FACULTY OF BIOSCIENCE ENGINEERING

DEVELOPMENT OF ONE-POT PHOSPHONYLATION REACTIONS FOR PYRIDINIUM SALTS AS POTENTIAL ENZYME INHIBITORS

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Preamble

Deze Master thesis werd geschreven tijdens de COVID-19 uitbraak van 2020. Naar aanleiding van de restricties opgelegd door de Belgische overheid werden alle wetenschappelijke praktijken van de SynBioC vakgroep van Universiteit Gent op 18 maart opgeschort. Bovendien werd verder contact met de promotor en tutors beperkt tot online communicatie via e-mail en Microsoft Teams. De impact van deze implicaties op dit eindwerk zal naar het einde van deze thesis verder worden besproken in Hoofdstuk 5. Deze preambule werd in overleg tussen de student en de promotor opgesteld en door beiden goedgekeurd.

This thesis was written during the COVID-19 outbreak of 2020. Following restrictions employed by the Belgian government, all scientific practices at the SynBioC research group from Ghent University were suspended as of March 18. In addition, further contact with the promotor and tutors was limited to online communication via e-mail and Microsoft Teams. The impact of these implications on this thesis will be further elaborated near the end of this thesis in Chapter 5. This preamble was drawn up in consultation between the student and the promotor and approved by both.

Abstract

In this Master's dissertation, a new method for the phosphonylation of azaheterocycles was developed and optimised. In recent decades, this class of compounds has sparked a great deal of interest due to its wide variety of applications among which its enzyme inhibiting capabilities, making it an attractive class for future medicinal and agricultural applications. In preliminary research at the SynBioC research group, one-pot diphosphonylation methods for α,β-unsaturated imines and quinolines were developed, characterised by high yields and low reaction times. Furthermore, the establishment of a one-pot reaction was until now barely explored for the synthesis of diphosphonylated azaheterocycles, leaving the door open for additional research.

Therefore, this Master's thesis focuses on a one-pot diphosphonylation method for pyridine and pyridinium salts. However, contrary to other phosphonylation reactions for pyridine and pyridinium salts, no substituents are applied to the pyridine ring and the addition of multiple phosphonate groups will occur in one pot without reactivating the nitrogen atom. Eventually, a one-pot triphosphonylation method was developed and optimised for N-benzylpyridinium bromide. This was rather unexpected as this compound was never before mentioned in literature. Unfortunately, due to the COVID-19 crisis, this method was only applied to 2 other pyridinium salts for which the results were inconsistent. For this reason, no conclusion can be drawn yet about this method's overall applicability towards pyridinium salts.

Preface

Where to begin. These past few years have been quite the roller coaster. I've made valuable friendships, gathered a great amount of valuable knowledge and skills and developed a passion for chemistry and the life sciences. For these reasons, I'm delighted to complete my studies as a Master in Bioscience Engineering and start a new and exciting chapter of my life. For starters, I would like to express my gratitude to my promotor, Prof. Dr. ir. Christian V. Stevens, for his excellent guidance and supervision. Your continuously positive attitude and advice greatly motivated me even at challenging times. Moreover, your interesting courses inspired me to choose a thesis at SynBioC of which I am very grateful. Thank you for everything!

Manuel, when you left Spain for Belgium you were probably thinking about all the new beers you were going to try. However, besides beer you also suddenly got assigned a Master student to help him with his thesis. And what a wonderful job you did! Your kind and approachable personality made me motivated to come to the lab in the morning and to stay till late at times. You were always eager to help and figure out every problem with me, no matter how challenging. Sadly, you had to go back home a few months before I could finish my thesis, but I am genuinely grateful for the knowledge and skills you passed down to me.

Andreas, when Manuel had to go back to Spain I first felt kind of alone. Luckily, I eventually had you to fall back on and even though your time as my tutor was rather brief due to the COVID-19 crisis, I want to thank you for helping me reach the finish line.

Furthermore, I would like to thank all my fellow thesis students at SynBioC. Together we made the overall atmosphere at the 4^{th} floor simply amazing. Giving me much joy to work along side you, helping each other out when necessary. Next, I would like to thank my brother for giving me the little push I needed to go and study Bioscience Engineering. At the time I didn't think this was in any way significant, but when looking back I'm really grateful for this. Thank you for all your advice, both related and unrelated to my studies. Also a big thanks to VLK, my student association, where I made friends that will remain with me for the rest of my life. I would like to thank them all for these 5 wonderful years. You know who you are!

Finally, I would like to thank all the amazing people I got to meet. This whole experience has impacted me greatly. Thank you for all the memorable moments. It was amazing!

Nick De Smedt, June 2020

Table of contents

Abbreviations

AHP	Azaheterocyclic phosphonates
AMPA	*R*-Amino-3-hydroxy-5-methylisoxazole-4-propionic acid
DAP	Dialkyl phosphite
DAPTMS	Dialkyl trimethylsilyl phosphite
DBU	1,8-Diazabicylo[5.4.0]undec-7-ene
DCM	Dichloromethane
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEP	Diethyl phosphite
DEPTMS	Diethyl trimethylsilyl phosphite
DMP	Dimethyl phosphite
DMPTMS	Dimethyl trimethylsilyl phosphite
dppp	1,3-Bis(diphenylphosphino)propane
IR	Infrared Spectroscopy
KF	Karl Fischer
KHDMS	Potassium bis(trimethylsilyl)amide solution
LC-MS	Liquid Chromatography–Mass Spectrometry
LDA	Lithium diisopropylamide
MCR	Multicomponent reactions
MeCN	Acetonitrile
MS	Mass Spectrometry

MW	Microwave
NMR	Nuclear Magnetic Resonance
PAP	3-Phosphonyl-aminoalkylphosphonate
PEM	Proton exchange membrane
PEMFC	Proton exchange membrane fuel cells
TEA	Triethylamine
THF	Tetrahydrofuran
TAP	Trialkyl phosphite
TEP	Triethyl phosphite
TLC	Thin Layer Chromatography
TMP	Trimethyl phosphite
TMSCl	Chlorotrimethylsilane
TS	Transition state

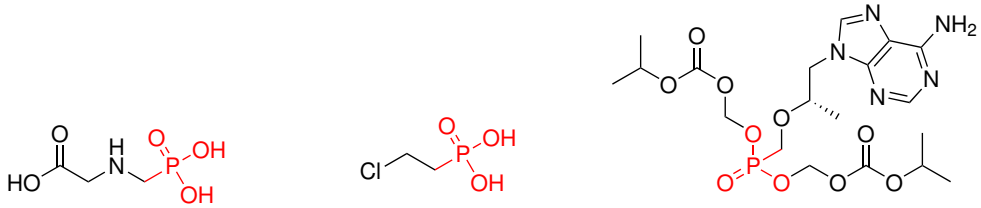
Chapter 1

Scope & Goal

1.1 Scope

With the discovery of phosphorus taking place in the 17th century and the development of its most important reactions at turn of the 20th century, phosphorus chemistry has been perceived as one of more fully developed branches of chemistry. Nonetheless, phosphorus remains one of the key compounds in life's processes. Therefore, this specialised branch of chemistry has had a rebirth in recent decades.[1] This thesis will focus on one specific class in organophosphorus chemistry: *phosphonates*.

Phosphonates are organophosphorus compounds characterised by a stable carbon-phosphorus or C-P bond, which usually resists biochemical, thermal, and photochemical decomposition and a $P(O)(OR)_2$ group (R = alkyl, aryl).[2] These compounds as well as their corresponding acids are used in a wide variety of industrial products which include chelating agents,[3] scale inhibitors,[4] personal care products, detergents and water treatment additives.[5] In addition, because of their important biological activity this class of compounds has also been of high interest in medicinal and agricultural sectors. Compounds such as glyphosate **1**,[6] the main active molecule in the famous herbicide "Roundup" from Monsanto© , or ethephon **2**,[7] a common regulator for plant growth by stimulating ethylene production, and tenofovir disoproxil (Viread) **3**,[8] an antiviral nucleotide analogue used in anti-HIV therapy, are just some famous examples of phosphonates.

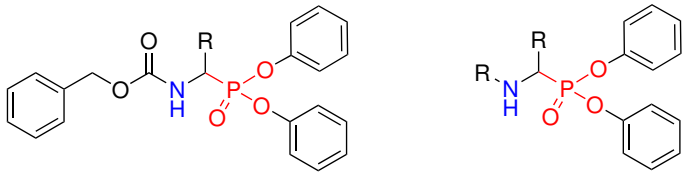


1 **2** **3**

Scheme 1.1: Examples of some of the more recognised phosphonates and phosphonic acids: glyphosate **1**, ethephon **2**, tenofovir disoproxil **3**.

1.1.1 α-Aminophosphonic acids and their esters

An important class of these phosphonates are the α-aminophosphonates and their corresponding acids. These are structural analogues of amino acids where the carboxylic acid has been replaced by a phosphonic acid or phosphonate group. Because of a similar tetrahedral structure, analogues to the transition state during peptide hydrolysis, these compounds are able to mimic this hydrolysis process and thus affect the activity of the cell by acting as an enzyme inhibitor.[9] As a consequence they are used as medicinal, antibacterial, plant growth regulatory and neuromodulatory compounds.[10] A good example of the inhibitory effects of this class are the diaryl esters of α-aminophosphonates (see Scheme 1.2).[11]



Scheme 1.2: Derivatives of α-aminophosphonate diaryl ester inhibitors which are known to potently and selectively inactivate serine proteases.[12]

The major applications for this type of phosphonate (**4**) arise from their ability to potently and selectively inhibit serine proteases.[12] This happens by irreversibly binding to the enzyme due to similarities with the transition state of peptide bond cleavage observed in enzymatic reactions. Their mode of action is shown below in Figure 1.1. Here, the α-aminoalkylphosphonate derivatives provide specific interactions with the protease due to the nucleophilic hydroxyl group of Ser195, located in the active site. This allows an attack on the electrophilic phosphorus of the inhibitor, thereby permanently blocking the active site and consequently causing irreversible inhibition of the enzyme.[11]

Due to their high potency and complete selectivity towards serine proteases, the demand for these types of phosphonates is increasing. In addition, the selectivity of these inhibitors can be easily adjusted by derivatisation. As a result, these α-aminophosphonates are already used as activity based probes for serine protease-like activity screening and as covalently reactive antigens for the development of catalytic antibodies.[13,14]

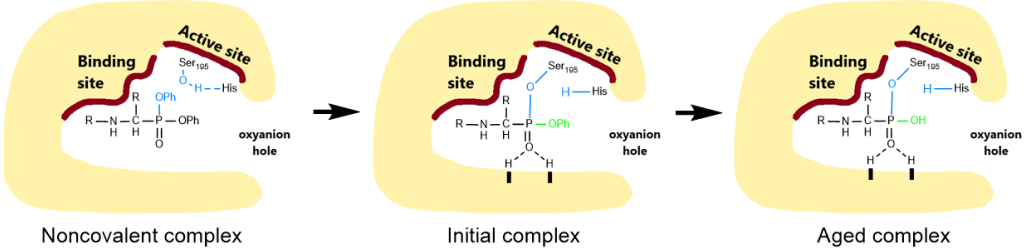
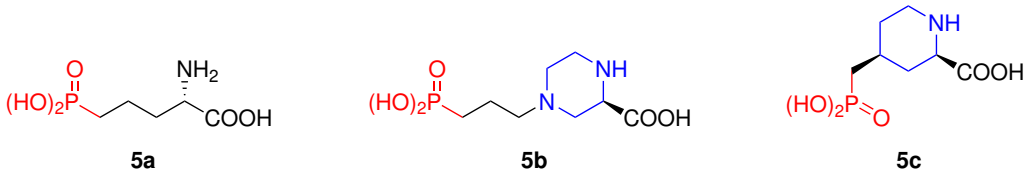


Figure 1.1: Mechanism of serine protease inhibition by α-aminoalkylphosphonate diphenyl esters.[11]

1.1.2 Azaheterocyclic phosphonates

Another group of phosphonates are the azaheterocyclic phosphonates or AHPs. These structures are ever-present in nature, hence they are often found in biologically active compounds with applications in agrochemical and medicinal chemistry.[15] The interest in AHPs came from synthesising phosphonylated analogues of glutamate, when a considerable enhancement of antagonist potency was achieved by synthesising conformationally restricted analogues of **5a**. One method incorporates the amino group in a ring structure to form a phosphonylated azaheterocycle (**5b** and **5c**), illustrating the potential of the class (see Scheme 1.3).[16,17]



Scheme 1.3: Phosphonylated analogues of glutamate: (*S*)-2-amino-5-phosphonopentanoate (**5a**), (*R*)-3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (**5b**) and *cis*-4-phosphonomethyl-2-piperidine carboxylic acid (**5c**).

Research in new AHPs and their biological activities have already been reviewed multiple times. Some enzyme inhibitors such as pyridylphosphonates have already been found, making them highly interesting compounds. Potential uses include antiproliferating and antiplatelet activating properties of 2-pyridylphosphonates. Also quinolylphosphonic acid derivatives with potent antagonistic activity against AMPA receptors have been found.[18] These *R*-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (or AMPA) receptors are ionotropic transmembrane receptors for glutamate that mediates fast synaptic transmission in the central nervous system.[19] Therefore, these quinolylphosphonic acid derivatives can be used as potential therapeutic agents in the acute treatment of stroke and head trauma.[18]

However the applications of AHPs are not limited to biological applications. To illustrate this, one can look at its use in proton exchange membrane fuel cells or PEMFCs.[20] These fuel cells are interesting because of their high power density and high power to weight ratio. Here, the key material for the operation of PEMFCs is the proton-exchange membrane or PEM. While usually these membranes are made of organic polymers, the proton transport properties of these membranes strongly depend on their water content. As a consequence, temperatures are limited to around 90°C thus lowering overall performance.

AHPs can offer a solution to this problem. Certain derivatives of azaheterocyclic aromatic diphosphonates of benzimidazole and benzotriazolepolybenzimidazole are already known to be promising proton carriers because of their good proton donating and accepting properties and have proven to be good alternatives for sulphonic acid groups due to their high proton conductivity, oxidation resistance and better thermal stability. The result is a fuel cell that is able to operate above 100°C which vastly improves the performance of the fuel cells due to faster electrode reaction without CO-poisoning of the Pt-electrocatalyst.

1.1.3 Biphosphonic acids and their esters

A final group worth mentioning are the biphosphonic acids. Due to the presence of 2 phosphonate groups, these compounds are known to prevent the loss of bone density, making it an ideal drug for Paget's disease, osteoporosis and similar diseases. Normally bone tissue undergoes a constant remodelling by osteoblasts, creating bone, and osteoclasts, removing bone. When this homeostasis is disrupted because of diseases, biphosphonates can be used to inhibit the osteoclasts and thereby stop or slow down the decrease of bone density, as is shown in Figure 1.2.

The 2 phosphonate groups arrive at the bone tissue by coordination with calcium ions. Since these ions are mostly transported to the bones, high accumulation of biphosphonates at the bone tissue is evident. After reaching the bone tissue, these molecules will attach themselves to the osteoclasts and enter them. Here, they start to disrupt intracellular enzymatic reactions needed for bone resorption.[21] Although their effects to inhibit these osteoclasts have been proven, these biphosphonates have been getting less popular because of some harmful side effects.[22] However, when a nitrogen is present in the molecule, these side effects are strongly reduced and the osteoclast inhibition is more potent.[23]

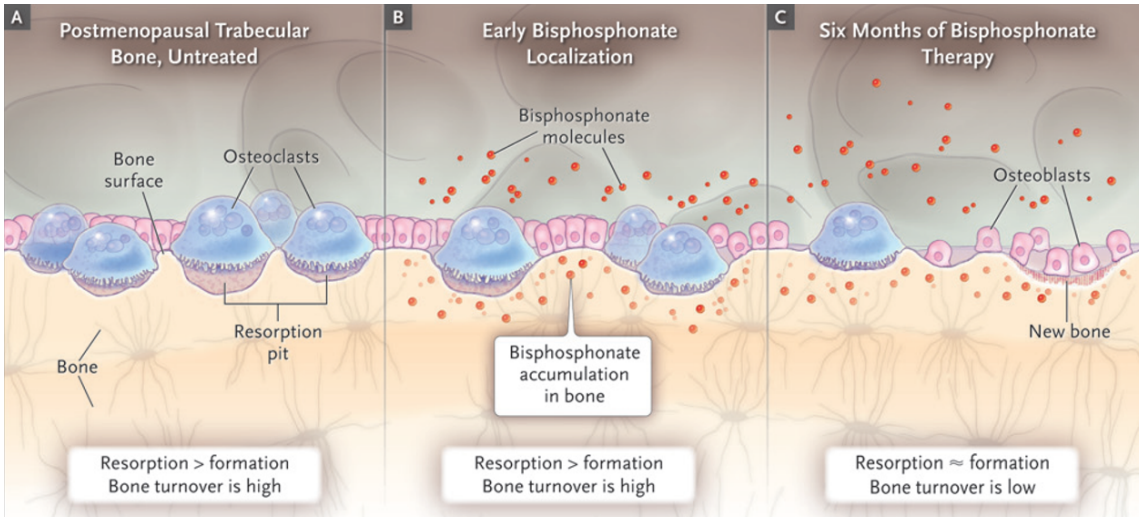


Figure 1.2: Cellular elements involved in postmenopausal trabecular bone turnover before and during bisphosphonate therapy.[21]

Besides applications concerning bone diseases, reports of modified biphosphonates have been published where they are shown to have strong actions against the in vitro proliferation of several protozoan parasites.[24] Other applications include antimalarial[25] , bactericidal[26] and anticancer activities.[27,28]

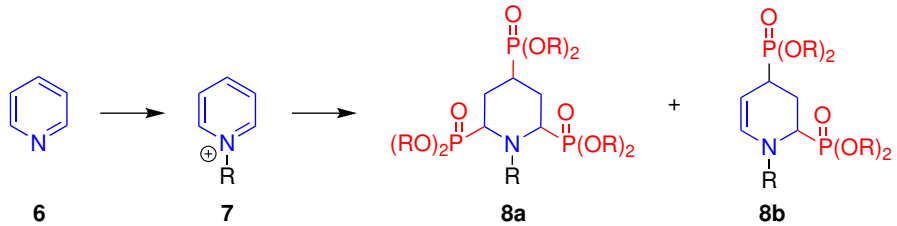
1.1.4 Conclusion

After reading the previous sections it should be clear that phosphonates can offer a wide variety of applications. All arising from its durability, stability and ability to mimic amino acids causing irreversible enzymatic inhibition. Since enzymes are characteristic to all life on earth, the areas of application are thus immense. With so many possibilities, there will always be room for improvement as long as the search for new types of phosphonates continues. The focus of this thesis will be on the merger of 2 important classes, *azaheterocyclic phosphonates* (see Section 1.1.2) and *biphosphonates* (see Section 1.1.3).

1.2 Goal

The need for producing new phosphonylated compounds has already been well established in the previous section and while the α-amino phosphonates have already extensively been researched, the interest in new azaheterocyclic compounds is still increasing. The amount of synthetic pathways leading to these compounds are already numerous and recently some attention has gone towards finding direct one-pot phosphonylation methods. As other pathways make use of permanent or temporary modification of the starting material to achieve phosphonylation. The straightforward one-pot approach has led to some major discoveries. Therefore, this master thesis will pick up on this research by developing and optimising a synthetic route for a fast one-pot multiphosphonylation of pyridine derivatives.

Pyridine (**6**) and was chosen as the starting material because of its, and the corresponding piperidine analogue, highly potent biological activity and is, as a result, often found in naturally occurring heterocycles. Tri- or diphosphonylated piperidine (**8a, 8b**) would make a valuable addition to the collection of AHPs as it might posses new activities. Aside from that, it would also be interesting from a preparatory point of view as pyridine is more readily accessible and cheaper to synthesise than other azaheterocycles.



Scheme 1.4: General reaction of a one-pot di-/triphosphonylation of pyridine with R = alkyl, aryl (see Section 2.3).

Chapter 2

Literature overview

As the topic of this thesis is the phosphonylation of pyridine, this literature overview will consist of different methods for synthesising phosphonylated pyridine rings, followed by a critical review of these types of reactions. However, since phosphorus chemistry holds a specific place within organic chemistry a small introduction into phosphorus chemistry will first be given as well as a summary of the more known phosphonylation methods. Finally, because this thesis is based on previous research, a short summary of this research will be given to provide more context around this topic.

2.1 An introduction to organophosphorus chemistry

Organophosphorus chemistry holds its own specific place in organic chemistry, with the carbon-phosphorus bond playing a crucial role. Due to the different physical and chemical properties of phosphorus, these compounds are found in a broad range of fields. These include variable oxidation states, multivalency, asymmetry and metal-binding properties. As a result, this class is considered as a versatile and unique branch in organic chemistry.[29]

Organophosphorus compounds are found in a variety of structures, which are centered around the phosphorus atom. These structures are characterised by their own specific coordination numbers (1 to 6) and oxidation states (III and V). The coordination number of an atom is the amount of atoms that are bound via a single bond or multiple bonds to a specific atom and differ from 1 to 6 for phosphorus. Secondly, the oxidation state of an atom is the degree of oxidation (i.e. loss of electrons) of an atom in a chemical compound.

An example is the oxidation of phosphines to phosphine oxides ($R_3P(O)$), changing the oxidation state of the phosphorus atom from trivalent P(III) to pentavalent P(V). Overall the oxidation from P(III) to P(V) is generally quite easy, but the reduction of the oxide back to the 3-coordinate form has proven to be more difficult.

Another aspect worth mentioning is the stability of the carbon-phosphorus bond (or C-P bond) in organophosphorus compounds.[29,30] Regardless of the functionality, these bonds are quite thermodynamically stable. The average heat of dissociation is 272kJ/mol for a tetracoordinated C-P bond. Especially phosphine oxides and phosphonates have proven to be very stable as they can be safely heated to temperatures of 150 to 200°C. However, this is not always the case: Tricoordinated compounds, while possessing stable C-P bonds, can be thermally unstable. Nevertheless, it can be stated that the C-P bond has a good stability in most chemical reactions so it would most likely survive the experimental conditions to which it is subjected, including acid and base hydrolysis.

To conclude the introduction into organophosphorus chemistry it is worth mentioning some analytical methods used for identifying these compounds. The most popular technique is nuclear magnetic resonance or NMR which can be measured by ^{1}H- and ^{13}C-NMR spectra. But because phosphorus itself is also NMR-active (^{31}P has a spin of $\frac{1}{2}$), ^{31}P-NMR is also possible. This gives much needed structural information in addition to ^{1}H- and ^{13}C-NMR spectra. In Figure 2.1, the ranges of the chemical shifts (δ) for several types of organophosphorus compounds are given (see also Figure A1, Appendix).[31]

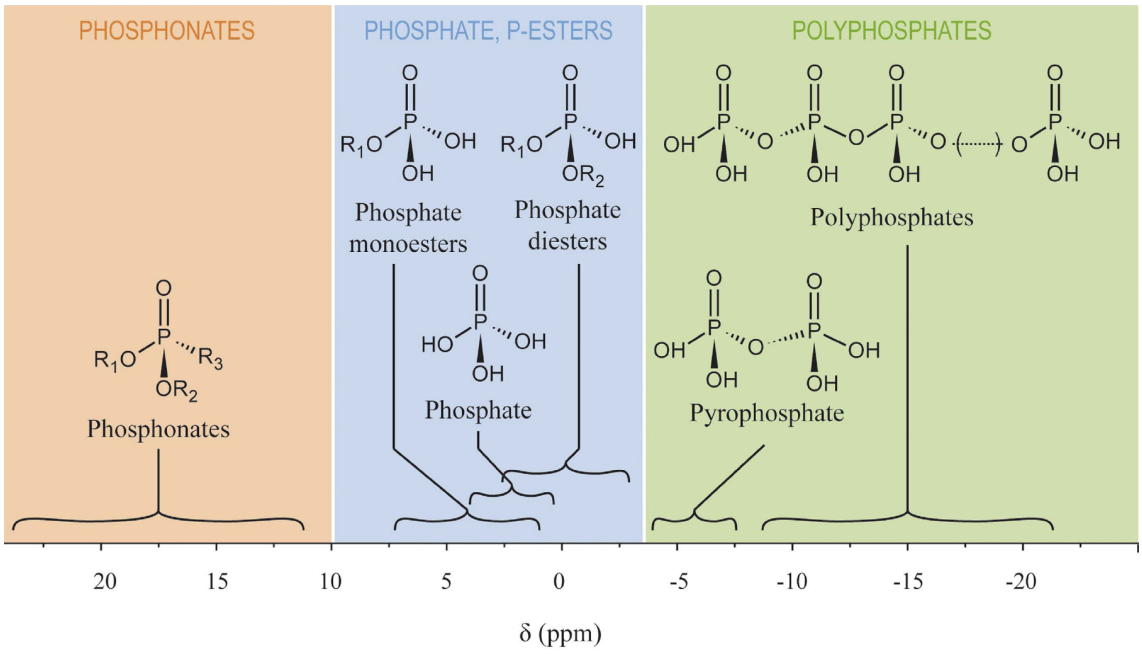


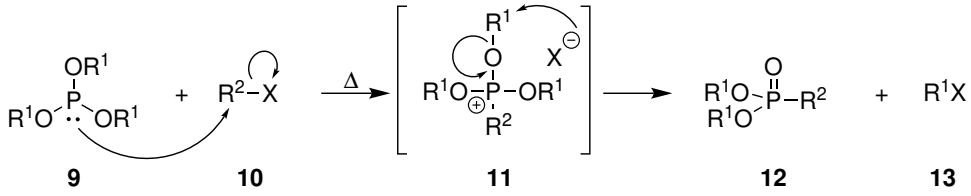
Figure 2.1: Chemical shifts (δ) for several types of organophosphorus compounds.[32]

2.2 Phosphonylation methods

When trying to synthesise phosphonylated azaheterocyclic compounds, 2 general approaches are possible: a direct phosphonylation of a heterocyclic system or a ring forming reaction where already phosphonylated substrates go trough a cyclisation reaction. For the direct phosphonylation reactions some general methods will be discussed which are not limited to azaheterocycles, followed by some ring forming reactions. In addition, some possible catalysts will be discussed for the phosphonylation of (aza)heterocycles.

2.2.1 Nucleophilic phosphonylation

Synthetic methods for producing phosphonates have been known since the end of the 19th century. The most well-known methods for the direct synthesis of phosphonates are the Michaelis-Arbuzov (or Arbuzov) and Michaelis-Becker reactions. The Arbuzov reaction consists of a nucleophilic substitution where a trivalent nucleophilic phosphite ester (**9**) initiates an attack on an electrophilic alkyl or aryl halide (**10**) to give a phosphonium salt (**11**). Next, this intermediate reacts with the remaining anion to produce the desired phosphonate (**12**) and a new alkyl or aryl halide (**13**) (see Scheme 2.1).[33–35]

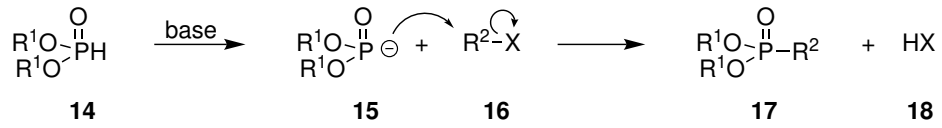


Scheme 2.1: General scheme of an Arbuzov reaction (R = alkyl, aryl).

The Arbuzov reactions are highly dependent on the substrate and the reagent. Primary alkyl halides are more reactive than secondary, while tertiary are almost non reactive. The best leaving group is iodide, followed by bromide and chloride. For the reagent, the most important factor is the presence of electron donating groups which speed up the reaction rate since the attack of the nucleophile is the rate determining step in the reaction. When aryl groups are used, stable phosphonium salts (**11**) can be formed and the reaction stops. Temperatures from 120°C to 160°C are considered normal for Arbuzov reactions.

For α-bromo- and α-chloroketones, side reactions such as the Perkow reaction can take place. In this case, the nucleophilic phosphorus atom (**9**) will interact with the carbonyl to form an enol phosphate. However, increasing the temperatures will often be enough to favour the Arbuzov product.

Similar to the Arbuzov is the Michaelis-Becker reaction, which starts from a dialkyl phosphite (**14**) which is deprotonated by a base to give a good nucleophile (**15**). Next, the nucleophilic reagent attacks on a n-alkyl halide (**16**) producing a phosphonate (**17**) and a hydrogen halide (**18**). However, when compared to the Arbuzov reaction the overall yields are often lower. Instead of deprotonating the dialkyl phosphites *in situ*, sodium or lithium salts of these phosphites can be used as effective nucleophiles. This removes the need for external bases.[36]

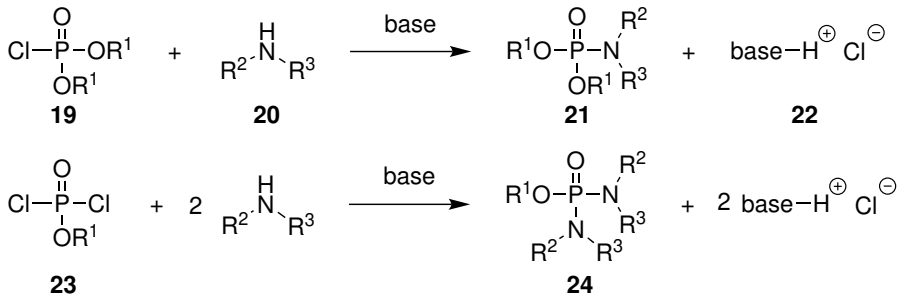


Scheme 2.2: General scheme of a Michaelis-Becker reaction (R = alkyl, aryl).

A final class of nucleophilic phosphonylating agents are the silylated phosphites, more specifically tris(trimethylsilyl) phosphites and dialkyl trimethylsilyl phosphites. Here, the trimethylsilyl group is used for stabilisation of the intermediate, vastly increasing the reaction time but the instability of the reagent as well. However, while being prone to hydrolysis, this rather unstable class of reagents has a very unique reactivity towards α,β-unsaturated imines and some azaheterocycles (e.g. quinoline). This because they are able to induce a 1,4-1,2 tandem addition of phosphonate groups in the presence of an acid (see Section 2.5). Because of their high reactivity, most phosphonylation reactions are performed at room temperature or even lower. However, it has to be noted that trialkyl phosphites have also been known to achieve some of these reactions.[37]

2.2.2 Electrophilic phosphonylation

Although most direct phosphonylating reactions start from the attack of a nucleophilic phosphonylating agent, electrophilic phosphonylation reactions are also possible. For these kind of reactions mostly mono- (**19**) or dichlorophosphates (**23**) are used. In the presence of a nucleophile (**20**) (e.g. alcohol or amine) phosphonates (**21,24**) are formed. The addition of a base is often used to deprotonate the nucleophile and facilitate the attack. Furthermore, some one-pot reactions have also been published where chlorophosphates are formed *in situ* from dialkyl phosphate, CCl_4 and TEA.[15] To avoid the use of the carcinogenic CCl_4, Brands et al. published a more environmental friendly method, where the mixing of NaOCl and NaOH with diisopropyl phosphite produced the desired chlorophosphate.[38]

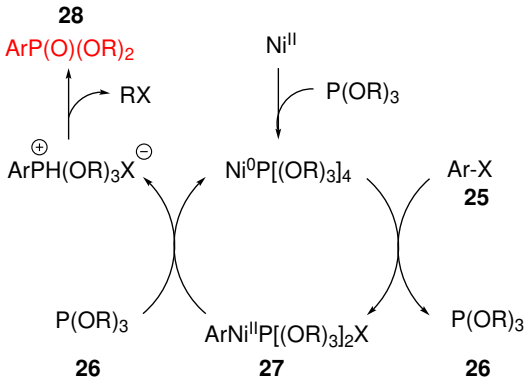


Scheme 2.3: Electrophilic phosphonylation reactions of mono- (**19**) and dichlorophosphates (**23**) with an amine (**20**) (R = alkyl, aryl).

2.2.3 Metal activated or catalysed phosphonylations

Phosphonylation reactions can also be achieved with catalysts. These involve aryl and alkenyl halide substitutions into phosphonates by transition metal complexes. Possible metal catalysts include nickel and palladium. In addition, metal complexes can also be used as activating groups using stoichiometric amounts.

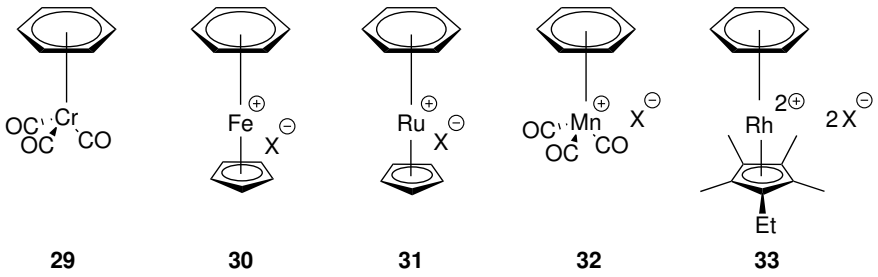
Phosphonylation reactions for aryls using nickel as a catalyst stem from a combination of a cross coupling and the Arbuzov reaction.[39,40] When optimising the catalyst, Zhao et al. found that using a [Ni(dppp)Cl_2] complex (dppp=1,3-bis(diphenylphosphino)propane) significantly lowered the reaction temperature and eliminated the need for an external reductans.[41] However, with the use of higher reaction temperatures, the need for such ligands could be avoided since the high temperatures promoted the reduction of Ni(II) to Ni(0) as well as the oxidative addition of the aryl halide (**25**). This meant nickel could be added as a salt (e.g. $NiCl_2$). After reduction to Ni(0), a new complex is formed by an oxidative addition which introduces an aryl and halide on the metal complex (**27**). Next, a nucleophilic cleavage of the metal carbon σ-bond takes place to produce an aryl phosphonate (**28**) as seen in the Arbuzov reaction.[40] In addition to trialkyl phosphites (**26**), this catalyst is also applicable with other types of phosphonylating agents, such as dialkyl phosphite. Besides nickel, there have also been publications where copper has been used as a catalyst for the phosphonylation of halo substituted aryls in a similar manner.[42]



Scheme 2.4: A nickel catalysed phosphonylation reaction of aryls using a cross coupling and the Arbuzov reaction.

Since the main problem with phosphonylation of pyridine is the high stability of the aromatic ring, which does not favour the addition of a nucleophile, activation of the nitrogen atom is necessary. To facilitate these reactions, a transition metal complex can be used to withdraw electrons from the pyridine ring promoting nucleophilic addition to the aryl. Compared to other electron withdrawing bonds attached via σ-bonds, these metal complexes have less influence on the regioselectivity of these reactions. For 6-membered rings, hexahapto ligands (η^6) are necessary since they fill 6 coordination sites through 1 point of attachment.

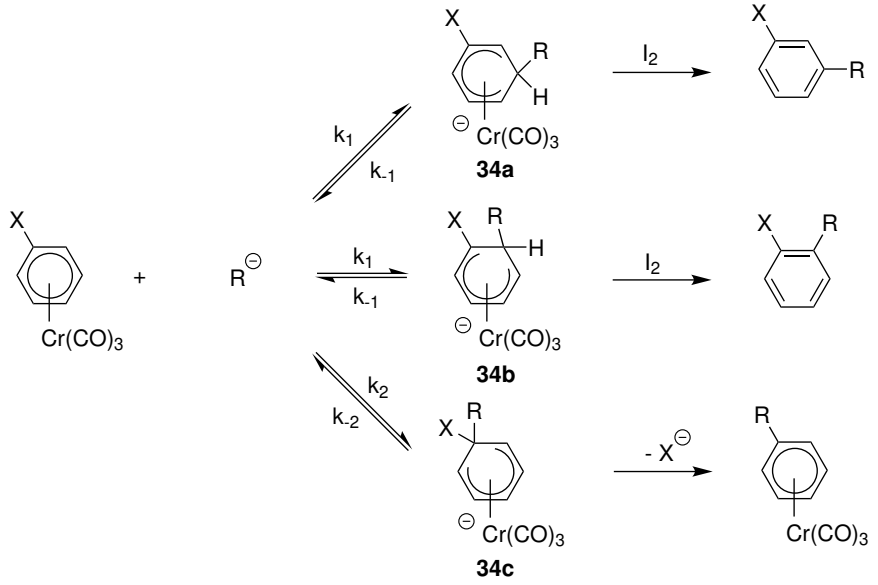
Some examples of these arene π-complexes are η^6-arene-$Cr(CO)_3$ (**29**), (η^5-c_p)Fe(II) (**30**), (η^6-arene)(η^5-c_p)Ru(II) (**31**), η^6-arene-$Mn(CO)_3$ (**32**), and (η^6-arene)(η^5-ethyltetramethyl-c_p)Rh(III) (**33**) (see Scheme 2.5).[43]



29 **30** **31** **32** **33**

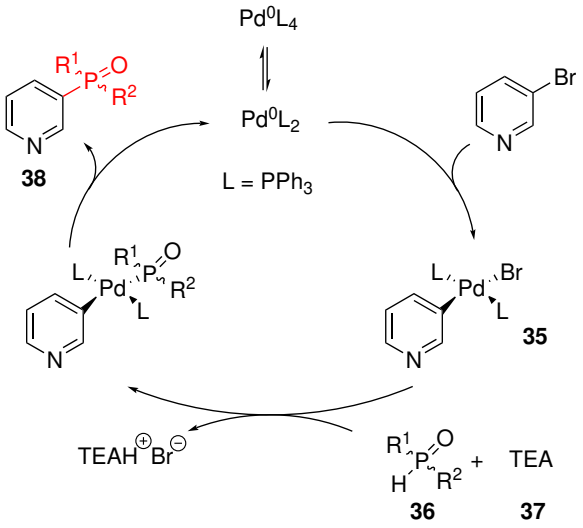
Scheme 2.5: Examples of arene-metal π-complexes.

These metal complexes can be divided into 3 classes: stabilised carbanions (pK_a<18), reactive carbanions(30>pK_a>20) and very reactive carbanions (pK_a>30). The stable carbanions are known to give easily reversible reactions, while more reactive carbanions give complete conversion at lower temperatures. Rearrangement occurs when the mixture is heated. Finally, carbanions can also be very reactive which leads to irreversible additions to the aryl (see Scheme 2.6). Overall it can be stated that an addition to an unsubstituted position (k_1; **34a, 34b**) is kinetically less favoured than addition to an already substituted position (k_2; **34c**).



Scheme 2.6: Possible equilibria during a nucleophilic addition to a haloarene-$Cr(CO)_3$ complex.

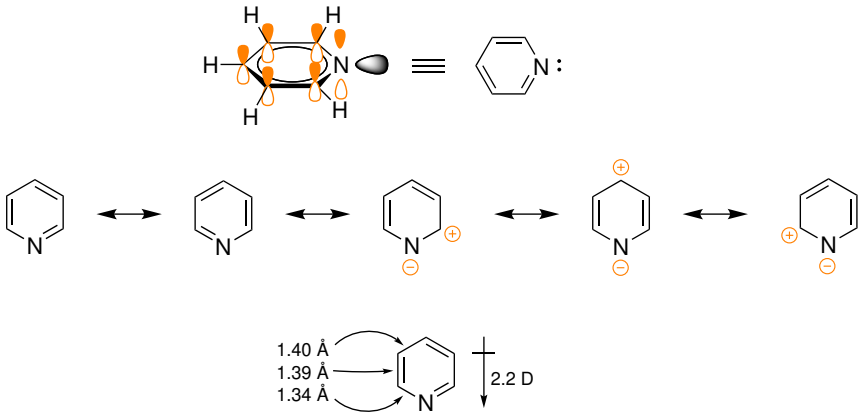
However, the most used catalysed reaction for introducing C-P bonds is via a palladium cross coupling (see Scheme 2.7).[44] After an oxidative addition of the palladium complex, the bromine atom in the palladium complex is substituted with a nucleophilic dialkyl phosphite (**36**) and regenerated with triethylamine (**37**). After a *cis-trans*-isomerisation and reductive elimination an arylphosphonate (**38**) is produced and the palladium is recovered. This coupling reaction was also tested for pyridine with the regioselectivity depending on the position of the halogen. In this way it was also possible to synthesise 3-phosphonylated pyridines.[45]



Scheme 2.7: Palladium catalysed cross coupling of halogenated pyridines to form pyridine phosphonates, with R^1 and R^2 alkyl or aryl groups.

2.3 Phosphonylation of pyridine

As the structure of pyridine is very similar to that of benzene, their reactivities are often compared to one another, both containing 6 delocalised π-electrons on 6 π-orbitals. However, the major difference between the 2 molecules is the presence of the nitrogen atom with a lone electron pair on an sp^2 orbital. This is in contrast to pyrrole where the lone pair of the nitrogen is involved in the aromaticity. It is because of this heteroatom that pyridine obtains some interesting properties. The nitrogen atom can act as an electron sink, draining electrons from the pyridine ring, thus making it more susceptible towards nucleophiles with preference towards the *ortho*- and *para*-positions.[46]



Scheme 2.8: Orbital positioning (top), resonance forms (middle), bond lengths and dipole moment (bottom) of pyridine.

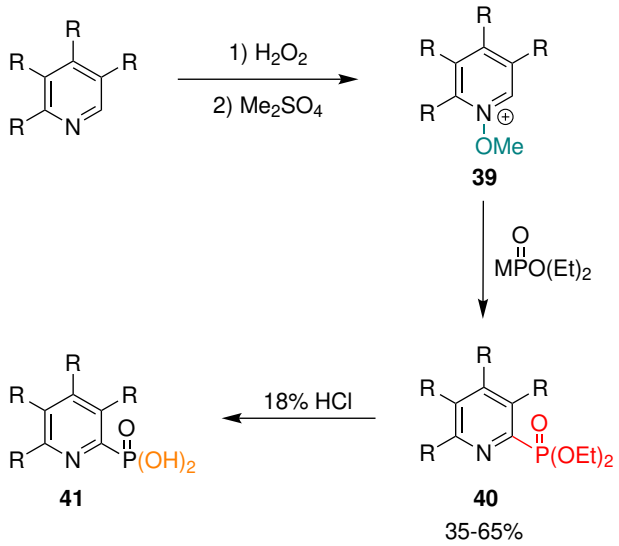
Pyridine is more known for its reactions with electrophiles. Electrophilic substitutions where the last step (i.e. the release of a proton) can happen very easily. However, this is not the case for nucleophilic substitutions. Here the last step, the hydride transfer, is a challenging step that often requires the use of an external oxidising agent as hydride acceptor. However, if the substituted atom is a good leaving group (e.g. Br, Cl, F) at the *ortho*- or *para*-position, this last step occurs more rapidly.[47]

Since most phosphonylation reactions make use of a nucleophilic phosphonylating agent, reactions with pyridine can be very challenging. Besides introducing a phosphorus nucleophile, activation of the nitrogen atom or the introduction of a good leaving group on the pyridine ring is necessary to achieve phosphonylation.

2.3.1 Nucleophilic addition to *N*-substituted pyridinium cations

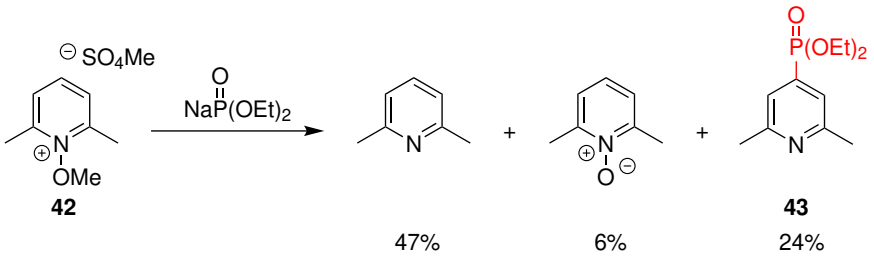
Phosphonylation of pyridine by nucleophilic attack of a phosphorus nucleophile is quite common. While having similar mechanisms they differ largely in the phosphorus nucleophile and the manner in which the nitrogen atom is activated to facilitate the nucleophilic attack of the phosphorus moiety.

This section will consist of the different phosphonylation methods of *N*-substituted pyridinium cations that have been developed over the years, categorised by pyridinium cation. These include *N*-alkoxy, *N*-triphenylmethyl, *N*-(4-pyridyl), *N*-(2,6-dimethyl-4-oxopyridin-1-yl), *N*-(2,5-dimethylpyrrol-1-yl), *N*-triflyl and *N*-alkyl substituents, but also acylation.[48]



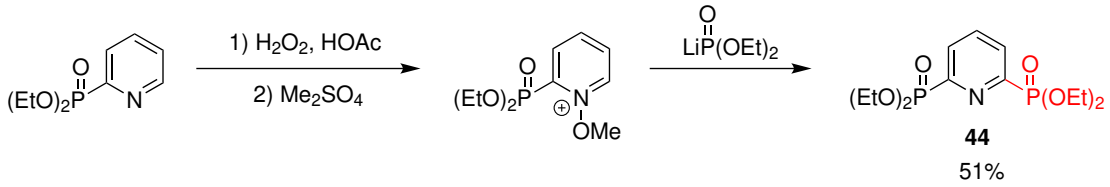
Scheme 2.9: Nucleophilic phosphonylation of *N*-alkoxypyridinium salts at position 2 (R = H, Me).

One possible way is the use of *N*-alkoxypyridinium salts (**39**). Using these salts, Redmore was able to synthesise a series of dialkyl pyridin-2-ylphosphonates (**40**) in 35-65% yield (see Scheme 2.9).[49] Metallic salts of dialkyl phosphites (e.g. lithium or sodium) were used as phosphorus nucleophiles. By hydrolysis with a strong acid (e.g. HCl) the corresponding pyridylphosphonic acids (**41**) were obtained.



Scheme 2.10: Phosphonylation of 2,6-dimethyl-*N*-alkoxypyridinium salts at position 4.

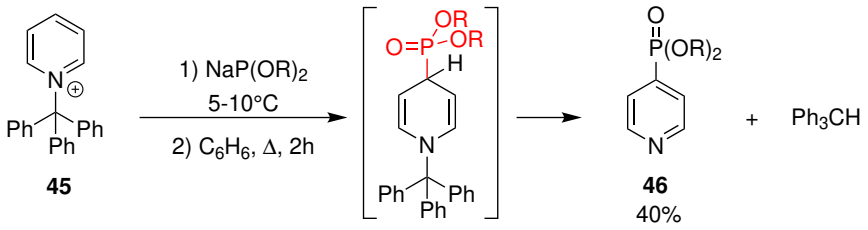
Furthermore, when positions 2 and 6 were substituted (**42**), phosphonylation at position 4 occurred (**43**) (see Scheme 2.10). However, some side products were also formed. Applying the same methodology, Boduszek was able to synthesise pyridin-2-ylphosphonylcarboxylic acids from *N*-oxides in yields of 41-49%.[50] In addition, the synthesis of 2,6-pyridyldiphosphonates (**44**) was possible by a two-step reaction, building further on the previous research of Chen et al. (see Scheme 2.11).[51]



Scheme 2.11: Two-step synthesis of 2,6-pyridyldiphosphonates from 2-pyridylphosphonates by activation with a methoxy group.

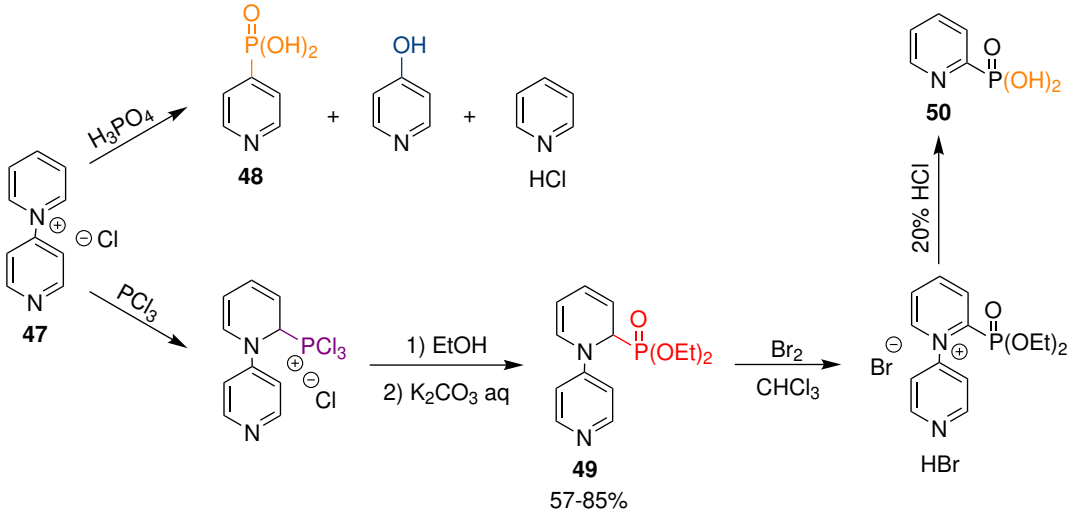
A synthetic route for diethyl 2-pyridylphosphonates was also achieved from an *N*-methoxypyridinium salt with diethyl phosphite by mixing the reagent with 1,8-diazabicylo[5.4.0]-undec-7-ene (DBU) prior to adding the pyridinium salt. This promoted the formation of the desired phosphonates (80%) and hydroxymethylphosphonate (20%) within 5 minutes, meaning that the nucleophilic attack happened much faster than without adding the DBU. However, when pyridine was first mixed with DBU, no reaction took place.[52]

By activating the nitrogen atom with a triphenylmethyl substituent (**45**), shielding of the *ortho*-positions occurred which lead to phosphonylation of the *para*-position (**46**). By using sodium dialkyl phosphite, yields between 28% and 53% were achieved (see Scheme 2.12).[53]



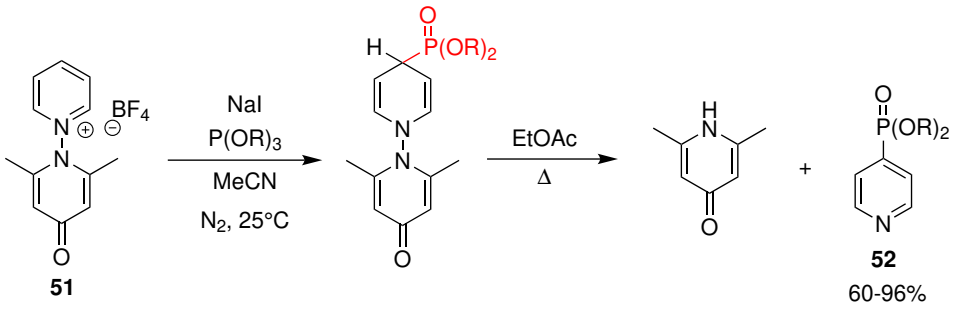
Scheme 2.12: Addition at the *meta*-position after activation with a triphenylmethyl group (R = Me, Et).

Next, 1-(4-pyridyl)-pyridinium salts (**47**) were investigated as possible starting material for phosphonylation. Reaction with phosphoric acid and heating for 8-10 h at 130-140°C yielded 25-28% pyridin-4-ylphosphonic acids (**48**).[54] Reaction of the pyridinium salt with phosphorus(III)chloride also lead towards phosphonylation after heating the salt with an excess of phosphorus reagent. After treatment with ethanol, diethyl-1-(4-pyridyl)-1,2-dihydropyridin-2-ylphosphonate (**49**) was produced in good yields (57-85%). Finally, the aromaticity could be reintroduced by adding bromine in chloroform. The corresponding phosphoric acids (**50**) could then be formed by treatment with 20% HCl (see Scheme 2.13).[55]



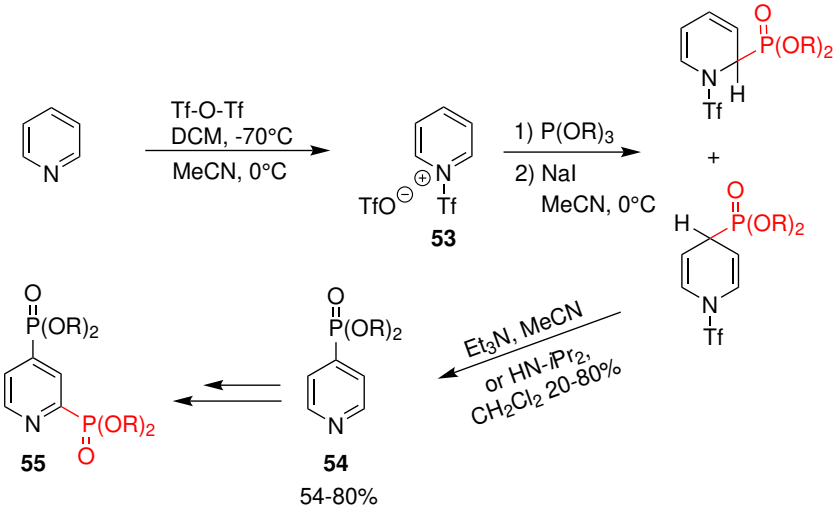
Scheme 2.13: Phosphonylation of 1-(4-pyridyl)-pyridinium salts with phosphorous acid or phosphorus(III)chloride.

N-(2,6-Dimethyl-4-oxopyridin-1-yl)pyridinium salts (**51**) have also been reported to be phosphonylated.[56,57] These salts are able to obtain a regioselective synthesis of a variety of 4-substituted pyridines. This selectivity is obtained by shielding the *ortho*-positions by introducing 2 methyl groups and causing 4-phosphonylation to take place. After isolation of this unstable compound, the substituent could be removed by heating in ethyl acetate (yields of 60-96%) (see Scheme 2.14). This reaction was also feasible with *N*-(2,5-dimethylpyrrol-1-yl)pyridinium iodide and while the intermediate proved to be much more stable, the end product only had a yield of 47%. Nevertheless, the main disadvantage of these strategies remains to be the special preparations of the starting materials to perform the phosphonylation.



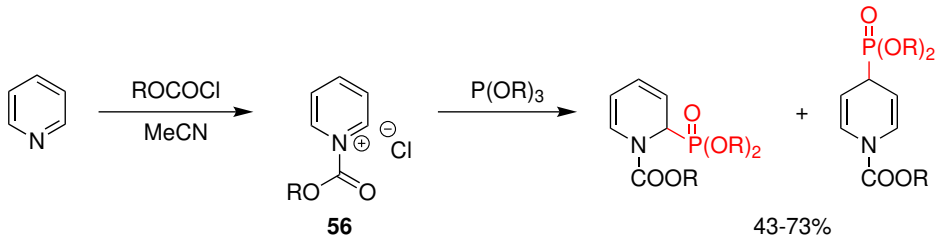
Scheme 2.14: Regioselective phosphonylation of pyridine at the *para*-position starting from a *N*-(2,6-dimethyl-4-oxopyridin-1-yl)pyridinium salt (R = Me, Et).

Activation of the nitrogen atom with triflyl (F_3CSO_2) gave *N*-triflylpyridinium triflate (**53**). This salt was able to be phosphonylated in a selective manner at the *para*-position.[58] This selectivity towards position 4 depended strongly on the nature of the R-group of the nucleophile ($P(OR)_3$): 95:5 for methyl, 100:0 for ethyl and 60:40 for isopropyl, with A:B the ratio of 4 to 2 phosphonylated pyridines. After reaction, deprotonation was only possible for the *para*-substituted moiety (**54**) (54-80% yield). Diphosphonylation in positions 2 and 4 (**55**) was also possible by repeating the reaction (see Scheme 2.15).



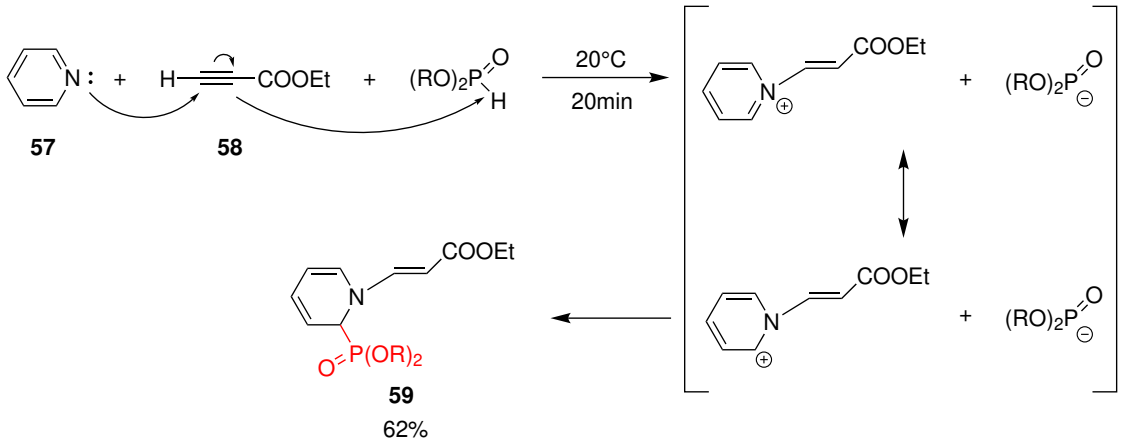
Scheme 2.15: Regioselective mono- and diphosphonylation of pyridine starting from *N*-triflylpyridinium triflate (R = Me, Et, *i*Pr).

Activation by acylation with an ethoxycarbonyl (**56**) lead to the synthesis of pure diisopropyl 1-(ethoxycarbonyl)-1,4-dihydropyridin-4-ylphosphonate (see Scheme 2.16). Other derivatives were also tested but always lead to an unpredictable mixture of *ortho*- and *para*-phosphonylated pyridines.[59]



Scheme 2.16: Phosphonylation of acylated pyridine (R = Me, Et, *i*Pr).

Finally, alkylated pyridines were also investigated as possible phosphonylation materials. In 1999, Albouy et al. published an addition reaction where the nitrogen of pyridine (**57**) was activated by reacting with ethyl propiolate (**58**) (see Scheme 2.17).[60] In the presence of a phosphonylating agent (DAP), a nucleophilic anion was then formed which was able to react with the pyridinium ring. Analysis by NMR showed dialkyl 1,2-dihydropyridine phosphonate (**59**) in moderate to good yields. Addition at position 4 did not seem to occur which excludes the formation of the 1,4-adduct. The addition rate was enhanced in the absence of solvent and by impregnation of the catalyst on a solid support (alumina). This heterogeneous mixture (dry media process) was stirred at 20°C or even higher temperatures for 20 min. However, when trying to validate this reaction, no phosphonylation was detected by ^{31}P-NMR or LC-MS.

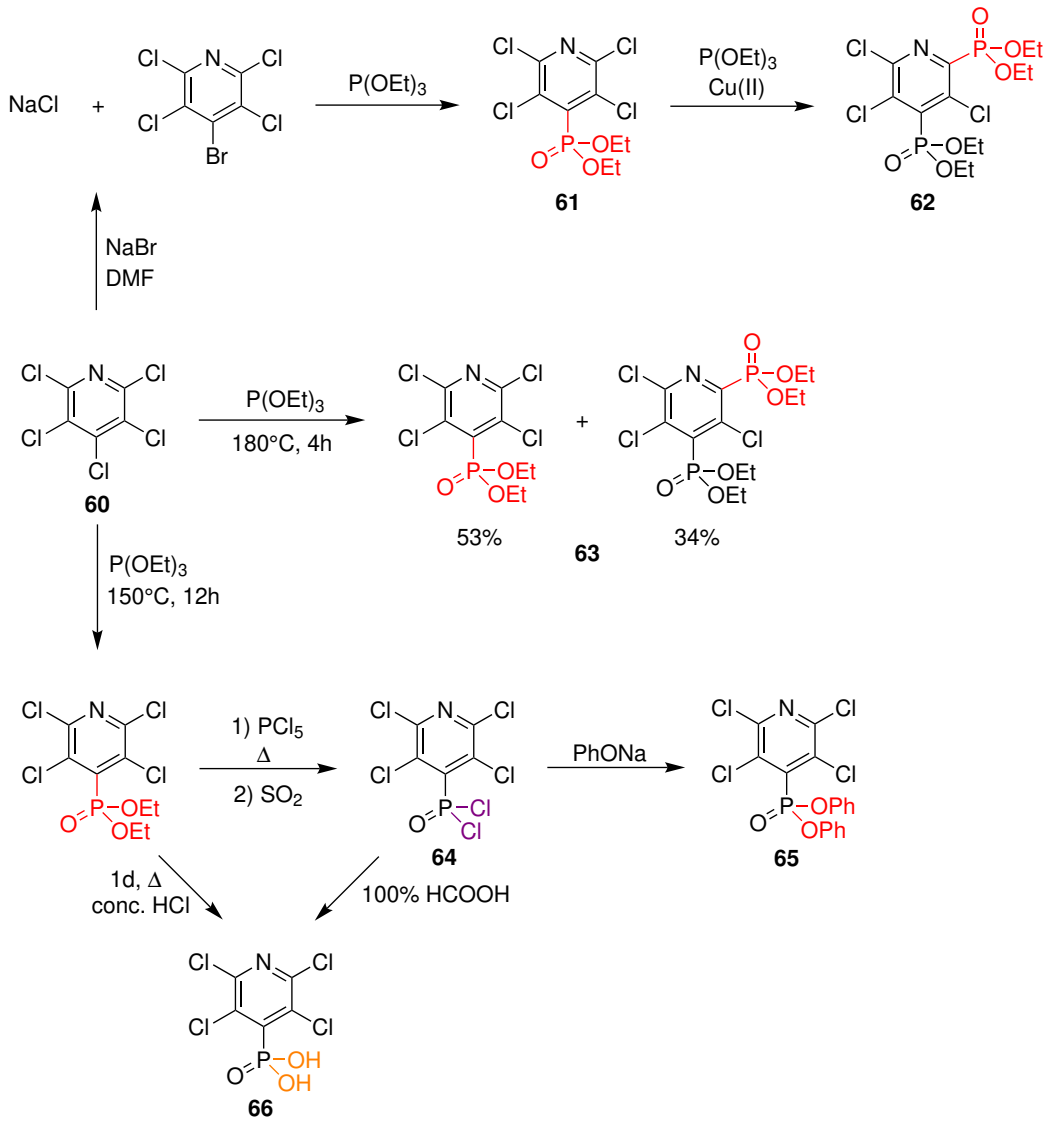


Scheme 2.17: One-pot phosphonylation of pyridine at the *ortho*-position by activation with ethylpropiolate.

2.3.2 Nucleophilic substitution of halides on pyridine

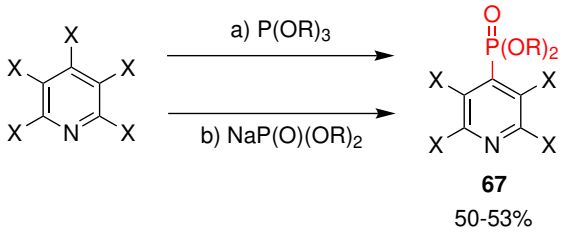
Pyridylphosphonates can also be synthesised by nucleophilic substitutions. These types of reactions generally require harsh conditions and involve mainly substitutions of halogens with nucleophilic phosphorus moieties. Pentachloropyridine (**60**) for example can be phosphonylated by the Michaelis-Arbuzov reaction.[61] By reaction with NaBr followed by $P(OR)_3$ at relatively high temperatures dialkyl pyridin-4-ylphosphonates (**61**) could be produced. Similar results could also be achieved with just $P(OR)_3$.

Increasing the temperature of the latter led to a mixture of mono- and diphosphonylated pyridines (**63**).[62] Treatment with PCl_5 followed by SO_2 gave the phosphonic dichloride (**64**), which upon reaction with PhONa gave the diphenyl phosphonate (**65**). By adding an acid to the phosphonate or phosphonic dichloride, it was possible to form the phosphonic acid of the pyridine (**66**). Furthermore it has to be noted that during these phosphonylation reactions 2,3,5,6-tetrachloropyridine was also formed by a side reaction involving protonation of the ion.[63] Finally, Tolmachev reported that reaction of 4-phosphonylated perchloropyridine with TEP in the presence of copper salts gave 2,4-diphosphonylated pyridine (**62**) (see Scheme 2.18). However, no further reaction details or yields are provided.[64]



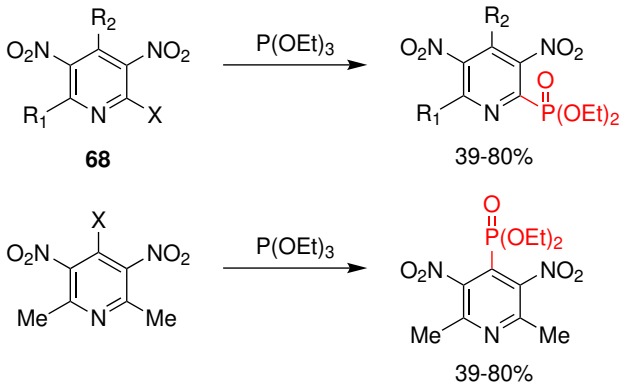
Scheme 2.18: Nucleophilic substitution methods for phosphonylation of pentachloropyridine.

Besides chloro-substituted pyridines, fluor can also be used as possible leaving group via 2 synthetic routes. The first one uses a simple reaction with TAP in methanol but can also be performed without solvent. The second route uses a Michaelis-Becker reaction with $NaP(O)(OR)_2$ and yields 50-53% of **67** (see Scheme 2.19).[65]

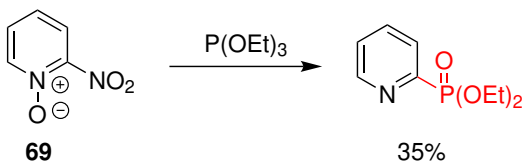


Scheme 2.19: Phosphonylation methods in the *para*-position for halo-substituted pyridines (X = Cl, F and R = Me, Et).

Since most substitution reactions prefer phosphonylation at position 4, it is also interesting to look towards reactions preferring position 2. One of these reactions uses chloro-3,5-dinitrodimethylpyridines (**68**), with the position of the chlorine atom determining the position of phosphonylation (see Scheme 2.20). The reactions were carried out for 0.5-3h at 120-130°C to obtain yields of 39 to 80%.[48] Furthermore, it was found that a nitro group could also function as a valuable leaving group, which is the case with 2-nitropyridine *N*-oxide (**69**) (see Scheme 2.21).[66]



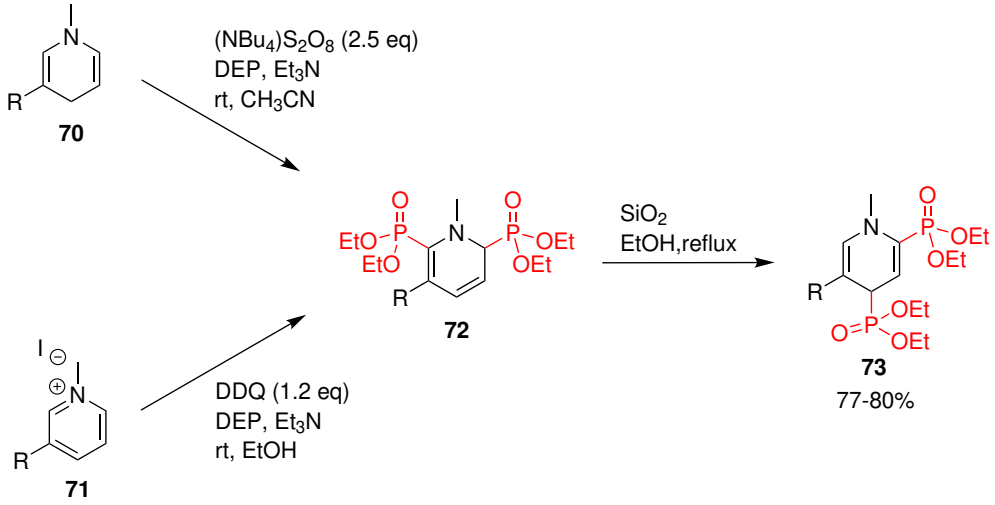
Scheme 2.20: Regioselective phosphonylation methods for the *para*- and *ortho*-positions starting from chloro-3,5-dinitrodimethylpyridines (R^1 = H, R^2 = Me or R^1 = Me, R^2 = H or R^1 = R^2 = H and X = Cl).



Scheme 2.21: Phosphonylation reaction using the NO_2-group as leaving group.

2.3.3 Oxidative diphosphonylation of 1,4-dihydropyridines and pyridinium salts

A method for synthesising diphosphonylated pyridines was achieved by Lavilla et al., starting from 1,4-dihydropyridine (**70**) and pyridinium salts (**71**).[67] Inspired by Effenbergers oxidative phosphonylation of arenes,[68] they were able to achieve a diphosphonylation of the pyridine ring by oxidation in the presence of Et_3N and diethyl phosphite (DEP). Triethyl phosphite was not useful under these conditions since high temperatures are needed. Finally, 2,6-diphosphonylated-1,2-dihydropyridines (**72**) were obtained by this one-pot reaction involving tandem nucleophilic addition/oxidation processes. By changing the solvent and oxidant they were able to optimise the reaction to yields of 77% from 1,4-dihydropyridine and 80% yield from the pyridinium salt. 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) seemed to be the best oxidant for the reaction. The reactions were all carried out under a nitrogen atmosphere at room temperature for 12 hours.



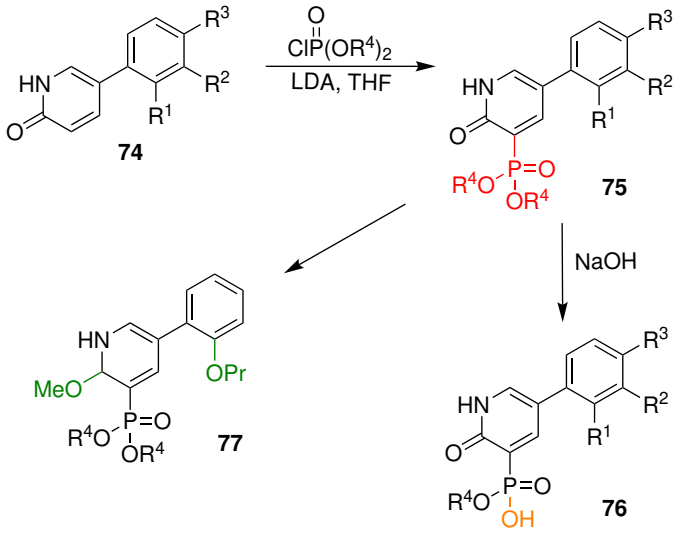
Scheme 2.22: Oxidative diphosphonylation of 1,4-dihydropyridines and pyridinium salts (R = CO_2CH_3).

Purification was done by normal phase column chromatography (SiO_2) with a mixture of DCM and ethanol to yield the diphosphonates. However, in some cases during chromatography, a new isomer, 2,4-diphosphonylated-1,4-dihydropyridine (**73**), was formed. This isomerisation could be performed in a more efficient manner (practically quantitative conversion) by refluxing in a suspension of SiO_2 and EtOH. The reaction mechanism consists of an oxidation of the dihydropyridine to the corresponding pyridinium salt. The nucleophilic addition of the phosphite may take place at the less encumbered α-position to give a second dihydropyridine, which would consume a second equivalent of oxidant to furnish a second pyridinium salt, ready to suffer a new R-addition. The unusual phosphonate shift leading to isomeric dihydropyridine may be explained by the reversibility of the phosphite addition and the improved stability. In fact, calculations showed a difference of 17.8 kcal/mol during the formation of the 2,6-diphosphonates and 2,4-diphosphonates, favouring the latter compound. To summarise, the reaction seems to be generally applicable and works well with dihydropyridines and pyridinium salts bearing electron-withdrawing substituents at the *meta*-position.

2.4 Other phosphonylation methods for pyridine

2.4.1 Phosphonylation of pyridinone derivatives

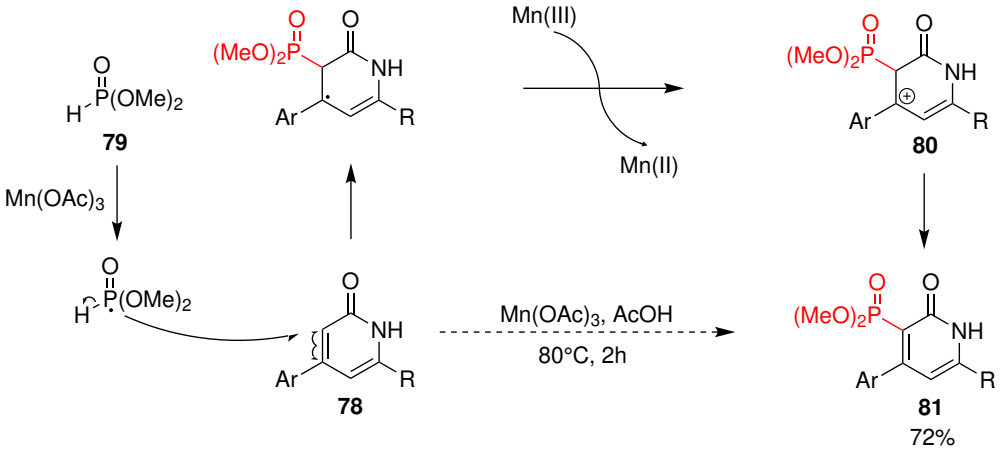
One particular group of pyridine derivatives that are also known to be phosphonylated are the pyridinones (**74**). By treatment of the starting material with dialkyl chlorophosphate and LDA in THF, the phosphonylated compounds (**75**) was made. Deprotection was possible after adding NaOH (**76**) or transformation to their methoxy analogues (**77**) (see Scheme 2.23).[69] However, no further reaction details or yields were provided.



Scheme 2.23: Electrophilic addition of dialkyl chlorophosphate to pyridinone derivatives with LDA to obtain phosphonates (R^1 = H, OPr, Br; R^2 = H, OPr, Br; R^3 = H, OPr, Br; R^4 = Et, Pr).

Another way to phosphonylate pyridinone derivatives is by a manganese(III) promoted direct phosphonylation of pyridinones with dialkyl phosphite (**79**) (see Scheme 2.24).[70] This $Mn(OAc)_3$-mediated selective reaction uses free radicals generated from the reaction of $Mn(OAc)_3$ and dimethylphosphite. The produced electrophilic phosphonyl radical attacks the *meta*-position of the pyridinone. The selectivity towards position 3 is due to its high electron density. Afterwards the compound is oxidised by the second equivalent of $Mn(OAc)_3$ to form a carbocation (**80**) followed by deprotonation to give the phosphonylated end product (**81**).

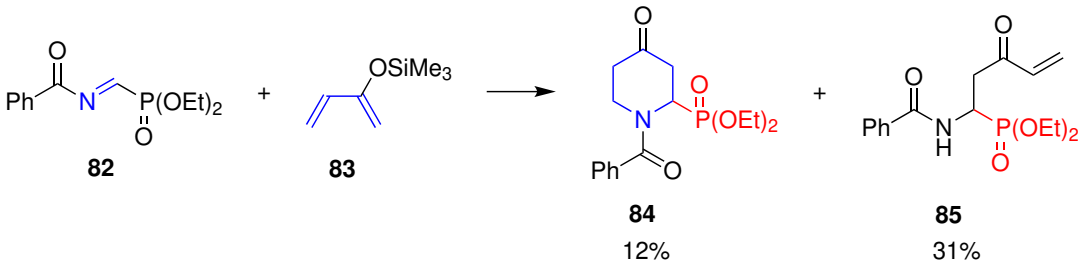
As starting material 4,6-diphenylpyridin-2(1H)-one (**78**) was used for developing the reactions and after optimising the reaction, Sun et al. concluded that the best solvent and reagents were acetic acid, dimethyl phosphite and manganese(III)acetate. In addition, they found that the addition of three equivalents of manganese(III)acetate in three instances gave the best results. Finally, after reacting for 2h at 80°C, the product was formed with a yield of 72% yield.



Scheme 2.24: $Mn(OAc)_3$-mediated selective free radical phosphonylation of pyridinones (R = Ph, Me, 4−$MeOC_6H_4$, 4−BrC_6H_4).

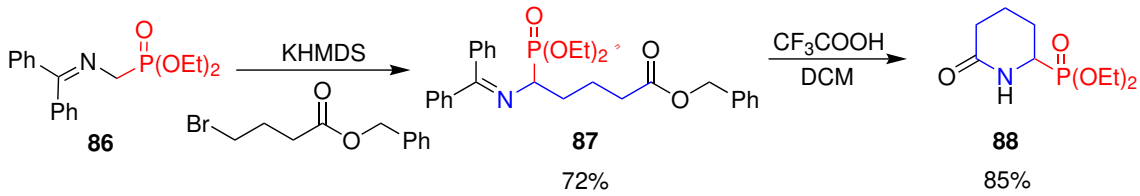
2.4.2 Ring forming reactions of phosphonylated substrates

Ring forming reactions of phosphonylated substrates holds a broad spectrum of synthetic routes and while being very different, most of these methods can be classified into 2 groups: cycloaddition reactions and ring closure reactions by intra- or intermolecular addition and elimination. In cycloadditions, two or more unsaturated molecules (or parts of the same molecule) react to form a cyclic adduct. One example for 6 membered rings is the reaction of a phosphonate dienophile (e.g. an acylimine (**82**)) and a diene (e.g. a silylated enol ether (**83**)). In this [4+2]-cycloaddition the most nucleophilic carbon of the diene attacks the α-position of the acylimine, resulting in a mixture of amino phosphonates in low yields (**84,85**) (see Scheme 25).[71]



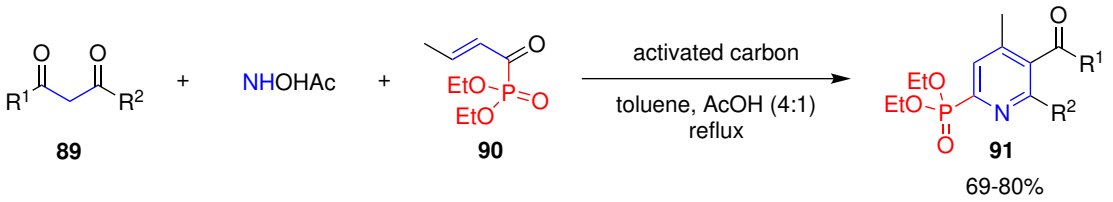
Scheme 2.25: [4+2]-Cycloaddition of acylimine and a silylated enol ether resulting in a mixture of amino phosphonates.

Ring closure reactions by intramolecular addition and or elimination typically involve the attack of a nucleophilic nitrogen onto a carbonyl. In order to achieve such a reaction, one of these nucleophiles needs to be deactivated. This is mainly the nitrogen since both groups are present in the same precursor. Afterwards the non-nucleophilic moiety is reconverted into a nucleophile to initiate ring closure.[15] In Scheme 26, an example is given for the hydrolysis of an imine.[72]



Scheme 2.26: Ring closure reaction to form diethyl (6-oxopiperidin-2-yl) phosphonate.

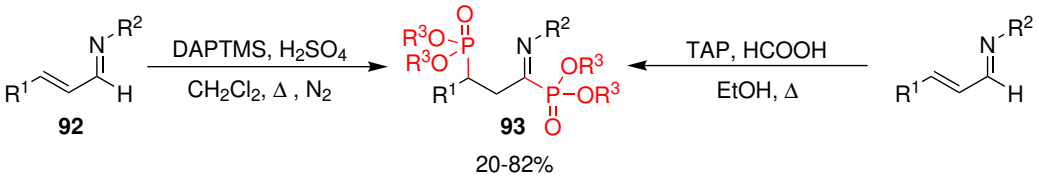
For pyridylphosphonates, an oxidative three-component reaction of α-ketophosphonates (**90**), ammonium acetate and 1,3-dicarbonyl compounds (**89**) has been described by Allais et al. (see Scheme 2.27).[73] This method allowed the synthesis of highly functionalised pyridylphosphonates (**91**) in 69–80% yields by refluxing in toluene/acetic acid 4:1 in the presence of molecular sieves (4Å).



Scheme 2.27: Oxidative three-component reaction of α-ketophosphonates, ammonium acetate and dialkyl-1,3-dicarbonyl compounds to give pyridylphosphonates ($R^1 = R^2 =$ alkyl).

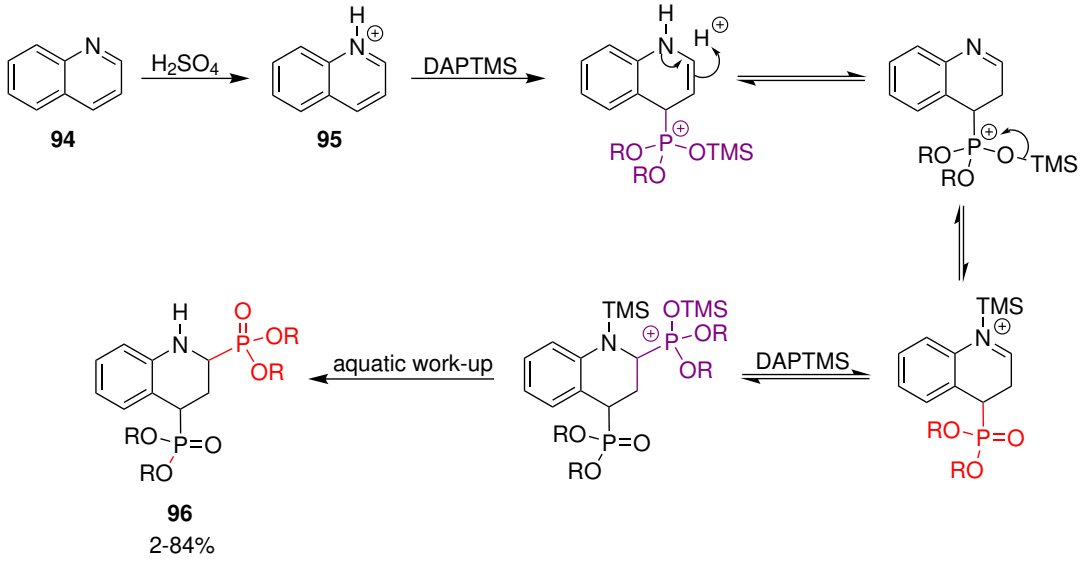
2.5 One-pot diphosphonylation

As stated in Section 1.1, phosphonylated α,β-unsaturated imines exhibit enzyme inhibitory effects as they are structural analogues of glutamic acid. In 2005, our research group published a method for a one-pot 1,4-1,2 diphosphonylation reaction of these α,β-unsaturated imines (**92**). By using a strong acid (H_2SO_4) to activate the nitrogen, diphosphonylation (**93**) was possible using a silylated reagent (DAPTMS).[74,75] In addition, another method was published which made use of a milder acid (HCOOH) and a less reactive phosphonylating reagent ($P(OR)_3$).[76] The latter was deemed more efficient with little sterically substituted nitrogen atoms. This is in contrast to the first route which works better with a highly sterically substituted nitrogen atoms.



Scheme 2.28: Diphosphonylation methods for α,β-unsaturated imines (R = Me, Et).

Building on this research, our research group applied the same methodology to quinoline (**94**) and its derivatives. They concluded that the combination of a strong acid (e.g. H_2SO_4) and a silylated phosphonylating reagent (DAPTMS) made it possible to diphosphonylate an azaheterocycle. Looking more into this reaction, this turned out to be a one-pot tandem 1,4-1,2-diphosphonylation. Starting from quinoline, the formed 2,4-diphospho--1,2,3,4-tetrahydroquinoline (**96**) was characterised as a new class of phosphonylated azaheterocycles.

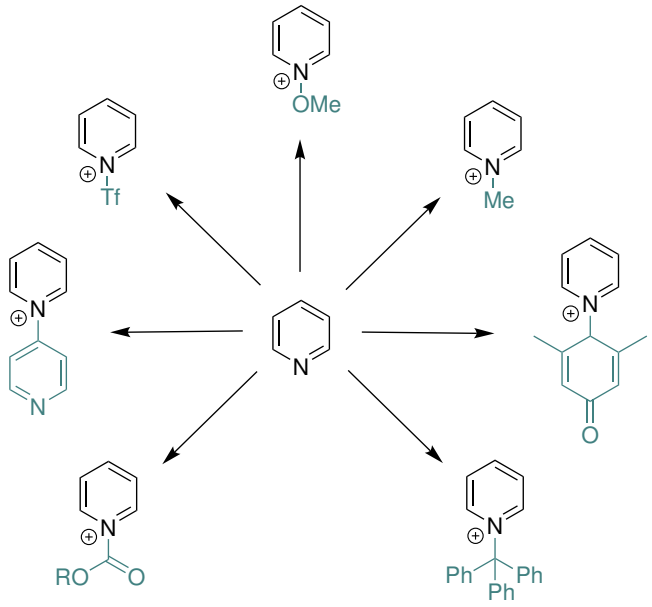


Scheme 2.29: Mechanism for the one-pot diphosphonylation of quinoline (R = Me, Et).

These one-pot reactions proved to be very efficient since full conversion was possible after a short time with minimal heating (3 hours in a microwave at 45°C). Purification could then easily be achieved by a series of extractions. However, when applying these methods to pyridine no phosphonylation was observed.

2.6 Conclusion

Diphosphonylated azaheterocycles, in this case pyridine, are linked to a variety of applications correlated with enzyme inhibition. When trying to phosphonylate a pyridine ring a variety of techniques are available: nucleophilic addition, electrophilic substitution, oxidation reactions and ring forming reactions, with nucleophilic addition being the most common method. However, this requires the pyridine ring to be activated by quaternisation of the nitrogen atom. In Scheme 36, an overview is given of different activating groups which have already been reported in literature. As phosphonylating reagent $P(OR)_3$ and $HP(OR)_2$ are used (with R = alkyl). As alternative, silylated derivatives could also be used. These tend to be more efficient and can be used at lower temperatures, but also tend to be more susceptible to hydrolysis.



Scheme 2.30: Summary of molecules used for activating a pyridine ring (R = alkyl, aryl).

When looking into diphosphonylation reactions for pyridine, it was noticeable that all diphosphonylations worked by reactivating the pyridine ring. When compared to the one-pot tandem diphosphonylations developed for α,β-unsaturated imines and quinolines, these are obviously less efficient and require an additional work-up as well as an excess of reagent. The result is a more time consuming and labour intensive method not favoured for industrial applications. The one-pot 1,4-1,2 tandem diphosphonylations (see Section 2.5) proved to be a better alternative.

Overall it can be stated that phosphonylation of pyridine is possible but remains very challenging. Since the aromatic ring is very unreactive, special activating groups or good leaving groups are often needed. Furthermore, phosphonylations tend to be characterised by long reaction times and low to medium yields, making room for improvement.

Chapter 3

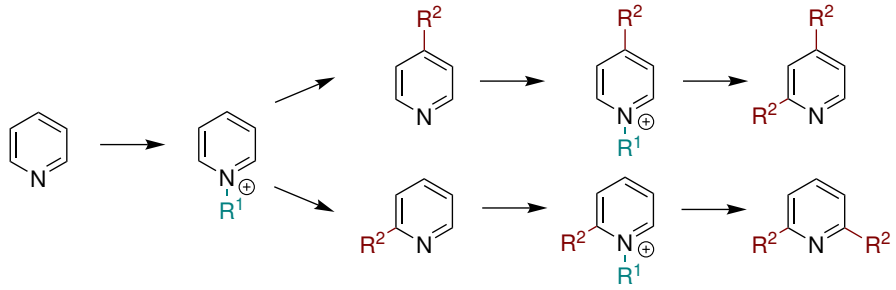
Results & Discussion

In this Master thesis, multiphosphonylation reactions for pyridine are further elaborated, focusing on high yielding one-pot reactions. Since multiple pathways were eventually explored for synthesising these compounds, this chapter will be divided in several sections. Using the most promising pathway, the scope was broadened by testing the reaction with new substrates. Having said that, this section will start by giving the general approach for these types of reactions.

3.1 Approach

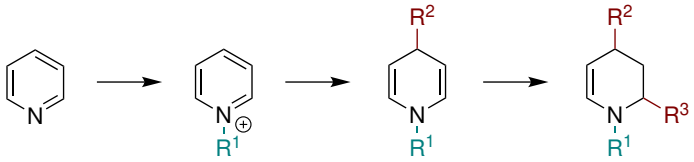
After reading Section 2.3, one might have noticed that most reactions are limited to monophosphonylated compounds. However, since this thesis will focus on diphosphonylation, it is of great importance to understand the mechanisms behind the known diphosphonylation reactions. One way is the reactivation of the nitrogen atom of the pyridine ring to make a new nucleophilic attack possible (see Scheme 2.11 & 2.15). However, this route requires the purification of the intermediate followed by a new reactivation of the nitrogen to achieve the diphosphonylated end product. Such multistep reactions can be very time consuming and labour intensive and are thus not favoured for industrial use.

A similar manner for diphosphonylation was seen in Section 2.3.3, where an oxidation reaction was used to achieve diphosphonylation by reoxidising the pyridine. In contrast to the reactions above, purification of the monophosphonylated compound was not necessary in this case and both phosphonate additions could be performed in one reactor. Important to add is the phosphonate shift to the *para*-position because of its higher stability. This is also seen in Section 2.5, where the phosphonate addition to the protonated quinoline happened first in the *para*-position.



Scheme 3.1: Multistep diphosphonylation of pyridine by reactivating the ring, with R^1 an activating group (e.g. OMe) and R^2 a phosphonate group.

The known diphosphonylation methods for pyridine are thus limited to multistep reactions. However, from the reactions with quinoline (see Scheme 2.29) one can get an idea of the mechanism it would follow. In short, it should encompass just one activation step of the nitrogen. Furthermore, the primary addition to the pyridine will most likely be in the *para*-position because of its higher stability and less sterically hindered position.

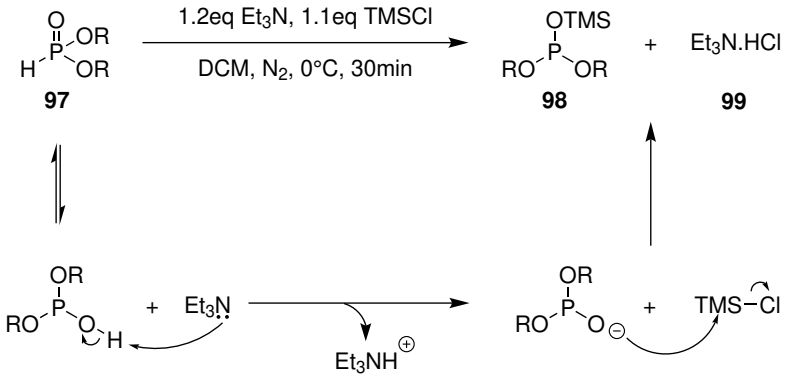


Scheme 3.2: Diphosphonylation method for quinoline applied to pyridine. With R^1 an activating group (e.g. H^+) and R^2 a phosphonate group.

3.2 Protonated pyridinium salts

A first strategy was based on the one-pot tandem 1,4-1,2-addition of phosphites to quinolines, using strong acids to activate the pyridine ring by protonation. In the case of quinoline, 0.5 eq. sulphuric acid was used together with 4.05 eq. of dialkyl trimethylsilyl phosphite (DAPTMS) as reagent. This reagent was chosen since it is known to be one of the more reactive reagents used for phosphite addition and because of its ability to be prepared *in situ*, allowing one-pot reactions. However, an adverse side-effect of this increased reactivity is its increased susceptibility to hydrolysis, forming dialkyl phosphites or other hydrolysates even at relatively low temperatures.

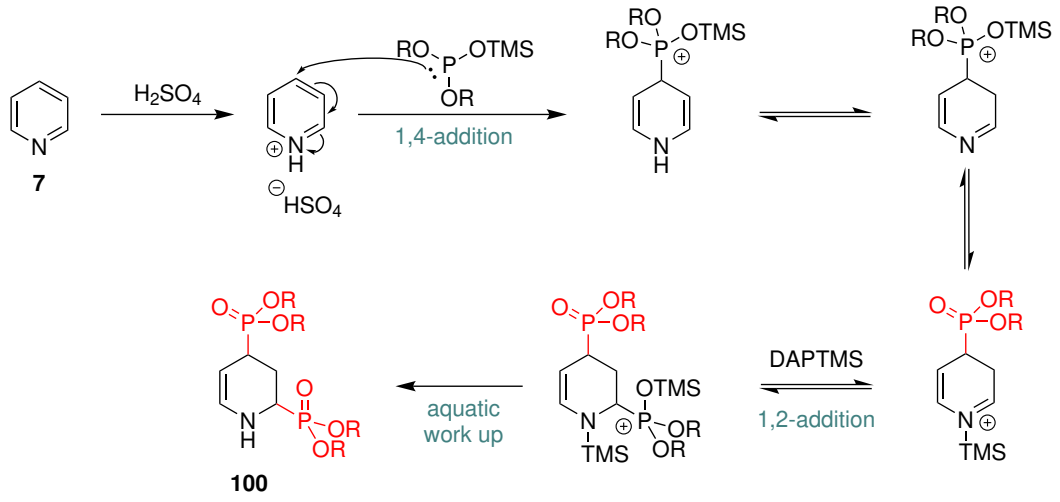
The synthesis of DAPTMS was performed by stirring dialkyl phosphite (DAP) with a base, like triethylamine (TEA), making it more nucleophilic and transforming the phosphorus from penta- to trivalent. When chlorotrimethylsilane is added, an exothermic reaction takes place, producing the desired DAPTMS as well as a white precipitate of $Et_3N.HCl$ which can easily be filtered off. All of this needs to happen under a dry atmosphere to keep hydrolysis to a bare minimum (see Scheme 3.3).



Scheme 3.3: Synthesis of dialkyl trimethylsilyl phosphite (DAPTMS), with R = alkyl.

With the expected reaction mechanism given (see Scheme 3.4), pyridine was submitted to the identical reaction conditions from the diphosphonylation of quinoline. But unfortunately no phosphonylation took place.

The problems with this phosphonylation reaction can be boiled down to 3 key issues: the unreactivity of pyridine, two-phase mixing problems and hydrolysis of the reagent, with the unreactivity of pyridine being already extensively discussed in Section 2.3. The mixing issue on the other hand emerges from the formation of an ionic liquid. Formed by combining pyridine with sulphuric acid, the generated ionic liquid (i.e. a liquid salt characterised by a very low vapour pressure, high viscosity and a low melting point) is known to be very stable.[77]



Scheme 3.4: Expected reaction mechanism for a one-pot tandem 1,4-1,2-diphosphonylation of pyridine using H_2SO_4 and DAPTMS, with R = alkyl.

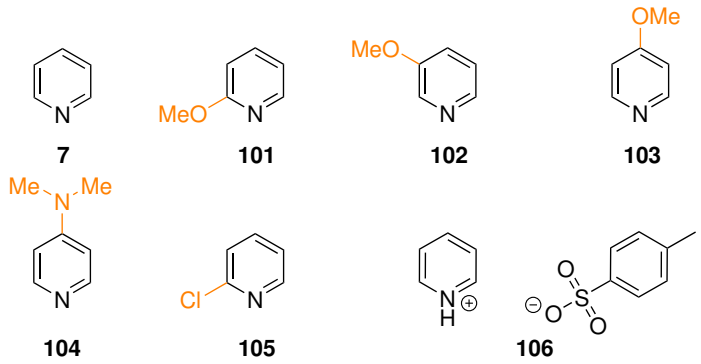
When mixing this viscous and polar liquid with a rather nonpolar reagent, 2 phases are formed which do not interact with each other. This is in contrast to the quinoline reaction where only one homogeneous phase is formed. However, the most important issue turned out to be the hydrolysis of DEPTMS. Even when working under a dry argon atmosphere in a flame dried flask, most DEPTMS (3.5 eq.) was immediately hydrolysed after mixing.

By analysing the mixture by ^{31}P-NMR to follow up the reaction, it was clear that all DEPTMS (127.6 ppm, $CDCl_3$) was hydrolysed into diethyl phosphite (7.2 ppm, $CDCl_3$) and other hydrolysates (10 to -15 ppm, $CDCl_3$) such as ethyl phosphite, phosphonic acid or phosphate by reacting with water and acid. Furthermore, protonation of pyridine was checked by ^{1}H-NMR in D_2O, since protonation of pyridine led to lower chemical shifts.[78]

Since azaheterocycles, such as pyridine and quinoline, can be protonated in an acidic medium to increase its polarity, its phosphonylated analogues are easily purified by means of a simple acid-base extraction. For diphosphonylated quinoline, this was achieved by evaporating the solvent from the reaction mixture and mixing the residue with diethyl ether (Et_2O) and 3N HCl (1:1 ratio). After multiple extractions with Et_2O, the aquatic phase was isolated and neutralised with 3N NaOH. Next, the aquatic phase was mixed with dichloromethane (DCM) (1:1 ratio), followed by multiple extractions with DCM and isolation of the organic phase. After drying the latter with magnesium sulphate ($MgSO_4$), filtering off its solids and evaporating the residual solvent, the diphosphonylated end product was obtained in high purity. Thus, finding a working method allowing the protonation of the nitrogen after the phosphonylation reaction would be very beneficial because of the simple work-up procedure. For this reason, pyridine was subjected to various conditions by using different reagents, acids, temperatures, substrates and solvents in order to achieve phosphonylation while keeping in mind the problems addressed earlier (see Table 3.1).

For the hydrolysis issue, it seemed useful looking into different reagents since DAPTMS is very susceptible to degradation. Experiments with other phosphonylating agents, such as the more stable triethyl phosphite in combination with formic acid, a method which was already successful for the diphosphonylation of α,β-unsaturated imines, and dimethyl trimethylsilyl phosphite (DMPTMS), a more reactive species, were concluded.[76] Furthermore, the strong acids which are added (e.g. H_2SO_4) are very hygroscopic and already contain a small amount of water which causes hydrolysis. Using weaker acids might thus effect the rate of hydrolysis. On the other hand, stronger acids (e.g. HCl) might increase reactivity. As a result, both types were tested. Next, several solvents and temperatures were tested in order to solve the mixing issues and increase the reactivity respectively. However, high temperatures were avoided since the silylated reagent (DAPTMS) is very unstable.

Finally, the influence of functional groups on the pyridine ring was tested as they might influence its reactivity and solve some of the mixing issues. For example, by donating electrons to the ring to promote the protonation of pyridine, so the substrate will be activated for the reaction with the nucleophile. (e.g. -OMe, -NMe_2). In addition, good leaving groups (e.g. halogens) were tested as well in order to see if they might have an effect on its reactivity towards nucleophilic addition. To conclude, an already protonated, non-ionic liquid, pyridinium salt was tested as well to avoid the mixing problems arising from phase separation.



Scheme 3.5: Overview of substrates used for phosphonylation by activation via protonation.

All of the parameters mentioned above were tested thoroughly by microwave heating for 4 hours since reflux reactions generally require a longer reaction time (3 days for quinoline). By using dry solvents, an inert dry atmosphere (argon or nitrogen) and flame dried flasks, hydrolysis was kept to a bare minimum. From the results in Table 3.1, one can notice that the use of stronger acids (e.g. H_2SO_4) causes the formation of ionic liquids, which impedes the reaction and increases hydrolysis. In contrast, weak acids (e.g. HCOOH) ensure less hydrolysis. However, phosphonylation did not occur and hydrolysis was still abundant (30-100%). No difference was noticed between DMPTMS and DEPTMS. Moreover, when comparing the silylated reagents to TEP, the latter showed reduced amounts of side reactions because of its higher stability. Increasing the temperature did not improve reactivity and led to degradation. Furthermore, the use of substituents did not show any clear effect on the reaction.

Finally, pyridinium *p*-toluenesulphonate was tested as starting material, in order to prevent the formation of an ionic liquid. When using dry dichloromethane (DCM) as solvent, analysis by ^{31}P-NMR showed small peaks in the region associated with phosphonate groups (30-20 ppm). However, due to the high amounts of side products and hydrolysis, further analysis was unfeasible. In addition, the diphosphonylation reaction for quinoline was performed as well to confirm the results published by De Blieck et al..[79] Similar results were found as the reaction gave a yield of 56% after 4 hours. It could thus be concluded that activation of the pyridine ring by protonation was not successful, contrary to quinoline, and other routes needed to be investigated.

Table 3.1: Tested conditions for the diphosphonylation of protonated pyridinium salts.

Substrate	**Acid**	**Reagent**	**Temp.**	**Solvent**	**Remarks***
Pyridine	H_2SO_4	DEPTMS	45°C	DCM	Yellow [a,b,c,d]
	H_2SO_4	DEPTMS	60°C	DCM	Yellow [a,b,c,d]
	H_2SO_4	DEPTMS	45°C	MeCN	Yellow [a,b,c,d]
	H_2SO_4	DEPTMS	45°C	$CHCl_3$	Yellow [a,b,c,d]
	H_2SO_4	DEPTMS	45°C	/	Yellow precipitate. [b,c]
	H_2SO_4	DMPTMS	45°C	DCM	Yellow [a,b,c,d]
	H_2SO_4	DMPTMS	60°C	DCM	Yellow [a,b,c,d]
	HCOOH	TEP	45°C	DCM	Clear [b,c]
	HCOOH	TEP	45°C	MeOH	Clear [b,c]
	HCOOH	TEP	60°C	MeOH	Clear [b,c]
	HCOOH	TEP	100°C	MeOH	Brown mixture implying degradation. [b,c]
	HCOOH	DEPTMS	60°C	DCM	Clear [b,c]
	CH_4O_3S	DEPTMS	60°C	DCM	Clear [a,b,c,d]
	HCl	DEPTMS	60°C	DCM	Yellow [a,b,c,d]
2-Methoxypyridine	H_2SO_4	DEPTMS	45°C	DCM	Yellow [a,b,c,d]
3-Methoxypyridine	H_2SO_4	DEPTMS	45°C	DCM	Yellow [a,b,c,d]
4-Methoxypyridine	H_2SO_4	DEPTMS	45°C	DCM	Yellow [a,b,c,d]
4-Dimethylamino-pyridine	H_2SO_4	DEPTMS	45°C	DCM	Yellow [a,b,c,d]
2-Chloropyridine	H_2SO_4	DEPTMS	45°C	DCM	Yellow [a,b,c,d]
Pyridinium *p*-toluenesulphonate	/	DEPTMS	45°C	/	White precipitate. [b,c]
	/	DEPTMS	45°C	DCM	Yellow mixture with signs of phosphonylation by ^{31}P-NMR. [b]
Quinoline	H_2SO_4	DEPTMS	45°C	DCM	Yellow oil. Full conversion into diphosphonylated product. Some hydrolysis.

**Results after a 4h reaction with microwave heating under an inert nitrogen atmosphere.*
[a]Phase separation, [b]reagent hydrolysis, [c]no phosphonylation,
[d]formation of an ionic liquid.

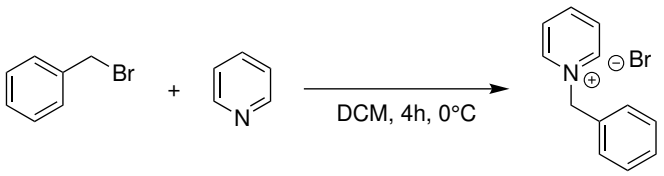
3.3 Phosphonylation of benzylated pyridinium salts

Another approach to initiate the phosphonylation reaction is by using alkylation of the pyridine ring to activate the nitrogen atom. This diverse class, *N*-alkylpyridinium halides, can be found in many natural products and bioactive pharmaceuticals as anti-microbial, anti-cancer, anti-malarial and anti-cholinesterase inhibitors. Moreover, several compounds with applications in materials science and biological issues related to gene delivery are also known.[80]

Quaternisation of pyridine is very dependent on the substituent as they might completely change the reactivity of the molecule. As a result, there is no specific condition that governs the preparation for every single one of these salts. This is specially true for its two types, substituted and unsubstituted rings, causing them to differ greatly in reactivity and synthesis.[81] With the most common synthesis of these unsubstituted *N*-alkyl or *N*-arylpyridinium salts being a S_{N2}-type reaction of pyridine with alkyl or aryl halides. Overall, their synthesis is characterised by high yields and generally simple work-up procedures.[80]

These *N*-alkylpyridinium salts were chosen in order to overcome some of the issues discussed earlier from using protonated pyridinium salts. And since no ionic liquid is formed here, mixing would already be greatly improved. In addition, hydrolysis would be reduced since no acids are required. However, because of the absence of a free nitrogen atom, finding an adequate purification method would be more challenging.

To start, *N*-benzylpyridinium bromide was chosen as primary substrate for the diphosphonylation. Synthesis was achieved by mean of an exothermic S_{N2}-type reaction generated by mixing benzyl bromide and pyridine for several hours at 0°C (see Scheme 3.6). Purification of the salt was performed by washing the salt, followed by evaporating the solvent and the residual reagents.



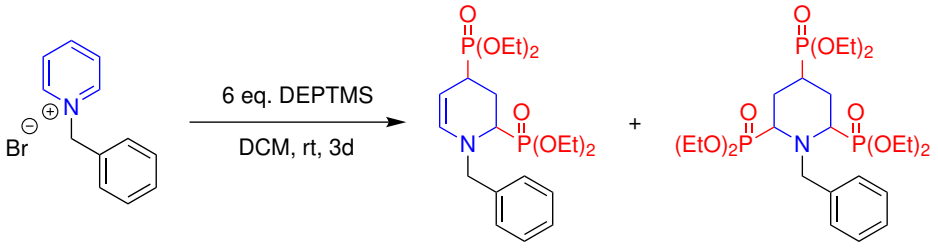
107 **7** **108**
92-98%

Scheme 3.6: Synthesis of *N*-benzylpyridinium bromide.

3.3.1 Development

The one-pot diphosphonylation of *N*-benzylpyridinium bromide was similar to that of protonated pyridine using the same reagent (DEPTMS) and conditions. However, due to equipment failure of the microwave, reflux reactions had to be used which required a much longer reaction time (i.e. up to several days for most phosphonylation reactions). Hence, the pyridinium salt was refluxed for 3 days with 6 eq. of DEPTMS in dry dichloromethane (DCM), an excess to compensate for the hydrolysis.

Afterwards, analysis was conducted by LC-MS and ^{31}P-NMR. For the latter, several peaks in the specific range associated with phosphonates (35 - 10 ppm) were observed as well as very large amounts of hydrolysis.[31] Furthermore, analysis by ^{1}H-NMR was pointless since, due to the high amount of hydrolysed reagent, almost no peaks could be recognised or integrated. Eventually it was possible to identify these compounds by LC-MS, which gave among others the following masses: 170, 446 and 584. Other masses were associated with the hydrolysis of the reagent or other side reactions (see Section 3.3.2.2). The masses previously given correspond with the compounds given in Scheme 3.7.



Scheme 3.7: Formed phosphonates by reacting DEPTMS with *N*-benzylpyridinium bromide, based on the results from LC-MS.

As one can see, not only the diphosphonylated product was formed but the triphosphonylated compound as well. A compound which was never previously mentioned in literature. Furthermore, it is also worth noting that the monophosphonylated compound was not found by LC-MS, suggesting that the addition of the second phosphonate group immediately follows the primary addition. This seems fairly obvious since the primary addition has to break the aromaticity of the pyridinium ring, making it the rate-determining step. However, the addition of the third phosphonate group does not share this fast reactivity, although it was more abundant.

Reaching full conversion:

Since the overall conversion was estimated to be around 45% (after 3 days), it seemed useful to first try to push the reaction to its maximal conversion in order to evaluate the final ratios of all compounds so the reaction could be further investigated. This was achieved by refluxing under a dry inert atmosphere using flame dried glassware. An additional in-line opening was used for taking samples to follow up the reaction. Due to hydrolysis and solvent escape over time, additional reagent and dry solvent was added at specific intervals in order to keep the concentrations from changing too much while also trying to avoid too much hydrolysis from external additions or contact with wet air (see Figure 3.1). Finally after 11 days, conversion stagnated around 95% and the compounds were isolated for further analysis.

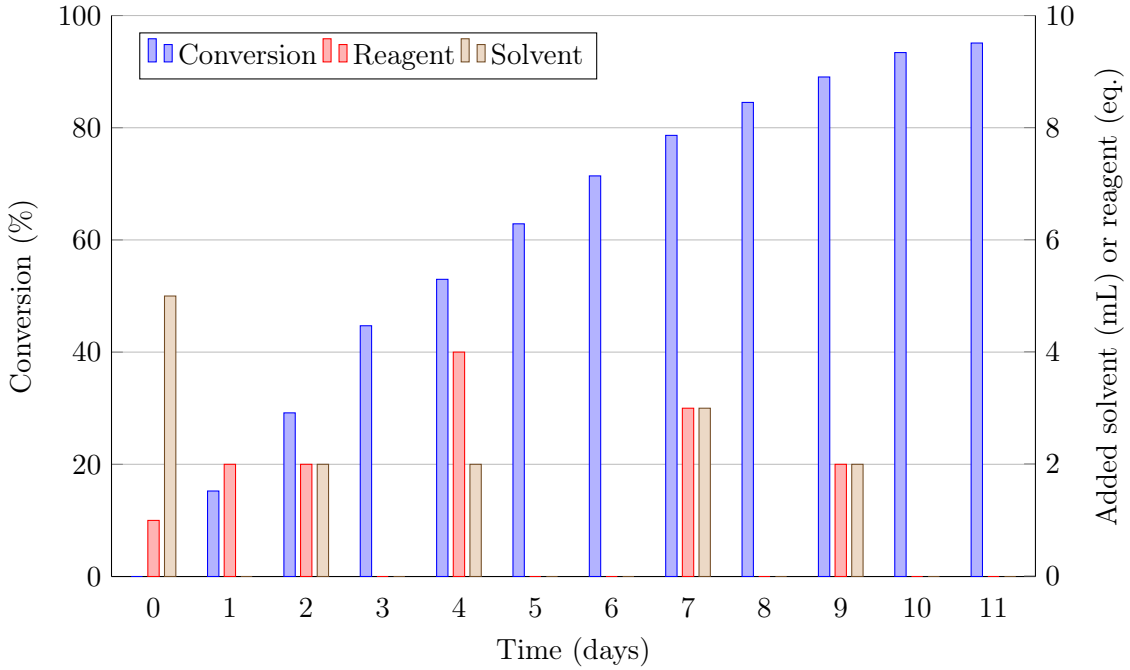
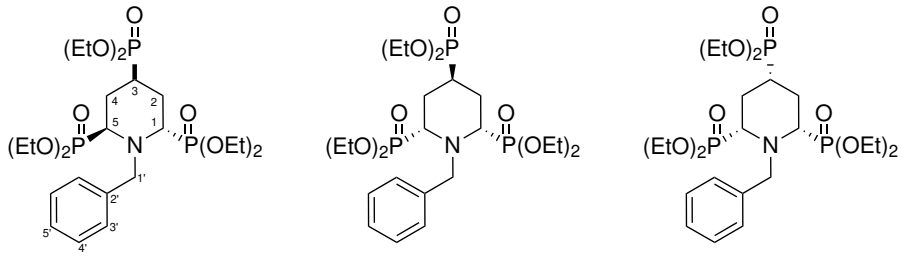


Figure 3.1: Reaction progression during the diphosphonylation of *N*-benzylpyridinium bromide.

Purification & analysis:

Purification proved to be very challenging as the acid-base extraction mentioned earlier in Section 3.2 was unsuited for this reaction. The most successful purification method turned out to be a normal phase column chromatography after a distillation to remove most hydrolysates, residual reagent and solvent in order to facilitate further purification. Mere distillation was not enough since the residual starting material and formed side products (see Section 3.3.2.2) had too high boiling points. Furthermore, no complete separation of the di- and triphosphonates was possible by chromatography due to the high similarities in structure and polarity. As a consequence a fraction of the triphosphonates had to be discarded to obtain a pure end product (35% yield). Purification of the diphosphonate was not feasible.

With further analysis of the purified triphosphonylated end product, it was possible to identify the different diastereomers by means of a two-dimensional NOESY NMR, where the proton-proton coupling is measured (see Scheme 3.8). Their specific ratios were however difficult to determine by ^{1}H-NMR due to peak overlap. For this reason only the ratio of the major (*M*) was distinguishable and estimated around 92%. However, further investigation into the selectivity of the diastereomers as well as the ratio between di- and triphosphonylation was certainly necessary.



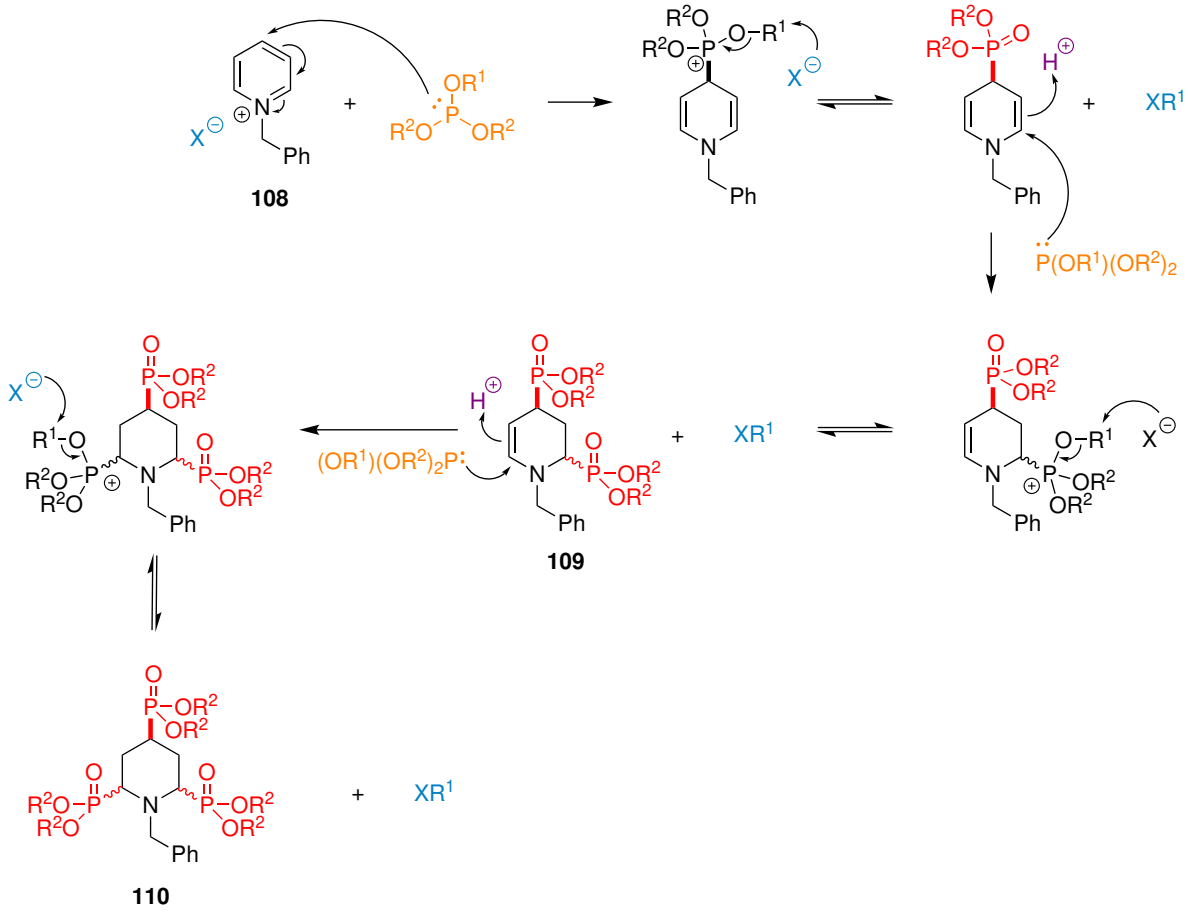
Scheme 3.8: The different diastereomers of *N*-benzyl-[2,4,6-tris(diethylphosphonyl)] piperidine.

3.3.2 Optimisation

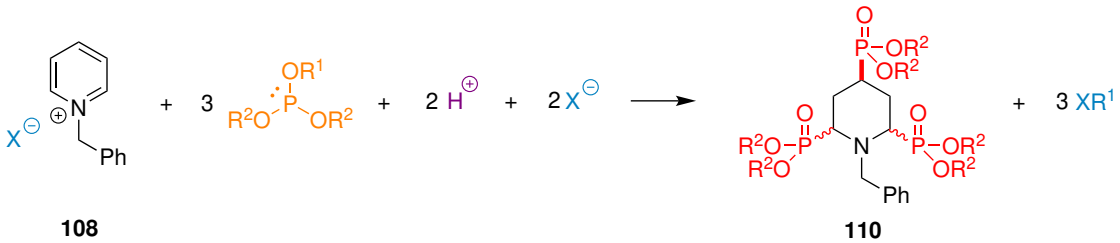
From the previous section it is clear that phosphonylation of pyridine was successful. However, before moving on to other substrates, it was crucial to further optimise the reaction since the reaction time was too long. In addition, specificity and overall yield needed to be improved as well. Optimisation was thus crucial before moving on to other substrates. Moreover, since both the di- and triphosphonylated compounds were produced, it was important to make the reaction more specific towards one compound. Since the triphosphonylated piperidine was the most abundant form, the first objective was pushing the reaction towards this product.

In Scheme 3.9, the overall reaction mechanism is given for the triphosphonylation of *N*-benzylpyridinium bromide for trialkyl phosphite (TAP) or dialkyl trimethylsilyl phosphite (DAPTMS). The reaction starts by the addition of a nucleophilic trivalent phosphonylating agent at the more accessible *para*-position. By interaction with a nucleophile, a phosphonate group is formed. This nucleophile is the counter anion, bromide, from the pyridinium salt. Next, the secondary addition of a nucleophilic species occurs. Since aromaticity is already broken, this step rapidly occurs and is accompanied by the incorporation of a hydrogen atom. This is repeated an additional time to produce the triphosphonylated product.

The overall reaction is shown in Scheme 3.10, giving the necessary reactants and formed compounds (not including side reactions). By analysing this reaction, several key aspects were identified which were crucial for the reaction optimisation: type of reagent, side reactions, solvent, temperature and degradation, acidity, possible catalysts and selectivity. Furthermore, the reaction was tested to see if a similar one-pot reaction was possible as already observed with the diphosphonylation of quinoline.



Scheme 3.9: Plausible mechanism for the triphosphonylation of *N*-benzylpyridinium bromide by TAP (R^1 = alkyl) or DAPTMS (R^1 = TMS), with R^2 = alkyl and X = nucleophile.



Scheme 3.10: General reaction overview for the triphosphonylation of *N*-benzylpyridinium bromide by TAP (R^1 = alkyl) or DAPTMS (R^1 = TMS), with R^2 = alkyl and X = nucleophile.

3.3.2.1 Phosphonylating agents

Other reagents besides DEPTMS were evaluated as well, i.e. dimethyl trimethylsilyl phosphite (DMPTMS) for its increased reactivity and trialkyl phosphites (TAP) for their increased stability. Dialkyl phosphites (DAP) were tested as well since these were already present in all the previous reactions due to hydrolysis of TAP and DAPTMS. The latter however did not show any signs of phosphonylation and could thus be ruled out.

In Figure 3.2, all of the above mentioned reagents were tested in dichloromethane at 40°C and acetonitrile at 80°C. After 3 days, the conversions were determined which are depicted in the graph below. From a first glance, DMPTMS seems to be the preferred reagent. However, due to more side reactions, the difficulty for the purification was increased immensely. As a result, DMPTMS was discarded as a potential reagent for the reaction. This was also the case for trimethyl phosphite (TMP). As a consequence, further optimisation continued with DEPTMS, although triethyl phosphite (TEP) could be of use as well because of its higher resistance to hydrolysis.

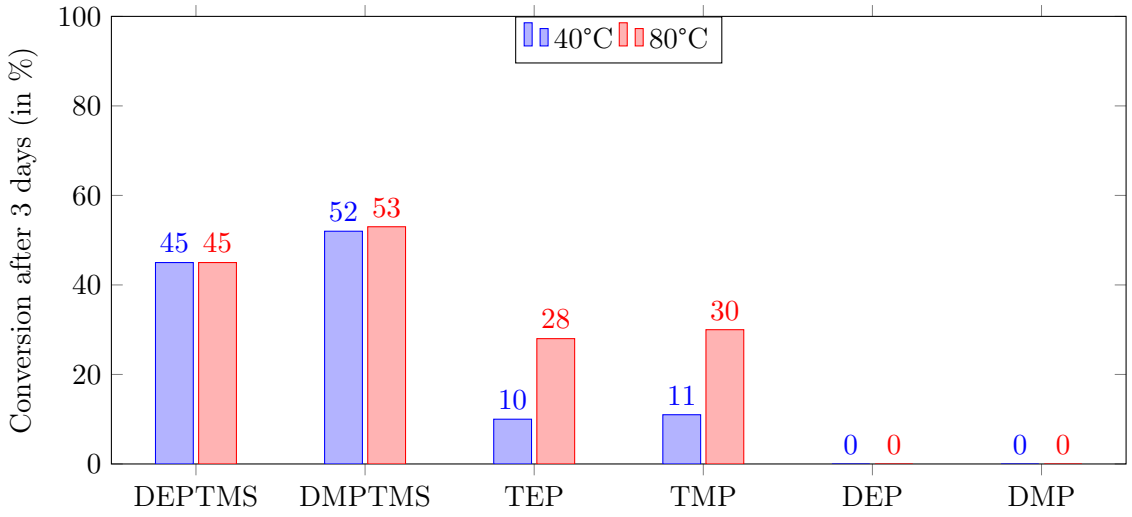
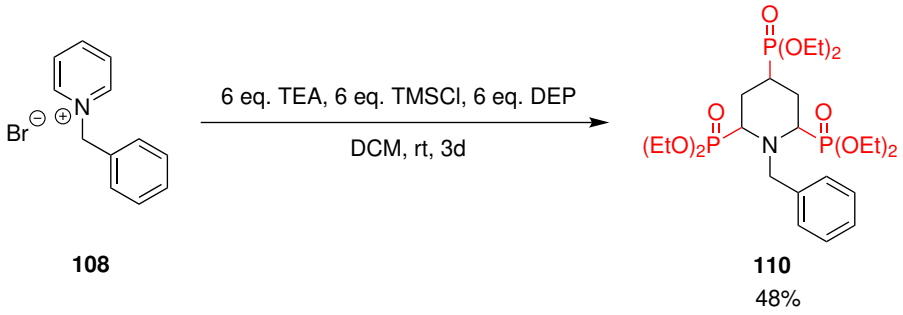


Figure 3.2: Influence of several reagents on the phosphonylation of *N*-benzylpyridinium bromide.

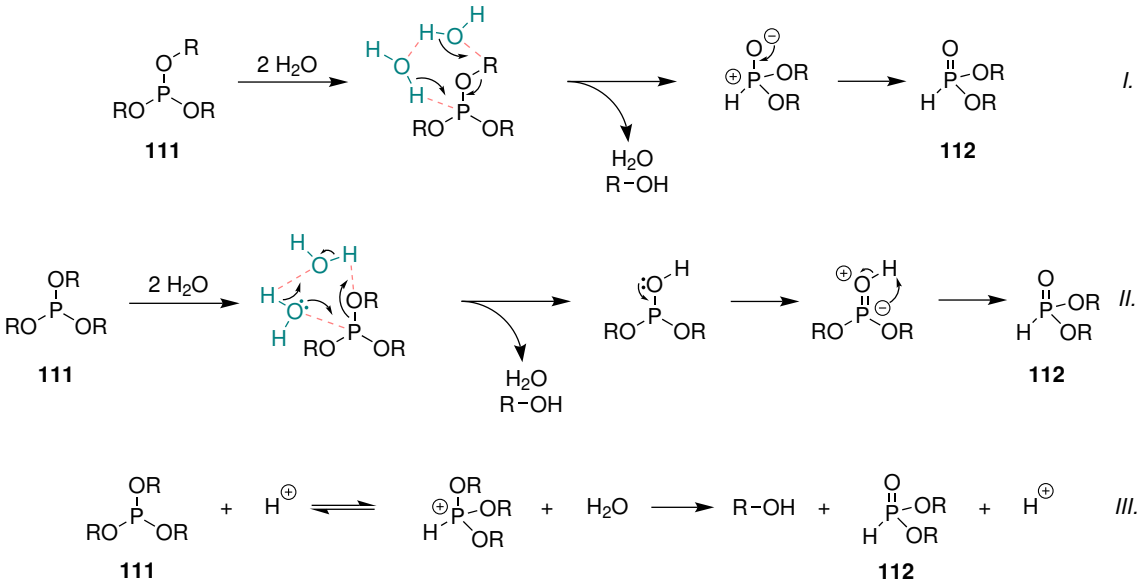
As previously seen in Section 2.5, the diphosphonylation of α,β-unsaturated imines and quinolines could be carried out by a simple one-pot reaction. These one-pot reactions are favoured because of their practicality and slightly lower sensitivity towards hydrolysis. For this reason, a one-pot reaction was also tested for *N*-benzylpyridinium bromide where DEPTMS was produced *in situ* (see Scheme 3.11). Analysis by ^{31}P-NMR showed a conversion of 48% after 3 days.



Scheme 3.11: One-pot synthesis using *in situ* produced DEPTMS.

3.3.2.2 Side reactions

The major side reaction occurring during phosphonylation is due to hydrolysis as previously mentioned. With the rate of hydrolysis being strongly dependent on the overall concentration of the reactants and possibly the acid catalysts. McIntyre and Alam concluded that hydrolysis of trialkyl phosphites (TAP) (**111**) followed a third-order rate at room temperature for all concentrations. However, at higher temperatures hydrolysis started to deviate exponentially from this behaviour and was best described by first-order kinetics.[82] Hydrolysis mechanisms for phosphite occurred via 3 possible mechanisms: Michaelis–Arbuzov (I), an analogue mechanism to the organic ester hydrolysis (II) or an acid-catalysed mechanism (III), with the latter two being the most dominant for phosphites (see Scheme 3.12). Further hydrolysis of diethyl phosphite (DEP) (**112**) was not very specific as many different hydrolysates were formed.

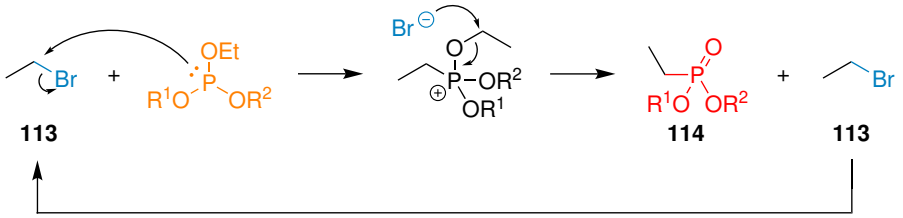


Scheme 3.12: Proposed mechanisms for the hydrolysis of TAP to DAP: (*I.*) the Michaelis–Arbuzov, (*II.*) the organic ester hydrolysis and (*III.*) the acid-catalysed mechanism, with R = alkyl.

However, since the previous mechanism is based on TAP, there will be a difference when comparing these to DEPTMS, which is much more sensitive towards nucleophiles. For this reason, even the ROH formed during hydrolysis will be able to react with the reagent. This also applies to other nucleophiles present in the reaction mixture such as the anion from the pyridinium salt for example.

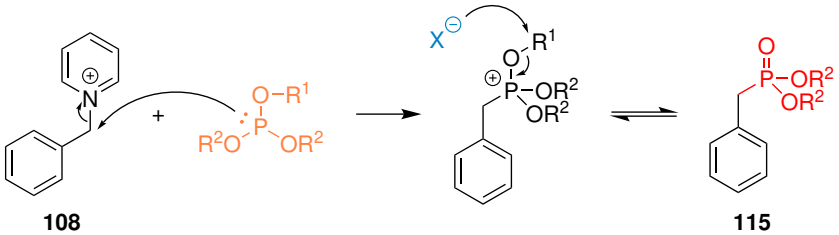
Besides hydrolysates, several undesired phosphonates are formed as well, with the most common ones being diethyl ethyl phosphonate and diethyl benzyl phosphonate for DEPTMS and TEP. These phosphonates can cause many problems later on during work-up as these compounds possess very high boiling points (>180°C). The produced amounts of both compounds can fluctuate heavily but remain under 4% (for T<100°C). Increasing the temperature above 100°C did however drastically increase their formation. [83]

Diethyl ethyl phosphonate (**114**) (^{31}P-NMR (162.0MHz): 33.4 ppm, $CDCl_3$) is formed by an Arbuzov reaction between DEPTMS/TEP and ethyl bromide (see Scheme 3.13). This ethyl bromide (**113**) is formed during the triphosphonylation reaction by the interaction of the counteranion bromine from the pyridinium salt with the added reagent (see Scheme 3.9). Since this Arbuzov reaction yields another ethyl bromide, one is dealing with an autocatalytic reaction. However, due to its high volatility, most of the ethyl bromide escapes to the gas phase where it does not pose a problem unless when using a closed vessel (e.g. microwave flask, pressure tubes) causing an increase in gas solubility due to pressure build up.



Scheme 3.13: Synthesis of diethyl ethyl phosphonate by means of an Arbuzov reaction using TEP (R^1 = R^2 = ethyl) or DEPTMS (R^1 = TMS, R^2 = ethyl).

Diethyl benzyl phosphonate (**115**) (^{31}P-NMR (162.0MHz): 26.15 ppm, $CDCl_3$) is formed due to a side reaction between the N-benzylpyridinium bromide (**108**) and the DEPTMS/TEP. The reagent interacts with the benzyl side group, producing diethyl benzyl phosphonate and pyridine (see Scheme 3.14). This side reaction can however simply be avoided by changing to other substrates such as 1-methylpyridinium salt for example.



Scheme 3.14: Synthesis of diethyl benzyl phosphonate by TEP (R^1 = R^2 = ethyl) or DEPTMS (R^1 = TMS, R^2 = ethyl), with X = nucleophile.

3.3.2.3 Solvent impact

Since the solvent affects the rate of reaction, finding the correct solvent can have a crucial effect on the reaction. For S_{N2}-type reactions, polar aprotic solvents are considered to be optimal as the solvent molecules will not interact too much with the nucleophile, thus not hindering its approach to the starting material.[84] For this reason, several solvents were tested for DEPTMS and TEP under reflux. In tetrahydrofuran (THF) and toluene, no reaction occurred since the pyridinium salt was insoluble. In addition, methanol caused complete hydrolysis of the reagent and the use of no solvent led to degradation of the reactants.

Dichloromethane (DCM), acetonitrile (MeCN), 1,2-dimethoxyethane (DME) and chloroform ($CHCl_3$) were considered to be the most promising solvents as they all showed very similar conversions for DEPTMS. However, due to the lower boiling points of DCM and $CHCl_3$ (40°C and 61°C respectively), the overall conversion for these 2 solvents was lower when compared to acetonitrile and 1,2-dimethoxyethane (DME) (82°C and 85°C respectively), indicating that the optimal reaction temperature will be around or above 80°C. Finally, after testing all solvents, acetonitrile was chosen for further analysis because of practical and environmental reasons.

As mentioned in Section 3.2, 2 phases are formed when mixing the nonpolar reagent with the protonated pyridinium salt. As a result, interaction between both is limited. This is also the case for *N*-benzylpyridinium bromide and DAPTMS or TAP. However, once the reaction took off, both phases started to mix until finally a homogeneous mixture was formed. Attempts were made to speed up this mixing by testing multiple solvents, but no significant improvements were noticed between DCM, MeCN, DME and $CHCl_3$. For this reason, it was clear that the phase mixing was mainly determined by the overall conversion.

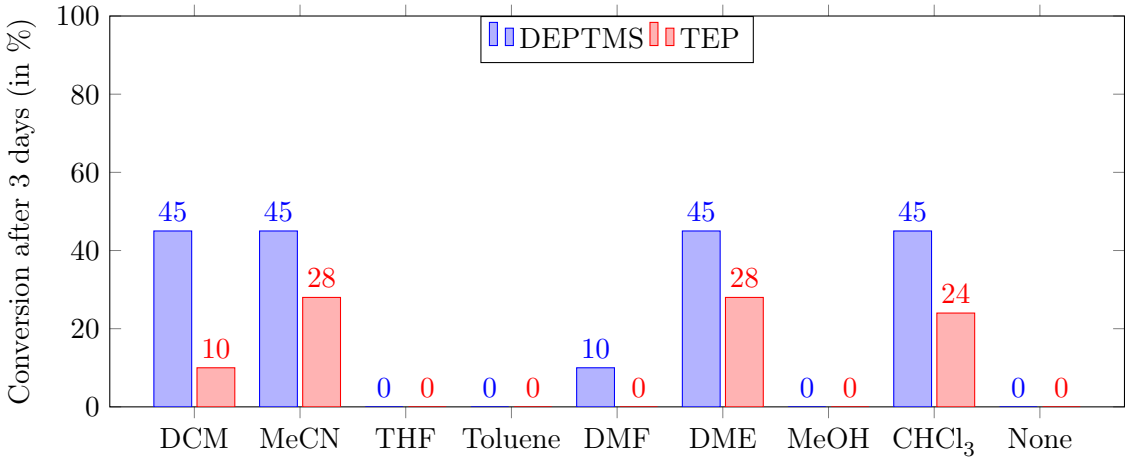


Figure 3.3: Influence of the solvent on the phosphonylation of *N*-benzylpyridinium bromide.

3.3.2.4 Influence of acidic conditions

When looking at the general reaction overview portrayed in Scheme 3.10, one can notice the incorporation of 2 external protons in the final compound. For this reason the influence of several acids was investigated at different concentrations. At first, DEPTMS was tested with various acids (HCOOH, H_2SO_4, CH_3SO_3H), however in all cases, analysis by ^{31}P-NMR showed full hydrolysis of the reagent and thus no reaction occurred. However, when using TEP as phosphonylating agent a clear effect was noticeable. Hydrolysis still occurred but was less abundant. A small excess (3.33 eq.) of the reagent would quickly solve this problem.

Unfortunately, due to solvent escaping from the reaction, the acquired results were very inconsistent as the acidic concentrations changed drastically. However, a proportional relationship was established between the amount of evaporated solvent and the overall conversion. When the reaction was then carried out without solvent, a significant increase in conversion was detected. This in contrast to the results found in Section 3.3.2.3, where no acids were used. The overall conversion was then increased drastically with full conversion after 3 days at 80°C with 2eq. of formic acid (HCOOH). Other concentrations of HCOOH did not achieve such high conversions and the use of other acids (H_2SO_4 and CH_3SO_3H) resulted only in full hydrolysis of the reagent.

3.3.2.5 Silica as a potential catalyst

In 2000, Lavilla et al. published an article regarding the oxidative diphosphonylation of substituted pyridinium salts. Here, they made use of silica (SiO_2) during the work-up for relocating the phosphonate groups from the *ortho*- to the more stable *para*-position (see Scheme 2.22). This unusual phosphonate shift may be explained by invoking the reversibility of the phosphite addition and its greater stability at the *para*-position.[67]

For this reason it was worth looking further into this by adding silica (0.25 mg) to the reaction mixture, now consisting of 1 mmol *N*-benzylpyridinium bromide, 10 mmol TEP, 2 mmol HCOOH and no solvent. Analysis by ^{31}P-NMR showed already full conversion after just one day, clearly showing the effect of silica on the reaction. In addition, the effect of silica was tested also without the use of acid, but this was without success (see Figure 3.4).

Considering the results from Figure 3.4, it can be stated that the silica is able to drastically improve selectivity towards the triphosphonylated compound, thus improving further purification and increasing the overall yield (80.5% triphosphonate, 3.5% diphosphonate). However, its exact mechanism is still unknown. Increasing the reaction time ($>$ 1 day) or catalyst quantity did, however, not improve the selectivity.

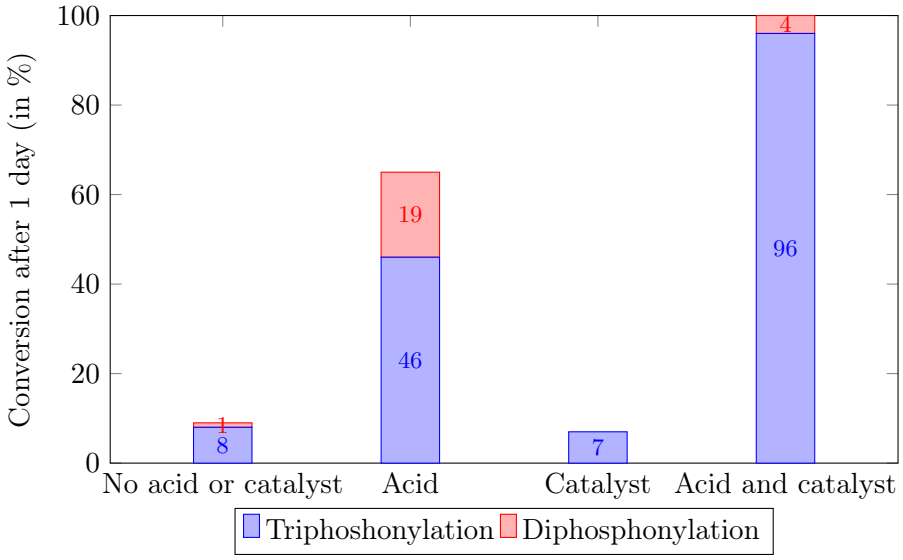


Figure 3.4: Difference in conversion when applying TEP with acid (HCOOH), catalyst (silica) or both. For comparison purposes, the results without silica and acid are given as well.

3.3.2.6 Thermal stability

Prior to testing the reaction time and conversion at different temperatures, it seemed best to start by investigating the thermal stability of each compound. These degradation experiments were performed for both reagents (TEP and DEPTMS) as well as the starting material.

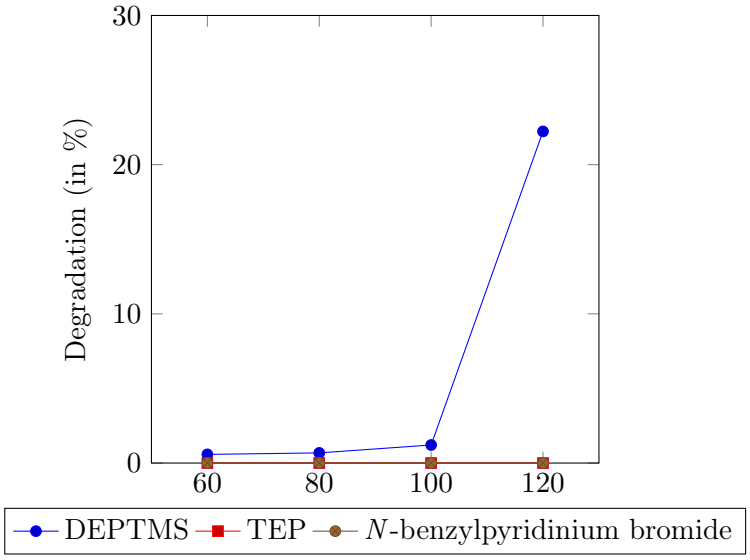


Figure 3.5: Thermal degradation of reactants after 3 hours by microwave heating.

For DEPTMS, temperatures till 100°C won't cause any major problems. However, when reaction times are extended to several days, these small percentages can become quite significant. Next, for TEP and *N*-benzylpyridinium bromide, no decomposition was noticeable under 120°C. Although, this does not mean that very high reaction temperatures

are possible. Since the boiling point of formic acid is situated at 101°C, this has to be taken into account as evaporation will cause HCOOH concentrations to drop, lowering overall conversion.[83] Furthermore, the reaction with TEP, HCOOH and SiO_2 was tested at 70, 80, 90 and 100°C, with the best result, by far, being with heating at 80°C.

3.3.2.7 Microwave *vs.* standard heating

As conventional heating usually involves using a furnace or oil bath to heat up the reactor from the outside by convection or conduction. This causes the core of the sample to take a longer time to achieve the targeted temperature. In comparison, microwave heating acts as internal heat source, where the target compounds or the solvents are heated by microwave absorption without external heating saving time and energy.[85] Different compounds convert microwave radiation to heat by different amounts. This selectivity allows some parts of the object to be heated more quickly or more slowly than others (particularly the reaction vessel) and can cause a significant difference for certain reactions. One example is the diphosphonylation of quinoline as previously mentioned. This reaction took over 3 days with conventional heating (oil bath) but only 3 hours with microwave heating. On top of that, the difference between an open or closed vessel can also have some influence.

For this reason, microwave heating was tested as well for the triphosphonylation of pyridine. At first, reactions using DEPTMS and TEP were tested without acid or silica. However, even though there was an improvement relative to conventional heating, the reaction still required multiple days. Next, phosphonylation was tested with the pyridinium salt mixed with 2 eq. of formic acid, 3.33 eq. of TEP and silica at 80°C. As expected, a significant difference was found between both types of heating as can be seen in Figure 3.6. Microwave heating caused a significant increase in reaction speed but at the expense of its selectivity. Significantly more side reactions took place as well as an increase in diphosphonylation ratio. Still, by microwave heating the shortest reaction time yet was established, even though the purification was more difficult. Unfortunately, due to often malfunctioning of the microwave equipment, further testing was halted and must be revisited in the future.

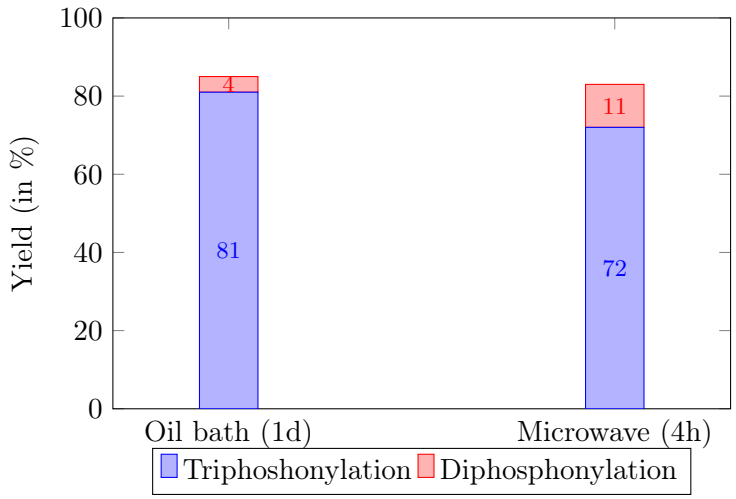
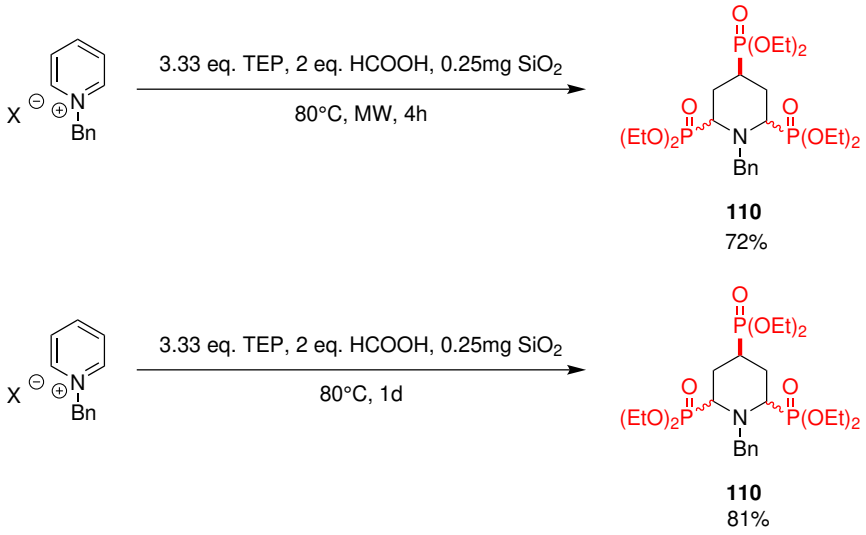


Figure 3.6: Difference in selectivity due to different heating methods.

3.3.3 Summary

The triphosphonylation of pyridine posed many challenges. The sensitive reagents, long reaction times, difficult purification procedures and mixing problems all needed to be tackled. But after a long optimisation phase the outcome was a big improvement. By microwave heating the fastest reaction time was established (4h) with a yield of 72%. However, conventional heating achieved a higher yield (81%), although having a longer reaction time of 1 day. By making the established procedure very accessible and easy to perform, it was possible to move on to other substrates. The final optimised reaction procedure is given in Scheme 3.15.

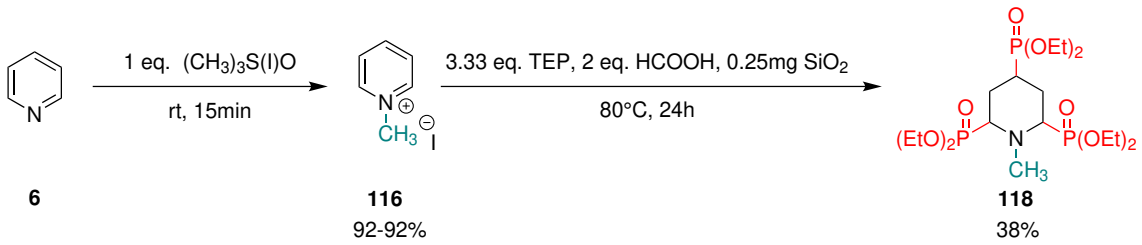


Scheme 3.15: General overview of the optimised triphosphonylation reaction for *N*-benzylpyridinium salts by microwave heating (top) and conventional heating (bottom).

3.4 Phosphonylation of other alkylated pyridinium salts

3.4.1 1-Methylpyridinium iodide

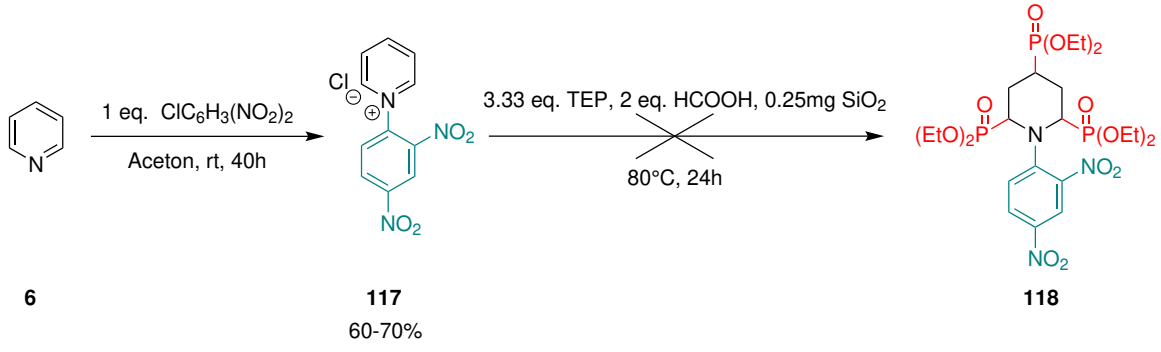
In order to avoid the formation of the byproduct diethyl benzyl phosphonate, 1-methylpyridinium iodide (**116**) was chosen as a starting material. Synthesis of this compound did however not follow the traditional S_{N2}-reaction of pyridine and an alkyl halide. Instead trimethylsulphoxonium iodide was used to alkylate pyridine (**6**). After purification, the acquired salt was phosphonylated according to the optimised method (see Scheme 3.15). After 1 day full conversion was reached, of which 74% consisted of triphosphonylated piperidine and 4% diphosphonylated piperidine. Meaning that over 20% of the starting material was converted into byproducts which interfere with further purification. For this reason the yield of the final step was only 38%. The use of a longer alkyl chain might improve this yield by increasing its solubility in TEP, thereby increasing phase mixing. Furthermore, NOESY NMR showed the same diastereomeric mixture as the benzylated product (see Scheme 3.8), with the ratio of the major being 94%.



Scheme 3.16: Triphosphonylation of *N*-methylpyridinium iodide.

3.4.2 *N*-(2,4-dinitrophenyl)pyridinium chloride (Zincke salt)

N-(2,4-dinitrophenyl)pyridinium chloride (**117**), more commonly known as Zincke salt, was investigated because of its highly electrophilic properties. By withdrawing electrons from the pyridine ring, it was made more susceptible towards the addition the TEP. Unfortunately, only decomposition of the starting material and reagent were detected by LC-MS or ^{31}P-NMR.



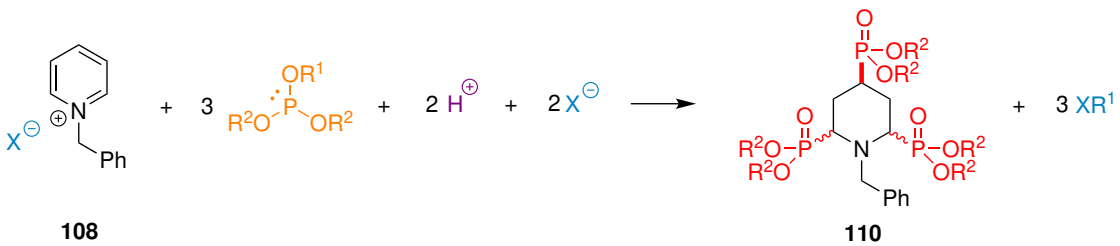
Scheme 3.17: Triphosphonylation of *N*-(2,4-dinitrophenyl)pyridinium chloride.

Chapter 4

Summary & Conclusion

In this Master thesis, one-pot multiphosphonylated piperidine analogues were synthesised as potential enzyme inhibitors, characterised by a low reaction time, high efficiency and yield. A big improvement relative to reactions described in literature (see Section 2), where often leaving groups or large activating groups are used, multiple steps are required and reaction times of several days are not uncommon.

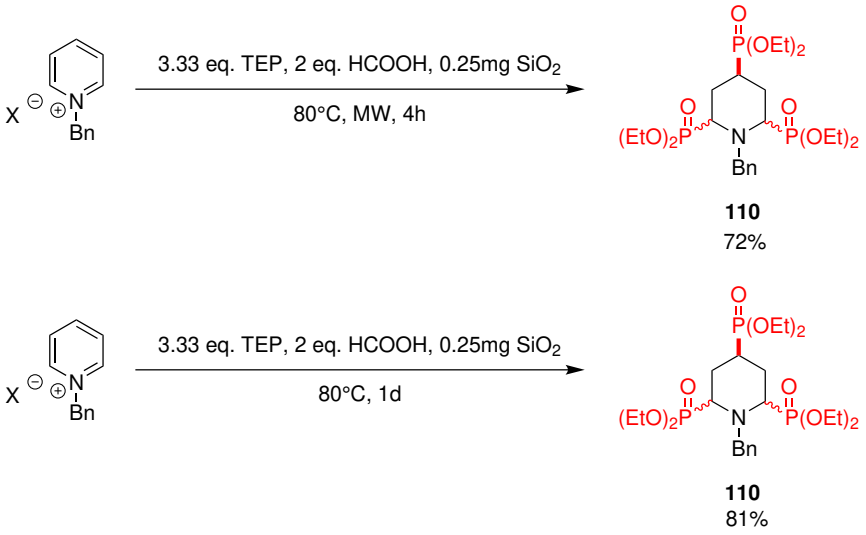
Initially, the reactions were based on the methods developed at the SynBioC research group regarding the one-pot diphosphonylation of α,β-unsaturated imines and quinoline. In this case, the nitrogen atom was activated by protonation, followed by multiple phosphite additions in a simple one-pot reaction (see Section 2.5). Unfortunately, when similar mechanisms were applied on pyridine, no phosphonylation occurred. Instead, activation of the nitrogen atom by alkylation (e.g. benzyl group) proved to be more successful. The result was a diastereomeric mixture of di- and triphosphonylated *N*-alkylpiperidines. This outcome was rather unexpected since triphosphonylation of pyridine was never mentioned in any literature study. Since it was more abundant than its diphosphonylated analogue, the reaction was pushed towards the triphosphonylated piperidine. However, due to low yields, long reaction times (*ca.* 11 days) and hydrolysis of the reagent, experiments on other substrates were halted until the overall efficiency was improved. The optimisation process included the influence of acids, heating, catalysts, reagents, solvents, control of the side reactions and containment of reagent hydrolysis.



108 **110**

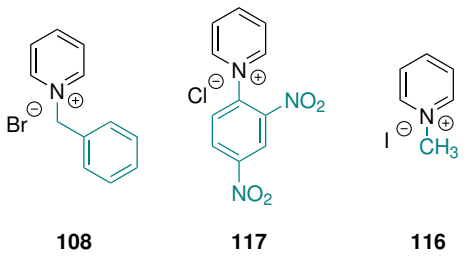
Scheme 4.1: General reaction overview for the triphosphonylation of *N*-benzylpyridinium bromide by TAP (R^1 = alkyl) or DAPTMS (R^1 = TMS), with R^2 = alkyl and X = nucleophile.

After thorough testing and analysis, the ideal reaction conditions were established. This method required the use of formic acid, silica, triethyl phosphite (TEP) and microwave heating (80°C) to obtain a yield of 72% in only 4 hours or 81% in 24 hours when conventional heating was used (see Scheme 4.2). Important to note here is the absence of solvent, making the reaction more attractive from an environmental and economical point of view. However, the exact influence of silica on the phosphonylation reaction is still unknown and will require further investigation.



Scheme 4.2: General overview of the optimised triphosphonylation reaction for pyridinium salts by microwave heating (top) and conventional heating (bottom), with R = alkyl, benzyl.

With the optimisation process finished, the applicability of this newly established method was investigated by testing other N-alkylpyridinium salts. However, due to the COVID-19 crisis the scope was limited to *N*-methylpyridinium iodide and *N*-(2,4-dinitrophenyl)pyridinium chloride (see Scheme 4.3). For this reason, no significant conclusions can be drawn about this methods applicability for the phosphonylation of pyridinium salts.



Scheme 4.3: Structures of *N*-benzylpyridinium bromide (left), *N*-(2,4-dinitrophenyl)pyridinium chloride (middle) and *N*-methylpyridinium iodide (right).

To conclude, one-pot phosphonylation of pyridinium salts was achieved by alkylating the nitrogen instead of protonation, resulting in a mixture of di- and triphosphonylated piperidines. Reaction optimisation gave a reasonable reaction time with high selectivity towards the triphosphonylated product and high yields for *N*-benzylpyridinium bromide. However, more investigation is still required for further optimisation of this phosphonylation method. Furthermore, a broadening of the scope is needed in order to assess its potential as a general triphosphonylation method for alkylated pyridinium salts.

Chapter 5

Future perspectives

As a direct result of the current COVID-19 crisis, the lab work for this Master thesis was discontinued earlier than foreseen. For this reason, several aspects of this thesis could not be completed in time and will be briefly explained in this section together with other future perspectives. These should hopefully be able to validate further research into the one-pot phosphonylation of pyridinium salts.

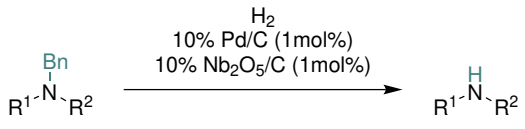
One aspect regards the effect of the counter anion of the pyridinium salt, as this anion might have a significant impact on the reaction speed by tilting the equilibrium as seen in Scheme 3.9. In addition, bromide also led to the formation of side reactions because of its high reactivity. It is thus certainly possible that the anion has a significant effect on the phosphonylation as well. For this reason, other counterions (e.g. halogens, ClO_4^-, BF_4^-, NO_3^-, bistriflimide) should be investigated as they might lead to higher yields, increased selectivity and/or a decrease in side reactions.

These experiments should be performed for a single pyridinium salt, using the optimised phosphonylation method from Scheme 3.15 in order to correlate every effect directly to the change in counter anion. Since *N*-benzylpyridinium bromide has been the most promising substrate so far, experiments should be performed using this pyridinium cation with a variety of anions. In order to synthesise this cation with various anions, an anion exchange should be performed using anion exchange chromatography. In an anion exchange column, the packing is positively charged, therefore retaining negatively charged compounds by Coulomb interaction. The bound molecules are then eluted using an anion gradient to obtain the desired pyridinium salt.[86] A more straightforward method, however, is to alter the reagent used for the synthesis of the pyridinium salt (e.g. benzyl bromide in case of *N*-benzylpyridinium bromide). Since a variety of different halide reagents are available on the market, this method will be less time consuming and more economic. However, some reagents will not share the high reactivity bromide does, while others might even be too unstable. For these instances, anion exchange chromatography will need to be used. This experiment was already performed for bromide and chloride. However, no significant differences were observed between these anions.

Secondly, since microwave reactions proved to have a large impact on the reaction time (see Section 3.3.2.7), it might be of interest to investigate this further by testing the reaction in a flow reactor, where heat is more evenly distributed and control over the temperature is much higher. In addition, phase mixing should be increased as well, possibly resulting in a much lower reaction time, less side reactions and less hydrolysis.

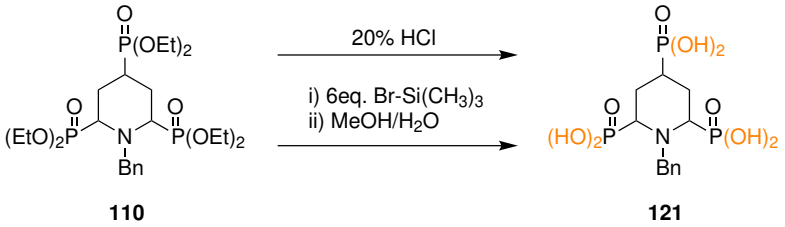
Furthermore, during this Master thesis the focus was mainly put on the triphosphonylation of pyridine as it is more abundantly formed than its diphosphonylated analogue. However, experiments regarding selectivity towards the diphosphonylated product were scheduled as well. Since the triphosphonylated compound was always more abundant, a substituent at the *ortho*-position (e.g. alkyl group) could be used to acquire only the diphosphonylated moiety. However, this would be contradictory to our goal (i.e. phosphonylation of unsubstituted pyridine with the exception of the nitrogen atom). Another approach might be the addition of a large substituent to the nitrogen causing steric hindrance to one of the *ortho*-positions. Possible substituents which might achieve this are *N*-(2-methylcyclopenta-2,4-dien-1-yl) (**123**) and *N*-(2-phenylcyclopenta-2,4-dien-1-yl) (**122**). Addition of a phosphonate group at one of the *ortho*-positions will inhibit other phosphonate groups from nearing the other *ortho*-position. Moreover, solely achieving diphosphonylation will also benefit further purification, since complete separation of the di- and triphosphonylated piperidines was not possible.

Another subject regards the removal of the substituent on the nitrogen atom of the phosphonylated piperidine. As phosphonylation by alkylation proved to be more suitable than acidification, an additional step was needed to form triphosphonylated piperidine. For *N*-benzyl-[2,4,6-tris(diethylphosphonyl)] piperidine, this meant a removal of the benzyl group. One possible method makes use of a palladium-on-carbon (Pd/C)-catalysed hydrogenation with the use of niobic acid-on-carbon (Nb_2O_5/C) (see Scheme 5.1). This is an acidic heterogeneous catalyst prepared from $NbCl_5$ and activated carbon, which is easily removed from the reaction mixture.[87]



Scheme 5.1: Debenzylation of tertiary amines by Pd-catalysed hydrogenation, with R = alkyl, aryl.[87]

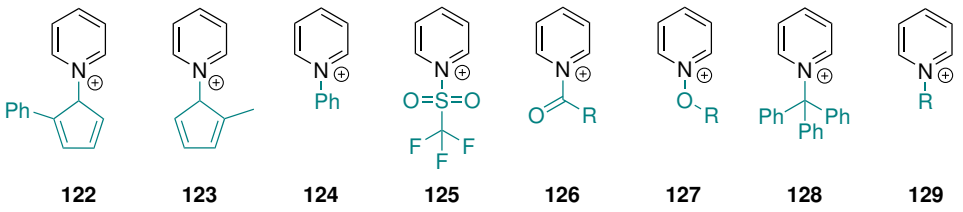
Besides azaheterocyclic phosphonates, their phosphoric acid analogues are also known to be effective enzyme inhibitors. For this reason it would be of interest to synthesise and characterise these compounds as well. Synthesis occurs by dealkylation of dialkyl phosphonates under either acidic conditions (20% HCl) or using the McKenna procedure (i.e. a two-step reaction that makes use of bromotrimethylsilane ($(CH_3)_3SiBr$) followed by methanolysis or hydrolysis) (see Scheme 5.2).[88]



Scheme 5.2: Dealkylation of dialkyl phosphonates under either acidic conditions (HCl) (top) or using the McKenna procedure (bottom).[88]

Another focus point is related to the specific role of silica (SiO_2) in the phosphonylation reaction and its influence on the selectivity towards the triphosphonylated product. In addition, it might be of interest to find out if this increased reactivity and selectivity is solely due to silica or if other catalysts, such as alumina (Al_2O_3), are also capable to achieve this. This might help to better understand the specific role this catalyst has on the reaction.

A final aspect concerns the scope of the optimised reaction. In order to evaluate the applicability of the phosphonylation method, a larger quantity of substrates will need to be tested. A possible candidate is the use of *N*-phenylpyridinium salt (**124**), as it avoids the formation of its side product diethyl benzylphosphonate. Other interesting *N*-substituents include triflyl (**125**), acyl (**126**), alkoxy (**127**), trityl (**128**) and longer alkyl chains (**129**) (see Scheme 5.3). Afterwards, once the scope of the reaction has improved and a variety of different pyridinium salts have been phosphonylated, biological testing can commence to evaluate the applicability of this class as enzyme inhibitors in medicine and or agriculture. Moreover, because of its high stability, other applications, such as flame retardants, might also be of interest.



Scheme 5.3: Pyridinium cations which could be used for further research, with R = alkyl.

Chapter 6

Experimental section

6.1 General analytical methods and laboratory equipment

6.1.1 Column Chromatography

Column chromatography is used for the purification/separation of compounds from a reaction mixture. This technique is performed by filling a glass column partially with silica gel (max height of 15-20cm), with a particle diameter between 0.035 and 0.07mm. The used eluens are determined in advance by thin layer chromatography (TLC). Besides silica, sand is also added to the column. A sand layer on the bottom ensures an even bedding for the stationary phase and acts as a filter to avoid the silica from going through, while the upper layer is used for protection of the well packed stationary phase.

6.1.2 Dry Solvents

To avoid hydrolysis of the water sensitive reagents, most chemical reactions were performed under dry conditions. The MBraun SPS-800 solvent purification system provided the following dry solvents: acetonitrile (MeCN), diethyl ether, tetrahydrofuran (THF), dichloromethane (DCM) and toluene. These were held in a safety closet in Pure-Pac storage tanks of 17L, under pressure with an inert gas (N_2) which is then send through 2 filtering/drying columns of stainless steel (1.4301 / US 304) with an internal volume of 4.8L. Each column possesses its own specific filtering material (molecular sieves) for the used solvent. In addition, THF was sent through an additional filtering material before it came in contact with the drying columns. Collection of the solvents occurred by creating a vacuum by means of a membrane pump (type PMC 301 Zp) in separate flasks. This way the dry solvents could be collected under an inert atmosphere (N_2).

Acetone was dried by adding an excess of calcium chloride $CaCl_2$ and stirring the mixture under a nitrogen atmosphere (N_2) for several days. Other Solvents such as DMF were dried over molecular sieves. Afterwards, their water content was determined by means of a Karl Fischer titration.

6.1.3 Infrared Spectroscopy (IR)

Infrared spectra were measured using the Shimadzu IRAffinity-1S, a Fourier transformed infrared spectrometer (FTIR) along with an attenuated total reflectance (ATR) crystal. By means of the coupled LabSolutions IR software the infrared spectra could be obtained.

6.1.4 Karl Fischer titration (KF)

The Karl Fischer titration is a method used for measuring the water content of chemical compounds by coulometric or volumetric titration. Measuring was performed with a Compact C10S titrator using a hydranal solution. After addition of the sample to the solvent, the titration will start and stop once an equilibrium is reached. By accurately measuring the time and current, the amount of water in the sample can then be calculated.

6.1.5 Liquid Chromatography-Mass Spectrometry (LC-MS)

Liquid chromatography-mass spectrometry or LC-MS was performed by an Agilent 1200 series LC/MSD SL-device with a Supelco Acentis Express C18 column (I.D. x L 4.6mm x 3cm, 2.7µm fused core particles with 90Å pore size). In addition, this device is also equipped with a UV detector, an Agilent 1100 series mass spectrometer with electron spray ionisation source (ESI, 4000V, 70eV) and a quadrupole detector. For analysis, a mobile phase consisting of water (5mM NH_4OAc) and acetonitrile was used, with their ratios depending on the selected method.

6.1.6 Mass Spectrometry (MS)

Mass spectrometry was carried out by an Agilent 1100 MSD SL series using an electron-spray ionisation source (ESI, 4000V) and a quadrupole detector for low resolution spectra. However, to obtain high resolution spectra an Agilent Technologies 6210 Series *Time-of-Flight* mass spectrometer was used.

6.1.7 Microwave reactor (MW)

A CEM focused Microwave Synthesis System (Model Discover) was used to perform microwave reactions. This system has an adaptable power input ranging from 0 to 300W, which is monitored by the Synergy software (version 1.32). Reaction mixtures were held in a 10mL Pyrex vial under continuous stirring, sealed with a snap-on PTFE-septum.

6.1.8 NMR Spectroscopy

^{1}H-NMR (400MHz), ^{13}C-NMR (100.6MHz), ^{31}P-NMR (162.0MHz), COSY, HSQC, NOESY and DEPT spectra were produced by a NMR spectrometer. More specifically a Bruker Avance Nanobay III NMR spectrometer equipped with a 5mm BBFO Z-gradient high resolution probe. By dissolving the compounds in a deuterated solvent (e.g. $CDCl_3$, D_2O, $DMSO-d_6$) their spectra could be obtained and each signal could be assigned. For $CDCl_3$, tetramethylsilane is used as an internal standard, while the others are adjusted by the signals of the solvents. The shift of every peak are then reported as a δ-value in ppm. Using the TopSpin software (version 3.5) all spectra could then be further analysed.

6.1.9 pH-Indicator

In order to measure the pH of a chemical mixture, pH-indicator strips were used. These strips are suited for all media in environmental analysis and in industrial in-process controls. The pH is then measured by visual comparison of the strip with the given colour scale.

6.1.10 Thin Layer Chromatography (TLC)

Thin layer chromatography or TLC is used to follow up the progress of reactions, to determine the suitable eluens for chromatographic purification or to analyse the different fractions during chromatography.

In order to perform a TLC, silica plates (Merck silica gel 60 F_{254}, precoated on glass, thickness 0.25mm) were used with an experimentally determined eluens. Detection of the compounds happened by using UV-light or colouring agents such as potassium permanganate ($KMnO_4$).

6.2 Safety

6.2.1 General safety aspects

At the beginning of this Master's dissertation, all students signed 3 documents following the health and safety measures drawn up by the SynBioC research group (Department of Green Chemistry and Technology, Faculty of Bioscience Engineering, UGent). The documents, which needed to be read and signed prior to the start of the thesis, are: 'Safety and hygiene in chemical laboratories', Safety instructions: how to work with chemicals' and 'Welzijns- milieugids UGent'. In addition, a presentation and tour of the laboratory were given with emphasis on safe handling of hazardous chemicals and devices. To conclude, an introduction test on safety was implemented to evaluate each student.

6.2.2 Specific safety risks

When working in the lab, the use of hazardous compounds is almost unavoidable. Minimising the danger by following the safety guidelines and reading the Material Safety Data Sheet (MSDS) of every compound prior to the experiment is obviously a necessity. In Table 6.1 and Table 6.2, a summary will be given of the most frequently used and/or most dangerous solvents and reagents as well as their corresponding hazard statements.

Table 6.1: Overview of the most frequently used hazardous solvents with their corresponding hazards statements.[89]

Solvents	**Hazard statements**
Acetonitrile (MeCN)	Causes eye irritation. May be harmful if swallowed, inhaled or absorbed through the skin. May cause skin and respiratory tract irritation. Metabolised to cyanide in the body, which may cause headache, dizziness, weakness, unconsciousness, convulsions, coma and possible death. May cause liver and kidney damage.
Chloroform ($CHCl_3$)	Causes eye, skin and respiratory tract irritation. May be harmful if swallowed or inhaled. May cause central nervous system depression. May cause cancer based on animal studies. May cause cardiac disturbances. This substance has caused adverse reproductive and fetal effects in animals. Light sensitive.
Dichloromethane (DCM)	Harmful if swallowed. Causes eye, skin and respiratory tract irritation. May be harmful if inhaled. Potential cancer hazard. This substance has caused adverse reproductive and fetal effects in animals. May cause central nervous system effects. May cause kidney damage.
Diethyl ether (Et_2O)	Extremely flammable liquid and vapour. Vapour may cause flash fire. Breathing vapours may cause drowsiness and dizziness. Harmful if swallowed. Repeated exposure may cause skin dryness or cracking. Causes eye and skin irritation. May cause respiratory tract irritation. Aspiration hazard if swallowed. Can enter lungs and cause damage. May form explosive peroxides. Hygroscopic (absorbs moisture from the air).
Methanol (MeOH)	May be fatal or cause blindness if swallowed. Harmful vapour. Flammable liquid and vapour. Harmful if swallowed, inhaled or absorbed through the skin. Causes eye, skin and respiratory tract irritation. May cause central nervous system depression.
Tetrathydrofuran (THF)	Highly flammable. Causes eye and respiratory tract irritation. May form explosive peroxides. Hygroscopic.

Table 6.2: Overview of the most frequently used hazardous reagents with their corresponding hazard statements.[89]

Reagents	**Hazard statements**
Chlorotrimethylsilane (TMSCl)	Causes burns by all exposure routes. Inhalation of high vapour concentrations may cause symptoms like difficulty breathing, headache, dizziness, tiredness, nausea and vomiting. Product is a corrosive material. Ingestion causes severe swelling, severe damage to the delicate tissue and danger of perforation.
Triethyl phosphite (TEP)	A flammable liquid and vapour. Stench. May cause eye and skin irritation. May cause respiratory and digestive tract irritation. May be harmful if swallowed.
Formic acid	Corrosive to the respiratory tract. Flammable liquid and vapour. Harmful if swallowed. Causes severe skin burns and eye damage.
Sulphuric acid	Causes eye and skin burns. Causes digestive and respiratory tract burns. May be fatal if mist inhaled. Strong inorganic acid mists containing sulphuric acid may cause cancer. Concentrated sulphuric acid reacts violently with water and many other substances under certain conditions. May cause lung damage. Hygroscopic. Corrosive to metal.
Pyridine	Causes severe eye and skin irritation with possible burns. Flammable liquid and vapour. Causes respiratory tract irritation. Stench. May be harmful if swallowed, inhaled, or absorbed through the skin. May cause central nervous system depression.
Quinoline	Cancer suspect agent. Possible risks of irreversible effects. May cause liver damage. Causes eye irritation and possible injury. Harmful in contact with skin and if swallowed. Causes skin and respiratory tract irritation.
Benzyl bromide	Combustible liquid and vapour. Toxic if inhaled. Causes eye, skin, and respiratory tract irritation.
Trimethylsulphoxonium iodide	Causes eye and skin irritation. Causes respiratory and digestive tract irritation. Light sensitive. The toxicological properties of this material have not been fully investigated.
Triethylamine (TEA)	Highly flammable liquid and vapour. Toxic if inhaled. Harmful if swallowed. Toxic in contact with skin. Causes severe skin burns and eye damage. Causes respiratory irritation. Causes drowsiness or dizziness.
1-Chloro-2,4-dinitrobenzene	Toxic by inhalation, in contact with skin and if swallowed. Danger of cumulative effects. Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment. Heat sensitive.

6.3 Description of experiments

6.3.1 Synthesis of diethyl trimethylsilyl phosphite (DEPTMS)

13.8 g (100 mmol) of diethyl phosphite is added to a flame dried 250 mL flask and dissolved in 150 mL of dry dichloromethane. Next, 12.15 g (120 mmol) triethyl amine is added to the mixture, which is kept stirring at 0°C under a nitrogen or argon atmosphere. Once the mixture is homogeneous, 11.94 g (110 mmol) chlorotrimethylsilane is added dropwise using a dry syringe. After 30 minutes at 0°C, a sample is taken to analyse the conversion by ^{31}P-NMR.

Once full conversion is achieved after 30-35 minutes, the formed salts ($Et_3N \cdot HCl$) are filtered off by means of a dry glassinterfilter and vacuum pump. After removal of the solvent by evaporation, the residual mixture is dissolved in dry diethyl ether and the residual salts are filtered off. This process is repeated until no more salts are observed.

The final compound (DEPTMS) is a clear and colourless liquid with a very characteristic smell. Its purity is checked by ^{31}P-NMR with yields between 75 and 80%. The product can be stored at -30°C for several months under an inert atmosphere.[79]

Diethyl trimethylsilyl phosphite (DEPTMS, $C_7H_{19}O_3PSi$)
^{31}P-NMR (162.0MHz, $CDCl_3$) δ: 127.5 ppm (1P, s)

6.3.2 Synthesis of dimethyl trimethylsilyl phosphite (DMPTMS)

Analogue to the synthesis of DEPTMS (see Section 6.3.1), 11 g (100 mmol) of dimethyl phosphite is used instead of diethyl phosphite. The final compound (DMPTMS) is a clear and colourless liquid with a very characteristic smell. Its purity is checked by ^{31}P-NMR with yields between 70 and 75%. The product can be stored at -30°C for one month under an inert atmosphere.[79]

Dimethyl trimethylsilyl phosphite (DMPTMS, $C_5H_{15}O_3PSi$)
^{31}P-NMR (162.0MHz, $CDCl_3$) δ: 128.3 ppm (1P, s)

6.3.3 Synthesis of 1-benzylpyridinium bromide

3 mL (37.2 mmol) of pyridine is dissolved in 6 mL of dichloromethane. While stirring at 0°C, 4.5 mL (36.6 mmol) of benzyl bromide is added dropwise to the reaction mixture. The reaction is kept stirring at 0°C for 4 hours, after which the conversion is checked by ^{1}H-NMR. Once full conversion is reached, the solvent is evaporated under vacuum. The formed salt, which is very hygroscopic, is then dried under high vacuum at 90°C (oil bath).

The final compound (1-benzylpyridinium bromide) is a yellow hygroscopic crystal. Its purity is checked by ^{1}H-NMR with a yield between 92 and 98%. The product can be stored at room temperature under an inert atmosphere.[90]

1-Benzylpyridinium bromide ($C_{12}H_{12}NBr$)
^{1}H-NMR (400.1MHz, $CDCl_3$) δ: 9.60 (2H, d, J = 6.08Hz); 8.42 (1H, t, J = 7.65Hz); 8.03 (2H, t, J = 6.82Hz); 7.70 (2H, m); 7.38 (3H, m); 6.37 (2H, s).

6.3.4 Synthesis of 1-benzylpyridinium chloride

The synthesis of 1-benzylpyridinium chloride is analogue to the preparation of 1-benzylpyridinium bromide as previously seen in Section 6.3.3. The only key difference, is a small temperature increase during reaction. 4 hours at 80°C ensured a full conversion.

The final compound (1-benzylpyridinium chloride) is a white hygroscopic crystal. Its purity is checked by ^{1}H-NMR with a yield between 92 and 98%. The product can be stored at room temperature under an inert atmosphere for at least several months.

1-Benzylpyridinium chloride ($C_{12}H_{12}NCl$)
^{1}H-NMR (400.1MHz, $CDCl_3$) δ: 9.81 (2H, d, J = 5.92Hz); 8.39 (1H, t, J = 7.59Hz); 8.02 (2H, t, J = 6.71Hz); 7.73 (2H, m); 7.36 (3H, m); 6.41 (2H, s).

6.3.5 Synthesis of 1-methylpyridinium iodide

1 g (4.55 mmol) of trimethylsulphoxonium iodide is dissolved in 5 mL of pyridine and refluxed (115°C) for 15 minutes. Afterwards, the reaction mixture is cooled to room temperature and the produced crystals are removed by filtration. Next, the filtrate is washed with diethyl ether and cold pyridine. This process is repeated 3 times, after which the salt is dried under high vacuum. The final compound (1-methylpyridinium iodide) is a white solid. Its purity is checked by ^{1}H-NMR with a yield between 92 and 97%.[91]

1-Methylpyridinium iodide (C_6H_8NI)
^{1}H-NMR (400.1MHz, DMSO$-d_8$) δ: 8.99 (2H, d, J = 5.93Hz); 8.58 (1H, t, J = 7.51Hz); 8.14 (2H, t, J = 8.12Hz); 4.36 (3H, s).

6.3.6 Synthesis of 1-(2,4-dinitrophenyl)pyridinium chloride (Zincke salt)

6,06 g (29.9 mmol) of 1-chloro-2,4-dinitrobenzene is dissolved in 20 mL of acetone and stirred to a homogeneous mixture. Next, 2.53 mL (31.4 mmol) of pyridine is added so a red/orange mixture is formed. After refluxing for 1 day, the mixture is cooled to room temperature and filtered. The filtrate is then washed 3 times with 50 mL acetone and 3 times with 50 mL pentane. After drying under high vacuum, the pure compound is obtained. The final compound (1-(2,4-dinitrophenyl)pyridinium chloride) is a yellow solid. Its purity is measured by ^{1}H-NMR with a yield between 60 and 70%.[92]

1-(2,4-Dinitrophenyl)pyridinium chloride ($C_{11}H_8ClN_3O_4$)
^{1}H-NMR (400.1MHz, DMSO$-d_8$) δ: 9.40 (2H, d, J = 5.93Hz); 9.14 (1H, m); 8.97 (2H, m); 8.45 (3H, m).

6.3.7 Synthesis of 2,4-diphosphono-1,2,3,4-tetrahydroquinoline

Batch reaction:
To a flame dried 25 mL flask under inert atmosphere, 0.26 g (2 mmol) of quinoline is added and dissolved in 15 mL of dry 1,2-dichloroethane. Afterwards, 0.5 eq. of sulphuric acid (0.10 g, 1 mmol) is added along with 2.05 eq. of DEPTMS (0.86 g, 4 mmol). The flask is then flushed again with nitrogen or argon and stirred at reflux temperature (83°C) for 3 days. A sample is taken and analysed by ^{31}P-NMR. Once full conversion is reached, the product is isolated by means of an acid-base extraction.

Microwave reaction:
To a flame dried microwave flask of 10mL under inert atmosphere, 0.26 g (2 mmol) of quinoline is dissolved in 7 mL of dry dichloromethane. While stirring, 0.10 g (1 mmol) of sulphuric acid is added followed by 0.36 g (2 mmol) of DEPTMS. After flushing the flask with nitrogen or argon, the flask is sealed and placed inside a microwave reactor at 45°C. After 30 minutes, an additional equivalent (0.36 g, 2 mmol) of DEPTMS is added to the reaction mixture. The flask is again placed in the microwave reactor for an additional hour. Afterwards, a sample is taken and analysed by ^{31}P-NMR. Once full conversion is reached after 4 hours, the product is isolated by means of an acid-base extraction.

Firstly, the solvent is evaporated under vacuum, while the residue is dissolved in 20 mL diethyl ether and 40 mL 3N HCl. After a triple extraction with diethyl ether, the aqueous phase is neutralised with 3N NaOH. The compound is then extracted with dichloromethane (3x20 mL) after which the organic phase is dried using $MgSO_4$. Removal of the solids by filtration and removal of the volatiles gave the desired end product.

The final compound (tetraethyl (1,2,3,4-tetrahydroquinoline-2,4-diyl)bis(phosphonate)) is a yellow oil, consisting of 2 diastereomers (95/5 ratio) which can be separated by chromatography using petroleum ether/ethyl acetate (50/50) followed by acetonitrile/dichloromethane/methanol (80/17/3). The purity is checked by ^{31}P-NMR and yields of 55-65%.[79]

Tetraethyl (1,2,3,4-tetrahydroquinoline-2,4-diyl)bis(phosphonate) ($C_{17}H_{29}NO_6P_2$)
^{31}P-NMR (162.0MHz, $CDCl_3$) δ: 26.78 ppm (1P, s); 24.55(1P, s).

6.3.8 Triphosphonylation of pyridinium salts using TEP

Batch reaction:
To a flame dried 10 mL flask under inert atmosphere, 1 mmol of pyridinium salt (250mg *N*-benzylpyridinium bromide or 221mg *N*-methylpyridinium iodide) is added along with 0.25 mg of dry silica, 2 eq. of formic acid (0.08 mL, 2 mmol) and an excess of triethyl phosphite (3.33 eq., 1.7 mL, 10 mmol). Afterwards, the flask is flushed again with an inert gas (e.g. nitrogen, argon) and heated at 80°C. After 1 day, a sample is taken and analysed by ^{31}P-NMR.

Microwave reaction:
To a flame dried 10mL microwave flask under inert atmosphere, 1 mmol pyridinium salt (250mg *N*-benzylpyridinium bromide or 221mg *N*-methylpyridinium iodide) is added along with 0.25 mg dry silica, 0.5 eq. formic acid (0.08 mL, 2 mmol) and a large excess of triethyl phosphite (3.33 eq., 1.7 mL, 10 mmol). Afterwards, the flask is flushed again with an inert gas (e.g. nitrogen or argon) and heated in a microwave reactor at 80°C. After 4 hours, a sample is taken and analysed by ^{31}P-NMR in order to calculate the conversion.

Once full conversion (after approximately 4 hours) is reached, the product is isolated by distillation (95°C at 13mPa) in order to remove most of the side products. The remaining mixture is dissolved in dichloromethane, after which the compound is separated by column chromatography. Separation of the di- and triphosphonylated piperidine proved to be rather challenging, but was possible using dichloromethane/methanol with a 95/5 ratio. Finally, the product was dried under high vacuum to remove any residual solvent or water.

For 1-benzylpyridinium, the final compound *N*-benzyl-[2,4,6-tris(diethylphosphonyl)] piperidine is a yellow oil. The purity is checked by ^{31}P-NMR with yields between 70 and 85%. Separation of the diastereomers was not possible.

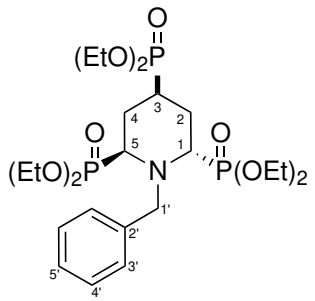
N-benzyl-[2,4,6-tris(diethylphosphonyl)] piperidine ($C_{24}H_{44}NO_9P_3$)
^{31}P-NMR (162.0MHz, $CDCl_3$) δ: 24.32 (1P, s, M); 24.78 (2P, s, *m*); 25.23 (1P, d, *J* = 16.90 Hz, M); 29.57 (1P, d, *J* = 16.90 Hz, M); 32.91 (1P, s, *m*).

For 1-methylpyridinium, the final compound *N*-methyl-[2,4,6-tris(diethylphosphonyl)] piperidine is a yellow oil. The purity is checked by ^{31}P-NMR with yields between 35 and 40%. Separation of the diastereomers was not possible. The synthesis of this compound was not possible by microwave.

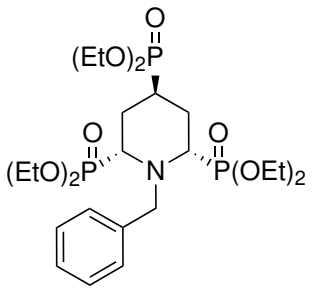
N-methyl-[2,4,6-tris(diethylphosphonyl)] piperidine ($C_{18}H_{40}NO_9P_3$)
^{31}P-NMR (162.0MHz, $CDCl_3$) δ: 24.08 (1P, s, M); 24.20 (2P, s, *m*); 24.88 (1P, d, *J* = 14.84 Hz, M); 29.55 (1P, d, *J* = 14.53 Hz, M); 32.14 (1P, s, *m*).

6.4 Characterisation

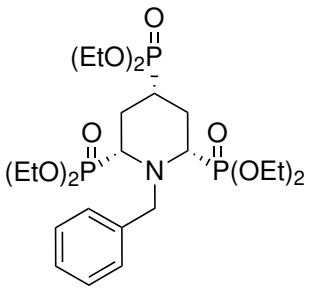
***N*-benzyl-[2,4,6-tris(diethylphosphonyl)] piperidine ($C_{24}H_{44}NO_4P_3$)**



Major (M)
110a



Minor (*m*[1])
110b



Minor (*m*[2])
110c

Yield: 81% (Yellow oil)

Chromatography: CH_2Cl_2MeOH: 95/5

IR: ν_{max}/cm^{-1} 1234.42 (P=O), 1045.42(P-O), 1016.49(P-O) and 947.05 (P-O).

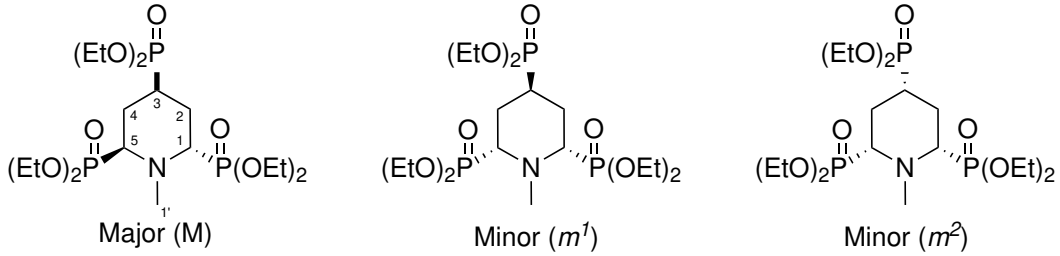
^{1}H-NMR: (400.1 MHz, $CDCl_3$) δ 1.17 (18H, txd, J = 7.32, 2.76 Hz, $OCH_2C\underline{H}_3$, m); 1.27 (18H, m, $OCH_2C\underline{H}_3$, M); 1.76-2.23 (4H, m, $C_2\underline{H}_2$, $C_4\underline{H}_2$, M); 2.59 (1H, m, $C_3\underline{H}$, M); 3.04 (1H, d, J = 24.40 Hz, $C_5\underline{H}$, M); 3.77 (1H, d, J = 13.73 Hz, C_1'$\underline{H}_a$, M); 3.82-3.95 (12H, m, $OC\underline{H}_2CH_3$, m); 3.95-4.20 (12H, m, $OC\underline{H}_2CH_3$, M); 4.35 (2H, dxd, J = 12.70 Hz, J = 11.02Hz, C_1'$\underline{H}_b$, $C_1\underline{H}$, M) ; 7.14-7.27 (3H, m, C_4'$\underline{H}$, C_5'$\underline{H}$, M); 7.32 (2H, d, J = 6.91 Hz, C_3'$\underline{H}$, M).

^{13}C-NMR: (100.6 MHz, $CDCl_3$) δ 15.43 (m, $OCH_2\underline{C}H_3$); 15.97 (s, $\underline{C}_2$); 18.23 (s, $\underline{C}_4$); 29.90 (dxd, J = 147.53, 14.55 Hz, $\underline{C}_3$); 50.48 (txd, J = 161.40, 14.71 Hz, $\underline{C}_5$); 52.32 (d, J = 16.65 Hz, $\underline{C}_1$'); 53.34 (dxd, J = 143.91 Hz, J = 16.0 Hz, $\underline{C}_1$); 60.43 (d, J = 7.09 Hz, $O\underline{C}H_2CH_3$); 60.82 (t, J = 5.65 Hz, $O\underline{C}H_2CH_3$); 61.33 (d, J = 6.92 Hz, $O\underline{C}H_2CH_3$); 61.89 (d, J = 6.92 Hz, $O\underline{C}H_2CH_3$); 126.34 (s, $\underline{C}_5$'); 127.21 (s, $\underline{C}_4$'); 128.13 (s, $\underline{C}_3$'); 137.58 (s, $\underline{C}_2$').

^{31}P-NMR: (162.0 MHz, $CDCl_3$) δ 24.32 (1P, s, M); 24.78 (2P, s, m); 25.23 (1P, d, J = 16.90 Hz, M); 29.57 (1P, d, J = 16.90 Hz, M); 32.91 (1P, s, m).

m/z (ESI, 70 eV): 584 ($[M+H]^+$, 100%)

***N*-methyl-[2,4,6-tris(diethylphosphonyl)] piperidine ($C_{18}H_{40}NO_9P_3$)**



Yield: 38% (Yellow oil)

Chromatography: CH_2Cl_2MeOH: 95/5

IR: ν_{max}/cm^{-1} 1242.68 (P=O), 1053.32(P-O), 1022.56(P-O) and 965.77 (P-O).

^{1}H-NMR: (400.1 MHz, $CDCl_3$) δ 1.37-1.28 (18H, m, $OCH_2C\underline{H}_3$, M + *m*); 1.94 (2H, m, $C_2\underline{H}_2$, M); 2.05 (2H, m, $C_4\underline{H}_2$, M); 2.52 (1H, m, $C_3\underline{H}$, M); 2.72 (3H, s, C_1'$\underline{H}_3$, M); 3.19 (1H, dxd, *J* = 22.39, 2.86 Hz, $C_5\underline{H}$, M); 4.05-3.97 (1H, m, $C_1\underline{H}$, M); 4.31-4.05 (12H, m, $OC\underline{H}_2CH_3$, M + *m*).

^{13}C-NMR: (100.6 MHz, $CDCl_3$) δ 16.43 (m, $OCH_2\underline{C}H_3$); 19.65 (m, $\underline{C}_4$); 20.03 (d, *J* = 4.17 Hz, $\underline{C}_2$); 29.67 (s, $\underline{C}_3$); 40.90 (d, *J* = 13.11 Hz, $\underline{C}_1$'); 53.88 (dxd, *J* = 159.90, 16.00 Hz, $\underline{C}_1$); 58.41 (dxt, *J* = 152.56 Hz, *J* = 14.73 Hz, $\underline{C}_5$); 61.08 (t, *J* = 15.38 Hz, $O\underline{C}H_2CH_3$); 61.88 (t, *J* = 15.38 Hz, $O\underline{C}H_2CH_3$); 62.52 (d, *J* = 7.02 Hz, $O\underline{C}H_2CH_3$); 63.33 (d, *J* = 7.02 Hz, $O\underline{C}H_2CH_3$).

^{31}P-NMR: (162.0 MHz, $CDCl_3$) δ 24.08 (1P, s, M); 24.20 (2P, s, *m*); 24.88 (1P, d, *J* = 14.84 Hz, M); 29.55 (1P, d, *J* = 14.53 Hz, M); 32.14 (1P, s, *m*).

m/z (ESI, 70 eV): 493 ($[M+H]^+$, 100%)

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Chapter 7

Appendix

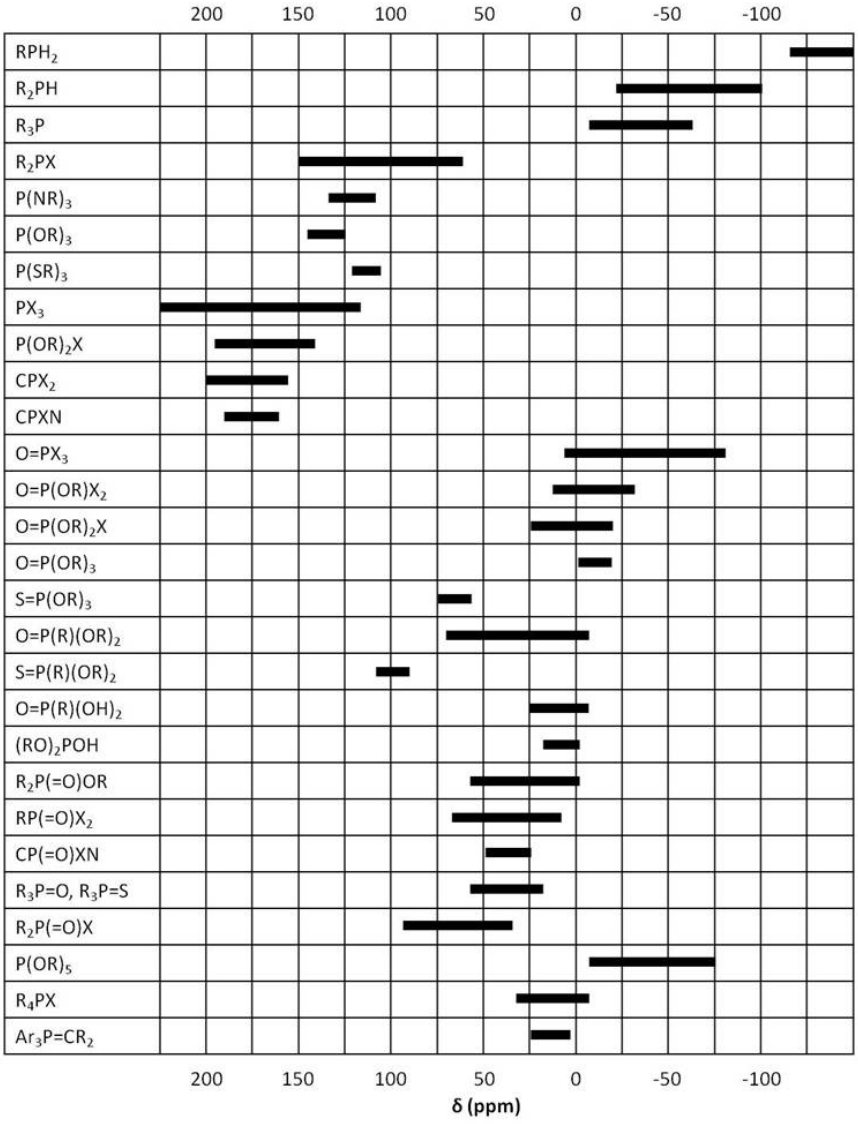

Figure A1: Chemical shift ranges of different types of phosphorus compounds.[31]