ESTERIFICATION OF CARBOXYLIC ACIDS FOR ANALYSIS VIA GAS CHROMATOGRAPHY USING SWELLABLE ORGANICALLY MODIFIED SILICA AS A NANO-REACTOR

by

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Honors Thesis

Appalachian State University

Submitted to the A.R. Smith Department of Chemistry and Fermentation Sciences
and The Honors College
in partial fulfillment of the requirements for the degree of

Bachelor of Science

August, 2019

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Acknowledgements

I would like to take this opportunity to say thank you to the people who made this project possible:

To my parents, thank you for supporting me always, but especially over the last four years. You have listened to me complain about this science more than I would like to admit, and you have always loved me through it.

To my second readers, Dr. Klima and Dr. Culpepper, thank you for agreeing to come on this journey with me.

To The Honors College, thank you for providing me with the platform to do this work and the support needed to finish it.

To the Shaw Research Group, thank you for allowing me to be myself and providing me with a family away from home. I could not have asked to be surrounded by better people during my time at Appalachian State University.

Finally, to Dr. Nicholas Shaw, thank you for trusting me to do this work. We have always known that the science done by the Shaw Research Group is much larger than any of us. As this science continues to grow (and you continue to chase it) know that you are a capable steward for this work and always remember one thing: Drink Water.

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Abstract

Gas chromatography is a popular method for the identification and quantification of organic mixtures. Currently, there are no simple methods for the quantitative analysis of carboxylic acids via gas chromatography. This research proposes an efficient universal method for the derivatization of carboxylic acids to methyl esters in the presence of an acid catalyst by using swellable organically modified silica (SOMS) as a nano-reactor. SOMS forces the esterification reaction toward completion in two ways: 1) by forcing reagents to interact and 2) by removing the water byproduct from the reaction vessel to invoke Le Chatelier's principle. This work has shown that esterification reactions of simple carboxylic acids in SOMS produce quantitative yields, efficiently, without excessive heat or expensive catalysts, making it an ideal choice for the chromatographic analysis of carboxylic acids. The esterification of a representative library of simple carboxylic acids using SOMS, along with spectral data collected from proton nuclear magnetic resonance spectroscopy and a proof of concept experiment using gas chromatography coupled to a flame ionization detector were utilized to validate the hypothesis set forth in this project. Through this endeavor, an experimental procedure was established that will set the groundwork for the eventual optimization and application of this esterification method to more complex molecules.

Chapter 1: Introduction

Gas Chromatography

Gas chromatography (GC) is a commonly used analytical technique that allows for the separation and identification of the compounds within a sample. As with all other chromatographic techniques, GC requires both a mobile phase and a stationary phase in order to achieve separation. In gas chromatography specifically, a sample is volatilized and carried by the mobile phase (an inert gas) through a column containing the stationary phase (which varies in its functionality depending on the type of column). The retention time of each component of the sample is dependent upon the strength of its interaction with the stationary phase and the oven parameters utilized in the experiment.[1] After traveling through the column, each component of the sample is registered by a detector. In the case of gas chromatography using a flame ionization detector (GC-FID), each of the compounds of interest is detected in the form of ions, which are created when the sample is combusted in a hydrogen flame. Since GC-FID requires the combustion of each sample component of interest, it is a method that is best suited for organic analytes.[1] Unfortunately, gas chromatography is not a suitable analytical technique for all organic molecules of interest. The majority of carboxylic acids are insufficiently volatile for analysis by GC, making them especially difficult to identify and quantify in samples.[2]

Historically, there have been a number of proposed solutions to this problem, each with their own unique shortcomings. The first possible solution is improving the stationary phase and/or column of the gas chromatogram. Although a few notable columns (including FFAP, OV-351, and SP-1000) with exceptionally polar stationary phases (polyethylene glycol esters) have been developed for the separation of short and medium chain underivatized aliphatic acids, the high polarities and boiling/volatilization points of most carboxylic acids (especially those

containing additional polar substituents) makes them unsuitable for any type of gas chromatography that is currently available.[2,3]

The second possible solution is derivatization, or converting the carboxyl group into a less polar substituent in order to improve the chromatographic properties of the molecule. The most popular derivatization for the purpose of gas chromatography is Fischer Esterification. Unfortunately, traditional methods of direct esterification, such as acid catalysis and more recently enzyme catalysis (Lesczak and Tran-minh), are not quantitative and can have yields as low as 4.4%.[4] This is a regrettable result, considering the fact that gas chromatography can be used to quantify the amount of each substance in a sample. If the carboxylic acid cannot be completely converted into an ester derivative, then GC can simply not be used for quantitative analysis. Additionally, the methods of direct esterification which do report quantitative yields require difficult to maintain conditions, including high temperatures, microwave irradiation (Hamzah *et al.*), and temperamental zeolites (Kirumakki *et al*.), making them less than ideal for most industrial and research applications.[5,6]

Traditional Organic Synthesis: Limitations and Proposed Solutions

Traditional methods of organic synthesis have scarcely changed since the initiation of the field. A synthetic organic chemist seeking to synthesize a compound must first combine the required reagents in a flask or reactor in the presence of a solvent. Molecular reactivity is thus dependent upon the diluted reactants colliding with sufficient velocity and in the correct three-dimensional orientation to produce the desired product. Although this method can be improved by heating, mixing, and/or adding catalysts, molecular reactivity is rarely achieved in an efficient timeframe or with a reliable yield.[7] Recent research in the field of molecular reactivity has yielded a few novel solutions to the age old problem of low synthetic yields. For instance, micro-

fluidic devices (Bogdan *et al*.) have been shown to increase the probability of successful collisions between reagents by using micro-liter reactors as a vessel for organic synthesis.[8] Additionally, solid phase catalysts such as a porous phenolsulphonic acid-formaldehyde resin (PAFR) (Baek *et al.*) have been shown to notably increase the esterification yields of alkyl chain and cyclohexyl carboxylic acids.[9]

Organic Synthesis Using Swellable Organically Modified Silicas

Since the collision of reagents is integral to molecular reactivity, restricting the reagents to a micro sized "reactor" forces them to collide and therefore provides a more efficient method of synthesis. Swellable organically modified silica (SOMS) selectively restricts organic reactants to "nano-reactors" that are suitable for organic synthesis. SOMS are generated from the poly-condensation reaction of bis(trimethoxysilylethyl)-benzene.[10] The SOMS structure (Figure 1.1) contains bridged silanes functionalized by an aromatic group which is covalently bound to the silicon center by way of rotationally flexible methylene or ethylene groups. The swellable nature of SOMS, at least in part, comes from the interconnected organosilicate structures that become crosslinked during the sol-gel process. Chemical modification of the unreacted silanol groups (SiOH) induces molecular order within the cross-linked structure that ultimately affords the SOMS nano-reactor matrix capable of encapsulating organic molecules.

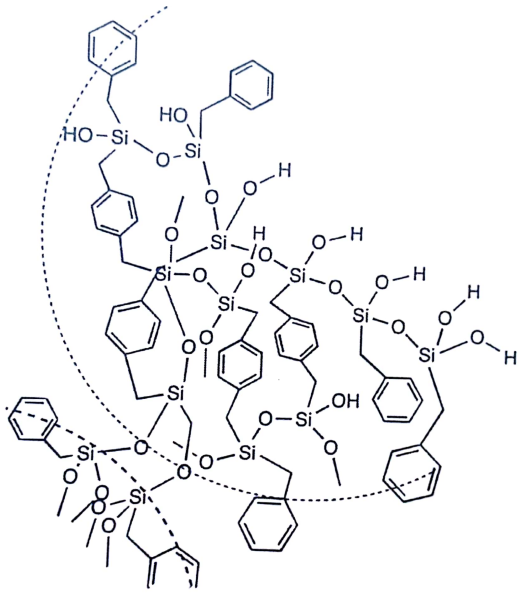


Figure 1.1: *Schematic depiction of the SOMS nano-reactor cross linked structure capable of encapsulating organic molecule*[10].

SOMS have three distinct characteristics that make them ideal nano-reactors for organic synthesis. First, SOMS nano-reactors can swell up to eight times their dry weight, allowing for the encapsulation of large organic reagents. Second, the porous organophilic inner matrix absorbs organic molecules, allowing for synthesis reagents to become trapped together. Finally, the hydrophobicity of the outer surface of SOMS prevents water from migrating inside of the inner cavities and forces any water produced during a reaction out of the reaction vessel.[10,11]

Precedence for the Use of Swellable Organically Modified Silicas in Esterification Reactions

Previous work in the Shaw Research Group, conducted at The University of Wooster, has yielded a general process for the esterification of carboxylic acid using SOMS as a nano-reactor (Figure 1.2). First, the carboxylic acid is dissolved in a suitable organic solvent and the solution is introduced to SOMS. The organic molecules induce mechanical expansion of the nano-reactors, allowing the carboxylic acid and solvent to migrate inside (open SOMS). Rotary evaporation of the solvent causes the matrix of the SOMS to collapse (close SOMS), effectively

trapping the carboxylic acid. Introducing an alcohol to the SOMS (containing carboxylic acid) allows it to reopen as the alcohol migrates inside of the reaction vessel. Since the alcohol acts as both a reagent and a solvent, the excess can be evaporated off while simultaneously forcing some molecules inside of the SOMS with the carboxylic acid. This encapsulation forces the two reagents to interact, effectively esterifying the carboxylic acid. The desired product can then be flushed from the SOMS with excess organic solvent, which can then be removed with a rotary evaporator to collect only the esterified product.[12]

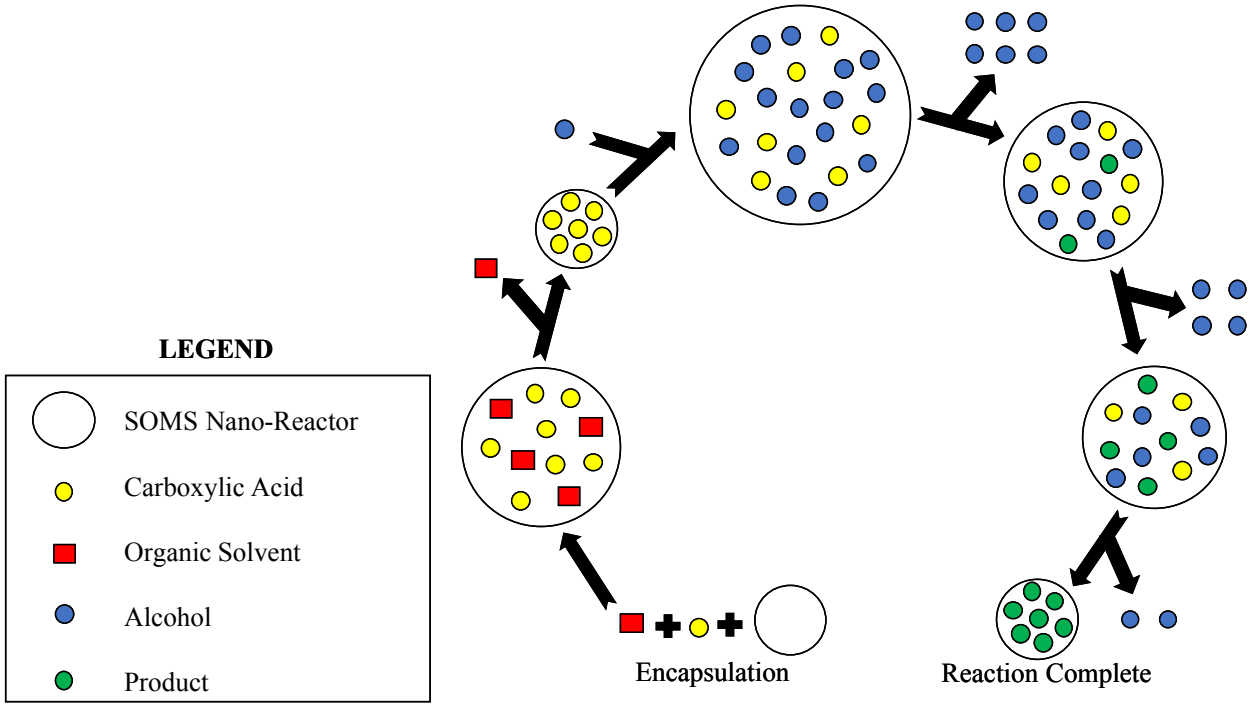


Figure 1.2: *Schematic diagram representing the encapsulation of carboxylic acid and methanol in SOMS in order to produce the desired product*

Preliminary experiments conducted by Hannah Huston at the College of Wooster have indicated that an acid catalyst is required for the esterification of carboxylic acids in SOMS.[12] In the esterification reaction of a carboxylic acid, the attacking alcohol is not a strong nucleophile. In this case, it becomes necessary to add a proton source to the reaction environment, allowing the carbonyl carbon of the carboxylic acid to become a stronger electrophile (Figure 1.3).

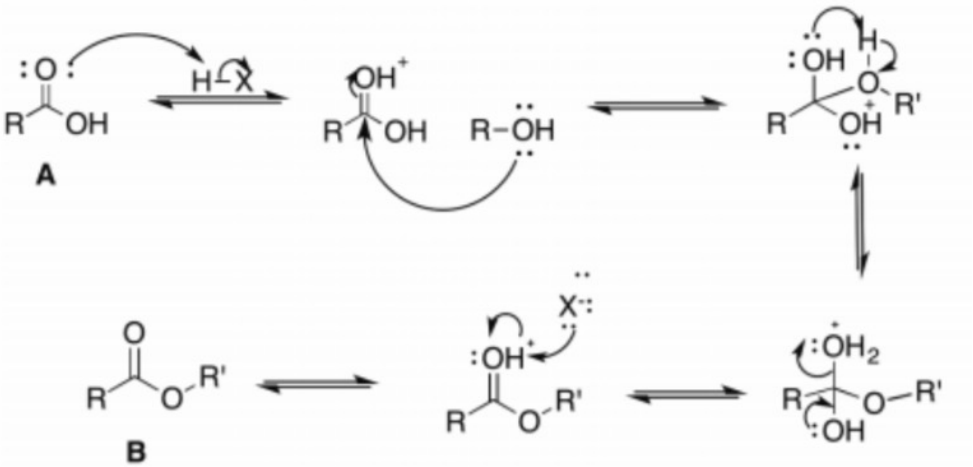


Figure 1.3: *Acid Catalyzed Esterification*[10]

Since SOMS is hydrophobic, and the water byproduct of the acid catalyzed esterification will be forced out of the reaction vessel, Le Chatelier's principle indicates that the reaction will continuously proceed toward the products until the reaction is complete. This principle is supported by preliminary experiments, once again conducted by Hannah Huston, in which pivalic acid, benzoic acid, and 4-methoxybenzoic acid were individually reacted with methanol in the presence of 4 N HCl in dioxane and yielded 100% conversion to their respective methyl esters.[12]

Research Objectives

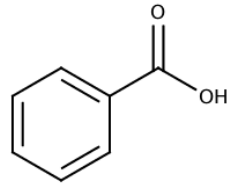
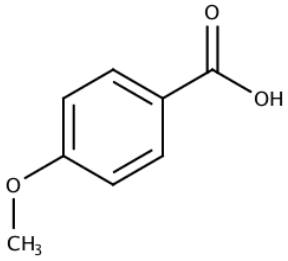
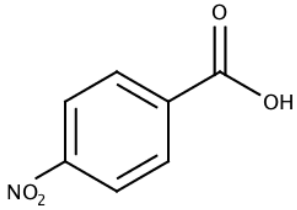
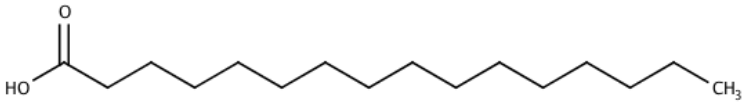
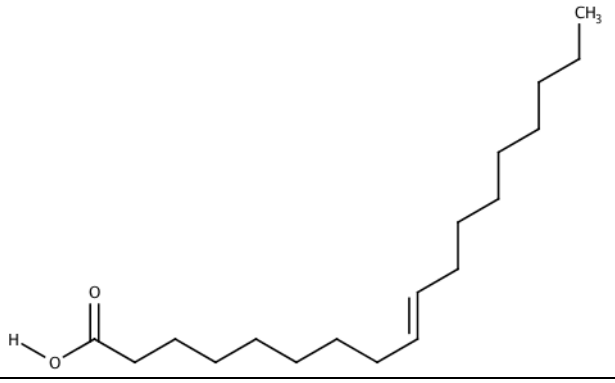
The ultimate goal of this research was to create a general procedure for the esterification of carboxylic acids using SOMS as a nano-reactor. This method, which should theoretically produce 100% conversion of each carboxylic acid, could then be used as a possible method of derivatization for the quantitative analysis of carboxylic acid samples via gas chromatography. Although a quantitative study of derivative carboxylic acids by gas chromatography is included in this research, the primary goal of this work was to set the organic synthetic groundwork for the quantitative conversion of carboxylic acids into their corresponding methyl esters using swellable organically modified silicas as a nano-reactor.

Chapter 2: The Use of Swellable Organically Modified Silicas in Esterification Reactions

General Procedure

For this work, five simple carboxylic acids were reacted individually with methanol in the presence of an acid catalyst in order to obtain the corresponding methyl esters. The library of simple carboxylic acids selected for this project are listed in Table 2.1.

Table 2.1. *Library of Simple Carboxylic Acids*

Type	Name	Structure
Aromatic	Benzoic Acid	
	4-Methoxybenzoic Acid	
	4-Nitrobenzoic Acid	
Aliphatic	Palmitic Acid	
	Oleic Acid	

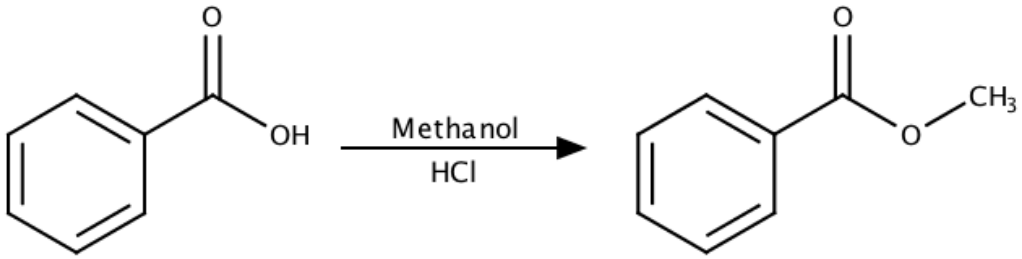
The conversion of each carboxylic acid to its corresponding methyl ester was completed using SOMS as a nano-reactor. A hotplate and Radley's™ Findenser™ were used as a "Flexing Station", to ensure the repeated removal and reintroduction of methanol to the SOMS. Dichloromethane was used for the encapsulation of each of the solid carboxylic acids in SOMS (i.e. palmitic acid, benzoic acid, 4-methoxybenzoic acid, and 4-nitrobenzoic acid). For the purpose of simplicity, a general procedure for the esterification of a solid carboxylic acid on a one-gram scale using SOMS as a nano-reactor is as follows:

In a 100-mL round bottom flask approximately 1 gram of a carboxylic acid dissolved in dichloromethane (20 mL) was added to approximately 5 grams of SOMS. The dichloromethane was then removed using a rotary evaporator (485 mbar, 40 °C) in order to encapsulate the carboxylic acid in the SOMS. An additional 10 mL of dichloromethane was then used to ensure a quantitative transfer of the carboxylic acid into round bottom flask. This additional volume of dichloromethane was also removed by pressure dependent evaporation. To the dried SOMS (containing the carboxylic acid), 8.00×10^2 μL of 3 N HCl in methanol was added in a dropwise fashion, followed by an excess of methanol (approximately 7.5 mL), until the SOMS had expanded, but was not visibly oversaturated. The round bottom flask was then placed on a flexing station (65 °C), which allowed the methanol to repeatedly evaporate and condense (effectively opening and closing the SOMS nano-reactors) for 24 hours. Upon completion of the 24-hour period, the product was removed from the SOMS by vacuum filtration using an excess of methanol. The product was then collected by removing the methanol through rotary evaporation (115 mbar, 40 °C). Percent yield was determined for each carboxylic acid by mass, and a ^{1}H-NMR spectrum was collected using a 300 MHz Varian Gemini 2000 to confirm product formation.

The remainder of this chapter will discuss the specific synthetic details of each experiment conducted for the five of the carboxylic acids of interest in this project. Unless these details are of interest to the reader, the general description of the process on a one-gram scale is sufficient, and the reader may wish to advance to Chapter 3 of this work.

Conversion of Benzoic Acid to Methyl Benzoate

i. Reaction Scheme



Scheme 2.1: *Acid Catalyzed Esterification of Benzoic Acid*

ii. Procedure: Small Scale

Before running esterification reactions on a one-gram scale, a small scale proof of concept experiment was run with benzoic acid. The procedure for this reaction was as follows: To a 50-mL round bottom flask containing SOMS (500 mg) was added 50 mg of benzoic acid dissolved in dichloromethane (20 mL). The dichloromethane was then removed from the mixture by rotary evaporation (485 mbar, 40 °C) in order to encapsulate the benzoic acid in the SOMS. To the dried SOMS (containing benzoic acid), 1.10×10^2 µL of 3 N HCl in methanol was added in a dropwise fashion, followed by an excess of methanol (1.5 mL). The round bottom flask was placed on a flexing station (65 °C), which allowed the methanol to repeatedly evaporate and condense (effectively opening and closing the SOMS nano-reactors) for 24 hours. Upon the completion of the 24-hour period, the product was removed from the SOMS by vacuum filtration using an excess of methanol (200 mL). The product was then collected by removing the

methanol through rotary evaporation (115 mbar, 40 °C). A proton NMR was collect to ensure complete conversion of the carboxylic acid into the methyl ester. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.05 (d, 2H), δ 7.54 (t, 1 H), δ 7.43 (t, 2 H), δ 3.91 (s, 3H)

iii. Results: Small Scale

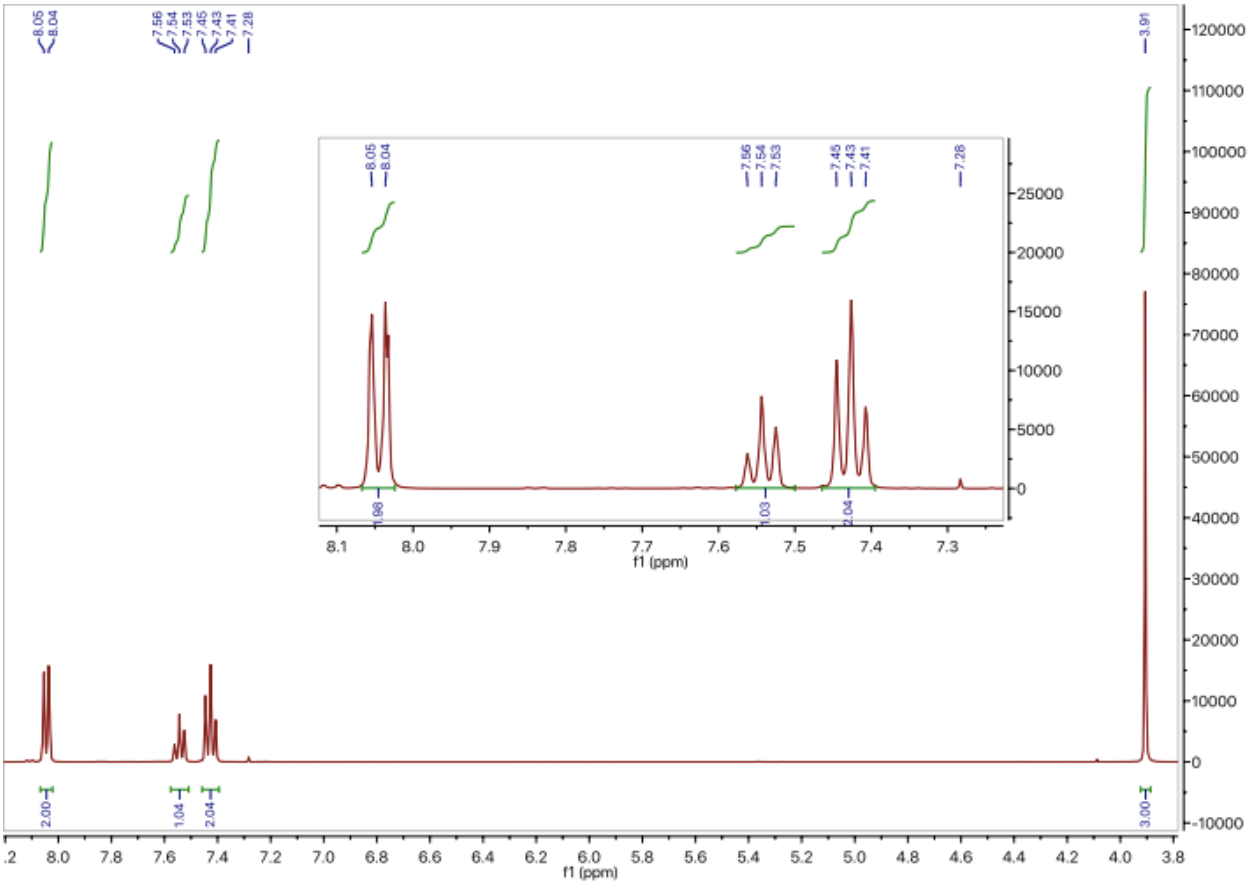


Figure 2.1: *^{1}H-NMR of Methyl Benzoate Product*

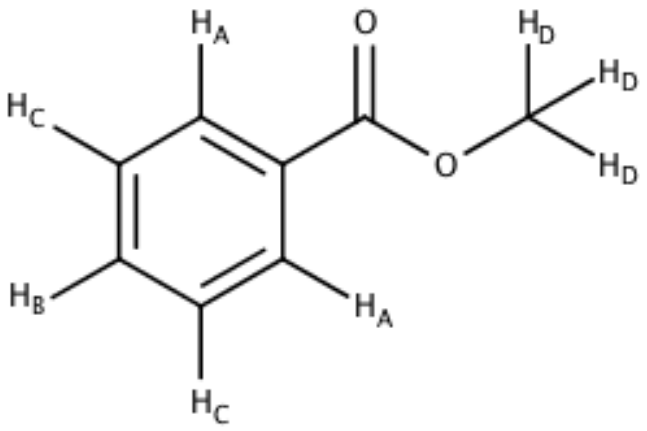


Figure 2.2: *Structure of Methyl Benzoate*

Table 2.2: *Proton Assignment for ^{1}H-NMR of Methyl Benzoate Product*

Assignment (H_x)	Chemical Shift (δ, ppm)	Splitting	Relative Integration	Observed Integration
A	8.05	doublet	2	2.00
B	7.54	triplet	1	1.04
C	7.43	triplet	2	2.04
D	3.91	singlet	3	3.00

The ^{1}H-NMR spectrum of the product which resulted from the esterification of benzoic acid using SOMS as a nano-reactor exhibited four unique peaks (Figure 2.1), three of which fell in the aromatic region. The most downfield of the peaks is a doublet with an integration of two at approximately 8.05 ppm. This peak represents two chemically equivalent hydrogens, each or which having one neighbor. These two hydrogen atoms are bound to the aromatic carbons that are closest to the only substituent on the aromatic ring in benzoic acid (i.e. H_A, Figure 2.2). The next peak, a triplet with an integration of one, occurred at 7.54 ppm. This peak represents the single hydrogen on the aromatic carbon directly across from the only substituent on the ring (i.e. H_B). Finally, a triplet with an integration of two appears 7.43 ppm. This peak represents the final two chemically equivalent hydrogens bound to the aromatic ring (i.e. H_c). Another peak, a singlet with an integration of three located at 3.91 ppm, represents the three hydrogens of the

newly formed methyl ester and is the definitive indictor that the desired product (methyl benzoate) has been formed. In addition to the previously assigned peaks, the solvent used in this analysis, $CDCl_3$, also displays a singlet at 7.28 ppm. A summary of the ^{1}H-NMR data for the product are given in Table 2.2.

iv. Discussion: Small Scale

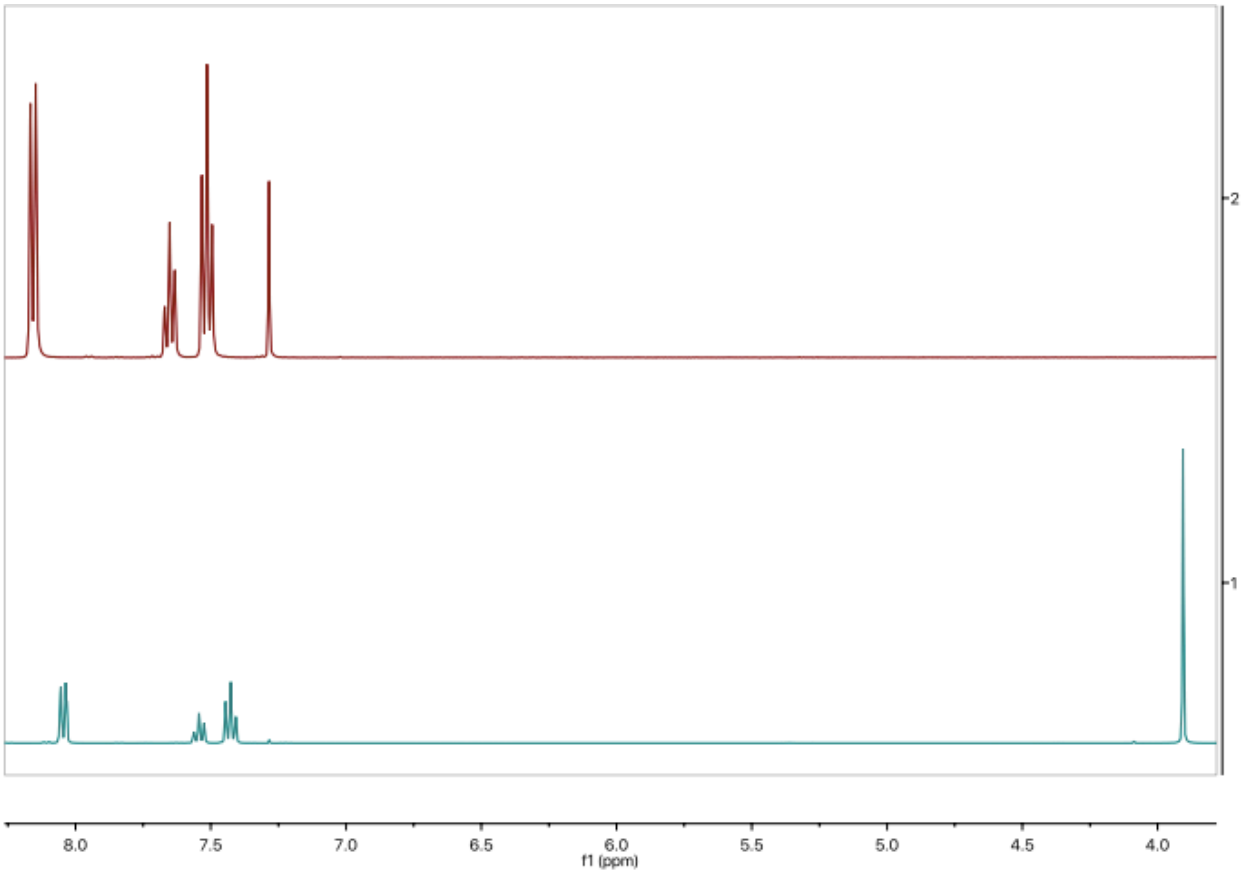


Figure 2.3: *Stacked ^{1}H-NMR of Benzoic Acid Starting Material (Top) and Methyl Benzoate Product (Bottom)*

Before benzoic acid has reacted with methanol to form methyl benzoate, the only peaks in the ^{1}H-NMR spectrum of the starting material can be found in the aromatic region (Figure 2.3, top). Once methyl benzoate has formed, a peak is observed further upfield, at 3.91 ppm (Figure 2.3, bottom). In this reaction, the ^{1}H-NMR spectrum provides two clues to suggest that complete conversion of the carboxylic acid into the methyl ester has occurred. First, the aromatic region in

the ^{1}H-NMR spectrum of the product exhibits clean and distinct peaks. Since the protons bound to the aromatic carbons of benzoic acid and methyl benzoate exist in slightly different chemical environments, the corresponding peaks between the two molecules occur at slightly different ppm values. If some of the starting material still existed in the product, its spectrum would exhibit messy, overlapping peaks in the aromatic region. Second, the peak representing the three hydrogens of the methyl ester in the product has a relative integration of three when compared to the known integrations of the aromatic region. If starting material still existed in the product, the relative integrations of the aromatic region would not correspond to the relative integration of the methyl ester peak in this clean ratio.

v. Procedure: One Gram Scale

In a 100-mL round bottom flask approximately 1.0042 grams of benzoic acid dissolved in dichloromethane (20 mL) was added to 5.0114 grams of SOMS. The dichloromethane was then removed using a rotary evaporator (485 mbar, 40 °C) in order to encapsulate the carboxylic acid in the SOMS. An additional 10 mL of dichloromethane was then used to ensure a quantitative transfer of the carboxylic acid into round bottom flask. This additional volume of dichloromethane was also removed by pressure dependent evaporation. To the dried SOMS (containing the carboxylic acid), 8.00 x 10^{2} μL of 3 N HCl in methanol were added in a dropwise fashion, followed by an excess of methanol (7.00 mL), until the SOMS had expanded, but was not visibly oversaturated. The round bottom was then placed on a flexing station (65 °C), which allowed the methanol to repeatedly evaporate and condense (effectively opening and closing the SOMS nano-reactors) for 24 hours. Upon completion of the 24-hour period, the resulting product was removed from the SOMS by vacuum filtration using 350 mL of methanol. The product

(1.1135 g) was then collected by removing the methanol through rotary evaporation (115 mbar, 40 °C). ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.05 (d, 2H), δ 7.54 (t, 1 H), δ 7.43 (t, 2 H), δ 3.91 (s, 3H)

vi. Results: One Gram Scale

Theoretical Yield (1:1)

$$1.0042 \text{ g Benzoic Acid} \times \frac{1 \text{ mole Benzoic Acid}}{122.12 \text{ g Benzoic Acid}} = 0.0082231 \text{ mol Benzoic Acid}$$

$$0.0082231 \text{ mol} \times \frac{136.15 \text{ g Methyl Benzoate}}{1 \text{ mol Methyl Benzoate}} = 1.1196 \text{ g Methyl Benzoate}$$

Percent Yield

$$\frac{1.1135 \text{ g Methyl Benzoate}}{1.1196 \text{ g Methyl Benzoate}} \times 100\% = 99.5\%$$

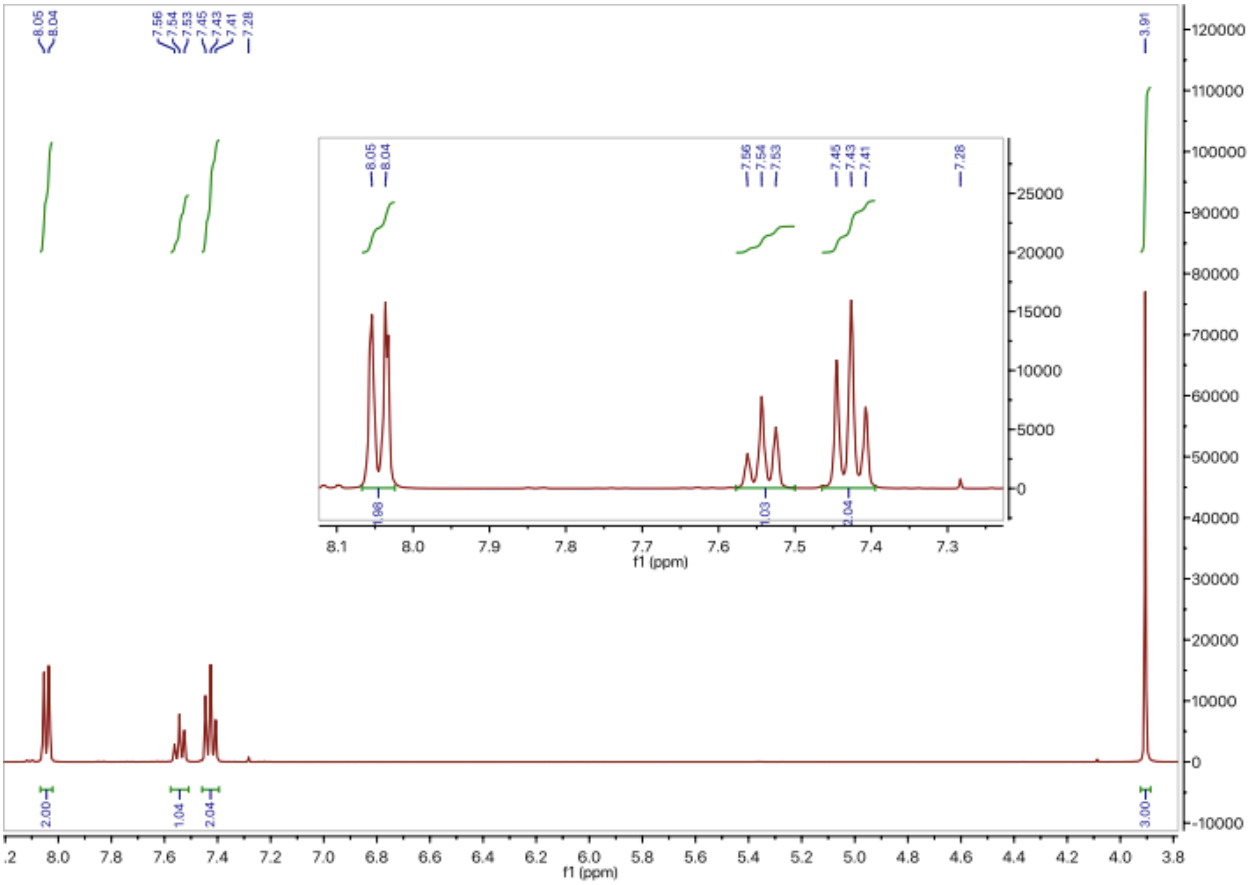


Figure 2.4: *^{1}H-NMR of Methyl Benzoate Product*

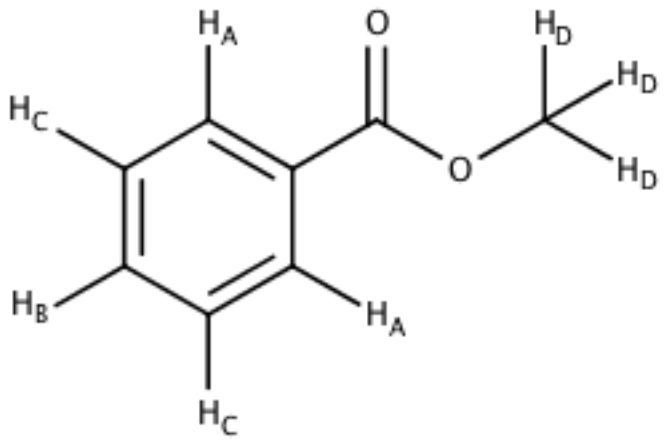


Figure 2.5: *Structure of Methyl Benzoate*

Table 2.3: *Proton Assignment for ^{1}H-NMR of Methyl Benzoate Product*

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The ^{1}H-NMR spectrum of the product which resulted from the esterification of benzoic acid using SOMS as a nano-reactor exhibited four unique peaks (Figure 2.4), three of which fell in the aromatic region. The most downfield of the peaks is a doublet with an integration of two at approximately 8.05 ppm. This peak represents two chemically equivalent hydrogens, each or which having one neighbor. These two hydrogen atoms are bound to the aromatic carbons that are closest to the only substituent on the aromatic ring in benzoic acid (i.e. H_A, Figure 2.5). The next peak, a triplet with an integration of one, occurred at 7.54 ppm. This peak represents the single hydrogen on the aromatic carbon directly across from the only substituent on the ring (i.e. H_B). Finally, a triplet with an integration of two appears 7.43 ppm. This peak represents the final two chemically equivalent hydrogens bound to the aromatic ring (i.e. H_c). Another peak, a singlet with an integration of three located at 3.91 ppm, represents the three hydrogens of the

newly formed methyl ester and is the definitive indictor that the desired product (methyl benzoate) has been formed. In addition to the previously assigned peaks, the solvent used in this analysis, $CDCl_3$ also displays a singlet at 7.28 ppm. A summary of the ^{1}H-NMR data for the product are given in Table 2.3.

vii. Discussion: One Gram Scale

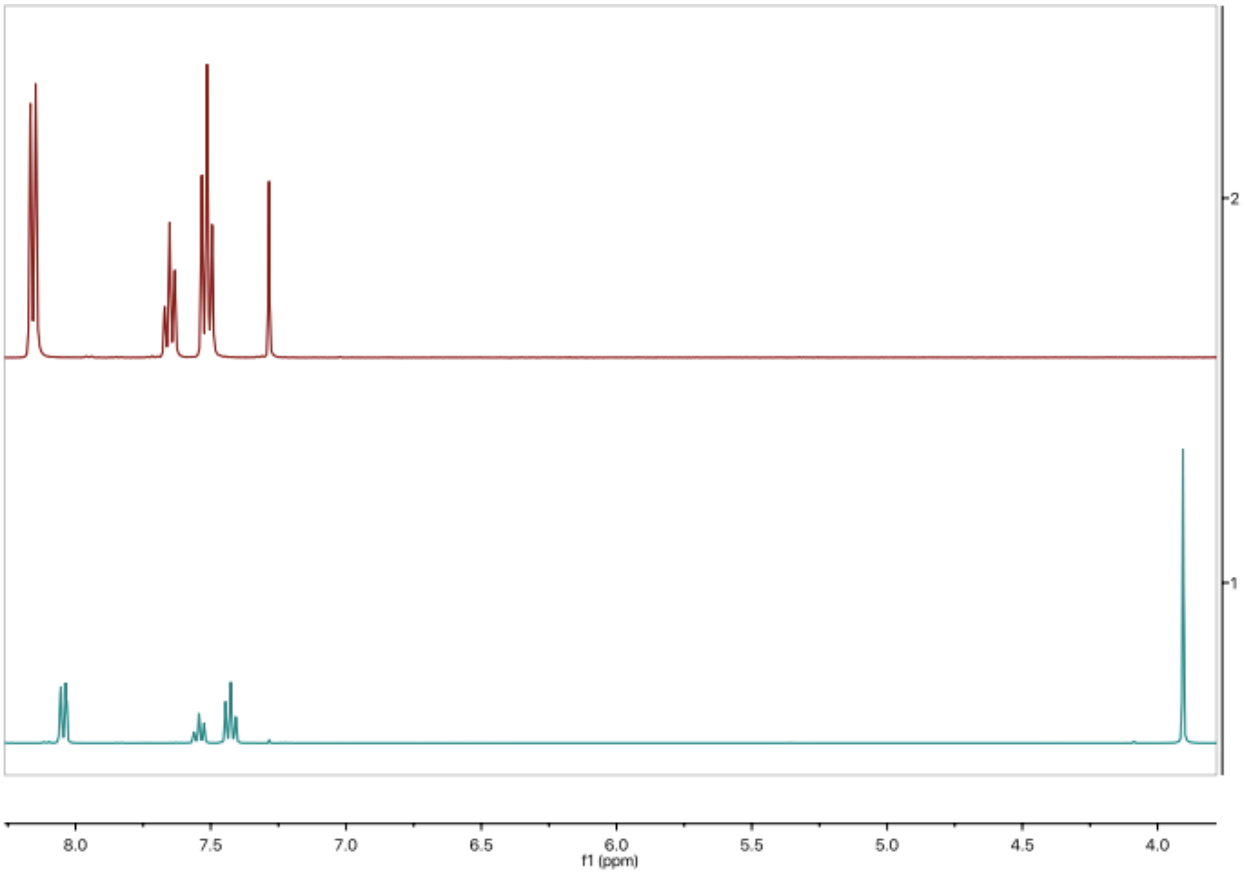


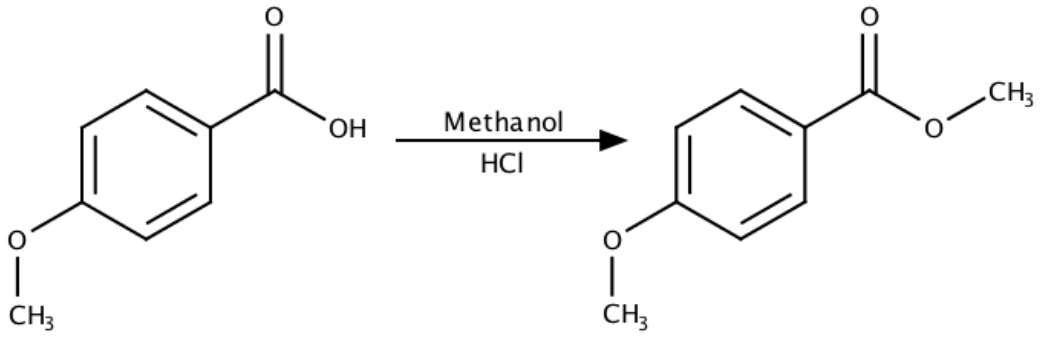
Figure 2.6: *Stacked ^{1}H-NMR of Benzoic Acid Starting Material (Top) and Methyl Benzoate Product (Bottom)*

As in the small scale proof of concept reaction, before benzoic acid has reacted with methanol to form methyl benzoate, the only peaks in the ^{1}H-NMR spectrum of the starting material can be found in the aromatic region (Figure 2.6, top). Once methyl benzoate has formed, a peak can be seen further upfield, at 3.91 ppm (Figure 2.6, bottom). In this reaction, the ^{1}H-NMR spectrum provides two clues to suggest that complete conversion of the carboxylic acid

into the methyl ester has occurred. First, the aromatic region in the ^{1}H-NMR spectrum of the product exhibits clean and distinct peaks. Since the protons bound to the aromatic carbons of benzoic acid and methyl benzoate exist in slightly different chemical environments, the corresponding peaks between the two molecules occur at slightly different ppm values. If some of the starting material still existed in the product, its spectrum would exhibit messy, overlapping peaks in the aromatic region. Second, the peak representing the three hydrogens of the methyl ester in the product has a relative integration of three when compared to the known integrations of the aromatic region. If starting material still existed in the product, the relative integrations of the aromatic region would not correspond to the relative integration of the methyl ester peak in this clean ratio. Since the ^{1}H-NMR data suggests that the methyl ester product is pure, the calculated percent yield of 99.5% becomes noteworthy, suggesting a quantitative conversion in this reaction.

Conversion of 4-Methoxybenzoic Acid into Methyl 4-Methyoxybenzoate

i. Reaction Scheme



Scheme 2.2: *Acid Catalyzed Esterification of 4-Methoxybenzoic Acid*

ii. Procedure: One Gram Scale

In a 100-mL round bottom flask 1.0028 grams of 4-methoxybenzoic acid dissolved in dichloromethane (20 mL) was added to 5.0023 grams of SOMS. The dichloromethane was then

removed using a rotary evaporator (485 mbar, 40 °C) in order to encapsulate the 4-methoxybenzoic acid in the SOMS. An additional 10 mL of dichloromethane was then used to ensure a quantitative transfer of the carboxylic acid into round bottom flask. This additional volume of dichloromethane was also removed by pressure dependent evaporation. To the dried SOMS (containing the 4-methoxybenzoic acid), 8.00 x 10^2 μL of 3 N HCl in methanol were added in a dropwise fashion, followed by an excess of methanol (6.50 mL), until the SOMS had expanded, but was not visibly oversaturated. The round bottom was then placed on a flexing station (65 °C), which allowed the methanol to repeatedly evaporate and condense (effectively opening and closing the SOMS nano-reactors) for 24 hours. Upon completion of the 24-hour period, the resulting product was removed from the SOMS by vacuum filtration using an excess of methanol (350 mL). The product (1.0842 g) was then collected by removing the methanol through rotary evaporation (115 mbar, 40 °C). ^{1}HNMR (400 MHz, $CDCl_3$) δ 7.98 (d, 2H), δ 6.90 (d, 2 H), δ 3.87 (s, 3H), δ 3.83 (s, 3H)

iii. Results: One Gram Scale

Theoretical Yield (1:1)

$$1.0028 \text{ g 4-Methoxybenzoic Acid} \times \frac{1 \text{ mole 4-Methoxybenzoic Acid}}{152.15 \text{ g 4-Methoxybenzoic Acid}}$$

$$= 0.0065909 \text{ mol 4-Methoxybenzoic Acid}$$

$$0.0065909 \text{ mol} \times \frac{166.17 \text{ g Methyl 4-Methoxybenzoate}}{1 \text{ mol Methyl 4-Methoxybenzoate}} = 1.0952 \text{ g Methyl 4-Methoxybenzoate}$$

Percent Yield

$$\frac{1.0042 \text{ g Methyl 4-Methoxybenzoate}}{1.0952 \text{ g Methyl 4-Methoxybenzoate}} \times 100\% = 99.0\%$$

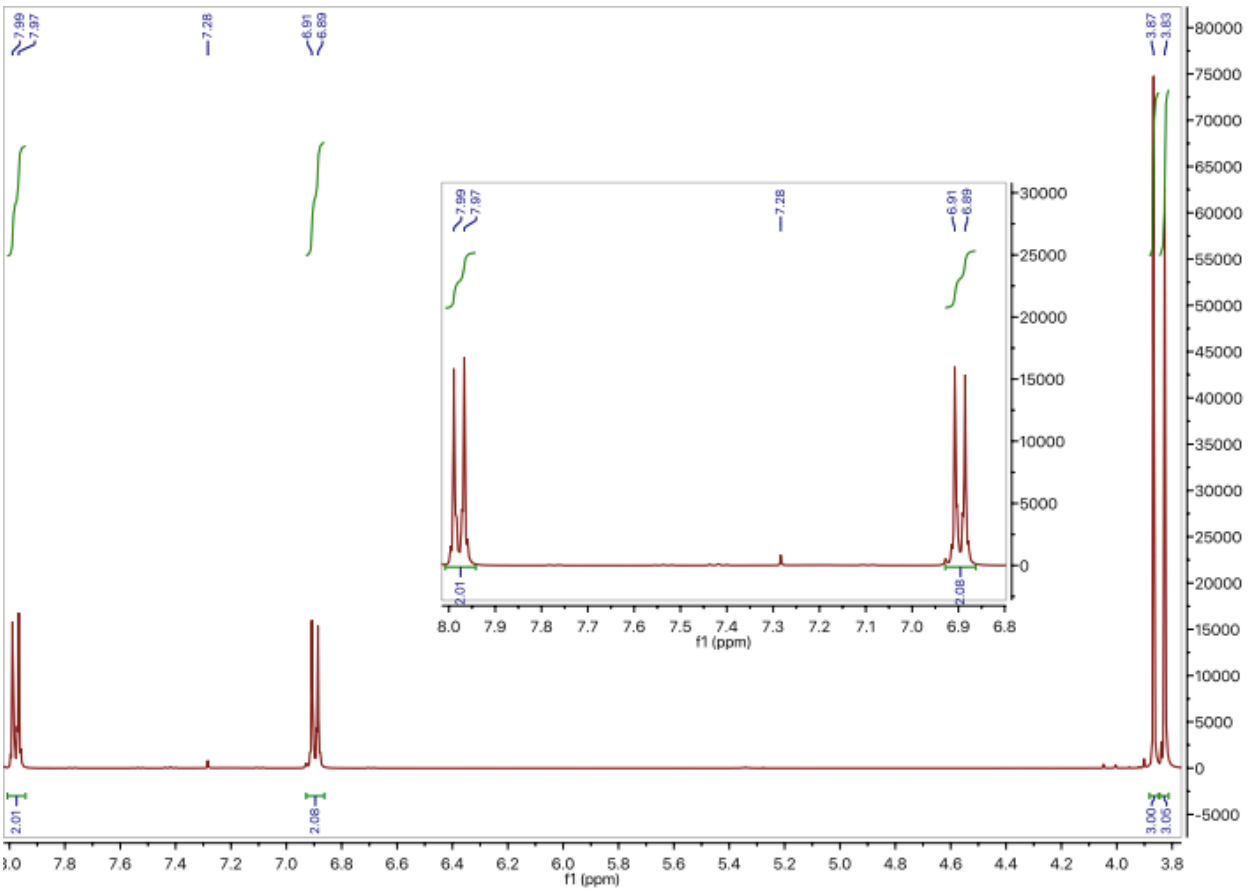


Figure 2.7: *^{1}H-NMR of Methyl 4-Methyoxybenzoate Product*

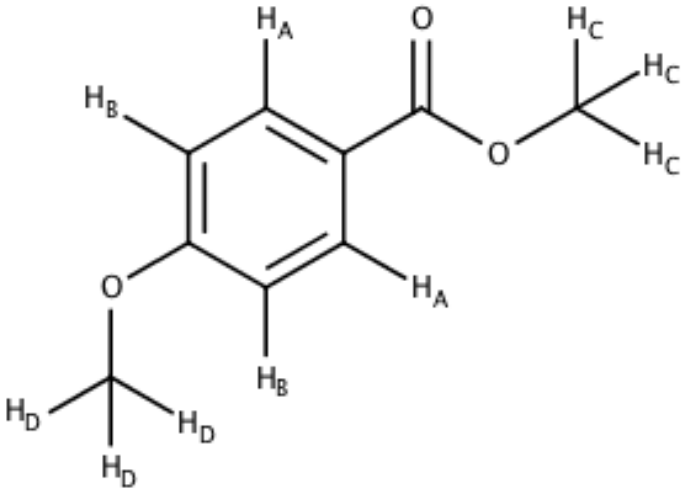


Figure 2.8: *Structure of Methyl 4-Methyoxybenzoate*

Table 2.4: *Proton Assignment for ^{1}H-NMR of Methyl 4-Methyoxybenzoate Product*

Assignment (H_x)	Chemical Shift (δ, ppm)	Splitting	Relative Integration	Observed Integration
A	7.98	doublet	2	2.01
B	6.90	doublet	2	2.08
C	3.87	singlet	3	3.00
D	3.83	singlet	3	3.05

The ^{1}H-NMR spectrum of the product which resulted from the esterification of 4-methoxybezoic acid using SOMS as a nano-reactor exhibited four unique peaks (Figure 2.7), two of which fell in the aromatic region. The two most downfield of the peaks, a pair of doublets each with an integration of two can be found at approximately 7.98 ppm and 6.90. These peak represent the two sets of two chemically equivalent hydrogens, each or which having one neighbor. These four hydrogen atoms are bound to the aromatic carbons of methyl 4-methoxybenzoate. The most downfield peak represents the two hydrogens that are closest to the carbonyl (i.e. H_A, Figure 2.8), while the slightly more upfield doublet represents the two hydrogens closest to the methoxy substituent of the ring (i.e. H_B). Further upfield, a singlet with an integration of three located at 3.87 ppm, represents the three hydrogens of the newly formed methyl ester (i.e. H_C) and is the definitive indictor that the desired product (methyl benzoate) has been formed. Additionally, a final singlet with an integration of three can be found at 3.83 ppm. This peak represents the three hydrogens of the methoxy group attached to the aromatic ring (i.e. H_D). In addition to the previously assigned peaks, the solvent used in this analysis, $CDCl_3$ also displays a singlet at 7.28 ppm. A summary of the ^{1}H-NMR data of the product are given in Table 2.4.

iv. Discussion: One Gram Scale

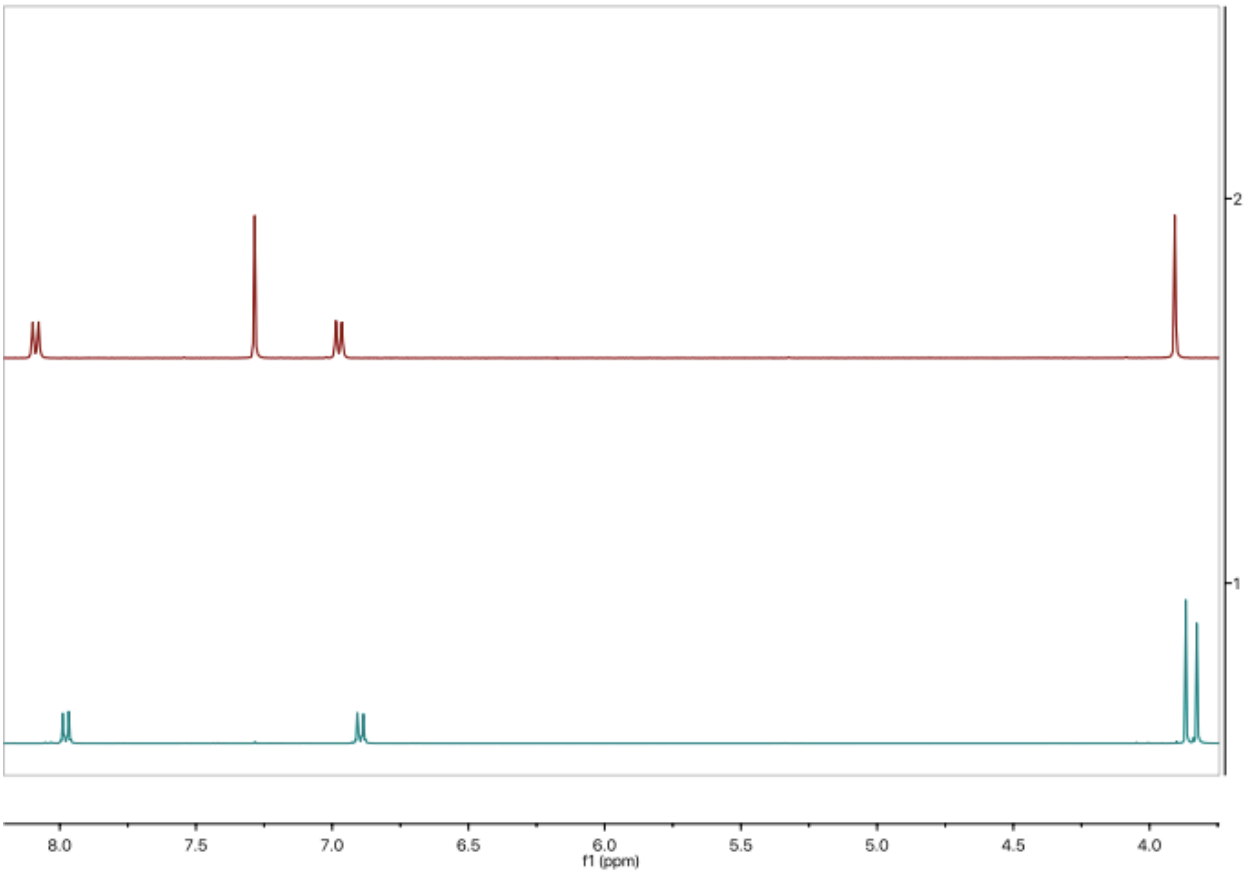


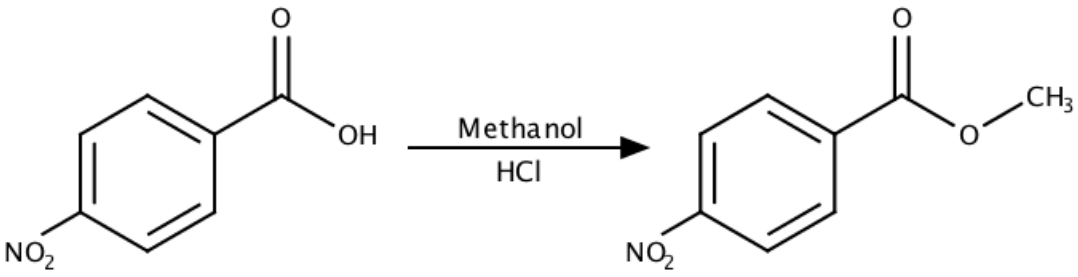
Figure 2.9: *Stacked ^{1}H-NMR of 4-Methoxybenzoic Acid Starting Material (Top) and Methyl 4-Methyoxybenzoate Product (Bottom)*

Before 4-methoxybenzoic acid has reacted with methanol to form methyl 4-methoxybenzoate, the ^{1}H-NMR spectrum of the starting material contains three distinct peaks, two of which are found in the aromatic region and the last of which is found further upfield (Figure 2.9, top). Once methyl 4-methoxybenzoate has formed, a fourth peak can be seen further upfield than any of the peaks of the starting material, at 3.83 ppm (Figure 2.9, bottom). As in the previous cases described in this project, the ^{1}H-NMR spectrum of the product provides two clues to suggest that complete conversion of the carboxylic acid into the methyl ester has occurred. First, the ^{1}H-NMR spectrum of the product exhibits clean and distinct peaks in both the aromatic and aliphatic regions. Since the analogous protons of 4-methoxybenzoic acid and methyl 4-

methoxybenzoate exist in slightly different chemical environments, the corresponding peaks between the two molecules occur at slightly different ppm values. If some of the starting material still existed in the product, the analogous peaks in the spectrum of the product would be messy and appears as if multiple peaks were overlapping. Second, the peak representing the three hydrogens of the methyl ester in the product has a relative integration of three when compared to the known integrations of the rest of the molecule. If starting material still existed in the product, the relative integrations of the hydrogens provided by the carboxylic acid starting material would not correspond to the relative integration of the methyl ester peak in this clean ratio. Since the ^{1}H-NMR data suggests that the methyl ester product is pure, the calculated percent yield of 99.0% becomes noteworthy, suggesting a quantitative conversion in this reaction.

Conversion of 4-Nitrobenzoic Acid into Methyl 4-Nitrobenzoate

i. Reaction Scheme



Scheme 2.3: *Acid Catalyzed Esterification of 4-Nitrobenzoic Acid*

ii. Procedure: One Gram Scale

In a 100-mL round bottom flask 1.0008 grams of 4-nitrobenzoic acid dissolved in dichloromethane (20 mL) was added to 5.1200 grams of SOMS. The dichloromethane was then removed using a rotary evaporator (485 mbar, 40 °C) in order to encapsulate the carboxylic acid in the SOMS. An additional 10 mL of dichloromethane was then used to ensure a quantitative transfer of the carboxylic acid into round bottom flask. This additional volume of

dichloromethane was also removed by pressure dependent evaporation. To the dried SOMS (containing the 4-nitrobenzoic acid), 8.00 x 10^2 μL of 3 N HCl in methanol were added in a dropwise fashion, followed by an excess of methanol (7.00 mL), until the SOMS had expanded, but was not visibly oversaturated. The round bottom was then placed on a flexing station (65 °C), which allowed the methanol to repeatedly evaporate and condense (effectively opening and closing the SOMS nano-reactors) for 24 hours. Upon completion of the 24-hour period, the product was removed from the SOMS by vacuum filtration using an excess of methanol (350 mL). The product (1.0855 g) was then collected by removing the methanol through rotary evaporation (115 mbar, 40 °C). ^{1}HNMR (400 MHz, $CDCl_3$) δ 8.32 (d, 2H), δ 8.24 (d, 2 H), δ 4.01 (s, 3H)

iii. Results: One Gram Scale

Theoretical Yield (1:1)

$$1.0008\text{ g 4-Nitrobenzoic Acid} \times \frac{1\text{ mole 4-Nitrobenzoic Acid}}{167.12\text{ g 4-Nitrobenzoic Acid}}$$

$$= 0.0059885\text{ mol 4-Nitrobenzoic Acid}$$

$$0.0059885\text{ mol} \times \frac{181.15\text{ g Methyl 4-Nitrobenzoate}}{1\text{ mol Methyl 4-Nitrobenzoate}} = 1.0848\text{ g Methyl 4-Nitrobenzoate}$$

Percent Yield

$$\frac{1.0855\text{ g Methyl 4-Nitrobenzoate}}{1.0848\text{ g Methyl 4-Nitrobenzoate}} \times 100\% = 100.1\%$$

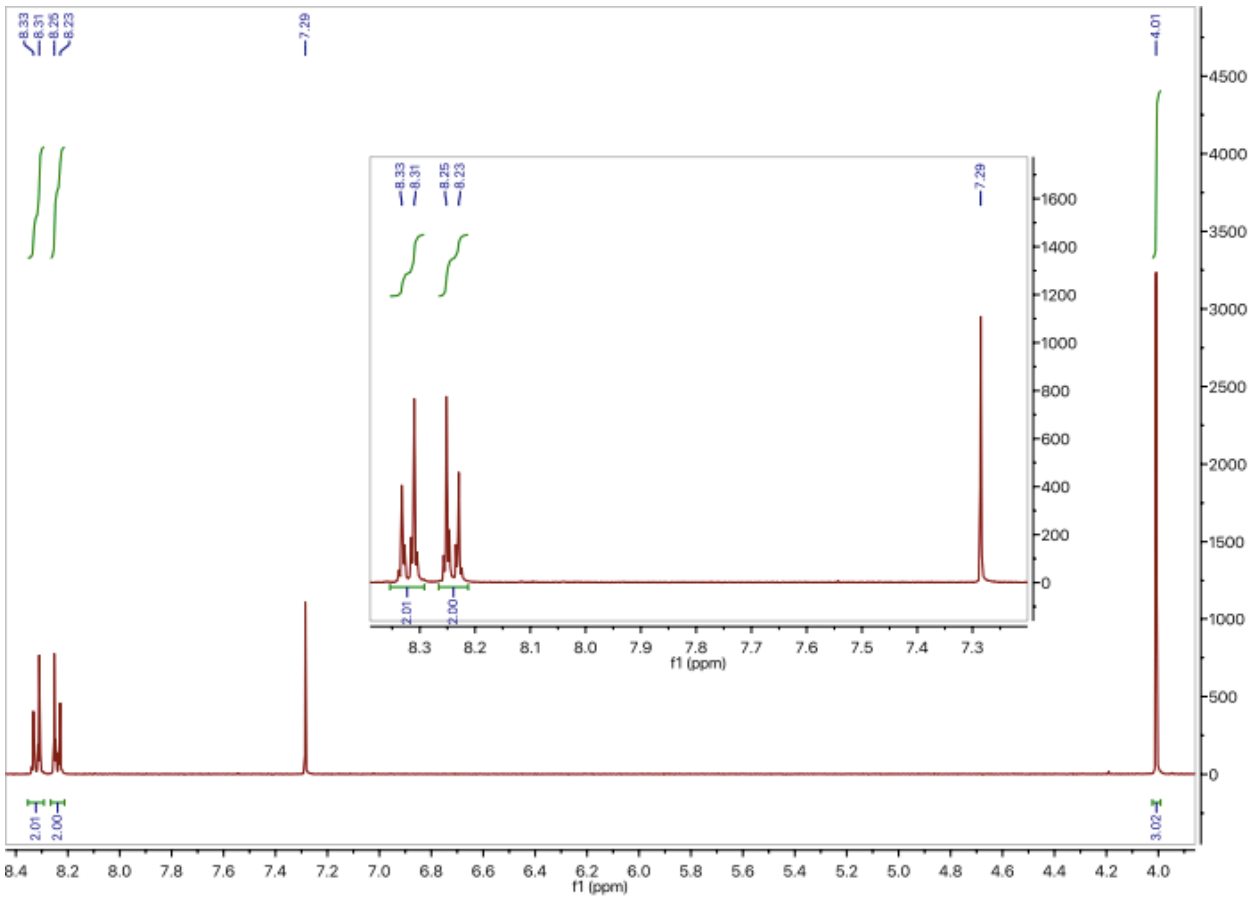


Figure 2.10: *^{1}H-NMR of Methyl 4-Nitrobenzoate Product*

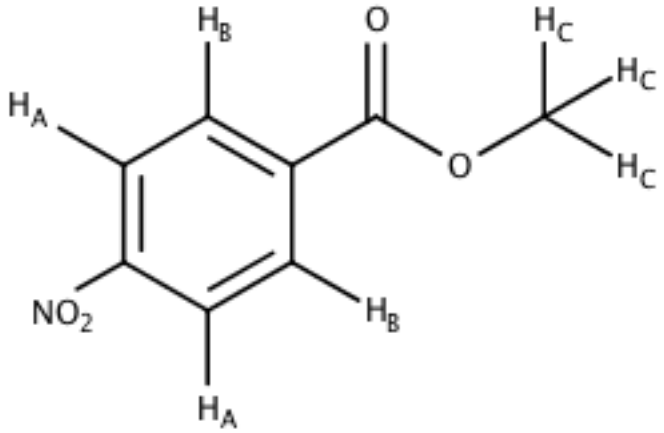


Figure 2.11. *Structure of Methyl 4-Nitrobenzoate*

Table 2.5: *Proton Assignment for ^{1}H-NMR of Methyl 4-Nitrobenzoate Product*

Assignment (H_x)	Chemical Shift (δ, ppm)	Splitting	Relative Integration	Observed Integration
A	8.32	doublet	2	2.01
B	8.24	doublet	2	2.0
C	4.01	singlet	3	3.02

The ^{1}H-NMR spectrum of the product which resulted from the esterification of 4-nitrobezoic acid using SOMS as a nano-reactor exhibited three unique peaks (Figure 2.10), two of which fell in the aromatic region. The two most downfield of the peaks, a pair of doublets each with an integration of two can be found at approximately 8.32 ppm and 8.24. These peak represent the two sets of two chemically equivalent hydrogens, each or which having one neighbor. These four hydrogen atoms are bound to the aromatic carbons of methyl 4-nitrobenzoate. The most downfield peak represents the two hydrogens that are closest to the nitro substituent of the aromatic ring (i.e. H_A, Figure 2.11), while the slightly more upfield doublet represents the two hydrogens closest to the carbonyl (i.e. H_B). Further upfield, a singlet with an integration of three located at 4.01 ppm, represents the three hydrogens of the newly formed methyl ester (i.e. H_C) and is the definitive indictor that the desired product (methyl 4-nitrobenzoate) has been formed. In addition to the previously assigned peaks, the solvent used in this analysis, $CDCl_3$ also displays a singlet at 7.28 ppm. A summary of the ^{1}H-NMR data of the product are given in Table 2.5.

iv. Discussion: One Gram Scale

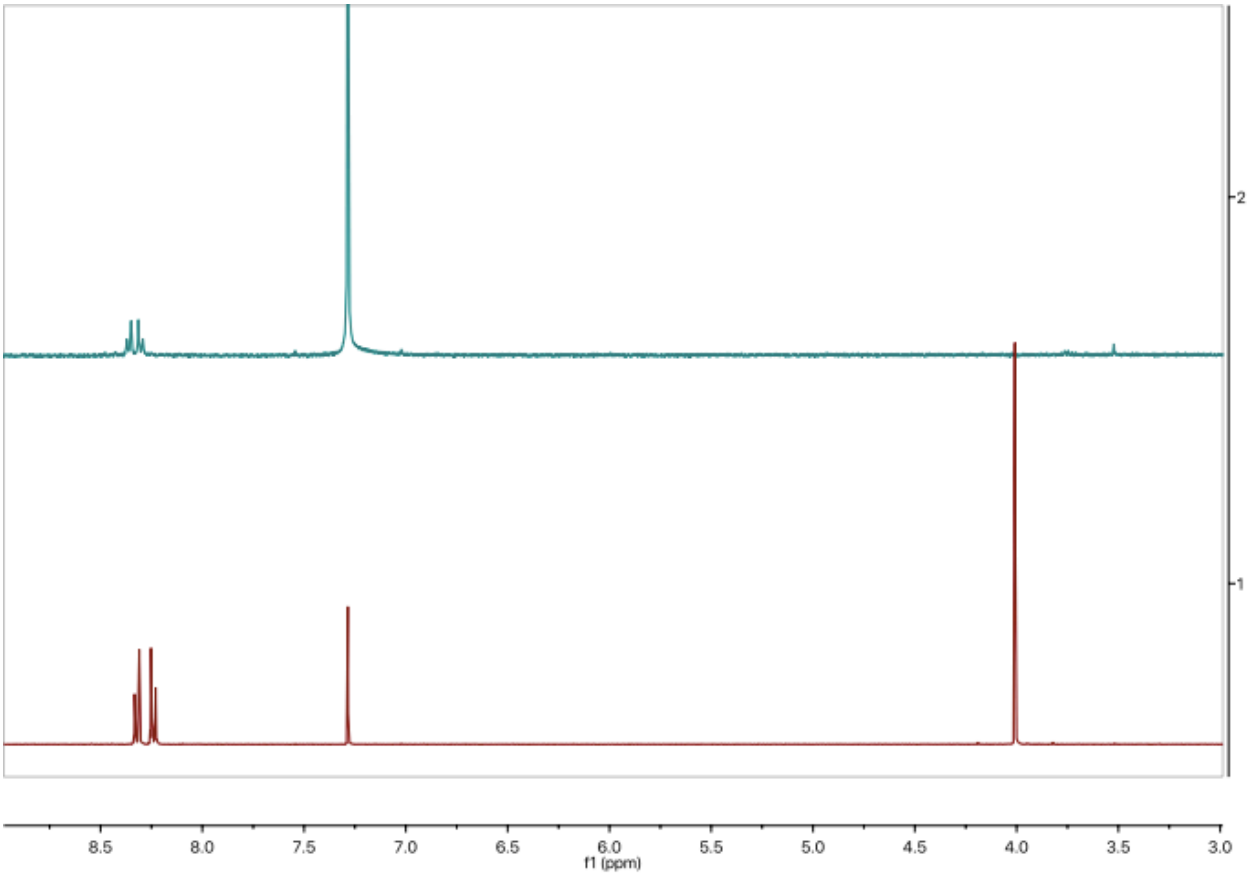


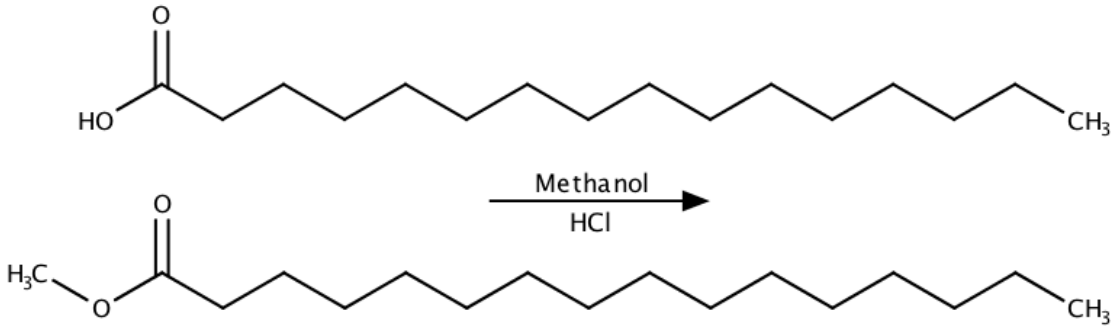
Figure 2.12: *Stacked ^{1}H-NMR of 4-Nitrobenzoic Acid Starting Material (Top) and Methyl 4-Nitrobenzoate Product (Bottom)*

Before 4-nitrobenzoic acid has reacted with methanol to form methyl 4-nitrobenzoate, the only peaks in the ^{1}H-NMR spectrum of the starting material can be found in the aromatic region (Figure 2.12, top). Once methyl benzoate has formed, a peak can be seen further upfield, at 4.01 ppm (Figure 2.12, bottom). In this reaction, like that of benzoic acid, the ^{1}H-NMR spectrum provides two clues to suggest that complete conversion of the carboxylic acid into the methyl ester has occurred. First, the aromatic region in the ^{1}H-NMR spectrum of the product exhibits two clean and distinct peaks. Since the four protons bound to the aromatic carbons of 4-nitrobenzoic acid and methyl 4-nitrobenzoate exist in slightly different chemical environments, the corresponding peaks between the two molecules occur at slightly different ppm values. If

some of the starting material still existed in the product, its spectrum would exhibit messy, overlapping peaks in the aromatic region. Second, the peak representing the three hydrogens of the methyl ester in the product has a relative integration of three when compared to the known integrations of the aromatic region. If starting material still existed in the product, the relative integrations of the aromatic region would not correspond to the relative integration of the methyl ester peak in this clean ratio. Since the ^{1}H-NMR data suggests that the methyl ester product is pure, the calculated percent yield of 100.1% becomes noteworthy, suggesting a quantitative conversion in this reaction.

Conversion of Palmitic Acid into Methyl Palmitate

i. Reaction Scheme



Scheme 2.4: *Acid Catalyzed Esterification of Palmitic Acid*

ii. Procedure: One Gram Scale

In a 100-mL round bottom flask 1.0020 grams of palmitic acid dissolved in dichloromethane (20 mL) was added to 5 grams of SOMS. The dichloromethane was then removed using a rotary evaporator (485 mbar, 40 °C) in order to encapsulate the carboxylic acid in the SOMS. An additional 10 mL of dichloromethane was then used to ensure a quantitative transfer of the carboxylic acid into round bottom flask. This additional volume of dichloromethane was also removed by pressure dependent evaporation. To the dried SOMS

(containing the palmitic acid), 8.00 x 10^2 µL of 3 N HCl in methanol were added in a dropwise fashion, followed by an excess of methanol (7.50 mL), until the SOMS had expanded, but was not visibly oversaturated. The round bottom was then placed on a flexing station (65 °C), which allowed the methanol to repeatedly evaporate and condense (effectively opening and closing the SOMS nano-reactors) for 24 hours. Upon completion of the 24-hour period, the product was removed from the SOMS by vacuum filtration using 350 mL of methanol. The product (1.0445 g) was then collected by removing the methanol through rotary evaporation (115 mbar, 40 °C). ^{1}HNMR (400 MHz, $CDCl_3$) δ 3.69 (s, 3H), δ 2.32 (m, 2 H), δ 1.64 (m, 2H), δ 1.27 (m, 24H), δ 0.90 (t, 3H)

iii. Results: One Gram Scale

Theoretical Yield (1:1)

$$1.0020 \text{ g Palmitic Acid} \times \frac{1 \text{ mole Palmitic Acid}}{256.43 \text{ g Palmitic Acid}}$$

$$= 0.0039075 \text{ mol Palmitic Acid}$$

$$0.0039075 \text{ mol} \times \frac{270.45 \text{ g Methyl Palmitate}}{1 \text{ mol Methyl Palmitate}} = 1.0568 \text{ g Methyl Palmitate}$$

Percent Yield

$$\frac{1.0445 \text{ g Methyl Palmitate}}{1.0568 \text{ g Methyl Palmitate}} \times 100\% = 98.8\%$$

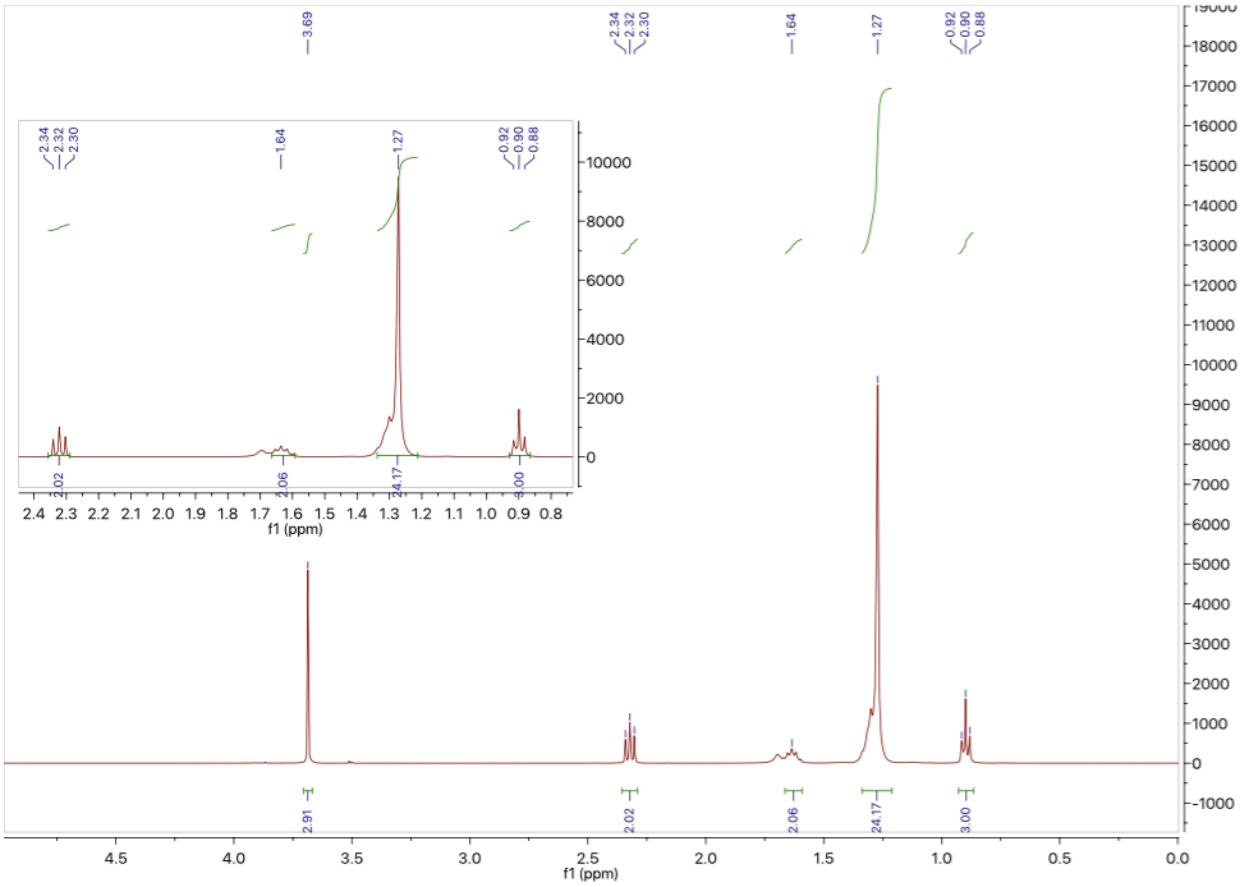


Figure 2.13: *^{1}H-NMR of Methyl Palmitate Product*

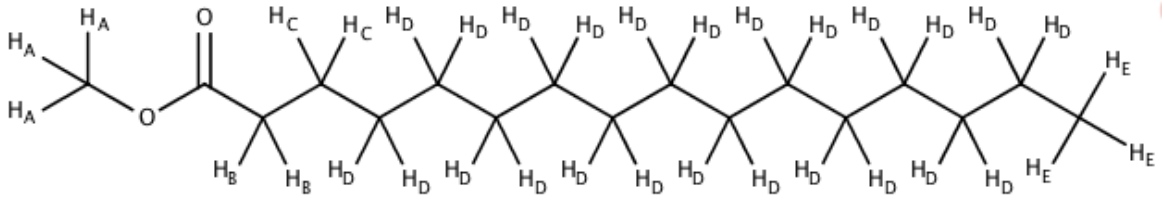


Figure 2.14: *Structure of Methyl Palmitate*

Table 2.6: *Proton Assignment for ^{1}H-NMR of Methyl Palmitate Product*

Assignment (H_x)	Chemical Shift (δ, ppm)	Splitting	Relative Integration	Observed Integration
A	3.69	singlet	3	2.91
B	2.32	triplet	2	2.02
C	1.64	multiplet	2	2.06
D	1.27	multiplet	24	24.17
E	0.90	triplet	3	3.00

The ^{1}H-NMR spectrum of the product which resulted from the esterification of palmitic acid using SOMS as a nano-reactor exhibited five unique peaks (Figure 2.13). Unlike the previously described aromatic carboxylic acids, the most downfield peak in this case, a singlet with an integration of three located at 3.69 ppm, represents the three hydrogens of the newly formed methyl ester (H_A, Figure 2.14). The next most downfield peak, a triplet (i.e. two neighboring hydrogens) with an integration of 2, represents the two hydrogens bound to the carbon of the hydrocarbon tail that is closest to the carbonyl (H_B). The next most downfield peak, a multiplet with an integration of two found at 1.64 ppm represents the two chemically equivalent hydrogens on the next carbon of the hydrocarbon tail (H_C). Yet another multiplet, with an integration of 24 and a chemical shift of 1.27 ppm, represents the nearly chemically equivalent hydrogens that are bound to the next 12 carbons of the hydrocarbon chain (H_D). The final peak, a triplet with an integration of 3, represents the three chemically equivalent hydrogens attached to the final carbon of the hydrocarbon chain (H_E). Its integration of three and triplet splitting pattern (indicating the existence of two hydrogen neighbors) confirm the identity of this peak. In addition to the previously assigned peaks, the solvent used in this analysis, $CDCl_3$ also displays a singlet at 7.28 ppm. A summary of the ^{1}H-NMR data of the product are given in Table 2.6.

iv. Discussion: One Gram Scale

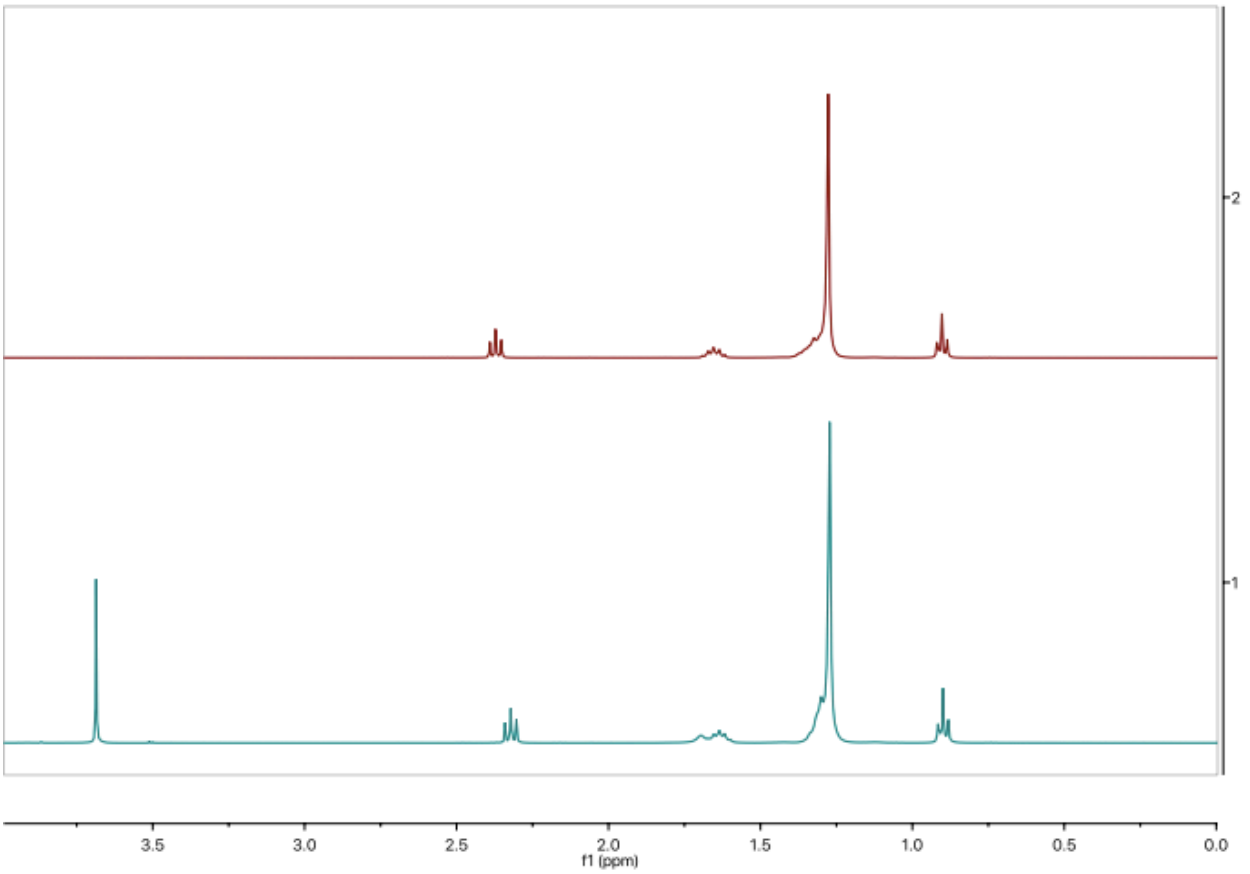


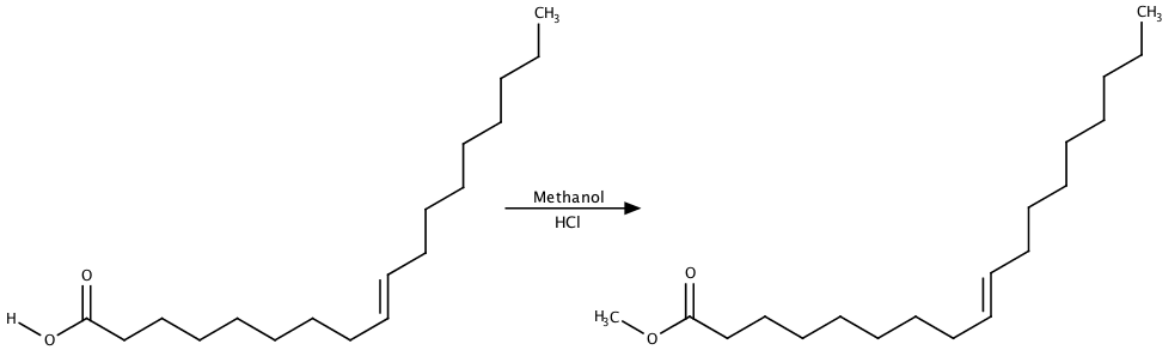
Figure 2.15: *Stacked ^{1}H-NMR of Palmitic Acid Starting Material (Top) and Methyl Palmitate Product (Bottom)*

Before palmitic acid has reacted with methanol to form methyl palmitate, ^{1}H-NMR spectrum of the starting material contains four peaks (Figure 2.15, top). Once methyl palmitate has formed, a fifth peak can be seen further downfield, at 3.69 ppm (Figure 2.15, bottom). In this reaction, like those of the aromatic carboxylic acids, the ^{1}H-NMR spectrum provides two clues to suggest that complete conversion of the carboxylic acid into the methyl ester has occurred. First, the two distinct triplets that are analogous in the ^{1}H-NMR spectra of the starting material and the product display clean and distinct peaks in both spectra. Since the protons represented by these peaks exist in slightly different chemical environments, the corresponding peaks between the two molecules occur at slightly different ppm values. If some of the starting material still existed in

the product sample that was analyzed, these triplets would appear as messy, overlapping peaks. Second, the peak representing the three hydrogens of the methyl ester in the product has a relative integration of three when compared to the known integrations of the resto of the molecule. Although, this relative integration is not as cleanly proportional as those of the previously studied aromatic carboxylic acids, this slight discrepancy is possibly due to the difficult integration of the relatively complex aliphatic region of the ^{1}H-NMR spectrum exhibited by methyl palmitate. Since the ^{1}H-NMR data suggests that the methyl ester product is pure, the calculated percent yield of 98.8% becomes noteworthy, suggesting a quantitative conversion in this reaction.

Conversion of Oleic Acid into Methyl Oleate

i. Reaction Scheme



Scheme 2.5: *Acid Catalyzed Esterification of Oleic Acid*

ii. Procedure: One Gram Scale

To the dried SOMS was added both 1.0193 grams of oleic acid (liquid) and 8.00 x 10^{2} μL of 3 N HCl in methanol, each in a dropwise fashion. These additions were followed by an excess of methanol (9.00 mL), until the SOMS had expanded, but was not visibly oversaturated. The round bottom was then placed on a flexing station (65 °C), which allowed the methanol to repeatedly evaporate and condense (effectively opening and closing the SOMS nano-reactors)

for 24 hours. Upon completion of the 24-hour period, the product was removed from the SOMS by vacuum filtration using 350 mL of methanol. The product (1.0769 g) was then collected by removing the methanol through rotary evaporation (115 mbar, 40 °C). ^{1}HNMR (400 MHz, $CDCl_3$) δ 5.32 (m, 2H), δ 3.64 (s, 3 H), δ 2.28 (m, 2H), δ 2.00 (m, 3H), δ 1.60 (m, 2H), δ 1.25 (m, 20H), δ 0.86 (t, 3H)

iii. Results: One Gram Scale

Theoretical Yield (1:1)

$$1.0193 \text{ g Oleic Acid} \times \frac{1 \text{ mole Oleic Acid}}{282.47 \text{ g Oleic Acid}}$$

$$= 0.0036085 \text{ mol Oleic Acid}$$

$$0.0036085 \text{ mol} \times \frac{296.49 \text{ g Methyl Oleate}}{1 \text{ mol Methyl Oleate}} = 1.0699 \text{ g Methyl Oleate}$$

Percent Yield

$$\frac{1.0769 \text{ g Methyl Oleate}}{1.0699 \text{ g Methyl Oleate}} \times 100\% = 100.7\%$$

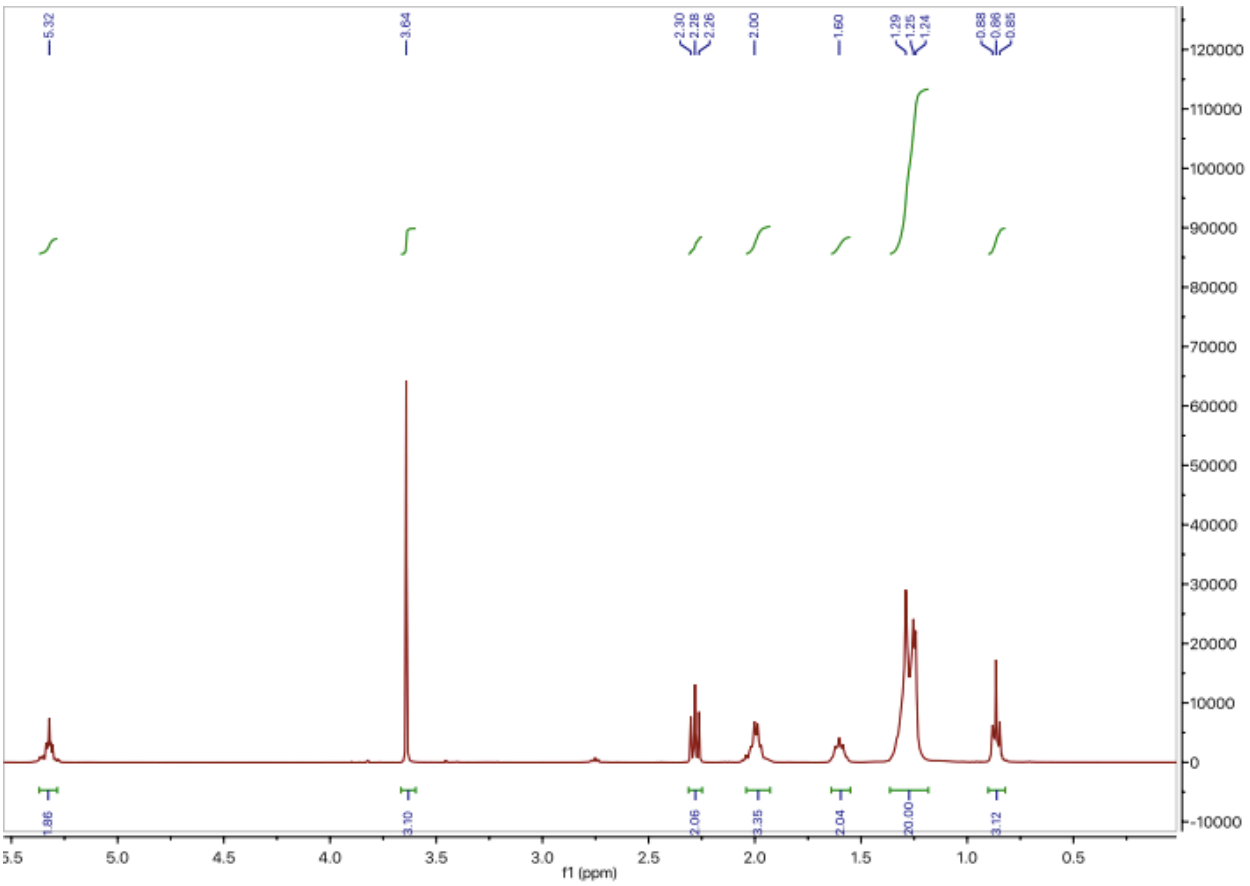


Figure 2.16: *^{1}H-NMR of Methyl Oleate Product*

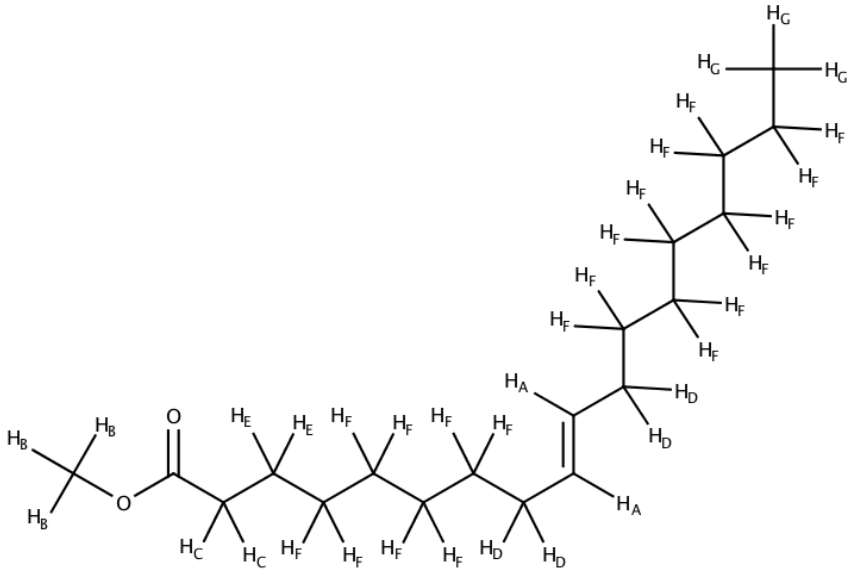


Figure 2.17: *Structure of Methyl Oleate*

Table 2.7: *Proton Assignment for ^{1}H-NMR of Methyl Oleate Product*

Assignment (H_x)	Chemical Shift (δ, ppm)	Splitting	Relative Integration	Observed Integration
A	5.32	multiplet	2	1.86
B	3.64	singlet	3	3.10
C	2.28	triplet	2	2.06
D	2.00	multiplet	4	3.35
E	1.60	multiplet	2	2.04
F	1.25	multiplet	20	20.00
G	0.86	triplet	3	3.12

The ^{1}H-NMR spectrum of the product which resulted from the esterification of oleic acid using SOMS as a nano-reactor exhibited seven unique peaks (Figure 2.16). The most downfield peak in this case, a multiplet with an integration of two located at 5.32 ppm, represents the two hydrogens attached to the double bonded carbons of the hydrocarbon chain (H_A, Figure 2.17). The next most downfield peak, a singlet with an integration of three, represents the three chemically equivalent hydrogens of the newly formed methyl ester (H_B). The next peak, a triplet with an integration of two and a chemical shift of 2.28 ppm, represents the two chemically equivalent hydrogens bound to the carbon of the hydrocarbon chain next to the carbonyl of the methyl ester (H_C). The triplet splitting pattern of this peak confirms that it has two neighbors on the hydrocarbon chain. Moving further upfield, the next peak of the spectrum is a multiplet with an integration of 4 located at 2.00 ppm. This multiplet represents the four nearly chemically equivalent hydrogens bound to the two carbons that surround the single carbon-carbon double bond within the hydrocarbon chain (H_D). The next most upfield peak, a multiplet with a chemical shift of 1.60 and an integration of 2, represents the two chemically equivalent hydrogens bound to the carbon that is one away from the carbonyl of the methyl ester (i.e. H_E, the neighbor of H_C). The second most upfield peak, a multiplet with an integration of 20 and a chemical shift of 1.25, represents the 20 hydrogens bound to the remaining 10 interior carbons of the hydrocarbon chain

(H_F). Finally, the most upfield peak, a triplet with an integration of 3, represents the three chemically equivalent hydrogens attached to the final carbon of the hydrocarbon chain (H_G). Its integration of three and triplet splitting pattern (indicating the existence of two hydrogen neighbors) confirm the identity of this peak. In addition to the previously assigned peaks, the solvent used in this analysis, $CDCl_3$ also displays a singlet at 7.28 ppm. A summary of the ^{1}H-NMR data of the product are given in Table 2.7.

iv. Discussion: One Gram Scale

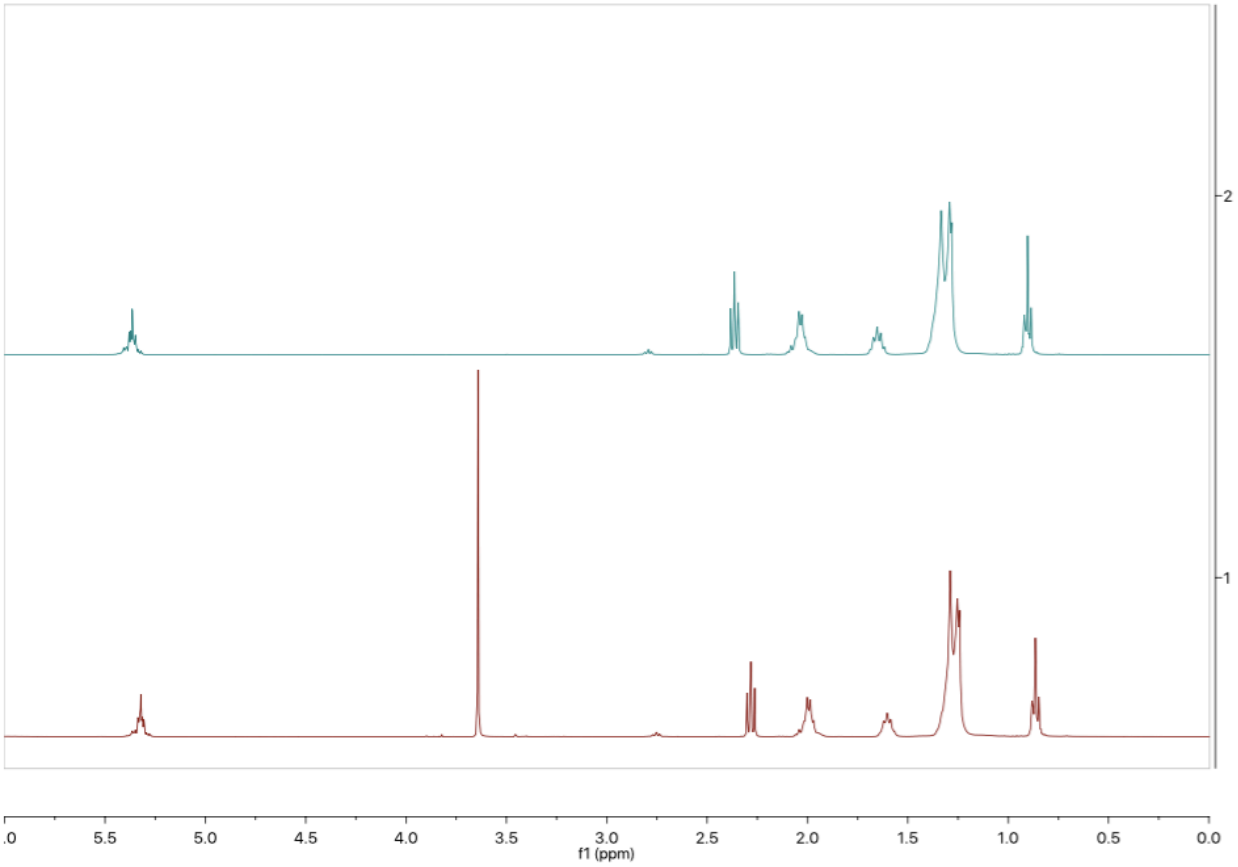


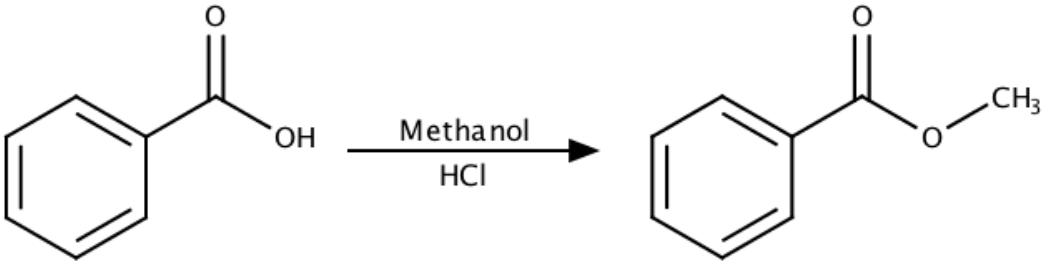
Figure 2.18: *Stacked ^{1}H-NMR of Oleic Acid Starting Material (Top) and Methyl Oleate Product (Bottom)*

Before oleic acid has reacted with methanol to form methyl oleate, ^{1}H-NMR spectrum of the starting material contains six peaks (Figure 2.18, top). Once methyl oleate has formed, a seventh peak can be seen at 3.64 ppm (Figure 2.18, bottom). In this reaction, like those of the

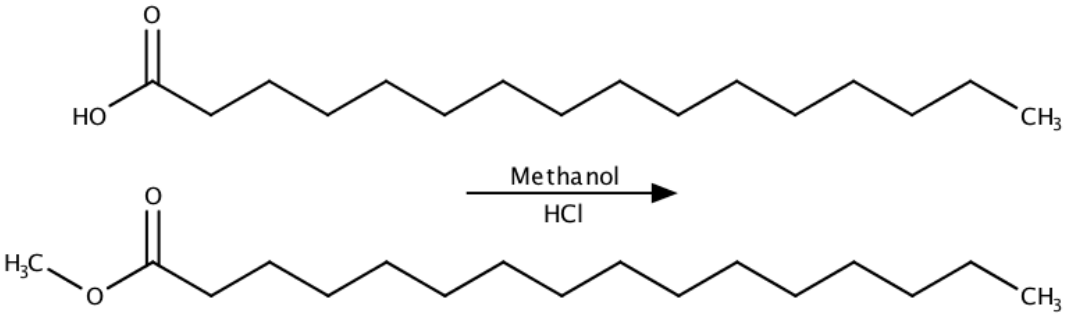
aromatic carboxylic acids, the ^{1}H-NMR spectrum provides two clues to suggest that complete conversion of the carboxylic acid into the methyl ester has occurred. First, the two distinct triplets that are analogous in the ^{1}H-NMR spectra of the starting material and the product display clean and distinct peaks in both spectra. Since the protons represented by these peaks exist in slightly different chemical environments, the corresponding peaks between the two molecules occur at slightly different ppm values. If some of the starting material still existed in the product sample that was analyzed, these triplets would appear as messy, overlapping peaks. Second, the peak representing the three hydrogens of the methyl ester in the product has a relative integration of three when compared to the known integrations of the resto of the molecule. Although, this relative integration is not as cleanly proportional as those of the previously studied aromatic carboxylic acids, this slight discrepancy is possibly due to the difficult integration of the relatively complex aliphatic region of the ^{1}H-NMR spectrum exhibited by methyl oleate. Since the ^{1}H-NMR data suggests that the methyl ester product is pure, the calculated percent yield of 100.7% becomes noteworthy, suggesting a quantitative conversion in this reaction.

Chapter 3: Conversion of a Mixed Sample of Carboxylic Acids to Esters Using SOMS

Reaction Schemes



Scheme 3.1: *Acid Catalyzed Esterification of Benzoic Acid*



Scheme 3.2: *Acid Catalyzed Esterification of Palmitic Acid*

Procedure

In a 100-mL round bottom flask 0.7496 g of benzoic acid and 0.2418 g of palmitic acid dissolved in dichloromethane (20 mL) were added to 5.0570 g of SOMS. The dichloromethane was then removed using a rotary evaporator (485 mbar, 40 °C) in order to encapsulate the carboxylic acids in the SOMS. An additional 10 mL of dichloromethane was then used to ensure a quantitative transfer of the carboxylic acids into round bottom flask. This additional volume of dichloromethane was also removed by pressure dependent evaporation. To the dried SOMS (containing the two carboxylic acids), 8.00 x 10^2 μL of 3 N HCl in methanol were added in a dropwise fashion, followed by an excess of methanol (7.50 mL), until the SOMS had expanded, but was not visibly oversaturated. The round bottom was then placed on a flexing station (65

°C), which allowed the methanol to repeatedly evaporate and condense (effectively opening and closing the SOMS nano-reactors) for 24 hours. Upon completion of the 24-hour period, the product mixture was removed from the SOMS by vacuum filtration using an excess of methanol (350 mL). The product was then collected by removing the methanol through rotary evaporation (115 mbar, 40 °C).

Results

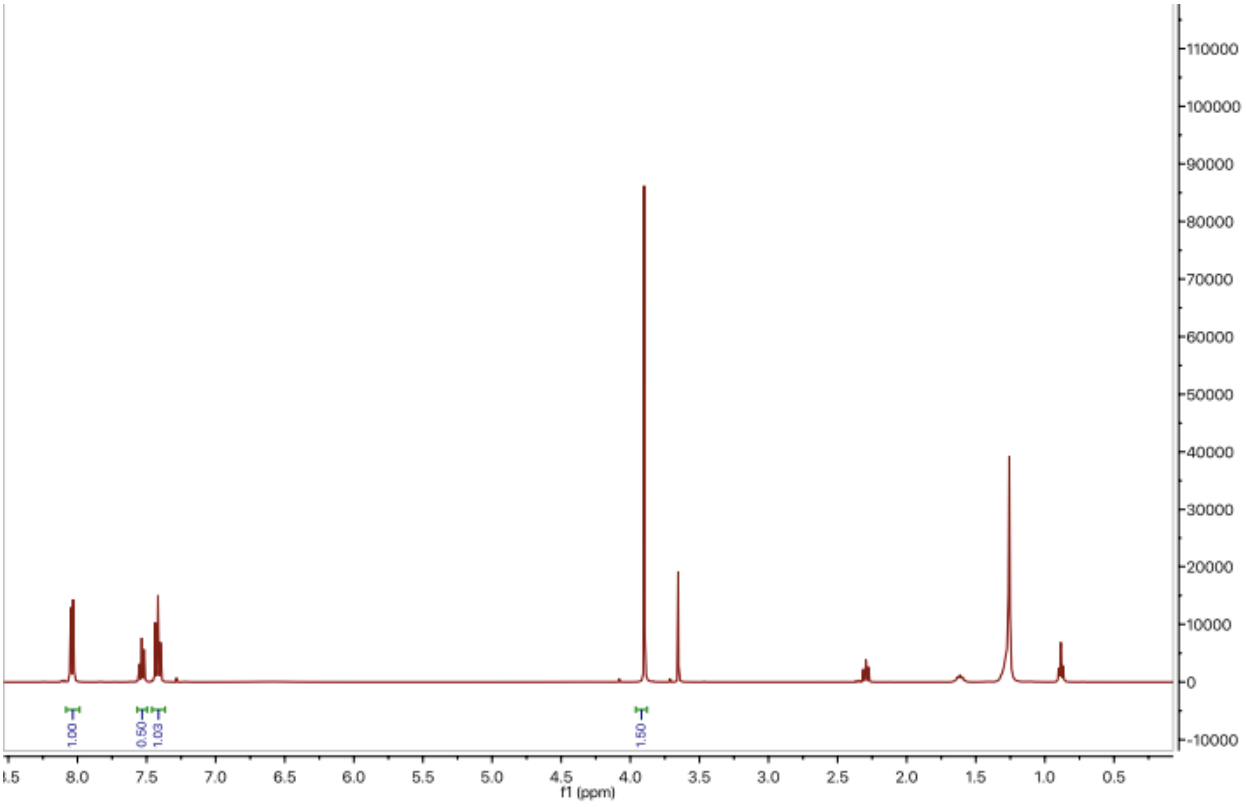


Figure 3.1: *^{1}H-NMR of Combined Methyl Benzoate and Methyl Palmitate Products*

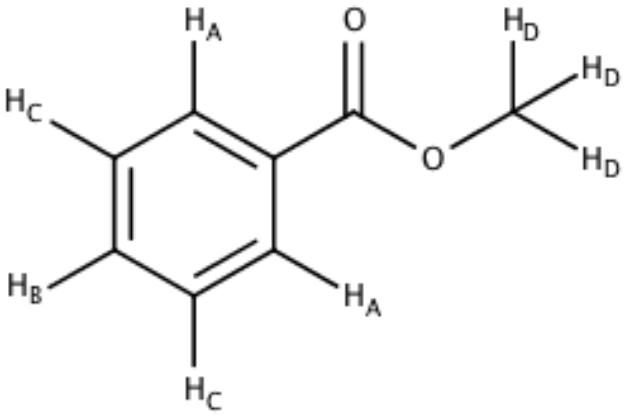


Figure 3.2: *Structure of Methyl Benzoate*

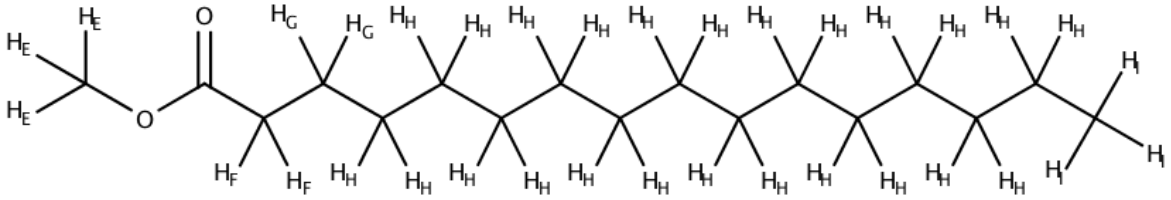


Figure 3.3: *Structure of Methyl Palmitate*

Table 3.1: *Proton Assignment for ^{1}H-NMR of Methyl Benzoate and Methyl Palmitate Products*

Assignment (H_x)	Chemical Shift (δ, ppm)	Splitting	Relative Integration
A Methyl Benzoate	8.05	doublet	2
B Methyl Benzoate	7.54	triplet	1
C Methyl Benzoate	7.43	triplet	2
D Methyl Benzoate	3.91	singlet	3
E Methyl Palmitate	3.69	singlet	3
F Methyl Palmitate	2.32	triplet	2
G Methyl Palmitate	1.64	multiplet	2
H Methyl Palmitate	1.27	multiplet	24
I Methyl Palmitate	0.90	triplet	3

As expected, the ^{1}H-NMR spectra of the product which resulted from the simultaneous esterifications of benzoic acid and palmitic acid using SOMS as a nano-reactor exhibited the diagnostic peaks of both methyl benzoate and methyl palmitate (Figure 3.1). Four peaks were assigned to methyl benzoate, three of which fell in the aromatic region. The most downfield of the peaks is a doublet with an integration of two at approximately 8.05 ppm. This peak represents two chemically equivalent hydrogens, each of which having one neighbor. These two hydrogen atoms are bound to the aromatic carbons that are closest to the only substituent on the aromatic ring in benzoic acid (i.e. H_A, Figure 3.2). The next peak, a triplet with an integration of one, occurred at 7.54 ppm. This peak represents the single hydrogen on the aromatic carbon directly across from the only substituent on the ring (i.e. H_B). Finally, a triplet with an integration of two appears 7.43 ppm. This peak represents the final two chemically equivalent hydrogens bound to the aromatic ring (i.e. H_c). Another peak, a singlet with an integration of three located at 3.91 ppm, represents the three hydrogens of the newly formed methyl ester and is the definitive indictor that the desired product (methyl benzoate) has been formed.

In addition to the diagnostic peaks of methyl benzoate, five distinct peaks were also assigned to methyl palmitate. Unlike the previously described aromatic carboxylic acid, the most downfield peak in this case, a singlet with an integration of three located at 3.69 ppm, represents the three hydrogens of the newly formed methyl ester (H_E, Figure 3.3). The next most downfield peak, a triplet with an integration of 2, represent the two hydrogens bound to the carbon of the hydrocarbon tail that is closest to the carbonyl (H_F). The next most downfield peak, a multiplet with an integration of two found at 1.64 ppm represents the two chemically equivalent hydrogens on the next carbon of the hydrocarbon tail (H_I). Yet another multiplet, with an integration of 24 and a chemical shift of 1.27 ppm, represents that nearly chemically equivalent hydrogens that are

bound to the next 12 carbons of the hydrocarbon chain (H_G). Finally, the triplet with an integration of 3 represents the three chemically equivalent hydrogens attached to the final carbon of the hydrocarbon chain (H_I). Its integration of three and triplet splitting pattern (indicating the existence of two hydrogen neighbors) confirm the identity of this peak. A summary of the ^{1}H-NMR data for the multicomponent product are given in Table 3.1.

Discussion

Before the mixture of benzoic acid and palmitic acid have reacted with methanol to form their corresponding methyl esters, the ^{1}H-NMR spectra of the two starting materials are missing the methyl ester peaks present at 3.91 ppm and 3.69 ppm. Once methyl benzoate and methyl palmitate have formed, these peaks become present in the ^{1}H-NMR spectra of the product. In this simultaneous esterification of benzoic acid and palmitic acid, as in each the individual reactions discussed previously, the ^{1}H-NMR spectrum provides two clues to suggest that complete conversion of the carboxylic acid into the methyl ester has occurred. First, the analogous peaks between each of the carboxylic acids and their corresponding methyl esters appear as clean and distinct peaks in the ^{1}H-NMR spectrum of the combined product. Since the analogous protons of each individual carboxylic acids and its corresponding methyl ester exist in slightly different chemical environments, the corresponding peaks between the two molecules occur at slightly different ppm values. If either of the starting material still existed in the product, the spectrum would contain messy, overlapping peaks in the aromatic region (residual benzoic acid), the aliphatic region (residual palmitic acid) or both. Second, the two peaks representing the three hydrogens of the methyl esters in both methyl benzoate and methyl palmitate have relative integrations of three when compared to the other peaks that correspond to each species. If either of the starting materials still existed in the product, the relative integrations of one or both of the

methyl ester peaks would not correspond to the relative integration of the other peak of the spectrum in this clean ratio.

Chapter 4: Analytical Study of a Mixed Sample of Esters (A Proof of Concept for Gas Chromatography)

Background

In order to support the hypothesis that the esterification of carboxylic acids in SOMS could be extended to the study of carboxylic acids via gas chromatography, a proof of concept experiment was designed. The product mixture synthesized in Chapter 3 of this work (i.e. the methyl ester derivatives of benzoic acid and palmitic acid), was used for this study. The experiment described in this section was intended to explore the possibility of methyl ester separation via gas chromatography and ensure that quantitative conversion of the carboxylic acids was being achieved through a desirable analytical method.

Procedure

i. Instrumentation

All of the single-component standards prepared in this experiment, as well as an laboratory fortified blank (LFB), and multicomponent carboxylic acid sample, were analyzed by an Agilent 6850 Series II Gas Chromatograph coupled to a flame ionization detector. A DB-WAX column with dimensions of 30m x 0.320 mm x 0.25 μM purchased from Agilent was utilized for these analyses and helium was the mobile phase. The injection volume was 1.0 μL at a 1:1 split ratio and an inlet temperature of 250 °C. Optimal separation of the three carboxylic acids was established using the following parameters: the oven temperature was first held at 100 °C for 1 minute, then ramped at 25 °C/min to 200 °C, then ramped at 100 ◦C/min to 240 °C, and finally held at 240 °C for 4 minutes, for a total method time of 9.40 minutes.

ii. Single-Component Standard Solutions

A single component standard solution was prepared for the two methyl esters of interest, as well as the methyl 4-cyanobenzoate internal standard (Table 4.1). A 1,530 ppm single component standard of methyl benzoate was prepared by dissolving 0.0153 g of the carboxylic acid in methanol in a 10.00 mL volumetric flask. A 1,030 ppm single component standard of methyl palmitate was prepared by dissolving 0.0130 g of the carboxylic acid in methanol in a 10.00 mL volumetric flask. Finally, a 1,040 ppm single component standard of the internal standard, methyl 4-cyanobenzoate, was prepared by dissolving 0.0104 g of the carboxylic acid in methanol in a 10.00 mL volumetric flask.

Table 4.1: *Methyl Ester Single-Component Standard Preparation*

Identity of Methyl Ester	Mass of Methyl Ester	Total Volume of Single Component Standard	Concentration of Single Component Standard	Identity of Solvent
Methyl Benzoate	0.0153 g	10.00 mL	1,530 ppm	Methanol
Methyl Palmitate	0.0103 g	10.00 mL	1,030 ppm	Methanol
Methyl 4-Cyanobenzoate	0.0104 g	10.00 mL	1,040 ppm	Methanol

iii. Multi-component Standard Stock Solution

A multicomponent stock solution containing both methyl benzoate and methyl palmitate each at a concentration of approximately 10,000 ppm was prepared by dissolving 0.5060 g of methyl benzoate and 0.5093 g of methyl palmitate in methanol in a 50.00 mL volumetric flask. The resulting stock solution had concentrations of 10,100 ppm methyl benzoate and 10,200 ppm methyl palmitate (Table 4.2).

Table 4.2: *Phthalate Multi-Component Stock Solution Preparation*

Identity of Methyl Ester	Mass of Methyl Ester	Total Volume of Multi-Component Stock Solution	Concentration of Each Methyl Ester in Multi-Component Stock Solution	Identity of Multi-Component Stock Solution Solvent
Methyl Benzoate	0.5060 g	50.00 mL	10,100 ppm	Methanol
Methyl Palmitate	0.5093 g		10,200 ppm	

iv. Internal Standard Stock Solution

A 9,980 ppm stock solution of the selected internal standard for this experiment, methyl 4-cyanobenzoate, was created by dissolving 0.2496 g of the methyl ester in methanol in a 25.00 mL volumetric flask.

v. External Calibration Standards

Five multi-component methyl ester standards with concentrations of each methyl ester ranging from approximately 1,000 ppm to 8,000 ppm were prepared from the multi-component methyl ester stock solution (Table 4.3). Additionally, a blank (0 ppm) was prepared. The multicomponent methyl ester stock solution with concentrations of 10,100 ppm methyl benzoate and 10,200 ppm methyl palmitate was dispensed into 10.00 mL volumetric flasks with volumetric pipettes. The standards were then diluted to their desired concentrations with methanol.

Table 4.3: *Methyl Ester Multi-Component External Calibration Standards Preparation*

Standard	Volume of Multi-Component Stock Solution Added	Total Volume of Multi-Component Calibration Standard	Final Concentration of Methyl Benzoate in Calibration Standard	Final Concentration of Methyl Palmitate in Calibration Standard
Blank	0.00 mL	10.00 mL	0 ppm	0 ppm
1	1.00 mL	10.00 mL	1,010 ppm	1,020 ppm
2	2.00 mL	10.00 mL	2,020 ppm	2,040 ppm
3	4.00 mL	10.00 mL	4,040 ppm	4,080 ppm
4	5.00 mL	10.00 mL	5,050 ppm	5,100 ppm
5	6.00 mL	10.00 mL	6,060 ppm	6,120 ppm

Each of the external standards were then transferred to gas chromatography vials in 1.00 mL portions. Each vial contained 0.050 mL of the 9,980 ppm internal standard stock solution. The resulting concentrations of each calibration standard are listed in Table 4.4.

Table 4.4: *Addition of Internal Standard to Methyl Ester Multi-Component External Calibration Standards*

Standard	Volume of Calibration Standard Added to GC Vial	Volume of Internal Standard Stock Solution in Calibration Standard	Final Concentration of Methyl Benzoate in Calibration Standard	Final Concentration of Methyl Palmitate in Calibration Standard	Final Concentration of Internal Standard in Calibration Standard
Blank	1.00 mL	0.050 mL	0 ppm	0 ppm	0 ppm
1	1.00 mL	0.050 mL	962 ppm	971 ppm	475 ppm
2	1.00 mL	0.050 mL	1,920 ppm	1,940 ppm	475 ppm
3	1.00 mL	0.050 mL	3,850 ppm	3,890 ppm	475 ppm
4	1.00 mL	0.050 mL	4,810 ppm	4,860 ppm	475 ppm
5	1.00 mL	0.050 mL	5,770 ppm	5,830 ppm	475 ppm

vi. Quality Assurance/Quality Control

In addition to the external standards, a laboratory fortified bank (LFB) was also prepared. The LFB was prepared by diluting 3.00 mL of the multicomponent methyl ester stock solution that contained 10,100 ppm methyl benzoate and 10,200 ppm methyl palmitate in methanol in a

10.00 mL volumetric flask, yielding a solution that was 3,030 ppm methyl benzoate and 3,060 ppm methyl palmitate. A 1.00 mL aliquot of the LFB was then transferred to a gas chromatography vial, where 0.050 mL of the 9,980 ppm internal standard stock solution was added to the LFB, yielding a final concentration of 2,890 ppm methyl benzoate, 2,910 ppm methyl palmitate, and 475 ppm internal standard.

vii. Sample Preparation

Two samples were prepared from the product described in Chapter 3 of this work. If both benzoic acid and palmitic acid underwent complete conversion to their respective methyl esters, which is likely the case according to the ^{1}H-NMR of the product, then the product mixture described in Chapter 3 should contain 76.62% methyl benzoate and 23.38% methyl palmitate. The first sample was created to ensure the concentration of methyl benzoate would fall within the range of concentrations given in the calibration curve, while the second sample was intended to have a similar result for methyl palmitate.

The methyl benzoate focused sample was prepared by dissolving 0.0089 grams of the methyl ester product mixture in methanol in a 2.00 mL volumetric flask, yielding a solution that contained 4,500 ppm of the product mixture. A portion of this solution (1.00 mL) was then transferred to a GC vial, and 0.050 mL of the 9,980 ppm internal standard stock solution was added, yielding a final solution that was 4,200 ppm of the methyl ester product mixture. If complete conversion from benzoic acid to methyl benzoate occurred, the product mixture should be 76.62% methyl benzoate, yielding a solution that contains 3,200 ppm methyl benzoate and 475 ppm internal standard.

The methyl palmitate focused sample was prepared by dissolving 0.0438 grams of the methyl ester product mixture in methanol in a 2.00 mL volumetric flask, yielding a solution that

contained 21,900 ppm of the product mixture. A portion of this solution (1.00 mL) was then transferred to a GC vial, and 0.05 mL of the 9,980 ppm internal standard stock solution was added, yielding a final solution that was 20,900 ppm of the methyl ester product mixture. If complete conversion from benzoic acid to methyl benzoate occurred, the product mixture should be 23.38% methyl palmitate, yielding a solution that contains 4,880 ppm methyl palmitate and 475 ppm internal standard.

vii. Data Analysis

The chromatograms of the three single component standards containing methyl benzoate, methyl palmitate, and methyl 4-cyanobenzoate were used to determine the retention time/elution order of each of the three methyl esters. Once these retention times for each of the three methyl esters had been established, these times were used to isolate the peaks of interest from the chromatograms of each of the external calibration standards, in addition to that of the blank, LFB, and multicomponent sample. The peak area for each of the methyl esters was then determined from the chromatogram of each of the multicomponent standards. An external calibration curve was then created for each of the two methyl esters of interest.

Results

The 5,000 ppm calibration standard was used to determine the oven parameters required to ensure separation of the three methyl esters (Figure 4.1). Once peak separation had been established, the retention times of the three methyl ester were determined by analyzing the gas chromatograms of the three single component standards. The retention times of methyl benzoate, methyl palmitate, and methyl 4-cyanobenzoate were found to be 5.19 min, 7.95 min, and 8.05 min, respectively, as listed in Table 4.5.

Table 4.5: *Retention Times of Methyl Esters of Interest*

Methyl Ester	Retention Time of Single Component Standard
Methyl Benzoate	5.19 min
Methyl Palmitate	7.95 min
Methyl 4-Cyanobenzoate	8.05 min

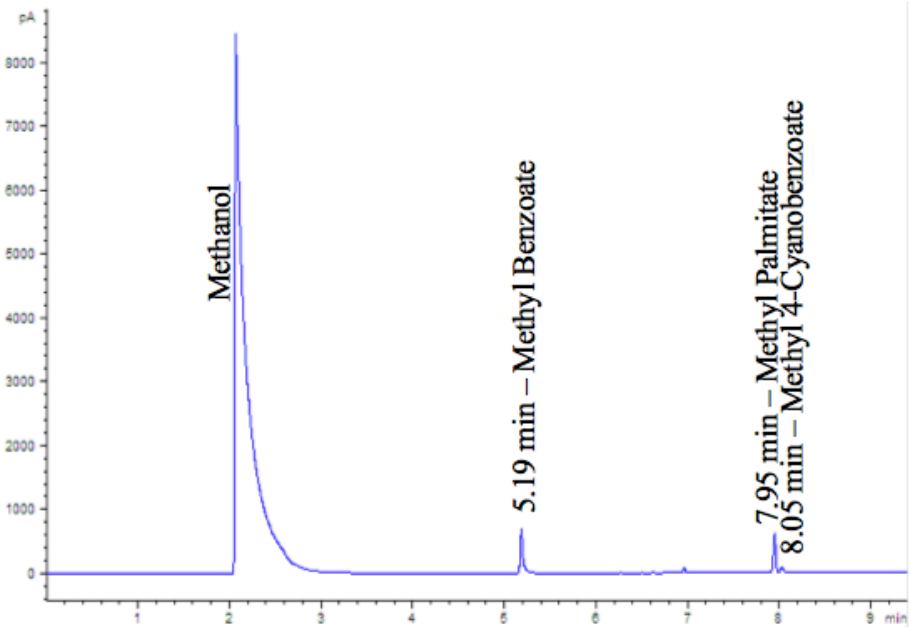


Figure 4.1 *Example Gas Chromatograph of a Multi-Component Methyl Ester External Calibration Standard*

Each of the five external calibration multicomponent methyl ester standards were analyzed. Data for the two methyl esters of interest, as well as the internal standard were organized using their respective retention times.

Methyl benzoate was found to have a retention time 5.19 minutes, while the internal standard was found to have a retention time of 8.05 minutes. The peak area for methyl benzoate was divided by the peak area of the internal standard for the triplicate trials of each standard. These values, as well as the average peak area, standard deviation, and relative standard deviation can be found in Table 4.6.

Table 4.6: *Methyl Benzoate External Calibration Peak Area from Gas Chromatography*

Methyl Benzoate Concentration (ppm)	Peak Area 1	Peak Area 2	Peak Area 3	Average Peak Area	Standard Deviation	Relative Standard Deviation
0 ppm (Blank)	Not Detected	Not Detected	Not Detected	N/A	N/A	N/A
962 ppm	1.44694	1.50714	1.40574	1.45	0.05	0.04
1,920 ppm	2.83295	2.78435	2.83260	2.82	0.03	0.01
3,850 ppm	5.94862	4.96799	5.52716	5.5	0.5	0.09
4,810 ppm	5.82037	6.62172	6.60176	6.3	0.5	0.07
5,770 ppm	8.26374	7.84782	6.27623	7	1	0.1

When plotting the methyl benzoate concentration of the multicomponent external standards against the average peak area (Figure 4.2), a trend line of $y = 0.00134 \pm 0.00003x$ can be found. The trend line has been forced through zero to afford the greatest possible coefficient of determination, 0.998.

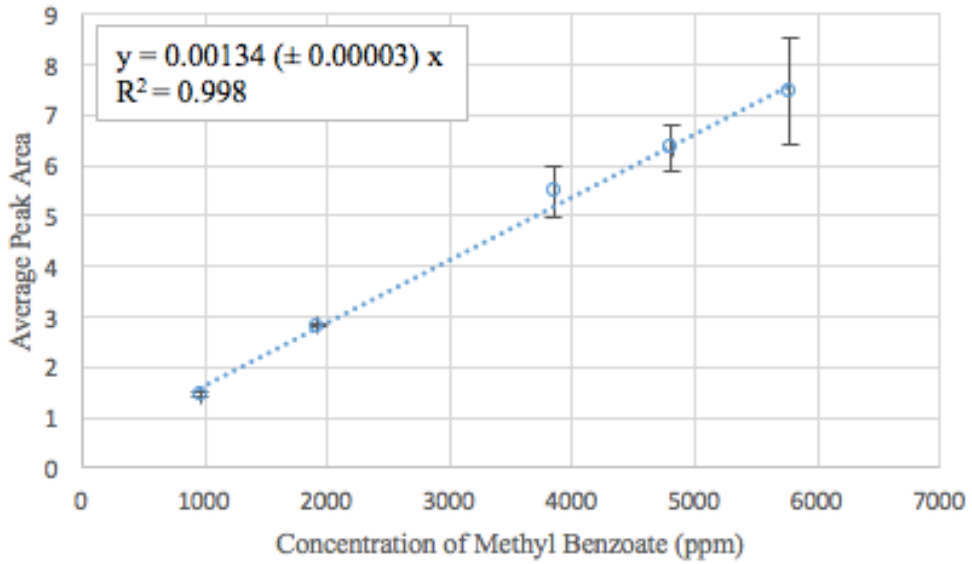


Figure 4.2: *Concentration of Methyl Benzoate vs. Peak Area (Analyte/IS) from GC-FID*

An LFB containing a known concentration of methyl benzoate (2,890 ppm) was analyzed in triplicate in order to determine to quality of the calibration curve shown in Figure 4.2. The

LFB exhibited an average peak area of 3.8 ± 0.3. According the line of best fit established from the calibration curve, the concentration of the LFB was 2800 ± 100 ppm, a percent yield of 96.9%.

Methyl palmitate was found to have a retention time 7.95 minutes, while the internal standard was found to have a retention time of 8.05 minutes. The peak area for methyl palmitate was divided by the peak area of the IS for the triplicate trials of each standard. These values, as well as the average peak area, standard deviation, and relative standard deviation can be found in Table 4.7.

Table 4.7: *Methyl Palmitate External Calibration Peak Area from Gas Chromatography*

Methyl Palmitate Concentration (ppm)	Peak Area 1	Peak Area 2	Peak Area 3	Average Peak Area	Standard Deviation	Relative Standard Deviation
0 ppm (Blank)	Not Detected	Not Detected	Not Detected	N/A	N/A	N/A
971 ppm	1.19475	1.33428	1.29078	1.27	0.07	0.06
1,940 ppm	2.10968	2.23208	2.21086	2.18	0.07	0.03
3,890 ppm	5.48862	4.08923	5.02524	4.9	0.7	0.1
4,860 ppm	5.78148	5.01123	6.73531	5.8	0.9	0.1
5,830 ppm	6.95676	8.95838	6.88748	8	1	0.2

When plotting the methyl palmitate concentration of the multicomponent external standards against the average peak area (Figure 4.3), a trend line of $y = 0.00125 \pm 0.00003x$ can be found. The trend line has been forced through zero to afford the greatest possible coefficient of determination, 0.998.

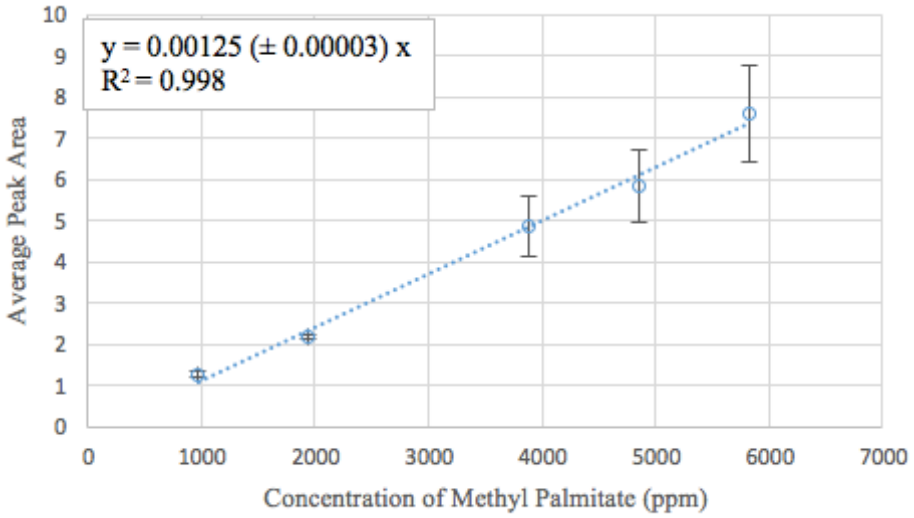


Figure 4.3: *Concentration of Methyl Palmitate vs. Peak Area (Analyte/IS) from GC-FID*

An LFB containing a known concentration of methyl palmitate (2,910 ppm) was analyzed in triplicate in order to determine to quality of the calibration curve shown in Figure 4.3. The LFB exhibited an average peak area of 3.8 ± 0.6. According the line of best fit established from the calibration curve, the concentration of the LFB was 3000 ± 100 ppm, a percent yield of 103.1%.

The two samples created from the methyl ester product synthesized in Chapter 3 were analyzed in triplicate by GC. The retention times and peak areas were then collected and examined using the calibration curves described above (Figures 4.2 and 4.3) the results are listed below.

Table 4.8: *Concertation of the Two Methyl Esters of Interest in their Respective Sample Solutions based on External Calibration*

Methyl Ester Sample	Peak Area 1	Peak Area 2	Peak Area 3	Average Peak Area	Concentration (Derived from Calibration Curves)
Methyl Benzoate (Sample Solution 1)	4.33656	4.73105	3.93471	4.3 ± 0.4	3,200 ± 100 ppm
Methyl Palmitate (Sample Solution 2)	4.56337	6.30042	6.19016	6 ± 1	4,500 ± 100 ppm

Discussion

Each of the two calibration curves generated in this experiment displayed coefficents of deterimination equal to 0.998. This value suggests that the calibrations standards were prepared efficiently and that the calibration curves are sufficiently linear for the examination of samples. That being said, it is worth mentioning that when analyzed, the final three standards for each calibration curves exhibited peak values with large relative standard deviations, indicating the possibility of some amount of error in the method of integration. Although these relative standard deviation values are worth noting, they are less than or equal to 20% in all cases, and are therefore deemed acceptable for the internal standard technique in gas chromatography.[13]

An LFB known to contain 2,890 ppm methyl benzoate and 2,910 ppm methyl palmitate was the primary source of quality control in this experiment and was intended to determine the accuracy of the calibration curves. After triplicate trials, the LFB was determined to contain 2800±100 ppm methyl benzoate and 3000±100 ppm methyl palmitate according to their respective calibration curves. These values represented percent yields of 96.6% and 103.3%, respectively. Since the known value of each of the species in the LFB falls within the uncertainty range of the concentration calculated from the line of best fit, it is known that the two calibration curves are accurate at their centroids.

Of the two samples analyzed, the methyl benzoate focused sample (which theoretically contained 3,200 ppm methyl benzoate) was found to contain 3,200 ± 100 ppm methyl benzoate according to the calibration curve shown in Figure 4.2, a percent yield of 100%. This result indicted that the esterification method described in Chapter 3 was effective at producing quantitative yields of the methyl ester from its corresponding carboxylic acid.

The methyl palmitate focused sample (which theoretically contained 4,880 ppm methyl palmitate was found to contain 4,500 ± 100 ppm methyl palmitate according to the calibration curve shown in Figure 4.3, a percent yield of 92.2%. Although this value is lower than the expected value, there is a possible source for this error, other than the incomplete conversion of palmitic acid into methyl palmitate, as described in Chapter 3. In the three peak areas collected from the triplicate analyses of this sample there is a large value of standard deviation. That being said, two of the values are quite similar, with one notable outlier. When the two similar peak values are averaged, the outcome is 6.2, a value with a corresponding concentration of 5,000 ± 100 ppm methyl palmitate. Although this idea is purely speculation, and would require additional testing to confirm, it is possible that the concentration discrepancy seen in methyl palmitate is due to instrumental error. This possibility could be confirmed by analyzing the methyl palmitate sample again, and using a Grubb's test to determine if the outlying trial could be eliminated. Unfortunately, due to time constraints, it was not possible to experimentally explore this hypothesis.

Chapter 5: Conclusions and Future Work

Gas chromatography is a popular method for the identification and quantification of sample mixtures. Currently, there are no simple methods for the direct analysis of carboxylic acids via gas chromatography. This research has proposed and experimentally supported an efficient universal method for the derivatization of simple carboxylic acids to methyl esters. This conversion has been seen to produce quantitative yields without excessive heat or expensive catalysts, making it an ideal choice for the analysis of carboxylic acids. Establishing the experimental procedure in this project has set the groundwork for the eventual optimization and application to more complex molecules.

Once the preliminary 'proof of concept' stage for this project has been completed, there is a great deal of potential for future work. Time dependent studies could be employed to determine the minimum time for 100% conversion of each species of carboxylic acid into its corresponding methyl ester. Additionally, the parameters of the experiment, such as amount of SOMS utilized and reaction temperatures, can be optimized in order to yield the most efficient reaction conditions (both in terms of reaction time and environmental impact). Additionally, the procedural basis that this work is setting can eventually be applied to more interesting molecules and mixtures.

Appendix

Abbreviations

Deuterated Chloroform	$CDCl_3$
Dichloromethane	DCM
Laboratory Fortified Blank	LFB
Proton Nuclear Magnetic Resonance	^{1}H-NMR
Round Bottom Flask	RBF
Swellable Organically Modified Silica	SOMS
Gas Chromatography	GC

Terminology

Open – The state of the SOMS nano-reactors after they have swelled with organic solvent

Close – The state of the SOMS nano-reactors once an experimentally added organic solvent has been removed.

Flex – The process of ‘opening’ and ‘closing’ the SOMS nano-reactors through the continuous evaporation and reintroduction of solvent.

Materials

Table 6.1: *List of Chemicals and Compounds Used During Experimentation*

Name	Additional Name or Abbreviation	CAS #	Vendor
Benzoic Acid	----------	65-85-0	Sigma Aldrich
4-Methoxybenzoic Acid	p-Anisic Acid	100-09-4	OxChem
4-Nitrobenzoic Acid	----------	62-23-7	Alfa Aesar
Palmitic Acid	Hexadecanoic Acid	57-10-3	Sigma Aldrich
Oleic Acid	cis-9-Octadecenoic Acid	112-80-1	Sigma Aldrich
Methyl Benzoate	----------	93-58-3	Sigma Aldrich
Methyl Palmitate	Methyl Hexadecanoate	112-39-0	Synthesized in Lab*
Methyl 4-Cyanobenzoate	----------	1129-35-7	Lancaster
3 N HCl in Methanol	----------	----------	Sigma Aldrich
Methanol	----------	----------	Alfa Aesar
Dichloromethane	Methylene Chloride, DCM	----------	Alfa Aesar
Deuterated Chloroform	$CDCl_3$	865-49-6	Alfa Aesar

***** The methyl palmitate used to create the standards for the gas chromatography portion of this experiment was synthesized in the Shaw Laboratory. It is acknowledged that this decision is not best practice due to the possibility of contamination in the standards. Should this work be continued, this experiment would need to be repeated with a pure standard purchased from a reliable source. The methyl palmitate used in this experiment was synthesized via traditional Fischer esterification, washed with 1 M sodium hydroxide to ensure that all starting material had been removed, and investigated by NMR to ensure that the product was pure.

Methods

Washing SOMS

The SOMS described in these experiments was reused throughout experimentation, and had been used for different experiment prior to the commencement of this study. In total, approximately 20 grams of SOMS was used in these experiments. Between trials, the SOMS would be rinsed with 200 mL of methanol, 200 mL of acetone, and 200 mL of dichloromethane via vacuum filtration. The SOMS would then be left to dry at least 24 hours before reuse.

NMR of Carboxylic Acid Starting Materials

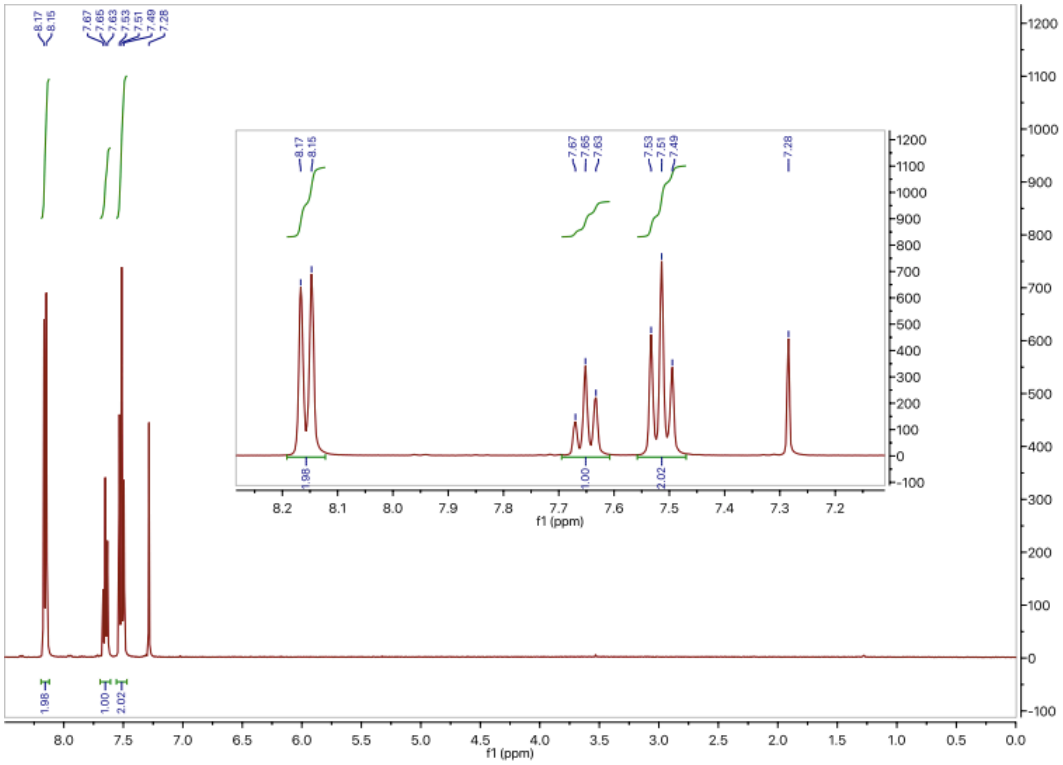


Figure 7.1: *^{1}H-NMR Benzoic Acid*

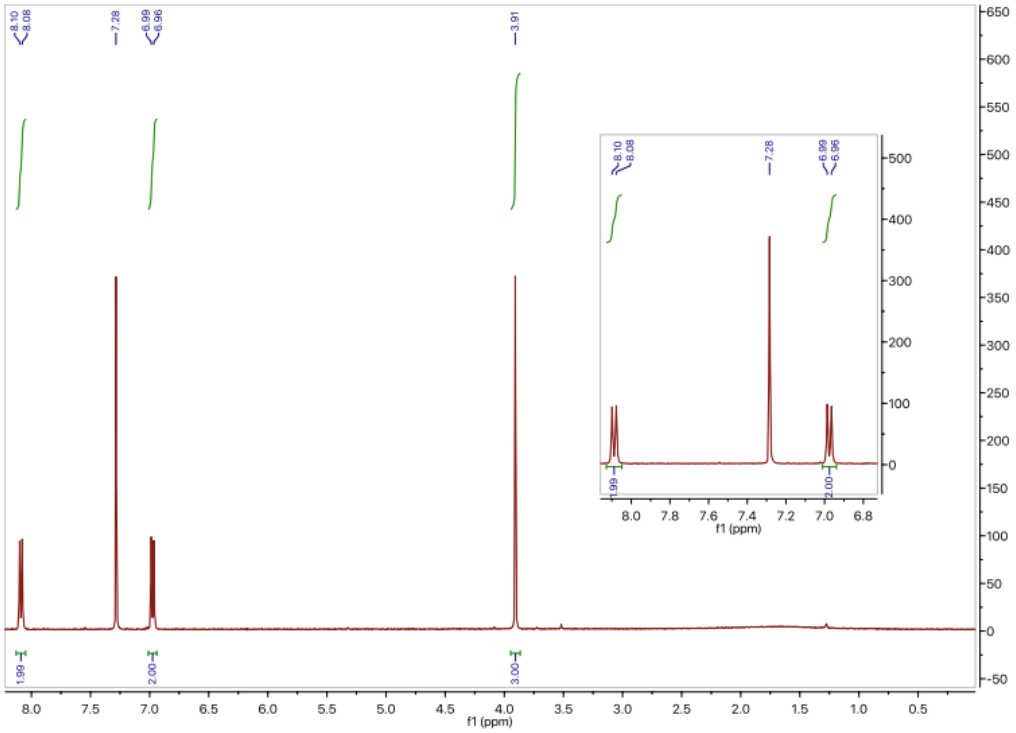


Figure 7.2: *[1]H-NMR 4-Methoxybenzoic Acid*

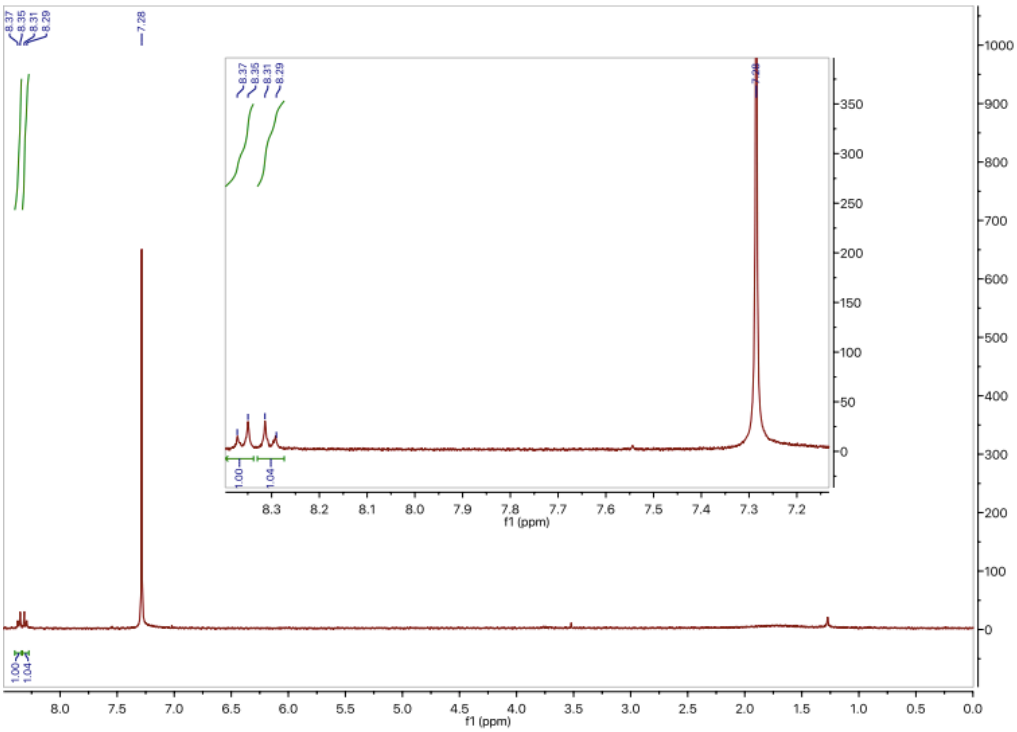


Figure 7.3: *[1]H-NMR 4-Nitrobenzoic Acid*

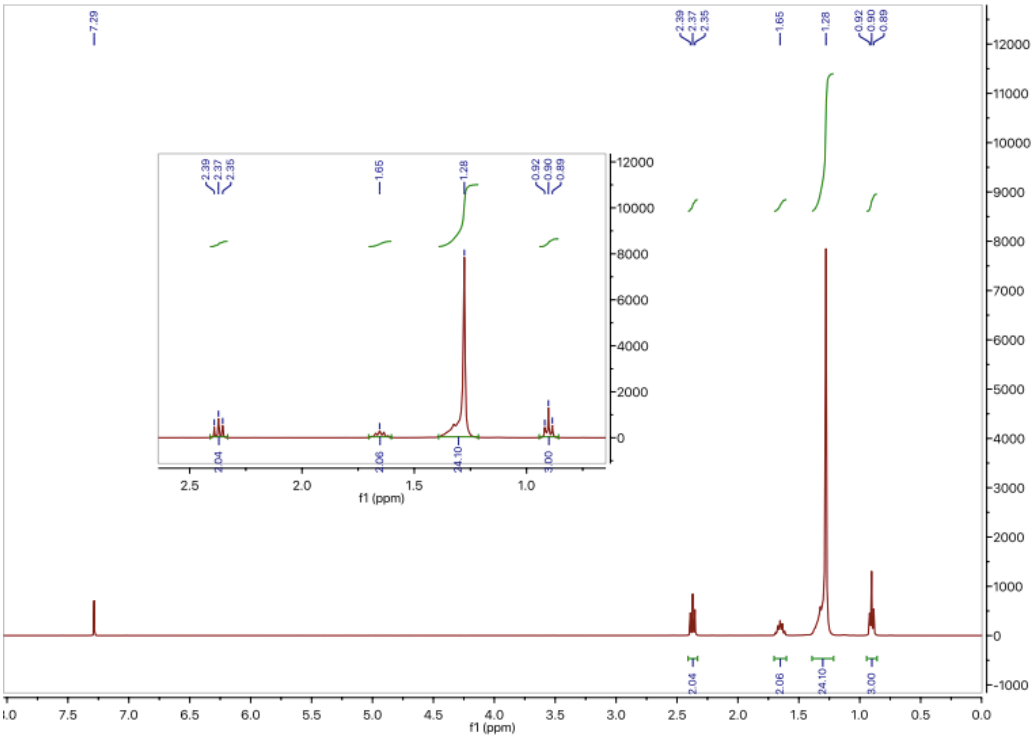


Figure 7.4: *[1]H-NMR Palmitic Acid*

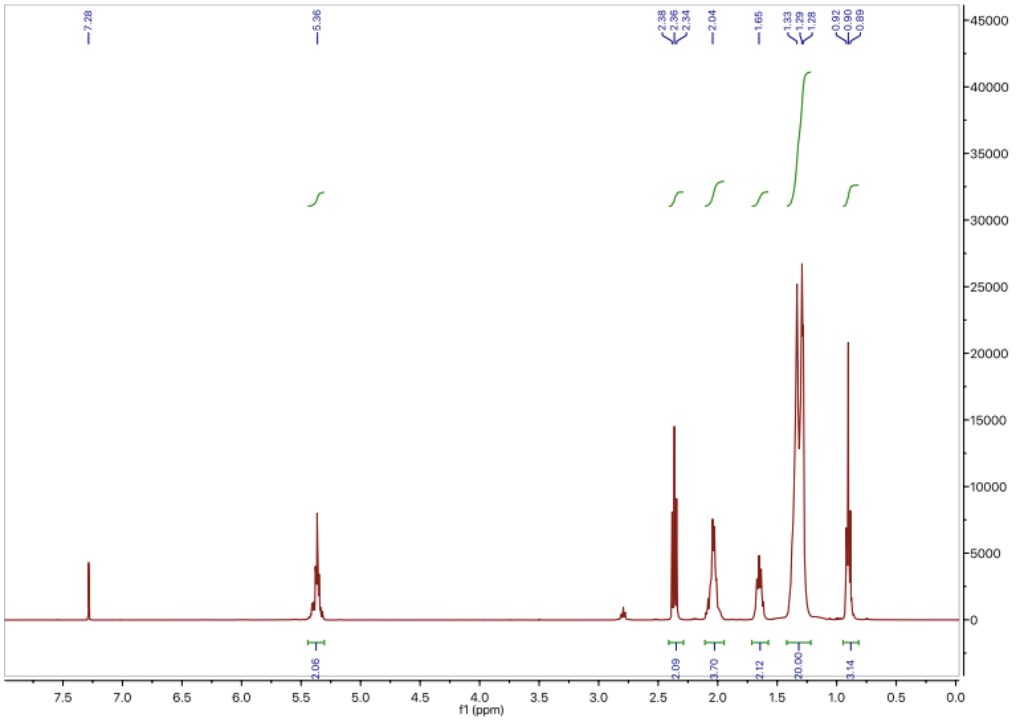


Figure 7.5: *[1]H-NMR Oleic Acid*

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