The Synthesis of Dendrimer-based Radioimaging Agents

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By

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Abstract

The synthesis of new macromolecular diagnostic imaging agents has been a growing field in polymeric chemistry research. Dendrimers provide a viable scaffold for such applications due to their unique, defined macromolecular architecture. The precise structural control afforded via the step-wise, systematic approach to dendrimer synthesis yields exceptional and precise macromolecules that can in turn be functionalized to include necessary imaging moieties with the same degree of precision.

The enhanced permeability and retention (EPR) effect is an observable occurrence that allows macromolecular structures present in the circulatory system to passively concentrate in tumour tissues. Covalent attachment of poly(ethylene glycol) (PEG), widely referred to as PEGylation, to macromolecular structures has been shown to significantly increase their blood circulation times. This, in turn, allows for significant concentration of these PEGylated macromolecules in tumour tissue over an extensive time frame. The use of PEGylated polymeric structures in imaging and chemotherapeutic drug-delivery applications reliant upon the EPR effect has seen a sharp increase in the past three decades, resulting in a staggering number of these conjugates entering current clinical trials.

We have herein contributed to this growing field by attempting the synthesis of a series of PEGylated poly(2,2-bis[hydroxymethyl]propanoic acid) PMPA dendrons using thiol-ene "click" chemistry to fully functionalize the periphery of the dendrons. The series consisted of three dendritic architectures peripherally functionalized with PEG chains of

varying length (n= 3, 8, 16), with the goal of determining the effect of PEG chain length on blood circulation times. Synthesis of these conjugates began first with functionalization of the dendron periphery to incorporate alkene functionalities using anhydride-mediated esterification chemistry. The core of the peripheral-alkene PMPA dendrons was then modified to introduce a metal chelating ligand using standard amidation chemistry. The ligand, a tri-nitrogen bearing bis-pyridyl functionality, has been observed to chelate the radionuclide technetium-99m (^{99m}Tc) with high binding affinity. ^{99m}Tc is the most widely used diagnostic radioisotope in diagnostic medicine due to its ideal isotopic properties, widespread availability, low cost, and its ability to be traced, in real time, *in vivo* using Single Photon Emission Computed Tomography (SPECT). The resulting bis-pyridyl core, alkene PMPA dendrons formed the basis of a novel imaging platform.

The peripheral alkenes of these core metal-chelating dendrons was then functionalized via thiol-ene "click" reaction with PEG thiols (PEG-SH) of the desired length. The strong reactivity of these alkenes was observed in their reaction with smaller PEG-SH chains (n=3, 8), however difficulty in fully functionalizing the periphery with the longest polymer chain was encountered.

Metallation of the core of each PEGylated dendron was then attempted according to literature procedures for ^{99m}Tc radiolabeling with the bis-pyridyl chelate. This was met with mixed results, however a viable candidate for *in vivo* imaging and biodistribution studies was successfully produced.

Acknowledgements

My deepest thanks go to my supervisor and mentor, Prof. Alex Adronov. I would not have had the slightest inclination to pursue such a rewarding project if not for his constant encouragement, mentorship and support. I am confident that working under his guidance has made me a much more critical thinker, and a more confident and capable scientist.

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This thesis would not have been possible without the love and support of my family. My father, Cecil Knight has always encouraged me to do my best to make the world a better place for every person, and supported me during both brief moments of success and months of failure. My mother, Nancy Kivett Knight, has remained a constant source of love and support even after her passing. Her long and brave battle with cancer has inspired me to keep pressing through the most difficult times of this project with the hope that someday we will find a cure for this terrible disease.

Lastly I thank my favourite person, Rachel Smith, for being herself. I love you.

Table of Contents

List of Figures and Schemes

List of Schemes **Page**

Chapter 1: Introduction

1.1. Introduction to Dendrimers

Branching patterns can be found almost everywhere in nature. Some notable examples include the way water flows to form complex systems of rivers, streams, tributaries and deltas, to how vegetation develops an incredibly branched system of roots to access the water supply, as well as the complex leafing patterns on trees to harvest sunlight for photosynthesis. In addition, animals rely on branched systems of arteries, capillaries and veins to supply organs with nutrients via the circulatory system. Our brains utilize our central nervous system, a branched network of neurons, to gather, store, and process information about our surroundings. The net result of branching in all of these examples is an increase in the efficiency of particular processes.

The properties of branched structures and networks have been a focus of attention in polymer chemistry for decades. It has led to the synthesis, development and discovery of many new and unique polymer architectures. The family of branched polymer architectures has grown to include graft copolymers, branched polymers, star polymers, hyperbranched polymers and dendrimers (Figure 1.1).

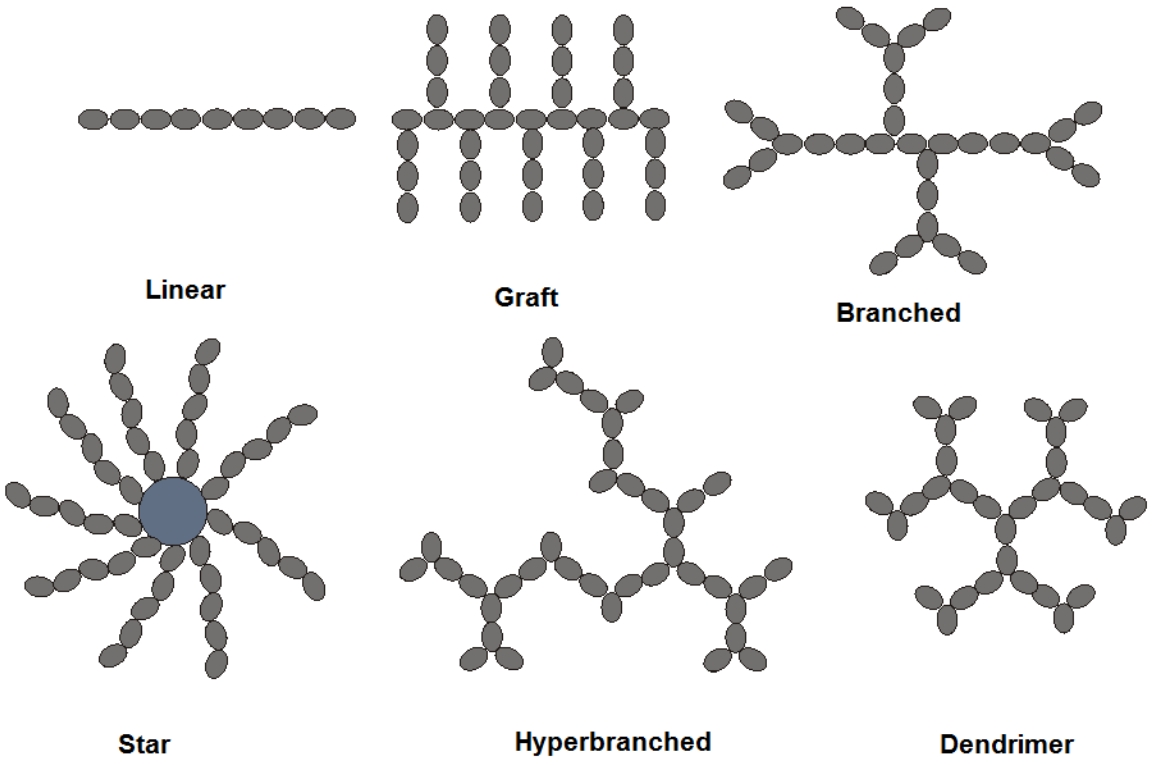


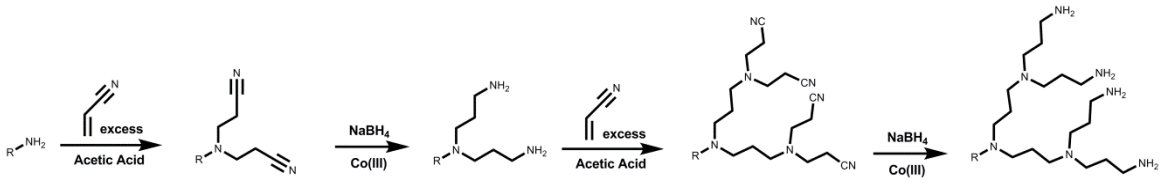
Figure 1.1. A schematic representation of a linear polymer versus common branched polymer architectures.

Dendrimers represent a unique class of highly branched and monodisperse polymers. Originally developed in the late 1970's and early 80's,[1–3] dendritic polymers have attracted much attention due to their distinctive architectures and potential applications in many fields of research. Characteristically, dendrimers are comprised of numerous layers of monomer units, referred to as dendrimer "generations" that create an increasingly branched system with every generation growth step. The formation of microenvironments within the dendritic network combined with the ease of

functionalization at the periphery and core have made these polymers particularly appealing for biomedical applications, including drug delivery[4–7] and imaging.[8]

1.2. Synthetic History of Dendrimers

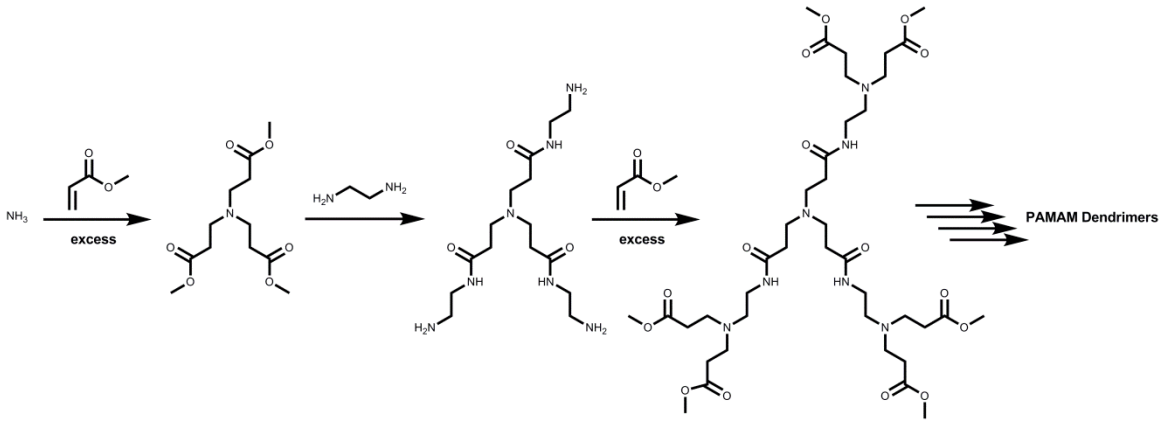
The first stepwise-controlled synthesis of branched polymers was successfully performed by Vogtle in 1978.[1] This synthesis began by combining a primary amine with an excess of acrylonitrile in a Michael addition to yield a bifunctional core. The peripheral nitriles were reduced in the presence of Co(III) and $NaBH_4$ (Scheme 1.1). Iterative repetition of these two steps resulted in a successful but low-yielding synthesis of a low molecular weight branched oligomer. These "cascade polymers", as Vogtle initially described them, mark the beginning of dendritic polymer chemistry due to their precise architecture and controlled stepwise synthesis.



Scheme 1.1. 1978 Synthesis of cascade polymers.

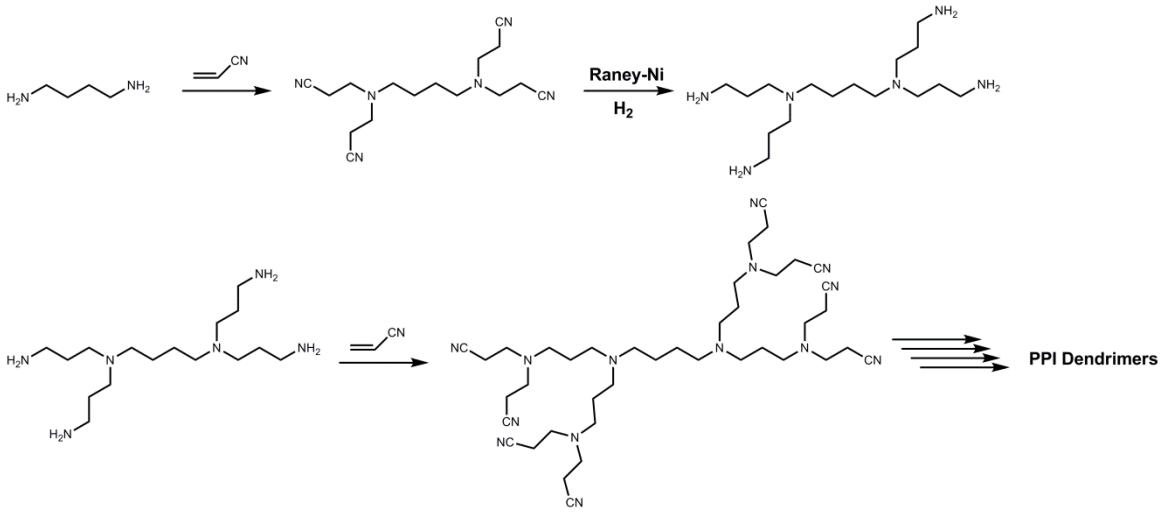
The term "dendrimer" was first used in a publication by Tomalia in 1985.[2] Building on Vogtle's initial synthesis of low-molecular weight dendritic polymers, ammonia was first combined with an excess of methyl acrylate to provide a trifunctional core via Michael addition. Amidation of the methyl esters with ethylene diamine provided reactive amines at the periphery for further Michael addition to methyl acrylate (Scheme 1.2). This iterative methodology provided the first dendrimers with molecular weights in

excess of 1 million Daltons,[9] and due to its simplicity has made polyamidoamine (PAMAM) dendrimers widely commercially available. PAMAM dendrimers remain the most widely used in research today.



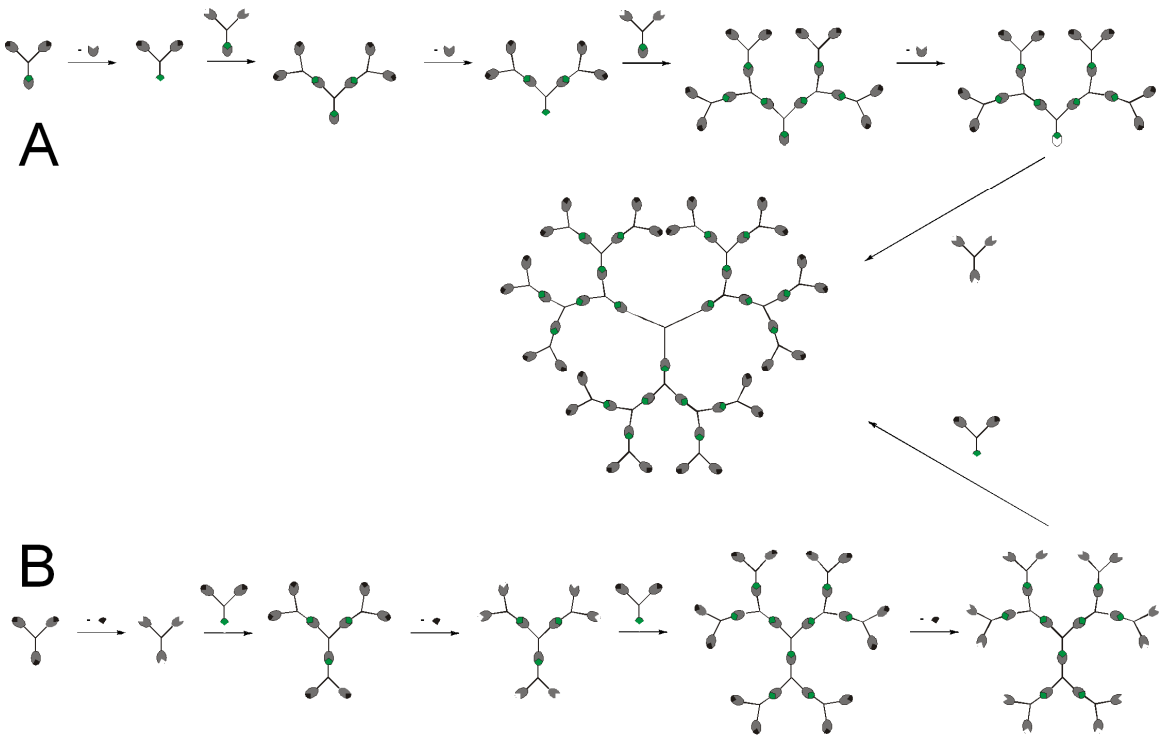
Scheme 1.2. PAMAM dendrimer synthesis.

Optimization Vogtle's original inefficient synthesis by Meijer in 1993 resulted in the preparation of another class of dendritic macromolecules, polypropyleneimine (PPI) dendrimers (Scheme 1.3).[10] The high yield and degree of purity obtained in this synthesis has made PPI dendrimers the second most popular architecture in dendrimer chemistry. They have also been produced on the industrial scale.



Scheme 1.3. Synthesis of PPI dendrimers.

The synthesis of dendrimers can be accomplished by two paths, convergently or divergently (Scheme 1.4). As described above, the earliest examples of dendrimers were synthesized via divergent means. Divergent synthesis begins with a central core molecule that reacts with monomers in layers in an outward pattern, from core to periphery, generation by generation (Scheme 1.4B). In order to prevent runaway polymerization, the monomer unit must have some means of peripheral protection. Growth initially occurs when the core of the dendron couples to a reactive moiety on the monomer unit. In preparation for additional monomer coupling and continued growth, any peripheral protection must then be removed to activate the periphery. The resulting reactive periphery is then combined with additional monomer to continue growth of the dendrimer.



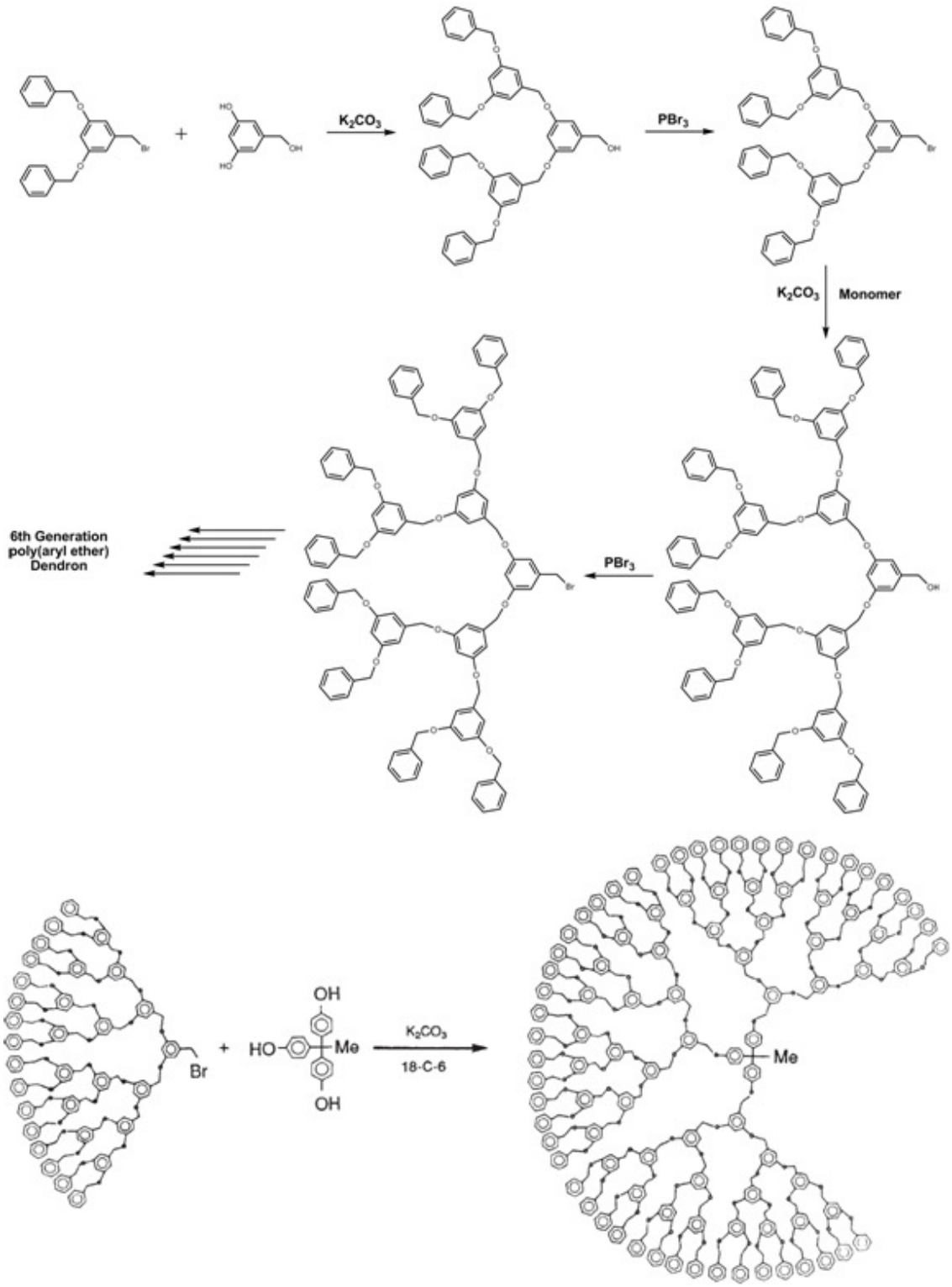
Scheme 1.4. Schematic representation of convergent (A) and divergent (B) synthesis of a dendrimer.

This synthetic strategy has proven useful for the synthesis of many types of dendritic polymers. However, obtaining higher generation dendrimers is limited by the efficiency of the reaction method as the number of reactive sites increases exponentially with each generation. The large number of reactive sites also increases the chance of partial functionalization and presence of defect sites at high generations. This requires that large excesses of monomer be used in each growth step to ensure complete functionalization of the periphery.

However, divergent synthetic methodologies typically result in very high yields due to lack of unfavourable steric interaction at peripheral reactive sites. Furthermore, the amount of material produced via divergent synthesis grows exponentially with increasing generation number. Dendrimers produced in this fashion are also simple to purify and separate from their starting materials.

While each of the previous breakthroughs in dendrimer chemistry are important, perhaps the most significant advance in dendrimer synthesis came from Hawker and Fréchet in 1990.[11] In their seminal paper, Hawker and Fréchet described their convergent synthesis of dendritic polymers (Scheme 1.5).[11,12] Unlike divergent methods which start from the core and build the dendrimer outwards, convergent synthesis begins with the exterior and builds inwards, with core functionalization as the final step (Scheme 1.4A). Synthesis of the periphery begins with two protected monomer units reacting with an orthogonally protected central monomer to yield a fragment of a whole dendrimer

referred to as a dendron. Once the core protecting group is removed from the linking monomer, two of these dendrons can then be linked through identical chemistry to yet another central monomer to yield larger and larger dendrons. Once the desired generation number has been achieved, the dendrons can then be coupled to the desired core through similar connectivity to yield the dendrimer. In the example by Hawker and Fréchet (Scheme 1.5) a 3,5-dihydroxy benzyl alcohol was used as an AB_2 monomer unit, where the phenol functionalities were protected as benzyl ethers. The core hydroxymethyl group was converted to a benzyl bromide using PBr_3, allowing further reaction with 3,5-dihydroxy benzyl alcohol. These iterative coupling and activation steps were repeated to build up the final dendrimers.



Scheme 1.5. First convergent synthesis of a dendrimer.[11]

This convergent synthesis strategy results in synthetically pure and monodisperse architectures. This is a direct result of the sharp decrease in the number of reactive sites in each growth step of the dendrons. With a typical bifunctional monomer, a fifth generation (G5) dendrimer has 32 reactive sites for continued growth by divergent synthesis. Conversely, a G5 dendron grown convergently has only one reactive site at its core. Thus if a coupling reaction fails to occur in convergent synthesis, the fully coupled product is easily separable from partially coupled products by column chromatography. allowing synthesis of pure, high-generation dendrimers.[11]

However, unlike divergent synthesis, dendrons and dendrimers synthesized by convergent means require extensive purification at each growth step. Furthermore, the reactivity of the periphery of a dendrimer to divergent synthesis remains constant throughout the growth of higher generations. As a consequence of sterics, as dendrons increase in generation in convergent synthesis their reactivity at the single interior monomer unit decreases sharply. Combined with the exhaustive purification necessary to yield pure dendrimers convergently, this results in poor overall yield compared to divergent synthesis.

1.3. Properties of Dendrimer Microenvironments

Dendrimers consist of three key microenvironments: a central core, the interior comprised of repeating monomer units, and the peripheral functionality.

The core of a dendritic polymer can be modified to include various moieties of interest before coupling/growth of the dendrons takes place, or before the dendrimer is

grown outwards. Subsequent functionalization of the periphery allows substantial modification of molecular properties, making dendrimers highly versatile for many potential applications. Unlike many conventional polymers, dendrimers have a polydispersity index (PDI) of 1.0 and do not exhibit a glass transition temperature (Tg).[13] The perfect monodispersity of these molecules is a direct consequence of controlled synthesis and purification at each generation. The absence of a Tg is the result of a lack of polymer chain interaction and entanglement within the system. Since dendrimers tend to adopt a spherical conformation and generally lack long polymeric chains, they cannot become entangled with other dendrimer molecules (Figure 1.2).[13]

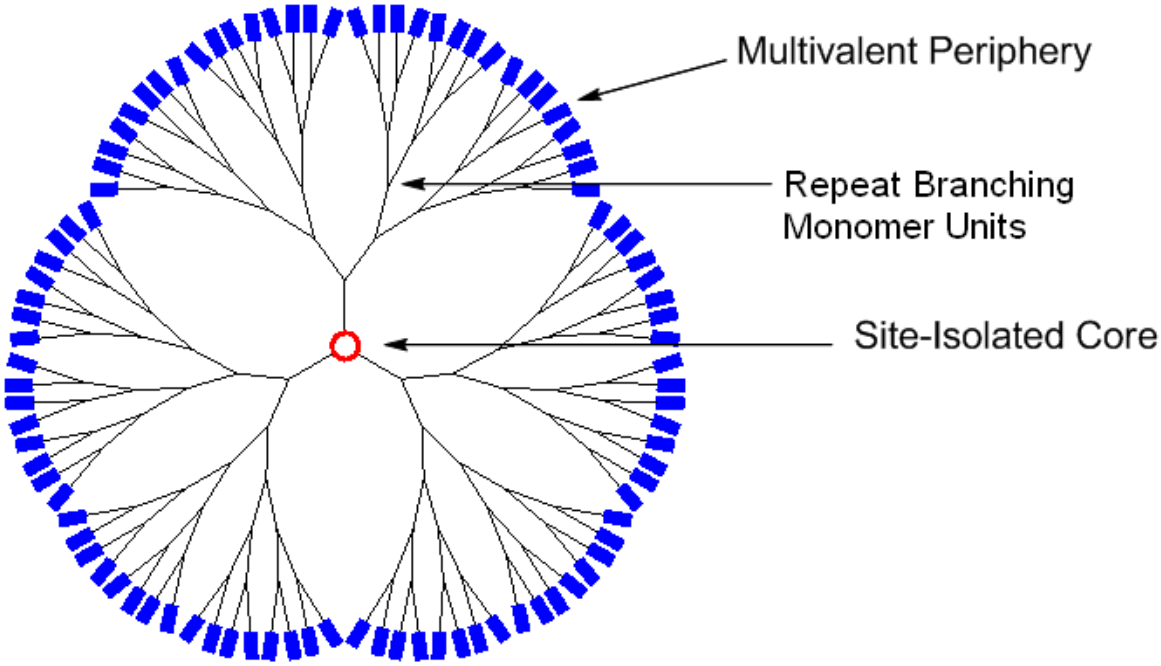


Figure 1.2. 2D representation of a dendrimer.[14]

1.4. Dendrimers as Imaging Agents:

Dendrimers have a wealth of properties that make them ideal macromolecules for radioimaging or drug-delivery applications. First and foremost, dendrimers are structurally perfect macromolecules. This is due to the high degree of structural control obtained using a step-wise synthetic methodology. Each successive growth of the dendrimer produces an exact, monodisperse structure. The monodispersity of these macromolecules makes them extremely valuable as drug delivery agents because the structure of each dendrimer is exact. This confidence in the structure of any dendrimer-radionuclide or dendrimer-drug conjugate vastly improves the probability of approval for human use by regulatory agencies such as Health Canada or the FDA in the United States.

The multivalent periphery of dendrimer molecules allows for extensive modification that expands exponentially with each progressive growth. This structural consequence of dendrimers makes them highly valuable as drug delivery agents. With the correct peripheral modification in place, dendrimer-drug conjugates can deliver a highly localized dose of a therapeutic agent, or take advantage of multivalent binding to a receptor target on a cell surface, exponentially expanding the effectiveness of the agent.[15–17] However, the periphery of a dendron is not the only place where modification can occur. At any stage in a dendrimer growth, the repeating monomer units may be exchanged for a variety of other structures, as long as the functional groups for dendrimer growth are retained.[18,19] For example, Parrott et al. produced dendrimers functionalized with lipophilic carborane structures (Figure 1.3), while maintaining the overall aqueous solubility of the product by dendronizing to a sufficient degree beyond the carborane unit.[20]

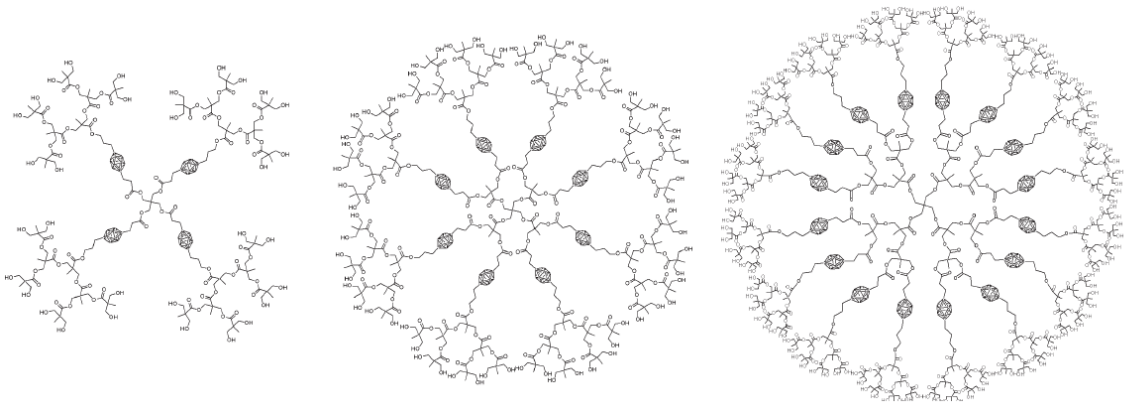


Figure 1.3. Carborane-containing, water-soluble polyester dendrimers.[20]

The site-isolated core of dendrimers also allows for ease of modification. The core of a dendrimer is chosen such that distinctly different chemistry can be performed at this site, relative to reactive peripheral functional groups. This allows for diverse modification of the macromolecule to capitalize on the benefits of multiple moieties within one molecular unit. Fréchet, Gillies, and Ihre have examined this property extensively through peripheral modification with poly(ethylene glycol) (PEG) chains (PEGylation) followed by core modification to attach drug conjugates.[6,21] The core of a dendrimer can also be modified to incorporate radionuclide tracers for imaging applications. Parrott et al. bound the γ-emitter, technetium-99m (^{99m}Tc) to the core of the poly[2,2-bis(hydroxymethyl) propanoic acid] (PMPA) dendron (Figure 1.4).[8]

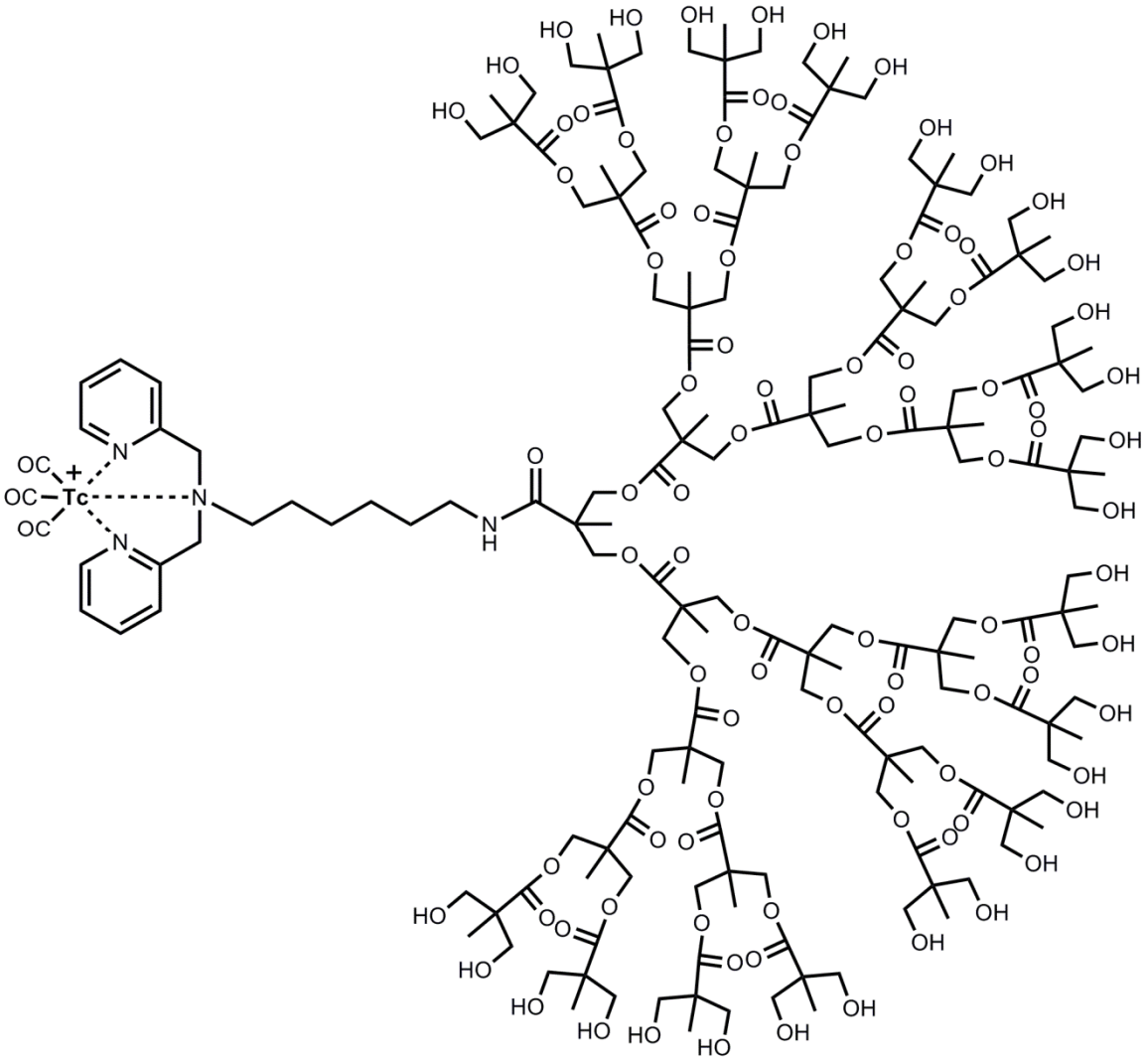


Figure 1.4. Technetiated fifth generation PMPA dendron for imaging applications.

Magnetic resonance imaging (MRI) contrast agents (CAs) are also a current focus of research in dendrimer-based imaging applications. Gd^{III}-based CAs currently dominate clinical use.[22–26] However, concerns have been raised regarding the safety of Gd^{III} for use in humans as well as its sustainability and environmental impact. Since Gd^{III} possesses a significant transverse spin-spin relaxation time (T_2), it could potentially be used in much lower concentrations due to increases in modern MRI instrument field strength.[27] In an

attempt to provide a safe, effective and sustainable lanthanide (Ln) based CA, Fréchet and coworkers recently incorporated a series of Ln^{III} complexes into the architecture of a polylysine dendrimer (Figure 1.5).[27] The T_2 relaxivity of most dendrimer-Ln^{III} complexes showed a significant increase. Furthermore, the biocompatibility of the PEGylated dendrimer negated any cytotoxicity from the Ln^{III} complex, and its long circulation time meant that lower amounts of the CA could be used in each trial. Accumulation of the CA in tumour tissue via the enhanced permeability and retention (EPR) effect, discussed later in this chapter, provides a potential application for targeted imaging of tumour tissues.

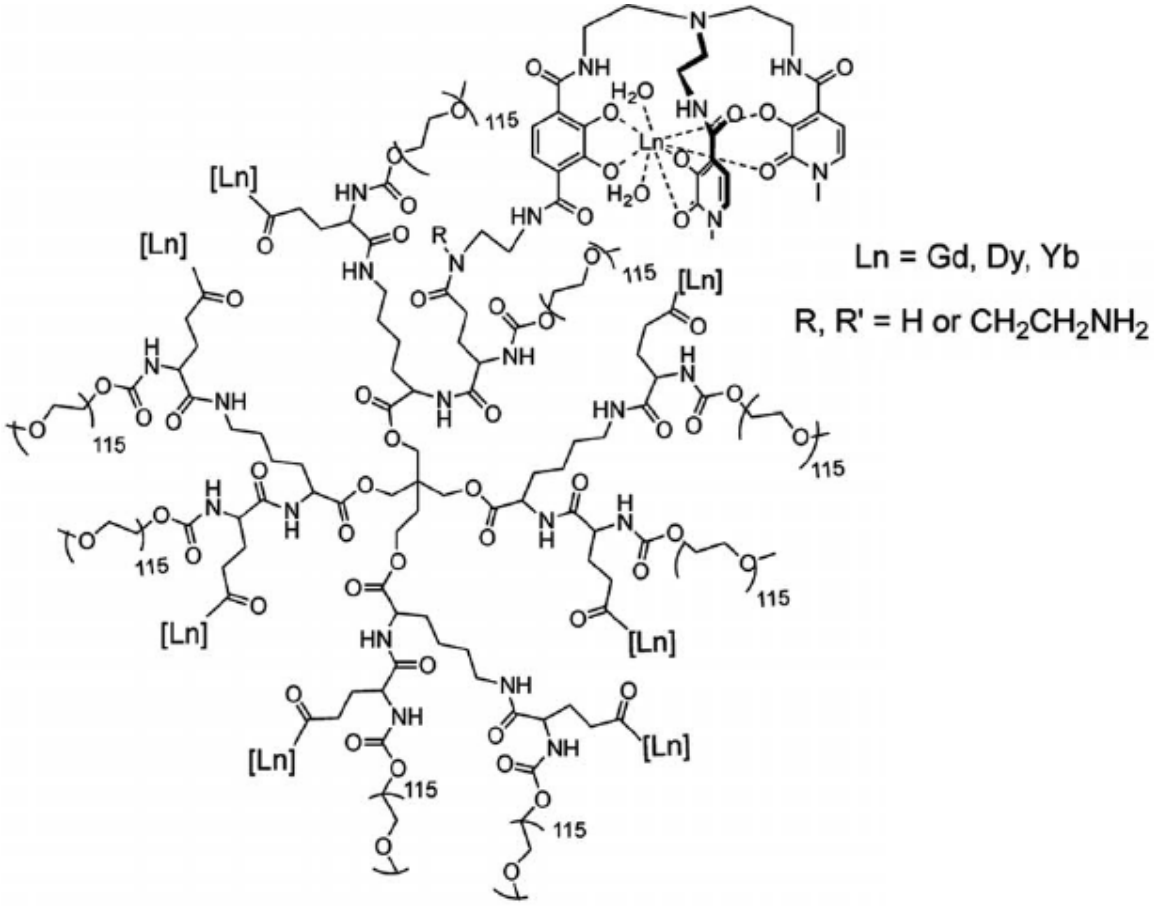


Figure 1.5. PEGylated Polylysine Dendrimer-Ln^{III} conjugate for MRI.[27]

Zhang and coworkers emulated the success of Fréchet's work by incorporating gold nanoparticles to the core of PAMAM dendrimers for X-ray computed tomography (CT) imaging applications.[28,29] These PEGylated, biocompatible dendrimer-nanoparticle conjugates relied on the EPR effect to passively target tumour tissues, providing effective CT imaging of the tumour model.[28,29]

The use of dendrimers in the development of new and effective imaging agents for use in humans cannot be ignored. The wealth of beneficial properties that stem from dendritic architecture make them an ideal candidate for imaging applications.

1.5. Dendrimers for Drug Delivery and Chemotherapy: The EPR Effect

The application of dendrimers in medical treatments has become an increasing area of interest because macromolecules have been identified as desirable compounds for chemotherapeutic drug-delivery systems. In comparison with smaller molecules, macromolecules have distinct advantages in specific targeting of tumour tissue, as well as efficacy of drug delivery and subsequent treatment.[30–32] The ability of macromolecules to selectively target tumour tissue is largely due to the EPR effect.[33–36] This phenomenon is a direct result of the mechanism of formation of blood vessels that compose tumour tissue (Figure 1.6.). It is currently understood that the rapid, disorganized growth of these blood vessels results in the observed, leaky vasculature.[30–34] This imperfect vasculature allows for the selective extravasation of macromolecules into the tumour tissue. In addition, the vascular structure of tumour tissue lacks the lymphatic drainage vessels found in normal, healthy tissue. Therefore, when macromolecules are absorbed into tumour tissue, they

cannot be drained via lymphatic means. This results in the tumour tissue acting as a concentration sink for macromolecules flowing through the bloodstream.

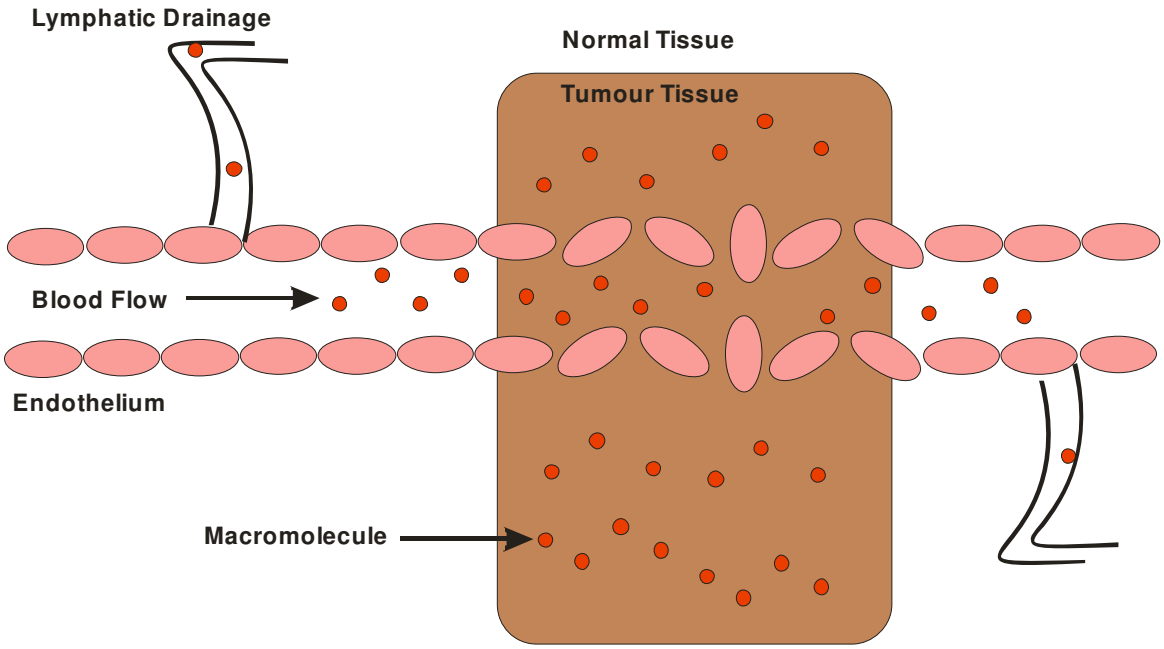


Figure 1.6. Absorption of macromolecules into tumour tissue via the EPR effect.

However, in order to experience this effect the *in vivo* circulation time of the macromolecules in the bloodstream must be sufficiently long. Rapid clearance from the body is not favourable in this case as an insufficient amount of target molecules will be absorbed into the tumour tissue.

Prior to the studies by Zhang[29] and Fréchet [27], Chang et al. took the use of dendrimers in creating viable MRI CAs one step further.[37] Magnetic iron oxide nanoparticles (IONPs) were incorporated to the core of a PEGylated PAMAM dendrimer loaded with acid-labile moieties of the anti-cancer drug doxorubicin (DOX). The resulting macromolecules were not only useful as MRI CAs, but passive targeting of tumour

tissues via the EPR effect provided a method of tumour-specific drug delivery. The hydrazone linkages used to attach DOX to the dendrimer were stable at physiological pH in the bloodstream but are cleaved in the acidic physiological pH of tumour tissues, providing a pH-sensitive delivery system. This delivery system for DOX was originally developed with great success by Fréchet and coworkers in 2006.[38] A bowtie PEGylated PMPA dendron - DOX-loaded PMPA dendron was synthesized utilizing hydrazone linkages similar to what was used by Chang et al (Figure 1.7). This PEGylated dendrimer-drug conjugate showed remarkable treatment of mice bearing C-26 colon carcinomas. Mice were inoculated with the tumour and given a single dose of either the dendrimer-DOX conjugate, free DOX-HCl, or the widely implemented chemotherapy drug Doxil. Control studies including the use of a dendrimer-DOX conjugate with a non-labile carbamate linkage were also performed.

It was observed that the mice were able to tolerate a dosage of dendrimer-DOX equivalent to 3.33 times the maximum safe dosage of free DOX-HCl. With the proper dosage, mice showed no appreciable weight loss and an outstanding 100% survival rate compared to both DOX-HCl and Doxil (Figure 1.8). The mice receiving an equivalent dosage of Doxil displayed dangerously high weight loss, and did not achieve similar survival rates.[38] In biodistribution studies of the dendrimer-DOX conjugate it was found that its *in vivo* circulation time overwhelmed the rapid clearance of DOX-HCl. With a circulation half-life of 31 ± 2 h, it remains in the circulatory system approximately 186 times longer than DOX-HCl.[39] Furthermore, its tumour concentrations were found to be approximately 9 times higher than those from DOX-HCl dosage.

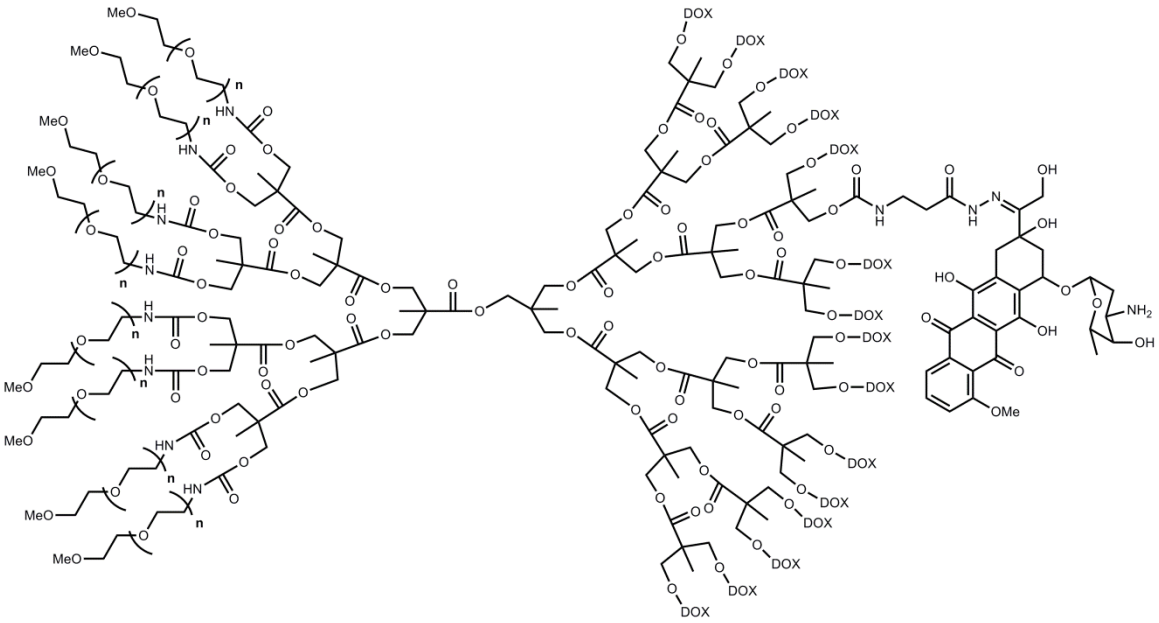


Figure 1.7. Fréchet's PEGylated dendrimer-drug conjugate loaded with doxorubicin.[38]

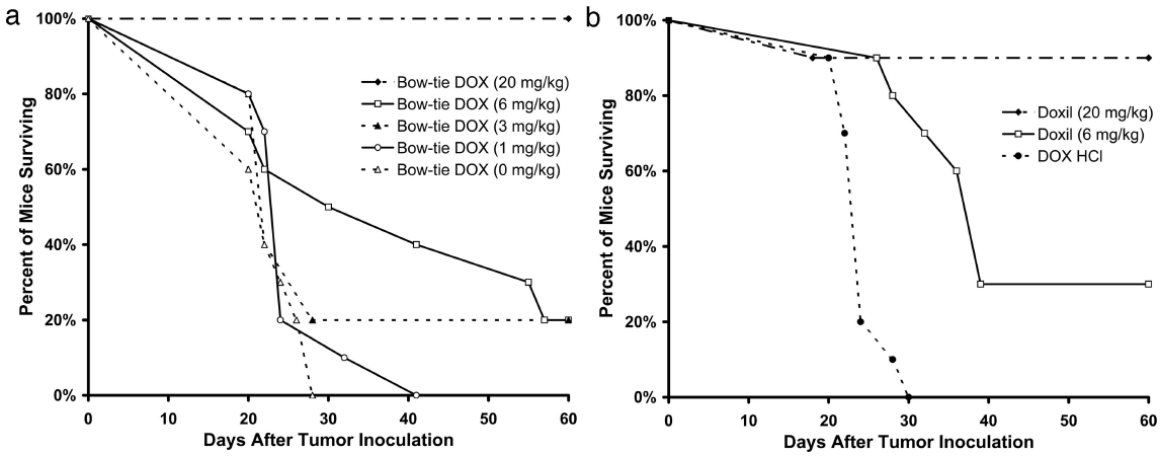


Figure 1.8. Survival rates of mice bearing C-26 colon carcinomas treated with single doses of dendrimer-DOX conjugate (A) and Doxil/DOX-HCl (B).[38]

This extraordinary work performed by Fréchet and coworkers is just one of many examples of delivery systems that have relied upon the EPR effect for chemotherapy applications with dendrimers. The effectiveness of this passive targeting method has been proven repeatedly in the literature. It is expected to remain a focus of targeted dendrimer-based chemotherapy applications for years to come.

1.6. PEGylation for Improved Imaging and Drug Delivery Applications

Ihre[15], Gajbhiye[16], and Kumar[17] have shown that peripheral PEGylation of dendrimers increases *in vivo* circulation of dendrimer-drug conjugates, and stabilizes drug release rates for prolonged delivery. Fréchet's 2006 PNAS article highlights these factors in great detail.[38] The hydrophilicity of PEG attracts water molecules to the exterior surface of the molecule, effectively masking it from any immune system response in the bloodstream.

The PMPA dendron has previously been examined as a viable template for chemotherapeutic drug delivery and imaging. The fifth to seventh generation dendrons were radiolabeled with ^{99m}Tc at their core and evaluated as potential tumour imaging agents, with a hypothesis that an increase in molecular size may have an impact on *in vivo* circulation time (Figure 1.9). However, the molecular weight of each dendritic scaffold alone was below the renal clearance cut-off of 40 – 60 kDa.[8]

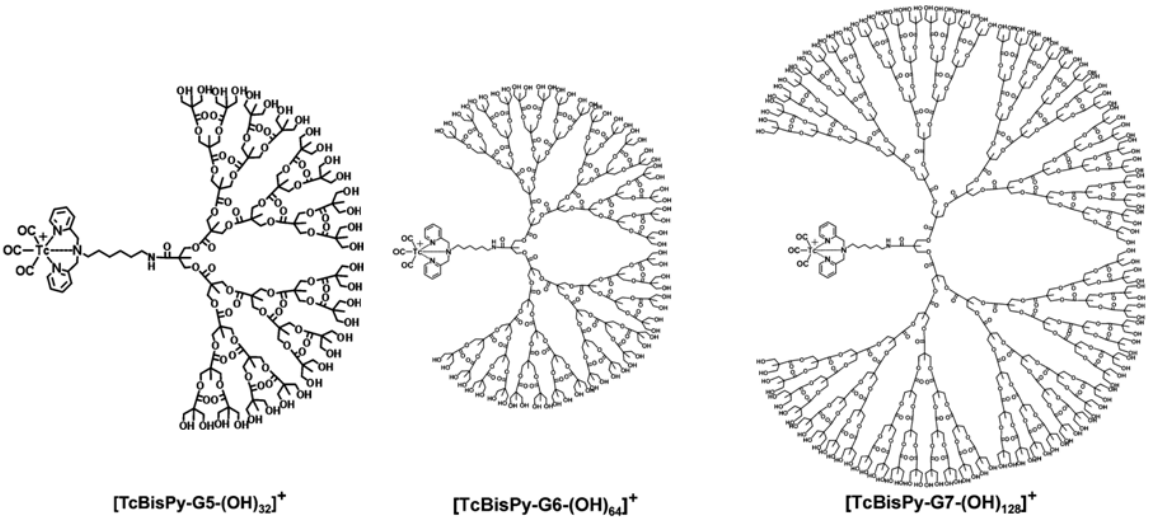


Figure 1.9. ^{99m}Tc-labeled PMPA dendrons.[8]

The results of *in vivo* biodistribution and Single Photon Emission Computed Tomography (SPECT) imaging of each generation showed little increase in circulation time with increasing generation number, and thus molecular size, for the PMPA dendron.[8] SPECT imaging showed that 98% of ^{99m}Tc radiolabeled PMPA dendrons injected into the test animal were found to be cleared into the bladder within ten minutes of exposure.[8] Biodistribution studies also confirm this result, and although no unfavourable liver uptake is observed, the molecule is cleared far too quickly to experience the EPR effect.

In order to combat this shortcoming, the peripheral PEGylation of the fifth generation PMPA dendron will be performed to increase the *in vivo* circulation time. By increasing the circulation time, its viability as a potential chemotherapy imaging and delivery agent can be increased. The circulation time of the PEGylated PMPA dendrons is expected to be significantly longer in comparison to previously prepared, non-PEGylated

PMPA dendrons of the same generation[8], and thus will take advantage of the EPR effect in tumour tissue.

It has yet to be explored whether PEG chain length has a direct effect on the circulation time of PEGylated dendrimer conjugates. We plan to examine the effect of varying PEG chain length on circulation time, to obtain optimal dendron-PEG conjugates, *in vivo* using SPECT imaging. Using various PEG chain lengths (n = 3, 8, 16), we will explore the effects of peripheral PEG chain length on biodistribution and circulation time *in vivo* via a systematic increase in polymer chain length in each dendron-PEG conjugate.

The main advantages for the use of dendrimers as drug-delivery agents include their multivalent periphery as well as their well-defined architecture that results from precise stepwise, divergent synthesis. The high degree of structural control in this manner of synthesis yields specific and reproducible architectures. Modification of these precise dendritic structures allows for control over both the functional groups at the periphery, in this case PEG, as well as the relative size of the drug-dendrimer conjugate. The resulting conjugate structure is a well-defined system with a favourably low PDI. Although the use of PEG chains will increase the PDI of the system, previous FDA approval for PEG has no ill effect with regards to obtaining approval for use in humans.

The monomer unit of choice is the 2,2-bis(hydroxymethyl)propanoic acid unit, as it exhibits a large number of favourable properties when applied to a dendritic architecture. The structure formed through dendronization is a water soluble polyester.[8] This solubility is granted by the peripheral hydroxyl groups, and although PEG chains are

eventually attached to the periphery, modification to impart water solubility is unnecessary. The aqueous solubility of the dendron in its uncharged state imparts a degree of biocompatibility to the system. Cationic[40] and anionic[41] systems have been shown to exhibit unfavourable toxicological properties *in vivo*. Cationic systems have shown high levels of toxicity and rapid clearance from the circulatory system. Anionic systems have exhibited unfavourable properties via poor uptake into cellular membranes, which would prevent any benefit granted by the EPR effect.

The polyester architecture of the dendron is also biodegradable, as the ester linkages are susceptibile to hydrolysis. However, the neo-pentyl ester linkages found in the PMPA dendrimer are sterically hindered, thus preventing both nucleophilic attack and acid-catalyzed hydrolysis. This property of the PMPA dendron makes it a valuable candidate for imaging and drug-delivery applications in that it remains stable *in vivo*[8], while still exhibiting favourable biodegradability properties.

1.7. Thiol-ene "Click" Chemistry for PEGylation

A variety of methods of PEGylating dendrimers and macromolecules have been employed extensively in the literature.[5,6,15–17,19,21,30,38,42–49] However, the majority of these methods result in a mixture of full and partial functionalization on multivalent peripheries, requiring extensive purification to isolate the desired product. In order to create a viable platform for a dendrimer-based imaging agent, a more efficient and robust synthetic methodology is necessary. Acid-chloride reactions could be employed, however

the potential sensitivity of the PMPA ester backbone means this class of reactions should be avoided.

Thiol-ene "click" chemistry (Scheme 1.6) has been previously explored as an incredibly effective method of functionalizing the multivalent peripheries in dendrimers, as well as synthesizing dendrimers themselves (Figure 1.10).[50–52]

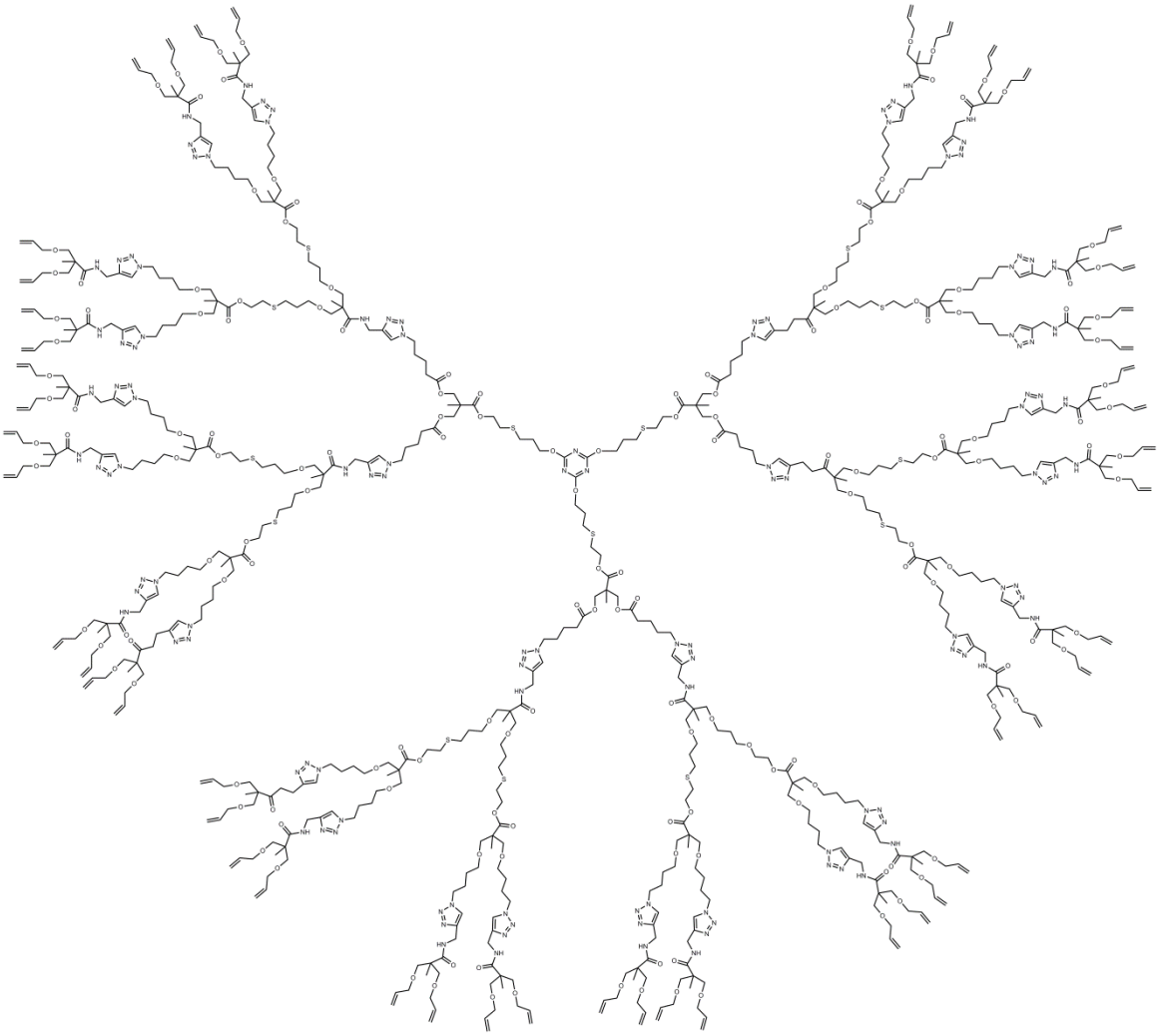
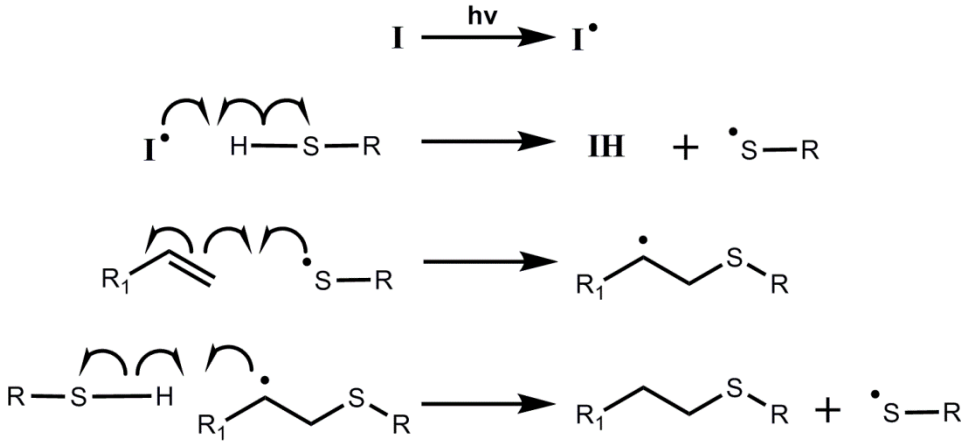


Figure 1.10. A dendrimer synthesized using thiol-ene "click" chemistry.[51]

The hydroxylated periphery of the PMPA dendron has proven to be highly reactive towards esterification using anhydrides.[8,15,20,21] This chemistry can be readily used to introduce alkenes at the dendrimer periphery via an alkene-derivatized synthon. The resulting alkene-functionalized dendrons can then be combined with a large excess of thiol-terminated PEG chains (which are both readily synthesized and commercially available) under photoinitiated reaction conditions to produce the desired PEGylated dendrons. Complete functionalization of the periphery is easily achieved through the robust reactivity of the thiol-ene "click" reaction and the product can be purified simply via column chromatography.



Scheme 1.6. General mechanism of the photoinitiated thiol-ene "click" reaction.

1.8. ^{99m}Tc Chelation for Imaging

The SPECT imaging and biodistribution analysis of PMPA dendrons was previously achieved via the chelation of ^{99m}Tc to the core of the dendron.[8] ^{99m}Tc is currently the most widely employed radionuclide for diagnostic imaging in medicine.[53,54]

Its γ emissions pass through organs and tissue with minimal radiation damage to the patient due to their γ-energy of 140 keV, similar to X-rays. This, combined with an ideal radionuclide half-life of 6 h and low production cost have contributed significantly to ^{99m}Tc's wide use in diagnostic imaging.[55]

Non-labile chelation of ^{99m}Tc to the core of each dendron has been achieved through the use of a tridentate bis-pyridyl (BisPy) binding ligand, which is irreversibly bound to the core of the dendron via an amide bond.[8] The BisPy ligand has proven valuable for chelation of ^{99m}Tc in a variety of imaging and targeted-delivery applications.[8,56–62] This method of radiolabeling will be employed in the PEGylated PMPA dendrons.

Chapter 2: Objectives

2.1. Synthesis of Alkene-Functionalized PMPA Dendrons

In order to prepare the PMPA dendron for thiol-ene "click" reactions, the hydroxyl groups on the periphery must be converted to alkenes. Simple anhydride esterification chemistry used for the growth of the PMPA dendrons themselves[8] was applied here. By replacing the benzylidene-protected bis-MPA anhydride with pentenoic anhydride (from pentenoic acid), identical reaction conditions were employed to produce alkene-terminated PMPA dendrons in very high yields at each generation up to G5. Prior to PEGylation necessary modifications to the core functionality were also performed to include the BisPy ligand.

2.2. PEGylation via Thiol-ene "click" Chemistry

Once alkene-terminated PMPA dendrons were prepared with the BisPy ligand at the core, thiol-terminated PEG (PEG-SH) was required. Varying lengths of PEG-SH were synthesized according to literature methods with ease,[63,64] although slight modifications were noted in order to improve overall yield and purity.

There are two main potential methods of initiation for thiol-ene "click" chemistry.[65,66] Thermally initiated reactions using initiators such as AIBN have proven relatively efficient, however photo-initiated thiol-ene "click" reactions generally result in higher yields of product.[65,66] In order to ensure maximum functionalization of the multivalent dendron periphery, photoinitiation is used. Efficient functionalization of

multivalent peripheries in synthesis and modification of dendritic structures via thiol-ene "click" chemistry was achieved previously using 2,2-Dimethoxy-2-phenylacetophenone (DMPA) as the photoinitiator.[50,51] By combining the alkene dendron with each desired chain length of PEG-SH in the presence of DMPA, an efficient synthesis of PEGylated PMPA dendrons was performed in sufficient yield.

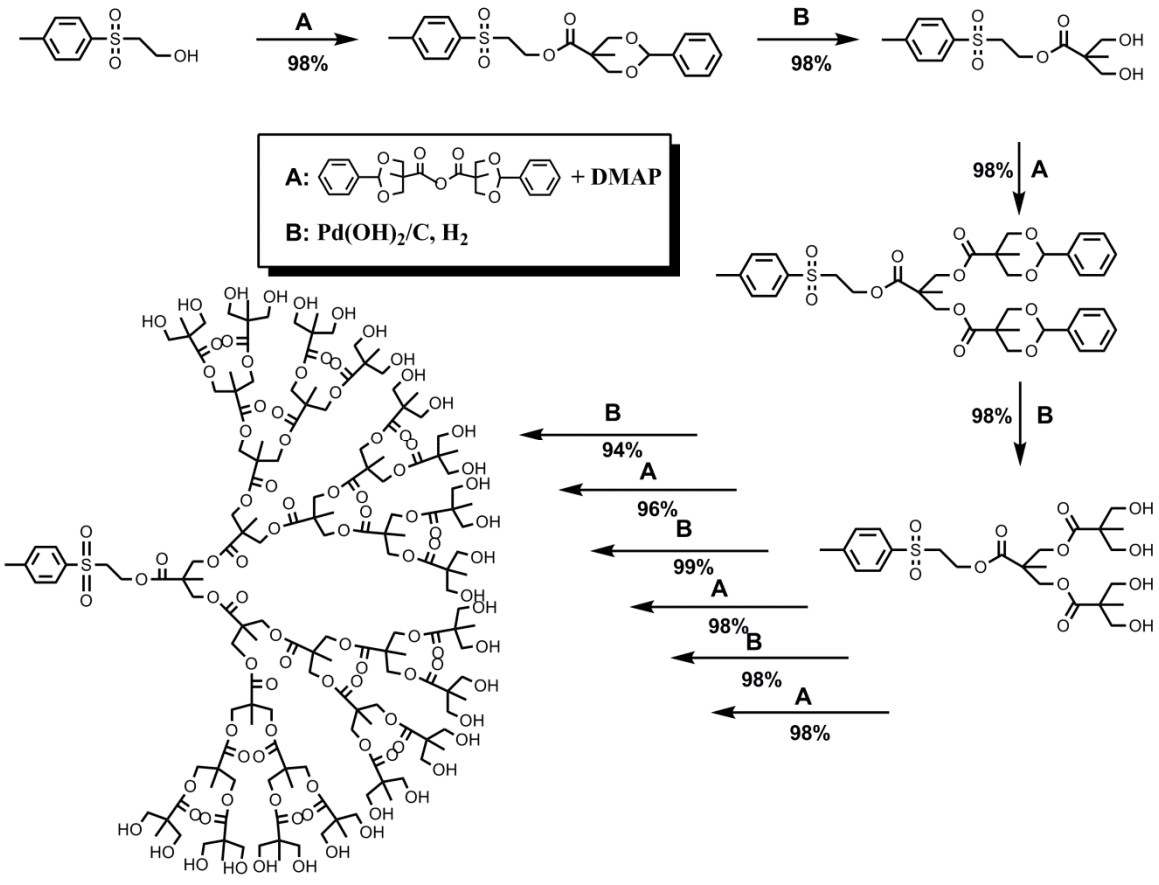
2.3. Radiolabeling of PEGylated PMPA Dendrons

With peripherally PEGylated PMPA dendrons prepared, the final step in the preparation of these imaging agents was attaching ^{99m}Tc to the chelating ligand at the core. This is performed according to slightly modified literature procedures[8] which are optimized to suit the requirements of each PEGylated PMPA dendron.

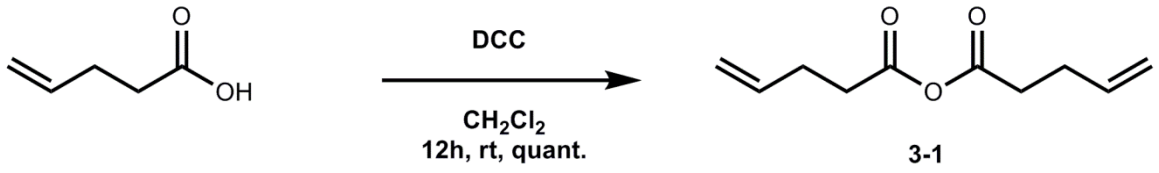
Chapter 3: Preparation of Peripherally Alkene-Functionalized PMPA Dendrons

3.1. Overview

Following literature procedures,[8] PMPA dendrons were synthesized up to the fifth generation with yields in excess of 94% (Scheme. 3.1). In preparation for further esterification at the periphery, pentenoic anhydride (**3-1**) was prepared from pentenoic acid (Scheme. 3.2). Each generation, one through five, of PMPA dendron was then combined with pentenoic anhydride using similar esterification chemistry to form alkene-functionalized PMPA dendrons in high yield. Each dendron was fully characterized by Nuclear Magnetic Resonance (NMR) spectroscopy and mass spectrometry. The core of these dendrons was then modified to include the BisPy ligand necessary for future radiolabeling and imaging. Since it has previously been observed that no appreciable increase in circulation time occurs between G5 to G7 for the PMPA dendron, synthesis of PMPA dendrons was only carried out to fifth generation.[8]



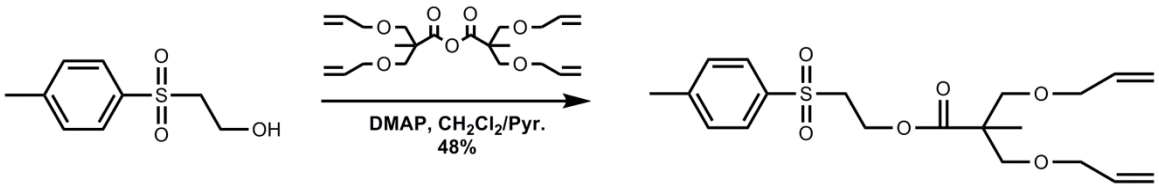
Scheme 3.1. PMPA dendron synthesis.



Scheme 3.2. Synthesis of **3-1**.

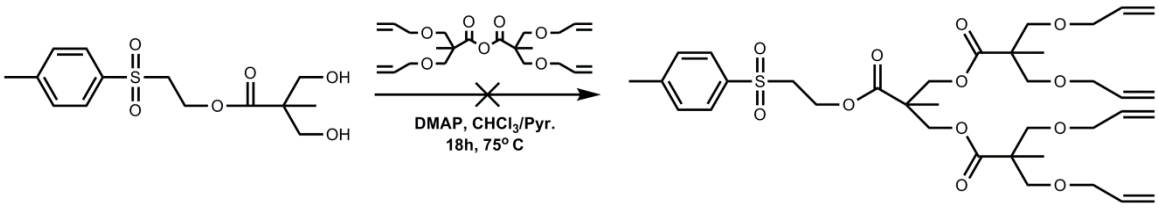
In preparation for thiol-ene "click" reactions, the hydroxylated periphery of the PMPA dendron was converted to an alkene functional group. Initially, it was thought that a bis-allyl MPA[51] anhydride (Scheme 3.3.) would be the best synthon for such an application. The anhydride was synthesized and used in an esterification reaction similar

to the growth of the dendron to yield allyl-functionalized PMPA dendrons while simultaneously increasing the generation number in a single step.



Scheme 3.3. Synthesis of bis-allyl MPA anhydride.

Although the synthesis of this anhydride was novel, its reactivity toward esterification with the dendron periphery was ineffective (Scheme 3.4), leading to low yields at G1 and no product at G2. Plagued by these low yields and difficult purification, this method for functionalizing the periphery with alkenes was abandoned in favour of a much simpler reaction.



Scheme 3.4. Attempted syntheses of allyl-functionalized dendrons.

3-1 was prepared via simple dicyclohexylcarbodiimide (DCC) mediated dimerization of pentenoic acid. This smaller, less complex anhydride was used for peripheral functionalization of PMPA dendrons under identical esterification conditions to those used in the dendron synthesis with remarkable results. Yields were typically in the mid 90% range. Although reactivity towards the periphery was greatly increased,

purification was still demanding. Quenching to remove excess anhydride after coupling to the periphery went to completion was ineffective. Thus, purification of each generation was accomplished via silica gel column chromatography. Nomenclature of these dendrons follows the same convention used by Parrott et al.[8] For example, a third generation pentenoate (PTA)-functionalized dendron with the TSe protecting group at the core would be referred to as TSe-G3-$(PTA)_8$.

The next step in developing an imaging platform with these dendrimers was to modify the core. The TSe core protecting group was removed using 1,8-Diazabicyclo[5.4.0]undec-7-ene (DBU).[8] This efficient procedure provided a reactive carboxylic acid-core dendron in high yield following purification by silica gel column chromatography to remove the alkene by-product.

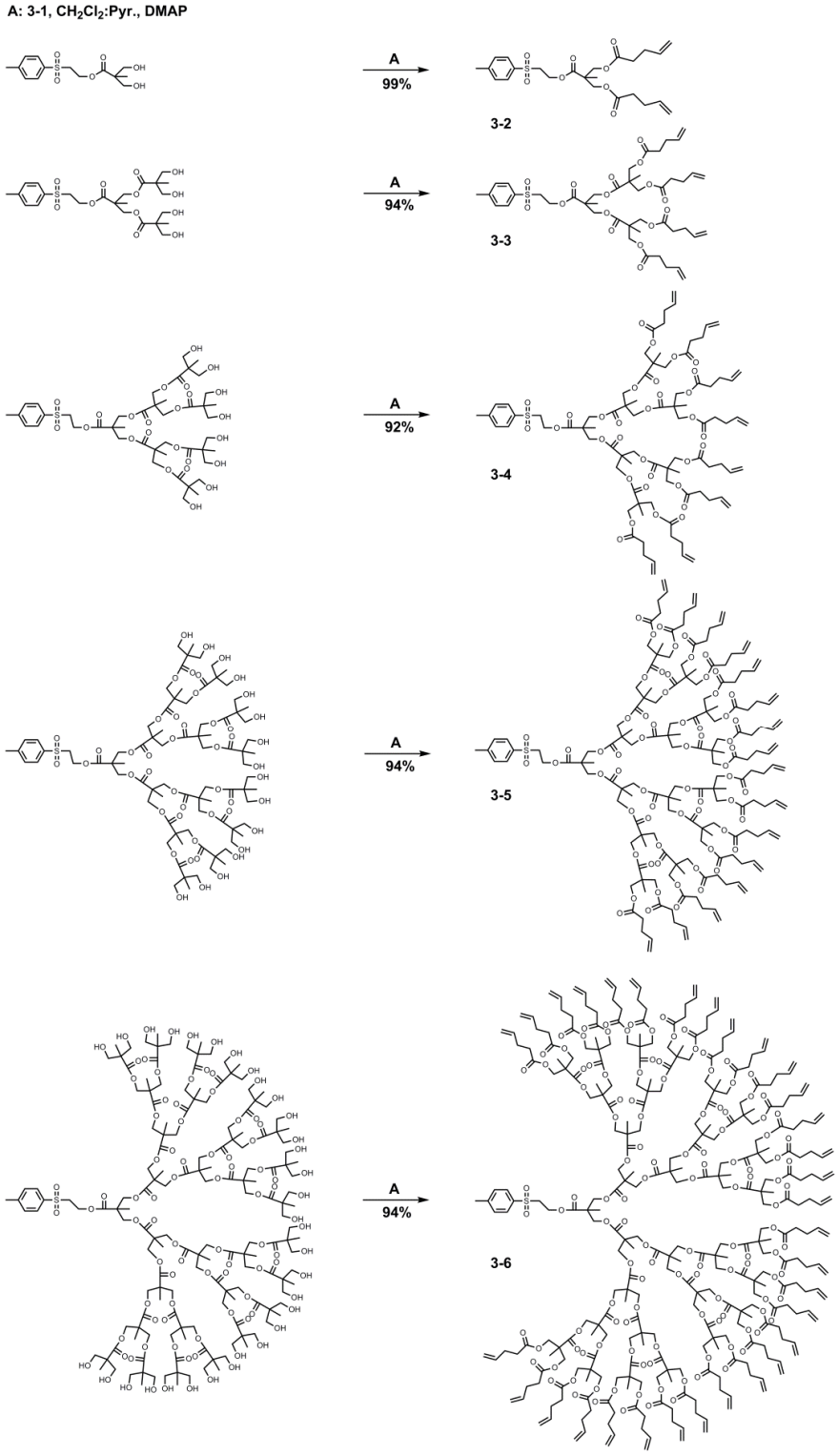
With the COOH-G5-$(PTA)_{32}$ synthesized, the final step prior to functionalizing the periphery with PEG was to couple a ^{99m}Tc-binding ligand to the core. The primary amine-bearing BisPy ligand was reacted with the carboxylic acid core in an amidation reaction.[8] Initially, yields of the product were low and coupling was attempted with benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) as well as hydroxybenzotriazole (HOBT)/ 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC). However, an optimized amidation reaction using standard HOBT/ O-(Benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate (HBTU) mediation proved to be most effective. Where other methods had failed to produce any product, this method provided the desired product with yields typically in the low to mid 90% range after purification by column chromatography. This synthetic methodology was

also successfully applied to the coupling of a cold-standard, rheniated BisPy (ReBisPy) ligand to the core of the dendron.

Isolation of BisPy-G5-$(PTA)_{32}$ from reaction mixtures was initially problematic. Full conversion to the product was not obtained, meaning some residual COOH-G5-$(PTA)_{32}$ remained. Due to their complementary acid and base core functionalities, co-elution of the two molecules was inevitable. However, with the addition of 1% acetic acid to the eluent, BisPy-G5-$(PTA)_{32}$ was slowed enough on the column to allow full displacement and elution of COOH-G5-$(PTA)_{32}$ to occur, providing pure BisPy-G5-$(PTA)_{32}$ in high yield. This multistep synthesis provides an efficient means of synthesizing a dendritic scaffold for ^{99m}Tc-based imaging applications.

3.2. Synthesis of TSe-Gx-$(PTA)_y$

The synthesis of pentenoate-functionalized dendrons was accomplished via reaction of the hydroxyl-terminated dendrons with **3-1** under basic conditions (Scheme 3.5). Surprisingly, product yields were high, ranging from 92 to 94%, even up to the fifth generation. Each of the product dendrons was easily isolated by column chromatography to yield light yellow, viscous oils.



Scheme 3.5. PTA-functionalized PMPA dendron synthesis.

Conversion of the hydroxylated periphery to the desired alkenes was easily observed by ^{1}H NMR spectroscopy (Figure 3.1).

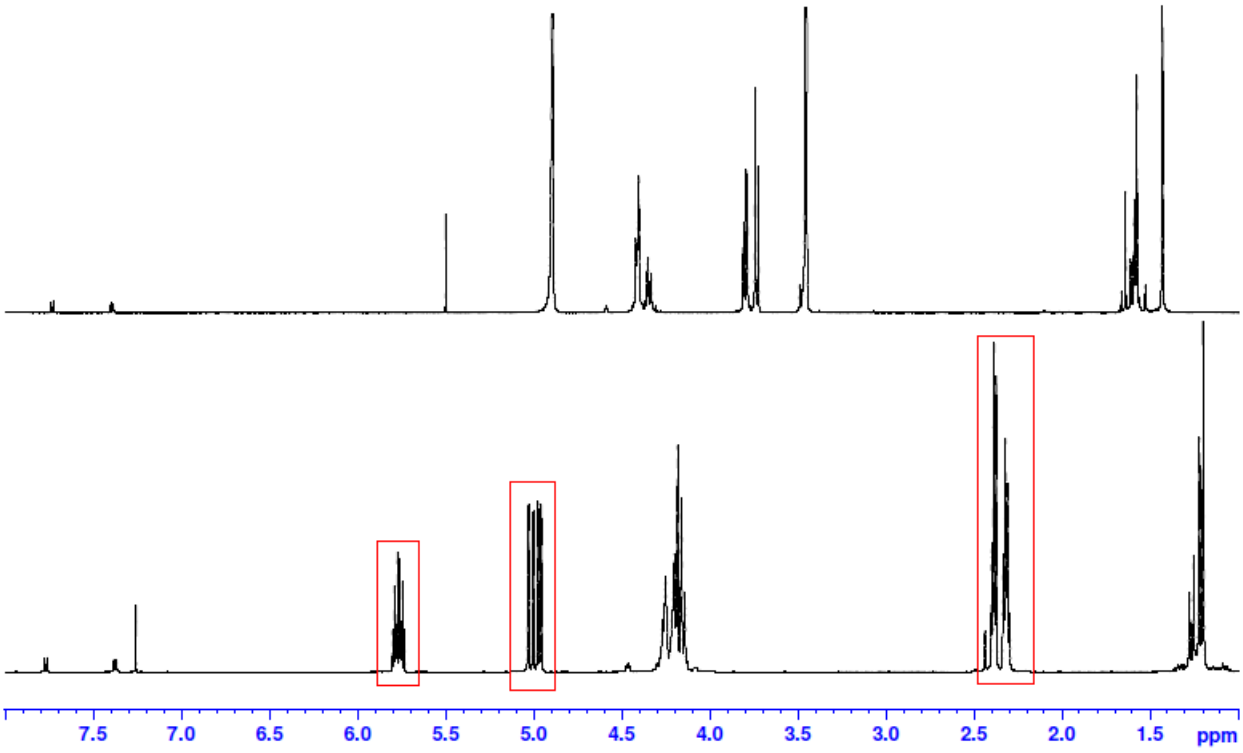


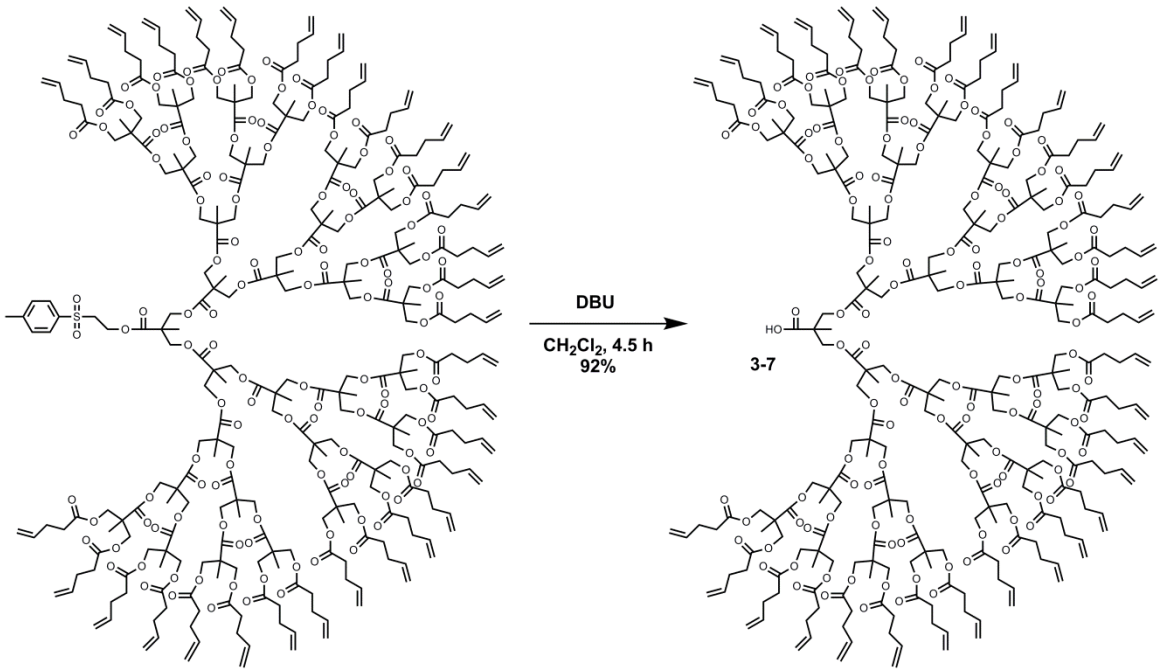
Figure 3.1. ^{1}H NMR spectra of TSe-G5-$(OH)_{32}$ (top, MeOD) versus TSe-G5-$(PTA)_{32}$ (**3-6**) (bottom, $CDCl_3$), PTA signals highlighted in red.

3.3. Core Functionalization of Alkene Dendrons

In order to simplify purification and isolation of the target dendrons, the core was then functionalized to include the desired BisPy ligand. Since the periphery of a dendron dictates its molecular properties, PEGylation at this stage would reach a dead end as molecules with the TSe core were found to be inseparable from their acid- and BisPy-core counterparts.

3.3.1. Conversion to COOH Core

Deprotection of the core was carried out using DBU, according to previously described procedures (Scheme 3.6).[8] The deprotected product was again isolated by column chromatography, producing a light yellow oil in greater than 90% yield



Scheme 3.6. Synthesis of COOH-G5-$(PTA)_{32}$ (**3-7**).

Once again, ^{1}H NMR spectroscopy was very indicative of the conversion from the protected TSe core to the reactive carboxylic acid core. A complete lack of any ^{1}H signals coming from the TSe core is observed in the NMR spectrum for **3-7** (Figure 3.2). The reaction can be monitored over time by observing the rapid decrease in these signals to confirm full conversion to the product.

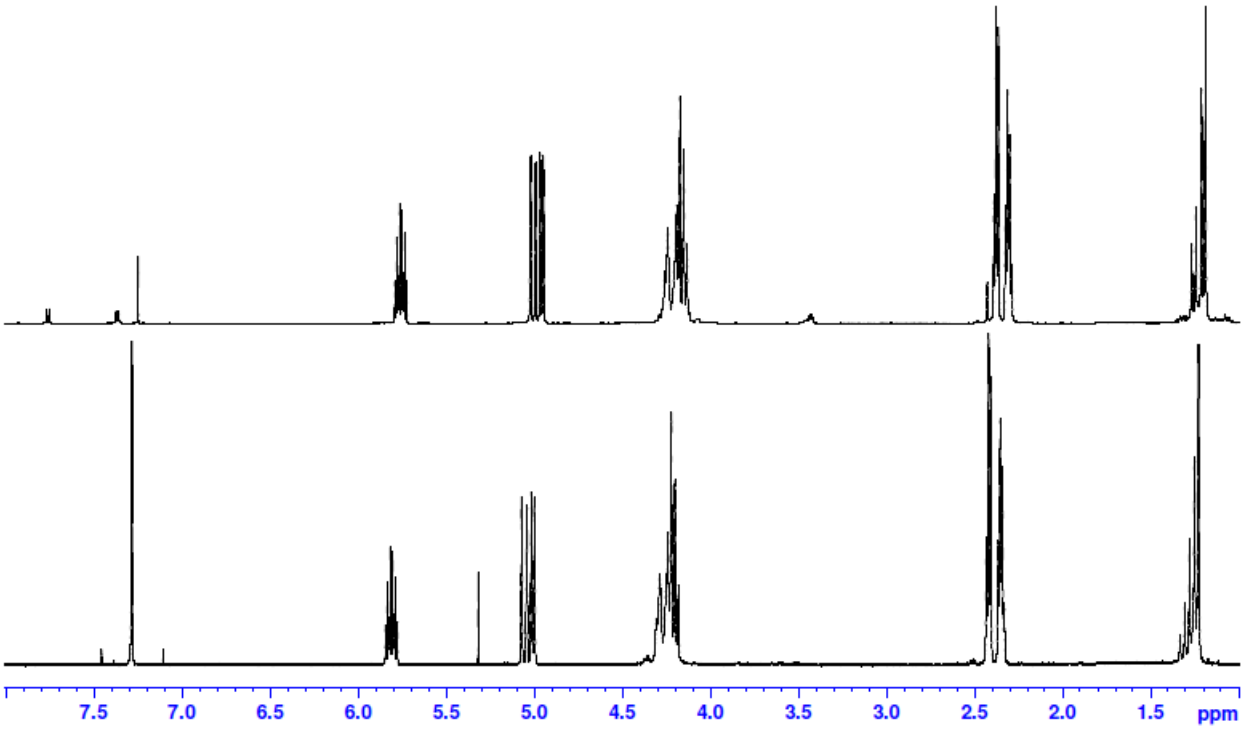
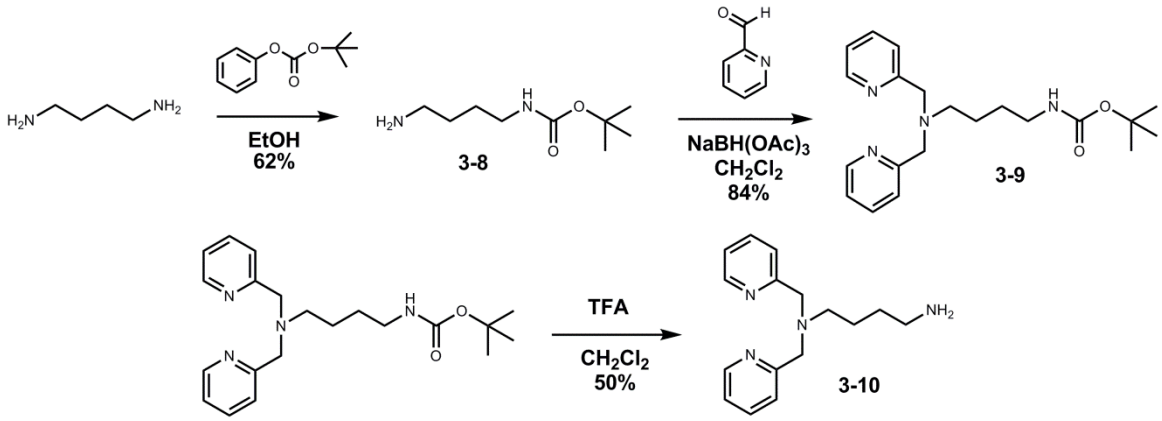


Figure 3.2. ^{1}H NMR spectra ($CDCl_3$) of **3-6** (top) and **3-7** (bottom).

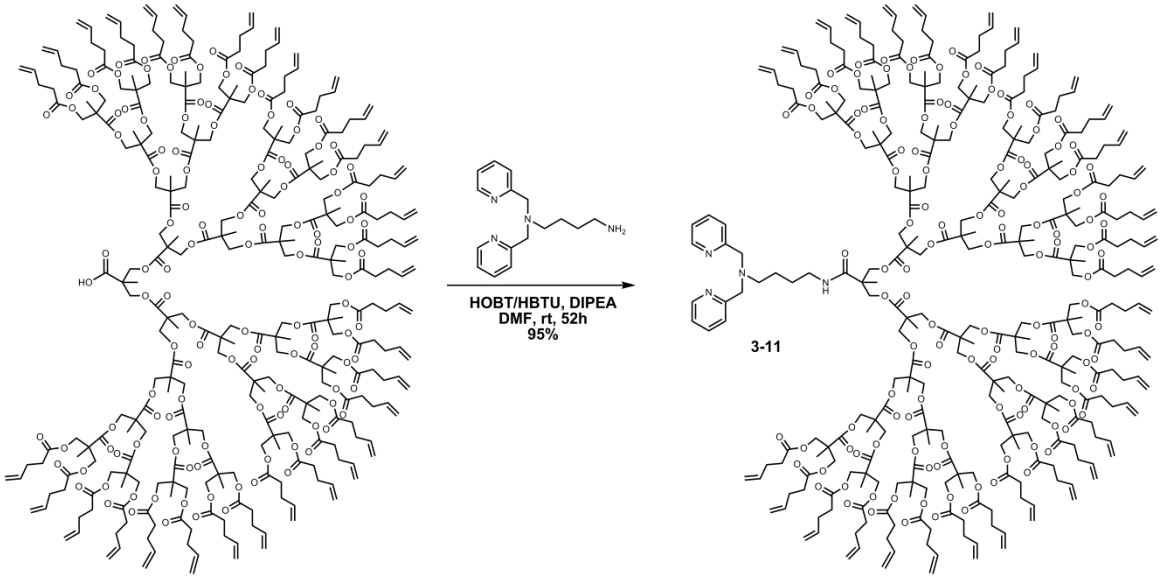
3.3.2. Introduction of Core BisPy Ligand

In order to provide a proper *in vivo* imaging platform for these dendrons, a ^{99m}Tc chelating BisPy ligand similar to that used previously on PMPA dendrons[8] was then synthesized (Scheme 3.7) and bound to the core of the alkene dendron using standard amidation chemistry.



Scheme 3.7. BisPy ligand, BisPy-$(CH_2)_4$-NH_2 (**3-10**), synthesis.[8,67]

Introduction of the BisPy ligand was performed using standard amidation reaction conditions (Scheme 3.8). The ligand-functionalized dendron was again isolated using column chromatography to produce clear, colourless oil in greater than 90% yield. Previous coupling of BisPy ligands to the core of PMPA dendrons have resulted in relatively low yields (55-75%) due to intensive purification methods such as HPLC[8]. However, with some slight modifications, this amidation reaction has provided consistently high yields of the desired product.



Scheme 3.8. BisPy-G5-$(PTA)_{32}$ (**3-11**) synthesis.

The conversion from carboxylic acid core to the desired BisPy ligand can also be confirmed by ^{1}H NMR spectroscopy. The appearance of characteristic aromatic signals from the ligand can be observed easily due to a lack of any aromatic protons in the starting material (Figure 3.3). In order to provide a cold standard for the eventually technetiated imaging agent, a rheniated analogue of **3-11** was also synthesized using a rheniated BisPy ligand (Scheme 3.9).

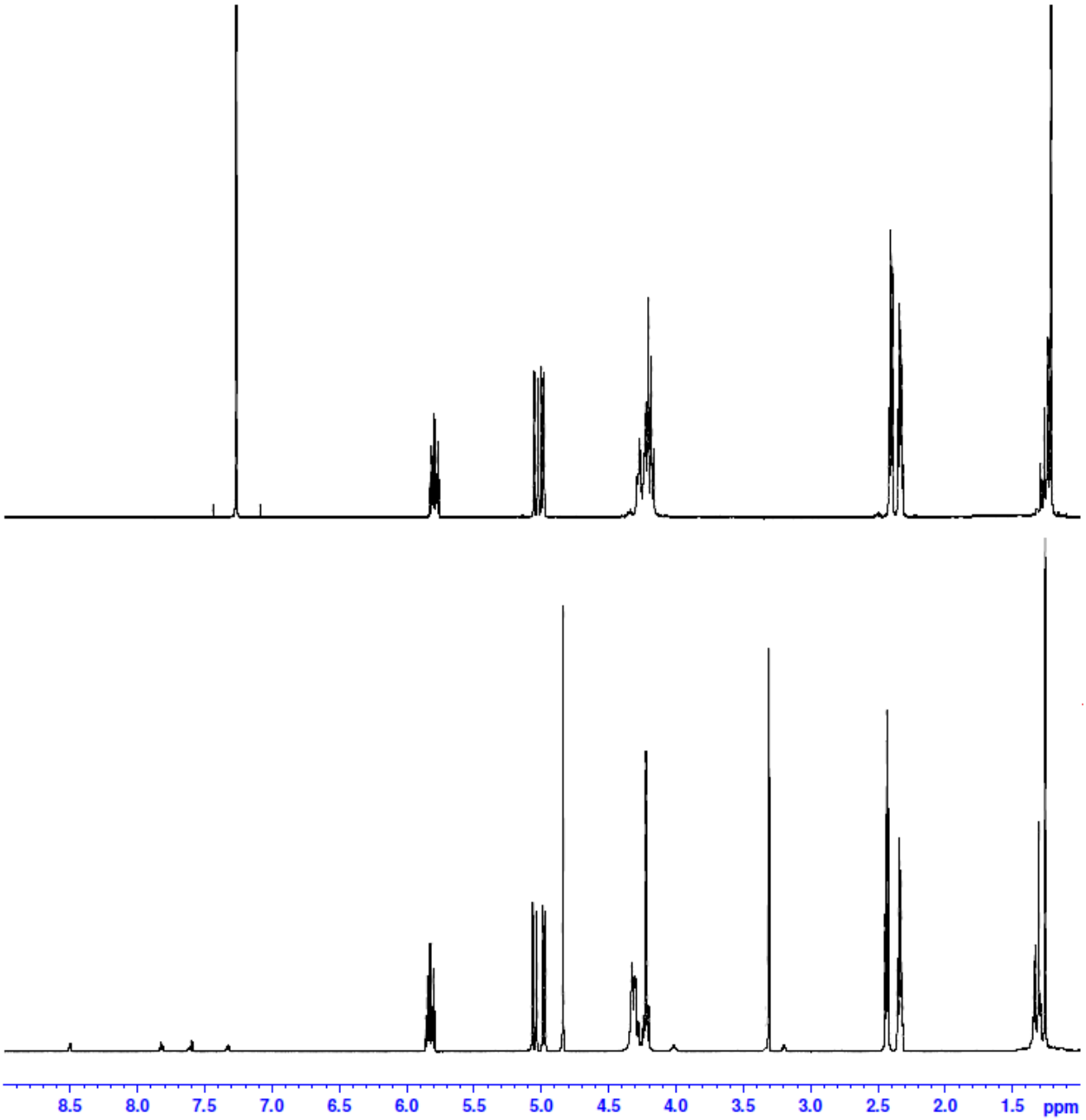
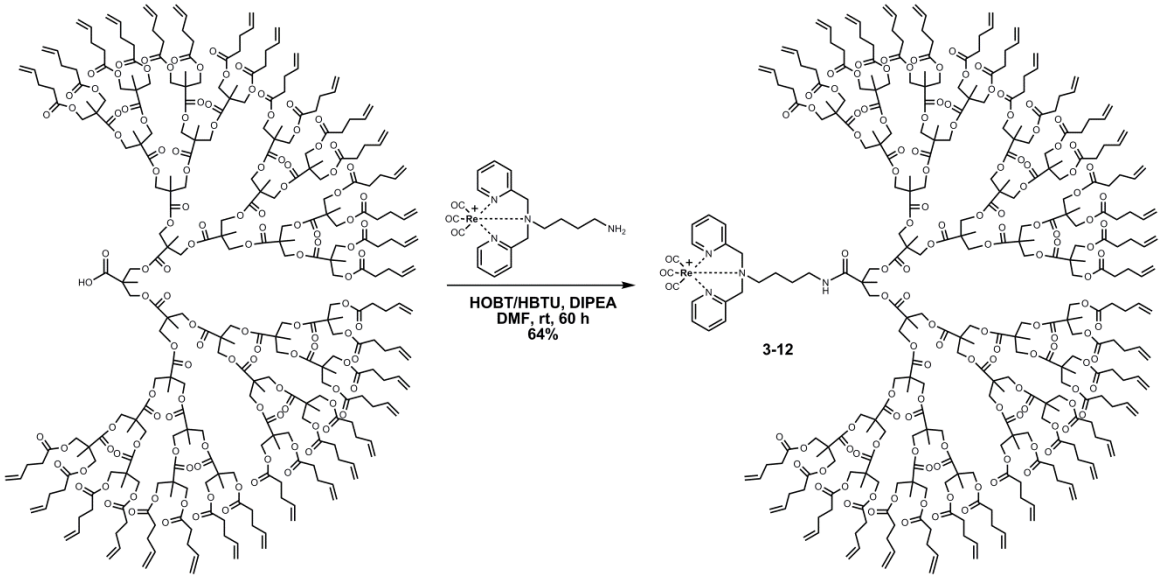


Figure 3.3. ^{1}H NMR spectra of **3-7** (top, $CDCl_3$) versus **3-11** (bottom, MeOD).



Scheme 3.9. Synthesis of ReBisPy-G5-$(PTA)_{32}$ (**3-12**).

With the synthesis of **3-11** accomplished and optimized to provide a high yield of product consistently, a viable imaging scaffold was developed. The reactive alkene periphery is a basis for the attachment of a variety of different moieties. The desired moiety can be attached via several synthetic methodologies including simple Michael addition, or a more robust thiol-ene "click" reaction.

3.4. Conclusion

Alkene-functionalized PMPA dendrons bearing a TSe-core protecting group were synthesized in high yields up to the fifth generation using anhydride esterification chemistry with pentenoic anhydride. The TSe protecting group was removed from **3-6** using DBU to yield a reactive carboxylic acid core dendron, **3-7**. The primary amine of the BisPy and ReBisPy ligands was reacted with this carboxylic acid core via an

amidation reaction with HOBT/HBTU. The product, **3-11**, provides a scaffold for ^{99m}Tc-based imaging with the BisPy binding ligand at the core. Synthesis of **3-12** provides a cold-standard for verifying coordination of ^{99m}Tc to the core of the dendron.

3.5. Experimental

General. Bis-MPA, pentenoic acid, DIPEA, DMAP, DBU, tert-butyl phenyl carbonate, 1,4-diaminobutane, sodium triacetoxyborohydride, and pyridine-2-carbaldehyde were all purchased from Aldrich and used without further purification. Na_2CO_3, $NaHSO_4$, and Pyridine were obtained from Fisher Scientific. HOBT and HBTU were purchased from Chem-Impex International. DCC was purchased from Acros Organics. Rheniated BisPy ligand, ReBisPy-$(CH_2)_4$-NH_2 was provided by Lukas Sadowski and used without further purification. All other reagents and solvents were purchased from commercial sources and either used as provided or dehydrated as necessary. NMR spectroscopy was performed on a Bruker Avance 600MHz Spectrometer. Electrospray MS was performed on a Micromass Quattro Ultima Spectrometer, MALDI MS was performed on a Micromass MALDI-TOF Spectrometer. MALDI MS of ReBisPy-G5-$(PTA)_{32}$ was performed by the University of Guelph Mass Spectrometry Facility.

Synthesis of 3-1:

In a flame-dried, 500 mL round-bottom flask equipped with stir bar under argon atmosphere, pentenoic acid (5.0 g, 49.9 mmol) was combined with DCC (5.15 g, 25 mmol) and dissolved in 300 mL CH_2Cl_2. The reaction mixture was stirred at room

temperature for 12 h. Urea byproduct was removed by vacuum filtration and solvent was removed in vacuo to yield product as a yellow, viscous oil in quantitative yield (4.551 g, 49.91 mmol). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 2.42 (dt, 4H, *J*= 7.2, 6.6), 2.56 (t, 4H, *J*= 7.5), 5.07 (m, 4H), 5.81 (m, 2H).

Synthesis of 3-2:

In a flame-dried, 100 mL round-bottom flask equipped with stir bar under argon atmosphere, pTSe-G1-$(OH)_2$ (0.200 g, 0.632 mmol) was combined with **3-1** (0.288 g, 1.58 mmol) and DMAP (0.200 g, 1.58 mmol), and dissolved in 10 mL of 3:2 (v/v) CH_2Cl_2: Pyridine. The reaction mixture was stirred at room temperature for 16 h, then washed with 1M $NaHSO_4$ (3 x 30 mL), 10% (w/v) Na_2CO_3 (3 x 30 mL) and brine (1 x 30 mL). The organic layer was removed and dried over $MgSO_4$. Drying agent was removed by vacuum filtration and solvent was removed in vacuo. Silica gel chromatography was performed using 99:1 (v/v) CH_2Cl_2:MeOH as the eluent to obtain the product as a clear, colourless oil (0.301 g, 0.626 mmol, 99%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 1.13 (s, 3H), 2.34 (m, 4H), 2.38 (m, 4H), 2.45 (s, 3H), 3.416 (t, 2H, *J*= 6.2), 4.11 (q, 4H, *J*= 11, 10), 4.44 (t, 2H, *J*= 6.2), 5.01 (m, 4H), 5.79 (m, 2H), 7.38 (d, 2H, *J*= 8), 7.80 (d, 2H, *J*= 8). ^{13}C NMR ($CDCl_3$, 600 MHz) δ = 17.57, 21.73, 28.77,33.37, 46.33, 55.08, 58.27, 65.12, 115.76, 128.19, 130.24, 136.28, 136.49, 145.36, 172.31, 172.45. LRMS (ES+) calculated for $C_{24}H_{32}O_8S$, found $[M+NH_4]^+$ 498.4.

Synthesis of 3-3:

In a flame-dried, 25 mL round-bottom flask equipped with stir bar under argon atmosphere, pTSe-G2-$(OH)_4$ (0.200 g, 0.365 mmol) was combined with **3-1** (0.332 g, 1.82 mmol) and DMAP (0.223 g, 1.82 mmol), and dissolved in 10 mL of 3:2 (v/v) CH_2Cl_2: Pyridine. The reaction mixture was stirred at room temperature for 16 h, then washed with 1M $NaHSO_4$ (3 x 150 mL), 10% (w/v) Na_2CO_3 (3 x 150 mL) and brine (1 x 150 mL). The organic layer was removed and dried over $MgSO_4$. Drying agent was removed by vacuum filtration and solvent was removed in vacuo. Silica gel chromatography was performed using 3:1 (v/v) Hexanes: EtOAc as the eluent to obtain the product as a clear, colourless oil (0.302 g, 0.343 mmol, 94%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 1.17 (s, 3H), 1.22 (s, 6H), 2.34 (m, 8H), 2.41 (m, 8H), 2.46 (s, 3H), 3.45 (t, 2H, *J*= 6), 4.18 (m, 12H), 4.47 (t, 2H, *J*= 6.1), 5.03 (m, 8H), 5.79 (m, 4H), 7.38 (d, 2H, *J*= 7.8), 7.81 (d, 2H, *J*= 7.8). ^{13}C NMR ($CDCl_3$, 600 MHz) δ = 17.35, 17.89, 21.74, 28.77, 33.33, 46.48, 46.65, 54.89, 58.39, 65.21, 65.54, 115.76, 128.20, 130.22, 136.52, 171.76, 172.07, 172.51. LRMS (ES+) calculated for $C_{44}H_{60}O_{16}S$, found $[M+NH_4]^+$ 894.5.

Synthesis of 3-4:

In a flame-dried, 100 mL round-bottom flask equipped with stir bar under argon atmosphere, pTSe-G3-$(OH)_8$ (0.900 g, 0.89 mmol) was combined with **3-1** (1.62 g, 8.89 mmol) and DMAP (0.11 g, 0.89 mmol), and dissolved in 50 mL of 3:2 (v/v) CH_2Cl_2: Pyridine. The reaction mixture was stirred at room temperature for 16 h, then washed with 1M $NaHSO_4$ (3 x 150 mL), 10% (w/v) Na_2CO_3 (3 x 150 mL) and brine (1 x 150 mL). The organic layer was removed and dried over $MgSO_4$. Drying agent was removed by vacuum filtration and solvent was removed in vacuo. Silica gel chromatography was

performed using 3:1 (v/v) Hexanes: EtOAc as the eluent to obtain the product as a clear, colourless oil (1.362 g, 0.82 mmol, 92%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 1.22 (m, 21H), 2.34 (m, 16H), 2.41 (m, 16H), 2.46 (s, 3H), 3.45 (t, 2H, *J*= 6), 4.22 (m, 28H), 4.48 (t, 2H, *J*= 6), 5.01 (m, 16H), 5.79 (m, 8H), 7.39 (d, 2H, *J*= 7.8), 7.81 (d, 2H, *J*= 7.8). ^{13}C NMR ($CDCl_3$, 600 MHz) δ = 17.30, 17.62, 17.92, 21.76, 28.79, 33.34, 46.49, 46.66, 46.84, 54.84, 58.44, 65.17, 65.40, 66.18, 115.76, 128.20, 130.25, 136.57, 171.56, 171.71, 172.13, 172.50. LRMS (ES+) calculated for $C_{84}H_{116}O_{32}S$, found $[M+NH_4]^+$ 1688.0.

Synthesis of 3-5:

In a flame-dried, 100 mL round-bottom flask equipped with stir bar under argon atmosphere, pTSe-G4-$(OH)_{16}$ (1.02 g, 0.52 mmol) was combined with **3-1** (3.06 g, 16.8 mmol) and DMAP (0.07 g, 0.52 mmol), and dissolved in 50 mL of 3:2 (v/v) CH_2Cl_2: Pyridine. The reaction mixture was stirred at room temperature for 16 h, then washed with 1M $NaHSO_4$ (3 x 150 mL), 10% (w/v) Na_2CO_3 (3 x 150 mL) and brine (1 x 150 mL). The organic layer was removed and dried over $MgSO_4$. Drying agent was removed by vacuum filtration and solvent was removed in vacuo. Silica gel chromatography was performed using 98:2 (v/v) CH_2Cl_2: MeOH as the eluent to obtain the product as a clear, colourless oil (1.59 g, 0.49 mmol, 94%).^{1}H NMR ($CDCl_3$, 600 MHz) δ = 1.24 (m, 48H), 2.32 (m, 32H), 2.41 (m, 32H), 2.46 (s, 3H), 3.46 (t, 2H, *J*= 6.2), 4.20 (m, 62H), 4.49 (t, 2H, *J*= 6.2), 4.99 (m, 32H), 5.79 (m, 16H), 7.38(d, 2H, *J*= 7.9), 7.81 (d, 2H, *J*= 7.8). ^{13}C NMR ($CDCl_3$, 600 MHz) δ = 17.23, 17.53, 17.63, 17.91, 28.78, 33.32, 46.46, 46.80, 54.79, 58.46, 65.13, 65.23, 65.78, 66.61, 115.75, 128.16, 130.23, 136.57, 171.46, 171.59,

171.72, 172.09, 172.47. LRMS (ES+) calculated for $C_{164}H_{228}O_{64}S$, found $[(M+2H)]^{2+}$ 1627.7.

Synthesis of 3-6:

In a flame-dried, 25 mL round-bottom flask equipped with stir bar under argon atmosphere, pTSe-G5-$(OH)_{32}$ (1.66 g, 0.44 mmol) was combined with **3-1** (5.10 g, 27.9 mmol) and DMAP (0.053 g, 0.44 mmol), and dissolved in 10 mL of 3:2 (v/v) CH_2Cl_2: Pyridine. The reaction mixture was stirred at room temperature for 16 h, then washed with 1M $NaHSO_4$ (3 x 150 mL), 10% (w/v) Na_2CO_3 (3 x 150 mL) and brine (1 x 150 mL). The organic layer was removed and dried over $MgSO_4$. Drying agent was removed by vacuum filtration and solvent was removed in vacuo. Silica gel chromatography was performed using 98:2 (v/v) CH_2Cl_2: MeOH as the eluent to obtain the product as a clear, colourless oil (2.57 g, 0.40 mmol, 94%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 1.23 (m, 93H), 2.32 (m, 64H), 2.39 (m, 64H), 2.44 (s, 3H), 3.44 (t, 2H, *J*= 6.3), 4.18 (m, 124H), 4.48 (t, 2H, *J*= 6.2), 5.01 (m, 64H), 5.76 (m, 32H), 7.37(d, 2H, *J*= 7.9), 7.76 (d, 2H, *J*= 7.8). ^{13}C NMR ($CDCl_3$, 600 MHz) δ = 17.10, 17.50, 17.60, 17.66, 17.93, 28.79, 33.32, 46.46, 46.76, 46.85, 53.55, 65.10, 65.41, 65.80, 115.76, 128.14, 130.26, 136.60, 171.43, 171.50, 171.59, 171.74, 172.09, 172.44. MALDI-TOF MS (lin +): $[M+Na]^+$ 6450.12, $[M+K]^+$ 6466.43

Synthesis of 3-7:

In a flame-dried, 10 mL round-bottom flask equipped with stir bar under argon atmosphere, **3-6** (0.246 g, 0.038 mmol) was dissolved in 5 mL of CH_2Cl_2. DBU (0.035 g,

0.23 mmol) was added and the reaction mixture was left to stir for 4.5 h at room temperature. The reaction mixture was washed with 1M $NaHSO_4$ (1 x 40 mL). Organic extracts were dried over Na_2SO_4 and the drying agent was removed by vacuum filtration. Solvent was removed by rotary evaporation and the product was isolated by silica gel chromatography using 99:1 CH_2Cl_2: MeOH as the eluent. The product was isolated as a clear, colourless oil (0.220 g, 0.035 mmol, 92%). ^{1}H NMR (CD_2Cl_2, 600 MHz) δ = 1.30 (m, 96H), 1.64 (m, 64H), 1.70 (m, 64H), 2.37 (t, 64 H, *J*= 7.1), 2.59 (t, 64H, *J*= 7.1), 2.72 (t, 64H, *J*=7.1), 3.37 (s, 96H), 3.54 (m, 64H), 3.61 (m, 260H), 4.20 (m, 124 H). ^{13}C NMR (CD_2Cl_2, 600 MHz) δ = 17.50, 17.60, 17.77, 17.85, 18.06, 24.29, 29.49, 30.01, 31.74, 32.33, 33.76, 46.65, 46.95, 58.97, 65.13, 70.63, 70.75, 70.82, 71.22, 72.26, 171.77, 171.98, 172.41, 172.96. MALDI-TOF MS (lin +): $[M+H]^+$ 6245.57.

Synthesis of BocNH-$(CH_2)_4$-NH_2, 3-8:

In a flame-dried, 50 mL round-bottom flask equipped with stir bar under argon atmosphere, 1,4-diaminobutane (0.441 g, 5.00 mmol) was dissolved in 20 mL of anhydrous EtOH. Tert-butyl phenyl carbonate (0.971 g, 5.00 mmol) was added dropwise over 15 minutes at room temperature. The reaction mixture was left to stir overnight and solvent was then removed by rotary evaporation. The crude mixture was purified by silica gel chromatography using 90:10 CH_2Cl_2: MeOH as the eluent. Pure product was isolated as a clear, colourless oil (0.580 g, 3.08 mmol, 62%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 1.44 (m, 13 H), 2.68 (t, 2H, *J*= 6.8), 3.08 (q, 2H, *J*= 6.2, 6.4). LRMS (ES+) calculated for $C_9H_{20}N_2O_2$, found $[M+H]^+$ 189.3.

Synthesis of BisPy-$(CH_2)_4$-NHBoc, 3-9:

In a flame-dried round bottom flask equipped with stir bar under argon atmosphere, **3-8** (0.580 g, 3.08 mmol) was combined with pyridine-2-carbaldehyde (0.990 g, 9.24 mmol) and dissolved in 15 mL anhydrous CH_2Cl_2. The reaction mixture was allowed to stir for 20 min at r.t. The mixture was then cooled to 0^oC and sodium triacetoxyborohydride (2.29 g, 10.8 mmol) was added. The reaction was left to stir for 5 h. The mixture was then diluted with CH_2Cl_2 and washed with 50 mL 1M NaOH. The organic layers were dried over $MgSO_4$ and the drying agent was removed by vacuum filtration. Solvent was removed by rotary evaporation and the product was isolated by silica gel chromatography using 95:5 CH_2Cl_2: MeOH as the eluent. Pure product was isolated as a light yellow oil (0.953 g, 2.59 mmol, 84%). ^1H NMR ($CDCl_3$, 600 MHz) δ = 1.44 (m, 13H), 2.61 (m, 2H), 3.06 (m, 2H), 3.86 (m, 4H), 7.16 (m, 2H), 7.54 (m, 2H), 7.66 (t, 2H, *J*= 7.3), 8.54 (d, 2H, *J*= 4.5). LRMS (ES+) calculated for $C_{21}H_{30}N_4O_2$, found $[M+H]^+$ 371.5.

Synthesis of 3-10:

TFA (30 mL) was added to a round bottom flask equipped with stir bar and allowed to stir at 0^oC. **3-9** (0.953 g, 2.57 mmol) was dissolved in a minimum of CH_2Cl_2 and added dropwise over 15 min. The solution was left to stir for 2 h. The pH was then adjusted to 14 by adding conc. NaOH and the product was extracted with CH_2Cl_2. The organic layers were dried over $MgSO_4$ and the drying agent was removed by vacuum filtration. Solvent was removed by rotary evaporation and product was isolated by silica gel chromatography using 90:10 CH_2Cl_2: MeOH as the eluent followed by a gradient to

79:20:1 CH_2Cl_2:MeOH:AcOH. The pure fractions of product were washed with 10% Na_2CO_3 to remove residual AcOH. The organic layer was dried over $MgSO_4$ and drying agent was removed by vacuum filtration. Solvent was removed by rotary evaporation to yield a light brown oil (0.348 g, 1.29 mmol, 50%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 1.46 (p, 2H, *J*= 6.9, 7.4), 1.59 (p, 2H, *J*= 6.9, 7.4), 2.54 (t, 2H, *J*= 7), 2.70 (t, 2H, *J*= 7), 3.79 (s, 4H), 7.15 (t, 2H, *J*= 7.4), 7.48 (d, 2H, *J*= 7.9), 7.64 (dt, 2H, *J*= 7.7), 8.55 (d, 2H, *J*= 6.0). LRMS (ES+) calculated for $C_{16}H_{22}N_4$, found $[M+H]^+$ 271.4.

Synthesis of 3-11:

In a flame-dried, 10 mL round-bottom flask equipped with stir bar under argon atmosphere, **3-7** (0.216 g, 0.035 mmol) was combined with HOBT (0.020 g, 0.121 mmol), HBTU (0.092 g, 0.242 mmol), BisPy-$(CH_2)_4$-NH_2 (0.028 g, 0.104 mmol) and 60 µL of DIPEA and dissolved in 2.2 mL anhydrous DMF. The reaction mixture was left to stir for 52 h and then washed with 1M $NaHSO_4$ (1x 40 mL) and 10% Na_2CO_3 (1x 40 mL). The organic layer was collected and solvent was removed by rotary evaporation. The resulting mixture was then purified by silica gel chromatography using 98:1:1 CH_2Cl_2:MeOH:AcOH as the eluent to remove unreacted starting material, followed by 95:5 CH_2Cl_2:MeOH to isolate the product as a clear colourless oil (0.214 g, 0.033 mmol, 95%). ^{1}H NMR (MeOD, 600 MHz) δ = 1.23 (m, 104H), 2.33 (m, 64H), 2.40 (m, 64H), 4.22 (m, 124H), 5.00 (m, 64H), 5.78 (m, 32H), 7.12 (t, 2H, *J*= 7.4), 7.35 (d, 2H, *J*= 7.9), 7.77 (m, 2H), 8.62 (d, 2H, *J*= 6.0). ^{13}C NMR (MeOD, 600 MHz) δ = 18.35, 18.41, 29.91, 34.29, 47.75, 48.06, 66.47, 66.61, 67.02, 67.15, 116.20, 138.03, 173.09, 173.15, 173.56, 173.86.

Synthesis of 3-12:

In a flame-dried, 10 mL round-bottom flask equipped with stir bar under argon atmosphere, **3-7** (0.096 g, 0.015 mmol) was combined with ReBisPy-$(CH_2)_4$-NH_2 (0.025 g, 0.046 mmol) and dissolved in 2 mL DMF. After stirring for 1 min, HOBT (0.006 g, 0.045 mmol), HBTU (0.017 g, 0.045 mmol), and 50 uL DIPEA were added to the reaction. The reaction was left to stir for 60 h. The reaction mixture was then washed with 1M $NaHSO_4$ (1x 40 mL), 10% Na_2CO_3 solution (1x 40 mL) and brine (1x 40 mL). The organic layers were dried over Na_2SO_4 and drying agent was removed by vacuum filtration. Solvent was removed by rotary evaporation to yield a crude mixture. The product was isolated as a bright orange oil by silica gel chromatography using 98:1:1 CH_2Cl_2: AcOH: MeOH, followed by 97:3 CH_2Cl_2: MeOH as the eluent (0.066 g, 0.009 mmol, 64%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 1.23 (m, 104H), 2.33 (m, 64H), 2.38 (m, 64H),3.37 (m, 2H), 3.86 (m, 2H), 4.20 (m, 124H), 5.01 (m, 64H), 5.77 (m 32H), 7.25 (t, 2H, *J*=7.1), 7.62 (d, 2H, *J*= 7.9), 7.87 (t, 2H, *J*=7.7), 8.70 (d, 2H, *J*=5.4). ^{13}C NMR ($CDCl_3$, 600 MHz) δ = 17.56, 17.66, 17.95, 28.81, 29.84, 33.34, 46.47, 46.77, 65.12, 65.44, 115.77, 136.63, 171.63, 172.13, 172.48. MALDI-TOF MS (lin +): $[M+H_2O]^+$ 6765.62.

Chapter 4: Synthesis of Thiol-terminated PEG Chains

4.1. Overview

Thiol-terminated PEG chains were required to efficiently PEGylate the periphery of the alkene dendrons. These were synthesized according to slightly modified literature procedures.[63,64] The terminal alcohol of PEG-monomethyl ether was first converted to either a tosylate or mesylate using p-toluenesulfonyl chloride (TsCl) or methane sulfonyl chloride (MsCl), accordingly. The resulting reactive leaving group was converted to a thiol in the presence of thiourea to yield MeO-PEG-SH of varying chain lengths.

As stated previously, PEGylated macromolecules have shown extended circulation times *in vivo*. This makes them ideal candidates for passive imaging of and delivery to tumour tissues via the EPR effect. With a viable imaging platform synthesized in **3-11**, the reactive alkene periphery is ideal for thiol-ene "click" chemistry.

Although commercially available, the high cost of PEG-SH's, combined with the large excess of thiol required for thiol-ene "click" reactions made the synthesis of PEG-SH a necessity. In order to avoid peripheral functionalization of multiple alkenes with the same PEG chain, PEG monomethyl ethers were used. Since such a large excess of thiol would be needed to fully functionalize the multivalent periphery of the alkene dendrons, an efficient synthetic method for producing a thiol from the terminal alcohol was necessary. Literature methods for this reaction tend to follow the same general steps, but

result in low overall yield.[63,64] However, optimization produced the desired PEG-SH's in high yield.

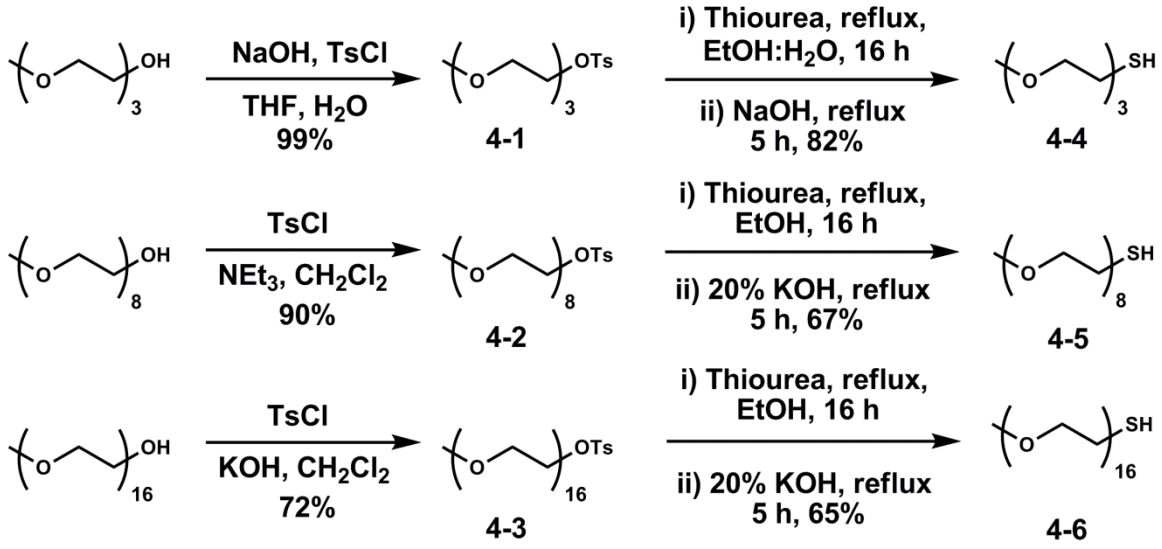
Alcohols are not inherently reactive towards substitution. The alcohol must first be converted to a good leaving group. This can be achieved by converting the alcohol to a tosylate. Literature examples have made this reaction with PEG very straightforward.[63,64] When combined with TsCl, MeO-PEG-OH readily forms the desired tosylate. Excess TsCl can be removed with an acid wash to yield pure product. Although the polymer chain lengths and sizes vary greatly from MeO-TEG-OH to MeO-PEG_{750}-OH, their reactivity towards these reactions was very similar. However, on occasion it was observed that full conversion to MeO-PEG_{750}-OTs (**4-3**) did not occur. Furthermore, its reaction with TsCl typically resulted in low yields due to the work-up procedures utilized. The high degree of aqueous solubility of PEG_{750} makes its extraction into organic solvent somewhat problematic. For these reasons, MeO-PEG_{750}-OH was combined with MsCl to form the corresponding mesylate. Utilizing a more reactive synthon in MsCl resulted in full conversion to MeO-PEG_{750}-OMs (**4-7**) with no further purification necessary.

The reactive tosylate and mesylate end-group functionalities of each polymer chain length were then converted to thiols using thiourea. Use of potassium thioacetate for this reaction was found to be ineffective and produced a mixture of products. When combined with thiourea, each PEG chain length showed a similar degree of reactivity. However, the conversion of **4-7** to MeO-PEG_{750}-SH (**4-6)** was found to produce a much higher yield of thiol than the corresponding tosylate. Purification of each PEG-SH was novel. Due to its low molecular weight, MeO-TEG-SH (**4-4**) could be purified by

fractional distillation. Column chromatography was required to purify MeO-PEG_{350}-SH (**4-5**) and **4-6**. The efficiency of the conversion from tosylate/mesylate to thiol made this purification simple due to a lack of any starting tosylate/mesylate. The resulting thiols were observed to be stable to oxidation, and resistant to disulfide formation over time.

4.2. Synthesis of PEG-tosylates/mesylate

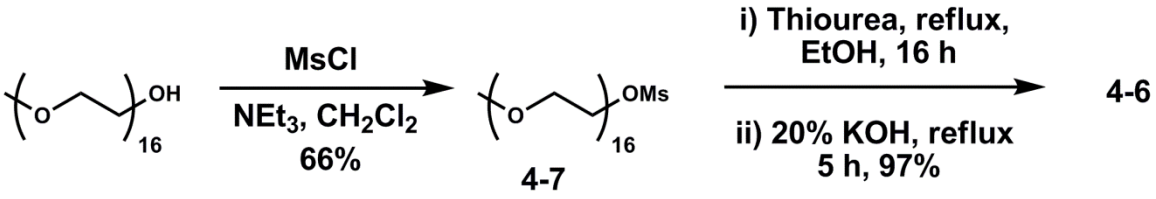
In order to provide reactive end-groups for conversion to thiols, PEG monomethyl ether was combined with TsCl or MsCl under standard tosylation conditions (Scheme 4.1). Products were isolated by simple liquid-liquid extraction to yield each product as either a clear, colourless oil or a waxy white solid depending on polymer chain length.



Scheme 4.1. Synthesis of PEG-tosylates and corresponding thiols.

Difficulty in obtaining **4-3** was observed between different syntheses and thus synthesis of **4-7** was also performed (Scheme 4.2). This change in the trend of reactivity

is likely a result of the increase in polymer chain length. Suprisingly, by exchanging the end-group tosylate functionality for a mesylate, the following conversion to the thiol produced a much more favourable yield.



Scheme 4.2. Synthesis of **4-6** from **4-7**.

Characterization of the tosylates and mesylate was simple and straightforward. The conversion from alcohol to the desired leaving group was observed by ^{1}H NMR spectroscopy. The appearance of characteristic aromatic signals from the tosylates / methyl signal from the mesylate can be recognized easily (Figure 4.1).

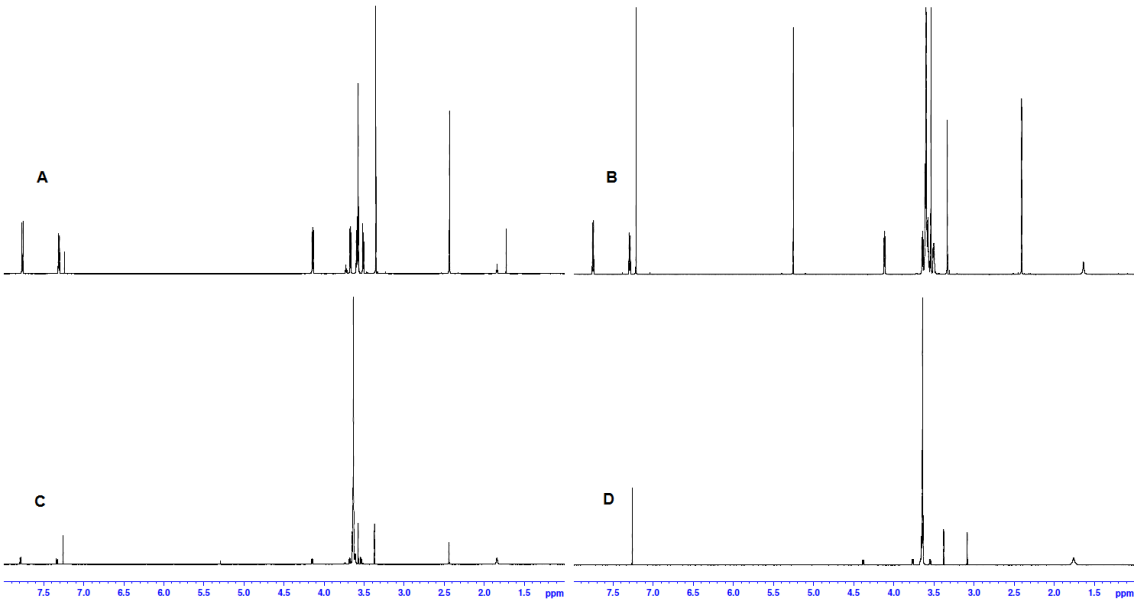


Figure 4.1. ^{1}H NMR Spectra ($CDCl_3$) of **4-1** (A), **4-2** (B), **4-3** (C), **4-7**(D).

4.3. Synthesis of PEG Thiols

Conversion of the reactive end-group to the desired thiol was performed by reacting PEG-OTs/Ms with thiourea (Scheme 4.1, 4.2). The products were extracted from the resulting mixture either by fractional distillation or silica gel chromatography, depending on the polymer chain length.

Once reactive leaving groups had been attached to the PEG chains, terminal thiols could be obtained either by reaction with potassium thioacetate[64] or thiourea.[63] While there are literature precedents for both pathways, reactions with thioacetate proved to provide a large number of impurities which made purification unnecessarily difficult. Each thiol was characterized by ^{1}H NMR spectroscopy (Figure 4.2), where characteristic peaks for tosylates/mesylates were observed to completely disappear. The significant shift in the signal for the terminal methylene group of each polymer chain was also noted in each case.

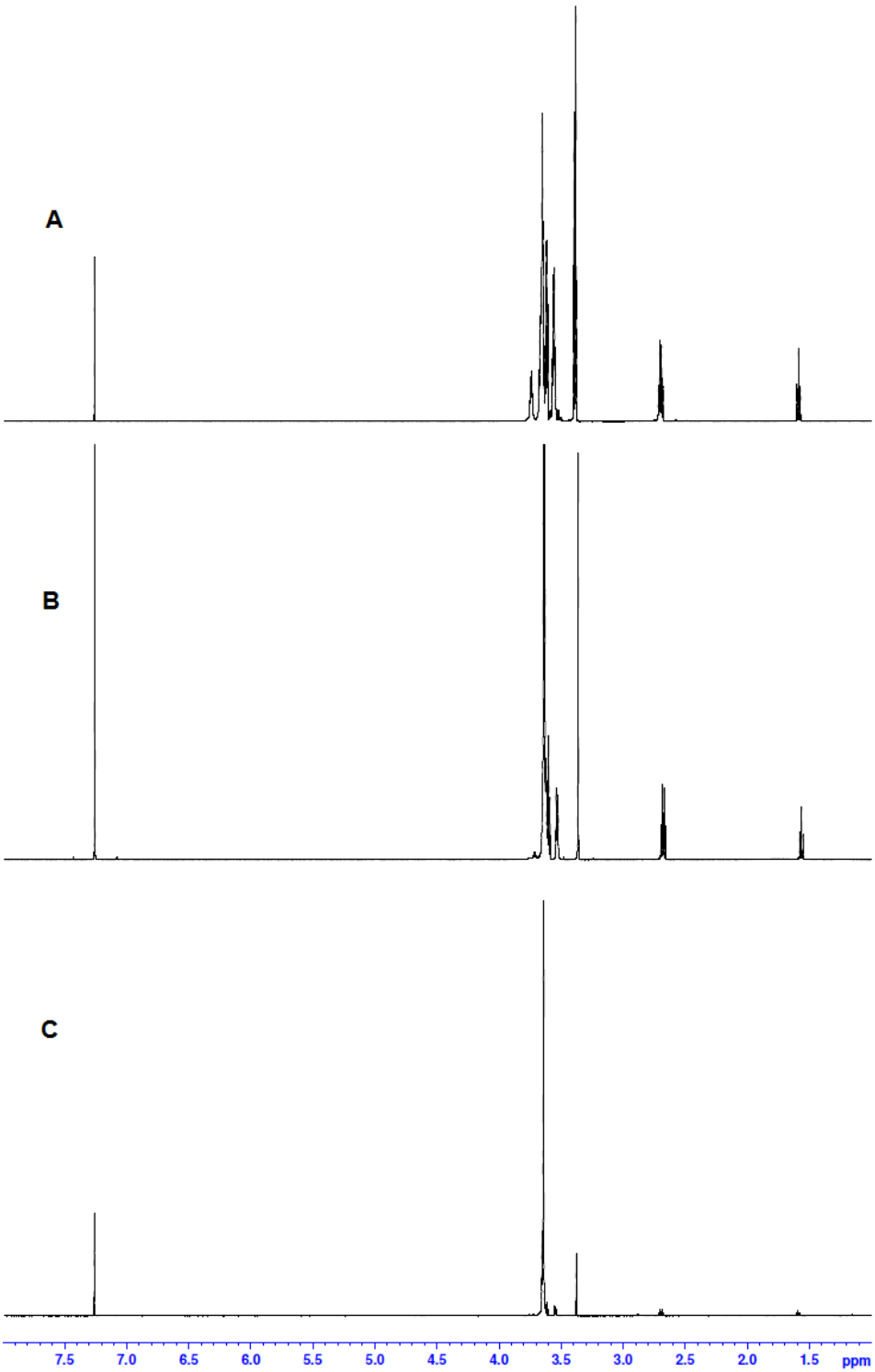


Figure 4.2. ^{1}H NMR spectra ($CDCl_3$) of **4-4** (A), **4-5** (B), **4-6** (C).

Once attached to the periphery, the three chain lengths of PEG-SH will provide a series of PEGylated dendrons that should provide a detailed study of the effect of polymer chain length on *in vivo* circulation time and biodistribution for a multivalent periphery.

4.4. Conclusion

PEG-monomethyl ether chains of varying lengths (n = 3, 8, 16) were converted to tosylates and mesylates in the presence of TsCl or MsCl as necessary. The resulting reactive end-groups were converted to thiols in the presence of thiourea in high yields. The systematic increase in polymer chain length within this series of PEG thiols provides the basis for a proper analysis of polymer chain length effect on *in vivo* circulation time once coupled to the periphery of the alkene dendrons.

4.5. Experimental

General. Tri(ethylene glycol) monomethyl ether (TEG), PEG Monomethyl ether (Mn= 350, 750), TsCl, MsCl, and thiourea were purchased from Aldrich and used without further purification. NaOH, KOH, HCl, $MgSO_4$ and Na_2SO_4 were purchased from Caledon Laboratory Chemicals and used as provided. All other reagents and solvents were purchased from commercial sources and were either used as provided or dried as necessary. NMR spectra were obtained on a Bruker Avance 600MHz spectrometer and were compared with literature references to confirm product purity.[63,64]

Synthesis of 4-1:

In a 100 mL round-bottom flask equipped with stir bar under argon atmosphere, tri(ethylene glycol) monomethyl ether (10.08 g, 60.9 mmol) was dissolved in 15 mL THF. NaOH (4.80 g, 109.6 mmol) dissolved in 15 mL H_2O was added to the reaction mixture slowly at room temperature and left to stir for 15 min. pTsCl (11.70 g, 60.9 mmol) was dissolved in 18 mL THF and added slowly to the stirring solution. The reaction mixture was left to stir for 16 h at room temperature. The reaction mixture was diluted with 50 mL H_2O and washed with CH_2Cl_2 (3 x 50 mL). The combined organic extracts were then backwashed with H_2O (3 x 50 mL) and dried over $MgSO_4$. Drying agent was removed by vacuum filtration and solvent was removed in vacuo to yield the product as a viscous, clear oil (19.45 g, 60.6 mmol, 99.5%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 2.44 (s, 3H), 3.36 (s, 3H), 3.51 (m, 2H), 3.58 (m, 6H), 3.68 (t, 2H, *J* = 4.8), 4.15 (t, 2H, *J* = 4.9), 7.33 (dd, 2H, *J* = 7.8), 7.78 (dd, 2H, *J* =7.8).

Synthesis of 4-4:

In a 50 mL round-bottom flask equipped with stir bar under argon atmosphere, **4-1** (3.65 g, 0.011 mol) was dissolved in 8 mL of a 5:3 mixture of EtOH:H_2O and combined with thiourea (0.874 g, 0.011 mol) and brought to reflux. The reaction mixture was refluxed overnight. After 16 h, NaOH (0.551 g, 0.014 mol) in 5 mL H_2O was added and the mixture was brought to reflux for an additional 6 h. The reaction mixture was then brought to pH 4 with conc. HCl, diluted with 20 mL H_2O and the product was extracted with CH_2Cl_2 (3 x 50 mL). The combined organic layers were backwashed with H_2O (2 x 30 mL) and dried over $MgSO_4$. Drying agent was removed by vacuum filtration and solvent removed in vacuo to yield a brown oil. Pure product was isolated by fractional

distillation (1 mBar, 80°C) as a clear, colourless oil (1.69 g, 0.009 mol, 82%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 1.57 (t, 1H, *J* = 8.2), 2.66 (q, 2H, *J* = 6.7, 7.1, 7.2), 3.35 (s, 3H), 3.56 (m, 10H).

Synthesis of 4-2:

In a round-bottom flask equipped with stir bar under argon atmosphere, MeO-PEG_{350}-OH (4.00 g, 14.3 mol) was combined with pTsCl (3.00 g, 15.7 mol) and dissolved in 7 mL THF. NEt_3 (2.5 mL, 14.3 mol) was added to the reaction mixture at room temperature. The reaction was left to stir overnight and the mixture was dissolved in 25 mL CH_2Cl_2 then washed with 10% HCl (3 x 30 mL). The organic layer was dried over Na_2SO_4 and drying agent was removed by vacuum filtration. The solvent was removed by rotary evaporation to yield the product as a clear colourless oil (6.49 g, 12.87 mol, 90%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 2.47 (s, 3H), 3.37 (s, 3H), 3.57 (m, 2H), 3.64 (m, 28 H), 3.68 (m, 2H), 4.12 (t, 2H, *J*= 4.9), 7.35 (dd, 2H, *J*= 8.1), 7.81 (dd, 2H, *J*= 8.2).

Synthesis of 4-5:

In a round-bottom flask equipped with a stir bar and condenser under argon atmosphere, **4-2** (10.66 g, 0.021 mol) was combined with thiourea (1.72 g, 0.022 mol) and dissolved in 20 mL EtOH and brought to reflux. The reaction mixture was left to reflux overnight. 51 mL of 20% KOH was added to the reaction. Reflux continued for another 5 h. The reaction mixture was then brought to a pH of 2-3 with conc. HCl, diluted with 50 mL H_2O and washed with CH_2Cl_2 (3 x 50 mL). The combined organic extracts were then backwashed with H_2O (3 x 35 mL) and dried over $MgSO_4$. The drying agent was

removed by vacuum filtration and solvent was removed by rotary evaporation. The product was extracted from the resulting mixture by silica gel chromatography using 98:2 CH_2Cl_2: MeOH as the eluent. Pure product was isolated as a viscous, light yellow oil (4.77 g, 0.014 mol, 67%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 1.55 (1H, t, *J*= 8.2), 2.65 (dt, 2H, *J*= 6.3, 8.2), 3.33 (s, 3H), 3.64 (m, 28 H).

Synthesis of 4-3:

In a flame-dried round-bottom flask equipped with a stir bar and condenser under argon atmosphere, MeO-PEG_{750}-OH (20.72 g, 27.6 mmol) was combined with pTsCl (10.52 g, 55.2 mmol) and dissolved in 60 mL dry CH_2Cl_2. KOH (8.03 g, 143.2 mmol) was added slowly and the reaction was stirred vigorously for 16 h. The reaction mixture was poured over an ice/water mixture and extracted with CH_2Cl_2. The organic extracts were backwashed with H_2O and dried over Na_2SO_4. Drying agent was removed by vacuum filtration and solvent was removed by rotary evaporation to yield a waxy white solid (17.897 g, 19.9 mmol, 72%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 2.40 (s, 3H), 3.38 (s, 3H), 3.60 (m, 62H), 4.11 (m, 2H), 7.28 (d, 2H, *J*= 7.8), 7.72 (d, 2H, *J*=7.8).

Synthesis of 4-6(1):

In a flame-dried 200 mL round bottom flask equipped with condenser and stir-bar under argon atmosphere, **4-3** (16.78 g, 18.6 mmol) was combined with thiourea (1.56 g, 20.4 mmol) and dissolved in 30 mL anhydrous EtOH. The reaction mixture was refluxed overnight and then 75 mL 20% KOH was added to the reaction. Reflux continued for another 5 h. The reaction mixture was then brought to a pH of 2-3 with conc. HCl and

washed with CH_2Cl_2 (3 x 150 mL). The combined organic extracts were then backwashed with H_2O (3 x 100 mL) and dried over Na_2SO_4. The drying agent was removed by vacuum filtration and solvent was removed by rotary evaporation. The product was extracted from the resulting mixture by silica gel chromatography using 97:3 CH_2Cl_2: MeOH followed by 92:8 CH_2Cl_2: MeOH as the eluent. Pure product was isolated as a light yellow, waxy solid (9.23 g, 12.09 mmol, 65%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 1.55 (1H, t, *J*= 8.2), 2.65 (dt, 2H, *J*= 6.3, 8.2), 3.33 (s, 3H), 3.64 (m, 62H).

Synthesis of 4-7:

In a flame-dried 100 mL round-bottom flask equipped with a stir bar and condenser under argon atmosphere, MeO-PEG_{750}-OH (10.00 g, 13.3 mmol) was combined with NEt_3 (2.70 g, 26.7 mmol) and dissolved in 50 mL anhydrous CH_2Cl_2. MsCl (2.29 g, 19.9 mmol) was added slowly and the reaction was stirred vigorously for 16 h. The reaction mixture was washed with 10% (v/v) HCl (3x 100 mL). The organic extracts were backwashed with H_2O (3x 50 mL) and dried over Na_2SO_4. Drying agent was removed by vacuum filtration and solvent was removed by rotary evaporation to yield a waxy white solid (6.62 g, 8.26 mmol, 66%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 3.11 (s, 3H), 3.42 (s, 3H), 3.58 (m, 62H), 4.09 (m, 2H).

Synthesis of 4-6(2):

In a flame-dried 100 mL round bottom flask equipped with condenser and stir-bar under argon atmosphere, **4-7** (8.96 g, 0.011 mol) was combined with thiourea (0.989 g, 0.013 mol) and dissolved in 20 mL anhydrous EtOH. The reaction mixture was refluxed

overnight and then 50 mL 20% KOH was added to the reaction. Reflux continued for another 5 h. The reaction mixture was then brought to a pH of 2-3 with conc. HCl and washed with CH_2Cl_2 (3 x 150 mL). The combined organic extracts were then backwashed with H_2O (3 x 100 mL) and dried over Na_2SO_4. The drying agent was removed by vacuum filtration and solvent was removed by rotary evaporation. The product was extracted from the resulting mixture by silica gel chromatography using 97:3 CH_2Cl_2: MeOH followed by 92:8 CH_2Cl_2: MeOH as the eluent. Pure product was isolated as a light yellow, waxy solid (8.04 g, 0.0107 mol, 97%). ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 1.55 (1H, t, *J*= 8.2), 2.65 (dt, 2H, *J*= 6.3, 8.2), 3.33 (s, 3H), 3.64 (m, 62H).

Chapter 5: Thiol-ene "Click" Coupling Reactions

5.1. Overview

3-11 and **3-12** were combined with varying chain lengths of PEG-SH. The mixture was placed in standard thiol-ene "click" reactions using DMPA photoinitiator in a UV reactor and irradiated with 365 nm light for varying amounts of time, dependent on polymer chain length. Note that since THF is used as the solvent, it must either by HPLC grade or purified to exclude any inhibitors present in commercially available THF. Each product was purified by column chromatography and characterized by NMR spectroscopy.

In order to ensure complete functionalization of the multivalent peripheries that exist in dendrimers, the reactions utilized must be robust, aggressive and efficient. There are few reactions that can satisfy these demanding conditions. However, click chemistry has proven to be a remarkable method of functionalization for the multivalent peripheries in dendrimers.[50–52,68–72] There is no doubt the thiol-ene click reaction has its drawbacks in that a large excess of thiol is needed to ensure each coupling goes to completion. However, the widespread commercial availability and low cost of poly(ethylene glycol) makes its use as the thiol in these reactions ideal.

The synthesis of the first target dendron, BisPy-G5-$(TEG)_{32}$ (**5-1)** was performed by combining **4-4** with **3-11**. DMPA photoinitiator was used due to its precedence as an incredibly efficient thiol-ene "click" initiator. The reactivity of the peripheral alkenes toward the thiol in click reaction conditions was outstanding. Allowing the reaction to

proceed for one hour left only one remaining alkene unfunctionalized by ^{1}H NMR spectroscopy. When the reaction time was extended to a mere two hours, complete functionalization of the periphery with TEG chains was observed by ^{1}H NMR. Identical results were observed for reactions with **3-12**. Purification of **5-1** was remarkably simple. Due to the relatively low polarity of all remaining starting materials, namely **4-4** and DMPA as well as a small amount of MeO-TEG-S-S-TEG-MeO disulfide formed during the reaction, column chromatography was performed with ease. Less polar molecules were removed, followed by an increase in eluent polarity to elute pure product in sufficient yield. Similar purification was performed on ReBisPy-G5-$(TEG)_{32}$ (**5-2)** with equal results.

The second target molecule, BisPy-G5-$(PEG_{350})_{32}$ (**5-3**) was synthesized in a similar manner to **5-1**. The only difference was the use of **4-5** in place of **4-4**. However, although the periphery of the dendron remained reactive towards the larger thiol, a reaction time of two hours was observed to be insufficient by ^{1}H NMR spectroscopy. Functionalization of approximately twenty-eight of thirty-two alkenes was typically observed under these conditions. The degree of functionalization was improved to 100% with a modest increase in reaction time to between sixteen and twenty-four hours. The exact same situations were observed in the synthesis of ReBisPy-G5-(PEG_{350}) (**5-4**). Purification of **5-3** was slightly more complicated than with its TEGylated counterpart. Column chromatography could still be performed to remove DMPA easily. However, the increased polarity of **4-5** and its disulfide byproduct caused a need for careful control over the eluent used in order to avoid co-elution of the desired product with these molecules.

Once this problem was solved, pure product was isolated in significant yield. This method of purification worked analogously with **5-4**.

Synthesis of the final target in the series, BisPy-G5-$(PEG_{750})_{32}$ (**5-5**) was much more problematic. When the shorter PEG-SH chains were substituted for **4-6** under identical reaction conditions, a complete lack of reactivity at the periphery was observed. Upon leaving the reaction to continue for forty-eight hours, formation of the product was still not visualized.

Optimization of the reaction was clearly necessary. Heating the reaction mixture to reflux was first attempted to mobilize the polymer chains in solution and potentially make the thiols more available to react. When no appreciable increase in reactivity was detected, it was hypothesized that the photoinitiator may be quenched too quickly during the reaction. To eliminate this potential shortcoming, an initiator with a longer half-life was employed. Azobisisobutyronitrile (AIBN) is a widely used thermal initiator for this exact reason. Reactions using AIBN was the radical initiator were conducted in refluxing THF, however no conversion to the desired product was observed. AIBN was determined to be an inefficient radical initiator in comparison to DMPA, therefore use of DMPA as the initiator was resumed. However, rather than relying on a set amount of initiator in the reaction flask throughout the reaction, a solution of additional DMPA in THF was prepared. By slowly adding the additional DMPA solution to the original reaction mixture over time via the use of a syringe pump, conversion to what appeared to be product was finally detected. Purification of this potential product was extremely problematic. Due to their high affinity for silica, co-elution of **4-6** and the potential product in column

chromatography was unavoidable regardless of the eluent used. Furthermore, the drastically higher affinity of PEG_{750}-functionalized dendron to silica resulted in a large amount of product remaining bound to the stationary phase. Isolated product was also observed to have only partial functionalization of the periphery with **4-6** by ^{1}H NMR.

Full characterization of each target molecule proved to be challenging. ^{1}H NMR spectra of each PEGylated molecule showed an absence of signals for the core BisPy ligand. In order to ensure that removal of the ligand from the core was not occurring under the reaction conditions, **3-11** was placed under identical thiol-ene click conditions, without the presence of any PEG-SH, and irradiated with 365 nm UV light for seventy-two hours. Upon extraction of **3-11** from the reaction, no appreciable decrease in signal from the BisPy ligand was observed (Figure 5.1). This indicated that the amide linkage binding the BisPy ligand to the dendron remains stable under the reaction conditions. The lack of the ligand ^{1}H signals in the PEGylated dendrons is potentially due to dendrimer micellization in solution.

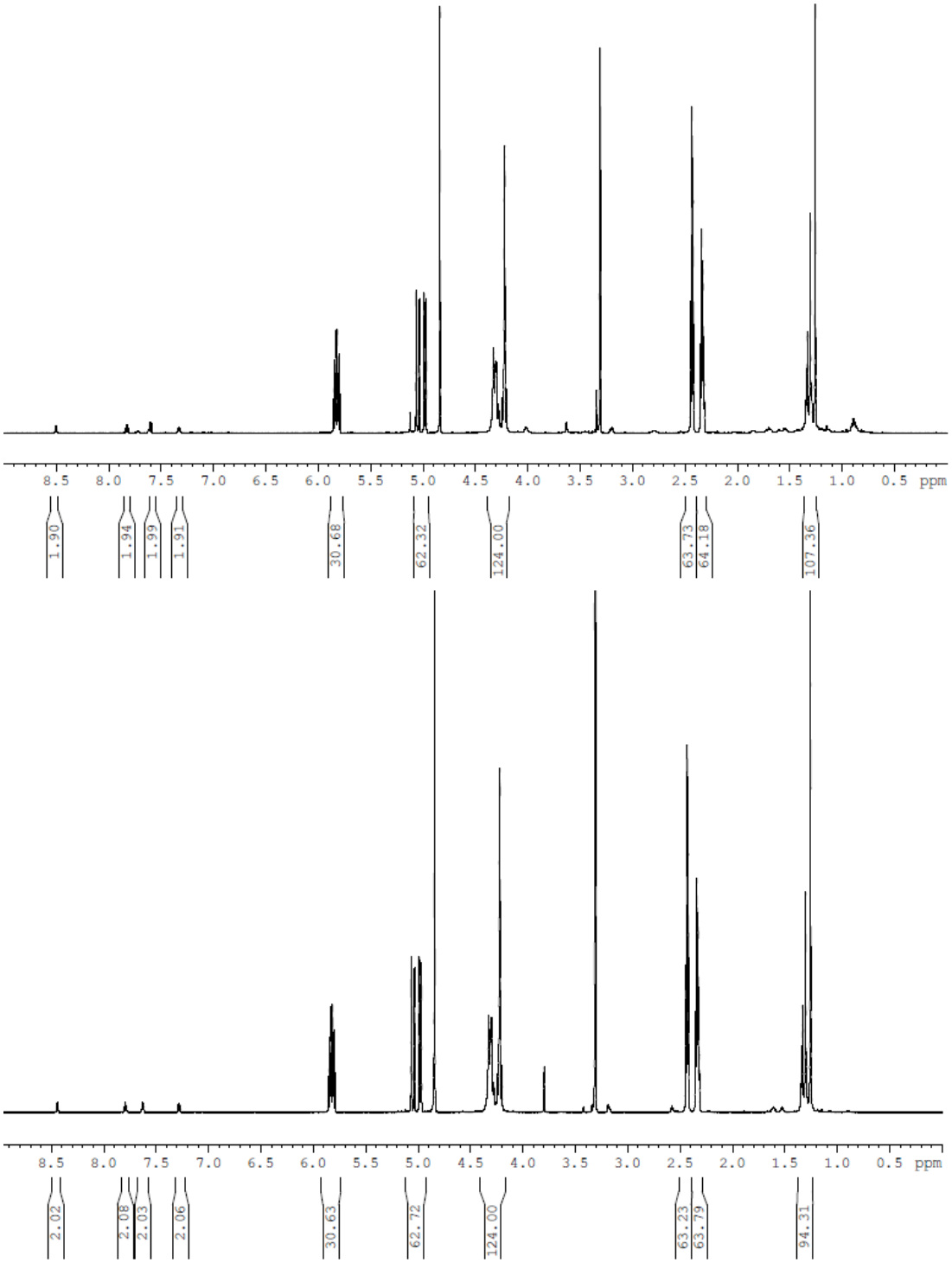
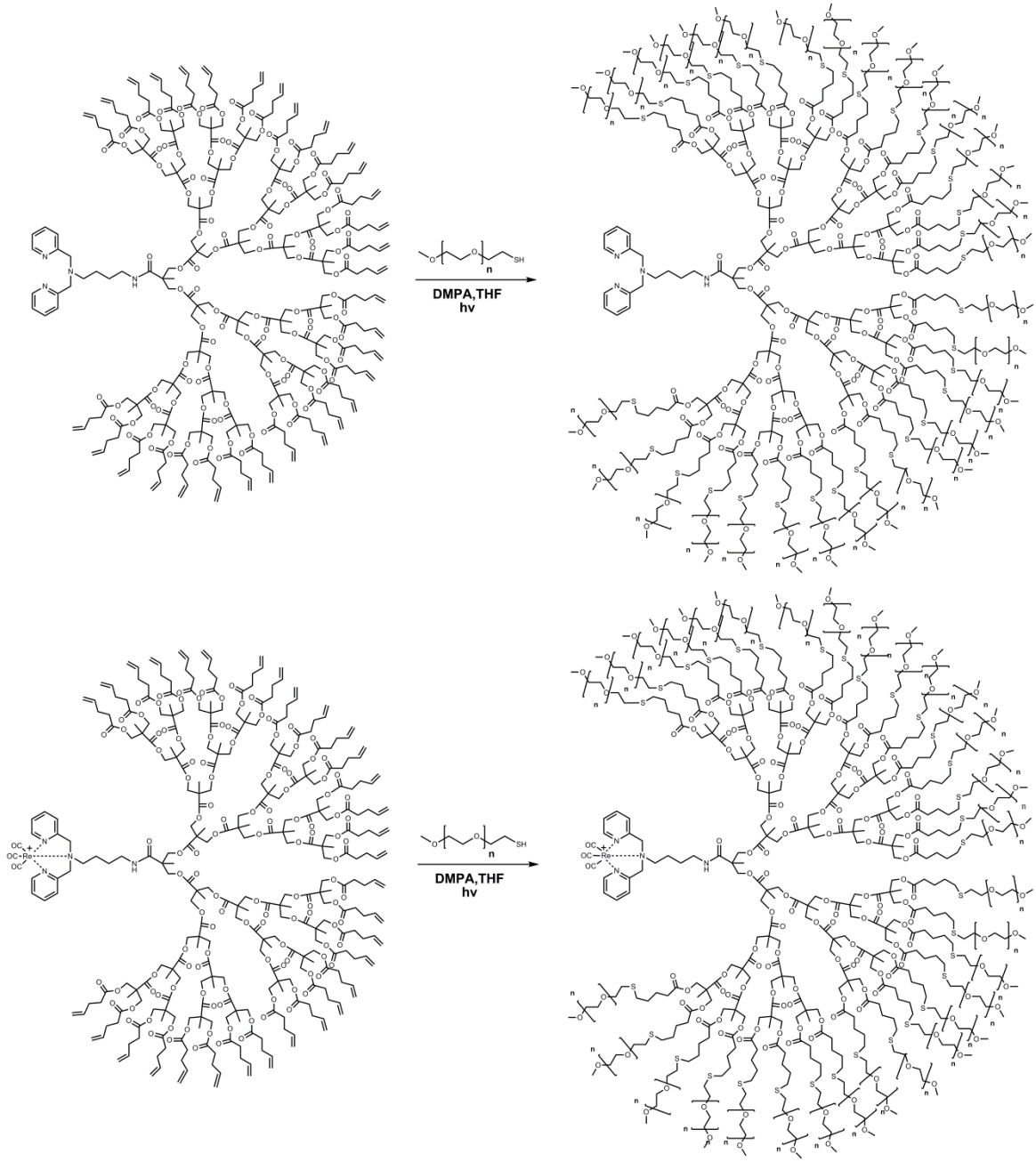


Figure 5.1. ^{1}H NMR (MeOD) of **3-11** before (top) and after (bottom) irradiation with UV light.

Mass spectral characterization of these targets also proved to be very problematic. Due to their large molecular weight, characterization by MALDI was a necessity. Multiple charging in electrospray ionization was insufficient. However, due to the macromolecular properties of each target and limitations of the MALDI-TOF instrument available, mass spectral data for each target was unobtainable.

5.2. Synthesis of PEGylated PMPA Dendrons

Functionalization of the periphery of **3-11** and **3-12** with PEG chains was achieved by combining the alkene dendrons with large excesses of each PEG-SH chain length. DMPA was used as a photoinitiator under standard thiol-ene "click" reaction conditions (Scheme 5.1). The reaction was irradiated at 365 nm for 2-48 hours, dependent on PEG chain length, in a custom-built UV reactor containing 4 sets of lamps. Due to the increased polarity of each product compared to its starting materials, isolation of each PEGylated dendron as a light yellow-dark orange, viscous oil was achieved by column chromatography.



Scheme 5.1. Synthesis of PEGylated BisPy and ReBisPy PMPA dendrons.

In order to ensure full functionalization with larger PEG-SH chains, increased reaction times were required. The reaction with **4-4** was quite rapid. Complete conversion to the desired product was observed by ^{1}H NMR spectroscopy after 2 h. The reaction

progress in each case could be monitored by ^{1}H NMR spectroscopy. The alkene proton signals in the starting material between 5 and 6 ppm disappeared as several characteristic methylene signals from the product were observed in the region of 1.5 to 3 ppm (Figure 5.2). This observation also leads to the realization that unfortunately, full functionalization of with **4-6** is not possible using this method. Almost identical percent conversion of starting material to product was observed by ^{1}H NMR spectroscopy between 48 h and 72 h reaction time (Figure 5.3). This is indicative of a maximum functionalization limit using this method.

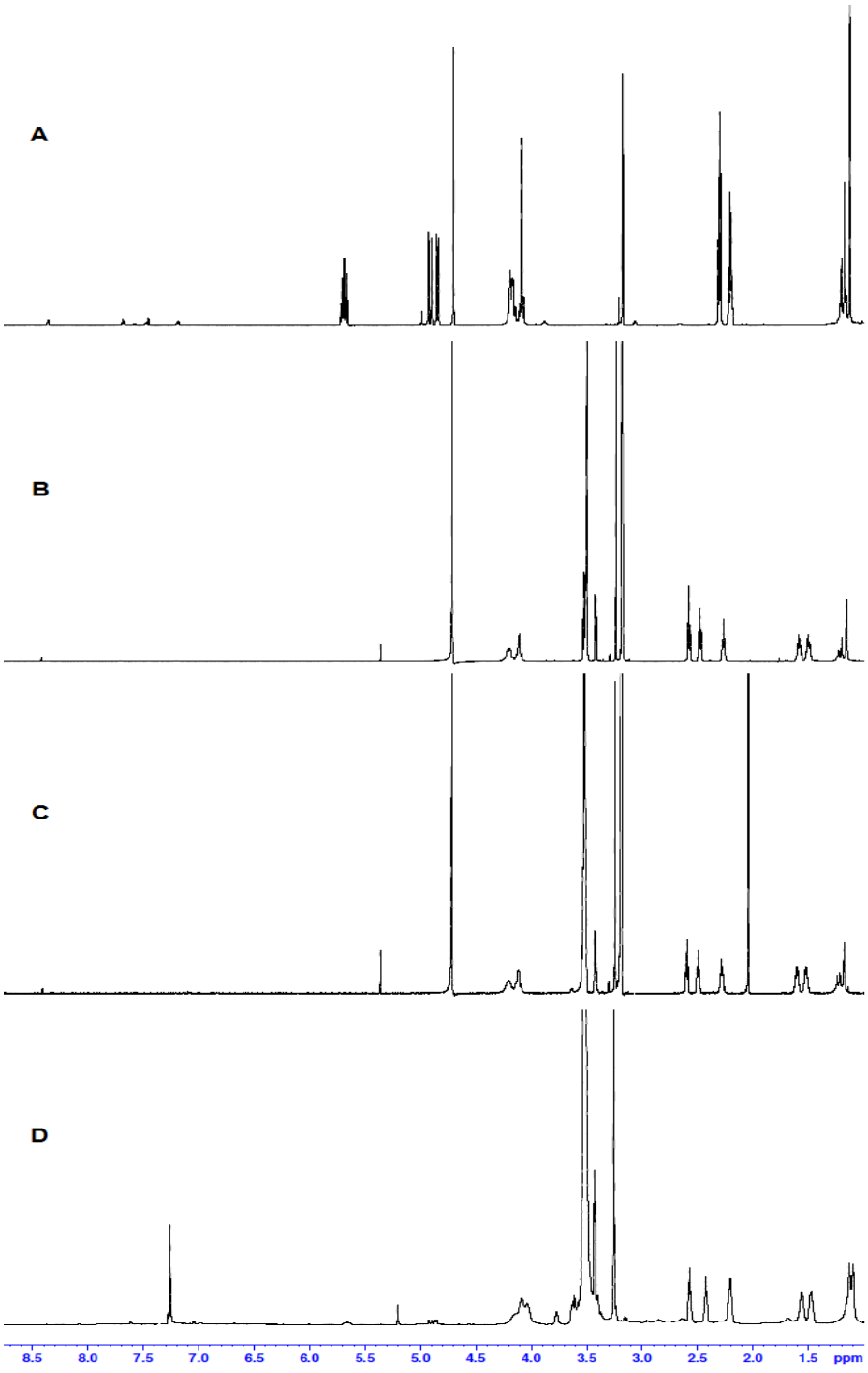


Figure 5.2. ^{1}H NMR spectra of **3-11** in MeOD (A), **5-1** in MeOD (B), **5-3** in MeOD (C), **5-5** in $CDCl_3$ (D).

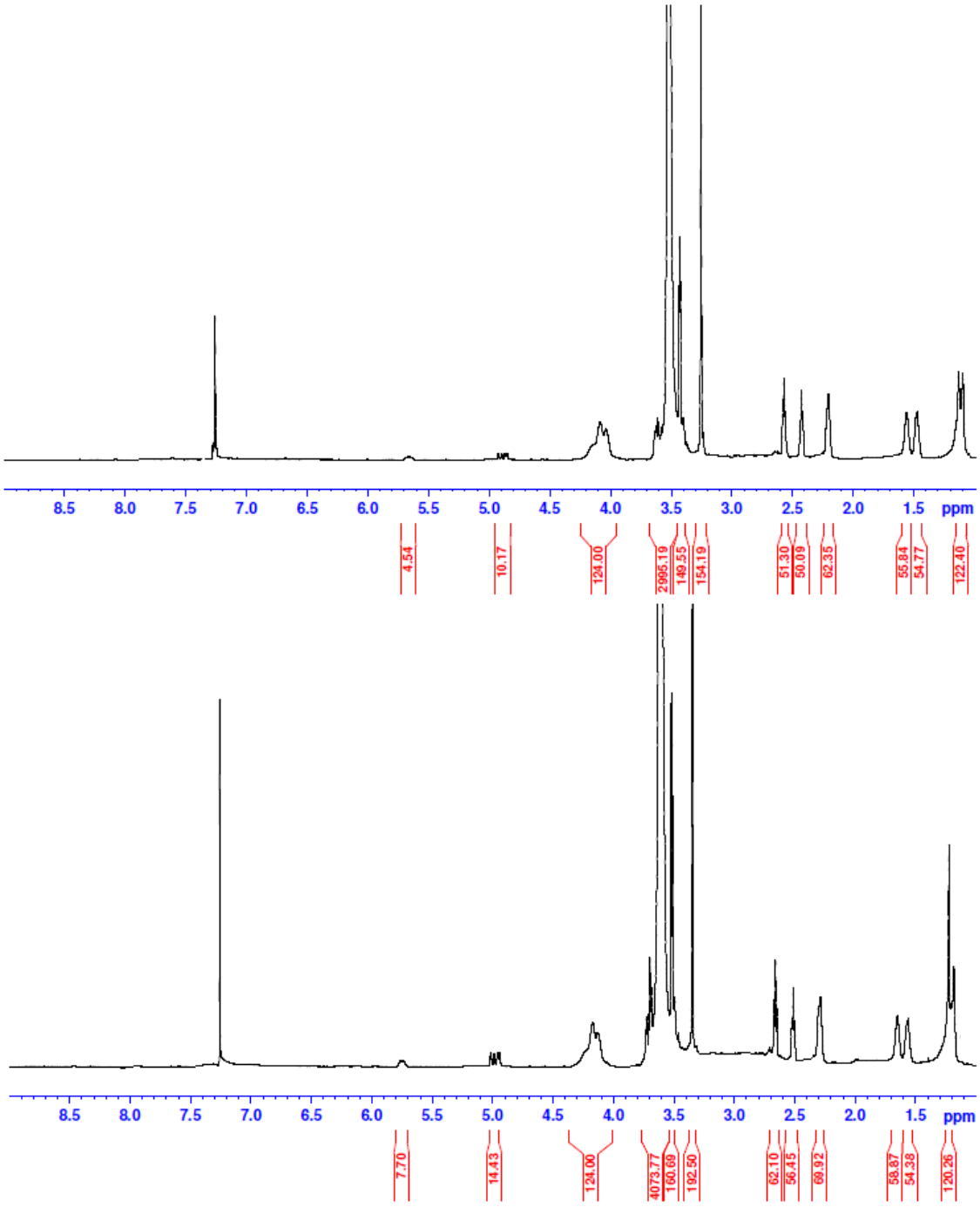


Figure 5.3. ^{1}H NMR spectra ($CDCl_3$) of isolated product from **5-5** synthesis after 48 h (top) and 72 h (bottom) (Note: Excess **4-6** remained in samples, NMR spectra are from a mixture of product and **4-6**).

5.3. Benefits and Drawbacks of Current Methodology

Although full functionalization of **3-11** with **4-6** was not achieved, valuable information about limitations to the reactivity of this alkene periphery was obtained. The obstacle in obtaining full conversion to a PEGylated periphery is most likely steric. The fully TEGylated product is obtained after 2 hours of reaction time, whereas fully PEGylated dendron using **4-5** typically requires 24 hours. Shorter reaction times in the **4-5** coupling did yield partially functionalized product, with 3-4 alkenes typically remaining. Both couplings yielded the desired product in appreciable yields. However, these partially functionalized products could be placed back in the original reaction mixture conditions, irradiated once more and fully functionalized product was obtained. Yet another advantageous component of this synthetic methodology was that purification via silica gel chromatography not only provided pure product, but also the ability to recollect and isolate pure PEG-SH starting materials. Although a large excess is required, a significant quantity was recovered and re-used in additional click reactions.

Coupling with **4-6** is not ideal for several reasons. Unlike couplings with **4-5**, isolated partially-functionalized products were not fully functionalized after being placed in original reaction conditions and irradiated further. Furthermore, purification via silica gel chromatography is not a viable option. Due to the high polarity of **4-6** as well as the target product, both components of the reaction mixture have a strong affinity for silica. In order to elute any significant amount of target molecule from the column, significant co-elution of **4-6** occurred. Even preparative TLC of the co-eluted components could not

fully separate excess **4-6** from the target molecule, as observed in figure 5.2. Although it will significantly affect the overall yield, in order to fully isolate pure PEGylated dendron, preparative HPLC will likely be required.

5.4. Conclusion

Synthesis of two target molecules in the series, **5-1** and **5-3**, was accomplished using the thiol-ene click method developed. The peripheral alkenes showed remarkable reactivity towards the smaller **4-4** and **4-5** thiols, fully functionalizing the periphery of the dendron in as little as two and sixteen hours, respectively. Purification of the two targets was accomplished by simple silica gel chromatography. Analogous results were observed for **5-2** and **5-4**. Each target was characterized by NMR spectroscopy. MALDI data was not obtainable.

Synthesis of the final target, **5-5** is currently under optimization. Current methodology yields partially functionalized dendrons that cannot be separated from all starting materials by silica gel chromatography and prep-TLC alone. HPLC will be required to purify and isolate this product.

5.5. Experimental

General. DMPA was purchased from Aldrich and used without further purification. HPLC-grade THF was purchased from Caledon Laboratory Chemicals and used as provided. All other solvents were purchased from commercial sources and used as provided. UV irradiation was carried out in a custom-built UV reactor containing 4

individual sets of 365 nm lamps. The interior of the reactor was coated with aluminum foil to provide full irradiation of the reaction mixture without any gaps in coverage. Proper precautions must be taken to avoid exposure to UV light as it can severely damage eyes and skin. All NMR spectra were recorded on a Bruker Avance 600MHz spectrometer.

Synthesis of 5-1:

In a flame-dried quartz round-bottom flask equipped with stir bar, **3-11** (0.095 g, 0.015 mmol) was combined with DMPA (0.019 g, 0.073 mmol) and **4-4** (0.842 g, 4.67 mmol) and dissolved in 15 mL THF. The reaction mixture was sparged with argon for 15 min at room temperature before being placed in a UV reactor and irradiated with 365 nm light for 2 h. Solvent was then removed by rotary evaporation and the product was isolated by silica gel chromatography using 97:3 CH_2Cl_2: MeOH as the eluent. The product was obtained as a light yellow, viscous oil (0.131 g, 0.011 mmol, 73%). ^{1}H NMR (MeOD, 600 MHz) δ = 1.33 (m, 104 H), 1.63 (m, 64 H), 1.71 (m, 64H), 2.39 (t, 64H, *J*= 7), 2.61(t, 64H, *J*= 7), 2.71(t, 64H, *J*= 7), 3.36 (s, 3H), 3.55 (t, 64H, *J*= 5), 3.64 (m, 256 H), 4.32 (m, 124H). ^{13}C NMR (MeOD, 600 MHz) δ = 14.42, 18.61, 25.21, 30.31, 30.71, 32.36, 32.97, 34.53, 47.80, 48.06, 59.25, 66.40, 71.36, 71.47, 71.66, 72.26, 73.05, 174.25.

Synthesis of 5-2:

In a flame-dried 25 mL quartz round-bottom flask equipped with stir bar under argon atmosphere, **3-12** (0.019 g, 0.003 mmol) was dissolved in 5 mL of HPLC grade THF and combined with **4-4** (0.163 g, 0.896 mmol) and DMPA (0.004 g, 0.013 mmol). The

reaction mixture was sparged with argon for 15 min, then irradiated with 365nm UV light for 2 h at room temperature while stirring. The resulting solution turned bright yellow. Solvent was removed by rotary evaporation and product was isolated as a slightly yellow oil by silica gel chromatography using 96:4 CH_2Cl_2 as the eluent (0.021 g, 0.002 mmol, 61%). ^{1}H NMR (CD_2Cl_2, 600 MHz) δ = 1.30 (m, 104H), 1.64 (m, 64H), 1.71 (m, 64H), 2.36 (t, 64H, *J* = 7.5), 2.59 (t, 64H, *J* = 7.3), 2.72 (t, 64H, *J* = 6.9), 3.34 (s, 96H), 3.52 (m, 64 H), 3.61 (m, 260H), 4.24 (m, 124H), 7.25 (t, 2H, *J* =7.1), 7.62 (d, 2H, *J* = 7.9), 7.87 (t, 2H, *J* =7.7), 8.70 (d, 2H, *J* =5.4). ^{13}C NMR (CD_2Cl_2, 600 MHz) δ = 17.78, 17.90, 18.12, 22.66, 24.35, 29.53, 30.07, 31.80, 32.39, 33.81, 46.69, 46.99, 59.01, 65.16, 70.66, 70.78, 70.86, 71.24, 72.29, 172.49, 173.03.

Synthesis of 5-3:

In a flame-dried 10 mL round-bottom flask equipped with stir bar under argon atmosphere, **3-11** (0.047g, 0.007 mmol) was combined with DMPA (0.012 g, 0.035 mmol) and **4-5** (1.024 g, 2.304 mmol) and dissolved in 5 mL of HPLC-grade THF. The reaction mixture was sparged with argon for 15 min at room temperature before being placed in a UV reactor and irradiated with 365 nm light for 24 h. Solvent was then removed by rotary evaporation and the product was isolated by silica gel chromatography using 96:4 CH_2Cl_2: MeOH as the eluent to remove all excess starting materials, followed by a gradient elution from 93:7 to 85:15 CH_2Cl_2:MeOH. The product was obtained as a dark orange, viscous oil (0.110 g, 0.005 mmol, 74%). ^{1}H NMR (MeOD, 600 MHz) δ = 1.33 (m, 104 H), 1.63 (m, 64 H), 1.71 (m, 64H), 2.39 (t, 64H, *J*= 7), 2.61(t, 64H, *J*= 7), 2.71(t, 64H, *J*= 7), 3.36 (s, 96H), 3.55 (t, 64H, *J*= 5), 3.64 (m, 1000 H), 4.32 (m, 124H).

^{13}C NMR (CD_2Cl_2, 600 MHz) δ = 17.76, 17.86, 18.09, 24.31, 29.49, 30.03, 31.77, 32.36, 33.76, 46.63, 46.93, 58.98, 65.06, 70.59, 70.71, 70.84, 71.14, 72.25, 171.96, 172.42, 172.96.

Synthesis of 5-4:

In a flame-dried quartz round bottom flask equipped with stir bar under argon atmosphere, **3-12** (0.0114 g, 0.0016 mmol) was combined with DMPA (0.007 g, 0.024 mmol) and **4-5** (0.228 g, 0.512 mmol) and dissolved in 2 mL THF. The reaction mixture was sparged with argon for 15 min at room temperature before being placed in a UV reactor and irradiated with 365 nm light for 30 h. Solvent was then removed by rotary evaporation and the product was isolated by silica gel chromatography to remove all excess starting materials using 96:4 CH_2Cl_2: MeOH as the eluent to remove all excess starting materials, followed by a gradient elution from 93:7 to 85:15 CH_2Cl_2: MeOH. The product was obtained as an orange, viscous oil (0.010 g, 0.0005 mmol, 28%). ^{1}H NMR (MeOD, 600 MHz) δ = 1.34 (m, 104 H), 1.62 (m, 64 H), 1.70 (m, 64H), 2.41 (t, 64H, *J*= 7), 2.62 (t, 64H, *J*= 7), 2.73 (t, 64H, *J*= 7), 3.38 (s, 96H), 3.60 (t, 64H, *J*= 5), 3.67 (m, 1090 H), 4.36 (m, 124H). ^{13}C NMR (CD_2Cl_2, 600 MHz) δ = 17.80, 17.88, 18.11, 24.31, 29.06, 29.50, 30.04, 31.79, 32.37, 33.51, 33.77, 46.64, 58.98, 65.07, 70.62, 70.75, 70.88, 71.17, 72.27, 115.68, 137.17, 171.93, 172.41, 172.94.

Synthesis of 5-5:

In a flame-dried 10 mL round-bottom flask equipped with condenser and stir bar under argon atmosphere, **3-11** (0.044g, 0.007 mmol) was combined with DMPA (0.027 g, 0.106

mmol) and **4-6** (1.62 g, 2.11 mmol) and dissolved in 6 mL of HPLC-grade THF. The reaction mixture was sparged with argon for 15 min under sonication at room temperature before being placed in a UV reactor and irradiated with 365 nm light. Additional DMPA (0.054 g, 0.212 mmol) was dissolved in 2 mL of HPLC-grade THF and placed in a 5 mL syringe. The syringe was placed in a syringe pump and covered in aluminum foil to ensure no irradiation of the photoinitiator occurred. The pump was set to deliver solution at a rate of 0.03 mL/hr to the reaction mixture. The reaction was left to stir for 48 h under reflux. Solvent was then removed by rotary evaporation and the product was isolated by silica gel chromatography using 96:4 CH_2Cl_2: MeOH as the eluent to remove all excess starting materials, followed by a gradient elution from 93:7 to 20:80 CH_2Cl_2:MeOH. A mixture of **4-6** and the desired product was isolated. Preparative TLC of the mixture was performed using a 20 cm x 20 cm, 1mm plate and an eluent of 92:8 CH_2Cl_2: MeOH. A thin, UV-absorbing band was isolated near the baseline of the TLC plate. Silica was removed from the TLC plate and placed in a mortar and ground with a pestle vigorously for 2 minutes. The silica was then placed in a 100 mL round-bottom flask and combined with 50 mL of 3:2 CH_2Cl_2: MeOH. This mixture was sonicated for 30 min, then silica was removed by vacuum filtration. The material obtained, a light brown oil, was observed to be a combination of **4-6** and BisPy-G5-$(PEG_{750})_{25\text{-}27}$ by ^{1}H NMR. This reaction was repeated and left for 72 h before isolation to yield a similar mixture of product and starting material. ^{1}H NMR ($CDCl_3$, 600 MHz) δ = 1.13 (m, 110 H), 1.47 (m, 56 H), 1.56 (m, 56H), 2.20 (m, 62H), 2.43(t, 56H, *J*= 6.3), 2.57(t, 56H, *J*= 6.6), 3.26 (s, 154H), 3.44 (t, 150H, *J*= 4.3), 3.52 (m, 2996 H), 4.09 (m, 124H), 4.89 (m, 10H), 5.67 (m, 5H).

Chapter 6:

Radiolabeling of PEGylated PMPA Dendrons with ^{99m}Tc

6.1. Overview

5-1 and **5-3** were combined with $[^{99m}Tc(CO_3)(H_2O)_3]^+$ (**6-1**) and heated in a microwave reactor to bind ^{99m}Tc to the core binding ligand of each target molecule using a methodology similar to that developed by Parrott et al.[8] The reaction was carried out in a combination of aqueous and organic solvents, optimized for the solubility of each dendron. This labeling technique met with mixed results.

Having achieved the preparation of peripherally PEGylated PMPA dendrons with a chelating BisPy ligand bound to the core, ^{99m}Tc could now be introduced. Using a methodology similar to previous labeling of PMPA dendrons,[8] a viable candidate for imaging could be produced. However, unlike the hydroxylated PMPA dendrons used previously for imaging studies, aqueous labeling conditions alone were found to be unsatisfactory for labeling of the PEGylated analogues. Solutions of **5-1** and **5-3** were prepared in distilled H_2O. When combined with the highly saline aqueous solution containing **6-1**, problems arose as the resulting solutions became cloudy and opaque as the dendrons emulsified. This is not an ideal condition for labeling as the dendrons should be completely soluble to achieve the best radiochemical yield possible. The opacity of these solutions was likely caused by the PEGylated periphery of the dendrons. Extraction of PEG from aqueous media is often performed by increasing the salinity of the solution.

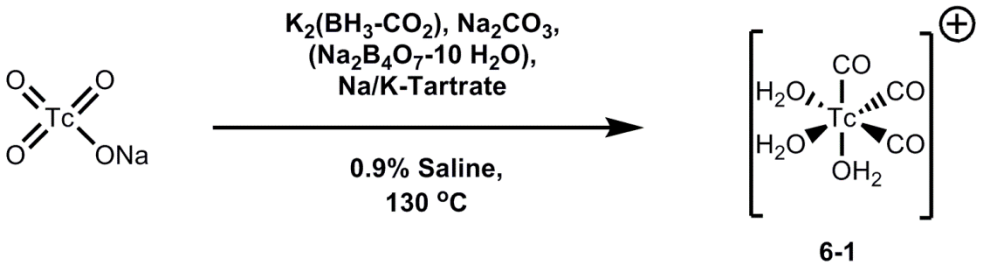
By introducing a strongly saline ^{99m}Tc solution, it was not surprising that emulsion formation was observed.

Labeling with ^{99m}Tc can also be carried out in mixtures of ethanol and distilled water. The introduction of ethanol did aid in solubilizing **5-3**, but **5-1** persistently emulsified upon addition of **6-1** solution. DMSO is another organic solvent that can be employed in labeling. Upon addition of DMSO to the ethanol/water mixture, complete solubility of **5-1** in the presence of **6-1** solution was achieved. However, results in attempted labeling of **5-1** did not show improvement in this new solubilizing solvent system. Gamma detection initially showed promising uptake of radioactivity in the reaction mixture. Once the reaction was heated with 2,2'-bipyridyl to remove unbound ^{99m}Tc however, all activity was bound to 2,2'-bipyridyl. This indicates that no ^{99m}Tc was bound to the core of **5-1**. The lack of labeling exhibited could be related to the potential micellization observed in ^{1}H NMR analysis of **5-1**.

Labeling experiments with **5-3** were much more promising. Initial labeling using a mixture of ethanol in aqueous solution was similar to that performed with **5-1** in that no uptake of activity was observed. The use of DMSO in combination with **6-1** solution greatly improved labeling results. Activity was retained on the dendron after heating with 2,2'-bipyridyl, indicating ^{99m}Tc was successfully bound to the core. This labeling procedure was repeated to ensure its viability, producing identical results each time. We have reached a stage where semi-prep HPLC isolation of $[TcBisPy\text{-}G5\text{-}(PEG_{350})_{32}]^{+}$ (**6-3)** will be performed in preparation for imaging and biodistribution studies similar to those performed by Parrott et al.[8]

6.2. Preparation of $[^{99m}Tc(CO_3)(H_2O)_3]^+$

Since ^{99m}Tc is only available commercially from $^{99}Mo/^{99m}Tc$ generators, a saline solution of potassium boranocarbonate ($K_2[BH_3{\bullet}CO_2]$), Na_2CO_3, Na/K-tartrate, and borax ($Na_2B_4O_7{\bullet}10\ H_2O$) was combined with a solution of $Na^{99m}TcO_4$ from the generator. The resulting solution was heated in a microwave reactor to convert $Na^{99m}TcO_4$ to the reactive species, $[^{99m}Tc(CO_3)(H_2O)_3]^+$ (Scheme 6.1). HPLC of the resulting solution revealed complete conversion to the desired product (Figure 6.1). On occasion it was observed that some residual $Na^{99m}TcO_4$ remained, however its presence had no negative effect on labeling or radiochemical yield.



Scheme 6.1. Synthesis of **6-1**.

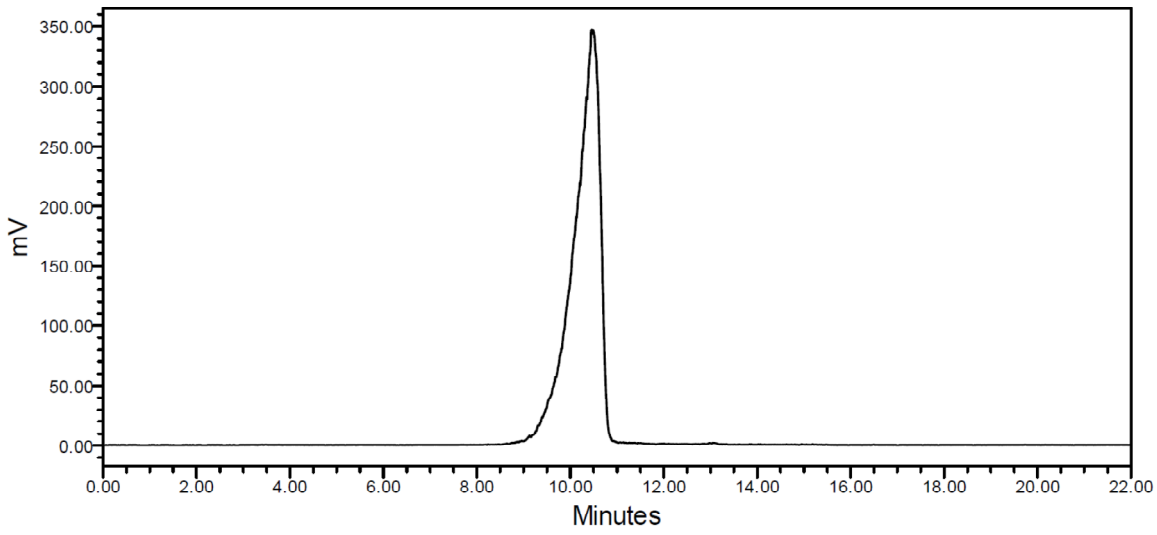
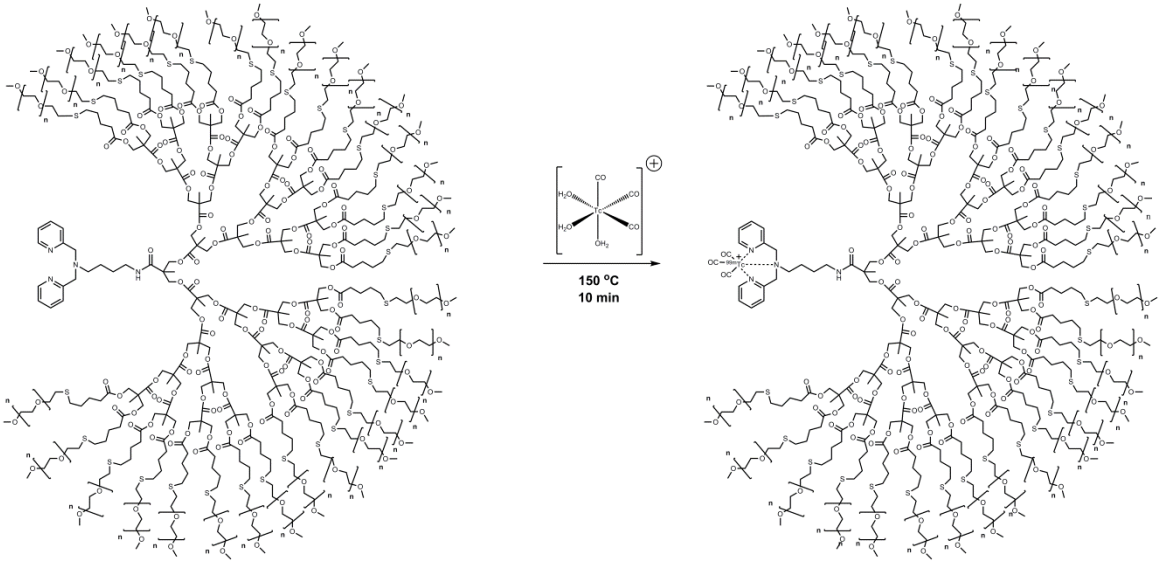


Figure 6.1. HPLC Chromatogram [γ] of **6-1** reaction mixture post-microwave activation.

6.3. Radiolabeling of PEGylated PMPA Dendrons

In a microwave vial, 200 μL of a 25-30 mg/mL solution of PEGylated PMPA dendron in DMSO was combined with saline solution containing **6-1**, DMSO and distilled H_2O, with an additional volume EtOH added for labeling of **5-1**. The solution was placed in a microwave and heated to bind the ^{99m}Tc chelate to the BisPy core (Scheme 6.2). In order to remove unbound ^{99m}Tc from the dendron, 2,2'-bipyridyl was added to the vial and it was heated in the microwave again to remove any free **6-1** from solution.



Scheme 6.2. Synthesis of $[TcBisPy\text{-}G5\text{-}(PEG)_{32}]^{+}$.

As mentioned above, entirely aqueous labeling conditions used previously with hydroxylated PMPA dendrons[8] were found to be unfavourable for PEGylated PMPA dendrons. This is due to the strong salinity of the labeling solution. PEG is typically extracted from aqueous media by increasing the salinity of the solution. This greatly decreases the solubility of PEG in the aqueous layer, allowing efficient extraction into organic solvent. This property of PEG was initially problematic in that solutions of the PEGylated dendrons, once combined with saline solution containing **6-1**, would become cloudly and emulsify. Attempts to label in this manner using microwave activation were unsuccessful.

Labeling can also be performed in the presence of ethanol, up to a 50:50 combination of EtOH:aqueous. Solutions of PEGylated PMPA dendron were prepared in EtOH and combined with saline solution containing the activity. When combining

solutions of **5-1** in EtOH with **6-1** solution, once again the dendron appeared to emulsify. Conversely, **5-3** solution in EtOH combined with the aqueous media to provide a clear, colourless solution. Unfortunately, after microwave activation of both mixtures, no appreciable amount of labeling was observed by gamma counter detection in HPLC.

Another organic solvent commonly used in labeling of less soluble organic molecules is DMSO. Once DMSO was introduced as the organic solvent for labeling, more favourable results were observed. A broad peak was observed by gamma detection in the HPLC run of the mixture obtained from labeling **5-3** indicating the presence of $[TcBisPy\text{-}G5\text{-}(PEG_{350})_{32}]^{+}$ (**6-3)** (Figure 6.2).

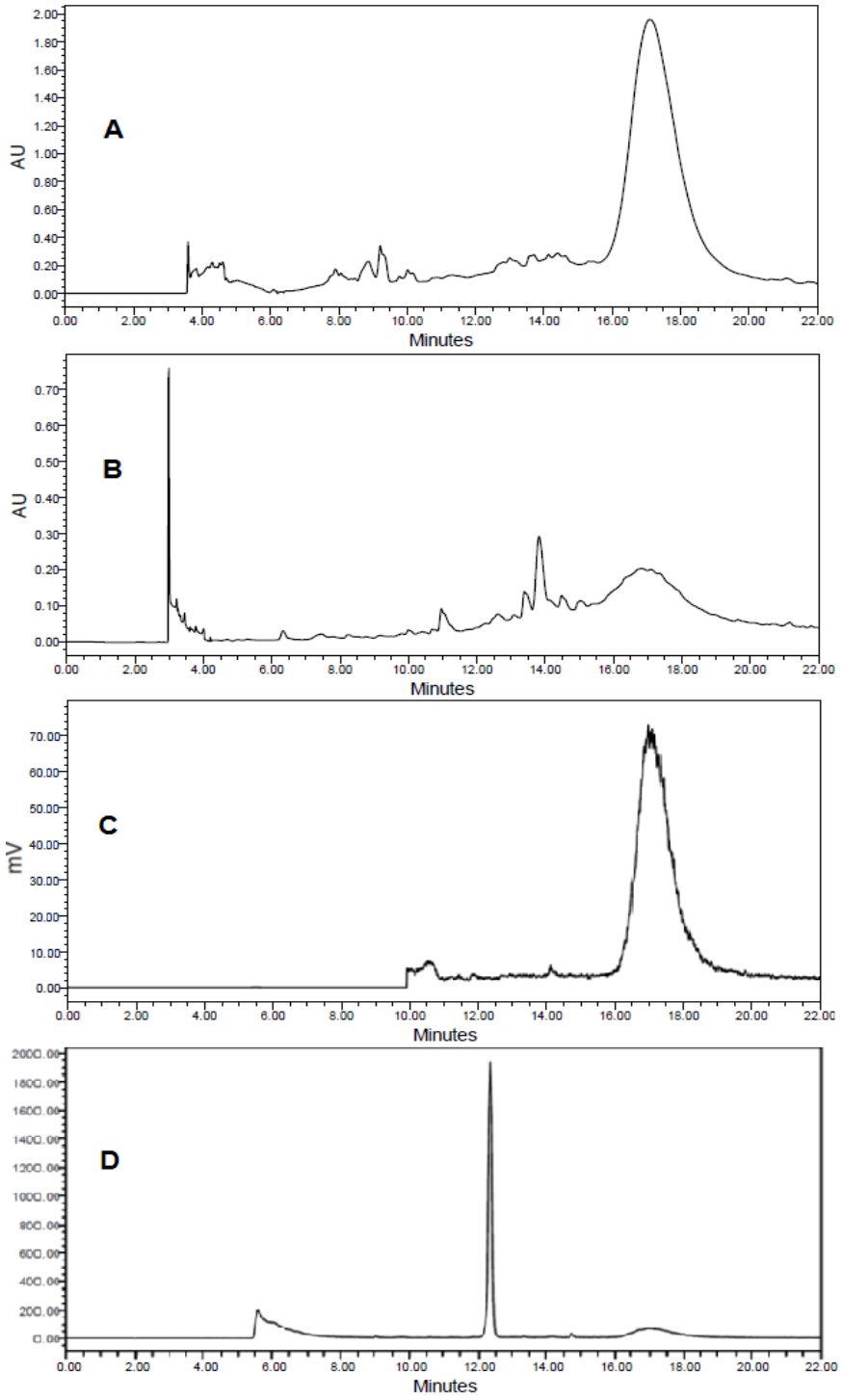


Figure 6.2. HPLC Chromatograms of (A) **5-3** [uv], (B) **5-4** [uv], (C) **6-3** prior to 2,2'-bipyridyl addition [γ], (D) **6-3** post 2,2'-bipyridyl addition [γ].

Collection of this peak from the HPLC elution was performed (Figure 6.3) and the radiochemical yield was determined to be 28%.

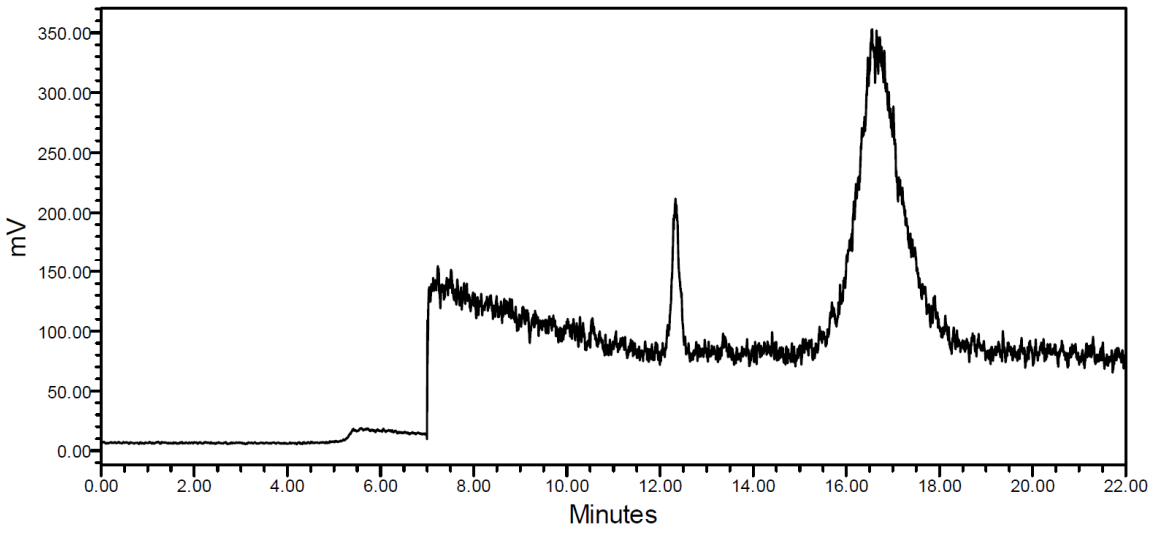


Figure 6.3. HPLC Chromatogram [γ] of isolated **6-3** peak with some residual labeled 2,2'-bipyridyl.

Labeling of **5-1** proved to be more problematic. When DMSO was used as the organic solubilizing component, an emulsion still formed once combined with saline solution containing activity. The addition of EtOH and H_2O to the organic component prior to combination with the **6-1** solution prevented the formation of any emulsion, providing a clear, colourless and transparent mixture. However, appreciable labeling of the compound was still not observed (Figure 6.4.).

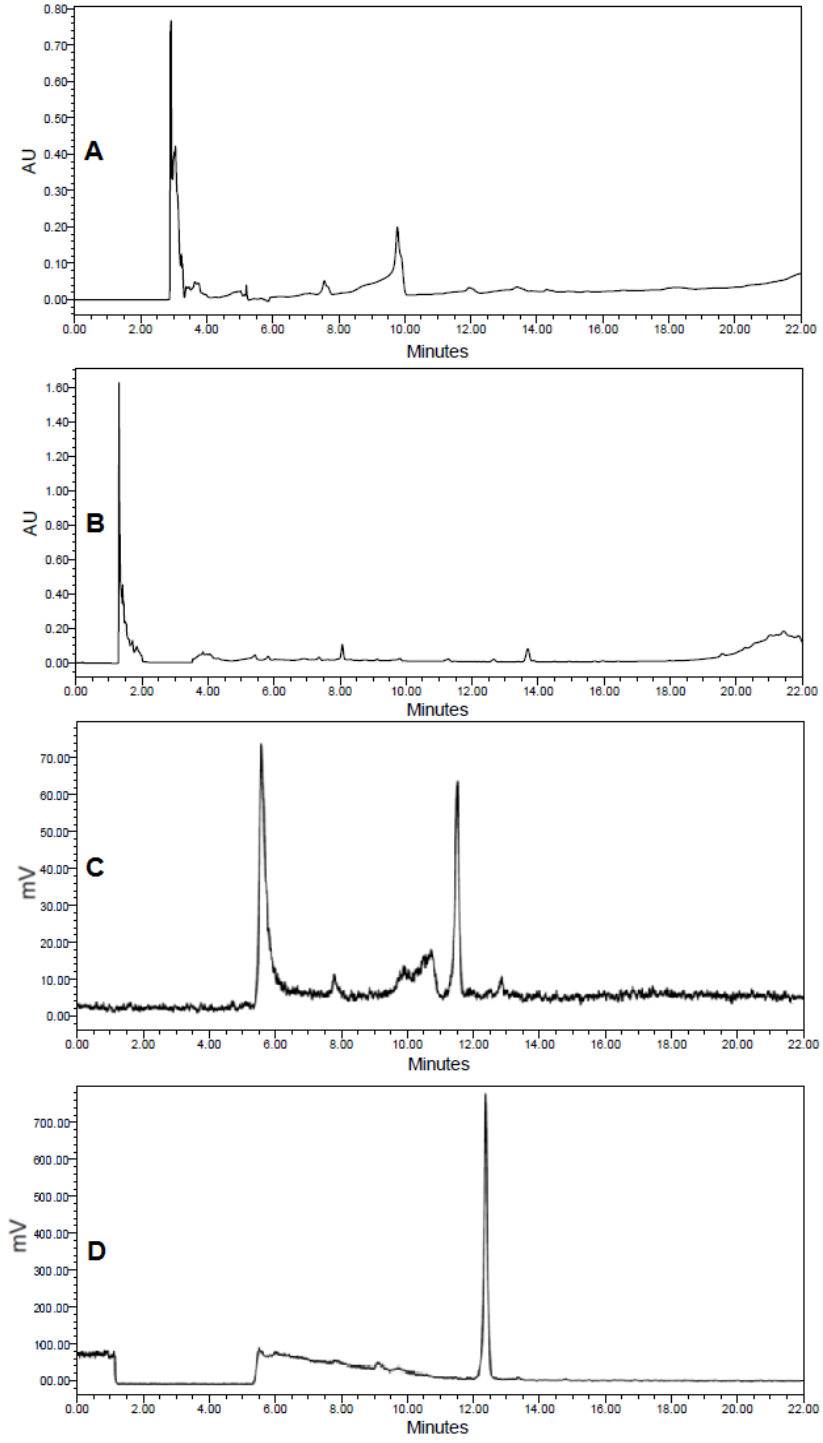


Figure 6.4. HPLC Chromatograms of (A) **5-1** [uv], (B) **5-2** [uv], (C) **6-2** mixture prior to 2,2'-bipyridyl addition [γ], (D) **6-2** mixture after 2,2'-bipyridyl addition [γ].

The low solubility of **5-1** in solution with **6-1** is likely to blame. Optimization of these labeling conditions, including emulsion reactor[73] labeling may be necessary to improve labeling and radiochemical yield of the desired product.

6.4. Conclusion

Radiolabeling experiments with **5-1** and **5-3** were met with mixed results. Successful labeling of **5-3** was achieved through slight modifications to literature procedures.[8] This new method for labeling has been repeated successfully and is ready for semi-prep isolation of **6-3** for future biodistribution and imaging studies.

Successful synthesis of $[TcBisPy\text{-}G5\text{-}(TEG)_{32}]^+$ (**6-2**) was not obtained using this method of labeling. This is likely due to micellization of the **5-1** molecules in solution. To decrease the degree of micellization and potentially expose the BisPy ligand at the core to external media, emulsion reactor radiolabeling will be explored in the near future.[73] Since pure **5-5** was not obtained, no labeling studies on this target have been performed to date.

6.5. Experimental

General. 2,2'-bipyridyl was purchased from Aldrich and used without further purification. 0.9% Saline solution was obtained in sealed 10 mL vials from Hospira. The Technelite $^{99}Mo/^{99m}Tc$ generator was obtained from Lantheus Medical Imaging and used as provided. All other reagents and solvents were obtained from commercial sources and used as provided. Microwave reactions were performed on a Biotage Initiator Eight instrument. HPLC was performed on a Waters 1525 Binary HPLC Pump connected to a

Waters 2998 Photodiode Array Detector and Bioscan Gamma Detector. The HPLC column was a Phenomenex Gemini C-18 5 micron, 250 x 4.6 mm column. Flow rate was set to 1 mL/min. Eluent was a combination of distilled H_2O with 0.1% TFA (Solvent A) and acetonitrile with 0.1% TFA (Solvent B). A gradient elution was performed, beginning with 95:5 (v/v) A:B. This adjusted to 61:39 (v/v) A:B over 8 minutes, 5:95 (v/v) A:B between 8-20 minutes, 95:5 (v/v) A:B between 20-21 minutes and remained constant at 95:5 (v/v) A:B) from 21-22 minutes. All experiments involving radioactivity were performed by Aimen Zlitni. ^{99m}Tc is a radioactive gamma emitter and should only be used in a licensed, approved facility with proper protective equipment in place.

Synthesis of 6-1 solution:

$K_2[BH_3{\bullet}CO_2]$ (9.0 mg, 66.2 μmol) was combined with Na_2CO_3 (4.2 mg, 39.6 μmol), Na/K-tartrate (16 mg), and borax (3.5 mg, 9.2 μmol) in a microwave vial and purged with argon for 10 minutes. 10 mL of Technelite 0.9% saline solution was eluted through a Technelite ^{99}Mo/^{99m}Tc generator to provide a solution of $Na^{99m}TcO_4$. 1 mL of this solution (68 mCi) was then added to the microwave vial. The resulting solution was heated in a microwave at 130 ^{o}C for 6 minutes. The pH of the solution was then adjusted to between 4-6 with HCl for labeling of the PMPA dendrons.

Synthesis of 6-2:

A 25 mg/mL solution of **5-1** was prepared in DMSO. 200 μL of this solution was added to a microwave vial. In the following order, 600 μL of EtOH, 200 μL DMSO and 200 μL distilled H_2O were added to the vial to ensure the dendron remained soluble. 200

μL of **6-1** solution was added dropwise with light shaking. The vial was sealed and purged with argon once more. The mixture was heated in a microwave at 150 ^{o}C for 10 minutes. An excess of 2,2'-bipyridyl was added to the vial, which was heated again in the microwave at 130 ^{o}C for 6 minutes. Aside from a peak for labeled 2,2'-bipyridyl, no activity to indicate synthesis of the product was observed in the HPLC chromatogram.

Synthesis of 6-3:

A 30 mg/mL solution of **5-3** was prepared in DMSO. 200 μL of this solution was added to a microwave vial. 300 μL DMSO, 200 μL distilled H_2O and 200 μL of **6-1** solution were added to the vial with light shaking. The vial was sealed and purged with argon once more. The mixture was heated in a microwave at 150 ^{o}C for 10 minutes. An excess of 2,2'-bipyridyl was added to the vial, which was heated again in the microwave at 130 ^{o}C for 6 minutes. An aliquot of the reaction mixture (390 μCi) was injected onto an HPLC column. An intense, broad peak was observed in the HPLC chromatogram between 14 and 16 minutes, indicating the presence of $[TcBisPy\text{-}G5\text{-}(PEG_{350})_{32}]^{+}$. This peak was isolated and its activity was found to be 110 μCi (radiochemical yield = 28%).

Chapter 7: Conclusions

In the preparation of a series of new, dendrimer-based radioimaging agents we have demonstrated a detailed and comprehensive synthesis of peripherally alkene-functionalized PMPA dendrons, from generations one through 5, using anhydride coupling chemistry with pentenoic anhydride. The core of the PMPA dendron was necessarily modified to incorporate a tridentate BisPy ligand in order to chelate ^{99m}Tc to the macromolecule. In preparation for reactions with the alkene periphery, a series of thiol-terminated PEG chains were also synthesized in significant yield after minor improvement to literature syntheses.

The strong reactivity of (Core)-G5-$(PTA)_{32}$ towards thiol-ene "click" chemistry was observed in its reaction with **4-4** and **4-5**. These click reactions were observed to fully functionalize the periphery of the dendron in a matter of hours. However, a limit to this molecules peripheral reactivity was observed when reacted with **4-6**. Near-complete functionalization was demonstrated by ^{1}H NMR, and the current synthetic methodology failed to separate the mostly-functionalized product **4-6**. We are, however, confident that more aggressive methods of purification such as HPLC should be able to provide pure, partially PEG_{750}-ylated PMPA dendron at a cost to overall yield of product.

Attempts at radiolabeling of the PEGylated PMPA dendrons were met with mixed results. **5-1** has proven problematic in labeling. While the issue of solubility in the reaction mixture appears to have been overcome, the chelation of ^{99m}Tc to the core of the dendron has yet to be observed. In the future, emulsion labeling may be necessary to

effectively radiolabel this target. The lack of ^{1}H signals for the ligand in NMR studies of **5-1** indicate potential micellization, which could make the ligand unavailable to the ^{99m}Tc in solution. Emulsion reactor labeling could be the solution to this problem.

5-3 has shown promising results in radiolabeling studies. The high radiochemical yield obtained after extraction of excess ^{99m}Tc with 2,2'-bipyridyl makes this a likely candidate for future *in vivo* imaging and biodistribution studies.

We are confident in the high-yielding overall synthesis of **3-11**, as well as its high reactivity toward thiols. These factors, combined with the syntheses of **5-1**, **5-3** and partial synthesis of **5-5**, provide the foundation for a series of commercially viable, dendrimer-based radioimaging agents.

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