SYNTHESIS OF POLYMERIC MATERIALS FOR DRUG DELIVERY AND INDUSTRIAL APPLICATIONS

Stephanie A. Kramer

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Approved by:
Michel R. Gagne
Cynthia K. Schauer
Joseph L. Templeton
Maurice S. Brookhart
Marcy L. Waters
ABSTRACT

Stephanie Ann Kramer: Synthesis of Polymeric Materials for Drug Delivery and Industrial Applications
(Under the direction of Wenbin Lin and Michel R. Gagne)

This dissertation reports the synthesis of polymeric materials for industrial and medicinal applications. Depending on their chemical structure, polymers have different properties and applications. Polyolefins, used in packaging and films, are one of the most highly produced polymeric materials in industry, whereas only a handful of polymeric materials are in the market for drug delivery.

The first part of this thesis presents a method to deoxygenate linear polyols producing branched polyolefins. In Chapter 2, linear polyols, such as poly(vinyl alcohol) (PVA), are hydrosilylatively deoxygenated using catalytic amounts of $\text{B(C}_6\text{F}_5)_3$ and a reducing hydrosilane. Following reduction, a highly branched, predominantly saturated structure is obtained. Depending on the alcohol-protecting group and the hydrosilane used, the branch numbers can exceed 200 branches per 1000 carbons. The branching microstructure is also dependent on the protecting group and silane. For instance, the deoxygenation of TMS-protected PVA with diethylsilane produced a polymer with different branch types when compared to the polymer that results from the deoxygenation of TES-protected PVA.

The $\text{B(C}_6\text{F}_5)_3$-catalyzed deoxygenation was applied to a triblock system wherein the central block was a 1,5-polyol structure to produce otherwise inaccessible triblock polymers with an amorphously branched interior block (Chapter 3). The starting polymer was obtained
by sequential hydroboration/oxidation of poly(styrene-\text{-}b\text{-}butadiene-\text{-}b\text{-}styrene) (SBS), which converts the polybutadiene block into a 1,5-polyol block. The hydroxylated SBS polymer was then completely deoxygenated to yield a novel triblock polymer with a highly branched interior, with the branching being predominantly butyl or longer chains as established by $^{13}$C-NMR spectroscopy. The structure-property relationships of this new triblock system still need to be investigated and compared to SBS. The second section of this thesis discusses an alternative drug delivery approach utilizing nanoscale coordination polymers (NCPs). Chapter 4 presents the synthesis of Ca(II) and Mn(II)-based NCP formulations containing a cisplatin prodrug. Coating the NCPs with a lipid bilayer stabilized both of these formulations. Drug release profiles demonstrated sustained cisplatin release from the NCPs. Drug loadings of 20\% for Ca-based NCP and 25\% for Mn-NCP were determined. Due to the exceptionally high drug loadings and nanoscale size, these cisplatin NCPs are promising drug delivery candidates.
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<th>Definition</th>
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<tr>
<td>Å</td>
<td>Angstrom</td>
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<tr>
<td>AA</td>
<td>Anisamide</td>
</tr>
<tr>
<td>aq.</td>
<td>Aqueous</td>
</tr>
<tr>
<td>9-BBN</td>
<td>9-Borabicyclo(3.3.1)nonane</td>
</tr>
<tr>
<td>Bodipy</td>
<td>Boron dipyrromethane</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine Serum Albumin</td>
</tr>
<tr>
<td>Ca-PtBP</td>
<td>Calcium-based platinum bisphosphonate particle</td>
</tr>
<tr>
<td>C</td>
<td>Celsius</td>
</tr>
<tr>
<td>c(RGDfk)</td>
<td>Cyclic peptide known to target the $\alpha_v\beta_3$ integrin</td>
</tr>
<tr>
<td>°</td>
<td>Degree</td>
</tr>
<tr>
<td>$d_1$</td>
<td>Delay time</td>
</tr>
<tr>
<td>@</td>
<td>Denotes one component inside another</td>
</tr>
<tr>
<td>SiEt$_2$H$_2$</td>
<td>Diethylsilane</td>
</tr>
<tr>
<td>SiMe$_2$EtH</td>
<td>Dimethylethylsilane</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>DSPE-PEG$_{2k}$</td>
<td>1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]</td>
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<td>DOPA</td>
<td>1,2-dioleoyl-sn-glycero-3-phosphate</td>
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<td>1,2-dioleoyl-sn-glycero-3-phosphocholine</td>
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<td>DOPE</td>
<td>1,2-dioleoyl-sn-glycero-3-phosphoethanolamine</td>
</tr>
<tr>
<td>DOTAP</td>
<td>1,2-dioleoyl-3-trimethylammonium-propane</td>
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<tr>
<td>DLS</td>
<td>Dynamic light scattering</td>
</tr>
<tr>
<td>EPR</td>
<td>Enhanced permeability and retention</td>
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<td>EtOH</td>
<td>Ethanol</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>GPC</td>
<td>Gel Permeation Chromatography</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>$t_{1/2}$</td>
<td>Half-life</td>
</tr>
<tr>
<td>Hz</td>
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</tr>
<tr>
<td>HDPE</td>
<td>High density polyethylene</td>
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</tr>
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<td>Hour</td>
</tr>
<tr>
<td>HT-29</td>
<td>Human colorectal adenocarcinoma cell line</td>
</tr>
<tr>
<td>A549</td>
<td>Human non-small cell lung carcinoma cell line</td>
</tr>
<tr>
<td>H460</td>
<td>Human non-small cell lung carcinoma cell line</td>
</tr>
<tr>
<td>PBD-OH</td>
<td>Hydroxylated polybutadiene</td>
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<td>SBS-OH</td>
<td>Hydroxylated styrene-$b$-butadiene-$b$-stylene</td>
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<tr>
<td>ICP-MS</td>
<td>Inductively-coupled plasma mass spectrometry</td>
</tr>
<tr>
<td>IC$_{50}$</td>
<td>Inhibitory concentration 50%</td>
</tr>
<tr>
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<tr>
<td>LDPE</td>
<td>Low density polyethylene</td>
</tr>
<tr>
<td>Mn-PtBP</td>
<td>Manganese-based platinum bisphosphonate particle</td>
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<tr>
<td>MIL</td>
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<tr>
<td>min</td>
<td>Minutes</td>
</tr>
<tr>
<td>M</td>
<td>Mega-</td>
</tr>
<tr>
<td>MOF</td>
<td>Metal organic framework</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Micro-</td>
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<td>m</td>
<td>Milli-</td>
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<td>Abbreviation</td>
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<td>M</td>
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<tr>
<td>MW</td>
<td>Molecular weight</td>
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<tr>
<td>MPS</td>
<td>Mononuclear phagocyte system</td>
</tr>
<tr>
<td>n</td>
<td>Nano</td>
</tr>
<tr>
<td>NCP</td>
<td>Nanoscale coordination polymer</td>
</tr>
<tr>
<td>N-BP</td>
<td>Nitrogen-containing bisphosphonates</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffer saline</td>
</tr>
<tr>
<td>PtBP</td>
<td>$c,c,t,-\text{Pt}(\text{NH}_3)_2(\text{phosphonocarbamate})_2$</td>
</tr>
<tr>
<td>PDI</td>
<td>Polydispersity index</td>
</tr>
<tr>
<td>PEG</td>
<td>Poly(ethylene glycol)</td>
</tr>
<tr>
<td>PS</td>
<td>Poly(styrene)</td>
</tr>
<tr>
<td>PVA</td>
<td>Poly(vinyl alcohol)</td>
</tr>
<tr>
<td>psi</td>
<td>Pounds per square inch</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>s</td>
<td>Second</td>
</tr>
<tr>
<td>SLB</td>
<td>Single lipid bilayer</td>
</tr>
<tr>
<td>SBS</td>
<td>Styrene-$b$-butadiene-$b$-styrene</td>
</tr>
<tr>
<td>SEBS</td>
<td>Styrene-$b$-ethylene/butadiene-$b$-styrene</td>
</tr>
<tr>
<td>TEOS</td>
<td>Tetraethyl orthosilicate</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TGA</td>
<td>Thermogravimetric analysis</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission electron microscopy</td>
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<td>Abbreviation</td>
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</tr>
<tr>
<td>SiEt₃H</td>
<td>Triethylsilane</td>
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<td>SiEt₃D</td>
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<tr>
<td>TMS</td>
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<td>B(C₆F₅)₃</td>
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<td>UV</td>
<td>Ultraviolet</td>
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<td>Vis</td>
<td>Visible</td>
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<tr>
<td>W</td>
<td>Water to surfactant ratio</td>
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<td>wt</td>
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Chapter 1. SYNTHESIS OF POLYMERIC MATERIALS FOR DRUG DELIVERY AND INDUSTRIAL APPLICATIONS

1.1 Introduction

The properties of polymers are highly varied, as are their applications, which range from synthetic plastics to rubbers to natural biopolymers (e.g. DNA) to lubricants. The properties and hence the application of polymers depends exquisitely on the polymer composition and microstructure. With few exceptions, one normally associates the monomer units with the composition of a polymer, though as described herein, this is not always the case. The microstructure on the other hand typically reflects how the monomers are arranged within the chain (e.g. tacticity), but this can also be ambiguous. This dissertation will discuss two different applications of polymeric materials (industrial polyolefins and drug delivery nanoscale coordination polymers), and in particular, the former materials in this chapter will be shown to significantly digress from their nominal “repeat unit” formulations.

1.2 Polymer Defunctionalization

1.2.1 Polyolefins

Polyolefins are used in a variety of applications, including industrial lubricants, grocery bags, engineering plastics, and adhesives. Despite their wide range of uses, polyolefins are composed only of carbon and hydrogen atoms, yet it is the connectivity and structure of the polymer that determines its properties, and consequently its use. Simply manipulating how the monomer units are bound in the chain can produce material with
wildly varying properties. For instance, low-density polyethylene (LDPE) contains short chain branches, giving it properties that make it useful in plastic wraps. On the other hand, the minimal short chain branches in high-density polyethylene (HDPE) produces a more dense material that finds use in plastic storage containers (e.g. milk jugs).

The synthesis of branched polyolefins typically involves polymerization of olefinic monomers using metal catalysts. Coordination catalysts, which enchain alkene monomers via coordination-insertion mechanisms allow for greater control over the polymer structure when compared to free radical initiators. Depending on the catalyst selected, control over the connectivity of the monomers, and therefore the polymer properties, can be achieved. Initial olefin polymerization catalysts were discovered in the 1950s by Ziegler and Natta (Ziegler-Natta catalysts) and by Hogan and Banks (Phillips catalysts). The extensive development and optimization of these and many other families of polymerization catalysts have fed the enormously important polyolefin business that exists today. Polymerization with coordination catalysts involves coordination of the monomer to the active metal site, followed by insertion into the metal-carbon bond at the active end of the polymer chain. The stereo- and regiochemistry of monomer coordination is what controls the propagation and structure of the resulting polymer chain.


Polymerization of ethylene, using different coordination catalysts, provides a synthetic route to produce polymers with unique microstructures and compatibility with polar solvents depending on the catalyst selected. Traditional approaches, such as Ziegler-Natta catalysis, of olefin polymerization allow for production of linear unbranched polymers at atmospheric pressures. In 1953, Ziegler discovered that a titanium catalyst together with an aluminum alkyl co-catalyst result in the polymerization of high molecular weight HDPE.\(^2\)

Later, Natta extended that this catalytic system to other olefins, such as polypropylene.\(^3,4,6\)

Second-generation catalysts were developed to increase the catalyst activity to efficiencies to 100-1000 kg polymer per gram of titanium, eliminating the need to remove residual catalysts from the final polymer.\(^7\)

Figure 1.1 presents a proposed general mechanism for the Ziegler-Natta catalysis of polyethylene.\(^8\)

The first step of the polymerization reaction is catalyst initiation, in which the alkyl aluminum co-catalyst serves to activate the titanium catalyst by acting as a Lewis acid to abstract a chloride, producing a vacant site for ethylene coordination. Although the exact mechanism is unknown, polymer chain growth is believed to occur at vacant Cl sites at the titanium surface through migratory insertion reactions. For migratory insertion reactions to occur, the polymerization catalyst must have an extra empty orbital after coordination of ethylene. The alkyl group forms an agostic C-H bond interaction with the empty orbital on the titanium metal tilting the sp\(^3\) orbital away from the metal to promote migratory insertion. The polymer chain is terminated through either \(\beta\)-hydrogen

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elimination to produce polyethylene with a terminal C=C bond or, more commonly, through the use of hydrogen as a chain transfer reagent.\textsuperscript{9}

\textbf{Initiation:}

\[
\text{L}_n\text{Ti-Cl} \xrightarrow{\text{AlR}_3} \text{L}_n\text{Ti-R} \\
\text{L}_n\text{Ti-R} \xrightarrow{-\text{AlR}_2\text{Cl}} \text{L}_n\text{Ti-R}
\]

\textbf{Propagation:}

\[
\text{Ti-CH}_3 \xrightarrow{\text{insertion}} \text{Ti-CH}_3 \\
\text{Ti-CH}_3 \xrightarrow{\text{insertion}} \text{Ti-CH}_3
\]

\textbf{Termination:}

\[
\text{Ti-H} + \text{H}_2 \rightarrow \text{Ti-H} + \text{H}_2
\]

\textbf{Figure 1.1.} Proposed mechanism of Ziegler-Natta polymerization of ethylene.

The controlled synthesis of branched polyethylene was first reported by Brookhart. Ni and Pd \(\alpha\)-diimine catalysts were developed to polymerize olefins in a living fashion to yield high molecular polymers with a narrow weight distribution.\textsuperscript{10} Incorporation of the bulky diimine ligands into the catalyst allowed for synthesis of highly branched polymers through a chain walking mechanism (Figure 1.2).\textsuperscript{10,11,12,13} It is well established that the metal is able to


\textsuperscript{11} Guan, Z.; Cotts, P.; McCord, E.; McLain, S. \textit{Science} \textbf{1999}, \textit{283}, 2059.

migrate or “walk” along the polymer chain through insertion and β-hydride elimination reactions. The two late transition metal catalysts demonstrate different polymerization behaviors. In the case of the Ni(II) system, the degree of branching and branching microstructure was manipulated by controlling the ethylene pressure and temperature during the polymerization reaction. Increasing the ethylene pressure during the reaction lead to a polymer with a decrease in branch number and a linear topology with mostly short branches. At reduced ethylene pressures, dense, hyperbranched polymers were produced. Depending on the reaction conditions selected, the synthesized polyethylene ranged from linear to a highly branched (>120 branches/1000 C) structure. When the Pd(II)-catalyst was used in ethylene polymerization, the degree of branching (100 branches/1000 C) was essentially the same for all reaction conditions.

![Mechanism of α-diimine Ni/Pd-catalyzed olefin polymerizations.](image)

**Figure 1.2.** Mechanism of α-diimine Ni/Pd-catalyzed olefin polymerizations.

Due to their importance in defining properties, methods for characterizing the defects or branches in a polyolefin, such as polyethylene, have been developed with $^1$H- and $^{13}$C-NMR spectroscopy being particularly useful. A branch is defined as the number of carbons from a methyl to a methine off of the backbone, and the number of these per 1000 C is a

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benchmark measure. This is typically quantitated in the $^1$H-NMR spectrum by integration of each proton type.\textsuperscript{14} For example, the degree of branching can be estimated from the ratio of methyl hydrogens ($\sim$0.85 ppm) to the total aliphatic hydrogens in the polymer by a specific formula. The equation used to calculate the degree of branching in this work is shown in Equation 1.1. A higher ratio of methyl to total hydrogens indicates a greater degree of branching.

\textbf{Equation 1.1.}

$$\frac{\text{# Branches}}{1000C} = \frac{CH_3}{3 \times \frac{CH + CH_2 + CH_3}{2}} \times 1000$$

Adding chromium(III) acetylacetonate to an NMR sample containing a polyethylene sample prior to obtaining the $^{13}$C-NMR spectrum aids in shortening relaxation times which consequently allows for quantitative NMR spectroscopy. Unique carbon resonances can be distinguished, and have been assigned by others, for short chain branches of polyethylene,\textsuperscript{15} with the key methyl resonances from methyl, ethyl, propyl, and butyl or longer branches being particularly valuable. The signal at $\sim$30 ppm in the $^{13}$C-NMR spectrum of branched polyethylene is due to the main CH$_2$ groups in the polymer backbone.

\textbf{1.2.2 Metal-Free Defunctionalization}

Defunctionalization of organic functional groups is an important process in synthetic chemistry, particularly in the reduction of the C-O functional group to a methyl group. The carbonyl functionality can be reduced through a Clemmensen\textsuperscript{16} reduction, which makes use

\textsuperscript{14} Daugulis, O.; Brookhart, M.; White, P. S. \textit{Organometallics} 2002, 21, 5935.

\textsuperscript{15} Cotts, P.; Guan, Z.; McCord, E.; McLain, S. \textit{Macromolecules} 2000, 33, 6945-6952.
of a strong acid and amalgamated zinc, or through the complementary Wolff-Kirschner reduction, which employs hydrazine and a strong base. These defunctionalization procedures require harsh conditions: high temperatures, high pressures, and strong acids or bases. The combination of a Lewis acid and a hydrosilane provides a milder approach for the reduction of carbonyl-containing compounds.

One defunctionalization reaction that has not received significant attention is the conversion of C-O bonds at the alcohol oxidation state to C-H bonds, i.e. alcohols to alkanes. The Lewis acid trispentafluorophenylborane \((B(C_6F_5)_3)\) has been investigated as a catalyst in the mild hydrosilylative reduction of C-O bonds because of its commercial availability, convenient handling, and comparable Lewis acidity to \(BF_3\).\(^{19}\) \(B(C_6F_5)_3\) is advantageous over \(BF_3\) because it avoids problems associated with reactive B-F bonds. Yamamoto first reduced primary alcohols using triethylsilane in the presence of catalytic amounts of \(B(C_6F_5)_3\).\(^{20}\) This was the first example of catalytic use of a Lewis acid in the reduction of alcohols. Addition of an equimolar amount of silane resulted in silyl-protection to quantitatively produce the alkyl silyl ether (with concomitant \(H_2\) evolution), while adding excess silane (3 equivalents) fully reduced a primary alcohol to the alkane (Scheme 1.1). Using triethylsilane, more bulky secondary and tertiary alcohols were not reduced and only the corresponding silyl ethers

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were obtained. Cleavage of linear and cyclic primary alkyl ethers was achieved in the presence of a stoichiometric amount triethylsilane and catalytic amounts of B(C₆F₅)₃ to give the corresponding alkane and silyl ether. Nimmagadda and McRae further expanded the reduction of alcohols to secondary and tertiary alcohols, though this required the use of more reactive hydrosilanes, such as diethylsilane or n-butylsilane. Further investigations by Yamamoto indicated that the relative reactivity of alcohols towards reduction was: primary >> secondary > tertiary.

**Scheme 1.1.** Treatment of a primary alcohol with HSiEt₃ and catalytic amounts of B(C₆F₅)₃.

Inspired by the mild B(C₆F₅)₃-reduction approach, Njardarson investigated the reduction of the C-O bonds in unsaturated cyclic ethers. First, the reduction of 2,5-dihydrofurans, which contain two allylic ethereal C-O bonds, was studied to compare the impact of steric and electronics in the reaction mechanism. Following the reduction of 2-heptyl-2,5-dihydrofuran (Scheme 1.2), the less sterically hindered C-O bond was cleaved, suggesting that steric congestion does impact the selectivity of reduction. However, B(C₆F₅)₃-reduction of 2-phenyl-2,5-dihydrofuran, shown in Scheme 1.3, indicates that other factors can influence the selectivity, for example when a cation-stabilizing group is present.

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The results suggest that both steric and electronic effects play a role in the cleavage of the C-O bonds. Secondly, the B(C₆F₅)₃-reduction of 3,6-2H-dihydropyrans was studied to compare the reduction of allylic vs. non-allylic C-O bonds. A range of substrates was reduced, and the allylic C-O bond was found to be more easily reduced over the non-allylic position in all cases. In the case of this class of substrates, this provides a controlled route to substituted homoallylic alcohols. The magnitude of this preference was demonstrated on a quaternary substituted allylic ether in Scheme 1.4. In this case, the more stabilized allylic position was preferentially reduced even though it is sterically congested. The work by Njardarson has shown that B(C₆F₅)₃ with a hydrosilane can selectively reduce the C-O bond of cyclic unsaturated ethers without affecting the initial olefin geometry. In general, allylic/benzylic C-O bonds will be preferentially reduced over less hindered, non-stabilized C-O bonds. It is proposed that the activated ether is reduced fast enough to suppress formation of a free allyl cation and prohibit scrambling of the initial olefin geometry.
Scheme 1.2. Catalytic reduction of a sterically hindered 2-heptyl-2,5-dihydrofuran C-O bond.

\[
\begin{align*}
\text{C}_7\text{H}_{15} & \xrightarrow{1.1 \text{ eq SiEt}_3\text{H}} \xrightarrow{5 \text{ mol\% B(C}_6\text{F}_5)_3} \xrightarrow{\text{CH}_2\text{Cl}_2, RT} \text{C}_7\text{H}_{15} \xrightarrow{\text{TBAF}} \text{OH} \\
\end{align*}
\]

Scheme 1.3. Catalytic reduction of a 2-phenyl-2,5-dihydrofuran C-O bond.

\[
\begin{align*}
\text{Ph} & \xrightarrow{1.1 \text{ eq SiEt}_3\text{H}} \xrightarrow{5 \text{ mol\% B(C}_6\text{F}_5)_3} \xrightarrow{\text{CH}_2\text{Cl}_2, RT} \text{Ph} \xrightarrow{\text{TBAF}} \xrightarrow{\text{OH}} \\
\end{align*}
\]

Scheme 1.4. Catalytic reduction of a 3,6-2H-dihydropyran C-O bond, demonstrating the electronic control on the reaction.

\[
\begin{align*}
\text{Ph} & \xrightarrow{1.1 \text{ eq SiEt}_3\text{H}} \xrightarrow{5 \text{ mol\% B(C}_6\text{F}_5)_3} \xrightarrow{\text{CH}_2\text{Cl}_2, RT} \xrightarrow{\text{TBAF}} \text{Ph} \xrightarrow{\text{HO}} \\
\end{align*}
\]

Based on the work from Yamamoto\textsuperscript{20,22}, McRae\textsuperscript{21}, Piers\textsuperscript{24}, a catalytic cycle for the reduction of alcohols with B(C\textsubscript{6}F\textsubscript{5})\textsubscript{3} is proposed in Scheme 1.5. The first step in this reaction is borane activation of the Si-H bond, which is believed to commence through coordination of the Si-H bond of the hydrosilane to the Lewis acidic boron. Nucleophilic attack of a silyl ether can then occur at the silicon atom by a process that has been shown to be invertive at Si.\textsuperscript{25} In this case, the oxygen atom of the silyl ether acts as the nucleophile. This results in the generation of a disilyl oxonium ion and a borohydride species. Lastly, hydride addition occurs to form the reduced species. In cases where alkoxide abstraction leads to stabilized carbocations (e.g. oxocarbenium ions), S\textsubscript{N}1-type processes may occur.\textsuperscript{22}


Scheme 1.5. Proposed B(C₆F₅)₃-catalytic cycle for the reduction of alcohols.

The proposed borane-silane adduct was recently studied and observed by Piers and Tuononen.²⁶ Up until this work, attempts to spectroscopically observe the Lewis acid/base adduct in solution using low temperature NMR spectroscopy failed.²⁷ These observations lead to the conclusion that the equilibrium lies away from formation of the B(C₆F₅)₃-silane adduct. To shift the equilibrium towards the adduct, a more Lewis acidic borane species was utilized. The anti-aromatic 1,2,3-tris(pentafluorophenyl)-4,5,6,7-tetrafluoro-1-boraindene is a stronger Lewis acid than B(C₆F₅)₃,²⁸ and its behavior in the presence of triethylsilane (Figure 1.3) was investigated by UV-Vis and NMR spectroscopy. The free boraindene is characterized by a weak absorption at 465 nm, which is disrupted upon complexation with Lewis bases, as observed by a color change from red to yellow that occurs on formation of

the borane-silane adduct. Variable temperature NMR spectroscopy experiments revealed the formation of the Lewis acid/base adduct. $^{19}$F-NMR analysis revealed a significant perturbation in the chemical shift of the fluorine atom that is ortho to the boraindene boron atom. Cooling the sample also revealed an upfield shift for the Si-H resonance in the $^{1}$H-NMR spectrum, as expected for a Si-H···B bridge. A crystal structure of the boraindene-silane adduct was obtained providing further evidence for the proposed Si-H···B bridge.

![Figure 1.3](image.png)

**Figure 1.3.** Lewis acid/base adduct formed between 1,2,3-tris(pentafluorophenyl)-4,5,6,7-tetrafluoro-1-boraindene and triethyilsilane.

Gagné and coworkers demonstrated the use of B(C$_6$F$_5$)$_3$ to catalyze the hydrosilylative reduction of monosaccharides and polysaccharides into hydrocarbons.$^{29}$ Moreover, the reaction conditions were tuned to selectively deoxygenate glucose (Scheme 1.6). These reactions were monitored using $^{13}$C NMR spectroscopy, as it allowed the disappearance of C-O bonds to be easily detected. For all substrates tested, complete deoxygenation was achieved when diethylsilane was selected as the hydride source. It was also noted that depending on the identity of the sugar substrate, the relative yields of alkane and alkene products was variable. For instance, the deoxygenation of 1-deoxyglucose led to >60% of the alkyl-shifted products, 2-methyl and 3-methylpentane. By contrast, the ring-opened sugar glucitol only provides <15% of the production distribution in the branched form. The

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generation of branched products was interpreted as likely reflecting the formation of cationic-intermediates in the deoxygenation sequence.

**Scheme 1.6.** B(C$_6$F$_5$)$_3$-catalyzed hydrosilylative deoxygenation of glucose into hexane and hexene isomers.

When a tertiary silane (dimethylethylsilane) was selected as the reducing agent, the reaction rate and yield were significantly reduced, which is likely a reflection of the larger steric congestion of the bulkier silane. For example, the reduction of Me$_2$EtSi-protected glucitol with dimethylethylsilane yields only partial deoxygenation even after a reaction time of 1 week.

### 1.2.3 Research Objectives

While inexpensive methods to synthesize branched polyolefins exist, the purpose of this research was to apply the B(C$_6$F$_5$)$_3$-catalyzed hydrosilylative deoxygenation methods to commercially available linear polyols determine if, and to what extent, branched polymer structures would ensue, in analogy to the formation of branched hexane. Initial work focused on deoxygenating commercially available linear polyols (of high MW), which were demonstrated to deoxygenate in a process that produces highly branched polyolefins with unique microstructures (Chapter 2). The chemistry was then applied to an industrially relevant triblock polymer as a means to synthesize a material containing blocks arranged in a linear-branched-linear architecture. Such materials display interesting phase separation properties (Chapter 3).
1.3 Nanoscale Coordination Polymers for Anticancer Drug Delivery

1.3.1 Nanoscale Coordination Polymers

Coordination polymers, also known as metal organic frameworks (MOFs), are inorganic compounds containing polymerized metal-ligand networks. The structure of coordination polymers is composed of metal ions connected to polydentate bridging ligands (Figure 1.4). The composition of the coordination can be easily tuned by simply changing the metal ion or the bridging ligand. The highly tunable nature of the material allows for different structures to be synthesized.

Figure 1.4. Self-assembly of metal ion and bridging ligand to form a metal-ligand network, known as a coordination polymer.

Published in 1960 by John Bailar, the first coordination polymer contained polymeric Schiff bases coordinated to various metal ions.\textsuperscript{30} Extensive research has since been conducted on the crystalline network form of various coordination polymers, yet the first nanoscale coordination polymer (NCP) was not demonstrated until 2005.\textsuperscript{31} The Mirkin group demonstrated that addition of an initiation solvent to an aqueous solution of metal ions and metalloligands resulted in spontaneous precipitation of spherical NCPs. The NCPs consisted


of a carboxylate-functionalized binapthyl bis-metallo-tridentate Schiff base units coordinated to a metal ion (either Zn$^{2+}$, Cu$^{2+}$, or Ni$^{2+}$) through the carboxylate functionalities. By adding a nonpolar solvent to the polar precursor solution, precipitation occurred due to the poor solubility of the NCPs.

1.3.2 Nanoscale Coordination Polymers in Drug Delivery

Since the initial discovery of NCPs, these materials have been investigated for use in drug delivery. Conventional small molecule therapeutics are limited by their rapid clearance and nonspecific distribution in the body, which leads to high doses and adverse side effects.\textsuperscript{32} Incorporation of the small molecule drug into a nanomaterial provides a route to overcome the side effects and to increase the circulation time. Considerable research is being conducted in designing nanoparticles that are capable of controllable release of an active drug at a targeted area of interest and selective delivery of the drug to the target tumor sites.\textsuperscript{33} Many nanoscale systems have been shown to load and release drugs; these systems include: liposomes\textsuperscript{34,35}, polymer micelles\textsuperscript{36}, dendrimers\textsuperscript{37}, inorganic particles\textsuperscript{38,39}, and mesoporous

\begin{thebibliography}{99}
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silica.\(^{40}\) Through extensive research in this area, successful translation into clinical studies has been achieved for few nanocarrier systems, such as doxorubicin (Doxil) and daunorubicin (Daunoxome).

Passive targeting of nanoparticles to the tumor region occurs due to the enhanced permeability and retention (EPR) effect (Figure 1.5).\(^{41}\) Newly formed tumor vessels are abnormal in form and architecture, leading to large fenestrations in which macromolecules can escape through. The EPR effect is known as passive targeting because nanoparticles are unable to penetrate through the tight junctions of normal blood vessels. Furthermore, the nanoparticles are retained in the tumor region due to poor lymphatic drainage. Over time, the concentration of nanoparticles in the tumor site can build up due to the lack of effective drainage.

![Figure 1.5](image)

**Figure 1.5.** The enhanced permeability and retention (EPR) effect which leads to passive tumor targeting by nanoparticles. Illustration provided by Demin Liu.

\(^{40}\) Lu, J.; Liong, M.; Zink, J.; Tamanoi, F. *Small* 2007, 3, 1341-1346.

Additionally, the surface of nanoparticles can be coated or functionalized with many different types of molecules for therapeutic applications. For instance, by incorporating poly(ethylene glycol) (PEG) onto the surface, nonspecific adsorption of proteins onto the nanoparticles can be minimized, increasing the blood circulation time.\(^4\) The hydrophilic nature of PEG attracts water molecules that shield the particle from protein adsorption. In other cases, coating nanocarriers with a lipid bilayer coating aids in creating a neutral surface charge to minimize nonspecific protein interactions. PEGylated liposomes can be utilized to coat the nanoparticles in a neutral bilayer and to increase the blood circulation time by preventing protein interaction with the nanoparticle surface. Also, pegylated liposomes are slow to degrade, resulting in a slow, sustained release of drug from the interior. Up until release, the drug is inert due to its entrapment. Alternatively, molecules can be directly attached to the particle surface to serve as targeting functionalities. Incorporation of a targeting molecule can play a role in cell uptake by specific cell types. Various molecules, including nucleic acid aptamers, antibodies, and small organic compounds, have been used to achieve active targeting of cancer cells. Introduction of functional groups (such as carboxylates) onto these targeting molecules allow for conjugation onto the nanoparticle.

The Lin group has set precedence in the development of NCPs for effective anticancer therapy. Using NCPs as an alternative approach to other nanocarriers provides many advantages due to the properties and characteristics of coordination polymers. NCPs can combine the advantages of both inorganic and organic nanoparticles to exhibit

\(^{42}\) Jokers, J.; Lobovkina, T.; Zare, R.; Gambhir, S. Nanomed. 2011, 6, 715-728.
compositional tunability, biodegradability, high drug loadings,\textsuperscript{43} surface functionalization, and well-defined morphologies.\textsuperscript{44,45} Incorporation of an anticancer therapeutic into the framework of the NCP allows for exceptionally high drug loadings. Secondly, adjusting the synthetic conditions can modify the composition, shape, and size of the NCPs. Additionally, the relatively labile metal-ligand bond makes NCPs biodegradable.

\subsection*{1.3.3 Initial Drug Delivery NCPs}

Direct incorporation of a drug into a NCP framework can be achieved through use of the active agent as the bridging ligand. A cisplatin prodrug, disuccinatocisplatin ($c,c,t$-[PtCl$_2$(NH$_3$)$_2$(O$_2$CCH$_2$CH$_2$CO$_2$H)$_2$]), was designed for use in a Tb(III) NCP.\textsuperscript{46} Dynamic light scattering (DLS) measured the hydrodynamic diameter of the NCPs to be ~58.3 nm in size. To increase the stability of the coordination polymer, the NCPs were encapsulated in silica. The resulting drug release exhibited a $t_{1/2}$ of 9 hours after silica coating, whereas the bare NCPs had a $t_{1/2}$ equal to 1 hour. Active targeting was achieved by functionalizing the Tb-based NCP surface with c(RGDfk), a peptide that targets the $\alpha_v\beta_3$ integrin overexpressed on certain cancer cell lines. The targeted NCPs displayed a higher cytotoxicity than cisplatin ($IC_{50}= 9.7 \mu$M and 13.0 $\mu$M, respectively) when tested against HT-29 human colorectal adenocarcinoma cells. The nontargeted version did not display any cytotoxic effects against


this cell line. The improved cytotoxicity of the c(RGDfk)-targeted NCPs suggest that active targeting can be achieved.

Another method to incorporate small molecules drugs into NCPs is through post-functionalization of the bridging ligands. Lin and coworkers synthesized Fe(III)-carboxylate NCPs with a MIL-101 structure by solvothermal methods (~200 nm in diameter by DLS). Covalent attachment of biologically relevant cargoes was achieved without altering the NCP structure. The NCP was successfully post-synthetically modified with an optical imaging dye (BODIPY) and a cisplatin prodrug. UV-Vis spectroscopy confirmed a BODIPY loading of up to 12 wt%, and ICP-MS confirmed a cisplatin loading of 13 wt%. Confocal microscopy revealed that HT-29 cells internalized the BODIPY-loaded NCPs. Silica coating was again used to increase the stability of the NCP system, which increased the $t_{1/2}$ from 1 hour to 14 hours. Post-functionalization with both an imaging agent and a therapeutic agent suggest that NCPs can be developed for theranostic applications.

1.3.4 Stabilization of NCPs

Although silica coating of NCPs aids in controlled release of the drug, the previously reported NCPs were not stable under physiological conditions. Therefore, alternative NCP systems encapsulated in a lipid bilayer were synthesized. First, NCPs were used to deliver nitrogen-containing bisphosphonates (N-BPs) to cancer cells. The structure of two different N-BPs, zoledronic acid and pamidronic acid, are shown in Figure 1.6. Currently used to treat osteoporosis, N-BPs are not effective anticancer drugs due to their high affinity towards bone.
absorption. By incorporating N-BPs into NCPs, targeted delivery of the drugs to cancer cells can be achieved. Both zoledronate and pamidronate were incorporated into the framework of NCPs through coordination to Ca\(^{2+}\) ions. The as-synthesized NCPs rapidly decomposed in solution (t\(_{1/2}\) = 6.3 h for pamidronate NCP). To control the release of the N-BPs, the surface of the NCPs was coated with single lipid bilayers. Following lipid coating, the drug release kinetics demonstrated a significant increase in the in vitro circulation half-life (t\(_{1/2}\) = 20.1 h for pamidronate NCP). Therefore, it was concluded that the presence of the single lipid bilayer significantly stabilized the NCP systems.

![Structures of two nitrogen-containing bisphosphonates, zoledronic acid and pamidronic acid.](image)

**Figure 1.6.** Structure of two nitrogen-containing bisphosphonates, zoledronic acid and pamidronic acid.

More recently, the self-assembly of two zinc bisphosphonate NCPs containing approximately 48 wt% cisplatin prodrug or 45 wt% oxaliplatin prodrug was reported.\(^4^9\) These NCPs possess hydrodynamic volumes in the range of 20-30 nm as determined by DLS. The NCPs were surrounded by a lipid bilayer and further PEGylated to increase the circulation times. Following PEGylation, the hydrodynamic diameters of the NCPs increased to 40-45 nm, indicating the presence of the lipid bilayer on the particle surface. In vitro stability studies revealed that the near-neutral surface charge of these systems minimized nonspecific protein interactions suggesting that uptake by the MPS can effectively be avoided. Additionally, no premature drug release was observed from these NCPs prior to being placed into a reducing environment similar to that of the tumor site. This suggests that the NCPs can

circulate the body with minimal drug release, but upon arrival at the tumor site, the active agent can be released. Further, the selective delivery of the zinc-based NCPs to solid tumors was achieved in several different mouse models.

1.3.5 Research Objectives

While cisplatin is an effective anticancer therapeutic, this research aimed to minimize the adverse side effects from the small molecule agent by incorporating a cisplatin prodrug into two new NCP platforms containing either calcium or manganese (Chapter 4). Calcium is a biocompatible metal making it a desirable metal for use in drug delivery NCPs. The first system used calcium as the metal ion and a cisplatin prodrug as the bridging ligand to form the NCP framework. The second system incorporated manganese into the cisplatin-based NCP to synthesize a theranostic agent. In vitro stability and cytotoxic assays were conducted on both particle systems.
Chapter 2. B(C₆F₅)₃-CATALYZED HYDROSILYLATIVE DEOXYGENATION OF POLYOLS TO FORM HIGHLY BRANCHED STRUCTURES

2.1 Introduction

The synthesis of branched polyolefins for industrial applications involves polymerization of olefin monomers using either free radical initiators or metal catalysts.¹ In contrast to free radical methods, coordination catalysts have demonstrated the ability to exercise significantly greater control of the polymer microstructure.⁵⁰ Depending on the catalyst selected, control over the both the regiochemistry and stereochemistry of monomer enchainment can be achieved, which provides for polymers with variable properties. The microstructure and molecular weight of a polymer controls the properties of the bulk material, which consequently influences the applications for which it may be used.

We are interested in developing alternative methods to synthesize branched polyolefins through defunctionalization of linear, high MW polyols. The Lewis acid trispentafluorophenylborane (B(C₆F₅)₃) has previously been used as a catalyst in the mild hydrosilylative reduction of C-O bonds.¹⁰,²²,²¹,²⁴ Typically, a hydrosilane, such as diethylsilane, is used as the hydride source in the reduction. Our group has utilized this chemistry to affect the deoxygenation of carbohydrates as a possible approach to biomass conversion to fuels.²⁹ When diethylsilane was combined with the B(C₆F₅)₃ catalyst, an aggressive reduction of glucose ensues to generate a mixture of products wherein hexane and

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hexene isomers were observed, the former being dominant. The formation of branched products (predominantly 2-methylpentane and 3-methylpentane) indicates that elimination and alkyl shifting occurs during the deoxygenation process. Interestingly, the product distribution was dependent on the silane and protecting group used for the glucose OH groups.

In this study, we applied the B(C₆F₅)₃-catalyzed hydrosilylative deoxygenation to linear polyols to explore how reduction would affect the structure of the reduced hydrocarbon polymer. The reduction protocol leads to completely deoxygenated, highly branched polyolefins. We investigated the effect of the silane and protecting group on the branching and amount of elimination that occurs during the deoxygenation. Both ¹H and ¹³C NMR spectroscopy were used as the primary methods to study the polymer structures post-deoxygenation.

2.2 Experimental

2.2.1 Materials

The deoxygenation reactions were performed inside a Vacuum Atmospheres glovebox under an argon atmosphere. All chemicals were purchased from Sigma Aldrich or Fischer Scientific unless otherwise noted. Tris(pentafluorophenyl) borane was purchased from Gelest, Inc. Methylene chloride was degassed via three freeze-pump-thaw cycles and dried over 4Å molecular sieves prior to use. Diethylsilane was dried over 4Å molecular sieves prior to use. Poly(vinyl alcohol) was purchased from Sigma Aldrich with a MW of 9-10,000.
2.2.2 Instrumentation and Analysis

NMR spectra were recorded using a Bruker Avance III spectrometer equipped with a cryoprobe operating at 600 MHz ($^1$H) or 150 MHz ($^{13}$C). NMR chemical shifts are reported in ppm and internally referenced to residual proton peaks ($^1$H) or the $^{13}$C resonances of the deuterated solvent ($^{13}$C). All NMR spectra were recorded in CDCl$_3$ or CD$_2$Cl$_2$. For $^{13}$C-NMR spectroscopy, chromium(III) acetate (Cr(acac)$_3$) was added as a relaxation agent to all polymer samples prior to collecting spectra ($T_1 = 1$ s).

Gel permeation chromatography (GPC) was performed using an Agilent Technologies 1260 Infinity separation module equipped with two Agilent 3 µm MIXED-E 30×7.5mm column and a Wyatt Optilab T-Rex refractive index detector. Polymer samples were prepared in HPLC grade tetrahydrofuran and filtered before injection. Tetrahydrofuran was used as the eluent with a flow rate of 1.0 mL/min at 25 °C. Retention times were calibrated against narrow molecular weight polystyrene standards.

2.2.3 Hydroboration-Oxidation of Polybutadiene

Polybutadiene was hydroborated with borane reagents followed by oxidation using a procedure similar to that previously reported.$^{51}$ A typical hydroboration-oxidation sequence was done as following: 0.5 g of polymer was combined with 50 mL of 0.5 M 9-9-borabicyclo[3.3.1]nonane (9-BBN) in THF under an N2 atmosphere. The reaction was stirred for 3 days at room temperature. To the resulting solution, a mixture of NaOH (3.0 M, 2.0 mL) and aqueous H2O2 (30%, 2.0 mL) was added at 0 °C. The reaction mixture was

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allowed to stir at room temperature overnight. The mixture was dialyzed against 1:1 MeOH:
water for 5 hours, or until pH was neutral. The MeOH: water was changed hourly. The
reaction mixture was then removed from the dialysis tubing, the insoluble fraction was
removed by filtration, and the soluble fraction was rotovapped to isolate the polyol (PBD-
OH, 52% yield). \(^1\)H NMR (600 MHz, Chloroform-\(d\)) \(\delta\) 3.81 – 3.78 (broad, m, 1H), 1.90 –
1.21 (broad, m, 6H). \(^{13}\)C-NMR (600 MHz, Chloroform-\(d\)) \(\delta\) 71.90, 36.43, 31.53, 20.17.

2.2.4 Protection of Polyols

PVA and hydroxylated-polybutadiene were protected with different silanes using the
following procedure: unprotected polymer (0.5 g) was dissolved in 20 mL pyridine and 0.05
moles of chlorosilane were added. The reaction was stirred for 3-4 days before workup. To
quench the silane, 50 mL of water was added. The protected polymer was extracted into
diethyl ether (3 x 50 mL). The ether layers were combined and washed with water (3 x 50
mL), brine (3 x 50 mL), and then dried over MgSO\(_4\). The solvent was removed by rotary
evaporation. The product was triturated with toluene to remove any remaining pyridine and
was dried on the vacuum line overnight. The materials were isolated as viscous oils.

\[
\begin{array}{c}
\text{OTMS} \\
\end{array}
\]

PVA-TMS: 90% yield; \(^1\)H NMR (600 MHz, Methylene chloride-\(d_2\)) \(\delta\) 3.87 (broad,
1H), 1.99*, 1.59 (broad, 2H), 0.12 (broad, 9H). \(^{13}\)C-NMR (600 MHz, Methylene chloride-\(d_2\))
\(\delta\) 170.99*, 67.74, 47.50, 21.63*, 1.63. *Indicates minor peaks in spectrum due to
unhydrolyzed poly(vinyl acetate) in starting material.
PVA-SiMe$_2$Et: $^1$H NMR (600 MHz, Chloroform-$d$) $\delta$ 3.85 (broad, 1H), 1.99*, 1.56 (broad, 2H), 0.92 (3H), 0.57 (2H), 0.09 (6H). $^{13}$C-NMR (600 MHz, Chloroform-$d$) $\delta$ 170.49*, 67.03, 46.90, 21.16*, 9.45, 7.15, 1.19. *Indicates minor peaks in spectrum due to unhydrolyzed poly(vinyl acetate) in starting material.

PVA-TES: $^1$H NMR (600 MHz, Chloroform-$d$) $\delta$ 3.86 (broad, 1H), 1.98*, 1.55 (broad, 2H), 0.93 (9H), 0.54 (6H). $^{13}$C-NMR (600 MHz, Chloroform-$d$) $\delta$ 170.57*, 67.07, 46.94, 21.03*, 9.45, 6.64, 5.88. *Indicates minor peaks in spectrum due to unhydrolyzed poly(vinyl acetate) in starting material.

PBD-OTMS: $^1$H NMR (600 MHz, Chloroform-$d$) $\delta$ 3.78 – 3.66 (m, 1H), 1.83 – 1.50 (m, 4H), 1.49 – 1.28 (m, 2H), 0.17 – 0.05 (s, 9H). $^{13}$C-NMR (600 MHz, Chloroform-$d$) $\delta$ 72.07, 36.47, 20.07, 0.04.

PBD-OSiMe$_2$Et: $^1$H NMR (600 MHz, Chloroform-$d$) $\delta$ 3.71 (m, 1H), 1.83 – 1.57 (m, 4H), 1.38 – 1.32 (m, 2H), 0.91 (t, 3H), 0.54 (q, 2H), 0.04 (s, 6H). $^{13}$C-NMR (600 MHz, Chloroform-$d$) $\delta$ 72.08, 36.56, 20.16, 8.38, 6.72, 2.24.
2.2.5 General Deoxygenation Procedure

In a nitrogen-filled glovebox, silyl-protected polymer (50 mg) and B(C₆F₅)₃ (5 mol% relative to monomer) was dissolved in 0.25 mL CD₂Cl₂ or CH₂Cl₂. Then, 0.25 mL of silane (diethylsilane, dimethylethylsilane, or triethyilsilane) was added. The solution was immediately capped with a vented cap and swirled. After gas evolution subsided, the reaction mixture was transferred to a septum-capped NMR tube and removed from the glovebox to continue reacting for 48 h at 35 °C. At this point, in situ NMR spectra were obtained if desired.

2.2.6 General Silane Removal Procedure

To remove excess silane and silyl ether from the deoxygenation reaction, the reaction mixture was added to a 15-mL plastic centrifuge tube. To the centrifuge tube, 0.80 mL of pyridine-HF was added. The reaction mixture was treated with pyridine-HF for 24 h. Silica was then added to remove any excess HF present. The silica was isolated by vacuum filtration, and the filtrate was rotovapped to yield the branched polyolefin.

2.2.7 General Hydrogenation Procedure

The branched polyolefin was dissolved in 2.0 mL of CH₂Cl₂ and transferred to a Fisher porter vessel. Wilkinson’s catalyst (1 mol%) was added, and the vessel was put under 40 psi of H₂. The reaction was allowed to stir at 40 °C for two days. The catalyst was removed by vacuum filtering over a silica plug, which was washed with 10 mL of CH₂Cl₂. The solvent was then removed by rotary evaporation to yield the hydrogenated, branched polyolefin.
2.3 Results and Discussion

2.3.1 Synthesis and Deoxygenation of TMS-PVA

During the deoxygenation process, the reaction conditions cause any of the free alcohols to become silylated, though the relative rates of OH silylation versus -OSiR₃ deoxygenation are difficult to assess. To aid in studying the deoxygenation and to increase the solubility of the polymer, poly(vinyl alcohol) (PVA, MW=9-10,000) was preprotected as a silyl ether. As shown in Scheme 2.1, this was accomplished with TMS-Cl in pyridine; 48 h reaction times ensured complete formation of silyl ethers, and the desired product was isolated in 90% yield.

**Scheme 2.1.** TMS-Protection of Poly(vinyl Alcohol).

![Reaction scheme](image)

Use of a catalytic amount of commercially available B(C₆F₅)₃ with diethylsilane as the reducing agent caused complete deoxygenation of PVA-TMS to occur (35 °C, 48 h, Scheme 2.2) as ascertained by NMR spectroscopy and gel permeation chromatography (GPC). Since the theoretical mass loss for the conversion of PVA-TMS to PE is 76%, GPC was used to check this loss of mass. This technique separates polymers based on size with high molecular weight polymers eluting first. Figure 2.1 shows the GPC chromatogram of TMS-protected PVA and the corresponding deoxygenated product. Qualitatively, the starting material elutes at a shorter retention time indicating that the product is indeed of lower molecular weight. The broad MW distribution additionally suggests that the deoxygenated polyolefin may have suffered chain scission during deoxygenation, may have become more heterogenous, and/or may have become branched.
Scheme 2.2. Deoxygenation of TMS-PVA with B(C₆F₅)₃ and diethylsilane.

![Scheme Diagram]

Figure 2.1. GPC chromatogram of the starting material (TMS-PVA, black) compared to the deoxygenated product (blue) confirming loss of mass following deoxygenation.

Particularly helpful was $^{13}$C-NMR analysis as this enabled the disappearance of the C-O bond to be followed. To perform quantitative $^{13}$C-NMR analysis, spectra were obtained by dissolving the polymer sample in a 0.05M solution of Cr(acac)$_3$ (relaxation agent) in CDCl$_3$ or CD$_2$Cl$_2$, and a 1 s relaxation delay between scans was used. $^1$H gated decoupling was performed to reduce NOE enhancement. The two characteristic peaks of the starting material at 67 (carbinol $\text{CHOSiR}_3$) and 47 ppm ($\text{CH}_2$) shift to an envelope of peaks upfield of 45 ppm. Figure 2.2 compares the $^{13}$C-NMR spectrum of the starting material (TMS-PVA) to the in situ derived deoxygenation reaction. Based on the in situ NMR spectrum, complete deoxygenation was achieved during this period of time, at least to the detection limit of the
spectra. The presence of excess silane used for the reduction and the \( \text{R}_3\text{Si-O-SiR}_3 \) complicates \(^{13}\text{C}\)-NMR analysis of the deoxygenated polymer structure. These interferents were removed from the deoxygenated polymer by treating with pyridine-HF, which breaks the Si-O bonds through formation of the stronger Si-F bond.\(^5\) Excess silica gel was added to quench any remaining HF. The deoxygenated polymer was isolated by filtration, and the solvent was removed.

Figure 2.2. $^{13}$C-NMR spectra (in CD$_2$Cl$_2$) of PVA-TMS (top) and in situ deoxygenation of PVA-TMS (bottom).

NMR analysis (Figure 2.3) was used to study the purified deoxygenated polymer structure. The $^{13}$C-NMR spectrum shows a complex, highly branched polyolefin. The peak at 68 ppm indicates that this sample remained partially oxygenated. Several peaks were observed in the 120-150 ppm region, indicative of alkene groups and the likelihood of elimination occurring during the deoxygenation process. Most characteristic were the many peaks observed in the 10-50 ppm region of the spectrum, consistent with a highly branched

---

microstructure post-deoxygenation. Since the starting polyol is a linear structure, it appears that the deoxygenation is accompanied by significant alkyl shifting, perhaps reasonably suggesting the intermediacy of carbocations.\textsuperscript{54} For the reduction of TMS-PVA, \textsuperscript{1}H-NMR spectroscopy was used to quantify the degree of branching and elimination in the resulting product. Modifications of the previously reported formula for calculating the number of branches in a polyethylene sample\textsuperscript{14}, Equation 2.1 and Equation 2.2 were used to estimate both the branch and elimination number. It is important to note that Equation 2.2 does not account for the presence of tri- and tetra- substituted alkenes in the elimination calculation, which consequently underestimates the number of alkenes per 1000 C in the product polymer. The expectation is that these would represent low probability events given the linear nature of the starting polymer, but it does represent an uncertainty. The branch numbers for the polyolefin were determined from the ratio of the integration of methyl groups to the integration of total carbons (methyl + methylene + methine + allyl + alkene) in the \textsuperscript{1}H-NMR spectrum. Integration of the methyl \textsuperscript{1}H resonances with respect to the total resonances and applying the formula in Equation 2.2 gave the notably high average degree of branching of $210 \pm 35$ per 1000 C (Table 2.2, Entry 1), which suggests that nearly one out of every two C$_2$ “ethylene” units contains a branch. Similarly, the number of alkenes per 1000 C can be determined by taking a ratio of the alkenes to the total carbon integration. This analysis suggested that the deoxygenated polyolefin possessed an average of around 17 alkenes per 1000 C (1 alkene = 2 alkene carbons).

Figure 2.3. $^{13}$C-NMR spectrum of partially deoxygenated TMS-PVA (in CD$_2$Cl$_2$) after treatment with pyridine-HF. The presence of peaks between 120-140 ppm indicates the likelihood of elimination occurring, whereas peaks between 15-50 ppm indicates the likelihood of rearrangement occurring during the deoxygenation reaction.

Equation 2.1.

$$\frac{\# \text{ Branches}}{1000C} = \frac{\frac{CH_3}{3}}{(2 \times CH_{\text{alkene}} + CH + CH_2 + CH_3) \times 1000}$$

Equation 2.2.

$$\frac{\# \text{ Alkenes}}{1000C} = \frac{\frac{CH_{\text{alkene}}}{2}}{(2 \times CH_{\text{alkene}} + CH + CH_2 + CH_3) \times 1000}$$

An example NMR analysis to determine the number of branches and alkenes present is shown below. The $^1$H-NMR spectrum shown below (Figure 2.4) is following deoxygenation (with borane and SiEt$_2$H$_2$) and pyridine-HF treatment of TMS-PVA. The integration values for each unique proton resonance are presented in Table 2.1.
Figure 2.4. $^1$H-NMR spectrum of deoxygenated, non-hydrogenated TMS-PVA (in CD$_2$Cl$_2$) after treatment with pyridine-HF. Analysis of the proton integrations using Equation 2.1 and Equation 2.2 determined a branch number of 210 and an alkene number of 30 (per 1000 C).

Table 2.1. $^1$H-NMR relative integration values for each unique proton resonance in the deoxygenated polymer produced from the reduction of TMS-PVA with borane and diethylsilane.

<table>
<thead>
<tr>
<th>Proton Type</th>
<th>Methyl</th>
<th>Methylene</th>
<th>Methine</th>
<th>Allyl</th>
<th>Alkene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relative Integration</td>
<td>3.15</td>
<td>7.60</td>
<td>1.23</td>
<td>1.00</td>
<td>0.34</td>
</tr>
</tbody>
</table>

Calculation 1. Determination of branch number for the polymer produced from deoxygenation of PVA-TMS.

$$\frac{\text{# Branches}}{1000C} = \frac{\frac{CH_3}{3}}{(2 \times CH_{\text{alkene}} + CH + CH_2 + CH_3)} \times 1000$$
\[
\frac{\# \text{ branches}}{1000C} = \frac{3.15}{3} \frac{1000}{(2 \times 0.34 + 1.23 + 7.60 + 3.15)} \times 1000
\]

\[
\frac{\# \text{ branches}}{1000C} = \frac{3.15}{3} \frac{1000}{12.66} = \frac{1.05}{6.33} \times 1000
\]

\[
\frac{\# \text{ branches}}{1000C} = 167 \approx 170
\]

**Calculation 2.** Determination of alkene number for the polymer produced from deoxygenation of PVA-TMS.

\[
\frac{\# \text{ Alkenes}}{1000C} = \frac{CH_{\text{alkene}}}{2} \frac{1000}{(2 \times CH_{\text{alkene}} + CH + CH_{2} + CH_{3})}
\]

\[
\frac{\# \text{ Alkenes}}{1000C} = \frac{0.34}{2} \frac{1000}{(2 \times 0.34 + 1.23 + 7.60 + 3.15)}
\]

\[
\frac{\# \text{ Alkenes}}{1000C} = \frac{0.34}{2} \frac{1000}{12.66} = \frac{0.17}{6.33}
\]

\[
\frac{\# \text{ Alkenes}}{1000C} = 27
\]

Even at this stage, the polyolefin structure was too complex to analyze the branching structure present by $^{13}$C NMR spectroscopy. The sample was further simplified by exhaustive hydrogenation of all alkenes using 1 mol% of freshly prepared Wilkinson’s catalyst at 40 psi hydrogen and 40 °C (2 days). A fresh preparation of Wilkinson’s catalyst was key to achieving complete reduction. To remove the catalyst, the mixture was passed through a silica plug eluting with hexanes, and the polymer was isolated by evaporation of the solvent. Figure 2.5 displays the $^{13}$C-NMR spectra of deoxygenated TMS-PVA before and after hydrogenation with Wilkinson’s catalyst, which shows exhaustive hydrogenation was
achieved as evidenced by the disappearance of alkene peaks at 120-140 ppm. The branch number on the hydrogenated polymer was again calculated using Equation 2.2 following hydrogenation, and no change in branch number was observed, confirming that the modified equation provides a reasonable branch number estimation. For convenience, all branch numbers in this chapter were thus calculated prior to hydrogenation of the polymer.

Figure 2.5. $^{13}$C-NMR spectrum of deoxygenated TMS-PVA after pyridine-HF treatment: before (top) and after (bottom) hydrogenation with Wilkinson’s catalyst. Following hydrogenation, the sample no longer appears to contain alkenes, which are characterized by peaks at 120-140 ppm.

Figure 2.6 shows the $^{13}$C-NMR spectrum obtained following exhaustive hydrogenation with Wilkinson’s catalyst. Based on previous literature, the carbon resonances can be assigned to unique types of branches.\textsuperscript{55,56,57} The peak at 30 ppm represents the

\textsuperscript{55} Gottfried, A.; Brookhart, M. Macromolecules \textbf{2003}, \textit{36}, 3085-3100.
methylenes in the middle of a long, straight chain (at least 4 methylene groups on each side).
The other carbon resonances, such as methines at a branch point, have been assigned by
Brookhart and the DuPont group.\textsuperscript{57} Comparison of the hydrogenated polyalkane to
previously reported branched polyethylene samples shows a number of structural similarities,
and some differences. The deoxygenated material contains methyl, propyl, butyl and longer
branches, but does not apparently contain ethyl branches, which are characterized by a
resonance at \textasciitilde11 ppm. In contrast to the branched polymers reported by Brookhart for Ni-
catalyzed ethylene polymerization, the methyl peaks (20 ppm) in the present case appear to
be more complex. Notably, the peak(s) at 46 ppm, which is characteristic of a methylene
between two branch points, is significant in the present samples, but completely absent in the
Ni-derived branched polyethylene.\textsuperscript{57,58}


\textsuperscript{58} Hsieh, E.; Randall, J. \textit{Macromolecules} \textbf{1982}, 15, 1402-1406.
The deoxygenation of poly(vinyl alcohol) samples by a combination of borane and diethylsilane leads to complete deoxygenation and the introduction of significant branching in the resulting polymer. Although this reactivity parallels observations on the conversion of glucose to hexanes, the amount of branching was surprisingly high.

2.3.2 Effect of Silane and Protecting Group

Previously, the Gagné lab demonstrated that both the protecting group and the silane affect the selectivity and reactivity in glucose deoxygenation chemistry.\(^{29}\) For instance, the rate and selectivity of the reduction of Me\(_2\)EtSi-protected glucitol were affected when the hydrosilane was changed from diethylsilane to dimethylethylsilane (Figure 2.6), indicating a
significant steric effect for this specific experiment. In the deoxygenation of Me$_2$EtSi-protected glucitol with dimethylethylsilane, only partial consumption of the C-O bonds was observed even after reaction times of 1 week. Under these reaction conditions, reduction of primary C-O bonds was kinetically favored. When diethylsilane was used as the hydrosilane, complete reduction of glucitol occurred within 2 h.\textsuperscript{29}

![Figure 2.7. Reduction of Me$_2$EtSi-protected glucitol with different hydrosilanes, demonstrating that the selectivity and reactivity can be altered through changing the deoxygenation conditions.](image)

Therefore, attempts were made to vary the protecting group or silane to see if either of these parameters affected the amount of branching and elimination that occurred during the deoxygenation. In my first set of experiments, the identity of the protecting group was held constant at TMS (i.e. TMS-PVA) and the reducing silane was varied from diethylsilane to dimethylethylsilane to triethylsilane. In small molecule studies, when hydrosilanes were employed as the reducing agent, the reactions tended to proceed at a slower rate. However, in the case of all polymeric samples, no difference in reaction rate was observed when the hydrosilane was varied. The experiments were monitored by $^{13}$C-NMR spectroscopy to ensure complete deoxygenation was achieved. For all three reaction conditions, the polymer was not fully deoxygenated until \(~48\) h reaction time was achieved. The samples were purified by treatment with pyridine-HF to remove excess silane and silyl ethers from the
deoxygenated materials. Following sample purification, $^1$H and $^{13}$C-NMR spectra were obtained on a Bruker Avance III 600 MHz spectrometer with CD$_2$Cl$_2$ or CDCl$_3$ as the solvent. For all $^{13}$C-NMR analyses, a 0.05 M solution of Cr(acac)$_3$, a relaxation agent, in the deuterated solvent was used to allow for quantitative analysis of the types of branches present in the structure. Each experiment was performed at least twice to obtain average branching and elimination values.

By changing the reducing silane to the bulkier dimethylethylsilane (Table 2.2, Entry 2), the calculated degree of branching decreased to about 150 branches per 1000 C, though the degree of elimination remained unchanged at ~12 alkenes per 1000 C. However, when triethylsilane was used (Table 2.2, Entry 3), the degree of branching was high (~260 branches per 1000 C), and the number of alkenes remained at about 14 alkenes per 1000 C.
Table 2.2. $^1$H-NMR analysis of branching and elimination present in deoxygenated PVA polymer samples.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Polymer$^a$</th>
<th>Silane$^b$</th>
<th># Branches (per 1000C)$^c$</th>
<th># Alkenes (per 1000C)$^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TMS-PVA</td>
<td>SiEt$_2$H$_2$</td>
<td>210 ± 35</td>
<td>17 ± 11</td>
</tr>
<tr>
<td>2</td>
<td>TMS-PVA</td>
<td>SiMe$_2$EtH</td>
<td>150 ± 5</td>
<td>12 ± 8</td>
</tr>
<tr>
<td>3</td>
<td>TMS-PVA</td>
<td>SiEt$_3$H</td>
<td>260 ± 5</td>
<td>14 ± 6</td>
</tr>
<tr>
<td>4</td>
<td>SiMe$_2$Et-PVA</td>
<td>SiEt$_2$H$_2$</td>
<td>200 ± 35</td>
<td>23 ± 17</td>
</tr>
<tr>
<td>5</td>
<td>SiMe$_2$Et-PVA</td>
<td>SiMe$_2$EtH</td>
<td>165 ± 35</td>
<td>24 ± 4</td>
</tr>
<tr>
<td>6</td>
<td>TES-PVA</td>
<td>SiEt$_2$H$_2$</td>
<td>205 ± 4</td>
<td>Trace</td>
</tr>
<tr>
<td>7</td>
<td>TES-PVA</td>
<td>SiMe$_2$EtH</td>
<td>200$^*$</td>
<td>Trace</td>
</tr>
</tbody>
</table>

$^a$ Prepared from 9-10,000 MW PVA.  
$^b$ Conditions for reductions: 5 mol% B(C$_6$F$_5$)$_3$, xs. silane, in CH$_2$Cl$_2$, 35 °C, 48 hr.  
$^c$ By $^1$H-NMR spectroscopy of non-hydrogenated polymer using Equation 2.1.  
$^d$ By $^1$H-NMR spectroscopy of non-hydrogenated polymer using Equation 2.2.  
* Only 1 run was performed under these conditions.

Additionally, the silyl protecting group was varied, and the branch and alkene numbers were calculated in the resulting deoxygenated polymers. As shown in Table 2.2, increasing the steric hindrance of the protecting group and silane did not lead to a clear trend in the branching or alkene number. Figure 2.7 shows the $^1$H-NMR spectra of the deoxygenated polymers resulting from reduction of TMS-PVA, SiMe$_2$Et-PVA, and TES-PVA. Each of the unique proton resonances were integrated and used in Equations 2.1 and 2.2 to determine the degree of branching and elimination present in the polymer following complete reduction. Further investigation into the polymer microstructures produced from the different hydrosilanes revealed very different branching structures. For instance, Figure 2.8 compares the branching microstructure of the polymers derived from the deoxygenation of TMS-PVA, SiMe$_2$Et-PVA, and TES-PVA.
Figure 2.8. $^1$H-NMR spectra of deoxygenated, non-hydrogenated TMS-PVA (top, in CDCl$_3$), SiMe$_2$Et-PVA (middle, in CDCl$_3$), and TES-PVA (bottom, in CD$_2$Cl$_2$) used to calculate branch and alkene number (using SiEt$_2$H$_2$ as the hydrosilane).
Figure 2.9. $^{13}$C-NMR spectra of deoxygenated, hydrogenated TMS-PVA (top, in CDCl$_3$), SiMe$_2$Et-PVA (middle, CDCl$_3$), and TES-PVA (bottom, in CD$_2$Cl$_2$). Deoxygenation of TMS-PVA results in a structure containing a high degree of methyl branches, whereas deoxygenation of TES-PVA results in a structure with ethyl yet no methyl branches.

From the $^{13}$C-NMR spectra in Figure 2.8, one can reconstruct representative structures for the three different branched polymers (Figure 2.9). All three samples have branch numbers exceeding 200 branches per 1000 C, but the type of branches vary considerably. Deoxygenation of TMS-PVA results in a branched microstructure containing a large number of methyl branches, as indicated by the characteristic peaks at around 20 ppm in the $^{13}$C-NMR spectrum. As indicated by a peak at 11 ppm, this sample was devoid of ethyl branches. When SiMe$_2$Et-PVA was reduced, the peak at ~20 ppm disappeared indicating a lack of methyl branches in the structure. Only long chain branches (C$_4+$) were present in this sample. In the TES-PVA case, no methyl branches were present following deoxygenation. However, ethyl branches were present as seen in the $^{13}$C-NMR spectrum. The peak at 11 ppm
is characteristic of the methyl at the end of an ethyl branch. All three samples contained butyl or longer chains.

**Figure 2.10.** Schematic representation of polymers obtained by deoxygenation of TMS- and TES-PVA as assessed by $^{13}$C-NMR analysis and comparing to the database of assignments compiled by Brookhart and McLain.\textsuperscript{11,13,14}

It should be noted that silyl exchange can occur between the protecting group and the hydrosilane in small molecule studies, the possibility of which complicates the analysis for these polymeric samples. For example, when starting with TMS-protected PVA and reducing with B(C$_6$F$_5$)$_3$ and dimethylethylsilane, protecting group exchange could occur to provide a partially SiMe$_2$Et-protected alcohol (Figure 2.10). Although one begins with a homogeneously protected polymer, the identity of the silyl-protecting group at the point of time that a specific C-O bond becomes reduced is ambiguous. In small molecule analogs, however, it has been observed that larger silicon-protecting groups tend to react slower.

**Figure 2.11.** Proposed exchange of silicon-protecting group that can occur during the B(C$_6$F$_5$)$_3$ reduction.

Complete deoxygenation of silyl-protected PVA results in a highly branched polyolefin with branch numbers ranging from 150-210 branches per 1000 carbons. The presence of branches and alkenes in the deoxygenated polymer reveals that rearrangement
and elimination occurs during the course of the reaction. However, attempts to study the mechanism in detail have proved difficult. The B(C₆F₅)₃-catalyzed reduction of small molecule substrates produced the reduced alkane, along with minor eliminated products. The physical properties of polymers in solution (such as chain entanglement) make this system difficult to model. Furthermore, silyl group exchange is likely occurring, compounding difficulties to determine the actual protecting group present at any given time during the reaction.

2.3.3 Synthesis and Deoxygenation of a PBD-OH

The methylene spacers between the hydroxyl groups were increased to study the deoxygenation of a 1,5-polyol (PBD-OH). A sample of polybutadiene was modified by a hydroboration-oxidation scheme to introduce the alcohol functionality. This is an alternative approach to copolymerizing ethylene and vinyl acetate (followed by hydrolysis), which would produce a similar polymer. However, polymerization of an olefin and vinyl acetate would lead to a random structure, and the alternating copolymerization of the two monomers in a precise 1:1 ratio is difficult to achieve. By hydroboration-oxidation of polybutadiene using a previously reported procedure,⁷ a regular alternating copolymer was synthesized (Scheme 2.3). First, polybutadiene was hydroborated with 9-BBN in THF. Subsequent H₂O₂ oxidation yielded the desired polyol. The $^{13}$C-NMR spectrum confirmed that the hydroxyl-functionality was present on every 4th carbon to form a 1,5-polyol (Figure 2.11). As shown in the $^{13}$C-NMR spectrum, minor signals were observed at ~35 ppm, perhaps resulting from regioirregularities (ie combinations of 1,3 and 1,5 diol arrays) or hydroxyl ethyl defects from hydroboration-oxidation of 1,2-insertion mistakes installed during the butadiene polymerization. Additionally, $^{13}$C-NMR analysis was used to determine the hydroxy-content; the isolated material was determined to be >98% hydroxylated.
Scheme 2.3. Hydroboration-oxidation of polybutadiene to form a 1,5-polyol.

Prior to deoxygenation, PBD-OH was silyl-protected with a range of protecting groups (TMS-, SiMe$_2$Et-, or TES-) by reacting with the corresponding silyl chloride for 2-3 days in the presence of pyridine. The silyl ethers were formed in good yields. The silyl protected-1,5-polyol was then deoxygenated using B(C$_6$F$_5$)$_3$ and various hydrosilanes (Scheme 2.4). Following deoxygenation and deprotection using pyridine-HF, both $^1$H- and $^{13}$C-NMR analysis was used to compare the branching and elimination in the polymer structure (Table 2.3). The protecting group used did not have an effect on the branch or alkene number as calculated from $^1$H-NMR spectra of non-hydrogenated polymers. In the case of the reducing silane, when the bulkier SiMe$_2$EtH silane was used, initial trends suggest a decrease in branch number was achieved. For instance, deoxygenation of PBD-OTMS
using SiEt₂H₂ resulted in about 195 branches per 1000C and minimal alkenes (Table 2.3, Entry 1). Changing the silane to SiMe₂EtH yielded a polyolefin with about 160 branches per 1000C (Table 2.3, Entry 2).

Scheme 2.4. General scheme for the deoxygenation of hydroxylated-polybutadiene.

![Scheme 2.4](image)

Table 2.3. ¹H-NMR analysis of branching and elimination present in deoxygenated PBD-OH polymer samples.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Polymer a</th>
<th>Silane b</th>
<th># Branches (per 1000C) c</th>
<th># Alkenes (per 1000C) d</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PBD-OTMS</td>
<td>SiEt₂H₂</td>
<td>195</td>
<td>trace</td>
</tr>
<tr>
<td>2</td>
<td>PBD-OTMS</td>
<td>SiMe₂EtH</td>
<td>160</td>
<td>trace</td>
</tr>
<tr>
<td>3</td>
<td>PBD-OSiMe₂Et</td>
<td>SiEt₂H₂</td>
<td>195</td>
<td>trace</td>
</tr>
<tr>
<td>4</td>
<td>PBD-OSiMe₂Et</td>
<td>SiMe₂EtH</td>
<td>160</td>
<td>trace</td>
</tr>
</tbody>
</table>

a Prepared from commercially available polybutadiene (MW=200-300,000).  
b Conditions for reductions: 5 mol% B(C₆F₅)₃, x.s. silane, in CH₂Cl₂, 35 °C, 48 hr.  
c By ¹H-NMR spectroscopy of non-hydrogenated polymer using Equation 2.1.  
d By ¹H-NMR spectroscopy of non-hydrogenated polymer using Equation 2.2.

Again, the polyolefins were hydrogenated using Wilkinson’s catalyst to facilitate the NMR analysis of branching structure. Following hydrogenation, the ¹³C- NMR spectrum showed the disappearance of alkene peaks and a more simplified alkane region. The hydroxylated-polybutadiene system produced similar results to PVA deoxygenation. Both high molecular weight polymers were reduced to a highly branched polyolefin. Figure 2.12 highlights the branched microstructure produced following deoxygenation of PBD-OTMS. The disappearance of the characteristic C-O peak at 72 ppm and the appearance of multiple peaks in the alkane region (10-45 ppm) were observed, indicating complete deoxygenation and skeletal rearrangement had occurred. Figure 2.14 highlights the difference in branching microstructure between deoxygenated PBD-OTMS and PVA-TMS. Comparison of the two
$^{13}$C-NMR spectra suggests that a branched polyalkane was obtained, but the types of branches present following deoxygenation varies.

**Figure 2.13.** Comparison of $^{13}$C-NMR spectra of PBD-OH (top) and deoxygenated PBD-OTMS (with SiEt$_2$H$_2$) after pyridine-HF treatment. Following complete deoxygenation, a branched hydrocarbon structure is observed.
Further experiments to decrease and possibly stop the branching are proposed. Investigations into acyclic diene metathesis (ADMET) polymerizations of alcohol-functionalized dienes to form other linear polyols would provide another route to spread out the alcohol functionalities, which may hinder any neighboring group participation. Additionally, the concentration of the polymer in solution may have an impact on the branch number observed. For instance, using a lower polyol concentration in the deoxygenation may result in fewer alkyl shifts between chains due to less chain entanglement.
2.3.4 Investigation of Small Molecule Models

The degree of branching for the deoxygenation of PVA-TMS with diethylsilane was determined to be approximately 170 branches per 1,000 carbons, which is equivalent to nearly one branch for every two monomer units. Therefore, it seems that the branching mechanism is occurring in a controlled manner. Small molecule model substrates were deoxygenated under the same reaction conditions to determine if similar rearrangement mechanisms were operative. First, 2-methyl-3-hexanol was selected as a model substrate because it would similarly contain an alcohol functionality next to a branch point. However, deoxygenation of 2-methyl-3-hexanol (Scheme 2.3) resulted in clean reduction of the alcohol to 2-methylhexane, i.e. no additional branching occurred. Next, the small molecule diols, 2,5-hexanediol and 2,4-pentanediol, were studied. After B(C₆F₅)₃-reduction with diethylsilane, the major product in both cases was the reduced n-alkane with traces of alkene (Scheme 2.4 and Scheme 2.5). Since screening of these small molecule substrate analogs failed to reproduce the branching behavior of the polymers, it suggests that the high MW polyols are inherently more complex. Key differences may include the entangled nature of polymers in solution, which may contribute to the alkyl shifting during the reaction mechanism.

Scheme 2.5. Deoxygenation of 2-methyl-3-hexanol with B(C₆F₅)₃ and diethylsilane.
Scheme 2.6. Deoxygenation of 2,5-hexanediol with B(C₆F₅)₃ and diethylsilane.

\[
\text{xs. SiEt₂H₂} \quad 5 \text{ mol\% B(C₆F₅)₃} \\
\text{CD₂Cl₂, RT} \\
\text{OH} \quad \text{OH} \\
\xrightarrow{16} + \\
\xrightarrow{3} + \\
\text{1} \\
>98\% \text{ conversion}
\]

Scheme 2.7. Deoxygenation of 2,4-pentanediol with B(C₆F₅)₃ and diethylsilane.

\[
\text{xs. SiEt₂H₂} \quad 5 \text{ mol\% B(C₆F₅)₃} \\
\text{CD₂Cl₂, RT} \\
\text{OH} \quad \text{OH} \\
\xrightarrow{3} + \\
\xrightarrow{1} \\
>98\% \text{ conversion}
\]

2.3.5 Mechanistic Insights

It is conceivable that the deoxygenation may proceed by a mechanism that exists on a continuum of pathways ranging from totally random deoxygenation or a blocky zipper-like process. If the deoxygenation occurs in a random manner, where any C-O site may be reduced, the intermediate structure will become more complex as the reaction initially proceeds (Figure 2.13). The starting PVA polymer is a simple, linear structure with two characteristic peaks at 67 and 47 ppm in the $^{13}$C-NMR spectrum. One might expect the structure to quickly increase in complexity if random C-O bonds are cleaved. If this were the case, the $^{13}$C-NMR spectrum of the partially deoxygenated material would show an immediate appearance of alkane branches in the 10-45 ppm region with the disappearance of the starting polymer peaks at 67 and 47 ppm. Additionally, the PVA peaks would become more complex, either through the appearance of additional peaks or an increase in broadness, with initial random deoxygenation.
Possible deoxygenation mechanism in which the deoxygenation proceeds through a random process. In this case, one would expect the sample to go from a simple to highly complex to lower complexity structure, as observed in the $^{13}$C-NMR spectrum. P indicates silyl protecting group.

Another pathway that the deoxygenation mechanism may proceed through is a “zipper” effect, in which deoxygenation begins at the least sterically hindered end of a polyol chain and reduces by walking down the chain reducing adjacent C-O bonds (Figure 2.14). In this scenario, the characteristic PVA peaks in the $^{13}$C-NMR spectrum should remain unchanged in complexity, but disappear as the deoxygenation proceeds. The alkane region would appear simpler with branches growing in as the reaction proceeded.
Figure 2.16. Possible deoxygenation mechanism in which the deoxygenation proceeds through a controlled, zippered process where deoxygenation starts at the least sterically hindered end and walks down the chain. P indicates the silyl protecting group.

To probe the deoxygenation mechanism, an experiment that followed the deoxygenation over time was designed. Samples that tracked the deoxygenation of TMS-PVA with B(C₆F₅)₃/diethylsilane were obtained by quenching with triethylamine at specific time points (15 min, 1 h, 5 h, and 48 h). After quenching, in situ $^{13}$C-NMR spectra were obtained to capture the loss of PVA units and growth of polyalkane units and to determine if random C-O bonds were being reduced or if a zipper effect was occurring (Figure 2.15). Following treatment with B(C₆F₅)₃ and diethylsilane for 15 minutes, the peak at 47 ppm from the methylenes of the starting PVA broadens and displays a new shoulder peak slightly upfield. This is accompanied by a significant number of peaks in the alkane region. Together, these observations therefore imply that the deoxygenation occurs in a mostly random manner. As random C-O bonds continue to be reduced, the structure grows in complexity (at
time points 1 h and 5 h), yet after complete deoxygenation, the branch points converge into a more simplified structure.

**Figure 2.17.** Time course $^{13}$C-NMR analysis of the deoxygenation of TMS-PVA monitoring disappearance of starting material and appearance of branched product. Peak at 47 ppm, indicative of methylenes in starting polymer, initially broadens and then disappears at later times. Multiple peaks increase in intensity in alkane region (15-45 ppm), showing an increase in the number and types of branches as the reaction proceeds.

Attempts to study the deoxygenation reaction through timed NMR experiments proved difficult. The initial broadening of peak at 47 ppm from the starting methylenes in TMS-PVA, suggests that the structure becomes more complex. However, as time proceeds,
the peak sharpens, indicating a more simplified polymer structure. The complexity of the reaction and the structure complicates analysis of the deoxygenation reaction.

2.4 Conclusions

In conclusion, the hydrosilylative reduction of polyols using B(C₆F₅)₃ and a hydrosilane to give a branched polymer has been demonstrated. Deoxygenation of linear polyols, such as PVA and PBD-OH, results in unsaturated branched polyolefins, where the branched microstructure is dependent on the silyl protecting group and the silane used as the hydride source. This makes it possible to develop polyolefins with unique branch structures.

2.5 Acknowledgements

The Army Research Office and the Department of Energy primarily supported this work. I would like to acknowledge Laura Adduci and Trandon Bender for their discussions and expertise.
Chapter 3. SYNTHESIS OF A LINEAR-BRANCHED-LINEAR TRIBLOCK POLYMER THROUGH HYDROSILYLATIVE DEOXYGENATION

3.1 Introduction

Block copolymers are a class of materials that have interesting properties due to the presence of different polymer blocks in the chain.\(^{59}\) Styrene-\textit{b}-butadiene-\textit{b}-styrene (SBS) and its hydrogenated version styrene-\textit{b}-ethylene/butadiene-\textit{b}-styrene (SEBS) are two widely used commercial triblock polymers.\(^{60,61}\) The combination of two incompatible materials allow for both elastic and thermoplastic behavior in the same material. By varying the length of the butadiene interior, different properties can be achieved.\(^{62}\) For instance, when a shorter butadiene block is present, the rigid material is used in plastic storage containers. Increasing the butadiene chain to a larger block size leads to a more rubbery material, which is ideal for use in applications like shoe soles. Hydrogenation of the butadiene interior block of SBS leads to a tougher material with greater weather resistance.


In the previous chapter, an alternative method to synthesize branched polyolefins through deoxygenation of linear polyols was reported. The Lewis acid trispentafluorophenylborane B(C₆F₅)₃ was used as a catalyst, along with silane, to reduce high MW polyols. Previous reports have used B(C₆F₅)₃ as a mild catalyst in the reduction of primary and secondary alcohols.²⁰,²²,²¹,²⁴ Typically, a hydrosilane, such as diethylsilane, is used as the hydride source in the reduction. Application of this chemistry to linear polyols resulted in highly branched polyolefins with branch numbers up to 236 (per 1000 C). The branching microstructure was dependent on the alcohol protecting group and silane used.

In this chapter, the synthesis of a hydroxylated-SBS (SBS-OH), in which the polybutadiene block has every point of unsaturation converted to an alcohol by a hydroboration-oxidation sequence, is reported. Reduction of SBS-OH was achieved using catalytic amounts of B(C₆F₅)₃ with hydrosilane. The material was completely deoxygenated to yield a new triblock polymer with a highly branched interior block. To the best of our knowledge, the synthesis of a linear-highly branched-linear triblock polymer has not yet been reported.

3.2 Experimental

3.2.1 Materials

The deoxygenation reactions were performed inside a Vacuum Atmospheres glovebox under an argon atmosphere. All chemicals were purchased from Sigma Aldrich or Fischer Scientific unless otherwise noted. Tris(pentafluorophenyl)borane was purchased from Gelest, Inc. Methylene chloride was degassed via three freeze-pump-thaw cycles and dried over 4Å molecular sieves prior to use. Diethylsilane was dried over 4Å molecular sieves prior to use.
3.2.2 Instrumentation and Analysis

NMR spectra were recorded using a Bruker Avance III spectrometer equipped with a cryoprobe operating at 600 MHz (\(^1\)H) or 150 MHz (\(^{13}\)C). NMR chemical shifts are reported in ppm and internally referenced using the residual proton peaks (\(^1\)H) or the \(^{13}\)C resonances of the deuterated solvent (\(^{13}\)C). All NMR spectra were recorded in CDCl\(_3\) or CD\(_2\)Cl\(_2\) unless otherwise noted. For \(^{13}\)C-NMR spectroscopy, chromium(III) acetate (Cr(acac)\(_3\)) was added as a relaxation agent to all polymer samples prior to collecting spectra (d\(_1\) = 1 s).

3.2.3 Synthesis of Poly(styrene-\(b\)-butanol-\(b\)-styrene)

\[
\text{SBS triblock polymers were hydroborated and oxidized using a similar procedure as previously reported.}^{51} \text{ A typical hydroboration-oxidation was done as following: 0.5 g of polymer was combined with 50 mL of 0.5 M 9-BBN in THF under an N}_2 \text{ atmosphere. The reaction was stirred for 3 days at room temperature. To the resulting solution, a mixture of NaOH (3.0 M, 2.0 mL) and aqueous H}_2\text{O}_2 \text{ (30\%, 2.0 mL) was added at 0 °C. The reaction mixture was allowed to stir at room temperature overnight. A white precipitate formed. The mixture, including the precipitate, was dialyzed against 1:1 MeOH: water for 5 hours, or until the pH was neutral. The MeOH: water was changed hourly. The reaction mixture was then removed from the dialysis tubing, the insoluble fraction was dried under vacuum, and the soluble fraction was rotovapped to isolate the soluble polyol fraction (SBS-OH).}
\]
3.2.4 General Deoxygenation Procedure

In a nitrogen-filled glovebox, SBS-OH (50 mg) and B(C₆F₅)₃ (5 mol% relative to monomer) was combined with 0.250 mL CD₂Cl₂ or CH₂Cl₂. Then, 0.250 mL of silane (diethylsilane, dimethylethylsilane, or triethylsilane) was added. The solution was immediately capped with a vented cap and swirled. After gas evolution subsided, the reaction mixture was transferred to a septum-capped NMR tube and removed from the glovebox to continue reacting for 48 h at 35 °C. At this point, NMR spectra were obtained if desired.

3.2.5 General Silane Removal Procedure

To remove excess silane and silyl ether from the deoxygenation reaction, the reaction mixture was added to a 15-mL plastic centrifuge tube. To the centrifuge tube, 0.80 mL of pyridine-HF was added. The reaction mixture was treated with pyridine-HF for 24 h. Silica was then added to remove any excess HF present. The silica was isolated by vacuum filtration, and the filtrate was rotovapped to yield the deoxygenated triblock polymer.

3.2.6 General Hydrogenation Procedure

The branched polyolefin was dissolved in 2.0 mL of CH₂Cl₂ and transferred to a Fisher-Porter vessel. Wilkinson’s catalyst (1 mol%) was added, and the vessel was put under 40 psi of H₂. The reaction was allowed to stir at 40 °C for two days. The catalyst was removed by vacuum filtering over a silica plug, which was washed with 10 mL of CH₂Cl₂. The solvent was then removed by rotary evaporation to yield the hydrogenated, branched polymer.
3.2.7 Hydrogenation of SBS

The SBS polymer was hydrogenated based on a previously reported procedure.\textsuperscript{63} In a Schlenk flask, SBS (0.5 g) was dissolved in 25 mL of \textit{p}-xylene, and 0.025 mL of BHT was added. Tosylhydrazide (1.9 g) was added to the flask, and the mixture was degassed twice by freeze-pump-thaw cycles. The solution was placed under a N\textsubscript{2} atmosphere, and a reflux condenser was attached to the Schlenk flask. The mixture was heated at 120 °C for 3 days. The solution was cooled to room temperature before pouring into rapidly stirring methanol to isolate the hydrogenated SEBS. The polymer was dried under vacuum (65% yield, 85% hydrogenated by NMR analysis).

3.3 Results and Discussion

3.3.1 Synthesis and Deoxygenation of Hydroxylated-SBS

The molecular weight of styrene-\textit{b}-butadiene-\textit{b}-styrene was to be 50,900 from by GPC. The triblock polymer contains 30 wt% styrene, which is equivalent to approximately roughly 140 PS and 650 butadiene repeat units. The interior butadiene block was post-synthetically modified to incorporate alcohol functionalities. SBS was hydroborated and oxidized using a previously reported procedure (Scheme 3.1). First, SBS was treated with 9-BBN to hydroborate the polymer. Subsequent H\textsubscript{2}O\textsubscript{2} oxidation led to formation of a white precipitate. The reaction mixture, including the precipitate, was dialyzed to remove any salts. The precipitate was then isolated by vacuum filtration. Attempts were made to dissolve the precipitated solid with no success. Therefore, it is suspected that a material with ether crosslinks formed.

\begin{footnote}{63}Wu, Z.; Grubbs, R. H. \textit{Macromolecules} \textbf{1995}, 28, 3502-3508.\end{footnote}
The crosslinked SBS-OH precipitate was deoxygenated using catalytic amounts of B(C₆F₅)₃ with diethyilsilane with a procedure that simultaneously protects the free alcohols as the silyl ethers. The reaction mixture was heated at 35 °C for 3 days to achieve complete deoxygenation. As the reaction proceeded and the proposed ether linkages were cleaved, the material was solubilized. ¹³C-NMR analysis was used to monitor the reaction progress through disappearance of the C-O bonds at ~70 ppm in the spectrum. As the material began to solubilize, a peak at ~70 ppm appeared in the spectrum indicative of remaining C-O bonds. However, following complete deoxygenation, this peak disappeared completely. To remove excess silane and silyl ethers from the deoxygenated polymer, the sample was treated with pyridine-HF, which breaks up the Si-O bonds through formation of the stronger Si-F bond. Excess silica gel was added to quench any remainder HF. The deoxygenated polymer was isolated by filtration, and the solvent was removed to isolate an oily substance.

Both ¹H- and ¹³C-NMR analysis were used to study the deoxygenated polymer structure. The ¹³C-NMR spectrum confirms that deoxygenation was in fact achieved and that skeletal rearrangement occurred during the reaction to produce a linear-branched-linear triblock system. In the case of a homopolymer, ¹H-NMR spectroscopy is typically used to
determine the branch number through comparative integration of the methyl protons of the branches relative to the total proton integration. However, the degree of branching could not be determined through similar analysis, as the protons of the styrene block tend to overlap with the methyl and methine protons of the branched polyolefin interior.

Figure 3.1. $^1$H-NMR analysis of (left) highly branched polyolefin synthesized through $\text{B(C}_6\text{F}_5\text{)}_3$/diethylsilane deoxygenation of PVA-TMS and of branched triblock polymer (right). The degree of branching cannot be determined through integration due to overlap of the PS protons.

Further analysis of the $^{13}$C-NMR spectrum was conducted to determine the types of branching present in the triblock polymer. In terms of the butadiene block, the resulting alkene can be formed in three different ways: cis, trans, and vinyl. The cis and trans isomers arise from 1,4 polymerization, whereas the vinyl form results from 1,2-

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polymerization of butadiene. The properties of the three different forms vary. Cis-polybutadiene has a high elasticity, whereas the trans-polybutadiene is rather straight, forming microcrystalline regions in the chain. The vinyl groups can appear as branch points in the deoxygenated structure. Therefore, it was necessary to hydrogenate SBS to provide a reference structure for comparison. SBS was hydrogenated through use of tosylhydrazide via in situ generation of a diimide to form SEBS (Scheme 3.3).

**Scheme 3.3.** Hydrogenation of SBS with tosylhydrazide in xylene to form SEBS.

As shown in Figure 3.1, the $^1$H-NMR spectrum of SEBS confirmed that the material was 85% hydrogenated. The allylic protons in the starting material appear at 1.96 ppm, and the methylene protons in the hydrogenated product appear at 1.25 ppm. The relative integrations of these peaks were used to determine the hydrogenation percentage. The $^{13}$C-NMR spectrum of SEBS (Figure 3.3) does show the presence of alkenes in the 120-140 ppm region, indicating that full hydrogenation was not achieved.
Figure 3.2. $^1$H-NMR spectrum of hydrogenated SBS (SEBS) in CDCl$_3$. Only 85% hydrogenation of the sample was achieved, as indicated by the residual alkene peak at 5.4 ppm.
Figure 3.3. $^{13}$C-NMR spectrum (CDCl$_3$, 0.5 M Cr(acac)$_3$) of SBS that is ~85% hydrogenated as calculated from $^1$H-NMR spectroscopy (Figure 3.2). The PS aromatic peaks are found at 145, 130, 128, and 125 ppm, while the PS backbone makeup the broad peak at 40 ppm. The peak at 29 ppm is characteristic of the methylene backbone of the hydrogenated interior block. The remaining peaks are indicative of defects from the polymerization.

The defects present in the butadiene interior block of SEBS were further analyzed by $^{13}$C-NMR spectroscopy. Figure 3.4 shows the alkane region of the NMR spectrum and provides further peak assignments based on the known defects present. The 15% of unhydrogenated polybutadiene exists as 1,4-cis, 1,4-trans, or vinyl groups. Because complete hydrogenation was not achieved, all three of these forms are observed in the $^{13}$C-NMR spectrum. For instance, the allylic carbon from 1,4-cis polymerization appears at 32.32 ppm, and the allylic carbon from 1,4-trans polymerization is observed at 26.91 ppm. The carbon at a vinyl branch appears at 38.53 ppm. After hydrogenation, the vinyl group becomes an ethyl branch off the polymer backbone, with peaks observed at 25.57 and 10.58 ppm.
The hydrogenated SEBS material was then compared to the deoxygenated triblock sample. Figure 3.5 overlays the $^{13}$C-NMR spectra of SEBS and the deoxygenated product. Not only are the expected defects from the butadiene polymerization observed in the NMR spectrum of the deoxygenated material, but additional peaks due to branching are observed. The branches were assigned based on previous assignments in the literature.$^{55,56,57}$ The branching observed is predominantly long chains. For instance, the peak at 14 ppm is indicative of methyls from butyl and larger chains, while the peak at 22.5 ppm indicates the 2$^{nd}$ carbon from the end of the chain for pentyl and larger branches. It should be noted that the broad peak from 40-41 ppm due to the polystyrene block is unchanged after the
hydrosilylative reduction, which suggests that the polystyrene unit is not rearranging during the reaction.

![Figure 3.5. $^{13}$C-NMR analysis of SEBS (top) and deoxygenated SBS-OH (bottom).](image)

Following B(C$_6$F$_5$)$_3$-catalyzed deoxygenation, a linear-branched-linear structure is formed.

### 3.4 Conclusions

In conclusion, the hydrosilylative reduction of SBS-OH using B(C$_6$F$_5$)$_3$ and a hydrosilane to yield a linear-branch-linear triblock polymer has been demonstrated. As discussed in the previous chapter, deoxygenation of linear polyols, such as PVA and PBD-OH, results in unsaturated branched polyolefins. This chemistry was applied to a triblock system as a way to synthesize an otherwise inaccessible linear-branched-linear material. Following B(C$_6$F$_5$)$_3$-catalyzed deoxygenation, the triblock branching architecture was analyzed by $^{13}$C-NMR. The predominant branches observed were of butyl and larger lengths. The glass transition temperatures and phase-separation properties are currently being
investigated. By varying the length of the polybutadiene block, materials with different properties may be obtained.

3.5 Acknowledgements

This work was primarily supported by the Army Research Office and the Department of Energy. I would like to acknowledge Laura Adduci and Trandon Bender for their discussions and expertise.
Chapter 4. PLATINUM BISPHOSPHONATE COMPLEXES AS THERANOSTIC AGENTS

4.1 Introduction

Cisplatin has been used in the clinic since 1978 to treat a variety of cancers, including testicular, lung, breast, and ovarian.\(^{65}\) The widespread success of cisplatin has resulted in the synthesis and study of many other platinum anticancer drugs, yet only two other platinum complexes, oxaliplatin and carboplatin, have been approved by the FDA.\(^{66,67}\) All three of these platinum complexes inhibit cancer cell growth by binding to DNA causing structural distortions that trigger programmed cell death.\(^{65,68}\) Although platinum anticancer drugs are still widely used in the clinic, the performances of the drugs are limited by their poor pharmacokinetic properties. The severe side effects, including nephrotoxicity and neurotoxicity, associated with platinum drugs reduce a patient’s quality of life.\(^{69}\) Furthermore, inherent or acquired tumor resistance limits the effectiveness of cisplatin.\(^{70}\)

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\(^{69}\) Wong, E.; Giandomenico, C. M. *1999*, 99, 2451-2466.

Therefore, strategies to effectively deliver active platinum drugs to the tumor were explored.

Ideally, an effective delivery system for anticancer drugs, such as cisplatin, would deliver and release the drug selectively to the tumor. Recent discoveries in the area of cancer nanotherapeutics provide promise for selective drug delivery to the tumor site. Nanoparticles, in the size range of 10 to 200 nm, have emerged as a class of cancer chemotherapeutics. Nanoparticle-based therapeutic agents have several advantages over traditional small-molecule agents, including high agent loading, tunable size, tailorable surface properties, controllable drug release kinetics, and improved pharmacokinetics.\textsuperscript{33,45,71,72,73,74,75}

Nanoparticles also tend to have increased accumulation in tumors as a result of the enhanced permeability and retention (EPR) effect that results from the leaky tumor neovasculatures and poor functioning lymphatic system. Additionally, nanoparticles can be specifically targeted to cancer cells by surface conjugation of an appropriate ligand to further enhance the accumulation of nanoparticles in tumors. The clinical success of nanoparticle-based therapeutics such as Doxil illustrates the potential of nanomaterials in anticancer drug delivery. The Lin group has previously developed nanoscale coordination polymers (NCPs) as a potential delivery vehicle for cisplatin prodrugs.\textsuperscript{46,47} These materials demonstrated high


agent loading and comparable cytotoxicity to cisplatin \textit{in vitro}. However, these materials readily degrade under physiological conditions, thus limiting their \textit{in vivo} efficacy. Therefore, alternative nanoparticle platforms that possess minimal drug release and nanoparticle decomposition when circulating in the bloodstream, but also readily release the drugs in the reducing tumor microenvironment or upon cellular internalization, were developed.

This chapter reports the development of cisplatin-based NCPs that exhibit controlled drug release. A platinum(IV) prodrug was synthesized, and then covalently incorporated into the nanoparticle through a reverse microemulsion method in which the platinum precursor binds to a metal center. Two different metal systems were studied: a calcium-based cisplatin nanoparticle and a manganese-based cisplatin nanoparticle. Calcium was selected due to its biocompatibility,\textsuperscript{48} and manganese was studied due to its applicability as a MRI contrast agent. The cisplatin-containing NCPs were then lipid coated to increase stability and blood circulation time (Scheme 4.1). Finally, the nanoparticles were \textit{in vitro} evaluated against human non-small cell lung cancer cell lines.
**Scheme 4.1.** Synthesis and lipid coating of PtBP nanoparticles containing either Ca\(^{2+}\) or Mn\(^{2+}\).

4.2 Materials and Methods

4.2.1 General Materials and Methods

All starting materials were purchased from either Fisher Scientific or Sigma-Aldrich and used without further purification unless otherwise noted. Cisplatin was purchased from AK Scientific. DOPC (DOPC= 1,2-dioleoyl-\(sn\)-glycero-3-phosphocholine) and DSPE-PEG\(_{2K}\) (DSPE-PEG\(_{2K}\)= 1,2-distearoyl-\(sn\)-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene...
glycol)-2000]) were purchased from Avanti Polar Lipids. Fetal bovine serum (FBS, Sigma), RPMI-1640 growth medium (Gibco), penicillin-streptomycin (Sigma), trypsin-EDTA (Sigma), and phosphate buffered saline (PBS, Gibco) were purchased from the Tissue Culture Facility in the Lineberger Comprehensive Cancer Center at UNC-Chapel Hill. $^1$H-NMR spectra were obtained on a 400 MHz Bruker DRX spectrometer. Thermogravimetric analyses (TGA) were performed on a Shimadzu TGA-50 equipped with a platinum pan at a sampling rate of 3 °C/min. A JEM 100CX-2 transmission electron microscope (TEM) was used to determine particle size and morphology. Dynamic light scattering (DLS) measurements were collected with a Malvern Zetasizer Nano Zs. Inductively-coupled plasma mass spectrometry (ICP-MS) was performed on a Varian 820-MS Inductively-Coupled Plasma Mass Spectrometry.

A549 human non-small cell lung carcinoma cells (ATCC# CCL-185) and NCI-H460 human non-small cell lung carcinoma cells (ATCC# HTB-177) were purchased from the Tissue Culture Facility of the Lineberger Comprehensive Cancer Center at the University of North Carolina at Chapel Hill. A549 cells were cultured in RPMI-1640 growth medium (Cellgro) supplemented with 10% fetal bovine serum (Sigma) and 2% penicillin-streptomycin (Sigma). H460 cells were cultured in RPMI-1640 growth medium (Cellgro) supplemented with 10%FBS (Mediatech) and 2% penicillin-streptomycin solution (Sigma).
4.2.2 Synthesis of Platinum Complexes

4.2.2.1 Synthesis of \(c,c,t\)-\(\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{OH})_2\)

A reported procedure was modified in order to synthesize \(c,c,t\)-\(\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{OH})_2\).\(^{76}\) Cisplatin (5.0298 g, 0.017 mol), water (100 mL), and 30% hydrogen peroxide (100 mL) were combined in a 500-mL round bottom flask. The reaction mixture was stirred in the dark at 50 °C for 2 hours. It was then cooled to room temperature and placed in the refrigerator overnight to encourage precipitation of the product out of solution. The bright yellow product was collected by vacuum filtration and washed with water, acetone, and ether (75% yield).

\(\text{Synthesis of } c,c,t,\text{-Pt}(\text{NH}_3)_2(\text{phosphonocarbamate ester})_2:\) A reported procedure was modified in order to synthesize \(c,c,t\)-\(\text{Pt}(\text{NH}_3)_2(\text{phosphonocarbamate ester})_2\).\(^{77}\) In a 20-dram vial, \(c,c,t\)-\(\text{Pt}(\text{NH}_3)_2\text{Cl}_2(\text{OH})_2\) (884.5 mg, 2.65 mmol) was combined with DMF (3 mL). In a separate vial, diethoxyphosphinyl isocyanate (1.630 mL, 10.6 mmol) was combined with DMF (2 mL). The isocyanate mixture was added dropwise, with stirring, to the platinum mixture. The reaction mixture was stirred overnight at room temperature in the dark. The resulting solution was then filtered. Diethyl ether was added to the filtrate to precipitate out the desired product. The yellow product was isolated via centrifugation and washed twice with diethyl ether (80% yield).


4.2.2.2 Synthesis of \( \text{c,c,t-} \text{Pt(NH}_3\text{)}_2(\text{phosphonocarbamate})_2 \) (PtBP)

Under an inert atmosphere, dry \( \text{c,c,t-} \text{Pt(NH}_3\text{)}_2(\text{phosphonocarbamate ester})_2 \) (1.465 g, 2.17 mmol) was dissolved in anhydrous DMF (3.0 mL). Then, TMS-Br (2.81 mL, 21.2 mmol, 10 equiv.) was added dropwise at 0°C. The reaction mixture was stirred overnight at room temperature in the dark under a N\(_2\) atmosphere. The product was crash-out overnight in the fridge after the addition of DCM. The product was isolated via centrifugation and washed twice with DCM. It was then dissolved in methanol (10 mL) and stirred overnight at room temperature in the dark. The light yellow product was crash-out with DCM, isolated via centrifugation, and washed twice with DCM (60% yield).

4.2.3 Nanoparticle Synthesis

4.2.3.1 Synthesis of Mn-PtBP and Ca-PtBP Nanoparticles

In a 20-dram vial, 3 M NaOH (183 µL, 0.55 mmol) and 3.017 mL of milliQ water were combined. Then, PtBP (80 mg, 0.15 mmol) was dissolved into the solution via sonification. In a 100-mL flask, 25 mL of 0.3 M tritonX-100/1.5 M 1-hexanol in cyclohexane was combined with 1.0 mL of an aqueous metal salt solution (either MnCl\(_2\) or CaCl\(_2\), 100 mg/mL). This solution was stirred rapidly for 10 minutes. In a separate 50-mL RB flask, 25 mL of tritonX-100/1.5 M 1-hexanol in cyclohexane was combined with 1.0 mL of the PtBP solution and 100 µL of DOPA (200 mg/mL in CHCl\(_3\)). This solution was stirred rapidly for 10 minutes before adding to the metal salt solution. Once the two solutions were combined, the microemulsion was stirred rapidly for 30 min in the dark at room temperature. Ethanol was added to the reaction mixture to induce precipitation of the nanoparticles overnight. The following day, the particles were isolated via centrifugation (13,000 rpm x 15 min). The particles were then redispersed into 15 mL of cyclohexane and crash-out with 10 mL of ethanol. The particles were isolated by centrifugation (13,000 rpm x 20 min). The particles
were then redispersed into 15 mL of THF and crashed out with 10 mL of ethanol. The particles were isolated by centrifugation (13,000 rpm x 15 min), and redispersed into 10 mL of THF for storage. The particles were allowed to settle overnight to remove any large aggregates. They were then filtered through a 0.45 um PFTE filter, followed by a 0.2 um filter.

4.2.3.2 Synthesis of Ca-Control and Mn-Control Nanoparticles

In a 100-mL flask, 0.3 M triton-100/1.5 M 1-hexanol (25.0 mL) was combined with 1.0 mL of an aqueous metal salt solution (either MnCl₂ or CaCl₂, 100 mg/mL in water), and stirred rapidly for 10 min. In a separate 50-mL flask, 0.3 M triton-100/1.5 M 1-hexanol (25.0 mL) was combined with sodium pyrophosphate (1.0 mL, 25 mg/mL in water) and DOPA (200 µL, 200 mg/mL in CHCl₃), and stirred rapidly for 10 min. The pyrophosphate solution was rapidly poured into the metal solution. The reaction mixture was stirred rapidly for 30 min in the dark at room temperature. Ethanol was added in order to precipitate out the particles. The following day, the nanoparticles were isolated by centrifugation, and washed with cyclohexane/ethanol followed by THF/ethanol. The nanoparticles were stored in THF.

4.2.4 Lipid Coating of Nanoparticles

4.2.4.1 Lipid Coating of Ca-PtBP, Mn-PtBP, Ca-Control, and Mn-Control

DOPC (5 mg/mL in THF), cholesterol (5 mg/mL in THF), and DSPE-PEG₂K (5 mg/mL in THF) were removed from the freezer and heated to 50 °C using a water bath. To a 1-dram vial was added 84 µL of DOPC, 42 µL of cholesterol, and 150 µL of DSPE-PEG. The volume of THF was evaporated to ~100 µL using a stream of nitrogen. Then, 0.5 mg of nanoparticles (stored in THF) was added, and the volume was evaporated back down to ~100 µL. Another 1-dram vial containing a stir bar was placed in the 50 °C water bath, and 0.5 mL
of 30% ethanol in water was added to it. The nanoparticle solution was slowly added dropwise with stirring to the 30% ethanol. Blowing a stream of nitrogen in the vial evaporated the THF off completely. The sample was incubated at 50 °C without stirring for 5 min. The sample was then cooled to room temperature. The free liposomes were removed by centrifuging at 13,000 rpm for 10 min. The lipid-coated particles were redispersed into PBS.

4.2.4.2 Anisamide Targeting of Ca-PtBP and Mn-PtBP

DOPC (5 mg/mL in THF), cholesterol (5 mg/mL in THF), DSPE-PEG\textsubscript{2K} (5 mg/mL in THF), and DSPE-PEG\textsubscript{2K}-AA (1 mg/mL in THF) were removed from the freezer and heated to 50 °C using a water bath. To a 1-dram vial was added 84 µL of DOPC, 42 µL of cholesterol, 150 µL of DSPE-PEG, and 30 µL of DSPE-PEG-AA. The volume of THF was evaporated to ~100 µL using a stream of nitrogen. Then, 0.5 mg of nanoparticles (stored in THF) was added, and the volume was evaporated back down to ~100 µL. Another 1-dram vial containing a stir bar was placed in the 50 °C water bath, and 0.5 mL of 30% ethanol in water was added to it. The nanoparticle solution was slowly added dropwise with stirring to the 30% ethanol. Blowing a stream of nitrogen in the vial evaporated the THF off completely. The sample was incubated at 50 °C without stirring for 5 min. The sample was then cooled to room temperature. The free liposomes were removed by centrifuging at 13,000 rpm for 10 min. The lipid-coated particles were redispersed into PBS.

4.2.5 Stability Assays

4.2.5.1 BSA Stability Assay of Ca-PtBP@Lipid and Mn-PtBP@Lipid

The lipid-coated particles were combined with 5 mg of BSA. The sample was sonicated briefly to dissolve the BSA. Size measurements were obtained on the DLS every
10 minutes for 12 hours to detect whether the albumin protein adsorbed onto the surface of the nanoparticle.

4.2.5.2 *Platinum Release from PtBP Nanoparticles*

5 mg of PtBP nanoparticles were prepared in 500 µL PBS and placed in dialysis tubing (MW cutoff = 10,000). The dialysis tubing was submerged in a beaker containing 400 mL 5 mM PBS at 37 °C. This concentration of PBS was chosen because it exceeds the concentration of phosphate in the blood. At selected time intervals, 1 mL of sample was removed from the beaker. The samples were digested in concentrated HNO$_3$ for several hours before diluting with H$_2$O. The diluted samples were then analyzed by ICP-MS for platinum content (in ppb). The platinum content was used to calculate the percent drug release from the nanoparticles.

4.2.6 *In Vitro Cell Viability Assays*

4.2.6.1 *A549 Cell Viability Assay using PtBP@Lipid Nanoparticles*

Confluent A549 cells were trypsinized and counted with a hematocytometer. Cells were plated in 96-well plates at a cell density of 1,000 cells/well and 100 µL of medium. Plates were incubated at 37°C, 5% CO$_2$, overnight. Media was removed from wells, and each well was washed with PBS. Drug/particle solutions were prepared in media containing 5% PBS. Aliquots of drug/particle solutions and media (5% PBS) were given to each well to result in cisplatin concentrations of 0, 0.75, 1.5, 3.75, 7.5 and 15 µM. Plates were incubated for 3 days, and viability was determined via the MTS assay.

4.2.6.2 *H460 Cell Viability Assay using PtBP@Lipid Nanoparticles*

Confluent H460 cells were trypsinized and counted with a hematocytometer. Cells were plated in 96-well plates at a cell density of 1,000 cells/well and 100 µL of medium.
Plates were incubated at 37°C, 5% CO₂, overnight. Media was removed from wells, and each well was washed with PBS. Drug/particle solutions were prepared in media containing 5% PBS. Aliquots of drug/particle solutions and media (5% PBS) were given to each well to result in cisplatin concentrations of 0, 0.75, 1.5, 3.75, 7.5 and 15 µM. Plates were incubated for 3 days, and viability was determined via the MTS assay.

4.2.6.3 H460 Cell Viability Assay using MnPtBP@LipidAA Nanoparticles

Confluent H460 cells were trypsinized and counted with a hematocytometer. Cells were plated in 96-well plates at a cell density of 1,000 cells/well and 100 µL of medium. Plates were incubated at 37°C, 5% CO₂, overnight. Media was removed from wells, and each well was washed with PBS. Drug/particle solutions were prepared in media containing 5% PBS. Aliquots of drug/particle solutions and media (5% PBS) were given to each well to result in a range of cisplatin concentrations. Plates were incubated for 3 days, and viability was determined via the MTS assay.

4.3 Results and Discussion

4.3.1 Synthesis of Platinum Complexes

The platinum(IV) complex c,c,t,-Pt(NH₃)₂(phosphonocarboxamate)₂ (PtBP) was synthesized starting from commercially available cisplatin (Scheme 4.2). First, cisplatin was oxidized using hydrogen peroxide to form the dihydroxy-platinum complex c,c,t- Pt(NH₃)₂Cl₂(OH)₂ in up to 80% yields. Diethoxypyrophosphinyl isocyanate was treated with the dihydroxy-platinum complex to form a carbamate with an 82% yield. The hydroxyl groups are known to act as a nucleophile and rapidly attack the electrophilic carbon atom of the
isocyanate group.\textsuperscript{78} The platinum-based ester was then hydrolyzed to form the desired PtBP in a 60% yield. The platinum complex has two orthogonal phosphonate groups to allow for coordination to a metal ion, in this case either Mn\textsuperscript{2+} or Ca\textsuperscript{2+}.

**Scheme 4.2.** Synthesis of \(c,c,t\)-Pt(NH\(_3\))\(_2\)(phosphonocarbamate)\(_2\) (PtBP): a) oxidation of cisplatin with hydrogen peroxide, b) formation of platinum-carbamate species through nucleophilic substitution, and c) hydrolysis of platinum-ester to form PtBP.

\[
\text{Cl}_2\text{Pt} \left(\text{NH}_3\right)_{3} + \text{H}_2\text{O} \xrightarrow{\text{H}_2\text{O}_2} \text{Cl}_2\text{Pt} \left(\text{OH}\right) \left(\text{NH}_3\right)_{3}
\]

\[
\text{Cl}_2\text{Pt} \left(\text{OH}\right) \left(\text{NH}_3\right)_{3} + \text{DMF} \xrightarrow{12 \text{ h}} \text{Pt} \left(\text{OH}\right) \left(\text{DMF}\right) \left(\text{NH}_3\right)_{3}
\]

\[
\text{Pt} \left(\text{OH}\right) \left(\text{DMF}\right) \left(\text{NH}_3\right)_{3} + \text{TMS-Br} \xrightarrow{\text{N}_2, 18 \text{ h, dark}} \text{MeOH} \xrightarrow{8 \text{ h}} \text{Pt} \left(\text{OH}\right) \left(\text{MeOH}\right) \left(\text{NH}_3\right)_{3}
\]

4.3.2 Synthesis of Control, Ca-PtBP, and Mn-PtBP Nanoparticles

A reverse, or water-in-oil, microemulsion was used to synthesize nanoparticles containing a metal ion (either Mn\textsuperscript{2+} or Ca\textsuperscript{2+}) and PtBP. **Ca-PtBP** and **Mn-PtBP** corresponds to the resulting nanoparticles synthesized using either Ca\textsuperscript{2+} or Mn\textsuperscript{2+}, respectively. To

synthesize the metal-PtBP nanoparticle, a reverse microemulsion of 0.3 M Triton X-100 and 1.5 M 1-hexanol in cyclohexane with a water to surfactant ratio ($W$ value) of 7.4 was used. The PtBP complex was dissolved into an aqueous basic solution to deprotonate the phosphonic acids, and the solution was added rapidly to the oil phase with stirring, followed by addition of an aqueous metal ion solution. A small amount of DOTAP (DOTAP= 1,2-dioleoyl-3-trimethylammonium-propane) was added to the reverse microemulsion in order to aid in nanoparticle stabilization. The DOTAP remains at the oil-water interface, with its hydrophobic tails in the oil phase and its polar head groups in the water phase. In the water phase, the phosphonate groups of either the PtBP complex or DOTAP coordinate to the metal ions, polymerizing to form a NCP (Scheme 4.3). Due to the hydrophobicity of the DOTAP chains, it is expected that the DOTAP will be on the surface rather than in the interior of the nanoparticle. The polymerization reaction was quenched by the addition of ethanol, and the particles were isolated by centrifugation. Washing with ethanol aids in removal of excess surfactant from the nanoparticles.
To characterize the Ca-PtBP and Mn-PtBP nanoparticles, images were obtained using TEM (Figure 4.1). Nanoparticle Ca-PtBP was spherical and approximately 15 nm in diameter, while nanoparticle Mn-PtBP was approximately 20-25 nm in diameter. Dynamic light scattering (DLS) measurements of Ca-PtBP and Mn-PtBP in THF had unimodal distributions that corresponded roughly to the TEM diameters (Figure 4.2, Table 4.1). Inductively coupled plasma-mass spectroscopy (ICP-MS) confirmed a cisplatin loading of 20% for Ca-PtBP and 25% for Mn-PtBP.
Figure 4.1. TEM images of Ca-PtBP (a) and Mn-PtBP (b). Scale bars equal 200 nm.

Figure 4.2. Number weighted DLS spectrum of Ca-PtBP (black) and Mn-PtBP (red).

Table 4.1. DLS measurements for Ca-PtBP and Mn-PtBP obtained in THF.

<table>
<thead>
<tr>
<th>Nanoparticle</th>
<th>Z Average (nm)</th>
<th>PDI</th>
<th>Number Average (nm)</th>
</tr>
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<tr>
<td>Ca-PtBP</td>
<td>88.50</td>
<td>0.276</td>
<td>30.22</td>
</tr>
<tr>
<td>Mn-PtBP</td>
<td>66.6</td>
<td>0.183</td>
<td>33.86</td>
</tr>
<tr>
<td>Ca-control</td>
<td>45.35</td>
<td>0.285</td>
<td>25.69</td>
</tr>
<tr>
<td>Mn-control</td>
<td>33.79</td>
<td>0.199</td>
<td>18.75</td>
</tr>
</tbody>
</table>

Using a similar procedure, cisplatin-free nanoparticles (Ca-control and Mn-control) were synthesized as a control. Instead of using PtBP as the linker, pyrophosphate was used (Scheme 4.4). The DLS measurements are reported in Table 4.1. These particles were
synthesized to ensure that no cell cytotoxicity occurs from the metal ions or phosphonate groups present in the nanoparticles.

**Scheme 4.4.** Synthesis of Ca-control and Mn-control nanoparticles.

4.3.3 Lipid Coating of Control, Ca-PtBP, and Mn-PtBP

To impart stability and biocompatibility, nanoparticles **Ca-control**, **Mn-control**, **Ca-PtBP**, and **Mn-PtBP** were coated with PEGylated liposomes (Scheme 4.5) to form **Ca-control@Lipid**, **Mn-control@Lipid**, **Ca-PtBP@Lipid**, and **Mn-PtBP@Lipid**, respectively. Liposomes act as a delivery vehicle for the nanoparticles, with the presence of PEG increasing blood circulation time by shielding the particles from the mononuclear phagocyte system (MPS). The particles were coated with a single lipid bilayer (SLB) containing 1:1:0.5 (by mol) DOPC/cholesterol/DSPE-PEG_{2K} (DOPC = 1,2-dioleoyl-sn-glycero-3-phosphocholine, DSPE-PEG_{2K} = 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000]). The lipid solution (in THF) was combined with a THF solution of nanoparticles, followed by removal of THF and dispersion in 30% ethanol in water. The presence of the lipid coating was confirmed by
TEM, DLS, TGA, and cisplatin release data. The TEM images of both Ca-PtBP@Lipid and Mn-PtBP@Lipid show monodisperse particles (Figure 4.3). Additionally, DLS measurements of the lipid-coated particles confirm an increase in size and a decrease in PDI due to the presence of the SLB (Table 4.2). For instance, after lipid coating nanoparticle Ca-PtBP, the PDI decreased from 0.281 to 0.155 indicating a more monodisperse sample. The lipid coating helped to reduce the amount of nanoparticle aggregation observed in solution.

Scheme 4.5. Lipid coating of PtBP nanoparticles with 1:1:0.5 (by mol) DOPC/cholesterol/DSPE-PEG$_{2K}$.
Figure 4.3. TEM images of (a) Ca-PtBP@Lipid and (b) Mn-PtBP@Lipid.

Table 4.2. DLS measurements for Ca-PtBP@Lipid and Mn-PtBP@Lipid obtained in 5 mM PBS.

<table>
<thead>
<tr>
<th>Nanoparticle</th>
<th>Z Average (nm)</th>
<th>PDI</th>
<th>Number Average (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca-PtBP@Lipid</td>
<td>130.8</td>
<td>0.155</td>
<td>81.44</td>
</tr>
<tr>
<td>Mn-PtBP@Lipid</td>
<td>141.6</td>
<td>0.087</td>
<td>103.7</td>
</tr>
<tr>
<td>Ca-control@Lipid</td>
<td>170.1</td>
<td>0.325</td>
<td>50.22</td>
</tr>
<tr>
<td>Mn-control@Lipid</td>
<td>156.5</td>
<td>0.411</td>
<td>46.43</td>
</tr>
</tbody>
</table>

4.3.4 BSA Stability Assay of Ca-PtBP@Lipid and Mn-PtBP@Lipid

Proteins found in the body tend to adsorb onto the surface of nanoparticles via non-specific adsorption limiting their use in vivo. The mononuclear phagocyte system (MPS) recognizes the protein-covered nanoparticle as a foreign body and immediately clears it from the body. In drug delivery, the nanoparticle needs to circulate the body until it reaches the desired target (i.e. the tumor region). PEG has been shown to reduce protein adsorption and thus inhibit macrophage uptake. The incorporation of PEG into the liposomal formulation should increase the stability of Ca-PtBP@Lipid and Mn-PtBP@Lipid in the presence of proteins. To confirm this, the particles were combined with bovine serum albumin (BSA), and DLS measurements were obtained over time. Albumin is the most abundant blood

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plasma protein, so it was selected as a model to study protein interaction with the synthesized nanoparticles. If BSA adsorbed onto the surface of the nanoparticles, an increase in particle size would be observed. To conduct this study, the nanoparticles were combined with BSA, and DLS measurements were recorded every 10 minutes for 12 hours. The BSA stability results for Mn-PtBP@Lipid are shown in Figure 4.4. The particle size remained relatively constant indicating the PEG-containing SLB prevented non-specific protein adsorption.

![Z Average (nm) of Mn-PtBP@Lipid as a function of BSA exposure time.](image)

**Figure 4.4.** Z Average (nm) of Mn-PtBP@Lipid as a function of BSA exposure time.

### 4.3.5 Cisplatin Release from Ca-PtBP and Mn-PtBP

Drug delivery nanoparticles must be stable enough to circulate the body, yet still release the active agent after reaching the tumor site. Incubating a known amount of particle in PBS allowed the *in vitro* particle degradation to be monitored. Nanoparticle samples were enclosed in dialysis bags (MW cut-off 10,000) and dialyzed in PBS at 37 °C. At predetermined time points, 500 µL of sample was removed from the incubation medium. To determine the amount of cisplatin released from the particles into the medium, the sample
was digested in 2% nitric acid and ICP-MS data was obtained. As shown in Figure 4.5 and Figure 4.6, the as-synthesized particles demonstrated fast drug release. However, after lipid coating, the particles were stabilized and cisplatin release occurred at a much slower rate.

Figure 4.5. Platinum release from Ca-PtBP and Ca-PtBP@Lipid.
4.3.6 Anisamide Targeting of PtBP Nanoparticles

A wide range of cancers, including non-small cell lung, breast, and prostate, overexpress sigma receptors on the cancer cell surface.\textsuperscript{80,81,82} The small molecule anisamide (AA) has been used as a ligand to target drugs to sigma receptors on cancer cells \textit{in vitro}.\textsuperscript{80} The targeting ligand AA was attached to the end of DSPE-PEG (MW= 2000) to form DSPE-PEG-AA. This tumor-targeting lipid was incorporated into the liposomal formulation used when coating the nanoparticles with SLBs. In the lipid coating procedure described previously, DSPE-PEG\textsubscript{2K} was substituted with DSPE-PEG\textsubscript{2K} and DSPE-PEG\textsubscript{2K}-AA in a 9:1 molar ratio forming particles \textbf{Ca-PtBP@LipidAA} and \textbf{Mn-PtBP@LipidAA}. As


demonstrated by TEM and DLS (Figure 4.7 and Table 4.3), both AA-targeted systems display similar sizes and morphologies to their non-targeted lipid coated analogs.

![TEM images of (a) Ca-PtBP@LipidAA and (b) Mn-PtBP@LipidAA.](image)

**Figure 4.7.** TEM images of (a) Ca-PtBP@LipidAA and (b) Mn-PtBP@LipidAA.

**Table 4.3.** DLS measurements for Ca-PtBP@Lipid and Mn-PtBP@Lipid obtained in 5 mM PBS.

<table>
<thead>
<tr>
<th>Nanoparticle</th>
<th>Z Average (nm)</th>
<th>PDI</th>
<th>Number Average (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ca-PtBP@LipidAA</td>
<td>121.5</td>
<td>0.166</td>
<td>72.58</td>
</tr>
<tr>
<td>Mn-PtBP@LipidAA</td>
<td>169.6</td>
<td>0.188</td>
<td>119.5</td>
</tr>
</tbody>
</table>

**4.3.7 In Vitro Cytotoxicity Assays**

The cytotoxicity of the synthesized control and PtBP nanoparticles was tested against human lung cancer cell lines. The lipid-coated PtBP systems were tested against both A549 (Figure 4.8) and H460 (Figure 4.9) non-small cell lung cancer cells. In both of these assays, the lipid-coated particles demonstrated decreased efficacy when compared to the small molecule counterparts, cisplatin and PtBP. As demonstrated by the drug release profile, the lipid-coated particles are expected to stable for a long period of time, which most likely leads to an increase in IC\textsubscript{50} values when compared to the small molecules. In the nanoparticle form, a sustained drug release is observed. Figure 4.10 shows the results of an assay against H460 cells using AA-targeted MnPtBP nanoparticles. In this assay, small molecule cisplatin and the monomer PtBP displayed similar efficacy, while **MnPt@Lipid** and **MnPtBP@LipidAA** were less effective (see Table 2.4 for IC\textsubscript{50} values). No enhanced
cytotoxicity was observed for the AA-targeted particles, indicating that targeting is not necessary when moving the particles into an *in vivo* model.

**Figure 4.8.** Cell viability assays of cisplatin (black), PtBP (red), MnPtBP@Lipid (green), and CaPtBP@Lipid (blue) evaluated against A549 human non-small cell lung cancer line.
Figure 4.9. Cell viability assays of cisplatin (black), PtBP (red), MnPtBP@Lipid (green), and CaPtBP@Lipid (blue) evaluated against H460 human non-small cell lung cancer line.

Figure 4.10. Cell viability assays of cisplatin (blue), PtBP (red), MnPtBP@Lipid (green), MnControl@Lipid (light blue), and MnPtBP@LipidAA (purple) evaluated against H460 human non-small cell lung cancer.
4.4 Conclusions

In conclusion, two PtBP nanoparticle systems were developed with high loadings of cisplatin-based chemotherapeutics (~20-25%). After lipid coating, these particles demonstrate slow drug release with $t_{1/2} > 70$ h for MnPtBP@Lipid and CaPtBP@Lipid. The slow drug release is ideal when translating the nanoparticles into in vivo applications because it demonstrates the potential to circulate the blood stream much longer than the small molecule agent. The Pt(IV) prodrug incorporated into the nanoparticle would be released once the nanoparticle reaches the reducing environment present in the tumor region. By incorporating PEG into the liposomal formulation, the blood circulation time would also increase due to shielding from the MPS. The presence of the AA-targeting ligand on the nanoparticle surface did not enhance the cytotoxic effects of the nanoparticle, indicating that targeting is not necessary for these systems.

4.5 Acknowledgements

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REFERENCES


