New Derivatization Strategies for Installation of Fixed, Permanent Charges onto Analytes of Biological and Environmental Concern

by

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Abstract

Mass spectrometry (MS) is a highly sensitive analytical technique that can be used to identify thousands of analytes from a single biological matrix. Chemical derivatization (CD) reagents can enhance analyte detection and quantification by adding a specific functional group that enhances ionization efficiency and stability. The goal of this project is to design a new CD reagent by synthesizing a Meldrum’s acid derivative containing a fixed permanent charge (MAD\(^+\)) to be used as a stable ketene precursor. Synthesis of a MAD\(^+\) was explored via alkylation and Knoevenagel condensation reactions but proved unfruitful. However, C-alkylation with 6-bromohexanoic acid via carbodiimide coupling, reduction and amine alkylation produced a MAD\(^+\) in generous yields. The reaction of this MAD\(^+\) with 3,5-di-tert-butylphenol demonstrated its ability to induce its fixed permanent charge onto analytes, allowing its detection in MS. Purification of the final MAD\(^+\) remained an issue and should be investigated and optimized in future works.
Acknowledgements

I would like to thank my supervisor, Jeff Manthorpe, for all his support and patience over the past two years. It was an incredible experience working and learning from you, who would have thought a MAD would drive us so mad haha. I will never forget our chalkboard sessions trying to come up with new ideas. I guess nineth times the charm, right?

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<tbody>
<tr>
<td>CID</td>
<td>Collision-induced dissociation</td>
</tr>
<tr>
<td>COSY</td>
<td>$^1$H–$^1$H Correlation spectroscopy</td>
</tr>
<tr>
<td>Da</td>
<td>Daltons</td>
</tr>
<tr>
<td>DBA</td>
<td>N,N-dimethylbenzylamine</td>
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<tr>
<td>DCM</td>
<td>Dichloromethane</td>
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<tr>
<td>DCU</td>
<td>1,3-Dicyclohexylurea</td>
</tr>
<tr>
<td>DIPEA</td>
<td>N-Diisopropylethylamine</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-Dimethylaminopyridine</td>
</tr>
<tr>
<td>EDC•HCl</td>
<td>1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray ionization</td>
</tr>
<tr>
<td>EtOAc</td>
<td>Ethyl acetate</td>
</tr>
<tr>
<td>Et$_2$O</td>
<td>Diethyl ether</td>
</tr>
<tr>
<td>FCC</td>
<td>Flash column chromatography</td>
</tr>
<tr>
<td>FVP</td>
<td>Flash vacuum pyrolysis</td>
</tr>
<tr>
<td>HSQC</td>
<td>Heteronuclear single quantum coherence</td>
</tr>
<tr>
<td>HMBC</td>
<td>Heteronuclear multiple bond correlation</td>
</tr>
<tr>
<td>iTrEnDi</td>
<td><em>in situ</em> trimethylation enhancement using diazomethane</td>
</tr>
<tr>
<td>LC</td>
<td>Liquid chromatography</td>
</tr>
<tr>
<td>MA</td>
<td>Meldrum’s acid</td>
</tr>
<tr>
<td>MAD</td>
<td>Meldrum’s acid derivative</td>
</tr>
<tr>
<td>MeCN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>MS/MS</td>
<td>Tandem mass spectrometry</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass-to-charge ratio</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>PSF</td>
<td>Pear-shaped flask</td>
</tr>
<tr>
<td>PILs</td>
<td>Protic ionic liquids</td>
</tr>
<tr>
<td>PyrrolIL</td>
<td>Pyrrolidinium ionic liquid</td>
</tr>
<tr>
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<td>Round bottom flask</td>
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</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
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Chapter 1: Introduction

1.1 Chemical Derivatization Strategies in the Omic Disciplines

The term “omic” has been added to numerous fields of molecular biology to denote its comprehensive assessment of a sample through a high-throughput, non-targeted and non-biased approach. Often the samples contain complex matrices with some low-abundance analytes, making their detection and identification difficult. There are many types of analytical techniques available to evaluate these complex samples but mass spectrometry (MS) is most commonly used. As a highly sensitive technique, MS measures the molecular mass of an analyte based on its mass-to-charge ratio ($m/z$). Analysis of complex matrices provides exact masses of thousands of analytes simultaneously over a wide mass range and can resolve small mass differences within the mixture.

However, detection and quantification via MS can be challenging for an analyte if it has low ionization efficiency, forms multiple charge states or forms multiple molecular ions. One method used to address detection and quantification challenges seen in MS are chemical derivatization (CD) reagents. CD reagents involve reacting a specific functional group of an analyte and results in structural alterations that increases ionization efficiency and stability and decrease volatility leading to enhanced MS detection and quantification.

In the omic disciplines, derivatization reagents can also be helpful when analyzing complex matrices to not only enhance detection of low-abundance analytes but also target specific functionalities within the matrix. For example, proteomic studies can analyze reactive cysteine residues that are prone to oxidation by reactive oxygen species by reacting free cysteines with CD reagents with and without isotope incorporation. The oxidation of cysteine on the protein can then be quantified by comparing the ratio of $^{12}$C and $^{13}$C labelled
CDs as seen in Figure 1.1 A which can be later used to understand common diseases and conditions.\textsuperscript{8} In targeted lipidomic studies, CD reagents incorporate isotopes to allow simultaneous analysis of multiple samples targeting free fatty acids as seen in Figure 1.1 B.\textsuperscript{9} For untargeted metabolomic studies, the metabolome is subdivided into multiple function-group based submetabolome which would then undergo derivatization with a CD reagent based on the targeted functional group as seen in Figure 1.1 C.\textsuperscript{10} The data is then combined to produce a thorough metabolome profile containing all targeted functional groups.\textsuperscript{10}
For biological samples, CD reactions targeting amines, carboxylic acids, alcohols, phenols, thiols, ketones, phosphates and aldehydes are used due to the presences of these functionalities in numerous biomolecules, as seen in Figure 1.2.⁴ Although there are numerous different types of CD reagents, the overall outcome of the derivatization can be divided into two separate categories. The first being the incorporation of easily ionizable groups, while the second results in a charged tag being fixed onto the analyte.³

---

**Figure 1.1.** Examples of the incorporation of CD reagents in omic technologies. A) Chemical derivatization of cysteine residues using isotope-coded affinity tag (ICAT) of proteins to determine the ratio of oxidized cysteines when under normal and oxidative stress conditions. Adapted by Leonard and Carroll.⁸ B) General workflow of multiplexed quantification of free fatty acids in lipids using different derivatization tags with and without isotope labelling. Adapted by Zhao.⁹ C) General workflow of untargeted LC-MS metabolome analysis using CD reagents. Adapted by Zhao and Li.¹⁰
Figure 1.2. Functional groups observed in biomolecules including amines, carboxylic acids, alcohols, phenols, thiols, ketones, phosphates and aldehydes.\textsuperscript{4,11–13}

1.1.1 CD reagents introducing easily ionizable groups

One category of CD reagents is the addition of a functional group that has a high proton affinity onto an analyte of interest.\textsuperscript{3} Often, these groups are highly basic to permit proton capture from solvent or during the desolvation process in the ionization chamber.\textsuperscript{3,14} One example from this classification is succinic acid 2-dimethylaminoethyl ester imidazolide as seen in Figure 1.3.\textsuperscript{15} The derivatization reaction occurs over four hours at 65 ^\textdegree C, however, the reagent must be prepared immediately before use.\textsuperscript{15} Successful derivatization was observed for cholesterol and phospholipids from a human serum extract.\textsuperscript{15} However,
multiple ion peaks corresponding to underivatized and derivatized phospholipids were observed, making quantification complex.\textsuperscript{15} Another CD reagent that contains a highly ionizable group is 4-amidinobenzoic acid (Aba) as seen in Figure 1.3.\textsuperscript{16} This reagent was highly successful at increasing sensitivity and generating d-ions of the peptide to allow differentiation of leucine and isoleucine residues.\textsuperscript{16} However, derivatization reactions with peptides occurred overnight at room temperature thus quick analysis of analytes was limited.\textsuperscript{16}

\begin{center}
\includegraphics[width=\textwidth]{figure1_3.pdf}
\end{center}

\textbf{Figure 1.3.} Chemical derivatization strategies containing an easily ionizable group including A) 2-dimethylamino ester imidazolide and B) 4-amidinobenzoic acid (Aba).\textsuperscript{15,16}

\subsection{CD reagents containing a fixed permanent charge}

While the use of CD reagents to incorporate an easily ionizable group can impart increased MS sensitivity, there can be simultaneous fragmentation of the substrate, resulting in the loss of the charge tag due to proton migration.\textsuperscript{3} Thus, a second approach is the use of CD reagents containing a fixed permanent charge.\textsuperscript{3} One CD reagent that results in a fixed permanent charge on an analyte is S,S-dimethyl-4-thiobutanoyl hydroxysuccinimide (DMBNHS) iodide, as seen in Figure 1.4 (A). This reagent is often used to modify lysine residues and the N-terminus of peptides and proteins as it reacts with primary amines.\textsuperscript{17}
During CID-MS/MS experiments, modified analytes in complex mixtures can be identified based on the neutral loss of dimethylsulfide from the derivatized analytes. However, the modification reaction takes 24 hours to complete, a mixture of charge states can be observed and there remains the potential for neutralization due to the acidic proton α to the sulfonium ion, though the latter is unlikely.

Another group of CD reagents that result in a fixed permanent charge are Girard’s reagents shown in Figure 1.4 (B). There are numerous variations of the Girard reagents but each contains a quaternary ammonium ion. Variants incorporating pyridine (P) and trimethylamine (T) are commercially available and are often used to derivatize steroids to enhance MS sensitivity and adjust chromatographic behaviour. The reaction results in a conversion of over 91%; however, the stability of the resulting derivatized analyte in one study was up to 18 hours at 4 °C, thus indicating a limited lifetime of these derivatives. Analysis of the peak area corresponding to the derivatized analytes had a relative standard deviation ranging from 6.4 – 12.2% during the 18 hour period so it was suggested that analysis be performed within this time frame. Furthermore, similar to DMBNHS iodide, there is also a potential for neutralization due to the presence of the acidic proton; however in this case, the α proton is also α to the carbonyl, resulting in a much lower proton affinity and thus greater potential for neutralization than in DMBNHS esters.
Aulenback; Derivatization Strategies for Fixed Permanent Charges

**Figure 1.4.** Chemical derivatization strategies inducing a fixed permanent charge including A) $S,S'$-dimethylthiobutanoylhydroxysuccinimide (DMBNHS) iodide, and B) Girard’s reagent T (i) and P (ii).\(^ {3,15,17,20}\)

Due to the observed issues of potential neutralization, multiple charge states and long reaction times for various fixed charged CD reagents, the Manthorpe and Smith groups of Carleton University decided to develop their own CD strategy called “TrEnDi” or trimethylation enhancement using diazomethane.\(^ {11,21-23}\)

TrEnDi was first reported in 2014 to enhance peptide sensitivity and quantification with mass spectrometry.\(^ {11,21-23}\) It uses CD reagent diazomethane, which is a methylating agent of functional groups with $pK_a$ values of up to 11 such as phosphate/phosphoric acid moieties, carboxylic acids and amines, resulting in a fixed permanent charge on the desired analyte.\(^ {11,21-23}\) This type of derivatization is unique as the CD reagent itself does not contain the fixed charge but induces it by complete methylation of all derivatization sites.\(^ {11,21-23}\)

Initial development of this strategy used an ethereal solution of diazomethane to react with peptides and glycerophospholipids, affording quaternary ammonium ions, as seen in Figure 1.5.\(^ {11,21}\) However, it was observed that fragmentation of TrEnDi-modified
phosphatidylethanolamine (PE) and TrEnDi-modified phosphatidylcholine (PC) would produce isobaric species.\textsuperscript{11,21} Thus, isotopic labelling was incorporated into TrEnDi to differentiate the species by synthesizing $^{13}$C labelled diazomethane.\textsuperscript{11,21}

One particular issue with the TrEnDi method is the safety concerns regarding diazomethane. Diazomethane is highly explosive and toxic, thus direct handling had to be conducted in an extremely safe manner to prevent detonation of the reagent. To address this issue, the method was further developed to the in situ TrEnDi strategy or iTrEnDi.\textsuperscript{22} An apparatus was constructed to allow the in situ generation of diazomethane gas, which is then delivered through PEEK tubing as a steady stream of nitrogen gas containing ether and diazomethane directly into the analyte solution.\textsuperscript{22} While this modification addressed the safety concerns regarding direct handling of diazomethane, the inconsistent full modification of functionalities with a $pK_a$ around or slightly higher than 11 remained an issue.\textsuperscript{22} This concern is illustrated nicely in the derivatization of sphingomyelin (SM) as singly and doubly methylated products were detected by MS, as seen in Figure 1.5.\textsuperscript{22} The identification of the second methylation site was suggested to be the allylic secondary alcohol rather than the secondary amide as it is more acidic.\textsuperscript{22} However, specific identification of which site was modified could not be confirmed with the data collected.\textsuperscript{22} For other experiments, splitting of modification states can limit the ability for the data to quantify analytes as all charged states would need to be identified and considered for the correlation.
A comparative study was performed to determine the sensitivity increase gained between the in-solution TrEnDi and iTrEnDi apparatus. As seen in Figure 1.6, derivatization of PE and PC resulted in a sensitivity increase compared to its unmodified counterpart. However, there was a significant loss in sensitivity when the iTrEnDi apparatus was used. Although it allows a safer method of derivatization, the decrease in MS sensitivity poses a concern as the ultimate goal of the CD reagent is to increase sensitivity as much as possible.
Thus, due to the issues regarding safety, decrease in sensitivity, production of isobaric species and inconsistent modification of functionalities with pK_a values higher than 11, it was decided to investigate new CD reagents. The goal is to develop a CD reagent containing a fixed permanent charge that is able to increase MS sensitivity similar to the original TrEnDi method while being functional, highly chemoselective and user friendly.

1.2 Click Chemistry

When designing a CD reagent, the synthesis of the reagent should be easy to achieve while maintaining high yields at all steps. One group of chemical reactions that could be incorporated into the synthetic design to achieve this goal are click chemistry reactions. The concept of click chemistry was first introduced back in 2001 by Kolb and Sharpless as a group of reactions that are easy to use, versatile, produce high yields and are stereospecific. In addition to this general criteria, to be considered a “click reaction" the
reaction must be neat or use a benign solvent and purification must also be achieved using non-chromatographic methods.\textsuperscript{25,26} The most common click reaction is the copper-catalyzed cycloaddition of azides and alkynes, as seen in Figure 1.7.\textsuperscript{25,26} Despite being a fast, simple and high yielding reaction, the safety of small molecule azides remained a concern.\textsuperscript{25,26} Certain small organic azides are explosive when heated, with the hazard related to the azide increasing if the azide group is attached to olefinic, aromatic or carbonyl groups.\textsuperscript{25} However, under standard conditions, they are stable and will remain “invisible” until contact with a dipolarophile, such as an alkyne.\textsuperscript{25,27,28} Other common click reactions include nucleophilic opening of ring structures and non-aldol type carbonyl chemistry as seen in Figure 1.7.\textsuperscript{25,26}

**Cu(I)-catalyzed azide-alkyne cycloaddition (CuACC)**

\[
\begin{align*}
\text{CuSO}_4/\text{THPTA} & \quad 6 \text{ h, } 40 \degree \text{C} \\
\text{O} & \quad \text{N} \\
\end{align*}
\]

**Nucleophilic ring opening**

**Oxime ether formation**

*Figure 1.7.* Common click reactions including copper catalyzed azide-alkyne cycloadditions, nucleophilic ring opening and oxime ether formation. Adapted from Kolb and Meng.\textsuperscript{27,28}

Due to the versatility and the wide range of starting material available either commercially or synthetically, it was decided to incorporate the copper-catalyzed click reaction into the design of the new CD reagent.
1.2.1 Ketenes

Click chemistry, in its simplest form, involves reactions that occur at room temperature, are quantitative and can occur without a solvent or catalyst.\textsuperscript{24} Although it is not considered a conventional click reaction, the [2+2] cycloaddition reaction and nucleophilic reactions of cumulenes can fall in this reaction category as they often occur without a solvent and produce quantitative yields.\textsuperscript{24} Cumulenes are defined by their distinctive bonding pattern including a central sp hybridized atom with double bonds to two other sp or sp\textsuperscript{2} hybridized atoms.\textsuperscript{29,30} However, the reactive intermediate ketenes are unique within the class as they contain two pi-bonds, alkene and carbonyl, to the same carbon.\textsuperscript{29,30}

1.2.2 Ketene reactivity

The reactivity of ketenes is highly diverse including reactions with amines (1\textdegree, 2\textdegree and 3\textdegree), alcohols (1\textdegree, 2\textdegree and 3\textdegree) and [2 + 2] cycloadditions, giving them great potential as new CD reagents.\textsuperscript{29,31} The versatility of ketenes has made them a useful synthetic building block in numerous applications such as the synthesis of natural products, drug development and polymer chemistry as seen in Figure 1.8.\textsuperscript{32–34}
Figure 1.8. Examples of ketene chemistry in various synthetic applications. A) Ketene intermediate accessed photochemically during the total synthesis of natural product (+)-psiguadial. B) Ketene intermediate accessed via its acid chloride counterpart when treated with N,N-diisopropylethylamine during the synthesis of non-toxic analogue of anesthetic drug Bupivacaine. C) Ketene intermediate utilized in living anionic polymerization with aldehydes to produce highly substituted polyesters.

Ketenes have been subjected to various studies to understand the electronics related to their unique chemistry. Studies investigating the dipole moment of the ketene found the compound resonates between various structures, with its most dominant forms illustrated in Figure 1.9. Studies have determined the resonance contributors to be structure i, ii and iii each with a percentage contribution of 37%, 32% and 29% respectively.
To understand the nucleophilic and electrophilic properties of the ketene, a study by Houk was performed to investigate the molecular orbital energies of the compound. It was found that their structure is highly unique with the lowest unoccupied molecular orbital (LUMO) located within the ketene plane and the highest unoccupied molecular orbital (HOMO) perpendicular to the ketene plane, illustrated in Figure 1.10. As a result, C2 has electrophilic properties while the carbonyl oxygen and C1 are nucleophilic, thus making ketenes ambiphilic.
However, it is noted that the substitution of the ketene can impact its electronic properties and thus its reactivity in these reactions. In general, substituents that are electropositive and \( \pi \) acceptors are more stabilizing, while electronegative and \( \pi \) donors are more destabilizing. Using \textit{ab initio} molecular orbital calculations, one study found that these substituents would delocalize the negative charge present on C1 when it was in resonance structure III, seen in Figure 1.9, resulting in a more stabilized ketene.\(^{40}\)

\begin{figure}
\centering
\includegraphics[width=\textwidth]{frontier_molecular_orbitals_ketene.png}
\caption{Frontier Molecular Orbital Energies of HOMO and LUMO of ketene measured in complete neglect of differential overlap (CNDO/2). Adapted from Houk.\(^{38}\)}
\end{figure}
1.2.3 **Ketene formation**

The synthesis of a ketene can be accomplished in many different ways. Meldrum's acid (2,2-dimethyl-1,3-dioxane-4,6-dione, 1) is a stable, user-friendly ketene precursor.\(^{29,32,41}\) As seen in Figure 1.11, Meldrum's acid derivatives (MADs) can undergo thermolysis or photolysis to produce the corresponding ketene which can then either dimerize or become trapped in situ by a nucleophile.\(^{29,32,41}\)

\[ \text{O} \quad \text{O} \\
\text{O} \quad \text{R} \quad \text{R'} \\
\text{O} \quad \Delta \text{ or } h\text{v} \
\text{R} \quad \text{R'} \
\]  

**Figure 1.11.** Formation of ketene from Meldrum's acid derivative via thermolysis or photolysis.\(^{29,32,41}\)

Synthesis of ketenes via MADs has its advantages over direct synthesis as it allows a wide diversity of ketene precursors to be easily accessible and the generation process does not produce reactive by-products.\(^{29}\) However, it is important to note the reactivity of MAD ketenes and the conditions required to produce them.

When designing a MAD as a ketene precursor, it was important to consider that disubstituted ketenes often require harsher conditions.\(^{42,43}\) As seen in Scheme 1.1, flash vacuum pyrolysis results in the formation of the corresponding ketene and co formation of acetone and carbon dioxide at temperatures over 200 °C.\(^{42,43}\) In comparison, enolizable MADs can undergo retro-hetero-Diels Alder cycloadditions either thermally (110–160 °C) or
photochemically to form the corresponding acyl-ketene as an intermediate.\cite{43,44} Once captured by the nucleophile, further adjustment of reaction conditions can allow the loss of carbon dioxide to form the final product.\cite{45} However, it is important to note that depending on the type of substitution present on the enolizable MAD, higher temperatures or longer reaction times may be required for complete modification.

Scheme 1.1. Ketene formation from Meldrum's acid and derivatives.\cite{42-44}

In addition to substitution pattern, the chain length of the substituent on the ketene must also be considered. This is due to their preference to undergo intramolecular Friedel-Crafts acylation reactions when the ring size is 6, 7 or 5 according to preference order as seen in
Figure 1.12. This reaction has been very useful when producing β-lactams and substituted indanones however, must be kept in mind when designing a MAD in the context of a CD reagent. If the chain length at the C5 position provides a 5-7 atom system, the resulting ketene will preferentially undergo an intramolecular Friedel-Crafts acylation reaction rather than reacting with the desired analyte.

**Figure 1.12.** Friedel-Crafts Acylation of enolizable Meldrum’s acid derivative catalyzed by Lewis acid TMSOTf. Atoms in six membered ring to participate in intramolecular Friedel-Crafts Acylation are highlighted in blue.

Adapted from Fillion.47

1.2.3.1 The Utility of Meldrum’s Acid

1.2.3.1.1 History

Meldrum’s acid (2,2-dimethyl-1,3-dioxan-4,6-dione, 1) was first synthesized by Andrew Meldrum in 1908, while investigating condensation reactions involving acetone and malonic acid.48 At the time, condensation reactions involving these reagents were examined by
various researchers including Massot, Meyenberg and Knoevenagel, summarized in Scheme 1.2.\textsuperscript{48–52}

**Massot (1894) and Knoevenagel (1905)**

\[
\text{HOOC-CHOH} + \text{O} \quad \xrightarrow{\text{or}} \quad \text{NH}_4^+ \quad \text{OOC-CHOH} \quad \xrightarrow{\text{or}} \quad \text{NH}_4^+
\]

\[
\text{decarboxylated } \alpha\text{-addition product}
\]

**Meyenberg (1895)**

\[
\text{OOC-CHOH} + \text{O} \quad \xrightarrow{\text{ZnCl}} \quad \text{OOC-CHOH-CH}_2\text{OOC-CHOH}
\]

\[
\alpha\text{-addition product}
\]

**Knoevenagel (1896)**

\[
\text{OOC-CHOH} + \text{CH}_2\text{CO} + \text{Pyridine} \quad \text{0}^\circ \text{C}
\]

\[
\text{bis-adduct}
\]

\[
\text{EtO}_2\text{C-CO} + \text{CH}_2\text{CO} + \text{Pyridine} \quad \text{20}^\circ \text{C}
\]

\[
\text{EtO}_2\text{C} + \text{CO} + \text{CH}_2\text{CO} + \text{Pyridine}
\]

**Scheme 1.2.** Precedent for condensation reactions studied by Meldrum in 1908.\textsuperscript{48–52}

Based on the literature at the time, the condensation reaction of malonic acid, acetone with catalytic sulfuric acid in acetic anhydride was expected to yield either the \(\alpha\)-addition product or decarboxylated \(\alpha\)-addition product.\textsuperscript{48} However, characterization of the product using
alkali titration reflected a monoprotic acid with the chemical formula $\text{C}_6\text{H}_8\text{O}_4$.\textsuperscript{48} This suggested that one of the carboxyl groups of malonic acid were unchanged, thus, Meldrum proposed a $\beta$-lactonic acid-based structure (Scheme 1.3), to which he named 'Meldrum's acid'.\textsuperscript{48}

This structure was accepted by the chemistry community until a paper was published by Davidson and Bernhard in 1948 who further investigated the proposed structure.\textsuperscript{53} Through a series of reactions with Meldrum’s acid demonstrated in Scheme 1.3, Davidson and Bernhard suggested that the consistent regeneration of acetone indicated the structure proposed by Meldrum was incorrect.\textsuperscript{53}

Scheme 1.3. Verification reactions performed by Davidson and Bernhard to investigate the structure of Meldrum’s acid. General reactions performed that consistently resulted in the generation of acetone and other side products.\textsuperscript{53}
It was also found troubling that upon treatment of Meldrum’s acid silver salt with methyl iodide, starting material, monomethyl and dimethyl variants were detected, and were assigned structures (i) and (ii) respectively in Scheme 1.4.53

Scheme 1.4. Treatment of Meldrum’s acid 1B with methyl iodide yielded starting material 1B, monomethyl and dimethyl variants.53

Upon analysis of the corresponding dimethyl variant (putative ii), ester characteristics were not reported.53 When subjected to pyrolysis, the dimethyl variant (putative ii) generated dimethylketene in addition to carbon dioxide and acetone.53 It was suggested that this derivative must be in relation to the compound dimethylmalonic acid.53 Davidson and Bernhard proposed that the methylene group in malonic acid is fully incorporated into the structure along with both carboxyl groups instead of just the one as Meldrum proposed.53 This idea was supported by the fact that cinnamalonic acid is able to form a condensation product with cinnamaldehyde despite not having any methylene groups for aldol condensations.53 Thus structure 1 in Scheme 1.5, a bifunctional ester, was suggested as the proper structure as it accounts for the constant generation of acetone and its acidity.53
Scheme 1.5. Synthesis of Meldrum's acid through the condensation reaction of acetone and malonic acid.

Correct Structure is 1; 1B is β-lactone structure proposed by Meldrum.

The compounds reactivity was compared to an analogous compound, dimedone, to further confirm the proposed structure. Similar to dimedone, Meldrum’s acid produced a purple product when treated with aqueous sodium nitrite, which is presumed to be sodium isopropylidene isonitrosomalonate. In addition, when titrated with bromine in acetic acid with three equivalents of potassium acetate, Meldrum’s acid reacted with two moles of bromine just as dimedone does. To provide further evidence that the proposed structure 1 was correct, Davidson and Bernhard demonstrated that the initially proposed structure for the dimethyl derivative of Meldrum’s acid (ii) was actually (iii) as seen in Figure 1.13. This was done by treating the dimethyl derivative with dilute HCl which produced acetone and dimethylmalonic acid.
1.2.3.1.2 Chemical Properties

Compared to other 1,3-dicarbonyl compounds, 1 is extremely unique due to its high acidity and complex reactivity. In comparison to homologous structures such as dimedone (6) and dimethyl malonate (7), 1 is significantly more acidic by orders of magnitude as seen in Scheme 1.6.\textsuperscript{54,55}

\begin{center}
\begin{tabular}{c|c|c|c}
 & pKa (DMSO at 25 °C) & & \\
\hline
1 & 7.32 & 6 & 11.24 \\
7 & 15.87 & & \\
\end{tabular}
\end{center}

\textbf{Scheme 1.6.} Acidity of Meldrum's acid (1) and homologous structures.\textsuperscript{55–57}

Studies have attributed this phenomenon to the geometric constraint presented in the six membered ring, forcing the two esters into an (E)-conformation as seen in Figure 1.14.\textsuperscript{55–57} Further investigation indicated an anomeric stabilization of the enolate anion due to the delocalization of electron density from the oxygen lone pair orbitals into the $\sigma^*$ orbitals between the ester oxygens and the C2 carbon.\textsuperscript{55,56}
1.2.3.1.3 Reactivity

Meldrum’s acid derivatives (MAD) are great building blocks in synthetic chemistry as they are easily synthesized from 1, a cheap precursor, and are bench-stable. A wide range of derivatives are possible as 1 has both electrophilic and nucleophilic properties. The carbonyls at positions C4 and C6 are highly susceptible to nucleophilic attack, often resulting in ring-opening with the loss of acetone. Upon deprotonation at C5, 1 becomes enolizable as seen in Scheme 1.7 and becomes a highly versatile nucleophile under both stoichiometric or catalytic conditions. It has been shown that derivatives can then be synthesized from nucleophilic 1 using various reactions including alkylation, Knoevenagel condensation and carbodiimide coupling.
1.3 Project Objectives

The overall goal for this project is to determine if a ketene can be used as a chemical derivatization agent to enhance detection of analytes using mass spectrometry. This will be tested by observing the sensitivity enhancement of 1-naphthol and 3,5-di-tert-butylphenol using ESI-MS. The ketene will be accessed through a Meldrum’s acid derivative which will be activated through heat.

The general synthetic plan for this project is outlined below in Figure 1.15. The synthetic route can be conveniently divided into two portions part A and B. In part A, Meldrum’s acid (1) is synthesized and is then transformed into a MAD that possesses a fixed positive
charge (i). In part B, the charged MAD (i) from part A is subjected to pyrolysis to produce the corresponding ketene (ii). As seen in B, ketene (ii) would then be able to react with a variety of functional groups as described previously, resulting in the fixed permanent charge being introduced onto the analyte. This includes alcohols (1°, 2° and 3°), alkenes and amines (1°, 2° and 3°).

Numerous different strategies were used in order to synthesize the desired MAD (ii). The initial synthetic design of MAD used an alkylation reaction, however, due to issues that will be described in Chapter 2.1, other synthetic strategies were considered. Chapter 2 will outline these alternative strategies including the Michael addition reaction and Knoevenagel
condensation reaction. Despite these alternative strategies, the most successful reaction used to synthesize the MAD including a fixed, permanent charge was to use carbodiimide coupling reagents as outlined in Chapter 3. The two variations and establishment of optimal conditions will be described in detail.
Chapter 2: Attempted Syntheses

2.1 Initial Synthetic Strategy via Monoalkylation at position C5

Based on the general synthetic strategy presented in Figure 1.15, the initial synthetic route for this project involved alkylating 1 at the C5 position using propargyl bromide. Once purified, the compound could undergo a click reaction with an azide containing a charged R group, denoted as R⁺, as seen below in Scheme 2.1.

![Scheme 2.1](image)

Scheme 2.1. Initial synthetic strategy of MAD⁺ using a propargyl handle and a charged azide group.

2.1.1 Synthesis of Meldrum’s Acid (1)

The first step in this synthetic strategy was to produce 1. Following a procedure written in German\(^6^3\) that was translated to English shown in Scheme 2.2, 1 was produced at 69% yield as white, needle-like crystals. Despite efforts to fully dry the crystals after recrystallization, residual acetone was still present resulting in a purity of ~97%.

![Scheme 2.2](image)

Scheme 2.2. Synthetic procedure for Meldrum’s acid (1) from acetone and malonic acid. Procedure was the English translation of the procedure outlined by Tietze and Eicher.\(^6^3\)
2.1.2 Synthesis of 2,2-dimethyl-5-(prop-2-yn-1-yl)-1,3-dioxane-4,6-dione (8)

The next step of the synthetic route was to add the propargyl handle to 1 through an alkylation reaction as seen in Scheme 2.3.

\[
\text{O} \quad + \quad \text{Br} \quad \xrightarrow{\text{K}_2\text{CO}_3, \text{DMF}, \text{RT}, 5 \text{ hr}} \quad \text{O} \quad + \quad \text{O}
\]

**Scheme 2.3.** Preparation of 2,2-dimethyl-5-(prop-2-yn-1-yl)-1,3-dioxane-4,6-dione (8). Procedure by Leibfarth\(^\text{41}\) was followed.

The procedure was a modification of one published by Leibfarth\(^\text{41}\) that originally used monomethylated 1 instead of 1 as the nucleophile. To minimize the known phenomenon of competitive dialkylation at the C5 position to produce 5,5-dimethyl-2,2-bis(2-propynyl)-1,3-cyclohexanedione (9), equivalents were adjusted to have 1 in significant excess.\(^\text{60,64}\) The reaction produced orange needle-like crystals which were then purified using flash column chromatography (FCC), isolating two compounds identified via TLC.

The first compound was isolated as white, circular crystals was identified as the dialkylated MAD (9) via \(^1\)H NMR spectroscopy. \(^1\)H NMR data of the second compound identified a mixture of Meldrum’s acid and the monoalkylated MAD (8). A second column using 25–100% EtOAc/hexanes was used in attempts to separate the two compounds. \(^1\)H NMR analysis identified fraction one as a mixture of 1 and 8 and fraction two as semi-pure 8.

Despite the successful synthesis of the monoalkylated MAD, aspects of this synthetic route posed a concern. First, adjusting reaction conditions was unable to limit the production of the dialkylated MAD. Based on \(^1\)H NMR data, it was determined that the dominant product of the reaction was 9 rather than the preferred product 8. It was suggested that
monoalkylated Meldrum’s acid is highly reactive and will outcompete MA, resulting in predominantly dialkylated species.\textsuperscript{60,64,65} Second, only \~33\% of MA was recovered from the reaction in either unreacted material or product. Thus, it would be expected to lose large amounts of material. Since this material is synthesized as step 1 in the synthetic route, it is not cost or time effective to be losing large amounts of material for such a low yield early in the synthetic route. Third, purification of monoalkylated Meldrum’s acid proved to be challenging. Due to the similarity in polarity (as well as structure and mass), separation of the monoalkylated MAD and MA was difficult. As seen in Figure 2.1, despite testing numerous solvent systems, the movement of both compounds are almost identical in TLC. When comparing \( R_f \) values of monoalkylated MAD and MA, the difference in \( R_f \) ranged from 0.03–0.07 with the exception of 30\% EtOAc/hexane which had a difference of 0.13. It is believed this large difference in \( R_f \) is due to the difference in concentration for the two compounds with MA highly concentrated and tailing and MAD highly dilute. With the small difference in \( R_f \), separation of the compounds via FCC would be extremely difficult. Thus, due to limited production of the monoalkylated MAD, large losses of MA and challenging purification, other synthetic routes were investigated.
Figure 2.1. TLC plates of various solvent systems to determine better solvent system for FCC in the purification of monoalkylated MAD from Meldrum's acid. Spots were visualized using ceric ammonium molybdate stain. Percentages relate to the percent composition of solvent one compared to solvent two in the solvent system. A) EtOAc/hexanes B) EtOAc/toluene, C) Et₂O/hexanes, D) DCM/hexane and E) MeOH/DCM

2.2 The One-Pot Method

A one-pot method for synthesizing thioesters using copper-catalyzed click chemistry outlined by Lu and Cai was the basis for the one-pot synthetic route. A thioester would be synthesized and once purified, it would undergo a click reaction with an azide containing a charged R group, denoted as R\(^{+}\). The thioester would then be reduced to the thiol. Then, based on the general synthetic strategy presented in Figure 1.15, the thiol would be added to 1 and isovaleraldehyde to produce the corresponding MAD in as seen in Figure 2.2 in a Michael addition to the corresponding alkylidene MAD.
Figure 2.2. Proposed one-pot synthetic method involving the synthesis of a thioester containing a charged R group denoted by R⁺ based on procedures outline by Lu and Cai. The thioester would be transesterfied to the thiol and undergo a reaction with 1 and isovaleraldehyde to produce the corresponding MAD⁺.

2.2.1 Synthesis of S-prop-2-ynyl benzothioate (10)

The first step in the one-pot synthetic route is to produce a thioester containing a propargyl handle that can be used for subsequent click reactions. The procedure for this reaction was modelled after a reaction outlined by Lu and Cai which involves the use of potassium carbonate and surfactant TX100 (also known as Triton X 100) to minimize hydrolysis of acyl halides caused by water molecules. For this reaction, Triton X 100 was substituted for Triton X 114 due to availability as outlined in Scheme 2.4.
Scheme 2.4. First step in one-pot method to synthesize thioester S-prop-2-ynyl benzothioate (10). Procedure was as modification of the procedure outlined by Lu and Cai.66

This reaction was attempted three times as outlined in Table 2.1. During the first attempt, a brown precipitate formed and became a clear liquid after filtration. However, analysis of the $^1$H NMR spectrum determined only solvent and water was detected. During attempt two, orange crystals formed after filtration and TLC indicated the synthesis of two new compounds. Comparison of the crude $^1$H NMR data to starting material indicated a reaction did occur. Analysis of the $^1$H NMR indicated two new species, one of which was the desired thioester based on the diagnostic peaks present at 2.23 ppm and 3.83 ppm. However, the signal intensity was significantly lower than residual solvents and other signals. Taking into account the mass of the crude sample, this was highly suggestive of a low yield. During the third attempt, the reaction was scaled up and left for a longer period. Red crystals were produced after filtration and TLC indicated the presence of two new compounds within the reaction mixture. Similar to the second attempt, signals for the desired thioester were of quite low intensity, again indicating a low yield.
Table 2.1. One pot synthetic method reaction attempts with reaction conditions and modifications to produce S-prop-2-ynyl-benzothioate following the procedure outlined by Lu and Cai.66

<table>
<thead>
<tr>
<th>Attempt</th>
<th>Condition/modification</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>• Original procedure</td>
<td>No observed product</td>
</tr>
<tr>
<td></td>
<td>• Heated to 60 °C</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>• Original procedure</td>
<td>Product observed at low intensity</td>
</tr>
<tr>
<td></td>
<td>• Heated to 30 °C</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>• 20x scale</td>
<td>Product observed at low intensity</td>
</tr>
<tr>
<td></td>
<td>• Heated to 60 °C for 6.5 hours</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Continued for additional 20 hours</td>
<td></td>
</tr>
</tbody>
</table>

Since this reaction is the first step in the synthetic route, it was determined the yields of this known reaction were insufficient to continue pursuing this route and thus, other routes were investigated.

2.3 The Knoevenagel Condensation Reactions

A common method for the alkylation of Meldrum’s acid is through the Knoevenagel condensation reaction with a carbonyl compound to yield an alkylidene or arylidene Meldrum’s acid derivative.61,64,67–70 Based on the general synthetic strategy presented in Figure 1.15, the Grignard synthetic route would begin with the common Knoevenagel condensation reaction between isovaleraldehyde and 1 as seen in Figure 2.3. Next, the resulting alkylidene MAD could undergo a Grignard addition to add a propargyl handle, which would react with an azide containing a charged R group, denoted as R⁺.
Figure 2.3. Proposed Grignard synthetic route involving the addition of ethynylmagnesium chloride to alkylidene MAD. The resulting MAD would undergo a click reaction to allow the addition of a charged azide group to produce MAD$^+$. 

2.3.1 Synthesis of pyrrolidinium acetate ionic liquid (PyrrIL)

The first step in this synthetic strategy involves a Knoevenagel condensation reaction between isovaleraldehyde and 1. When performing this reaction specifically with Meldrum’s acid, the presence of catalytic amounts of pyridine and pyrrolidinium acetate has often been used.$^{68,70}$ A greener alternative is to use ionic liquids (IL), specifically pyrrolidinium-based protic ILs as they are relatively low cost and low toxicity.$^{61}$ A procedure outlined by Anouti was followed to produce the pyrrolidinium acetate ionic liquid (PyrrIL) as seen in Scheme 2.5.$^{61}$
During the dropwise addition of acetic acid to pyrrolidine, large amounts of smoky vapor were produced and left the system. As a result, the yield for this reaction was approximately 68% of an orange liquid. Analysis of the $^1$H NMR showed an overlap of the singlet for the $\alpha$-carbon on the acetate component with the $\beta$-carbons on the pyrrolidinium at 1.91 ppm.

2.3.2 Synthesis of 2,2-dimethyl-5-(3-methylbutylidene)-1,3-dioxane-4,6-dione (11)

The next step involved a Knoevenagel condensation with isovaleraldehyde with MA (1) in PyrrIL as seen in Scheme 2.6. During the reaction, an extremely chunky, sticky, brown mixture formed, and the stir bar was sticking consistently throughout the 1.5-hour reaction time. Analysis of the $^1$H NMR data for the crude reaction mixture identified peaks associated with the alkylidene MAD and residual 1. Peaks associated with isovaleraldehyde were not observed.
The crude material was then purified via FCC using a 5% EtOAc/hexanes solvent system. It was noted that the alkylidene MAD moved as a yellow band throughout the column and produced yellow fractions. The alkylidene MAD was isolated as a yellow oil with a purity of ~91% with residual EtOAc and hexanes identified via $^1$H NMR. The total yield for this reaction was 58%, which is much lower than the expected 90% described by Sobrinho.\textsuperscript{61} Despite the lower yield, sufficient alkylidene MAD was isolated and purified to continue with the synthetic route. A standard solution of the alkylidene MAD was prepared in benzene and stored in the freezer for future use in subsequent reactions.

### 2.3.3 Synthesis of 2,2-dimethyl-5-(5-methylhex-1-yn-3-yl)-1,3-dioxane-4,6-dione (12)

The next step in this synthetic sequence was to perform a Grignard addition to the alkylidene MAD using ethynylmagnesium chloride. A procedure for this reaction specifically was described by Krabbe as seen in Scheme 2.7.\textsuperscript{71} Modifications to the original procedure included starting with the alkylidene MAD 2,2-dimethyl-5-(3-methylbutylidene)-1,3-dioxane-4,6-dione (11), reducing the scale by a factor of 3, ethynylmagnesium chloride provided as a 0.5 M solution in THF and placing the RBF in the oven rather than flame-drying it.

![Scheme 2.7](image)

**Scheme 2.7.** Attempted Grignard addition of ethynylmagnesium chloride to 11 to produce 2,2-dimethyl-5-(5-methylhex-1-yn-3-yl)-1,3-dioxane-4,6-dione (12).\textsuperscript{71}
This reaction was attempted on four separate occasions, as summarized in Table 2.2. During attempt one of this reaction, 11 was dried down and mixed with dry THF. The resulting solution was then added to the Grignard via a cannula transfer and produced a yellow solution. $^1$H NMR analysis of the crude material indicated the presence of starting material and no trace of 12. It was then thought that the presence of toluene in the reaction would contribute to its success since Krabbe used a 0.6 M solution of ethynylmagnesium chloride in THF/Toluene. Thus, for attempt two, 11 was dissolved in 1:1 THF and toluene. In addition, the Grignard was added to the solution via cannula transfer and no colour change was observed. It was then hypothesized that the Grignard reagent may be lost during the addition through cannula transfer so for attempt three, the reagent was added to the reaction directly from the bottle. Due to availability, the reaction occurred under argon gas rather than the traditional nitrogen gas, but this is inconsequential. In the last attempt for the reaction, the RBF was flame dried under vacuum and the reaction was heated to varying temperatures over a 48-hour period as outlined in Table 2.2. Despite the modifications to the original procedure, $^1$H NMR analysis only demonstrated starting material for all four attempts. Thus, other synthetic routes were then investigated.

Table 2.2. Grignard synthetic method reaction attempts with reaction conditions and modifications to produce 2,2-dimethyl-5-(5-methylhex-1-yn-3-yl)-1,3-dioxane-4,6-dione (12) following the procedure by Krabbe.$^{71}$

<table>
<thead>
<tr>
<th>Attempt</th>
<th>Condition/modification</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Original procedure</td>
<td>No reaction</td>
</tr>
<tr>
<td>2</td>
<td>• Added toluene as solvent (1:1 dry THF) • Flip addition (Grignard to substrate)</td>
<td>No reaction</td>
</tr>
<tr>
<td>3</td>
<td>• Added Grignard directly to MA/THF from bottle dropwise • Argon gas</td>
<td>No reaction</td>
</tr>
<tr>
<td>4</td>
<td>• Flame-dried RBF • Heated to 50 °C for 19 hrs and then increased to 65 °C for 24 hr • quenched with citric acid and sat. NH$_3$Cl</td>
<td>No reaction</td>
</tr>
</tbody>
</table>
2.3.4 Modification of the Knoevenagel Condensation reaction

Of the many reactions performed until this point of the project, the one that was the most consistently successful was the Knoevenagel condensation reaction of an aldehyde to 1. Due to the difficulties observed trying to add the alkyne handle to an alkylidene MAD through the Grignard reaction, it was then hypothesized that the propargyl handle could be incorporated onto the aldehyde prior to coupling, and then add the charged R group (R⁺) at the last step before ketene formation. Thus, the next synthetic route was designed to synthesize 4-(prop-2-ynyloxy)butan-1-ol which contained the propargyl handle and then oxidize it to 4-(prop-2-ynyloxy)butanal using the Swern oxidation reaction. Then, based on the general synthetic strategy presented in Figure 1.15, the aldehyde would be coupled to 1 through the Knoevenagel condensation reaction and reduced from the alkylidene MAD to the monoalkylated MAD. Once reduced, the monoalkylated MAD will then undergo a click reaction with an azide containing a charged R group, denoted as R⁺ as seen in Figure 2.4 to produce the charged MAD.
2.3.4.1 Synthesis of 4-(prop-2-yn-1-yloxy)butanal (14)

The first step in this synthetic route is to synthesize 4-(prop-2-yn-1-yloxy)butanal containing the propargyl handle to be used for click reactions later in the synthesis. This aldehyde can be synthesized through a Swern oxidation of the corresponding alcohol, 4-(prop-2-ynloxy)butan-1-ol (13). A procedure outlined by Yamamoto was modified to synthesize 4-(prop-2-ynloxy)butan-1-ol as outlined below in Scheme 2.8.\textsuperscript{72}
Scheme 2.8. Synthesis of 4-(prop-2-ynyloxy)butan-1-ol (13) following a procedure outlined by Yamamoto.\textsuperscript{72}

During the addition of propargyl bromide to the reaction mixture, the reaction turned from a milky white solution to brown. After 26 hours, TLC indicated the synthesis of a new compound within the reaction mixture. $^1$H NMR analysis of the crude orange oil indicated the successful synthesis of 13 and absence of residual propargyl bromide. Purification of the crude via FCC resulted in an orange oil with a yield of 39\% with residual EtOAc present within the sample. Despite having a low yield, enough material was produced for step 2 of the synthetic route.

The second step for this synthetic route was to perform a Swern oxidation to oxidize 13 into its corresponding aldehyde, 4-(prop-2-ynyloxy)butanal (14). A procedure outlined by Yamamoto was modified to achieve this as outlined below in Scheme 2.9.\textsuperscript{72}

Scheme 2.9. Synthesis of 4-(prop-2-ynyloxy)butanal (14) following a procedure outlined by Yamamoto.\textsuperscript{72}

Once concentrated, 170 mg of an orange oil was observed. Analysis of the crude $^1$H NMR indicated a successful reaction with the diagnostic peak at 9.78 ppm for the aldehyde proton present however, when comparing the ratio of the alcohol to aldehyde the yield was approximately 92\%.\textsuperscript{72} Despite having residual 13 in the crude material, the next step of the synthetic route was performed.
2.3.4.2 **Synthesis of 2,2-dimethyl-5-(3-(prop-2-yn-1-yloxy)propylidene)-1,3-dioxane-4,6-dione (15)**

The next step in the synthetic route was the Knoevenagel condensation of 14 with 1 as seen in Scheme 2.10. Despite the purity of 14 being low, the crude reaction mixture was used for this reaction.

![Scheme 2.10. The Knoevenagel condensation of 14 with 1 to produce 2,2-dimethyl-5-(3-(prop-2-yn-1-yloxy)propylidene)-1,3-dioxane-4,6-dione (15). Procedure by Sobrinho was followed with the crude material of 14.](image)

The crude mixture produced an orange-brown mushy solid after 1.5 hours. During the reaction, the mixture became difficult to stir due to a sticky consistency observed at the bottom of the mixture. TLC analysis of the reaction demonstrated some leftover starting material, but a new compound was observed. Analysis of the crude $^1$H NMR indicated leftover 1, residual 14 and the desired MAD 15 characterized by the diagnostic peak at 7.95 ppm. When comparing the diagnostic 15 peak to the leftover 1 in the reaction, it was found there was a 1:3 ratio, suggesting a poor yield. Despite the low yield, the crude material was then purified using FCC. However, purification resulted in the isolation of an unknown
species with negligible amounts of desired product. Due to the excess 1 being added to the reaction, it was suggested that the unknown product may be the bis-adduct MAD. A known phenomenon of the Knoevenagel condensation with 1 is the formation of the bis-adduct product which is the result of a Michael addition of 1 to an alkylidene MAD as seen in Figure 2.5.69

![Figure 2.5](image.jpg)

**Figure 2.5.** Formation of bis-Meldrum's acid adducts via Michael addition to alkylidene Meldrum's acid derivatives. Figure is an adaptation of a figure depicted by Dumas.69

With the addition of a slight excess of 1 to the reaction, the formation of the bis-adduct is possible. However, analysis of the compounds purified do not suggest the formation of this compound due to the absence of a quintet peak coupling to a doublet and a quartet.

### 2.3.5 Evaluation of Knoevenagel Condensation Conditions

Upon reviewing the results observed for all Knoevenagel condensation reactions, it was found that solubility issues were a constant hurdle, as mixtures would often stick as they were stirring. In addition, yields of the desired alkylidene MAD were around 60% and the potential for bis-adduct MADs were considered a probable loss of product. Other Knoevenagel condensation protocols suggested that the formation of the desired alkylidene MAD over the bis-adduct MAD could be favoured by performing the reaction in a dilute
organic solvent.\textsuperscript{69} This is due to the favoured formation of the bis-adduct MAD in polar and non-polar solvents as it precipitates out of solution.\textsuperscript{69} Thus performing reactions in dilute organic solvents can push the equilibrium shown in Figure 2.5 between the MADs to the desired alkylidene MAD as the dominant product.\textsuperscript{69} However, Bigi suggests performing the reaction in water without a catalyst does not result in the formation of the bis-adduct MAD.\textsuperscript{70} Thus, a comparative study was performed to determine the best conditions to perform the Knoevenagel condensation reaction at in hopes to reattempt the condensation between 14 and 1.

Three different conditions were then determined to be compared as outlined below in Scheme 2.11 using cyclohexane carboxyaldehyde as the proof-of-concept aldehyde. Condition A was a modification of the PyrrIL Knoevenagel condensation reaction conditions used until this point described by Sobrinho except with the addition of benzene as the solvent in combination with the work up procedure outlined by Dumas.\textsuperscript{61,69} Conditions B was based on Dumas to use 0.2 M benzene, while condition C was based on Bigi to use water.\textsuperscript{69,70}
Original caption: Derivatization Strategies for Fixed Permanent Charges

**Scheme 2.11.** Optimization of solvent and temperature conditions of Knoevenagel condensation reaction for the synthesis of 5-(cyclohexylmethylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (16). Conditions and procedures are modifications of procedures outlined by Bigi, Dumas and Sobrinho.\(^{61,69,70}\)

Initial analysis of the crude mixtures by \(^1\)H NMR indicated the production of 5-(cyclohexylmethylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (16) and the presence of an aldehyde peak at 9.60 ppm. Comparison of this peak to cyclohexane carboxyaldehyde suggested it was not leftover starting material as its aldehyde peak was observed at 9.54 ppm. However, it is possible the aldehyde is experiencing some hydrogen bonding to cause it to be more deshielded and thus appear at a higher chemical shift. As no other aldehydes were introduced to the system, it was assumed the 9.60 ppm peak corresponded to hydrogen bonded cyclohexane carboxyaldehyde. The ratio of 16 to the aldehyde was compared between the three conditions and it was observed that condition C had preferential production of 16 as seen in Table 2.3. It was suggested in literature that the desired product could be purified either by FCC or recrystallization. The crude mixture for condition B was purified using recrystallization from methanol, however, 16 was not isolated. Thus, purification for the other two conditions was done using FCC which resulted in the isolation of multiple products. The desired 16 was isolated from reaction mixtures A
and C however, some residual impurities were observed. In addition, the unidentified compound purified via recrystallization from condition B was also isolated from the column from reaction C. There is no spectroscopic evidence to suggest the formation of the bis-adduct in this reaction.

**Table 2.3.** Comparison of the Knoevenagel condensation reaction conditions to produce 5-(cyclohexylmethylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (16). Conditions and procedures are modifications of procedures outlined by Bigi, Dumas and Sobrinho.\(^61,69,70\) Ratio of 16 to hydrogen-bonded cyclohexane carboxyaldehyde is based on the ratio of \(^1\)H NMR peaks 7.70 ppm to 9.60 ppm.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Crude Alkyldene MAD: Aldehyde</th>
<th>Pure?</th>
</tr>
</thead>
<tbody>
<tr>
<td>A) PyrrIL (10 mol %) Benzene, 0 °C, 23 hr</td>
<td>1 : 0.31</td>
<td>No</td>
</tr>
<tr>
<td>B) PyrrIL (10 mol %) 0.2 M Benzene, RT, 25 hr</td>
<td>1 : 0.30</td>
<td>N/A(^a)</td>
</tr>
<tr>
<td>C) PyrrIL (10 mol %) dH(_2)O, RT, 25 hr</td>
<td>1 : 0.12</td>
<td>No</td>
</tr>
</tbody>
</table>

\(^a\) Yield was not calculated due to product being lost during recrystallization attempt

In this project, various synthetic methods to produce monoalkylated MADs were attempted with the Knoevenagel condensation reaction being the most successful method. Analysis of the crude material via \(^1\)H NMR indicated the successful production of desired alkyldene MADs on numerous occasions. However, due to issues with unidentified side products, optimization and purification issues this approach was not continued, and other methods of alkylation were investigated.
Chapter 3: The Coupling Route

3.1 Introduction

A common method of monoalkylation includes the coupling of carboxylic acids to MA using carbodiimides and then reducing the corresponding acyl-MAD. A paper by Senaweera and Weaver highlighted a one-pot variation of this reaction using carbodiimide coupling agent $N,N'$-dicyclohexylcarbodiimide (DCC), resulting in moderate yields of monoalkylated MADs as seen in Scheme 3.1. It was highlighted by the paper that use of the cyclohexyl variant of Meldrum’s acid was favoured over the traditional dimethyl MA, as the former is more soluble in most organic solvents.

\[
\begin{align*}
\text{Scheme 3.1. One-pot monoalkylation of cyclohexyl MA described by Senaweera and Weaver.}^{73}
\end{align*}
\]

A new synthetic strategy was then developed as seen in Figure 3.1 using the one-pot monoalkylation procedure described by Senaweera and Weaver as the main reaction. Based on the general synthetic strategy presented in Figure 1.15, this strategy would use the procedure described by Senaweera and Weaver to couple a carboxylic acid containing a fixed permanent charge, denoted as $R^+$, to the cyclohexyl MA variant which could then go on to form the corresponding ketene.\textsuperscript{73}
Figure 3.1. Synthetic strategy using a one-pot monoalkylation procedure of cyclohexyl MA using DCC coupling. Coupling and reducing procedure is based on the procedure described by Senaweera and Weaver.\textsuperscript{73}

3.2 Synthesis of 1,5-dioxaspiro[5.5]undecane-2,4-dione (17)

The first step of this synthetic strategy is to synthesize the cyclohexyl variant of Meldrum's acid, 1,5-dioxaspiro[5.5]undecane-2,4-dione (17). As seen in Scheme 3.2, the conditions of the reaction are similar to those outlined in Scheme 2.2 except \textit{para}-toluenesulfonic acid monohydrate is used as the catalyst and the reaction is run for 7 days.
Scheme 3.2. Synthesis of cyclohexyl Meldrum’s acid variant, 1,5-dioxaspiro[5.5]undecane-2,4-dione (17).

Procedure outlined by Kimmel was followed. When all the reagents were added, the reaction mixture started as a light beige colour. However, over 2.5 hours on the first day, it turned into a black solution as seen in Figure 3.2. Upon addition of water on day seven, red precipitate was observed and moved about the water phase as seen in Figure 3.2. After isolation and recrystallization, it was found that this red precipitate was 17. The red colour of the crystals persisted but $^1$H NMR spectroscopy did not reveal any obvious impurities, resulting in an overall yield of 50%.

Figure 3.2. Colour change observed during the synthesis of 17. A) Time zero of the reaction. B) 45 minutes after time zero. C) 2.5 hours after time zero. D) Addition of water to reaction on day seven.

It is noted, however, that there was an issue of stability of 17 when stored on the bench. As seen in Figure 3.3, storing 17 at room temperature on the bench top in a scintillation vial sealed with parafilm over 5 months resulted in new peaks being observed in the $^1$H NMR.
Attempts to remove the new peaks included recrystallizing the stored product using the same procedure outlined by Kimmel during the initial isolation. This was able to reduce the number of impurities detected but trace amounts were still present in the sample.

**Figure 3.3.** NMR comparison of 17 at 5 months (A) to initial synthesis (B). Both NMR spectra were acquired in CDCl₃ and calibrated to the residual CHCl₃ peak at 7.26 ppm.

Analysis of the sample via $^{13}$C NMR spectroscopy indicated the presence of a new carbonyl peak at 181 ppm that correlates to the triplet at 2.92 ppm as observed in the HMBC spectrum. One suggestion for the identification of these new peaks is the formation of
acylated MAD derivatives due to the reactivity of MA during storage. It is hypothesized that over time, storage of large amounts of MA resulted in the formation of the acylated variation of the desired product. This can be due to the keto–enol tautomerism of MA and MADs. When this occurs, it is possible that self-reactivity can occur as illustrated in Figure 3.4 to form the acylated MAD variant.

![Figure 3.4. Self-reaction of MA with enol variant of MA to form acylated MAD in storage.](image)

Thus, to minimize this phenomenon from occurring it is suggested to store samples in a fridge or freezer.

### 3.3 Proof-of-concept coupling of hexanoic acid to synthesize 3-hexyl-1,5-dioxaspiro[5.5]undecane-2,4-dione (18)

The next step of this desired route is to couple a charged carboxylic acid with 17. To verify all conditions outlined by Senaweera and Weaver, hexanoic acid was used as the proof-of-concept carboxylic acid as seen in Scheme 3.3.
When performing this reaction exactly as outlined, coupling of hexanoic acid appeared to be successful via $^1$H NMR with the production of a triplet at 2.97 ppm corresponding to the intermediate acyl-MAD proton. After the reduction, TLC of the final crude material indicated the presence of two new compounds and residual 17. Analysis of the material via $^1$H NMR proved to be difficult as peaks associated with 1,3-dicyclohexylurea (DCU) masked diagnostic peaks of the desired product, specifically a triplet at 3.51 ppm corresponding to the C5 proton of 18. As well, leftover 17 and hexanoic acid were observed, suggesting a suboptimal reaction. The procedure was then modified in attempts to both increase the reaction efficiency and to reduce the amount of DCU present within the reaction mixture when performing the reduction. This was done by first implementing a stirring period of 15 minutes of hexanoic acid, 4-dimethylaminopyridine (DMAP) and DCC in acetonitrile. This then allows maximal production of the intermediate fatty O-acylisourea and minimize the nucleophilic attack of 17 to DCC.\textsuperscript{75} After the 15 minutes, 17 was then added to the reaction with $N,N$-diisopropylethylamine (DIPEA) to continue to coupling reaction. Once complete, the crude reaction mixture was then filtered and concentrated in vacuo in attempts to remove any precipitated DCU from the reaction.\textsuperscript{76}
Implementation of the filtering step proved to be successful as the diagnostic peak at 3.51 ppm was visible in the crude $^1$H NMR, however, leftover 17 was observed at a 1:1.13 ratio to 18. Upon purification using FCC, 18 was obtained resulting in a 53% yield. It is noted that pure leftover 18 was not isolated from the column. Due to the inability to recover pure leftover 18 upon purification, other carbodiimide coupling agents were evaluated.

### 3.3.1 Efficiency of other carbodiimide coupling agents

One major issue that was observed when using the protocol described by Senaweera and Weaver was the persistence of DCU throughout the reaction. Although it can be reduced by filtering the crude mixture, doing so runs the risk of losing desired product and decreasing yields. Other carbodiimide coupling agents were then compared to DCC including 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC•HCl) and $N,N$-diisopropylcarbodiimide (DIC). EDC•HCl was selected to be the new carbodiimide coupling agent since the resulting urea by-product and excess reagent can be more easily removed via acidic and basic wash steps. As seen in Scheme 3.4, the reaction was then split into two separate reactions rather than performing it as a one-pot synthesis as was previously done. The coupling reaction was based on a protocol outlined by Bruckner while the reduction was based on conditions outlined by Winkler.
Scheme 3.4. Synthesis of 3-hexyl-1,5-dioxa[5.5]undecane-2,4-dione (18) using a two-step approach with coupling agent EDC•HCl. A) Coupling of 1,5-dioxa[5.5]undecane-2,4-dione to hexanoic acid using EDC•HCl coupling agent as outlined by Bruckner to produce 3-(1-hydroxyhexylidene)-1,5-dioxa[5.5]undecane-2,4-dione (19). B) Reduction of 19 to produce 18 as outlined by Winkler. Coupling hexanoic acid to 17 using the procedure outlined in Scheme 3.4 (A) was determined to be successful via $^1$H NMR analysis of the crude yellow oil. The diagnostic peak for intermediate 3-(1-hydroxyhexylidene)-1,5-dioxa[5.5]undecane-2,4-dione (19) at 3.05 ppm was easily visible along with diagnostic peaks for residual 17 and hexanoic acid. However, no obvious peaks correlating to ECU were observed indicating its removal was successful. When comparing the ratio of the acylated MAD to residual hexanoic acid, it was determined that the reaction was 82% successful. The crude material was then carried to the reduction as outlined in Scheme 3.4 B. Analysis of the crude material via $^1$H NMR.
demonstrated a 100% conversion of the 19 to 18 indicated by the new triplet at 3.51 ppm and residual 17 and hexanoic acid was noted. Purification of the crude mixture resulted in 18 being isolated with residual amounts of hexanoic acid and an unknown species at 3.67 ppm detected in the $^1$H NMR. This resulted in an approximate yield of 68% and thus this procedure was to be used when coupling future charged carboxylic acids to 17.

3.4 Synthesis of cationic carboxylic acid salts

With the confirmation that the coupling chemistry worked as expected and the monoalkylated MAD can be purified with high purity and yield, options for cationic carboxylic acid salts were evaluated. Three specific procedures and cationic carboxylic acid salts as outlined in Scheme 3.5 were selected and attempted.

**Scheme 3.5.** Synthetic procedures to produce charged carboxylic acid salts. A) Synthesis of $N$-5-carboxy-$N,N,N$-trimethylpentan-1-aminium bromide salt (20) using a procedure outlined by Ruff. B) Synthesis of $N$-(5-carboxypentyl)-$N,N$-dimethylbenzammonium bromide salt (21) using a procedure outlined by Szafran. C) Synthesis of 21 using a procedure outlined by Oyervides-Muñoz.
Numerous attempts were made for all three reactions outlined in Scheme 3.5 but were either unsuccessful or unreproducible. For the reaction outline in Scheme 3.5 A, crude $^1$H NMR analysis indicated only the starting material present when the reaction was run both for 3 hours or 48 hours, thus this strategy was not continued further. The first attempt for reaction B in Scheme 3.5 produced white, sticky crystals after the addition of acetone to the reaction mixture. TLC indicated a new species was produced and $^1$H NMR analysis suggested a species resembling of 21 was produced with a new triplet at 3.9 ppm. MS analysis of the sample indicated the desired product was produced with characteristic diagnostic peak at $m/z$ 236.1 as seen in Figure 3.5. However, this procedure was irreproducible, thus alternative synthetic strategies were considered. A new procedure by Oyervides-Muñoz was then used in attempts to synthesize 21, however, production and isolation of crystals and reproducibility remained a challenge.82
Figure 3.5. Mass spectrum (A) and MS/MS (B) of \(N\)-(5-carboxypentyl)-\(N, N\)-dimethylbenzammonium bromide salt (21). Both spectra were obtained on a Sciex QSTAR XL mass spectrometer fitted with a nano-ESI emitter in positive ion mode. Peaks noted with an asterix are common backgrounds ions observed in ESI.

3.5 Coupling commercially available cationic carboxylic acid salts

Due to the challenges observed synthesizing and isolating charged carboxylic acids, it was decided to use commercially available cationic carboxylic acid salts to couple to 17. Thus, betaine was selected as the charged acid of choice to undergo coupling to 17 using the EDC•HCl coupling protocol that was previously verified using hexanoic acid as seen in Scheme 3.6. The difference between the reactions was to remove the intermediate workup of reaction A due to concern of potential removal of the desired acylated MAD from the organic layer. Thus, similar to the procedure outlined by Weaver, the procedure would be performed by a “one-pot” type method.
Aulenback; *Derivatization Strategies for Fixed Permanent Charges*

Scheme 3.6. Synthesis of 2-(2,4-dioxo-1,5-dioxaspiro[5.5]undecane-3-yl)-N,N,N-trimethylethan-1-aminium chloride salt (23) A) Coupling of 1,5-dioxaspiro[5.5]undecane-2,4-dione to betaine using EDC•HCl coupling agent as outlined by Bruckner to produce 2-(2,4-dioxospiro[5.5]undecan-3-ylidene)-2-hydroxy-N,N,N-trimethylethan-1-aminium chloride (22) without workup.\(^{78}\) B) Reduction of 22 to produce 23 as outlined by Winkler.\(^{79}\)

Analysis of the crude material via \(^1\)H NMR after the coupling reaction did not suggest a successful reaction as there was no acylated OH group in the higher region of the spectrum. The remainder of the spectrum appeared too complex to make any distinct conclusions, but the reduction was attempted. Analysis of the \(^1\)H NMR of the crude material did not suggest that 23 was present due to the absence of a triplet around 3.5 ppm correlating to an alkane chain, thus attempts were not made to purify the mixture. The \(^1\)H NMR spectra did not contain any peaks resembling the expected peak pattern or correlations of 23 or its acylated variant 22.

### 3.6 Re-evaluation of the synthetic approach using carbodiimide coupling reagents

Due to the difficulties seen trying to couple a charged species to 17, the approach to this synthetic strategy was re-evaluated. Looking at the mechanism related to the first reaction
as seen in Figure 3.6, it is believed the issue being observed is the unlikelihood of the charged carboxylic acid to react with the carbodiimide coupling reagent. The moment a charged carboxylic such as betaine gets deprotonated by a base, such as DMAP, it forms a zwitterionic species, (i). The likelihood of this species continuing in the desired reaction is highly unlikely as it is quite stable.

Figure 3.6. Formation of zwitterionic species of charged carboxylic acids during carbodiimide coupling reactions.

The synthetic strategy was then rearranged to avoid this potential issue from occurring. As seen in Figure 3.7, the strategy was modified to couple the 6-bromohexanoic acid to 17 then reduce it. Once purified, the charge would be added using previously attempted procedures outlined in Scheme 3.5 using an amine base.
Figure 3.7. Proposed synthetic strategy to couple 6-bromohexanoic acid to 17. Once reduced to the monoalkylated MAD, it will undergo an amine alkylation reaction previously described to input a fixed permanent charge.

3.6.1 Synthesis of 3-(6-bromohexyl)-1,5-dioxaspiro[5.5]undecane-2,4-dione (24)

The first step in this modified synthetic route was to couple 6-bromohexanoic acid to 17. To confirm the probability of a halogenated carboxylic acid being able to couple to 17 using EDC•HCl, literature was reviewed for any similarly preceded reactions. As a result, a protocol outlined by Hashimoto described coupling long chained chlorinated carboxylic acids to the dimethyl Meldrum’s acid variant 1. Thus this protocol was adapted and modified as seen in Scheme 3.7 with the addition of a stirring period for all reagents except 17.
Protocol outlined by Hashimoto was adapted.\textsuperscript{83}

One observation made throughout the coupling reactions when using both DCC and EDC•HCl was that residual 17 was always detected via $^1$H NMR once reduction had occurred. Thus, it is suggested that the residual 17 may be due to the conditions for the carbodiimide coupling reaction being insufficient to obtain full acylation. As previously stated, one way to maximize coupling of a carboxylic acid to 17 is to have a stirring period in the absence of 17 and thereby maximize production of the fatty O-acylisourea intermediate.

It was then decided to perform time trials to determine what the optimal stir time is to maximize the carbodiimide coupling reaction. A series of reactions were then performed using the conditions outlined in Scheme 3.7 except varying amounts of incubation periods were tested ranging from 0.5 to 24 hours. As seen Figure 3.8, the mole ratio of starting material for each of the starting materials was then compared to 24 by comparing integrations in the $^1$H NMR spectrum of the crude reaction products. It is noted that at the 3-hour incubation period, minimal amounts of both 17 and 6-bromohexanoic acid were observed. Thus, going forward, all reactions outlined in Scheme 3.7 will be performed with a

\begin{scheme}
\centering
\includegraphics[width=\textwidth]{scheme3.7.png}
\caption{Synthesis of 3-(6-bromo-1-hydroxyhexylidene)-1,5-dioxaspiro[5.5]undecane-2,4-dione (24).}
\end{scheme}
3-hour incubation period of all reagents excluding 17 instead of a 30 min incubation period as previously done.

![Bar chart showing mole ratio of starting material vs incubation period for EDC-HCl, cat. DMAP, and NEt₃ in DCM](image)

**Figure 3.8.** Comparison of incubation periods of EDC-HCl, cat. DMAP and NEt₃ in DCM prior to the addition of 17. Reaction conditions described by Hashimoto was used with the addition of an incubation period of all reagents excluding 17. Mole ratio of starting material was calculated by comparing the ratio of the diagnostic peak for 24 at 3.07 ppm to either 3.61 ppm for 17 or 2.37 ppm for 6-bromohexanoic acid and normalizing the ratio to the sum of both integrals. All ¹H NMR spectra were performed in CDCl₃, calibrated to the corresponding solvent peak at 7.26 ppm and integrals were normalized to 2.37 ppm with an integration of 2 protons.

Once coupling of 6-bromohexanoic acid was complete, the corresponding acylated MAD was then reduced using as protocol outlined by Winkler as seen in Scheme 3.8.
Scheme 3.8. Reduction of 24 to produce 3-(6-bromohexyl)-1,5-dioxaspiro[5.5]undecane-2,4-dione (25). Procedure outlined by Winker was followed.79

Analysis of the crude reaction mixture indicated 100% conversion of the acylated MAD to its corresponding monoalkylated MAD. Purification of the mixture by FCC resulted in white crystals with a yield of ~16.9%. It is noted however that residual unidentified alkane peaks were present within the spectra impacting the purity and the yield of the reaction. It is suggested to perform a gradient elution during FCC in order to increase the purity of isolated 25.

3.6.2 Amine alkylation of 3-(6-bromohexyl)-1,5-dioxaspiro[5.5]undecane-2,4-dione

To perform an amine alkylation of 25, the procedure outlined by Szafran was followed with N,N-dimethylbenzylamine (DBA) as seen in Scheme 3.9.81 The reaction was monitored via TLC and was removed after product was visible. Verification of a complete reaction was done via $^1$H NMR. If starting material was still visible, the reaction would be repeated until $^1$H NMR confirmed the absence of the characteristic 3.41 ppm triplet of the protons $\alpha$ to the bromine, suggesting full amine alkylation of 25.
Scheme 3.9. Amine alkylation of 25 using N,N-dimethylbenzylamine to produce N-benzyl-6-(2,4-dioxo-1,5-dioxaspiro[5.5]undecan-3-yl)-N,N-dimethylhexan-1-aminium bromide (26). Procedure outlined by Szafran was followed and was deemed complete when $^1$H NMR confirmed the absence of 3.41 ppm peak.$^{81}$

The first reaction performed used equal equivalents of 25 to DBA. Synthesis of 26 resulted in an off-white, oily residue and was stored immediately in the freezer. Analysis via $^1$H NMR demonstrated excess DBA and a new aromatic system that integrated for two and three protons, which corresponded to the new aromatic system produced in 26. It is noted that the remainder of the spectrum demonstrated limited information as peaks within the alkane region did not show splitting patterns and were presented either as a singlet or a highly broad peak. Further characterization of the reaction was done by comparing the NMR to the starting materials as well as using COSY, $^{13}$C NMR, HMBC and HSQC data.

As noted previously, the characteristic peak correlating to 25 was the triplet at 3.41 ppm. It was hypothesized that this triplet would then shift in chemical shift slightly as it goes from being associated with a bromine to a quaternary nitrogen. Analysis of the $^1$H NMR did not show a triplet in this region but a singlet at 3.57 ppm. However, COSY data showed that the 3.57 ppm peak was correlated to a peak at 1.87 ppm which the expected chemical shift of the $\beta$ proton to the quaternary nitrogen based on the POC-MAD chemical shifts. It was then
hypothesized that the triplet is being masked by the residual DBA peak as the CH$_2$ alpha to the nitrogen is detected in this region. This is further supported by HSQC data showing this peak is on a carbon at 64.0 ppm, which is in the region of a carbon bond to a nitrogen. In addition, HMBC data shows the 3.57 ppm peak correlating to carbons within the aromatic region and at 49.6 ppm, further suggesting overlapping peaks. Analysis of the carbon peak at 49.6 ppm demonstrated its correlation to a proton peak at 3.26 ppm that integrates for approximately 6 protons. Based on this information it is suggested that this peak correlates to the two methyl groups present on the quaternary nitrogen. HMBC data demonstrated a correlation of the 49.6 ppm carbon to the proton peak at 4.97 ppm which integrates for approximately two protons, suggesting the protons correspond to the CH$_2$ group between the aromatic system and quaternary amine of the desired product. This was further supported by its correlation to a 67.6 ppm carbon via HSQC data and its correlation to aromatic carbons via the HMBC data. Based on this information, the $^1$H and $^{13}$C NMR data were assigned to the desired product as seen in Figure 3.9 thus suggesting the desired product was synthesized.
Figure 3.9. $^1$H and $^{13}$C chemical shifts assigned to diagnostic structural functions of 26. A) Structure of 26 with diagnostic peaks A to F highlighted. B) Table outlining diagnostic structural functions A to F with their corresponding proton and carbon chemical shifts. All characterization was done using $^1$H NMR, $^{13}$C NMR, COSY, DEPT135, HSQC and HMBC data.

To further confirm the amine alkylation reaction was successful, the crude reaction mixture was analyzed via mass spectrometry. As seen in Figure 3.10, the most abundant peaks within the spectrum corresponded to 26 as well as common fragments of 26. Tandem MS of the desired product 26 at $m/z$ 402.2 further confirmed that the desired product had been made as seen in Figure 3.11.

<table>
<thead>
<tr>
<th></th>
<th>$^1$H chemical shift (ppm)</th>
<th>$^{13}$C chemical shift (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.87</td>
<td>22.4</td>
</tr>
<tr>
<td>B</td>
<td>3.57</td>
<td>64.0</td>
</tr>
<tr>
<td>C</td>
<td>3.26</td>
<td>49.6</td>
</tr>
<tr>
<td>D</td>
<td>4.97</td>
<td>67.6</td>
</tr>
<tr>
<td>E</td>
<td>7.47</td>
<td>130.9, 129.4</td>
</tr>
<tr>
<td>F</td>
<td>7.65</td>
<td>133.3</td>
</tr>
</tbody>
</table>
Figure 3.10. Mass spectrum of 26 obtained on a Sciex QSTAR XL mass spectrometer fitted with a nano-ESI emitter in positive ion mode. Characteristic fragments corresponding to abundant peaks in spectrum are presented with predicted m/z values. Peaks noted with an asterix are common backgrounds ions observed in ESI.
Figure 3.11. MS/MS of 26 obtained on a Sciex QSTAR XL mass spectrometer fitted with a nano-ESI emitter in positive ion mode. Characteristic fragments corresponding to abundant peaks in spectrum are presented with predicted m/z values.

After storage in the freezer for over a month, purification of the crude was investigated. Prior to purification, $^1$H NMR analysis of the stored crude was performed to verify the stability of the compound. As seen in Figure 3.12, new peaks had formed while others had disappeared. The most notable peak was the broadened triplet at 3.57 ppm and new singlet at 3.79 ppm. Recall previously this peak was a singlet that corresponded to both excess DBA as well as the characteristic triplet of the $\alpha$ proton to the quaternary amine on the alkane chain side.
When analyzing the peaks of the $^1$H NMR of 2-month-old 26, the 3.57 ppm triplet still correlates to the 1.87 ppm proton and is on a carbon at 64.2 ppm. In comparison, the new peak at 3.79 ppm maintains a 2:6 ratio with the proton peak at 2.47 ppm and is on a carbon at 63.4 ppm. In addition, HMBC data demonstrates a correlation between the 3.71 ppm peak with aromatics at approximately 130 ppm. It is noted that the aromatic region at approximately 7.4 ppm, which correlates to carbons at approximately 130 ppm, have shifted.
downfield and demonstrate a different splitting pattern compared to when the crude mixture was initially analyzed. Based on this information, it is suggested that the excess DBA present within the sample has formed its conjugate acid, which would explain the deshielding effect seen on the aromatic peaks and CH$_2$ group compared to DBA. In addition, a new peak at 2.14 ppm correlating to the 1.87 ppm peak is noted, suggesting that the proton source for DBA could be the 26 which is forming its carboxylate counterpart. The same peak pattern of 3.57 ppm triplet and a singlet was observed when this reaction was repeated with excess DBA however the singlet shifted from 3.71 ppm to 3.79 ppm in order to ensure full modification of 25. To verify this theory, the crude sample with excess DBA was analyzed via mass spectrometry. As seen in Figure 3.13, an abundance of m/z 136.16 was detected which could correspond to a N,N-dimethylbenzylamine conjugate acid (i.e., N,N-dimethylbenzylammonium ion). Peaks corresponding with the desired 26 were also detected. It should be noted that not all peaks were assigned, as this was a crude sample.
Figure 3.13. Mass spectrum of 26 crude material performed with excess DBA. Spectrum was obtained on an AB Sciex 4000 QTRAP hybrid triple quadrupole linear ion trap mass spectrometer equipped with an online nanoESI ionization source via direct injection. Performed EMS scan with a declustering potential of 40 eV. Once it was confirmed that the reaction was successful in producing 26 based on NMR and MS data, purification was attempted. Based on the procedure by Szafran, purification was attempted by recrystallization using acetone and Et<sub>2</sub>O at room temperature. The crude mixture was able to dissolve in the acetone as an orange liquid and turned cloudy once Et<sub>2</sub>O was added. The sample was then left at room temperature overnight and an orange oil with a few crystals observed. The recrystallization process was attempted a second time.
with acetone and methyl t-butyl ether and stored in the fridge overnight. Again, an orange oil with one or two crystals was observed, thus deeming it unsuccessful.

Since the main impurity present in the reaction mixture was DBA, it was thought that an acid-base liquid-liquid extraction would be able to remove and purify the sample. However, washing with 1 M HCl produced a new compound that did not resemble the starting material via $^1$H NMR analysis. The mixture was then analyzed via TLC to determine if FCC would be a viable method for purifying the reaction mixture. Due to the presence of the fixed permanent charge on 26, conditions for FCC must be selected carefully to prevent its retention on the column. Common methods for purifying polar species by FCC include using methanol and DCM as the solvent system or performing it on a reverse phase column.

### 3.6.2.1 Alternative amines for amine alkylation reaction

Due to the purification issues previously discussed, the amine alkylation was repeated with the more volatile amine trimethylamine as seen in Scheme 3.10. This amine was selected as it is highly volatile and can be easily removed from the reaction by *in vacuo* concentration thus simplifying the purification issues.
Scheme 3.10. Amine alkylation of 25 using trimethylamine to produce 6-(2,4-dioxo-1,5-dioxaspiro[5.5]undecan-3-yl)-N,N,N-trimethylhexan-1-aminium bromide (27). Procedure outlined by Szafran was followed with the exception of refluxing and was deemed finished when \(^1\)H NMR spectroscopy confirmed the absence of 3.41 ppm peak.\(^{81}\)

Due to its volatile nature, excess equivalents had to be used to push the reaction forward. Analysis of the crude after 12 hours and 6 equivalents of TMA demonstrated a similar peak pattern seen in 26 with a triplet at 3.56 ppm as seen in Figure 3.14. However, MS data could not confirm that 27 was produced when analyzed a week later. Verification of the \(^1\)H NMR of the crude demonstrated the compound had decomposed during this short period of time despite being stored in the freezer as seen in Figure 3.14.
Figure 3.14. Comparison of $^1$H NMR of 27 crude material on day 1 (A) to 26 (B) and day 7 (C).

It is possible that the compound could have undergone a Hofmann elimination reaction resulting in the loss of the trimethylamine from 27. However, analysis of the $^{13}$C NMR does not reveal any alkene carbons thus further investigation of the formation and stability of this alternative MAD$^+$ should be investigated.
Chapter 4: Ketene reactions

4.1 Proof of concept ketene reactions

To determine optimal reaction conditions and to evaluate the chemistry of the reaction with phenols, ketene reaction studies were initially performed using the POC-MAD, 18. As seen in Scheme 4.1, 1-naphthol was selected as the test phenol due to its structural similarity to steroids, a biologically relevant analyte. The desired outcome from this reaction is to produce the corresponding phenyl ester. However, it is noted that the reaction proceeds through a β-ester acid intermediate prior to the formation of the desired phenyl ester as seen in Scheme 4.1.45

![Scheme 4.1](image)

Scheme 4.1. Ketene reaction of 18 with analyte of interest 1-naphthol to produce naphthalen-1-yl octanoate.

Procedure outlined by Wolffs was modified.84

A procedure reported by Wolffs was modified to perform the reaction with equal equivalents of the POC-MAD 18 and 1-naphthol.84 Both compounds melted immediately upon entering the 190 °C oil bath to produce a dark orange-red solution. Condensation around the upper
parts of the reaction vessel was also observed within a few minutes of the reaction mixture melting. Analysis of the crude mixture via $^1$H NMR spectroscopy indicated a new aromatic system formed and the diagnostic peaks of 18 were absent. In addition, peaks corresponding to cyclohexanone were identified, suggesting a pyrolysis reaction of 18 did occur. Analysis of the $^{13}$C NMR spectrum indicated the presence of multiple carbonyl peaks that could correspond to an ester. HMBC data demonstrated a correlation of the carbonyls to two distinct triplets at 2.73 ppm and 2.34 ppm which could correspond to alpha hydrogens of an ester. However, the signal to noise ratio for these carbonyl peaks was too high, thus other procedures and reaction conditions were investigated. It was thought that the high temperature could be too aggressive for future analytes, particularly biologically relevant molecules, which tend to be sensitive to thermal or oxidative degradation, both of which are accelerate as temperature increases. Hence, a longer reaction time at lower temperatures was considered. Temperatures of 100 °C, 130 °C and 160 °C with a reaction time of 80 minutes were compared to the initial 30-minute reaction at 190–200 °C reaction. As seen in Figure 4.1, peaks corresponding to cyclohexanone were present in all reactions except for the 100 °C reaction. This suggests that the reaction was successful under all conditions except for the 100 °C reaction. However, it is noted there is an inconsistent peak pattern in the aromatic region and inconsistent intensity of the triplet at 2.73 ppm. The reactions were also evaluated via TLC and compared to 18. As seen in Figure 4.1 B, up to 7 different spots were visualized in the reaction lanes.
Figure 4.1. A) Comparison of ketene reactions of 18 with 1-naphthol. (i) 190 °C 20 mins then 205 °C for 10 mins, (ii) 160 °C for 80 mins, (iii) 130 °C for 80 mins and (iv) 100 °C for 80 mins. Peaks highlighted with an (*) correspond to peaks in relation to cyclohexanone. Reaction conditions were modified procedures by Wolffs and Li. 45,84 B) TLC of ketene reactions i–iv in 25% EtOAc/hexanes compared to 18 (M). Plate was visualized using ceric ammonium molybdate stain.

The purpose of these initial reactions was to investigate how the ketene reactions would behave and develop optimal conditions that could be translated to the ketene reactions with MAD⁺. However, full characterization of the resulting products from these reactions was highly complex, making the development of reaction conditions challenging. Since 1-naphthol is an unsubstituted phenol, C-acylation at the ortho, meta and para position are
potential side-products to the desired O-acylation product and its corresponding \( \beta \)-ester acid products in this reaction. Thus, it was decided to use a more substituted phenol that would promote O-acylation over C-acylation to make the characterization of the reaction less complex.

4.1.1 Study of O-acylation of substituted phenols using POC-MAD

To investigate and characterize the O-acylation of substituted phenols using the POC-MAD, 3,5-di-tert-butylphenol was selected as the replacement analyte of interest. The bulky tert-butyl groups were believed to prevent C-acylation from occurring during the reaction and promote the formation of the desired phenyl ester as seen in Scheme 4.2. Based on the ketene studies performed with 1-naphthol, the reaction was performed at 160 °C over 80 minutes, as it produced the triplet at 2.73 ppm, which is believed to correspond to the \( \alpha \) hydrogen of the ester in the desired product.

\[ \begin{align*}
&\text{18} & + & \text{3,5-di-tert-butylphenol} \\
\rightarrow & & \text{29} \\
\end{align*} \]

\text{Scheme 4.2. Ketene reaction of 18 with 3,5-di-tert-butylphenol to produce 3,5-di-tert-butylphenyl octanoate (29). Intermediate \( \beta \)-ester acid product shown.}
Similar to the ketene reactions with 1-naphthol, condensation around the vial was observed as seen in Figure 4.2 A and produced a yellow oil. $^1$H NMR analysis of the reaction indicated the presence of three separate aromatic systems, one of which corresponded to the initial analyte 3,5-di-tert-butylphenol. Within the alkane region, peaks corresponding to cyclohexanone were present in addition to a triplet at 2.55 ppm which is believed to be the $\alpha$-hydrogen of the ester 29 shown in Scheme 4.2. Analysis of the $^{13}$C NMR indicated the presence of two carbonyl peaks with a third detected at a lower intensity. None of the chemical shifts of the carbonyl peaks corresponded to 18 and the absence of the quaternary carbon on 18 at 105.7 ppm, further confirmed a reaction occurred. HMBC analysis of the reaction mixture indicated the correlation of three triplets to each of the carbonyl peaks, all of which could correspond to an $\alpha$ proton of an ester as seen in Figure 4.2.
Figure 4.2. Proof of concept reaction of 18 with 3,5-di-tert-butylphenol. A) Close up of reaction vial to depict formation of condensation around reaction vessel. B) HMBC correlations between distinguishing triplets and three carbonyl peaks.

It is noted that 2.34 ppm peak also corresponds to cyclohexanone, which was suggested to be in the reaction mixture with the presence of the other proton peaks and the ketone carbon peak at 212.8 ppm. HSQC data of the 2.34 ppm peak indicated its correlation to two carbon peaks at 42.0 ppm and 34.0 ppm. It is possible this triplet could also be corresponding to a product but is being shielded by the overwhelming presence of cyclohexanone within the sample.
Based on the presence of three carbonyls correlating to three triplets, it was believed that the second product could potentially be the intermediate β-ester acid as demonstrated in Scheme 4.2. The correlation of an extra triplet could indicate possible E/Z confirmations of the desired ester product or the presence of an alternative product. One product that could be formed in addition to the β-ester acid is an ortho or para hydroxyphenol ketone via the Friese rearrangement as depicted in Figure 4.3. If a C-alkylated product was detected, it would be expected that the protons related to that particular aromatic system would have a 1:1 ratio and would be less deshielded than the desired product and 3,5-di-tert-butylphenol. This is based on the comparison 1H NMR analysis of the aromatic peaks of 3,5-diisopropylphenyl acetate and 3,4,5-trimethyl-phenol as seen in Figure 4.4. However, for this reaction the integration ratio of the two sets of aromatic peaks remained 2:1, thus suggesting two different species of O-acylation rather than a potential C-acylation product.
Figure 4.3. Possible C-acylation products formed during ketene reaction due to Fries rearrangement. Figure adapted from Korb and Lang.\textsuperscript{88}

\begin{align*}
\text{A)} & \quad \text{B)} \\
\begin{array}{l}
6.94 \ (s, \ 1H) \\
6.75 \ (d, \ J = 1.4 \ Hz, \ 2H)
\end{array} & \\
6.48 \ (2H, \ s)
\end{align*}

Figure 4.4. Comparison of $^1$H NMR chemical shifts and splitting pattern of the aromatic peaks of (A) 3,5-diisopropylphenyl acetate and (B) 3,4,5-trimethyl-phenol. Values are shown in chemical shifts ($\delta$).\textsuperscript{86,87}
It was then decided to perform the ketene reaction at various reaction times to see if the reaction could be pushed to a single product. In addition, to limit the transfer of cyclohexanone into the NMR sample, the reaction was to be performed under nitrogen and NMR samples would be prepared only using the reaction mixture and not a rinse of the reaction vessel. The ketene reaction was then performed at reaction times ranging from 80 minutes to 6 hours. As seen in Figure 4.5, the presence of 3,5-di-tert-butylphenol at 6.69 ppm decreased, however, the reaction was unable to be pushed to a single product. It is noted at a reaction time of 80 minutes, the reaction favours one aromatic system significantly over another while at the 4-hour reaction time, the preference is reversed.
Figure 4.5. $^1$H NMR comparison of aromatic region of POC ketene reaction between 18 and 3,5-di-tert-butylphenol with reaction times of A) 80 minutes, B) 2 hours, C) 4 hours and D) 6 hours. Ratio of each doublet to each other is written below with normalization to 3,5-di-tert-butylphenol. It is noted that the corresponding triplet to each doublet maintains the same ratio to one another.
Characterization of the various products was attempted via NMR and IR analysis. As discussed previously, three separate triplets at 3.81 ppm, 2.55 ppm and 2.34 ppm at varying integrations found within the $^1$H spectrum could potential correspond to the $\alpha$ ester proton. Using COSY and HMBC data, each of these triplets had their own chains of correlations and correlated to a separate carbonyl peak present within the $^{13}$C spectrum at 179 ppm, 172 ppm and 168 ppm. The 3.81 ppm triplet could correspond to the $\beta$–ester acid product as it is on a CH/CH$_3$ carbon at 52.4 ppm as determined by DEPT135 and HSQC analysis and correlates to the 168 ppm carbon. In comparison, the 2.55 ppm and 2.34 ppm triplets each correspond to CH$_2$ carbons at 34.8 ppm and 34.0 ppm respectively corresponding to the 172 ppm and 179 ppm. Based on the chemical shifts of both triplets and their corresponding carbons, both could correspond to the desired phenyl ester. To further investigate the potential identities of both products, IR analysis was performed. Comparison of the diagnostic region of the ketene reaction, 3,5-di-tert-butylphenol and cyclohexanone via IR are summarized in Table 4.1.
Table 4.1. IR analysis of the diagnostic region of the ketene reaction, 3,5-di-tert-butylphenol and cyclohexanone. Experimental peaks are based on figures B4 and B5 in Appendix B. Peaks associated with 3,5-di-tert-butylphenol and cyclohexanone are from SDBS. It is noted that experimental data was obtained using the NMR sample prepared in CDCl₃, cyclohexanone was obtained using CCl₄ solution and 3,5-di-tert-butylphenol was obtained using nujol mull.

<table>
<thead>
<tr>
<th></th>
<th>Cyclohexanone Peaks (cm⁻¹)¹</th>
<th>3,5-di-tert-butylphenol Peaks (cm⁻¹)²</th>
<th>Ketene Reaction A Peaks (cm⁻¹)</th>
<th>Ketene Reaction C Peaks (cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH organic</td>
<td>2940 - 2867</td>
<td>2924 - 2955</td>
<td>2862 - 2959</td>
<td>2862 - 2964</td>
</tr>
<tr>
<td>Alcohol (OH)</td>
<td>-</td>
<td>3073 - 3617</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Unidentified OH</td>
<td>-</td>
<td>-</td>
<td>3484</td>
<td>3474</td>
</tr>
<tr>
<td>Ketone C=O</td>
<td>1717</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Unidentified carbonyl C=O</td>
<td>-</td>
<td>-</td>
<td>1756, 1711</td>
<td>1712 - 1760</td>
</tr>
<tr>
<td>Aromatic C=C</td>
<td>-</td>
<td>1597, 1464</td>
<td>1592 - 1611</td>
<td>1588 - 1611, 1464 - 1478</td>
</tr>
</tbody>
</table>

The purpose of analysing the crude mixture via IR spectroscopy is to determine if a carboxylic is present within the sample. Its detection would indicate that one of the products could be the β-ester acid product illustrated in Scheme 4.2. An OH peak is detected in the diagnostic region however it does not take up the whole OH region, allowing the detection of the CH stretch, suggesting this is an alcohol OH group rather than a carboxylic acid OH. However, as seen in Table 4.1 the detected OH alcohol stretch is similar to the OH alcohol stretch detected in 3,5-di-tert-butylphenol, suggesting this peak could also correspond to leftover 3,5-di-tert-butylphenol that was detected via NMR. In addition, it is possible that the acid of the β-ester acid is present within this range but is present in a low concentration that it is not taking up the entirety of the diagnostic region as expected for a carboxylic acid OH.

¹ Data retrieved from SDBS database CCl₄ solution (SDBS 571)
² Data retrieved from SDBS database nujol mull (SDBS 12434)
When analyzing the carbonyl and aromatic region of the IR spectrum a carbonyl peak is detected. Comparison of this peak to the original POC-MAD 18 seen in Figure 4.6 demonstrated a shift from 1790 cm\(^{-1}\) and 1749 cm\(^{-1}\) to a broad carbonyl peak ranging from 1760 cm\(^{-1}\) to 1712 cm\(^{-1}\). The carbonyl peak at 1760 cm\(^{-1}\) is most likely to correspond to an ester carbonyl peak that experiences conjugation with the single-bonded oxygen of the ester group.\(^{89}\) The peak at 1712 cm\(^{-1}\) could potentially correspond to the β-ester acid.

Analysis of the aromatic region in the crude reaction mixture from both reactions indicates multiple aromatic systems as double signals are detected at around 1600 cm\(^{-1}\) and 1465 cm\(^{-1}\). This further suggests the formation of a new aromatic product was produced during the reaction.
Figure 4.6. Comparison of IR spectra of 18 (black), ketene reaction A (red) and Ketene reaction C (blue) from 4000 cm$^{-1}$ to 1300 cm$^{-1}$.

To further aid in the identification of the types of products produced in this reaction, a literature search was performed to determine trends of phenyl esters with varying chain
lengths as well as diagnostic peaks of $\beta$–ester acid systems. The IR peaks, $^1$H chemical shifts and $^{13}$C chemical shifts of these systems are summarized in Table 4.2.

Table 4.2. Summary of IR peaks (cm$^{-1}$), $^1$H chemical shifts (ppm) and $^{13}$C chemical shifts (ppm) for various phenyl esters and $\beta$-ester acid variants.

<table>
<thead>
<tr>
<th></th>
<th>IR (cm$^{-1}$)</th>
<th>$^1$H NMR (ppm)</th>
<th>$^{13}$C NMR (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-Naphthyl acetate$^{90,91}$</td>
<td>2926, 1745, 1600, 1494, 1369</td>
<td>7.80-7.85 (m, 2H) 7.70 (d, $J$ = 8.4 Hz, 1H) 7.38-7.49 (m, 3H) 7.20 (d, $J$ = 7.6 Hz, 1H) 2.41 (s, 3H) 200 MHz, CDCl$_3$</td>
<td>169.78 (C=O) 146.79 134.85 128.27 126.97 126.67 (2 C) 126.27 125.62 121.33 118.30 21.24 (CH$_3$) 50 MHz, CDCl$_3$</td>
</tr>
<tr>
<td>Phenyl acetate$^{90,91}$</td>
<td>3060, 2926, 1745, 1595, 1494, 1369</td>
<td>7.33 (t, $J$ = 7.2 Hz, 2H) 7.17 (t, $J$ = 7.6 Hz, 1H) 7.03/7.04 (2 d overlapped, $J$ = 8.6, 8.2 Hz, 2H) 2.24 (s, 3H) 200 MHz, CDCl$_3$</td>
<td>169.73 (C=O) 152.20 129.64 (2) 126.04 121.78 (2) 21.35 (CH$_3$) 50 MHz, CDCl$_3$</td>
</tr>
<tr>
<td>Phenyl propanoate$^{92}$</td>
<td>3068, 2885, 1761, 1593, 1488, 1461, 1422</td>
<td>7.38 (m, 2H) 7.23 (m, 1H) 7.09 (m, 2H) 2.60 (q, $J$ = 7.6 Hz, 2H) 1.27 (t, $J$ = 7.6 Hz, 3H) 400.1 MHz, CDCl$_3$</td>
<td>173.1 (C=O) 150.9 129.5 125.8 121.7 (2) 27.9 (CH$_2$) 9.2 (CH$_3$) 100.6 MHz, CDCl$_3$</td>
</tr>
<tr>
<td>Phenyl butyrate$^{93,94}$</td>
<td>1770 (C=O), 1205 (C-O)</td>
<td>7.5-7.0 (m, 5H) 2.50 (t, $J$ = 7.2 Hz) 1.7 (sextet, $J$ = 7.2 Hz) 0.98 (t, $J$ = 7.2 Hz) 90 MHz, CDCl$_3$</td>
<td>171.8 (C=O) 151.1 121.6 129.4 125.6 36.3 (CH$_2$) 18.5 (CH$_2$) 13.6 (CH$_3$) 22.63 MHz, CDCl$_3$</td>
</tr>
</tbody>
</table>
Based on the literature data collected in Table 4.2, the following trend is observed. For phenyl esters with a non-substituted ring, the carbonyl peak demonstrates an IR peak at 1745 cm\(^{-1}\) and a \(^{13}\)C chemical shift of 169 ppm and increases into the late 1700 cm\(^{-1}\) and 170 ppm range as the chain increases. When a 1,3,5-substitution occurs on the phenyl ring, a short chain ester will have a carbonyl IR peak around 1768 cm\(^{-1}\) and a \(^{13}\)C chemical shift of 169 ppm. It is noted that the \(\alpha\) proton of the ester is within the 2.0 ppm range when a
non-substituted or 1,3,5-trisubstituted phenyl ring is present. For β-ester acids formed during a reaction with MA or a MAD, the corresponding carbonyl IR peaks occur in the late 1770 cm⁻¹ and early 1700 cm⁻¹ for the phenyl ester and carboxylic acid respectively. The alpha proton of the β-ester acid occurs in the late 3.0 ppm range.

Based on this information, it is proposed that one potential product observed in this reaction is the β-ester acid as seen in Figure 4.7 with the diagnostic 3.81 ppm triplet. As stated previously, this triplet demonstrated an HMBC correlation to the diagnostic 168.2 ppm carbonyl peak. However, a correlation to a second carbonyl peak was not observed. It is noted that the absence of correlation does not necessarily mean there is no correlation occurring. It is possible that the correlation is not detectable in a crude mixture thus a purified sample may provide a strong enough signal to determine the secondary carbonyl peak. The remaining two carbonyls are proposed to correspond to the E and Z isomers of the desired product due to their similarity in chemical shift in both the ¹H and ¹³C NMR spectra. These isomers may not necessarily produce different chemical environments for the phenyl group thus can correlate to the same aromatic system. The suggested characterization in ¹H NMR and ¹³C NMR of both of these products are outlined in Figure 4.7. However, it is noted that the exact characterization and identification of these products is not finalized. It is suggested to purify each of the products in order for full characterization of each to be accurate and complete.
Figure 4.7. Proposed chemical structures with corresponding $^1$H and $^{13}$C chemical shifts of A) β-ester acid product and B) desired phenyl ester product.

4.2 Ketene reactions with MAD$^+$

Although full characterization of the different products produced during the ketene reaction with 3,5-di-tert-butylphenol was not complete, ketene reaction conditions were adequate to attempt ketene reactions with MAD$^+$ 26 as seen in Scheme 4.3.
Scheme 4.3. Ketene reaction of 26 with 3,5-di-tert-butylphenol to produce N-benzyl-8-(3,5-di-tert-butylphenoxy)-N,N-dimethyl-8-oxooctan-1-aminium bromide (30).

This reaction was performed in triplicate with a different reaction vessel for each including a microwave vial, 10 mL two-neck pear shaped flask (PSF) and 100 mL two-neck round bottom flask (RBF). It is noted that for the reactions performed in the PSF and RBF, 3,5-di-tert-butylphenol was added to the reaction vessel in which 26 was synthesized as illustrated in Scheme 3.9. Each of the three reactions produced an orange, sticky oil with condensation observed around the walls of the reaction vessels. As seen in Figure 4.8, analysis of the aromatic region indicated two separate aromatic systems correlating at a 2:1 ratio not aligning with the 3,5-di-tert-butylphenol suggesting two O-acylation products were produced during the reaction. The reaction performed in the microwave vial, reaction one, preferred one product while the reaction performed in the RBF, reaction three, preferred the other. It is also noted that the aromatic peaks corresponding to 26 changed in peak splitting suggesting an alteration to the system and that a reaction has occurred.
Analysis of the $^{13}$C NMR of reaction one and three as seen in Figure 4.9, depicted two carbonyl carbons at varying intensities. However, peaks corresponding to the quaternary carbons of the original 3,5-di-tert-butylphenol portion of the compound goes from two sets in reaction one to one set in reaction three. This suggests that in reaction one, two different
types of systems related to 3,5-di-tert-butylphenol were observed that differ enough electronically to shift the peaks corresponding to the two sets of aromatic protons. In addition, the two compounds produced in reaction three differ slightly in the alkane chain portion of the compound to produce the same aromatic environment for the t-butylphenyl portion of the compound.

Figure 4.9. Comparison of $^{13}$C NMR of ketene reaction 1 (A) to ketene reaction 3(B).
Using HMBC analysis, it was found that the carbonyl peak at 176 ppm correlated to a broad peak at 2.30 ppm while a triplet at 2.53 ppm corresponded to the 172 ppm carbonyl peak. The broad peak at 2.30 ppm correlated to a CH₂ carbon at 34.6 ppm in reaction one and three, however in reaction one an additional CH/CH₃ carbon at 42.1 ppm correlated to this peak. In comparison, the triplet 2.53 ppm corresponded to a CH₂ carbon at 34.3 ppm in both reactions. Thus, both peaks could possibly correspond to the α proton of the desired phenyl ester.

Comparison of the alkane region of both reactions to 26 indicated new peaks in the 5.0 ppm region and two sets of doublets around 3.30 ppm. HSQC data indicated that the 5.0 ppm doublet like peak correlated to a carbon at 67.6 ppm which could be the CH₂ group between the quaternary ammonium and the phenyl ring of the DBA portion of the desired compound. HMBC further suggested this assignment as it indicated a correlation to the 133 ppm aromatic peak believed to belong to the phenyl ring of the DBA portion of this compound in addition to a carbon at 49.7 ppm. HSQC analysis of this peak correlated it to the 3.26 ppm doublet like peak suggesting its identification to be the methyl groups of the quaternary ammonium.

To further investigate the possible products produced in this reaction, an IR spectrum for each of the reactions was acquired in addition to 26. The carbonyl peak corresponding to 26 at 1737 cm⁻¹ does not really shift and the 2191 cm⁻¹ peak corresponding potentially to the quaternary ammonium portion of the compound shifts higher to 2198 cm⁻¹. However, the aromatic peaks around 1560 cm⁻¹ and 1450 cm⁻¹ have more of a double peak pattern compared to a single peak with a slight shoulder as observed in 26. This suggests that a
new aromatic system is present and that the carbonyl is most likely not corresponding to the β-ester acid.

Final characterization of the crude materials was done via mass spectrometry. As seen in Figure 4.10, various ions were detected within the crude material, however, ion $m/z$ 466.40 was detected with reasonable counts. Tandem MS of the ion indicated characteristic fragments corresponding to 30.

![Figure 4.10](image.png)

**Figure 4.10.** Mass spectrum (A) and MS/MS (B) of 30 with characteristic fragments (C). Both spectra were acquired on an AB Sciex 4000 QTRAP hybrid triple quadrupole linear ion trap mass spectrometer equipped with an online nanoESI ionization source via direct injection.
Based on the information gathered via NMR, IR and MS, it is suggested that the desired phenyl ester 30 was produced in some capacity within the three ketene reactions performed with 26. Correlation of the aromatic peaks of the DBA portion of the compound to new peaks at 5.0 ppm suggests that a reaction did occur with 26. The detection of a new aromatic system corresponding to the 3,5-di-tert-butyl portion of this compound with a 2:1 ratio suggests that an O-acylation product was being detected. The identification of different proton peaks corresponding to different carbonyls suggest that possible E/Z isomers of this product was detected. The IR spectrum indicated new aromatic systems was introduced in comparison to the IR spectrum of 26. However, the detection of a carbonyl peak at 1736 cm⁻¹ does not follow the trend of a phenyl ester peak as described previously in Table 4.2. In comparison, mass spectrometry analysis of the crude material does suggest that 30 was detected with the m/z 466.4 ion with characteristic fragmentation that could correspond to the desired phenyl ester. To better characterize and identify the products produced in this reaction, it is suggested to investigate purification methods of both the starting material and the reaction. Isolation of the individual products will allow full identification to verify the suggested trends described above support the identification of the desired phenyl ester.
Chapter 5: Future Works

This project proposes the use of ketenes as a new CD agent to enhance detection of analytes using mass spectrometry. The ketenes will be accessed through MADs by thermal activation to induce a fixed permanent charge onto the desired analyte. The synthesis of a MAD containing a fixed permanent charge proved to be challenging however, the synthesis was ultimately successful through the use of carbodiimide coupling agent EDC•HCl as outlined in Figure 5.1 to produce MAD⁺ 26 using a three-step synthesis. Initial verification of the reaction conditions of reactions A and B were done using hexanoic acid as a proof-of-concept carboxylic acid to produce POC-MAD 18.

Figure 5.1. Synthesis of POC-MAD (18) and MAD⁺ (26) using carbodiimide coupling reagent EDC•HCl. A) Coupling of carboxylic acid to cyclohexyl-MA (17) using EDC•HCl. B) Reduction of acylated MADs to monoalkylated MADs using sodium borohydride. C) Amine alkylation reaction of MAD 25 with DBA to produce MAD⁺ (26). Note: reaction A only contained NEt₃ when coupling 6-bromohexanoic acid to produce acylated MAD 24.
Although the synthesis of MAD$^+$ 26 was successful as determined by MS and NMR analysis, purification of the desired MAD was unsuccessful despite attempts using recrystallization as the purification method.

Reaction conditions for the ketene reaction were determined using POC-MAD 18 to verify that the desired O-acylation product was being produced. Initial studies with 1-naphthol as a test analyte suggest that additional C-acylation products may have been forming. Thus, 3,5-di-tert-butylphenol was used for all proceeding ketene reactions as the t-butyl groups would promote the formation of the desired ester and prevent the C-acylation reactions from occurring. With this adjustment, it was found that two products in addition to excess 3,5-di-tert-butylphenol was being detected via NMR. Through careful analysis of the NMR data, it is suggested that the β-ester acid intermediate and possible E/Z conformers of the desired ester 29 were being detected. However, purification of these products should be done to verify the identities through characterization studies.

Despite MAD$^+$ 26 not being fully purified, ketene studies were performed with the crude reaction mixture in triplicate with 3,5-di-tert-butylphenol as a test analyte. All three reactions suggest that a reaction did occur and indicate that the desired phenyl ester is present within the reaction mixture via MS analysis as seen in Figure 4.10. NMR analysis further suggests that a carbonyl containing product is produced but confirmation of desired ester 30 could not be done. However, similar to the POC-MAD ketene reactions, purification of the various products should be done to confirm their identification.
As a CD method, the aspects of this work show promise but optimization of conditions including purification of MAD\(^+\) and single product production through ketene reactions needs to be addressed.

As described previously, one limitation of this synthetic strategy is the challenges observed when purifying 26 prior to performing the ketene reaction. One way to address this challenge is to screen other types of amines that can be used to induce the fixed permanent charge. The MAD\(^+\) produced a salt, thus purification via recrystallization would be the best method to purify the compound. \(N,N\)-dimethylaniline and DBA were first chosen due to their incorporation of a phenyl group within their structure. This functional group allows increased crystallinity of the MAD\(^+\) and aids in its purification via recrystallization. However, due to this characteristic the boiling point of the selected amines is increased thus removal by simple methods such as in vacuo are not an option. At the other end, TMA is composed of methyl groups which does reduce the crystalline property of the MAD\(^+\). However, with its lower boiling point removal of excess TMA can be done in vacuo. As described in Chapter 3.6.2.1, this then introduces stability issues of MAD\(^+\) and thus premade MAD\(^+\) cannot be stored for use prior. The ability to store CD reagents is a desired property as it reduces experimental preparation when performing the CD reaction for a MS based experiment. Thus, it is suggested to screen various amines to determine if there is a good compromise between ease of removal and storage of MAD\(^+\). One possible candidate could be \(N\)-methylpyrrolidine as its boiling point is low enough to be removed via in vacuo methods while high enough to not readily evaporate at room temperature as seen in Figure 5.2.
Amines tested and suggested with corresponding boiling points for amine alkylation with 25.

Amines include \( N,N \)-dimethylaniline, DBA, \( N \)-methylpyrrolidine and TMA.

Due to the obvious challenges observed when trying to produce a monoalkylated MAD, an alternative to this synthetic method is the incorporation of the charged \( R^+ \) group at either C6 or C4 to produce a modified dioxinone.\(^{97–99}\) As seen in Figure 5.3, the Meldrum’s acid method can be modified to produce a dioxinone compound by altering the substitution on the \( \beta \)-keto ester.\(^{97–99}\) Similar to Meldrum’s acid, upon heating dioxinones produce their corresponding acetylketene which then can be trapped by a nucleophile.\(^{97–99}\) The benefit of these compounds though is that the formation of the corresponding acetylketene can be done at temperatures closer to 100 °C.\(^{97–99}\) The challenge however is the synthesis of the desired \( \beta \)-keto ester.\(^{97–99}\) In a study by Peixoto, a variety of \( \beta \)-keto ester were synthesized from their corresponding carboxylic acids via their respective Weinreb amides.\(^{98}\) However, it is proposed to investigate synthesizing a \( \beta \)-keto ester with the incorporation of a charged R group to be used to synthesize the corresponding charged dioxinone compound.

**Figure 5.2.** Amines tested and suggested with corresponding boiling points for amine alkylation with 25.

<table>
<thead>
<tr>
<th>Amine</th>
<th>Boiling Point</th>
</tr>
</thead>
<tbody>
<tr>
<td>( N,N )-dimethylaniline</td>
<td>194 °C</td>
</tr>
<tr>
<td>DBA</td>
<td>180 °C</td>
</tr>
<tr>
<td>( N )-methylpyrrolidine</td>
<td>81 °C</td>
</tr>
<tr>
<td>TMA</td>
<td>2.9 °C</td>
</tr>
</tbody>
</table>

\( \text{Figure 5.2.} \) Amines tested and suggested with corresponding boiling points for amine alkylation with 25.
Figure 5.3. Synthesis of modified dioxinones. A) Modification of Meldrum’s acid synthesis with the use of modified β-keto esters. B) Synthesis of modified β-keto esters from their corresponding carboxylic acid. Figures adapted by Fuse and Piexoto.97,98
Chapter 6: Experimental Procedures

6.1 General

All reagents were purchased from commercial sources and were used as received, without purification unless otherwise noted. Alkyl halides were purified by passage through a Pasteur pipette prepared with a cotton plug and a short column of activated basic alumina. TLC monitoring of reactions was done using glass-backed extra hard (60 Å) TLC plates from SiliCycle and visualized by ultraviolet (UV) light and/or ceric ammonium molybdate stain (CAM, Hanessian’s stain). FCC purification of products was performed using Silia-P Flash silica gel from Silicycle using a forced flow of eluent by the method of Still.\textsuperscript{100} In vacuo concentration refers to rotary evaporation at the appropriate pressure for the given solvent with a 40 °C water bath. Yields refer to purified and spectroscopically pure compounds unless otherwise indicated as crude or partially purified. NMR spectra were obtained on a Brucker AVANCE 300 MHz or JEOL ECZS 400 MHz spectrometer and chemical shifts are reported in parts per million (ppm). \textsuperscript{1}H NMR spectra referenced to the internal residual chloroform (7.26 ppm) or deuterated dimethyl sulfoxide-d\textsubscript{5} (2.50 ppm) signals and \textsuperscript{13}C NMR spectra were referenced to the deuterated solvent signal. Infrared (IR) spectra were recorded on an ABB Bomem MB series spectrometer and absorptions are given in wavenumber (cm\textsuperscript{−1}). Mass spectrometry (MS) was performed on a SciEx QSTAR XL mass spectrometer fitted with a nano-ESI emitter in positive ion mode and an AB SciEx 4000 QTRAP hybrid triple quadrupole linear ion trap mass spectrometer equipped with an online nanoESI ionization source.
6.2 Experimental Procedures Related to Initial Synthetic Strategy

6.2.1 Synthesis of Meldrum’s Acid (2,2-dimethyl-1,3-dioxane-4,6-dione) (1)

The following procedure was adapted from a translation of the procedure outlined by Tietze and Eicher.\textsuperscript{63}

A suspension of malonic acid (5.20 g, 49.9 mmol, 1 equiv.) in acetic anhydride (7.2 mL, 64.6 mol, 1.3 equiv.) was placed on an ice bath for 30 minutes, then H\textsubscript{2}SO\textsubscript{4} (18 M, ca. 1mL) was added dropwise via Pasteur pipette while stirring. The ice bath was removed, and the solution was left to stir for 5 minutes. Acetone (7.65 mL, 104.1 mmol, 2.0 equiv.) was added dropwise to the stirring solution, resulting in a yellow tinted solution. The mixture was left to stir at RT for 30 minutes and was then transferred to the freezer for 24 hr.

The resultant crystalline mass was crushed in its containment with a spatula and washed with ice cold 0.5 M sulfuric acid\textsubscript{aq} (approximately 3 mL, 3 pipettes) and then with ice cold distilled water (30 mL). The crystals were left under vacuum and open to air for 2 hours. The crystals were then recrystallized using acetone and left on ice for 3 hours open to air to produce white, needle-like crystals (4.99 g, 69.3%)

\textit{R}r 0.23 (20% EtOAc/hexanes; UV) \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 300 MHz): \( \delta \) 3.62 (s, 2H), 1.78 (s, 6H).

Spectroscopic data consistent with previously reported literature.\textsuperscript{63}
6.2.2 Synthesis of 2,2-dimethyl-5-(prop-2-ynyl)-1,3-dioxane-4,6-dione (8) and 5,5-Dimethyl-2,2-bis(2-propynyl)-1,3-cyclohexanedione (9)

The following procedure was a modification of the procedure outlined by Leibfarth. Meldrum’s acid (1) (1.51 g, 10.47 mmol, 2.65 equiv.) was dissolved in DMF (10 mL), then K$_2$CO$_3$ (1.53 g, 11.07 mmol, 2.80 equiv.) was added. After filtration, propargyl bromide (0.3 mL, 3.95 mmol, 1.0 equiv.) was added dropwise to the mixture of 1 and DMF, producing a dark orange solution. The solution was left to stir at RT for 5 hours. The solution was transferred to 1 N HCl$_{aq}$ (75 mL), resulting in the formation of foam. The solution with foam was transferred to a separatory funnel and extracted with Et$_2$O (40 mL). The aqueous phase was further extracted two times with Et$_2$O (25 mL). The resulting organic phases were combined and washed with brine (25 mL), dried over anhydrous MgSO$_4$, filtered, and concentrated in vacuo to produce yellow, needle-like crystals. The crude sample was purified by flash chromatography using 15% EtOAc/hexanes to obtain 9 (.17 g, 19.4%) and a mixture of 8 and 1. The mixture was then further purified by flash chromatography using 15–100% EtOAc/hexanes to obtain 8 (.15 g, 16.1%) and a mixture of 8 and 1 (0.37 g).

8: $R_f$ 0.2 (25% EtOAc/hexanes; UV) $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 3.70 (t, $J = 4.9$ Hz, 1H), 3.02 (dd, $J = 2.6$, 4.9 Hz, 2H), 2.05 (t, $J = 2.6$ Hz, 2H), 1.80 (d, $J = 6.1$ Hz, 6H). $^{13}$C NMR (CDCl$_3$, 300 MHz): $\delta$ 164.1, 105.5, 70.8, 46.0, 28.6, 27.0, 16.6.
9: \( R_t \) 0.28 (15% EtOAc/hexanes; UV) \(^1\)H NMR (CDCl\(_3\), 300 MHz): \( \delta \) 2.87 (d, \( J = 2.7 \) Hz, 4H), 2.19 (t, \( J = 2.7 \) Hz, 2H), 1.83 (s, 6H). \(^{13}\)C NMR (CDCl\(_3\), 300 MHz): \( \delta \) 167.10, 107.1, 73.68, 53.53, 30.07, 27.92.

Spectroscopic data consistent with previously reported literature.\(^{101}\)

6.3 Experimental Procedures Related to the One-Pot method

6.3.1 Synthesis of S-(prop-2-yn-1-yl) benzothioate (10)

The following procedure was a modification of the procedure outlined by Lu and Cai.\(^{66}\)

At RT, propargyl bromide (750 \( \mu \)L, 10 mmol, 1.0 equiv.), thiourea (1.52 g, 20.0 mmol, 2 equiv.), benzoyl chloride (2.4 mL, 20.6 mmol, 2.06 equiv.) and potassium carbonate (4.14 g, 30.0 mmol, 3 equiv.) were stirred with 2\% (w/w) aqueous Triton X-114 (~0.3 mL by Pasteur pipette) under nitrogen. The reaction was heated to 80 \( ^\circ \)C then cooled to 60 \( ^\circ \)C and left to stir for 6 hr, resulting in the formation of orange precipitates. The heat was removed, and the reaction was left to stir overnight at RT. After 26 hr at RT, the mixture was diluted with EtOAc (80 mL) and then filtered through a bed of silica gel layered over Celite. The resulting solution was concentrated in vacuo to produce red crystals.

Characterization of this product was not performed.
6.4 Experimental procedures related to the Knoevenagel Condensation Reactions

6.4.1 The Grignard Method

6.4.1.1 Preparation of Pyrrolidinium acetate ionic liquid (PyrrIL)

\[
\text{NH} \quad + \quad \overset{0 - 23 \, ^{\circ}C}{\text{O}} \quad \overset{0 - 23 \, ^{\circ}C}{\text{OH}} \quad \overset{0 - 23 \, ^{\circ}C}{\text{PyrrIL}}
\]

The following procedure was a modification of the procedure outlined by Anouti.\textsuperscript{102}

Pyrrolidine (4.5 mL, 5.39 mmol, 1 equiv.) was added to a three-neck RBF equipped with a reflux condenser, dropping funnel and a thermometer, and placed in an ice bath. Under vigorous stirring, acetic acid (5.42 mmol, 1 equiv.) was added dropwise over 36 minutes to the flask, ensuring the temperature remained below 25 °C for the duration of the addition. The ice bath was removed after the last drop and left to stir at RT for 4 hours. The sample was concentrated \textit{in vacuo} to produce a viscous, orange oil (4.83 g, 68%).

\textbf{\textsuperscript{1}H NMR} (CDCl$_3$, 300 MHz): $\delta$ 10.59 (s, 2H), 3.14 (t, $J = 6.9$ Hz, 4H), 1.91 (m, 8H)

Spectroscopic data consistent with previously reported literature.\textsuperscript{102}
6.4.1.2 Synthesis of 2,2-dimethyl-5-(3-methylbutylidene)-1,3-dioxane-4,6-dione (11)

The following procedure was a modification of the procedure outlined by Sobrinho.\textsuperscript{61}

Isovaleraldehyde (1.1 mL, 10.25 mmol, 1 equiv.) and Meldrum’s acid (1) (2.16 g, 14.9 mmol, 1.45 equiv.) were combined and cooled to 0 °C. Then PyrIL (135 µL, 1.01 mmol, 0.098 equiv.) was then added to the stirring solution and allowed to stir for 1.5 hours at 0°C. The resulting sticky and chunky orange crude material was then purified via flash chromatography using 5% EtOAc/hexanes to produce a yellow oil (1.38 g, 58%).

\[ R_f \text{ 0.41 (20\% EtOAc/hexanes; CAM)} \]

\[ ^1\text{H NMR (CDCl}_3, \text{ 300 MHz)}: \delta \text{ 7.95 (t, } J = 7.5 \text{ Hz, 1H), 2.85 (dd, } J = 6.9, 7.5 \text{ Hz, 2H), 1.95 (sept, } J = 6.7 \text{ Hz, 1H), 1.00 (d, } J = 6.6 \text{ Hz, 6H)} \]

\[ ^{13}\text{C NMR (CDCl}_3, \text{ 300 MHz)}: \delta \text{ 168.0, 161.9, 159.9, 118.7, 104.9, 39.8, 28.7, 27.74, 22.61.} \]

Spectroscopic data consistent with previously reported literature.\textsuperscript{61}
6.4.2 The O-Link route

6.4.2.1 Synthesis of 4-(prop-2-ynyloxy)butan-1-ol (13)

The following procedure was a modification of the procedure outlined by Yamamoto.\textsuperscript{72}

Sodium hydride (95% dry, 1.32 g, 0.055 mmol, 0.91 equiv.) was mixed in DMF (10 mL) for 80 minutes at 0 °C to produce a milky white suspension. Then, a solution of 1,4-butanediol (5.3 mL, 0.06 mmol, 1 equiv.) in DMF (5 mL) was added dropwise to the RBF at 2 °C. After 30 minutes of stirring, a filtered solution of propargyl bromide (1.14 mL, 0.015 mmol, 0.25 equiv.) in DMF (5 mL) was added at 4 °C to produce a brown solution. The mixture was allowed to warm to RT and stir for 26 hours under nitrogen. The reaction was quenched with dH\textsubscript{2}O (20 mL). The reaction was then washed with EtOAC (5x 10 mL, 1x 5 mL), brine (10 mL) and dried over anhydrous MgSO\textsubscript{4}, filtered and concentrated in vacuo to produce an orange oil. The crude product was purified via FCC using 35% EtOAC/hexanes to afford a yellow oil (0.78 g, 39%).

\textit{R} \textsubscript{f} 0.29 (35% EtOAc/hexanes; CAM) \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 300 MHz): \( \delta \) 4.15 (d, \( J = 2.3 \) Hz, 2H), 3.66 (t, \( J = 5.6 \) Hz, 2H), 3.56 (t, \( J = 5.8 \) Hz, 2H), 2.42 (t, \( J = 2.3 \) Hz, 1H), 1.81 (s, 1H), 1.61-1.76 (m, 4H).

Spectroscopic data consistent with previously reported literature.\textsuperscript{72}
6.4.2.2 Synthesis of 4-(prop-2-yn-1-yloxy)butanal (14)

The following procedure was a modification of the procedure outlined by Yamamoto.\textsuperscript{72}

DMSO (500 µL, 7.74 mmol, 2.13 equiv.) in DCM (1 mL) was added dropwise to a solution of oxalyl chloride (380 µL, 4.49 mmol, 1.23 equiv.) in DCM (5 mL) at -78 °C. The solution was left to stir for 15 minutes at -78 °C and then a solution of 13 (460 mg, 3.63 mmol, 1 equiv.) in DCM (10 mL) was added via cannula transfer. The transfer flask was rinsed with DCM (5 mL) and added to the reaction mixture via cannula transfer. After 15 minutes of stirring at -78 °C, triethylamine (2.45 mL, 17.57 mmol, 4.8 equiv.) was added to the reaction mixture. The mixture was allowed to reach 0 °C slowly and then ether (15 mL) was added. The mixture was then wash with 1 M HCl (2x 15 mL), H\textsubscript{2}O (3x 15 mL), and brine (2x 15 mL). The organic phase was then dried over anhydrous MgSO\textsubscript{4}, filtered and concentrated in vacuo to produce an orange oil.

\[ R = 1.9 \text{ (20\% EtOAc/hexanes; CAM)} \]

\textsuperscript{1}H NMR crude (CDCl\textsubscript{3}, 300 MHz): \( \delta \) 9.78 (t, \( J = 1.4 \) Hz, 1H), 4.12 (d, \( J = 2.4 \) Hz, 2H), 3.55 (t, \( J = 6.0 \) Hz, 2H), 2.55 (dt, \( J = 1.4, 7.1 \) Hz, 2H), 2.42 (t, \( J = 2.3 \) Hz, 1H), 1.93 (tt, \( J = 6.0, 7.0 \) Hz, 2H).

Spectroscopic data consistent with previously reported literature.\textsuperscript{72}
6.4.2.3 Synthesis of 2,2-dimethyl-5-(3-(prop-2-yn-1-yl)oxy)propylidene)-1,3-dioxane-4,6-dione (15)

The following procedure was a modification of the procedure outlined by Sobrinho.\textsuperscript{61} The crude material of 14 (150 mg, 1.23 mmol, 1 equiv.) and Meldrum’s acid (1) (285 mg, 1.98 mmol, 1.59 equiv.) were combined and cooled to 0 °C. Then PyrrIL (46 µL, 0.35 mmol, 0.28 equiv.) was then added to the stirring solution and allowed to stir for 1.5 hours at 0°C. The resulting sticky and chunky orange crude material was then purified via FCC using 20% EtOAc/hexanes but product was not isolated.
6.4.3 Optimization of Knoevenagel Condensation Reaction

6.4.3.1 Synthesis A of 5-(cyclohexylmethylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (16)

The following procedure was a modification of the procedure outlined by Sobrinho and Dumas.\textsuperscript{61,69}

Cyclohexane carboxaldehyde (0.59 g, 5.3 mmol, 1 equiv.) and Meldrum's acid (1) (1.15 g, 7.9 mmol, 1.49 equiv.) were added to benzene (34 mL) and cooled to 0 °C. Then PyrrIL (65 μL, 0.49 mmol, 0.098 equiv.) was added to the stirring solution and allowed to warm to RT. The reaction mixture was left under N\textsubscript{2} gas to stir overnight. The reaction was then diluted with EtOAc (10 mL) and washed with sat. sodium bicarbonate (3x 15 mL), brine (2x 10 mL), dried over anhydrous MgSO\textsubscript{4}, filtered and concentrated in vacuo to produce white, prismatic crystals with a yellow oil. The crude material was then purified via FCC using 10% EtOAc/hexanes to afford partially-pure 16 as orange, prism crystals (0.27 g, ~21.3%).

R\textsubscript{f} 0.25 (10% EtOAc/hexanes; CAM) \textsuperscript{1}H NMR partially-pure (CDCl\textsubscript{3}, 300 MHz): δ 7.70 (d, J = 10.5 Hz, 1H), 3.50 (qt, J = 2.9, 10.8 Hz, 1 H), 1.74 (m, 12H), 1.02-1.66 (m, 10H).

Spectroscopic data consistent with previously reported literature.\textsuperscript{68}
6.4.3.2 Synthesis B of 5-(cyclohexylmethylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (16)

The following procedure was a modification of the procedure outlined by Dumas.\(^6^9\)

To a stirring solution of cyclohexane carboxaldehyde (0.90 g, 8.02 mmol, 1 equiv.), Meldrum’s acid (1) (1.15 g, 7.9 mmol, 0.98 equiv.) and 0.2 M benzene\(_{aq}\) (40 mL), PyrrIL (106 µL, 0.80 mmol, 0.1 equiv.) was added dropwise at RT. The reaction mixture was left under N\(_2\) gas to stir for 24 hr. The reaction was then diluted with EtOAc (50 mL) and washed with sat. sodium bicarbonate (3x 30 mL), brine (2x 30 mL), dried over anhydrous MgSO\(_4\), filtered and concentrated in vacuo to produce a yellow oil. The crude material was then recrystallized using methanol but did not isolate 16.
6.4.3.3 Synthesis C of 5-(cyclohexylmethylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (16)

The following procedure was a modification of the procedure outlined by Dumas. To a stirring solution of cyclohexane carboxaldehyde (0.90 g, 8.02 mmol, 1 equiv.), Meldrum’s acid (1) (1.15 g, 7.9 mmol, 0.98 equiv.) and dH₂O (40 mL), PyrrIL (106 µL, 0.80 mmol, 0.1 equiv.) was added dropwise at RT. The reaction mixture was left under N₂ gas to stir for 24 hr. The reaction was then diluted with EtOAc (80 mL) and washed with sat. sodium bicarbonate (3x 30 mL), brine (3x 30 mL), dried over anhydrous MgSO₄, filtered and concentrated in vacuo to produce a yellow oil. The crude material was then purified via FCC using 10% EtOAc/hexanes to afford 16 as white, prism crystals (0.26 g, 13.8%).

R<sub>f</sub> 0.67 (30% EtOAc/hexanes; CAM) <sup>1</sup>H NMR (CDCl₃, 300 MHz): δ 7.68 (d, J = 10.5 Hz, 1H), 3.49 (qt, J = 10.0, 3.2 Hz, 1H), 1.74 (m, 12H), 1.16-1.44 (m, 6H).

Spectroscopic data consistent with previously reported literature. 
6.5 Experimental Procedures Related to The Coupling Route

6.5.1 Synthesis of 1,5-dioxaspiro[5.5]undecane-2,4-dione (17)

The following procedure was a modification of the procedure outlined by Kimmel.\textsuperscript{74}

Malonic acid (5.02 g, 48.3 mmol, 1 equiv.), p-toluenesulfonic acid monohydrate (0.23 g, 1.20 mmol, 0.02 equiv.), cyclohexanone (5 mL, 50.9 mmol, 1.05 equiv.) and acetic anhydride (7.8 mL, 82.5 mmol, 1.70 equiv.) was added to a three-neck RBF. The mixture was stirred for approximately 5 hours until the solution turned black. The stirring was stopped, and the reaction mixture was left to sit at RT for 7 days. On the 7\textsuperscript{th} day, dH\textsubscript{2}O (238 mL) was added and the solution was stirred until compound precipitated. The flask was then moved to an ice bath for 1 hr to cool. The resulting crystals were then filtered on a Buchner funnel, washed with hot hexanes (100 mL) and left to dry on the filter for 1 hr. The resulting crystals were then recrystallized using 5 mL/g of hot 5:2 hexanes/ethanol (200 proof). The crystals were then washed with hot hexanes to produce red crystals of 17 (4.45 g, 50.3%).

\textit{R}r 0.67 (30\% EtOAc/hexanes; CAM) \textsuperscript{1}H NMR (CDCl\textsubscript{3}, 300 MHz): \(\delta 3.61\) (s, 2H), 1.97 (m, 4H), 1.74 (m, 4H), 1.50 (m, 2H). \textsuperscript{13}C NMR (CDCl\textsubscript{3}, 300 MHz): \(\delta 163.12, 107.26, 36.5, 36.4, 24.0, 22.2\).

Spectroscopic data consistent with previously reported literature.\textsuperscript{103}
6.5.2 Synthesis 3-hexyl-1,5-dioxaspiro[5.5]undecane-2,4-dione (18)

6.5.2.1 One-pot coupling using DCC modification

![Reaction Scheme]

The following procedure was a modification of the procedure outlined by Brinkerhoff, Weaver and Tsukamoto.\textsuperscript{73,75,76}

Hexanoic acid (62 µL, 0.49 mmol, 1 equiv.), DCC (0.15 g, 0.72 mmol, 1.46 equiv.) and DMAP (1 crystal) were added to MeCN (25 mL) and stirred at RT for 15 minutes to produce a cloudy mixture. 17 (104 mg, 0.56 mmol, 1.1 equiv.) was then added to the stirring mixture followed by DIPEA (280 µL, 1.6 mmol, 3.28 equiv.) dropwise. The reaction mixture was left to stir overnight at RT under N\textsubscript{2} gas. Solid DCU was removed by filtration and the resulting mixture was concentrated in vacuo to produce an orange, sticky oil. The crude was then dissolved in DCM (25 mL) and cooled to 0 °C. Acetic acid (280 µL, 4.66 mmol, 9.51 equiv.) was then added dropwise to the stirring solution followed by sodium borohydride (50 mg, 1.49 mmol, 3.04 equiv.) portion wise. The reaction was allowed to warm to RT and left to stir overnight. The reaction mixture was then quenched with water (25 mL) and acidified with 1 M HCl. The reaction mixture was transferred to a separatory funnel to separate the layers. The organic layer was then washed with water (12 mL) and brine (13 mL) combined, brine (3x 25 mL), dried over anhydrous MgSO\textsubscript{4}, filtered and concentrated in vacuo to
produce an orange crystals and oil. The crude material was then purified via FCC using 7.5% EtOAc/hexanes to afford partially purified 18 as white, prism crystals (0.07 g, 53.2%).

$R_f$ 0.69 (25% EtOAc/hexanes; CAM) $^1$H NMR partially purified (CDCl$_3$, 300 MHz): $\delta$ 3.51 (t, $J = 4.9$ Hz, 1H), 2.09 (m, 2H), 1.96 (m, 4H), 1.73 (m, 6H), 1.15-1.54 (m, 24H), 0.82 (m, 8H)

6.5.2.2 Two-step coupling using EDC•HCl

The following procedure was a modification of the procedure outlined by Bruckner and Winkler.$^{78,79}$

**Step A)** Hexanoic acid (110 µL, 0.87 mmol, 1 equiv.), EDC•HCl (0.22 g, 1.14 mmol, 1.31 equiv.) and DMAP (0.2 g, 1.63 mmol, 1.87 equiv.) were added to DCM (21 mL) and stirred at RT for 30 minutes. 17 (170 mg, 0.92 mmol, 1.06 equiv.) was then added to the stirring
The reaction mixture was left to stir overnight at RT under N₂ gas. The reaction mixture was then diluted with 50/50 dH₂O/brine (20 mL total) and transferred to a separatory funnel. The layers were separated and the organic layer was then washed with 1 M HCl (3x 20 mL), brine (2x 20 mL), dried over anhydrous MgSO₄, filtered and concentrated in vacuo to produce yellow crystals in a yellow oil. The crude material was then verified using ¹H NMR to confirm 19 was produced based on proton at 3.05 ppm (~82% yield by ¹H NMR comparing 19 to 1).

**Step B)** The crude was then dissolved in DCM (10 mL) and acetic acid (300 µL, 5.26 mmol, 10.7 equiv.) was then added dropwise to the stirring solution. The reaction mixture was then cooled to 0 °C by sitting in an ice bath for 30 minutes. Sodium borohydride (50 mg, 1.49 mmol, 3.04 equiv.) was then added portion wise and the vial was rinsed with DCM (3 mL) and added to the reaction mixture. The reaction was allowed to warm to RT and left to stir overnight. The reaction mixture was then quenched with water (25 mL). The reaction mixture was then separated to a separatory funnel to separate the layers. An additional 10 mL of DCM was then added to the mixture. The layers were separated, and the aqueous layer was washed with DCM (3x 25 mL). The combined organic layers were then washed with brine (25 mL), dried over anhydrous MgSO₄, filtered and concentrated in vacuo to produce yellow crystal. The crude material was then purified via FCC using 7.5% EtOAc/hexanes to afford 18 as white, prism crystals (0.16 g, 68.5% over two steps).

Rᵣ 0.5 (20% EtOAc/hexanes; CAM) ¹H NMR (CDCl₃, 300 MHz): δ 3.50 (t, J = 5.0 Hz, 1H), 2.09 (m, 2H), 1.96 (m, 4H), 1.73 (m, 4H), 1.24-1.54 (m, 10H), 0.87 (t, J = 6.6 Hz, 3H). ¹³C NMR (CDCl₃, 300 MHz): δ 165.9, 105.7, 46.5, 37.0, 36.2, 31.5, 29.8, 29.2, 27.0, 26.6, 24.2, 22.7, 22.6, 21.9, 14.1. FT-IR (NaCl): cm⁻¹ 2947, 2852, 1790, 1749, 993.
6.5.3 Synthesis of cationic carboxylic acid salts

6.5.3.1 Synthesis of N-(5-carboxypentyl)-N, N-dimethylbenzammonium bromide salt (21)

The following procedure was a modification of the procedure outlined by Szafran.\(^1\)

\(N,N\)-dimethylaniline (1.3 mL, 0.01 mmol, 1 equiv.) was mixed with 6-bromohexanoic acid (2.04 g, 0.01 mmol, 1 equiv.) forming a black, heterogenous mixture. The reaction mixture was left to stir overnight and was stopped and left at RT for 13 days. Drops of acetone were added to the mixture until crystals appeared. The reaction mixture was then left to stir for 20 minutes and placed in the freezer for 6 days. The resulting crystals were then filtered and washed with cold acetone on a cold Buchner funnel to produce white, fluffy crystals. Yield for this reaction was not calculated.

\(^1\)H NMR (DMSO, 300 MHz): \(\delta\) 7.93 (d, \(J = 7.8\) Hz, 2H), 7.5-7.67 (m, 3H), 3.94 (m, 2H), 3.62 (s, 6H), 2.13 (t, \(J = 7.2\) Hz, 2H), 1.12-1.45 (m, 6H). \(^{13}\)C NMR (DMSO, 300 MHz): \(\delta\) 174.2, 144.5, 130.1, 130.0, 121.2, 67.7, 53.7, 33.2, 24.8, 23.7, 22.4.

MS (ESI): \(m/z\) calcd for \(C_{14}H_{22}NO^+\): 236.1650; observed 236.1406.
6.5.4 Synthesis of 2-(2,4-dioxo-1,5-dioxaspiro[5.5]undecane-3-yl)-N,N,N-trimethylethan-1-aminium chloride salt (23)

A)  
\[
\begin{align*}
\text{O} & \quad \text{H} & \quad \text{O} \\
17 & \quad \text{+} & \quad \text{HO-}\text{C-}\text{N}^+\text{Cl}^- \\
& & \text{EDC-}\text{HCl} \quad \text{DMAP} \\
& & \text{DCM} \quad \text{RT, 24 hr} \\
& & \quad \text{Cl}^- \\
\text{O} & \quad \text{O} \\
22 &
\end{align*}
\]

B)  
\[
\begin{align*}
\text{O} & \quad \text{OH} & \quad \text{N}^+\text{Cl}^- \\
\text{Cl}^- \\
\text{NaBH}_4 & \quad \text{AcOH} \\
& & \text{DCM} \\
\text{O} & \quad \text{O} \\
23 &
\end{align*}
\]

The following procedure was a modification of the procedure outlined by Bruckner and Winkler.\textsuperscript{78,79}

**Step A)** Betaine hydrochloride (130 mg, 0.84 mmol, 1 equiv.), EDC•HCl (240 mg, 1.25 mmol, 1.48 equiv.) and DMAP (110 mg, 0.90 mmol, 1.07 equiv.) were added to DCM (11.5 mL) and stirred at RT for 30 minutes. 17 (180 mg, 0.97 mmol, 1.15 equiv.) was then added to the stirring mixture. The reaction mixture was left to stir overnight at RT under N\textsubscript{2} gas. The reaction mixture was then concentrated in vacuo.

**Step B)** The crude was then dissolved in DCM (5 mL) and acetic acid (480 µL, 8.41 mmol, 10.0 equiv.) was then added dropwise to the stirring solution. The reaction mixture was then cooled to 0 °C by sitting in an ice bath for 30 minutes. Sodium borohydride (120 mg, 3.17 mmol, 3.77 equiv.) was then added portion wise and the vial and reaction flask was rinsed with DCM (9 mL) and added to the reaction mixture. The reaction was allowed to warm to
RT and left to stir overnight. The reaction mixture was then quenched with dH₂O (10 mL) and brine (6 mL) and acidified using 1 M HCl. The reaction mixture was transferred to a separatory funnel to separate the layers. The layers were separated, and the aqueous layer was washed with DCM (10 mL). The combined organic layers were then washed with water (2x 10 mL), brine (26.5 mL), dried over anhydrous MgSO₄, filtered and concentrated in vacuo to produce an orange oil. Purification and characterization of this compound was not performed.

6.5.5 Coupling halogenated acid then performing amine alkylation route

6.5.5.1 Synthesis of 3-(6-bromohexyl)-1,5-dioxaspiro[5.5]undecane-2,4-dione (24)

The following procedure was a modification of the procedure outlined by Hashimoto.⁸³ 6-bromohexanoic acid (100 mg, 0.51 mmol, 1 equiv.), EDC•HCl (110 mg, 0.57 mmol, 1.11 equiv.), triethylamine (155 µL, 1.10 mmol, 2.15 equiv.) and DMAP (20 mg, 0.16 mmol, 0.31 equiv.) were added to DCM (10 mL) and stirred at 0 °C for 3³ hours. 17 (107 mg, 0.58 mmol, 1.13 equiv.) was then added to the stirring mixture. The reaction mixture was left to stir overnight at RT under N₂ gas. The reaction mixture was concentrated in vacuo and then resuspended in EtOAc (20 mL). 1 M HCl (20 mL) was added to the reaction mixture and stirred for a few minutes. The entire solution was then transferred to a separatory funnel.
The layers were separated, and the organic layer was then washed with water (3x 20 mL), brine (20 mL), dried over anhydrous MgSO₄, filtered and concentrated in vacuo to produce yellow crystals in a yellow oil. The crude material was then verified using ¹H NMR to confirm 24 was produced based on proton at 3.07 ppm (~77% yield by ¹H NMR comparing 24 to 6-bromohexanoic acid).

α Incubation period was tested varying from 30 minutes to 6 hours. Optimal stirring period was determined to be 3 hours.

### 6.5.5.2 Synthesis of 3-(6-bromohexyl)-1,5-dioxaspiro[5.5]undecane-2,4-dione (25)

The following procedure was a modification of the procedure outlined by Winkler. The crude of 24 (0.07 g, 0.51 mmol, 1 equiv) was dissolved in DCM (10 mL) and acetic acid (315 μL, 5.52 mmol, 10.8 equiv.) was then added dropwise to the stirring solution. The reaction mixture was then cooled to 0 °C by sitting in an ice bath for 30 minutes. Sodium borohydride (89 mg, 2.35 mmol, 4.62 equiv.) was then added portion wise and the vial and reaction flask was rinsed with DCM (5 mL) and added to the reaction mixture. The reaction was allowed to warm to RT and left to stir overnight. The reaction mixture was then quenched with dH₂O (25 mL). The reaction mixture was transferred to a separatory funnel to separate the layers. The aqueous layer was washed with DCM (3x 25 mL).
combined organic layers were then washed with brine (30 mL), dried over anhydrous MgSO₄, filtered and concentrated in vacuo to produce yellow crystals in a yellow oil. The crude material was then purified via FCC using 10% EtOAc/hexanes to afford 25 as white crystals (30 mg, ~16.9%b over two steps).

a Calculations of equivalents for this reaction were based on assumed “full coupling” in the synthesis of 24. It is acknowledged this reaction is not complete.

b Complete purification of 25 was not obtained, unidentified residues are present within the sample.

Rf 0.5 (20% EtOAc/hexanes; CAM) ¹H NMR (CDCl₃, 300 MHz): δ 3.51 (t, J = 5.0 Hz, 1H), 3.39 (t, J = 6.7 Hz, 2H), 2.08 (m, 2H), 1.96 (m, 4H), 1.63-1.90 (m, 7H), 1.30-1.53 (m, 9H).

¹³C NMR (CDCl₃, 300 MHz): δ 165.7, 105.8, 46.4, 37.0, 36.1, 33.9, 32.6, 28.7, 27.8, 26.6, 26.3, 24.1, 22.6, 21.8. FT-IR (NaCl): cm⁻¹ 2934, 2856, 1787, 1749, 1296, 1269.
6.5.5.3 Synthesis of $N$-benzyl-6-(2,4-dioxo-1,5-dioxaspiro[5.5]undecan-3-yl)-$N,N$-dimethylhexan-1-aminium bromide (26).

![Chemical diagram]

The following procedure was a modification of the procedure outlined by Szafran.\(^{81}\)

25 (70 mg, 0.20 mmol, 1 equiv.) was dissolved in MeCN (5 mL) in a multi-neck RBF equipped with a reflux condenser. DBA (60 µL, 0.39 mmol, 1.99 equiv.) was added dropwise to the solution and then the reaction mixture stirred for 1 hr at RT under $N_2$ gas. After 1 hr, the reaction mixture was refluxed for 4 hr and produced an orange oil. The addition of DBA and refluxing was repeated until diagnostic peaks of 25 were no longer visible via $^1$H NMR. Purification and characterization of this compound was not completed.
6.5.5.4 Synthesis of 6-(2,4-dioxo-1,5-dioxaspiro[5.5]undecan-3-yl)-N,N,N-trimethylhexan-1-aminium bromide (27)

The following procedure was a modification of the procedure outlined by Szafran.\textsuperscript{81}  

25 (50 mg, 0.15 mmol, 1 equiv.) was dissolved in MeCN (5 mL). TMA (75 µL, 0.31 mmol, 2.1 equiv.) was added dropwise to the solution and then the reaction mixture stirred for 2 hr under N\textsubscript{2} gas. After 2 hr, additional TMA (150 µL, 0.63 mmol, 4.2 equiv.) was added dropwise to the solution and the reaction mixture was heated to 40 °C overnight. Purification and characterization of this compound was not completed.
6.6 Experimental procedures related to POC ketene reactions

6.6.1 Synthesis of naphthalen-1-yl octanoate (28)

6.6.1.1 Variation 1

The following procedure was a modification of the procedure outlined by Wolffs.\textsuperscript{84}

Stoichiometric amounts of 18 and 1-naphthol were added to a microwave vial that was sealed and equipped with a venting needle. The vial was placed into a pre-heated oil bath at 190 °C for 20 minutes while stirring. The bath was then heated to 205 °C for 10 minutes. The vial was then removed and opened to atmosphere and allowed to cool for 20 minutes prior to $^1$H NMR analysis. Purification and characterization of this compound was not completed.

6.6.1.2 Variation 2

The following procedure was a modification of the procedure outlined by Li.\textsuperscript{45}
Stoichiometric amounts of 18 and 1-naphthol were added to a microwave vial that was sealed and equipped with a venting needle. The vial was placed into a pre-heated oil bath at $X$ °C for 1.33 hr while stirring. The vial was then removed and opened to atmosphere and allowed to cool prior to $^1$H NMR analysis. Purification and characterization of this compound was not completed.

$X$ – temperatures performed for this reaction include 100 °C, 130 °C and 160 °C.

**6.6.2 Synthesis of 3,5-di-tert-butylphenyl octanoate (29)**

The following procedure was a modification of the procedure outlined by Li.$^{45}$

Stoichiometric amounts of 18 and 3,5-di-tert-butylphenol were added to a microwave vial that was sealed and under N$_2$ gas. The vial was placed into a pre-heated oil bath at 160 °C for $X$ hr while stirring. The vial was then removed and opened to atmosphere and allowed to cool prior to $^1$H NMR analysis. Purification and characterization of this compound was not completed.

$X$ – reaction times for this reaction include 1.33 hr, 2 hr, 4 hr and 6 hr.
6.7 Experimental procedures related to MAD+ ketene reactions

6.7.1 Synthesis of $N$-benzyl-8-(3,5-di-tert-butylphenoxy)$-N,N$-dimethyl-8-oxooctan-1-aminium bromide (30)

The following procedure was a modification of the procedure outlined by Li.\textsuperscript{45}

Stoichiometric amounts of 26 and 3,5-di-tert-butylphenol were added to a containment\textsuperscript{a} that was sealed and under N\textsubscript{2} gas. The containment was placed into a pre-heated oil bath at 160 °C for $X$ hr while stirring. The vial was then removed and opened to atmosphere and allowed to cool prior to $^1$H NMR analysis. Purification and characterization of this compound was not completed.

\textsuperscript{a} containment refers to a microwave vial, multi-neck RBF or a multi-neck PSF.
Appendices

Appendix A

This is Appendix A containing all NMR data.

A.1 NMR spectra related to the initial synthetic strategy

Figure A 1. $^1$H NMR of Meldrum’s acid (2,2-dimethyl-1,3-dioxane-4,6-dione, 1) in CDCls.
Figure A 2. $^1$H NMR of 5,5-Dimethyl-2,2-bis(2-propynyl)-1,3-cyclohexanedione (9) in CDCl$_3$.

Figure A 3. $^{13}$C NMR of 5,5-Dimethyl-2,2-bis(2-propynyl)-1,3-cyclohexanedione (9) in CDCl$_3$. 
Figure A 4. $^1$H NMR of 2,2-dimethyl-5-(prop-2-yn-1-yl)-1,3-dioxane-4,6-dione (8) in CDCl$_3$.

Figure A 5. $^{13}$C NMR of 2,2-dimethyl-5-(prop-2-yn-1-yl)-1,3-dioxane-4,6-dione (8) in CDCl$_3$. 
Figure A 6. DEPT135 NMR of 2,2-dimethyl-5-(prop-2-yn-1-yl)-1,3-dioxane-4,6-dione (8) in CDCl₃.
Figure A 7. HSQC NMR of 2,2-dimethyl-5-(prop-2-yn-1-yl)-1,3-dioxane-4,6-dione (8) in CDCl₃.
A.2 NMR spectra related to the One-pot method

Figure A 8. $^1$H NMR of crude material from attempted synthesis of S-prop-2-ynyl benzothioate (10) in CDCl$_3$. 
A.3 NMR spectra related to the Knoevenagel condensation reactions

Figure A 9. $^1$H NMR of pyrrolidinium acetate ionic liquid (PyrrIL) in CDCl$_3$.

Figure A 10. $^1$H NMR of 2,2-dimethyl-5-(3-methylbutyldiene)-1,3-dioxane-4,6-dione (11) in CDCl$_3$. 
Figure A 11. $^{13}$C NMR of 2,2-dimethyl-5-(3-methylbutylidene)-1,3-dioxane-4,6-dione (11) in CDCl$_3$.

Figure A 12. $^1$H NMR of crude material from attempted synthesis of 2,2-dimethyl-5-(5-methylhex-1-yn-3-yl)-1,3-dioxane-4,6-dione (12) in CDCl$_3$. 
Figure A 13. $^1$H NMR of 4-(prop-2-yn-1-yloxy)butanol (13) in CDCl$_3$.

Figure A 14. $^1$H NMR of crude material of 4-(prop-2-yn-1-yloxy)butanal (14) in CDCl$_3$. 
Figure A 15. $^1$H NMR expansion at 2-4 ppm of crude material of 4-(prop-2-yn-1-yl oxy)butanal (14) in CDCl$_3$. 
Figure A 16. $^1$H NMR of crude material of 2,2-dimethyl-5-(3-(prop-2-yn-1-yloxy)propylidene)-1,3-dioxane-4,6-dione (15) in CDCl$_3$.

Figure A 17. $^1$H NMR of reaction A crude material of 5-(cyclohexylmethylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (16) in CDCl$_3$. 
Figure A 18. $^1$H NMR of reaction A partially purified 5-(cyclohexylmethylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (16) in CDCl$_3$.

Figure A 19. $^1$H NMR of reaction B crude material of 5-(cyclohexylmethylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (16) in CDCl$_3$. 
Figure A 20. $^1$H NMR of reaction B purified unknown compound in CDCl$_3$.

Figure A 21. $^1$H NMR of reaction C crude material of 5-(cyclohexylmethylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (16) in CDCl$_3$. 
Figure A22. $^1$H NMR of reaction C purified 5-(cyclohexymethylene)-2,2-dimethyl-1,3-dioxane-4,6-dione (16) in CDCl$_3$. 
A.4 NMR spectra related to the coupling route

Figure A 23. $^1$H NMR of pure 1,5-dioxaaspiro[5.5]undecane-2,4-dione (17) in CDCl₃.

Figure A 24. $^{13}$C NMR of recrystallized 5 month old 1,5-dioxaaspiro[5.5]undecane-2,4-dione (17) in CDCl₃.
Figure A 25. $^1$H NMR of 5 month old 1,5-dioxaspiro[5.5]undecane-2,4-dione (17) in CDCl$_3$.

Figure A 26. $^1$H NMR of 5 month old 1,5-dioxaspiro[5.5]undecane-2,4-dione (17) in CDCl$_3$. 
Figure A 27. $^1$H NMR of partially purified 3-hexyl-1,5-dioxaspiro[5.5]undecane-2,4-dione (18) in CDCl$_3$.

Figure A 28. $^1$H NMR of pure 3-hexyl-1,5-dioxaspiro[5.5]undecane-2,4-dione (18) in CDCl$_3$. 
Figure A 29. $^{13}$C NMR of pure 3-hexyl-1,5-dioxaspiro[5.5]undecane-2,4-dione (18) in CDCl$_3$.

Figure A 30. DEPT135 NMR of pure 3-hexyl-1,5-dioxaspiro[5.5]undecane-2,4-dione (18) in CDCl$_3$. 
Figure A 31. COSY NMR of pure 3-hexyl-1,5-dioxaspiro[5.5]undecane-2,4-dione (18) in CDCl₃.
Figure A 32. HSQC NMR of pure 3-hexyl-1,5-dioxaspiro[5.5]undecane-2,4-dione (18) in CDCl₃.
Figure A 33. $^1$H NMR of pure $N$-(5-carboxypentyl)-$N$, $N$-dimethylbenzammonium bromide salt (21) in DMSO.

Figure A 34. $^{13}$C NMR of pure $N$-(5-carboxypentyl)-$N$, $N$-dimethylbenzammonium bromide salt (21) in DMSO.
Figure A 35. $^1$H NMR of attempted synthesis of 2-(2,4-dioxospiro[5.5]undecan-3-ylidene)-2-hydroxy-N,N,N-trimethylethan-1-aminium chloride (22) in DMSO.

Figure A 36. $^1$H NMR of attempted synthesis of 2-(2,4-dioxo-1,5-dioxa[5.5]undecane-3-yl)-N,N,N-trimethylethan-1-aminium chloride salt (23) in DMSO.
A.5  NMR spectra related to coupling halogenated acid then performing amine alkylation route

**Figure A 37.** $^1$H NMR of 0.5 hr incubation of all reagents except 1 in the synthesis of 3-(6-bromo-1-hydroxyhexylidene)-1,5-dioxaspiro[5.5]undecane-2,4-dione (24) in CDCls.

**Figure A 38.** $^1$H NMR of 1 hr incubation of all reagents except 1 in the synthesis of 3-(6-bromo-1-hydroxyhexylidene)-1,5-dioxaspiro[5.5]undecane-2,4-dione (24) in CDCls.
Figure A 39. $^1$H NMR of 3 hr incubation of all reagents except 1 in the synthesis of 3-(6-bromo-1-hydroxyhexylidene)-1,5-dioxaspiro[5.5]undecane-2,4-dione (24) in CDCl₃.
Figure A 40. COSY NMR of 3 hr incubation of all reagents except 1 in the synthesis of 3-(6-bromo-1-hydroxyhexylidene)-1,5-dioxaspiro[5.5]undecane-2,4-dione (24) in CDCl₃.
Figure A 41. $^1$H NMR of 6 hr incubation of all reagents except 1 in the synthesis of 3-(6-bromo-1-hydroxyhexylidene)-1,5-dioxaspiro[5.5]undecane-2,4-dione (24) in CDCl$_3$.

Figure A 42. $^1$H NMR of 24 hr incubation of all reagents except 1 in the synthesis of 3-(6-bromo-1-hydroxyhexylidene)-1,5-dioxaspiro[5.5]undecane-2,4-dione (24) in CDCl$_3$. 
Figure A 43. $^1$H NMR of pure 3-(6-bromohexyl)-1,5-dioxaspiro[5.5]undecane-2,4-dione (25) in CDCl$_3$.

Figure A 44. $^1$H NMR expansion of pure 3-(6-bromohexyl)-1,5-dioxaspiro[5.5]undecane-2,4-dione (25) in CDCl$_3$. 
Figure A 45. $^{13}$C NMR of pure 3-(6-bromohexyl)-1,5-dioxaspiro[5.5]undecane-2,4-dione (25) in CDCl$_3$.

Figure A 46. DEPT135 NMR of pure 3-(6-bromohexyl)-1,5-dioxaspiro[5.5]undecane-2,4-dione (25) in CDCl$_3$. 
Figure A 47. COSY NMR of pure 3-(6-bromohexyl)-1,5-dioxaspiro[5.5]undecane-2,4-dione (25) in CDCl₃.
Figure A 48. HSQC NMR of pure 3-(6-bromohexyl)-1,5-dioxaspiro[5.5]undecane-2,4-dione (25) in CDCl₃.
Figure A 49. $^1$H NMR of crude $N$-benzyl-6-(2,4-dioxo-1,5-dioxaspiro[5.5]undecan-3-yl)-$N, N$-dimethylhexan-1-aminium bromide (26) made with one equivalent DBA in CDCl$_3$.

Figure A 50. $^{13}$C NMR of crude $N$-benzyl-6-(2,4-dioxo-1,5-dioxaspiro[5.5]undecan-3-yl)-$N, N$-dimethylhexan-1-aminium bromide (26) made with one equivalent DBA in CDCl$_3$. 
Figure A 51. HSQC NMR of crude N-benzyl-6-(2,4-dioxo-1,5-dioxaspiro[5.5]undecan-3-yl)-N,N-dimethylhexan-1-aminium bromide (26) made with one equivalent DBA in CDCl₃.
Figure A 52. $^1$H NMR of crude N-benzyl-6-(2,4-dioxo-1,5-dioxaspiro[5.5]undecan-3-yl)-N,N-dimethylhexan-1-aminium bromide (26) made with two equivalents DBA in CDCl$_3$. 
Figure A53. COSY NMR of crude N-benzyl-6-(2,4-dioxo-1,5-dioxaspiro[5.5]undecan-3-yl)-N,N-dimethylhexan-1-aminium bromide (26) made with two equivalents DBA in CDCl₃.
Figure A 54. $^1$H NMR of crude 6-(2,4-dioxo-1,5-dioxaspiro[5.5]undecan-3-yl)-N,N,N-trimethylhexan-1-aminium bromide (27) in CDCl$_3$ on day one.

Figure A 55. $^1$H NMR of crude 6-(2,4-dioxo-1,5-dioxaspiro[5.5]undecan-3-yl)-N,N,N-trimethylhexan-1-aminium bromide (27) in CDCl$_3$ on day seven.
Figure A 56. $^{13}C$ NMR of crude 6-(2,4-dioxo-1,5-dioxaspiro[5.5]undecan-3-yl)-N,N,N-trimethylhexan-1-aminium bromide (27) in CDCl$_3$ on day seven.
A.6 NMR spectra related to POC ketene reactions

Figure A 57. $^1$H NMR of crude Naphthalen-1-yl octanoate (28) in CDCl$_3$.

Figure A 58. $^1$H NMR of crude 3,5-di-tert-butylphenyl octanoate (29) from 1.33 hr reaction in CDCl$_3$. 
Figure A 59. $^{13}$C NMR of crude 3,5-di-tert-butylphenyl octanoate (29) from 1.33 hr reaction in CDCl$_3$.

Figure A 60. HMBC NMR of crude 3,5-di-tert-butylphenyl octanoate (29) from 1.33 hr reaction in CDCl$_3$. 
Figure A 61. $^1$H NMR of crude 3,5-di-tert-butylphenyl octanoate (29) from 2 hr reaction in CDCl₃.

Figure A 62. $^1$H NMR of crude 3,5-di-tert-butylphenyl octanoate (29) from 4 hr reaction in CDCl₃.
Figure A 63. $^{13}$C NMR of crude 3,5-di-tert-butylphenyl octanoate (29) from 4 hr reaction in CDCl$_3$.

Figure A 64. HSQC NMR of crude 3,5-di-tert-butylphenyl octanoate (29) from 4 hr reaction in CDCl$_3$. 
Figure A 65. HMBC NMR of crude 3,5-di-tert-butylphenyl octanoate (29) from 4 hr reaction in CDCl₃.
A.7 NMR spectra related to MAD\textsuperscript{+} ketene reactions

Figure A 66. \textsuperscript{1}H NMR of crude \textit{N}-benzyl-8-(3,5-di-\textit{tert}-butylphenoxy)-\textit{N},\textit{N}-dimethyl-8-oxooctan-1-aminium bromide (30) from reaction one in CDCl\textsubscript{3}.

Figure A 67. \textsuperscript{13}C NMR of crude \textit{N}-benzyl-8-(3,5-di-\textit{tert}-butylphenoxy)-\textit{N},\textit{N}-dimethyl-8-oxooctan-1-aminium bromide (30) from reaction one in CDCl\textsubscript{3}.
Figure A 68. $^1$H NMR of crude $N$-benzyl-8-(3,5-di-tert-butylphenoxy)-$N,N$-dimethyl-8-oxooctan-1-aminium bromide (30) from reaction two in CDCl$_3$.

Figure A 69. $^1$H NMR of crude $N$-benzyl-8-(3,5-di-tert-butylphenoxy)-$N,N$-dimethyl-8-oxooctan-1-aminium bromide (30) from reaction three in CDCl$_3$. 
**Figure A 70.** COSY NMR of crude \(N\)-benzyl-\(8\)-(3,5-di-\textit{tert}-butylphenoxy)-\(N\),\(N\)-dimethyl-\(8\)-oxooctan-1-aminium bromide (30) from reaction three in CDCl₃.
Figure A 71. $^{13}$C NMR of crude $N$-benzyl-8-(3,5-di-tert-butylphenoxy)-$N,N$-dimethyl-8-oxooctan-1-aminium bromide (30) from reaction three in CDCl$_3$. 
Figure A 72. HSQC NMR of crude $N$-benzyl-$8$-(3,5-di-tert-butyloxy)-$N,N$-dimethyl-$8$-oxooctan-1-aminium bromide (30) from reaction three in CDCl$_3$. 
Figure A 73. HMBC NMR of crude N-benzyl-8-(3,5-di-tert-butylphenoxy)-N,N-dimethyl-8-oxooctan-1-aminium bromide (30) from reaction three in CDCl₃.
Appendix B

This is Appendix B containing all IR spectra.

B.1 IR spectra of MADs

Figure B 1. IR of 3-hexyl-1,5-dioxaspiro[5.5]undecane-2,4-dione (18) using NaCl cell method.
Figure B 2. IR of 3-(6-bromohexyl)-1,5-dioxaspiro[5.5]undecane-2,4-dione (25) using NaCl cell method.

Figure B 3. IR of N-benzyl-6-(2,4-dioxo-1,5-dioxaspiro[5.5]undecan-3-yl)-N,N-dimethylhexan-1-aminium bromide (26) using NaCl cell method.
B.2 IR spectra of POC ketene reactions

Figure B 4. IR of crude material of 3,5-di-tert-butylphenyl octanoate (29) from 1.33 hr reaction using NaCl cell method.

Figure B 5. IR of crude material of 3,5-di-tert-butylphenyl octanoate (29) from 4 hr reaction using NaCl cell method.
B.3 IR spectra of MAD<sup>+</sup> ketene reactions

**Figure B 6.** IR of crude N-benzyl-8-(3,5-di-tert-butylphenoxy)-N,N-dimethyl-8-oxooctan-1-aminium bromide (30) from reaction one using NaCl cell method.

**Figure B 7.** IR of crude N-benzyl-8-(3,5-di-tert-butylphenoxy)-N,N-dimethyl-8-oxooctan-1-aminium bromide (30) from reaction two using NaCl cell method.
Figure B 8. IR of crude N-benzyl-8-(3,5-di-tert-butylphenoxy)-N,N-dimethyl-8-oxooctan-1-aminium bromide (30) from reaction three using NaCl cell method.
Appendix C

This is Appendix C containing all MS spectra.

C.1 Mass spectra of cationic carboxylic acids

Figure C 1. Mass spectrum of $N$-(5-carboxypentyl)-$N,N$-dimethylbenzammonium bromide salt (21) at $m/z$ 236.1. Spectrum was obtained on a Sciex QSTAR XL mass spectrometer fitted with a nano-ESI emitter in positive ion mode.
Figure C.2. MS/MS of N-(5-carboxypentyl)-N,N-dimethylbenzammonium bromide salt (21) at m/z 236.1.
Spectrum was obtained on a Sciex QSTAR XL mass spectrometer fitted with a nano-ESI emitter in positive ion mode.
C.2 Mass spectra of MADs containing a fixed permanent charge (MAD$^+$)

Figure C 3. Mass spectrum of $N$-benzyl-6-(2,4-dioxo-1,5-dioxaspiro[5.5]undecan-3-yl)-$N,N$-dimethylhexan-1-aminium bromide (26) obtained on a Sciex QSTAR XL mass spectrometer fitted with a nano-ESI emitter in positive ion mode.
Figure C 4. MS/MS of N-benzyl-6-(2,4-dioxo-1,5-dioxaspiro[5.5]undecan-3-yl)-N,N-dimethylhexan-1-aminium bromide (26) obtained on a Sciex QSTAR XL mass spectrometer fitted with a nano-ESI emitter in positive ion mode.
Figure C 5. Mass spectrum of N-benzyl-6-(2,4-dioxo-1,5-dioxaspiro[5.5]undecan-3-yl)-N,N-dimethylhexan-1-aminium bromide (26) crude material performed with excess DBA. Spectrum was obtained on an AB Sciex 4000 QTRAP hybrid triple quadrupole linear ion trap mass spectrometer equipped with an online nanoESI ionization source via direct injection. Performed EMS scan with a declustering potential of 40 eV.
Figure C 6. MS/MS of N-benzyl-6-(2,4-dioxo-1,5-dioxaspiro[5.5]undecan-3-yl)-N,N-dimethylhexan-1-aminium bromide (26) crude material performed with excess DBA. Spectrum was obtained on an AB Sciex 4000 QTRAP hybrid triple quadrupole linear ion trap mass spectrometer equipped with an online nanoESI ionization source via direct injection.
Figure C 7. Mass spectrum of 6-(2,4-dioxo-1,5-dioxaspiro[5.5]undecan-3-yl)-N,N,N-trimethylhexan-1-aminium bromide (27) crude material performed with excess DBA. Spectrum was obtained on an AB Sciex 4000 QTRAP hybrid triple quadrupole linear ion trap mass spectrometer equipped with an online nanoESI ionization source via direct injection.
C.3  Mass spectra of ketene reactions with MAD$^+$

Figure C 8. Mass spectrum 50-1000 Da of N-benzyl-8-(3,5-di-tert-butyloxy)-N,N-dimethyl-8-oxooctan-1-aminium bromide (30). Spectrum was acquired on an AB Sciex 4000 QTRAP hybrid triple quadrupole linear ion trap mass spectrometer equipped with an online nanoESI ionization source via direct injection.
Figure C.9. Mass spectrum 50-500 Da of \(N\)-benzyl-8-(3,5-di-tert-butylphenoxy)-\(N, N\)-dimethyl-8-oxooctan-1-aminium bromide (30). Spectrum was acquired on an AB Sciex 4000 QTRAP hybrid triple quadrupole linear ion trap mass spectrometer equipped with an online nanoESI ionization source via direct injection.
Figure C 10. MS/MS of N-benzyl-8-(3,5-di-tert-butylphenoxy)-N,N-dimethyl-8-oxooctan-1-aminium bromide (30). Spectrum was acquired on an AB Sciex 4000 QTRAP hybrid triple quadrupole linear ion trap mass spectrometer equipped with an online nanoESI ionization source via direct injection.
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