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Self-immolative linkers for chemical amplification application

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Self-immolative linkers for chemical amplification application

by

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CHAPTER 1: INTRODUCTION

Molecules are increasing in complexity in order to gain a wide variety of functionality useful for many applications. Many challenges accompany these increasing complexities, particularly synthetic challenges. Old synthetic methods often have to be used in innovative new ways to gain success. Increasing chemical complexity, and meeting the challenges of higher difficulty synthesis, has the potential for exciting and rewarding payouts.

Chemical machines or molecular machines are a class of molecule, often with many complex "moving" parts that have high functionality.[1-5] Like a mathematical function, a chemical machine has the ability to transform an input into an entirely different output. In the case of chemical machines, this is usually through a quasi mechanical movement that are easy to imagine in a macro-world and that often emulate the form of macro machines.[6] The ability of chemical machines to do complex tasks makes the challenge of difficult target molecule synthesis one worth pursuing.

One example of a chemical machine is a chemical amplifier. These chemical amplifiers are structures that translate a single bond-breaking event into release of numerous chemical outputs. In this way, a single bond cleavage input reaction (e.g. a reaction triggered by an analyte, a photon, or an enzyme) can be translated into the release of numerous output chemical cargoes.[7-11] Outputs can take the form of reporting molecules (e.g. fluorescent dyes), biomolecules, or drugs. This kind of chemical amplifier has numerous applications in chemical sensing, in drug delivery and a variety of other highly sophisticated tasks, especially when combined with other chemical functionalities as part of increasingly complex chemical machines.

We investigated a chemical amplifier based on hexaesters of mellitic acid. This class of molecule is difficult to synthesize and transform, requiring a variety of techniques. Because of the synthetic challenges, careful selection of a target was investigated. A self-immolative linker system, based on anhydride formation of a 1,2 carboxylic acid of a six member ring, was chosen to be the target of interest because of a number of reasons: benign byproducts, availability of starting materials, and kinetics of release. An unprecedented oxidative aromatization was used to synthesize these mellitic acid hexaesters.

CHAPTER 2: OXIDATIVE AROMATIZATION

Introduction:

In the course of attempting to synthesize chemical amplifiers, several unexpected synthetic challenges were encountered. Most notably, direct esterification of mellitic acid proved difficult. Although mellitic acid could be esterified to the hexamethyl ester derivative using standard ester coupling reactions, in the case of esterification with phenols dozens of coupling conditions were unsuccessfully attempted. To overcome the synthetic difficulty of a direct esterification, we considered a synthetic approach involving synthesis of non-aromatic esters that could then be transformed to the desired aryl esters. One approach that we considered was to esterify cyclohexane hexacarboxylic acid, and then aromatize the aliphatic cyclohexane ring to the heavily substituted benzene. However, no such reaction exists in literature.

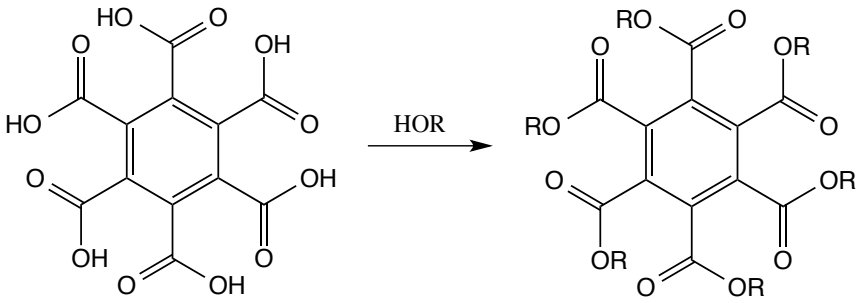


Figure 1: Mellitic acid direct esterification attempts, all of which proved unsuccessful, when R=an aromatic ring or system.

Aromatization of a cyclohexane is not a trivial transformation often requiring expensive catalysts or electrochemical techniques, and, because of expense, is not suitable to larger scale synthesis and lack of diversity of substituents that can be attached

to the ring.[12] We found that if the molecule is sufficiently substituted with certain carboxylate groups, however, the cyclohexane can be oxidized to the aromatic derivative on treatment with phosphorus pentachloride. Depending on work-up conditions, aromatic esters or aromatic carboxylic acids could be prepared in this fashion. Initial investigations and intuition lead us to believe that the reaction is undergoing a Hell-Volhard-Zelinsky (HVZ) type reaction to form alpha halogenated compounds. After halogenation, the molecules can undergo beta elimination to generate the aromatic compound.

The HVZ reaction prepares alpha-halogen carboxylic acids and has been well known for over a century.[13-15] Since, in the traditional variation of the HVZ reaction, an alpha-halo acyl halide intermediate is generated, alpha-halo esters, thioesters or amides can readily be prepared through this route.[16] In general, the HVZ reaction requires relatively harsh conditions with temperatures in excess of 100 °C and long reactions times.[17, 18] Elimination happens fairly readily following the halogenations, particularly at higher temperatures.[19] That makes this particular technique attractive for highly functionalized aromatic esters synthesis. However, no literature precedence exists for use of the HVZ reaction, followed by same pot elimination, in order to create aromatic molecules.

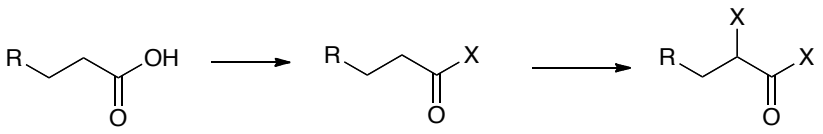


Figure 2: A traditional HVZ reaction scheme

Computational Methods:

In order to gain insight into the feasibility of the elimination reactions that might occur following alpha halogenation, computations were carried out to assess the pK_a values of the cyclohexane haloesters.

All the molecular geometries of the electronic states of all molecules were optimized under the DFT level of theory using the B3LYP functional and the 3-21+G* basis set.[20] The stationary points were found to have zero imaginary frequencies, and all energies contain a correction for the zero-point energy. All the single-reference computations were computed with Gaussian03/09.[21] The hybrid B3LYP functional used consists of the Becke 3-parameter exchange[22, 23] functional with the correlation functional of Lee, Yang, and Parr [24]. This and related DFT functionals have been shown to give quite reasonable geometries for ground state molecules.[25-27] A polarizable continuum model (PCM) was used to approximate solvent conditions in DMSO.

The pK_a calculations followed the proton exchange or relative method. The general approach of the proton exchange scheme is to consider some acid/base reaction with a reference molecule.[28] The relative pK_a values used were in DMSO and set to pyridine/ pyridinium acid/base pair. Choosing pyridine as the reference base may, however lead to some degree of error because it is a nitrogen based base compared to the carbon acids that we are investigating. Furthermore, since the majority of reactions were done in aprotic solvents, explicit molecule shells were not considered. However, an advantage of the relative method is an inherent cancelation of errors that makes this approach reasonable.

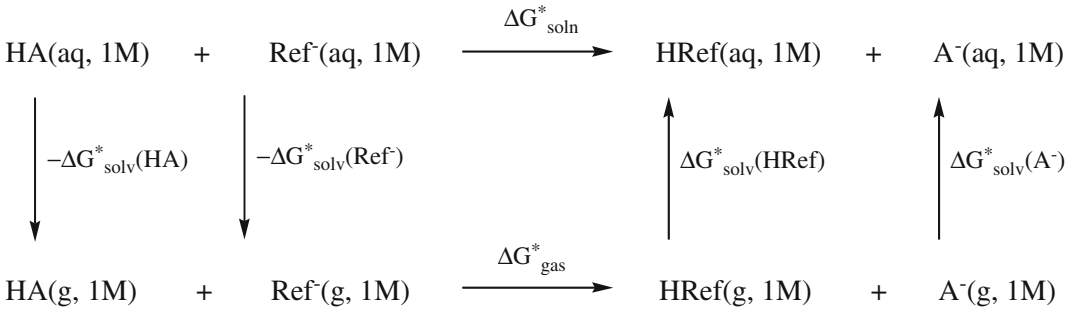


Figure 3: The thermodynamic cycle considered for the relative method of determining pK_a

The pK_a could be calculated by the Gibbs free energy equation shown in Figure 3. To calculate pK_a the following relations were applied:

$$pK_a = -\log K_a \qquad (1)$$

$$\Delta G_s = -RT \ln K_a \qquad (2)$$

$$pK_a = \frac{\Delta G_s}{RT \ln(10)} \qquad (3)$$

Final pKa calculations are based on the following modification of equation 3:

$$pK_a = \frac{c_1(G_s(A^-) - G_s(AH))}{RT \ln(10)} + c_2 \qquad (4)$$

Where c_1 is the slope of the standardized pK_a graph (figure 3) and c_2 is the experimental value of the pK_a of pyridine in DMSO. By plotting several molecules with known pK_a's we can get not only the c_1 and c_2 values, but also an idea of the accuracy of this method. The r^2 value of our data (0.9949) with a wide variety of carbon based acids give a good

indication that our method is valid. The plot below uses the reference molecules of: 2, 4-pentadione, propane, 2-methylpropane, phenol, acetic acid, benzoic acid, cyclohexane, cyclohexanone, propene, 1,4-pentadiene, cyclopentane, hydrochloric acid, nitrous acid, and p-nitro benzoic acid primarily using the acidities compiled by Bordwell in DMSO.[29] The results of these pK_a studies are listed below in the "pK_a results and discussion" section.

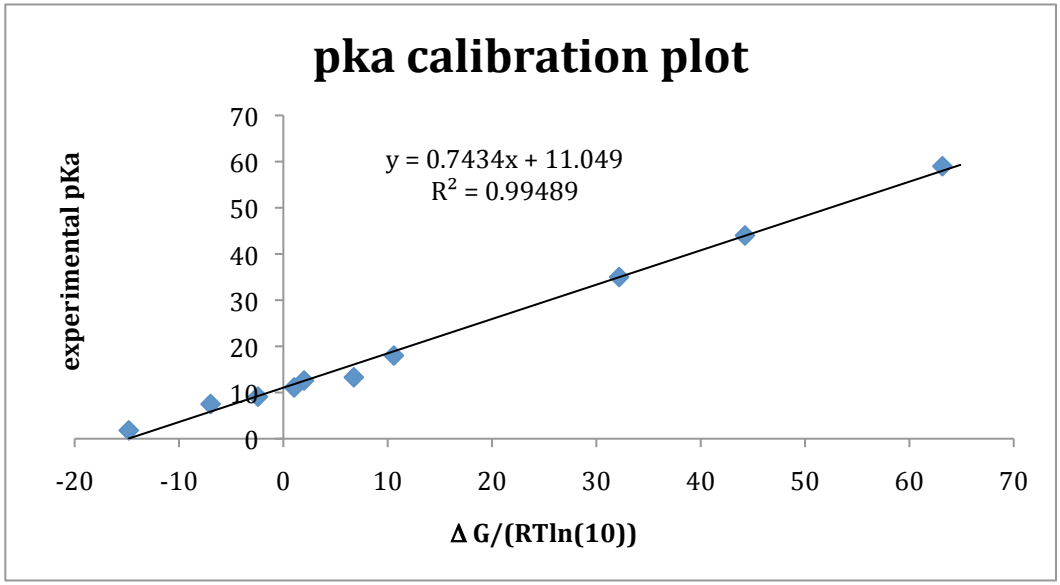


Figure 4: pKa calibration plot for fitting calculated pKa's using the relative method

Experimental:

HVZ aromatizations are still being optimized but below are shown conditions that give the listed product:

General procedure for synthesis of benzene derivatives:

To the carboxylic acid or ester substituted cyclohexene was added phosphorus pentachloride (equivalents dependant on number of functional groups) and the reaction was heated to 120-140 °C for 1-4 hours. Alcohol or water was added in large excess to give either the carboxylic acid or ester. Pyridine was added dropwise until formation of pyridinium chloride salt was no longer observed. Typical work-up of esters was dilution with methylene chloride and washing with copious water. Concentration of organic layer gave a solid suspended in viscous oil in most cases. Filtration with a fine borosilicate glass fritted filter followed by recrystalization in THF/hexanes yielded pure product. For full synthetic procedures, see chapter 7.

Results and discussion:

The compounds shown below, in figure 5, were all successfully synthesized by the oxidative aromatization method, although not all of them have been purified in good yield; as of yet, structural determination is limited solely to ^{1}H-NMR. This set of reactions begins to probe at the overall diversity of this reaction.

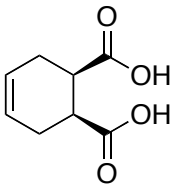
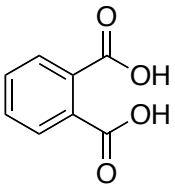
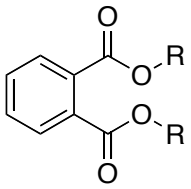
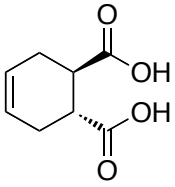
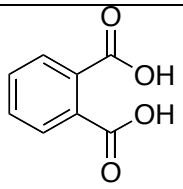
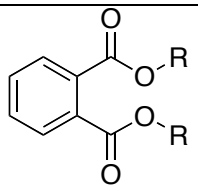
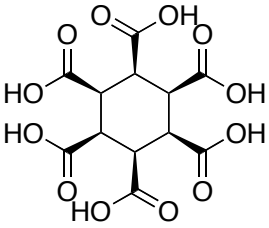
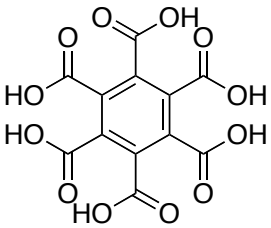
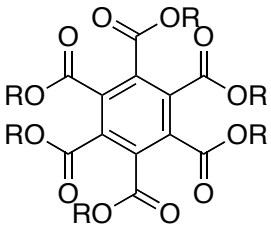
Starting Material	Acid workup	Alcohol Workup
		
		
		

Figure 5: Successful oxidative aromatization performed so far. The alcohol work-up can yield either aromatic or aliphatic esters.

The key to our reaction scheme is the source of chlorine. Esterifications of the cyclohexane carboxylic acids investigated in this experiment could be done by first producing the acid chloride with thionyl chloride, but with this reagent, no aromatic product was formed. However, when an excess of PCl_5 is used, some aromatic product is formed. In fact, even when 1 or 2 equivalents amount of PCl_5 is used, some aromatic product is formed, although yield suffers. It is known that PCl_5 exists in equilibrium with PCl_3 and chlorine at higher temperatures.[30] The supposition of the source of chlorine being from the PCl_5 is consistent with our finding that, in most cases, temperatures in

excess of 120 °C are required for this reaction to proceed. However, temperatures in excess of 140 °C seem to cause some unwanted degradations, so increasing the temperature beyond this point does not give greater yield.

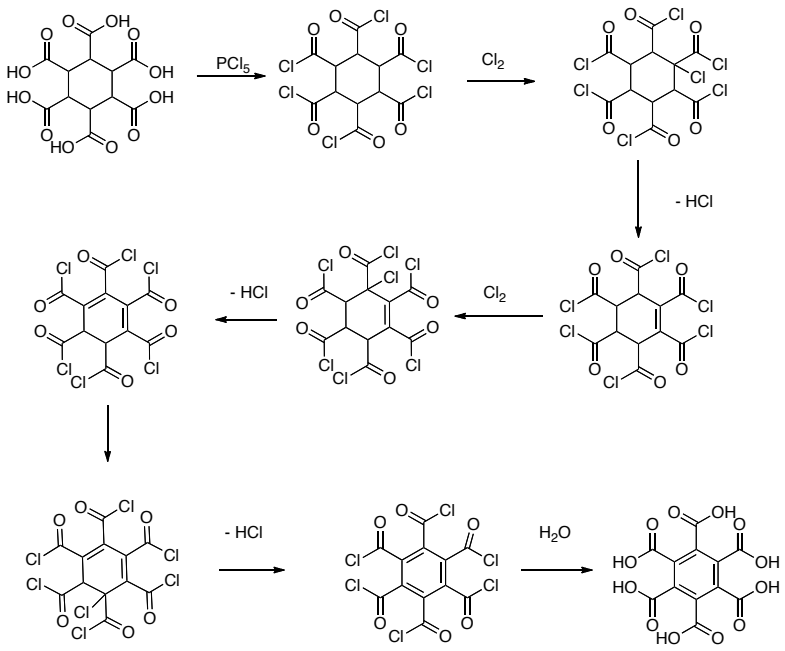


Figure 6: Proposed synthetic pathway of formation of mellitic acid. In the case of the hexacyl chloride, the chloride ion is a strong enough base to drive the reaction

The dimethyl phthalate that was synthesized with our oxidative aromatization did show some product yielded with temperatures never exceeding 90 °C, but this reaction seems to be the exception and our intial investigations indicate that yields are higher at 120 °C than 90°C. Even though the cyclohex-4-ene-1,2-dicarboxylic acid did react with PCl_5 at room temperature, the esterification gave no aromatic product under these conditions. For the hexacarboxylic acid cyclohexane derivative, the reaction with PCl_5 did not take place until 120 °C, so it is difficult to draw conclusions on the effect of lower

temperatures on the reaction, but so far it appears that higher temperatures give more favorable results up to degradation temperatures.

Phosphorus oxychloride ($POCl_3$) is a side product of the reaction that was intentionally not distilled off before addition of alcohol or water. On addition of water, the phosphoryl chloride reacts and becomes phosphoric acid, which is easily removed. On addition of alcohols, when the ester products are desired, a phosphate ester is produced. This phosphate ester is useful in the work-up since the product is insoluble in the phosphate ester oil, which was yielded on concentration of methylene chloride. This makes filtration a viable and easy purification at the end of the reaction.

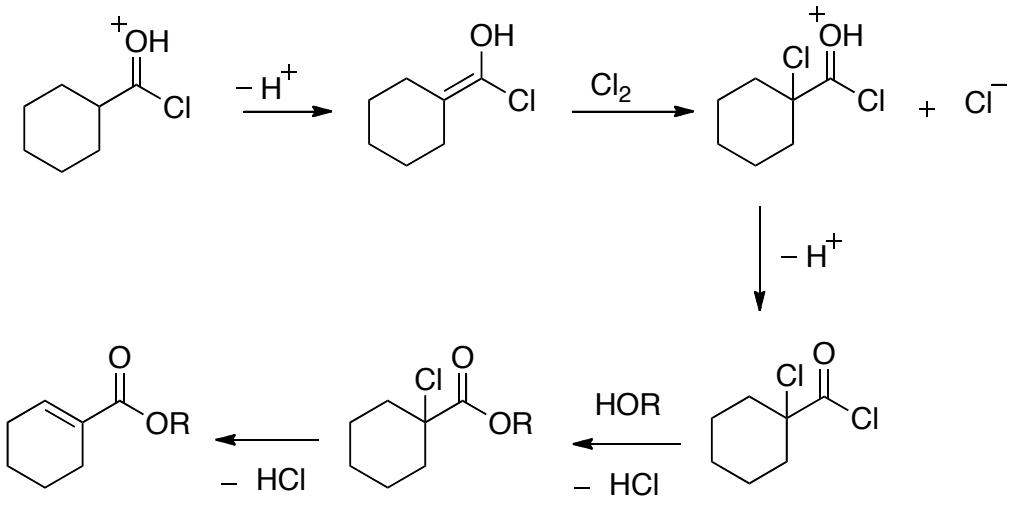


Figure 7: Possible scheme for oxidative HVZ reactions to form esters.

Optimizations are currently being done focusing on the 1,2,3,4,5,6 hexacarboxylic acid cyclohexane because this has the most potential utility for use as an amplifier. Thus far, the only phenols that have been used to successfully create the hexaester have been

phenol and p-methoxy phenol. Aliphatic esters have been shown to be able to be synthesized using the oxidative aromatization as well, however.

Mellitic acid can be reacted with phosphorus pentachloride at very high temperatures to yield the acyl chloride. On addition of the alcohol or alcohol/catalytic pyridine, however, the ester is not produced. This suggests that the esterification happens before the elimination of the chloride as shown in Figure 7.

It is possible that the reaction is not solely undergoing HVZ for chlorination. If the hexaester of the saturated cyclohexane is treated with PCl_5 and subjected to high temperatures, for an extended period, some aromatic product is formed. It could be that the chlorination can proceed through a radical mechanism, since HVZ type halogenation is unlikely with the ester (see pK_a study below). These yields are low, however, and require even harsher conditions (140-180°C and 12 hours). Therefore we do not believe that this is the predominate pathway, but rather a competing reaction.

pK_a results and discussion:

The pK_a data was computed as described above and this data are summarized in Figure 9. Given the low computed pK_a's, it seems reasonable to expect that elimination can happen readily with even fairly mild conjugate bases. Obviously, the deprotonation barrier of the beta hydrogens are important for rate of elimination and for determining under what conditions the elimination will happen. Less obviously, the low pK_as are important for understanding the initial halogenation. If HVZ halogenation happens to the enolate form of the carboxyl derivatives, having fairly acidic protons indicate low barriers for the tautomerization. This is why it is generally accepted that the HVZ

reaction happens more readily with the acid chloride than with the ester or carboxylic acid.[31] Since our system is particularly acidic, the enol formation must also be investigated and such studies are forthcoming.

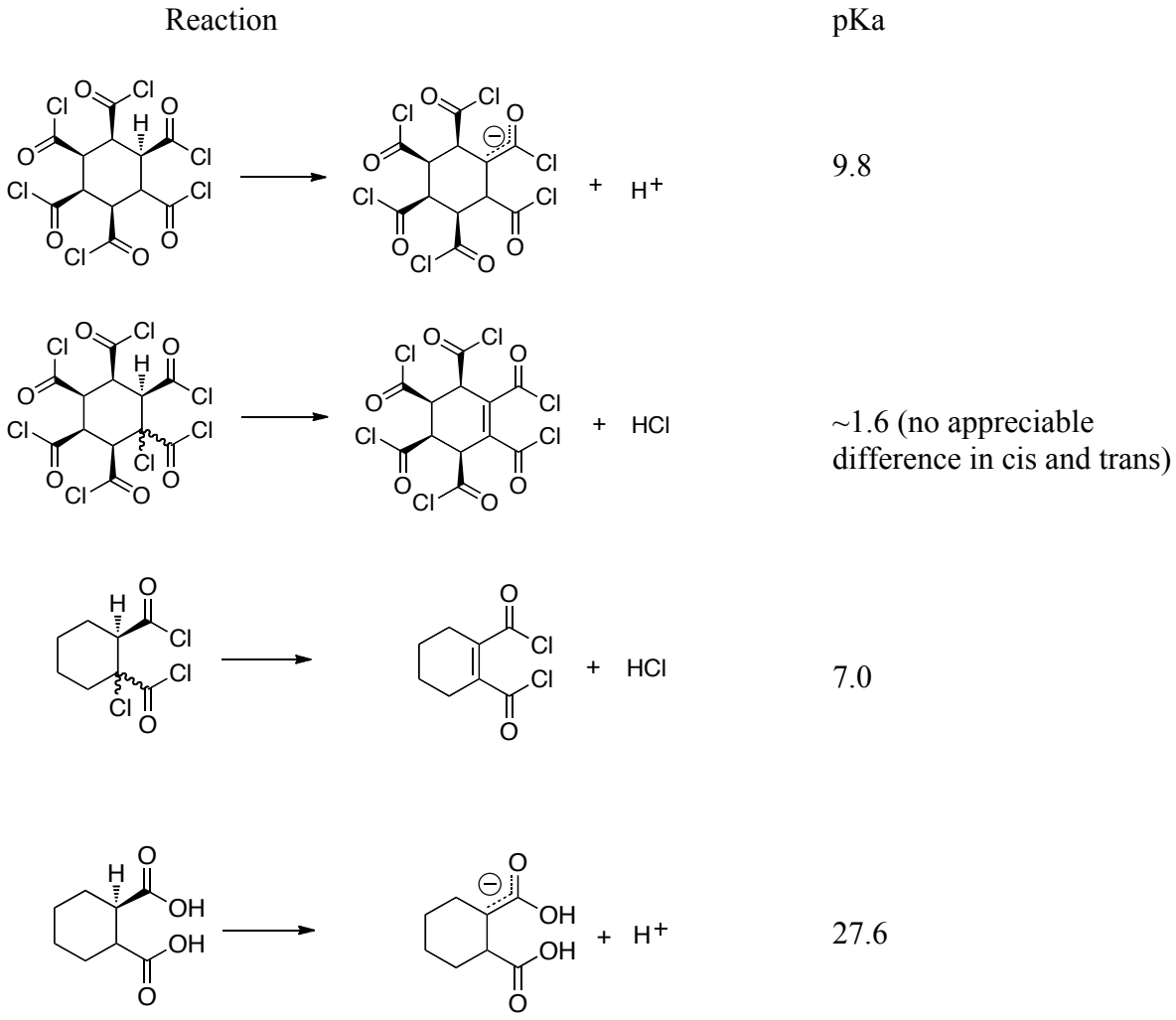


Figure 8: some of the pK_a calculated in this study

It is also noteworthy that the more substituted the cyclohexane is, the lower the pK_a. It is still unclear as to why this is, however, it is possible that this is because there is

significant axial strain that is being relieved by the deprotonation. This is in keeping with experimental results of epimerization being rapid in the case of the hexasubstutited cylohexanes. Unsurprisingly, the more unsaturated the compound is, the lower the pK_a's are. A possible consequence of this is that the reaction may be accelerating toward aromatization. Therefore, even if the initial halogenation of the HVZ may be disfavored, this reaction may proceed anyway, especially since we expect the aromatic product to be significantly more stable.

Conclusions:

Several ester substituted aromatic compounds were synthesized and characterized by NMR and mass spectrometry to show the successful oxidative aromatization. The HVZ mechanism, followed by elimination is consistent with our results and is thus the suggested mechanism, although further computation detail should be considered for a more conclusive reaction scheme and mechanism. Most of these reactions are low yielding, so the synthetic utility is limited to situations where synthesis would otherwise be difficult.

The phenolate esters were not able to be prepared from the direct esterification of mellitic acid, showing the HVZ aromatization to be useful for creating highly esterified benzene derivatives. This is particually useful for our purposes of synthesizing our amplifier. The scope and limitations of this reaction are still being investigated with particular attention being paid to increasing reaction yields.

CHAPTER 3: SELF-IMMOLATIVE CHEMICAL LINKER

Introduction:

Many properties of pharmacological drugs can be improved with the careful implementation of proper drug delivery systems.[32] One area where improvement could be used is the chemical linker by which the payload is delivered.[33] The ideal chemical linker should have a switch that, when activated, releases a chemical payload or cargo and meet certain criteria for practical use.[34] For our purposes, the linker is subject to the following criteria: kinetics faster than those previously reported (τ<1 hour), stable in water for long enough for the payload to be delivered (τ>1 day), with benign byproducts, and a synthetic scheme that would allow for placement of drug or reporter molecules as a payload. Proper selection of the chemical linker could then be used in more advanced molecules and molecular machines, such as a chemical amplifier.

Self-immolative linkers have become indispensible molecules for connecting a cleavable mask to an output cargo molecule.[35-37] Upon an input reaction that cleaves the mask, self-immolative linkers release their output cargo, and the molecule "self-destructs" into harmless byproducts. Self-immolative linkers have proven to be extremely useful in enzyme-activated prodrugs,[38-43] chemical sensors,[44-46] traceless linkers,[47-50] biological probes,[51-54] and degradable polymers.[55-57] Released chemical cargoes are often biomolecules, drugs, or reporters such as fluorescent dyes. Linker structure can aid prodrugs by improving stability, solubility, biodistribution, pharmacokinetics, bioavailability and activation.

The ideal self-immolative linker is simple, stable, compatible with water, and transforms into a benign byproduct upon releasing the output cargo. Furthermore, such linkers should be easy to synthesis, readily adaptable to a variety of inputs and outputs, and quickly release the output cargo upon the input reaction. In particular, some common self-immolative linkers suffer from slow release of their output cargo. New linkers that incorporate these desirable features would be highly useful.

Experimental:

Phenyl hydrogen phthalate, was synthesized according to a known procedure.[58] Synthesis of both cis and trans- 2-((p-methoxyphenoxy)carbonyl)cyclohexanecarboxylic acid and p-methoxy phenyl hydrogen phthalate were prepared by adapting the method described in literature.[58] Phenyl hydrogen phthalate and p-methoxy phenyl hydrogen phthalate had ^{13}C-NMR and ^{1}H-NMR in good agreement with literature values.[34] The cis and trans cyclohexane 1,2 p-methoxy phenolate carboxylate had ^{13}C-NMR and ^{1}H-NMR in good agreement with expected values. For full synthetic procedures, see chapter 7.

Kinetics:

Kinetic experiments were performed using the Agilent 8453 UV-Visable spectrometer and plotted using Kaleida graph vesion 4.1.1 plots were fitted to find the rate constant (k) or $1/k=\tau$. Phthalic anhydride was monitored at 300 nm, phenol was monitered at 270 nm and p-methoxy phenol was monitored at 285. All studies were done at 10 mM concentrations in phosphate buffered solutions prepared with ultra pure water.

Computational methods:

All the molecular geometries of the electronic states of all molecules were optimized and transition states were found under the DFT level of theory using the B3LYP or M06-2X functionals and the 3-21+G* or 6-311++G* basis set.[20] The stationary points were found to have zero imaginary frequencies, and all energies contain a correction for the zero-point energy. All the single-reference computations were computed with GAMESS suit.[21] The hybrid B3LYP functional used consists of the Becke 3-parameter exchange[22, 23] functional with the correlation functional of Lee, Yang, and Parr. [24] This and related DFT functionals have been shown to give quite reasonable geometries for ground state molecules.[25-27] The M06-2X is a meta hybrid GGA functional with double exchange energy (54% hartree fock exchange energy) that has been shown to be a very good functional for main group elements and kinetics. Polarizable continuum model (PCM) was used to approximate solvent conditions in water. Further solvent approximations were attempted but were too computationally expensive to be studied to satisfaction.

Results and Discussion:

There are three parts to the selected linker: the trigger, the base system, and the payload released. The phenyl hydrogen phthalate has been shown to be a good candidate for a system and is the metric to which other linkers are compared. Shown below is the proposed mechanism, that was computationally investigated. pH was considered by protonating in logically consistent ways for neutral pH systems. Given this mechanism, we would expect, and we observe, a pH dependence on the system.

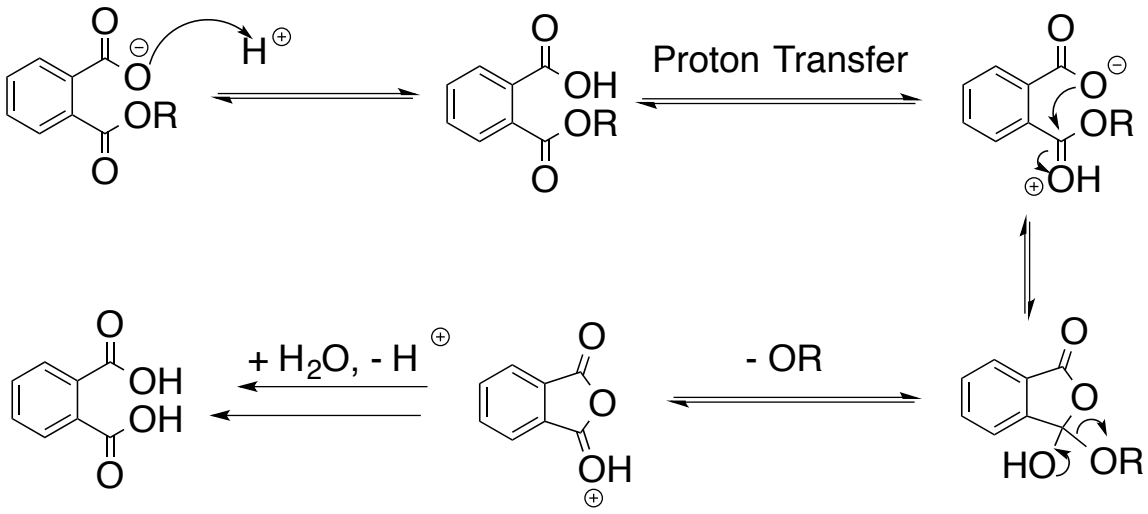


Figure 1: Reaction mechanism for carboxylate assisted release of alcohol

Cargo:

The ultimate payload release should have some sort of functionality or emulate some functionality. However, for our purposes of a proof of concept study, any alcohol that is easily observable and that demonstrates appropriate kinetics could be considered. Aliphatic alcohols were for the most part not extensively explored because of the known slow reaction times, even in the hydrogen phthalate case.[59] Molecules useful for chemical sensing, such as coumarin and coumarin derivatives have been shown to be appropriate, particularly in the hydrogen phthalate case[34], but are not discussed here. In particular, phenol and the phenolic derivative p-methoxy phenol were used. Phenol serves as a good analogue because several drugs contain a phenolic alcohol group that could be used as the esterified alcohol in our linker system. P-methoxy phenol was used

to show that even with some electron donating character (which we expect to, and was observed to, slow down the reaction), phenolic esters could still be useful.

After synthesis of p-methoxy phenolate hydrogen phthalate and phenyl hydrogen phthalate, the two alcohols could be compared for potential use. In the p-methoxy phenol case, a UV-wavelength of 285 was selected because it is conveniently far from any other species' wavelength, thus the kinetic plots show only growth that corresponds to the alcohol. The anhydride formation was not observed because, unlike the aromatic case, the aliphatic cyclohexanes do not strongly absorb UV-light. For the phenol case, phenol has a UV-spectrum that overlaps with the phthalic ester, therefore the formation of the anhydride was the species observed, as the anhydride absorbs at a sufficiently different wavelength than any other species. Thus we can see the release of phenol (growth in the plot corresponding to the formation of the anhydride) and the anhydride ring opening to the pthalic acid (decay of the curve).

In both instances it can be shown that the release is pH dependent. For both phenol and p-methoxy phenol, neutral pH (7.0 phosphate buffer) showed faster kinetics for release of the payload than lower pH (5.1 phosphate buffer). This is consistent with the computational data observation that the protenation accompanied with a lower pH raises the barrier of activation. In the case of the phenol, the release of the alcohol was so fast that we were unable to observe the kinetics at neutral pH, only the reopening of the anhydride was observed. Unsurprisingly the p-methoxy phenol was slower than the phenol, as ether groups (the para substituted methoxy) are electron donating, thus destabilizing the released phenolate.

It should be noted that amide linkers were investigated. However, amide linkers are slow to release the amine payloads at neutral pH. In very acidic conditions, (such as those that might be found in the stomach), the release kinetics were much faster. For the purposes of our chemical amplifier, the amide was not further considered since we deemed it to be unsuited to our criteria of appropriate kinetics at neutral pH. However, for other applications, amides may be considered, especially in the context of a pH sensitive linker.

Base system:

The hydrolysis of hydrogen phthalate is a classic case of neighboring group participation. The phenyl hydrogen phthalate has particularly been extensively investigated because of its fast kinetics of release in water at neutral pH, the mechanism of which has seen previous investigation.[60-63] It is also shown that the mechanism is pH dependant, such that the phenyl hydrogen phthalate is a shelf-stable compound when stored away from moisture, but this compound hydrolyzes rapidly in water at neutral pH. We discuss a more generalized case of the phthalate system with different chemical payloads and bases, as it pertains to potential use in further functionalized systems. It has been determined that the fast ester hydrolysis of this compound is a case of intramolecular catalysis wherein the neighboring carboxylate group displaces the alcohol to generate a water-unstable anhydride that in turn spontaneously hydrolyzes to phthalic acid. These factors make the phthalate system ideal for the fast release of a chemical payload in further functionalized systems.

Although the phthlalic acid system is a good candidate because of its kinetics, increasing the number of carboxylate groups on the ring system makes the aromatic base

system difficult to work with and transform because of the complex electronic and steric environment involved. A number of unpublished experiments were done demonstrating this fact, even with as few as three carboxylate groups. Because of this, cyclohexane-1,2-dicarboxylic acid was investigated for potential use in both the cis and trans forms.

We identify our target kinetics to have tau values of less than 1 hour at room temperature and under neutral conditions in order to be competitive with other published amplifiers. In the case of the trans base, the tau values were so high as to be difficult to obtain accurately in the timeframe we investigated for the p-methoxy phenol. When a better leaving group of phenol was used, the tau value was still 15,625 s^{-1} (see figure 2). Given this, it was determined that the trans-cyclohexanes carboxylic acid would be poor candidates for linkers since these values fell outside of the range of interest. The high tau values are likely a result of the trans-five member ring junction that would be required for the neighboring group participation required to kick off the phenolic alcohol.

The cis cyclohexane dicarboxylic acid was also investigated for potential use as a linker. The all cis-cyclohexane hexacarboxylic acid is commercially available and can be esterified through simple Fischer esterifcation methods, making it a convenient candidate. Additionally, the model studies conducted were promising from a kinetics point of view. The tau value of the phenol was 127 s^{-1} and the p-methoxy phenol was 692 s^{-1}. Both of these values were with the acceptable range for kinetic, even though they were considerably slower than the hydrogen phthalate case. Of concern is cis-trans epimerization with the cis cyclohexane carboxylic acid and its ester derivatives. At neutral conditions and low heat, epimerization is slow, but at either basic or acidic conditions, epimerization happens more readily to the less sterically strained trans

cyclohexanes. This is especially evident as the number of carboxylic acids on the ring increases. For purposes of use as an amplifier, the cis bases system was not investigated for this reason.

However, even though the cis base system may not be an ideal candidate for the use in an amplifier system, it may be useful for other applications. Particularly, if some binding event needs to happen before release, relatively slow hydrolysis-even after activation may be desirable. For drug delivery systems, this may be especially important. Such application is currently being investigated.

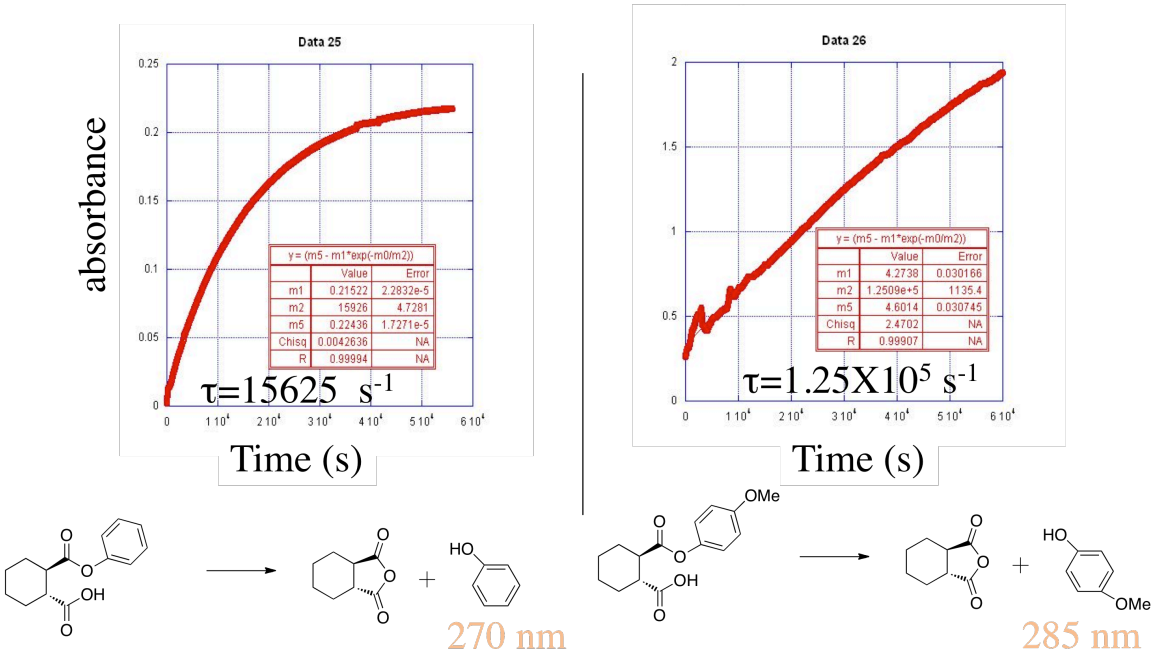


Figure 2: UV plots of the trans phenol (left) and trans p-methoxy phenol (right). Both are very slow, indicating that the trans may not be suitable for a base system.

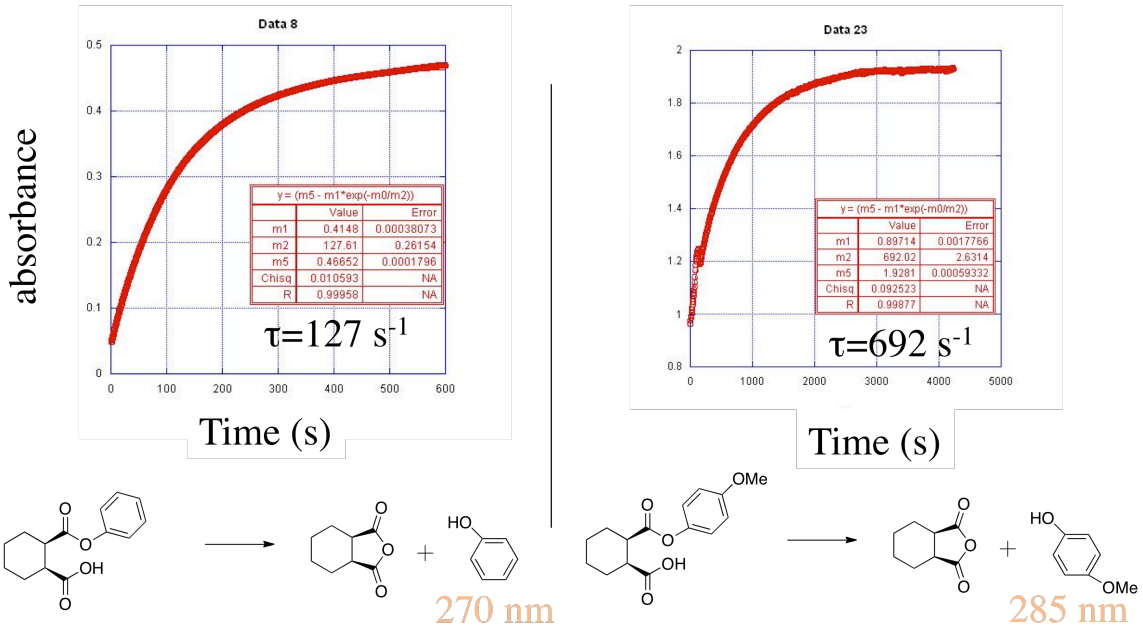


Figure 3: UV plots of the cis phenol (left) and cis p-methoxy phenol (right). Both are on a kinetics scale that makes them appropriate for use.

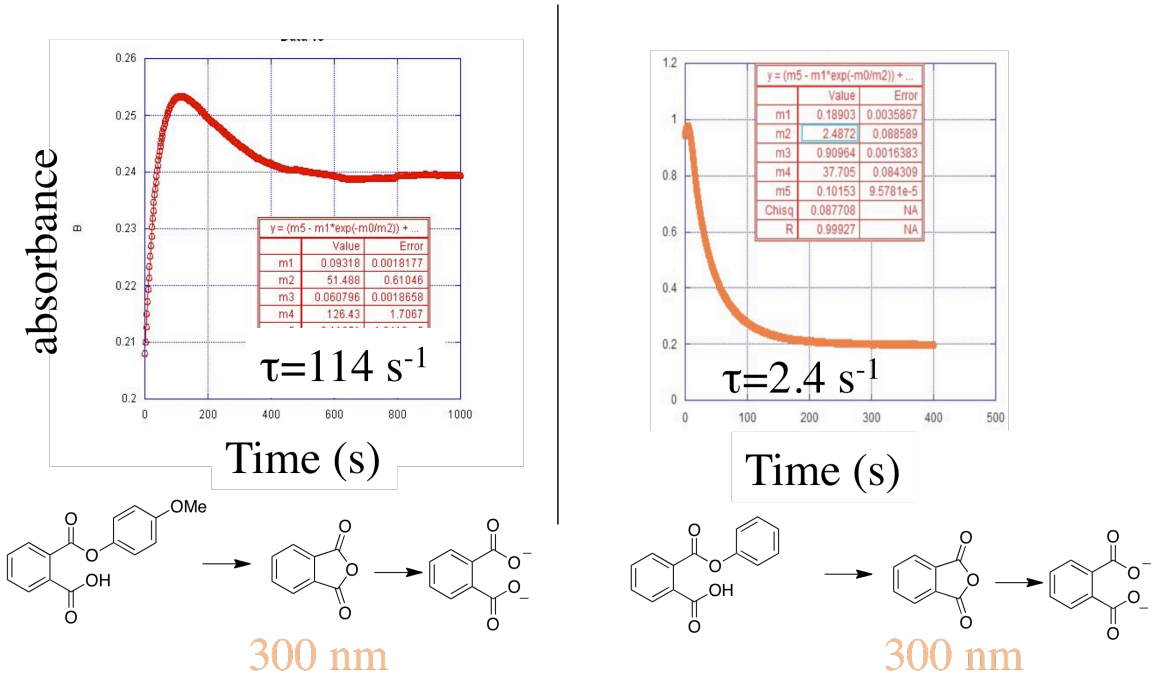


Figure 4: UV plots of the phtalate phenol (left) and phthalate p-methoxy phenol (right) Both are quick and very desirable for kinetics purposes.

Tau values (s^{-1})				
	pH7 Phthalic	pH 7 trans	pH 7 cis	pH 4 Pthalic
Phenol	2.4	125000	127	50 (pH 5)
p-methoxy phenol	50.2	16000	692	114
p-nitro aniline	Very high	Not measured	Not measured	347

Figure 5: A summary of the tau values of relevant kinetics (as determined by UV) in different pHs. Other, amide linkers were also investigated but were too slow to measure over the indicated pH ranges

The Trigger:

Two triggers that have been previously investigated have been 2-(trimethylsilyl) ethanol (TMSE) and 2-nitro benzyl alcohol.[34] The TMSE group is sensitive to the fluoride ion and the 2-nitro benzyl alcohol is sensitive to UV-light irradiation. Both triggers have proven to be compatible with the phthalic system and the triggers are not further discussed here. These two triggers serve mostly as a proof of concept.

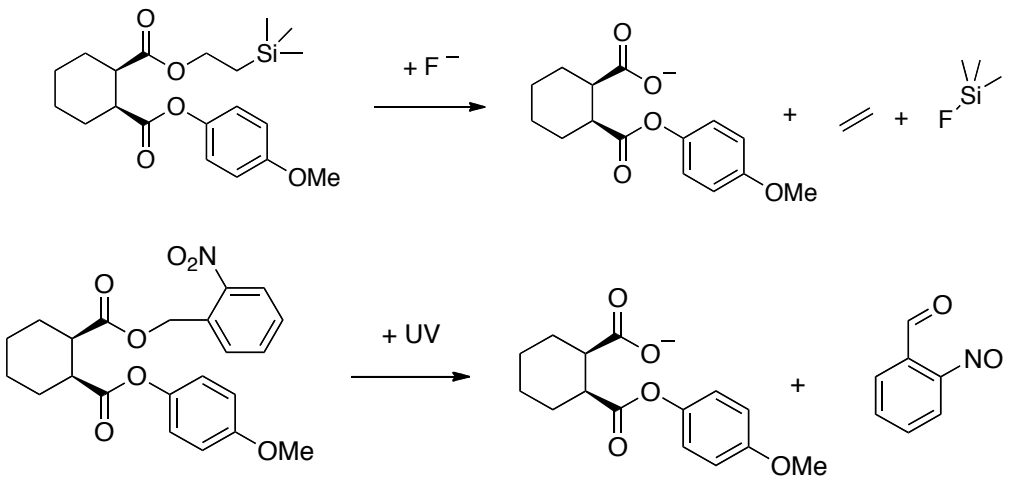


Figure 6:
Top: A TMSE trigger, activated by fluorine
Bottom: A 2-nitrobenzyl alcohol trigger, activated by UV-light

Conclusion:

Using a fluoride-sensitive 2-(trimethylsilyl)ethyl ester group to mask the catalytic carboxyl group, in combination with phenolic cargos, we find that aryl phthalate esters can indeed be exploited as self-immolative linkers. The saturated analogues of phthalic esters, cis and trans cyclohexane carboxylic acid, were also investigated. In the case of the cis, stability is an issue that must be considered when moving forward with more substituted ring systems. In the case of the trans, we did find this system to have many of the criteria for a good linker, however, the kinetics made it less than ideal for investigation for more functionalized chemical machines, particularly chemical amplifiers.

We show that these linkers can be synthesized easily starting from phthalic anhydride or cis and trans cyclohexane anhydride, cheap convenient starting materials in the manufacture of plastics, and "self-immolate" to ultimately yield biologically benign byproducts upon release of the phenolic outputs.

CHAPTER 4: SELF-IMMOLATIVE CHEMICAL AMPLIFIERS

Introduction:

A single molecule containing multiple functionalities is nothing new in chemistry. Total synthesis groups have been creating just such molecules for just about as long as the field of synthetic organic chemistry has existed.[64-66] However, using these functionalities in order to complete a specified task is still a field with many new exciting possibilities. One such possibility is the design of dendritic chemical amplifiers.

Dendritic systems have been investigated for chemical amplification with some previous success.[67-71] Unlike most dendritic systems that rely solely on the number of functional groups, dendrimers for chemical amplification also rely heavily on their structural relationship in order to undergo some transformation.[35] This transformation can then be used to do some useful "work". We can therefore define these dendritic amplifiers as chemical machines capable of performing tasks such as chemical gating, signal amplification (taking one signal and transforming it into another, stronger signal) or drug delivery.[35, 70, 72]

The existing systems studied are limited in scope of what they are able to release, as they require specific molecules to act as triggers and specific linking systems. We propose a proof of concept of an amplification system that can accommodate a variety of prodrugs and have precisely controlled kinetics of release. Based on the discussion in chapter 3, we determined that mellitic acid with aromatic leaving groups as analogues for potential prodrug payloads with a single trigger attached would be the ideal target.

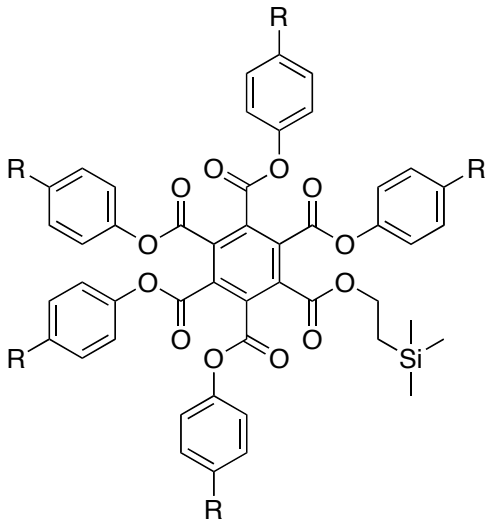


Figure 1: Target molecule where R=H, OMe or NO_2

Mellitic acid provides several challenges as a base system, however, particularly synthetically. Mellitic acid cannot be easily esterified, even with the use of common coupling reagents, because of complex electronics and high steric hindrance.[33] Two techniques that have been shown to be successful in the synthesis of these systems are the cyclotrimerization of diester butynes and the previously discussed oxidative aromatization.[73] Both the cyclotrimerization and oxidative aromatization synthetic methods are discussed below for use as potential amplifier synthesis pathways.

Experimental:

Syntheses of the benzene hexaesters were prepared via the oxidative aromatization as described in chapter 2. Transesterifications were done under high pressure and with increased temperatures to give modest yields. Cyclotrimerization was done catalytically with a bis-allyl ruthenium (IV) precatalyst that showed good tolerance for sterically hindered butyne diesters.[73] Stoichiometric cyclotrimerizations were done

with a zirconacyclopentadiene reagent using a modified literature preparation.[74] For exact synthetic procedures, see chapter 7.

Results and discussion:

We were able to successfully synthesize the hexakis benzyl esters using two different methodologies, each with its own advantages. For the cyclotrimerization synthetic route, a dicarboxylate butyne would give the desired benzylic system. This was successfully done in high yields to give hexakis (4-methoxy phenyl) benzene-1,2,3,4,5,6-hexacarboxylate. Although the catalytic cyclotrimerization gave good yields and nearly pure product, (figure 2) they are limited by the fact that only the homosubstituted esters could be created, or at least with no selectivity in the hexaester system. Using this method, it would be difficult to synthetically dictate where a single trigger molecule would be placed on our system without having multiple trigger substitutents . We have also shown that with a stoichiometric cyclotrimerization agent, selectable substituted benzenes hexacarboxylates could be synthesized (Figure 3). Unfortunately this synthetic pathway is limited by the dicarboxylate butynes synthesized. So far the only dibutyl carboxylates synthesized are the methyl, ethyl, and p-methoxy derivatives. The synthesis of the dicarboxylate butynes is a several step synthesis, which makes creating a library of suitable compounds more difficult. This reaction path does add possible versatility, however, and is still being investigated with different ester groups.

Also considered was making benzylic ethers and then oxidizing to the appropriate esters. Although the benzylic ether could be fairly simply synthesized by the William

ether sythesis, the oxidation to the esters did not readily happen. Again this indicates that the electronics and/or sterics of the hexa-substituted benzyl esters are complicated.

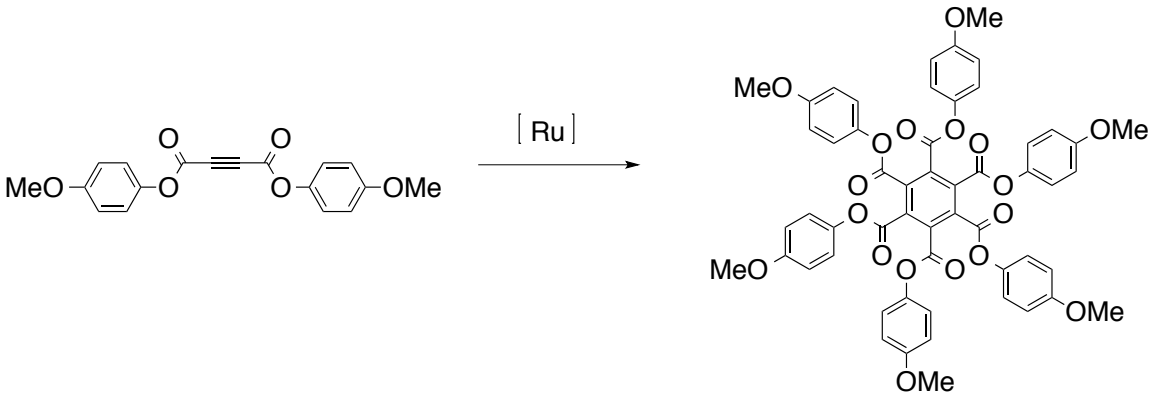


Figure 2: Catalytic cyclotrimerization demonstrating the ability of cyclotrimerization to produce sterically hindered hexaesters

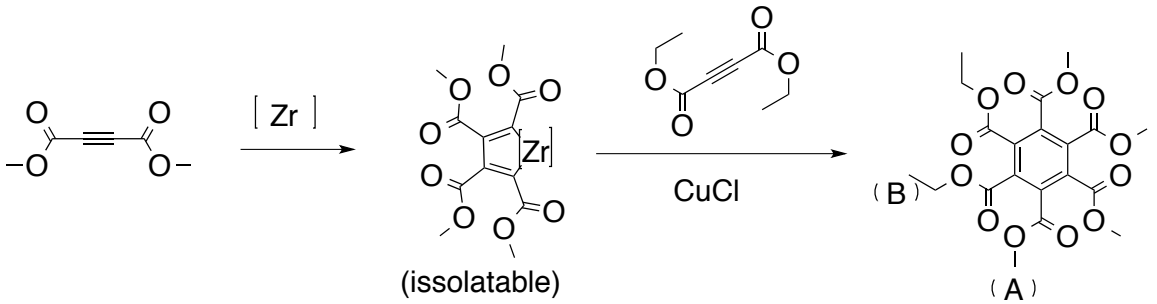


Figure 3: Cyclotrimerization using stoichiometric zirconium reagent that gives selective substitution

As discussed in chapter 2, hexester systems could also be prepared by our oxidative aromatization technique. Since this is a one pot synthesis, a wider variety of molecules could be synthesized much quicker than with the cyclotrimerization

techniques. Like the catalytic trimerization, however, selectivity is difficult in this synthetic pathway, without previously functionalizing our system.

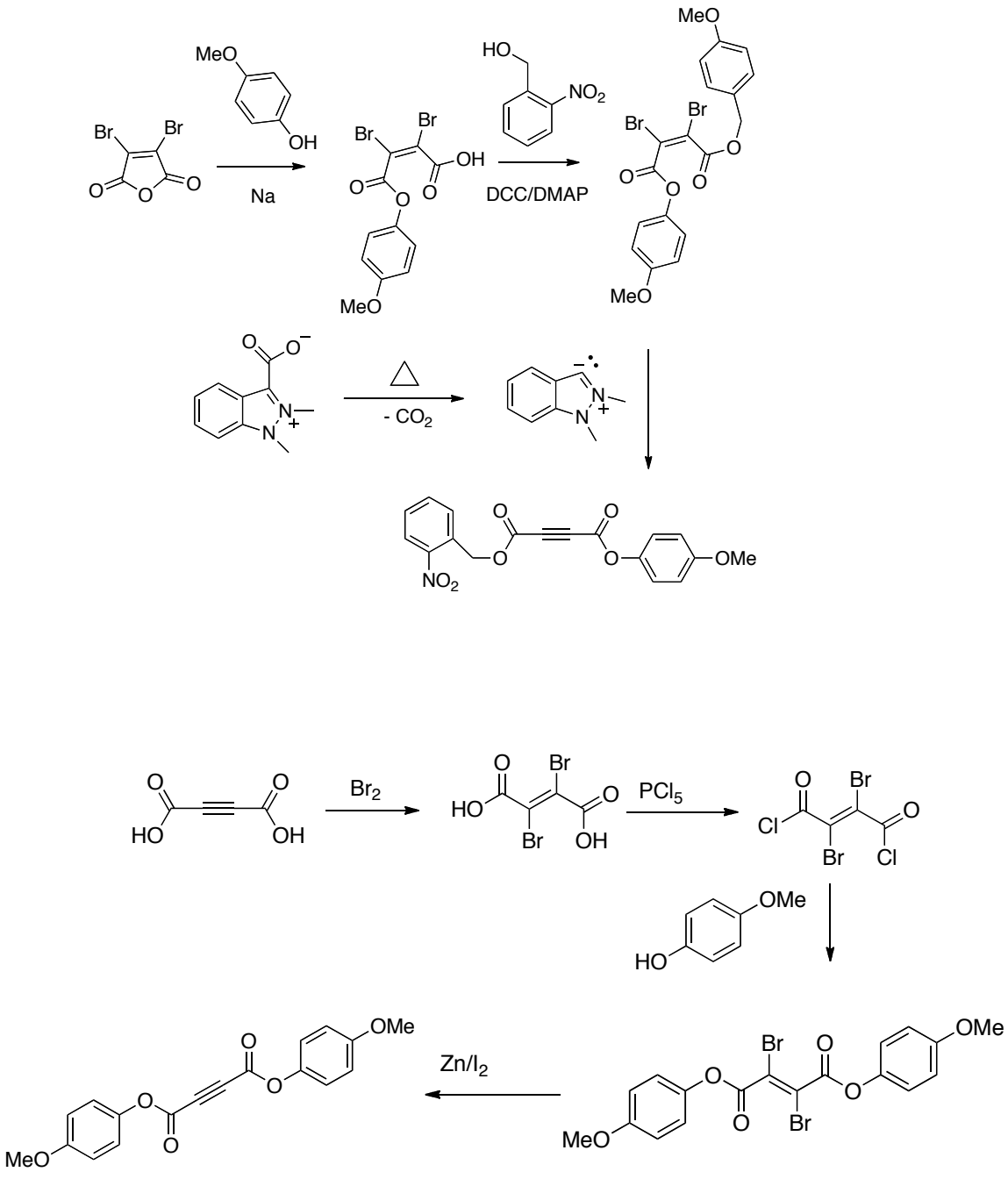


Figure 4: Current proposed synthetic pathway for the formation of the necessary dicarboxylate butynes

The transesterification reactions were non-trivial due to the restrictive steric system and the complicated electronics that made the direct esterification of the mellititc acid derivatives difficult. However, high-pressure systems have been shown to give moderate yields in sterically hindered transesterifications.[75] Even with the high pressure and increased temperature, a labile leaving group was necessary. The transesterification reaction failed with the hexkis (4-methoxy phenyl) benzene 1,2,3,4,5,6 hexacarboxylate, and provided only modest yields even with the high pressure transesterification of the phenolate derivative. Further transesterification with better leaving groups (such as p-nitrophenol or coumarins) are expected to give even higher yields.

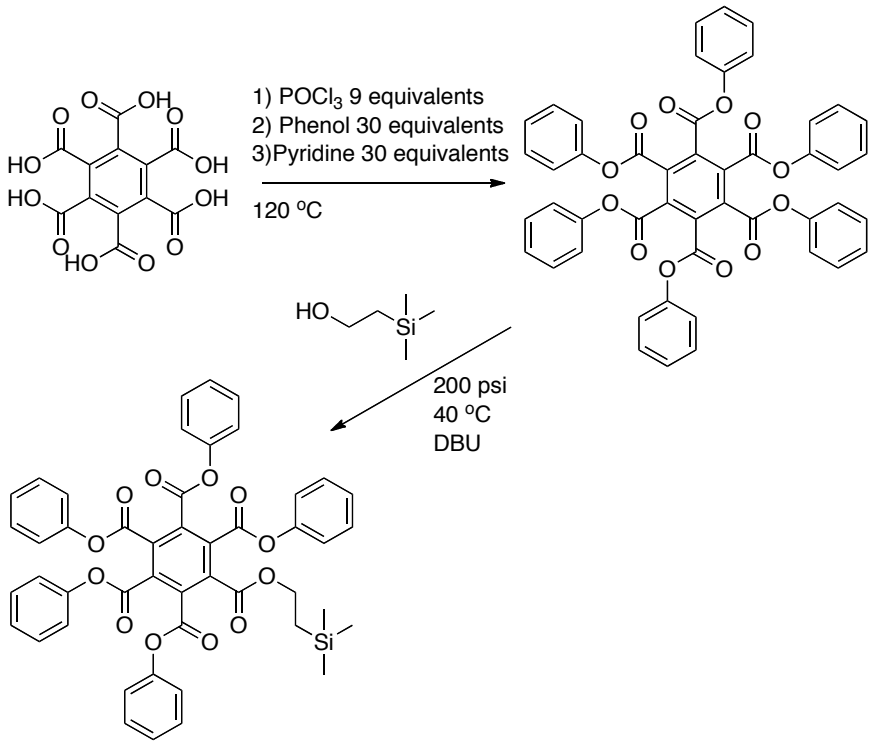


Figure 5: Synthesis of the chemical amplifier

Additionally, the transesterification using the 2-nitrobenzyl alcohol photocage as the nucleophile proved unsuccessful. It is conceivable that with a better leaving group, the transesterification may be done with the photocage, however, previously reported studies of sterically hindered transesterification showed difficulty with benzyl type alcohols.[75] Otherwise, cyclotrimerization may be a viable alternative for obtaining amplifier systems with different triggers.

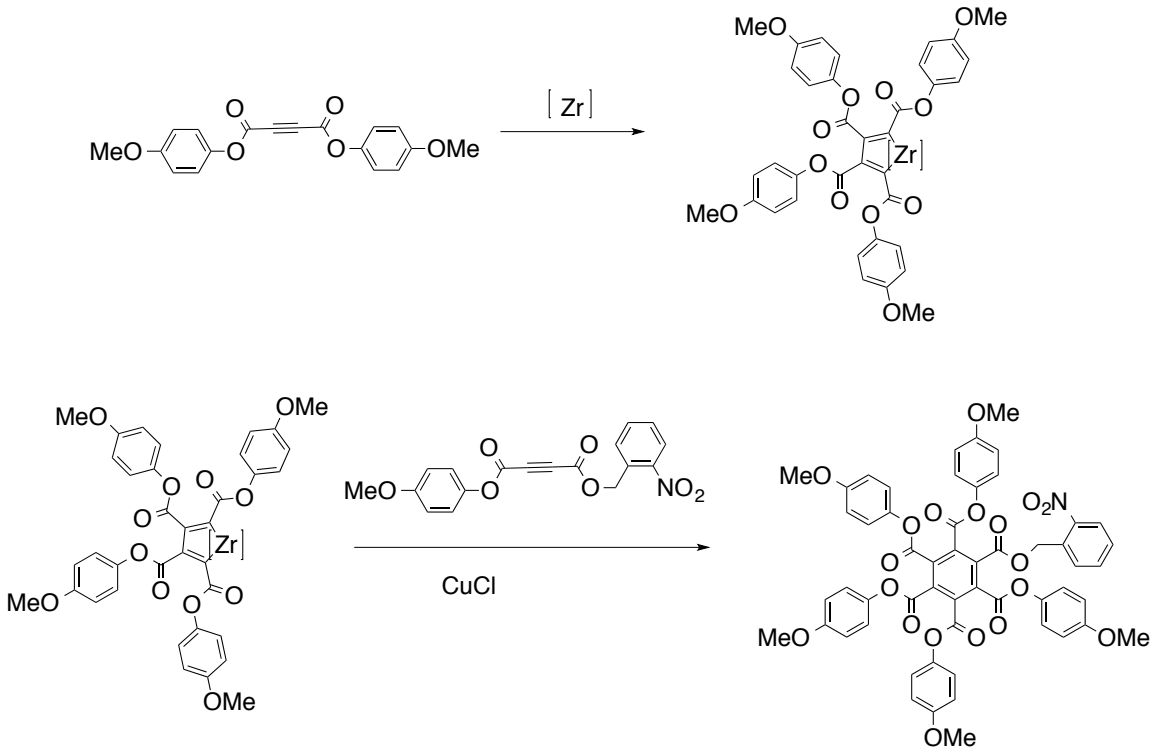


Figure 6: Proposed scheme for synthetic route for cyclotrimerization approach to making chemical amplifier

To test the stability to unwanted hydrolysis of the hexaester, 10 mg of the hexakis 4-methoxy phenyl benzene-1,2,3,4,5,6-hexacarboxylate was dissolved in 100 μL of dioxane. 10 μL of this solution was placed into 1 mL of D_2O. The D_2O/dioxane solution was monitored by ^{1}H-NMR for appearance of phenol. The sample, in an NMR tube and

without stirring, was kept in a 37 °C water bath between NMR runs. No phenol was observed over the first 24 hours, demonstrating that the substrate is suitably water stable for our purposes. A small amount of phenol was detected after 48 hours indicating a small, but acceptable amount of hydrolysis. To help with solubility, the synthesis is being repeated with nitro groups added to the alcohol (eg. p-nitro phenol). Adding the nitro groups should also increase the rate of kinetics. Alternatively, with lower concentrations and purer product, kinetic studies could be done via UV-vis spectroscopy in a water/dioxane mixture.

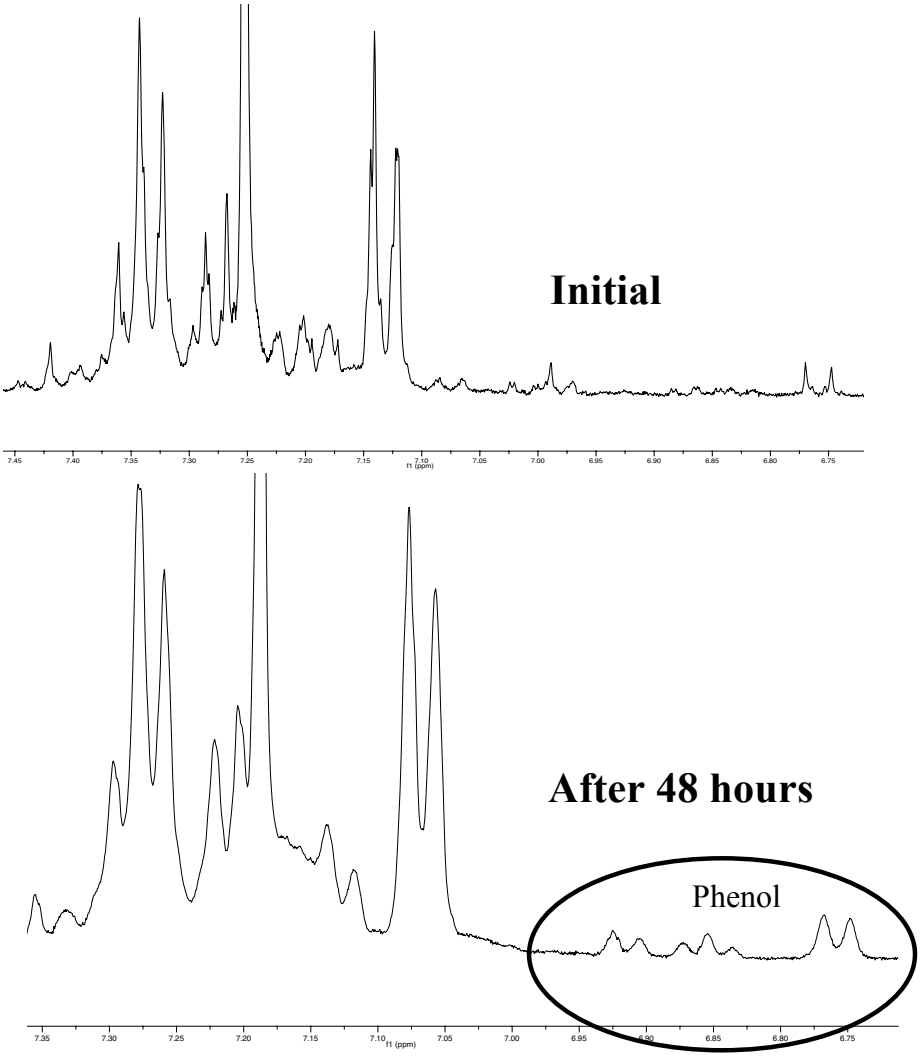


Figure 7: Hydrolysis of the phenol hexaester after 48 hours at 37°C in a dioxane/water solution (some chloroform impurity as well)

Studies testing the effect of the amplifier are currently ongoing. However, initially studies seem to be promising. The 1,2,3,4,5-pentaphenyl 6-(2-(trimethylsilyl)ethyl) benzene-1,2,3,4,5,6-hexacarboxylate, synthesized by the transesterification method and with impurities, was dissolved in THF and subjected to tetra-n-butylammonium fluoride in order to activate the trigger. After 1 hour the reaction mixture was placed in pH 7 phospate buffered water and reaction was monitored by UV. Release of phenol was nearly immediately observed by UV, on addition of the mixture to the water, indicating that our system retains the quick kinetics of the model linker system.

Conclusion:

Using our newly developed oxidative aromatization technique we were able to quickly synthesize several hexaester benzyl derivatives. Using criteria that we laid out in chapter 3, and with the transesterification goal in mind, hexakis phenyl benzene-1,2,3,4,5,6-hexacarboxylate was synthesized and transesterified with a fluorine sensitive trigger, which has been shown to work well with similar systems.[34] Current work is in purification of the amplifier and studies that prove the amplifier effect. After proving release on activation of a trigger, a variety of other alcohols, such as p-nitro phenol and 7-hydroxy coumarin, could be considered. These have better utility as reporter molecules because of their inherent fluorescence.

CHAPTER 5: CONCLUSIONS

This thesis was designed to show the synthesis of a molecular amplifier and the process involved in said synthesis. Special attention was paid to repurposing a well known reaction for new application, groundwork done to demonstrate a good linker system, and criteria for selection of the amplifier.

Chapter 2 involved a discussion of the Hell-Volhard-Zelinsky reaction. Particularly an application of the HVZ reaction was explored where halogenation was followed by elimination in order to yield a substituted aromatic compound from a saturated or partially unsaturated cyclohexane derivative in a one-pot synthesis. Although currently this reaction has heavy limitations due to the specific substitution patterns needed for successful aromatization, and gives low yields, this approach seems to have great promise for an array of specific reactions. In particular, this approach has been shown to be applicable for synthesis of hexakis substituted benzene esters. Since the direct synthesis of hexakis substituted benzene esters through the esterification of mellitic acid proved to be a challenging synthesis that is too restrictive for our purposes of possible chemical amplifiers, the oxidative aromatization has great utility.

Within Chapter 3 was a discussion of self-immolating chemical linkers. Three parts of chemical linkers were discussed: the trigger, the base, and the chemical cargo. Investigations regarding the trigger, and potential applications are ongoing but a fluoride sensitive and light sensitive trigger were shown to be appropriate for a proof of concept study. The three base systems we discussed were a cis cyclohexane, trans cyclohexane, and phthalic (aromatic) system. All three demonstrated potential for use, however with regards to kinetics we found the trans cyclohexane to not meet our target. The cis

cyclohexane was also appropriate from a kinetics point of view, but readily epimerized to the trans under certain conditions, so we deemed this system to be “unstable”. Finally in regards to the alcohol released, the stability of the conjugate base is the primary factor for determining rate of release (assuming neutral pH or within a few pH units of neutral). Aromatic alcohols were found to be appropriate, even those with electron donating groups, such as p-methoxy phenol, even though these are slower than a molecule without electron withdrawing groups or with extended conjugation (such as phenol or 7-hydroxy coumarin).

Finally in chapter 4, the synthesis of the actual amplifier was discussed. Although two routes have shown potential for synthesis, cyclotrimerization and the oxidative aromatization discussed in chapter 2, only the oxidative aromatization has so far led to successful synthesis of an amplifier (after a high pressure transesterifcation as well). This thesis represents a divergent project in an example of a successful synthesis of a chemical machine, a chemical amplifier. Work is ongoing in the characterization of the amplifier as well as potential implementation. Future studies will especially be concerned with the trigger and release of possible reporter molecules, for practical applications.

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SELECTED SUPPORTING SYNTHESIS

Synthesis of hexakis phenyl benzene-1,2,3,4,5,6-hexacarboxylate:

cis-1,2,3,4,5,6 cyclohexane hexacarboxylic acid (1g) was added to phosphorus pentachloride (4g) and reaction mixture was heated to 140°C and stirred for 1 hour. Phenol (10g) was added to reaction mixture and heating was maintained with stirring for another 4 hours. 20 mL of 5% w/v sodium bicarbonate in water was added to reaction mixture and reaction was refluxed for 3 hours. After cooling, solid was filtered out. Recrystalization in THF/hexanes gave pure product.

Synthesis of hexaxis(4-methoxy phenyl) benzene-1,2,3,4,5,6-hexacarboxylate (oxidative aromatization):

cis-1,2,3,4,5,6 cyclohexane hexacarboxylic acid (1g) was added to phosphorus pentachloride (4g) and reaction mixture was heated to 140 and stirred for 1 hour. P-methoxy phenol (13g) was added to reaction mixture and heating was maintained with stirring for another 4 hours. Pyridine (5mL) was added dropwise over 2 hours. Reaction mixture was cooled to room temperature and oil was taken up in methylene chloride. Organic layer was washed with copious water and concentrated, yielding a solid suspended in viscous oil. Filtration with a fine borosilicate glass fritted filter yielded impure solid product. Recrystalization in THF/hexanes gave pure product.

Synthesis of hexaxis(4-methoxy phenyl) benzene-1,2,3,4,5,6-hexacarboxylate (cyclotrimerization): 100 mg of bis(4-methoxyphenyl) but-2-ynedioate was dissolved in 10% dioxane/water mixture and was heated to 70 °C. ~5 mg of dichlorobis(μ-

chloro)bis[(1,2,3,6,7,8-n)-2,7-dimethyl-2,6-octadien-1,8-diyl] diruthenium (IV) was added and the reaction mixture was stirred for 12 hours. Mixture was filter and aqueous solution was collected. Product was extracted with methylene chloride. Organic layer was concentrated giving mostly pure product. An analytical amount was recrystalized from THF/hexanes.

Synthesis of mellitic acid:

Cis-1,2,3,4,5,6 cyclohexane hexacarboxylic acid (1g), was added to phosphorus pentachloride (4g) and reaction mixture was heated to 140 and stirred for 4 hours. 20 mL of water was added and mixture was refluxed for one hour. After cooling, pure, solid product was filtered out.

Synthesis of trans- 2-((p-methoxyphenoxy)carbonyl)cyclohexanecarboxylic acid:

5g of 1,2-cyclohexane, trans anhydride was dissolved in 30 mL of DI water. 5g of p-methoxy phenol was added and mixture was heated to 35 °C for 30 minutes. Mixture was acidified to pH 1-2 with dilute HCl and mixture was cooled slowly to 5 °C. Pure product crystals were collected via vacuum filtration and used without further purification.

Synthesis of cis- 2-((p-methoxyphenoxy)carbonyl)cyclohexanecarboxylic acid:

5g of 1,2-cyclohexane, cis anhydride was dissolved in 30 mL of DI water. 5g of p-methoxy phenol was added and mixture was heated to 35 °C for 30 minutes. Mixture was acidified to pH 1-2 with dilute HCl and mixture was cooled slowly to 5 °C. Pure

product crystals were collected via vacuum filtration and used without further purification.

Synthesis of p-methoxy phenyl hydrogen phthalate:

5g of 1,2-cyclohexane, cis anhydride was dissolved in 30 mL of DI water. 5g of p-methoxy phenol was added and mixture was heated to 35 °C for 30 minutes. Mixture was acidified to pH 1-2 with dilute HCl and mixture was cooled slowly to 5 °C. Pure product crystals were collected via vacuum filtration and used without further purification.

Synthesis of 4-methoxyphenyl (2-(trimethylsilyl)ethyl) phthalate:

1.5 g of cis- 2-((p-methoxyphenoxy)carbonyl)cyclohexanecarboxylic acid, 1 mL of 2-trimethylsilylethanol, and 0.085 g of 4-*N,N*-Dicyclohexylcarboiimide were dissolved in 4 mL of dry DMF and stirred until complete solvation was observed. 1.54 g of *N,N*-Dicyclohexylcarbodiimide was dissolved in 2 mL of dry DMF and added to the reaction mixture. The reaction was stirred under an argon atmosphere overnight. The dicyclohexylurea biproduct was filtered off. The solvent was removed under reduced pressure. Purification was done by flash chromatography (Hex/EtOAc 90:10) to give pure product as a clear yellow oil.

Synthesis of 1,2-diethyl 3,4,5,6-tetramethyl benzene-1,2,3,4,5,6-hexacarboxylate:

580 mg of bis(cyclopentadienyl)zirconium dichloride was cooled to -78°C. 2.4 mL of 2.5 M n-butyl lithium in hexanes was added dropwise and mixture was stirred for 1 hour. 1

mL of acetylenedicarboxylate was added and mixture was stirred for 1 hour. 324 mg of copper I chloride was added to the flask. Immediately after, 0.600 mL of diethyl acetylene dicarboxylate was added. Reaction was stirred for 10 hours. 30 mL of .1 M HCl was added to the reaction and stirred until heat stopped evolving. 50 mL of water was added and product was extracted by ether. Concentrating the ether gave crude product.

Synthesis of 1,2,3,4,5-pentaphenyl 6-(2-(trimethylsilyl)ethyl) benzene-1,2,3,4,5,6-hexacarboxylate:

100 mg of the hexakis phenyl benzene 1,2,3,4,5,6 hexa carboxylic acid was dissolved in 10 mL of chloroform. 100 μL of 2-(trimethylsiyls)ethanol and 16 μL of DBU was added and reaction was placed in a pressure vessel. Reaction was heated to 40 °C, pressurized to 200 psi and allowed to proceed for 4 hours. Chloroform layer was washed with copious water. The organic layer was concentrated down yielding impure product as an oil. Product was placed under a reduced atmosphere (~200 microns) for 2 days to yield a yellow, crude product. ^{1}H-NMR revealed expected peaks for a 1:5 amplifier. Further purifications will be done by preparative HPLC.