Building Biodegradable Antimicrobial Poly(caprolactone) Materials using Oxime Chemistry

An essay submitted in partial fulfillment of

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Biology

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Introduction:

Antimicrobials, agents that work against microbial organisms, are used extensively in our world today. From agriculture to human medicine, antimicrobials help prevent developing infections and treat those already ill. One practical use of antimicrobials that has been and continues to be utilized by humans is the addition of antimicrobials to textiles. Whether it be clothing, bandages, or shower curtains, the addition of antimicrobials help fight or prevent the growth of microorganisms (1). Though antimicrobials have proven to be very useful, bacteria have started to develop resistance against some of the known antimicrobials. This is an issue because bacteria have many diverse pathways in which they can affect the human body. For example, bacteria can be transferred through the consumption of other animals, additionally, some bacteria can be transmitted into other food products or surrounding environments that humans are exposed to (2). This ever growing resistance of antimicrobials yields a need for further antimicrobial research. For the purpose of this research, there was a focus on the utilization of antimicrobials in the textile industry. To find better antimicrobials for textiles, the antimicrobial must fit the criteria that prove antimicrobial viability including “efficacy against microorganisms, suitability for textile processing, durability, and a favorable safety and environmental profile” (1).

Antimicrobial Agents:

There are many different classes of antimicrobial agents that are utilized in the textile industry. Some of these antimicrobial agents include Triclosan, polybiguanides, zinc pyrithione and silver-based compounds, and *N*-halamines derivatives. Not enough research or studies have been completed to distinguish any of these antimicrobial agent superior over the other (1). On the other hand, it is known that some of these antimicrobial agents are considered safer and more environmentally friendly than others.

A particular concern has been raised with the use of metal based antimicrobial agents, such as silver nanoparticles. Though effective, silver nanoparticle additions have been found to be linked with leaching, short-term activity, and toxicity problems (3). When in contact with humans, silver nanoparticles can cause an inflammatory response (4). Additionally, nanoparticles within the human body have the ability to concentrate and potentially generate reactive oxygen species, which can disrupt the functionality of biomolecules (DNA, proteins, and enzymes) (5).

Other concerns surround alternate antimicrobial agents. The absorption of Triclosan through the skin has caused various health concerns for humans such as skin irritation and increased allergic reactions (6). Furthermore, polybiguanides have been utilized in a multitude of ways, including combating microbial contamination on sterile dressings. Alongside Triclosan, polybiguanides have been connected with cases of skin irritation and sensitization (7). Last, *N*-halamine structures have recently been conjugated with polymers, which can give way to the formation of antimicrobial agents. Though these agents are being generated, some problems have occurred: the covalent binding of the polymer and the *N*-halamine have proven difficult, and if successful, the binding of the *N*-halamine could cause deterioration of the polymer (8). For this

experiment, there was a focus on the addition of a hydantoin-based *N*-halamine to a polymer that not only has biodegradable properties, but also has antimicrobial characteristics.

Importance of an Antimicrobial Polymer

When looking at antimicrobial synthesis, one of the primary ways to which antimicrobials are made is through the addition of antimicrobial *N*-halamine derivatives to polymer chains. Antimicrobial polymer compounds are utilized to fight/prevent microbial infection; these compounds are especially connected to everyday practical items such as drugs, water purification systems, textiles, and food packaging and storage equipment. Furthermore, these antimicrobial polymer compounds can also be found on more important tools such as hospital or dental surgery equipment. Not only do antimicrobial polymer compounds assist in fighting/preventing microbial infection, but they also combat the issues concerning the low molecular weight that antimicrobial agents have. Low molecular weight antimicrobial agents are known for causing environmental toxicity and only have short-term abilities (9). By using a polymer compound, a large molecular weight for the antimicrobial is achieved. These polymer compounds contribute to many advantages necessary for a viable and effective antimicrobial. Such contributions include the minimization of environmental toxicity, increasing efficiency and selectivity, and establishing an increased lifetime of the antimicrobial agents. (9).

Hydantoin Based N-halamine

A *N*-halamine compound is a compound that has one or more nitrogen-halogen covalent bond(s) that is/are formed by the halogenation of an imide, amide, or amine group (10). Specific cyclic compounds within the *N*-halamine group have complementing properties with antimicrobials. Two of these cyclic compounds are hydantoin derivatives, 5-membered rings containing 2 cyclic nitrogens, and uracil derivatives, 6-membered rings containing 2 cyclic

nitrogens (11). *N*-halamine materials have been recognized and studied in response to their stabilities and regenerabilities when combating microorganisms. Additionally, when the hydantoin derivative is conjugated onto a polymer, it adds a hydrophobicity characteristic. This hydrophobicity has been known to increase the disinfection rate of cells (12). Working with the cyclic hydantoin compound, this research focused on manipulating the hydantoin derivative in order to create a derivative that could later be attached to a polymer backbone chain. The utilization of "click" chemistry allowed the conjugation of the hydantoin derivative to the polymer backbone chain. Moreover, the byproducts generated through "click" chemistry can be easily removed by benign solvents (13). With the successful conjugation of the polymer, textiles in the health care system could prevent infection of patients if the materials were coated with a hydantoin-based *N*-halamine antimicrobial agent.

Current Research

This Bachelor's Essay primary focus was on the synthesis of a polyester polymer, the synthesis of hydantoin compounds, and the conjugation of hydantoin compounds onto polyester polymers.

Prior to this study, a hydantoin-propyl bromide compound was used as the base of the hydantoin derivative. To incorporate a better leaving group and obtain higher yields, further manipulation of the hydantoin derivative was conducted: a substitution reaction of iodine for bromide was completed. Additionally, "Click" chemistry of the *O*-(2-iodobenzyl)hydroxylamine onto the polymer backbone was conducted to create a potential antimicrobial agent.

Results and Discussion:

A Baeyer-Villager oxidation reaction was conducted for the TOSUO monomer sysnthesis. A carbon and an oxygen was added onto 1,4-dioxaspiro[4,5]decan-8-one by using meta-chloroperoxybenzoic acid. Sodium carbonate and sodium bisulfite were used to work up the solution. The final product of 1,4,8-trioxaspiro[4.6]-9-undecanone was isolated by extraction, as seen in **Figure 1**. The ring-opening polymerization reaction of 1,4,8-trioxaspiro[4.6]-9-undecanone and ε-caprolactone was performed with tin (II) 2-ethylhexanoate as the catalyst to prepare a copolymer. With ^{1}H NMR analysis, it was confirmed that the copolymer consisted of 22.5 ε-caprolactone units and 3.5 TOSUO units. Trityltetrafluoroborate was used to removed the ketal group of P($CL_{22.5}$-co-$TOSUO_{3.5}$) and was converted into a ketone functional group as a site for monoiodohydroxylamine conjugation. The formation of P($CL_{22.5}$-co-$OPD_{3.5}$) was confirmed by the presence of two triplets at 2.70 and 2.51 ppm from the hydrogens alpha to the carbonyl and the lack of a singlet from the ketal hydrogens at 3.93 ppm in **Figure 2.**

KOH pellets were used to removed the amine hydrogen between the two carbonyl groups of 5,5-dimethylhydantoin. This was done in order to reduce the acidity of the hydrogen coming from the neighboring carbonyl groups. In a hot oil bath, the bromide on 1-chloro-3-bromopropane conjugated onto the hydantoin derivative, replacing the leftover electrons. The new hydantoin propyl chloride derivative was extracted, rotovaped, and dried on a high vacuum line. ^{1}H NMR spectroscopy confirmed a complete reaction by the downfield shift of the multiplet from 2.25 ppm on 5,5 dimethylhydanotin to 2.13 ppm on the chlorinated hydantoin in **Figure 3.**

Further manipulation of the hydantoin compound was completed including a Finkelstein reaction on the chlorinated hydantoin compound, which replaced chlorine with iodine. This was completed in order to generate a better leaving group on the hydantoin compound. Last, click

chemistry of the hydantoin compound and polymer P(CL_{50}-CO-OPD_6) was performed in order to get a functionalized polymer. The ^{1}H NMR spectra showed that the reaction was considered successful by the presence of an upfield shift in peaks, seen in **Figure 4.**

Summary and Conclusions:

There are a wide variety of antimicrobial agents utilized today. Though a variety of these agents are effective in preventing microbial growth, many of them have unfavorable health side-effects or are connected with toxicity, non-biodegradability, or deterioration. The use of a hydantoin based *N*-halamine compound could generate an antimicrobial agent that helps fight against microorganisms, provide a sustainable/durable material for textile processing, and develop a compound that is environmentally friendly. Through monomer synthesis, polymerization, manipulation of a hydantoin derivative, and the use of “click” chemistry, a polymer with antimicrobial properties can be created. The functional polymer can then be implemented and processed in the textile industry to help prevent the spreading and developing infections, specifically in a health care setting.

Materials:

Meta-chloroperoxybenzoic acid (*m*-CPBA), 1,4-cyclohexandione monomethylene ketal, 2-iodobenzylbromide, *N*-hydroxyphthalamide and hydrazine monohydrate were purchased from Sigma Aldrich. Sodium bisulfate, sodium carbonate, and anhydrous sodium sulfate were purchased from Fischer Scientific. Anhydrous sodium iodide was purchased from Chem-IMPEX International Inc. Anhydrous 5,5-dimethylhydantoin was purchased from Alfa Aesar. Sodium hydroxide and potassium hydroxide pellets were purchased from Ward's Science. Toluene (Sigma Aldrich) was dried by heating at reflux over sodium and distilled under nitrogen prior to use. All other solvents were purchased from Sigma Aldrich, Fisher, or VWR in bulk: ethyl acetate (EtOAc), hexanes, acetone, methanol (MeOH), dichloromethane (CH2Cl2), deuterated chloroform (CDCl3), and tetrahydrofuran (THF). ε-Caprolactone (CL) (Sigma Aldrich) and benzyl alcohol (BnOH) were distilled from calcium hybrid (Ca_2H) and stored under nitrogen prior to use.

Instrumentation:

A 400 MHz Varian Mercury 300 Vx NMR spectrometer was utilized to obtain proton and carbon nuclear magnetic resonance (^{1}H and ^{13}C NMR). Samples were acquired in deuterated chloroform for nt=32 or 128 for proton experiments of small molecules and polymers. MestreNova freeware was used to create NMR figures.

(need more here).

Experimental:

TOSUO Monomer Synthesis

Solid 1, 4-dioxasprio[4,5]decan-8-one was dissolved in 40mL of dichloromethane in a 200-mL round bottom flask and stirred with a magnetic stir bar. Over 30 minutes, *meta*-chloroperoxybenzoic acid (9.86g, 41.62mmol, 1.3 eq) added scoop-wise from a 50-mL beaker. The beaker was washed with 20 mL of dichloromethane, and added to the round bottom flask. The round bottom flask was capped and left stirring over night. This solution was poured into a 1000-mL Erlenmeyer flask with the addition of 100mL dichloromethane and 100mL of water. Sodium bisulfite (6.50 g, 63.42 mmol, 1.11 eq) was added scoop-wise over five minutes. The solution was left stirring for an hour. Sodium bicarbonate (5.30 g, 63.10 mmol, 1.11 eq) was added scoop-wise to the flask. The flask was covered with foil and and left to stir overnight. The Erlenmeyer flask was uncovered and 50-mL of dichloromethane and 50-mL of water were added. This solution was poured into to a 1000-mL separatory funnel. The organic layer was drained into a 500-mL Erlenmeyer flask, and the aqueous was washed with 2 x 50 mL of dichloromethane. The organic layer was washed with 2 x 50 mL sodium bisulfite. The solution was shaken before drained into a 500 mL Erlenmeyer flask. The new organic layer was washed with 3 x 50 mL sodium bicarbonate, and was shaken alongside careful ventilation. The organic layer was dried with 50 mL of brine and added to a 500 mL Erlenmeyer flask. Add approximately 20g of anhydrous sodium sulfate to finish drying the solution. This solution was filtered with a funnel into a 200 mL round bottom flask and dried using rotary evaporation. Yield: 5.07g, (92% yield), ^{1}H NMR (400 MHz, CDCl3, δ): 1.68 (t, 2H, -COCH2C**H**$_2$-), 2.02 (t, 2H, -COOCH2C**H**$_2$-), 2.34 (t, 2H, -COC**H**$_2$-), 4.09 (s, 4H, acetal -OC**H**$_2$C**H**$_2$O-), 4.20 (t, 2H, -COOC**H**$_2$-) ppm.

Deprotection of P($CL_{22.5}$-CO-$TOSUO_{3.5}$) using Trityltetrafluoroborate.

Solid P($CL_{22.5}$-CO-$TOSUO_{3.5}$) (0.5 g, .153 mmol, 1 eq) was dissolved in 18 mL of dichloromethane in a 250 mL round bottom flask and stirred with a magnetic stir bar. Trityl tetrafluoroborate (0.27 g, 0.804 mmol, 1.5 eq) was added to the round bottom flask. The solution was left to stir for an hour. This solution was poured into a 1000 mL Erlenmeyer flask with a magnetic stir bar. Eight hundred milliliters Methanol was added to the Erlenmeyer flask. The Erlenmeyer flask was covered with foil and left to stir over night. Yield: 0.4252g (89 % yield). ^{1}H NMR (400 MHZ, $CDCl_3$, δ): 7.34 (m, 2H, Ar **H**), 6.98 (t, 1H, Ar **H**), 5.05 (s, 2H, benzyl -C**H**$_2$-), 4.27 (t, 2H, -CH_2C**H**$_2$O-), 3.99 (t, 2H, -CH_2C**H**$_2$O-), 2.70 (m, 4H, -C**H**$_2$COOC**H**$_2$-), 2.51 (t, 2H, -COOC**H**$_2$-), 2.23 (t, 2H, -COOC**H**$_2$-), 1.58 (m, 4H, -C**H**$_2$$CH_2$C**H**$_2$), 1.32 (m, 2H, -$CH_2$C**H**$_2$$CH_2$-) ppm.

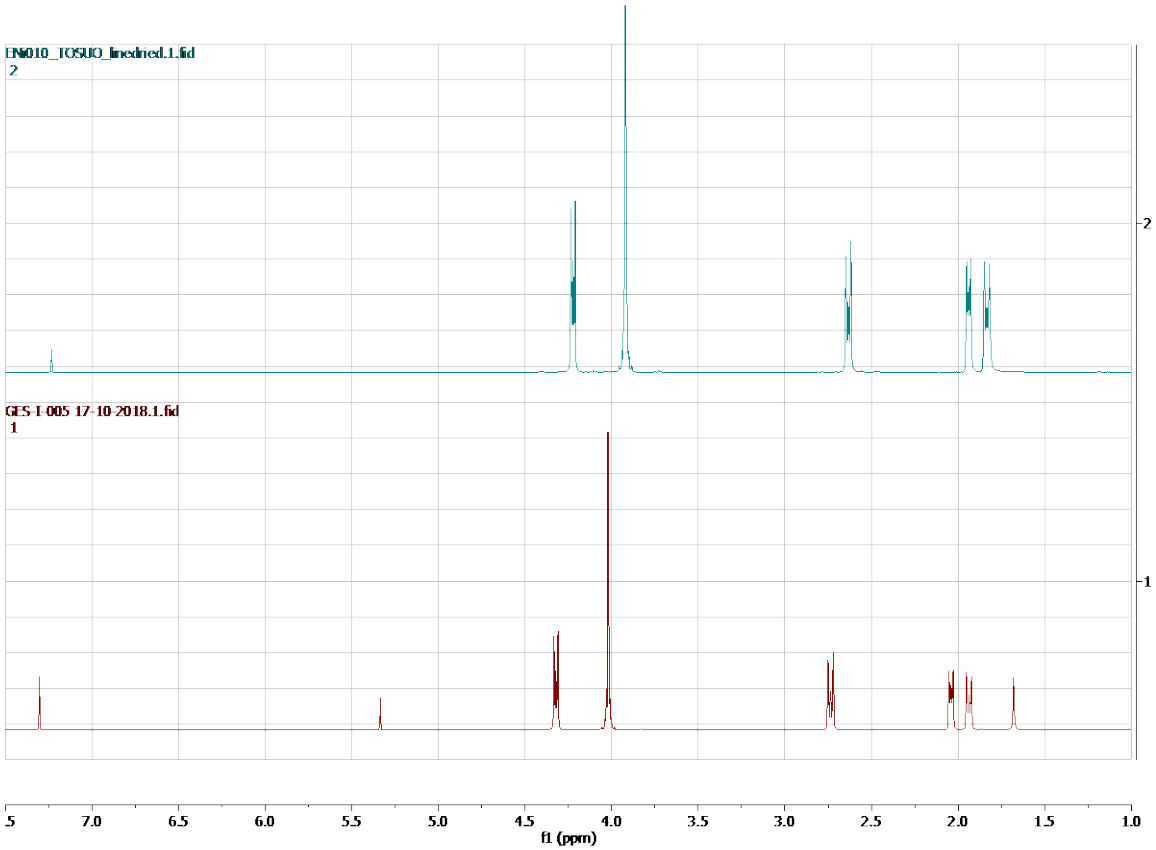
Synthesis of Hydantoin-Propyl Chloride.

Solid KOH pellets (0.88 g, 15.6 mmol, 1 eq) and 30 mL of EtOH were added to a beaker with a stir bar. The solution was stirred until all of the pellets were dissolved. Solid 5,5-dimethylhydantoin (2.00 g, 15.6 mmol, 1eq) was added to a 100 mL round bottom flask. Transfer the dissolved solution to the 100 mL round bottom flask. Cap the flask and leave to stir for 20 minutes. The flask was set in an 80^{o}C silicone oil bath and liquid 1-bromo-3-chloropropane (3.00 mL, 31.2 mmol, 2.0 eq) was added drop wise to the flask. The solution turned cloudy white and the reaction refluxed for 24 hours.

The solution was transferred to a 200 mL round bottom flask with MeOH and CH_2Cl_2. The excess solvent was rotovaped off, leaving a thick white residue. Fifty milliliters of both EtOAc and H_2O were added to the round bottom flask. The solution was poured into a 500 mL

separatory. The bottom aqueous layer was drained into its respective labeled Erlenmeyer flask. The top organic layer was drained into its respective Erlenmeyer flask. The aqueous layer was poured back into the separatory funnel and washed with 25 mL of EtOAc. The organic layer was poured back into the separatory funnel and washed with 2 x 25 mL of $NaHCO_3$. The aqueous layer layer was drained into its respective flask, and the organic layer was drained into its respective flask. The organic layer was dried with 8 g of anhydrous $NaSO_4$ for 20 minutes. The organic solution was filtered into a 250 mL round bottom flask and rotovaped to get the excess solution off. White crystals were produced in the rotovaped process and were dried on a high vacuum line. Yield: . ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.05 (s, 1 H, -N**H**-), 3.67 (t, 2H, -NC**H$_2$**CH2CH2Cl-), 3.55 (t, 2H, -NCH2CH2C**H$_2$**Cl-), 2.13 (m, 2H, -NCH2CH2C**H$_2$**Cl-), 1.45 (s, 6H, -CC**H$_2$**C**H$_2$**-) ppm.

Figure 1: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f1 (ppm)

Figure 2.

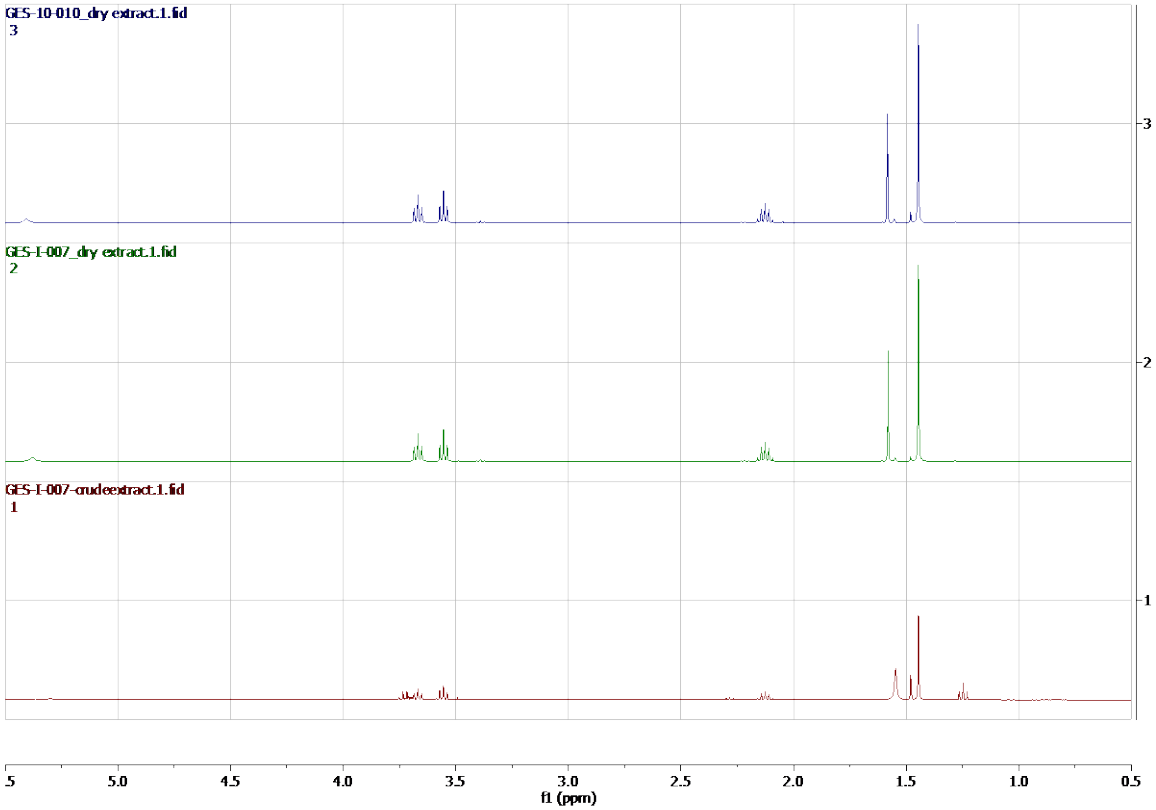
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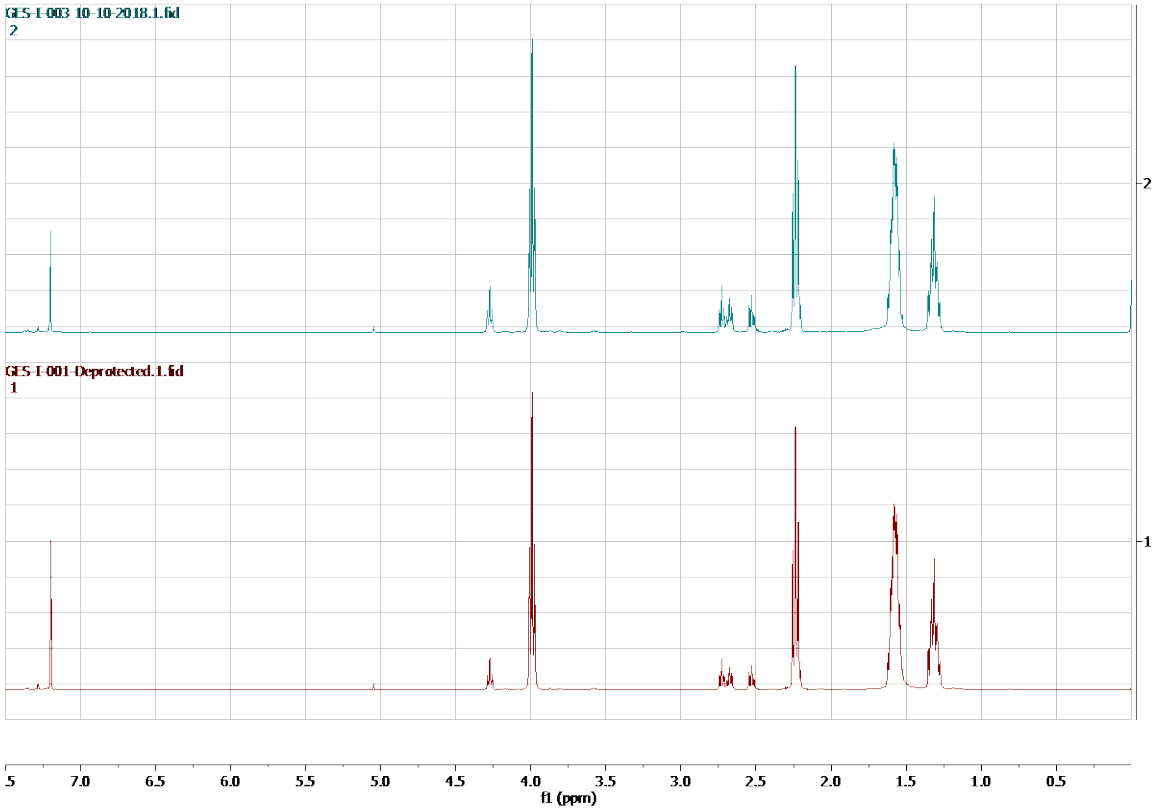
Figure 3.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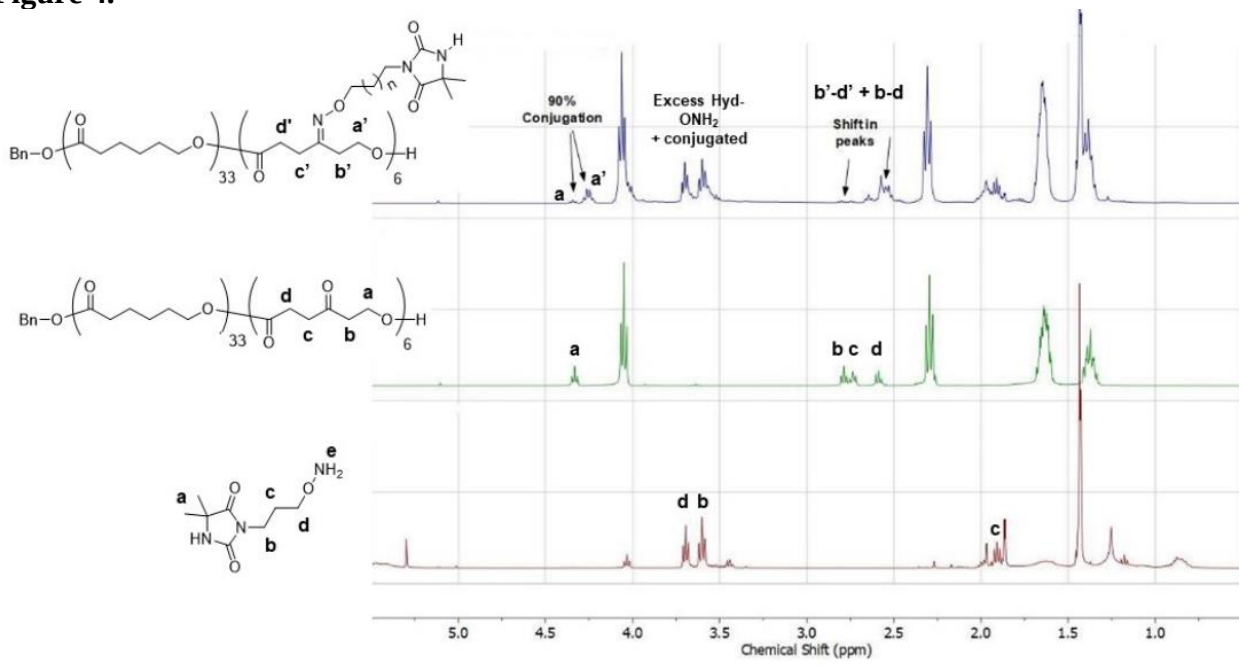
f1 (ppm)

Figure 4.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Excess Hyd-ONH2 + conjugated
b'-d' + b-d
Shift in peaks
a
a'
d'
a'
c'
b'
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Bn–O
H
d
a
c
b
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d b
c
e
NH2
HN
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Chemical Shift (ppm)

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