universität wien

# MASTERARBEIT

Titel der Masterarbeit

„Synthesis of (*R*)-phosphaproline, (*R*)-phosphaproline analogues and a zoledronic acid derivative“

verfasst von

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**DANKE!**

You miss 100% of the shots you don´t take [Wayne Gretzky]

Alle sind verrückt und ich bin ein Flugzeug (Všetkým šibe iba ja som lietadlo) [Petra Križková]

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# 1. **Organophosphorus compounds**

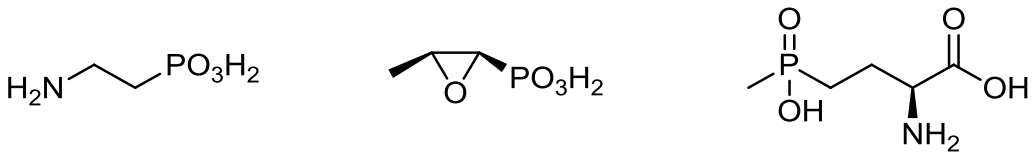
All living organisms mainly consist of proteins, nucleic acids, polysaccharides and lipids. All of these compounds are built up by different subunits. Proteins consist of 22 different proteinogenic amino acids, polysaccharides of sugar molecules and nucleic acids of eight different nucleotides.[1] DNA is also comprised of a phosphate ester **1.1** (e.g.: C-O-P, *Figure 1*) moiety, which in addition to being important for genetic information, is essential for the synthesis of cellular fuel, ATP.

**1.1** **1.2**

*Figure 1.1. General structure of phosphates **1.1** and phosphonates **1.2**.*

This subject has been researched a lot over the last years.[2] However, there is an assumption that when the Earth was young, phosphonates **1.2** (e.g.: C-P, *Figure 1*) have been more dominating than phosphates due to low oxygen concentration.[3] Over the last decades phosphonates have gained much attention, due to their versatile applications from medicine to agriculture.

2-Aminoethylphosphonic acid (**1.3**) (*Figure 1.2*) was first synthesized in 1943[4], but it was not possible to identify this organophosphorus compound until 1959, when Horiguchi was the first to extract it from *rumen Protozoa*.[5]



**1.3** **1.4** **1.5**

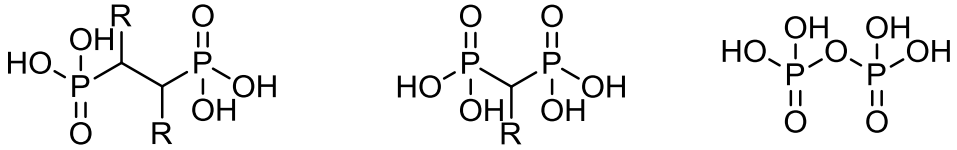
*Figure 1.2. Structure of 2-aminoethylphosphonic acid (**1.3**), fosfomycin (**1.4**) and phosphinothricin (**1.5**).*

Since then, other phosphonates have been isolated from different organisms. For instance, lower marine invertebrates have a higher phosphonates to phosphates ratio than any other organism.[6] Usually, phosphonates are found as side groups on saccharides and glycoproteins. This could be due to the fact that the C-P bond is stable in presence of phosphatases, and thus are not degraded.[7]

Natural occurring phosphonates which possess antibiotic properties are isolated from bacteria. Fosfomycin (**1.4**), an antibacterial agent, as well as phosphinothricin (**1.5)**, a herbicide, are isolated from *Streptomyces* spp. (*Fig 1.2*).[8]

Compounds **1.3** and **1.5** might be considered to be phosphorylated analogues of amino acids, which can be used as inhibitors of metabolic processes, neuroactive compounds, anticancer drugs and pesticides.[9] Due to their high structural identity to amino acids, aminophosphonic acids have been researched for the last decades.

However, organic compounds may contain more than one phosphorus. *Fig 1.3* shows different diphosphorus compounds: diphosphonates **1.6**, bearing two phosphorus moieties on different carbon atoms.




**1.6** **1.7** **1.8**

*Fig 1.3. General structure of diphosphonates **1.6**, bisphosphonates **1.7** and pyrophosphate **1.8**.*

Bisphosphonates (BP) **1.7**, which are structural analogues of pyrophosphate (PP) **1.8**, have these phosphorus functionalities on the same carbon atom.

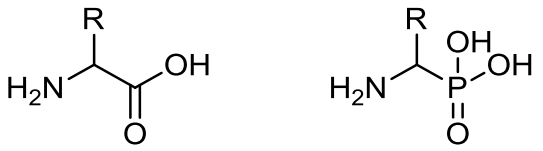
Bisphosphonates are well known compounds since the 1860s. Their early use was not for medicinal purposes, but rather for textile and oil industries or as corrosion inhibitors. [10] Like all other organophosphorus compounds, bisphosphonates are more or less stable against enzymatic hydrolysis. They have a high affinity to calcium in bone tissue, but their interactions with cells might be of greater interest. They are not only provided due to the bisphosphonic moiety, but also due to the side chains carrying a hydroxyl or nitrogen-containing group. [11] Due to their high affinity to bone tissue, bisphosphonates are capable of modulate calcification *in vivo* as well as *in vitro*. In comparison to pyrophosphate, bisphosphonates are active as oral drugs to prevent pathological bone demineralization. [12]

With all these considerations in mind, bisphosphonates extended their application from industrial use to medicine. Nowadays they are used for treatment of bone diseases where osteoclasts are important, like Paget´s disease, hypercalcemia of malignancy or osteoporosis.

# 2. α-Aminophosphonic acids

## 2.1 General remarks

As already mentioned, aminophosphonic acids **2.1** are analogues of amino acids **2.2** (*Fig 2.1*).

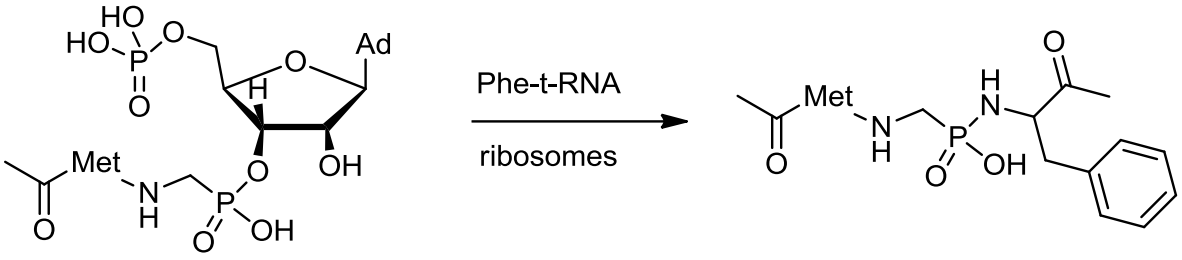



**2.2** **2.1**

*Fig 2.1. General structure of amino acids **2.2** and aminophosphonic acids **2.1**.*

They can act as "false" substrates by competing with the carboxylic counterpart in the active site of an enzyme.9 This is surprising because the phosphonic group is different in size, shape and acidity from the carboxylic group.

However, there are many enzymes that recognize phosphonic acids in addition to their carboxylic counterpart. Up to now, inhibition of many enzymes like glutamine synthetase[13], alanine racemase[14] and phenylalanine ammonia lyase [15] was discovered. In these cases, the structural differences between the phosphonic and carboxylic groups are not important, because the acid functionality is not participating in the reaction in the active site. Until 1994 only one exception was reported, namely, the direct P-N bond formation of phosphonamide **2.4** using a phosphonopeptide **2.3** and phenylalanine t-RNA[16] from *Escherichia coli* (*Scheme 2.1*).



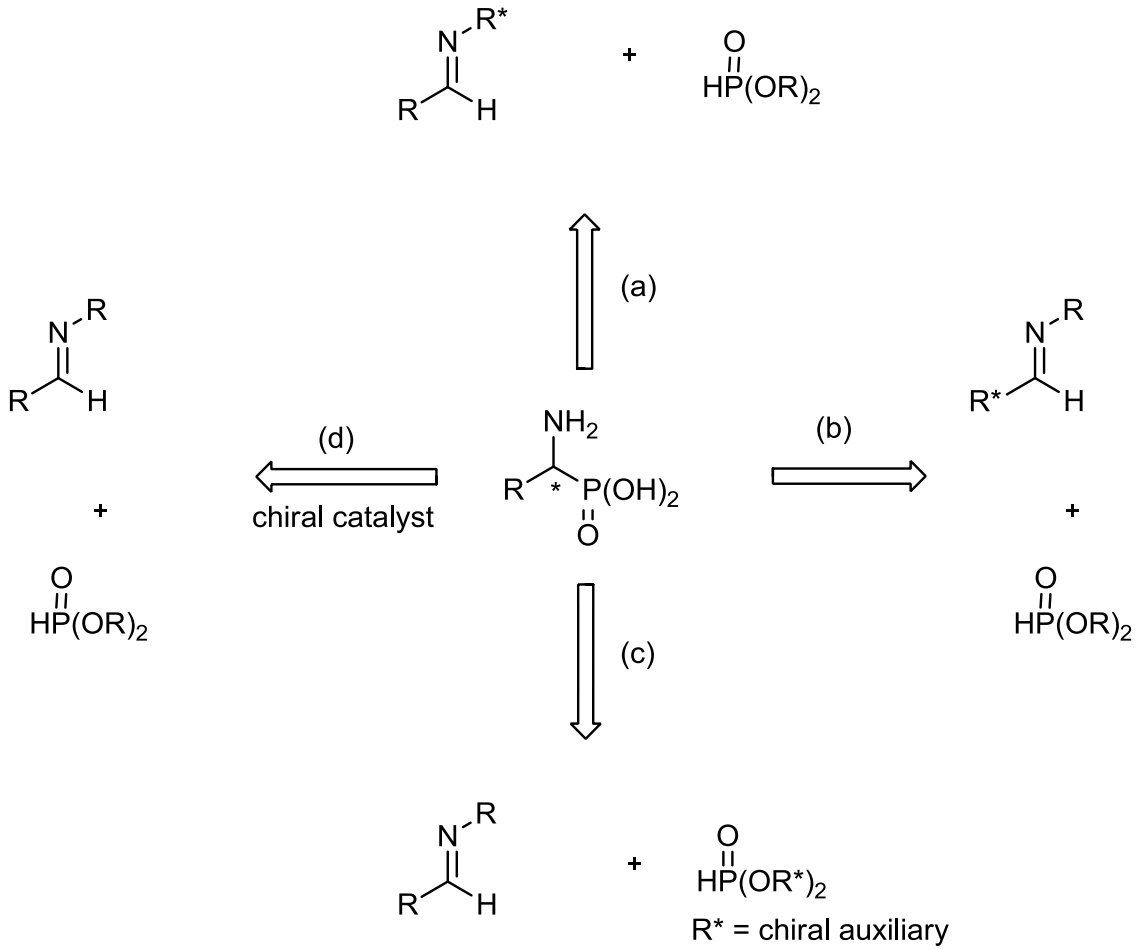

**2.3** **2.4**

*Scheme 2.1. Synthesis of phosphonamide **2.4**.*

## 2.2 Synthesis of α-aminophosphonic acids and their derivatives

### 2.2.1 Asymmetric formation of C-P compounds

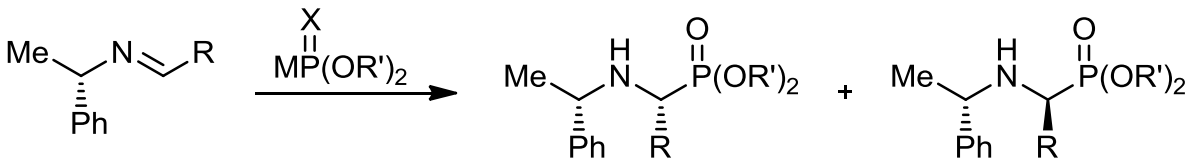
There are several methods for alkylation of phosphorus compounds. The Pudovik reaction[17] is one of the most important methods to prepare α-aminophosphonic acids. Related to it, there are four methods to form α-aminophosphonates asymmetrically: (a) via nucleophilic addition of dialkyl phosphites to chiral imines obtained by condensation of chiral amines and non-chiral aldehydes; (b) condensation of non-chiral amines and chiral aldehydes followed by addition of dialkyl phosphites; (c) addition of chiral alkyl phosphites to non-chiral imines and (d) addition of non-chiral dialkyl phosphites to non-chiral imines using an asymmetric catalyst (*Scheme 2.2*).[18]




*Scheme 2.2. Overview of synthetic strategies to obtain α-aminophosphonic acids.*

**2.2.1.1 Nucleophilic addition of alkyl phosphites to chiral imines**

Gilmore and McBride[19] were the first to synthesize enantiomerically pure α-aminophosphonic acids, starting from (*S*)-**2.5a**, which was phosphorylated using diethyl phosphite to obtain (*R,S*)-**2.6a** and (*S,S*)-**2.7a**, with a diastereomeric ratio of 66:34 (*Scheme 2.3*).[20]



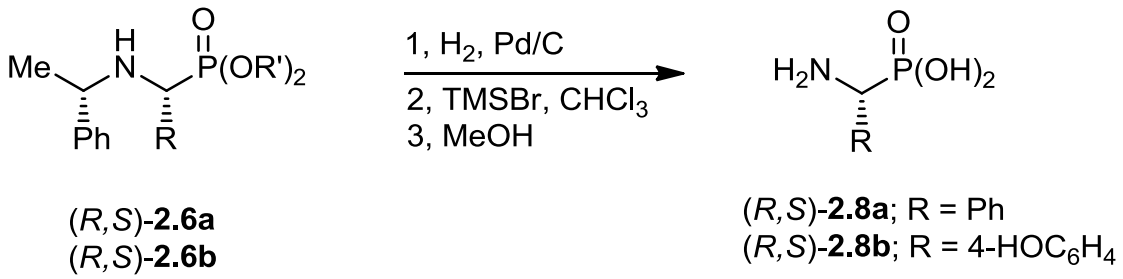

(*S*)-**2.5a-c** (*R,S*)-**2.6a-d** (*S,S*)-**2.7a-d**

| Product | R | R' | X | M | yield (%) | (*R,S*):(*S,S*) |
|---|---|---|---|---|---|---|
| a | Ph | Et | O | H | n.a. | 66:34 |
| b | 4-$HOC_6H_4$ | Et | O | Na | 98 | 97.5:2.5 |
| c | *c*-Hexyl | Et | O | H | 70 | 83:17 |
| d | Ph | Me | S | H | 64 | 76:24 |

*Scheme 2.3. Synthesis of protected α-aminophosphonic acids.*

To increase the moderate diastereomeric ratio, the addition of diethyl phosphite to the imine (*S)*-**2.5c**, synthesized from the cyclohexanecarbaldehyde, was performed to obtain (*R,S*)-**2.6c** and (*S,S*)-**2.7c** with a distereomeric ratio of 83:17.[21] Using an excess of sodium diethyl phosphite with imine (*S*)-**2.5b** led to α-aminophosphonates (*R,S*)-**2.6b** and (*S,S*)-**2.7b** in 98% yield and 97.5:2.5 diastereomeric ratio.[22]

(*R,S*)-**2.6a** and (*R,S*)-**2.6b** were easily converted to the deprotected α-aminophosphonic acids **2.8** using Pd/C/$H_2$ for reductive removal of the benzyl group from the amine (*Scheme 2.4*).



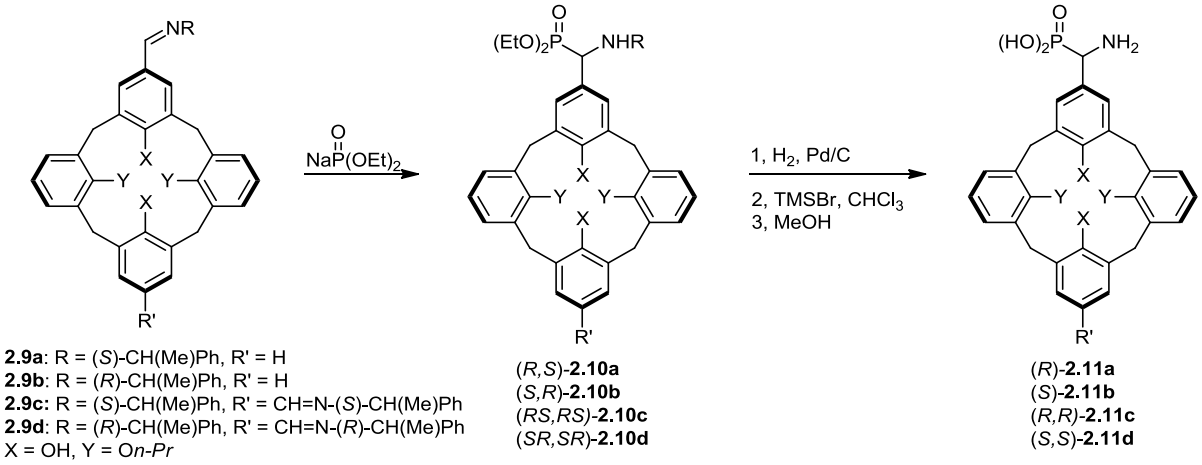

(*R,S*)-**2.6a**
(*R,S*)-**2.6b**

(*R,S*)-**2.8a**; R = Ph
(*R,S*)-**2.8b**; R = 4-$HOC_6H_4$

*Scheme 2.4. Deprotection of α-aminophosphonic acids.*

Trimethylsilyl bromide was necessary to deprotect the phosphonate group to obtain the enantiomerically pure compound **2.8**.

The same procedure has been used for the synthesis of calix[4]arene α-aminophosphonic acids **2.11**. Addition of the diethyl phosphite to the iminocalix[4]arenes **2.9a-d** gave the phosphonates **2.10a-d** in good yields (60-80%) and 75-85% diastereomeric excess (*Scheme 2.5*).

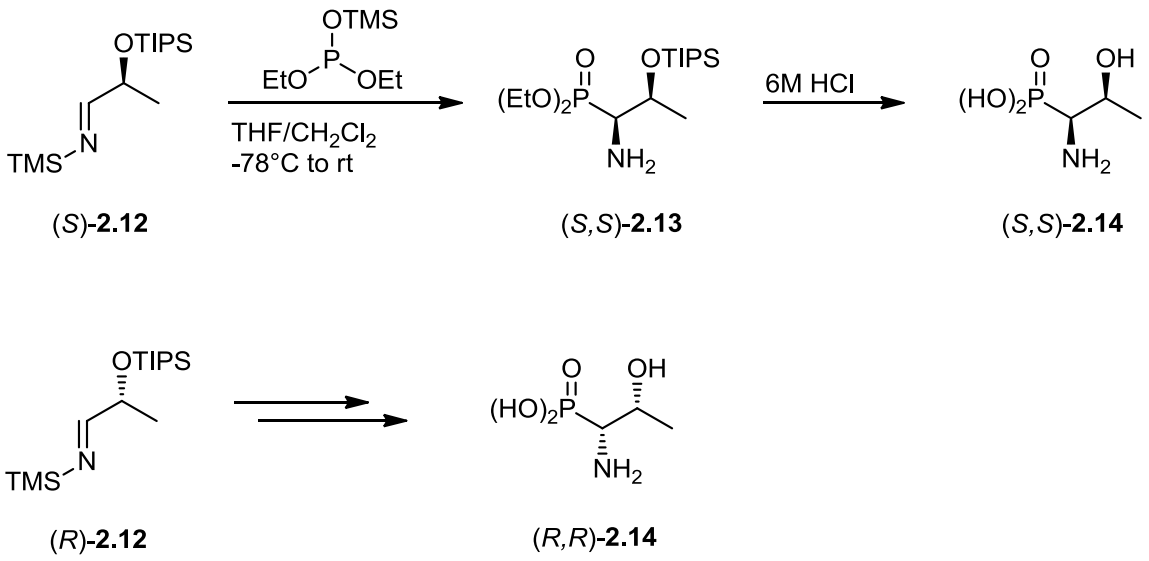



*Scheme 2.5. Synthesis of calix[4]arene α-aminophosphonic acids.*

Removing the chiral benzyl group from the nitrogen atom by hydrogenolysis and removal of the ethyl groups from phosphorus with trimethylsilyl bromide gave the enantiomerically pure α-aminophosphonic acids **2.11a-d** in quantitative yields. Their ability to inhibit porcine kidney alkaline phosphatase (PKAP) is highly dependent on the stereochemistry. Therefore, the $K_i$ of (*S*)-**2.11b** is only half of the $K_i$ of its enantiomer. Also the binding strength of (*R,R*)-**2.11c** to PKAP is about 50 times higher than that of (*S,S*)-**2.11d** enantiomere.[22]

## 2.2.1.2 Nucleophilic addition of dialkyl phosphites to imines synthesized from chiral ketones and aldehydes

In 1996, Bongini et al.[23] were the first to report the synthesis of diastereomerically pure phosphonothreonine. Addition of diethyl trimethylsilyl phosphite to imine (*S*)-**2.12** gave the aminophosphonate (*S*)-**2.13** with a diastereomeric ratio of >98:2 and 85% yield (*Scheme 2.5*).




*Scheme 2.5. Synthesis of phosphonothreonine.*

Deprotection of the hydroxyl and the phosphonate moieties using HCl (6 M) gave the (*S,S*)-phosphonothreonine **2.14** in quantitative yields. The same procedure can be used for the (*R,R*)-enantiomer starting from the imine (*R*)-**2.12**.

To explain the high diastereoselectivity computational studies were performed. It has been found, that there are two reasons for the high *syn* diastereoselectivity for the addition of a dialkyl trimethylsilyl phosphite to an imine: a) the α-silyloxy group induced a high degree of *syn* diastereoselectivity, without a Lewis acid and b) due to the bulkiness of the silyl group (*Figure 2.2*).

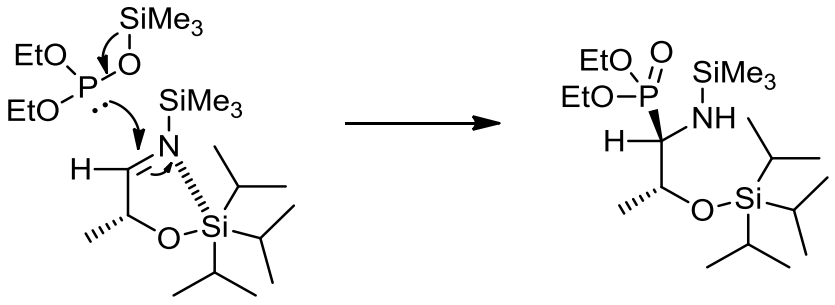
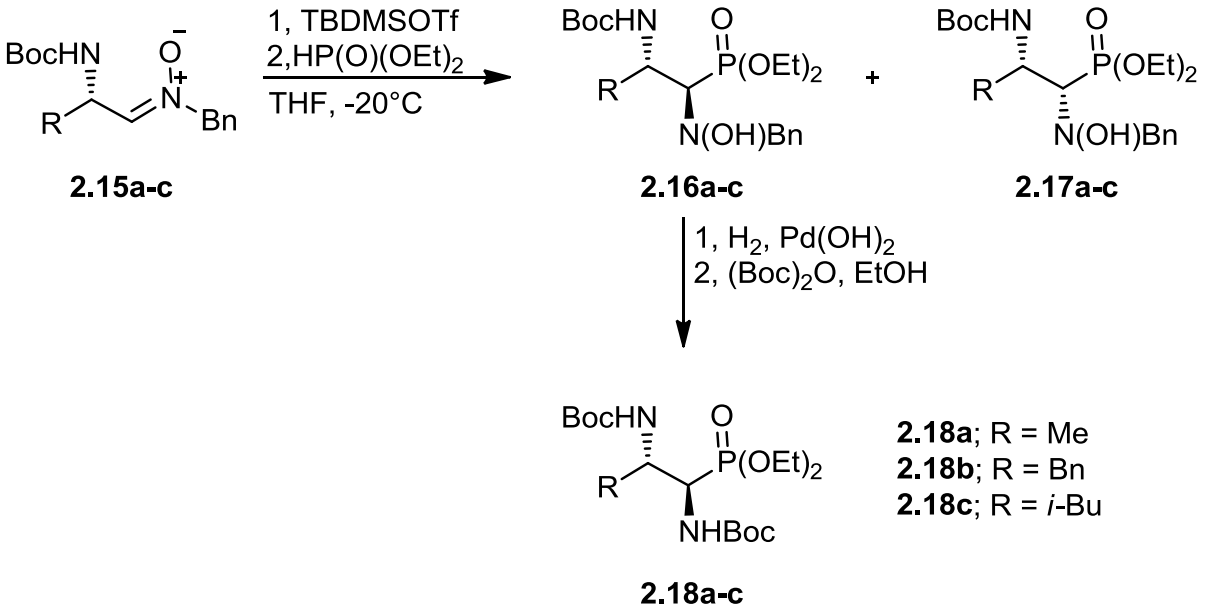



*Fig 2.2. Transition state for the addition of $(EtO_2)POSiMe_3$ to (S)-**2.12**.*

Since the reactions were carried out at -78°C, where the tautomeric structure of $(EtO)_2PO$-$SiMe_3$ is stabilized, a concerted [2+3] cycloaddition was observed.

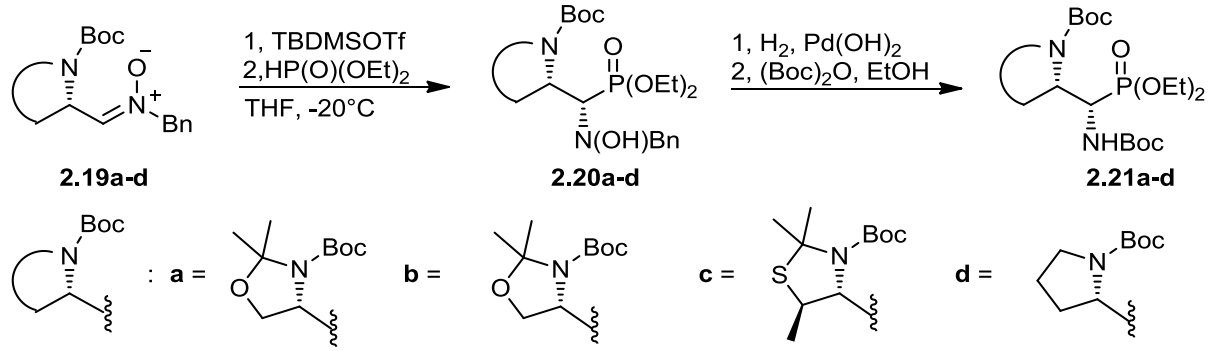
Another way of synthesizing α-aminophosphonic acids via nucleophilic addition of dialkyl phosphites to imines derived from carbonyl compounds, is using *N*-benzyl nitrones **2.15a-c**. Complexation of nitrones with *tert*-butyldimethylsilyl triflate (TBDMSOTf) followed by addition of diethyl phosphite led to *α,β*–aminophosphonates **2.16a-c** and **2.17a-c** in 80% yield and 95:5 diastereomeric ratio in favour of the *anti*-product (*Scheme 2.6*).




*Scheme 2.6. Synthesis of α-aminophosphonic acids via N-benzylnitrones.*

Hydrogenation with $H_2/Pd(OH)_2$ and protection of the amines using $(Boc)_2O$ gave the *N,N*-diprotected *α,β*-diaminophosphonates **2.18a-c** in moderate yields (60-66%).[24]

The same method has been used for the *N*,*N*-diprotected nitrones **2.19a-d**. Treating these compounds with TBDMSOTf, followed by addition of diethyl phosphite led to the *syn*-products **2.20a-d** with good yields (70-88%) and a diastereomeric excess of 100% (*Scheme 2.7*).




*Scheme 2.7. Synthesis of α-aminophosphonic acids via N,N´-disubstituted benzylnitrones.*

Catalytic reduction of the hydroxylamine to the amine which was protected gave the *α,β*-diaminophosphonates in moderate yields (60-80%).[25]

To explain the stereoselectivity of this reaction two possible transition states have been reported.[25] In both cases the silyl group coordinates to the nitrone oxygen atom and the carbonyl group of the Boc group. As shown in *Fig 2.3*, the addition of silylated diethyl phosphite to *N*-monosubstituted compounds **2.15a-c** appears to be from the less hindered side of transition state **2.22**, resulting in the *anti*-adducts **2.16a-c**.

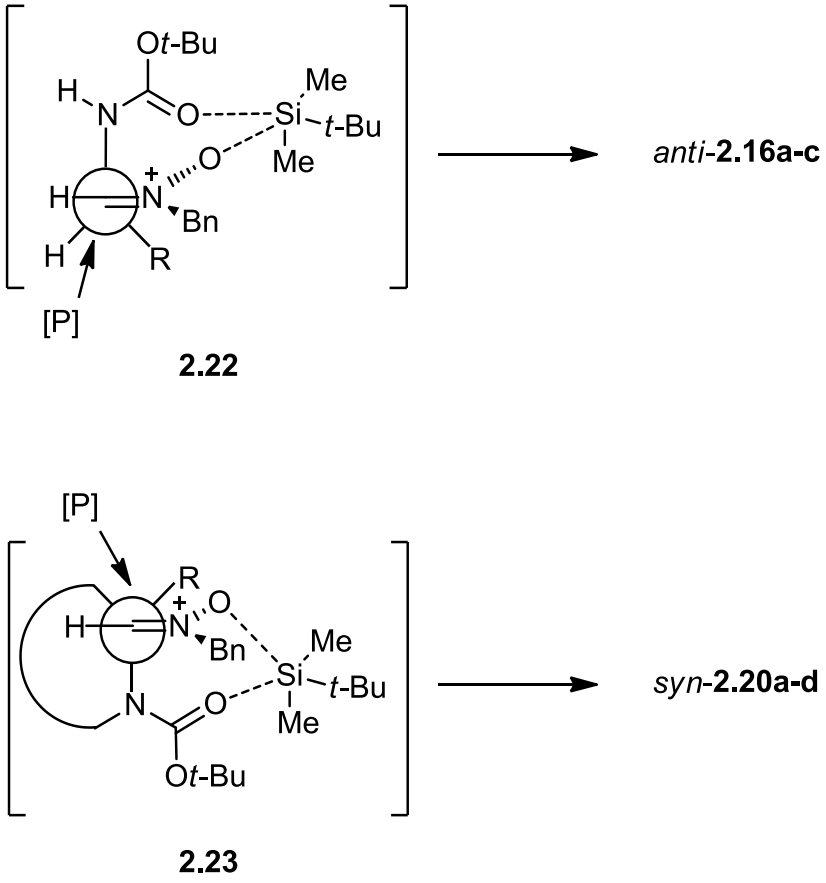


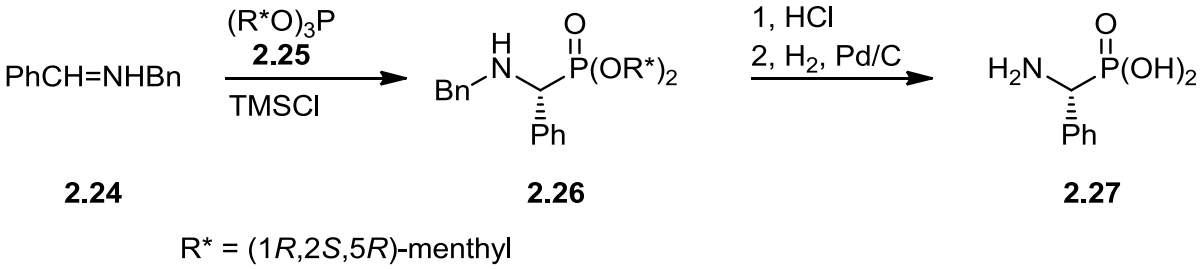

*Fig 2.3. Transition states for the addition of $(EtO)_2POSiMe_3$ to **2.16** and **2.20**.*

For *N,N´-disubstituted* derivatives **2.19a-d** only the *re*-attack should be possible, due to the less hindered transition state **2.23**, leading to the *syn*-products **2.20a-d**.

**2.2.1.3 Nucleophilic addition of asymmetric alkyl phosphites to chiral and non-chiral imines**

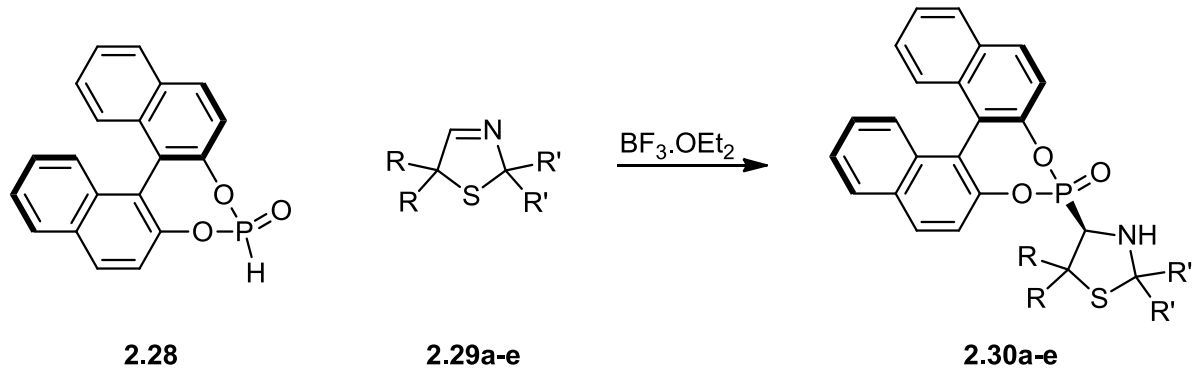
Stereochemistry does not have to be necessarily introduced with a chiral imine, but can be inserted via the phosphonic moiety. Kolodiazhnyi et al.[26] used a chiral trialkyl phophite for the asymmetric synthesis of α-aminophosphonic acids. Nucleophilic addition of tris[(1*R*,2*S*,5*R*)-menthyl] phosphite (**2.25**) to imine **2.24** with TMSCl gave the protected phosphonate **2.26** in good yield with diastereomeric excess of 50%. Diastereomerically pure compound **2.26** was

hydrolysed with HCl, followed by hydrogenolysis using $H_2$/Pd/C to give the α-aminophosphonic acid **2.27** with 95% ee (*Scheme 2.8*).




*Scheme 2.8. Asymmetric synthesis of α-aminophosphonic acids via chiral phosphite.*

Chiral auxiliaries can be removed by hydrolysis of the phosphonic moiety. Therefore the addition of BINOL-phosphite **2.28** to non-chiral 3-thiazolines **2.29a-e** leads to the corresponding phosphonates **138a-e** with diastereoselectivities greater 95% when $BF_3.OEt_2$ is added (*Scheme 2.9*).

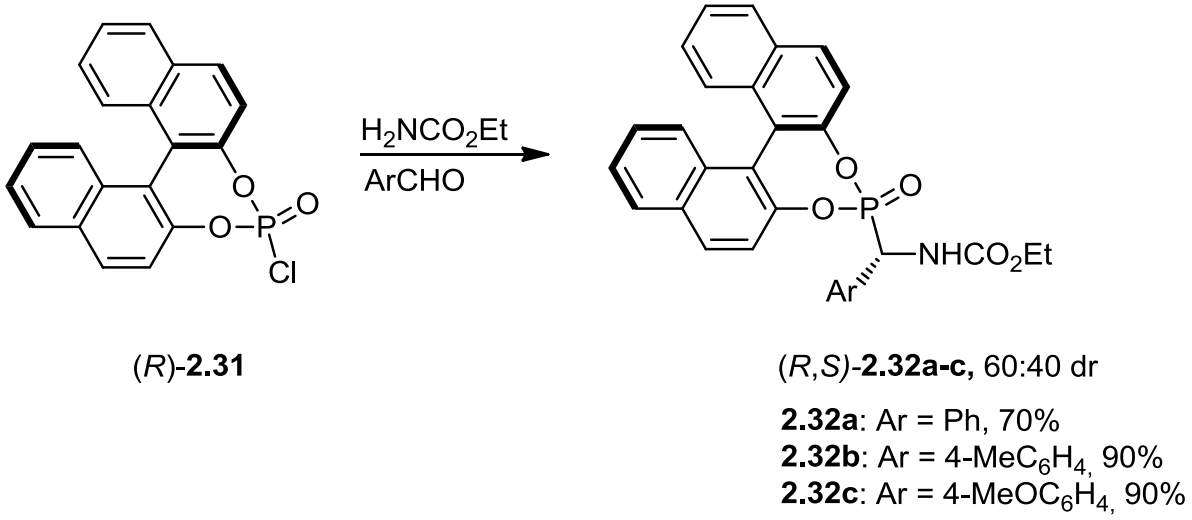



| Product | *R/R* | R'/R' | yield (%) | dr |
|---|---|---|---|---|
| **2.30a** | Me/Me | Me/Me | 47 | 83:17 |
| **2.30b** | Me/Me | $-(CH_2)_5-$ | 47 | >95:5 |
| **2.30c** | $-(CH_2)_5-$ | Me/Me | 37 | 80:20 |
| **2.30d** | $-(CH_2)_5-$ | $-(CH_2)_5-$ | 68 | >95:5 |
| **2.30e** | H/H | $-(CH_2)_5-$ | 30 | >95:5 |

*Scheme 2.9. Synthesis of α-aminophosphonic acids using BINOL-phosphite.*

The R substituent seems to have no influence on the diastereomeric ratio, whereas the R' substituent on the *N,S*-acetalic carbon atom affects the stereoselectivity in a greater degree.[27] Afterwards the chiral auxiliary and the acetal can be cleaved by hydrolysis to obtain the *α*-aminophosphonic acid.[28]

*α*-Aminophosphonates can also be synthesised by a solvent-free one-pot reaction using BINOL-Cl **2.31**, urethane and the arylaldehydes (*Scheme 2.10*).




(*R*)-**2.31**

(*R,S)*-**2.32a-c,** 60:40 dr

**2.32a**: Ar = Ph, 70%
**2.32b**: Ar = 4-$MeC_6H_4$, 90%
**2.32c**: Ar = 4-$MeOC_6H_4$, 90%

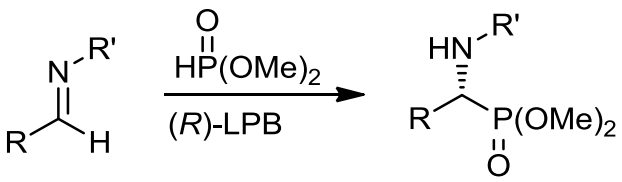
*Scheme 2.10. Synthesis of α-aminophosphonates using BINOL-Cl.*

The resulting products **2.32a-c** are obtained in high yields and a 60:40 diastereomeric ratio, with preference for the (*R*,*S*)-diastereomer.[29]

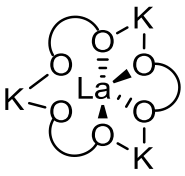
### 2.2.1.4 Asymmetric nucleophilic addition of dialkyl phosphites to non-chiral imines using chiral catalysts

Over the past decades, catalytic synthesis has gained more attention due to high efficiency of enantioselective synthesis for different compounds.[30]

Therefore the synthesis of (*R*)-α-aminophosphonates **2.34a-f** was discovered using non-chiral imines **2.33a-f**, dimethyl phosphite and a lanthanoid/potassium/BINOL complex (*R*)-LPB. The phosphonates were obtained in moderate yields but high enantiomeric excess (*Scheme 2.11*).[31]

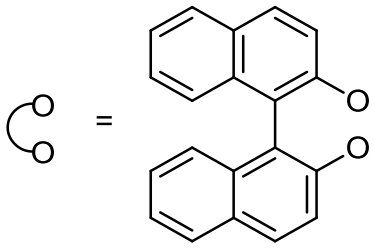



**2.33a-f** **2.34a-f**



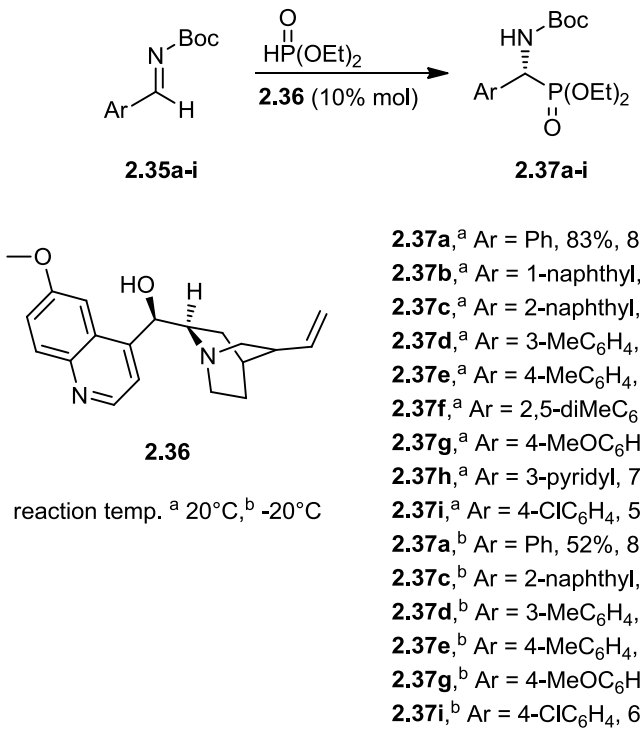

(*R*)-LPB

**2.34a**, R = *i*-Pr, R' = DAM, 70%, 96% ee
**2.34b**, R = *i*-Pr, R' = $CHPh_2$, 82%, 92% ee
**2.34c**, R = Me, R' = $CHPh_2$, 73%, 75% ee
**2.34d**, R = Et, R' = $CHPh_2$, 80%, 91% ee
**2.34e**, R = *c*-hexyl, R' = $CHPh_2$, 87%, 85% ee
**2.34f**, R = *c*-hexyl, R' = 4-$MeOC_6H_4$, 71%, 49% ee




*Scheme 2.11. Enantioselective catalysis of α-aminophosphonic acids using (R)-LPB.*

Another example of enantioselective catalysis is shown in *Scheme 2.12*. The addition of diethyl phosphite to imines **2.35a-i** gave (*R*)-aminophosphonates **2.37** in moderate yields and good enantiomeric excess. In this case quinine **2.36** was used as a chiral catalyst. To investigate the effect on enantioselectivity, the reaction was not only carried out at 20°C, but also at -20°C. [32]




*Scheme 2.12. Enantioselective synthesis of α-aminophosphonic acids using quinine.*

The authors postulated a reaction mechanism to explain the high chiral induction. The imine could be activated by a hydrogen bond to the quinine and if the equilibrium between the phosphite and phosphonate lies on the side of the phosphite, the attack onto the azomethine carbon atom should be simplified (*Figure 2.4*).[32]

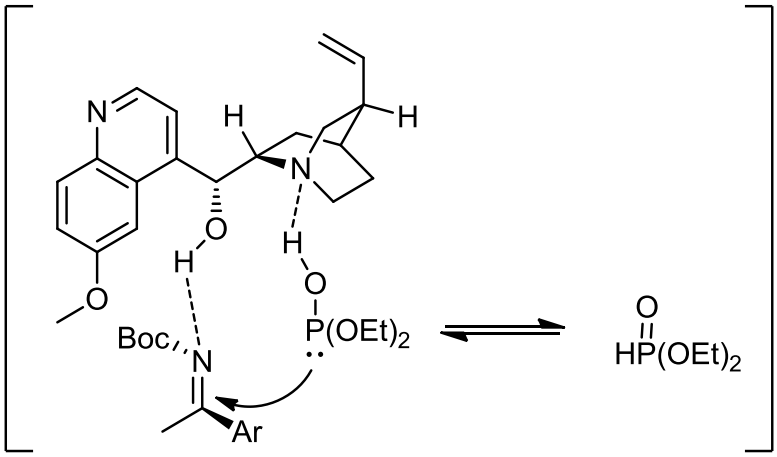
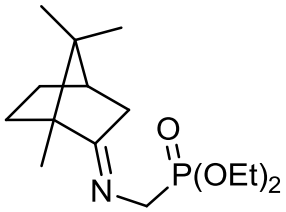



*Figure 2.4. Transition state for the addition of $(EtO)_2POSiMe_3$ to **2.35**.*

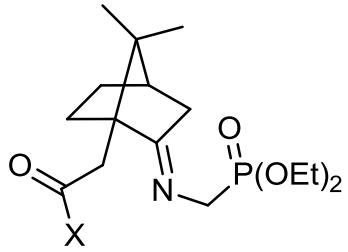
## 2.2.2 Stereoselective formation of C-C bonds

### 2.2.2.1 Alkylation of phosphonoglycine derivatives

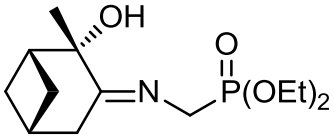
*α*-Aminophosphonic acids can be easily obtained by a stereoselective C-C coupling compounds derived from phosphonoglycine and chiral carbonyl compounds. The resulting chiral Schiff bases **2.38-2.41** found widespread use in organic chemistry (*Fig 2.5*). [33,34,35,36,37]



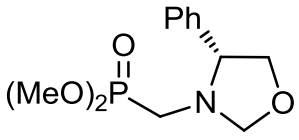

**2.38**




**2.39a**: X = OH
**2.39b**: X = *i*-PrNH
**2.39c**: X = 3-$HOC_6H_4NH$



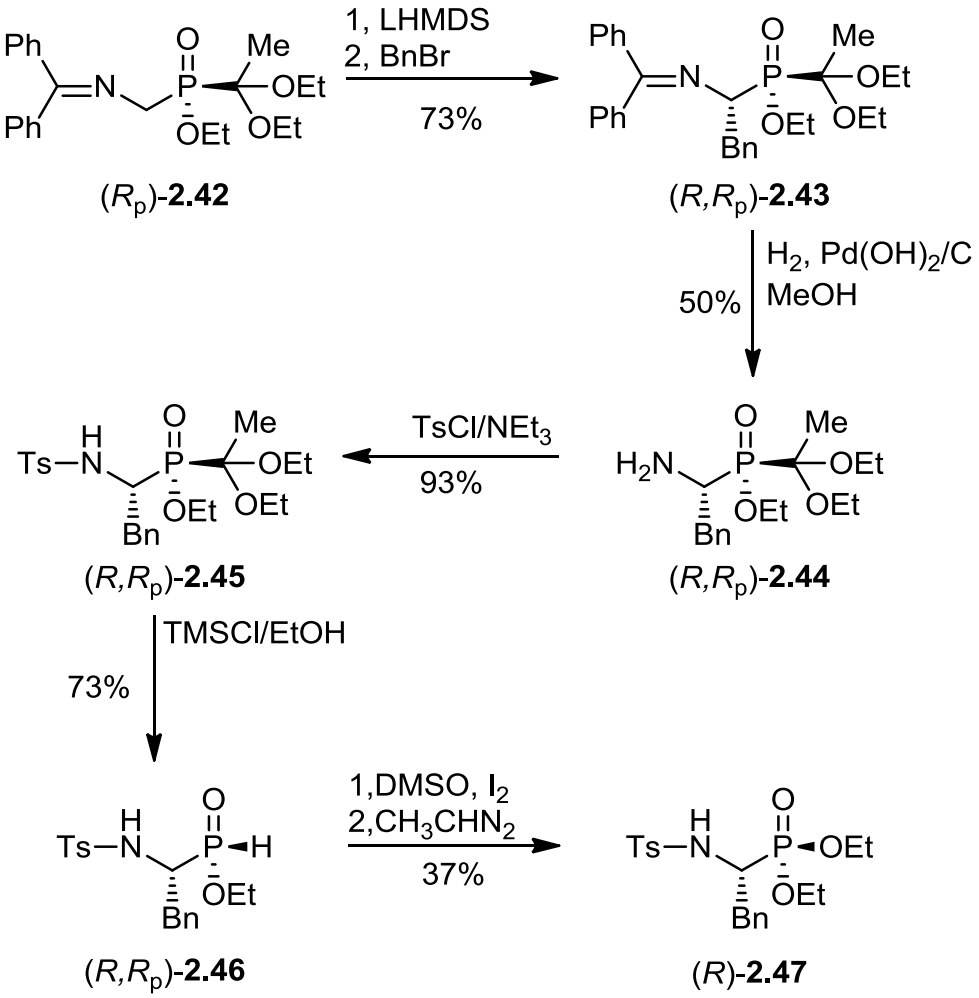

**2.40**




**2.41**

*Fig 2.5. Different Schiff bases used for coupling.*

These Schiff bases have been used in different syntheses, an example is shown in *Scheme 2.13,* where the imine **2.42** was treated with LHMDS and benzyl bromide to obtain the benzylated product **2.43** in a 10:1 dr and 73% yield.

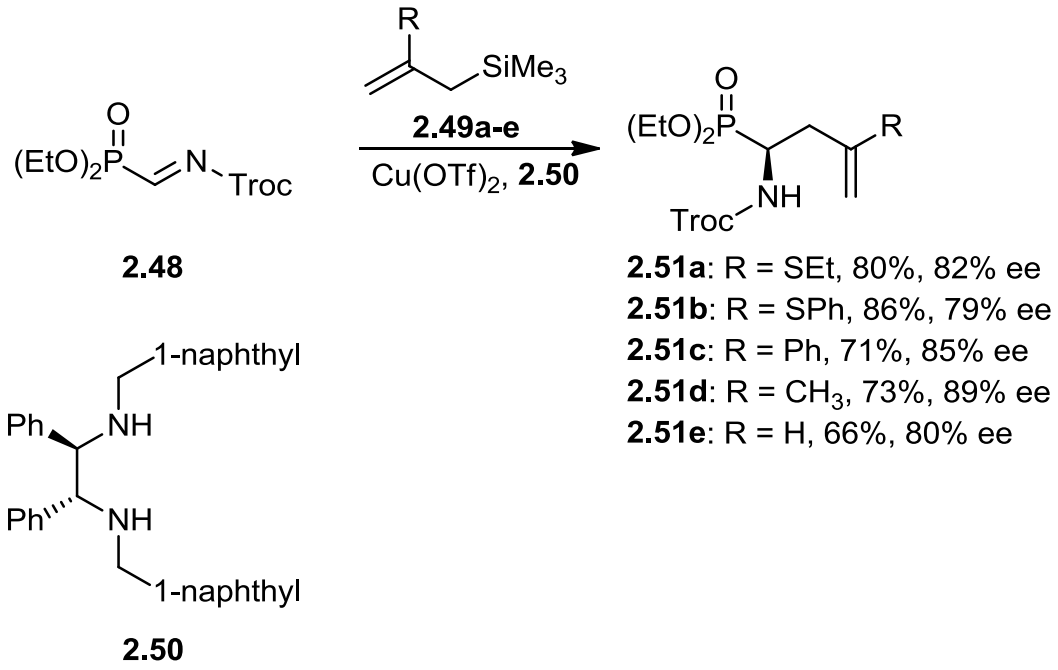



*Scheme 2.13. Synthesis of benzylated aminophosphonic acids.*

The ($S_p$)-enantiomer can be obtained in the same way, also with a diastereomeric ratio of 10:1. Hydrolysis with $H_2$, $Pd(OH)_2/C$ gave the ($R,R_p$)-amine **2.44** in 50% yield, which was then treated with Tosyl-Cl under basic conditions to obtain the tosylated compound **2.45** in 93% yield. Cleavage of the ketal with TMSCl and EtOH was necessary to yield ($R,R_p$)-**2.46** in 73%. Before esterification of the phosphorus moiety with diazoethane, the tosylate needed to be oxidized with DMSO and $I_2$. The enantiomerically pure compound **2.47** was obtained in 37% yield.

### 2.2.2.2 Nucleophilic addition to iminophosphonates

Another way to synthesise *α*-aminophosphonates is shown in *Scheme 2.14*. Treatment of iminophosphonate **2.48** with allylsilanes **2.49a-e** using $Cu(OTf)_2$ as a catalyst and chiral amine **2.50** gave the allylic *α*-aminophosphonates in good yields and good enantiomeric ratios.[38]

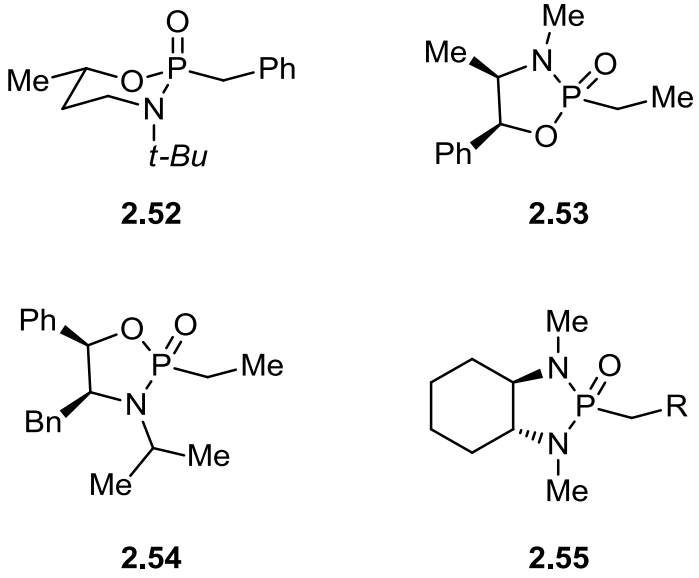



*Scheme 2.14. Synthesis of α-aminophosphonates using iminophosphonates as starting materials.*

## 2.2.3 Stereoselective formation of C-N bonds

### 2.2.3.1 Stereoselective electrophilic amination

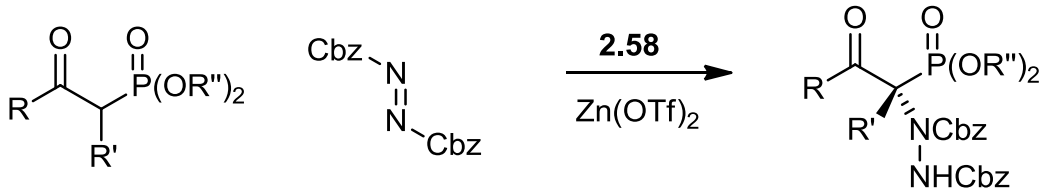
*α*-Aminophosphonic acids might also be synthesised using electrophilic amination of *α*-phosphate carbanions. Key elements in those reactions are oxazaphosphorinanes **2.52**[39], oxazaphospholanes **2.53**[40] and **2.54**[41] as well as diazaphospholanes **2.55**[42] (*Figure 2.6*).



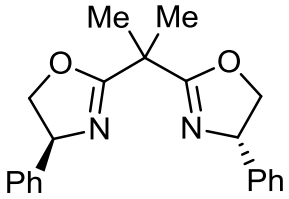

**2.52** **2.53** **2.54** **2.55**

*Figure 2.6. General structure of oxazaphosphorinanes **2.52**, oxazaphospholanes **2.53, 2.54** and diazaphospholanes **2.55**.*

Enantioselective synthesis of compound **2.59** was performed using *β*-ketophosphonates **2.56** and dibenzyl azodicarboxylate **2.57** where $Zn(OTf)_2$ and bisoxazoline **2.58** formed an *in* situ catalyst (*Scheme 2.15*). The afforded products have good yields and excellent enantioselectivity.




**2.56** **2.57** **2.59**




**2.58**

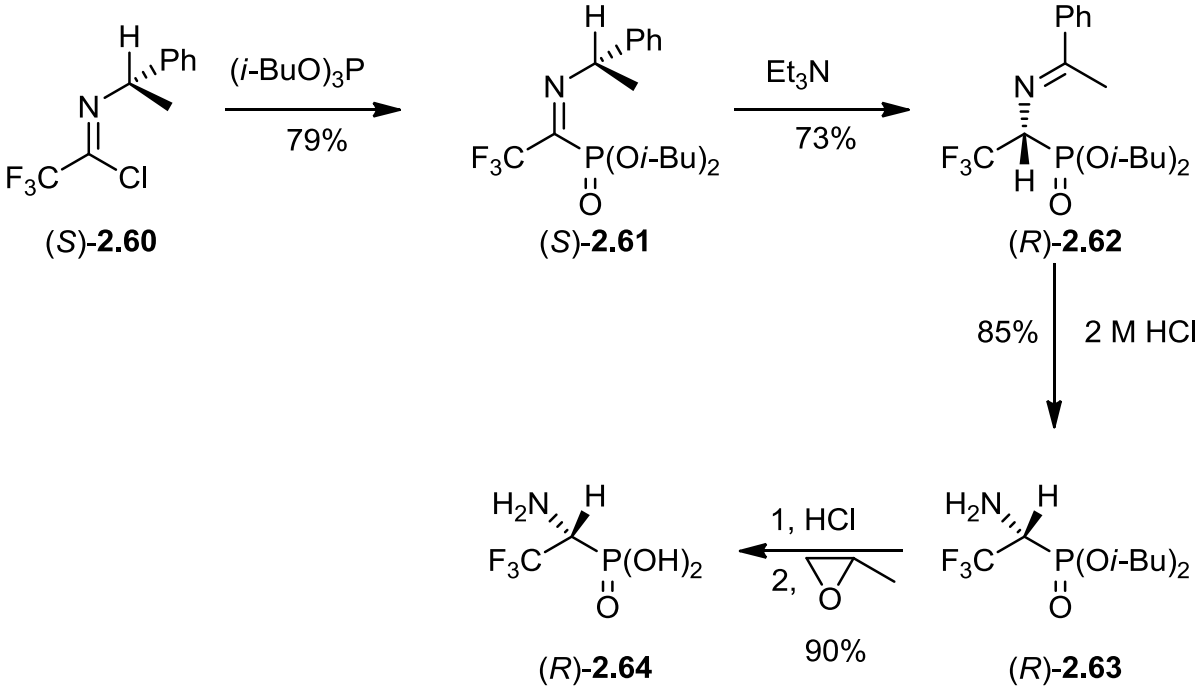
| R | R' | R'' | yield (%) | ee (%) |
|---|---|---|---|---|
| Ph | Me | Et | 85 | 92 |
| 2-Napthyl | Me | Et | 93 | 92 |
| Bn | Me | Et | 60 | 95 |
| Me | Me | Et | 75 | 85 |
| Ph | Allyl | Et | 85 | 98 |
| Ph | Me | Me | 97 | 94 |
| $-(CH_2)_3-$ | | Et | 98 | 95 |
| $-(CH_2)_4-$ | | Et | 98 | 94 |

*Scheme 2.15. Synthesis of oxazolidines.*

## 2.2.4 Stereoselective formation of C-H bonds

### 2.2.4.1 Catalytic hydrogenation of dehydroaminophosphonates

Many different catalysts have been reported for the synthesis of enantiomerically pure *α*-aminophosphonic acids via dehydroaminophosphonates.[43] Related, the synthesis of phosphonic acid (*R*)-**2.64** has been achieved by a base-catalysed 1,3-proton shift (*Scheme 2.16*). [44]

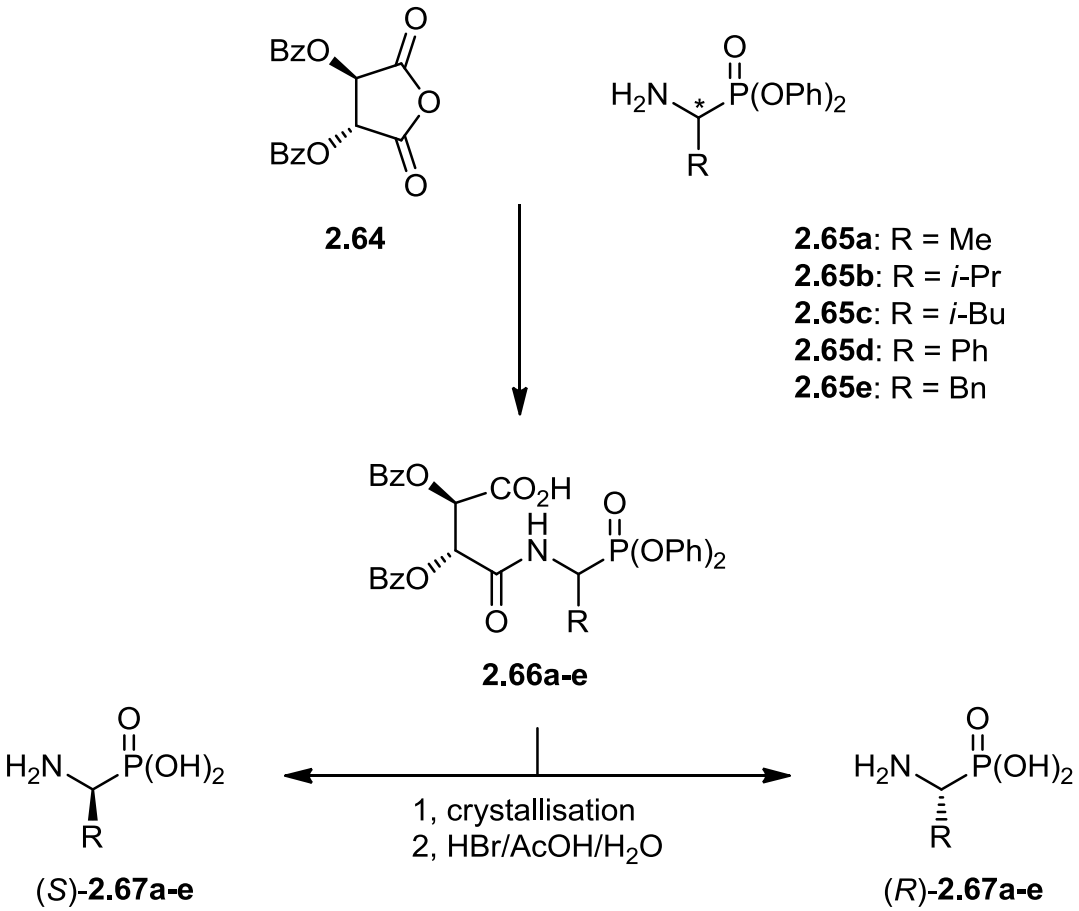



*Scheme 2.16. Synthesis of 1-amino-2,2,2-trifluoroethylphosphonic acid.*

(*S*)-**2.60** has been treated with triisobutyl phosphite in 79% yield, followed by a 1,3-proton shift under basic conditions using triethylamine. The resulting (*R*)-enantiomer **2.62**, which was obtained in 73% yield and with an ee of 67%, was deprotected with HCl to give the free amine (*R*)-**2.63** in 85% yield. To obtain the free aminophosphonic acid (*R*)-**2.64** in 90% yield, the amine was treated with concentrated HCl and propylenoxide.[45]

## 2.2.5 Resolutions

Another way to synthesise chiral *α*-aminophosphonic acids is by resolution. As shown in *Scheme 2.17*, the (*R*)- and (*S*)-enantiomers can be separately obtained.

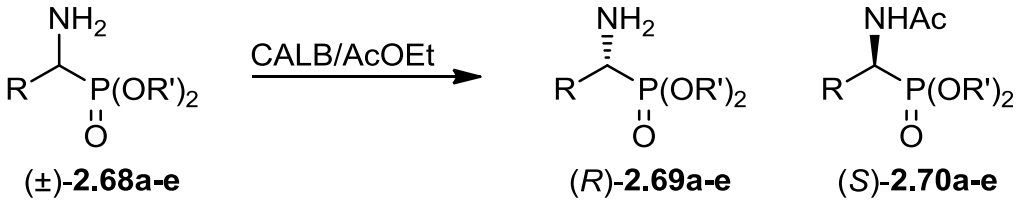



*Scheme 2.17. Resolution of α-aminophosphonates.*

In this context, treatment of diphenyl α-aminophosphonic acids (±)-**2.65a-e** with tartaric anhydride **2.64** gave amides **2.66a-e**. Fractional crystallization followed by deblocking furnished the enantiomerically pure *α*-aminophosphonic acids (*S*)- and (*R*)-**2.67a-e** in high yields.[46]

Biocatalytic resolution has gained more and more attention over the past years.[47] Therefore the separation of enantiomerically pure *α*-aminophosphonic acids using CALB as a catalyst cannot stay unmentioned. Racemic phosphonates **2.68a-e** undergo an enantioselective acetylation in

presence of CALB to give optically pure amine (*R*)-**2.69a-e** and amide (*S*)-**2.70a-e** in good yields with a high enantiomeric excess (*Scheme 2.18*).[48]



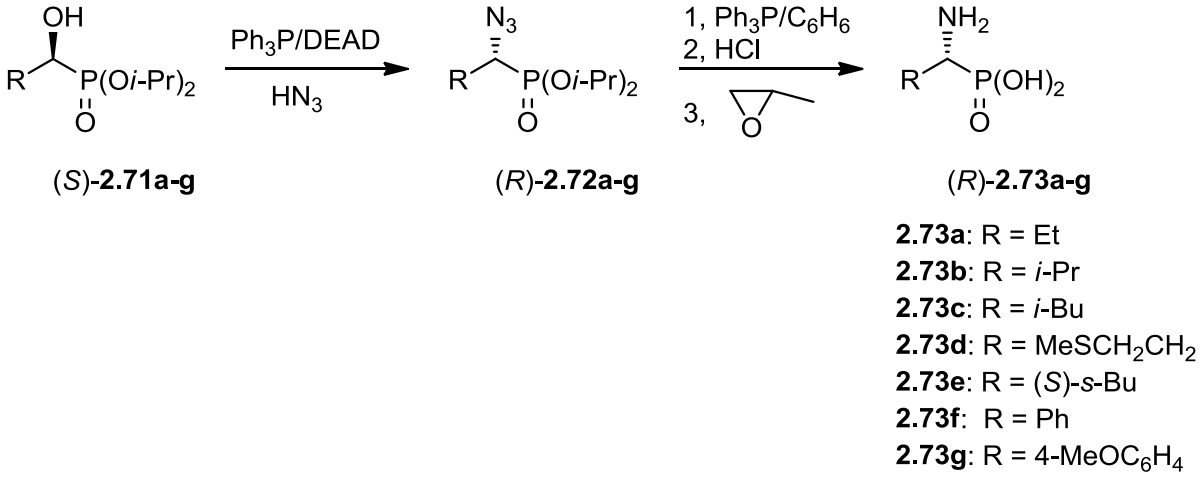

(±)-**2.68a-e** (*R*)-**2.69a-e** (*S*)-**2.70a-e**

| Substrate | R | R‘ | **2.69** | | **2.70** | |
|---|---|---|---|---|---|---|
| | | | yield (%) | ee (%) | yield (%) | ee (%) |
| **2.68a** | Me | Et | 41 | 99.7 | 48 | 90 |
| **2.68b** | Me | *n*-Pr | 42 | 90 | 42 | 98 |
| **2.68c** | Me | *i*-Pr | 44 | 96 | 43 | 98 |
| **2.68d** | Et | Et | 73 | 18 | 10 | 100 |
| **2.68ae** | $CF_3$ | Et | - | - | - | - |

*Scheme 2.18. Biocatalytic resolution of α-aminophosphonates.*

## 2.2.6 Chiral pool

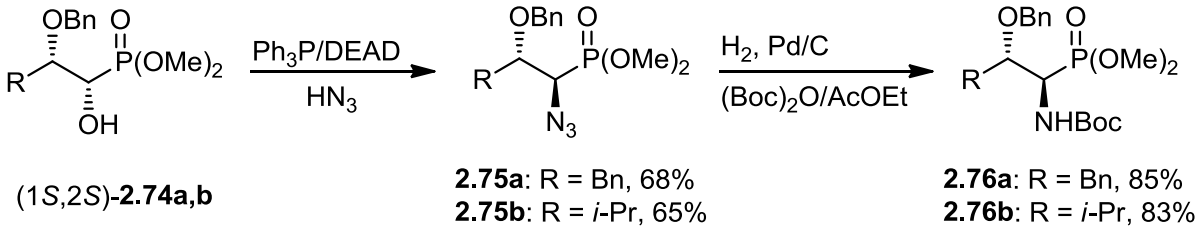
*α*-Aminophosphonic acids can be easily obtained starting from the corresponding α-hydroxyalkylphosphonates using the Mitsunobu reaction to introduce the amine functionality.[49] Accordingly, in order to obtain (R)-azidophosphontes **2.72a-**g in good yields and with high ee (70-90%), (*S*)-hydroxyphosphonates **2.71a-g** (92-99% ee) were treated with $Ph_3P$/DEAD/$HN_3$ (*Scheme 2.19*).




*Scheme 2.19. Synthesis of α-aminophosphonic acids using Mitsunobu conditions.*

Reduction of the azides with $Ph_3P$, deprotection with boiling HCl and and treatment of the crude hydrochloride with propylene oxide gave the (*R*)-aminophosphonic acids **2.73a-g** in good yields.[50]

The same methodology can be used to synthesise *α*-amino-*β*-hydroxyphosphonic acids. Therefore, monoprotected dihydroxyphosphonates (1*S*,2*S*)-**2.74a,b** were converted to azidophosphonates **2.75a,b** using $Ph_3P$/DEAD/$HN_3$ (*Scheme 2.20*).



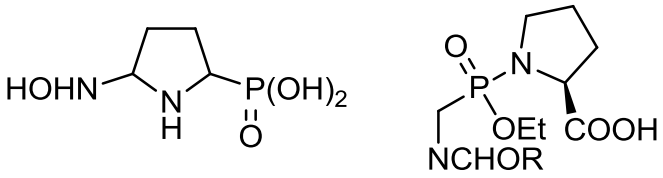

*Scheme 2.20. Synthesis of protected α-amino-β-hydroxyphosphonates.*

Catalytic reduction of the azides with Pd/C/$H_2$ to amines, which were reacted with $(Boc)_2O$, gave the protected *α*-aminophosphonates **2.76a,b** in good yields.[51]

## 2.3 Azaheterocyclic phosphonates

### 2.3.1 General remarks

Five-membered azaheterocycles were found to be more useful in organic and medicinical chemistry than their corresponding three or four-membered counterparts.[52] Therefore the focus will stay on the five-membered rings. The synthesis of different aminophosphonic acid analogues of proline will be discussed in this chapter. Due to their structural analogy, many peptides have been synthesized containing phosphonoproline, which are used as antiviral agents, for HIV protease[53,54,55], or as inhibitors for the dipeptidyl peptidase IV. Moreover phosphonoproline derivatives have certain activity as bactericide, fungicide and herbicide (**2.77**, *Figure 2.7*).[56]



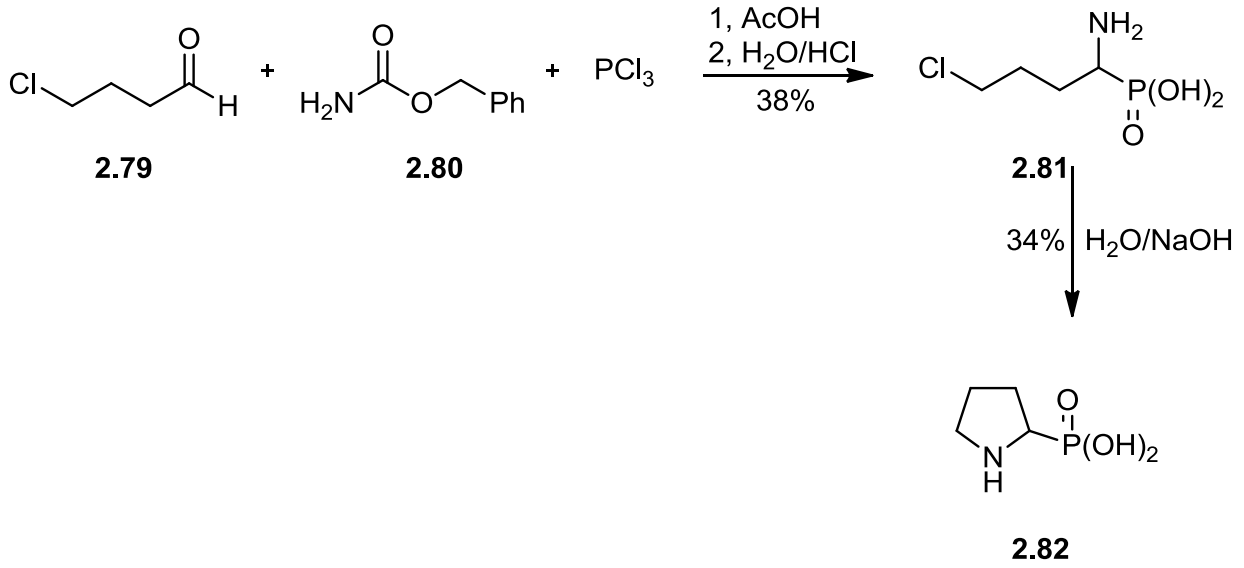

**2.77** **2.78**

*Figure 2.7. Different phosphonoproline derivatives.*

Also *N*-phosphonoproline derivative **2.78** can inactivate the class C, which catalyses the hydrolysis of penicillin, for the *β*-lactamase from *Enterobacter cloacae P99*.[57]

## 2.3.2 Nucleophilic substitution as a useful tool for ring closure

Phosphonoproline (**2.82**) can be obtained, in moderate yields, under basic conditions using aminobutylphosphonic acid **2.81**, which was synthesised by condensation of 4-chlorobutanal (**2.79)**, benzyl carbamate (**2.80**) and $PCl_3$ (*Scheme 2.21*).[58]

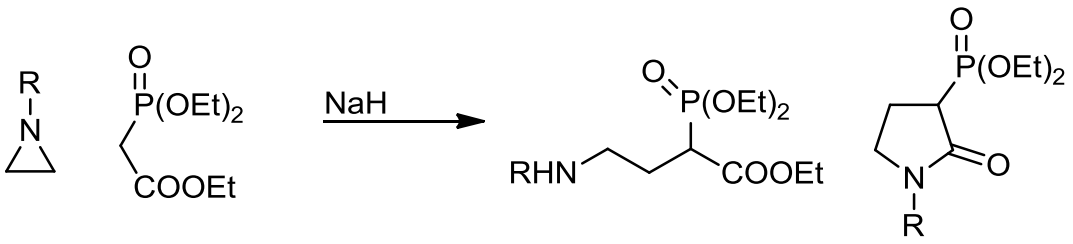



**2.79** **2.80** **2.81** **2.82**

*Scheme 2.21. Synthesis of racemic phoshonoproline.*

## 2.3.3 Ring expansions of aziridines

Pyrrolidinones **2.86a-e** can be prepared using aziridines **2.83a-e** and phosphonoactetates **2.84** under basic conditions. The cyclization is strongly influenced by steric hinderance. Nonetheless the lifetime of the intermediate anion might also play an important role (*Scheme 2.22*).[59]



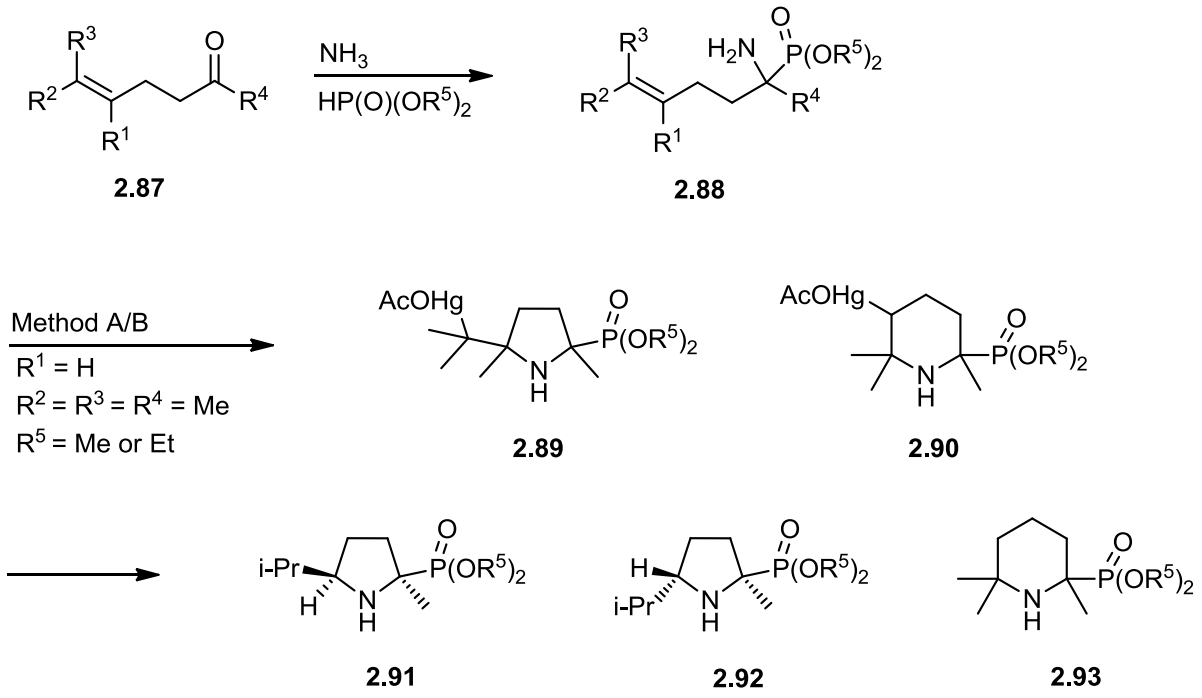

**2.83a-e** **2.84** **2.85a-e** **2.86a-e**

| Product | R | **2.85** yield (%) | **2.86** yield (%) |
|---|---|---|---|
| **a** | (1-adamantyl)CO | 85 | 0 |
| **b** | COPh | 62 | 0 |
| **c** | COOEt | 0 | 28 |
| **d** | $CONPh_2$ | 22 | 41 |
| **e** | Ts | 0 | 53 |

*Scheme 2.22. Ring expansion of aziridines.*

## 2.3.4 Intramolecular aminomercuration

For the synthesis of heterocyclic amines, intramolecular aminomercuration is a viable option. Starting from *γ*-alkenyl carbonylic compounds **2.87**, either aldehydes or ketones, *α*-amino-*δ*-alkylenylphosphonates **2.88** are accessible by bubbling ammonia through the solution (*Scheme 2.23*).




Method A: 1, $Hg(OAc)_2$, acetone; 2, $NaBH_4$, $CH_2Cl_2$
Method B: 1, $Hg(OAc)_2$, THF/water; $NaBH_4$, THF/water

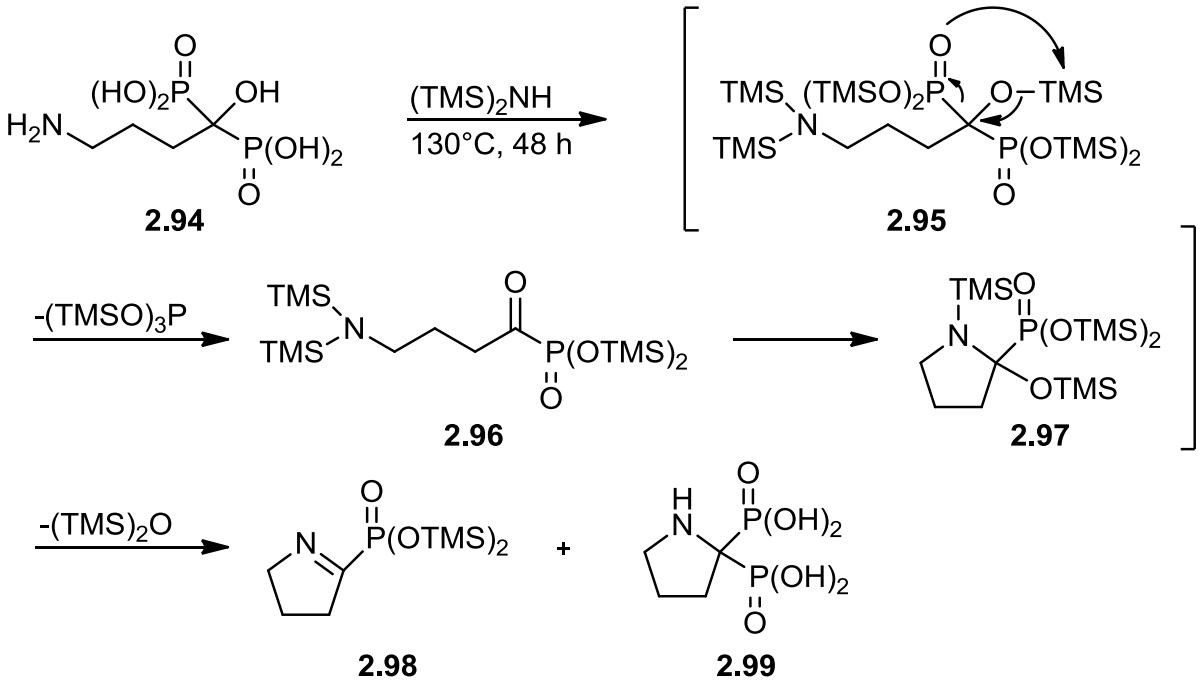
| entry | method | $R_5$ | **2.91** yield (%) | **2.92** yield (%) | **2.93** yield (%) |
|---|---|---|---|---|---|
| 1 | A | Me | 12 | 88 | 0 |
| 2 | A | Et | 10 | 83 | 7 |
| 3 | B | Me | 88 | 12 | 0 |
| 4 | B | Et | 80 | 17 | 3 |

*Scheme 2.23. Synthesis of heterocyclic amines using mercuric acetate as a useful tool.*

Addition of $Hg(OAc)_2$ to the double bond will initiate the cyclization, followed by nucleophilic attack of the amino functionality. By extending the carbon chain, six membered rings can be easily obtained in nearly the same yields (55%). Only in one case ($R^1$ = H, $R^2$ = R3 = $R^4$ = Me) the reaction is not regiospecific[60,61,62], therefore the formation of the corresponding six-membered rings occurs in 3-7% yield when diethyl phosphonate derivatives are used. Reductive removal of mercury with $NaBH_4$ gave the desired heterocyclic phosphonates **2.91-2.93**. Stereoselectivity is highly dependent on the solvent used for the cyclisation and the reduction. [61,62]

## 2.3.5 Rearrangement of bisphosphonic acids

Bisphosphonic acid **2.94** was reacted with $(TMS)_2NH$ and then heated at low pressure. The silylated compound **2.95** decomposed with loss of tris(trimethylsilyl) phosphite to α-ketophosphonate **2.96**, which cyclised to silylated phosphonate **2.97** (*Scheme 2.24*).




*Scheme 2.24. Rearrangement of bisphosphonic acid **2.94**.*

Elimination of hexamethylsiloxane gave imine **2.98**, which added the tris(trimethylsily) phosphite. Hydrolytic removal of the silyl groups furnished the bisphosphonic acid analogue of proline.[63]

# 3. Biosynthesis of L-proline

## 3.1 Biosynthesis in plants

The biosynthesis of L-proline, a compatible solute which can be accumulated in plant cells in high concentrations without disrupting its cellular activity, is a valuable target for manipulation. Their ability to lower the water potential and maintain turgor under dry and saline circumstances makes compatible solutes important for the survival of plants. To improve the agricultural gain, the effect of water stress should be decreased by development of drought-tolerant plants either with conventional or transgenic strategies. It was already observed in experiments with *E.coli*, that proline overproducing mutants can tolerate higher osmotic pressures, therefore modifications of genes responsible for the proline biosynthesis and catabolism has a high potential to lead to stress resistant plants.[64]

In plants, L-proline is synthesized by two different pathways, either starting from glutamate or ornithine whereas in humans only through glutamate. The glutamate pathway is identical to that found in *E.coli*, whereas the ornithine pathway has not been fully clarified until now. The key enzyme in the glutamate biosynthetic pathway of proline is $\Delta^1$-pyrroline-5-carboxylate synthetase, a bifunctional enzyme. It first phosphorylates glutamate using ATP as a phosphate source, the resulting γ-glutamyl phosphate is then converted with the NADPH dependent GSA reductase to glutamic γ-semialdehyde (GSA). After spontaneous cyclization of GSA, $\Delta^1$-pyrroline-5-carboxylate is reduced to proline with NADPH-depending $\Delta^1$-pyrroline-5-carboxylate synthetase (*Scheme 3.1*).[64]

For the synthesis of proline via L-ornithine, two routes are proposed, where either $\Delta^1$-pyrroline-5-carboxylate or $\Delta^1$-pyrroline-2-carboxylate are the final intermediates. However, ornithine-δ-aminotransferase activity has been found in moth bean *Vigna aconitifia*, which hints to $\Delta^1$-pyrroline-5-carboxylate as an intermediate in the biosynthesis of proline from ornithine in plants.

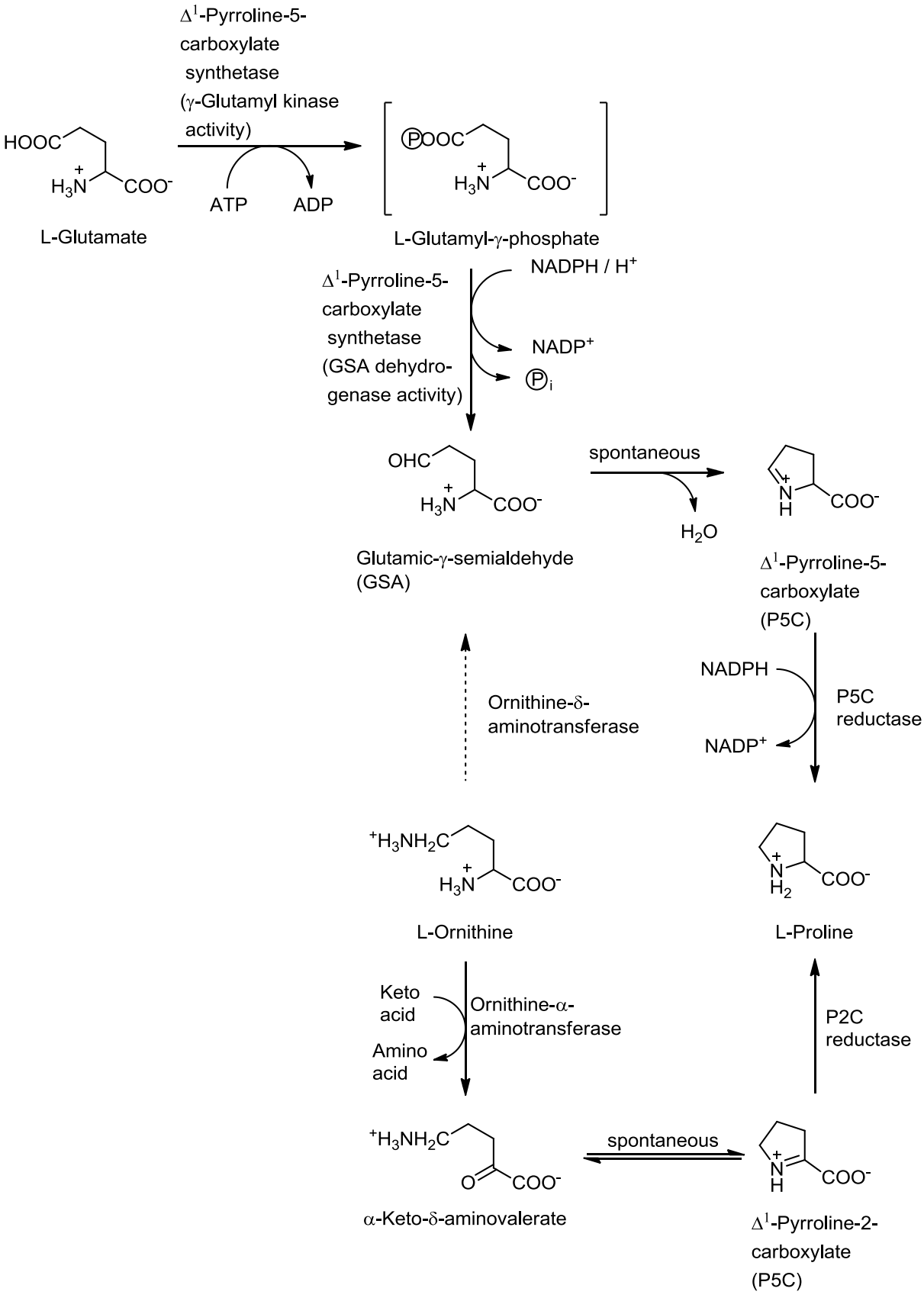
## 3.2 Advantages of proline compared to other amino acids

The proline metabolism seems to be relatively sensitive to hostile environmental conditions. In comparison to most of the other amino acids, proline is the terminal product of a short and

highly regulated pathway. Therefore proline accumulation has a far smaller effect on other metabolisms than for example the multi-use substrate glutamate, which participates in different reactions in diverse pathways.[65]

No enzyme in the biosynthetic pathway of proline is reversible, therefore the final product of this metabolism is not equilibrated with any intermediate. Another advantage of proline is its secondary amine functionality. It is not likely to undergo decarboxylation and transamination reactions, which are observed with many other amino acids.[66]

Accumulation of proline is important for the survival of plants. During its degradation, high amounts of energy are released, because one molecule of proline produces 30 molecules of ATP.[67] This characteristic makes the accumulation of proline a viable option for plants either in "stress" situations or upon relief. The benefit might not be the accumulation of proline itself, but its regulatory effect on other biosynthetic pathways even under stress conditions. However, it is suggested that proline only contributes to the survival of the plant right after stress situations. [68,69]



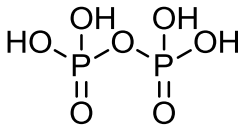

*Scheme 3.1. Biosynthesis of L-proline*

# 4. Bisphosphonic acids

## 4.1 General remarks

As mentioned in chapter **1**, bisphosphonates made their way from being of industrial use to being important in medicine, especially for treatment of calcification inducing diseases. In this chapter the focus will stay on the medicinal uses and applications of bisphosphonates (BP).

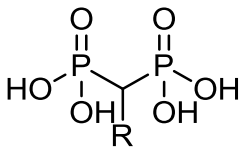
Studies about the mechanism of calcification induced by collagen have been reported since the early 1960s.[70] Surprisingly, inhibitors for calcification have been found in humans, mainly in plasma and urine. At this time, polyphosphates were known inhibitors of crystallisation of calcium salts, therefore the idea was raised that compounds with related structure would be able to regulate calcification under physiological conditions. It was soon found that pyrophosphate (PP, **1.8**), which is a byproduct in different metabolisms and can be found in different bodily fluids like plasma and urine, was capable of binding calcium and therefore preventing calcification in different tissues (*Figure 4.1*). [71,72]



**1.8**

*Figure 4.1. Structur of pyrophosphate.*

Utilization of pyrophosphate and other polyphosphates for the inhibition of calcification was only possible if the drug was administered intravenously.[73] Oral application did not give the desired effects, maybe due to hydrolysis in the gastrointestinal tract. Therefore derivatives with the same properties and insensitive to hydrolysis were needed. It turned out that bisphosphonates **1.7** were capable of just that (*Figure 4.2*).



**1.7**

*Figure 4.2. General structure of bisphosphonates.*

They had a high affinity to bone material as well as the capability to prevent the formation of calcium crystals.[74] In comparison to pyrophosphate, bisphosphonates were able to prevent calcification *in vivo* and *in vitro*, as well as pathological calcification in rats when applied orally which led to further investigations of their potential use in humans.[75]

## 4.2 Pharmacologic effects of bisphosphonates in *in vivo* and *in vitro* systems

### 4.2.1 Calcification inhibitors

Experimentally induced calcification in soft tissues like skin and blood vessels can be easily prevented using bisphosphonates. The inhibition mechanism does not lie in direct interactions with the matrix, but in the impairment of the calcification process, therefore the occurrence of displaced formations and bone mineralisation are also inhibited. For the inhibition of soft tissues doses of 1 mg BP/ kg weight are administered, which might affect the normal mineralisation of bones or cartilage.[76]

### 4.2.2 Bone resorption inhibitors for *in vivo* and *in vitro* systems

Different studies showed the inhibition of osteoclast mediated bone resorption by bisphosphonates of normal as well as increased bone resorption. To increase the bone resorption experimentally, calcitriol, vitamin D or retinoids were used. Systems based on retinoids to induce hypercalcaemia were used to produce assays for new compounds.[77,78]

Studies show, that bisphosphonates accumulate in bones only up to a certain level, regardless of how much is administered and over what period of time.[79] Therefore long term

administration is less important than the administered dose.[80] The excess of bisphosphonates is buried in the bones and stays inactive until it is needed. Nonetheless, there is small chance of bone turnover, even with the appropriate medication, which increases bone fragility and might lead to osteoporosis.

### 4.2.3 Bone resorption inhibitors in relation to cancer

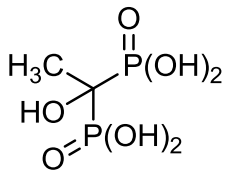
Hypercalcaemia and bone loss go along with many cancers. This occurs due to tumour released proteins such as parathyroid-hormone-related peptides or interleukin 6. The ability of bisphosphonates to prevent bone turnover induced by tumours is not completely elucidated until now, but a reasonable explanation is the release of growth factors, which stimulate the tumour cell growth during bone depletion.[81,82] Furthermore there is a possibility for bisphosphonates to have a direct impact on the tumour itself by attaching to the cell interface and inducing apoptosis, but further investigations need to be done on this research topic.

## 4.3 Clinical applications

After it was proven that bisphosphonates are effective against bone diseases in experimental systems, it took decades for them to become established as drugs. They became popular for diseases where no treatment was established. Therefore, ailments like Paget´s disease, metastatic and osteolytic bone diseases and osteoporosis, which have excessive osteoclast activity, can now be cured.

### 4.3.1 Calcification inhibitor

For the inhibition of calcification, etidronate (**4.1**), which can be administered for different diseases, plays an important role (*Figure 4.3*).



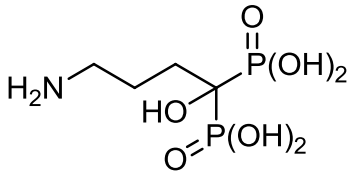
**4.1**

*Figure 4.3. Structure of etidronate.*

For example, etidronate (**4.1**) was applied after hip replacement to enhance mobility and avoid heterotopic ossification.[83] Furthermore, prevention of ectopic ossification and calcification for people suffering after spinal cord injuries is another field of application for etidronate (**4.1**). It should be mentioned that the doses of etidronate (**4.1**) used for treatment of the before mentioned diseases, need to be much higher than the recommended doses for treatment of osteoporosis.

### 4.3.2 Paget´s disease

Paget´s disease and its characteristics, like bone pain, fractures and skeletal deformation, are due to either high osteoclast activity or high number of osteoclasts. Therefore, a dose dependent application of bisphosphonates, especially pamidronate (**4.2**)[84], seems to be important for its treatment (*Figure 4.4*).[85,86,87]



**4.2**

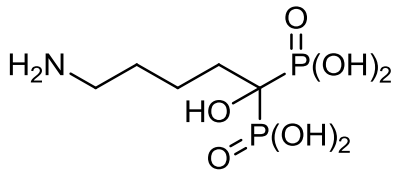
*Figure 4.4. Structure of pamidronate.*

In Paget`s disease, osteoclasts build up to larger clusters, which might be due to abnormal apoptosis. The reason for this stays unclear up to now, but a viral etiology has been suggested.

Bisphosphonates seem to influence apoptosis of osteoclasts and can be seen as bone selective drugs, which are capable for initiating apoptosis.

### 4.3.3 Osteoporosis

In the last decades osteoporosis has become more of a threat for mankind. Therefore understanding of its epidemiology, pathogenesis, as well as its diagnosis and evaluation is an important point for the treatment of osteoporosis, either postmenopausal or other forms. In this regard, bisphosphonates, especially etidronate (**4.1**) and alendronate (**4.3**), are well known drugs for all forms of osteoporosis (*Figure 4.5*).[88,89,90,91,92]




**4.3**

*Figure 4.5. Structure of alendronate.*

They both are able to increase the bone mass as well as reduce fracture rates in spines and hips. The reduction of fractures might not only be due to the increase in bone mass, but also due to enhanced osteon mineralisation.[93] Another advantage of etidronate (**4.1**) and alendronate (**4.3**) is the decrease in bone turnover by administration of glucocorticosteroids.[94,95] In addition to these effects, pamidronate (**4.2**) was found to be effective in increasing bone mass in children with osteogenesis imperfect.[96]

## 4.4 Clinical pharmacology

Bisphosphonates have a low absorption rate in the gastrointestinal tract, but their retention in bones is quite high. Until now, no significant side effects have been observed. [97,98,99] Since bisphosphonates are similar to each other, any disease related to bone resorption, can be

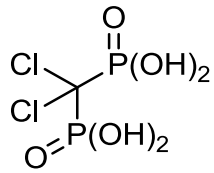
treated with any bisphosphonate. Although in practise some compounds are favoured for some diseases.

For the clinical application some issues should be considered, like the therapeutic regimen for example: intermittent or continuous dosing, intravenous versus oral therapy and length of therapy. Another noteworthy issue is the combination with other drugs such as oestrogens and their indications for glucocorticosteroid-assosiated osteoporosis, male osteoporosis, childhood osteopenic disorders or arthritis. Therefore improvement of existing drugs and/or introducing new ones is important for humans.[100]

## 4.5 Structural relationships of bisphosphonates in biological systems

After bisphosphonates made their way into medicinal use, the search for new compounds with high antiresorptive activity and without the ability to inhibit mineralisation started. Therefore hundreds of new bisphosphonates were synthesized and analysed for their potential. As a result, about a dozen compounds are used for treatment of different bone diseases nowadays.

The structural properties of bisphosphonates that exhibit biological activity have been well defined. For the binding to the crystals which are located in bones, the P-C-P moiety is of great importance. To synthesize different bisphosphonates, the substitution of the $R^1$ and the $R^2$ is necessary. To increase the binding to bones, namely to hydroxyapatite, a hydroxyl group in the $R^1$ side chain is desired, like etidronate (**4.1**), in contrast to a halogen atom like chlorine in clodronate (**4.4**) (*Figure 4.6*).




**4.4**

*Figure 4.6. Structure of clodronate.*

The hydroxy moiety in the $R^1$ position increases the affinity to bones by chelating calcium in a tridentate complex, rather than in a bidentate complex when no alcohol is present.[101]

The P-C-P structure is also important for the inhibition of bone resorption *in vivo* as well as *in vitro*. P-C-C-P, P-N-P and monophosphonates are ineffective in this regard. The anti-resorptive effect of bisphosphonates might be due to their ability to interfere directly with the osteoclast, rather than adsorption of bisphosphonates to bone material or simple physicochemical mechanisms.

After the successful introduction of etidronate (**4.1**) and clodronate (**4.4**) in the 1970s and 80s, different bisphosphonates have been synthesized containing different $R^2$ side chain, where $R^1$ remained the same. This led to discovery of more active bisphosphonates like pamidronate (**4.2**) and alendronate (**4.3**), containing a primary nitrogen moiety in the $R^2$ side chain, which were found to be 10-100 times more potent in comparison to etidronate (**4.1**) and clodronate (**4.4**). Later, synthesis was focused on new compounds with greater effect on the calcium metabolism, which could be more effective inhibitors for bone resorption. As a result, compounds with a tertiary nitrogen moiety, namely ibandronate (**4.5**)[102] and olpadronate (**4.6**)[103], were found to be effective in inhibiting bone resorption. During the era of finding new bisphosphonates, heterocyclic compounds containing a nitrogen atom, like risedronate (**4.7**)[104] and zoledronate (**4.8**)[105] have been found to be up to 10000 times more potent against resorption, compared to etidronate (**4.1**) (*Figure 4.7*).

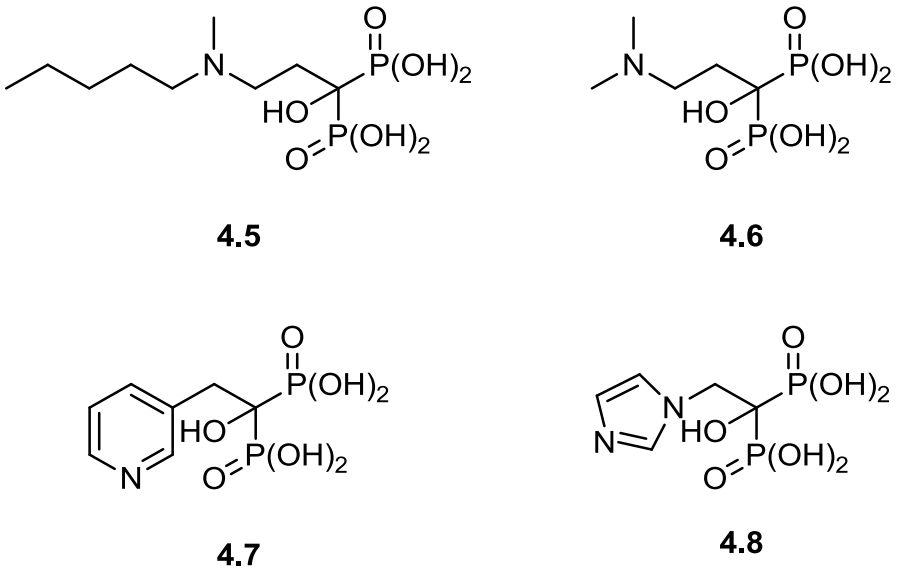



*Figure 4.7. Structure of ibandronate, olpadronate, risedronate and zoledronate.*

Another important feature of bisphosphonates responsible for the anti-resorptive properties is the spatial arrangement of the nitrogen atom to be in a certain distance from the P-C-P moiety as well as in an optimal spatial configuration.[106] However, not only the side chains on the carbon atom are important for the biological activity, but also the phosphonate groups. Replacement of

one or both phosphonate groups lead to less affinity to hydroxyapatite and therefore those derivatives are less potent for bone diseases.

If one hydroxyl group is replaced with a methyl group, generating a phosphophosphinate, bone affinity and anti-resorptive potency is reduced. When both hydroxyl groups are replaced, resulting in a bisphosphinate, anti-resorptive activity as well as bone affinity is lost *in vivo*. Some bisphosphonate analogues, for example phosphonophosphinates and phosphonocarboxylates, might have nearly the same affinity to bones, but completely different anti-resorptive properties. Therefore the assumption was made, that the two phosphonate groups are responsible for bone affinity as well as for the anti-resorptive interactions. This might be due to their similarity to pyrophosphate-containing compounds.

## 4.6 Bone resorption mechanism

The value of bisphosphonates in medicine lies in their high affinity to bone tissue, in comparison to other tissues. Since bisphosphonates are adsorbed in bone tissue, on a mineral surface, they might interfere with the osteoclasts. Osteoclast-mediated bone resorption can be influenced by bisphosphonates in different ways, namely by effects on osteoclast recruitment, differentiation and resorptive activity. Moreover, bisphosphonates can participate in biochemical processes where pyrophosphate (**1.8**) is a substrate, because of their related structural properties. Therefore, metabolism can be disrupted, which would lead to apoptosis.

Bisphosphonates are able to inhibit bone resorption by preventing the osteoclast formation, which takes place after fusion of mononuclear precursors. They are also capable of preventing those osteoclast cell formations *in vitro*, with application of the correct pharmacological dose.[107] Furthermore, production of mature osteoclasts might be inhibited by some bisphosphonates.[108,109]

### 4.6.1 Effects on osteoclasts in bone

Endocytic activity and the accumulation in bones are mainly the reasons why bisphosphonates interact with osteoclasts more likely than with other cell types. The environment becomes acidic during the process of bone turnover, which causes hydroxyapatite to dissolve.[110] Osteoclasts are exposed to high concentrations of unbound bisphosphonates when they are released under acidic conditions.[111,112] In this context, application of a pharmacological dose of alendronate

(**4.3**), which inhibits bone resorption *in vivo*, can give a 1 mM concentration at the specific active site. Therefore apoptosis is induced due to this high concentration of bisphosphonates.[113]

### 4.6.2 Apoptosis as a key event?

The question remains whether apoptosis is the main factor for the action of bisphosphonates. Studies up to now are showing contradicting results. Some of them found no bisphosphonate toxicity to osteoclasts[114], whereas others showed reduced number of osteoclasts[115] and possibly induced apoptosis.[116] Some features, like the changes in morphology, chromatin condensation and fragmentation, sequence of biochemical events, and intranucleosomal DNA cleavage following the activation of an endonuclease, were discovered to be associated with bisphosphonate induced apoptosis. They were found in murine osteoclasts as well as in mice which were treated with bisphosphonates. These effects were observed regardless of whether the bisphosphonates contained an amine moiety or a halogen.[117] However, it is not clarified until now, whether bisphosphonate induced apoptosis is crucial for the inhibition effect or not.

Moreover, in *in vitro* systems, such as human myeloma cell lines[118,119] and macrophages[120,121], apoptosis was also observed during treatment with nitrogen containing bisphosphonates. This led to further clarification of their mechanism and the discovery of their potential usefulness as anti-tumour agents. In conclusion, it seem that bisphosphonates inhibit bone turnover due to osteoclast apoptosis, but up to now it remains unclear whether it is the only reason for their effectiveness as drugs. It is possible that bisphosphonates are active in the bone metabolism in the first place and apoptosis is a secondary effect.

# 5. Results and discussion

## 5.1. Aim of the thesis

My Master thesis comprises two parts. The main part is the synthesis of different aminophosphonic acids which are structural analogues of amino acids. They have different applications ranging from medicine to agriculture, as was already discussed in previous chapters.

The main focus was the synthesis of proline analogues (*R*)-**2.82**, (*R*)-**5.1 and** (*R*)-**5.2**, as well as the synthesis of the phosphonic acid analogue (*R*)-**5.3** of glutamic acid in racemic and chiral, nonracemic form (*Figure 5.1*).

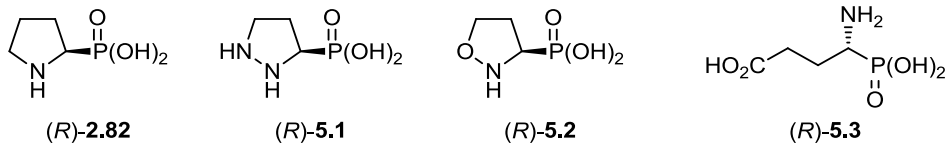



*Figure 5.1. Structure of target compounds.*

L-Proline is produced via two distinct pathways in plants, namely via glutamine or ornithine. In order to inhibit its biosynthesis, both of these pathways have to be blocked. Both routes use the same enzyme at some point, namely $\Delta^1$-pyrroline-5-carboxylate reductase (P5C), what makes it a perfect target for inhibition. Phosphaproline and its analogues, especially those synthesised during this Master thesis, have the potential to interact with P5C reductase and therefore inhibit the proline biosynthesis, which would lead to plant death.

The second part of my thesis is the synthesis of a zoledronate derivative and its coupling to DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) to give **5.4** (*Figure 5.2*).

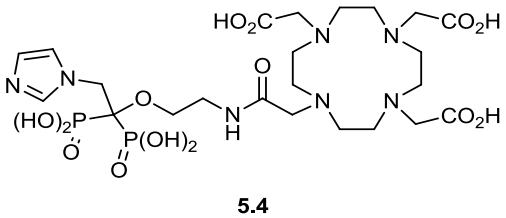



*Figure 5.2. Structure of desired bisphosphonate **5.4***

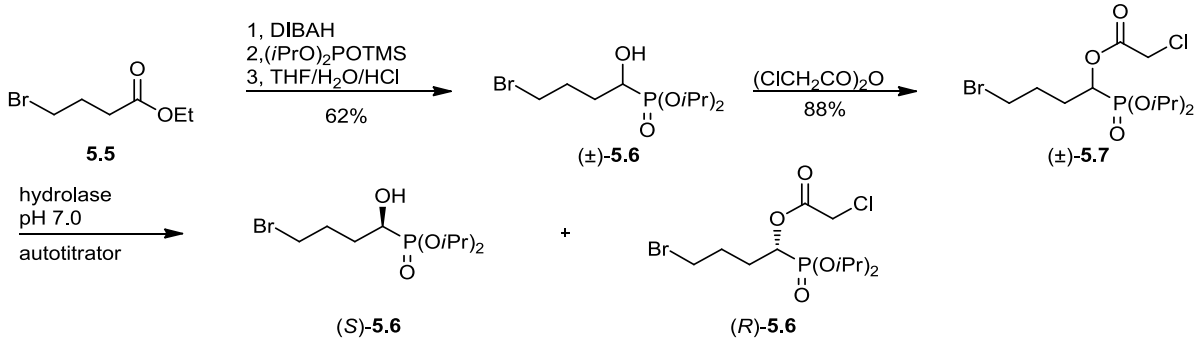
As already discussed in chapter 4, bisphosphonates are able to interact with hydroxyapatite in bones and are therefore useful drugs against bone demineraliation. They are also able to chelate radioactive isotopes, namely $^{99m}$Technetium, $^{186}$Rhenium, $^{68}$Gallium and many more, which makes them valuable as skeletal radiochemical tracers.

In the early 1970s, the first skeletal imaging agents bearing a long polyphosphate chain were introduced.[122] As time went by, new side chains like pyrophosphate and nowadays the bone-seeking bisphosphonates have been introduced. After the uptake of bisphosphonates, different predictions can be made. High local uptake may signalize sites where injuries are likely to occur or where primary or metastatic tumors which induce rapid bone turnover are located. Moreover, some radiolabeled bisphosphonates are able to highlight pathological tissues for the diagnosis of meningiomas[123], cirrhosis of liver[124], cerebral infarction[125], myocardial infarction[126], osteosarcoma[127], breast cancer[128] and others.

With those considerations in mind, the synthesis of new radiolabeled bisphosphonates is important. Therefore, the synthesis of a radiolabeled zoledronate derivative which could be useful for imaging and its bone seeking properties might be of great interest.

## 5.2 Synthesis of racemic and enantiomerically pure (R)-pyrrolidin-2-ylphosphonic acid

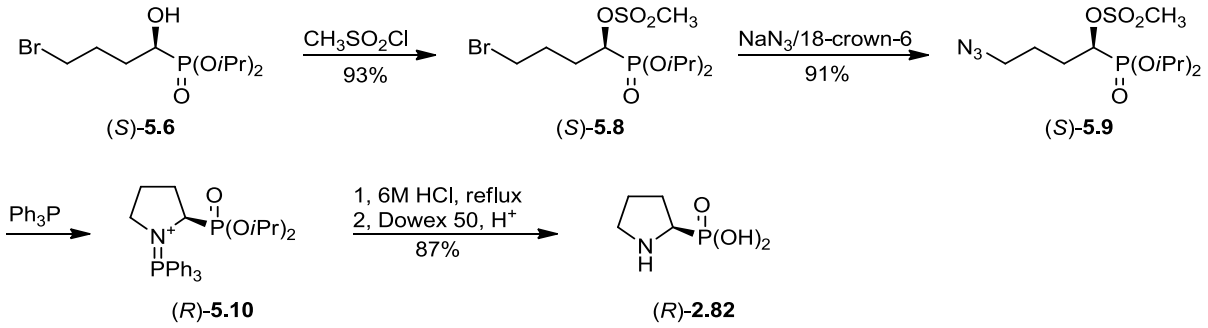
The synthesis of enantiomerically pure phosphaproline (*R*)-**2.82** was already developed by a former group member.[129] The synthesis started from ethyl 4-bromobutanoate (**5.5**), which was reduced with DIBALH in toluene at -78°C (*Scheme 5.1*).




*Scheme 5.1. Synthesis of enantiomerically pure α-hydroxyphosphonate (S)-**5.6**.*

After 2h of stirring, diisopropyl trimethylsilyl phosphite was added and the cooling bath was removed. After stirring overnight and acidic workup the racemic hydroxyphosphonate (±)-**5.6** was obtained. It was converted to its corresponding chloroacetate (±)-**5.7**, using chloroacetic anhydride and pyridine in order to perform a kinetic enzyme-catalysed resolution. The hydrolysis was performed in a biphasic system of hexanes/*tert*-butyl methyl ether (1:1) and phosphate buffer (25 mM) at pH 7 with the help of an autotitrator. At a conversion of 45%, hydrolysis was stopped by addition of HCl (2 M) to lower the pH to 4.0. Workup and purification by flash chromatography furnished enantiomerically pure (*S*)-α-hydroxyphosphonate (*S*)-**5.6** and chiral, nonracemic (*R*)-chloroacetate (*R*)-**5.7**.

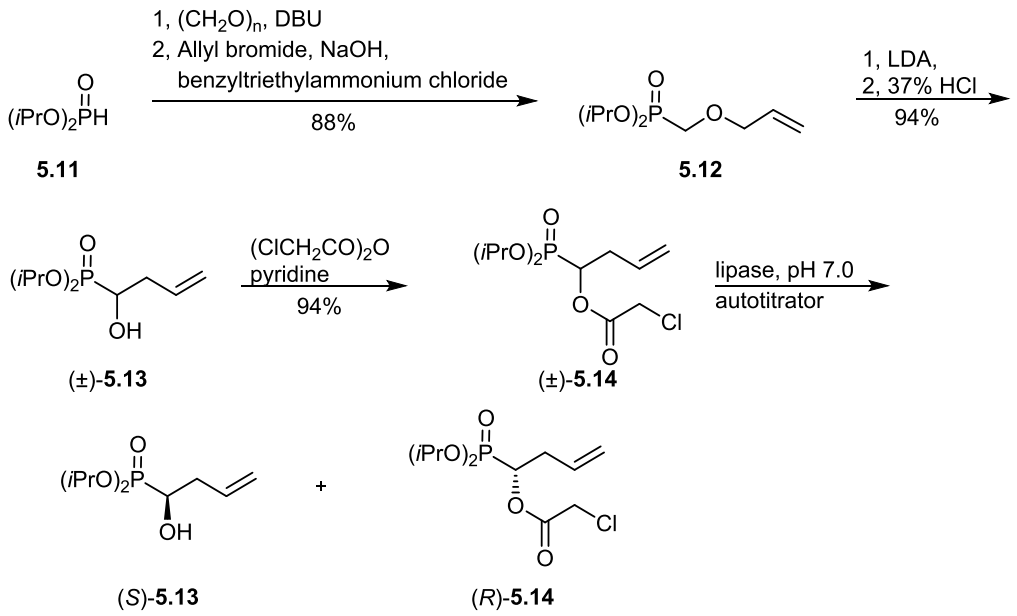
After obtaining the (*S*)-hydroxyphosphonate (*S*)-**5.6** in high yield and ee >99%, treatment with methanesulfonyl chloride/base gave the (*S*)-mesylate (*S*)-**5.8** in excellent yield (*Scheme 5.2*).




*Scheme 5.2. Synthesis of (R)-phosphaproline (R)-**2.82**.*

Reaction with $NaN_3$ and 18-crown-6 yielded the (*S*)-azidophosphonate (*S*)-**5.9**. Staudinger reaction with $PPh_3$ gave an iminophosphorane which cyclised to compound (*R*)-**5.10**. Hydrolysis with 6 M HCl and purification by ion-exchange chromatography furnished (*R*)-phosphaproline (*R*)-**2.82**.[129]

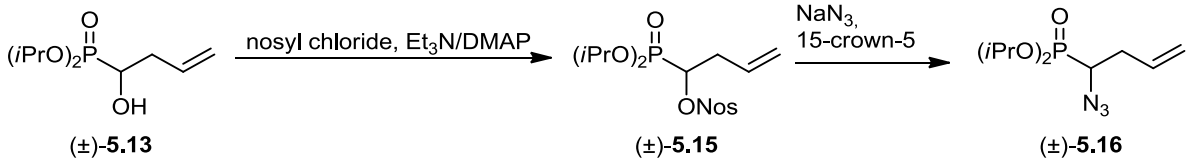
I tried to optimize the synthesis in order to increase the yield and reduce the number of steps. I proposed a new synthesis based on a precursor **5.13** already synthesised by a former group member by addition of diisopropyl phosphite to paraformaldehyde catalysed by DBU (*Scheme 5.3*).




*Scheme 5.3. Synthesis of (S)-diisopropyl but-3-enylphosphonate [(S)-**5.13**].*

The intermediate hydroxymethylphosphonate was not isolated but directly allylated with allyl bromide in the presence of sodium hydroxide and benzyltriethylammonium chloride as a phase-transfer catalyst.[140] Allyl ether **5.12** underwent a [2,3]-sigmatropic rearrangement upon treatment with LDA and the subsequent acidic workup gave (±)-hydroxyphosphonate (±)-**5.13** in good yield. To obtain the enantiomerically pure compounds, the racemic chloroacetate was synthesised followed by hydrolysis with the lipase from *Thermomyces lanuginosus* (≥100,000 U/g, from Aldrich) under the same conditions as outlined above. Precursor (*S*)-5.13 was then used for the synthesis of different aminophosphonic acids.[140]

With this precursor in hand, I prepared (±)-1-azidophosphonate (±)-**5.16** from nosylate (±)-**5.15** with $NaN_3$ and 15-crown-5 at first (*Scheme 5.4*).




*Scheme 5.4. Synthesis of α-azidophosphonate (±)-**5.16**.*

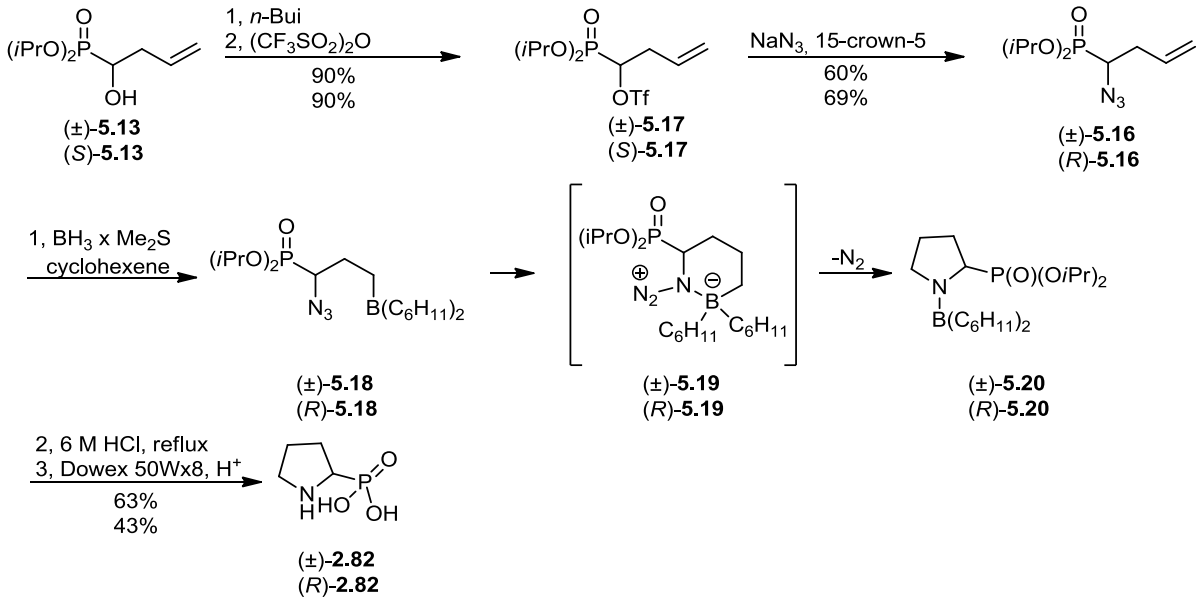
The yield of this reaction was 87% after 96 h at 60°C as determined by $^1$H NMR spectroscopy, but separation of starting nosylate (±)-**5.15** and azidophosphonate (±)-**5.16** by flash chromatography was not possible.

I decided to introduce a different leaving group in order to facilitate isolation of the product. In this regard, the trifluoromethanesulfonate (±)-**5.17** gave the necessary $R_f$ difference for the separation of ester and azide. However, the preparation of the triflate itself was not as easy as imagined. Various reaction times and temperatures as well as bases and solvents were tested (*Table 5.1*).

| Entry | Base | Solvent | Temp. [°C] | Time [h] | Yield [%] |
|---|---|---|---|---|---|
| 1 | pyridine | $CH_2Cl_2$ | -35 | 3 | n.d. |
| 2 | pyridine | $CH_2Cl_2$ | -25 | 2 | n.d. |
| 3 | *n*-BuLi | $Et_2O$ | -78 | 0.75 | 42 |
| 4 | *n*-BuLi | $Et_2O$ | -78 | 1.5 | 90 |

*Table 5.1. Reaction conditions for the preparation of triflate (±)-**5.17**.*

The reaction did not work with pyridine as a base and only starting material was observed, even when both, time and temperature, were varied. Hence, trying a different base and varying the reaction conditions was necessary. The best yield was obtained when the α-hydroxyphosphonate (±)-**5.13** was converted to the lithium alkoxide first (*Scheme 5.5*).

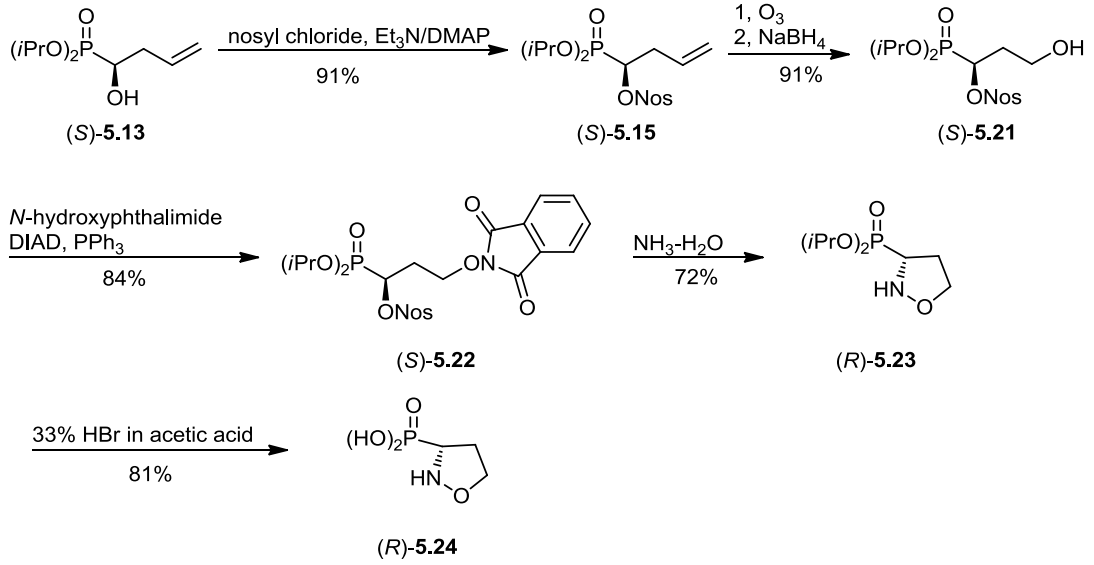



*Scheme 5.5. Synthesis of (±)- and (R)-phosphaproline [(±)-**2.82** and (R)-**2.82**].*

Therefore, *n*-BuLi was added to a solution of the hydroxyphosphonate in $Et_2O$ at -78°C and the reaction mixture was stirred for 5 min. Trifluoromethanesulfonic anhydride was slowly added. Workup and purification gave the triflate (±)-**5.17** in 90% yield. The activated hydroxyphosphonate (±)-**5.17** was then converted to the racemic azide (±)-**5.16** in 69% yield as outlined above. The cyclisation of homoallyl azide (±)-**5.16** to phosphaproline is reminiscent of the preparation of chiral, non-racemic substituted pyrrolidines by Salmon and Carboni[130]. Dicyclohexylborane prepared from borane dimethyl sulfide and cyclohexene was added to the alkene regioselectively. The trialkylborane (±)-**5.18** underwent a sequence comprising a cyclisation to compound (±)-**5.19**, migration of an alkyl group and loss of nitrogen. The intermediate aminoborane (±)-**5.20** was deprotected with refluxing HCl and the (±)-phosphaproline (±)-**2.82** was isolated by ion-exchange chromatography in an overall yield of 43% over these three steps (*Scheme 5.5*). The same procedure was used for the preparation of the chiral phosphaproline (*R*)-**2.82** (63% yield), which should have the same ee (97%) as the starting α-hydroxyphosphonate (S)-**5.13**.

## 5.3 Synthesis of racemic and enantiomerically pure isoxazolidin-5-ylphosphonic acid

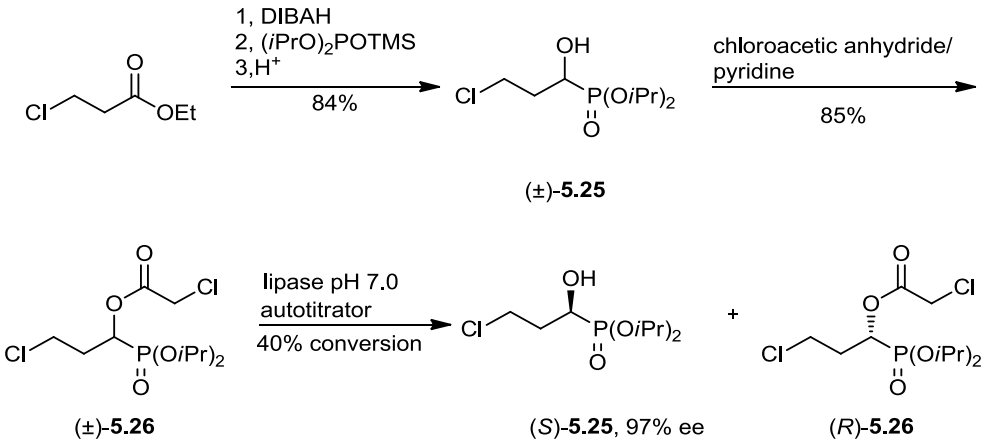
Isoxazolidin-3-ylphosphonic acid (*R*)-**5.24** was prepared by a former group member.[140] The synthesis started from enantiomerically pure (*S*)-α-hydroxyphosphonate (*S*)-**5.13** (synthesis shown in chapter 5.2), which was converted to the corresponding (*S*)-nosylate (*S*)-**5.15** using nosyl chloride/$Et_3N$/DMAP in $CH_2Cl_2$ in 91% yield (*Scheme 5.6*).




*Scheme 5.6. Synthesis of (R)-isoxazolidin-3-ylphosphonic acid [(R)-**5.24**].*

Ozonolysis followed by reductive work up with $NaBH_4$ gave the (*S*)-γ-hydroxyphosphonate (*S*)-**5.21** in excellent yield (91%). The hydroxyl group was replaced by the phthalimidooxy group in 84% yield under Mitsunobu conditions using DIAD, $PPh_3$ and *N*-hydroxyphthalimide. When phthalimido derivative (*S*)-**5.22** was treated with aqueous ammonia, the amino group was partially or fully deblocked. It attacked as nucleophile at C-1 to replace the nosyloxy group with inversion of configuration. The isooxazolidin-3-ylphosphonate (*R*)-**5.23** formed had to be deprotected with HBr (33%) in acetic acid at ambient temperature. The enantiomerically pure (*R*)-isoxazolidin-3-ylphosphonic acid [(*R*)-**5.24**] was isolated by ion exchange chromatography in 81% yield. When refluxing HCl or TMSBr were used for deprotection of phosphorus at 50 °C, side products resulted and the desired product formed in low yield (by NMR) could not be isolated in homogenous form. The same procedure was used for the synthesis of the racemic compound (±)-**5.24**.

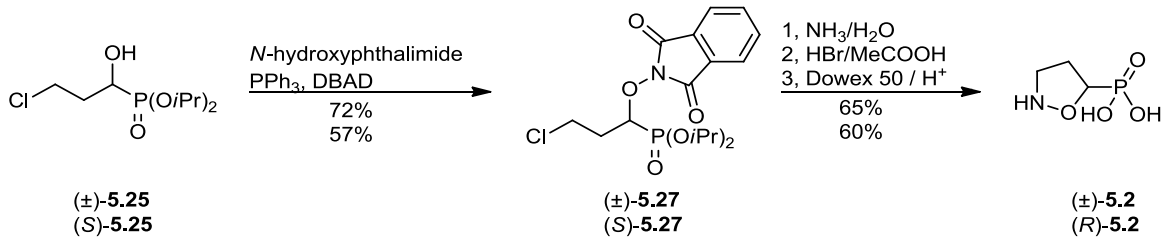
The permuted analogue, namely (*R*)-isoxazolidin-5-ylphosphonic acid [(*R*)-**5.2**], seems to be another valuable phosphonic acid analogue of L-proline, which could be tested for its inhibitory properties against P5C reductase. Therefore, α-hydroxyphosphonate (±)-**5.25** was prepared from ethyl 3-chloropropionate and diisopropyl trimethylsilyl phosphite in 84% yield (*Scheme 5.7*).




*Scheme 5.7. Lipase-catalysed kinetic resolution of chloroacetate (±)-**5.26**.*

It was chloroacetylated using chloroacetic anhydride and pyridine as a base. Afterwards, enzyme-catalysed resolution (conversion 40%) of the chloroacetate (±)-**5.26** with lipase from *Thermomyces lanuginosus* gave the enantiomerically pure (ee 97%) (*S*)-α-hydroxyphosphonate (*S*)-**5.25** and the chiral, nonracemic (*R*)-chloroacetate (*R*)-**5.26**.

The synthesis of the isoxazolidin-5-ylphosphonic acid was optimised in the racemic series. The (±)-hydroxyphosphonate (±)-**5.25** was treated with *N*-hydroxyphthalimide, di-*tert*-butyl azodicarboxylate and $PPh_3$ under Mitsunobu conditions to obtain the (±)-phthalimidooxyphosphonate (±)-**5.27** in 57% yield (*Scheme 5.8*).




*Scheme 5.8. Synthesis of isoxazolidin-5-ylphosphonic acid [(R)-**5.2**].*

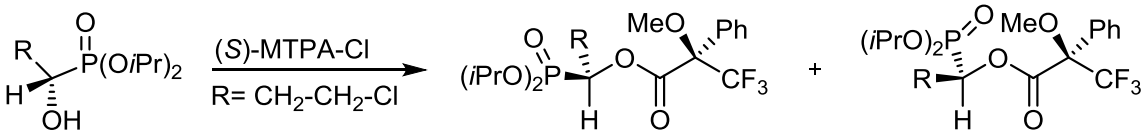
When tris(4-chlorophenyl)phosphine was used instead of triphenylphosphine, the yield was increased by 10% as determined by $^1$H NMR, but a separation problem appeared. Therefore $PPh_3$ was used due to the higher overall yield after chromatography. Afterwards, aqueous $NH_3$ was added and the solution was allowed to stir overnight. The crude product was hydrolysed with HBr (33%) in acetic acid at room temperature followed by ion-exchange chromatography to obtain the (±)-isoxazolidinylphosphonic acid (±)-**5.2** in 60% yield. The (R)-enantiomer was prepared similarly with comparable yields. Its ee should be the same as that of (*R*)-**5.27** (ee 96%).

Several methods have been used for the determination of the enantiomeric excess and the configuration of α-hydroxyphosphonates. For the (+)-α-hydroxyphosphonate **5.25**, the ee and (S)-configuration were determined either with (*S*)-Mosher chloride (*S*)-**5.28** or the chiral solvating agent (*R*)-**5.29** (CSA) (*Figure 5.3*).


*Figure 5.3. Chemical shift reagents.*

The CSA (*R*)-**5.29** was synthesised using a method reported in literature.[134] Since no derivatisation is necessary for the determination of the enantiomeric excess, it is a rapid and clean method. This chiral solvating agent was used for the determination of ee of different compounds such as phosphinic amides, phosphinates, phosphine oxides and many more.[131] The enantiomeric excess of secondary[132] as well as tertiary[133] α-hydroxyphosphonates was determined via this method as well. Hammerschmidt *et al*.[134] were the first to determine the enantiomeric excess and the absolute configuration of α-hydroxyphosphonates via $^{31}$P NMR spectroscopy. They found for a series of α-hydroxyphosphonates that the complexes with CSA (*S*)-**5.29** for the (*R*)-configured α-hydroxyphosphonates resonated at lower field than for the (*S*)-configured ones. However, when (*R*)-CSA is used, (*S*)-configured phosphonates resonate at lower fields than the (*R*)-configured ones. This prediction was confirmed via $^{31}$P NMR spectroscopy of (+)-α-hydroxyphosphonate **5.25**, which showed a major singlet at 22.90 ppm and a minor one at 22.69 ppm. This α-hydroxyphosphonate has therefore (*S*)-configuration and the ee (97%) was calculated from the signal areas.

This difference of chemical shift was also observed when the (*S*)-α-hydroxyphoshonate (*S*)-**5.25** was esterified with α-methoxy-α-(trifluoromethyl)phenylacetic chloride [(*S*)-**5.28**] ((*S*)-MTPACl) to obtain two diastereomeric esters (*Scheme 5.9*)




*Scheme 5.9. Conformation model for (R)-Mosher esters.*

As proven by Li and Hammerschmidt[135], the absolute configuration can be easily determined using $^{31}$P NMR spectroscopy. They found for a series of chiral, nonracemic α-hydroxyphoshonates (*S*)-5.**30** that the phosphorus atoms of the (*R*)-Mosher esters derived from the (*S*)-hydroxyphosphonates resonated at lower field than those derived from the (*R*)-hydroxyphosphonates. The same was found for the (*R*)-Mosher esters derived from (*S*)- and (*R*)-**5.25**. This might be due to the fact that the phosphorus atom in (*R*)-MTPA esters is shielded by the phenyl group when the alcohol has (*R*)-configuration based on the conformation model for Mosher ester (*Scheme 5.9*).

Therefore, (*S*)-configuration can be assigned to (+)-α-hydroxyphosphonate **5.25** on the basis of the $^{31}$P NMR spectrum: major signal (integration 1.000) at 16.00 ppm and minor signal (0.024) at 15.40 ppm; ee 95%.

## 5.4 Synthesis of racemic and chiral, nonracemic pyrazolidin-3-ylphosphonic acid of high enantiomeric excess

After the synthesis of phosphaproline (*R*)-**2.82** and isoxazolidin-5-ylphosphonic acid [(*R*)-**5.2**], the synthesis of another L-proline analogue, namely pyrazolidin-3-ylphosphonic acid [(*R*)-**5.1**], was planned (*Figure 5.4*).

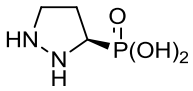
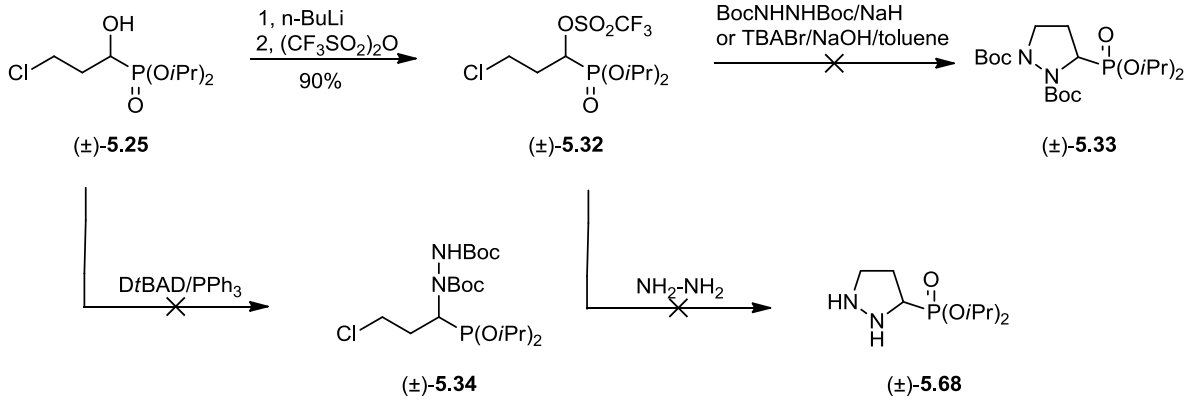


*Figure 5.4. Desired analogue of phosphaproline (R)-**5.1**.*

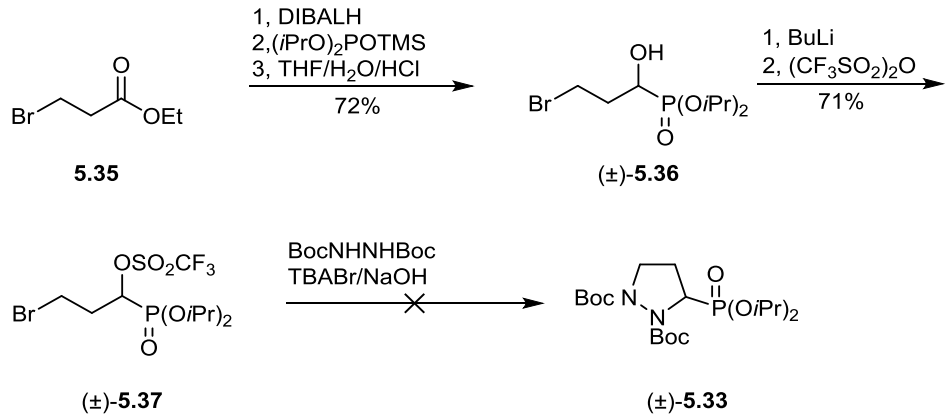
As before, the synthesis of the pyrazolidin-3-ylphosphonic acid [(±)-**5.**1] was optimised in the racemic series. On the first thought, this cyclic hydrazinophosphonic acid should be easily prepared starting from the same precursor as the isoxazolidin-3-ylphosphonic acid [(*R*)-**5.2**]. Treatment of α-hydroxyphosphonate (±)-**5.25** with di-*tert*-butyl azodicarboxylate and $PPh_3$ in toluene under Mitsunobu conditions did not yield the desired hydrazine derivative (±)-**5.34** (*Scheme 5.10*).




*Scheme 5.10. First attempts to synthesise pyrazolidin-3-ylphosphonic acid [(±)-**5.1**].*

To accomplish the addition of hydrazine or its di-Boc-protected derivative, introduction of a leaving group was necessary. Since the trifluoromethanesulfonyloxy group was used as leaving group for the preparation of phosphaproline (±)-**2.82**, it seemed obvious to use it for this derivative too. The introduction of the leaving group was easy, using $(CF_3SO_2)_2O$ in $Et_2O$ and *n*-BuLi as a base to obtain the triflate (±)-**5.32** in 91% yield. Unfortunately, treatment of the triflate with hydrazine or the anion derived from the di-Boc protected hydrazine did not yield the cyclised product at all. The failure of the reactions was attributed to the chloride, which is a bad leaving group, and the notorious sluggish $S_N2$ reactions at C-1, which will be attacked easily intramolecularly. It was hoped that bromide would facilitate substitution at C-3 and then the cyclisation should proceed smoothly.

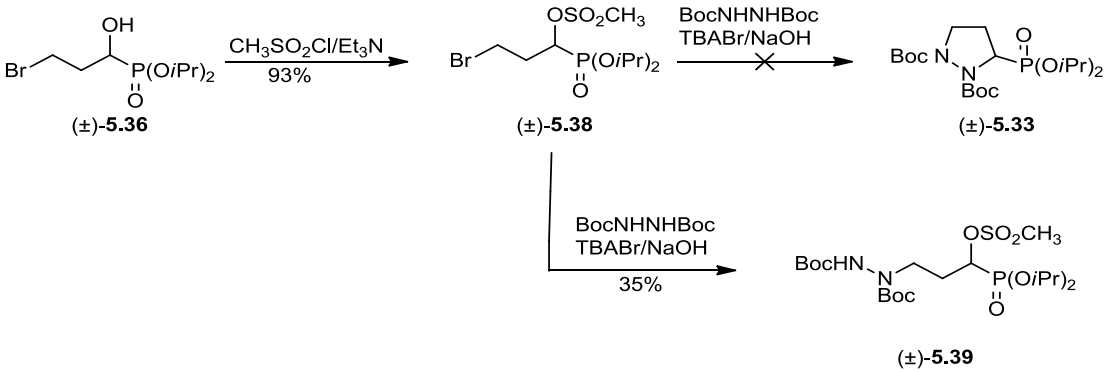
Therefore, the 3-bromo-1-hydroxyphosphonate (±)-**5.36** was envisaged as an alternative and better starting material than the chloro analogue, as bromide is a better leaving group than chloride. It was obtained in 72% yield from ethyl 3-bromopropionate **5.35** which was reduced with DIBALH to the corresponding aldehyde. Addition of diisopropyl trimethylsilyl phosphite furnished the silylated α-hydroxyphosphonate which was deprotected by acidic workup (*Scheme 5.11*).




*Scheme 5.11. First attempt for the synthesis of (±)-**5.33** with bromo-α-hydroxyphosphonate (±)-**5.36**.*

The first attempt with the bromo-hydroxyphosphonate (±)-**5.36** was the formation of the triflate in 72% yield under the same conditions as outlined for (±)-**5.25**. However, treatment of the activated hydroxyphosphonate (±)-**5.37** with the di-Boc-protected hydrazine in a biphasic system with a phase transfer catalyst did not yield the desired product. This led to the consideration that the triflate (±)-**5.37** was possibly too reactive, since the $^{31}P$ NMR spectrum showed plenty of signals.

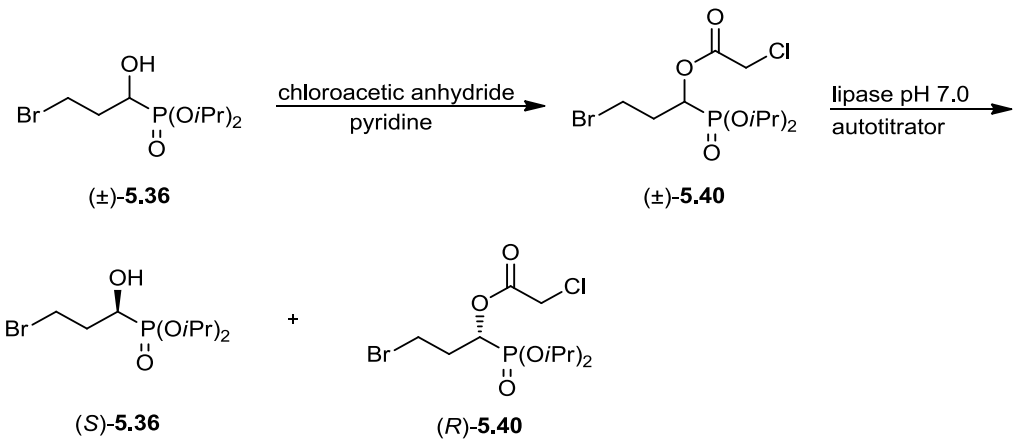
Next, the mesyloxy group, which is less reactive than the trifluoromethanesulfonyloxy group as leaving group at C-1, was used. The α-hydroxyphosphonate (±)-**5.36** was treated with $CH_3SO_2Cl$ and $NEt_3$ in $CH_2Cl_2$ to give mesylate (±)-**5.38** in 93% yield (*Scheme 5.12*).




*Scheme 5.12. Attempted conversion of mesylate (±)-**5.38** to pyrazolidin-3-ylphosphonate (±)-**5.33**.*

It was reacted with the protected hydrazine, tetrabutylammonium bromide and NaOH at 50°C in a mixture of water/toluene, but did not yield the pyrazolidine (±)-**5.33**. However, careful investigation of the reaction products by NMR spectroscopy in combination with flash chromatography delivered substituted hydrazine (±)-**5.39** in 35% yield, surprisingly. Evidently, this product did not cyclise under the basic reaction conditions even when heated at higher temperatures (60°C). Clearly, a more reactive leaving group than the methanesulfonyloxy (mesyloxy) group, but less reactive group than the trifluoromethanesulfonyloxy group was necessary. The p-nitrobenzenesulfonyloxy (nosyloxy) group seemed to be best suited for that purpose.

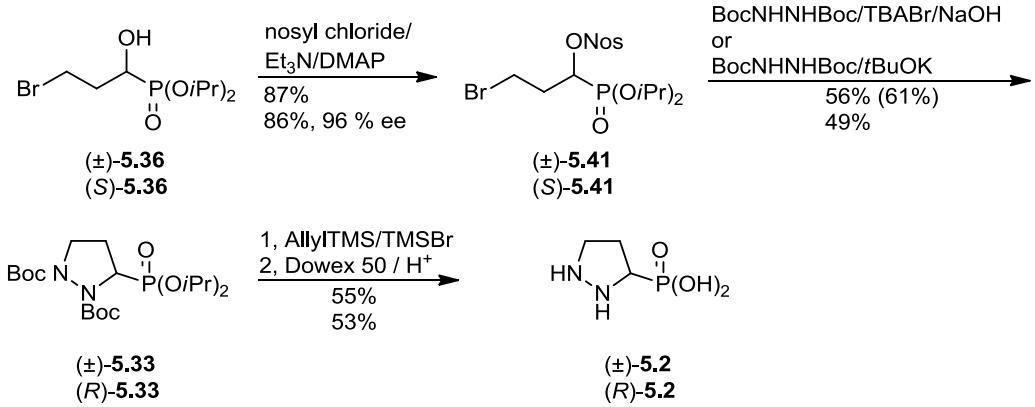
The racemic α-hydroxyphosphonate (±)-**5.36** was resolved by lipase-catalysed enantioselective hydrolysis as outlined in chapter 5.3. The hydroxyphosphonate was chloroacetylated with chloroacetic anhydride in the usual way and hydrolysed with lipase from *Thermomyces lanuginosus* in a biphasic system (phosphate buffer pH 7.0/hexanes and t-butyl methyl ether) (*Scheme 5.13*).




*Scheme 5.13 Lipase-catalysed resolution of racemic hydroxyphosphonate (±)-**5.36**.*

When the reaction was stopped at a conversion rate of 40%, the enantiomerically pure (95%) (*S*)-α-hydroxyphosphonate (*S*)-**5.36** and chiral, non-racemic (*R*)-chloroacetate (*R*)-**5.40** were obtained.

First, the synthesis of the pyrazolidin-3-ylphosphonic acid [(±)-**5.1**] was optimised in the racemic series. The α-hydroxyphosphonate (±)-**5.36** was treated with 4-nitrobenzensulfonyl chloride with $Et_3N$/DMAP as bases to obtain the (±)-nosylate (*S*)-**5.41** (*Scheme 5.14*).



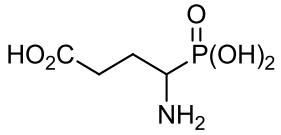

*Scheme 5.14. Synthesis of racemic and (R)-pyrazolidin-3-ylphosphonic acid [(±)- and (R)-**5.2**].*

The nosylation did not work as well as triflation, but 86% yield was perfect. To synthesise the cyclised compound (±)-**5.33**, two different methods were used, either treatment of the nosylate (±)-**5.41** with di-Boc-protected hydrazine, tetrabutylammonium bromide in aqueous

NaOH/toluene (method A) or with the di-Boc-protected hydrazine and *tert*-BuOK in dry DMF at 60°C (method B). Both methods gave similar yields (56 and 61%). Finally, with the cyclised product (±)-**5.33** in hand, deprotection of the nitrogens as well as of the phosphorus was supposed to be relatively simple and straightforward. However, this was not the case. At first, I tried to globally deported the pyrazolidin-3-ylphosphonate (±)-**5.33** hydrolytically with HCl (6M) under reflux conditions, but $^{31}$P NMR spectroscopy showed the formation of phosphate and other side products, which led to the conclusion that the substituted pyrazolidine (±)-**5.33** was not stable under these harsh reaction conditions. The second reagent evaluated for deprotection was HBr (33%) in acetic acid. In this case however, all protective groups were removed but one of the nitrogens was acetylated surprisingly. This resulted in a 1:1 mixture of N-acetylated pyrazolidin-3-ylphosphonic acids. To obtain the free phosphonic acid, hydrochloric acid could have been used, but to keep the number of steps needed for the synthesis of this phosphonic acid as low as possible, deprotection with allyltrimethylsilane and trimethylsilyl bromide was used. After successful global deprotection, ion-exchange chromatography was performed, which led to the free (±)-pyrazolidin-3-ylphosphonic acid [(±)-**5.1**] in 55% yield. The (*R*)-enantiomer of **5.1** was prepared in the same way as the racemic compound in yield similar to those of the racemic series (see Scheme 5.14). It is assumed that the ee of (*R*)-**5.1** is that same as that of the nosylate (ee 96%) as determined by HPLC on a chiral stationary phase. This value agreed with the ee of 95% of the starting hydroxyphosphonate (*S*)-**5.35** which was determined by using $^{31}$P NMR spectroscopy of the (*R*)-Mosher-ester (*R*)-**5.28** and the diasteromeric complexes formed with CSA (*R*)-**5.29** (chapter 5.3, especially *Figure 5.3*).

## 5.5 Towards the synthesis of racemic 4-amino-4-phosphonobutanoic acid (phosphaglutamic acid)

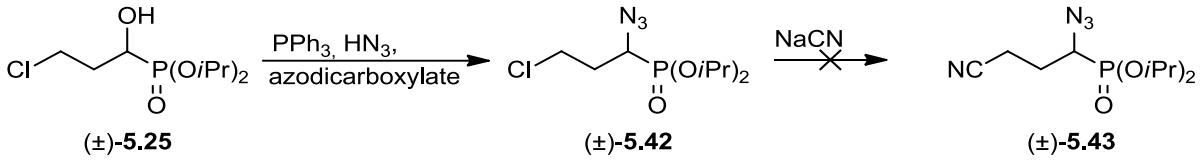
With the 3-chloro-1-hydroxyphosphonate (±)-**5.25** in hands, the idea was born to synthesise the phosphonic acid analogue (±)-**5.3** of glutamic acid, at first in racemic and then in chiral, nonracemic form of high ee (*Figure 5.5*).




(±)-**5.3**

*Figure 5.5. Structure of phosphaglutamic acid (±)-**5.3**.*

The first approach started from already available hydroxyphosphonate (±)-**5.25** which was synthesized for the isoxazolidin-5-ylphosphonic acid [(±)-**5.2**]. Mitsonobu reaction with $PPh_3$, $HN_3$ and dialkyl azodicarboxylate gave the azidophosphonate (±)-**5.42** (*Scheme 5.15*).




(±)-**5.25** (±)-**5.42** (±)-**5.43**

*Scheme 5.15. First attempts to synthesise phosphaglutamic acid (±)-**5.3**.*

Different reaction conditions have been used for its formation (*Table 5.2*).

| Entry | Azodicarboxylate | Time [h] | Yield [%] |
|---|---|---|---|
| 1 | DIAD | 16 | 39 |
| 2 | DIAD | 8 | 55 |
| 3 | D*t*BAD | 3 | 61 |

*Table 5.2. Azodicarboxylates and reaction time used for formation of (±)-**5.42**.*

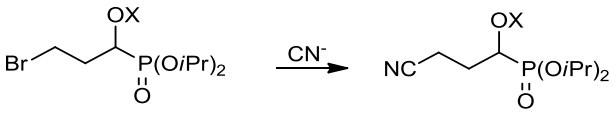
The best result was achieved using di-*tert*-butyl azodicarboxylate and holding the reaction time as short as possible. Treatment with NaCN and different additives, as shown in *Table 5.3*, did not yield the desired azidonitrile (±)-**5.43**.

| Entry | Additive | Crown ether | Time [h] | Yield |
|---|---|---|---|---|
| 1 | NaI | 18-crown-6 | 72 | n.d. |
| 2 | NaI | 15-crown-5 | 28 | n.d. |
| 3 | NaBr | 15-crown-5 | 28 | traces |

*Table 5.3. Reaction conditions for the introduction of the cyano group (n.d.: not detected).*

Because of these poor results, the synthesis needed to be reconsidered. Chloride was replaced by bromide to increase the reaction rate for the substitution by cyanide. However, the use of the bromophosphonate was not compatible with the Mitsunobu reaction, which would give beside the desired azido-bromophosphonate also the diazidophosphonate as side product as found by Wuggenig[136] for the racemic diisopropyl 4-bromo-1-hydroxypentylphosphonate.

Therefore, the azido group had to be introduced after the cyano group. Starting from γ-bromo-phosphonates (±)-**5.37, 5.38** and **5.41** with different leaving groups OX at C-1, many reaction conditions were tested (*Scheme 5.16, Table 5.4*).




(±)-**5.37** X = Tf
(±)-**5.38** X = Ms
(±)-**5.41** X = Nos

(±)-**5.44** X = Tf
(±)-**5.45** X = Ms
(±)-**5.46** X = Nos

*Scheme 5.16. Introduction of the cyano group.*

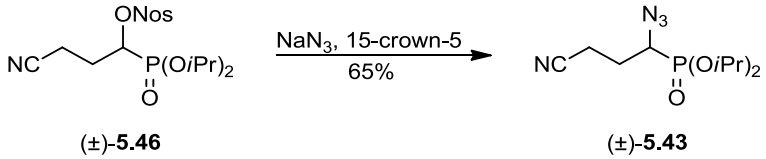
| Entry | X | Solvent | Crown ether | Cyanide | Temp. [°C] | Time [h] | Yield [%] |
|---|---|---|---|---|---|---|---|
| 1 | Ms | DMSO | 15-crown-5 | NaCN | r. t. | 17 | 79 (+SM) |
| 2 | Ms | DMSO | 15-crown-5 | NaCN | 50 | o. n.[a] | n. d.[b] |
| 3 | Tf | DMSO | 15-crown-5 | NaCN | r. t. | o. n. | n. d. |
| 4 | Nos | DMSO | 15-crown-5 | NaCN | r. t. | o. n. | n. d. |
| 5 | Nos | DMF | 15-crown-5 | NaCN | -50 | o. n. | 34 |
| 6 | Nos | DMF | 15-crown-5 | NaCN | -45 | 6 | 44 |
| 7 | Nos | DMF | 15-crown-5 | NaCN | -50 | 7 | 32 |
| 8 | Nos | DMF | 18-crown-6 | KCN | -20 | 24 | 45 |

*Table 5.4. Reaction conditions for the introduction of the cyano group ([a]o. n.: over night; [b]n. d.: not detected; SM = starting material).*

The first experiment performed with mesylate (±)-**5.38** looked promising (Entry 1). However, the separation of product from starting material was not possible to accomplish and a mixture of product and starting material (±)-**5.36** was isolated by flash chromatography (ratio by $^{31}$P NMR: 79:21). Therefore, changing the reaction conditions and the starting material was necessary to

obtain the desired product (±)-**5.44-46**. Acceptable yields were only obtained when the nosylate (±)-**5.41** was used as starting material for the substitution reaction (Entries 5-8). The best yield was obtained when it was reaction with KCN in dry DMF at -20 °C in the presence of 18-crown-6 to complex the potassium ion (Entry 8). After stirring for 24 h at -20°C the nitrile (±)-**5.46** was isolated in 45% yield.

The next step towards the synthesis of the phosphaglutamic acid (±)-**5.3** was the introduction of the azido group using $NaN_3$ and 15-crown-5 (*Scheme 5.17*).

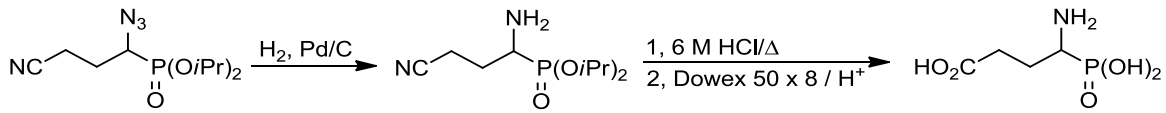



(±)-**5.46** (±)-**5.43**

*Scheme 5.17. Introduction of the azido group.*

This nucleophilic substitution was carried out in DMF at 70°C over 5 h. The separation of starting material form the product seemed to be difficult at first, but after some tries I was able to obtain the azide (±)-**5.43** in 65% yield.

This was the last step of the synthesis I performed due to lack of time. Nonetheless, I will just give a short overview of what the finishing steps would have been. Azido-nitrile (±)-**5.43** would have been reduced to the amine (±)-**5.47** with $H_2$/Pd/C (*Scheme 5.18*).



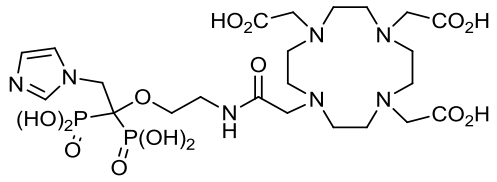

(±)-**5.43** (±)-**5.47** (±)-**5.3**

*Scheme 5.18. Outlook for the final steps of the synthesis of phosphaglutamic acid [(±)-**5.3**].*

Treatment with HCl (6 M) would have converted the cyano into a carboxyl group and at the same time the phosphorus would have been deprotected to give the racemic phosphaglutamic acid (±)-**5.3**. Similarly, the (*R*)-enantiomer (*R*)-**5.3** would be obtained by starting from (*S*)-3-bromo-1-hydroxyphosphonate (*S*)-**5.36**.

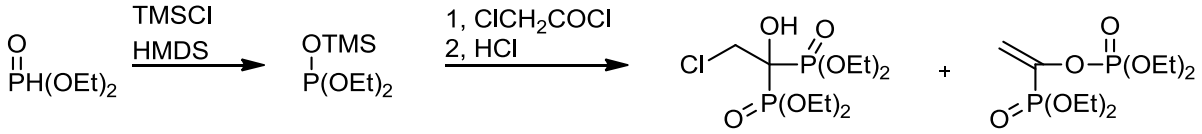
## 5.6 Towards the synthesis of a DOTA-zoledronate derivative

As already mentioned, another part of my thesis was the synthesis of a DOTA-zoledronate derivative **5.4**, which can be used as a skeletal imaging agent (see *Figure 5.2*). It was planned to synthesise a hydroxybisphosphonate containing a leaving group for the introduction of the imidazolyl group. The hydroxyl group could be used to attach via a short spacer the DOTA.



**5.4**

The synthesis started with diethyl phosphite **5.48**, which was converted to the silylated phosphite **5.49** in 81% yield (*Scheme 5.19*).



**5.48** **5.49** **5.50** **5.51**

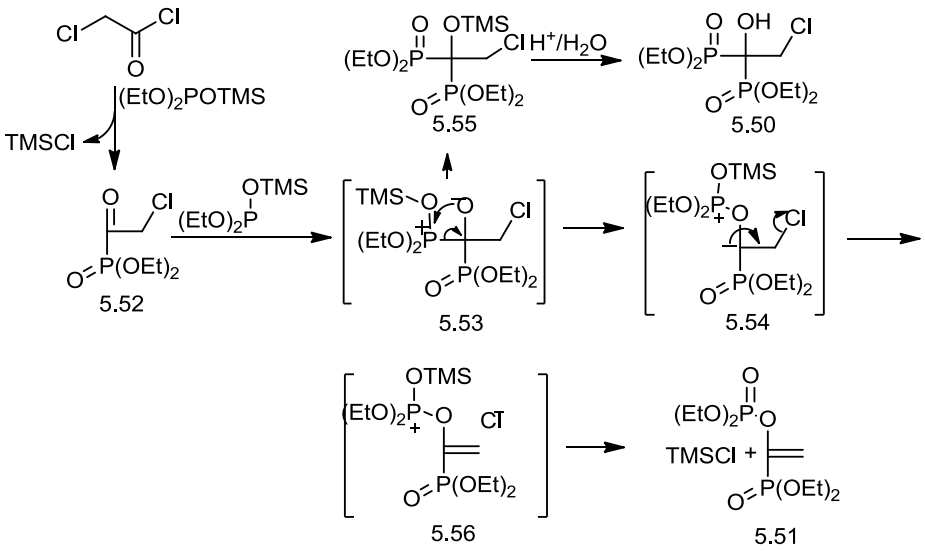
*Scheme 5.19. Synthesis of bisphosphonate **5.50**.*

The next step was more complicated than I thought. Treatment of diethyl trimethylsilyl phosphite (**5.49**) with 2-chloroacetyl chloride gave the bisphosphonate **5.50** only in 39% yield at best. Changing the reaction conditions did not improve the yield at all (*Table 5.5*), due to the generation of the enol phosphate **5.51**.

| Entry | Solvent | Temp. [°C] | Time [h] | Yield |
|---|---|---|---|---|
| 1 | $CH_2Cl_2$ | -78 | 1.75 | 20 |
| 2 | THF | -78 | 2 | 39 |
| 3 | THF | -90 | 3 | 35 |
| 4 | THF | -90 | 2.25 | 31 |

*Table 5.5. Reaction conditions for the synthesis of bisphosphonate **5.50**.*

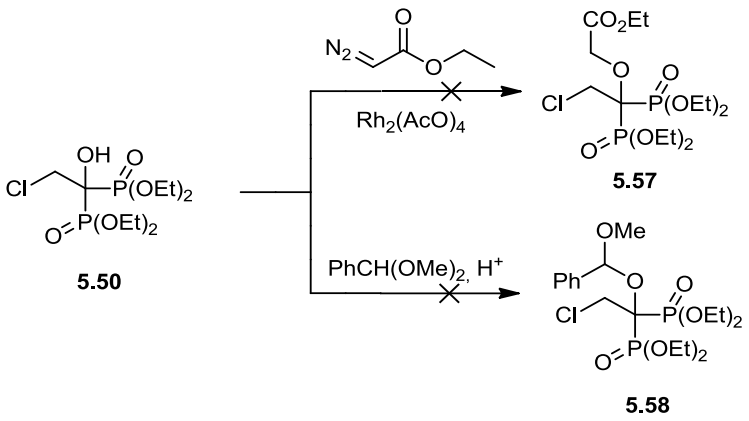
A tentative mechanism for this transformation is given in *Scheme 5.20*.




*Scheme 5.20. Mechanism for the formation of enol phosphate **5.51**.*

The silylated phosphite **5.49** added to the $\alpha$-oxophosphonate **5.52** to give intermediate **5.53**, which could follow two reaction pathways. It ended up as silylated hydroxybisphoshonate **5.55** by migration of TMS from the oxygen atom at phosphorus or as alkoxonium intermediate **5.54** by a phosphonate-phosphate like rearrangement[137], followed by elimination of chloride. The intermediate **5.56** gave TMSCl and the enol phosphate as side product. The silylated hydroxybisphosphonate **5.55** was deprotected under the acidic conditions of work up.

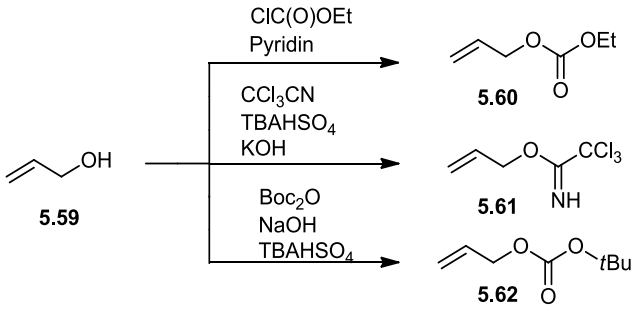
With the bisphosphonate **5.50** in hands, the next step would have been the etherification of the hydroxyl group. For the first attempt, bisphosphonate **5.50** was treated with $Rh_2(OAc)_4$ and ethyl 2-diazoacetate at room temperature (*Scheme 5.21*).[138]




*Scheme 5.21. Attempted synthesis of ether **5.57** and mixed acetal **5.58** from the hydroxybisphosphonate **5.50**.*

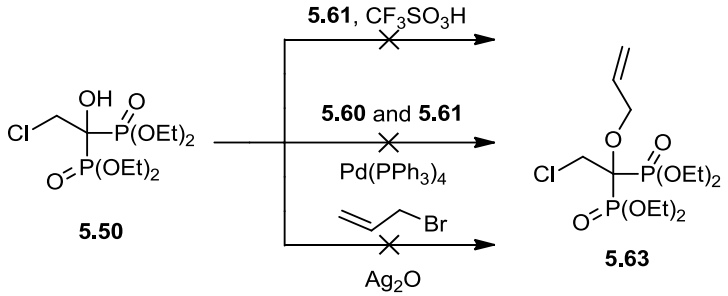
Despite a reaction time of five days no desired reaction product was observed. It could have been converted to an aminoethyl group to attach DOTA via amide bond formation. Therefore another method was tried. The next attempt was the treatment of bisphosphonate **5.50** with benzaldehyde dimethylactal and p-toluenesulfonic acid. Since no conversion was observed after 24 h, a few drops of trifluoromethanesulfonic acid were added. This did not turn out to be a good idea since the reaction mixture turned black and only the elimination product **5.51** was observed.

With this knowledge, I needed to reconsider the synthetic strategy. Therefore, allylation of the hydroxyl group seemed to be the way to go. Various derivatives of allyl alcohol **5.59** were prepared, namely allyl ethyl carbonate[144] **5.60**, trichloroacetimidate[146] **5.61** and allyl *tert*-butyl carbonate[147] **5.62**, which could be used for the allylation under appropriate conditions (*Scheme 5.21*).




*Scheme 5.22. Synthesis of different derivatives of allyl alcohol.*

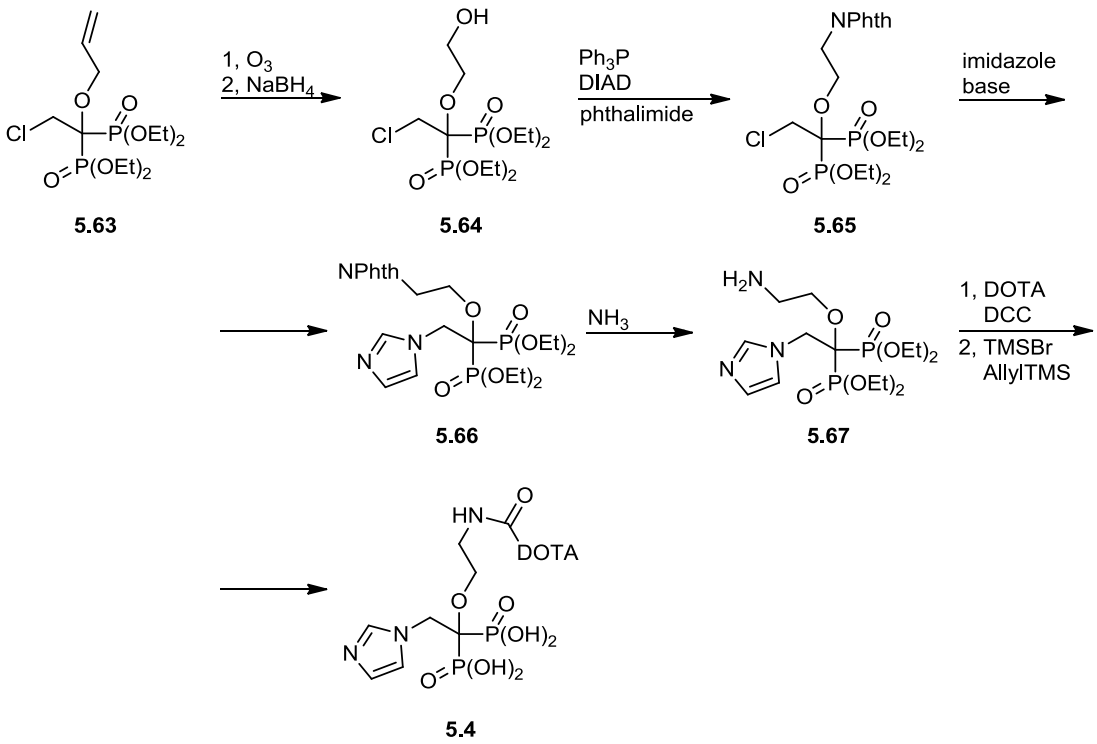
Treatment of the hydroxybisphosphonate **5.50** with trichloroacetimidate **5.61**/acid did not yield any product at all, even when Lewis acids such as camphorsulfonic acid or trifluoroacetic acid were used (*Scheme 5.23*).




*Scheme 5.23. Attempts towards the allylation of hydroxybisphosphonate **5.50**.*

Furthermore, treatment of the hydroxybisphosphonate **5.50** with $Pd(PPh_3)_4$ and the allyl carbonate **5.60** and **5.62** at 50°C, according to the method from Stoner *et al.*[139], did not yield better results. The last attempt towards the preparation of the DOTA-zoledronate derivate was the allylation using $Ag_2O$ and allyl bromide. However, not surprisingly at all at this time, only the rearranged product **5.51** was observed (*Scheme 5.23*).

Due to lack of time and the low success on this project I stopped at this point of the synthesis. In *Scheme 5.24* I will give a short outlook for this project, as soon as I find a method for the alkylation of the hydroxyl group of the hydroxybisphosphonate.




*Scheme 5.24. Outlook for the synthesis of DOTA-zoledronate derivative **5.4**.*

O-Allylated hydroxybisphosphonate **5.63** would be ozonolysed, followed by reduction to the primary alcohol **5.64**. Further treatment with $Ph_3P$, DIAD and phthalimide under Mitsunobu conditions would be necessary to obtain the chlorobisphosphonate **5.65**. After introduction of the imidazolyl group, the amino moiety would be deprotected with $NH_3$ to obtain the free amine **5.67**. To complete the synthesis, coupling of the amine and DOTA using DCC followed by deprotection of the phosphorus moiety would lead to the DOTA-zoledronate derivative **5.4**.

# 6.Experimental Section

## 6.1 Remarks

Data were collected as follows:

NMR: Bruker DRX 600 ($^{1}$H: 600.13 MHz, $^{13}$C: 150.92 MHz, $^{31}$P: 242.92 MHz)

Bruker Avance DRX 400 ($^{1}$H: 400.13 MHz, $^{13}$C: 100.61 MHz, $^{31}$P: 161.98 MHz)

Bruker AV 400 ($^{1}$H: 400.27 MHz, $^{13}$C: 100.65 MHz, $^{31}$P: 162.03 MHz)

Solvent peaks: $CHCl_3$: $\delta_H$ = 7.24 ppm, HOD: $\delta_H$ = 4.80 ppm, $d_8$-toluene $CHD_2$: $\delta_H$ = 2.05 ppm,

$CDCl_3$: $\delta_C$ = 77.00 ppm, $d_8$-toluene $CHD_2$: $\delta_c$ = 21.40 ppm

| | |
|---|---|
| IR Spectra: | Vertex 70 IR spectrometer as ATR spectra |
| Optical Rotation: | Perkin-Elmer 341 polarimeter (d = 1 dm, at given temperature) |
| Chromatography: | Merck silica gel 60 (230-400 mesh) |
| TLC: | Merck silica gel 60 $F_{254}$ plates (0.25 mm thick) |
| Dyeing reagents: | Molybdate solution [23 g $(NH_4)_6Mo_7O_{24}$ x 4 $H_2O$, 1 g $Ce(SO_4)_2$ x 4 $H_2O$ in 500 ml 10% aqueous $H_2SO_4$] |
| | Ninhydrin solution (0.2% ninhydrin in ethanol 98%) |
| | Iodine |
| | UV detection |
| Melting point: | Leica Galen III Reichert Thermovar |
| Chemicals: | $Et_2O$ was distilled over $LiAlH_4$, and THF over potassium. |
| | Chemicals bought from Sigma-Aldrich, ABCR, Fluka or Acros were used without any further purification. |
| HPLC: | Self-assembled HPLC from SHIMADZU (LC 20AD, LC 20AT, Autosampler SIL 20A HT, UV-detector SPD-20A) |
| | Columns: Chiralpak AS-3 150x4.6 mm ID; Chiralpak IA 250x4.6 mm (particle size 5 µm) |
| | All measurements were performed at 25°C. |

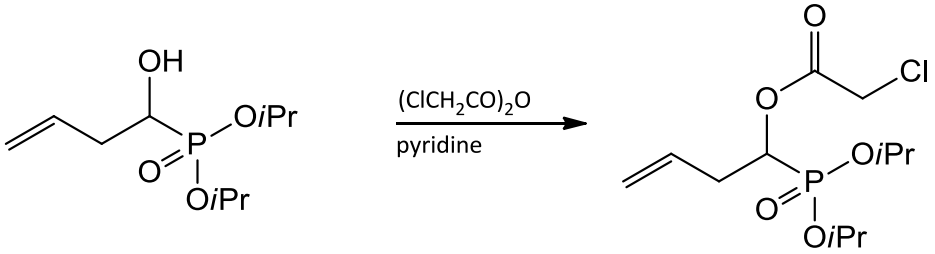
## 6.2 Procedure:

### 6.2.1 Preparation of (*R*)-Mosher ester (general procedure A):

A solution of (*S*)-MTPACl (0.331 M in dry $CH_2Cl_2$, 2.5 equiv.) was added to a solution of 1-hydroxyphosphonate (racemic or optically active, approximately 15 mg) in dry pyridine (0.20 ml, 2.47 mmol) and dry $CH_2Cl_2$ (0.5 ml). The reaction mixture was left over night at r.t. (TLC control) before a few drops of water were added. Volatile components were removed under reduced pressure. Water (5 ml) and $CH_2Cl_2$ (10 ml) were added to the residue. The organic phase was separated and the aqueous one was extracted with another portion of $CH_2Cl_2$ (10 ml). The combined organic layers were washed with HCl (2M, 5 ml), water (5 ml), $NaHCO_3$ (sat., 5 ml), dried ($Na_2SO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography.

### 6.2.2 Synthesis of (±)- and (*R*)-pyrrolidin-2-ylphosphonic acid

#### 6.2.2.1 (±)-Diisopropyl 1-(chloroacetoxy)but-3-enylphosphonate [(±)-5.13]



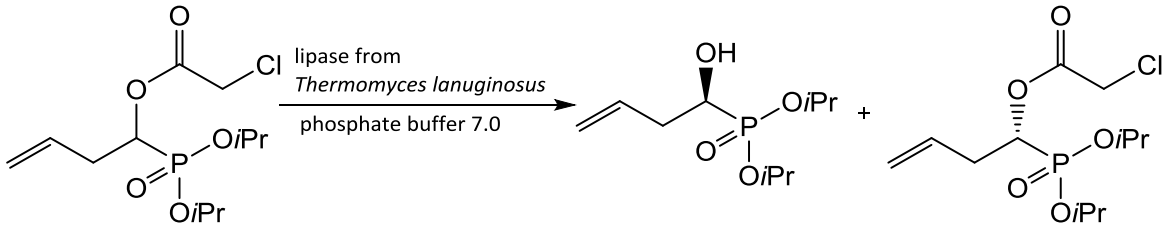

(±)-**5.13** (±)-**5.14**

The α-hydroxybut-3-enylphosphonate (±)-**5.13** (5.670 g, 24 mmol) was dissolved in dry $CH_2Cl_2$ (40 ml) and pyridine (5.695 g, 72 mmol, 3 equiv.) was added under Ar atmosphere. A solution of chloroacetic anhydride (6.326 g, 37 mmol, 1.54 equiv.) in dry $CH_2Cl_2$ (20 ml) was slowly added at 0°C. After 3 h, TLC showed no starting material and the reaction was quenched with water (3 ml). The layers were separated and the aqueous phase was extracted with $CH_2Cl_2$ (30 ml). The combined organic phases were washed with HCl (2M, 20 ml), water (10 ml), $NaHCO_3$ (sat., 10 ml), dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash

chromatography (hexanes:EtOAc = 1:2, $R_f$ = 0.74) to obtain the chloroacetoxyphosphonate (±)-**5.14** (5.351 g, 17.1 mmol, 69%) as a colourless oil.

Spectroscopic data are identical to those reported in literature.[140]

### 6.2.2.2 (*S*)-(+)-Diisopropyl 1-hydroxybut-3-enylphosphonate [(*S*)-5.13] and (*R*)-(-)-diisopropyl-1-(chloroacetoxy)but-3-enylphosphonate [(*R*)-5.14]

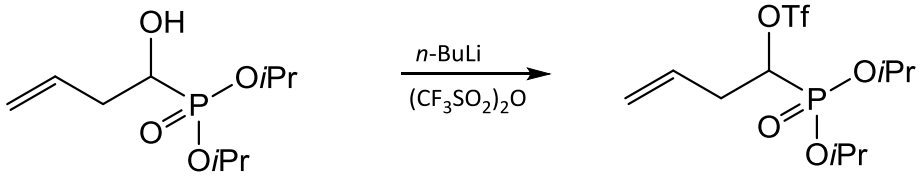



(±)-**5.14** (*S*)-**5.13** (*R*)-**5.14**

(±)-Diisopropyl-1-hydroxybut-3-enylphosphonat [(±)-**5.14**] (5.351 g, 17.1 mmol) was dissolved in a mixture of *tert*-butyl methyl ether and hexanes (30 mL, ratio= 1:1) and phosphate buffer (25mM, 100 mL). After the pH had been adjusted to 7 using the autotitrator, lipase from *Thermomyces lanuginosus* (0.45 ml, SP 524, ≥ 100 000 U/g, [3.1.1.3] Sigma) was added. The mixture was stirred vigorously and the pH kept constant by addition of NaOH (0.5M). At a conversion of 42%, hydrolysis was stopped and the pH was brought to 4 by addition of HCl (2M). The reaction mixture was extracted with EtOAc (4x 30 ml). The combined organic phases were washed with sat. aqueous $NaHCO_3$, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc = 1:2, ester: $R_f$ = 0.45, hydroxyphosphonate: $R_f$ = 0.19) to give the hydroxyphosphonate (*S*)-**5.13** {1.421 g, 6.02 mmol (42% conv.), 78%, ee 97% by $^{31}P$ NMR of Mosher ester, $[\alpha]_D^{20}$ = +21.76 (c = 1.25, acetone)} and the (*R*)-(-)-chloroacetate (*R*)-**5.14** (2.422 g, 7.7 mmol, 82%) as colourless oils.

Spectroscopic data are identical to those of the racemic compounds.[140]

**6.2.2.3 (±)-Diisopropyl 1-(trifluoromethanesulfonyloxy)but-3-enylphosphonate [(±)-5.17]**




(±)-**5.13** (±)-**5.17**

The α-hydroxyphosphonate (±)-**5.13** (0.925 g, 3.91 mmol) was dissolved in dry diethyl ether and cooled to -78°C under Ar atmosphere. *n*-BuLi (1.56 ml, 4.3 mmol, 2.5M in hexanes, 1.1 equiv.) was added and the solution was stirred for 5 min before addition of trifluoromethanesulfonic anhydride (1.213 g, 0.72 ml, 4.3 mmol, 1.1 equiv.). Stirring was continued for 90 min until TLC showed complete conversion of starting material to product. Water (5 ml) was added to the reaction mixture. The phases were separated and the aqueous one was extracted with EtOAc (3 x 10 ml). The combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc = 1:1, $R_f$ = 0.72) to yield triflate (±)-**5.17** (1.296 g, 3.51 mmol, 90%) as a yellowish oil.

$^1$H NMR ($CDCl_3$; 7.24 ppm; 400.27 MHz): $\delta$ = 5.86-5.74 (m, 1H, $CH_2$=C<u>H</u>), 5.26-5.19 (m, 2H, C<u>H$_2$</u>=CH), 4.93 (td, *J* = 8.3 Hz, *J* = 4.6 Hz, 1H, P-CH), 4.86-4.72 (m, 2H,2x O-C<u>H</u>), 2.86-2.74 (m, 1H, C<u>H$_2$</u>-CH-P), 2.73-2.60 (m, 1H, C<u>H$_2$</u>-CH-P), 1.362 (d, *J* = 6.2 Hz, 3H, $CH_3$), 1.360 (d, *J* = 6.2 Hz, 3H, $CH_3$), 1.356 (d, *J* = 6.2 Hz, 6H, 2x $CH_3$).

$^{13}$C NMR ($CDCl_3$, 77.00 ppm, 100.61 MHz): $\delta$ = 130.68 (d, *J* = 9.8 Hz, 1C, $H_2$C=<u>C</u>H), 120.47 (s, 1C, $H_2$<u>C</u>=CH), 81.52 (d, *J* = 169.5, 1C, C-P), 73.11 (d, *J* = 7.0 Hz, 1C, O-CH), 73.00 (d, *J* = 7.3 Hz, 1C, O-CH), 35.00 (s, 1C, <u>C</u>$H_2$-CH-P), 24.07 (d, *J* = 3.8 Hz, 1C, $CH_3$), 23.98 (d, *J* = 3.9 Hz, 1C, $CH_3$), 23.91 (d, *J* = 5.0 Hz, 1C, $CH_3$), 23.71 (d, *J* = 5.1 Hz, 1C, $CH_3$).

$^{31}$P NMR ($CDCl_3$, 162.04 MHz): $\delta$ = 12.25 (s, 1P).

IR (ATR): ν = 2985, 1416, 1204, 1179, 1141, 999, 912 $cm^{-1}$.

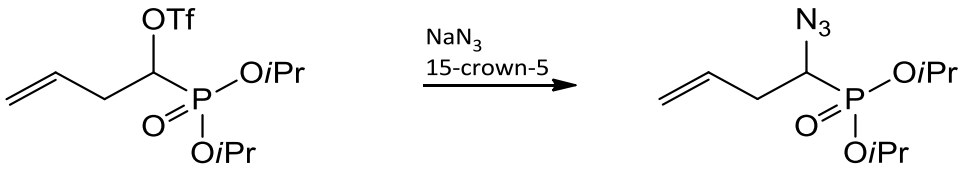
m/z (ESI) calc: 391.0568 (+Na); found: 391.0560 (+Na).

### 6.2.2.4 (*S*)-(+)-Diisopropyl 1-(trifluoromethanesulfonyloxy)but-3-enylphosphonate [(*S*)-5.17]

(*S*)-(+)-Diisopropyl 1-(trifluoromethanesulfonyloxy)but-3-enylphosphonate [(*S*)-**5.17**] {0.663 g, 1.80 mmol, 90%; $[\alpha]_D^{22}$ = +28.2 8 (c = 0.987, acetone)} was synthesised from (*S*)-(+)-diisopropyl 1-hydroxybut-3-enylphosphonate [(*S*)-**5.13**] (0.472 g, 2 mmol, ee 97%) using the same prodcedure as used for the racemate.

Spectroscopic data are identical to those of the racemate.

### 6.2.2.5 (±)-Diisopropyl 1-azidobut-3-enylphosphonate [(±)-5.16]




(±)-**5.17** (±)-**5.16**

The triflate (±)-**5.17** (1.291g, 3.5 mmol) was dissolved in dry acetonitrile (14 ml) and cooled to 0°C under Ar atmosphere. After addition of 15-crown-5 (0.192g, 0.87 mmol) the reaction mixture was stirred for 5 min. $NaN_3$ (0.455g, 7.01 mmol) was added and stirring was continued for 16 h with gradual warming of the cooling bath to r.t. The solvent was removed and the residue was taken up in water/EtOAc (1:1, 20 ml). The organic phase was separated and the aqueous one extracted with EtOAc (3x 20 ml). The combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc = 3:2, $R_f$ = 0.43) to obtain the desired azide (±)-**5.16** (0.633g, 2.53 mmol, 69%) as a yellowish oil.

$^1$H NMR ($CDCl_3$; 7.24 ppm; 400.27 MHz): $\delta$ = 5.84 (dddd, *J* = 17.1 Hz, *J* = 10.2 Hz, *J* = 7.2 Hz, *J* = 6.4 Hz, 1H, $CH_2$=C<u>H</u>), 5.20 (qd, *J* = 17.1 Hz, *J* = 1.5 Hz, 1H, <u>C</u>$H_2$=CH), 5.16 (broadened d, *J* = 10.2 Hz, 1H, <u>C</u>$H_2$=CH), 4.83-4.71 (m, 2H, O-CH), 3.36 (ddd, *J* =12.0 Hz, *J* = 11.2 Hz, *J* = 3.5 Hz, 1H, CH-P), 2.67-2.56 (m, 1H, <u>C</u>$H_2$-CH-P), 2.46-2.33 (m, 1H, <u>C</u>$H_2$-CH-P), 1.35 (d, *J* = 6.2 Hz, 9H, 3x $CH_3$), 1.34 (d, *J* = 6.2 Hz, 3H, $CH_3$).

$^{13}$C NMR ($CDCl_3$, 77.00, 100.61 MHz): $\delta$ = 133.46 (d, *J* = 15.2 Hz, 1C, $CH_2$=<u>C</u>H), 118,45 (s, 1C, <u>C</u>$H_2$=CH), 71.88 (d, *J* = 7.4 Hz, 1C, O-CH), 71.80(d, *J* = 7.1 Hz, 1C, O-CH), 57.27 (d, *J* = 160.9, 1C, CH-P), 33.04 (s, 1C, <u>C</u>$H_2$-CH-P), 24.15 (d, *J* =3.5 Hz, 1C, $CH_3$), 24.14 (d, *J* = 3.6 Hz, 1C, $CH_3$), 23.98 (d, *J* = 4.6 Hz, 2C, 2x $CH_3$).

$^{31}$P NMR ($CDCl_3$, 162.04 MHz): $\delta$ = 19.42 (s, 1P)

IR (ATR): ν = 2982, 2936, 2121, 1387, 1376, 1260, 1179, 1143, 1105, 987 $cm^{-1}$.[141]

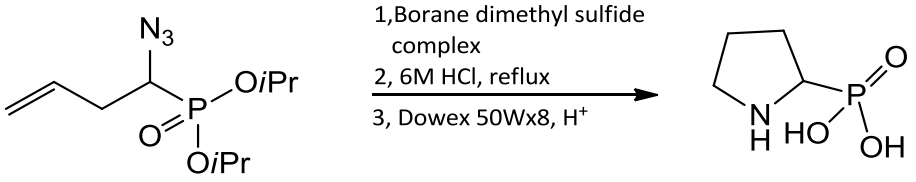
Anal. cald. for $C_{10}H_{20}N_3O_3P$ (261.25): C 45.97%, H 7.72%, N 16.08%; found: C 46.12%, H 7.73%, N 15.90%.

### 6.2.2.6. (*R*)-(-)-Diisopropyl 1-azidobut-3-enylphosphonate [(*R*)-5.16]

(*S*)-(+)-Diisopropyl 1-(trifluoromethanesulfonyloxy)but-3-enylphosphonate [(*S*)-**5.17**] (0.366 g, 0.99 mmol) was converted to the (*R*)-(+)-azide (*R*)-**5.16** {0.155 g, 0.59 mmol, 60%, $[\alpha]_D^{19}$ = -31.25 (c = 1.2, acetone), lit.[141] for (*S*)-enantiomer: $[\alpha]_D^{20}$= +32.04 (c = 1.08, acetone)} using the same procedure as for the racemate.

Spectroscopic data collected for the enantiomeric compound were identical to those of the racemate.

### 6.2.2.7 (±)-Pyrrolidin-2-ylphosphonic acid [(±)-5.16]




(±)-**5.16** (±)-**2.82**

Dry 1,2-dimethoxyethane (5 ml) was cooled to 0°C under Ar atmosphere. Borane dimethyl sulfide complex (0.309 g, 4 mmol) and cyclohexene (0.328 g, 8 mmol) were added and the reaction mixture was stirred for 15 min at 0°C and then the cooling bath was removed. After

stirring for 45 min at r.t. the racemic azide (±)-**5.16** (0.533 g, 2 mmol, dissolved in 3 ml of dry 1,2-dimethoxyethane) was added. Stirring was continued for 2 h and then the reaction was quenched with acetic acid (0.5 ml) at 0°C. The solution was taken up in HCl (2M)/$CH_2Cl_2$ (10 ml, 1:1). The phases were separated and the organic layer was extracted with HCl (2M, 2x 5 ml). The combined aqueous layers were concentrated under reduced pressure, the residue was taken up in water/HCl (conc., 5 ml, 1:1) and refluxed for 20 h. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in water and applied to a column filled with Dowex 50Wx8, $H^+$ (elution with water, 10 ml fractions were collected). Fractions containing the aminophosphonic acid (TLC silica: *I*PrOH:$H_2O$:$NH_3$ = 6:3:1, detection with $I_2$) were pooled, concentrated under reduced pressure and crystallised ($H_2O$/EtOH) to give the desired aminophosphonic acid (±)-**2.82** (0.129 g, 0.85 mmol, 43%) as colourless needles.

$^1$H NMR ($D_2O$; 4.80 ppm; 400.27 MHz): $\delta$ = 3.61 (q, *J* = 9.1 Hz, 1H, CH-P), 3.48-3.37 (m, 2H, $CH_2$-N), 2.41-2.30 (m, 1H, C<u>H</u>$_2$-CH-P), 2.24-2.01 (m, 3H, $CH_2$-C<u>H</u>$_2$-$CH_2$, C<u>H</u>$_2$-CH-P).

$^{13}$C NMR ($D_2O$; 100.61 MHz): $\delta$ = 58.46 (d, *J* = 143.7 Hz, 1C, CH-P), 49.41 (d, *J* = 6.1 Hz, 1C, $CH_2$-N), 28.94 (s, 1C, <u>C</u>$H_2$-CH-P), 26.48 (d, *J* = 8.5 Hz, 1C, $CH_2$-<u>C</u>$H_2$-$CH_2$).

$^{31}$P NMR ($D_2O$; 162.04 MHz): $\delta$ = 14.88 (s, 1P).

Anal. cald. for $C_4H_{10}NO_3P$ (151.10): C 31.80%, H 6.67%, N 9.27%; found: C 31.72%, H 6.50%, N 9.05%

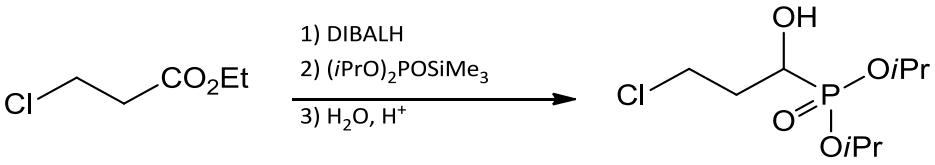
#### 6.2.2.8 (*R*)-(-)-Pyrrolidin-2-ylphosphonic acid [(*R*)-2.82]

The (*R*)-enantiomer of pyrrolidin-2-ylphosphonic acid [(*R*)-**2.82**] {0.191 g, 1.26 mmol, 63%, $[\alpha]_D^{22}$ = -46.6 {c = 0.5, NaOH 1M), lit.[142] $[\alpha]_D^{21}$ = -49.1 {c = 1.1, NaOH 1M)} was prepared from (*R*)-diisopropyl 1-azidobut-3-enylphosphonate [(*R*)-**5.16**] {0.533 g, 2 mmol, $[\alpha]_D^{19}$ = -31.25 (c = 1.2, acetone)} using the same method as for the racemic compound.

The NMR spectra are identical to those of the racemate.

## 6.2.3 Synthesis of (±)- and (*R*)-isoxazolidin-5-ylphosphonic acid

### 6.2.3.1 (±)-Diisopropyl 3-chloro-1-hydroxypropylphosphonate [(±)-5.25]




(±)-**5.25**

Ethyl 3-chloropropionate (**6.1**) (4.097 g, 30 mmol) was dissolved in dry toluene (40 ml) and cooled to –78°C under Ar atmosphere. A solution of DIBALH (33 ml, 1 M, toluene, Sigma) was added drop wise over 10 min. After 2 h of additional stirring diisopropyl trimethylsilyl phosphite (7.150 g, 36 mmol) was added. The cooling bath was removed and the reaction mixture was stirred at r.t. over night. HCl (2 M, 10 ml, exothermic!) was added dropwise and after 10 min more HCl (90 ml) were added and stirring was continued for 30 min (TLC: the silylated hydroxyphosphonate should be absent). The organic phase was separated and the aqueous one extracted with EtOAc (3 x 50 ml). The combined organic layers were washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc = 1:3; $R_f$ = 0.24) to give the racemic $\alpha$-hydroxyphosphonate (±)-**5.25** (6.558 g, 25.3 mmol, 84%) as a colorless oil.

$^1$H NMR ($CDCl_3$; 7.24 ppm; 400.27 MHz): $\delta$ = 4.80-.4.67 (m, 2H, 2x O-CH), 4.06-3.98 (m, 1H, CH-P), 3.78-3.67 (m, 2H, Cl-$CH_2$), 3.21-3.11 (s, 1H, -OH), 2.16-2.07 (m, 2H, Cl-$CH_2$-$CH_2$-CH), 1.325 (d, *J* = 6.2 Hz, 3H, $CH_3$), 1.33 (d, *J* = 6.2 Hz, 3H, $CH_3$), 1.322 (d, *J* = 6.2 Hz, 3H, $CH_3$), 1.32 (d, *J* = 6.2 Hz, 6H, 2x $CH_3$).

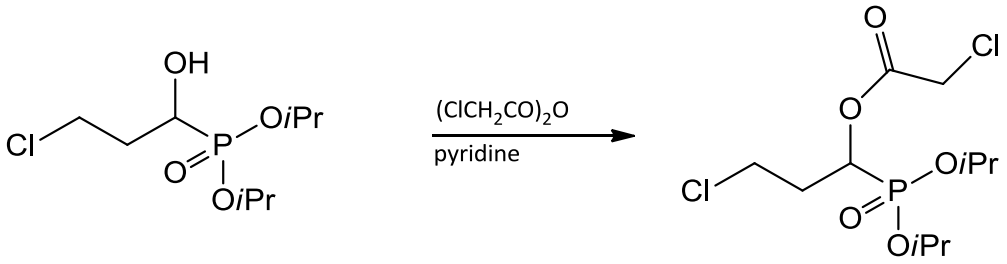
$^{13}$C NMR ($CDCl_3$, 77.00, 100.61 MHz): $\delta$ = 71.56 (d, *J* = 7.2 Hz, 1C, O-CH), 71.38 (d, *J* = 7.3 Hz, 1C, O-CH), 64.84 (d, *J* = 164.8 Hz, 1C, CH-P), 41.12 (d, *J* = 16.5, 1C, Cl-$CH_2$), 34.32 (d, *J* = 2.0 Hz, 1C, $CH_2$-$CH_2$-CH), 24.12 (d, *J* = 3.5 Hz, 1C, $CH_3$), 24.09 (d, *J* = 3.6 Hz, 1C, $CH_3$), 23.97 (d, *J* = 4.6 Hz, 2C, 2x $CH_3$).

$^{31}$P NMR ($CDCl_3$, 162.04 MHz): $\delta$ = 22.43 (s, 1P).

IR (ATR): ν = 3272, 2979, 1386, 1376, 1222, 1078, 984 $cm^{-1}$.

Anal. cald. for $C_9H_{20}ClO_4P$ (258.07): C 41.79%, H 7.79%, O 24.74%; found: C 41.84%, H 7.76%, O 24.84%.

### 6.2.3.2 (±)-Diisopropyl 3-chloro-1-(chloroacetoxy)propylphosphonate [(±)-5.26]




(±)-**5.25** (±)-**5.26**

Diisopropyl 3-chloro-1-hydroxypropylphosphonate [(±)-**5.25**] (4.967 g, 19.2 mmol) was dissolved in dry $CH_2Cl_2$ (30 ml) under Ar atmosphere. After cooling to 0°C dry pyridine (3.03 g, 38.4 mmol, 3.09 ml, 2 equiv.) was added, followed by chloroacetic anhydride (4.924 g, 28.8 mmol) dissolved in dry $CH_2Cl_2$ (16 ml). The reaction mixture was stirred. When the starting material was consumed (TLC, 2.5 h) the reaction was quenched with water (4 ml) and stirring was continued for 5 min before more water (20 ml) was added. The organic phase was separated and the aqueous one was extracted with EtOAc (30 ml). The combined organic phases were washed with HCl (30 ml, 2M), water (10 ml), $NaHCO_3$ (sat., 20 ml), dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc = 1:1, $R_f$ = 0.33) to give the chloroacetate (±)-**5.26** (5.463 g, 16.3 mmol, 85%) as a colorless oil.

$^1$H NMR ($CDCl_3$, 7.24 ppm, 400.27 MHz): $\delta$ = 5.41 (td, $J$ = 9.2 Hz, $J$ = 4.0 Hz, 1H, CH-P), 4.75 (m, 2H, O-CH), 4.09 (AB-system, $J$ = 15.0 Hz, 2H, C(O)-$CH_2$-Cl), 3.65-3.47 (m, 2H, $CH_2$-Cl), 2.39-2-23 (m, 2H, Cl-$CH_2$-C$\underline{H}_2$), 1.37 (d, $J$ = 6.2 Hz, 3H, $CH_3$), 1.374 (d, $J$ = 6-4 Hz, 3H, $CH_3$), 1.37 (d, $J$ = 6.2 Hz, 3H, $CH_3$), 1.36 (d, $J$ = 6.4 Hz, 3H, $CH_3$).

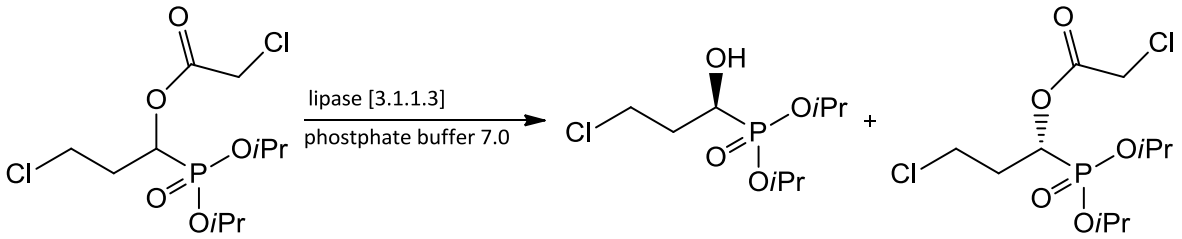
$^{13}$C NMR ($CDCl_3$, 77.00 ppm, 100.61 MHz): $\delta$ = 166.19 (d, $J$ = 5.0 Hz, 1C, C=O), 72.28 (d, $J$ = 6.8 Hz, 1C, O-CH), 72.09 (d, $J$ = 7.2 Hz, 1C, O-CH), 67.64 (d, $J$ = 171.5 Hz, 1C, CH-P), 40.51 (s, 1C, C(O)-$CH_2$-Cl), 40.13 (d, $J$ = 14.2 Hz, 1C, Cl-$CH_2$), 32.68 (s, 1C, Cl-$CH_2$-$\underline{C}H_2$-CH), 24.14 (d, $J$ =3.3 Hz, 1C, $CH_3$), 24.02 (d, $J$ = 4.0 Hz, 1C, $CH_3$), 23.98 (d, $J$ = 4.9 Hz, 1C, $CH_3$), 23.84 (d, $J$ = 5.0 Hz, 1C, $CH_3$).

$^{31}$P NMR ($CDCl_3$, 162.04 MHz): $\delta$ = 16.21 (s,1P).

IR (ATR): ν = 2981, 2938, 1770, 1387, 1377, 1250, 1160, 1104, 989 $cm^{-1}$.

Anal. cald. for $C_{11}H_{21}Cl_2O_5P$ (335.16): C 39.42%, H 6.32%, O 23.87%; found: C 39.39%, H 6.23%, O 24.06%.

### 6.2.3.3 Lipase-catalysed resolution of racemic diisopropyl 3-chloro-1-(chloroacetoxy)-propylphosphonate [(±)-5.26]: (*S*)-(+)-diisopropyl 3-chloro-1-hydroxypropylphosphonate [(*S*)-5.25] and (*R*)-(-)-diisopropyl 3-chloro-1-chloroacetoxypropylphosphonate [(*R*)-5.26]




(±)-**5.26** (*S*)-**5.25** (*R*)-**5.26**

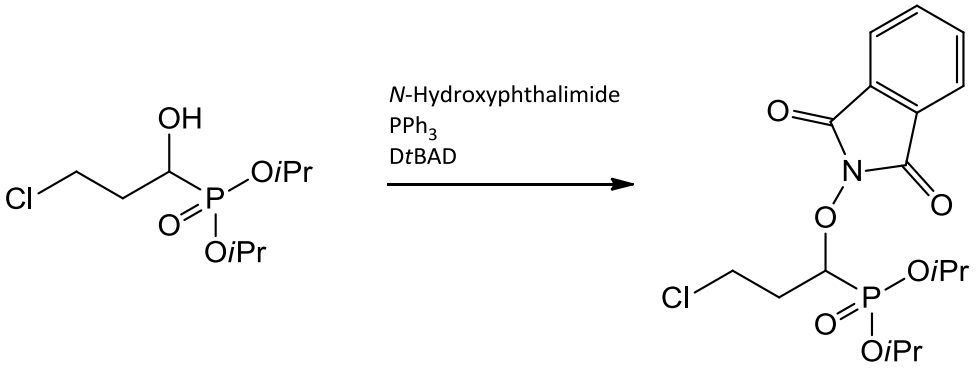
Analytical experiment: (±)-Diisopropyl 3-chloro-1-(chloroacetoxy)propylphosphonate [(±)-**5.26**] (0.670 g, 2 mmol) was dissolved in a mixture of *tert*-butyl methyl ether and hexanes (4 mL, ratio = 1:1) and phosphate buffer (25mM, 15 mL). After the pH had been adjusted to 7 using the autotitrator, lipase from *Thermomyces lanuginosus* (≥ 100000 U/g, [3.1.1.3], Sigma) was added. The mixture was stirred vigorously and the pH kept constant by addition of NaOH (0.5M). At a conversion of 40% (by consumption of base) the pH was brought to 4 by addition of HCl (2M). The reaction mixture was extracted with EtOAc (3x20 ml). The combined organic phases were washed with $NaHCO_3$ (10 ml), dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc=1:2, ester: $R_f$ = 0.44; hydroxyphosphonate: $R_f$ = 0.15) to give the (*S*)-(+)-hydroxyphosphonate (*S*)-**5.25** {0.138 g, 0.53 mmol, 66%, $[\alpha]_D^{21}$ = +31.65 (c = 1.67, acetone)} and the (*R*)-(-)-chloroacetate (*R*)-**5.26** {0.273 g, 0.8 mmol, 67%, $[\alpha]_D^{21}$ = -29.40 (c = 1.01, acetone)} as colourless oils.

Preparative experiment: The same procedure as used for the analytical experiment was performed with racemic chloroacetate (±)-**5.26** (5.463 g, 16.3 mmol) to obtain (*S*)-(+)-hydroxyphosphonate (*S*)-**5.25** {1.332 g, 5.14 mmol, 79%, $[\alpha]_D^{21}$ = +31.65 (c = 1.67, acetone)} and (*R*)-(-)-chloroacetate (*R*)-**5.26** {2.918 g, 8.71 mmol, 89%, $[\alpha]_D^{21}$ = -21.40 (c = 1.01, acetone)}.

Spectroscopic data of the isolated products were identical to those of the racemic forms.

Determination of ee using (*R*)-(+)-(Ph)(*t*Bu)P(O)(SH) as CSA: 97% by $^{31}P$ NMR spectroscopy: major singlet (1.00) at 22.90 ppm and minor one (0.015) at 22.69 ppm.

**6.2.3.4 (±)-Diisopropyl 3-chloro-1-(phthalimidooxy)propylphosphonate [(±)-5.27]**




**(±)-5.25** **(±)-5.27**

*N*-Hydroxyphthalimide (0.977 g, 5.99 mmol, 2 equiv.) and $PPh_3$ (1.571 g, 5.99 mmol, 2 equiv.) were added to a solution of (±)-diisopropyl 3-chloro-1-hydroxypropylphosphonate [(±)-**5.25**] (0.776 g, 2.9 mmol) in dry THF (15 ml) under Ar atmosphere. After stirring the solution for 5 min, di-*tert*-butyl azodicarboxylate (1.379 g, 5.99 mmol, 2 equiv.) in dry THF (5 ml) was added and the mixture was stirred for 6 h at 60°C. After cooling to room temperature, MeOH (10 drops) was added. The solvent was removed under reduced pressure and the residue was purified by flash chromatography (hexanes:EtOAc = 1:1, $R_f$ = 0.33) to give (±)-diisopropyl 3-chloro-1-(phthalimidooxy)propylphosphonate [(±)-**5.27**] (0.662 g, 1.64 mmol, 57%) as colourless crystals, mp 94-95°C (hexanes/$CH_2Cl_2$).

$^1H$ NMR ($CDCl_3$; 7.24 ppm; 400.27 MHz): $\delta$ = 7.83-7.78 (m, 2H, 2x $CH_{arom}$), 7.74-7.68 (m, 2H, 2x $CH_{arom}$), 5.05-4.92 (m, 1H, O-CH), 4.80-4.68 (m, 2H, O-CH, CH-P), 3.99-3.91 (m, 1H, Cl-$CH_2$), 3.88-3.80 (m, 1H, Cl-$CH_2$), 2.45-2.31 (m, 2H, C$\underline{H}_2$-CH-P), 1.38 (d, *J* = 6.2 Hz, 3H, $CH_3$), 1.33 (d, *J* = 6.3 Hz, 3H, $CH_3$), 1.325 (d, *J* = 6.2 Hz, 3H, $CH_3$), 1.32 (d, *J* = 6.9 Hz, 3H, $CH_3$).

$^{13}$C NMR ($CDCl_3$, 77.00 ppm, 100.61 MHz): $\delta$ = 163.2 (s, 2C, C=O,), 134.63 (s, 2C, $C_{arom}$), 129.00 (s, 2C, $C_{q,arom}$), 123.69 (s, 2C, $C_{arom}$), 79.41 (d, *J* = 164.0 Hz, 1C, CH-P), 72.60 (d, *J* = 6.7 Hz, 1C, O-CH), 72.03 (d, *J* = 7.1 Hz, 1C, O-CH), 40.83 (d, *J* = 12.4 Hz, 1C, Cl-$CH_2$), 33.12 (s, 1C, $\underline{C}H_2$-CH-P), 24.37 (d, *J* = 3.1 Hz, 1C, $CH_3$), 24.13 (d, *J* = 3.5 Hz, 1C, $CH_3$), 24.10 (d, *J* = 4.8 Hz, 1C, $CH_3$), 23.87 (d, *J* = 5.4 Hz, 1C, $CH_3$).

$^{31}$P NMR ($CDCl_3$, 162.04 MHz): $\delta$ = 15.03 (s, 1P).

IR (ATR): ν = 2980, 1732, 1468, 1375, 1249, 1187, 980 $cm^{-1}$.

Anal. cald. for $C_{17}H_{23}ClNO_6P$ (403.80): C 50.57%, H 5.74%, O 23.77%, N 3.47%; found: C 50.69%, H 5.52%, O 23.79%, N 3.45%.

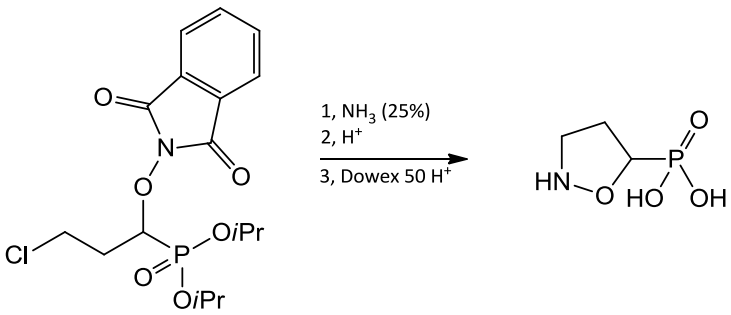
### 6.2.3.5 (R)-(-)-Diisopropyl 3-chloro-1-(phthalimidooxy)propylphosphonate (*R*)-5.27

(*R*)-(-)-Diisopropyl 3-chloro-1-(phthalimidooxy)propylphosphonate [(*R*)-**5.27**] {0.727 g, 1.8 mmol, 72%, $[\alpha]_D^{15}$ = -28.75 (c = 2.4, acetone), mp 92-93°C (hexanes/EtOAc)} was prepared from (*S*)-diisopropyl 3-chloro-1-hydroxypropylphosphonate [(*S*)-**5.25** (0.575 g, 2.5 mmol, ee = 96%) using the same method as for the racemic compound.

Determination of ee by HPLC: Chiralpak AS-3, *n*-heptane+0.1% *i*PrOH:*i*PrOH = 9:1; 0.5 ml/min flow rate; $t_R$ of major peak: 15.97 min, $t_R$ of minor peak: 18.50 min, ee = 96%.

Spectroscopic data matches those of the racemic compound.

### 6.2.3.6 (±)-Isoxazolidin-5-ylphosphonic acid [(±)-5.2]




(±)-**5.27** (±)-**5.2**

The protected $\alpha$-aminooxyphosphonate (±)-**5.27** (0.344 g, 0.85 mmol) was dissolved in EtOH (5 ml). After addition of $NH_3$ (0.5 ml, 25%) the solution was stirred for 17 h at r.t. Afterwards the solvent was removed and the residue was taken up in $H_2O$ (10 ml) and HCl (2M, 5 ml), followed by extraction with EtOAc. The aqueous layer was concentrated under reduced pressure. The residue was stirred for 23 h with HBr (33% in AcOH, 3 ml). Volatile components were removed under reduced pressure and the residue was purified by ion-exchange chromatography (Dowex 50Wx8, $H^+$, elution with water, TLC: silica plate, *i*PrOH:$H_2O$:$NH_3$ = 6:3:1) to give isoxazolidin-5-ylphosphonic acid [(±)-**5.2**] (0.078 g, 0.51 mmol, 60%) as fawn crystals. mp 192°C ($H_2O$/EtOH) (decomposition).

$^1$H NMR ($D_2O$, 4.80 ppm, 400.27 MHz): $\delta$ = 4.47 (dd, $J$ = 9.5 Hz, $J$ = 7.0 Hz, 1H, CH-P), 3.77 (AB-system, A-part: $J_{AB}$ = 10.9 Hz, $J$ = 8.5 Hz, $J$ = 4.2 Hz; B-part: $J$ = 11.0 Hz, $J_{AB}$ = 10.9 Hz, $J$ = 8.2 Hz, 2H, N-$CH_2$), 2.89-2.77 (m, 1H, C$\underline{H}_2$-CH-P), 2.67-2.51 (m, 1H, C$\underline{H}_2$-CH-P).

$^{13}$C NMR ($D_2O$, 100.61 MHz): $\delta$ = 81.17 (d, $J$ = 155.8 Hz, 1C, CH-P), 49.75 (d, $J$ = 7.7 Hz, 1C, C-N), 32.64 (s, 1C, $CH_2$).

$^{31}$P NMR ($D_2O$, 162.04 MHz): $\delta$ = 10.78 (s, 1P).

IR (ATR): v = 3034, 2608 (very broad), 2105 (very broad), 1580, 1243, 1135, 1077, 928, 907, 890 $cm^{-1}$.

Anal. cald. for $C_3H_8NO_6P$ (153.07): C 23.54%, H 5.27%, N 9.15%, O 41.81%; found: C 23.57%, H 5.23%, N 9.01%, O 41.38%.

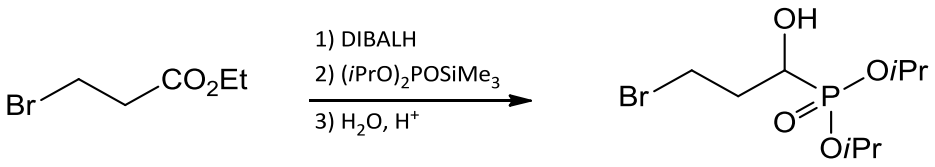
### 6.2.3.7 (*R*)-(+)-Isoxazolidin-5-ylphosphonic acid [(*R*)-5.2]

The protected (*R*)-(-)-$\alpha$-aminooxyphosphonate (*R*)-**5.27** (0.662 g, 1.6 mmol) was converted to the (*R*)-(+)-isoxazolidin-5-ylphosphonic acid [(*R*)-**5.2**] {0.159 g, 1.04 mmol, 65%, $[\alpha]_D^{15}$ = +20.29 (c = 1.67, $H_2O$), mp 192°C ($H_2O$/EtOH) (decomposition)} by the same procedure as used for the racemic compound.

Spectroscopic data identical to those of the racemic form.

## 6.2.4 Synthesis of (±)- and (*R*)-pyrazolidin-3-ylphosphonic acid

### 6.2.4.1 (±)-Diisopropyl 3-bromo-1-hydroxypropylphosphonate [(±)-5.36]




**5.35** (±)-**5.36**

Ethyl 3-bromopropionate (**5.35**) (5.431 g, 30 mmol) was dissolved in dry toluene (40 ml) and cooled to –78°C under Ar atmosphere. A solution of DIBALH (33 ml, 1 M, toluene, Sigma) was added drop wise over 10 min. After 2 h of additional stirring diisopropyl trimethylsilyl phosphite (8.580 g, 36 mmol) was added. The cooling bath was removed and the reaction mixture was stirred at r. t. over night. HCl (2 M, 10 ml, exothermic!) was added dropwise and after 10 min more HCl (90 ml) was added and stirring was continued for 30 min (TLC: the silylated hydroxyphosphonate should be absent). Brine was added and the organic phase was separated and the aqueous one extracted with EtOAc (3 x 50 ml). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc = 1:3, $R_f$ = 0.24) to give the racemic $\alpha$-hydroxyphosphonate (±)-**5.36** (6.558 g, 21.63 mmol, 72%) as a colourless oil.

$^1$H NMR ($CDCl_3$; 7.24 ppm; 400.27 MHz): $\delta$ = 4.80-4.67 (m, 2H, 2x O-CH), 4.04-3.96 (m, 1H, CH-P), 3.59 (dd, *J* = 6.8 Hz, *J* = 6.5 Hz, 2H, Br-$CH_2$), 3.29 (broad s, 1H, -OH), 2.23-2.15 (m, 2H, $\underline{C}H_2$-CH-P), 1.323 (d, *J* = 6.2 Hz, 6H, 2x $CH_3$), 1.32 (d, *J* = 6.2 Hz, 6H, 2x $CH_3$).

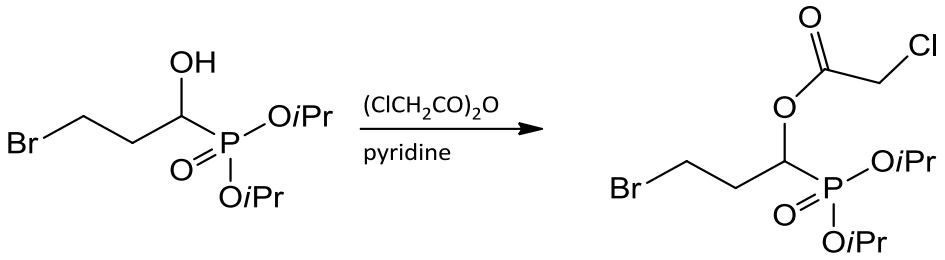
$^{13}$C NMR ($CDCl_3$, 77.00, 100.65 MHz): $\delta$ = 71.56 (d, *J* = 7.3 Hz, 1C, O-CH), 71.41 (d, *J* = 7.3 Hz, 1C, O-CH), 65.87 (d, *J* = 164.4 Hz, 1C, CH-P), 34.46 (d, *J* = 2.5 Hz, 1C, Br-$CH_2$), 29.80 (d, *J* =16.9 Hz, 1C, $\underline{C}H_2$-CH-P), 24.12 (d, *J* = 3.3 Hz, 1C, $CH_3$), 24.09 (d *J* = 3.3 Hz, 1C, $CH_3$), 23,99 (d, *J* =3.4 Hz, 1C, $CH_3$), 23.97 (d, *J* = 4.8 Hz, 1C, $CH_3$).

$^{31}$P NMR ($CDCl_3$, 162.04 MHz): $\delta$ = 22.28 (s, 1P).

IR (ATR): ν = 3267, 2979, 1386, 1376, 1253, 1222, 1106, 987 $cm^{-1}$.

Anal. cald. for $C_9H_{20}BrO_4P$ (303.13): C 35.66%, H 6.65%; found: C 35.91%, H 6.50%.

## 6.2.4.2 (±)-Diisopropyl 3-bromo-1-(chloroacetoxy)propylphosphonate [(±)-5.40]




(±)-**5.36** (±)-**5.40**

Racemic diisopropyl 3-bromo-1-hydroxypropylphosphonate [(±)-**5.36**] (7.578 g, 25.0 mmol) was dissolved in dry toluene (30 ml) under Ar atmosphere. After cooling the stirred solution to -30°C, dry pyridine (3.955 g, 50.0 mmol, 2 equiv.) was added, followed by chloroacetic anhydride (6.409 g, 37.5 mmol, 1.5 equiv.) dissolved in dry toluene (20 ml) and the reaction mixture was stirred. After completion (TLC, 1.5 h) the reaction was quenched with water (5 ml) and stirring was continued for 5 min. More water (20 ml) was added, the organic phase was separated and the aqueous one was extracted with EtOAc (3 x 30 ml). The combined organic layers were washed with HCl (2M, 25 ml), water (25 ml) and $NaHCO_3$ (25 ml, sat.), dried ($MgSO_4$) and concentrated under reduced pressure. The residue was flash chromatographed (hexanes:EtOAc = 1:1, $R_f$ = 0.33) to give the racemic chloroacetate (±)-**5.40** (7.022 g, 18.5 mmol, 74%) as a colourless oil.

$^1$H NMR ($CDCl_3$; 7.24 ppm; 400.27 MHz): $\delta$ = 5.39 (ddd, $J$ = 9.1 Hz, $J$ = 8.1 Hz, $J$ = 5.2 Hz, 1H, CH-P), 4.81-4.69 (m, 2H, O-CH), 4.09 (AB-system: $J_{AB}$ = 14.9 Hz, 2H, C(O)-$CH_2$-Cl), 3.41 (AB-system: A-part: $J_{AB}$ = 10.3 Hz, $J$ = 6.6 Hz, $J$ = 6.0 Hz, $J$ =1.0 Hz, B-part: $J_{AB}$ = 10.3 Hz, $J$ = 2x 7.5 Hz, 2H, $BrCH_2$), 2.44-2.35 (m, 2H, C$H_2$-CH-P), 1.33 (d, $J$ = 6.2 Hz, 3H, $CH_3$), 1.327 (d, $J$ = 6.3 Hz, 3H, $CH_3$), 1.32 (d, $J$ = 6.2 Hz, 3H, $CH_3$), 1.31 (d, $J$ = 6.3 Hz, 3H, $CH_3$).

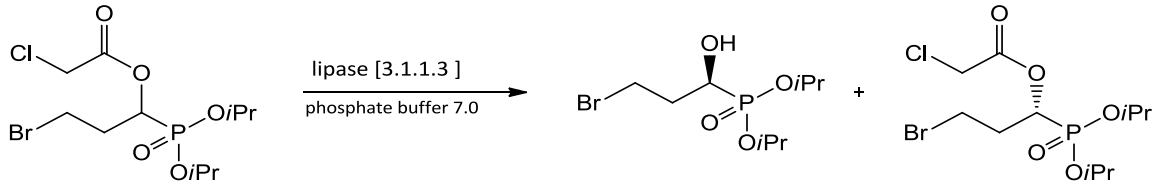
$^{13}$C NMR ($CDCl_3$, 77.00 ppm, 100.65 MHz): $\delta$ = 166.20 (d, $J$ = 5.1 Hz, 1C, C=O), 72.24 (d, $J$ = 6.6 Hz, 1C, O-CH), 72.05 (d, $J$ = 7.2 Hz, 1C, O-CH), 68.65 (d, $J$ = 171.1 Hz, 1C, CH-P), 40.50 (s, 1C, $CH_2Cl$), 32.88 (d, $J$ = 14.6 Hz, 1C, C$H_2$-CH-P), 27.77 (d, $J$ = 12.6 Hz, 1C, $CH_2Br$), 24.14 (d, $J$ = 3.5 Hz, 1C, $CH_3$), 24.02 (d, $J$ = 3.3 Hz, 1C, $CH_3$), 23.99 (d, $J$ = 4.5 Hz, 1C, $CH_3$), 23.84 (d, $J$ = 5.1 Hz, 1C, $CH_3$).

$^{31}$P NMR ($CDCl_3$, 162.04 MHz): $\delta$ = 16.03 (s, 1P).

IR (ATR): ν = 2981, 1725, 1387, 1377, 1181, 995 $cm^{-1}$.

Anal. cald. for $C_{11}H_{21}BrClO_5P$ (379.61): C 34.80%, H 5.58%; found: C 34.52%, H 5.36%.

### 6.2.4.3 Lipase-catalysed resolution of racemic diisopropyl 3-bromo-1-(chloroacetoxy)-propylphosphonate [(±)-5.40]: (*S*)-(+)-diisopropyl 3-bromo-1-hydroxypropylphosphonate [(*S*)-5.36] and (*R*)-(-)-diisopropyl 3-bromo-1-(chloroacetoxy)propylphosphonate [(*R*)-5.40]




(±)-**5.40** (*S*)-**5.36** (*R*)-**5.40**

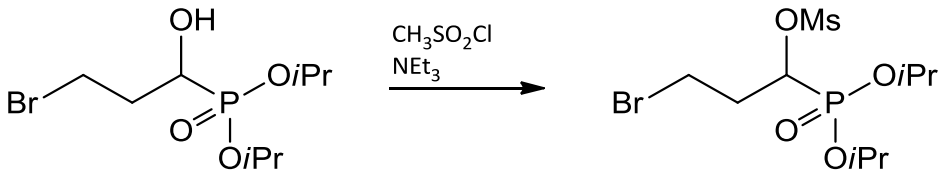
(±)-Diisopropyl 3-bromo-1-(chloroacetoxy)propylphosphonate [(±)-**5.40**] (8.321 g, 21.9 mmol) was dissolved in a mixture of *tert*-butyl methyl ether and hexanes (48 mL, ratio= 1:1) and phosphate buffer (50mM, 100 mL). After the pH was set to 7, lipase from *Thermomyces lanuginosus* (≥ 100000 U/g, [3.1.1.3], Sigma) was added and the mixture was autotitrated. At a conversion of 40% by consumption of base (NaOH, 0.5M) hydrolysis was stopped and the pH was brought to 4 by addition of HCl (2M). The reaction mixture was extracted with EtOAc (3x 40 ml). The combined organic phases were washed ($NaHCO_3$ sat., 2x 30 ml), dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc = 1:2, ester: $R_f$ = 0.44, hydroxyphosphonate: $R_f$ = 0.15) to give the (*S*)-(+)-hydroxyphosphonate (*S*)-**5.36** {1.856 g, 6.12 mmol, 70%, ee : 95% by $^{31}P$ NMR spectroscopy of (*R*)-(+)-Mosher ester; $[\alpha]_D^{18}$ = +30.5 (c = 0.95, acetone)} and the (*R*)-(-)-chloroacetate (*R*)-**5.40** {4.356 g, 11.4 mmol, 87%, $[\alpha]_D^{18}$ = -29.4 (c = 1.01, acetone)} as colourless oils.

Spectroscopic data are identical to those of the racemic compounds.

Determination of ee by $^{31}P$ NMR spectroscopy ($CDCl_3$, 162.04 MHz) of (*R*)-(+)-Mosher-ester of 3-bromo-1-hydroxyphosphonate prepared according to general procedure A: major signal (integration 1.000) at 16.00 ppm and minor signal (0.024) at 15.40 ppm, ee = 95 %.

Significant signal of (*R*)-(+)-Mosher ester in $^1H$ NMR ($CDCl_3$, 7.24 ppm, 400.27 MHz): major signal at 5.55 (td, *J* = 8.5 Hz, *J* = 5.6 Hz, 1H, CH-P), minor signal at 5.49 (td, *J* = 8.7 Hz, *J* = 4.9 Hz, 1H, CH-P), partly overlapping.

**6.2.4.4 (±)-Diisopropyl 3-bromo-1-(methanesulfonyloxy)propylphosphonate [(±)-5.38]**




(±)-**5.36** (±)-**5.38**

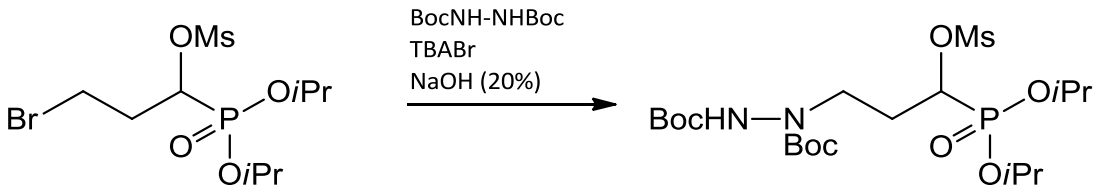
(±)-Diisopropyl 3-bromo-1-hydroxypropylphosphonate [(±)-**5.36**] (3.031 g, 10.0 mmol) was dissolved in dry $CH_2Cl_2$ under Ar atmosphere. $NEt_3$ (1.517 g, 2.08 ml, 15.0 mmol, 1.5 equiv.) was added and the reaction mixture was cooled to 0°C before $CH_3SO_2Cl$ (1.718 g, 1.16 ml, 15.0 mmol, 1.5 equiv.) was added. After 1 h of stirring the cooling bath was removed and HCl (2M, 10 ml) was added. The phases were separated and the aqueous layer was extracted with $CH_2Cl_2$ (2x 30 ml). The combined organic phases were washed with water (30 ml), brine (25 ml), dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc = 1:2, $R_f$ = 0.54) to yield the mesyloxyphosphonate (± )-**5.38** (3.535 g, 9.27 mmol, 93%) as a colourless oil.

$^1$H NMR ($CDCl_3$; 7.24 ppm; 400.27 MHz): $\delta$ = 4.91 (td, $J$ = 8.4 Hz, $J$ = 4.8 Hz, 1H, CH-P), 4.84-4.70 (m, 2H, 2x O-CH), 3.60-3.52 (m, 1H, $CH_2$-Br), 3.49-3.42 (m, 1H, $CH_2$-Br), 3.21 (s, 3H, S-$CH_3$), 2.42-2.32 (m, 2H, $CH_2$-CH-P), 1.36 (d, $J$ = 6.2 Hz, 9H, 3x $CH_3$), 1.35 (d, $J$ = 6.2 Hz, 3H, $CH_3$).

$^{13}$C NMR ($CDCl_3$, 77.00, 100,61 MHz): $\delta$ = 75.16 (d, $J$ = 171.7 Hz, 1C, CH-P), 72.74 (d , $J$ = 6.8 Hz, 1C, O-CH), 72.53 (d, $J$ = 7.1 Hz, 1C, O-CH), 39.18 (s, 1C, S-$CH_3$), 34.16 (s, 1C, Br-$CH_2$), 27.78 (d, $J$ =14.7 Hz, 1C, $\underline{C}H_2$-CH-P), 24.15 (d, $J$ =3.6 Hz, 1C, $CH_3$), 24.03 (d, $J$ = 3.7 Hz, 1C, $CH_3$), 23.99 (d, $J$ = 4.8 Hz, 1C, $CH_3$), 23.86 (d, $J$ = 4.9 Hz, 1C, $CH_3$).

$^{31}$P NMR ($CDCl_3$, 162.04 MHz): $\delta$ = 14.93 (s, 1P).

**6.2.4.5 (±)-Diisopropyl 3-{(*N*,*N´*-di-tert-butoxycarbonyl)hydrazino}-1- (methansulfonyloxy) propylphosphonate [(±)-5.39]**



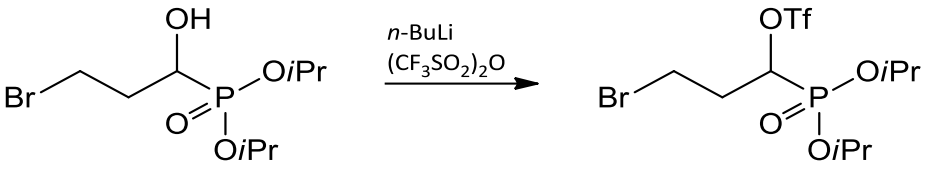

(±)-**5.38** (±)-**5.39**

BocHN-NHBoc (**6.3**) (1.466 g, 6.3 mmol) was dissolved in dry toluene (12 ml), NaOH (6 ml, 20 w/w%) and tetrabutylammonium bromide (0.189 g, 0.58 mmol, 0.14 equiv.) were added. After stirring for 5 min, (±)-diisopropyl 3-bromo-1-(methanesulfonyloxy)propylphosphonate [(± )-**5.38**](1.605 g, 4.2 mmol) was added and the reaction mixture was stirred at 50°C for 48 h. The solution was taken up in EtOAc (20 ml), washed with $NaHCO_3$ (20 ml), water (10 ml) and brine (15 ml). The organic phase was dried ($MgSO_4$) and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (hexanes:acetone = 4:1, $R_f$ = 0.29) to obtain the hydrazinophosphonate (±)-**5.39** (0.782 g, 1.47 mmol, 35%) as a colourless gum.

$^1$H NMR ($CDCl_3$; 7.24 ppm; 400.27 MHz): $\delta$ = 6.71-6.03 (2 overlapping broad s, 1H, NH), 4.90-4.68 (m, 3H, 2x O-CH, CH-P), 3.80-3.62 (broad s, 1H, N-$CH_2$), 3.62-3.48 (broad m, 1H, N-$CH_2$), 3.19 (s, 3H, $CH_3$-S), 2.38-2.18 (broad s, 1H, C$\underline{H}_2$-CH-P), 2.15-2.02 (m, 1H, C$\underline{H}_2$-CH-P), 1.45 (broad s, 18H, 2x C(C$\underline{H}_3$)$_3$), 1.344 (d, $J$ = 6.0 Hz, 6H, CH-(C$\underline{H}_3$)$_2$), 1.341 (d, $J$ = 6.0 Hz, 6H, CH-(C$\underline{H}_3$)$_2$).

$^{31}$P NMR ($CDCl_3$, 162.04 MHz): $\delta$ = 15.76 (s, 1P).

## 6.2.4.6 (±)-Diisopropyl 3-bromo-1-(trifluoromethanesulfonyloxy)propylphosphonate [(±)-5.37]



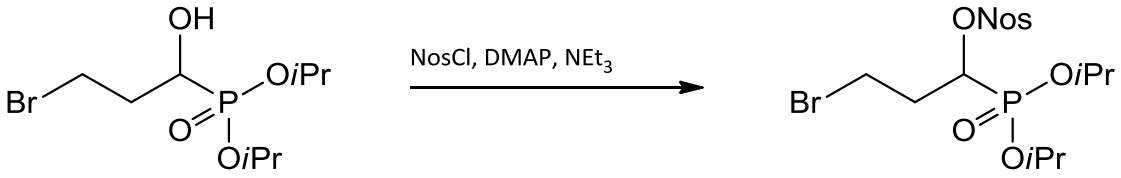

(±)-**5.36** (±)-**5.37**

(±)-Diisopropyl 3-bromo-1-hydroxypropylphosphonate [(±)-**5.36**] (1.515 g, 5.0 mmol) was dissolved in dry diethyl ether (20 ml) and cooled to -78°C. *n*-BuLi (2.25 ml, 2.5M in hexanes) was added and the solution was stirred for 5 min. Afterwards $(CF_3SO_2)_2O$ (1.551 g, 0.93 ml, 5.5 mmol) was added and the reaction was stirred for 2 h at -78°C. The reaction mixture was quenched with water (5 ml) and stirring was continued for 5 min. EtOAc (20 ml) was added and the phases were separated. The aqueous layer was extracted with EtOAc (2x 15 ml), the combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc = 1:3, $R_f$ = 0.53) to yield (±)-diisopropyl 3-bromo-1-(trifluoromethanesulfonyloxy)propylphosphonate [(±)-**5.37**] (1.554 g, 3.57 mmol, 71%) as a yellowish oil.

$^1$H NMR ($CDCl_3$; 7.24 ppm; 400.27 MHz): $\delta$ = 5.12 (td, $J$ = 8.2 Hz, $J$ = 5.2 Hz, 1H, CH-P), 4.87-4.73 (m, 2H, O-CH), 3.51 (AB-system: A-part: $J_{AB}$ = 10.6 Hz, $J$ = 6.8 Hz, $J$ = 6.0 Hz, $J$ = 1.0 Hz, B-part: $J_{AB}$ = 10.6 Hz, $J$ = 2x 7.3 Hz, 2H, Br-$CH_2$), 2.61-2.41 (m, 2H, C$\underline{H}_2$-CH-P), 1.37 (d, $J$ = 5.8 Hz, 3H, $CH_3$), 1.367 (d, $J$ = 6.2 Hz, 6H, 2x $CH_3$), 1.36 (d, $J$ = 5.2 Hz, 3H, $CH_3$).

$^{31}$P NMR ($CDCl_3$, 162.04 MHz): $\delta$ = 11.77 (s, 1P).

## 6.2.4.7 (±)-Diisopropyl 3-bromo-1-(4-nitrobenzenesulfonyloxy)propylphosphonate [(±)-5.41]




(±)-**5.36** (±)-**5.41**

Racemic diisopropyl 3-bromo-1-hydroxypropylphosphonate [(±)-**5.36**] (1.515 g, 5 mmol) was dissolved in dry $CH_2Cl_2$ (20 ml) and cooled to 0°C under Ar atmosphere. $NEt_3$ (1.214 g, 1.67 ml, 12 mmol) was added and the reaction mixture was allowed to stir for 5 min. After addition of a solution of *p*-nitrobenezenesulfonyl chloride (1.329 g, 6 mmol, = NosCl) and DMAP (40 mg) in dry $CH_2Cl_2$ (5 ml) the reaction mixture was stirred for 4.5 h at 0°C. The reaction was quenched with $H_2O$ (5 ml) and HCl (conc., 1 ml) and the phases were separated. The aqueous layer was extracted with $CH_2Cl_2$ (2x 20ml). The combined organic phases were washed with $NaHCO_3$ (sat., 30 ml), dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc=1:1, $R_f$ = 0.22) to give the nosylate (±)-**5.41** (2.148 g, 4.3 mmol, 86%) as a yellowish oil.

$^1$H NMR ($CDCl_3$; 7.24 ppm; 400.27 MHz): $\delta$ = 8.40-8.35 (m, 2H, $CH_{arom.}$), 8.22-8.17 (m, 2H, $CH_{arom.}$), 5.06 (ddd, *J* = 9.4 Hz, *J* = 8.4 Hz, *J* = 4.6 Hz, 1H, CH-P), 4.76-4.58 (m, 2H, O-CH), 3.59-3.51 (m, 1H, $CH_2Br$), 3.41-3.32 (m, 1H, $CH_2Br$), 2.46-2.29 (m, 2H, $C\underline{H_2}$-CH-P), 1.31 (d, *J* = 6.2 Hz, 3H, $CH_3$), 1.30 (d, *J* = 6.2 Hz, 3H, $CH_3$), 1.27 (d, *J* = 6.2 Hz, 3H, $CH_3$), 1.24 (d, *J* = 6.2 Hz, 3H, $CH_3$).

$^{13}$C NMR ($CDCl_3$, 77.00 ppm, 100.61 MHz): $\delta$ = 150.82 (s, 1C, $C_{arom}$-$NO_2$), 142.16 (s, 1C, $C_{q\ arom}$), 129.55 (s, 2C, $C_{arom}$), 124.22(s, 2C, $C_{arom}$), 75.61 (d, *J* = 172.2 Hz, 1C, CH-P), 72.71 (d, *J* = 6.8 Hz, 2C, O-CH), 33.78 (s, 1C, $BrCH_2$), 27.85 (d, *J* = 13.0 Hz, 1C, $\underline{C}H_2$-CH-P), 24.01 (d, *J* = 4.0 Hz, 1C, $CH_3$), 23.96 (d, *J* = 4.9 Hz, 1C, $CH_3$), 23.89 (d, *J* = 4.9 Hz, 1C, $CH_3$), 23.70 (d, *J* = 4.87Hz, 1C, $CH_3$).

$^{31}$P NMR ($CDCl_3$, 162.04 MHz): $\delta$ = 13.88 (s, 1P).

IR (ATR): ν = 2982, 1536, 1404, 1377, 1261, 1188, 992 $cm^{-1}$.

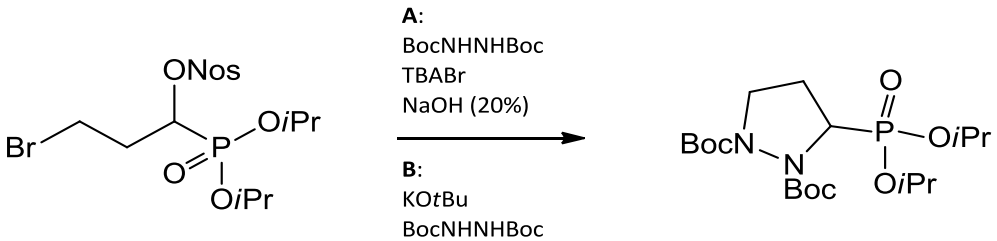
Anal. cald. for $C_{15}H_{23}BrNO_8PS$ (488.28): C 36.90%, H 4.75%, S 6.57%; found: C 37.09%, H 4.75%, S 6.59%.

### 6.2.4.8 (*S*)-(+)-Diisopropyl 3-bromo-1-(4-nitrobenzenesulfonyloxy)propylphosphonate [(*S*)-5.41]

The (*S*)-(+)-nosyloxyphosphonate (*S*)-**5.41** {2.313 g, 4.7 mmol, 87%, ee determination by chiral HPLC (Chiralpak IA) 96% , $[\alpha]_D^{23}$ = +19.73 (c = 1.86, acetone)} was produced from (*S*)-(+)-diisopropyl 3-bromo-1-hydroxypropylphosphonate [(*S*)-**5.36**] {1.649 g, 5.44 mmol, ee = 95%, ; $[\alpha]_D^{18}$ = +30.5 (c = 0.95, acetone)} under the same conditions as the racemic compound.

Spectroscopic data were identical to those of the racemic compound.

### 6.2.4.9 (±)-Diisopropyl (1,2-di-tert-butoxycarbonyl)pyrazolidin-3-ylphosphonate [(±)-5.33]




(±)-**5.41** (±)-**5.33**

Method A:

(±)-Diisopropyl 3-bromo-1-(4-nitrobenzenesulfonyloxy)propylphosphonate [(±)-**5.41**] (1.464 g, 3 mmol) was dissolved in dry toluene (10 ml) under Ar atmosphere. Afterwards a solution of NaOH (6 ml, 20w/w%), BocNH-NHBoc (1.393 g, 6 mmol) and tetrabutylammonium bromide (0.290 g, 0.9 mmol, dissolved in 5 ml of dry toluene) were added at r.t. Heating and stirring at 50°C for 7 h were necessary to complete the cyclisation. Citric acid was added until solution was acidic (pH 5). The mixture was taken up in EtOAc (10 ml) and the aqueous layer was extracted with EtOAc (2x 10 ml). The combined organic layers were washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc = 1:1, $R_f$ = 0.15) to yield pyrazolidinylphosphonate (±)-**5.33** (0.728 g, 1.66 mmol, 56%) as a yellowish gum.

$^{1}$H NMR ($CDCl_3$; 7.24 ppm; 400.13 MHz, 50°C): $\delta$ = 4.90-4-78 (m, 1H, O-CH), 4.77-4.64 (m., 1H, O-CH), 4.42 (td, *J* = 9.6 Hz, *J* = 3.3 Hz, 1H, CH-P), 3.97 (td, *J* = 9.8 Hz, *J* = 6.9 Hz, 1H, $CH_2$-N), 3.21 (td, *J* = 9.8 Hz, *J* = 5.3 Hz, 1H, $CH_2$-N), 2.44-2.30 (m, 1H, C$\underline{H}_2$-CH-P), 2.30-2.15 (m, 1H , C$\underline{H}_2$-CH-P), 1.46 (s, 9H, C(C$\underline{H}_3$)$_3$), 1.45 (s, 9H, C(C$\underline{H}_3$)$_3$), 1.31 (d, *J* = 5.5 Hz, 3H, $CH_3$), 1.30 (d, *J* = 6.1 Hz, 6H, 2x$CH_3$), 1.29 (d, *J* = 6.2 Hz, 3H, $CH_3$).

$^{13}$C NMR ($CDCl_3$, 77.00, 100.61 MHz, 50°C): $\delta$ = 156.57 (s, 1C, C=O), 156.46 (s, 1C, C=O), 81.84 (s, 1C, $\underline{C}(CH_3)_3$), 80.69 (s, 1C, $\underline{C}(CH_3)_3$), 71.99 (d, *J* = 7.0 Hz, 1C, O-CH), 70.78 (d, *J* = 7.2 Hz, 1C, O-CH), 54.13 (d, *J* = 173.3 Hz, 1C, CH-P), 45.32 (br. s, 1C, $CH_2$-N), 28.32 (s, 3C, ($\underline{C}H_3$)$_3$C), 28.13 (s, 3C, ($\underline{C}H_3$)$_3$C), 27.25 (br. s, 1C, $\underline{C}H_2$-CH-P), 24.37 (d, *J* = 2.4 Hz, 1C, $CH_3$-CH), 24.07 (d, *J* = 4.8 Hz, 1C, $CH_3$-CH), 243.97 (d, *J* = 3.6 Hz, 1C, $CH_3$-CH), 23.72 (d, *J* = 6.1 Hz, 1C, $CH_3$-CH).

$^{31}$P NMR ($CDCl_3$, 162.04 MHz, 50°C): $\delta$ = 21.75(s, 1P).

IR (ATR): ν = 2977, 1702, 1366, 1252, 1166, 1141, 986 $cm^{-1}$.

Anal. cald. for $C_{19}H_{37}N_2O_7P$ (436.48): C 52.28%, H 8.54%, N 6.42%; found: 51.99%, 8.40%, N 6.34%.

Method B:

Di-*tert*-butyl hydrazine-1,2-dicarboxylate (1.161 g, 5 mmol, 2.5 equiv.) and KO*t*Bu (0.561 g, 5 mmol, 2.5 equiv.) were dissolved in dry DMF (5 ml) under Ar atmosphere. (±)-Diisopropyl 3-bromo-1-(4-nitrobenzenesulfonyloxy)propylphosphonate [(±)-**5.41**] (0.976 g, 2 mmol) dissolved in dry DMF (5 ml) was added at 0°C and the reaction mixture was stirred for 1.5 h at 0°C and at r. t. until the starting material was virtually consumed (1.5 h).

Work up: Acetic acid (6 drops) was added and volatile components were removed under reduced pressure (0.5 mbar). The residue was taken up in $H_2O$/EtOAc (30 ml, 1:1). The organic phase was separated and the aqueous one was extracted with EtOAc (2x 10 ml). The combined organic phases were washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure. Flash chromatography (hexanes:EtOAc = 3:2, $R_f$ = 0.23) of the residue gave the desired pyrazolidinylphosphonate (±)-**5.33** (0.570 g, 1.30 mmol, 65%) as a yellowish oil.

Cyclisation with alternative work up: bromo nosylate (±)-**5.41** (2.300 g, 4.71 mmol) was cyclized as before, but worked up differently. When cyclisation was finished, HCl (2M, 25 ml) and water (25 ml) were added (colour changed from dark red/brown to yellow). The mixture was extracted

with EtOAc (4x 25 ml). The combined organic phases were washed with HCl (1M, 2x 20 ml), $NaHCO_3$ (sat., 10 ml), dried ($MgSO_4$) and concentrated under reduced pressure. Flash chromatography of the residue gave the desired pyrazolidinylphosphonate (±)-**5.33** (1.250 g, 2.86 mmol, 61%) as a yellowish oil.

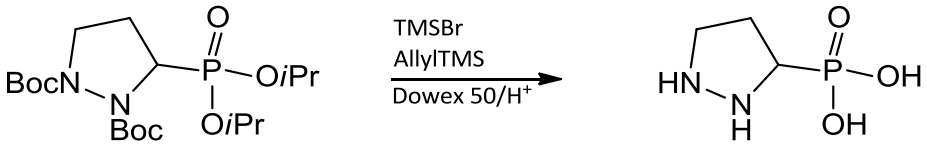
Spectroscopic data are identical to those of compound prepared by Method A.

### 6.2.4.10 (*R*)-Diisopropyl (2,3-di-tert-butoxycarbonyl)pyrazolidin-3-ylphosphonate [(*R*)-5.33]

The (*R*)-enantiomer of protected pyrazolidinylphosphonate (*R*)-**5.33** {0.635 g, 1.45 mmol, 49%, $[\alpha]_D^{26}$ = -25.50 {c = 1.40, acetone)} was obtained from the (*S*)-bromo nosylate (*S*)-**5.41** {1.464 g, 3 mmol, $[\alpha]_D^{23}$ = +19.73 (c = 1.86, acetone)} using method A.

Spectroscopic data are identical to the ones of the racemic compound.

### 6.2.4.11 (±)-Pyrazolidin-3-ylphosphonic acid [(±)-5.1]




(±)-**5.33** (±)-**5.1**

The cyclized product (±)-**5.33** (1.700 g, 3.89 mmol) was dissolved in dry 1,2-dichloroethane (20 ml) under Ar atmosphere. Allyltrimethylsilane (1.333 g, 1.85 ml, 11.67 mmol, 3 equiv.) was added before addition of bromotrimethylsilane (5.955 g, 5.13 ml, 38.9 mmol, 10 equiv.). Stirring at 50°C for 14 h completed the reaction. Afterwards volatile components were removed under reduced pressure (0.5 mbar, volative components were collected in a trap cooled with liquid $N_2$). The residue was dissolved in 1,2-dichloroethane (10 ml) and the solvent was again removed under reduced pressure (0.5 mbar). Afterwards water (20 ml) was added to the residue. After stirring the mixture for 10 min the mixture was extracted with EtOAc (2x 15 ml). The aqueous phase was concentrated (5 ml) and applied to a column filled with Dowex 50Wx8, $H^+$ (column diameter: 1.5 cm, height: 14 cm). The column was eluted with water and fractions (15 x 25 ml)

containing product (TLC: silica gel plates, *i*PrOH:$H_2O$:$NH_3$ = 6:3:1, $R_f$ = 0.42) were pooled to give phosphonic acid (±)-**5.1** (0.323 g, 2.14 mmol, 55%), mp 129-131 °C ($H_2O$/EtOH).

$^1$H NMR ($D_2O$; 4.80 ppm; 600.13 MHz): $\delta$ = 3.48 (td, $J$ = 8.8 Hz, 8.2 Hz, 1H, CH-P), 3.42 ( AB-System: A-part: $J_{AB}$ = 11.3 Hz, $J$ = 9.2 Hz, $J$ = 3.6 Hz; B-part: $J_{AB}$ = 11.3 Hz, 2x 8.2 Hz, 2H, $CH_2$-NH), 2.50-2.42 (m, 1H, $CH_2$-CH-P), 2.27-2.17 (m, 1H, $CH_2$-CH-P).

$^{13}$C NMR ($D_2O$, 150.92 MHz): $\delta$ = 56.15 (d, $J$ = 151.5 Hz, 1C, CH-P), 46.81 (d, $J$ = 8.2 Hz, 1C, $CH_2$-N), 27.82 (s, 1C, $\underline{C}H_2$-CH-P).

$^{31}$P NMR ($D_2O$, 242.94 MHz): $\delta$ = 14.87 (s, 1P).

IR (ATR): ν = 3277, 1626, 1446, 1234, 1190, 1145, 1028, 957, 924, 894 cm$^{-1}$.

Anal. cald. for $C_3H_9N_2O_3P$ (152.08): C 23.69%, H 5.96%, N 18.42%; found: C 23.60%, H 5.70%, N 18.34%.

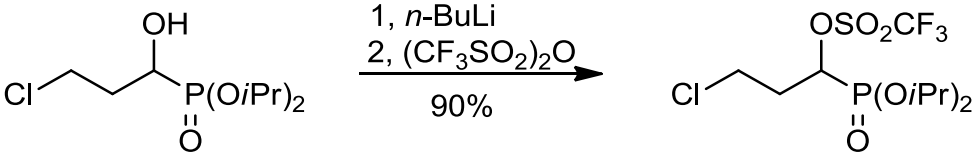
### 6.2.4.12 (*R*)-(+)-Pyrazolidin-3-ylphosphonic acid [(*R*)-5.1]

(*R*)-(-)-Diisopropyl-(2,3-di-tert-butoxycarbonyl)pyrazolidine-3-ylphosphonate [(*R*)-**5.33**] {0.739 g, 1.69 mmol, $[\alpha]_D^{26}$ = -25.50 {c = 1.40, acetone)} was converted, by the procedure used for the racemic compound, to (*R*)-(+)-pyrazolidine-3-ylphosphonic acid [(*R*)-**5.1**] {0.136 g, 0.90 mmol, 53%, $[\alpha]_D^{18}$ = + 5.09 (c = 0.51, $H_2O$)}. mp 118-119 °C ($H_2O$/EtOH)

IR (ATR): ν = 3184, 1621, 1588, 1453, 1237, 1141, 1046, 1025, 946, 912 cm$^{-1}$.

NMR data were identical to those of the racemate.

### 6.2.4.13 (±)-Diisopropyl 3-chloro-1-(trifluoromethanesulfonyloxy)propylphosphonate [(±)-5.32]



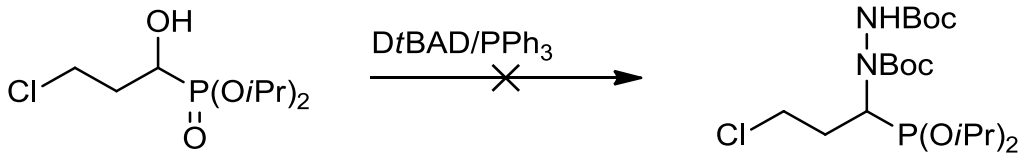

(±)-**5.25** (±)-**5.32**

The α-hydroxyphosphonate (±)-**5.25** (0.517 g, 2 mmol) was dissolved in dry diethyl ether and cooled to -78°C under Ar atmosphere. *n*-BuLi (0.88 ml, 2.2 mmol, 2.5M in hexanes, 1.1 equiv.) was added and the solution was stirred for 5 min before addition of trifluoromethanesulfonic anhydride (0.620 g, 0.37 ml, 2.2 mmol, 1.1 equiv.). Stirring was continued for 90 min until TLC showed complete conversion of starting material to product. Water (5 ml) was added to the reaction mixture. The phases were separated and the aqueous one was extracted with EtOAc (3 x 10 ml). The combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc = 1:3, $R_f$ = 0.72) to yield the triflate (±)-**5.32** (0.713 g, 1.82 mmol, 91%) as a yellowish oil.

$^1$H NMR ($CDCl_3$; 7.24 ppm; 400.27 MHz): $\delta$ =5.16 (td, *J* = 8.2 Hz, *J* = 5.0 Hz, 1H, CH-P), 4.88-4,74 (m, 2H, O-CH), 3.78-3.71 (m, 1H, Cl-$CH_2$). 3.64-3.57 (m, 1H, Cl-$CH_2$), 2.52-2.34 (m, 2H, C$\underline{H_2}$-CH-P), 1.38 (d, *J* = 2.4 Hz, 3H, $CH_3$), 1.37 (d, *J* = 2.9 Hz, 3H, $CH_3$), 1.36 (d, *J* = 2.4 Hz, 3H, $CH_3$), 1.35 (d, *J* = 2.9 Hz, 3H, $CH_3$).

$^{31}$P NMR ($CDCl_3$, 162.04 MHz): $\delta$ = 11.93 (s, 1P).

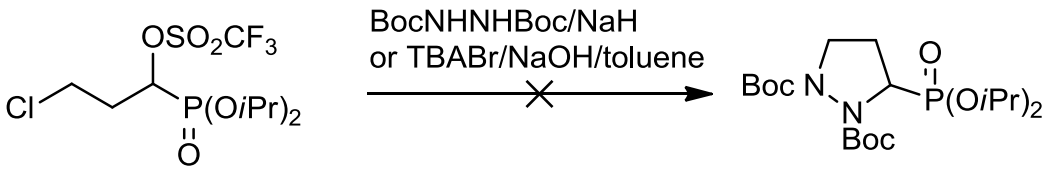
**6.2.4.14 Attempted conversion of (±)-diisopropyl 3-chloro-1-hydroxypropylphosphonate [(±)-5.25] to hydrazinophosphonate [(±)-5.34]**




(±)-**5.25** (±)-**5.34**

$PPh_3$ (0.393 g, 1.5 mmol, 1.5 equiv.) and α-hydroxyphosphonate (±)-**5.25** (0.258 g, 1 mmol) were dissolved in dry toluene under Ar atmosphere. D*t*BAD (0.345 g, 1.5 mmol, 1.5 equiv.) was added at 0°C and the reaction was monitored by TLC and NMR spectroscopy. After 72 h the reaction mixture was discarded, because no hydrazinophosphonate (±)-**5.34** could be detected.

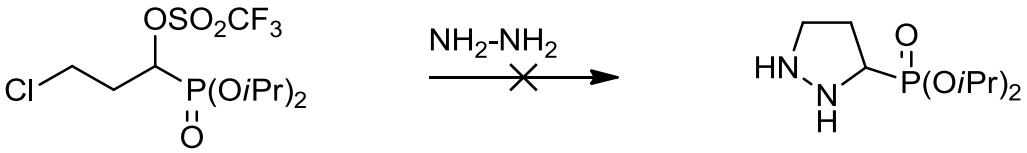
**6.2.4.15 Attempted conversion of triflate (±)-5.32 to (±)-diisopropyl (1,2-di-tert-butoxycarbonyl)pyrazolidin-3-ylphosphonate [(±)-5.33]**




(±)-**5.32** (±)-**5.33**

BocHN-NHBoc (0.232 g, 1 mmol) was dissolved in dry toluene (5 ml), NaOH (20 w/w%) and tetrabutylammonium bromide (0.045 g, 0.14 mmol, 0.14 equiv.) were added. After stirring for 5 min, (±)-diisopropyl 3-bromo-1-(methanesulfonyloxy)propylphosphonate [(± )-**5.38**] (0.390 g, 1 mmol) was added and the reaction mixture was heated to reflux. After 48 h decomposition was observed and the reaction mixture was discarded.

**6.2.4.16 Attempted conversion of triflate (±)-5.32 to pyrazolidinylphosphonate (±)-5.68**

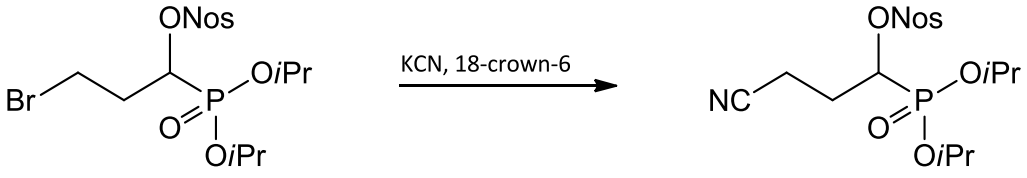



(±)-**5.32** (±)-**5.68**

The triflate (±)-**5.32** was dissolved in THF (5 ml) and cooled to 0°C under Ar atmosphere. Hydrazine (5 ml, 5 mmol, 1M in THF) was added and the reaction mixture was stirred overnight. Since TLC only showed starting material the reaction mixture was heated to 50°C and stirring was continued for 24 h. The solvent was removed and the residue was taken up in diethyl ether and water and the phases were separated. The aqueous phase was extracted with diethyl ether (4x 10 ml), the combined organic phases were washed with brine (2x 20 ml), dried ($MgSO_4$) and concentrated under reduced pressure. NMR only showed starting material and therefore the residue was discarded.

## 6.2.5 Towards the synthesis of (±)-4-amino-4-phosphonobutanoic acid (phosphaglutamic acid)

### 6.2.5.1 (±)-Diisopropyl 3-cyano-1-(4-nitrobenzenesulfonyloxy)propylphosphonate [(±)-5.46]




(±)-**5.38** (±)-**5.46**

The phosphonate (±)-**5.38** (1.464 g, 3.00 mmol) was dissolved in dry DMF (6 ml) and cooled to -40°C (acetone/dry ice) under Ar atmosphere. Potassium cyanide (0.390 g, 6 mmol, 2 equiv.) and 18-crown-6 (0.158 g, 0.6 mmol, 0.2 equiv.) were added and the reaction mixture was allowed to warm to 0°C in the cooling bath and was then kept at 0°C (ice/water). After a total of 48 h the reaction mixture was taken up in EtOAc/water (20 ml, 1:1, workup has to be performed in a well vented hood, because of the cyanide!) and the aqueous layer was extracted with EtOAc (2x 10 ml). The combined organic layers were washed with HCl (2M, 10 ml), water (10 ml), dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc = 1:1, $R_f$ = 0.23) to yield the cyanophosphonate (±)-**5.46** (0.584 g, 1.34 mmol, 45%) as a yellow oil.

$^1$H NMR ($CDCl_3$; 7.24 ppm; 400.27 MHz): $\delta$ = 8.47-8.42 (m, 2H, $CH_{arom.}$), 8.25-8.21 (m, 2H, $CH_{arom.}$), 4.91 (ddd, $J$ = 10.8 Hz, $J$ = 8.2 Hz, $J$ = 4.5 Hz, 1H, CH-P), 4-80-4.63 (m, 2H, O-CH), 2.66 (AB-System, A-part: $J_{AB}$ = 17.0 Hz, $J$ = 8.4 Hz, $J$ = 5.8 Hz; B-part: $J_{AB}$ = 17.0 Hz, $J$ = 7.9 Hz, $J$ = 7.3 Hz, 2H, NC-$CH_2$), 2.43-2.30 (m, 1H, C$\underline{H_2}$-CH-P), 2.30-2.17 (m, 1H, C$\underline{H_2}$-CH-P), 1.34 (d, $J$ = 6.3 Hz, 6H, 2x $CH_3$), 1.32 (d, $J$ = 6.8 Hz, 3H, $CH_3$), 1.29 (d, $J$ = 6.1 Hz, 3H, $CH_3$).

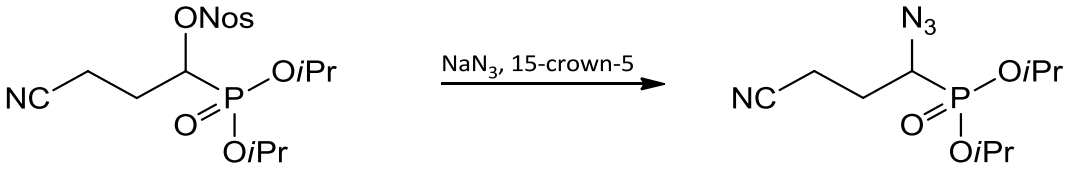
$^{13}$C NMR ($CDCl_3$, 77.00, 100.65 MHz): $\delta$ = 151.23 (s, 1C, $C_{arom.,q}$), 141.69 (s, 1C, $C_{arom.,q}$), 129.62 (s, 2C, $C_{arom.}$), 124.34 (s, 2C, $C_{arom.}$), 118.16 (s, 1C, CN), 75.26 (d, $J$ = 172.7 Hz, 1C, CH-P), 73.08 (d, $J$ = 6.8 Hz, 1C, O-CH), 72.98 (d, $J$ = 7.0 Hz, 1C, O-CH), 27.13 (s, 1C, $\underline{C}H_2$-CH-P), 24.26 (d, $J$ = 3.7 Hz, 1C, $CH_3$), 23.94 (d, $J$ = 3.4 Hz, 1C, $CH_3$) 23.93 (d, $J$ = 5.4 Hz, 2C, $CH_3$), 23.72 (d, $J$ = 5.0 Hz, 1C, $CH_3$), 13.87 (d, $J$ = 12.0 Hz, 1C, NC-$CH_2$).

$^{31}$P NMR ($CDCl_3$, 162.04 MHz): $\delta$ = 12.95 (s, 1P).

IR (ATR): v = 2983, 2250 (very weak), 1533, 1375, 1350, 1252, 1185, 983, 927 $cm^{-1}$.

Anal. cald. for $C_{16}H_{23}N_2O_8PS$ (434.40): C 44.24%, H 5.34%, O 29.46%; found: C 43.92%, H 5.29%, O 29.49%.

### 6.2.5.2 (±)-Diisopropyl 1-azido-3-cyanopropylphosphonate [(±)-5.43]




(±)-**5.46** (±)-**5.43**

The cyanonosylate (±)-**5.46** (0.384 g, 0.88 mmol) dissolved in dry DMF (3 ml) was added to a mixture of $NaN_3$ (0.114 g, 1.76 mmol, 2 equiv.) and 15-crown-5 (0.036 g, 0.176 mmol, 0.2 equiv.) in dry DMF (4 ml) under Ar atmosphere. The reaction mixture was stirred at 70°C for 5 h and then cooled. The solvent was removed under reduced pressure (0.5 mbar) and the residue was taken up in water/EtOAc (20 ml, 1:1). The aqueous layer was separated and extracted with EtOAc (3x 10 ml). The combined organic layers were dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes:acetone = 4:1, $R_f$ = 0.30) to give the azidophosphonate (±)-**5.43** (0.158 g, 0.57 mmol, 65%) as a yellowish oil.

$^1$H NMR ($CDCl_3$; 7.24 ppm; 400.27 MHz): $\delta$ = 4.84-4.71 (m, 2H, O-CH), 3.53 (ddd, *J* = 12.1 Hz, *J* = 10.7 Hz, *J* = 4.0 Hz, 1H, CH-P), 2.63-2.47 (m, 2H, NC-$CH_2$), 2.19-2.06 (m, 1H, C$\underline{H}_2$-CH-P), 1.95-1.82 (m, 1H, C$\underline{H}_2$-CH-P), 1.34 (d, *J* = 6.2 Hz, 6H, 2x $CH_3$), 1.33 (d, *J* = 6.2 Hz, 2x $CH_3$).

$^{13}$C NMR ($CDCl_3$, 77.00, 100,61 MHz): $\delta$ = 118.27 (s, 1C, NC), 72.64 (d, *J* = 6.0 Hz, O-CH), 72.57 (d, *J* = 6.1 Hz, 1C, O-CH), 56.10 (d, *J* = 158.1 Hz, 1C, CH-P), 25.24 (s, 1C, $\underline{C}H_2$-CH-P), 24.14 (d, *J* = 3.7 Hz, 1C, $CH_3$), 24.03 (d, *J* = 3.7 Hz, 1C, $CH_3$), 23.95 (d, *J* = 3.1 Hz, 1C, $CH_3$), 23.91 (d, *J* = 3.0 Hz, 1C, $CH_3$), 14.52 (d, *J* = 14.1 Hz, 1C, $\underline{C}H_2$-CH-P).

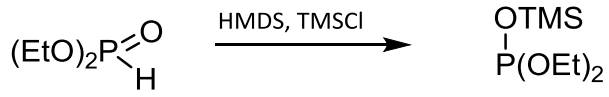
$^{31}$P NMR ($CDCl_3$, 162.04 MHz): $\delta$ = 18.02 (s, 1P).

IR (ATR): v = 2982, 2935, 2103, 1387, 1377, 1247, 1104, 988, $cm^{-1}$.

Anal. cald. for $C_{10}H_{19}N_4O_3P$ (274.25): C 43.79%, H 6.98%; found: C 43.13%, H 6.89%.

## 6.2.6 Towards the synthesis of a DOTA-zoledronate derivative

### 6.2.6.1. Diethyl trimethylsilyl phosphite [5.49]

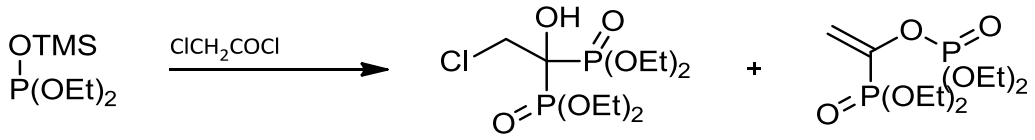



**5.48** **5.49**

A mixture of diethyl phosphite (**5.48**) (20.715 g, 150 mmol), hexamethyldisilazane (12.104 g, 75 mmol) and trimethylsilyl chloride (8.148 g, 75 mmol) in hexanes (75 ml) was refluxed for 1 h under Ar atmosphere. The formed $NH_4Cl$ was removed by filtration through celite. Hexanes was removed at normal pressure and the product **5.49** (23.656 g, 112 mmol, 75%, 34°C/9 mmHg, colourless oil) was fractionated.

Spectroscopic data were identical to those of the literature.[143]

### 6.2.6.2 Tetraethyl (2-chloro-1-hydroxyethane-1,1-diyl)bisphosphonate [5.50]




**5.49** **5.50** **5.51**

Diethyl trimethylsilyl phosphite (**5.49**) (5.257 g, 25 mmol) was dissolved in dry THF (40 ml) and cooled to -90°C under Ar atmosphere. 2-Chloroacetyl chloride (1.129 g, 10 mmol) was added slowly over 10 min and the reaction mixture was allowed to warm to r.t. gradually (2.5 h). Addition of HCl (2M, 10 ml) and stirring for 1 h was necessary to deprotect the silylated hydroxybispohosphonate (at the end TLC showed no silylated compound). The solvent was removed under reduced pressure and the reaction mixture was purified by flash chromatography (EtOAc:EtOH = 20:1, bisphosphonate: $R_f$ = 0.18; phosphonophosphate: $R_f$ = 0.30) to give the bisphosphonate **5.50** (1.093 g, 3.09 mmol, 31%) and the phosphonophosphate **5.51** (1.145 g, 3.62 mmol, 36%) as colourless gums.

**Hydroxybisphosphonate 5.50:**

$^{1}$H NMR ($CDCl_3$; 7.24 ppm; 400.27 MHz): $\delta$ = 4.32-4.21 (m, 8H, 4x O-$CH_2$), 4.04 (dd, *J* = 12.0Hz, *J* = 11.3 Hz, 2H, Cl-$CH_2$), 3.90 (broad s, 1H, OH), 1.35 (td, *J* = 7.0 Hz, *J* = 1.1 Hz, 12H, 4x $CH_3$).

$^{13}$C NMR ($CDCl_3$; 77.00 ppm, 100.65 MHz): $\delta$ = 74.31 (t, *J* = 152.0 Hz, 1C, C-P), 64.50 (d, *J* = 3.3 Hz, 1C, O-$CH_2$), 64.47 (d, *J* = 3.3 Hz, 1C, O-$CH_2$), 64.40 (d, *J* = 3.3 Hz, 1C, O-$CH_2$), 64.36 (d, *J* = 3.3 Hz, 1C, O-$CH_2$), 47.00 (s, 1C, $ClCH_2$), 16.44 (d, *J* = 2.9 Hz, 1C, $CH_3$), 16.41 (d, *J* = 2.9 Hz, 2C, 2x $CH_3$), 16.38 (d, *J* = 3.2 Hz, 1C, $CH_3$).

$^{31}$P NMR ($CDCl_3$, 162.04 MHz): $\delta$ = 16.72 (s, 1P).

IR (ATR): ν = 3202 (very broad), 2982, 1242, 1022, 975 $cm^{-1}$.

**Phosphonophosphate 5.51:**

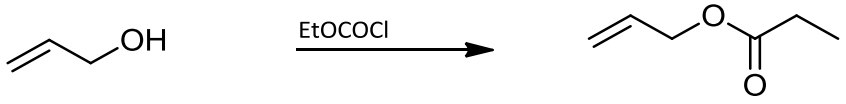
$^{1}$H NMR ($CDCl_3$; 7.24 ppm; 400.27 MHz): $\delta$ = 5.85 (ddd, *J* = 35.1 Hz, *J* = 2.7 Hz, *J* = 1.2 Hz, 1H, $CH_2$=C), 5.78 (ddd, *J* = 11.3 Hz, *J* = 2x 2.7 Hz, 1H, $CH_2$=C), 4.25-4.07 (m, 8H, O-$CH_2$), 1.35 (td, *J* = 7.1 Hz, *J* = 1.0 Hz, 6H, 2x $CH_3$), 1.34 (td, *J* = 7.1 Hz, *J* = 0.5 Hz, 6H, 2x $CH_3$).

$^{31}$P NMR ($CDCl_3$, 162.04 MHz): $\delta$ = 7.09 (d, *J* = 27.5 Hz, 1P, C-P), -6.54 (d, *J* = 27.5, 1P, C-O-P).

$^{13}$C NMR ($CDCl_3$; 77.00 ppm, 100.65 MHz): $\delta$ = ($C_q$ n.d.), 114.74 (dd, *J* = 24.3 Hz, 3.7 Hz, 1C, $\underline{C}H_2$=C), 64.77 (d, *J* = 6.3 Hz, 2C, 2x O-$CH_2$), 63.04 (d, *J* = 5.2 Hz, 2C, 2x O-$CH_2$), 16.23 (d, *J* = 6.4 Hz, 2C, 2x $CH_3$), 16.00 (d, *J* = 6.7 Hz, 2C, 2x $CH_3$).

IR (ATR): ν =2984, 1620, 1268, 1220, 1165, 1022, 998 $cm^{-1}$.

## 6.2.6.3 Allyl ethyl carbonate (5.60)

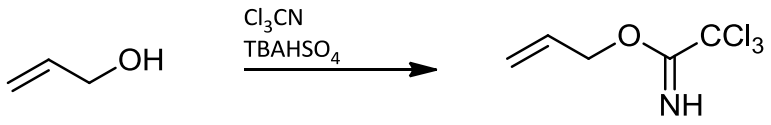



**5.59** **5.60**

Prop-2-en-1-ol (**5.59**) (0.580 g, 10 mmol) was dissolved in diethyl ether (20 ml), pyridine (1.186 g, 15 mmol) was added and the reaction mixture was cooled to 0°C under Ar atmosphere. Ethyl chloroformate (1.593 g, 13 mmol) was added slowly over 10 min and the solution was allowed to warm to r.t. After 3 h the reaction was quenched with $NH_4Cl$, the combined organic phases were washed with water (30 ml), brine (30 ml), dried ($MgSO_4$) and concentrated under reduced pressure. The residue was purified by flash chromatography (hexanes:EtOAc = 3:1, $R_f$ = 0.64) to give the desired ester **5.60** (0.913 g, 8.00 mmol, 80%) as a colourless oil.

Spectroscopic data and procedure are identical to those in the literature.[144]

### 6.2.6.4 Allyl 2,2,2-trichloroacetimidate [5.61]

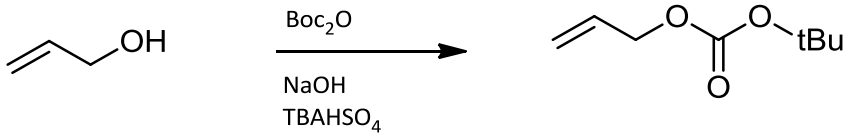



**5.59** **5.61**

Prop-2-en-1-ol (**5.59**) (1.00 g, 17.2 mmol) was dissolved in dry $CH_2Cl_2$ (10 ml), KOH (50w/w%, 5 ml) and tetra-*n*-butylammonium hydrogensulfate (0.015 g, 0.04 mmol) were added. The reaction mixture was cooled to -20°C and stirred for 5 min. Afterwards $Cl_3CCN$ (1.980 g, 20.6 mmol) was slowly added and stirring was continued for 35 min at -15 to -10°C. The cooling bath was removed and stirring was continued for 0.5 h. The layers were separated, the aqueous layer was extracted with $CH_2Cl_2$ (2x 30 ml). The combined organic phases were dried ($MgSO_4$) and concentrated under reduced pressure to 1/3 of its volume. The residue was filtered over celite pad and the solvent was removed under reduced pressure to give the allylic imidate **5.61** (3.406 g, 16.8 mmol, 97%) as a colourless oil.

Method[145] and spectroscopic data[146] have been the same as reported in literature.

**6.2.6.5 Allyl tert-butyl carbonate [5.62]**




**5.59** **5.62**

Prop-2-en-1-ol (**5.59**) (1.834 g, 31 mmol) was added to a solution of di-*tert*-butyl dicarbonate (8.271 g, 37.9 mmol) and tetra-*n*-butylammonium bromide (0.315 g, 0.93 mmol) in dry $CH_2Cl_2$ (15 ml) under Ar atmosphere. The solution was cooled to 0°C and cold NaOH (30w/w%, 15 ml) was added. After 1.5 h of stirring the reaction mixture was extracted with $CH_2Cl_2$ (2x 20 ml) and the combined organic layers were washed with brine (2x 15 ml), dried ($MgSO_4$) and the solvent was concentrated under reduced pressure. The residue was bulb to bulb distilled (104-114°C, 68 mmHg) to obtain the carbonate **5.62** (3.218 g, 20.3 mmol, 64%) as a colourless oil.

Method and spectroscopic data identical to those in the literature.[147]

# 6. Summary

Phosphonates, compounds with a direct P-C bond, might exist since primitive earth. Up to now, different P-C compounds were found to be useful. α-Aminophosphonates are capable of inhibition of different biosynthetic pathways in plants and therefore work as herbicides. Moreover, they also found use as drugs against osteoclast induced bone turnover, as well as importance for the oil industry.

During my master thesis I synthesised different α-aminophosphonic acids, which might have inhibitory effects onto the P5C reductase, but the evaluation belongs to the co-operation partners.

The phosphorus analogue of proline was synthesised starting from cheap paraformaldehyde, allylbromide and diisopropyl phosphite. The resulting (±)-diisopropyl 1-hydroxybut-3-enylphosphonate was converted to the corresponding chloroacetate, which was chemoenzymatically hydrolysed to obtain the (*S*)-hydroxyphosphonate. After activation of the hydroxyl functionality with $(CF_3SO_2)_2O$, the triflate was converted to the (*R*)-azide. Cyclisation with borane dimethyl sulfide complex, hydrolysis with HCl followed by ion-exchange chromatography gave phosphaproline in its enantiomerically pure (ee: 97%) and its racemic form. The synthesis was accomplished in six and eight steps for the racemic and enantiomerically pure compound in an overall yield of 14%, respectively.

Isoxazoldine-4-yl phosphonic acid was synthesised in enantiomerically pure and racemic form. The cheap starting material, ethyl 3-chloropropionate, was treated with diispropyl phosphite to give the α-hydroxyphosphonate, which could be converted to the chloroacetate. Chemoenzymatic hydrolysis, using lipase from *Thermomyces lanuginosus*, gave the (*S*)-phosphonate with an ee of 97%. Further treatment with *N*-hydroxyphthalimide with inversion of configuration gave the (*R*)-phthalimido compound. Cyclisation with $NH_3$ and hydrolysis with HBr/AcOH followed by ion-exchange chromatography gave the desired isoxazoldine-4-yl phosphonic acid either racemic or enantiomerically pure in a total of three or five steps with an overall yield of 16%, respectively.

Pyrazolidin-3-ylphosphonic acid was synthesised using ethyl 3-bromopropionate and diisopropyl phosphite. The resulting α-hydroxyphosphonate was chemoenzymatically resolved and afterwards activated with the introduction of a nosyloxy moiety. Cyclisation with di-*tert*-butyl hydrazine-1,2-dicarboxylate, hydrolysis with allyltrimethylsilane and trimethylsilyl bromide, followed by ion-exchange chromatography gave the desired proline analogue racemic and enantiomerically pure in a four or six steps synthesis with an overall yield of 11%, respectively.

For the synthesis of the phosphono glutamic acid only a few steps were performed. Starting from the racemic nosylated compound, which was already used for the synthesis of pyrazolidin-3-yl phosphonic acid, the nitrile functionality was introduced followed by the conversion to the azide with $NaN_3$. Only the racemic compounds were generated and the overall yield for the four steps is 18%.

The last project was the synthesis of a DOTA-zoledronate derivative for skeletal imaging purpose. The generation of the bisphosphonic compound was possible using diethyl (trimethylsilyl) phosphite and 2-chloroacetyl chloride. However, further efforts to elongate the bisphosphonate did not succeed at all.

# 7. Zusammenfassung

Phosphonate sind Verbindungen mit einer direkten P-C-Bindung die sehr stabil sind. Man vermutet das sie ihren Ursprung in der sauerstoff-armen und daher reduzierenden Uratmosphäre haben. Bisher erwiesen sich verschiedene P-C-Verbindungen als nützlich. Substituierte Phosphonsäuren können Enzyme inhibieren und sind daher von biologischer Bedeutung, z. B. als Herbizide. Bisphoshonate werden in der Medizin als Medikamente gegen Knochenschwund und in der Industrie als Antikorrosiva eingesetzt.

Während meiner Master-Arbeit synthetisierte ich verschiedene α-Aminophosphonsäuren, die vermutlich die P5C-Synthase, ein Schlüsselenzym des Prolinmetabolismus in Pflanzen, inhibieren können. Die Inhibitorwirkung soll mit Hilfe von Pflanzenenzymen durch Giuseppe Forlani, einen italienischen Kooperationspartner überprüft werden. Das Phosphonatanalogon des Prolins wurde aus Paraformaldehyd, Allylbromid und Diisopropylphosphit hergestellt. Das resultierende (±)-Diisopropyl-1-hydroxybut-3-enylphosphonate wurde zuerst in das entsprechende Chloracetat überführt und enantioselektiv mit einer Lipase hydrolysiert, um das (*S*)-Hydroxyphosphonat zu erhalten. Die Hydroxylgruppe der racemischen Verbindung und des (*S*)-Enantiomers dieses α-Hydroxyphosphonats wurde mit Trifluormethansulfonylchlorid verestert. Diese Triflate lieferten jeweils das racemische und das (*R*)-Azid. Eine durch Dicyclohexylboran-induzierte Cyclisierung, vollständige Entschützung mit HCl in der Siedehitze, gefolgt von einer Ionenaustauschchromatographie lieferten das (±)- und das (*R*)-Phosphaprolin (ee 97%). Die Synthese konnten in sechs bzw. acht Schritten durchgeführt werden.

Des weiteren wurde die racemische als auch die enantiomerenreine Form der Isoxazolidin-4-ylphosphonsäure hergestellt. Das billige Ausgangsmaterial, das Ethyl-3-chlorpropionat, wurde mit DIBAH zum Aldehyd reduziert und mit Diisopropyl-trimethylsilyphosphit zum 3-Brom-1-hydroxyphosphonat umgesetzt, das chloracetyliert wurde. Enzymatische Hydrolyse des Chloracetats mit einer Lipase aus *Thermomyces lanuginosus* lieferte das enantiomerenreine (*S*)-Hydroxyphosphonat (ee 97%). Eine Mitsunobu-Reaktion mit *N*-Hydroxyphthalimid führte unter Inversion der Konfiguration zum (*R*)-1-Phthalimidooxyphosphonat. Ammoniak-vermittelte Zyklisierung, gefolgt von einer vollständiger Entschützung mit HBr/AcOH und zuletzt Ionenaustauschrchromatographie lieferten schließlich die gewünschte Isoxazolidin-4-ylphosphonsäure in racemischer oder enantiomerenreiner Form in einer Gesamtaubeute von 16% in vier bzw. sechs Schritten.

Pyrazolidin-3-ylphosphonsäure wurde ausgehend von 3-Brompropionsäureethylester und Diisopropyl-trimethylsilylphosphit synthetisiert. Beim entstandenen α-Hydroxyphosphonat wurde eine enzymatische Racematspaltung durchgeführt. Das racemische und das (*S*)-α-Hydroxyphosphonat wurden zur Aktivierung in die jeweiligen Nosylate überführt. Umsetzung

mit Di-*tert*-butyl-hydrazin-1,2-dicarboxylat in Gegenwart von Kalium-*tert*-butoxid lieferten die cyclischen Hydrazinderivate. Die Schutzgruppen wurden mit Bromtrimethylsilan/Allyltrimethylsilan entfernt und das Rohprodukt wurde durch Ionenaustauschchromatographie gereinigt, um die gewünschte Pyrazolidin-3-ylphosphonsäure in racemischer und enantiomerenreiner Form in einer Gesamtausbeute von 11% in einer vier- bzw. sechsstufigen Synthese zu gewinnen.

Für die Synthese der Phosphaglutaminsäure wurden nur wenige Reaktionsschritte durchgeführt. Ausgehend vom Nosylat, das sich vom Diisopropyl-3-brom-1-hydroxyphoshonat ableitet, wurde die Cyanogruppe eingeführt, gefolgt von der Umwandlung mit $NaN_3$ in das Azid. Es wurden nur die racemischen Zwischenprodukt hergestellt. Die Gesamtausbeute über vier Schritte betrug 18%.

Das letzte Projekt meiner Masterarbeit war die Synthese eines DOTA-Zeledronsäure- Derivates. Die Herstellung eines Hydroxybisphosphonats als Zwischenprodukt aus Diethyl-trimethylsilylphosphit und 2-Chloracetylchlorid gelang. Jedoch konnte die Hydroxylgruppe nicht funktionalisiert werden, obwohl verschiedene Methoden getestet wurden.

# 8. Curriculum Vitae

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Degree: Bakk. rer. nat.

Date of birth: 19.11.1986

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| **Education** | |
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| *Sept. 1992 - June 1997* | Volksschule Kleistgasse 12, A-1030 Wien |
| *Sept. 1997 - Oct. 2005* | Realgymnasium BRG 3 Radetzkystrasse 2A, A-1030 Wien |
| *Oct. 2006 - Feb. 2012* | Bachelor´s programm in Chemistry, Universität Wien |
| | Faculty for Chemistry, Währingerstrasse 38-42, A-1090 Wien |
| | Titel of Bachelor Thesis: „*Synthese höherer Aminozucker aus Aminosäuren, durch Indium gestützte Kohlenstoff-Kohlenstoff Verknüpfung*“ |
| | Supervisor: Univ.-Prof. Dr. Walther **Schmid** |
| *Feb .2012 - Jan .2015* | Master´s programm in chemistry, Universität Wien |
| | Faculty for Chemistry, Währingerstrasse 38-42, A-1090 Wien |
| | Titel of Master Thesis: „Synthesis of (*R*)-phosphaproline, (*R*)-phosphaproline analogues and a zoledronate acid derivative” |
| | Supervisor: Ao. Univ.-Prof. Dr. Friedrich **Hammerschmidt** |
| **Experience** | (with relevance for chemistry) |
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| *Mar. 2011 - June 2011* | - |
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