New Methods and Reagents for Small Scale Synthesis of Phosphor Organic Compounds With Focus on the Phosphonic Acids and Their Analogues

Rikard Wärme
Title

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Abstract

The development of a synthetic method of radiolabelled methylphosphono-fluoridates on a milligram scale is presented. The aim of this method is, besides affording high yield, to choose reaction pathways and reagents so that handling and transfer of labelled toxic substances is minimised, thereby reducing the risk of exposure as much as possible. The only substituent that is stable enough to be labelled is the methyl group, directly bonded to phosphorus. A drawback when labelling the methyl group is that it requires the label to be introduced early in the synthesis since the carbon-phosphorus bond of the methyl substituent usually has to be synthesized a few steps ahead of the final product.

Two new classes of reagents for halogenation of phosphorus oxyacids have been developed. Firstly, four different analogues of α-chloroenamines and α-fluoroenamines were evaluated. Secondly, cyanuric fluoride was assessed in solution, but more importantly, as a resin-bound reagent. The reagents are evaluated for halogenation of phosphinic, phosphonic and phosphoric acids. Cyanuric fluoride is also successfully loaded on a polystyrene resin and used as a solid-phase reagent. The reagents produce high yields and low levels of impurities on a milligram scale.

Furthermore, a new method for the preparation of mono-alkylated phosphonic acids on a small scale has been developed. The new method utilises the crystal water bound to certain salts to liberate limited amounts of water in a controlled manner. Phosphonic dichlorides are in this way reacted with water to form anhydrides. The anhydride is then cleaved with an appropriate alcohol to produce mono-alkylated phosphonic acids.

Keywords

Micro scale, phosphorus oxyacids, solid phase, chlorination, fluorination, radiolabelling
This Thesis is Dedicated to My Dear Daughter

Marta
1. List of Papers


II Norlin Rikard; Juhlin Lars; Lind Per; Trogen Lars. α-Haloenamines as 
   Reagents for the Conversion of Phosphorus Oxyacids to Their 
   Halogenated Analogues. 

III Wärme Rikard; Juhlin Lars. A New Micro Scale Method for the 
   Conversion of Phosphorus Oxyacids to Their Fluorinated Analogues, 
   using Cyanuric Fluoride in Solution and on Solid Support. 
   *Phosphorus Sulphur Silicon Relat. Elem. Accepted*, **2010**.

IV Wärme Rikard, Juhlin Lars. A New One-Pot Micro Scale Synthesis of 
   Alkyl Alkylphosphonic Acids Utilizing Water Release of Inorganic 
   Salts. 
   *(Manuscript)*

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3. Abbreviations

AChe  Acetylcholinesterase
Agent Orange A fifty-fifty mixture of 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid
BC  Before Christ
Borax  Sodium tetraborate decahydrate
BPR  Berry pseudorotation
Brimstone  Sulfur, elemental
cAMP  cyclic Adenosine mono-phosphate
CBRN  Chemical, Biological, Radiological and Nuclear
CIMS  Chemical ionisation mass spectrometry
CNS  Central nervous system
CW  Chemical weapons
CWA  Chemical weapons agents
CWC  Chemical Weapons Convention
DCM  Dichloromethane
DIPEA  N,N-Diisopropyl-ethylamine
DMF  Dimethylformamide
DNA  Deoxyribonucleic acid
Ea  Energy of activation
EIMS  Electro spray ionisation mass spectrometry
FOI  Swedish Defence Research Agency/
     Totalförsvarets forskningsinstitut
h  Hours
HMPA  Hexamethyl phosphoramide
Hz  Hertz
Kg  Kilograms
MS  Mass spectrometry
Mustard gas  Bis(2-chloroethyl)sulfide
NMR  Nuclear magnetic resonance
NOE  Nuclear overhauser effect
OPCW  The Organisation for the Prohibition of
       Chemical Weapons
Phosgene  Carbonyl dichloride
ppm  Parts per million
RT  Room temperature
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<tr>
<td>ΔS</td>
<td>Entropy of activation</td>
</tr>
<tr>
<td>Sarin</td>
<td>Isopropyl methylphosphonofluoridate</td>
</tr>
<tr>
<td>Ser</td>
<td>Serine</td>
</tr>
<tr>
<td>Soman</td>
<td>Pinaocolyl methylphosphonofluoridate</td>
</tr>
<tr>
<td>T₁</td>
<td>Spin-lattice relaxation time</td>
</tr>
<tr>
<td>t₁/₂</td>
<td>The time it takes for half of the enzymes to become aged.</td>
</tr>
<tr>
<td>Tabun</td>
<td>O-ethyl N,N-diethyl phosphoramidocyanidate</td>
</tr>
<tr>
<td>TBP</td>
<td>Trigonal bipyramid</td>
</tr>
<tr>
<td>TEA</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TR</td>
<td>Turnstile rotation</td>
</tr>
<tr>
<td>UN</td>
<td>United Nations</td>
</tr>
<tr>
<td>US</td>
<td>United States of America</td>
</tr>
<tr>
<td>Vp</td>
<td>Vapour pressure</td>
</tr>
<tr>
<td>VR</td>
<td>S-2-(diethylamino)ethyl O-2-methylpropyl methylphosphonothiolate</td>
</tr>
<tr>
<td>VX</td>
<td>S-2-(diisopropylamino)ethyl O-ethyl methylphosphonothiolate</td>
</tr>
<tr>
<td>Zyklon B</td>
<td>Hydrogen cyanide</td>
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4. Introduction

It is important to get a thorough background of the subject and intention of this thesis before discussing the chemistry and research undertaken. The production and use of lethal compounds goes way back in human history, as briefly referred to in the fifth chapter. The production of highly toxic compounds is still a controversial subject and some believe that the production of such substances should always be banned. This is a matter of politics not touched upon in this thesis, why this subject is left at this stage. However, in the thesis the reasons for developing methods for the synthesis of these compounds will be clarified to the extent possible.

Today, there is still a significant risk of incidents with chemical weapons (CW), for the military as well as for the civil society. Terrorists, criminals and dictators are among the threats commonly mentioned in this context. The production of chemical weapons agents (CWA) is, at present, strictly regulated by international treaties and national laws and may only be conducted for protective purposes by approved laboratories. Today, there are 27 such facilities in 22 countries in the world and the Swedish Defence Research Agency (FOI), the Division of CBRN Defence and Security, is one of them. Protective purposes enclose several areas of research, for instance; chemical analysis, research on protective material, detection instrument research, medical research, biochemical research, toxicological research. Most of these research areas only require micrograms of CWA in each experiment. Thereby, the limiting factors for the amount that is produced frequently rise from practical considerations taken during synthesis and purification. This situation is generating a waste of resources and, consequently, money as well as exposing the chemist performing the synthesis for unnecessary risks.

During the synthesis of toxic compounds, the perspective of occupational safety is of outmost importance. Accidents might possibly lead to severe injuries or fatalities since even very small amounts are potentially life threatening. New methods allowing synthesis to be performed on a smaller scale considerably reduce the inevitable risks involved for the persons handling the substances. Preferably the compounds should be synthesized in such small amounts that lethal doses are avoided. The new method should also be safer compared to prior ones in the way it is performed, in that also
contributing to reducing the risks. This includes, for instance, less transfers of toxic compounds during work-up or avoiding pressurised equipment. Furthermore, there is a demand in some situations to rapidly produce a substance in order to use it as an analytical reference or to study its chemical properties. For instance, this is the case in the work supporting the verification regime of the Chemical Weapons Convention (CWC). Also, there are environmental and economic reasons for scaling down the chemical reactions, although not as important as the safety issues in this case. The observant reader/chemist should also note that these new methods are neither suited nor developed for large scale synthesis, which is not a coincident. For such applications, there are already methods available.
5. History

5.1. Introduction

The connection between the production and use of thousands of tons of CWA in warfare and the micro scale synthesis currently conducted is not obvious. In order to understand the reasons for performing small scale synthesis of CWA for research purposes, describing how chemicals have been used in the past is important. There is no guarantee that it will not happen again, therefore a short chapter on the history of CW will be presented.

5.2. Ancient times

For well over ten thousand years, mankind has been using chemical substances in weapons in order to increase their effectiveness. There are numerous examples, and one of the earliest ones might be when hunters in southern Africa used poison arrows during the late Stone Age (approx. 10 000 BC)\(^1\). It is, however, difficult to know whether the arrows were used in warfare and not only as a way of improving the outcome of hunting.

Some of the oldest writings on the subject are Chinese. They contain recipes for the production of primitive CW and date back to approximately 1000 BC\(^2,3\). These military records describe not only how to use these methods and substances in war, but also give account for their use\(^4\). One of the earliest unsophisticated weapons, used on several occasions, was an aerosol of lime that could be used as an incapacitating agent. Another example is the “soul hunting fog”, i.e. a smoke composed by compounds containing arsenic, mainly used for disrupting the enemy when they were digging tunnels.

As early as in the 5\(^{th}\) century BC, records prove the use of poisons in Europe to harm the enemy in warfare\(^5\). It was in ancient Greece, during the Peloponnesian War between Sparta and Athens, as besieging Spartan forces set fire to wood, tar and brimstone, causing a cloud of sulphur dioxide to drift in over the city of Plataia. It is not clear whether the tactics were invented intentionally, or if the use of tar and sulphur was only a way to make the fire more effective. Nevertheless, the walls of Plataia did not hold for the huge fire and crumbled, but the wind eventually turned and the cloud of sulphur

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dioxide drifted away. The citizens managed to endure the siege and Plataia
did not fall.

Interestingly, even the great Leonardo da Vinci did suggest an arsenic-
containing powder to be used as a CWA in the 15th century1. Every now and
then, incidents with CW have occurred throughout history and in practically
all parts of the world. In general, the use was rather small scale, relatively
unsophisticated and ineffective until World War I.

5.3. Post industrialisation

The rapid development of the chemical industry during the industrialisation
had suddenly made it possible to produce large amounts of chemicals at a
reasonable cost. Up until then, the production, transportation and
infrastructure could not easily handle large quantities of chemicals. Neither
dispersal techniques nor delivery systems were well developed before this
time period. Prior to the industrialisation, the chemicals had to be delivered
by simple means such as, for instance, fire or smoke, but the delivery
systems could at the end of the 19th century also be developed on an
industrial scale. The development of CWA as well as delivery systems was
pursued even though the very first Hague Convention of 1899 had banned
the use of “poisonous projectiles”. The effectiveness of the new weapons was
probably more important than were the legal or moral aspects of using CWA.
Artillery shells were among the first projectiles to be developed especially
for delivering toxic chemicals in warfare.

Figure 1: Aerial photo of a CW attack during World War I using gas cylinders. (Copyright,

During World War I, chemicals were first employed by the French forces
who shelled the German forces with munitions filled with an irritating agent6.
The military significance of these shells was small, but the historical impact
was enormous. These incidents gave the other forces participating in the war
a reason to start developing and using chemicals as well. Soon, literally all nations involved in the war had started programs for the production of CW, not only irritating agents. Within a year, they were all, to various degrees, using different CWA in their arsenals (Figure 1).

The history of CW was revolutionised at approximately 17:00 on the 22nd of April in 1915, when the Germans after vast preparations released approximately 150 000 kilograms of chlorine over 6.5 kilometres of the front at Ypres in Belgium⁷-⁹. The cloud of chlorine, which is heavier than air, slowly drifted by the wind and swept in over the allied lines. The allied forces were taken by surprise and lacked equipment to protect themselves from the gas that was inescapably filling the trenches. It has been estimated that, within the first ten minutes, approximately 6 000 troops died and many more were wounded. Even the Germans were taken by surprise by the devastating effect and did not have the resources to exploit the effects militarily and break the enemy lines.

Once this step had been taken, there was no going back. Soon, new and even more toxic chemicals were introduced by the fighting nations and the moral aspects were forgotten or neglected. The ways of delivering the CWA were rapidly developed and already towards the end of the first year, phosgene-filled artillery shells were used. The “King of Warfare Agents”, the mustard gas, was introduced in 1917 (Scheme 1). However, already in 1860 mustard gas was prepared by Frederick Guthrie. He noticed the blistering effect it had on his hands but he had probably not foreseen the possibilities of its use¹⁰.

![Scheme 1: The chemical structure of mustard gas, the chemical agent that was used in vast amounts during World War I.](image)

The mustard gas is a severe blistering agent which attacks eyes, skin and lungs. Even though mustard gas is not directly killing the victims, many of the casualties later die as a consequence of complications. The mustard gas has a long persistency as well as a delayed effect lasting for hours, properties that made it very effective as a CWA.

By the end of World War I, the casualties due to CW had well passed one million including 100 000 deceased⁹. The horrific losses and the mutilations of soldiers made the public opinion against CW grow strong resulting in the Geneva Protocol in 1925 prohibiting the use of chemicals in war¹¹. However, producing and stockpiling was still allowed and pursued by many nations, often said to be needed as means of retaliation.
5.4. World War II and up until today

The next mayor step in the history of CW was not taken until on the day before Christmas Eve in 1936 when the nerve agents were discovered\textsuperscript{12,13}. It was Dr Schrader, working at a German chemical company called I.G. Farben, when conducting research on insecticides, who discovered the nerve agent tabun (Scheme 2). However, tabun was not a suitable insecticide since it could kill the farmer just as well. During this time, discoveries of possible interest to the armed forces, for instance the discovery of the nerve agents, were immediately reported to the military.

\begin{center}
\textbf{Scheme 2:} The chemical structure of tabun, the first nerve agent to be discovered.
\end{center}

Tabun was soon produced on a large scale for military use and filled into artillery shells and bombs. Also, the Germans did discover two more nerve agents before the end of World War II which were even more potent than tabun. Fortunately, the Germans did not know that they were the only forces having knowledge of nerve agents\textsuperscript{13}. Although they had more than 10 000 tons of nerve agents in weapons ready to use towards the end of the war, they did not dare to use them. During a testimony after the war, Albert Speer said that this was mainly due to fear of a massive retaliation, but it has been speculated that since Hitler himself was a victim of CW during World War I, he disapproved of this method of warfare\textsuperscript{12}.

The German national socialists did, however, use chemical agents for killing people in vast amounts during World War II, but never on the battlefield. The holocaust is probably the most repulsive example of use of chemicals for killing humans. The chemical that has become synonymous with the use in concentration camps is hydrogen cyanide, or Zyklon B as it was denounced by the national socialists. Hydrogen cyanide inhibits the oxygen exchange on a cellular level which causes immediate suffocation.
After the war the conquering nations took samples of the agents and transferred them to their own countries for research. The research was so successful that it resulted in the development of yet another class of nerve agents, both more toxic as well as more stable. VX was invented in 1952 by the British and is still today one of the most toxic compounds made by man (Scheme 3)\(^1\). Theoretically, only 20 kg is enough to eliminate the whole population of Sweden, but in reality, the payload of a single bomber could cover an area of roughly 300 km\(^2\).\(^8,13\). The United States (US) had until recently approximately 20 000 tons stored ready for use and Russia approximately 30 000 tons of their version of VX, Russian-VX (VR). Both countries have started to destroy their stocks of VX, but not all of them have been destructed yet\(^11\). Fortunately, to date, V agents have not yet been used in warfare.

**Figure 2:** U.S. aircrafts on a defoliant spray round during the Vietnam War in 1962-71 (Copyright, National Museum of the U.S. Air Force).
One of the largest uses of chemicals in warfare was the US usage of defoliants during the war in Vietnam in 1962-1971. Approximately 80 million litres of defoliants were sprayed over the forests to deprive the Vietnamese soldiers of their hiding places (Figure 2). The defoliant agent that became best known to the public was “Agent Orange” as it was named due to the colour of the barrels in which it was shipped (Scheme 4). However, there were several other agents used as well, although not to the same extent. The military benefit has been questioned and the public opinion in the US finally grew too strong and the spraying was eventually stopped. The use of such chemicals in warfare was not prohibited and banned at the time, and is still today not covered by the CWC.

Iraq became the first nation to deploy nerve agents, in an actual war and in a combat situation, during the war against Iran in 1983-1988. The most well-known incident was the Iraqi attack on the domestic village of Halabja in 1988 where 5000 citizens were killed, mostly civilian Iraqi Kurds. There is a report issued by the Iran Photo Foundation which describes the situation in the village shortly after the attack.

On a later occasion, nerve agents were used by the Japanese sect Aum Shinrikyo for yet another purpose. After this incident, CWA had for the first time been used in terrorism thus expanding the horizon of misuse even further. The sect’s attack in the Tokyo subway station was fortunately not well executed, and the effects not as horrific as they could have been. Still, twelve persons died immediately and many thousands were injured, some crippled for life.

Scheme 4: The chemical structure of the two ingredients of Agent Orange, 2,4-dichlorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid. The formula contained a fifty-fifty mixture of the two substances.
6. The Chemical Weapons Convention

6.1. Introduction

A more positive date than the ones mentioned so far is the 29th of April, 1997, when the Chemical Weapons Convention (CWC) entered into force. The full name of the treaty is “The Convention on the Prohibition of the Development, Production, Stockpiling and Use of Chemical Weapons and on Their Destruction”. It is the first multilateral disarmament agreement covering all aspects of CW and it took almost 20 years of negotiations to finalise the treaty. Today, all research concerning CWA is regulated through the CWC, in most countries. In fact, some of the research is directly contributing in upholding the treaty. Therefore, a brief discussion on the CWC will be addressed in this chapter.

6.2. Overview of the convention

The first attempt to prohibit CW dates back to 1675 when Germany and France agreed not to use poison bullets. Again, in the Brussels Convention of 1874, the "employment of poison or poisoned weapons" is condemned. Unfortunately, the Brussels Convention never entered into force. Also, the Hague Convention of 1899 was trying to ban the use of new technologies in war, and the use of CW was one of them. The Hague Convention of 1899 was followed by the 1925 Geneva Protocol which came in the wake of World War I and of the massive use of CWA and the casualties they caused. However, the Geneva Protocol did not prohibit the development, production or stockpiling of CW, simply their use in war. Another aspect that even further weakened the treaty was that many countries signed the protocol with the reservation that they would retaliate chemical attacks using the same means. Unfortunately, none of the treaties above were effective in abolishing the CW.

Finally, at the end of the 20th century, the military significance of CW had become marginalized and it was politically achievable to negotiate a disarmament treaty. Development of better protective equipment for the troops, together with new conventional weapons that were becoming so efficient that the use of CW was more or less outdated, certainly contributed to the progress, not only the moral aspects.
The CWC defines CW in much more general terms than earlier treaties such as the 1925 Geneva Protocol. According to CWC, CW are defined as “toxic chemicals and their precursors, except when intended for use not prohibited by the convention”. Also, “munitions and devices specially designed” for release of toxic chemicals are prohibited. The CWC was much more comprehensive than earlier attempts to prohibit the CW. However, the real difference from previous treaties is that a regime to monitor the implementation of the CWC was established.

Figure 3: A map of the countries that have signed and ratified the CWC (in green) and those who have not (in brown or yellow) (Copyright, OPCW).

The heart of the new convention was the establishment of a new organisation to “achieve the object and purpose of the Chemical Weapons Convention”\textsuperscript{11}. The organisation was named “The Organisation of the Prohibition of Chemical Weapons” (OPCW) and is independent of, although having a relationship agreement with, the United Nations (UN). The organisation, situated in The Hague in the Netherlands, was given the authority as well as the means to inspect that the treaty is fully implemented among the member states. This includes the inspection teams that can be sent to various sites to verify the type of production or handling of chemicals. For instance, industry producing chemicals possible to use as precursors for CWA and plants producing discrete organic chemicals (DOCS) can be subject to inspections, should the production exceed certain limits. Also, declared facilities for small scale production of CWA, for protective purposes, are inspected regularly. These systematic inspections can be executed without informing the facility and the country to be inspected until 48 hours in advance. Hence, the Swedish Defence Research Agency (FOI), the Division of CBRN Defence and Security is regularly inspected by OPCW on short notice.
The OPCW also monitors that adequate adjustments of national legislations are undertaken by the member states in order to comply with the CWC. Today, 188 countries have ratified the convention, but still some nations have not (Figure 3). To mention the most interesting ones, Egypt, Israel, North Korea and Syria have yet to ratify the convention.

6.3. Verification

As mentioned above, to control the compliance by the member states, the OPCW was given a mandate to, at any time, inspect chemical industries handling chemical compounds related to CW. In order to be able to verify alleged use of CW, or to verify the legit processing of chemicals in large plants, the OPCW requires the help of analytical laboratories. Therefore, laboratories in the member states, in different parts of the world, which can perform analysis of various samples from chemical plants, stockpiles or war zones are designated.

Accordingly, the samples can be anything from neat agents to waste water samples and, also, contaminated materials. By issuing yearly proficiency tests, the OPCW appoints designated laboratories having documented skills in analysing chemicals related to chemical weapons. In order to sustain a designated laboratory status, one annual test must be undertaken and the spiked chemicals correctly reported.

The Swedish Defence Research Agency (FOI), the Division of CBRN Defence and Security, is one of these designated laboratories. Currently, there are 19 designated laboratories in the world, but the number can differ from year to year depending on the results of the annual tests. The CWC lists hundreds of specific chemicals as well as generic groups of chemicals and the designated laboratories are required to have the ability to detect and report these substances and their related degradation products. All in all, a designated laboratory must be able to identify hundreds of thousands of compounds.

For the absolute identification of novel compounds that are covered by the CWC, syntheses of reference substances are necessary. Even though the compound is not novel, the use of reference compounds makes the analysis secure and reliable. These references must be analysed within a very short time due to the fact that the OPCW needs the results of the analysis rapidly. In fact, a written report should be returned within two weeks from the date of arrival of the samples. Hence, reference chemicals must be synthesized quickly. The number of products synthesized during a proficiency test can exceed 20 compounds within a single week, not taking into account precursors or intermediates. The compounds can, on the other hand, be
produced on an analytical scale and a micro scale synthesis is generally sufficient.

Thus, it is of importance that new methods are developed which are more suited for fast micro scale syntheses and work-up. It is also important, from an occupational safety point of view, to minimize the amounts produced and handled since even very small amounts of CWA could be lethal. These are, as previously mentioned, two of the reasons to why the research was carried out for the development of new synthetic methods which can be performed quickly on a small scale. Hence, the small scale synthesis of CWA is contributing to uphold the CWC and secure its verification regime.
7. General phosphorus Chemistry

7.1. Introduction

Phosphorus was discovered by Henning Brandt in 1669 as he was trying to make gold out of urine. The chemistry of phosphorus is a very wide field of research covering, for instance, DNA, safety matches, pesticides, flame retardants, fertilizers, detergents, cellular membranes and many more areas. This thesis will, however, only give an overview of some general aspects of phosphorus chemistry and phosphor organic chemistry in particular.

7.2. Bonding of phosphorus

Phosphorus can be directly bonded to between one and six different atoms. This is relatively uncommon, at least for organic chemists accustomed to carbon chemistry. Furthermore, the number of bonds connecting phosphorus to other atoms in the molecule can be anything from one up to six bonds. If the possible number of directly bound atoms and the possible number of bonds to the phosphorus atom are combined, the result will be 10 known combinations or classes (Table 1). These classes are, in turn, often divided into subclasses depending on the nature of the substituents.

The use of oxidation numbers in phosphorus chemistry is limited which complicates the classification of phosphorus compounds. Historically, all phosphorus compounds are classified by the number of coordinated substituents. For instance, three-coordinate compounds are designated P(III) in the literature. However, by calculating oxidation numbers the usual way by counting oxygen as (-II) and hydrogen as (+I), trimethylphosphite would be assigned (+III) and triphenylphosphine would be assigned (–III).

In order to bring some order among the classes of phosphorus compounds, the symbols sigma (σ) and lambda (λ) have been introduced. Sigma describes the number of coordinated atoms, or the coordination number, and lambda describes the number of bonds to phosphorus. For example, sarin is designated σ4λ5 since sarin has four groups, or substituents, connected to phosphorus with five bonds.
Table 1: The different classes of compounds if characterized by the number of substituents and the number of bonds to phosphorus. The structures below are only examples and it should be noted that this is not a complete list of possible compounds. R= Alkyl, H etc; X= Halogens.

7.3. The phosphoryl group

There are several functionalities correlated to phosphorus chemistry, but among them, the phosphoryl group has a special position. The phosphoryl structure is a common entity in phosphorus chemistry and is of utmost importance, chemically as well as biologically. The phosphoryl group is very stable and the entity is easily formed\textsuperscript{25}. The stability of the phosphoryl group is probably one reason to why phosphates are the “backbone” of DNA as well as part of other biological functions such as phospholipids in the cellular membranes and adenosine triphosphate (ATP) for energy transfer\textsuperscript{26}. All nerve agents also contain phosphoryl groups.
The presence of a phosphoryl group or the possibility to form one can influence reactions. The phosphoryl group is commonly, or almost always, drawn as having a double bond (P=O) although it is known that it is not a true double bond. Much research has been conducted on this matter but still no consensus has been reached about the true nature of the bond\textsuperscript{27-29}. It seems as the bond has some ionic properties/contribution but it does not behave like a true ionic bond\textsuperscript{30-32}. However, since there is no better representation proposed, as of today, the double bond notation will be used to illustrate the phosphoryl group in this thesis.

\begin{center}
\includegraphics[width=0.5\textwidth]{phosphoryl_group.png}
\end{center}

\textbf{Scheme 5:} The phosphoryl group can sometimes adopt a structure that resembles that of enols of carbon chemistry.

The phosphoryl group can, as the ketones, occur in a type of enol form (Scheme 5). The equilibrium is very much shifted to the phosphoryl form and has been compared to that of acetone\textsuperscript{24}, but can in spite of this be of importance in reactions. The phosphoryl oxygen can act as a nucleophilic centre in many cases and most often it is the enol form that reacts\textsuperscript{25}. The very polar phosphoryl group has a powerful hydrogen bonding capability and this fact influences for instance solubility\textsuperscript{33}. Phosphine oxides are good examples of water soluble compounds and hexamethyl phosphoramide (HMPA) is a well known highly polar aprotic solvent with a phosphoryl group used by organic chemists in the past (Scheme 6).

\begin{center}
\includegraphics[width=0.5\textwidth]{phosphine_oxides.png}
\end{center}

\textbf{Scheme 6:} The chemical structures of trimethylphosphinoxide and HMPA.
7.4. Nucleophilic substitution reactions at phosphorus esters

Stereochemistry of nerve agents has been found to strongly affect the toxicity\textsuperscript{34}. Furthermore, the hydrolysis of nerve agents might be influenced by the mechanisms of nucleophilic substitutions in phosphorus chemistry\textsuperscript{35}. The nucleophilic substitution at phosphorus can be divided into three different mechanisms; the concerted (S\textsubscript{N}2), the dissociative (S\textsubscript{N}1(P)) and the associative (S\textsubscript{N}2(P))\textsuperscript{36-38} (Scheme 7). This is, of course, a generalized view, but the subject is more easy to discuss if the limiting cases are discussed one at a time\textsuperscript{39}. Much has been written on this subject and below is an overview of some of the findings and published results.

\textbf{Scheme 7:} The limiting cases for the mechanisms of substitution reactions at phosphate esters. 
\( Z = O, S; X = \text{alkyl, O-alkyl etc.; } N = \text{nucleophile; } L = \text{leaving group.} \)

In the concerted mechanism (S\textsubscript{N}2), the approach of the nucleophile and the departure of the leaving group take place at the same time and rate. The concerted mechanism, which has no intermediate, seems to be preferred by, for instance, the mono-substituted phosphate esters\textsuperscript{40}. Studies, which were originally intended for investigating the dissociative mechanism, concluded that concerted mechanisms were favoured if the leaving group is good and that they can appear as dissociative mechanisms should the data not be properly evaluated\textsuperscript{41,42}.
The dissociative mechanism has been the subject of many discussions and experiments. The metaphosphate, which would be the intermediate of the dissociative mechanism, has only been generated a few times when unambiguous proof of its existence have been demonstrated. The conditions under which it has been generated have been rather extreme, raising questions about how general it would be as an intermediate. The species have been generated in gas-phase in the ion source of mass spectrometers, but until now, proof of its existence in solution is vague.

However, the thiometaphosphates where phosphorus is bonded to three sulphur atoms, do form stable salts under some conditions. Contrary to the oxygen analogues, it has been demonstrated that thiometaphosphates are intermediates in reactions. Other investigations, implicating the dissociative mechanism, are fragmentation studies which can present first order kinetics that probably would not suit any of the other mechanisms. The hypothesis is further supported by stereochemical data and calculations. For instance, it has been shown that the entropy of activation (ΔS) is close to zero which could indicate dissociation, i.e. an increase in the disorder in the rate determining step. However, the calculated data have been questioned recently since energy of activation (Ea) seem to be fairly similar between mechanisms. Hence, there is no conclusive evidence of the existence of free metaphosphate in solution, only circumstantial data.

The addition-elimination mechanism is based on several experimental evidences. The mechanism is also named the SN2(P) mechanism and is, as the name indicates, a nucleophilic substitution reaction unique for phosphorus. The ligand positions are not equivalent in these substitution reactions at phosphorus and this fact influence the reactions. The mechanism has been extensively studied and resulted in “Westhemiers guidelines” which can be used to predict the outcome of a reaction. The substitution at phosphorus proceeds through the apical positions, the nucleophile attacks and the leaving group leaves from these positions (Scheme 8). However, if for instance, steric reasons make it more or less impossible equatorial positions might become involved in the substitution. The SN2(P) does also, unlike the SN2 mechanism, form intermediates. The intermediate formed is pentacoordinated and has the geometry of a trigonal bipyramid (TBP).

However, more importantly the intermediate can rearrange the substituents in a process called the pseudorotation, which will be discussed in more detail in the following chapter. In order to predict the outcome of a reaction there are, as discussed above, several factors that have to be taken into account. Some factors are for instance; from which position the nucleophile will attack due to steric- or electronic effects, which substituent is the most potent leaving group, which substituent has the highest apicophilicity and which orientation does the substituents adopt in the intermediate and finally, will the
intermediate pseudorotate? Understandably, the number of possible products can be numerous and the outcome of the reaction difficult to predict.

### 7.5. The trigonal bipyramid and 5-coordinated phosphorus

Already in 1948, it was established that phosphorus can acquire both 5-coordinated and 6-coordinated states\(^{63}\). The geometry of the 5-coordinated phosphorus has also been known for a long time and has, as previously mentioned, the geometry of a trigonal bipyramid\(^ {35}\) (Scheme. 8). The TBP has three bonds in the equatorial plane and two bonds in the so called apical positions situated at the top of the pyramids. The bond angels are thus not equal between all bonds, but 120° between bonds in the equatorial plane and 90° between an equatorial bond and an apical one. Moreover, the bonds to the apical positions are also longer and more polarisable than those at equatorial sites.

![Scheme 8: The trigonal bipyramid and the geometry of the 5-coordinated phosphorus.](image)

Those differences in properties lead to a preference of different ligands to different bonding positions. The experience from experiments over the years, especially by Trippett\(^ {64-66}\) and Holmes\(^ {67,68}\) who have made vast empirical studies on the subject, has made it possible to predict the preference of many common substituents\(^ {69}\). The term apicophilicity was introduced to describe the apical preference of substituents and is shown for some of them in Figure 4.

**Figure 4:** The domestic apicophilicity of some common ligands to phosphorus, where fluorine has the highest apical preference. It should be noted that, for instance, pH can alter the domestic apicophilicities.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Apicophilicity</th>
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</thead>
<tbody>
<tr>
<td>F</td>
<td>Highest</td>
</tr>
<tr>
<td>H</td>
<td></td>
</tr>
<tr>
<td>CF(_3)</td>
<td></td>
</tr>
<tr>
<td>PhO</td>
<td></td>
</tr>
<tr>
<td>Cl</td>
<td></td>
</tr>
<tr>
<td>MeS</td>
<td></td>
</tr>
<tr>
<td>MeO</td>
<td></td>
</tr>
<tr>
<td>(\text{Me}_2\text{N})</td>
<td></td>
</tr>
<tr>
<td>Me</td>
<td></td>
</tr>
<tr>
<td>Ph</td>
<td></td>
</tr>
</tbody>
</table>

There are some factors known to influence apicophilicity, e.g. size, electronegativity and \(\pi\)-conjugative effects and these factors direct the ligands towards apical or equatorial positions. Large bulky ligands, for example, quite understandably prefer the equatorial positions since there is more space there\(^ {35}\). Electronegative substituents on the other hand have a preference for the apical positions since the bonds are slightly longer and the distance...
between the electronegative substituent and phosphorus is greater. π– conjugative effects direct the substituents towards equatorial positions if the substituent is a π–donor and towards apical positions if the substituent is a π–acceptor. The π–donors are believed to interact with an acceptor orbital perpendicular to the equatorial plane. The apicophilicity is, however, still not fully explored and understood.

If phosphorus is incorporated in a ring system, the size of the ring will to some extent determine the geometry. Five-membered species prefer to be positioned apical-equatorial and six-membered rings prefer a diequatorial positioning. Five-membered di-ester rings are more easily cleaved than six-membered di-ester rings and acyclic ones, which is suggested to be mainly due to ring strain. It is easily foreseen that it can be difficult to predict what contribution to the conformation of the TBP intermediate that will dominate, considering the influence different conformations of the ring itself will have upon the TBP. In the case of a six-membered ring where chair- and boat-conformations compete, this is particularly complicated.

Figure 5: The two proposed mechanisms of the pseudo rotation; the Berry pseudo rotation (BPR) at the top and the Turnstile Rotation (TR) at the bottom.

However, the geometry around phosphorus is not rigid, but dynamic, and the atoms can switch positions. The rearrangement is known as the pseudorotation and the positions are rotated in a certain pattern. There are two theories on how the mechanism of the rearrangement proceeds, the “Berry Pseudorotation” (BPR) and the “Turnstile Rotation” (TR) (Figure 5). The BPR has found general acceptance, but there are cases when BPR is physically impossible. In those cases the TR provides a reasonable explanation, even though the energy of activation is higher for the TR than for the BPR. When pseudorotation occurs, one substituent does not “rotate”, i.e. the pivot of rotation.
It could be interesting to mention that most enzymatic nucleophilic reactions at phosphorus do not involve pseudorotation. This means that the reaction between nerve agents and AChE does probably not involve pseudorotation in the majority of cases. There are, however, other biological substitutions at phosphorus that have been observed to undertake pseudorotation, for example hydrolysis of adenosine cyclic mono-phosphate (cAMP).

### 7.6. $^{31}$P Nuclear Magnetic Resonance Spectroscopy

This chapter will provide a brief overview of the benefits and limitations of $^{31}$P-nuclear magnetic resonance (NMR) spectroscopy, and some of the differences compared to the more common $^1$H-NMR and $^{13}$C-NMR techniques.

The naturally occurring phosphorus isotope has a 100% natural abundance and a spin quantum number of $\frac{1}{2}$. This achieves a relatively strong signal as all phosphorus nuclei contribute to the signal in NMR. This can be compared to $^{13}$C-NMR where only about 1% of the naturally occurring carbon nuclei are magnetic, the other 99% are $^{12}$C and non-magnetic.

Phosphorus has rather low sensitivity compared to proton, but high sensitivity compared to carbon. The sensitivity is thus normally not a problem when analysing products or reaction mixtures due to the relative high concentrations obtained in such samples. Also, through the nuclear overhauser effect (NOE), decoupling of the protons does in some cases assist to increase the sensitivity.

Phosphorus compounds, such as the phosphorus oxyacids, are strongly affected by, for instance, hydrogen bonding or other solvent effects. Therefore, it is of importance that the solvent and the conditions are reported along with the spectral data. Currently, the reference used in $^{31}$P-NMR is 85% phosphoric acid and shifts down-field are assigned positive values and shifts up-field are assigned negative values. However, this has not always been the case. In the early days of $^{31}$P-NMR spectroscopy, the opposite assignment was utilized and values reported before the 80’s may have to be recalculated in order to accord with tables of today.

Coupling constants between phosphorus and proton nuclei show values varying from only a few Hertz over a few covalent bonds to several hundred Hertz for direct P-H bonds. Since the $^{31}$P-NMR spectra are generally $^1$H-decoupled, the J values are commonly obtained in the $^1$H-NMR spectra. The splitting of the signals are recognised as being larger than J-couplings between protons, generally between 10 – 20 Hz for a methyl group directly bonded to phosphorus. There are other cases in which couplings are...
revealed in the $^{31}$P-NMR spectra, and the most common situation with phosphorus compounds related to CWA is when fluorine is present in the molecule. Fluorine is also a spin $\frac{1}{2}$ nucleus with an abundance of 100%. The couplings between $^{31}$P and $^{19}$F are in the range of 900 – 1100 Hz if the coupling is over one bond and the couplings are recognised in both $^{31}$P- as well as $^{19}$F-NMR spectra. These couplings make the fluorinated phosphorus analogues easily analysed in $^{31}$P-NMR spectra since they are the only species in which these couplings are present. The number of attached fluorines is also easily determined according to the splitting pattern.

The possibility to quantify the signals depends on the relaxation times ($T_1$) and NOE. In order to compare quantitative data of different phosphorus compounds, it is important to use long delays (5 x $T_1$ is generally recommended for 90° pulses) in order to minimize the effect of different relaxation times between signals. Alternatively, a relaxation agent such as Cr(acac)$_3$ can be added to the sample in order to shorten the relaxation times. The NOE can be eliminated by running the instrument in the inverse gated $^1$H-decoupling mode. The decoupling is turned on only during the acquisition time and there is no time for distortion of the population ratios between relevant energy levels, thereby eliminating the NOE. When the compounds to be compared are similar, NOE is not considered to significantly influence the quantification. However, it is important that the number of protons in the close vicinity of the phosphorus atom is similar.

Most $^{31}$P-NMR shifts are found between -200 ppm and +300 ppm but they actually span over a range of about 2000 ppm. Within this region, different functional groups and classes of phosphorus compounds have their own spectral ranges. There is, of course, some overlap between these sub-regions, and the identification of an unknown compound can rarely be carried out based solely upon $^{31}$P-NMR chemical shifts. For the organic chemist who needs to know whether a certain chemical transformation has been successful, the $^{31}$P-NMR can be an extremely powerful spectroscopic method.
8. Chemistry of phosphonofluoridates

8.1. Introduction

The majority of known nerve agents belong to the phosphonofluoridates. There are several research areas where these substances are of interest, but in the end it all comes down to molecules and the importance of understanding the chemistry. This chapter will give an overview on the chemistry of phosphonofluoridates.

8.2. Structure

Phosphorus, as mentioned earlier, is literally part of the backbone of life and can even be part of the chemistry of death. All nerve agents have some general structural relationships which will be discussed in the following section (Scheme 9). First of all, there is a phosphorus atom constituting the centre of the molecule.

\[
\text{Scheme 9: The general structure of nerve agents of G-type. } L = \text{leaving group, } R = \text{alkyl, } R' = \text{alkyl.}
\]

Different ligands are attached to the phosphorus atom, all of which, to a varying degree, influence the properties of the molecule. According to the system referred to in Chapter 7.2., nerve agents are designated \(\sigma^4\lambda^5\), which means that phosphorus has five bonds to four other atoms. The first of those ligands is a carbon directly attached to phosphorus, usually a methyl group (R in Scheme 9). If this group is too large, the toxicity is drastically lowered and this is probably one of the reasons to why the CWC only considers alkyl groups up to propyl as relevant for CW11. The second ligand is an O-alkyl group. The most common distinctions between different agents are variations of this second substituent. How the properties of the substances are affected as a result of this will be further developed in Chapter 8.3., in which physical properties are discussed. The third group is the “double-bonded” oxygen in the phosphoryl group. The final and fourth group, i.e. leaving group, is commonly a fluorine atom. It is called the leaving group because of the fact...
that when the nerve agent reacts with its main biological target in the nervous system, this group leaves the molecule. This nucleophilic substitution reaction binds the phosphorus atom of the nerve agent to the target protein. Also, there are other classes of nerve agents that do not completely match the description above, e.g. tabun, which has no carbon directly bonded to phosphorus, but which nevertheless generally constitutes of similar groups connected to phosphorus (Scheme 2).

8.3. Physical properties

A large number of nerve agents with different properties have been developed in order to be able to use CW in any part of the world, and in any climate. In this context there are some physical properties of importance to discuss.

The nerve agents are all, despite the fact that they are frequently called nerve gases, clear colourless liquids at room temperature (RT). If the agent is coloured, it will raise concern regarding the purity since the impurities are the ones most likely causing the colour. There are a few reports of the scents of nerve agents; some are said to smell like flowers and others like fruit. However, it is difficult to verify these statements. Since there is a smell, there is a vapour pressure (vp) too, and the vp varies considerably between agents. For instance, Sarin has a vp of 2.9 mmHg whereas VX’s vp is only 0.0007 mmHg.

The sensitivity to hydrolysis and the heat stability are other examples of differentiating properties. The nerve agents with bulky O-alkyl groups (R’ in Scheme 9) seem to have better stability to hydrolysis, which might be due to both steric and hydrophobic factors. The stability to heat is most important when choosing the method of dispersal since, for instance, the heat generated in the explosion of a grenade might destroy the agent instead of dispersing it.

The solubility in water can be another important property which is greatly dependent on the size of the hydrocarbon chains in the molecule. All nerve agents are soluble in water but to different extents; some are barely soluble and others are freely soluble. The solubility is a factor of great importance, when the aim is to decontaminate personnel or equipment and a suitable method must be chosen.
8.4. Mechanism of action

The primary target for the nerve agents is the enzyme acetylcholinesterase (AChE) (Figure 6) and other cholinesterases. AChE is an enzyme engaged in the nervous system of humans, and all other higher animals. The enzyme is situated in the synapses between neighbouring nerve cells. The role of AChE is to hydrolyse the neurotransmitter acetylcholine when the nerve signal is terminated (Scheme 10). The hydrolysis of acetylcholine is an extremely rapid reaction and AChE is considered as one of the fastest enzymes known. The substrate turnover rate is approximately 15000 molecules / second.

![Figure 6: X-ray structure of AChE inhibited by sarin (Copyright, Fredrik Ekström).](image)

Scheme 10: The native transmitter substance of nerve signals, acetylcholine, which the nerve agents mimic in order to destroy the enzyme and consequently impact the whole CNS.

The nerve agents are designed to fit the enzyme’s active site in order to compete with the substrate. The nerve agent reacts with, and binds covalently to the catalytic site of the enzyme, making AChE irreversibly inhibited (Scheme 11). The inhibition of AChE leads to accumulation of transmitter substances and overstimulation of the neurones. The overstimulation is causing the collapse of the CNS and as a consequence, death within minutes.
Scheme 11: The nerve agents covalently bond to an amino acid (ser203) in acetylcholinesterases active site, here exemplified by sarin. The serine moiety is part of the catalytic centre of the enzyme which becomes inhibited by this addition.

For most nerve agents it is possible to reactivate the enzyme to some extent if an antidote is administered quickly. However, tabun is one of the agents which are more resistant to reactivation. If the enzyme is not reactivated without delay, another reaction takes place, i.e. the aging. Depending on which agent that is causing the intoxication, the rate of the aging varies considerably. For example, soman has a $t_{1/2}$ of approximately 6 min and has thereby one of the shortest $t_{1/2}$ among the nerve agents\textsuperscript{86}. The aging of the enzyme occurs when the phosphorus atom in the nerve agent is hydrolysed while the covalent bond to the enzyme remains intact (Scheme 12). The reactivity of the formed enzyme-nerve agent complex is drastically lowered and it becomes impossible to reactivate the enzyme. The only way for the organism to repair this damage is to replace the whole enzyme, which is a process that takes at least two weeks\textsuperscript{13}.

Scheme 12: The aging of the enzyme is permanently destroying the catalytic site, again exemplified with sarin.

Today, there are several known antidotes, which, if administered quickly could reactivate AChE to some extent, but none with truly high efficiency (Scheme 13). The role of the antidote is to disconnect the nerve agent and release the enzyme by a nucleophilic attack on the phosphorus atom of the nerve agent - enzyme complex (Scheme 14).
Scheme 13: The most common reactivators currently in use by armed forces and rescue services to medicate intoxication of nerve agents.

The protective factor of reactivators is today about 10 units at the best, which means that you will approximately survive with 10 times a deadly dose of nerve agent if treated with a reactivator. This is still only about 50 mg of VX if the skin has been contaminated, much less if the agent has been inhaled. In addition, the antidote has to be administered shortly after exposure, if not, the aging will take place instead. Currently, some research efforts are devoted to finding new antidotes and to understand the mechanism and interactions of the inhibition\(^\text{87}\).

Scheme 14: The schematic reactivation of sarin inhibited AChE. In this case illustrated with the HI-6 antidote.
8.5. Synthesis of G-agents

Synthesis has ever since Gerhard Schrader’s times been conducted using almost the same methods. The production methods are often industrial large scale ones not suited for small scale synthesis. It might be for good reasons to why the research in this field has not been published in open literature, as in other fields. Nevertheless, for the purpose of, for instance, verification or research for novel antidotes there is a need to develop new methods for small scale synthesis of these substances. The amounts needed for such research are generally much smaller than what is possible to synthesise. This makes the method of production the factor that decides the amount being produced, not what is required for the imminent research. One example is low dose experiments in which approximately 5 µg/kg/24h is a significant amount for rodents, but the actual synthesis generally produce at least 10 mg – 1 g of CWA. The following section will briefly describe a common synthetic route, available in open literature, on the synthesis of Sarin.

A simple scheme for the synthesis of sarin is shown below, deliberately truncated (Scheme 15). There are of course other synthetic routes, but these are common sequences. Note: For references on Chapter 8.5., please contact the author.

Scheme 15: Common route to synthesize sarin, a nerve agent developed by the Germans during World War II.

37
Nowadays, the starting point for almost any phosphorus containing chemicals is phosphorus containing minerals. These minerals are common in practically all parts of the world and many countries have the possibility to mine them. Fluoroapatite (Ca_{10}(PO_{4})_6F_{2}) is one of the more common minerals used in the production of P_4. The mineral is, by chemical transformations, converted into elemental white phosphorus, P_4 (Figure 7). The process consumes large amounts of carbon in the presence of quartz and can certainly not be considered climate neutral.

\[ 4 \text{Ca}_{5}(\text{PO}_{4})_{3}\text{F} + 21 \text{SiO}_{2} + 30 \text{C} \rightarrow 20 \text{CaSiO}_{3} + 30 \text{CO} + \text{SiF}_{4} + 3 \text{P}_{4} \]

Figure 7: The production of white phosphorus produces vast amounts of carbon monoxide (CO). Every mole of produced P_4 generates 10 mole of CO.

A stream of chlorine is lead over the heated white phosphorus and the product, phosphorus trichloride is suitably captured as condensed fumes. PCl_3 is collected as a liquid together with the by-product PCl_5, which is a solid compound. Contamination of PCl_5 is managed by adding more white phosphorus to the mixture in order to transform the impurity to product which is then redistilled. PCl_3 is central in nerve agent synthesis, and is the first chemical in the reaction chain related to CWA that is controlled by OPCW and the CWC. After this step, all intermediates are scheduled compounds according to the CWC, and should be reported to the national authority if they are produced, consumed, imported, exported or stored.

By treating methanol with PCl_3 and a base, trimethylphosphite is formed by a nucleophilic substitution reaction. The hydrochloric acid formed is simply removed by leading a stream of nitrogen gas through the solution.

The next step is to attach a methyl group directly to the phosphorus atom. At this stage of the reaction sequence, the aim of the synthesis becomes evident. There are practically no other phosphonates but nerve agents, at least not commercially handled, that has a methyl group attached directly to the phosphorus atom.

The trimethylphosphite is reacted with a methyl halide in the Michaelis-Arbuzov reaction to produce dimethyl methylphosphonate. The Michaelis-Arbuzov reaction is an autocatalytic reaction and care must be taken in order not to loose control over it. The reaction is very reliable, if handled correctly, and produces high yields with good purity under controlled conditions. The alkyl halide formed is easily evaporated along with the solvent.
Following the Michaelis-Arbuzov reaction, the methoxy groups of the dimethyl methylphosphonate are substituted by chlorines using a potent chlorinating reagent such as phosgene.

The next step in the sequence is the introduction of the fluorine atom which is classically carried out using quite simple chemicals such as hydrogen fluoride (HF) or sodium fluoride (NaF). The reaction of HF generates product in higher yield, but the complications of using the highly toxic gaseous reagent makes NaF a competitive alternative.

The final step is to attach the O-propyl group. The transformation is commonly executed using 2-propanol and a base. The reaction mixture is washed, evaporated and distilled under reduced pressure to isolate the product.
9. Paper I

9.1. $[^{14}\text{C}]$ Radiolabelling of phosphonofluoridates

The mechanisms of intoxication by nerve agents in the human body are, even today, not fully understood in detail$^{89}$. It is well known that the organophosphorus compounds inhibit the enzyme acetylcholinesterase by forming a covalent bond to the catalytic site of the enzyme. In the catalytic site, the amino acid serine (Ser203) catalyses the hydrolysis of acetylcholine and it is to this amino acid the nerve agent becomes attached. If the enzyme is not treated with a reactivating agent rapidly, nerve agents are also known to cause aging of the enzyme. However, there is little known about alternative target molecules and through what pathways degradation products are eliminated by the living organism$^{90}$. In order to study the process of aging in more detail and of the fate of the nerve agent and its metabolites within the organism, the use of radiolabelled compounds is a powerful tool.

Since the toxicity of the nerve agents is extremely high, the dose used in experiments in vivo becomes very low. This is evident in low dose experiments when cells or organisms are expected to stay alive throughout the test period$^{88}$. In order to obtain sufficient radioactivity to be able to detect the compound or its degradation products after exposure, the activity of the labelled compound must be very high. Since the amount of sarin needed for an experiment is in the range of a few µg of agent per kg of organism or cell, the concentration of a specific radiolabelled metabolite in, for instance, blood becomes extremely low. The percent of labelling was in this synthesis as high as was possible to achieve and no dilution with cold material was sought. As a consequence, the specific activity becomes as high as 2 GBq for a synthesis of about 80 mg of the labelled product.

Not only is the compound itself extremely reactive and toxic, but also radiolabelled. This implies that the compound can phosphorylate many tissues or sites within the body and covalently bond the irradiating nucleus to living tissue. Incorporation of irradiating nuclei in living tissue could over time give a substantial dose of radiation and possibly cause cancer. Furthermore, with a half-life for the $[^{14}\text{C}]$ nucleus of about 5730 years$^{91}$, contamination of lab equipment will probably mean that it has to be discarded since it is most likely made unusable for regular synthetic work.
On the other hand, radiolabelled compounds are easily detected using a Geiger meter. Thus, a possible contamination can be investigated fairly easy.

When synthesising radiolabelled compounds, the labelled reagent is preferably added as late as possible in the reaction sequence in order to minimize handling of labelled compounds, but also to minimize the loss of radiolabel. Loss of label during the synthesis means more radiolabel in the waste which, in turn, implicates more work and that care must be taken managing the waste. However, the label must be situated in a part of the molecule that can be anticipated to remain in the most interesting degradation products, or else it will be of little use. For instance, it would be relatively easy to label the leaving group since it is commonly attached late in the synthesis of nerve agents, but it would also be detached as soon as it reacts with AChE or other nucleophiles during the metabolism. Furthermore, the fluorine, which is normally the leaving group in these molecules, would have to be replaced with, for instance, a cyano moiety (CN) since there are no suitable fluorine isotopes available. This could, in turn, alter the chemical properties and possibly even the metabolism within the organism.

In this case it was desired to label the methyl group, which means that the labelled atom is directly bonded to the phosphorus atom. This methyl group is firmly attached to the phosphorus atom in the centre of the molecule, and will probably be the last substituent to be detached from phosphorus. The drawback of using this strategy for the labelling is that the label is inserted rather early in the synthetic route. Hence, several steps must be undertaken using already labelled compounds. There are known methods that incorporate the radiolabelled atom later in the sequence, but these methods are less compatible with different phosphorus ester groups. Since it was desired for the method to be universal for all nerve agents of this type, the following strategy became our choice.

The preferred synthetic route begun with the earlier mentioned classic Michaelis-Arbuzov reaction. However, triethylphosphite and methyl iodide were used instead of trimethylphosphite and methyl iodide, which usually would be the first choice (Scheme 16). The first reason for this is that the reaction product from the first reaction step is an alkylhalide, and using trimethylphosphite would generate methyl iodide without radiolabelled carbon. The unlabelled methyl iodide would compete with the labelled one and create a product without any radiolabel. By using triethylphosphite, the by-product formed is instead ethyl iodide. The radiolabelled methyl iodide reacts much faster than, and effectively competes with, the formed ethyl iodide. Secondly, the by-product formed by ethyl iodide reacting is disfavoured in the following reaction sequence which further reduces contamination.
It is of great importance that the proper amounts of triethylphosphite and methyl iodide are used. The amount of methyl iodide must be used in slight excess, otherwise the formed ethyl iodide will react with triethylphosphite to produce unwanted by-product. The use of triisopropylphosphite was evaluated as well and the purity after the Michaelis-Arbuzov reaction was found to be even better than when triethylphosphite was used, but this route produced lower total yields. Especially the second reaction step, the hydrolysis, suffered from low yields and this alternative was considered slightly less efficient. In order to avoid loss of the volatile radiolabelled methyl iodide both the methyl iodide and the triethylphosphite was quickly cooled before the reagents were opened. An ampoule of methyl iodide (1.85 GBq) was opened and triethylphosphite added. The mixture was transferred to a screw cap vial and heated at 100 °C for 2 h without any additional solvent. After the completion of the reaction the ethyl iodide was removed by evaporation under reduced pressure. Crude product (131 mg, 93 % from [14C]-methyl iodide and 96 % from triethylphosphite) was obtained and used without further purification.

Instead of chlorinating the diethyl methylphosphonate directly as in the common sequence mentioned in Chapter 8.5., an extra reaction step was introduced. This procedure might seem a bit awkward, but was carefully evaluated. The direct chlorination of diethyl methylphosphonate was found to produce some by-products that had to be removed before continuation of the synthesis. The by-products were otherwise anticipated to interfere with forthcoming reactions, which would cause loss of yield. This difficulty would make a thorough work-up necessary before continuing with the next
reaction step. Since one of the aims was to avoid unnecessary handling, other options were investigated.

![Chemical structures](image)

**Scheme 17:** The synthetic route for soman is essentially the same as for sarin since universality was one of the benefits of the method.

The hydrolysis reaction was known to be reliable and was thus assessed as an option. The advantages of hydrolysing the reaction mixture were several as it turned out. Firstly, the reaction produced quantitative yields. Secondly, impurities and by-products formed during the hydrolysis reaction as well as the Michaelis-Arbuzov reaction could be removed easily by heating the product for 20 h at 100°C. The melting point of methylphosphonic acid is approximately 105°C, if pure, which means that it is barely liquefied at these temperatures. The phosphonic acids have very low vp and high temperature stability and are not affected by the high temperature, whereas by-products are evaporated. Thirdly, chlorination of the methylphosphonic acid afforded product of higher purity than direct chlorination of diethyl methylphosphonate. The reaction vessel containing the diethyl methylphosphonate was also used for the hydrolysis reaction. Aqueous hydrochloric acid was added in one portion and the reaction heated at 150°C for 24 h. The water was removed by purging with argon while the reaction was heated to 60°C, after which the phosphonic acid was placed in a thermostat at 100°C for 12 h. The yield of methyl phosphonic acid was 80.5 mg. (97%).

Since the reactivity to chlorination was higher for phosphonic acids than it was for phosphonates, the relatively mild oxalyl chloride was chosen. By introducing a hydrolysis step, it was possible to avoid other harsher chlorinating reagents. The by-products from oxalyl chloride are low volatile compounds which are easily removed. The methyl phosphonic acid was a solid compound and was not easily solvated in the solvents preferred for the
chlorination reaction. It was therefore important to maximise the surface of the phosphonic acid before the chlorination. This was facilitated when the phosphonic acid was removed from the heating, by slowly rotating the reaction vessel as the product was solidifying. In this way, a thin film of phosphonic acid on the glass wall of the reaction vessel was achieved, and the reaction could proceed using shorter reaction time and higher yield was afforded. In order to improve the reaction, diethyl formamide (DMF) and triethyl amine (TEA) were evaluated as catalysts\(^3\). We did not notice any difference in the yields between the two catalysts, and together with the fact that TEA was used later in the synthesis as well, is why we selected the more volatile TEA for the reaction. Still using the same vial, deuterated chloroform was added. TEA in analytical amounts was added followed by oxalyl chloride in one portion. The reaction vessel was heated at 70ºC for 6 h. The progress of the reaction could be visually followed by observing the consumption of the film of phosphonic acid on the glass ware. The yield obtained was 100.2 mg, 90%.

For the fluorination, the method of using hydrogen fluoride was chosen. The hydrogen fluoride is a difficult chemical to work with, not only due to the fact that it is gaseous, but also due to its severe toxicity and the corrosive effects it has on the skin. On the other hand, excess hydrogen fluoride was easily removed by bubbling argon gas through the reaction medium. Furthermore, the reactivity is moderate, which means that excess of reagent is of no concern. However, most importantly, the hydrogen fluoride produces product of high purity. The hydrogen chloride formed as a by-product was removed using the same method, and basically, no other compounds except for the target molecule are left in solution. The fluorination reaction was the first reaction that demanded a change of reaction vessel. The reaction mixture of the chlorination reaction was transferred to a plastic vial and used without further purification. The solution was bubbled with hydrogen fluoride (HF) for 10 s at 20ºC and then sealed for an additional reaction time of 30 min. Most of the excess of HF was removed with reduced pressure. No analysis of the \(^{14}\text{C}\)-product was executed at this stage, but from preparing experiments using unlabelled materials the yields from this reaction are known to be in the range of 90-95%.

The final step, the addition of the O-alkyl group, was conducted by adding triethylamine (TEA) until the solution became neutral. A small amount of methyl red was used to indicate when the solution reached the right pH. One additional equivalent of TEA, calculated from the expected yield, was added in order to eliminate the amount of HF expected to be formed during the substitution of fluorine by 2-propyl alcohol. The solution was stirred for 18 h at 20ºC, followed by washing with slightly acidified brine and drying with Mg SO\(_4\). The individual yield of the last reaction step could not be calculated
since the starting material was not isolated, but from experiments with unlabelled material it was found to be in the range of 80-90%. The total yield of $[^{14}\text{C}]-\text{Isopropyl methylphosphonofluoridate}$ was 79.4 mg rendering a total yield calculated from $[^{14}\text{C}]-\text{methyl iodide}$ of 61%.

Figure 8: The $^{31}\text{P}-\text{NMR}$ spectra of soman synthesized by the method described.

As earlier mentioned, the method should be generic which was demonstrated by the synthesis of soman (Scheme 17). Soman was also synthesized using the same protocol with the evident exception of the last step, in which the alcohol used was pinacolyl alcohol (1,2,2-trimethylpropanol). The soman synthesis produced slightly lower yield. The pinacolyl alcohol is significantly more sterically hindered than the 2-propanol and was expected produce somewhat lower yields, 55 % in total, calculated from methyl iodide.
9.2. Conclusions

A method for synthesising highly toxic compounds, and at the same time handle 2 GBq, is presented. The method is focusing on maximising the yield, and at the same time minimising both the handling of labelled material, but also the amount of waste. The method presented is producing a total yield of 61% over five reaction steps, which can be compared to earlier published methods where the yield is between 30 – 50%\textsuperscript{94,95}. The individual yields of the reactions are all but the last one above 90%. The work-up procedures are minimized as is the handling of waste and labelled compounds. The method is generic and can be used for all agents in the G-series. This was demonstrated by synthesising soman with the same method. Soman was afforded in a total yield of 55%, which was, even though not as good as for sarin, still acceptable. Especially since the purity of the afforded soman was extraordinary, this can be seen in Figure 8.
10. Paper II

10.1. Enamines as halogenating reagents for phosphorus

Investigations in order to find new reagents for small scale synthesis of phosphorus halides eventually led to α-haloenamines (Scheme 18). α-Haloenamines, although known coupling reagents for years, had previously not been used in the field of phosphorus chemistry\textsuperscript{96-100}. The common way to produce fluorinated phosphorus compounds is to begin by synthesising the chlorinated analogue which subsequently is fluorinated. The aim was to investigate if it was possible to use mono-alkylated phosphonic acids as starting material for both the phosphono chloridates as well as the phosphono fluoridates directly. In addition to being stable and easily stored compounds the phosphorus oxyacids are, furthermore, straightforwardly synthesized. In that perspective the phosphorus oxyacids are tempting starting materials in micro scale synthesis. In this way, it would be possible to produce the phosphono fluoridates using one reaction less than in usual methods. This, in turn, shortens the time it would take to produce the chemical, which can be of utmost importance when synthesising, for instance, analytical references.

Scheme 18: The general structure of α-haloenamines. X = Cl, F; R = methyl, isopropyl.

The substance class that was most interesting in the context of CW was the mono-alkylated phosphonic acids, but to assess the scope of the reaction other compounds were added to the matrix. Twelve different compounds were evaluated as starting materials (Table 2) among which phosphinic-, phosphonic-, as well as phosphoric acids are represented. Seven of the phosphonic acids had to be synthesized since only the ethyl methylphosphonic acid was commercially available. The syntheses of the phosphonic acids were conducted based on a reliable method\textsuperscript{101} which is further described in paper IV.
Table 2: The phosphorus oxyacids that were converted and the yields of respective analogues.

<table>
<thead>
<tr>
<th>Phosphorus Oxyacids</th>
<th>Phosphorus oxy chlorides</th>
<th>Yield</th>
<th>Phosphorus oxy fluorides</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Structure" /></td>
<td><img src="image2" alt="Structure" /></td>
<td>88%</td>
<td><img src="image3" alt="Structure" /></td>
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<td><img src="image4" alt="Structure" /></td>
<td><img src="image5" alt="Structure" /></td>
<td>86%</td>
<td><img src="image6" alt="Structure" /></td>
<td>Quant</td>
</tr>
<tr>
<td><img src="image7" alt="Structure" /></td>
<td><img src="image8" alt="Structure" /></td>
<td>90%</td>
<td><img src="image9" alt="Structure" /></td>
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<td>81%</td>
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<td>87%</td>
</tr>
<tr>
<td><img src="image13" alt="Structure" /></td>
<td><img src="image14" alt="Structure" /></td>
<td>95%</td>
<td><img src="image15" alt="Structure" /></td>
<td>77%</td>
</tr>
<tr>
<td><img src="image16" alt="Structure" /></td>
<td><img src="image17" alt="Structure" /></td>
<td>91%</td>
<td><img src="image18" alt="Structure" /></td>
<td>75%</td>
</tr>
<tr>
<td><img src="image19" alt="Structure" /></td>
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<td><img src="image30" alt="Structure" /></td>
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<td>22%</td>
</tr>
<tr>
<td><img src="image34" alt="Structure" /></td>
<td><img src="image35" alt="Structure" /></td>
<td>57%</td>
<td><img src="image36" alt="Structure" /></td>
<td>52%</td>
</tr>
</tbody>
</table>

It is usually problematic to start with mono-alkylated phosphonic acids to produce the halogenated analogue. This is because the acid used as starting material is rather nucleophilic and the sought product of the reaction is equipped with a good leaving group, especially the chlorinated analogues.
The by-products of starting material reacting with desired product are pyrophosphonates which is the phosphorus analogue to carboxylic anhydrides (Scheme 19). The pyrophosphonates are easily formed and not easily avoided. The formation of one unit of pyrophosphonate is consuming two units of possible product, which means that the pyrophosphonates are severely affecting the yield if their formation is favoured. Discouragingly the first chlorination experiment that was conducted with this new reagent produced large amounts of pyrophosphonate.

Scheme 19: Pyrophosphonates are common by-products in the syntheses of phosphonic halides. R = alkyl.

After experimenting with different temperatures and, it became evident that the reaction had to be heated to a certain temperature to perform well and produce desired product. On the other hand, if the reaction temperature was lowered, using for instance an ice bath, the reaction produced pyrophosphonates in good yields with only traces of halogenated phosphonic acids. In other words, by choosing the right temperature either one of these products could be obtained. However, when this threshold temperature was reached, the reaction reached completion within minutes with high purity and good yields of the phosphono chloridates (Figure 9). The threshold temperature was different between the different classes of phosphorus oxyacids. The phosphinic acids had the lowest threshold temperature and the phosphoric acids the highest one, the phosphonic acids being in between. After a small study of different solvents and by comparing with published results, chlorinated organic solvents were chosen. For the chlorination reactions of the phosphonic acids, chloroform was appropriate. The phosphoric acids had a threshold temperature of 120°C, which made tetrachloroethane a more suitable solvent.

Scheme 20: The general reaction of α-haloamine and phosphonates. R = alkyl, X = halogen.

The general procedure for preparing the chlorinated analogues was performed by dissolving the α-chloroamine (1-Chloro-N,N, 2-trimethyl-1-propenylamine) in dry chloroform at 50°C with stirring. The phosphonic acid
was then added under an argon atmosphere and allowed to react for two minutes (Scheme 20). The product was analysed by $^{31}$P-NMR and $^1$H-NMR to establish the outcome of the reaction. Out of the chlorinated compounds that were synthesized, five were novel. A full characterisation was made of those analogues using, besides NMR spectroscopy, also mass spectrometry (EIMS and CIMS).

Figure 9: The $^{31}$P-NMR spectra of the crude benzyl isopropylphosphonochloridate.

The general procedure for the fluorinated analogues was to a large extent similar to the procedure for the chlorinated $\alpha$-haloenamines but the temperature was raised to 120°C and the reaction time extended to five minutes. However, the fluoroenamine was not commercially available and had to be synthesized starting from the chloro analogue.$^{104}$ To a slurry of dry CsF in 1,3-dichlorobenzene, 1-Chloro-N,N,2-trimethyl-1-propenylamine is added. The reaction is heated to 110°C for 36 h under argon atmosphere. The product was distilled directly from the reaction vessel in moderate yields (79%).
The fluorinated products were analysed using the same techniques as for the chloro analogues, but in addition, \(^{19}\text{F}-\text{NMR}\) was used for the characterization. Among the fluorinated products, six novel compounds were fully characterized since there was no previous publication describing their properties. Interestingly, the fluoro analogues of the phosphorus oxyacids did not suffer from pyrophosphonate formation to the same extent as the chloro analogues, but did still, in general, produce lower yields, with a few exceptions (Table 2). Some degree of over-fluorination where the O-alkyl group had been replaced by a second fluorine atom was achieved. The formation of phosphonic difluoride proved to be a competitive reaction rather than a degradation reaction of the product since the amount formed was neither dependent on the reaction times nor the amount of reagent used. Regrettably, the two side reactions complemented each other. The pyrophosphonates were favoured by lower temperatures and the phosphonic difluorides by higher temperature and no centre point temperature could be found were none of the by-products troubled the yields.

![Scheme 21: The various enamines evaluated for the halogenation reactions at phosphorus.](image)

Since the fluorinated analogues, and especially the dibutyl fluorophosphate, resulted in slightly lower yield than the chlorinated one, an alternative was evaluated. In the literature it was indicated that analogues of the \(\alpha\)-haloenamines with larger carbon chains perform better than the commercially available enamine in other applications\(^9\). Therefore, the \(\alpha\)-haloenamines with isopropyl groups on the nitrogen were synthesized (N, N-diisopropyl-1-fluoro-2-methylpropenamine) (Scheme 21). Since the chloro analogue was used as starting material for the fluoro enamine, it was synthesized and evaluated as well even though the chlorination reactions generally performed adequately. The new enamines were synthesized starting from isobutyric acid chloride and diisopropylamine in dichloromethane (DCM) at 0°C. The formed amide was chlorinated by dissolving POCl\(_3\) and DMF (~0.1 eq) in DCM and adding the amide dropwise at 0 °C. The synthesis of \(\alpha\)-fluoroenamine, starting from the \(\alpha\)-chloroenamine was conducted using the same conditions and method earlier mentioned. The evaluation did, however, prove that the new reagents were drastically less efficient than the ones first assessed. The typical yields were in the order of a few %. This was seen in the chlorination reactions as well as in the fluorinations.

In an attempt to improve the reagent even further, the enamines were investigated as solid phase reagents by synthesising them directly on a
polystyrene resin. The synthesis started with a Merrifield resin in chloroform to which an amine linker was added\textsuperscript{105}. N, N-Dimethylethylenediamine was selected partially since the resulting polymer-linker could be used for the continuation directly following the reaction without any modifications. Isobutyric acid chloride was reacted to the amine to form an amide. The amide was then chlorinated using oxaly chloride\textsuperscript{106} or phosphorus oxychloride\textsuperscript{107}. Both methods worked well, but for analytical reasons, the oxaly chloride had an advantage. Phosphorus oxychloride and its reaction products were leaving signatures in the $^{31}$P-NMR spectra, if not fully removed, which complicated the analysis of the products. The resin was characterised using IR spectroscopy and by weighing of the resin.

Using the carefully synthesized reagent for the chlorination of phosphorus oxyacids did turn out to be a disappointment. Despite rather extensive laboratory work in changing the reaction conditions, the main product was always the pyrophosphonate, sometimes in almost quantitative yields. One possible explanation might be that it was not possible to reach the threshold temperature for the reaction to proceed smoothly. It is quite common that reactions perform somewhat slower when applied to solid phase chemistry\textsuperscript{108}. Since it was indicated already in the solution experiments that the rate of the reaction was critical, this could be a probable cause for the failure. When it turned out that the stability of the resin bound reagent was poor as well, it was decided not to pursue this path any further.

### 10.2. Conclusions

The $\alpha$-haloamines are shown to be useful and rapid reagents for the conversion of phosphorus oxyacids to the chloro- as well as fluoro analogues, sometimes with reaction times of less than a minute. The scale can be smaller than in earlier methods thus resulting in safer working conditions as well as in faster production of agents aimed at, for instance, chemical analysis or biochemical research. Conducting syntheses using these reagents on a micro scale are convenient and produce good yields. However, the control of temperature at the time of addition of reagent is crucial. The temperature must be preset before mixing the reagent and starting material or yield will be lost.

Interestingly, it is possible to entirely change the product of the synthesis to pyrophosphorus compounds solely by lowering the reaction temperature. The conducted solid phase experiments demonstrated that there are several problems in using $\alpha$-haloamines as solid phase reagents.
11. Paper III

11.1. Cyanuric fluoride as fluorinating reagent for phosphorus

The next reagent to be evaluated was cyanuric fluoride. Cyanuric fluoride (2,4,6, trifluoro[1, 3, 5] triazine) (Scheme 22) has been known for some time, and primarily been used for fluorination of carboxylic acids and for peptide coupling reactions\(^{109-112}\). The reagent is a cyclic compound containing three fluorines which all can participate in the reaction (Scheme 23). Cyanuric fluoride is a clear liquid at RT. One advantage of cyanuric fluoride worth mentioning is that it has long shelf-stability compared to that of the earlier mentioned \(\alpha\)-haloenamines. This might be due to the fact that the degradation product from hydrolysis, cyanuric acid, precipitates from the reagent and does hence not significantly influence the purity. One further advantage is that cyanuric fluoride does not form water when it is consumed; the hydroxyl from the phosphonic acid is incorporated within the cyanuric acid.

![Scheme 22: Cyanuric fluoride (2, 4,6, trifluoro[1, 3, 5] triazine).](image)

The reagent was added at RT in the first experiment. However, there was almost no conversion, although by raising the reaction temperature, the reaction proceeded slowly. For the reaction to work properly the temperature had to be raised to 70 - 100°C. The reaction did also proceed without the formation of any pyrophosphonate as by-product. Hence, the need to find conditions that afforded product rapidly was not as pronounced as for the \(\alpha\)-haloenamines (Paper II). The reaction could be left at the appropriate temperature to reach completion.

Investigations of the reagent showed that in the reaction mixture there was either intact cyanuric fluoride or cyanuric acid. The intermediate species containing one or two fluorines were only present at low concentrations according to \(^{19}\)F-NMR investigations. One hypothesis is that the cyanuric fluoride is activated when the first fluorine is reacted and that the other
species react quickly once they are formed. However, the subject has not been fully investigated with regard to mechanisms, and it is not at present possible to definitely determine the mechanism of the reaction.

\[
\begin{align*}
\text{F}_3 & \quad + \quad 3 \quad \text{POH} & \quad \rightarrow \quad \text{OH} \\
\text{N} & \quad \text{N} & \quad \text{O} & \quad \text{N} & \quad \text{N} & \quad \text{OH} \\
\text{F} & \quad \text{O} & \quad \text{F} & \quad \text{OR} & \quad \text{OR} & \quad \text{OR}
\end{align*}
\]

Scheme 23: All three fluorines in the cyanuric fluoride molecule can be transferred to phosphorus during the reaction. R = alkyl.

The selection of phosphorus oxyacids that were used in the α-haloamine studies were used again to evaluate the fluorinating ability of cyanuric fluoride (Table 3). A general procedure for the fluorination of phosphonic acids with cyanuric fluoride in solution is as follows; to dry 1,2-dichloroethane phosphorus oxyacid was added, the solution was then stirred with a magnetic stirring bar under argon atmosphere until the material was dissolved, cyanuric fluoride was then added from a freshly prepared stock solution of cyanuric fluoride (20%) in 1,2-dichloroethane and the argon filled vial was sealed, and finally, the reaction was heated under stirring at 100 °C for 20 h. Conveniently, the reaction can be followed by visually observing the precipitation of cyanuric acid. After the reaction had been cooled to RT, the mixture was filtered through a short column of citric acid and the product collected in the filtrate. The filtration removed all cyanuric acid in the reaction mixture, and the citric acid functioned as a scavenger for unreacted cyanuric fluoride. The products were characterised by $^{31}$P-NMR and the yields are given in Table 3.

Especially in micro scale synthesis, it is convenient that there are few by-products to take into account during the work-up procedure. Since the reaction between cyanuric fluoride and a phosphorus oxyacid does not produce any impurities in significant amounts, the only compounds to consider during work-up was superfluous reagent or unreacted phosphorus oxyacid. The cyanuric fluoride is added in slight excess, but traces of water could consume some reagent if it is not properly handled, for instance, if equipment, solvent or starting material are improperly dried. Phosphonic acids are hygroscopic and might contain considerable amounts of water if measures are not taken to dry them properly.
Table 3: The fluorinated compounds and the yields after reaction with cyanuric fluoride in solution as well as loaded on resin.

<table>
<thead>
<tr>
<th>Phosphorus oxyacids</th>
<th>Phosphorus oxy fluorides</th>
<th>Yield in Solution</th>
<th>Yield on Resin</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1" alt="Phosphorus oxyacids" /></td>
<td><img src="image2" alt="Phosphorus oxy fluorides" /></td>
<td>quant.</td>
<td>quant.</td>
</tr>
<tr>
<td><img src="image3" alt="Phosphorus oxyacids" /></td>
<td><img src="image4" alt="Phosphorus oxy fluorides" /></td>
<td>quant.</td>
<td>quant.</td>
</tr>
<tr>
<td><img src="image5" alt="Phosphorus oxyacids" /></td>
<td><img src="image6" alt="Phosphorus oxy fluorides" /></td>
<td>quant.</td>
<td>quant.</td>
</tr>
<tr>
<td><img src="image7" alt="Phosphorus oxyacids" /></td>
<td><img src="image8" alt="Phosphorus oxy fluorides" /></td>
<td>89%</td>
<td>quant</td>
</tr>
<tr>
<td><img src="image9" alt="Phosphorus oxyacids" /></td>
<td><img src="image10" alt="Phosphorus oxy fluorides" /></td>
<td>77%</td>
<td>quant</td>
</tr>
<tr>
<td><img src="image11" alt="Phosphorus oxyacids" /></td>
<td><img src="image12" alt="Phosphorus oxy fluorides" /></td>
<td>30%</td>
<td>quant</td>
</tr>
<tr>
<td><img src="image13" alt="Phosphorus oxyacids" /></td>
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<td>quant</td>
</tr>
<tr>
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<td>quant</td>
</tr>
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<td>0%</td>
<td>quant</td>
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</tbody>
</table>
The presence of water will consume more cyanuric fluoride than anticipated, and unreacted phosphonic acid will be present after the completion of the reaction. Therefore, a different purification strategy must be chosen. Excess of phosphorus oxyacid is in this scenario easily collected in a short column of alumina through which the product passes fairly quickly but the phosphoric acid does not.

The general procedure above is applicable for most phosphonic acids. The reaction times can be slightly different if, for instance, there are bulky groups giving rise to steric hindrance. However, when the starting material was changed to phosphinic acids, the reaction times could be reduced to about 8 h. By changing the starting material to phosphonic acids, the reaction times were considerably increased to about 72 h.

There is also a third strategy for the purification of the reaction. By connecting the reagent to a solid phase it becomes possible to simply filter the reaction mixture and collect the product in the filtrate. In order to assess the possibilities of making the solid phase reagent, cyanuric fluoride was connected to a Wang resin. This was achieved by connecting one of the fluorine positions directly to the resin\textsuperscript{113}. The cyanuric fluoride was added to the Wang resin in dry chloroform and the vial was sealed. N-Diisopropyl-ethylamine (DIPEA) was added and the reaction was stirred at 20°C for 24 h. The resin was washed 3 - 4 times with dry chloroform, 3 - 4 times with tetrahydrofuran (THF) and evaporated to dryness, affording yields between 80 - 90%. The resin was briefly characterised by IR and by weight of the polymer.

Interestingly, it was found that when the solid phase reagent was reacted with a phosphonic acid, the solid phase reagent was actually even more potent than the reagent in solution. Contrary to what usually takes place, the reaction temperature could be lowered and the reaction times shortened compared to the reaction in solution. The yields were in the range from 95% to quantitative. If the yields were not quantitative it was mainly due to improperly dried starting material. The work-up only involves a filtration and an evaporation of the solvent to isolate the product.

The general procedure for the resin-bound reagent was used under similar conditions as for the solution-based reagent. Although the reagent was used in more excess, generally 5 to 10 eq, since superfluous reagent is of no concern during work-up. Phosphorus oxyacid was dissolved in 2 ml of dry chloroform. The resin-bound cyanuric fluoride was added under argon atmosphere and the reaction was heated to 70°C. After 20 h of slow stirring, the resin was removed by filtration and the product was collected in the filtrate.
11.2. Conclusions

A new reagent for the conversion of phosphonic acids to their fluorinated analogues was developed. The method was exceptionally valuable for small scale synthesis, particularly in the solid phase application. Procedures for the work-up in the case of excess or deficit of reagent in solution, but also for the solid phase application were developed. The method is fast, reliable and produces product of high quality on a micro scale. The occupational safety is significantly improved with the solid phase application due to the ease of the work-up of highly toxic products.
12. Paper IV

12.1. Controlled release of water in small amounts

The phosphonic acids used as starting material in the halogenation reactions in papers II-III above would sometimes preferably be synthesized on micro scale. In this way the synthesized phosphonic acid could be further fluorinated on the same scale and possibly even without purification. Furthermore, the phosphonic acids are interesting compounds in the context of being possible degradation products of CWA. The analyzing and verifying of the presence of such compounds in, for instance, environmental samples can be important proofs of illegal activities concerning CW. These are the reasons to why a method was pursued that could produce the alkyl alkylphosphonic acids, quickly in small scale yet with high purity.

A synthetic method, starting with the alkyl phosphonic dichloride in toluene, and adding one equivalent of water was used for 100 mg scale synthesis. This method was for instance used in the syntheses of the starting materials for papers II and III. The corresponding phosphonic dichloride was dissolved in dry toluene at RT. Water was added in an equimolar amount. The stirring was crucial to the outcome of the reaction and had to be kept at a maximum. Since water was scarcely soluble in toluene in the proportions used during this synthesis, the water would form a droplet at the bottom of the vessel if the stirring was not vigorous. The stirring had to be fast enough to whip the water into tiny droplets or the phosphonic dichloride was hydrolysed into phosphonic acid at the surface of the drop of water, with loss of yield as a consequence.

Scheme 24: The structure of the anhydride intermediate in the synthesis of alkyl alkylphosphonic acids.

Water and phosphonic dichloride forms a cyclic intermediate (Scheme 24) during two hours of stirring. The intermediate is, in the case of
methylphosphonic acids, not freely soluble in toluene and precipitates as thick oil on the glass surface. This was visually observed for the methyl analogues but the ethyl, propyl and isopropyl analogues are more difficult to monitor. The intermediate was reacted with an alcohol which was added in one portion and the temperature was raised to 100°C at first and after 2 h to 110°C. The temperature was kept at 110°C for 2.5 h, meanwhile the mono-alkylated phosphonic acid was formed. This method was producing excellent yields on a 100 mg scale synthesis but if the reaction was downscaled it was difficult to manage. This was mainly due to problems concerning the addition and distribution of the water. There are other methods but they too suffer from poor correlation with small scale synthesis\textsuperscript{116-121}. The new method is also much more rapid than any of the others, an important property when synthesising on a tight time schedule. The analytical references are required in short notice, as earlier mentioned.

It is difficult to add the precise amounts of water when the reaction is downscaled to just a few milligrams. Excess of water will hydrolyze the phosphonic dichloride to phosphonic di-acid with loss of yield and purity as a consequence. The di-acid formed reacts with unreacted dichloride and produces pyrophosphonates. This is also the case if the water is readily solvated. On the other hand, if the water is not soluble enough, the reaction will not proceed, or the reaction will only take place on the surface of water droplets in the organic solvent at a slow rate. The problem is delicate, and experimenting with a few solvents showed that only a few solvents were useful. Most other solvents produce lower yields than toluene, for instance benzene or chloroform. Nitro methane and nitro ethane were found to possess the right properties and were further evaluated. The nitro ethane was found to afford slightly higher yields but was on the other hand more toxic than nitro methane.

There is, however, a way of circumventing the problem of addition of petite amounts of water. Crystal water in inorganic salts may be used to achieve a controlled addition of water. The problem is to find suitable salt / solvent combinations. Eventually, it was found that magnesium sulfate heptahydrate (MgSO\textsubscript{4}\textcdot 7 H\textsubscript{2}O) and sodium tetra borate decahydrate (borax\textcdot 10 H\textsubscript{2}O) were good sources of water. The magnesium sulfate proved to be slightly more efficient than borax. However, magnesium sulfate broadened the signals in NMR-spectroscopy which could obstruct the analysis of the product. The water can conveniently be released by raising the temperature; the first molecule of water departures from the salt at a relatively low temperature and the second one at a slightly higher temperature and so forth. By starting at RT and raising the temperature from RT – to 40°C, the water is released in the right amounts and time.
Table 4: The various phosphonic acids synthesized and the respective yields.

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<tr>
<th>19</th>
<th>28</th>
<th>46</th>
<th>59</th>
<th>71</th>
<th>42</th>
<th>52</th>
<th>71</th>
<th>72</th>
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<td>52</td>
<td>59</td>
<td>49</td>
<td>69</td>
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<td>69</td>
<td>53</td>
<td>23</td>
<td>11</td>
<td>3</td>
<td>38</td>
<td>62</td>
<td>60</td>
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</table>
By this precise control of the addition of water the formation of the intermediate can be controlled. After the first reaction step, the formation of the cyclic intermediate, the alcohol was added. More reactive alcohols generated some dialkyl alkylphosphonate impurities. Since this was not observed to the same extent if more sterically hindered alcohols were used, a probable explanation might be found in the cleavage of the intermediate. The first alcohol reacts with, and opens, the cyclic intermediate. If the alcohol is bulky, the phosphorus atom to which the new entity is attached becomes significantly more sterically hindered than the other phosphorus atoms of the intermediate (Scheme 24). This directs the nucleophilic attack of the second alcohol to a second phosphorus atom etc. If, on the other hand, the alcohol is not bulky the steric difference between the phosphorus atoms is not large enough to firmly direct the nucleophile to a second phosphorus atom. This leads to the formation of alkylphosphonic di-acid and of dialkyl alkylphosphonate. The problem is similar to the pyrophosphonate formation discussed in papers II and III resulting in the loss of two equivalents of possible product.

The general procedure for the synthesis of alkyl alkylphosphonic acids is as follows. The alkylphosphonic dichloride was dissolved in dry nitroethane. Magnesium sulfate heptahydrate was added and the reaction vessel was closed. The reaction was heated to 40°C for 15 min in an oil bath. The vessel was then opened but care had to be taken due to the risk of overpressure. Dry alcohol was added, the vial closed and the reaction allowed to proceed for 120 min at 60°C. The solvent was removed by evaporation and diisopropylether is added. The mixture was filtered through a small column of alumina (40 mg) to remove residual alkylphosphonic acid. The filter was washed with diisopropylether and the combined ether solutions are filtered once more through deactivated alumina (20 mg). The solvent was removed by evaporation and the crude product isolated.

The products were formed in moderate to good yields with high purity, with a few exceptions. The isopropylphosphonates suffer from lower yields than the other phosphonates and the pattern seen in the table indicate that steric reasons are most probable (Table 4). This was despite of the fact that the reaction temperature of the second reaction step, in the case of isopropylphosphonic acids, was increased to 90°C. However, when the reaction times were significantly increased for the second reaction step the yield of, for instance, 1,2,2-trimethylpropyl isopropylphosphonic acid became 85%. The reaction time was in this case 20 h. But since the aim of this method primarily was to develop a fast one-pot synthesis, the reaction times were not optimised with respect to the yields. Several of the entries in Table 4 can though be anticipated to afford higher yields if allowed to react for 20 h or longer.
12.2. Conclusions

A method to synthesise alkyl alkylphosphonic acids of high purity in a short time is demonstrated. The yields are moderate, but in this context, high yields are not as important as they might be in other situations. The need for a generic and reliable method that affords the reference substances quickly and with control of the by-products is the main issue. Furthermore, the yields can be significantly increased for several entries if the reaction times are optimised individually.

The difficulty of adding the precise amount of water when only a few μL are required is circumvented. The release of crystal water is superior compared to addition of free water in two ways. It is easier to add the right amount of water and the release can be controlled in a more precise manner.
13. Prospective research

There is always a potential to improve synthetic methods and it is certainly true even for these substances. The optimization of the crystal water method (Paper IV) with respect to yields instead of reaction time is one example. The solid phase chemistry is another possible area in which there might be opportunities for continued research. A major step forward in the synthesis of highly toxic compounds would be to synthesize the nerve agents on a solid support and to release them when they are to be used in research, in the amounts needed. The occupational safety would certainly gain from such a development. This could, for instance, diminish the need to keep stocks of CWA in the facility which, besides the issue of occupational safety, can be foreseen to have other advantages. The HR-MAS technique in $^{31}$P-NMR could be extremely powerful in a solid phase synthesis in order to analyze the outcome of reactions directly on the resin. Since there is no phosphorus in most resins, the spectra can be anticipated to be fairly easy to assign.

There are also the obvious challenges in developing new methods for the micro scale synthesis of substance classes such as the V-compounds or the tabun analogues. The tabun analogues are interesting compounds to study due to the difficulties in the treatment and reactivation of inhibited AchE. The VX analogues are interesting from the perspective of their extreme toxicity and that even a single mg can potentially be lethal. These substances have a slightly different chemistry, tabun being a phosphoramidate and VX being a phosphonothiolate. This means that other methods need to be developed for the small scale synthesis of these compounds compared to the phosphonofluoridates. To the best of my knowledge, the synthetic methods are scarcely explored on micro scale.

However, all new methods should be carefully evaluated not to be convenient large scale methods. Furthermore, the developed methods should not facilitate the circumvention of export control and the schedules of chemicals of CWC. The issues of proliferation must always be taken seriously and proper measures taken to avoid spreading sensitive material.
14. Concluding remarks

This thesis describes some new and improved methods for the small scale synthesis of phosphonic acids and some of their halogenated analogues. The methods contribute in making the occupational safety higher as well as producing the substances on a smaller scale. A generic high yielding method for synthesis of radiolabelled alkyl methylphosphonofluoridates was developed. The method is focuses on high yield and on minimizing the handling of the radiolabelled materials. α-Chloroenamines and α-fluoroenamines were assessed as halogenating reagents for phosphorus oxyacids. The reagents are extremely fast and the reactions are completed within minutes. Cyanuric fluoride was utilized to fluorinate phosphorus oxyacids with good results in solution. The reagent was also loaded on a resin and used as solid phase reagent. The solid phase method is not only yielding product of exceptional quality but also increases the occupational safety considerably. The alkyl alkylphosphonic acids were synthesized with a new method of adding water to the reaction mixture. By using the crystal water bound to certain inorganic salts, the release of petit amounts of water can be controlled.

If the intent is to produce these highly toxic compounds in amounts above 1 g, the methods developed since long time are both cheaper and easier to utilize. However, if the purpose is to synthesize analytical references, substances to be used in enzyme inhibition experiments, or in toxicological experiments on a milligram scale, the methods presented are superior to other published methods.

Finally, I truly hope that sometime in the future, the protective research regarding CWA will not be necessary, and that no one will have to synthesize such toxic compounds. I am, however, afraid that this is just a dream.
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16. References


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