universität wien

MASTERARBEIT

Titel der Masterarbeit

„Synthesis of phosphonic acid analogues of 2-AEP and 1-OH-2-AEP as substrates for PhnY and PhnZ“

verfasst von

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angestrebter akademischer Grad

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Studienrichtung lt. Studienblatt:	Masterstudium Chemie
Betreut von:	Ao. Univ.-Prof. Dr. Friedrich Hammerschmidt

Wie *Albert Einstein* bereits sagte:

Zwei Dinge sind zu unserer Arbeit nötig:
Unermüdliche Ausdauer und die Bereitschaft,
etwas, in das man viel Zeit und Arbeit gesteckt hat,
wieder wegzuwerfen.

Gib nie etwas auf, was du wirklich willst.
Jemand mit großen Träumen ist mächtiger
als jemand mit all den Fakten.

Probleme kann man niemals mit derselben Denkweise lösen, durch die sie entstanden sind.

Um ein tadelloses Mitglied einer Schafherde sein zu können,
muss man vor allem ein Schaf sein.

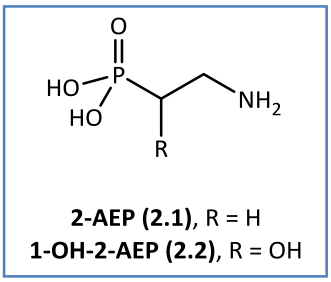
1. Table of contents

2. Aim of Master thesis

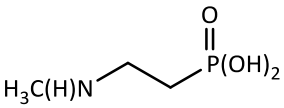
Today, there are three different routes known for phosphonate degradation – P_i-dependent catabolism by C-P lyase, P_i-insensitive breakdown by different C-P hydrolyses and oxidative cleavage. But it is assumed that there are further, yet uncharacterised pathways. Previously discovered enzymes like PhnZ also have to be investigated further, because not all of their mechanisms are elucidated. On the one hand, the regulation of organophosphonate degradation pathways is extensively studied to understand regulatory differences in diverse microorganisms for the same substrate. On the other hand, better strategies for genome sequencing and expansion of genomic libraries will contribute to optimise the application of synthetic organophosphonates. Improved methods for detecting and monitoring phosphonates and breakdown products or the development of recycling strategies will have significant ecological benefits [1].

Attention is actually paid to a pair of coupled enzymes, PhnY and PhnZ, known for catalysing the oxidative P-C bond cleavage of 2-AEP using a mixed-valent cofactor and dioxygen. PhnY is an enzyme catalysing the hydroxylation at the α-C position of (2-aminoethyl)phosphonic acid (2-AEP) to produce (*R*)-(2-amino-1-hydroxyethyl)phosphonic acid [(*R*)-1-OH-2-AEP]. The latter is then cleaved by the enzyme PhnZ catalysing the oxidative P-C bond cleavage.

Different analogues of the naturally occurring phosphonic acids 2-AEP (**2.1**) and 1-OH-2-AEP (**2.2**) will be synthesised in order to study the degradation mechanism of PhnY and PhnZ, respectively (*Figure 1*). Small modifications by introducing functional groups will be carried out to investigate the broadness of substrate specificity of both enzymes. Different substituents will be introduced in α-position [(±)-**2.5**, (±)-**2.6**] or β-position [(*R*)- and (*S*)-**2.12**] to phosphorus, modifications on nitrogen [**2.3**, (±)-**2.4**] will be made or protective groups on phosphorus [(±)-**2.7**, **2.8**] will be altered to investigate their influence on reactivity. Other target molecules contain an extended alkyl chain (**2.9**) compared to 2-AEP or are unsaturated (**2.13**). Furthermore, C-2 of 2-AEP is going to be replaced by an oxygen (**2.10**) or nitrogen atom (**2.11**). The obtained compounds will be tested as enzyme substrates for PhnY/PhnZ in cooperation with the group of Dr. David L. Zechel (Queen's University, Ontario, Canada). The tests will show whether the substances can be metabolised by PhnY and/or PhnZ. Further investigations will show if the compounds possibly act as inhibitors of the enzymes. ^{31}P NMR spectroscopy is used to study whether the tested compounds are hydroxylated by PhnY, which results in a chemical shift compared to the starting material, and/or degraded by PhnZ by detecting the signal of released P_i. The analogues of 2-AEP will be tested as putative substrates for PhnY. Phosphonic acids analogous to 1-OH-2-AEP will bring insight into the relevance of the α-hydroxy group for the P-C bond cleavage itself. The most promising substrates [(*R*)- and (*S*)-**2.12**] will be prepared in enantiomerically pure form.



2-AEP (2.1), R = H
1-OH-2-AEP (2.2), R = OH



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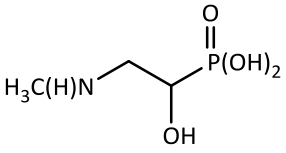
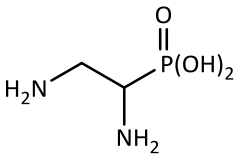
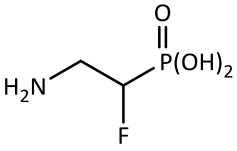


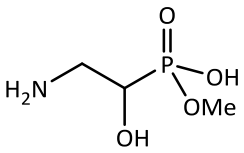
(±)-**2.4**



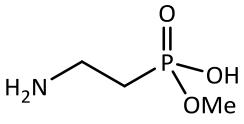
(±)-**2.5**



(±)-**2.6**



(±)-**2.7**



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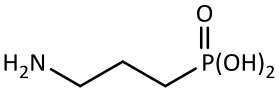

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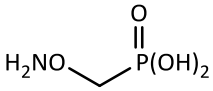

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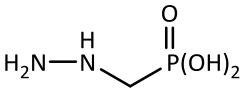

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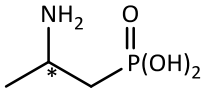
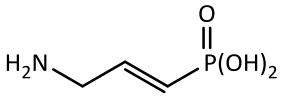


(*R*)- and (*S*)-**2.12**



2.13

Figure 1: 2-AEP, 1-OH-2-AEP and their synthesised analogues as target molecules.

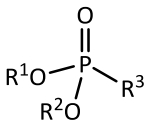
3. Theoretical background

3.1. Organophosphonates

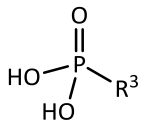
In the biosphere, phosphorus usually occurs in the oxidation state +5 in the form of inorganic phosphate P_i, which is used as P source by living organisms. This is the highest possible oxidation state for this element and found in phosphoric acid esters, the most common form of P_i [3]. However, under the reducing atmosphere on primitive Earth, phosphonates were possibly the predominant phosphorus-containing species [2, 3]. With increasing oxygen concentrations, phosphonates were increasingly replaced by phosphates. Also, enzymes catalysing P-C bond formation and cleavage were replaced by enzymes catalysing P-O bond formation and cleavage [2]. Investigations on the Murchinson meteorite in 1969 supported the theory that phosphonates stem from times of primitive Earth [3].

Excursus:

The general use of names often varies to the actual IUPAC recommendations (2005), especially in older publications. The following Scheme will give an overview on the nomenclature of P compounds used in this thesis, regardless of IUPAC recommendations.

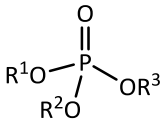


Phosphonate

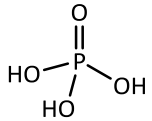


Phosphonic acid

Phosphonates are esters of phosphonic acid. They have a direct P-C bond and a +3 oxidation state.

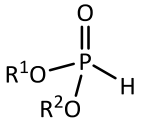


Phosphate ester

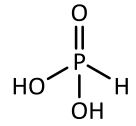


Phosphoric acid

Phosphates are esters of phosphoric acid. They have P-O bonds and a +5 oxidation state.

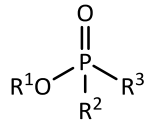


Phosphite

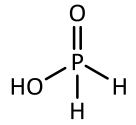


Phosphorous acid

Phosphites are esters of phosphorous acid. They have a +3 oxidation state.

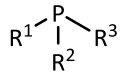


Phosphinate

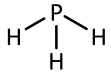


Phosphinic acid

Phosphinates are esters of phosphinic acid. They have a +1 oxidation state.



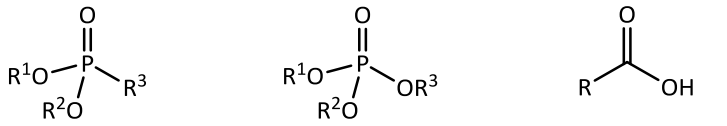
Organophosphine



Phosphine

Organophosphines are analogues of phosphines (-3 oxidation state) in which one or more hydrogen bonds are replaced by organic substituents.

Organophosphonates **3.1** are structural analogues of phosphate esters **3.2** and carboxylic acids **3.3** with a direct, highly stable P-C bond instead of a P-O-C bond [1, 3] (*Figure 2*). Both bonds have the



Phosphonate **3.1** | **Phosphate ester** **3.2** | **Carboxylic acid** **3.3**

Figure 2: Structural analogues: phosphonates, phosphates and carboxylic acids.

same dissociation energy of 70 kcal·mol^{-1}. However, the cleavage of the P-C bond has a significantly higher activation energy than the cleavage of the more labile P-O bond of phosphates [3]. Phosphonates are stable towards chemical hydrolysis by strong acids or bases [4]. They are also more resistant to different forms of degradation, such as thermal decomposition and photolysis [1]. Their stability to the action of phosphatases may be a reason why phosphonates are often found as components of macromolecules such as phosphonopeptides, phosphonoglycans, phosphonolipids or phosphonopolysaccharides [3, 4]. They protect cells against enzymatic breakdown and contribute to their rigidity [3]. Phosphonates have useful biological properties due to their structural similarity to phosphate esters and carboxylic acids. Phosphonates are potent competitive enzyme inhibitors. The affinity of phosphonates to the enzyme active site is often very high [4]. There are numerous potential targets for inhibition considering that phosphate esters are ubiquitous and very important for all living organisms [1, 4].

The inert nature of P-C bonds, the favourable biological properties and the similarity of phosphonic acids to phosphoric acids make them potent antimetabolites [3]. Therefore, synthetic phosphonates find an increasing range of applications. They are used as insecticides, fungicides, herbicides, antibiotics and antivirals [1, 3]. To name but a few, fosfomycin (**3.4**) was the first antibiotic which contained a P-C bond. It was isolated in 1969 from the culture broth of *Streptomyces fradiae* and showed activity in mice against numerous gram-positive and gram-negative microorganisms by interfering with their cell wall biosynthesis [10]. FR-900098 (**3.5**) was tested as potent antimalarial agent, which blocks the non-mevalonate pathway of isoprenoide biosynthesis. It shows low toxicity in both animals and humans and good antibacterial activity against numerous gram-negative bacteria [4]. The non-selective herbicide phosphinothricin (**3.6**) [10] is the only known naturally occurring phosphinate. Phosphinates are phosphate analogues, with C-P-C bonds instead of O-P-O bonds. Phosphinothricin was discovered as a component of an antibiotic isolated from *Streptomyces viridochromogenes* (phosphinothricin tripeptide, PTT) and *Streptomyces hygroscopicus* (bialaphos) [4]. The most widespread and applied synthetic herbicide is glyphosate (**3.7**), making up about 25% of the global herbicide market. In 1970 this phosphonate was introduced by E. Franz of Monsanto as a broad-spectrum herbicide named Roundup. It interferes with the shikimate pathway, which yields the aromatic amino acids in plants, by inhibiting the key enzyme 5-enolpyruvylshikimate-3-phosphate synthase. Doubts about the

extensive use of glyphosate have come up in the recent years, considering its enormous negative effects on the environment [1] (*Figure 3*).

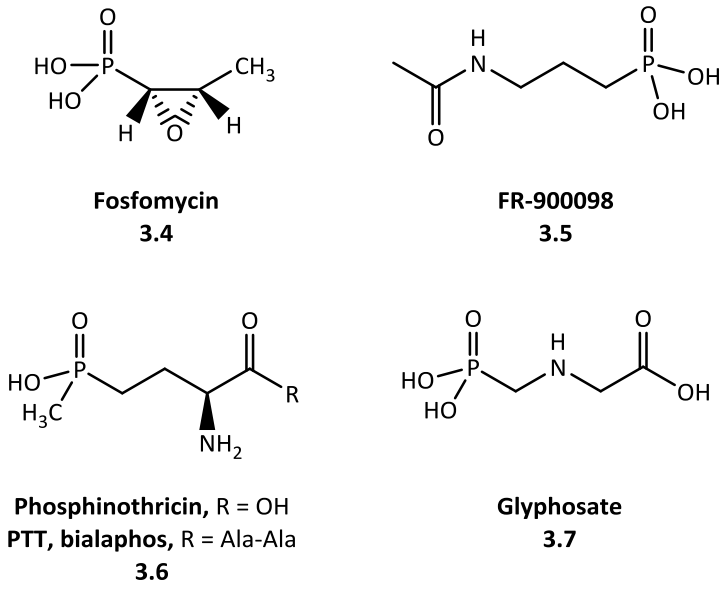


Figure 3: Small molecules with large impact – structure of important phosphonates and phosphinates.

The existence of organophosphonates of biogenic origin was proved with the isolation of **2.1** in sheep rumen protozoa in 1959. 2-AEP (**2.1**) and *N*-methyl AEP (**2.3**) were found to be the prevalent P-C compounds in nature [1].

Nature has recognised the value of phosphonates much earlier than humans [4]. In the biosphere, phosphonates are widely distributed [3] and in some organisms they are the prevalent P source. About 20-30% of the dissolved phosphorus in oceans stem from organophosphonates [4]. They are increasingly paid attention to [3] as they provide alternative sources of nutrients for organisms in oligotrophic environments, such as in marine ecosystems [1]. Nevertheless, the role of organophosphonates in the biogeochemical phosphorus cycle is poorly understood as there are also anthropogenic sources of P-C compounds coming into the environment [3].

Even though the elucidation of phosphonate metabolism is of great scientific importance still only a few biological pathways are characterised completely [4].

3.1.1. Biosynthesis of phosphonates

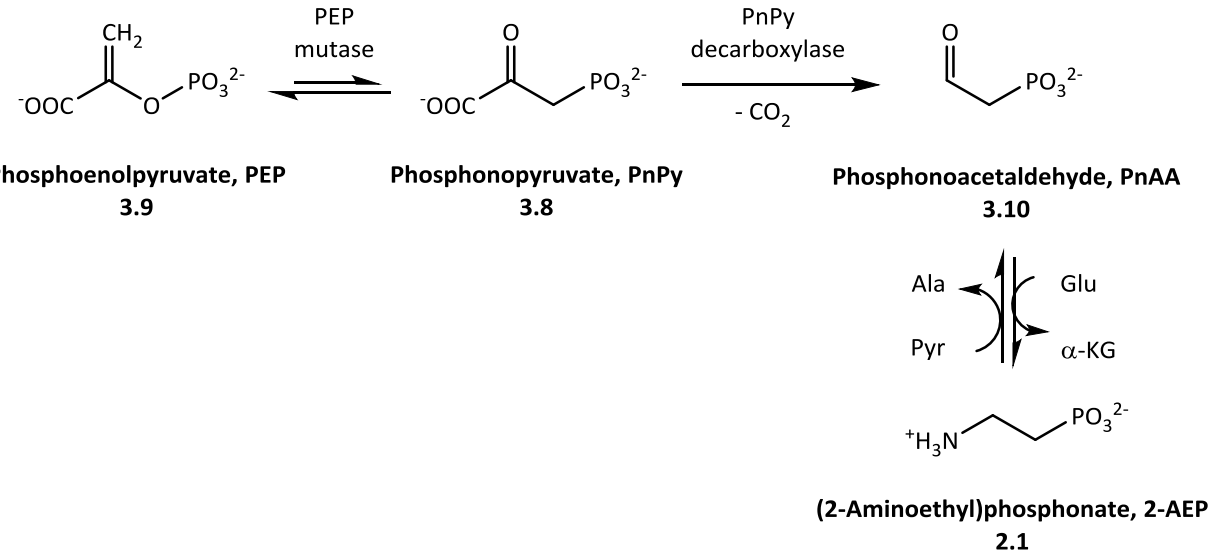
P-C compounds are synthesised by microorganisms in different pathways [3]. Most share the same initial step – a central P-C forming reaction [3, 4], first discovered by analysing the biosynthesis of 2-AEP in *Tetrahymena.* Phosphonopyruvate (PnPy) (**3.8**) is the common intermediate in the biosynthesis of natural phosphonates [4] (*Scheme 1*). It is formed through intramolecular rearrangement of phosphoenolpyruvate (PEP) (**3.9**). This equilibrium reaction, responsible for the

initial formation of a P-C bond, is catalysed by phosphoenolpyruvate phosphomutase (PEP mutase) [1]. The formation of **3.8** is thermodynamically unfavourable [11]. Therefore, the subsequent reaction, leading to different biogenic phosphonates, has to be highly exergonic [4]. There are two general transformations that **3.8** undergoes which will be described in the next sections [13] based on the biosynthesis of important phosphonates.

3.1.1.1. Decarboxylation

Biosynthesis of 2-AEP

2-AEP (**2.1**) has the shortest known biosynthesis of all natural phosphonates [4] (*Scheme 1*). The

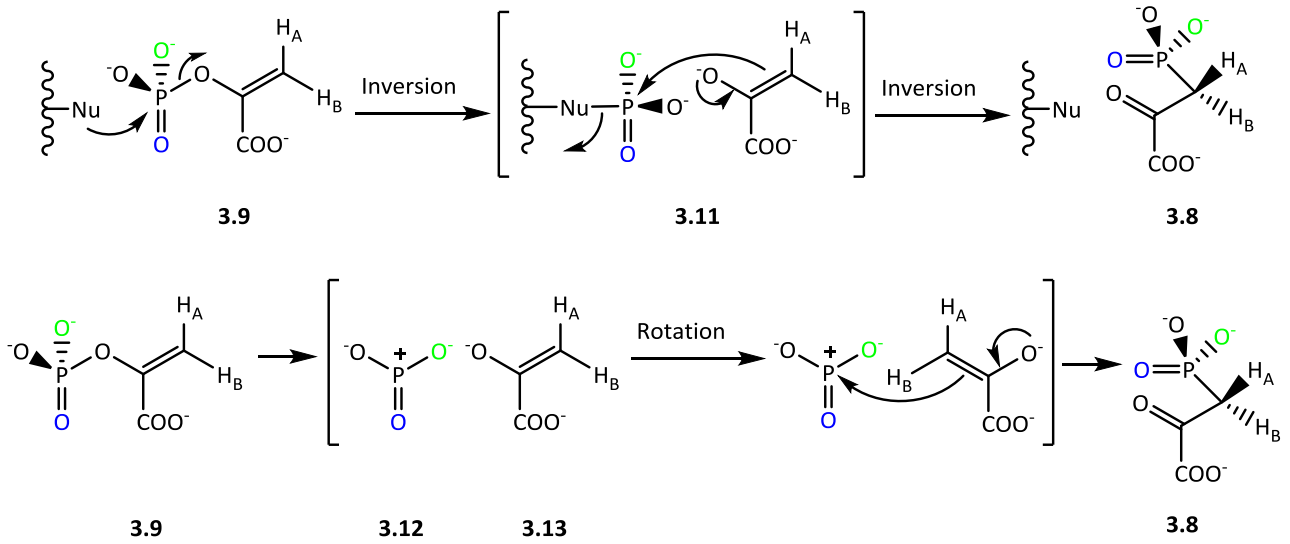


Scheme 1: Biosynthesis of PnAA by PEP mutase and PnPy decarboxylase; modified from [1, 4, 11].

intramolecular transfer of the phosphoryl group in **3.9** gives **3.8** [3]. The coupled enzyme phosphonopyruvate decarboxylase (PnPy decarboxylase) removes the unfavoured metabolite **3.8** from the reaction equilibrium by decarboxylation. This irreversible step [1] drives the formation of **3.8** forward [11]. Decarboxylation is the most common way [13] to transform **3.8** into different phosphonate secondary metabolites [2]. Studies on PnPy decarboxylase have shown that this enzyme plays a role in the biosynthesis of fosfomycin (**3.4**) and bialaphos (**3.6**) [11]. 2-AEP is formed by subsequent transamination of the decarboxylation product, namely phosphonoacetaldehyde (PnAA) (**3.10**) [3].

Labelling studies with *Tetrahymena pyriformis* revealed that the intramolecular phosphoryl transfer by PEP mutase proceeds with retention of configuration at the P atom [2]. Originally, the mechanism was thought to occur through a phosphoenzyme intermediate (**3.11**). It was proposed that the enzymebased intermediate was formed by nucleophilic attack of an enzyme residue on PEP (**3.9**) with inversion of configuration. Consequently, the pyruvate enolate would attack the

phosphoenzyme with inversion of configuration leading to a net retention. However, this theory was not confirmed. A more recent mechanism suggests that the PEP mutase reaction follows a dissociative process. Metaphosphate (**3.12**) would be formed as intermediate and fixed by interaction with enzyme active site residues. After rotation of the C-1/C-2 bond of the pyruvate enolate (**3.13**), the C-3 attacks the metaphosphate to give **3.8**. This matches the observed stereochemistry at the P atom as well as at C-1 of PnPy (**3.8**) [4] (*Scheme 2*).



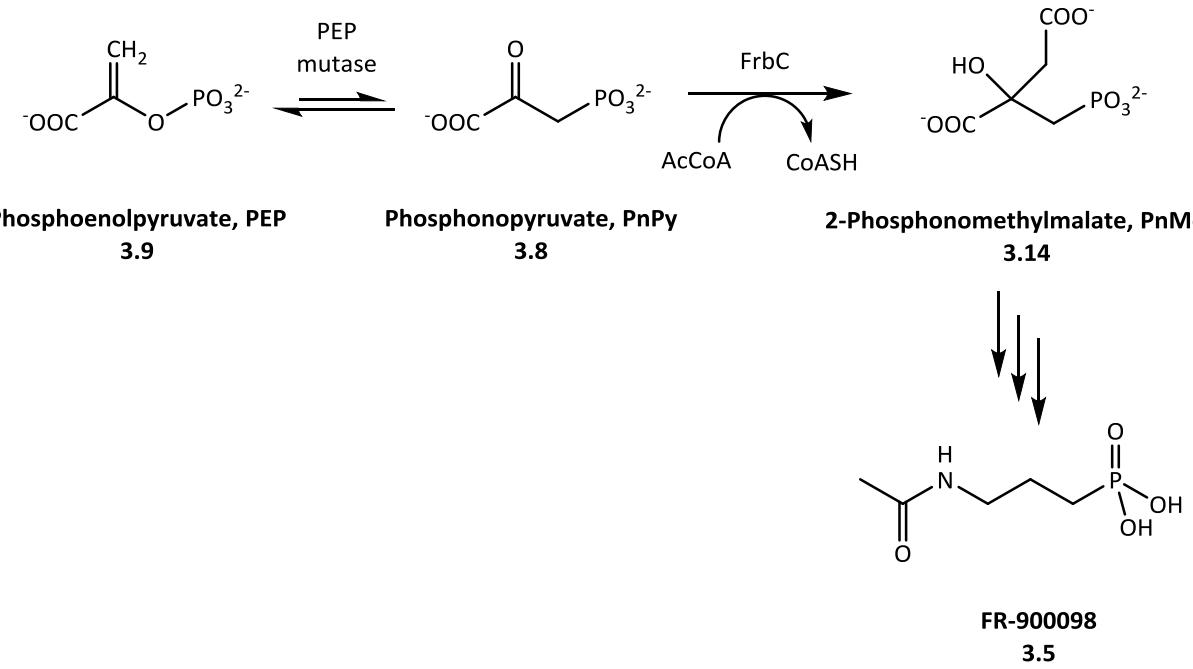
Scheme 2: Labelling studies to unravel stereochemistry at the P atom; modified from [4].

In *T. pyriformis*, the PnPy decarboxylase enzyme is membrane-bound and therefore difficult to isolate and to characterise [4, 11]. It is certain that in pathways going through decarboxylation, PEP mutase and PnPy decarboxylase cooperate to form PnAA (**3.10**) as common precursor, requiring thiamine pyrophosphate and divalent metal ions as cofactors [11]. Also the enzyme responsible for the transamination of **3.10** is membrane-bound in *T. pyriformis*. Nevertheless, it was demonstrated that transamination is pyridoxal phosphate-dependent [11]. For the formation of 2-AEP (**2.1**), L-Glu acts as amino group donor yielding α-ketoglutarate [22]. The reverse reaction uses pyruvate as NH_2 acceptor to give L-Ala and **3.10** [12] (*Scheme 1*). The three enzymes of 2-AEP biosynthesis were found as orthologs in several organisms, but there is no characterisation of all enzymes together in one organism [4].

3.1.1.2. Condensation with acetyl-CoA

Biosynthesis of FR-900098

Unlike the pathway shown above, the driving force of the second pathway is condensation of PnPy (**3.8**) with acetyl-CoA to form 2-phosphonomethylmalate (PnMeM) (**3.14**), which is exergonic. The enzyme that catalyses the addition of the acetate to PnPy is FrbC, a homocitrate synthase homolog [4, 13]. Subsequent transformations lead to FR-900098 (**3.5**) [4] (*Scheme 3*).



Scheme 3: Biosynthesis of FR-900098; modified from [4].

It is likely that there are several other possibilities to shift the equilibrium of the highly unfavourable first step towards the phosphonate [4].

3.1.2. Biodegradation of phosphonates

In many ecosystems phosphorus is a growth limiting nutrient. Therefore, several lower organisms developed different ways to obtain P_i as a breakdown product of biogenic phosphonates [13]. The degradation of organophosphonates by microorganisms was formerly thought to occur just in case of phosphate shortage. The degradation is catalysed by the C-P lyase multienzyme system and controlled by the (phosphate) *Pho*-regulon. More recent studies have revealed that microbial phosphonate mineralisation can be *Pho*-independent [3]. Considering the enormous strength of a P-C bond, it is remarkable that nature developed ways for microbial cleavage [1]. So far, three ways have been found, which will be described shortly. But first, a short overview about some biological terms will be given in a short excursus.

Excursus:

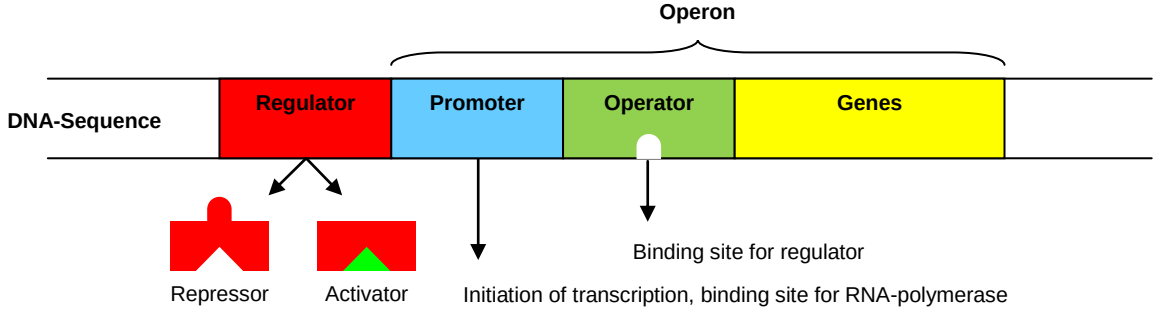


Figure 4: Explanation of biological terms in graphic form; modified from [16, 17].

A regulon contains a group of operons or genes that are regulated in coordination of each other [16]. In an operon a single promoter is responsible for its transcription [1].

3.1.2.1. C-P lyase – microbial phosphonate catabolism under Pho-regulon control

P_i is the favoured phosphorus source for cellular growth in most organisms [3]. Phosphorus uptake and metabolism in microbial cells are mostly controlled by the *Pho*-regulon [1, 5]. The *Pho*-regulon consists of numerous genes, which are co-regulated by environmental phosphate levels. Under *Pho*-regulon control P_i shortage activates the expression of *Pho*-genes whose products are responsible for the supply and uptake of phosphate [3]. If enough phosphate is present, gene expression is down regulated [5]. Signal transduction in cells takes place over a membrane-associated complex containing seven proteins. It includes two types of two-component system (TCS) proteins – the integral membrane protein PhoR and the transcription factor PhoB. Further, an ATP-binding cassette (ABC) is present, which has four transporter units called PstA/B/C/S. PstS serves as binding protein for P_i and is saturated when enough phosphate is present. There is also an inhibitor for the PhoR/PhoB TCS named PhoU [5]. Phosphate limitation leads to the activation of the system by decoupling of PstS. It induces a conformational change in the signal complex, which is transmitted from PhoU to PhoR. The histidine kinase PhoR can act both as repressor ($PhoR^I$) and as activator ($PhoR^A$) for PhoB [1]. Under phosphate starvation PhoB is autophosphorylated by $PhoR^A$, leading to a conformational change in PhoB [5]. It can then bind to a specific and characteristic DNA site in the *Pho*-regulon, the so-called *Pho*-box [1, 3] (*Figure 5*).

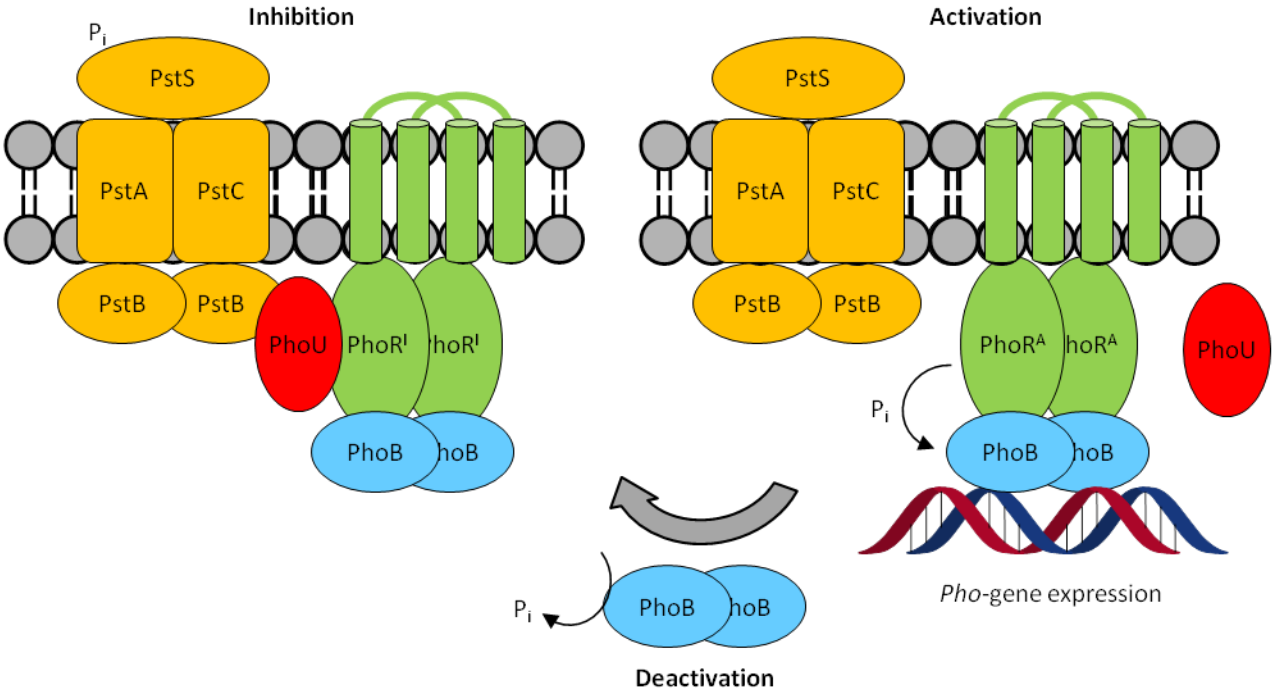
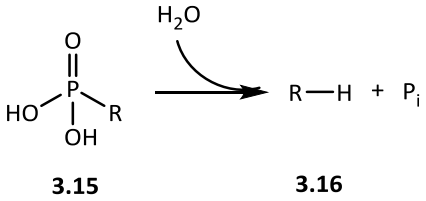


Figure 5: Cellular signal transduction regulated by environmental P_i; modified from [5].

Consequently, the formation of a transcription complex in the promoter region of the regulon is induced, enabling an efficient use of organic and inorganic phosphorus [1].

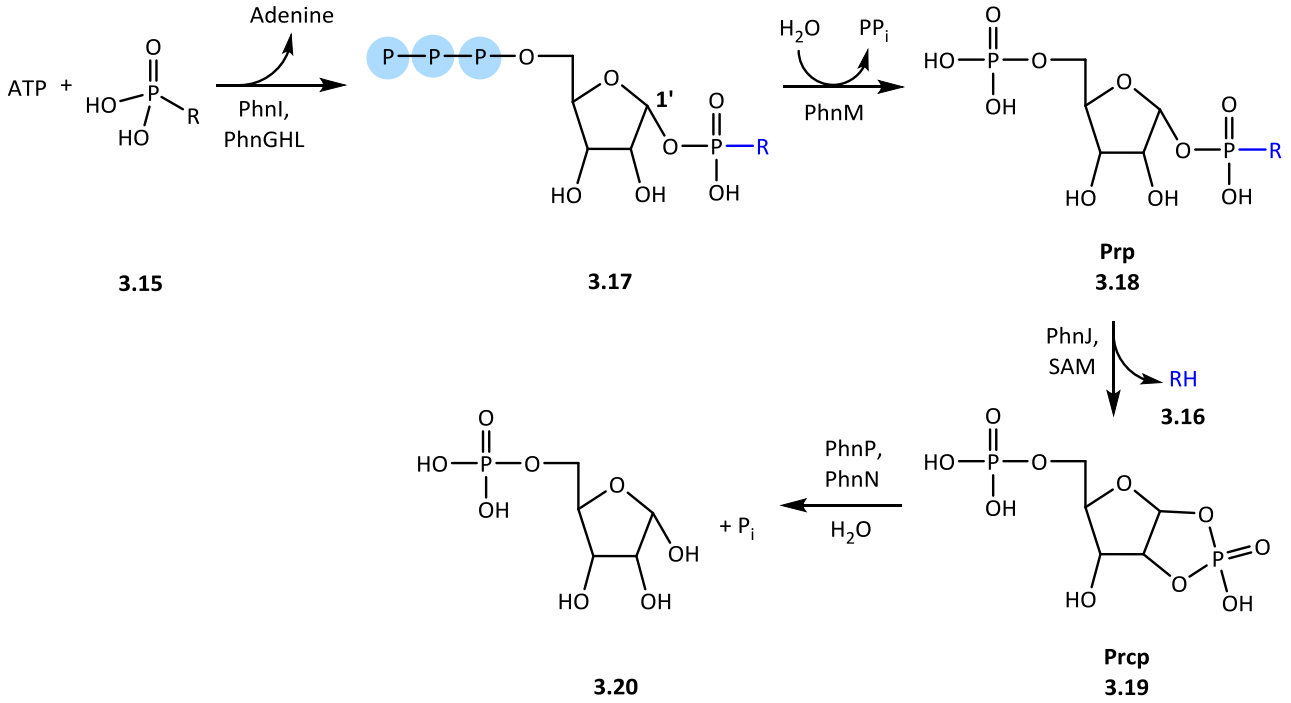
Many previous studies upon the utilisation of organophosphonates by microorganisms [6] suggested that phosphonate mineralisation is generally controlled by the *Pho*-regulon [1] and therefore depends on the cell's and its environment's phosphate status [8]. The consequence is that phosphonates (in this case) can only serve as phosphorus source, but not as carbon or nitrogen source for microbial growth, because the catabolically released phosphate inhibits further mineralisation.

C-P lyase catalyses the direct dephosphonylation of phosphonates [9]. It converts alkylphosphonates **3.15** to the corresponding alkanes **3.16** and releases P_i [1] (*Scheme 4*).



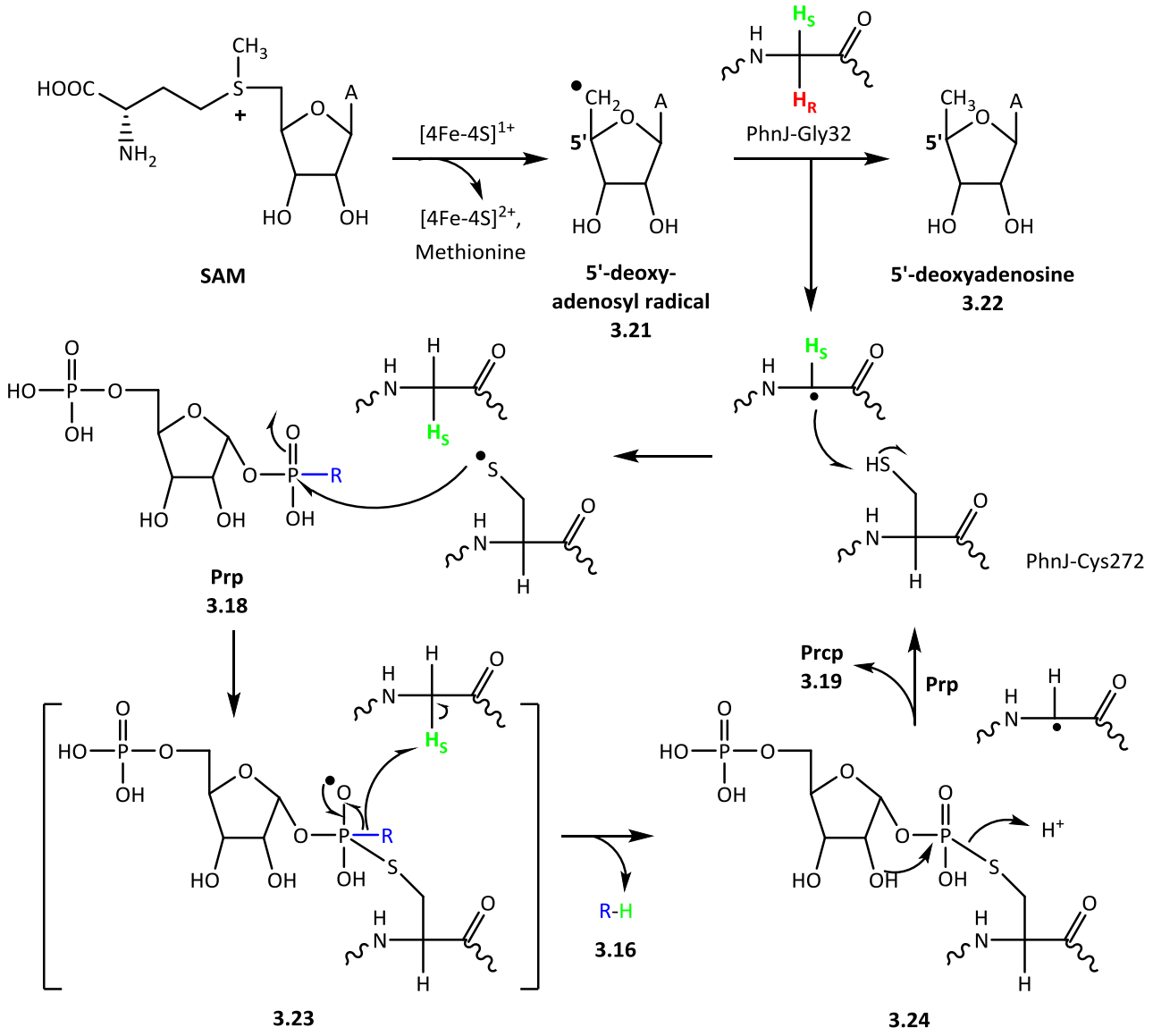
Scheme 4: Phosphonate biodegradation catalysed by C-P lyase gives alkanes and P_i; modified from [1, 7].

The reconstruction of C-P lyase activity *in vitro* was difficult, as components of the multienzyme complex are membrane-bound [3, 13]. Although C-P lyase was already discovered in the 1970s the mechanism of the enzymatic degradation of alkylphosphonates has just been resolved recently [1]. The genetic analysis of the regulatory region of the *Pho*-regulon, called *Pho*-box, provided a mechanistic basis for the cleavage reaction. In *E. coli*, 14 genes located in the *Pho*-box region encode the uptake of phosphonates and lyase activity [3]. This gene cluster is also called the *Phn*-operon (*PhnCDEFGHIJKLMNOP*) [14, 15]. The enzymes encoded by the individual genes or groups of them have different functions in phosphonate catabolism. PhnCDE act as phosphonate transporter [15]. PhnF has a regulatory function as *Phn*-operon repressor [1, 15]. *PhnO* encodes a *N*-acetyltransferase. The first phosphonate biodegradation step is the phosphorylation of the ATP 1' position, catalysed by the nucleotide phosphorylase PhnI in presence of PhnGHL. Phosphonate monoester **3.17** is formed and adenine is released. The following hydrolysis with release of pyrophosphate, catalysed by phosphohydrolase PhnM, gives 5-phosphoribosyl-1-phosphonate (Prp) (**3.18**). It is the substrate for PhnJ, which is responsible for the actual P-C bond cleavage [1]. The reaction follows a radical mechanism and needs *S*-adenosylmethionine (SAM) and a redox-active iron-sulphur cluster [18]. It produces the corresponding alkane **3.16** of the starting phosphonate and 5-phosphoribosyl-1,2-cyclic phosphate (Prcp) (**3.19**). The latter is finally hydrolysed by the phosphodiesterase PhnP to give **3.20** and inorganic phosphate. The action of the ribose bisphosphokinase PhnN and phosphoribosyltransferases makes P_i accessible for further microbial metabolism (*Scheme 5*). The function of PhnK is still unknown [1].



Scheme 5: Biodegradation mechanism under *Pho*-regulon control; modified from [1].

The mechanism of the P-C bond cleavage by PhnJ was proposed in 2013 by Raushel *et al.* PhnJ contains four cysteines. It is well known that radical SAM enzymes require three cysteines for the formation of an $[4Fe\text{-}4S]^{1+}$ cluster. Mutation experiments revealed that Cys-241, Cys-244 and Cys-266 in PhnJ were needed for the formation of this iron-sulphur cluster. The fourth cysteine residue, Cys-272, is essential for the main catalytic reaction. The $[4Fe\text{-}4S]^{1+}$ cluster cleaves SAM, a cosubstrate, reductively, forming a 5'-deoxyadenosyl radical (**3.21**) with release of methionine. The radical was shown to abstract the *pro-R* hydrogen of the Gly-32 located in the active-site of PhnJ, giving 5'-deoxyadenosine (**3.22**). The resulting glycyl radical then stereospecifically attacks the Cys-272 and abstracts the hydrogen atom of the SH group, giving regenerated Gly-32 and a thiyl radical. It is assumed that the thiyl radical attacks the phosphorus atom of the phosphonate derivative Prp (**3.18**), generating radical intermediate **3.23**. With the Gly-32 *pro-S* hydrogen atom a C-H bond is formed in place of the P-C bond. The initial thiophosphonate intermediate is converted to thiophosphate **3.24**, which gives Prcp (**3.19**) by intramolecular attack of the 2'-OH on the P atom. Cys-272 is regenerated. Only the first cycle is SAM-dependent, while all following cycles can cleave substrate without using further SAM molecules (*Scheme 6*). However, the formation of a thiophosphate intermediate could yet not be confirmed. The given reaction mechanism was proposed on the basis of methylphosphonate catabolism to explain the paradoxical phenomenon of aerobic methane production [18].

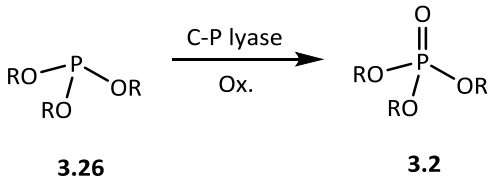


Scheme 6: Mechanism of P-C bond cleavage by PhnJ; modified from [13, 18].

Excursus:

The C-P lyase enzyme is the reason for the paradoxical phenomenon that methane concentrations are high in the oxygen-rich ocean surface waters. Methane production normally occurs under anaerobic conditions [13]. Nevertheless, the potent greenhouse gas methane is produced in considerable amounts by aerobic marine microorganisms that are deficient in phosphorus as nutrient source. Microorganisms containing C-P lyase are able to degrade methylphosphonate to methane and P_i. The *Pho*-regulon gene product PhnJ is responsible for the P-C bond cleavage [18]. However, it is still unclear if methylphosphonic acid occurs in nature, as its detection has not been successful in natural systems. Nevertheless, genome mining of *Nitrosopumilus maritimus* revealed genes encoding a pathway for the biosynthesis of methylphosphonate, which may include the synthesis of (2-hydroxyethyl)phosphonate (2-HEP) (**3.25**), a widespread intermediate in the biosynthesis of organophosphonates [28].

C-P lyase has a broad substrate specificity [9], which is due to the fact that there are different types of the enzyme [3]. It is able to cleave activated but also unactivated P-C bonds [1]. Studies on *Phn*-operon mutants also showed that the genes were not just required for P-C bond cleavage, but also for the oxidation of phosphite **3.26** (+3) to phosphate **3.2** (+5) (*Scheme 7*). Therefore,



3.26 **3.2**

Scheme 7: Oxidation of phosphite to phosphate.

phosphites can be used as sole phosphorus source, too [14].

The C-P lyase pathway was thought to be the only major route for microorganisms to catabolise phosphonates [1]. If C-P lyase was the only route for degrading biogenic organophosphonates, the contribution to the global phosphorus cycle would be limited due to P_i based inhibition [3]. Recent studies revealed alternative pathways for phosphonate biodegradation, which do not depend on environmental phosphate concentrations [1]. Studies by McGrath *et al.* (1997) revealed that 15 of 19 biogenic and synthetic organophosphonates were used as P sources just in the absence of P_i. Four biogenic phosphonates also acted as carbon and energy source for microbial growth. They were completely mineralised with release of P_i. Those substrates were β-oxoalkylphosphonates which have a polarised P-C bond. The *Pho*-independent degradation of some of the most widespread biogenic phosphonates will be discussed next [3].

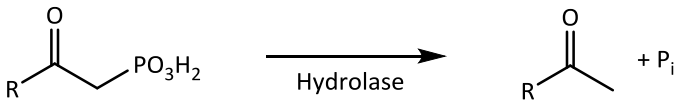
3.1.2.2. C-P hydrolases – microbial phosphonate catabolism independent of Pho-regulon control

2-AEP (**2.1**), *N*-methyl AEP (**2.3**), phosphonoacetic acid (**3.27**) and phosphonoalanine (**3.28**) are the most widely distributed phosphonic acids in nature. Their biodegradation is catalysed by different metal ion dependent phosphonohydrolases (C-P hydrolases) which belong to distinct enzyme superfamilies and have unique substrate specificities. Organophosphonates themselves are responsible for their degradation by inducing the expression of the required enzymes. Phosphonates act as coinducers for different bacterial transcriptional regulators of the LysR family (LTTRs). The LTTRs of different bacterial strains show high sequence similarity in the *N*-terminal region that acts as DNA binding site. However, there is low similarity in the *C*-terminal region, which is responsible for coinducer recognition. The diversity of C-P hydrolases may have developed on primitive Earth because of the structural diversity of organophosphonates, which were there the predominant P species [3].

Contrary to C-P lyases the expression of C-P hydrolases is not under *Pho*-regulon control, which means that they are also active under conditions where C-P lyases are not active. C-P hydrolases

are responsible for P_i release when phosphonates are the only carbon and energy source for microbial growth. This fact plays an important role for the contribution of phosphonates to the global P cycle. Phosphonohydrolases show detectable activity *in vitro*, enabling full biochemical characterisation [3].

Phosphonates undergoing hydrolytic P-C bond cleavage have usually an electron-withdrawing carbonyl group in β-position to the P atom [23], which is called "β electron sink" [10]. The mechanism which uses the β-carbonyl moiety varies in different enzymes [23]. The hydrolysis of β-ketophosphonates like PnAA (**3.10**), phosphonoacetate (PnA) (**3.27**) and PnPy (**3.8**) (*Scheme 8*)



β-keto phosphonate

R = H, phosphonoacetaldehyde, PnAA, **3.10**
R = OH, phosphonoacetate, PnA, **3.27**
R = COOH, phosphonopyruvate, PnPy, **3.8**

R = H, acetaldehyde, **3.29**
R = OH, acetate, **3.34**
R = COOH, pyruvate, **3.36**

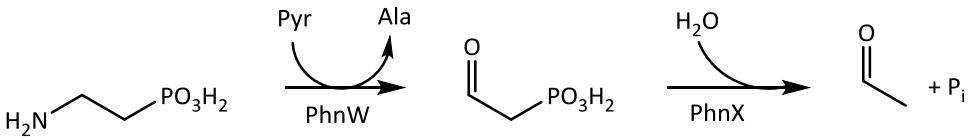
Scheme 8: Degradation of β-ketophosphonates by hydrolases; modified from [21].

involves the formation of a Schiff base intermediate, labilising the P-C bond for nucleophilic attack by either an enzyme active site residue or a water molecule at the P centre. The negative charge induced in this step is also stabilised by the formation of a Schiff base or by metal ions [12, 21, 23], making β-elimination of the phosphonate group easier [10].

(2-Aminoethyl)phosphonic acid

2-AEP predominates within the diversity of biogenic phosphonic acids [3]. There are at least four pathways for its degradation. In diverse microorganisms, 2-AEP catabolism can be controlled either by C-P lyase or by different phosphonohydrolases. The reason for regulatory differences is yet unknown [1].

As already described, 2-AEP (**2.1**) is formed by decarboxylation of PnPy (**3.8**) to PnAA (**3.10**), followed by transamination [3]. La Nauze & Rosenberg in 1967 and Lacoste & Neuzil in 1969 described a catabolic pathway for 2-AEP including two steps [1]. The first step is transamination catalysed by 2-AEP pyruvate aminotransferase, an enzyme encoded by the gene *PhnW* to give **3.10** [3]. The reaction uses pyridoxal phosphate as cofactor and pyruvate as amino group acceptor, giving L-Ala [10]. In the next step, the P-C bond of **3.10** is cleaved by phosphonoacetaldehyde hydrolase (phosphonatase), giving acetaldehyde (**3.29**) and P_i (*Scheme 9*). Phosphonatase is encoded by the *PhnX* gene and found in various bacterial strains [3] such as *Pseudomonas putida, Pseudomonas aeruginosa* and *Bacillus cereus*. In *Enterobacter aerogenes* and *Salmonella typhimurium* 2-AEP degradation was found to be under *Pho*-regulon control [1, 10].



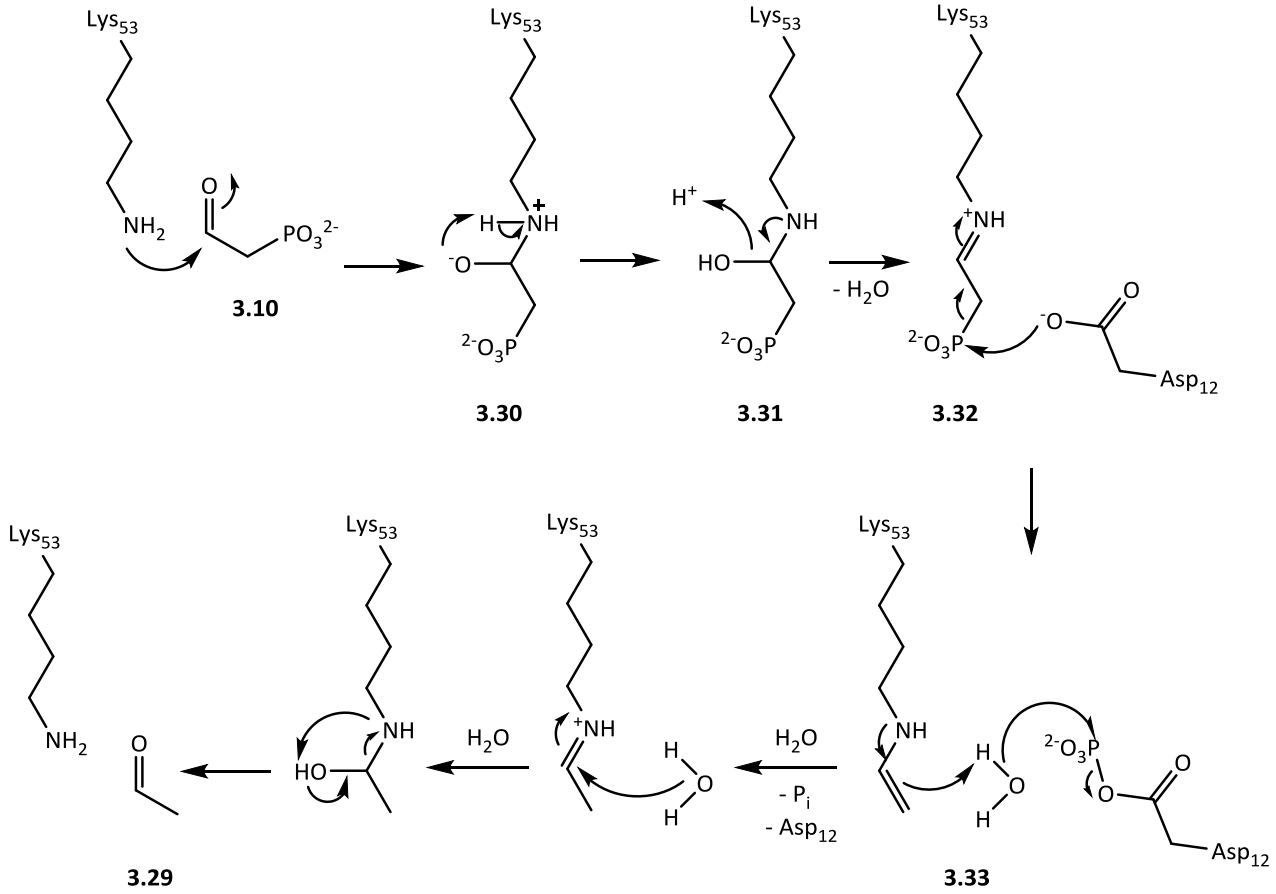
(2-Aminoethyl)phosphonate, 2-AEP
2.1

Phosphonoacetaldehyde, PnAA
3.10

Acetaldehyde
3.29

Scheme 9: Biodegradation of 2-AEP; modified from [1, 3].

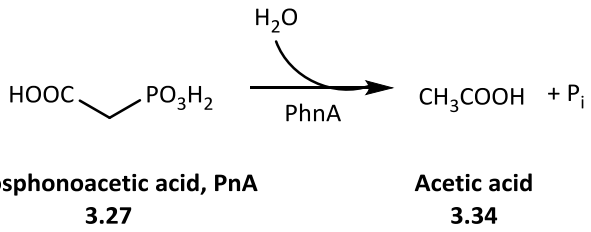
PhnX belongs to the haloacid dehalogenase superfamily, depending on Mg^{2+} for catalytic activity [1]. Hydrolysis proceeds through a Schiff base intermediate formed between the PnAA carbonyl group and the Lys-53 active site residue of the PhnX enzyme [3, 25]. This reaction is facilitated by the active site residues His-56 and Met-49 in the close vicinity of Lys-53. Together with H_2O they build a hydrogen bond network. Catalysis starts with the deprotonation of the Lys ε-amino group to facilitate nucleophilic attack on the β-carbonyl carbon atom of **3.10**, giving **3.30**. The following proton transfer forms a carbinolamine **3.31**. The latter is then converted into an iminium ion **3.32**, enabling the nucleophilic attack of Asp-12 on the P atom. Dephosphonylation of the Schiff base leads to a phosphoenzyme intermediate **3.33**, which finally undergoes hydrolytic cleavage releasing acetaldehyde (**3.29**) and P_i [25] (*Scheme 10*).



Scheme 10: Catalytic mechanism of the hydrolysis of PnAA catalysed by PhnX; modified from [22, 25, 26].

Phosphonoacetic acid

Catabolism of phosphonoacetic acid (**3.27**) is catalysed by phosphonoacetate hydrolase, a *PhnA* gene product, which forms acetic acid (**3.34**) and P_i [1] (*Scheme 11*). The enzyme was first

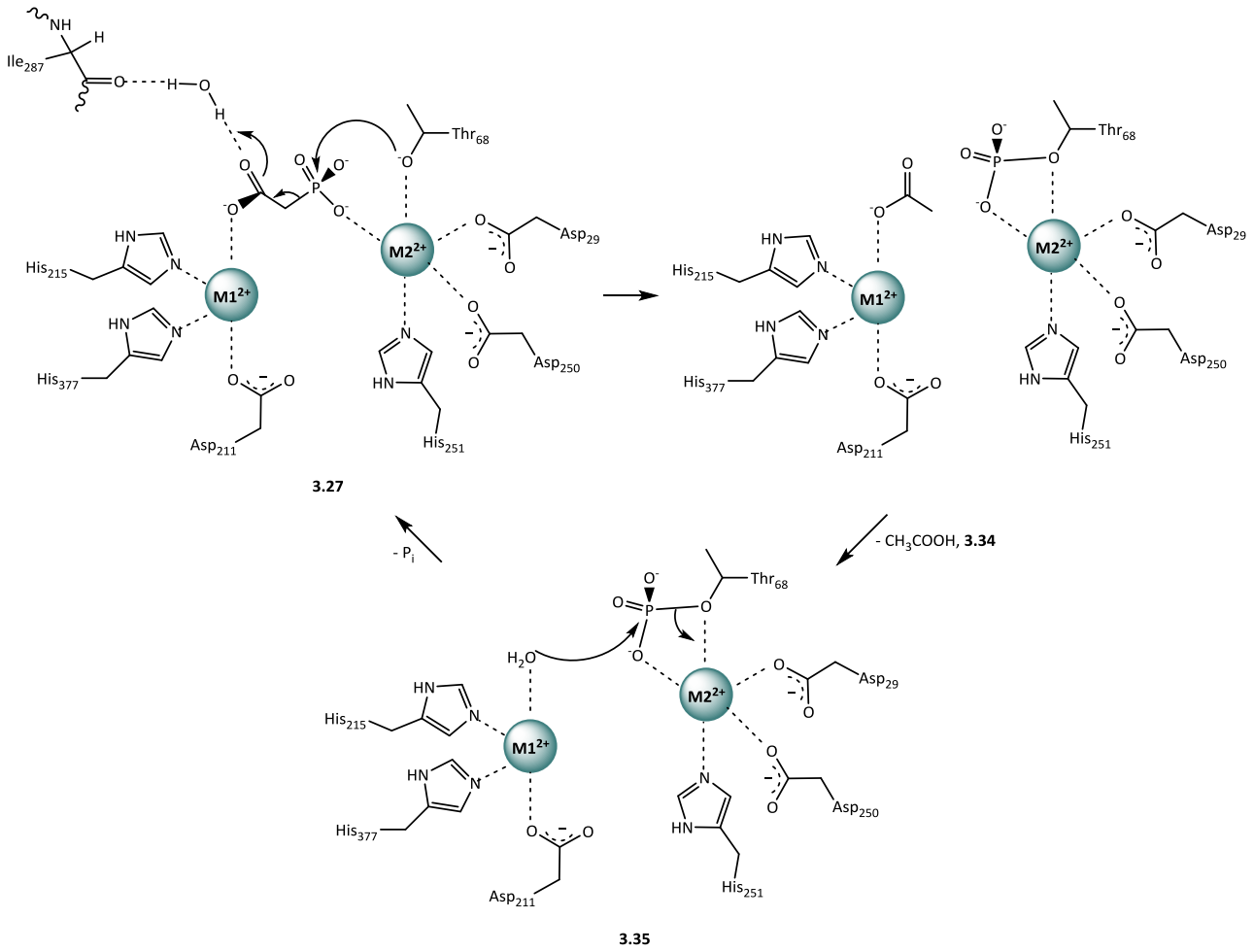


Scheme 11: Biodegradation of phosphonoacetic acid; modified from [1].

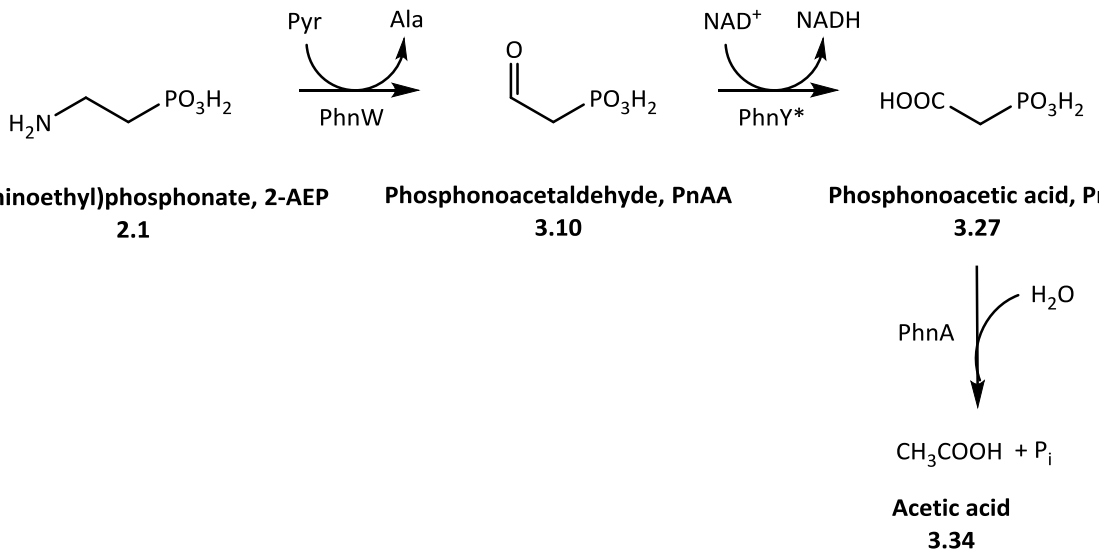
described in 1992 by McMullan *et al.* in *Pseudomonas fluorescens* strain 23F [3].

PhnA belongs to the alkaline phosphatase (AP) superfamily, characterised by two metal ions, M1 and M2, in the catalytic active centre. PhnA has two zinc ions, coordinated by highly conserved protein ligands. M1 ligands are Asp-211, His-215 and His-377. M2 is coordinated by Asp-29, Asp-250 and His-251. Furthermore, the enzyme requires Ser or Thr residues for its catalytic activity to form phosphoenzyme intermediate **3.35**. For P-C bond cleavage, M2 may activate one of these amino acid residues to form the corresponding alkoxides. Nucleophilic attack on the P centre releases acetate (**3.34**). The phosphoenzyme intermediate **3.35** is expected to be attacked by a M1 activated water molecule to displace P_i (*Scheme 12*). The metal ions are responsible for the stabilisation of negative charge formed in the course of the hydrolysis [23].

PhnA was found in an operon also encoding PhnW and the phosphonoacetaldehyde dehydrogenase PhnY*. It has to be noted that PhnY* is not the same as PhnY, which will be discussed in the next chapter. Both enzymes have completely different functions. PhnY* catalyses hydrolytic cleavage of PnAA (**3.10**) using NAD^+ as cofactor to give phosphonoacetic acid (**3.27**) (*Scheme 13*). This enables 2-AEP to be degraded in an alternative pathway compared to the PhnX pathway [1].



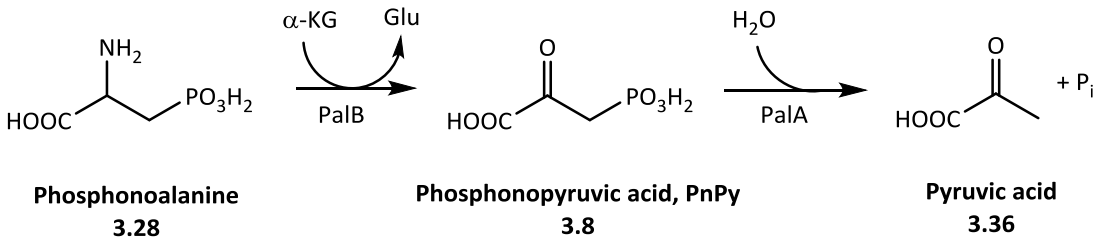
Scheme 12: Proposed mechanism for the hydrolysis of PnA catalysed by PhnA; modified from [23].



Scheme 13: Alternative route for the biodegradation of 2-AEP; modified from [1].

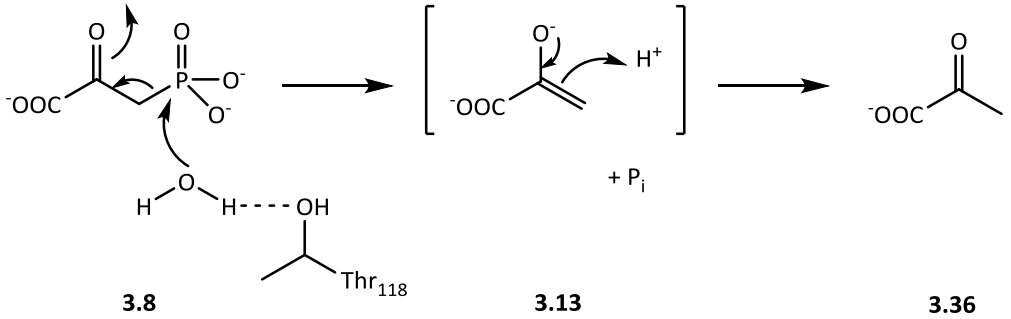
Phosphonoalanine

2-Amino-3-phosphonopropionic acid, also called phosphonoalanine (**3.28**), is formed by transamination of PnPy (**3.8**), especially in marine invertebrates [3]. Its structural analogue is L-Asp. Microbial utilisation of **3.28** was first described in 1998 by Ternan & Quinn in *Burkholderia* sp. [1]. Degradation starts with transamination to give **3.8**. This reaction is catalysed by phosphonoalanine transaminase (PalB) using α-ketoglutarate as amino group acceptor, giving L-Glu. The following P-C bond cleavage catalysed by phosphonopyruvate hydrolase (PalA) yields pyruvate (**3.36**) and P_i [3] (*Scheme 14*).



Scheme 14: Biodegradation of phosphonoalanine; modified from [1].

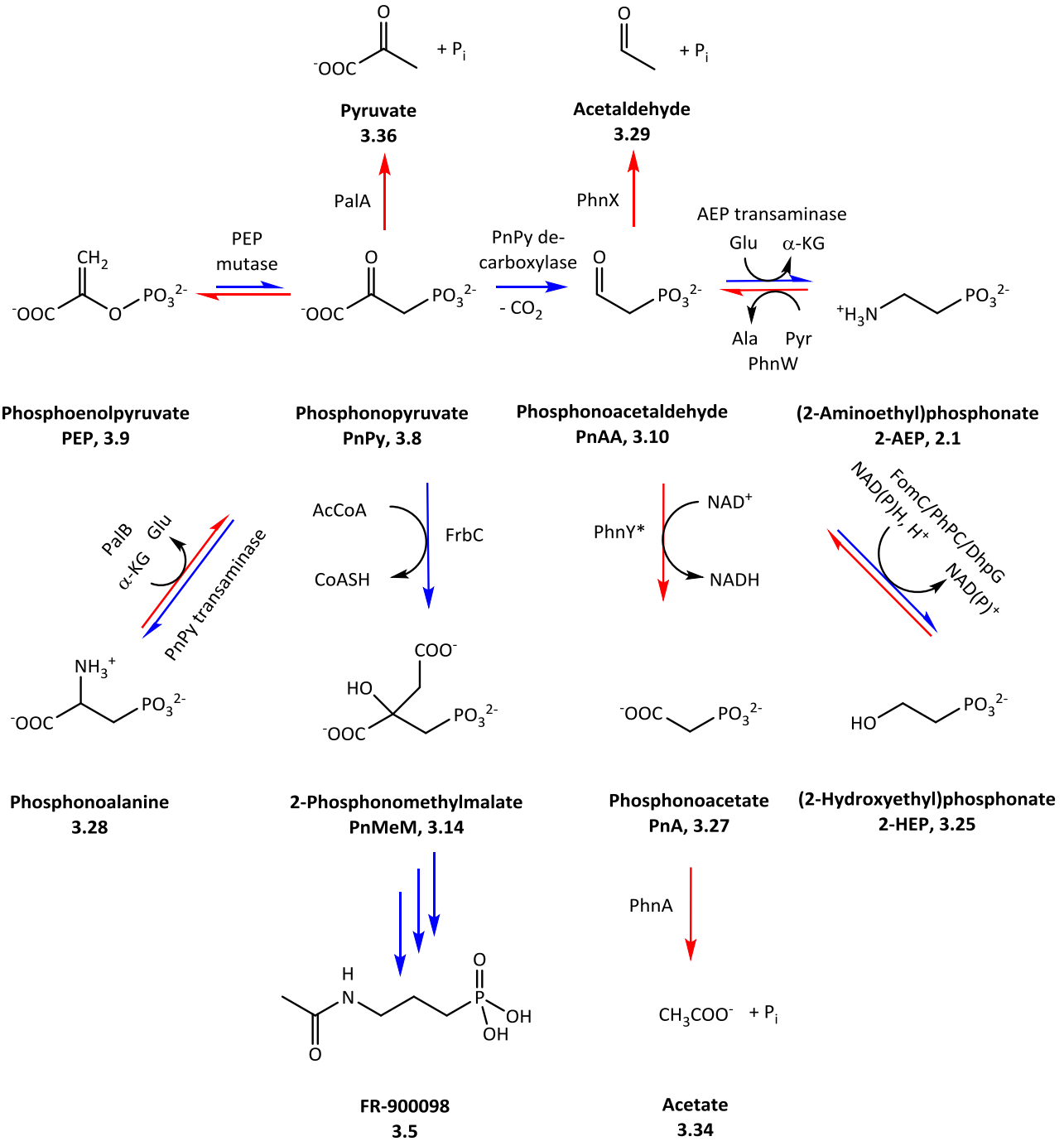
PalA belongs to the phosphoenolpyruvate phosphomutase (PEP mutase)/isocitrate lyase (ICL) superfamily [1]. 40% of PalA is identical with PEP mutase [1, 27]. However, PalA neither shows mutase activity, nor PEP mutase shows hydrolase activity. Kinetic studies showed that PalA is also able to catalyse the hydrolysis of PEP (**3.9**), but at a significantly lower rate than PnPy (**3.8**). PEP mutase and PalA have the same turnover rate for **3.8** and both enzymes have lower affinity for **3.9**. The main functional difference between the two enzymes may be based on the substitution of the Asn-122 active site residue by Thr-118 in PalA. For catalysis, which depends on Mg^{2+} as cofactor, the Thr-118 residue may be used to activate a water molecule for the hydrolysis of **3.8**. The reaction was proposed to have a pyruvate enolate intermediate (**3.13**) [27] (*Scheme 15*).



Scheme 15: Proposed mechanism for the hydrolysis of PnPy catalysed by PalA; modified from [27].

Overview of the hydrolytical catabolic pathways of phosphonates

Scheme 16 gives an overview of phosphonate metabolism by C-P hydrolases. Summarising the above reactions, it can be said that degradation by non-specific C-P lyase or by substrate specific phosphonohydrolases converts different phosphonate substrates into a form of N, C and P that microorganisms can use as nutrient and energy source [24]. As 2-AEP is the most abundant organophosphonate in nature it is the most important P_i source [27].



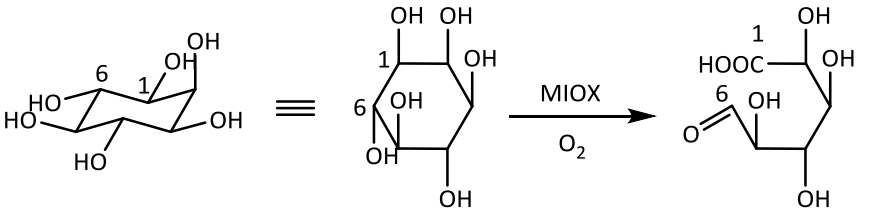
Scheme 16: Phosphonate metabolism, biosynthesis pathways are marked in blue, degradation pathways are marked in red; modified from [4, 27].

3.1.2.3. PhnY and PhnZ – phosphonate catabolism by oxidative P-C cleavage

The last of the three known phosphonate degrading pathways using P-C compounds as P_i source in phosphate deficient ecosystems is catalysed by a two-component enzyme system called PhnY & PhnZ.

Proteomics is a common and modern method in protein chemistry to identify and classify proteins and to assign interactions and functions. Comparison with existing sequences or structures may help to allocate uncharacterised proteins to known superfamilies and to predict their function. However, this does not lead necessarily to correct interpretations as new functions are based on divergent evolution. The purpose of an enzyme can vary within a superfamily, resulting for example in different substrate specificities or even in different reaction types. The histidine-aspartate (HD) protein superfamily was discovered by sequence analysis in 1998. It comprises over 37.000 proteins which are distributed in numerous organisms. Members show a characteristic H-HD-D sequence motif able to bind a divalent metal ion, for example Zn^{2+}, Mg^{2+} and Mn^{2+}. Not many HD proteins are biochemically characterised. However, they are known for their ability to catalyse exclusively the hydrolysis of phosphoesters, therefore named phosphohydrolases. Their wide distribution suggests that there exist further activities within this superfamily [29].

The *myo*-inositol oxygenase (MIOX) is an HD protein providing a special catalytic function. Its mechanism differs a lot from other members of the superfamily. MIOX is an enzyme with a dinuclear metal centre resulting from two additional His in the sequence motif (H-HD-H-H-D). Mixed-valent Fe^{2+}/Fe^{3+} ions act as cofactor to catalyse the $4e^-$ oxidative cleavage of the *myo*-inositol (MI) (**3.37**) C-C bond to give D-glucuronate (**3.38**) [29] (*Scheme 17*). Fe^{3+} is responsible for



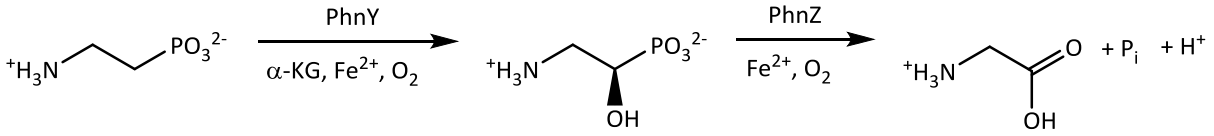
Scheme 17: Oxidative C-1/C-6 bond cleavage of *myo*-inositol by MIOX; modified from [32, 33].

the coordination of the bidentate MI substrate through its vicinal C-1 and C-6 hydroxyl groups [29, 30, 31]. It serves as Lewis acid to activate the substrate. Fe^{2+} activates O_2 to give superoxide oxygen [29, 31]. In this substrate binding mode the reactive C-1 position projects the H-bond towards the superoxide. The C-1-OH group is furthermore bound to the Lys-127 active site residue, which is essential for catalytic activity [31].

Excursus:

Myo-inositol is widespread in mammalian tissues and involved in various cellular regulation mechanisms like signal transduction. MIOX is of special physiological interest as it catalyses the initial step in the only degradation pathway for *myo*-inositol, which occurs mainly in the kidney. The reaction is therefore used to adjust inositol levels *in vivo*. MI is excreted in considerable amounts in the urine of patients with diabetes mellitus. This may be due to a hyperglycaemic state as isomeric glucose inhibits MI transport and further MIOX activity is decreased. Different MI tissue levels may also contribute to diabetic neuropathy. Therefore the understanding on how MIOX expression is regulated would be an important step forward in the aetiology of diabetes and enable the control of key signalling pathways [32].

A new HD protein, the non-haem diiron-dependent oxygenase PhnZ, was discovered recently. Together with the coupled enzyme PhnY it represents a further pathway for the degradation of 2-AEP [29, 30, 31]. PhnY and PhnZ were discovered by screening for genes enabling an *E. coli* strain to grow on phosphonates despite missing C-P lyase activity [1, 13]. Both represent completely new types of enzymes in the field of phosphonate chemistry [13]. PhnY is an α-ketoglutarate/non-haem Fe^{2+}-dependent dioxygenase, catalysing the stereospecific hydroxylation of the α-position next to P of 2-AEP (**2.1**) [29, 30]. This activation enables the coupled enzyme PhnZ to catalyse the $4e^-$, O_2-dependent oxidative cleavage of the P-C bond of 1-OH-2-AEP (**2.2**), yielding glycine (**3.39**) and P_i [29] (*Scheme 18*). The degradation through an oxidative pathway is notable, as PhnZ



(2-Aminoethyl)phosphonate, 2-AEP
2.1

(*R*)-(**2-Amino-1-hydroxy-ethyl)phosphonate**
2.2

Glycine
3.39

Scheme 18: Biodegradation of 2-AEP: stereospecific hydroxylation by PhnY and oxidative P-C bond cleavage by PhnZ; modified from [29, 31].

belongs to the HD phosphohydrolase superfamily [31].

The affiliation to the HD superfamily, the conservation of six metal-coordinating residues, the coordination geometry, the binding mode of the substrate, the iron dependence and the analogy of the oxidation reaction – those similarities to MIOX suggest the abundance of a mixed-valent diiron cofactor for the catalysis of the oxygenation [29, 31]. The presence of such a cofactor was indeed confirmed by EPR and Mössbauer spectroscopy and by X-ray crystallography. It showed the same substrate binding mode like in MIOX. The mechanism of MIOX and PhnZ may be even more widespread in at present unknown reactions catalysing the oxidative cleavage of different types of

C-X bonds. Also genome mining of the HD protein superfamily suggests the existence of more mixed-valent diiron oxygenases [29].

PhnZ appears as a monomer in solution and as a crystallographic dimer, consisting of 190 amino acids [29, 31]. It is smaller than MIOX which contains 285 amino acids. In the conserved core of the enzyme six iron-coordinating residues, His-34 and 58, Asp-59, His-80 and 104 and Asp-161 make up the characteristic HD scaffold [29] (*Figure 6*). The two iron ions (Fe1 and Fe2) in the metal

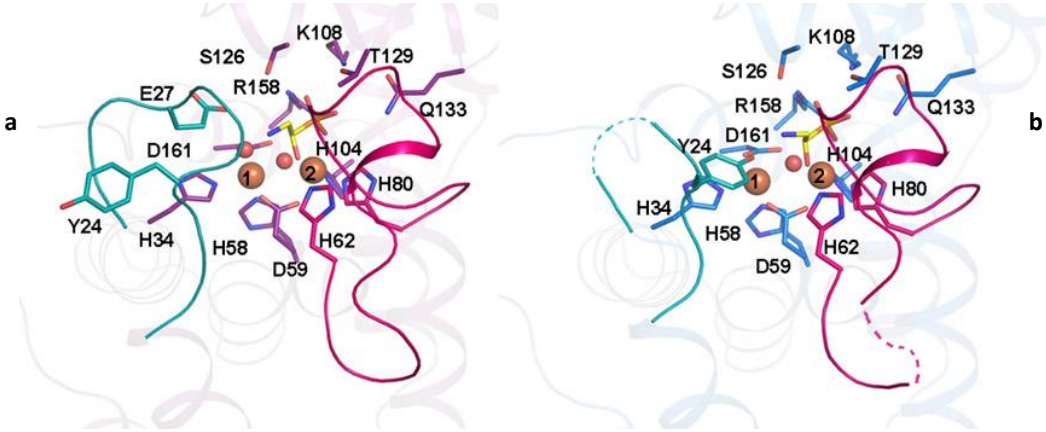


Figure 6: a) nearly closed form of the active site, b) more open form of the active site; from [31].

centre, which are bridged by the Asp-59 residue and by a water molecule, show both an inactive +3 oxidation state, which can be transformed into the catalytically active mixed-valent form by L-ascorbate [29, 31]. Fe1 shows a distorted octahedral geometry. It is coordinated by Tyr-24, His-34, His-58, Asp-59, Asp-161 and the water molecule bridging to Fe2. Fe2 shows an octahedral geometry. It is coordinated by Asp-59, His-80, His-104 and also the water molecule. The two remaining coordination sites are responsible for binding the bidentate substrate [31]. Only the (*R*)-enantiomer of **2.2** is accepted as substrate. It coordinates to the Fe2 ion with the O-atom of the α-hydroxy group and with one phosphonyl oxygen, forming a five-membered ring [29, 31]. The phosphonyl oxygens of the substrate are furthermore bound by a polar pocket (Lys-108, Ser-126, Thr-129, Gln-133, Arg-158) of the enzyme. The C-1-OH is additionally bound over a hydrogen bond to a fifth PhnZ histidine residue, His-62, which occurs only within HD Phn enzymes and not in other members of the superfamily [31]. His-62 helps to guarantee the right orientation of the PhnZ substrate and supports the proton abstraction from the C-1-OH [29, 31]. It is therefore essential for catalytic activity. The O_2 necessary for oxidation is bound to Fe1 by replacing its Tyr-24 ligand to form a superoxo-Fe^{3+}/Fe^{3+} complex [29]. The conformation of PhnZ makes only the (*R*)-substrate accessible to O_2 [31]. The Fe2 bound 1-OH-2-AEP is orientated in a way that the α-C hydrogen, which has to be abstracted during the stereospecific reaction, faces the Fe1-O_2 [29, 31]. That is the reason why PhnZ is unreactive towards the (*S*)-enantiomer and explains why experiments with racemic (±)-**2.2** resulted in just 50% conversion. Studies also showed that 2-AEP itself could not be used as substrate by PhnZ and that the product of the coupled enzyme PhnY

has to be the (*R*)-enantiomer [30, 31]. Further investigations on the PhnZ reaction revealed that complete conversion of **2.2** to P_i was only successful if iron as cofactor was used [30].

The interaction between the enzyme and the substrate follows an induced-fit model (*Figure 7*).

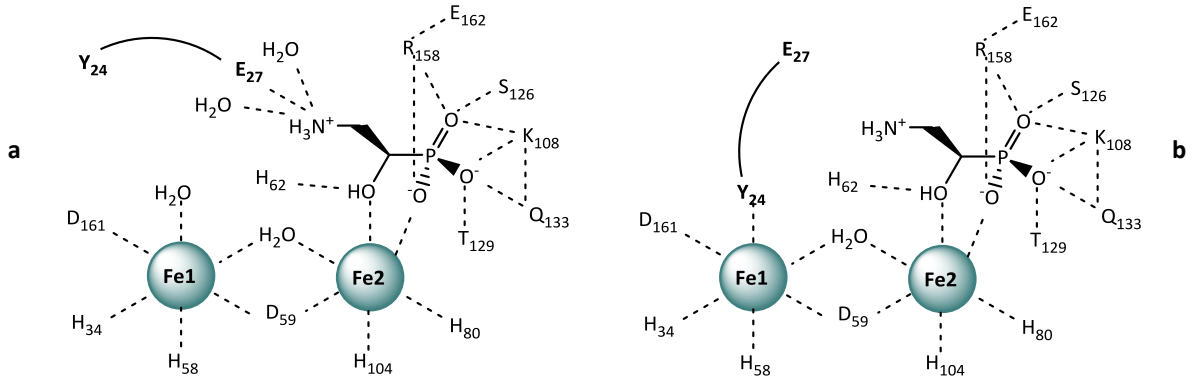
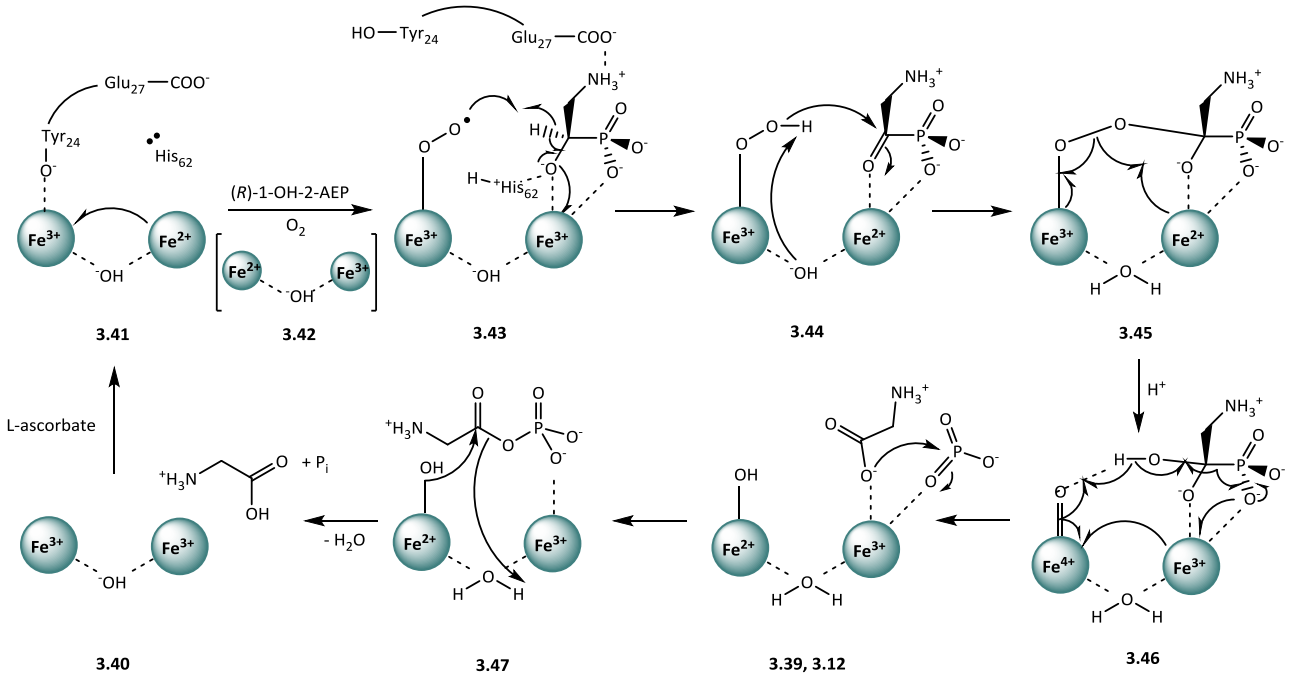


Figure 7: Substrate binding follows an induced-fit model, a) closed form of the active site, b) open form of the active site; modified from [31].

When (*R*)-**2.2** is bound to PhnZ, several conformational changes are induced in the active site. They cause the movement of two loops (His-62–His-80 and Asp-21–Asn-30) opening the catalytic centre. The conformation changes between a nearly closed and a more open form of the active site. The result is a strong difference in the environment of the (*R*)-**2.2** amino group and in the ligand state of the Fe1 ion. In the closed state **a**, the positively charged amino group may be solvated by two H_2O molecules and electrostatically interact with the loop Glu-27 residue. In the more open state **b** there are no interactions and the Asp-21–Asn-30 loop is very mobile. Also the His-62–His-80 loop is here more flexible. The ligand state of the Fe1 ion directly depends on the environment of the amino group. The motion of the Glu-27 residue is inevitably coupled to the motion of the Fe1-ligand Tyr-24, as they are connected through a short loop region. Glu-27 interacting with the amino group leads to the displacement of the Tyr-24 ligand from the Fe1 centre. Its position is replaced by a H_2O molecule, situated near the α-C of the substrate in reactive distance if water is replaced by O_2. It was proposed that the closed form was the active one essentially depending on the amino group of the substrate, while the more open form was an inactive intermediate. This intermediate with Tyr-24 bound to Fe1 avoids the premature activation of O_2 in the absence of the right substrate. In any case, Glu-27 and Tyr-24 are highly conserved. Although, catalysis does not critically depend on Tyr-24 as it just occupies the Fe1 ligand site until molecular O_2 is bound [31].

Except MIOX, dinuclear non-haem iron-dependent dioxygenases generally use the metal cofactor in their reduced form, which is necessary to activate molecular O_2. The mixed-valent oxidation states are normally of limited stability. As MIOX supports the activation of both substrate and O_2 by a mixed-valent cofactor, it stabilises this oxidation state. The same is assumed for PhnZ. The inactive +3 oxidation state (**3.40**) is reduced to the catalytically active mixed-valent form by L-

ascorbate. This state is regenerated after an enzymatic cycle, as charge is balanced between the oxidation of (*R*)-**2.2** and the 4e$^-$ reduction of O_2 [29]. Therefore, PhnZ does not depend on external reducing agents for multiple catalytic cycles [30]. The PhnZ mechanism uses the Fe1 ion in its ferrous form (Fe^{2+}) to reduce O_2 to superoxide. The Fe2 ion is used in its ferric form (Fe^{3+}) to bind the substrate, as the greater positive charge better binds the negative charge of the phosphonyl oxygen and further supports ionisation of the C-1 hydroxyl group. The latter facilitates hydrogen abstraction by resonance stabilisation. Initially, Tyr-24 binds to the Fe1 ion in a +3 oxidation state, while Fe2 is in oxidation state +2 (**3.41**). When (*R*)-**2.2** binds to Fe2 the ion serves as electron-source for the reduction of Fe1, resulting in reversed oxidation states (**3.42**). This electron transfer supports the expulsion of Tyr-24 as well as the reduction of O_2. Glu-27 electrostatically interacts with the amino group of the substrate and Tyr-24 is removed from the enzyme's active site, enabling O_2 to bind (**3.43**). The formed superoxide Fe^{3+}-$O_2^{\bullet-}$ then abstracts the hydrogen from the α-C next to P and thereby initiates the cleavage of the P-C bond [31]. The hydrogen abstraction may cause a 1e$^-$ oxidation of the substrate forming an α-ketophosphonate intermediate (**3.44**). Subsequently, the Fe^{3+}-hydroperoxide acts as nucleophile to attack the α-carbonyl group, assisted by the hydroxide which is bridging the metal ions. Then, the O-O bond in **3.45** was suggested to undergo homolytic cleavage, resulting in a Fe^{4+}-oxo species and a diol intermediate (**3.46**). In the following step the P-C bond of the substrate is cleaved, initiated by the abstraction of the hydroxyl proton by the ferryl oxygen, to give glycine (**3.39**) and metaphosphate (**3.12**). The Fe^{2+} oxidation state is regenerated. It was suggested that a glycyl phosphate (**3.47**) was formed, which could not be observed by NMR spectroscopy until now, as it may be immediately hydrolysed to **3.39** and P_i [30] (*Scheme 19*).



Scheme 19: Proposed mechanism for PhnZ; modified from [30, 31].

As already mentioned above, not many HD proteins are yet biochemically characterised. But it is very likely that more mixed-valent diiron oxygenases (MVDOs) exist, which are capable of catalysing the oxidative cleavage of a C-X bond [29]. There are numerous microbial enzymes that show the characteristic HD scaffold and a dimetal centre [29, 31]. To be able to identify potential MVDO candidates within the HD superfamily the question whether MVDOs specifically stabilise the mixed-valent state or if HD proteins generally are responsible for stabilisation has to be answered. If the first assumption was true, new HD proteins could be assigned functions. If the latter was the case, hydrolases and oxygenases would be the result of divergent evolution within the HD superfamily [29].

3.1.3. The contribution of organophosphonates to the global P cycle

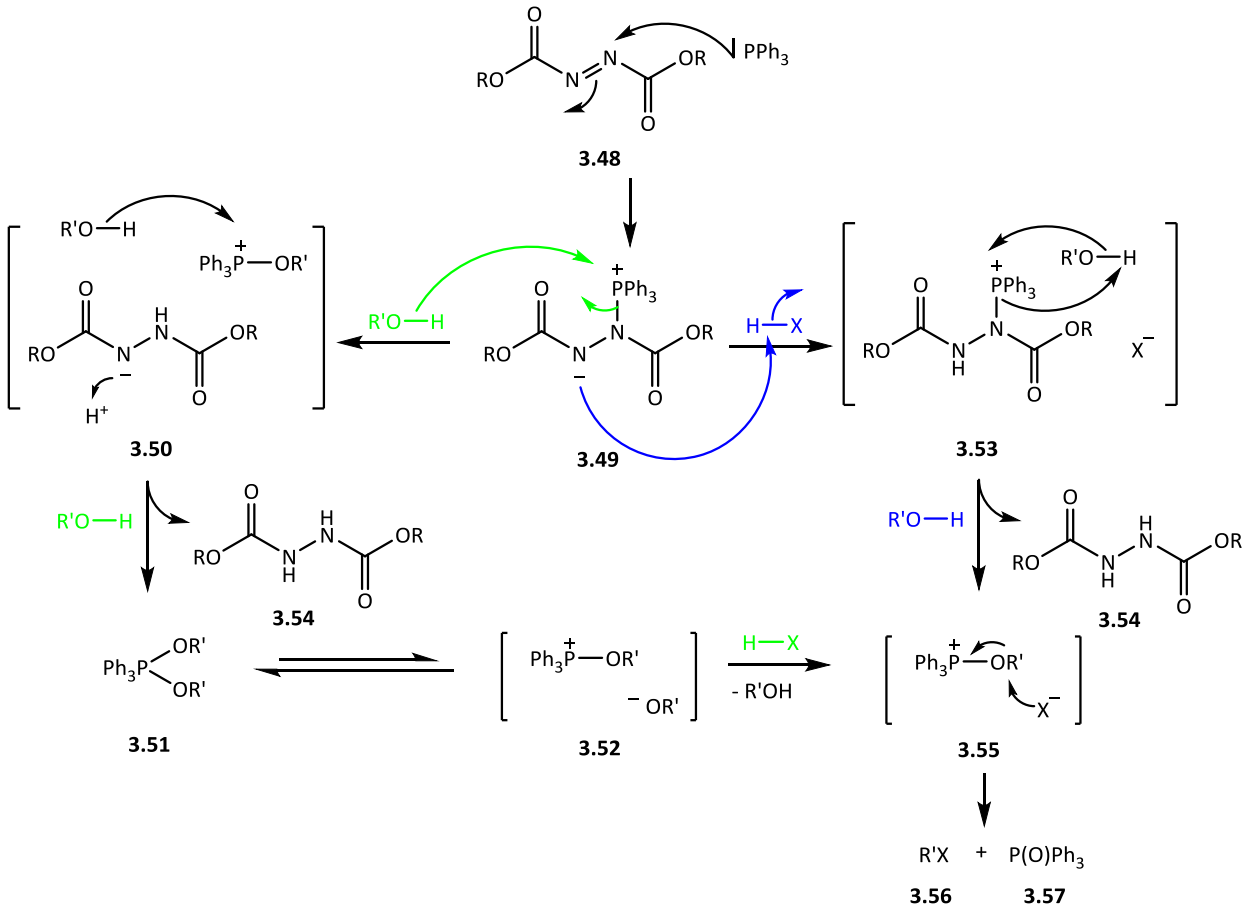
Phosphorus is the 11th most frequently occurring element on Earth. Nevertheless, it is the main limiting nutrient for many, especially marine microorganisms. The availability of phosphorus can vary a lot in marine ecosystems [1]. In the terrestrial biosphere, P_i shortage is rare and therefore will not be discussed [3]. In the biosphere P_i is the most frequent P species, however, it is often difficult to acquire [3, 20]. Marine productivity decisively depends on P availability, which is in turn connected to C and N cycling in the oceans. P_i concentrations are low in oligotrophic environments but also seasonally in different surface waters. Concentrations are often close to the detection limit (<1 nM). Recent climatic changes are not helping either. Correlated with this phenomenon is an increase of dissolved organic phosphorus (DOP) levels relative to P_i levels. Recent studies revealed that in marine environments considerable amounts of phosphorus stem from DOP [20], in some regions up to 80% of the total soluble phosphorus [1, 20]. DOP is a complicated mixture of different organic components including various forms of phosphates and phosphonates [1]. It results from excretion, decomposition of dead organisms and autolysis [34], but also organophosphonates of anthropogenic origin enter soils and waters in diluted form. However, there is little understanding of the extent to which they contribute to the P cycle [3]. It is known that phosphonates make up about 25% of DOP in the oceans and therefore are an important alternative source of phosphorus for marine microorganisms [1, 3]. Further, genomic analysis revealed that possible pathways for the biosynthesis of phosphonates are encoded in 10% of the examined bacterial genomes and that even 30% encode diverse degradation pathways for phosphonates. Therefore, it is not surprising that nature developed different pathways for the biosynthesis and biodegradation of phosphonates. The most prominent example for the diversity of phosphonate biosynthesis and catabolism is 2-AEP. Its catabolic routes are predominant among marine microorganisms. The importance of organophosphonates for the global biogeochemical P cycle is increasingly recognized. But still the definitive quantitative contribution and the qualitative composition of phosphonates remain unknown, as research is difficult due to analytical limitations in complex matrices like sea water. There is extensive research involving genetic, chemical and biochemical analyses to better understand the different phosphonate degrading pathways and their regulation as well as to find new biosynthetic routes. This field of research is of economic interest in order to elucidate worldwide P cycling and to identify potential candidates for commercial applications [1].

3.2. Phosphorus reactions

3.2.1. Mitsunobu reaction

The Mitsunobu reaction is a S_N2 reaction which was discovered by Oyo Mitsunobu in 1981 [35]. The hydroxyl group of primary or secondary alcohols is substituted by diverse nucleophiles with inversion of configuration at the carbon centre. Triphenylphosphine (PPh_3) and a dialkyl azodicarboxylate **3.48** are needed for the reaction. The latter is typically diethyl azodicarboxylate (DEAD) or diisopropyl azodicarboxylate (DIAD) [36]. In this redox reaction, PPh_3 is oxidised to triphenylphosphine oxide $P(O)Ph_3$ (**3.57**), while the dialkyl azodicarboxylate is reduced to the corresponding hydrazine dicarboxylate **3.54** [35]. The reaction outcome strongly depends on the nature of the substrate and the nucleophile, while the type of azodicarboxylate is of minor importance [36]. To reduce side reactions the dehydrating reagents are activated first, generating an intermediate phosphonium salt [35, 36]. The Mitsunobu reaction is a mild and effective method to introduce different functional groups [36].

The reaction starts with the nucleophilic attack of PPh_3 on one of the azodicarboxylate nitrogens, forming a zwitterionic phosphonium salt **3.49**. This intermediate can follow two pathways. In both cases the alcohol is activated by the formation of an oxyphosphonium salt [36] (*Scheme 20*).



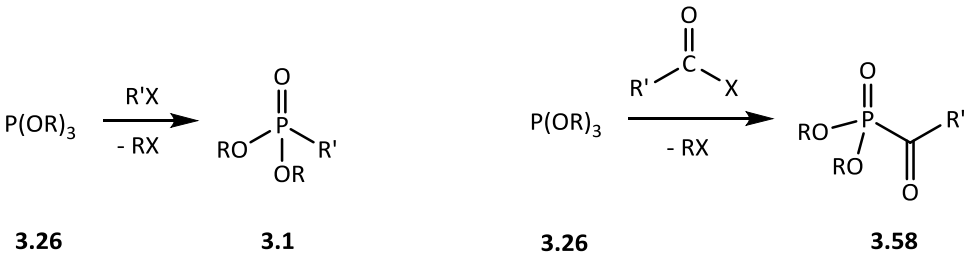
Scheme 20: Proposed reaction mechanism for the Mitsunobu reaction; modified from [36, 37].

Weakly acidic substances like HN_3, carboxylic acids, phenols, imides, thiols, thioamides and β-dicarbonyl compounds can be used as nucleophiles in the Mitsunobu reaction [38].

3.2.2. Michaelis-Arbuzov reaction

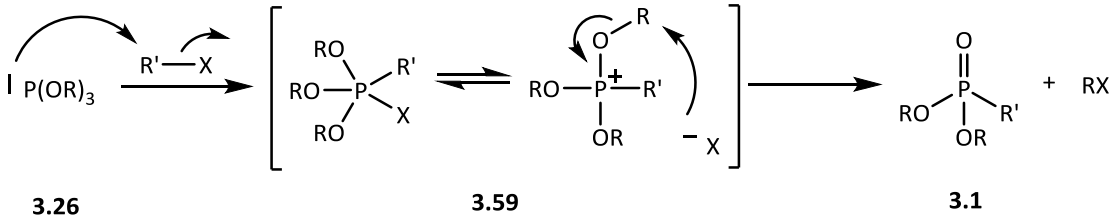
In organic synthesis P-C bond forming reactions are very common. They include the Michaelis-Arbuzov reaction, the Pudovik reaction and the Abramov reaction.

The Michaelis-Arbuzov reaction was discovered by August Michaelis in 1898 and further investigated by Aleksandr Arbuzov [48]. This reaction converts trialkyl phosphites **3.26** to alkyl phosphonates **3.1** using alkyl halides [44]. A variant of the Arbuzov reaction converts trialkyl phosphites to acylphosphonates **3.58** using acyl halides [51] (*Scheme 21*).



Scheme 21: Overall Scheme of two variants of the Arbuzov reaction.

The efficiency of the reaction depends on the type of alkyl halide. Reactivity increases in the order X = Cl^- < Br^- < I^-. Furthermore, primary alkyl halides give higher yields than secondary ones [45]. The reaction is a two step process. First, an intermediate **3.59** is formed by nucleophilic attack of the trialkyl phosphite on the alkyl group of R'X. In a S_N2 type reaction the formed trialkoxyphosphonium salt gives the desired phosphonate under release of alkyl halide RX [44, 45] (*Scheme 22*). Normally, step one is completed before the alkyl halide RX can disturb the reaction.

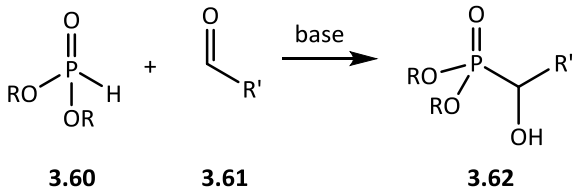


Scheme 22: Proposed reaction mechanism for the Arbuzov reaction; modified from [44, 45].

A competition would just take place if RX was more reactive than R'X [44].

3.2.3. Pudovik reaction

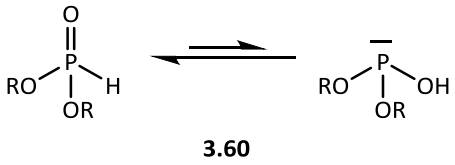
The Pudovik reaction was discovered by Arkady Pudovik in 1948 [46]. It is a reaction where aldehydes **3.61** are hydrophosphonylated with dialkyl phosphites **3.60**. The reaction is used to generate α-hydroxyphosphonates **3.62** in organic synthesis [39] (*Scheme 23*). Enantiopure forms



Scheme 23: Overall Scheme of the Pudovik reaction.

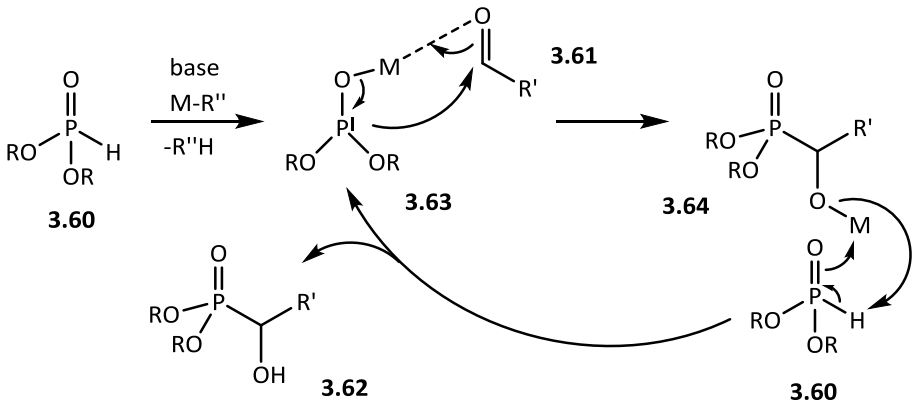
of α-hydroxyphosphonates have a great potential due to their biological activity [39].

The unstable oxyacid easily tautomerises to dialkyl phosphite. As the latter is the predominating but unreactive equilibrium form (*Scheme 24*) the reaction requires base catalysed activation [39].



Scheme 24: Equilibrium reaction between the dialkyl phosphite form and the oxyacid tautomer; modified from [41].

It is assumed that a catalytic amount of organometallic or organic base first deprotonates the phosphite to give an alkali metal salt **3.63** which attacks the carbonyl compound [42, 43, 47]. The reaction intermediate **3.64** is a strong base which metallates the phosphite [39, 42] (*Scheme 25*).

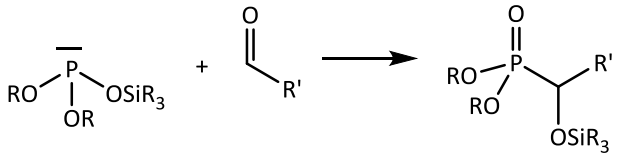


Scheme 25: Proposed reaction mechanism for the Pudovik reaction with an organometallic base M-R'', where M acts as electrophile and R'' acts as nucleophile [49]; modified from [46, 47].

As efficient proton abstraction is crucial for the reaction, electron withdrawing alkyl substituents on the phosphorus facilitate the reaction [43]. Dialkyl phosphites are stable against oxidation, in contrast to trialkyl phosphites, and do not react with halides [42]. Aldehydes, ketones, α,β-unsaturated carbonyl compounds and aldimines can be hydrophosphonylated by the Pudovik reaction [40, 46].

3.2.4. Abramov reaction

The Abramov reaction was discovered by Vasily Abramov in 1947. In the Abramov reaction aldehydes **3.61** are phosphonylated with dialkyl silyl phosphites **3.65**, which show excellent reactivity under redox conditions [39, 49] (*Scheme 26*).

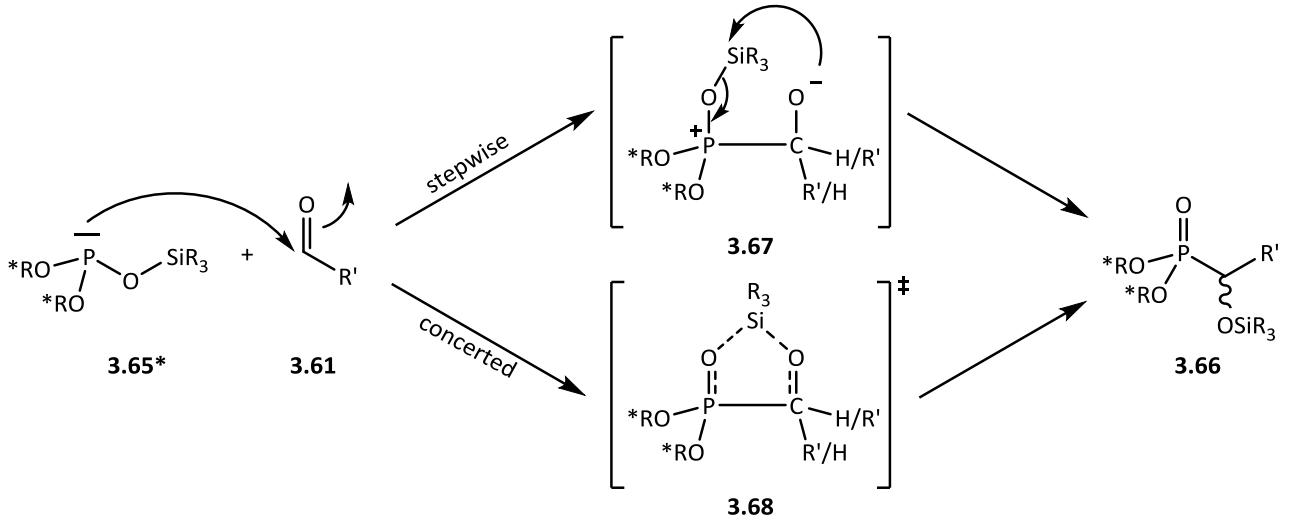


3.65 **3.61** **3.66**

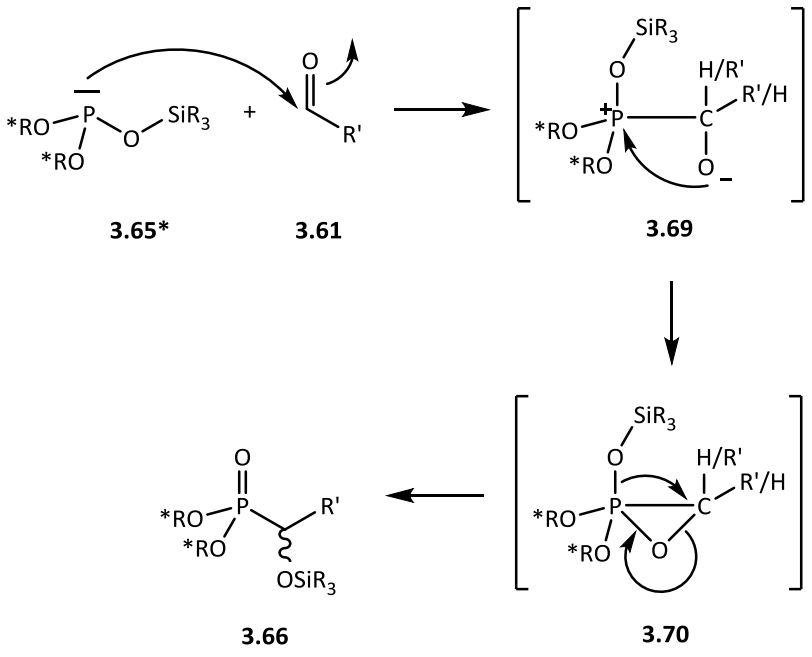
Scheme 26: Overall Scheme of the Abramov reaction.

α-Siloxy-phosphonates **3.66** are stable compounds and silylation can be used to protect α-hydroxyl groups in phosphonates. Deprotection under mild conditions gives equivalent products to the Pudovik reaction. For a long time, enantioselective forms of this reaction were unknown. Recently a catalytic asymmetric Abramov reaction was developed by Guin *et al.* using a chiral disulfonimide as catalyst. The substituents at phosphorus have a strong influence on reactivity and stereoselectivity. Phosphites with sterically less demanding substituents such as OMe, OEt and O*i*Pr groups or mixed variants therefore give excellent yields, whereas phosphites with bulkier *t*Bu groups are not reactive at all. Other silyl groups than TMS with higher steric hindrance like TBS were shown to decrease the yield. In any case, the results of control experiments with unsilylated phosphites proved the requirement for silylated nucleophiles [39, 40, 50]. Steric and electronic factors of the carbonyl substrate also influence reactivity. Nucleophilic attack on the carbon centre is easier when the substituents are small and electron poor. The P atom acts as a nucleophile, the Si atom acts as an electrophile, while the O atom serves as a spacer between them. It enables electron transmission and therefore influences reactivity as nucleophilicity rises with electrophilicity. Therefore, such P-O-Si reagents are well suited for the phosphonylation of prochiral carbonyl compounds. The Abramov reaction is an attractive and mild method facilitating the introduction of functionalities into the α-position of phosphonates [49]. Two possible mechanisms were proposed for the intramolecular silyl group transfer. It could be a stepwise process involving polar intermediate **3.67** or a concerted process with pericyclic transition state **3.68** (*Scheme 27*). However, the former seems more likely [49, 50]. In an asymmetric variant of the Abramov reaction this reaction mechanism would result in net retention of configuration at the involved carbon stereocentre. If the mechanism involved an epoxy intermediate **3.70**, the product

would show inversion of configuration (*Scheme 28*). Labelling studies showed that the oxo group on the P atom of the phosphonate is derived from the oxygen atom of the siloxy group, which supports the retentive mechanism [49].



Scheme 27: Proposed reaction mechanism for the Abramov reaction. Asymmetric variants (R* = chiral auxiliaries) would result in overall retention at the carbon stereocentre; modified from [49, 50].



Scheme 28: Proposed alternative reaction mechanism for the Abramov reaction. Asymmetric variants (R* = chiral auxiliaries) would result in overall inversion at the carbon stereocentre; modified from [49].

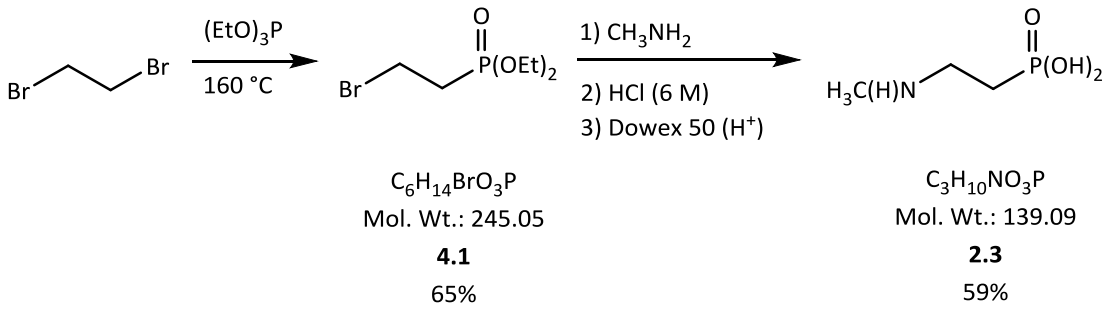
Ketones and α,β-unsaturated carbonyl compounds also can be phosphonylated in the Abramov reaction [50].

4. Results and discussion

To map the active sites of PhnY and PhnZ, a series of analogues of their substrates, 2-AEP and 1-OH-2-AEP, were prepared and sent to Canada for evaluation with these enzymes by the cooperation partner.

4.1. (2-(Methylamino)ethyl)phosphonic acid (2.3)

The *N*-monomethylated form of 2-AEP (**2.1**) is a naturally occurring compound and therefore possibly a substrate for the coupled enzymes PhnY & PhnZ. (2-(Methylamino)ethyl)phosphonic acid (**2.3**) was synthesised in a two step sequence (*Scheme 29*).



Scheme 29: Synthesis of (2-(methylamino)ethyl)phosphonic acid (**2.3**).

In the first step triethyl phosphite was reacted with an excess of 1,2-dibromoethane in an Arbuzov reaction with elimination of ethyl bromide. The crude product was purified by fractional vacuum distillation. Excess 1,2-dibromoethane was recycled. Investigation by NMR spectroscopy showed that the desired bromoethylphosphonate **4.1** contained small amounts of two side products. The first one was diethyl ethylphosphonate formed by the reaction of ethyl bromide with triethyl phosphite. The other one was triethyl phosphate. In the following S_N2 reaction, bromide **4.1** was reacted with a large excess (12 eq.) of methylamine. The ethyl protective groups on phosphorus were removed with boiling 6 M HCl. The desired acid **2.3** was purified by ion exchange chromatography using Dowex 50Wx8, H^+ and elution with water. At first, ethylphosphonic acid, phosphoric acid and HCl were eluted, followed by the desired product at neutral pH. Finally, aminophosphonic acid **2.3** was crystallised from water/EtOH. When the S_N2 reaction was performed with a lower excess of methylamine, 8 eq. instead of 12 eq., and using a longer reaction time, 48 h instead of 3 h, the yield was worse than before. The yields for the two steps were 65% and 59%, respectively.

The *N*-methylaminophosphonic acid **2.3**, a structural analogue of 2-AEP, was incubated with PhnY by the cooperation partner in Canada. The progress of the reaction was monitored by ^{31}P NMR spectroscopy.

Surprisingly, the test results showed that (2-(methylamino)ethyl)phosphonic acid (**2.3**) is not hydroxylated by PhnY. The hydroxylated phosphonate would give another signal increasing in intensitiy with time (*Table 1*). One possible explanation for the fact that the *N*-methyl analogue of

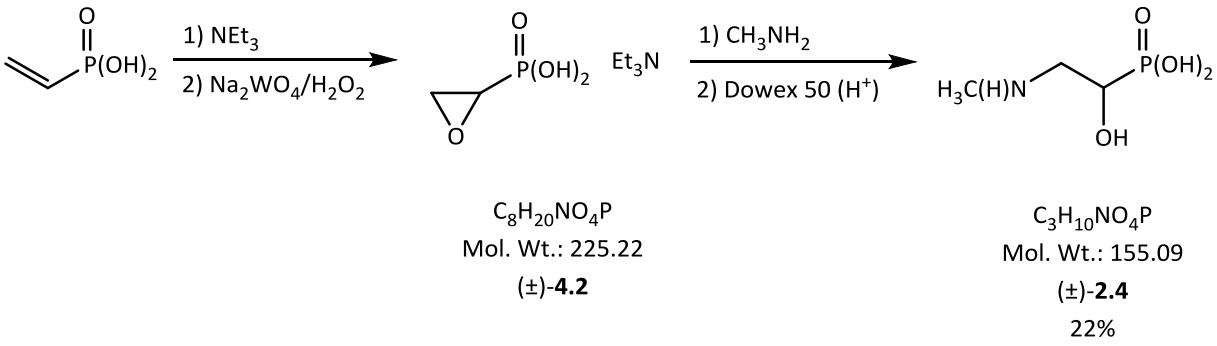
***δ* [ppm] – control**	***δ* [ppm] – PhnY**
17.94	17.39

Table 1: Test results for compound **2.3**, left column: chemical shift for ^{31}P signal for enzyme free control, right column: chemical shift for ^{31}P signal for enzyme containing reaction mixture.

2-AEP is not accepted as substrate for PhnY is the occurrence of a degradation pathway different from the one used for 2-AEP.

4.2. (±)-(1-Hydroxy-2-(methylamino)ethyl)phosphonic acid [(±)-2.4]

(±)-(1-Hydroxy-2-(methylamino)ethyl)phosphonic acid [(±)-**2.4**] was synthesised in a two step sequence from vinylphosphonic acid (*Scheme 30*). As 1-OH-2-AEP (**2.2**) is the substrate for PhnZ, it was reasonable to assume that the naturally occurring *N*-monomethylated 2-AEP (**2.3**) is possibly hydroxylated and then used as substrate by PhnZ.



$C_8H_{20}NO_4P$
Mol. Wt.: 225.22
(±)-**4.2**

$C_3H_{10}NO_4P$
Mol. Wt.: 155.09
(±)-**2.4**
22%

Scheme 30: Synthesis of (±)-(1-hydroxy-2-(methylamino)ethyl)phosphonic acid [(±)-**2.4**].

The epoxidation of the triethylammonium salt of vinylphosphonic acid was performed with H_2O_2 catalysed by Na_2WO_4 to give racemic epoxyphosphonate (±)-**4.2**. The reaction was optimised and the results can be seen in *Table 2*.

Entry	**Temperature [°C]**	**Reaction time [h]**	**Yield (%)**
1	20	72	n.d.
2	25	120	n.d.

Table 2: Optimisation of the epoxidation, n.d. = not determined.

Under the conditions given in Entry 1, the reaction mixture contained 39% of unreacted starting material as determined by ^{31}P NMR spectroscopy. When the reaction temperature and time were increased (Entry 2), no starting vinylphosphonate was left, but small amounts of side products, possibly diol and phosphate, were detected. Excess H_2O_2 was destroyed with MnO_2. The crude epoxide was regioselectively ring opened by methylamine in a S_N2 reaction at the sterically less hindered C-2 carbon atom to give the corresponding racemic 1-hydroxyphosphonate. Investigation of the reaction mixture by ^{31}P NMR spectroscopy showed that the epoxyphosphonate was completely consumed under the reaction conditions given in Entry 2. Hydroxyphosphonate (±)-**2.4** was purified by ion exchange chromatography using Dowex 50Wx8, H^+ and elution with water. At first, acidic components such as the diol derived from the epoxyphosphonic acid were eluted. Further elution with water – pH of eluate was 4-5 – yielded the desired phosphonic acid (±)-**2.4** in an overall yield of 22% after crystallisation from water/EtOH.

Incubation tests with PhnZ showed that (±)-(1-hydroxy-2-(methylamino)ethyl)phosphonic acid [(±)-**2.4**] is a substrate for this enzyme (*Table 3*, *Figure 8*, *Figure 9*). However, only half of the

***δ* [ppm] – control**	***δ* [ppm] – PhnZ**
13.37	13.38; 2.23

Table 3: Test results for compound (±)-**2.4**, left column: chemical shift for ^{31}P signal for enzyme free control, right column: chemical shift for ^{31}P signal for enzyme containing reaction mixture.

racemate, probably the (*R*)-enantiomer, was consumed, due to the fact that only the (*R*)-enantiomer of 1-OH-2-AEP (**2.2**) can be metabolised. It is surprising that **2.3** is not hydroxylated by PhnY to (±)-**2.4**, which could be metabolised by PhnZ. This might indicate that the *N*-methylated 2-AEP is degraded by a different pathway or that it is hydroxylated by a specific enzyme with a mechanism different from that of PhnY.

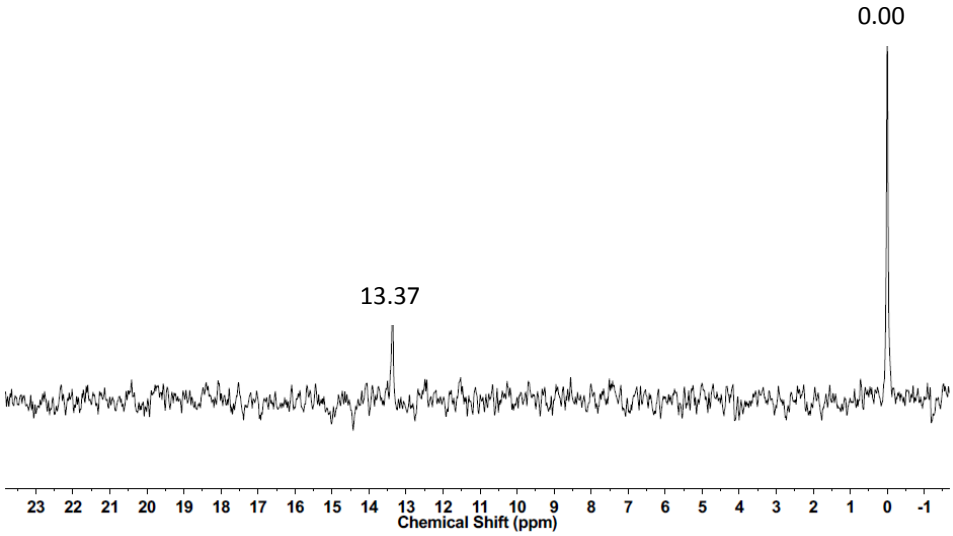


Figure 8: ^{31}P NMR spectrum of the reaction mixture containing (±)-**2.4** as substrate in the absence of PhnZ.

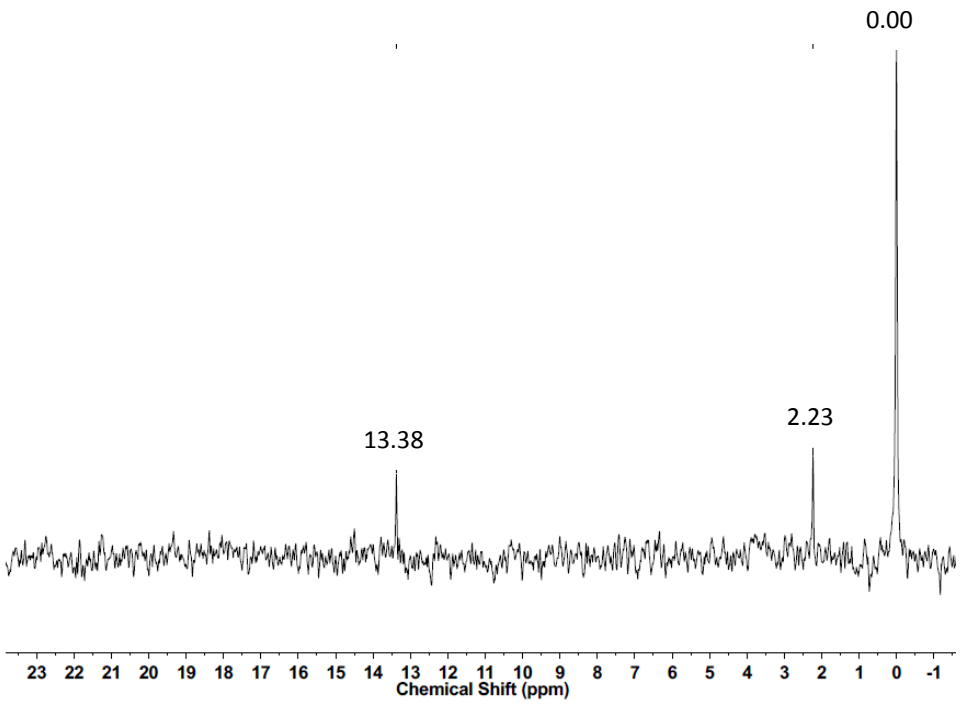
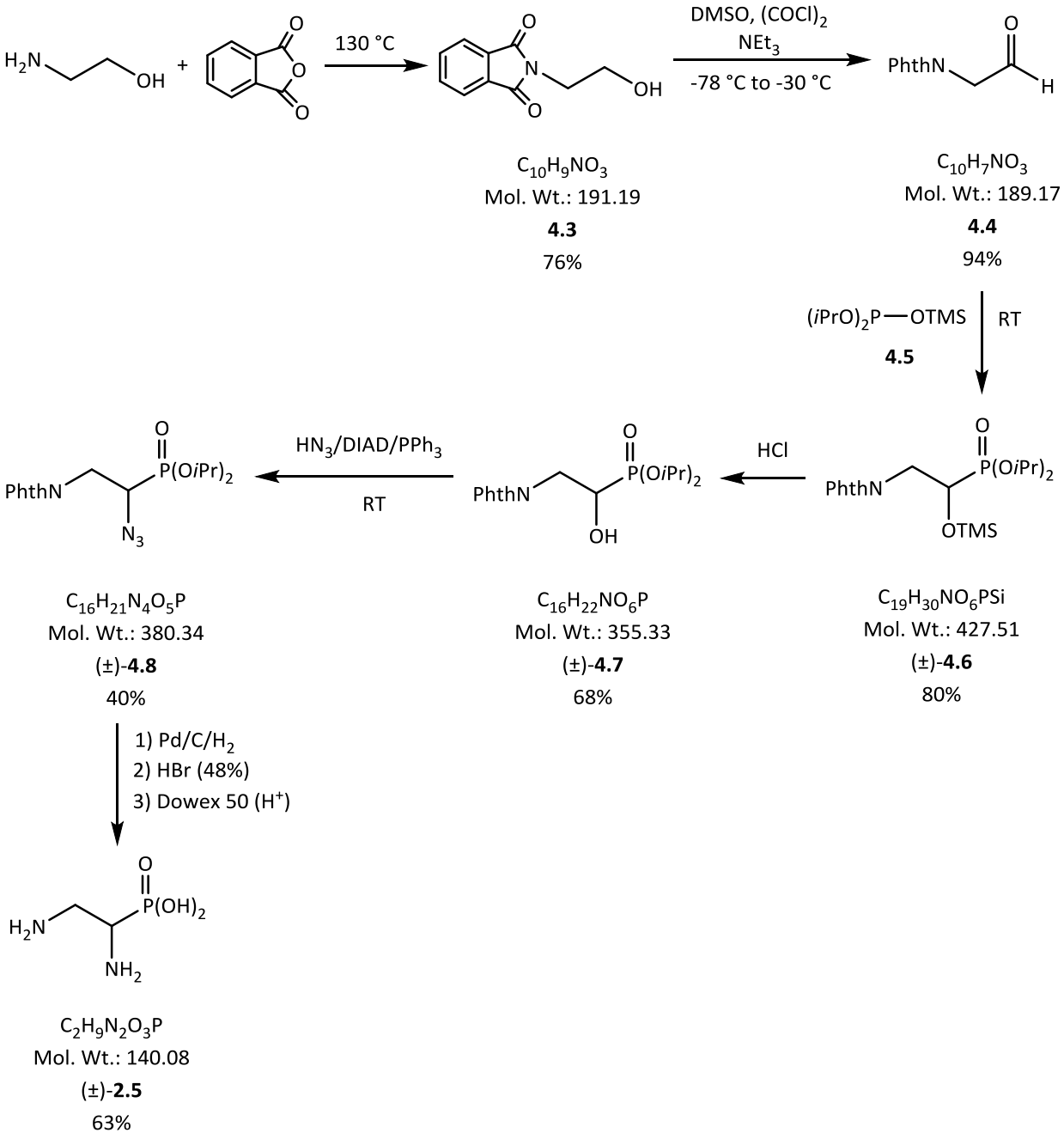


Figure 9: ^{31}P NMR spectrum of the reaction mixture containing (±)-**2.4** as substrate in the presence of PhnZ.

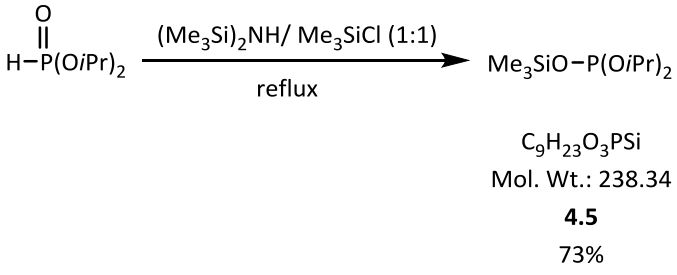
4.3. (±)-(1,2-Diaminoethyl)phosphonic acid [(±)-2.5]

(±)-(1,2-Diaminoethyl)phosphonic acid [(±)-**2.5**] was synthesised in a five step sequence starting from ethanolamine (*Scheme 31*). It is the 1-amino analogue of 1-OH-2-AEP (**2.2**), which was prepared in order to investigate whether an amino group instead of a hydroxyl group in α-position is tolerated in the substrate for PhnZ.

Ethanolamine was coupled with phthalic anhydride to form 2-phthalimidoethanol (**4.3**) to protect the amino group for the following reactions. The crude alcohol was oxidised to aldehyde **4.4** using the Swern oxidation. Excess triethylamine was removed by HCl. Alternatively, oxidation of alcohol **4.3** by DMP/CH_2Cl_2 was attempted, but failed possibly because the reagent did not dissolve in CH_2Cl_2. Subsequently, the crude aldehyde was phosphonylated to phosphonate (±)-**4.6** with diisopropyl trimethylsilyl phosphite (**4.5**) in an Abramov reaction. Chromatography had to be performed quickly because of the lability of the TMS protective group on silica gel. The silylated phosphite **4.5** should be prepared freshly and stored under exclusion of moisture (*Scheme 32*).

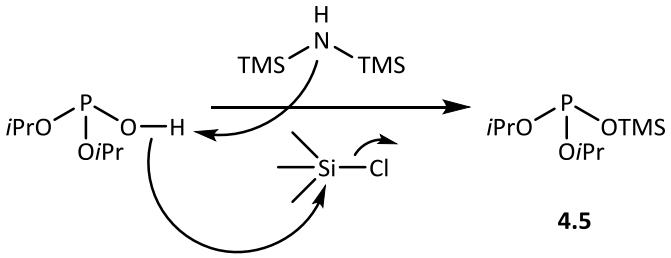


Scheme 31: Synthesis of (±)-(1,2-diaminoethyl)phosphonic acid [(±)-**2.5**].



Scheme 32: Preparation of diisopropyl trimethylsilyl phosphite (**4.5**).

The reaction mechanism for the synthesis of the silylation reagent is given in *Scheme 33*.



Scheme 33: Diisopropyl phosphite is deprotonated by HMDS. The conjugated base is then silylated with TMSCl [50]. α-Siloxy-phosphonates are stable under different conditions but easily hydrolysed. Chromatography on silica gel has to be performed quickly.

The TMS protective groups were removed by 2 M HCl. The alcohol was purified by flash chromatography and crystallisation. The following reaction was a Mitsunobu reaction to convert alcohol (±)-**4.7** to azide (±)-**4.8**, which was also purified by flash chromatography and crystallisation. The azide was reduced to the corresponding amine by catalytic hydrogenation in a Parr apparatus using a Pd/C catalyst. All protective groups were removed by refluxing 48% HBr in the last step. The product was isolated by ion exchange chromatography using Dowex 50Wx8, H^+. The column was washed with water until neutral to remove acids. The diaminophosphonic acid (±)-**2.5** was eluted with NH_3(25%)/water (1:4) and crystallised from hot water. The combustion analysis revealed that the crystalline compound contained one mole of crystal water.

Although (±)-(1,2-diaminoethyl)phosphonic acid [(±)-**2.5**] is comparable in size to 1-OH-2-AEP (**2.2**), the ^{31}P NMR spectra show that it is not accepted as substrate by PhnZ as no signal in addition to that of the substrate appeared (*Table 4*). The amino group is probably protonated and therefore

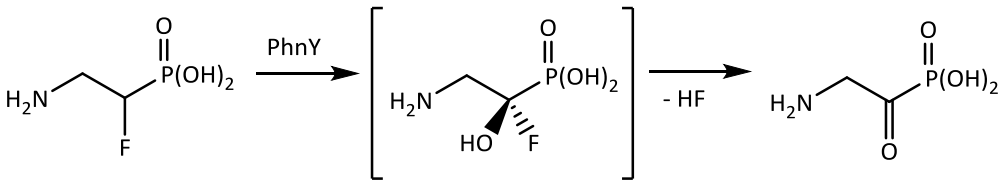
***δ* [ppm] – control**	***δ* [ppm] – PhnZ**
12.76	12.81

Table 4: Test results for compound (±)-**2.5**, left column: chemical shift for ^{31}P signal for enzyme free control, right column: chemical shift for ^{31}P signal for enzyme containing reaction mixture.

does not bind to the Fe^{3+} in the active site of the enzyme, a prerequisite for the P-C bond cleavage.

4.4. (±)-(2-Amino-1-fluoroethyl)phosphonic acid [(±)-2.6]

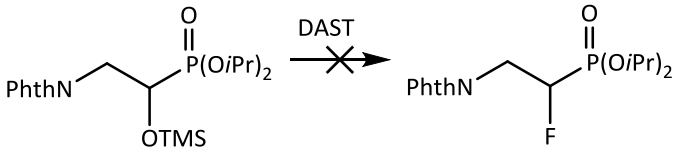
(±)-(2-Amino-1-fluoroethyl)phosphonic acid [(±)-**2.6**], an analogue of 1-OH-2-AEP (**2.2**), was synthesised in order to investigate whether a substrate with a fluorine in place of the hydroxyl group in α-position is accepted as substrate either by both PhnY and PhnZ or one of them. The compound was expected to be transformed by PhnY into unstable intermediate (*R*)-**4.9**, which will easily eliminate HF to give α-ketophosphonate **4.10** (*Scheme 34*).



(±)-**2.6** (*R*)-**4.9** **4.10**

Scheme 34: Expected metabolism of compound (±)-**2.6** by PhnY.

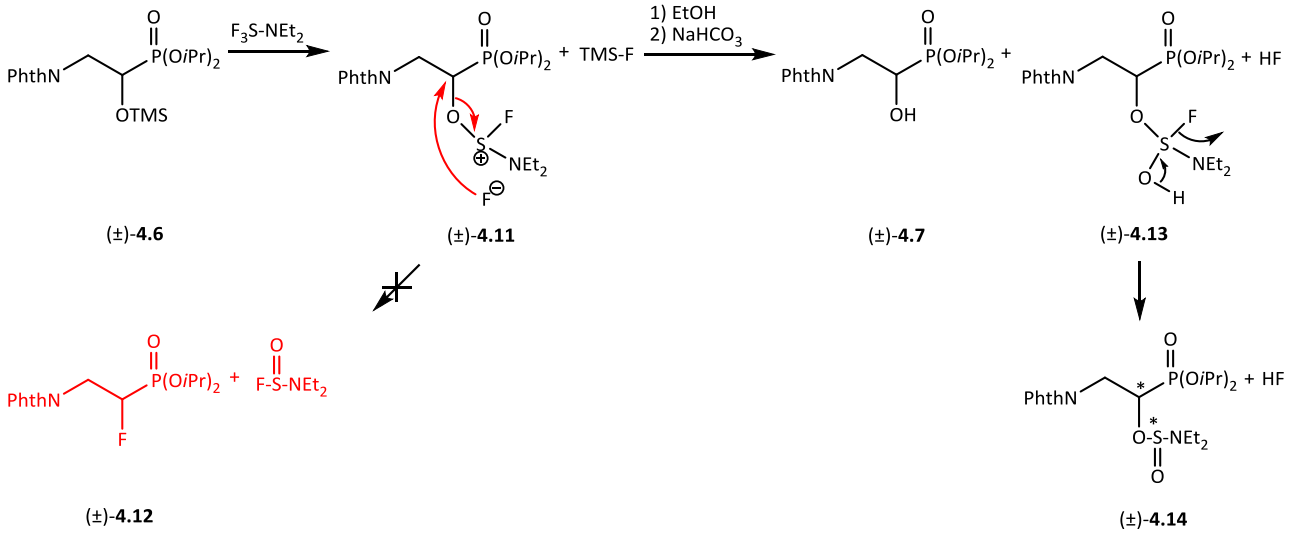
First, I tried to introduce the fluorine in the α-position of phosphonate (±)-**4.6** by substitution with DAST (*Scheme 35*). The OTMS group was considered to be a good leaving group, but the



$C_{11}H_{26}O_4PSi$
Mol. Wt.: 281.38
(±)-**4.6** (±)-**4.12**

Scheme 35: α-Fluorination by DAST.

substitution was not successful. Instead, an unexpected side product was obtained, in which DAST was bound covalently by its sulphur atom to the oxygen atom as described in the following Scheme (*Scheme 36*). The side product (±)-**4.14** was discovered by NMR spectroscopy based on the

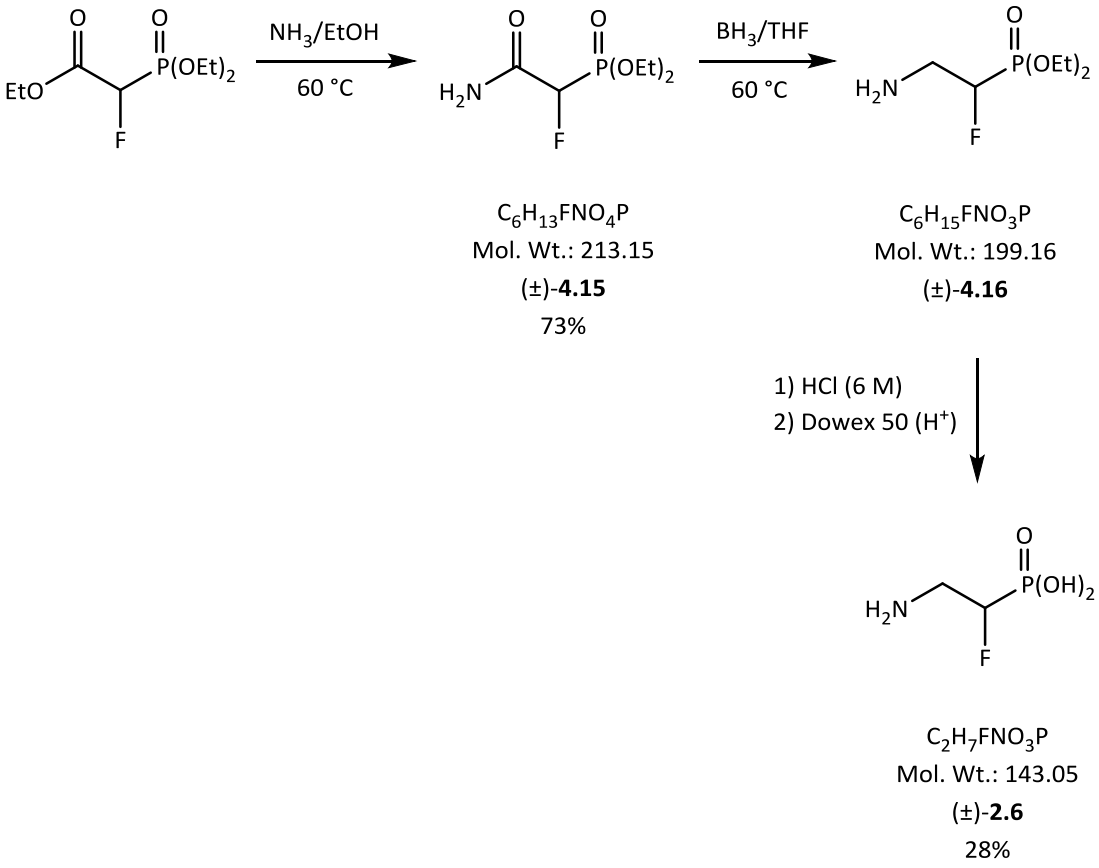


(±)-**4.6** (±)-**4.11** (±)-**4.7** (±)-**4.13**

(±)-**4.12** (±)-**4.14**

Scheme 36: Interaction mode of DAST and formation of side product.

two pairs of diastereomers caused by two chiral centres (C*, S*). Different solvents and reaction temperatures were tried, but the desired product did not form. Therefore, a different approach was attempted and was successful luckily (*Scheme 37*). (±)-(2-Amino-1-fluoroethyl)phosphonic



Scheme 37: Synthesis of (±)-(2-amino-1-fluoroethyl)phosphonic acid [(±)-**2.6**].

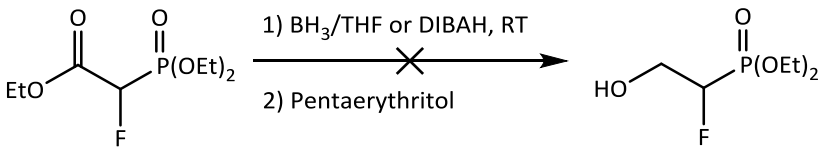
acid [(±)-**2.6**] was synthesised in a three step sequence starting from commercially available monofluorinated phosphonoacetate, which was converted into the corresponding amide (±)-**4.15** by heating with NH_3/EtOH. The first step resulted in 73% yield. The reaction was carried out in a sealed tube to avoid evaporisation of NH_3. The amide forming reaction was modified until it was successful. The modifications can be seen in *Table 5*.

Entry	Temperature [°C]	Reaction time [h]	Solvent	Yield (%)
1	110	18	NH_3/THF	-
2	160	18	NH_3/EtOH	-
3	150	2	NH_3/EtOH	-
4	100	1	NH_3/EtOH	-
5	80	2	NH_3/EtOH	-
6	60	2	NH_3/EtOH	73

Table 5: Modification of the amide forming reaction.

Amide (±)-**4.15** was reduced to amine (±)-**4.16** with BH_3·THF in dry THF. Excess borane was destroyed with glacial acetic acid. Unfortunately, the yield of the reduction was very low as judged by ^{31}P NMR spectroscopy of the crude product and could not be improved. The desired aminophosphonate (±)-**4.16** was not isolated. The crude mixture was refluxed with 6 M HCl to remove the ethyl protective groups from phosphorus. The fluorinated aminophosphonic acid (±)-**2.6** was isolated as usually by ion exchange chromatography and crystallisation, albeit in 28% overall yield.

Alternatively, I tried to reduce the fluoro ester with BH_3·THF and DIBAH to get the corresponding alcohol (±)-**4.17** for substitution of the hydroxyl by the amino group, but it did not work (*Scheme 38*) [52].



$C_6H_{14}FO_4P$
Mol. Wt.: 200.15
(±)-**4.17**

Scheme 38: Direct reduction of the fluoro ester with BH_3·THF or DIBAH.

Test results show that (±)-(2-amino-1-fluoroethyl)phosphonic acid [(±)-**2.6**] is neither hydroxylated by PhnY nor accepted as substrate by PhnZ as no signal for a reaction product appeared in the ^{31}P NMR spectra (*Table 6*). This may not be attributed to the size of fluorine comparable

***δ* [ppm] – control**	***δ* [ppm] – PhnY**
8.49	8.48
***δ* [ppm] – control**	***δ* [ppm] – PhnZ**
8.50; 8.14	8.49; 8.14

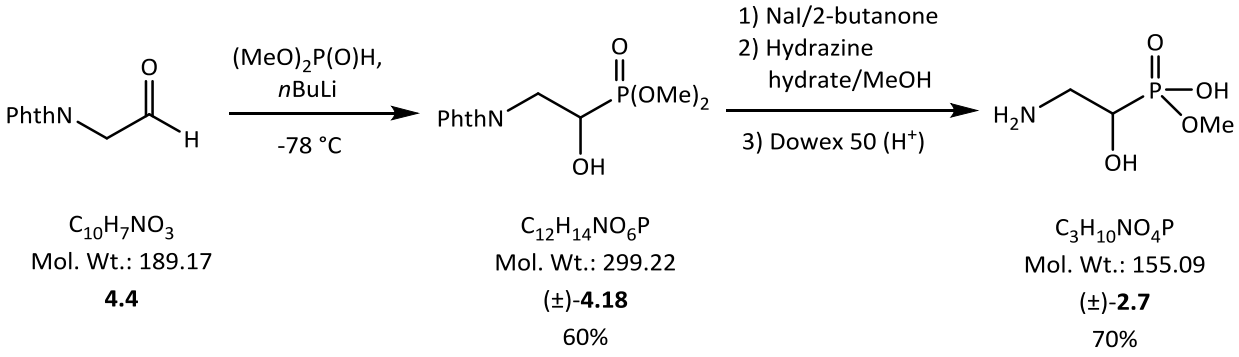
Table 6: Test results for compound (±)-**2.6**, left column: chemical shift for ^{31}P signal for enzyme free control, right column: chemical shift for ^{31}P signal for enzyme containing reaction mixture.

in size to a hydrogen atom but its electron withdrawing properties. Consequently, the phosphonic acid group will become more acidic and the amino group less basic. The latter could be decisive as the protonated amino group in 1-OH-2-AEP (**2.2**) binds to an active site residue of PhnZ.

4.5. (±)-Methyl hydrogen (2-amino-1-hydroxyethyl)phosphonate [(±)-2.7]

A phosphonic acid monoester of 1-OH-2-AEP (**2.2**) was synthesised to study whether a phosphonic acid group with just one negative charge instead of two is still accepted as substrate by PhnZ. To

keep the steric influence of the alkyl group on one of the oxygen atoms as small as possible, the methyl group was selected. Therefore, (±)-methyl hydrogen (2-amino-1-hydroxyethyl)phosphonate [(±)-**2.7**] was prepared in two steps (*Scheme 39*).



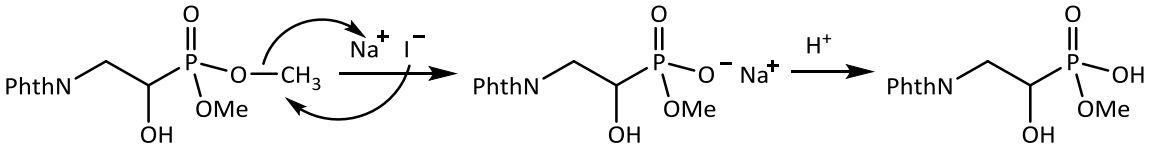
$C_{10}H_7NO_3$
Mol. Wt.: 189.17
4.4

$C_{12}H_{14}NO_6P$
Mol. Wt.: 299.22
(±)-**4.18**
60%

$C_3H_{10}NO_4P$
Mol. Wt.: 155.09
(±)-**2.7**
70%

Scheme 39: Synthesis of (±)-methyl hydrogen (2-amino-1-hydroxyethyl)phosphonate [(±)-**2.7**].

Aldehyde **4.4** was hydrophosphonylated with dimethyl phosphite in a Pudovik reaction catalysed by *n*BuLi as base. The α-hydroxyphosphonate (±)-**4.18** was purified by crystallisation. The removal of just one methyl group was accomplished by dealkylation with sodium iodide as described in *Scheme 40*. A phosphonic acid monomethyl ester cannot be selectively generated from the



(±)-**4.18**

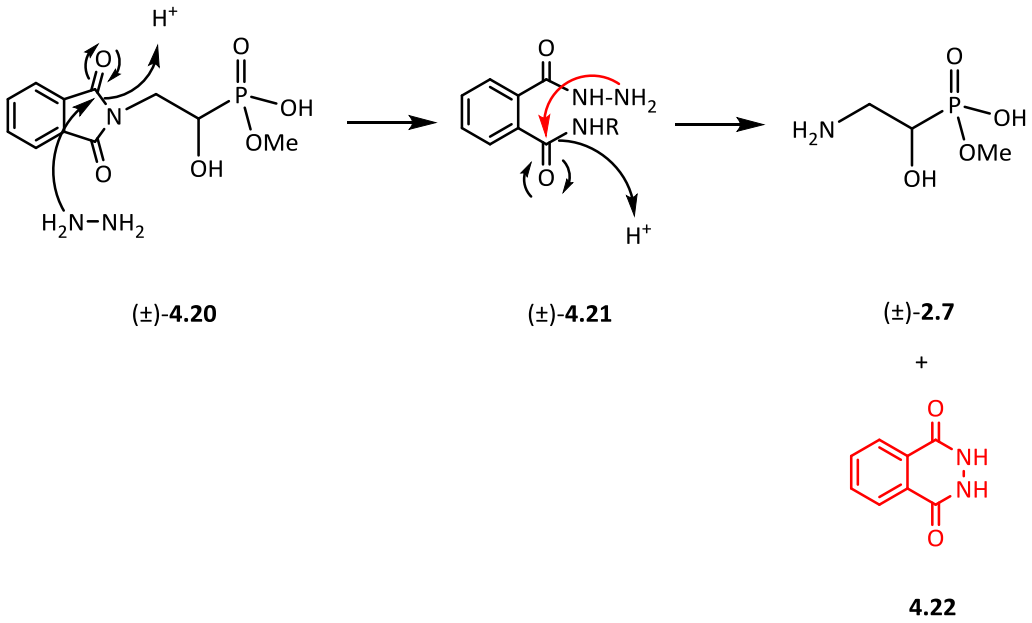
(±)-**4.19**

(±)-**4.20**

Scheme 40: Reaction mechanism of a single methyl group cleavage.

corresponding dimethyl ester by acid hydrolysis, but only by dealkylation. Monodealkylation of just methyl and benzyl esters occurs by attack of good nucleophiles such as iodide ions in polar aprotic solvents, giving the corresponding sodium salt and methyl iodide and benzyl iodide, respectively. Salts of phosphonic acid monoesters can be obtained in 60-95% yield [54], matching with the yield of the above performed conversion. The amino group was deprotected by hydrazinolysis [53]. It was difficult to separate the product from the 2,3-dihydrophthalazine-1,4-dione (**4.22**) by chromatography on Amberlite (*Scheme 41*). Several columns and large amounts of Amberlite (at least 50 mL/mmol of salt) were necessary to remove the impurities. In comparison, deblocking of the amino group with NH_3 (25%) resulted in a limited turnover despite a far longer reaction time. The sodium salt can be transformed into the phosphonic acid monoester by acidification [54]. Here, an aqueous solution of the salt was passed through a column filled with

Dowex 50Wx8, H^+. Fractions containing (±)-**2.7** were pooled, concentrated under reduced pressure and purified by crystallisation. The overall yield was 70%.



Scheme 41: Hydrazinolysis and formation of the side product 2,3-dihydrophthalazine-1,4-dione (**4.22**).

The test results show that (±)-methyl hydrogen (2-amino-1-hydroxyethyl)phosphonate [(±)-**2.7**] is not metabolised by PhnZ as no signal for a reaction product is observed in the ^{31}P NMR spectrum (*Table 7*). Possible explanations for this result are the missing second negative charge at the

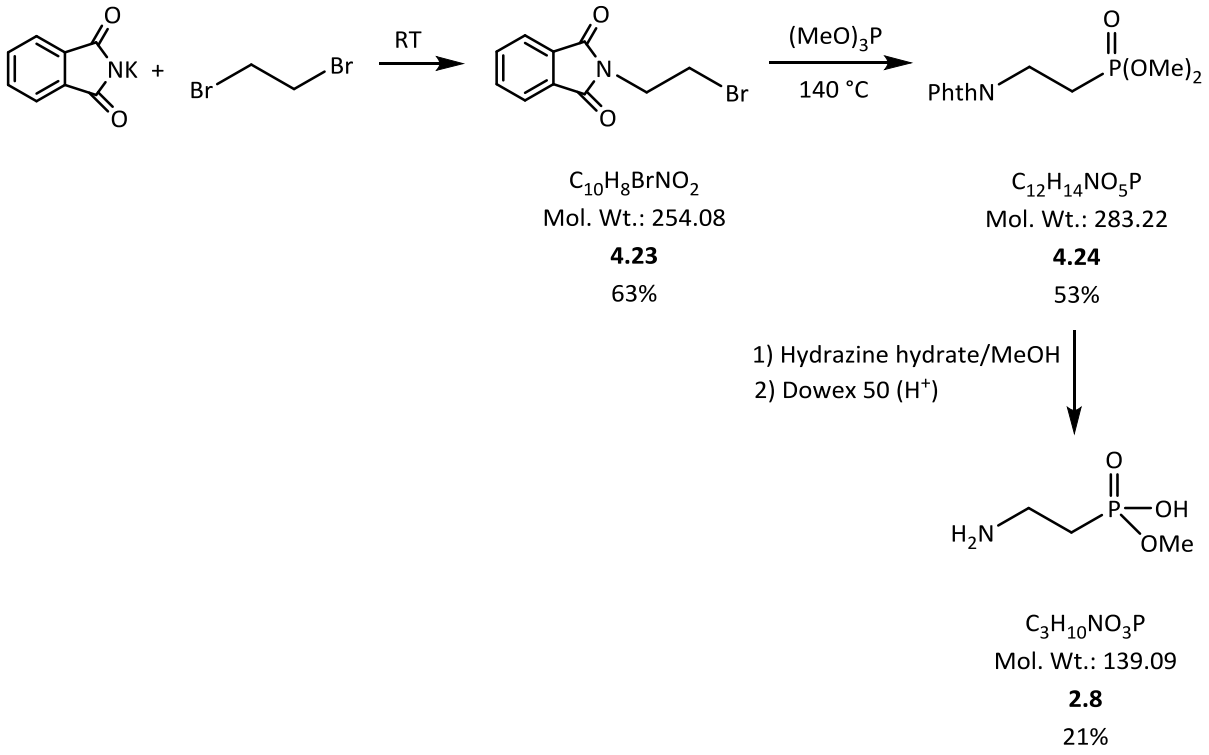
***δ* [ppm] – control**	***δ* [ppm] – PhnZ**
17.80	17.79

Table 7: Test results for compound (±)-**2.7**, left column: chemical shift for ^{31}P signal for enzyme free control, right column: chemical shift for ^{31}P signal for enzyme containing reaction mixture.

phosphonate group or the steric requirements of the methyl group compared to 1-OH-2-AEP (**2.2**).

4.6. Methyl hydrogen (2-aminoethyl)phosphonate (2.8)

A phosphonic acid monoester of 2-AEP (**2.1**) was synthesised to investigate whether a phosphonic acid with just one negative charge instead of two on the phosphonate group is accepted as substrate by PhnY. Therefore, methyl hydrogen (2-aminoethyl)phosphonate (**2.8**) was synthesised in a three step sequence (*Scheme 42*).



Scheme 42: Synthesis of methyl hydrogen (2-aminoethyl)phosphonate (**2.8**).

Potassium phthalimide was *N*-alkylated with 1,2-dibromoethane to yield bromide **4.23**, which was purified by flash chromatography and crystallisation. It was converted to phosphonate **4.24** by an Arbuzov reaction with excess trimethyl phosphite. The components were combined and heated in a round bottomed flask fitted with an open air condenser to allow low boiling methyl bromide to escape (Entries 1 and 2, *Table 8*). Under these conditions air could enter and oxidise phosphite to phosphate. When the air condenser was sealed with a septum cap and fitted with a needle for the escaping methyl bromide and when then reaction time was increased to 48 h, the yield increased to satisfying 53% (Entries 3 and 4, *Table 8*). Phosphonate **4.24** was purified by flash

Entry	Temperature [°C]	Reaction time [h]	Eq. $(MeO)_3P$	Air condenser	Yield (%)	Remarks
1	140	12	3.3	open	-	83% SM left
2	140	55	9.9	open	15	side products
3	140	24	6.0	septum+needle	44	side products
4	140	48	6.0	septum+needle	53	side products

Table 8: Optimisation of the Arbuzov reaction, SM = starting material.

chromatography and crystallisation. Removal of the side product after deprotecting the amino group by hydrazinolysis caused the same problems as described in the previous experiment. Hydrazinolysis was again the most effective method for removing the protective group. The

phosphonic acid monomethyl ester **2.8** was purified by ion exchange chromatography using Dowex 50Wx8, H^+. After crystallisation the desired compound was obtained in 21% overall yield.

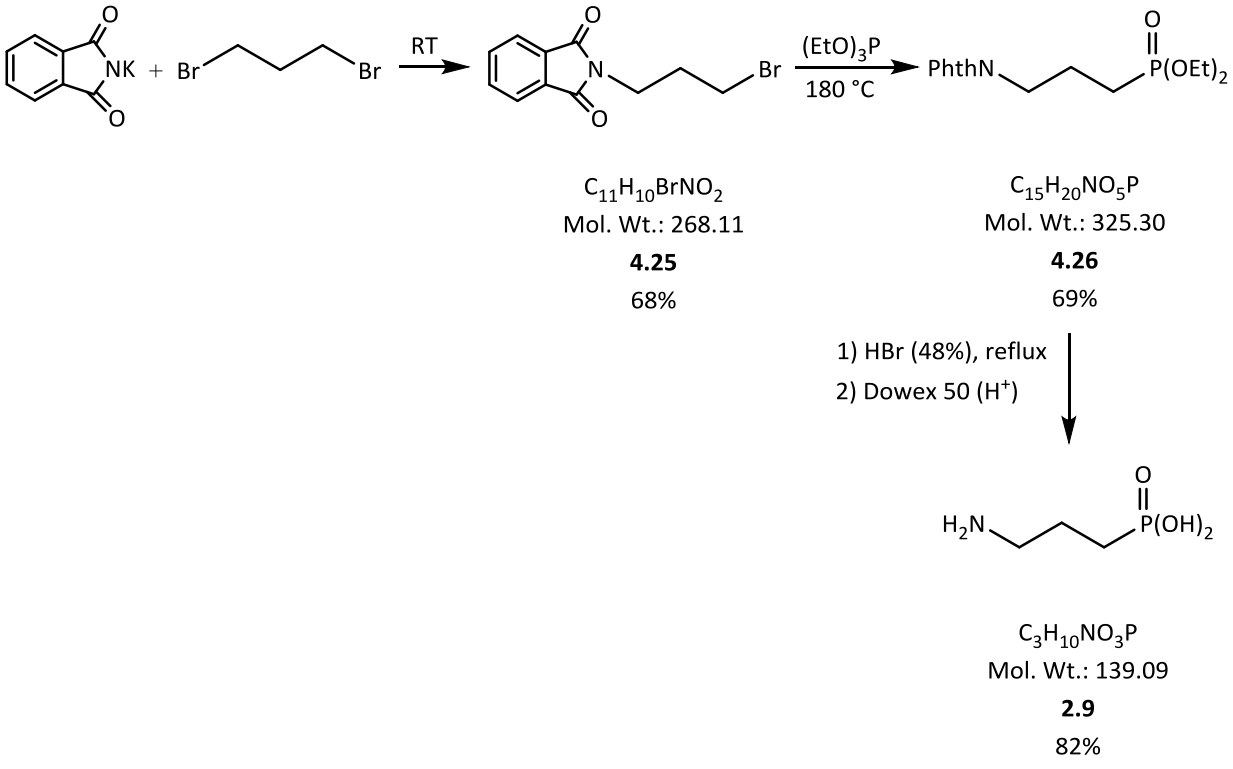
The test results show that methyl hydrogen (2-aminoethyl)phosphonate (**2.8**) is not hydroxylated by PhnY as no additional signal for a reaction product appeared in the ^{31}P NMR spectrum (*Table 9*).

δ [ppm] – control	**δ [ppm] – PhnY**
22.53	22.60

Table 9: Test results for compound **2.8**, left column: chemical shift for ^{31}P signal for enzyme free control, right column: chemical shift for ^{31}P signal for enzyme containing reaction mixture.

4.7. (3-Aminopropyl)phosphonic acid (2.9)

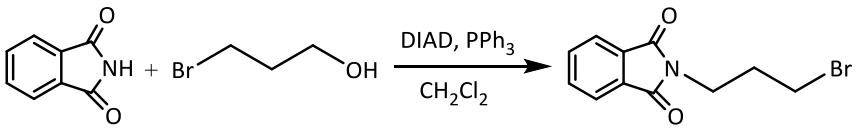
(3-Aminopropyl)phosphonic acid (**2.9**) is a homologue of 2-AEP (**2.1**) with an additional CH_2 group. The compound was synthesised to investigate whether the elongated alkyl chain can be hydroxylated by PhnY. The desired compound was prepared in a three step sequence (*Scheme 43*).



Scheme 43: Synthesis of (3-aminopropyl)phosphonic acid (**2.9**).

Potassium phthalimide was *N*-alkylated with 1,3-dibromopropane. Phthalimido-substituted propylbromide **4.25** formed by nucleophilic substitution was purified by flash chromatography and crystallisation. The substance seemed to crystallise from diisopropyl ether/hexanes only below

30 °C. Bromide **4.25** was also prepared from 3-bromopropanol and phthalimide using the Mitsunobu reaction (*Scheme 44*). The low yield of 10% may be caused by the inadequate workup.



$C_{11}H_{10}BrNO_2$
Mol. Wt.: 268.11
4.25
10%

Scheme 44: Synthesis of *N*-(3-bromopropyl)phthalimide (**4.25**) by Mitsunobu reaction.

The reaction mixture was concentrated under reduced pressure to remove CH_2Cl_2. Hexanes instead of a mixture of diethyl ether/hexanes (1:1) used normally were added to the residue to precipitate $P(O)Ph_3$ and hydrazo ester, but not the desired bromide **4.25**. Unfortunately, its solubility in hexanes is low and it was coprecipitated with the side products. Bromide **4.25** was subjected to an Arbuzov reaction with triethyl phosphite to get phosphonate **4.26**. After purifying by flash chromatography it was globally deprotected by refluxing 48% HBr. The (3-aminopropyl)-phosphonic acid (**2.9**) was purified by ion exchange chromatography using Dowex 50Wx8, H^+ and crystallisation. The phosphonic acid could not be eluted from the resin with water or 5% acetic acid, but 5% ammonia solution.

Test results show that (3-aminopropyl)phosphonic acid (**2.9**) is not hydroxylated by PhnY as no additional signal for the hydroxylated form could be observed in the ^{31}P NMR spectrum (*Table 10*).

δ [ppm] – control	**δ [ppm] – PhnY**
22.80	22.50

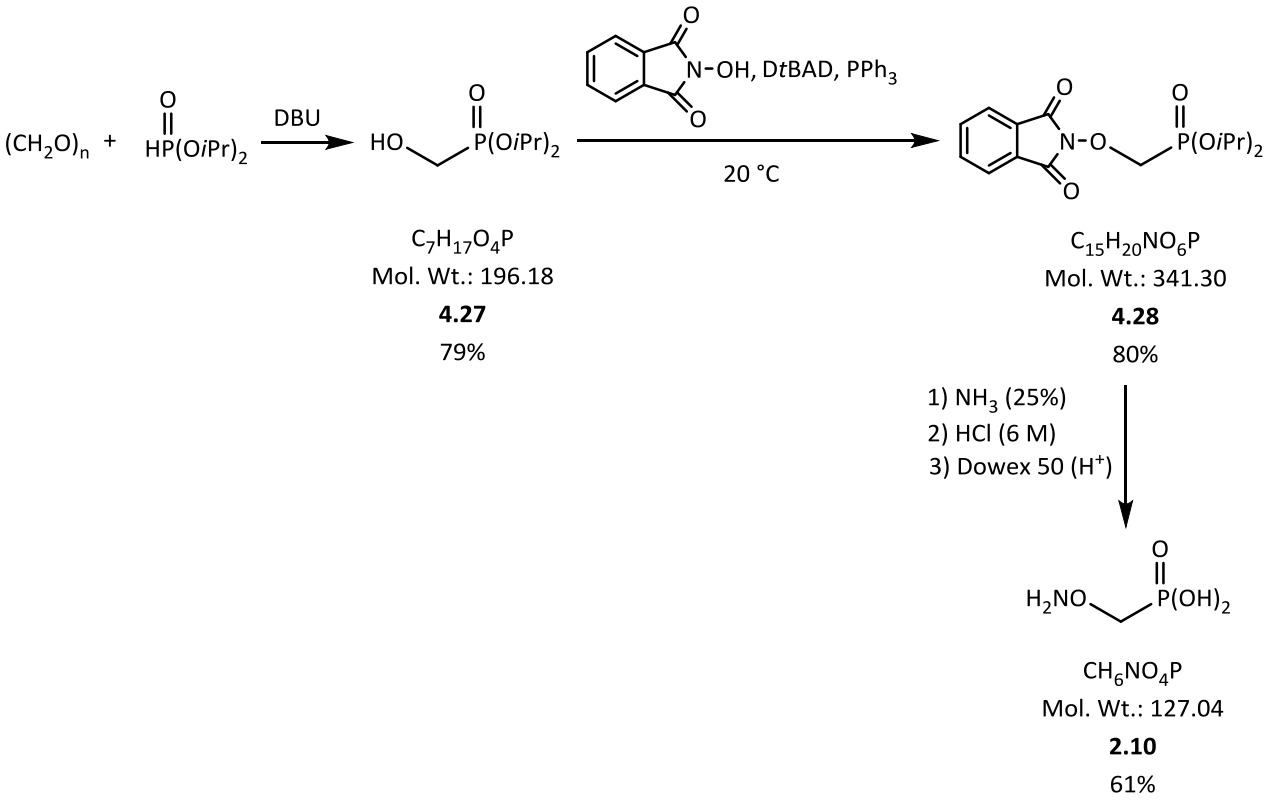
Table 10: Test results for compound **2.9**, left column: chemical shift for ^{31}P signal for enzyme free control, right column: chemical shift for ^{31}P signal for enzyme containing reaction mixture.

4.8. (Aminooxymethyl)phosphonic acid (2.10)

The carbon atom two of 2-AEP (**2.1**) was replaced by an oxygen atom to investigate whether it can be metabolised. To do so (aminooxymethyl)phosphonic acid (**2.10**) was synthesised in a three step sequence (*Scheme 45*).

Phosphonate **4.27** was generated by a Pudovik reaction from paraformaldehyde and diisopropyl phosphite in 79% yield, using DBU as base. The hydroxymethylphosphonate **4.27** was purified by bulb to bulb distillation. The following smooth Mitsunobu reaction with *N*-hydroxyphthalimide as nucleophile furnished protected aminooxyphosphonate **4.28** which was purified by flash

chromatography and crystallisation. Finally, the phthalimide protective group was removed by ammonia and the isopropyl protective groups by refluxing 6 M HCl. The aminooxyphosphonic acid **2.10** was purified as usual by ion exchange chromatography and crystallisation. The yields for the last two steps were 80% and 61%, respectively.



Scheme 45: Synthesis of (aminooxymethyl)phosphonic acid (**2.10**).

The test results show that (aminooxymethyl)phosphonic acid (**2.10**) is neither hydroxylated by PhnY nor accepted as substrate by PhnZ as no signal for a reaction product could be observed in the ^{31}P NMR spectra (*Table 11*).

δ [ppm] – control	**δ [ppm] – PhnY**
12.54	12.47
δ [ppm] – control	**δ [ppm] – PhnZ**
11.80	11.85

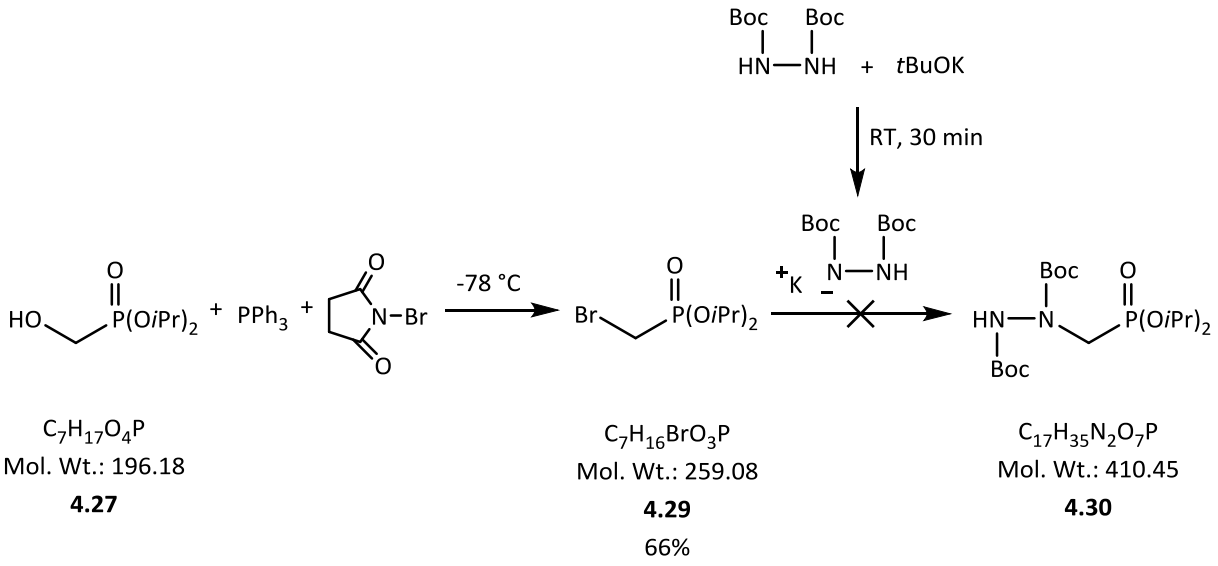
Table 11: Test results for compound **2.10**, left column: chemical shift for ^{31}P signal for enzyme free control, right column: chemical shift for ^{31}P signal for enzyme containing reaction mixture.

4.9. (Hydrazinomethyl)phosphonic acid (2.11)

The carbon atom two of 2-AEP (**2.1**) was replaced by a NH group to investigate whether the respective (hydrazinomethyl)phosphonic acid (**2.11**) can be hydroxylated by PhnY. Although it is a

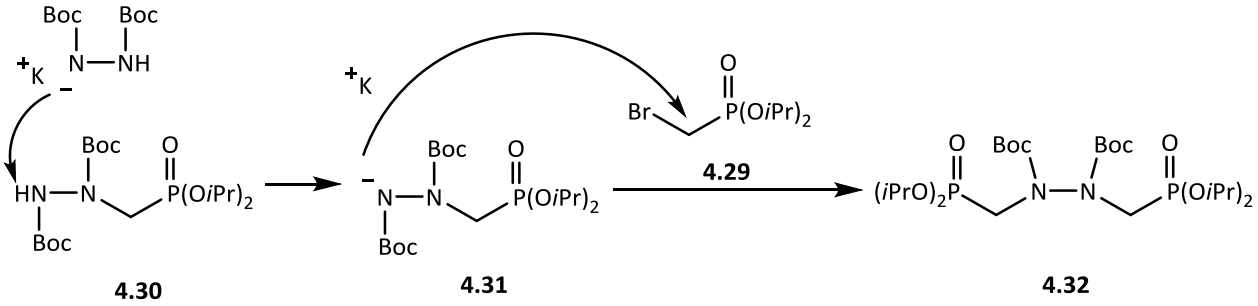
literature known compound, the preparation of this small molecule was really challenging.

In the first approach, bromomethylphosphonate **4.29** was formed in a Mitsunobu reaction and purified by bulb to bulb distillation (*Scheme 46*). Bromide despite being a good leaving group could



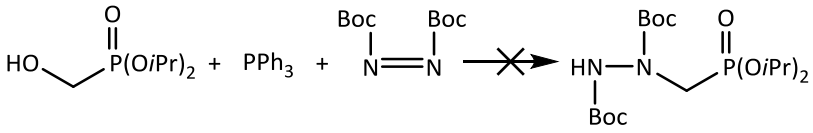
Scheme 46: Attempted preparation of protected hydrazinomethylphosphonate **4.30**.

not be substituted by the anion generated from the *N,N'*-di-Boc-substituted hydrazine and *t*BuOK [55]. For the formation of this nucleophile an excess of *t*BuOK was used to avoid the formation of diphosphonate **4.32** (*Scheme 47*).



Scheme 47: Formation of a diphosphonate side product.

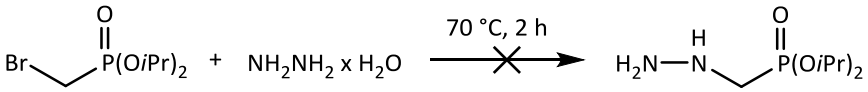
The reaction did not proceed in THF, even after addition of tetrabutylammonium iodide to substitute bromide with iodide to introduce a better leaving group. Therefore, another approach had to be developed. Direct substitution of the hydroxyl group of **4.27** with the anion derived in the Mitsunobu reaction from D*t*BAD was also not successful (*Scheme 48*). Direct substitution of bromide **4.29** with hydrazine monohydrate did not yield the desired product either (*Scheme 49*).



$C_7H_{17}O_4P$
Mol. Wt.: 196.18
4.27

$C_{17}H_{35}N_2O_7P$
Mol. Wt.: 410.45
4.30

Scheme 48: Attempted Mitsunobu reaction with D*t*BAD.

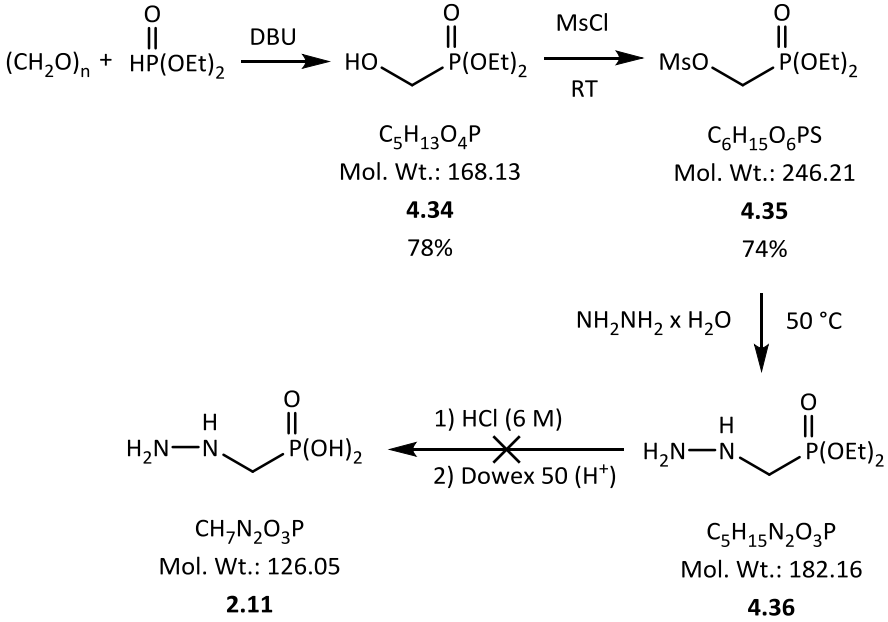


$C_7H_{16}BrO_3P$
Mol. Wt.: 259.08
4.29

$C_7H_{19}N_2O_3P$
Mol. Wt.: 210.21
4.33

Scheme 49: Attempted S_N2 substitution with hydrazine monohydrate.

As the failure was attributed to the bulky isopropyl protective groups blocking substitution of the hydroxyl group by a bulky nucleophile the ethyl analogue of **4.27** was prepared. Hydroxymethylphosphonate **4.34** was successfully reacted with mesyl chloride to form mesyloxyphosphonate **4.35**, which required only purification by extractive workup (*Scheme 50*).



$C_5H_{13}O_4P$
Mol. Wt.: 168.13
4.34
78%

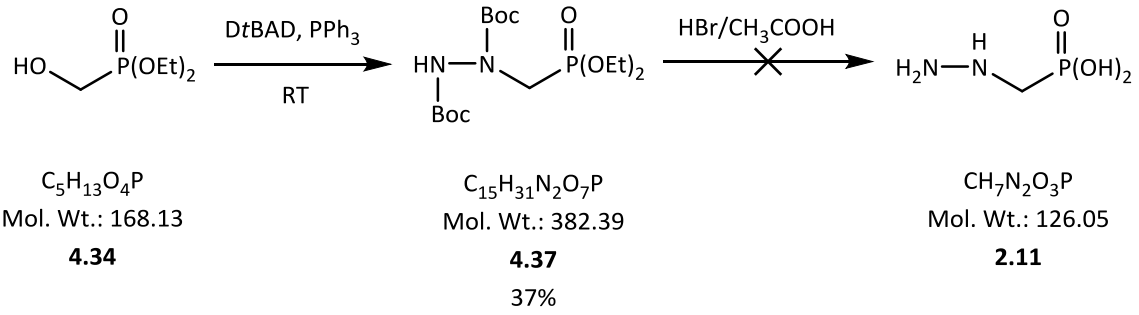
$C_6H_{15}O_6PS$
Mol. Wt.: 246.21
4.35
74%

$CH_7N_2O_3P$
Mol. Wt.: 126.05
2.11

$C_5H_{15}N_2O_3P$
Mol. Wt.: 182.16
4.36

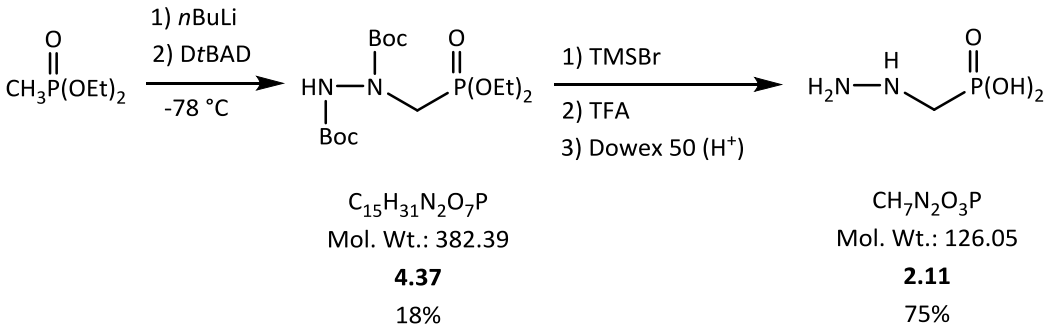
Scheme 50: Attempted synthesis of (hydrazinomethyl)phosphonic acid (**2.11**) via diethyl (hydrazinomethyl)-phosphonate (**4.36**).

The substitution with hydrazine hydrate [85] followed by deprotection was unfortunately unsuccessful again. Mitsunobu reaction of hydroxymethylphosphonate **4.34** with D*t*BAD worked in low yield, but deprotection with HBr in AcOH gave a *N*-monoacetylated derivative of the desired product (*Scheme 51*). This method was thus unsuitable for the preparation of **2.11**. The ^{31}P NMR



Scheme 51: Synthesis of diethyl (*N,N'*-di-Boc-hydrazinomethyl)phosphonate (**4.37**).

spectrum of Boc-protected phosphonate **4.37** recorded at 25 °C showed two signals, but only one, when the spectrum was recorded at 80 °C, indicating the presence of two conformers. Finally, hydrazinophosphonate **4.37** was obtained by an alternative approach using lithiated methylphosphonate and D*t*BAD (*Scheme 52*). Although the yield was worse than before,



Scheme 52: Synthesis of (hydrazinomethyl)phosphonic acid (**2.11**).

purification by flash chromatography was much easier. Global deprotection was successful with TMSBr [56] and TFA and gave phosphonic acid **2.11**. TFA was used to guarantee removal of the Boc protective groups, which might have survived TMSBr. Ion exchange chromatography on Dowex 50Wx8, H^+ and crystallisation furnished (hydrazinomethyl)phosphonic acid (**2.11**) in 75% yield.

The test results show that (hydrazinomethyl)phosphonic acid (**2.11**) is not hydroxylated by PhnY as beside the signal for the substrate no signal for a product was visible in the ^{31}P NMR spectrum (*Table 12*).

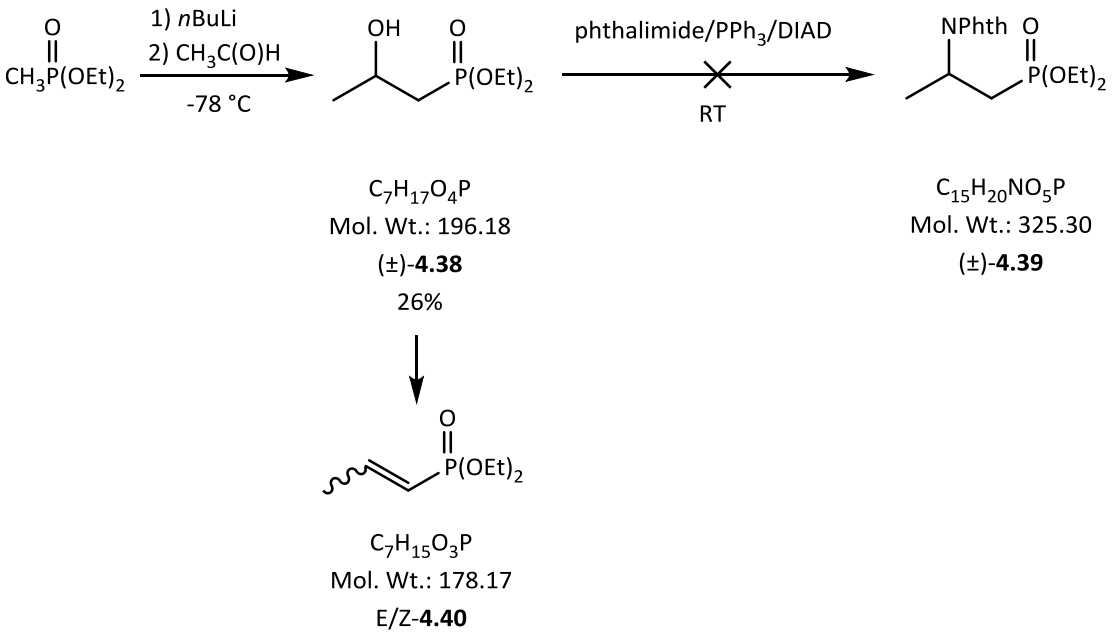
δ [ppm] – control	δ [ppm] – PhnY
13.31	13.27

Table 12: Test results for compound **2.11**, left column: chemical shift for ^{31}P signal for enzyme free control, right column: chemical shift for ^{31}P signal for enzyme containing reaction mixture.

4.10. (R)- and (S)-(2-aminopropyl)phosphonic acid [(R)- and (S)-2.12]

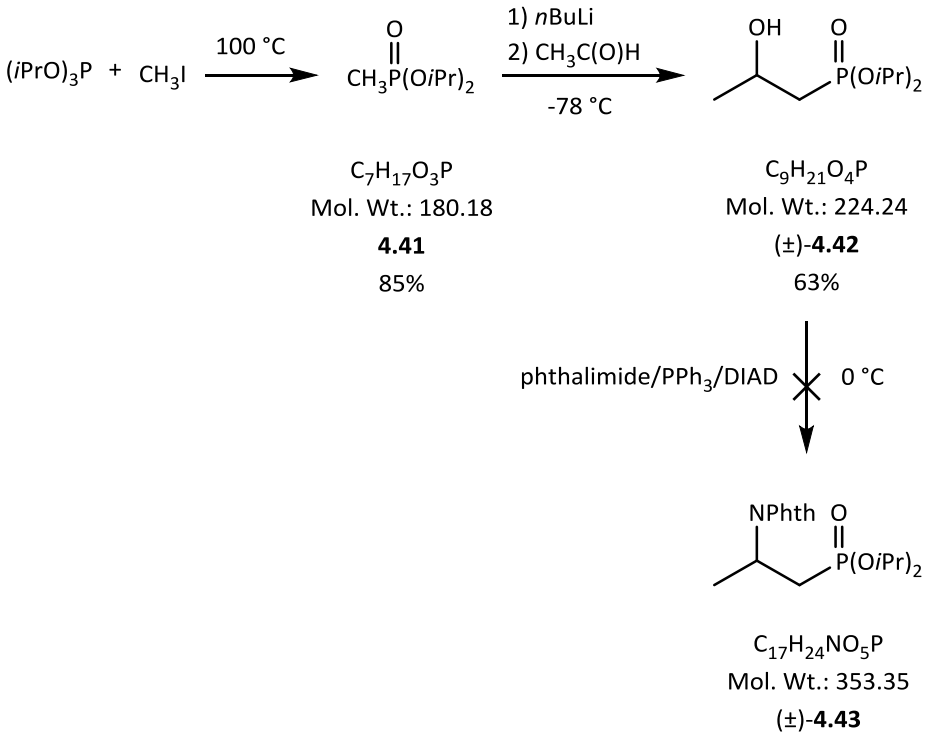
Analogues of 2-AEP (**2.1**) with a methyl group in β-position and having either (*R*)- or (*S*)-configuration, were synthesised to map the active site of PhnY. Only the enantiomers were tested, but not the racemic form. I wanted to see whether analogues of 2-AEP with a methyl group are accepted as substrates by PhnY. Furthermore, I wanted to know which enantiomer is hydroxylated more rapidly. The two enantiomers, (*R*)- and (*S*)-(2-aminopropyl)phosphonic acid [(*R*)- and (*S*)-**2.12**], were synthesised in a five step sequence (*Scheme 55*). As I envisaged separation of a racemate by HPLC on a chiral stationary phase to obtain the enantiomers, the phthalimido group was selected as substituent.

Diethyl methylphosphonate was lithiated and reacted with acetaldehyde to give hydroxyphosphonate (±)-**4.38**, which was purified by flash chromatography. The following Mitsunobu reaction with phthalimide was not successful. Only the elimination products, the *E/Z*-alkenes **4.40**, were formed (*Scheme 53*). Therefore, isopropyl protective groups were used instead



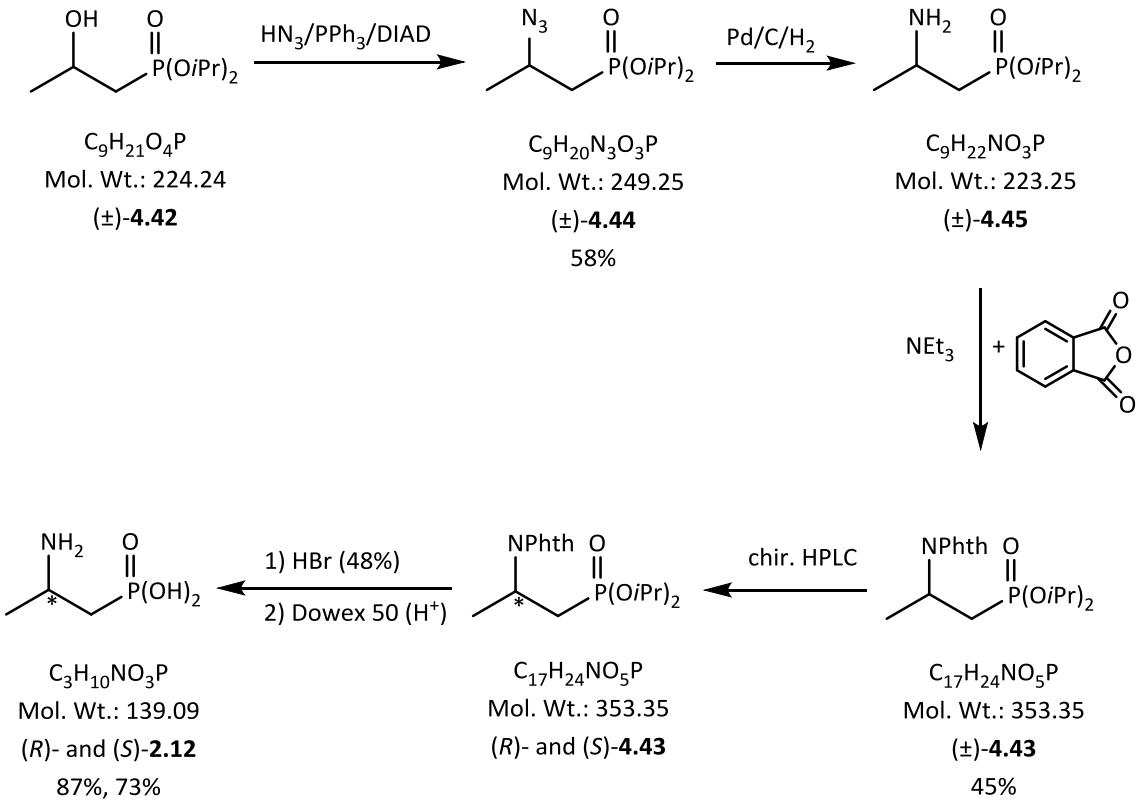
Scheme 53: Attempted synthesis of (±)-diethyl (2-phthalimidopropyl)phosphonate [(±)-**4.39**].

of ethyl groups to better shield the α-CH_2 group against deprotonation. Diisopropyl methylphosphonate (**4.41**) was prepared by an Arbuzov reaction from triisopropyl phosphite and methyl iodide, followed by purification by bulb to bulb distillation (*Scheme 54*). Lithiation and



Scheme 54: Attempted synthesis of (±)-diisopropyl (2-phthalimidoypropyl)phosphonate [(±)-**4.43**].

reaction with acetaldehyde furnished hydroxyphosphonate (±)-**4.42** in 63% yield. It was converted into the corresponding phthalimide derivative (±)-**4.43** by a Mitsunobu reaction. The desired product could not be isolated in homogeneous form but in admixture with one of the olefins and $P(O)Ph_3$. Therefore, (±)-**4.43** was synthesised via the azide (±)-**4.44**, which was obtained from hydroxyphosphonate (±)-**4.42** by a Mitsunobu reaction with HN_3. The azide was catalytically reduced to amine (±)-**4.45**. The crude ammonium hydrochloride was used for the following amide formation. It was treated with NEt_3 and phthalic anhydride in toluene to form the phthalimido derivative (±)-**4.43**. It was purified by flash chromatography and crystallisation. Resolution of the racemate by HPLC on a chiral stationary phase furnished enantiomers (*R*)- and (*S*)-**4.43** of 99% ee. Both were purified by crystallisation, globally deprotected with refluxing 48% HBr and finally purified by ion exchange chromatography and crystallisation (*Scheme 55*). The enantiomers (*R*)- and (*S*)-**2.12** were obtained in 87% and 73% yield, respectively.



Scheme 55: Synthesis of (*R*)- and (*S*)-(2-aminopropyl)phosphonic acid [(*R*)- and (*S*)-**2.12**].

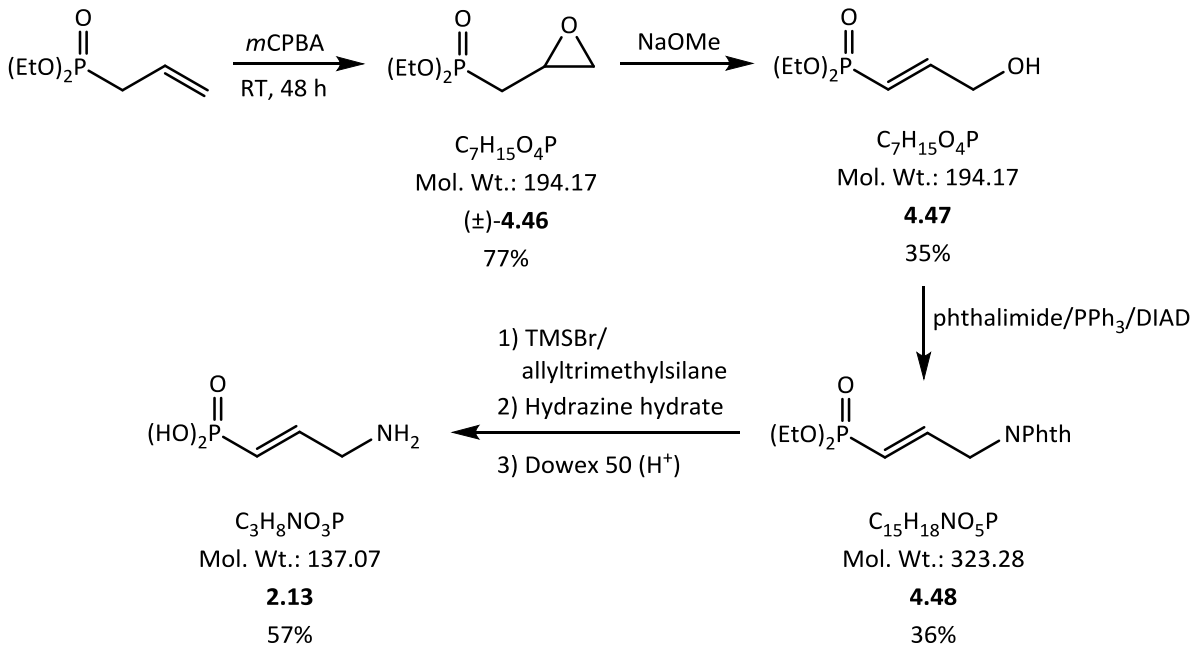
The test results of the incubation of both enantiomers with PhnY show that neither the (*R*)- nor the (*S*)-enantiomer of (2-aminopropyl)phosphonic acid (**2.12**) was hydroxylated as no signal for a reaction product could be observed in the ^{31}P NMR spectra (*Table 13*).

Enantiomer	*δ* [ppm] – control	*δ* [ppm] – PhnY
(*S*)	16.86	16.85
(*R*)	16.84	16.86

Table 13: Test results for compounds (*R*)- and (*S*)-**2.12**, left column: chemical shift for ^{31}P signal for enzyme free control, right column: chemical shift for ^{31}P signal for enzyme containing reaction mixture.

4.11. (3-Aminoprop-1-enyl)phosphonic acid (2.13)

(3-Aminoprop-1-enyl)phosphonic acid (**2.13**) was synthesised in a four step sequence and evaluated with PhnY as substrate for metabolisation (*Scheme 56*). It is known that hydroxylation normally occurs by insertion of oxygen into a C-H bond. As this way of reaction is not possible for this substrate, epoxidation is envisaged as alternative.



Scheme 56: Synthesis of (3-aminoprop-1-enyl)phosphonic acid (**2.13**).

Oxirane (±)-**4.46** was formed by epoxidation of the starting allyl phosphonate with *m*CPBA in 77% yield. The crude product was ring opened to hydroxy phosphonate **4.47** using NaOMe/MeOH. It was purified by flash chromatography and used for the following Mitsunobu reaction with phthalimide to yield phthalimidophosphonate **4.48** in low yield (36%). The product had to be purified by HPLC, because flash chromatography was not successful. Finally, the ethyl protective groups were removed with TMSBr/allyltrimethylsilane and the phthalimide protective group was cleaved with hydrazine monohydrate. Aminophosphonic acid **2.13** was purified by chromatography on Amberlite and Dowex 50Wx8, H^+ and final crystallisation.

The test results show that (3-aminoprop-1-enyl)phosphonic acid (**2.13**) is not hydroxylated as no signal for a reaction product shows up in the ^{31}P NMR spectrum (*Table 14*).

δ [ppm] – control	**δ [ppm] – PhnY**
8.45	8.43

Table 14: Test results for compound **2.13**, left column: chemical shift for ^{31}P signal for enzyme free control, right column: chemical shift for ^{31}P signal for enzyme containing reaction mixture.

4.12. Final conclusions of the test results

Small modifications were carried out to investigate the broadness of substrate specificity of PhnY and PhnZ. In nature these enzymes are yet known to accept only one organophosphonate as substrate, 2-AEP and 1-OH-2-AEP, respectively. This does not necessarily mean that there do not exist further substrates, but maybe not naturally occurring ones. It was surprising that none of the synthesised compounds was hydroxylated by PhnY and that solely (±)-(1-hydroxy-2-(methylamino)ethyl)phosphonic acid was accepted as substrate by PhnZ. This compound is not formed from (2-(methylamino)ethyl)phosphonic acid by PhnY to serve as further substrate for PhnZ. Therefore, (±)-(1-hydroxy-2-(methylamino)ethyl)phosphonic acid may coincidentally belong to the substrate broadness of PhnZ. Possibly, the substance is not a naturally occurring compound as it was not yet detected in nature. However, as *N*-methyl AEP indeed occurs in nature there may be another enzymatic way for hydroxylation than via PhnY. Its degradation pathway is yet unknown. Although initial expression problems of the enzymes could be solved the results should be dealt with caution considering the instability of the enzyme systems. Possibly, a modified experimental procedure would deliver positive results. However, it is also possible that the enzymes have a high substrate specificity and that PhnY accepts only 2-AEP as substrate, the oldest known phosphonate in the evolution history of P-C compounds. The question about substrate specificity could yet not be clarified. Further enzymatic tests will have to be performed.

5. Experimental part

5.1. General characterisation methods and determination of optical purity

All synthesised compounds were identified by NMR spectroscopy. In addition, new substances were characterised by IR spectroscopy, combustion analysis and determination of melting point. The optical purity was determined by HPLC on a chiral stationary phase. Furthermore, the specific optical rotation of chiral, nonracemic compounds was determined.

5.2. General procedures

Chemicals were obtained from Merck, Sigma-Aldrich, Fluka, Alfa-Aesar or Acros and used without further purification.

For thin layer chromatography plates (TLC Silica gel 60, F_{254}, 0.2 mm) from Merck were used. Spots were detected by UV light (254 nm, R_f*) and/or by using a staining reagent with subsequent heating with a heat gun. Phosphonates were detected with molybdate-blue containing $[(NH_4)_4Mo_7O_{24} \cdot 4\ H_2O]$ (25 g) and $[Ce(SO_4)_2 \cdot 4\ H_2O]$ (1 g) in H_2SO_4 (500 mL, 10% aqueous solution), aminophosphonic acids were detected with ninhydrin-solution (0.2% ninhydrin in EtOH 98%) or iodine. The mobile phases for TLC of phosphonates were different mixtures of organic solvents (acetone, dichloromethane, ethyl acetate, hexanes), for phosphonic acids a mixture of *i*PrOH/H_2O/NH_3(25%) (6:3:2 or 6:3:1) was used. For flash column chromatography Silica gel 60 (0.040-0.063 mm, 230-400 mesh ASTM) from Merck was used. Dowex 50Wx8-100 (H^+) from Sigma-Aldrich was used as resin for cation exchange chromatography. Further purification of phosphonic acids was carried out with Amberlite XAD-4 (nonionic polymeric adsorbent).

NMR spectra were recorded either on a Bruker AV 400 (^{1}H: 400.27 MHz, ^{13}C: 100.65 MHz, ^{31}P: 162.03 MHz), DRX 400 (^{1}H: 400.13 MHz, ^{13}C: 100.61 MHz, ^{31}P: 161.98 MHz) or DRX 600 (^{1}H: 600.13 MHz, ^{13}C: 150.92 MHz, ^{31}P: 242.92 MHz), unless given otherwise. The spectra were referenced to: δ_H = 7.24 ($CHCl_3$), δ_H = 4.80 (HOD), δ_H = 4.78 (CD_3OH), δ_H = 2.05 (toluene-d_8 CHD_2) and δ_C = 77.00 ($CDCl_3$), δ_C = 21.40 (toluene-d_8). ^{31}P NMR chemical shifts in the spectra obtained by the group of Prof. David Zechel are referenced to internal 85% phosphoric acid. Chemical shifts (δ) are given in ppm and coupling constants (J) in Hz.

IR spectra of analytically pure samples were measured on a Bruker VERTEX 70 IR spectrometer in ATR mode. Wave numbers of the most intensive bands are given in cm^{-1}.

Optical rotations were measured on a Perkin-Elmer 341 polarimeter at 589 nm (NaD-line) in a 1 dm cell at ambient temperature (~20 °C). The specific optical rotations were calculated according to the following equation:

$$[\alpha]_D^T = \frac{\alpha_{D\ measured}\ [°] \times 100}{l\ [dm] \cdot c\ [g/100\ mL]}$$

Melting points of crystalline compounds were measured either on a Leica Galen III Reichert Thermovar or on a Büchi B-540 and are uncorrected.

Analytical HPLC was performed on a SHIMADZU system (pump: LC-20AT, solenoid valves: LPGE unit, column oven: CTO-20AC, system controller: CBM-20A, UV detector: SPD-20A, auto sampler: SIL-20AHT). Preparative HPLC was also run on a SHIMADZU system (pump: LC-8A, rotary valves: FCV-20AH$_2$, system controller: CBM-20A, UV detector: SPD-20A, auto sampler: FRC-10A). Characteristics of the used columns are given each time in the experimental part.

Dry THF was obtained by refluxing over potassium and distillation before use.

5.3. Enzyme tests with PhnY and PhnZ

The synthesised compounds given in *Figure 1* were tested as enzyme substrates for PhnY/PhnZ.

Reactions catalysed by PhnY were carried out by the group of Prof. Zechel as follows:

Reaction mixture:	substrate	1	mM	
	$Fe(SO_4)_2(NH_4)_2$	0.1	mM	
	α-KG	2	mM	
	ascorbate	0.2	mM	
	PhnY	10	µM	in 25 mM Tris pH 7.5
Incubation:	28 °C, 240 rpm, 16 h			
Termination:	sodium dithionite	10	mM	
	EDTA	50	mM	
	D_2O	20	% (v/v)	

Reactions catalysed by PhnZ were carried out by the group of Prof. Zechel as follows:

Reaction mixture:	substrate	1	mM	
	$Fe(SO_4)_2(NH_4)_2$	0.1	mM	
	PhnZ	5	µM	in 25 mM Tris pH 7.5

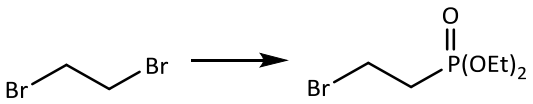
Incubation and termination as described above.

^{31}P NMR (400 MHz) spectra of the reaction mixtures with the substrate analogues used in enzyme tests were performed in presence and absence of enzyme (control).

Test results, NMR spectra and data and the above given parameters were obtained from Prof. David Zechel.

5.4. Synthesis of (2-(methylamino)ethyl)phosphonic acid (2.3)

5.4.1. Diethyl (2-bromoethyl)phosphonate (**4.1**)



4.1

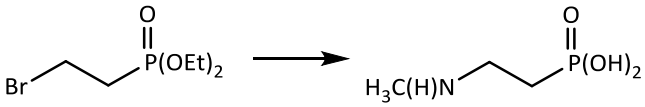
Synthesis was performed analogously to literature [57].

1,2-Dibromoethane (48.67 g, 259.1 mmol, 22.3 mL, 7.9 eq.) and triethyl phosphite (5.45 g, 32.8 mmol, 5.6 mL, 1.0 eq.) were mixed and refluxed (160 °C bath temperature) for 4 h. The crude product was purified by fractional vacuum distillation (2.69 g, 11.0 mmol, 34%; 10 mm Hg, 90 °C). A fraction of lower purity was also collected (2.52 g, 10.3 mmol, 31%; 10 mm Hg, 60-80 °C). Diethyl (2-bromoethyl)phosphonate (**4.1**) was obtained as colourless oil. Its spectroscopic data were in accordance with literature data [57, 58].

^{1}H NMR ($CDCl_3$, 400.27 MHz, 4Apr1014/60 [MV001/1/Fr4]): δ = 4.18-4.02 (m, 4H, OCH_2), 3.55-3.47 (m, 2H, CH_2Br), 2.42-2.31 (m, 2H, CH_2P), 1.32 (t, J = 7.1 Hz, 6H, CH_3).

^{31}P NMR ($CDCl_3$, 162.03 MHz, 4Apr1014/61 [MV001/1/Fr4]): δ = 25.54 (s).

5.4.2. (2-(Methylamino)ethyl)phosphonic acid (**2.3**)



4.1 **2.3**

Diethyl (2-bromoethyl)phosphonate (**4.1**) (1.21 g, 4.9 mmol, 1.0 eq.) was dissolved in EtOH (5 mL). After cooling the solution in an ice bath methylamine (1.83 g, 58.8 mmol, 2.0 mL, 12.0 eq., 40% aqueous solution) was added and stirred at 0 °C for 5 min and at RT for 3 h (TLC: EtOAc). Concentration under reduced pressure furnished crude diethyl (2-(methylamino)ethyl)phosphonate as colourless oil, which was refluxed (120 °C bath temperature) with HCl (20 mL, 6 M) for 24 h. After concentrating the solution under reduced pressure, the residue was dried in a vacuum desiccator over KOH. The crystalline product was dissolved in water and applied to a column (Ø1x24 cm) filled with Dowex 50Wx8, H^+ for ion exchange chromatography with water as eluent. Ninhydrin-positive fractions (TLC: R_f = 0.25, iPrOH/H_2O/NH_3(25%) 6:3:2) were pooled and concentrated under reduced pressure. The residue was crystallised from water/EtOH to give (2-(methylamino)ethyl)phosphonic acid (**2.3**) as white solid (0.41 g, 2.9 mmol, 59%); mp. 275-278 °C (lit. [59] 290-291 °C). The spectroscopic data were in accordance with literature data [59, 60].

^{1}H NMR (D_2O, 400.27 MHz, Apr3014/20 [MV001/2/rein]): δ = 3.33-3.24 (m, 2H, CH_2N), 2.79 (s, 3H, CH_3N), 2.09-1.98 (m, 2H, CH_2P).

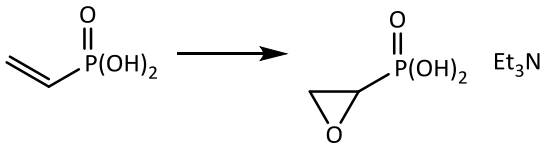
^{31}P NMR (D_2O, 161.98 MHz, Apr3014/22 [MV001/2/rein]): δ = 19.48 (s).

^{13}C NMR (D_2O, 100.61 MHz, Apr3014/21 [MV001/2/rein]): δ = 45.30 (s, 1C, CH_2N), 32.89 (s, 1C, CH_3N), 24.66 (d, J = 131.9 Hz, 1C, CH_2P).

IR (ATR, [MV001-2-rein]): ν = 3099, 2880, 2687, 1679, 1511, 1255, 1132, 1066, 975 cm^{-1}.

5.5. Synthesis of (±)-(1-hydroxy-2-(methylamino)ethyl)phosphonic acid [(±)-2.4]

5.5.1. (±)-(Epoxyethyl)phosphonic acid, triethylammonium salt [(±)-**4.2**]

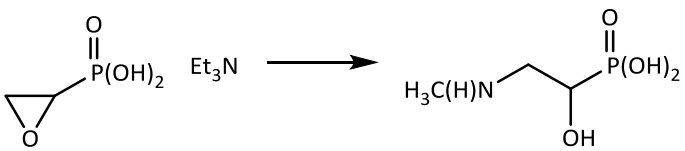


(±)-**4.2**

Synthesis was performed analogously to literature [31].

After dissolving vinylphosphonic acid (4.00 g, 37.0 mmol, 1.0 eq., 90% in water) in isopropyl alcohol (18 mL) triethylamine (4.49 g, 44.4 mmol, 6.2 mL, 1.2 eq.) was added at RT, followed by $K_3EDTA \cdot 2\ H_2O$ (0.089 g, 0.2 mmol), $Na_2WO_4 \cdot 2\ H_2O$ (0.627 g, 1.9 mmol) and H_2O_2 (9.5 mL, 30% in water). After stirring for 4 h, more H_2O_2 (9.5 mL, 30% in water) was added. Stirring was continued at ~25 °C for 5 d. Then, the reaction mixture was cooled in an ice bath and three spatula tips of MnO_2 were added. The mixture was stirred for 3 h to destroy excess H_2O_2. The amount of (±)-(epoxyethyl)phosphonic acid triethylammonium salt [(±)-**4.2**] was determined by NMR spectroscopy and compared with literature data [31]. The mixture was used in the next step without further purification.

5.5.2. (±)-(1-Hydroxy-2-(methylamino)ethyl)phosphonic acid [(±)-**2.4**]



(±)-**4.2** (±)-**2.4**

Synthesis was performed analogously to literature [31].

To the epoxide solution was added excess methylamine (9.19 g, 296.0 mmol, 10.2 mL, 8.0 eq., 40% aqueous solution) at RT and stirred for 24 h. The mixture was concentrated under reduced pressure and the residue was dissolved in water (20 mL). After filtration through Celite moistened with water the filtrate was concentrated to a small volume, applied to a column (Ø2x28 cm) filled with Dowex 50Wx8, H^+ for ion exchange chromatography and eluted with water. Ninhydrin-positive fractions (TLC: R_f = 0.12, *i*PrOH/H_2O/NH_3(25%) 6:3:2) were pooled and concentrated under reduced pressure. The residue was crystallised from water/EtOH to give (±)-(1-hydroxy-2-(methylamino)ethyl)phosphonic acid [(±)-**2.4**] as a white solid (1.13 g, 7.3 mmol, 22%); mp. 240-245 °C.

^{1}H NMR (D_2O+NaOD, 400.13 MHz, May0914/20 [MV002/4/rein2]): δ = 3.99 (dt, J_{HH} = 3.3 Hz, J_{HH} = J_{HP} = 10.0 Hz, 1H, CHP), 3.33 (AB part of an ABXP system, J_{AB} = 13.1 Hz, J_{AX} = 3.3 Hz, J_{AP} = 6.5 Hz, J_{AB} = 13.1 Hz, J_{BP} = 6.1 Hz, J_{BX} = 10.0 Hz, 2H, CH_2N), 2.82 (s, 3H, CH_3N).

^{31}P NMR (D_2O+NaOD, 161.98 MHz, May0914/21 [MV002/4/rein2]): δ = 15.37 (s).

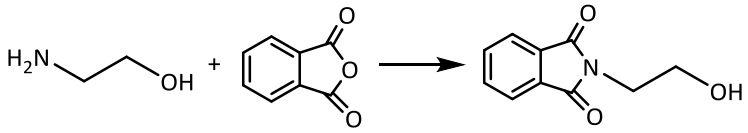
^{13}C NMR (D_2O+NaOD, 100.61 MHz, May0914/22 [MV002/4/rein2]): δ = 65.26 (d, J = 150.4 Hz, 1C, CHP), 51.56 (d, J = 8.0 Hz, 1C, CH_2N), 33.20 (s, 1C, CH_3N).

IR (ATR, [MV002-4-rein]): ν = 3193, 2997, 2705, 2460, 1632, 1481, 1227, 1122, 997 cm^{-1}.

Anal. calc. for $C_3H_{10}NO_4P$: C 23.23%, H 6.50%, N 9.03%, O 41.26%; found: C 23.04%, H 6.11%, N 8.92%, O 41.23%.

5.6. Synthesis of (±)-(1,2-diaminoethyl)phosphonic acid [(±)-2.5]

*5.6.1. N-(2-Hydroxyethyl)phthalimide (**4.3**)*



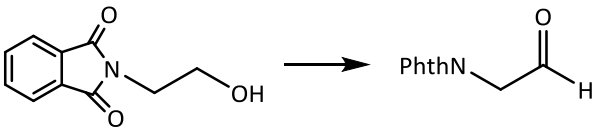
4.3

Synthesis was performed analogously to literature [61, 62].

In an open flask phthalic anhydride (10.0 g, 67.5 mmol, 1.0 eq.) and ethanolamine (4.95 g, 81.0 mmol, 4.9 mL, 1.2 eq.) were mixed and heated at 130 °C for 2 h. After cooling to RT the solidified melt was dissolved in hot EtOH (~40 mL). The solution was cooled to RT, giving *N*-(2-hydroxyethyl)phthalimide (**4.3**) as colourless crystals (9.81 g, 51.3 mmol, 76%).

^{1}H NMR ($CDCl_3$, 400.27 MHz, 4Apr3014/130 [MV004/5]): δ = 7.86-7.80 (m, 2H, H_{ar}), 7.73-7.67 (m, 2H, H_{ar}), 3.91-3.82 (m, 4H, CH_2N+CH_2O), 2.25 (brs, 1H, OH).

5.6.2. (2-Phthalimido)acetaldehyde (**4.4**)



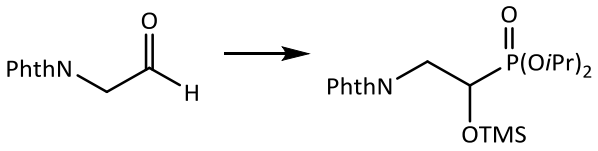
4.3 **4.4**

Synthesis was performed analogously to literature [63 ,64].

A round bottomed flask was loaded with a solution of oxalyl chloride (2.28 g, 18.0 mmol, 1.5 mL, 1.2 eq.) in dry CH_2Cl_2 (25 mL) under argon atmosphere. After cooling to -78 °C a solution of dry DMSO (2.81 g, 36.0 mmol, 2.6 mL, 2.4 eq.) in dry CH_2Cl_2 (20 mL) was added dropwise, followed by a solution of *N*-(2-hydroxyethyl)phthalimide (**4.3**) (2.87 g, 15.0 mmol, 1.0 eq.) in dry CH_2Cl_2 (60 mL) 15 min later. Stirring of the mixture was continued for 20 min, until a solution of triethylamine (4.10 g, 40.5 mmol, 5.6 mL, 2.7 eq.) was added dropwise. The reaction mixture was warmed to -30 °C within 2 h (TLC: R_f = 0.59*, CH_2Cl_2/EtOAc 5:1) before HCl (20 mL, 2 M) was added. After warming the mixture to RT, the layers were separated. The aqueous layer was extracted several times with CH_2Cl_2. The combined organic layers were washed with water, dried (Na_2SO_4), filtered and concentrated under reduced pressure to give (2-phthalimido)acetaldehyde (**4.4**) as a yellow solid (2.66 g, 14.1 mmol, 94%). Its spectroscopic data were in accordance with literature data [63]. The substituted acetaldehyde **4.4** was used in the next step without further purification.

^{1}H NMR ($CDCl_3$, 400.27 MHz, 4May0714/200 [MV004/6/Swern]): δ = 9.64 (s, 1H, CHO), 7.91-7.85 (m, 2H, H_{ar}), 7.77-7.72 (m, 2H, H_{ar}), 4.54 (s, 2H, CH_2).

5.6.3. (±)-Diisopropyl (2-phthalimido-1-(trimethylsiloxy)ethyl)phosphonate [(±)-**4.6**]



4.4 (±)-**4.6**

Synthesis was performed analogously to literature [64].

A round bottomed flask was loaded with a solution of (2-phthalimido)acetaldehyde (**4.4**) (0.83 g, 4.4 mmol, 1.0 eq.) in dry CH_2Cl_2 (13 mL) under argon atmosphere. After cooling to 0 °C, a solution of diisopropyl trimethylsilyl phosphite (**4.5**) (1.26 g, 5.3 mmol, 1.2 eq.) in dry CH_2Cl_2 (5.3 mL) was added dropwise. The colourless reaction mixture was stirred at RT for 30 min (TLC: hexanes/EtOAc 2:1), concentrated under reduced pressure and purified by flash chromatography (Ø3x25 cm, hexanes/EtOAc 3:1 → hexanes/EtOAc 1:1, TLC: R_f (3:1) = 0.11*) to give (±)-diisopropyl (2-

phthalimido-1-(trimethylsiloxy)ethyl)phosphonate [(±)-**4.6**] as colourless oil (1.48 g, 3.5 mmol, 80%).

^{1}H NMR ($CDCl_3$, 400.13 MHz, Jun1214/30 [MV006/7/rein]): δ = 7.86-7.77 (m, 2H, H_{ar}), 7.73-7.66 (m, 2H, H_{ar}), 4.81-4.66 (m, 2H, OCH), 4.37-4.27 (m, 1H, CHP), 4.06-3.95 (m, 1H, CH_2N), 3.91-3.82 (m, 1H, CH_2N), 1.40-1.25 (overlapping d, 12H, CH_3), -0.03 (s, 9H, $SiMe_3$).

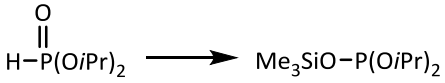
^{31}P NMR ($CDCl_3$, 161.98 MHz, Jun1214/31 [MV006/7/rein]): δ = 19.54 (s).

^{13}C NMR ($CDCl_3$, 100.61 MHz, Jun1214/32 [MV006/7/rein]): δ = 168.03 (s, 2C, CO), 134.00 (s, 2C, C_{ar}), 132.05 (s, 2C, *C*CO), 123.18 (s, 2C, C_{ar}), 71.90 (d, *J* = 6.8 Hz, 1C, OCH), 71.10 (d, *J* = 7.4 Hz, 1C, OCH), 66.40 (d, *J* = 169.9 Hz, 1C, CHP), 40.18 (d, *J* = 8.5 Hz, 1C, CH_2N), 24.34 (d, *J* = 3.2 Hz, 1C, CH_3), 24.01 (d, *J* = 4.8 Hz, 1C, CH_3), 23.99 (d, *J* = 3.6 Hz, 1C, CH_3), 23.76 (d, *J* = 5.3 Hz, 1C, CH_3), -0.18 (s, 3C, $SiMe_3$).

IR (ATR, [MV006-7]): ν = 3278, 2979, 2937, 1774, 1710, 1468, 1396, 1251, 1083, 977 cm^{-1}.

Anal. calc. for $C_{19}H_{30}NO_6PSi$: C 53.38%, H 7.07%, N 3.28%, O 22.45%; found: C 53.02%, H 7.07%, N 3.28%, O 22.25%.

*5.6.3.1. Diisopropyl trimethylsilyl phosphite (**4.5**)*



4.5

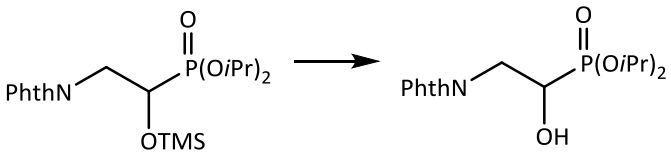
Synthesis was performed analogously to literature [64].

A mixture of diisopropyl phosphite (49.85 g, 300.0 mmol, 1.0 eq.), hexamethyl disilazane (24.21 g, 150.0 mmol, 31.0 mL, 0.5 eq.) and chlorotrimethylsilane (16.30 g, 150.0 mmol, 19.2 mL, 0.5 eq.) in hexanes (150 mL) was refluxed for 1 h under argon atmosphere. After cooling the mixture to RT it was filtered twice through Celite moistened with hexanes. Hexanes were removed under reduced pressure. Diisopropyl trimethylsilyl phosphite (**4.5**) was obtained by fractional vacuum distillation as a colourless liquid (52.02 g, 218.3 mmol, 73%; 20 mm Hg, 72-80 °C). The spectroscopic data were in accordance with literature data [64].

^{1}H NMR (toluene-d_8, 400.27 MHz, 4May1214/220 [MV005/10/Fr.2]): δ = 4.39 (dsept, J_{HH} = 6.2 Hz, J_{HP} = 9.3 Hz, 2H, OCH), 1.13 (dd, J_{HP} = 1.2 Hz, J_{HH} = 6.2 Hz, 12H, CH_3), 0.18 (s, 9H, $SiMe_3$).

^{31}P NMR (toluene-d_8, 162.03 MHz, 4May1214/221 [MV005/10/Fr.2]): δ = 128.76 (s).

5.6.4. (±)-Diisopropyl (1-hydroxy-2-phthalimidoethyl)phosphonate [(±)-**4.7**]



(±)-**4.6** (±)-**4.7**

To (±)-diisopropyl (2-phthalimido-1-(trimethylsiloxy)ethyl)phosphonate [(±)-**4.6**] (2.57 g, 6.0 mmol) was added HCl (10 mL, 2 M) and the mixture was stirred vigorously for 20 min (TLC: hexanes/EtOAc 1:1). It was extracted with EtOAc. The combined organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (Ø3x29 cm, EtOAc, TLC: R_f = 0.45) and crystallised from 1,2-dichloroethane/diisopropyl ether to give (±)-diisopropyl (1-hydroxy-2-phthalimidoethyl)phosphonate [(±)-**4.7**] as colourless crystals (1.44 g, 4.1 mmol, 68%); mp. 120 °C (lit. [31] 120-122 °C). The spectroscopic data were in accordance with literature data [31].

^{1}H NMR ($CDCl_3$, 400.13 MHz, Jun1214/40 [MV006/9/rein]): δ = 7.87-7.81 (m, 2H, H_{ar}), 7.73-7.67 (m, 2H, H_{ar}), 4.82-4.69 (m, 2H, OCH), 4.22-4.04 (m, 2H, CH_2N), 4.02-3.93 (m, 1H, CHP), 3.19 (brs, 1H, OH), 1.35 (d, *J* = 6.2 Hz, 3H, CH_3), 1.323 (d, *J* = 6.2 Hz, 3H, CH_3), 1.316 (d, *J* = 6.3 Hz, 3H, CH_3), 1.30 (d, *J* = 6.3 Hz, 3H, CH_3).

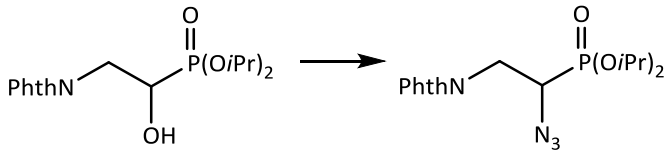
^{31}P NMR ($CDCl_3$, 161.98 MHz, Jun1214/41 [MV006/9/rein]): δ = 20.26 (s).

^{13}C NMR ($CDCl_3$, 100.61 MHz, Jun1214/42 [MV006/9/rein]): δ = 168.53 (s, 2C, CO), 134.11 (s, 2C, C_{ar}), 131.99 (s, 2C, *C*CO), 123.42 (s, 2C, C_{ar}), 71.85 (d, *J* = 3.7 Hz, 1C, OCH), 71.78 (d, *J* = 3.8 Hz, 1C, OCH), 66.70 (d, *J* = 161.7 Hz, 1C, CHP), 40.04 (d, *J* = 7.1 Hz, 1C, CH_2N), 24.11 (d, *J* = 3.0 Hz, 1C, CH_3), 24.08 (d, *J* = 3.1 Hz, 1C, CH_3), 23.93 (d, *J* = 4.8 Hz, 1C, CH_3), 23.85 (d, *J* = 4.8 Hz, 1C, CH_3).

IR (ATR, [MV006-9]): ν = 3276, 2980, 1774, 1710, 1469, 1396, 1251, 1209, 1083, 978 cm^{-1}.

Anal. calc. for $C_{16}H_{22}NO_6P$: C 54.08%, H 6.24%, N 3.94%, O 27.02%; found: C 54.17%, H 6.33%, N 3.94%, O 26.89%.

5.6.5. (±)-Diisopropyl (1-azido-2-phthalimidoethyl)phosphonate [(±)-**4.8**]



(±)-**4.7** (±)-**4.8**

Synthesis was performed analogously to literature [65].

In a round bottomed flask diisopropyl (1-hydroxy-2-phthalimidoethyl)phosphonate [(±)-**4.7**] (0.71 g, 2.0 mmol, 1.0 eq.) and triphenylphosphine (0.79 g, 3.0 mmol, 1.5 eq.) were dissolved in a mixture of dry toluene (15 mL) and dry CH_2Cl_2 (3 mL) under argon atmosphere. After cooling to 0 °C HN_3 (0.13 g, 3.0 mmol, 1.8 mL, 1.5 eq., 1.7 M solution in toluene) was added, immediately followed by DIAD (0.61 g, 3.0 mmol, 0.6 mL, 1.5 eq.). The solution was stirred at 0 °C for 30 min and then at RT for 24 h (TLC: hexanes/EtOAc 1:1). The reaction was quenched with MeOH (0.2 mL) and the solution was concentrated under reduced pressure. The residue was purified by flash chromatography (Ø3x28 cm, hexanes/EtOAc 1:1, TLC: R_f = 0.36) and crystallisation from diisopropyl ether (5 mL) gave (±)-diisopropyl (1-azido-2-phthalimidoethyl)phosphonate [(±)-**4.8**] as colourless crystals (0.30 g, 0.8 mmol, 40%); mp. 92 °C.

^{1}H NMR ($CDCl_3$, 400.27 MHz, 4Aug0514/200 [MV008/10/umkrist]): δ = 7.89-7.83 (m, 2H, H_{ar}), 7.76-7.69 (m, 2H, H_{ar}), 4.89-4.76 (m, 2H, OCH), 4.08-3.89 (m, 3H, CH_2N+CHP), 1.399 (d, *J* = 6.2 Hz, 3H, CH_3), 1.395 (d, *J* = 6.2 Hz, 3H, CH_3), 1.37 (d, *J* = 6.6 Hz, 3H, CH_3), 1.36 (d, *J* = 6.8 Hz, 3H, CH_3).

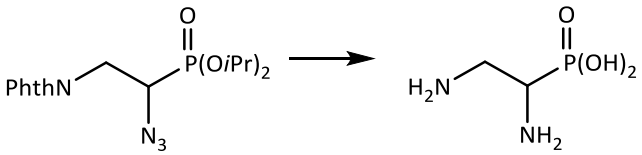
^{31}P NMR ($CDCl_3$, 162.03 MHz, 4Aug0514/201 [MV008/10/umkrist]): δ = 16.29 (s).

^{13}C NMR ($CDCl_3$, 100.65 MHz, 4Aug0514/202 [MV008/10/umkrist]): δ = 167.81 (s, 2C, CO), 134.21 (s, 2C, C_{ar}), 131.89 (s, 2C, *C*CO), 123.52 (s, 2C, C_{ar}), 72.56 (d, *J* = 2.3 Hz, 1C, OCH), 72.49 (d, *J* = 2.4 Hz, 1C, OCH), 55.49 (d, *J* = 152.5 Hz, 1C, CHP), 37.12 (d, *J* = 5.7 Hz, 1C, CH_2N), 24.16 (d, *J* = 3.4 Hz, 1C, CH_3), 24.12 (d, *J* = 3.5 Hz, 1C, CH_3), 23.96 (d, *J* = 5.2 Hz, 1C, CH_3), 23.90 (d, *J* = 5.2 Hz, 1C, CH_3).

IR (ATR, [MV008-10]): ν = 2986, 2939, 2106, 1773, 1716, 1469, 1389, 1254, 1118, 970 cm^{-1}.

Anal. calc. for $C_{16}H_{21}N_4O_5P$: C 50.53%, H 5.57%, N 14.73%, O 21.03%; found: C 50.51%, H 5.61%, N 14.58%, O 21.02%.

*5.6.6. (±)-(1,2-Diaminoethyl)phosphonic acid [(±)-**2.5**]*



(±)-**4.8** (±)-**2.5**

Synthesis was performed analogously to literature [66].

(±)-Diisopropyl (1-azido-2-phthalimidoethyl)phosphonate [(±)-**4.8**] (0.30 g, 0.8 mmol) was dissolved in dry EtOH (15 mL) and hydrogenated in a Parr apparatus for 3 h at 50 PSI (3.5 bar) using Pd(10%)/C as catalyst (90 mg). The mixture was filtered through Celite moistened with EtOH and the clear eluate was concentrated under reduced pressure. To the residue was added HBr (10 mL, 48% aqueous solution) and heated at 120 °C for 30 min. The reflux condenser was shortly removed to allow isopropyl bromide to evaporate. Then the reaction mixture was refluxed (160 °C bath temperature) for 5 h and concentrated in vacuo. The residue was dissolved in water (10 mL)

and extracted with EtOAc. The aqueous layer was concentrated and applied to a column (Ø1.5x15 cm) filled with Dowex 50Wx8, H^+, which was first washed with water until neutral and then eluted with NH_3(25%)/water 1:4. Ninhydrin-positive fractions (TLC: R_f = 0.28, *i*PrOH/H_2O/NH_3(25%) 6:3:2) were pooled and concentrated under reduced pressure. The residue was dissolved in hot water and on cooling (±)-(1,2-diaminoethyl)phosphonic acid [(±)-**2.5**] was obtained as white powder (64 mg, 0.5 mmol, 63%); decomposition at 180 °C (lit. [67] 269-271 °C). The spectroscopic data were in accordance with literature data [67].

^{1}H NMR (D_2O, 400.27 MHz, 4Sep1714/100 [MV008/11/umkrist]): δ = 3.51-3.32 (m, 3H, CH_2+CH).

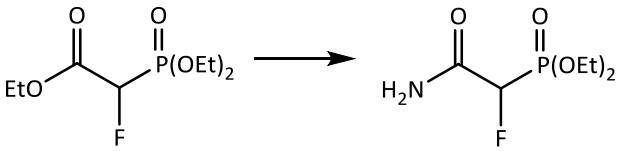
^{31}P NMR (D_2O, 162.03 MHz, 4Sep1714/101 [MV008/11/umkrist]): δ = 9.46 (s).

Anal. calc. for $C_2H_9N_2O_3P{\cdot}H_2O$: C 15.19%, H 7.01%, N 17.72%, O 40.48%; found: C 15.13%, H 6.89%, N 17.18%, O 40.65%.

The combustion analysis showed that the compound crystallised with one equivalent of crystal water per molecule.

5.7. Synthesis of (±)-(2-amino-1-fluoroethyl)phosphonic acid [(±)-2.6]

*5.7.1. (±)-Diethyl 2-fluoro-2-phosphonoacetamide [(±)-**4.15**]*



(±)-**4.15**

A solution of triethyl 2-fluoro-2-phosphonoacetate (0.99 g, 4.1 mmol) in EtOH containing ammonia (10 mL, 13 w/w%) was heated in a sealed tube at 60 °C for 2 h. The reaction mixture was cooled to RT and concentrated under reduced pressure. The residue was dissolved in dry THF, again concentrated under reduced pressure and purified by flash chromatography (Ø3x40 cm, EtOAc/acetone, TLC: R_f = 0.26, iodine for detection) to give (±)-diethyl 2-fluoro-2-phosphonoacetamide [(±)-**4.15**] as colourless oil (0.64 g, 3.0 mmol, 73%).

^{1}H NMR ($CDCl_3$, 400.27 MHz, 4Jul2314/560 [MV009/12/gross2gesFr36+37.2]): δ = 6.43 (brs, 1H, NH_2), 5.87 (brs, 1H, NH_2), 5.13 (dd, J = 11.9 Hz, J = 47.0 Hz, 1H, CHF), 4.32-4.17 (m, 4H, OCH_2), 1.360 (dt, J = 0.5 Hz, J = 6.9 Hz, 3H, CH_3), 1.357 (dt, J = 0.5 Hz, J = 6.9 Hz, 3H, CH_3).

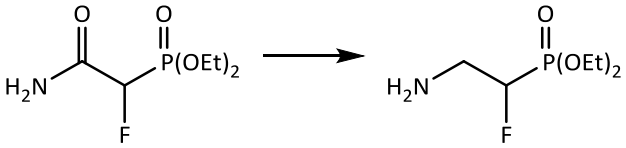
^{31}P NMR ($CDCl_3$, 162.03 MHz, 4Jul2314/561 [MV009/12/gross2gesFr36+37.2]): δ = 11.02 (d, J = 68.1 Hz).

^{13}C NMR ($CDCl_3$, 100.65 MHz, 4Jul2314/562 [MV009/12/gross2gesFr36+37.2]): δ = 166.39 (d, J = 20.4 Hz, 1C, CO), 86.31 (dd, J = 159.2 Hz, J = 198.8 Hz, 1C, CHF), 64.44 (d, J = 6.6 Hz, 1C, OCH_2), 64.33 (d, J = 6.6 Hz, 1C, OCH_2), 16.34 (d, J = 5.8 Hz, 2C, CH_3).

IR (ATR, [MV009-12]): ν = 3189, 2986, 2934, 1686, 1608, 1393, 1249, 1163, 1010 cm^{-1}.

Anal. calc. for $C_6H_{13}FNO_4P$: C 33.81%, H 6.15%, N 6.57%, O 33.02%; found: C 33.54%, H 6.02%, N 6.43%.

5.7.2. *(±)-Diethyl (2-amino-1-fluoroethyl)phosphonate [(±)-**4.16**]*

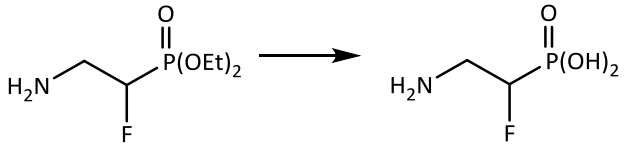


(±)-**4.15** (±)-**4.16**

Synthesis was performed analogously to literature [68].

A round bottomed flask was loaded with (±)-diethyl 2-fluoro-2-phosphonoacetamide [(±)-**4.15**] (0.38 g, 1.8 mmol, 1.0 eq.) in dry THF (1.8 mL) and cooled in a water bath. When BH_3·THF complex (0.46 g, 5.4 mmol, 5.4 mL, 3.0 eq., 1.0 M in THF) was added dropwise, gas evolved. The solution was stirred at RT for 30 min and at 60 °C for 2 h. The reaction progress was monitored by NMR spectroscopy and showed that just half of the starting material had reacted. Even under different reaction conditions the yield could not be improved. After cooling the solution in an ice bath, the reaction was quenched with glacial acetic acid (16.2 mmol, 0.9 mL, 9.0 eq.) and water (1 mL). The mixture was stirred for 5 min, when evolution of gas and a formation of a white precipitant could be observed. The mixture was concentrated under reduced pressure to give a white solid containing (±)-diethyl (2-amino-1-fluoroethyl)phosphonate [(+)-**4.16**], which was used in the next step without further purification.

5.7.3. *(±)-(2-Amino-1-fluoroethyl)phosphonic acid [(±)-**2.6**]*



(±)-**4.16** (±)-**2.6**

The crude solid containing (±)-**4.16** was refluxed (130 °C bath temperature) with HCl (15 mL, 6 M) for 24 h. The solution was concentrated under reduced pressure. The residue was applied to a column (Ø1x38 cm) filled with Dowex 50Wx8, H^+ for ion exchange chromatography with water as

eluent. At first, contaminating boric acid and HCl were eluted at pH 1, followed by the desired product at increased pH (pH ~4-5). Ninhydrin-positive fractions (TLC: R_f = 0.31, *i*PrOH/H_2O/NH_3(25%) 6:3:2) were pooled and concentrated under reduced pressure. The residue was crystallised from water/EtOH to give (±)-(2-amino-1-fluoroethyl)phosphonic acid [(±)-**2.6**] as colourless crystals (67 mg, 0.5 mmol, 28%); mp. 256 °C (lit. [69] 283-284 °C). The spectroscopic data were in accordance with literature data [69].

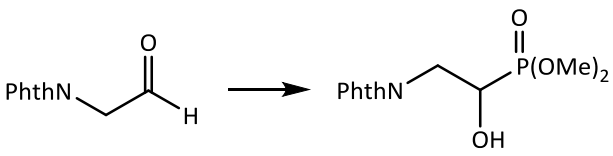
^{1}H NMR (D_2O, 400.27 MHz, 4Jul2314/30 [MV009/14/umkrist2]): δ = 4.97 (dddd, J = 2.8 Hz, J = 7.2 Hz, J = 9.7 Hz, J = 48.1 Hz, 1H, CHF), 3.65-3.42 (m, 2H, CH_2N).

^{31}P NMR (D_2O, 162.03 MHz, 4Jul2214/323 [MV009/14/umkrist]): δ = 8.49 (d, J = 62.8 Hz).

^{13}C NMR (D_2O, 100.65 MHz, 4Jul2214/324 [MV009/14/umkrist]): δ = 87.11 (dd, J = 156.2 Hz, J = 177.5 Hz, 1C, CHF), 40.39 (dd, J = 7.1 Hz, J = 19.8 Hz, 1C, CH_2N).

5.8. Synthesis of (±)-methyl hydrogen (2-amino-1-hydroxyethyl)phosphonate [(±)-2.7]

*5.8.1. (±)-Dimethyl (1-hydroxy-2-phthalimidoethyl)phosphonate [(±)-**4.18**]*



4.4 (±)-**4.18**

Synthesis was performed analogously to literature [70].

A round bottomed flask was loaded with dimethyl phosphite (0.83 g, 7.5 mmol, 0.7 mL, 1.1 eq.) and dry THF (3 mL) under argon atmosphere. After cooling the solution to -78 °C *n*BuLi (0.04 g, 0.7 mmol, 0.3 mL, 0.1 eq., 2.5 M in hexanes) was added dropwise. After stirring for 5 min, a solution of (2-phthalimido)acetaldehyde (**4.4**) (1.28 g, 6.8 mmol, 1.0 eq.) in dry THF (7 mL) was added dropwise. The cooling bath was removed and the reaction mixture was stirred for 1 h (TLC: R_f = 0.23*, EtOAc) until the reaction was quenched with HCl (10 mL, 1 M). The layers were separated and the aqueous layer was extracted three times with EtOAc (15 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was crystallised from 1,2-dichloroethane/diisopropyl ether to give (±)-dimethyl (1-hydroxy-2-phthalimidoethyl)phosphonate [(±)-**4.18**] as colourless crystals (1.22 g, 4.1 mmol, 60%); mp. 161-163 °C (lit. [70] 148-149 °C).

^{1}H NMR ($CDCl_3$, 400.27 MHz, 4Jul0414/50 [ZH891/6a/umkrist]): δ = 7.87-7.81 (m, 2H, H_{ar}), 7.74-7.68 (m, 2H, H_{ar}), 4.29 (ddd, J = 3.3 Hz, J = 8.5 Hz, J = 9.3 Hz, 1H, CHP), 4.06 (AB part of an ABXP system, J_{AB} = 14.5 Hz, J_{HP} = 7.0 Hz, J_{HH} = 9.3 Hz, J_{AB} = 14.5 Hz, J_{HH} = 3.3 Hz, J_{HP} = 7.7 Hz, 2H, CH_2N), 3.82 (d, J = 10.5 Hz, 3H, OCH_3), 3.81 (d, J = 10.5 Hz, 3H, OCH_3), 3.38 (brs, 1H, OH).

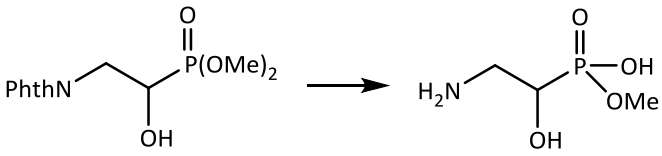
^{31}P NMR ($CDCl_3$, 162.03 MHz, 4Jul0414/51 [ZH891/6a/umkrist]): δ = 22.95 (s).

^{13}C NMR ($CDCl_3$, 100.65 MHz, 4Jul0414/52 [ZH891/6a/umkrist]): δ = 168.52 (s, 2C, CO), 134.17 (s, 2C, C_{ar}), 131.92 (s, 2C, *C*CO), 123.51 (s, 2C, C_{ar}), 66.31 (d, *J* = 161.7 Hz, 1C, CHP), 53.69 (d, *J* = 6.9 Hz, 1C, OCH_3), 53.52 (d, *J* = 7.1 Hz, 1C, OCH_3), 39.96 (d, *J* = 7.7 Hz, 1C, CH_2N).

IR (ATR, [MV010-15]): ν = 3242, 2962, 2856, 1774, 1709, 1469, 1395, 1220, 1055, 1019 cm^{-1}.

Anal. calc. for $C_{12}H_{14}NO_6P$: C 48.17%, H 4.72%, N 4.68%, O 32.08%; found: C 48.09%, H 4.46%, N 4.67%.

*5.8.2. (±)-Methyl hydrogen (2-amino-1-hydroxyethyl)phosphonate [(±)-**2.7**]*



(±)-**4.18** (±)-**2.7**

Synthesis was performed analogously to literature [71].

To a solution of (±)-dimethyl (1-hydroxy-2-phthalimidoethyl)phosphonate [(±)-**4.18**] (0.80 g, 2.7 mmol, 1.0 eq.) in 2-butanone (8 mL) was added sodium iodide (0.48 g, 3.2 mmol, 1.2 eq.). The mixture was refluxed (80 °C bath temperature) for 3 h (TLC: R_f = 0.00, EtOAc/acetone 1:1) and cooled. The crystalline residue was collected, washed with 2-butanone and dried in a vacuum desiccator. A mixture of this salt, dry MeOH (5 mL) and hydrazine monohydrate (1.19 g, 23.8 mmol, 1.2 mL, 8.8 eq.) was heated in a sealed tube at 100 °C for 21 h. After cooling, the mixture solidified. To transfer it from the sealed tube into a flask the solid was dissolved in water (15 mL). Residual hydrazine was removed under reduced pressure. Four subsequent columns filled with Amberlite XAD-4 (each 50 mL/mmol, washed neutral with water) were necessary to remove the side product 2,3-dihydrophthalazine-1,4-dione, which was difficult to get rid of. The product was eluted with water each time, concentrated and finally, applied to a column (Ø1x40 cm) filled with Dowex 50Wx8, H^+ for ion exchange chromatography with water as eluent. Ninhydrin-positive fractions (TLC: R_f = 0.70, *i*PrOH/H_2O/NH_3(25%) 6:3:2) were pooled and concentrated under reduced pressure. For crystallisation, the residue was dissolved in a minimum amount of hot water and EtOH was allowed to diffuse into the cold solution over several days to give (±)-methyl hydrogen (2-amino-1-hydroxyethyl)phosphonate [(±)-**2.7**] as white solid (0.29 g, 1.9 mmol, 70%); mp. 236-239 °C.

^{1}H NMR (D_2O, 400.27 MHz, 4Jan0715/200 [MV010/16/Wdhumkr]): δ = 4.10 (dt, *J* = 3.3 Hz, *J* = 9.9 Hz, 1H, CHP), 3.71 (d, *J* = 10.1 Hz, 3H, OCH_3), 3.32 (AB part of an ABXP system, J_{AB} = 13.3 Hz, J_{HH} = 3.3 Hz, J_{HP} = 6.6 Hz, J_{AB} = 13.3 Hz, J_{HP} = 6.1 Hz, J_{HH} = 9.9 Hz, 2H, CH_2N).

^{31}P NMR (D_2O, 162.03 MHz, 4Jan0715/201 [MV010/16/Wdhumkr]): δ = 17.64 (s).

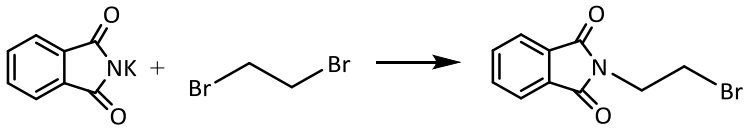
^{13}C NMR (D_2O, 100.65 MHz, 4Jan0715/202 [MV010/16/Wdhumkr]): δ = 64.32 (d, J = 157.7 Hz, 1C, CHP), 52.27 (d, J = 5.9 Hz, 1C, OCH_3), 41.36 (d, J = 8.9 Hz, 1C, CH_2N).

IR (ATR, [MV010-16]): ν = 3133, 2840, 2661, 2075, 1634, 1541, 1390, 1170, 1025 cm^{-1}.

Anal. calc. for $C_3H_{10}NO_4P$: C 23.23%, H 6.50%, N 9.03%, O 41.26%; found: C 23.25%, H 6.36%, N 8.97%, O 41.05%.

5.9. Synthesis of methyl hydrogen (2-aminoethyl)phosphonate (2.8)

5.9.1. N-(2-Bromoethyl)phthalimide (**4.23**)



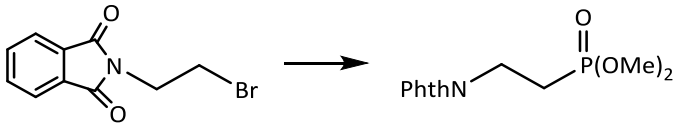
4.23

Synthesis was performed analogously to literature [72].

A round bottomed flask was loaded with potassium phthalimide (5.00 g, 27.0 mmol, 1.0 eq.), dry DMF (10 mL) and 1,2-dibromoethane (15.22 g, 81.0 mmol, 7.0 mL, 3.0 eq.). The reaction mixture was stirred at RT for 24 h under argon atmosphere and concentrated under reduced pressure. The residue was dissolved in EtOAc and extracted with water. The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (Ø3x60 cm, CH_2Cl_2, TLC: R_f = 0.37*) and crystallised from CH_2Cl_2/hexanes to yield *N*-(2-bromoethyl)phthalimide (**4.23**) as colourless crystals (4.35 g, 17.1 mmol, 63%); mp. 83 °C (lit. [73] 84 °C). Its spectroscopic data were in accordance with literature data [73].

^{1}H NMR ($CDCl_3$, 400.27 MHz, 4Jun2614/230 [ZH891/1]): δ = 7.89-7.83 (m, 2H, H_{ar}), 7.76-7.70 (m, 2H, H_{ar}), 4.10 (t, J = 6.7 Hz, 2H, CH_2Br), 3.60 (t, J = 6.7 Hz, 2H, CH_2N).

5.9.2. Dimethyl (2-phthalimidoethyl)phosphonate (**4.24**)



4.23 **4.24**

Synthesis was performed analogously to literature [74].

N-(2-Bromoethyl)phthalimide (**4.23**) (1.83 g, 7.2 mmol, 1.0 eq.) and trimethyl phosphite (5.36 g, 43.2 mmol, 5.1 mL, 6.0 eq.) were mixed and heated at 140 °C (bath temperature) for 48 h (TLC: R_f

= 0.33*, EtOAc). Volatile components were removed by bulb to bulb distillation (1 mbar, 100 °C) to give a yellow oil as residue. It was flash chromatographed (Ø3x34 cm, EtOAc → EtOAc/acetone 1:1, TLC: R_f = 0.51*) and crystallised from CH_2Cl_2/hexanes to give dimethyl (2-phthalimidoethyl)phosphonate (**4.24**) as colourless crystals (1.07 g, 3.8 mmol, 53%); mp. 127-130 °C.

^{1}H NMR ($CDCl_3$, 400.27 MHz, 4Jul0414/362 [ZH891/3/umkrist]): δ = 7.86-7.80 (m, 2H, H_{ar}), 7.72-7.66 (m, 2H, H_{ar}), 3.98-3.89 (m, 2H, CH_2N), 3.73 (d, J = 10.9 Hz, 6H, OCH_3), 2.26-2.15 (m, 2H, CH_2P).

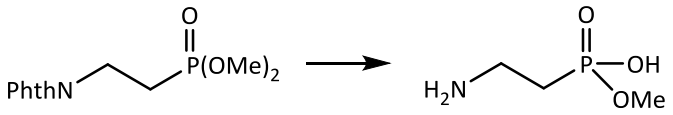
^{31}P NMR ($CDCl_3$, 162.03 MHz, 4Jul0414/363 [ZH891/3/umkrist]): δ = 29.31 (s).

^{13}C NMR ($CDCl_3$, 100.65 MHz, 4Jul0414/364 [ZH891/3/umkrist]): δ = 167.75 (s, 2C, CO), 134.04 (s, 2C, C_{ar}), 132.03 (s, 2C, *C*CO), 123.34 (s, 2C, C_{ar}), 52.53 (d, J = 6.4 Hz, 2C, OCH_3), 32.05 (d, J = 1.5 Hz, 1C, CH_2N), 23.78 (d, J = 140.1 Hz, 1C, CH_2P).

IR (ATR, [MV011-18]): ν = 3460, 2953, 1764, 1703, 1400, 1362, 1267, 1218, 1049, 1020 cm^{-1}.

Anal. calc. for $C_{12}H_{14}NO_5P$: C 50.89%, H 4.98%, N 4.95%, O 28.24%; found: C 51.02%, H 4.65%, N 4.93%.

5.9.3. Methyl hydrogen (2-aminoethyl)phosphonate (**2.8**)



4.24 **2.8**

A solution of dimethyl (2-phthalimidoethyl)phosphonate (**4.24**) (1.33 g, 4.7 mmol, 1.0 eq.) and hydrazine monohydrate (2.07 g, 41.4 mmol, 2.0 mL, 8.8 eq.) in dry MeOH (8 mL) was heated in a sealed tube at 100 °C for 20 h. After cooling, the solidified mixture was dissolved in water (20 mL), transferred into a round bottomed flask and concentrated under reduced pressure. 2,3-dihydrophthalazine-1,4-dione was removed by centrifugation and by chromatography on Amberlite XAD-4 (each 50 mL/mmol, washed neutral with water). Two subsequent columns filled with Amberlite were necessary to remove the side product. The product was eluted with water and applied to a column (Ø1x56 cm) filled with Dowex 50Wx8, H^+ for ion exchange chromatography with water as eluent. Ninhydrin-positive fractions (TLC: R_f = 0.56, *i*PrOH/H_2O/NH_3(25%) 6:3:2) were pooled and concentrated under reduced pressure. For crystallisation, the residue was dissolved in a minimum amount of water and EtOH was allowed to diffuse into the cold solution over several days. Methyl hydrogen (2-aminoethyl)phosphonate (**2.8**) was obtained as colourless crystals (0.14 g, 1.0 mmol, 21%); mp. 221-224 °C.

^{1}H NMR (D_2O, 400.27 MHz, 4Nov1114/80 [MV011/19/Wdh2umkr]): δ = 3.64 (d, J = 10.6 Hz, 3H, OCH_3), 3.29-3.20 (m, 2H, CH_2N), 2.11-1.99 (m, 2H, CH_2P).

^{31}P NMR (D_2O, 162.03 MHz, 4Nov1114/81 [MV011/19/Wdh2umkr]): δ = 22.48 (s).

^{13}C NMR (D_2O, 100.65 MHz, 4Nov1114/82 [MV011/19/Wdh2umkr]): δ = 51.33 (d, J = 5.8 Hz, 1C, OCH_3), 35.23 (s, 1C, CH_2N), 23.82 (d, J = 133.8 Hz, 1C, CH_2P).

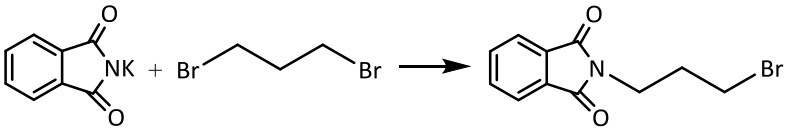
IR (ATR, [MV011-19]): ν = 2832, 2727, 2580, 2456, 2167, 1644, 1535, 1269, 1182, 1036 cm^{-1}.

Anal. calc. for $C_3H_{10}NO_3P$: C 25.91%, H 7.25%, N 10.07%, O 34.51%; found: C 25.92%, H 7.07%, N 9.61%, O 34.23%.

5.10. Synthesis of (3-aminopropyl)phosphonic acid (2.9)

*5.10.1. N-(3-Bromopropyl)phthalimide (**4.25**)*

Alternative A:



4.25

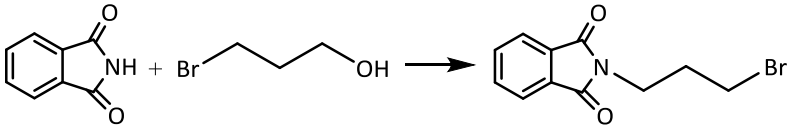
Synthesis was performed analogously to literature [75].

Under argon atmosphere potassium phthalimide (2.50 g, 13.5 mmol, 1.0 eq.) was suspended in dry DMF (5 mL) and 1,3-dibromopropane (8.18 g, 40.5 mmol, 4.1 mL, 3.0 eq.) was added. The mixture was stirred at RT for 24 h and concentrated under reduced pressure. The residue was dissolved in EtOAc, washed twice with water, once with a saturated aqueous solution of NH_4Cl and brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure. The residue was purified by flash chromatography (CH_2Cl_2/hexanes 1:1) and crystallised from diisopropyl ether/hexanes. *N*-(3-Bromopropyl)phthalimide (**4.25**) was obtained as colourless crystals (2.47 g, 9.2 mmol, 68%). The spectroscopic data were in accordance with literature data [76].

^{1}H NMR ($CDCl_3$, 400.27 MHz, 4Jun2314/100 [ZH888-2-umkrist]): δ = 7.87-7.81 (m, 2H, H_{ar}), 7.74-7.68 (m, 2H, H_{ar}), 3.82 (t, J = 6.8 Hz, 2H, CH_2N), 3.40 (t, J = 6.8 Hz, 2H, CH_2Br), 2.25 (q, J = 6.8 Hz, 2H, CH_2C).

Anal. calc. for $C_{11}H_{10}BrNO_2$: C 49.28%, H 3.76%, N 5.22%, O 11.93%; found: C 49.28%, H 3.70%, N 5.17%.

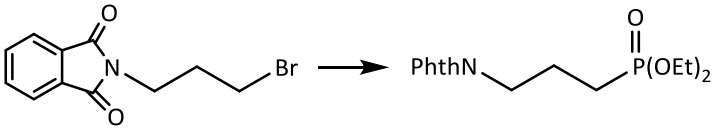
Alternative B:



4.25

In a round bottomed flask a mixture of 3-bromopropanol (0.69 g, 5.0 mmol, 1.0 eq.) and triphenylphosphine (1.57 g, 6.0 mmol, 1.2 eq.) was cooled to 0 °C under argon atmosphere. A solution of DIAD (1.21 g, 6.0 mmol, 1.2 eq.) and phthalimide (0.88 g, 6.0 mmol, 1.2 eq.) in dry CH_2Cl_2 (4 mL) was added dropwise and stirring was continued for 3 h (TLC: hexanes/ CH_2Cl_2). The reaction was quenched with MeOH (0.2 mL) and concentrated under reduced pressure. Hexanes was added to the residue to precipitate $P(O)Ph_3$ and the hydrazo ester, which were then filtered through Celite. The concentrated filtrate was purified by flash chromatography (CH_2Cl_2, TLC: R_f = 0.73*, iodine for detection) and crystallised from diisopropyl ether/hexanes. *N*-(3-Bromopropyl)phthalimide (**4.25**) was obtained as colourless crystals (0.13 g, 0.5 mmol, 10%). The spectroscopic data were in accordance with literature data [76].

5.10.2. Diethyl (3-phthalimidopropyl)phosphonate (**4.26**)

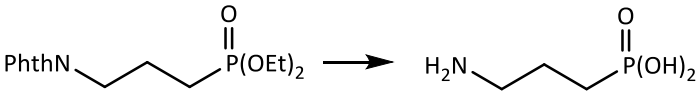


4.25 **4.26**

Synthesis was performed analogously to literature [74].

A mixture of *N*-(3-bromopropyl)phthalimide (**4.25**) (2.47 g, 9.2 mmol, 1.0 eq.) and triethyl phosphite (5.05 g, 30.4 mmol, 5.2 mL, 3.3 eq.) was heated at 180 °C for 6 h. Afterwards, volatile components were removed under reduced pressure. The residue was purified by flash chromatography (hexanes/EtOAc 1:2, TLC: R_f = 0.49) to give diethyl (3-phthalimidopropyl)phosphonate (**4.26**) as yellowish oil (2.07 g, 6.4 mmol, 69%). The spectroscopic data were in accordance with literature data [77].

5.10.3. (3-Aminopropyl)phosphonic acid (**2.9**)



4.26 **2.9**

Synthesis was performed analogously to literature [74].

To diethyl (3-phthalimidopropyl)phosphonate (**4.26**) (1.26 g, 3.9 mmol) was added HBr (20 mL, 48% aqueous solution) and the mixture was stirred at 120 °C for 30 min. Meanwhile, the reflux condenser was removed several times to allow bromoethane to escape. Then the reaction mixture was refluxed (180 °C bath temperature) for 6 h and concentrated in vacuo. The residue was dissolved in water, extracted with EtOAc, concentrated to a small volume and applied to a column

(Ø1.5x40 cm) filled with Dowex 50Wx8, H^+. After elution with water until neutral the product was eluted with NH_3 (5% aqueous solution). Ninhydrin-positive fractions (TLC: R_f = 0.24, *i*PrOH/H_2O/NH_3(25%) 6:3:2) were pooled and concentrated under reduced pressure. The residue was crystallised from water/EtOH to give (3-aminopropyl)phosphonic acid (**2.9**) as white solid (0.44 g, 3.2 mmol, 82%). The spectroscopic data were in accordance with literature data [78].

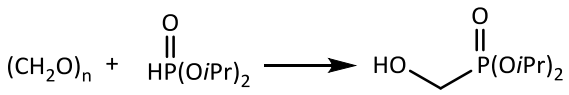
^{1}H NMR (D_2O, 400.27 MHz, 4Jul1414/50 [ZH888/9/umkrist]): δ = 3.13 (t, J = 7.4 Hz, 2H, CH_2N), 2.02-1.88 (m, 2H, CH_2Br), 1.77-1.65 (m, 2H, CH_2).

^{31}P NMR (D_2O, 162.03 MHz, 4Jul1414/51 [ZH888/9/umkrist]): δ = 23.64 (s).

^{13}C NMR (D_2O, 100.65 MHz, 4Jul1414/52 [ZH888/9/umkrist]): δ = 40.20 (d, J = 17.5 Hz, 1C, CH_2N), 24.97 (d, J = 134.6 Hz, 1C, CH_2P), 21.51 (d, J = 4.2 Hz, 1C, CH_2C).

5.11. Synthesis of (aminooxymethyl)phosphonic acid (2.10)

*5.11.1. Diisopropyl (hydroxymethyl)phosphonate (**4.27**)*



4.27

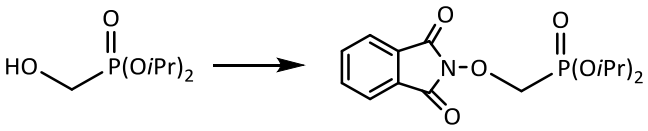
Synthesis was performed analogously to literature [79].

A round bottomed flask was loaded with paraformaldehyde (0.93 g, 31.0 mmol, 1.0 eq.) and diisopropyl phosphite (5.15 g, 31.0 mmol, 1.0 eq.) and DBU (20 drops) was added (no cooling, strong exothermal reaction!). The mixture was stirred vigorously. After 2 h, when the reaction mixture had cooled to RT, CH_2Cl_2 (35 mL) and HCl (2 mL, 2 M) were added. The layers were separated and the aqueous layer was extracted twice with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The crude product was purified by bulb to bulb distillation (0.26 mbar, 90-110 °C) to yield diisopropyl (hydroxymethyl)-phosphonate (**4.27**) as colourless oil (4.83 g, 24.6 mmol, 79%; TLC: R_f = 0.23, CH_2Cl_2/acetone 5:1). The spectroscopic data were in accordance with literature data [79].

^{1}H NMR ($CDCl_3$, 400.27 MHz, 4Aug0814/180 [MV018/23/dest]): δ = 4.72 (dsept, J = 6.2 Hz, J = 7.4 Hz, 2H, OCH), 3.81 (d, J = 6.3 Hz, 2H, CH_2P), 3.15 (brs, 1H, OH), 1.32 (dd, J = 0.7 Hz, J = 6.2 Hz, 12H, CH_3).

^{31}P NMR ($CDCl_3$, 162.03 MHz, 4Aug0814/181 [MV018/23/dest]): δ = 22.25 (s).

5.11.2. Diisopropyl (1-phthalimidooxymethyl)phosphonate (**4.28**)



4.27 **4.28**

Synthesis was performed analogously to literature [80].

In a round bottomed flask a mixture of diisopropyl (hydroxymethyl)phosphonate (**4.27**) (0.78 g, 4.0 mmol, 1.0 eq.), *N*-hydroxyphthalimide (0.98 g, 6.0 mmol, 1.5 eq.) and triphenylphosphine (1.57 g, 6.0 mmol, 1.5 eq.) was dissolved in dry THF (10 mL) under argon atmosphere. A solution of D*t*BAD (1.38 g, 6.0 mmol, 1.5 eq.) in try THF (6 mL) was added dropwise and stirred at 20 °C (water bath) for 21 h. The reaction was quenched with water and concentrated under reduced pressure. The crude product was purified by flash chromatography (CH_2Cl_2/acetone 8:1, TLC: R_f = 0.68*) and crystallised from 1,2-dichloroethane/diisopropyl ether to give diisopropyl (1-phthalimidooxy-methyl)phosphonate (**4.28**) as white solid (1.09 g, 3.2 mmol, 80%); mp. 89-93 °C.

^{1}H NMR ($CDCl_3$, 400.27 MHz, 4Jul0314/90 [ZH892/2/umkr]): δ = 7.85-7.79 (m, 2H, H_{ar}), 7.76-7.70 (m, 2H, H_{ar}), 4.84 (dsept, J = 6.2 Hz, J = 7.5 Hz, 2H, OCH), 4.51 (d, J = 9.0 Hz, 2H, CH_2P), 1.40 (d, J = 6.2 Hz, J = 10.5 Hz, 6H, CH_3), 1.37 (d, J = 6.2 Hz, J = 10.5 Hz, 6H, CH_3).

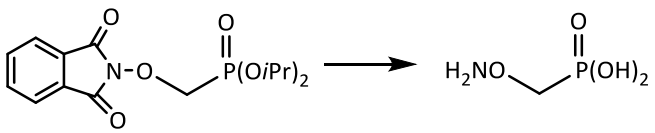
^{31}P NMR ($CDCl_3$, 162.03 MHz, 4Jul0314/91 [ZH892/2/umkr]): δ = 12.89 (s).

^{13}C NMR ($CDCl_3$, 100.65 MHz, 4Jul0314/92 [ZH892/2/umkr]): δ = 162.63 (s, 2C, CO), 134.59 (s, 2C, C_{ar}), 128.86 (s, 2C, *C*CO), 123.63 (s, 2C, C_{ar}), 72.24 (d, J = 6.5 Hz, 2C, OCH), 71.13 (d, J = 157.9 Hz, 1C, CH_2P), 24.10 (d, J = 3.8 Hz, 2C, CH_3), 23.89 (d, J = 4.9 Hz, 2C, CH_3).

IR (ATR, [MV013-24]): ν = 2986, 2938, 1787, 1726, 1382, 1250, 1187, 1141, 973 cm^{-1}.

Anal. calc. for $C_{15}H_{20}NO_6P$: C 52.79%, H 5.91%, N 4.10%, O 28.13%; found: C 52.79%, H 5.67%, N 4.13%.

5.11.3. (Aminooxymethyl)phosphonic acid (**2.10**)



4.28 **2.10**

Synthesis was performed analogously to literature [80].

To a solution of diisopropyl (1-phthalimidooxymethyl)phosphonate (**4.28**) (0.96 g, 2.8 mmol) in MeOH (2 mL) was added NH_3 (2.0 mL, 25% aqueous solution) and stirred at RT for 6 h (TLC: R_f = 0.41, EtOAc). After concentration under reduced pressure the residue was taken up in HCl (15 mL, 2 M) and extracted with EtOAc. The aqueous layer was concentrated under reduced pressure. To the residue was then added HCl (10 mL, 37% aqueous solution) and stirred at 140 °C for 3.5 h. The solution was concentrated under reduced pressure, redissolved in water, applied to a column (Ø1x23 cm) filled with Dowex 50Wx8, H^+ for ion exchange chromatography with water as eluent. Ninhydrin-positive fractions (TLC: R_f = 0.47, *i*PrOH/H_2O/NH_3(25%) 6:3:2) were pooled and concentrated under reduced pressure. The residue was crystallised from water/EtOH to give (aminooxymethyl)phosphonic acid (**2.10**) as colourless crystals (0.22 g, 1.7 mmol, 61%); mp. 198 °C (lit. [81] 207-208 °C). The spectroscopic data were in accordance with literature data [81].

^{1}H NMR (D_2O, 400.27 MHz, 4Jul0814/100 [ZH892/24/umkrist]): δ = 4.27 (d, J = 10.0 Hz, 2H, CH_2P).

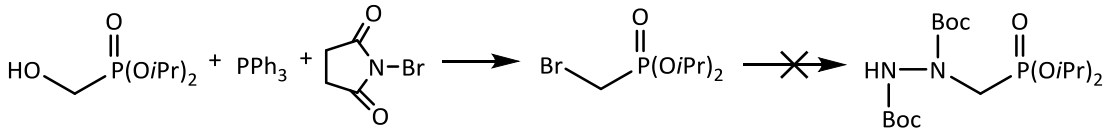
^{31}P NMR (D_2O, 162.03 MHz, 4Jul0814/101 [ZH892/24/umkrist]): δ = 9.86 (s).

^{13}C NMR (D_2O, 100.65 MHz, 4Jul0814/102 [ZH892/24/umkrist]): δ = 70.75 (d, J = 148.7 Hz, 1C, CH_2P).

5.12. Synthesis of (hydrazinomethyl)phosphonic acid (2.11)

Alternative A:

*5.12.1. Diisopropyl (bromomethyl)phosphonate (**4.29**)*



4.27 **4.29** **4.30**

Synthesis was performed analogously to literature [82].

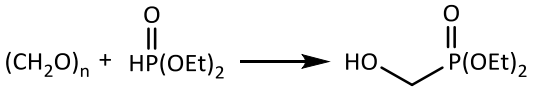
A suspension of *N*-bromosuccinimide (2.47 g, 13.9 mmol, 1.2 eq.) in dry CH_2Cl_2 (41 mL) was added to a solution of triphenylphosphine (3.65 g, 13.9 mmol, 1.2 eq.) in dry CH_2Cl_2 (29 mL) at -78 °C under argon atmosphere. The mixture was stirred until NBS was completely dissolved. A solution of diisopropyl (hydroxymethyl)phosphonate (**4.27**) (2.28 g, 11.6 mmol, 1.0 eq.) in dry CH_2Cl_2 (17 mL) was added dropwise. The reaction was stirred at RT for 2 h and at 40 °C for 2 h (TLC: hexanes/EtOAc 1:2). The reaction mixture was concentrated under reduced pressure and the residue was purified by flash chromatography (Ø2x40 cm, hexanes/EtOAc 1:2, TLC: R_f = 0.63). Bulb to bulb distillation (0.5 mbar, 75-80 °C) provided diisopropyl (bromomethyl)phosphonate (**4.29**) as colourless oil (1.97 g, 7.6 mmol, 66%). The spectroscopic data were in accordance with literature data [83].

^{1}H NMR ($CDCl_3$, 400.27 MHz, 4Jul1014/50 [ZH892/26/Fr6-9destill]): δ = 4.76 (dsept, J = 6.2 Hz, J = 7.5 Hz, 2H, OCH), 3.22 (d, J = 9.7 Hz, 2H, CH_2Br), 1.34 (dd, J = 1.0 Hz, J = 6.2 Hz, 12H, CH_3).

^{31}P NMR ($CDCl_3$, 162.03 MHz, 4Jul1014/51 [ZH892/26/Fr6-9destill]): δ = 16.38 (s).

Alternative B:

5.12.2. Diethyl (hydroxymethyl)phosphonate (**4.34**)



4.34

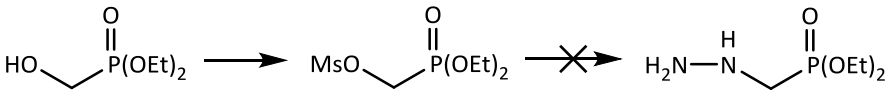
Synthesis was performed analogously to literature [79].

Diethyl phosphite (4.28 g, 31.0 mmol, 1.0 eq.) and DBU (10 drops) were added to paraformaldehyde (0.93 g, 31.0 mmol, 1.0 eq.) (no cooling, strong exothermal reaction!). The mixture was stirred vigorously. 2 h later, after cooling down to RT, CH_2Cl_2 (35 mL) and HCl (2 mL, 2 M) were added. The layers were separated and the aqueous layer was extracted twice with CH_2Cl_2. The combined organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The crude product was purified by bulb to bulb distillation (0.26 mbar, 100-120 °C) to yield diethyl (hydroxymethyl)phosphonate (**4.34**) as a colourless oil (4.08 g, 24.3 mmol, 78%; TLC: R_f = 0.20, EtOAc). The spectroscopic data were in accordance with literature data [84].

^{1}H NMR ($CDCl_3$, 400.27 MHz, 4Aug0814/170 [MV019/28/dest]): δ = 4.20-4.08 (m, 4H, OCH_2), 3.88 (d, J = 6.2 Hz, 2H, CH_2P), 3.33 (brs, 1H, OH), 1.32 (t, J = 7.1 Hz, 6H, CH_3).

^{31}P NMR ($CDCl_3$, 162.03 MHz, 4Aug0814/171 [MV019/28/dest]): δ = 24.08 (s).

5.12.3. Diethyl (mesyloxymethyl)phosphonate (**4.35**)



4.34 **4.35** **4.36**

Synthesis was performed analogously to literature [85].

Triethylamine (1.11 g, 11.0 mmol, 1.5 mL, 1.1 eq.) was added to diethyl (hydroxymethyl)phosphonate (**4.34**) (1.68 g, 10.0 mmol, 1.0 eq.) in dry CH_2Cl_2 (10 mL) and the mixture was stirred at 0 °C for 10 min, followed by mesylchloride (1.26 g, 11.0 mmol, 0.9 mL, 1.1 eq.). After stirring at RT for further 2 h the reaction was quenched with HCl (5 mL, 2 M). The layers were separated and the

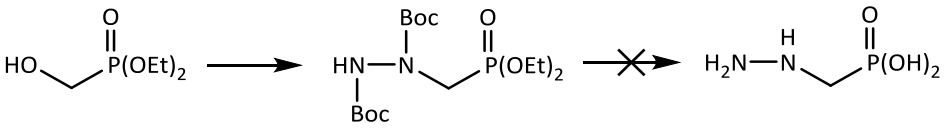
aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with water, a saturated aqueous solution of $NaHCO_3$, dried (Na_2SO_4), filtered and concentrated under reduced pressure. Diethyl (mesyloxymethyl)phosphonate (**4.35**) was obtained as yellowish oil (1.83 g, 7.4 mmol, 74%).

^{1}H NMR ($CDCl_3$, 400.27 MHz, 4Aug1114/100 [MV019/30]): δ = 4.40 (d, J = 8.8 Hz, 2H, CH_2P), 4.20 (qd, J = 7.1 Hz, J = 8.3 Hz, 4H, OCH_2), 3.11 (s, 3H, CH_3S), 1.36 (td, J = 0.4 Hz, J = 7.1 Hz, 6H, CH_3).

^{31}P NMR ($CDCl_3$, 162.03 MHz, 4Aug1114/101 [MV019/30]): δ = 15.63 (s).

Alternative C:

5.12.4. Diethyl (N,N'-di-Boc-hydrazinomethyl)phosphonate (**4.37**)

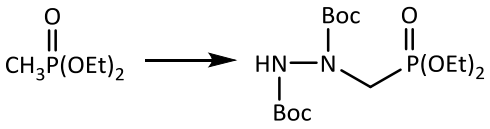


4.34 **4.37** **2.11**

A round bottomed flask was loaded with diethyl (hydroxymethyl)phosphonate (**4.34**) (0.87 g, 5.2 mmol, 1.0 eq.) and triphenylphosphine (2.05 g, 7.8 mmol, 1.5 eq.) under argon atmosphere and suspended in dry THF (1 mL). A solution of D*t*BAD (1.80 g, 7.8 mmol, 1.5 eq.) in dry THF (6 mL) was added and stirring was continued at 50 °C for 23 h (TLC: EtOAc). The reaction was quenched with water. After concentration under reduced pressure a mixture of diethyl ether/hexanes (1:1) (20 mL) was added to the residue to precipitate triphenylphosphine oxide and the hydrazo ester. The mixture was filtered through Celite moistened with hexanes and concentrated under reduced pressure. The residue was purified by flash chromatography (Ø3x40 cm, EtOAc, TLC: R_f = 0.67) and crystallised from diisopropyl ether/hexanes (1:10) to give diethyl (*N,N'*-di-Boc-hydrazinomethyl)phosphonate (**4.37**) as colourless crystals (0.74 g, 1.9 mmol, 37%).

Alternative D:

5.12.5. Diethyl (N,N'-di-Boc-hydrazinomethyl)phosphonate (**4.37**)



4.37

Synthesis was performed analogously to literature [86].

Diethyl methylphosphonate (0.76 g, 5.0 mmol, 1.0 eq.) was dissolved in dry THF (10 mL) and cooled to -78 °C under argon atmosphere. A solution of *n*BuLi (0.35 g, 5.5 mmol, 2.2 mL, 1.1 eq., 2.5 M in hexanes) was added dropwise and stirred for 10 min, followed by a solution of D*t*BAD (0.58 g, 2.5 mmol, 0.5 eq.) in dry THF (2.5 mL). After 5 min the reaction was quenched with glacial acetic acid (6.0 mmol, 0.3 mL) in dry THF (1.5 mL). The cooling bath was removed and a saturated aqueous solution of $NaHCO_3$ (20 mL) was added. The mixture was extracted three times with EtOAc and the combined organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The reaction was investigated by TLC (EtOAc). The residue was purified by flash chromatography (Ø2x40 cm, EtOAc, TLC: R_f = 0.51) and crystallised from diisopropyl ether/hexanes (1:10) to yield diethyl (*N,N'*-di-Boc-hydrazinomethyl)phosphonate (**4.37**) as colourless crystals (0.33 g, 0.9 mmol, 18%); mp. 91 °C (lit. [86] 75 °C). The spectroscopic data were in accordance with literature data [86]. The NMR spectra recorded at RT showed signals for two conformers of the compound, and one signal at 80 °C.

^{1}H NMR (toluene-d_8, RT, 400.13 MHz, Oct2914/10 [MV027/34/umkr.RT]): δ = 4.13-3.75 (m, 6H, OCH_2, CH_2P), 1.37 (brs, 9 H, Me_3C), 1.33 (s, 9 H, CH_3), 1.00 (brt, J = 7.0 Hz, 6H, CH_3).

^{31}P NMR (toluene-d_8, RT, 161.98 MHz, Oct2914/12 [MV027/34/umk.RT]): δ = 23.40 (s), 23.25 (brs).

^{1}H NMR (toluene-d_8, 80 °C, 400.13 MHz, Oct2914/13 [MV027/34/umkr.353K]): δ = 6.67 (brs, 1H, NH), 3.97-3.79 (m, 6H, OCH_2+CH_2P), 1.36 (s, 9 H, Me_3C), 1.33 (s, 9 H, Me_3C), 1.02 (t, J = 7.0 Hz, 6H, CH_3).

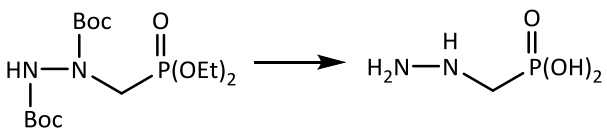
^{31}P NMR (toluene-d_8, 80 °C, 161.98 MHz, Oct2914/15 [MV027/34/umkr.353K]): δ = 23.04 (s).

^{13}C NMR (toluene-d_8, 80 °C, 100.61 MHz, Oct2914/14 [MV027/34/umkr.353K]): δ = CO n.d., 82.45 (s, 1C Me_3C), 81.73 (s, 1C Me_3C), 63.01 (d, J = 6.3 Hz, 2C, OCH_2), 29.45 (s, 3C, Me_3C), 29.34 (s, 3C, Me_3C), 17.48 (d, J = 5.6 Hz, 2C, CH_3), CH_2P n.d.

IR (ATR, [MV027-34]): ν = 3347, 3181, 2981, 2933, 1735, 1365, 1224, 1155, 1022 cm^{-1}.

Anal. calc. for $C_{15}H_{31}N_2O_7P$: C 47.12%, H 8.17%, N 7.33%, O 29.29%; found: C 47.13%, H 7.93%, N 7.32%, O 29.41%.

5.12.6. (Hydrazinomethyl)phosphonic acid (**2.11**)



4.37 **2.11**

To diethyl (*N,N'*-di-Boc-hydrazinomethyl)phosphonate (**4.37**) (0.31 g, 0.8 mmol, 1.0 eq.) dissolved in dry 1,2-dichloroethane (3 mL) was added bromotrimethylsilane (1.22 g, 8.0 mmol, 1.0 mL, 10.0 eq.) under argon atmosphere. After stirring the mixture at 50 °C for 5 h excess TMSBr was removed in vacuo (0.5 mbar). The residue was again dissolved in 1,2-dichloroethane and

concentrated. Then, the residue was dissolved in water (10 mL) and extracted once with EtOAc. The aqueous layer was concentrated under reduced pressure and TFA (1 mL) was added. After stirring at RT for 1.5 h the acid was removed under reduced pressure. The crude product was applied to a column (Ø1x23 cm) filled with Dowex 50Wx8, H^+ for ion exchange chromatography with water as eluent. Molybdate-positive fractions (TLC: R_f = 0.43, *i*PrOH/H_2O/NH_3(25%) 6:3:2) were pooled and concentrated under reduced pressure. The residue was crystallised from water/EtOH to give (hydrazinomethyl)phosphonic acid (**2.11**) as yellowish crystals (72 mg, 0.6 mmol, 75%); decompositon at 170 °C (lit. [87] 196-198 °C).

^{1}H NMR (D_2O, 400.27 MHz, 4Oct3114/150 [MV027/29/umkr]): δ = 3.27 (d, *J* = 13.1 Hz, 2H, CH_2P).

^{31}P NMR (D_2O, 162.03 MHz, 4Oct3114/151 [MV027/29/umkr]): δ = 12.12 (s).

^{13}C NMR (D_2O, 100.65 MHz, 4Oct3114/152 [MV027/29/umkr]): *δ* = 47.32 (d, *J* = 140.7 Hz, 1C, CH_2P).

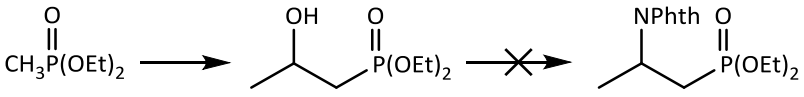
IR (ATR, [MV027-29]): ν = 3254, 2979, 2687, 2294, 2103, 1639, 1532, 1226, 1130, 1031 cm^{-1}.

Anal. calc. for $CH_7N_2O_3P$: C 9.53%, H 5.60%, N 22.22%, O 38.08%; found: C 9.53%, H 5.41%, N 22.15%, O 38.23%.

5.13. Synthesis of (R)- and (S)-(2-aminopropyl)phosphonic acid [(R)- and (S)-2.12]

Alternative A:

*5.13.1. (±)-Diethyl (2-hydroxypropyl)phosphonate [(±)-**4.38**]*



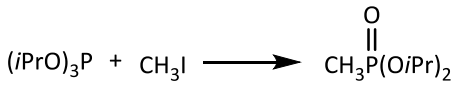
(±)-**4.38** (±)-**4.39**

Synthesis was performed analogously to literature [89].

A round bottomed flask was loaded with diethyl methylphosphonate (0.76 g, 5.0 mmol, 1.0 eq.) in dry THF (10 mL) under argon atmosphere. After cooling to -78 °C a solution of *n*BuLi (0.35 g, 5.5 mmol, 2.2 mL, 1.1 eq., 2.5 M in hexanes) was added. After stirring for 20 min a solution of acetaldehyde (0.24 g, 5.5 mmol, 0.3 mL, 1.1 eq.) in dry THF (1 mL) was added and stirred for further 10 min. The reaction was quenched with glacial acetic acid (6.0 mmol, 0.3 mL) in dry THF (1.5 mL). The cooling bath was removed and a saturated aqueous solution of $NaHCO_3$ (20 mL) was added. The mixture was extracted with EtOAc. The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (Ø3x40 cm, EtOAc/acetone 1:1, TLC: R_f = 0.39) to give (±)-diethyl (2-hydroxypropyl)phosphonate [(±)-**4.38**] as a colourless oil (0.25 g, 1.3 mmol, 26%).

Alternative B:

5.13.2. Diisopropyl methylphosphonate (**4.41**)

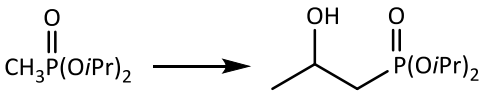


4.41

Synthesis was performed analogously to literature [88].

A mixture of triisopropyl phosphite (20.82 g, 100.0 mmol, 25.0 mL, 1.0 eq.) and methyl iodide (14.19 g, 100.0 mmol, 6.3 mL, 1.0 eq.) was gradually heated to 100 °C within 2 h (+10 °C/15 min) and finally kept for 1 h at that temperature. The product was purified by fractional vacuum distillation. Isopropyl iodide was removed at atmospheric pressure (62-80 °C). The product was obtained as a colourless liquid (6 mm Hg, 64-86 °C) still containing diisopropyl phosphite, which was destroyed by the addition of NH_3 (20 mL, 25% aqueous solution) and EtOH (5 mL). The reaction mixture was stirred vigorously at RT for 2 d. The solution was concentrated under reduced pressure. To the residue was added water (20 mL) and the mixture was extracted with CH_2Cl_2. The dried organic layer was purified by bulb to bulb distillation to give diisopropyl methylphosphonate (**4.41**) as colourless oil (15.29 g, 84.9 mmol, 85%; 0.40 mbar, 40-95 °C). A small amount of triisopropyl phosphate could not be removed. The spectroscopic data were in accordance with literature data [44].

5.13.3. (±)-Diisopropyl (2-hydroxypropyl)phosphonate [(±)-**4.42**]



4.41 (±)-**4.42**

Synthesis was performed analogously to literature [89].

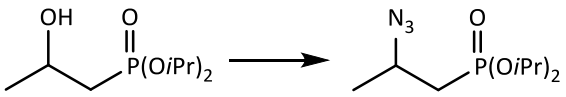
A round bottomed flask was loaded with diisopropyl methylphosphonate (**4.41**) (3.60 g, 20.0 mmol, 1.0 eq.) in dry THF (40 mL) under argon atmosphere. After cooling to -78 °C a solution of *n*BuLi (1.41 g, 22.0 mmol, 8.8 mL, 1.1 eq., 2.5 M in hexanes) was added, followed by a solution of acetaldehyde (0.97 g, 22.0 mmol, 1.2 mL, 1.1 eq.) in dry THF (4 mL) 20 min later and by glacial acetic acid (24.2 mmol, 1.4 mL) in dry THF (3 mL) 10 min later. The cooling bath was removed and a saturated aqueous solution of $NaHCO_3$ (40 mL) was added, followed by extraction with EtOAc. The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure. The crude product was purified by flash chromatography (Ø3x50 cm, EtOAc, TLC: R_f = 0.19) to give (±)-

diisopropyl (2-hydroxypropyl)phosphonate [(±)-**4.42**] as a colourless oil (2.82 g, 12.6 mmol, 63%). The spectroscopic data were in accordance with literature data [89].

^{1}H NMR ($CDCl_3$, 400.27 MHz, 4Dec1214/120 [MV032/39/Fr19-38]): δ = 4.78-4.62 (m, 2H, OCH), 4.21-4.06 (m, 1H, C*H*OH), 3.62 (brs, 1H, OH), 1.94-1.76 (m, 2H, CH_2P), 1.32 (d, J = 6.2 Hz, 3H, CH_3), 1.314 (d, J = 6.2 Hz, 6H, CH_3), 1.310 (d, J = 6.2 Hz, 3H, CH_3), 1.25 (dd, J = 2.5 Hz, J = 6.2 Hz, 3H, CH*CH*$_3$).

^{31}P NMR ($CDCl_3$, 162.03 MHz, 4Dec1214/121 [MV032/39/Fr19-38]): δ = 28.19 (s).

*5.13.4. (±)-Diisopropyl (2-azidopropyl)phosphonate [(±)-**4.44**]*



(±)-**4.42** (±)-**4.44**

Synthesis was performed analogously to literature [89].

(±)-Diisopropyl (2-hydroxypropyl)phosphonate [(±)-**4.42**] (1.19 g, 5.3 mmol, 1.0 eq.) was dried azeotropically with toluene. Triphenylphosphine (1.83 g, 6.9 mmol, 1.3 eq.) and dry toluene (10 mL) were added under argon atmosphere. The solution was cooled to 0 °C. HN_3 (0.30 g, 6.9 mmol, 4.1 mL, 1.3 eq., 1.7 M) was was added, followed immediately by DIAD (1.40 g, 6.9 mmol, 1.4 mL, 1.3 eq.). After stirring at RT for 1.5 h (TLC: hexanes/EtOAc 1:2) the reaction was quenched with MeOH (0.2 mL) and concentrated under reduced pressure. A mixture of hexanes/diethyl ether (4:1) was added to the residue to precipitate triphenylphosphine oxide and the hydrazo ester, which were then removed by filtration through Celite moistened with hexanes. The concentrated filtrate was purified by flash chromatography (Ø2x47 cm, hexanes/EtOAc 1:2, TLC: R_f = 0.30) to yield (±)-diisopropyl (2-azidopropyl)phosphonate [(±)-**4.44**] as a colourless oil (0.77 g, 3.1 mmol, 58%).

^{1}H NMR ($CDCl_3$, 400.27 MHz, 4Jan0815/20 [MV032/41/sauberes Azid]): δ = 4.77-4.63 (m, 2H, OCH), 3.86-3.74 (m, 1H, CHN_3), 1.92 (AB part of an ABXP system, J_{AB} = 15.3 Hz, J_{AX} = 6.4 Hz, J_{AP} = 19.0 Hz, J_{AB} = 15.3 Hz, J_{BX} = 7.4 Hz, J_{BP} = 18.4 Hz, 2H, CH_2P), 1.37 (dd, J = 0.7 Hz, J = 6.6 Hz, CH_3), 1.31 (brd, J = 6.2 Hz, 12H, CH*CH*$_3$).

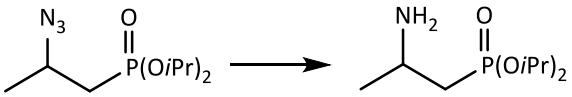
^{31}P NMR ($CDCl_3$, 162.03 MHz, 4Jan0815/21 [MV032/41/sauberes Azid]): δ = 24.44 (s).

^{13}C NMR ($CDCl_3$, 100.65 MHz, 4Jan0815/22 [MV032/41/sauberes Azid]): δ = 70.53 (d, J = 6.7 Hz, 1C, OCH), 70.45 (d, J = 6.7 Hz, 1C, OCH), 53.35 (s, 1C, CHN_3), 34.27 (d, J = 142.0 Hz, 1C, CH_2P), 24.06 (d, J = 3.4 Hz, 1C, CH*CH*$_3$), 24.02 (d, J = 3.9 Hz, 1C, CH*CH*$_3$), 24.00 (d, J = 4.4 Hz, 2C, CH*CH*$_3$), 20.84 (d, J = 8.7 Hz, 1C, CH_3).

IR (ATR, [MV032-41]): ν = 2978, 2934, 2104, 1454, 1383, 1347, 1245, 1107, 980 cm^{-1}.

Anal. calc. for $C_9H_{20}N_3O_3P$: C 43.37%, H 8.09%, N 16.86%, O 19.26%; found: C 43.43%, H 8.14%, N 16.86%, O 18.93%.

5.13.5. (±)-Diisopropyl (2-aminopropyl)phosphonate [(±)-**4.45**]

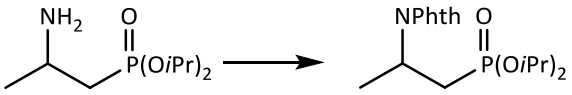


(±)-**4.44** (±)-**4.45**

Synthesis was performed analogously to literature [66].

(±)-Diisopropyl (2-azidopropyl)phosphonate [(±)-**4.44**] (0.77 g, 3.1 mmol) was dissolved in dry isopropyl alcohol (20 mL) and hydrogenated in a Parr apparatus for 3 h at 50 PSI (3.5 bar) using a Pd(10%)/C catalyst (0.19 g, 60 mg/mmol) and HCl (0.12 g, 3.4 mmol, 0.3 mL, 10 M, 1.1 mmol/mmol azide). The mixture was filtered through Celite moistened with isopropyl alcohol and the clear filtrate was concentrated under reduced pressure. The crude (±)-diisopropyl (2-aminopropyl)phosphonate [(±)-**4.45**] as hydrochloride was used in the next step without further purification.

5.13.6. (±)-Diisopropyl (2-phthalimidopropyl)phosphonate [(±)-**4.43**]



(±)-**4.45** (±)-**4.43**

Synthesis was performed analogously to literature [90].

A mixture of (±)-diisopropyl (2-aminopropyl)phosphonate [(±)-**4.45**] as hydrochloride (0.69 g, 3.10 mmol, 1.0 eq.), phthalic anhydride (0.51 g, 3.41 mmol, 1.1 eq.), triethylamine (9.3 mmol, 1.3 mL, 3.0 eq.), dry toluene (25 mL) and molecular sieves (3 g) was refluxed (120 °C bath temperature) for 4 h (TLC: hexanes/EtOAc 1:2). The reaction mixture was filtered and the molecular sieves were washed with CH_2Cl_2. The concentrated filtrate was purified by flash chromatography (Ø2x50 cm, hexanes/EtOAc 1:2, TLC: R_f = 0.25*) and crystallised from CH_2Cl_2/hexanes to give (±)-diisopropyl (2-phthalimidopropyl)phosphonate [(±)-**4.43**] as colourless crystals (0.49 g, 1.4 mmol, 45%); mp. 104-106 °C.

^{1}H NMR ($CDCl_3$, 400.27 MHz, 4Jan0715/150 [MV031/40/umkr]): δ = 7.83-7.77 (m, 2H, H_{ar}), 7.70-7.64 (m, 2H, H_{ar}), 4.80-4.68 (m, 1H, CHN), 4.66-4.53 (m, 2H, OCH), 2.72 (ddd, J = 10.0 Hz, J_{AB} = 15.5 Hz, J = 16.5 Hz, 1H, CH_2P), 2.10 (ddd, J = 5.0 Hz, J_{AB} = 15.5 Hz, J = 19.7 Hz, 1H, CH_2P), 1.53 (dd, J =

2.1 Hz, *J* = 7.0 Hz, 1C, CH_3), 1.23 (d, *J* = 6.2 Hz, 1C, CH*CH*$_3$), 1.21 (d, *J* = 6.2 Hz, 1C, CH*CH*$_3$), 1.15 (d, *J* = 6.2 Hz, 1C, CH*CH*$_3$), 1.09 (d, *J* = 6.2 Hz, 1C, CH*CH*$_3$).

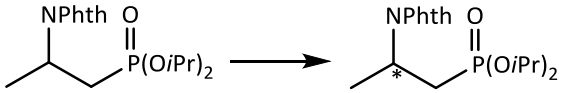
^{31}P NMR ($CDCl_3$, 162.03 MHz, 4Jan0715/151 [MV031/40/umkr]): δ = 24.97 (s).

^{13}C NMR ($CDCl_3$, 100.65 MHz, 4Jan0715/152 [MV031/40/umkr]): δ = 167.96 (s, 2C, CO), 133.78 (s, 2C, C_{ar}), 132.19 (s, 2C, *C*CO), 123.08 (s, 2C, C_{ar}), 70.20 (d, *J* = 7.0 Hz, 1C, OCH), 70.12 (d, *J* = 7.4 Hz, 1C, OCH), 42.22 (d, *J* = 5.2 Hz, 1C, CHN), 31.18 (d, *J* = 142.1 Hz, 1C, CH_2P), 23.96 (d, *J* = 3.7 Hz, 1C, CH*CH*$_3$), 23.92 (d, *J* = 3.7 Hz, 1C, CH*CH*$_3$), 23.80 (d, *J* = 4.9 Hz, 1C, CH*CH*$_3$), 23.79 (d, *J* = 3.8 Hz, 1C, CH*CH*$_3$), 20.58 (d, *J* = 14.6 Hz, 1C, CH_3).

IR (ATR, [MV031-40]): ν = 2973, 2933, 1767, 1701, 1610, 1468, 1370, 1243, 1086 978 cm^{-1}.

Anal. calc. for $C_{17}H_{24}NO_5P$: C 57.79%, H 6.85%, N 3.96%, O 22.64%; found: C 57.96%, H 6.61%, N 4.00%, O 22.60%.

*5.13.6.1. (R)- and (S)-diisopropyl (2-phthalimidopropyl)phosphonate [(R)- and (S)-**4.43**]*



(±)-**4.43** (*R*)- and (*S*)-**4.43**

The racemic product (±)-**4.43** was resolved by HPLC on a chiral stationary phase at RT.

Parameters preparative HPLC:

column:	Chiralpack IC 250x20 mm
particle size:	5 µm
solvent:	hexanes/EtOH 85:15
flow:	15 mL/min

Parameters analytical HPLC:

column:	Chiralpack IC 250x4.6 mm
particle size:	5 µm
solvent:	*n*-heptane + 0.1% *i*PrOH/EtOH 8:2
flow:	0.7 mL/min
retention time:	13.2 min (E1), 16.0 min (E2)

The first enantiomer (E1)-**4.43** was recrystallised from CH_2Cl_2/hexanes and gave large, colourless crystals (0.31 g, 0.9 mmol, ee >99%, $[\alpha]_D^{20}$ = +23.11 (*c* 1.10, acetone), mp. 101 °C). According to literature [91] this enantiomer had (*S*) configuration.

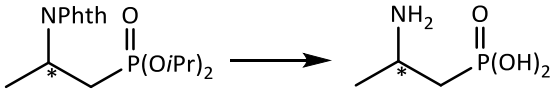
IR (ATR, [MV032-40-E1]): ν = 2974, 2933, 1765, 1700, 1611, 1469, 1370, 1244, 978 cm^{-1}.

The second enantiomer (E2)-**4.43** was recrystallised from CH_2Cl_2/hexanes and gave large, colourless crystals (0.55 g, 1.6 mmol, ee >99%, $[\alpha]_D^{20}$ = -23.68 (*c* 1.06, acetone), mp. 100 °C). According to literature [91] this enantiomer had (*R*) configuration.

IR (ATR, [MV032-40-E2]): ν = 2974, 2933, 1765, 1700, 1611, 1469, 1370, 1244, 978 cm^{-1}.

Spectroscopic data of the enantiomers were identical with those of the racemate (±)-**4.43**.

5.13.7. *(R)- and (S)-(2-aminopropyl)phosphonic acid [(R)- and (S)-**2.12**]*



(*R*)- and (*S*)-**4.43** (*R*)- and (*S*)-**2.12**

To (*S*)-**4.43** (0.38 g, 1.08 mmol) was added HBr (7.0 mL, 48% aqueous solution) and heated at 120 °C for 30 min. The reflux condenser was shortly removed to allow isopropyl bromide to escape. Then the reaction mixture was refluxed (160 °C bath temperature) for 5 h. The solution was concentrated in vacuo. The residue was dissolved in water and applied to a column (Ø1x18 cm) filled with Dowex 50Wx8, H^+ for ion exchange chromatography with water as eluent. Ninhydrin-positive fractions (TLC: R_f = 0.20, *i*PrOH/H_2O/NH_3(25%) 6:3:1) were pooled and concentrated under reduced pressure. Crystallisation from water/EtOH yielded (*S*)-**2.12** as colourless crystals (0.11 g, 0.79 mmol, 73%); $[\alpha]_D^{20}$ = +6.86 (*c* 1.02, water), $[\alpha]_D^{22}$ lit. [91] = +4.80 (*c* 0.78, NaOH); mp. 317 °C (lit. [92] 302-302 °C). The spectroscopic data were in accordance with literature data [91].

^{1}H NMR (D_2O, 400.27 MHz, 4Jan2615/290 [MV032/37/E1umkr]): δ = 3.74-3.61 (m, 1H, CHN), 1.99 (dd, *J* = 6.9 Hz, *J* = 17.6 Hz, 2H, CH_2P), 1.47 (d, *J* = 6.7 Hz, 3H, CH_3).

^{31}P NMR (D_2O, 162.03 MHz, 4Jan2615/291 [MV032/37/E1umkr]): δ = 18.62 (s).

^{13}C NMR (D_2O, 100.65 MHz, 4Jan2615/292 [MV032/37/E1umkr]): δ = 44.82 (s, 1C, CHN), 32.86 (d, *J* = 131.2 Hz, 1C, CH_2P), 19.30 (d, *J* = 9.4 Hz, 1C, CH_3).

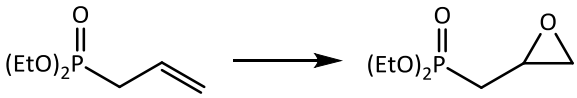
IR (ATR, [MV032-37-E1]): ν = 2940, 2838, 2737, 2646, 25654, 2164, 1628, 1067, 1004 cm^{-1}.

Anal. calc. for $C_3H_{10}NO_3P$: C 25.91%, H 7.25%, N 10.07%, O 34.51%; found: C 25.96%, H 7.27%, N 9.96%, O 34.38%.

Similarly, (*R*)-**4.43** (0.61 g, 1.73 mmol) was converted to (*R*)-**2.12**. Recrystallisation from water/EtOH gave (*R*)-**2.12** as colourless crystals (0.21 g, 1.51 mmol, 87%); $[\alpha]_D^{20}$ = -7.48 (*c* 1.07, water), $[\alpha]_D^{20}$ = -5.33 (*c* 1.07, 1 M NaOH); mp. 319 °C. The analytical data of the (*R*)-enantiomer were identical with those of the (*S*)-enantiomer.

5.14. Synthesis of (3-aminoprop-1-enyl)phosphonic acid (2.13)

*5.14.1. (±)-Diethyl (2,3-epoxypropyl)phosphonate [(±)-**4.46**]*

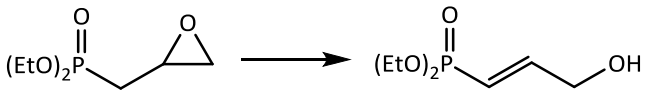


(±)-**4.46**

Synthesis was performed analogously to literature [93].

To an ice cooled solution of diethyl (prop-2-enyl)phosphonate (1.78 g, 10.0 mmol, 1.0 eq.) in dry CH_2Cl_2 (20 mL) was added a solution of *m*CPBA (2.59 g, 15.0 mmol, 1.5 eq.) in dry CH_2Cl_2 (30 mL) under argon atmosphere and stirred for 3 h. After removing the ice bath stirring was continued at RT for 48 h (TLC: EtOAc, R_f = 0.15). To destroy the excess of *m*CPBA a 10% (w/w) aqueous solution of sodium thiosulfate (10 mL) was added. The organic phase was washed with a saturated aqueous solution of $NaHCO_3$ and brine, dried (Na_2SO_4) and concentrated under reduced pressure. (±)-Diethyl (2,3-epoxypropyl)phosphonate [(±)-**4.46**] (1.50 g, 7.7 mmol, 77%) was used in the next step without further purification.

*5.14.2. Diethyl (3-hydroxyprop-1-enyl)phosphonate (**4.47**)*



(±)-**4.46** **4.47**

Synthesis was performed analogously to literature [94].

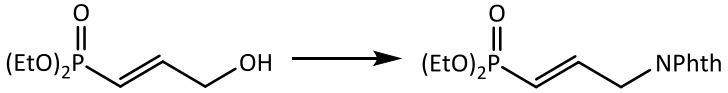
(±)-Diethyl (2,3-epoxypropyl)phosphonate [(±)-**4.46**] (1.50 g, 7.7 mmol, 1.0 eq.) was dissolved in MeOH (5 mL) and a solution of NaOMe (34 mg sodium dissolved in MeOH, 1.5 mmol, 0.2 eq.) was added at 0 °C. After stirring at RT for 2.5 h (TLC: EtOAc/acetone 1:1) Dowex 50Wx8, H^+ (0.40 g) was added. The mixture was filtered 15 min later, the resin was washed with MeOH and the filtrate was concentrated under reduced pressure. The crude product was purified by flash chromatography (Ø2x50 cm, EtOAc/acetone 1:1, TLC: R_f = 0.31) to give diethyl (3-hydroxyprop-1-enyl)phosphonate (**4.47**) as a colourless oil (0.52 g, 2.7 mmol, 35%). The spectroscopic data were in accordance with literature data [94].

^{1}H NMR ($CDCl_3$, 400.27 MHz, 4Dec1714/50 [MV033/44/Fr30]): δ = 6.85 (tdd, *J* = 3.4 Hz, *J* = 17.2 Hz, *J* = 22.5 Hz, 1H, CH=), 5.99 (tdd, *J* = 2.2 Hz, *J* = 17.2 Hz, *J* = 20.5 Hz, 1H, CH=), 4.30 (td, *J* = 2.2 Hz, *J* = 3.4 Hz, 2H, *CH*$_2$OH), 4.11-4.02 (m, 4H, OCH_2), 2.31 (brs, 1H, OH), 1.31 (td, *J* = 0.3 Hz, *J* = 7.0 Hz, 6H, CH_3).

^{31}P NMR ($CDCl_3$, 162.03 MHz, 4Dec1714/51 [MV033/44/Fr30]): δ = 18.75 (s).

^{13}C NMR ($CDCl_3$, 100.65 MHz, 4Dec1714/52 [MV033/44/Fr30]): δ = 151.62 (d, *J* = 5.4 Hz, 1C, *C*H=CH-P), 115.39 (d, *J* = 190.1 Hz, 1C, CH=*C*H-P), 62.60 (d, *J* = 22.3 Hz, 1C, CH_2OH), 61.81 (d, *J* = 5.7 Hz, 2C, OCH_2), 16.35 (d, *J* = 6.5 Hz, 2C, CH_3).

5.14.3. Diethyl (3-phthalimidoprop-1-enyl)phosphonate (**4.48**)



4.47 **4.48**

A mixture of diethyl (3-hydroxyprop-1-enyl)phosphonate (**4.47**) (0.49 g, 2.5 mmol, 1.0 eq.), triphenylphosphine (0.79 g, 3.0 mmol, 1.2 eq.) and phthalimide (0.44 g, 3.0 mmol, 1.2 eq.) was dissolved in dry toluene (2.5 mL) and dry CH_2Cl_2 (0.25 mL) under argon atmosphere. The solution was cooled to 0 °C and DIAD (0.61 g, 3.0 mmol, 1.2 eq.) in dry toluene (3 mL) was added. After stirring for 30 min the cooling bath was removed and stirring was continued at RT for 30 min (TLC: EtOAc/acetone 1:1). The reaction was quenched with MeOH (0.2 mL) and the mixture was concentrated under reduced pressure. The following purification by flash chromatography (Ø2x65 cm, CH_2Cl_2/acetone 4:1, TLC: R_f = 0.55*) gave only one fraction of homogenous product, which was crystallised from CH_2Cl_2/hexanes. Diethyl (3-phthalimidoprop-1-enyl)phosphonate (**4.48**) was obtained as colourless crystals (0.29 g, 0.9 mmol, 36%); mp. 83 °C. The remaining fractions containing also triphenylphosphine oxide were purified by HPLC (Macherey Nagel Nucleosil 50-5, 250x21 mm, 5 µm, hexanes/*i*PrOH 80:20, 10 mL/min).

^{1}H NMR ($CDCl_3$, 400.27 MHz, 4Jan0815/260 [MV033/45/umkr]): δ = 7.89-7.83 (m, 2H, H_{ar}), 7.76-7.71 (m, 2H, H_{ar}), 6.72 (tdd, *J* = 4.9 Hz, *J* = 17.1 Hz, *J* = 22.1 Hz, 1H, CH=), 5.75 (tdd, *J* = 1.7 Hz, *J* = 17.1 Hz, *J* = 18.0 Hz, 1H, CH=), 4.42 (ddd, *J* = 1.8 Hz, *J* = 2.9 Hz, *J* = 4.8 Hz, 2H, CH_2N), 4.11-3.97 (m, 4H, OCH_2), 1.29 (td, *J* = 0.2 Hz, *J* = 7.1 Hz, CH_3).

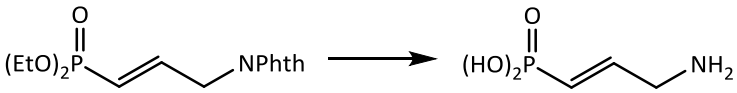
^{31}P NMR ($CDCl_3$, 162.03 MHz, 4Jan0815/261 [MV033/45/umkr]): δ = 16.79 (s).

^{13}C NMR ($CDCl_3$, 100.65 MHz, 4Feb0915/280 [MV033/45/umkr2]): δ = 167.45 (s, 2C, CO), 144.94 (d, *J* = 5.9 Hz, 1C, *C*H=CH-P), 134.24 (s, 2C, C_{ar}), 131.89 (s, 2C, *C*CO), 123.51 (s, 2C, C_{ar}), 119.45 (d, *J* = 188.2 Hz, 1C, CH=*C*H-P), 61.92 (d, *J* = 5.9 Hz, 2C, OCH_2), 39.57 (d, *J* = 25.1 Hz, 1C, CH_2N), 16.30 (d, *J* = 6.4 Hz, 2C, CH_3).

IR (ATR, [MV033-45]): ν = 2986, 2916, 1711, 1421, 1391, 1233, 1113, 1047, 1018, 955 cm^{-1}.

Anal. calc. for $C_{15}H_{18}NO_5P$: C 55.73%, H 5.61%, N 4.33%, O 24.74%; found: C 55.78%, H 5.57%, N 4.52%, O 24.79%.

5.14.4. (3-Aminoprop-1-enyl)phosphonic acid (**2.13**)



4.48 **2.13**

Allyltrimethylsilane (0.16 g, 1.4 mmol, 2.0 eq.) and TMSBr (7.7 mmol, 1.18 g, 1.0 mL, 11 eq.) were added to a solution of diethyl (3-phthalimidoprop-1-enyl)phosphonate (**4.48**) (0.23 g, 0.7 mmol, 1.0 eq.) in dry 1,2-dichloroethane (3 mL). The reaction mixture was stirred at RT for 18 h. The solvent was removed in vacuo (0.5 mbar), the residue was dissolved in 1,2-dichloroethane and concentrated again. EtOH (3 mL) and water (3 mL) were added to the residue, stirred for 5 min and concentrated under reduced pressure. A solution of the residue and hydrazine monohydrate (0.28 g, 5.6 mmol, 0.3 mL, 8.0 eq.) in dry MeOH (3 mL) was heated in a sealed tube at 100 °C for 18 h. On cooling to RT the solution solidified. The solid was dissolved in water (10 mL), transferred into a round bottomed flask and concentrated under reduced pressure. Water was added to the residue and the major portions of the insoluble components were removed by filtration. Remaining impurities were removed by a column filled with Amberlite XAD-4 (40 mL, 50 mL/mmol, washed neutral with water). The product was eluted with water. Finally, the crude product was applied to a column (Ø1x15 cm) filled with Dowex 50Wx8, H^+ for ion exchange chromatography with water as eluent. Ninhydrin-positive fractions (TLC: R_f = 0.16, *i*PrOH/H_2O/NH_3(25%) 6:3:2) were pooled and concentrated under reduced pressure. The residue was crystallised from water/EtOH to give (3-aminoprop-1-enyl)phosphonic acid (**2.13**) as white solid (54 mg, 0.4 mmol, 57%); decomposition at 240 °C (lit. [95] decomposition at 225 °C).

^{1}H NMR (D_2O, 400.27 MHz, 4Jan2015/280 [MV033/46/umkr]): δ = 6.46 (tdd, *J* = 5.6 Hz, *J* = 17.4 Hz, *J* = 20.3 Hz, 1H, CH=), 6.22-6.11 (m, 1H, CH=), 3.82-3.77 (m, 2H, CH_2N).

^{31}P NMR (D_2O, 162.03 MHz, 4Jan2015/281 [MV033/46/umkr]): δ = 9.98 (s).

^{13}C NMR (D_2O, 100.65 MHz, 4Jan2015/282 [MV033/46/umkr]): δ = 135.06 (d, *J* = 5.0 Hz, 1C, *C*H=CH-P), 128.57 (d, *J* = 174.5 Hz, 1C, CH=*C*H-P), 41.08 (d, *J* = 23.3 Hz, 1C, CH_2N).

IR (ATR, [MV033-46]): ν = 2841, 2752, 2649, 2190, 1636, 1549, 1437, 1255, 1125, 1061 cm^{-1}.

Anal. calc. for $C_3H_8NO_3P$: C 26.29%, H 5.88%, N 10.22%, O 35.02%; found: C 26.42%, H 5.55%, N 9.98%, O 34.75%.

6. Summary

Organophosphorus compounds have useful biological properties due to a direct and stable P-C bond. They find numerous applications as antibiotics and herbicides in the medicine and agriculture, for example. Natural phosphonates are widespread in the environment, especially in marine ecosystems as they present an important alternative source of phosphorus. Its bioavailable form, inorganic phosphorus P_i, can be a limiting nutrient for microbial growth in certain habitats. Considering the occurrence of phosphonates it is not surprising that there exist many pathways for their biosynthesis and biodegradation. However, just a little is known about the mechanisms of enzymatic breakdown, but efforts are undertaken to unravel the underlying mechanisms. Attention is actually paid to a pair of coupled enzymes, PhnY and PhnZ, known for catalysing the oxidative P-C bond cleavage of 2-AEP using a mixed-valent cofactor and dioxygen. PhnY stereospecifically hydroxylates at the α-C position of 2-AEP to produce (*R*)-1-OH-2-AEP. The latter is then used in a stereospecific reaction by the coupled enzyme PhnZ, which catalyses the oxidative P-C bond cleavage of the (*R*)-enantiomer. The specific recognition of the (*R*)-enantiomer is based on its orientation in the active site of the enzyme [31].

To study the degradation mechanism of 2-AEP and 1-OH-2-AEP by PhnY and PhnZ, respectively, eleven analogues of these substrates were synthesised and tested in cooperation with the group of Dr. David L. Zechel (Queen's University, Ontario, Canada). Syntheses were carried out by the easiest possible way and involved basic organic reactions as well as special phosphorus name reactions like the Mitsunobu, Arbuzov, Abramov and Pudovik reaction. The synthesised substrates were identified by NMR spectroscopy. In addition, new compounds were also characterised by IR spectroscopy, combustion analysis, the melting point and the specific optical rotation. The optical purity was determined by chiral HPLC.

To test the compounds as substrates for PhnY and PhnZ, they were incubated with these enzymes. ^{31}P NMR spectra were recorded before and after the addition of enzyme to detect signals of reaction products.

7. Zusammenfassung

Organophosphor-Verbindungen verfügen über eine direkte, stabile P-C Bindung. Sie finden zahlreiche Anwendungen als Antibiotika und Herbizide in der Medizin und Landwirtschaft. Natürlich vorkommende Phosphonate sind in der Umwelt, vor allem in marinen Ökosystemen, weit verbreitet und stellen eine wichtige alternative Quelle für Phosphor dar. Seine biologisch verfügbare Form, anorganisches Phosphat, ist ein wichtiger Nährstoff für mikrobielles Wachstum und der limitierende Faktor in bestimmten Habitaten. Betrachtet man die Häufigkeit von Phosphonaten, ist es nicht überraschend, dass viele Wege für deren Biosynthese und Bioabbau existieren. Über die genauen enzymatischen Abbaumechanismen ist jedoch wenig bekannt, wodurch sich dieses Thema zu einem wichtigen und aktuellen Forschungsgebiet entwickelt hat. Augenmerk liegt derzeit auf einem Paar gekoppelter Enzyme, PhnY und PhnZ, von denen bekannt ist, unter Verwendung eines gemischtwertigen Cofaktors und Luftsauerstoff die oxidative Spaltung der P-C Bindung zu katalysieren. PhnY ist für die stereospezifische Hydroxylierung der α-Position von 2-AEP verantwortlich. Die auf diesem Weg produzierte (*R*)-1-OH-2-AEP dient als Substrat für das gekoppelte Enzym PhnZ, welches anschließend die oxidative Spaltung der P-C Bindung katalysiert. Die spezifische Erkennung des (*R*)-Enantiomers basiert auf dessen Orientierung im aktiven Zentrum des Enzyms.

Um den Abbaumechanismus von 2-AEP und 1-OH-2-AEP durch PhnY beziehungsweise PhnZ zu untersuchen, wurden elf Analoga dieser Substrate synthetisiert und in Kooperation mit der kanadischen Arbeitsgruppe von Dr. David L. Zechel (Queen's University, Ontario, Kanada) getestet. Die Synthese der Zielverbindungen erfolgte auf einfachst möglichem Wege und bediente sich sowohl grundlegender organischer Reaktionen als auch spezieller Reaktionen der Phosphorchemie. Dabei kamen Namensreaktionen wie die Mitsunobu-, Arbuzov-, Abramov- und Pudovik-Reaktion zum Einsatz. Die synthetisierten Substrate wurden mittels NMR-Spektroskopie identifiziert. Neue Substanzen wurden zudem mittels IR-Spektroskopie, Elementaranalyse, Schmelzpunkt und Drehwert charakterisiert. Die optische Reinheit wurde mittels chiraler HPLC bestimmt.

Um die Verbindungen als Substrate für PhnY und PhnZ zu testen, wurden sie mit diesen Enzymen inkubiert. ^{31}P NMR-Spektren wurden vor und nach der Zugabe von Enzym zur Reaktionsmischung aufgenommen, um Signale für Reaktionsprodukte festzustellen.

8. Acknowledgements/Danksagung

Für meine liebe Familie,

ein Dankeschön wäre nicht genug...

So einige Jahre sind vergangen,
seit ich mit meinem Studium habe angefangen.
Für eure Unterstützung möchte ich mich heute bedanken,
darum hier, ein paar meiner Gedanken.
An meiner Schwester, Kristina, schätze ich besonders ihr offenes Ohr,
und dass sie nie ein schlechtes Urteil über etwas verlor.
Für das, was meine Mama leistet, reichen kaum Worte.
Sie kümmert sich einfach um alles und Probleme jeder Sorte.
Meinem Vati danke ich für die große Unterstützung meiner Finanzen,
dem Taxiservice und seine Art im Ganzen.
Dass ihr immer für mich da seid, weiß ich sehr zu schätzen,
und dass ich mein Vertrauen kann stets in euch setzen.
Mit Zusammenhalt, Rückhalt und auch Spaß,
da schafft man im Leben leichter was.
Denn ohne euch wäre ich nie
Master of Science der Chemie!

Lieber Fritz!
Ich möchte mich vielmals für die Möglichkeit bedanken, Teil deiner Arbeitsgruppe gewesen sein zu dürfen. Ich habe deine kompetente, fachliche Unterstützung, dein scheinbar endloses Wissen, deine Geduld, deine Gelassenheit, dein Verständnis und deinen Sinn für Humor immer sehr geschätzt. Ich fand es toll, dass du im Labor direkt mit uns zusammen gearbeitet hast und wir so viel von dir lernen konnten.

Liebe Kathi, liebe Petra, lieber Renzhe, lieber Thomas!
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"Nichts ist wertvoller als ein guter Freund, außer ein Freund mit Schokolade." – Charles Dickens.

Dear David!
I wanna thank you and your team for the great cooperation and for testing my substances!

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9. Curriculum Vitae

PERSONAL INFORMATION

MARGRET VOGT, BSC

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Sex Female
Date of birth 16/08/1989
Nationality Austria

COURSE OF STUDY

2012-2015 Master studies in Chemistry

University of Vienna

Study focus:

- Bioanalytical Chemistry
- Bioinorganic Chemistry
- Environmental Chemistry
- Complex- and Radiochemistry
- Biochemistry and Structure Biology
- Bioorganic Chemistry

Title of Master thesis:

Synthesis of phosphonic acid analogues of 2-AEP and 1-OH-2-AEP as substrates for PhnY and PhnZ; *Supervisor:* Ao. Univ.-Prof. Dr. Friedrich Hammerschmidt, *Planned degree:* MSc (Master of Science)

2008-2012 Bachelor studies in Chemistry

University of Vienna

Title of Bachelor thesis:

Torfmoor-Huminstoffe als kolloidale Eisenträger unter mesomixohalinen Bedingungen; *Supervisor:* Ao. Univ.-Prof. Mag. Dr. Regina Krachler, *Degree:* BSc (Bachelor of Science)

Teaching Tutor in the lab course „Biologisch-chemisches Praktikum, Teil B“ from summer semester 2013 until winter semester 2014

EDUCATION

2003-2008 High-school diploma — ISCED 3A

Commercial college *BHAK Gänserndorf*

1999-2003 Music junior high school *Musikhauptschule Dürnkrut* — ISCED 2A

1995-1999 Primary school *Volksschule Jedenspeigen* — ISCED 1

WORK EXPERIENCE

2015 CTA (10 h)

University of Vienna, Department of Biological Chemistry

Responsibilities:

- Solid phase peptide synthesis on the synthesisers Liberty Blue™ from CEM and Tribue™ from PTI
- Analytical and preparative HPLC

2005-2013 **Internships:**

- Siemens Enterprise Communications GmbH
- IBM Österreich Internationale Büromaschinen GmbH
- Habring Uhrentechnik OG
- Wiener Zeitung GmbH

PUBLICATIONS

Krachler, R.; von der Kammer, F.; Jirsa, F.; Süphandag, A.; Krachler, R. F.; Plessl, C.; Vogt, M.; Keppler, B. K.; Hofmann, T. *Global Biogeochem. Cycles* **2012**, *26*, GB3024/1-GB3024/9.

10. Abbreviations

1-OH-2-AEP	(2-Amino-1-hydroxyethyl)phosphonic acid
2-AEP	(2-Aminoethyl)phosphonic acid
2-HEP	(2-Hydroxyethyl)phosphonate
ABC	ATP-binding cassette
AcCoA	Acetyl coenzyme A
ADH	Alcohol dehydrogenase
Ala, A	Alanine
AP	Alkaline phosphatase
Ar	Aromatic
Arg, R	Arginine
Asn, N	Asparagine
Asp, D	Aspartate
ATP	Adenosine triphosphate
ATR	Attenuated total reflectance
Boc	*tert*-butoxycarbonyl
c	Concentration
Cys, C	Cysteine
DAST	Diethylaminosulfur trifluoride
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DIAD	Diisopropyl azodicarboxylate
DMF	Dimethylformamide
DMP	Dess–Martin periodinane
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DOP	Dissolved organic phosphorus
D*t*BAD	Di-*tert*-butyl azodicarboxylate
E. coli	*Escherichia coli*
EDTA	Ethylenediaminetetraacetic acid
ee	Enantiomeric excess
EPR	Electron paramagnetic resonance
Eq.	Equivalents
Et	Ethyl
EtOAc	Ethyl acetate
EtOH	Ethanol
Gln, Q	Glutamine
Glu, E	Glutamate
Gly, G	Gylcine
HD	Histidine-aspartate
His, H	Histidine
HMDS	Hexamethyl disilazane

HPLC	High performance liquid chromatography
Hz	Hertz
*i*Pr	Isopropyl
*i*PrOH	Isopropyl alcohol
IR	Infrared spectroscopy
kcal mol^{-1}	Kilocalorie per mol
LTTRs	Transcriptional regulators of the LysR family
Lys, K	Lysine
M	Molarity
*m*CPBA	*meta*-Chloroperoxybenzoic acid
Me	Methyl
MeOH	Methanol
Met, M	Methionine
MI	*Myo*-inositol
MIOX	*Myo*-inositol oxygenase
mp.	Melting point
Ms	Mesylate (methanesulfonyl)
MsCl	Mesylchloride
MVDO	Mixed-valent diiron oxygenase
M*X*	Metal ion
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NaOMe	Sodium methoxide
NBS	*N*-bromo succinimide
*n*BuLi	*n*-Butyllithium
NEt_3	Triethylamine
nm	Nanometers
NMR	Nuclear magnetic resonance
Nu	Nucleophile
P	Phosphorus
$P(O)Ph_3$	Triphenylphosphine oxide
PEP	Phosphoenolpyruvate
PEP mutase	Phosphoenolpyruvate phosphomutase
Pho	Phosphate
Phth	Phthalimido
P_i	Inorganic phosphate
PnA	Phosphonoacetate
PnAA	Phosphonoacetaldehyde
PnMeM	2-Phosphonomethylmalate
PnPy	Phosphonopyruvate
PnPy decarboxylase	Phosphonopyruvate decarboxylase
PPh_3	Triphenylphosphine

PP_i	Pyrophosphate
ppm	Parts per million
Prcp	5-phosphoribosyl-1,2-cyclic phosphate
Prp	5-phosphoribosyl-1-phosphonate
PSI	Pound-force per square inch
PTT	Phosphinothricin tripeptide
Pyr	Pyruvate
R	Residue
R_f	Retardation factor
rpm	Revolutions per minute
RT	Room temperature
SAM	*S*-adenosylmethionine
Ser, S	Serine
*t*BuOK	Potassium *tert*-butoxide
TCS	Two-component system
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
Thr, T	Threonine
TLC	Thin-layer chromatography
TMS	Trimethylsilyl
TMSBr	Bromotrimethylsilane
Tris	Tris(hydroxymethyl)aminomethane
Tyr, Y	Tyrosine
UV	Ultraviolet
α-KG	α-Ketoglutarate

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