Developing Novel Polymer Architectures For Applications In Magnetic Resonance Imaging And Self-assembly

by

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ABSTRACT

Macromolecular scaffolds for drug delivery, self-assembly, and imaging applications have attracted significant attention over the last several decades. As polymerization techniques become more sophisticated, it becomes possible to create polymeric architectures with increasing control over structure, molecular weight, and mass dispersity. Herein, ring-opening metathesis polymerization (ROMP) is paired with highly efficient synthetic methods to create functional bottle-brush polymers for MRI imaging and self-assembly applications. In this "graft-through" approach, bivalent macromonomers bearing a terminal *exo*-norbornene group were synthesized and polymerized to yield bottle-brush polymers with controlled molar masses and low dispersities. This approach is first utilized in the development of organic radical contrast agents (ORCAs) for magnetic resonance imaging (MRI). These ORCAs are composed of macromonomers bearing long polyethylene glycol chains and sterically hindered bis(spirocyclohexyl) nitroxide free radicals. This approach enables facile tuning of nitroxide loading percentages and molecular size. Bottle-brush ORCAs displayed high r_1 and r_2 relaxivities suggesting that they have potential for further *in vivo* MRI studies. Next, bottle-brush copolymers composed of multiple polymeric domains are synthesized by ROMP. We propose that these multi-block bottle-brush polymers will self-assemble into interesting solution and bulk architectures. Several approaches to synthesizing tetrablock bottle-brush polymers were explored by combining "graft-through" and "graft-from" polymerization methods.

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Chapter I: Development of a Tunable, All-Organic MRI Contrast Agent

Introduction

Magnetic resonance imaging (MRI) is a powerful, noninvasive tool for studying biological systems *in vivo* without the use of ionizing radiation. Much like nuclear magnetic resonance (NMR) spectroscopy, MRI employs low-intensity radiofrequency electromagnetic pulses within a strong magnetic field. An MRI image gains its contrast from the regional variations in the relaxation of water molecules in body tissues. These variations are mainly brought about by differences in the intrinsic relaxation parameters, T_1 and T_2, of different tissues. The sensitivity of this technology is therefore limited by the ability to detect subtle relaxivity differences. To address this limitation, molecular probes known as contrast agents (CAs) are injected intravenously in order to alter the T_1 and T_2 of imaged tissues. These probes exaggerate the visual contrast between tissues with different water concentrations.[1] The efficiency of contrast agents is quantified by the relaxivity parameters r_1 and r_2, which measure the enhancement in T_1 and T_2, respectively, per millimole of agent.

The relaxivity parameters are affected by several factors such as hydration environment, tumbling motions of the contrast agent, and the number of unpaired electrons of the CA.[1] Common contrast agents in clinical use include chelated paramagnetic lanthanide ions such as gadolinium 3^+ (Gd(III)) and paramagnetic manganese 2^+ (Mn(II)). The many unpaired electrons of these CAs (7 and 5, respectively) lead to high r_1 values making them especially well suited for T_1-weighted imaging.

Unfortunately, T_1-weighted CAs display decreased relaxivity with increasing

magnetic field strength.[2] In contrast, T_2-weighted CAs display a direct relationship between field strength and relaxivity. As clinical MRI instruments move toward higher field strengths, T_2 agents could become the preferred CAs.[3] Currently, superparamagnetic iron oxide nanoparticles (SPIONs) are the primary commercially available CAs for T_2 weighted imaging.[4] SPIONs have excellent r_2 values and their magnetic properties are more easily tuned than Gd(III) CAs by manipulating the core size and coating composition. SPIONs, however, may pose long term health risks.[5]

Due to concerns about the safety of Gd(III) for patients with low renal function, there has been increased interest in developing CAs with higher relaxivity values so as to decrease the high doses of metal required for medical diagnostics. This effort has led to the investigation of macromolecular chelating agents including biomolecules, dendrimers, and nanoparticles.[3, 6] Overall, adding synthetic polymers such as poly(ethylene glycol) (PEG) to ligand scaffolds increases the biocompatibility and solvation of metal chelates and enhances r_1 and r_2 values significantly.[4] In addition, this macromolecular approach has opened the door to structures that tether several paramagnetic centers together leading to an additive increase in relaxivity.[7]

To further address the issue of metal toxicity, organic radical contrast agents (ORCAs) have emerged as a possible alternative to metal-based compounds. Specifically, nitroxide radicals are an attractive option. Simple nitroxide small molecules display r_1 values around 0.14 mM^{-1} s^{-1}, which is enough to give noticeable contrast enhancement.[7] For practical purposes, this value should be maximized. Furthermore, most small molecule nitroxides are poorly soluble in water, and rapidly reduced *in vivo* to spin-inactive

hydroxylamines.

A recent report by Professor Rajca et. al. at the University of Nebraska outlines the synthesis of multi-generation polyamidoamine (PAMAM) dendrimers decorated with reduction resistant bis-spirocyclohexyl nitroxide radicals and short solubilizing PEG chains.[4] Conjugation of the nitroxides to this macromolecular scaffold increases their water solubility and inhibits their reduction *in vivo*. Furthermore, linking several radicals into one molecule maximizes the molecular relaxivity, which can minimize the lowest effective dose required for successful imaging.

Though they have shown potential, ORCAs based on this dendrimeric scaffold face limitations. For example, the PEG chains and radical centers must compete for reactive sites during synthesis, which leads to an intrinsic loss of potential spin concentration. Furthermore, dendrimer synthesis is notoriously tedious and large generations are subject to steric crowding on the surface, which further reduces the degree of functionalization.[8] In addition, in Rajca's study, only the fourth generation dendrimers displayed r_1 values comparable to commercial Gd agents. This fact limits the utility of these ORCAs to one structure of a single size and functionality. Since *in vivo* nanoparticle distribution is tied to molecular size and is likely patient specific, it would be ideal to identify a class of molecular agents that display similar relaxivity values regardless of overall size.

Previous work from our lab has shown that TEMPO nitroxide radicals conjugated to macromonomers within bottle-brush polymers were resistant to reduction by reducing agents while still maintaining a hydrated environment.[5] These observations were attributed to the shielding of the radical center by attached PEG chains. In collaboration

with Professor Racja, we proposed translating our nitroxide-labeled bivalent-bottle-brush scaffold to MRI applications in order to provide increased versatility compared to dendrimers and answer key questions about the relationship of particle size to contrast enhancement, bioavailability, and excretion.

Results and Discussion

Bis-spirocyclohexyl nitroxide *N*-hydroxysuccinimidyl (NHS) ester **1** was provided by the Rajca group. Compound **1** was first coupled to two distinct azide linkers: a hydrophilic tetraethylene glycol chain (**2**) and a hydrophobic alkyl chain (**3**), which provided nitroxide-azides **4** and **5** respectively. The linkers **2** and **3** were prepared according to known literature procedures.[9, 10] These linkers were chosen to study the effect of local hydrophilicity on relaxivity.

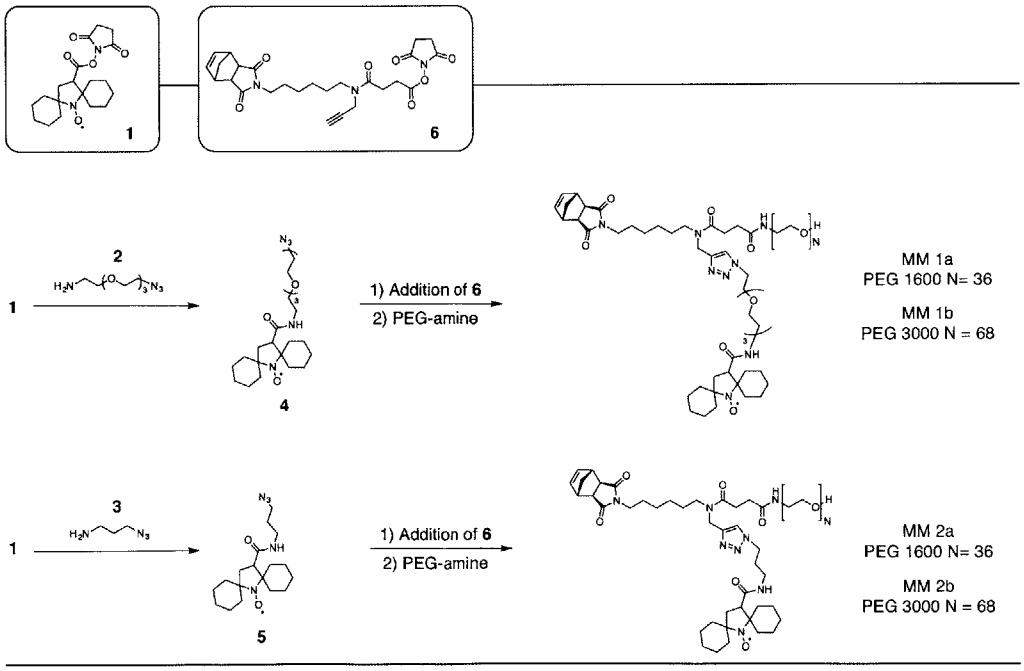


Figure 1. Synthesis of PEG-*branch*-nitroxide macromonomers (MMs).

PEG-*branch*-nitroxide macromonomers (MMs) were prepared using a streamlined one-pot procedure that improved upon a procedure previously reported in our group **(Figure 1).**[11, 12] First, nitroxide azides **4** and **5** were coupled to norbornene derivative **6** via copper-catalyzed azide-alkyne cycloaddition (CuAAC) click chemistry. These reactions were performed in dichloromethane using copper(I) acetate as the catalyst. Under these conditions, rapid coupling was achieved without the need for exogenous reducing agent, the latter of which could lead to reductive degradation of the nitroxide. After complete CuAAC conversion (as monitored by liquid chromatography/mass spectrometry, LC/MS) PEG monoamine (PEG-NH_2) was added directly to the reaction mixture. An excess of azide and NHS ester **6** was used to avoid unreacted amino-PEG impurities that could interfere with the ruthenium catalyst for ROMP. The entire reaction mixtures were subjected to high-performance liquid chromatography (HPLC) to provide pure MMs. The MMs and azide intermediates were characterized by NMR, mass spectrometry, and LC/MS.

Nitroxide-loaded bottle-brush polymers of various sizes were prepared by direct polymerization of the MMs above using Grubb's third generation catalyst. Samples of the crude reaction mixtures were subjected to gel permeation chromatography (GPC) analysis. The remaining reaction mixtures were dialyzed against deionized water overnight to remove residual MM and catalyst. The polymer solutions were then lyophilized to dryness. Relaxivity values for these purified materials are reported in Figure 2. The samples were also characterized by dynamic light scattering (DLS). The spin concentrations of these bottle-brush polymers were characterized by electron paramagnetic resonance (EPR) spectroscopy. The uniformly high spin concentrations (>90%) show that the nitroxide molecules within the polymers are stable towards ROMP conditions and dialysis.

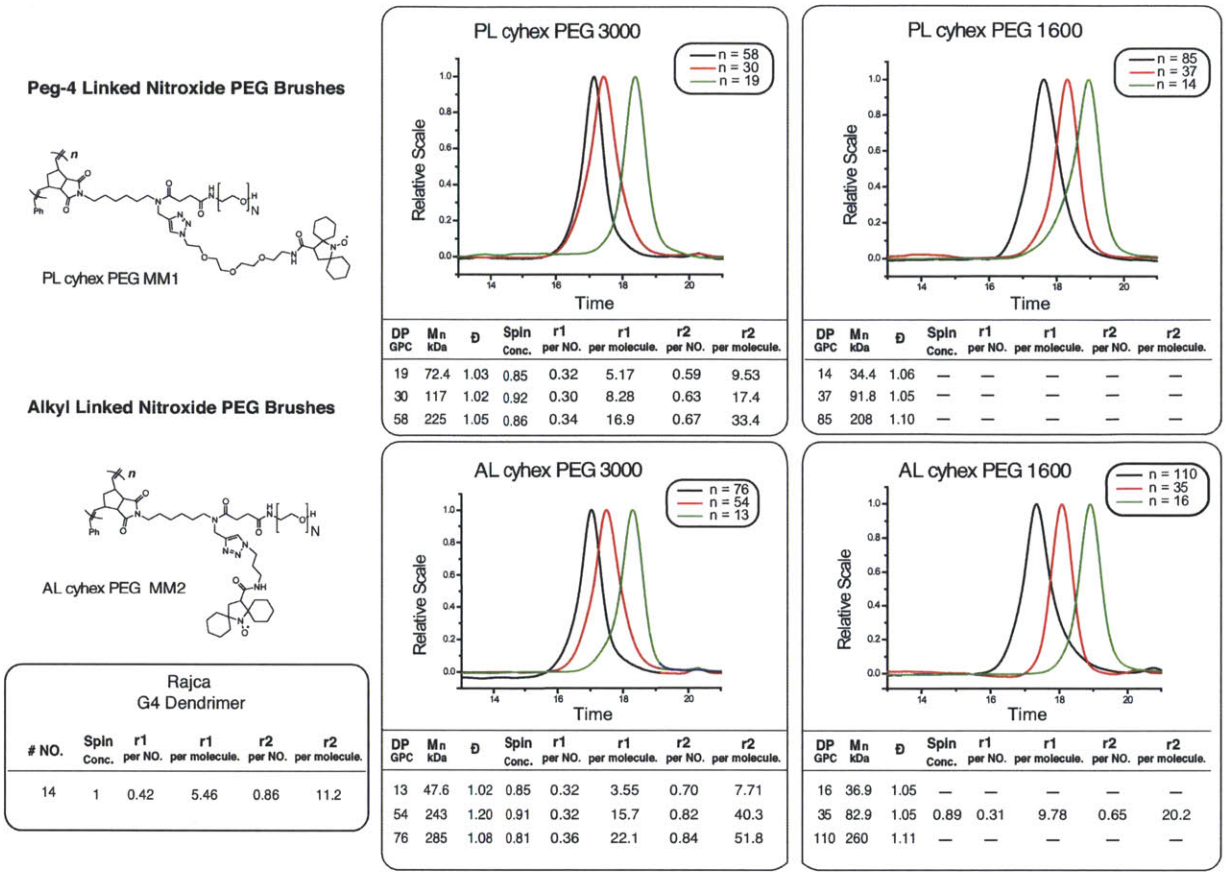


Figure 2. Gel permeation chromatography (GPC) and relaxivity data for bottle-brush polymers synthesized from nitroxide macromonomers. Tables show the degree of polymerization (DP), the number average molar mass (M_n), the dispersity index ($Đ$), the spin concentration of the brushes, and the r_1 and r_2 values per nitroxide and per entire molecule.

Rajca and coworkers measured the relaxivity values for these materials using a 7 T magnet at the University of Nebraska. Surprisingly, the alkyl-linked nitroxide bottle-brush polymers showed the highest relaxivity in both r_1 and r_2, with per nitroxide values comparable to Rajca's fourth generation dendrimers. Due to the larger number of nitroxides per particle, however, these values lead to a larger overall molecular relaxivity when compared to the dendrimers. These high molecular relaxivity values are encouraging, and make these ORCAs promising new CAs for MRI applications.

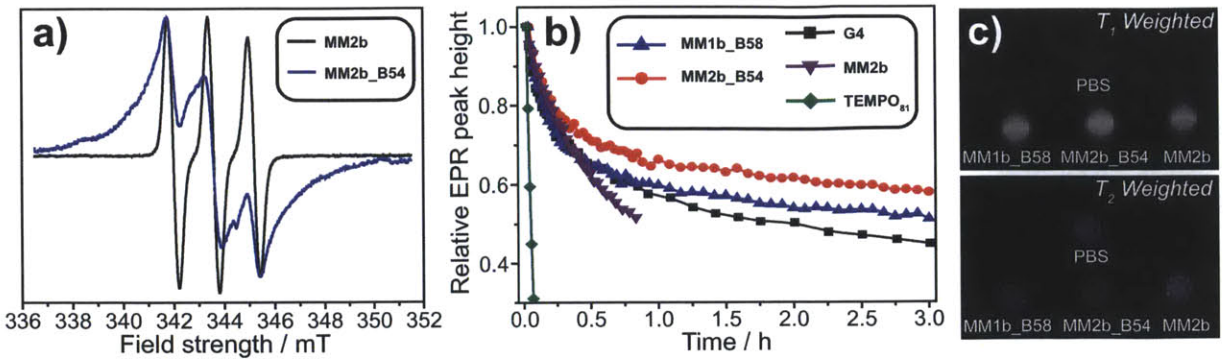


Figure 3. a) EPR spectra for MM2b and a bottle-brush polymers prepared from MM2b b) Ascorbate-Nitroxide quenching kinetics, c) MRI phantoms.

Figure 3 shows that the EPR spectrum for a bottle-brush polymer with degree of polymerization (DP) of 54 (**MM2b_B54**) is much broader than for the alkyl-linked macromonomer (**MM2b**). This broadening is due to increased reduced nitroxide rotational freedom and increased spin-spin interactions within the particle core. Figure 3b shows the kinetics of quenching by ascorbate. Both the alkyl-linked (**MM2b_B54**) and PEG-linked (**MM1b_B58**) bottle-brush polymers showed increased resistance to quenching when compared to Rajca's G4 dendrimer. The macromonomer and a small molecule nitroxide are rapidly quenched. Figure 3c shows MRI phantom images for 4 mM solutions of **MM2b_B54**, **MM1b_B58**, and **MM2b** in PBS buffer. The T_1 weighted images show enhanced positive contrast for the ORCA solutions versus the PBS control. The T_2 weighted images show a corresponding negative contrast.

Conclusion

Organic radical contrast agents (ORCAs) were synthesized via ring-opening metathesis polymerization (ROMP) using bi-functional *exo*-norbornene terminated macromonomers. This approach allowed for the facile synthesis of a library of bottle-brush

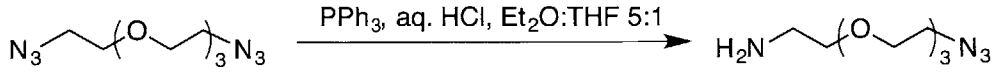
polymers with controlled molecular weights and low dispersities. The ORCAs presented in this chapter achieved enhanced r_1 and r_2 relaxivities that were demonstrated using MRI phantoms. These preliminary results strongly suggest that these ORCAs are suitable candidates for further *in vivo* studies.

Experimental Methods

All reagents and solvents were purchased from Aldrich or VWR and used as supplied unless otherwise noted. Dichloromethane (DCM) and tetrahydrofuran (THF) were degassed and purified by passing through solvent purification columns prior to use.[13] Gel permeation chromatography (GPC) was performed using two Shodex KD-806M GPC columns along with a DAWN EOS 18 angle laser light scattering (MALLS) detector and a T-rEX refractive index detector (Wyatt Technology). Experiments were performed at 60 °C at a flow rate of 1 mL / min using 0.025 M LiBr in *N,N*-dimethylformamide (DMF). Molar masses were calculated using dn/dc values that were obtained assuming 100% mass recovery from the columns. Dynamic light scattering (DLS) measurements were performed at room temperature using a Wyatt DynaPro Titan with a temperature controlled microsampler. Samples were dissolved in millipore water at a concentration of ~1 mg / mL. A fresh, clean, quartz cuvette was used to remove dust. The sample solution was passed through a 0.4 μm Teflon syringe filter directly into the cuvette; the cuvette was capped and placed in the DLS instrument for particle sizing. At least 3 measurements were made per sample and average hydrodynamic diameters were calculated by fitting the DLS correlation function using Dynamics 6.9.2.11 software. Nuclear magnetic resonance (NMR) experiments were performed on either a VARIAN Mercury 300 MHz spectrometer or a

VARIAN Inova-500 MHz spectrometer. NMR spectra were analyzed using MestreNova NMR 8.1.2-11880 software. Analytical high-performance liquid chromatography mass spectrometry (HPLC-MS or LC-MS) data were obtained using an Agilent 1260 series HPLC system equipped with a variable wavelength ultraviolet-visible (UV-Vis) detector and an Agilent 6130 single quadrupole mass spectrometer. This system was used along with an Agilent Zorbax SB-C18 rapid resolution HT column (LC/MS) with mobile phase gradients using 0.1% acetic acid in water and acetonitrile. Experiments were performed at room temperature with a flow rate of 1.0 mL / min. High Pressure Reverse Phase Liquid Chromatography (HPLC) was performed using an Agilent Zorbax SB-C18 column with Beckman-Coulter System Gold preparative HPLC system using a gradient HPLC grade acetonitrile (MeCN) in millipore purified water containing 0.1% Acetic Acid at a flow rate of 20 mL / min. High-resolution mass spectrometry (HRMS) data were obtained on a Bruker Daltonics APEXIV 4.7 Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR-MS).

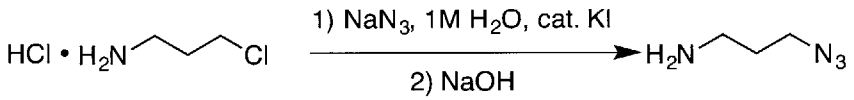
Synthetic Procedures:



2

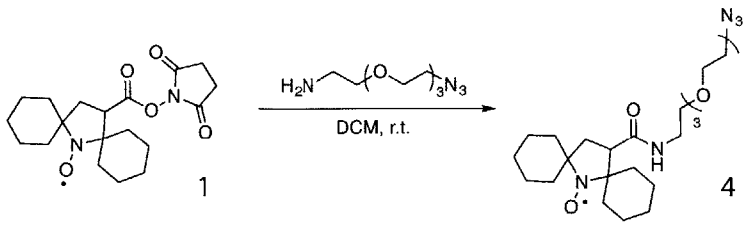
1-amino-11-azido-3,6,9-trioxaundecane ***(2)***: This molecule was prepared according to a modified literature procedure.[9, 10] Spectral data matched that of the literature. 1,11-diazido-3,6,9-trioxaundecane (5.5 g, 22.5 mmol) was dissolved in 30 mL 5:1 Et_2O:THF in a 250mL round-bottomed flask. 100 mL of a freshly prepared 1M solution of HCl was added. A solution of PPh_3 (5.9 g, 22.5 mmol) dissolved in diethyl ether (75 mL) was added

dropwise over an hour while stirring vigorously. After 2 hours, the organic layer was decanted and extracted with aqueous HCl. The aqueous layers were combined and a 50% NaOH solution was used to bring the pH to 14. This solution was extracted 3 times with diethyl ether. The organic portions were collected and dried over Na_2SO_4, filtered, and concentrated. This compound was further purified by column chromatography using a gradient of 1-5% MeOH in DCM to afford compound 2 as a clear, slightly viscous oil (2.2 g, 40% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.70 – 3.59 (m, 10H), δ 3.50 (t, *J* = 5.3 Hz, 2H), δ 3.38 (t, *J* = 5.0 Hz, 2H), δ 2.86 (t, *J* = 5.2 Hz, 2H), δ 1.39 (s, 2H)

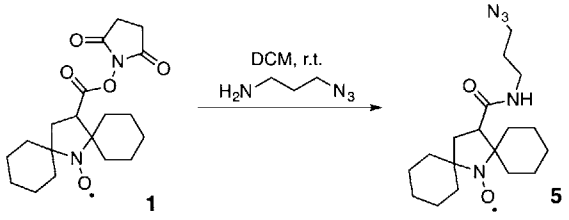


3

*1-azido-3-propylamine **(3)**:* Sodium azide (12.5 g, 192 mmol) was added to a solution of 3-chloropropylamine HCl (5 g, 38.5 mmol) in of deionized water (40 mL). A pinch of potassium iodide was added and the reaction was stirred for 72 hours at 90 °C under reflux conditions. NaOH pellets were added until pH 11 was reached. Sodium chloride was added until the solution was saturated and then the mixture was extracted several times with toluene. The organic layers were dried with $MgSO_4$ and filtered. The solution in toluene was *carefully* (no heating; the product is somewhat volatile) concentrated to 2 M as confirmed by ^{1}H NMR. ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.46 (t, *J* = 6.7 Hz, 2H), δ 2.90 (t, *J* = 6.9 Hz, 2H), δ 1.83 (m, *J* = 6.7 Hz, 2H), δ 1.14 (s, 2H).



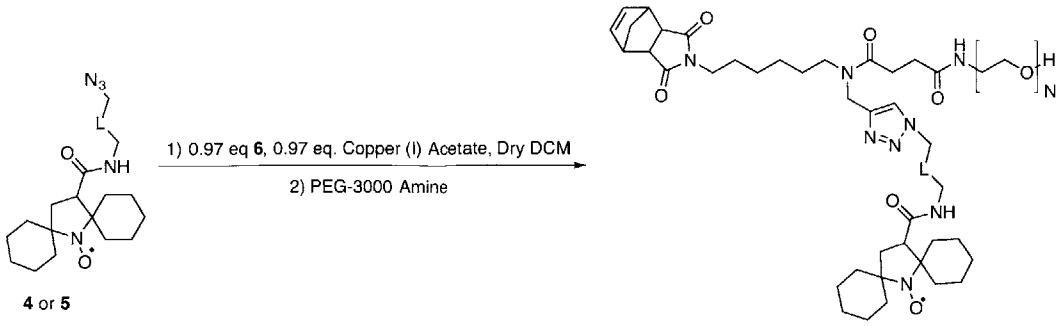
Bis-spirocyclohexylnitroxide-PEG(4)-Azide ***(4):*** Dry DCM (1 mL) was added to a vial containing compound **1** (40mg, 0.11 mmol), and 1-amino-11-azido-3,6,9-trioxaundecane (48 mg, 0.22 mmol). The solution was stirred for 12 hours before the reaction mixture was pipetted onto a silica column and purified via flash chromatography using a gradient of 1%-3% MeOH in DCM. Product-containing fractions were determined by LCMS, combined, dried over $MgSO_4$, and condensed on a rotary evaporator. The yellow residue was dried under vacuum to provide pure **4** (42.2 mg, 83% yield). The product identity and purity were determined by LC/MS. The NMR spectrum is complicated due to the paramagnetic nature of **4** (See Appendix).



Bis-spirocyclohexylnitroxide-Propyl-Azide ***(5):*** Dry DCM (1 mL) was added to a vial containing compound **1** (40mg, 0.11 mmol). A 2 M solution of 1-amino-3-propylazide in toluene was then added (0.87 mL 0.17 mmol). The solution was stirred for 12 hours, then the reaction mixture was transferred to a Biotage column and purified via flash chromatography using a gradient of 1%-3% MeOH in DCM. Product-containing fractions were determined by LC/MS, combined, dried over $MgSO_4$, and condensed on a rotary evaporator. The yellow residue was dried under vacuum to give pure **5** (32.4 mg, 82% yield). The product identity and purity were determined by LC/MS. The NMR spectrum is complicated due to the paramagnetic nature of **5** (See Appendix).

6

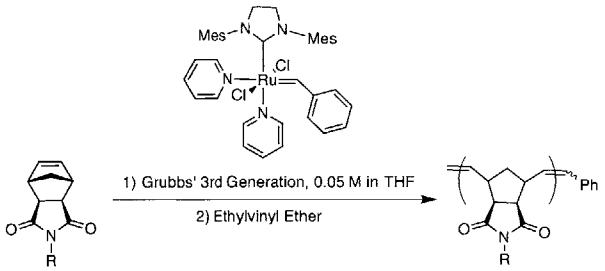
Norbornene-alkyne-NHS ***(6)****:* This compound was prepared according to a published literature procedure with slight modifications.[14] Anhydrous DCM was added to a vial containing norbornene-alkyne-COOH (3.8g, 9.40 mmol) and a stir bar. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (2.2g, 14.1 mmol) was added followed by 4-dimethylaminopyridine (DMAP, 115 mg, 0.943 mmol). *N*-hydroxy succinimide (1.6 g, 14.1 mmol) was then added. When the reaction was deemed complete by LCMS, the reaction mixture was concentrated and purified by column chromatography in a gradient of 50-100% ethyl acetate in hexanes. Compound **6** was isolated as a sticky glass (3.3 g, 71% yield). ^{1}H NMR (500 MHz, Chloroform-*d*) δ 6.23 (s, 2H), δ 4.17 (d, *J* = 2.3 Hz, 1H), δ 4.07 (dd, *J* = 7.0, 8.6 Hz, 1H), δ 3.98 (broad m, 1H), δ 3.38 (q, *J* = 7.6 Hz, 4H), δ 3.21 (s, 2H), δ 2.95 (t, *J* = 6.9 Hz 2H), δ 2.79 (m, 5H), δ 2.68 (t, *J* = 6.9 Hz, 1.49H), δ 2.62 (m, 2H), δ 2.29 (t, *J* = 2.4 Hz 0.4H), δ 2.18 (t, *J* = 2.4 Hz 0.6H), δ 1.99 (m, 1H), δ 1.65-1.39 (m, 6H), δ 1.38-1.08 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.0, 169.6, 169.3, 169.0, 168.4, 137.8, 78.94, 78.41, 72.85, 71.82, 60.33, 47.74, 46.87, 46.64, 42.69, 38.50, 38.34, 37.36, 34.42, 28.05, 27.95, 27.76, 27.26, 26.59, 26.51, 26.23, 26.16, 25.56, 21.02, 14.17.



4 or **5**

General Procedure for Synthesis of Nitroxide Macromonomers:

A vial containing Cu(I)acetate was evacuated and backfilled with nitrogen. The vial was then charged with compound **6** and either compound **4** or **5** in a solution of anhydrous DCM (1 mL). The reaction immediately turned light green. The reaction was monitored by LC/MS. After 5 minutes, the reaction was deemed complete. PEG amine was then added and the reaction was allowed to stir for 2 hours. After the reaction was deemed complete by LCMS, the reaction was filtered through a 0.4 μm Teflon syringe, concentrated, dissolved in methanol, and purified by preparative HPLC (gradient of 95:5 0.1% AcOH-H_2O: MeCN to 5:95 0.1% AcOH-H_2O: MeCN over 9 minutes). Fractions containing pure macromonomer were combined and condensed with a rotary evaporator. The resulting residue was re-dissolved in DCM, dried over Na_2SO_4, re-condensed, and dried overnight under vacuum. The macromonomers were characterized by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry, GPC, and NMR.



General Procedure for Synthesis, Purification, and Characterization of Bottle-brush Polymer ORCAs: Macromonomers were weighed into 0.5 dram vials with stir bars and taken into the glove box. THF was added and the mixtures were stirred until all of the MM dissolved. The stirring solution was then charged with the appropriate equivalents of a 0.002 g/mL solution of Grubb's third generation initiator in THF (catalyst equivalents chosen to

provide the desired bottle-brush polymer degree of polymerization). The polymerization was allowed to stir for 60-90 minutes. A drop of ethyl vinyl ether was added to quench the polymerization and the molecular weight was obtained by GPC as presented in Figure 2. The remaining THF solution containing the crude bottle-brush polymer was diluted with deionized water and transferred to a dialysis bag that was clipped on both sides and allowed to gently agitate in 500 mL of water overnight with several exchanges of the external water solution. The dialysis bag was then removed from the water and the contents were decanted into a 20 mL scintillation vial. This solution was frozen in liquid nitrogen and lyophilized to dryness over a period of three days.

CW X-band EPR spectra for spin quantitation of nitroxide radicals and kinetics of reduction were acquired on a Bruker EMX instrument at the University of Nebraska equipped with a frequency counter and nitrogen flow temperature control (120–300 K). The spectra were obtained using a Bruker TE102 cavity. DPPH powder (g = 2.0037) was used as a g-value reference. The spin concentration of low molecular weight nitroxide radicals and of nitroxide radicals in the bottle-brush ORCAs were measured in dichloromethane and PBS buffer at room temperature (295 K), respectively; 4-oxo- TEMPO and/or 3-carboxy-PROXYL in respective solvents were used as intensity references. For low molecular weight nitroxide radicals, typical parameters were: modulation amplitude, 0.2 G; sweep width, 100 G.

Chapter II: Synthesis of Tetrablock Bottle-Brush Copolymers

Introduction

The self-assembly of polymers is a fascinating field that is driven by the tendency of unlike macromolecules to spontaneously segregate into discreet domains.[15] The self-assembly of linear block copolymers (BCPs) has been extensively studied, leading to new applications in nanolithography, photonics, and semi-conductors.[16] Many opportunities exist to push the boundaries of multi-block copolymer synthesis in order to obtain self assembled architectures of increasing complexity.[16b] Bottle-brush polymers are especially promising in this field due to their intrinsically reduced chain entanglement when compared with that of high molecular weight linear BCPs.[17] The extreme steric crowding of side chains forces the polymer backbone to adopt an elongated conformation, enhancing the rate of self-assembly and allowing for the formation of larger domain sizes which are necessary for photonic applications.[16b]

Grubbs and coworkers have recently demonstrated the synthesis of bottle-brush block and random copolymers via sequential and simultaneous, respectively, ROMP of norbornene-terminated MMs.[18] The diblock bottle-brush polymers prepared via sequential addition of two MMs were shown to self assemble into one dimensional lamella with large phase domains (>100nm). These self-assembled structures showed photonic crystal properties due to the alternating refractive indices of the periodic domains. These photonic crystals were able to reflect light at a variety of wavelengths, including those in the near infrared region. The latter is a feat that was unobtainable using linear BCP systems.

Inspired by these one-dimensional assemblies, we envisioned the synthesis of higher-order tri- and tetra-block bottlebrush copolymers that could self assemble into multi-dimensional architectures.

Results and Discussion

Starting with our bivalent *exo*-norbornene scaffold, several branched macromonomers were designed that feature two attached polymer chains or a polymer chain and an initiator for polymer growth. We reasoned that two of these branched macromonomers could then be sequentially polymerized by ROMP to yield diblock of diblocks, i.e., tetrablock, bottle-brush copolymers. Alternatively, bottle-brush polymers synthesized from only one monomer could be end functionalized with either an alkyne or an azide. Then, two different diblock copolymers bearing these functionalities could be covalently linked by a CuAAC reaction.

To achieve symmetric assemblies, the four polymer domains must be of equivalent volume fractions. This requires tailoring of individual polymer lengths according to their densities. Polystyrene (PS), for example, is much denser than polyethylene glycol (PEG), requiring a higher degree of polymerization.[19] The four polymers must also possess appropriate Flory-Huggins interaction parameters (χ) to assure phase segregation.[15] This parameter is complex, however, and depends on various factors such as temperature, concentration, and solvent. In future studies, the versatility of the macromonomer syntheses described below will enable facile substitution of the four different polymer blocks to achieve the best segregation.

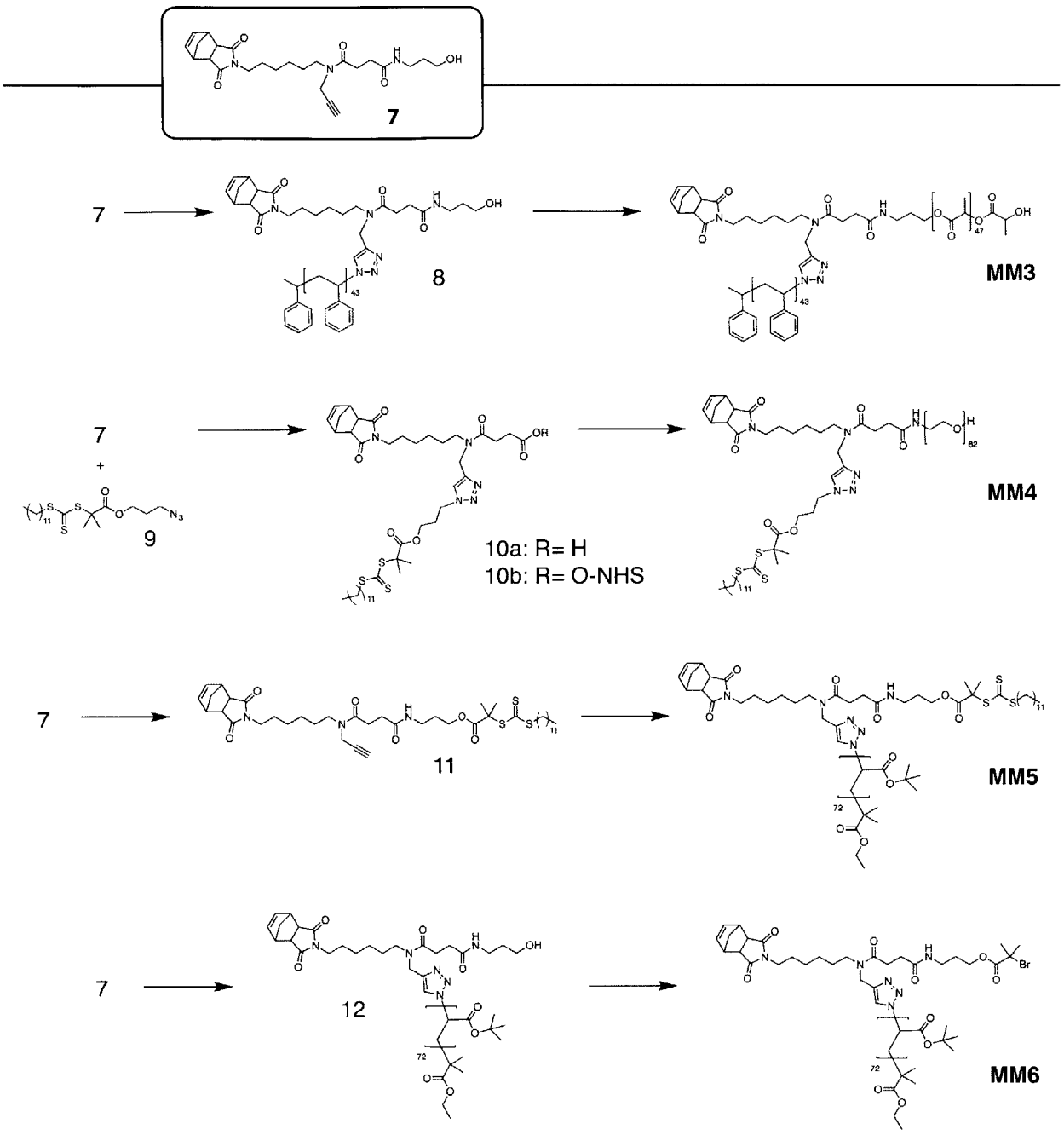


Figure 4. Synthesis of macromonomers 3 through 6 using an alkyne and alcohol functional bivalent *exo*-norbornene scaffold (7). (See experimental section for synthetic details.)

MM3 features polystyrene (PS) and poly(lactic acid) (PLA) chains. An azide functional polystyrene chain was "clicked" to the bivalent scaffold **7** via CuAAC, and the terminal alcohol group was used to grow PLA from lactide monomer using tin(II)-catalyzed

ring-opening polymerization. MM4 and MM5 were designed to feature one long polymer chain (PEG or PTBA, respectively) tethered to a trithiocarbonate chain-transfer agent (CTA) for post-ROMP growth of various acrylates or acrylamides using reversible addition-fragmentation chain-transfer polymerization (RAFT). We chose *N*-isopropyl acrylamide (NiPAAm) as the target monomer due to its ability to undergo a lower critical solution temperature phase transition. When heated above 32 °C in water, NiPAAm polymers transition from a hydrated state to a collapsed dehydrated state.[20]

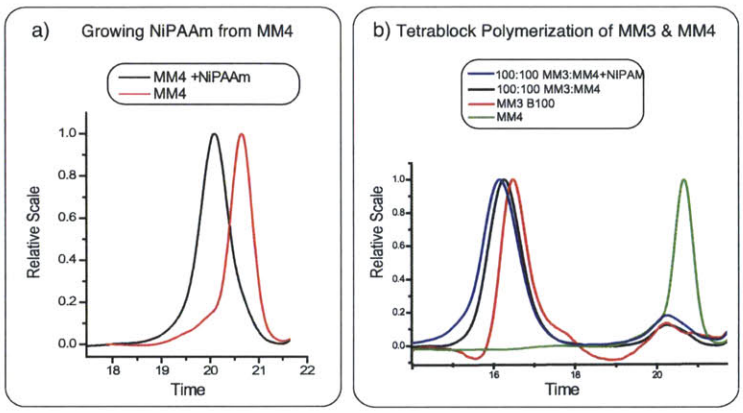


Figure 5. a) NiPAAm photo-polymerization study from MM4. b) Sequential polymerization of MM3 followed by MM4 into a bottle-brush polymer. This bottle-brush polymer was then subjected to photo-polymerization of NiPAAm from chain transfer agents (CTA) within the brush.

The original design of MM4 paired the CTA with a 3000 Da PEG chain. First, **9** was synthesized from a commercially available CTA ester.[21] This product was then "clicked" onto the alkyne of our *exo*-norbornene macromonomer **1**. The carboxylic acid of **10** was activated for nucleophilic attack via transformation into a NHS ester. This NHS ester was stirred with amine terminated PEG in DMF and precipitated in cold diethyl ether to afford MM4 as a white solid.

Preliminary diblock polymerizations were carried out by sequentially polymerizing

MM3 and MM4 (Figure 5b). Growth of NiPAAm from the brush polymers was studied using MM3 in conjunction with photo-initiated RAFT polymerization techniques recently reported by Dr. Huaxing Zhou of our group (Figure 5a).[22] When compared to radical initiators such as AIBN, photo-initiation is desirable for the purpose of this synthesis. Radical initiators inherently result in early termination of some chains leading to incomplete functionalization of brushes, possibly impairing self-assembly. Figure 5 shows an initial polymerization study of RAFT growth of NiPAAm from brushes. To obtain a NiPAAm chain of ~4000 Da, 250 equivalents of monomer per CTA was used. Growth attempts from the diblock bottle-brush gave modest results (Figure 5b).

To match the volume fraction of the PEG to that of potentially longer PS domains, we attempted to increase the MW of the PEG chain to 3000 to 5000 Da. Following the procedure above, a PEG 5000 analogue of MM4 was synthesized. The larger PEG chain, however, did not ROMP well. Thus, poly(*tert*-butyl acrylate) (PTBA) was substituted for PEG in the synthesis of MM5. MM5 was synthesized from a commercially available CTA carboxylic acid and **7** by the way of EDC coupling followed by the attachment of azide functionalized-PTBA using "click" chemistry. The decreased polarity of PTBA when compared with PEG also allowed for facile purification by column chromatography.

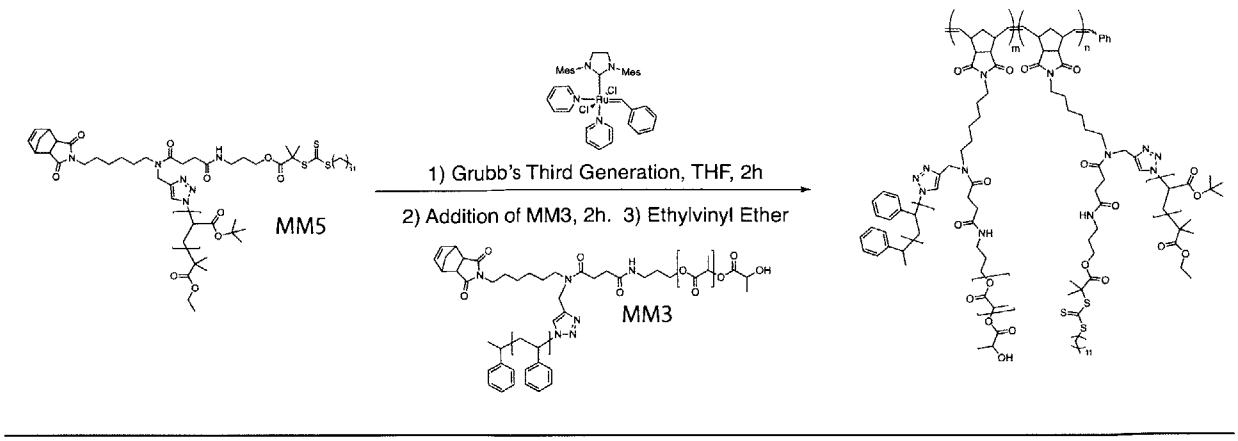


Figure 6. Sequential ROMP of MM5 and MM3 to create diblock bottle-brush polymers.

Diblock polymers were synthesized from MM3 and MM5 using ROMP (Figure 6, Figure 7b). Conversions were not as high as in studies carried out with MM3 and MM4. Figure 7b shows the growth of a 50:50 PTBA-CTA:PS-PLa brush with >90% conversion of MM3 followed by >80% conversion of MM3. While the photo-polymerization of NiPAAm was successful when conducted with MM5 (Figure 7a), attempts to polymerize NiPAAm from diblock polymers or even from homo-bottle-brushes composed of MM5 were met with frustrations (Figure7c). Thermal polymerizations of NIPAAM using AIBN as an initiator were also fruitless and resulted in zero growth as observed by GPC. These poor results may be due to incompatibilities of RAFT polymerization in sterically crowded environments such as that of the CTA molecules buried in the bottle-brush core.

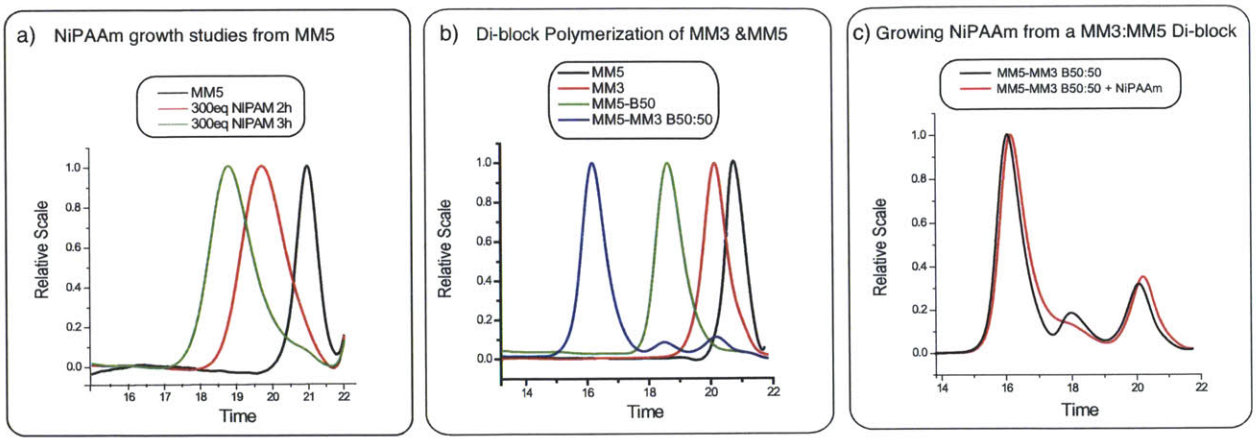


Figure 7. a) NiPAAM growth studies using MM5. b) Sequential ROMP of MM5 and MM3 to create a diblock with PTBA, PS, PLA, and CTA functionalities. Incomplete conversion is observed in the resulting bottle-brush. c) Attempts to polymerize NiPAAm from a diblock bottle-brush polymer composed of MM5 and MM3.

To address the issues of low conversion and difficult RAFT polymerizations, a new synthetic scheme was envisioned (Figure 8). First, since atom transfer radical polymerization (ATRP) has been more widely used in the "graft-from" synthesis of bottle-brushes, the RAFT CTA in MM5 was replaced with an ATRP initiator in MM6.[23] Second, instead of relying on diblock copolymerization, homopolymers can be synthesized and end-functionalized with an activated ester that can be displaced by functional amines.[24] Using this design, two homopolymers can be functionalized with either azide or alkyne groups that can then be "clicked" together to afford a larger tetrablock polymer. Another advantage is that "graft-from" ATRP can be carried out on a homopolymer synthesized from MM6 before clicking.

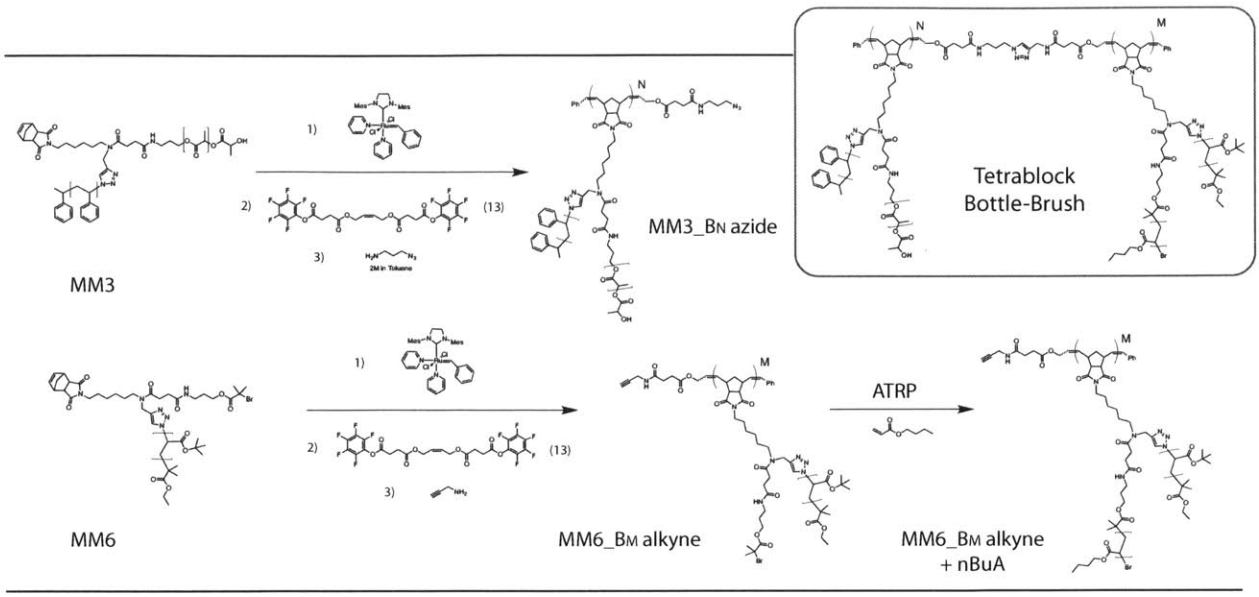


Figure 8. Synthesis of tetrablock copolymers via "click" chemistry. MM3 and MM6 were polymerized into bottle-brush polymers that are end functionalized with an alkyne and an azide. These "clickable" bottle-brush polymers were subjected to copper(I)bromide / PMDETA "click" conditions in DMF to afford tetrablock copolymers.

PS-*co*-PLA homopolymers synthesized from MM3 were functionalized with an azide group according to Figure 8 to create MM3_B97_N_3. PTBA-ATRPi homopolymers bearing pendant alkynes were synthesized from MM6 to create MM6_B179_alkyne. These two brushes were combined in a 1:1 ratio and subjected to "click" conditions using copper (I) bromide and PMDETA in DMF. Alkyne and azide resin was added after the reaction was complete to facilitate purification. The resulting diblock polymer eluted on the GPC as intermediate between the two brushes (Figure 9a). This exciting result, which suggested successful coupling of the large azide and alkyne terminated bottle-brush polymers, was corroborated by ^{1}H NMR.

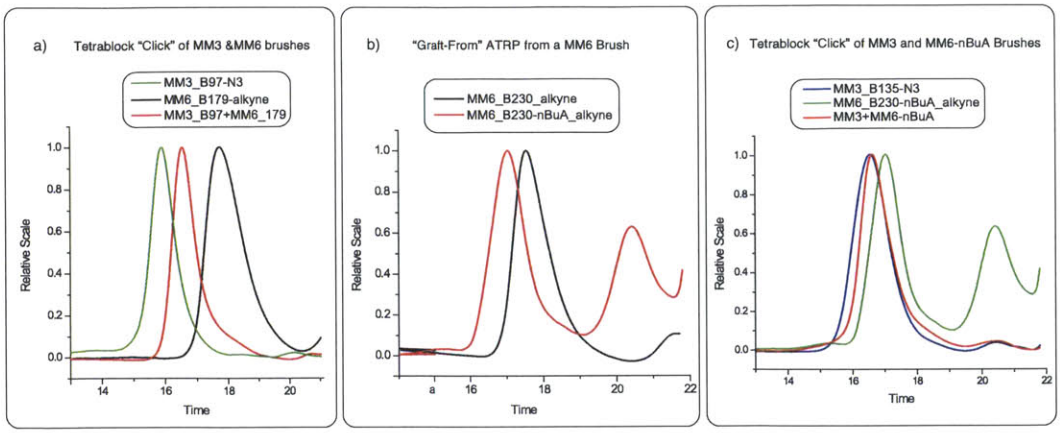


Figure 9. a) Preliminary tetrablock "click" experiment featuring the synthesis of a tetrablock polymer bearing PS, PLA, PTBA, and ATRPi functionalities. b) ATRP "graft-from" polymerization of n-Butyl Acrylate from a bottle-brush composed of MM6. c) Tetrablock "click" reaction of MM3_B135 azide and MM6_B230 alkyne after n-Butyl Acrylate growth.

To fill in the fourth polymer domain, a homopolymer of MM6, MM6_B230-alkyne was synthesized and *n*-butyl acrylate was polymerized from the brush. (Figure 9b). This polymer was then combined with MM3_B135-N_3 in a 1:1 ratio and subjected to the same "click" conditions. The resulting tetrablock polymer was analyzed by GPC and ^{1}H NMR (Figure 9c, Figure 10). The ^{1}H NMR spectra, however, shows a decreased PLA resonance suggesting that the PLA domain degraded at some point during the CuAAC coupling and/or purification.

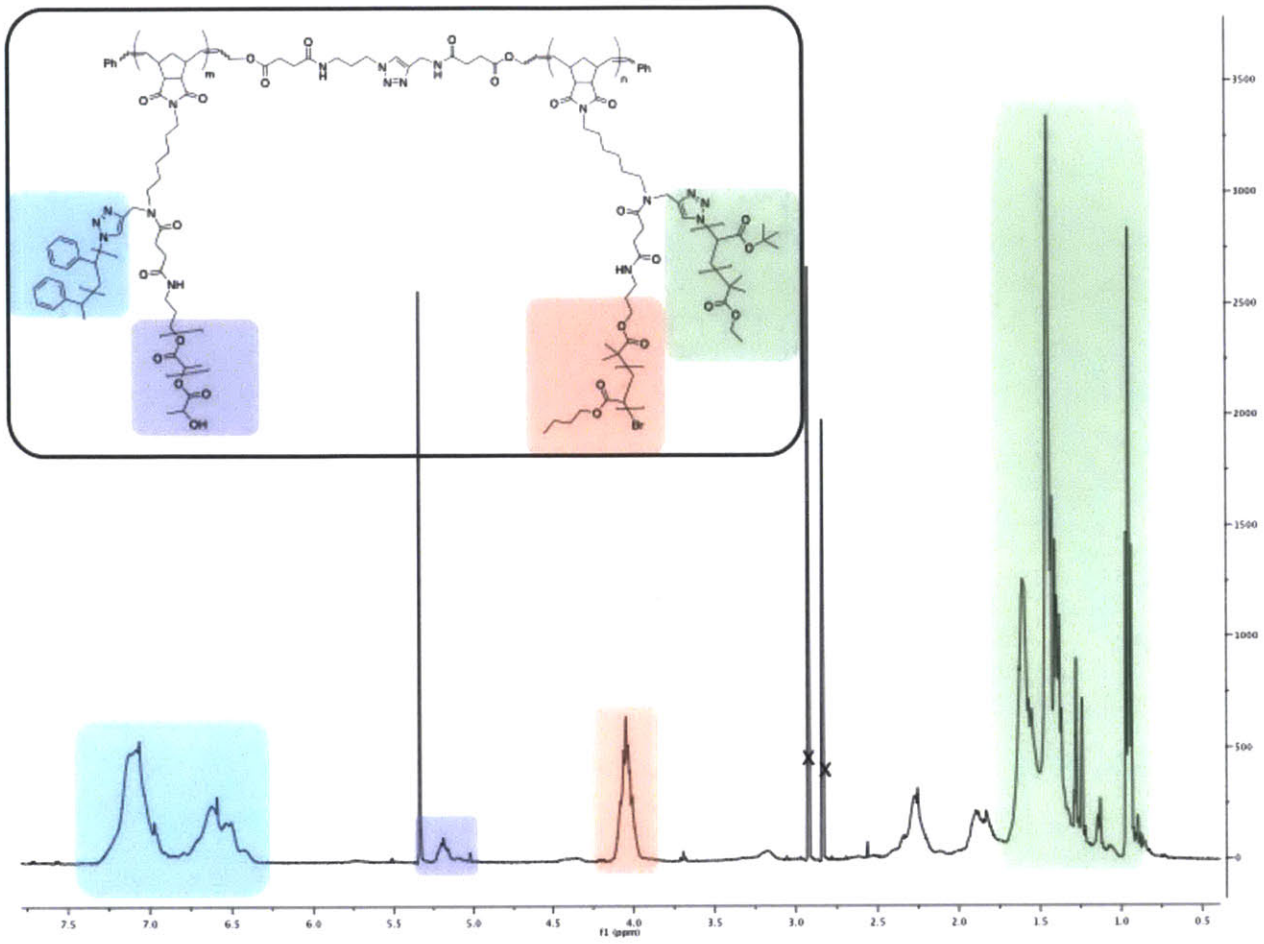


Figure 10. ^{1}H NMR spectra of tetrablock bottle-brush polymer showing peaks for all four polymer domains.

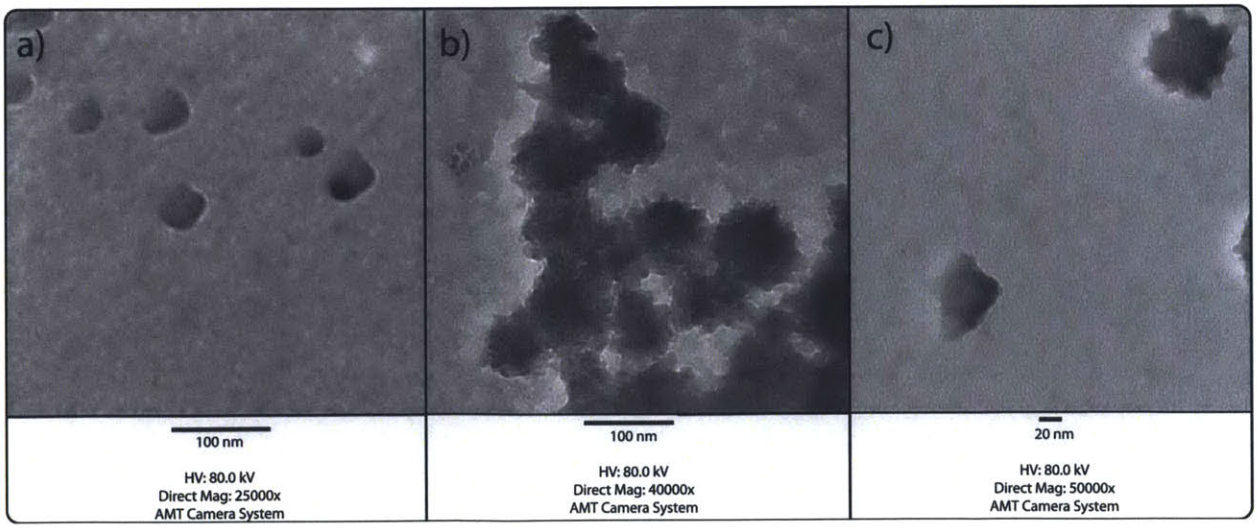


Figure 11. TEM images of tetrablock polymers at varying magnification.

TEM images in Figure 10 show spherical particles with diameters from 30-50 nm with areas of lighter and darker shades. These images may suggest that there is phase segregation in the tetrablock polymers though additional studies must be conducted to determine the extent of segregation.

Conclusion

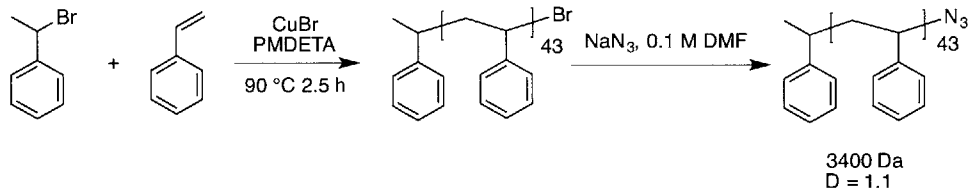
This chapter outlined the development of a synthetic scheme for the synthesis of tetrablock bottle-brush polymers containing 4 discrete polymer domains. This approach combined controlled polymerization techniques such as atom-transfer-radical polymerization (ATRP), reversible addition-fragmentation chain-transfer polymerization (RAFT), and ring-opening metathesis polymerization (ROMP) as well as several highly efficient synthetic techniques such as copper "click" chemistry and nucleophilic additions to activated esters.

Experimental Methods

All reagents and solvents were purchased from Sigma-Aldrich or VWR and used as supplied unless otherwise noted. Norbornene-Alkyne-COOH was prepared according to literature procedures.[14] 3-azidopropyl 2-(((dodecylthio)carbonothioyl)thio)-2-methylpropanoate **(9)** was prepared according to a reported literature procedure.[25] (Z)-O,O'-(but-2-ene-1,4-diyl) bis(perfluorophenyl) disuccinate **(13)** was prepared according to a reported literature procedure.[24] Degassed tetrahydrofuran (THF) was passed through solvent purification columns prior to use.[13] *N*-Butyl Acrylate monomer was purified by passing through a column of basic aluminum oxide. *Tert*-Butyl Acrylate and styrene monomers were purified by distillation *in vacuo.*

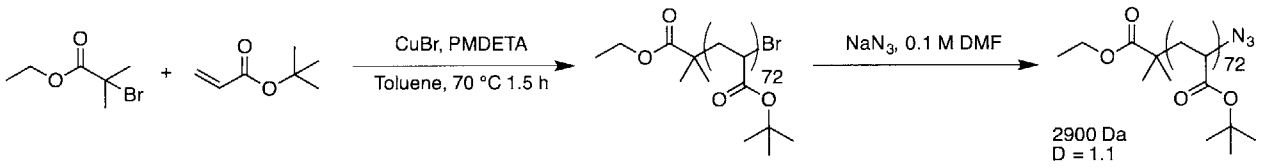
This chapter utilizes all the instrumentation from Chapter I with one addition: TEM images were obtained at the MIT Center for Materials Science and Engineering (CMSE) on a JEOL 200CX TEM instrument with a 1k x 1k CCD camera. Sample preparation was as follows: 5.0 μL of a 0.05 mg / mL solution of tetrablock polymer in DCM was deposited onto a carbon film-coated 200-mesh copper grid placed on a piece of parafilm. The sample was allowed to dry and then placed carbon-coated side up inside of a LC/MS vial. The LC/MS vial was placed inside a 20mL scintillation vial. About 0.30 mL of a 0.5% $RuO_{4(aq}$ solution was carefully added to the scintillation vial around the smaller vial. The scintillation vial was capped and allowed to stand for 30 min. The grid was removed and then analyzed for TEM imaging.

Synthetic Procedures:



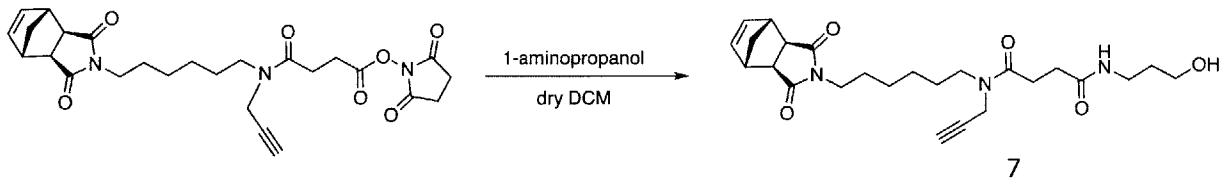
Polystyrene azide: Copper(I)bromide (131mg, 0.91 mmol) was added to a 3-neck 50-mL round-bottomed flask. The flask was placed under vacuum and refilled with nitrogen. The flask was then charged with distilled styrene (11 mL, 96 mmol), 2-Phenyl-2-bromoethane (328 μL, 2.4 mmol), and *N,N,N',N'',N''*-pentamethyldiethylenetriamine (PMDETA, 501 μL, 2.4 mmol). After the addition of PMDETA, the flask was one freeze-pump-thaw cycle. The reaction was then heated to 90 °C in a preheated oil bath and allowed to stir for 2.5 hours. The reaction was quenched by submerging the flask in liquid nitrogen and opening it to air. Once the mixture had warmed to room temperature, it was diluted with THF and poured

onto a column filled with basic alumina. The filtrate was collected, partially condensed and precipitated into methanol. The precipitate was collected on a nylon membrane and washed with methanol (250 mL, x3). Polystyrene-bromide was collected as a white powder and placed under vacuum overnight to dry (4.8 g). This powder was then dissolved in anhydrous DMF (20 mL) and sodium azide (312 mg, 4.8 mmol) was added. This mixture was allowed to stir overnight at 50 °C after which time the reaction was diluted in diethyl ether and washed with water (200 mL, x6). The organic layer was dried with Na_2SO_4, filtered and then partially concentrated and precipitated in 500 mL methanol. The precipitate was collected and washed with methanol (250 mL, x3) and dried *in vacuo.* (GPC: M_n = 3,400 Da, $Đ$ = 1.1).



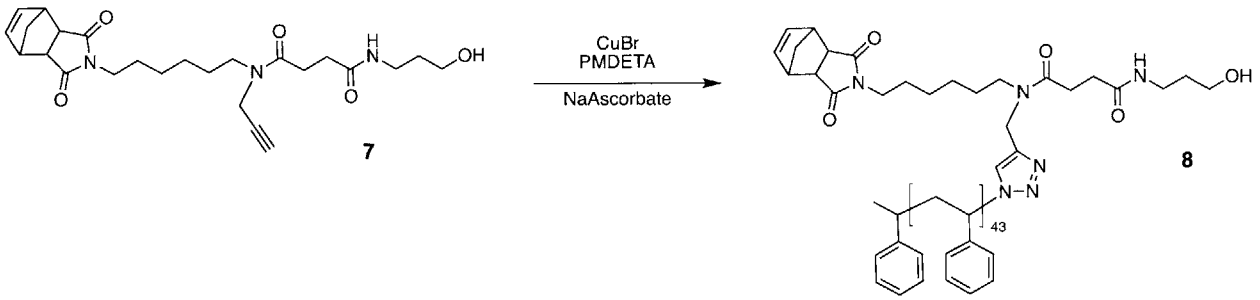
Poly(tert-butyl acrylate)azide: Copper(I)bromide (131mg, 0.91 mmol) was added to a 3-neck 50 mL round-bottomed flask. The flask was placed under vacuum and refilled with nitrogen. The flask was then charged with distilled *tert*-butyl acrylate (8 mL, 55 mmol), ethyl α-bromoisobutyrate (102 μL, 0.91 mmol), anhydrous Toluene (4mL), and PMDETA (1.27 mL, 6.1 mmol). After the addition of PMDETA, the flask was subjected to one cycle of freeze-pump-thaw. The reaction was then heated to 70 °C in a preheated oil bath and allowed to stir for 90 minutes. The reaction was quenched by submerging the flask in liquid nitrogen and opening it to air. Once the mixture had warmed to room temperature, it was diluted with THF and poured onto a column filled with basic alumina. The filtrate was collected, partially condensed and precipitated into ice cold water : methanol (40:60 v/v)

as a sticky glass. The precipitate was collected and dissolved in ether, dried over Na_2SO_4 and filtered into a round-bottomed flask. This liquid was condensed to near dryness and then placed under vacuum overnight. The resulting PTBA-bromide (3.7 g) was dissolved in anhydrous DMF (5mL) and sodium azide (213 mg, 3.3 mmol) was added. This mixture was allowed to stir overnight at 50 °C after which time the reaction was diluted in diethyl ether and washed with water (200 mL, x6). The organic layer was dried with Na_2SO_4, filtered and then concentrated. The product was then purified by flash chromatography in a gradient of 0-2% ethyl acetate in hexanes to afford PTBA-azide (GPC: M_n =2,900 Da, $Đ$ = 1.1).

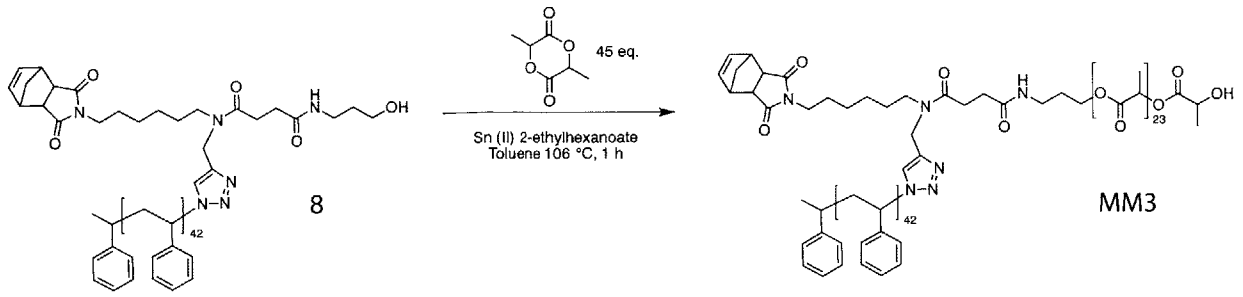


7

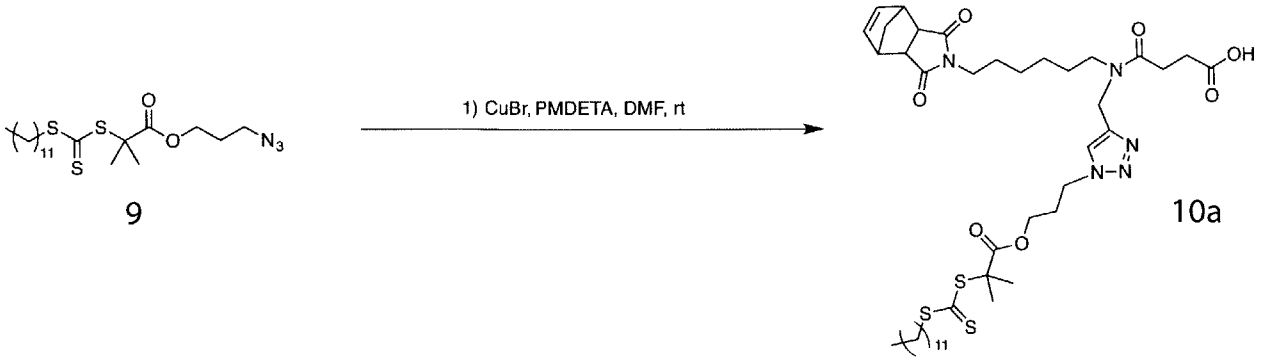
Norbornene-alkyne-alcohol **(7)**- In a 20 mL scintillation vial, compound **2** (250 mg, 0.50 mmol) was diluted with anhydrous DCM. 3-Aminopropanol (39 µL, 0.51 mmol) was then added and the reaction was allowed to stir for 6 hours. Once the reaction was deemed complete by LC/MS, the reaction was purified by flash chromatography in 2 % MeOH in DCM to yield a clear sticky oil (183 mg, 70% yield). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.00 (s, 1H), 6.17 (s, 2H), 3.99 (dd, *J* = 19.0, 2.5 Hz, 2H), 3.47 (t, *J* = 5.7 Hz, 2H), 3.37 – 3.17 (m, 6H), 3.13 (s, 2H), 2.65 (t, *J* = 6.6 Hz, 1H), 2.59 (s, 4H), 2.48 – 2.36 (m, 2H), 2.27 (t, *J* = 2.4 Hz, 0H), 2.14 (t, *J* = 2.4 Hz, 1H), 1.61 – 1.34 (m, 7H), 1.28 – 1.02 (m, 5H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 178.02, 173.53, 172.35, 172.03, 171.68, 137.63, 78.84, 77.16, 71.69, 59.14, 47.62, 44.96, 42.57, 38.38, 38.26, 36.12, 34.64, 31.80, 30.98, 28.45, 27.94, 27.37, 27.07, 26.36, 26.03, 25.31. HRMS: calcd. for $C_{25}H_{35}N_3O_5$ [M + H]: 458.2649; found, 458.2638.



Norbornene-polystyrene-alcohol **(8)**- Copper(I)bromide (175 mg, 1.2 mmol), sodium ascorbate (242 mg, 1.2 mmol), and ~3000 Da polystyrene azide (3.6 g, 1.0 mmol) were weighed into a 150 mL 2-neck round-bottomed flask that was then placed under vacuum and backfilled with nitrogen. 30 mL anhydrous DMF was charged to the flask. Once the contents were dissolved, PMDETA (1.27 mL, 6.1 mmol) was added and the reaction was frozen in liquid nitrogen and evacuated, then refilled with nitrogen. The reaction was allowed to return to room temperature before heating to 50 °C for 24 hrs. A spatula tip of azide-resin and alkyne-resin was added to the reaction and allowed to stir overnight. The reaction mixture was then cooled to room temperature and diluted with THF and poured onto a short aluminum oxide column that was washed with 300 mL of THF. The filtrate was collected and partially concentrated. This viscous solution was precipitated in 20% water in methanol. The precipitate was filtered using a nylon filter paper and washed with cold 20:80 water : methanol (250 mL, x3). The white powder was dissolved in DCM and purified by flash chromatography in a gradient of 0-2% MeOH in DCM. The polymer containing fractions were combined and concentrated to dryness before being re-dissolved in minimal THF. The precipitation procedure was then repeated and the white polymer was allowed to dry overnight under vacuum.

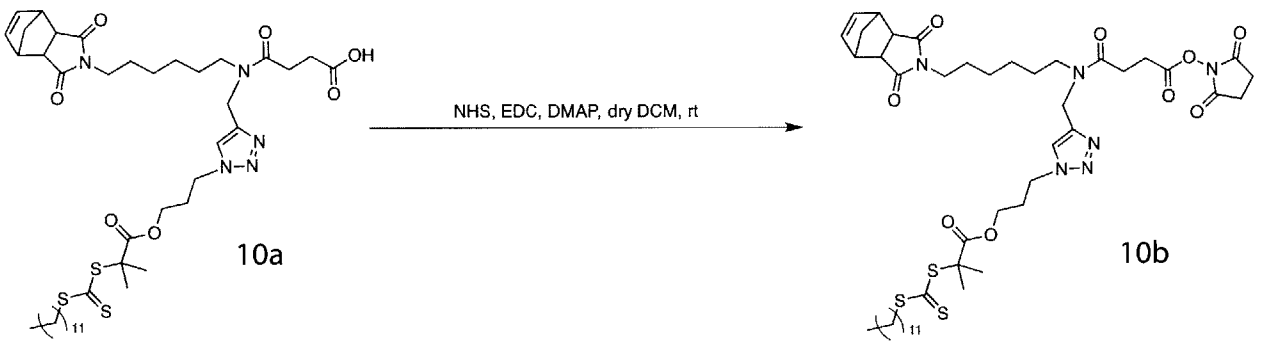


Norbornene-polystyrene-poly(lactic acid) **(MM3)**: 3,6-dimethyl-1,4-dioxane-2,5-dione (1.15g, 8.0 mmol) and compound **8** (897 mg, 0.23 mmol) were weighed into a vial that was brought into the glove box. A vial containing a known amount of tin(II)ethylhexanoate was also brought into the glove box and diluted with a known amount of toluene so as to create a standard solution. The reaction mixture was dissolved in 8 mL of toluene (2 M) and tin(II)ethylhexanoate (9.23 mg, 0.02 mmol) was added from the standard solution. The reaction was then placed in a sand bath preheated to 106 °C and was allowed to stir for 1 h. After this time, the reaction was quenched with cold methanol with 3 drops of 1M HCl and the vial was placed in a -80 °C freezer for 20 minutes. The methanol was then decanted and the solution was dissolved in a minimal amount of THF before precipitating into an ice-cold solution 30% water in methanol. The precipitate was filtered over a nylon membrane and washed with ice cold 30% water in methanol (200 mL, x3). The white powder was dried under vacuum. (See Appendix for ^{1}H NMR spectra)

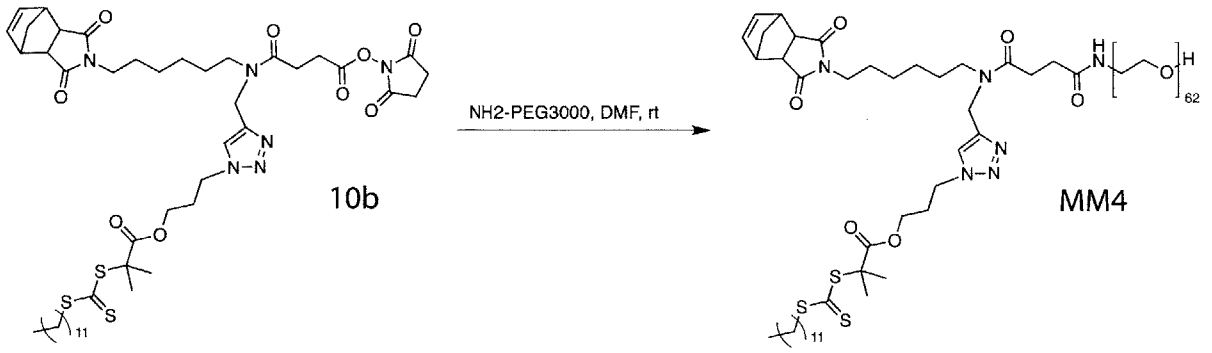


Norbornene-CTA-COOH ***(10a)***: 3-azidopropyl 2-(((dodecylthio)carbonothioyl)thio)-2-methylpropanoate (**9,** 129.8 mg 0.29 mmol), copper (I) bromide (40.2 mg, 0.28 mmol), and

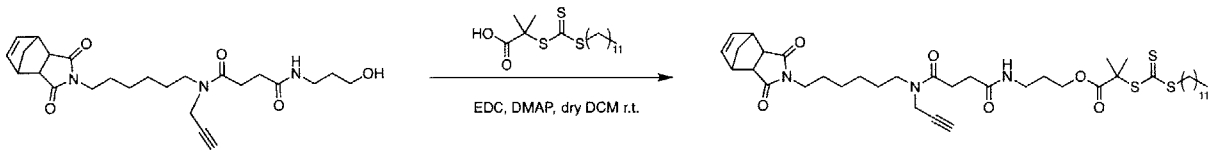
sodium ascorbate (277.4 mg, 1.4 mmol) were weighed into a 50 mL 3-neck round-bottomed flask that was placed under vacuum and back filled with nitrogen. Norbornene-alkyne-COOH (113.5 mg, 0.28 mmol) was diluted with 1.5 mL anhydrous DMF and transferred to the reaction vessel. PMDETA (293 μL, 1.4 mmol) was then added and the flask which was then subjected to one cycle of freeze-pump-thaw. The reaction was then allowed to stir overnight at room temperature. The reaction was then diluted with DCM and washed with water, saturated aqueous EDTA, and brine. The organic fractions were dried over Na_2SO_4, filtered, concentrated, and then purified by flash chromatography in 3% MeOH in DCM to afford **10a** (196 mg, 79%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.58 (s, 1H), δ 6.28 (s, 2H), δ 4.57 (m, J = 6.3 Hz, 2H), δ 4.36 (d, J = 7.1 Hz, 2H), δ 4.10 (t, J = 6.3 Hz, 2H), δ 3.50 (m, 4H), δ 3.26 (m, 4H), δ 2.67 (m, 6H), δ 2.25 (m, J = 6.1, 6.9, 6.3 Hz, 2H), δ 1.78-1.40 (m, 14H), δ 1.41-1.11 (m, 23H), δ 0.87 (t, J = 6.7 Hz, 3H).



*Norbornene-CTA-NHS **(10b)**:* Anhydrous DCM was added to a vial containing norbornene-CTA-COOH (**10a,** 65.0 mg, 0.08 mmol) and a stir bar. 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (11.9 mg, 0.08 mmol) was added followed by DMAP (4.70 mg, 0.04 mmol). *N*-hydroxy succinimide (8.86 mg ,0.08 mmol) was then added. When the reaction was deemed complete by LC/MS, the reaction mixture was concentrated and purified by column chromatography in a gradient of 2-20% acetone in hexanes. **10b** was isolated as a yellow oil.



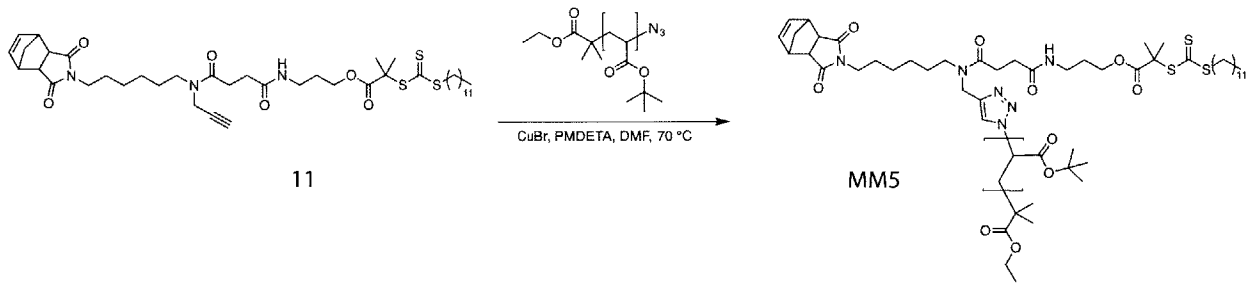
Norbornene-CTA-PEG3000 ***(MM4)****:* Norbornene-CTA-NHS was diluted in minimal anhydrous DMF and transferred to a 20 mL scintillation vial. Peg amine was dissolved in minimal DMF with gentle heating and added dropwise to the reaction mixture. The reaction was allowed to stir overnight or until the reaction was deemed complete by TLC. The reaction mixture was then pipetted into cold diethyl ether. This suspension was centrifuged after which the supernatant was decanted and the precipitate was re-suspended in cold ether. This process was repeated three times before the precipitate was collected and dried *in vacuo.* The resulting product was a waxy light-yellow powder. (See Appendix for ^{1}H NMR).



7 11

Norbornene-alkyne-CTA ***(11)****:* 2-(Dodecylthiocarbonothioylthio)propionic acid (175 mg, 0.48 mmol), EDC (111 mg, 0.72 mmol), and DMAP (29.3 mg, 0.24 mmol) were weighed into a vial containing compound **7** (219.4 mg 0.48 mmol) in 1mL anhydrous DCM. This reaction was stirred for 12 hours while being monitored by TLC. The product was purified by flash chromatography in a gradient of 0-50% ethyl acetate in hexanes. The second fraction was collected and concentrated to afford compound **11** as a yellow oil (256 mg, 67% yield). ^{1}H

NMR (500 MHz, $CDCl_3$) δ 6.27 (s, 2H), δ 6.21 (m, 1H), δ 4.16 (d, *J* = 2.5 Hz, 1H), δ 4.11 (t, *J* = 6.0 Hz, 2H), δ 4.02 (d, *J* = 2.3 Hz, 1H), δ 3.41 (m, 4H), δ 3.25 (m, 6H), δ 2.73 (t, *J* = 6.4 Hz, 1H), δ 2.66 (m, 3H), δ 2.51 (t, *J* = 6.4 Hz, 2H), δ 2.28 (t, *J* = 2.5 Hz, 0.4H), δ 2.17 (t, *J* = 2.5 Hz, 0.6H), δ 1.90-1.75 (m, 2H), δ 1.73-1.43 (m, 14H), δ 1.41-1.15 (m, 22H), δ 0.85 (t, *J* = 6.7 Hz, 3H). ^{13}C NMR (101 MHz, CD_2Cl_2) δ 222.1, 178.3,173.2,172.5,171.9, 171.5, 138.0, 79.41, 71.79, 64.13, 56.27, 48.02, 45.37, 42.96, 38.65, 37.25, 36.77, 34.81, 32.12, 31.62, 31.58, 29.84, 29.77, 29.67, 29.56, 29.32, 29.16, 28.05, 26.81, 25.57, 22.91, 14.37 HRMS: calcd. for $C_{42}H_{65}N_5O_7S_3$ [M + H]: 848.4119; found, 848.4129.



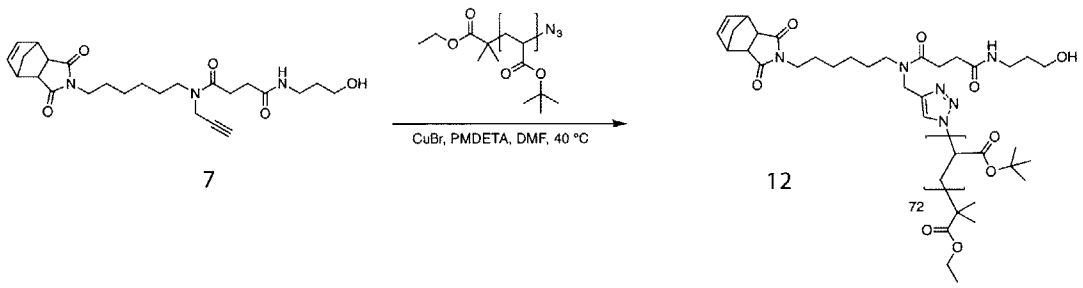
11 MM5

Norbornene-PTBA-CTA ***(MM5)****:* PTBA-azide (500mg, 0.12 mmol), compound **11** (99.7mg 0.12 mmol), copper (I) bromide (17.2 mg 0.12 mmol), and sodium ascorbate (11.9mg, 0.60 mmol) were weighed into a 50 mL round-bottomed flask. Anhydrous DMF was added followed by PMDETA (126 μL, 0.60 mmol). Immediately after the addition of PMDETA, the flask was subjected to one cycle of freeze-pump-thaw. The reaction was then heated to 50 °C for 10 minutes, while being monitored by TLC. Once the reaction was complete, azide and alkyne resin were added carefully and the reaction was allowed to heat for another five minutes. The reaction mixture was then diluted with THF and passed through a column of basic aluminum oxide. The filtrate was collected and concentrated to dryness before purification via flash chromatography in a gradient of 0-20% DCM in hexanes containing

1% MeOH. The product containing fractions were collected and condensed to afford MM5 as a yellow solid. (See Appendix for ^{1}H NMR).

General procedure for photo-growth of N-isopropylacrylamide from chain transfer agents:

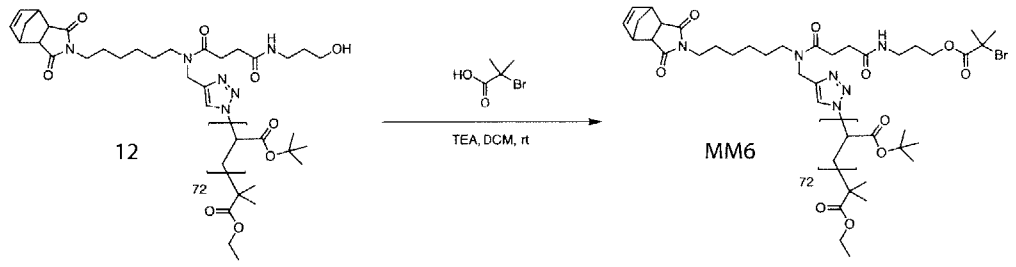
1 equivalent of chain transfer agent was added to a small vial followed by 200-300 equivalents of NiPAAm monomer. This vial was brought into the glove box where acetonitrile was added to a concentration of 1M with respect to the monomer. The vial was sealed and brought outside of the glove box where it was placed 10 cm away from a 365 nm UV light for a predetermined amount of time. A sample of the reaction mixture was then diluted in DMF containing 0.025 M lithium bromide, filtered through a 0.42 micron PTFE filter and then analyzed by GPC and ^{1}H NMR.



7 12

Norbornene-PTBA-alcohol ***(12)*****:** PTBA-azide (1.49 g, 0.48 mmol), and norbornene-alkyne-alcohol (**7,** 229 mg, 0.50 mmol) were added as standard solutions in DCM before being concentrated to dryness in a 50 mL 2-neck round-bottomed flask. Copper(I)bromide (55.0 mg, 0.38 mmol) and sodium ascorbate (76.0 mg, 0.38 mmol) were then added and the flask was evacuated and refilled with nitrogen. The reagents were diluted in 5 mL anhydrous DMF and PMDETA (401 μL, 1.9 mmol) was added. The flask was subjected to one cycle of freeze-pump-thaw before heating to 40 °C for 10 minutes. A spatula tip of azide-resin and

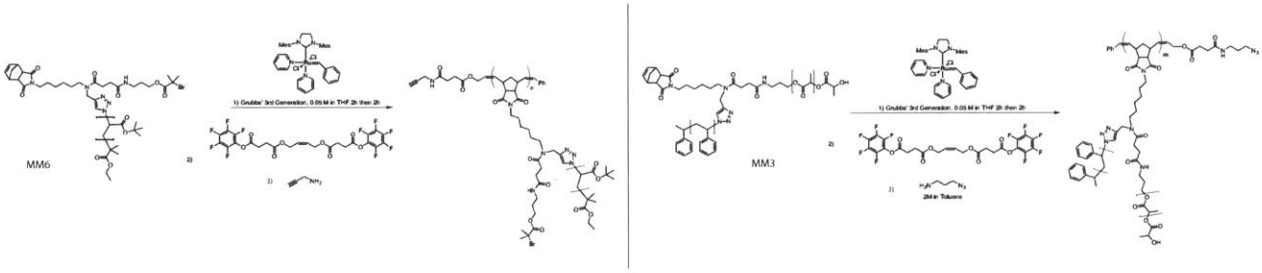
alkyne-resin was then carefully added to the reaction, which was allowed to stir for 10 additional minutes. The reaction mixture was then cooled to room temperature and diluted with THF and poured onto a short aluminum oxide column that was washed with 100 mL of THF. The filtrate was collected and concentrated. The product was purified by flash chromatography using a gradient of 20-80% DCM in hexanes to afford **12** as a yellow oil (784 mg, 44% yield).



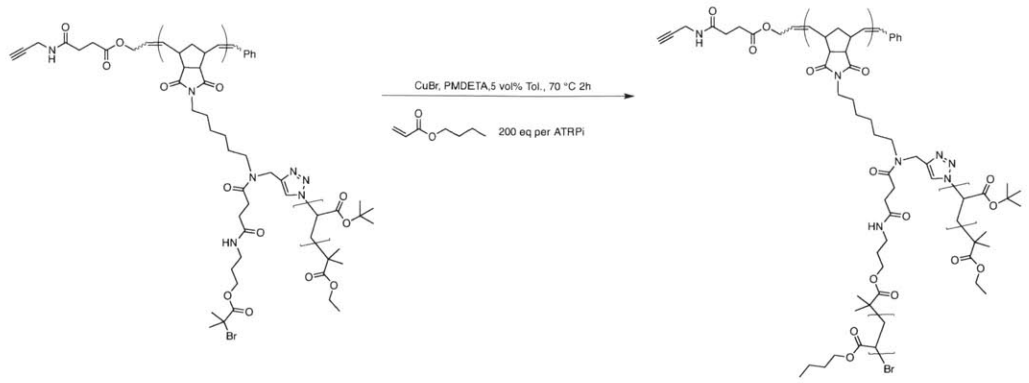
Norbornene-PTBA-ATRPi **(MM6):** Norbornene-PTBA-OH (**12,** 784 mg 0.22 mmol) was added to a vial and diluted with anhydrous DCM (1 mL). Freshly distilled triethylamine (61.0 μL, 0.44 mmol) was added via syringe. 2-Bromo-2-methylpropionyl bromide (54.0 μL, 0.44 mmol) was then added and the reaction was allowed to stir overnight. The reaction mixture was diluted with diethyl ether, washed with 1M HCl (100 mL) followed by 1M NaOH (100 mL) and brine (100 mL). The organic fractions were dried over $MgSO_4$, filtered, and concentrated. The product was purified by flash chromatography in a gradient of 0-2% MeOH in DCM (519 mg, 64% yield).

Representative procedure for diblock polymerization via ROMP: MM5 (50 mg, 0.0102 μmol) was added to a 0.5 dram vial with a stir bar. THF (0.05 M , 130 mL) was added to the vial and the mixture was allowed to stir until dissolved. A 0.002 g/mL solution of Grubb's third

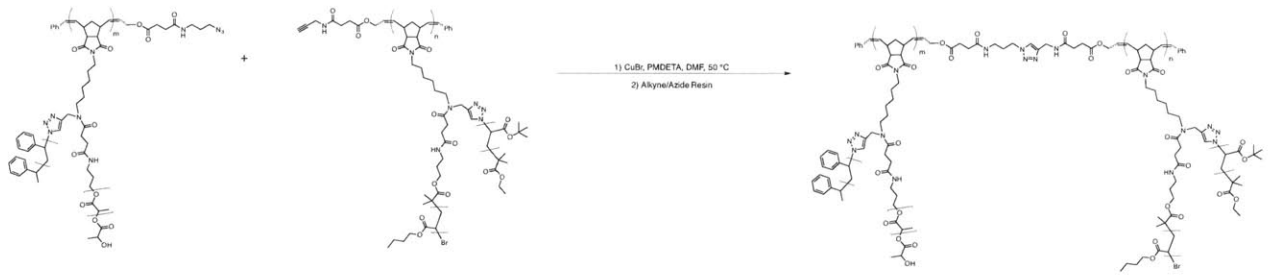
generation catalyst (G3) in THF was prepared in a separate vial. An aliquot of this G3 solution (74 μL) was added to the stirring solution of macromonomer. The polymerization was allowed to stir for 120 min and was monitored by GPC. After complete consumption of MM5, a solution of MM3 (112 mg, 0.0102 μmol) was prepared in THF (204 μL) and added to the stirring reaction. After another 120 minutes, a drop of ethyl vinyl ether was added to quench the polymerization. GPC characterization data is provided in Figure 7b.



General procedure for end functionalized bottle-brush polymers: Macromonomer (MM) was added to a 0.5 dram vial with a stir bar. THF (0.05 M) was added to the vial and the mixture was allowed to stir until dissolved. A 0.002 g/mL solution of Grubb's third generation initiator in THF was prepared in a separate vial. An aliquot of this G3 solution was added to the stirring solution of macromonomer. The polymerization was allowed to stir for 120 min and was monitored by GPC. After complete consumption of MM, 0.1 mg of (*Z*)-*O*,*O*'-(but-2-ene-1,4-diyl) bis(perfluorophenyl) disuccinate in 10μL of THF was added from a standard solution. This solution was allowed to stir for 3 hours before the vial was removed from the glove box and a drop of ethyl vinyl ether was added. A few drops of the appropriate amine (1-aminopropyl-3-azide or propargyl amine) were added and the vial was capped and allowed to stir overnight. The resulting mixture was purified either by precipitation in methanol (MM3_B_N_3) or by washing with water and evaporating (MM6_B_alkyne).

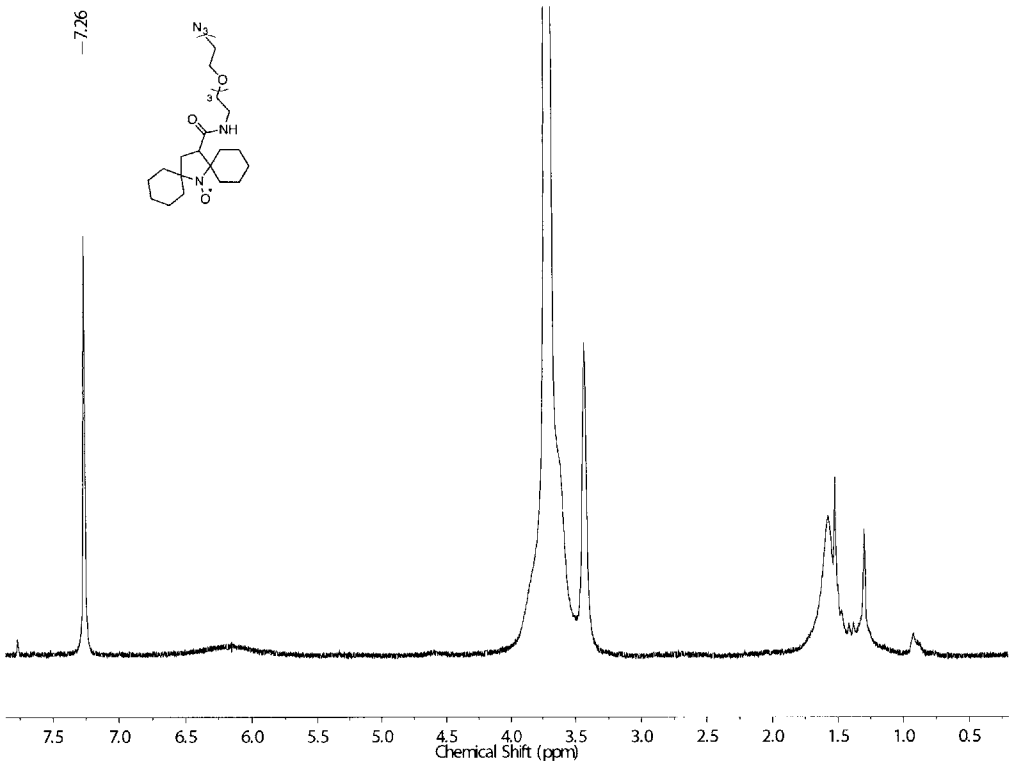


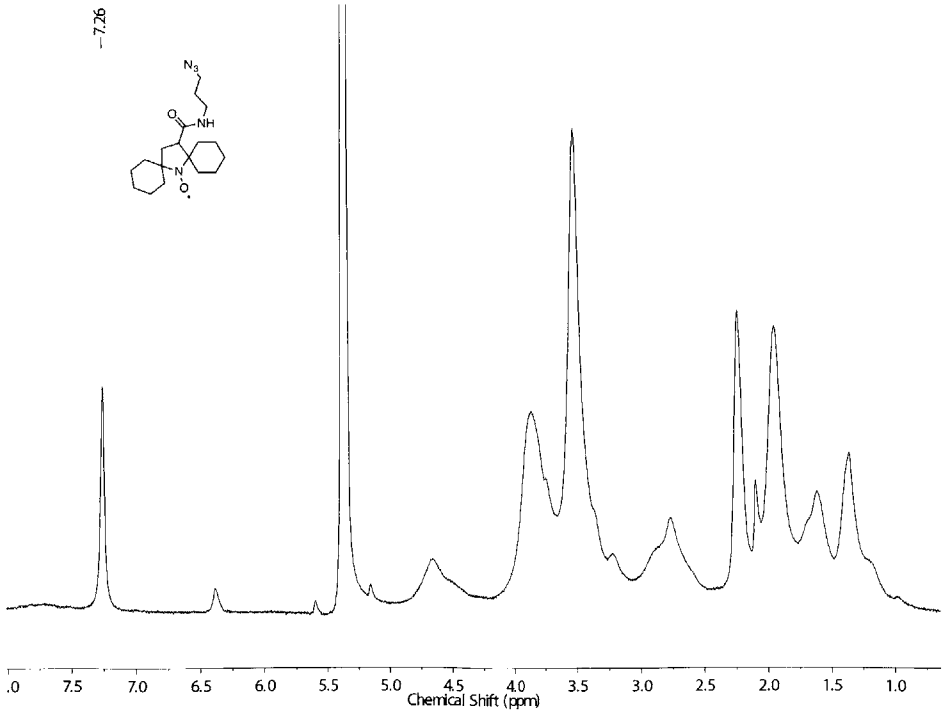
Growth of n-Butyl Acrylate from MM6_B230: 1 eq. copper(I)bromide was weighed into a vial containing **MM6_B230**. The vial was evacuated and refilled with nitrogen. N-Butyl Acrylate and 5% (v/v) toluene was added to the vial via syringe followed by PMDETA (4 eq.). The vial was then frozen in liquid nitrogen under vacuum. The vial was backfilled with nitrogen and allowed to warm to room temperature before being placed in a preheated oil bath set at 70 °C. The reaction was allowed to proceed for two hours before being quenched by exposure to oxygen and by freezing in liquid nitrogen. The reaction mixture was allowed to warm to room temperature before being diluted in THF and filtered through a pipette of basic aluminum oxide. The filtrate was collected, concentrated and dried on a vacuum line overnight. The resulting polymer was a reddish-brown oil. (GPC characterization in Figure 9b.)

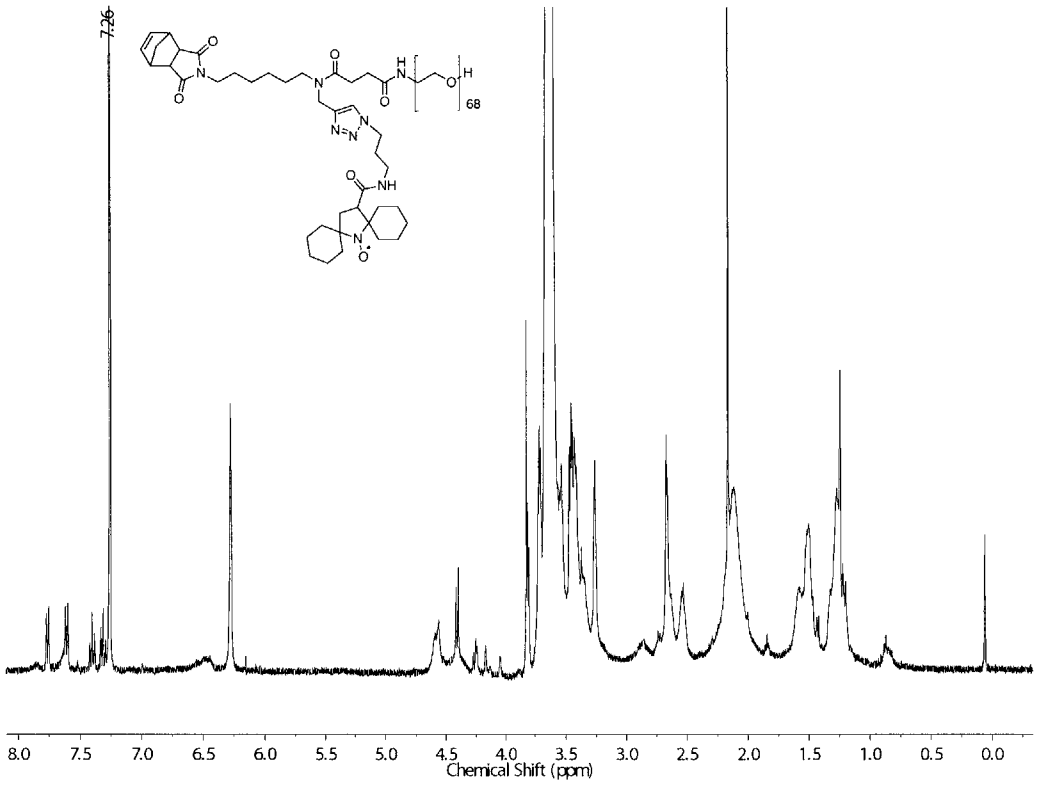


General procedure for CuAAC synthesis of tetrablock bottle-brush: MM3_B_N_3 and MM6_B_alkyne were combined in a small vial in a 1:1 ratio along with 1 eq. copper(I)bromide and 1 eq. sodium ascorbate. The vial was sealed with a rubber septum, evacuated and refilled with nitrogen. Anhydrous DMF was then added to a concentration of 1M followed by 5 eq. PMDETA. Immediately after addition of the ligand, the vial was frozen in liquid nitrogen under vacuum before being refilled with nitrogen. The vial was allowed to warm to room temperature before heating to 50 °C. The reaction was allowed to stir overnight after which a small amount of azide and alkyne resin was added and the vial was resealed and the reaction was allowed to stir for an additional three hours. The reaction mixture was then diluted with THF and passed through a pipette filled with neutral aluminum oxide. The solvent was evaporated off and the resulting reddish-brown residue was analyzed by GPC and ^{1}H NMR (Figure 8, 9).

Chapter III: Appendix



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68
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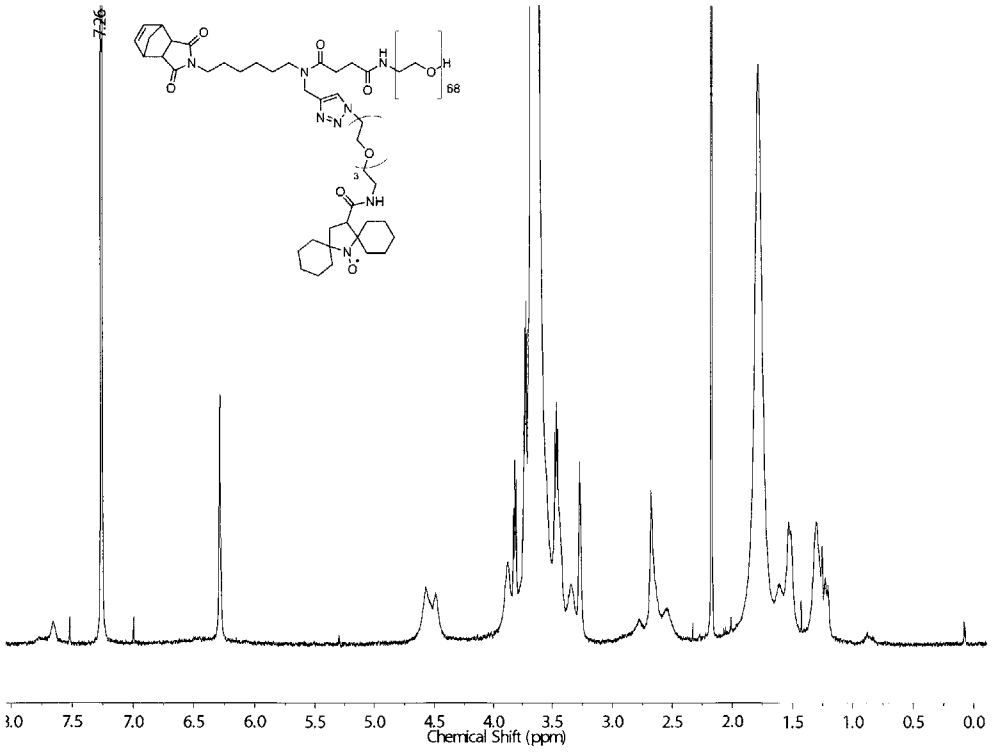
Chemical Shift (ppm)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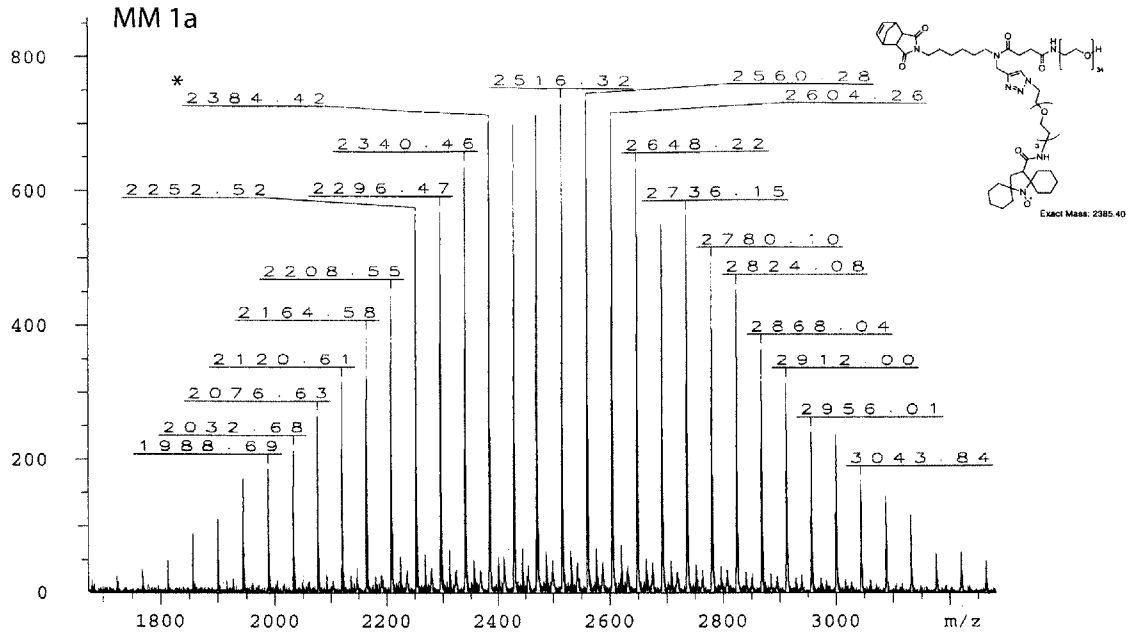
Chemical Shift (ppm)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m/z
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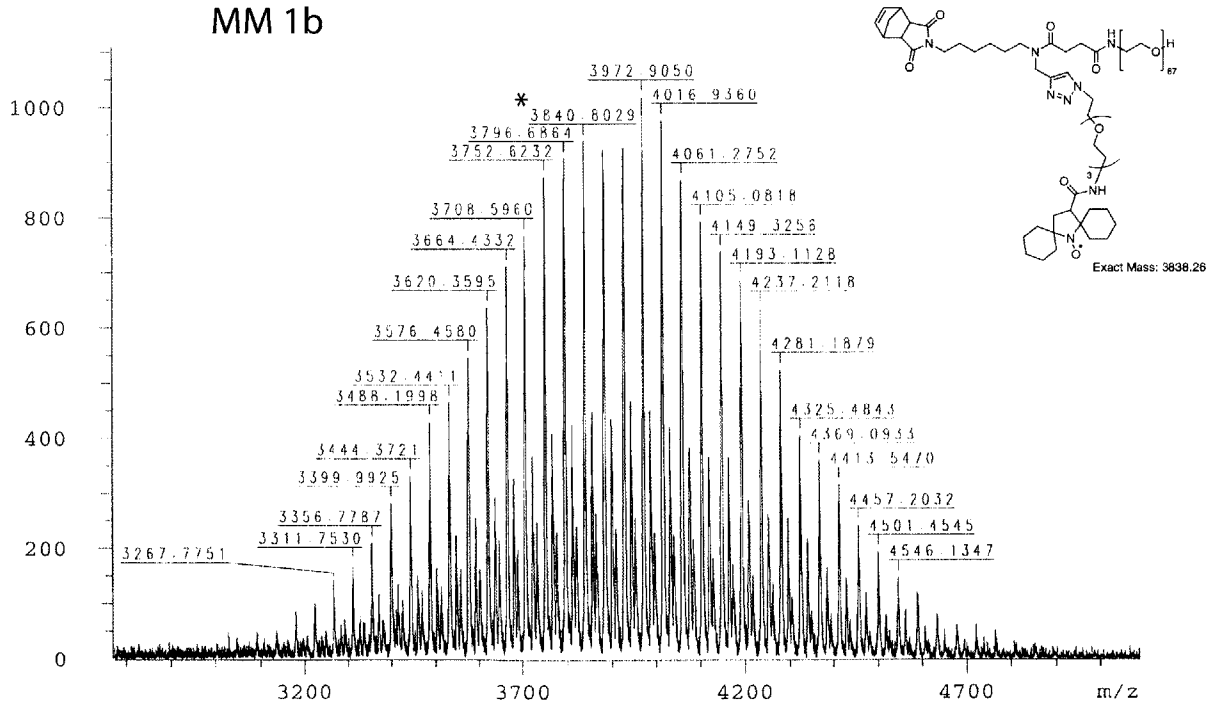
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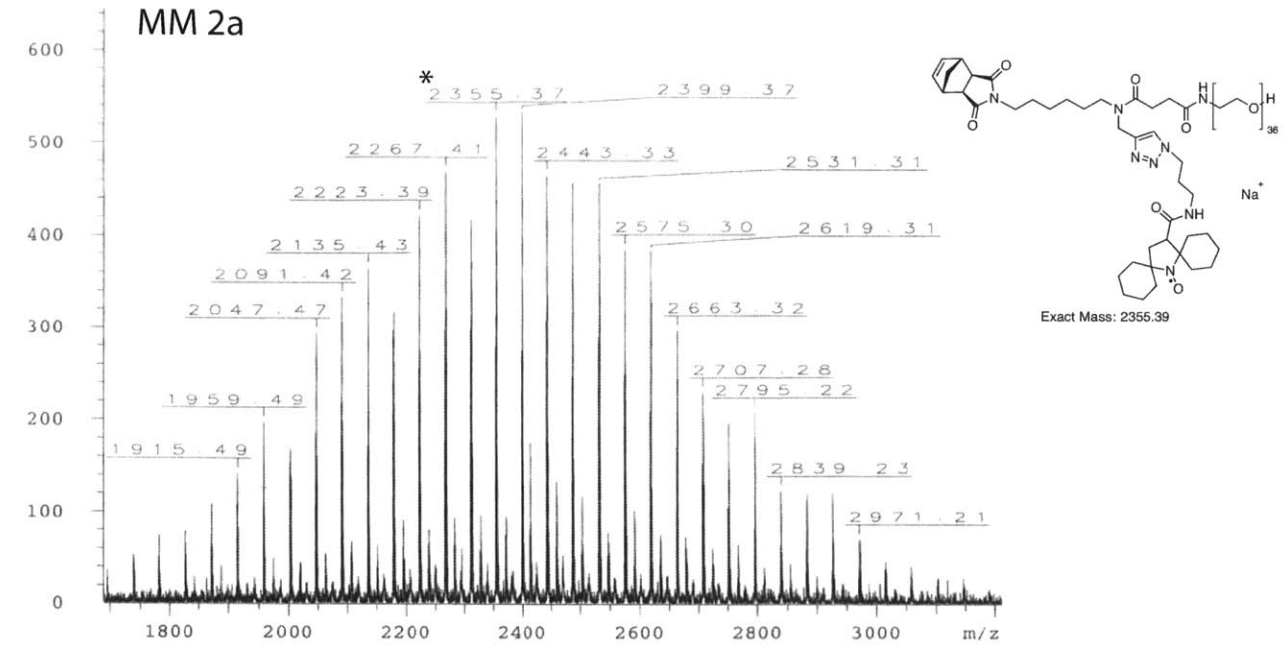
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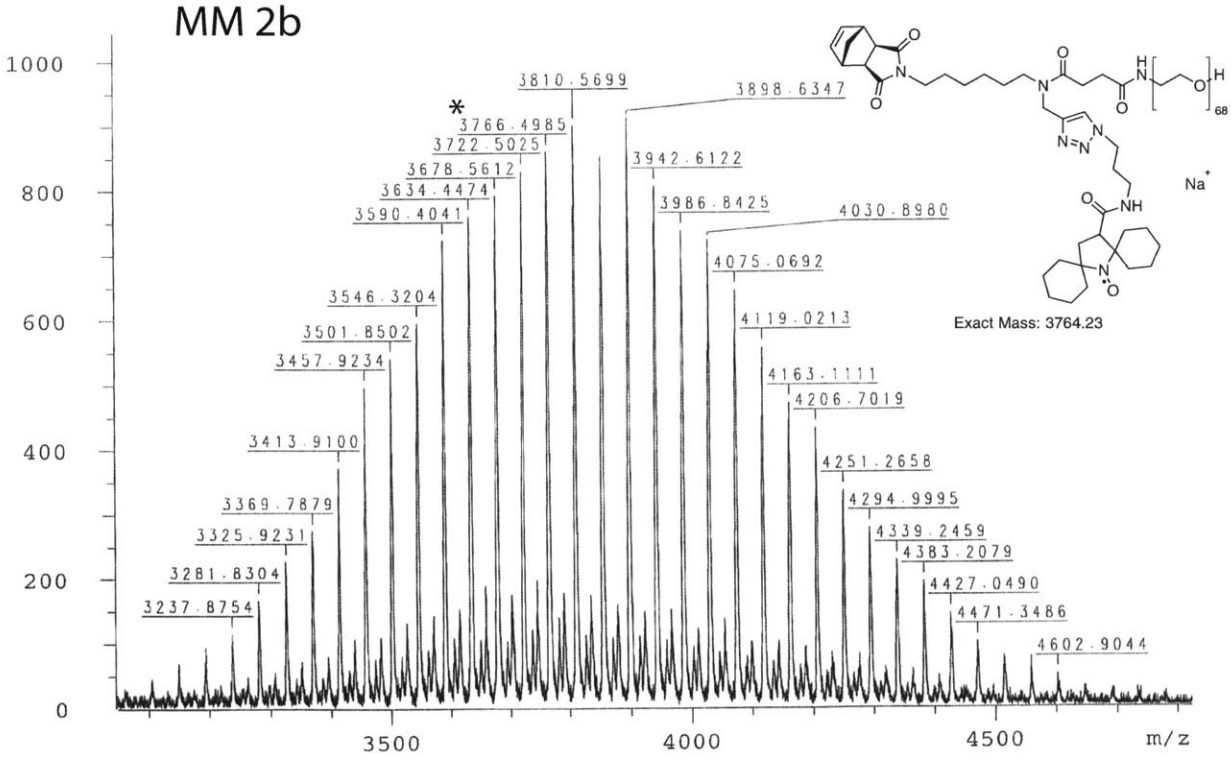
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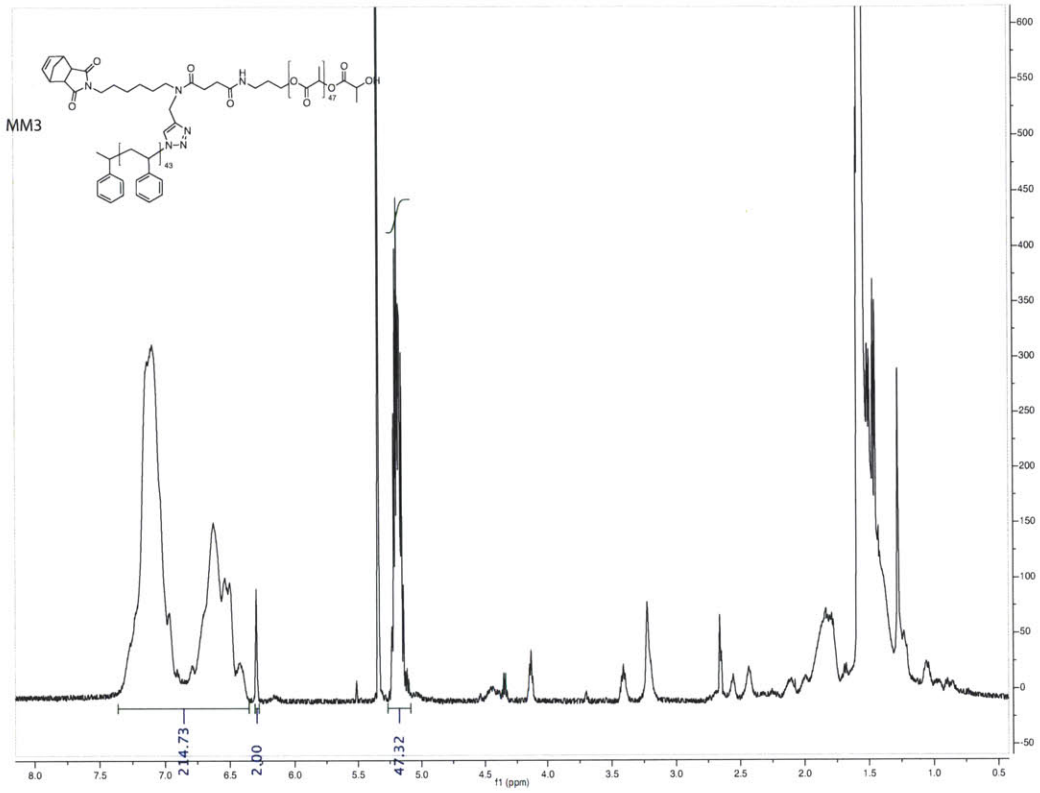
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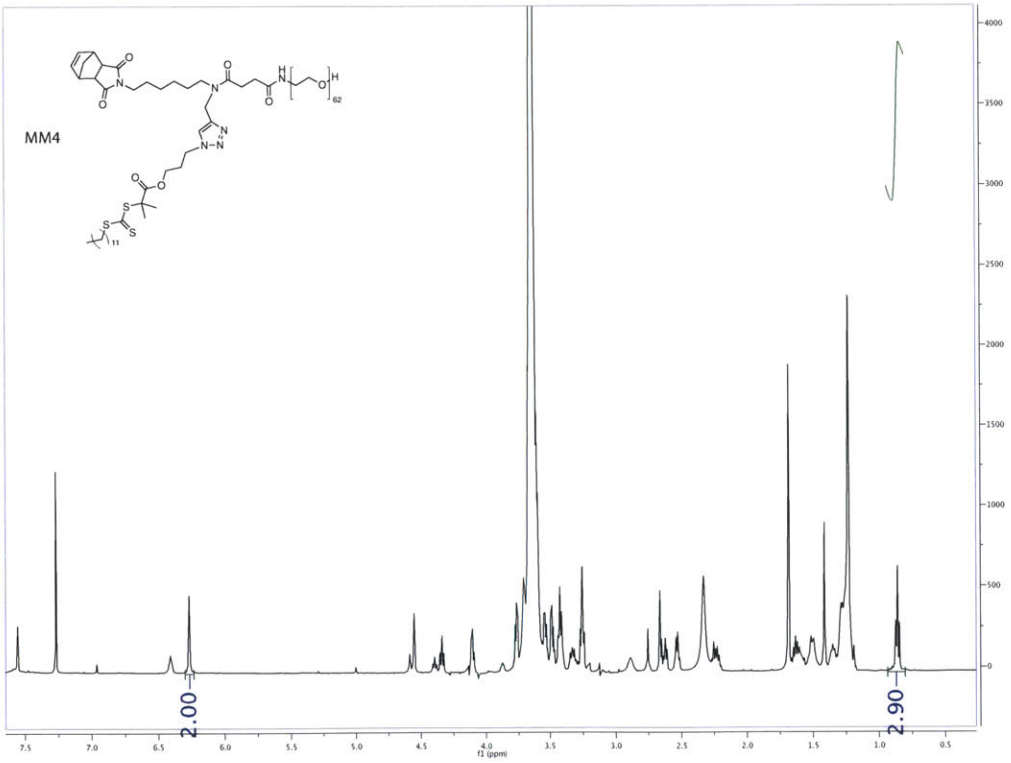
47.32
f1 (ppm)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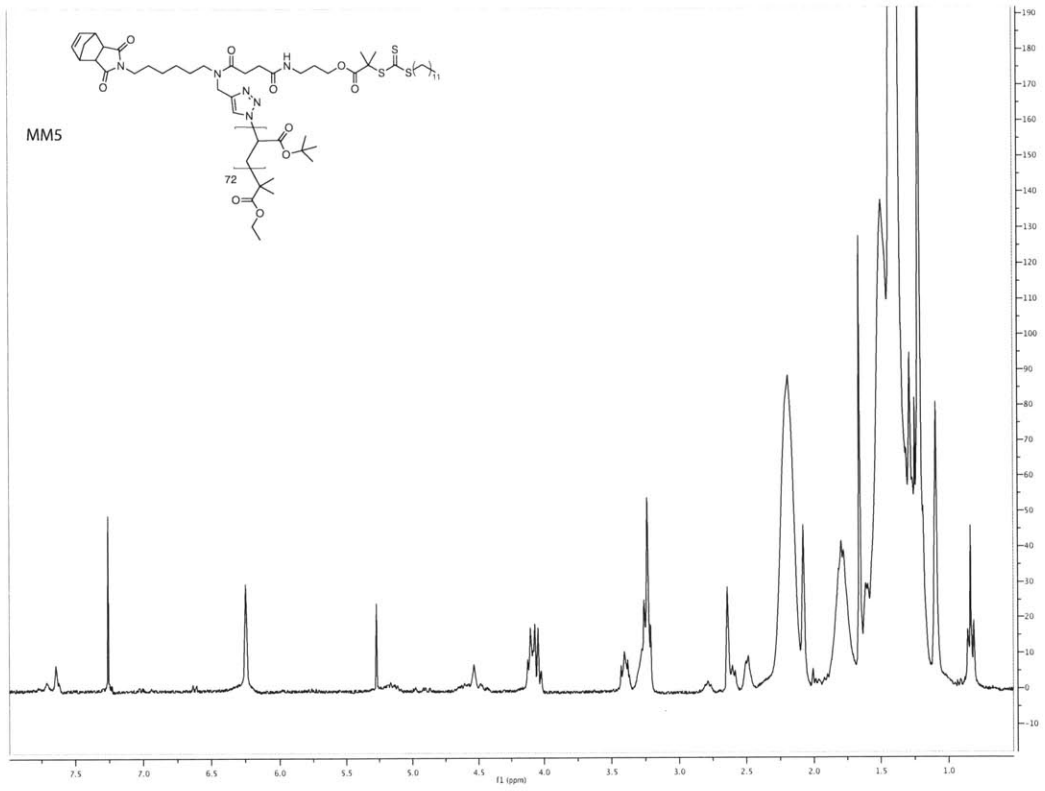
f1 (ppm)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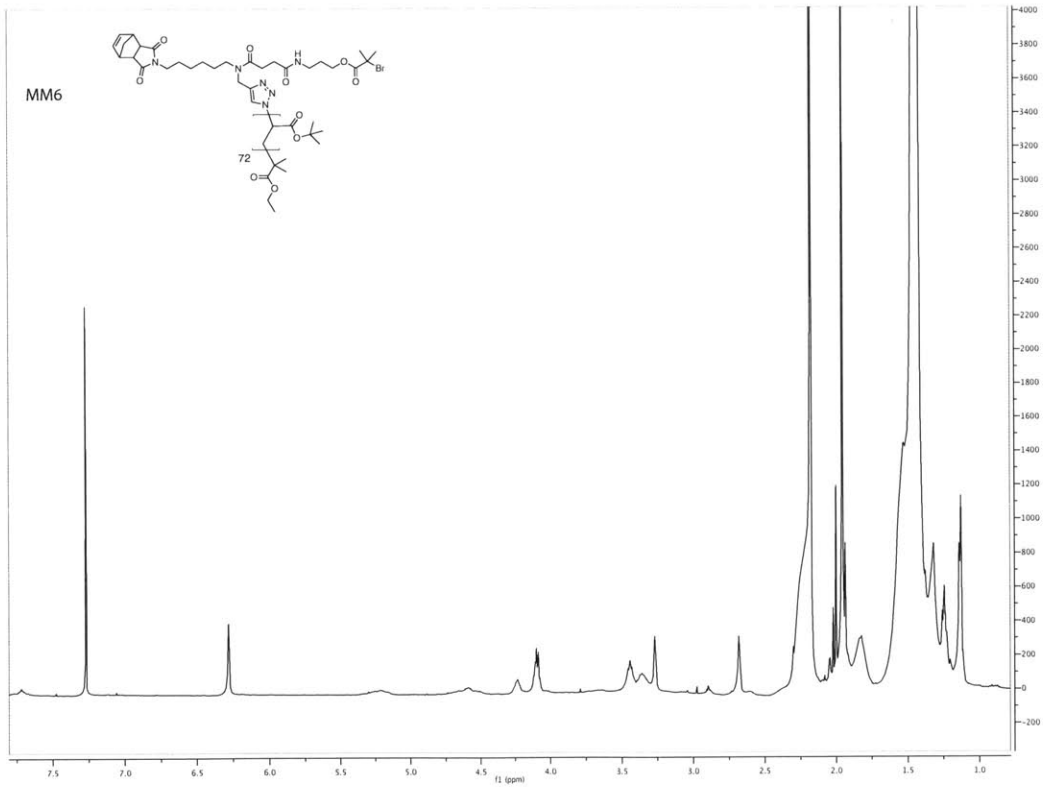
MM6
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f1 (ppm)

Chapter IV. References

1. Terreno, E.; Castelli, D. D.; Viale, A.; Aime, S., Challenges for molecular magnetic resonance imaging. *Chemical Reviews* **2010,** *110* (5), 3019-42.
2. (a) Caravan, P.; Ellison, J. J.; McMurry, T. J.; Lauffer, R. B., Gadolinium(III) Chelates as MRI Contrast Agents: Structure, Dynamics, and Applications. *Chemical Reviews* **1999,** *99* (9), 2293-2352; (b) Klemm, P. J.; Floyd Iii, W. C.; Andolina, C. M.; Fréchet, J. M. J.; Raymond, K. N., Conjugation to Biocompatible Dendrimers Increases Lanthanide T2 Relaxivity of Hydroxypyridinone Complexes for Magnetic Resonance Imaging. *European Journal of Inorganic Chemistry* **2012,** *2012* (12), 2108-2114.
3. Klemm, P. J.; Floyd III, W. C.; Andolina, C. M.; Fréchet, J. M. J.; Raymond, K. N., Conjugation to Biocompatible Dendrimers Increases Lanthanide T2 Relaxivity of Hydroxypyridinone Complexes for Magnetic Resonance Imaging. *European Journal of Inorganic Chemistry* **2012,** *2012*, 2108-2114.
4. LaConte, L. E. W.; Nitin, N.; Zurkiya, O.; Caruntu, D.; O'Connor, C. J.; Hu, X.; Bao, G., Coating thickness of magnetic iron oxide nanoparticles affects R2 relaxivity. *Journal of magnetic resonance imaging : JMRI* **2007,** *26* (6), 1634-41.
5. Kodali, V.; Littke, M. H.; Tilton, S. C.; Teeguarden, J. G.; Shi, L.; Frevert, C. W.; Wang, W.; Pounds, J. G.; Thrall, B. D., Dysregulation of Macrophage Activation Profiles by Engineered Nanoparticles. *ACS nano* **2013**.
6. Kálmán, F. K.; Geninatti-Crich, S.; Aime, S., Reduction/Dissolution of a β-MnOOH Nanophase in the Ferritin Cavity To Yield a Highly Sensitive, Biologically Compatible Magnetic Resonance Imaging Agent. *Angewandte Chemie International Edition* **2010,** *49* (3), 612-615.
7. Rajca, A.; Wang, Y.; Boska, M.; Paletta, J. T.; Olankitwanit, A.; Swanson, M. a.; Mitchell, D. G.; Eaton, S. S.; Eaton, G. R.; Rajca, S., Organic radical contrast agents for magnetic resonance imaging. *Journal of the American Chemical Society* **2012,** *134*, 15724-7.
8. Dykes, G. M., Dendrimers: a review of their appeal and applications. *Journal of Chemical Technology & Biotechnology* **2001,** *76* (9), 903-918.
9. Bakleh, M. E.; Sol, V.; Estieu-Gionnet, K.; Granet, R.; Déléris, G.; Krausz, P., An efficient route to VEGF-like peptide porphyrin conjugates via microwave-assisted 'click-chemistry'. *Tetrahedron* **2009,** *65* (36), 7385-7392.
10. Wen, W.-H.; Lin, M.; Su, C.-Y.; Wang, S.-Y.; Cheng, Y.-S. E.; Fang, J.-M.; Wong, C.-H., Synergistic effect of zanamivir-porphyrin conjugates on inhibition of neuraminidase and inactivation of influenza virus. *Journal of medicinal chemistry* **2009,** *52* (15), 4903-10.
11. Johnson, J. A.; Lu, Y. Y.; Burts, A. O.; Lim, Y. H., Core-Clickable PEG-Branch-Azide Bivalent-Bottle-Brush Polymers by ROMP: Grafting-Through and Clicking-To. *Journal of the* **2011,** *133* (3), 559-566.
12. Xia, Y.; Li, Y.; Burts, A. O.; Ottaviani, M. F.; Tirrell, D. a.; Johnson, J. a.; Turro, N. J.; Grubbs, R. H., EPR study of spin labeled brush polymers in organic solvents. *Journal of the American Chemical Society* **2011,** *133* (49), 19953-9.
13. Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J., Safe and Convenient Procedure for Solvent Purification. *Organometallics* **1996,** *15* (5), 1518-1520.

14. Johnson, J. a.; Lu, Y. Y.; Burts, A. O.; Xia, Y.; Durrell, A. C.; Tirrell, D. a.; Grubbs, R. H., Drug-loaded, bivalent-bottle-brush polymers by graft-through ROMP. *Macromolecules* **2010,** *43* (24), 10326-10335.
15. Bates, F. S.; Hillmyer, M. a.; Lodge, T. P.; Bates, C. M.; Delaney, K. T.; Fredrickson, G. H., Multiblock polymers: panacea or Pandora's box? *Science (New York, N.Y.)* **2012,** *336* (6080), 434-40.
16. (a) Gotrik, K. W.; Hannon, A. F.; Son, J. G.; Keller, B.; Alexander-Katz, A.; Ross, C. a., Morphology control in block copolymer films using mixed solvent vapors. *ACS nano* **2012,** *6* (9), 8052-9; (b) Edrington, a. C.; Urbas, a. M.; DeRege, P.; Chen, C. X.; Swager, T. M.; Hadjichristidis, N.; Xenidou, M.; Fetters, L. J.; Joannopoulos, J. D.; Fink, Y.; Thomas, E. L., Polymer-Based Photonic Crystals. *Advanced Materials* **2001,** *13* (6), 421-425; (c) Choi, H. K.; Gwyther, J.; Manners, I.; Ross, C. a., Square arrays of holes and dots patterned from a linear ABC triblock terpolymer. *ACS nano* **2012,** *6* (9), 8342-8.
17. Gu, W.; Huh, J.; Hong, S. W.; Sveinbjornsson, B. R.; Park, C.; Grubbs, R. H.; Russell, T. P., Self-assembly of symmetric brush diblock copolymers. *ACS nano* **2013,** *7* (3), 2551-8.
18. (a) Miyake, G. M.; Piunova, V. a.; Weitekamp, R. a.; Grubbs, R. H., Precisely tunable photonic crystals from rapidly self-assembling brush block copolymer blends. *Angewandte Chemie (International ed. in English)* **2012,** *51* (45), 11246-8; (b) Sveinbjörnsson, B. R.; Weitekamp, R. a.; Miyake, G. M.; Xia, Y.; Atwater, H. a.; Grubbs, R. H., Rapid self-assembly of brush block copolymers to photonic crystals. *Proceedings of the National Academy of Sciences of the United States of America* **2012,** *109* (36), 14332-6; (c) Miyake, G. M.; Weitekamp, R. a.; Piunova, V. a.; Grubbs, R. H., Synthesis of isocyanate-based brush block copolymers and their rapid self-assembly to infrared-reflecting photonic crystals. *Journal of the American Chemical Society* **2012,** *134* (34), 14249-54.
19. Brandrup, J.; Immergut, E. H.; Grulke, E. A.; Abe, A.; Bloch, D. R., Polymer Handbook (4th Edition). John Wiley & Sons.
20. Hchild, H. G., POLY (N-ISOPROPYLACRYLAMIDE): EXPERIMENT , THEORY AND APPLICATION. *Prog. Polym. Sci.* **1992,** *17*, 163-249.
21. Mansfeld, U.; Pietsch, C.; Hoogenboom, R.; Becer, C. R.; Schubert, U. S., Clickable initiators, monomers and polymers in controlled radical polymerizations – a prospective combination in polymer science. *Polymer Chemistry* **2010,** *1* (10), 1560-1560.
22. Zhou, H.; Johnson, J. a., Photo-controlled growth of telechelic polymers and end-linked polymer gels. *Angewandte Chemie (International ed. in English)* **2013,** *52* (8), 2235-8.
23. Rzayev, J., Synthesis of Polystyrene–Polylactide Bottlebrush Block Copolymers and Their Melt Self-Assembly into Large Domain Nanostructures. *Macromolecules* **2009,** *42* (6), 2135-2141.
24. Madkour, A. E.; Koch, A. H. R.; Lienkamp, K.; Tew, G. N., End-functionalized ROMP polymers for Biomedical Applications. *Macromolecules* **2010,** *43* (10), 4557-4561.
25. Vora, A.; Singh, K.; Webster, D. C., A new approach to 3-miktoarm star polymers using a combination of reversible addition–fragmentation chain transfer (RAFT) and ring opening polymerization (ROP) via "Click" chemistry. *Polymer* **2009,** *50* (13), 2768-2774.