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Synthesis of Multifunctional Polyacrylates and a Binding Group to Hemoglobin for the Treatment of Traumatic Brain Injuries

An Honors Thesis submitted in partial fulfillment of the requirements for Honors in Chemistry.

By
Marina E. Michaud

Under the mentorship of Dr. Hans J. Schanz

Abstract

Hemoglobin based oxygen carriers (HBOCs) hold promise as an effective emergency treatment of severe traumatic brain injuries (TBI). In the latest generation of HBOCs, polynitroxyl-pegylated hemoglobin (PNPH), cell-free hemoglobin is modified with TEMPO and PEG which reduce the toxicities associated with earlier generations of HBOCs. In our efforts to optimize the economic and therapeutic impacts of PNPH's we have synthesized polydimethylaminoethyl methacrylate (poly-DMAEMA) under controlled living conditions via reverse addition-fragmentation chain transfer (RAFT) polymerization. The poly-DMAEMA was then successfully functionalized via quaternization of its NMe₂ groups using chloroacetate derivatives of the TEMPO and PEG. This process was quantitative and the ratio of the functional groups could be controlled. In order to bond the functionalized poly-DMAEMA to the hemoglobin protein, we have explored the synthesis of maleic acid based derivatives. These binding groups contain a maleic acid and benzyl bromide functionalities to link the cysteine mercaptan groups of the hemoglobin shell to the NMe₂ groups of the functionalized poly-DMAEMA, respectively.

Thesis Mentor: _____
Dr. Hans J. Schanz

Honors Director: _____
Dr. Steven Engel

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Department of Chemistry
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Georgia Southern University

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INTRODUCTION

Traumatic Brain Injury

Traumatic brain injuries (TBI) represent a leading cause of morbidity and mortality in the United States, affecting 1.7 million Americans annually. Of those cases, 275,000 result in hospitalization and 52,000 result in mortality. Of those surviving TBI, more than 80,000 cases annually result in a permanent TBI-related disability.¹ Furthermore, the World Health Organization (WHO) predicts TBI will become the leading cause of morbidity and mortality globally by the year 2020.² The detrimental rate of morbidity and mortality in TBI patients arises largely from secondary complications, namely cerebral ischaemia and inflammation, as a result of hemorrhaging and cortical impact associated with the injury. In TBI patients, cerebral ischaemia caused by low cerebral blood flow (CBF) leads to inadequate oxygenation (hypoxia) and impaired metabolic function in the brain tissue, producing long term neuropsychological impairment.³ Along with ischaemia, TBI further induces an inflammatory response as a result of the release of cellular mediators such as proinflammatory cytokines, prostaglandins, and free radicals upon initial injury, causing further neuronal damage.⁴ Current TBI research therefore aims to develop treatments that restore normal cerebral blood flow to reduce the degree of secondary injury resulting from the initial trauma.

Hemoglobin-Based Oxygen Carriers

Hemoglobin-based oxygen carriers (HBOCs) have demonstrated promise as a TBI treatment by exploiting the oxygen transport mechanism of extracellular hemoglobin (Hb) to deliver oxygen to injured brain tissue. Hemoglobin is the active tetrameric protein

component of the red blood cell (RBC), containing iron-porphyrin rings that bind molecular oxygen in the lungs to deliver to tissues throughout the body (Figure 1).

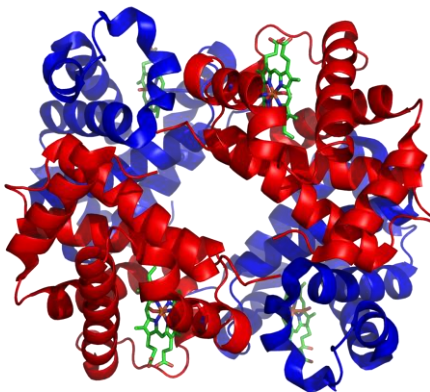


Figure 1. Crystal structure of the tetrameric protein, hemoglobin. Prosthetic porphyrin rings residing in each subunit are pictured in green.⁵

By utilizing the Hb units from lysed RBCs, HBOCs harness the oxygenation capabilities of the hemoglobin without the complications associated with traditional packed red blood cell (PRBC) transfusions typically administered to TBI patients. Namely, PRBC transfusions rely on viable blood donations and donor-recipient immunocompatibility due to the antigens coating the RBC membrane, hindering both the availability and use of the treatment.⁵ HBOCs, however, utilize hemoglobin purified from readily available human and bovine blood sources, producing abundant and universally immunocompatible oxygen carrying units.⁶ Furthermore, Hb units are comparably one-seventieth the size of an RBC, allowing for the units to effectively infiltrate and oxygenate swollen tissue that RBCs fail to reach.⁵

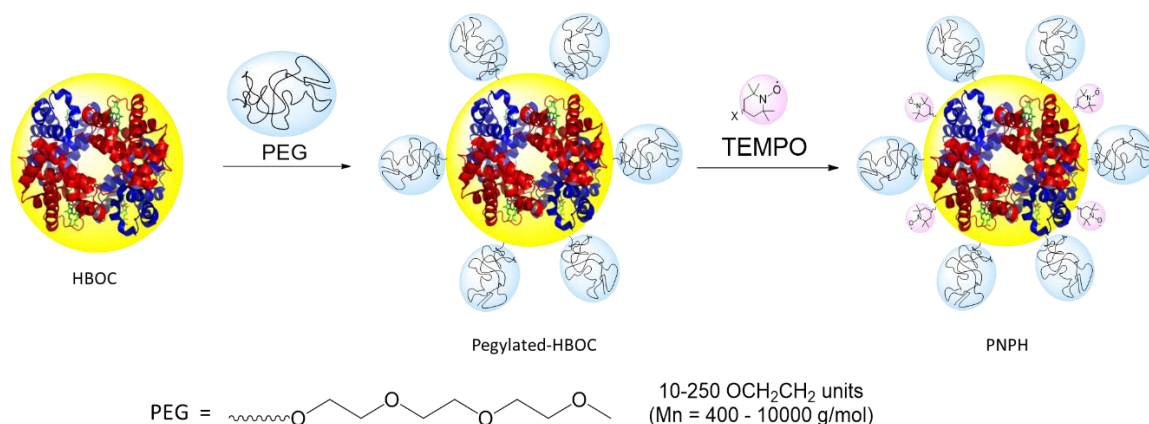
Despite the inherent advantages of extracellular Hb, first generation HBOCs shed light on the biophysiological complications that arise from a lack of the regulatory control mechanisms intrinsic to the RBC. Explicitly, when the tetrameric cell-free Hb units

naturally present in RBCs at a 5mM concentration are released into the blood at μM concentrations during HBOC treatments, the Hb units begin to rapidly dissociate into dimeric subunits that are unregulated in the body, causing unwanted peripheral damage. The decreased molecular weight of dimeric Hb units causes the units to undergo unnecessary glomerular filtration, resulting in nephrotoxicity. These dimeric units also exhibit stronger oxygen affinity and noncooperation with allosteric factors that initiate oxygen release in the tissue, causing an increase in oxygen binding coupled with a decrease in peripheral oxygen delivery. Further, the ferrous iron atoms of cell-free Hb dimers have been shown to be oxidized more readily than those of their tetrameric counterpart, inhibiting the ability of the porphyrin prosthetic group to bind oxygen. The ferric state of the dimer moreover induces a cascade of reactive oxygen species (ROC) generating reactions that cause oxidative stress in the body. Lastly, dimers have been shown to extravasate and scavenge nitric oxide (NO) produced by the endothelium, inhibiting proper vasoregulation. By reducing NO availability in the bloodstream, cell-free Hb causes vasoconstriction that further impedes proper tissue perfusion and oxygenation.⁵

Polynitroxyl-Pegylated Hemoglobin

The adverse effects of first generation HBOCs prompted the development of a wide range of modified HBOCs that seek to reduce the nephrotoxicity, oxidative stress, and NO scavenging properties of cell-free Hb by artificially preventing the breakdown of the tetrameric protein into its dimeric form. One such type of modified HBOC is polynitroxyl-pegylated hemoglobin (PNPH), a bovine-based Hb unit grafted with polyethylene glycol

(PEG) and (2,2,6,6-Tetramethylpiperidin-1-yl)oxyl (TEMPO) functionalities to prevent dimer formation and subsequent toxicity and vasoconstriction (Scheme 1).



Scheme 1. PNPH molecule synthesized via the conjugation of PEG and TEMPO moieties to amino acids on the shell of cell-free Hb.

In PNPH molecules, PEG, a widely used polymer in biomedical applications, effectively increases the size and stability of the Hb unit in a process called PEGylation. Through PEGylation, the chemically inert polymers are conjugated to amino groups of the primary protein structure, thus increasing the molecular weight of the protein and subsequently decreasing glomerular filtration and nitric oxide scavenging while preserving the functional integrity of the protein.⁷ Additionally, the TEMPO moiety, a stable nitroxide radical, possesses redox metabolic capabilities that mimic the antioxidative control mechanisms of RBC enzymes to effectively neutralize ROS produced by cell-free Hb, reducing oxidative stress.⁸ Studies of PNPH employed for the treatment of TBI have validated the efficacy of these modifications. *In vitro* studies that administered PNPH treatment revealed reduced intracranial pressure, improved cerebral perfusion, and attenuated edema as compared to traditional PRBC transfusions for TBI treatment. Furthermore, PNPH requires one sixth of the resuscitation volume of a PRBC transfusion to achieve the same effects, due largely in part to the hypercolloidal properties of the polar

PEG moieties. Decreased resuscitation volume is particularly advantageous in the context of TBI treatment as high resuscitation volumes further aggravate cerebral edema, preventing oxygenation.⁹ The success of *in vitro* studies has led to the FDA approval of various PNPH treatments for clinical testing.

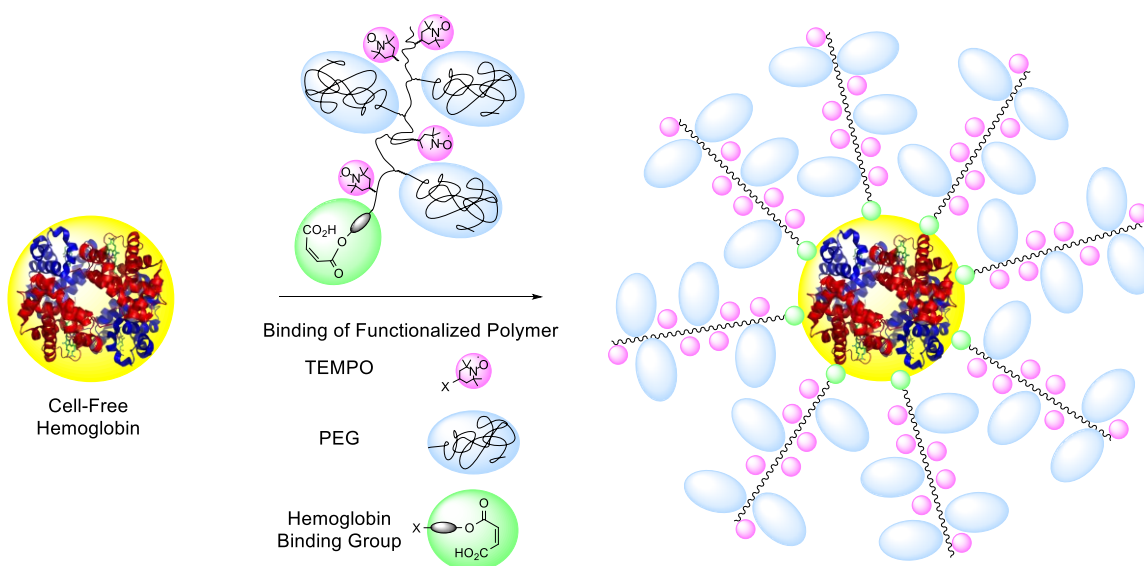
PURPOSE

Although PNPH holds great promise as an effective blood substitute, a cost-effective and practical synthesis of PNPH for pharmaceutical manufacturing has yet to be developed. Our research aims to develop a novel and cost-effective synthesis of a PNPH molecule for pharmaceutical application via the creation of multifunctional polyacrylates with neuroprotective properties for the functionalization of cell-free Hb.

Current synthetic strategies for PNPH molecules involve the conjugation of PEG and TEMPO moieties to the amino acid residues of the Hb unit via synthetic Hb binding groups. Initial synthetic approaches lacked amino acid site-selective control of conjugation, increasing moiety dispersity and interfering with the vasoregulatory, antioxidative, and colloidal properties of the functional Hb molecules. In revised approaches, development of binding groups with high site specificity permitted control of the patterns of conjugation and subsequent functionality of the molecule. Synthesizing these site-specific binding groups for both PEG and TEMPO moieties currently requires numerous steps in a costly procedure in order to yield the final PNPH molecule, limiting widespread pharmaceutical application.⁷

Our proposed synthetic strategy reduces the length and overall cost of PNPH synthesis for optimal pharmaceutical application by modifying cell-free Hb with functionalized polyacrylates. In this novel synthesis, acrylate polymers, widely known as acrylics, are functionalized with PEG and TEMPO moieties as well as site-specific hemoglobin binding groups for the creation of a multifunctional polyacrylate that can be attached to the cysteine mercapto groups of the outer hemoglobin shell. The polyacrylate can be functionalized with all three moieties in varying ratios in a single step process. The functionalized

polyacrylate can then be subsequently grafted onto the Hb unit, resulting in a novel PNP molecule with neuroprotective properties (Scheme 2).

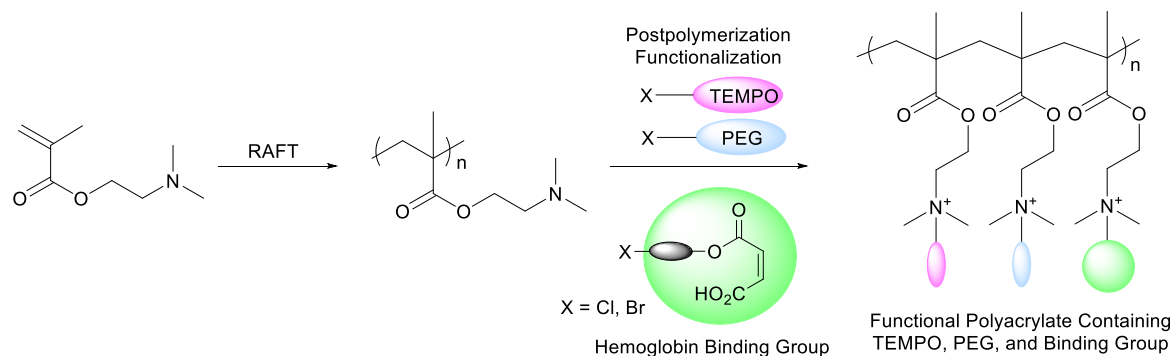


Scheme 2. Modification of cell-free Hb through the conjugation of polyacrylates functionalized with PEG and TEMPO moieties via a Hb binding group.

SYNTHETIC APPROACH

Synthesis of Well-Defined Polyacrylates via RAFT

Homogeneity of PNPH molecules is crucial for pharmaceutical application due to strict quality regulations set forth by the Federal Drug Administration (FDA). For this reason, the synthesis of polyacrylates with controlled molecular weight and low polydispersity is imperative to our design of the multifunctional polyacrylate. Reversible Addition-Fragmentation Chain Transfer (RAFT) polymerization specifically allows for the control of molecular weight and polydispersity via the use of a Chain Transfer Agent (CTA) that mediates the living polymerization. For this novel synthesis, we polymerize the methyl methacrylate monomer 2-(Dimethylamino)ethyl methacrylate (DMAEMA) via RAFT to yield a well-defined polymethyl methacrylate (poly-DMAEMA) for functionalization (Scheme 3). In order to facilitate RAFT, a CTA containing a phenol group adjacent to a thiocarbonylthio ($S=C-S$) bond was synthesized to provide stabilization of the adduct radical during polymerization propagation. RAFT polymerization of DMAEMA using the CTA and azobisisobutyronitrile (AIBN) radical initiator yields a poly-DMAEMA with low polydispersity and controlled molecular weight that can be confirmed via gel permeation chromatography (GPC). These well-defined polymers can then be functionalized with TEMPO, PEG, and binding group moieties in a single post-polymerization functionalization reaction.



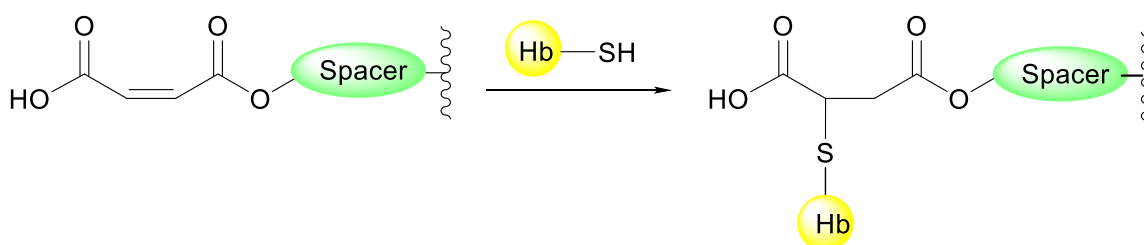
Scheme 3. Polymerization of DMAEMA via RAFT to yield a polyacrylate for post-polymerization functionalization with TEMPO, PEG, and binding group moieties.

Post-Polymerization Functionalization of Polyacrylates

To further reduce the cost and length of the PNPB synthesis, our novel synthetic strategy seeks to functionalize the polymer in a single post-polymerization reaction. In this post-polymerization reaction, TEMPO and PEG chloroacetate derivatives as well as synthetic hemoglobin binding group moieties quaternize the nitrogens of the DMAEMA nitrogen dimethyl (NMe_2) groups to create a functionalized polyacrylate (Scheme 3). This synthetic strategy permits for control of the ratio of TEMPO and PEG functionalities to achieve an optimal density that reduces the oxidative stress and subsequent toxicity associated with cell-free Hb. The poly-DMAEMA can be functionalized with 50 – 80% loading density of TEMPO and PEG to target an average of 10 – 300 moieties per polymer. Each hemoglobin molecule, containing ten cysteine binding sites on its shell for the binding group moiety, will hence be able to bind up to 100 – 3000 TEMPO and PEG functionalities per unit, in addition to those of crosslinked polymers. Confirmation of the attachment of the moieties to the polymer in the desired ratio can be confirmed via ^1H NMR.

Synthesis of a Binding Group for Attachment to Hemoglobin

This novel synthesis further reduces the complexity of current PNPH synthetic strategy by utilizing a maleic anhydride derived hemoglobin binding group to conjugate the functionalized polymers to the cell-free Hb (Scheme 4). An activated alkyl halide of the binding group quaternizes the nitrogen of the DMAEMA to attach the moiety to the polymer. The functionalized binding group then exploits the alkene of the maleic acid in order to undergo conjugate addition to the cysteine residues of the hemoglobin shell. It is imperative therefore that the synthesis of the binding group must yield a molecule that possesses the ability to bind to both the cysteine residues of the hemoglobin as well as the NMe₂ groups of the polymer. The ability of the binding group to attach to the polymer can also be confirmed via ¹H NMR. The conjugation of the functionalized polymer to the cysteine residues via the binding group can be confirmed via EPR.

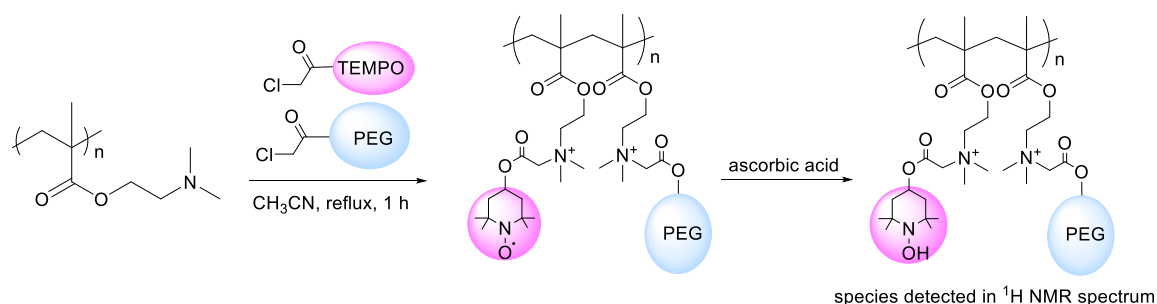


Scheme 4. Conjugate addition of the maleic acid of the binding group to the thiol of the cysteine residue of the Hb shell. Spacer represents an alkyl halide that can quaternize the NMe₂ groups of the polyacrylate to functionalize the polymer.

RESULTS AND DISCUSSION

Post-Polymerization Functionalization with TEMPO and PEG

The poly-DMAEMA has been successfully functionalized with TEMPO and PEG chloroacetate derivatives in a targeted 60% overall loading based on the number of NMe₂ groups available for quaternization, as confirmed by ¹H NMR. In order to yield a functionalized copolymer, TEMPO and PEG were both treated with chloroacetyl chloride to yield TEMPO chloroacetate and PEG chloroacetate, which were then reacted with the poly-DMAEMA by reflux for 24 h at 95 °C. Following treatment with ascorbic acid in order to visualize the TEMPO moieties, the quaternization of the NMe₂ groups by both functionalities was confirmed via ¹H NMR (Figure 2). This process was successfully repeated using varying ratios of TEMPO and PEG [1:1, 5:1, 1:5] respectively, demonstrating the ability to control the functional density of the polyacrylates. After the binding group is successfully synthesized and isolated, it can be included in this post-polymerization reaction with the same degree of control.



Scheme 5. Controlled functionalization of the polyacrylate with TEMPO and PEG chloroacetate derivatives in an overall targeted 60% loading ratio based upon the number of NMe₂ available for quaternization.

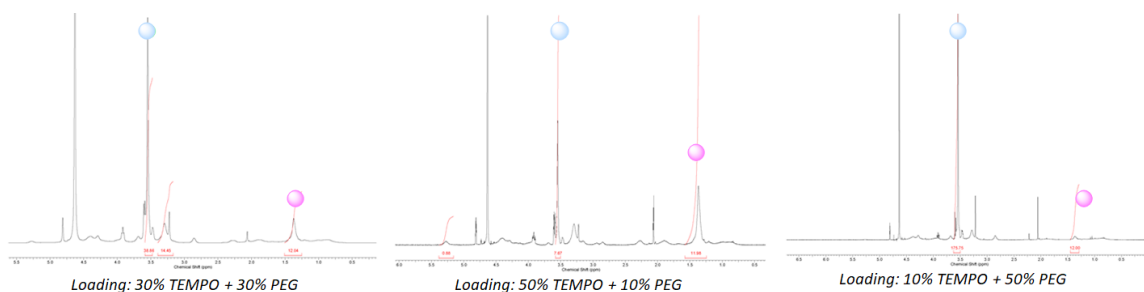
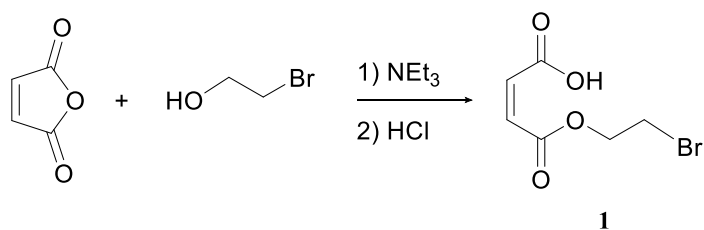


Figure 2. Synthesis of TEMPO and PEG functionalized copolymers in varying ratios confirmed via ^1H NMR.

Binding Group Synthesis

In an initial approach to the synthesis of the binding group, bromoethanol was reacted with maleic anhydride in the presence of triethylamine at room temperature with subsequent acid addition to yield a pure bromoethylmaleate product **1** (Scheme 6). Using ^1H NMR, the presence of the isolated product **1** containing both the maleate and activated alkyl bromide for binding to hemoglobin shell and poly-DMAEMA respectively was confirmed (Figure 3); hydrogen atom a at 6.44 ppm, hydrogen b at 6.47 ppm, hydrogens c at 4.58 ppm, and hydrogens d at 3.57 ppm. However, the attachment of the synthesized binding group **1** to the NMe_2 groups of the polymer via the alkyl bromide required a temperature of 120°C (Scheme 7). Under such harsh conditions, significant decomposition of the bromoethylmaleate binding group polymer **2** was observed, rendering the binding group ineffective for this purpose.



Scheme 6. Synthesis of bromoethylmaleate binding group from maleic anhydride and 2-bromoethanol.

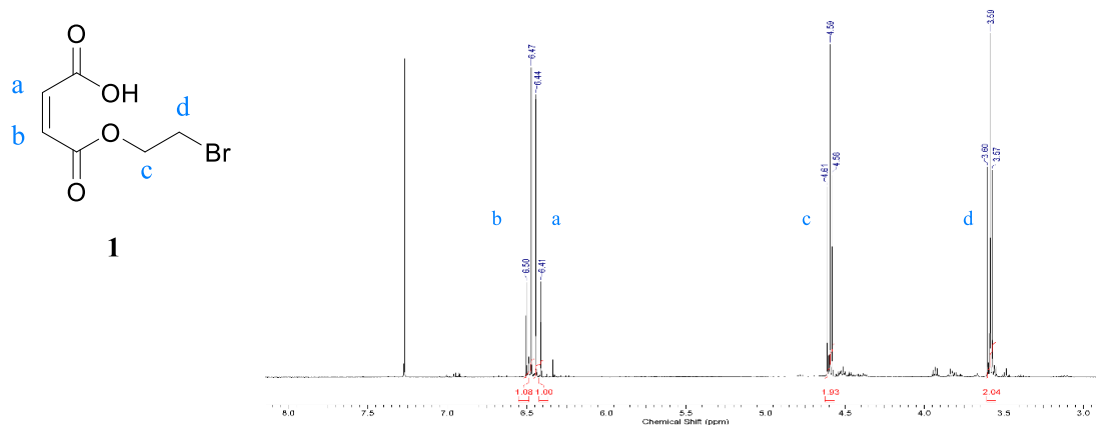
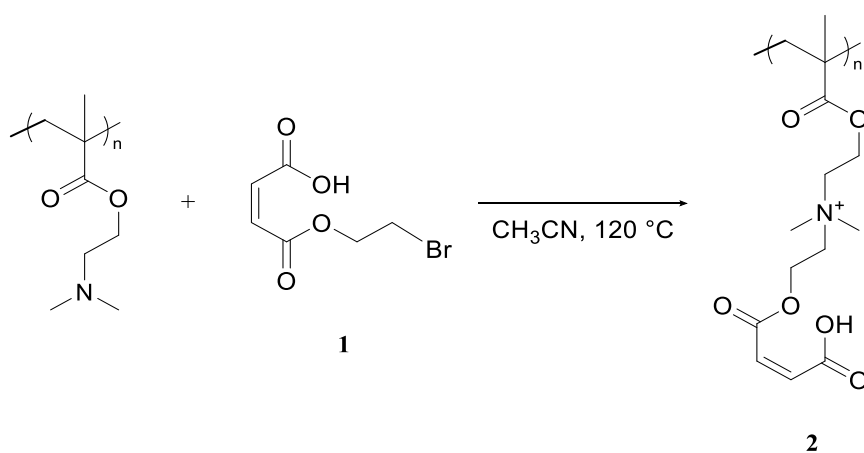


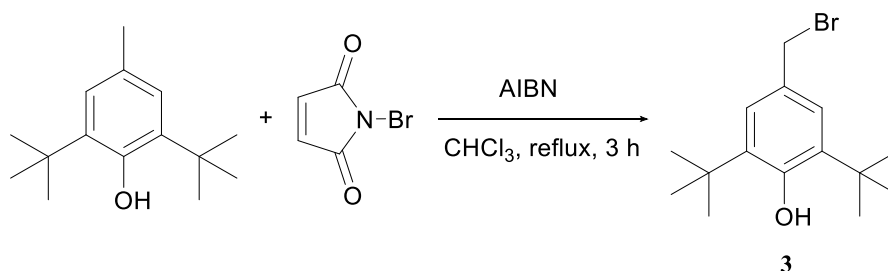
Figure 3. ^1H NMR confirmation of bromoethylmaleate binding group.



Scheme 7. Quaternization of NMe_2 groups of the controlled poly-DMAEMA with the bromoethylmaleate binding group.

To prevent polymer decomposition during functionalization, a new approach was developed in which the alkyl bromide was replaced with a more reactive benzylic bromide that can facilitate the reaction between the binding group and polymer at lower temperatures. In this synthetic approach, 2,6-di-tert-butyl-4-methylphenol was first reacted with N-bromosuccinimide (NBS) via radical substitution initiated by azobisisobutyronitrile (AIBN) in acetonitrile under reflux for 3 h to yield the desired intermediate product **3** containing a benzyl bromide with increased reactivity (Scheme 8). The brominated 2,6-di-tert-butyl-4-methylphenol **3** precipitated cleanly from the reaction solution and was

subsequently filtered to isolate the pure desired intermediate as confirmed by ^1H NMR (Figure 4); hydrogen atoms a at 4.53 ppm, hydrogens b at 7.22 ppm, hydrogens c at 1.46 ppm, and hydrogen d at 5.32 ppm in expected ratios.



Scheme 8. Synthesis of brominated 2,6-di-tert-butyl-4-methylphenol.

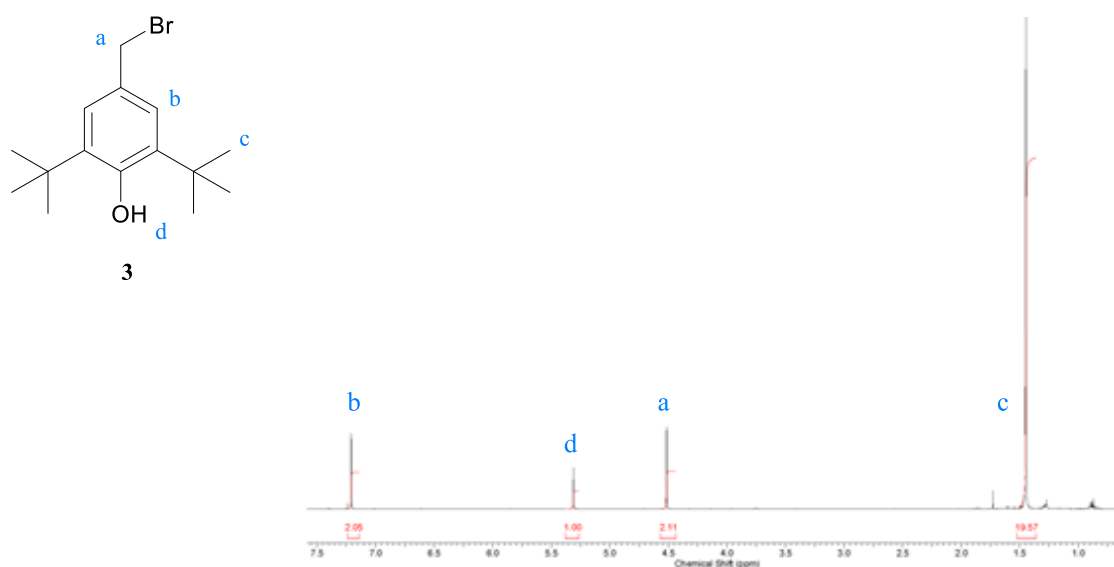
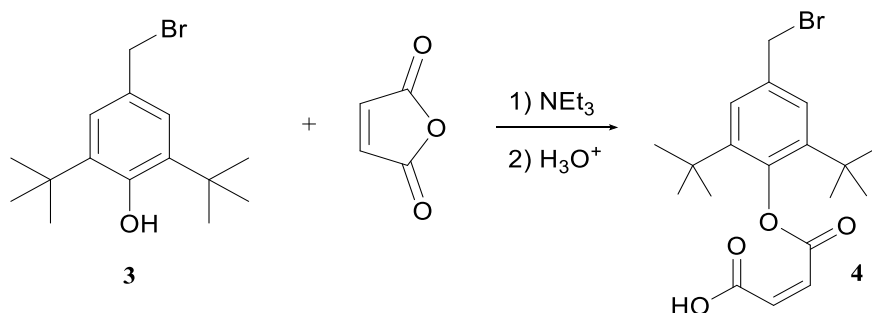


Figure 4. ^1H NMR confirmation of the isolated brominated 2,6-di-tert-butyl-4-methylphenol.

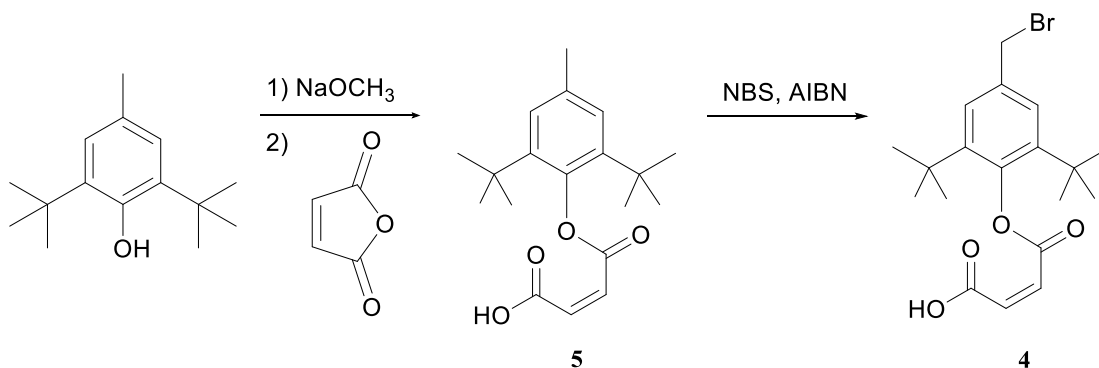
Difficulties arose, however, in functionalizing the brominated 2,6-di-tert-butyl-4-methylphenol **3** with maleic anhydride in order to yield the overall desired binding group **4** with both the benzylic bromide and maleate functionalities. An attempt to react the hydroxyl group of intermediate **3** with maleic anhydride to create the desired maleate was

made by stirring compound **3** at room temperature with maleic anhydride in the presence of triethylamine (Scheme 9); however, no reaction was observed.



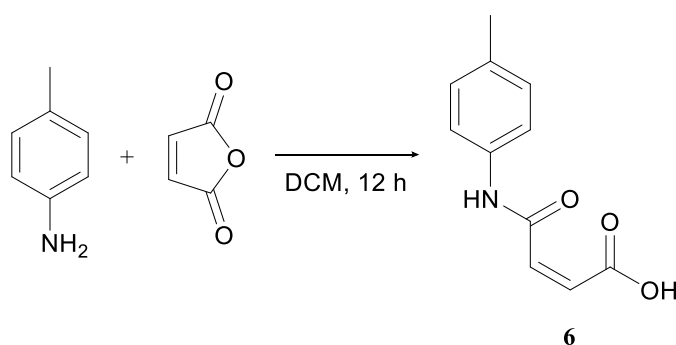
Scheme 9. Functionalization of brominated 2,6-di-tert-butyl-4-methylphenol using maleic anhydride.

The approach was subsequently revised to create a more nucleophilic phenolate that could be reacted with the maleic anhydride to create a maleate intermediate **5** which could then be functionalized using NBS to yield the desired binding group **4** (Scheme 10). Therefore, 2,6-di-tert-butyl-4-methylphenol was reacted with sodium methoxide to quantitatively yield the 2,6-di-tert-butyl-4-methylphenolate, which was then reacted with maleic anhydride at room temperature. Despite the deprotonation of the phenol to promote the nucleophilic attack of the maleic anhydride, no reaction was observed.



Scheme 10. Functionalization of 2,6-di-tert-butyl-4-methylphenolate using maleic anhydride.

Due to the difficulties encountered while attempting to functionalize the phenol using maleic anhydride, a new synthetic approach was designed in which the phenol was replaced with an aniline possessing a primary amine that could be reacted with the maleic anhydride to yield a maleimide derivative (Scheme 11). Combining 4-methylaniline and maleic anhydride at room temperature, the amino group was successfully functionalized to yield the pure desired maleimide product **6**, as confirmed by ^1H NMR (Figure 5).



Scheme 11. Functionalization of 4-methylaniline using maleic anhydride.

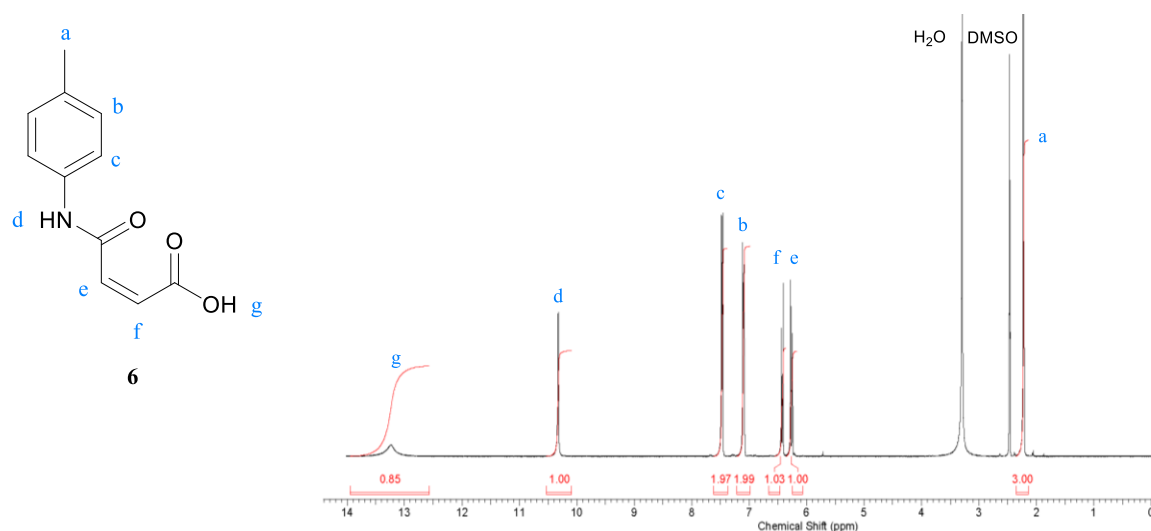
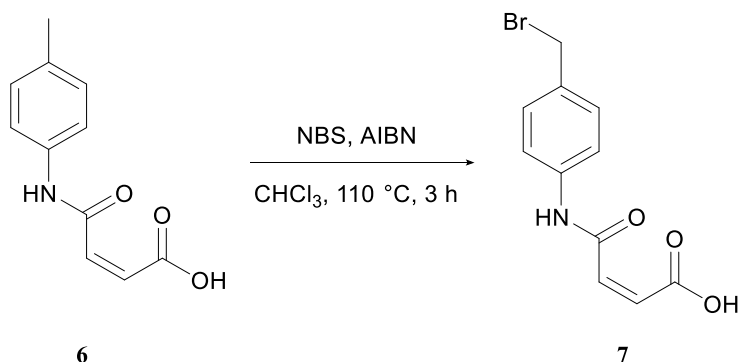


Figure 5. ^1H NMR confirmation of the isolated pure maleimide derivative.

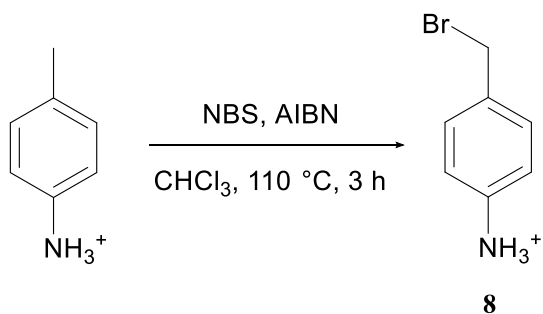
In the proceeding reaction, the installment of a benzylic bromide was attempted via a radical bromination reaction. The maleimide derivative **6** was reacted with NBS in the

presence of AIBN in a pressure vessel at 110 °C in order to dissolve all the starting materials (Scheme 12). However, the desired brominated binding group **7** was not observed via ^1H NMR.



Scheme 12. Radical bromination of the amide derivative using NBS and AIBN.

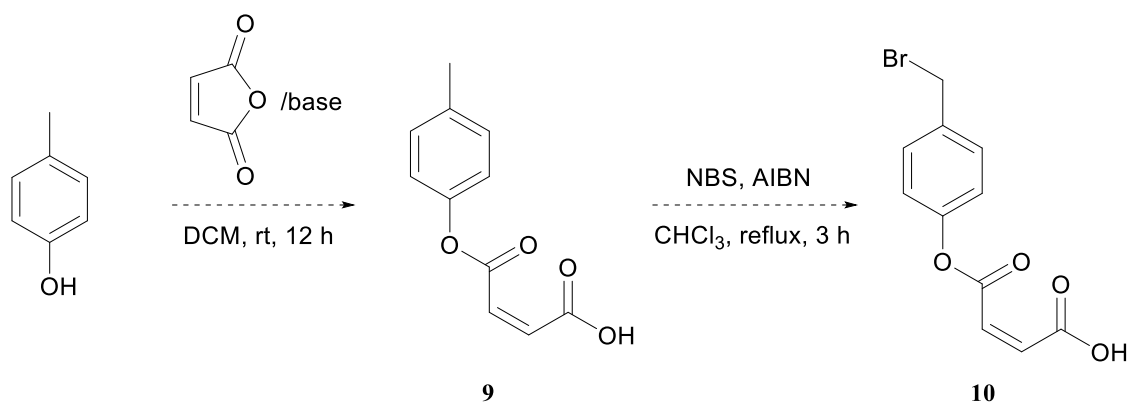
In an alternative attempt to brominate the benzylic position of the aniline, the approach was modified slightly by using 4-methylanilinium chloride in place of 4-methylaniline. The 4-methylanilinium chloride was reacted with NBS in the presence of AIBN in a pressure vessel at 110°C (Scheme 12). However, the desired brominated anilinium **8** was still not observed via ^1H NMR.



Scheme 12. Radical bromination of the anilinium using NBS and AIBN.

In the latest revised approach, the phenol derived binding group will be revisited. In place of 2,6-di-tert-butyl-4-methylphenol, *p*-cresol will be used, effectively eliminating any steric hindrance that may have prevented maleate formation in the final step of the

original synthetic approach. The phenol will first be reacted with maleic anhydride to create the maleate intermediate **9** that can be subsequently reacted with NBS in the presence of AIBN to yield the desired maleate binding group **10** with the reactive benzylic bromide for functionalization of the poly-DMAEMA (Scheme 13).



Scheme 13. Proposed synthesis of the maleate binding group from *p*-cresol.

SUMMARY AND OUTLOOK

Significant progress was achieved towards the synthesis of a binding group for the attachment of the functionalized polyacrylate to the hemoglobin shell. It was found that an alkyl bromide is not sufficiently reactive with the NMe₂ groups of the poly-DMAEMA under mild conditions. The synthetic approach has therefore been modified to incorporate a benzylic bromide which will allow for the functionalization of the NMe₂ groups of the polymer at lower temperatures. Multiple synthetic approaches were explored to synthesize a binding group possessing both the maleate and benzyl bromide functionalities critical to design of the binding group. In the first approach, 2,6-di-tert-butyl-4-methylphenol was successfully brominated to install a benzylic bromide, however, functionalization with maleic anhydride was unsuccessful, likely due to steric hindrance of the tert-butyl groups. In the second approach, the phenol was replaced with an aniline derivative that was contrarily easily functionalized with maleic anhydride, however, unfortunately unsuccessful in bromination of the benzylic carbon. In the latest revised approach, it is suggested that the phenol derived binding group be revisited, replacing the 2,6-di-tert-butyl-4-methylphenol with *p*-cresol to eliminate the steric hindrance that prevented maleic acid functionalization in the original approach. This latest synthetic approach is promising in yielding our desired binding group with both the maleate and benzylic bromide functionalities. The binding group, TEMPO, and PEG moieties will be able to be incorporated into the post-polymerization functionalization process to yield functionalized polyacrylates with controlled functional densities. With

the functionalized polyacrylates in hand, the attachment of the multi-functional polymer to the hemoglobin shell can then be studied.

In conclusion, we have refined the synthetic approach to the synthesis a binding group moiety for the attachment of multi-functional polyacrylates to cell-free Hb. These results bring us one step closer to the finalization of a new generation of PNPH type molecules that are both more cost effective and synthetically practicable for widespread pharmaceutical application.

EXPERIMENTAL

Materials:

All starting materials were purchased from commercial sources and solvents were used as they came. Nuclear magnetic resonance (NMR) spectra were obtained using an Agilent Technologies 400 MHz NMR.

Experimental:

Synthesis of bromoethylmaleate binding group 1:

To a round bottom flask equipped with stir bar was added maleic anhydride (1.05 g, 10.7 mmol) in 35 mL DCM. To the flask, triethylamine (1.63 g, 16.1 mmol) and 2-bromoethanol (1.32 g, 10.6 mmol) were added. The reaction was stirred overnight at room temperature. The reaction mixture was then extracted three times using 0.5 mL HCl (0.35 M) in 30 mL of DI water. The extracted organic (DCM) layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure to yield the desired bromoethylmaleate binding group (0.91 g, 4.1 mmol, 38.4%).

Radical bromination of 2,6-di-tert-butyl-4-methylphenol:

To a round bottom flask equipped with stir bar was added 2,6-di-tert-butyl-4-methylphenol (2.00 g, 9.1 mmol) in 30 mL chloroform. To the flask, N-bromosuccinimide (1.68 g, 9.4 mmol) and 2,2'-azobis(2-methylpropionitrile) (0.04 g, 0.24 mmol) were added, and the reaction was refluxed overnight. The chloroform was removed under reduced pressure and the desired product was precipitated from 30 mL of

heptane. The precipitate was isolated using vacuum filtration to yield the pure brominated 2,6-di-tert-butyl-methylphenol product (2.72 g, 9.1 mmol, 73.9%).

Addition of maleic anhydride to compound 2 under basic conditions:

To a round bottom flask equipped with stir bar, maleic anhydride (0.92 g, 9.4 mmol) in 30 mL DCM was added. To the flask, triethylamine (0.93 g, 9.2 mmol) and compound 2 (2.77 g, 9.3 mmol) were added, and the reaction was stirred at room temperature overnight. The DCM was then removed under reduced pressure and the product was precipitated from 80 mL of t-butyl methyl ether. The precipitate was then isolated via vacuum filtration to yield a white solid (1.42 g).

Addition of maleic anhydride to phenolate derivative:

To a round bottom flask equipped with stir bar, 2,6-di-tert-butyl-4-methylphenol (2.35 g, 10.7 mmol) was added in 30 mL of methanol. To the flask, sodium methoxide (0.59 g, 10.9 mmol) was added and the reaction was stirred for 5 minutes. The methanol was removed under reduced pressure to yield 2,6-di-tert-butyl-4-methylphenolate as a green solid (3.8 g, 17.5 mmol). To the phenolate flask, was added maleic anhydride (1.75 g, 17.9 mmol) in 30 mL of DCM. The reaction was stirred at room temperature overnight. The reaction mixture was then extracted using 0.5 M NaHCO₃ (30 mL), and the organic (DCM) layer was dried over anhydrous sodium sulfate and concentrated under reduced pressure to yield a yellow solid (2.32 g).

Addition of maleic anhydride to 4-methylaniline:

To a round bottom flask equipped with stir bar, maleic anhydride (1.37 g, 14.0 mmol) in 75 mL DCM was added. To the flask, 4-methylaniline (1.47 g, 13.7 mmol) was added and the reaction was stirred at room temperature overnight. The product precipitated and was subsequently isolated via vacuum filtration. The desired maleimide product was a yellow solid (3.01 g, 14.7 mmol, 106%).

Radical bromination of compound 6:

To a pressure vessel equipped with stir bar, compound **6** (0.54 g, 2.6 mmol) in 40 mL of chloroform was added. To the flask, N-bromosuccinimide (0.92 g, 5.2 mmol) and 2,2'-azobis(2-methylpropionitrile) (0.03 g, 0.18 mmol) were added, and the reaction was stirred overnight at 110 °C. The product precipitated and was subsequently isolated via vacuum filtration to yield a yellow solid (0.74 g).

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