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Emily P. Wein **April 20, 2011**

A Thorough Investigation of the Characteristic Phosphorus Couplings and ^{31}P NMR Analysis in Attempts to Synthesize an Ifosfamide Analog

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A thesis submitted to the Faculty of Emory College of Arts and Sciences of
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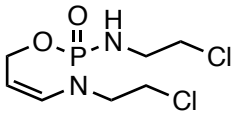
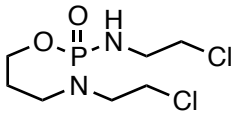
Abstract

A Thorough Investigation of the Characteristic Phosphorus Couplings and a ^{31}P NMR Analysis in Attempts to Synthesize an Ifosfamide Analog

By Emily P. Wein

Cancer remains one of the most prominent global health concerns in today's society. According to the International Journal of Cancer, there were an estimated 12.7 million newly reported cancer cases and 7.6 million cancer deaths in 2008.[1] The Cancer Journal for Clinicians indicated that, in the United States, one in four deaths is caused by cancer.[2] A common treatment for cancer is the use of nitrogen mustard chemotherapy treatments that function by alkylating two different positions of a DNA strand, causing intrastrand crosslinks (ISCs) or kinks in the DNA structure, as well as severing the base pairs from the DNA backbone. These chemotherapy treatments, however, have severe side effects due to their lack of selectivity in the body and their inability to differentiate between cancerous and healthy dividing cells. This investigation focused on a specific nitrogen mustard chemotherapy drug, ifosfamide (shown below on the left), in efforts to synthesize an analog of ifosfamide with the added functionality of a double bond in its ring structure (shown below on the right*).*





Once functionality is established in the ring, it opens the possibility of attaching other ligands to the double bond site on the ring, ligands that could selectively bind the drug to desired regions in the body.

[1] Ferlay, Jacques, et. al. "Estimates of Worldwide Burden of Cancer in 2008: GLOBOCAN 2008." International Journal of Cancer. **15 December 2010**, 127 (12), 2893.

[2] Jemal, Ahmedin, et. al. "Cancer Statistics, 2008." Cancer Journal for Clinicians. **2008**, 58, 71.

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I would like to give a tremendous thank you to my research advisor, Dr. Frank McDonald, for all of his wisdom and expertise, as well as his encouragement and patience as stumbled through my first moderately independent research experience. I am so extremely grateful for and appreciative of all that he has done to make this work possible, and the great wealth of knowledge and experience he has given me through providing me this research opportunity.

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1. Introduction

Cancer remains one of the most prominent global health concerns in today's society. According to the International Journal of Cancer, there were an estimated 12.7 million newly developed cancer cases and 7.6 million cancer deaths reported in 2008 alone.[1] The Cancer Journal for Clinicians indicated that in the United States specifically one in four deaths is caused by cancer. Furthermore, the probability of any individual developing caner in their lifetime is 45% for men and 38% for women, however, with women having a greater probability of developing cancer earlier in life (before the age of sixty) due to the early onset of breast cancer, the most common cancer developed in women worldwide. [2] Why cancer is so prominent and difficult to treat has to do with the way in which cancer develops. Otto Warburg, in 1956, was the first to show that cancer cells could live and develop in the absence of oxygen. This led him to reject the notion that viruses caused cancer. After many years of investigation into Warburg's claim, it is now widely accepted that cancer is neither a bacterial nor a viral infection and thus is not exposed to the body like most other illnesses. Cancer develops in the body on a cellular level. Unlike the regulated production of normal cells in the body that divide and are reproduced as needed when older cells die, cancerous cells proliferate at an uncontrolled and rapid rate, as well as remain longer in the body. Cancer cells develop as a result of mutations that damage or change the genetic material (DNA) of the reproducing cells.[3] These mutations in DNA sequence generally occur through one of two processes: damage to DNA caused by

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[1] Ferlay, Jacques, et. al. "Estimates of Worldwide Burden of Cancer in 2008: GLOBOCAN 2008." International Journal of Cancer. **15 December 2010**, 127 (12), 2893.

[2] Jemal, Ahmedin, et. al. "Cancer Statistics, 2008." Cancer Journal for Clinicians. **2008**, 58, 71.

[3] Voet, Donald, Judith G. Voet, and Charlotte W. Pratt. Fundamentals of Biochemistry: Life at the Molecular Level. Hoboken, NJ: John Wiley and Sons, Inc., 2006.

environmental agents, such as UV light, nuclear radiation, or chemical exposure or natural errors in the DNA replication process. Exposure to harmful environmental factors can alter the structure of a DNA base pair to appear to the DNA polymerase (who reads the DNA strand and creates the daughter strand) to be a different base entirely. Additionally, nuclear radiation can also break phosphorous-oxygen bonds that hold the deoxyribose backbone together.[4] The then fragmented DNA strands attempt to re-attach to other nearby strands, altering the sequence of the DNA, a process called translocation. Naturally occurring DNA errors, on the other hand, take place about once every 100,000,000 base pairs. As the human genome contains 24 chromosomes, each with somewhere between 50 million and 250 million base pairs, the result can be large errors in replication.[5] Although DNA repair proteins repair many of these replication mistakes, these proteins do not catch every DNA sequencing error. In order for a cell to undergo a malignant transformation (become a cancer cell), the cell must undergo an average of five genetic mutations, reflecting DNA complexity and explaining why cancer incidents increase with age. The resulting masses of excess cancer cells that collect in the body as cancer cells rapidly multiply form invasive, malignant tumors.[6]

Although there is no cure for cancer, there are many treatments that are employed to fight the disease. One of the primary initial treatments for cancer is the use of chemotherapy, a remedy that exposes chemicals to the body that are designed to damage large productions of dividing cells. As cancer cells are the most rapidly reproducing cells in a person's body, the expectation is that the drug will destroy most if not all of the cancerous

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[4] Hamminiki, Kari. "DNA adducts, mutations, and cancer." Carcinogenesis. **1993,** 14 (10), 2007.
[5] Lawley, P.D. "Mutagens as carcinogens: Development of Current Concepts. Mutation Research. **1989**, 213 (1), 3.
[6] Voet, 2006

replication sites in the body. As the toxic drug circulates through a person's blood stream, however, it destroys all dividing cells that it encounters, cancerous and healthy alike. Because of the inevitable damage to normal cells, these chemotherapy treatments severely weaken the patient's immune system, causing many harmful side effects, such as low white and red blood cell counts, decreased bone marrow count, impaired renal function, nausea, vomiting, hair loss, etc., and in severe cases may even have neurological manifestation, including somnolence, confusion, and hallucination. In some alarming incidents, the chemotherapy treatment has caused a patient to go into a coma.[7] In efforts to lessen the severity of the harmful side effects of chemotherapy treatments, researches have begun investigating methods of creating chemotherapy drugs that have the potential to selectively bind to desired sites of the body where the cancer cells are most prominent, thereby drastically reducing the toxic effects of the drug on the overall health of the patient.

This investigation chose to focus its attention on a particular chemotherapy drug called ifosfamide (*Figure 1)*.

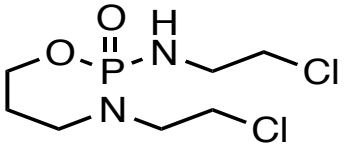

Figure 1: Ifosfamide: Nitrogen Mustard Drug

Ifosfamide is an anti-cancer drug used clinically in the chemotherapy treatment of a wide variety of cancer tumors, ranging from mild cases of soft tissue sarcomas to more severe lymphomas (cancer in the lymphatic cells of the immune system), and is an effective treatment for both adults and children. It is administered to the patients' body through

[7] Brade, W. P., k. Herdrich, U. Kachel-Fischer, and C. E. Araujo. "Dosing and side-effects of ifosfamide plus mesna." Journal of Cancer Research and Clinical Oncology. **1991**, 117, 164.

infusion into a vein (Intravenous, IV). Ifosfamide, which was introduced to clinical trials in the 1970s, had initial setbacks due to severe hemorrhagic cystitis, a highly toxic urinary infection that causes dysuria (painful urination), hematuria (red blood cells in the urine), and hemorrhage (bleeding). These initial complications, however led to the development of sodium mercaptoethanesulfonate (mesna), which can be supplemented with the ifosfamide drug to protect the body from its urotoxic effects.[8]

Ifosfamide is one of a family of drugs called nitrogen mustards, which have similar chemical structure to that of mustard gas (*Figure 2*), except that the drugs has a nitrogen atoms in place of the sulfur atom of mustard gas.[9]

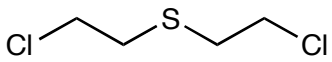

Figure 2: Mustard Gas

Mustard gas was first used on a large scale by the German army in World War I. It was one of the most toxic and powerful chemicals employed in warfare particularly because of its odorless nature. Furthermore, only a small amount of the relatively easily synthesized chemical was needed in an explosive shell for the chemical to be effective. Unlike nerve gas that causes instant death, mustard gas causes delayed effects and incapacitates far more people than it kills. Mustard gas takes approximately 12 hours to take effect on the body, yet can remain dangerous in soil for several weeks. Mustard gas causes severe blistering, sore eyes, and vomiting. The most severely injured organs were the wet and warm areas of the body, such as the scrotum, axillae, penis, and buttocks, as well as the trunk, dorsum of

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[8] Deroussent, Alain, et. al. "Quantification of dimethyl-ifosfamide and its N-deschloropropylated metabolites in mouse plasma by liquid chromatography-tandem mass spectrometry." Journal of Chromatography B **2001**, 879 (11-12), 743.

[9] Dirven, Hurbert A. A. M., Ben van Ommen, and Peter J. van Bladeren. "Glutathione Conjugation of Alkylating Cytostatic Drugs with a Nitrogen Mustard Group and the Role of Glutathione S-Transferases." Chemical Research in Toxicology. **1996**, 9(2), 351.

hands and forearms. In more severe cases, mustard gas exposure caused internal and external bleeding and difficulty breathing due to stripping of the mucous membrane in the bronchial tubes. The fatal cases following attack by mustard gas were primarily due to respiratory complications. The average time for healing was 19 excruciatingly painful days, however in more unfortunate cases, the effects could last for months. Interestingly enough, the main long-term effect of mustard gas exposure is its carcinogenic qualities[10]

Mustard gas functions in the body through means of a similar mechanism as nitrogen mustards, as both function by alkylating DNA. Nitrogen mustards initiate their neurotoxic effects through the process of self-cyclization, forming aziridinium rings (*Figure 3*).

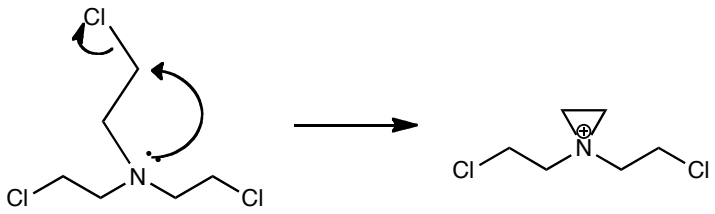

Figure 3: Formation of Aziridinium Ring

[10] Wormser, Uri. "Toxicology of Mustard Gas." Trends in Pharmacological Sciences. **1991**, 12, 164.

The N-7 atom of guanine in the DNA helix can then attack the aziridinium ring, attaching the drug to the DNA (*Figure 4*).

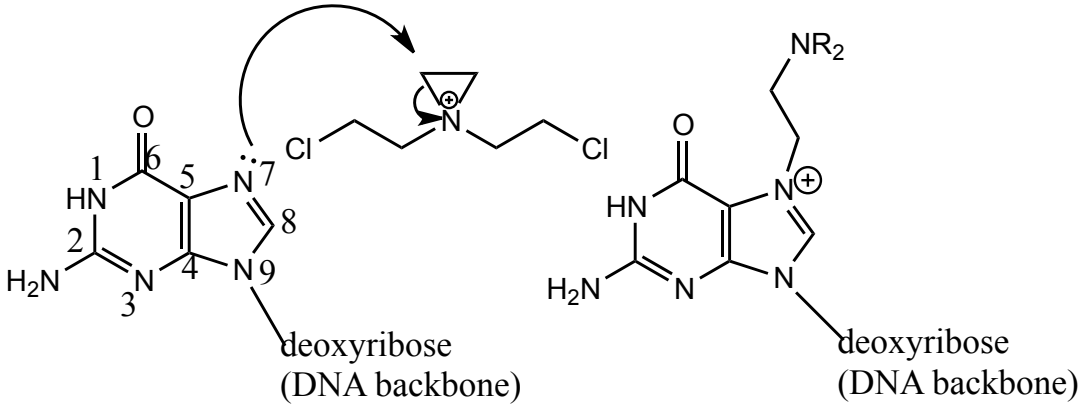

Figure 4: Guanine Attacking Aziridinium Ring

The nitrogen then forms another aziridinium ring on a second chain, so that an N-7 from a different guanine base of DNA can attack the second aziridinium ring. This process of attaching the drug to another location in the DNA helix causes a kink to form in the DNA strand.[11] As DNA replication requires the ability to separate DNA strands in order for the DNA polymerase to utilize each single DNA strand as a template from which new daughter DNA is synthesized, this type of DNA linkage, properly termed a intrastrand cross link (ISC), completely obscures the cells replication process.[12]

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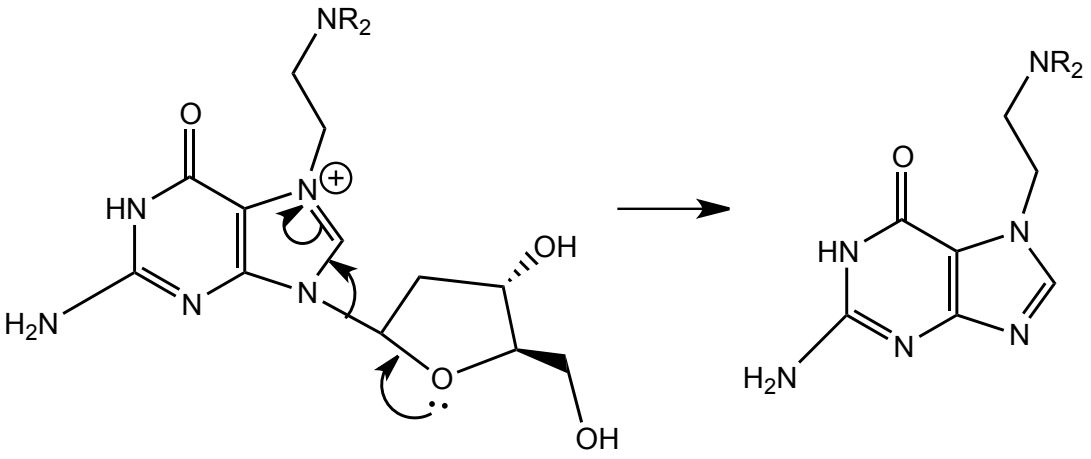
[11] Mann, David J. "Aziridinium Ion Ring Formation from Nitrogen Mustards: Mechanistic Insights from Ab Initio Dynamics." The Journal of Physical Chemistry A **2010**, 114 , 4486.

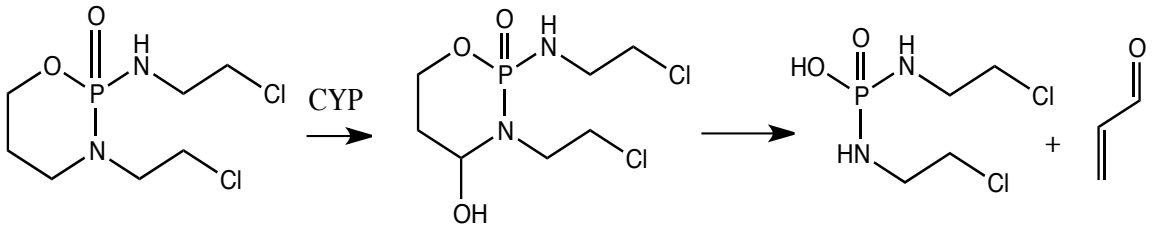
[12] Rajski, Scott R., and Robert M. Williams. "DNA Cross-Linking Agents as Antitumor Drugs." Chemical Reviews **1998**, 98 (8), 2723-2796.

The drugs destructive action, however, proceeds further as it then completely cleaves the guanine base pair from the DNA backbone, as shown in *scheme 1* below.



Scheme 1: Nitrogen Mustard Cleaving Guanine Base Pair from Deoxyribose (DNA) Backbone

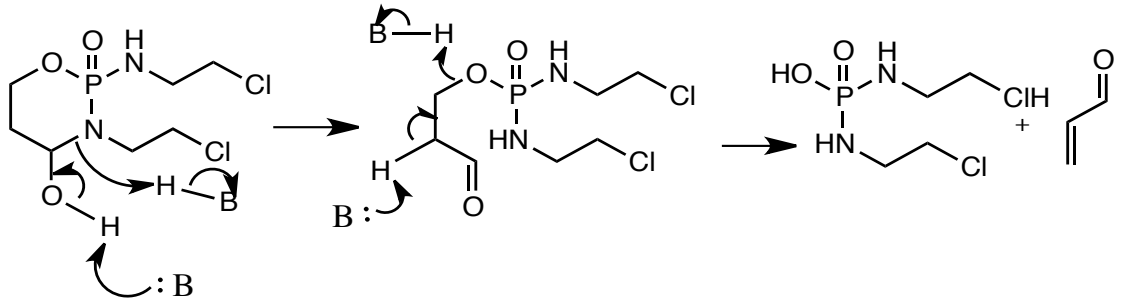
Ifosfamide is clinically administered as a prodrug, and thus it must be oxidized by the enzyme cytochrome p450 (CYP) before its neurotoxic effects can be activated. The CYP enzyme adds an alcohol to the ring, which results in a cleavage of two of the carbons in the ring structure, leaving the freely moving cytotoxic component (see *scheme 2*).[13]



Scheme 2: Ifosfamide Decomposition to Cytotoxic Molecule

[13] Deroussent, 743.

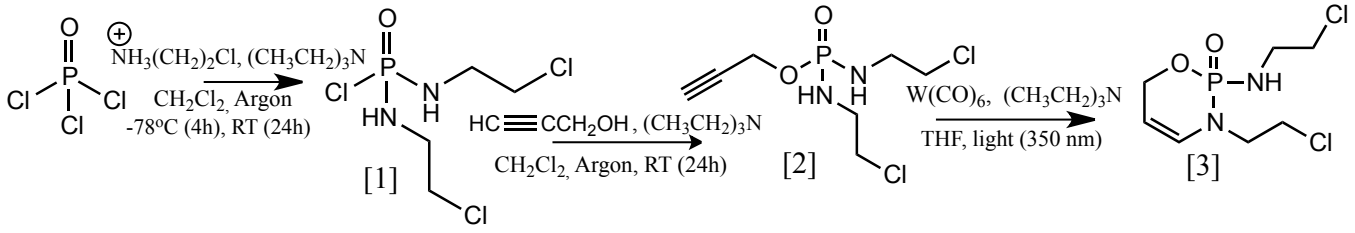
The proposed mechanism for the conversion of the oxidized ifosfamide to the cytotoxic drug is shown below (*scheme 3*). As acids and bases are readily available in the body, protonation and de-protonation are shown in the same mechanistic step.



Scheme 3: Mechanism of Conversion of Oxidative Ifosfamide to Cytotoxic Molecule

The current investigation sought to add an alkene selectively to an area of the ring of ifosfamide structure that is not involved in the alkylation of DNA. The purpose of adding functionality to the ring is to explore the possibility of adding other functional groups synthetically to the ifosfamide that could selectively bind to designated areas of interest in the body. The overarching goal is to create greater selectivity in chemotherapy treatments that employ the ifosfamide drug.

The proposed synthesis of the ifosfamide analog was a three-step process as shown in the following scheme (*scheme 4).*



Scheme 4: Overall Summary of Ifosfamide analog synthesis

The nitrogen amine groups that are necessary for the primary chemotherapy functionality of the nitrogen drug we proposed could be added by substitution for the electronegative chlorine atoms on a posphoryl chloride. We then suggest adding a propargyl alcohol group whose triple bond could provide adequate functionality for a tungsten catalyzed phosphoryl cyclization when irradiated (ultraviolet light) to cyclize the desired ring structure with the preferred double bond.

2. Results and Discussion

2.1 Ifosfamide characterization

Ifosfamide was purchased and characterized to help identify the special phosphorus couplings that might occur in the desired structure. The ifosfamide only partially dissolved in chloroform, with the NMR spectrum showing many multiplets that contained overlapping signals. The signals separate out some when dissolved in deuterated water (D_2O), but the cleanest spectra resulted when the ifosfamide was dissolved in d_6-acetone. For this reason, acetone was chosen as the starting solvent for all the NMRs taken for the reactions completed in this experiment.

The phosphorus-31 component of the molecule created additional coupling in the ^1H nuclear magnetic resonance (NMR) spectra. Coupling in an NMR results from interaction of different spin states through the chemical bond of a molecule, which creates the splitting of NMR signals. These couplings occur whenever there is a nucleus that has a spin ½ signal that interacts with another nucleus that also has a spin ½ signal. Typically in an ^1H NMR signal, hydrogen's coupling indicates the number of hydrogen atoms that are adjacent to it (whose nucleus its spin signal interacts with). Because phosphorus-31 also exists as spin ½ nuclei, it too can couple with hydrogen atoms in an ^1H NMR spectrum. Previous research has indicated that P-H coupling is clearly pronounced in two and three bond couplings and tends to be larger than H-H coupling.[14] However, caution should be taken when applying any three-bond hydrogen-phosphorus coupling constants ($^3J_{PH}$) universally, as they differ greatly depending on different structural compounds and especially regarding different phosphorus oxidation states. In all cases, maximum $^3J_{PH}$ values are observed at 0^o and 180^o

[14] Sharma, Mamta, et. al. "Direct Detection of Alkylphosphonic Acids in Environmental Matrices by Proton Coupled Phophorous NMR." Magnetic Resonance in Chemistry. **12 March 2009**, 47 (6), 478.

dihedral angles (the angle between the phosphorus-carbon-hydrogen atoms in the three bond coupling), while minimum values are found at 90°.[15] For example, the gauche conformation (P-H_A in the figure below) for a POCH bond only exhibits coupling constants averaging from 1.5-2.3 Hz, while trans conformations (P-H_B in *figure 5*) typically display hertz values averaging from 22-28 Hz (typical H-H coupling values average approximately 7 Hz).

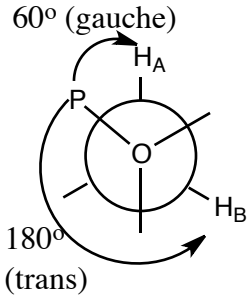


Figure 5: Newman Project of POCH Bond Designating Gauche and Trans Positions of Hydrogen Atoms in Relation to Phosphorus

These Hertz ranges, however, reference specifically acyclic compounds. For cyclic compounds, such as the ifosfamide structure, it has been shown that the J_{gauche} and J_{trans} display approximately even coupling values, rather than the large gap shown in acyclic compounds. These coupling constants are not usually as large as the maximum trans conformation value, nor are they as small as the gauche value.

[15] Nelson, John H. Nuclear Magnetic resonance Spectroscopy. Upper Saddle River, New Jersey: Pearson Education, Inc., 2003.

This intermediate coupling value has to do with the fact that the chair has two different conformations (shown below) in equilibrium.

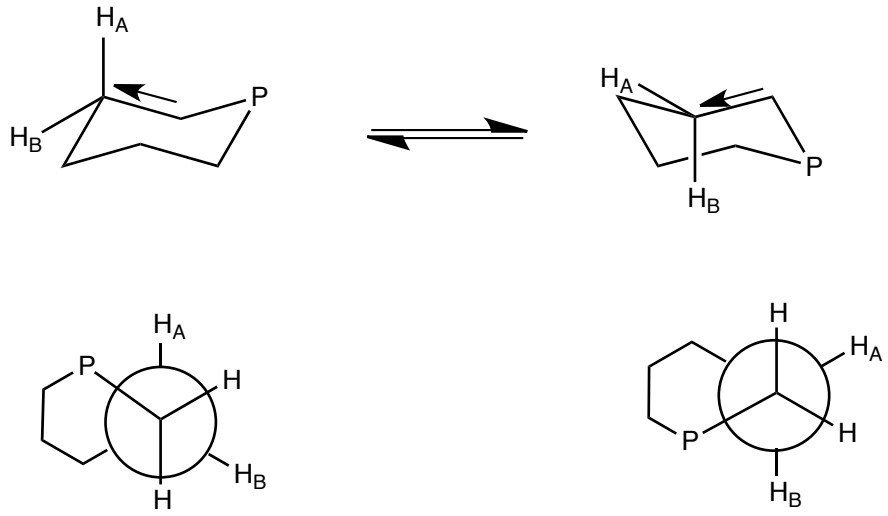


Figure 6: Cyclohexane Chairs in Equilibrium, Each With Their Newman Projections Below When Viewed Down the Arrow Shown on Each Chair

As shown above, the H_A is shown as Gauche to the phosphorus in the left conformation and trans to the phosphorus in the conformation on the right. The same phenomenon appears with the other hydrogen on the same carbon atom (both three bond coupling with the phosphorus), H_B, which lays trans to the phosphorus on the left and axial to the phosphorus on the right.[16]

[16] Kainosho, Masatsune, Asao Nakamura, and Masamichi Tsuboi. Phosphorus-proton Spein-sping Coupling in the P-O-C-H Group. A Comparison of Cyclic and Acyclic Systems. Bulletin of the Chemical Society of Japan. **June 1969**, 42, 1713.

Four bond $^4J_{PH}$ coupling for a molecule not in an allylic position or stuck in a rigid, planar conformation can typically only be seen if the molecule exists the conformations shown below and only as a small coupling constant (2-3 Hz).

Figure 7: Orientation for Maximum Four-Bond Phosphorus Coupling

Although these figures have been called a "w" conformation in the literature, only the figure on the right actually exists in a "w" conformation. The figure on the left, although not actually a "w" conformation when viewed three-dimensionally, does appear in the literature to exhibit four-bond coupling with the phosphorus.[17,18]

Because of the great complexity of the ifosfamide structure due to its multiple couplings, a heteronuclear multiple quantum coherence (HMQC) was conducted to supplement the ^{1}H and ^{13}C NMR results. An HMQC is a 2-dimensional inverse H-C correlation technique that provides information for determination of carbon (or other spin ½ heteroatom)-hydrogen one bond connectivity.[19] The data from all three tests was summarized in the chart on the next page (*Table 1)*, using the numbering system shown on the ifosfamide (*Figure 8*), depicted in its more natural cyclohexane form, the chair structure, to help assist in the interpretation of hydrogen and phosphorus couplings.

[17] Nelson, 2003.
[18] Kainosho, 1713.
[19] Nelson, 2003.

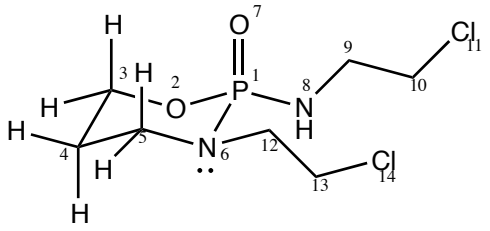


Figure 8: Ifosfamide in chair form and numbered (arbitrary numbering)

Number on Diagram	δ_H mult. (*J*, Hz)	δ_C
3	4.23 dt (16.8, 7.4)	67.889
4	a 1.84-1.88 m b 1.90-1.96 m	20.375
5, 9, 12	3.17-3.34 m	50.964, 48.404, 43.970
8	2.86 b	n/a
10	3.63 t (8.6)	40.404
13	a 3.70 app. dt	42.973

Table 1: Summary of HMQC and NMR Data for Ifosfamide

The large coupling constant from the doublet at position 3 (16.8 Hz) is a result of the hydrogen's three-bond coupling to phosphorus. As can been seen at position 10, however, where the phosphorus is at a position four bonds away (and not in a "W confirmation"), there is no phosphorus coupling. Unlike the clean signals for the hydrogens at positions 3 and 10, however, the remaining hydrogens in the molecule show more complex ^{1}H NMR signals.

At position 4, the two hydrogens are no longer identical and they give two signals next to each other that appear to be mirror image multiplets of one another (seen below in the bottom left blown in picture in *figure 9, E*), coupling with one another ($^{2}J_{HH}$ coupling)

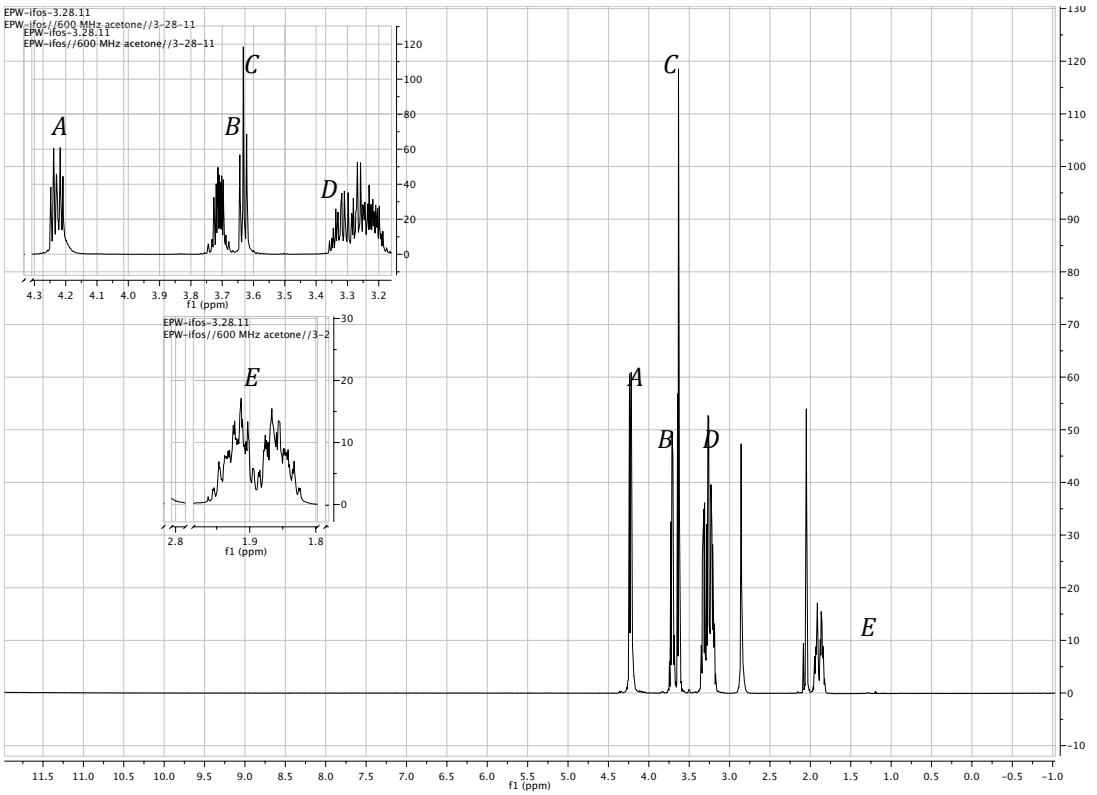


Figure 9: Ifosfamide ^{1}H NMR Spectra with characteristic sections expanded in upper left

Although the hydrogen at the equatorial position does lie in the characteristic confirmation for four-bond coupling with the phosphorus, the existence of the four-bond phosphorus coupling cannot be confirmed or denied due to the complexity of the coupling pattern. All three positions next to the nitrogen (5,9, and 12) resonate so closely on the spectra that they appear as one large complex multiplet whose individual signals could not be differentiated. We suggest that increased rigidity of the alkene chain attached to the nitrogen in the ring (12-14) creates diastereotopic hydrogens for carbons in this chain that do not exist in the other nitrogen chain (8-11).

The coupling at the carbon numbered 13 was particularly difficult to characterize, and it was determined that the two hydrogen atoms exist in magnetic nonequivalence. What this means is that because the two hydrogen atoms are diastereotopic, they do not show up as the same peak on an NMR spectra, however their chemical shifts are so nearly chemically equivalent that they exhibit a unique symmetry effect. As a consequence of their magnetic nonequivalence, these two hydrogen signals, which if accurately each, as we suggest, a doublet of triplets (the doublet from the coupling with the other hydrogen and the triplet from the coupling with the adjacent hydrogens on carbon 12) should theoretically show only ten lines where a standard two hydrogens that each give a signal of doublet of triplets would show a total of 12 lines. This apparent image of magnetic nonequivalence was observed in the 600 MHz ^{1}H NMR spectra shown below (see *figure 10)* on the left-hand side.

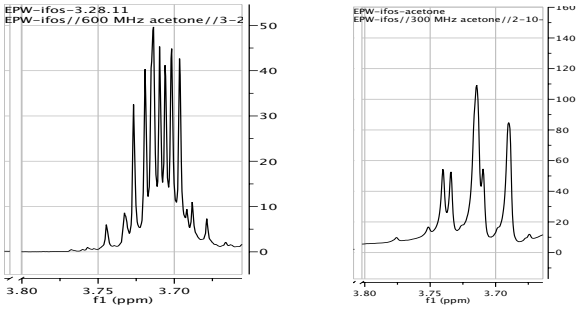


Figure 10: NMR of Magnetic Nonequivalence hydrogens seen on 600 MHz (right) and 400 MHz (left)

This phenomenon of magnetic nonequivalence is further verified by the fact that when the frequency was shifted from 600MHz to 400 MHz to 300 MHz respectively, the multiplet began to look more and more like a pure doublet of triplets, as can be seen in the figure above when comparing the 600 MHz picture (on the left) to the 300 MMHZ signal (to the right)[20].

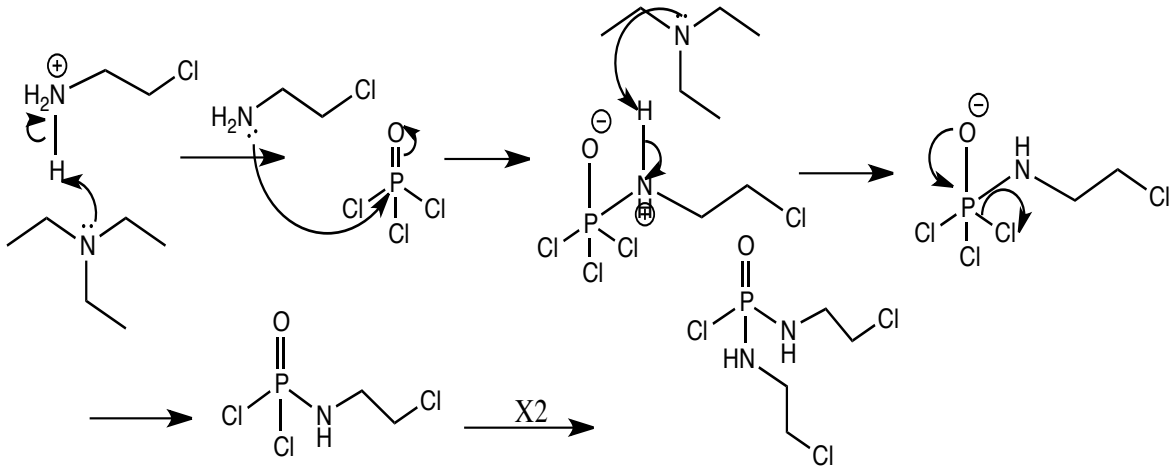
[20] Crews, Phillip, Jaime Rodriguez, and Marcel Jaspers. Organic Structure Analysis. New York: Oxford University Press, 2010.

2.2 Addition of two equivalents of chloroethylamine salts

The initial step of the ifosfamide analog synthesis was developed referencing a similar synthesis proposed in a literature procedure[21]. Two chloroethylamine groups were attached to the phosphorus (via the nitrogen). The substance appeared as a mixture of white crystals and a light yellowish-brown oil. The solution vacuum filtrated and then washed with ice-cold saturated sodium chloride to remove the ammonium salts. The solvent was mixed with toluene (which has a boiling point of 110.6 ^{o}C) before removal to ensure that all of the $POCl_3$ (which has a boiling point of 105-110^{o}C) and triethylamine (with a boiling point of 89-90 ^{o}C) was removed in the process, as dichloromethane, with a boiling point of only 40 ^{o}C, would be removed at a much more rapid rate and would potentially leave behind much greater concentrations of amine and excess starting materials. The resulting structure was confirmed with ^{1}H, ^{13}C and ^{31}P NMR spectra and mass spectrometry results (*see experimental section).* NMRs were taken of both the solid and the oil product independently to determine that they produced the same NMR signals.

[21] Glazier, Arnold. "Tumor Protease Activated Prodrugs of Phosphoramide Mustard Analogs." European Patent Office (espacenet database) **24 October 1996** , WO9633198.

The mechanism for the conversion to [1] can be seen in following scheme.



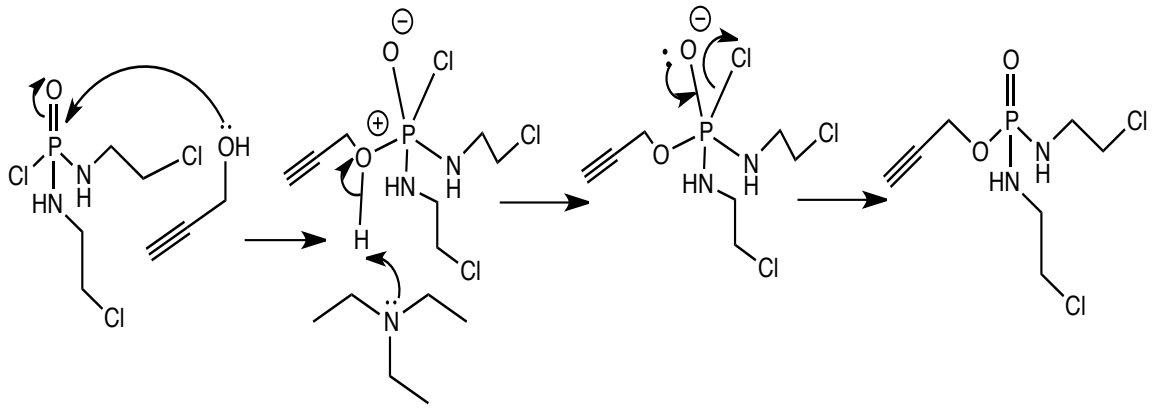
Scheme 5: Mechanism for synthesizing of N,N'-Bis(2-chloro-2-ethyl)phosphorodiamidic chloride [1]

2.3 Addition of propargyl alcohol

The second reaction synthesis procedure referenced the same literature as the first step[22], except that excess propargyl alcohol was used (4 equivalents) instead of the recommended one equivalent of alcohol. This excess was used due to the inexpensive and easily accessible nature of propargyl alcohol to ensure that all of the substrate would receive the added propargylic ester component (necessary for the final cyclization step). The oxygen of the alcohol group replaced the final chlorine on the phosphorus, forming a

[22] Glazier, WO9633198.

brown oily substance (*mechanism shown in scheme 5*).



Scheme 6: Mechanism for synthesis of N,N'-Bis(2-chloro-2-ethyl)phosphorodiamidicpropargylic ester [2]

The solution was washed with ice-cold saturated sodium chloride to remove the ammonium salts. It was then washed sequentially with a 10% citric acid solution to remove the base and saturated sodium bicarbonate to neutralize the solution. This step was not completed in the first step to prevent hydrolysis of the chlorine bonds. The solution was here too mixed with toluene before removal of the solvent under reduced pressure, not only to remove the triethylamine, as in the first step, but also to remove the excess propargyl alcohol (which has a boiling point of 114-115 ^{o}C , slightly higher than that of toluene). A greater purity of substance was obtained when no work-up was conducted in between the first two steps (see section *2.5 Phosphorus NMR Data as Used to Investigate Cyclization Starting Material [2] Side Products*). The resulting structure was confirmed with ^{1}H, ^{13}C and ^{31}P NMR images and mass spectrometry results (*see experimental section).*

2.4 Flash Chromatography and TLC (Thin-Layer Chromatography) difficulties

Neither product of the first and second step initially chromatographed because the substances were too polar and were more strongly dissolved in the polar silica gel than any of the solvent systems initially attempted to wash them out. The product of the first reaction [1] was successfully moved from the baseline of a TLC plate (Rf=0.63) using a solvent system of 9:1 dichloromethane: methanol, however when the system was chromatographed, no product was recovered, even after washing the silica gel with pure methanol. A small spot of the second product [2] was moved on a TLC plate using a 95:5 ratio of dichloromethane: methanol (Rf= 0.33), but a larger spot remained close to the baseline. Upon chromatography, no product or starting material was recovered. We suggest that perhaps methanol is too polar of a solvent to use during chromatography, as it too appeared to get absorbed in the polar silica gel material. Solutions of hexane and ethyl acetate were then attempted, however the second product [2] could not be moved off of the baseline on a TLC plate, even at a 4:1 ratio of ethyl acetate: hexane. Running a TLC plate fully through a 1:9 triethylamine: dichloromethane solution prior to TLC analysis was also attempted for purposes of neutralizing the acidic sites on the silica plate that could potentially be interacting with the amine group on the complex (and perhaps keeping the structure from moving up the silica plate). However, when the sample [2] was added to the neutralized TLC plate and run in a 4:1 solution of ethyl acetate: hexane, the sample still did not move from the baseline.

TLC analysis was further complicated by the fact that both substrates [1] and [2] when spotted on a TLC plate, placed in p-anisaldehyde, and heated showed up as a white spot (due to the phosphorus). This created greater difficulty for chromatography attempts,

as when nothing is in solution, it also appears as a white spot on the TLC plate stained with p-anisaldehyde and heated. Therefore it was impossible to determine when the substrate appeared in a solution by simply spotting the fractions during a chromatography.

All of the struggles with chromatography and TLC analysis, created difficulties in the development of the final experimental step, the cyclization of the compound to produced the desired ifosfamide analog, as there was no immediate method to determine if and when the starting material was converted to a product other than to stop the reaction at some arbitrary time, work up the solution, take an NMR, and attempt to characterize the new spectra (if there was a new spectra), for a molecule whose spectra is notably complex.

After the initial trial of the cyclization step (converting to [3]), a chromatography was attempted, referencing previous literature[23] using a 1:1 ratio of chloroform: acetone solution to determine if fractions would provide a more visible picture of any small changes that might have occurred in the spectra. After chromatography, however, no trace of starting or potentially cyclized material could be recovered, even when washed excessively with 100% acetone.

It was not until chromatography attempts were primarily abandoned and the experiment had progressed to the alternative cyclization synthesis that a paper[24] was found suggesting an unconventional, highly polar chromatography solution of 95:5 ethyl acetate: ethanol mixture. This solution was the first solution to move [2] from the baseline of the TLC plate. A chromatography was attempted for the second cyclization procedure

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[23] Hohorst, Hans-J., Gernot Peter and Robert F. Struck. "Synthesis of 4-Hydroperoxy Derivatives of Ifosfamide and Trofosfamide by Direct Ozonation and Preliminary Antitumor Evaluation in Vivo." Cancer Research **1976**, 36, 2278.

[24] Springer, James C., O. Michael Colvin, and Susan M. Ludeman. "Synthesis of (^{3}H, ^{33}P)- phosphoramide and –isophosphoramide mustards and metabolites (^{3}H)- chlroethylaziridine and –aziridine for studies of DNA alkylation." Journal of Labelled Compounds and Radiopharmaceuticals. **2007**, 50, 79.

product using initially pure ethyl acetate and then a 95:5 ethyl acetate: ethanol mixture and washing the solution with a 9:1 ethyl acetate: ethanol mixture. It appears from TLC analysis that this chromatography was able to separate something in solution, but as the NMR was too complex to characterize, it is unclear what products this chromatography was able to remove from solution.

The separation efforts were then shifted back to purifying the starting material. A solution of pure ethyl acetate was able to separate the two spots of [2] on a TLC plate, giving Rf factors of 0.2 and 0.6 respectively. Additionally, it was determined that potassium permanganate when used as a dye instead of p-anisaldehyde showed [2] as a brown substance due to the triple bond in its structure, while nothing when spotted still showed a white spot. The starting material, $POCl_3$ also displayed a white spot when dyed in potassium permanganate.. This dye, then, was used to differentiate spots where the substrate was present from spots where nothing was present during chromatography. A chromatography of [2] was attempted using initially pure ethyl acetate and then a 95:5 ethyl acetate: ethanol mixture and washing the solution with a 90:10 ethyl acetate: ethanol mixture. The substrate decomposed on the silica gel upon chromatography as shown by the more complex TLC spots post-chromatography, NMR analysis, and running a two dimensional TLC plate and having the spots curve rather than run diagonally (TLC spots that do not decompose would run straight up and then straight sideways when the TLC plate was turned on its side and run a second time).

2.5 Phosphorus NMR Data as Used to Investigate Cyclization Starting Material [2] Side Products

A ^{31}P NMR, much like that of a ^{13}C NMR, utilizes heteronuclear decoupling to remove multiplicities caused by spin-spin couplings between the phosphorus (or carbon) and other spin ½ nuclei. This effect is caused by continuous saturation of the proton spin system (as opposed to typical saturation only during acquisition) that produces an enhanced signal called the nuclear Overhauser effect. This signal can be used to provide more accurately a structural understanding of the spacing of nuclei.[25]

The phosphorus peak of ifosfamide was used as a reference point to compare the ^{31}P NMR spectra in this investigation because typical NMR solvents rarely if ever contain phosphorus atoms, and thus there are no generalized referenced points available to use in a ^{31}P spectra. As seen below (*figure 11*), the ifosfamide ^{31}P signal was referenced to 0ppm.

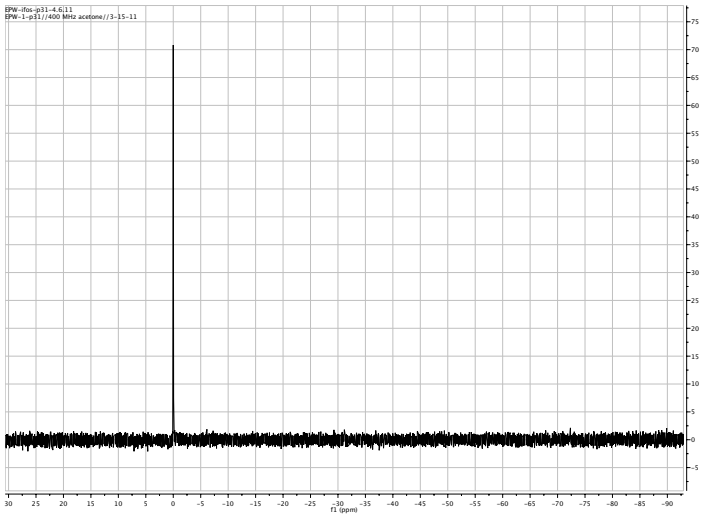


Figure 11: Ifosfamide ^{31}P NMR Spectra

Because of the proton decoupling, the phosphorus NMR structure only shows a single peak (representing the lone phosphorus in the ifosfamide molecule). Similarly, the effects of the

[25] Nelson, 2003.

heteronuclear decoupling of the synthetic materials' phosphorus NMRs allow these spectra to indicate exactly how many side products are made in each synthetic step (as any minor product would shown its own unique peak). These can then be combined with the ^{1}H NMR data to investigate what minor products are being produced.

For the formation of [2], the substrate was made through two distinct procedures. In one case, [2] was made by forming substrate [1], following the work-up procedures, and then forming substance [2] with its own work-up procedure as two separate steps. Additionally, [2] was also made by the process of forming substance [1] and then adding the reactants for the synthesis of substance [2] without removing [1] from solution (only working up the solution after both reactions were completed). These two reaction pathways resulted in different minor products and percentages.

The reaction attempted sequentially, without working up the solution in between steps, showed only two products. The phosphorus NMR shown below (to the left) also has ifosfamide in it, whose peak is the large center peak referenced to 0 ppm (parts per million).

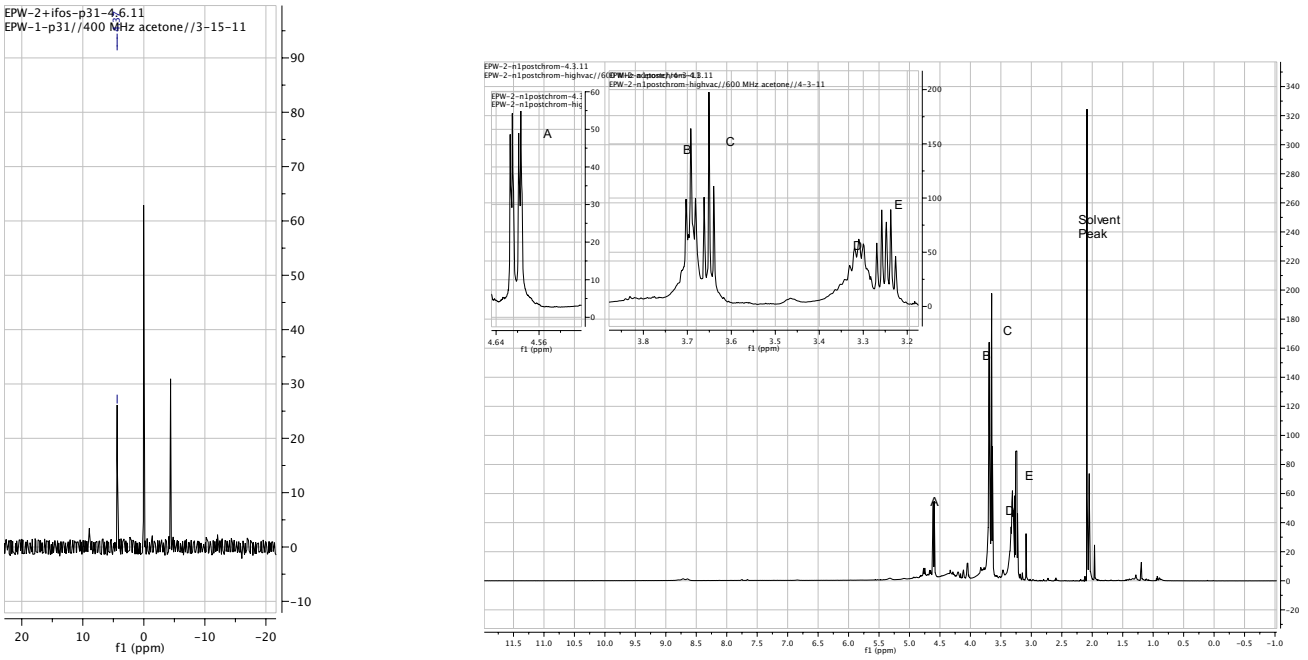


Figure 12: ^{31}P (to the left) and ^{1}H (to the right) for N,N'-Bis(2-chloro-2-ethyl)phosphorodiamidicpropargylic ester[2] made in sequential steps (no interlude work-up)

The ^{31}P spectrum shows two major products that are fairly closely aligned with few other impurities in solution. When this knowledge is compared to its ^{1}H NMR data (shown to the right) it can been seen that the two major products are the initial material [1] and the final material [2] with the added propargyl alcohol.

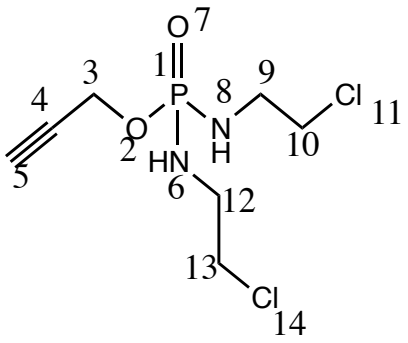


Figure 13: Numbered N,N'-Bis(2-chloro-2-ethyl)phosphorodiamidicpropargylic ester[2]

The two hydrogens at position 3 (shown above) display a doublet of doublets signal in the ^{1}H NMR (with a three bond coupling to both the hydrogen at position 5 and the phosphorus at position1) shown in the ^{1}H NMR spectra as the large peak to the far left (A). This integrates as a 1:2 ratio with the doublet of triplets on the far right of the blown up picture (E, which is positions 8 and 6) and as a 1:2 ratio to the triplet to the right of the middle section of signals on the blown up portion(C, which is positions 10 and 11). The other two peaks, which look very similar, but slightly shifted to the left (B and D) are signals of the initial starting material [1] without the propargyl alcohol. They are shifted to the left because the chlorine atom is more electronegative than the oxygen and thus causes a small downfield shift. The increased electronegativity decreases the electron density surrounding the protons, causing them to resonate at a lower field and increasing its δ_H values (shifting to the left).[26] This shifting affect can be confirmed when comparing the this ^{1}H NMR spectra to that of the original N,N'-Bis(2-chloro-2-ethyl)phosphorodiamidic chloride [1] ^{1}H NMR to shown that the peak of [1] (δ3.699 and δ3.354 respectively) are

[26] Nelson, 2003.

located at about the same position as the peaks shifting to the left in the ^{1}H NMR of [2] (δ3.69 and δ3.310)

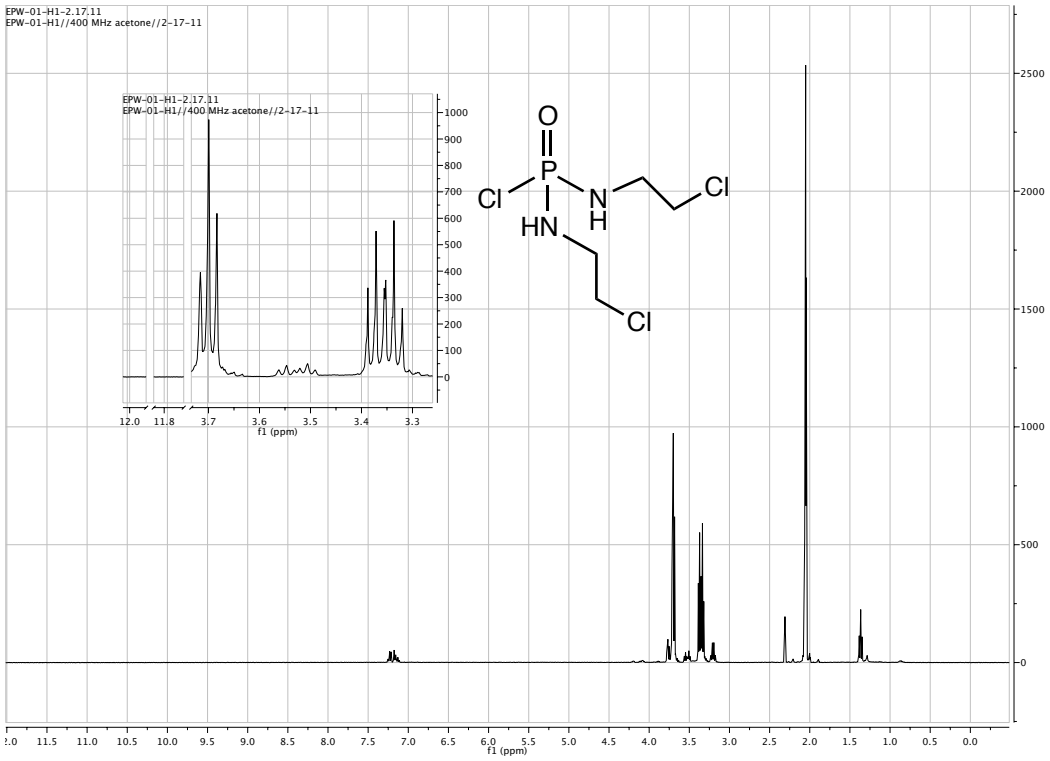


Figure 14: ^{1}H NMR of N,N'-Bis(2-chloro-2-ethyl)phosphorodiamidic chloride [1]

Revisiting the phosphorus NMR, it can now be concluded that the desired material [2] is the peak to the right of the ^{31}P spectra, for in phosphorus NMR structures, electronegative substituent cause a downfield shift (provided the phosphorus oxidation state remains the same, which it does here).[27] Thus, since the desired product has a less electronegative atom, oxygen, than the starting material, with its more electronegative chlorine atom, the starting material [1] has a downfield shift (to the left).

[27] Nelson, 2003.

When the two steps were done separately with a work-up in between them, more all the starting material [1] was converted to product [2] (*figure 15*), but there were also a greater number of minor products seen both in the ^{31}P and in the ^{1}H spectra.

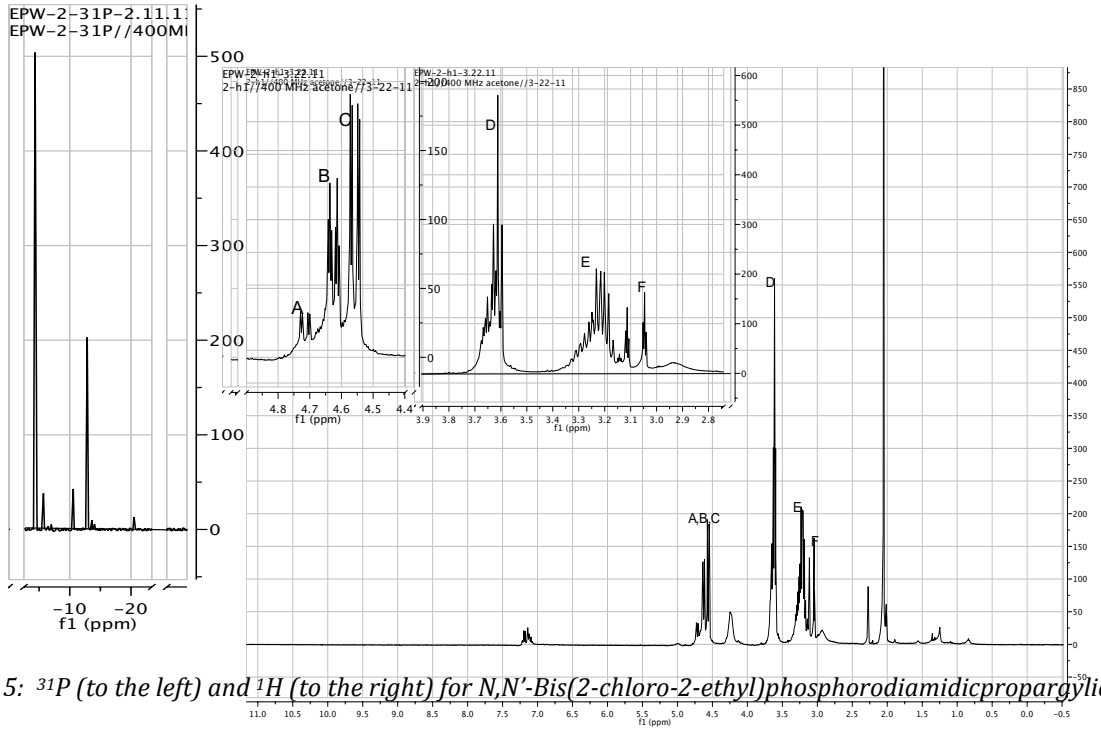


Figure 15: ^{31}P (to the left) and ^{1}H (to the right) for N,N'-Bis(2-chloro-2-ethyl)phosphorodiamidicpropargylic ester[2] made as separate steps (with individual work-ups)

This spectrum shows one major product, one significant minor product, and two smaller minor products. Based on the location of the major peak in the ^{31}P spectra, the major product appears to be [2]. The signals congruent with [2] (C, D, E) appear to be the most prominent signals in the ^{1}H spectra as well and thus supports the notion of it being the major product.

When compared with the ^{1}H NMR spectra of the previous procedure (with no intermediate work-up) there are more significant signals in this ^{1}H NMR to the left of (downwards shifted from) the propargyl alcohol hydrogens (position 3). Although these appear on cursory glance to show a similar doublet of doublets, but slightly shifted signal to the desired propargyl hydrogen (position 3) signal, the new signal is actual a doublet of triplets, rather than a doublet of doublets, and therefore is more complicated than a mere shift (*Figure 16).*

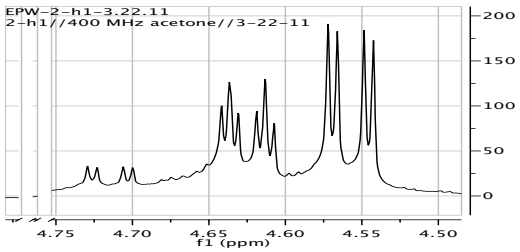


Figure 16: Downshifted ^{1}H peaks of [2] when doing the first two steps independently

Additionally, it is interesting that there are two triplets (as opposed to one in former procedure's ^{1}H NMR structure on page 29) around 3-3.1ppm (labeled F) that is the characteristic external hydrogen of the propargyl alcohol peak, suggesting perhaps that two different molecules with alcohol attached exist in the mixture of compounds. However the greater complexity of products results from this procedure has been difficult to characterize with certainty. It has been observed, however, that allowing the first procedure to run for 48 hours, as opposed to 24, created greater purity of product of [1] and perhaps because the amine salts were removed from solution at 24 hours in this procedure, there was greater complexity of starting material for this conversion that created the greater complexity of products.

2.6 Attempts at Cyclization

The initially attempted sequence was developed referencing previous experimentation[28],[29]. As this investigation contains a substrate with markedly different elements from previous explorations, it is important to explore the elements of previously verified tungsten catalyzed alkynyl cyclization experiments to determine if cyclization might be possible in this scenario. Balthaser explored oxazine formations varying solvents, tungsten molar percentages, and reaction time to obtain various percentages of the products he found (reaction scheme shown below)

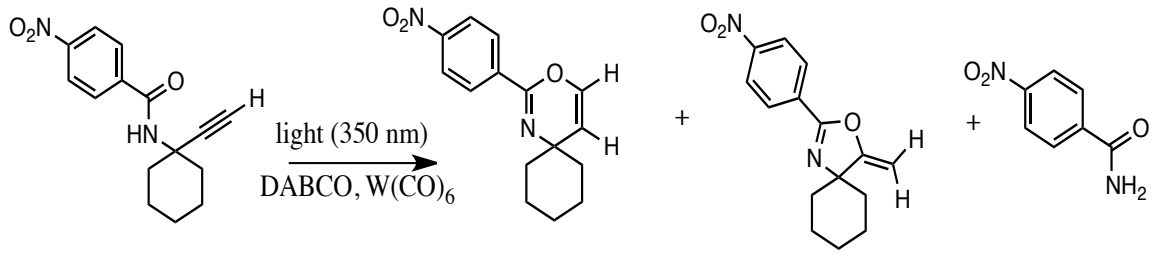


Figure 17: Balthaser's Tungsten Catalyzed Alkynyl Cyclization with a Carbonyl

[28] Balthaser, Bradley R. "Synthesis of Those Sectors of Saccharomicin A and B Containing Saccharosamine and the Discovery of Novel 4-H-1, 3-Oxazine Synthesis". Diss. Emory University, 2009.

[29] Alcazar, Eva, Joseph M. Pletcher, and Frank E. McDonald. "Synthesis of Seven-Membered Ring Glycals Via Endo-Selective Alkynol Cycloisomerization." Organic Letters. **2004**, 6 (21), 3877.

Balthaser also conducted tungsten catalyzed alkynyl cyclization reactions using alcohol instead of the former carbonyl group, as shown below (*figure 18*).

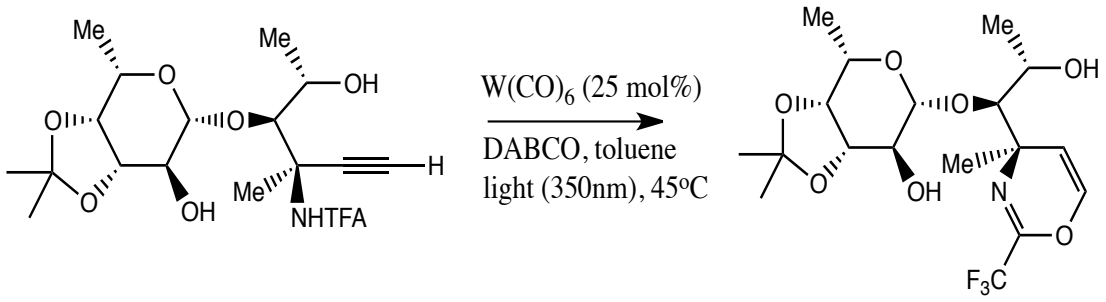
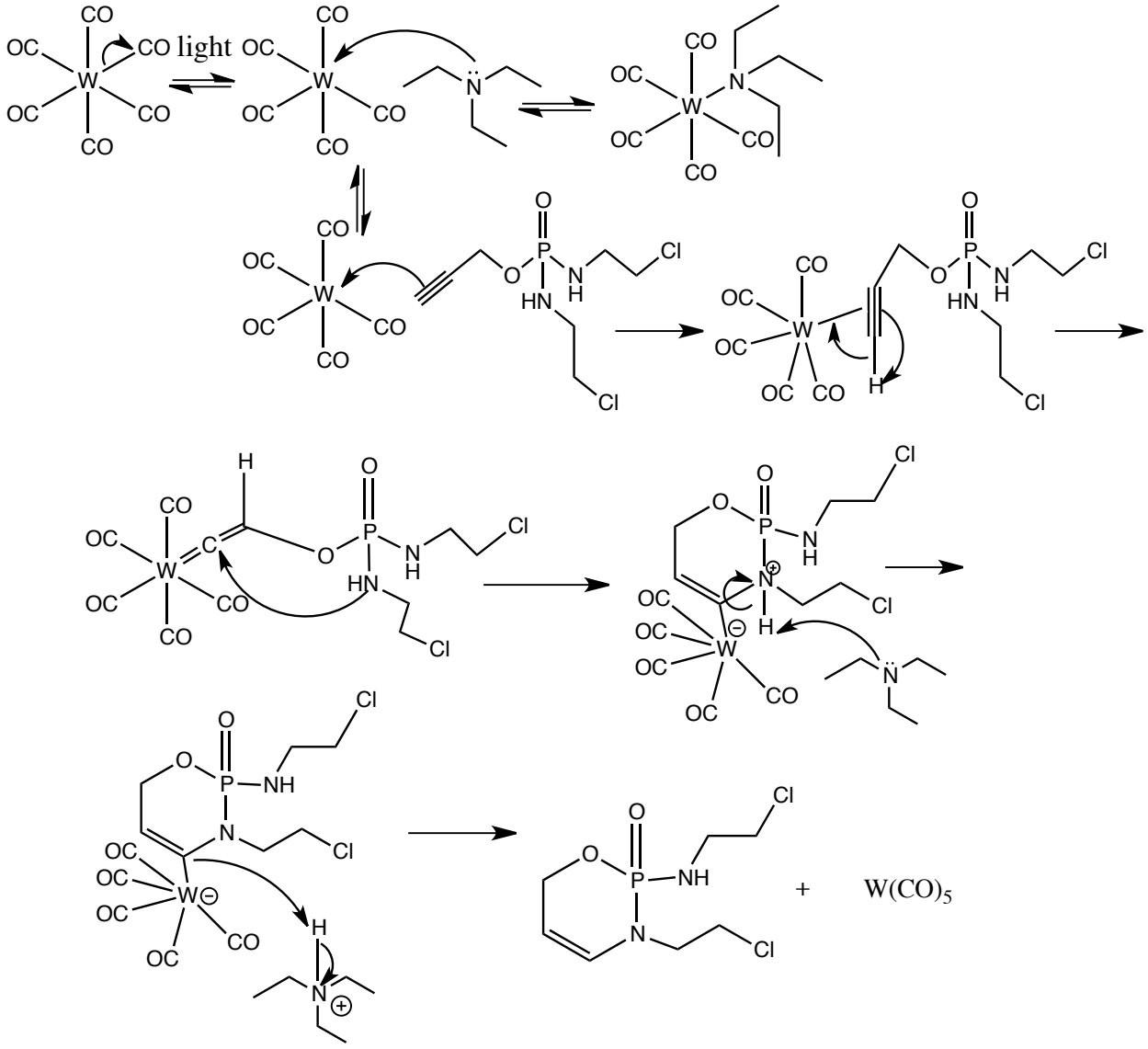


Figure 18: Balthaser's Tungsten Catalyzed Alkynyl Clclization with an Alcohol

It has been observed that this cyclization cannot be completed without some electronegative oxygen in close proximity. We suggest that it is possible that some form of hydrogen bonding between the migrating hydrogen and the electronegative atom allows the hydrogen to migrate.

The two main differences between the previously successful cyclizations and those attempted in this investigation is the substitution of the phosphorus that is double bonded to the oxygen instead of a carbon atom and that the desired product in this investigation requires the nitrogen to attack as opposed to the oxygen attack (since there is no nitrogen present in position to attack in Balthaser's examples).

The proposed mechanism for this investigation is shown in the scheme below.



Scheme 7: Proposed Mechanism for Ifosfamide Analog Cyclization (Conversion of [2] to [3])

The hexacarbonyl dissociates upon exposure to heat and irradiation into pentacarbonyl and carbon monoxide due to the carbonyl groups strong electronegativity. This dissociation, however, converts the tungsten into a more unstable 16-electron complex (as opposed to its former, stable 18 electron count molecule). This pentacarbonyl complex can then conjugate with either the base (shown in the mechanism as triethylamine) or the

substrate to reform its stable 18-electron count complex. The important step in this synthesis necessary for the cyclization to proceed is the migration of the external hydrogen to the internal position (the arrows in the mechanism are drawn arbitrarily to show where the hydrogen moves, and not necessarily the exact method for how it does so).

The seemingly small change of the phosphorus atom rather than a carbon atom can have large consequences on the reactivity of the compound. An important factor to consider is that phosphorus- oxygen bonds are much stronger than carbon-oxygen bounds. This may result in less of the electrons resonating into the oxygen (a more equal sharing of electrons) in the phosphorus-oxygen bond than occurs in a carbon-oxygen bond, which might cause problems for the important step of the migration of the hydrogen from the external to the internal position.

The desired product for this investigation, however, is not the only product that we suggest can theoretically occur. Due to the basic nitrogen and oxygen in solution, it is possible that either one can attack to cyclize the ring and form alternative products (shown below).

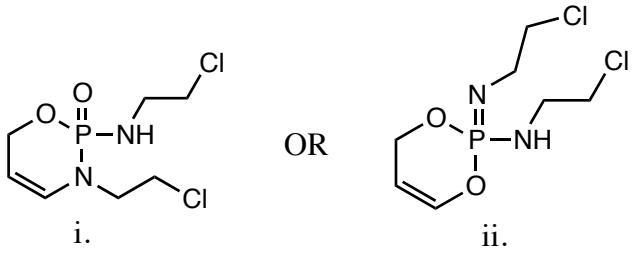


Figure 19: Possible six-membered ring cyclization possible products via (i) nitrogen or (ii) oxygen attack.

Similarly, the nucleophile can attack the tungsten complex at the internal rather than the external position, forming five membered rings (as shown below).

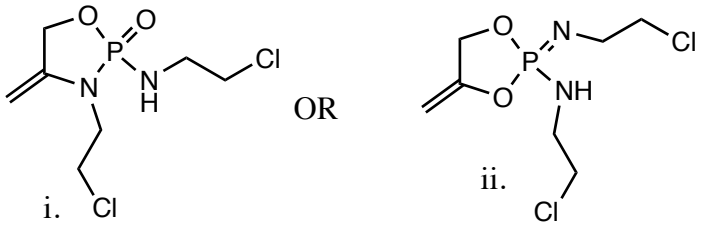


Figure 20: Possible five-membered ring cyclization products via (i) nitrogen or (ii) oxygen attack.

It is the expectation that the nitrogen will be the greater nucleophilic atom in this reaction because the nitrogen is more basic than the oxygen and because in order for either ring to form via the oxygen, the oxygen-phosphorus double bond has to convert to an oxygen-phosphorus single bond, which is unlikely considering the great affinity and stability of phosphorus-oxygen bonds. However, all the previously literature encountered has not reported successful results using this tungsten catalyzed cyclization reaction without some oxygen atom present (either an alcohol or a carbonyl group), and we suggests that it is the oxygen-hydrogen interaction that allows for the necessary migration of the hydrogen. If the conditions were such that they are kinetic, rather than thermodynamic condition, then it is possible that it would be more likely that the cyclization occurs via the oxygen.

Previous investigations of other tungsten-catalyzed cyclizations[30],[31] using a triple bond suggest that adjustment of conditions, such as base, solvent, and temperature could maximize certain products.[32] A control experiment was run using all the materials except the substrate to observe results. A bright yellow solid was easily dissolved and the NMR showed that it was primarily DABCO salt, but a dark brown solid could not be removed

[30] Balthaser, 2009.
[31] Alcazar, 3877.
[32] Balthaser, 2009.

from the flask, as it would not dissolve in any deuterated solvent attempted, even after placing the sample in a Cole Parmer ultrasonic cleaner (a machine that uses ultrasound waves, 50-60 Hz, typically to clean glassware, but it can also be beneficial for dissolving solid into the desired solvent). Some data from initial results attempts are shown below.

	Trial 1	Trial 2	Trial 3	CONTROL (No substrate)
$W(CO)_6$ Mol %	49 mol % (0.0688 g, 0.196 mmol, 0.49 equiv)	45 mol % (0.0818 g, 00.232 mmol, 0.45 equiv)	41 mol % (0.0636 g, 0.181 mmol, 0.41 equiv)	50 mol % (0.0688 g, 0.196 mmol, 0.50 equiv)
Base Used	DABCO	DABCO	DABCO	DABCO
Solvent Used	Toluene	Toluene	Toluene	Toluene
Reaction Time (hr)	1	2.5	5	2
Work-up	Rotavapor	Rotavapor	Rotavapor	Rotavapor
NMR Solvent	Acetone	Acetone	Acetone	$CDCl_3$
Starting Material present in NMR	Yes	Yes	Yes	Visible DABCO peak and a few impurities. Dark solid could not be removed from flask using any solvent.
Trace of Cyclized material?	No	No	No	N/A

Table 2: First Round of Cyclization Attempts

After the first few trials, it was observed that the starting material dissolved more completely in tetrahydrofuran (THF) than toluene, so the solvents was changed. Initial testing used a work-up of the solution that solely removed the solvent with a rotavapor under reduced pressure. Some solid was dissolved in acetone and an NMR could be taken, but there was always some dark substance that could not be removed from the flask, as it would not dissolve in any of the solvents attempted. From the previous control experiment, it was suspected that the tungsten was present in the undissolved solid component, and perhaps some final product could be coordinated to the tungsten, and therefore unable to come out of the flask. Because of this issue, the base was switched to triethylamine in efforts to decrease the amount of unwanted solid in the final product (see *Table 3*), as triethylamine can easily be removed with the solvent under reduced pressure.

	Trial 4	EXCESSIVE BASE TEST	Trial 5	Trial 6 (Aliquot 1)	Trial 6 (Aliquot 2)	Trial 6 (Aliquot 3)	Trial 6 (Aliquot 4)
W(CO)6 Mol %	45 mol % (0.0646 g, 0.1824 mmol, 0.45 equiv)	46 mol % (0.0664 g, 0.189 mmol, 0.46 equiv)	94 mol % (0.1334 g, 0.379 mmol, 0.94 equiv)	95 mol % (0.1360 g, 0.386 mmol, 0.95 equiv)	95 mol % (0.1360 g, 0.386 mmol, 0.95 equiv)	95 mol % (0.1360 g, 0.386 mmol, 0.95 equiv)	95 mol % (0.1360 g, 0.386 mmol, 0.95 equiv)
Base Used	DABCO	Et_3N	Et_3N	Et_3N	Et_3N	Et_3N	Et_3N
Solvent Used	THF	THF	THF	THF	THF	THF	THF
Reaction Time (hr)	3	6	3	6	6	6	6
Work-up	Stirred overnight with excess base then rotavaped	Rotavaped	Stirred overnight with excess base. Filtered through short plug of celite with ethyl acetate. Rotavaped	Stirred overnight with excess base. Filtered through short plug of celite with ethyl acetate. Rotavaped	Stirred overnight with excessive base. Diluted with excess Ethyl acetate. Filter through short plug of celite with ethyl acetate. Rotavapor	Stirred overnight with excessive base. Diluted with excess Ethyl acetate. Filter through short plug of celite with ethyl acetate. Filtered again with methanol. Rotavapor	Stirred overnight with excessive base. Diluted with excess Ethyl acetate. Filter through short plug of celite with ethyl acetate. Filtered again with methanol. Rotavapor
NMR Solvent	Acetone	Acetone	Acetone	Methanol (CD_3OD)	Acetone	Acetone	Di-methyl sulfoxide (DMSO)
Starting Material present in NMR	Yes	Yes	Yes	No	No	No	No
Trace of Cyclized Material	No	No	No	No	No	No	No

Table 3: Second Round of Cyclization Attempts with THF

A test trial was run with excessive base for 6 hours and showed only starting material, possibly indicating that excessive base competes with the substrate for coordination with the tungsten. Thus, we tried to use excessive base as a means of uncoordinating the substrate from the tungsten. As excessive base continued to show no product, further work-up procedures were attempted. It was attempted to remove the tungsten by flash chromatography in a Pasteur pipette using celite as the stationary phase. As was shown in trial 5, the starting material, when uncoordinated with the tungsten, did come through celite. After six hours of irradiation, mixing the solution overnight with excess base, and removing the tungsten with celite, no starting material or product remained. This indicated that either the starting material and/or product was entirely coordinated to the tungsten and thus could not pass through the celite, that all product was formed that could not pass through or was absorbed in the celite, or that somehow the starting material disintegrates from prolonged exposure to heat and radiation. Attempts at washing the celite with different solvents proved futile, as no starting material or product could be observed from any NMRs taken of any experiment run for six hours and filtered through celite.

Due to the lack of success with the above procedure, an alternative route was attempted for cyclization following procedures from literature[33], a method that entirely removing the base from the cyclization procedure. The experiment was also conducted in a much more strongly dilute solution. In its former investigations, this procedure isolated a carbene structure of cyclized product coordinated to the tungsten complex. The theoretical ifosfamide carbene structure using this procedure is shown below (*Figure 21)*.

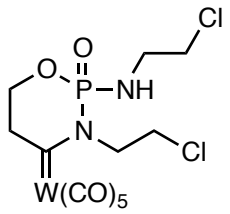


Figure 21: Carbene Produce of alternate procedure

Aliquots of the solution were removed and investigated and many time intervals, however because there was so much in the solution, the crude spectral data could not be characterized. The large amount of solid that could not be dissolved also contributed to the difficulty in getting clear spectral data. Upon freezing the large solution, a white crystal structure crashed out of solution. With NMR analysis, it was determined that the solid (which could only be dissolved in acetone after placed in the ultrasonic cleaner) was a tungsten-THF complex. The remaining solution was mixed with excess base in attempts to remove the substrate from the tungsten and then solvent was removed with a rotavapor under reduced pressure. Because some literature[34] suggested that the cyclized product might be volatile if solvent was removed under vacuum (0.1 mm Hg), aliquots of solution were taken both before and after solution was placed on this vacuum to determine if any

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[33] McDonald, Frank and Jason L. Bowman. "Tungsten Carbonyl- Induced Cyclizations of Alkynyl Alcohols to Dihydropyranylidene Carbenes and α-Stannyl Dihydropyrans." Tetrahedron Letters. **1996**, 37, 4675.
[34] McDonald, 4675.

desired peaks were lost. The spectra indicated that primarily solvent peaks were removed when placed on a highly pressurized (0.1 mm Hg) vacuum, however it was difficult to be certain due to the complexity of the spectra. The solution was chromatographed in ethyl acetate- ethanol mixture, however the spectra remained too complex to characterize even after apparent separation. It was shown by TLC analysis that some substances were separated, however, none of the fractions could be identified (which could perhaps have only been further complicated by disintegration on the silica gel that has occurred throughout this investigation). ^{1}H NMRs were taken both before and after chromatographed solutions were placed on the 0.1 mm Hg pressured vacuum, yielding no greater assistance in its identification, even referencing the ranges in which the peaks have been shown to be visible in the literature material.[35]

Because of the complexity of the results and spectral data associated with this synthetic procedure, this process was abandoned for the time being and further attempts were made utilizing at the first cyclization procedure. This time, instead of increasing tungsten percentages used in efforts to ensure the cyclization occurred, minimal tungsten was used (0.05 mole percent) and the experiment followed by TLC analysis in efforts to determine the exact point when the structure began to convert to its cyclized form in hopes of getting a visible NMR structure of where peaks began to change. The experiment was run for two hours when TLC suggested perhaps that something was changing. An NMR was taken, however, it appeared that only starting material was present. The solution was left stirring overnight at room temperature and another NMR was taken, similarly showing only starting material. The substrate was then irradiated for an additional two hours, yet

[35] McDonald, 4675.

not yielding anything new in the NMR spectra. All of these NMR solutions seemed to have a significant amount of solid more so than the minimal amounts of tungsten solid that was used in this reaction. The d_6-acetone solvent was removed under reduced pressure and the product was dissolved completely in DMSO. There were some peaks that appeared in the alkene region that were not previously seen in the starting material NMR in d_6-acetone, however when a sample of the starting material was dissolved in d_6-DMSO, these alkene region peaks appeared as something characteristic of the starting material, rather than a result of cyclization. This phenomenon was quite strange considering that the starting material appeared to dissolve completely in acetone, and typically if everything is dissolved in solution, the spectra should show all peaks. Although indiscernible by NMR analysis what the significant amount of solid was, it is possible, as seen in prior investigation, that the solid substance was a tungsten-THF complex.

The DMSO spectra showed more impurities than could be seen in the acetone, so chromatography and TLC analysis was again attempted for [2]. Attempts to form entirely pure starting material with which to further attempt the cyclization were unsuccessful. However, referencing the ^{31}P data, it may be possible to follow the reaction to completion (using TLC analysis, since the two products show different spots on a 95:5 ethyl acetate: ethanol TLC plate and confirming aliquots on ^{31}P NMR prior to stopping the reaction) without the use of chromatography.

3. Conclusions and Future Research

The polarity of the desired ifosfamide analog and the synthetic material created in the process of obtaining that analog created great difficulty in the progress of this investigation. The additional phosphorus coupling provided unique and complex data that was both helpful and problematic as the research progressed. As such a tungsten-catalyzed cyclization using a phosphorus- oxygen bond has yet to be confirmed in the literature, it was difficult to determine proper procedures and conditions that might allow for this conversion. Much knowledge was attained about the complex phosphorus couplings and phosphorus NMR spectra used throughout this investigation, but work towards the creation of the desired product is still underway.

For future investigations, it has been shown that coordinating phosphorus to a transition metal has been shown to bring about a low-field shift in the phosphorus resonance structure called a coordinating chemical shift. However, if the phosphorus contains electronegative electron donating groups, such as oxygen, nitrogen, and chlorine, coordination to transition metal often causes an upfield shift in a phosphorus resonance structure.[36] It would be interesting to try to follow the cyclization by ^{31}P NMR analysis, since there are much less peaks in that spectra than the ^{1}H spectra, to see if that would give a clearer representation of a cyclized product being formed.

Furthermore, if we return to using DABCO as the base, as it has been shown to give a greater percentage of the six membered ring than the five[37], we would add the workup to

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[36] Nelson, 2003.

[37] B Balthaser, 2009.

remove the salt referencing literature[38] of similar cyclization product that dissolved the crude product in dichloromethane, washed it with saturated ammonium chloride, and extracted the aqueous layer. The aqueous layer was dried with magnesium sulfate and after filtration, the volatiles were evaporated under reduced pressure.

Additionally, a previous investigation has discovered a method of tungsten catalyzed cycloisomerization without the use of irradiation, using a tungsten Fischer carbene precatalyst, and perhaps these conditions are more suitable for the conversion of this molecule into its cyclized form.[39]

Due to the difficulty in chromatographic procedures using these extremely polar compounds, a reverse-phase chromatography would be another alternative for separation. A reverse phase chromatography uses Sephadex for the mobile phase instead of silica gel. Sephadex is composed of macroscopic beads synthetically derived from polysaccharide dextran. These organic chains are cross-linked to give a three dimensional network with functional ionic groups attached and ether linkages to glucose. It uses mixtures of water or aqueous buffers and organic solvents to elute analytes from reverse phased column (commonly acetonitrile, methanol or THF) and the most polar compounds are eluted from the column first.[40]

Finally, using less complex phosphorus starting materials or less polar starting materials to test the cyclization activity of a molecule with a phosphorus-oxygen bond (rather than the carbon-oxygen bond that past literature has confirmed) would help

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[38] Boone, Matthew and Frank E McDonald. "Compounds, intermediates, and methods of preparing the same." European Patent Office: Espacenet. **7 July 2010,** WO 2010078396.

[39] Koo, BonSuk and Frank E. McDonald. "Fisher Carbene Catalysis of Alkynol Cycloisomerization: Application to the Synthesis of the Altromycin B Disaccharide." Organic Letters, **2007**, 9 (9), 1737.

[40] Beijer, K. and E. Nystrom. "Reversed-phase chromatography of fatty acids on hydrophobic Sephadex." Analytical Biochemistry. **Jul 1972**, 48(1), 1.

identify what conditions allows this cyclization to occur and which products seem to be the most prominent.

4. Experimental Section

4.1 General Procedures

^{1}H NMR , ^{13}C NMR, and ^{31}P NMR spectra were recorded on a Varion INOVA-400 spectrometer (400 MHz ^{1}H, 100 MHz ^{13}C) at room temperature in deuterated acetone [$CO(CD_3)_2$] with internal $CO(CH_3)_2$ as a reference (2.05 ppm for ^{1}H). Chemical shift values (δ) were reported in parts per million (ppm) and coupling constants (J values) in Hertz. Multiplicity is indicated using the following abbreviations: s= singlet, d= doublet, t= triplet, q= quartet, qn= quintet, m= multiplet, b= broad signal. High-resolution mass spectra were obtained using a Thermo Electron Corporation Finigan LTQFTMS (at the Mass Spectrometry Facility, Emory University), and we acknowledge the use of shared instrumentation provided by grants from the NIH and the NSF. Analytical thin layer chromatography (TLC) was performed on precoated glass backed silica gel plates purchased from Whatman (silica gel 60F254; 0.25 mm thickness). Visualization was accomplished with UV light or either ethanolic anisaldehyde or potassium permanganate followed by heating. Flash column chromatography was carried out with silica gel 60 (230-400 mesh ASTM) from EM science. All reactions were carried out with anhydrous solvents in oven-dried or flame-dried and argon-charged glassware. All anhydrous solvents were dried with activated 4 Å molecular sieves purchased from Sigma-Aldrich. Solvents for workup, extraction, and column chromatography were used as received from commercial suppliers. All reagents were purchased from Sigma- Aldrich and used as received unless otherwise noted. Tungsten catalysts were purchased from Sigma-Aldrich and used as received.

4.2 Synthesis of N,N'-Bis(2-chloro-2-ethyl)phosphorodiamidic chloride [1]

Chloroethylamine hydrochloride salt (2.38 g, 20.5 mmol, 3.00 equiv) and a magnetic stir bar were added quickly to an oven dried 200 mL roundbottom flask previously purged with nitrogen gas to avoid as much air exposure as possible. Phosphorus trichloride (0.597 mL, 6.6 mmol, 1.00 equiv) and 91.3 mL dichloromethane (5 M solution) were added under argon via cannula to the flask. The flask was placed in a -78^{o}C dry ice/acetone bath and triethylamine (3.62 mL, 26.1mmol, 4.00 equiv) was added over the course of four hours (under argon). After four hours, the solution was allowed to warm to room temperature and was left stirring for 48 hours at room temperature to ensure that it reached completion. The solution was vacuum filtrated and washed with ice-cold saturated sodium chloride. After extraction of the organic layer, the solution was dried with magnesium sulfate as needed. The organic layer was then rotavaped three times with toluene under reduced pressure. ^{1}H NMR (400 MHz, CD_3COCD_3) δ 3.699 (t, 1H, J=6.4 Hz), 3.354 (dt, 1H, J=14.4, 6.4 Hz); ^{13}C NMR (100 MHz, CD_3COCD_3) δ 43.950, 44.963, 45.0351; HRMS (+ ESI) calculated for $C_4H_{10}O_1N_2{}^{35}Cl_3P_1$ 237.96, found 238.96691 $[M+H]^+$.

4.3 *Synthesis of N,N'-Bis(2-chloro-2-ethyl)phosphorodiamidicpropargylic ester[2]*

Excess propargyl alcohol (1.403 mL, 24.01 mmol, 4.00 equiv) was then added to the solution in the presence of triethylamine (0.835 mL, 6.02 mL, 1.00 equiv) under argon and stirred for 24 hours. The solution was washed with ice-cold saturated sodium chloride. The solution was then washed sequentially with 10 % citric acid and saturated sodium bicarbonate. The organic layer was extracted from solution and dried with magnesium sulfate. After vacuum filtration, the organic solution was then placed on a rotavapor under reduced pressure three times under reduced pressure to remove the solvent and yield the

compound. ^{1}H NMR (400 MHz, CD_3COCD_3) δ 4.604 (dd, 1H, J= 9.6, 2.4), 3.651 (t, 2H, J= 6.6 Hz), 3.248 (dt, 2H, J=12, 6.6 Hz); ^{13}C NMR (100 MHz, CD_3COCD_3) δ 43.950, 44.963, 45.0351; ^{31}P NMR (100 MHz , CD_3COCD_3) (steps together) δ 4.296, -4.348 (steps separate) δ -4.345, -5.710, -10. 568, -12.853; HRMS (+ESI) calculated for $C_7H_{13}O_2N_2{}^{35}Cl_2P_1$ 258.01, found 259.0163 $[M+H]^+$.

4.4 Spectral Data for Ifosfamide

^{1}H NMR (600 MHz, CD_3COCD_3) δ 4.23 (dt, 2H, J=16.8, 7.4Hz), 3.70 (app. dt 2H), 3.63 (t, 2H, 8.6), 3.17-3.34 (m, 6H), 2.86 (b, 1H), 1.90-1.96 (m, 1H), 1.84-1.88 (m, 1H); ^{31}P NMR (100 MHz , CD_3COCD_3) δ 0.

4.5 Initial Attempt at Cyclization

In an oven dried Schlenk flask, the substrate (0.1 g, 0.411 mmol, 1.00 equiv) was added with 1,4-diazabicyclo[2.2.2.]octane (0.087 g, 0.775 mmol, 2.00 equiv) or triethylamine (0.107 mL, 0.775 mmol, 2.00 equiv) and a tungsten hexacarbonyl complex (using varying mol percentages) in toluene or tetrahydrofuran to make a 0.2 M solution. A condensing coil was attached to the top of the flask and the reaction was irradiated by UV light of 350nm at 60-65C for varying hours under argon (see *table 1*).

4.6 Second Cyclization Procedure

The tungsten carbonyl (0.766 g, 2.178 mmol, 5.00 equiv) was added to an oven-dried Schlenk flask fitted with a reflux condenser and purged with nitrogen gas. Tetrahydrofuran (45 mL, 0.06 M) was added via cannula and the solution was irradiated at 350 nm in a Rayonet photoreactor for five hours. The substrate was added via cannula and the solution was stirred in dark conditions for 48 hours.

4.7 Final Attempt at Cyclization

In an oven dried Schlenk flask, the substrate (0.1 g, 0.411 mmol, 1.00 equiv) was added with triethylamine (0.005 mL, 0.019 mmol, 0.10 equiv) and a tungsten hexacarbonyl complex (0.05 equiv) in toluene or tetrahydrofuran to make a 0.2 M solution. A condensing coil was attached to the top of the flask and the reaction was irradiated by UV light of 350nm at 60-65C and followed by TLC analysis for first sign of change.

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