COUPLING REACTIONS AND SEPARATIONS FOR IMPROVED SYNTHETIC PROCESSES

A Thesis
Presented to
the Academic Faculty

By

Reagan Charney

In Partial Fulfillment
Of the Requirements for the Degree
Doctor of Philosophy in Chemistry

Georgia Institute of Technology

December, 2008

COUPLING REACTIONS AND SEPARATIONS FOR IMPROVED SYNTHETIC PROCESSES

Approved by:

Dr. Charles Liotta, Advisor
School of Chemistry and Biochemistry
Georgia Institute of Technology

Dr. Charles Eckert, Co-Advisor
School of Chemical and Biomolecular Engineering
Georgia Institute of Technology

Dr. David Collard
School of Chemistry and Biochemistry
Georgia Institute of Technology

Dr. Facundo Fernandez
School of Chemistry and Biochemistry
Georgia Institute of Technology

Dr. Rigoberto Hernandez
School of Chemistry and Biochemistry
Georgia Institute of Technology

Date Approved: October 22, 2008

This work is dedicated to my husband David Charney. Thank you for all your love and support!

ACKNOWLEDEMENTS

It is said that no man is an island and no where is that more true than in researching and writing a PhD thesis. First, I would like to thank my advisors, Drs. Liotta and Eckert for their guidance and assistance with my research. I would also like to thank my committee members (past and present) for their help and contributions to my research.

I would like to thank the National Science Foundation for my graduate research fellowship.

I would also like to thank my collaborators. On the continuous flow reactor project, I would like to thank American Pacific and especially Kent Richman for their support. On the PTC project, I would like to thank Dow Corning for their support and Dr. Bommarius for his help. Lastly, on the metabolomics project, I would like to thank our collaborator Dr. Fernandez for his help.

I am also very thankful to my research group (past and present) for listening to ideas, watching presentations, and being encouraging. I am especially thankful to three people who contributed greatly to this thesis: Pamela Pollet, Michelle Kassner, and Stuart Terrett. As Helen Keller said, “Alone we can do so little; together we can do so much." Without your guidance and assistance, I know this thesis would not be what it is today.

I would also like to thank God. Without my relationship with God, this degree would have presented insurmountable hurdles.

Lastly, I would like to thank my family (both mine and my in-laws) and my husband, David Charney. His ability to keep everything in perspective and good advice were key ingredients to my completing this degree. I would like to thank my grandparents, Marvin and Margie Prochnow. Your love and support during my undergraduate degree enabled me to reach this next level in my education. I would also like to thank my parents, Mark and Koreen Hughes, and David's parents, John and Kathy Charney, for all of their love and support over the course of this degree.

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LIST OF SYMBOLS OR ABBREVIATIONS

AIDS:	Acquired immunodeficiency syndrome
Aliquat 336:	Trioctylmethylammonium chloride
AMPAC:	American Pacific Corporation
$BnNH_2$:	Benzylamine
BnSiPTC:	Benzyl-tris-[3-(1,1,3,3,3-pentamethyl-disiloxanyl)-propyl]-ammonium; chloride
^{13}C NMR:	Carbon – 13 Nuclear magnetic resonance spectroscopy
CMC:	Critical micelle concentration
CMK:	Chloromethylketone
CTFE:	Chlorotrifluoroethylene
DBU:	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC:	1,3-Dicyclohexylcarbodiimide
DHU:	1,3-Dicyclohexyl-urea
DMF:	*N*,*N*-dimethyl formamide
DMSO:	Dimethylsulfoxide
DSC:	Differential scanning calorimeter
DVDS-Pt:	Platinum (0)-1,3-divinyl-1,1,3,3-tetramethyldisiloxane
EA:	Elemental analysis
ESI:	Electrospray ionization
EtOAc:	Ethylacetate
GC:	Gas chromatography
GC-FID:	Gas chromatography-flame Ionization Detector
GC-MS:	Gas chromatography-mass spectrometry
^{1}H NMR:	Proton nuclear magnetic resonance spectroscopy
HIV:	Human immunodeficiency virus
HP:	Hewlett-Packard
HPLC:	High performance liquid chromatography
IR:	Infrared spectroscopy
ISCO:	Brand name of syringe pump
LC:	Liquid chromatography
LC-MS:	Liquid chromatography-mass spectrometry
LC-UV:	Liquid chromatography-ultraviolet spectrometry
m-CPBA:	*m*-chloroperbenzoic acid
MeOH:	Methanol
mp:	Melting point
MS:	Mass spectroscopy

MeSiPTC:	Methyl-tris-[3-(1,1,3,3,3-pentamethyl-disiloxanyl)-propyl]-ammonium; chloride
MTO:	Methyltrioxorhenium
NMP:	*N*-Methylpyrrolidone
PDMS:	Polydimethylsiloxane
pKa:	Acid dissociation constant
Ppm:	parts per million
PS:	Piperylene sulfone
Psi:	Pound per square inch
PTC:	Phase transfer catalyst
PTSA:	*p*-toluene sulfonic acid
RT:	Room temperature
$scCO_2$:	Supercritical carbon dioxide
SDS:	Sodium dodecylsulfate
SFC:	Supercritical fluid chromatography
S_N2:	Bimolecular nucleophilic substitution
TBACl:	Tetrabutylammonium chloride
TEA-HCl:	Triethylamine-hydrochloride salt
TFA:	Trifluoroacetic acid
TGA:	Thermogravimetric analyzer
THF:	Tetrahydrofuran
TLC:	Thin layer chromatography
UV-Vis:	Ultraviolet-Visible spectroscopy

SUMMARY

This thesis showcases a work that focused on developing processes with improved economic and environmental signatures. It illustrates the strengths of chemists and chemical engineers working together towards sustainable solutions. The joint collaboration between Drs. Liotta and Eckert allows the combination of disciplines to overcome economic and environment obstacles. This thesis depicts the application of chemical engineering and chemistry for industrial processes towards reducing cost and environmental impact.

In chapter 2, a synthetic sequence yielding a pharmaceutical precursor was optimized for continuous processing. The precursor was for the pharmaceutical drug Ro 31-8959, which acts as a human immunodeficiency virus (HIV) protease inhibitor. A continuous flow reactor was designed, built and utilized successfully for the two-step reaction of the diazoketone pharmaceutical precursor, (1-benzyl-3-chloro-2-hydroxy-propyl)-carbamic acid tert-butyl ester. The best configuration for the continuous flow reactor involved a single and double coiled stainless steel reactor packed with glass beads. The yield obtained for the diazoketone was quantitative.

In chapter 3, the cleavable surfactant (cleavable surfactants decompose in non-surface active ingredients upon stimulus), *n*-octyl thiirane oxide was

synthesized, characterized and its surface activity and loss of surface activity upon heating was demonstrated. The *n*-octyl thiirane oxide surfactant activity was measured using a dye, Suddan III, and compared to a commercially available surfactant sodium dodecyl sulfate.

In chapter 4, 5-amino-1H-tetrazole was synthesized using two novel synthetic routes starting from benign chemicals. Both routes involved Sharpless click chemistry in the first step to form the tetrazole ring. Both routes also used hydrogen transfer as the last step for the formation of the 5-amino-1H-tetrazole. These syntheses eliminated the use of highly toxic and/or explosive chemicals such as cyanamide, hydrazoic acid, and hydrazine.

Finally in chapter 5, phase transfer catalysis was used as a means to improve reaction rates and yields between a siloxylated reagent (in the liquid phase) and insoluble ionic reagents (in the solid phase). The activity of commercial phase transfer catalysts like tetra-*n*-butylammonium bromide was compared to the activity of two novel custom-made siloxylated phase transfer catalysts. Surprisingly, the tetra-*n*-butylammonium resulted in superior rate constants to the custom made siloxylated phase transfer catalysts.

CHAPTER 1: INTRODUCTION

The efficient technology transfer of a process from laboratory to industrial scale can only be done when chemists and chemical engineers work in synergy. Chemists and chemical engineers are trained in different but complementary ways and combining the two disciplines can allow for unique improvements that would not be possible with only one discipline. The relationship between the chemists and chemical engineers can help to overcome economic and environmental obstacles to make processes more sustainable. Benefiting from the unique collaboration between Drs. Liotta and Eckert, this thesis involves developing processes that are relevant to industry and that minimize cost, waste production, energy consumption while optimizing product yield and quality. The common theme throughout this thesis is joining chemists and chemical engineers to solve problems relevant to industry towards more sustainable processes.

In chapter 2, a multi-step synthesis was optimized from a batch to a continuous flow reactor. Continuous flow reactors allow for excellent temperature control and safety capabilities, often resulting in higher yields, higher product quality, less by-products, less waste and lower costs.[1] After much optimization, an optimal coiled flow reactor was designed and built. This study was particularly successful: the model two-steps synthesis was carried out in the

coiled reactor, yielding the quantitative conversion of the L-boc-phenylalanine to the desired product, the corresponding diazoketone.

Industry currently uses surfactants for a variety of processes like enhanced oil recovery and nanoparticles synthesis. However, separating the surfactants from the products remains both a cost- and waste- demanding step. Cleavable surfactants are surfactants that decompose into non-surface active fragments upon application of an external stimulus, easing the product separation step drastically. In Chapter 3, *n*-octyl thiirane oxide was synthesized and its ability to act as a cleavable surfactant was demonstrated. Its synthesis, surfactant activity, and loss of surfactant activity upon application of a stimulus are discussed.

Currently, 5-amino-1H-tetrazole is being utilized as a replacement for sodium azide in airbags and is a valuable starting material for the synthesis of pharmaceutical and explosive ingredients.[2] Previous syntheses to prepare 5-amino-1H-tetrazole have used hydrazoic acid, hydrazine, or cyanamide. These chemicals however are highly toxic and/or explosive. Two synthetic routes to the 5-amino-1H-tetrazole that eliminated the use of these compounds was designed and explored. Developing a safer process to synthesize 5-amino-1H-tetrazole is very attractive to industry, along with minimizing cost, safety hazards and potential product contamination with toxic chemicals.

In chapter 5, phase transfer catalysis was explored as a means to improve reaction rate and yields between a siloxylated reagent (liquid phase) and

immiscible ionic reagents (solid phase). Phase transfer catalysis is used to facilitate the reaction between reactants in different phases.[3] Although siloxylated derivatives have many applications, phase transfer catalysis was never reported to facilitate reactions involving siloxylated and ionic reagents that are hindered by reagents being in two different phases. The syntheses of a siloxylated substrate and two novel siloxylated phase transfer catalysts are reported. The reaction between a siloxylated model compound and potassium acetate is reported in detail. Other ionic reagents like potassium cyanide, potassium thiocyanate and L-lysine were also investigated. Four phase transfer catalysts were tested: aliquot 336, tetra-*n*-butylammonium chloride and the two novel siloxylated catalysts. Surprisingly, the tetra-*n*-butylammonium chloride performed the best in various conditions.

In summary, all of my projects have the potential to improving industrial processes. Most of these improvements were obtained through the synergy of the disciplines of chemistry and chemical engineering.

(1) Ehrfeld, W., Hessel, V., and Lowe, H. *Microreactors: New Technology for Modern Chemistry*; Wiley-VCH: Weinheim, 2000.
(2) *Angew. Chem. Int. Ed* **2008**, *47*, 3330-3347.
(3) Starks, C. M., Liotta, Charles L., Halpern, Marc *Phase Transfer Catalysis*; Chapman & Hall Inc, 1994, p1-22.

CHAPTER 2: APPLICATION OF A KINETIC STUDY TO A SMALL SCALE CONTINUOUS REACTOR TO PRODUCE (1-BENZYL-3-DIAZO-2-OXO-PROPYL)-CARBAMIC ACID ISOPROPYL ESTER, A DIAZOKETONE PHARMACEUTICAL INTERMEDIATE

2.1 Introduction

Continuous flow reactors have been finding increased application in the pharmaceutical industry due to their superior heat transfer capabilities leading to reduced waste and improved product quality and safety. This project focused on a synthetic sequence, which is a part of a multistep synthesis in the preparation of an active ingredient for the treatment of HIV. Specifically, the sequence involved three reactions: 1) the formation of a mixed anhydride, 2) formation of the corresponding diazoketone and 3) the HCl hydrolysis yielding to the α-chloroketone. First, the formation of the mixed anhydride was optimized. For analysis purposes, the temperature-sensitive mixed anhydride was quenched with an amine to form the corresponding amide. The second part of the project involved forming the diazoketone from the mixed anhydride and trimethylsilyl diazomethane.

The reaction conditions were also altered and optimized to fit the needs of a continuous process. Four continuous flow reactors configurations were

designed, built and tested throughout the project in order to optimize the conditions to this specific synthesis.

2.2 Background

2.2.1 Advantages of Continuous Flow Reactors

Transforming a reaction from batch to a continuous process can have many benefits. Although continuous flow reactors are often used for large scale processes, they can also be very small, either microreactor reactor scale or slightly larger. In the literature, small continuous flow reactors have been shown to have characteristics similar to continuous flow reactors in several areas such as heat exchange, safety, and scale out ability.[1,2] Microreactors are usually defined as miniaturized reaction systems with dimensions in the sub-micrometer to the sub-millimeter range.[3] Small scale continuous flow reactors, as we defined them, are slightly larger in the millimeter range. First, both have a high surface area to volume ratio due to their small size.[3] The high surface area then leads to a high heat-exchange efficiency, which results in rapid heating or cooling.[4] The small volumes resulting from the small size makes it easier to control process parameters, decreasing the potential hazard of explosive or extremely exothermic reactions. The small dimensions prevent the common mechanistic explosion pathways by suppressing radical chains and thermal build up.[4,5] The use of stainless steel continuous flow reactors also allows for better containment of any

potential explosions.[5] The increased safety of these systems is desirable with reactions that use diazomethane or other highly explosive compounds.[6] Lastly, the scale out ability means that additional reactors can be added to increase production rather than having to scale up the reaction.[1] This ability can not only save time, but also makes the process easier to adapt to production needs.

The reaction sequence involves a temperature unstable intermediate, a potentially explosive reagent (diazomethane) and reactions that can be highly exothermic. The advantages of continuous flow reactors can overcome these reaction characteristics by significantly increasing heat transfer, mass transfer, safety and overall performances.

2.2.3 Potential Applications of (1-Benzyl-3-chloro-2-hydroxy-propyl)-carbamic acid tert-butyl ester

Human immunodeficiency virus (HIV), encodes three enzymes and the inhibition of these enzymes could be a possible route to treat acquired immunodeficiency syndrome (AIDS). The product in Figure 2-1 is a precursor to a pharmaceutically active compound known as Ro 31-8959[7] that acts as a human immunodeficiency virus (HIV) protease inhibitor. Modern HIV inhibitors use a central three-carbon piece that contains two chiral carbons.[7,8] This synthesis uses the L-boc-phenylalanine to set the chirality of the first carbon center and asymmetrically reduces the ketone to give the other chiral center.

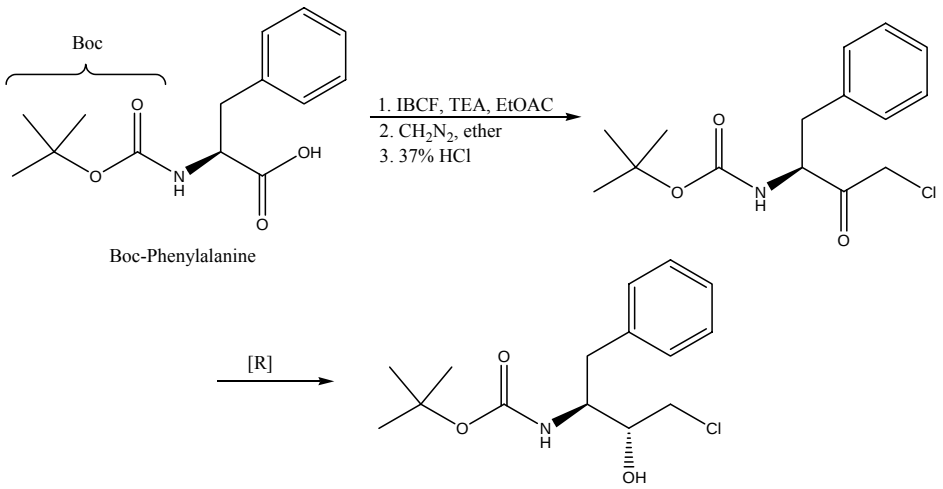


Figure 2-1: Synthetic route previously used [8]

2.2.4 Trimethylsilyl diazomethane

The classic procedure used diazomethane as a reactant in the second step of the synthesis. Diazomethane is extremely reactive, highly toxic, thermally labile, and potentially explosive. In contrast, trimethylsilyl diazomethane has been used as a safer substitute for diazomethane because it is non-explosive, non-mutagenic, and can be used by industry without hazard.[9] In addition, it has been widely used as a diazomethane substitute for a variety of reactions.[10] One specific example where trimethylsilyl diazomethane has been used as a diazomethane substitute is in the Arndt–Eistert synthesis. This synthesis involves the of conversion of an activated carboxylic acids to diazoketones by the action of diazomethane, followed by a Wolff rearrangement.[11] Currently, Cesar *et al* used

trimethylsilyl diazomethane in the synthesis in Figure 2-2.[10] They were able to obtain a 78% isolated yield by using trimethylsilyl diazomethane compared to 76% isolated yield using diazomethane.

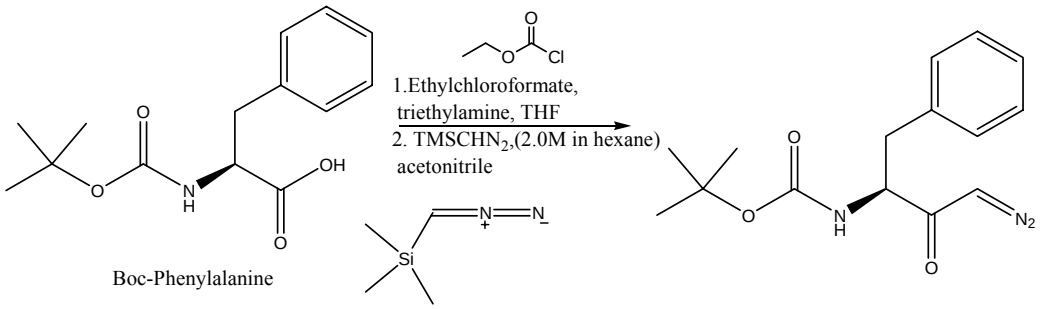


Figure 2-2: Diazoketone synthesis from L-boc-phenylalanine [10]

2.3 Results and Discussion

2.3.1 Optimize Model Reaction for Use in Continuous Flow Reactor

The first reaction to be optimized in the continuous flow reactor was the formation of the mixed anhydride from L-boc-phenylalanine and isobutylchloroformate in the presence of triethylamine in ethylacetate (Figure 2-3). The mixed anhydride, however, is quenched with a primary amine instead of reacting with diazomethane to form the diazoketone (Figure 2-1).

First, benzylamine was used as the primary amine for the quench of the mixed anhydride as shown in Figure 2-3 to obtain (1-benzylcarbamoyl-2-phenyl-ethyl)-carbamic acid tert-butyl ester. The reaction was run using the triethylamine as the HCl scavenger to determine the maximum yield of the mixed anhydride. I made a 20 wt % solution of L-boc-phenylalanine (1 equiv) in ethyl acetate. The reaction was cooled to -30ºC. Isobutylchloroformate (1.3 equiv) and triethylamine (1.3 equiv) were added and allowed to stir at -30ºC for 1 hour, analogous to the industrial procedure.[8] The benzylamine (1.3 equiv) was then added to quench the solution. The product was characterized by ^{1}H and ^{13}C NMR, MS, and elemental analysis.

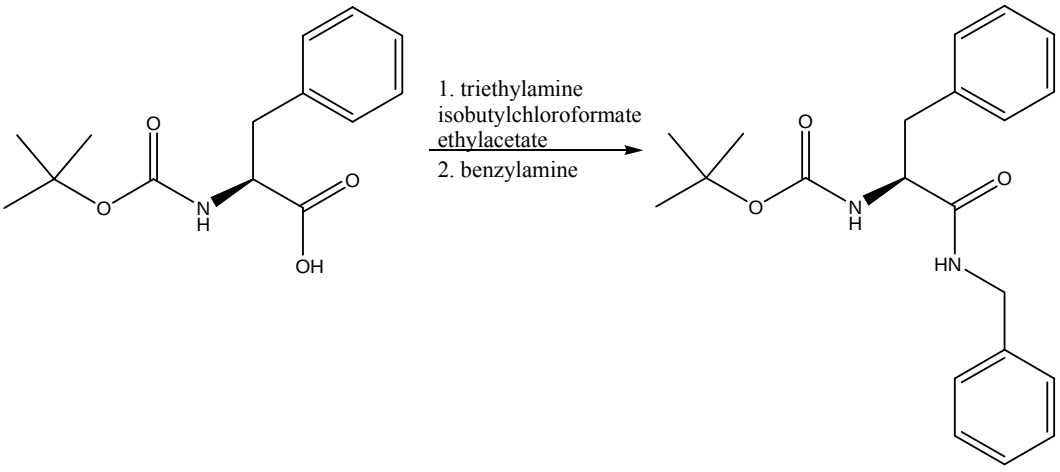


Figure 2-3: Previous reaction using benzylamine quench in place of continuing to the second step

With this reaction, the triethylamine acts as an HCl scavenger and forms a salt that precipitates out of the reaction. In a batch reaction, a salt that can be filtered is desirable because of the ease of removal for purification. However, a precipitate could clog the continuous flow reactor because of the small tubing. I needed to find a secondary or tertiary amine that would act as an HCl scavenger but would not precipitate out of the solution and would not quench the reaction. Pyridine, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), piperidine, tripropylamine, tributylamine were tested by adding one mL of HCl (37% reagent grade) to a solution of the amine (1 g) in ethylacetate (10 mL). All the amines except tripropylamine and tributylamine formed a precipitate that was visible to the eye.

The reaction to make (1-benzylcarbamoyl-2-phenyl-ethyl)-carbamic acid tert-butyl ester using the benzylamine quench shown in Figure 2-3 was then repeated using tripropylamine instead of triethylamine. All the conditions were the same as previously mentioned except the substitution of the triethylamine with the tripropylamine (1.3 equiv). However, the tripropylamine formed a precipitate visible to the eye under the reaction conditions.

The reaction to make (1-benzylcarbamoyl-2-phenyl-ethyl)-carbamic acid tert-butyl ester using the benzylamine quench shown in Figure 2-3 was repeated using tributylamine. All the conditions were the same as previously mentioned except the substitution of the triethylamine with the tributylamine (1.3 equiv). No

precipitate was formed. The starting material was not observed in an aliquot of the reaction solution by ^{1}H NMR.

2.3.2 Calibration for Batch Reaction Results

The reaction was to be monitored in the continuous flow reactor by LC-UV to determine conversions. In a batch setting, the reaction was allowed to proceed for one hour. Calibration curves of the starting material, L-boc-phenylalanine and the product, (1-benzylcarbamoyl-2-phenyl-ethyl)-carbamic acid tert-butyl ester were prepared.

First, a calibration curve of the starting material, L-boc-phenylalanine , was made using the LC-UV as shown in Figure 2-4.

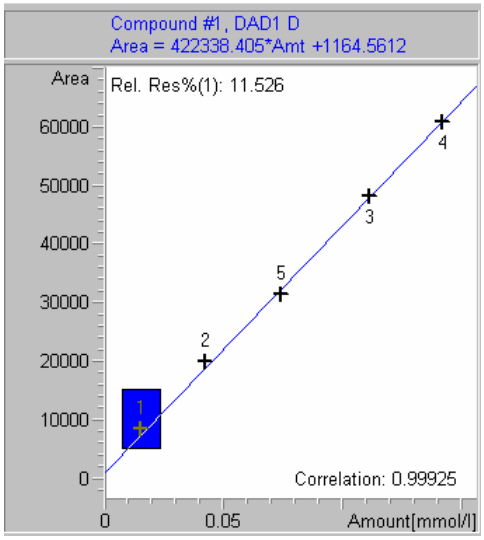


Figure 2-4: Calibration curve of L-boc-phenylalanine on LC-UV

The LC-UV of the pure product, (1-benzylcarbamoyl-2-phenyl-ethyl)-carbamic acid tert-butyl ester showed no peak (at similar concentration that the concentration used for the starting material). The UV maximum for (1-benzylcarbamoyl-2-phenyl-ethyl)-carbamic acid tert-butyl ester was determined to be 229 nm and the LC-UV was run with diode array detector set at 229 nm wavelength. There was still no peak observed for the (1-benzylcarbamoyl-2-phenyl-ethyl)-carbamic acid tert-butyl ester. A GC-MS was run on the pure product (1-benzylcarbamoyl-2-phenyl-ethyl)-carbamic acid tert-butyl ester as well. The sample contained a decane standard, (1-benzylcarbamoyl-2-phenyl-ethyl)-carbamic acid tert-butyl ester product, and benzylamine. The mole ratio of benzylamine to product was 1:1 and of product or benzylamine to decane was 1:4. However, the GC-MS area ratios were 1:12 product to decane while the benzylamine to decane ratio was 1:5 (Figure 2-5). These ratios show that the product was decomposing in the GC-MS. Clearly, the product was difficult to analyze by the methods that we had available in our laboratory.

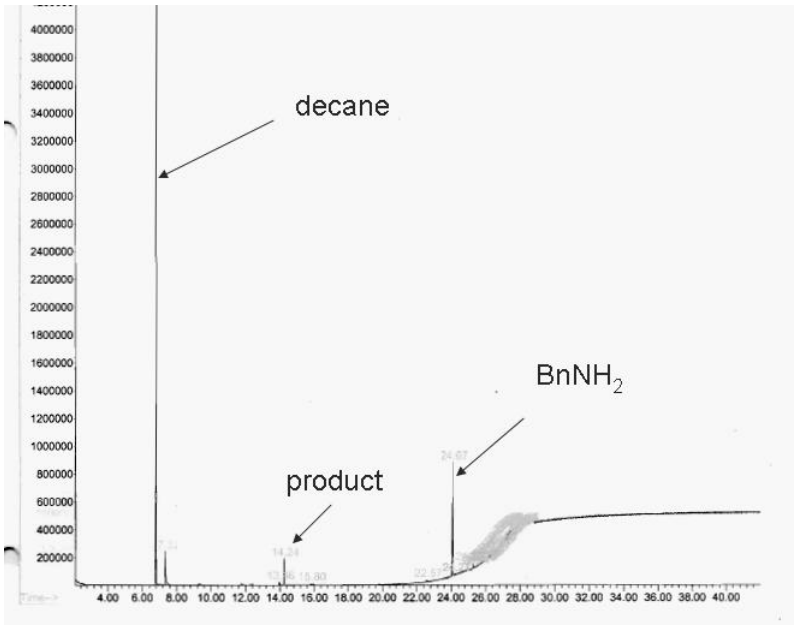


Figure 2-5: GC-MS of decane standard, benzylamine, and (1-benzylcarbamoyl-2-phenyl-ethyl)-carbamic acid tert-butyl ester product

The benzylamine was replaced by propylamine to now form (2-phenyl-1-propylcarbamoyl-ethyl)-carbamic acid tert-butyl ester. The reaction conditions were the same except the propylamine was substituted for the benzylamine (1.5 equiv). The TEA-HCl salt was filtered and the product was purified and isolated. The (2-phenyl-1-propylcarbamoyl-ethyl)-carbamic acid tert-butyl ester was characterized using ^{1}H and ^{13}C NMR and elemental analysis. A peak in the LC (at 229 nm wavelength) was easily detected (Figure 2-6). Therefore, propylamine was used consistently as the quench amine for the rest of the project. The (2-phenyl-1-propylcarbamoyl-ethyl)-carbamic acid tert-butyl ester was then synthesized using tributylamine rather than triethylamine as the HCl scavenger. The product was isolated, purified and characterized using ^{1}H and ^{13}C NMR and elemental analysis. The characterization results of the (2-phenyl-1-propylcarbamoyl-ethyl)-carbamic acid tert-butyl ester from the use of the triethylamine and the tributylamine were the same.

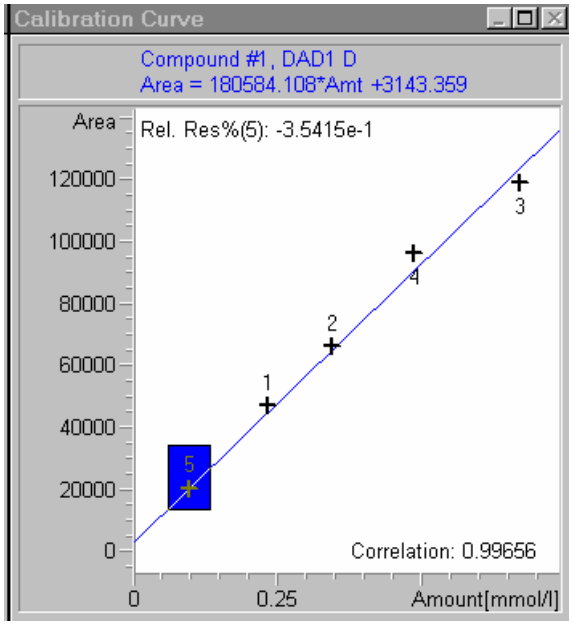


Figure 2-6: LC-UV calibration curve of product (2-phenyl-1-propylcarbamoyl-ethyl)-carbamic acid tert-butyl ester

2.3.3 Design and Use of 1st Generation Continuous Flow Reactor

The first continuous flow reactor was built using parts available in the laboratory. It had two streams (A & B) entering the continuous flow reactor that would mix and go to C (Figure 2-7). At C, the reactant stream would drip into a flask containing the propylamine quench in an ice bath.

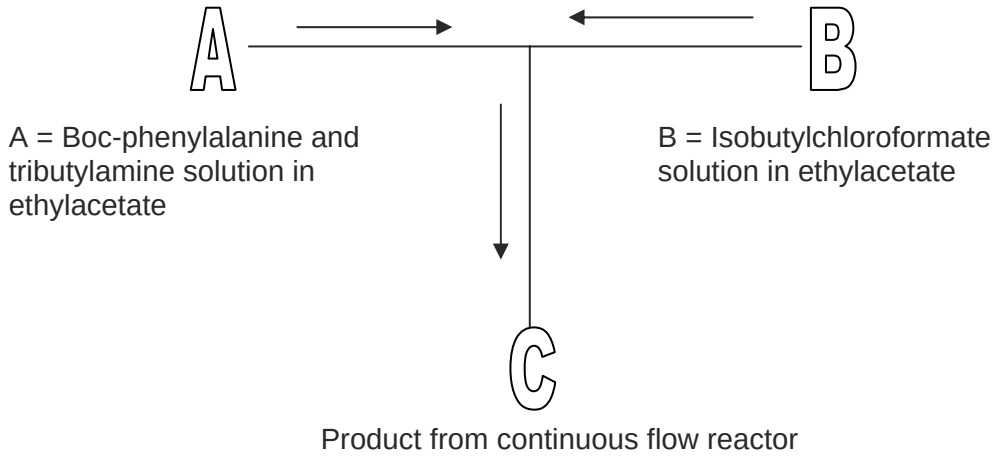


Figure 2-7: Planned flow for continuous flow reactor setup

The reactants at A (L-boc-phenylalanine , tributylamine in ethylacetate) are pumped into the continuous flow reactor using an HPLC pump. The L-boc-phenylalanine (0.75 g) and dry tributylamine (0.7 mL) were combined in dry ethylacetate (75 mL) to make a 0.04 M solution. The reactant at B (isobutylchloroformate in ethylacetate) was added to the continuous flow reactor using an ISCO. The isobutylchloroformate (2.4 mL) was combined with dry ethylacetate (450 mL) to make a 0.04 M solution. The reagents, ISCO, and continuous flow reactor were kept cool using a chiller that circulated at 5 L/min set at -20ºC. The same chiller was used throughout the project. The reactants were combined at a T-fitting. The continuous flow reactor itself was 6 ft long with an inner diameter of 0.06" and a thermocouple to measure the temperature at

the end. A picture of the 1st generation continuous flow reactor is shown in Figure 2-8. The continuous flow reactor was run using a 0.04 M reactant solution, rather than the higher concentration of 0.75 M used in the batch reaction, due to concerns of the reaction being exothermic when the mixed anhydride is formed. The quench solution, at the end of C in Figure 2-7, contained propylamine (0.16 mL) in ethylacetate (5 mL). The propylamine amount is based on having 1.5 equiv after a 10 min run with a flow rate of 3.3 mL/min. The flow rate for the ISCO was 3.3 mL/min to match the flow rate measured for the HPLC pump. The product stream was analyzed using LC-UV and ^{1}H NMR. No product was observed for either run. The thermocouple on the end of the continuous flow reactor read -7.3ºC compared to the initial temperature of -20ºC. I calculated that the exotherm from the reaction was not this large so the cooling of the continuous flow reactor was not efficient. It was originally hypothesized that the reason product was not observed was of the inefficient cooling.

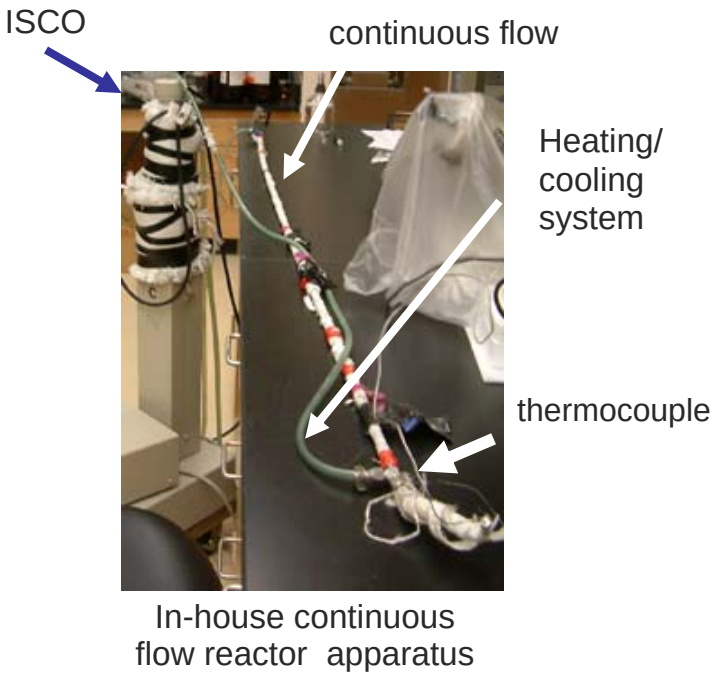


Figure 2-8: 1st generation continuous flow reactor

2.3.4 Design and Use of 2nd Generation Continuous Flow Reactor

The goal with the 2nd generation continuous flow reactor was to improve heat transfer. I designed and built a more compact continuous flow reactor utilizing stainless steel tubing. The whole reactor could fit inside a chiller for higher temperature control (Figure 2-9). I also added the quench stream directly to the continuous flow reactor by using a second HPLC pump. This would give more control over the rate of the addition of the quench. Another benefit of the 2nd generation system was the addition of the thermocouple at the cross fitting, which was the mixing point of the two reagents streams, providing a more

accurate reading of the potential exothermicity of the reaction. The inner diameter of the stainless steel tubing was 7 mm. The lengths of tubing were chosen from a calculation of how long it would take for a room temperature liquid to cool to -20ºC. The tubing was coiled to enhance the mixing and to allow for placement in the chiller. A picture of the 2nd generation continuous flow reactor can be seen in Figure 2-10.

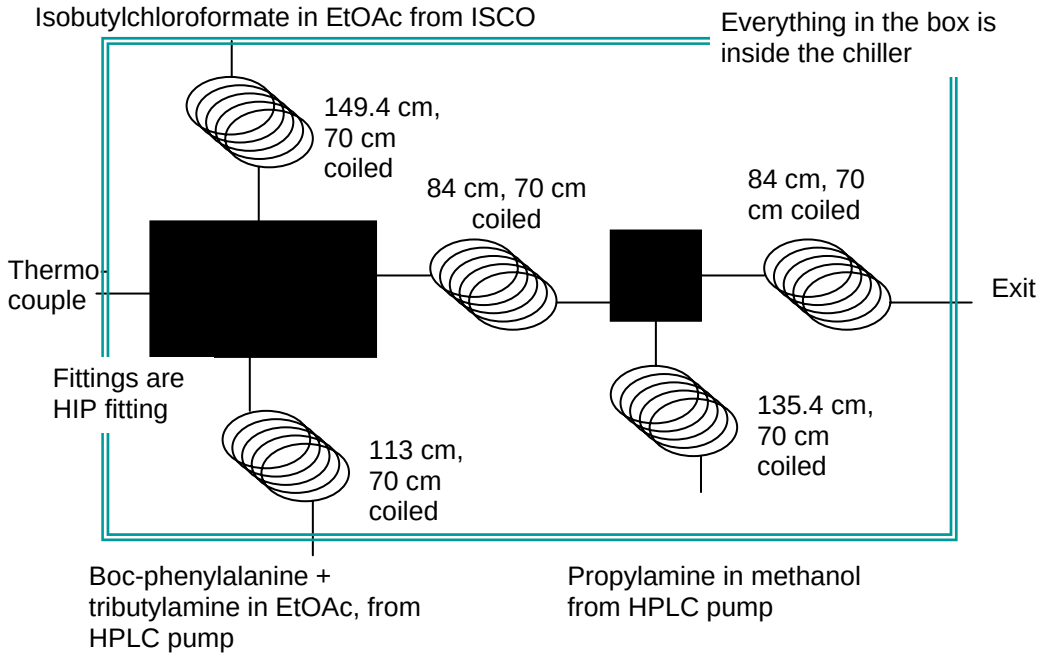


Figure 2-9: Specifications for 2nd generation continuous flow reactor

Previously, when the propylamine was added to the batch reaction, a white precipitate would form in ethyl acetate. To avoid the formation of a precipitate, methanol was used as solvent.

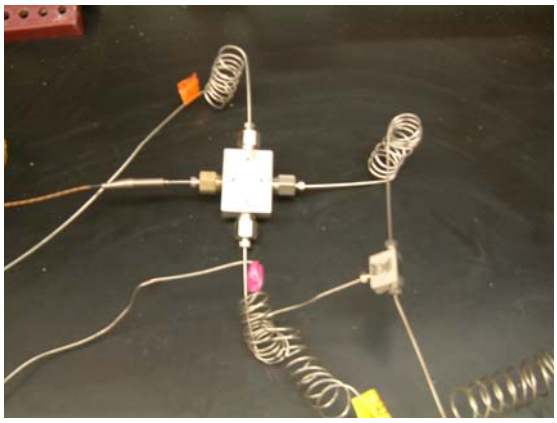


Figure 2-10: Picture of 2nd generation continuous flow reactor

The 2nd generation continuous flow reactor was run five times using conditions summarized in Figure 2-11 below. Experiments were run with the 2nd generation continuous flow reactor placed within the chiller set at -20ºC. The two reactant solutions of L-boc-phenylalanine , tributylamine in ethylacetate and isobutylchloroformate in ethylacetate were 0.04 M. The propylamine quench was added in excess (0.3 M) for the first three runs and then at 1.5 equiv for run 4 and 5 (adjusted for the difference in flow rate). Runs 2-4 were rotavapped after they

were collected and analyzed using ^{1}H NMR. Run 5 was the only run that was worked up like the batch reactions, using a saturated aqueous sodium bicarbonate wash, water wash, and brine wash. The flow rates of the L-boc-phenylalanine , tributylamine in ethylacetate solution and isobutylchloroformate in ethylacetate solution were set to match at 2.4 mL/min. The quench of propylamine in methanol was set at 2.0 mL/min. During run 2, the end solution was collected for 6 minutes. During run 3-5, the end solution was collected for 20 minutes. Regardless of the settings, the temperature at the mixing point of the two reagent streams did not change.

Run	Concentration (M)				Flowrate (mL/min)			Temp (C)	Time
	Boc-PA	TBA	IBCF	Propyla	B+T	IBCF	Quench		
1	0.04	0.04	0.04	0.3	2.4	2.4	2.0	-19.6	Pump broke
2	0.04	0.04	0.04	0.3	2.4	2.4	2.0	-19.7	6 min
3	0.04	0.04	0.04	0.3	2.4	2.4	2.0	-19.7	20 min
4	0.04	0.04	0.04	0.072	2.4	2.4	2.0	-19.9	20 min
5	0.04	0.04	0.04	0.072	2.4	2.4	2.0	-20.2	20 min

Figure 2-11: 2nd generation continuous flow reactor runs 1-5 with concentrations and flow rates

The desired amide product was not seen by ^{1}H NMR. Interestingly, all the ^{1}H NMRs were consistent from batch to batch. The ^{1}H NMR of the product, (2-phenyl-1-propylcarbamoyl-ethyl)-carbamic acid tert-butyl ester, and the

continuous flow reactor results are shown in Figure 2-12 and 2-13, respectively. The ^{1}H NMR of the continuous flow reactor results shows the three places at which the spectrum is dissimilar by the boxes.

To determine why the continuous flow reactor and batch results were different, the reaction was run in batch mode with stainless steel tubing to determine if the stainless steel was interfering with the reaction. I was able to obtain a yield of 52% for the 0.04 M concentration compared to the optimal isolated yield of 62% for the 0.04 M concentration. This was not a significant difference and therefore the stainless steel was not believed to interfere with the reaction's reagents and/or intermediates. Another considerations was that the residence time in the continuous flow reactor might have been too short, not providing enough time for the reaction to take place. Therefore, the reaction time was studied on batch-mode reactions using the 0.04 M reaction concentration.

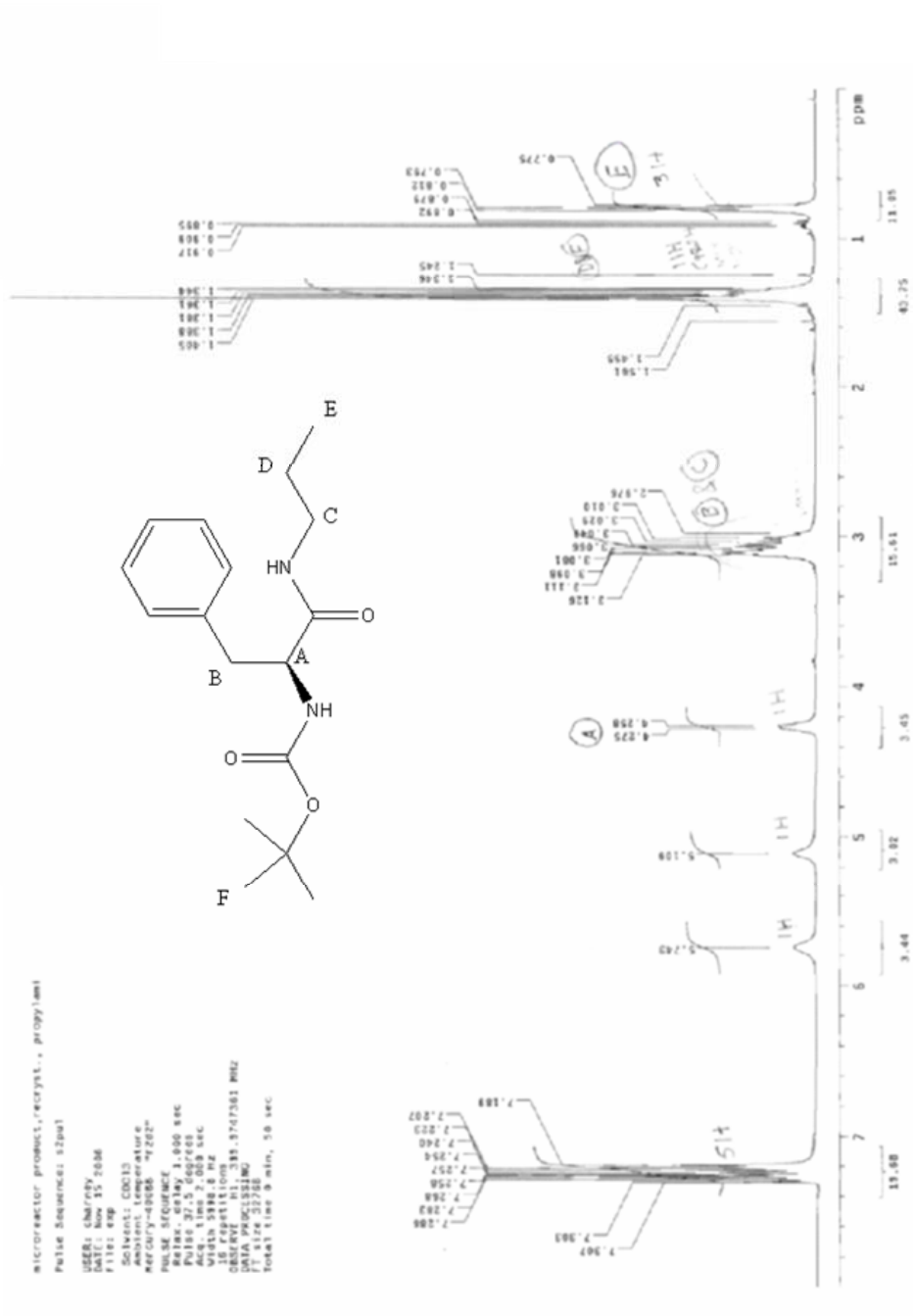


Figure 2-12: (2-Phenyl-1-propylcarbamoyl-ethyl)-carbamic acid tert-butyl ester ^{1}H NMR

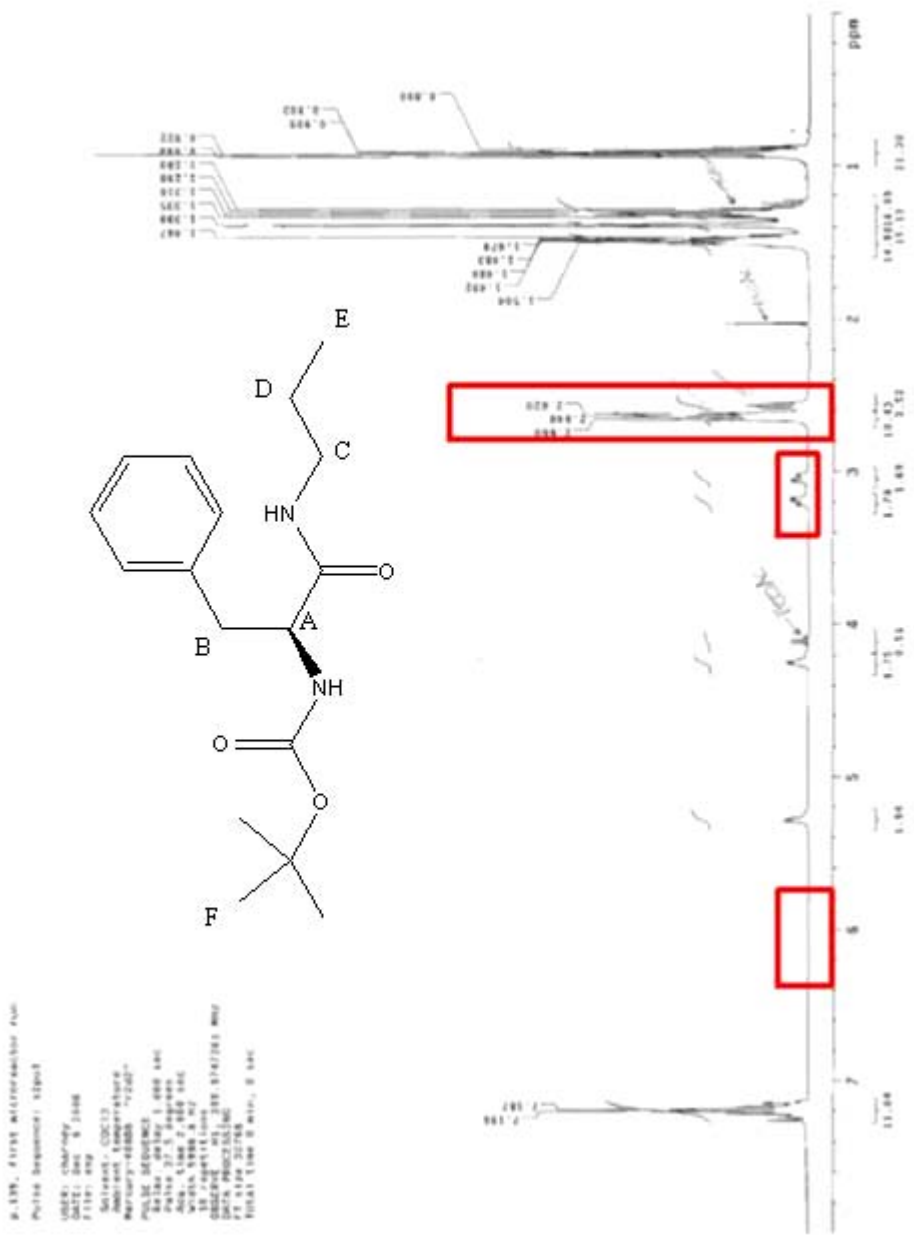


Figure 2-13: 2nd generation continuous flow reactor results by ^{1}H NMR

I made a solution of the L-boc-phenylalanine (0.75 g), tributylamine (0.7 mL), isobutylchloroformate (0.4 mL) in dry ethylacetate (75 mL), making a 0.04 M concentration and cooled in a -30ºC bath under argon. I made a separate solution of propylamine (0.1 mL, 1.5 equiv) in dry methanol (20 mL), for a 0.06 M concentration. I put 0.5 mL of the propylamine solution into 12 vials, which were all placed in an ice bath. An 0.5 mL aliquot of the reaction solution was removed at various time intervals, 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 45, 60 minutes and added to the propylamine vials. The quenched aliquots were analyzed on the LC-UV using the previously mentioned calibration curves to determine the product to starting material ratio. I also analyzed the 4 minutes and the 30 minutes samples by ^{1}H NMR to confirm the LC-UV results. A graph of the appearance of the product and disappearance of the starting material as a function of time is shown in Figure 2-14.

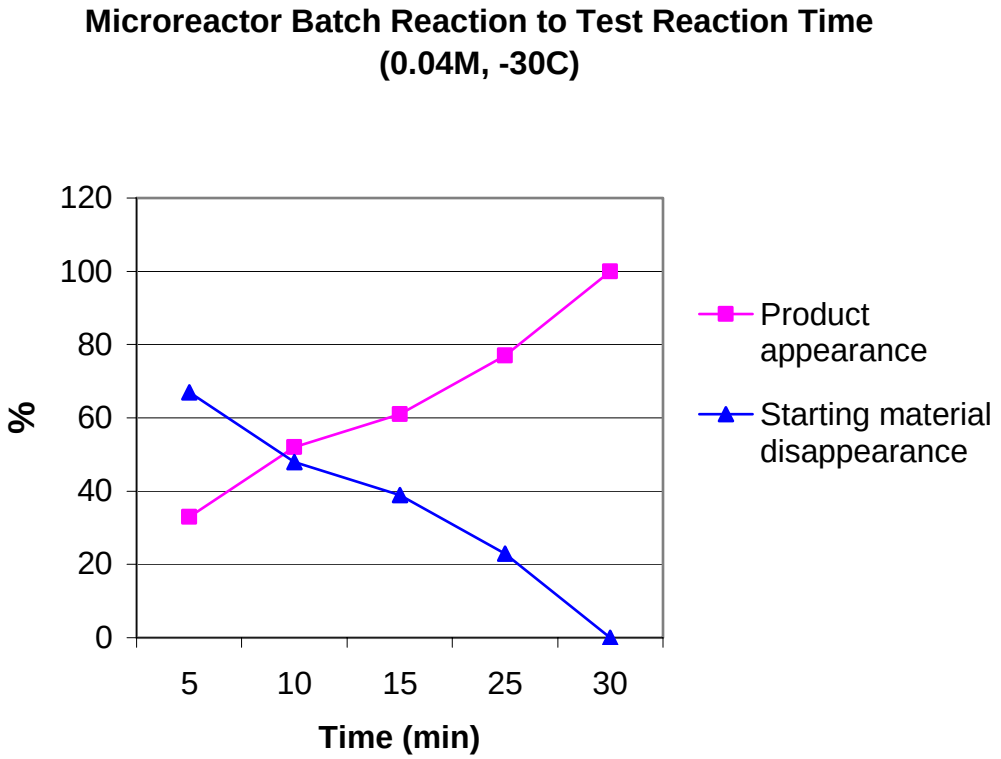


Figure 2-14: Percent of product and percent of starting material vs. time, Batch reaction at 0.04M quenched at various times and tested using LC-UV

After 30 minutes at 0.04 M, all the starting material had reacted and only product was observed. Prior to 5 minutes, the analyses show only starting material. Since the residence time in the continuous reactor was estimated to be less than four minutes, it is possible that the reaction did not have time to take place. Therefore, the reaction time was studied using solution at concentration of 0.75 M (instead of 0.4 M). The reaction was monitored as previously using the

LC-UV. The appearance of product was observed after only fifteen seconds. Subsequently, the reaction was still monitored by LC-UV but the isolated yields were also obtained for each reaction time. The maximum yield was about 76% as shown in Figure 2-15.

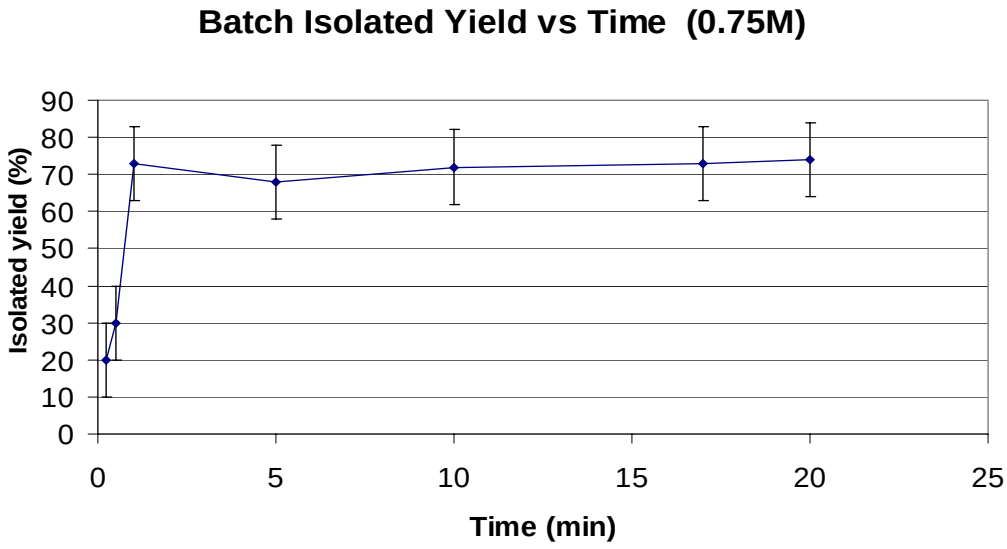


Figure 2-15: Isolated yield vs. time of batch reaction from 0.75M at -30ºC

Experimentally, I made a stock solution (0.75 M) of L-boc-phenylalanine and tributylamine in dry ethyl acetate. For each reaction, I used 2.8 mL of stock solution. I added isobutylchloroformate (0.15 mL) to each reaction. I let the reaction proceed for 5, 15, 30 seconds and 1, 5, 10, 20 minutes before adding the quench solution. The quench solution was a 1.1 M propylamine in dry methanol.

I used 1.4 mL of the quench solution for each reaction. Each experiment was run in triplicate. The isolated yield was determined for each experiment. Melting point and ^{1}H NMR were used to confirm product purity. I did not see any product formation at 5 seconds. I observed incomplete reaction at 15 and 30 seconds for a 20% and 30% isolated yield, respectively. I was able to obtain the maximum yield of 76% after 1 minute of reaction time.

I calculated the tubing length needed for 1 minute of residence time with the current continuous flow reactor design to be 526 cm. The tubing length was increased to 583 cm and three runs were performed. For each of the runs, the L-boc-phenylalanine , tributylamine in dry ethyl acetate (0.75 M) were added by an HPLC pump. The isobutylchloroformate in dry ethylacetate (0.75 M) was added by a second HPLC pump. The propylamine in dry methanol (1.35 M, adjusted concentration for the different flow rate) was added downstream in the continuous flow reactor as the quench stream by an HPLC pump. The reactants streams were run for 2 minutes through the continuous flow reactor before beginning collection. The reaction mixture was then collected for 5 minutes. The chiller was set at -20ºC for runs 1 and 2 and increased to -10ºC for run 3. After run 1, the flow rate was decreased to 0.8 mL/min from 2.4 mL/min for the reactant streams to increase the residence time from 1 min to 3.4 min (Figure 2-16). For the first time, product was detected by ^{1}H NMR for all three runs. The isolated yield was 2% with run 2.

Run	Flow rate (mL/min)	Temp (ºC)	Residence time (min)
1	2.4	-20	1
2	0.8	-20	3.4
3	0.8	-10	3.4

Figure 2-16: 2nd generation continuous flow reactor runs 1-3 with 583 cm reaction tubing. Product observed with all runs and best isolated yield was 2% with run 2

In light of the last results, two options were considered to improve the formation of the product: 1) increase the tubing length (and therefore residence time) or 2) decrease the inner diameter of the tubing to be in a microreactor regime. A 3rd generation continuous flow reactor was built.

2.3.5 Design and Use of 3rd Generation Continuous Flow Reactor

In building the 3rd generation continuous flow reactor, HPLC tubing with an inner diameter of 0.17 mm (compared to the 7 mm inner diameter for the 2nd generation) was used (Figure 2-17). HPLC tubing offered many advantages: their inner diameter is guaranteed by the manufacturer, they are very flexible and are cleaned of any particles, helping to minimize potential clogging. Also, a cleaning kit could be bought if a clog did occur.

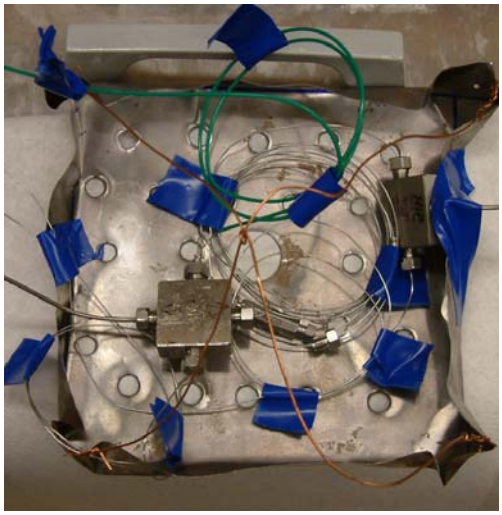


Figure 2-17: Photo of 3rd generation continuous flow reactor

The basic schematic of the 2nd generation continuous flow reactor was retained for the 3rd generation continuous flow reactor (Figure 2-18). The L-boc-phenylalanine and tributylamine in ethyl acetate and the isobutylchloroformate in ethylacetate are both added by separate streams to the cross fitting. There is a thermocouple in the cross fitting to measure the temperature at the mixing point. The propylamine quench in methanol is added by a third stream through a T-fitting. I originally started with 400 cm of tubing between the cross fitting where the reactants meet and the T-fitting where the quench is added. At the beginning of the reactant stream tubing, I added an HPLC mixer, which contains stainless steel beads, to improve the mixing.

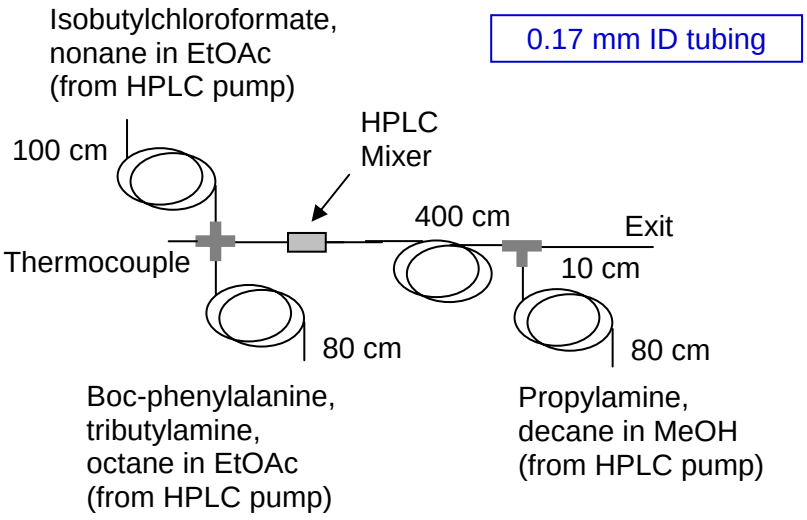


Figure 2-18: Schematic of 3rd generation continuous flow reactor

Since the HPLC pumps have a dial with number settings that do not always correlate with the flow rate, the flow rates of the three HPLC pumps were calibrated. I then set the reactant pumps both to a 0.6 mL/min flow rate. The quench pump was set to a flow rate of 1.0 mL/min. With the pressure drop, the overall flow rate was measured to be 1.7 mL/min. This flow rate resulted in a 5.4 second residence time. I then ran the continuous flow reactor using the 0.75 M combined concentration of the L-boc-phenylalanine, tributylamine, and isobutylchloroformate in dry ethylacetate and using a concentration of 1.5 M of propylamine in dry methanol as the quench. I set the chiller at seven different temperatures, -20ºC, -10ºC, 0ºC, 10ºC, 20ºC, 25ºC, 50ºC to determine the effect of temperature on the yield. The reactants and quench were flushed through the

continuous flow reactor for 2 minutes before each temperature change. The product stream was collected in duplicate for each temperature. The purity of the isolated product was confirmed using melting point and ^{1}H NMR. The -20ºC showed trace amounts of product in the ^{1}H NMR. The rest of the temperatures had measurable isolated yield and these results are summarized in the Figure 2-19. These results illustrate that isolated yield increases with increasing temperature for this continuous flow configuration.

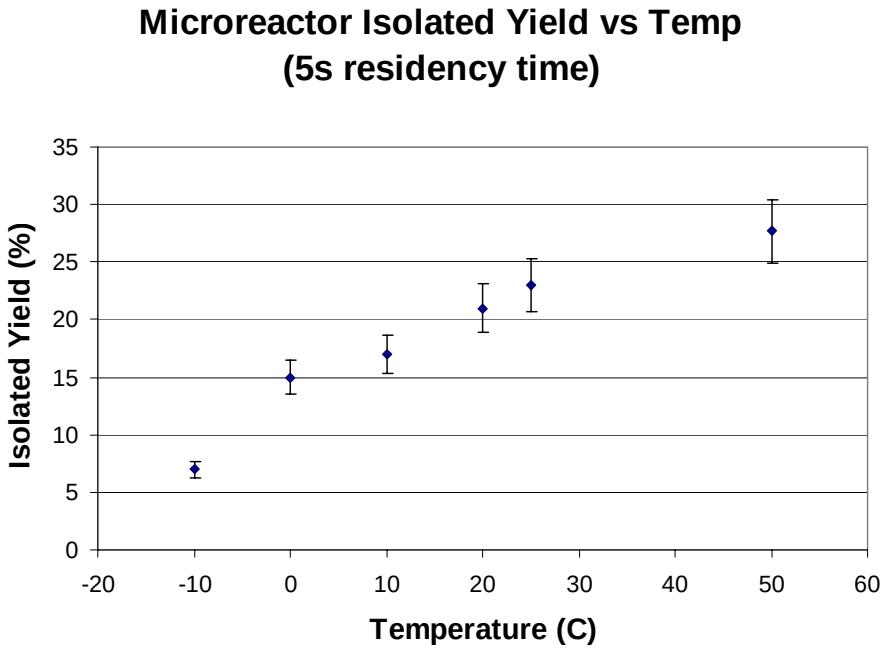


Figure 2-19: 3rd generation continuous flow reactor isolated yield results with 5 sec residence time at various temperatures

However, the increase in temperature from 25 to 50ºC brings an increase in yield that was within error whereas the increase in temperature from -20 to 25 ºC resulted in a definitive and large increase in yield. The ability to increase the reaction temperature above -20ºC is significant. In a batch reactor, the optimum temperature is -20ºC. This temperature effect is attributed to the fact that the mixed anhydride intermediate is very temperature sensitive. Being able to perform the reaction at room temperature could reduce energy costs. In addition, the yields of the 3rd generation continuous flow reactor are significantly better (about 25 %) than the yields of the 2nd generation continuous flow reactor (2%). However, the still low yield clearly indicates that the process was not yet fully optimized.

The length of tubing was increased from 400 cm to 720 cm (Figure 2-20). I ran experiments in the continuous flow reactor at 10ºC, 25ºC, and 50ºC using the 0.75 M concentration and isolated the yields. The reactants and product streams were flushed through the continuous flow reactor for 2 minutes for each temperature. The product stream was collected in duplicate for three minutes at each temperature. The isolated yields for the 720 cm continuous flow reactor were lower than the isolated yield for the 400 cm continuous flow reactor. As an example, the yield at 50ºC for 720 cm was 18+/-5% compared with the 28+/-% isolated yield for 400 cm. These results strongly suggested that other factors than tubing length were in play.

First, the individual flow ratios of each stream were measured. Alkanes were used as traces in each stream, namely, octane, nonane, and decane. The exit stream was analyzed using GC-MS and the area of the hydrocarbon peaks to determine the ratios. Theoretically, the relative ratio of the octane and nonane peaks should each be 25% and the decane, from the quench pump, should be 50%. Practically, the flows were adjusted to obtain octane 22%, nonane 28%, and decane 50%.

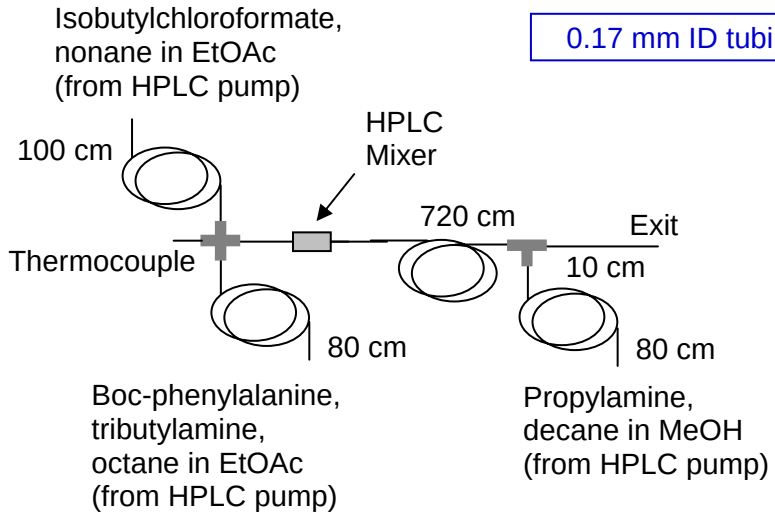


Figure 2-20: Schematic of 3rd generation continuous flow reactor with 720 cm tubing

After optimizing the pumps using traces of hydrocarbons, I resumed working with the reagent streams to which were added 1% by volume

hydrocarbon trace as an internal standard. The difference in viscosity between the actual reactants and the pure hydrocarbons required me to optimize the pumps again at 25ºC by changing the settings five times to get 27% octane, 28% nonane, and 45% decane (Figure 2-21).

I ran experiments in the continuous flow reactor at 25ºC using the optimum flow rates and isolated the product. One reactant stream was L-boc-phenylalanine, tributylamine and octane (1% vol) in dry ethylacetate and the other reactant stream was isobutylchloroformate and nonane (1% vol) in dry ethylacetate which combined gave a concentration of 0.75 M. The quench stream was propylamine and decane (1% vol) in methanol, giving a 1.5 M stream. The residence time was measured at 19.2 seconds. The continuous flow reactor was flushed with the quench and reactants for 3 minutes. Then, 5 mL of the product stream was collected and this in duplicate. The ratios of the hydrocarbons in the streams were systematically monitored by GC-FID and determined to be 26% (octane), 27% (nonane), and 47% (decane). The isolated yield was 30 +/-5% compared to 20 +/- 5% for the 400 cm tubing and 5.4 sec residence time at 25ºC. Although the residence time was 4 times longer with this set-up, the yield only increased by 50%. These results strongly suggested that the limiting factor was mixing.

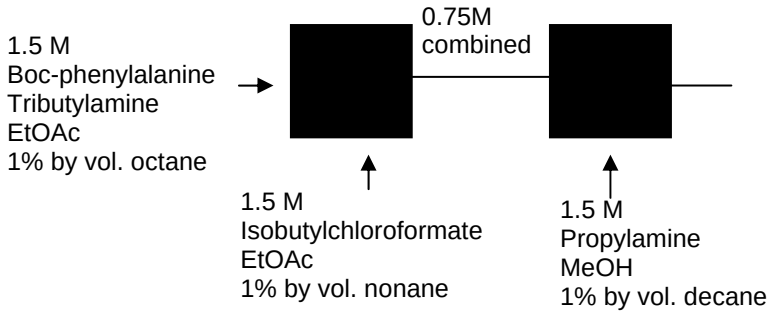


Figure 2-21: Optimize pumps by flow rate ratios using a hydrocarbon trace

To improve the mixing, a HPLC column (25 cm) repacked with 3 mm glass beads (Figure 2-22) were introduced. The tubular reactor was added after 320 cm of tubing, approximately in the middle of the system. The continuous flow reactor was run used the same concentrations, flow rates, and experimental procedures as mentioned previously. The residence time was measured to be 10.2 seconds and the hydrocarbon trace showed that the flow rates were 17% octane, 23% nonane, and 60% decane. The isolated yield was 40+/-5% compared to 30+/-5% previously.

Literature reports have mentioned using sonication as a means to increase mixing in microreactors.[12] The continuous flow reactor was placed in a sonicator. An experiment was run with the same conditions but with simultaneous sonication. The isolated yield from the sonicator was 30+/-5%, which was not an improvement. This option was not investigated further.

I also tried putting 0.5 mm glass beads in the cross fitting to improve the mixing where the streams first come into contact. However, the beads clogged the system so this route was abandoned.

Smaller diameter beads (0.5 mm instead of 3 mm) were used to repack the tubular reactor. Smaller bead size could induce more turbulence and therefore better mixing. The isolated yield increased to 47+/-5% at room temperature which was approximately a 5% improvement over the 3 mm glass beads.

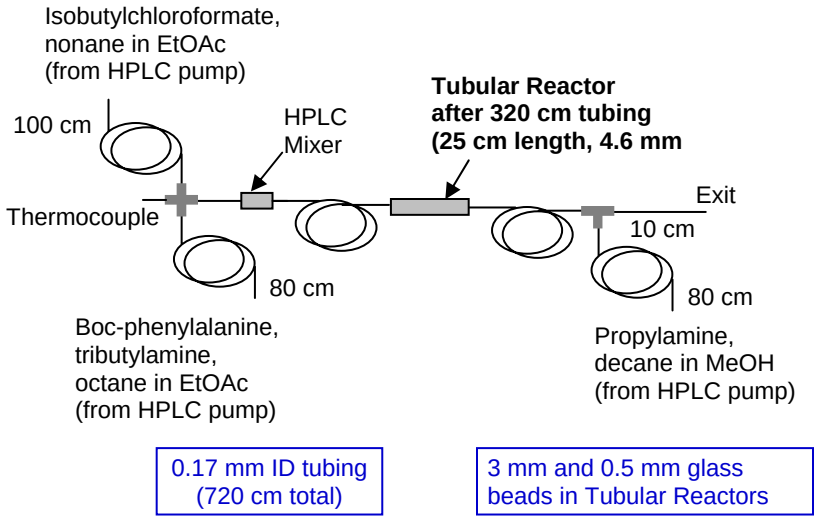


Figure 2-22: Schematic with tubular reactor added to the 3rd generation continuous flow reactor

Since the tubular reactor filled with glass beads seemed to have the largest mixing effect, a second tubular reactor was added to the system (Figure 2-23). The tubular reactor was 15 cm long and had an inner diameter of 4 mm. The silica inside an HPLC column was removed and the column was repacked with 0.5 mm glass beads. This tubular reactor was added after 240 cm of tubing and the first tubular reactor was moved to after 480 cm of tubing as seen in Figure 2-23. Experiments in the continuous flow reactor were run with the same concentrations, flow rates, and experimental procedures mentioned previously. The flow rates were optimized for the system at room temperature and the best isolated yield of 60+/-5% was obtained using a 0.1 mL/min flow rate (Figure 2-24). The improvement from 0.3 mL/min to 0.1 mL/min was probably due to a longer residence time with the slower flow rate. The improvement from 0.05 to 0.1 mL/min is probably due to better mixing with the faster flow rate. The balance between these two factors demonstrated the need to optimize both flow rate and mixing.

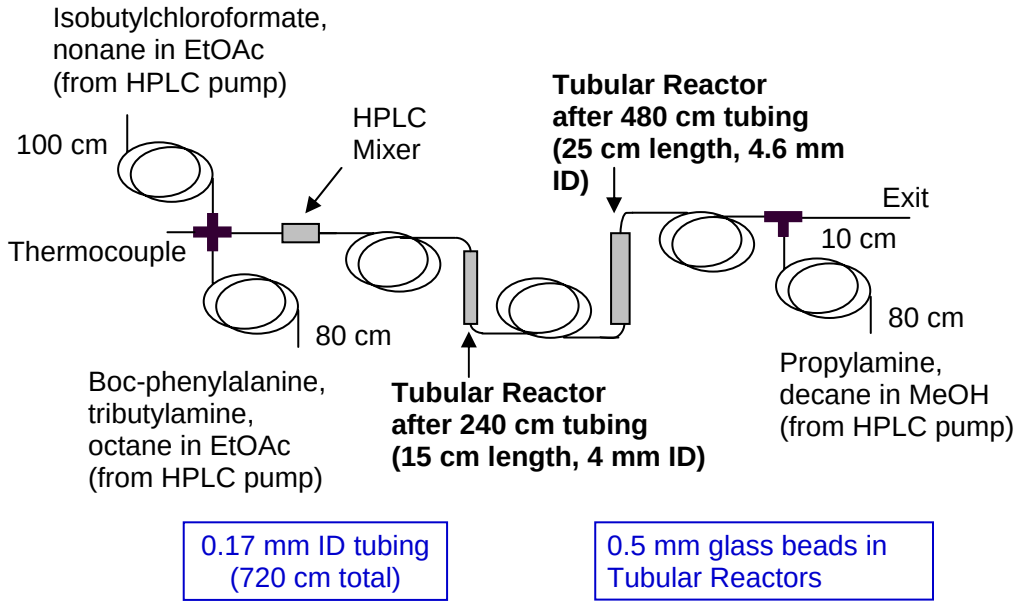


Figure 2-23: Continuous flow reactor schematic with 2 tubular reactors filled with 0.5 mm glass beads

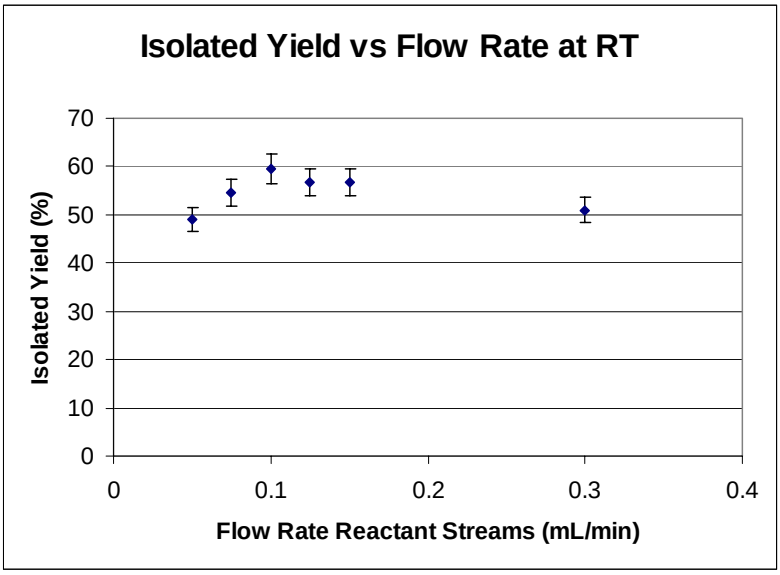


Figure 2-24: Optimized flow rate for reactants for 2 tubular reactors continuous flow reactor system at room temperature

Bends have been shown to induce chaotic mixing in systems with laminar flow.[13] One cm sharp bends to an 80 cm piece of HPLC tubing were created. The bent tubing was added after 160 cm of tubing and a flow rate of 0.3 mL/min for the reactants was used. Experiments in the continuous flow reactor were run using the same concentrations and experimental procedures. The isolated yield was 55+/-5% compared to 51+/-5% without the bent tubing at room temperature. The slight improvement in yields is not significant and is within experimental error.

Two additional tubular reactors were built, packed with glass beads and added to the system (Figure 2-25). Both tubular reactors were 20 cm in length

and had an inner diameter of 4.6 mm. They were filled with 0.5 mm glass beads and the beads were packed using a vibrator. One tubular reactor was added after 320 cm of tubing and the other tubular reactor was added after 560 cm of tubing. The original two tubular reactors were left on the system in their original position. Various flow rates between 0.1 and 0.4 mL/min were run and the product isolated (Figure 2-26). The best flow rate was 0.2 mL/min, giving an isolated yield of 53+/-5% at room temperature. It was not expected that the yield would decrease upon extending residence time and increasing mixing. With a longer residence time, the product may start degrading. When the experiments were repeated at lower temperatures of -20ºC and 0ºC, the isolated yields increased. This result was expected since at lower temperature the intermediate decomposition will indeed be minimized (Figure 2-27).

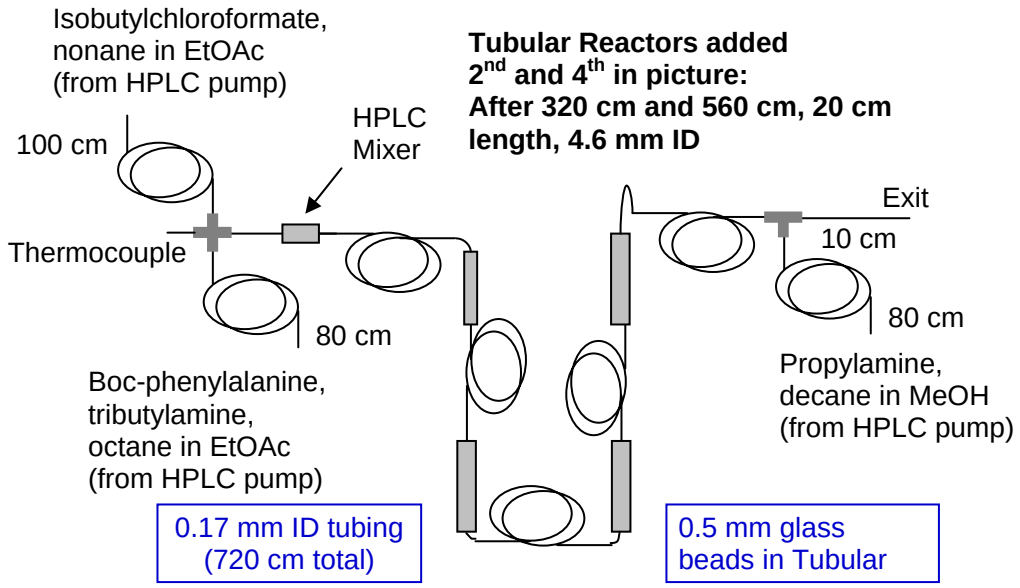


Figure 2-25: Continuous flow reactor schematic of 4 tubular reactor system filled with 0.5 mm glass beads

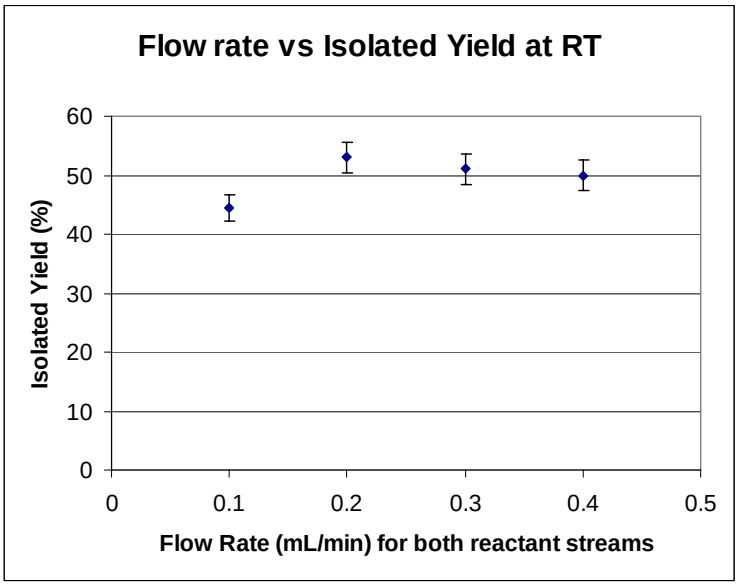


Figure 2-26: Flow rate vs. isolated yield for the 4 tubular reactor system with 0.5 mm glass beads

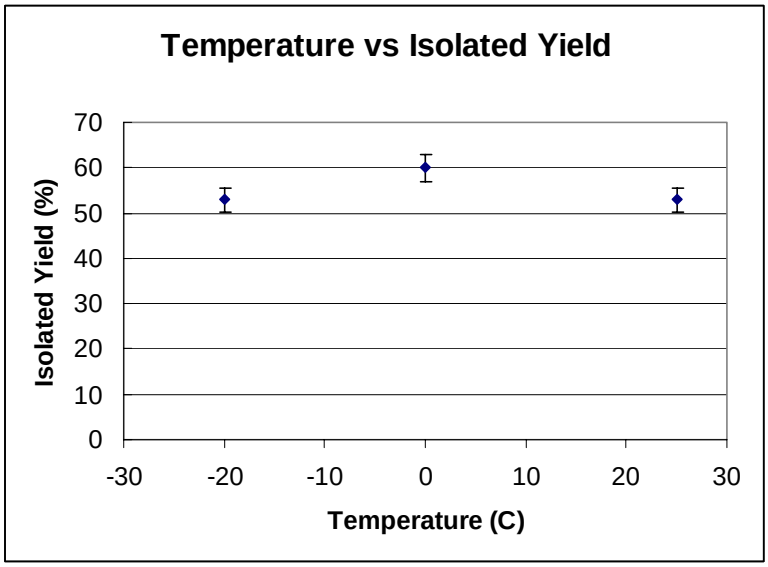


Figure 2-27: Temperature vs. isolated yield on the 4 tubular reactor system

With the 4 tubular reactor system, the best isolated yield was 60+/-5% at 0ºC. The batch reaction was repeated at room temperature instead of -20ºC as it has been originally studied. As shown in Figure 2-19, when the continuous flow reactor was tested at various temperatures, the yield increased as the temperature increased. This trend was not expected and was attributed to the excellent heat transfer capabilities and mixing of the continuous flow reactor. Nonetheless, reactions were repeated in a batch-mode at 25ºC instead of -20ºC. The first reaction was quenched after 16 minutes to mimic the residence time of the 2 tubular reactor continuous flow reactor and gave an isolated yield of 37+/-5%. The second reaction was quenched after 1 hour, like the original reaction

conditions, and gave an isolated yield of 27+/-5%. The drop in isolated yield from 16 minutes to 1 hour confirmed that the mixed anhydride intermediate decomposed over time at room temperature. This experiment clearly demonstrated that the 2 tubular reactor continuous flow reactor is superior to the batch reaction at 25ºC because the continuous flow reactor gave an isolated yield of 60+/-5%. The unique heat transfer capabilities of the continuous flow reactor are clearly making a difference on the reaction performances when compared to a batch-mode process.

Finally, it is worth mentioning that increasing the equivalents of isobutyl chloroformate (2x and 3x excess) showed no effect on the isolated yield.

2.3.6 Coiled Continuous Flow Reactor

The continuous flow reactor although performing well became quite complex throughout the generations. However, in light of the last results it was clear that the HPLC tubing was not playing a major role and could be eliminated. By designing a coiled continuous flow reactor, the performances were expected to be maintained yet the system will be simpler. Besides keeping benefits like superior heat transfer and improved safety, other benefits like reduction in cost and clogging were added. The coiled continuous flow reactor was made out of stainless steel tubing with 45 cm length with an inner diameter of 4.6 mm and

filled it with 0.5 mm diameter glass beads (Figure 2-28). A photograph of the coiled continuous flow reactor can be seen in Figure 2-29.

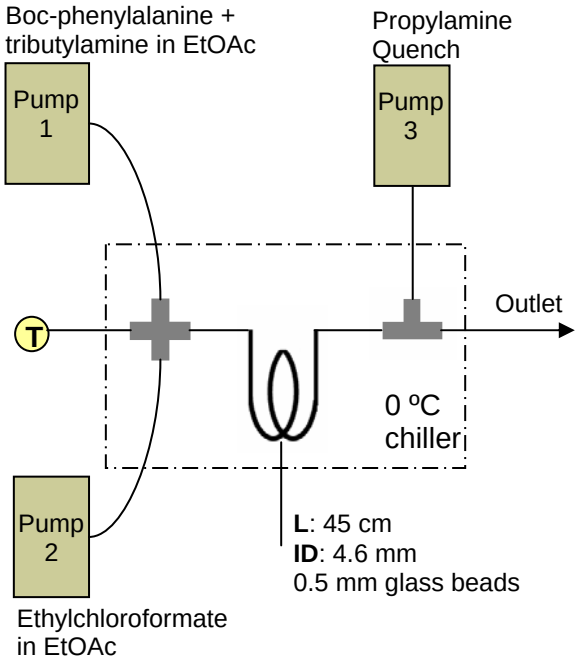


Figure 2-28: Schematic of coiled continuous flow reactor using propylamine quench

Figure 2-29: Photograph of the coiled continuous flow reactor

At this point the reaction was also to be carried out a step further, up to the formation of the diazoketone. Before doing any experiments in the continuous reactor, the reaction of the mixed anhydride with trimethylsilyl diazomethane was carried out in a batch mode. Originally, the synthesis used diazomethane, however we chose to use trimethylsilyl diazomethane, a safe alternative to diazomethane. I used a procedure analogous to one reported in the literature, that first formed the mixed anhydride that then reacts with trimethylsilyl diazomethane to form a diazoketone (Figure 2-30).[10]

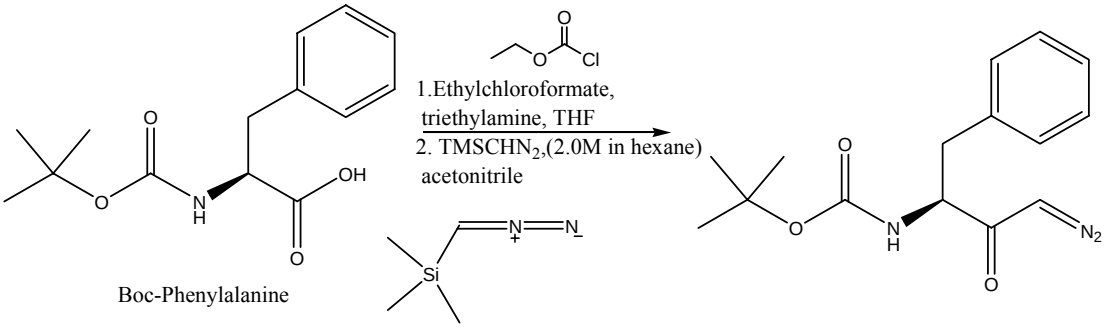


Figure 2-30: Diazoketone synthesis

The synthesis was first performed using L-boc-phenylalanine (1 equiv), isobutylchloroformate (1.3 equiv), tributylamine (1.3 equiv) in THF (0.2 M) then trimethylsilyl diazomethane (3 mL of 2.0 M solution in hexane) in acetonitrile (0.6 M). I obtained a 32% isolated yield of the 1-(benzyl-3-diazo-2-oxo-propyl)-carbamic acid tert-butyl ester. I then changed various reaction conditions as shown in Figure 2-31. The biggest effect was switching from using isobutyl chloroformate to ethyl chloroformate, resulting in a 78% isolated yield. It was hypothesized that the increase in yield was due to the smaller size of the ethyl group versus the isobutyl group, reducing the steric hindrance and making it easier for addition from the trimethylsilyl diazomethane. After obtaining a similar yield than reported in the literature (78%), I optimized the reaction for use in a coiled continuous flow reactor. Again, the triethylamine was avoided and tributylamine was preferred as its hydrochloride salt would not crash out in the

reaction mixture. I also changed the solvent to ethyl acetate since that is what I used for the previous mixed anhydride synthesis (Figure 2-32).

Isolated Yield	Variable changed
32%	Standard reaction using isobutylchloroformate
23%	Trimethylsilyl diazomethane purchased as 2.0M in diethyl ether
25%	Trimethylsilyl diazomethane 3 equivalents (9 mmol)
78%	Ethyl chloroformate
66%	Tributylamine with ethyl chloroformate
61%	Ethyl acetate with ethyl chloroformate (instead of THF)

Figure 2-31: Variables changed in synthesis of diazoketone

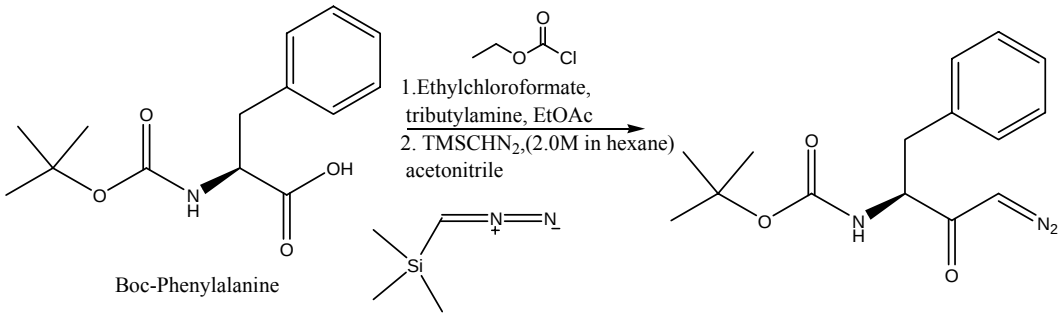


Figure 2-32: Diazoketone synthesis with reactants used for batch reactions

Before running the reaction in the coiled continuous flow reactor, an estimation of the optimum reaction time was required to set up flow rates. Since

the first step was changed from using isobutyl chloroformate to ethyl chloroformate, I needed to monitor both the first step with the propylamine quench and the second step with the addition of the trimethylsilyl diazomethane. As before, calibration curves on the LC-UV of the propylamine quench product, (2-phenyl-1-propylcarbamoyl-ethyl)-carbamic acid tert-butyl ester, and the diazoketone, (1-benzyl-3-diazo-2-oxo-propyl)-carbamic acid tert-butyl ester were prepared. I tested the reaction with ethyl chloroformate and the propylamine quench at 16 and 30 minutes. Both gave a peak on the LC-UV that correlated to the concentration that indicated 100% yield. I then ran the first step on the coiled continuous flow reactor shown in Figure 2-28. The ethyl chloroformate, anisole, an internal standard, and nonane in ethyl acetate in a 1.5 M solution were added by a pump to the cross fitting. The coiled continuous flow reactor was run using the same experimental procedures and concentrations as mentioned previously. The cross valve was connected to the continuous flow reactor which was in a chiller set at 0ºC. The results were monitored by LC-UV and the theoretical concentration was determined using the hydrocarbon trace ratios measured by GC-FID. The residence time of the coiled continuous flow reactor was estimated to be 16 minutes. With this system, the yield was quantitative (by LC-UV).

I monitored the reaction time of the diazoketone reaction using the LC-UV and sampling at various time intervals. The maximum yield of 100% was obtained after 2 hours and plateaued after this point within error (Figure 2-33). I

had been using the concentration (0.2 M) for the L-boc-phenylalanine, tributylamine, and ethyl chloroformate in ethylacetate in the literature, not the concentrations used for the previous mixed anhydride with the propylamine quench (0.75 M). By increasing the concentration for the first step, I could accelerate the reaction. I kept the concentration of the trimethylsilyl diazomethane in acetonitrile the same for safety considerations.

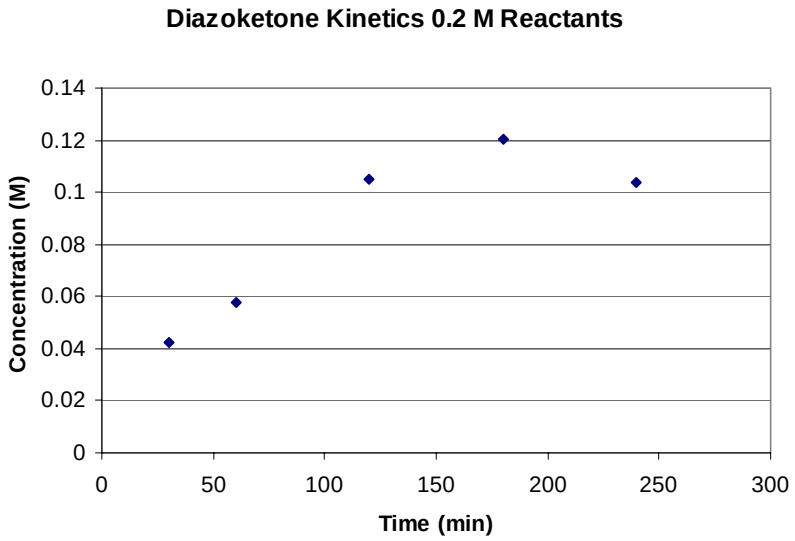


Figure 2-33: Diazoketone monitoring reaction by LC-UV for 0.2 M reactant concentration

An experiment was run with the concentration of the reagents streams being 0.75 M. Aliquots were taken at 10, 20, 30, 45, 90, and 120 minutes and analyzed by LC-UV. An internal standard, anisole, was added. Quantitative yields in the diazoketone product (within the 3% error) were obtained after as little as 10 minutes.

The continuous flow reactor, shown in Figure 2-28, was slightly modified to allow for the addition and reaction of the trimethylsilyl diazomethane. Since the trimethylsilyl diazomethane needed a longer residence time for reaction than the propylamine quench, a second coiled reactor identical to the first reactor was built and added to the original set-up. I added this second reactor after the T-fitting (Figure 2-34).

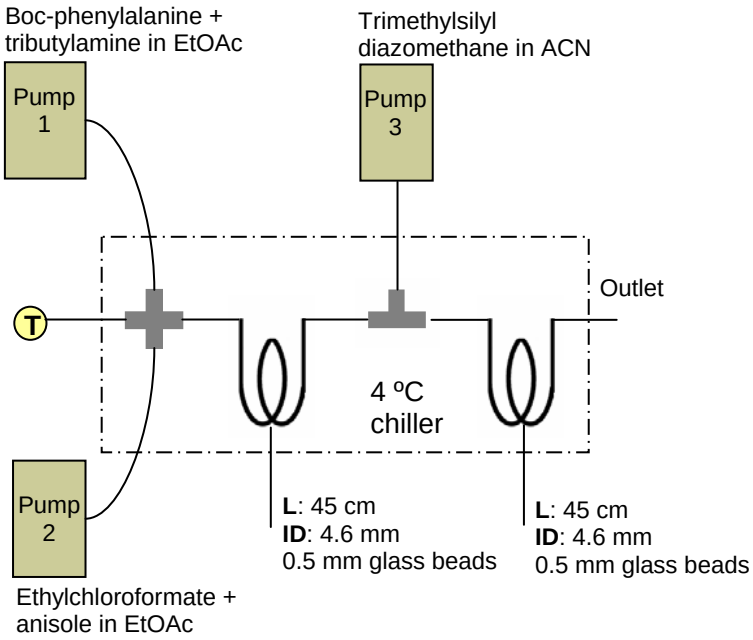


Figure 2-34: Continuous flow reactor added after T-fitting for addition of trimethylsilyl diazomethane

The L-boc-phenylalanine, tributylamine, and octane, as the hydrocarbon trace, in ethyl acetate in a 1.5 M solution were added by one pump. The ethyl chloroformate, anisole, as an internal standard, and nonane in ethyl acetate in a 1.5 M solution were added by another pump to the cross fitting. Both coiled flow reactors were placed in the chiller at 4ºC. The trimethylsilyl diazomethane and decane in acetonitrile in a 0.6 M solution were added by the T-fitting. The results were monitored by LC-UV and the theoretical concentration was determined using the hydrocarbon trace ratios measured by GC-FID. The residence time of each continuous flow reactor was estimated to be 16 minutes, for a total of 32

minutes. For this system, the yield in diazoketone product was quantitative (by LC-UV). This is remarkable as the simple design of two coiled continuous flow reactors allows for carrying out a two steps synthesis involving a very temperature-sensitive intermediate with quantitative yield.

2.4 Conclusion

The reaction of L-boc-phenylalanine with alkyl chloroformate to form a mixed anhydride followed by reaction with trimethylsilyl diazomethane was explored in a continuous flow reactor. In a batch mode, the first step of the reaction is carried at the optimum temperature -20ºC because the mixed anhydride is temperature sensitive (and decomposes readily above 0ºC). The best overall yield reported in the literature for this sequence was 78 %. During this research, several reactor configurations were built. The final configuration that involves two coiled continuous microreactors packed with glass beads is both simple and extremely efficient. The reaction sequence was carried out at 4ºC with quantitative yield in the diazoketone product. This result is remarkable. It clearly demonstrates that the continuous process not only improves yields (and product quality) over a batch process, it also utilizes cheaper and safer reagents (ethylchloroformate *vs.* isobutylchloroformate and trimethylsilyl diazomethane *vs.* diazomethane), and reduces energy intake by eliminating the need for low reaction temperatures.

2.5 Experimental

All chemicals were ordered from Aldrich or VWR and used as received, unless noted. ^{1}H and ^{13}C NMR spectra were recorded using a Varian Mercury Vx 400 spectrometer using residual $CDCl_3$ peak as an internal reference. Mass spectrometry samples were submitted to the mass spectrometry lab and used ESI-MS. GC-MS analysis was done on a HP GC 6890/ HP MS 5973. GC-FID analysis was done on a HP GC 6890 with FID detector. Elemental analyses were submitted to Atlantic Microlabs, Inc. Melting points were determined on Mettler-Toledo capillary apparatus and were uncorrected. LC-UV analysis was done on an Agilent 1100 Series LC with UV detector. UV-visible spectra were recorded on a Hewlett-Packard 8453 spectrometer. All the error bars were calculated from the standard deviation. The isolated yield of the propylamine quench were consistently +/-5% throughout the project.

Synthesis of (1-benzylcarbamoyl-2-phenyl-ethyl)-carbamic acid tert-butyl ester using triethylamine

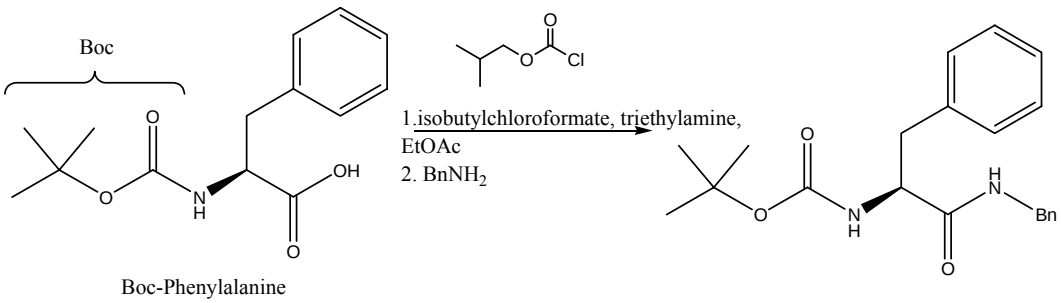


Figure 2-35: Triethylamine as HCl scavenger and benzylamine quench

L-Boc-phenylalanine (3.0 g, 0.0114 mol, 1 equiv) was added to dry ethylacetate (15 mL, 20 % wt solution). The solution was put under nitrogen and in a $CaCl_2$/ice/water bath (-30ºC). To the cold solution, isobutyl chloroformate (1.8 g, 0.015 mol, 1.3 equiv) was added. Then triethylamine (1.5 g, 0.015 mol, 1.3 equiv) was added drop-wise and a white precipitate (TEA-HCl salt) formed during the addition. The reaction was stirred for 1 hour at -30ºC. Then benzylamine (1.5 mL, 1.2 equiv) was added to quench the reaction. The reaction was allowed to warm to room temperature overnight. To work up the reaction, the TEA-HCl salt was removed by filtration and washed with cold ethylacetate. The ethylacetate solution was washed with saturated aqueous $NaHCO_3$, water, and saturated aqueous NaCl. The solution was dried over magnesium sulfate and the solvent was removed under reduced pressure. The white solid was characterized and used for a calibration curve to be used with the continuous flow reactor.

(1-Benzylcarbamoyl-2-phenyl-ethyl)-carbamic acid tert-butyl ester: ^{1}H NMR ($CDCl_3$) ppm: 1.39 (9, s), 3.06 (2, m), 4.37 (3, d), 5.02 (1, s), 6.01 (1, s), 7.10 (10, m). ^{13}C NMR ($CDCl_3$, ppm): 28.24, 38.52, 43.47, 44.73, 56.05, 126.96, 127.48, 127.46, 127.67, 128.61, 128.73, 129.34, 137.62, 170.94. GC-MS analysis was done on a HP GC 6890/ HP MS 5973. MS(m/z): 281 (loss O-C$(CH_3)_3$). EA: calculated C, 71.16%, H, 7.39%, N, 7.90%. Found C, 71.27%, H, 7.46%, N, 7.84%**.**

Solubility test of amines

Pyridine, DBU, piperidine, tripropylamine, tributylamine were all tested to determine if they formed a visible precipitate upon the addition of HCl (37% reagent grade). For all the amines, 1 g was added to 10 mL of ethylacetate. Then 1 mL of HCl was added drop wise and compared visibly to a control of triethylamine (1g), ethylacetate (10mL), and HCl (1mL). Pyridine, DBU, piperidine all showed a significant amount of precipitate. Tripropylamine and tributylamine did not show a precipitate upon the addition of HCl.

Synthesis of (1-benzylcarbamoyl-2-phenyl-ethyl)-carbamic acid tert-butyl ester using tripropylamine

L-Boc-phenylalanine (0.5 g, 0.0019 mol) was dissolved in ethylacetate (2.5 mL) to make a 20 wt % solution. The solution was put under nitrogen and

cooled in a $CaCl_2/H_2O$/ice bath (-30ºC). To the solution, isobutylchloroformate (0.3 g, 0.0025 mol) was added. Then the tripropylamine (0.36 g, 0.0025 mol) was added to the solution. However, a white precipitate formed upon the addition of the tripropylamine so this synthetic method was not pursued further.

Synthesis of (1-benzylcarbamoyl-2-phenyl-ethyl)-carbamic acid tert-butyl ester using tributylamine

L-Boc-phenylalanine (0.5 g, 0.0019 mol) was dissolved in ethylacetate (2.5 mL) to make a 20 wt % solution. The solution was put under nitrogen and cooled in a $CaCl_2$/ice/water bath (-30ºC). Isobutylchloroformate (0.3 g, 0.0025 mol) was added to this solution. Tributylamine (0.46 g, 0.0025 mol) was then added and a precipitate did not form. The reaction was allowed to continue for one hour. Then 7 mL of a 5 wt % solution of benzylamine in ethylacetate (1.0 g in 20 mL) was added to the reaction solution. The reaction was allowed to warm to room temperature overnight. No starting material was observed in ^{1}H NMR. I did not continue to purify because only wanted to confirm that a precipitate did not form in the first step.

Calibration curve of L-boc-phenylalanine

Five different amounts (0.0152 g, 0.0422 g, 0.0740 g, 0.1107 g, 0.1418 g) of L-boc-phenylalanine were used to make a calibration curve on the LC-UV at

wavelength 230 nm. The method used was RCBoc and the L-boc-phenylalanine was dissolved in 1 mL of methanol. A standard of 0.0646 g L-boc-phenylalanine in 1 mL of methanol was used to test the calibration curve. The LC calibration curve gave 0.0687 g, which was determined to be close to the actual amount of L-boc-phenylalanine used.

Synthesis of (2-phenyl-1-propylcarbamoyl-ethyl)-carbamic acid tert-butyl ester using triethylamine

L-Boc-phenylalanine (3 g) was added to dry ethylacetate (15 mL) to make a 20 wt % solution. The solution was put under nitrogen and in a $CaCl_2$/water/ice bath (-30ºC). Triethylamine (2 mL, 1.3 equiv) and isobutylchloroformate (1.8 mL, 1.3 equiv) were added to the solution. The solution was stirred for 1 hour. The propylamine (1.4 mL, 1.5 equiv) was then added and the solution was stirred for another hour. The reaction was then filtered and the solid, TEA-HCl salt, was washed with ethylacetate. The organic phase was washed with saturated aqueous $NaHCO_3$, water twice, saturated aqueous NaCl and dried over magnesium sulfate. The solvent was then removed under reduced pressure. The white solid was stirred with cold hexane and filtered (76% yield).

(2-Phenyl-1-propylcarbamoyl-ethyl)-carbamic acid tert-butyl ester using tributylamine: ^{1}H NMR ($CDCl_3$) ppm: 0.88 (3, m), 1.34 (11, m), 3.05 (4, m), 4.25 (1, m), 5.11 (1, s), 5.74 (1, s), 7.25 (5, m). ^{13}C NMR ($CDCl_3$) ppm: 11.215, 22.56,

28.25, 38.76, 41.11, 56.09, 80.10, 136.89, 128.63, 129.30, 136.86, 155.370, 170.94. EA: calculated C, 66.64%, H, 8.55%, N, 9.14%. Found C, 66.76%, H, 8.61%, N, 9.12%.

Calibration curve of (2-phenyl-1-propylcarbamoyl-ethyl)-carbamic acid tert-butyl ester

The LC-UV at 229 nm was used to make a calibration curve of the product. The method name was RCMICROP. Different amounts of product (0.356 g, 0.0527 g, 0.1028 g, 0.0744 g, 0.0148 g) were added to five vials and 0.5 mL of methanol was added. The concentration (0.2324 M, 0.3440 M, 0.6710 M, 0.4856 M, 0.0966 M) was calculated and used to make the calibration curve. The calibration curve was tested using a standard of 0.2304 M. The calibration curve gave 0.231 M, which was within error to the known standard concentration.

Synthesis of (2-phenyl-1-propylcarbamoyl-ethyl)-carbamic acid tert-butyl ester using tributylamine

L-Boc-phenylalanine (0.5 g, 0.0019 mol) was dissolved in dry ethylacetate (2.5 mL) to make a 20 wt % solution. The solution was put under nitrogen and cooled in a $CaCl_2$/ice/water bath (-30ºC). Isobutylchloroformate (0.3 g, 0.0025 mol) was added to this solution. Dry tributylamine (0.46 g, 0.0025 mol) was then added. The reaction was allowed to continue for one hour. Dry propylamine

(0.23 mL, 0.0029 mol, 1.5 equiv) in dry ethyl acetate (1 mL) was added to the solution. The reaction was allowed to warm to RT overnight. The reaction solution was washed with saturated aqueous $NaHCO_3$, water, and saturated aqueous NaCl solution. The ethyl acetate layer was dried over magnesium sulfate and solvent was reduced under pressure. The resulting white solid (76% yield) was characterized.

(2-Phenyl-1-propylcarbamoyl-ethyl)-carbamic acid tert-butyl ester using tributylamine: ^{1}H NMR ($CDCl_3$) ppm: 0.88 (3, m), 1.34 (11, m), 3.05 (4, m), 4.25 (1, m), 5.11 (1, s), 5.74 (1, s), 7.25 (5, m). ^{13}C NMR ($CDCl_3$) ppm: 11.215, 22.56, 28.25, 38.76, 41.11, 56.09, 80.10, 136.89, 128.63, 129.30, 136.86, 155.370, 170.94. EA: calculated C, 66.64%, H, 8.55%, N, 9.14%. Found C, 66.76%, H, 8.61%, N, 9.12%**.**

1st generation continuous flow reactor

The continuous flow reactor was run using 0.04 M solutions. The L-boc-phenylalanine solution was L-boc-phenylalanine (0.75 g) and dry tributylamine (0.7 mL) in dry ethylacetate (75 mL). This solution was pumped into the system using an HPLC pump from a round bottom in the chiller. The isobutylchloroformate (2.4 mL) solution was in ethylacetate (450 mL) and was pumped into the system using an ISCO. The propylamine solution was propylamine (0.16 mL) in ethylacetate (5 mL) which was based on having 1.5

equiv of propylamine after a 10 min run at 3.3 mL/min. The chiller was set at -20ºC. The L-boc-phenylalanine solution alone was run through the system for 2 minutes, giving a reading of -7.8ºC on the thermocouple. The ISCO was set for 3.3 mL/min to match the HPLC pump flow rate. All the reactants were run through the continuous flow reactor for 2 min to flush the system. Then the reactant solution was dripped into the flask containing the propylamine solution for 10 min. The thermocouple reading increased to -7.3ºC during the run. The propylamine solution with the reactants was put into a round bottom and the solvent was removed under reduced pressure, resulting in an oil. The NMR peaks and the LC-UV retention time did not correlate with the product. The experiment was repeated with the same results.

2nd generation continuous flow reactor specifications

The cross fitting and T-fitting are HIP fittings made of stainless steel. The tubing has an inner diameter of 7 mm and was made of stainless steel. The HPLC pumps were from Eldex. The tubing lengths can be seen in Figure 2-9.

2nd Generation Continuous flow reactor 0.04M

Run 2-3 (pump broke with run 1)

L-Boc-phenylalanine (0.75 g) and dry tributylamine (0.7 mL) were added to dry ethylacetate (75 mL) to make an 0.04 M solution. Isobutylchloroformate

(0.6 mL) was added to dry ethylacetate (112 mL) to make an 0.04 M solution. Dry propylamine (2 mL) was added to dry methanol (75 mL) to make an 0.3 M solution which was a large excess of propylamine for the quench. The chiller was set to -20ºC and the thermocouple read -19.7ºC. The reactant flows were run and collected for 6 minutes for run 2 and for 20 minutes for run 3 and the solvent was removed under reduced pressure, giving an oil. The NMR did not show any product formation.

Run 4

The L-boc-phenylalanine solution and the isobutylchloroformate solution are the same as for runs 1-3. The propylamine concentration was reduced to 1.5 equiv which was 0.072 M when corrected for the slower flow of the quench pump. Propylamine (0.44 mL) was added to dry methanol (75 mL). The system was flushed with all the reactants for 1 minute. Then the solution was collected for 20 min and the solvent was removed under reduced pressure. The NMR did not show any product formation.

Run 5

The solutions were made the same way as run 4. The system was flushed for 1 minute. Then the solution was collected for 20 min. The solution was then worked up the same way as the batch reaction, first the solution was washed with water, saturated aqueous $NaHCO_3$, and saturated aqueous NaCl. The organic

phase was dried over magnesium sulfate and solvent was removed under reduced pressure. The resulting oil did not show any product by NMR.

Batch reaction at 0.04 M

L-Boc-phenylalanine (0.75 g) was dissolved in dry ethylacetate (75 mL). The reaction was put under nitrogen and in a $CaCl_2$/water/ice bath (-30ºC). Tributylamine (0.7 mL) and isobutylchloroformate (0.4mL) were added to make an 0.04 M solution. The reaction was stirred for 1 hour. Then dry methanol (20 mL) and propylamine (0.1 mL) were added to have a 0.06 M solution of propylamine and the reaction was stirred at room temperature overnight. To workup the reaction mixture, the organic phase was washed with saturated aqueous $NaCO_3$, water twice, and saturated aqueous NaCl. The organic phase was dried over magnesium sulfate and the solvent was removed under reduced pressure. To purify the resulting white solid, the solid was washed with cold hexane and filtered. The solid was pure by ^{1}H NMR with a 62% yield.

Batch reaction at 0.04 M with steel

The reaction conditions were the same as above except a small piece of stainless steel tubing was added to the round bottom. This was to determine if the stainless steel was hindering the reaction in the continuous flow reactor. The product was pure by ^{1}H NMR with a 52% yield.

Test Reaction time using 0.04M batch reactions

L-Boc-phenylalanine (0.75 g) was dissolved in dry ethylacetate (75 mL). The solution was put under argon and in a $CaCl_2$/water/ice bath (-30ºC). Tributylamine (0.7 mL) and isobutylchloroformate (0.4 mL) were added to the solution to form a concentration of 0.04 M. A separate propylamine solution was made with propylamine (0.1 mL) in dry methanol (20 mL) to form a 0.06M concentration, which resulted in 1.5 equiv of propylamine. Vials were made with the propylamine solution (0.5 mL) and placed in an ice batch. At 1, 2, 3, 4, 5, 10, 15, 20, 25, 30, 45, 60 minutes, the L-boc-phenylalanine solution (0.5 mL) was removed and put into the vial containing the propylamine quench. All the samples were run on the LC-UV. The method used was RCMICROP. In addition, the sample taken at 4 minutes and 30 minutes were tested by ^{1}H NMR. The sample at 4 minutes showed partial conversion to the product. The sample at 30 minutes showed only product peaks in the ^{1}H NMR.

Test reaction time using 0.75M batch reactions (analysis by LC-UV)

L-Boc-phenylalanine (5 g) was dissolved in dry ethylacetate (25 mL). The solution was put under argon and in a $CaCl_2$/water/ice bath (-30ºC). Tributylamine (3.0 mL) and isobutylchloroformate (1.5 mL) were added to the solution to form a concentration of 0.75 M. A separate propylamine solution was

made with propylamine (1.8 mL) in dry methanol (20 mL) to form a 1.1 M concentration which results in 1.5 equiv of propylamine. Vials were made with the propylamine solution (0.5 mL) and placed in an ice batch. At 1, 2, 3, 4, 5, 10, 15, 20, 25 and 30 minutes, the L-boc-phenylalanine solution (0.5 mL) was removed and put into the vial containing the propylamine quench. All the samples were run on the LC-UV. The method used was RCMICROP. Product began to appear in as little as 1 minute by LC-UV.

Test reaction time using 0.75M batch reactions (isolated yield)

A stock solution was made with L-boc-phenylalanine (5 g), tributylamine (6 mL), and dry ethylacetate (50 mL). For each test, 2.8 mL of stock solution was used. A separate stock solution of quench was made with propylamine (2.4 mL) and dry methanol (26 mL). For each test, 1.4 mL of the quench stock solution was used. To the 2.8 mL, isobutylchloroformate (0.15 mL) was added to form a 0.75 M concentration. At 5, 15, and 30 sec, 1, 5, 10, and 20 minutes, the quench was added. Each time was done in triplicate. The solutions were worked up by washing with saturated aqueous $NaHCO_3$, twice with water, and saturated aqueous NaCl. The solutions were dried over magnesium sulfate and the solvent was removed under reduced pressure. The 5 second time sample did not show any product formation by ^1H NMR. The 15 and 30 second time sample gave 20%

and 30% isolated yield, respectively. Any time after 1 minute gave the maximum possible isolated yield of 76+/-5%.

2nd generation continuous flow reactor 0.75M

Run 1

L-Boc-phenylalanine (14.9 g) and tributylamine (13.3 mL) were added to dry ethylacetate (75 mL) to make an 0.75 M solution. Isobutylchloroformate (7.3 mL) was added to dry ethylacetate (75 mL) to make a second 0.75 M solution. Propylamine (8.3 mL) was added to dry methanol (75 mL) to make a 1.35 M solution so there were 1.5 equiv of propylamine in solution from the slower pump. Both HPLC pumps were set to 1 and the ISCO, containing the isobutylchloroformate, was set to 2.4 mL/min. The chiller was set to -20ºC. The residence time was 1 min. Three runs were performed with a 2 min flush of reactants then 5 min collecting product for each run. The thermocouple read -20.3ºC during the runs. The three runs were then worked up using the same procedure as the batch reactions. Trace amounts of product was seen by ^{1}H NMR.

Run 2 -3

The solutions were made the same way as with run 1. The flow rates were reduced with this run to 0.8 mL/min for the HPLC pumps and the ISCO was set to 0.8 mL/min. The residence time was 3.4 minutes. The chiller was set to -20ºC

for run 2 and -10ºC for run 3. Two runs were collected for each temperature. The continuous flow reactor was flushed with reactants for 5 min and was collected for 5 min for each run. All of the runs were worked up using the same procedure as the batch reaction. In run 2, 2% product was isolated. In run 3, trace product was observed by ^{1}H NMR.

3rd generation continuous flow reactor specifications:

The HPLC mixer used was 6 cm long and the specifications stated that it could hold 420 μL. The part number for the mixer was G1312-87330. It contained stainless steel beads. The tubing was purchased from Agilent. Each piece was 80 cm in length, had an inner diameter of 0.17 mm, and was made of stainless steel. The cross fitting and T-fitting were HIP fittings made of stainless steel. Three Eldex Recipro Model AA stainless steel pumps were used until partway through the 3rd generation system. Then, two Eldex Recipro Optos 2SM pumps were used for the reactant streams and an Eldex Recipro Model A pump was used for the quench.

3rd generation continuous flow reactor flow rates

All pumps were also tested individually to determine individual flow rates. The last setting with all three pumps combined was a 15% pressure drop. At 0.01 setting, the boc pump should pump 0.6 mL/min, at 0.25 setting the iso pump

should pump 0.6 mL/min, and the propyl pump should pump at 1 mL/min. Settings and flow rates in Figure 2-36.

Settings				Flow rate			Settings	
Boc pump	Iso pump	mL	min		Boc pump	propyl		
1	0.75	2.4	1		3	1	Flow rate	
		2.4	1				mL	min
		2.4	1				6.8	1
		2.4	1		1	1	6.8	1
1	0.5	1.8	1				6.8	1
		2	1				4.4	1
		2.4	1		0.5	0.5	4.4	1
		2.2	1				4.4	1
		2.4	1				2.6	1
0.5	1	1.4	1				2.6	1
		1.4	1				2.6	1
		1.4	1					
3	3	4.4	1					
		4.4	1					
		4.4	1					
Switch Iso and propyl pumps								
Settings		Flow rate						
Boc			New iso	mL	min			
0.01	0.25	0.8	1					
		0.8	1					
Settings			Flow rate					
Boc	Iso	propyl	mL	min				
0.01	0.25	0.02	1.2	1				
			1.2	1				
0.01	0.25	0.25	1.6	1				
			1.8	1				
raise propyl pump								
0.01	0.25	0.25	1.6	1				
				1.7	1			
			1.8	1				

Figure 2-36: Flow rate 3rd generation continuous flow reactor

3rd generation continuous flow reactor 400 cm tubing

L-Boc-phenylalanine (6 g) and tributylamine (5.4 mL) were added to dry ethylacetate (15 mL) to make a 1.5 M solution. The isobutylchloroformate (2.9 mL) was added to dry ethylacetate (15 mL) to make a 1.5 M solution. The two solutions combined to make a 0.75 M solution of all the reactants. Propylamine (2.8 mL) was added to dry methanol (30 mL) to make a 1.125 M solution, which was 1.5 equiv of propylamine compared to the L-boc-phenylalanine . The L-boc-phenylalanine pump was set at 0.01 which correlates to a flow rate of 0.6 mL/min. The isobutylchloroformate pump was set at 0.25, which correlated to a flow rate of 0.6 mL/min. The propylamine pump was set at 0.25 which correlates to a flow rate of 1.0 mL/min. With the pressure drop, the overall flow rate should be 1.7 mL/min. The chiller was set to -20ºC, -10ºC, 0ºC, 10ºC, 20ºC, 25ºC, and 50ºC with the thermocouple reading -20.4ºC, -10.5ºC, -0.5ºC, 10.0ºC, 19.7ºC, 24.8ºC, and 49.9ºC, respectively. The reactants were flushed in the continuous flow reactor for 2 minutes before each temperature change. For each temperature, the reactants were collected in duplicate for a 2 min run with -20ºC, -10ºC, 0ºC, and for a 3 min run with 10ºC, 20ºC, 25ºC and 50ºC. The reactants were worked up as previously described. The product was isolated and the melting point determined of each sample and compared to the product melting point of 111.4ºC. The -20ºC produced an oil but showed trace amounts of product. The isolated yield of the -10ºC was 6 +/-5%, for 0ºC was 10+/-5%, for 10ºC was 17+/-5%, for

20ºC was 20 +/-5%, for 25ºC was 23+/-5%, and for 50ºC was 25 +/-5%. See Figure 2-19 for the graph.

3rd generation continuous flow reactor 720 cm tubing optimize pumps for pressure drop

The same quantities of all the reactants were used as for the 400 cm tubing length continuous flow reactor. The L-boc-phenylalanine pump was set to 0.01 for a flow rate of 0.6mL/min and the isobutyl chloroformate pump was set to 0.25 for a flow rate of 0.6 mL/min. The propylamine pump was set to 0.50 for a flow rate of 1.2 mL/min. All of these flows should give a total flow rate of 1.8 mL/min with a 15% flow rate reduction. The continuous flow reactor was flushed with reactants for 2 minutes before each temperature change and the reactants were collected in duplicate for 3 minutes each for the various temperatures. The chiller was set to 10ºC, 25ºC, and 50ºC with the thermocouple reading 10.1ºC, 25.2ºC, and 50.3ºC, respectively. The reactants were collected and worked up the same way as the batch reaction. The isolated yield for the 10ºC was 6+/-5%, for 25ºC was 16+/-5%, for 50ºC was 18+/-5%.

3rd generation continuous flow reactor flow ratios:

Pure hydrocarbon (at 25°C)

pump settings			hydrocarbon ratios (average of 2 runs)		
Boc	Iso	propyl	octane	nonane	decane
0.02	0.25	0.2	23.5	13	63
0.01	0.25	0.1	27	16	57
0.01	0.25	0.05	26.5	30	43.5
0.01	0.27	0.08	21	34	45
0.01	0.25	0.09	22	31	47
0.01	0.23	0.09	22	28	50
0.01	0.24	0.09	22	29.5	48.5

Reactants with 1%vol hydrocarbons (at 25°C)

pump settings			hydrocarbon ratios (average of 2 runs)		
Boc	Iso	propyl	octane	nonane	decane
0.01	0.23	0.09	11	32.5	56.5
0.01	0.2	0.09	10.5	30.5	59
0.03	0.2	0.09	17	32	51
0.05	0.2	0.09	24.5	35	40.5
0.04	0.2	0.09	27.5	39.5	33

Figure 2-37: Hydrocarbon flow rates and pump settings

For the pure hydrocarbon flow ratios, octane was used with the L-boc-phenylalanine pump, nonane was used with the isobutylchloroformate pump, and decane was used with the propylamine pump. For each setting, two samples were collected of 1 mL each and run on the GC-MS to determine the peak area. The results are shown in Figure 2-37.

For the 1% vol hydrocarbon trace, L-boc-phenylalanine (6 g), tributylamine (5.4 mL), and octane (0.15 mL, 1% vol) were added to dry ethylacetate (15 mL). Isobutylchloroformate (2.9 mL) and nonane (0.15 mL, 1% vol) were added to dry ethylacetate (15 mL). Propylamine (2.8 mL) and decane

(0.30 mL, 1% vol) were added to dry methanol (30 mL). The continuous flow reactor was flushed for 2 min before each collection and two samples of 1 mL each were collected and run on the GC-FID to determine the ratios by the peak area. The method used was MK-RC. The results are shown in Figure 2-37.

3rd generation continuous flow reactor: 720 cm tubing optimizing pumps with hydrocarbon trace

L-Boc-phenylalanine (6 g), tributylamine (5.4 mL), and octane (0.15 mL, 1% vol) were added to dry ethylacetate (15 mL). Isobutylchloroformate (2.9 mL) and nonane (0.15 mL, 1% vol) were added to dry ethylacetate (15 mL). Propylamine (2.8 mL) and decane (0.30 mL, 1% vol) were added to dry methanol (30 mL). The chiller temperature was set to 25ºC. The L-boc-phenylalanine pump was set to 0.04, the isobutylchloroformate pump was set to 0.15, and the propylamine pump was set to 0.09. The continuous flow reactor was flushed with reactants for 3 minutes then 5 mL reactant was collected in duplicate. The residence time was measured to be 19.2 sec. The ratios of the octane, nonane, and decane were measured on the GC-FID to be 26%, 27%, and 47%, respectively. The duplicate runs were worked up using the same procedure as the batch reaction. The isolated yield was 30+/-5%. The product purity was measured by melting point and ^{1}H NMR.

3rd generation continuous flow reactor: 720 cm tubing with sonicator

L-Boc-phenylalanine (6 g), tributylamine (5.4 mL), and octane (0.15 mL, 1% vol) were added to dry ethylacetate (15 mL). Isobutylchloroformate (2.9 mL) and nonane (0.15 mL, 1% vol) were added to dry ethylacetate (15 mL). Propylamine (2.8 mL) and decane (0.30 mL, 1% vol) were added to dry methanol (30 mL). The L-boc-phenylalanine pump was set to 0.04, the isobutylchloroformate pump was set to 0.15, and the propylamine pump was set to 0.09. The continuous flow reactor was placed in the sonicator for this run. The sonicator temperature was measured using the thermocouple and was maintained between 24-27ºC by adding ice periodically. The reactants were flushed through the continuous flow reactor for 5 min then 7 mL of reactants was collected in duplicate. The ratios of the octane, nonane, and decane were measured on the GC-FID to be 19%, 28%, and 53%. The duplicate runs were worked up using the same procedure as the batch reaction. The isolated yield was 30+/-5% and melting point and ^{1}H NMR were used to verify purity.

3rd generation continuous flow reactor: 720 cm tubing with tubular reactor

L-Boc-phenylalanine (6 g), tributylamine (5.4 mL), and octane (0.15 mL, 1% vol) were added to dry ethylacetate (15 mL). Isobutylchloroformate (2.9 mL) and nonane (0.15 mL, 1% vol) were added to dry ethylacetate (15 mL). Propylamine (2.8 mL) and decane (0.30 mL, 1% vol) were added to dry methanol

(30 mL). The L-boc-phenylalanine pump was set to 0.04, the isobutylchloroformate pump was set to 0.15, and the propylamine pump was set to 0.09. The chiller was set to 25ºC. The HPLC column was filled with 3 mm glass beads and added after 4-80 cm tubing connections. The HPLC column dimensions are 25 cm long and 4.6 mm ID. The continuous flow reactor was flushed with reactants for 5 min and 8 mL of reactants were collected in duplicate. The ratios of the octane, nonane, and decane were measured on the GC-FID to be 15%, 20%, and 65%, respectively. The residence time was measured to be 10.2 sec. The duplicate runs were worked up using the same procedure as the batch reaction. The isolated yield was 40+/-5% and melting point and ^{1}H NMR were used to verify purity.

3rd generation continuous flow reactor: beads in cross fitting

I tried adding 0.5mm glass beads inside the cross fitting where the reactant streams of L-boc-phenylalanine, tributylamine, octane in ethylacetate and isobutylchloroformate and nonane in ethylacetate combined. The beads were added to increase mixing at the addition point. The beads clogged the system so this mixing option was abandoned.

3rd generation continuous flow reactor: smaller beads in tubular reactor

The 3 mm glass beads were removed from the tubular reactor and replaced with 0.5 mm glass beads. The L-boc-phenylalanine and isobutylchloroformate pumps were set to 0.3 mL/min. The propylamine pump was optimized using the GC-FID and hydrocarbon trace. The optimization resulted in the propylamine pump being set at 0.03 for the decane to be 50%. L-Boc-phenylalanine (6 g), tributylamine (5.4 mL), and octane (0.15 mL, 1% vol) were added to dry ethylacetate (15 mL). Isobutylchloroformate (2.9 mL) and nonane (0.15 mL, 1% vol) were added to dry ethylacetate (15 mL). Propylamine (2.8 mL) and decane (0.30 mL, 1% vol) were added to dry methanol (30 mL). The chiller temperature was not set and was left at room temperature. The thermocouple read 21.3ºC. The continuous flow reactor was flushed with the three streams for seven minutes. Three samples of 5 mL each were collected. The samples took between 4 min and 4:30 min to collect. One mL was removed from each sample to be run on the GC-FID to determine the percentage of octane, nonane, and decane. The percentages for the three samples were 19% octane, 27% nonane, and 54% decane. The remaining four mL of the product stream was worked up the next day the same way as the batch reaction. The white solid was dried in a vacuum oven overnight and purity was confirmed by melting point and ^{1}H NMR. The average isolated yield was 47 +/-5%.

<u>3rd generation continuous flow reactor: bent tubing</u>

The tubular reactor containing 0.5 mm glass beads was left on the system. An 80 cm piece of 0.17 mm ID HPLC tubing was bent to have 1 cm sharp angles to induce chaotic mixing. The bent tubing replaced the third section of unbent tubing. The L-boc-phenylalanine and isobutylchloroformate pumps were set to 0.3 mL/min. The propylamine pump was optimized using the GC-FID and hydrocarbon trace. The optimization resulted in the propylamine pump being set at 0.03 for the decane to be 50%. L-Boc-phenylalanine (6 g), tributylamine (5.4 mL), and octane (0.15 mL, 1% vol) were added to dry ethylacetate (15 mL). Isobutylchloroformate (2.9 mL) and nonane (0.15 mL, 1% vol) were added to dry ethylacetate (15 mL). Propylamine (2.8 mL) and decane (0.30 mL, 1% vol) were added to dry methanol (30 mL). The chiller temperature was not set and was left at room temperature. The thermocouple read 21.5ºC. The continuous flow reactor was flushed with the three streams for seven minutes. Three samples of 5 mL each were collected. The samples took between 4 min and 4:30 min to collect. One mL was removed from each sample to be run on the GC-FID to determine the percentage of octane, nonane, and decane. The percentages for the three samples were 21% octane, 28+/-2% nonane, and 51+/-2% decane. The remaining four mL of the product stream was worked up the same way as the batch reaction. The white solid was dried in a vacuum oven overnight and purity was confirmed by melting point and ^{1}H NMR. The average isolated yield was 47 +/-5%.

3rd generation continuous flow reactor: reduce flow

The bent tubing was removed and replaced with non-bent tubing in the 3rd position. The tubular reactor was left on with 0.5 mm beads. The L-boc-phenylalanine and isobutylchloroformate pumps were set to 0.1, 0.01, and 0.05 mL/min. The propylamine pump set to its lowest setting of 0.01. Since the propylamine pump pumps faster than the other two pumps at this setting, this resulted in a larger percentage of the propylamine stream. I first tested if the quench propylamine stream would overwhelm the other two streams. L-Boc-phenylalanine (6 g), tributylamine (5.4 mL), and octane (0.15 mL, 1% vol) were added to dry ethylacetate (15 mL). Isobutylchloroformate (2.9 mL) and nonane (0.15 mL, 1% vol) were added to dry ethylacetate (15 mL). Propylamine (2.8 mL) and decane (0.30 mL, 1% vol) were added to dry methanol (30 mL). The chiller temperature was not set and was left at room temperature. The thermocouple read 20.9ºC. The continuous flow reactor was flushed with the three streams for four minutes. Two samples of one mL each were collected to be run on the GC-FID. For the 0.01 mL/min, the octane and nonane were not observed by GC-FID. For the 0.05 mL/min, the percentages were 4% octane, 2% nonane, and 94% decane. For the 0.1 mL/min, the percentages were 10% octane, 12% nonane, and 78% decane.

Since the 0.1 mL/min flow rate seemed to give acceptable percentages of the hydrocarbon trace, I ran the system using this flow rate. The continuous flow reactor was flushed with the three streams for 20 minutes. Triplicate samples of 10 mL were collected with each taking between 14:30 to 15 min to collect. One mL was removed from each sample to be run on the GC-FID to determine the percentage of octane, nonane, and decane. The percentages for the first sample were 10% octane, 9% nonane, and 81% decane. The percentages for the second and third samples were 11% octane, 15+/-1% nonane, and 74+/-1% decane. Because the percentages were not acceptable for the 1st sample, that sample was not worked up. The remaining nine mL of the product stream was worked up the next day the same way as the batch reaction. The white solid was dried in a vacuum oven overnight and purity was confirmed by melting point and ^{1}H NMR. The average isolated yield was 48 +/-5%.

3rd generation continuous flow reactor: Adding a second tubular reactor

A second HPLC column with the silica removed was added to the system. The HPLC column dimensions were 4 mm ID and 15 cm long. The tubular reactor was filled with 0.5 mm glass beads. This tubular reactor was added between the 3rd and 4th HPLC tubing in the continuous flow reactor. The L-boc-phenylalanine and isobutylchloroformate pumps were set to 0.3 and 0.1 mL/min. The propylamine pump was put at the lowest setting of 0.01. L-Boc-

phenylalanine (6 g), tributylamine (5.4 mL), and octane (0.15 mL, 1% vol) were added to dry ethylacetate (15 mL). Isobutylchloroformate (2.9 mL) and nonane (0.15 mL, 1% vol) were added to dry ethylacetate (15 mL). Propylamine (2.8 mL) and decane (0.30 mL, 1% vol) were added to dry methanol (30 mL). The chiller temperature was not set and was left at room temperature. The thermocouple read 21.2ºC. The continuous flow reactor was flushed with the three streams for twenty minutes. Three samples of 5 mL for the 0.3 mL/min and three samples of 10 mL for the 0.1 mL/min each were collected. The samples took between 4:30 min and 4:38 min to collect for the 0.3 mL/min and 15:42 min for the 0.1 mL/min. One mL was removed from each sample to be run on the GC-FID to determine the percentage of octane, nonane, and decane. The percentages for the 0.3 mL/min were 19% octane, 29% nonane, and 51% decane. The percentages for the 0.1 mL/min were 10% octane, 16% nonane, and 74% decane. The remaining four or nine mL of the product stream was worked up the same way as the batch reaction. The white solid was dried in a vacuum oven overnight and purity was confirmed by melting point and ^{1}H NMR. The average isolated yield was 51 +/-5% for the 0.3 mL/min and 60+/-5% for the 0.1 mL/min.

3rd generation continuous flow reactor: Adding a second tubular reactor with the bent tubing

The bent tubing was again added in place of the third piece of HPLC tubing. The L-boc-phenylalanine and isobutylchloroformate pumps were set at

0.3 mL/min with the propylamine pump set at 0.01. All other reaction conditions were the same as above. Two samples of four mL were collected and worked up in the same method as above. The percentages were 17+/-1% octane, 29+/-2% nonane, and 54+/-3% decane. The isolated yield was 56+/-5%. This was an improvement over the 51+/-5% isolated yield without the bent tubing.

3rd generation continuous flow reactor: Optimizing flow

L-Boc-phenylalanine (6 g), tributylamine (5.4 mL), and octane (0.15 mL, 1% vol) were added to dry ethylacetate (15 mL). Isobutylchloroformate (2.9 mL) and nonane (0.15 mL, 1% vol) were added to dry ethylacetate (15 mL). Propylamine (2.8 mL) and decane (0.30 mL, 1% vol) were added to dry methanol (30 mL). The chiller temperature was not set and was left at room temperature. The L-boc-phenylalanine and isobutylchloroformate pumps were set using the flow rates below. The propylamine pump was set at 0.01. The flush times, amount of product stream worked up, collection times, percentages from the GC-FID and isolated yield were presented in Figure 2-38 and a graph in Figure 2-24. The product stream was worked up and purified using the method described previously. The purity was confirmed using melting point and ^{1}H NMR.

Flow rate (mL/min)	Flush (min)	workup (mL)	collect time (min)	octane %	nonane %	decane %	Isolated Yield
0.05	60	25	60	8	9+/-2	83+/-2	48+/-5
0.15	45	9	13:45-15	12	18	70	57+/-5
0.125	45	9	14:16-14:58	11	17	72	57+/-5
0.075	60	9	16:43-17:09	8	12	80	55 +/-5

Figure 2-38: Optimizing flow rate on the 2 tubular reactor system

3rd generation continuous flow reactor: Add two additional tubular reactors

Two additional tubular reactors were built and added to the continuous flow reactor. Both tubular reactors were made of stainless steel, 20 cm in length, 4.6 mm inner diameter, and were filled with 0.5 mm glass beads. The glass beads were packed using a vibrator. One tubular reactor was added after 320 cm of HPLC tubing and the other tubular reactor was added after 560 cm of tubing. All the reaction conditions with the reagents were the same. The propylamine pump was set at 0.01. The L-boc-phenylalanine and isobutylchloroformate pumps are set at the same flow rate. The chiller was left at room temperature and the thermocouple read 23.2ºC throughout the experiment. The first tubular reactor (after 320 cm) was added and run at 0.1 mL/min giving an isolated yield of 49+/-5%. Then the second tubular reactor (after 560 cm) was added and run at 0.1 mL/min, giving an isolated yield of 57+/-5%. The flow rates were then optimized. Reaction specifications were presented in Figure 2-39 and a graph in Figure 2-26. The product stream was worked up and purified using the method

described previously. The purity was confirmed using melting point and ^{1}H NMR.

Flow rate (mL/min)	Flush	workup	collect time	octane %	nonane %	decane %	Isolated Yield
0.1	60 m	9 mL	15:39	10	11	78	49+/-5
0.1	60 m	9.5 mL	8:46	6	7	87	44+/-5
0.2	60 m	9.5 mL	7:37	10	13	77	53+/-5
0.3	30 m	9.5 mL	7:08	8	19	73	51+/-5
0.4	30 m	9.5 mL	6:10	15	21	64	50+/-5

Figure 2-39: Experimental Data for 4 tubular reactor system at room temperature

3rd generation continuous flow reactor: Different temperatures on 4 tubular reactor system

The four tubular reactor system was used for these experiments. All the reaction conditions with the reagents were the same. The propylamine pump was set at 0.01. The L-boc-phenylalanine and isobutylchloroformate pumps were set at the same flow rate of 0.2 mL/min. The temperatures of the chiller were -20ºC and 0ºC. The product stream was worked up and purified using the method described previously. The purity was confirmed using melting point and ^{1}H NMR. The details were listed in Figure 2-40 and a graph in Figure 2-27.

Temp (ºC)	Flush	workup	collect time	octane %	nonane %	decane %	Isolated Yield
-20	35 min	9.5 mL	7:20	7	11	82	53+/-5
0	35 min	9.5 mL	7:28	8	10	82	60+/-5

Figure 2-40: Different temperatures on 4 tubular reactor system

3rd generation continuous flow reactor: Different equivalents of isobutyl chloroformate

The four tubular reactor system was used for these experiments. The L-boc-phenylalanine, tributylamine, octane in ethyl chloroformate and the propylamine in methanol were the same as the previous experiments (1.5 M). Both reactant pumps were set at 0.2 mL/min and the propylamine pump was set at 0.01. The chiller was left at room temperature and the thermocouple read 21.2ºC. The isobutylchloroformate equivalents were 2 times excess and 3 times excess based on the L-boc-phenylalanine. The product stream was worked up and purified using the method described previously. The purity was confirmed using melting point and ^{1}H NMR. The reaction details were in Figure 2-41.

Equivalents	Flush	workup	collect time	octane %	nonane %	decane %	Isolated Yield
2x excess	35	9.5 mL	7:53	8	9	83	54+/-5
3x excess	35	9.5 mL	7:23	9	9	82	48+/-5

Figure 2-41: Excess isobutylchloroformate used in the 4 tubular reactor system

Batch propylamine quench at room temperature

The same solutions as the continuous flow reactor of L-boc-phenylalanine, tributylamine in ethylacetate (1.5 M), isobutyl chloroformate in ethyl acetate (1.5 M), propylamine in methanol (1.2 M) were used. The L-boc-phenylalanine solution (2 mL) and isobutyl chloroformate solution (2 mL) were combined. The quench solution (4 mL) was added after 16 minutes and 1 hour. Each time was done in triplicate. The product stream was worked up and purified using the method described previously. The purity was confirmed using melting point and ^{1}H NMR. The 16 minute quench time gave an isolated yield of 37+/-5% and the 1 hour quench time gave an isolated yield of 27+/-5%.

Diazoketone ((1-benzyl-3-diazo-2-oxo-propyl)-carbamic acid tert-butyl ester): From isobutylchloroformate

L-Boc-phenylalanine (0.8 g, 3 mmol) was dissolved in anhydrous THF (15 mL), cooled to -15ºC, and put under argon. Triethylamine (0.43 mL, 3.1 mmol) was added. Isobutylchloroformate (0.4 mL, 3.1 mmol) was combined with 2.5 mL anhydrous THF and added slowly to the solution. The reaction was allowed to react for 30 minutes. The triethylamine hydrochloride salt was filtered while keeping the filtrate cold. The trimethylsilyl diazomethane (2.0 M in hexane, 3 mL, 6 mmol) was combined with anhydrous acetonitrile (10 mL) and added slowly to the reaction solution. The reaction was warmed to 4ºC and allowed to react for 24 hours. The reaction was worked up by adding diethyl ether (40 mL),

washing 10% aqueous citric acid (30 mL), saturated aqueous $NaHCO_3$ (30 mL), saturated aqueous NaCl (30 mL), and dried over magnesium sulfate. The solvent was removed under reduced pressure. The product was purified by a silica gel column was run using 1/2=ethylacetate/ hexane giving a yellow solid (32% yield). These results were repeated and gave the same yield.

(1-Benzyl-3-diazo-2-oxo-propyl)-carbamic acid tert-butyl ester:

^{1}H NMR ($CDCl_3$) ppm: 1.398 (s, 9H), 3.05 (m, 2H), 4.40 (br s, 1H), 5.10 (br s, 1H), 5.20 (br s, 1H), 7.27 (m, 5H). ^{13}C NMR ($CDCl_3$) ppm: 28.2, 38.5, 54.4, 58.4, 80.0, 126.9, 128.6, 129.3, 136.3, 155.1, 193.3. MS(m/z) 290 (M+1) EA: calculated C, 62.27%, H, 6.62%, N, 14.52%. Found C, 62.25%, H, 6.65%, N, 14.32%**.**

Isolated Yield	**Variable changed**
32%	Standard reaction using isobutylchloroformate
23%	Trimethylsilyl diazomethane purchased as 2.0M in diethyl ether
25%	Trimethylsilyl diazomethane 3 equivalents (9 mmol)
75%	Ethyl chloroformate[10]
66%	Tributylamine with ethyl chloroformate
61%	Ethyl acetate with ethyl chloroformate (instead of THF)

Figure 2-42: Isolated yield obtained for diazoketone

Diazoketone ((1-benzyl-3-diazo-2-oxo-propyl)-carbamic acid tert-butyl ester): Using ethyl chloroformate[10]

L-Boc-phenylalanine (0.8 g, 3 mmol) was dissolved in anhydrous THF (15 mL), cooled to -15ºC, and put under argon. Triethylamine (0.43 mL, 3.1 mmol) was added. Ethyl chloroformate (0.3 mL, 3.1 mmol) was combined with 2.5 mL anhydrous THF and added slowly to the solution. The reaction was allowed to react for 30 minutes. The triethylamine hydrochloride salt was filtered while keeping the filtrate cold. The trimethylsilyl diazomethane (2.0 M in hexane, 4.5 mL, 9 mmol) was combined with anhydrous acetonitrile (10 mL) and added slowly to the reaction solution. The reaction was warmed to 4ºC and allowed to react for 24 hours. The reaction was worked up by adding diethyl ether (40 mL), washing 10% aqueous citric acid (30 mL), saturated aqueous $NaHCO_3$ (30 mL), saturated aqueous NaCl (30 mL), and dried over magnesium sulfate. The solvent was removed under reduced pressure. The product was purified by a silica gel column was run using 1/2 ethylacetate/ hexane giving a yellow solid (75+/-3% yield).

Diazoketone ((1-benzyl-3-diazo-2-oxo-propyl)-carbamic acid tert-butyl ester) calibration curve

Pure diazoketone, (1-benzyl-3-diazo-2-oxo-propyl)-carbamic acid tert-butyl ester, was dissolved in methanol to form various concentrations. These solutions were run on the LC-UV to form a calibration curve. The method used

was C18CMKA. The solution concentration and areas were shown below in Figure 2-43. The calibration curve was shown in Figure 2-44.

sample	Conc (M)	area
1	0.01	21747.7
2	0.00648	15612.8
3	0.00324	9609
4	0.00162	6771.3
5	0.00081	5052

Figure 2-43: Calibration curve diazoketone concentration and area

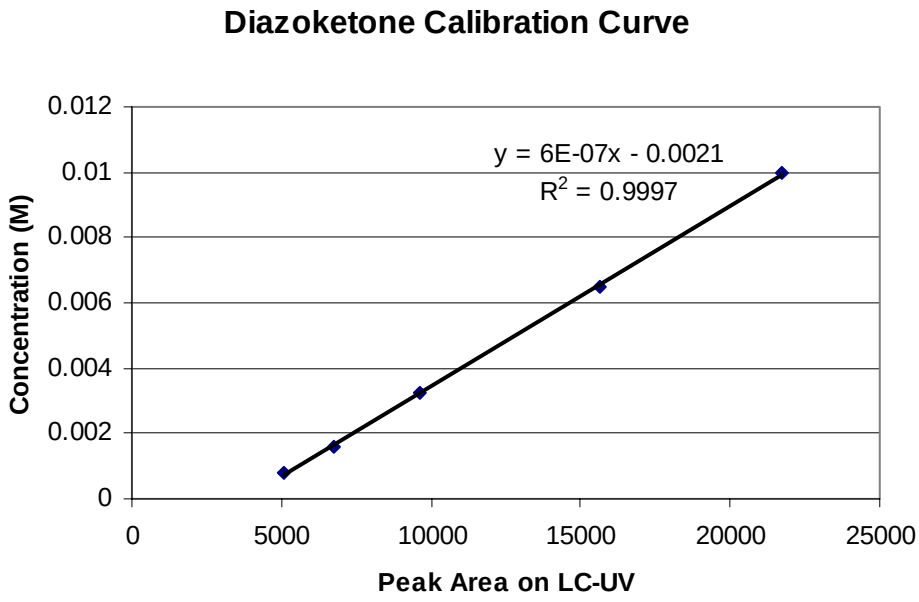


Figure 2-44: Calibration curve diazoketone, (1-benzyl-3-diazo-2-oxo-propyl)-carbamic acid tert-butyl ester

2-Phenyl-1-propylcarbamoyl-ethyl)-carbamic acid tert-butyl ester calibration curve

Pure 2-phenyl-1-propylcarbamoyl-ethyl)-carbamic acid tert-butyl ester was dissolved in methanol to form various concentrations. These solutions were run on the LC-UV to form a calibration curve. The method used was C18CMKA. The solution concentration and areas were shown below in Figure 2-45. The calibration curve is shown in Figure 2-46.

sample	Conc (M)	area
1	0.0102	16600.2
2	0.005106	10913.4
3	0.002553	7986.4
4	0.001277	5385.7

Figure 2-45: Calibration concentration and area for 2-phenyl-1-propylcarbamoyl-ethyl)-carbamic acid tert-butyl ester

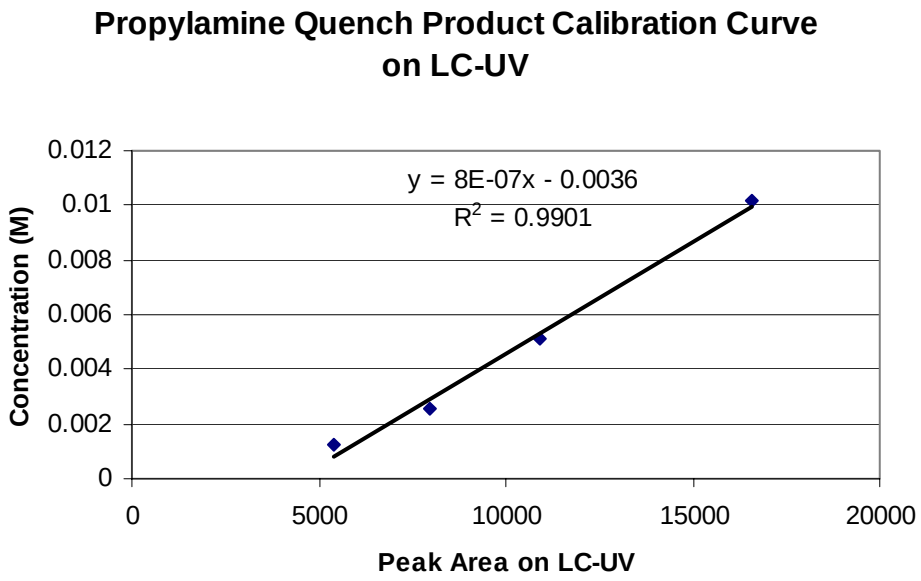


Figure 2-46: Calibration curve for 2-phenyl-1-propylcarbamoyl-ethyl)-carbamic acid tert-butyl ester

2-Phenyl-1-propylcarbamoyl-ethyl)-carbamic acid tert-butyl ester: Ethyl chloroformate batch

A stock solution was made with L-boc-phenylalanine (2.5 g), tributylamine (1.5 mL), and dry ethylacetate (12.5 mL). For each test, 2.8 mL of stock solution was used. A separate stock solution of quench was made with propylamine (1.2 mL) and dry methanol (13 mL). For each test, 1.4 mL of the quench stock solution was used. To the 2.8 mL solution, ethyl chloroformate (0.2 mL) was added to form a 0.75 M concentration. The times tested were 1, 16, 30 minutes. Using the calibration curve of 2-phenyl-1-propylcarbamoyl-ethyl)-

carbamic acid tert-butyl ester, the reaction solutions were run on the LC-UV. The method used was C18CMKA. The area of the peak that corresponded to 100% yield was observed for 16 and 30 minutes. The 30 minute sample was worked up by adding ethylacetate then washing with aqueous saturated $NaHCO_3$, water, and brine. The reaction solution was dried over magnesium sulfate and the solvent removed under reduced pressure. The product was isolated using a silica gel column (2/1=hexane/ethylacetate) giving a white solid (49% yield).

Diazoketone, (1-benzyl-3-diazo-2-oxo-propyl)-carbamic acid tert-butyl ester), monitoring reaction rate

L-Boc-phenylalanine (0.8 g, 3 mmol) was dissolved in anhydrous ethyl acetate (15 mL), cooled to -8ºC, and put under argon. Tributylamine (0.7 mL, 3.1 mmol) was added. Ethyl chloroformate (0.3 mL, 3.1 mmol) was combined with 2.5 mL anhydrous ethyl acetate and added slowly to the solution. The reaction was warmed to -5ºC and allowed to react for 30 minutes. The trimethylsilyl diazomethane (2.0 M in hexane, 3 mL, 6 mmol) was combined with anhydrous acetonitrile (10 mL) and added slowly to the reaction solution. The reaction was warmed to 4ºC and was sampled at 15, 30, 45, 60 min, 2, 4, 22 hours. A 50 μL sample was removed from the reaction solution and combined with 1.3 mL of methanol. The samples were run on the LC-UV and the calibration curve of the diazoketone was used to determine the product concentration at various time

intervals. The method used was C18CMKA. For this system, the quantitative yield was obtained after 2 hours and plateaued (Figure 2-31) (by LC-UV). The points after two hours were within error of the maximum yield.

Diazoketone, (1-Benzyl-3-diazo-2-oxo-propyl)-carbamic acid tert-butyl ester), monitoring reaction rate (0.75 M)

L-Boc-phenylalanine (0.8 g, 3 mmol) was dissolved in anhydrous ethyl acetate (4 mL), cooled to -8ºC, and put under argon. Tributylamine (0.7 mL, 3.1 mmol) and ethyl chloroformate (0.3 mL, 3.1 mmol) were added. The reaction was warmed to -5ºC and allowed to react for 30 minutes. The trimethylsilyl diazomethane (2.0 M in hexane, 3 mL, 6 mmol) was combined with anhydrous acetonitrile (10 mL) and added slowly to the reaction solution. Anisole (1.6 mL, 0.75 M) as an internal standard was added. The reaction was warmed to 4ºC and was sampled at 10, 20, 30, 45, 60, 90, 120 minutes. A 15 μL sample was removed from the reaction solution and combined with 1 mL of methanol. The samples were run on the LC-UV and the calibration curve of the diazoketone was used to determine the product concentration at various time intervals. The method used was C18CMKA. For this system, the yield was quantitative (by LC-UV). Times shorter than 10 minutes were not tested.

Single coiled continuous flow reactor using ethyl chloroformate:

L-Boc-phenylalanine (18 g), tributylamine (16.2 mL), and octane (0.45 mL, 1% vol) were dissolved in dry ethyl acetate (45 mL) to make a 1.5 M solution. Ethyl chloroformate (12.76 mL), anisole (4.4 mL), and nonane (0.66 mL, 1% vol) were added to dry ethyl acetate (30 mL) to make a 3.3 M solution to compensate for the slower pump. Propylamine (5.6 mL) and decane (0.6 mL, 1% vol) were added to dry methanol to make a 1.5 M solution. A continuous flow reactor was constructed using of 45 cm length and 4.6 mm inner diameter stainless steel tubing (SS 314) and filled with glass beads (0.5 mm). For the single and double coiled continuous flow reactors, an Eldex Recipro A pump made from chlorotrifluoroethylene (CTFE) was used for the isobutylcholoroformate stream, and the two Optos pumps were utilized for the other streams. The CTFE was used because it is resistant to HCl which is formed from the acid chloride. The tubing was packed using a vibrator and bent using a tubing bender. The L-boc-phenylalanine pump and propylamine pumps were set at 0.2 mL/min and the ethylchloroformate pump was set at 1.0. The chiller was set at 0ºC and the thermocouple read 0.2ºC. The system was flushed with reactants for 30 minutes then 3 samples of 3.5 mL were collected. For the GC-FID, 0.5 mL sample was added to 0.5 mL methanol. For the LC-UV, 10 μL of sample was added to 1 mL of methanol. The method used was C18CMKA. The hydrocarbon trace from the GC-FID was used to calculate the maximum concentration of product. For this system, the yield was quantitative (by LC-UV).

Double coiled continuous flow reactor using trimethylsilyl diazomethane:

The same system as above was used as the first step of the reaction. The same concentrations of the L-boc-phenylalanine and ethyl chloroformate solutions were used. The propylamine solution was replaced with trimethylsilyl diazomethane (9 mL), decane (0.3 mL, 1 % vol) in dry acetonitrile (30 mL) for a 0.6 M solution. A second continuous flow reactor, identical to the 1st continuous flow reactor, was added after the T-fitting adding the trimethylsilyl diazomethane. The flow rates used were the same. The chiller was set to 0ºC. The system was flushed with reactants for 30 minutes then 2.5 mL samples were collected. For the GC-FID, 0.5 mL sample was added to 0.5 mL methanol. For the LC-UV, 10 μL of sample was added to 1 mL of methanol. The method used was C18CMKA. The hydrocarbon trace from the GC-FID was used to calculate the maximum concentration of product. For this system, the yield was quantitative (by LC-UV).

2.6 References

(1) Yang, D., Le, L. A., Martinez, R. J., Currier, R. P., Spencer, D. F., Deppe, G. *Energy & Fuels* **2008**, *22*, 2649-2659.

(2) Wang, D., Humphrey, A. E., Eagleton, L. C. *Biotechnology and Bioengineering* **1964**, *6*, 367-379.

(3) Ehrfeld, W., Hessel, V., and Lowe, H. *Microreactors: New Technology for Modern Chemistry*; Wiley-VCH: Weinheim, 2000.

(4) Jahnisch, K., Hessel, V., Lowe, H., and Baerns, M. *Angew. Chem. Int. Ed.* **2004**, *43*, 406-446.

(5) Proctor, L. D., Warr, A. J. *Org. Proc. Res. Dev.* **2002**, *6*, 884-892.

(6) Mason, B., Price, K, Steinbacher, J., Bogdan, A., and McQuade, D. *Chemical Reviews* **2007**, *107*, 2300-2318.

(7) *J. Org. Chem* **1994**, *59*, 3656-3664.

(8) US, 2003; Vol. 6,605,732.
(9) Aoyama, T., Shioiri, Takayuki *Chem. Pharm. Bull.* **1981**, 3249-3255.
(10) Cesar, J., Dolenc, M. S. *Tett. Lett.* **2001**, *42*, 7099-7102.
(11) Arndt, F., Eistert, B., Partale, W., *Ber. Dtch. Chem. Ges.* **1927**, *60*, 1364-1370.
(12) Ahmed-Omera, B., Barrowb, David, Wirth, Thomas *Chem. Eng. J.* **2008**, *135S*, S280-S283.
(13) Song, H., Bringer, Michelle, Tice, Joshua, Gerdts, Cory, Ismagilova, Rustem *Appl. Phys. Lett* **2003**, *83*, 4664.

CHAPTER 3: CLEAVABLE, *N*-OCTYL THIIRANE OXIDE, SURFACTANT AND REVERSIBLE SULFOLENE SURFACTANTS

3.1 Introduction

Cleavable surfactants are defined as surfactants that can ***irreversibly*** convert into fragment molecules with either reduced or virtually zero surface activity. In contrast, ***switchable*** surfactants can ***reversibly*** convert into fragment molecules with either reduced or virtually zero surface activity. In both cases, the conversion from the surfactant to the non-surface active fragments is typically accomplished by means of chemical, thermal or photochemical triggers.

The synthesis of a novel cleavable surfactant was conducted, and its surface activity and cleavage ability were demonstrated. Attempted syntheses of a novel switchable surfactant are also reported.

3.2 Background

Surface active agents (surfactants) are ubiquitous. Surfactants are present in many commercial products, such as paints, detergents, inks, adhesives, agrochemical formulations, tertiary oil recovery, and cosmetics.[1] Surfactants are also key additives in the manufacture of many chemicals such as polymers by emulsion polymerization.[2] The emerging nanotechnology field is also dependent

on surface active compounds since surfactants often control the size and size distribution of nanoparticles.[3]

Although surface active compounds are critical to many industries, they are sometimes difficult to separate from the products and to subsequently dispose of. Currently, most surfactants are removed from the desired product by repetitive washings.[4] This creates a significant amount of contaminated waste water. In addition to the obvious economic disadvantages, surfactants have been targeted by environmental groups as a source of water-borne pollution.[4] The desire to reduce waste and to facilitate the removal of surfactants from products has led several research groups to investigate the synthesis and use of cleavable and reversible surfactants.[5]

3.2.1 Prior Art

3.2.1.1 Cleavable Surfactants

Cleavable surfactants can be irreversibly converted into one or more molecules with either reduced or essentially zero surface activity. The "cleavability" property is conferred to the surfactant by incorporating cleavable bonds between the hydrophilic head group and the hydrophobic tail.[5] The earliest example was in 1966 by Distler who reported the trimethyl-[2-(4-octyl-phenoxysulfonyl)-ethyl]-ammonium methylsulfonate that displayed surfactant

properties under acidic or neutral conditions.[6] However, in the presence of a base, such as sodium hydroxide, an elimination reaction took place to produce two fragments with reduced surface activity: ethenyl-4-octyl-phenylsulfonate and trimethylamine (Figure 3-1). Since, several other groups have developed cleavable surfactants and studied their surface tension, micelle formation, and decomposition.[7-10] A range of possible "triggers" for the decomposition have been reported; these include pH[7-10], ozone[11], UV[12-16], and heat[17].

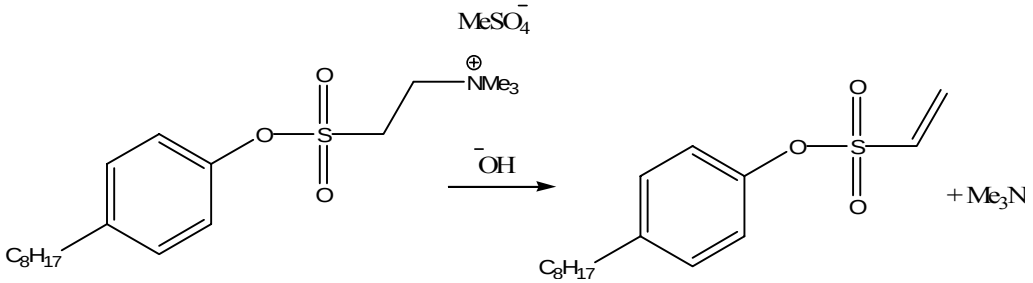


Figure 3-1: Distler's cleavable surfactant. Decomposition into ethenyl-4-octyl-phenylsulfonate and trimethylamine.

3.2.1.2 Switchable Surfactants

Switchable surfactants have several advantages over cleavable surfactants. Switchable surfactants can change between active and inactive forms to stabilize then break emulsions. In addition, their activity can be delayed until needed and then be recovered and reused.[18] Two switchable surfactants (compounds 1 and 2 in Figure 3-2) were reported by McElhanon *et al.*[19] Their critical micelle

concentration ranges was reported 0.6-2.5mM and 1.4-9.3mM (respectively). Upon retro Diels-Alder the surfactants formed two non-surface active fragments. The retro Diels-Alder was triggered by heat (90ºC for a minimum of 1.5 hours).

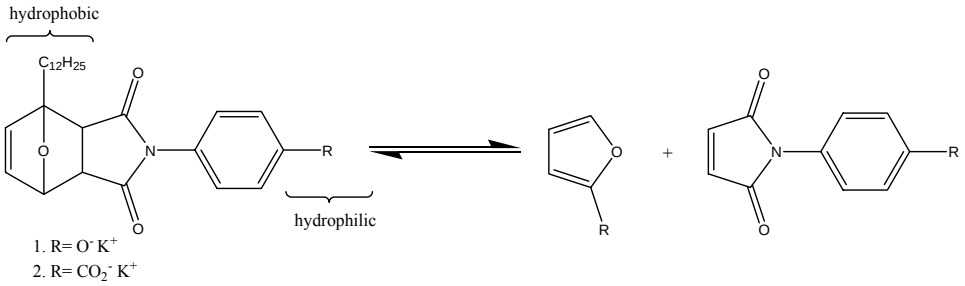


Figure 3-2: Exo-4dodecyl-7-oxabicyclo-[2.2.1]hept-5-ene-2,3-dicarboxy-N-(4-hydroxyphenyl)imide (1); exo-4dodecyl-7-oxabicyclo-[2.2.1]hept-5-ene-2,3-dicarboxy-N-(4-carboxyphenyl)imide (2)

Recently, a surfactant that uses CO_2 or air as the trigger to switch "on" or "off" was synthesized by the Jessop group as shown in Figure 3-3.[18] The group used an amidine that would switch to a charged amidinium bicarbonate with the addition of water and carbon dioxide and switch back again by bubbling nitrogen or air through the neat solution. The amidine system has negligible surface activity and water solubility while the amidinium bicarbonate has surfactant activity. This switchable surfactant was used as a demulsifier for light crude oil and for an emulsion polymerization of latex.[18]

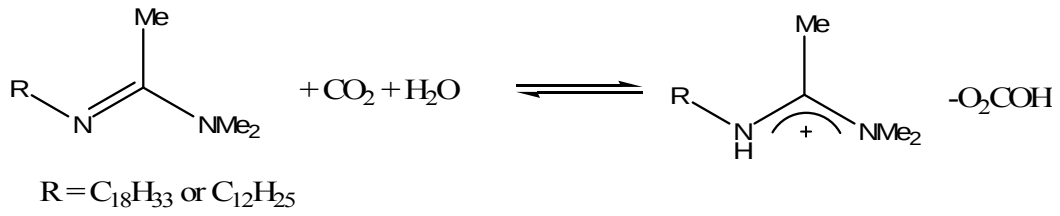


Figure 3-3: Switchable surfactant using amidine motif by Jessop group

The nature of the trigger for both cleavable and switchable surfactants can significantly limit their practical viability. Triggers based on addition of acids, bases, or oxidants are economic and environmental costly. In addition, they can potentially lead to product contamination and/or undesired side reaction(s). The photochemical approach is often limited due to the opacity of many emulsions.

3.3 Results and Discussion

3.3.1 Cleavable Surfactants

Thiirane oxide can undergo a retro-cheletropic reaction with heat to give sulfur monoxide and an ethylene (Figure 3-4). By incorporating an octyl alkyl chain onto the thiirane oxide ring the resulting molecule can act as a cleavable surfactant, containing a "built-in" thermal switch to turn "off" the surfactant activity. The *n*-octyl thiirane oxide can decompose to form two products with essentially zero surface activity, 1-decene and sulfur monoxide (Figure 3-5).[20,23]

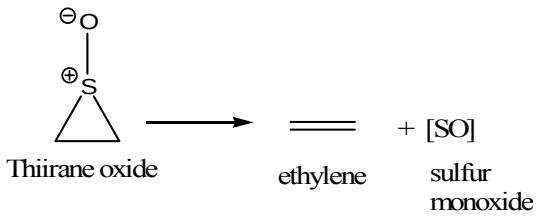
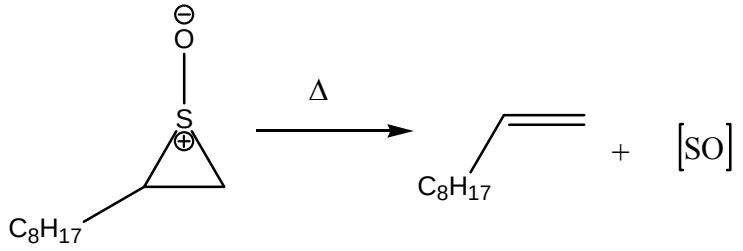


Figure 3-4: Thiirane oxide retro-cheletropic reaction to give sulfur monoxide and ethylene



n-octylthiirane oxide 1-decene sulfur monoxide

Figure 3-5: *n*-octyl thiirane oxide surfactant undergoing retro-cheletropic decomposition to 1-decene and the unstable sulfur monoxide (brackets to indicate sulfur monoxide disproportionate)

The *n*-octyl thiirane oxide was first synthesized, according to literature as shown in Figure 3-6.[21-23] The 1-decene underwent epoxidation using methyltrioxorhenium (MTO), hydrogen peroxide, and 2,2'-bipyridine-N,N'-dioxide. The thiirane ring was then formed upon reaction with sodium thiocyanate. Then the thiirane oxide was prepared from the oxidation of the thiirane ring with *m*-chloroperoxybenzoic acid (mCPBA). Finally, the *n*-octyl

thiirane oxide was characterized by ^{1}H NMR, ^{13}C NMR, IR, MS, and elemental analysis.

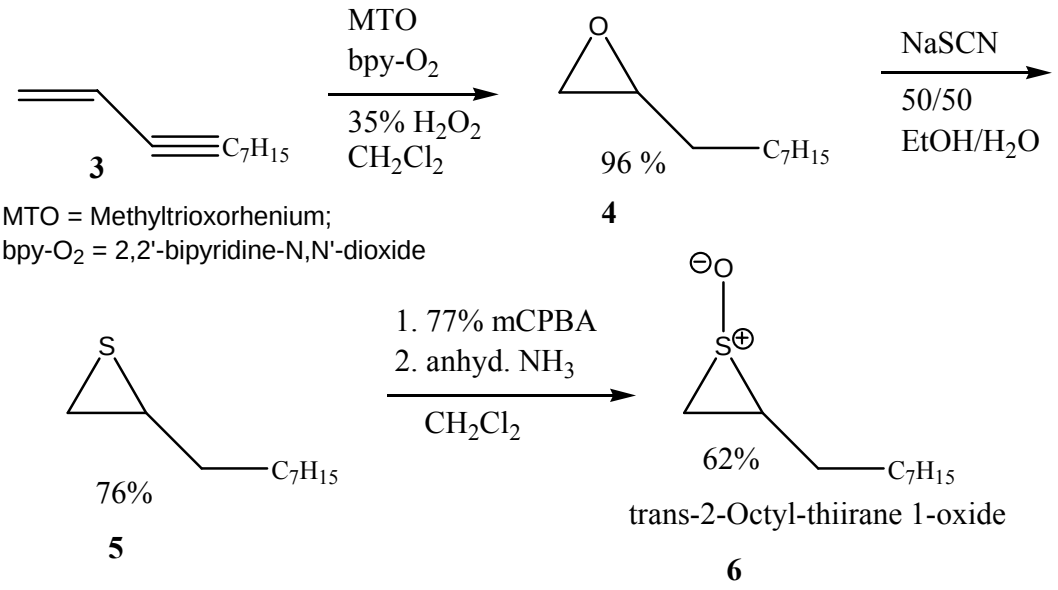


Figure 3-6: Synthetic scheme for *n*-octyl thiirane oxide

The critical micelle concentration (CMC)[24] was determined by two methods: dye solubility and capillary rise. The dye solubility method monitors the concentration of Sudan III in the solution as a function of surfactant concentration. The Sudan III, a dye, is not soluble in aqueous media unless it is supported by micellar formation.[18,25] Therefore, as soon as micelles form (above the CMC), the concentration of Sudan III in solution rises and allows the

determination of the CMC of the surfactant. Figure 3-7 plots the dye absorbance (right axis) versus concentration of *n*-octylthiirane oxide. An increase in concentration of Suddan III is observed at *n*-octylthiirane oxide concentration of 6.4 mM.

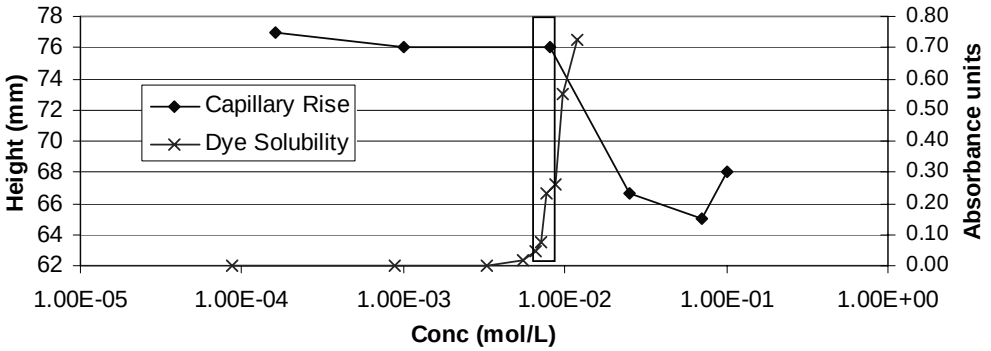


Figure 3-7: CMC determination by capillary rise & dye solubility

Capillary rise uses the height of a solution in a capillary measured in mm and plotted versus the concentration of *n*-octylthiirane oxide (Figure 3-7, left axis).[26] A sharp drop from 76 mm to 66 mm is observed at a concentration of 8.0 mM. From the data of both methods, the critical micelle concentration range of *n*-octylthiirane oxide was determined to be 6.4-8.0 mM. Using the same methods of analysis, the commercially available surfactant sodium dodecylsulfate (SDS)

exhibited a critical micelle concentration of 6-8 mM consistent with the range reported in the literature.[27]

The rate of the retro-cheletropic reaction of *n*-octylthiirane oxide was studied by quantitative ^{13}C NMR. The signal of the tertiary carbon in the thiirane oxide ring is seen at 50.3 ppm. The signal of this same carbon in 1-decene, now a sp^2 carbon, is observed at 140 ppm (Figure 3-8).

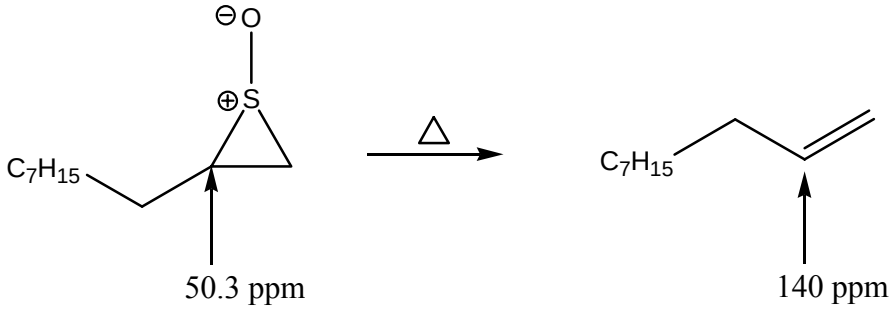


Figure 3-8: Retro-cheletropic decomposition noted with relevant chemical shift

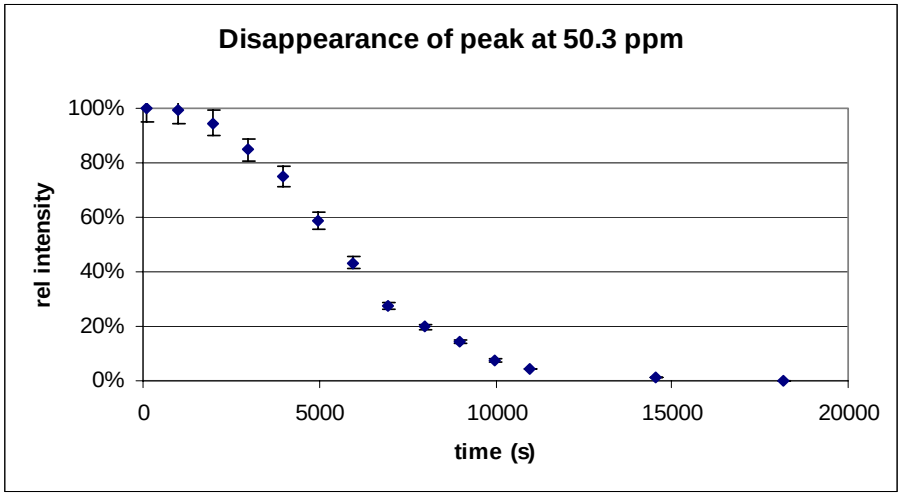


Figure 3-9: Intensity of peak at 50.3 ppm vs. time. (estimated 5% error shown)

A solution of *n*-octylthiirane oxide in d-chloroform was placed in a closed NMR tube, which was then introduced into the pre-heated NMR probe (70ºC). When the temperature of the solution inside the NMR tube reached equilibrium, the NMR spectrum was recorded every 16.6 min (996 seconds), and the peak intensity (at 50.3 ppm) was plotted versus time as shown in Figure 3-9. After 183 minutes, the ^{13}C NMR showed that all of the *n*-octylthiirane oxide has fully decomposed. The resulting kinetic rate profile in Figure 3-9 is consistent with a reaction with an induction period or an autocatalysis reaction.

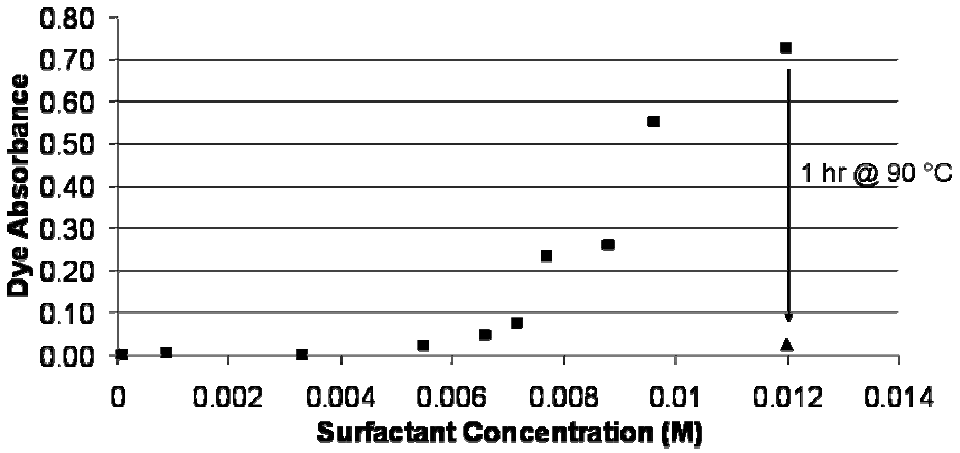


Figure 3-10: Dye solubility as a function of concentration. Triangle indicates dye solubility after heating at 90 °C

The loss of surface activity of *n*-octylthiirane oxide upon application of heat was then investigated. In Figure 3-10, the squares show the absorbance of the Sudan III aqueous emulsion versus the *n*-octylthiirane oxide surfactant concentration. At a concentration of 120 mM of surfactant (well above CMC), the emulsion of Sudan III was heated at 90ºC for one hour. As can be seen in Figure 3-10, the absorbance drops from about 0.70 to close to zero (shown by the triangle) indicating the loss of the dye's solubility in the water solution. It is known that emulsions can be broken solely by heat; therefore, we needed to compare the *n*-octylthiirane oxide behavior with the already mentioned commercially available sodium dodecylsulfate (SDS). The first two bars in Figure 3-11 correspond to the absorbance of the Sudan III and SDS saturated emulsion in water. Upon heating for

one hour, both the emulsions of the Sudan III in water with SDS (on the left in Figure 3-11) and *n*-octylthiirane oxide (in the middle in Figure 3-11) broke. However, once the mixtures were cooled down, both flasks were shaken/mixed again. The SDS-based emulsion re-formed instantaneously as seen by the second bar (white). In contrast, the emulsion did not reform in the flask which initially contained the *n*-octylthiirane oxide as seen by the second bar (white). This confirms the irreversible loss of the surface active character. On the right side of Figure 3-11, the same experiment was repeated with Sudan III, *n*-octylthirane oxide as surfactant, except that the heating lasted only 10 min instead of one hour. The loss of surface active character was again observed. It should be noted that the time required to lose the surface activity may be even less than ten minutes, but has not yet been tested.

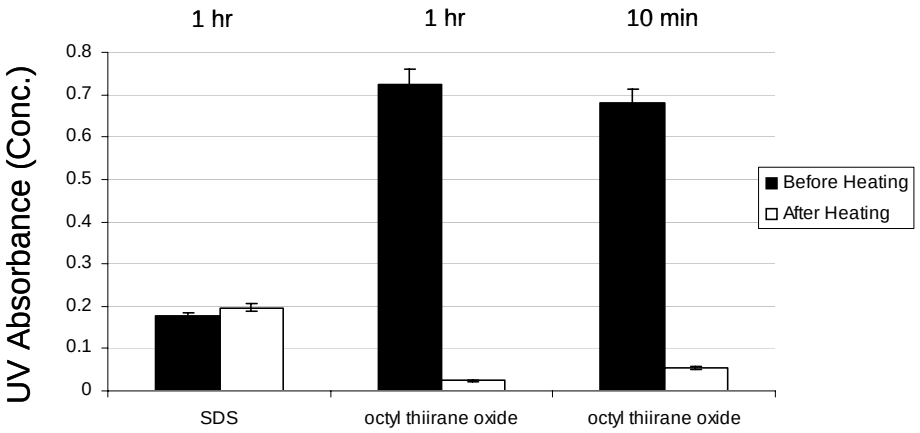


Figure 3-11: Dye solubility before (white) and after (black) heating at 90 °C and cooling. SDS shown as a control

3.3.2 Switchable Surfactants

Upon heat, piperylene sulfone reversibly decomposes into two gas, piperylene and SO_2 (Figure 3-12). The recombination of piperylene and SO_2 allows for the reformation of piperylene sulfone. By analogy, it was hypothesized that an alkyl substituted sulfolene can act as a switchable surfactant (Figure 3-13).

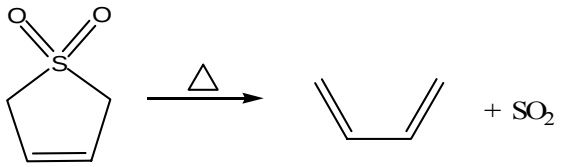


Figure 3-12: Piperylene sulfone decomposes into piperylene and sulfur dioxide

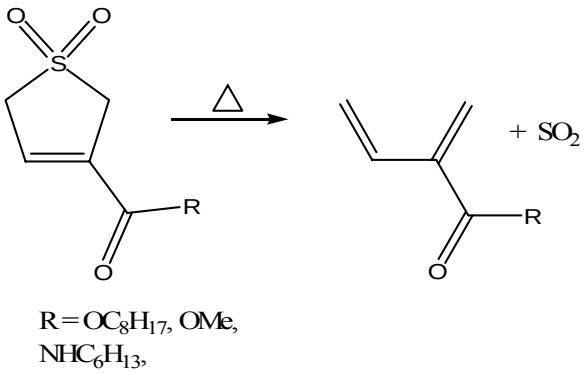


Figure 3-13: Sulfolene surfactant decomposes into a butadiene with an ester or amide group and sulfur dioxide

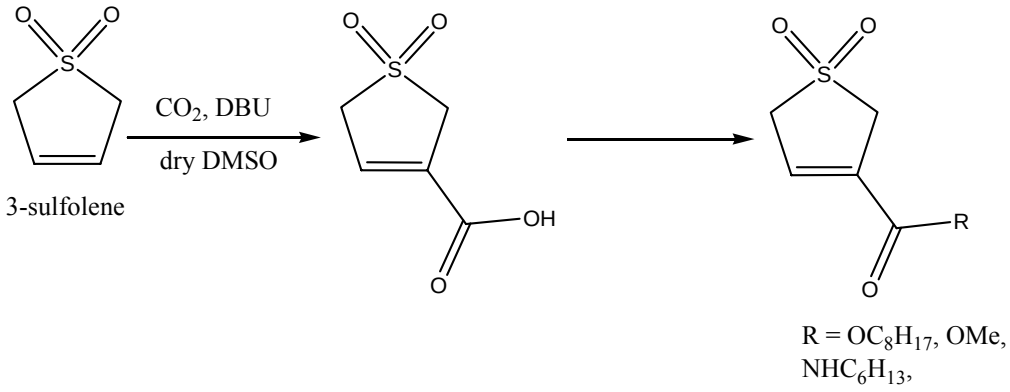


Figure 3-14: 3-sulfolene to carboxylic acid then to ester or amide link with alkyl chain

The 3-sulfolene carboxylic acid was known in the literature.[28] The strategy was therefore to prepare the 3-sulfolene carboxylic acid and subsequently form an ester as seen in Figure 3-14. The 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid was synthesized using a literature procedure, shown in Figure 3-15.[28] The carboxylic acid was formed by reacting 3-sulfolene with carbon dioxide (50 psi) and DBU (2 equiv) to form the DBU salt of the carboxylic acid. The salt was then protonated by HCl to form 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid which was purified using a silica gel plug and acetone for a white solid with a 66% yield. The 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid was characterized using ^{1}H NMR, ^{13}C NMR in d-DMSO and elemental analysis and was consistent with the literature.[28] The DSC/TGA of the 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid is shown in Figure 3-16. The DSC shows an endothermic peak at 183ºC which indicates a melting point. The

endothermic peak in the DSC occurs at the same temperature as when the weight loss begins in the TGA. This indicates that the melting point is also the decomposition temperature. The TGA first shows a weight percent loss of 67.20% then a loss of 24.27%. This seems to indicate that the carbon dioxide from the carboxylic acid is lost after the decomposition of the sulfolene.

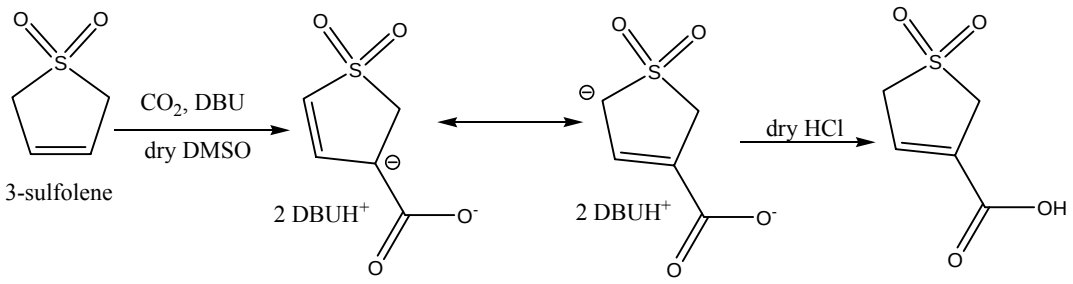


Figure 3-15: Synthesis of 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid

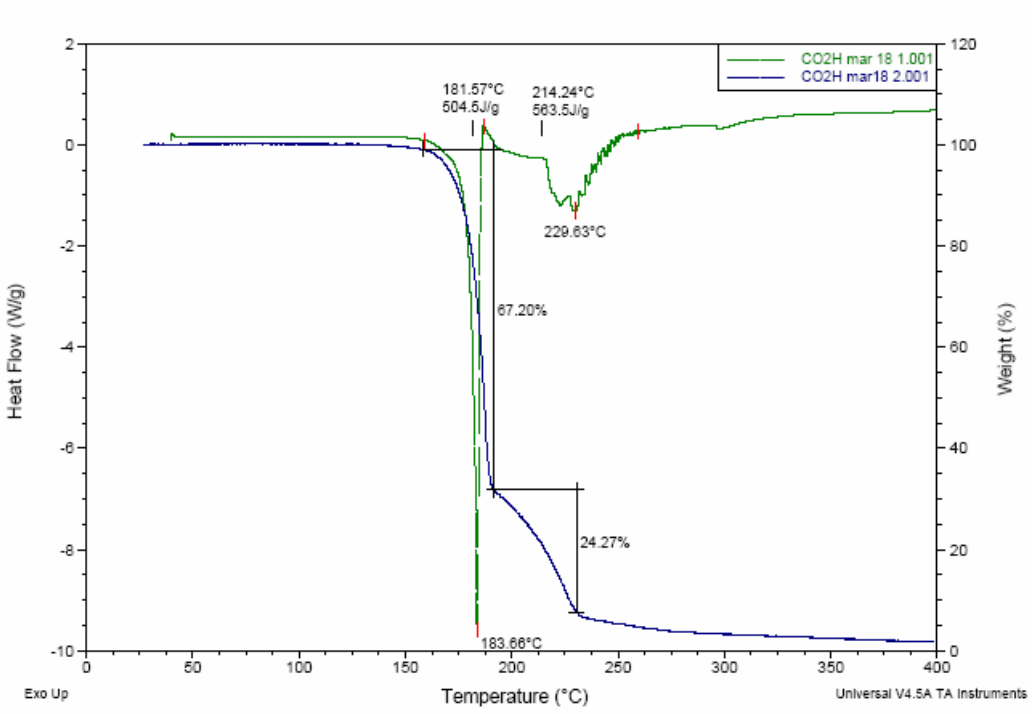


Figure 3-16: DSC/TGA of 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid

The 3-methoxycarbonyl-3-sulfolene was synthesized from 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid using PTSA (10 mol %), as the acid catalyst, and excess methanol at room temperature for 72 hours (Figure 3-17). The work-up yielded a white solid (yield 79%). The 3-methoxycarbonyl-3-sulfolene was characterized using ^{1}H and ^{13}C NMR, and DSC/TGA. The DSC/TGA of the 3-methoxycarbonyl-3-sulfolene is seen in Figure 3-18. The DSC shows an endothermic peak, indicating melting, at 62.25ºC which is consistent with the literature melting point.[29,30] The TGA shows an initial weight loss of 89.47%. The remaining weight percent could correlate with the methyl from the methyl ester.

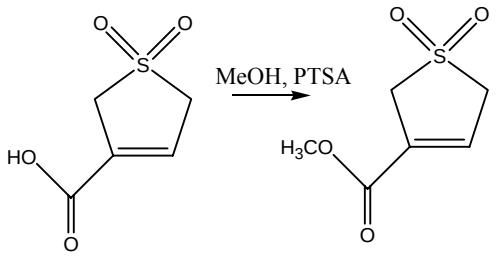


Figure 3-17: Synthesis of 3-methoxycarbonyl-3-sulfolene

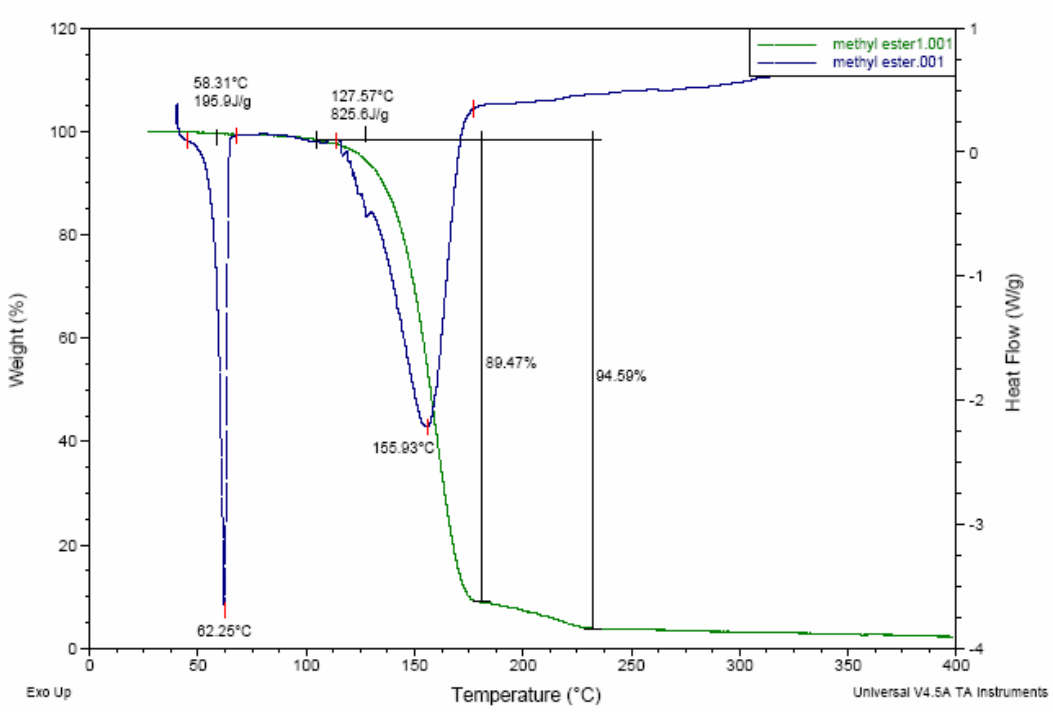


Figure 3-18: DSC/TGA of 3-methoxycarbonyl-3-sulfolene

The preparation of the octyl ester was attempted by means of 1) fisher esterification, 2) formation of the acid chloride followed by reaction with alcohol, 3) reaction with alkyl halide under various basic conditions, 4) acid catalyzed trans-esterification from the methyl ester, and 5) trans-esterification from the methyl ester catalyzed by *Candida Antarctica Lipase B*. All attempts were unsuccessful and detailed procedures are reported in the experimental section.

The reaction dimethyl amine and sulfolene 3-carboxylic acid to form the dimethyl amide was reported as seen in Figure 3-19.[31] By analogy, the reaction

of the 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid with hexyl amine based on the reported procedure was attempted. The product however was the product of the acid-base reaction, the ammonium carboxylate salt of the amine and carboxylic acid. The ammonium carboxylate salt was characterized by ^{1}H NMR, ^{13}C NMR, IR, DSC/TGA and elemental analysis. All attempts to form the amide were unsuccessful and are detailed in the experimental section.

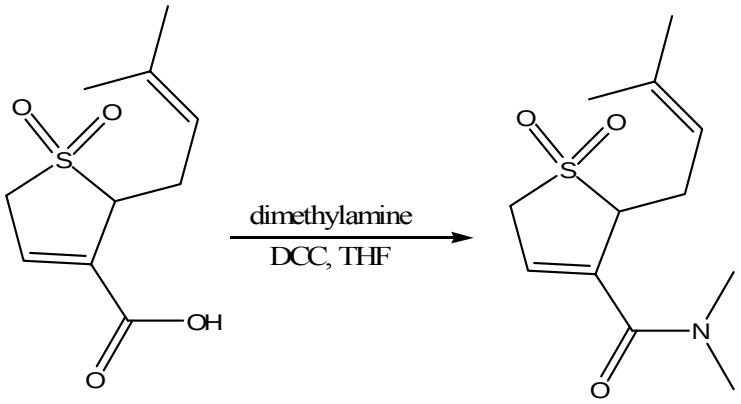


Figure 3-19: Synthesis of amide from carboxylic acid in literature

3.4 Conclusions

n-Octyl thiirane oxide was successfully synthesized and its surface active property was determined. The irreversible decomposition upon heating of n-octyl thiirane oxide to surface inactive fragments was demonstrated.

The synthesis of a sulfolene based switchable surfactant was unsuccessful although the synthesis of the sulfolene methyl ester was successfully.

3.5 Experimental

All chemicals were ordered from Aldrich or VWR and used as received, unless noted. ^{1}H and ^{13}C NMR spectra were recorded using a Varian Mercury Vx 400 spectrometer using residual DMSO peak as an internal reference. Mass Spectrometry samples were submitted to Mass Spectrometry Lab and used Micromass Quattro LC to perform ESI-MS. Elemental analyses were submitted to Atlantic Microlabs, Inc. Thermal analyses studies were performed on TA instruments Differential Scanning Calorimeter (DSC) Model Q20 and Thermogravimetric Analyzer (TGA) Model Q50. Samples were heated at 5°C/ min for both DSC and TGA analyses. DSC experiments were performed in standard sealed pans.

Synthesis of *n*-octyl epoxide:[21]

Methyltrioxorhenium (MTO) (12.4 g, 0.5 mmol) and bipyridine-*N,N'*-dioxide (11.3g, 0.6 mmol) were added to CH_2Cl_2 (20 mL) in a flask flushed with nitrogen. Then, 35 wt % H_2O_2 (7.0 mL, 0.08 mol) was added to the flask. After allowing the solution to stir for five minutes, 1-decene (**3**) (10 mL, 0.053 mmol) was added. The reaction was then stirred for sixteen hours at rt. The reaction

mixture was washed with water (3 x 20mL), dried over magnesium sulfate, and filtered. Finally, the solvent was removed under reduced pressure to yield a yellow oil (96.2%).

n-Octyl epoxide:

^{1}H NMR (ppm): 2.89 (m), 2.74 (m), 2.46 (m), 1.39 to 1.55 (m), 1.27 (br m), 0.85 (t). ^{13}C NMR (ppm): 52.4, 47.1, 32.5, 31.8, 29.5, 29.4, 29.2, 26.0, 22.6, 14.0. EA: calcd: C 76.86, H 12.92; found: C 76.44, H 13.15.

n-Octyl ethylene episulfide:[22]

Sodium thiocyanate (7.4 g, 0.09 mol) was added to a solution of ethanol and water (100 mL, 50/50 v/v). The *n*-octyl ethylene epoxide (**4**) (7.94 g, 0.05 mol) was slowly added to the solution over three hours and stirred over night at room temperature. The reaction was extracted with CH_2Cl_2 (3x20 mL). The organic phases were combined, dried over $MgSO_4$, filtered and the solvent removed under reduced pressure to yield a yellow oil. The oil was purified by silica gel column chromatography using hexane and ethyl acetate (50/50) (yield 64%).

n-Octyl ethylene episulfide:

^{1}H NMR (ppm): 2.87 (m), 2.50 (d), 2.15 (d), 1.39 to 1.54 (m), 1.27 (br m), 0.87 (t). 1^{3}C NMR: 52.2, 46.9, 36.3, 31.6, 29.2, 29.1, 29.0, 25.7, 22.4, 13.9. EA: calcd C 69.70 H 11.70 found: C 69.13 H 11.85.

n-Octyl thiirane oxide:[23]

The *n*-octyl episulfide (**4**) (7.88 g, 0.046 mol) was added to CH_2Cl_2 (25 mL). A solution of *m*-chloroperbenzoic acid (mCPBA) (11.34 g. 0.051 mol) in CH_2Cl_2 (100 mL) was prepared and added to the episulfide solution over three hours at rt. The resulting solution was stirred for an additional hour. Gaseous anhydrous ammonia (6 mL, 0.5 mol) was condensed using a dry ice/acetone cooled condenser and added to the reaction mixture. The precipitate of the ammonium salt formed from the benzoic acid by-product and ammonia was removed from the solution by filtration. The solution was dried over magnesium sulfate. After filtration, the solvent was removed under reduced pressure. The product was purified by silica gel column chromatography using hexane and ethyl acetate (50/50) to yield a clear, colorless oil (79.7%).

n-octyl thiirane oxide:

^{1}H NMR (ppm): 2.92 (m), 2.66 (dd), 1.99 (dd), 1.51 (m), 1.27 (m), 0.87 (t). ^{13}C NMR: 50.3, 41.5, 31.7, 29.4, 29.2, 29.0, 28.9, 27.5, 22.5, 14.0. ESI-MS: m/z = 189 (M+1). EA: calcd: C 63.77, H 10.70; found C 63.63, H 10.86. IR: 1067 cm^{-1} (υS=O).

CMC Determination

Six solutions of different concentrations of surfactant were made in water. Solutions of the surfactant in water were made gravimetrically; no stock solutions, or dilutions thereof, were made.

Dye Solublization/Sudan III

McElhanon *et al.* have published this method.[19] Samples of all concentrations were removed from the standards and placed in vials. Excess Sudan III was added to each of the dilutions in small vials. The vials were sonicated for 30 minutes, allowed to settle ft least two hours before filtering through a 0.2 μm, 13 mm PTFE syringe filter. Analysis by UV-vis spectroscopy gives an absorbance proportional to dye concentration. Experiments run in duplicate.

Capillary Rise

5 μL capillary pipettes were placed in six standard vials. The height of the liquid was marked and measured. Experiments were run in triplicate with new capillaries each time.

Synthesis of 2,5-Dihyrothiophene-1,1-dioxide-3-carboxylic acid[28]

A solution of 3-sulfolene (4.02 g, 34.0 mmol) in 1,8-diazabicyclo[5.4.0]undec-7-ene (10.2 mL, 68.2 mmol) was placed in a pressure reactor apparatus. The pressure reactor apparatus was purged three times with CO_2 and dry DMSO (10 mL) was added to the reaction. Approximately 50 psi of CO_2 was added to the pressure vessel and stirred for 72 hours. The product in the form of a DBU salt was precipitated using acetone (50 mL) and filtered. The salt

was dissolved in methylene chloride (200 mL). Dry HCl was bubbled through the solution until the pH reached four. The solvent was removed under reduced pressure. The resulting oil was dissolved in acetone (25 mL) and the solution was eluted through a silica plug. The plug was then flushed with additional acetone (75 mL). The solvent was removed under reduced pressure to give 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid as an off-white solid. The solid was dried in a vacuum oven at 40ºC for 48 hours to remove any remaining water. The product was isolated as a white solid at 66% yield.

2,5-Dihyrothiophene-1,1-dioxide-3-carboxylic acid:

^{1}H NMR (DMSO, ppm): 3.98 (m, 2H), 4.12 (m, 2H), 6.95(m, 1H), 13.22 (s, 1H). ^{13}C NMR (DMSO, ppm): 55.32, 58.24, 130.79, 134.99, 164.35. EA: calculated, C, 37.03%, H, 3.73%, S, 19.77%; found C, 36.85%, H, 3.65%, S, 19.77%. DSC/TGA shown in Figure 3-16.

Fisher Esterification:

For all the Fisher esterification methods tried, all the carboxylic acid was observed to be reacted by TLC and ^{1}H NMR. When a silica column was tried to isolate the product, the octanol was not successfully removed.

Excess octanol and sulfuric acid

An excess of dry octanol (5 mL) was added to dry 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid (0.5 g, 4.84 mmol). To the solution, sulfuric acid (0.5 mL) was added dropwise. The reaction was heated gradually to 60ºC and allowed to reflux for 48 hours. When the reaction did not show product, 1,3-dicyclohexylcarbodiimide (DCC) (1.0 g) was added as a drying agent. To work up the reaction, ethyl acetate (50 mL) was added and the solution was washed with saturated $NaHCO_3$ (3 x 75 mL). The solution was then washed once with water and washed once with saturated NaCl. The solution was then dried over magnesium sulfate and the solvent was removed under reduced pressure. A silica column was packed using hexane and the solute was 90/10 hexane/EtOAc as solute. The fractions collected still contained octanol.

Octanol and PTSA

1,3-Dicyclohexylcarbodiimide (DCC) (1.5893 g, 1 equiv, 7.27 mmol) was added to a round bottom flask. 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid (0.5 g, 4.85 mmol) was added. The round bottom was put under argon and octanol (0.8 mL, 1 equiv) was added. Then THF (5 mL) was added. To this solution, the *p*-toluene sulfonic acid (PTSA) (0.15 g) was added. The solution was heated gradually to 60ºC and was heated overnight. To work up the reaction, ethyl acetate (40 mL) was added and the solution was washed with saturated sodium bicarbonate (2 x 50 mL), water (1 x 50 mL), and brine (1 x 50 mL). The

solution was then dried over magnesium sulfate and the solvent was removed under reduced pressure. No product was observed by ^{1}H NMR.

Acid chloride as step 1 then ester from alcohol and base

2 equiv pyridine, 1 equiv octanol:

First, 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid (0.5 g, 4.85 mmol) was added to an excess of thionyl chloride (2 mL) at room temperature under argon. The reaction was monitored by TLC. After 2 hours, the excess thionyl chloride was removed under reduced pressure. After the removal of the thionyl chloride, the reaction was put back under argon. Anhydrous octanol (0.76 mL, 1 equiv) was added to the solution at 0ºC. Then, anhydrous pyridine (0.78 mL, 2 equiv) was added dropwise to the solution at 0ºC and 5 mL of anhydrous THF was added after 30 minutes. The solution was allowed to warm to room temperature over 1 hour. The reaction was then heated to 50ºC overnight. To work up the reaction, the solution was cooled to room temperature and ethyl acetate (40 mL) was added. The solution was then washed with water (4 x 75 mL), saturated aqueous $NaHCO_3$ (2 x 75 mL), and brine (1 x 75 mL). The solution was dried over magnesium sulfate, filtered and the solvent was removed under reduced pressure to give a dark brown solution. From the ^{13}C NMR, there was no carbonyl peak leading us to believe that no reaction occurred.

1.2 equiv pyridine, 1.2 equiv octanol:

First, 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid (0.5 g, 4.85 mmol) was added to an excess of thionyl chloride (2 mL) at room temperature under argon. After 3.5 hours at 40ºC, the excess thionyl chloride was removed under reduced pressure. After the removal of the thionyl chloride, the reaction was put back under argon. To the reaction mixture, dry THF (10 mL) was added. Anhydrous octanol (0.76 mL, 1 equiv) was added to the solution at 0ºC. Then, anhydrous pyridine (0.47 mL, 1.2 equiv) was added dropwise to the solution at 0ºC. The solution was allowed to warm to room temperature overnight. To work up the reaction, the reaction mixture was poured onto dichloromethane (50 mL) and ice water (100 mL). The dichloromethane layer was removed and the ice water mixture was extracted with more dichloromethane. The dichloromethane layers were combined and washed with brine. The solution was dried over magnesium sulfate and the solvent was removed under reduced pressure. The resulting ^{1}H NMR showed octanol but no carboxylic acid so it may have decomposed in the first step.

1.2 equiv pyridine, 1.2 equiv octanol with the first step at 0ºC

First, 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid (0.5 g, 4.85 mmol) was added to an excess of thionyl chloride (2 mL) at 0ºC under argon. After 1 hour at 0ºC, the excess thionyl chloride was removed under reduced pressure. After the removal of the thionyl chloride, the reaction was put back

under argon. To the reaction mixture, THF (10 mL) was added. Anhydrous octanol (0.76 mL, 1 equiv) was added to the solution at 0ºC. Then, anhydrous pyridine (0.47 mL, 1.2 equiv) was added dropwise to the solution at 0ºC. The solution was allowed to warm to room temperature overnight. To work up the reaction, the reaction mixture was poured onto dichloromethane (50 mL) and ice water (100 mL). The dichloromethane layer was removed and the ice water mixture was extracted with more dichloromethane. The dichloromethane layers were combined and washed with brine. The solution was dried over magnesium sulfate and the solvent was removed under reduced pressure. The resulting ^{1}H NMR showed octanol but no carboxylic acid so it may have decomposed in the first step.

Base and Octyl Iodide

Cesium carbonate

First, 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid (0.5 g, 4.85 mmol) was added under argon to dry THF (10 mL). Then cesium carbonate (1.4205 g, 1.5 equiv) was added. Then aliquot 336 (0.13 mL, 10 mol %), a phase transfer catalyst, was added After 2 hours, the octyl iodide was added (1.05 mL, 1.1 equiv) and allowed to react overnight at room temperature. The reaction was filtered. The reaction mixture was poured onto dichloromethane (50 mL) and ice water (100 mL). The dichloromethane layer was removed and the ice water

mixture was extracted with more dichloromethane. The dichloromethane layers were combined and washed with brine. The solution was dried with magnesium sulfate and the solvent was removed under reduced pressure. No reaction by ^{1}H NMR.

Pyridine

First, 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid (0.5 g, 4.85 mmol) was added under argon to 10 mL of THF from the still. Then pyridine (0.41 mL, 1.05 equiv) was added. After 1 hour, the octyl iodide was added (1.05 mL, 1.1 equiv) and allowed to react overnight at 40ºC. The reaction mixture was poured onto dichloromethane (50 mL) and ice water (100 mL). The dichloromethane layer was removed and the ice water mixture was extracted with more dichloromethane. The dichloromethane layers were combined and washed with brine. The solution was dried over magnesium sulfate and the solvent was removed under reduced pressure. No reaction by ^{1}H NMR.

DBU

First, 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid (0.3876 g, 3.76 mmol) was added under argon to dry THF (10 mL). Then DBU (0.59 mL, 1.05 equiv) in 5 mL of dry THF was added dropwise, forming a white precipitate. After 1 hour, the octyl iodide was added (0.75 mL, 1.1 equiv) and allowed to react

overnight at room temperature. The reaction mixture was poured onto dichloromethane (50 mL) and ice water (100 mL). The dichloromethane layer was removed and the ice water mixture was extracted with more dichloromethane. The dichloromethane layers were combined and washed with brine. The solution was dried over magnesium sulfate and the solvent was removed under reduced pressure. No reaction by ^{1}H NMR.

DBU salt carboxylic acid

A solution of 3-sulfolene (4.02 g, 34.0 mmol) in 1,8-diazabicyclo[5.4.0]undec-7-ene (10.2 mL, 68.2 mmol) was placed in a pressure reaction apparatus. The pressure reaction apparatus was purged three times with CO_2 and dry DMSO (10 mL) was added to the reaction. Approximately 50 psi of CO_2 was added to the pressure vessel and stirred for 72 hours. The mixture was diluted with acetone (50 mL) and filtered. ^{13}C NMR (DMSO, ppm): 9.63, 14.63, 22.78, 26.03, 28.59, 28.72, 29.27, 30.57, 31.91, 33.62, 55.16, 58.12, 65.63, 135.67. Elemental analysis: calculated, C, 59.51%; H, 8.45%; N, 10.68%; found: C, 59.27%; H, 8.00%; N, 10.46%.

DBU salt and 1.2 equiv octyl iodide

The DBU carboxylic acid salt was dried in a vacuum oven overnight at 40ºC. The DBU carboxylic acid salt (1.5 g) was dissolved in dichloromethane (50

mL). Then octyl iodide (0.8 mL, 1.2 equiv) was added to the reaction mixture. The reaction was left overnight at room temperature. No reaction by ^{1}H NMR.

DBU salt and 3.5 equiv octyl iodide

The resulting DBU carboxylic acid salt was dried in a vacuum oven overnight at 40ºC. Then part of the resulting DBU carboxylic acid salt (1.5 g) was dissolved in 50 mL of dichloromethane. Then octyl iodide (2.3 mL, 3.5 equiv) was added to the reaction mixture. The reaction was left overnight at room temperature. No reaction by ^{1}H NMR.

Transesterification

2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid methyl ester

An excess of methanol (5 mL) was added to dry 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid (0.1 g, 0.96 mmol) and PTSA (0.02 g, 10 mol %). The reaction was put under argon at room temperature over the weekend. The solution was then extracted using ice, saturated aqueous $NaHCO_3$, and dichloromethane. The solution was then dried over magnesium sulfate and the solvent was removed under reduced pressure. The methyl ester was a white solid (0.0894g, 79 % yield).

2,5-Dihyrothiophene-1,1-dioxide-3-carboxylic acid methyl ester: ^{1}H NMR (DMSO, ppm): 3.72 (s, 3H), 4.03 (m, 2H), 4.14 (m, 2H), 7.04 (s, 1H). ^{13}C

(DMSO, ppm): 52.31, 54.43, 57.36, 129.46, 135.25, 156.60. mp = 62.25ºC.[29,30] The DSC/TGA is shown in Figure 3-18.

Octyl ester from methyl ester using PTSA

An excess of octanol (5 mL) was added dropwise to dry 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid methyl ester (0.3 g, 2.56 mmol) in dry THF (2 mL), molecular sieves, and PTSA (0.04 g, 10 mol %). The reaction was put under argon at room temperature over 2 weeks. The solution was then extracted with ice, saturated aqueous $NaHCO_3$, and dichloromethane. The solution was then dried over magnesium sulfate and the solvent was removed under reduced pressure. No reaction by ^1H NMR.

Octyl ester from methyl ester using enzyme and excess octanol[32]

The 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid methyl ester (0.11 g) was added to anhydrous octanol (3 mL). Molecular sieves and the enzyme *Candida Antarctica Lipase B* immobilized on resin (50 mg) were added to the reaction. The reaction was put under nitrogen, wrapped in foil, and heated to 40ºC overnight. No reaction by ^1H NMR.

Octyl ester from methyl ester using enzyme and 3 equiv octanol

The 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid methyl ester (0.1 g, 0.85 mmol) was dissolved in distilled acetone (2 mL). Molecular sieves (200 mg) and the enzyme *Candida Antarctica Lipase B* immobilized on resin (50 mg) were added to the solution and the solution was put under nitrogen. Octanol (0.4 mL, 3 equiv) was added to the solution. The round bottom flask was covered with foil and heated to 40ºC. After a week, ^{1}H NMR showed no more starting material of the methyl ester. The acetone was removed under reduced pressure. Product was not observed by ^{1}H NMR.

Octyl ester from methyl ester using enzyme and 3 equiv methyl ester

The 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid methyl ester (0.4 g, 0.00227 mol, 3 equiv) was added to acetone (1 mL) and molecular sieves. The reaction was put under nitrogen and the round bottom flask was covered with foil. The *Candida Antarctica Lipase B* (50 mg) was added. The octanol (0.1 mL, 1 equiv) was added. The reaction was heated to 40ºC. After 2 days, the NMR showed some possible product peaks and the octanol peak had disappeared by TLC (99/1 EtOAc/Hex), showing the reaction may be complete. However, both the product and the methylester were on the baseline of the TLC. The immobilized enzyme and molecular sieves were removed by filtration and washed with acetone. The unreacted methylester seemed to precipitate out as a fine white

solid in the fridge. However, after a week in the fridge not all of the methylester had precipitated out so this route was abandoned.

Amide

Amide synthesis in dichloromethane

Dry 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid (0.3780 g, 0.00233 mol) and 1,3-dicyclohexylcarboiimide (DCC) (0.37 g, 0.0018 mol) were combined and dichloromethane (12 mL) was added until all of the reactants dissolved. The reaction solution was put under nitrogen and wrapped in foil. The hexyl amine (0.2 mL, 0.0018 mol) was added to the reaction solution. The reaction proceeded at room temperature and was monitored by NMR for 5 days until all of the starting material was reacted. The dichloromethane was removed under reduced pressure. Then ethylacetate (10 mL) was added and a white precipitate formed. The precipitate (0.5725g) was removed and dissolved in chloroform (10 mL). The chloroform was washed 3x with water. An emulsion formed that made the extraction difficult. The water layers were combined and back extracted with 3x chloroform. No emulsion was formed during the back extraction. Both the extractions were dried with magnesium sulfate and the solvent was removed. NMR only showed hexyl amine in the ethylacetate filtrate. Tried fractional crystallization by adding ethylacetate until all the solid is dissolved, then added 2 drops more. The solution was put into the fridge

overnight for 2 days and filtered. The yellow oil was still impure by NMR. The impurity is 1,3-dicyclohexyl-urea (DHU) which is the DCC reacted with the water. To try to remove the DHU, the yellow oil was dissolved in ethylacetate (5 mL) and washed with water (5 x 10 mL). The water layer was then back extracted with ethylacetate (1 x 7 mL). Both extractions were dried over magnesium sulfate and the ethylacetate was removed under reduced pressure. The resulting yellow solid was still impure with DHU and no clear product peaks were observed by ^{1}H NMR.

Amide in ether

Dry 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid (0.5 g, 0.0031 mol) and 1,3-dicylcohexylcarboimide (DCC) (0.5 g, 0.0024 mol) were combined in a round bottom flask. To the reaction mixture, THF (15 mL) was added. Since the reactants did not dissolve, ether (16 mL) was added. Since the reactants, still did not dissolve, the reaction was heated to 35ºC but the reactants still did not dissolve so the heating was stopped. The hexyl amine (0.24 g, 0.0024 mol) was added to the reaction solution, causing an increase in temperature of 1ºC (from 26.6ºC to 27.7ºC). The reaction was allowed to continue overnight. In the morning, the NMR showed no more starting material carboxylic acid. The solvent was removed under reduced pressure, giving a yellow solid. The DHU

could not be successfully removed and we believe that the salt of the amine and carboxylic acid formed.

2,5-Dihyrothiophene-1,1-dioxide-3-carboxylatehexyl-ammonium

The 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid (0.25 g, 0.0015 mol, 1.3 equiv) was dissolved in acetonitrile (15 mL). The hexyl amine (0.15 mL, 0.0012 mol) was added dropwise. The reaction was put under nitrogen. The reaction vessel was wrapped in foil and stirred at room temperature until solid formation was observed. The solvent was removed under vacuum, resulting in a pure white solid with quantitative yield.

2,5-Dihyrothiophene-1,1-dioxide-3-carboxylatehexyl-ammonium ^{1}H NMR (DMSO, ppm): 6.35 (1H, s), 3.94 (2H, s), 3.78 (2H, s), 2.72 (2H, t), 1.48 (2H, m), 1.26 (6H, m), 0.85 (3H, t). ^{13}C NMR (DMSO, ppm): 13.91, 21.95, 25.56, 27.13, 30.75, 38.61, 56.27, 57.88, 126.29, 128.51, 164.75. MS (M+1-SO_2) 184.1. EA: calculated C, 50.17%, H, 8.04%, N, 5.32%, S, 12.18%; found: C, 50.19%, H, 8.07%, N, 5.32%, S, 12.16%.

2,5-Dihyrothiophene-1,1-dioxide-3-carboxylateoctyl-ammonium

The 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid (0.5 g, 0.0031 mol, 1.3 equiv) was dissolved in acetonitrile (30 mL). The octyl amine (0.4 mL, 0.0024 mol) was added dropwise. The reaction was put under nitrogen. The reaction

vessel was wrapped in foil and stirred at room temperature until solid formation was observed. The solvent was removed under vacuum, resulting in a pure white solid with quantitative yield.

2,5-Dihyrothiophene-1,1-dioxide-3-carboxylateoctyl-ammonium: ^{1}H NMR (DMSO, ppm): 6.35 (1H, s), 3.94 (2H, s), 3.77 (2H, s), 2.72 (2H, t), 1.50 (2H, m), 1.23 (6H, m), 0.84 (3H, t). ^{13}C NMR (DMSO, ppm): 13.91, 21.95, 25.56, 27.13, 30.75, 38.61, 56.27, 57.88, 126.29, 128.51, 164.75. MS (octyl salt fragment) 171.1. EA: calculated C, 53.58%, H, 8.65%, N, 4.81%, S, 11.00%; found C, 53.20%, H, 8.25%, N, 4.92%, S, 10.98%.

Amide in acetonitrile and DCC [21]

The 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid (0.5 g, 1 equiv) was dissolved in distilled acetonitrile (15 mL). Hexyl amine (0.41 mL, 1 equiv) and 1,3-dicyclohexylcarbodiimide (DCC) (0.8 g, 1.2 equiv) were added. The reaction was put under nitrogen, wrapped in foil, and stirred at room temperature. The reaction was performed twice with the reaction proceed for 24 hours and then for 5 days. Both reactions did not show evidence of product by ^{1}H NMR but both showed formation of the 2,5-dihyrothiophene-1,1-dioxide-3-carboxylic acid and hexyl amine salt.

3.6 References

(1) *Other Types of Surfactants-Cationic Surfactants*; Farn, R., Ed.; Blackwell Publishing: Oxford, 2006.
(2) Riess, G. *Macrommol. Rapid Commun.* **2004**, *25*, 401-435.
(3) S. Hua; Zheng, G. Z., Y.;Liu, R.;Li, G. *Journal of Dispersion Science and Technology* **2006**, *27*, 311-315.
(4) *Reactions and Synthesis in Surfactant Systems*; Texter, J., Ed., 2001; Vol. 100.
(5) Holmberg, K. In *Reactions and Synthesis in Surfactant Systems*; Marcel Dekker: New York, 2001.
(6) Distler, H.; USA, 1966; Vol. 19620412.
(7) Jaeger, D. *Langmuir* **1990**, *6*, 547-554.
(8) Iyer, M. *Langmuir* **2001**, *17*, 6816-6821.
(9) Jaeger, D. *Langmuir* **1998**, *14*, 1940-1.
(10) Jaeger, D. *JACS* **1989**, *111*, 3001-6.
(11) Masuyama, A. *Langmuir* **2000**, *16*, 368-373.
(12) Nuyken, O. *Journal of Photochemistry and Photobiology, A: Chemistry* **1994**, *81*, 45-53.
(13) Epstein, W. *Analytical Biochemistry* **1982**, *119*, 304-12.
(14) Mezger, T. *Progress in Organic Coating* **1996**, *29*, 147-157.
(15) Nuyken, O. *Journal of Photochemistry and Photobiology, A: Chemistry* **1995**, *85*, 291-8.
(16) Metzner, B. *Journal of Photochemistry and Photobiology, A: Chemistry* **1994**, *83*, 129-40.
(17) Hayashi, Y. *J. Am. Oil Chem. Soc* **1985**, *62*, 555-557.
(18) Yingxin Liu, P. J., Michael Cunningham, Charles Eckert, Charles Liotta *Science* **2006**, *313*, 958-960.
(19) McElhanon, J. R. *Langmuir* **2005**, *21*, 3259-3266.
(20) Sulfur monoxide is an unstable gas, which quickly disproportionates into numerous sulfur compounds, making this a one way process a one way process.
(21) Nakjima, M. *Tetrahedron Letters* **1998**, *39*, 87-88.
(22) Snyder, H. R. *J. Am. Chem. Soc* **1947**, *69*, 2672-4.
(23) Kondo, K. *Tetrahedron Letters* **1969**, *29*, 2461-2464.
(24) *The critical micelle concentration (CMC) is the concentration at which a surfactant begins to form micelles*.
(25) *Dye Solublization/Sudan III: Excess Sudan III was added to various concentrations in small vials. The vials were sonicated for 30 minutes, allowed to settle for at least two hours before filtering through a 0.2 µm, 13 mm PTFE syringe filter. Analysis by UV-Vis spectroscopy gives an*

absorbance proportional to dye concentration. Experiments run in duplicate.
(26) *The rise of water in a capillary tube is directly related to the surface tension, which is disrupted in the presence of surface active agents. Experimentally, surfactants reduce the surface tension of the water, causing the height of capillary rise to drop once the critical micelle concentration has been reached*.
(27) Bales, B. M., L.; Vidal, A.; Peric, M.; Nascimento, O.; *J. Phys. Chem. B* **1998**, *50*, 10347.
(28) *Synthetic Communications* **2003**, *33*, 3643-3650.
(29) www.vwr.com.
(30) McIntosh, J. M. *J. Org. Chem.* **1978**, *43*, 4431.
(31) *J. Chem. Soc. Perkin Trans. 1* **1994**, 2359.
(32) Yan, Y. B., U.; and Schmid, R.; *Biotechnology Letters* **1999**, *21*, 1051.

CHAPTER 4: SYTHESIS OF 5-AMINO-1H-TETRAZOLE

4.1 Introduction

5-Amino-1H-tetrazole is a gas-generating molecule and is a valuable starting material for the synthesis of many tetrazole derivatives used in pharmaceutical, pyrotechnique and propellant technologies.[1-7] Commonly, the 5-amino-1H-tetrazole is synthesized from amino guanidinium nitrate and nitrous acid or from cyanamide and hydrazoic acid or some modified procedure of these syntheses. In this chapter, two novel syntheses of 5-amino-1H-tetrazole are reported avoiding the use of hazardous and/or toxic materials. Efforts focused on providing a safer, cost-efficient synthesis that yields a high quality 5-amino-1H-tetrazole product, free of potential toxic traces impurities.

4.2 Background

4.2.1 Uses of 5-Amino-1H-tetrazoles

Tetrazoles are unsaturated 5-membered ring heterocycles containing four nitrogen atoms and one carbon atom. The hydrolysis of tetrazoles forms the corresponding carboxylic acid making tetrazoles a stable and water soluble mimic of carboxylic acid in the pharmaceutical field. From an energetic material standpoint, it is the high nitrogen content of tetrazoles that is the most attractive

feature. For example, the nitrogen content of the unsubstituted tetrazole is about 80 wt.% while for the 5-amino-1H-tetrazole it is about 82 wt.%. Upon decomposition tetrazole molecules produce two moles of N_2 per tetrazole ring, making them attractive constituent for pyrotechnique, propellants and airbag compositions.

Specifically, the 5-amino-1H-tetrazole is a replacement for sodium azide (NaN_3) in the inflating airbag technology.[8] In contrast with sodium azide, the 5-amino-1H-tetrazole is aromatic and therefore is relatively stable, an advantage for processing, transport and storage. In addition, 5-amino-1H-tetrazole is a valuable starting material for the synthesis of many tetrazole derivatives used in pharmaceutical, pyrotechnique and propellant technologies.

4.2.2 Previous Synthesis of 5-Amino-1H-tetrazole

Previously, 5-amino-1H-tetrazole was synthesized using sodium azide and cyanamide. With the addition of acid, the sodium azide forms hydrazoic acid (HN_3). This reacts with the cyanamide to form 5-amino-1H-tetrazole as seen in Figure 4-1.[9] The disadvantage of this synthesis was that the amount of hydrazoic acid was controlled only by the rate of the addition of the acid. Hydrazoic acid is a gas, explosive, and toxic so it is advantageous to bypass its formation.

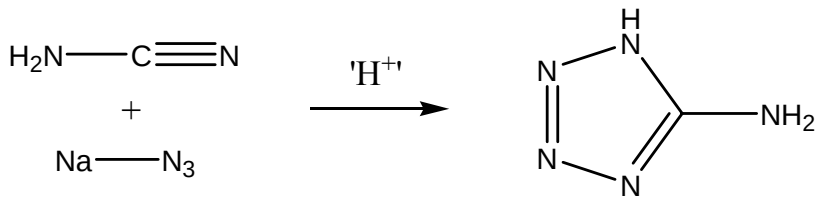


Figure 4-1: Previous Synthesis of 5-amino-1H-tetrazole by J.S.Mihina, R.M.Herbst

Another synthetic route for 5-amino-1H-tetrazole was developed in 1995. This synthesis built on the synthesis by Mihina and Herbst shown in Figure 4-1, using sodium azide and cyanamide with acid addition to form the 5-amino-1H-tetrazole.[10] The preferred acid was boric acid and the reaction solution was kept between a pH of 6-8. After reaction completion, they were able to precipitate the 5-amino-1H-tetrazole and control the crystal morphology by lowering the pH. The main difference from the previous synthesis was the use of neutral pH conditions during the reaction, which reduced the hydrazoic acid production. However, the use of hydrazoic acid was not eliminated so the procedure remained a high risk process.[10] In 1997, a procedure was developed that eliminated the use of hydrazoic acid. Instead it used cyanamide and hydrazine as reactants to form the aminoguanidine intermediate. The aminoguanidine is diazotized using hydrochloric acid and sodium nitrite and cyclized upon addition of sodium hydroxide to form the 5-amino-1H-tetrazole, as seen in Figure 4-2. Although the

procedure does eliminate the use of hydrazoic acid, it uses hydrazine and cyanamide which are also toxic chemicals.[11]

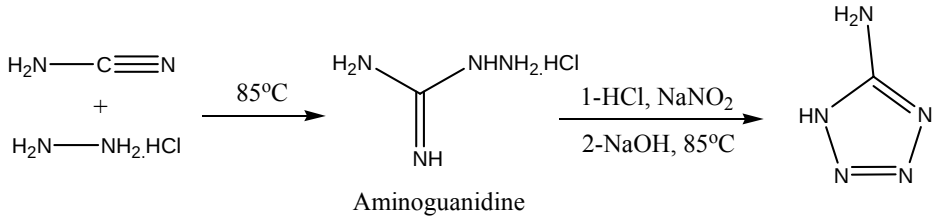


Figure 4-2: 1997 synthesis of 5-amino-1H-tetrazole by Masahiro

It is important to emphasize that the synthesis that was designed in our laboratory was intended for potential industrial process. Considerations like safety, cost, and efficiency were inherent to the development of the synthesis. First, the use of highly toxic and/or explosive chemicals was avoided to lessen the safety hazards for personnel and the cost associated with handling such chemicals. For example, hydrazoic acid is highly explosive at concentration as little as a few percent (in the gas phase) and can cause convulsions, coma, pulmonary edema, severe hypotension (shock) at doses greater than 10 mg/kg. Cyanamide is reported unstable at temperatures above 104ºF (40ºC) potentially yielding to violent thermal decomposition.[12,13]

4.2.3 Click Chemistry and Tetrazole

Click chemistry is the joining together of small units with heteroatom linkers.[14] The tetrazole is formed by [2,3] cycloaddition between organic azides and cyanides. This synthesis is analogues to the direct Huisgen 1,3-dipolar cycloaddition used to synthesize triazoles.[15] However, in the click chemistry cycloaddition, the electron withdrawing group bound to the cyanide lowers the activation barrier and makes the formation of the tetrarazole possible. This chemistry has been developed by Sharpless and was used to synthesize various tetrazole derivatives, an example is shown in Figure 4-3. These reactions are usually quantitative in yield, solventless, and therefore do not need extensive purification. In addition, the organic azide is substituted for increased reactivity.[16,17] Click-chemistry was never applied for the synthesis of the 5-amino-1H-tetrazole despite providing a distinctive opportunity to develop a benign yet efficient synthesis of the tetrazole ring.

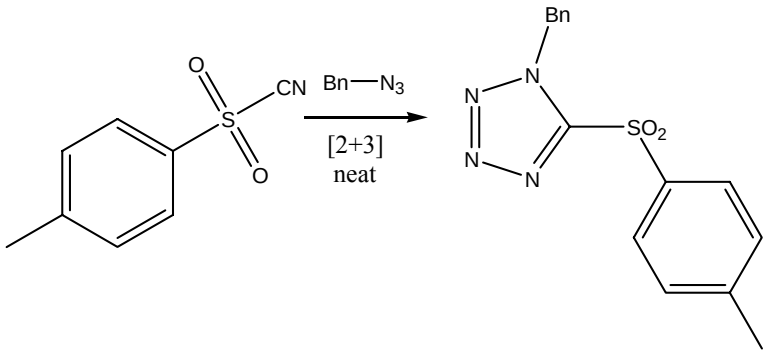


Figure 4-3: Example of Click Chemistry by Sharpless

4.3 Results and Discussion

4.3.1 Step-wise Synthesis Containing 1-Benzyl-5-benzylaminotetrazole

A three step synthesis for 5-amino-1H-tetrazole was developed that used click chemistry in the 1st step as seen in Figure 4-4.

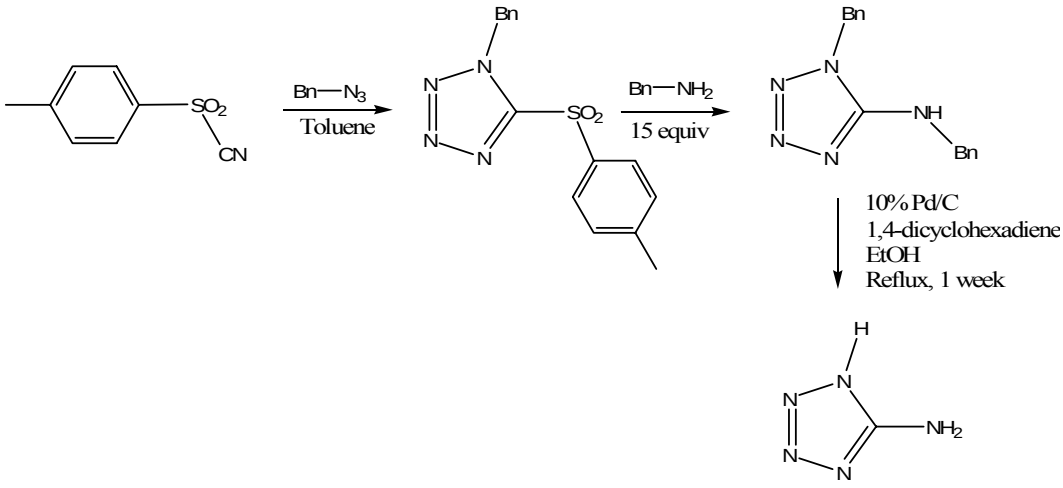


Figure 4-4: Three step synthesis for 5-amino-1H-tetrazole

In the first step, an equimolar mixture of benzyl azide and *p*-toluenesulfonyl cyanide was reacted to form 1-benzyl-5-sulfonyltoluene tetrazole. Initially, the cycloaddition was attempted neat at 80°C as reported by Sharpless and Demko.[16] However, in order to better control the viscosity and the heat diffusion, the cycloaddition reaction was then performed in toluene. The yields were identical although the reaction was slower (16 hour neat versus 24-30 hours

in toluene). The reaction mixture was homogeneous at 80°C, however, the 1-benzyl-5-sulfonyltoluene tetrazole precipitated upon cooling to room temperature, resulting in a very facile separation by filtration.

In the second step, the sulfonyl toluene group on 1-benzyl-5-sulfonyltoluene tetrazole was displaced with an amino benzyl group by using benzylamine as both the reactant and solvent to form 1-benzyl-5-benzylaminotetrazole. The separation of the product was trivial since its precipitation was induced upon the addition of water and the 1-benzyl-5-benzylaminotetrazole collected by simple filtration. Although the conversion was close to quantitative, the isolate yields were about 70 %—most probably due to the water solubility of the product. Introducing a solvent to the reaction to reduce the excess of benzylamine for higher atom efficiency and reach higher isolated yields met limited success. The results are summarized in Table 4-1. The conversions obtained in highly polar solvents like DMSO were high but unfortunately the isolation of the 1-benzyl-5-benzylaminotetrazole was extremely strenuous and difficult.

Table 4-1: Substitution by the aminobenzyl group in different solvents

Solvent	BnNH2 (eq)	Conversion (%)	Yield (%)
Benzylamine	--	>95	65
Toluene	15	--	67
Acetonitrile	5	0	--
Xylenes	2-5	0	--
DMSO	2-5	100	--
NMP	2-5	80	--
THF	2-5	0	--

The Liotta-Eckert group has developed a volatile mimic of DMSO, piperylene sulfone, that can be decomposed by mild heating and therefore easily removed.[18] Piperylene sulfone's solvent properties being almost identical to DMSO, this reaction may take advantage of its easier separation ability. First, a solution of 1-benzyl-5-benzylaminotetrazole in piperylene sulfone was heated at 110ºC to initiate decomposition. It was critical to verify that the tetrazole can sustain the decomposition temperature (110ºC) as tetrazoles are known to decompose with temperature. The ^{1}H NMR showed that the piperylene sulfone was successfully removed without decomposition of the 1-benzyl-5-benzylaminotetrazole. Next, the reaction of 1-benzyl-5- sulfonyltoluene tetrazole with benzylamine in piperylene sulfone was performed at 50ºC. After 24 hrs, an aliquot was taken and analyzed by ^{1}H NMR. Only the signals of the 1-benzyl-5-sulfonyltoluene tetrazole starting material were observed. The temperature was increased to 70ºC for 24 hrs and a second aliquot was taken and analyzed by ^{1}H

NMR. At this point, decomposition of the starting material was seen. In addition, it appears that piperylene sulfone was undergoing a side-reaction. Benzylamine was dissolved in piperylene sulfone and heated to 50ºC for 24 hrs. A white precipitate was formed. Clearly, benzylamine and piperylene sulfone reacted with each other, precluding the use of piperylene sulfone in this case.

The deprotection of the 1-benzyl-5-benzylaminotetrazole to yield to the 5-amino-1H-tetrazole was then investigated. Finnegan *et al*. reported the hydrogenation of 1-methyl-5-benzylaminotetrazole in acetic acid using palladium oxide as the catalyst to produce the corresponding 1-methyl-5-amino-1H-tetrazole.[19] The Finnegan procedure was repeated with the 1-benzyl-5-benzylaminotetrazole. Unfortunately, the conditions were ineffective on the 1-benzyl-5-benzylaminotetrazole regardless of the time, solvent, catalyst load or hydrogen pressure. Several attempts were made using activated palladium on carbon as catalyst. The catalyzed hydrogenation of the benzyl groups failed using either 5 % Pd/C or 10% Pd/C. Again, the modifications of solvents, time, and catalyst load did not affect the benzyl deprotection. Heterogeneous catalyzed hydrogenations using either the activated palladium on carbon or the palladium oxide were repeated at 50ºC. None of these attempts were successfully concluded.

At that point, hydrogen transfer using 1,4-cyclohexadiene as a hydrogen source and activated palladium on carbon as the catalyst was investigated. The reaction appeared slow, but for the first time cleavage was observed. The reaction

was run in ethanol at reflux and was followed by ^{13}C NMR. After one week under reflux with regular addition of 1,4-cyclohexadiene and catalyst, the 1-benzyl-5-benzylaminotetrazole was quantitatively hydrogenated to yield the target product: 5-amino-1H-tetrazole.

During this synthesis, each step was isolated and purified before proceeding on to the next step. A "one-pot" synthesis it was then investigated.

4.3.2 One Pot Synthesis Containing 1-Benzyl-5-benzylaminotetrazole

First, the optimum temperature for each steps was determined. The results are summarized in Table 4-2. The optimum temperature was 100ºC for the first step (T_1) and 90ºC for the second step (T_2) giving the highest isolated yield of 58% over the two steps. This yield is comparable to the isolated yield using the step wise synthesis (Figure 4-5 and Table 4-2).

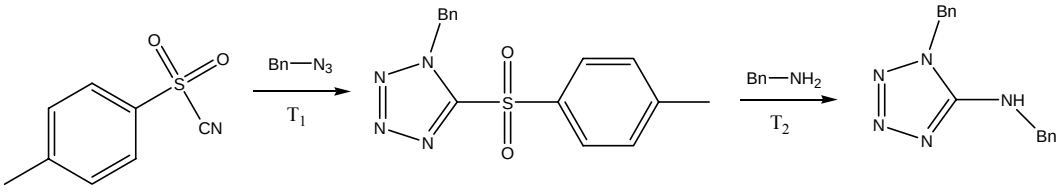


Figure 4-5: One pot synthesis in toluene

Table 4-2: One pot synthesis in toluene

Temperature T_1 (^oC)	Temperature T_2 (^oC)	x eq $BnNH_2$	Time (h)	Yield %
80	70-75	1.5	36	0
80	80	7	48	38
80	80	15	36	51
80	120	15	24	35
120	90	15	24	25
100	100	15	36	34
80	80	15	36	54
100	90	15	48	58

When adding the third step to the "one-pot" synthesis, the benzylamine present in large excess inhibited the cleavage of the benzyl groups. Previously, water was added after the second step to cause the precipitation of the 1-benzyl-5-benzylaminotetrazole. In the "one-pot" synthesis, the reaction mixture is composed of the 1-benzyl-5-benzylaminotetrazole, benzylamine, and toluene. As a consequence, the 1-benzyl-5-benzylaminotetrazole no longer precipitated from the reaction solution upon addition of water.

I tried various methods to selectively remove the benzylamine. First, fractionated precipitation was explored. Upon addition of acid, amines form the corresponding ammonium hydrochloride salts that may be precipitated selectively based on their pKa. The pKa of benzylamine is 9.33 and the pKa of 5-amino-1H-

tetrazole is 6.8. Even with a careful control of pH upon addition of hydrochloric acid, the 1-benzyl-5-benzylaminotetrazole and the benzylamine co-precipitated.

Next, an excess of toluene was added to the reaction solution causing a precipitation. However, this precipitation was also not selective because the by-product, toluene-4-sulfonatebenzylammonium precipitated along with the product (Figure 4-6). This un-desired salt is formed by the reaction of *p*-toluenesulfonic acid, which was formed along the product, with the benzylamine present in large excess.

Finally, the toluene was simply removed under reduced pressure and the 1-benzyl-5-benzylaminotetrazole was isolated by precipitation upon the addition of water, as was done in the step-wise synthesis. I was able to obtain an isolated yield of 58% for the first two steps using this method.

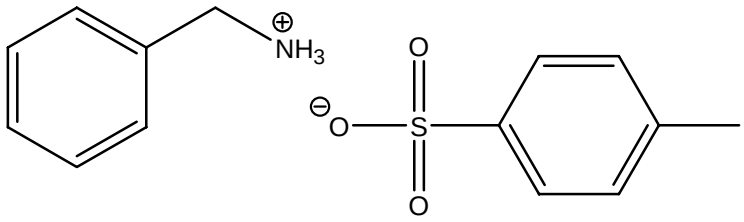


Figure 4-6: Toluene-4-sulfonatebenzyl-ammonium by-product salt

The three-step synthesis of the 5-amino-1H-tetrazole from benign and activated starting materials was successful. The product was synthesized in high purity with 48 % overall yield. Simple purifications were devised and the two first-steps could be combined in a “one-pot” step, without loss of yield. However, the last step was a hurdle. After optimization, the hydrogenation still takes a full week to proceed. This is a limitation for the potential transfer of this synthesis to a commercial process.

In the last step, the cleavage of the benzyl groups, it was observed that the first benzyl group was difficult to cleave. However, when the first cleavage takes place then the cleavage the second benzyl group was much faster. We hypothesized that steric effect may be detrimental for the reaction and that a benzyl-monosubstituted tetrazole intermediate may not exhibit such resistance to cleavage.

4.3.3 Alternative Synthetic Route Containing 5-Azido-1-Benzyltetrazole

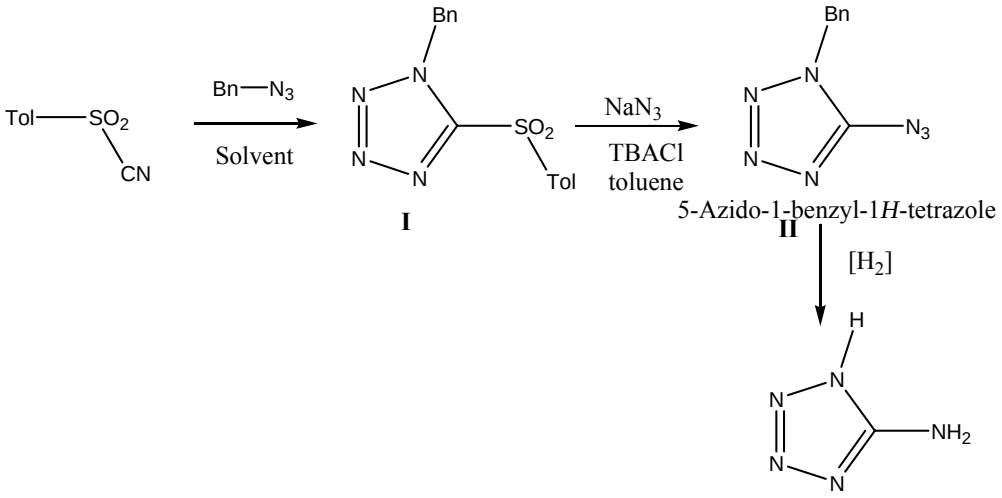


Figure 4-7: New synthetic scheme for 5-amino-1H-tetrazole with 5-azido-1-benzyltetrazole as the second step

In the light of the findings from our previous synthesis, the synthetic scheme was modified. Namely, the sulfonyltoluene group of the intermediate **I** was displaced with an azide (in place of benzylamine previously) to form the 5-azido-1-benzyltetrazole intermediate. Again, this intermediate can undergo hydrogenation to form the 5-amino-1H-tetrazole. The azide has several advantages, it is a good nucleophile, it is a cheap reagent and it is easily hydrogenated to form amines (generally at 1 atm within a couple of hours). First, 1-benzyl-5-sulfonyltolueneaminotetrazole was reacted with sodium azide in the

presence of tetrabutylammonium chloride, to act as a phase transfer catalyst, in toluene at 80ºC. The reaction was monitored using ^{1}H NMR and took 5 days for reaction completion. The ^{1}H NMR showed near quantitative conversion by disappearance of the starting material. The 5-azido-1-benzyltetrazole was successfully synthesized and was characterized using ^{1}H NMR, ^{13}C NMR, IR, MS, and DSC/TGA. A small amount of impurity (~1%) remained in the elemental analysis regardless the purifications method (water washes, cold water washes, or a silica-gel column using various solvent mixtures).

Deviating from the synthesis for a moment, the 1-benzyl-5-azide tetrazole was reacted with p-toluenesulfonyl cyanide in toluene to yield the bis-tetrazole derivative (Figure 4-8). This was an interesting opportunity to form the bis-tetrazole derivative, which has numerous applications in the inflating and energetic materials technologies.

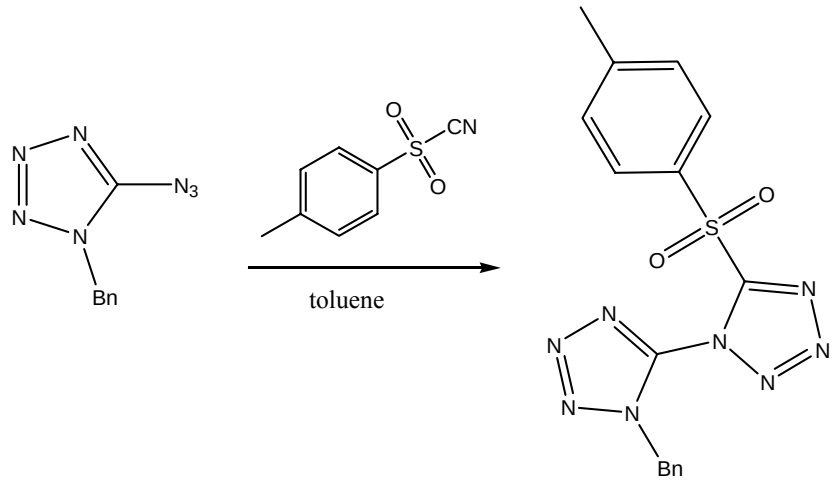


Figure 4-8: 1-benzyl-5-azide tetrazole to form di-tetrazole

The reaction was run at 60ºC for 3 days. The ^{1}H and ^{13}C NMR showed no reaction so the temperature was increased to 80ºC for 4 days. The ^{1}H and ^{13}C NMR still showed no reaction the temperature was finally increased to 100ºC for 7 days. Regardless the changes, the reaction did not proceed. The steric hindrance and the electronic effect (the azide is deactivated by the tetrazole ring) are believed to be detrimental. In fact, both electronic and steric effects have been shown by Sharpless *et al.* to hinder the cycloaddition of substituted azides to the *p*-toluenesulfonyl cyanide.[16] This reaction was not investigated further.

Finally, the 5-azido-1-benzyltetrazole was reacted using the same hydrogen transfer conditions as the 1-benzyl-5-benzylaminotetrazole to form the 5-amino-1H-tetrazole. The 5-azido-1-benzyltetrazole was combined with 1,4-cyclohexadiene as a hydrogen source and activated palladium on carbon as the

catalyst. The reaction was run in ethanol at reflux with regular addition of 1,4-cyclohexadiene and catalyst. The reaction was monitored by ^{1}H NMR. After one month, the benzyl peak was still present. After isolating the product, it was seen that the azide, but not the benzyl, had undergone hydrogen transfer to form the amine. The 5-amino-1-benzyl-tetrazole was characterized using ^{1}H NMR, ^{13}C NMR, IR, MS, and melting point.[20] The ^{1}H NMR showed near quantitative conversion by disappearance of the starting material.

Sajiki reported that adding mineral acid can facilitate the cleavage of the benzyl group in the presence of an amine.[21] The paper stated that the acid needed to be strong enough to protonate the amine. With this in mind, trifluoroacetic acid (pKa of 0.5) was added to the hydrogenation reaction (0.2 mL, 0.5 equiv).[10] The pH of the solution was monitored during the reaction and remained constant throughout. The reaction was again run in ethanol at reflux with regular addition of 1,4-cyclohexadiene and catalyst and followed using ^{1}H NMR. After three weeks, complete disappearance of the benzyl peak was observed in NMR and the 5-amino-1H-tetrazole was isolated. The 5-amino-1H-tetrazole was characterized by ^{13}C NMR, MS, IR, and melting point.[22] The ^{1}H NMR showed near quantitative conversion by disappearance of the starting material.

4.4 Conclusions

In conclusion, 5-amino-1H-tetrazole was synthesized using two novel synthetic routes. Both routes made use of Sharpless' click chemistry to form the tetrazole ring. By using not only masked but activated cyanide and azide derivatives, the 5-amino-1H-tetrazole was successfully synthesized in good yields. This novel process allowed efficient reactions (with minimum by-product formation) and easy isolation of the intermediates. For the first time, the hydrogen transfer of the 1-benzyl-5-benzylaminotetrazole has been investigated and successfully concluded. I also synthesized a novel compound, 1-benzyl-5-azido tetrazole. The hydrogen transfer of the 5-azido-1-benzyltetrazole to form the 5-amino-1-benzyl-tetrazole and the 5-amino-1H-tetrazole was successfully concluded. The last step, the hydrogenation of the protecting groups, takes a week to go to completion in the first synthesis and three weeks in the second synthesis. This is a limitation for the potential transfer of this synthesis to a commercial process. Nonetheless, these syntheses minimized, if not eliminated, the potential contamination of the 5-amino-1H-tetrazole by toxic and/or un-desired catalyst or by-products. It also showed improvements over the existing methods in terms of safety concerns.

4.5 Experimental

All chemicals were ordered from Aldrich or VWR and used as received, unless noted. ^{1}H and ^{13}C NMR spectra were recorded using a Varian Mercury Vx 400 spectrometer using residual DMSO peak as an internal reference. Mass Spectrometry samples were submitted to Mass Spectrometry Lab and used a Micromass Quattro LC to perform ESI-MS. Elemental analyses were submitted to Atlantic Microlabs, Inc. Melting points were determined on Mettler-Toledo capillary apparatus and were uncorrected.

Synthesis of benzyl azide[23]

Benzyl chloride (10 g) was dissolved in ethanol (60 mL) and water (10.5 mL) at 0ºC. Sodium azide (5.13 g) was added to the reaction. The reaction was allowed to warm to room temperature and then heated at 60ºC overnight. To work up the reaction, the reaction was first allowed to cool to room temperature and filtered. Dichloromethane was added to the organic phase and was washed with water three times and with saturated aqueous $NaHCO_3$ one time. The organic phase was then dried over magnesium sulfate and the solvent was removed under reduced pressure.

Benzyl azide: ^{1}H NMR ($CDCl_3$, ppm): 4.35 (s, 2H), 7.37 (m, 5H).

Synthesis of 1-benzyl-5- sulfonyltoluenetetrazole

The benzyl azide (0.5 g, 4.06 mmol) was dissolved in toluene and then the *p*-toluene sulfonylcyanide (0.73 g, 1.0 eq) was added and the reaction mixture heated progressively to 80ºC. The reaction was run behind a blast-shield. The reaction progress was followed by TLC or NMR (H^1 or C^{13}). Once complete, the reaction mixture was cooled to 0ºC and the resulting precipitate was filtered to give white needles of 1-benzyl-5-benzylaminotetrazole in 90-95 % yield.

1-Benzyl-5-sulfonyltoluene aminotetrazole:[16] mp = 136.5ºC. ^{1}H NMR (CDCl3, ppm): 2.43 (s, 3H, CH3), 5.93 (s, 2H, CH2), 725-7.36 (m, 7H), 7.74 (d, 2H,). ^{13}C NMR (CDCl3, ppm): 22.25, 53.42, 128.65, 129.26, 129.29, 129.36, 130.46, 132.96, 134.31, 127.89, 155.00. IR (Toluene, cm^{-1}): 1353 and 1157. MS(m/z): 315.1 (M+1). Elemental analysis: calculated C, 57.31, H 4.49, N 17.82, S 10.20%; found C 57.50, H 4.73, N 17.58, S 10.01%. IR (MeOH, cm^{-1}): 1353.10, 1157.57 (sulfones), 1080.63(tetrazole), DSC/TGA is shown in Figure 4-9.

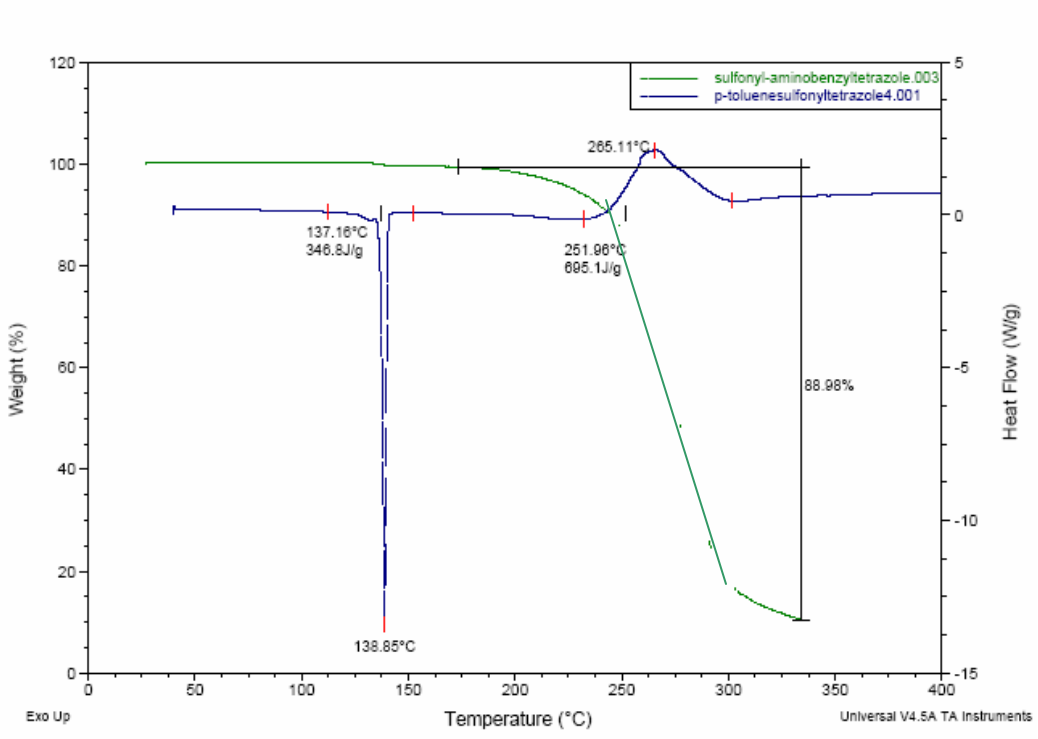


Figure 4-9: DSC/TGA of 1-benzyl-5-sulfonyltolueneaminotetrazole

Synthesis of 1-benzyl-5-benzylaminotetrazole

The 1-benzyl-5-sulfonyltoluenetetrazole (0.3 g, 0.95 mmol) was dissolved in benzylamine (5 ml). The reaction was allowed to react at 90ºC for 24h. After cooling the reaction mixture, water (5 ml) was added and the resulting precipitate was filtered off. The 1-benzyl-5-benzylaminotetrazole was isolated in 70% yield as a white powder.

1-Benzyl-5-benzylaminotetrazole: [24] mp = 166.0ºC. ^{1}H NMR (DMSO, ppm): 4.48 (d, 2H, J= 6Hz, CH2), 5.42 (s, 2H, CH2), 7.19-7.35 (m, 9H), 7.62 (t, 1H). ^{13}C

NMR (DMSO, ppm): 47.61, 48.37, 127.65, 127.88, 128.05, 128.57, 128.89, 129.33, 135.89, 139.61, 156.15. IR (DMSO, cm^{-1}): 1494.29, 1460.12, 1080.55. MS (m/z): 265 (M).

Preparation of the 1-benzyl-5-benzylaminotetrazole (II)"one pot" procedure:

The benzyl azide (0.5 g, 4.06 mmol) was dissolved in toluene. Then *p*-toluene sulfonylcyanide (0.73 g, 1.0 eq) was added and the reaction mixture heated progressively to 100ºC. The reaction progress was followed by TLC or NMR (^{1}H or ^{13}C). Once complete, the reaction was cooled to room temperature and benzylamine was added (6.51 g, 60.9 mmol, 15 equiv). The reaction mixture was then heated progressively to 90^{o}C. The reaction progress was followed by NMR (^{1}H or ^{13}C). Once complete, the reaction was cooled to room temperature and the toluene was removed by vacuum. Then, 10 mL of water was added and the reaction was cooled overnight. The resulting precipitate was filtered to give a white powder of 1-benzyl-5-benzylaminotetrazole in 58% overall yield.

5-amino-1H-tetrazole:

The 1-benzyl-5-benzylaminotetrazole (0.48 g, 1.81 mmol) was dissolved in ethanol (5 ml) and a slurry of 10 % Pd on activated carbon (0.1 g) in ethanol was added. Then 1,4-cyclohexadiene (0.51 ml, 3.0 eq) was added at 0ºC. The reaction mixture was progressively warmed to reflux.. The reaction was followed by ^{13}C

NMR. Portions of 1,4-dicyclohexane (0.2 ml, 1.5 eq) were added every day. Additional catalyst (0.1 g) was added after 72 hours and again after 96 hours. After 7 days, the starting material was undetectable by NMR and only the 5-amino-1H-tetrazole was observed. The reaction mixture was then cooled down and filtered on a celite pad. The filtrate was evaporated to dryness to give 5-amino-1H-tetrazole as white powder (90 % yield).

5-amino-1H-tetrazole:[22] mp (decomp) =191.9 ^{o}C**.** ^{13}C NMR ($CDCl_3$, ppm) 157.40.

Synthesis of 1-benzyl-5-benzylaminotetrazole in piperylene sulfone

1-benzyl-5-sulfonyltoluenetetrazole (0.1 g, 1 equiv) was dissolved in piperylene sulfone (2 mL). Benzylamine (0.06 mL, 1 equiv) was added. The reaction was put under nitrogen and heated to 50ºC overnight. The reaction was tested by ^{1}H NMR and no product was observed. The reaction temperature was increased to 70ºC overnight. The reaction was tested by ^{1}H NMR and the starting material had decomposed and the piperylene sulfone had rearranged.

5-azido-1-benzyltetrazole:

1-benzyl-5-sulfonyltoluenetetrazole (0.05 g, 1 equiv) was dissolved in toluene (5 mL). Sodium azide (0.03 g, 3 equiv) and tetrabutylammonium chloride (0.0025 g, 5% wt) were added. Since the product and sodium azide are potentially

explosive, the reaction was done behind a blast shield. The reaction was heated to 80ºC and monitored by ^{1}H NMR. After 5 days, all the starting material was observed to be reacted. To work up the reaction, the sodium azide was filter off and ether (20 mL) was added to the reaction mixture. The organic phase was washed with water (3 x 20 mL) and dried over magnesium sulfate. The ^{1}H NMR showed near quantitative conversion by disappearance of the starting material.

5-Azido-1-benzyltetrazole: ^{1}H NMR (DMSO, ppm): 5.39(s, 2H), 7.31 (m, 3H), 7.38 (m, 2H). ^{13}C NMR (DMSO, ppm): 49.32, 127.77, 128.28, 128.66, 133.68, 151.99. IR (Tolune, cm^{-1}): 2150. MS(m/z): 202.1 (M+1). DSC/TGA shown in Figure 4-10. IR (MeOH, cm^{-1}): 2150.06 (azide), 1109.58 (tetrazole).

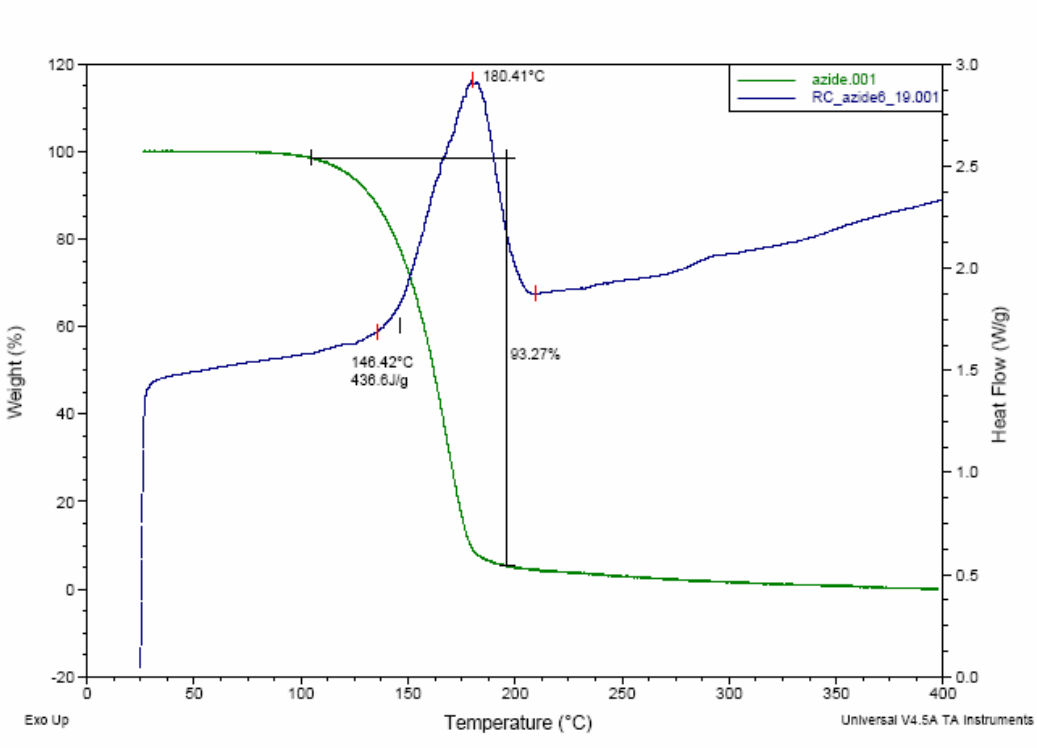


Figure 4-10: DSC/TGA of 5-azido-1-benzyltetrazole

5-amino-1-benzyltetrazole

The 5-azido-1-benzyltetrazole (0.96 g, 0.005 mol) in 15 mL of toluene was added to ethanol (5 ml) and a slurry of 10 % Pd on activated carbon (0.2 g) in ethanol was added. Then, 1,4-cyclohexadiene (1.4 ml, 3.0 eq) was added at 0ºC. The reaction mixture was progressively warmed up to reflux. Portions of 1,4-dicyclohexane (0.7 ml, 1.5 eq) were added every day. Additional catalyst (0.1 g) was added every three days. After 20 days, the reaction was stopped. The reaction mixture was then cooled down and filtered on a celite pad. The filtrate was

evaporated to dryness to give 5-amino-1-benzyl-tetrazole. The ^{1}H NMR showed near quantitative conversion by disappearance of the starting material.

5-Amino-1-benzyl-tetrazole:[20] mp(decomp): 179ºC. ^{1}H NMR (DMSO, ppm): 5.344 (s, 2H), 7.334 (m, 5H). ^{13}C NMR(DMSO, ppm): 47.48, 127.48, 127.88, 128.650, 135.39, 155.50. MS(m/z): 176.1 (M+1). IR (DMSO, cm^{-1}): 1004.90.

5-amino-1H-tetrazole

The 5-azido-1-benzyltetrazole was in 70 mL of toluene and had an estimated mass of 0.96 g. The exact mass was not determined since the 5-azido-1-benzyltetrazole is not isolated except in small quantities. Ethanol (5 ml) and a slurry of 10 % Pd on activated carbon (0.2 g) in ethanol were added. Then 1,4-cyclohexadiene (1.4 ml, 3.0 eq) was added at $0^{o}C$. The reaction mixture was progressively warmed to reflux. The reaction was followed by ^{1}H NMR. Portions of 1,4-dicyclohexane (0.7 ml, 1.5 eq) were added every day. Additional catalyst (0.2 g) was added every other day. After 21 days, the benzyl peak on the starting material was undetectable by ^{1}H NMR. The reaction mixture was then cooled down and filtered on a celite pad. The filtrate was evaporated to dryness and recrystallized in water to give 5-amino-1H-tetrazole as white powder. The ^{1}H NMR showed near quantitative conversion by disappearance of the starting material.

5-Amino-1H-tetrazole:[22] mp (decomp) =201-203 ^{o}C. ^{13}C NMR (DMSO, ppm) 157.31. MS(m/z): 85.8 (M^{+}) IR (DMSO, cm^{-1}): 1004.80.

4.6 References

(1) Brown, M. US, 1967; Vol. 3,338,915.
(2) *Chem. Abstr.* **1968**, 87299.
(3) *Chem. Abstr.* **1980**, *92*, 8480.
(4) Henry, R. A. US, 1963; Vol. 3,096,312.
(5) Bradbury, R. H. *J. Med. Chem.* **1993**, *36*, 1245.
(6) Carini, D. J. *J. Med. Chem.* **1991**, *34*, 2525.
(7) Tarver, C. M. Proc. Symp. Int. Detonation 6th, 231, ACR (US), 1967.
(8) Wood, J. US, 2001; Vol. 6328830.
(9) Mihina, J. S., Herbst, R.M., *J. Org. Chem.* **1950**, *15*, 1082-1092.
(10) Highsmith, T. K., Lund, G.K., US, 1995; Vol. 5451682.
(11) Masahiro, M. US, 1997; Vol. 5594146.
(12) www.sciencelab.com/xMSDS-Sodium_azide-9927588, 2008.
(13) www.sciencelab.com/xMSDS-Cyanamide-9923608, 2008.
(14) Kolb, H., Finn, M. G., Sharpless, K. Barry *Angew. Chem. Int. Ed* **2001**, *40*, 2004-2021.
(15) Sharpless, K. B., Demko, Z. P., *Angew. Chem. Int. Ed* **2002**, *41*, 2110-2113.
(16) Demko, Z. P., Sharpless, K. Barry *Angew. Chem. Int. Ed* **2002**, *41*, 2113-2115.
(17) *Chem. Commun* **2007**, 1427-1429.
(18) Finnegan, W. G., Henry, R. A., Lieber, E. *J. Org. Chem.* **1953**, *779-786*.
(19) Carbrechtard, W. L., Herbst, R. M. *J. Org. Chem.* **1953**, 1022.
(20) Sajiki, H., Hirota, K. *Tetrahedron* **1998**, *54*, 13981-13996.
(21) www.aldrich.com, 2008.
(22) Ohtani, N., Murakawa, S., Watanabe, K., Tsuchimoto, D., Sato, D., *J. Chem. Soc. Perkin Trans.* **2000**, *2*, 1851-1856.
(23) Henry, R. A., Finnegan, W.G. *J. Am. Chem. Soc.* **1954**, *76*, 923-6.

CHAPTER 5: HETEROGENEOUS REACTIONS BETWEEN NUCLEOPHILIC SALTS AND SILOXYLATED ELECTROPHILE UNDER PHASE TRANSFER CATALYSIS CONDITIONS. COMPARISION OF HYDROCARBON AND SILOXYLATED PHASE TRANSFER CATALYSTS

5.1 Introduction

Phase-transfer catalysis (PTC) is a well-proven technique that facilitates reactions between reactants located in different phases.[1-3] Classic phase-transfer catalysis generally involves immiscible liquid-liquid (e.g. organic-aqueous) or liquid-solid (e.g. organic-salt) phases. Quaternary ammonium salts, such as tetra-*n*-butylammonium chloride, represent the most common types of phase-transfer catalysts.[3] They have been found to operate effectively in a wide variety of heterogeneous processes including substitution, addition, elimination and polymerization reactions.[3-5] Over the years, phase-transfer catalysts have been structurally customized to address specific reactions.[3] In contrast to the wide variety of heterogeneous systems reported, phase transfer catalysis has never been reported for a system in which one of the phases was a partially or highly siloxylated medium and an immiscible solid phase. This is of great importance since siloxylated materials are useful in numerous applications, ranging from health products, such as lotions and make-up, to industrial fluids, such as surfactants and lubricants.[6,7] However, the reaction of siloxylated reagents with

ionic substrates is difficult because these reactants are not sufficiently miscible with one another or in a common solvent. For example, the reaction of *p*-[1&2-(1,1,3,3,3-pentamethyldisiloxane)-ethyl]-benzyl chloride, **1A & 1B** (called herein siloxylated benzylchloride) with L-lysine in an acetonitrile-methanol mixed solvent exhibited a slow reaction rate with only 70% conversion after 22 hours**.** The mass spectroscopy of the coupled product contained peaks of molecular mass corresponding to the mono, di-, tri- and tetra-substitution products (Figure 5-1).[8] In the present report, various phase transfer catalytic systems were investigated in order to improve reaction rates and conversions for the reaction of a siloxylated reagent and a series of ionic substrates. Specifically, a detailed investigation of the nucleophilic substitution of siloxylated benzyl chloride with potassium acetate is reported (Figure 5-2). Experiments dealing with inorganic ionic nucleophiles (i.e. potassium cyanide and potassium thiocyanate) and the amino acid L-lysine are also reported. Tetra-*n*-butylammonium chloride (TBACl) and two custom-made siloxylated phase-transfer catalysts (Figure 5-3) were tested. It was anticipated that the siloxylated catalysts would facilitate the reaction between the siloxylated reagent in the liquid phase and the ionic substrate in the solid phase. A comparison of activity between the classic TBACl and the two custom designed siloxylated PTCs is reported.

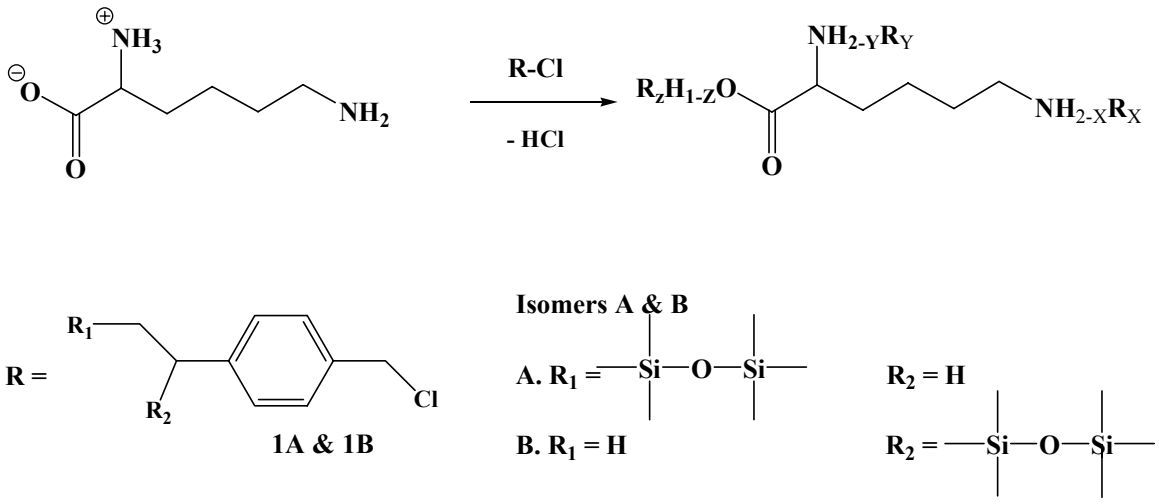


Figure 5-1: Reaction of L-Lysine with Siloxylated Benzyl Chloride (1A & 1B)

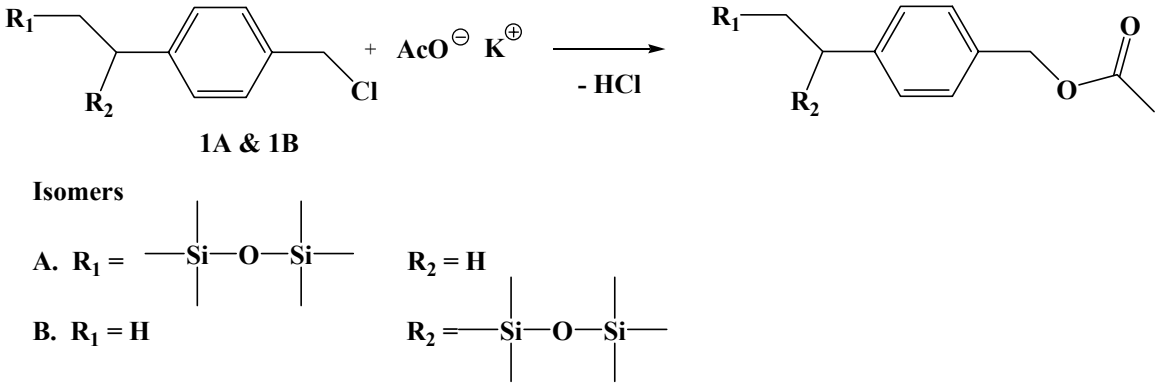
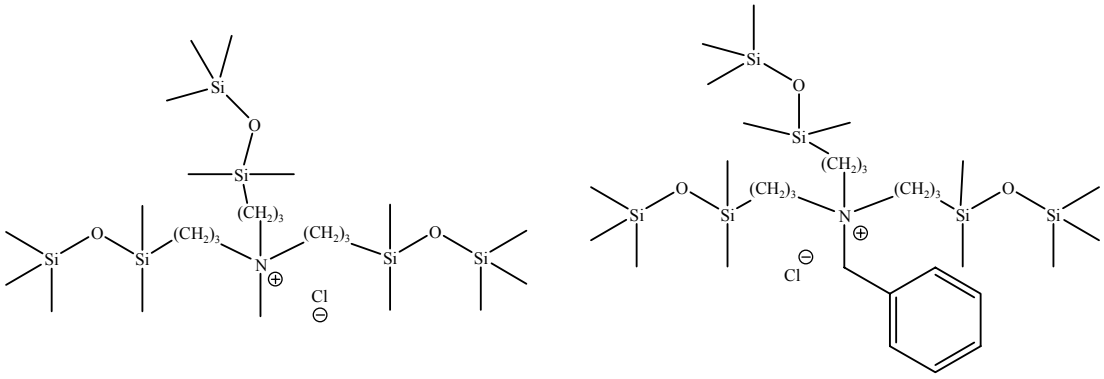


Figure 5-2: Reaction of Potassium Acetate with Siloxylated Benzyl Chloride (1A & 1B)



2 **3**

Figure 5-3. Novel Siloxane PTCs: Left: Methyl-tris-[3-(1,1,3,3,3-pentamethyl-disiloxanyl)-propyl]-ammonium chloride (2) and Right: Benzyl-tris-[3-(1,1,3,3,3-pentamethyl-disiloxanyl)-propyl]-ammonium chloride (3)

5.2 Background

5.2.1 Phase Transfer Catalysis

Phase-transfer catalysis (PTC) is a well-proven technique that facilitates reactions between reactants located in different phases.[1-3] The principle behind phase transfer catalysis is that "phase-transfer agents" facilitate the transport from one phase into another phase. The most common type of phase-transfer catalysts are quaternary ammonium salts, such as tetra-*n*-butylammonium chloride.

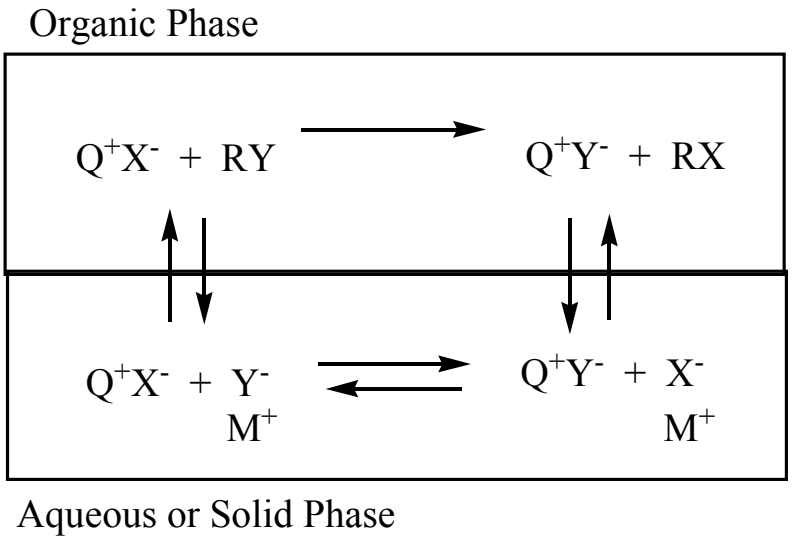


Figure 5-4: Phase Transfer Catalyst

A symbolic illustration of how phase transfer catalysis works can be seen in Figure 5-4. The Q^+ represents the phase transfer catalyst. The X^- and Y^- represent the anions being transferred between phases. The M^+ represents the counter cation. The RY is the starting material and RX is the product. The Q^+ transfers the X^- from the aqueous or solid phase into the organic phase. When the Q^+X^- is in the organic phase, the X^- can displace the Y and bond to R. Q^+ then transfers the displaced Y^- to the aqueous or solid phase. The Q^+ undergoes an anion exchange to switch the Y^- and X^-. The cycle then begins again with the Q^+ transferring X^- to the organic phase. The transfer of Q^+ with X^- or Y^- across the phase boundary and the anion exchange are reversible.

Classic phase-transfer catalysis generally involves two immiscible liquid-liquid (e.g. organic-aqueous) or liquid-solid (e.g. organic-salt) phases. I planned to use phase transfer catalysis for a siloxane liquid phase and an immiscible solid phase. Using phase transfer catalysis in this way has not previously been reported.

5.2.2 Previous Work

In previous work, the siloxylated benzylchloride was coupled to L-lysine (Figure 5-5). The reaction was performed in 1:1 (v:v) methanol:acetonitrile solvent mixture with equimolar reagents. The L-lysine remained a separate solid phase with the siloxylated benzylchloride in the organic phase. The reaction exhibited a slow reaction rate with only 70% conversion in 22 hours.[8] We speculated the slow reaction rate was due to phase contact issues between the solid phase, L-lysine, and the organic phase, siloxylated benzylchloride. The phase contact issues and slow reaction rate should be overcome by using a PTC.

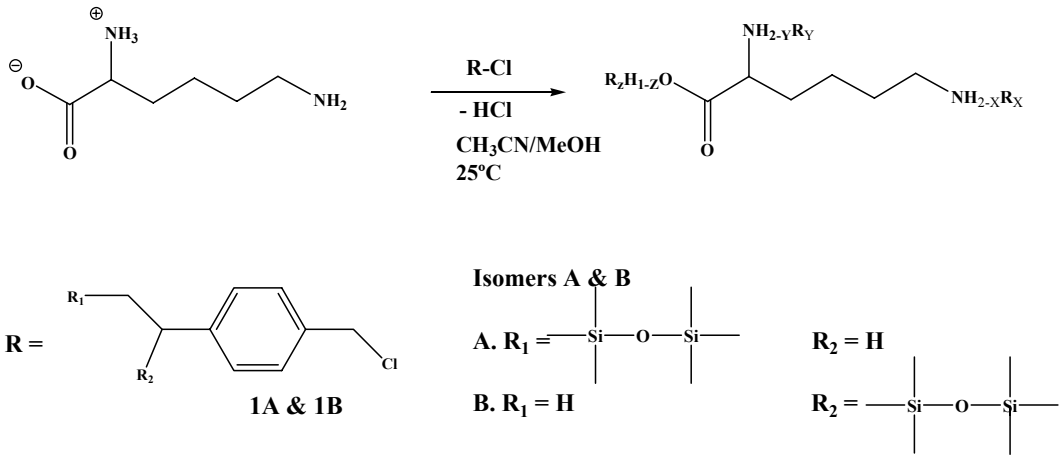


Figure 5-5: Previous work coupling L-lysine with siloxylated benzylchloride without phase transfer catalyst

5.2.3 Applications of Siloxylated Compounds

Siloxylated compounds are frequently used in personal care products such as soaps, deodorants, and cosmetics. They are attractive for use in these products because of their low heat of vaporization and smooth, silky feel.[9] In 1993, 89,000 metric tons of polydimethylsiloxane (PDMS)-based elastomers were produced or imported in the United States, representing 50% of all organosilicon products.[10] The siloxylated benzylchloride coupled to L-lysine has potential use as an ingredient in personal care products.[8] The siloxylated phase transfer catalysts (**2** and **3** in Figure 5-3) have potential applications as surface active agents, emulsions, and/or antimicrobial agents.[11]

5.3 Results and Discussion

5.3.1 Synthesis of Siloxylated Phase Transfer Catalysts

The common siloxylated amine, tris-[3-(1,1,3,3,3-pentamethyl-disiloxanyl)-propyl]-amine, was prepared by reacting the triallylamine with pentamethyl disiloxane in the presence of the catalyst, platinum(0)-1,3-divinyl-1,1,3,3-tetramethyl disiloxane complex (3 wt % xylene) (DVDS-Pt) (Figure 5-6).[12] The two novel siloxylated ammonium quaternary salt phase transfer catalysts, **2** and **3**, were synthesized from this common siloxylated amine. Methyl-tris-[3-(1,1,3,3,3-pentamethyl-disiloxanyl)-propyl]-ammonium chloride, **2** was synthesized by reacting the tris-[3-(1,1,3,3,3-pentamethyl-disiloxanyl)-propyl]-amine with methylchloride in THF under pressure (50 psi, 40ºC, 0.1 mol) with quantitative yields (Figure 5-7). Benzyl-tris-[3-(1,1,3,3,3-pentamethyl-disiloxanyl)-propyl]-ammonium chloride, **3** was synthesized by reacting the tris-[3-(1,1,3,3,3-pentamethyl-disiloxanyl)-propyl]-amine with benzylchloride as the reactant and solvent at 65ºC for 5 days with quantitative yields (Figure 5-8). The products were characterized using ^{1}H and ^{13}C NMR, MS, and elemental analysis.

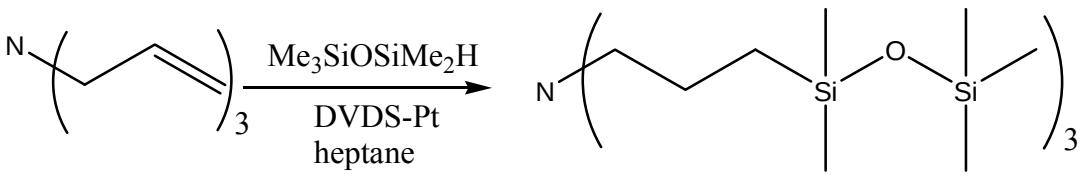


Figure 5-6: Tris-[3-(1,1,3,3,3-pentamethyl-disiloxanyl)-propyl]-amine synthesis

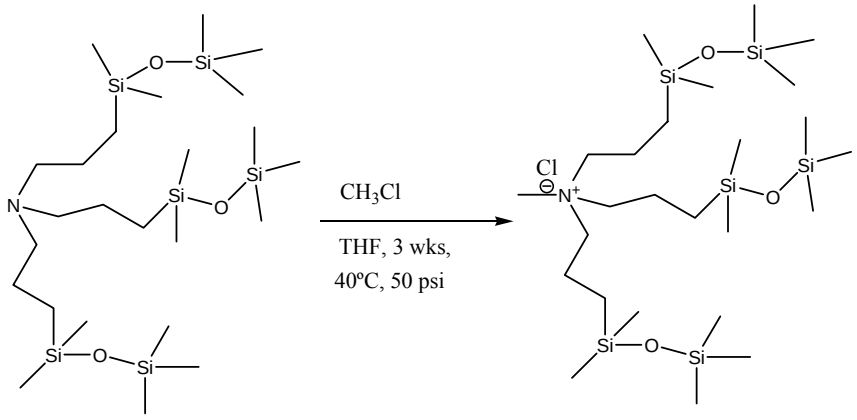


Figure 5-7: Synthesis methyl-tris-[3-(1,1,3,3,3-pentamethyl- disiloxanyl)-propyl]-ammonium chloride

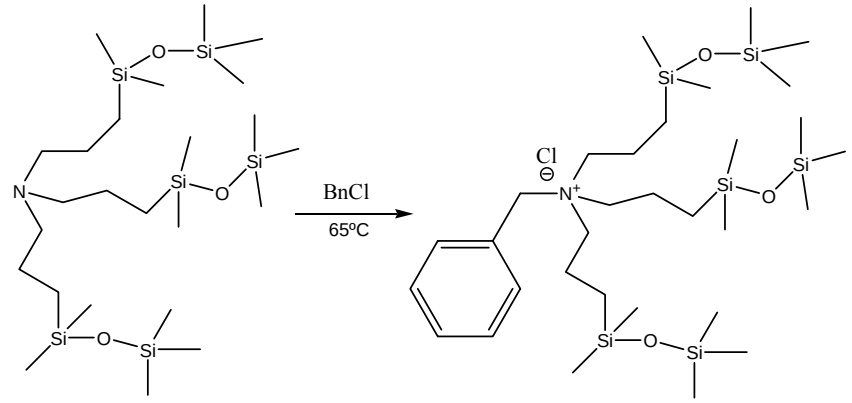


Figure 5-8: Synthesis benzyl-tris-[3-(1,1,3,3,3-pentamethyl-disiloxanyl)-propyl]-ammonium chloride

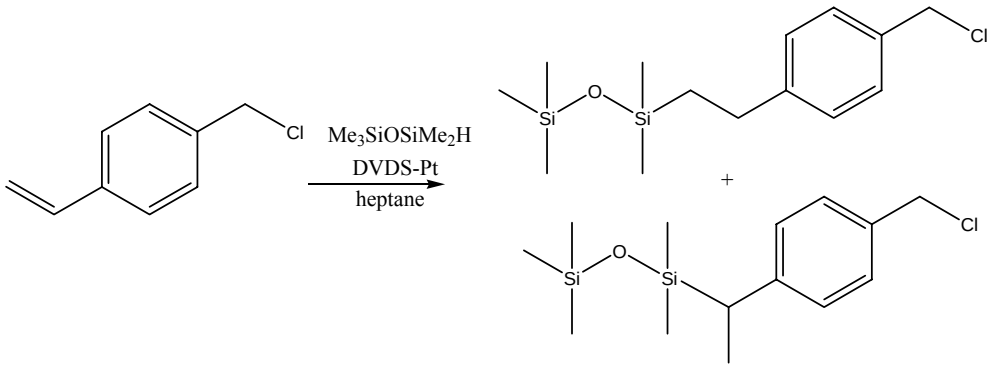


Figure 5-9: Synthesis of *p*-[1 & 2-(1,1,3,3,3-pentamethyldisiloxane)-ethyl]-benzyl chloride with both isomers (A & B) shown

5.3.2 Synthesis of Siloxylated Reactant

The siloxylated reactant, *p*-[1 & 2-(1,1,3,3,3-pentamethyldisiloxane)-ethyl]-benzyl chloride, was synthesized from 4-vinyl-benzylchloride using the same reaction conditions as the synthesis of tris-[3-(1,1,3,3,3-pentamethyl-disiloxanyl)-propyl]-amine.[12] The pentamethyl disiloxane was coupled to the 4-vinyl benzylchloride using platinum (0)-1,3-divinyl-1,1,3,3-tetramethyldisiloxane (DVDS-Pt) in heptane as shown in Figure 5-9. The synthesis resulted in two isomers as seen in Figure 5-9 which were used together for the kinetic studies. The two isomers were formed by the pentamethyl disiloxane coupling to the first and second carbons of the alkene. I believe this did not occur with the triallyl amine because steric hindrance made the second inner carbon inaccessible.

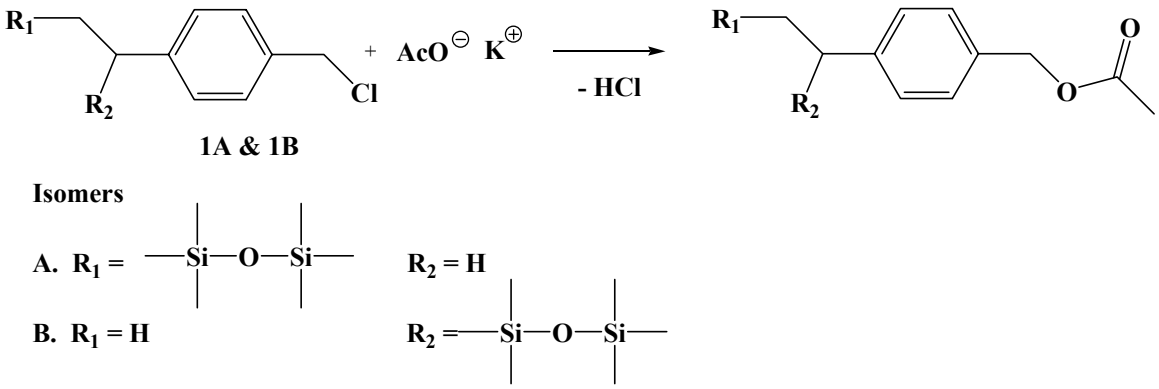


Figure 5-10: Couple *p*-[1 & 2-(1,1,3,3,3-pentamethyldisiloxane)-ethyl]-benzyl chloride with potassium acetate as sample displacement

5.3.3 Kinetic Studies

The nucleophilic substitution of the siloxylated benzyl chloride with potassium acetate was performed with and without phase-transfer catalysts (Figure 5-10). Other nucleophiles like potassium cyanide and potassium thiocyanate were tested. Because the reaction rates with these nucleophiles were similar although slightly slower to the one with potassium acetate, this chapter is focusing on the model case potassium acetate. The results for potassium thiocyanate and potassium cyanide are shown in Table 5-1 for both isomers. The reactions were carried out in ethyl acetate with the tetra-*n*-butylammonium

chloride and the two siloxane-containing PTCs, **2** and **3**. Aliquat 336 was also tested (Table 5-1) but showed slower rates than the tetra-*n*-butylammonium chloride and was not investigated further. The disappearance of the starting materials and the appearance of the products were monitored by GC-MS.

Table 5-1: Pseudo-first order rate constants for the reaction of potassium acetate with siloxane electrophile and various PTCs at 70 °C and 900 rpm stirring. Both isomers shown.

	Pseudo-First Order Rate Constant * 10^5 (s^{-1})			
PTC (5%)	**KSCN isomer A**	**KSCN isomer B**	**KCN isomer A**	**KCN isomer B**
None	0.80 ± 0.08	0.77 ± 0.05	No Rxn	No Rxn
TBACl	2.6 ± 0.1	2.7 ± 0.2	1.76 ± 0.05	2.5 ± 0.5
Aliquat 336	2.6 ± 0.2	2.6 ± 0.2	0.18 ± 0.03	0.25 ± 0.04
MeSiPTC ***(2)***	1.9 ± 0.1	1.9 ± 0.2	0.12 ± 0.01	0.251 ± 0.006
BnSiPTC ***(3)***	1.17 ± 0.02	1.26 ± 0.03	0.75 ± 0.03	0.90 ± 0.03

In the absence of catalyst, no reaction occurs (Table 5-2 and Figure 5-11). In contrast, reaction does take place using as little as 1 mol % of catalysts. However, the optimum catalyst loading for the reaction was 5 mol %. Table 5-2 and Figure 5-11 also summarize the pseudo-first order rate constant for four catalyst

loadings: 0, 1, 5 and 10 mol %. The rate increases more dramatically with increasing the catalyst loading from 1 mol % to 5 mol % than with increasing the catalyst loading from 5 mol % to 10 mol % as seen in Figure 5-12. The 5 mol % optimum catalyst loading was used consistently afterward. The pseudo-first order rate plots in the cases of the three catalysts (TBACl, **2** and **3**) are shown in Figure 5-13 and the rate constants are listed in Table 5-3. The rate constants for both isomers were identical.

Table 5-2: Reaction of KOAc with siloxane electrophile and various amounts of TBACl PTC at 70°C and 900rpm Ethyl acetate was the solvent. Rates for both isomers were identical.

% TBACl	**Pseudo-first order rate constant (10^5, s^{-1})**
0	-
1	16 ± 1
5	65 ± 4
10	120 ± 7

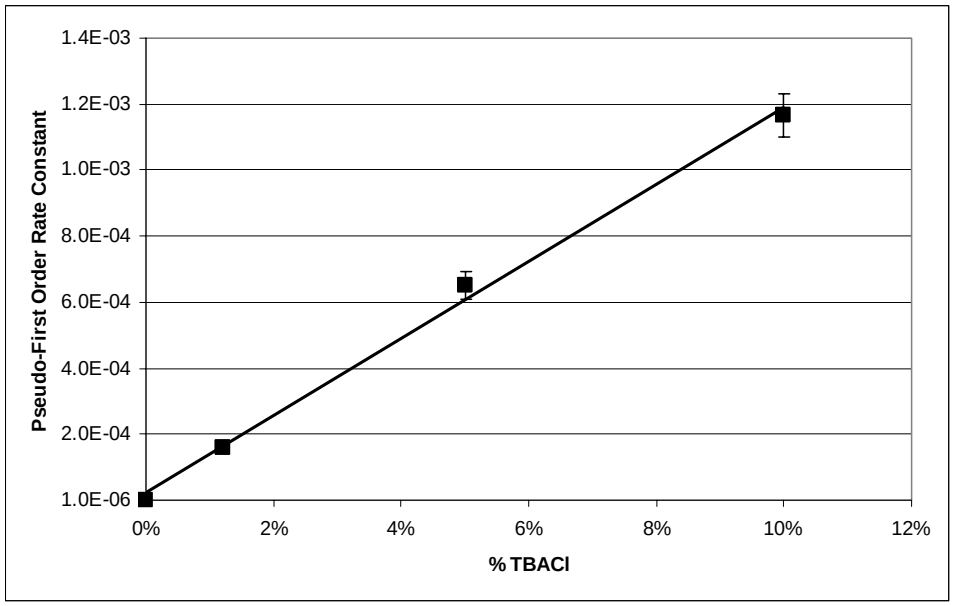


Figure 5-11: Effect of catalyst loading on conversion for reaction of KOAc with siloxane electrophile and various amounts of TBACl PTC at 70°C. Ethyl acetate was the solvent. Rates for both isomers were identical.

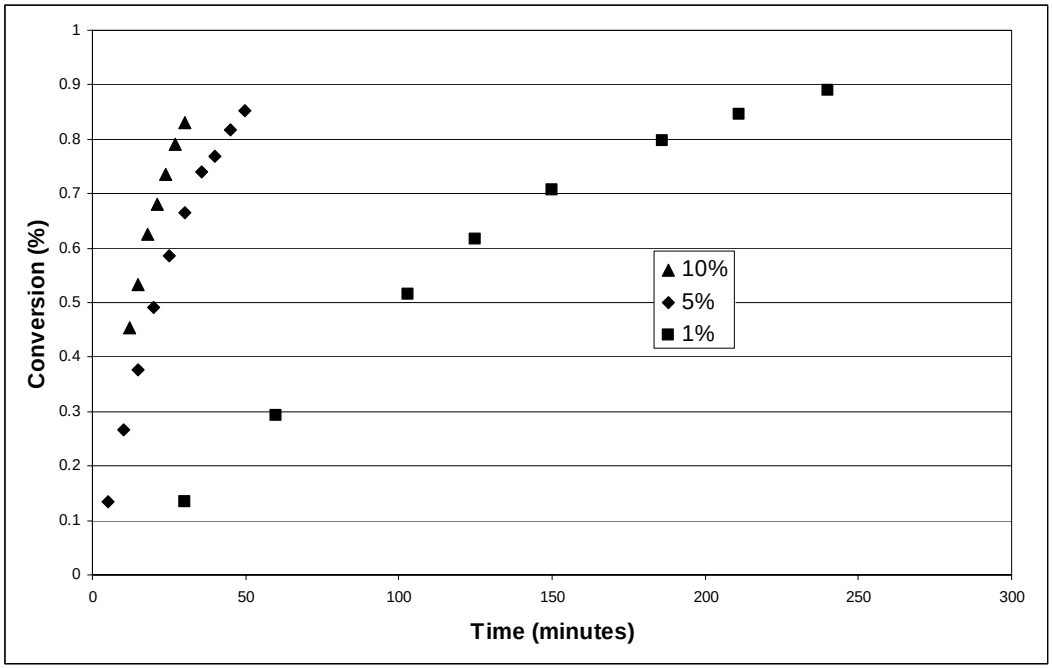


Figure 5-12: Time-dependent behavior for reaction of KOAc with siloxane electrophile and various amounts of TBACl PTC at 70°C and 900 rpm in ethyl acetate. Rates for both isomers were identical.

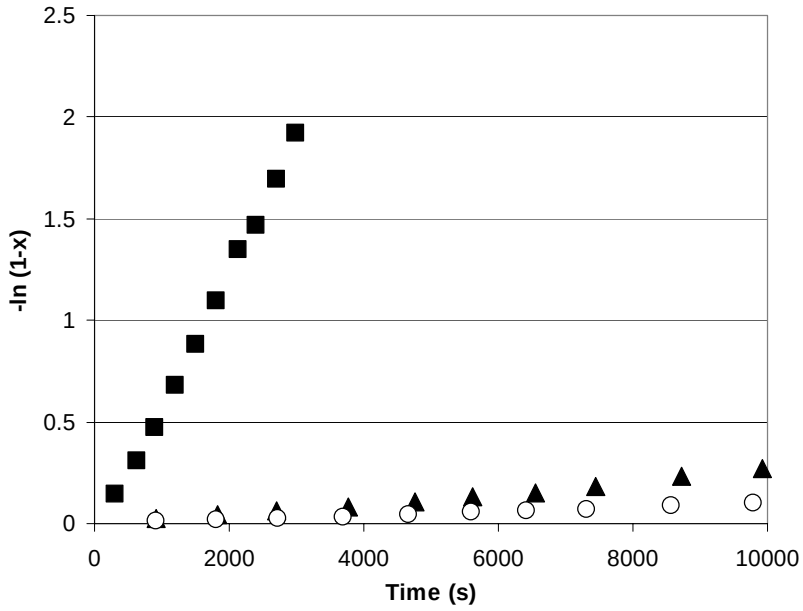


Figure 5-13: Time-dependent behavior of potassium acetate with siloxane electrophile and various PTCs at 70 °C and 900 rpm stirring (■): TBACl, (▲): SiMePTC (2), (○): SiBnPTC (3). Rates for both isomers were identical.

Table 5-3: Pseudo-first order rate constants for the reaction of potassium acetate with siloxane electrophile and various PTCs at 70 °C and 900 rpm stirring. Rates for both isomers were identical.

	Pseudo-First Order Rate Constant * 10^5 (s^{-1})
PTC (5%)	**KOAc**
None	-
TBACl	65 ± 4
2	2.33 ± 0.04
3	0.99 ± 0.04

Inspection of the results in Table 5-3 and Figure 5-13 indicates that tetra-*n*-butylammonium chloride significantly outperforms the siloxane-containing PTCs. The tetra-*n*-butylammonium shows a thirty fold enhancement of the rate constant over the siloxylated PTCs. Under these conditions, it seems that the siloxane electrophile does not benefit from the "like-like" interactions with the specialty catalysts. It was surprising that the siloxylated catalysts were outperformed by the classic TBACl. In light of these results, it was concluded that ethyl acetate may generate a predominantly organic environment minimizing the influence of the siloxylated character of the substrate and specialty catalysts.

Table 5-4 presents data for the KOAc displacement using 5% of various PTCs in ethyl acetate from 30-70°C. At each temperature, the rate constants decreased in the order TBACl > methyl PTC > benzyl PTC. The Arrhenius plots for these data are provided as Figure 5-14. Activation energies ranged from 95 kJ/mol for TBACl to 132 kJ/mol for the benzyl siloxylated PTC. No conversion was observed in 1100 minutes without PTC.

Table 5-4: Reaction of KOAc with siloxane electrophile and 5% of various PTCs in ethyl acetate at various temperatures. 5x excess KOAc is used in all conditions.

	Pseudo-First Order Rate Constant * 10^5 (s^{-1})				Activation Energy (kJ/mol)
PTC (5%)	**30°C**	**50°C**	**60°C**	**70°C**	
None	No Rxn	No Rxn	No Rxn	No Rxn	
TBACl	0.70 ± 0.06	6.59 ± 0.06	-	57 ± 3	95
SiMePTC	-	0.18 ± 0.02	1.46 ± 0.1	2.0 ± 0.2	116
BnMePTC	-	0.047 ± 0.005	0.35 ± 0.05	0.80 ± 0.03	132

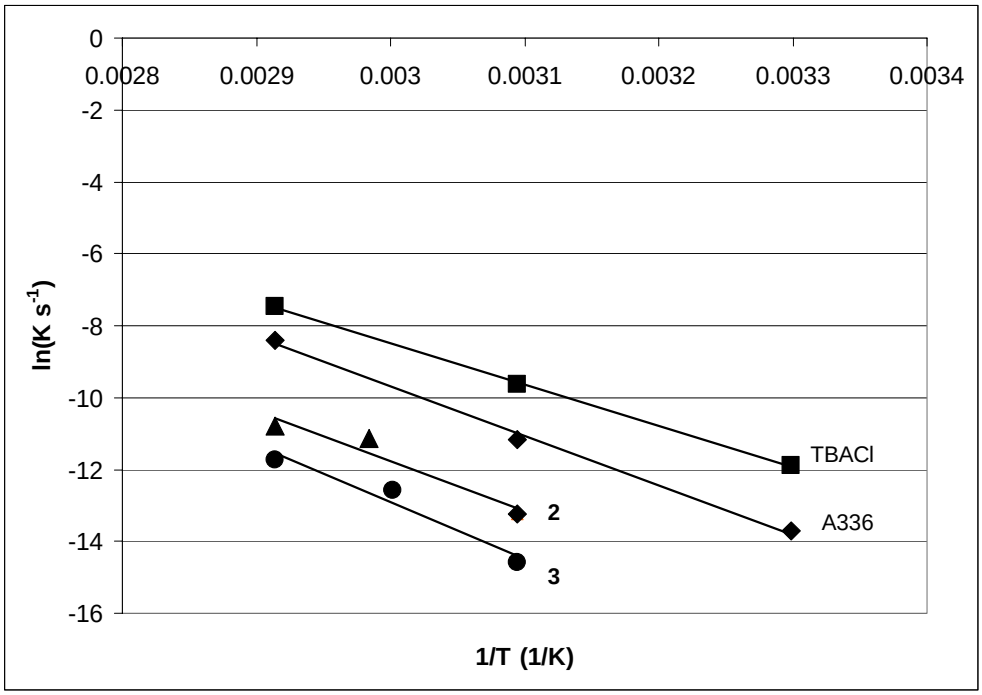


Figure 5-14: Arrhenius plots for reaction of KOAc with siloxane electrophile and 5% of various PTCs in ethyl acetate at various temperatures. 5x excess KOAc is used in all conditions.

I hypothesized that in a more siloxylated medium the differences in the phase-transfer catalyst structure (classic *vs.* siloxylated) may be more distinctive. In order to explore the effect of the siloxylated character of the solvent on the PTC process, three solvent systems were compared: 1) ethyl acetate ("organic"), 2) 50 % ethylacetate/50 % PDMS ("50 % Si) and 3) 100% PDMS ("100% Si"). The pseudo-first order rate constants for tetra-*n*-butylammonium chloride, **2**, and **3** are shown in Figure 5-15 and Table 5-5.

Both Figure 5-15 and Table 5-5 show definitive changes in the rates for TBACl and the siloxylated PTC **2** when the siloxane-character of the organic phase changes. The activity of the siloxylated PTC **3** remained constant throughout. As previously discussed for the organic case, TBACl has the highest rate with at least one order of magnitude improvement over **2** and **3**. With 50% PDMS, TBACl shows a drop in reaction rate, lowering by approximately two-thirds of its original value. At the same time, the siloxylated PTC **2** show a 6 fold increase in rate constant. At 100% PDMS, the TBACl rate constant remains approximately equal to the 50 % PDMS case, while the rate constant for **2** is slightly reduced.

Table 5-5: Pseudo-first order rate constants for the reaction of potassium acetate with siloxane electrophile and various PTCs at 70 °C and 900 rpm stirring in various solvent systems. The rates for both isomers were identical.

	Pseudo-First Order Rate Constant * 10^5 (s^{-1})		
PTC (5%)	**EtOAc**	**50 % Si**	**100% Si**
TBACl	65 ± 4	18 ± 3	20 ± 3
2	2.33 ± 0.04	12 ± 1	4.7 ± 0.6
3	0.99 ± 0.04	1.4 ± 0.2	1.41 ± 0.06

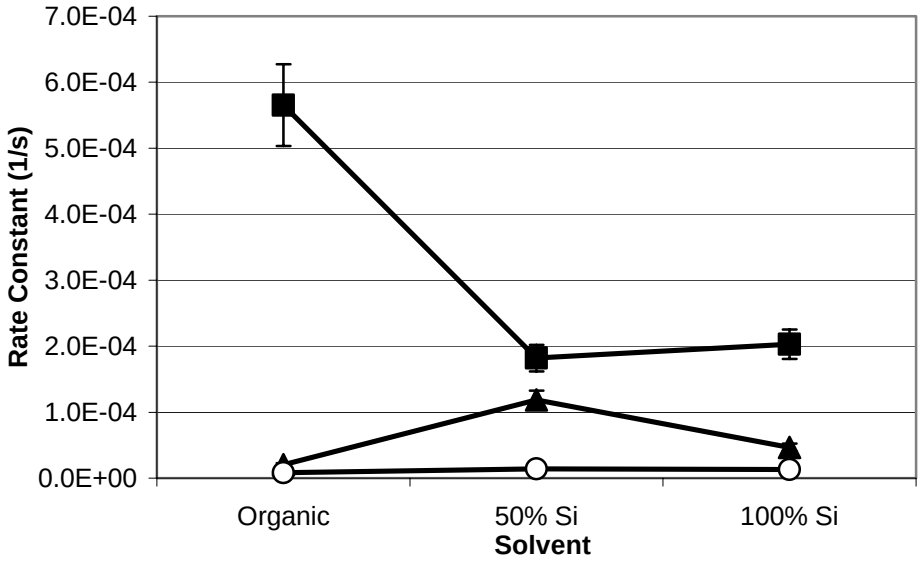


Figure 5-15: Solvent dependence of SiBnCl and KOAc reaction at 70ºC and 5% PTC (■): TBACl, (▲): SiMePTC (2), (○): SiBnPTC (3). The rates for both isomers were identical.

Tetra-*n*-butylammonium bromide shows unexpected versatility to enhance the reaction between a siloxylated reagent in the liquid phase and ionic substrates in the solid phase. TBACl outperformed the custom-made siloxylated catalysts when the liquid phase was ethyl acetate. In a more siloxylated media like PDMS, TBACl and the siloxylated catalyst **2** exhibited closer activity. In either case, the excellent performance of TBACl does not justify the use of a more sophisticated custom-made siloxylated catalyst.

Using the results from the reaction of the siloxylated benzyl chloride with potassium acetate, I explored the model reaction of siloxylated benzyl chloride with the amino acid L-lysine (Figure 5-16). Efficiently reacting siloxylated reagents with peptides would open access to many siloxylated compounds with wide applications such as personal care products. Up to now, however, the reaction is inhibited by the reagents, the siloxylated starting material and the peptide, being in different phases. The model reaction of siloxylated benzyl chloride with the amino acid L-lysine were carried out in ethyl acetate with the three phase-transfer catalysts previously reported (TBACl and the two specialty siloxylated PTCs). The pseudo-first order rate constants for both isomers were the same and are enumerated in Table 5-6.

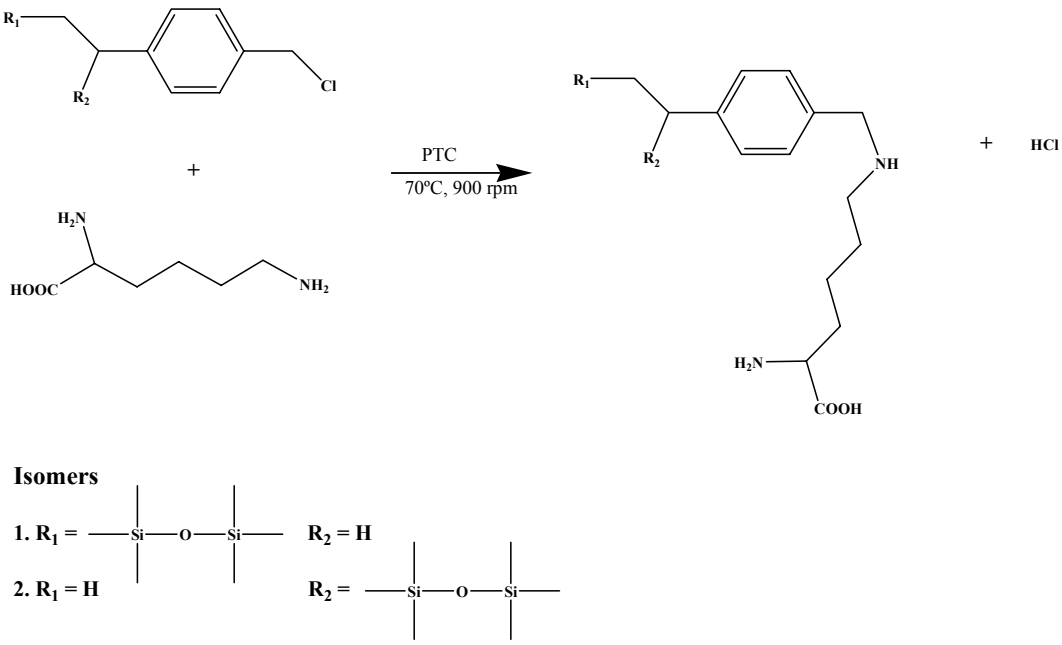


Figure 5-16: Reaction of L-lysine and siloxylated benzylchloride

Table 5-6: Pseudo-first order rate constants for the reaction of L-lysine with siloxane electrophile and various PTCs at 70 °C and 900 rpm stirring. Rates for both isomers were identical.

	Pseudo-First Order Rate Constant * 10^5 (s^{-1})
PTC (5%)	**L-lysine**
None	-
TBACl	1.35 ± 0.01
2	0.86 ± 0.01
3	0.57 ± 0.08

In the absence of PTC, no reaction occurred between the L-lysine and the siloxylated benzyl chloride. In contrast, under phase transfer catalysis conditions the reaction does take place. In this case, the tetra-*n*-butylammonium chloride slightly outperformed the siloxane-containing PTCs for the L-lysine. The pseudo-first order rate constants with tetra-*n*-butylammonium chloride is least 1.5 times the siloxylated PTCs pseudo-first order rate constants.

A qualitative mass spectroscopy analysis suggested that the product distribution of the phase transfer-catalyzed reaction was comparable to the one reported when the reaction was run in a mixed methanol-acetonitrile solvent. Specifically, peaks of molecular mass corresponding to the mono, di-, tri- and tetra-substitution products were observed.

5.4 Conclusion

Phase-transfer catalysis is an effective technique for coupling siloxane-containing compounds with non-siloxylated reagents. The model reaction between a *p*-[2-&1-(1,1,3,3,3-pentamethyldisiloxane)-ethyl]-benzyl chloride and potassium acetate and L-lysine was reported. Three phase-transfer catalysts were tested: the commercially available tetra-*n*-butylammonium chloride and the two specialty siloxylated PTCs **2** and **3**. Quite surprisingly, the tetra-*n*-butylammonium chloride showed superior activity to the custom-made siloxylated compounds in a variety of solvents. When the siloxylated benzylchloride was

reacted with potassium acetate in ethyl acetate, TBACl drastically outperformed the siloxylated catalysts. However, in a siloxylated solvent like PDMS or with L-lysine as reagent, the difference of activity between the three catalysts was slight. In each case, the unexpected performance and versatility of TBACl does not justify the need for a more expensive and less accessible siloxylated phase transfer catalysts.

5.5 Experimental

All chemicals were ordered from Aldrich or VWR and used as received, unless noted. ^{1}H and ^{13}C NMR spectra were recorded using a Varian Mercury Vx 400 spectrometer using the $CDCl_3$ peak as an internal reference. Mass Spectrometry were recorded using a HP GC 6890/ HP MS 5973 or were performed by Georgia Institute of Technology Bioanalytical Mass Spectrometry Facility using a Micromass Quattro LC to perform ESI-MS. Elemental analyses were submitted to Atlantic Microlabs, Inc.

Synthesis of tris-[3-(1,1,3,3,3-pentamethyl-disiloxanyl)-propyl]-amine

Tris-[3-(1,1,3,3,3-pentamethyl-disiloxanyl)-propyl]-amine was synthesized using a method from patent 5,654,374.[12] A 250 mL round-bottom flask under argon was fitted with a magnetic stir bar, a reflux condenser and an addition funnel. The flask was charged with triallyl amine (6.5 g, 0.47 mol) and

heptane (20 mL). Platinum (0)-1,3-divinyl-1,1,3,3-tetramethyldisiloxane (DVDS-Pt) 3% wt in xylenes complex (2.19 g, 1% wt based on amine) was added to this mixture, which was then heated to 85°C. Pentamethyldisiloxane (21.12 g, 0.14 mol) in heptane (20 mL) was added slowly through the addition funnel to the stirred mixture. After the addition was complete, it was observed to be reddish-brown in color. The mixture was stirred for three hours at 70ºC. After three hours, colloidal clay was added. The reaction was allowed to cool to room temperature and stir overnight. The reaction mixture was then filtered and the heptane removed under reduced pressure. A short silica plug with hexane as the eluent was used to purify the product. The product amine was a light yellow (35% yield).

Tris-[3-(1,1,3,3,3-pentamethyl-disiloxanyl)-propyl]-amine: ^{1}H NMR ($CDCl_3$) ppm: 0.053 (45H, m, CH_3-Si), 0.449 (6H, t, CH_2-CH_2-Si), 1.444 (6H, m, CH_2-CH_2-CH_2), 2.396 (6H, t, CH_2-N). ^{13}C NMR ($CDCl_3$) ppm: 0.751, 2.428, 16.439, 21.059, 57.980. MS(m/z): 406 (M^+-$CH_2CH_2Si(CH_3)_2OSi(CH_3)_3$). EA: calculated C, 48.70%, H, 10.87%, N, 2.30%. Found C, 48.47%, H, 10.66%, N, 2.47%.

Synthesis of methyl-tris-[3-(1,1,3,3,3-pentamethyl-disiloxanyl)-propyl]-ammonium chloride:

Tris-3-(1,1,3,3,3-pentamethyl-disiloxanyl)-propyl-amine (2.4187 g, 0.0042 mol) was added to 10 mL of THF. The solution was added to a pressure vessel with methyl chloride at 50 psi and 40ºC (5 g, 0.1 mol). The reaction was

allowed to proceed for three weeks. At the end of three weeks, the pressure vessel was vented to remove the methyl chloride and the THF was removed under reduced pressure. The crude was then dried in the vacuum oven at 40°C overnight. The resulting thick brown liquid was quantitative in yield.

Methyl-tris-[3-(1,1,3,3,3-pentamethyl-disiloxanyl)-propyl]-ammonium chloride: ^{1}H NMR ($CDCl_3$) ppm: 0.045 (45H, m, CH_3-Si), 0.528 (6H, m, CH_2-CH_2-Si), 1.628 (6H, m, CH_2-CH_2-CH_2), 3.319 (9H, m, CH_2-N). ^{13}C NMR ($CDCl_3$) ppm: 2.322, 15.339, 17.031, 48.960, 64.648. MS (m/z): 596.4 (M^+-Cl). EA: calculated C, 47.45%, H, 10.51%, N, 2.21%. Found C, 47.12%, H, 10.26%, N, 2.25%.

Synthesis of benzyl-tris-[3-(1,1,3,3,3-pentamethyl-disiloxanyl)-propyl]-ammonium chloride

Tris-3-(1,1,3,3,3-pentamethyl-disiloxanyl)-propyl-amine (1.1205 g, 0.001717 mol) was put under nitrogen. To the amine, benzyl chloride (10 mL) was added to act as solvent and reactant. The solution was heated gradually to 65°C and allowed to react until ^{1}H NMR showed reaction completion (five days). The resulting product was a brown thick liquid and yield was quantitative. *Benzyl-tris-[3-(1,1,3,3,3-pentamethyl-disiloxanyl)-propyl]-ammonium chloride:* ^{1}H NMR ($CDCl_3$) ppm: 0.046 (45H, m, CH_3-Si), 0.501 (6H, m, CH_2-CH_2-Si), 1.800 (6H, m, CH_2-CH_2-CH_2), 3.200 (6H, m, CH_2-N), 4.568 (2H, s, benzyl CH_2), 7.355 (5H, m, benzyl ring). ^{13}C NMR ($CDCl_3$) ppm: 2.051, 15.417, 17.086,

46.284, 132.332, 128.539. MS (m/z): 672.4 (M^+-Cl). EA: calculated C, 52.53%, H, 9.95%, N, 1.98%. Found C, 52.79%, H, 9.56%, N, 2.35%.

Synthesis of *p*-[1 & 2-(1,1,3,3,3-pentamethyldisiloxane)-ethyl]-benzyl chloride:

The 4-vinyl-benzyl chloride (5.0 g, 0.0337 mol) was added to 20 mL of heptane under nitrogen. The mixture was heated to 75ºC. The catalyst platinum(0)-1,3-divinyl-1,1,3,3-tetramethyl disiloxane complex (3 wt % xylene) (DVDS-Pt) (1.7 g, 1%wt) was added to the solution. The pentamethyl disiloxane (5.75 g, 0.0388 mol, 1.15 equiv) in heptane (5 mL) was added drop wise. The solution changed from a light yellow to a dark brown upon addition and the addition was stopped whenever the reaction temperature increased by more than 2ºC. After the addition was complete, the temperature was reduced to 70ºC. After 3 hours at 70ºC, the reaction was allowed to cool to room temperature and was stirred overnight. The heptane was removed under reduced pressure. A column chromatography on silica gel with hexane as eluent was run and all the fractions combined. The hexane was removed under reduced pressure to give a clear liquid. Yield was 50% with two isomers.

p-[2-(1,1,3,3,3-pentamethyldisiloxane)-ethyl]-benzyl chloride and p-[1-(1,1,3,3,3-pentamethyldisiloxane)-ethyl]-benzyl chloride: ^{1}H NMR ($CDCl_3$) ppm: 0.1 (15H, m, CH_3-Si), 0.9 (2H, m, CH_2 isomer), 1.3 (3H, d, CH isomer), 2.3 (2H, q, CH_3 isomer), 2.7 (2H, m, CH_2 isomer), 4.6 (2H, s, benzyl CH_2), 7.2 (4H, m, benzyl

ring). ^{13}C NMR ($CDCl_3$) ppm: 1.516-2.156, 20.39, 29.2533; 46.49, 129.42-127.44, 134.50, 145.56. MS(m/z): 300 (M^+). EA: calculated, C, 55.87%, H, 8.41%. Found, C, 55.82%, H, 8.41%.

Acetic acid 4-[2-(1,1,3,3,3-pentamethyl-disiloxanyl)-ethyl]-benzyl ester:

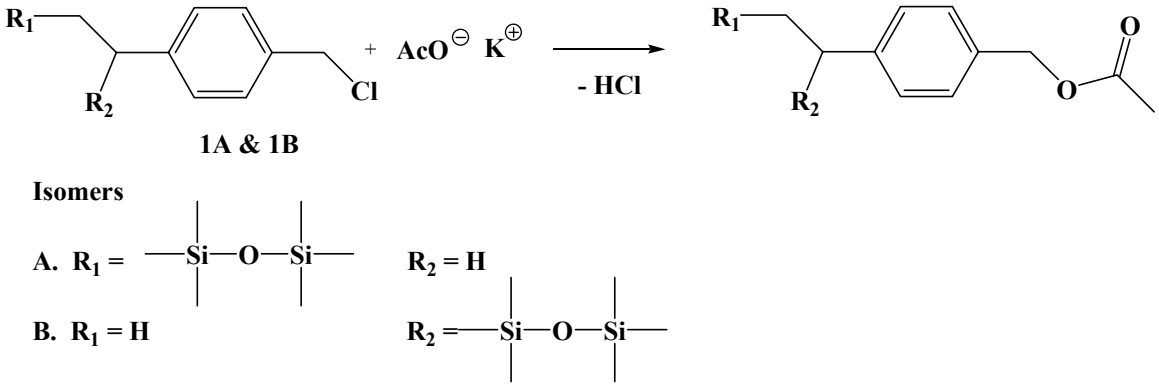


Figure 5-17: Acetic acid 4-[2-(1,1,3,3,3-pentamethyl-disiloxanyl)-ethyl]-benzyl ester synthesized from KOAc and siloxane electrophile.

p-[1 & 2-(1,1,3,3,3-pentamethyldisiloxane)-ethyl]-benzyl chloride (0.4 g, 1 equiv) was added to ethyl acetate (4 mL). To the solution, tetrabutylammonium chloride (0.02 g, 5 mol %) and potassium acetate (0.7 g, 5 equiv) were added. The solution was heated to 60ºC for 48 hours. The reaction was worked up by

washing with saturated aqueous $NaHCO_3$, water and saturated aqueous NaCl. The organic phase was dried over magnesium sulfate and the solvent was removed under reduced vacuum. The orange liquid was purified by silica gel chromatography ethyl acetate/hexane=1/9 to give a clear liquid. Both isomers of the original *p*-[1& 2-(4-chloromethyl-phenyl)-ethyl]-1,1,3,3,3-pentamethyldisiloxane were observed in the product.

Acetic acid 4-[2-(1,1,3,3,3-pentamethyl-disiloxanyl)-ethyl]-benzyl ester: ^{1}H NMR ($CDCl_3$, ppm): 0.084 (15H, m, CH_3-Si), 0.8 (2H, m, CH_2 isomer), 1.4 (1H, m, CH isomer,), 2.1 (3H, s, CH_3-C=O), 2.2 (3H, m, CH_3 isomer), 2.6 (2H, m, CH_2 isomer), 5.1 (2H, s, benzyl CH_2), 7.2 (4H, m, benzyl ring). ^{13}C NMR ($CDCl_3$, ppm):-1.73, -1.46, 0.28, 1.82, 1.98, 14.33, 20.32, 21.05, 29.15, 31.19, 66.29, 66.34, 127.45, 127.99, 128.21, 128.50, 131.73, 133.00, 145.48, 145.57, 170.95. IR ($CDCl_3$, cm^{-1}): 1740.03 (C=O), 1046.06 (SiOSi). MS (m/z): 270 (M^+-$COCH_3$+Na). EA: calculated: C, 59.21%, H, 8.70%. Found: C, 59.49%, H, 8.87%.

Experimental Procedure – Kinetics

The siloxane electrophile, *p*-[1 & 2-(1,1,3,3,3-pentamethyldisiloxane)-ethyl]-benzyl chloride were reacted with potassium acetate, potassium thiocyanate, potassium cyanide and L-lysine hydrate at 70ºC. The reactions took place in ethyl acetate with four phase transfer catalysts, tetra-*n*-butylammonium

chloride, aliquat 336, methyl-tris-[3-(1,1,3,3,3-pentamethyl-disiloxanyl)-propyl]-ammonium chloride and benzyl-tris-[3-(1,1,3,3,3-pentamethyl-disiloxanyl)-propyl]-ammonium chloride. The reactions were carried out in a 25 mL round bottom flask immersed in an oil bath at the appropriate temperature. The system was stirred at 900 rpm with a Teflon coated magnetic stir rod. A typical reaction consisted of 5 mol % PTC based on the electrophile, 0.1 mL siloxane electrophile, 5 equivalents of nucleophile, 5 times excess potassium chloride (only salt reactions), 3 mL ethyl acetate, and 0.1 mL decane, as an internal standard. The potassium chloride was included to maintain a constant concentration solution for accurate kinetics and the decane was added as an internal standard. The reaction mixture was sampled at varying intervals by removing 0.05 to 0.075 mL aliquots, which were immediately quenched in ethyl acetate and analyzed by GC-MS. The two isomers were measured using the same reactions but had different retention times on the GC-MS and occasionally different reaction rates. The products of each reaction were isolated after reaction completion with three extractions with ethyl acetate. The solution was evaporated under reduced pressure to remove the solvent and the resulting product was analyzed by NMR. The L-lysine products were confirmed by ^{1}H NMR. The salt products were confirmed by GC-MS and ^{1}H NMR.

5.6 References

(1) Yadav, G. D. *Topics in Catalysis* **2004**, *29*, 145-161.
(2) Dehmlow, E. V.; Dehmlow, S. S. *Phase Transfer Catalysis*; 3rd ed.; VCH: New York, 1993.
(3) Starks, C. M.; Liotta, C. L.; Halpern, M. *Phase Transfer Catalysis: Fundamentals, Applications, and Industrial Perspectives*; Chapman & Hall: New York, 1994, p1-22.
(4) Beltrame, P., Biale, G., Lloyd, D. J., Parker, A. J., Ruane, M., Winstein, S. *J. Am. Chem. Soc.* **1972**, *94*, 2240.
(5) Takeishi, M., Naito, Y., Okawa, M. *Angewandte Makromolekulare Chemie* **1973**, *28*, 119-121.
(6) Drake, R.; Postiaux, S. WO, 2007; Vol. 2007141565.
(7) Lane, T. H.; Liotta, C. L.; Brandstadt, K. F.; Hand, J. H.; Charney, R.; Bommarius, A. S.; Eckert, C. A.; Polizzi, K. M.; Pollet, P.; Hallett, J. P. WO, 2008; Vol. 2008033908.
(8) Lane, T. H., Brandstadt, K. F., Hand, J. H.; Dow Corning, 2003.
(9) http://www.dowcorning.com/content/sitech/sitechapps/, 2008.
(10) *Handbook of Elemental Speciation II: Species in the Environment, Food, Medicine, and Occupational Health*; Cornelis, R., Ed.; Wiley, 2005.
(11) R. Solaro; D'Antone, S., Chiellini, E.; *J. Org. Chem* **1980**, *45*, 4179.
(12) Arren, D. H. C., Coggio, W. D., Parker, D. S. US, 1997; Vol. 5,654,374.

CHAPTER 6: CONCLUSIONS & RECOMMENDATIONS

6.1 Conclusions & Recommendations for Chapter 2: Continuous Flow Reactor

The reaction of L-boc-phenylalanine with alkyl chloroformate to form a mixed anhydride followed by reaction with trimethylsilyl diazomethane was explored in a batch reactor and in a continuous flow reactor. In a batch mode, the first step of the reaction was carried at the temperature -20ºC because the mixed anhydride is temperature sensitive (and decomposes readily above 0ºC). Fundamental studies were accomplished on the batch reaction to determine the critical factors (i.e. reaction time, structures of reagents, reaction temperature, and solvents). The best overall yield reported in the literature for this sequence for the synthesis of the diazoketone was 78 %, which matched my best overall yield.

During this research, several continuous reactor configurations were built. The final configuration that involves two coiled continuous microreactors packed with glass beads is both simple and extremely efficient. The reaction sequence was carried out at 4ºC with quantitative yield in the diazoketone product. This result is remarkable. It clearly demonstrates that the continuous process that was developed in our laboratory improves yields (and product quality) utilizes cheaper and safer reagents (ethylchloroformate *vs.* isobutylchloroformate and

trimethylsilyl diazomethane *vs*. diazomethane), and reduces energy intake by eliminating the need for low reaction temperatures (4ºC vs. -20ºC).

Recommendations can be made from the results reported in this chapter. First, it was observed that the diazoketone product yield was quantitative by LCMS analysis. However the isolated yield was between 60-70 %. This discrepancy was attributed to the diazoketone decomposing during the purification, namely the silica-gel column. Although this diazoketone is not isolated in the real process, the potential decomposition could be confirmed by adding a silica column before the column on the LC-UV. Additionally, it would be very interesting to study the last step of the three-step sequence, the HCl step, yielding to the α-chloroketone by adding it onto the continuous flow reactor current process. If this will be pursued, the corrosive character of concentrated HCl will be an important factor to take into consideration. Currently, the continuous reactor and the pump's core are made of stainless steel that can be corroded by HCL and will need to be modified in order to sustain the use of concentrated HCl. Lastly, the benefits of the continuous flow reactor design that was developed could be used advantageously for different types of multi-step reactions.

6.2 Conclusions and Recommendations for Chapter 3: Cleavable, n-octyl-thiirane oxide, Surfactant and Reversible Sulfolene Surfactants

n-Octyl thiirane oxide was successfully synthesized and its surface active property was determined. The irreversible decomposition upon heating of n-octyl thiirane oxide to surface inactive fragments was demonstrated and occurred in less than 10 min at 110ºC. .

The synthesis of a sulfolene based switchable surfactant was unsuccessful although the synthesis of the sulfolene methyl ester was successfully achieved.

The cleavable surfactant, *n*-octyl thiirane oxide may be useful in my research group synthesis of nanoparticles. Currently, a reversible ionic liquid is being tested. A comparison of the nanoparticle size resulting from the cleavable surfactant and the reversible ionic liquid may be interesting to study.

The synthesis of a reversible surfactant from the carboxylic acid to form an ester or amide would be very interesting (Figure 6-1). I recommend that my research group continues to remain aware of new research that could overcome the synthetic hurdles so far encountered.

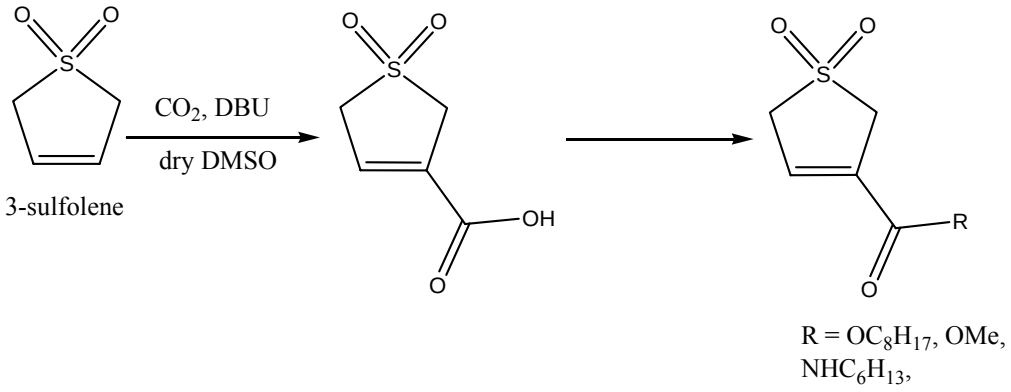


Figure 6-1: Synthesis of reversible surfactant

6.3 Conclusions & Recommendations for Chapter 4: Synthesis of 5-aminotetrazole

5-amino-1H-tetrazole was synthesized using two novel synthetic routes. Both routes made use of Sharpless' click chemistry to form the tetrazole ring. By using not only masked but activated cyanide and azide derivatives, the 5-amino-1H-tetrazole was successfully synthesized in good yield (60%). This novel process allowed efficient reactions (with minimum by-product formation) and easy isolation of the intermediates. For the first time, the hydrogen transfer of the 1-benzyl-5-benzylaminotetrazole has been investigated and successfully concluded. I also synthesized a novel compound, 1-benzyl-5-azido tetrazole. The reduction reactions of the 5-azido-1-benzyltetrazole to form the 5-amino-1-benzyl-tetrazole and the 5-amino-1H-tetrazole were successfully concluded. The last step, the hydrogenation of the protecting groups, takes a week to go to completion in the first synthesis and three weeks in the second synthesis. This is a

limitation for the potential transfer of these syntheses to a commercial process. Nonetheless, these syntheses minimized, if not eliminated, the need and/or potential contamination of the 5-amino-1H-tetrazole by toxic and/or un-desired catalyst or by-products. It also showed improvements over the existing methods in terms of safety concerns.

A possible next step for this project could be the development and optimization of a "one-pot" synthesis with the 5-azido-1-benzyltetetrazole as the second step. Modifying conditions (phase transfer catalyst, stirring rate, solvent) for the phase transfer catalyzed nucleophilic displacement of benzylsulfonyl by the azide could improve a currently relatively slow reaction (few days). In addition, the hydrogenation reaction to cleave the benzyl group is by far the main limitation for both syntheses. Although the reaction is quantitative, it can take as much as three weeks to reach completion. The hydrogenation can be attempted at higher pressure (up to 150 psi) and temperature up to 80ºC. Other metal catalysts like Raney nickel and solvent system can also be investigated. After optimizing these steps, a "one-pot" synthesis could be then developed for the formation of the 5-aminotetrazole.

6.4 Conclusions and Recommendations for Chapter 5: Phase Transfer Catalysis for Reaction between a Siloxylated Electrophile and Insoluble Nucleophilic Salt

Phase-transfer catalysis is an effective technique for coupling siloxane-containing compounds with non-siloxylated reagents. The model reaction between a *p*-[2-&1-(1,1,3,3,3-pentamethyldisiloxane)-ethyl]-benzyl chloride and potassium acetate and L-lysine was reported. Three phase-transfer catalysts were tested: the commercially available tetra-*n*-butylammonium chloride and the two specialty siloxylated PTCs **2** and **3**. Quite surprisingly, the tetra-*n*-butylammonium chloride showed superior activity to the custom-made siloxylated compounds in a variety of solvents. When the siloxylated benzylchloride was reacted with potassium acetate in ethyl acetate, TBACl drastically outperformed the siloxylated catalysts. However, in a siloxylated solvent like PDMS or with L-lysine as reagent, the difference of activity between the three catalysts was slight. In each case, the unexpected performance and versatility of TBACl does not justify the need for a more expensive and less accessible siloxylated phase transfer catalysts.

This project was done in collaboration with Dow Corning. Since the project was successfully concluded, no future work is currently planned. Recommendations if this work was to continue will be to extend the work started with L-Lysine. L-lysine is the model for reaction of amino acids with siloxylated reagents by means of phase transfer catalysis. Preliminary data showed that multiple substitutions can take place. For future work, the regioselectivity of the reactions and the product distribution should be determined quantitatively. The

information from this model reaction (L-Lysine with siloxylated benzyl) can then be used to study reactions with more complex reagents like bipeptide and tripeptide. Phase transfer catalysis can open new avenues to prepare siloxylated substituted polypeptides and has yet been fully explored. In addition, a more siloxylated electrophile could be synthesized and tested with the siloxylated phase transfer catalysts. A more siloxylated electrophile may show a better reaction rate when using a siloxylated phase transfer catalyst

APPENDIX A: HYDROLYZABLE AZIDES

A.1. Introduction

Hydrolyzable azides have potential as an anti-biofouling agent. The aim of this project was to synthesize various azides and test their hydrolysis in water and salt water to determine their usefulness as an anti-biofouling agent.

A.2. Background

Marine biofouling occurs when barnacles attach to the hull or rudder of a boat. Barnacles attach to the boat by producing an epoxy-like cement that can stick to even Teflon. The barnacles increase corrosion and drag resistance causing a problem that costs the maritime industry billions of dollars a year. One of the most recent treatments used tributyltin (TBT) which has been shown to be toxic to marine animals. An ideal anti-biofouling agent would be easy to apply, inexpensive, nontoxic, and long lasting. Currently sodium azide is used as a biocide in agricultural for pest control. Since azides are already used as a biocide, it was thought that a hydrolyzable azide would be able to prevent biofouling. The hydrolyzable azide would release the inorganic azide slowly, creating an azide layer around the marine vessel.

A.3. Previous Work

Previously, the hydrolysis of various organic azides were tested by A. Szewczuk.[1] Szewczuk found that the release of inorganic azide from organic

acid azide proceeded very slowly in an aqueous neutral solution at room temperature. In Figure A-1, a table is given that showed the hydrolysis of organic azides as measured by absorptivity. Of particular interest to my research were the compounds phenylacetyl azide, diphenylphosphoryl azide, and the phenylmethanesulfonyl azide.

Table I. Absorptivities of Some Azides after Hydrolysis with KOH and Complexing with Fe^{3+} at the Condition Given in Analytical Procedure

compound	absorptivity[a] molar[b]	relative, %
sodium azide	4100	100
phenylacetyl azide	3772	92
phenoxyacetyl azide	4059	99
phenylmethanemalonyl diazide	3936	96
6-aminohexanoyl azide	3731	91
N-benzyloxycarbonyltyrosyl-glycylglycyl azide	3997	97.5
N-benzyloxycarbonyltyrosyl azide	4038	98.5
phenylmethanesulfonyl azide	3936	96
diphenylphosphoryl azide	3075	75
phenyl azide	0	0
phenylmethane azide	0	0
n-hexyl azide	0	0

[a] Absorptivities were mean calculated from three to five determinations. [b] For all acid azides except phenylmethanesulfonyl and diphenylphosphoryl azides, the data were calculated from the amount of proper hydrazide used for their synthesis.

Figure A-1: Table of hydrolysis of various organic acid azides as shown by absorptivity with sodium azide as standard [1]

A.4. Results and Discussion

A.4.1. Synthesis of the Hydrolyzable Azides

For this project, I investigated the synthesis of two different hydrolyzable azides: an acyl and a sulfonyl. The phenyl-acetyl azide synthesis was synthesized using a method from literature.[2] The synthetic scheme is shown in Figure A-2. However, the phenyl-acetyl azide did not show complete reaction in ^{1}H NMR, giving a ratio of 15:1 product to starting material and this avenue was not pursued further.

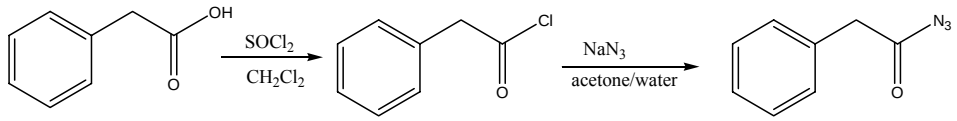


Figure A-7-1: Synthesis of phenyl-acetyl azide from phenyl acetic acid

The 4-methyl-benzenesulfonyl azide was synthesized using a method by McManus *et al.* (Figure A-3).[3] The yield was quantitative and the product was characterized by ^{1}H and ^{13}C NMR and elemental analysis. This compound was used for the rest of the experiments as a proof of principle.

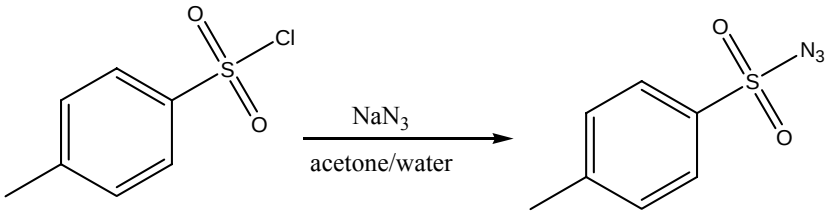


Figure A-7-2: Synthesis of 4-methyl-benzenesulfonyl azide from *p*-toluene sulfonyl chloride

A.4.2 Hydrolysis of Sulfonyl Azide

Since I planned on measuring the hydrolysis of the sulfonyl azide by LC-UV, I first made a calibration curve of the sodium azide and of 4-methyl-benzenesulfonyl azide at various concentrations. (Figure A-4 and Figure A-5, respectively)

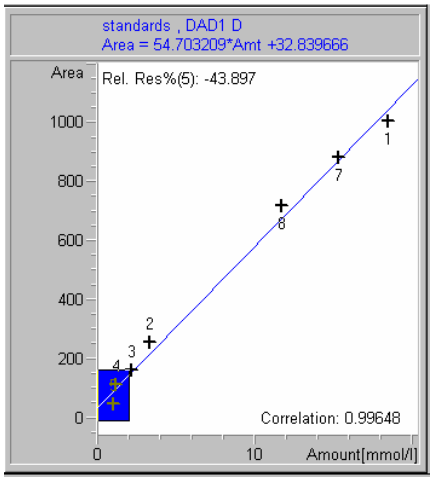


Figure A-7-3: Calibration curve of sodium azide on LC-UV, UV at 230 nm

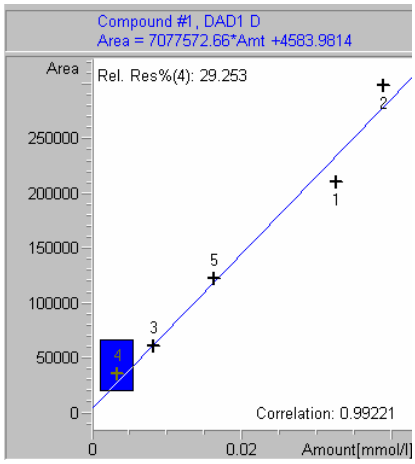


Figure A-7-4: Calibration curve of 4-methyl-benzenesulfonyl azide on LC-UV, UV at 230 nm

By using a calibration curve, I was able to determine the concentration of 4-methyl-benzenesulfonyl azide and released sodium azide during the hydrolysis.

Another important aspect to consider is that the mechanism of the release of azide will be different in pure water and in salt water. The 4-methyl-benzenesulfonyl azide should react with the water to release hydrogen azide. (Figure A-6) Since this azide is acidic, the hydrolysis was also monitored using pH paper. Due to the low concentrations of sulfonyl azide used, I did not anticipate that the acid would affect the pH. However, if I had observed a rise in pH, I would have buffered the solution.

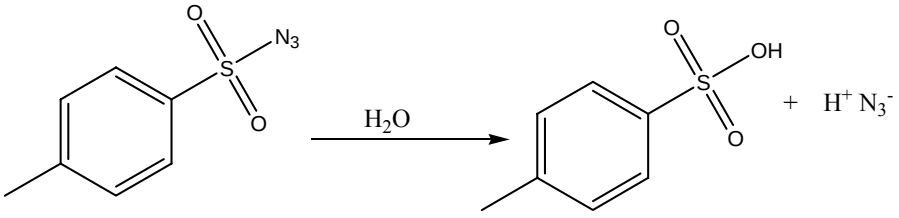


Figure A-7-5: Reaction of 4-methyl-benzenesulfonyl azide and water

In the salt solution, I believed that the sodium chloride should react with the 4-methyl-benzenesulfonyl azide first to form *p*-toluene sulfonyl chloride and sodium azide. The sulfonyl chloride will then react with the water to form

hydrochloric acid.(Figure A-7) Because this reaction should also form acid, I monitored the acidity of the reaction using pH paper and would have buffered the solution if the pH rose.

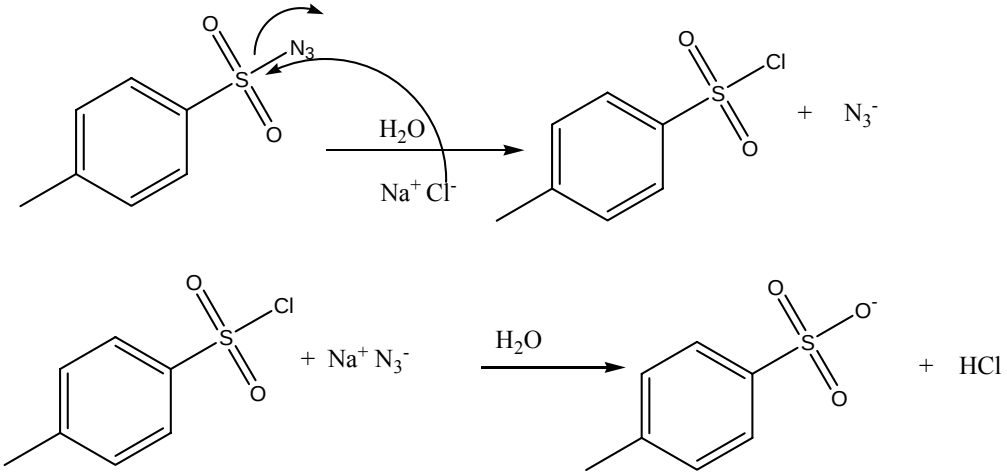


Figure A-7-6: Mechanism of 4-methyl-benzenesulfonyl azide with water and sodium chloride

To test the hydrolysis of the 4-methyl-benzenesulfonyl azide in water, a solution of water and 4-methyl-benzenesulfonyl azide was made. Since the 4-methyl-benzenesulfonyl azide formed a layer separate from the water, methanol was added until the two layers were miscible. An aliquot was taken periodically and tested by LC-UV to determine the amount of hydrolysis (Figure A-8). When

each aliquot was taken, the pH was also measured to confirm that there was not an excess of acid formation. During the experiment, the pH remained neutral.

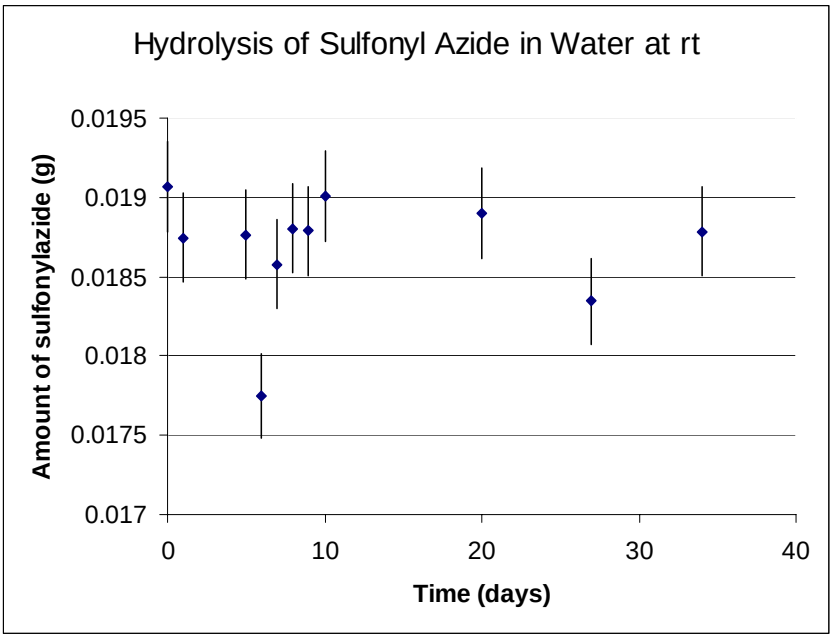


Figure A-7-7: Hydrolysis of sulfonyl azide in water at room temperature over 35 days

During the experiment, the hydrolysis of the 4-methyl-benzenesulfonyl azide in water remained negligible. In addition, after the 35 day, more samples were taken until the 75th day and still the hydrolysis of 4-methyl-benzenesulfonyl azide in water remained negligible.

The hydrolysis of 4-methyl-benzenesulfonyl azide in sea water was performed in a similar way to the hydrolysis in water. The 4-methyl-benzenesulfonyl azide was added to a mixture of sea water and methanol until the phases are miscible. An aliquot of the solution was taken periodically and measured by LC-UV (Figure A-9). The pH was also monitored and remained neutral throughout the experiment. Over the course of the thirty-five days, the change in concentration of the sulfonyl azide and thus the hydrolysis of the 4-methyl-benzenesulfonyl azide remained negligible. More samples were taken on this experiment until the 75^{th} day with no change in results.

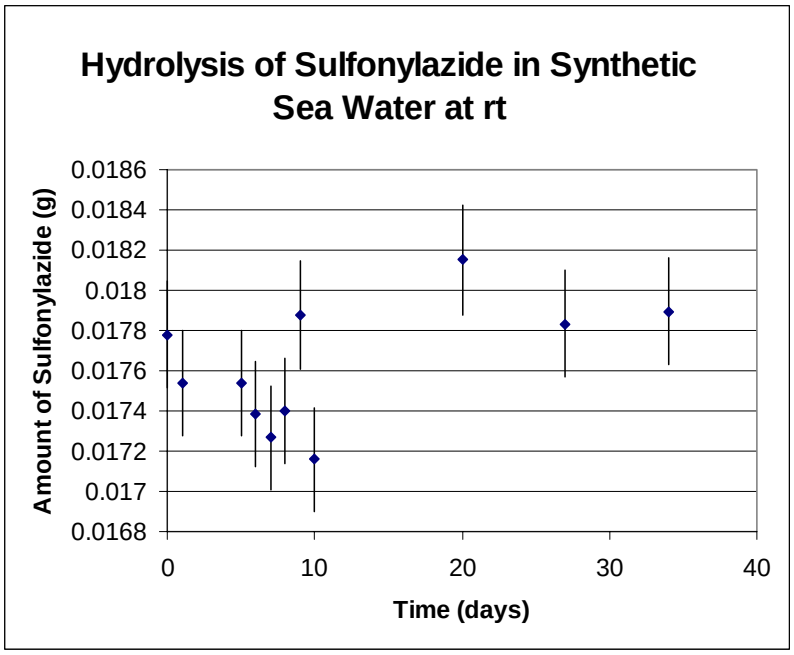


Figure A-7-8: Hydrolysis of 4-methyl-benzenesulfonyl azide in sea water at room temperature over 35 days

Due to the lack of positive results, this project was not pursued further. However, if this project were to be reinvestigated, the hydrolysis could be done with heating. By heating the hydrolysis solution, the rate of hydrolysis can be calculated during a much shorter amount of time.

A.5 Conclusion

This work investigated two potentially hydrolyzable organic azide compounds. During this project, the hydrolysis of 4-methyl-benzenesulfonyl azide over a 75 day period in water and salt water was investigated.

A.6 Experimental

All chemicals were ordered from Aldrich or VWR and used as received, unless noted. ^{1}H and ^{13}C NMR spectra were recorded using a Varian Mercury Vx 400 spectrometer using the $CDCl_3$ peak as an internal reference. Mass Spectrometry were recorded using a HP GC 6890/ HP MS 5973 or were performed by Georgia Institute of Technology Bioanalytical Mass Spectrometry Facility using a Micromass Quattro LC to perform ESI-MS. Elemental analyses were submitted to Atlantic Microlabs, Inc. LC-UV analysis was done on an Agilent 1100 Series LC with UV detector.

Phenyl-acetyl azide:

Phenyl acetic acid (1 g, 0.0073 mol) was added to dry dichloromethane (5 mL) under nitrogen. Thionyl chloride (3.2 mL, 0.043 mol) was added slowly to the reaction solution. The solution was heated to 45ºC and allowed to reflux for 18 hours. The solvent and excess thionyl chloride was removed under reduced pressure. Dry acetone (3 mL) was added to the reaction solution. Sodium azide (1.9 g) in water (10 mL) was added slowly to the solution at 0ºC. The reaction was allowed to warm to room temperature overnight and a color change to orange was observed. To work up the reaction, the solution was extracted using diethyl ether. The organic layer was washed with water and brine, and then dried over magnesium sulfate. The solvent was not removed under reduced pressure and

was stored in the fridge, wrapped in foil. An aliquot was analyzed by ^{1}H NMR and showed a ratio of 15:1 product to starting material. This synthetic route was not pursued further.

4-Methyl-benzenesulfonyl azide:

Sodium azide (0.44 g, 0.0068mol, 1.3 equiv) was dissolved in 3 mL water and cooled in an ice-salt bath under nitrogen. *p*-Toluene sulfonyl chloride (1 g, 0.0052 mol) was dissolved in 4 mL of a 95% acetone-water mixture. The *p*-toluene sulfonyl chloride solution was added slowly to the cold sodium azide solution causing the reaction to turn orange. The resulting solution was stirred for 30 min cold and 30 min at room temperature. The reaction was then worked up by adding 5 mL of water and extracting with ether (2 x 25 mL). The combined ether layers were washed with water (4 x 50 mL) and dried over magnesium sulfate. The solvent was not removed for safety reasons. The solution was stored in the fridge in a vial wrapped in foil.

4-Methyl-benzenesulfonyl azide: ^{1}H NMR ($CDCl_3$, ppm): 7.93 (1H, d), 7.85 (1H, d), 7.42 (2H, d), 2.49 (3H, s). ^{13}C ($CDCl_3$, ppm): 130.62, 130.02, 114.13, 114.08, 111.40, 110.92, 5.96. Elemental analysis: calc: C 42.63, H 3.58, N 21.31, found: C 42.80, 3.58, 21.36.

Calibration curve of 4-methyl-benzenesulfonyl azide:

To make the calibration curve, five samples of the 4-methyl-benzenesulfonyl azide with masses of 0.0326 g, 0.0390 g, 0.0651 g, 0.0854 g, 0.1190 g were dissolved in 1 mL of methanol. A standard of 0.0427 g in 1 mL methanol was tested using the calibration curve. The LC-UV gave a reading of 0.0416 g which demonstrated the accuracy of the calibration curve.

Hydrolysis of 4-methyl-benzenesulfonyl azide:

The 4-methyl-benzenesulfonyl azide (0.49g) was added to 10 mL water and 45 mL of methanol for the water hydrolysis experiment. The sulfonyl azide (0.47 g) was added to 10 mL of sea water from Aldrich and 46 mL of methanol. Both solutions were in closed containers and wrapped in foil. The solutions were stirred at the same rate at room temperature. Periodically, a 0.5 mL aliquot was removed and analyzed on the LC-UV. Both were allowed to stir for 75 days. The results are shown in Figure A-8 and A-9.

A.7 Reference

(1) *Anal. Chem* **1982**, *54*, 846-847.
(2) *J. Org. Chem.* **2005**, *70*, 2701-2707.
(3) *J. Org. Chem.* **1984**, *49*, 683-687.

APPENDIX B: GREEN METABOLOMICS: SILYLATED AMINO ACIDS FOR SEPARATION ON SUPERCRITICAL FLUID CHROMATOGRAPHY

B.1 Introduction

The detection of metabolites can be used as an early disease diagnostic tool. Unfortunately, the detection of metabolites is an immature field with a small upper range in detection and identification. Currently, GC-MS and LC-MS are used for metabolite detection but using SFC coupled with a MS detector could be a potentially superior method. SFC has higher efficiency and resolution but is limited by the solubility of the metabolites. The primary issue is the lack of solubility in $scCO_2$ of the polar amino acids. To make SFC a more useful detection tool, the methodology needs to be modified to increase the solubility of the polar amino acids. This could be done by changing the amino acid itself or by changing the SFC mobile phase. The addition of a silyl group to an amino acid has been shown to increase its solubility in $scCO_2$. The addition of a co-solvent or additive has been shown to modify the SFC mobile phase to increase the solubility amino acids. In this project, I pursued both avenues of increasing the solubility of amino acids for use in SFC separations. This project was done in collaboration with Dr. Fernandez.

B.2 Background

B.2.1 Metabolites

The term metabolomics (or metabonomics) began being used in the 1990s to describe approaches to measure metabolites.[1] Metabolites are the end products of cellular regulatory processes. They are present within a cell, tissue, or organism during a genetic modification or physiological stimulus.[1] Metabolomics can reflect the pathological state of various organs and can aid in the early detection of disease. For example, metabolites are sensitive to a number of subtle genetic modifications including a silent mutation in yeast.[1] However, metabolite identification and quantification has an upper range in the order of hundreds. Comparing metabolomics to the thousands of proteins that can be analyzed by proteomic approaches, illustrates that the study of metabolomics is a less mature field and that there is a need for an increase in the development in tools to identify and quantify metabolites.

B.2.2 Supercritical Fluid Chromatography (SFC)

Currently, metabolites are analyzed using liquid chromatography-mass spectrometry (LC-MS) or gas chromatography-mass spectrometry (GC-MS).[1] These techniques are usually used in conjunction with pattern recognition. Metabolites currently rely heavily on chemical separation. Supercritical Fluid Chromatography (SFC) separates molecules based on their volatility in

supercritical carbon dioxide ($scCO_2$). Using SFC for molecule separation instead of LC or GC has several advantages which include higher efficiency and higher resolution.[2] In addition, the reduced use of organic solvents minimizes waste and undesirable interferences with the mass spectrometry analysis. The current problem with SFC is that it has been traditionally limited to relatively non-polar compounds.[2] The SFC mobile and stationary phases do not allow the separation of ionic species which limits its use to hydrophobic peptides. The insufficient solubility of polypeptides has resulted in the SFC currently being limited to separating polypeptides less than 5000 Da.[2] There are three methods that have been used to modify the SFC mobile phase to allow the more polar molecules to be separated. The first method is to use a more polar pure fluid such as SO_2 or N_2O, rather than CO_2. Another method involves adding a polar organic solvent (known as a modifier) to increase the solubility in $scCO_2$. Lastly, a highly polar or ionic compound (known as an additive) can be added to the mobile phase. The last example has been done using trifluoroacetic acid (TFA) in a CO_2/methanol mobile phase to elute 40 mer peptides. The TFA suppresses deprotonation of the peptide carboxylic acid and protonates the amino groups.[2] An alternative to changing the SFC mobile phase through use of a different gas, a modifier, or an additive is to change the metabolite or peptide itself to increase its solubility in $scCO_2$.

B.2.3 Increase Solubility in SFC

Several functional groups, when added to a compound, can increase the CO_2 solubility of that compound. Those functional groups, known as CO_2-philic, are fluoroether, fluoroalkyl, fluoroacrylate, and siloxane.[3,4] Currently, silyl groups have been added to increase the volatility of the 18 common amino acids in gas chromatography.[5] After the addition of a silyl group, several hydroxy and amino compounds that were previously nonvolatile and unstable at 200-300ºC have been successfully chromatographed. The silyl group makes these compounds more volatile by replacing hydrogens that participate in hydrogen bonding. This replacement reduces the polarity of the compound and decreases the hydrogen bonding which increases the volatility. The silyl group has been added and analyzed without product isolation using gas chromatography (GC), mass spectrometry (MS), or a combination of the two. The silyl group that has been the most useful for GC and MS is *t*-butyldimethyl silyl. This silyl is usually added to a hydroxide using chloro(dimethyl)t-butyl silane in the presence of a base such as imidazole and pyridine in a solvent such as *N, N*-dimethyl formamide (DMF). Having the *t*-butyldimethyl silyl group is preferred over the trimethyl silyl group because the *t*-butyldimethyl silyl ether is more stable to alkaline conditions, to hydrogenolysis, and to solvolysis than the trimethyl silyl ether.[5,6]

B.3 Results and Discussion

B.3.1 Synthesis of Silylated Amino Acid

The first step of this project was to synthesize an amino acid derivatized with silylation reagents. Since the SFC only has a UV-Vis detector, I needed to synthesize a UV-Vis active molecule. This meant that I was either limited to UV-Vis active amino acids such as tyrosine and phenylalanine or was limited to silylation reagents containing a UV active group.

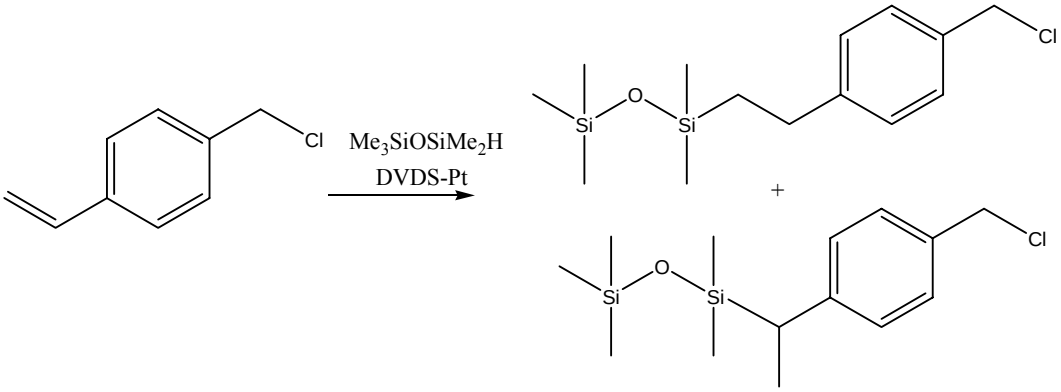


Figure B-2-1: Synthesis of *p*-[1 & 2-(1,1,3,3,3-pentamethyldisiloxane)-ethyl]-benzyl chloride with isomers A & B shown

I began the project using the same synthesis as was used in the siloxylated phase transfer catalyst (PTC) chapter. First, I synthesized *p*-[1 & 2-(1,1,3,3,3-pentamethyldisiloxane)-ethyl]-benzyl chloride from 4-vinylbenzylchloride and pentamethyldisiloxane using platinum(0)-1,3-divinyl-1,1,3,3-tetramethyl

disiloxane complex (3 wt % xylene) catalyst (Figure B-1) by the same procedure that I developed for the PTC project.[6] I reacted the *p*-[1 & 2-(1,1,3,3,3-pentamethyldisiloxane)-ethyl]-benzyl chloride with cysteine (5 equiv) in ethylacetate in the presence of a commercially available PTC, tetra-*n*-butylammonium chloride (10 mol %) at 70ºC for 48 hours (Figure B-2). No reaction was observed so triethylamine (0.1 mL) was added to act as an HCl scavenger and allowed to react for 5 days. I observed a shift in the benzyl CH_2 peak from 4.6 to 5.1 ppm in the ^{1}H NMR. I tried a silica column with using ethyl acetate and hexane which was unsuccessful. I reacted the *p*-[1 & 2-(1,1,3,3,3-pentamethyldisiloxane)-ethyl]-benzyl chloride with lysine (5 equiv) in ethylacetate in the presence of a commercially available PTC, tetra-*n*-butylammonium chloride (5 mol %) at 70ºC for 24 hours (Figure B-3). I observed the same shift in the ^{1}H NMR of the benzyl CH_2 peak. I tried a silica-gel column using ethyl acetate and hexane which was unsuccessful. In addition, I became concerned that the benzyl group and siloxane chain may hinder the volatility of the amino acid because of the large molecular mass. I decided to shift the focus to a procedure in the literature that used distillation for purification and had a lower molecular mass.

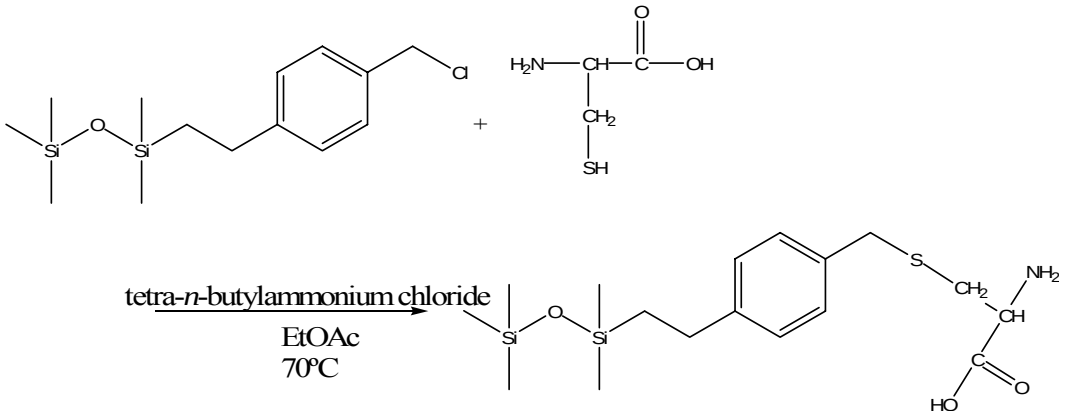


Figure B-2-2: Cysteine + benzylchloride disiloxane

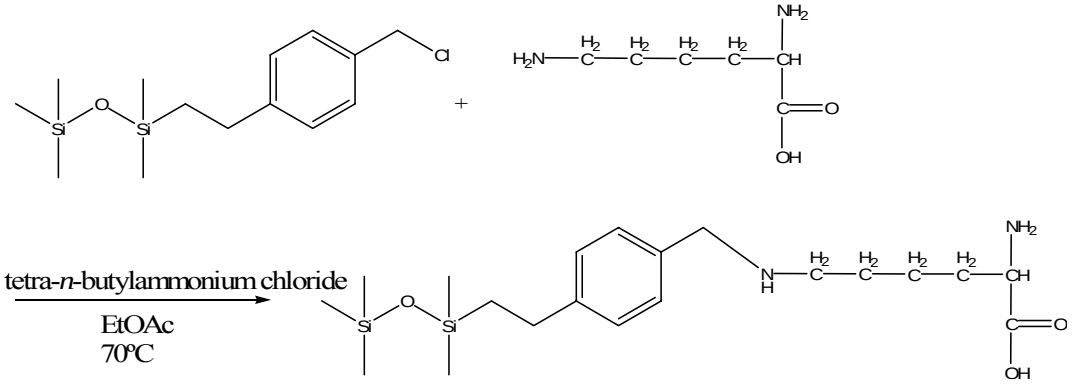


Figure B-2-3: Lysine + benzylchloride disiloxane

I moved on to a modification of a procedure found in the Journal of Organic Chemistry, which involved combining chloro(methyl)diphenyl silane with cyclohexyl-methanol in the presence of imidazole in DMF.[7]

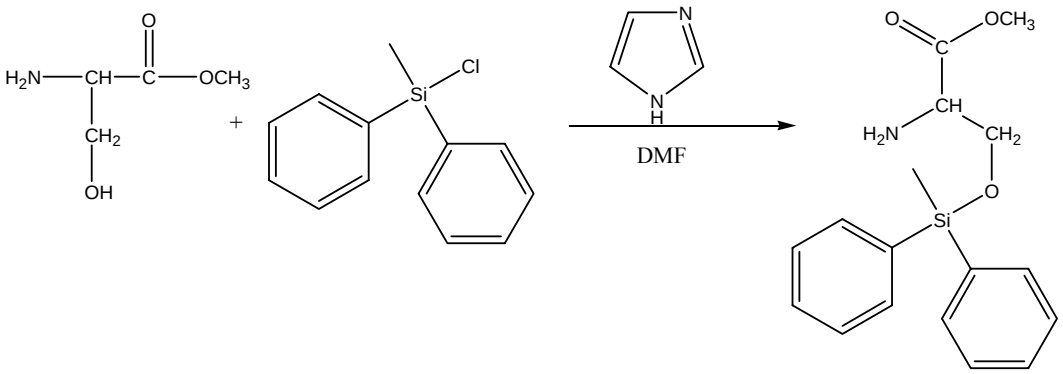


Figure B-2-4: Synthesis of 2-amino-3-(methyl-diphenyl-silanyloxy)-propionic acid

I tried to add chloro(methyl)diphenyl silane (1.1 equiv) to serine methylester hydrochloride (1 equiv) with imidazole (2 equiv) as an HCl scavenger in DMF at room temperature. GC-MS result of the crude product showed 70% conversion. The procedure used distillation to purify the product, however, I was unable to remove all trace amounts of the starting material. The starting material observed in the GC-MS was methyl-diphenyl-silanol which is formed from reaction between the chloro(methyl)diphenyl silane and water during the workup.

I also tried switching the limiting reagent to the chloro(methyl)diphenyl silane (1 equiv) and use an excess of the serine (1.5 equiv) which could be removed by a water wash. However, the GC-MS results still showed starting material, methyl-diphenyl-silanol, so this route was abandoned.

I then decided to change the amino acid to an UV active amino acid and use a lighter, non-UV active silane. I hypothesized that a lighter silane would offer a more facile removal and product purification.

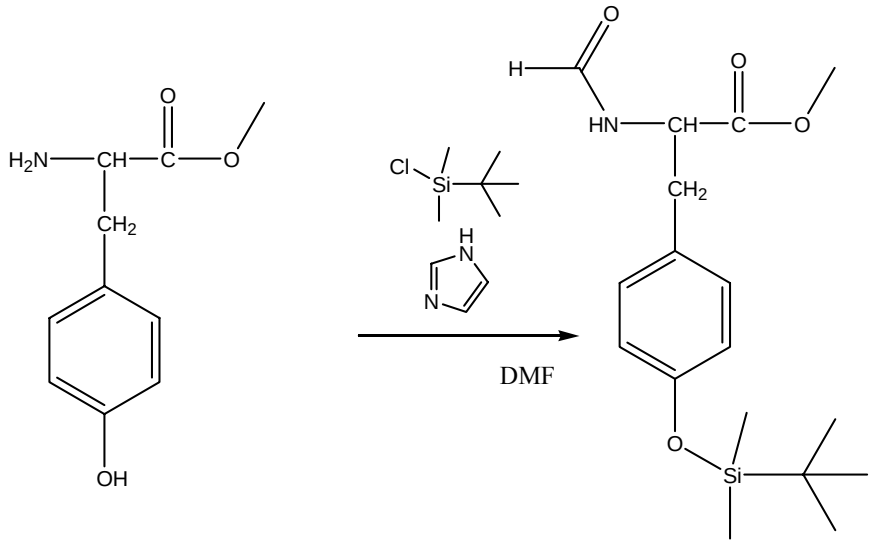


Figure B-2-5: Synthesis of 3-[4-(t-butyl-dimethyl-silanyloxy)-phenyl]-2-formylamino-propionic acid methyl ester

I used chloro(dimethyl)t-butylsilane since it forms a siloxane bond that is stable in water. I reacted L-tyrosine methylester hydrochloride (1 equiv), chloro(dimethyl)t-butylsilane (2.2 equiv), and imidazole (3.3 equiv) in DMF at room temperature for 5 days (Figure B-5). I used a procedure modified from a JOCs paper that reacts chloro(dimethyl)t-butylsilane with *N*-(tert-butoxycarbonyl)-L-tyrosine.[8] The reaction was worked up like the literature procedure but a short silica plug was needed to purify the compound (90/10= CH_2Cl_2/EtOAc to remove the silyl impurity, then a 100% ethylacetate flush to obtain pure product). The tyrosine was found to have reacted with the DMF resulting in a new compound (Figure B-5). The modification of the amine is not expected to be a problem because this should increase the CO_2-philicity and volatility in the SFC by decreasing the hydrogen bonding from the amine.

B.3.2 Rebuilding the Supercritical Fluid Chromatograph (SFC)

There is a SFC in the laboratory, but over the years it has been modified for use as a makeshift analytical instrument in other types of experiments, such as Taylor-Aris Dispersion. The focus of this stage of the project was to rebuild the chromatograph and optimize the conditions for the separation of amino acids. The details of the rebuilding of the SFC are contained in the theses of Michelle Kassner and Stuart Terrett.[9,10]

B.3.3 Preliminary results

Initial injections of small aromatic molecules such as toluene were made into the SFC in an effort to test the ability of the detector and column before the amino acids were introduced. The analytes were detected by the UV-Vis detector, but were also seen on subsequent injections of pure methanol. The prolonged recurrence of these compounds implied that the analyte was strongly retained on the column during the experiments. Multiple injections of methanol were required to clean the column in preparation for another analyte injection. Mixed injections of small aromatic molecules, such as toluene and acetophenone, were performed in an effort to observe a separation. No separation was observed with these molecules, and the issue of the strong retention remained. Because the column was designed for amino acids and peptides, it was postulated that the small size of the molecules being used was one of the underlying issues, and for all subsequent trials amino acids were used exclusively.

B.3.3 Initial injection and analysis of amino acids

Siloxylated tyrosine and neutralized tryptophan were injected into the SFC both alone and as a mixture. Peaks in the UV-Vis spectrum were detected for each compound. In two separate trials, a small separation was seen between the siloxylated tyrosine and tryptophan amino acids that were injected. The peak sizes for both of these compounds were much smaller than expected, implying a lower

solubility of the amino acids in $scCO_2$ than expected. This implied a disparity between the actual pressure through the column and detector and the pressure that was measured by our pressure transducer at the inlet. This led to a closer inspection of the flow path of the SFC.

B.3.4 Further Investigation of SFC Flow Path

On further investigation, a second pressure gauge was added to the flow path at the outlet of the detector. This pressure gauge showed a pressure drop of approximately 1800 psi from the inlet, where the original pressure gauge is located, to the outlet. The outlet pressure of approximately 600 psig under these conditions demonstrated that the CO_2 at the outlet was not supercritical and cast doubts on the exact conditions of the mobile phase both in the detector itself and in the column during the separation. An effort was made to increase the outlet pressure of the mobile phase by increasing the inlet pressure to the ISCO's maximum pressure of 3500 psig and by eliminating as much tubing and as many valves as possible to reduce the pressure drop. These measures increased the outlet pressure to approximately 1000 psig; however, CO_2 must be in excess of 1400 psi to ensure sufficient density to assume that the solutes stay in solution (Figure B-6).[11]

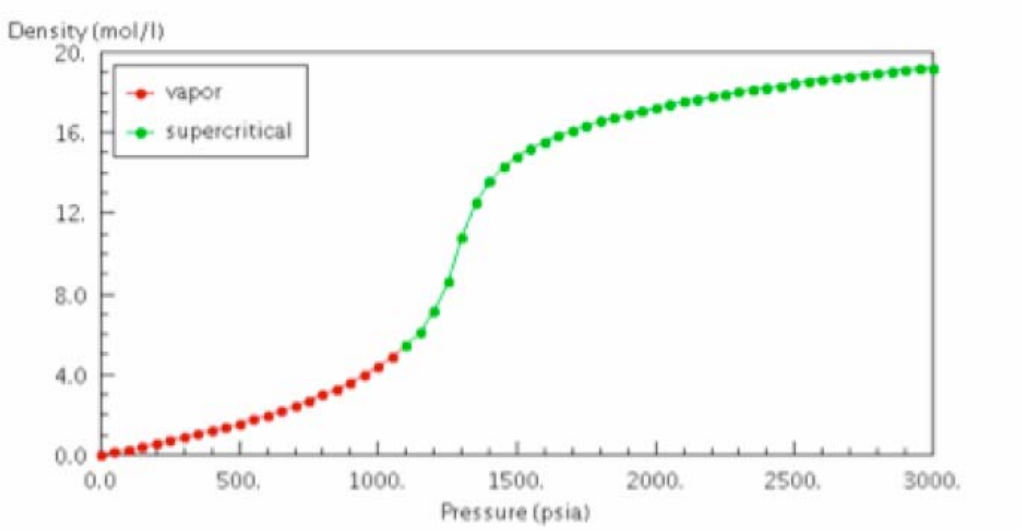


Figure B-2-6: Density and phase behavior of carbon dioxide at 40ºC. [11]

After the outlet pressure increased to 1000 psig, an existing leak in the UV-Vis flow cell became both visibly and audibly present. This leak accounts for a significant amount of the pressure drop, but its location in the window of the cell required extensive repair or the fabrication of a new detector assembly.

B.3.5 Fabricating a new detector

A new detector was fabricated in lab using fiber-optic UV-Vis light sources and detectors. The design was originally created by Dr. Frank Bright, who we have collaborated before. The fiber-optics was mounted in a stainless

steel cross valve capable of withstanding the pressure of $scCO_2$, and the signal was monitored via a software program on a stand-alone computer. The fiber-optic cables was mounted along one axis of the cross valve while the $scCO_2$ and analytes flowed across the other axis. This is diagrammed in Figure B-7.

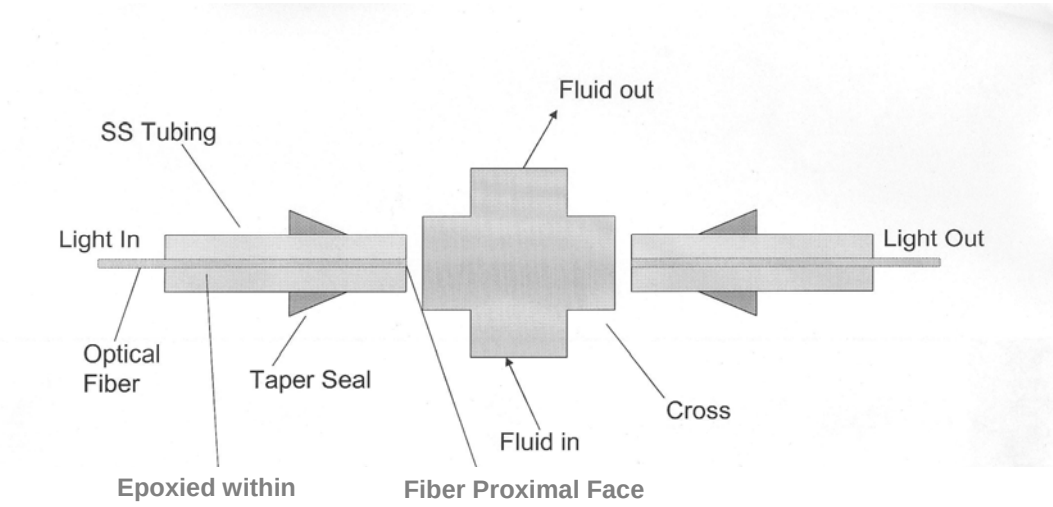


Figure B-2-7: Schematic of the fiber-optic UV-Vis detector apparatus

The actual UV-Vis detector, which was attached to the light out line above to detect the intensity of UV-Vis radiation after it passed through the sample, was provided by Ocean Optics, as was the software used to monitor the intensity.

B.3.6 Injection and analysis of amino acids with new detector

As an initial test of our new detector, I combined L-boc-phenylalanine, siloxylated tyrosine, and tryptophan in methanol (2 mL). Since this was only an initial test to determine if the cell was holding pressure, the mass of the amino acids was not measured. As can be seen in the Figure B-8 below, I was able to see peaks in the UV-Vis spectrum. After obtaining this spectrum, I had several questions to answer: 1) Why did the base line shift upwards for the 215 nm wavelength? 2) What are the concentrations? 3) Can full separation be achieved 4) What is the eluation order?

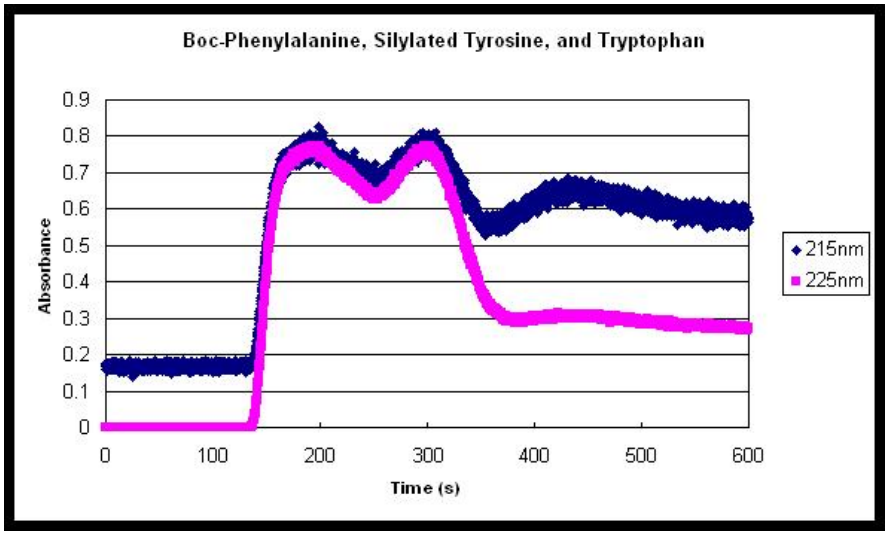


Figure B-2-8: UV-Vis results from injecting L-boc-phenylalanine, siloxylated tyrosine, and tryptophan

I tested the various amino acids separately to determine the elution order and the cause of the upward base line shift. Tryptophan (0.06 g in 1 mL of methanol) was run through the SFC and gave a small peak in the UV-Vis spectrum as can be seen in Figure B-9. L-Boc-phenylalanine (saturated in 1 mL of methanol) was run through the SFC and gave a peak in the UV-Vis spectrum as can be seen in Figure B-10. The peak was not well resolved and a trailing peak could be seen as time continued. The peak in the UV-Vis spectrum took over 600 seconds to return to the base line. The lack of resolution and the upward baseline shift indicated that the L-boc-phenylalanine was "sticking" to the column. I also ran the siloxylated tyrosine (0.07 g in 1 mL of methanol). As can be seen in Figure B-11, the siloxylated tyrosine did not show a UV-Vis peak using only $scCO_2$ but did show a UV-Vis peak with the methanol wash. Since the absorbance was above 0.1, the concentration of the siloxylated tyrosine was reduced by half (0.035 g siloxylated tyrosine in 1 mL of methanol) and run again on the SFC. As can be seen in Figure B-12, the initial injection using $scCO_2$ did not give a UV-Vis peak. However with a methanol wash, a UV-Vis peak was observed. A second methanol wash also showed a UV-Vis peak. These results demonstrated that the siloxylated tyrosine was "sticking" to the column. Since the siloxylated tyrosine came off the column with a methanol wash, this seemed to indicate that a co-solvent was needed and that $scCO_2$ may not solubilize the siloxylated tyrosine sufficiently. Another possibility was that the amino acids

were interacting with the ethylpyridine-bonded silica stationary phase in the column. The UV-Vis spectrum of the L-boc-phenylalanine indicated that either the lack of solubility $scCO_2$ or interaction with the column was also a problem with non-polar amino acids. Due to the lack of solubility of both the modified and non-modified amino acids, it was decided that a co-solvent or additive was necessary to continue with this project. The addition of a co-solvent made the silylation of the amino acids unnecessary because the polar amino acids were adequately soluble in $scCO_2$ and a co-solvent such as methanol.

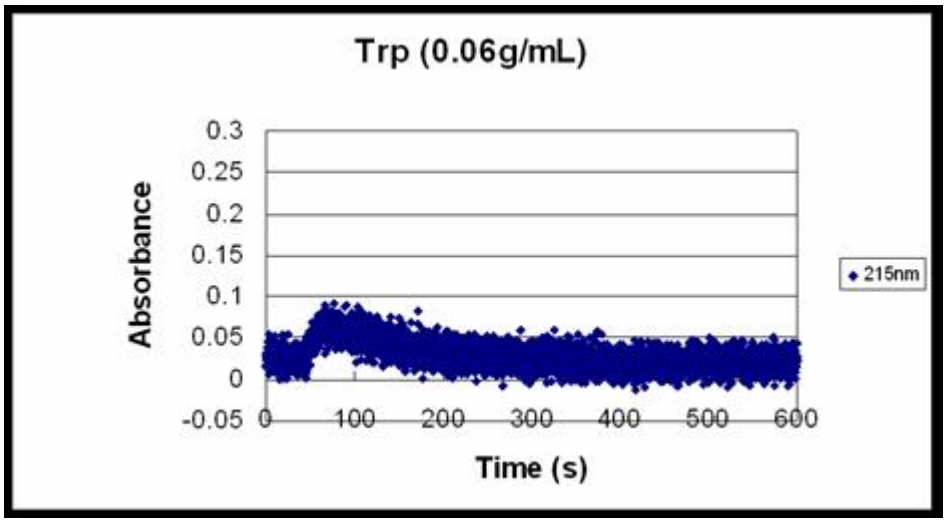


Figure B-2-9: Tryptophan UV-Vis spectrum on SFC

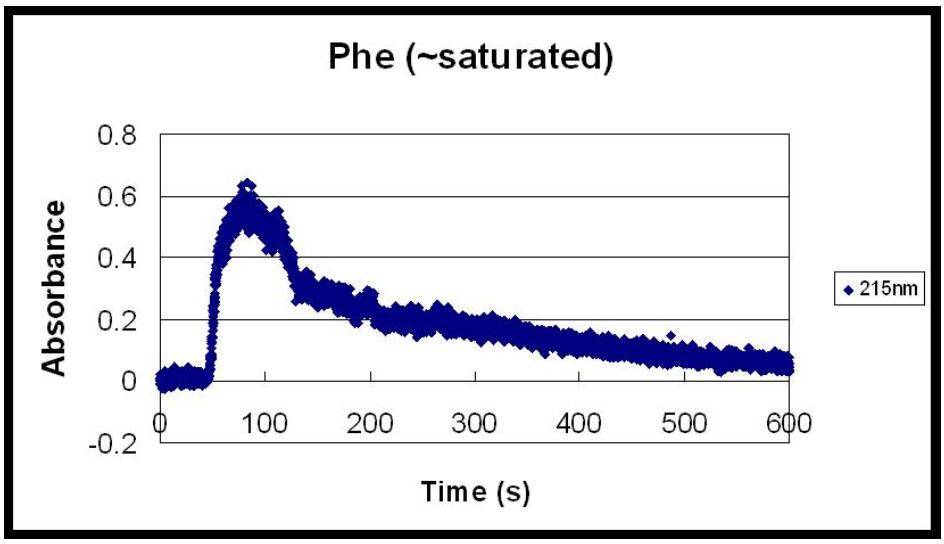


Figure B-2-10: L-Boc-phenylalanine UV-Vis spectrum on SFC

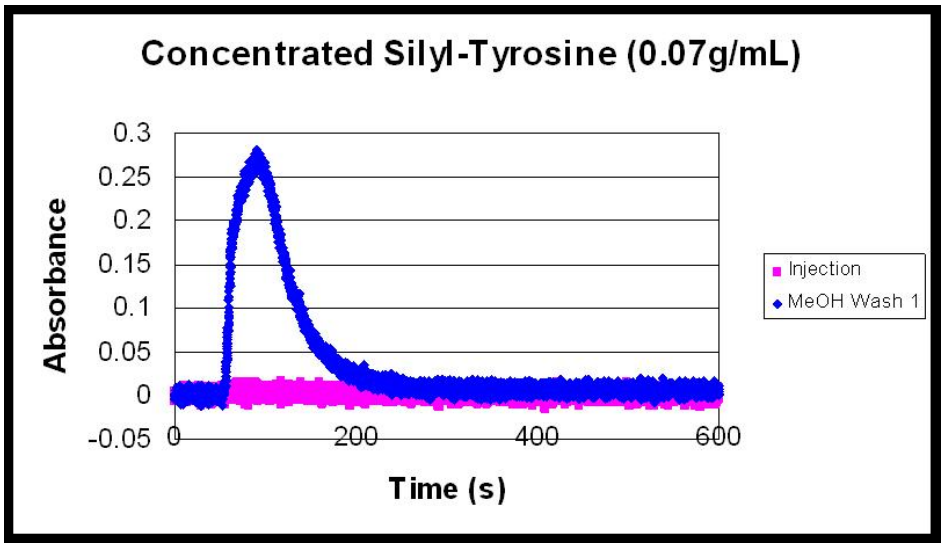


Figure B-2-11: Silyl tyrosine UV-Vis spectrum on SFC

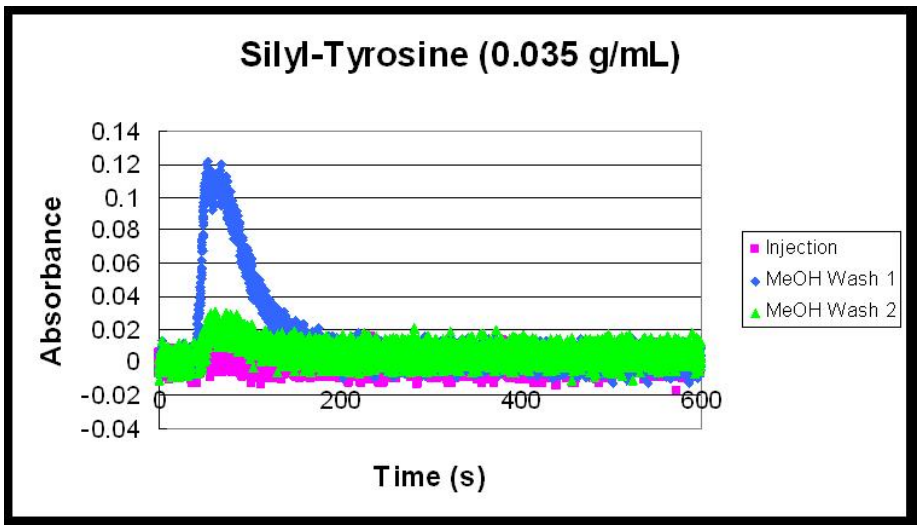


Figure B-12: Silyl-tyrosine (reduced concentration) UV-Vis spectrum on SFC

B.5 Conclusions

In conclusion, a new silylated amino acid was successfully synthesized, purified, and characterized. The amino acid chosen was a tyrosine, allowing for UV detection on the SFC and the silyl group chosen was a *t*-butyl dimethyl to form a relatively stable siloxane bond. The rebuilding of the SFC was also a substantial and successful part of this project. The most important aspect that changed on the SFC was a new detector fabricated in lab using fiber-optic UV-Vis light sources and detectors. After rebuilding the SFC, three amino acids, L-boc-phenylalanine, tryptophan, and siloxylated tyrosine were injected and analyzed. All three amino acids demonstrated a trailing peak in the UV-Vis spectrum which could indicate that the amino acids were "sticking" to the column. This was also demonstrated by injecting methanol after the injection of

the siloxylated tyrosine which resulted in a peak in the UV-Vis spectrum. These results led us to conclude that the system needed a co-solvent or additive to increase the solubility of the amino acids in $scCO_2$. Since the addition of a silyl group to an amino acid was to increase the solubility in $scCO_2$, the need of a co-solvent or additive makes the use of the silyl group redundant. At this point the silylation of amino acids for analysis by SFC was stopped while the project continued to investigate the use of co-solvents for increasing the solubility of amino acids in the mobile phase.

B.6 Experimental

All chemicals were ordered from Aldrich or VWR and used as received, unless noted. ^{1}H and ^{13}C NMR spectra were recorded using a Varian Mercury Vx 400 spectrometer using residual $CDCl_3$ peak as an internal reference. GC-MS analysis was done on a Hewlett-Packard GC 6890/ Hewlett-Packard MS 5973 equipped with a HP-5MS (Agilent, 5% phenyl-methylpolysilane) column or were performed by Georgia Institute of Technology Bioanalytical Mass Spectrometry Facility using a Micromass Quattro LC to perform ESI-MS. Elemental analyses were submitted to Atlantic Microlabs, Inc.

Synthesis of *p*-[1 & 2-(1,1,3,3,3-pentamethyldisiloxane)-ethyl]-benzyl chloride:[6]

The 4-vinyl-benzyl chloride (5.0 g, 0.0337 mol) was added to heptane (20 mL) and put under nitrogen. The mixture was heated to 75ºC. The catalyst platinum(0)-1,3-divinyl-1,1,3,3-tetramethyl disiloxane complex (3 wt% xylene) (DVDS-Pt) (1.7 g, 1 %wt) was added to the solution. The pentamethyl disiloxane (5.75 g, 0.0388mol, 1.15 equiv) in 5 mL of heptane was added slowly. The solution changed from a light yellow to a dark brown upon addition and the addition was stopped whenever the reaction temperature increased by more than 2ºC. After the addition was complete, the temperature was reduced to 70ºC and the reaction was heated for 3 hours. After 3 hours, the reaction was allowed to cool to room temperature and was stirred overnight. To work up the reaction, the heptane was removed under reduced pressure. A column of silica gel in hexane was run and all the fractions combined. The hexane was removed under reduced pressure to give a clear liquid. Yield was 50%.

p-[1 & 2-(1,1,3,3,3-pentamethyldisiloxane)-ethyl]-benzyl chloride: ^{1}H NMR ($CDCl_3$, ppm): 0.1 (15, m), 0.9 (2, m), 1.3 (2, m), 2.3 (2, m), 2.7 (2, m), 4.6 (2, s), 7.2 (4, m). ^{13}C NMR ($CDCl_3$, ppm): 1.516-2.156, 20.39, 29.2533; 46.49, 129.42-127.44, 134.50, 145.56. MS(m/z): 300 (M^+). EA: calculated, C, 55.87%, H, 8.41%. Found, C, 55.82%, H, 8.41%.

Synthesis of 2-amino-3-{4-[2-(1,1,3,3,3-pentamethyl-disiloxanyl)-ethyl]-benzylsulfanyl}-propionic acid:

Cysteine (0.33 g, 5 equiv) and tetra-*n*-butylammonium chloride hydrate (0.014 g, 10 mol%) were added to ethylacetate (3 mL). The solution was put under nitrogen. *p*-[1 & 2-(1,1,3,3,3-Pentamethyldisiloxane)-ethyl]-benzyl chloride (0.3 mL) was added and the solution was heated to 70ºC for 48 hours. The reaction was tested by ^{1}H NMR and no reaction was observed. Triethylamine (0.1 mL) was added as an HCl scavenger and let run 5 more days until reaction was observed to be complete by ^{1}H NMR. To work up the reaction, the excess cysteine was filtered off. The organic phase was washed with water three times and dried over magnesium sulfate. The solvent was removed under reduced pressure. The benzyl CH_2 peak appears to have shifted from 4.6 to 5.1 ppm in the ^{1}H NMR. However, I was unable to isolate the product by a silica gel column using 50/50 EtOAc/Hex and flushing using 100% hexane, 100% ethylacetate, and 100% methanol.

Synthesis of 2-amino-6-{4-[2-(1,1,3,3,3-pentamethyl-disiloxanyl)-ethyl]-benzylamino}-hexanoic acid:

p-[1 & 2-(1,1,3,3,3-Pentamethyldisiloxane)-ethyl]-benzyl chloride (1.2 mL) was added to ethylacetate (12 mL) and put under nitrogen. L-Lysine hydrate (1.6 g, 5 equiv) and tetra-*n*-butylammonium chloride (0.0494 g, 5 mol %) were added. The reaction was heated to 70ºC overnight. To workup the reaction, the lysine

was removed by filtration. The organic phase was washed with water three times and dried over magnesium sulfate. The solvent was removed under reduced pressure. The benzyl CH_2 peak appears to have shifted from 4.6 to 5.1 ppm in the ^1H NMR. However, we were unable to isolate the product by a silica column using a 25/75 hexane and ethylacetate.

Synthesis 2-amino-3-(methyl-diphenyl-silanyloxy)-propionic acid (Figure 6-4):

Serine methylester hydrochloride (0.5 g, 1 equiv) was dissolved in DMF (4 mL). Imidazole (0.4 g, 2 equiv) and chloro(methyl)diphenylsilane (0.8 g, 1.1 equiv) were added. The reaction was put under nitrogen and stirred overnight at room temperature. To work up the reaction, water (25 mL) was added to the reaction. The water was extracted with ether (3 x 50 mL). The ether layers were combined, washed with saturated aqueous NaCl, and dried over magnesium sulfate. The solvent was removed under reduced pressure. GC-MS showed 70% product, 2-amino-3-(methyl-diphenyl-silanyloxy)-propionic acid, (13 min) and 30% starting material, chloro(methyl)diphenylsilane which becomes methyl-diphenyl-silanol after a water wash. The product and starting material mixture were distilled at 250ºC using vacuum. The majority of the starting material was removed from the product but trace amounts of starting material could not be removed.

Different ratios were used of the starting materials to make the chloro(methyl)diphenylsilane the limiting reagent since it was difficult to remove and the excess serine can be removed by a water wash. Serine methylester hydrochloride (0.4 g, 1.5 equiv) was dissolved in DMF (6 mL) and put under nitrogen. Imidazole (0.3 g, 2 equiv) and chloro(methyl)diphenylsilane (0.5 mL, 1 equiv) were added. The reaction was put under nitrogen and stirred overnight at room temperature. To work up the reaction, water (25 mL) was added to the reaction. The water was extracted with ether (3x50 mL). The ether layers were combined, washed with saturated aqueous NaCl, and dried over magnesium sulfate. The solvent was removed under reduced pressure. The GC-MS still showed the starting material, methyl-diphenyl-silanol, so this route was abandoned.

Synthesis of 2-amino-3-(4-trimethylsilanyloxy-phenyl)-propionic acid methyl ester:

L-tyrosine methylester hydrochloride (1.4 g, 1.5 equiv) was dissolved in DMF (6 mL). Imidazole (0.5 g, 2 equiv) and chlorotrimethyl silane (0.5 mL, 1 equiv) were added. The reaction was put under nitrogen and stirred overnight at room temperature. To work up the reaction, water (25 mL) was added to the reaction. The water was extracted with ether (3 x 50 mL). The ether layers were combined, washed with saturated aqueous NaCl, and dried over magnesium

sulfate. The solvent was removed under reduced pressure. Concern over the stability of the siloxane group in water caused us to try a different silane.

Synthesis of 3-[4-(*t*-butyl-dimethyl-silanyloxy)-phenyl]-2-formylamino-propionic acid methyl ester

L-tyrosine methylester hydrochloride (0.5 g, 1 equiv) was dissolved in DMF (10 mL). Chlorodimethyl *t*-butyl silane (0.7 g, 2.2 equiv) and imidazole (0.5 g, 3.3 equiv) were added. The reaction was put under nitrogen and stirred at room temperature for 5 days. The reaction solution turned yellow. To work up the reaction, ether (40 mL) was added and was washed with water (5 x 50 mL). The organic phase was dried over magnesium sulfate and the solvent was removed under reduced pressure. A silica gel plug of 90/10=CH_2Cl_2/EtOAc was used to remove the silyl impurity. A 100% ethylacetate flush of the silica gel plug eluted the pure product, as a yellow oil (32% yield) without optimization.

3-[4-(t-Butyl-dimethyl-silanyloxy)-phenyl]-2-formylamino-propionic acid methyl ester: ^{1}H NMR ($CDCl_3$, ppm): 0.18 (s, 6H), 0.97 (s, 9H), 3.1 (d, 2H), 3.73 (s, 3H), 4.9 (m, 1H), 6.05 (s, 1H), 6.7 (d, 2H), 6.9 (d, 2H), 8.1 (s, 1H). ^{13}C NMR ($CDCl_3$, ppm): -4.46, 18.16, 25.62, 36.99, 51.86, 52.41, 120.19, 127.93, 130.22, 154.88, 160.38, 171.57**.** MS(m/z): 338.0 EA: calculated C, 60.50%, H, 8.06%, N, 4.15%. Found C, 60.37%, H, 8.22%, N, 3.99%.

Experiments on SFC:

The separation column was a SFC 2-ethylpyridine column (150 mm length, 5 μm particle size, 4.6 mm ID) purchased from Princeton Chromatography. L-Boc-phenylalanine, siloxylated tyrosine, and tryptophan (neutralized) were added to methanol (2 mL) and run on the SFC to determine if a UV-Vis spectrum can be obtained. Tryptophan (0.06 g) was added to methanol (1 mL) and run on the SFC to obtain a UV-Vis spectrum. L-Boc-phenylalanine was added to methanol (1 mL) to the saturation point and run on the SFC to obtain a UV-Vis spectrum.

Siloxylated tyrosine (0.07g) was added to methanol (1 mL) and run on the SFC to obtain a UV-Vis spectrum. Siloxylated tyrosine (0.035 g) was added to methanol (1 mL) and run on the SFC to obtain a UV-Vis spectrum. The concentration was reduced by half because the absorbance was higher than the linear range of the UV-Vis detector. Once the absorbance gets over 0.1, it becomes exponential and becomes hard to model.

B.5 References

(1) *Phil. Trans. R. Soc. B* **2006**, *361*, 147-161.
(2) Zheng, J. D., Pinkston, P. H., Zoutendam, Taylor, J., *Anal. Chem.* **2006**, *78*, 1535-1545.
(3) *Science* **1992**, *24*, 162-166.
(4) *Ind. Eng. Chem. Res.* **1996**, *35*, 3644-3652.
(5) Mawhinney US, 1984; Vol. 4,467,037.
(6) US, 1997; Vol. 5,654,374.
(7) *J. Org. Chem* **1987**, *52*, 165-168.

(8) *J. Org. Chem* **1985**, 945-950.
(9) Kassner, M., Georgia Institute of Technology, 2008.
(10) Terret, S., Georgia Institute of Technology, 2008.
(11) Linstrom, P. J., Mallard, W. G., *NIST Chemistry WebBook*; National Institute of Standards and Technology: Gaithersburg, MD 20899, 2005.