Synthesis of an Unnatural Phospholipid
for use in Pulmonary Surfactant Therapy

by

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SYNTHESIS OF AN UNNATURAL PHOSPHOLIPID FOR USE IN PULMONARY SURFACTANT THERAPY

Natasha L. Best
University of Guelph, 2012

Neonatal respiratory distress syndrome (RDS) is a disease that affects premature infants born prior to 32 weeks gestation. The main cause is a deficiency in pulmonary surfactant due to immature type II pneumocyte cells found in the alveoli. These cells are not capable of producing the required surfactant which normally functions to reduce the surface tension at the air-liquid interface of the lungs, as well as reduce the work of breathing and prevent alveolar collapse. A current treatment method for RDS is exogenous surfactant replacement therapy involving application of an exogenous surfactant preparation directly into the lungs of premature infants. Current surfactant preparations are animal-derived and very costly. Synthetic preparations, on the other hand, are an attractive alternative. The goal of this research is to synthesize a diether phosphonolipid analogue of dipalmitoyl phosphatidylcholine (DPPC), designated DEPN-8. When incorporated into a synthetic exogenous surfactant mixture, DEPN-8 exhibits greater adsorption and surface activity compared to its natural counterpart, DPPC. The synthesis of several components related to the re-tailored synthesis of DEPN-8 will be presented and discussed below.
ACKNOWLEDGEMENTS

I’d like to begin by first acknowledging my advisor and mentor, Dr. Adrian Schwan. Thank you for seeing my potential and giving me this great opportunity to work under your supervision. There are not enough words to express how grateful I am for all that you have done. It hasn’t been easy but I feel I have learned the good, the bad, and the ugly sides of research; all of which have helped me grow into a stronger person and chemist. Your guidance and encouragement is greatly appreciated.

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<tbody>
<tr>
<td>ALI</td>
<td>acute lung injury</td>
</tr>
<tr>
<td>ARDS</td>
<td>acute respiratory distress syndrome</td>
</tr>
<tr>
<td>Bn</td>
<td>benzyl</td>
</tr>
<tr>
<td>CLSE</td>
<td>calf lung surfactant extract</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>DEPN-8</td>
<td>trimethyl (3-phosphonopropyl) ammonium mono-(2,3-bis-hexadecyloxy)-propyl ester</td>
</tr>
<tr>
<td>DHP</td>
<td>3,4-dihydro-2H-pyran</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>DPPC</td>
<td>dipalmitoyl phosphatidylcholine</td>
</tr>
<tr>
<td>GC-MS</td>
<td>gas chromatography – mass spectrometry</td>
</tr>
<tr>
<td>GPC</td>
<td>glycerclyphosphorylcholine</td>
</tr>
<tr>
<td>HMD</td>
<td>hyaline membrane disease</td>
</tr>
<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>KHMDS</td>
<td>potassium hexamethyldisilazide</td>
</tr>
<tr>
<td>LPC</td>
<td>lysophosphatidylcholine</td>
</tr>
<tr>
<td>LPL</td>
<td>lysophospholipid</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>PAF</td>
<td>platelet activating factor</td>
</tr>
<tr>
<td>PC</td>
<td>phosphatidylcholine</td>
</tr>
<tr>
<td>PE</td>
<td>phosphatidylethanolamine</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>PG</td>
<td>phosphatidylglycerol</td>
</tr>
<tr>
<td>PI</td>
<td>phosphatidylinositol</td>
</tr>
<tr>
<td>PL</td>
<td>phospholipase</td>
</tr>
<tr>
<td>ppm</td>
<td>parts per million</td>
</tr>
<tr>
<td>PPTS</td>
<td>pyridinium p-toluenesulfonate</td>
</tr>
<tr>
<td>PrG</td>
<td>protecting group</td>
</tr>
<tr>
<td>PS</td>
<td>phosphatidylserine</td>
</tr>
<tr>
<td>RDS</td>
<td>respiratory distress syndrome</td>
</tr>
<tr>
<td>sn</td>
<td>stereochemical number</td>
</tr>
<tr>
<td>SP</td>
<td>surfactant protein</td>
</tr>
<tr>
<td>SPH</td>
<td>sphingomyelin</td>
</tr>
<tr>
<td>TBAI</td>
<td>tetrabutylammonium iodide</td>
</tr>
<tr>
<td>TBDPS</td>
<td>tert-butyl diphenylsilane</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>THP</td>
<td>tetrahydropyranyl</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>trimethylsilyl</td>
</tr>
<tr>
<td>TMSBr</td>
<td>bromotrimethylsilane</td>
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<tr>
<td>Tr</td>
<td>trityl</td>
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CHAPTER 1:

INTRODUCTION
1.0 Introduction

The lungs are a very complex organ which allows oxygen to enter our bodies and carbon dioxide to exit; a crucial factor in mammalian survival. As we breathe air in through our nose or mouth, it travels through a complex network of airways known as bronchial tubes ending in small air sacs called alveoli (Figure 1).\(^1\) The average person has 300 million alveoli within their lungs, each measuring approximately 75 to 300 microns in diameter. This leads to an internal surface area of approximately 1 m\(^2\) per kg of body weight when the lungs are fully expanded. For example, the lungs of a 70 kg (~ 150 lb) person would have an internal surface area roughly equal to the size of a badminton court.\(^1\) The following chapter will outline pertinent information regarding lung function and biochemistry, pulmonary surfactant, associated diseases, and current treatment methods.

![Figure 1: Bronchial tubes and alveoli, adapted from the Medical Encyclopedia.\(^2\)](image)

1.1 Surface Tension

The lungs, and ultimately the alveoli, function as the main site for gas exchange within
mammals.\textsuperscript{1} As a person inhales, the alveoli expand and as they exhale, the alveoli contract. It’s easy to see that work is required to inflate the lungs. To accommodate this, each alveolus is coated with a thin liquid film, known as the alveolar hypophase.\textsuperscript{1} The hypophase covers the pulmonary epithelial cells within the alveoli and thus creates a gas-liquid interface within the lungs. This interface is the main source of surface tension since the components at the interface act differently than those in the bulk hypophase. The work of breathing is due to expanding the alveoli against this surface tension force during inhalation/expansion.\textsuperscript{1} Surface active agents (surfactants) are present in the hypophase and are capable of quickly adsorbing to the interface to help regulate these tension forces, reduce the work of breathing, and stabilize the alveoli against collapse.\textsuperscript{1,3}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{forces.png}
\caption{Forces felt by molecules in a solution.}
\end{figure}

To better understand surface tension, envision a beaker of water (Figure 2). The water molecules in the bulk solution are attracted equally from all directions and thus feel a net force of
zero. The molecules at the surface will feel a strong pull from the bulk solution below but only a slight pull from the gas phase above, creating an overall downward force. This force is known as surface tension and can be physically visualized by the formation of a meniscus at the surface of the water. Surface tension can be thought of as a thermodynamic force which can be measured for a surface film and has units of milliNewtons per meter (mN/m).\textsuperscript{1}

1.2 Background

Surface behaviour has been studied for centuries. It dates back to the Ancient Greeks and their use of oil to calm rough seas.\textsuperscript{1} In 1765, Benjamin Franklin demonstrated that oil could calm the roughness on Clapham pond,\textsuperscript{4} and in 1774, he used the same method to measure the rate and extent of spreading of oil over the pond.\textsuperscript{1} Lord Rayleigh, in 1890, determined that an oil film spread on water had dimensions on the molecular level and with the help of contributions from Agnes Pockels, published an article on her behalf in \textit{Nature} in 1891 on surface tension.\textsuperscript{1,4,5} However, it wasn’t until the 20\textsuperscript{th} century that most of the findings regarding lung function and physiology were studied and reported.

Significant pulmonary breakthroughs of the 20\textsuperscript{th} century begin with Kurt von Neergaard in 1929.\textsuperscript{3,4,6,7} His studies of isolated porcine lungs involved comparing the pressure needed to inflate the lungs with air or with a saline solution. This gave insight into surface tension forces that are present at the air-tissue interface, but which are not present at the liquid-tissue interface. His concluding remarks were that lower surface tension is beneficial to the respiratory mechanism and that surface tension is the force that counteracts breathing and should be investigated further, especially in newborns.\textsuperscript{6} In 1947, Peter Gruenwald came to similar conclusions while repeating von Neergaard’s experiments.\textsuperscript{6} Surface active agents were shown to
reduce the pressure needed to expand the lungs. In the 1950’s, Richard Pattle discovered that pulmonary edema foam was present in rabbit lungs that were exposed to nerve gas.\textsuperscript{3,4,6,7} This foam remained unusually stable for hours, even after treating with antifoaming agents. Pattle concluded that a substance present in the lungs must be covering the air bubbles which helps to stabilize them.\footnote{Absence of this substance was also speculated to be a difficulty that premature infants have to contend with.\textsuperscript{6} Around the same time, the surface tension of films taken from the lungs of rats, cats, and dogs were reported by John Clements.\textsuperscript{6} He also made similar conclusions to Pattle regarding the lungs, and through a series of experiments, determined that the surface area of the lungs was large and that surface tension was at its lowest when the lungs were deflated.\textsuperscript{3,4,9}} In the late 1950’s, Mary Ellen Avery made the connection between hyaline membrane disease (HMD) in newborns and abnormal surface tension through analyzing the lungs of infants who had recently died from HMD.\textsuperscript{3,4,6,7} Along with Jere Mead, Avery published an article stating that infants with HMD showed an absence of surface active agents in their lungs which normally function to lower the surface tension in healthy patients.\textsuperscript{10}

Up until the 1960’s, the research regarding lung surfactants was not very extensive nor had many clinical trials been attempted to resolve the issues around HMD. A few major discoveries were made regarding the lungs and its function, however, one event really brought light onto the subject. On August 7, 1963, Patrick Bouvier Kennedy was born 5 to 6 weeks premature to former USA President John F. Kennedy and his wife, Jacqueline Bouvier Kennedy. A few days later, the infant passed away from the effects of HMD at Boston Children’s Hospital. The obituary stated that, ‘the battle for the Kennedy baby was lost only because medical science has not yet advanced far enough’.\textsuperscript{11} HMD, now known as Respiratory Distress Syndrome (RDS), had gained the public’s attention and within a few years, treatments were becoming available.
1.3 **Surfactant Properties**

Knowing that surfactant has a crucial role in the development and function of the lungs, it’s important to understand exactly what surfactant is and how it functions. Surfactant is synthesized, stored and secreted by type II pneumocyte cells within the alveoli of the lungs (Figure 3).\(^1\) It is stored within the alveolar type II cells as lamellar bodies and within the alveolar hypophase as tubular myelin, vesicles, and other large aggregate forms until it is required.\(^1,3\)

**Figure 3:** General characteristics of a pulmonary alveolus, adapted from Hawgood and Clements.\(^12\)

It has been identified that pulmonary surfactant components are ‘surface active’, meaning they adsorb quickly to the air-liquid interface where they form a film which is one molecule thick, known as a monolayer (Figure 4). These molecules have been identified to consist mainly of phospholipids having an amphipathic structure: a polar, hydrophilic head group at one end in contact with the liquid phase and nonpolar, hydrophobic chains at the other end extending towards the gas phase. Since these molecules are capable of interacting with both phases present at the interface, they are capable of reducing the surface tension forces felt during the expansion
and contraction cycles of breathing.

Another important feature surfactant components possess is the capability of re-entering a monolayer film once ejection has occurred. As the lungs contract, the monolayer compresses and components will occasionally get ‘squeezed out’ if there is not enough room to accommodate all of the surfactant present. The components that have a lower affinity for the monolayer will be preferentially lost and may not be capable of effectively ‘respreading’ into the monolayer when the lungs once again fill with air and expand. This can therefore result in the depletion or enrichment of certain components present at the interface.

![Surfactant monolayer](image)

**Figure 4:** Surfactant monolayer present at the air-liquid interface.

For pulmonary surfactant films to achieve a low surface tension of less than 1 mN/m, they must have a significant amount of rigid, saturated phospholipids which are capable of forming tightly packed, solid films when the alveoli are contracted.\(^1\) Thus, phospholipids with long, saturated chains and zwitterionic headgroups are typically employed.\(^13\) Although such lipids are effective at reducing the surface tension, they do not always effectively respread once ejected from the monolayer.\(^1\) The ability of surfactant components to adsorb quickly to the interface, as well as effectively respreading into the monolayer, are also key factors associated with high surface activity.\(^14\text{--}16\) Unsaturated phospholipids, on the other hand, respread much
better, but do not reduce the surface tension as noticeably.\textsuperscript{1} Therefore, surfactant mixtures must be complex since any one natural phospholipid on its own doesn’t seem to fulfil all the requirements of an effective surfactant.

The actual composition of pulmonary surfactant has, in fact, proven to be quite sophisticated. It consists of a mixture of lipids (~ 90%) and proteins (~ 10%).\textsuperscript{1,3} The lipid component consists of mainly phospholipids with only 5% being neutral lipids, such as cholesterol. Most phospholipids contain the general backbone shown in Figure 5, but differ based on the length and saturation of their fatty acid chains, R\textsuperscript{1} and R\textsuperscript{2}, attached at the sn-1 and sn-2 position of the glycerol backbone, respectively, as well as the corresponding headgroup component, X, attached at the sn-3 position of the glycerol backbone.

![Phospholipid Structure](image)

**Figure 5**: General phospholipid structure, where R\textsuperscript{1} and R\textsuperscript{2} are fatty acid chains and X is the corresponding headgroup component.

Interestingly, endogenous pulmonary surfactant consists of approximately 80% phosphatidylcholine (PC) derivatives, of which roughly half exists as 1,2-dipalmitoyl-sn-3-
phosphatidylcholine (DPPC).\textsuperscript{1,14-17} The structure of DPPC is shown in Figure 6. It contains two saturated palmitoyl (C16:0) fatty acid chains which allows it to form tightly packed films at the air-liquid interface.\textsuperscript{1} Although effective at reducing the surface tension, it has been shown that DPPC has poor respreading and adsorption behaviours.\textsuperscript{1,18} The remaining lipid component of endogenous surfactant contains mainly the anionic lipids phosphatidylglycerol (PG), phosphatidylinositol (PI) and phosphatidylserine (PS) with a small amount of the other zwitterionic lipids such as phosphatidylethanolamine (PE) and sphingomyelin (SPH).\textsuperscript{16} SPH is a phosphosphingolipid consisting of a PC headgroup and a ceramide-derived hydrophobic region.\textsuperscript{1}

![Figure 6: Phospholipase (PL) cleavage sites on DPPC.](image)

Compared to the lipid component of endogenous pulmonary surfactant, the protein component is minor and consists of only four proteins. The most abundant protein, surfactant protein A (SP-A), is a hydrophilic protein which accounts for roughly half of the protein component.\textsuperscript{1,14,19} It’s main function is to aid in host defense, however, this protein also plays an important role in promoting adsorption of lipids to the interface, and is typically isolated in association with surfactant lipids.\textsuperscript{17} SP-B and SP-C are both hydrophobic proteins, each accounting for \( \sim 15\% \) of the total protein component, and are also isolated in association with
Surfactant lipids. SP-B aids in increasing phospholipid aggregates near the interface and promoting lipid insertion to the film, while SP-C acts to disrupt fatty acid chains to promote lipid insertion to the monolayer. The fourth protein, SP-D, is also a hydrophilic protein but is not a functional biophysical component of surfactant. It’s main role is to help defend against incoming pathogens within the lungs. Of the four proteins, SP-B and SP-C are both crucial for surface activity and a lack of either protein at birth can produce lethal and irreversible respiratory failure. Due to this, these two proteins are typically incorporated into exogenous pulmonary surfactant mixtures, while the hydrophilic proteins, SP-A and SP-D, are not.

Surfactant has a very important role within the lungs and is naturally kept in a high concentration within the hypophase of healthy individuals. However, within the alveolar hypophase are also enzymes that degrade phospholipids. These enzymes, known as phospholipase (PL) enzymes, are more concentrated in individuals with inflamed or injured lungs. PL enzymes are capable of hydrolyzing phospholipids, forming by-products such as lysophospholipids (LPL) and free fatty acids. These by-products function to digest other lipids and, as they accumulate in the hypophase, the natural phospholipid concentration decreases. These harmful species will therefore decrease the overall surface activity of endogenous surfactant, ultimately raising the surface tension at the alveolar interface and making it very difficult for the individual to breathe.

There are four main pulmonary phospholipase enzymes, each of which cleaves at a specific site on the target phospholipid. PL-A and PL-A specifically cleave the carbon-oxygen bond of the ester linkage in the fatty acid chains attached at the sn-1 and sn-2 positions, respectively (Figure 6 above). These generate the harmful LPL and free fatty acids mentioned previously. PL-C and PL-D cleave the phosphorus-oxygen (P-O) bond on either side of the
phosphate moiety in the headgroup, which is attached to the sn-3 position. Thus, chemical degradation, inactivation, and enzymatic inhibition are all mechanisms that reduce surface activity of surfactant mixtures. When inflammation is present in the lungs, these pathways are particularly concerning since their effects can be more detrimental to the individual.

1.4 Associated Diseases

In a healthy individual, surfactant is plentiful. It functions to reduce surface tension in the lungs, overcome the work of breathing, and prevent alveolar collapse.\(^1\) However, when pulmonary surfactant is deficient or dysfunctional, major problems arise. Infants that are born prior to 32 weeks gestation typically experience a disease called Respiratory Distress Syndrome (RDS), formerly known as Hyaline Membrane Disease (HMD).\(^{14\sim17,25,26}\) The lungs of premature infants have under-developed type II pneumocyte cells, which are responsible for production and secretion of surfactant.\(^{1,27,28}\) Thus, little or no surfactant is produced from these cells in premature infants which can lead to a low concentration of surfactant within the hypophase. Due to this, surfactant is not able to cover the surface of each alveolus completely and breathing problems tend to arise due to abnormal surface tension within the lungs.

Mortality rates among premature infants were exceptionally high, around 66\% in 1961, shortly after RDS was first discovered and studied.\(^{29}\) However, mortality rates were reported to have dropped below 20\% in 1998.\(^{29}\) On average, the direct in-hospital care costs for an infant born less than 25 weeks gestation can exceed $100,000, with additional indirect costs adding 30\% more to this value. Infants with a birth weight under 750 grams face, on average, 40 days on mechanical ventilation in-hospital, while infants weighing between 1500 – 2500 grams face 5 days on this type of ventilation.\(^{29}\) More recently, a study performed by the Canadian Neonatal
Network in 2010 reported a mortality rate of only 3.4% for RDS patients among 27 different sites across Canada.30

Other issues related to surfactant dysfunction include Acute Respiratory Distress Syndrome (ARDS) and Acute Lung Injury (ALI).14,16,17,25,26,31 Both of these can affect people of all ages and are typically caused by severe injury to the lungs. When the lungs are injured, inflammation occurs which increases the concentration of phospholipase and other inhibitory enzymes, making breathing more difficult. Lung injury can occur by both direct and indirect methods, including the following: smoking, near-drowning incidences, bacterial or viral infections, physical trauma, and meconium aspiration at birth.31,32 Also important to note is that other organs may be involved or feel the effects of the injury, not just the lungs. High mortality rates, nearing 42%, are also reported for ARDS which can range from 50,000 to 190,000 reported cases per year.31,32

1.5 Current Treatment Methods

With the high mortality rates seen for RDS, as well as ARDS and ALI, a treatment regime that involves physically ventilating the lungs and/or administering a surfactant mixture is ideal. A method commonly known as exogenous pulmonary surfactant replacement therapy has already proven effective as it maintains the lungs open while surfactant is administered.17 On average, the required dose for an infant is 100 mg of surfactant per kilogram of body weight. The surfactant administered complements the natural surfactant found in the lungs, thus helping to decrease surface tension and allow the infant to breathe more easily. In the case of infants with RDS, this method may be administered via an endotracheal tube until the infant begins to produce natural surfactant on their own (ie: the type II pneumocyte cells have developed and
begun to produce and secrete natural surfactant).\textsuperscript{15,19,27,28} Survival rates among infants treated with exogenous pulmonary surfactant has significantly increased and the symptoms of RDS have been shown to slowly disappear with time.\textsuperscript{1,33}

The era of exogenous pulmonary surfactant replacement therapy began with the first published trials involving aerosolized DPPC in 1964 and 1967, both of which were sadly disappointing.\textsuperscript{4,34,35} After initial unsuccessful attempts, Enhorning and Robertson successfully administered surfactant obtained from adult rabbits into the lungs of premature rabbits via tracheal instillation.\textsuperscript{4,36} Several years later, Fujiwara and colleagues reported human clinical trials using their own ‘artificial surfactant’, which was simply material from the lungs of cows.\textsuperscript{4,7,37} Although the initial human trials did not adhere to specific guidelines, it still stimulated many researchers to publish useful data they obtained from controlled clinical trials investigating surfactant replacement therapy.\textsuperscript{4,38,39} By the 1990’s, it was estimated that approximately 500 hospitals around the world had enrolled nearly 30,000 infants in clinical trials to evaluate and optimize the use of pulmonary surfactant.\textsuperscript{4}

1.6 Surfactant Preparations & Techniques

Since the early beginnings of pulmonary surfactant replacement therapy, many clinical surfactant preparations have become commercially available. They can be divided into three basic groups:\textsuperscript{7}

- Group I: Surfactant extracts from lavaged mammalian lungs.
- Group II: Surfactant extracts from processed mammalian lungs, with or without synthetic additives.
- Group III: Fully synthetic preparations.
Group I preparations, also referred to as natural surfactants, closely resemble human endogenous surfactant and are obtained from the alveolar lung lavage fluid of the mammal it is derived from. These preparations typically contain all the surfactant phospholipids plus the two hydrophobic proteins SP-B and SP-C. A few commercially available preparations include bLES®, Infasurf®, and Alveofact®, all of which are obtained from lavaged bovine lungs.

Group II preparations are obtained from the processed lung tissue of animals and therefore also contain surfactant phospholipids and one or both of the hydrophobic proteins. Since the lungs are processed, other cellular lipids and/or protein fragments may be included as well. A major downfall of this method is that processing affects the total protein content of the preparation, and therefore synthetic additives may be required. A few examples include Surfacten® and Survanta®, both obtained from processed bovine lungs, and Curosurf®, obtained from processed porcine lungs.

Group III preparations are fully synthetic mixtures which do not include any natural surfactant obtained from mammals. Since they are manufactured, there are many advantages for their use over other preparations. Some advantages include the high purity obtained, the ease of reproducibility, and the option for synthetic scale-up. Since these preparations are not animal-derived, they are free from the religious and cultural issues surrounding such preparations and the risk of prion transmission is also eliminated. A few examples of commercially available preparations include ALEC®, Exosurf®, Surfacin®, and Venticute®.

The debate on whether to use animal-derived surfactant versus synthetic surfactant is an on-going battle. Animal-derived mixtures closely resemble our own pulmonary surfactant; however, animal sacrifice raises many issues around the world. The high cost of raising animals and acquiring animal-derived surfactant is also a major issue, which can be eliminated with the
use of synthetic surfactants. The challenge for scientists is to synthesize a surfactant mixture that can compete with the animal-derived preparations currently available and used worldwide.

To test the surface activity of a surfactant preparation, the sample is measured using a pulsating bubble surfactometer (Figure 7).\textsuperscript{16,40,41} An air bubble, of known radius, is formed at the end of a capillary tube, which is in contact with the surfactant mixture. The air bubble can be pulsated at a specific rate in order to mimic the alveoli during the expansion and contraction cycles of breathing. This is done by varying the pressure within the sample cuvette by means of a precision pulsator. The radius of the air bubble can be monitored via microscope to ensure the minimum and maximum bubble sizes remain constant throughout pulsation. The pressure is measured by a pressure transducer, and therefore surface tension can be calculated by rearranging the Young-Laplace equation. This equation, $\Delta P = 2\sigma/R$, relates the pressure difference measured across the bubble to its radius and surface tension.

![Figure 7: General apparatus of a pulsating bubble surfactometer, based on Zuo et al.\textsuperscript{16}](image-url)
CHAPTER 2:
SYNTHETIC STRATEGIES
2.0 Synthetic Strategies

With the increase in animal-derived surfactant mixtures currently on the market, there is an overwhelming demand for a synthetic preparation which is superior to all those currently available. The benefits of a purely synthetic mixture include alleviating the ethical and moral issues surrounding animal-derived preparations, as well as potentially reducing the overall cost for RDS treatment. Since surfactant contains both lipids and proteins, it would be beneficial if all these components were characterized and synthetically available; something to possibly look forward to in the future. The following section will outline techniques and significant synthetic approaches used in the preparation of phospholipids, with a focus on the preparation of PC lipids and specific modifications thereof. For a more complete history of synthetic approaches towards phospholipids, several reviews are available.42-46

2.1 Microwave Synthesis

A useful piece of equipment in the synthetic preparation of phospholipids is a microwave reactor. The basic concept of microwave heating is based on the application of microwaves, specifically at 2.45 GHz, to a sample in order to generate a flow of electrons.47,48 This ultimately produces both a magnetic and an electric field. The amount of energy delivered to a reaction at this frequency is roughly 0.037 kcal/mol and thus interacts with the sample at the molecular rotational level.47 As microwaves are applied, the molecules rotate within the vessel. The constant energy applied keeps the molecules in motion, leading to ‘super heating’ within the vessel which ultimately increases the overall reaction temperature.

The use of microwave heating provides a more consistent heat source compared to conventional heating methods which use an oil bath and hot plate.47 These conventional methods
rely on transferring heat to a reaction vessel by means of convection currents and thermal conductivity. This requires that the actual reaction vessel be heated in addition to its contents. The potential for hot spots within the reaction mixture are also more probable, as well as the possible decomposition of reactants and/or products. This occurs since the reaction vessel is essentially hotter than the bulk reaction mixture contained inside. In microwave synthesis, the use of Teflon or borosilicate glass reaction vessels eliminates these undesired outcomes. Vessels made of such materials do not absorb the microwaves applied, and can therefore remain cooler than the mixture within.

Another limiting factor in microwave synthesis is the choice of solvent. More polar solvents cause increased rotational movement within the sample because of their stronger dipole moments. This generates more heat within the reaction vessel. A few examples include methanol and dimethyl sulfoxide (DMSO). Less polar solvents, on the other hand, do not absorb as much energy and therefore do not contribute to heating the reaction as much. These can also be classified as ‘microwave transparent’ and include dichloromethane (DCM), chloroform, and ether, to name a few.

In addition, the rate at which the temperature can change within a microwave reactor is also much greater compared to an oil bath. The microwave reactor is capable of reaching the set temperature within minutes and when the reaction is complete, cool air can be applied to lower the temperature quickly. With an oil bath, usually an extended amount of time is required to heat the oil. This forces the chemist to begin warming it possibly several hours prior to starting the reaction, depending on the temperature desired.

In general, the use of a microwave reactor in organic synthesis provides increased product yields, decreased reaction times, and cleaner and more reproducible reactions.
2.2 Phospholipid Synthesis

Since the discovery of lecithin (now commonly known as phosphatidylcholine) in egg yolk, ox, and man by Gobley in 1846,\textsuperscript{43,45,46} routes towards phospholipid synthesis have been modified and optimized as chemistry, in general, has become more advanced. Researchers attempted to synthesize phospholipids following that initial discovery,\textsuperscript{43} however, it wasn’t until 1947 that the first reliable synthesis of a phospholipid was developed by Rose.\textsuperscript{49} With plenty of room for improvement, more research began in the field of phospholipid synthesis.

By analyzing the phospholipid structure, it is evident that it consists of three major portions: the glycerol backbone, the phosphate moiety, and the headgroup component. Early articles regarding phospholipid synthesis focused on ways to access the glycerol backbone having fatty acid chains of varying length and saturation. In 1939, Fischer and Baer proposed that D-acetone glycerol (solketal) can be used as an appropriate starting compound for the glycerol backbone (Scheme 1).\textsuperscript{46,50,51} This compound is synthetically accessible through acetonation of D-mannitol with zinc chloride and acetone and subsequent oxidative cleavage with lead tetra-acetate to afford D-glyceraldehyde. This can then be catalytically reduced via hydrogen gas in the presence of nickel to provide the desired starting compound.

![Scheme 1: Fischer and Baer’s synthesis of solketal.](image)
With the starting compound in hand, the next step would focus on producing the acylated glycerol. A simple procedure to obtain saturated diacyl glycerols was proposed by Fischer and Sowden in 1941 (Scheme 2). It begins with the benzyl (Bn) protection of solketal using benzyl bromide in the presence of sodium. The isopropylidene group is removed via mild acid hydrolysis and the resulting diol can be diacylated with the desired fatty acid chloride in pyridine. The benzyl protecting group is then cleaved by catalytic hydrogenolysis to produce the desired D-α,β-glyceride.

Scheme 2: Fischer and Sowden’s synthesis of D-α,β-glyceride.

Once the desired saturated glyceride is synthesized, it can be phosphorylated and transformed into the corresponding phospholipid through introduction of the phosphate moiety and headgroup. Baer and Kates proposed a synthetic pathway in 1950 which involves phosphorylation of D-α,β-glyceride with phenyl phosphoryl dichloride in the presence of pyridine in chloroform (Scheme 3). The resulting mixture is used without purification and is esterified with choline chloride in pyridine to afford the choline ester, which is isolated as its reineckate salt. After transformation to the corresponding sulfate, the protecting phenyl group is cleaved via catalytic hydrogenolysis to afford the desired PC lipid after crystallization.

This method is effective for the preparation of saturated diacyl PC lipids, but is unsuitable for the preparation of similar unsaturated compounds. Problems arise from the catalytic hydrogenolysis of the protecting phenyl group, which is also known to reduce the
unsaturation in the fatty acid chains.\textsuperscript{46}

\begin{center}
\begin{align*}
\text{R(CO)O} & \quad \text{Cl} \quad \text{Cl} \quad \text{P-OCl} \quad \text{Ph} \\
\text{R(CO)O} & \quad \text{pyridine} \quad \text{chboroform} \\
\text{R(CO)O} & \quad \text{Cl} \quad \text{Cl} \quad \text{P-OCl} \quad \text{Ph} \\
\text{R(CO)O} & \quad \text{pyridine} \quad \text{chboroform} \\
\text{R(CO)O} & \quad \text{O} \quad \text{P-O} \quad \text{Cl} \\
\text{R(CO)O} & \quad \text{N}^+\text{Me}_3\text{Cl}^- \\
\text{R(CO)O} & \quad \text{O} \quad \text{P-O} \quad \text{Cl} \\
\text{R(CO)O} & \quad \text{N}^+\text{Me}_3\text{Cl}^- \\
\text{R(CO)O} & \quad \text{O} \quad \text{P-O} \quad \text{Cl} \\
\text{R(CO)O} & \quad \text{N}^+\text{Me}_3\text{Cl}^- \\
\end{align*}
\end{center}

\textbf{Scheme 3}: Baer and Kates’ synthesis of saturated diacyl PC.

An alternate synthesis for unsaturated diacyl PC lipids was proposed in 1956 by Baer, Buchnea and Newcombe (Scheme 4).\textsuperscript{54} It involves the direct phosphorylation of solketal with phenyl phosphoryl dichloride in quinoline followed by reaction with ethylene chlorohydrin in pyridine. The product then undergoes catalytic hydrogenolysis at this point to remove the protecting phenyl group, as well as mild acid hydrolysis to obtain the free diol. The product is converted to its barium salt via barium hydroxide, which is then diacylated with the desired unsaturated fatty acid chloride in pyridine and \( N,N \)-dimethylformamide (DMF). Formation of the choline headgroup is carried out by reaction with trimethylamine.

It wasn’t until a few years later that a synthetic route was developed that could be used to prepare both saturated and unsaturated PC lipids.\textsuperscript{46} The synthetic route, proposed by Baer and Buchnea in 1959, begins with L-\( \alpha \)-glycerolphosphorylcholine (L-\( \alpha \)-GPC) (Scheme 5).\textsuperscript{55} L-\( \alpha \)-GPC is accessible from solketal, a procedure developed by Baer and Kates in 1948.\textsuperscript{56} The first step involves the reaction of L-\( \alpha \)-GPC with cadmium chloride to obtain the cadmium chloride complex of L-\( \alpha \)-GPC. Cadmium chloride is said to form a complex with the quaternary
ammonium, which allows acylation to occur quickly at low temperatures. Acylation is achieved through reaction of the desired saturated or unsaturated fatty acid chloride in the presence of pyridine.

**Scheme 4**: Baer, Buchnea, and Newcombe’s synthesis of unsaturated diacyl PC.

Similarly, Hirt and Berchtold developed a procedure for the synthesis of PC lipids in 1958 (Scheme 6). This method involves the coupling of diacyl glycerol with 2-bromoethyl phosphoric acid dichloride in the presence of triethylamine. Subsequent amination with trimethylamine provides the desired PC lipid. Simple modifications were made by Hansen in 1982, specifically to the choice of solvent, which helped reduced reaction times and eliminate
several unwanted side products.\textsuperscript{57}

\begin{center}
\begin{align*}
\text{R(CO)O} & \text{O} \text{H} + \text{ClPOOBr} & \text{Et}_3\text{N} \\
\text{Me}_3\text{N} & \rightarrow \text{R(CO)O} & \text{POO}^- \\
& \text{R(CO)O} & \text{POO}^- \text{NMe}_3
\end{align*}
\end{center}

\textbf{Scheme 6}: Hirt and Berchtold’s synthesis of PC lipids.

The synthetic methods mentioned so far allow for PC lipids to be synthesized with two identical fatty acid chains. However, in nature, lipids can also exist with two non-identical fatty acid chains, varying in both length and saturation. These chains can also vary in the way they are connected to the glycerol backbone. For instance, typical phospholipids contain ester linkages to the glycerol backbone, although ether linkages have also been identified in nature as early as the 19\textsuperscript{th} century.\textsuperscript{46,58} A significant advantage of ether linkages in phospholipids is the resistance to phospholipase degradation.\textsuperscript{1} Since PL-A\textsubscript{1} and PL-A\textsubscript{2} attack the ester linkages of the respective fatty acid chains (refer to Figure 6), the ether linkage will remain unaffected. The methods mentioned previously for preparation of PC lipids containing acyl chains can also be altered to accommodate the preparation of similar compounds with alkyl chains. Preparation of mixed chain lipids require a few extra precautions than those mentioned above.

In 1964, Baumann and Mangold proposed a procedure to obtain 1-O-alkylglycerols beginning with deprotonation of solketal by reaction with either potassium in benzene or potassium hydroxide in xylene (Scheme 7).\textsuperscript{58} Further alkylation using the appropriate alkyl methanesulfonate and subsequent hydrolysis of the isopropylidene with mild acid produces the
1-\(O\)-alkylglycerols in moderate yields.

Scheme 7: Baumann and Mangold’s synthesis of 1-\(O\)-alkylglycerol.

With 1-\(O\)-alkylglycerol in hand, synthetic methods could be developed towards the isolation of mixed chain glycerols. A procedure proposed by Baumann and Mangold in 1966 involves trityl (Tr) protection of 1-\(O\)-alkylglycerol, followed by acylation of the resulting secondary alcohol (Scheme 8).\(^{59}\) Similarly, alkylation procedures could be employed to obtain mixed chain dialkyl derivatives. Detrylation with boron trifluoride in methanol provides the desired mixed chain glycerol.\(^{60}\) This can be further transformed into the desired PC lipid by methods similar to those mentioned above (Schemes 3 and 6).\(^{53}\)

Scheme 8: Baumann and Mangold’s synthesis of mixed acid glycerol.

In the 1970’s, Benveniste discovered a natural PC lipid isolated from rabbit platelets which contained an alkyl chain at \(sn\)-1 and an acyl chain at \(sn\)-2.\(^{61,62}\) Its structure, 1-\(O\)-alkyl-2-acetyl-\(sn\)-glyceryl-3-phosphorylcholine, was elucidated in 1979 by Demopoulos as being identical to that of platelet activating factor (PAF), an important activator and mediator of
phospholipids related to immune system functions.\textsuperscript{63} A synthetic route towards its synthesis was proposed in 1984 by Eibl, which requires 2,5-dibenzyl-3,4-isopropylidene-D-mannitol as the starting precursor (Scheme 9).\textsuperscript{45} Alkylation is achieved using the desired alkyl bromide in a mixture of tetrahydrofuran (THF) and potassium \textit{tert}-butoxide. Through a series of steps, the mannitol derivative is cleaved to provide the respective glycerol derivative, which can be phosphorylated with bromoethyl phosphoric acid dichloride in THF and triethylamine. After the methyl ester is obtained, it is debenzylated and subsequently acetylated at the \textit{sn}-2 position. PAF is obtained through amination and demethylation using trimethylamine in chloroform/isopropanol.

\textbf{Scheme 9}: Eibl’s synthesis of PAF.

In addition to the methods mentioned above for isolating PC lipids (Schemes 3 and 6), cyclic phosphorylating agents have also been identified.\textsuperscript{64,65} A simple procedure reported by Eibl in 1978 begins with the production of the respective phosphatidic acid dichloride by reaction of phosphorus oxychloride with the desired alcohol (Scheme 10).\textsuperscript{65} Further reaction with ethylene
glycol in the presence of triethylamine provides the dioxaphospholane derivative, which can be reacted with trimethylamine to open the ring and produce the respective PC lipid.

**Scheme 10**: Eibl’s synthesis of PC via a dioxaphospholane intermediate.

Alternative methods have focused on synthesizing the dioxaphospholane portion separately followed by combination with the glycerol backbone (Scheme 11). The phosphorylating agent, 2-chloro-2-oxo-1,3,2-dioxaphospholane, is easily prepared from barium bromoethyl phosphate according to the procedure of Kumamoto et al., however, this compound is now commercially available. Coupling of the glycerol backbone with the dioxaphospholane derivative is followed by transformation to the PC lipid as described previously using trimethylamine (Scheme 10).

**Scheme 11**: PC synthesis via coupling of 1,2-diradylglycerol and 2-chloro-2-oxo-1,3,2-dioxaphospholane.
2.3 Phosphonolipid Synthesis

Since the discovery of phospholipids containing diacyl chains connected to a phosphate moiety and a polar headgroup, many lipid analogues have been discovered. The existence of phospholipids with dialkyl chains, as well as mixed acid chains, has led to many new synthetic protocols over the years. More recently, natural lipids have been isolated containing a carbon-phosphorus bond between the phosphorus moiety and the headgroup. These lipids have been named ‘phosphonolipids’ as they contain a phosphonic acid functionality instead of phosphoric acid. The stability of these compounds differs from that of regular phospholipids; the presence of the carbon-phosphorus bond allows phosphonolipids to be highly resistant to both acid and base hydrolysis. For example, phosphonolipids exposed to 6M hydrochloric acid at temperatures nearing 110 °C for 48 hours remained intact and were able to be separated by column chromatography from other phospholipids which had been digested under the acidic conditions. Other reports suggest that the carbon-phosphorus bond of phosphonolipids is resistant to the effects of PL-D, which can be quite advantageous in many scenarios.

The first natural phosphonate, 2-aminoethyl phosphonic acid, was discovered in sheep rumen by Horiguchi and Kandatsu in 1959. The presence of natural phosphonates was later confirmed in 1962 by Kittredge et al. when a similar compound was isolated from the sea anemone Anthopleura elegantissima. The synthetic protocols described previously for the synthesis of phospholipids were found to be unacceptable for producing the desired phosphonolipids under similar conditions. Installation of the headgroup containing a carbon-phosphorus bond was proving difficult as the final step. In order to overcome this set-back, a synthetic protocol was developed that simply involved a reversal of the synthetic steps. This allowed Baer and Stanacev to publish the first synthetic route towards the isolation of
phosphonates in 1964. The PE phosphonolipid analogue, 1,2-dipalmitoyl-sn-glycero-3-(2-aminoethyl)phosphonate, was prepared by reaction of the diacyl glycerol with the protected 2-aminoethylphosphonic acid monochloride in the presence of triethylamine (Scheme 12). In the specific case of PE derivatives, the protecting group (PrG) is used to avoid possible interference from the amine group during the phosphonylation procedure. For similar PC compounds, these protection strategies are not required as the quaternary ammonium is not reactive under the required reaction conditions.

Scheme 12: Baer and Stanacev’s synthesis of PE-derived phosphonates; PrG = protecting group.

Following the isolation and synthesis of several PE-derived phosphonates, the possible existence of other phosphonates in nature sparked an interest amongst researchers. The first synthetic approach towards PC-derived phosphonates was proposed in 1965 by Baer and Stanacev. Similar in preparation to PE-derived phosphonates, the general trend is to synthesize the glycerol backbone component and the phosphonic acid component separately. The desired glycerol backbone is easily prepared as previously described (Scheme 2, 8). Baer and Stanacev proposed that the phosphonylating agent be prepared from diethyl 2-bromoethylphosphonate. Compounds of this nature can be easily prepared through the Michaelis-Arbuzov reaction (Scheme 13) or the Michaelis-Becker reaction (Scheme 14).
In general, the Michaelis-Arbuzov reaction proceeds by heating an electrophilic alkyl halide with the desired trialkyl phosphite at temperatures ranging from 120 – 180 °C. This causes a nucleophilic substitution to occur, and the resulting halide anion aids in dealkylating the phosphonium intermediate, which ultimately provides the desired phosphonate diester. Alternatively, the Michaelis-Becker reaction proceeds by treatment of the respective H-phosphonate with a base, such as sodium hydride, which generates the salt of the dialkyl phosphonate. This is then reacted with an electrophilic alkyl halide, generating the desired phosphonate diester via P-alkylation. The phosphonate diester can be converted into a useful phosphorylating agent by conversion to its phosphonic acid derivative, which is followed by transformation to either its monochloride or dichloride derivative with the use of chlorinating agents such as phosphorus pentachloride, oxalyl chloride, or thionyl chloride.

Baer and Stanacev proposed the phosphonate diester could be transformed into the respective phosphonic acid monochloride before being coupled to the glycerol component. These two compounds are brought together in the presence of triethylamine and subsequently
transformed into the PC-derived phosphonate with the use of trimethylamine (Scheme 15).\(^7^1\)

\[
\begin{align*}
R^1(CO)O\underset{\text{Et}_3N}{\text{O}}\underset{\text{R}^2(CO)O}{\text{OH}} + \underset{\text{Cl-P}}{\text{HO}}\underset{\text{Br}}{\text{Br}} & \rightarrow R^1(CO)O\underset{\text{Et}_3N}{\text{O}}\underset{\text{R}^2(CO)O}{\text{O}}\underset{\text{Me}_3N}{\text{PO}}\underset{\text{N}\text{Me}_3}{\text{OH}} \\
\end{align*}
\]

**Scheme 15**: Baer and Stanacev’s synthesis of PC-derived phosphonates.

Many similar procedures have also been reported which demonstrate how to access other phosphonates containing diacyl, dialkyl, and mixed acid chains.\(^6^7\) Of particular interest is the preparation of dialkyl phosphonocholesterol lipids. In 1966, Baer and Rao described the synthesis of several diether PC-derived phosphonates (Scheme 16).\(^7^7\) The procedure they used is similar to that described previously by Baer and Stanacev for the preparation of PC-derived phosphonates (Scheme 15).\(^6^9,^7^4\) They begin by transforming diethyl 2-bromoethylphosphonate into the corresponding phosphonic acid monochloride. This can then be combined with the desired dialkyl glycerol in the presence of triethylamine. Further transformation into the desired dialkyl PC-derived phosphonate occurs, as described above, by treatment with trimethylamine.

\[
\begin{align*}
\text{EtO-P} & \underset{\text{Et}_3N}{\text{O}}\underset{\text{Br}}{\text{Br}} & \rightarrow & \text{Cl-P} & \underset{\text{OH}}{\text{HO}}\underset{\text{Br}}{\text{Br}} & + & \underset{\text{RO}}{\text{O}}\underset{\text{OH}}{\text{OH}} \\
\text{EtO-P} & \underset{\text{Me}_3N}{\text{O}}\underset{\text{Br}}{\text{Br}} & \rightarrow & \underset{\text{RO}}{\text{O}}\underset{\text{OH}}{\text{OH}}\underset{\text{Br}}{\text{Br}} & \rightarrow & \underset{\text{RO}}{\text{O}}\underset{\text{OH}}{\text{OH}}\underset{\text{N}\text{Me}_3}{\text{N}\text{Me}_3} \\
\end{align*}
\]

**Scheme 16**: Baer and Rao’s synthesis of dialkyl PC-derived phosphonates; \(R = \text{C}_{18}\text{H}_{37}, \text{C}_{16}\text{H}_{33},\) or \(\text{C}_{14}\text{H}_{29}\).
Many other procedures have since been reported for the synthesis of phospholipids and phosphonolipids alike. The increasing popularity of phosphonolipids, especially those containing diether linkages, is perhaps due to their interesting surface behaviours and/or structural similarities to natural lipids. The benefits of small structural changes become noticeable when lipids are arranged in a monolayer and surface activity is measured. Such results will be discussed in the subsequent section as they apply specifically to the diether phosphonolipid prepared in this thesis. Research regarding the inclusion of this compound into synthetic preparations used to treat RDS has already begun by collaborators at the University of Rochester under the supervision of Dr. Robert Notter. Various results will be discussed below that help illustrate how this lipid has increased surface activity and stability when compared to its phospholipid counterparts.
CHAPTER 3:

RESULTS AND DISCUSSION
3.0 Results and Discussion

The primary component of endogenous pulmonary surfactant is DPPC, which accounts for approximately 40% of the total lipid component.\(^1\) Synthetic preparations for RDS treatment have focused on utilizing this lipid as the main component in their mixtures; however, there are many downfalls. The lifetime of DPPC in the monolayer is very short due to increased phospholipase degradation (refer to Figure 6) within the inflamed lungs of RDS patients.\(^{18, 78}\) DPPC is also known to exhibit poor adsorption behaviour and is ineffective at continually reducing surface tension when used alone as an exogenous surfactant.\(^1, 18\) Surface films consisting of DPPC are more solid and tightly packed than natural films. In addition, DPPC exhibits poor resspreading behaviour after it is squeezed out of the monolayer.\(^1, 79\) The solution to this problem was to slightly alter the structure of DPPC in order to obtain an analogue that would maintain surface activity while avoiding degradation by phospholipase enzymes. The diether phosphonolipid analogue of DPPC fulfilled these requirements and is named DEPN-8; trimethyl (3-phosphonopropyl) ammonium mono-(2,3-bis-hexadecyloxy)-propyl) ester (Figure 8).

The structure of DEPN-8 is similar to that of DPPC, with a few modifications: the ester linkages between the fatty acid chains and the backbone are replaced with ether linkages, and a methylene-for-oxygen substitution occurs in the choline headgroup. Both of these alterations have significant benefits. The ether linkages are more hydrophobic and allow for greater flexibility of the fatty acid chains which helps promote adsorption into the monolayer.\(^{24, 80}\) Monolayer films containing DEPN-8 are also capable of packing tightly together since the ether linkages do not sterically hinder the molecule to the extent that ester linkages do. This helps to improve not only the resspreading behaviour of the lipid but also the overall surface activity.\(^1, 24\) Removal of the ester linkages eliminates possible degradation by both PL-A\(_1\) and PL-A\(_2\), and the
methylene replacement in the headgroup eliminates PL-D degradation.\textsuperscript{18,24,25} It has also been shown that the phosphonate headgroup is more hydrophobic than the phosphate headgroup which affects both bond angles and orientations when incorporated into a monolayer film.\textsuperscript{81} The proper balance of hydrophobic and hydrophilic regions in a phospholipid is essential in determining the stability of the lipid at the alveolar interface.\textsuperscript{81}

\textbf{Figure 8:} Structure of DEPN-8 identifying alterations from DPPC.

The following sections will give insight towards the potential incorporation of DEPN-8 into exogenous pulmonary surfactant preparations, followed by the synthetic work that has been performed towards its synthesis.

\textbf{3.1 Preliminary Synthetic Surfactant Results}

Initial attempts at preparing surfactant mixtures effective at reducing surface tension within the lungs typically involved incorporation of DPPC, as well as one or more other components. In 1979, Hildebran \textit{et al.} demonstrated that a mixture consisting of 90\% DPPC and up to 10\% cholesterol or monounsaturated PC could function as pulmonary surfactant.\textsuperscript{3,82} A few years later, Morley \textit{et al.} reported an effective surfactant that consisted of 70\% DPPC and 30\% PG.\textsuperscript{3,83} Tests performed on premature rabbits showed positive results and the surfactant
preparation was later tested on a group of premature infants. All of the infants showed improvements following the application of the surfactant preparation.  

Since the initial reports of synthetic pulmonary surfactant preparations, surfactant mixtures have become increasingly complex. Mixtures can now include, but are not limited to, a variety of phospholipids and phosphonolipids, as well as specific surfactant proteins or functional derivatives thereof. Surfactant proteins can be obtained from mammalian lung lavage extracts using centrifugation and extraction techniques, which will provide both SP-B and SP-C. Another route is the synthetic preparation of proteins and derivatives, such as Mini-B, prepared by Walther et al.  

This synthetic protein is an SP-B derivative which is truncated to 34 amino acids. The residues incorporated can be found in the amphipathic regions of the natural protein which are located in both the N- and C-terminals. Results demonstrate that this protein derivative strongly interacts with phosphonolipids at the molecular level which helps to improve surface activity.  

Previous research reported by Schwan and Notter compares surface tension data obtained from calf lung surfactant extract (CLSE), DPPC, and DEPN-8 (with and without added surfactant proteins). Measurements were recorded at minimum bubble radius using a pulsating bubble surfactometer (Table 1). CLSE, which is equivalent to the commercially available exogenous surfactant Infasurf®, has already proven to be an effective surfactant. CLSE is capable of reducing the surface tension to values below 1 mN/m in less than one minute. On the other hand, a surfactant preparation consisting of purely DPPC is very ineffective based on the results below. Even after 20 minutes of continual cycling, surface tension remains above 20 mN/m. It can therefore be concluded that DPPC alone should not be used exclusively in surfactant preparations. However, based on the data below, the phosphonolipid analogue of
DPPC shows promising results. Used alone as a surfactant, it is capable of reducing the surface tension to values below 1 mN/m within 15 minutes of continual cycling. However, when 1.5% SP-B and SP-C are also incorporated, which mimics the amount found in endogenous pulmonary surfactant, surface tension falls below 1 mN/m within 5 minutes. This confirms that DEPN-8 becomes competitive with CLSE at reducing surface tension when 1.5% SP-B and SP-C are added to the surfactant mixture.\textsuperscript{18,24,25}

**Table 1:** Dynamic surface tensions (mN/m) of various surfactant mixtures.\textsuperscript{18,24,25}

<table>
<thead>
<tr>
<th>Surfactant Mixture</th>
<th>Dynamic Surface Tension(a) (mN/m) and Corresponding Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td>CLSE</td>
<td>5 ± 2</td>
</tr>
<tr>
<td>DPPC</td>
<td>66 ± 1</td>
</tr>
<tr>
<td>DEPN-8</td>
<td>32 ± 2</td>
</tr>
<tr>
<td>DEPN-8 + 1.5% SP-B/C</td>
<td>12 ± 0</td>
</tr>
</tbody>
</table>

\(a\) Surface tension was measured at minimum bubble radius on a pulsating bubble surfactometer for 20 minutes with continual cycling.

Other encouraging results include the effect of PL-A\(_2\) on surfactant preparations containing CLSE and DEPN-8 (Table 2).\textsuperscript{18} As CLSE is obtained from the lung lavage extracts of calf lungs, it contains a variety of natural phospholipids with ester linkages between the glycerol backbone and the fatty acid chains. Its ability to continually reduce the surface tension to low values is thus compromised in the presence of PL-A\(_2\) as it can be readily degraded by this enzyme. The activity of surfactant preparations in the presence of PL-A\(_2\) can thus be monitored by the generation of LPL; in this case, specifically lysophosphatidylcholine (LPC).\textsuperscript{18} Upon exposure to PL-A\(_2\), the weight percent of LPC in the CLSE preparation is shown to increase, while the weight percent of PC decreases. This suggests that PL-A\(_2\) is actively degrading the
Table 2: Effect of PL-A₂ exposure on CLSE and DEPN-8 preparations.¹⁸

<table>
<thead>
<tr>
<th>Surfactant Componentᵃ</th>
<th>Surfactant Preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CLSE</td>
</tr>
<tr>
<td>LPC</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>PC</td>
<td>84.4 ± 0.4</td>
</tr>
</tbody>
</table>

ᵃ Surfactant components are reported as weight percents relative to the total phospholipid (or phosphonolipid) weight.ᵇ PL-A₂ concentration was 0.1 units/mL (U).

The surface tension of these two preparations after exposure to PL-A₂ was also measured and is summarized in Table 3.¹⁸ As mentioned above, CLSE is readily degraded by PL-A₂, which jeopardizes its surface activity. After exposure to the phospholipase, the CLSE preparation can no longer actively reduce the surface tension and values remain above 10 mN/m after 20 minutes of continuous cycling. Thus, it can be concluded that CLSE is not an effective surfactant when its activity is compromised by the presence of phospholipase enzymes. This will therefore have drastic effects if administered to an infant with RDS since the natural abundance of phospholipase enzymes is increased within the lungs of such patients.¹⁸,²⁵,²⁶ On the other hand, the DEPN-8 preparation retains its surface activity and can still effectively reduce the surface tension to below 1 mN/m within 5 minutes, making it the perfect candidate for RDS treatment.¹⁸

It can therefore be concluded, as demonstrated above, that the incorporation of DEPN-8 into a surfactant preparation can be very promising in the treatment of RDS. The lungs of
premature infants suffering from RDS produce little to no surfactant and thus, the application of a surfactant preparation that can maintain its surface activity under the harsh conditions present in the infants’ lungs is highly favourable. The tremendous benefit of incorporating a diether phosphonolipid that resists degradation seems to be a step in the right direction and, in time, will possibly become the standard in RDS treatment.

Table 3: Dynamic surface tensions (mN/m) of CLSE and DEPN-8 preparations upon exposure to PL-A$_2$.$^{18}$

<table>
<thead>
<tr>
<th>Surfactant Mixture</th>
<th>Dynamic Surface Tension$^a$ (mN/m) and Corresponding Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td>CLSE + 0.1U PL-A$_2$</td>
<td>24 ± 2</td>
</tr>
<tr>
<td>DEPN-8 + 1.5% SP-B/C + 0.1U PL-A$_2$</td>
<td>15 ± 3</td>
</tr>
</tbody>
</table>

$^a$ Surface tension was measured at minimum bubble radius on a pulsating bubble surfactometer for 20 minutes with continual cycling. $^b$ The PL-A$_2$ concentration was 0.1 units/mL (U).

3.2 Synthetic Results

As mentioned above, the target compound, DEPN-8, is a diether phosphonolipid analogue of DPPC. Since the beginning of the Schwan group’s collaboration with Notter in synthesizing and testing DEPN-8 as a potential surfactant component, there has been a high demand for production of this phosphonolipid from our laboratory. Previous synthetic methods towards its isolation were performed by Davy of the Schwan group, but involved several unnecessary steps, low yields and long reaction times.$^{84}$ Due to the demand, many of Davy’s procedures have been repeated while exploring other synthetic routes simultaneously so that 40 grams of DEPN-8 could be prepared and shipped to our collaborators.$^{79}$ Modifications focused
on overall production efficiency, reaction optimization, reducing the amount of purification required and scale-up of many reactions. All reaction performed will be detailed below.

The general retrosynthetic approach towards DEPN-8 (1) is illustrated below in Scheme 17. The first apparent disconnection can be made in the headgroup component of DEPN-8. Preparation of DEPN-8 is achieved through treatment of 3-bromopropylphosphonate 2 with aqueous trimethylamine, which completes the formation of the choline headgroup. Purification with Amberlite® ion exchange resin is required to ensure the formation and isolation of the zwitterionic product. The next disconnection to be made generates the glycerol backbone 3 and the phosphonate headgroup 4. These two compounds are combined through reaction with triethylamine to produce phosphonate 2. The two starting compounds, 3 and 4, are also synthesized from basic compounds and their synthesis will be discussed below.

Scheme 17: Retrosynthesis of DEPN-8.

3.2.1 Synthesis of the Glycerol Backbone

The desired glycerol backbone 3 can be prepared using two different methods. The first method attempted is similar to that outlined by Bauman and Mangold, and has been previously used by Schwan, Notter and co-workers to prepare the glycerol backbone for DEPN-8 containing two hexadecyl chains (Scheme 18). It requires that solketal be dissolved in a basic
solution of potassium hydroxide suspended in DMSO, which allows for the alkylation to occur at the \textit{sn}-1 position with the addition of bromohexadecane. The isopropylidene group is then cleaved by mild acid to provide diol 6. After recrystallization of the diol, trityl chloride is used to selectively protect the \textit{sn}-3 primary alcohol, leaving the secondary \textit{sn}-2 alcohol unaffected. Compound 7 is then alkylated at the \textit{sn}-2 position with bromohexadecane as before and subsequently detritylated to provide the dihexadecyl glycerol 3.

![Scheme 18](image)

\textbf{Scheme 18}: First strategy for the synthesis of the glycerol backbone.

Although this strategy seems acceptable, many problems were encountered when the reactions were repeated. The first step, involving preparation of diol 6, required recrystallization before proceeding with the tritylation. Yields obtained after recrystallization were always low; averaging 27\%. The $^1$H-NMR was also obtained after this step to confirm its formation. The five backbone protons and the two methylene protons attached to C1 of the alkyl chain appear as three separate multiplets ranging from 3.90 to 3.40 ppm. The C2 methylene protons of the alkyl chain appear at 1.53 ppm, while the majority of the remaining protons appear as a large, broad resonance at 1.23 ppm, with the terminal C16 protons appearing as a triplet at 0.85 ppm. The \textit{sn}-2 and \textit{sn}-3 hydroxyl protons appear at 2.55 and 2.11 ppm, respectively. The \textit{sn}-2 alcohol appears as a doublet since it couples to only one backbone proton, while the \textit{sn}-3 alcohol appears as a
doublet of doublets, although a triplet would be anticipated. This pattern occurs since it couples differently to each of the respective backbone protons since they are in slightly different environments. Although the reaction is low yielding, enough of the diol was prepared to proceed. Following tritylation, product 7 is ideally purified using column chromatography. Loss of the product on the silica gel or simply the unsuccessful isolation of a pure compound were problems encountered at this stage. A mixture of the product and starting material would typically be recovered from the column since these two compounds have similar R_f values, regardless of the solvent system employed. Notable peaks in the ^1H-NMR spectrum of this compound include the downfield shift of the sn-2 backbone proton since the attachment of the trityl group to the sn-3 position produces a deshielding effect on this proton. The other backbone protons are all shifted slightly upfield. An explanation for these outcomes can be described by the anisotropic effect that is created by the phenyl rings of the trityl group. Upon constructing a molecular model, it is evident that steric effects play a major role in determining the overall conformation. Due to space limitations, the phenyl rings of the trityl group must be arranged at slight angles to each other. Thus, the attached glycerol backbone protons at the sn-1 and sn-3 positions extend into the anisotropic cone of the phenyl groups and therefore experience a shielding effect and appear more upfield than in diol 6. The proton at the sn-2 position extends away from the anisotropic cone of the phenyl groups which aids in deshielding this proton. As purification was difficult at this stage, the crude product was used for the subsequent alkylation reaction and once again, undesired outcomes were obtained. The crude ^1H-NMR shows a low yield of the desired product 8, however, purification of this compound by column chromatography results in a loss of the product. Due to the many disadvantages that are evident for the preparation of the glycerol backbone utilizing this pathway, this method was abandoned in hopes of a more promising
synthesis that could accommodate large scale preparation.

Since DEPN-8 contains two identical chains on its glycerol moiety, it was thought that methods similar to those described by Fischer and Sowden in Scheme 2 could be employed (Scheme 19).  

**Scheme 19**: Second strategy for the synthesis of the glycerol backbone.

Since two hydroxyl groups are protected in solketal, the free alcohol is selectively protected using benzyl bromide. This alkylation is confirmed by $^1$H-NMR spectroscopy as the appearance of a multiplet at 7.33 ppm representing the five aromatic protons, and an AB quartet centred at 4.57 ppm accounting for the two methylene protons of the benzyl functionality. The two geminal methylene protons show coupling to each other due to the distinct chemical environment imposed by the aromatic ring. The isopropylidene group is then cleaved by mild acid hydrolysis to yield diol 9. The $^1$H-NMR spectrum of this compound shows the loss of a doublet at 1.39 ppm, which confirms the loss of the isopropylidene functionality. This is replaced by a broad singlet at 2.74 ppm for the two hydroxyl groups that were subsequently formed. When the isopropylidene group is removed, the signals for the four backbone protons at the $sn$-1 and $sn$-3 positions become overlapped and form a multiplet centred at 3.63 ppm. The lone backbone proton at the $sn$-2 position forms a distinct quintet at 3.74 ppm. This overlap is due to
the fact that diol 9 is no longer locked into a specific conformation, allowing rotation to occur throughout the molecule and thus causing the signals to become less resolved.

Once diol 9 is obtained, it is dialkylated with bromohexadecane in a basic solution to afford compound 10. This is further purified by column chromatography since two spots appear on the thin layer chromatography (TLC) plate of the crude reaction mixture. Purification is ideal at this stage since the unwanted by-product observed by TLC is the alkene generated from the competitive E2 elimination of H-Br from bromohexadecane during the alkylation process. The $^1$H-NMR spectrum of compound 10 features, as described above, peaks for the five backbone protons and the benzyl functionality, as well as additional signals for the long alkyl chains that are now attached. These signals appear at the following chemical shifts: a multiplet centred at 3.53 ppm for the four C1 protons which overlap the multiplet seen for the five backbone protons; a multiplet at 1.56 ppm for the four C2 protons; a singlet at 1.28 ppm for 52 protons on C3 to C15; and a triplet at 0.88 ppm for the terminal C16 protons.

The next step, benzyl deprotection, proceeds very well and only requires recrystallization to afford moderate to excellent yields of pure product 3. The distinct difference in the $^1$H-NMR spectrum is the absence of the benzyl signals at 7.33 ppm and 4.57 ppm and the appearance of the hydroxyl proton as a doublet of doublets at 2.16 ppm. The backbone and C1 methylene proton signals appearing near 3.53 ppm become more resolved, as four separate multiplets can be identified in the spectrum of the glycerol backbone 3. The signals for the remaining alkyl chain protons appear in the same regions described previously.

There are many obvious benefits of using the second pathway which include reducing the total number of synthetic steps as well as the amount of time spent on purification. There is also an overall improvement on the yield of the dihexadecyl glycerol backbone. In addition,
production of the glycerol backbone on large scale seems appropriate with this procedure. Reactions on the 20 gram scale have been performed and work well, especially when the appropriate size glassware is available. Other encouraging results include those obtained from reusing the palladium catalyst required for benzyl deprotection in the last step (Table 4). Once the catalyst, 10% palladium on activated carbon, is used and rinsed well with chloroform, it can be reused in subsequent reactions. Yields, although sometimes inconsistent, seem to range from moderate to good when the catalyst is reused up to 5 times. It seems the only cost is the loss of roughly 0.2 grams of the catalyst which cannot be recovered due to filtration methods.

Table 4: Yields obtained after reusing the palladium catalyst in the formation of the glycerol backbone 3 from precursor 10.

<table>
<thead>
<tr>
<th>No. Of Times Catalyst Was Used</th>
<th>Yield&lt;sup&gt;a&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>88 – 98</td>
</tr>
<tr>
<td>2</td>
<td>60 – 89</td>
</tr>
<tr>
<td>3</td>
<td>78 – 98</td>
</tr>
<tr>
<td>4</td>
<td>68 – 84</td>
</tr>
<tr>
<td>5</td>
<td>95&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Range of yields obtained from several repeated trials.  <sup>b</sup> This specific reaction was only performed once.

The ease of product purification is also an added benefit of using benzyl protection. The by-product generated from the hydrogenolysis of the benzyl group is toluene. This volatile compound is more readily removed since both rotary evaporation and recrystallization methods require heating. Alternatively, trityl deprotection generates trityl alcohol as the by-product, which is not a volatile compound. Removal of this compound is more tedious and time
consuming as both recrystallization and column chromatography are typically required to isolate all of the desired product. In summary, the glycerol backbone 3 is now prepared in four steps, totalling a span of three days with an overall yield of 30%. If diol 9 is purchased, on the other hand, two synthetic steps would be required to produce the glycerol backbone, totalling a span of one day. Individual reaction yields are typically moderate to good, with the exception being the alkylation step. Future improvements to this reaction could make the whole process more efficient on large scale. However, with this compound in hand, production of the phosphonylating agent would be the next focus.

3.2.2 Preparation of the Phosphonylating Agent

By analyzing the structure of DEPN-8, it is evident that the phosphonylating agent required needs to have one of two basic structures (Figure 9). Compound 4 is the acyclic phosphonic acid dichloride. This compound has been previously utilized in the preparation of DEPN-8 by Schwan, Notter and co-workers. Compound 11 is the cyclic chloropropylphosphonate. This compound is of particular interest for the re-tailored synthesis of DEPN-8 and other similar phosphonocholine derivatives.

Figure 9: Structure of the phosphonylating agent.

The methods utilized by Schwan, Notter and co-workers to access compound 4 were
repeated so that production of DEPN-8 could move forward (Scheme 20). This route begins with the Michaelis-Arbuzov reaction: triethyl phosphite is combined with 1,3-dibromopropane, using conventional oil bath heating or microwave heating, to produce diethyl 3-bromopropylphosphonate 12. Since an excess of 1,3-dibromopropane is necessary, purification by vacuum distillation is required to remove (and recover) the excess reagent. Once the excess reagent is removed, phosphonate 12 is distilled and collected as a pure fraction.

Scheme 20: Preparation of 3-bromopropyl phosphonic dichloride.

It is worthwhile to mention here that $^{31}\text{P}$ is an NMR active nucleus and therefore it participates in spin-spin coupling with $^1\text{H}$ and $^{13}\text{C}$ nuclei that are nearby in the compound. Due to this, both $^1\text{H}$-NMR and $^{13}\text{C}$-NMR spectrums will show additional J coupling to the $^{31}\text{P}$ nucleus when it is within three bond lengths away. This is evident in the $^1\text{H}$-NMR spectrum of phosphonate 12, where the CH$_2$ protons of the ethyl groups would normally appear as a quartet at 4.10 ppm, but due to $^{31}\text{P}$ coupling, the quartet gets further split. The appearance should resemble two quartets of doublets overlapping each other; however, such a resonance will often be described as a multiplet because of the limited resolution and difficulty in determining $^{31}\text{P}$-$^1\text{H}$ coupling constants. The signal for the CH$_3$ protons will appear as a triplet at 1.32 ppm, as it typically does not couple to the $^{31}\text{P}$ nuclei, which is four bonds away. The proton signals for the bromopropyl functionality typically all show coupling to the $^{31}\text{P}$ nucleus. The closest protons will have the largest coupling constants and those at a distance will have weaker coupling or
none at all. The two methylene groups closest to the phosphorus appear as complex multiplets at 2.15 and 1.88 ppm, while the methylene closest to the bromine appears as a triplet of doublets at 3.46 ppm. The $^4J_{P,H}$ coupling for these protons is actually obtainable from the spectrum; however, it only measures 1 Hz since it is located four bonds away from the $^{31}P$ atom.

With phosphonate 12 in hand, transformation to a useful phosphorylating agent is achieved by first treatment with bromotrimethylsilane (TMSBr), which produces the silylated derivative of compound 12, followed by addition of phosphorus pentachloride to obtain phosphonic dichloride 4 (Scheme 20 above). This compound is typically generated in situ immediately before reaction with the glycerol backbone and its formation is confirmed by analyzing a small aliquot by $^1H$-NMR. The spectrum should only contain three signals which account for the bromopropyl protons. The preparation of this compound could also be confirmed by GC-MS as the incorporation of two chlorine atoms in the molecule would produce a unique isotope effect. The naturally occurring isotopes of chlorine are $^{35}Cl$ and $^{37}Cl$, with natural abundances of 75% and 25%, respectively. Therefore, if two chlorine atoms should exist within the same compound, there would be three peaks corresponding to the following possibilities: $^{35}Cl + ^{35}Cl$, $^{35}Cl + ^{37}Cl$, and $^{37}Cl + ^{37}Cl$. The first of these represents the molecular ion peak and will have the highest peak height of the three since $^{35}Cl$ is the most abundant isotope of chlorine. The second and third peaks are represented by [M+2] and [M+4], simply due to the fact that these peaks appear two or four mass units heavier than the molecular ion peak due to the incorporation of $^{37}Cl$. The peak height ratio will therefore be 9:6:1, based on the natural abundances of the chlorine isotopes.

As mentioned, there are two heating methods available to perform the Michaelis-Arbuzov reaction stated in Scheme 20. Conventional oil bath heating requires a consistent temperature of
150 °C for roughly 3 to 4 hours; until TLC shows complete consumption of the triethyl phosphite reagent. Attempted reactions gave moderate yields of phosphonate 12; typically averaging 76% after distillation to remove the excess 1,3-dibromopropane reagent. Microwave heating, on the other hand, is a much quicker method. It requires that a microwave reactor maintains a consistent temperature of 180 °C for 1 minute. The downfall to this method is the microwave reactor of interest can only facilitate a 10 or 35 mL vessel which makes large scale preparation of this component very challenging utilizing this particular device. To ensure the pressure build up within the vessel does not exceed the maximum allowed by the reactor, the contents of each vessel is limited to 50% of its maximum (ie: a limit of 5 mL of reactants in a 10 mL vessel, or approximately 17 mL in a 35 mL vessel). Attempted reactions often gave low yields; averaging only 41% after short path distillation and purification by silica plug.

Thus, each method has its own benefits and downfalls. Conventional oil bath heating requires longer reaction times but it affords the product in better yield and can be used to generate large quantities of this product at once. To generate the same amount of product using microwave heating would require multiple reactions to be subsequently performed, followed by short path distillation of the combined vessels, as well as purification by elution through a silica plug. The amount of time and effort spent on microwave synthesis exceeds that of oil bath heating, and thus, conventional heating methods have been currently employed for the preparation of phosphonate 12, especially on large scale.

As stated, the current methods used by Schwan, Notter and co-workers to obtain compound 4 include treating phosphonate 12 with TMSBr and phosphorus pentachloride. Although a reliable method, due to the amount of equivalents required as well as the high cost of TMSBr, its use is highly unfavourable, especially for large scale preparation. The current market
price of 100 mL of TMSBr from Sigma-Aldrich is roughly $415,\textsuperscript{91} thus making it the most expensive reagent used in the preparation of DEPN-8. The previously proposed synthesis of DEPN-8, which utilizes phosphonic dichloride \textit{4}, requires a total of four equivalents of TMSBr for the production of one equivalent of DEPN-8. In addition, half of the required amount of the phosphonylating agent is sacrificed in the production of the phosphonolipid. Alternative methods would therefore include limiting or avoiding the use of TMSBr altogether.

With that being said, one practical solution initiated by Davy,\textsuperscript{84} was the preparation of chloro phostonate \textit{11} (Scheme 21). The first step involves production of phostonate \textit{13} from phosphonate \textit{12} with the use of a microwave reactor. Davy determined that simply heating phosphonate \textit{12} for 10 minutes in a microwave reactor with a consistent temperature of 180 °C produced the cyclized phostonate \textit{13}. Repeated reactions were disappointing since low yields, averaging 38%, were typically obtained. The \textit{1}H-NMR spectrum of the crude reaction mixture obtained for phostonate \textit{13} shows the generation of both the product and the ethyl bromide by-product. Purification is achieved by eluting through a two inch plug of silica gel with 80% ethyl acetate and 20% hexanes. The key identifying feature in the \textit{1}H-NMR spectrum of phostonate \textit{13} is the absence of the triplet of doublets at 3.46 ppm since this particular signal was due to the methylene protons adjacent to the bromine atom. When the compound is cyclized, ethyl bromide is lost, which forms the phostonate.

![Scheme 21: Synthesis of chloro propylphostonate.](image-url)
Other recent attempts to cyclize phosphonate 12 that utilize older methods involving oil bath heating weren’t as successful.\textsuperscript{92} It was found that if the temperature of the oil bath rises above 185 °C, the product formed is gelatinous in texture and does not dissolve in any solvent. Characterization of this gelatinous compound has thus been impossible. Therefore, care must be taken in ensuring the temperature remains at a reasonable level when using oil bath heating. When this is considered, the product can be isolated after four to size hours, based on the disappearance of starting material monitored by TLC. Based on this, the use of the microwave reactor seems most beneficial as it reduces the amount of time spent obtaining the product even though yields aren’t ideal.

Many reactions were attempted for the second step involving transformation of phostonate 13 to the cyclic phosphonylating agent 11 (Scheme 21 above). Initial efforts included treatment of phostonate 13 with 4 equivalents of oxalyl chloride in DCM (with and without catalytic DMF) or in chloroform and allowing it to stir overnight at room temperature.\textsuperscript{93,94} The reactions were monitored by TLC and \textsuperscript{1}H-NMR spectroscopy. It was anticipated that TLC would show consumption of the starting material as well as formation of the product. The \textsuperscript{1}H-NMR spectrum was expected to show the loss of a 2H multiplet centred at 4.10 ppm and a 3H triplet at 1.34 ppm, as these signals correlate to the protons of the ethoxy functionality. However, based on the data obtained, no reaction had occurred. Other attempts that followed include the reaction of phostonate 13 with phosphorus pentachloride in solvents such as ethanol-free chloroform, commercial chloroform, and benzene.\textsuperscript{89,95,96} A variation containing phosphorus pentachloride and phosphorus oxychloride in chloroform was also attempted.\textsuperscript{97} All reactions were heated to 50 °C for 1.5 hours followed by removal of the solvent via an aspirator fitted with a calcium chloride drying tube. Since acid chloride compounds can be quite unpredictable, in hopes of
intercepting chloro phostonate 11 in situ, each mixture was used immediately in an alkylation reaction involving triethylamine and dodecanol in the respective solvent (Scheme 22). However, results were disappointing and the desired dodecyl propylphostonate (14) was not observed. One particularly notable observation was found in the $^1$H-NMR of what was hoped to be chloro phostonate 11. When ethyl propylphostonate (13) is reacted with phosphorus pentachloride in ethanol-free chloroform as described above, the product isolated shows two peaks at 4.10 and 1.34 ppm corresponding to the ethoxy CH$_2$ and CH$_3$ protons, respectively, from the starting phostonate. The integration of these two peaks is different compared to the starting compound and there is also the appearance of a triplet at 3.61 ppm in the spectrum. Further characterization also confirms the presence of the ethoxy group. Therefore, the reaction must not be proceeding as hoped and the ethoxy group is remaining intact.

It wasn’t until the compound was analyzed by gas chromatography and mass spectrometry (GC-MS) that a conclusion was drawn. The addition of phosphorus pentachloride must be acting on phostonate 13 to open the ring and form compound 15 (Scheme 23). Subsequent alkylation with triethylamine and dodecanol produced compound 16 as the major product. Injection of compound 15 into the GC-MS confirmed the incorporation of two chlorine atoms. The spectrum shows the molecular ion peak at 205 m/z, as well as peaks corresponding to [M+2] and [M+4] at 207 and 209 m/z, respectively. The peak heights were also in a ratio of
9:6:1, thus confirming the inclusion of two chlorine atoms in compound 15. After the alkylation is performed, the GC-MS spectrum of product 16 shows peaks for the molecular ion at 355 m/z and [M+2] at 357 m/z, with peak heights in the ratio of 3:1. Thus, treatment of ethyl propylphostonate 13 with phosphorus pentachloride reacts to open the ring instead of transforming it into the desired chloro propylphostonate (11).

Scheme 23: Unexpected reaction of ethyl propylphostonate with PCl$_5$.

The conversion of the ethoxy group to a chloride seems to be in competition with ring opening since there are two possible P-O bonds that can be broken. Based on a hydrolysis model, reaction with phosphorus pentachloride promotes a trigonal bipyramidal transition state where (i) apical positions are occupied by atoms with higher electronegativities, (ii) substituents that are anionic in nature will occupy equatorial positions, and (iii) if a five-membered ring is present, the more electronegative substituent will occupy an apical position while the other portion will be equatorial. Based on these rules, Figure 10 illustrates a proposed transition state for phostonate 13, which would lead to a relief of ring strain. This is an important factor in determining which P-O bond is cleaved. Pseudorotation can occur when an incoming nucleophile enters an apical position. Exocyclic and endocyclic cleavage are both possible and are competitive processes. Since ring strain is relieved when the trigonal bipyramidal transition state is formed, it is more difficult for the intermediate to undergo pseudorotation to cleave the exocyclic P-O bond without breaking the rules mentioned above that pertain to the five-
membered ring.\textsuperscript{115} Also, the orientation of the two lone pairs on the oxygen atoms in the transition state affect which bond is targeted. The non-bonding orbitals need to be antiperiplanar to the desired P-O bond that will ultimately be broken.\textsuperscript{100} Further investigations should ideally be performed in hopes of preferentially cleaving the exocyclic P-O bond.

Figure 10: Proposed trigonal bipyramidal transition state for reaction of phostonate 13 with phosphorus pentachloride.

Another approach, initially performed by Davy, was to access phostonate 11 through the lithiated derivative 18 (Scheme 24). The first step involves hydrolysis of the ester, which produces phosphonic acid 17. The \textsuperscript{1}H-NMR spectrum of this compound is simple as the bromopropyl protons appear in similar positions as described for the starting material, phosphonate 12. This is then made alkaline by addition of an aqueous 1M lithium hydroxide solution which produces the cyclized lithiated derivative 18 in good yield. The difference in the chemical shift of the protons in phosphonic acid 17 compared to the lithiated derivative 18 helped to confirm the conversion. The methylene protons attached to the bromine in phosphonic acid 17 are shifted downfield in phostonate 18 to 3.95 ppm since they are now directly attached to an ester oxygen atom, causing the protons to be further deshielded. The other two signals are both shifted apart from each other somewhat; one slightly downfield and the other slightly upfield, reflective of their asymmetric environment. \textsuperscript{31}P coupling is also seen in the \textsuperscript{1}H-NMR of this compound for all three methylene signals.

Previous methods reported by Davy of the Schwan group use TMSBr to cleave the
phosphonate ester which generates the silylated derivative. This is followed by quenching with water to isolate phosphonic acid 17. After the two steps, the yield of compound 17 averages 93%. However, preparation of this compound with the use of TMSBr is still highly unfavourable. Other attempts to produce phosphonic acid 17 without using TMSBr follow literature procedures utilizing hydrobromic acid and hydrochloric acid, however, these reactions were unfortunately also unsuccessful. Another downfall is the extremely low yields Davy reports for the transformation of the lithiated derivative 18 into the corresponding chloro phostonate 11. Similar attempts performed for this transformation were also unsuccessful. Compound 18 is ultimately transformed into an ester derivative through conversion of the phosphorus to an electrophilic species followed by treatment with an appropriate alcohol. With this unfortunate luck at producing our cyclic phosphonylating agent, it was ‘back to the drawing board’ for a new route to access the cyclic phostonate 11.

![Scheme 24: Preparation of lithium propylphostonate.](image)

It was at this point that the Michaelis-Becker reaction was looked at more carefully. It was envisioned that dibenzyl phosphonate 19 or 20 (Figure 11) could function in place of phosphonate 12 as the precursor to phosphonylating agent 4 in the synthesis of phosphonolipids. Transformation to compound 4 is hypothesized to occur through cleavage of the benzyl esters of 19 via hydrogenolysis and further treatment with a chlorinating agent such as phosphorus pentachloride, oxalyl chloride, or thionyl chloride. It was also thought that phosphonates 19 or
20 could function as precursors for the cyclized phostonate 21 (Figure 11). Production of compound 21 is envisioned to occur through either heating phosphonate 19 in a microwave reactor, as seen above for production of phostonate 13 from phosphonate 12 (Scheme 21), or by deprotection of the tetrahydropyranyl (THP) group in phosphonate 20, which will ideally displace benzyl alcohol to generate phostonate 21.

![Figure 11: Structures of phosphorylating agent precursors](image)

Preparation of these compounds begins with generation of the starting material, dibenzyl H-phosphonate 22 (Scheme 25). Although commercially available, preparation of this compound is convenient due to its elevated cost. Its synthesis requires that a toluene solution containing benzyl alcohol and dimethylaniline be added dropwise to a solution of phosphorus trichloride in toluene over a period of one hour. After additional stirring and work-up, phosphonate 22 is obtained in both good yield and chemical purity. The $^1$H-NMR spectrum gives signals for the ten benzyl aromatic protons centred at 7.34 ppm and the four methylene protons at 5.05 ppm. The proton attached directly to the phosphorus atom appears as a doublet centred at 6.92 ppm with a $^1$J$_{P,H}$ coupling of 707 Hz, which is typical for H-phosphonates.

![Scheme 25: Synthesis of dibenzyl H-phosphonate](image)
With starting phosphonate 22 in-hand and no particular procedure in mind for the synthesis of compounds 19 or 20, a few test reactions were performed using iodomethane as the alkyl halide (Scheme 26). This inexpensive reagent is used to determine appropriate reaction conditions that can ideally be applied to the preparation of phosphonates 19 and 20. Initially, the sodium hydride used was purchased as a 60% dispersion in mineral oil, which had to be rinsed with hexanes prior to its use. This purification was tedious and time consuming, so dry sodium hydride powder was alternatively used in subsequent reactions. Sodium hydride is the base used to produce the sodium salt of dibenzyl H-phosphonate, which can be further alkylated at phosphorus with an electrophilic alkyl halide, such as iodomethane. The $^1H$-NMR spectrum of this compound shows the benzyl protons as described above for the starting compound, however, the doublet observed at 6.92 ppm is relocated to 1.45 ppm, with a significantly smaller $J_{P,H}$ coupling value. This is due to the fact that the $J_{P,H}$ coupling is now through two bonds instead of one since the methyl group is now attached. This reduces the magnitude of the coupling constant as it is farther away from the $^{31}P$ nucleus. Although this reaction was never optimized, with the formation of the desired product 23, it seems reasonable to apply this procedure to the formation of phosphonates 19 and 20. If the desired product is detectable, even in small quantities, further optimizations will be completed at that time.

![Scheme 26](image)

**Scheme 26:** Michaelis-Becker reaction of dibenzyl H-phosphonate with iodomethane.

The respective alkyl halides to be used in the production of compounds 19 and 20
include: 1,3-dibromopropylene, which is commercially available, and 2-(3-bromopropoxy)tetrahydro-2H-pyran (24), which can be easily prepared from 3-bromo-1-propanol (Scheme 27). The THP protection of 3-bromo-1-propanol is achieved by reaction with 3,4-dihydro-2H-pyran (DHP) in the presence of catalytic amounts of pyridinium p-toluenesulfonate (PPTS) in DCM. The solution is allowed to stir at room temperature for up to three hours. Moderate yields of compound 24 are obtained after purification by column chromatography, eluting with a solvent system of 15% ether in hexanes. The formation of the product was confirmed by $^1$H-NMR spectroscopy and GC-MS. With the attachment of the THP functionality, a very characteristic triplet for the lone tertiary proton is observed at 4.60 ppm, which is significantly downfield due to the two neighbouring ether functionalities. The other proton signals appear as expected at reasonable chemical shifts. The GC-MS spectrum also helps to confirm the structure since there is a bromine present in compound 24. The natural isotopes of bromine are $^{79}$Br and $^{81}$Br which have natural abundances of 51% and 49%, respectively. Since the natural abundances of the two isotopes are roughly the same, the molecular ion peak and the [M+2] peak will be in a peak height ratio of 1:1, which is evident in the GC-MS spectrum of compound 24.

![Scheme 27: THP protection of 3-bromo-1-propanol.](image)

In addition to this alkyl halide species, compound 24 was also transformed into the iodo derivative 25 by methods similar to those described by Weyna and co-workers (Scheme
This was achieved through reaction of the bromo derivative 24 with sodium iodide in acetone. With minimal exposure to light, the solution is stirred overnight at room temperature before being filtered through Celite® and washed with acetone. Purification by column chromatography produces moderate yields of iodo derivative 25. The 1H-NMR spectrum of this compound is very similar to the bromo derivative 24; however, with the incorporation of iodine instead of bromine, a few peaks appear slightly shifted and more resolved. The proton signals for the THP protecting group appear at the same chemical shifts as seen for compound 24, whereas the proton signals for the newly formed iodopropyl portion are slightly different. The multiplet integrating for 4H near 3.52 ppm in compound 24 becomes separated into two signals in the spectrum of iodo derivative 25, with each signal integrating for 2H and appearing slightly more upfield. This can be explained by the different negative inductive effects experienced by each halogen. Since a halogen is more electronegative than carbon, it can essentially pull electron density away from the carbon when they are bonded together. The comparatively small bromine nucleus is close to the bonding pair of electrons and thus holds the electrons close to its centre which causes them to appear more downfield (deshielded) in the 1H-NMR spectrum. The larger atomic radius of iodine creates an environment where the nucleus is farther away from the bonding pair of electrons and thus creates a more shielding effect. This causes the protons to appear more upfield in the 1H-NMR spectrum.

Scheme 28: Finkelstein halogen exchange reaction.
Since the atomic radius of halogens plays an important part in their reactivity, the reasoning behind the halogen exchange was to ensure the compounds are given a reasonable chance to react under the conditions described above for the initial Michaelis-Becker reaction (Scheme 26). Although iodomethane was used as our test alkyl halide, the hope was that the bromo derivative 24 would suffice and the reaction would produce our desired product under the specified conditions. However, no product was isolated and it was therefore further transformed into the iodo derivative 25. A consequence of iodine’s large radius is the fact that it forms essentially weaker bonds between atoms more electropositive than itself. This allows it to function as a good alkylating agent and the hope was that it would react preferentially under the reaction conditions.

Other alcohol protecting groups that were considered include trimethylsilane (TMS) and tert-butyl diphenylsilane (TBDPS). Access to the TMS-protected derivative was sought through reaction of oxetane with either (i) iodo(trimethyl)silane or (ii) sodium iodide and chlorotrimethylsilane.\textsuperscript{111} The TBDPS-protected derivative was attempted by reaction of oxetane with sodium iodide and tert-butyl chlorodiphenylsilane.\textsuperscript{111,112} Unfortunately, no product was ever isolated from these reactions and thus preparation of these compounds was disregarded considering compounds 24 and 25 should be sufficient enough for the preparation of the phosphorylating precursor 20.

Initial attempts at preparing compounds 19 and 20 followed the procedure described in Scheme 26 for the isolation of dibenzyl methylphosphonate (23);\textsuperscript{106} a base is reacted with phosphonate 22 in a variety of solvents and alkylation is achieved by treatment with the respective alkyl halide (Scheme 29). A summary of the attempted reaction conditions is given in Table 5.
**Table 5:** Reaction conditions attempted for the Michaelis-Becker reaction of dibenzyl H-phosphonate with several alkyl halides.

![Scheme 29: Michaelis-Becker reaction of dibenzyl H-phosphonate with a variety of bases, solvents and alkyl halides.](image)

<table>
<thead>
<tr>
<th>Trial</th>
<th>Equivalents of 22</th>
<th>Solvent</th>
<th>Base and Equivalents</th>
<th>Alkyl Halide and Equivalents</th>
<th>Product (#, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.00</td>
<td>THF</td>
<td>dry NaH</td>
<td>1.00 Br___OTHP</td>
<td>20, 0</td>
</tr>
<tr>
<td>2</td>
<td>1.00</td>
<td>toluene</td>
<td>dry NaH</td>
<td>1.30 Br___OTHP</td>
<td>20, 0</td>
</tr>
<tr>
<td>3</td>
<td>1.15</td>
<td>toluene</td>
<td>dry NaH</td>
<td>1.15 Br___OTHP</td>
<td>20, 0</td>
</tr>
<tr>
<td>4</td>
<td>1.15</td>
<td>toluene</td>
<td>dry NaH</td>
<td>1.15 I___OTHP</td>
<td>20, 0</td>
</tr>
<tr>
<td>5</td>
<td>1.44</td>
<td>THF</td>
<td>dry NaH</td>
<td>1.40 I___OTHP</td>
<td>20, 0</td>
</tr>
<tr>
<td>6</td>
<td>1.44</td>
<td>THF</td>
<td>dry NaH</td>
<td>1.40 Br___OTHP</td>
<td>20, 0</td>
</tr>
<tr>
<td>7</td>
<td>1.00</td>
<td>toluene</td>
<td>dry NaH</td>
<td>1.30 Br___OH</td>
<td>20b, 0</td>
</tr>
<tr>
<td>8</td>
<td>1.00</td>
<td>DMF</td>
<td>dry NaH</td>
<td>1.30 Br___OH</td>
<td>20b, 0</td>
</tr>
<tr>
<td>9</td>
<td>1.00</td>
<td>THF</td>
<td>dry NaH</td>
<td>1.30 Br___OH</td>
<td>20b, 0</td>
</tr>
<tr>
<td>10</td>
<td>1.00</td>
<td>toluene</td>
<td>KHMDS</td>
<td>1.00 Br___OTHP</td>
<td>20, 0</td>
</tr>
<tr>
<td>11</td>
<td>1.00</td>
<td>DMF</td>
<td>Cs₂CO₃, TBAI</td>
<td>3.00 Br___Br</td>
<td>19, 56</td>
</tr>
<tr>
<td>12</td>
<td>1.00</td>
<td>DMF</td>
<td>Cs₂CO₃, TBAI</td>
<td>3.00 Br___OTHP</td>
<td>20, 47</td>
</tr>
</tbody>
</table>

The first attempts at production of phosphonate 20 focused on varying the equivalents of dry sodium hydride powder in a variety of solvents, and also utilized both bromo and iodo derivatives, 24 and 25, respectively (Trials 1-6, Table 5). However, upon completion of the
reaction, benzyl alcohol, the respective alkyl halide, and other inseparable side products were typically recovered. These reactions involving the use of dry sodium hydride powder did not seem to be proceeding smoothly and many undesired side products were formed when the mixture was visualized by TLC. This made the reaction mixtures difficult to separate by column chromatography since multiple compounds with similar Rf values eluted together from the column, regardless of the solvent system used. Similar reactions were performed in a variety of solvents with the unprotected 3-bromo-1-propanol as well, yet still no product was formed (Trials 7-9, Table 5). With no success at preparing phosphonate 20 yet, it was decided that an alternative choice of base was needed.

Other procedures which prepare similar reagents to those desired have used potassium hexamethyldisilazide (KHMDS) as the base.113 This strong, non-nucleophilic base is a good alternative to sodium hydride as its bulkiness may be beneficial in our desired reaction. With high hopes of isolating compound 20, it was unfortunately disappointing that similar results were obtained to those above, and none of the desired product was formed based on characterization data (Trial 10, Table 5).

With a number of failed reactions to account for, it was now time to head back to the drawing board. It was at this point that a procedure was stumbled upon originally developed by the Salvatore group which utilized cesium carbonate in the presence of tetrabutylammonium iodide (TBAI) to generate the dialkyl phosphonate salt.114 TBAI is believed to act as a phase transfer catalyst which helps to promote the alkylation reaction. The authors performed two reactions simultaneously in the presence and absence of this catalyst utilizing the optimized conditions. It was found that yields were dramatically improved when TBAI was incorporated as a reagent. The Salvatore group also screened several dialkyl H-phosphonates and an extensive
number of alkyl halides under the optimized conditions and it was found that the desired product was isolated in moderate to high yields in all cases. Thus, it seemed possible that application of this pathway could indeed produce the desired phosphonate products 19 and 20.

The reaction begins with the addition of cesium carbonate and TBAI to a DMF solution containing dibenzyl H-phosphonate 22. This is allowed to react at room temperature under a nitrogen atmosphere for one hour. At this point, the desired alkyl halide is added: 1,3-dibromopropane for preparation of phosphonate 19, or either alkyl halide derivative 24 or 25 for the preparation of phosphonate 20. This solution is then stirred at room temperature for up to five days. TLC of the crude reaction mixture is generally cluttered; therefore purification by column chromatography is required to isolate the product. Eluting with a solvent system of 70% ethyl acetate (EtOAc) and 30% hexanes, the product is typically isolated as the fourth compound eluting from the column, having an Rf value near 0.4 or 0.3 for compounds 19 and 20, respectively. Moderate to low yields are generally obtained after column chromatography, averaging 56% for product 19 and 47% for product 20 (Trials 11-12, Table 5). The 1H-NMR spectra of these compounds are particularly interesting with respect to the coupling patterns observed. When the desired product, 19 or 20, is formed, the methylene protons of the benzyl functionality appear as a doublet of an AB quartet centred near 5.01 ppm. The geminal protons experience coupling to each other, which produces the AB quartet pattern. The addition splitting into a doublet occurs since the 31P nucleus is in close vicinity and will further split the specified proton signals. The other proton signals for the aromatic and propyl portion of the compound appear as expected at reasonable chemical shifts.

With the phosphonylating precursors 19 and 20 prepared, efforts could now focus on preparation of the desired phosphonate 21. An attempt at cyclization of phosphonate 19 utilized
similar microwave conditions described earlier for the transformation of phosphonate 12 to phostonate 13 (Scheme 20 above). Thus, phosphonate 19 was heated neat under argon atmosphere in a microwave reactor set to 180 °C for 5 minutes (Scheme 29). The TLC of the crude reaction mixture, checked in 70% ethyl acetate and 30% hexanes, showed consumption of the starting material. The crude \( ^1 \text{H}-\text{NMR} \) spectrum resembles that of the starting compound with slight differences, signifying the possible preparation of phostonate 21. Column chromatography was performed to purify the phostonate; however, the desired product was not isolated from the column. The cyclized product was either never prepared through the reaction described, or was lost during column chromatography. With time running short, further attempts at this cyclization were not performed; however it may be worthwhile to re-visit this pathway in the future.

![Scheme 30: Attempted cyclization of dibenzyl phosphonate 19.](image)

Although there was an unfortunate outcome from the cyclization, the ultimate goal if it would have been prepared would be to use it in the preparation of chloro phostonate 11. This was envisioned to proceed by the use of the following conditions: (i) hydrogen in the presence of 10% palladium on carbon and/or (ii) a chlorinating agent, such as phosphorus pentachloride or oxalyl chloride. The first step was thought to be useful in deprotection of the benzyl group, which would expose the phosphonic acid derivative. The second step would aid in conversion to the desired chloro phostonate 11 through the use of a chlorinating agent as listed above.

Although the cyclization was not attempted under any other conditions, it was also
envisioned that phosphonylating precursor 20 could be used to produce phostonate 21. It would first need to undergo deprotection of the THP functionality which would hopefully induce cyclization to produce the desired product. The possibility of heating the reaction would also be considered to help promote formation of the cyclized product.

The preparation of the cyclic phosphonylating agent 11 through the Michaelis-Becker reaction described above looks like a promising alternative to the methods currently employed. The reagents are all reasonably priced, or easily prepared, and further investigations into reaction optimizations and preparation of these compounds should be performed in the future. Avoiding the use of TMSBr in the preparation of the phosphonylating agent is a crucial factor in being able to perform large scale synthesis of the desired phosphonolipid, DEPN-8.

Since the desired cyclic phosphonylating agent 11 was never successfully prepared, the alkylation of this compound and subsequent choline headgroup formation could never be fully assessed. As well, other unsuccessful results were obtained from attempting the isolation of phosphonylating agent 4 without the use of TMSBr.

### 3.2.3 Preparation of DEPN-8

As the demand for DEPN-8 was high, the phosphonolipid was prepared using the procedures described previously by Davy (Scheme 31). The first steps involve transformation of diethyl phosphonate 12 into the corresponding phosphonylating agent 4. This is achieved by cooling a DCM solution containing the phosphonate to -40 °C and slowly adding TMSBr. After this is warmed to room temperature, it is stirred for three hours then the solvent is removed via an aspirator fitted with a calcium chloride drying tube. This is left under high vacuum for one and a half hours before proceeding with the chlorination. The residue is dissolved in ethanol-free
chloroform and phosphorus pentachloride is added at room temperature. The solution is heated to 50 °C for 1.5 hours, at which point the solvent is carefully removed as before. The phosphonylating agent 4 is left under high vacuum for 1.5 hours. When the solvent is removed, care must be taken to ensure the product does not ‘bump’ over as well. From experience, the process of removing the solvent is usually most sensitive when the solvent first begins to distil and therefore should be monitored closely. Once the phosphonylating agent 4 is prepared, it is dissolved in ethanol-free chloroform once again and cooled to 0 °C. A solution containing the glycerol backbone 3 and triethylamine in ethanol-free chloroform is added dropwise to the solution containing phosphonylating agent 4, which is further warmed to room temperature and allowed to stir for 48 hours. At this point, the reaction is quenched with water and eventually stirred with Amberlite® ion exchange resin. An extremely gentle extraction from DCM is performed at this point which isolates phosphonate 2. The final reaction step involves conversion to the phosphonocholine through treatment with a 40% aqueous trimethylamine solution. This is followed once again by purification with Amberlite® ion exchange resin and gentle extraction from DCM. Further purification is obtained by column chromatography as well as recrystallization of the product from acetone/chloroform (5:2 v/v). The final step involves warming the lipid with a Kugelrohr apparatus to 50 °C under high vacuum in the presence of phosphorus pentoxide. This is performed until the mass has stabilized, which ensures the lipid is completely free of moisture. Although yields can vary tremendously, typical yields obtained from this reaction are generally over 50%.

The techniques involved in the preparation of DEPN-8 are quite challenging. The general chemistry is straight forward and easy to understand, however, the actual preparation is where a little skill is required. Simple carelessness is extremely detrimental to the formation of the
product. Much patience is required as many of the steps require constant monitoring every few hours. Preparation of the phosphonylating agent 4 is possibly the most time consuming as the sequence of reactions takes almost a full 12 hours before it is combined with the glycerol backbone and allowed to react for the next two days. Removal of the solvent at numerous steps is also very sensitive. The lipid behaves very differently from other compounds when the solvent is removed by rotary evaporation and thus the solution frequently ‘bumps’. Constant supervision is required at the beginning of this procedure since bumping results in loss of the lipid, and re-isolation of the contaminated fractions proves even more challenging and time consuming.

![Scheme 31: Synthesis of DEPN-8.](image)

### 3.3 Conclusions

In summary, the preparation of DEPN-8 has proven to be quite challenging. The formation of the glycerol backbone has been improved and is now more applicable to large scale synthesis. Although the formation of the phosphonylating agent precursor 4 has not been accomplished without the use of TMSBr, the formation of phosphonates 19 and 20 might prove to be useful in the future for preparation of this component.

The preliminary results obtained for the surface activity of DEPN-8 are also very promising. They suggest that diether phosphonolipids might actually be superior to endogenous
phospholipids at reducing the surface tension within the lungs of infants suffering from RDS. Data to support this includes the better adsorption and respreading data obtained when compared to DPPC and other animal-derived surfactant mixtures such as CLSE. Since an increased amount of phospholipase enzymes and other harmful by-products are present in the lungs of such infants, the use of lipid analogues that show resistance to these compounds is extremely beneficial. The added structural freedom from the ester to ether conversion, as well as the phosphate to phosphonate conversion, are both beneficial to the surface activity of DEPN-8 and other phosphonolipids alike.

3.4 Future Work

Since the discovery that neonatal RDS and pulmonary surfactant deficiency are directly connected, an extensive amount of research has been performed in hopes of finding a cure to this fatal disease. With the incredible results seen for pulmonary surfactant replacement therapy, the race to develop a purely synthetic surfactant preparation with the highest surface activity has already begun. The use of phosphonolipids, such as DEPN-8, as alternatives to phospholipids in synthetic preparations seems to be a turn in the right direction. The possible incorporation of other phosphonolipids, such as diether phosphonoglycerol, as well as synthetic proteins, such as Super Mini-B, have already been considered and testing is underway. Initial results are extremely encouraging.

The synthesis of DEPN-8 described above still requires improvement. The lower yields obtained from the alkylation step in the preparation of the glycerol backbone require possible optimization to further increase the product yield. As well, the preparation of phosphonylating agent without the use of TMSBr is also an area that requires further research. In a recent
literature search, a paper published by Bryant et al. in 2009 suggests that mono-dealkylation of H-phosphonate esters (ie: dibenzyl H-phosphonate 22) is possible by refluxing in excess tert-butylamine.\textsuperscript{116} The authors applied these conditions to several dialkyl H-phosphonates containing the following alkyl groups: methyl, ethyl, isopropyl, n-butyl, and benzyl. All reactions were reported to produce high yields of the desired product. As an extension to this method, potential application of it should be considered for the dealkylation of phosphonates 12, 19, or 20, however, with the propyl functionality present in these compounds, it might prove to be more challenging than with dialkyl H-phosphonates.

In addition, the overall synthesis of phosphonolipids may need to be reconsidered all together. One possibility is that the H-phosphonates be dealkylated by the conditions described by Bryant et al.\textsuperscript{116} and further coupled to the glycerol backbone prior to installing the phosphonocholine headgroup portion. An appropriate procedure could be very different from that describe above for the synthesis of DEPN-8 and may be worth looking at in the future.

Another area that requires attention is the preparation of the cyclic phosphonylating agent 11 and its further reaction with an alcohol, such as the glycerol backbone. Once this is formed, ring opening with trimethylamine to form the choline headgroup will also need to be evaluated. Since the methods attempted above to cleave the phosphonate ester group and convert it to the acid chloride species all failed, different methods will have to be evaluated.

Research in the field of RDS treatment is only just beginning. It will be interesting to see where scientific advances in the fields of chemistry and medicine will take us and what new preparations will be discovered along the way. One hope is that DEPN-8 and other phosphonolipids will eventually be incorporated into commercially available synthetic surfactant/peptide preparations that treat infants suffering from RDS.
CHAPTER 4:

EXPERIMENTAL
4.0 Instrumentation and General Methods

Dimethylsulfoxide (DMSO) was stored over 4 Å molecular sieves and used as is. Methanol was purchased from Sigma Aldrich and used as is. Tetrahydrofuran (THF) was freshly distilled from sodium and benzophenone. Toluene was dried over calcium hydride then distilled and stored over 4 Å molecular sieves under argon atmosphere. Ethanol-free chloroform was obtained by washing commercial chloroform several times with water, distilling from calcium chloride, and storing it in a dark bottle away from the light over 4 Å molecular sieves. Acetone, dichloromethane (DCM), and N,N-dimethylformamide (DMF) were obtained from an LC Technology Solutions SP-1 Stand Alone Solvent Purification System. Microwave reactions were carried out in a CEM Discover S-class reactor in a 10 or 35 mL microwave vessel. Flash chromatography was performed on SiliaFlash P60 230-400 mesh silica gel. Melting points were determined using a MEL-TEMP melting point apparatus. Infrared (IR) spectra were obtained using a Bruker Alpha FT-IR spectrometer. Nuclear magnetic resonance (NMR) spectra are reported in parts per million (ppm) and were calibrated to either tetramethylsilane (0.00 ppm), deuterated chloroform (CDCl₃) (7.26 ppm), deuterated methanol (CD₃OD) (3.30 ppm) in the case of a CDCl₃/CD₃OD mixture, or an external standard of 85% phosphoric acid (0.00 ppm) in the case of ³¹P-NMR. ¹H-NMR and ¹³C-NMR spectra were recorded on a Bruker spectrometer at 300 and 75 MHz, 400 and 100 MHz, or 600 and 151 MHz, respectively. ³¹P-NMR spectra were recorded on a Bruker 400 MHz spectrometer at 162 MHz. GC-MS spectra were recorded on a Varian CP-3800 gas chromatograph/Varian Saturn 2200 GC/MS. The following abbreviations are used to describe the signals in ¹H-NMR spectroscopy: bs, broad singlet; d, doublet; dd, doublet of doublets; dABq, doublet of AB quartet; m, multiplet; q, quartet; s, singlet; t, triplet; td, triplet of doublets.
4.1 Synthetic Procedures

(6) 3-(Hexadecyloxy)-propane-1,2-diol$^{84}$

The following compound was synthesized by modifying the methods previously described by Davy.$^{84}$ KOH (7.40 g, 132 mmol, 3.5 eq) was ground to a fine powder and suspended in DMSO (75 mL). Solketal (4.70 mL, 37.8 mmol, 1.0 eq) was added and allowed to stir for 10 minutes. After the addition of bromohexadecane (23.9 mL, 78.3 mmol, 2.07 eq), the reaction was stirred for 18 hours at room temperature. Following this, the reaction was heated to 60 °C for 1.5 hours. Upon cooling, water (135 mL) was added and the product was extracted with 50% ethyl acetate in hexanes (3 × 75 mL). The combined organic layers were washed with water (3 × 75 mL), dried over MgSO$_4$, filtered, and concentrated under vacuum. The crude product was then dissolved in 10% HCl in methanol (75 mL) and stirred at room temperature for 18 hours. Ether (200 mL) was added and the mixture was washed with an aqueous NaHCO$_3$ solution (3 × 100 mL) followed by a water wash (3 × 100 mL). The organic layer is then dried over MgSO$_4$, filtered, and concentrated under vacuum. Recrystallization from hexanes provided 7.89 g (66%) of product 6.

$^1$H-NMR (400 MHz, CDCl$_3$), δ: 3.86 – 3.80 (m, 1H), 3.73 – 3.60 (m, 2H), 3.54 – 3.42 (m, 4H), 2.55 (d, J = 5.1 Hz, 1H), 2.11 (dd, J = 5.1 Hz, 7.1 Hz, 1H), 1.58 – 1.52 (m, 2H), 1.23 (s, 26H), 0.85 (t, J = 6.8 Hz, 3H) ppm; $^{13}$C-NMR (100 MHz, CDCl$_3$), δ: 72.5, 71.8, 70.4, 64.3, 31.9, 29.7, 29.6, 29.5, 29.4, 29.3, 22.7, 14.1 ppm.

(7) 1-(Hexadecyloxy)-3-(trityloxy)propan-2-ol$^{84}$

The following compound was synthesized by modifying the methods described by Davy.$^{84}$ 3-(Hexadecyloxy)-propane-1,2-diol (6) (9.37 g,
29.6 mmol, 1.0 eq) was dissolved in 1:4 v/v THF/ACN solution (150 mL). Triethylamine (4.93 mL, 35.5 mmol, 1.2 eq) was added, followed by the addition of trityl chloride (9.90 g, 35.5 mmol, 1.2 eq). This solution was refluxed for 24 hours, and upon cooling, the precipitate was removed by filtration and washed with ethyl acetate (50 mL). The organic filtrate was washed with the following series of solutions: dilute HCl (50 mL), saturated NaHCO₃ (50 mL), water (2 × 50 mL), and brine (2 × 50 mL). The combined organic layers were dried over MgSO₄, filtered, and concentrated under vacuum to afford 9.37 g (57%) of crude product 7.

1H-NMR (300 MHz, CDCl₃), δ: 7.42 – 7.28 (m, 15H), 3.97 – 3.91 (m, 1H), 3.55 – 3.40 (m, 4H), 3.21 - 3.18 (m, 2H), 2.40 (d, J = 4.6 Hz, 1H), 1.53 (m, 2H), 1.25 (s, 26H), 0.88 (t, J = 6.6 Hz, 3H) ppm; 13C-NMR (75 MHz, CDCl₃), δ: 143.8, 128.6, 127.9, 127.0, 86.8, 71.9, 71.6, 69.8, 64.6, 31.9, 29.7, 29.5, 29.3, 26.0, 22.6, 14.1 ppm.

(9) 3-(Benzyloxy)-propane-1,2-diol

The following compound was synthesized by modifications made to the methods of Menger et al. KOH (90.2 g, 1.61 mol, 10.0 eq) was ground to a fine powder and suspended in DMSO (250 mL). Solketal (20.0 mL, 0.16 mol, 1.0 eq) was added, followed by the addition of benzyl bromide (21.0 mL, 0.17 mol, 1.1 eq). The reaction was stirred overnight at room temperature. Ethyl acetate (200 mL) and 10% aq. HCl (200 mL) were added and the aqueous layer was extracted with ethyl acetate (3 × 100 mL). The combined organic layers were washed with water (2 × 100 mL) and brine (100 mL), dried over MgSO₄, filtered and concentrated under vacuum. The crude oil was then mixed with 15% aq. acetic acid (200 mL) and stirred at 80-95 °C (2-3 hours) until homogeneous. After cooling to room temperature, the aqueous layer was extracted with ethyl acetate (3 × 75 mL) and the
combined organics were washed with water (2 × 75 mL) and brine (75 mL). The solution was dried over MgSO₄, filtered, and concentrated under vacuum to give 25.8 g (88%) of product 9, which was used without further purification.

**1H-NMR** (300 MHz, CDCl₃), δ: 7.38 – 7.29 (m, 5H), 4.55 (s, 2H), 3.92 – 3.86 (m, 1H), 3.73 – 3.50 (m, 4H), 2.50 (bs, 2H) ppm; **13C-NMR** (100 MHz, CDCl₃), δ: 137.6, 128.5, 127.9, 127.8, 73.6, 71.8, 70.6, 64.0 ppm; **IR** (neat), ν max: 3423, 3054, 2986, 2924, 2868, 1453, 1422, 1265, 1112, 1069, 896, 739, 704 cm⁻¹.

**((2,3-bis(hexadecyloxy)propoxy)methyl)benzene**

By modifying the methods of Schwan and Notter et al.,³⁻⁸⁻¹⁸ 3-(benzyloxy)-propane-1,2-diol (9) was alkylated as follows. 3-(Benzyloxy)-propane-1,2-diol (9) (25.8 g, 0.14 mol, 1.0 eq) was added to a stirred solution of ground KOH (47.7 g, 0.85 mol, 6.0 eq) suspended in DMSO (200 mL). After 20 minutes, bromohexadecane (108 mL, 0.35 mol, 2.5 eq) was added and the reaction was left to stir at room temperature overnight. Ethyl acetate (100 mL) and 5% aq. HCl (100 mL) were added and the aqueous layer was extracted with ethyl acetate (3 × 75 mL). The combined organic layers were washed with water (2 × 100 mL) and brine (100 mL), dried over MgSO₄, filtered and concentrated under vacuum. The product was purified by flash chromatography, eluting with 2% ethyl acetate in hexanes, to give 40.3 g (45%) of product 10.

**1H-NMR** (400 MHz, CDCl₃), δ: 7.36 – 7.28 (m, 5H), 4.55 (s, 2H), 3.61 – 3.40 (m, 9H), 1.58 – 1.52 (m, 4H), 1.25 (s, 52H), 0.88 (t, J = 6.5 Hz, 6H) ppm; **13C-NMR** (100 MHz, CDCl₃), δ: 138.4, 128.3, 127.6, 127.5, 77.9, 73.3, 71.6, 70.7, 70.6, 70.3, 31.9, 30.0, 29.7, 29.6, 29.5, 29.3, 26.1, 26.0, 22.7, 14.1 ppm; **IR** (neat), ν max: 3054, 2986, 2927, 2854, 1639, 1454, 1422, 1265,
The following was adapted from the methods of Menger et al.\textsuperscript{85} 10% palladium on activated carbon (5.2 g, 50% w/w) was suspended in THF/MeOH (100 mL, 1:1 v/v) in a flame dried round bottom flask. After purging the solution with hydrogen, ((2,3-bis(hexadecyloxy)propanoxy)methyl)benzene (10) (10.3 g, 16.3 mmol, 1.0 eq) was added and was allowed to stir at room temperature under H\textsubscript{2} balloon until TLC showed full conversion (2-3 hours). The catalyst was filtered and washed with chloroform and the solvent was removed under vacuum. The crude product was recrystallized from acetone to produce 7.05 g (80\%) of product 3, melting at 56-57 °C (lit. 59 °C).\textsuperscript{117}

\textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3}), δ: 3.72 – 3.70 (m, 1H), 3.62 – 3.57 (m, 2H), 3.54 – 3.39 (m, 6H), 2.15 (t, J = 6.1 Hz, 1H), 1.58 – 1.54 (m, 4H), 1.25 (s, 52H), 0.88 (t, J = 6.8 Hz, 6H). \textsuperscript{13}C-NMR (100 MHz, CDCl\textsubscript{3}), δ: 78.2, 71.8, 70.9, 70.4, 63.1, 31.9, 30.1, 29.6, 29.4, 29.3, 26.0, 22.6, 14.1 ppm; IR (neat), ν max: 3454, 2957, 2918, 2850, 1464, 1113, 1081 cm\textsuperscript{-1}.

Diethyl 3-bromopropylphosphonate was prepared by two methods. \textbf{Method A.}\textsuperscript{89} Triethyl phosphite (15.0 mL, 86.2 mmol, 1.0 eq) and 1,3-dibromopropane (44.5 mL, 435.4 mmol, 5.0 eq) were added to a round bottom flask fixed with a Dean Stark apparatus and a water condenser. The mixture was heated to 150 °C until TLC showed the disappearance of triethyl phosphite (3-4 hours). Fractional distillation was used to remove the excess 1,3-dibromopropane as well as to purify the product, giving 17.0 g (76\%) of
pure compound 12. **Method B:** In a 35 mL flame dried microwave vessel under argon atmosphere, triethyl phosphite (3.0 mL, 17.2 mmol, 1.0 eq) and 1,3-dibromopropane (8.9 mL, 87.1 mmol, 5.0 eq) were added. A microwave reactor was used to apply 150 W of power, holding the temperature at 180 °C for 1 minute. Short path distillation (55 °C, 0.2 mm Hg) was then used to remove the excess 1,3-dibromopropane. The product was purified by elution through a silica gel plug using 75% ethyl acetate in hexanes, to give 1.84 g (41%) of product 12.

**1H-NMR** (300 MHz, CDCl₃), δ: 4.19 – 4.07 (m, 4H), 3.52 – 3.45 (td, J = 6.4 Hz, J₁P-H = 1.0 Hz, 2H), 2.22 – 2.04 (m, 2H), 1.95 – 1.87 (m, 2H), 1.37 – 1.30 (m, 6H) ppm; **13C-NMR** (100 MHz, CDCl₃), δ: 61.6 (J₁P-C = 6.5 Hz), 33.5 (J₁P-C = 18.7 Hz), 25.9 (J₁P-C = 4.4 Hz), 24.4 (J₁P-C = 142.5 Hz), 16.4 (J₁P-C = 6.0 Hz) ppm; **31P-NMR** (162 MHz, CDCl₃), δ: 30.9 ppm; **IR** (neat), ν max: 3154, 2984, 2908, 1474, 1440, 1391, 1262, 1244, 1223, 1164, 1030 cm⁻¹.

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**Ethyl propylphostonate**

In a 35 mL flame dried microwave vessel under argon atmosphere was added neat diethyl 3-bromopropanephosphonate (12) (1.3 g, 5.15 mmol). A microwave reactor was used to apply 150 W of power while holding the temperature at 180 °C for 10 minutes. The product was purified by elution through a silica gel plug, using 80% ethyl acetate in hexanes, to give 0.29 g (38%) of product 13.

**1H-NMR** (400 MHz, CDCl₃), δ: 4.26 – 4.07 (m, 4H), 2.34 – 2.17 (m, 2H), 1.93 – 1.76 (m, 2H), 1.34 (t, J = 7.0 Hz, 3H) ppm; **13C-NMR** (100 MHz, CDCl₃), δ: 67.1 (J₁P-C = 10.9 Hz), 62.2 (J₁P-C = 6.6 Hz), 23.9 (J₁P-C = 2.6 Hz), 18.6 (J₁P-C = 122.5 Hz), 16.4 ppm; **31P-NMR** (162 MHz, CDCl₃), δ: 49.5 ppm; **IR** (neat), ν max: 3054, 2986, 1421, 1265, 1047, 727, 705 cm⁻¹.
(15) **Ethyl 3-chloropropylphosphonochloridate**

In a flame dried round bottom flask fitted with a condenser, ethyl propylphostonate (13) (0.8 g, 5.35 mmol, 1.0 eq) was dissolved in ethanol-free CHCl₃ (20 mL/g of phostonate). PCl₅ (1.5 g, 6.95 mmol, 1.3 eq) was added at room temperature and the reaction was heated to 50 °C for 1.5 hours. The solvent was removed via a water aspirator fitted with a CaCl₂ drying tube and was kept at 50 °C under vacuum for 1 hour. Product 15 was immediately used without purification.

**^1H-NMR** (400 MHz, CDCl₃), δ: 4.14 – 4.07 (m, 2H), 3.61 (t, J = 6.3 Hz, 2H), 2.14 – 2.04 (m, 2H), 1.96 – 1.88 (m, 2H), 1.34 (t, J = 7.0 Hz), ppm; **^13C-NMR** (151 MHz, CDCl₃), δ: 61.4 (Jₚ₋C = 6.6 Hz), 44.7 (Jₚ₋C = 18.3 Hz), 25.6 (Jₚ₋C = 3.7 Hz), 23.3 (Jₚ₋C = 146.2 Hz), 16.3 (Jₚ₋C = 6.2 Hz) ppm. **^31P-NMR** (162 MHz, CDCl₃), δ: 33.9 ppm; **IR** (neat), ν max: 2985, 2907, 1475, 1442, 1196, 1044, 987, 732 cm⁻¹; **GC-MS** (8.3 min, EI), m/z (%): 209 (10), 207 (70), 205 (M⁺, 100), 141 (6), 115 (13).

(16) **Dodecyl ethyl 3-chloropropylphosphonate**

Ethanol-free CHCl₃ (5 mL/g of phostonate) was added to the crude ethyl 3-chloropropylphosphonochloridate (15) and the reaction was cooled to 0 °C. A mixture of 1-dodecanol (4.83 mL, 34.74 mmol, 6.5 eq) and Et₃N (7.89 mL, 34.74 mmol, 6.5 eq) in ethanol-free CHCl₃ (5 mL/g of phostonate) was then added dropwise. After the addition, the reaction was warmed to room temperature and stirred for 48 hours. Water (2 mL/g of alcohol) was added and the reaction was stirred for 30 minutes. The organic phase was washed with water (2 × 10 mL) and brine (10 mL) and then back-extracted with CHCl₃ (20 mL). After drying with MgSO₄ and filtering, the solvent was removed under vacuum and the product
was purified by flash chromatography, eluting with 50% ethyl acetate in hexanes to give 1.02 g (54%) of product 16.

$^1$H-NMR (400 MHz, CDCl$_3$), δ: 4.17 – 3.95 (m, 4H), 3.59 (t, J = 6.0 Hz, 2H), 2.12 – 2.03 (m, 2H), 1.93 – 1.85 (m, 2H), 1.68 – 1.61 (m, 2H), 1.31 (t, J = 7.0 Hz, 3H) 1.25 (s, 18H), 0.87 (t, J = 6.8 Hz, 3H) ppm; $^{13}$C-NMR (100 MHz, CDCl$_3$), δ: 65.7 (J$_{P-C}$ = 6.7 Hz), 61.6 (J$_{P-C}$ = 6.5 Hz), 44.9 (J$_{P-C}$ = 18.1 Hz), 31.8, 30.5 (J$_{P-C}$ = 6.1 Hz), 29.6, 29.5, 29.4, 29.3, 29.1, 25.8 (J$_{P-C}$ = 4.2 Hz), 25.5, 23.7, 22.6, 22.3, 16.5, 14.1 ppm; $^{31}$P-NMR (162 MHz, CDCl$_3$), δ: 30.9 ppm; IR (neat), ν max: 2925, 2854, 1466, 1441, 1236, 1044, 1008, 963, 830, 722 cm$^{-1}$. GC-MS (19.4 min, EI), m/z (%): 357 (13), 355 (M$^+$, 42), 187 (100), 159 (46), 123 (18), 97 (10), 65 (6), 55 (15).

(17) 3-Bromopropylphosphonic acid$^{84}$

TMSBr (2.2 mL, 16.8 mmol, 2.2 eq) was added dropwise to a neat solution of diethyl 3-bromopropyl phosphonate (12) (1.5 mL, 7.72 mmol, 1.0 eq) at 0 °C. The reaction was stirred for 4 hours at room temperature, after which the volatile compounds were removed by rotary evaporation. Water (1.5 mL) was added and the mixture was stirred for 20 minutes. Rotary evaporation was used to remove the water (~ 60 °C water bath). The diacid was then dissolved in chloroform and cooled in the freezer (~ -22 °C) for 72 hours, at which point the contents were filtered to obtain 0.75 g (51%) of pure product 17, melting at 104 – 107 °C (lit. 107 – 108 °C).$^{102}$

$^1$H-NMR (400 MHz, CDCl$_3$/CD$_3$OD), δ: 3.41 (t, J = 6.4 Hz, 2H), 2.13 – 2.03 (m, 2H), 1.85 – 1.80 (m, 2H) ppm; $^{13}$C-NMR (100 MHz, CDCl$_3$/CD$_3$OD), δ: 33.5 (J$_{P-C}$ = 19.3 Hz), 26.1 (J$_{P-C}$ = 10.1 Hz), 24.7 ppm; $^{31}$P-NMR (162 MHz, CDCl$_3$/CD$_3$OD), δ: 29.8 ppm; IR (neat), ν max: 2968, 2715, 2304, 1298, 1245, 1009, 952, 766, 730 cm$^{-1}$. 77
(18) Lithium propylphosphonate\textsuperscript{92}

By following the procedure of Eberhard \textit{et al.},\textsuperscript{92} 3-bromopropylphosphonic acid (17) (0.5 g, 2.43 mmol, 1.0 eq) was dissolved in deionized water (20 mL). The pH was brought up to 9-10 using an aqueous 1M LiOH solution (~ 10 mL). The reaction was then stirred for 20 hours at room temperature, at which point the water was removed under vacuum (water bath ~ 60 °C). The white solid was then filtered and rinsed with acetone, discarding the filtrate. MeOH was then used to wash the white solid and the filtrate was collected and concentrated under vacuum. The product was purified by elution through a silica gel plug using 100% MeOH to give 0.26 g (88%) of product 18.

\textsuperscript{1}H-NMR (400 MHz, CD\textsubscript{3}OD/CDCl\textsubscript{3}), δ: 3.95 (dt, J = 6.4 Hz, J\textsubscript{P-H} = 10.8 Hz, 2H), 2.07 – 1.97 (m, 2H), 1.55 (dt, J = 7.6 Hz, J\textsubscript{P-H} = 13.4 Hz, 2H) ppm; \textsuperscript{13}C-NMR (100 MHz, CD\textsubscript{3}OD/CDCl\textsubscript{3}), δ: 65.8 (J\textsubscript{P-C} = 9.8 Hz), 25.4 (J\textsubscript{P-C} = 2.5 Hz), 20.3 (J\textsubscript{P-C} = 120.1 Hz) ppm; \textsuperscript{31}P-NMR (162 MHz, CD\textsubscript{3}OD/CDCl\textsubscript{3}), δ: 43.8 ppm; IR (neat), ν max: 2964, 2942, 2871, 1259, 1182, 1168, 1038, 775 cm\textsuperscript{-1}.

(22) Dibenzyl H-phosphonate\textsuperscript{104}

By following the procedure from Perruchon \textit{et al.},\textsuperscript{104} dibenzyl H-phosphonate (22) was prepared as follows. Phosphorus trichloride (5.0 mL, 57.3 mmol, 1.0 eq) was added to dry toluene (150 mL) and the solution was cooled to 0 °C. In an attached dropping funnel, benzyl alcohol (11.9 mL, 114.6 mmol, 2.0 eq) and \textit{N,N}-dimethylaniline (15.7 mL, 123.8 mmol, 2.2 eq) were mixed with dry toluene (40 mL). This mixture was slowly added in a dropwise fashion to the reaction flask over 1 hour. After addition was complete, the reaction was allowed to stir for 1 hour while warming to room temperature. Water was then added to the
mixture and was allowed to stir for 1 hour. Extraction of the aqueous layer with ether (2 × 100 mL) was followed by a water wash (100 mL) and the organic layer was dried over MgSO₄, filtered and concentrated under vacuum to afford 11.1 g (74%) of product 22.

**1H-NMR** (400 MHz, CDCl₃/CD₃OD), δ: 6.92 (d, Jₚ-H = 706.8 Hz, 1H), 7.38 – 7.29 (m, 10H), 5.10 – 4.99 (dABq, J = 11.8 Hz, Jₚ-H = 9.6 Hz, 4H) ppm; **13C-NMR** (100 MHz, CDCl₃), δ: 135.4 (Jₚ-C = 6.2 Hz), 128.6, 127.9, 126.9, 67.2 (Jₚ-C = 5.6 Hz) ppm; **31P-NMR** (162 MHz, CDCl₃), δ: 8.18 ppm; **IR** (neat), ν max: 3054, 2986, 2441, 1497, 1456, 1262, 979, 896, 752 cm⁻¹

(23) **Dibenzyl methylphosphonate**

![Dibenzyl methylphosphonate](image)

By following the procedure of Meyer et al., dry NaH powder (0.2 g, 8.83 mmol, 1.1 eq) was suspended in freshly distilled THF (30 mL) at 0 °C. Dibenzyl H-phosphonate (22) (1.8 mL, 8.03 mmol, 1.0 eq) was added slowly via syringe. The reaction was allowed to stir for approximately 1 hour, at which point iodomethane (0.5 mL, 8.03 mmol, 1.0 eq) was slowly added. The reaction was then stirred for 2 hours, while it warmed to room temperature. The reaction was quenched with water (25 mL), extracted with ether (3 × 25 mL), dried over MgSO₄, filtered and concentrated under vacuum. The product was purified by flash chromatography, eluting with 20% ethyl acetate in hexanes, to produce 0.74 g (33%) of product 23.

**1H-NMR** (400 MHz, CDCl₃): 7.36 – 7.29 (m, 10H), 5.07 – 4.93 (dABq, J = 11.8 Hz, Jₚ-H = 8.4 Hz, 4H), 1.45 (d, Jₚ-H = 17.6 Hz, 3H) ppm; **13C-NMR** (100 MHz, CDCl₃), δ: 136.2 (Jₚ-C = 6.1 Hz), 128.5, 128.3, 127.8, 67.0 (Jₚ-C = 6.1 Hz), 11.5 (Jₚ-C = 144.4 Hz) ppm; **31P-NMR** (162 MHz, CDCl₃), δ: 32.2 ppm; **IR** (neat), ν max: 3090, 3065, 3034, 2954, 2927, 2893, 1587, 1455, 1312, 1233, 997, 923, 829, 733, 697 cm⁻¹.
(24) 2-(3-Bromopropoxy)tetrahydro-2H-pyran\textsuperscript{107}

The following compound was prepared by altering the procedure from De Feyter and van Esch \textit{et al.}\textsuperscript{107} 3-Bromo-1-propanol (1.3 mL, 14.4 mmol, 1.3 eq) was added to DCM (50 mL) at room temperature. DHP (1.0 mL, 11.1 mmol, 1.0 eq) and pyridinium \textit{p}-toluene sulfonate (0.17 g, 0.66 mmol, 0.06 eq) were added and the reaction was left to stir for 3 hours. Ether (100 mL) was added and the organic layer was washed with brine (2 \times 50 mL), dried over MgSO\textsubscript{4}, filtered and concentrated under vacuum. The product was purified by flash chromatography, eluting with 15\% ether in hexanes, to provide 1.81 g (73\%) of product 24.

\textbf{\textsuperscript{1}H-NMR} (400MHz, CDCl\textsubscript{3}), \(\delta\): 4.60 (t, \(J = 3.4\) Hz, 1H), 3.90 – 3.83 (m, 2H), 3.56 – 3.48 (m, 4H), 2.17 – 2.09 (m, 2H), 1.84 – 1.66 (m, 2H), 1.61 – 1.50 (m, 4H) ppm; \textbf{\textsuperscript{13}C-NMR} (100 MHz, CDCl\textsubscript{3}), \(\delta\): 98.8, 64.8, 62.2, 32.8, 30.6, 30.5, 25.4, 19.4 ppm; \textbf{IR} (neat), \(\nu\) max: 2948, 2872, 1265, 1134, 1118, 1075, 813, 739 cm\textsuperscript{-1}; \textbf{GC-MS} (8.3 min, EI), m/z (%): 223 (50), 221 (M\textsuperscript{+}, 58), 207 (8), 143 (8), 85 (100).

(25) 2-(3-Iodopropoxy)tetrahydro-2H-pyran\textsuperscript{109,110}

By following the procedure from Weyna \textit{et al.},\textsuperscript{110} bromine to iodine exchange was performed as follows. NaI (0.89 g, 5.94 mmol, 1.3 eq) was dissolved in acetone (10 mL). 2-(3-Bromopropoxy)tetrahydro-2H-pyran (24) (1.0 g, 4.56 mmol, 1.0 eq) was added at room temperature and the reaction was left to stir over night, with protection from ambient light. After filtering through Celite\textsuperscript{TM} and washing with acetone, the solvent was removed under vacuum. The product was purified by flash chromatography, eluting with 15\% ether in hexanes, to provide 0.87 g (71\%) of product 25.
**1H-NMR** (400 MHz, CDCl₃), δ: 4.58 (t, J = 3.4 Hz, 1H), 3.90 – 3.76 (m, 2H), 3.53 – 3.40 (m, 2H), 3.30 – 3.26 (m, 2H), 2.10 – 2.01 (m, 2H), 1.82 – 1.67 (m, 2H), 1.60 – 1.50 (m, 4H) ppm;

**13C-NMR** (100 MHz, CDCl₃), δ: 98.9, 66.8, 62.3, 33.5, 30.6, 25.4, 19.4, 3.4 ppm; **IR** (neat), ν max: 3054, 2986, 1264, 895, 747 cm⁻¹; **GC-MS** (9.3 min, EI), m/z (%): 269 (M⁺, 64), 143 (54), 85 (100).

(19) **Dibenzyl 3-bromopropylphosphonate**¹¹⁴

![Dibenzyl 3-bromopropylphosphonate](image)

The following compound was synthesized by modifying the methods from Cohen et al.¹¹⁴ Dibenzyl H-phosphonate (22) (0.85 mL, 3.81 mmol, 1.0 eq) was added to DMF (30 mL) at room temperature in a flame dried round bottom flask under nitrogen atmosphere. Cs₂CO₃ (3.7 g, 11.4 mmol, 3.0 eq) and TBAI (4.2 g, 11.4 mmol, 3.0 eq) were then added and allowed to stir for 1 hour at room temperature. 1,3-Dibromopropane (1.2 mL, 11.4 mmol, 3.0 eq) was then added and the reaction was allowed to stir at room temperature for 4 days. Water (30 mL) was added and the product was extracted with ethyl acetate (3 × 30 mL). The combined organic layers were washed with water (2 × 30 mL) and brine (30 mL), dried over MgSO₄, filtered, and concentrated under vacuum. The product was purified by flash chromatography, eluting with 70% ethyl acetate in hexanes, to give 0.81 g (56%) of product 19.

**1H-NMR** (400 MHz, CDCl₃), δ: 7.38 – 7.31 (m, 10H), 5.08 – 4.94 (dABq, J = 11.8 Hz, Jₚ₋H = 8.6 Hz, 4H), 3.38 (t, J = 6.5 Hz, Jₚ₋H = 1.2 Hz, 2H), 2.15 – 2.01 (m, 2H), 1.94 – 1.85 (m, 2H) ppm; **13C-NMR** (100 MHz, CDCl₃), δ: 136.1 (Jₚ₋C = 5.8 Hz), 128.6, 128.5, 127.9, 67.2 (Jₚ₋C = 6.5 Hz), 33.3 (Jₚ₋C = 19.1 Hz), 25.7 (Jₚ₋C = 4.1 Hz), 24.8 (Jₚ₋C = 40.1 Hz) ppm; **31P-NMR** (162 MHz, CDCl₃), δ: 32.1 ppm; **IR** (neat), ν max: 3154, 3091, 3068, 3035, 2962, 2895, 1456, 1378, 1262, 1227, 998, 911, 744, 649 cm⁻¹.
Dibenzyl H-phosphonate (22) (0.20 mL, 0.76 mmol, 1.0 eq) was added to DMF (30 mL) at room temperature in a flame dried round bottom flask under nitrogen atmosphere. Cs$_2$CO$_3$ (0.75 g, 2.29 mmol, 3.0 eq) and TBAI (0.84 g, 2.29 mmol, 3.0 eq) were then added and allowed to stir for 1 hour at room temperature. 2-(3-Bromopropoxy)tetrahydro-2$H$-pyran (24) (0.51 g, 2.29 mmol, 3.0 eq) was then added and the reaction was allowed to stir at room temperature for 4 days. Water (30 mL) was added and the product was extracted with ethyl acetate (3 $\times$ 30 mL). The combined organic layers were washed with water (2 $\times$ 30 mL) and brine (30 mL), dried over MgSO$_4$, filtered, and concentrated under vacuum. The product was purified by flash chromatography, eluting with 70% ethyl acetate in hexanes, to give 0.15 g (47%) of product 20.

$^1$H-NMR (400 MHz, CDCl$_3$), $\delta$: 7.35 – 7.30 (m, 10H), 5.08 – 4.94 (dABq, J = 11.8 Hz, J$_{P-H}$ = 8.7 Hz, 4H), 4.51 (t, J = 3.5 Hz), 3.81 – 3.68 (m, 2H), 3.48 – 3.35 (m, 2H), 1.90 – 1.63 (m, 6H), 1.57 – 1.47 (m, 4H) ppm; $^{13}$C-NMR (100 MHz, CDCl$_3$), $\delta$: 136.4 (J$_{P-C}$ = 6.0 Hz), 128.5, 128.3, 127.8, 98.6, 67.0 (J$_{P-C}$ = 6.7 Hz), 66.8, 62.1, 30.5, 25.3, 22.9 (J$_{P-C}$ = 142.2 Hz), 22.8 (J$_{P-C}$ = 4.8 Hz), 19.4 ppm; $^{31}$P-NMR (162 MHz, CDCl$_3$), $\delta$: 33.7 ppm; IR (neat), $\nu$ max: 3090, 3064, 3033, 2943, 2871, 1244, 1134, 1119, 1025, 995, 815, 735, 697 cm$^{-1}$.

(1) **DEPN-8$^{18,24,84}$**

In a flame dried round bottom flask under Argon atmosphere, diethyl 3-bromopropyl phosphonate (12) (2.4 g, 9.26 mmol, 1.0 eq) was dissolved in dry DCM (10 mL/g). The solution was cooled to -40 °C and TMSBr (2.64 mL, 20.4 mmol, 1.1 eq/ester) was
added over 10 minutes. This was stirred for 20 minutes at -40 °C before warming to room temperature, at which point it was stirred for 3 hours. The solvent was removed by an aspirator fixed with a CaCl$_2$ drying tube and subsequently put under high vacuum for 1.5 hours. The residue was dissolved in ethanol-free chloroform (20 mL/g) and PCl$_5$ (7.4 mL, 22.2 mmol, 2.4 eq) was added in one portion at room temperature. The solution was heated to 50 °C for 1.5 hours, at which point the solvent was again removed by an aspirator fixed with a CaCl$_2$ drying tube and put under high vacuum for 1.5 hours at 50 °C. The residue, which corresponds to 3-bromopropylphosphonic acid dichloride (4), was once again dissolved in ethanol-free chloroform (10 mL/g) and cooled to 0 °C. A mixture of Et$_3$N (0.68 mL, 9.26 mmol, 1.0 eq) and 2,3-bis(hexadecyloxy)propan-1-ol (3) (2.5 g, 4.63 mmol, 1.0 eq) in ethanol-free chloroform (10 mL/g) was added dropwise. After the addition, the solution was warmed to room temperature, where it was allowed to stir for 48 hours. Water (2 mL/g of alcohol) was added to the flask and stirred for 1 hour before removing the solvent under vacuum (~ 60 °C water bath). The residue was dissolved in 10:10:1 CHCl$_3$:MeOH:H$_2$O (10 mL/g) and stirred with Amberlite$^\text{TM}$ ion exchange resin (20 mL/g) for 1.5 hours. The solution was filtered and washed with the same solvent, and the filtrate was concentrated by rotary evaporation. The residue was then taken up in DCM (20 mL/g), gently washed with water (10 mL/g) and brine (10 mL/g), dried over MgSO$_4$, filtered and concentrated under vacuum. The residue was re-dissolved in 10:17:17 CHCl$_3$:i-PrOH:MeCN (25 mL/g) and 40% aq. Me$_3$N (9.3 mL, 139.8 mmol, 15.1 eq) and allowed to stir for 48 hours at 60 °C. After cooling and concentrating the solution under vacuum, it was once again dissolved in 10:10:1 CHCl$_3$:MeOH:H$_2$O (10 mL/g) and stirred with Amberlite$^\text{TM}$ ion exchange resin (20 mL/g) for 1 hour. After filtering and rinsing with the same solvent, the filtrate was concentrated under vacuum, taken up in DCM (25 mL/g), gently washed with water (10
mL/g) and brine (10 mL/g), dried over MgSO4, filtered and concentrated by rotary evaporation. Flash chromatography (65:35:5 CHCl3:MeOH:H2O) was used to purify the crude DEPN-8, which was recrystallized from 5:2 acetone/CHCl3 and subsequently dried to constant weight using a Kugelrohr, warming to 50 °C in the presence of P2O5 to provide 1.6 g (49%) of pure product 1.

**1H-NMR** (600 MHz, CDCl3/CD3OD), δ: 3.93 – 3.86 (m, 2H), 3.59 – 3.51 (m, 4H), 3.46 – 3.38 (m, 5H), 3.09 (s, 9H), 2.00 – 1.92 (m, 2H), 1.64 – 1.59 (m, 2H), 1.53 – 1.49 (m, 4H), 1.22 (s, 52H), 0.83 (t, J = 7.0 Hz, 6H) ppm; **13C-NMR** (100 MHz, CDCl3/CD3OD), δ: 78.5 (J_{P-C} = 6.9 Hz), 72.0, 70.9 (J_{P-C} = 3.8 Hz), 67.2 (J_{P-C} = 10.6 Hz), 63.7, 53.2, 32.2, 30.3, 30.0, 29.9, 29.8, 28.6, 26.4 (J_{P-C} = 4.8 Hz), 22.9, 17.0, 14.2 ppm; **31P-NMR** (162 MHz, CDCl3/CD3OD), δ: 24.0 ppm.
CHAPTER 5:

REFERENCES
5.0 References


16. Zuo, Y. Y.; Veldhuizen, R. A. W.; Neumann, A. W.; Petersen, N. O.; Possmayer, F.


   (accessed March 11, 2012).


6.0  List of Numbered Compounds

1. \( \text{H}_{33}\text{C}_{16} \text{O} \text{O} \text{H}_{33}\text{C}_{16} \text{O} \text{PO}_{2}^{-} \text{NMMe}_{3} \)

2. \( \text{H}_{33}\text{C}_{16} \text{O} \text{O} \text{POH} \text{H}_{33}\text{C}_{16} \text{O} \text{Br} \)

3. \( \text{H}_{33}\text{C}_{16} \text{O} \text{OH} \text{H}_{33}\text{C}_{16} \text{O} \)

4. \( \text{Cl} \text{PO} \text{Br} \)

5. \( \text{HO} \text{OP} \text{O} \)

6. \( \text{H}_{33}\text{C}_{16} \text{O} \text{OH} \text{H}_{33}\text{C}_{16} \text{O} \)

7. \( \text{H}_{33}\text{C}_{16} \text{O} \text{OH} \text{H}_{33}\text{C}_{16} \text{O} \text{OTr} \)

8. \( \text{H}_{33}\text{C}_{16} \text{O} \text{OH} \text{H}_{33}\text{C}_{16} \text{O} \text{OTr} \)

9. \( \text{HO} \text{OBn} \text{OH} \)

10. \( \text{H}_{33}\text{C}_{16} \text{O} \text{OBn} \text{H}_{33}\text{C}_{16} \text{O} \)

11. \( \text{Cl} \text{PO} \)

12. \( \text{EtO} \text{PO} \text{Br} \)

13. \( \text{EtO} \text{PO} \)

14. \( \text{H}_{26}\text{C}_{12} \text{O} \text{PO} \)

15. \( \text{EtO} \text{PO} \text{Cl} \)

16. \( \text{EtO} \text{PO} \text{Cl} \text{H}_{25}\text{C}_{12} \text{O} \)

17. \( \text{HO} \text{PO} \text{Br} \)

18. \( \text{LiO} \text{PO} \)

19. \( \text{BnO} \text{PO} \text{Br} \text{BnO} \)

20. \( \text{BnO} \text{PO} \text{O} \text{THP} \text{BnO} \)

21. \( \text{BnO} \text{PO} \)

22. \( \text{BnO} \text{PO} \text{H} \text{BnO} \)

23. \( \text{BnO} \text{PO} \text{CH}_{3} \text{BnO} \)

24. \( \text{Br} \text{O} \text{O} \text{O} \)

25. \( \text{I} \text{O} \text{O} \text{O} \)