Design, Synthesis and Investigation into the Applications of Phospholipid Tubules



A Senior Comprehensive Project
Allegheny College
Meadville, PA 16335
Presented by:
Brennan Cahill

April 13th, 2018

Submitted to the Chemistry Department, Allegheny College
in partial fulfillment of the requirements for the degree of
BACHELOR OF SCIENCE

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ACKNOWLEDGEMENTS

To Dr. Persichini, thank you for always challenging me in the lab. This year has not only taught me to grow as a chemist, but also as a person. I will always be grateful that you let me join your lab my junior year. Thank you for always being there for when I had any questions. You were a great mentor to have through my time here at Allegheny and I will always appreciate everything you have done for me here.

To Dr. Hersh, thank you for your help through the comp process. I appreciate your contributions and questions that you asked during my defense.

To Dr. Deckert, thank you for making me a better student. I was glad to see that you were on my committee because I knew that you would push me to work harder. From having class with you I know your expectations are high but fair, and hope that I have done a good job from your perspective.

To. Dr. Chapp, thank you for your help and guidance in lab. I really appreciate you helping me even though I was never a student of yours. Your help let me learn new lab techniques that I will never forget.

To Bob Caskey, thank you for putting up with me bothering you at random times throughout the day. Steffee would be nothing without you keeping the labs in check. Your positive words of encouragement to keep up the good work really kept me going.

To Ben Bloom, the O-chem God. Thank you for everything. You have been so helpful not only as a classmate but as a friend. Your knowledge and love of chemistry is truly unmatched. I wish you the best after Allegheny.

To Katie, thank you for somehow putting up with me. I know I do not make that easy sometimes. I would not be where I am if it wasn't for you. I'm happy how much we have grown together this year. I know you will do great next year and have a fun senior year. Thank you for everything, you don't know how much I truly appreciate you being there for me through it all.

To Cala, Maria and Lindsay, thank you guys for being you. I'm really glad with how close we have gotten just over this past year. You guys helped to cheer me up when I was worried about comping or just other work. I know we will stay in touch over the years and wish you guys nothing but the best.

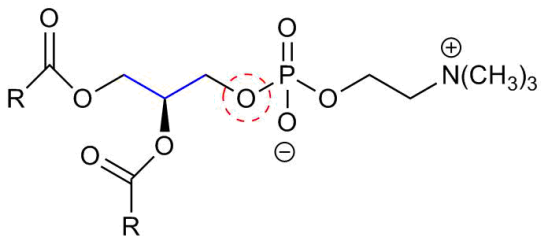
To the tower, Jimmy and Nick, thank you for all the support. You guys are the best people I know and am honored to be friends with each of you. I don't know what I would do without you guys. Our friendship over the years has made us brothers. Good luck to all of you in the future, I know you all will do great things.

To Mom and Dad, thank you for everything. I work as hard as I do for you guys. You guys inspire me to be a better person. Your love and support always gets me through the tough times and I know that I can go to you guys for anything.

ABSTRACT

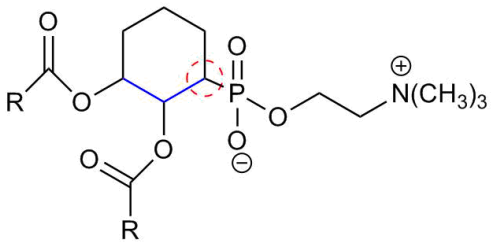
Phospholipid tubules have surfaced as a primary target to help with many potential medical and technological applications. Previous work focused on modifications to the nonpolar fatty acid side chains and the polar head group of the phospholipid. Currently, modifications to the glycerol backbone of phospholipids are being investigated. This modification helps define the molecular scope of the phospholipid's ability to form tubules. Potential new tubule-forming lipids in which the "C-O-P" glycerol backbone linkage of the typical tubule forming phospholipid, 1-2-bis(10,12-tricosadiynoyl)-*sn*-glycerol-3-phosphocholine, otherwise known as $DC_{8,9}PC$, is replaced by a cyclohexene ring linkage. We hypothesize that this modification will not affect the ability for this "monomer" to form tubules relative to $DC_{8,9}PC$. Experiments are currently underway to prove this modification will result in tubule formation.

$DC_{8,9}PC$



$(CH_2)_8—C\equiv C—C\equiv C—(CH_3)_9CH_3$

New tubule-forming Analog



$(CH_2)_8—C\equiv C—C\equiv C—(CH_3)_9CH_3$

INTRODUCTION

Overview of Phospholipids

Lipids are a broad ranging group of compounds that, along with proteins, nucleic acids and carbohydrates, make up the four building blocks of life. Oils, fats and waxes are molecules that comprise a diverse class of naturally occurring lipids.[1] These molecules are made mostly of long, nonpolar hydrocarbon chains called fatty acids. Fatty acid chains can bond to varying molecules to form different types of lipids. The unique structure of lipids correspond to the diverse roles they can play in organisms. The main biological functions of lipids include acting as structural constituents of cell membranes and serving as a major energy source.[2] The lipid structure of interest is the phospholipid.

Phospholipids are fundamental components of all cell membranes.[3] They are amphipathic molecules consisting of hydrophilic regions that interact with water and hydrophobic regions which repel water. The orientation of their hydrophilic and hydrophobic regions direct their packing into membrane bilayers. The structure of the phospholipid consists of a phosphate group, most commonly called the head group, and the tail group consisting of chains of fatty acids. The fatty acid chain can vary in length and may have varying degrees of saturation. The middle portion of phospholipids contain a glycerol group, which serves as the backbone structure that connects the head and the tail groups (Figure 1). The distinguishing polar head consists of a basic group attached to diacylglycerol-3-phosphate or 1,2-diacylglycerol which constitutes the hydrophilic portion of the molecule.[4] The fatty acid chains embody the opposing hydrophobic portion of the molecule.[3]

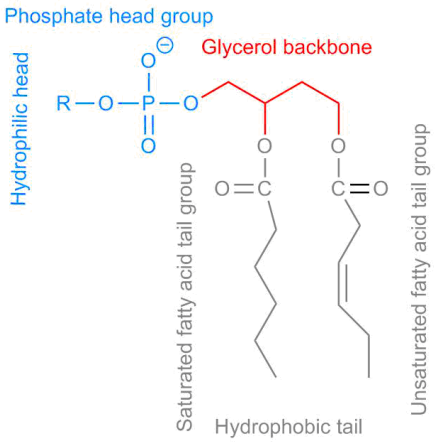


Figure 1. This structure shows a generic phospholipid. The hydrophilic head group, on the left, is directly attached to the glycerol backbone. The glycerol backbone serves as the connector of the head group to the fatty acid side chains, extending downwards from the backbone, making up the hydrophobic tail.

Modified phospholipids have been used in a variety of technological applications, ranging from medical encapsulation to control and release systems.[5-7] The specific change to the structure of a phospholipid determines the extent to which it can be utilized. Understanding the exact functionality of phospholipids is key in determining the best way to optimize their morphology for given applications.[5,8] For example, the length and diameter of a modified phospholipid will have a direct effect on the rate of diffusion of the particular encapsulated material.[8] The wall thickness of a phospholipid is equally as crucial because certain lipids may be coated if they have multiple bilayers, and if the layer is thin, this would not serve the required properties. Additional research must be done in order to appropriately identify the capability of delivery, minimize material loss during therapeutic applications, and further explore the carrying capacity of the phospholipid.[5,8,9]

Behavior of Phospholipids

The environment surrounding lipids determines their orientation. By having lipids in an aqueous medium, they have a tendency to self-assemble into a variety of mesophase structures which include monolayers, micelles, and bilayers.[10] The behavior of lipids is dictated by many

internal parameters such as lipid polarity of either the head group or fatty acid chain. One of the mesophase structures that forms when lipids are dispersed in an aqueous medium are micelles. These structures are spherical aggregates commonly formed by single-tailed lipids such as surfactants molecules (Figure 2).[10,11] A micelle in aqueous medium forms an aggregate with the hydrophilic polar head regions in contact with the surrounding solvent. This forces the nonpolar hydrophobic single-tail regions to point towards the center of the cell.[10]

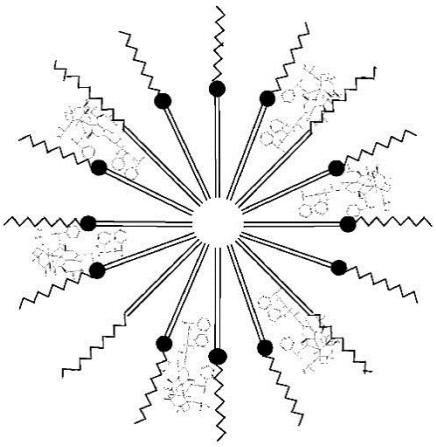


Figure 2. Representation of a micelle. This micelle is capable of encapsulating poorly soluble anticancer drugs. The polar head group of the micelle, depicted by the dark circles, will interact with the surrounding solvent. The nonpolar tails will self-assemble and fold inwards to form a cluster.

Studies have shown that this behavior exhibited by micelles may be utilized to help in drug delivery.[11] A study conducted by Zhonggao et al showed that when you have medicine that is poorly soluble in water, micelles have the capability to act as effective encapsulating agents.[11] The encapsulation method works due to the ability of the micelle to form a compartment that is able to trap and hold the medicine. This event occurs when the micelle is exposed to the aqueous medium and hydrophobic tails cluster to form the center core. The center core now acts as the perfect storage space for the medicine. This method of drug delivery by micelles may be a convenient solution for drugs that are poorly water soluble.[10-14]

Typically, when the structure of acyl chains exceeds eight carbon atoms, the micelle structure is no longer favorable, resulting in the formation of another mesophase structure known as the phospholipid bilayer. The bilayer is a necessary structure because it is the barrier that marks the boundaries of our cells, preventing contents of the cell from diffusing into areas where they do not belong. The bilayer is able to control cell shape, as well as other necessary functions including storage of compounds, ion transport, and metabolism.[10] The phospholipids organize themselves into a bilayer in order to hide their hydrophobic tail regions and expose their hydrophilic regions to water (Figure 3).[10,15] This orientation is similar to the micelles in the way that the nonpolar hydrophobic tail is oriented towards the interior of the membrane while the polar hydrophilic head is pointed towards the aqueous medium.[12]

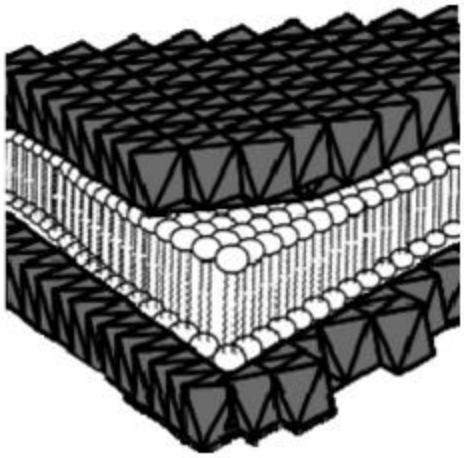


Figure 3. The lighter region, found in the middle of the figure, is representative of the lipid bilayer. The hydrophobic portion makes up the core of the bilayer. The hydrophilic portion, represented by the outward facing circular heads, interacts with the hybrid material.

A study by Begu et al investigated the lipid bilayer as a utility of drug encapsulation.[15] The way in which the bilayer folds when interacting with the aqueous medium is the exact same as the micelle. The lipid bilayer will form a center core that is able to trap the medicine from the aqueous medium. The experiment found that the phospholipid bilayer may also serve as an acceptable storage and release pathway for drugs that are poorly water-soluble. Additional

experiments will need to be conducted in order to find out the exact storage and release parameters of bilayers.[15-17]

Understanding the properties behind this interaction of hydrophobic and hydrophilic regions serve as an important concept moving forward. In order to correctly form an aggregate structure, such as the micelle or lipid bilayer, the hydrophobic region and the surrounding aqueous medium must be in the proper orientation. It is crucial that the modified phospholipid has a nonpolar region that will readily self-assemble when interacting with an aqueous medium so that the correct structure may be achieved.

Applications of Phospholipids

Phospholipids are being altered to employ in real life applications. The first modification of the phospholipid was done by inserting diacetylene fatty acid chains in the tail region to self-assemble tubules that are stable, hollow, crystalline vesicles with cylindrical microstructures.[8] This was first observed by Yager and co-workers.[8,18-21] The successful synthesis of tubules has led to the investigation of other regions of the phospholipids to see if the tubule-forming capacity will change. The specific alterations that we are interested in are those to the glycerol backbone, first investigated by Thomas et al.[8] They altered the phospholipid backbone to produce changes in the mesophase structure. Results indicated that tubule formation was not affected. The growing interest of phospholipids stems from the versatility of these structures to be used in measureless applications.

Lipid tubules have demonstrated to be effective carriers for a wide variety of drugs, proteins, peptides, and DNA due to their ability to encapsulate and control release of material over time.[23] For example, Yui et al was able to encapsulate an iron storage protein in a hollow

nanotube.[22,23] Several other studies were done to demonstrate that proteins such as nerve growth factors and bone morphogenetic proteins can also be encapsulated.[23]

Phospholipids are still being considered for their full capability as drug and release carriers. A delivery technique that is currently being investigated is liposome encapsulation technology. A liposome is a vesicle that has at least one lipid bilayer. This way of transport can get the medicine to the location of interest. These liposomes work in a manner in which they protect the encapsulated medicine by forming a compact barrier. The idea is that by having this barrier, the medicine will be protected from degradation. The liposome will be undamaged until the inside content is delivered to the desired location. This method of delivery assures that the location of interest will get the full contents of the medicine.[24]

There are different ways in which the liposomes may receive the contents they carry. Medicine can be loaded either during or after the liposome is formed. The drug being loaded during liposome formation has shown to be the superior method. This is because the contents that would be added after liposome formation would dispense out very quickly if not for entrapping tools such as pH gradients. As for the drugs loaded during formation, the liposomes would be wrapping the drugs in the middle of the layers, protecting them from dispersing out. The effectiveness of the drug to trap the medicine is often one hundred percent, however, the actual percentage depends on the solubility of the drug in the membrane.[24]

After liposomes have been administered, the drawback as drug carriers depends on the interactions with particular cells. *In vivo* and *in vitro* studies have shown that there are two main types of limiting interactions that lipids face when encountering cells. The first is by absorption, which could be due to like charges or a difference in charges. The second interaction implies that the lipid could be degraded by neutrophils or macrophages. Determining which specific

interaction may be the cause of the lipid not arriving at the correct location needs to be solved in order to make this mode of drug delivery more reliable.[24]

While the focus of lipids and tubules has been on the medical field, usefulness of these structures are not limited to this area. Further use for phospholipids is in controlled release applications. Price et al used tubules with tetracycline inside which was released at a specified rate over a period of time (Figure 4).[25]

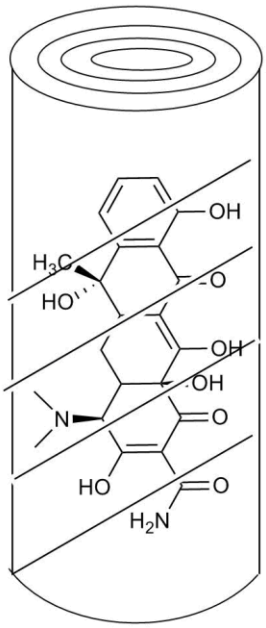


Figure 4. This figure illustrates a cartoon representation of a tubule encapsulating tetracycline. This method of delivery served to be an appropriate measure to deal with marine fouling. The diffusion rate of tetracycline is much slower when dispensed out of a tubule, thereby providing more protection from degradation.

This was used to help with marine fouling, which is the build-up of organisms on material exposed to the marine environment. This example used metal rods to indicate degradation by fouling, which could then be applied on a larger scale to help reduce degradation to underwater structures. They showed that the rate of diffusion was affected by the length of the encapsulating tubule. Tubules with a larger diameter may be able to release more of the tetracycline and in turn stop fouling for a longer period of time. In comparison, when tetracycline was mixed directly in

the paint of the rod, it was released almost immediately and fouling began to take place sooner. From this experiment, they were able to demonstrate that the rate of release could be controlled in tubules.[7,25]

Tubule formation

In order for phospholipids to be used as transport and release systems, they must be able to form tubules. The parameters that must be controlled for these applications to be implemented are the tubules' capacity to dispense the material, the diffusion rate of the material, and the ability to place the tubule in the desired location of the body.[8] Most of all, the specific applications of these tubules depends largely on their diameter. Self-assembled tubule structures are typically tens of microns in length and roughly 1 micron in diameter (Figure 5).[8,20] Self-assembly occurs due to the amphipathic nature of the molecule.

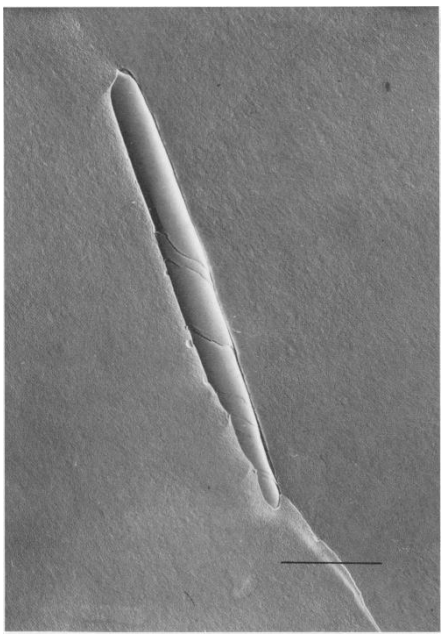


Figure 5. This is a free fractured replica of a tubule showing helical wrapping. This tubule is "upside-down" since the ribbon that it is formed from is at the bottom right hand corner. This exhibits a right handed sense of winding. The scale bar is set to 1 micron.

The typical "monomer" that is investigated is the tubule-forming molecule, 1,2-bis(10,12-tricosadiynoyl)-sn-glycero-3-phosphocholine ($DC_{8,9}PC$), which is able to form tubules consisting

of multiple bilayers (Figure 6).[8] This molecule is named this way because it contains eight methylenes between the ester and diacetylene groups, and nine methylenes between the diacetylene and terminal methyl groups.[16]

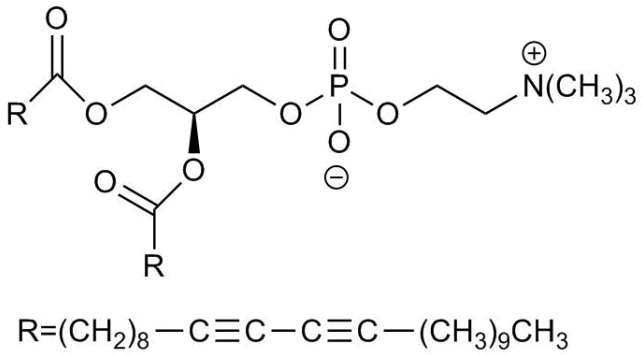


Figure 6. 1,2-bis(10,12-tricosadiynoyl)-sn-glycero-3-phosphocholine

When modifications are made to this molecule, such as inserting diacetylene groups midway in the hydrocarbon tails, this is where we see cylindrical, hollow vesicles form. These hollow cylinders possess an exterior helical trace similar to that found on a paper drinking straw (Figure 7).[20]

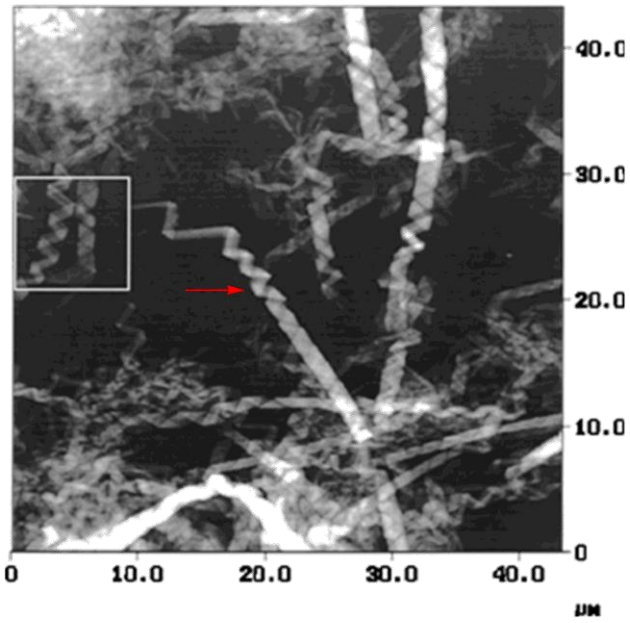
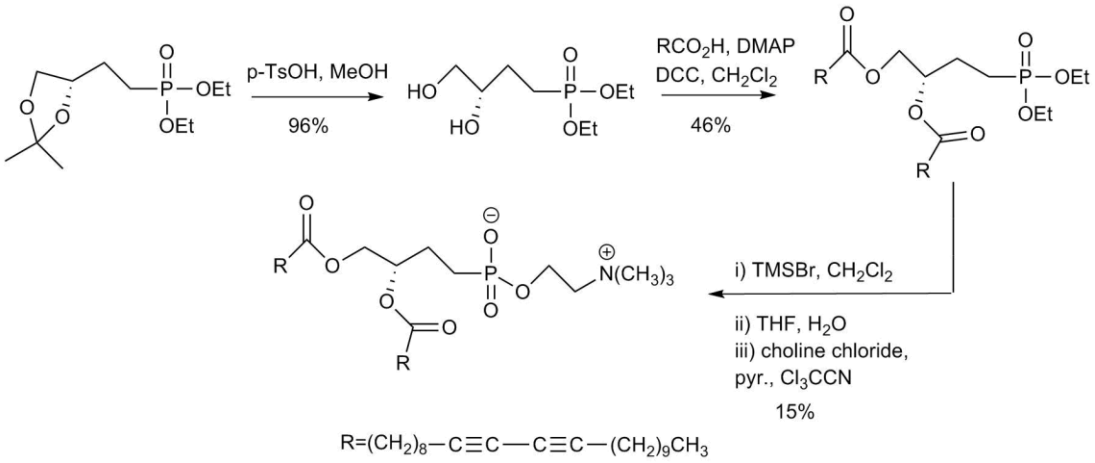


Figure 7. Right handed helices of the C4 phosphonate shown by contact mode atomic force microscopy. The helical wrapping is indicated by the box to the left, and a right handed outer layer is shown by the red arrow.

Previous Modifications to Glycerol Backbone

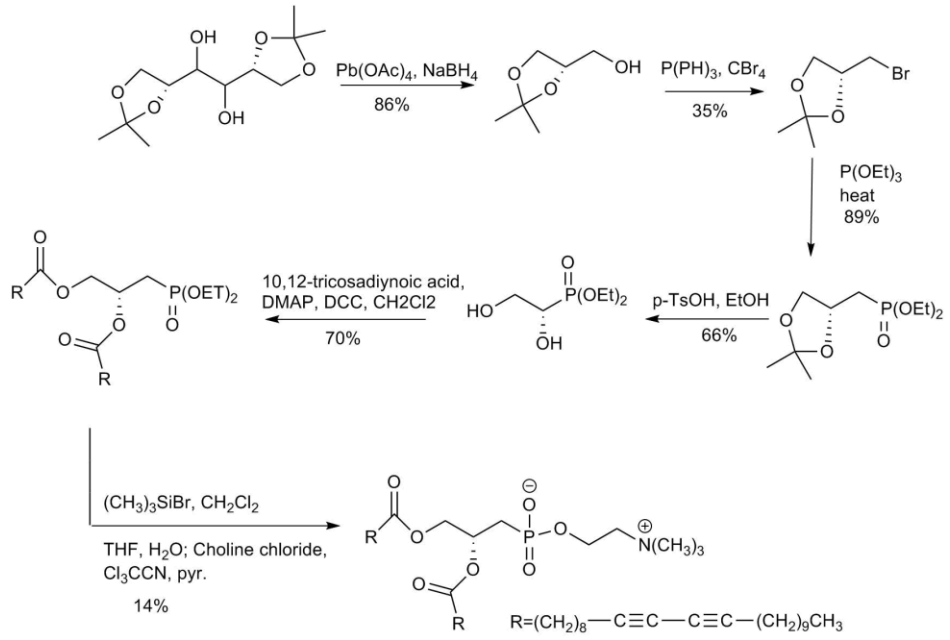
Work done by Persichini et al reported the effects of modifying the glycerol backbone of the $DC_{8,9}PC$ monomer. Their results showed a two-fold increase in diameter, more flexibility in the helices and thinner walls when $DC_{8,9}PC$ analogs were synthesized. This derivative was synthesized through the replacement of the oxygen linkage to the phosphate group with a methylene group (-CH_2-). This $DC_{8,9}PC$ analog is referred to as "C4" phosphonate because the backbone of the molecule has been extended from a three-carbon glycerol backbone to a four-carbon backbone.[8] This was the first monomer of the protypical $DC_{8,9}PC$ that was synthesized. This work showed that modifications to the glycerol backbone would not affect the tubule forming process (Scheme 1).[8]



Scheme 1. Synthesis of Phosphonate Analog 4

The successful synthesis of the C4 phosphonate analog motivated the synthesis of additional analogs of $DC_{8,9}PC$, further modifying the glycerol backbone. The linking phosphoryl oxygen atom was removed. This resulted in the polar headgroup being moved closer to the stereogenic center contained within the glycerol backbone. This modification resulted in a 1.94-

fold increase in tubule diameter and a decrease in tubule length. This compound was ultimately referred to as C3 phosphonate due to the three-carbon glycerol backbone (Scheme 2).[26]



Scheme 2. Synthesis of Phosphonate Analog 3

Current Modifications to Glycerol Backbone

The overall goal of this ongoing project is to synthesize phospholipid derivatives of $DC_{8,9}PC$. The variations of the analogs will be synthesized by modifying the glycerol backbone. This new modification will be adding a cyclohexene structure to see if the tubule-forming capacity is enhanced. To our knowledge, no modification such as this one has ever been made to the glycerol backbone. This synthesis adds a new dimension as there is now more steric hindrance added to the already bulky fatty acid chain. We hypothesize that this new alteration will not affect the ability for this "monomer" to form tubules relative to previous analogs. This new alteration is most closely related to the phosphonate analog 3 (Scheme 3).

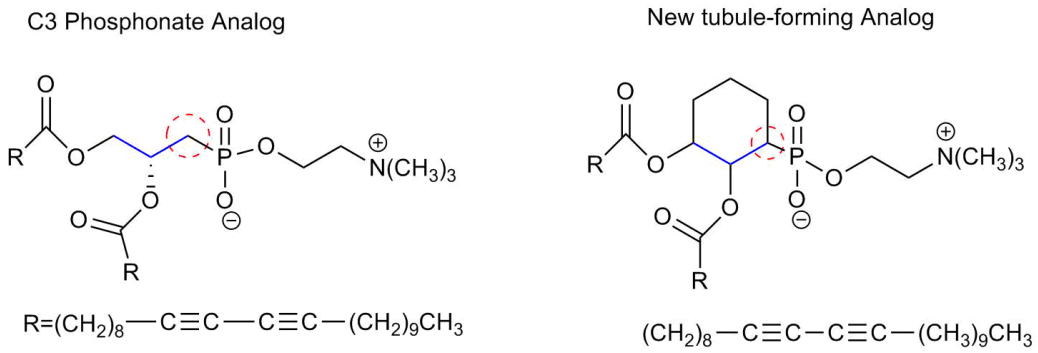
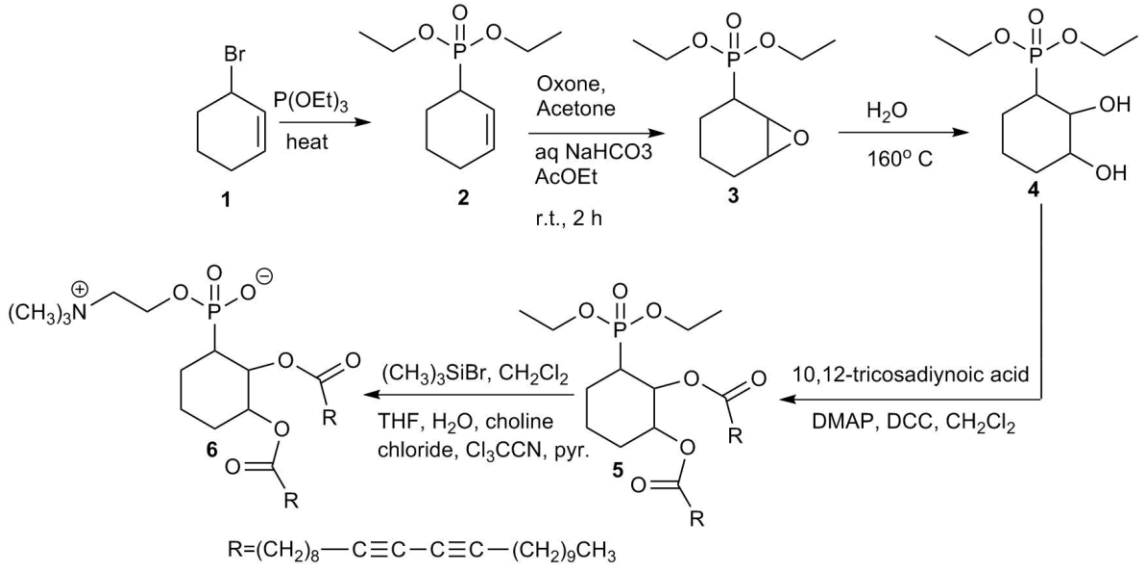


Figure 8. Comparison between the C3 phosphonate analog and the new tubule-forming analog

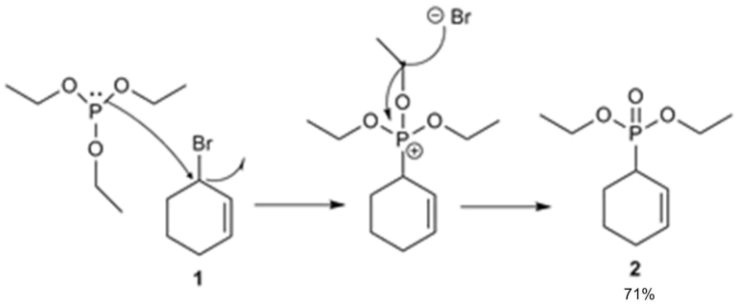
RESULTS AND DISCUSSION

The goal of this investigation was to make a new modification to the glycerol backbone. This was motivated by the successful synthesis of previous analogs. Due to what was commercially available, we selected 3-bromocyclohexene as a starting point. The literature provided ways to begin the synthesis of this monomer. This information was altered as necessary to find the pathway that would lead to the product of interest. The synthesis is shown below (Scheme 3).



Scheme 3. Synthesis of new tubule-forming Analog

The synthesis of this phospholipid tubule involved five key steps. The first reaction starts with a solution of 3-bromocyclohexene in triethyl phosphite which undergoes a typical substitution at an allylic center. This is followed by an interesting SN2 reaction instigated by a nucleophilic bromine on one of the ethoxy groups to form (**2**) in high yield (71%) (Scheme 4).



Scheme 4. Mechanism for the formation of (2)

Substitution

The synthesis of (**2**) was performed by two methods. The literature provided a procedure of the synthetic pathway to the phosphonate.[8,27] The literature pathway stated that 3-bromocyclohexene (3.28 g,20 mmol) in triethyl phosphite (7.04 mL,60.0 mmol) was heated and stirred at 160 ° C for 14 hours.[27] This reaction was monitored by gas chromatography to show no starting material was left. The conditions of this experimental were altered as necessary, and the synthesis was carried forward on a smaller scale.

The first method used a biotage microwave with excess triethyl phosphite. The reaction was monitored by TLC, however, this proved to be an inefficient analysis of starting and reactant material. The reaction was monitored by ^{1}H NMR, ^{13}C NMR, ^{31}P NMR and gas chromatography-mass spectrometry (GCMS) and structures confirmed by NMR. The reaction mixture was heated for 30 minutes at 210° C. The residual triethyl phosphite was reduced under pressure using short path distillation to obtain a yellow oil of (**2**) (71%). The second reaction was ran under conditions of equal amounts of 3-bromocyclohexene and triethyl phosphite. This time, the reaction mixture was concentrated so this was reacted in the anton paar monowave 50 for 15 minutes at 180° C**.** Just as before, the residual triethyl phosphite was reduced under pressure to

give a yellow oil of (**2**) (68%). GC analysis in comparison to standards indicated the starting material was consumed, indicating the reaction was run to completion (Figure 9).

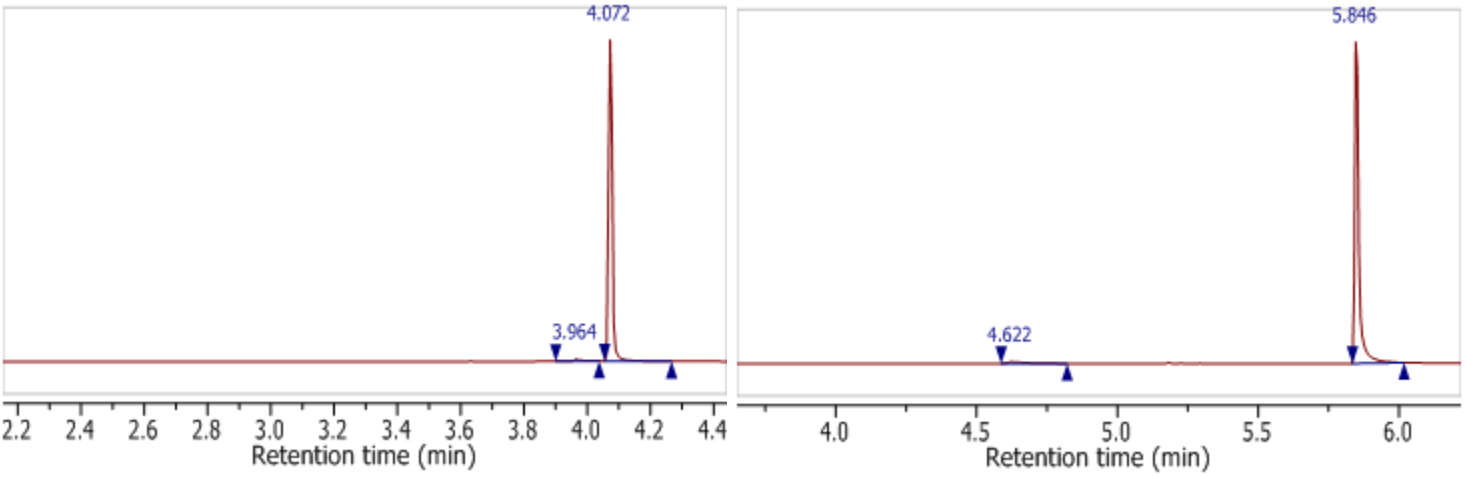


Figure 9. Analysis from the GCMS. The retention times of 3-bromocyclohexene (left) and the phosphonate (right). This illustrates that the starting material peak at 4.072 minutes is completely gone, while there is a new peak at 5.846 minutes. Further analysis proved that this peak was of the desired product.

By comparing the ^{1}H NMR spectra of the starting material to that of the product, we can see that the peak at 5.84ppm moved to 5.73ppm, the peak at 4.85ppm from the starting material is gone, and there is a new peak at 4.1ppm for the product (Figure 10).

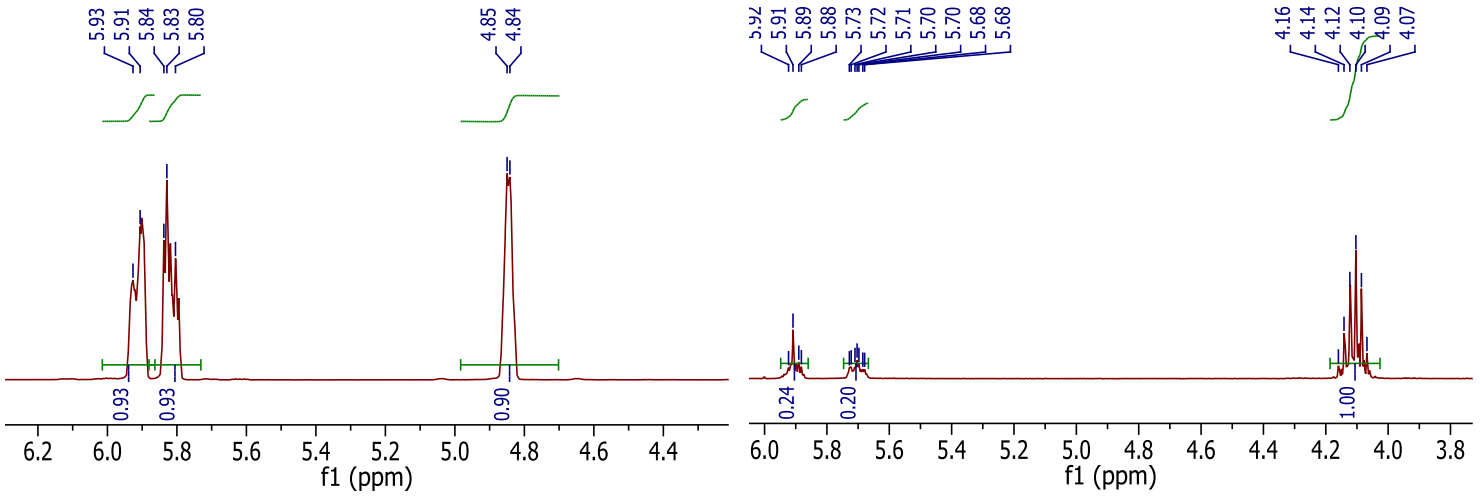


Figure 10. The ^{1}H NMR data of 3-bromocyclohexene (left) and the phosphonate (**2**) (right).

Additionally, the ^{1}H NMR spectra of the triethyl phosphite and the phosphonate were compared (Figure 11). We can see there is a peak at 3.8 for triethyl phosphite. We would expect the peak for diethyl ether to be around 3.4, but when attached to phosphorus this will appear more downfield. Therefore, the peak of 3.8 moving to 4.1 for the phosphonate is an indication that the peak of the diethyl ether are now attached to a cyclic structure, becoming more electronegative, and has moved more downfield. The peaks of the ^{1}H NMR were compared to that of the literature. All the peaks matched up except for a minor impurity peak around 7.67.[27]

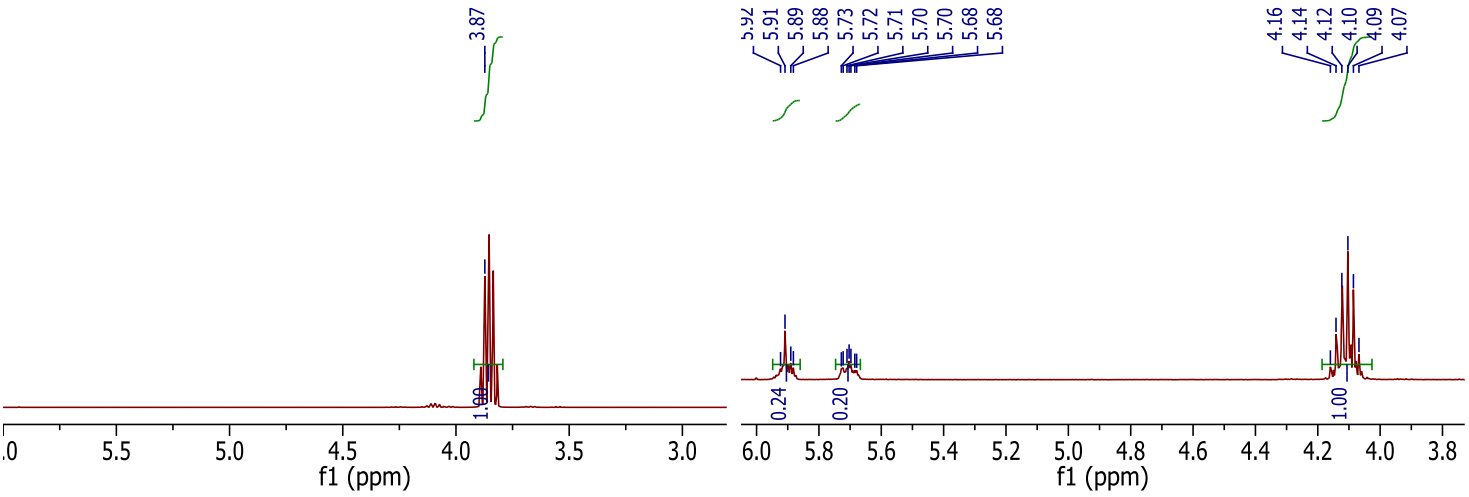


Figure 11. The ^{1}H NMR spectra of triethyl phosphite (left) and the phosphonate (**2**) (right)

Looking at the ^{13}C NMR spectra of the starting and product material, there are noticeable differences in the spectra. The peaks of the phosphonate (**2**) ^{13}C NMR were compared to that of the literature and matched perfectly.[27] When comparing the starting material to the product, we see there are new peaks at 121, 62, 35, 20 and 16 (Figure 12).

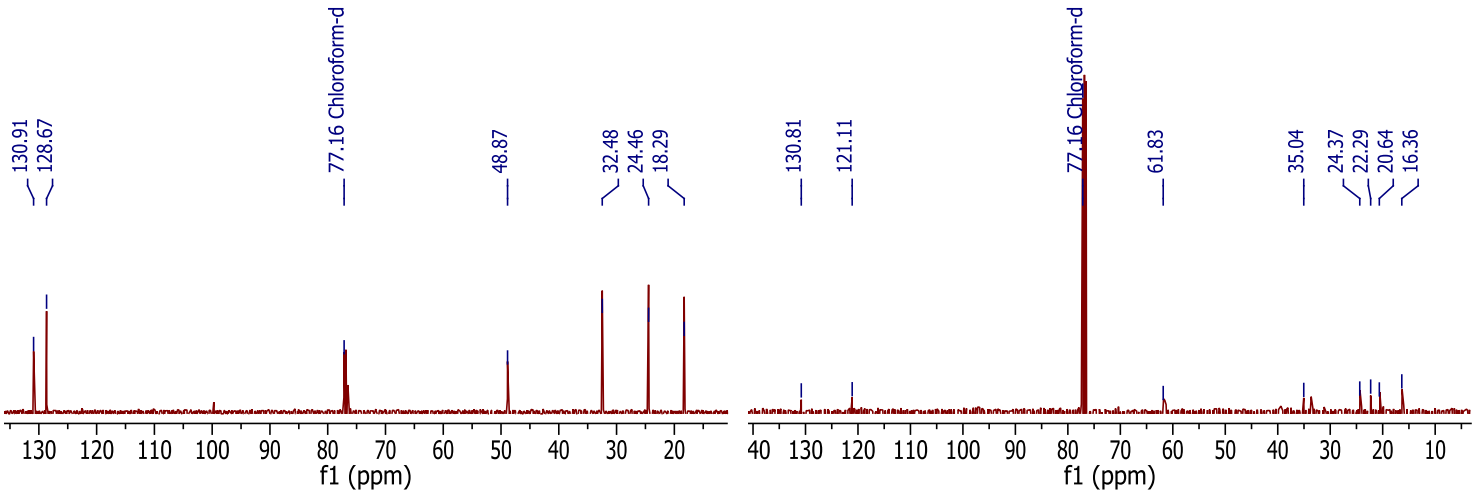


Figure 12. The ^{13}C spectra for 3-bromocyclohexene (right) and the phosphonate (**2**) (right)

As for the ^{31}P NMR data, looking at the starting material and product, the starting material peak at 139ppm is clearly gone in the product spectra, indicating the triethyl phosphite has been fully reacted. There are new peaks for the product at 34, 31 and 0 (ppm)(Figure 13). The ^{31}P NMR data was compared to the literature and the peak at 31 is the product. The other two peaks are minor impurities due to oxidation.[27]

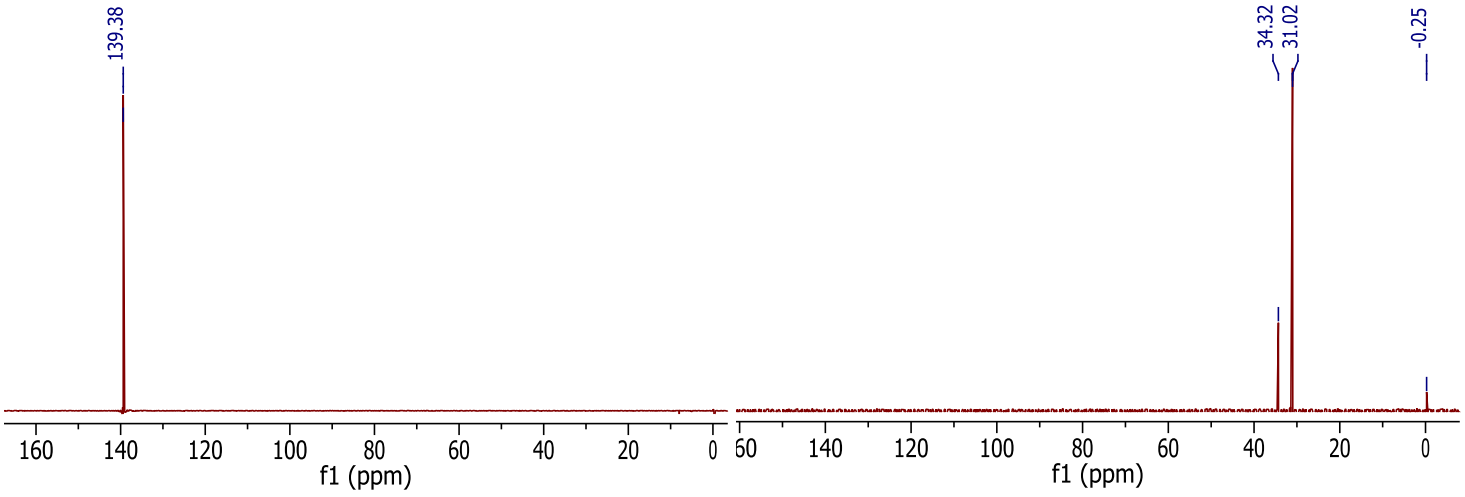
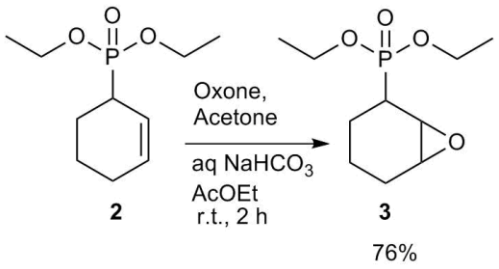


Figure 13. The ^{31}P spectra for triethyl phosphite (left) and the phosphonate (**2**) (right)

The second step is an epoxidation reaction. The synthesis for reaction was done by reacting (**2**) with Oxone while using ethyl acetate, acetone and water as solvents (Scheme 5).



Scheme 5. Epoxidation of Phosphonate

Epoxidation

The epoxidation of phosphonate was done at room temperature. The literature provided the necessary information for this synthesis, and the reaction was carried forward on a smaller scale.[28] The reaction was monitored by GCMS, ^{1}H NMR, ^{13}C NMR, and ^{31}P NMR and structures confirmed by NMR. A sample was taken for analysis by GCMS at hour one. This showed a roughly 1:1 ratio of starting material and product material (Figure 14).

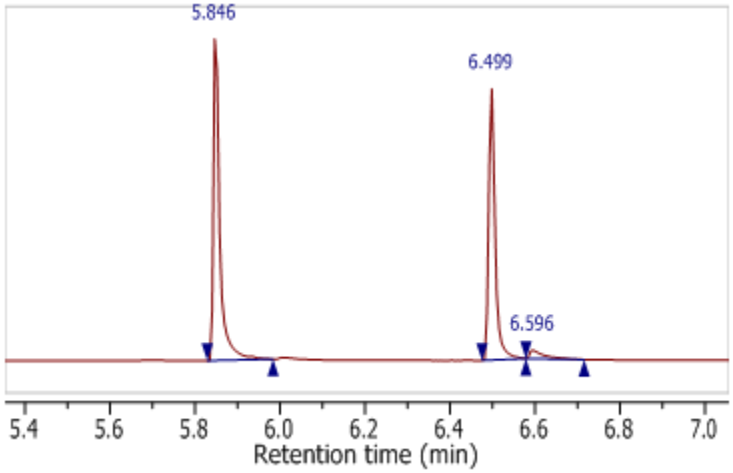


Figure 14. Representation of a 1:1 ratio between the phosphonate (**2**) (5.84min) and the epoxide (**3**) (6.50min) retention times

The reaction mixture was left to stir for an additional hour. At two hours, the reaction was monitored again by GCMS to show there was no starting material, indicating the reaction was run to completion (Figure 15). The material was separated, washed with brine and evaporated to give (**3**) (76%).

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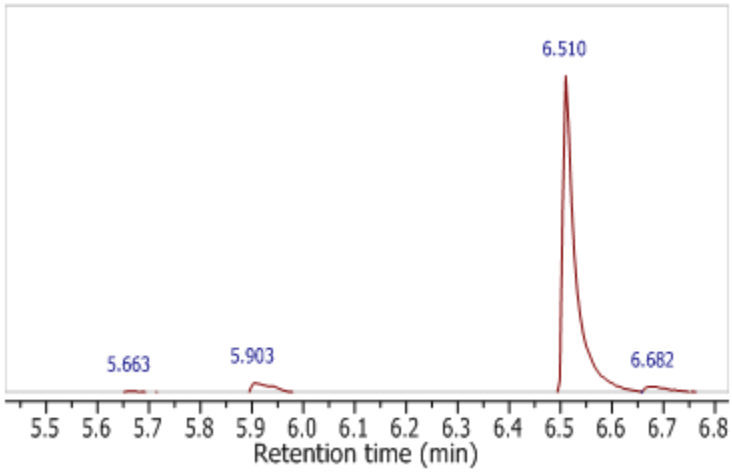


Figure 15. Representation of the reactant material no longer visible, indicating the reaction was run to completion. Further analysis indicated the 6.51min retention time peak was the epoxide (**3**)

The material was separated, washed with brine and evaporated to give (**3**) (76%).

By contrasting the ^{1}H NMR data we are able to see that the 5.92ppm and 5.73ppm are gone, which is exactly what we would expect. These protons are associated with the double bond character of the cyclohexene. A few new peaks appear in the product. A new peak arises at 4.38ppm, 3.33ppm, and at 3.20ppm. The two new peaks at 3.33ppm and 3.20ppm are the protons associated with the carbons closest to the epoxide. This data suggests the epoxide product was achieved (Figure 16).

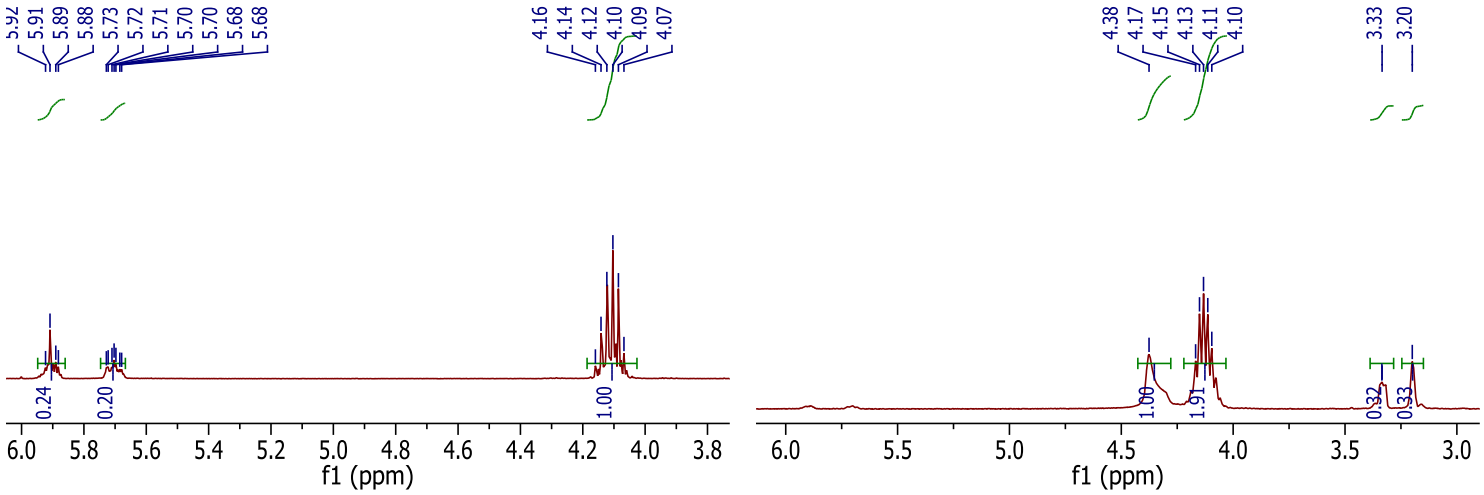


Figure 16. [1]H NMR of the phosphonate (**2**) (left) and the epoxide (**3**) (right).

Looking at the differences in the [13]C NMR showed that there were many new peaks. Additionally, the peaks for the phosphonate at 130 and 121 are now gone. The new peaks appeared at 52,50,34,32 and 23 (Figure 17).

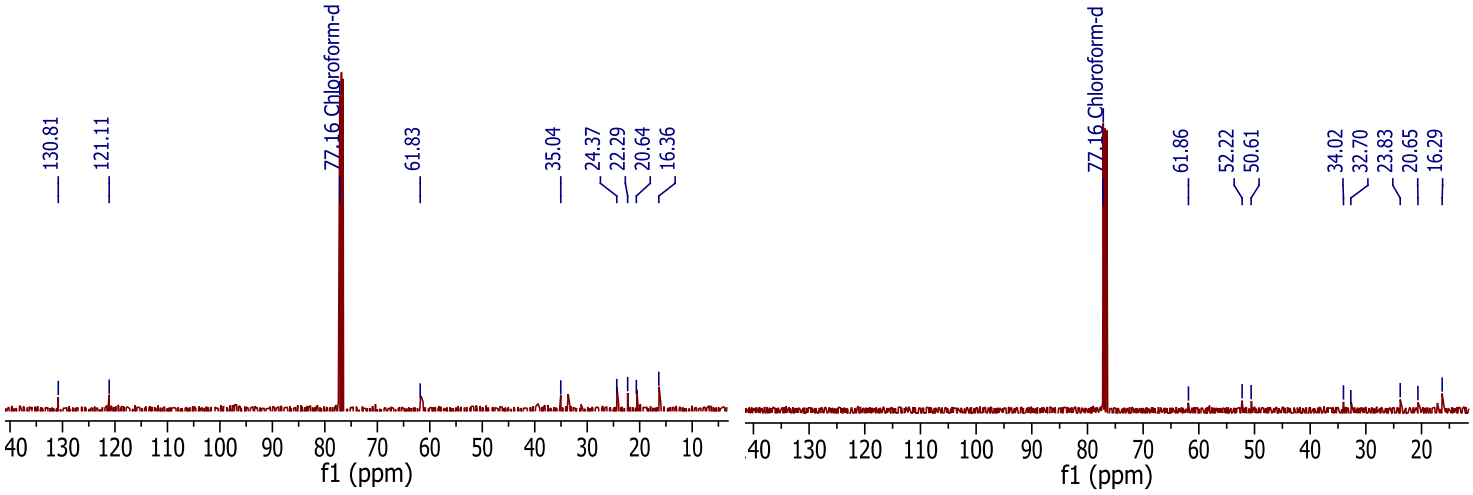


Figure 17. [13]C NMR of the phosphonate (**2**) (left) and the epoxide (**3**) (right)

The NMR spectra of ^{31}P was exactly the same for the phosphonate and for the epoxide. This is what you would expect since the reaction did not interact with the phosphate group (Figure 18).

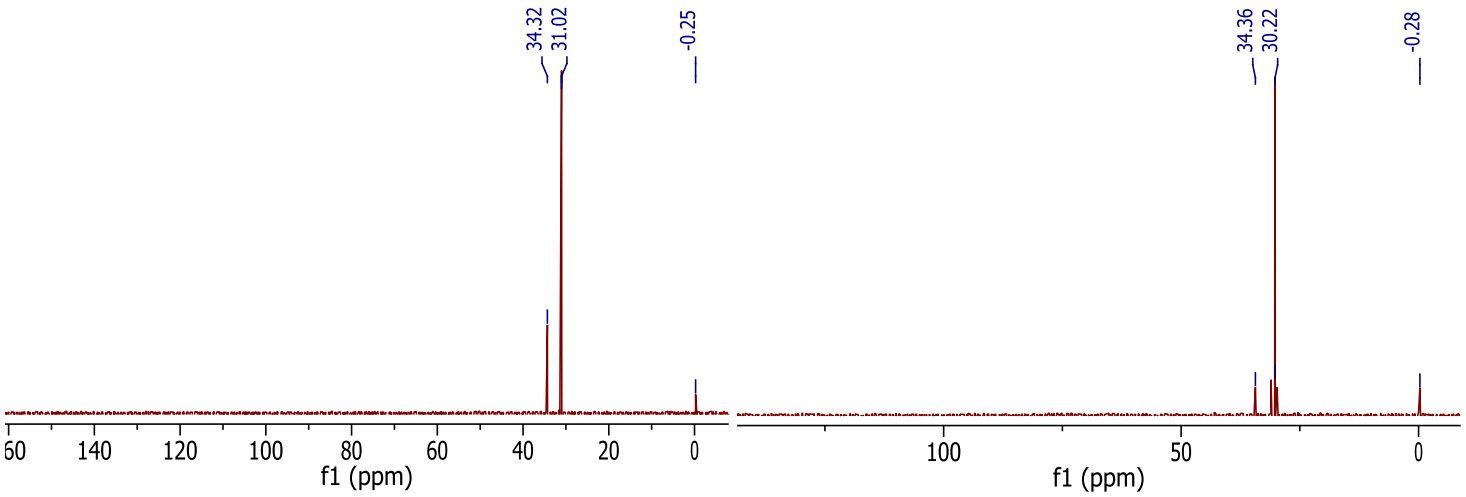
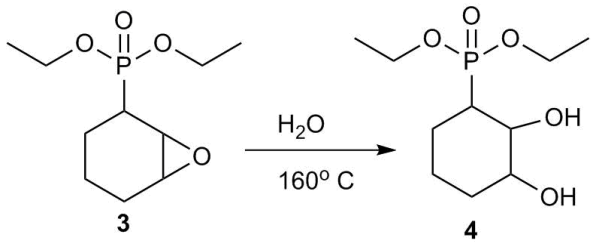


Figure 18. ^{31}P NMR of the phosphonate (**2**) (left) and the epoxide (**3**) (right)

The third step uses water to break the epoxide and form the diols (Scheme 6).



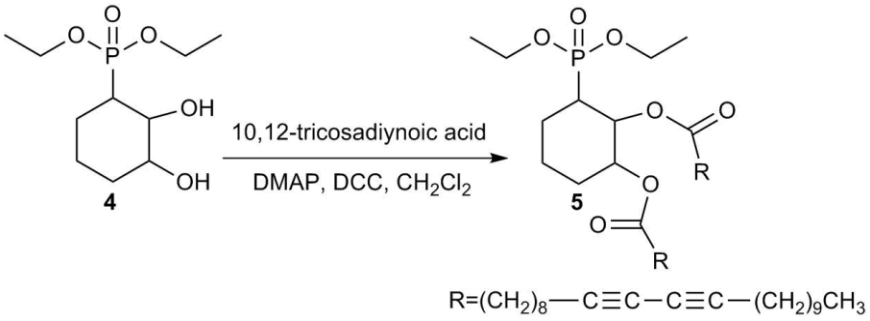
Scheme 6. Ring Opening of Epoxide

Diol Formation

The synthesis of (**4**) was designed and altered from literature.[29] A solution of (**3**) was microwaved for 10 minutes at 160° C. After completion, the mixture was extracted, washed and

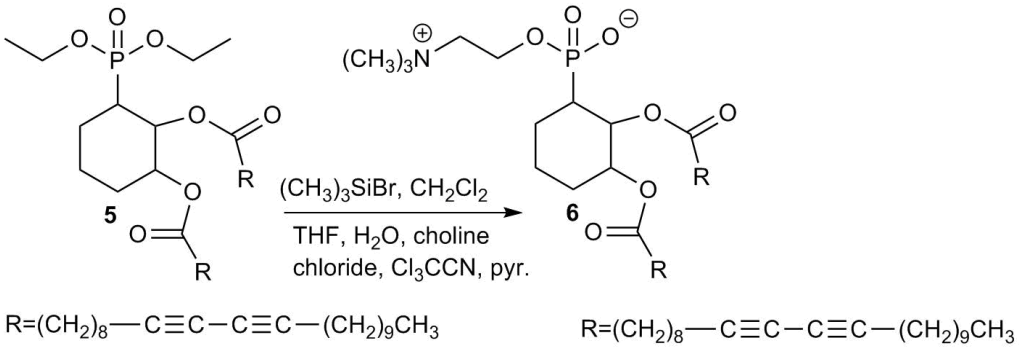
dried. At this time, the reaction is being analyzed by GCMS to distinguish if the reaction was run to completion and if the product was synthesized. Data showed that there is a new peak that is further along in retention time than the epoxide (**3**), but this peak has not yet been identified as the product.

The fourth step is a DCC esterification (Scheme 7).



Scheme 7. DCC esterification

The fifth step ends the synthesis with a routine hydrolysis and esterification. The end result that we are left with is a phosphatidylcholine derivative (Scheme 8).



Scheme 8. Routine hydrolysis and esterification

CONCLUSIONS AND FUTURE WORK

The results of this project have provided further direction investigating the alterations to the glycerol backbone. The results have shown that literature methods working with similar analogs serve to be appropriate templates for the synthesis of the new monomer. The results thus far are promising that tubule-formation can still be achieved with this monomer. Currently, the synthesis is ongoing to prove this hypothesis. This work can be taken on in the future to keep working towards the final goal. There is as much information included in this paper as possible in order to have this research continued and successfully completed one day.

EXPERIMENTAL

General procedures:

Triethyl phosphite was distilled under reduced pressure by short path distillation prior to use. All other chemicals were used without any further purification. 10,12-tricosadiynoic acid was purchased from Lancaster. All of the other chemicals were purchased directly from Sigma Aldrich.

Equipment:

^{1}H (400MHz), ^{13}C (100MHz) and ^{31}P (400MHz) NMR spectra of products were recorded on a JOEL 400MHz spectrometer. All spectra were obtained in $CDCl_3$ solutions at room temperature.

An agilent technologies 6850 network gas chromatography-mass spectrometry (GCMS) system was used to monitor reactants and products.

The reactants were heated using a biotage microwave and anton paar monowave 50 respectively.

Synthesis of (2):

Trial One: Diethyl (2-cyclohexen-1-yl) phosphonate (2)

A solution of 3-bromocyclohexene (586mg, 3.0mmol) in triethyl phosphite (3.5mL , 20mmol) was put into the microwave under the conditions of 206 degrees Celsius for 30 minutes. The crude product was purified by reducing the residual triethyl phosphite under reduced pressure to obtain a yellow oil of diethyl (2-cyclohexen-1-yl) phosphonate (**2)** (345mg, 71.43%). ^{1}H NMR ($CDCl_3$) δ 7.67 (s,1H), 5.92 (s,2H), 4.10 (m,4H), 2.60 (md,1H), 2.03 (m,2H),

1.84 (m,3H), 1.50 (m,1H), 1.30 (m,1H). ^{13}C NMR ($CDCl_3$) δ 131.07, 121.37, 61.80, 35.31, 24.60, 22.60, 20.81, 16.57. ^{31}P ($CDCl_3$) δ 34, 31, 0. MS m/z 218 (44), 190 (5), 175 (1), 162 (4), 150 (1), 139 (59), 138 (38), 111 (100), 93 (12), 83 (96), 82 (34), 81 (73), 80 (91).

Trial Two: Diethyl (2-cyclohexen-1-yl) phosphonate (2)

A solution of 3-bromocyclohexene (0.700g, 3.5mmol) in triethyl phosphite (0.75mL, 4.40mmol) was put into the monowave under the conditions of 180 degrees Celsius for 15 minutes. The crude product was purified by reducing the residual triethyl phosphite under reduced pressure to obtain a yellow oil of diethyl (2-cyclohexen-1-yl) phosphonate **(2)** (383mg, 68.03%). ^{1}H NMR ($CDCl_3$) δ 7.67 (s,1H), 5.92 (s,2H), 4.10 (m,4H), 2.60 (md,1H), 2.03 (m,2H), 1.84 (m,3H), 1.50 (m,1H), 1.30 (m,1H); ^{13}C NMR ($CDCl_3$) δ 131.07, 121.37, 61.80, 35.31, 24.60, 22.60, 20.81, 16.57. ^{31}P ($CDCl_3$) δ 34, 31, 0. MS m/z 218 (44), 190 (5), 175 (1), 162 (4), 150 (1), 139 (59), 138 (38), 111 (100), 93 (12), 83 (96), 82 (34), 81 (73), 80 (91).

Synthesis of (3):

Trial One: 7-oxabicyclo(4.1.0)hept-2-yl-diethyl ester (3)

To a 50-mL three necked flask were added sodium bicarbonate (355mg, 0.00423mol), water (4 mL), acetone (490 mg, 0.00846mol), ethyl acetate (4 mL), and diethyl 2-cyclohexenylphosphonate (200mg, 0.000846mol) and were stirred vigorously. An aqueous solution of monopersulfate compound (Oxone 520 mg, 0.000846 mol, water 3.5mL) was added dropwise for an hour at room temperature. Following Oxone addition, the reaction mixture was stirred for an addition hour. The organic layer was separated and washed with 20% (w/v) aqueous sodium chloride and was rotovapped to obtain a liquid of (**3**) (150mg,76%). ^{1}H NMR ($CDCl_3$) δ 4.39 (d,1H), 4.15 (m,4H), 3.35 (s,1H), 3.20 (d,1H), 2.33 (d,1H), 2.08 (d,1H), 1.71

(d,6H), 1.35 (q,6H); ^{13}C NMR ($CDCl_3$) δ 7.26, -7.97, -17.68, -19.34, -35.88, -37.26, -46.07, -49.25, -52.76, -53.61. . ^{31}P ($CDCl_3$) δ 34, 31, 0. MS m/z 233 (3), 218 (4), 206 (6), 191 (5), 178 (25), 165 (100), 150 (9), 138 (35), 135 (10), 122 (19), 111 (70), 97 (24), 96 (35), 81 (39), 67 (25), 55 (8).

Synthesis of (4):

Trial One: 2,3-dihydroxycyclohexyl-diethyl ester (4)

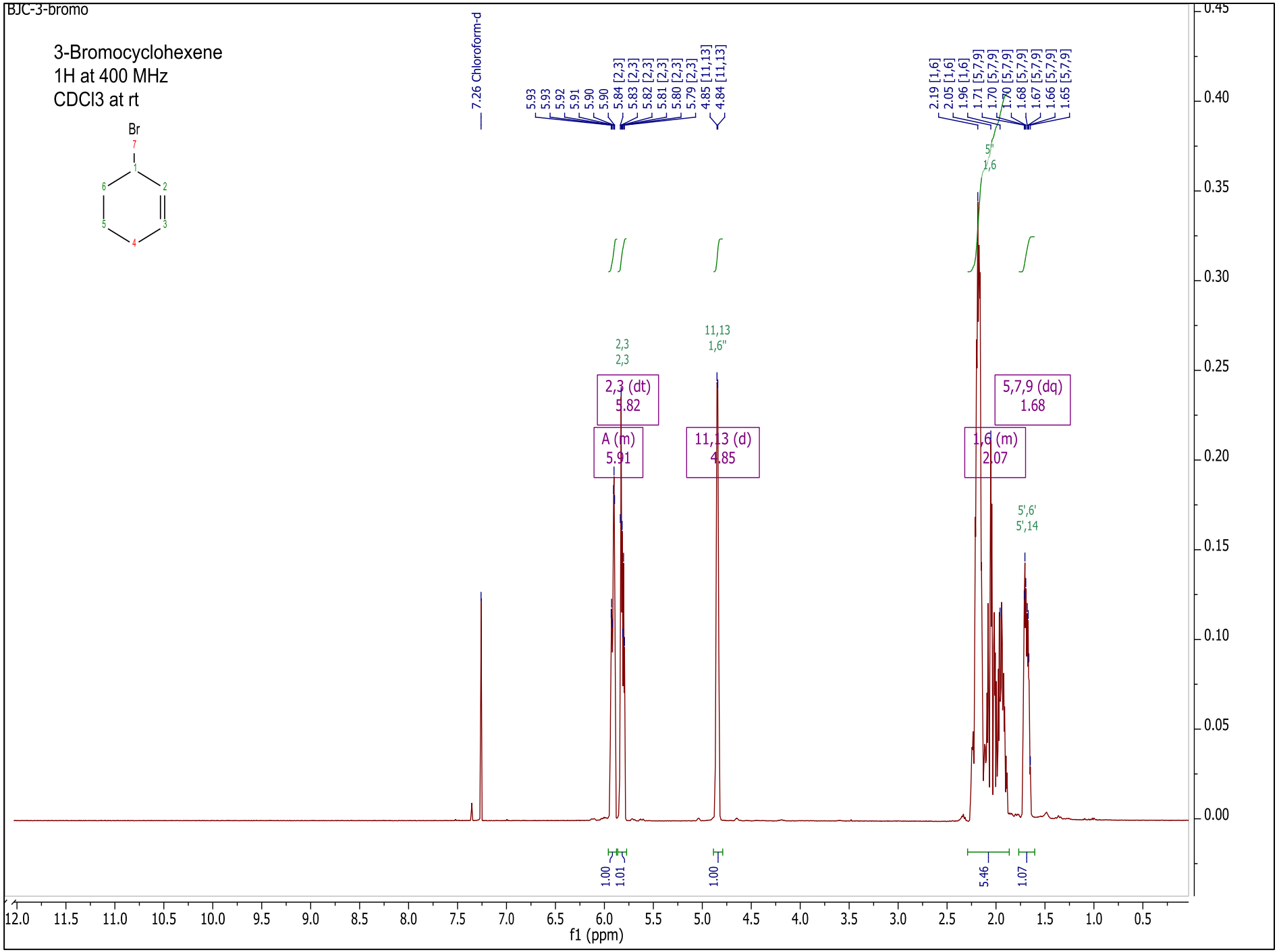
A solution of (**3**) (1mmol, 220mg) in distilled water (5mL) was added to a vial and put into the microwave for 10 minutes at 160° C. After completion, the mixture was extracted with ethyl acetate, washed with brine and dried with magnesium sulfate. This reaction is currently being analyzed by GCMS to distinguish if the reaction has been run to completion and if the product was synthesized.

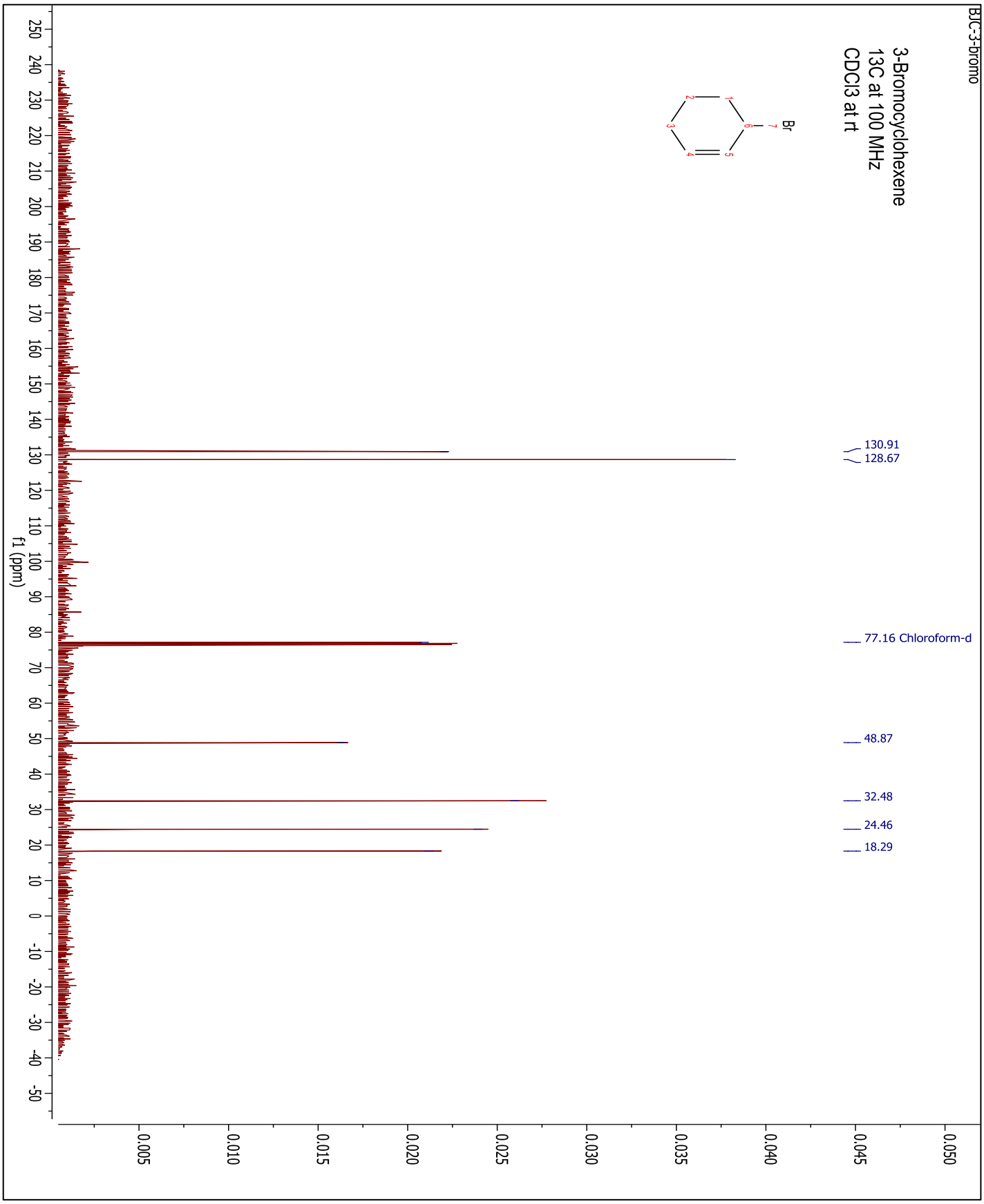
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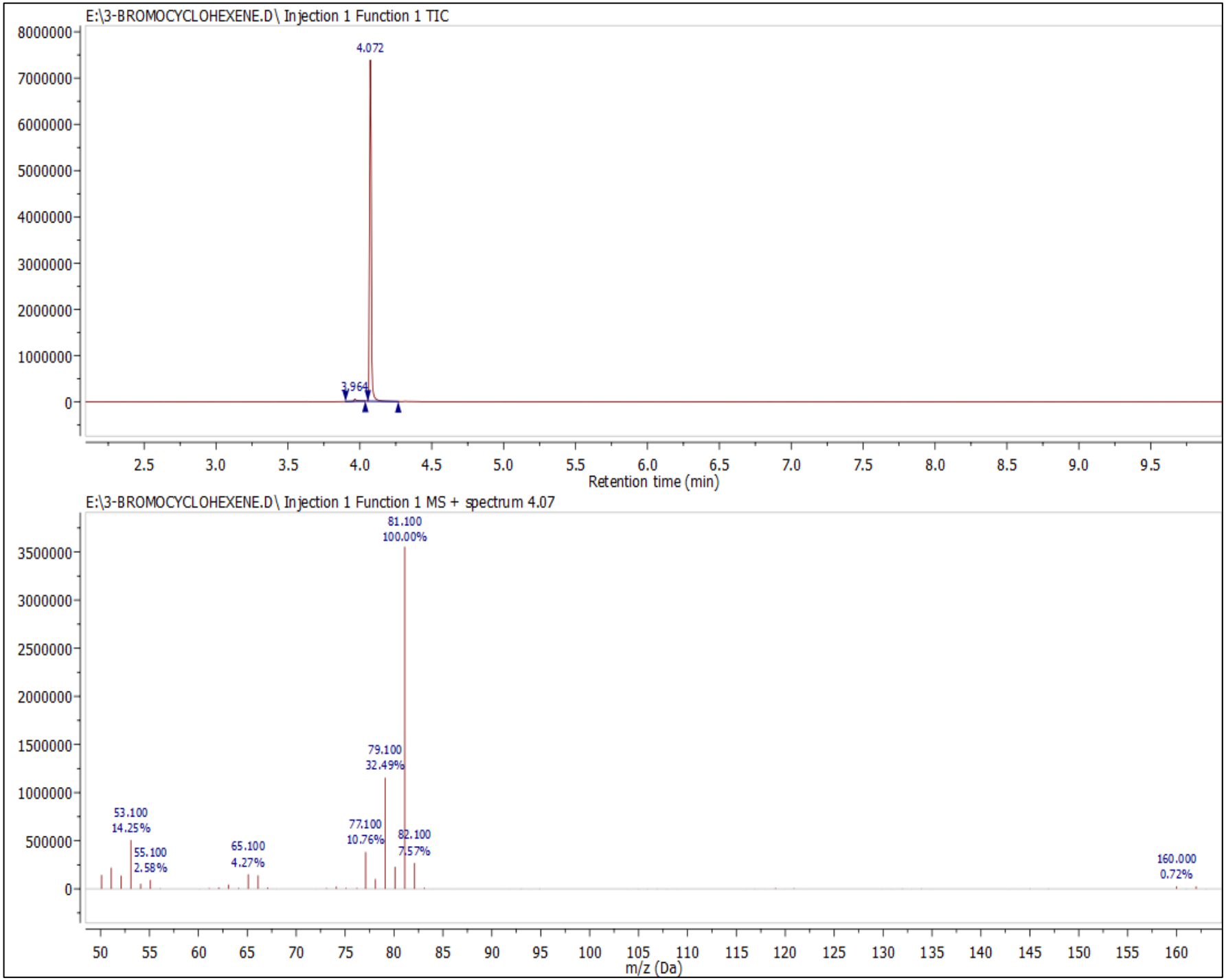
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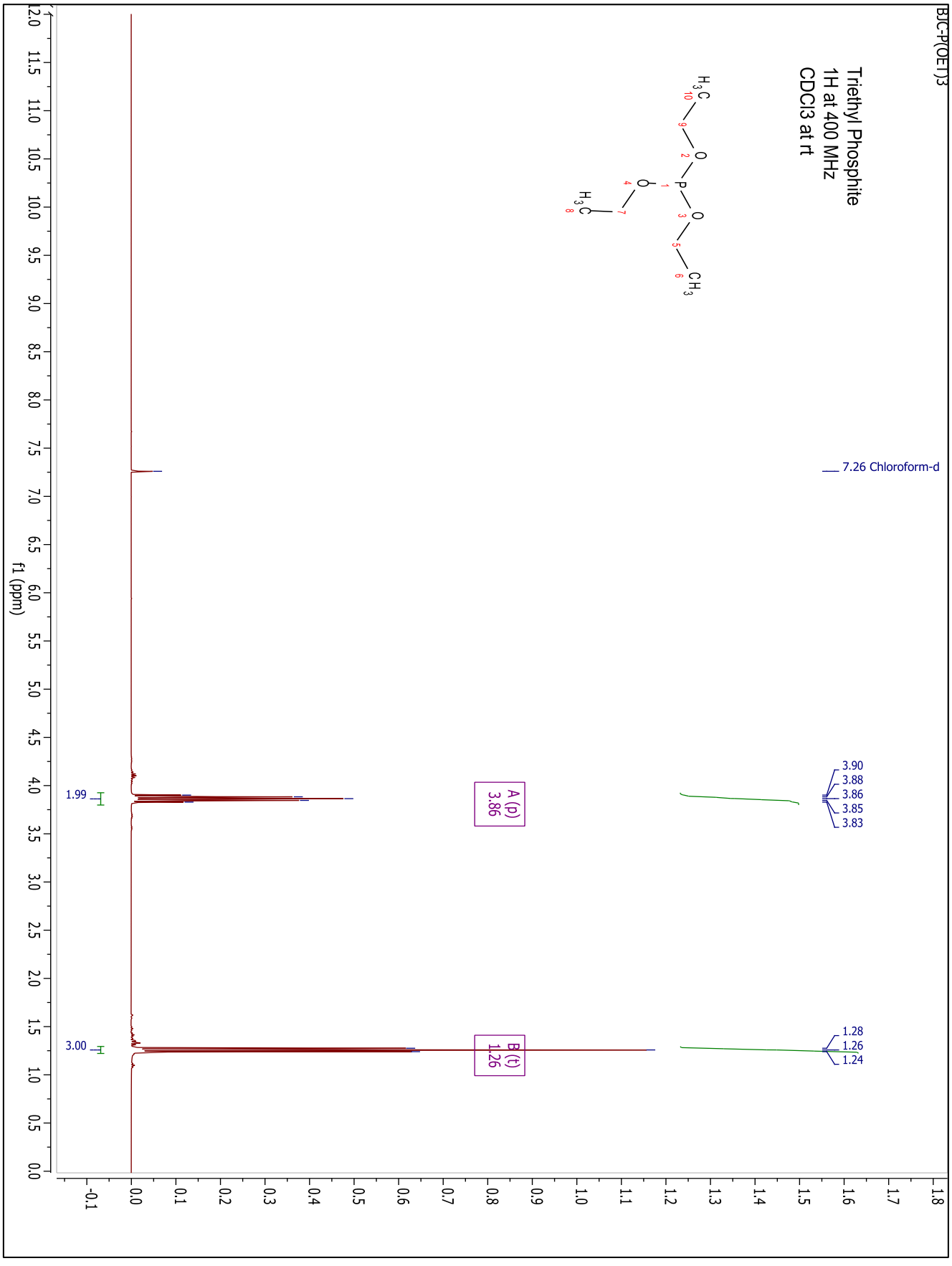
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APPENDIX


BJC-3-bromo
3-Bromocyclohexene
1H at 400 MHz
CDCl3 at rt
Br
7
1
2
3
4
5
6
7.26 Chloroform-d
5.93
5.93
5.92
5.91
5.90
5.90
5.84 [2,3]
5.83 [2,3]
5.82 [2,3]
5.81 [2,3]
5.80 [2,3]
5.79 [2,3]
4.85 [11,13]
4.84 [11,13]
2.19 [1,6]
2.05 [1,6]
1.96 [1,6]
1.71 [5,7,9]
1.70 [5,7,9]
1.70 [5,7,9]
1.68 [5,7,9]
1.67 [5,7,9]
1.66 [5,7,9]
1.65 [5,7,9]
5'
1,6
2,3
2,3
11,13
1,6"
2,3 (dt)
5.82
A (m)
5.91
11,13 (d)
4.85
5,7,9 (dq)
1.68
1,6 (m)
2.07
5',6'
5',14
1.00
1.01
1.00
5.46
1.07
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

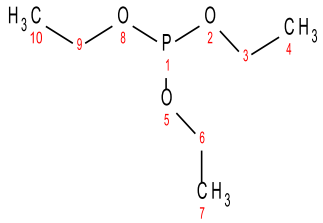

BJC-3-bromo
3-Bromocyclohexene
13C at 100 MHz
CDCl3 at rt
Br
130.91
128.67
77.16 Chloroform-d
48.87
32.48
24.46
18.29
f1 (ppm)


E:\3-BROMOCYCLOHEXENE.D\ Injection 1 Function 1 TIC
4.072
3.964
Retention time (min)
E:\3-BROMOCYCLOHEXENE.D\ Injection 1 Function 1 MS + spectrum 4.07
81.100
100.00%
79.100
32.49%
53.100
14.25%
55.100
2.58%
65.100
4.27%
77.100
10.76%
82.100
7.57%
160.000
0.72%
m/z (Da)


BJC-P(OET)3
Triethyl Phosphite
1H at 400 MHz
CDCl3 at rt
7.26 Chloroform-d
3.90
3.88
3.86
3.85
3.83
1.28
1.26
1.24
A (p)
3.86
B (t)
1.26
1.99
3.00
f1 (ppm)

BJC-P(OET)3

Triethyl Phosphite
13C at 100 MHz
CDCl3 at rt

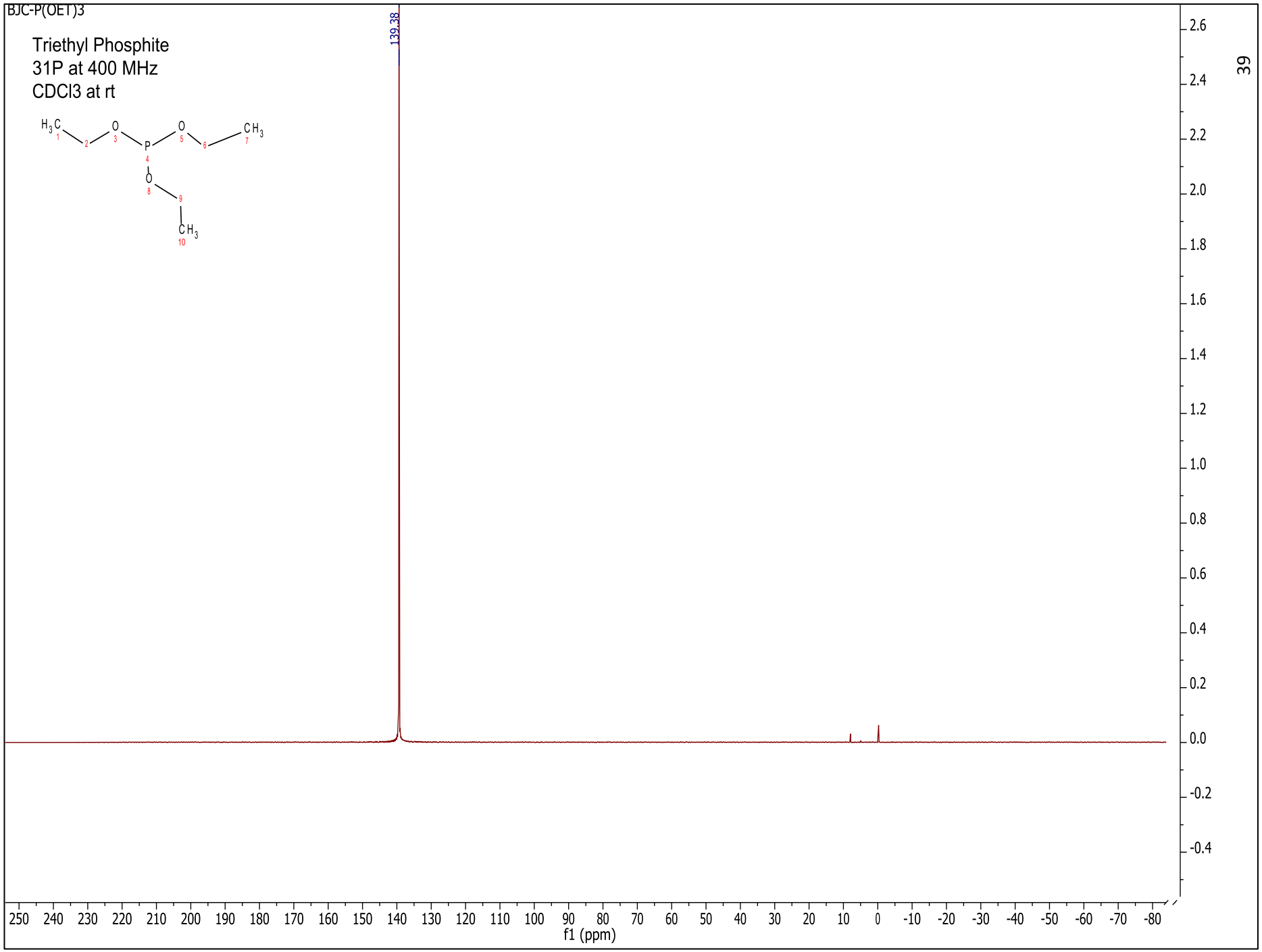


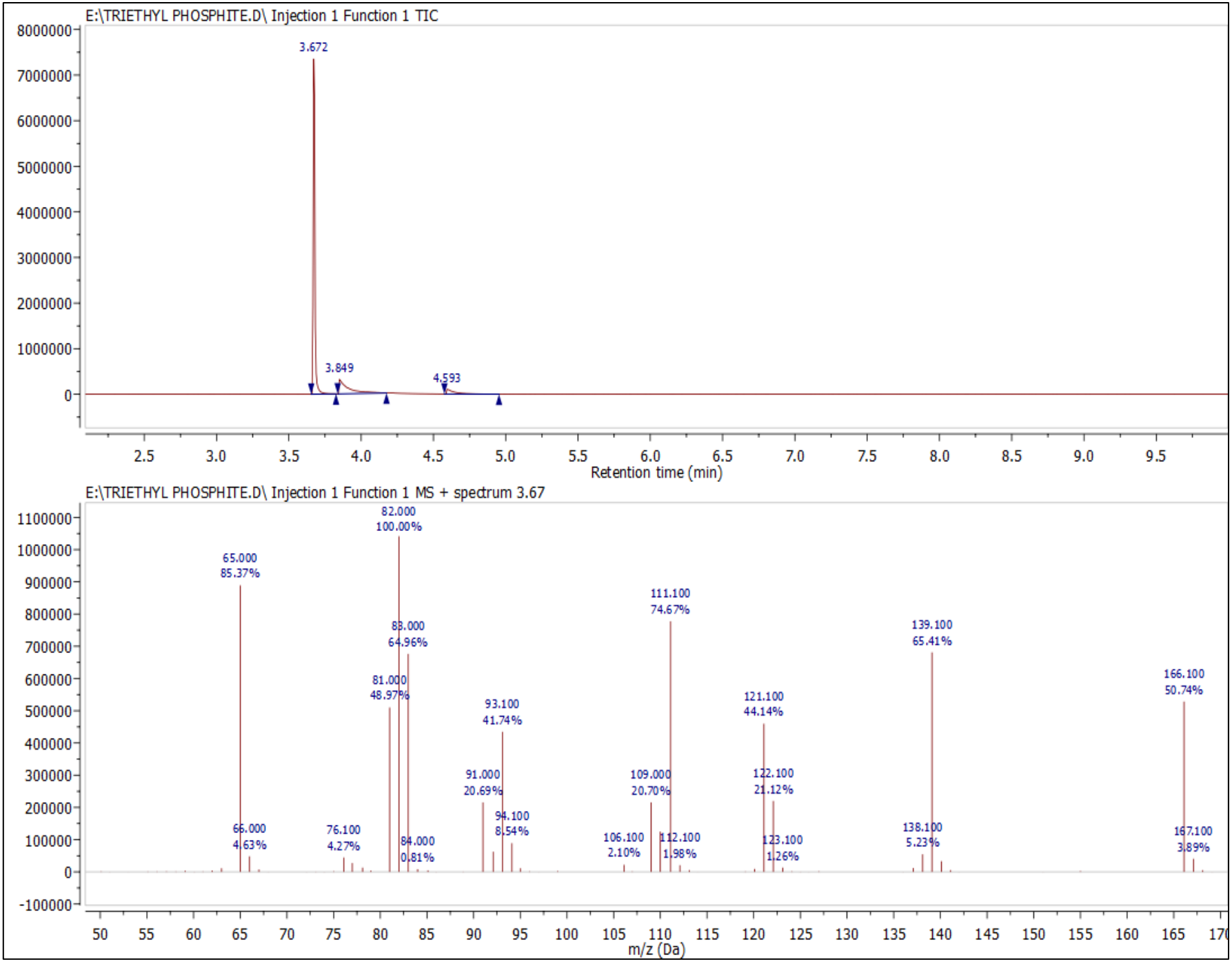
77.16 Chloroform-d
57.74
16.69

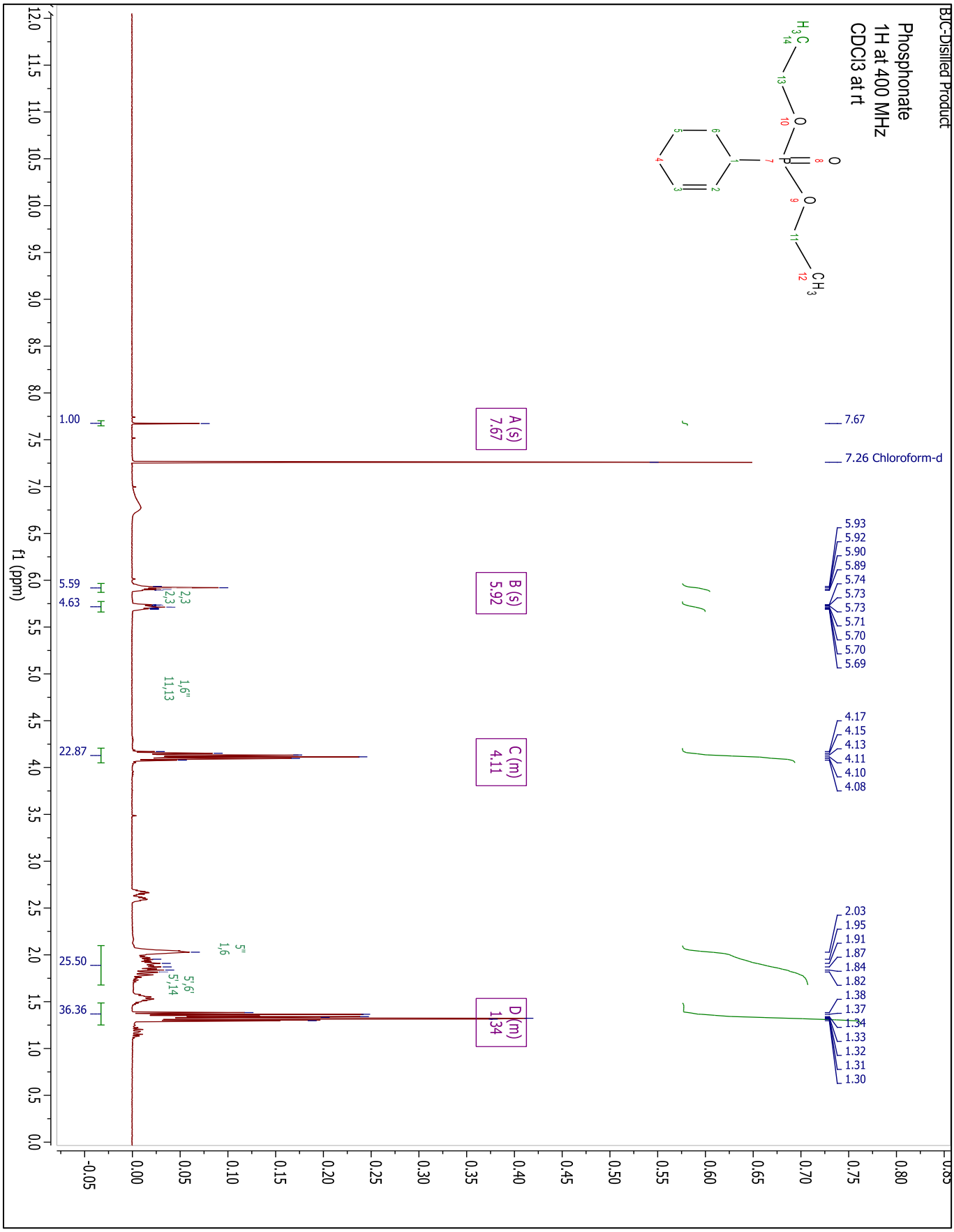
0.050
0.045
0.040
0.035
0.030
0.025
0.020
0.015
0.010
0.005

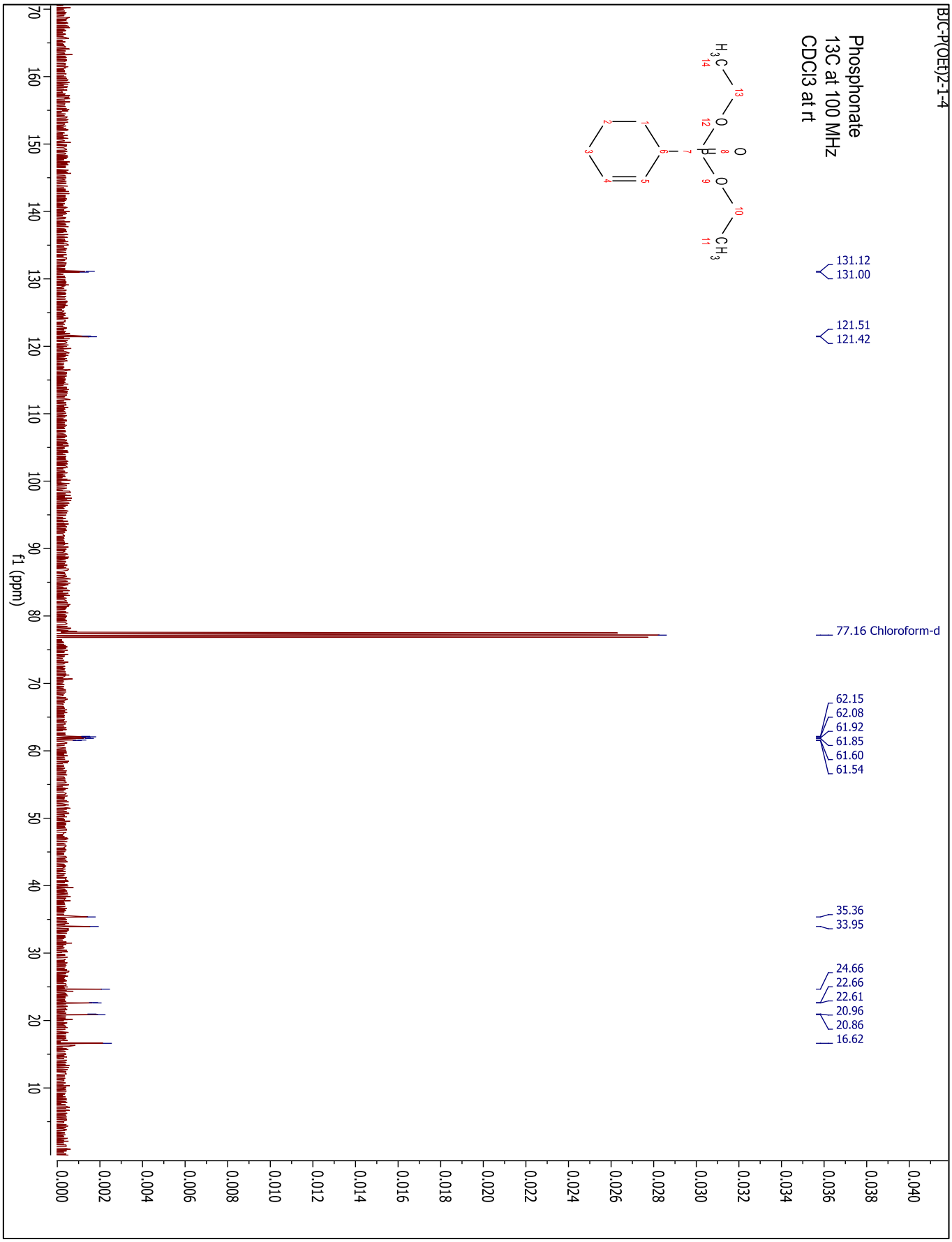
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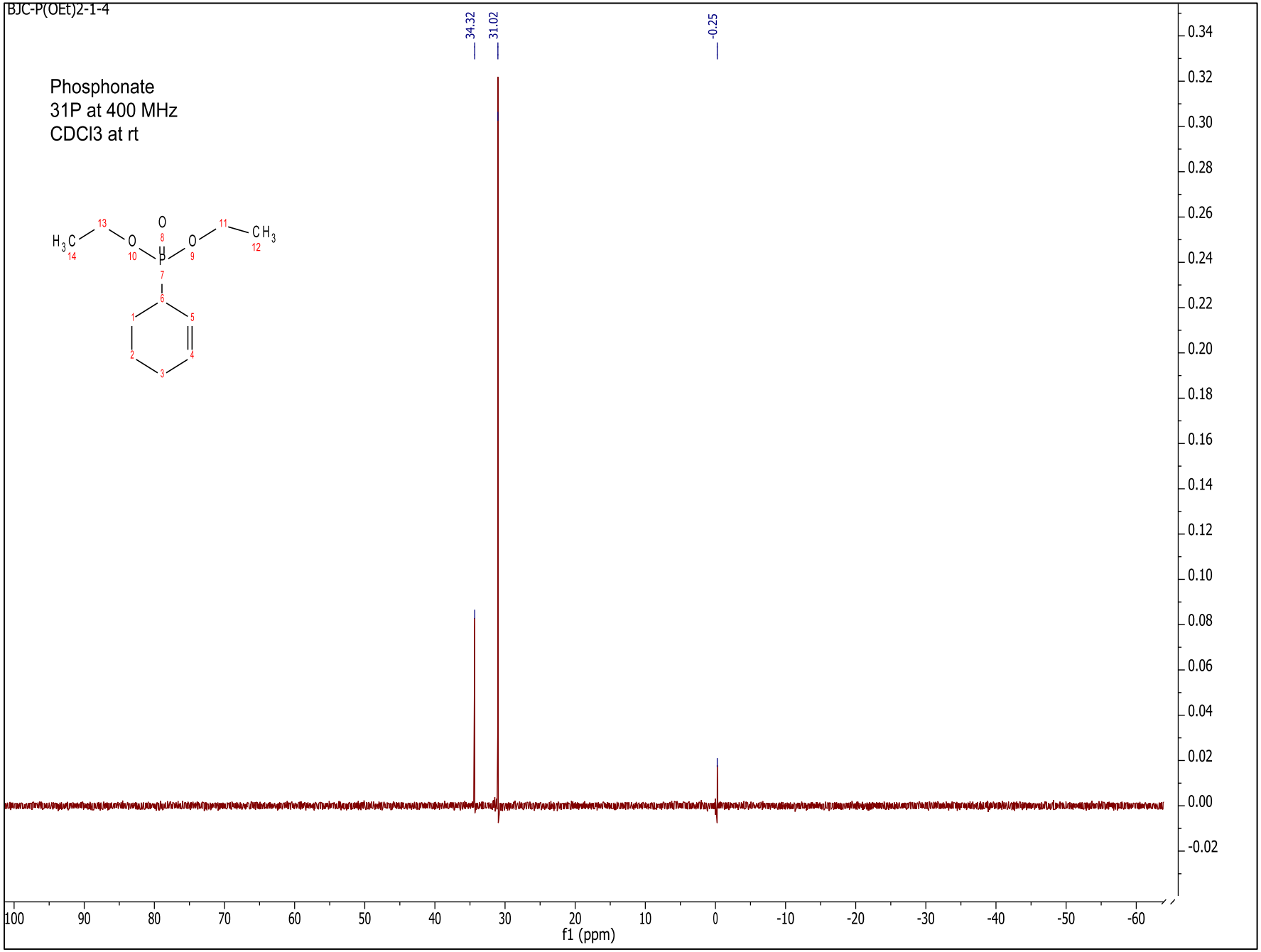
f1 (ppm)

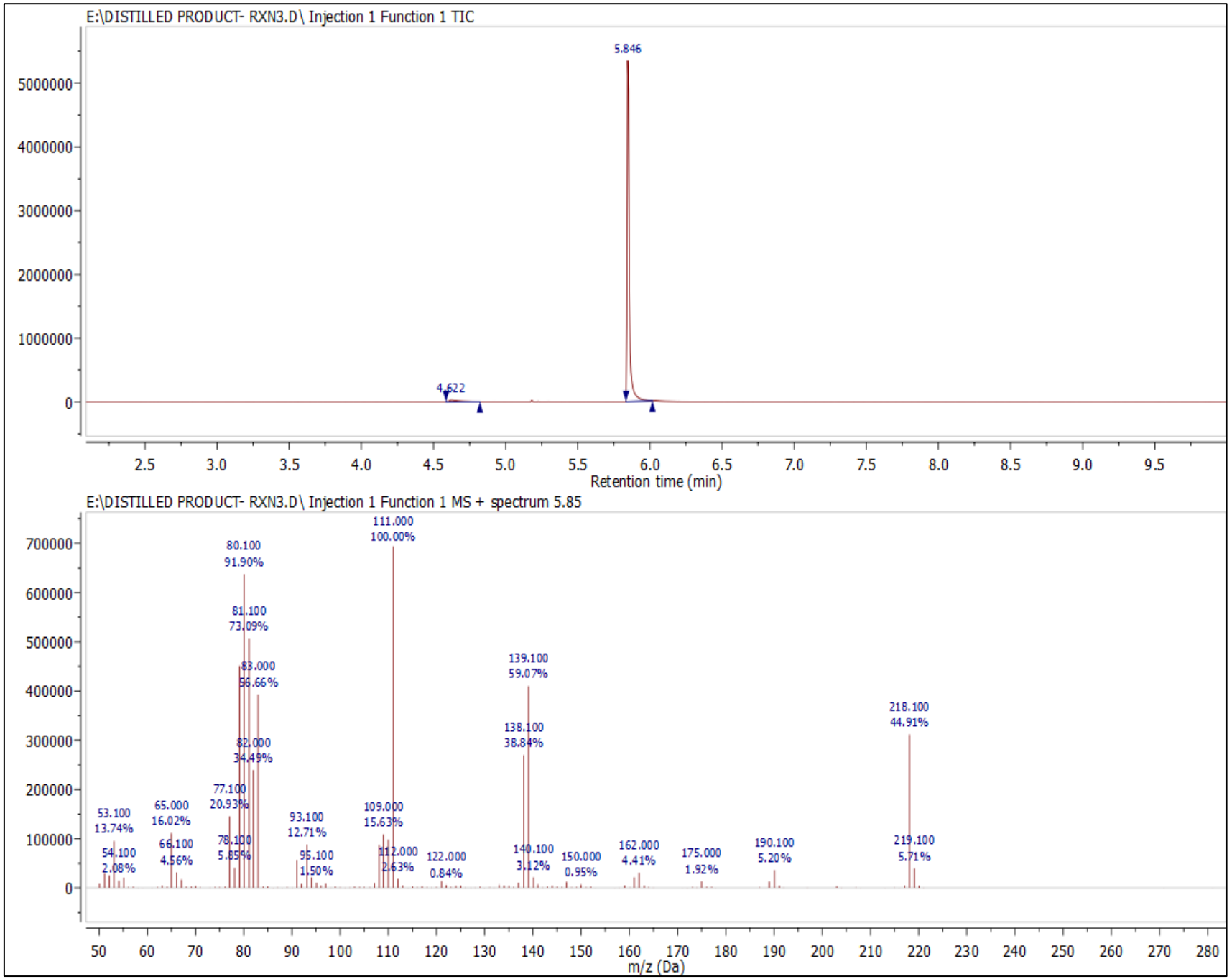

BJC-P(OET)3
Triethyl Phosphite
31P at 400 MHz
CDCl3 at rt
139.38
39
f1 (ppm)

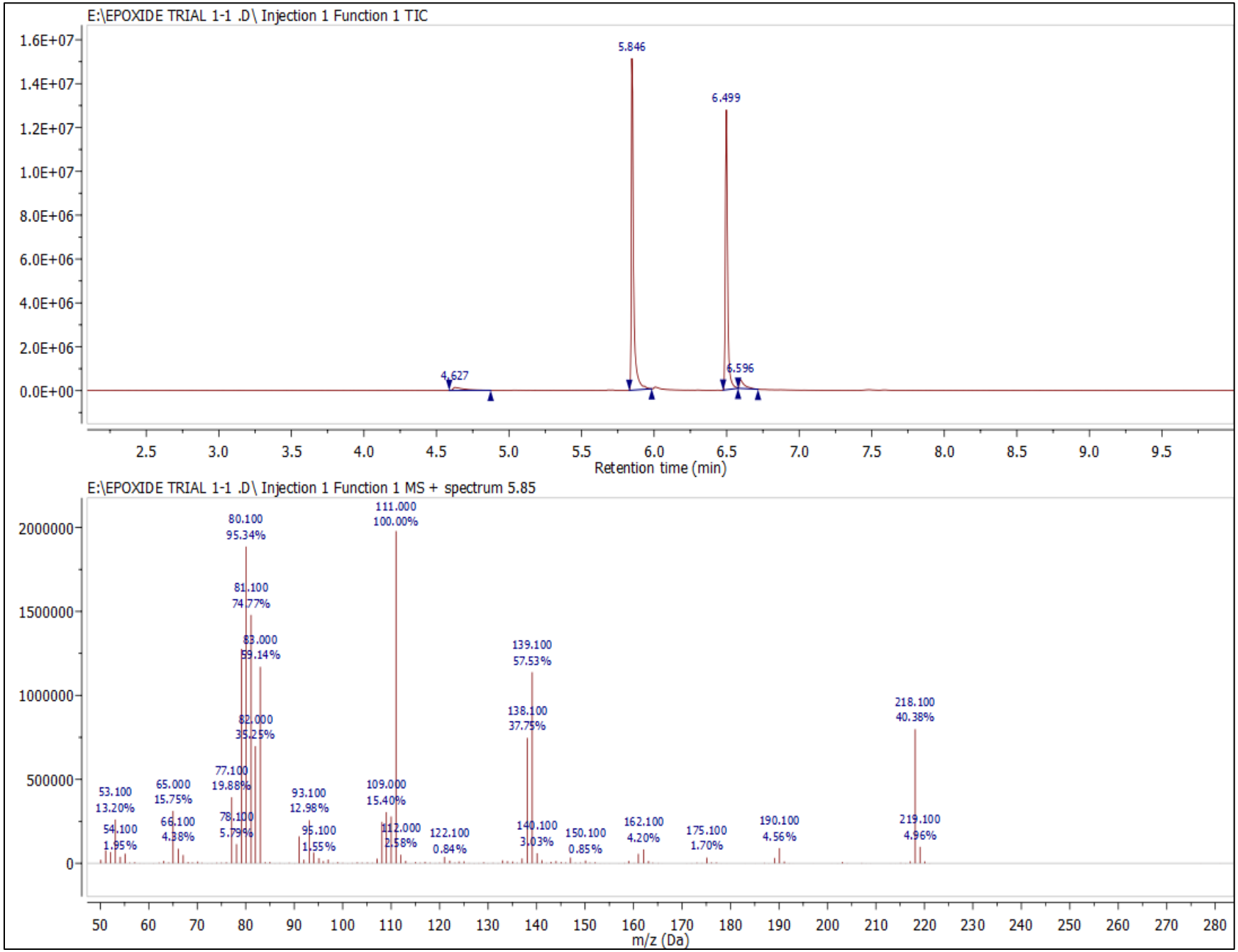

E:\TRIETHYL PHOSPHITE.D\ Injection 1 Function 1 TIC
3.672
3.849
4.593
Retention time (min)
E:\TRIETHYL PHOSPHITE.D\ Injection 1 Function 1 MS + spectrum 3.67
82.000
100.00%
65.000
85.37%
111.100
74.67%
83.000
64.96%
139.100
65.41%
81.000
48.97%
166.100
50.74%
93.100
41.74%
121.100
44.14%
91.000
20.69%
109.000
20.70%
122.100
21.12%
94.100
8.54%
66.000
4.63%
76.100
4.27%
84.000
0.81%
106.100
2.10%
112.100
1.98%
123.100
1.26%
138.100
5.23%
167.100
3.89%
m/z (Da)

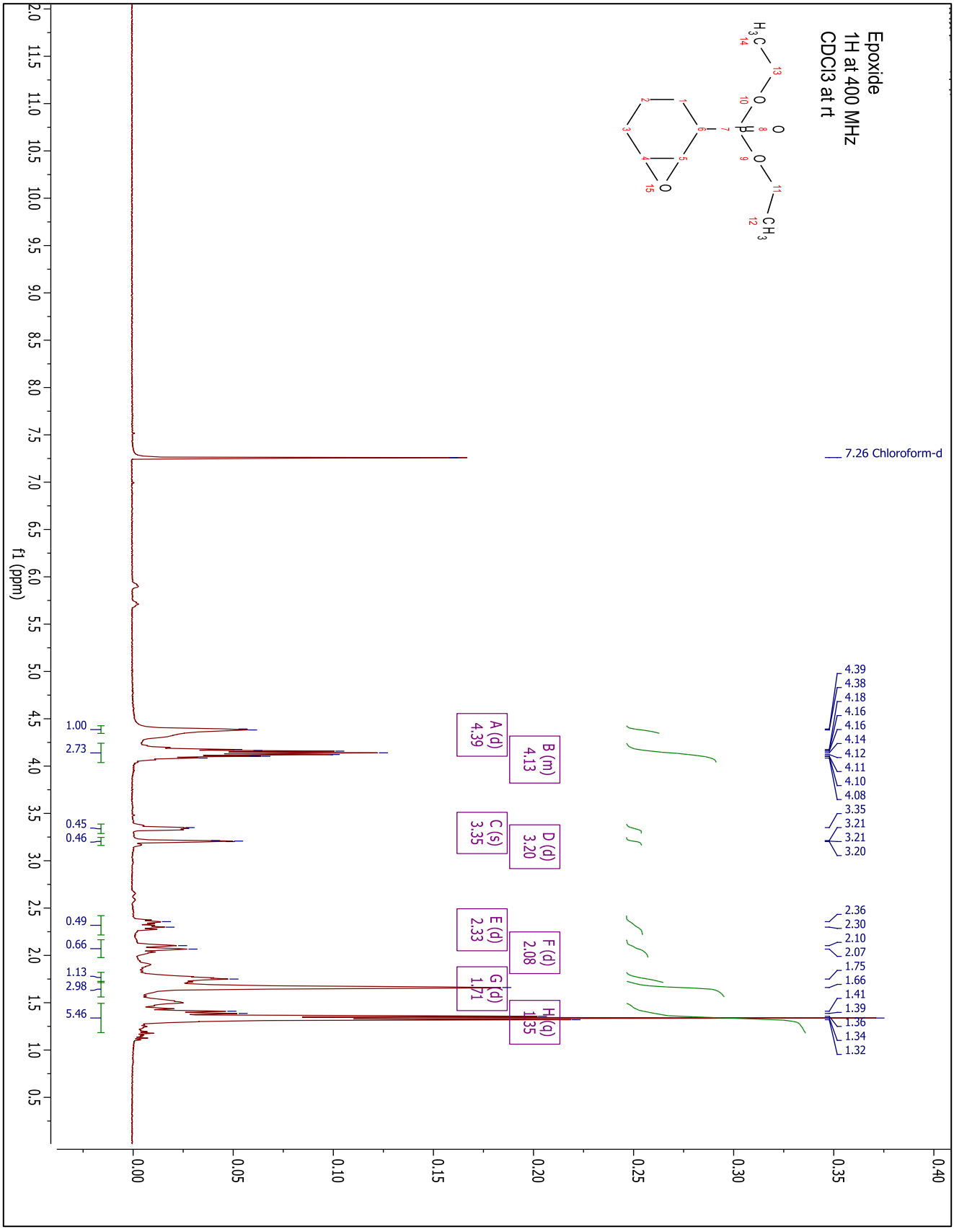

BJC-Distilled Product
Phosphonate
1H at 400 MHz
CDCl3 at rt
A (s)
7.67
B (s)
5.92
C (m)
4.11
D (m)
1.34
7.67
7.26 Chloroform-d
5.93
5.92
5.90
5.89
5.74
5.73
5.73
5.71
5.70
5.70
5.69
4.17
4.15
4.13
4.11
4.10
4.08
2.03
1.95
1.91
1.87
1.84
1.82
1.38
1.37
1.34
1.33
1.32
1.31
1.30
1.00
5.59
4.63
22.87
25.50
36.36
f1 (ppm)


BJC-P(OEt)2-1-4
Phosphonate
13C at 100 MHz
CDCl3 at rt
131.12
131.00
121.51
121.42
77.16 Chloroform-d
62.15
62.08
61.92
61.85
61.60
61.54
35.36
33.95
24.66
22.66
22.61
20.96
20.86
16.62
f1 (ppm)

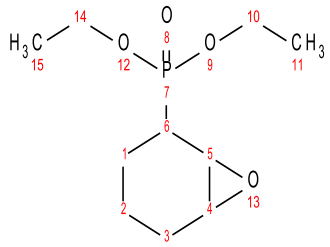

BJC-P(OEt)2-1-4
34.32
31.02
-0.25
Phosphonate
31P at 400 MHz
CDCl3 at rt
f1 (ppm)

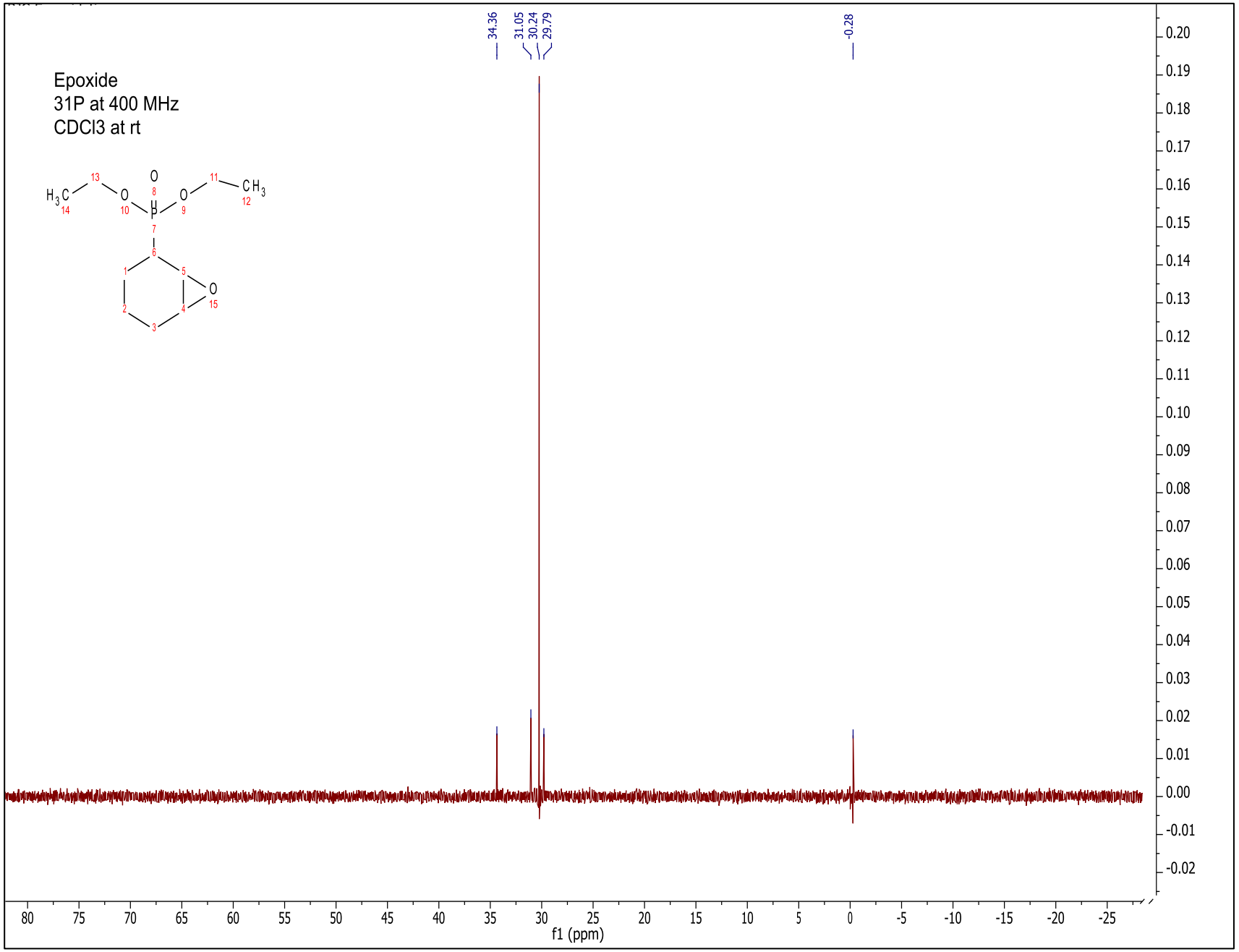

E:\DISTILLED PRODUCT- RXN3.D\ Injection 1 Function 1 TIC
5.846
4.622
Retention time (min)
E:\DISTILLED PRODUCT- RXN3.D\ Injection 1 Function 1 MS + spectrum 5.85
111.000
100.00%
80.100
91.90%
81.100
73.09%
83.000
56.66%
82.000
34.49%
139.100
59.07%
138.100
38.84%
218.100
44.91%
77.100
20.93%
65.000
16.02%
53.100
13.74%
93.100
12.71%
109.000
15.63%
78.100
5.85%
66.100
4.56%
54.100
2.08%
95.100
1.50%
112.000
2.63%
122.000
0.84%
140.100
3.12%
150.000
0.95%
162.000
4.41%
175.000
1.92%
190.100
5.20%
219.100
5.71%
m/z (Da)

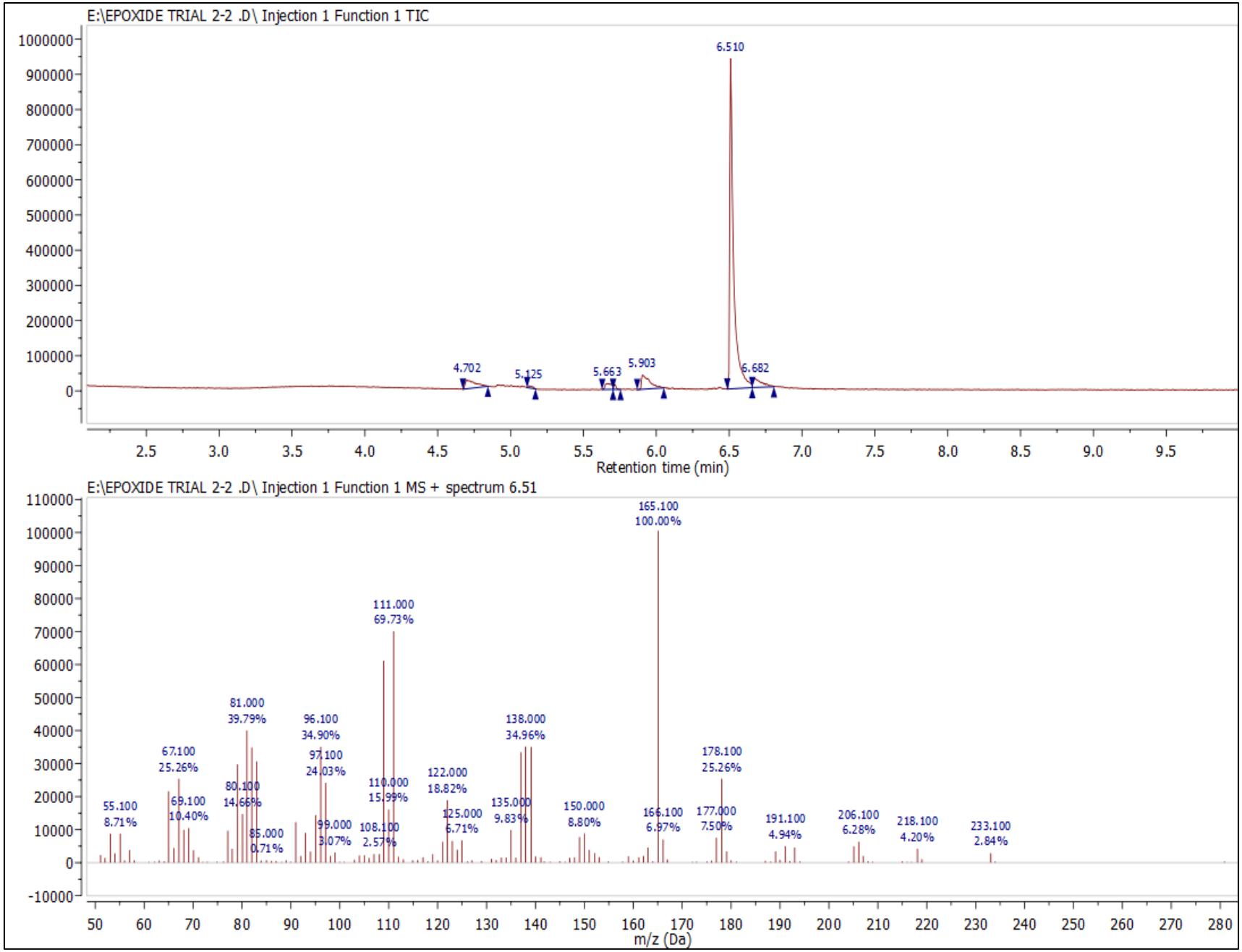

E:\EPOXIDE TRIAL 1-1 .D\ Injection 1 Function 1 TIC
1.6E+07
1.4E+07
1.2E+07
1.0E+07
8.0E+06
6.0E+06
4.0E+06
2.0E+06
0.0E+00
5.846
6.499
4.627
6.596
2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5
Retention time (min)
E:\EPOXIDE TRIAL 1-1 .D\ Injection 1 Function 1 MS + spectrum 5.85
2000000
1500000
1000000
500000
0
111.000 100.00%
80.100 95.34%
81.100 74.77%
83.000 59.14%
82.000 35.25%
139.100 57.53%
138.100 37.75%
218.100 40.38%
77.100 19.88%
65.000 15.75%
53.100 13.20%
93.100 12.98%
109.000 15.40%
78.100 5.79%
66.100 4.38%
54.100 1.95%
95.100 1.55%
112.000 2.58%
122.100 0.84%
140.100 3.03%
150.100 0.85%
162.100 4.20%
175.100 1.70%
190.100 4.56%
219.100 4.96%
50 60 70 80 90 100 110 120 130 140 150 160 170 180 190 200 210 220 230 240 250 260 270 280
m/z (Da)


Epoxide
1H at 400 MHz
CDCl3 at rt
7.26 Chloroform-d
A (d) 4.39
B (m) 4.13
C (s) 3.35
D (d) 3.20
E (d) 2.33
F (d) 2.08
G (d) 1.71
H (q) 1.35
1.00
2.73
0.45
0.46
0.49
0.66
1.13
2.98
5.46
f1 (ppm)

Epoxide
13C at 100MHz
CDCl3 at rt




Epoxide
31P at 400 MHz
CDCl3 at rt
34.36
31.05
30.24
29.79
-0.28
f1 (ppm)


E:\EPOXIDE TRIAL 2-2 .D\ Injection 1 Function 1 TIC
6.510
4.702
5.125
5.663
5.903
6.682
Retention time (min)
E:\EPOXIDE TRIAL 2-2 .D\ Injection 1 Function 1 MS + spectrum 6.51
165.100
100.00%
111.000
69.73%
81.000
39.79%
96.100
34.90%
97.100
24.03%
67.100
25.26%
138.000
34.96%
178.100
25.26%
122.000
18.82%
110.000
15.99%
80.100
14.66%
55.100
8.71%
69.100
10.40%
135.000
9.83%
150.000
8.80%
166.100
6.97%
177.000
7.50%
191.100
4.94%
206.100
6.28%
218.100
4.20%
233.100
2.84%
125.000
6.71%
108.100
2.57%
99.000
3.07%
85.000
0.71%
m/z (Da)