than dendrimers constructed from di- or trifunctional cores. The core molecule has terminal alcohol groups, since we wished to employ click chemistry not only in the functionalization of our dendrimers, but also in their construction, our first step was to attach alkyne groups through the use of an etherification reaction (Figure 2.1).

Huisgen’s 1,3-dipolar cycloaddition of an azide to an alkyne is a reaction that has been around since the late 1960’s. As a consequence of the lack of regioselective control over the configuration of the resulting triazole ring, it is a reaction that was underused until it was improved by Sharpless’ group about a decade ago. Sharpless et al. introduced the use of a copper(I) catalyst, which allows only the formation of the 1,4-disubstituted product and not the 1,5-regioisomer. The CuAAC reaction, as it is now known, is part of the class of reactions coined as “click” chemistry by Sharpless et al. in 2001. Click reactions are characterized by high yields, a tolerance to a wide range of substituent groups and benign reaction conditions. Since the synthesis of dendrimers is a cascade process necessitating the use of diverse moieties, click chemistry is ideally suited for this purpose.

The synthesis of dendrimers reported here required the design of multifunctional building blocks which allow the structure to become more hyperbranched with each increasing generation. Towards this goal we selected a backbone molecule with a diol at one end and an azide at the other (Figure 2.1). The azide is clicked to the peripheral alkynes of the growing dendrimer,
regenerating the original alcohol functionality. The sequence can then be repeated to construct subsequent generations in a divergent manner.

The acetylene-terminated dendrimers can be subsequently functionalized upon reaction with an appropriate azide molecule through the use of click chemistry. The hydrophilic azide terminated molecules selected for this purpose were either a phosphonate azide, to confer aqueous solubility and anti-scalant effect, or a cationic amino azide, for aqueous solubility and bactericidal effect. As previously discussed, small molecules and polymers functionalized with phosphonate groups are known to be effective at controlling the build-up of low-solubility inorganic salts, known as scale, in industrial processes employing water, such as water treatment and pulp and paper. Cationic amino groups are well known to exert a bactericidal effect by disrupting the integrity of bacterial cell membranes eventually leading to cell lysis and death.

**2.2 - Results and Discussion**

The synthesis of dendrimers requires the selection of appropriate building blocks with compatible functionalities. Since we wished to employ click
chemistry both in the synthesis and functionalization of our dendrimers, it was necessary to select two building blocks: one with an acetylene moiety and another with an azide group. Towards this goal, we prepared an azide functionalized 2,2-bis(hydroxymethyl)propanoic acid (bis-MPA) molecule as our azide building block (Scheme 2.1). The latter was prepared starting from bis-MPA (1) and bromoethanol (3). The diol terminal of the bis-MPA unit was first protected with an acetonide group, to yield (2) which could be purified simply by first neutralizing the p-toluenesulfonic acid, followed by a filtration. In parallel, bromoethanol (3) was azidified through the use of sodium azide to yield (4), which was purified by extraction. The two could then be coupled together through the use of N,N'-dicyclohexylcarbodiimide (DCC)/4-dimethylaminopyridine (DMAP), to yield (5), a molecule with a protected diol at one end and an azide at the other. Molecule (5) required column chromatography for its purification, which was made more challenging by the fact that it is not UV-active. Compound (5) was then deprotected to yield (6), with azide and diol terminals. Since the protecting group is volatile once removed, the purification of (6) necessitated only a filtration and evaporation. We selected propargyl bromide

Scheme 2.1 – Synthesis of Backbone Unit
as our acetylene-terminated building block. It was coupled through its bromo
group to the selected pentaerythritol core through an etherification with its
terminal alcohol groups, yielding the tetra-acetylene-terminated structure, \( \text{G0} \to \equiv \) (Scheme 2.2), which was purified by column chromatography. During a
repetition of the synthesis of \( \text{G0} \to \equiv \), a calculation error unexpectedly led to the
construction of a trifunctionalized structure, \( 3\text{G0} \to \equiv \), which could be employed
to construct asymmetrical dendritic structures. The synthetic methodologies for
all of the molecules discussed to this point have been published elsewhere,\(^{40,41}\) facilitating their preparation and characterization. Characterization of the new
dendrimers discussed below was achieved by comparison with the known spectra
of the starting materials.

The azide functionalized bis-MPA unit (5) was then clicked to \( \text{G0} \to \equiv \)
through the use of the CuAAC reaction to yield the \( \text{G1} \) dendrimer with eight
terminal hydroxide groups (Scheme 2.2), which was purified by column
chromatography. Due to its hydrophilic backbone and multiple terminal
hydroxide groups, it is both quite polar and hydrophilic, making it only slightly
soluble in most organic solvents. This makes loading it on a column in anything
other than pure methanol (which is far too polar to achieve good separation in)
quite difficult. Conversely, \( \text{G1} \) is unfortunately soluble enough that precipitation
and washing steps were not a facile process. Because of the difficulty in purifying
\( \text{G1} \), a protected version of the azide functionalized bis-MPA molecule was
clicked onto \( \text{G0} \to \equiv \) instead. The resulting protected hydroxide-terminated
dendrimer, pG1, was then deprotected to yield the hydroxide-terminated dendrimer, which was far easier than G1 to purify by column chromatography.
Scheme 2.2 – Synthesis of Dendrimers
Many varied attempts to attach propargyl bromide to \( G_1 \) using similar procedures to that used to prepare \( G_0 \) failed to generate the desired \( G_1 \) dendrimer. Since the important component was that the structure have acetylene termini, and not the ether linkage, we considered a variation on this structure. Thus, an esterification with 4-pentynoic acid, followed by column chromatography, was used to prepare \( G_1\text{-ester} \), with eight terminal acetylene groups. Molecule (6) was then clicked onto the growing dendrimer to yield \( pG_2 \), with sixteen terminal protected hydroxides, which was purified by dialysis. A deprotection step with the same cationic resin used for the deprotections of \( pG_1 \) and (5) failed to generate the desired hydroxide terminated \( G_2 \). Thus a deprotection using bismuth trichloride was employed instead. A simple filtration to remove the remaining salts was the only purification necessary to yield pure \( G_2 \) with sixteen terminal hydroxides. Another esterification with 4-pentynoic acid was then used to generate \( G_2\text{-ester} \), with sixteen terminal acetylenes. The purification of \( G_2\text{-ester} \) was quite simple, employing only washing and filtration steps. A click reaction was then used to attach an additional layer of (5) onto \( G_2\text{-ester} \), generating \( pG_3 \), with thirty two terminal protected hydroxide groups. The purification of \( pG_3 \) was conducted by precipitation. The compound was then deprotected using the same cationic resin as for the deprotections of (5) and \( pG_1 \), to yield \( G_3 \) with thirty two terminal hydroxides. A subsequent esterification with 4-pentynoic acid was then employed to generate \( G_3\text{-ester} \), with thirty two terminal acetylene groups. The purification of \( G_3\text{-ester} \) presented quite a challenge. The crude product mixture was first dissolved in
dichloromethane (DCM) and filtered to remove the salts. The solvent was evaporated and the product mixture was dialyzed overnight. $^1$H NMR taken at this point revealed that in addition to all of the expected product peaks, there were two extra peaks in the methyl and triazole regions of the spectrum. Since the $^1$H NMR indicated that the product may not yet be pure, the mixture was then dialyzed for another night. $^1$H NMR taken the next day revealed the same extra peaks, so an alumina column purification was attempted. The column was first flushed with DCM and the product subsequently eluted in methanol. $^1$H NMR again revealed the same two extra peaks, so another alumina column was undertaken, this time in hexanes/ethyl acetate. The product would only elute if an extremely large volume of pure ethyl acetate was used. Again, the same extra peaks were present in the $^1$H NMR spectrum. By this point so much of the product had been lost in attempted purification that the synthesis had to be repeated. Again the crude product was dissolved in DCM and filtered to remove the salts. This time the product was precipitated out of DCM using hexanes. Ether and hexanes washes were then carried out. While all of the above mentioned purification did increase the purity of the product, the $^1$H NMR still contained the same two extra peaks. A $^{13}$C{$^1$H} NMR taken at this point indicated the presence of all of the product peaks. A MALDI-TOF MS analysis of the product revealed a broad distribution of peaks centered around the theoretical mass of the product. Our results suggest that G3-ester is sufficiently pure, and that the two extra peaks may be caused by immobility of the
internal structure of the dendrimer which differentiates some regions from the equivalent outer regions.

We subsequently made an attempt to synthesize \( pG4 \) by clicking (5) onto \( G3\)-ester\( \equiv \equiv \), using the same general procedure employed for the synthesis of \( pG1 \), \( pG2 \) and \( pG3 \). Purification was carried out using two separate alumina columns in i) DCM and methanol and ii) hexanes and ethyl acetate. We did not obtain a pure sample of \( pG4 \) despite a subsequent attempt at dialysis. It turned out that some indicative peaks in the \( ^1H \) NMR were those of the starting material, (5). The only logical possibility at this juncture was that \( G3\)-ester\( \equiv \equiv \) is too crowded for complete functionalization upon reaction with an azide. Despite the fact that the spectra obtained of the impure mixture did indicate the presence of \( G3\)-ester\( \equiv \equiv \), two variations on the synthetic conditions were undertaken: the ratios of reagents were kept the same but the solvent system and temperature were varied. The crude mixtures of both reactions were dialyzed overnight. \( ^1H \) NMR spectra taken the next day were almost identical to those obtained previously. We are continuing our efforts to resolve the problems associated with the synthesis of \( pG4 \).

Once synthesized, the acetylene-terminated dendrimers (\( G0\equiv \equiv \), \( G1\)-ester\( \equiv \equiv \) and \( G2\)-ester\( \equiv \equiv \)) were functionalized with a protected phosphonate azide (Scheme 2.3) and a protected amino azide (Scheme 2.4), respectively. The
protected phosphonate azide was synthesized from the commercially available phosphonate bromide (Scheme 2.3). The bromo group of (7) was azidified with sodium azide to yield (8), which was purified by extraction. The reaction was easy to follow by $^{31}$P{$_1^1$H} NMR as the peak at 26 ppm (7) disappeared and the peak at 30 ppm (8) grew. Compound (8) was then clicked to the periphery of any of the acetylene-terminated dendrimers. Deprotection of the resulting structures was intended to yield the desired phosphonate terminated dendrimers. Unfortunately, two different literature procedures used in an attempt to deprotect G2-PO(OEt)$_2$ did not result in the desired –PO(OH)$_2$ structures. This difficulty with the deprotection reactions prevented us from performing anti-scalant testing on these molecules.

The protected amino azide (12) was synthesized starting from the commercially available amino alcohol (9) (Scheme 2.4). The amine group of (9) was first protected with a t-butoxycarbonyl (BOC) group to yield (10) which was purified by washing with a basic solution, followed by an acidic solution, brine and finally water. The alcohol group of (10) was then converted to a mesylate through the use of methane sulfonyl chloride to yield (11) which was purified by washing with water and brine. The mesylate group of (11) was subsequently
azidified to afford (12), which was purified by extraction. After the functionalization of $G_0$ with (12), a literature search revealed another amino azide which could be made in three steps instead of four, and at a lower cost. This second amino azide was constructed from the commercially available amino bromide (13). The bromo group of (13) could then immediately be taken to the desired azide moiety to yield (14), which was purified by a basic work-up, followed by extraction (Scheme 2.4). The amino moiety of (14) was protected with a BOC group to yield (15) which was purified by column chromatography, because it facilitated the purification of the resulting dendrimers. The higher generation dendrimers, $G_{1}$-ester and $G_{2}$-ester were both functionalized with this molecule (14). The only difference between the two final amino azide structures is that the latter has one extra carbon atom between the amine and azide functionalities. After being clicked to the acetylene-terminated dendrimers ($G_0$, $G_{1}$-ester and $G_{2}$-ester), the resulting structures were deprotected using trifluoroacetic acid (TFA) to yield the desired cationic amino-terminated macromolecules ($G_0$-$\text{NH}_3^+$, $G_{1}$-$\text{NH}_3^+$ and $G_{2}$-$\text{NH}_3^+$). The effectiveness of these dendrimers as bactericides was then tested; the results are described in Chapter 3.
2.3 - Conclusions

In conclusion, click chemistry is an effective tool both for the construction and functionalization of dendrimers. Through the use of versatile, compatible building blocks, we have prepared dendrimers with a high density of end groups (4-32), including hydroxide and acetylene. These dendrimers of generations 0-3, with 4, 8, 16 and 32 terminal acetylene groups, respectively, can be functionalized with a wide variety of surface groups through the use of the CuAAC reaction. We have demonstrated this by functionalizing generations 0-2 with phosphonate groups, for potential anti-scalant applications, and cationic amino groups, for bactericidal activity. The potential of these functionalized dendrimers for bactericidal applications is explored in Chapter 3.

2.4 - Experimental

2.4.1 - Synthesis of Building Blocks

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

Para-toluenesulfonic acid (0.598g, 0.00314mol) was added to a stirred solution of 2,2-bis(hydroxymethyl)propanoic acid (1) (8.43g, 0.0628mol) in acetone (34mL), under nitrogen, in a 250mL round bottom flask. 2,2-dimethoxypropane (9.81g, 0.0942mol) and magnesium sulfate (0.756g, 0.00628mol) were then added to the flask. The reaction mixture was then left to stir, under nitrogen, for 48h. A solution of ammonia in dioxane (6.27mL, 0.5M) was then added to neutralize the acid. The crude mixture was then filtered, and the solvent evaporated to yield the product as a white powder (9.30g, 0.0534mol, 85% yield). $^1$H NMR (400MHz, CDCl$_3$): $\delta = 1.20$ (s, 3H, -CO-C-CH$_3$), 1.40 (s,
3H, -O-C-CH₃), 1.43 (s, 3H, -O-C-CH₃), 3.67 (t, 2H, -O-CH₂-C-CO-), 4.18 (d, 2H, -O-CH₂-C-CO-) ppm. $^{13}$C\{¹H\} NMR (300MHz, CDCl₃): $\delta$ = 18.42 (-CO-C-CH₃), 22.03 (-O-C-CH₃), 25.07 (-O-C-CH₃), 41.71 (-CO-C-), 65.81 (-O-CH₂-C-), 98.28 (-O-C-(CH₃)₂), 180.15 (-CO-) ppm.

Synthesis of Azidoethanol (4)

A mixture of bromoethanol (3) (2.37g, 0.0190mol) and sodium azide (2.47g, 0.0380mol) in water (4mL) was left stirring overnight at 70°C. The reaction mixture was then extracted with DCM, the organic layer was then isolated and dried with magnesium sulfate. The solvent was subsequently evaporated to yield the product as a yellow oil (1.36g, 0.0156mol, 82% yield). $^1$H NMR (400MHz, CDCl₃): $\delta$ = 3.46 (t, 2H, N₃-CH₂-CH₂-), 3.79 (q, 2H, N₃-CH₂-CH₂-) ppm. $^{13}$C\{¹H\} NMR (300MHz, CDCl₃): $\delta$ = 53.6 (N₃-CH₂-CH₂-), 61.6 (N₃-CH₂-CH₂-) ppm.

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

A solution of azidoethanol (4) (0.74g, 0.0085mol), 2,2,5-trimethyl-1,3-dioxane-5-carboxylic acid (2) (2.22g, 0.0128mol) and DMAP (0.519g, 0.00425mol) in anhydrous DCM (10mL) was left stirring, under nitrogen, for 5min. DCC (2.10g, 0.0102mol) was then added to the reaction mixture, which was then left stirring under nitrogen, at room temperature, overnight. The precipitate was then filtered off and the solvent was evaporated to yield a residue that was purified by column chromatography (1:7 ethyl acetate:hexanes) to yield the product as a white solid (1.25g, 0.00514mol, 60% yield). $^1$H NMR (400MHz, CDCl₃): $\delta$ = 1.21 (s, 3H, -CO-C-CH₃), 1.39 (s, 3H, -O-C-CH₃), 1.44 (s, 3H,
-O-C-CH₃), 3.49 (t, 2H, N₃-CH₂-CH₂-), 3.68 (d, 2H, -O-CH₂-C-CO-), 4.21 (d, 2H, -O-CH₂-C-CO-), 4.33 (t, 2H, N₃-CH₂-CH₂-) ppm. ¹³C{¹H} NMR (300MHz, CDCl₃): δ = 18.5 (-CO-C-CH₃), 22.3 (-O-C-CH₃), 24.9 (-O-C-CH₃), 42.0 (-C-CH₃), 49.8 (N₃-CH₂-CH₂-), 63.6 (N₃-CH₂-CH₂-), 65.9 (-O-CH₂-C-), 98.1 (-C-CH₃), 174.0 (-CO-) ppm.

Synthesis of 2-azidoethyl 3-hydroxy-2-(hydroxymethyl)-2-methylpropanoate (6)

A spoonful of Dowex cationic resin was added to a solution of (5) (0.301g, 0.00173mol) in methanol (6mL). The mixture was then left stirring overnight at room temperature. The supernatant was then decanted off and the resin was rinsed several times with methanol. The supernatant was then combined with the rinsings and the solvent was evaporated to yield the product as a colourless oil (0.238g, 0.00117mol, 95% yield). ¹H NMR (400MHz, CDCl₃): δ = 1.09 (s, 3H, -CO-C-CH₃), 2.47 (s, 2H, -OH), 3.41 (m, 2H, N₃-CH₂-CH₂-), 3.50 (dd, 2H, -CH₂-OH), 3.80 (dd, 2H, -C-CH₂-OH), 4.28 (t, 2H, N₃-CH₂-CH₂-) ppm. ¹³C{¹H} NMR (300MHz, CDCl₃): δ = 17.0 (CH₂-C-), 49.3 (CH₃-C), 49.8 (N₃-CH₂-CH₂-), 63.5 (N₃-CH₂-CH₂-), 68.2 (CH₂-OH), 175.5 (-CO-) ppm.⁴⁴

2.4.2 - Synthesis of Backbone

Synthesis of G₀

Anhydrous DMF (25mL) was added by syringe to a round bottom flask containing pentaerythritol (G₀) (2.00g, 0.0147mol) and KOH (12.50g, 0.223mol), with stirring, under nitrogen. The mixture was left stirring for 5min, under nitrogen, at 0°C. A solution of propargyl bromide (20.00g, 0.168mol) in toluene (80%) was then added dropwise to the reaction mixture over 30min. The reaction
was then heated at 40°C overnight, under nitrogen. Water (100mL) was added to the mixture, which was then extracted with diethyl ether (3x50mL). The organic layers were isolated, combined, and washed with water (3x50mL) and brine (3x50mL). The organic layer was isolated and dried with sodium sulfate. The solvent was then removed to yield an orange oil which was purified by column chromatography (2:8 ethyl acetate:hexanes) to give the product as an orange-brown solid (1.75g, 0.00606mol, 84% yield). $^1$H NMR (400MHz, CDCl$_3$): δ = 2.40 (t, 4H, CH-C-CH$_2$-), 3.53 (s, 8H, -C-CH$_2$-O-), 4.12 (d, 8H, CH-C-CH$_2$-) ppm. $^{13}$C{$_1^1$H} NMR (300MHz, CDCl$_3$): δ = 44.7 (-C-CH$_2$-O-), 58.7 (CH-C-CH$_2$), 69.0 (-C-CH$_2$-O-), 74.0 (CH-C-CH$_2$), 80.0 (CH-C-CH$_2$) ppm. ESI-MS: m/z = 311.1 [M + Na$^+$].

**Synthesis of 3G0**

During the synthesis of G0, a calculation error led to the unexpected synthesis of a similar molecule where only 3 of the four hydroxide groups were reacted with propargyl bromide, hereafter known as 3G0. This synthesis was achieved by the same method outlined above for G0 with the exception that the masses of propargyl bromide and KOH were inverted. Thus for the same amount of pentaerythritol (2.00g, 0.0147mol), 12.50g of propargyl bromide (0.105mol) and 20.00g of KOH (0.357mol) were used. The same purification techniques were used as with the synthesis of G0 to yield 3G0 as an orange-brown solid (0.661g, 0.00264mol, 18% yield). $^1$H NMR (400MHz, CDCl$_3$): δ = 2.42 (t, CH-C-CH$_2$-, 3H), 3.56 (s, C-CH$_2$-O-,8H), 3.68 (d, C-CH$_2$-OH, 2.5H), 4.12 (d, CH-C-CH$_2$-, 7H) ppm. $^{13}$C{$_1^1$H} NMR (300MHz, CDCl$_3$): δ =
44.6 (C-CH₂-O-), 58.7 (CH-C-CH₂), 65.0 (C-CH₂-OH), 70.1 (C-CH₂-O-), 74.5 (CH-C-CH₂), 79.6 (CH-C-CH₂) ppm.

Synthesis of pG1

A solution of CuSO₄·5H₂O (0.019g, 0.0000767mol) in water (0.5mL) was added to a round bottom flask containing a stirred solution of G₀ (0.077g, 0.000266mol) and (5) (0.268g, 0.00110mol) in tetrahydrofuran (THF) (0.5mL). Sodium ascorbate (0.032g, 0.000160mol) was then added and the mixture was allowed to react overnight. The product was purified by column chromatography (100% methanol) to yield a yellow oil (0.278g, 0.000220mol, 83% yield).

¹H NMR (400MHz, CD₃OD): δ = 1.00 (s, 3H, -CO-C-CH₃), 1.22 (s, 3H, -O-C-(CH₃)₂), 1.39 (s, 3H, -O-C-(CH₃)₂), 3.44 (s, 2H, -C-CH₂-O-CH₂), 3.63 (dd, 2H, -CO-C-CH₂-O-), 4.07 (dd, 2H, -CO-C-CH₂-O-), 4.51 (s, 2H, -O-CH₂-C-N-), 4.54 (t, 2H, -N-CH₂-CH₂-O-), 4.72 (s, 2H, -N-CH₂-CH₂-O-), 8.04 (s, 1H, -C-CH₃) ppm.

¹³C{¹H} NMR (300MHz, CD₃OD): δ = 17.19 (-C-CH₃), 20.52 (-O-C-(CH₃)₂), 24.93 (-O-C-(CH₃)₂), 41.78 (-C-CH₃), 45.11 (-C-CH₂-O-), 48.90 (-N-CH₂-CH₂-O-), 62.63 (-N-CH₂-CH₂-O-), 64.06 (-CH₂-O-CH₂-C-N-), 65.47 (-C-CH₂-O-C-), 68.65 (-C-CH₂-O-CH₂-C-N-), 97.97 (-O-C-(CH₃)₂), 124.07 (-C-CH-N-), 145.00 (-C-N-), 173.80 (-O-CH₂-) ppm. MALDI-MS: m/z = 1267.3 [M + Li⁺]

Synthesis of G1

From G₀ → G₁:

A solution of CuSO₄·5H₂O (0.019g, 0.0000767mol) in water (0.5mL) was added to a round bottom flask containing a stirred solution of G₀ → (0.077g, 0.000266mol) and (5) (0.268g, 0.00110mol) in tetrahydrofuran (THF) (0.5mL). Sodium ascorbate (0.032g, 0.000160mol) was then added and the mixture was allowed to react overnight. The product was purified by column chromatography (100% methanol) to yield a yellow oil (0.278g, 0.000220mol, 83% yield).
0.000266mol) and (6) (0.260g, 0.00128mol) in THF (0.5mL). Sodium ascorbate (0.032g, 0.000160mol) was then added and the mixture was allowed to react overnight. The product was purified by column chromatography (100% methanol) to yield an orange oil (0.148g, 0.000134mol, 50% yield).

This reaction was repeated at an elevated temperature (65°C) and the yield obtained was even poorer (40%).

From pG1:

*Using Dowex Cationic Resin:*

Dowex Cationic Resin (6.72g) was added to a solution of pG1 (9.69g, 0.0077mol) in methanol (485mL). The mixture was then left stirring overnight. The resin was then filtered off and the solvent evaporated to yield G1 as a viscous orange oil (6.68g, 0.0061mol, 79% yield)

*Using BiCl₃:*

BiCl₃ (0.0023g, 0.00000729mol) and a drop of D₂O were added to a solution of pG1 (0.044g, 0.0000349mol) in deuterated acetonitrile (1g). The reaction mixture was then left stirring for 30min and a ¹H NMR was taken. The mixture had not reacted at all and thus more BiCl₃ (0.003g, 0.0000095mol) was added and the mixture was left stirring for another hour. Another ¹H NMR revealed that a reaction had still not taken place.

¹H NMR (400MHz, CD₃OD): δ = 1.08 (s, 3H, -CO-C-CH₃), 3.45 (s, 2H, -C-CH₂-O-), 3.61 (q, 4H, -C-CH₂-OH), 4.51 (t, 4H, -O-CH₂-C-N-), 4.52 (s, 2H, -N-CH₂-CH₂-O-), 4.70 (t, 2H, -N-CH₂-CH₂-O-), 8.04 (s, 1H, -C-CH-N-) ppm. ¹³C{¹H}
NMR (300MHz, CD$_3$OD): $\delta = 17.40$ (-C-CH$_3$), 46.53 (C-CH$_2$-O-), 50.42 (-C-CH$_3$), 51.78 (-N-CH$_2$-CH$_2$-O-), 63.86 (-N-CH$_2$-CH$_2$-O-), 65.40 (-O-CH$_2$-C-N-), 65.85 (-C-CH$_2$-OH-), 70.09 (C-CH$_2$-O-), 125.77 (-C-CH-N-), 146.35 (-O-CH$_2$-C-N-), 176.06 (-C-CO-O-) ppm. MALDI-MS: m/z = 1107.6 [M + Li$^+$].

**Attempted Synthesis of G1**

**Using KOH**

Anhydrous DMF (5mL) was added by syringe to a round bottom flask containing G1 (0.050g, 0.0000454mol) and KOH (0.076g, 0.00136mol), with stirring, under nitrogen. The mixture was left stirring for 5min, under nitrogen, at 0°C. A solution of propargyl bromide (0.119g, 0.0010mol) in toluene (80%) was then added dropwise to the reaction mixture over 30min. The reaction was then heated at 40°C overnight, under nitrogen.

**Using K$_2$CO$_3$**

G1 (0.050g, 0.000045mol), K$_2$CO$_3$ (0.20g, 0.0015mol) and a solution of propargyl bromide (0.119g, 0.0010mol) in toluene (80%) were dissolved in anhydrous DMF (3mL) in a round bottom flask, under nitrogen. The reaction mixture was then left stirring, under nitrogen, overnight at 50°C.

**Using NaH**

32 equiv NaH, 32 equiv propargyl bromide

Anhydrous THF (2mL) was added to NaH (0.038g, 0.00157mol) in a round bottom flask, under nitrogen, with stirring. A solution of G1 (0.054g, 0.000049mol) in dry THF (2.5mL) and dry DMF (0.5mL) was then added dropwise over 30min. The reaction mixture was then left stirring, under nitrogen,
for 3h. A solution of propargyl bromide (0.186g, 0.00157mol) in toluene (80%) was then dissolved in dry THF (2mL) and added dropwise to the reaction mixture over 20min. The mixture was then left stirring, under nitrogen, overnight.

8.4 equiv NaH, 32 equiv propargyl bromide

Anhydrous THF (1mL) was added to a solution of NaH (0.008g, 0.000343mol) in mineral oil (60%) in a round bottom flask, under nitrogen, with stirring. A solution of \(G1\) (0.045g, 0.0000409mol) in dry THF (1.5mL) and dry DMF (0.5mL) was then added dropwise over 30min. The reaction mixture was then left stirring, under nitrogen, for 3h. A solution of propargyl bromide (0.186g, 0.00157mol) in toluene (80%) was then dissolved in dry THF (2mL) and added dropwise to the reaction mixture over 20min. The mixture was then left stirring, under nitrogen, overnight.

Thin layer chromatography (TLC) and \(^1\)H NMR spectra taken of the products of the above discussed reactions revealed that none of them resulted in the desired \(G1\) structure. Since the important component was that the structure have acetylene termini, and not the ether linkage, we moved on to an esterification with 4-pentynoic acid instead of an etherification with propargyl bromide (Scheme 2.2).

**Synthesis of \(G1\)-ester**

DMAP (2.97g, 0.0243mol) was added to a stirred mixture of \(G1\) (6.68g, 0.0061mol) in anhydrous DCM (267mL), under nitrogen, in a 500mL round bottom flask. DCC (15.02g, 0.0728mol), pyridine (133mL) and 4-pentynoic acid (7.14g, 0.0728mol) were then added to the flask. The reaction mixture was then
left stirring, under nitrogen, overnight. The crude mixture was then filtered and the solvent evaporated. The product was then purified by column chromatography (ethyl acetate) to yield the product as a yellow oil (7.39g, 0.0042mol, 70% yield). $^1$H NMR (400MHz, CD$_3$OD): $\delta = 1.19$ (s, 3H, -C-CH$_3$), 2.26 (t, 2H, -C-CH), 2.46-2.53 (m, 8H, -O-C-CH$_2$-CH$_2$-C-), 3.45 (s, 2H, -C-CH$_2$-O-CH$_2$-), 4.22 (q, 4H, -C-(CH$_2$-O-C)-), 4.54 (s, 2H, -C-CH$_2$-O-CH$_2$-C), 4.55 (t, 2H, -CH-N-CH$_2$-CH$_2$-), 4.73 (t, 2H, -C-CH-N-CH$_2$-), 8.01 (s, 1H, -N-CH$_2$-) ppm.

$^{13}$C{$^1$H} NMR (300MHz, CD$_3$OD): $\delta = 15.10$ (-CH$_2$-C-CH), 18.20 (-C-CH$_3$), 34.31 (-CH$_2$-CH$_2$-C-CH), 46.57 (-C-CH$_2$-O-CH$_2$-C-N-), 47.70 (-C-CH$_3$), 50.22 (-N-CH$_2$-CH$_2$-O-), 64.51 (-N-CH$_2$-CH$_2$-O-), 65.59 (-C-CH$_2$-O-CH$_2$-C-N-), 66.61 (-C-CH$_2$-O-C-), 70.19 (-C-CH$_2$-O-CH$_2$-C-N-), 70.55 (-C-CH), 83.54 (-C-CH), 125.49 (-C-CH-N-), 146.51 (-C-CH-N-), 172.84 (-O-CH$_2$-), 173.72 (O-CH$_3$-) ppm.

MALDI-MS: m/z = 1747.5 [M + Li$^+$$]$

**Synthesis of pG2**

A solution of CuSO$_4$·5H$_2$O (0.146g, 0.000586mol) in water (10mL) was added to a round bottom flask containing a stirred solution of G1-ester $\equiv$ (1.70g, 0.000977mol) and (5) (2.28g, 0.00938mol) in THF (10mL). Sodium ascorbate (0.232g, 0.00117mol) was then added and the mixture was allowed to react overnight. The product mixture was evaporated, dissolved in a minimum of methanol and run through a silica plug to remove the copper salts. The product was then purified by dialysis (MWCO = 1000Da, methanol) to yield a brown solid (2.05g, 0.000556mol, 57% yield). $^1$H NMR (400MHz, CD$_3$OD): $\delta = 1.02$ (s, 6H, CH$_3$-C-O-CH$_2$-C-CH$_3$), 1.10 (s, 3H, C-C-O-CH$_2$-C-CH$_3$), 1.28 (s, 6H, CH$_3$-
Synthesis of G2

BiCl₃ (0.0009g, 0.0000029mol) and a drop of water were added to a stirred solution of pG2 (0.025g, 0.00000678mol) in acetonitrile (1.1mL). The reaction mixture was then left stirring for 60h at 45°C. The product mixture was then evaporated, dissolved in methanol, and filtered to remove the bismuth salts. The product was then evaporated to yield a brown oil (0.020g, 0.00000604mol, 3710.1[M + Na⁺].
89% yield). $^1$H NMR (400MHz, CD$_3$OD): $\delta$ = 1.10 (s, 9H, -C-CH$_3$), 2.69 (t, 4H, -C-CH$_2$-CH$_2$-C-N-), 2.95 (t, 4H, -C-CH$_2$-CH$_2$-C-N-), 3.43 (s, 2H, -C-CH$_2$O-CH$_2$-C-N-), 3.60 (q, 8H, -C-CH$_2$-OH), 4.10 (s, 4H, -C-CH$_2$O-C-), 4.48 (m, 8H, -N-CH$_2$-CH$_2$O-C-C-CH$_3$-CH$_2$-OH, -O-CH$_2$-CH-N-CH$_2$-CH$_2$-OH), 4.66 (m, 4H, -N-CH$_2$-CH$_2$O-C-C-CH$_3$-CH$_2$-OH), 4.71 (s, 2H, -O-CH$_2$-CH-N-CH$_2$-CH$_2$O-, -O-CH$_2$-CH-N-CH$_2$-CH$_2$O-), 7.86 (s, 2H, -CH$_2$-CH$_2$-C-N-), 8.03 (s, 1H, -O-CH$_2$-C-CH-N-) ppm. $^{13}$C{$^1$H} NMR (300MHz, CD$_3$OD): $\delta$ = 17.41 (-C-H$_3$-C-CH$_2$-OH), 18.08 (-C-O-CH$_2$-C$_3$H$_3$), 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$_2$-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$_2$-), 64.45 (-O-CH$_2$-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$_3$), 70.13 (-C-CH$_2$O-CH$_2$-C-N-), 124.35 (-C-CH$_2$-CH$_2$-C-CH-N-), 125.61 (-C-CH$_2$O-CH$_2$-C-CH-), 146.37 (-C-CH$_2$-O-CH$_2$-C-CH-), 147.58 (-C-CH$_2$-CH$_2$C-CH-N-), 173.47 (-O-C-CH$_2$-O-C-CH$_3$), 173.70 (-C-CH$_2$-O-C-CH$_2$-), 176.08 (-O-C-CH$_2$-CH$_2$-C-) ppm. MALDI-MS: m/z = 3388.5 [M + K$^+$].

**Synthesis of G2-ester**

DMAP (0.159g, 0.00130mol) was added to a stirred mixture of G2 (0.273g, 0.0000811mol) in anhydrous DCM (6.6mL), under nitrogen. 1-ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (0.373g, 0.00195mol), pyridine (3.3mL) and 4-pentynoic acid (0.191g, 0.00195mol) were then added to the flask. The reaction mixture was then left stirring, under nitrogen, overnight.

The crude product mixture was then washed with methanol, dissolved in
chloroform and filtered to yield the product as a brown oil (0.27g, 0.000058mol, 63% yield). \(^1\)H NMR (400MHz, CDCl\(_3\)): \(\delta = 1.12 \ (s, \ 3H, \ -N-C-CH_2-CH_2-C-O-CH_2-C-CH_3), \ 1.20 \ (s, \ 6H, \ -CH-C-CH_2-CH_2-C-O-CH_2-C-CH_3), \ 1.98 \ (s, \ 4H, \ -C-CH), \ 2.50 \ (m, \ 16H, \ -C-CH_2-CH_2-C-CH), \ 2.70 \ (t, \ 4H, \ -O-C-CH_2-CH_2-C-N-), \ 2.97 \ (t, \ 4H, \ -O-C-CH_2-CH_2-C-N), \ 3.42 \ (s, \ 2H, \ -C-CH_2-O-CH_2), \ 4.10 \ (m, \ 4H, \ -C-CH_2-O-C-CH_2-CH_2-C-CH), \ 4.51 \ (m, \ 8H, \ -CH_2-CH_2-C-N-N-CH_2-CH_2-O-, \ -C-CH_2-O-CH_2-C-N-CH_2-CH_2-), \ 4.59 \ (m, \ 4H, \ -C-CH_2-O-CH_2-C-N-C-N-C-CH_2-O-CH_2-C-N-), \ 7.48 \ (s, \ 2H, \ -O-C-CH_2-CH_2-C-N-), \ 7.78 \ (s, \ 1H, \ -CH_2-O-CH_2-C-N-) \ ppm. \(^{13}\)C\(^{1}\)H NMR (300MHz, CDCl\(_3\)): \(\delta = 14.26 \ (\ -C-H_2-C-CH), \ 17.58 \ (\ -N-C-CH_2-CH_2-C-O-CH_2-C-CH_3), \ 17.68 \ (\ CH-C-CH_2-CH_2-C-O-CH_2-C-CH_3), \ 20.76 \ (\ -O-C-CH_2-CH_2-C-N-), \ 33.11 \ (\ -C-H_2-CH_2-C-CH), \ 33.15 \ (\ -O-C-CH_2-CH_2-C-N-), \ 43.19 \ (\ -C-H_2-CH_2-C-N-), \ 45.17 \ (\ -CH_2-CH_2-C-CH-N-CH_2-CH_2-), \ 46.27 \ (\ -N-C-CH_2-CH_2-C-O-CH_2-C-CH_3), \ 46.29 \ (\ CH-C-CH_2-CH_2-C-O-CH_2-C-CH_3), \ 48.67 \ (\ -N-CH_2-CH_2-CH_2-O-C-CH_2-O-CH_2-C-CH_2-C-CH), \ 63.10 \ (\ -N-CH_2-CH_2-CH_2-O-C-CH_2-O-CH_2-C-CH), \ 64.75 \ (\ -CH_2-O-CH_2-C-CH-N-CH_2-CH_2-), \ 65.10 \ (\ -N-C-CH_2-CH_2-C-O-CH_2-C-CH_3), \ 65.25 \ (\ CH-C-CH_2-CH_2-C-O-CH_2-C-CH_3), \ 69.02 \ (\ -C-H_2-O-CH_2-), \ 69.34 \ (\ -C-H_2-C-CH), \ 77.20 \ (\ -C-H_2-O-CH_2-), \ 82.26 \ (\ -CH_2-C-CH), \ 121.87 \ (\ -O-C-CH_2-CH_2-C-CH-N-), \ 123.31 \ (\ -CH_2-O-CH_2-C-CH-N-), \ 145.29 \ (\ -CH_2-O-CH_2-C-CH-N-), \ 171.09 \ (\ -O-C-CH_2-CH_2-C-CH), \ 171.98 \ (\ -O-C-CH_2-CH_2-C-CH), \ 172.09 \ (\ -O-C-CH_2-CH_2-C-CH), \ 172.14 \ (\ -O-C-CH_2-O-CH_2-C-CH_2-C-N-) \ ppm. MALDI-MS: m/z = 4669.1 [M + Na\(^{+}\)].
Synthesis of pG3

A solution of CuSO$_4$·5H$_2$O (0.008g, 0.00059mol) in water (2mL) was added to a round bottom flask containing a stirred solution of G2-ester$\equiv$ (0.126g, 0.0000272mol) and (5) (0.127g, 0.000521mol) in THF (2mL). Sodium ascorbate (0.013g, 0.000065mol) was then added and the mixture was allowed to react overnight. The product mixture was evaporated, dissolved in a minimum of methanol and run through a silica plug to remove the copper salts. The product was then precipitated out of chloroform with hexanes. The product was isolated and the remaining solvent evaporated to yield a yellow oil (0.067g, 0.0000079mol, 30% yield). $^1$H NMR (400MHz, CDCl$_3$): $\delta = 1.04$ (s, 12H, CH$_3$-C-O-CH$_2$-C-CH$_3$), 1.11 (s, 3H, C-C-O-CH$_2$-C-CH$_3$), 1.12 (s, 6H, C-C-O-CH$_2$-C-CH$_3$), 1.33 (s, 12H, CH$_3$-C-O-CH$_2$-C-CH$_3$), 1.41 (s, 12H, CH$_3$-C-O-CH$_2$-C-CH$_3$), 2.69 (t, 12H, -O-C-CH$_2$-CH$_2$-), 2.97 (t, 12H, -O-C-CH$_2$-CH$_2$-), 3.40 (s, 2H, C-CH$_2$-O-CH$_2$-C-CH$_3$), 4.10 (s, 12H, -C-CH$_2$-O-C-CH$_3$), 4.12 (dd, 8H, -C-CH$_2$-O-C-CH$_3$), 4.40-4.75 (m, 30H, -CH$_2$-CH$_2$-N-N-N-CH$_2$-CH$_2$-O-, -C-CH$_2$-O-CH$_2$-C-CH-N-CH$_2$-CH$_2$-) ppm.

$^{13}$C{$^1$H} NMR (300MHz, CDCl$_3$): $\delta = 17.48$ (CH$_3$-C-CH$_2$-O-CO-), 18.12 (CH$_3$-C-CH$_2$-O-C-), 20.71 (-CH$_2$-CH$_2$-C-N-), 20.86 (-C-((CH$_3$)$_2$), 26.22 (-C-(CH$_3$)$_2$), 33.05 (-CH$_2$-CH$_2$-C-N-), 41.98 (-N-CH$_2$-CH$_2$-O-CO-C-CH$_2$-O-C-), 46.19 (-N-CH$_2$-CH$_2$-O-CO-C-CH$_2$-O-C-), 48.68 (-C-CH$_2$-O-CH$_2$-C-N-), 48.99 (-CO-C-CH$_3$), 62.82 (-N-CH$_2$-CH$_2$-O-CO-C-CH$_2$-O-C-), 62.94 (-N-CH$_2$-CH$_2$-O-CO-C-CH$_2$-O-
CO-), 65.04 (CH$_3$-CH$_2$-O-CO-), 65.85 (CH$_3$-CH$_2$-O-C-), 68.41 (-C-CH$_2$-O-CH$_2$-C-N-), 77.20 (-C-CH$_2$-O-CH$_2$-C-N-), 98.05 (-O-C-CH$_3$), 122.22 (-C-CH-N-), 146.71 (-C-CH-N-), 171.86 (-CO-C-CH$_2$-O-C-), 171.98 (-CO-C-CH$_2$-O-C-), 172.05 (-CO-C-CH$_2$-O-C-), 173.70 (CH$_3$-C-CH$_2$-O-CO-) ppm. MALDI-MS: m/z = 8580.6 [M + K$^+$].

**Synthesis of G3**

Dowex cationic resin (0.063g) was added to a solution of pG3 (0.153 g, 0.0000179mol) in methanol (8.5mL). The mixture was then left stirring for three days at 45°C. The resin was then filtered off and the solvent evaporated to yield the product as a yellow oil (0.098g, 0.000012mol, 71%yield). $^1$H NMR (400MHz, CD$_3$OD): $\delta = 1.09$ (s, 21H, -C-CH$_3$), 2.69 (m, 12H, -O-C-CH$_2$-CH$_2$-C-N-), 2.95 (m, 12H, -O-C-CH$_2$-CH$_2$-C-N-), 3.43 (s, 2H, -C-CH$_2$-O-CH$_2$-C-), 3.60 (q, 16H, -C-CH$_2$-OH), 4.10 (m, 12H, -C-CH$_2$-O-C-), 4.48 (m, 16H, -C-CH$_2$-O-C-), 4.66 (m, 14H, -N-CH$_2$-CH$_2$-O-), 7.82-8.07 (m, 7H, -C-CH-N-) ppm. $^{13}$C{$^1$H} NMR (300MHz, d$_6$-dimethylsulfoxide (DMSO)): $\delta = 16.65$ (CH$_3$-C-CH$_2$-OH), 16.85 (-C-CH$_2$-O-CH$_2$-C-CH-N-CH$_2$-CH$_2$-O-C-CH$_3$), 16.89 (CH$_3$-C-CH$_2$-O-CO-CH$_2$-CH$_2$-C-CH-N-CH$_2$-CH$_2$-O-C-C-CH$_2$-OH), 20.38 (-CH$_2$-CH$_2$-C-N-), 32.69 (-CH$_2$-CH$_2$-C-N-), 45.77 (-C-CH$_2$-O-CH$_2$-C-CH-N-CH$_2$-CH$_2$-O-C-CH$_2$-), 45.80 (-N-CH$_2$-CH$_2$-O-CO-C-CH$_2$-O-CO-CH$_2$-CH$_2$-C-CH-N-CH$_2$-CH$_2$-O-C-CH$_2$-OH), 48.05 (-C-CH$_2$-O-CH$_2$-C-CH-N-CH$_2$-CH$_2$-O-C-), 48.34 (CH$_3$-C-CH$_2$-OH), 48.53 (CH$_3$-C-CH$_2$-O-CO-CH$_2$-CH$_2$-C-CH-N-CH$_2$-CH$_2$-O-C-O-C-CH$_2$-OH), 50.24 (-N-CH$_2$-CH$_2$-O-CO-C-CH$_2$-OH), 51.25 (-C-CH$_2$-O-
CH$_2$-C-N-), 62.24 (-N-CH$_2$-CH$_2$-O-CO-C-CH$_2$-OH), 62.94 (-C-CH$_2$-O-CH$_2$-C-N-), 63.06 (-N-CH$_2$-CH$_2$-O-CO-C-CH$_2$-O-CO-), 63.68 (-C$_2$-OH), 63.84 (-C-CH$_2$-O-CH$_2$-C-N-), 64.82 (CH$_3$-C-CH$_2$-O-CO-), 122.31 (-C-CH-N-CH$_2$-CH$_2$-O-CO-C-CH$_2$-CH$_2$-O-CO-C-CH$_2$-OH), 122.44 (-C-CH-N-CH$_2$-CH$_2$-O-CO-C-CH$_2$-OH), 123.98 (-C-CH$_2$-O-CH$_2$-C-CH$_2$-N-), 144.08 (-C-CH$_2$-O-CH$_2$-C-CH$_2$-N-), 145.22 (-C-CH-N-CH$_2$-CH$_2$-O-CO-C-CH$_2$-OH), 145.26 (-C-CH-N-CH$_2$-CH$_2$-O-CO-C-CH$_2$-CH$_2$-O-CO-C-CH$_2$-CH$_2$-O-CO-C-CH$_2$-OH), 171.50 (CH$_3$-C-CH$_2$-O-CO-), 171.83 (-C$_2$-C-CH$_2$-O-CO-), 174.20 (-C$_2$-O-C-CH$_2$-OH) ppm. MALDI-MS: m/z = 7894.2 [M + H$^+$].

**Synthesis of G3-ester**

DMAP (0.027g, 0.00022mol) was added to a stirred mixture of G3 (0.071g, 0.0000068mol) in anhydrous DCM (3.3mL), under nitrogen. EDC (0.063g, 0.00033mol), pyridine (1.7mL) and 4-pentynoic acid (0.080g, 0.00033mol) were then added to the flask. The reaction mixture was then left stirring, under nitrogen, overnight. The crude product mixture was then dissolved in DCM and filtered to remove the salts. The solvent was evaporated and the crude product mixture was dialyzed (MWCO=1000, DCM) overnight. The mixture was then dialyzed for another night (MWCO=1000, chloroform). An alumina column was then attempted (methanol). Another alumina column was then undertaken (ethyl acetate).

The synthesis was then repeated. The crude product was dissolved in DCM and filtered to remove the salts. The product was then precipitated out of DCM with hexanes. Ether and hexanes washes were then performed.
Two variations on the synthetic conditions were then undertaken. The same ratios of reagents were used but the solvent system and temperature were altered. In one reaction, a 1:1 ratio of pyridine:DCM was used instead of 1:2. In the other, DMF was employed instead of DCM and the reaction was heated to 50°C. After allowing them to react overnight, the crude mixtures were evaporated until only the DMF remained. Water was then added to the reaction performed in DMF and the product was extracted with DCM. After drying with magnesium sulfate and evaporation of the solvent, the crude mixtures of both reactions were dialyzed overnight (MWCO = 1000Da, DCM). ¹H NMR spectra taken the next day were almost identical to those taken of the products of the previous synthesis of G3-ester.

¹H NMR (400MHz, CDCl₃): δ = 1.07-1.24 (m, 32H (theor. = 21), -CH₃), 1.99 (s, 8H, -CH), 2.43-2.55 (m, 32H, -CO-CH₂=CH₂-C-H), 2.71 (m, 12H, -CO-CH₂=CH₂-C-N-), 2.96 (m, 12H, -CO-CH₂=CH₂-C-N-), 3.42 (s, 2H, -C-CH₂-O-CH₂-C-N-), 4.11 (s, 12H, CH₃-C-CH₂-O-CH₂-CH₂-C-N-), 4.17-4.26 (m, 16H, CH₂-C-CH₂-O-CH₂-CH₂-C-CH), 4.49-4.66 (m, 30H, -N-CH₂=CH₂-O-, -C-CH₂-O-CH₂-C-N-), 7.48-7.80 (m, 7H, -C-CH-N-) ppm. ¹³C{¹H} NMR (300MHz, CDCl₃): δ = 14.29 (-CH₃-C-CH), 17.29 (-C-CH₂-O-CH₂-C-CH-N-CH₂-CH₂-O-C-C-CH₃), 17.62 (CH₃-C-CH₂-O-CH₂-CH₂-C-CH-N-CH₂-CH₂-O-C-C-CH₃), 17.72 (CH₃-C-CH₂-O-CH₂-CH₂-C-CH), 20.9 (-CH₂-CH₂-C-N-), 29.69 (-CH₂-CH₂-C-N-), 33.14 (-CH₂-CH₂-C-CH), 43.60 (-C-CH₂-O-CH₂-C-N-), 45.22 (-C-CH₂-O-CH₂-C-N-N-C-CH₂-CH₂-), 46.33 (-C-CH₃),
ppm. MALDI-MS: m/z = 10 000 - 11 000 (theoretical mass = 10 456).

**Attempted Synthesis of pG4**

An attempt was made to click (5) onto the impure mixture of G3-ester, using the same general procedure employed for the synthesis of pG1, pG2
and pG3, in the hopes that the resulting pG4 structure would be easier to purify than G3-ester. Alumina columns were run on the resulting mixture (DCM and methanol, hexanes and ethyl acetate), but no pG4 was obtained despite a subsequent attempt at dialysis (MWCO=1000Da, methanol).

2.4.3 - Phosphonate Functionalization

Synthesis of diethyl (2-azidoethyl)phosphonate (8)

Sodium azide (0.744g, 0.0114mol) was added to a stirred solution of diethyl (2-bromoethyl)phosphonate (7) (0.467g, 0.00191 mol) in water (5mL). The reaction was left stirring at 80°C overnight. The crude mixture was then extracted with DCM. The organic layer was isolated, dried with magnesium sulfate and the solvent evaporated to yield the product as a yellow oil (0.32g, 0.0015mol, 81% yield). $^{31}$P{$^1$H} NMR (200MHz, CD$_3$OD): δ = 29.90 ppm. $^1$H NMR (400MHz, CDCl$_3$): δ = 1.33 (t, 6H, -CH$_3$), 2.05 (m, 2H, -P-CH$_2$-), 3.54 (m, 2H, -N-CH$_2$-), 4.12 (m, 4H, -CH$_2$-CH$_3$) ppm. $^{13}$C{$^1$H} NMR (300MHz, CDCl$_3$): δ = 16.40 (-CH$_3$), 26.01 (-P-CH$_2$-), 45.38 (N$_3$-CH$_2$-), 61.92 (-CH$_2$-CH$_3$) ppm. $^{45}$

Synthesis of G0-PO(OEt)$_2$

A solution of CuSO$_4$·5H$_2$O (0.008g, 0.000032mol) in water (1mL) was added to a round bottom flask containing a stirred solution of G0 (0.023g, 0.00008mol) and (8) (0.100g, 0.000483mol) in THF (2mL). Sodium ascorbate (0.013g, 0.000064mol) was then added and the mixture was allowed to react overnight. The solvent was evaporated and the product purified by column chromatography (1:1 methanol:acetone) to yield a sticky orange solid (0.089g, 0.000080mol, 99% yield). $^1$H NMR (400MHz, CDCl$_3$): δ = ppm. $^{31}$P{$^1$H} NMR
(200MHz, CDCl₃): δ = 26.82 ppm. ^1^H NMR (400MHz, CDCl₃): δ = 1.30 (t, 6H, -CH₂-CH₃), 2.45 (m, 2H, -P-CH₂-), 3.45 (s, 2H, -C-CH₂-O-CH₂-C-N), 4.09 (q, 4H, -O-CH₂-CH₃), 4.54 (s, 2H, -C-CH₂-O-CH₂-C-N), 4.63 (m, 2H, -N-CH₂-), 7.67 (s, 1H, -CH-) ppm. ^1^C{^1^H} NMR (300MHz, CDCl₃): δ = 16.26 (-C₃H₃), 27.50 (-P-C₂H₂), 44.37 (-N-C₂H₂), 45.13 (-C-CH₂-O-CH₂-C-N), 62.03 (-CH₂-CH₃), 64.77 (-C-CH₂-O-CH₂-C-N), 69.08 (-C-CH₂-O-CH₂-C-N), 122.82 (-CH-), 145.13 (-C-CH-) ppm. MALDI-MS: m/z = 1123.2 [M + Li⁺].

**Synthesis of G1-PO(OEt)₂**

A solution of CuSO₄·5H₂O (0.005g, 0.000021mol) in water (1mL) was added to a round bottom flask containing a stirred solution of G1-ester (0.045g, 0.000026mol) and (8) (0.065g, 0.00031mol) in THF (2mL). Sodium ascorbate (0.008g, 0.000042mol) was then added and the mixture was allowed to react overnight. The product mixture was evaporated and then filtered in THF to remove the salts. The solvent was then evaporated and the product mixture washed five times with ether to yield a viscous yellow oil (0.030g, 0.000088mol, 34% yield). ^3^P{^1^H} NMR (200MHz, CD₃OD): δ = 28.30 ppm. ^1^H NMR (400MHz, CD₃OD): δ = 1.10 (s, 3H, -C-CH₃), 1.17 (t, 12H, -P-CH₂-CH₃), 2.51 (m, 4H, -CH₂-P-O-), 2.68 (t, 4H, -O-C-CH₂-CH₂-C-N), 2.94 (t, 4H, -O-C-CH₂-CH₂-C-N), 3.42 (s, 2H, -C-CH₂-O-CH₂), 4.08 (m, 12H, -C-CH₂-O-C-, -P-O-CH₂-CH₃), 4.50 (m, 4H, -O-CH₂-C-CH-N-CH₂-CH₂), 4.61 (m, 4H, -N-CH₂-CH₂-P), 4.71 (t, 2H, -O-CH₂-C-CH-N-CH₂-CH₂), 7.82 (s, 2H, -CH-N-CH₂-CH₂-P), 8.05 (s, 1H, -O-CH₂-C-CH-N-) ppm. ^1^C{^1^H} NMR (300MHz, CD₃OD): δ = 16.77 (-CH₂-CH₃), 18.09 (-C-CH₃), 21.80 (-C-CH₂-CH₂-C-N), 27.25 (-CH₂-P-),
34.17 (-C-C-H2-CH2-C-N-), 45.49 (-N-C-H2-CH2-O-), 47.64 (-CH2-CH2-P-), 48.66 (-C-CH3), 50.22 (-C-CH2-O-CH2-C-N-), 63.70 (-CH2-CH3), 64.46 (-N-CH2-CH2-O-), 65.57 (-C-CH2-O-CH2-C-N-), 66.44 (CH3-C-CH2-O-), 70.16 (-C-CH2-O-CH2-C-N-), 123.69 (-C-CH-N-CH2-CH2-P-), 124.14 (-O-CH2-C-CH-), 144.90 (-N-CH2-C-CH-), 146.11 (-CH-N-CH2-CH2-P-), 171.91 (-O-O-CH2-), 172.22 (-O-O-C-) ppm. MALDI-MS: m/z = 3419.2 [M + Na+].

Synthesis of **G2-PO(OEt)2**

A solution of CuSO4·5H2O (0.087g, 0.00035mol) in water (15mL) was added to a round bottom flask containing a stirred solution of **G2-ester** (1.35g, 0.000291mol) and (8) (1.16g, 0.00559mol) in THF (15mL). Sodium ascorbate (0.139g, 0.000699mol) was then added and the mixture was allowed to react for 40h. The solvent was evaporated and the product mixture filtered in methanol to remove the salts. The product was then purified by dialysis (MWCO=1000Da, methanol) to yield a viscous yellow oil (1.29g, 0.000164mol, 56% yield). $^{31}$P{1H} NMR (200MHz, CD3OD): δ = 28.25 ppm. $^1$H NMR (400MHz, d6-dimethyl sulfoxide (d6-DMSO): δ = 1.02 (s, 3H, -O-CH2-C-N-N-CH2-CH2-O-C-C-CH3), 1.04 (s, 6H, CH3-C-CH2-O-C-CH2-CH2-C-N-N-CH2-CH2-P-), 1.18 (t, 24H, -P-O-CH2-CH3), 2.40 (m, 8H, -CH2-CH2-P-), 2.61 (t, 12H, -O-C-CH2-CH2-C-N-), 2.82 (t, 12H, -O-C-CH2-CH2-C-N-), 3.30 (s, 2H, -C-CH2-O-CH2-), 3.96 (q, 16H, -P-O-CH2-CH3), 4.04 (s, 12H, CH3-C-CH2-O-), 4.43 (m, 16H, -N-CH2-CH2-), -CH2-O-CH2-C-N-N-CH2-CH2-O-), 4.57 (t, 4H, -CH2-CH2-C-N-N-CH2-CH2-O-), 4.62 (s, 2H, -C-CH2-O-CH2-), 7.86 (s, 2H, -CH2-CH2-O-), 7.90 (s, 4H, -CH-N-CH2-CH2-P-), 8.07 (s, 1H, -CH2-O-
\(^{13}\)C\{\(^{1}\)H\} NMR (300MHz, CD\(_3\)OD): \(\delta = 15.36\) (CH\(_2\)-CH\(_3\)), 16.64 (\(\text{CH}_3\)-CH\(_2\)-O-CO-CH\(_2\)-CH\(_2\)-C-CH-N-CH\(_2\)-CH\(_2\)-P-), 16.71 (\(\text{CH}_3\)-C-CH\(_2\)-O-CO-CH\(_2\)-CH\(_2\)-C-CH-N-CH\(_2\)-CH\(_2\)-O-), 20.40 (O-CO-CH\(_2\)-CH\(_2\)-C-N-), 25.01 (\(\text{CH}_2\)-P), 32.75 (O-CO-CH\(_2\)-CH\(_2\)-C-N-), 44.07 (\(\text{CH}_2\)-CH\(_2\)-O-CO-CH\(_2\)-CH\(_2\)-C-CH-N-CH\(_2\)-CH\(_2\)-P-), 45.10 (CH\(_2\)-O-CO-CH\(_2\)-CH\(_2\)-C-N-), 46.20 (\(\text{CH}_2\)-CH\(_2\)-P), 48.73 (CH\(_3\)), 48.86 (CH\(_2\)-O-CO-CH\(_2\)-C-N-), 62.20 (CH\(_2\)-CH\(_3\)), 63.03 (N-CH\(_2\)-CH\(_2\)-O-), 64.19 (CH\(_2\)-O-CO-CH\(_2\)-C-N-), 65.04 (CH\(_3\)-C-CH\(_2\)-), 68.76 (O-CO-CH\(_2\)-CH\(_2\)-C-N-), 122.61 (CH\(_2\)-N-), 146.30 (CH\(_2\)-N-), 171.92 (O-CO-CH\(_2\)-CH\(_2\)-C-N-), 172.27 (O-CO-C-) ppm. MALDI-MS: m/z = 7985.7 [M + Na\(^+\)].

**Attempted Synthesis of G\(_2\)-PO(OH)\(_2\)**

**In chloroform**

Trimethylsilyl bromide (TMSBr) (0.2mL) was added dropwise, through a syringe flushed with nitrogen, to a stirred solution of G\(_2\)-PO(OEt)\(_2\) (0.100 g, 0.0000127mol) in chloroform (5mL), under nitrogen. The reaction mixture quickly turned from yellow to purple as the TMSBr was added. After 5min a black precipitate began to form, and after 20min a colourless, transparent solution and an oily, dark purple precipitate remained. The reaction was left stirring, under nitrogen for another 30min after this occurred. The solvent was evaporated and a \(^{31}\)P\{\(^{1}\)H\} NMR spectrum was taken (200MHz, d\(_6\)-DMSO): \(\delta = 24.09, 27.54\) ppm. The peak at 27.54ppm (G\(_2\)-PO(OEt)\(_2\)) was large and sharp, while the one at 24.09ppm was small and broad, indicating that the mixture contained mostly
unreacted $\text{G2-PO(OEt)}_2$. A literature search undertaken at this time revealed anhydrous DCM to be a more suitable solvent.\textsuperscript{46}

In anhydrous DCM

TMSBr (0.2mL) was added dropwise, through a syringe flushed with nitrogen, to a stirred solution of $\text{G2-PO(OEt)}_2$ (0.100g, 0.000127mol) in dry DCM (5mL), under nitrogen. The reaction mixture quickly turned from yellow to purple as the TMSBr was added. This time no precipitation occurred and the reaction mixture was left stirring under nitrogen for one hour. The solvent was evaporated and a $\textsuperscript{31}P\text{\{}^1\text{H}\text{}}$ NMR spectrum was taken (200MHz, d\textsuperscript{6}-dimethyl sulfoxide), unfortunately, no peaks were visible even after the spectrum was run for 30min. Another $\textsuperscript{31}P\text{\{}^1\text{H}\text{}}$ NMR spectrum was taken (200MHz, CD\textsubscript{3}OD), this time >90% of the product mixture was dissolved in the NMR sample, but still no signal could be obtained.

2.4.4 - Amine Functionalization

Synthesis of tert-butyl (2-hydroxyethyl)carbamate (10)

Di-tert-butyl dicarbonate (BOC\textsubscript{2}O) (7.86g, 0.036mol) was added slowly in a portionwise fashion to a stirred solution of ethanolamine (9) (2.0g, 0.033mol) in DCM and triethylamine (5mL), at 0°C. The mixture was then allowed to react overnight, at room temperature. The crude mixture was then washed with an aqueous potassium carbonate solution, followed by an aqueous hydrochloric acid solution, brine, and finally water. The organic phase was isolated, dried with magnesium sulfate, and the solvent evaporated to yield the product as a colourless
oil (3.70g, 0.023mol, 70% yield). \(^1\)H NMR (400MHz, CDCl\(_3\)): \(\delta = 1.47\) (s, 9H, -C-(CH\(_3\))\(_3\)), 3.26 (s, 2H, -CH\(_2\)-NH-), 3.67 (s, 2H, -CH\(_2\)-OH), 5.04 (s, 1H, -NH-) ppm. \(^1\)C\({}\(^1\)H\) NMR (300MHz, CDCl\(_3\)): \(\delta = 28.6\) (s, -C-(CH\(_3\))\(_3\)), 43.4 (s, -CH\(_2\)-NH-), 62.8 (s, -CH\(_2\)-OH), 79.9 (C-(CH\(_3\))\(_3\)), 157.1 (-NH-CO-O-) ppm.\(^{47}\)

**Synthesis of 2-((tert-butoxycarbonyl)amino)ethyl methanesulfonate (11)**

Methanesulfonyl chloride (9.42g, 0.0822mol) was added very slowly in a dropwise fashion to a stirred solution of (10) (12.87g, 0.0798mol) in anhydrous DCM (500mL) and triethylamine (20mL) at 0°C, under nitrogen. The reaction was left stirring, under nitrogen at room temperature overnight. The crude mixture was then washed with water (2x250mL) and brine (2x250mL). The organic layer was isolated, dried with magnesium sulfate and the solvent evaporated to yield the product as a yellow oil (17.95g, 0.075mol, 94% yield). \(^1\)H NMR (400MHz, CDCl\(_3\)): \(\delta = 1.44\) (s, 9H, -C-(CH\(_3\))\(_3\)), 3.03 (s, 3H, -S-C\(_3\)H\(_3\)), 3.46 (m, 2H, -CH\(_2\)-NH-), 4.28 (m, 2H, -CH\(_2\)-O-), 4.92 (s, 1H, -NH-) ppm. \(^1\)C\({}\(^1\)H\) NMR (300MHz, CDCl\(_3\)): \(\delta = 28.6\) (-C-(CH\(_3\))\(_3\)), 37.6 (-CH\(_2\)-NH-), 40.2 (-S-CH\(_3\)), 69.1 (-CH\(_2\)-O-), 80.2 (-C-(CH\(_3\))\(_3\)) 158 (-NH-CO-O-) ppm.\(^{47}\)

**Synthesis of tert-butyl (2-azidoethyl)carbamate (12)**

Sodium azide (27.30g, 0.420mol) was added to a stirred solution of (11) (19.98g, 0.0835mol) in DMF (100mL) and water (100mL), under nitrogen. The reaction was left stirring, at 80°C overnight, under nitrogen. The crude product mixture was then extracted with ethyl acetate (3x100mL), the organic layers were isolated and combined and washed with water (3x100mL) and brine (2x100mL). The organic layer was then isolated, dried with magnesium sulfate and the solvent
evaporated to yield the product as a yellow oil (9.93g, 0.0533mol, 64% yield).

$^1$H NMR (400MHz, CDCl$_3$): $\delta = 1.44$ (s, 9H, -C-(CH$_3$)$_3$), 3.28 (m, 2H, -CH$_2$-NH-), 3.40 (m, 2H, N$_3$-CH$_2$-), 4.82 (s, 1H, -NH-) ppm. $^{13}$C{$^1$H} NMR (300MHz, CDCl$_3$): $\delta = 28.3$ ( -C-(CH$_3$)$_3$), 40.0 ( -CH$_2$-NH-), 51.2 ( -CH$_2$-N$_3$), 79.8 ( -C-(CH$_3$)$_3$), 155.70 (-NH-CO-O-) ppm.$^{47}$

**Synthesis of 3-azidopropylamine (14)**

A solution of sodium azide (3.39g, 0.0522mol) in water (11mL) was added to a stirred solution of 3-bromopropylamine hydrobromide (13) (3.42g, 0.0157mol) in water (8mL). The reaction was then refluxed overnight. In the morning 2/3 of the solvent was evaporated and the crude mixture was placed in an ice bath. Potassium hydroxide (4.2g, 0.0749mol) and ether (50mL) were then added, the aqueous phase was isolated and washed with ether (3x35mL). The organic phases were isolated, combined and dried with magnesium sulfate to yield the product as a yellow oil (1.38g, 0.0138mol, 88% yield). $^1$H NMR (400MHz, CDCl$_3$): $\delta = 1.42$ (s, 2H, -NH$_2$), 1.72 (q, 2H, -CH$_2$-CH$_2$-CH$_2$-), 2.79 (t, 2H, NH$_2$-CH$_2$-CH$_2$-CH$_2$-N$_3$), 3.36 (t, 2H, NH$_2$-CH$_2$-CH$_2$-CH$_2$-N$_3$) ppm. $^{13}$C{$^1$H} NMR (300MHz, CDCl$_3$): $\delta = 30.54$ (-CH$_2$-CH$_2$-CH$_2$-), 36.13 (N$_3$-CH$_2$-CH$_2$-CH$_2$-NH$_2$), 51.12 (N$_3$-CH$_2$-CH$_2$-CH$_2$-NH$_2$) ppm.$^{42}$

**Synthesis of tert-butyl (3-azidopropyl)carbamate (15)**

A solution of BOC$_2$O (1.09g, 0.0050mol) in methanol (6mL) was added dropwise to a solution of (14) (0.500g, 0.0050mol) in triethylamine (3.48mL) and methanol (20mL). The reaction was left stirring, overnight at room temperature. The product mixture was then filtered, and the solvent evaporated. The mixture
was then purified by column chromatography (chloroform) to yield the product as a yellow oil (0.546g, 0.00273mol, 55% yield). $^1$H NMR (400MHz, CDCl$_3$): $\delta =$ 1.41 (s, 9H, -C-(CH$_3$)$_3$), 1.74 (q, 2H, -CH$_2$-CH$_2$-CH$_2$-), 3.17 (t, 2H, -CH$_2$-CH$_2$-CH$_2$-N$_3$), 3.33 (t, 2H, -CH$_2$-CH$_2$-CH$_2$-N$_3$), 3.75 (t, 2H, -CH$_2$-CH$_2$-CH$_2$-N$_3$), 4.75 (s, 1H, -NH-) ppm. $^{13}$C{$^1$H} NMR (300MHz, CDCl$_3$): $\delta =$ 28.39 (C-(C$_3$H$_3$)$_3$), 29.87 (-CH$_2$-CH$_2$-CH$_2$-), 48.13 (N$_3$-CH$_2$-CH$_2$-CH$_2$-NH-), 53.93 (N$_3$-C$_2$H$_2$-CH$_2$-CH$_2$-NH-), 79.74 (-C-(CH$_3$)$_3$), 155.47 (-NH-COO-) ppm.

**Synthesis of G0-NHBOC**

A solution of CuSO$_4$·5H$_2$O (0.075g, 0.00030mol) in water (2.5mL) was added to a round bottom flask containing a stirred solution of G0 (0.300g, 0.00104mol) and (12) (0.853g, 0.00458mol) in THF (2.5mL). Sodium ascorbate (0.124g, 0.000624mol) was then added and the mixture was allowed to react overnight. The product mixture was evaporated and purified by column chromatography (acetone) to yield an orange solid (0.904g, 0.000875mol, 97% yield). $^1$H NMR (400MHz, CDCl$_3$): $\delta =$ 1.36 (s, 9H, -C-(CH$_3$)$_3$), 3.41 (s, 2H, -C-CH$_2$-O-), 3.55 (q, 2H, -N-CH$_2$-CH$_2$-NH-), 4.43 (t, 2H, -N-CH$_2$-CH$_2$-NH-), 4.47 (s, 2H, -C-CH$_2$-O-CH$_2$-), 5.62 (t, 1H, -NH-), 7.56 (s, 1H, -C-CH-N-) ppm. $^{13}$C{$^1$H} NMR (300MHz, CDCl$_3$): $\delta =$ 28.66 (-C-(C$_3$H$_3$)$_3$), 40.29 (-CH-N-CH$_2$-CH$_2$-), 46.47 (-C-CH$_2$-O-CH$_2$-C-N-), 48.52 (-CH-N-CH$_2$-CH$_2$-), 65.44 (-C-CH$_2$-O-CH$_2$-C-N-), 70.25 (-C-CH$_2$-O-CH$_2$-C-N-), 80.21 (-C-(CH$_3$)$_3$), 126.40 (-C-), 147.20 (-C-CH-), 154.93 (-CO-) ppm. ESI-MS: m/z = 1056.5 [M + Na$^+$].

**Synthesis of G1-NHBOC**
A solution of CuSO$_4$$\cdot$5H$_2$O (0.0081g, 0.000032mol) in water (2mL) was added to a round bottom flask containing a stirred solution of G1-ester (0.099g, 0.000057mol) and (15) (0.166g, 0.000680mol) in THF (2mL). Sodium ascorbate (0.013g, 0.000068mol) was then added and the mixture was allowed to react overnight. The product mixture was evaporated and purified by column chromatography (acetone) to yield a sticky yellow solid (0.095g, 0.000028mol, 50% yield). $^1$H NMR (400MHz, CD$_3$OD): $\delta$ = 1.09 (s, 3H, -C-C-CH$_3$), 1.42 (s, 18H, -C-(CH$_3$)$_3$), 2.03 (m, 4H, -N-CH$_2$-CH$_2$-CH$_2$-NH-), 2.68 (t, 4H, -O-C-CH$_2$-CH$_2$-C-N-), 2.94 (t, 4H, -O-C-CH$_2$-CH$_2$-C-N-), 3.05 (t, 4H, -N-CH$_2$-CH$_2$-CH$_2$-NH-), 3.42 (s, 2H, -C-CH$_2$-O-), 4.09 (q, 4H, CH$_3$-C-CH$_2$-O-), 4.38 (t, 4H, -N-CH$_2$-CH$_2$-CH$_2$-NH-), 4.51 (m, 4H, -CH$_2$-O-CH$_2$-C-CH-N-CH$_2$-), 4.71 (t, 2H, -N-CH$_2$-CH$_2$-O-), 6.89 (t, 2H, -NH-), 7.77 (s, 2H, -CH-N-CH$_2$-CH$_2$-CH$_2$-), 8.03 (s, 1H, -CH$_2$-O-CH$_2$-C-CH-N-) ppm. $^{13}$C{$^1$H} NMR (300MHz, CDCl$_3$): $\delta$ = 18.08 (C$_3$H-C-CH$_2$-), 21.84 (-CH$_2$-C$_2$H$_2$-C-N-), 28.87 (-C-(CH$_3$)$_3$), 31.67 (-CH$_2$-C$_2$H$_2$-NH-), 34.22 (-CH$_2$-C$_2$H$_2$-C-N-), 38.52 (-C$_2$H$_2$-NH-), 46.55 (-C-CH$_2$-O-CH$_2$-C-N), 47.67 (-CH$_2$-C-CH$_3$), 49.90 (-N-CH$_2$-CH$_2$-O-), 50.16 (-N-CH$_2$-CH$_2$-CH$_2$-NH-), 64.45 (-N-CH$_2$-CH$_2$-O-), 65.58 (-C-CH$_2$-O-CH$_2$-C-N), 66.48 (-C$_2$H$_2$-C-CH$_3$), 70.18 (-C-CH$_2$-O-CH$_2$-C-N), 80.12 (-C-(CH$_3$)$_3$), 123.73 (-C$_2$H$_2$-N-CH$_2$-CH$_2$-CH$_2$-), 125.52 (-C-CH$_2$-O-CH$_2$-C-CH-N-), 146.45 (-C-CH$_2$-O-CH$_2$-C-CH-N-), 147.43 (-C-CH-N-CH$_2$-CH$_2$-CH$_2$-), 158.45 (-CO-O-C-(CH$_3$)$_3$), 173.43 (-O-C=O), 173.67 (-O-C=O) ppm. MALDI-MS: m/z = 3365.1 [M + Na$^+$].

**Synthesis of G2-NHBOC**
A solution of CuSO₄·5H₂O (0.006g, 0.000023mol) in water (1mL) was added to a round bottom flask containing a stirred solution of G₂-ester (0.091g, 0.000020mol) and (15) (0.094g, 0.00047mol) in THF (1mL). Sodium ascorbate (0.0093g, 0.00004698mol) was then added and the mixture was allowed to react overnight. The product mixture was evaporated and filtered in DCM to remove the salts. The product was then precipitated out of DCM using ether, isolated and dried to yield a viscous yellow oil (0.043g, 0.0000055mol, 28% yield). ¹H NMR (400MHz, CDCl₃): δ = 1.11 (s, 9H, -O-C-C(CH₃)₃), 1.41 (s, 36H, -C-(CH₃)₃), 2.04 (q, 8H, -N-CH₂·CH₂·CH₂-NH·), 2.69 (t, 12H, -O-C·CH₂·CH₂·C-N·), 2.97 (t, 12H, -O-C·CH₂·CH₂·C-N·), 3.10 (m, 8H, -N-C·CH₂·CH₂·N-N-C·CH₂·O-N·), 3.46 (s, 2H, -C·CH₂·O·CH₂·), 4.10 (m, 12H, CH₃-C·CH₂·O·C·), 4.36 (t, 8H, -N·CH₂·CH₂·CH₂·NH·), 4.48 (m, 8H, -CH₂·CH₂·C-N·N·N·CH₂·CH₂·O·), 4.58 (t, 4H, -CH₂·O·CH₂·C·N·N·N·CH₂·CH₂·O·), 4.65 (s, 2H, -C·CH₂·O·CH₂·), 5.14 (s, 4H, -NH·), 7.47 (s, 4H, -CH-N·CH₂·CH₂·CH₂-NH·), 7.54 (s, 2H, -C·CH₂·CH₂·C·CH·N·CH₂·CH₂·O·), 7.83 (s, 1H, -O·CH₂·C·CH·N·) ppm. ¹³C {¹H} NMR (300MHz, CDCl₃): δ = 17.58 (-CH₂·C·CH₃·), 20.76 (-CO·CH₂·CH₂·C·CH·N·CH₂·CH₂·O·), 20.81 (-CH₂·C·CH·N·CH₂·CH₂·CH₂·), 28.36 (-C·(CH₃)₃), 30.62 (-N·CH₂·CH₂·CH₂·), 33.17 (-CO·CH₂·CH₂·C·CH·N·CH₂·CH₂·O·), 33.28 (-CH₂·CH₂·C·CH·N·CH₂·CH₂·CH₂·), 37.36 (-N·CH₂·CH₂·CH₂·), 45.18 (-CH₂·O·CH₂·C·N·), 46.26 (-CH₂·O·CH₂·C·CH·N·CH₂·CH₂·), 46.30 (-CO·CH₂·CH₂·C·CH·N·CH₂·CH₂·O·), 47.47 (-N·CH₂·CH₂·CH₂·), 48.68 (-CH₂·O·CO·CH₂·CH₂·C·CH·N·CH₂·CH₂·O·), 48.75 (-CH₂·O·CO·CH₂·CH₂·C·CH·N·CH₂·CH₂·O·), 63.05 (-CO·CH₂·CH₂·C·CH·N·CH₂·CH₂·O·), 63.11 (-CH₂·O·CH₂·
C-CH-N-CH$_2$-CH$_2$), 64.75 (-C-CH$_2$-O-CH$_2$-C-N-), 65.14 (-C-CH$_2$-O-CO-CH$_2$-CH$_2$-C-CH-N-CH$_2$-CH$_2$-CH$_2$-), 69.05 (-C-CH$_2$-O-CH$_2$-C-N-), 70.50 (-C-CH$_2$-CO-CH$_2$-CH$_2$-C-CH-N-CH$_2$-CH$_2$-O-), 79.32 (-C-(CH$_3$)$_3$), 121.67 (-C-CH-N-CH$_2$-CH$_2$-CH$_2$-), 122.10 (-CO-CH$_2$-CH$_2$-C-CH-N-CH$_2$-CH$_2$-O-), 123.50 (-C-CH$_2$-O-CH$_2$-C-CH-N-CH$_2$-CH$_2$-O-), 146.01 (-CO-CH$_2$-CH$_2$-C-CH-N-CH$_2$-CH$_2$-O-), 146.15 (-C-CH-N-CH$_2$-CH$_2$-CH$_2$-), 149.93 (-C-CH$_2$-O-CH$_2$-C-CH-N-), 156.12 (-C-O-O-C-(CH$_3$)$_3$), 171.96 (-CO-CH$_2$-CH$_2$-C-CH-N-CH$_2$-CH$_2$-O-), 172.01 (-CO-CH$_2$-CH$_2$-C-CH-N-CH$_2$-CH$_2$-O-), 172.12 (-CO-CH$_2$-CH$_2$-C-CH-N-CH$_2$-CH$_2$-O-), 172.15 (-C-CH$_2$-O-CH$_2$-C-CH-N-CH$_2$-CH$_2$-O-), ppm. MALDI-MS: m/z = 7881.9 [M + Na$^+$].

Synthesis of **G0-NH$_3^+$**

TFA (5mL) was added dropwise to a solution of **G0-NHBOC** (0.904g, 0.000875mol) in DCM (5mL). The reaction was left stirring for 5min, the solvent was evaporated and the product was dissolved in methanol. The methanol was then evaporated, bringing the remaining TFA with it. This methanol addition and evaporation cycle was then repeated several times to ensure that all remaining TFA had been evaporated. The product was obtained as a sticky orange solid (0.800g, 0.000735mol, 84% yield). $^1$H NMR (400MHz, CD$_3$OD): $\delta$ = 1.80 (s, 3H, -NH$_3$), 3.47 (s, 2H, -C-CH$_2$-O-), 3.59 (t, 2H, -N-CH$_2$-CH$_2$-NH$_3$), 4.53 (s, 2H, -C-CH$_2$-O-CH$_2$-), 4.76 (t, 2H, -N-CH$_2$-CH$_2$-NH$_3$), 8.06 (s, 1H, -C-CH-N-) ppm. $^{13}$C{$^1$H} NMR (300MHz, CD$_3$OD): $\delta$ = 40.30 (-CH-N-CH$_2$-CH$_2$-), 46.48 (-C-CH$_2$-O-CH$_2$-C-N-), 48.54 (-CH-N-CH$_2$-CH$_2$-), 65.46 (-C-CH$_2$-O-CH$_2$-C-N-),
70.27 (-C-CH₂-O-CH₂-C-N-), 119.20 (-CF₃), 126.40 (-CH-), 147.22 (-C-CH-), 162.95 (-CO-) ppm. ESI-MS: m/z = 633.3 [M - 4TFA + H⁺].

Synthesis of G₁-NH₃⁺

TFA (0.5mL) was added dropwise to a solution of G₁-NHBOC (0.05g, 0.000015mol) in DCM (0.5mL). The reaction was left stirring for 5min, the solvent was evaporated and the product was dissolved in methanol. The methanol was then evaporated, bringing the remaining TFA with it. This methanol addition and evaporation cycle was then repeated several times to ensure that all remaining TFA had been evaporated. The product was obtained as a sticky yellow solid (0.041g, 0.000012mol, 80% yield).

¹H NMR (400MHz, CD₃OD): δ = 1.10 (s, 3H, -C-CH₃), 2.27 (q, 4H, -N-CH₂-CH₂-O-CH₂-C-N-), 2.95 (t, 4H, -C-CH₂-CH₂-C-N-), 3.00 (t, 4H, -N-CH₂-CH₂-CH₂-NH₃), 3.42 (s, 2H, -C-CH₂-O-CH₂-), 4.08 (s, 4H, CH₃-C-CH₂-O-), 4.51 (t, 8H, -CH₂-O-CH₂-C-), 4.71 (t, 2H,-N-CH₂-CH₂-O-), 7.80 (s, 2H, -CH₂-NH₂), 8.04 (s, 1H,-CH₂-O-CH₂-C-) ppm. ¹³C¹H NMR (300MHz, CD₃OD): δ = 18.03 (CH₃-C-CH₂-), 21.74 (-CH₂-CH₂-C-N-), 29.18 (-CH₂-CH₂-NH₃), 34.14 (-CH₂-CH₂-C-N-), 38.17 (-CH₂-NH₃), 46.55 (-CH₂-CH₂-O-CH₂-C-N-), 47.63 (-CH₂-C-CH₃), 49.90 (-N-CH₂-CH₂-O-), 50.20 (-N-CH₂-CH₂-CH₂-NH₃), 64.44 (-N-CH₂-CH₂-O-), 65.48 (-CH₂-O-CH₂-C-N-), 66.41 (-CH₂-C-CH₃), 70.15 (-CH₂-O-CH₂-C-N), 119.08 (-F₃), 123.91 (-CH₂-N-CH₂-CH₂-CH₂-), 125.64 (-CH₂-O-CH₂-C-C-N-), 146.39 (-CH₂-O-CH₂-C-CH₃-N-), 147.69 (-CH₂-N-CH₂-CH₂-CH₂-), 162.57 (-CO-O-C-F₃), 173.46 (-O-CO-CH₂-), 173.69 (-O-CO-C-) ppm. MALDI-MS: m/z = 2563.6 [M - 8TFA + Na⁺].
Synthesis of G2-NH$_3^+$

TFA (1mL) was added dropwise to a solution of G2-NHBOC (0.043g, 0.0000055mol) in DCM (1mL). The reaction was left stirring for 5min, the solvent was evaporated and the product was dissolved in methanol. The methanol was then evaporated, bringing the remaining TFA with it. This methanol addition and evaporation cycle was then repeated several times to ensure that all remaining TFA had been evaporated. The product was obtained as a sticky yellow solid (0.034g, 0.0000042mol, 77% yield). $^1$H NMR (400MHz, CD$_3$OD): δ = 1.10 (s, 9H, -O-C-C-CH$_3$), 2.27 (q, 8H, -N-CH$_2$-CH$_2$-CH$_2$-NH$_3$), 2.68 (t, 12H, -O-C-CH$_2$-CH$_2$-C-N-), 2.97 (t, 12H, -O-C-CH$_2$-CH$_2$-C-N-), 3.01 (m, 8H, -N-CH$_2$-CH$_2$-CH$_2$-NH$_3$), 3.41 (s, 2H, -C-CH$_2$-O-CH$_2$-), 4.08 (m, 12H, CH$_3$-C-CH$_2$-O-C-), 4.50 (m, 8H, -CH$_2$-O-CH$_2$-C-N-N-N-C-CH$_2$-CH$_2$-O-), 4.66 (t, 4H, -CH$_2$-O-CH$_2$-C-N-N-N-C-CH$_2$-CH$_2$-O-), 4.88 (s, 12H, -NH$_3$), 7.82 (s, 4H, -CH-N-CH$_2$-CH$_2$-CH$_2$-NH-), 7.86 (s, 2H, -C-CH$_2$-CH$_2$-C-CH-N-CH$_2$-CH$_2$-O-), 8.07 (s, 1H, -O-CH$_2$-O-CH$_2$). $^{13}$C-$^1$H NMR (300MHz, CD$_3$OD): δ = 16.55 (-CH$_2$-CH$_2$-CH$_2$-N-N-C-CH$_2$-CH$_2$-CO-O-CH$_2$-C-CH$_3$), 16.63 (O-CH$_2$-CH$_2$-N-N-C-CH$_2$-CH$_2$-CO-O-CH$_2$-C-CH$_3$), 20.30 (-CH$_2$-C-CH-N-CH$_2$-CH$_2$-CH$_2$-), 20.35 (-CO-CH$_2$-CH$_2$-C-N-CH$_2$-CH$_2$-CH$_2$-O-), 27.71 (-N-CH$_2$-CH$_2$-CH$_2$-), 32.70 (-CO-CH$_2$-CH$_2$-C-N-), 36.72 (-N-CH$_2$-CH$_2$-CH$_2$-), 45.05 (-C-CH$_2$-O-CH$_2$-C-N-), 46.17 (-CO-CH$_2$-CH$_2$-C-CH-N-CH$_2$-CH$_2$-O-), 46.19 (-C-CH$_2$-O-CH$_2$-C-CH-N-CH$_2$-CH$_2$-), 46.85 (-N-CH$_2$-CH$_2$-CH$_2$-), 48.73 (CH$_3$-C-CH$_2$-O-CO-), 62.97 (-N-CH$_2$-CH$_2$-O-), 64.05 (-C-CH$_2$-O-CH$_2$-C-N-), 64.97 (-C-CH$_2$-O-CO-CH$_2$-CH$_2$-C-CH-N-CH$_2$-CH$_2$-CH$_2$-), 65.05 (-C-CH$_2$-
O-CO-CH₂-CH₂-C-CH-N-CH₂-CH₂-O-, 68.75 (-C-H₂-O-CH₂-C-N-), 118.50 (-CF₃), 122.48 (-C-H-N-), 146.08 (-C-CH-N-), 161.39 (-C-O-CF₃), 171.98 (-C-O-O-CH₂-C-CH₃), 172.24 (-CO-CH₂-CH₂-C-CH-N-CH₂-CH₂-O-CO-), 172.29 (-C-H₂-CH₂-C-CH-N-CH₂-CH₂-O-CO-) ppm. MALDI-MS: m/z = 6248.2 [M - 8TFA + H⁺].

2.5 - References

(35) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. Angew. Chem. Int. Ed. 2002, 41, 2596.
Chapter 3: An Evaluation of the Bactericidal Activity of Dendrimers

3.1 - Introduction

As discussed in Chapters 1 and 2, dendrimers are employed for a wide range of applications due mainly to their tailorability, as well as their unique globular structure leading to among other things, a high density of surface groups. A distinctive combination of properties in dendrimers means that a large number of desired functionalities can be presented in a single macromolecule. A variety of synthetic methodologies have been developed in the recent past which have provided access to varied backbones and surface functionalities. However, one of the most exciting developments in dendrimer synthesis within the last decade has been the introduction of click chemistry by Sharpless’ group in 2001, in particular, the copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) reaction. This simple, versatile, high yielding reaction is an ideal candidate for dendrimer synthesis and subsequent functionalization. Through the use of the CuAAC reaction, acetylene-terminated dendrimers can be prepared and functionalized upon reaction with an array of azide terminated molecules, in order to impart a variety of properties including fluorescence, solubility, anti-scaling and bactericidal action. We have been interested in the development of dendrimers functionalized with cationic moieties at their periphery for use as bactericides. Our synthetic efforts in this regard were elaborated in Chapter 2, in which we demonstrated the potential of click chemistry for the construction and post-functionalization of dendrimers. The
alkyne-terminated dendrimers developed in Chapter 2 provide a useful platform for their extension into materials for bactericidal action, which form the focus of this chapter.

The azide-terminated molecule selected for coupling and subsequent bactericidal action as desired in this project was a cationic amino azide, designed to impart the dendrimers with bactericidal properties as well as aqueous solubility. Due to the increasing amount of resistance to various antimicrobial agents encountered in numerous fields, there is an ever escalating need for the development of new bactericidal compounds. The mechanism of action of many small biocidal molecules is well known, and it follows that dendrimers functionalized with these moieties would disrupt bacteria in a similar fashion. Cations and polycations are thought to disrupt the bacterial membrane structure by displacing the divalent counterions that help to hold the phospholipid bilayer together. Since the divalent cations can assist in holding together two negatively charged phospholipid groups, they are necessary for maintaining the integrity of the membrane. This substitution leads to an increase in membrane permeability and the subsequent leakage of intracellular materials such as potassium ions and nucleic acids. These modifications to membrane structure can be either bacteriostatic or bactericidal, depending on the concentration of bactericide employed. Dendrimers functionalized with cationic groups are known to function by this same mechanism, while dendrimers functionalized with anionic groups are believed to behave as polyanions, complexing with the divalent cations instead of displacing them, and also resulting in membrane disruption.
Dendrimers with terminal hydroxide groups have been shown to hydrogen bond to O-antigens in the bacterial membrane, leading to an increase of membrane permeability, as discussed above. The two main factors governing the bactericidal effect of dendrimers are the number of bactericidal end groups and the biopermeability. Higher generation dendrimers present more bactericidal end groups, but are also less able to arrive at the bacterial membrane, their site of action, as a consequence of their size. The reverse can be said of lower generation dendrimers, they are small enough to easily enter the bacterial structure, but are less effective. These two opposing trends mean that a peak bactericidal efficacy is often reached for a given dendrimer backbone at an intermediate generation number, with both higher and lower generations being less effective.

Bactericidal efficacy can be assessed by measuring two relevant parameters, the minimum inhibitory concentration (MIC), and the minimum bactericidal concentration (MBC). The MIC is defined as: “the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation”, while the MBC, which for a given species is always larger than the MIC, is defined as “the lowest concentration of an antimicrobial that will prevent the growth of an organism after sub-culture onto an antibiotic-free media”.

This chapter explores the bactericidal efficacy of the dendrimers described in Chapter 2. We had originally set out to prepare and test cationic amino-terminated structures, however, it is known that hydroxide-terminated structures may also show bactericidal effect. So we decided to examine the bactericidal
properties of the generation one hydroxide-terminated dendrimer (G1-OH), as well as the cationic amino-terminated structures (G0-NH$_3^+$, G1-NH$_3^+$ and G2-NH$_3^+$). The structures of the dendrimers are presented in Figure 3.1. Dendrimer G1-OH was found to be water soluble without the need for any further surface modification.

3.2 - Results and Discussion

The synthesis of the dendrimers examined here for their bactericidal action is described in Chapter 2. Aqueous solutions of the dendrimers with a range of different concentrations were prepared and then added to aliquots of a bacteria
Figure 3.1 – Structures of G1-OH, G0-NH$_3^+$, G1-NH$_3^+$ and G2-NH$_3^+$ Dendrimers

solution. The initial optical density (OD) of the solutions was measured, and they were subsequently incubated overnight. After 18-24h had elapsed, the OD was measured again. A graph showing the relative absorbance of the solutions as a function of bactericide concentration was then prepared and the MIC determined as the lowest concentration point where the curve drops sharply to zero (Figure 3.2). The MIC was reported as a range for structures where the sharp drop did not
correspond to a zero value on the curve (Figure 3.2). When possible, further testing was subsequently performed in order to more precisely determine the MIC. The same dendrimer/bacteria solutions were then plated onto agar and allowed to incubate overnight. The MBC was taken as the lowest concentration showing no visible growth after 18-24h of incubation. Preliminary testing was done with solutions having dendrimer concentrations in the range of 0.025-64 mg/L, with subsequent tests performed to narrow in on the exact MIC value.

Four different dendrimers were tested for their bactericidal activity (Table 3.1), three of them were functionalized with cationic amino groups (G0-NH$_3^+$, G1-NH$_3^+$ and G2-NH$_3^+$), while the fourth presented hydroxide surface groups (G1-OH). The latter was not found to be bactericidal at any of the concentrations tested (MIC >64 mg/L, 8 OH groups, Table 3.1), which clearly suggests the importance of the cationic surface groups employed in the other structures for bactericidal action.

![Figure 3.2 – MIC Determination of G2-NH$_3^+$](image)
Of the cationic amino-terminated structures, the least effective bactericide was the smallest structure tested, G0-NH$_3^+$ (MIC = 32-64 mg/L, 4 NH$_3^+$ groups, Table 3.1). Although G0-NH$_3^+$ is a more effective bactericide than the hydroxide-terminated structure, G1-OH (MIC >64 mg/L, 8 OH groups, Table 3.1), the two larger cationic amino-terminated structures were found to be more effective. The most effective bactericide was the generation one cationic amino-terminated dendrimer, G1-NH$_3^+$ (MIC = 0.9 mg/L, 8 NH$_3^+$ groups, Table 3.1). G1-NH$_3^+$ is a more effective bactericide than both the lower and higher generation structures with the same backbone and type of end groups, indicating that for these dendrimers G1-NH$_3^+$ is the best balance between the number of bactericidal end groups and the biopermeability of the structure. The larger dendrimer, G2-NH$_3^+$ (MIC = 1-16 mg/L, 16 NH$_3^+$ groups, Table 3.1), was more effective than G0-NH$_3^+$ (MIC = 32-64 mg/L, 4 NH$_3^+$ groups, Table 3.1), but less effective than G1-NH$_3^+$ (MIC = 0.9 mg/L, 8 NH$_3^+$ groups, Table 3.1). These results may suggest that the generation two structure is past the tipping point where the gain in bactericidal activity obtained from additional charged end groups is outweighed by the loss of biopermeability.

### Table 3.1 – MIC and MBC Values of Dendrimers

<table>
<thead>
<tr>
<th>Structure</th>
<th>Type of End Groups</th>
<th>No. of End Groups</th>
<th>MW (g/mol)</th>
<th>MIC (mg/L)</th>
<th>MIC (µM)</th>
<th>MBC (mg/L)</th>
<th>MBC (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1-OH</td>
<td>-OH</td>
<td>8</td>
<td>1101.1</td>
<td>&gt;64</td>
<td>&gt;58</td>
<td>&gt;64</td>
<td>&gt;58</td>
</tr>
<tr>
<td>G0-NH$_3^+$</td>
<td>NH$_3^+$ TFA'</td>
<td>4</td>
<td>1088.5</td>
<td>32-64</td>
<td>29-59</td>
<td>&gt;64</td>
<td>&gt;59</td>
</tr>
<tr>
<td>G1-NH$_3^+$</td>
<td>NH$_3^+$ TFA'</td>
<td>8</td>
<td>3454.9</td>
<td>0.9 ±0.1</td>
<td>0.26 ±0.03</td>
<td>4-8</td>
<td>1.1-2.3</td>
</tr>
<tr>
<td>G2-NH$_3^+$</td>
<td>NH$_3^+$ TFA'</td>
<td>16</td>
<td>8075.0</td>
<td>1-16</td>
<td>0.12-2</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
The only structure for which an accurate MBC could be determined was $\text{G1-NH}_3^+$ (MBC = 4-8 mg/L, 8 NH$_3^+$ groups, Table 3.1), the value followed the expected pattern of MBC>MIC for a given structure. Because the MIC values of both $\text{G1-OH}$ and $\text{G0-NH}_3^+$ were greater than or equal to the highest concentration tested, we were unable to assess the MBC. Difficulty with the sensitive plating procedure prevented us from obtaining accurate results for the $\text{G2-NH}_3^+$ dendrimer.

In order to better evaluate the bactericidal efficacy of our dendrimers, a comparison with dendrimers which have already been tested in the literature for bactericidal activity was carried out and the data is presented in Table 3.2. As discussed in Chapter 1, the mechanism by which dendrimer bactericides function is highly dependent on the nature of their end groups. In order to facilitate direct comparison, the data presented in Table 3.2 corresponds only to dendrimers that function by the membrane disruptive mechanism (as opposed to anti-adhesive), where an MIC was measured.$^{16}$ Our best performing dendrimer, $\text{G1-NH}_3^+$ (8 NH$_3^+$ groups), is a more effective bactericide than any of the dendrimers (4-32 end groups) presented in Table 3.2. Comparable both in size and number of charged end groups to the generation two carbosilane dendrimer$^{18}$ (MIC = 64 mg/L, 8 NMe$_3^+$ groups, Table 3.2), $\text{G1-NH}_3^+$ (MIC = 0.9 mg/L, 8 NH$_3^+$ groups, Table 3.1) is a much more effective bactericide, by almost two orders of magnitude. Equally comparable in size and number of charged end groups are the generation one carbosilane dendrimer (MIC = 4 mg/L, 4 NMe$_3^+$ groups, Table 3.2), and $\text{G0-NH}_3^+$ (MIC = 32-64 mg/L, 4 NH$_3^+$ groups, Table 3.1). Interestingly,
however, the trend is reversed in this case, with the generation one carbosilane
dendrimer showing about an order of magnitude more bactericidal effect than $\text{G0-NH}_3^+$. Since both sets of structures present the same number of charged amino
groups, approximately the same molecular weight, and were tested against the
same bacteria, the only major difference between the two dendrimer backbones
seems to be in their hydrophilicity. It is known that structures which present more
hydrophobic units can more easily permeate the bacterial structure to arrive at
their site of action.$^{16}$ These results may suggest that for dendrimers with
hydrophilic backbones, such as the ones prepared in this project, the peak
bactericidal efficiency is reached at a higher generation than for dendrimers with
hydrophobic backbones, such as carbosilane.

<table>
<thead>
<tr>
<th>Table 3.2 - MIC Values of Literature Dendrimers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Generation &amp; Backbone</td>
</tr>
<tr>
<td>------------------------</td>
</tr>
<tr>
<td>G3 PAMAM$^{19}$</td>
</tr>
<tr>
<td>G5 PAMAM$^{19}$</td>
</tr>
<tr>
<td>G1 Carbosilane$^{18}$</td>
</tr>
<tr>
<td>G2 Carbosilane$^{18}$</td>
</tr>
</tbody>
</table>

The bactericidal efficacy of the neutral amino-terminated generation three
(MIC = 6.3 mg/L, 31 NH$_2$ groups Table 3.2) and five (12.5 mg/L, 110 NH$_2$
groups Table 3.2) PAMAM dendrimers both fall into the wide range determined
for the $\text{G2-NH}_3^+$ (MIC = 1-16 mg/L, 16 NH$_3^+$ groups, Table 3.1) dendrimer. The
molecular mass of $\text{G2-NH}_3^+$ is comparable to that of the generation three
PAMAM dendrimer. It is difficult to draw definitive conclusions about these results as the structures have different backbones, number of end groups, type of end groups, and were tested against different bacteria. Interestingly, the neutral structures that were previously tested (generation three and five PAMAM dendrimers) both performed better than the only neutral structure tested in this project, G1-OH (MIC > 64 mg/L, 8 OH groups, Table 3.1). Although, again, it is difficult to draw conclusions in this case due to the differences in backbone, surface groups and bacteria.

Our results, as well as those for the dendrimers previously evaluated,\textsuperscript{18,19} indicate that higher generation dendrimers may not be a necessary and important factor for bactericidal action. Higher generation dendrimers were found to be similar or less effective than lower generation ones. Although a detailed evaluation is necessary in order to determine structure-property relationships, it is noted that the largest structure, the generation five PAMAM dendrimer with 110 peripheral amino groups, is the least effective bactericide per mole of bactericidal surface group. This suggests that a balance between the size of the dendrimer and the number of end groups is a key factor.

3.3 - Conclusions

In conclusion, the efficacy of dendrimer based bactericides is governed by two main factors, the number of bactericidal surface groups and the biopermeability, which are at odds with one another as the generation number of the dendrimer increases. This often leads to a peak efficacy at a certain generation number for a given backbone and end groups, with both larger and
smaller structures being less effective. These results indicate that for the
dendrimers reported here with cationic amino surface groups, this peak efficacy is
reached at $\text{G1-NH}_3^+$, with both $\text{G0-NH}_3^+$ and $\text{G2-NH}_3^+$ being less effective. Our
results also suggest the importance of the cationic amino surface groups, as $\text{G1-OH}$ with hydroxide surface groups was not bactericidal at any concentration
tested. The most effective dendrimer bactericide, $\text{G1-NH}_3^+$, was more effective
than several dendrimers reported in the literature.\textsuperscript{18,19} Further studies to explore
their detailed potential as bactericides are currently being pursued. This includes
an examination of the hydroxide-terminated dendrimers, $\text{G1-OH}$, $\text{G2-OH}$ and
$\text{G3-OH}$, as well as the generation three dendrimer functionalized with cationic
end groups.

3.4 - Experimental

Bactericidal activity experiments were performed in collaboration with
Cristina Motillo and Tiffany Hua, honours research students in the laboratories of
Professors Kakkar and van de Ven. Procedures for the MIC and MBC
determination were followed as described by Andrews.\textsuperscript{17} The water used was
deionized, followed by ion-exchange and filtration steps (Milli-Q; Millipore
GmbH).

3.4.1 - Determination of MIC

Aqueous solutions with dendrimer concentrations ranging from 0.025-64
mg/L were prepared. Initial testing was performed with these solutions with
subsequent testing performed in a more narrow range around the initially
determined MIC, in order to increase accuracy. Mueller Hinton Broth (MHB)
(85mL) was inoculated with one colony of *Escherichia coli* (*E. coli*) ATCC11229 and incubated with shaking (150rpm) at 37°C, for 18-24h. Fresh MHB (85mL) was then inoculated with overnight culture (300 µL) and incubated for 2h, with shaking (150rpm), at 37°C. The OD of the culture was then measured at 625nm. The bacterial solution was subsequently diluted in water until its OD lay in the range of 0.08-0.13, and then 100-fold in 15%MHB/85%H₂O. This diluted solution of bacteria (3mL) was then added in a 1:1 ratio to the prepared dendrimer solutions, in duplicate. The initial OD at 625nm of each dendrimer treated bacteria sample was then measured. Subsequently, the samples were incubated with shaking (150rpm) at 37°C, for 18-24h, when the OD was again measured. Bacterial controls ensured that each sample contained 5x10⁵ colony forming units (CFU). Experiments were repeated to ensure reproducibility. The OD values of the bacterial solutions taken 18-24h after dendrimer addition relative to the initial OD were plotted as a function of dendrimer concentration. The MIC was evaluated as the concentration where the relative OD drops sharply to zero.

### 3.4.2 - Determination of MBC

The same dendrimer treated bacterial samples used to evaluate the MIC were also employed to determine the MBC. After their OD had been measured at 625nm, 18-24h after exposure, the samples were plated on agar. The MBC was determined as the lowest concentration showing no bacterial growth after incubation for 18-24h at 37°C.

### 3.5 - References

(8) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. Angew. Chem. Int. Ed. 2002, 41, 2596.
Chapter 4: Conclusions and Future Outlook

4.1 - Summary and Conclusions

Dendrimers represent a valuable platform to address key issues in many fields, including the pulp and paper industry. While limited study has been carried out on the suitability of dendrimers for pulp and paper applications, further exploration is needed to fully assess their potential. In addition, although bactericides are routinely used within the pulp and paper industry to control the build-up of biofilms on machinery, dendrimer-based bactericides have not yet made their impact in this area. One of the key challenges related to the exploitation of dendrimer properties for these applications, is their easy and large scale synthesis, their post-functionalization with desired moieties, as well as the requirement that the final dendrimer structure must be water soluble.

In this thesis, we have developed a synthetic methodology towards water soluble dendrimers that can be easily functionalized with a variety of surface groups including cationic amino moieties for application as bactericides, and phosphonate groups, for potential application as anti-scalants. We employed a tetrafunctional core, as compared to more traditional bi- and trifunctional cores, since it allowed us access to a higher density of surface groups at a lower generation. For instance, the generation three acetylene-terminated dendrimer reported here, G3-ester, presents 32 terminal acetylene moieties, while a generation three dendrimer prepared from a trifunctional core will have only 24.
The synthesis and post-functionalization of our dendrimers, detailed in Chapter 2, was carried out using the copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) reaction. In order to employ the CuAAC reaction for this purpose, we designed and synthesized appropriate building blocks containing acetylene and azide end groups. The acetylene-terminated building block contained a carboxylic acid moiety at its other end, available for coupling to the hydroxide-terminated dendrimers and leaving them decorated with acetylene surface groups. The azide-terminated building block was designed with a protected diol at its other end. After this building block was coupled to the acetylene-terminated dendrimers, the diol was deprotected; doubling the number of surface groups as the generation number increases, and regenerating the hydroxide functionality. This repetitive sequence allowed us to synthesize dendrimers of generations 0-3 with 4-32 surface groups using a divergent synthetic methodology. We then demonstrated the significance of acetylene-terminated dendrimers by attaching phosphonate and cationic amino moieties to the periphery of \( \text{G0} \quad \equiv \equiv \), \( \text{G1-ester} \equiv \equiv \) and \( \text{G2-ester} \equiv \equiv \) using “click” chemistry. The bactericidal activity of the cationic amino-terminated dendrimers, \( \text{G0-NH}_3^+ \), \( \text{G1-NH}_3^+ \) and \( \text{G2-NH}_3^+ \), as well as one of the hydroxide-terminated structures, \( \text{G1} \), was then evaluated. The most bactericidal dendrimer tested here, \( \text{G1-NH}_3^+ \), was found to be more potent than many previously evaluated dendrimer bactericides. Difficulty with the deprotection of our phosphonate-terminated dendrimers prevented us from evaluating their efficacy as anti-scalants.
The results presented in this thesis highlight the importance of i) the selection of an appropriate core molecule in the design of dendrimers, which can reduce the number of generations required to obtain the desired number of functional groups at their periphery. Consequently, it also reduces the number of steps required in their synthesis, and thus facilitating their large scale preparation for industrial applications; ii) “click” chemistry in the synthesis of dendrimers, as well as their post-functionalization. We demonstrated the latter by functionalizing these dendrimers with cationic end groups, for applications as bactericides, and phosphonate groups, for use as antiscalants. However, the scope of functionalization is not limited to these moieties, and these easily synthesized dendrimers offer an advantageous platform for covalent linking with any desired functional groups for intended applications in a variety of fields.

4.2 - Future Outlook

4.2.1 - Evaluation of Anti-Scalant Behaviour

With the success in the synthesis of generations 0-3 acetylene-terminated dendrimers, the next logical step is to elaborate their functionalization with phosphonate groups. More specifically, the deprotection of the phosphonate terminated dendrimers from their ethyl esters to the corresponding alcohols, followed by an evaluation of their anti-scalant behaviour. Considering that these dendrimers are built on a four arm core, compared to more commonly employed three arm cores, it will be interesting to evaluate the role of the evolved structure in these dendrimers on their antiscalant behaviour. This will be essential in
developing structure-property relationships in dendrimers for anti-scalant behaviour.

4.2.2 - Further Evaluation of Bactericidal Properties

Since in this thesis only one OH-terminated dendrimer was tested, it would be interesting to evaluate the bactericidal activity of other such dendrimers. In addition to the first generation dendrimer, the second and third generation hydroxide-terminated dendrimers should be tested for their bactericidal action. This will be essential in better evaluating the role of the nature of the terminal groups on the bactericidal properties of dendrimers.

Another important venue to explore will be to evaluate the effect of the counter ion employed in the cationically charged structures on the bactericidal efficacy of the dendrimer. This could also help to optimize their performance as bactericides. Many of the previously evaluated cationically charged dendrimer bactericides have quaternary amine surface groups, unlike the primary amines employed in this project. Further studies could focus on functionalizing the acetylene-terminated dendrimers with quaternary amines instead of primary ones. An evaluation of the effect of these changes in counter ion and surface groups on the bactericidal properties of our dendrimers, could lead to more precise structure-activity relationships which would be invaluable in the design of future dendrimer bactericides.

4.2.3 - Elaboration of the Synthesis of Dendrimers

The synthesis of a fourth generation acetylene-terminated dendrimer would represent a noteworthy accomplishment. Provided that the periphery of the
largest structure prepared to date, **G3-ester**, is not too crowded to achieve complete reaction with our azide terminated building block. A fourth generation dendrimer with a tetrafunctional core, such as the one employed in this project, presents 64 terminal-acetylene groups. This represents a major increase over the 48 surface groups presented by a fourth generation dendrimer with a trifunctional core. It would be interesting to see the effect of this significant increase in surface group density on the properties of the fourth generation dendrimer.