Collège Doctoral du Languedoc-Roussillon

THÈSE

Pour obtenir le grade de

Docteur

Délivré par l'**Ecole Nationale Supérieure de Chimie de Montpellier**

Préparée au sein de l'école doctorale **Sciences Chimiques Balard**
Et de l'unité de recherche **Institut Charles Gerhardt Montpellier**

Spécialité : **Chimie organique, minérale et industrielle**

Présentée par **Vincent Blanchard**

Synthèse de composés phosphorés chélatants à visée phytosanitaire

Soutenue le 4 décembre 2015 devant le jury composé de

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Pr David Virieux, Professeur à l'ENSCM	Directeur de thèse
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ENSCM CHIMIE Montpellier

Synthesis of chelating phosphorus containing compounds for agrochemistry

Remerciements

Tout d'abord je tiens à remercier le Pr Jean-Marc Campagne qui dirige le laboratoire AM2N « Architectures moléculaires et matériaux nanostructurés » de m'avoir permis d'y réaliser ces travaux.

J'adresse mes remerciements aux personnes qui ont accepté de juger mon travail : Monsieur Thierry Durand et Monsieur Jean-Pierre Vors ainsi que les rapporteurs de mon mémoire Madame Annie-Claude Gaumont et Madame Catherine Grosdemange-Billiard.

Mes remerciements les plus chaleureux vont à mes encadrants David Virieux et Jean-Luc Pirat pour leurs conseils avisés, pour m'avoir guidé et fait confiance pendant ces 3 années. Je n'oublie pas non plus mes supérieurs de Bayer CropScience Francfort Messieurs Ralf Braun et Lothar Willms pour les discussions pendant les réunions et qui malheureusement n'ont pu être là pour la soutenance.

Je remercie également le groupe des Basiles pour tous les moments que l'on a pu passer tous ensemble que ce soit au moment de l'école ou même ensuite. Vivement les prochains rendez-vous !!

Pendant ces 3 années, beaucoup de personnes sont arrivées et parties, j'espère ne pas en oublier. Je tiens à remercier Coralie qui a su m'accueillir au sein du laboratoire et écouter les plaintes d'un pauvre 1ère année. Le 3ème étage de souillons, Jean-Simon et Poupette, on a été dans la galère en même temps, on a réussi à former un groupe soudé de personnes et à se soutenir mutuellement. Merci pour votre bonne humeur et vos conneries qui m'auront bien faites rire.

Les compagnons de thèse de pharma, Claire et Satan. Merci à toi Claire pour ton éternel sourire, l'aviron même si je n'ai pas été aussi assidu que toi, pour les BD que je dévore désormais, les randos, les bières et j'en passe. Satan tu ne cesseras jamais de m'ébahir par le nombre de blagues que tu peux sortir (Brutus, Mirador, Candy Raton etc). Tu es toujours prêt à sortir, à discuter, à rigoler et je te jure que ça fait un bien fou de connaître quelqu'un comme toi. Heureusement la relève à pharma est asurée par ma petite gueule Mélissa ! On a mis du temps à se comprendre mais cette fois c'est bon ma couille.

Ensuite il y a eu l'arrivée de mon frère de thèse Juju, mon demi-cerveau ! Heureusement que tu es venu faire ta thèse ici. Merci mon petit pour tout le soutien que tu as pu m'apporter sans forcément t'en rendre compte, que ce soit en proposant une pause café-clope, une bière (en général plusieurs se terminant à la terrasse du 11 rue cité Bousquet…) ou juste pour discuter de tout et de rien et parfois de chimie. Le duo a été complété par Rémi, merci à toi pour toutes ces soirées, ces discussions, les whisky-cigares…

Enfin, des rencontres tardives mais belles en fin de thèse. Renaud, Eline et Damien ! Eline merci pour ta gentillesse, tu es adorable. Ca fait plaisir de voir des gens qui gardent toujours le sourire comme tu le fais. Renaud, quel dommage qu'on ait dû partir juste après l'ouverture de la Barbote. J'espère qu'on se trouvera encore l'occasion de partir au ski tous ensemble pour que je puisse perfectionner mon planter de bâton ! Damien, merci à toi mon co-lab d'un peu moins d'un an pour les disussions, les soirées, les BD et ton humour !

Les derniers mots iront pour mes parents. Merci de m'avoir laissé le choix de faire ce que je voulais et de m'avoir toujours soutenu sur tous les plans.

Table of contents

Abbreviations

[U-$^{14}C_6$]-glucose	Uniformly ^{14}C labelled glucose
11-βHSD1	11-β-hydroxysteroid dehydrogenase
A_{340}	Absorbance at 340 nm
Ac	Acetyl
ACN	Acetonitrile
ADMET	Absorption, distribution, metabolism, excretion and toxicity
AD-mix	Asymmetric dihydroxylation mixture
AHAS	Acetohydroxy synthase
AIBN	Azobisisobutyronitrile
AL	Acetolactate
ALS	Acetolactate synthase
ATPase	Adenylpyrophosphatase
BCAA	Branched-chain amino acids
Bn	Benzyl
Bz	Benzoyl
CCK-B	Cholecystokinin type B
*c*Pr	Cyclopropyl
dba	Dibenzylideneacetone
DBU	1,8-Diazabicycloundec-7-ene
DCM	Dichloromethane
DIPEA	*N*,*N*-diisopropylethylamine or Hünig's base
DKN	Diketonitrile
DMF	Dimethylformamide
DNA	Deoxyribonucleic acid
dppb	1,4-Bis(diphenylphosphino)butane
dppp	1,3-Bis(diphenylphosphino)propane
DXR	1-deoxy-d-xylulose 5-phosphate reductoisomerase
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
EDTA	Ethylenediaminetetraacetic acid
Et	Ethyl
Et_2O	Diethylether
Et_3N	Triethylamine
EtOH	Ethanol
FAD	Flavine adenine dinucleotide
FEN1	Flap endonuclease 1
FPGS	Folylpolyglutamate synthase
GABA	γ-butyric acid
GC-MS	Gaz chromatography coupled to mass spectroscopy
HIV	Human immunodeficiency virus
Hoe 704	2-(dimethylphosphinoyl)-2-hydroxyacetic acid
HPPD	hydroxyphenylpyruvate deoxygenase
IC_{50}	Half maximum inhibitory concentration
IN	Enzyme integrase
INI	Enzyme integrase inhibitor
IpOHA	*N*-hydroxy-*N*-isopropyloxamic acid
*i*Pr	Isopropyl
KARI	Ketol-acid reductoisomerase

k_{cat}	Catalytic constant
K_i	Inhibition constant
K_m	Michaelis constant
KP	Ketopentoate
LiHMDS	Lithium hexamethyldisilazane
log P	Partition constant
m-CPBA	m-chloroperbenzoic acid
Me	Methyl
MeOH	Methanol
MMP	Matrix metalloprotinease
MTX	Methotrexate
MW	Microwave
NADH	Nicotinamide adenine dinucleotide
NADPH	Nicotinamide adenine dinucleotide phosphate
NMR	Nuclear magnetic resonance
*n*Pr	Linear propyl
NuH	Nucleophile
OD	Optical density
ON	Overnight
PepM	Phosphomutase
Ph	Phenyl
pH	Hydrogen potential
pKa	Acidity constant
PTSA	p-toluenesulfonic acid
PyBOP	Benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
QC	Glutaminyl cyclase
RT	Room temperature
SAHA	Suberoylanilide hydroxamic
SEM	2-trimethylsilylethyoxymethyl
TBAF	Tetrabutylammonium fluoride
TBDMS	*t*-butyldimethylsilyl
*t*Bu	*t*-butyl
THF	Tetrahydrofuran
ThPD	Thiamine diphosphate
TMS	Trimethylsilyl
U	Enzyme unit
VSEPR	Valence Shell Electron Pairs Repulsion
W	Watt
ΔF_{460}	Variation of fluorescence at 460 nm

Introduction

The use of crop protection products improved a lot the global living status since World War II. Indeed, for example the production losses due to low growth yields or bad transport conditions have been reduced by 42% concerning the major cultures, thanks to phytosanitary treatments as depicted in Figure 1.

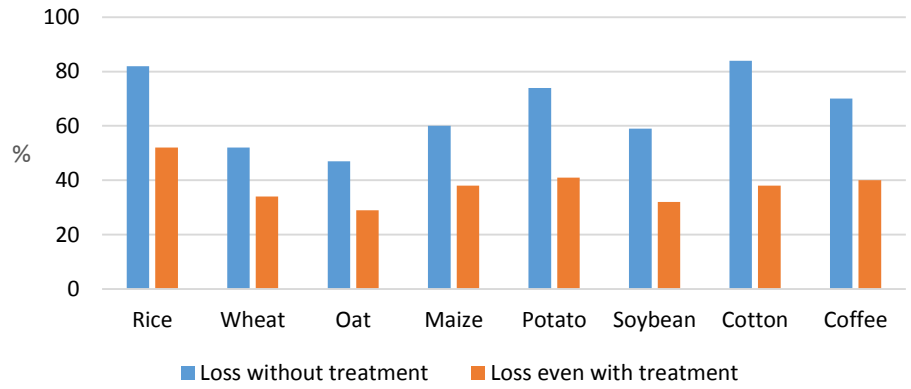


Figure 1: Impact of phytosanitary treatment on cultures (FAO 2005)

Since the 60s, the total area of arable land on earth has stagnated around 13 900 000 km² whereas the population has more than doubled, from 3 billion to nearly 7 billion people in 2010. As shown in Figure 2, the area of farmable land per person has been divided by more than two in less than 50 years, meaning that we need to produce more with the same available surface.

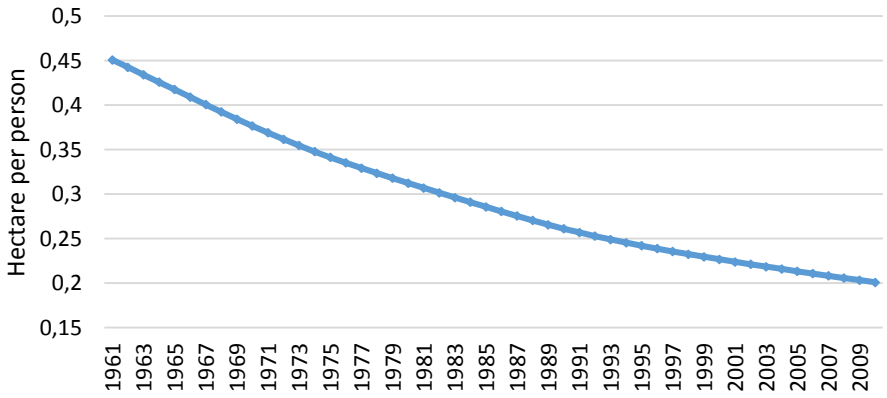


Figure 2: Chronological evolution of arable surface per human (FAO 2013)

This major problem may find an answer thanks to the use and development of agrochemicals.

Herbicides are one pesticide class which are widely used on fields in order to control weed growth and thus avoid competition between weeds and the ones farmers are producing. Since the beginning of the industrial production of herbicides in the 50s, their total consumption has skyrocketed. Figure 3 presents an overview of the increasing use of herbicides on a 20-year period of time.

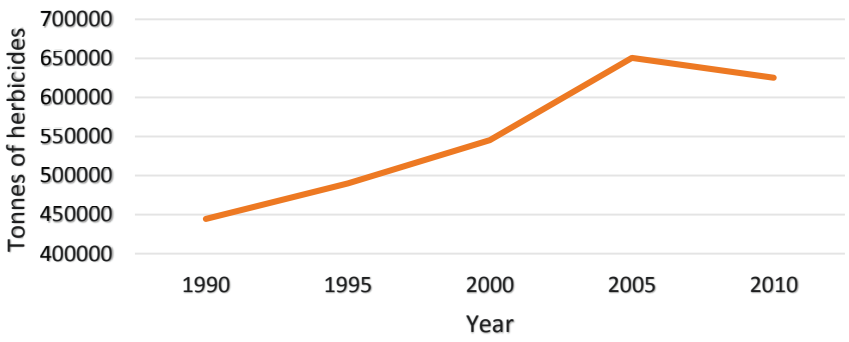
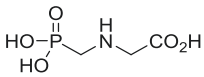


Figure 3: Evolution of the worldwide use of herbicides (FAO 2013)

Two types of herbicides exist, one is very selective of the crops it affects, the other one is called non-selective as it kills all the weeds of the treated area. It is the case of glyphosate (*N*-(phosphonomethyl)glycine) **1**, the most wide spread non-selective herbicidal compound.



1

In order to illustrate its spreading, in 2004 glyphosate was applied on 87% of the whole soybean growing fields in the US which is one the major culture there.[1] However the lack of rotation of herbicide chemistry because of an over-reliance on one technique or the reduction of dose rates are drivers for the selection of herbicide resistances towards glyphosate or other compounds.

As a way to avoid more herbicide-resistant plants to appear or spread, new modes of action of herbicides have to be investigated. The branched-chain amino acids biosynthesis is a metabolic pathway comprising successive enzyme catalysed steps for the synthesis of essential amino acids which are valine, leucine and isoleucine. This biosynthesis pathway is not present in mammals nor insects which makes it interesting to explore for the development of new herbicides. The enzyme acetohydroxyacid synthase, which catalyses the first step of the biosynthesis, has already been fully studied, which led to two major herbicide families: the sulfonylureas and the imidazolinones. The *in vitro* inhibition of ketol-acid reductoisomerase (KARI), the following enzyme, has shown some promising results with the synthesis of two potent inhibitors: Hoe 704 and IpOHA.

The aim of this manuscript is dedicated to the synthesis of new inhibitors of the metalloenzyme ketol-acid reductoisomerase. At first, an overview on phosphorus compounds in medicinal chemistry and agrochemistry will be broached, followed by the explanation of the branched-chain amino acids metabolic pathway. Then the metalloenzyme of interest ketol-acid reductoisomerase will be described in order to understand its functioning and how the inhibition occurs.

[1] National Agricultural Statistics Service, Agricultural Chemical Usage, **2005**, Field and Vegetable Crops Chemical Distribution Rate (U.S. Dept. Agric. Washington DC)

The five other chapters will be dedicated to the synthesis of different families of compounds based on the structure relationship activity of the inhitors Hoe 704 and IpOHA:

- the first one concerns the prodrug approach,
- the second one deals with the variation of the chelation ring size to fit in the catalytic site,
- the third chapter is about HPPD inspired compounds as triketones which are well-known chelating herbicides,
- the fourth one focuses on the bioisosterism principle to design new drugs,
- finally the last chapter treats the synthesis of uracil derivative compounds and hydroxamic moiety bearing molecules as chelating units.

Chapter I: Phosphonates and phosphinates for medicinal chemistry and agrochemistry

I. Historical aspects

1) Discoveries of phosphorus-containing compounds in biology

Phosphorus is an important constituent of living beings starting from unicellular species to more complex entities as plants, insects or animals. To be specific, a human body incorporates more than 450 g of phosphorus element incorporated in biomolecules that are essential to various metabolic pathways. Two major biological structures have been discovered in the XVIIIth and XIXth centuries and revealed to be composed partially of phosphorus.

Phospholipids were discovered by the French chemist and pharmacist Theodore Gobley in 1847. It is a class of lipids that are a major component of all cell membranes as they can form bilayers. Schematically they can be viewed as a hydrophilic head with a phosphate moiety on which are branched hydrophobic tails, generally fatty acids as shown in Figure 4.

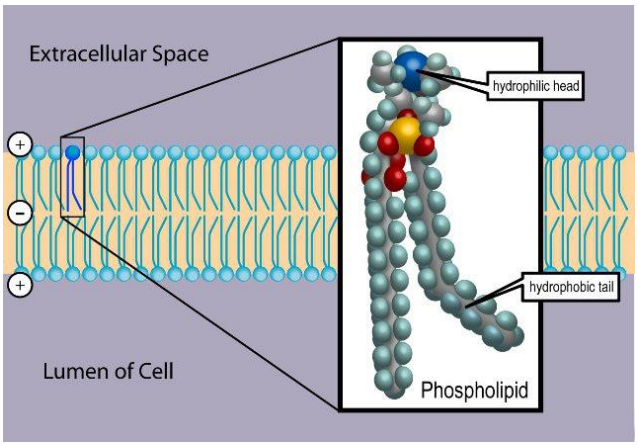


Figure 4: general structure of phospholipid bilayers (File:Phospholipid TvanBrussel.jpg)

The hydrophilicity comes from the negatively charged oxygen on the phosphorus atom and the possibility to form hydrogen bonds with water molecules aside. Moreover according to the VSEPR theory, phosphates have a tetrahedral geometry. Thus all the phosphorus-oxygen single bonds are repulsed towards the same side, meaning that the hydrophobic fatty acids on the phosphate are organised more or less as parallel chains leading to this bilayer organisation of the phospholipids in hydrophilic media.

The other natural product containing phosphorus as phosphate groups is well-known as its structure's elucidation is considered as one of the major scientific discovery in biology of the XXth century. Deoxyribonucleic acid's (DNA) structure, presented in Figure 5, was described by the American James Watson and the British Francis Crick in 1953. However the discovery of the molecule is prior to its structure's understanding, as the first mention of DNA dates from 1869 by the Swiss physicist Frederick Miescher. He isolated a high phosphorus-containing substance from white blood cell nuclei.

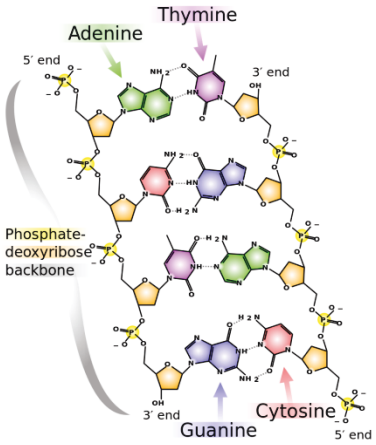


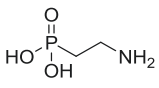
Figure 5: DNA structural organisation

The phosphate groups link the 3' and 5' hydroxyl groups between ribose sugars. This sequence constitutes the sugar-phosphate backbone on which are borne the different nucleobases.

Many more natural products, such as adenosyl triphosphate or nicotinamide adenine dinucleotide phosphate, have been discovered during the XIXth and XXth century. Nevertheless, they all contain phosphate moieties as a phosphorus group.

2) Natural C-P and C-P-C molecules

In 1959, M. Horiguchi and M. Kandatsu managed to isolate a new natural phosphorus compound from rumen Protozoa, 2-aminoethylphosphonic acid **2** also known as ciliatine[2]. The particularity of this molecule is that it doesn't contain a phosphate group but a phosphonic acid group. It was the first time a phosphonic acid bearing natural molecule was described. The biological pathways leading to natural phosphonates and their metabolism were yet totally unknown and its discovery established a cornerstone to future studies on this class of molecules.



2

Although scientists have discovered until recently the presence of C-P containing compounds, nature has long recognised their value as demonstrated by Figure 6. The timeline shows the array of small natural phosphonates and phosphinates extracted and purified since the 60s.[3]

[2] M. Horiguchi, M. Kandatsu, *Nature*, **1959**, *184*, 901-902
[3] K-S. Ju, J. R. Doroghazi, W. W. Metcalf, *J. Ind. Microbiol. Biotechnol.*, **2014**, *41*, 345-356

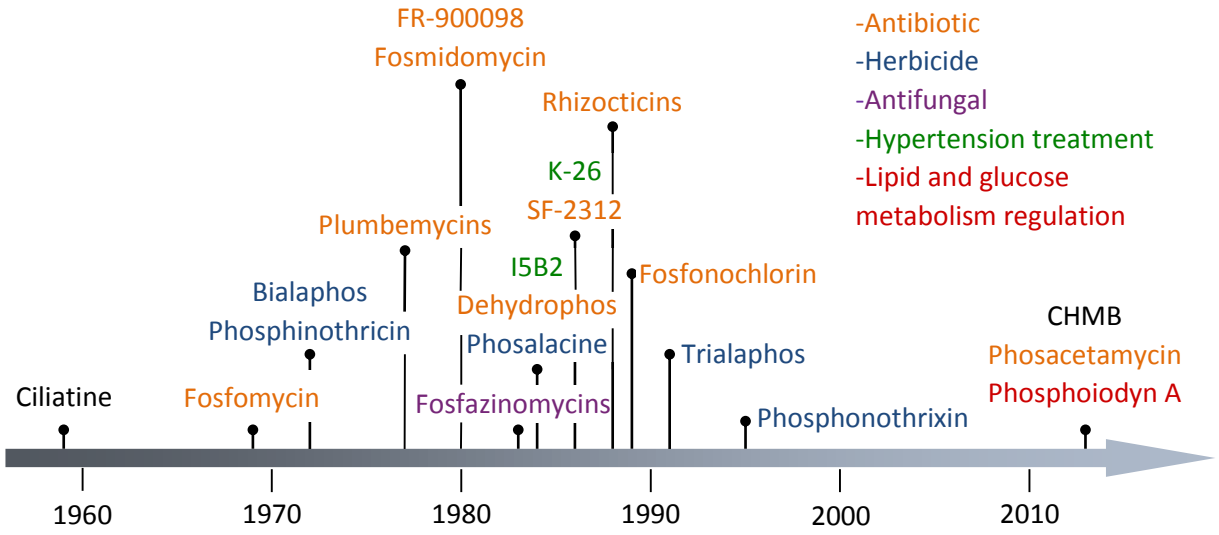
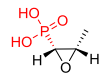
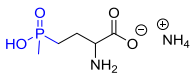
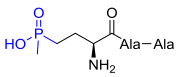
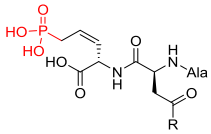
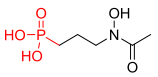
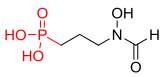
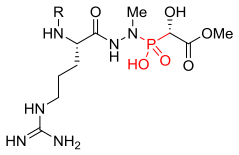
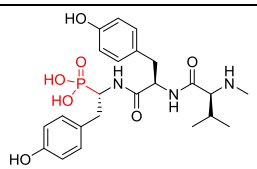
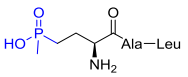
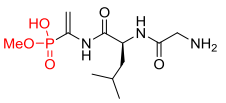
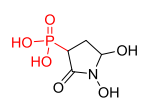
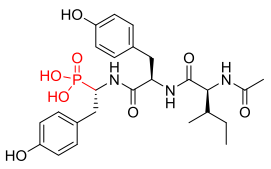
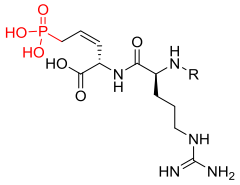
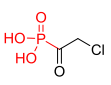
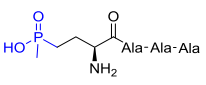


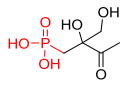
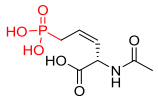
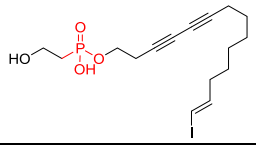
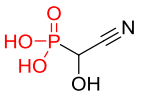
Figure 6: C-P compounds dates of discoveries[3]

Moreover these molecules show some fascinating bioactivities ranging from antibiotics to herbicides and include antifungals, antihypertensive or metabolism regulators. Their structures are presented in Table 1.

Table 1: structures of the discovered natural bioactive phosphonates and phosphinates

				
Fosfomycin	Phosphinothricin	Bialaphos	Plumbemycins	FR-900098
Antibiotic	Herbicide	Herbicide	Antibiotic	Antibiotic
				
Fosmidomycin	Fosfazinomycins	I5B2	Phosalacine	Dehydrophos
Antibiotic	Antifungal	Antihypertensive	Herbicide	Antibiotic

				
SF-2312	K-26	Rhizocticin	Fosfonochlorin	Trialaphos
Antibiotic	Antihypertensive	Antibiotic	Antibiotic	Herbicide

			
(±)-Phosphonothrixin	Phosacetamycin	Phosphoiodyn A	CHMB
Herbicide	Antibiotic	Metabolism regulation	

Many small phosphonates or phosphinates may have been missed during screenings because of the bioassays being used. Most phosphonate natural products are highly hydrophilic, and thus, are not enough soluble in organic partitioning solvents. This makes them less likely to be extracted during the usual protocols for purification and characterisation of molecules present in crude microbial or plant extracts. It raises a key question that needs to be answered in order to find new biological targets for medicine or agriculture: how many different natural products remain to be discovered?

II. Biological pathway

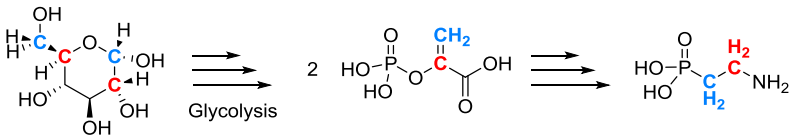
1) Biosynthesis of 2-aminoethanephosphonic acid 2 and bialaphos

The enthusiasm for the discovery of natural phosphonates and phosphinates and the use in medicine or agriculture have led spontaneously scientists to study and understand the biological pathways at stake.

Logically the first molecule to have its biosynthesis studied is 2-aminoethylphosphonic acid **2**. At the end of the 60s, Dr Horiguchi *et al.*, published a paper on the putative bio precursors of **2** thanks to the ^{14}C incorporation technique[4]. They used different ^{14}C-labelled compounds as metabolic sources of at least one carbon unit. Uniformly ^{14}C-labelled glucose, [U-^{14}C]glucose and [3-^{14}C]pyruvate showed interesting percentage of incorporated activity and an equal distribution of radioactivity on **2**, between C-1 and C-2. Thus, they appeared to be good precursors to ciliatine **2**. Pyruvate is the result of degradation of glucose **3** and they both intervene in the same metabolic pathway called glycolysis. The only intermediate that would introduce both carbon atoms and the phosphorus moiety would be phosphoenolpyruvate **4**.

The authors also demonstrated with located ^{14}C-labelling that the **C-2** from glucose was found at **C-2** on **2** and **C-3** from phosphoenolpyruvate **4** gave **C-1** on **2**. As glycolysis yields two molecules of **4** starting from one **3** and the carbon atoms have been traced during all the biological process, it is possible to attribute the carbons from **2** to the ones on **3** and **4** as described in Scheme 1.

[4] M. Horiguchi, J. S. Kitterdge, E. Roberts, *Biochim. Biophys. Acta*, **1968**, *165*, 164-166

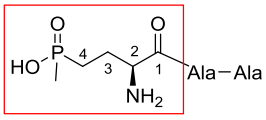


3 **4** **2**

Scheme 1: Summary of the biosynthesis of ciliatine 2

2) Common biological pathway for C-P biomolecules

In 1982, H. Seto *et al.*[5], studied the biosynthesis of bialaphos, purified from *Streptomyces hygroscopicus*, by incorporation of ^{13}C- and ^{2}H-labelled precursors. Bialaphos derives from phosphinothricin with two alanine residues. They considered only the phosphinothricin moiety as the main subject of interest.



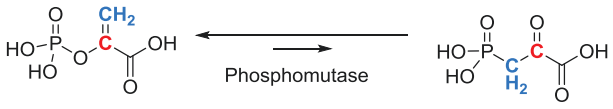
Bialaphos and Phosphionothricin

As it looks like an amino acid with four carbon atoms, the biosynthetic studies were initiated by incorporating ^{13}C-labelled related amino acids such as methionine, aspartic acid or glutamic acid to the fermentation medium. However no radiolabelled atom addition was observed in bialaphos.

Another postulate was experimented by using sodium [1,2-$^{13}C_2$]-acetate. Through the incorporation of the labelled-acetate in the acetyl-CoA pathway, the biologically produced bialaphos exhibited some ^{13}C-^{13}C coupling of C-1 and C-2 by ^{13}C NMR analysis. It provided an undeniable proof that these two carbon atoms are furnished by acetic acid. Concerning the rest of the molecule, they suggested that the CH_2-CH_2-P moiety came from glucose **3** as was postulated for 2-aminoethanephosphonic acid **4** in 1968. It was confirmed by the use of [U-$^{13}C_6$]-glucose during the bacterial fermentation. The coupling between ^{13}C-3 and ^{13}C-4 in ^{13}C NMR, undoubtedly confirmed the incorporation of the phosphorus and carbon atoms sequence from glucose molecules, thanks to the phosphoenolpyruvate biological pathway. Later, the methyl group borne by the phosphorus atom was proven to come from methionine after an experiment with CD_3-methionine.

The incorporation of **3** for the CH_2-CH_2-P sequence of other natural C-P compounds was studied and has been proven right, especially for fosfomycin. Consequently there may be a common biosynthetic pathway starting from phosphoenolpyruvate **4** leading to the formation of C-P compounds in some living organisms. Indeed in the late 80s a publication[6] demonstrated the intervention of an enzymatic step for the transposition of the phosphate **4** to the corresponding phosphonate, Scheme 2.

[5] H. Seto, S. Imai, T. Tsuruoka, A. Satoh, M. Kojima, S. Inouye, T. Sasaki, N. Ōtake, *J. Antibiot.*, **1982**, *35*, 1719-1721
[6] E. Bowman, M. McQueney, R. J. Barry, D. Dunaway-Mariano, *J. Am. Chem. Soc.*, **1988**, *110*, 5575-5576



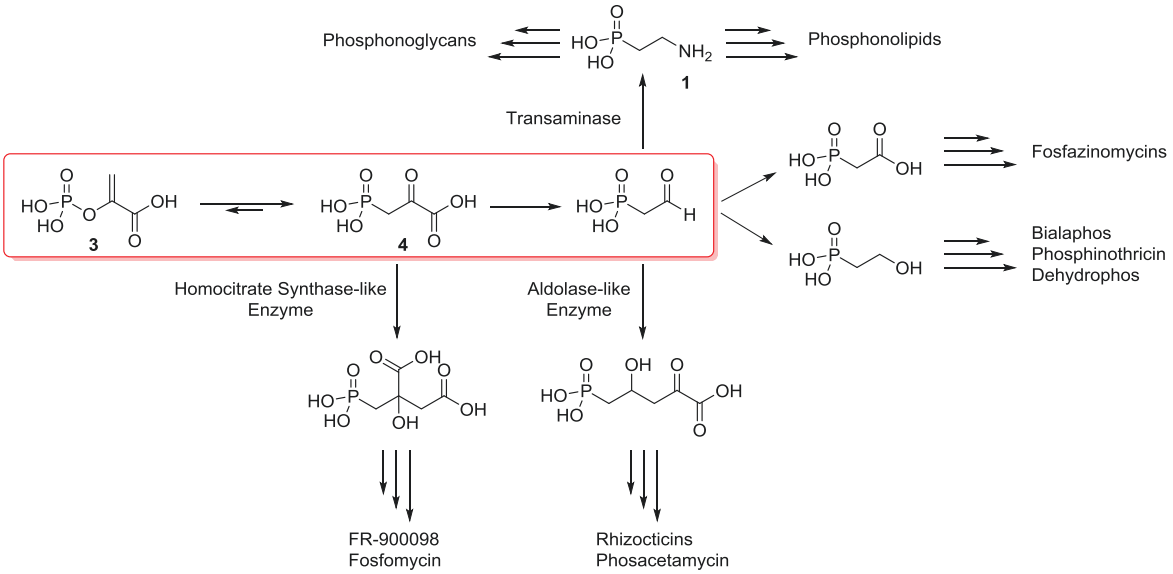
4 **5**

Scheme 2: phosphate to phosphonate enzymatic catalysed transposition

The phosphomutase *PepM* was extracted and purified from *Tetrahymena pyriformis* and catalyses the migration of the phosphorus moiety from the oxygen of the enol on the terminal chain carbon in presence of Mg^{2+}.

Surprisingly when P-O bonds and P-C bond energies are compared, the values range from 10-17 $kcal.mol^{-1}$ in favour of the P-O bond, thus this enzymatic step goes in opposition to the thermodynamic laws explaining why the equilibrium between **4** and **5** is far in favour of the formation of the phosphate **4**. This observation may have contributed to the difficulty of explaining the natural formation of phosphonates and discovering this pathway.

The enzyme has then been purified from other bacteria, thus proving the initial idea of a common enzymatic pathway from phosphoenolpyruvate **4** to synthesise phosphonates and phosphinates as summarised in Scheme 3.



Scheme 3: Common steps in the biological pathway of phosphonates and phosphinates

III. Interest of C-P and C-P-C molecules in agrochemistry and pharmacochemistry

1) Natural abundance

Early studies to discover the source of environmental phosphonates relied on chemical analysis of entire organisms or tissue samples which present a major drawback by limiting the scope. Indeed, the limitation comes from the inability to sample a significant fraction of the organisms present in any given environment. Moreover, the presence of phosphonates was determined by ^{31}P NMR which consists in searching for chemical shifts corresponding to C-P compounds between +5 to +45 ppm. However some cyclic phosphate esters have chemical shifts belonging to the range given above and this technique proves to be not enough sensitive for compounds present at a low abundance.

Another approach emerged from the fact that natural phosphonates and phosphinates are derived from phosphoenolpyruvate **4**, which is the substrate of the enzyme *PepM*[7]. The gene encoding this specific peptide belongs to a gene cluster comprising other genes coding for enzymes playing a role in phosphonates and phosphinates synthesis. As the biosynthesis of many P-C and C-P-C compounds have been understood and the enzymes involved have been genetically characterised, it opens a door to gene-based methods for assessing the abundance and identify the biological producers of these types of molecules.

PepM is a member of the large isocitrate lyase superfamily. Starting from the phosphomutases discovered in different microbes, in which phosphonates and phosphinates have been encountered, a highly conserved catalytic amino acids motif stands out. The amino acid sequence is highly specific from phosphomutase and distinguishes *PepM* from the other enzymes of the superfamily. Different databases of genomes and mutagenomes have been screened for *PepM*-coding genes homologs. The comparisons indicate that phosphomutase coding genes occur in a mean of 7.6 % of the genomes studied, considering that one database includes more than ten thousands of entries from microorganisms living in several environments. Moreover, many gene clusters containing the phosphomutase analogues, also include genes coding for yet unknown proteins, meaning unknown phosphonate and phosphinate compounds **3**. Thus, after screening for microorganisms synthesising natural P-C and C-P-C molecules, in-depth studies have to be carried out in order to discover and understand the function of new enzymes participating in the metabolic pathways of phosphonates and phosphinates.

The enzymes may be probable new biological targets in order to trigger interesting effects. It may be either inhibiting or activating them with new compounds for agrochemistry and/or pharmacochemistry. Moreover the unidentified natural phosphonates and phosphinates may also be a wide pool of potential new drugs.

[7] X. Yu, J. R. Doroghazi, S. C. Janga, J. K. Zhang, B. Circello, B. M. Griffin, D. P. Labeda, W. W. Metcalf, *PNAS*, **2013**, *110*, 20759-20764

2) Bioisosteres

Phosphonates and phosphinates have long been used as bioisosteres of phosphate and carboxylate groups.[8,9] Bioisosterism is a concept derived from isosterism which was introduced in 1919 by Langmuir.[10] He noticed that atoms or small molecules having the same number of electrons have very similar physical properties as illustrated below with a comparison between N_2O and CO_2 (**Table 2**). These two molecules hold both 22 electrons in their orbitals.

Table 2 Experimental data from Landolt-Börnstein used by Langmuir

Properties	N_2O	CO_2
Critical pressure (atm)	75	77
Critical temperature (°C)	35.4	31.9
Viscosity at 20°C ($m^2.s^{-1}$)	148×10^{-6}	148×10^{-6}
Heat conductivity at 100°C ($W.m^{-1}.K^{-1}$)	0.0506	0.0506

The concept has been extended by H. G. Grimm with his law of hydride displacement.[11] He introduced the idea of pseudo atoms. It stands on the idea that an addition of hydrogen to an atom will result with similar properties to the atom of the next highest atomic number. For example, NH is isosteric with O.

Then, appeared the notion of bioisosterism with Erlenmeyer.[12] It includes the pseudoatom rule, the fact that all atoms from the same column in the periodic table are all isosters from each other and the ring equivalence (for example benzene and thiophene are considered as equivalent). The last point was critical to the relevance of medicinal chemistry. Medicinal chemistry as well as agrochemistry excessively introduces bioisosteres in bioactive molecule structures, to achieve a better activity and/or selectivity on a target.

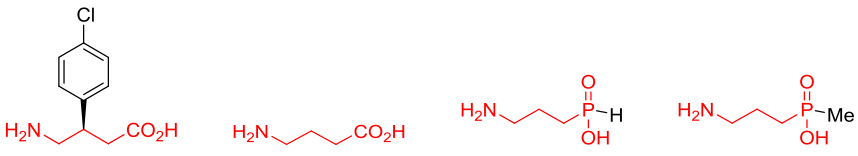
Nowadays, other parameters emerged to define the bioisosteric link between two chemical groups. The chemical proprieties, such as pKas, log P or geometry, should be in the same range. The interest in finding bioisosteres of phosphates and carboxylates is motivated by the fact that numerous regulatory events are controlled by phosphorylation (phosphate chemistry) and proteolysis (carboxylate chemistry) and they may be subject to interferences by C-P compounds.

Carboxylic acids have pKa range from 1 to 5 in water so at physiological pH ≈ 7, they are deprotonated to give the negatively charged corresponding moieties with a delocalisation due to resonance. Their geometry is planar because of the sp^2 hybridation carbon and they can act as a hydrogen bond accepting group. Phosphonates and phosphinates meet some of these criteria. Generally their pKas are more or less in the same range as carboxylic acids, from 2 to 8 for alkyl phosphonic acids and from 1 to 3 concerning phosphinic acids. Thus by modifying the substituents on the phosphorus atom, it

[8] Phosphate bioisosteres: T. S. Elliott, A. Slowney, Y. Ye, S. J. Conway, *Med. Chem. Commun.*, **2012**, *3*, 735-751
[9] Example of phosphonates as carboxylate bioisosteres: J-J. Shie, J-M., Fang, S-Y. Wang, K-C. Tsai, Y-S. Cheng, A-S. Yang, S-C. Hsiao, C-Y. Su, C-H. Wong., *J. Am. Chem. Soc.*, **2007**, *129*, 11892-11893
[10] I. Langmuir, *J. Am. Chem. Soc.*, **1919**, *41*, 1543-1559
[11] H. Grimm, *Z. Elektrochem.*, **1925**, *31*, 474-580
[12] H. Erlenmeyer, M. Leo, *Helv. Chem. Acta*, **1932**, *15*, 1171-1186

may be possible to increase the pKa meaning having a hydrogen acceptor and donor at the same time at physiological pH.

As illustrated in the search for $GABA_B$ agonists, the replacement of the carboxylic acid group by differently substituted phosphinic acids resulted in an important increase of the affinity of the substrate towards the enzyme.[13] $GABA_B$ receptors are activated by an antispastic and muscle relaxant agent (*R*)-baclofen **6**. The authors chose to study the inhibition of binding of baclofen to the enzyme by the natural substrate GABA **7** and phosphinic acid analogues CGP27492 **8** and CGP35024 **9**.



6 **7** **8** **9**

The results are expressed in IC_{50}, corresponding here to the concentration at which 50% of **6** is displaced from the enzyme. The lower the IC_{50}, the better the affinity to $GABA_B$.

Table 3: IC_{50} of GABA and phosphinic acids for $GABA_B$

Agonists	IC_{50} (pM)
GABA (natural substrate)	25
8	2.4
9	6.6

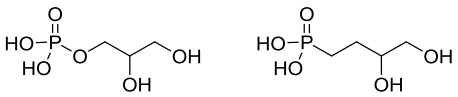
As shown in Table 3 phosphinic acids **8** and **9** are 4 to 10 times more potent agonists than GABA itself. It demonstrates the efficiency in some cases to replace carboxylic acids groups by their corresponding bioisosteric phosphonates or phosphinates.

Carboxylic acids are not the only functional group that phosphonates or phosphinates can replace, phosphates too can be changed to P-C or C-P-C compounds.[14] The C-P bond is very stable towards classic enzymatic hydrolysis compared to phosphate esters. The use of phosphonic or phosphinic analogues may enhance the lifetime and therefore the integrated activity of the drug. Moreover, both classes of compounds have the same range of pKa, whether it is the first acidity or the second one. The other important property is the log P constant which is very similar between carboxylic acids containing molecules and their phosphonic acid or phosphinic acid analogues.[15]

[13] W. Froestl, S. J. Mickel, R. G. Hall, G. von Sprecher, D. Struch, P. A. Baumann, F. Bruger, C. Gentsch, J. Jaekel, H-R. Olpe, G. Rihs, A. Vassout, P. Waldmeier, H. Bittiger, *J. Med. Chem.*, **1995**, *38*, 3297-3312
[14] R. Engel, *Chem. Rev.*, **1976**, *77*, 349-367
[15] R. G. Franz, *AAPS Pharmsci.*, **2001**, *3*, 1-13

However if a phosphate group is to be replaced by a phosphonate or a phosphinate, the global size and geometry of the molecule vary. The distances between the phosphoryl oxygen and other possible interaction sites are significantly changed. This could result in variations of the physiological activity.

The inhibition of glycerol-3-phosphate acyltransferase was studied by using phosphonate analogues.[16] The enzyme catalyses the first step of the phosphoglyceride biosynthesis. One of the most potent inhibitor tested is the direct bioisostere of the enzyme substrate, glycerol-3-phosphate **10** which has been replaced by the corresponding methylenephosphonate in 3,4-dihydroxybutylphosphonate **11**, in order to conserve the molecule size and geometry.



10 **11**

Phosphonate **11** is a competitive inhibitor of **10** with a Ki = 1.80 mM demonstrating that phosphonates can readily replace phosphates and exhibit biological activity.

3) Mimics of tetrahedral enzymatic intermediates

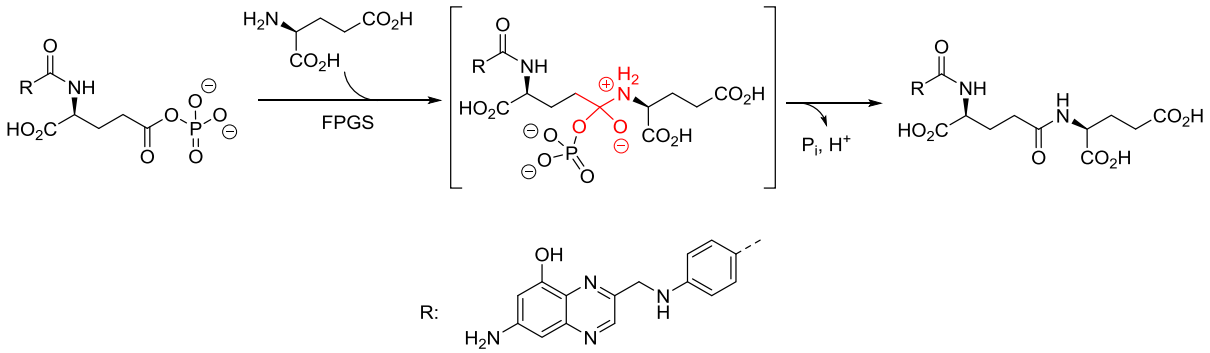
According to the VSEPR theory, phosphonates and phosphinates both have a tetrahedral geometry which can be an interesting point to design tetrahedral intermediates of enzymatic reaction involving a nucleophilic attack on a carbonyl.[17]

This rational design has been exploited especially to synthesise new inhibitors of the human folylpolyglutamate synthetase (FPGS).[18] The enzyme catalyses the ligation of glutamate moieties to folate and folate derivatives as detailed below in Scheme 4.

[16] T. Stein, R. Engel, B. E. Tropp, *Biochim. Biophys. Acta*, **1992**, *1123*, 249-256

[17] For a review about transition state analogues including phosphonates and phosphinates see : A. Mucha, P. Kafarski, Ł. Berlicki, *J. Med. Chem.*, **2011**, *54*, 5955-5980

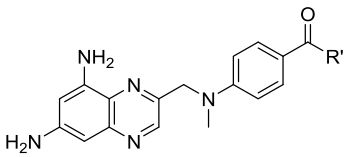
[18] T. Tsukamoto, W. H. Haile, J. McGuire, J. K. Coward, *Arch. Biochem. Biophys.*, **1998**, *355*, 109-118

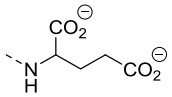
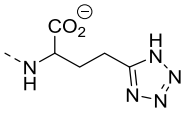
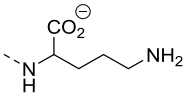
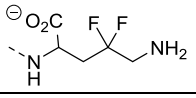
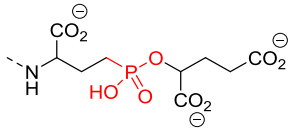


Scheme 4: Mechanism of FPGS

In order to mimic the tetrahedral intermediate in bracket, the tetravalent carbon in red (in the transition state intermediate above) has been replaced by a phosphonate which conserves the spatial organisation around this specific carbon. Analogues of methotrexate, MTX, an antitumor deriving from folic acid, have been synthesised and biologically tested to determine their respective IC_{50} described in Table 4.

Table 4: IC_{50} of different inhibitors of FPGS



Name	R'	IC_{50} (µM)
Glu (MTX)		49
γ-tetrazoyl-Glu		51
Orn		3.2
4,4-F_2Orn		>150
Glu-γ-[ψ-{P(O)(OH)-O}]glutarate		0.12

Glu-γ-[ψ-{P(O)(OH)-O}]glutarate was identified as the most potent inhibitor of FPGS at the end of the 90s proving that emulating a tetrahedral intermediate with a phosphonate or phosphinate is an efficient and interesting way in designing new drugs.

Many factors draw interest in the increasing introduction of phosphonates and phosphinates in agrochemistry and pharmacochemistry. On one hand, a great variety of natural P-C and C-P-C compounds still remain to be discovered along with their corresponding enzymes synthesising them in order to propose new biological targets and drugs. On the other hand, rational design and structure activity relationship may take advantage of the replacement of carboxylates or phosphates by phosphonates and phosphinates to develop more efficient bioactive molecules.

Chapter II: Biosynthesis of branched-chain amino acids

I. Branched-chain amino acids pathway

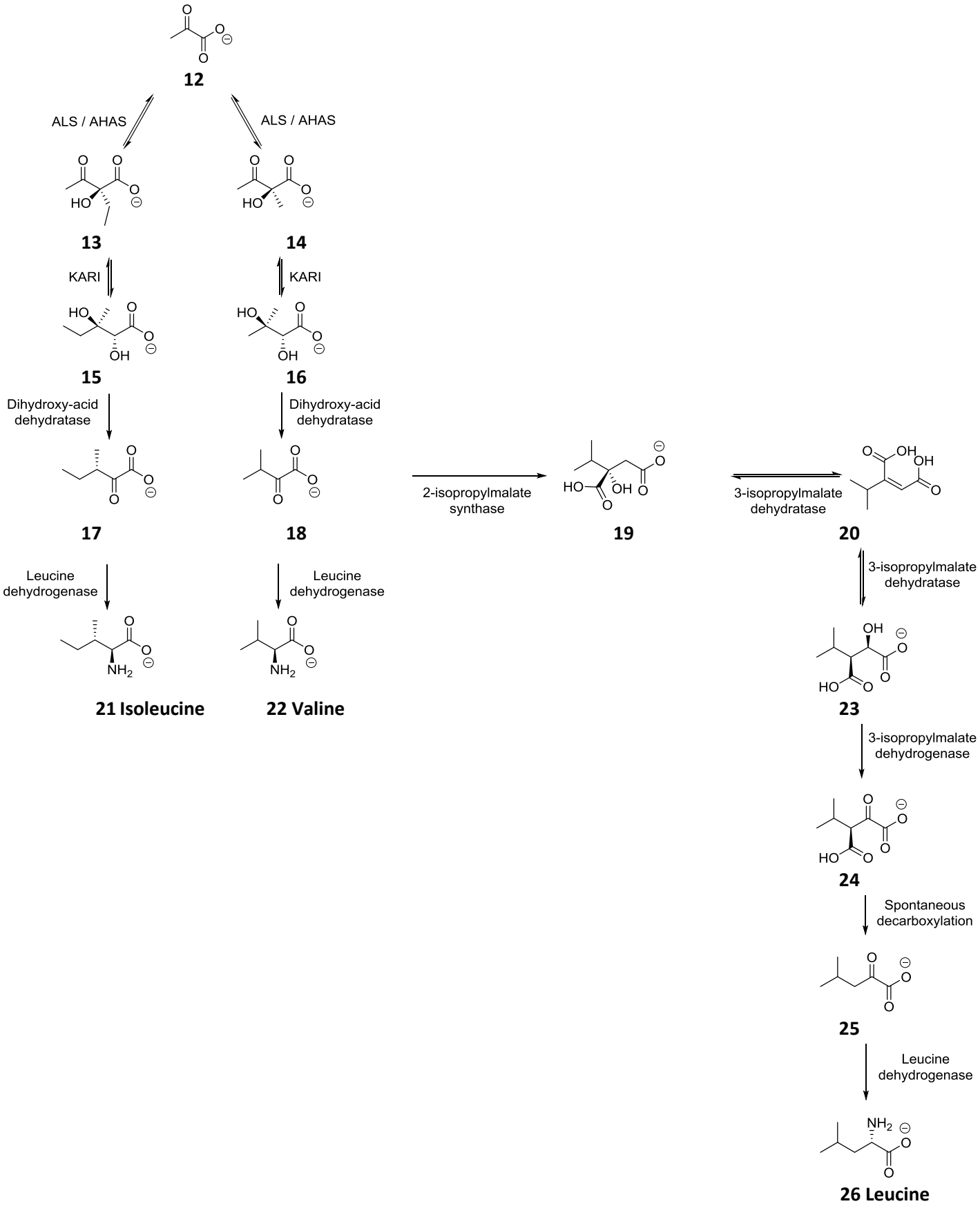
The branched-chain amino acids (BCAA) pathway is a cornerstone for many living organisms. Only fungi, algae, bacteria and plants have the ability to synthesise valine **22**, leucine **26** and isoleucine **21** which are also known as essential amino acids.[19] Animals and insects totally depend on their micro-organisms and plants intakes in order to metabolise these essential amino acids into their own biosynthetic pathways.

The BCAA pathway involves a large number of enzymes and starts from pyruvate **12**, as shown in Scheme 5.

The acetolactate synthase (ALS), also referred to as the acetohydroxy synthase (AHAS), catalyses the condensation of one 2-oxopropanoate **12** and 2-ketobutyrate or two molecules of **12**, both accompanied with a loss of carbon dioxide. It either leads to (*S*)-2-aceto-2-hydroxybutyrate **13** or (*S*)-2-acetolactate **14**. Afterwards, they undergo a double-reaction step catalysed by ketol-acid reductoisomerase (KARI). It consists in a 1,2-anionic alkyl migration followed by a reduction of the ketone newly created, resulting in (*R*)-2,3-dihydroxy-3-methypentanoate **15** and (*R*)-2,3-dihydroxy-3-methylbutanoate **16**. Both vicinal diols **15** and **16** are dehydrated into (*S*)-3-methyl-2-oxopentanoate **17** and 2-oxoisovalerate **18** by dihydroxyacid dehydratase. The last step to the amino acids **21** and **22** is catalysed by leucine dehydrogenase through a reductive amination reaction of the ketone borne by **17** and **18** with the transfer of an amino group from a glutamate molecule.

The synthesis of leucine **26** differs from the two other amino acids and brings 5 more enzymes into play. (*S*)-2-isopropylmalate **19** is synthesised from **18**, thanks to 2-isopropylmalate synthase, which transfers a carboxylic acid group on the ketone. Then 3-isopropylmalate dehydratase isomerises **19** by formally transferring the hydroxyl moiety from the third position to the second position on the longest alkyl chain giving (2*R*,3*S*)-3-isopropylmalate **23** with 2-isopropylmaleate **20** as an intermediate. The transferred alcohol group is oxidised into a ketone by 3-isopropylmalate dehydrogenase. It is followed by a spontaneous decarboxylation reaction of (*S*)-2-isopropyl-3-oxosuccinate **24** formed previously, in order to obtain 4-methyl-2-oxopentanoate **25**. The last step of the leucine **26** synthesis is common to isoleucine **21** and valine **22**.

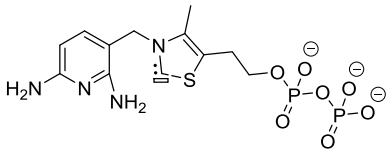
[19] S. Pang, R. Duggleby, *J. Biochem. Mol. Biol.*, **2000**, *33*, 1-36



Scheme 5: Branched-chain amino acids pathway

II. Mechanism of AHAS/ALS

AHAS and ALS, catalysing the first step of the BCAA pathway, have been studied closely since the end of the 50s[20]. A slight difference exists between AHAS and ALS, the former requires flavine adenine dinucleotide (FAD) as a cofactor unlike ALS. The use of FAD has not been understood yet as no oxidation nor reduction occur during the reaction; it explains why AHAS and ALS are generally referred to as the same enzyme. They both require thiamine diphosphate (ThDP) **27** and a divalent metal ion, usually Mg^{2+}, as cofactors. The magnesium ion helps anchoring ThPD to the protein by coordinating the diphosphate group and some amino acids of the protein chain.

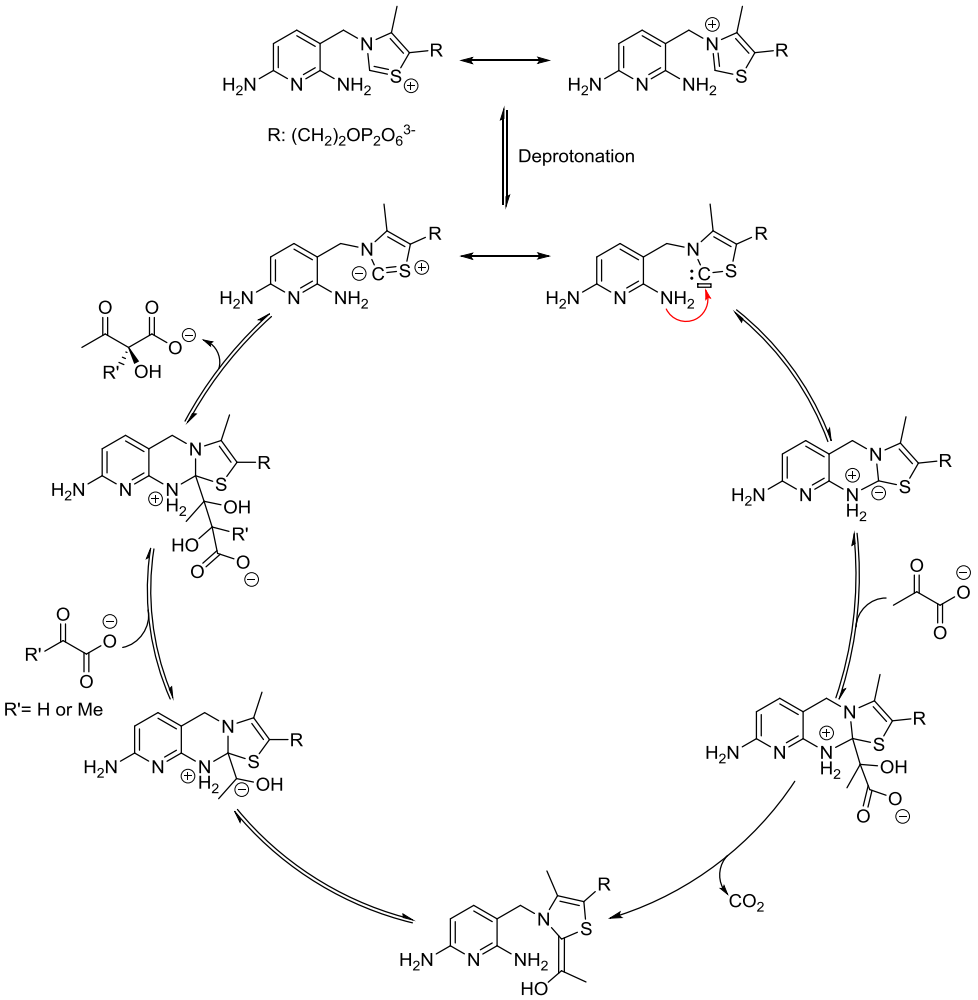


27

The following scheme, Scheme 6 shows the mechanism of AHAS reaction with ThPD.[21] The first step involves the deprotonation of the 2-carbon of the thiazolium ring of ThPD to form an ylide which is in equilibrium with the carbene mesomeric form. The nitrogen atom from the diaminopyridine and the 2-carbon are in the same plane, therefore the amine may participate in the stabilisation of the carbene species. The ionisation is followed by the addition of the 2-carbanion to the carbonyl group of the pyruvate with a loss of carbon dioxide. The enamine is converted to the corresponding carbanion and then either reacts with another molecule of pyruvate (R'= H) or with 2-oxobutyrate (R'= Me) to give acetolactate **14** or acetohydrobutyrate **13** respectively.

[20] Y. S. Halpern, H. E. Umbarger, *J. Biol. Chem.*, **1959**, *234*, 3067-3071

[21] S. Pang, R. Duggleby, R. Schowen, L. Guddat, *J. Biol. Chem.*, **2004**, *279*, 2242-2253



Scheme 6: AHAS mechanism

III. Inhibition of AHAS/ALS

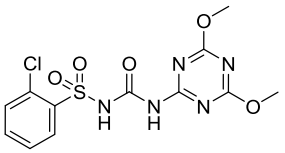
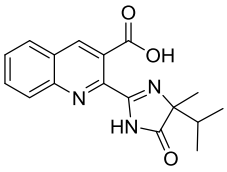
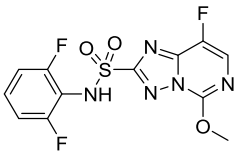
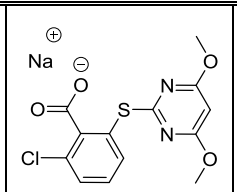
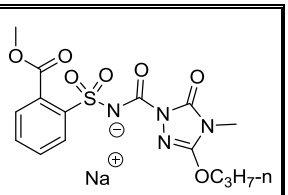
The enzyme gathered more attention on its active site when some small synthetic organic molecules have been discovered to be inhibiting its activity. Agrochemical companies have therefore developed herbicides since the last 30 years. AHAS/ALS inhibitors have proven to be the most used herbicides with more than fifty compounds commercialised. Indeed, they require low dosage, present low mammalian toxicity and are effective on a wide variety of crops. Five main classes have been discovered thanks to a screening strategy as shown in Table 5. The first commercial example is Glean® (Chlorsulfuron **28**) synthesised by DuPont and introduced on the market in 1982.[22] It belongs to the sulfonylurea family. Almost simultaneously, American Cyanamid discovered a structurally distinct family of inhibitors with the imidazolinones. The leading compound being Imazaquin **29**.[23]

[22] P. B. Sweetser, G. S. Schow, J. M. Hutchison, *Pestic. Biochem. Physiol.*, **1982**, *17*, 18-23
[23] D. Shaner, P. Anderson, M. Stadham, *Plant Physiol.*, **1984**, *76*, 545-546

Since then, three additional classes have been introduced including:

- Triazolopyrimidines with Florasulam **30** from the Dow Company,
- Pyrimidinyl(thio)benzoates with Pyrithiobac-sodium **31** developed by Kumiai
- Sulfonylaminocarbonyltriazolinones with Attribut® **32** from Bayer CropScience.

Table 5: IC_{50} of some inhibitors of AHAS/ALS

AHAS/ALS Inhibitor examples					
	28	**29**	**30**	**31**	**32**
IC_{50} / K_i	IC_{50}= 18.5 – 35.9 nM K_i= 54.6 nM	IC_{50} = 0.1 – 10 µM K_i= 3 µM	IC_{50} = 0.6 nM	IC_{50} = 32.4 nM	No data available

Schloss *et al.* explained in 1988 the origin of the inhibition of AHAS/ALS by some new classes of herbicides.[24] The aim of the study was to understand why the three main classes of herbicides inhibiting AHAS/ALS were so effective even if they do not share any similarities with the substrates (pyruvate or 2-oxobutyrate), the cofactors (FAD, ThPD and magnesium) or allosteric effectors (**21**, **22** and **26**). The explanation for the anomalous sensitivity of the enzyme to the herbicides mentioned above came with the discovery of the DNA sequence similarities between different genes. The gene coding for pyruvate oxidase, *poxB*, is comparable to *ilvB*, *ilvG* and *ilvI*, all coding for the large subunits of the major isozymes of ALS in bacteria, as well as the genes from yeast and higher plants. Pyruvate oxidase binds *in vivo* an additional cofactor in a special pocket, ubiquinone-40. There are some surprising interaction parallels between the binding of ubiquinone-40 in pyruvate oxidase and that of herbicides with AHAS/ALS. It suggests the herbicides don't bind in the active site of the enzyme but in a distinct site which might be a vestige from the quinone pocket of *poxB*.

IV. Resistance to herbicides

In 1987, the first example of resistance to **28** was observed in the USA in monoculture cereal-growing areas. The fields had been treated with this sulfonylurea-type herbicide for five years continuously. By 1992 there were numerous examples of weeds that had developed resistance to AHAS/ALS inhibiting herbicides. In 2007, 93 weed species were reported to be resistant to AHAS/ALS inhibitors. The

[24] J. V. Schloss, L. M. Ciskanik, D. E. Dyk, *Nature*, **1988**, *331*, 360-362

development of the resistance resulted from the conjugation of different factors.[25] First AHAS/ALS is the single site of action of the herbicides; moreover the enzyme may mutate in multiple areas on the protein conferring resistance. The semidominant mutated trait is carried on a nuclear gene which is transmitted to the lineage and the mutant enzyme can possess full catalytic activity. All these circumstances can lead to resistant weeds that are fit. The apparition of plants resistant to these herbicides makes attractive the development of new products.

[25]M. Hartnett, C. Chui, C. Mauvais, R. McDevitt, S. Knowlton, J. Smith, S. Falco, B. Mazur., Herbicide – Resistant Plants Carrying Mutated Acetolactate Synthase Genes in *Managing Resistance to Agrochemicals*, Ed. M. Green, H. LeBaron, W. Moberg, Washington DC: American Chemical Society **1990**, chap 31

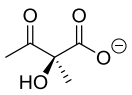
Chapter III: Inhibition of KARI

KetolAcid ReductoIsomerase is the second enzyme intervening in the Branched-Chain Amino-Acids (BCAA) pathway. According to the enzyme classification, KARI (EC 1.1.1.86) belongs to the group of oxidoreductase acting on the CH-OH moiety of an acceptor with nicotinamide adenine dinucleotide (NADH) or nicotinamide adenine dinucleotide phosphate (NADPH) as donor molecules. Surprisingly it is one of the very few enzymes catalysing two very different reactions in a row (not to be mistaken with multistep reactions such as the ones catalysed by proteases). The only other example of reductoisomerase is the 1-Deoxy-d-Xylulose 5-Phosphate Reductoisomerase (DXR) which has also been studied as a biological target for herbicides.[26]

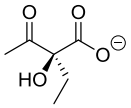
I. Mechanism of action

1) General equation

As mentioned earlier, the natural substrates of KARI are acetolactate **33a** and 2-aceto-2-hydroxybutyrate **33b** with respective Km values[27] of 25 µM and 37 µM in spinach chloroplasts.



33a

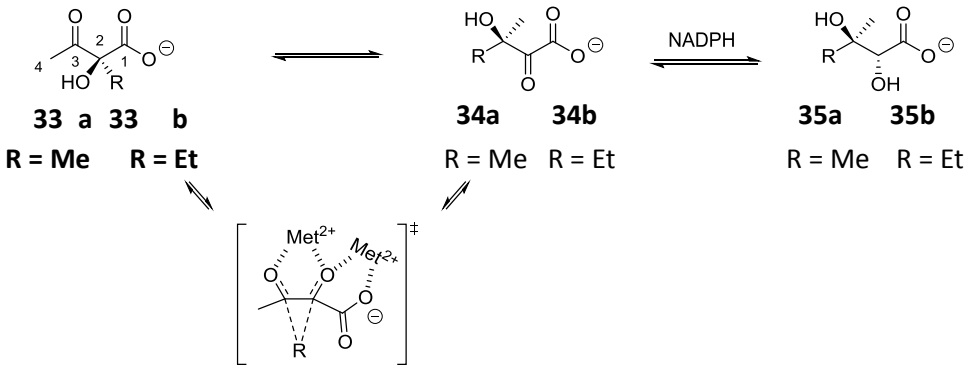


33b

The enzyme catalyses two very different reactions as detailed in Scheme 7 below. At first, the substrates undergo a 1,2-anionic alkyl migration of the R group which leaves a carbonyl on the C-2 position and to an alcohol on the third one. NADPH then reduces the alpha ketone giving the two expected diol products 2,3-dihydroxy-3-methylbutyrate **35a** and 2,3-dihydroxy-3-methylpentanoate **35b**. The metallic cations involved in the active site of the enzyme, act as Lewis acids to promote substrate isomerisation by polarising the ketone, thus inducing a partial positive charge on the carbon. Similarly the coordination of the reaction intermediate by the cations polarises the carbonyl formed on C-2, after the alkyl group transposition, and creates a partial positive charge on the carbon facilitating the hydride transfer from NADPH.

[26] S. Montel, C. Midrier, J-N. Volle, R. Braun, K. Haaf, L. Willms, J-L. Pirat, D. Virieux, *Eur. J. Org. Chem.*, **2012**, 3237-3248

[27] R. Dumas, J. Joyard, R. Douce, *Biochem. J.*, **1989**, *262*, 971-976



33 a 33 b **34a 34b** **35a 35b**

R = Me R = Et R = Me R = Et R = Me R = Et

36

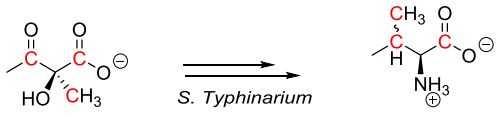
Scheme 7: mechanism of the reactions catalysed by KARI

Armstrong *et al.* reported in 1974 the stereospecificity of the enzyme towards the *(S)*-enantiomers of **33a-b** after studying the activity of the enzyme on a racemic mixture of **33a**.[28] They discovered they had done a chiral resolution of the *(R)* and *(S)* substrates by observing the formation of the diol **35a** from the *(S)*-derivative, the *(R)*-substrate remaining in the medium.

2) Stereospecific [1,2]-sigmatropic rearrangement

In addition to the stereospecificity, KARI catalyses enantioselective reactions. After the first step corresponding to the migration of the alkyl chain, the R group occupies the *pro-R* position on the C-3 position. The enantioselective isomerisation has been explained through a study with ^{13}C-labelled carbon atom and ^{13}C NMR.[29]

A racemic mixture of [1,3,5-$^{13}C_3$]-acetolactate was prepared (^{13}C-labelled carbons are represented in red on the molecule drawings). The enantiomers don't need to be separated from each other as the enzyme only reacts with the (*2S*) one, as shown above. The ^{13}C-labelled acetolactate **33a** is then converted into [1,3,4-$^{13}C_3$]-valine **37** by incubating the substrate with a cell-free system prepared from the bacterium *Salmonella typhinarium* as shown in Scheme 8.



33a **37**

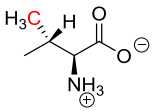
Scheme 8: [1,3,4-$^{13}C_3$]-valine biosynthesis

To determine whether the ^{13}C-labelled methyl group which migrated occupies the (*R*) or (*S*) position, the authors have based their study on a previous one[30] linking the carbons of (*2S,3S*)-[4-^{13}C]-valine **38** to their respective chemical shifts in ^{13}C NMR.

[28] F. Armstrong, C. Hedgehock, J. Reary, D. Whitehouse, D. White, *J. Chem. Soc. Chem. Commun.*, **1974**, *1974,* 351-352

[29] D. Crout, C. Hedgehock, E. Lipscomb, F. Armstrong, *Eur. J. Biochem.*, **1980**, *110*, 439-444

[30] H. Kluender , C. H. Bradley , C. J. Sih , P. Fawcett , E. P. Abraham, *J. Am. Chem. Soc.*, **1973**, *95*, 6149-6150

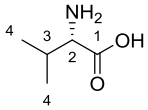


38

(*2S*,*3S*)-[4-^{13}C]-Valine **38** has been prepared in such a way to be enantiomerically pure. After observing the ^{13}C NMR spectrum of the structure, an intense signal stands out at 17.6 ppm, corresponding to the ^{13}C enriched carbon of the methyl group having the (*S*) position. The signal of the unlabelled carbon of the other methyl group appears at 18.8 ppm.

Knowing this information, it is now possible to understand how the alkyl migration occurs. All ^{13}C NMR chemical shifts are reported in Table 6 below in order to demonstrate that the migrating ^{13}C-labelled methyl moiety occupies the (*S*) position on C-3.

Table 6: ^{13}C chemical shift of Valine and ^{13}C-labelled Valine

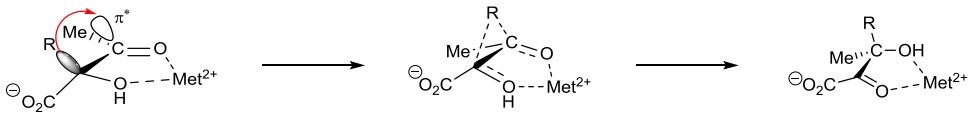


Atom	(±)-Valine		[1,3,4-$^{13}C_3$]-Valine	
	δ (ppm)	Type	δ (ppm)	Type ($^2J_{CC}$, Hz)
C-1	175.1	s	175.1	s
C-2	61.2	s	61.3	s
C-3	29.9	s	30.0	d (34.8)
C-4-*(R)*	18.8	s	18.8	s
C-4-*(S)*	17.6	s	17.6	d (34.8)

s: singulet, d: doublet

As the ^{13}C-4-*(S)* is coupling with C-3 and knowing that the labelled C-4 was at the beginning borne by C-2, the methyl group is formerly migrating on the same face of the molecule also known as a suprafacial migration. The alkyl adds itself on the *re* face of the ketone. The same study has been carried out concerning the ethyl group of **33b**. Therefore according to the Cahn-Ingold-Prelog priority rules, the ^{13}C-4 *(S)* is occupying the *pro-R* position when it is not isotopically labelled.

A mechanism has been therefore postulated which involves a three-centre pericyclic transition state **36** starting from the substrates **33a** or **33b** and leading to the α-ketoacids **34a-b** as detailed in Scheme 9.



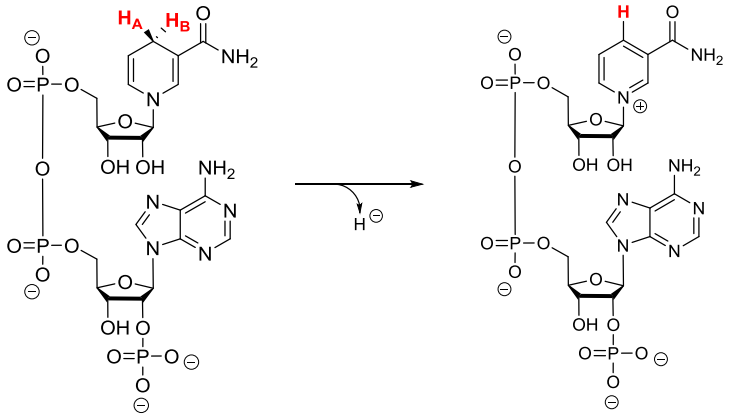
33 **36** **34**

Scheme 9: Suprafacial [1,2]-sigmatropic rearrangement

3) Stereospecific reduction with NADPH

The second step of KARI's catalysis is the reduction of the carbonyl of the α-ketoacids **34a-b** by a hydride transfer from NADPH. This reaction is enantioselective as well, as the migration during the first step.

Once again, the isomeroreductase of *Salmonella typhinurium* was purified and used to understand the conformation of the molecules inside the active site of the enzyme and explain the stereopecificity of the reduction.[31] On the reduced form NADPH, two hydrides may be transferred on the carbonyl as shown on its structure below.



H_A is considered to be the *pro-R* hydrogen, consequently H_B is at the *pro-S* position. The stereospecificity of the isomeroreductase towards the reduction was determined by incubating the α-acetohydroxy acids **34a-b** with stereospecifically ^{3}H-labelled NADPH: NADPH-4A-^{3}H and NADPH-4B-^{3}H, prepared from commercially available NADP$^+$-4-^{3}H after reduction with specific enzymes to obtain the tritium in the wished configuration.

With the ease for radioactivity detection, tritium can be spotted on molecules especially on NADPH or the produced diols **35a-b**. Table 7 shows the percentage of tritium detected either on the reducing agent or the product.

Table 7: stereoselectivity of the reduction with ^{3}H-labelled NADPH

Substrate	Cofactor	Tritium recovered in NADP$^+$	Tritium recovered in product
(*2S*)-acetolactate **33a**	NADPH-4A-^{3}H	100%	0%
(*2S*)-acetolactate **33a**	NADPH-4B-^{3}H	42%	58%
(*2S*)-2-aceto-2-hydroxybutyrate **33b**	NADPH-4A-^{3}H	100%	0%
(*2S*)-2-aceto-2-hydroxybutyrate **33b**	NADPH-4B-^{3}H	54%	46%

[31] S. Arfin, H. E. Umbarger, *J. Biol. Chem.*, **1969**, *244*, 1118-1127

No tritium hydride has been transferred from the 4A-labelled position, all the tritium was recovered in the oxidised form $NADP^+$, after the reduction step. Furthermore the only tritiums found in the diol products **35a-b** were obtained from NADPH-4B-^{3}H. These results established that the hydride transferred to the substrates **33a** and **33b** came specifically from position 4B, *pro-S*, of NADPH regardless from the substrate.

The stereospecificity is confirmed by a modelisation of KARI's active site which has been crystallised with two Mg^{2+}, NADPH and a specific inhibitor, IpOHA as shown in Figure 7

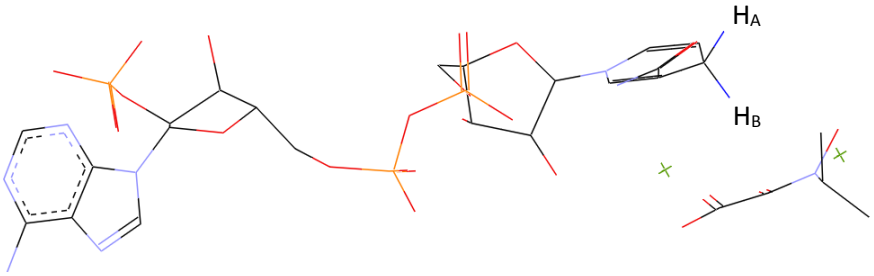


Figure 7: Modelisation of the active site from X-ray structure,[32] considering only Mg^{2+}, NADPH and IpOHA

As the NADPH is located above the substrate which is complexed by the two divalent cations, only the hydrogen H_B can be transferred to the carbonyl which confirms the radio labelled study from Arfin and Umbarger.[31]

4) Cofactors

In order to be effective, KARI absolutely requires two divalent cations and an electron donor species. The natural enzyme purified was crystallised with two magnesium ions and NADPH in its active site. The question that can be asked is why KARI has such an affinity for these cofactors and not other divalent metal ions or reducing agents such as NADH. For plant-descent KARI, it is worth observing the very low K_m values concerning NADPH and Mg^{2+} which are 3.5 µM and 6 µM respectively.

First of all, the surprise comes from the absence of cobalamin which is a typical cofactor involved in alkyl migration. In general adenosylcobalamin or coenzyme B_{12} catalyses unusual rearrangements that may include carbon-containing groups such as in the glutamate mutase for example which transfers a glycine moiety from one side of 2-aminopentanedioic acid to the other side of the carbon chain to yield 3-methylaspartic acid.[33]

[32] V. Biou, R. Dumas, C. Cohen-Addad, R. Douce, D. Job, E. Pebay-Peyroula, *Embo J.*, **1997**, *16*, 3405-3415
[33] E. Marsh, C. Drennan, *Curr. Opin. Chem. Biol.*, **2001**, *5*, 499-505

a) NADPH

Some studies have compared the affinity and the catalytic activity of the wild type IlvC of KARI, extracted and purified from *Escherichia Coli.* Table 8 presents the main enzymatic constants in order to compare NADH and NADPH.[34]

Table 8: enzymatic constants for the wild type IlvC

KARI type	U/mg		K_M [µM]		K_{cat} [s^{-1}]		K_{cat}/K_M ($s^{-1}.M^{-1}$)	
	NADH	NADPH	NADH	NADPH	NADH	NADPH	NADH	NADPH
IlvC	0.08	1.00	1080	40	0.3	3.6	300	87300

The enzyme activities were determined in 250 mM potassium phosphate (pH = 7) with 1 mM dithiothreitol, 200 µM NADPH or NADH, 10 mM of **33a** and 10 mM $MgCl_2$. The enzymatic activity U is defined as the quantity of substrate converted per unit of time. With NADPH, the enzyme activity is 12 times higher than with NADH. Moreover the Michaelis constant, K_M, shows that 27 times more NADH is needed compared to NADPH in order to achieve half of the maximal speed of the enzymatic reaction. It translates a better affinity of the NADPH towards KARI than NADH. The K_{cat} constant also displays a higher turnover with NADPH as a cofactor than with NADH. K_{cat}/K_M presents the global efficiency of the enzyme between NADPH and NADH. The higher the ratio, the more efficient the enzyme.

Globally, all the biochemical constants calculated above are clearly in favour of NADPH which has more affinity with the enzyme and promotes the best enzymatic activity compared to NADH.

b) Metal ion requirement

KARI includes two cationic magnesium in its active site as chelators of the substrate. Some other divalent or trivalent cations had been studied as substitutes for magnesium.

Chunduru *et al.* attempted to substitute Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Ca^{2+} and Co^{3+} for Mg^{2+}.[35] The study was carried out on the isomeroreductase purified from *Escherichia Coli*. NADPH absorbs light at 340 nm, which can be an easy measuring method to view a conformational change of the enzyme in presence of NADPH and **33a** in a standard buffer at pH 8.0. It is clear that a metal ion is required as there was no change in A_{340} when only KARI, NADPH and **33a** were incubated, without any metal. At concentrations up to 10 mM in each metal ion, no change in the A_{340} could be detected when the incubation included the substrate and the other cofactor. However, with **33a** as substrate, Mn^{2+} did behave as an inhibitor with respect to Mg^{2+}. Indeed when the reduction of hydroxyketoisovalerate **34a** was studied, Mn^{2+} was found to be as efficient as Mg^{2+} in catalysing this part of the reaction. The magnesium may be substituted by manganese for the reduction however the alkyl migration is specific to Mg^{2+}.

Another family of KARI enzyme has been studied in order to confirm the results above.[36] This time the enzyme is purified from spinach. The authors followed nearly the same hypothesis as translated in Table 9. Instead of measuring the absorbance they preferred to observe the difference of optical density (OD). Formerly, the OD is the amount of attenuation that occurs when light passes through an optical component.

[34] S. Bastian, X. Liu, J. Meyerowitz, C. Snow, M. Chen, F. Arnold, *Metab. Eng.*, **2011**, *13*, 345-352
[35] S. Chunduru, G. Mrachko, K. Calvo, *Biochemistry*, **1989**, *28*, 486-493
[36] K. Thomazeau, R. Dumas, F. Halgand, E. Forest, R. Douce, V. Biou, *Acta Crystallogr. D*, **2000**, *56*, 389-397

Table 9: Comparison of enzyme activities as a function of metal ions

Cation	ΔOD/min	Theoretical time required to oxidise all of NADPH	Enzyme activity (%)
None	8.7×10^{-4}	34 h	0.057
Mg^{2+}	1.98	54 s	100
Mn^{2+}	3.5×10^{-2}	52 min	2.4
Ni^{2+}	4.5×10^{-4}	67 h	0.023
Zn^{2+}	4.2×10^{-4}	72 h	0.019

Even with a different origin for KARI, the results are consistent with the previous ones presented. Although manganese ion shows some little enzyme activity and is still better than nickel or zinc ion, the magnesium ion is the most specific to both reactions catalysed by the isomeroreductase.

5) Structure of the protein

According to different crystallographic studies,[37] the enzyme is a tetramer, however in the case of a plant extracted KARI, it is a homodimer weighing between 114 and 116 kDa as pictured in Figure 8. Each monomer is composed of two domains corresponding to the N-terminal region and the C-terminal one. The active site is nested at the interface of these domains. The plant KARI will be mainly described, as it is the strain coinciding with our further interest.

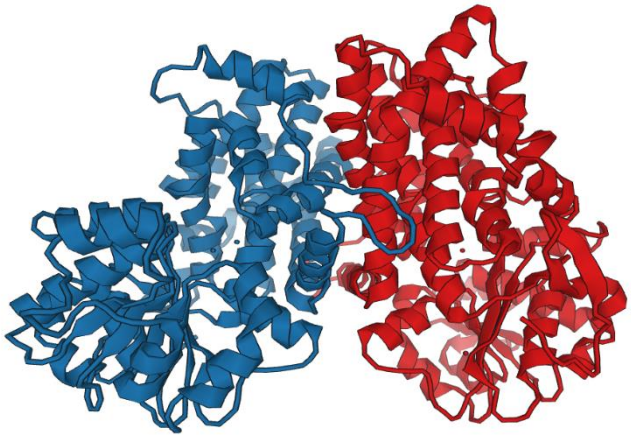


Figure 8: KARI's dimer structure modelisation from japanese rice KARI's X-ray structure

a) Identification of the different regions

In order to identify essential domains or residues of the enzyme that participate in metal and NADPH cofactor binding, the amino acid sequence of the protein purified from *Spinacia oleracea* has been aligned and compared to other known sequences corresponding to enzymes from fungi and bacteria.

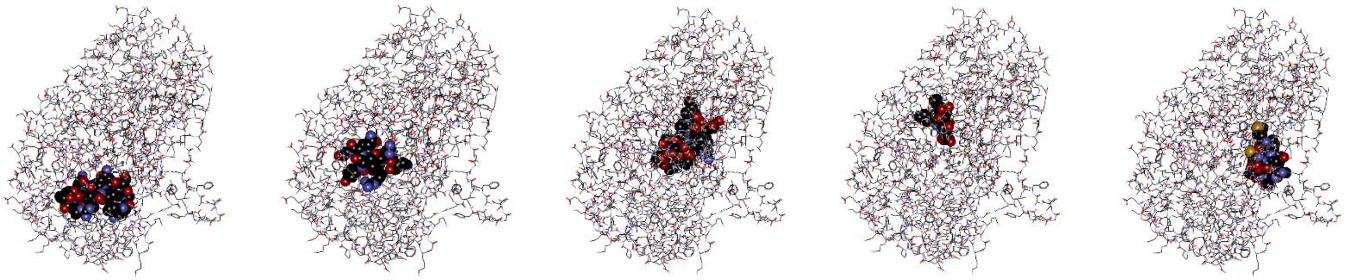
[37] For an example of the plant enzyme crystal, see: R. Dumas, M-C. Butikofer, D. Job, R. Douce, *Biochemistry*, **1995**, *34*, 6026-6036, and for micro-organism crystallographic data: R. Tyagi, S. Duquerroy, J. Nazava, L. W. Guddat, R. G. Duggleby, *Protein Sci.*, **2005**, *14*, 3089-3100

This comparison indicated five regions of similarity that are mentioned as regions I-V presented in Table 10. The numbers in bracket under the region name are the positions of the identical amino acids identified in the protein sequences. Only the similar amino acids are mentioned, the hyphens correspond to varying amino acids.

Table 10: Comparison of peptide sequence from different KARI strains

KARI species	Region I (132-146)	Region II (255-265)	Region III (311-325)	Region IV (492-496)	Region V (513-524)
S. oleracea	G-G-Q---------D	G--VR-----G	E---D--GE---L-G	E---E	M----S-TA--G
A. thaliana	G-G-Q---------D	G--VR-----G	E---D--GE---L-G	E---E	M----S-TA--G
S. cerevisiae	G-G-Q---------D	G--VR-----G	E---D--GE---L-G	E---E	M----S-TA--G
N. crassa	G-G-Q---------D	G--VR-----G	E---D--GE---L-G	E---E	M----S-TA--G
L. lactis	G-G-Q---------D	G--VR-----G	E---D--GE---L-G	E---E	M----S-TA--G
Synechocystis sp.	G-G-Q---------D	G--VR-----G	E---D--GE---L-G	E---E	M----S-TA--G
R. melitoti	G-G-Q---------D	G--VR-----G	E---D--GE---L-G	E---E	M----S-TA--G
E. coli	G-G-Q---------D	G--VR-----G	E---D--GE---L-G	E---E	M----S-TA--G

Thanks to molecular modelisation from X-ray crystal structures of KARI, the different domains were identified and highlighted on one dimer as shown in Scheme 10.



Region I Region II Region III Region IV Region V

Scheme 10: Regions identification on molecular modelisation of KARI's monomer

Just by analysing the series of amino acids of region I from the N-terminal domain, it presents some resemblance with NADPH-binding sites of a large number of NADPH-dependent oxidoreductases.[38] Indeed, it matches with a *βαβ*-fold, known as the Rossmann fold which has been empirically identified as a binding motif of nucleotides, especially NADPH. Region III is a putative candidate for metal ion binding, as it is very similar to another magnesium-site found in the catalytic subunit of vacuolar ATPases.[39] Site-directed mutagenesis is an efficient technique in order to determine the role of the regions. It consists in inducing mutations of one amino acid at a time on a specific position of the peptide chain and analysing the change in the enzymatic activity and/or the affinity of the cofactors

[38] R. Dumas, M. Lebrun, R. Douce, *Biochem. J.*, **1991**, *277*, 469-475
[39] M. Yoshida, J. W. Poser, W. S. Allison, *J. Biol. Chem.*, **1981**, *256*, 148-153

and substrate with the enzyme. The mutations were introduced by the polymerase chain reaction technique.[40]

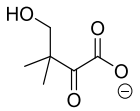
Regions III and IV were studied first by steady-state kinetic analyses to identify them as putative magnesium-binding sites. Table 11 presents the change in the affinities of the metallic ion, NADPH cofactor and the substrate **34b** towards KARI and the relative enzyme activity. The enzyme activity is expressed as µmol of NADPH reduced per min and mg of protein. The progression is followed by the decrease in absorbance of NADPH at 340 nm.

Table 11: Influence of mutations on the enzyme's proprieties

Mutations (Region-[mutation type and position])	K_M^{Mg2+} **(µM)**	K_M^{NADPH} **(µM)**	$K_M^{substrate}$ **(µM)**	**Relative enzyme activity**
None, wild-type enzyme	6	3.5	20	100
II-[R259K]	6	-	-	-
III-[E311D]	117	5	19.5	16.7
IV-[E488D]	82	4	10	48.2

Mutant II-[R259K] displays an identical affinity towards Mg^{2+} as the wild-type enzyme. As there is no change in the K_M, this region is not considered thereafter as a putative magnesium binding site. However, when regions III and IV are considered as candidates for mutation, relevant increases of the respective magnesium Michaelis constants are observed despite no changes in the affinity for the substrate and the cofactor, compared to those of the wild-type enzyme. Along with the downfall of the affinity for magnesium in mutants, there is a correlation with the drop of the enzyme activity. What is interesting to note is even with the mutations, the enzyme conserved the same homodimer structure profile as the natural strain. These results strongly suggest that, unlike region II, both III and IV play a major role in magnesium binding.

The magnesium ions participate and facilitate the two half-reactions by binding to the substrate and to the protein at the same time in regions III and IV. So, these domains may be the nests for the isomerisation and the reduction. To assign a role to each region, mutants that were unable to catalyse the whole reaction were further analysed for their ability to catalyse the reduction of the intermediate. For this purpose, ketopentoate (KP) **39** was used as a mimic of the intermediates **34a-b**.



Ketopantoate (KP) 39

The same studies were carried out with NADPH, Mg^{2+} and **39** as a substrate with different mutants. The enzyme activities of the overall reaction and the reduction step were compared function of the mutant strain, in Table 12.

[40] R. Dumas, M-C. Butikofer, D. Job, R. Douce, *Biochemistry*, **1995**, *34*, 6026-6036

Table 12: Enzyme activity function of the substrate and the mutation

KARI type	Substrate	Relative enzyme activity
wild-type	2-aceto-2-hydroxybutyrate **33a**	100
	ketopentoate **39**	11
III-[E311D]	**33a**	16.7
	39	7.9
III-[D315E]	**33a**	0
	39	0
III-[E319D]	**33a**	0
	39	0
IV-[E488D]	**33a**	48.2
	39	4.2
IV-[E492D]	**33a**	1.7
	39	23.5

With mutations III-[D315E] and III-[E319D], the enzyme is totally inactive, no NADPH has been oxidised. Although, mutant IV-[E492D] suffers from a depletion of its overall activity, its relative activity starting from **39** has even increased compared to the wild-type KARI. Thus there are formerly two distinct regions in which each half step takes place. It appears that Asp-315 and Glu-319 from region III are necessary to the reduction step, whereas region IV Glu-492 participates in the substrate isomerisation.

Among region III, three conserved amino acids, one aspartate and two glutamates can interact with the metallic ions through coordination with their carboxylate moieties. Likewise in region IV the two glutamates can coordinate the metal cation with their carboxylates. Moreover both regions define a hydrophilic pocket surrounded by a hydrophobic one.

The other regions, II and V have been determined to have mostly structural roles.[32] However region V binds to the carboxylate moiety of the substrate or the intermediate thanks to a serine.

Each region has its proper role and works in synergy as summarised in Table 13 below.

Table 13: Summary of each region's utility

Region	Role
I	binding NADPH
II	structural role
III	binding both Mg^{2+}
IV	binding Mg^{2+} through water interactions
V	binds substrate carboxylate moiety

b) Conformational changes

Dumas *et al.* wished to characterise directly the interaction between enzyme and substrate or cofactor by monitoring the absorbance and fluorescence of NADPH when interacting with the spinach

chloroplast strain enzyme purified from *E. Coli*. The nucleotide emits fluorescence at 460 nm upon excitation at 370 nm. [41]

At first, only the interaction between KARI and NADPH is analysed:

$$E + NADPH \rightleftharpoons E{\cdot}NADPH$$

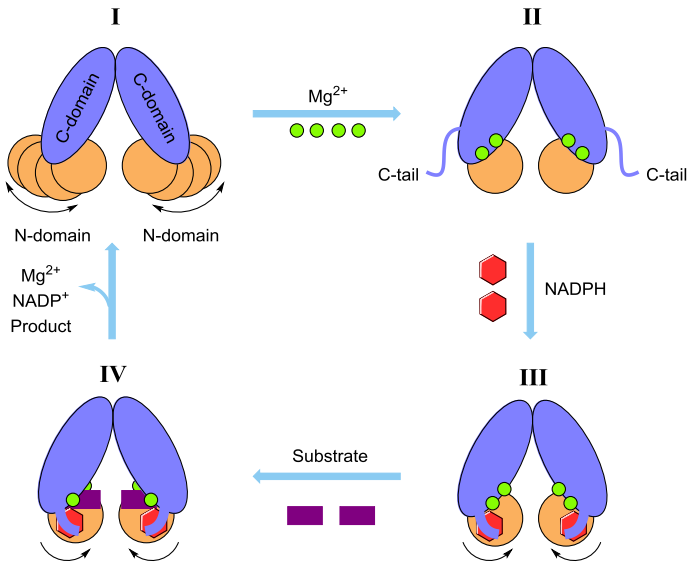
The variation in the fluorescence ΔF_{460} was plotted in function of the quantity of NADPH, [NADPH]. Without the enzyme, ΔF_{460} increases linearly versus [NADPH]. At a fixed quantity of enzyme, ΔF_{460} first increases as [NADPH] rises until reaching a steady sate. This enhancement of ΔF_{460} of NADPH with the enzyme indicates there exists some interactions between the protein and the cofactor by the formation of a binary complex. An experiment was carried out with a prior addition of Mg^{2+} to see whether NADPH would bind as efficiently to the enzyme or not. There were no significant differences when the assays contained magnesium or not.

In their following publication, the same authors proceeded to a similar fluorescence study about the equilibrium of magnesium binding to the binary complex E·NADPH. [40]

$$E{\cdot}NADPH + Mg^{2+} \rightleftharpoons E{\cdot}NADPH{\cdot}Mg^{2+}$$

When Mg^{2+} is added to the complex, a decrease in the emitted fluorescence was observed, indicating the formation of a ternary complex between KARI, NADPH and Mg^{2+}. It also may express that binding of the metal cations may trigger some conformational changes as NADPH is less fluorescent. Moreover, there is not a specific order in the bindings of the metal or the nucleotide to the peptide.

Conformational changes of the enzyme were highlighted by comparing three different crystal structures: rice KARI-Mg^{2+}, rice KARI-Mg^{2+}-NADPH and spinach KARI-Mg^{2+}-NADPH-IpOHA. IpOHA is an analogue to the α-ketoacid intermediates **34** of KARI. These comparisons led to a putative mechanism of the structural modifications of the enzyme as shown in Scheme 11. [42]



Scheme 11: Structural changes of the enzyme

[41] R. Dumas, D. Job, J. Ortholand, G. Emeric, A. Greiner, R. Douce, *Biochem. J.*, **1992**, *288*, 865-874
[42] E. W.W. Leung, L. W. Guddat, *J. Mol. Biol.*, **2009**, *389*, 167-182

The most obvious conformational differences between rice KARI-Mg^{2+}(corresponding to conformation II), rice KARI-Mg^{2+}-NADPH (III) and spinach KARI-Mg^{2+}-NADPH-IpOHA (IV) is that the N-domain is oriented in different angles relative to the C-domain. In the first complex, the active site is completely exposed to the solvent used for crystallisation, however upon addition of NADPH and then IpOHA, the active site becomes almost completely closed. This rotation of the N-domain towards the C-domain, also brings some structural modification of the active site as the two magnesium ions move closer to each other after addition of the intermediate analogue. At first they are separated by 5.3 Å, after the addition they are only 3.5 Å apart. In complex III an ordered C-terminal tail is observed which binds tightly the N-domain with the C-domain, favouring the closing of the space between the two domains.

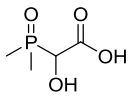
The first biological mechanism was explained by Emil Fischer at the end of the XIXth century with the "lock-key" model. It is based on a complementary active site for the substrate to bind to. In the beginning of the XXth century, Koshland improved the model and proposed an induce-fit mechanism. It is typically the case of KARI as the enzyme has to adopt a certain conformation in order for the substrate to bind and the reaction to occur. However KARI is still apart from the enzyme classified in the induce-fit model as it has to employ a multitude of structural variations to ensure an efficient substrate binding.

6) Inhibitors

Up to now, no inhibitors of KARI have been developed as commercial herbicides. Though two inhibitors have been synthesised in the 80s and beginning of the 90s, they exhibit only weak herbicidal activity.

a) Hoe 704

In 1982 a patent was filed by Hoechst AG on the preparation of functional derivatives of acetic acid containing phosphorus with the aim to develop a new class of nonselective herbicides against both monocotyledonous and dicotyledonous plants, such as wheat and soybean respectively. One compound drew the attention as it presented some very interesting inhibiting proprieties.[43]



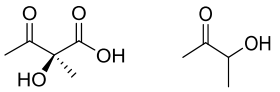
2-(dimethylphosphinoyl)-2-hydroxyacetic acid (Hoe 704) 40

The interest about 2-(dimethylphosphinoyl)-2-hydroxyacetic acid also known as Hoe 704 **40** appeared when herbicidal activity assays were carried out using it and the plant responded with a rapid and complete arrest of growth while other symptoms emerged few days after the treatment. Surprisingly the symptoms were very similar to those observed after exposure of plants to common herbicides specific from the BCAA pathway. Moreover a study performed on *Lemna gibba* or duckweed, showed that the inhibition of growth by **40** was pre-empted by the addition of the three banched-chain amino

[43] K. Bauer, H. Bieringer, H. Burstell, J. Kocur, **1984**, patent number EP0106114

acids valine, leucine and isoleucine.[44] From these observations raised the question of how Hoe 704 inhibits the BCAA pathway and which enzyme is reached.

During *in vitro* tests, nothing was observed on the acetoalactate synthase activity upon addition of **40**. However, it was found that plants treated had a massive accumulation of acetolactate (AL) **33a** and acetoin **41**, which is the product of decarboxylation of acetolactate, as presented in Table 14.



33a **41**

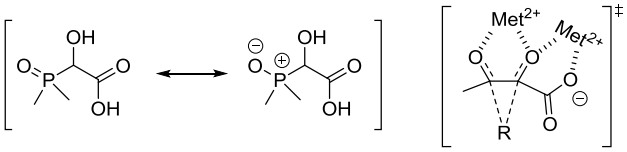
Table 14: accumulation of acetolactate and acetoin in corn plants with Hoe 704

	Acetoin **41** (nmol/100 mg of leaf)	AL **33a** (nmol/100 mg of leaf)
Control plant	1.17	not detectable
Treated plant	1150	350

These results strongly suggests that KARI, the second enzyme from BCAA pathway, is ineffective as the acetolactate, which is its substrate, is not consumed when the plant is treated with **40**. Therefore, it would be rational to say that KARI is inhibited by **40**.

A more specific test has been performed, solely on the isomeroreductase extracted and purified from *D. carota*. The oxidation of NADPH was followed in time by measuring the decrease of absorbance at 340 nm. The control was done without adding any acetolactate to the mixture KARI, NADPH, Mg^{2+}. At 0 µM of inhibitor, the oxidation of NADPH is shown by the rapid decrease of the absorbance. In presence of increasing concentrations of **40**, the rate of the oxidation of the dinucleotide is slower, thus proving **40** is an inhibitor of KARI with a $K_i \approx 0.8$ µM.

Moreover, on a structural point of view the bond between the oxygen and the phosphorus is dative as shown in the two mesomeric structures below.[45] In the second mesomeric form, the phosphorus has a positive charge and the oxygen a negative one. The bond doesn't have a real double-bond character neither a real sigma character. Formally, calculation studies suggest the existence of the sigma bond between phosphorus and oxygen and a back bonding behaviour from the charged oxygen atom. By analogy, **40** can be compared to the three-centre transition state **36** and has to be considered as a competitive inhibitor.[46]

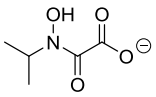


40 **36**

[44] A. Schulz, P. Spönemann, H. Köcher, F. Wengenmayer, *FEBS*, **1988**, *238*, 375-378
[45] D. Gilheany, *Chem. Rev.*, **1994**, *94*, 1339-1374
[46] D. Chestnut, A. Savin, *J. Am. Chem. Soc.*, **1999**, *121*, 2335-2336

b) IpOHA

Few years later, novel analogues of the transition state of the rearrangement state of KARI have been described belonging to the oxalyl hydroxamate family. One of them, N-hydroxy-N-isopropyloxamate IpOHA **42**, showed some interesting proprieties as another potent inhibitor of the reductoisomerase.[47]



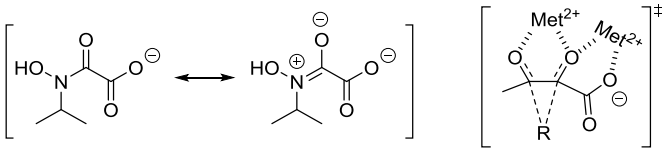
42

The authors discovered that **42** is a potent bacteriostatic as at a concentration of 100 nM, it prevented the growth of *E. coli* for 30 h on minimal media. In the presence of valine, isoleucine and **42**, the growth of the bacterial colonies were not disrupted. As IpOHA did not inhibit the other proteins from the BCAA pathway, it has been deduced that the oxamate **42** acts on KARI.

They also studied the binding characteristics of **42** and the enzyme activity in different conditions. *E. coli* extracted and purified enzyme was incubated with 10 mM of a solution of $MgCl_2$, 480 µM of substrate and 200 µM of NADPH to be at the maximum rate of activity. Different assays were conducted with increasing concentration of **42** starting from none. The change in absorbance was measured at 340 nm corresponding to the absorbing wavelength of NADPH. The results demonstrate that with an increasing amount of oxamate, the change in absorbance was less and less significant compared to the control test without **42**. It means with concentrations up to 4 µM of **42**, the enzyme activity is strongly disrupted as the NADPH is not oxidised to $NADP^+$. The K_i of the inhibitor was determined to be around 160 nM.

Tightest binding of **42** requires Mg^{2+} and NADPH. In the absence of metal, the complex between the enzyme and IpOHA is rapidly reversible. When the oxamate is in presence of the protein and Mg^{2+}, the complex releases the inhibitor with a half-time of two hours, but in presence of both cofactors (Mg^{2+} and NADPH), the release of **42** is slower with a half-time of six days.

Similarly to **40**, **42** is also an analogue to the rearrangement transition state **36**. Even if the predominant form of the inhibitor does not resemble the previously determined three-centre pericyclic transition state, its iminol form has a number of structural aspects in common.



42 **36**

Even though two potent inhibitors of KARI have been discovered at the end of the 80s, they were not developed as commercial herbicides because they both have very low herbicidal activity. In the case of IpOHA **42**, Wittenbach *et al.* discovered that the comound translocates within plants and penetrates

[47] A. Aulabalaugh, J. V. Schloss, *Biochemistry*, **1990**, *29*, 2824-2830

plastids where KARI is localised.[48] No further explanations have been given for the low herbicidal activity of the two molecules **40** and **42**. It hence leaves a wide field of research for the design of new potential herbicidal families of compounds with IpOHA, Hoe 704 and the transition state **36** as sources of inspiration.

[48] V. A. Wittenbach, A. Aulabaugh, J. V. Schloss in *Proceedings of the Seventh International Congress of Pesticides*, Ed. H. Frehse, Hamburg, VCH Publishers, **1990**, p151-160

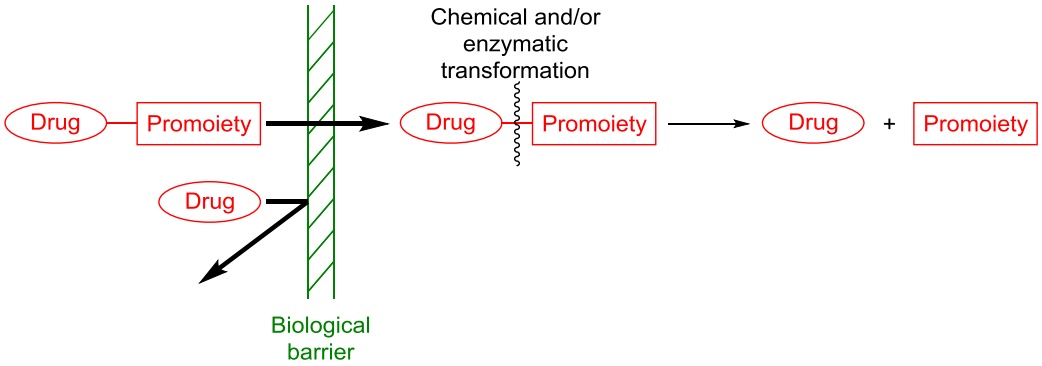
Chapter IV: Synthesis of Hoe704 prodrugs

I. Principle and examples in drug design

1) Definition

Prodrug can be defined as a drug substance which is inactive against the biological target aimed and must undergo *in vivo* chemical or enzymatic transformations in order to present the desired activity.[49] The concept appeared in the middle of the XXth century with the intentional modification of an antibiotic, chloramphenicol, to improve its organoleptic properties and its water solubility. Indeed, the need to design and produce a prodrug is often related to issues such as Absorption, Distribution, Metabolism, Excretion and unwanted Toxicity (ADMET properties). The active drug may present poor aqueous solubility, poor absorption, formulation difficulties or a short half-life due to instability.

Some functionalities of the active compounds are modified or simply masked by chemical groups called promoieties in order to improve the physiochemical, biopharmaceutical or pharmacokinetic properties of the potent molecules. The most classic problem encountered in medicine or phytosanitary would be to penetrate the targeted cell, meaning going through biological barriers. Thanks to the prodrug concept, a molecule can be chemically modified to ensure a better membrane permeability and then be chemically or biologically transformed to release the active form of the drug as shown in Scheme 12.



Scheme 12: general overview of the prodrug concept

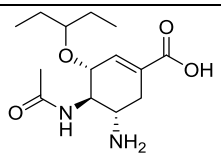
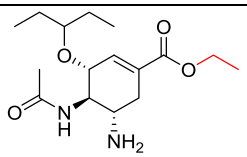
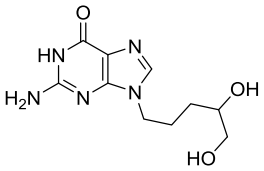
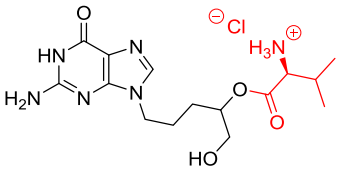
The most common functional groups that are modified with the prodrug strategy are carbonyls, carboxylic acids, hydroxyls, amines and phosphates/phosphonates.[50] Esters are very frequent prodrugs, it is estimated to be 49% of all marketed prodrugs. Furthermore they can easily be cleaved and release the active drug through hydrolysis by esterase enzymes.

[49] K-M. Wu, *Pharmaceuticals*, **2009**, *2*, 77-81
[50] J. Rautio, H. Kumpulainen, T. Heimbach, R. Oliyai, D. Oh, T. Järvinen, J. Savolainen, *Nat. Rev. Drug Discov.*, **2008**, *7*, 255-270

2) Prodrug examples in medicines

The improvements of bioavailability can be illustrated by two examples: Oseltamivir and Valganciclovir. The esterification of both, the anti-influenza and the antiviral, brought respectively a fifteen-fold and tenfold improvement in the oral bioavailability of the active compounds. Moreover by choosing the *L*-valine as the carboxylate moiety of the ester for Valganciclovir, the transportation of the prodrug is facilitated by hPEPT1. It is a membrane transport protein located in epithelial cells in the intestine and it facilitates the uptake of small peptides and peptidomimetic drugs.

Table 15: Prodrug strategy for Oseltamivir and Valganciclovir

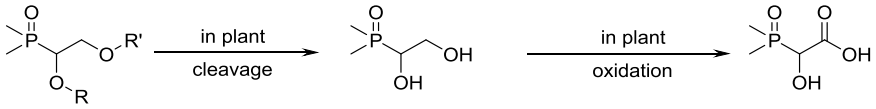
Prodrug name	Drug structure	Prodrug structure	Drug release means	Strategy
Oseltamivir			Esterases	Increase of oral bioavailability from 5% in rat to 80% in humans
Valganciclovir			Esterases	Transportation by hPEPT1 Increase of oral bioavailability from 6% to 61%

The prodrug concept may be applied as a strategy to develop new molecules to target KARI in order to improve the biological properties of new compounds. The idea is to design them to be hopefully the best competitive inhibitors against the natural substrates of the enzyme.

II. Target molecules

A first hypothesis is based on the development of prodrug molecules which already embed oxygen atom in a complexing system. As the sequence depicted in Scheme 13 involves two chemical steps, two different types of enzymes are required. Carboxylic acid ester, carbonic esters and acetals are privileged for R and R' groups as they are generally cleaved respectively by esterases or water. The oxidation of the primary alcohol function could be accomplished by alcohol dehydrogenase and followed by aldehyde dehydrogenase which is present for example in tobacco pollen.[51]

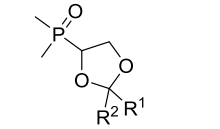
[51] R. G. L. OpdenCamp, C. Kuhlemeier, *Plant Mol. Biol.*, **1997**, *35*, 355-365.



43 **40**

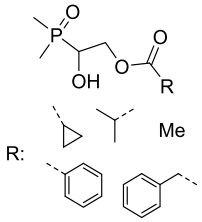
Scheme 13: Potential drug release pathway

As a consequence, the following compounds can be proposed as target molecules. The association of a vicinal diol motif with the dimethyl phosphine oxide group should deeply enhance the effectiveness of the complexing unit and therefore would lead to better substrates or Hoe 704 **40**-like inhibitors **44**-**48**.

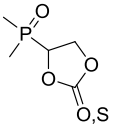


R^1/R^2: H, -$(CH_2)_4$-, CH_3
or
R^1=Me and R^2= H

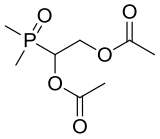
44
4-dimethylphosphoryl-2,2-alkyl-1,3-dioxolane derivatives



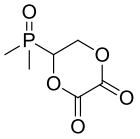
45
2-(dimethylphosphinoyl)-2-hydroxyethyl acetate derivatives



46
4-(dimethylphosphinoyl)-1,3-dioxolan-2-one derivatives

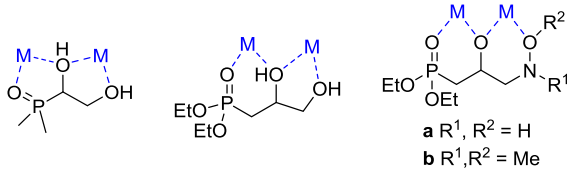


47
1-(dimethylphosphinoyl)ethane-1,2-diyl diacetate



48
5-(dimethylphosphinoyl)-1,4-dioxane-2,3-dione

In **43** and its suggested derivatives, the double pincer chelating the metal cations would adopt a side by side double 5-membered rings conformation. Analogues may be prepared in order to modify the size of the complexing rings. Two modifications may be interesting for analogues preparation, the first one being a methylene group could serve as a linker between the phosphorus moiety and the first alcohol, the other one would consist in exchanging the primary alcohol by a hydroxylamine. The prodrug family may be extended to derivatives of diethyl (2,3-dihydroxypropyl)phosphonate **49** and **50**.

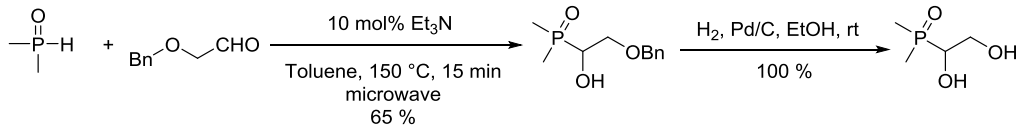


43 **49** **50**

Scheme 14: Chelating ring size changes

1) Synthesis of (1,2-dihydroxyethyl)dimethylphosphine oxide

This family may be synthesized from the Pudovik reaction of dimethylphosphine oxide **51a** with benzyloxyacetaldehyde **52** to give the addition product **53**.[52] This reaction worked well under microwave activation (15 min, 200 W, 150 °C) with a mean yield of 65 %. In the last step, **53** was deprotected through a palladium-catalysed hydrogenation leading to the (1,2-dihydroxy-ethyl)dimethylphosphine oxide **43** (Scheme 15). The diol **43** can be used further for the synthesis of the other prodrugs. This strategy appears synthetically short and affordable.

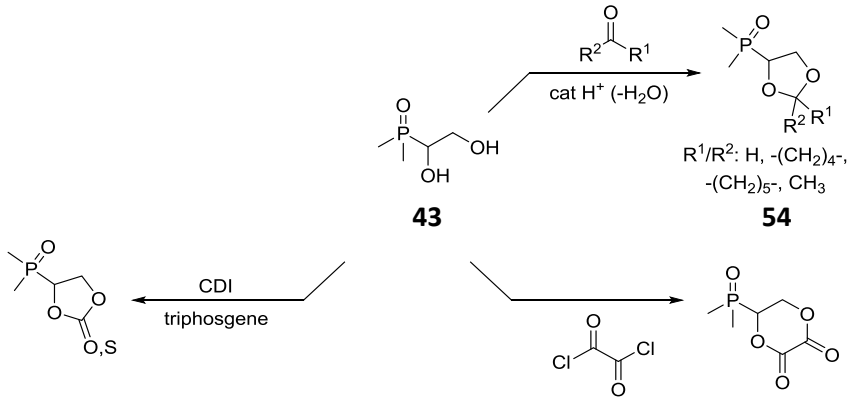


51 a **52** **53** **43**

Scheme 15: Preparation of (1,2-dihydroxyethyl)dimethylphosphine oxide 43

2) Functionalisation of (1,2-dihydroxyethyl)dimethylphosphine oxide

1,2-dihydroxyethyldimethylphosphine oxide **43**, the key building block, will permit the functionalisation of the two hydroxyl groups respectively by ketones or aldehydes giving the corresponding acetals **54**. In another way, reaction with triphosgene, CDI or oxalyl chloride will lead to the targeted esters **55-56** (Scheme 16).



43 **54**

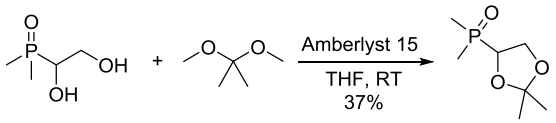
55 **56**

Scheme 16: functionalisation of 43

[52] T. Hanaya, A. Miyoshi, A. Noguchi, H. Kawamoto, M-A. Armour, A. Hogg, H. Yamamoto, *Bull. Chem. Soc. Jpn.*, **1990**, *63*, 3590-3594.

a) Synthesis of acetal derivatives

One acetal analogue **55** synthesis was achieved with $R^1 = R^2 = Me$ thanks to 2,2-dimethoxypropane **57** and acidic catalyst (Scheme 17).

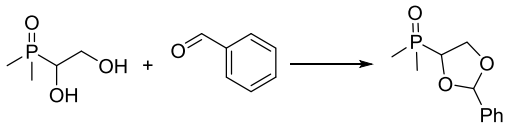


43 **57** **54**

Scheme 17: dimethyl acetal preparation

The product was obtained in 37% isolated yield. 84% conversion of **43** to **54** was observed by ^{31}P NMR. The low yield obtained after column chromatography may be due to the fact that the product was not stable on silica.

In addition to classical conditions as Brønsted acid along with dessicants, the acetal synthesis have been explored also with exotic conditions appealing to different Lewis acids such as copper sulfate with sulfuric acid[53], zinc chloride[54] or alumina[55]. In order to synthesise other acetal derivatives, a scope of different conditions was assayed without success. Benzaldehyde **58** was chosen as the test substrate. The observations are compiled in Table 16.



43 **58** **59**

Scheme 18: phenyl acetal synthesis

Table 16: Acetal synthesis with Brønsted acids and/or Lewis acids

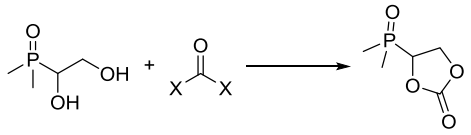
Conditions	Solvent	Temperature	Time	Yields and observations
PTSA (10 mol%)	Toluene	110 °C (Dean-Stark)	Overnight (ON)	43% conversion
PTSA (10 mol%), $MgSO_4$ (2 eq)	Toluene	110 °C	ON	No reaction
PTSA (10 mol%), $MgSO_4$ (2 eq)	Toluene	MW 150 °C, 200 W	20 min	No reaction
$CuSO_4$ (2 eq) H_2SO_4 (10 mol%)	Neat	RT	ON	No reaction
$ZnCl_2$ (1.5 eq)	DCM	RT	ON	No reaction
Al_2O_3 (10 eq)	Toluene	110 °C	ON	59% dimethylphosphinic acid, 17.4% **43**, 19% **59** and 4.6% **51a**, aldehyde recovered

[53] D. Koth, A. Fiedler, S. Scholz, M. Gottschaldt, *J. Carbohydr. Chem*, **2007**, *26*, 267-278
[54] S. Mons, L. Lebeau, C. Mioskowki, *Tet. Lett.*, **1998**, *39*, 9183-9186
[55] Y. Kamitori, M. Hojo, R. Masuda, T. Yoshida, *Tet. Lett.*, **1985**, *26*, 4767-4770

Even with different conditions including Lewis or Brønsted acids mediated reactions, microwave activation or conventional heating, the phenyl acetal appears difficult to synthesise. Dean-Stark apparatus was tried to trap the water formed during the reaction and thus avoid the counter reaction of hydrolysis as it is an equilibrium. Even magnesium sulfate was added as a desiccant, unluckily it didn't help the reaction.

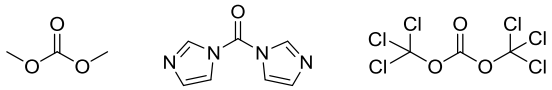
b) Synthesis of the carbonate and replacement of the carbonyl

Concerning the carbonate **55**, it may be prepared from the direct carbonylation of the diol **43** by carbonyl sources (Scheme 19). Some trials were made with dimethyl carbonate **60**, carbonyl diimidazole **61** and triphosgene **62** starting from **43** but they were not conclusive (Table 17).



43 **60-62** **55**

Scheme 19: Carbonate synthesis from 43



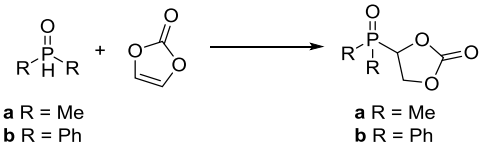
60 **61** **62**

Table 17: Carbonate synthesis attempts

Reactant	Solvent	Conditions	Duration	Observations
Dimethylcarbonate	THF	66 °C	2 days	Cleavage of P-C bond, formation of dimethylphosphinic acid **63**
Dimethylcarbonate	Triethylamine	100 °C (µW, 150 W)	20 min	7 products with a majority of dimethyllphosphinic acid **63**
Carbonyl diimidazole	Acetonitrile	80 °C	1 day	Separation problem with imidazole
Triphosgene	DCM	2 eq pyridine, RT	6h	Dimethylphosphinic acid through an unidentified P(III) intermediate

63

In parallel, hydrophosphorylation of vinylene carbonate **64** was also investigated as shown in Scheme 20.



51 **64** **55**

Scheme 20: general scheme for the hydrophosphorylation reactions

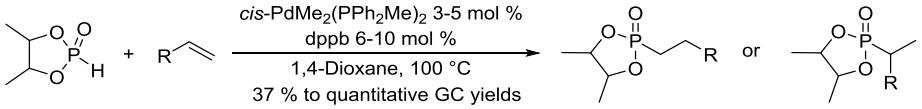
The first set of conditions to be tested is the activation of the carbon-carbon double bond with an organic Brønsted acid such as dried PTSA. Radical reactions[56] turn out to be vastly in use for hydrophosphorylation. AIBN[57] and triethylborane[58] appear to be interesting radical initiators for the reaction.

Table 18: Radical and acid catalysed hydrophosphinylation of 64

Conditions	Phosphorus Compound	Catalyst or radical initiator	Solvent	Heating	Duration	Observations
Radical	**51a**	AIBN (2 mol%)	Toluene	110°C	3 days	**63** as main product
Radical	**51a**	Triethylborane	THF	RT	14h	Mainly **63**
Acidic catalysis	**51a**	PTSA anh (2 mol%)	DCM	RT	2 days	**63** only

As the first attempts with classical conditions didn't yield any interesting addition product, metal-catalysed hydrophosphorylation are investigated. Three main types of organometallics are described.[59] The first one concerns $Pd^{(0)}$, the second one $Pd^{(II)}$ and the last studies $Rh^{(I)}$.

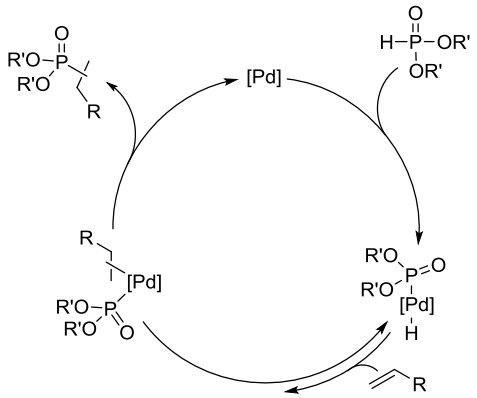
The hydrophosphorylation of alkenes have been studied mostly by M. Tanaka in the beginning of the 2000s. He established a new pallado-catalysed method for the addition of H-phosphonate on unactivated alkenes[60]. Until then, metal catalysed hydrophosphorylation often studied alkynes as substrates rather than alkenes, because of the difficulty of the reaction on the latter. However he demonstrated that the reaction was phosphite dependent as different H-phosphonates have been screened. Surprisingly only the pinacol phosphate displayed some reactivity towards the hydrophosphorylation reaction, compared to 6-membered cyclic phosphites and noncyclic ones. Either *cis*-$PdMe_2(PPh_2Me)_2$ ($Pd^{(II)}$) or Pd_2dba_3 ($Pd^{(0)}$) were active palladium sources with diphenylphosphinobutane as a ligand, as showed in Scheme 21.



Scheme 21: conditions developed by Tanaka's group

[56] A. Peterson, S. Levsen, S. Cremer, *Phosphorus Sulfur Silicon Relat. Elem.*, ***1996**, 115, 241-254.*
[57] H. Hays, *J. Org. Chem.*, **1968**, *33*, 3690-3694
[58] S. Deprele, J-L. Montchamp, *J. Org. Chem.*, **2001**, *66*, 6745-6755
[59] For a review on this topic see: L. Coudray, J-L. Montchamp, *Eur. J. Org. Chem.*, **2011**, 5860–5878
[60] L-B. Han, F. Mirzaei, C-Q. Zhao, M. Tanaka, *J. Am. Chem. Soc.*, **2000**, *122*, 5407-5408

The mechanism is rather classical as depicted in Scheme 22. It involves an oxidative addition of the H-P bond on the palladium complex, then the metal coordinates the alkene followed by a hydropalladation and to finish, the adduct is formed after the reductive elimination from the complex.



Scheme 22: Pallado-catalysed hydrophosphorylation of alkene

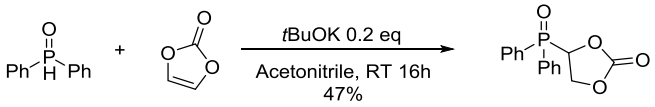
Later, in 2001, Reichwein *et al.* described a rhodium (I)-catalysed hydrophosphorylation of alkenes in order to replace the $Pd^{(II)}$ complex previously used which is air-sensitive and not commercially available.[61] The Wilkinson's catalyst, $Rh(PPh_3)_3Cl$, happened to be a good alternative as it gave good to excellent yields in presence of dppb with the same phosphite used by Tanaka. In the beginning of the investigation, dimethylphosphine oxide **51a** was used in the reactions, especially for the radical ones but this reactant is nonetheless expensive. Therefore, the trials were continued using the cheaper diphenylphosphine oxide **51b** as described in Table 19.

Table 19: Different assays with radical, organometallic, acidic and basic conditions

Reaction type	Phosphorus Compound	Catalyst or radical initiator	Solvent	Heating	Duration	Observations
Organometallic	**51b**	$Pd(PPh_3)_4$ (2 mol%)	Acetonitrile	80 °C	5 days	No reaction
Organometallic	**51b**	Pd_2dba_3 (1% mol) Xantphos (2.2 mol%)	Acetonitrile	80 °C	6 days	56 % conversion but many products
Organometallic	**51b**	$RhCl(PPh_3)_3$ (2.5 mol%)	Dioxane	80 °C	5.5 days	73 % conversion but many products
Organometallic	**51b**	Pd/C (5 mol%) Xantphos (10 mol%)	Acetonitrile	80 °C	3 days	No reaction
Organometallic	**51b**	$PdCl_2PPh_3$ (2 %mol) Et_3N (1 eq)	Acetonitrile	80 °C	6 days	58 % conversion but many products
Organometallic	**51b**	$PdCl_2PPh_3$ (2 mol%)	Acetonitrile	80 °C	3 days	No reaction

A trial was done by using diphenylphosphine oxide **51b** with 20 mol% of potassium *tert*-butoxide at room temperature giving **55b** in 47% yield (Scheme 23).

[61] J. F. Reichwein, M. C. Patel, B. L. Pagenkopf, *Org. Lett.*, **2001**, *3*, 4303-4306



51b **64** **55b**

Scheme 23: base-catalysed hydrophophinylation of vinylene carbonate

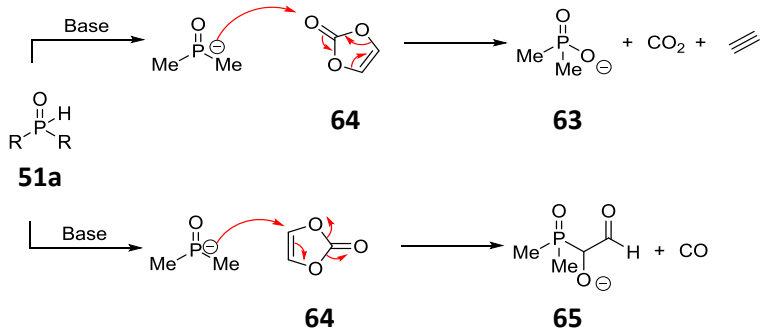
After this successful result, the transposition of these conditions of reaction with dimethylphosphine oxide **51a** was investigated. Unfortunately, the reaction gave no interesting product at room temperature even at reflux of the solvent. It may be attributed to the difference of pKa between the two phosphine oxides.[62]

51a
pKa (in DMSO): 26.9

51b
pKa (in DMSO): 20.6

According to the pKa, a stronger base must be used. Potassium *t*butoxide was replaced by 20 mol% of lithium diisopropylamine in THF but nothing changed in the results.

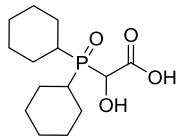
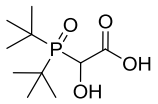
Side reactions may occur, which can explain the aspect of the ^{31}P NMR spectrum of the reaction mixture. The starting material, dimethylphosphine oxide **51a**, was the major peak even after a certain time and heating of the reaction along with other products (Scheme 24). The presence of electron-donating methyl group makes the phosphorus atom a harder nucleophile. Consequently, a nucleophilic attack on the oxygen atom from the carbonate would afford dimethylphosphinic acid **63** with the release of acetylene and carbon dioxide. Or else, the phosphorus nucleophile may attack a carbon of **64** leading to electron pair displacements and also to the release of carbon monoxide through the opening of the cyclic carbonate. The aldehyde compound **65** would be observed by ^{31}P NMR analysis around or above 60 ppm, according to literature dealing with similar compounds **66** and **67** (Table 19), corresponding to the chemical shifts range where most of the peaks were witnessed.[63]



Scheme 24: Potential degradation under basic conditions

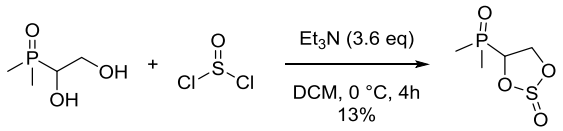
[62] L. Jia-Ning, L. Lei, F. Yao, G. Qing-Xiang, *Tetrahedron*, **2006**, *62*, 4453-4462.
[63] N. Peulecke, M. K. Kindermann, M. Köckerling, J. Heinicke, *Polyhedron*, **2012**, *41*, 61-69

Table 20: Chemical shifts of compounds similar to 65

		
	66	**67**
δ (in ppm, ^{31}P NMR)	58.2	68.3

Alternatives to carbonate derivative **55** were considered. Instead of having a carbonyl, it may be replaced by a sulfoxide or a phosphoryl group.

For the introduction of a sulfoxide moiety as for the sulfite **69**, the diol **43** was reacted with thionyl chloride in presence of triethylamine in dichloromethane at 0 °C (Scheme 25).



43 **68** **69**

Scheme 25: Sulfite synthesis

After 4h of stirring and evaporation of the medium, the crude was purified by column chromatography. Even if the reaction achieved completion, the poor yield (13 %) may be explained by the degradation of the product during the column as the formation of dimethylphosphinic acid **63** was observed.

The other moiety which would replace the carbonyl of the carbonate derivative is the phosphoryl which would lead to a cyclic phosphate compound. It was attempted to be synthesised starting from the diol **43** and ethyl dichlorophosphate **69** as described in Scheme 26.

43 **70** **71**

Scheme 26: synthesis of the phosphate

Many assays were tried with different conditions, summarised in below.

Table 21: Attempts of synthesising phosphate 71

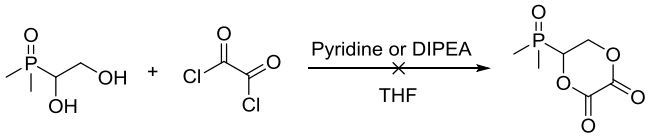
Base	Solvent	Temperature	Time	Observations
Triethylamine	Toluene	0°C to 80°C	30 min	Complex mixture of products without **71**
Triethylamine	DCM / THF	0°C to 60°C	4h	Complex mixture of products without **71**
Pyridine	Neat	0°C to RT	4h	Complex mixture of products, **71** observed

At first the system toluene/triethylamine was used.[64] ^{31}P NMR showed no more starting **43** nor **70**. However a complex mixture of phosphorus compounds appeared without signs of **71**. **71** would be characterised by a doublet around 15 ppm with $^{3}J_{PP} \approx 20$ Hz and another one at around 45 ppm with the same coupling constant.

The problem of using toluene was that the diol **43** wasn't soluble directly in the solvent, so THF was then preferred for the next trial. THF didn't reveal to be better but as a co-solvent with dichloromethane, **43** was completely dissolved. Nonetheless the mixture obtained didn't show the characteristic NMR signals awaited. The last conditions tried were directly in pyridine as a base and solvent. This time the two doublets were observed, showing that the product was synthesised but unfortunately it was in minority even at full consumption of **43**. Some purifications have been tested by cristallisation or column chromatography, nothing gave the pure expected phosphate **71**.

c) Synthesis of the oxalyl derivative

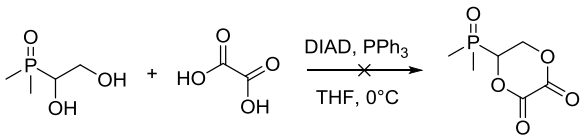
The last prodrug analogue is the oxalyl derivative **56**. During the beginning of the 90s, Itaya studied the synthesis of cyclic oxalates from 1,2-diols.[65] However the substrates only included simply substituted diols, with alkyl or aromatic groups. No publication reported diols substituted with heteroatom at the α position of an alcohol moiety The most common conditions used an amine as a base in THF at 0 °C and with oxalyl chloride **72**. It was tested with diol **43** as a substrate (Scheme 27), unfortunately it didn't give any product.



43 **72** **56**

Scheme 27: Direct double esterification with oxalyl chloride

Even at low temperature, to avoid the bolting of the reaction, some side reactions happened as observed by ^{31}P NMR analysis. Another approach in almost neutral conditions was envisaged by doing a double Mitsunobu reaction between **43** and oxalic acid **73** (Scheme 28).[66] This reaction gave 49% of dimethylphosphinic acid **63** and the starting material **43**.



43 **73** **56**

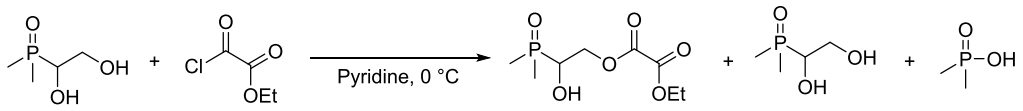
Scheme 28: Double Mitsunobu reaction assay

[64] C. Garner, C. McWhorter, A. Goerke, *Tet. Lett.*, **1997**, *38*, 7717-7720

[65] a) T. Iida, T. Itaya, *Tetrahedron*, **1993**, *49*, 10511-10530; b) T. Itaya, T. Iida, *J. Chem. Soc., Perkin Trans. 1*, **1994**, 1671-1672

[66] L. Maier, H. Spörri, *Phosphorus Sulfur Silicon Relat. Elem.*, **1992**, *70*, 49-57.

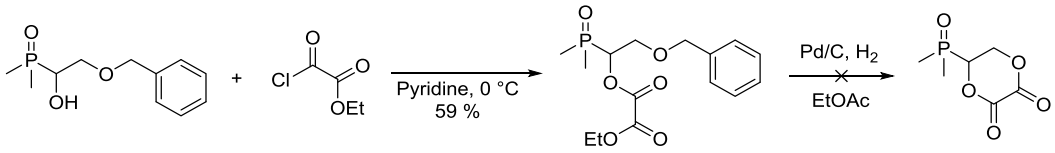
So far, the different one-step reactions tried did not give the right compound. So why not try a two-step reaction with hopefully the primary alcohol reacting first on ethyl chloro oxalate **74** followed by a transesterification including the secondary alcohol (Scheme 29). The two trials of reaction between **43** and **74** gave dimethylphosphinic acid **63** (57%), the starting material **43** (30%) and a bit of the desired product **75** (13%), observed by ^{31}P NMR. The first one was conducted in THF with pyridine as a base, and the second one with pyridine as the solvent and the base. The best results were in the pyridine, neat at 0 °C (Scheme 29).



43 **74** **75** **43** **63**

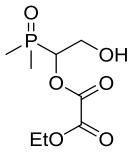
Scheme 29: Esterification of the primary alcohol

The reaction on the diol **43** was not clean enough. So ethyl chloro oxalate **74** can also be used on the protected diol **53**. The oxalate derivative **76** was isolated in 59% yield from pyridine. Then, a one-pot deprotection and transesterification can be performed which would afford **57** (Scheme 30). Unfortunately, even if the hydrogenation worked, the transesterification didn't occur. Only the free primary alcohol was obtained with the ester of the secondary alcohol **77** with 65% yield.



53 **74** **76** **57**

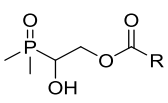
Scheme 30: Esterification of the monoprotected diol



77

d) Synthesis of mono and diesters

Mono esters of the diol **43** may be interesting compounds as only one position must be hydrolysed to free **43** in the plant.

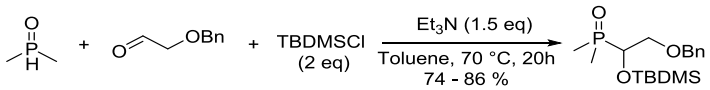


a R = Ph
b R = Ac
c R = CH_2Ph
d R = *c*-Pr
e R = *i*-Pr

78

In order to obtain only the terminal ester without a mixture of diesters along with the other ester on the α position of the phosphorus, the alcohol resulting from the Pudovik reaction must be protected. The protecting group must be chosen so that it is orthogonal to the benzyl ether group from benzyloxyacetaldehyde **52**. A silyl moiety would be meeting this requirement.

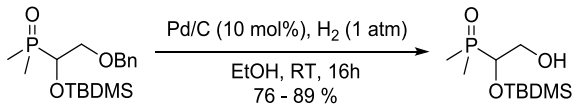
A one-pot synthesis was developed by Gallagher *et al.* in 1996 with *t*-butyldimethylsilyl chloride (TBDMSCl) **79**, a P-H compound and a protected glyceraldehyde.[67] This method was adapted to our molecules. At first TBDMSCl **79** and dimethylphosphine oxide **51a** were reacted together with triethylamine in toluene, then benzyloxyacetaldehyde **52** were added in order to synthesise 2-(dimethylphopshinoyl)-2-[(*tert*-butyldimethylsilyl)oxy]-*O*-benzylethanol **80** in good yields ranging from 74 to 86% as detailed in Scheme 31.



51a **52** **79** **80**

Scheme 31: Pudovik reaction for the orthogonally protected diol

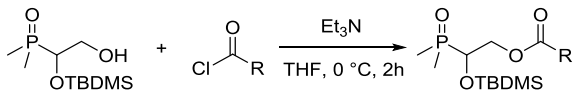
The terminal benzyl ether was deprotected thanks to a hydrogenation reaction, in yields above 76%, leading to the common mono-protected alcohol intermediate **81** (Scheme 32) for the synthesis of the mono-esters **78**.



80 **81**

Scheme 32: Benzyl hydrogenation

Thereafter, the first esterification starting from the corresponding acyl chlorides **82** had been attempted. They were conducted in THF at 0 °C with triethylamine (Scheme 33).



a R = Ph
b R = Ac
c R = CH_2Ph

a R = Ph
b R = Ac
c R = CH_2Ph

81 **82** **83**

Scheme 33: Esterification from acyl chlorides

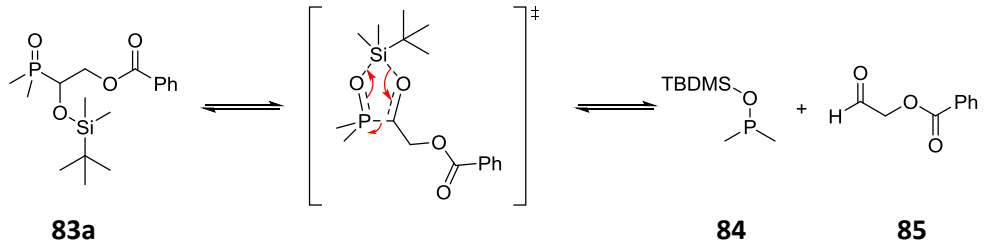
The results of the esterification reactions are compiled in Table 22.

[67] M. J. Gallagher, M. G. Ranasinghe, *J. Org. Chem.*, **1996**, *61*, 436-437

Table 22: Esterification with acyl chlorides and triethylamine

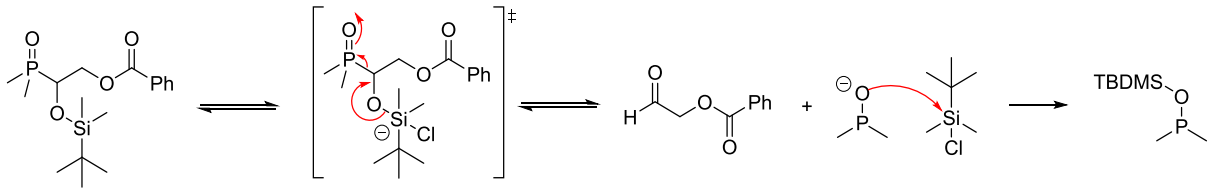
Products	Yields and observations
83a	10% of a $P^{(III)}$ compound, 63% of supposed **83a**, 22% of starting material **81** and 5% of unknown product
83b	**81** recovered
83c	69% of **81** and 31% of **83c**

The conditions were not efficient to provide the products correctly. Moreover, when benzoyl chloride **82a** was used, a P(III) compound was observed by ^{31}P NMR at 100.6 ppm. According to Sekine, Nakajima and Hata who worked on phosphoryl rearrangements involving silylphosphites,[68] it may correspond to dimethyl-*O*-*t*-butyldimethylsilylphosphinite **84**. As the TBDMS group is sensitive to acidic conditions with hydrogen chloride, the rearrangement may be due to the presence of the chloride ion which may activate the silicon through a pentavalent silicon intermediate leading to a retro-Pudovik type reaction.



83a **84** **85**

Scheme 34: Rearrangement supposed by Sukine *et al.*



83a **85** **84**

Scheme 35: Rearrangement through pentavalent anionic silicon

The structure of **84** has been confirmed after a reaction between dimethylphosphine oxide, triethylamine and TBDMSCl. It afforded the phosphinite **84** which appeared at 105 ppm by ^{31}P NMR.

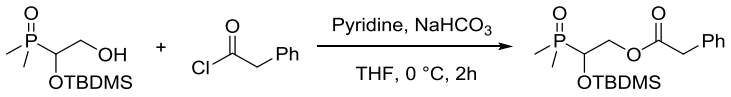
Pyridine later replaced triethylamine as a base but also as an acylium ion transfer agent in order to facilitate the reactions. Unfortunately it didn't give more positive results, as shown in Table 23.

Table 23: Esterification with acyl chlorides and pyridine

Products	Yields and observations
83a	No reaction, **81** recovered
83b	No reaction, **81** recovered

[68] M. Sekine, M. Nakajima, T. Hata, *J. Org. Chem.*, **1981**, *46*, 4030-4034

To avoid the activation of the silyl ether by the chloride ion, the latter must be trapped. The previous conditions with pyridine were used once again but sodium hydrogenocarbonate was added, hopefully to form the NaCl salt.



81 **82c** **83c**

Scheme 36: Esterification from acyl chloride, attempt to precipitate the chloride ion

The new set of conditions was tried with phenylacetyl chloride. It showed only 28% conversion of the starting material **81**, which was already better than recovering the mono-protected diol as before. The reaction was repeated, but this time the medium was heated to 70 °C and left to stir for 2 days. Total conversion was reached unfortunately there is only 46% of product in the medium among 7 other phosphorus containing compounds. The desired product **83c** was isolated in 17% yield.

The conditions were used with the other acyl chlorides as shown in Table 24, with the objective of obtaining **83a**, **83b**, **83d** and **83e.**

83a **83b** **83d** **83e**

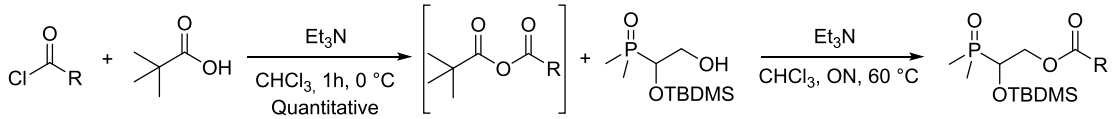
Table 24: Esterification with acyl chlorides with pyridine $NaHCO_3$ and heating

Products	Yields and observations
83a	30 % conversion in 5 phosphorus containing compounds
83b	60 % conversion in **83b**
83d	No reaction
83e	No reaction

It seems, the reactions works with small acyl chlorides such as acetyl chloride **82b**. There may be a problem of steric hindrance coming from the TBDMS group lessening the free space around the non-protected alcohol.

In order to avoid the use of acyl chlorides and having problems of retro-Pudovik, the former were replaced by mixed anhydrides with a pivaloyl counterpart. As the pivaloyl moiety is very bulky, there is a preference for primary alcohols to attack on the other carbonyl. Mixed anhydrides were prepared *in situ*, controlled by GC-MS analysis and directly used for the esterification reactions as presented in Scheme 37, the results are described in Table 25.[69]

[69] H. Arai, H. Nishioka, S. Niwa, T. Yamanaka, Y. Tanaka, K. Yoshinaga, N. Kobayashi, N. Miura, Y. Ikeda, *Chem. Pharm. Bull.*, **1993**, *41*, 1583-1588



a R = Ph
b R = Ac
c R = CH_2Ph
d R = c-Pr
e R = i-Pr

82 **86** **87** **81** **83**

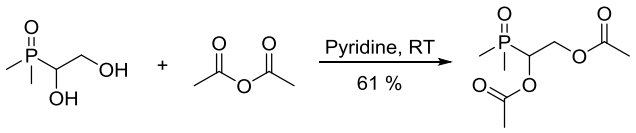
Scheme 37: Esterification with mixed pivalic anhydrides

Table 25: Esterification with mixed pivalic anhydrides

Products	Yields and observations
83a	32 % conversion, not purified
83b	93 % conversion in **83b** but degraded during purification
83c	83 % conversion in **83c** but degraded during purification
83d	81 % conversion in **83d** but degraded during purification
83e	54 % conversion in **83e**, 2 % yield (degradation of the product on the column)

This method of esterification, seemed to be efficient according to the conversion rates of the reactions. However, whenever the crude was purified by column chromatography, the products were degraded and couldn't be recovered except for a little of **83e**.

After investigating the synthesis of the mono esters from **81**, the preparation of the symmetric diester was considered starting from the diol **43**.



43 **88** **47**

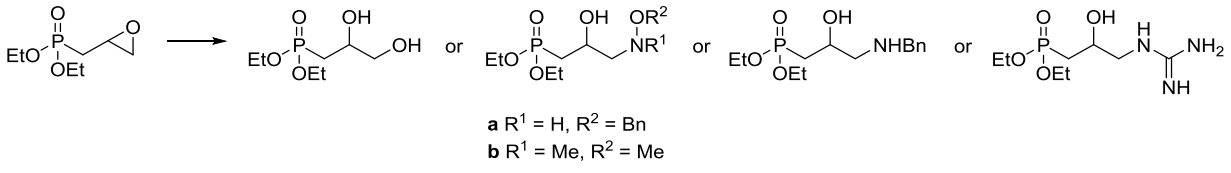
Scheme 38: Synthesis of diester

The preparation of diester **47** was quite easier. The acetylation of phosphine oxide **43** with acetic anhydride **88** in pyridine at room temperature afforded **47** in 61 % isolated yield (Scheme 38).[70]

[70] H. Yamamoto, Y. Nakamura, S. Inokawa, M. Yamashita, M. A. Armour, T. T. Nakashima, *J. Org. Chem.*, **1984**, *49*, 1364-1370

3) Diethyl (2,3-dihydroxypropyl)phosphonate and its derivatives

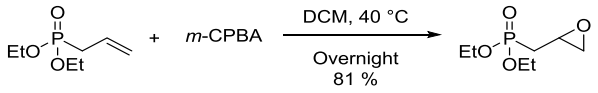
The opening of an epoxide would be the most logical common reaction to synthesise vicinal diols, amino alcohols or alcohol and hydroxylamines in a [2,3]-position.



89 **90** **91** **92** **93**

Scheme 39: Synthetic plan for epoxide opening

The common epoxide **89** was readily synthesised from the corresponding alkene, diethyl allylphosphonate, and *m*-CPBA in dichloromethane.[71]

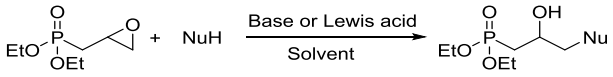


94 **95** **89**

Scheme 40: Epoxidation of diethyl allylphosphonate

The epoxidation reaction was very efficient as a mean yield of 81% was obtained from the different batches.

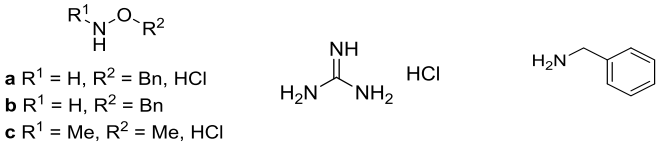
The first attempts to synthesise **91** were tried by direct opening of the epoxide by a nucleophile with a Brønsted base or a Lewis acid either at room temperature or while heating.



89 **90** **91** **92** **93**

Scheme 41: Direct epoxide opening

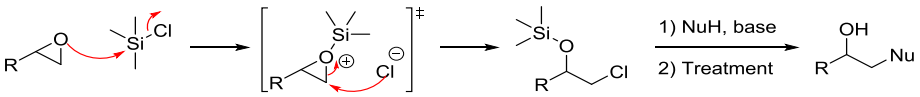
The nucleophiles are listed below:



96 **97** **98**

[71] P. Mitula, C. Wawrzenczyk, *Arkivoc*, **2012**, *iv*, 216-232

Typically, the opening of epoxide ring occurs in basic conditions usually with amine-containing Brønsted bases. In Table 26 triethylamine, 1,8-diazabicycloundec-7-ene (DBU) or Hünig's base have been tested for this purpose. However copper iodide has also been used as a Lewis acid to activate the oxygen and weaken a carbon-oxygen bond thus facilitating the addition of a nucleophile on the aforementioned carbon atom.[72] Another method for activating the oxirane has proven its efficiency by using trimethylsilyl chloride which would lead to the formation of the trimethylsilyl *O*-protected vicinal halohydrin intermediate which would undergo a nucleophilic substitution and a silyl ether cleavage in the following treatments as depicted in Scheme 42.[73]



Scheme 42: Mechanism of the activation of an epoxide by trimethylsilyl chloride

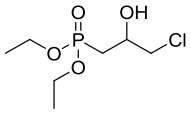
Table 26: Direct opening of the epoxide by nitrogen nucleophiles

Product	Nucleophile	Reagent	Solvent	Temperature	Time	Yield and observation
91a	**96a**	K_2CO_3	EtOH / H_2O	RT	24h	Starting materials recovered
91a	**96b**	Et_3N	Acetonitrile	60 °C	ON	No reaction
91a	**96b**	Et_3N	EtOH / H_2O	RT	24h	76 % of rearrangement
91a	**96b**	Et_3N	EtOH / H_2O	70 °C	24h	55 % of rearrangement
91a	**96b**	Et_3N	Neat	60 °C	ON	No reaction
91b	**96c**	Et_3N	EtOH / H_2O	RT	4 d	Yield 21 %
92	**98**		Neat	60 °C	ON	No reaction
92	**98**	CuI	Neat	50 °C	20h	No reaction
91b	**96c**	CuI	DCM	60 °C	18h	43 % yield in chlorohydrin
91b	**96c**	DBU	THF	RT	18h	No reaction
91b	**96c**	DIPEA	Toluene	60 °C	18h	No reaction
91a	**96b**	CuI, Et_3N	DCM	60 °C	18h	No reaction
93	**97**	CuI, Et_3N	DCM	60 °C	18h	No reaction
91a	**96b**	DIPEA	Toluene	60 °C	18h	No reaction
93	**97**	DIPEA	Toluene	60 °C	18h	No reaction
91a	**96b**	Et_3N, TMSCl	DCM	RT	4 d	33 % chlorohydrin

The direct opening of the epoxide **89** with nitrogen-containing nucleophiles with CuI as a Lewis acid reagent or trimethylsilyl chloride and Brønsted bases only give once the expected product. It worked concerning *N*-methyl-*O*-methylhydroxylamine hydrochloride **96c** with triethylamine in a mixture of water and ethanol at room temperature after stirring for 4 days.[74] However the yield is very low. With two other sets of conditions, another product was obtained. It has been identified as a chlorohydrin **99**. It resulted from the opening of epoxide **89** by a chloride ion, either coming from the

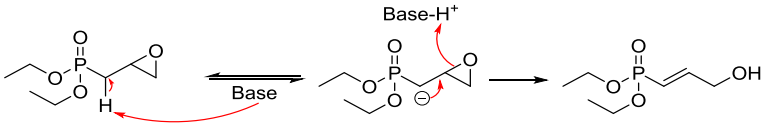
[72] A. Wróblewski, A. Hałajewska-Wosik, *Synthesis*, **2006**, *6*, 989-994
[73] G. C. Andrews, T. C. Crawford, L. G. Contillo Jr., *Tet. Lett.*, **1981**, *22*, 3803-3806
[74] A. E. Wroblewski, A. Halajewsa-Wosik, *Tetrahedron: Asymm.*, **2004**, *15*, 3201-3205

hydrochloride form of the nucleophile or from trimethylsilyl chloride. The byproduct has been identified by A. E. Wroblewski and A. Halajewsa-Wosik who also worked on the opening of **89**.[72]



99

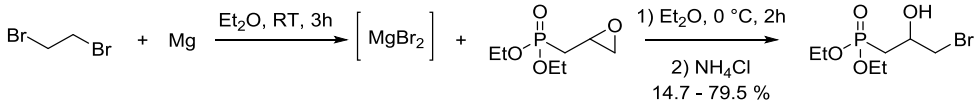
The last byproduct observed during these attempts was a rearrangement of the epoxide due to deprotonation at the α position of the phosphonate electron-withdrawing group leading to diethyl (3-hydroxyprop-1-en-1-yl)phosphonate **100** as detailed in Scheme 43.



89 **100**

Scheme 43: Epoxide rearrangement

Another method was developed by A. E. Wroblewski, A. Halajewsa-Wosik in 2002.[75] It consists in the preparation of a bromohydrin **104** from the epoxide **89** thanks to 1,2-dibromoethane **101** and magnesium turnings.



101 **102** **103** **89** **104**

Scheme 44: Bromohydrin preparation

The bromohydrin **104** has the particularity to be stabilised by an intramolecular hydrogen bond within a six-membered ring which tends to displace the equilibrium reaction, between the epoxide closing and its opening, towards the opening.

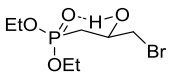
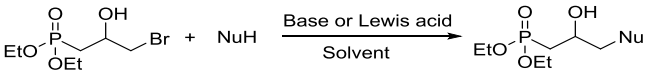


Figure 9: intramolecular H-bond stabilisation of 104

Then different substitution reactions were conducted using diverse conditions and nucleophiles as detailed in scheme 33.

[75] A. E. Wroblewski, A. Halajewsa-Wosik, *Eur. J. Org. Chem.*, **2002**, 2758-2763



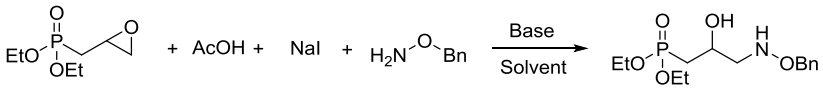
104 **91-93**

Scheme 45: Substitution on bromohydrin

Table 27: Substitution reactions on bromohydrin

Product	Nucleophile	Reagent	Solvent	Temperature	Time	Yield and observation
91a	**96a**	K_2CO_3	ACN	RT	ON	Starting material and epoxide recovered
93	**97**	K_2CO_3	ACN	80 °C	ON	70 % of rearrangement **100**
92	**98**	Et_3N	DCM	RT	24h	Majority of epoxide
92	**98**	DBU	DCM	RT	24h	43 % epoxide, 57 % **92** by GC-MS
91b	**96b**	Et_3N	Neat	100 °C	24h	No reaction

The bromohydrin method didn't seem to be efficient with these conditions. Another halohydrin have been used, the iodohydrin as the iodide would be assumed to be a better leaving group than bromide. However for a problem of less stable halohydrin, the iodohydrin was prepared and used *in situ* for substitution reactions (Scheme 46) with *O*-benzylhydroxylamine **96b**, the results are summarised in Table 28.



89 **96b** **91a**

Scheme 46: In situ synthesis of iodohydrin

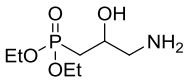
Table 28: Substitution reactions from *in situ* iodohydrin synthesis

Reagent	Solvent	Temperature	Time	Yield and observation
Et_3N	THF	80 °C	2 d	44 % of iodohydrin
	neat	100 °C	17h	No reaction product apart from epoxide
Et_3N	MeOH	100 °C	4h	26 % conversion in **91a**
Et_3N	MeOH	100 °C	24h	No more epoxide but no **91a**
Et_3N	EtOH / H_2O	RT	24h	Majority of epoxide

Even by changing the nature of the halogen group of the halohydrin, it didn't improve the synthesis of **90**-**92**. Moreover surprisingly while heating longer the reaction using the triethylamine in methanol conditions the product **91a** was degraded. After 24h of heating, no more epoxide **89** was monitored by GC-MS but benzyl alcohol and benzaldehyde appeared.

The N-O bond on benzylhydroxyamine was thermally cleaved, leaving the free amine as observed for instance by G. Laus *et al.* in 2013.[76] The 2,3-aminoalcohol **105**, analogous to **91a** was observed.

[76] G. Laus, V. Kahlenberg, K. Wurst, T. Müller, H. Kopacka, H. Schottenberger, *Z. Naturforsch.*, **2013**, *68b*, 1239-1252

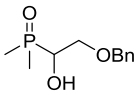


105

The oxidation of the benzyl alcohol previously cleaved may be explained by the presence of diiode in the medium. As the reaction wasn't conducted under inert atmosphere, the oxygen can oxidise the iodide into diiode.

III. Conclusion

The prodrug concept is an interesting one, justifying the synthesis of a family of potential KARI's inhibitors. The different compounds were the results of the functionalisation of the key intermediate diol **43** resulting from a Pudovik reaction.



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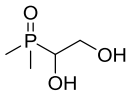
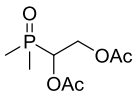


43

In general, the esterifications leading to the compounds of interest were not very efficient as for the oxalyl derivative **57** or the mono ester ones **78**. Either the double esterification didn't occur or the monoester with a silylether on the α position made the products sensitive and readily degradable. However some products have been synthesised and sent for biological tests.



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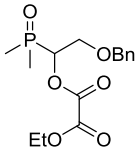

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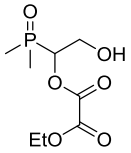

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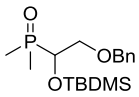

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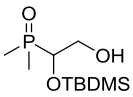
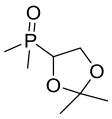


81

Cyclic derivatives have also been prepared with an acetonide moiety **54**, a thionyl one **69** and a carbonate which revealed to be very tricky to prepare. Only a base mediated hydrophosphorylation of vinylene carbonate with diphenylphosphine oxide lead to the cyclic carbonate **55b**.



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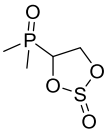

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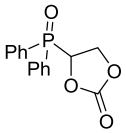
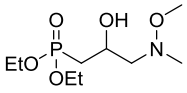


55b

In a second part of the prodrug family synthesis, a homologated derivative of **43** have been synthesised in the form of the epoxide **89**. Multiple conditions were explored in order to open the oxirane ring with nucleophiles, thus create half of the chelating pincer needed. Unfortunately, the epoxide was surprisingly stable and only phosphonate **91a** was successfully synthesised.



91a

Chapter V: Variations of chelating ring size

I. Interest of variations of the chelation ring sizes

As detailed in the explanation of the binding mode of KARI's substrate to its catalytic site (see Inhibition of KARI, I) Mechanism of action, Metal ion requirement on page 44), the complexation to the two magnesium cations involves two 5-membered chelating rings. Indeed, in order to achieve high binding efficiency of potential inhibitors inside of the enzyme, size matters.

A general study from Dr Martell *et al.* in 1994, reports their observations of the enthalpy energy variations between different ethylenediaminetetraacetic acid (EDTA) analogs adopting diverse chelation ring sizes with different metallic ions.[77] A trend shows the increase of the enthalpy energy when the chelation cycles are including more and more atoms meaning less affinity, however they notice two size exceptions. 5-membered cycle complexes and the 6-membered ones are the most efficient ones regardless the metallic ions radii.

In addition, a chelate ring size study was published in 2012 on the influence of the size of the double pincer from HIV-1 integrase inhibitors on their respective efficacies.[78] Raltegravir was established as being the reference compound, intervening as a blocker of the retroviral DNA strand-transfer within the host cell DNA. The inhibitor displayed a strand-transfer IC_{50} value of 10 nM. It possesses a double chelating pincer creating a 5-membered ring and a 6-membered ring.

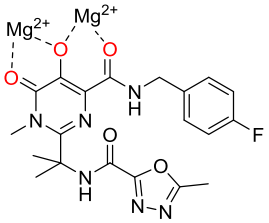
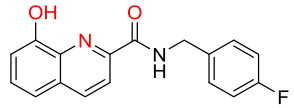
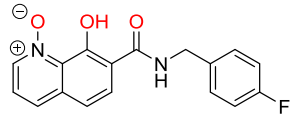
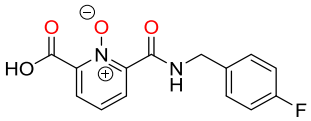


Figure 10: Raltegravir and its binding mode to HIV-1 integrase magnesiums

Other complexing units have been synthesised and studied to determine the most potent ones compared to Raltegravir. They include either two 5-membered chelate rings or two 6-membered ones.

[77] A. E. Martell, R. D. Hancock, R. J. Motekaitis, *Coord. Chem. Rev.*, **1994**, *133*, 39-65
[78] A. Agrawal, J. DeSoto, J. L. Fullagar, K. Maddali, S. Rostami, D. D. Richman, Y. Pommier, S. M. Cohen, *PNAS*, **2012**, *109*, 2251-2256

Table 29: Influence of the chelate ring sizes on the strand-transfer IC_{50}

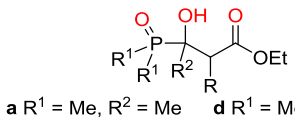
Raltegravir analogs	Chelate ring size	Strand-transfer IC_{50} (µM)
	5-, 5-	> 100
	6-, 6-	3.8
	6-, 6-	9.2

Even if the three highlighted Raltegravir analogs are more than 1000-fold less efficient, the influence of the chelate ring sizes is still noticeable as the one displaying a 5-, 5- motif is inefficient compared to the two 6-, 6- analogs.

The change in the chelate ring sizes from the double chelating pincer may be a key to synthesise potent inhibitors of KARI.

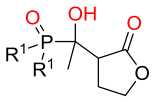
II. Target molecules

The objective is to synthesise 3-(diorganylphosphoryl)-3-hydroxybutanoate **106** or constrained cyclic analogues **107**-**109**. The interest of cyclic analogues lies in the fact that the chelating functional groups are all forced on the same side of the molecule. They all display a potential chelating motif involving a 5-membered ring aside a 6-membered cycle.



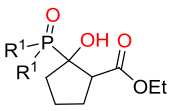
a R^1 = Me, R^2 = Me **d** R^1 = Me, R^2 = iPr
b R^1 = Ph, R^2 = Me **e** R^1 = Ph, R^2 = iPr
c R^1 = OEt, R^2 = Me **f** R^1 = OEt, R^2 = iPr

106



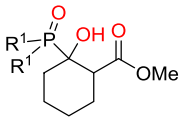
a R^1 = Me
b R^1 = Ph
c R^1 = OEt

107



a R^1 = Me
b R^1 = Ph
c R^1 = OEt

108

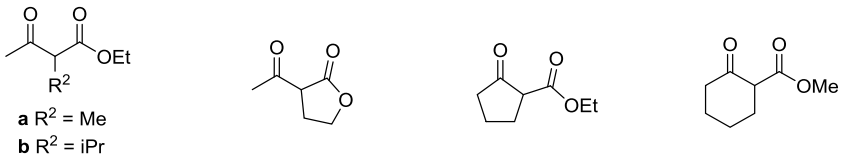


a R^1 = Me
b R^1 = Ph
c R^1 = OEt

109

Scheme 47: Biological targets

In order to synthesise these compounds, the respective β-ketoesters **110**-**113** were reacted with different P-H reagents thanks to Pudovik reactions (Scheme 48).

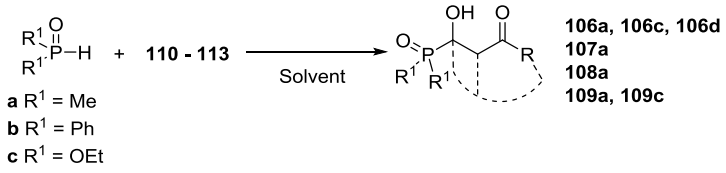


110 **111** **112** **113**

Scheme 48: β-ketoesters

1) Synthesis from Pudovik reactions

Different sets of conditions were tried ranging from base or Lewis acids mediated reactions to silylphosphite $P^{(III)}$ reagents.



51

Scheme 49: synthesis of the biological targets by Pudovik reactions

a) Trialkylamine Brønsted base mediated reactions

At first, triethylamine or DBU have been tried as a base, the results are detailed in Table 30 below. DBU has been used for a long time as a base for Pudovik reactions because of the reported faster reaction duration and its easy handling.[79] In neat conditions triethylamine has proven to be an efficient base for the addition of dimethyl phosphite on simple ketones.[80]

Table 30: amine base mediated Pudovik assays

Product	Base	Solvent	Temperature	Time	Yield and observation
106c	DBU	Toluene	RT	2 days	No reaction
109c	Et_3N	Neat	40 °C	overnight	4% conversion in **109c**
109c	Et_3N	Toluene	150 °C MW (250 W)	10 min	No reaction

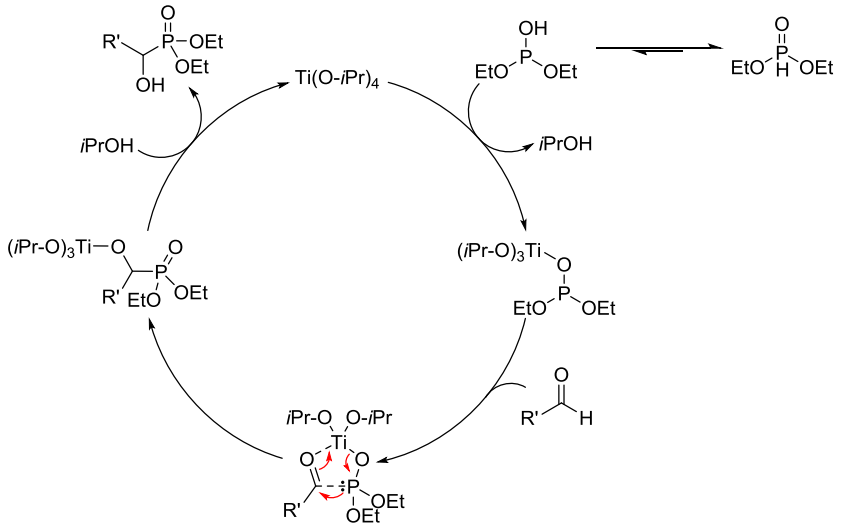
With DBU, no reaction occurred at all, as for microwave irradtion at 150°C for 10 min. However 4% conversion could be assessed by ^{31}P NMR spectroscopy when triethylamine was the base but without any solvent. The Pudovik reaction seems difficult to happen.

[79] a) M. Yamashita, K. Tsunekawa, M. Sugiura, T. Oshikawa, *Synthesis*, **1985**, *1985*, 65-66. b) O. Pàmies, J. Bäckvall, *J. Org. Chem.*, **2003**, *68*, 4815-4818

[80] C. Wang, J. Zhou, X. Lu, J. Wen, H. He, *Phosphorus Sulfur Silicon Relat. Elem.*, **2013**, *188*, 1334-1339

b) Titanium tetraisopropoxide

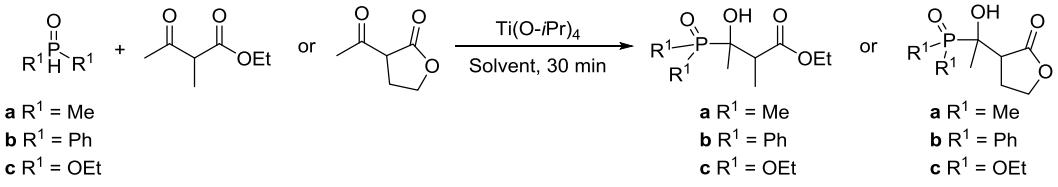
Titanium tetra-*iso*-propoxide is known to favour the formation of a titano-$P^{(III)}$ complex which would be more reactive than the corresponding $P^{(V)}$ / amine base system assayed above, because of the free lone electron pair borne by the phosphorus atom. The mechanism is explained in Scheme 50 drawn below.



Scheme 50: Ti(O-*i*Pr)$_4$ mediated Pudovik reaction mechanism

This system was developed for Pudovik reactions on aldehydes. A ligand exchange occurs on the titanium tetraalkoxide with diethyl phosphite in the $P^{(III)}$ tautomer form. After formation of the complex with the carbonyl of an aldehyde, the Pudovik reaction happens.[81] This procedure combines two properties favouring the Pudovik reaction. The formation of the $P^{(III)}$ intermediate makes it more nucleophile in addition to the complexing of the carbonyl by titanium which brings the reactants closer.

In 2009, Dr X. Zhou *et al.* were the first group to develop a Lewis-catalysed hydrophosphonylation of ketones with titanium tetraisopropoxide.[82] This method was employed with our substrates.



51 **110a** **111** **106** **107**

Scheme 51: Ti(O-*i*Pr)$_4$ mediated Pudovik reactions

[81] T. Yokomatsu, T. Yamagishi, S. Shibuya, *J. Chem. Soc., Perkin Trans. 1*, **1997**, 1527-1534

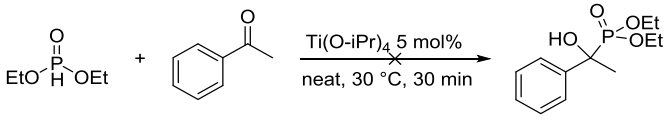
[82] X. Zhou, Y. Liu, L. Chang, J. Zhao, D. Shang, X. Liu, L. Lin, X. Feng, *Adv. Synth. Catal.*, **2009**, *351*, 2567-2572

Table 31: Ti(O-*i*Pr)$_4$ mediated Pudovik reactions

Product	Ti(O-*i*Pr)$_4$ equivalent	Solvent	Temperature	Yield and observation
107a	0.1	neat	60 °C	No reaction
107b	0.1	THF	60 °C	No reaction
107c	0.1	neat	60 °C	No reaction
106c	1	neat	MW 100 °C	10 phosphorus containing molecules.

THF was used as a solvent when diphenylphosphine oxide **51b** was the reagent, otherwise it would have been a heterogeneous medium. The reactions with 10 mol% of titanium (IV) didn't give any product, however when an equivalent was introduced, a mixture of 10 products was obtained.

The conditions of the publication used for **107a** and **107c** were verified by trying to reproduce one of their reaction involving acetophenone **114**.



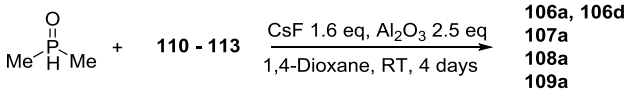
51c **114** **115**

Scheme 52: Reproduction of one publication's reaction

No expected Pudovik adduct **115** was obtained nor observed although the authors described a 96 % yield on this reaction.

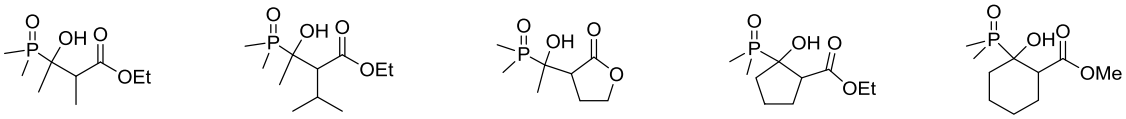
c) CsF-Al_2O_3 conditions

The Pudovik protocol developed by Dr F. Texier-Boullet was adapted by using an equimassic mixture of caesium fluoride and alumina in 1,4-dioxane (Scheme 53).[83,84] The results are reported in Table 32.



51a

Scheme 53: Texier-Boullet Pudovik conditions



106a **106d** **107a** **108a** **109a**

[83] F. Texier-Boullet, M. Lequitte, *Tet. Lett.*, **1986**, *27*, 3515-3516
[84] F. Texier-Boullet, A. Foucaud, *Synthesis*, **1982**, *1982*, 165-166

Table 32: Pudovik mediatedby CsF and Al_2O_3

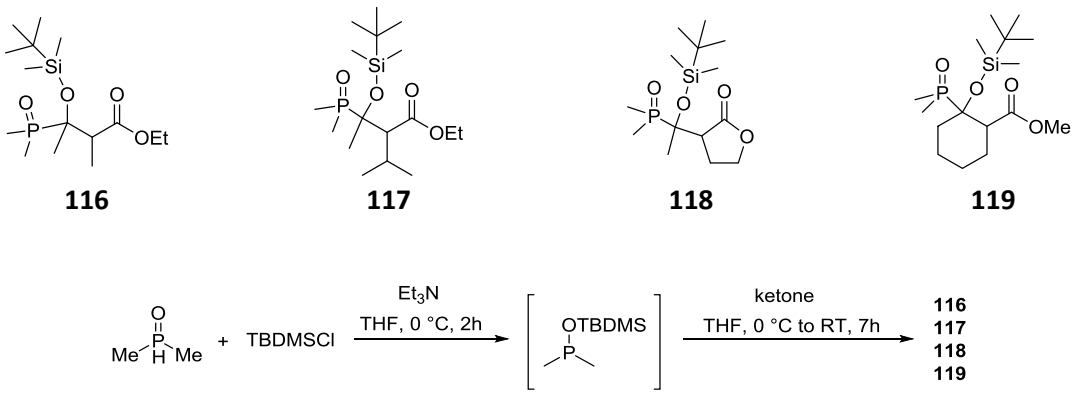
Product	Yield and observation
106a	66 % conversion in 7 P-containing compounds
106d	18 % conversion in 6 P-containing compounds
107a	56 % of dimethylphosphinic acid and 33 % of **51a**
108a	65 % conversion in 7 P-containing compounds
109a	38 % conversion in **109a** among 6 other P-containing compounds

Even after 4 days at room temperature, full conversion of dimethylphosphine oxide **51a** wasn't achieved. Moreover, the reactions gave significant amounts of dimethylphosphinic acid **63** among many other side products. The ketones may not be electrophilic enough letting side reactions to occur. The nucleophilicity of the P-H reagents may be strengthened by converting them into $P^{(III)}$ silylphosphinites.

d) Silylphosphinite method

There is no example in the literature of Pudovik reactions with silylphosphinite intermediates and a few concerning trimethylsilylphopshite on aromatic aldehydes.[85] Our substrates are admittedly different but silylphosphinite derivative are a source of nucleophilic $P^{(III)}$ reactants.

Dimethylphosphine oxide **51a** was converted to the corresponding silylphosphinite **120** by reacting with *t*-butyldimethylsilyl chloride and triethylamine in THF at 0 °C before adding the ketones **110a-b**, **111** and **113** which led to the Pudovik adducts protected with a *t*-butyldimethylsilyl group (**116**-**119**).



Scheme 54: $P^{(III)}$ silylphosphite Pudovik reactions

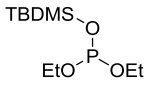
[85] A. A. Prischchenko, M. V. Livantsov, O. P. Novikova, L. I. Livantsova, A. V. Maryashkin, *Russ. J. Gen. Chem.*, **2005**, *75*, 1965-1967

Table 33: $P^{(III)}$ silylphosphite Pudovik reactions

Product	Yield and observation
116	54 % conversion to **116**
117	No reaction
118	36 % yield
119	Full conversion but degradation during treatment: 45 % yield

According to the results in Table 33, this method has proven to be more efficient than the conditions tried before. There was however an exception with **117**, it seemed that the isopropyl group of **110b** blocked the attack of the hindered *t*-butyldimethylsilyl dimethylphosphinite **120** on the carbonyl.

To continue with the same idea, the conditions were tested with diethyl phosphite **51c** with the same ketones. Surprisingly for each reaction, the *t*-butyldimethylsilyl diethyl phosphite **121** is observed by ^{31}P NMR spectroscopy along with diethyl phosphite but no product resulting from the Pudovik reactions on the ketones appeared.



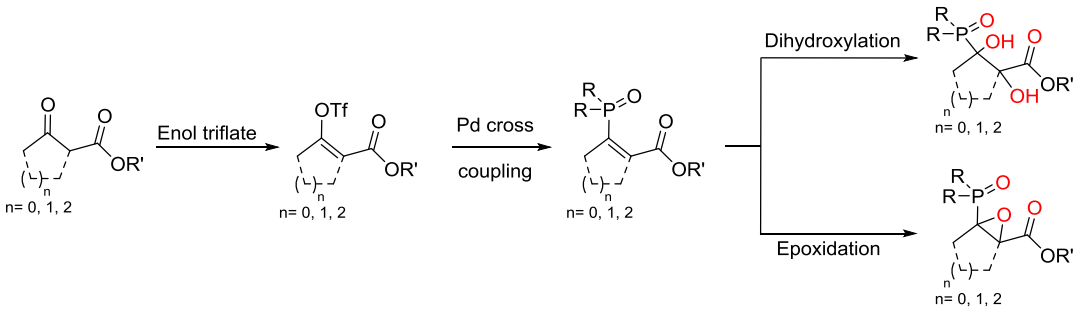
121

This method wasn't convenient for all the P-H compounds. As the previous methods tested were not conclusive either, the target compounds have been modified in order to facilitate the synthesis.

III. New target molecules

Instead of adding the phosphorus group on ketones, the carbonyl was blocked in its enol tautomeric form thanks to a triflate group. A pallado-catalysed cross coupling reaction was planned to obtain a vinyl phosphine or vinyl phosphonate. The vinyl compound would undergo either an epoxidation or a dihydroxylation reaction as summarised in Scheme 55.

Moreover it allows the diols form either two 5-membered chelation rings or two 6-membered ones and the oxirane cycle may act as a prodrug by ring opening.

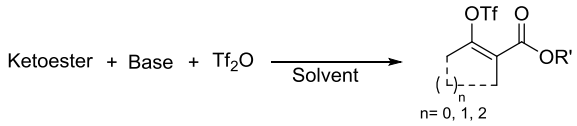


Scheme 55: Synthetic plan

All the ketones **110**-**113** were used, along with dimethylphosphine oxide **51a**, diphenylphosphine oxide **51b** or diethylphosphite **51c**.

1) Synthesis of enol triflates

First of all, the enol triflates have been prepared from triflic anhydride and an amine base as shown in Scheme 56 and Table 34. Different bases and solvents were applied to the reaction depending on the substrate.



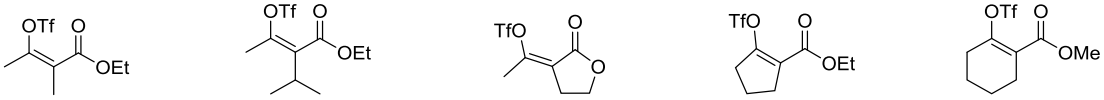
110-113 **122-126**

Scheme 56: Enol triflate preparation

Table 34: Enol triflate preparation

Product	Ketone	Base	Triflic anhydride	Solvent	Temperature	Time	Yield and observation
122	**110a**	DIPEA (5 eq)	1.2 eq	DCM	-78 °C to RT	ON	71 %
123	**110b**	LiHMDS (2 eq)	1 eq	THF	-78 °C to RT	2h	65 %
124	**111**	Et_3N (1 eq)	1.2 eq	DCM	0 °C to RT	30 min	82 %
125	**112**	DIPEA (5 eq)	1.2 eq	DCM	-78 °C to RT	ON	71 %
126	**113**	DIPEA (5 eq)	1.2 eq	DCM	-78 °C to RT	ON	84 %

Here are the enol triflates **122**-**126** prepared from the three different conditions.

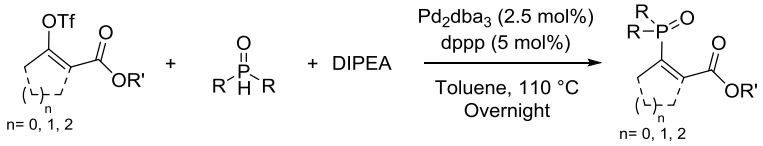


122 **123** **124** **125** **126**

The same conditions couldn't give all the products, three set of conditions had to be employed. The ones giving most of the products used DIPEA as a base in dichloromethane.[86] Unfortunately when ethyl 2-isopropyl-3-ketobutanoate **110b** or 2-acetylbutyrolactone **111** were considered for the triflation reaction with DIPEA, it didn't give the expected enol triflates. For **110b** lithium hexamethyldisilazane in solution in THF was considered as a base,[87] and concerning **111**, 1 equivalent of triethylamine was sufficient for the reaction.[88]

2) Palladium-cross coupling

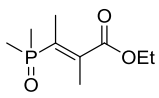
Then, after having prepared the enol triflates, they underwent a cross-coupling reaction with different phosphorus sources **51** catalysed by a $Pd^{(0)}$ with diphenylphosphinopropane as a ligand in toluene (Scheme 57).[89]



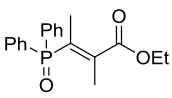
122-126 **51** **127-141**

Scheme 57: Pallado-catalysed cross coupling of enol triflates with phosphorus sources

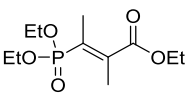
The coupling reactions worked very well as they all lead to the expected products in medium to good yields (36-93%). In order to have a better visualisation of the products synthesised, they are detailed below.



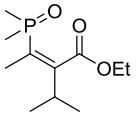
127
73 %



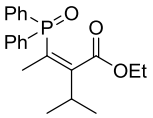
128
89 %



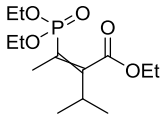
129
66 %



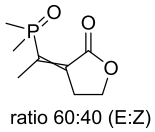
130
70 %



131
60 %

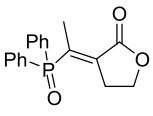


132
43 %

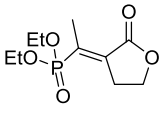


ratio 60:40 (E:Z)

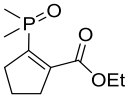
133
56 %



134
36 %

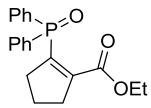


135
38 %

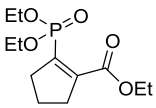


136
40 %

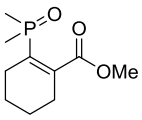
[86] S. V. Gagnier, R. C. Larock, *J. Am. Chem. Soc.*, **2003**, *125*, 4804-4807
[87] P. Maity, S. D. Lepore, *J. Org. Chem.*, **2009**, *74*, 158-162
[88] R. Pouwer, H. Schill, C. Williams, P. V. Bernhardt, *Eur. J. Org. Chem.*, **2007**, 4699-4705
[89] S. Ishikawa, K. Manabe, *Tetrahedron*, **2010**, *66*, 297-303



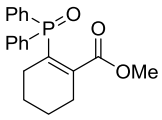
137
80 %



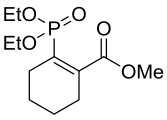
138
93 %



139
62 %



140
60 %

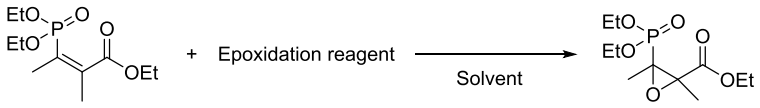


141
63 %

The alkenes obtained were later reacted with epoxidation or dihydroxylation reagents.

3) Epoxidation

The substrates for the epoxidation reactions are electron deficient alkenes. Therefore they are typical reagents for the Weitz-Scheffer epoxidation reaction generally using hydrogen peroxide and a base. However other classical conditions, such as *m*-CPBA were tested and were quickly followed by more exotic ones involving sodium hypochlorite alone, in an acidic solution or with metal mediation. All the reactions were conducted with the alkene **129**.



129 **142**

Scheme 58: Epoxidation reactions

Table 35: Epoxidation reactions attempts

Epoxidation reagent	Solvent	Temperature	Time	Yield and observation
H_2O_2 + Na_2CO_3	MeOH	RT	2 days	No reaction
tBuO_2H + *t*BuOK	THF	RT	1 day	No reaction
m-CPBA	THF	RT	1 day	No reaction
NaOCl	Dioxane	0 °C to RT	ON	No reaction
NaOCl + H_2SO_4 (pH = 5 – 6)	Acetonitrile	RT	ON	No reaction
NaOCl + Al_2O_3	Acetonitrile	RT	ON	No reaction
NaOCl + $Ni(OAc)_2$ 2.5 mol%	DCM	RT	ON	No reaction

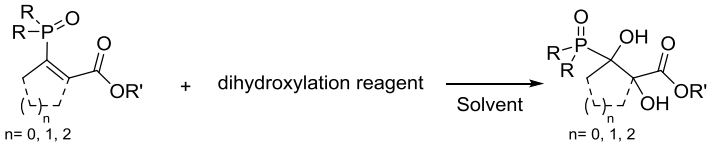
Table 35 unfortunately shows that the epoxidation of this kind of alkene is very difficult. From mild conditions with *m*-CPBA to more classical harsh conditions with hydrogen peroxide, nothing afforded the oxirane. Sodium hypochlorite has been investigated either alone[90] or with alumina which is a system that was designed for the epoxidation of electron poor alkenes[91] or the last conditions with nickel diacetate which has proven its efficacy for the epoxidation of α,β-unsaturated carboxylic acids[92]

[90] D. Lawrence, J. Zilfou, C. Smith, *J. Med. Chem.*, **1999**, *42*, 4932-4941
[91] A. Foucaud, M. Bakouetila, *Synthesis*, **1987**, *1987*, 854-856
[92] J. Grill, J. Ogle, S. Miller, *J. Org. Chem.*, **2006**, *71*, 9291-9296

but once again the epoxidation didn't occur at all. Each time the starting alkene was observed by ^{31}P NMR and GC-MS analysis.

4) Dihydroxylation

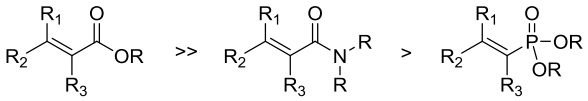
The dihydroxylation reactions were conducted with AD-mix α as a classical dihydroxylation reagent and other conditions involving manganese catalysed reactions[93] or potassium permanganate[94] were also considered. **129** and **138** were used as substrates at the beginning.



129 or **138**

Scheme 59: Dihydroxylation assays

In a publication from Dr P. Dupau published in 2002 on the synthesis of vicinal diol from di- and tetrasubstituted alkenes bearing at least one electron withdrawing group, the reactivity of the C-C double bond towards dihydroxylation adopted the following order: the way more reactive type of alkene being the α,β-unsaturated esters, then the α,β-unsaturated amides and to finish the less or even non-reactive are α,β-unsaturated phosphonates.[95]



Scheme 60: Alkene reactivity towards dihydroxylation function of the electron withdrawing group

Table 36: Dihydroxylation assays

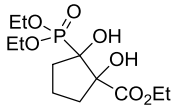
Alkene	Dihydroxylation reagents	Solvent	Temperature	Time	Yield and observation
129	AD-mix α + methylsulfonamide	*t*Butanol / H_2O (1 / 1)	RT	2 days	No reaction
129	H_2O_2 + nicotinic acid + $MnCl_2$ + AcONa	Acetone	RT	16 h	No reaction
138	$KMnO_4$ + AcOH + piperidine	Acetone	RT	16 h	28.5 %

[93] P. Saisaha, D. Pijper, R. Van Summeren, R. Hoen, C. Smit, J. de Boer, R. Hage, P. Alsters, B. Feringa, W. Browne *Org. Biomol. Chem.*, **2010**, *8*, 4444-4450

[94] F. Fernandez, G. Garcia, J. Rodriguez, *Synthetic Commun.*, **1990**, *20*, 2837-2847

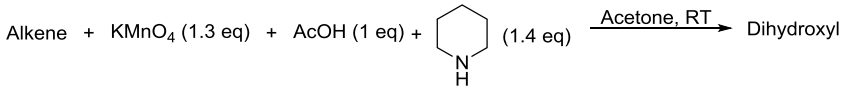
[95] P. Dupau, R. Epple, A. Thomas, V. V. Fokin, K. B. Sharpless, *Adv. Synth. Catal.*, **2002**, *344*, 421-433

Fortunately, the last conditions using potassium permanganate with acetic acid and piperidine in acetone at room temperature gave a positive result by leading to the dihydroxylation product **143** from alkene **138** in 28.5 % yield.



143

In order to exploit the reaction's conditions efficacy, the other alkenes were submitted to these conditions (Scheme 61). The results are reported in Table 37.



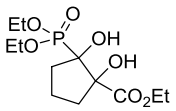
144 **145** **146** **147-155**

Scheme 61: Exemplification of the dihydroxylation reaction

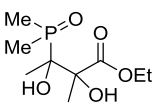
Table 37: Exemplification of the dihydroxylation reaction

Product	Alkene	Yield and observation
147	**127**	No reaction
148	**128**	9.4 %
149	**129**	No reaction
150	**133**	No reaction
151	**134**	No reaction
152	**136**	7 %
153	**137**	No reaction
154	**139**	6 mg of impure **154**
155	**141**	No reaction

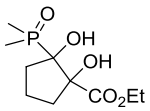
The attempts of dihydroxylation of alkenes under these conditions didn't give many interesting products as could have been expected even if some of them have been obtained pure. Only three dihydroxylation products have been isolated.



143



148

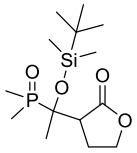


152

IV. Conclusion

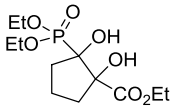
This family of potential KARI inhibitors was inspired by the variation of the double chelating pincer motif. The first pathway thought of was based on Pudovik reactions between branched-chain linear β-ketoesters **110** or cyclic β-ketoesters **112**-**113** or lactone **111** and different P-H reactants **51**.

The Pudovik reactions proved to be inefficient with classical conditions using amine Brønsted bases or more original systems involving $Ti(O\text{-}iPr)_4$ or silyldimethylphosphinite $P^{(III)}$ derivative except for one reaction which gave the Pudovik product **118**.

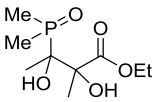


118

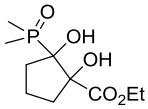
The targets were then modified in order to facilitate the synthesis and provide the compounds for biological testing, starting from the same β-ketoesters **110**-**113**. The P-H reagents **51** are successfully and easily introduced on the corresponding enol triflates **127**-**141** thanks to a pallado-catalysed cross coupling reaction. The alkenes thus obtained were submitted to epoxidation and dihydroxylation reactions. Unfortunately the epoxidation didn't work at all, however the dihydroxylations gave some of the expected products by using potassium permanganate but in very small yields due to very harsh conditions.



143



148

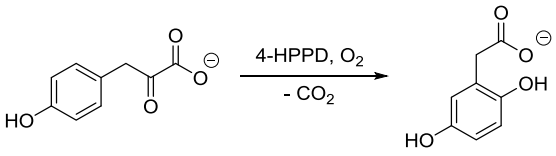


152

Chapter VI: Synthesis of HPPD-like potential inhibitors

I. Triketone as potent herbicides

4-hydroxyphenylpyruvate deoxygenase (4-HPPD) intervenes in the tyrosine degradation pathway which is widespread in nature. 4-HPPD catalyses the transformation of 4-hydroxyphenylpyruvate **156**, deriving from tyrosine, in homogentisate **157** with molecular oxygen, in presence of a ferrous Fe^{2+} cofactor through a decarboxylation and a rearrangement step (Scheme 62).[96] The substrate has a K_M = 5 – 8 µM.

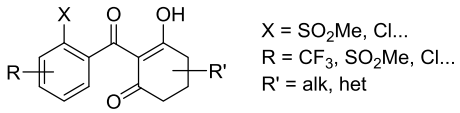


156 **157**

Scheme 62: biotransformation catalysed by 4-HPPD

Even if all aerobic organisms including animals and plants produce this enzyme, the latter are far more sensitive to specific inhibitors because in addition to the tyrosine degradation pathway, 4-HPPD participates also in the biosynthesis of plastoquinones and tocopherols. These two molecule families are respectively essentials in the photosynthetic electron transport chain and the antioxidative systems. The failure of the first biological function leads to the plant's death. The discovery and understanding of this interesting biological target let researchers develop a new class of herbicides.

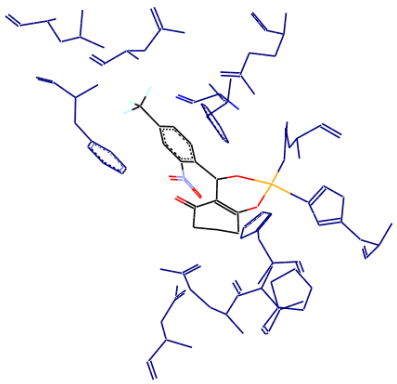
The major family of inhibitors designed to jam the biotransformation by 4-HPPD and ensure the leaf bleaching, by the release of chlorophyll from chloroplasts, is the triketone class of herbicides. It was studied and synthesised since the beginning of the 80s and their generic structure is shown below:



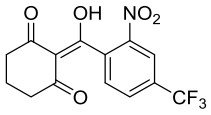
Scheme 63: generic structure of triketone herbicides

Dr C-S. Wu *et al.* determined in 2002 their mode of action thanks to structural activity relationship studies of the inhibitors and their respective affinity with the iron cation. The inhibition is based on the chelation of the ferrous ion in the active site in order to block the 4-HPPD protein.[97]

[96] I. Garcia, M. Rodgers, R. Pepin, T-F. Hsieh, M. Matringue, *Plant Physiol.*, **1999**, *119*, 1507-1516
[97] C-S. Wu, J-L. Huang, Y-S. Sun, D-Y. Yang, *J. Med. Chem.*, **2002**, *45*, 2222-2228



Brownlee and his colleagues purified the 4-HPPD protein from the bacterium *Streptomyces avermitilis* in 2004 and studied the crystal structure of the enzyme with the inhibitor 2-[2-nitro-4-(trifluoromethyl)benzoyl]-1,3-cyclohexanedione (NTBC) **158**.[98] The mode of action of the inhibitor, through chelation of the iron cation in yellow, is noticeable in the catalytic site modelisation on the left. This triketone compound has an IC_{50} = 40 nM for rat liver 4-HPPD. [99]



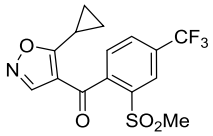
158

Figure 11: Modelisation of NTBC in *S. Avermitilis* 4-HPPD catalytic site from X-ray crystal structure

II. Biological targets

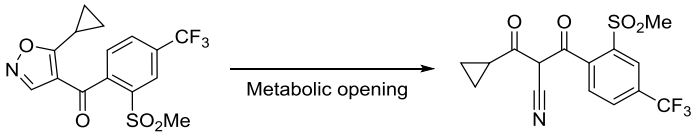
The aim of this compound family is to mimic triketone structures to exploit their good chelation properties which would be applicable to KARI.

One of the good representatives of the triketone family is the isoxaflutole **159**, which was first synthesised in the 90s by Rhône-Poulenc and is now part of a Bayer formulation.



159

This structure is not the active form of the molecule, it is considered as a prodrug or a pro-herbicide as it undergoes a metabolic opening of the isoxazole ring, leading to the diketonitrile (DKN) derivative **160**.[100]



159 **160**

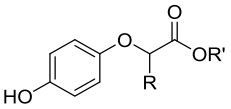
Scheme 64: Formation of the active diketonitrile principle

[98] J. Brownlee, K. Jonshon-Winters, D. H. T. Harrison, G. R. Moran, *Biochem.*, **2004**, *43*, 6370-6377

[99] M. K. Ellis, A. C. Whitfield, L. A. Gowans, T. R. Auton, W. McLean Provan, E. A. Lock, L. L. Smith, *Toxicol. Appl. Pharm.*, **1995**, *113*, 12-19

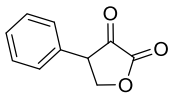
[100] K. E. Pallett, J. P. Little, M. Sheekey, P. Veerasekaran, *Pestic. Biochem. Phys.*, **1998**, *62*, 113-124

In 2002, Dr Y.-L. Lin *et al.* designed some new 4-HPPD inhibitors deriving from the enzyme substrate **156**.[101] They have shown that by keeping the α-ketoester moiety, the chelation of the inhibitor to the iron cation in the catalytic site is still strong, thus improving the inhibition compared to other molecules tested as reported in **Table 38**.



a R = H, R' = Et
b R = Me, R' = Et
c R = H, R' = H
d R = Me, R' = H

161

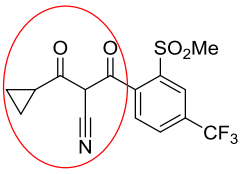


162

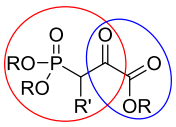
Table 38: IC_{50} of some 4-HPPD susbtrate analogues

Compounds	IC_{50} (µM)
161a	73
161b	588
161c	6
161d	93
162	0.5

The α-ketolactone **162** exhibits a promising IC_{50} on 4-HPPD. It may be a base structure, as DKN **160**, in order to develop some new compounds. The chelating unit from **160** including the cyano group may be united to the ketoester from the substrate analogue **162** in order to obtain the double chelating-pincer necessary for KARI's inhibition. Furthermore, in **160** an alkyl group is located on the α position of the ketone, it would thus be interesting also to replace the cyano by a methyl group.

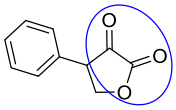


160



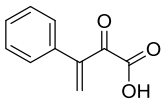
a R = Et, R' = CN
b R = Et, R' = Me
c R = Me, R' = H

163



162

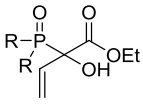
Similarly, other compounds could be designed and synthesised by adapting the intermediate **164** from the synthesis of **162** which spontaneously cyclised.



164

[101] Y-L. Lin, J-L. Huang, C-S. Wu, H-G. Liu, D-Y. Yang, *Bioorg. Med. Chem. Lett.*, **2002**, *12*, 1709-1713

In order to obtain small molecules to fit in KARI's catalytic site, the phenyl group may be avoided and a phosphorus moiety introduced to achieve the double chelating pincer.

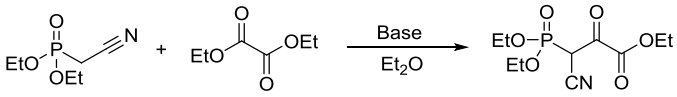


a = Me
b = Ph
c = OEt

165

1) Phosphonate diketonitrile analogue

The DKN analogue **163a** may be synthesised from the corresponding diethyl cyanomethylphosphonate **166** and diethyl oxalate **167** with a base in diethyl ether (Scheme 65).[102] It proceeds through a SN_2t (addition-elimination reaction) mechanism with the attack of the diethyl cyanomethylphosphonate carbanion on one of the carbonyls of diethyl oxalate.



166 **167** **163a**

Scheme 65: DKN analogue synthesis

The protons on the methylene moiety are rather acidic with a pKa = 17 in DMSO, due to the presence of two electron-withdrawing groups. Two Brønsted bases were tested: sodium hydride and potassium *tert*-butoxide with 18-crown-6 ether to enhance its basicity, as summarised in Table 39.

Table 39: DKN analogue 163a synthesis assays

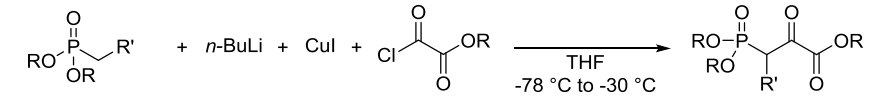
Bases	Temperature	Time	Observations
NaH	RT	2 h	No reaction
*t*BuOK, 18-crown-6	RT	2 h	Diethyl phosphate and starting phosphonate are observed by ^{31}P NMR

Unfortunately the two sets of conditions didn't give any expected DKN **163a**. When sodium hydride was the base, the reaction was monitored by ^{31}P NMR showing neither no evolution of the starting phosphonate **166**, nor the appearance of a new phosphorus containing product. However with potassium *tert*-butoxide and 18-crown-6 ether, diethyl phosphonic acid was observed along with the starting material **166**. The presence of diethylphosphonic acid may be explained by some Horner-Wadsworth-Emmons reaction occurring between two molecules of **163a** or one equivalent of product **163a** and one from the starting **166**.

[102] W. Grell, H. Machleidt, *Justus Liebigs Ann. Chem.*, **1966**, *693*, 134-157

2) Phosphonate ketoester

In order to synthesise **163b** and **163c** another methodology was applied. Starting from diethyl ethanephosphonate **168a** or dimethyl methanephosphonate **168b** and ethyl chlorooxoacetate with an organocuprate as one of the reaction intermediates.[103]



a R = Et, R' = Me
b R = Me, R' = H

168

a R = Et
b R = Me

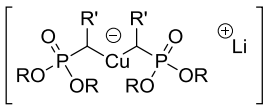
169

b R = Et, R' = Me
c R = Me, R' = H

163

Scheme 66: Phosphonate ketoesters synthesis

The organocuprate **170** was synthesised by deprotonation of the phosphonates with butyllithium followed by a metal exchange reaction between lithium and copper (I).



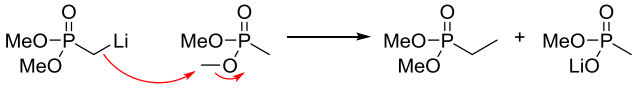
a R = Et, R' = Me
b R = Me, R' = H

170

Table 40: Organocuprate methodology assays

Products	Time	Observations
163b	1 h 20	68% yield
163c	Overnight	No expected product

163b was easily obtained in 68% yield, which wasn't the case for the synthesis of **163c**. When monitored by GC-MS the addition product **163c** wasn't observed at all, instead the autocondensation of the anion **171** from dimethyl methanephosphonate **168b** was observed (Scheme 67). Indeed, the lithium carbanion **171** is too reactive even at low temperatures such as -60 °C. The metal exchange is too slow to occur before, therefore leading to a mixture of dimethyl ethanephosphonate **172** and lithium monomethyl methanephosphonate **173**.



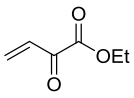
171 **168b** **172** **173**

Scheme 67: Autocondensation of dimethyl methanephosphonate

[103] P. Coutrot, P. Savignac, *Synthesis*, **1978**, *1978*, 36-38

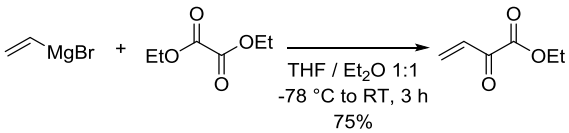
3) Vinylic derivatives

This vinylic derivative may be synthesised starting from ethyl 2-oxobutenoate **174** and phosphorus sources thanks to Pudovik reactions.



174

Ethyl 2-oxobutenoate **174** was prepared from the reaction of vinyl magnesium bromide on diethyl oxalate in a mixture of THF and diethyl ether (Scheme 68).[104]

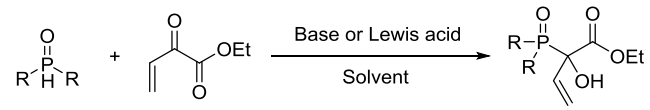


175 **176** **174**

Scheme 68: Ethyl 2-oxobutenoate preparation

174 was prepared very easily in 75% yield and could then be further used for the Pudovik reactions.

Dimethylphosphine oxide **51a**, diphenylphosphine oxide **51b** and diethyl phosphite **51c** were assayed for the reactions. Pudovik reactions were attempted by transposing previously applied conditions such as triethylamine in toluene with microwave activation, caesium fluoride-alumina heterogenous system or calcium oxide (Scheme 69). The two first sets of conditions have proven their efficiencies on saturated carbonyls but calcium oxide has been studied specifically on α,β-unsaturated carbonyls.[105] The authors have shown that depending on the type of carbonyls, either aldehydes, ketones or esters, the selectivity of the addition of the P-H compounds was different. When α,β-unsaturated ketones or aldehydes were the starting materials, they observed only a 1,2-addition, which was not the case on unsaturated esters for which it was a 1,4-addition.



a R = Me
b R = Ph
c R = OEt

51 **174** **165**

Scheme 69: DKN vinyl analogue synthesis

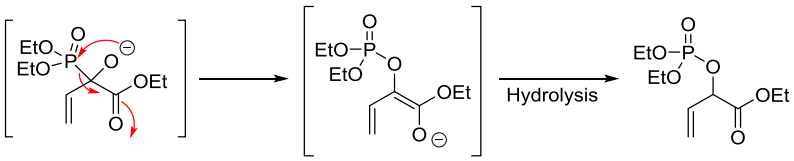
[104] M. Rambaud, M. Bakasse, G. Duguay, J. Villieras, *Synthesis*, **1988**, 564-566
[105] E. Martinez-Castro, O. Lopez, I. Maya, J. G. Fernandez-Bolaños, M. Petrini, *Green Chem.*, **2010**, *12*, 1171-1174

The results of the different reactions are summarised in Table 41.

Table 41: Pudovik reactions to prepare the vinyl analogue of DKN

Phosphorus	Base or Lewis acid	Solvent	Temperature	Time	Observation
51a	Al_2O_3-CsF	Dioxane	RT	3 h 30	No reaction, **51a** recovered
51b	Al_2O_3-CsF	Dioxane	RT	3 h 30	67 % of starting **51b**, 24 % of diphenylphosphinic acid
51c	Al_2O_3-CsF	Dioxane	RT	3 h 30	Phosphates and diethylphosphinic acid
51c	Et_3N	Toluene	Microwave, 150 °C, 200 W	15 min	No reaction, **51c** recovered
51c	CaO	Neat	RT	1 day	No more **51c** but only phosphates

Surprisingly either the reactions didn't occur with the different phosphorus sources **51** either the phosphorus compounds were degraded into their corresponding acids. However with diethyl phosphite, different signals observed by ^{31}P NMR translated the appearance of phosphate esters. It may be explained by a [1,2]-phospha Brook rearrangement (formerly a 1,2-Wittig rearrangement involving heteroatoms) of the intermediate during the Pudovik reaction as depicted in Scheme 70.



Scheme 70: [1,2]-phospha Brook rearrangement to phosphate

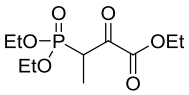
This type of rearrangement has been documented since the end of the 90s in apolar solvents and at room temperature with amine Brønsted base on systems including ketones deactivated by fluorinated electron withdrawing groups on the α position.[106] Later it has been studied on more similar molecules to ethyl 2-oxobutenoate **174**, either on ketoesters or ketoamides.[107]

[106] M. Kuroboshi, T. Ishihara, T. Ando, *J. Fluorine Chem.*, **1988**, *39*, 293-298
[107] M. Hayashi, S. Nakamura, *Angew. Chem. Int. Ed.*, **2011**, *50*, 2249-2252

III. Conclusion

The triketone family of compounds have proven its efficiency for nearly thirty years in the inhibition of HPPD by complexation within the catalytic site of the enzyme. Different structures were the results of the inspiration from this class of herbicides.

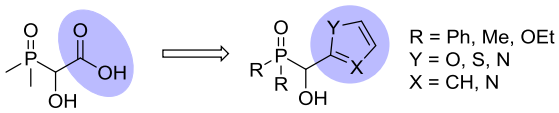
Different analogues from DKN were tried to be synthesised. Although the majority of the synthesis has shown to be unsuccessful, one compound, **163b**, has been fortunately obtained.



163b

Chapter VII: Bioisosteres and mimics of carboxylic acids

As explained in a previous chapter (cf Inhibition of KARI, I) Mechanism of action, 6) Inhibitors p45), Hoe 704 is a potent *in vitro* herbicide, not *in vivo*. Thus Hoe 704 may be a good basis for designing new structures. A way of developing new potential inhibitors would be to keep the phosphoryl and the hydroxyl groups and focus on the carboxylic acid part by introducing planar 5-membered heterocycles (Scheme 71).



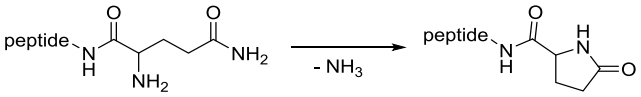
Scheme 71: Carboxylic acid replacement by heterocycles

Small heterocycles have found a great interest in drug design because of their hydrogen bond donor and acceptor capacity, their ability to participate in π-stacking interactions and also thanks to the metal chelation properties of some of them. Moreover they keep the same planar geometry as carboxylic acids and their derivatives. By combining these properties, 5-membered heterocycles would be interesting groups to introduce in order to replace the carboxylic acid one.

I. Heterocycles as chelating moieties in drug design

1) Imidazoles and other heterocycles as inhibitors of Glutaminyl Cyclase

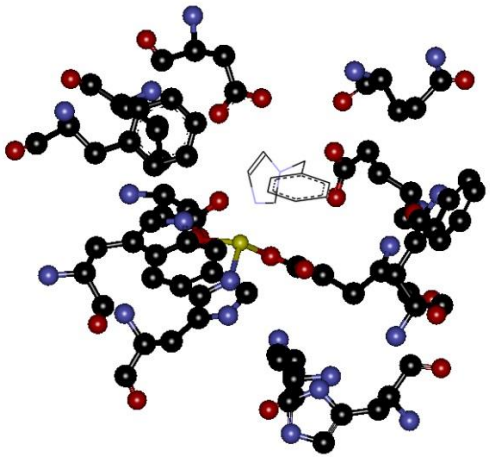
The human glutaminyl cyclase (QC) has been identified as a metalloenzyme. It belongs to the acyltransferase family and catalyses the conversion of N-terminal glutaminyl residues into pyroglutamic acid as shown in Scheme 72. Schilling *et al.* identified imidazole derivatives as potent inhibitors of QC by competitively binding to the catalytic site bearing a divalent cation.[108]



Scheme 72: Reaction catalysed by human QC

According to microbiology assays and crystal structures observations, the potent inhibitors imidazole derivatives are indeed chelating the metal cation in the catalytic site as depicted in Figure 12. The *N*[1]-benzylimidazole, co-crystallised with the enzyme, is the most potent one as it inhibits the protein with a K_i = 7.1 µM.

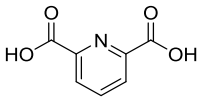
[108] S. Schilling, A. J. Niestroj, J-U. Rahfeld, T. Hoffmann, M. Wermann, K. Zunkel, C. Wasternack, H-U. Demuth, *J. Biol. Chem.*, **2003**, *278*, 49773-49779



In this simplified representation of the catalytic site of human QC, obtained from an X-ray crystal structure, N^1-benzylimidazole in thin lines clearly has his N^2 pointing towards the zinc cation in gold colour.[109]

Figure 12: Modelisation, from X-ray structure, of human QC catalytic site

In addition to imidazole derivatives, dipicolinic acid **177** has been tested successfully as a competitive inhibitor of human QC[108], which highlights also pyridine derivatives as interesting chelating heterocycles.



177

2) Heterocycles as effective metal chelators of HIV-1 integrase

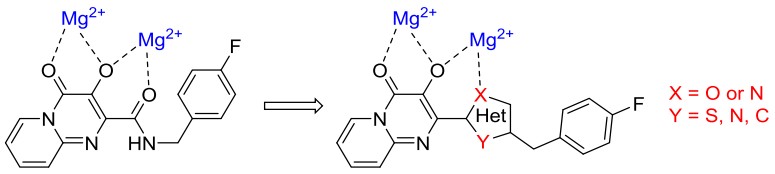
HIV-1 integrase is a key enzyme in the human immune-deficiency virus type 1 as it participates in the integration of the viral DNA strand inside the host genome leading to acquired immune-deficiency syndrome, better known as AIDS.

HIV infection and the establishment of proviral latency depend critically on the insertion of viral DNA into host genome. This integration process happens through two distinct steps. The virally encoded enzyme integrase (IN) catalyses both reactions and thus represents an important target for HIV treatment. Inhibitors with a novel structure core are essential for combating resistance associated with known IN inhibitors (INIs).

This enzyme shares the same particularity as KARI. They both bind two metal cations in their catalytic sites in order to be functional and effective.[110] Through rational design, a chelating triad capable of binding two Mg^{2+} ions showed promising activities.

[109] K-F. Huang, Y-L. Liu, W-J. Cheng, T-P. Ko, A. H-J. Wang, *PNAS*, **2005**, *102*, 13117-13122
[110] D. Rhodes T. Peat, N. Vandergraaff, D. Jeevarajah, G. Le, E. Jones, J. Smith, J. Coates, L. Winfield, N. Thienthong, J. Newman, D. Lucent, J. Ryan, G. Savage, C. Francis, J. Deadman, *Antiviral Chem. Chemother.*, **2011**, *21*, 155-168

J. J. Deadman *et al.* reported in 2010 a new scaffold designed as a HIV-1 integrase inhibitor.[111] They have developed new bicyclic pyrimidine derivatives **178**. The same year, after working on the scaffold in order to have a structural base to work on, the researchers designed metal chelators by including nitrogen containing heterocycles **179** to mimic the amide function as depicted in Scheme 73.[112]

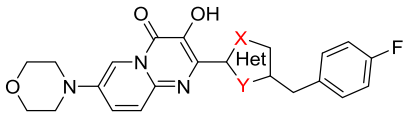


178 **179**

Scheme 73: Amide replacement by heterocycles

Nitrogen-containing heterocycles are known to simulate amide functional groups. Different 5-membered ring have been tested, especially imidazole, 1,3-thiazole, and 1,3-oxazole (Table 42).

Table 42: Difference of bioactivity between heterocycles



Heterocycle	IC_{50} (nM)
X = N, Y = S thiazole	20
X = N, Y = O oxazole	59
X = N, Y = N imidazole	45

The five-membered heterocycles have proven that they can easily replace some chelating functional groups by preserving the chelation properties in the designed molecule. In addition, their geometry and thermodynamic properties make them good carboxylic acids bioisosteres.

[111] E. D. Jones, N. Vandergraaff, G. Le, N. Choi, W. Issa, K. Macfarlane, N. Thienthong, L. J. Winfield, J. A. V. Coates, L. Lu, X. Li, X. Feng, C. Yu, D. I. Rhodes, J. J. Deadman, *Bioorg. Med. Chem. Lett.*, **2010**, *20*, 5913-5917
[112] G. Le, N. Vandergraaff, D. I. Rhodes, E. D. jones, J. A. V. Coates, N. Thienthong, L. J. Winfield, L. Lu, X. Li, C. Yu, X. Feng, J. J. Deadman, *Bioorg. Med. Chem. Lett.*, **2010**, *20*, 5909-5912

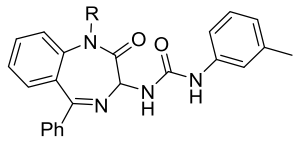
II. Carboxylic acids and derivatives replacement by heterocycles

1) 1,4-benzodiazepine derivatives as Cholecystokinin type B (CCK-B) antagonists

Cholecystokinin type B (CCK-B) is a peptide hormone activating the CCK-B receptor which is coupled to a G protein at the cell surface. When activated, the CCK-B receptor stimulates the digestion of fat and protein in the intestine by causing the release of bile and digestive enzymes. However when activated in the brain it regulates the dopamine release. The development of CCK-B receptor antagonists would help people suffering from irritable bowel syndrome which can be triggered by stress for example.

In 1993 M. Bock *et al.* reported the development of new non-peptide ligands of the CCK-B receptor showing antagonist activity.[113] The 1,4-benzodiazepine based structure has been substituted differently on fixed positions with acidic moieties, in order to find the compound showing the best activity. Among the substituents tested were the carboxylic acid substituted molecule and the imidazole one as detailed in Table 43.

Table 43: Bioactivity of two different 1,4-benzodiapine on CCK-B receptor



R group	IC_{50} (µM) on CCK-B receptor
$-CH_2COOH$	0.38
	0.059

The imidazole substituted 1,4-benzodiazepine displays a 6.4 time decreased IC_{50} compared to the carboxylic acid counterpart. The imidazole ring seems to be a good alternative for replacing the carboxylic acid group and affording more efficiency to the antagonist molecule.

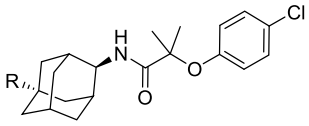
2) 11β-hydroxysteroid dehydrogenase inhibitors (11β-HSD1)

11β-hydroxysteroid dehydrogenase catalyses the conversion in the liver of biologically inactive 11-ketosteroids such as cortisone into their active 11β-hydroxy derivatives. This biological target has proven to be interesting to inhibit, in order to find treatments against nowadays metabolic diseases, diabetes and obesity.

[113] M. Bock, R. DiPardo, B. Evans, K. Rittle, W. Whitter, V. Garsky, K. Gilbert, J. Leighton, K. Carson, E. Mellin, D. Veber, R. Chang, V. Lotti, S. Freedman, A. Smith, S. Patel, P. Anderson, R. Freidinger *J. Med. Chem.*, **1993**, *36*, 4279-4292

J. Lee and his group studied *in silico*, the docking of 70 compounds that have already proven their biological activity as inhibitors of 11β-HSD1.[114] On one general structure deriving from adamantine, two structures are interesting to be compared to (Table 44).

Table 44: IC_{50} of two inhibitors of 11β-HSD1



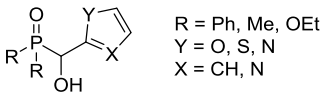
R group	IC_{50} (nM)
$-CH_2COOH$	44.7
	10

As shown previously, once again replacing the carboxylic acid by a 5-membered ring such as imidazole proved to be beneficial for the overall activity of the compound.

Carboxylic acids and derivatives such as esters or amides are planar functional groups due to resonance forms. The moiety replacing the carboxylic acid group of Hoe 704 should also be planar in order to respect the geometry of the molecule and potentially insure a bioactivity. 5-membered heteroatom containing rings answer well to the awaited expectations on geometry and combine chelation properties with good bioisosteric characteristics of carboxylic acid derivatives. Therefore introducing heterocyles may be an interesting way of developing potential inhibitors of KARI.

III. Target compounds.

In order to prepare the target compounds **180**, Pudovik reactions are involved between dimethylphosphie oxide **51a**, diphenylphosphine oxide **51b** or diethyl phosphite **51c** and different heterocyclic carboxaldehydes.



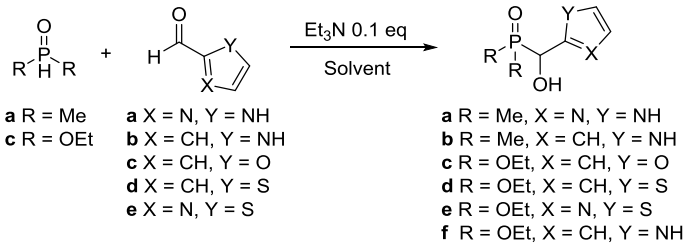
180

Different set of conditions have been tested, at first simple classical basic catalysis with triethylamine.

[114] J. H. Lee, N. S. Kang, S-E. Yoo, *Bioorg. Med. Chem. Lett.*, **2008**, *18*, 2479-2490

1) Triethylamine catalysis for Pudovik reactions.

The catalysis of Pudovik reactions by an amine base such as triethylamine has long proven its efficiency. This set of conditions has been tried for preparing 1-(dimethylphosphinoyl)-1-hydroxy-2-*O*-benzylpropane. It has been applied to different 2-carboxaldehyde heterocycles **181** with dimethylphosphine oxide **51a** or diethyl phosphite **51c** (Scheme 74). The results are detailed in Table 45.



51 **181** **180**

Scheme 74: Triethylamine catalysed Pudovik reaction

Table 45: Attempts to prepare different Pudovik adducts 180, catalysed by triethylamine

Product	Phosphorus Derivative	Aldehyde	Solvent	Temperature	Time	Observations
180a	**51a**	**181a**	Toluene	MW 150 °C 200 W	30 min	No reaction
180b	**51a**	**181b**	Toluene	MW 150 °C 200 W	30 min	No reaction
180c	**51c**	**181c**	1,4-dioxane	RT	2 h	No reaction
180d	**51c**	**181d**	1,4-dioxane	RT	2 h	No reaction
180e	**51c**	**181e**	1,4-dioxane	RT	Overnight	52 % conversion
180f	**51c**	**181b**	1,4-dioxane	RT	2 h	No reaction

The reactions weren't effective no reaction occurred except for **180e** for which 52 % conversion were observed but after a long reaction time.

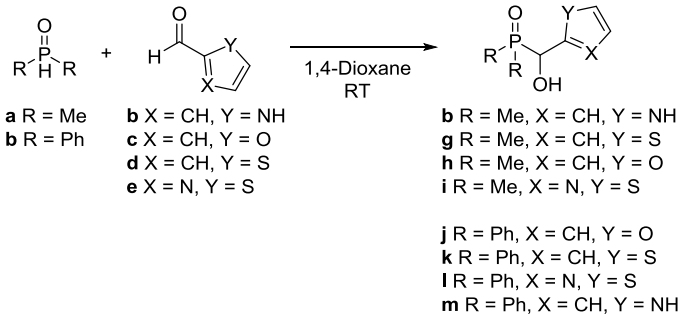
In another attempt to synthesise **180b**, triethylamine was replaced by polyvinylpyridine, a polymer supported pyridine. The reaction was conducted in 1,4-dioxane at room temperature during 1h30. However as for the previous assay with triethylamine, no expected product could be observed by ^{31}P NMR spectroscopy, only the starting **51a**.

Amine bases do not seem to be the best reactants to make the reactions occur. As these conditions haven't been effective, another way must be found.

2) Base-free Pudovik reactions

While working on the Pudovik reaction of diorganylphosphine oxides and 1-organyl-2-carboxaldehydeimidazoles, N. K. Gusarova *et al.* developed in 2002 a very simple procedure which affords the Pudovik adducts in good yields.[115] It consists in introducing at once one equivalent of diorganylphosphine oxide and one of 1-organyl-2-carboxaldehydeimidazole in dioxane and let the medium be stirred at room temperature for an hour. The driving force of the reaction is the formation of precipitates corresponding to the products.

All the products successfully synthesised by using this method were solids that precipitated in 1,4-dioxane. Scheme 75 summarises the different 2-carboxaldehyde heterocycles that have been reacted with dimethylphosphine oxide or diphenylphosphine oxide, the results are enclosed in Table 46.



51 **181** **180**

Scheme 75: Pudovik reaction in 1,4-dioxane

Table 46: Pudovik reactions in 1,4-dioxane at room temperature

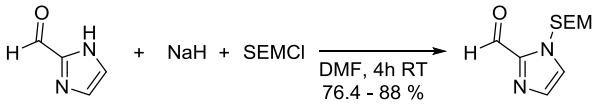
Products	Time	Yields and observations
180b	1h30	No reaction
180g	1h30	65 % conversion with 17 % remaining **51a** and 8 % dimethylphosphinic acid
180h	1h30	58 %
180i	1 h	No reaction
180j	1h30	70 %
180k	1h30	65 - 83 %
180l	1h	73 %
180m	1h	74 %

This simple method provided in medium to good yields the expected products except for **180b** and **180i**. As mentioned by R. Boobalan and C. Chen in 2013, *N*-non protected pyrrole-2-carboxaldehyde **181b** wasn't prone to the Pudovik reaction until they established a new catalytic system involving

[115] N. K. Gusarova, S.N. Arbuzova, A. M. Reutskaya, N. I. Ivanova, L. V. Baikalova, L. M. Sinegovskaya, N. N. Chipanina, A. V. Afonin, I. A. Zyryanova, *Chemistry Heterocycl Compd*, **2002**, *38*, 65-70

$Fe^{(III)}$.[116] This statement is confirmed also by literature search which has only resulted in this publication without further explanations. Surprinsingly when *N*-non protected **181b** was reacted with diphenylphosphine oxide **51b**, the reaction yielded **180m** in good yield. N. K. Gurasova *et al.* only worked on *N*-protected **181b**[115], this idea is followed for the next Pudovik reactions.

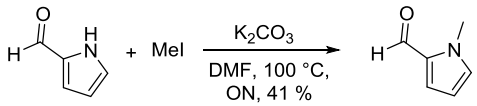
As suggested in the publication of Gurasova, the acidic proton from imidazole-2-carboxaldehyde **181a** have been masked. One of the nitrogen had its hydrogen replaced by a methyl group (Scheme 77) and at the same time the nitrogen atom was protected with a [2-(trimethylsilyl)ethoxy]methyl acetal (SEM) group (Scheme 76), hoping for an easy cleavage after the Pudovik reaction in order to have the free nitrogen back.



181a **181f**

Scheme 76: SEM protection of imidazole

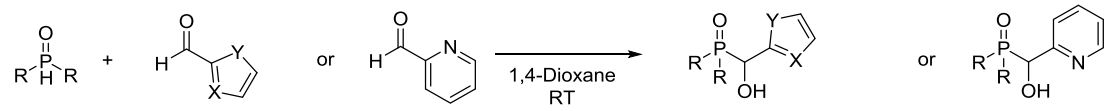
For **181b**, the nitrogen was functionalised by a methyl group thanks to a substitution reaction with iodomethane affording *N*-methylpyrrole 2-carboxaldehyde **181g** in 41 % yield.



181b **181g**

Scheme 77: N methylation of 181b

Afterwards, the Pudovik reactions were conducted as previously with the functionalised and protected heterocycles **181f** and **181g**. In order to complete the family, pyridine-2-carboxaldehyde **182** is added to the scope (Scheme 78).



a R = Me
b R = Ph

f X = N, Y = N-SEM
g X = CH, Y = N-Me

n R = Me, X = N, Y = N-SEM
o R = Me, X = CH, Y = N-Me
p R = Ph, X = N, Y = N-SEM
q R = Ph, X = CH, Y = N-Me

a R = Me
b R = Ph

51 **181** **182** **180** **183**

Scheme 78: Pudovik reaction in 1,4-dioxane

When the acidic proton of the nitrogen-containing heterocycles is hidden, all the Pudovik reactions worked successfully as described in Table 47.

[116] R. Boobalan, C. Chen, *Adv. Synth. Catal.*, **2013**, *355*, 3443-3450

Table 47: Pudovik reactions in 1,4-dioxane at room temperature

Products	Time	Yields and observations
180n	1h30	63 – 96 %
180o	1h30	31 %
180p	1h30	50 – 70 %
180q	1h	71 %
183a	1h	32 %
183b	1h	72 %

Following the synthesis of **180n** and **180p**, SEM deprotection reactions were attempted. Either the SEM-nitrogen bond is cleaved with tetrabutylammonium fluoride (TBAF) in THF or with an excess of trifluoacetic acid in a mixture of ethanol and dichloromethane (Scheme 79).

n R = Me
p R = Ph

180

a R = Me
r R = Ph

180

Scheme 79: SEM deprotection

Both deprotection conditions are compared in Table 48.

Table 48: Comparison of SEM deprotection conditions

Products	Reagent	Solvent	Time	Temperature	Observations
180r	TBAF	THF	Overnight	RT	No reaction, **180p** recovered
180r	Trifluoroacetic acid	EtOH / DCM	Overnight	RT	Quantitative
180a	Trifluoroacetic acid	EtOH / DCM	Overnight	RT	61 %

The first trial with TBAF didn't give the expected product, so trifluoroacetic acid was tested as a deprotection reagent. In order to achieve complete deprotection of SEM-imidazole for **180r** a considerable excess of trifluoroacetic acid, up to 30 equivalents, had to be introduced. The diphenylphosphinoyl group of **180p** was stable enough under these conditions unlike the dimethylphosphinoyl in **180n** which gave the corresponding dimethylphosphinic acid **63**.

In parallel, other conditions involving caesium fluoride and alumina have been tested as Pudovik conditions especially with diethyl phosphite **51c**.

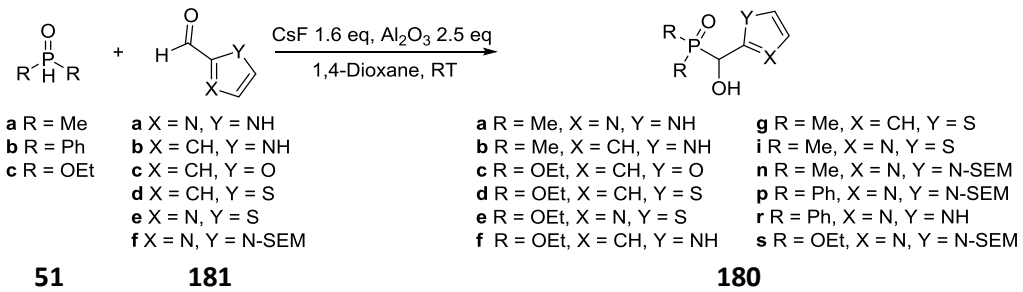
3) $CsF\text{-}Al_2O_3$ as a heterogenous basic system for Pudovik reactions

In 1984 D. Villemin and M. Ricard studied the activation of weak acid C-H bond by adsorption on the heterogenous system $KF\text{-}Al_2O_3$.[117] They showed that this inorganic mixture is:

- basic enough to deprotonate terminal aromatic alkyne (pKa = 18.5 in water) or methyl phenyl sulfoxide (pKa = 30 in water) to achieve condensation with benzaldehyde
- complexing the carbonyl and the alkoxide during the condensation.

Later, F. Texier-Boullet published a paper on the synthesis of 1-hydroxyalkane phosphonic esters thanks to Pudovik reactions between dialkylphosphites and non-activated ketones with the $KF\text{-}Al_2O_3$ system.[83] Moreover in the beginning of the 80s, it has been demonstrated that CsF is regarded as the most active fluoride source compared to KF.[118]

Properties of CsF as more active fluoride anion than KF and the strong basicity and complexing capacities of the $KF\text{-}Al_2O_3$ system are combined in $CsF\text{-}Al_2O_3$ used here for the Pudovik reactions between **51a-c** and different carboxaldehyde heterocycles (Scheme 80).



Scheme 80: $CsF\text{-}Al_2O_3$ mediated Pudovik reactions

The results of the different Pudovik assays are presented below in Table 49.

Table 49: Results of $CsF\text{-}Al_2O_3$ mediated Pudovik reactions

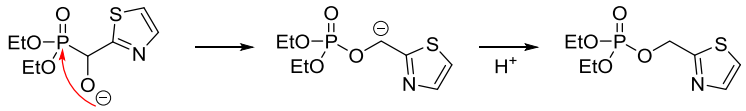
Products	Time	Observations and yields
180a	2h	31 % conversion in **180a** and 69 % of dimethylphosphinic acid **63**
180b	2h	94 %
180c	4h30	61 – 85 %
180d	4h30	78 %
180e	4h30	No **180e** but rearrangement product
180f	4h30	No reaction
180g	2h	Quantitative
180i	2h	51 %
180n	3 days	79 %
180p	3 days	51 %

[117] D. Villemin, M. Ricard, *Tet. Lett.*, **1984**, *25*, 1059-1060
[118] J. H. Clark, *Chem. Rev.*, **1980**, *80*, 429-452

Products	Time	Observations and yields
180r	2h	No reaction
180s	3 days	43 %

These conditions seem to be pretty efficient to synthesise the Pudovik adducts. They have been a good alternative to the simple conditions presented previously with no other reactant than the p-H compound and the carboxaldehyde heterocycles. For **180b** or **180i** it has been the only pathway to obtain the expected molecules.

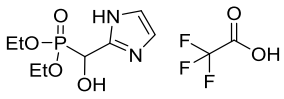
Surprisingly **180e** wasn't obtained at the end of the reaction between diethyl phosphite **51c** and thiazole 2-carboxaldehyde **181e**. The product observed instead, according to the ^{1}H NMR and ^{31}P NMR spectra, may be resulting from a Brook-type [1,2]-rearrangement leading to the phosphate **184** as explained in Scheme 81.[119]



180e **184**

Scheme 81: Brook-type [1,2]-rearrangement

The compound **180s** has been deprotected with the same conditions as for **180a** and **180r**, with trifluoroacetic acid in a mixture DCM/ethanol. **180t** was obtained with 59% yield.



180t

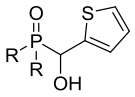
[119] Example of phospha-Brook rearrangement in synthesis: M. Hayashi, S. Nakamura, *Angew. Chem. Int. Ed.*, **2011**, *50*, 2249-2252

IV. Conclusion

According to the literature, by relying on the similar chelating properties and the similar spatial organisation of heterocycles compared to carboxylic acid derivatives, the former have proven to be good mimics and bioisosteres of carboxylic acids in drug design.

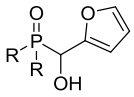
Potential analogs of Hoe 704 have been successfully synthesised thanks to Pudovik reactions between heterocycles 2-carboxaldehyde and P-H compounds. To achieve good yields, three sets of conditions have been tested including microwave activation, base-free conditions and the last ones which are heterogenous reactions with basic CsF-Al_2O_3. Unfortunately the microwave activation didn't give any positive results whereas base free conditions and F. Texier-Boullet's heterogenous system were very efficient.

15 products have been obtained as summarised below:



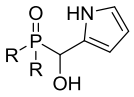
R = OEt, Me, Ph

180d **180g** **180k**



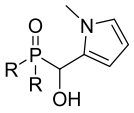
R = OEt, Me, Ph

180c **180h** **180j**



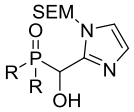
R = Me, Ph

180b **180m**



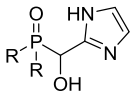
R = Me, Ph

180o **180q**



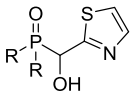
R = Me, Ph, OEt

180n **180p** **180s**



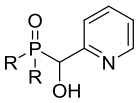
R = Me, Ph, OEt

180a **180r** **180t**



R = Me, Ph

180i **180l**



R = Me, Ph

183a **183b**

Some difficulties have been encountered on the imidazole derivative as the acidic proton had to be masked by a SEM group in order to do the Pudovik reaction. Moreover the deprotection needed harsh conditions which deteriorated **180n** into dimethylphosphinic acid. Concerning the thiazole derivative, the expected product **180e** seems to be just an intermediate as it undergoes a phospha-Brook rearrangement to give the corresponding phosphate **184**.

Chapter VIII: *N*-hydroxyimido derivatives and hydroxamate derivatives as complexing units

The *N*-hydroxyimido moiety and the hydroxamate derivatives are widely employed chelating functional groups in biochemistry to develop bioactive compounds.

The interest in N^3-hydroxypyrimidine-2,4-dione ring as a complexing group in inhibitor's structures emerged in the middle of the 2000s. Since then, more than 200 molecules have been developed for trying to inhibit only 3 types of enzyme: integrases with a particular insight on HIV integrase, reverse transcriptases and endonucleases.

Along with N^3-hydroxypyrimidine-2,4-dione rings, the biological activity of hydroxamate derivatives have been known for a long time but a renewal occurred at the end of the 90s and beginning of the 2000s. The principal biological targets of the compounds bearing these moieties are matrix metalloprotinease (MMP). More than a third of the molecules listed in the literature, meaning more than 25 000 compounds, targets this type of enzyme.

All the enzymes from these families need one or more metallic cofactors for their biological activity such as KARI. Studying the compounds designed as inhibitors of these enzymes may be a starting point to develop a new family of complexing molecules for KARI.

I. Uracil and thymine derivatives

1) N^3-hydroxypyrimidine-2,4-dione ring as a moiety for HIV-1 integrase inhibitors

As it has been already mentioned in the chapter Bioisosters and mimics of carboxylic acids, in the part on Heterocycles as effective metal chelators of HIV-1 integrase p108, KARI shares the same particularity as HIV-1 integrase as it complexes the inhibitors thanks to two metal cations in the active site.

N^3-hydroxypyrimidines have been developed as inhibitors of HIV-1 integrase as shown in Figure 13.

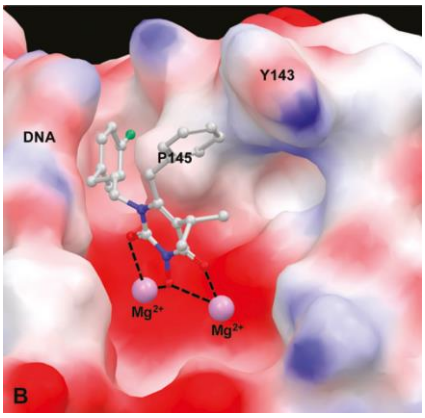
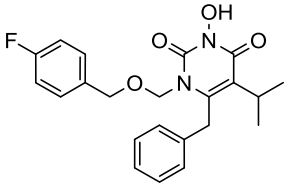


Figure 13: docking model of 185 in IN catalytic site

Tang *et al.* developed new integrase inhibitors (INIs) constructed around a *N*-hydroxypyrimidine-2,4-dione ring.[120] As shown in the docking model, the most potent inhibitor **185** of the series (IC_{50} = 0.44 µM) fits perfectly into the IN binding site by chelating the two Mg^{2+} cations.

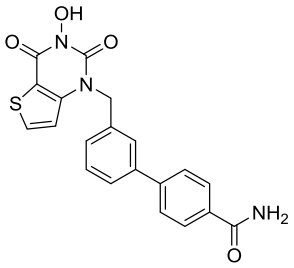


185

[120] J. Tang, K. Maddali, M. Metifiot, Y. Y. Sham, R. Vince, Y. Pommier, Z. Wang, *J. Med. Chem.*, **2011**, *54*, 2282-2292.

2) *N*-hydroxypyrimidine-2,4-dione ring as a moiety for Flap endonuclease-1 inhibitor

Such motif was also found in the inhibition of Flap endonuclease-1 (FEN1) which is a key divalent metallo-enzyme that exhibits both DNA endonuclease and exonuclease activity. This enzyme is involved in base excision repair, a process used by mammalian cells to repair damaged DNA strands. Sensitization to DNA damaging agents may improve the therapeutic window of classical chemotherapeutics by lowering the minimum effective dose.

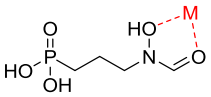


186

In 2005, Tumey *et al.* developed selective small-molecule inhibitors of FEN1 for use as chemopotentiating agents.[121] They synthesised a novel and potent series of FEN1 inhibitors, the most active **186** possessed an IC_{50} equal to 3 nM. They postulated that this compound inhibits the endonuclease activity by coordination of the two divalent metals required for catalysis. These two metals are essential for catalytic turnover of FEN1.

3) *N*-hydroxypyrimidine-2,4-dione ring as moiety central core for Fosmidomycin analogs

The 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR) is the second enzyme intervening in the non-mevalonate pathway of synthesis of isoprenoids. This biosynthesis operates in plants, green algae, protozoa and bacteria which makes it an interesting inhibition target to develop herbicides. As KARI, DXR requires two cofactors: a metal cation (magnesiumor manganese) and NADPH. Fosmidomycin **187** has proven to be a good inhibitor of different DXR strains, especially *E. coli* DXR with an IC_{50} = 8.2 nM.[122]



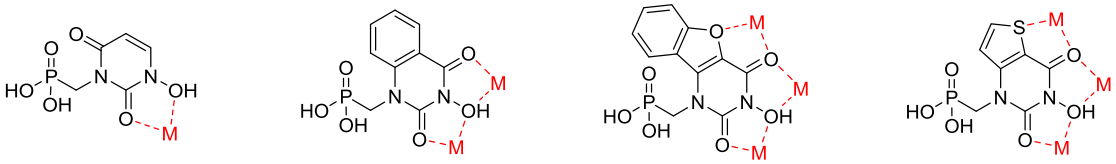
187

In the course of her PhD thesis, Sonia Montel developed the synthesis of Fosmidomycin analogues (Scheme 82) based on N^1-hydroxyuracil and N^3-hydroxyuracil motifs.[123]

[121] L. N. Tumey, D. Bom, B. Huck, E. Gleason, J. Wang, D. Silver, K. Brunden, S. Boozer, S. Rundlett, B. Sherf, S. Murphy, T. Dent, C. Leventhal, A. Bailey, J. Harrington, Y. L. Bennani, *Bioorg. Med. Chem. Lett.*, **2005**, *15*, 277-281.

[122] T. Kuzuyama, T. Shimizu, S. Takahashi, H. Seto, *Tet. Lett.*, **1998**, *39*, 7913-7916

[123] S. Montel, *The 1-Deoxy-D-Xylulose-5-Phosphate Reductoisomerase, a target metalloenzyme for the elaboration of chelation-based inhibitors*, **2012**, Ecole Nationale Supérieure de Chimie de Montpellier



188 **189** **190** **191**

Scheme 82: N^1 and N^3-hydroxyuracil analogs of Fosmidomycin

4) Target compounds

For our concern, we target the synthesis of N^3-hydroxyuracil and N^3-hydroxythymine derivatives.

192 **193**

Scheme 83: targets for potential KARI inhibitors

a) N^1-alkyl-N^3-hydroxyuracil and N^1-alkyl-N^3-hydroxythymine with R = Me, Et and *n*Pr

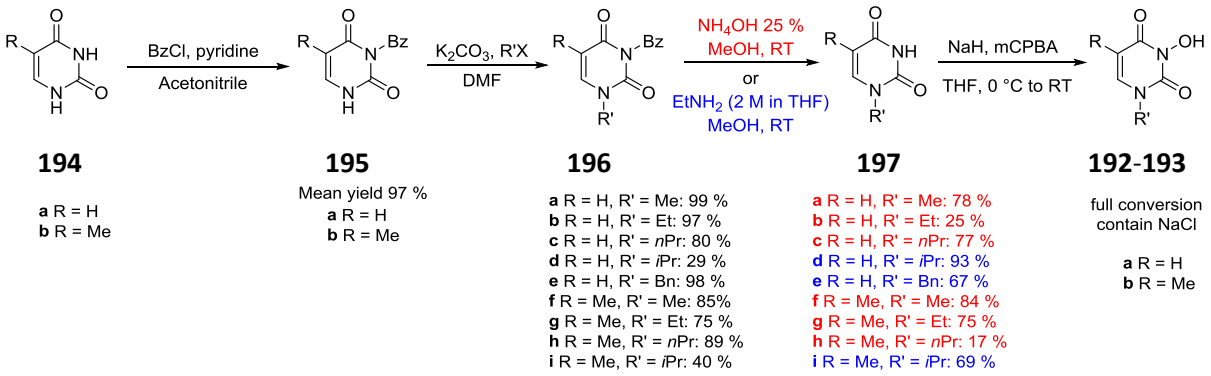
At first uracil and thymine are di-protected on the N^1 and N^3 positions through a diacylation reaction followed by the mono deprotection of N^1 with a solution of potassium carbonate in a mixture water / 1,4-dioxane.[124] N^3-alkylation on N^1-benzoyluracil is performed using the desired alkyl halides in the presence of potassium carbonate at 80 °C in DMF.[125] Then N^3 is deprotected by a transamidation of the benzoyl thus releasing the nitrogen of the dihydroxypyrimidine. The last step of the sequence consists in the oxidation of the imido nitrogen N^3 atom by *m*-CPBA[126] or by benzoylperoxide[127] as shown in Scheme 84.

[124] K. Cruickshank, J. Jiricny, C. Reese, *Tet. Lett.*, **1984**, *25*, 681-684

[125] Y. Abe, O. Nakagawa, R. Yamaguchi, S. Sasaki, *Bioorg. Med. Chem.*, **2012**, *20*, 3470-3479

[126] J. Tang, K. Maddali, M. Metifiot, Y. Y. Sham, R. Vince, Y. Pommier, Z. Wang, *J. Med. Chem.*, **2011**, *54*, 2282-2292

[127] A. M. Berman, J. S. Johnson, *J. Org. Chem.*, **2006**, *71*, 219-224.



Scheme 84: preparation of the uracil and thymine derivatives

N^1-alkyl pyrimidinedione **197** have been prepared with R^1 = Me, Et, *n*Pr, *i*Pr and Bn. An attempt of N^3-hydroxylation has been performed by oxidizing the nitrogen by using benzoylperoxide in slight excess with dipotassium phosphate in DMF. These conditions have proven to be inefficient twice. Milder conditions were tried instead of peroxides by using oxone and sodium bicarbonate.[128] In a following trial sodium carbonate was replaced by sodium hydride because the former might not be basic enough. However when *m*-CPBA and sodium hydride were used as oxidizing agents, the N^1-alkyl-N^3-hydroxyuracils **192** and N^1-alkyl-N^3-hydroxythymines **193** were obtained.

An important amount of sodium chloride salt was generated from the reaction work-up with hydrogen chloride. Methanol washings were experimented to get rid of it but at each step some would still remain. The washings were tried with other polar solvents such as acetonitrile or ethyl acetate but methanol proved to be the best one to dissolve the compounds **192**-**193**. During each washing, some sodium chloride was dissolved along with the product probably due to the complexing abilities of these targets. As all the salt could not get rid of in each cases, only total conversions of the N^1-alkyluracil and N^1-alkylthymine **197** were given.

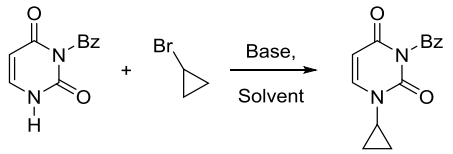
b) N^1-cyclopropyl-N^3-hydroxyuracil and N^1-cyclopropyl-N^3-hydroxythymine

The alkylation of N^1 with the cyclopropyl moiety (Scheme 85) was the second issue for the synthesis of N^1-cyclopropyl-N^3-hydroxyuracil **198a** and N^1-cyclopropyl-N^3-hydroxythymine **198b**.

198a **198b**

Different trials were accomplished by using conditions favouring an S_N2 mechanism on bromocyclopropane **199** were tried (Table 50).

[128] For a review on the different applications of oxone see: H. Hidayat, I. R. Green, A. Ishtiaq, *Chem. Rev.*, **2013**, *113*, 3329-3371



195 **199** **200**

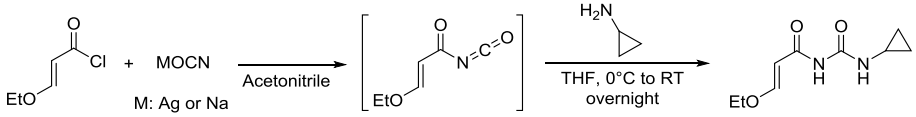
Scheme 85: N^1-cyclopropyl-N^3-benzoyluracil synthesis

Table 50: Attempts to synthesise 200

Eq of 199	Base	Solvent	Heating	Duration	Observations
1.1	K_2CO_3 (1 eq)	DMF	2 h at 100 °C	3 days	Starting materials
1.1	K_2CO_3 (1 eq)	DMF	RT	2 days	Starting materials
1.1	*t*BuOK (1.5 eq)	DMF	RT and 100 °C MW (200 W)	7 days then 40 min µW	A product appeared among many others
0.5	KOH (3.5 eq) 18-crown-6 (10 mol%)	THF	85 °C	16 h	Starting **195**

When using *t*BuOK as a base in DMF, after analysing the ^{1}H NMR spectra, there was no sign of **200**. The product observed was the deprotection of the benzoyluracil by dimethylamine coming from the decomposition of DMF at high temperature. It seems **199** is not stable under these conditions because no peak corresponding to a cyclopropyl moiety was observed in NMR.

The classical path used before to obtain the N^1-alkyl-N^3-hydroxyuracils **192** or N^1-alkyl-N^3-hydroxythymines **193** does not seem to be efficient for the synthesis of **198a** or **198b**. The best way may not be to start from the preformed uracil or thymine heterocycles, but to completely synthesise the dioxodihydropyrimidine and to introduce the cyclopropyl ring during the heterocycle formation (Scheme 86).



201 **202** **203** **204**

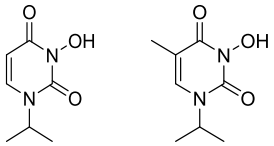
Scheme 86: preparation of *N*-(cyclopropylcarbamoyl)-3-ethoxyacrylamide 204

The 3-ethoxyacryloyl isocyanate **203** is formed *in situ* with the addition of silver or sodium isocyanide **202** on ethoxyacryloyl chloride **201**. **203** is directly used in order to be trapped by cyclopropylamine in a THF solution and then obtain *N*-(cyclopropylcarbamoyl)-3-ethoxyacrylamide **204**. The cyclisation into the dioxodihydropyrimidine can be performed thanks to concentrated sulphuric acid in ethanol.[129] It has not been done because *N*-(cyclopropylcarbamoyl)-3-ethoxyacrylamide **204** could not be isolated by column chromatography, the product deteriorates during purification.

[129] A. Ezzitouni, P. Russ and V. E. Marquez, *J. Org. Chem.*, **1997**, *62*, 4870-4873

c) N^1-isopropyl-N^3-hydroxyuracil and N^1-isopropyl-N^3-hydroxythymine

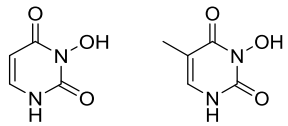
As a mimic for the cyclopropyl, the N^1-isopropyl-N^3-hydroxyuracil **192d** and N^1-isopropyl-N^3-hydroxythymine **193d** have been prepared using the same procedure as for **192-193** with isopropyl iodide as the alkyl halide. However a problem of purification happened during the benzoyl deprotection step. Then, instead of using ammonia in water/methanol mixture as usual, the deprotection was carried out with a solution of ethylamine in THF. *N*-ethylbenzamide is formed as the byproduct of the reaction and is easier to separate from the uracil or thymine derivatives compared to the benzamide. Thereafter, the N-hydroxylation reaction yielded **192d** but unfortunately when the thymine derivative was engaged in the reaction, no **193d** could be observed.



192d **193d**

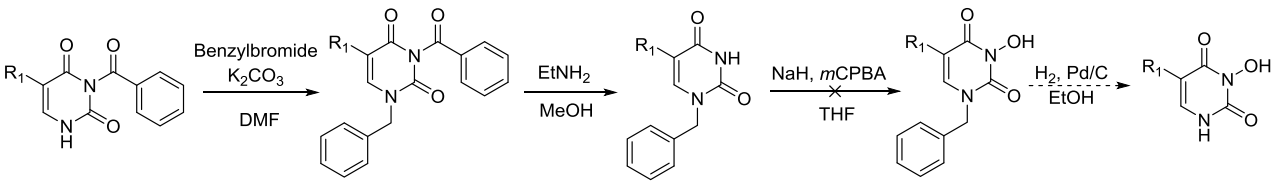
d) N^3-hydroxyuracil and N^3-hydroxythymine

In order to complete the family, N^1-H-N^3-hydroxyuracil **192f** and N^1-H-N^3-hydroxythymine **193f** must be synthesised. Unfortunately, with the classical amine hydroxylation conditions used, the reactions from uracil or thymine didn't afford any of the products awaited.



192f **193f**

As the direct *N*-hydroxylations of uracil and thymine didn't afford the respective products, another way of synthesis has been tried. By playing with orthogonal protecting groups such as a benzoyl and a benzyl one, it may be possible to oxidize only the N^3 position (Scheme 87).



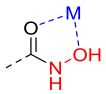
195a-b **196e** 98% **196j** 96% **197e** 67% **197j** 43% **192e** **193e** **192f** **193f**

Scheme 87: Synthesis of N^3-hydroxyuracil and N^3-hydroxythymine

Unfortunately, the oxidation step didn't afford the awaited products **192e** and **193e**.

II. 2-(dimethylphosphoryl)-*N*-hydroxyacetamide

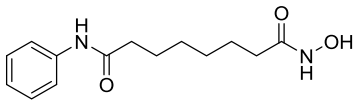
Hydroxamic acids **205** are bioisosters of the hydroxyl part of the carboxylic acid function.[130] They exhibit moderately acidic characteristics with pKa values included between 8 and 9.[131] They have a high chelating power[132] (chelation of Fe^{3+} [133], Cu^{2+} [133, 134], Ni^{2+} [133], Zn^{2+} [133], Mg^{2+} [135], Mn^{2+} [136]) have been reported), and also have a wide application in enzyme inhibition.



205

1) Histone deacetylase inhibitor

Suberoylanilide hydroxamic (SAHA) **206** is an anticancer drug and belongs to the histone deacetylase inhibitor family.[133] Histone deacetylase proteins catalyse the deacetylation of lysine residues on chromatin leading to transcription repression. Cristallographic studies of SAHA with various metallic ions show how good of a complexant is the hydroxamic acid moiety.

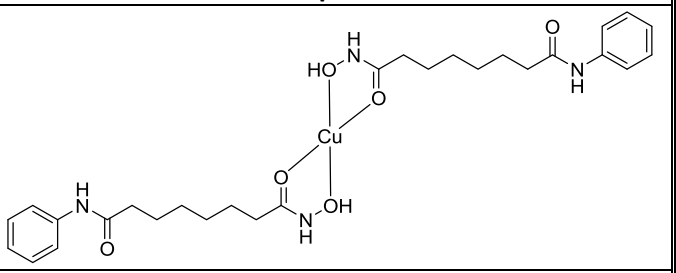
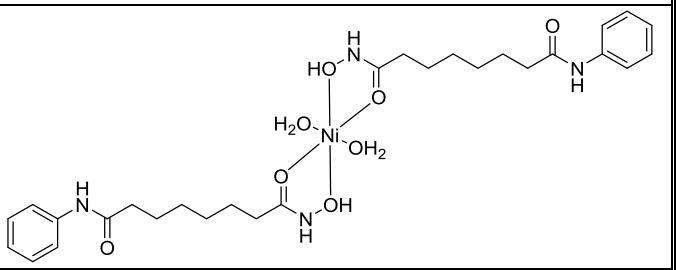
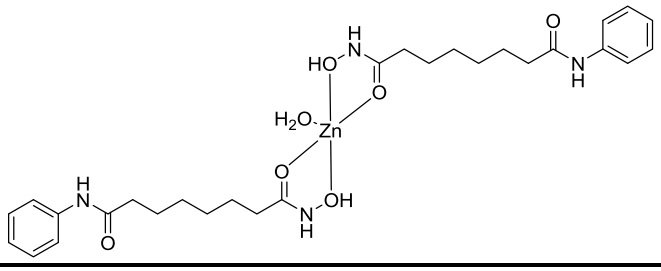


206

The complexes were synthesised starting mainly from metals in the second oxidation state (Table 51).

[130] R. J. Almquist, W. R. Chao, C. Jennings-White, *J. Med. Chem.*, **1985**, *8*, 1067–1071
[131] O. N. Ventura, J. B. Rama, L. Turi, J. J. Dannenberg, *J. Am. Chem. Soc.*, **1993**, *115*, 5754-5761
[132] A. Mai, "Hydroxamic acids: biological properties and potential uses as therapeutic agents", *The Chemistry of Hydroxylamines, Oximes and Hydroxamic Acids*, Ed. Z. Rappoport, J. Liebman, Hoboken NJ: Wiley **2009**, 731-806
[133] D. M. Griffith, B. Szőcs, T. Keogh, K. Y. Suponitsky, E. Farkas, P. Buglyó, C. J. Marmion, *J. Inorg. Biochem.*, **2011**, *105*, 763-769
[134] M. J. Haron, H. Jahangirian, M. H. S. Ismail, R. Rafiee-Moghaddam, M. Rezayi, K. Shameli, Y. Gharayebi, Y. Abdollahi, M. Peyda, B. Mahdavi, *Asian J. Chem.*, **2013**, *25*, 4183-4188
[135] S. Yajima, K. Hara, D. Iino, Y. Sasaki, T. Kuzuyama, K. Ohsawa, H. Seto, *Acta Cryst.*, **2007**, *F63*, 466-470
[136] C. Björkelid, T. Bergfors, T. Unge, S. L. Mowbay, T. A. Jones, *Acta Cryst.*, **2012**, *D68*, 134-143

Table 51 Examples of complexes with hydroxamic acids

Metals	Complexes
Copper	
Nickel	
Zinc	

2) Matrix metalloproteinase inhibitor

The main subject of interest about hydroxamates as inhibitors is matrix metalloprotinease (MMP). MMP is a wide family of proteins containing 23 different types in human beings for example. They participate in the degradation of extra-cellular membrane and some of them are involved in the metastatic tumour dispersion.

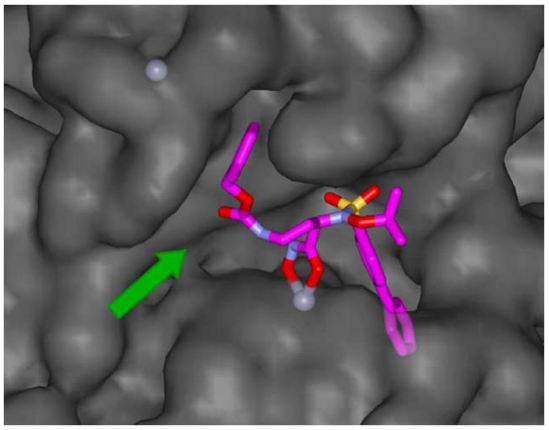
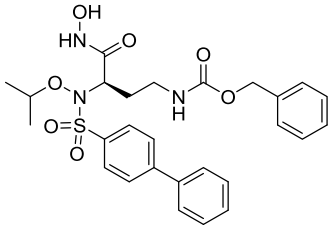


Figure 14: docking model of 207 in MMP-2 site

In 2005, Rossello *et al.* published the results of the development of new hydroxamates containing compounds, inhibiting selectively MMP-2 with an IC_{50} = 0.41 nM.[137] The docking model on the left shows how **207** chelates the zinc cation (in grey) inside the catalytic site.



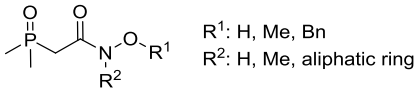
207

[137] A. Rossello, E. Nuti, P. Carelli, E. Orlandini, M. Macchia, S. Nencetti, M. Zandomeneghi, F. Balzano, G. Barretta, A. Albini, R. Benelli, G. Cercignani, G. Murphy, A. Balsamo, *Bioorg. Med. Chem. Lett.*, **2005**, *15*, 1321-1326

In light of the hydroxamate derivatives biological properties reported in literature, the development of compounds bearing this functional group may be of interest for synthesising potential KARI inhibitor.

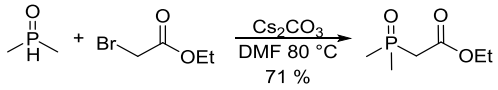
3) Target compounds

a) Ethyl 2-(dimethylphosphinoyl)acetate



208

The first approach for synthesising this family was a nucleophilic substitution from dimethylphosphine oxide **51a** on ethyl bromoacetate **209** providing ethyl 2-(dimethylphosphinoyl)acetate **210** in 71% yield (Scheme 88).

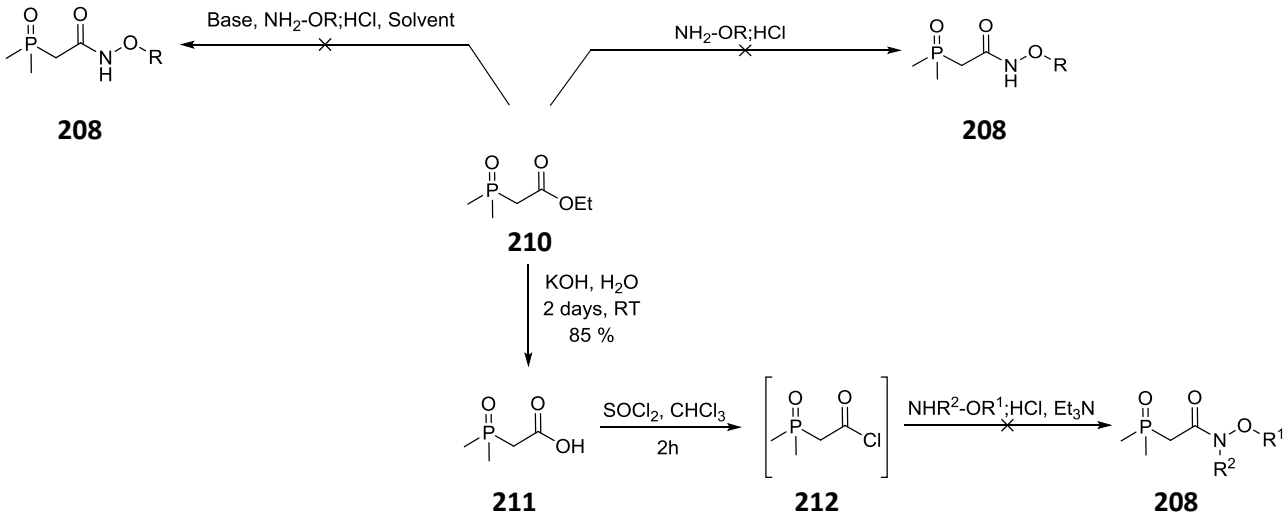


51a **209** **210**

Scheme 88: ethyl dimethylphosphinoylacetate preparation

b) Amidation from the ester

Then the alkyloxyamine, in general methoxyamine hydrochloride, would be reacted with different conditions giving **208** in few steps (Scheme 89).



208 **208** **210** **211** **212** **208**

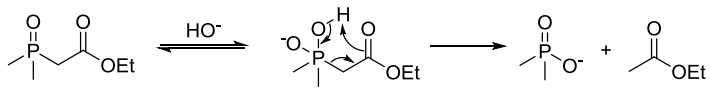
Scheme 89: (dimethylphosphoryl)acetohydroxamic acid derivatives synthesis routes

The direct synthesis of the hydroxamic acid **208** from the ethyl ester **210** was not conclusive, no new product could be observed even after different set of conditions (Table 52).

Table 52: Direct synthesis of hydroxamic acid 208

Methoxyamine equivalent	Base	Solvent	Temperature	Time	Yield
1	KOH (4N) 2 eq	H_2O	100 °C	5 h	Only dimethylphosphinic acid **63**
1.55	*t*BuMgCl (1 M in THF, 3 eq)	THF	0 °C to RT	3h	57 % of **210** and dimethylphosphinic acid **63**
1	Et_3N (1.5 eq)	THF	RT	3h	No reaction
1	Et_3N (2.1 eq)	DMF	RT	1 day	18 % of **210** and dimethylphosphinic acid **63**

So the one-pot ester hydrolysis and addition of the methoxyamine was investigated with a potassium hydroxide solution (4 N, 2 eq) as base at 100 °C during 5 hours. This time the conditions were too harsh for **210**. The phosphine oxide was cleaved into phosphinic acid and ethyl acetate through probably the mechanism described in Scheme 90.



210 **213** **63** **214**

Scheme 90: Potential degradation of ethyl (dimethylphosphoryl)acetate in basic aqueous conditions

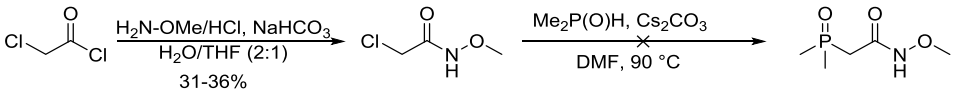
After the nucleophilic addition of the hydroxide ion on the phosphine **210**, the pentacoordinated phosphorus compound **213** may undergo an intramolecular rearrangement to give dimethylphosphinate **63** and ethyl acetate **214**.

A milder ester hydrolysis was done at room temperature in 85 % yield, but the carboxylic acid **211** was not reactive enough to afford the hydroxamic acid. It must be transformed into an acyl chloride **212** with thionyl chloride. **212** was not isolated and was directly used for the next step. Methoxyamine was then added to the acyl chloride but only gave dimethylphosphinic acid.

These methods could not afford any product. For each attempt, the ^{31}P NMR spectra showed many phosphorus compounds which are difficult, or even impossible, to separate due to their similar polarities. Aqueous washings could not be used because all the products are soluble in water. Purification on silica-gel columns is not a solution either because phosphorus containing molecules tend to get stuck and drag on silica. Moreover they are missing UV absorbent groups which make them impossible to spot by UV light. The most viable solution seemed to be the Kugelrohr distillation. An easier way of synthesis must be found.

c) Amidation then introduction of the phosphorus moiety

That is the case with commercially available chloroacetyl chloride **215**. The latter can be reacted with various *O*-alkylhydroxylamines followed by the reaction of dimethylphosphine oxide **51a** affording the targeted phosphine oxides **208** (Scheme 91). In the purpose of doing some substitutions on the nitrogen atom, the study has been done with methoxyamine hydrochloride all along.

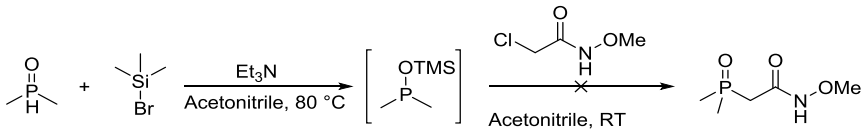


215 **216** **208**

Scheme 91: Synthesis of (dimethylphosphoryl)acetohydroxamic acid

The nucleophilic substitution by dimethylphosphine oxide **51a** using caesium carbonate, as a base, did not give any product, even at 90 °C during 13 days. The phosphorus atom may not be nucleophilic enough to attack the carbon atom bearing the chloride.

In order to improve the reaction, a variation was made on the classical Arbuzov reaction. First **51a** is transformed into trimethylsilyl dimethylphosphinite **218**, thanks to trimethylsilyl bromide **217**, followed by the silyl-Arbuzov reaction between the $P^{(III)}$ compound **218** and the methyl chloroacetohydroxamate **216** (Scheme 92).

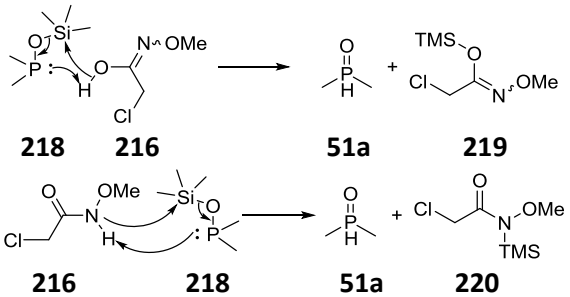


51a **217** **218** **208**

Scheme 92: Synthesis of (dimethylphosphoryl)acetohydroxamic acid derivatyive with a P(III) intermediate

Other attempts were made with HMDS and proved to be unsuccessful. Either only the starting material or both the starting material and dimethylphosphinic acid are observed by ^{31}P NMR.

A trial was done to see whether if the phopshinite **218** was formed and reacts with **216**. A peak at 104.7 ppm (4.7 %) was observed by ^{31}P NMR analysis, among the starting material (33.8 %), phosphinic acid (3.3 %), a major peak at 41 ppm (46.3 %) and other products (21.9 %). To explain why we got dimethylphosphine oxide **51a** after the addition of **216** we can suppose the existence of an exchange of the trimethylsilyl group from the phosphinite **218** towards the oxygen atom of the imidate form of the hydroxamate group or to the nitrogen atom of the oxamate form (Scheme 93).

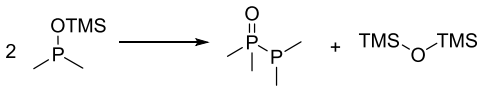


218 **216** **51a** **219**

216 **218** **51a** **220**

Scheme 93: Trimethylsilyl exchanges

218 is a very sensitive reactant which must not be heated over 80 °C or else a disproportionation reaction would occur leading to the creation of a P-P bond and other nucleophilic phosphorus species (Scheme 94).[138]

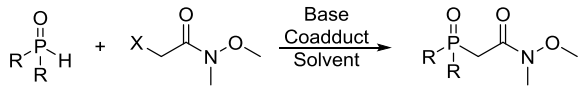


218 **221**

Scheme 94: dimethylphosphinous trimethylsilyl ester disproportionation

Bromoacetyl bromide can replace the chloroacetyl chloride which does not appear to be reactive enough. In order to avoid the formation of the *O*-trimethylsilylacetimidate **219** the hydrogen borne by the nnitrogen in **216** must be replaced by a protecting group such as benzyl, trimethylsilyl or an alkyl group. The following scope of conditions was done with 2-halogeno-*N*-methyl-*O*-methylacetohydroxamate **222** and **223**. 2-chloro-*N*-methyl-*O*-methylacetohydroxamate **222** and 2-bromo-*N*-methyl-*O*-methylacetohydroxamate **223** were prepared with the same procedure as for **216** but with *N*-methyl-*O*-methylhydroxylamine hydrochloride and chloroacetyl chloride or bromoacetyl bromide respectively.

Different phosphorus sources were tried in order to check if the reaction depends on the substituent on the phosphorus atom, along with two different *N*-methyl-*O*-methylacetylhydroxamates **222** and **223**. Modifications of bases and solvents are also listed in Scheme 95 and Table 53.



R: Me **51a** X: Cl **222** R: Me **208**
R: Ph **51b** X: Br **223** R: Ph **224**
R: OEt **51c** R: OEt **225**

Scheme 95: Hydroxamate derivatives preparation from halogenoacetohydroxamic acid

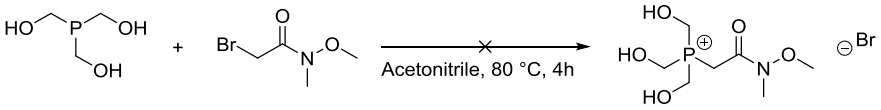
Table 53: Nucleophilic substitution with the phosphorus source to synthesise 208

Phosphorus reagent	X	Base	Co-reagent	Solvent	Temperature	Time	Yield
51a	Cl	NEt_3	TMSBr	CH_3CN	RT	16h	Mainly **63**
51a	Br	Cs_2CO_3	TBAI	DMF	RT	2 days	many products
51a	Br	NaH		THF	0°C to RT	1 day	Mainly **63**
51b	Cl	NaH		THF	0°C to RT	3 days	many prducts
51b	Br	*t*BuOK		CH_3CN	RT	2 days	many products
51c	Br	NaH		THF	0°C to RT	1 day	Recovery of starting material
$P(OEt)_3$	Br			Neat	155°C	1h	many products
$P(CH_2OH)_3$	Br			CH_3CN	80°C	8h	many products

[138] M. Volkholz, O. Stelzer, R. Schmutzler, *Chem. Ber.*, **1978**, *111*, 890-900

According to the literature, it does not seem complicated to prepare this type of compound. The recurrent conditions appearing on publications are those for the Arbuzov reaction between the bromo or chloro-*N*-methyl-*O*-methylacetylhydroxamate **222** or **223** and triethyl phosphite with heating. A trial has been done with microwave irradiation at 180 °C during 30 min between triethylphosphite and **223**, neat. The transformation of the phosphite was total but the ^{31}P NMR spectrum showed a too complex mixture of products.

Tris(hydroxymethyl)phosphine **226** was also used as a nucleophilic phosphorus species. The reaction was carried out by simply mixing this reagent with the bromo *N*-methyl-O-methylacetylhydroxamates **223** at reflux of acetonitrile (Scheme 96).



226 **223** **227**

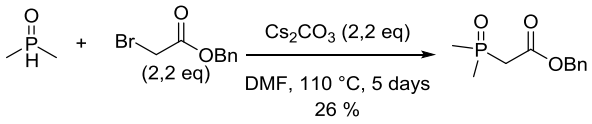
Scheme 96: tris(hydroxymethyl)(2-(methoxy(methyl)amino)-2-oxoethyl)phosphonium bromide

The reaction gave a complex mixture of 8 different phosphorus products with one major product at 26.8 ppm (38.4 %) by ^{31}P NMR spectrum which may correspond to the chemical shift of this type of phosphonium salt.[139]

Due to the possibility of many side-products, this method should not be used for the formation of *N*-methyl-*O*-methylacetylhydroxamate bearing a phosphorus atom on the alpha position of the carbonyl.

d) Benzyl ester: obtaining the acid without any hydrolysis step

To avoid the deterioration of ethyl 2-(dimethylphosphinoyl)acetate **210** during the saponification step, as explained in Scheme 90, the ethyl ester may be replaced by a benzyl ester which would be deprotected thanks to a pallado-catalysed hydrogenation step. However, the nucleophile substitution of benzyl bromoacetate by dimethylphosphine oxide **51a** in presence of caesium carbonate as a base, in DMF for five days at 110 °C gave the expected product in poor yield (Scheme 97).

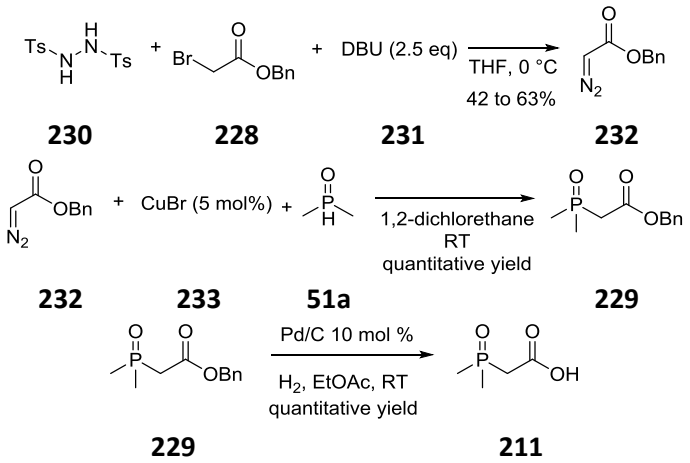


51a **228** **229**

Scheme 97: benzyl (2-dimethylphosphoryl)acetate synthesis assay

[139]V. Mark, C. Dungan, M. Crutchfield, J. Van Wazer, Compilation of ^{31}P NMR in *^{31}P Nuclear Magnetic Resonance, Topics in Phosphorus Chemistry*, Ed. M. Grayson, E. Griffith, Wiley-Interscience Publishers, London, UK, 1967, Volume 5, p383

Better and milder reaction conditions have been explored which involves the synthesis of benzyl diazoacetate **232**[140] and then a copper-catalysed cross coupling reaction[141] with dimethylphosphine oxide **51a** as presented in Scheme 98.



230 **228** **231** **232**

232 **233** **51a** **229**

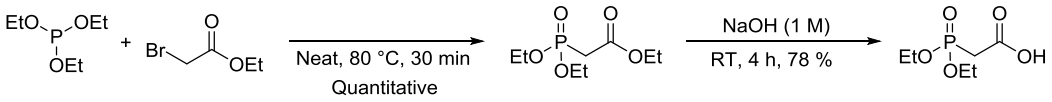
229 **211**

Scheme 98: Dimethyphophorylacetic acid synthesis

The *N,N'*-ditosylhydrazine **230** is prepared from *p*-toluenesulfonylhydrazine and *p*-toluenesulfonyl chloride in presence of pyridine. **230** reacts with benzyl bromoacetate **228** in a nucleophile substitution to afford the diazoacetate derivative **232** with yields from 42 to 63 %. Then the phosphorus moiety is introduced on **232** by using a cross coupling reaction with **51a** catalysed by copper (I) bromide with quantitative yields. Benzyl (2-dimethylphosphoryl)acetate **229** is hydrogenated with classical conditions leading to the corresponding carboxylic acid **211**. Therefore instead of hydrolysing the ester as seen in Scheme 90, the synthesis of the carboxylic acid implies milder conditions without any aqueous steps including treatments.

e) 2-(diethylphosphoryl)acetic acid

Simultaneously the diethyl phosphonate version of **210**, ethyl diethylphosphonoaetate **236**, was prepared by a simple Michaelis-Arbuzov reaction between triethyl phosphite **234** and ethyl bromoacetate **235** in neat conditions at 80 °C. The saponification proceeds easily with a 1 M solution of sodium hydroxide at room temperature affording the acid **237** with 78 % yield.



234 **235** **236** **237**

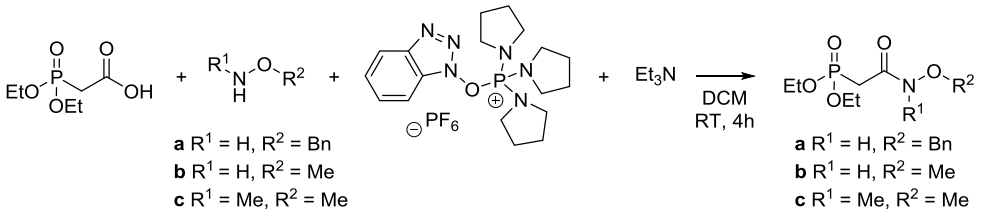
Scheme 99: Diethylphosphonoacetic acid synthesis

[140] T. Toma, J. Shimokawa, T. Fukuyama, *Org. Lett.*, **2007**, *9*, 3195-3197

[141] H. Jiang, H. Jin, A. Abdukader, A. Lin, Y. Cheng, C. Zhu, *Org. Biomol. Chem.*, **2013**, *11*, 3612-3615

f) Peptidic couplings

Some peptidic couplings have been tried by using the benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate reagent **239** (PyBOP) with different hydroxylamine **238** sources in order to access diethyl phosphonoacetohydroxamic acid derivatives **240** (Scheme 100).[142]

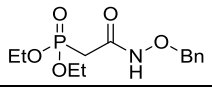
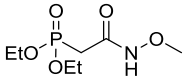
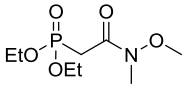


237 **238** **239** **240**

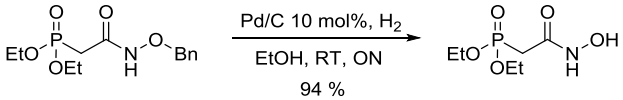
Scheme 100: PyBOP coupling

As described in Table 54, the peptidic coupling reaction afforded two hydroxamic acid derivatives **240a** and **240c**, respectively with 65 % and 50 % yields. However, when **238b** was used, the carboxylic acid **237** was converted to 56 % of **240b** and 44 % of diethyl phosphonic acid. The hydroxamic acid obtained was too hydrophilic and couldn't be separated by extraction from the other byproducts, nor by column chromatography.

Table 54: Assays of peptidic coupling with PyBOP

Hydroxylamine sources	Awaited product		Observations, yields
238a		**240a**	65 - 66 %
238b		**240b**	56 % conversion
238c		**240c**	50 %

The last step concerning the derivative **240a** is the pallado-catalysed cleavage of the benzyl protective group on the hydroxamic acid moiety. The free hydroxamic acid **240d** was obtained very easily in a good yield (Scheme 101).

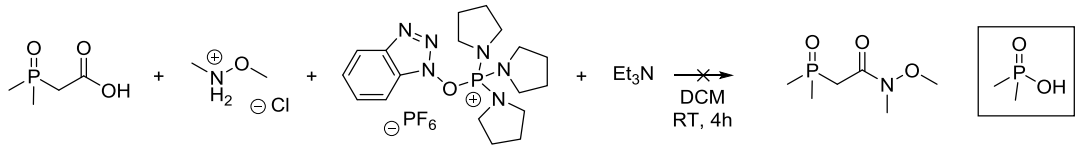


240a **240d**

Scheme 101: benzyl deprotection

[142] N. Barthes, C. Grison, *Bioorg. Chem.*, **2012**, *40*, 48-56

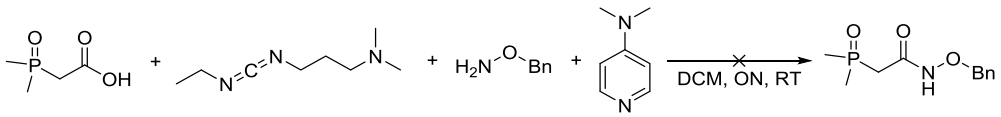
As the peptidic couplings proved to be efficient with (diethylphophonyl)acetic acid **237**, the conditions have been transposed to (2-dimethylphosphoryl)acetic acid **210** with *N*-methyl-*O*-methylhydroxylamine hydrochloride **238c**, unfortunately without success (Scheme 102). The molecule is very hydrophilic and as the treatment requires acidic washings, the hydroxamic derivative was deteriorated in its dimethylphosphinic acid **63** counterpart.



210 **238c** **239** **208** **63**

Scheme 102: PyBOP mediated (2-dimethylphosphoryl)acetohydroxamate preparation

Other peptidic coupling conditions may be applied, for example EDCI.[143] Even if the carboxylic acid has not been degraded as with the PyBOP conditions, no reaction occurred, the acid **210** was fully recovered (Scheme 103).



210 **241** **238a** **242** **243**

Scheme 103: EDCI mediated (2-dimethylphosphoryl)acetohydroxamate preparation

[143] S. Ladame, M. Barder, J. Périé, M. Willson, *Bioorg. Med. Chem.*, **2001**, *9*, 773-783

III. Conclusion

According to the literature, N^3-hydroxyimide and hydroxamic acid derivatives are highly complexing units in inhibitors of metalloenzymes. This has been the key property to develop new potential inhibitors of KARI.

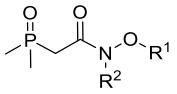
Starting from commercially available uracil and thymine, variations have been introduced on the N^1 position of the pyrimidine derivatives' rings through N^1 protection, N^3 alkylation and N^1deprotection steps. The oxidation of the N^3 nitrogen was trickier and didn't give all the expected products either because the reaction didn't work at all or it produced many side products that were not separable. However 7 products (Scheme 104) have been successfully.

192 **193**

R' = Me, Et, *n*Pr, *i*Pr (only for **192**)

Scheme 104: targets for potential KARI inhibitors

In the second direction, 2-(dimethylphosphinoyl)acetohydroxamate derivatives **208** have been tried to be synthesised.



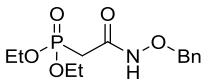
R^1: H, Me, Bn
R^2: H, Me, aliphatic ring

208

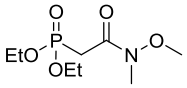
The direct synthesis from ethyl 2-(dimethylphosphinoyl)acetate **210** or its carboxylic acid counterpart **211** and hydroxylamines didn't afford any expected product because several problems have been encountered. The first one was the deterioration of the ester during saponification which has been bypassed by the preparation of benzyl 2-(dimethylphosphinoyl)acetate **229** thanks to a cross coupling reaction. The other issue came from the poor reactivity of the hydroxylamines on this type of ester or carboxylic acid even with activating reagents.

The way of synthesis has then been reversed, meaning that the phosphorus group has been tried to be introduced on a 2-halogenoacetohydroxamate derivative, which unfortunately wasn't successful either.

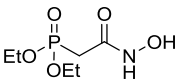
Fortunately, some investigations on ethyl 2-(diethylphosphoryl)acetate **236** proved to be successful after saponification and peptidic couplings using PyBOP activating reagent. Three compounds have been synthesised as detailed in Scheme 105



240a



240c



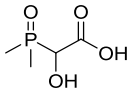
240d

Scheme 105: 2-(diethylphosphoryl)acetohydroxamate derivatives synthesised

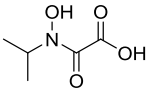
Conclusion

The branched-chain amino acid biosynthesis is present in plants, fungi, algae and bacteria but does not exist at all in mammals nor insects. This distribution among living beings is the crucial point for the development of new herbicides, by shutting down this pathway. It has already been the case with the design and synthesis of AHAS inhibitors, sulfonylureas and imidazolinones. However the unique properties of ketol-acid reductoisomerase (KARI) and its place within the biological pathway makes it an attractive target for drug design.

Two potent *in vitro* inhibitors of KARI have already been found: Hoe 704 **40** and IpOHA **42**. The remaining issue is now to design and synthesize not only *in vitro* inhibitors but more importantly *in vivo* active molecules that could be used in fields.



Hoe 704 40

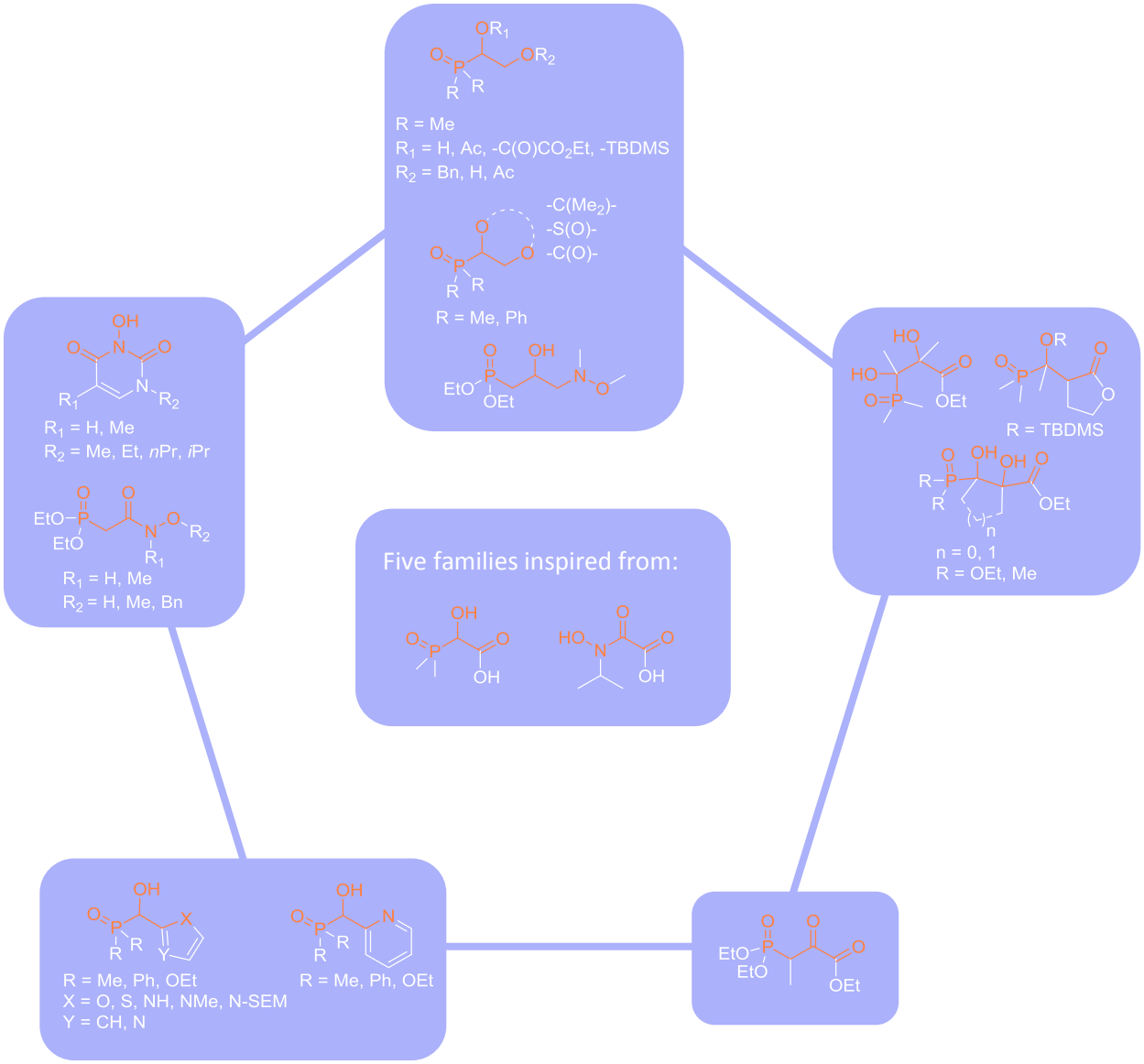


IpOHA 42

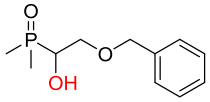
The conformational studies of KARI by the observation of its X-ray diffraction experiment led to the discovery of regions within the protein, each with specific functions. It helped to understand the position and interactions between the substrate and the catalytic site, thus unveiling the complete mechanism of how the 1,2-alkyl migration followed by the NADPH mediated reduction occurs. Moreover, the enzyme must include metal cations cofactors in order to be fully active. The chelation of the cations and mimicking the rearrangement transition state with analogues are two complementary ways of designing new potent inhibitors. However KARI presents some important conformational rearrangement steps induced by the ligands in order to catalyse the reactions, therefore the design of new active inhibitors represents a challenge to rise to.

Besides that, this challenge appears even greater when all the environmental factors that face a plant protection agent to reach its biological target are considered (UV degradation, micro-fauna and bacteria, plant metabolism, leaching …). For all these reasons, our products have been tested *in vivo* directly on the plant.

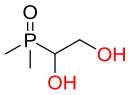
During this work, different approaches have been thought of in order to find new potent KARI inhibitor families. They are all summarized with their corresponding yields in the scheme depicted below.



The prodrug family was synthesised from the same intermediate resulting from the Pudovik reaction between dimethylphoshine oxide and benzyloxyacetaldehyde. By keeping the protecting benzyl group or cleaving it by palladium-catalysed hydrogenation, the following functionalization could be adapted either on one alcohol or both.

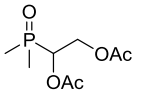


53 (65 %)

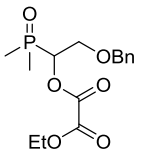


43 (100 %)

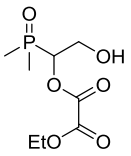
Starting from the mono protected diol of the free one, the mono and double esterifications have been done along with the acetal and the sulfite preparations.



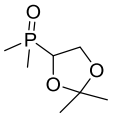
47 (61 %)



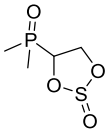
76 (59 %)



77 (100 %)

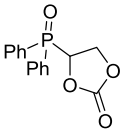


54 (37 %)



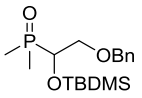
69 (13 %)

However the carbonate was not obtained starting from the diol. After many reactions with different set of conditions, only a hydrophosphorylation of vinylene carbonate with diphenylphosphine oxide worked.

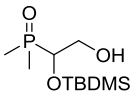


55 (47 %)

After having functionalised either both alcohols or only the secondary one, esterification on the primary alcohol was considered thanks to a one-pot *t*-butyldimethylsilyl chloride mediated Pudovik and protection reaction. Different esterification conditions were assayed without success.

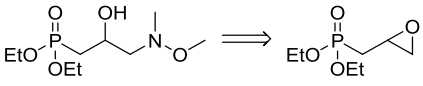


80 (86 %)



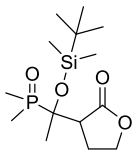
81 (89 %)

A homologated version of the diol has been synthesised by the opening of the corresponding epoxide with *N,O*-dimethylhydroxylamine. Other hydroxylamines were tested but the epoxide was relentlessly not reactive.



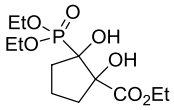
91b (21 %) **89** (81 %)

In a second family, the chelation ring size was varied in addition to the rigidification of the complexing unit by working with ring-containing reactants. The first methodology was to synthesise the products of interest thanks to Pudovik reactions on β-ketoesters, unfortunately only one could be obtained with a t-butyldimethysilyl protection and the ketone outside the ring.

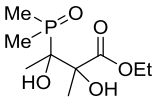


118 (36 %)

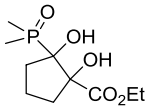
Another approach was considered by coupling the phosphorus sources to the enol triflates of the corresponding β-ketoesters, leading to tetrasubstituted alkenes with two electron-withdrawing groups. Epoxidation assays were attempted without any positive results. It was followed by dihydroxylation of each alkene with harsh conditions. Three products could be isolated in low yields.



143 (29 %)

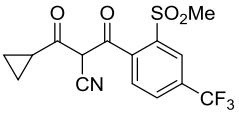


148 (9 %)

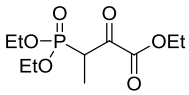


152 (7 %)

Then, analogues to HPPD's main inhibitors family, triketones, were tried to be synthesised. Only one product could be isolated, inspired from the diketonitrile **160** resulting from isoxaflutole's hydrolysis.

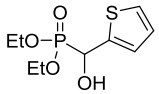


160

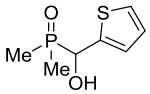


163b (68 %)

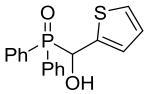
While struggling on the first families, the compounds designed by biosisosterism to mimic the carboxylic acid function was easier to obtain. They all derive from Pudovik reactions between different phosphorus sources and 2-carboxaldehyde heterocycles, giving 17 compounds. Three sets of conditions have been investigated, only base-free conditions in 1,4-dioxane and Dr Texier-Boullet's conditions ($CsF\text{-}Al_2O_3$ in 1,4-dioxane) gave significant results.



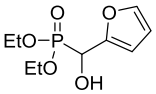
180d (78 %)



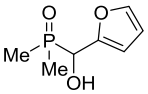
180g (quantitative)



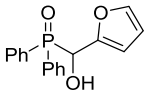
180k (83 %)



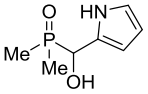
180c (85 %)



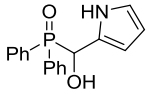
180h (58 %)



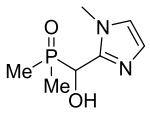
180j (74 %)



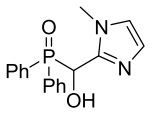
180b (94 %)



180m (74 %)



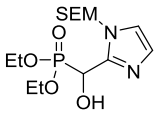
180o (31 %)



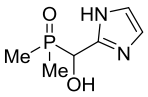
180q (71 %)

180n (96 %)

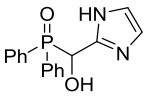
180p (70 %)



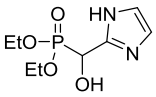
180s (43 %)



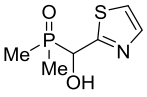
180a (61 %)



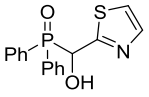
180r quantitative



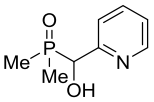
180t (59 %)



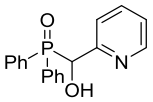
180i (51 %)



180l (73 %)

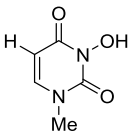


183a (32 %)

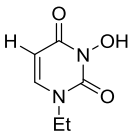


183b (72 %)

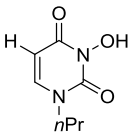
The last family included N-hydroxyimido and hydroxamate moieties as complexing units. The *N*-hydroxyimido compounds were prepared from N^1-alkylated uracils and thymines by using an N-oxidation reaction based on m-CPBA and sodium hydride. The major problem came from the purification of the products which were soluble only in methanol or water.



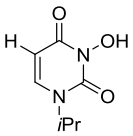
192a



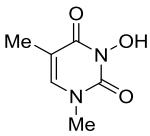
192b



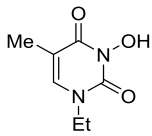
192c



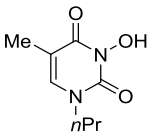
192d



193a

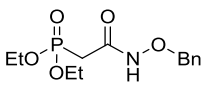


193b

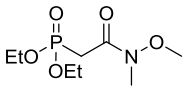


193c

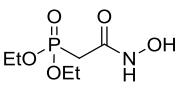
In a second part, as an analogue to the *N*-hydroxyimido compounds, hydroxamate derivatives have been synthesised, by peptidic coupling conditions with different hydroxylamines. Only diethylphosphonoacetohydroxamates have been prepared because with the dimethylphosphoryl moiety on the acetic acid part, no coupling occurred.



240a (66 %)



240c (50 %)



240d (94 %)

To date, unfortunately all the compounds sent to biological test in Bayer CropScience did not show any activity. However during this PhD only the chelation aspect of the potential inhibitors have been investigated, another aspect may be studied by trying to mimic the NADPH cofactor. It may be interesting to continue working on the complexing unit and in parallel introduce a unit imitating the NADPH in order to increase the interactions between the potential inhibitor and the catalytic site of the enzyme. Thus, it would mean increasing the chance of blocking the protein in a certain conformation without letting a natural substrate molecule undergo the biotransformation.

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Experimental section

Generalities

^{1}H NMR spectra were recorded on a BRUKER Ultra shield 400 plus (400.13 MHz) instrument. The chemical shifts are expressed in parts per million (ppm) referenced to residual chloroform-*d1* ($CDCl_3$, 7.27 ppm), DMSO-*d6* ($(CD_3)_2SO$, 2.50 ppm), D_2O (4.79 ppm), methanol-*d4* (CD_3OD, 3.31 ppm) or acetonitrile-*d3* (CD_3CN, 1.94 ppm). Coupling constants are expressed in Hertz (Hz). These abbreviations are used to express the multiplicity: s (singulet), d (doublet), t (triplet), q (quadruplet), quint (quintuplet), h (hexuplet), hept (heptuplet), m (multiplet), br (broad signal).
^{13}C NMR spectra were recorded on the same instrument at 100.6 MHz. The chemical shifts are expressed in parts per million (ppm), reported from the central of chloroform-*d1* ($CDCl_3$, 77.16 ppm), DMSO-*d6* ($(CD_3)_2SO$, 39.52 ppm), methanol-*d4* (CD_3OD, 49.00 ppm) or acetonitrile-*d3* (CD_3CN, 118.26, 1.32 ppm). Coupling constants are expressed in Hertz (Hz). These abbreviations are used to express the multiplicity: s (singulet), d (doublet), t (triplet).
^{31}P NMR spectra were recorded on the same instrument at 161.99 MHz. The chemical shifts are expressed in parts per million (ppm), reported from H_3PO_4 85%. These abbreviations are used to express the multiplicity: s (singulet), d (doublet).
^{19}F NMR spectra were recorded on the same instrument at 376.4 MHz. The chemical shifts are expressed in parts per million (ppm), reported from $CFCl_3$. The abbreviation used to express the multiplicity: s (singulet).
The assignment was obtained using one dimension NMR techniques (^{1}H, $^{13}C_{APT}$, $^{31}P_{CPD}$, ^{31}P, $^{19}F_{CPD}$). The assignment was made according to the numbering indicated in each figure.
Mass Spectra (MS): For low and high resolution spectra, the spectrometers used were electrospray ionization (ESI) WATERS Micromass Q-Tof spectrometer with as internal reference H_3PO_4 (0.1 % in water/ acetonitrile, 1 : 1).

Chromatography

Thin Layer Chromatography (TLC) was performed on precoated plates of silica gel 60 F254 Merck. Visualisation was performed with UV light then phosphomolybdic acid solution or permanganate solution followed by heating as developing agents.

Flash Chromatography was performed manually with silica gel 60 Å 35-70 µm SDS or using a CombiFlash Companion/TS with prepacked column (4 to 120 g scale) with 35-70 µm.

Purification of solvents and reagents

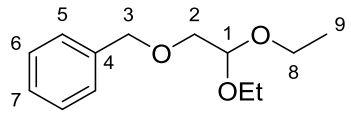
Solvent were purified before use by classical techniques under nitrogen:
- Tetrahydrofuran, diethyl ether, toluene, dichloromethane and acetonitrile were obtained from a dry solvent dispenser.
- Dimethylformamide was dried over activated 4 Å molecular sieves.

All air and/or water sensitive reactions were carried out under nitrogen atmosphere with dry, freshly distilled solvents and reagents when possible. All corresponding glassware was carefully dried under vacuum with a flameless heat gun.

Synthesis of Hoe 704 prodrugs

2-benzyloxyacetaldehyde diethyl acetal:

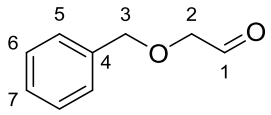
In a 250 mL two necked flask equipped with a condenser, dried and flushed with N_2, to a suspension of sodium hydride (60% dispersion in mineral oil, 2.24 g, 55.8 mmol, 1.1 eq) in THF (45 mL), benzyl alcohol (5.8 mL, 55.8 mmol, 1.1 eq) was added dropwise with stirring under nitrogen. The resulting solution was stirred for 5 min at room temperature and 2-bromoacetaldehyde diethyl acetal (7.6 mL, 50.7 mmol, 1 eq) was added dropwise followed by a solution of tetrabutylammonium iodide (940 mg, 2.54 mmol, 5 mol%) in THF (40 mL). The solution was then heated at reflux for 24h. The reaction was quenched with water (100 mL) and the product was extracted by ethyl acetate (3 x 200 mL), the organic layers were combined and dried over $MgSO_4$ and removed under reduced pressure. The crude product was purified by distillation under reduced pressure (Kugelrohr) b.p. 117 °C at 4 mbar, leading to 5.68 g of a colour less oil in 50% yield.

	$C_{13}H_{20}O_3$ M = 224.30 g.mol^{-1} 50%

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.41 – 7.25 (m, 5H, **CH_{Ar}**), 4.69 (t, $^3J_{HH}$ = 5.3 Hz, 1H, **^1CH**), 4.61 (s, 2H, **$^3CH_2$**), 3.77 – 3.55 (m, 4H, **$^8CH_2$**), 3.54 (d, $^3J_{HH}$ = 5.3 Hz, 2H, **$^2CH_2$**), 1.24 (t, $^3J_{HH}$ = 7.1 Hz, 6H, **$^9CH_3$**).

2-benzyloxyacetaldehyde 52:

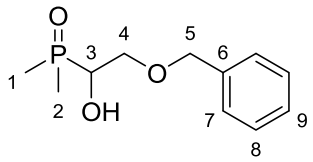
In a 250 mL flask equipped with a condenser was introduced a solution of 2-benzyloxyacetaldehyde diethyl acetal (5.66 g, 25.2 mmol, 1 eq) in THF (75 mL) and HCl (1 M, 63 mL, 63 mmol, 2.5 eq). The resulting solution was refluxed for 1h, then cooled and diluted with water (45 mL). The product was extracted with ethyl acetate (3 x 50 mL). The combined organic layers were dried over $MgSO_4$ and removed under reduced pressure. The crude product was purified by distillation under reduced pressure (Kugelrohr), b.p. 92 °C / 4 mbar leading to 3.29 g of 2-benzyloxyacetaldehyde as a colourless liquid in 87% yield.

	$C_9H_{10}O_2$ M = 150.18 g.mol^{-1} 87%

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.73 (t, $^3J_{HH}$ = 0.8 Hz, 1H, **^1CH**), 7.43 – 7.32 (m, 5H, **CH_{Ar}**), 4.65 (s, 2H, **$^3CH_2$**), 4.12 (d, $^3J_{HH}$ = 0.9 Hz, 2H, **$^2CH_2$**).

(2-benzyloxy-1-hydroxyethan-1-yl)dimethylphosphine oxide 53:

In a microwave sealed tube flushed with N_2 was introduced dimethylphosphine oxide (500 mg, 6.4 mmol, 1 eq), toluene (4.5 mL), 2-benzyloxyacetaldehyde (769 mg, 5.1 mmol, 0.8 eq) and triethylamine (71 µL, 0.51 mmol, 0.08 eq). The mixture was homogenised for 5 min by stirring at room temperature. Then it was heated at 150 °C with stirring for 15 min (200 W, 3 bar). The completion of the reaction was controlled by ^{31}P CPD NMR. The solvent was evaporated under reduced pressure. The crude was diluted with dichloromethane and washed twice with distilled water. The aqueous phase was evaporated to give 757 mg of a colourless oil in 65% yield.

	$C_{11}H_{17}O_3P$ M = 228.23 g.mol^{-1} 65%

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.37 – 7.26 (m, 5H, **CH_{Ar}**), 4.56 (s, 2H, **$^5CH_2$**), 4.02 (dd, $^3J_{HH}$ = 10.5 Hz, $^3J_{HH}$ = 4.9 Hz, 1H, **^{3}CH**), 3.89 – 3.77 (m, 2H, **$^4CH_2$**), 1.53 (d, $^2J_{PH}$ = 12.4 Hz, 3H, **$^1CH_3$**,), 1.47 (d, $^2J_{PH}$ = 12.4 Hz, 3H, **$^2CH_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 47.97 (s).

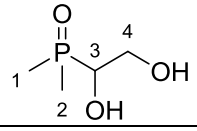
^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 137.46 (s, **^{6}C**), 128.48 (s, **^{7}CH**), 127.92 (s, **^{8}CH**), 127.89 (s, **^{9}CH**), 73.66 (s, **$^5CH_2$**), 69.63 (d, $^2J_{PC}$ = 3.8 Hz, **$^4CH_2$**), 69.61 (d, $^1J_{PC}$ = 83.1 Hz, **^{3}CH**), 13.91 (d, $^1J_{PC}$ = 67.0 Hz, **$^1CH_3$**), 13.17 (d, $^1J_{PC}$ = 65.7 Hz, **$^2CH_3$**).

MS (ES+) *m/z* = 229.1 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_{11}H_{18}O_3P$ 229.0994, found 229.0978.

(1,2-dihydroxyethan-1-yl)dimethylphosphine oxide 43:

In a 100 mL flask was introduced (2-benzyloxy-1-hydroxyethan-1-yl)dimethylphosphine oxide (2.25 g, 9.4 mmol, 1 eq), ethanol (30 mL) and 10% Pd/C (1 g, 0.94 mmol, 0.1 eq). Then the flask was let under hydrogen atmosphere (1 bar) with a rubber balloon at room temperature for 4h under agitation. The mixture was then filtered through a celite pad and the solvent removed under vacuum to give 1.08 g of white solid with 83% yield.

	$C_4H_{11}O_3P$ M = 138.10 g.mol^{-1} 83%

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.86 (s, 2H, **OH**), 4.06 – 3.85 (m, 3H, **^{3}CH**, **$^4CH_2$**), 1.60 (d, $^2J_{PH}$ = 12.9 Hz, 6H, **$^1CH_3$**), 1.54 (d, $^2J_{PH}$ = 12.9 Hz, 2H, **$^2CH_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 50.08 (s).

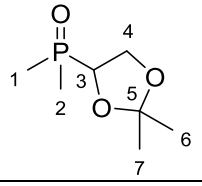
^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 71.33 (d, $^{1}J_{PC}$ = 83.5 Hz, **^{3}CH**), 62.00 (d, $^{2}J_{PC}$ = 3.6 Hz, **$^{4}CH_2$**), 14.02 (d, $^{1}J_{PC}$ = 66.2 Hz, **$^{1}CH_3$**), 12.98 (d, $^{1}J_{PC}$ = 66.0 Hz, **$^{2}CH_3$**).

MS (ES+) *m/z* = 139.05 ($M+H^+$).

HRMS (ES+): *m/z*: [M+H] calculated for $C_4H_{12}O_3P$ 139.0524, found 139.0524.

4-dimethylphosphoryl-2,2-dimethyl-1,3-dioxolane 54:

In a dry and nitrogen flushed 25 mL flask was introduced a solution of (1,2-dihydroxyethan-1-yl)dimethylphosphine oxide (200 mg, 1.45 mmol, 1 eq) in THF (5 mL) and 2,2-dimethoxypropane (223 mg, 1.6 mmol, 1.1 eq). Then, vacuum dried Amberlyst 15 (tip of spatula) was introduced in the medium which was stirred for 2 days at room temperature. The reaction was then submitted to microwave irradiation (90 °C with stirring for 20 min, 200 W). The suspension was filtered and directly evaporated under vacuum. The crude oil was purified by flash column chromatography (ethyl acetate/ethanol 80/20) to give 96 mg of an opaque and colourless oil in 37% yield.

	$C_7H_{15}O_3P$ M = 178.17 g.mol^{-1} 37%

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.31 – 4.15 (m, 3H, **^{3}CH**, **$^{4}CH_2$**), 1.52 (d, $^{2}J_{PH}$ = 13.1 Hz, 3H, **$^{1}CH_3$**), 1.43 (d, $^{2}J_{PH}$ = 13.0 Hz, 3H, **$^{2}CH_3$**), 1.39 (s, 3H, **$^{6}CH_3$**), 1.30 (s, 3H **$^{7}CH_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 46.44 (s).

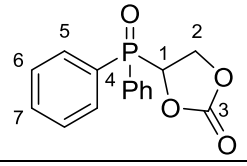
^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 111.09 (s, **^{5}C**), 72.21 (d, $^{1}J_{PC}$ = 88.8 Hz, **^{3}CH**), 64.13 (s, **$^{4}CH_2$**), 25.94 (s, **$^{6}CH_3$**), 24.08 (s, **$^{7}CH_3$**), 14.50 (d, $^{1}J_{PC}$ = 67.9 Hz, **$^{1}CH_3$**), 11.42 (d, $^{1}J_{PC}$ = 68.84 Hz, **$^{2}CH_3$**).

MS (ES+) *m/z* = 179.08 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_7H_{16}O_3P$ 179.0837, found 179.0838.

4-diphenylphosphoryl-2,2-dimethyl-1,3-dioxolane-2-one 55b:

In a dry and nitrogen flushed V-shaped 5 mL Wheaton tube, diphenylphosphine oxide (200 mg, 0.99 mmol, 1 eq) was introduced to a solution of vinylene carbonate (63 µL, 0.99 mmol, 1 eq) in acetonitrile (1 mL). Potassium *tert*-butoxide (22 mg, 0.19 mmol, 0.2 eq) was then added to the solution which was stirred at room temperature for 16h. The reaction was quenched by 4 mL of distilled water and extracted by 3 x 3 mL of dichloromethane. The organic phase was dried by $MgSO_4$ and evaporated under vacuum. The crude was purified by a flash column chromatography with ethyl acetate/heptane (50/50 to 100/0) leading to 134 mg of product as a white solid with 47% yield.

	$C_{15}H_{13}O_4P$ M = 288.24 g.mol^{-1} 47%

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.82 (m, 2H, **CH_{Ar}**), 7.80 (m, 2H, **CH_{Ar}**), 7.65 – 7.37 (m, 6H, **CH_{Ar}**), 5.30 (dt, $^{2}J_{PH}$ = 17.9 Hz, $^{3}J_{HH}$ = 8.9 Hz, 1H, **^{1}CH**), 4.74 – 4.53 (m, 2H, **$^{2}CH_2$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 26.10 (s).

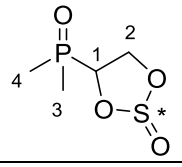
^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 153.58 (s, **^{3}C**), 133.56 (d, $^{4}J_{PC}$ = 2.7 Hz, **^{7}CH**), 133.34 (d, $^{4}J_{PC}$ = 2.7 Hz, **^{7}CH**), 132.22 (d, $^{2}J_{PC}$ = 9.2 Hz, **^{5}CH**), 131.17 (d, $^{2}J_{PC}$ = 9.6 Hz, **^{5}CH**), 129.23 (d, $^{3}J_{PC}$ = 12.2 Hz, **^{6}CH**), 129.20 (d, $^{3}J_{PC}$ = 12.0 Hz, **^{6}CH**), 128.98 (d, $^{1}J_{PC}$ = 103.1 Hz, **^{4}C**), 126.09 (d, $^{1}J_{PC}$ = 100.6 Hz, **^{4}C**), 71.90 (d, $^{1}J_{PC}$ = 82.1 Hz, **^{1}CH**), 64.54 (s, **$^{2}CH_2$**).

MS (ES+) *m/z* = 289.06 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_{15}H_{14}O_4P$ 289.0630, found 289.0634.

4-dimethylphosphoryl-1,3,2-dioxathiolane 69:

In a dry and nitrogen flushed V-shaped 5 mL Wheaton tube, thionyl chloride (126 µL, 1.74 mmol, 1.2 eq) was added to a solution of (1,2-dihydroxyethan-1-yl)dimethylphosphine oxide (200 mg, 1.45 mmol, 1 eq) in a mixture of dichloromethane (1 mL) and triethylamine (720 µL). The medium was stirred overnight at room temperature and evaporated under vacuum. The crude was dissolved in diethyl ether during which a solid precipitated and was filtered. The solid was purified by a flash column chromatography with ethyl acetate/ethanol (80/20) as eluent which gave 35 mg of the product in 13% yield as a mixture of diastereoisomers (1:1).

	$C_4H_9O_4PS$ M = 184.15 g.mol^{-1} 13%

^{1}H NMR (400 MHz, $CDCl_3$δ (ppm): 5.02 – 4.89 (m, 1H, **^{1}CH**), 4.83 – 4.65 (m, 1H, **$^{2}CH_2$**), 4.48 (m, 1H, **$^{2}CH_2$**), 1.67 (d, $^{2}J_{PH}$ = 13.4 Hz, 1H, **$^{3}CH_3$**, dia 1), 1.62 (d, $^{2}J_{PH}$ = 13.2 Hz, 6H, **$^{3}CH_3$**, **$^{4}CH_3$**, dia 2), 1.47 (d, $^{2}J_{PH}$ = 13.1 Hz, 1H, **$^{4}CH_3$**, dia 1).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 44.66 (s, dia 1), 43.38 (s, dia 2).

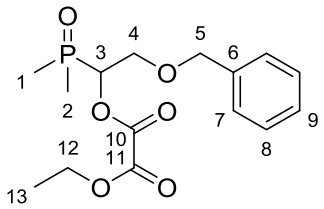
^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 77.56 (d, $^{1}J_{PC}$ = 77.1 Hz, **^{1}CH**), 73.83 (d, $^{1}J_{PC}$ = 79.3 Hz, **^{1}CH**), 68.02 (s, **$^{2}CH_2$**), 67.71 (s, **$^{2}CH_2$**), 16.12 (d, $^{1}J_{PC}$ = 70.5 Hz, **$^{3}CH_3$**), 15.15 (d, $^{1}J_{PC}$ = 70.0 Hz, **$^{3}CH_3$**), 11.92 (d, *J* = 69.7 Hz, **$^{4}CH_3$**), 11.47 (d, *J* = 70.5 Hz, **$^{4}CH_3$**).

MS (ES+) *m/z* = 185.00 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_4H_{10}O_4PS$ 185.0037, found 185.0037.

(1-(ethyl oxalyl)-2-benzyloxyethan-1-yl)dimethylphosphine oxide 76:

In a dried and nitrogen flushed 10 mL flask was prepared a solution of (2-benzyloxy-1-hydroxyethan-1-yl)dimethylphosphine oxide (200 mg, 0.87 mmol, 1 eq) in pyridine (2 mL) and cooled to 0 °C using an ice bath. Then followed the addition dropwise of ethyl chlorooxoacetate (196 µL, 1.75 mmol, 2 eq) at 0 °C. The mixture was stirred for 2h from 0 °C to room temperature. The suspension was filtered and the filtrate evaporated under vacuum to give 180 mg of the corresponding ester in 63% yield as a brownish oil.

	$C_{15}H_{21}O_6P$ M = 328.3 g.mol^{-1} 63%

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.38 – 7.30 (m, 5H, **CH_{Ar}**), 5.52 – 5.43 (m, 1H, **^{3}CH**), 4.59 (s, 2H, **$^{5}CH_2$**), 4.39 (q, $^{3}J_{HH}$ = 7.1 Hz, 2H, **$^{12}CH_2$**), 4.11 – 3.95 (m, 2H, **$^{4}CH_2$**), 1.64 (d, $^{2}J_{PH}$ = 13.3 Hz, 3H, **$^{1}CH_3$**), 1.56 (d, $^{2}J_{PH}$ = 13.3 Hz, 3H, **$^{2}CH_3$**), 1.41 (t, $^{3}J_{HH}$ = 7.1 Hz, 3H, **$^{13}CH_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 42.62 (s).

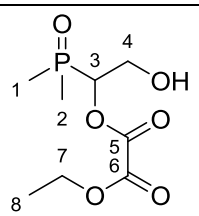
^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 157.10 (s, **^{10}C**), 156.97 (s, **^{11}C**), 137.20 (s, **^{6}C**), 128.49 (s, **^{7}CH**), 127.93 (s, **^{8}CH**), 127.71 (s, **^{9}CH**), 73.48 (s, **$^{5}CH_2$**), 73.05 (d, *J* = 79.9 Hz, **^{3}CH**), 66.85 (s, **$^{4}CH_2$**), 63.58 (s, **$^{12}CH_2$**), 15.33 (d, *J* = 69.2 Hz, **$^{1}CH_3$**), 14.38 (d, *J* = 68.9 Hz, **$^{2}CH_3$**), 13.90 (s, **$^{13}CH_3$**).

MS (ES+) *m/z* = 329.11 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_{15}H_{22}O_6P$ 329.1154, found 329.1158.

1-(ethyl oxalyl)-2-hydroxyethan-1-yl)dimethylphosphine oxide 77:

In a 5 mL flask was introduced (1-(ethyl oxalyl)-2-benzyloxyethan-1-yl)dimethylphosphine oxide (100 mg, 0.30 mmol, 1 eq) and ethanol (1 mL). Palladium on charcoal (10% Pd on C, 32 mg, 0.03 mmol, 0.1 eq) was then added to the solution. The flask was charged with hydrogen with a rubber balloon and the medium was stirred at room temperature overnight. The heterogenous mixture was filtered through a celite pad and the filtrate evaporated under vacuum to give 47 mg of product as a colourless oil in 65% yield.

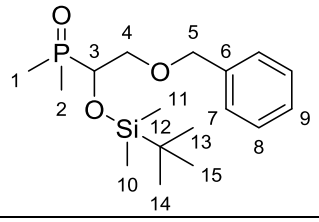
	$C_8H_{15}O_6P$ M = 238.18 g.mol^{-1} 65%

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.26 (q, $^{3}J_{HH}$ = 7.1 Hz, 2H, **$^{7}CH_2$**), 3.94 (m, 3H, **^{3}CH**, **$^{4}CH_2$**), 1.57 (d, $^{2}J_{PH}$ = 13.1 Hz, 3H, **$^{2}CH_3$**), 1.56 (d, $^{2}J_{PH}$ = 13.1 Hz, 3H, **$^{1}CH_3$**), 1.30 (t, $^{3}J_{HH}$ = 7.1 Hz, 3H, **$^{8}CH_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 54.76 (s).

(2-benzyloxy-*O*-(*tert*-butyldimethylsilyl)ethanol-1-yl)dimethylphosphine oxide 80:

In dry and nitrogen flushed two necked 250 mL flask was introduced dimethylphosphine oxide (1 g, 12.8 mmol, 1 eq), toluene (100 mL) followed by 2-benzyloxyacetaldehyde (1.92 g, 12.8 mmol, 1 eq), *tert*-butyldimethylsilyl chloride (2.89 g, 19.2 mmol, 1.5 eq) and triethylamine (2.7 mL, 19.2 mmol, 1.5 eq). The medium was stirred for 15h at 70 °C. The reaction was quenched by adding methanol (2.6 mL). The solids were filtered off, the filtrate was evaporated under vacuum. The crude oil was purified by a flash column chromatography with ethyl acetate/ethanol (90/10) as eluent to give 3.77 g of product as a colourless oil in 86% yield.

	$C_{17}H_{31}O_3PSi$ M = 342.49 g.mol^{-1} 86%

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.35 – 7.10 (m, 5H, **CH_{Ar}**), 4.45 (q, *J* = 11.8 Hz, 2H, **$^{5}CH_2$**), 4.04 – 3.93 (m, 1H, **^{3}CH**), 3.85 – 3.75 (m, 1H, **$^{4}CH_2$**), 3.75 – 3.59 (m, 1H, **$^{4}CH_2$**), 1.49 – 1.33 (m, 6H, **$^{1}CH_3$**, **$^{2}CH_3$**), 0.80 (s) 9H, **$^{13}CH_3$**, **$^{14}CH_3$**, **$^{15}CH_3$**), -0.00 (s, 6H, **$^{10}CH_3$**, **$^{11}CH_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 47.27 (s).

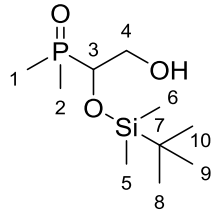
^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 137.80 (s, **^{6}C**), 128.33 (s, **^{7}CH**), 127.71 (s, **^{8}CH**), 127.64 (s, **^{9}CH**), 73.51 (s, **$^{5}CH_2$**), 71.62 (d, $^{1}J_{PC}$ = 89.6 Hz, **^{3}CH**), 70.33 (d, $^{2}J_{PC}$ = 4.7 Hz, **$^{4}CH_2$**), 25.74 (s, **$^{13}CH_3$**, **$^{14}CH_3$**, **$^{14}CH_3$**), 18.00 (s, **^{12}C**), 14.72 (d, $^{1}J_{PC}$ = 67.9 Hz, **$^{1}CH_3$**), 12.83 (d, $^{1}J_{PC}$ = 66.5 Hz, **$^{2}CH_3$**), -4.16 (s, **$^{10}CH_3$**), -5.16 (s, **$^{11}CH_3$**).

MS (ES+) *m/z* = 343.19 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_{17}H_{32}O_3PSi$ 343.1858, found 343.1865.

(2-hydroxy-*O*-*tert*-butyldimethylsilylethanol-1-yl)dimethylphosphine oxide 81:

In a 10 mL flask was introduced 2-benzyloxy-O-(tert-butyldimethylsilyl)-1-dimethylphosphorylethanol (154 mg, 0.45 mmol, 1 eq) and ethanol (4.5 mL). Palladium on charcoal (10% Pd on C, 48 mg, 0.045 mmol, 0.1 eq) was then added to the solution. The flask was charged with hydrogen with a rubber balloon and the medium was stirred at room temperature for 4h. The heterogenous mixture was filtered through a celite pad and the filtrate evaporated under vacuum to give 98mg of white solid corresponding to the debenzylated product with 86% yield.

	$C_{10}H_{25}O_3PSi$ M = 252.37 g.mol^{-1} 86%

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.94 – 3.75 (m, 3H, **^{3}CH**, **^{4}CH$_2$**), 1.48 – 1.39 (m, 6H, **^{1}CH$_3$**, **^{2}CH$_3$**), 0.80 (s, 9H, 1 **^{8}CH$_3$**, **^{9}CH$_3$**, **^{10}CH$_3$**), 0.06 (s, 6H, **^{5}CH$_3$**, **^{6}CH$_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 46.84 (s).

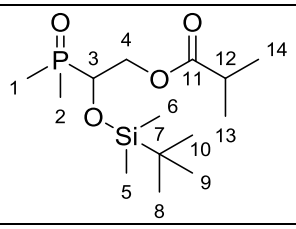
^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 70.77 (d, $^1J_{PC}$ = 89.2 Hz, **^{3}CH**), 62.74 (s, **^{4}CH$_2$**), 25.84 (s, **^{8}CH$_3$**, **^{9}CH$_3$**, **^{10}CH$_3$**), 18.23 (s, **^{7}C**), 14.89 (d, $^1J_{PC}$ = 66.3 Hz, **^{1}CH$_3$**), 14.08 (d, $^1J_{PC}$ = 66.8 Hz, **^{2}CH$_3$**), -4.27 (s, **^{5}CH$_3$**), -5.50 (s, **^{6}CH$_3$**).

MS (ES+) *m/z* = 253.14 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_{10}H_{26}O_3PSi$ 253.1389, found 253.1388.

(2-hydroxy-*O*-*tert*-butyldimethylsilylethan-1-yl isopropylate)dimethylphosphine oxide 83e:

In a dry and nitrogen flushed Radley™ tube glass reaction tube, isopropyl chloride (101 µL, 1.42 mmol, 1.2 eq) was added dropwise to a solution of pivalic acid (146 mg, 1.42 mmol, 1.2 eq) with trimethylamine (198 µL, 1.42 mmol, 1.2 eq) in chloroform (2.4 mL) at 0 °C. The reaction mixture was stirred for 1 h and the formation of the mixed anhydride was monitored by GC-MS. After the complete formation of the mixed anhydride, a solution of (2-hydroxy-*O*-*tert*-butyldimethylsilylethanol-1-yl)dimethylphosphine oxide (300 mg, 1.19 mmol, 1 eq) with pyridine (10 µL, 0.12 mmol, 0.1 eq), trimethylamine (165 µL, 1.19 mmol, 1 eq) in chloroform (2.4 mL) was added to the previous medium. The reaction mixture was stirred at 60 °C for 4 h and the reaction was monitored by GC-MS. At completion the medium was quenched with 10 mL of a citric acid solution at 30% w/w. The phases were separated and the organic one was washed with 10 mL of saturated solution of $NaHCO_3$. The organic phase was dried on $MgSO_4$ and evaporated under vacuum to lead to 297 mg of yellow oil. The crude oil was purified by flash column chromatography with ethyl acetate/ethanol (80/20) to give 7 mg of product as a colourless oil in 2% yield.

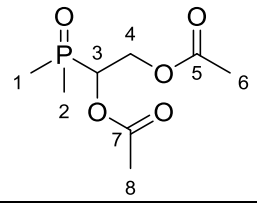
	$C_{14}H_{31}O_4PSi$ M = 322.46 g.mol^{-1} 2%

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.50 (ddd, *J* = 15.8, 12.2, 3.3 Hz, 1H), 4.12 (dt, *J* = 12.2, 4.5 Hz, 1H), 4.02 – 3.95 (m, 1H), 2.43 (hept, *J* = 7.0 Hz, 1H, **^{12}CH**), 1.41 (d, $^2J_{PH}$ = 13.3 Hz, 3H, **^{1}CH$_3$**), 1.39 (d, $^2J_{PH}$ = 12.9 Hz, 3H, **^{2}CH$_3$**) 1.04 (d, $^3J_{HH}$ = 7.0 Hz, 3H, **^{14}CH$_3$**), 1.03 (d, $^3J_{HH}$ = 7.0 Hz, 3H, **^{13}CH$_3$**), 0.75 (s, 9H, **^{8}CH$_3$**, **^{9}CH$_3$**, **^{10}CH$_3$**), -0.00 (s, 3H, **^{5}CH$_3$**), -0.02 (s, 3H, **^{6}CH$_3$**).

^{31}P NMR CDP (162 MHz, $CDCl_3$) δ (ppm): 47.03 (s).

(Ethan-1-yl-1,2-diyl diacetate)dimethylphosphine oxide 47:

In a dry and nitrogen flushed 10 mL flask, acetic anhydride (144 µL, 1.51 mmol, 2.1 eq) was added dropwise to a solution of (1,2-dihydroxyethan-1-yl)dimethylphosphine oxide (100 mg, 0.72 mmol, 1 eq) in pyridine (2.5 mL) at room temperature. The reaction was monitored by ^{31}P CPD NMR and GC-MS. When it reached completion after 3h of stirring, the medium was evaporated under vacuum to lead to 189 mg of a pale yellow oil. The crude oil was purified by a flash column chromatography with ethyl acetate/ethanol (80/20) to give 98 mg of product a colourless oil in 61% yield.

	$C_8H_{15}O_5P$ M = 222.18 g.mol^{-1} 61%

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 5.35 (ddd, *J* = 7.0, 6.1, 3.1 Hz, 1H, **^{3}CH**), 4.63 (td, *J* = 12.4 Hz, 3.1 Hz, 1H, **^{4}CH$_2$**), 4.33 (ddd, *J* = 12.6, 6.1, 4.0 Hz, 1H, **^{4}CH$_2$**), 2.09 (s, 3H, **^{8}CH$_3$**), 2.02 (s, 3H, **^{6}CH$_3$**), 1.54 (d, $^2J_{PH}$ = 13.3 Hz, 3H, **^{1}CH$_3$**), 1.49 (d, $^2J_{PH}$ = 13.0 Hz, 3H, **^{2}CH$_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 42.74 (s).

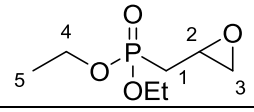
^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 170.31 (s, **^{5}C**), 169.61 (s, **^{7}C**), 69.18 (d, $^1J_{PC}$ = 81.7 Hz, **^{3}CH**), 61.37 (d, $^2J_{PC}$ = 3.8 Hz, **^{4}CH$_2$**), 20.71 (s, **^{6}CH$_3$**), 20.65 (s, **^{8}CH$_3$**), 15.00 (d, $^1J_{PC}$ = 69.2 Hz, **^{1}CH$_3$**), 14.02 (d, $^1J_{PC}$ = 68.7 Hz, **^{2}CH$_3$**).

MS (ES+) *m/z* = 223.0 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_8H_{16}O_5P$ 223.0735, found 223.0723.

Diethyl (2,3-epoxypropyl)phosphonate 89:

In a dry and nitrogen flushed 500 mL two necked flask equipped with a condenser and a 250 mL pressure-equalising dropping funnel, a solution of *m*-CPBA (4.35 g, 25.3 mmol, 1.5 eq) in dichloromethane (100 mL) was added dropwise over 3h to a solution of diethyl allylphosphonate (2.9 mL, 16.8 mmol, 1 eq) in dichloromethane (116 mL) at room temperature. At the end of the addition, the mixture was heated to 50 °C overnight. The reaction was monitored by GC-MS analysis. *m*-CPBA (2.9 g,16.8 mmol, 1 eq) was added directly to the medium to achieve completion. At completion, the medium was quenched and washed by 2 x 25 mL of a 10% solution of sodium thiosulfate. The organic phase was washed with 2 x 50 mL of 10% solution of sodium carbonate. The organic phase was dried with $MgSO_4$ and evaporated under vacuum leading to 3.16 g of pure epoxide as a colourless oil in 97% yield.

	$C_7H_{15}O_4P$ M = 194.17 g.mol^{-1} 97%

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.17 – 4.04 (m, 4H, **$^4CH_2$**), 3.18 – 3.11 (m, 1H, **^2CH**), 2.84 – 2.77 (m, 1H, one of **$^3CH_2$**), 2.55 (dd, *J* = 4.8, 2.5 Hz, 1H, one of **$^3CH_2$**), 2.17 (ddd, *J* = 18.3, 15.2, 5.6 Hz, 1H, one of **$^1CH_2$**), 1.83 (ddd, *J* = 19.9, 15.2, 6.5 Hz, 1H, one of **$^1CH_2$**), 1.31 (t, $^3J_{HH}$ = 7.1 Hz, 6H, **$^5CH_3$**).

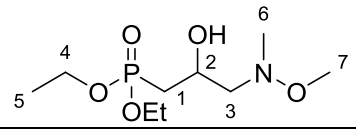
^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 26.27 (s).

MS (ES+) *m/z* = 195.08 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_7H_{16}O_4P$ 195.0786, found 195.0786.

Diethyl 3-(*N,O*-dimethylhydroxyamino)-2-hydroxypropylphosphonate 91b:

In a V-shaped 5 mL Wheaton vial, a mixture of diethyl (2,3-epoxypropyl)phosphonate (184 mg, 0.95 mmol, 1 eq), *N,O*-dimethylhydroxylamine hydrochloride (139 mg, 1.42mmol, 1.5 eq) and triethylamine (263 µL, 1.89 mmol, 2 eq) in ethanol/water (1:1, v/v, 1.74 mL) was stirred at room temperature for 5 days. The reaction mixture was extracted with dichloromethane (3 x 3 mL), the organic extracts were dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by a flash column chromatography with ethyl acetate/ethanol (95/5) to give 51 mg of the more polar phosphonate as a colourless oil in 21% yield.

	$C_9H_{22}NO_5P$ M = 255.25 g.mol^{-1} 21%

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.20 – 3.98 (m, 5H, **$^4CH_2$**, **^2CH**), 3.46 (s, 3H,**$^7CH_3$**), 2.66 (d, $^3J_{HH}$ = 4.6 Hz, 2H, **$^3CH_2$**), 2.56 (s, 3H, **$^6CH_3$**), 2.06 – 1.94 (m, 1H, one of **$^1CH_2$**), 1.88 (m, 1H, one of **$^1CH_2$**), 1.27 (t, $^3J_{HH}$ = 7.1 Hz, 6H).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 29.58 (s).

^{13}C APT NMR (63 MHz, $CDCl_3$) δ (ppm): 66.49 (d, $^2J_{PC}$ = 15.1 Hz, **$^4CH_2$**), 64.10 (s, **$^7CH_3$**), 61.88 (d, $^2J_{PC}$ = 23.8 Hz, **^2CH**), 59.86 (s, **$^3CH_2$**), 45.13 (s, **$^6CH_3$**), 31.49 (d, $^1J_{PC}$ = 140.4 Hz, **$^1CH_2$**), 16.31 (d, $^3J_{PC}$ = 6.1 Hz, **$^5CH_3$**).

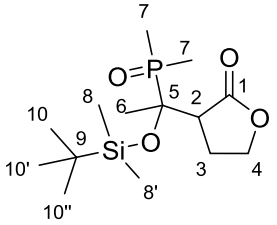
MS (ES+) *m/z* = 256.13 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_9H_{23}NO_5P$ 256.1314, found 256.1318.

Variations of chelating ring size

[1-(*tert*-butyldimethylsilyloxy)-1-(dihydrofuran-2(3H)-one-3-yl)ethan-1-yl]dimethylphosphine oxide 118:

In a 10 mL two-necked flask dried and flushed with N_2, to a solution of dimethylphosphine oxide (100 mg, 1.28 mmol, 1 eq) in THF (5 mL) was added, *tert*-butyldimethylsilyl chloride (290 mg, 1.92 mmol, 1.5 eq) and triethylamine (266 µL, 1.92 mmol, 1.5 eq). The medium was stirred at 0 °C for 2h. 2-acetylbutyrolactone (138 µL, 1.28 mmol, 1 eq) was then added to the medium at 0 °C, stirring continued for 7h. The heterogeneous medium was then filtered through a celite pad. The filtrate was evaporated and diluted with diethyl ether (10 mL), the resulting solid triethylammonium chloride was filtered through a celite pad and the filtrate was evaporated under reduced pressure. The crude oil was purified by a flash column chromatography with ethyl acetate/ethanol (90/10) to give 147 mg of a mixture of two diastereoisomers, in 36% yield.

	$C_{14}H_{29}O_4PSi$ M = 320.44 g.mol^{-1} (mixture 13/87 of diastereoisomers)

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.19 (t, $^3J_{HH}$ = 8.9 Hz, 4H, 2 isomers, **$^4CH_2$**), 4.06 – 3.88 (m, 4H, 2 isomers, **$^4CH_2$**), 2.94 – 2.80 (m, 2H, 2 isomers, **^2CH**), 2.50 – 2.25 (m, 2H, 2 isomers, **$^3CH_2$**), 1.84 (d, $^3J_{PH}$ = 15.6 Hz, 3H, 1 isomer, **$^6CH_3$**), 1.77 (d, $^3J_{PH}$ = 14.7 Hz, 3H, 1 isomer, **$^6CH_3$**), 1.49 (d, $^2J_{PH}$ = 14.7 Hz, 3H, 1 isomer, **$^7CH_3$**), 1.44 (d, $^2J_{PH}$ = 13.0 Hz, 3H, 1 isomer, **$^{7'}CH_3$**), 1.38 (d, $^2J_{PH}$ = 12.4 Hz, 3H, 1 isomer, **$^7CH_3$**), 1.33 (d, $^2J_{PH}$ = 12.0 Hz, 3H, 1 isomer, **$^{7'}CH_3$**), 0.71 (s, 9H, 2 isomers, **$^{10}CH_3$**, **$^{10'}CH_3$**, **$^{10''}CH_3$**), 0.04 (s, 6H, 1 isomer, **$^8CH_3$**, **$^{8'}CH_3$**), -0.00 (s, 6H, 1 isomer, **$^8CH_3$**, **$^{8'}CH_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 54.91 (s, 13 %), 54.42 (s, 87 %).

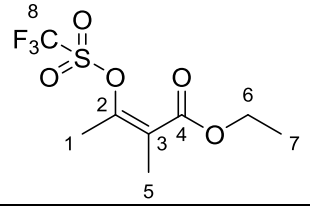
^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 177.60 (s, 1 isomer, **^1C**), 177.44 (s, 1 isomer, **^1C**), 77.90 (s, 1 isomer, **^5C**), 76.99 (s, 1 isomer, **^5C**), 67.95 (s, 1 isomer, **$^4CH_2$**), 67.26 (s, 1 isomer, **$^4CH_2$**), 49.77 (d, $^2J_{PC}$ = 5.7 Hz, 1 isomer, **$^6CH_3$**), 46.80 (d, $^2J_{PC}$ = 6.6 Hz, 1 isomer, **$^6CH_3$**), 27.63 (s, 2 isomers, **$^{10}CH_3$**), 27.09 (s, 2 isomers, **$^3CH_2$**), 24.03 (d, $^2J_{PC}$ = 6.1 Hz, 1 isomer, **^2CH**), 22.13 (d, $^2J_{PC}$ = 3.4 Hz, 1 isomer, **^2CH**), 20.17 (s, 1 isomer, **^9C**), 16.03 (d, $^1J_{PC}$ = 64.2 Hz, 2 isomers, **$^7CH_3$**), 15.20 (d, $^1J_{PC}$ = 64.8 Hz, 2 isomers, **$^{7'}CH_3$**), 0.00 (s, 2 isomers, **$^8CH_3$**), -0.20 (s, 2 isomers, **$^{8'}CH_3$**).

MS (ES+) *m/z* = 321.17 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_{14}H_{30}O_4PSi$ 321.1651, found 321.1654.

Ethyl 2-methyl-3-[(trifluoromethylsulfonyl)oxo]but-2-enoate 122:

In a 100 mL flask dried and flushed with N_2, to a solution of ethyl 2-methyl-3-oxobutanoate (2.8 mL, 20.8 mmol, 1 eq) in dichloromethane (52 mL) at -78 °C was introduced *N,N*-diisopropylethylamine (18.1 mL, 104 mmol, 5 eq). Trifluoromethanesulfonic anhydride (4.2 mL, 45 mmol, 1.2 eq) was added dropwise at -78 °C. At the end of the addition the medium was let to heat up to room temperature and stirred for 2h30. The completion of the reaction was controlled by GC-MS analysis. The reaction was quenched by distilled water (52 mL). The organic phase was separated and washed twice with HCl 1N (75 mL each time). The organic phase was dried over $MgSO_4$ and evaporated under reduced pressure. The brown oil crude was purified by a flash column chromatography with ethyl acetate/heptane (10/90), leading to 4.06 g of yellow oil in 71% yield.

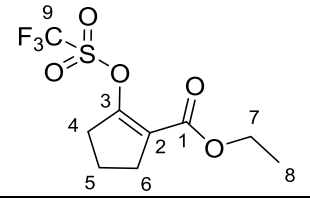
	$C_8H_{11}F_3O_5S$ M = 276.23 g.mol^{-1} 71%

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.26 (q, $^3J_{HH}$ = 7.1 Hz, 2H, **$^6CH_2$**), 2.42 (s, 3H, **$^1CH_3$**), 2.00 (s, 3H, **$^5CH_3$**), 1.33 (t, $^3J_{HH}$ = 7.1 Hz, 3H, **$^7CH_3$**).

^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 166.38 (s, **^{4}C**), 154.56 (s, **^{2}C**), 122.80 (s, **^{3}C**), 118.27 (d, $^1J_{CF}$ = 319.7 Hz, **^{8}C**), 61.52 (s, **$^6CH_2$**), 19.06 (s, **$^1CH_3$**), 14.05 (s, **$^7CH_3$**), 13.85 (s, **$^5CH_3$**).

Ethyl 2-[(trifluoromethyl)sulfonyloxy]cyclopent-1-ene-1-carboxylate 125:

The synthesis of ethyl 2-[(trifluoromethyl)sulfonyloxy]cyclopent-1-ene-1-carboxylate was performed according to the previous procedure starting from ethyl 2-oxocyclopentanecarboxylate (1 g, 6.4 mmol) and trifluoromethanesulfonic anhydride (1.26 mL, 7.68 mmol).

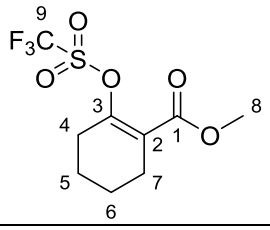
	$C_9H_{11}F_3O_5S$ M = 288.24 g.mol^{-1} 71% (1.31 g) Colourless oil

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.28 (q, $^3J_{HH}$ = 7.1 Hz, 2H, **$^7CH_2$**), 2.80 – 2.69 (m, 4H, **$^4CH_2$**, **$^6CH_2$**), 2.08 – 1.99 (m, 2H, **$^5CH_2$**), 1.34 (t, $^3J_{HH}$ = 7.1 Hz, 3H, **$^8CH_3$**).

^{19}F CPD NMR (376 MHz, $CDCl_3$) δ (ppm): -74.49 (s).

Methyl 2-[(trifluoromethyl)sulfonyloxy]cyclohex-1-ene-1-carboxylate 126:

The synthesis of methyl 2-[(trifluoromethyl)sulfonyloxy]cyclohex-1-ene-1-carboxylate was performed according to the previous procedure starting from methyl 2-oxocyclohexanecarboxylate (3 g, 19.2 mmol) and trifluoromethanesulfonic anhydride (3.8 mL, 23.1 mmol).

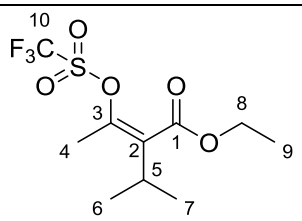
	$C_9H_{11}F_3O_5S$ M = 288.24 g.mol^{-1} 84% (4.64 g) Colourless oil

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.79 (s, 3H, **$^8CH_3$**), 2.52 – 2.44 (m, 2H, **$^4CH_2$**), 2.44 – 2.35 (m, 2H, **$^7CH_2$**), 1.82 – 1.73 (m, 2H, **$^5CH_2$**), 1.73 – 1.64 (m, 2H, **$^6CH_2$**).

^{19}F CPD NMR (376 MHz, $CDCl_3$) δ (ppm): -74.87 (s).

Ethyl 2-isopropyl-3-[(trifluoromethylsulfonyl)oxo]but-2-enoate 123:

In a 50 mL flask dried and flushed with N_2, a solution of LiHMDS (1 M in THF, 4 mL, 4 mmol, 2 eq) was diluted with THF (7 mL). The resulting solution was cooled to -78 °C and ethyl 2-isopropyl-3-oxobutanoate (358 µL, 2 mmol, 1 eq) was introduced. After 45 min of stirring at -78 °C trifluoromethanesulfonic anhydride (336 µL, 2 mmol, 1 eq) was added dropwise. The reaction was stirred for 2h while warming up to room temperature. The completion was controlled by GC-MS analysis. The reaction was quenched by the addition of a solution of saturated NH_4Cl (7 mL). The organic phase was separated. The aqueous fraction was extracted with diethyl ether. The organic phases were combined, dried over $MgSO_4$ and evaporated under reduced pressure. The crude oil was purified by a flash column chromatography with ethyl acetate/heptane (10/90) to give 396 mg of a yellow oil in 65% yield.

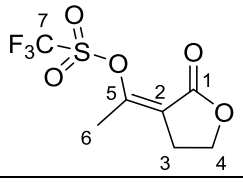
	$C_{10}H_{15}F_3O_5S$ M = 304.28 g.mol^{-1} 65%

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.10 (q, $^3J_{HH}$ = 7.1 Hz, 2H, **$^8CH_2$**), 2.51 (hept, $^3J_{HH}$ = 7.0 Hz, 1H, **^5CH**), 1.95 (s, 3H, **$^4CH_3$**), 1.16 (t, $^3J_{HH}$ = 7.2 Hz, 3H, **$^9CH_3$**), 0.99 (d, $^3J_{HH}$ = 6.9 Hz, 6H, **$^6CH_3$**, **$^7CH_3$**).

^{19}F CPD NMR (377 MHz, $CDCl_3$) δ (ppm): -74.86 (s).

1-(2-oxodihydrofuran-3(2H)-ylidene)ethyl trifluoromethanesulfonate 124:

In a 100 mL flask dried and flushed with N_2, to a solution of 2-acetylbutyrolactone (2.52 mL, 23.4 mmol, 1 eq) and triethylamine (3.3 mL, 23.4 mmol, 1 eq) in dichloromethane (37 mL) at 0 °C was introduced trifluoromethanesulfonic anhydride (4.6 mL, 28.1 mmol, 1.2 eq) dropwise. The reaction was stirred at 0 °C for 30 min. The completion of the reaction was monitored by GC-MS. The reaction was quenched by a mixture of brine (15 mL) and saturated solution of sodium hydrogenocarbonate (15 mL). The organic phase was separated and the aqueous phase was extracted by dichloromethane. The organic phases were combined, washed by HCl 1 N and dried over $MgSO_4$ before being evaporated under vacuum. The brown oil obtained was pure in product with 82% yield.

	$C_7H_7F_3O_5S$ M = 260.18 g.mol^{-1} 82%

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.44 – 4.33 (m, 2H, **$^4CH_2$**), 3.15 – 3.09 (m, 2H, **$^3CH_2$**), 2.55 (t, $^5J_{HH}$ = 2.6 Hz, 3H, **$^6CH_3$**).

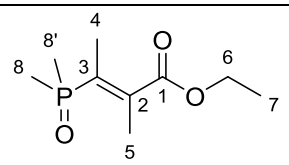
General procedure for the Pd-catalysed cross coupling of phosphorus with enol triflates:

In a two necked flask equipped with a condenser, dried and flushed with N_2, to a solution of enol triflate (1 eq) in toluene (0.2 mol.L^{-1}) were added $Pd_2(dba)_3$ (2.5 mol%), dppp (5 mol%), the phosphorus source (1.2 eq) and DIPEA (1.5 eq). The medium was stirred overnight at 110 °C. The completion of the reaction were monitored by GC-MS. The reaction mixture was quenched by HCl 1 N and extracted with ethyl acetate. The organic phase was dried over $MgSO_4$ and evaporated under vacuum. The product was purified by a flash column chromatography (conditions specified for each products).

((*E*)-ethyl 2-methylbut-2-enoat-3-yl)dimethylphosphine oxide 127:

The synthesis of ((*E*)-ethyl 2-methylbut-2-enoat-3-yl)dimethylphosphine oxide was performed according to the general cross coupling procedure, starting from 1.0 g of ethyl 2-methyl-3-[(trifluoromethylsulfonyl)oxo]but-2-enoate and 339 mg of dimethylphosphine oxide. The product wasn't in the organic phase but in the aqueous one, which was evaporated and purified with a flash column chromatography with ethyl acetate/ethanol (80/20).

The stereochemistry of the C-C double bond was determined by ^{13}C NMR thanks to the comparison of $^3J_{PC,cis}$ and $^3J_{PC,trans}$ coupling constants of **^1C** and **$^5CH_3$**.

	$C_9H_{17}O_3P$ M = 204.21 g.mol^{-1} 73% (541 mg) White solid

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 4.28 (q, $^3J_{HH}$ = 7.1 Hz, 2H, **$^6CH_2$**), 2.31 (dq, $^4J_{PH}$ = 3.1 Hz, $^5J_{HH}$ = 1.5 Hz, 3H, **$^5CH_3$**), 1.91 (dd, $^3J_{PH}$ = 13.8 Hz, $^5J_{HH}$ = 1.6 Hz, 3H, **$^4CH_3$**), 1.70 (d, $^1J_{PH}$ = 13.1 Hz, 6H, **$^8CH_3$**, **$^{8'}CH_3$**), 1.33 (t, $^3J_{HH}$ = 7.1 Hz, 3H, **$^7CH_3$**).

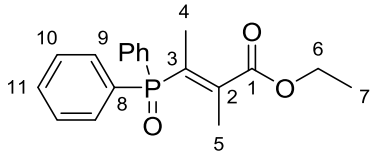
^{31}P CPD NMR (162 MHz, CD_3OD) δ (ppm): 44.70 (s).

^{13}C APT NMR (101 MHz, CD_3OD) δ (ppm): 170.60 (d, $^3J_{PC,trans}$ = 21.9 Hz, **^1C**), 144.63 (d, $^2J_{PC}$ = 7.7 Hz, **^2C**), 131.05 (d, $^1J_{PC}$ = 88.5 Hz, **^3C**), 62.34 (s, **$^6CH_2$**), 17.84 (d, $^3J_{PC,cis}$ = 13.2 Hz, **$^5CH_3$**), 17.56 (d, $^2J_{PC}$ = 7.0 Hz, **$^4CH_3$**), 16.39 (d, $^1J_{PC}$ = 71.5 Hz, **$^8CH_3$**, **$^{8'}CH_3$**), 14.47 (s, **$^7CH_3$**).

((*E*)-ethyl 2-methylbut-2-enoat-3-yl)diphenylphosphine oxide 128:

The synthesis of ((*E*)-ethyl 2-methylbut-2-enoat-3-yl)diphenylphosphine oxide was performed according to the general cross coupling procedure, starting from 1.0 g of ethyl 2-methyl-3-[(trifluoromethylsulfonyl)oxo]but-2-enoate and 878 mg, of diphenylphosphine oxide. The product was purified by flash column chromatography with ethyl acetate/heptane (75/25).

The stereochemistry of the C-C double bond was determined by ^{13}C NMR thanks to the comparison of $^3J_{PC,cis}$ and $^3J_{PC,trans}$ coupling constants of **^1C** and **$^5CH_3$**.

	$C_{19}H_{21}O_3P$ M = 328.35 g.mol^{-1} 89% (1.06 g) Orange oily solid

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.73 – 7.63 (m, 4H, **CH_{Ar}**), 7.58 – 7.50 (m, 2H, **CH_{Ar}**), 7.50 – 7.50 (m, 4H, **CH_{Ar}**), 4.24 (q, $^3J_{HH}$ = 7.1 Hz, 2H, **$^6CH_2$**), 2.19 (s, 3H, **$^5CH_3$**), 1.73 (d, $^2J_{PH}$ = 14.1 Hz, 3H, **$^4CH_3$**), 1.30 (t, $^3J_{HH}$ = 7.1 Hz, 3H, **$^7CH_3$**).

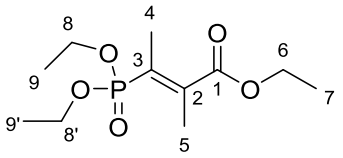
^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 31.34 (s).

^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 169.22 (d, $^3J_{PC,trans}$ = 22.2 Hz, **^1C**), 145.25 (d, $^2J_{PC}$ = 8.5 Hz, **^2C**), 132.50 (d, $^1J_{PC}$ = 102.5 Hz, **^8C**), 131.92 (d, $^4J_{PC}$ = 2.2 Hz, **^{11}CH**), 131.56 (d, $^2J_{PC}$ = 9.7 Hz, **^9CH**), 130.04 (d, $^1J_{PC}$ = 92.1 Hz, **^3C**), 128.67 (d, $^3J_{PC}$ = 12.0 Hz, **^{10}CH**), 61.06 (s, **$^6CH_2$**), 20.15 (d, $^3J_{PC,cis}$ = 12.1 Hz, **$^5CH_3$**), 18.60 (d, $^2J_{PC}$ = 7.6 Hz, **$^4CH_3$**), 14.17 (s, **$^7CH_3$**).

Diethyl ((*E*)-ethyl 2-methylbut-2-enoat-3-yl)phosphonate 129:

The synthesis of diethyl ((*E*)-ethyl 2-methylbut-2-enoat-3-yl) phosphonate was performed according to the general cross coupling procedure, starting from 1.0 g of ethyl 2-methyl-3-[(trifluoromethylsulfonyl)oxo]but-2-enoate and 560 µL of diethyl phosphite. The product was purified by flash column chromatography with ethyl acetate/ethanol (90/10).

The stereochemistry of the C-C double bond was determined by ^{13}C NMR thanks to the comparison of $^{3}J_{PC,cis}$ and $^{3}J_{PC,trans}$ constants of **^{1}C** and **$^{5}CH_3$**.

	$C_{11}H_{21}O_5P$ M = 264.26 g.mol^{-1} 66% (633 mg) Yellow oil

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.22 (q, $^{3}J_{HH}$ = 7.1 Hz, 2H, **$^{6}CH_2$**), 4.13 – 4.01 (m, 4H, **$^{8}CH_2$**, **$^{8'}CH_2$**), 2.24 (dq, $^{4}J_{PH}$ = 3.2 Hz, $^{5}J_{HH}$ = 1.5 Hz, 3H, **$^{5}CH_3$**), 1.91 (dq, $^{3}J_{PH}$ = 14.1Hz, $^{5}J_{HH}$ = 1.5 Hz, 3H, **$^{4}CH_3$**), 1.34 – 1.25 (m, 9H, **$^{7}CH_3$**, **$^{9}CH_3$**, **$^{9'}CH_3$**).

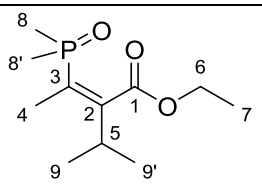
^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 18.53 (s).

^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 169.31 (d, $^{3}J_{PC,trans}$ = 31.7 Hz, **^{1}C**), 143.61 (d, $^{2}J_{PC}$ = 13.3 Hz, **^{2}C**), 126.53 (d, $^{1}J_{PC}$ = 175.2 Hz, **^{3}C**), 61.66 (d, $^{2}J_{PC}$ = 5.6 Hz, **$^{8}CH_2$**, **$^{8'}CH_2$**), 60.97 (s, **$^{6}CH_2$**), 17.99 (d, $^{2}J_{PC}$ = 7.0 Hz, **$^{4}CH_3$**), 17.84 (d, $^{3}J_{PC,cis}$ = 9.3 Hz, **$^{5}CH_3$**), 16.24 (d, $^{3}J_{HH}$ = 6.3 Hz, **$^{9}CH_3$**, **$^{9'}CH_3$**), 14.11 (s, **$^{7}CH_3$**).

((*Z*)-ethyl 2-isopropylbut-2-enoat-3-yl)dimethylphosphine oxide 130:

The synthesis of ((*Z*)-ethyl 2-isopropylbut-2-enoat-3-yl)dimethylphosphine oxide was performed according to the general cross coupling procedure, starting from 920 mg of ethyl 2-isopropyl-3-[(trifluoromethylsulfonyl)oxo]but-2-enoate and 283 mg of dimethylphosphine oxide. The product wasn't in the organic phase but in the aqueous one, which was evaporated and purified with a flash column chromatography with ethyl acetate/ethanol (80/20).

The stereochemistry of the C-C double bond was determined by ^{13}C NMR thanks to the comparison of $^{3}J_{PC,cis}$ and $^{3}J_{PC,trans}$ coupling constants of **^{1}C** and **^{5}CH**.

	$C_{11}H_{21}O_3P$ M = 232.26 g.mol^{-1} 70% (494 mg) White solid

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.23 (q, $^{3}J_{HH}$ = 7.1 Hz, 2H, **$^{6}CH_2$**), 2.92 (hept, $^{3}J_{HH}$ = 6.0 Hz, 1H, **^{5}CH**), 1.81 (d, $^{3}J_{PH}$ = 13.2 Hz, 3H, **$^{4}CH_3$**), 1.54 (d, $^{2}J_{PH}$ = 12.9 Hz, 6H, **$^{8}CH_3$**, **$^{8'}CH_3$**), 1.30 (t, $^{3}J_{HH}$ = 7.1 Hz, 3H, **$^{7}CH_3$**), 1.09 (d, $^{3}J_{HH}$ = 6.9 Hz, 6H, **$^{9}CH_3$**, **$^{9'}CH_3$**).

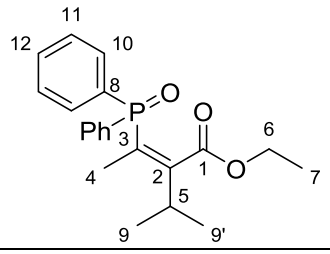
^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 36.72 (s).

^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 168.60 (d, $^{3}J_{PC,cis}$ = 8.9 Hz, **^{1}C**), 152.58 (d, $^{2}J_{PC}$ = 1.6 Hz, **^{2}C**), 126.78 (d, $^{1}J_{PC}$ = 88.8 Hz, **^{3}C**), 60.88 (s, **$^{6}CH_2$**), 29.92 (d, $^{2}J_{PC}$ = 11.1 Hz, **$^{4}CH_3$**), 20.23 (s, **$^{9}CH_3$**, **$^{9'}CH_3$**), 18.54 (s, **$^{7}CH_3$**) 16.23 (d, $^{1}J_{PC}$ = 70.5 Hz, **$^{8}CH_3$**, **$^{8'}CH_3$**), 13.92 (d, $^{3}J_{PC,trans}$ = 12.9 Hz, **^{5}CH**).

((*Z*)-ethyl 2-isopropylbut-2-enoat-3-yl)diphenylphosphine oxide 131:

The synthesis of ((*Z*)-ethyl 2-isopropylbut-2-enoat-3-yl)diphenylphosphine oxide was performed according to the general cross coupling procedure, starting from 920 mg of ethyl 2-isopropyl-3-[(trifluoromethylsulfonyl)oxo]but-2-enoate and 734 mg of diphenylphosphine oxide. The product was purified with a flash column chromatography with ethyl acetate/heptane (75/25).

The stereochemistry of the C-C double bond was determined by ^{13}C NMR thanks to the comparison of $^{3}J_{PC,cis}$ and $^{3}J_{PC,trans}$ coupling constants of **^{1}C** and **^{5}CH**.

	$C_{11}H_{21}O_3P$ M = 232.26 g.mol^{-1} 60% (650 mg) Light orange oil

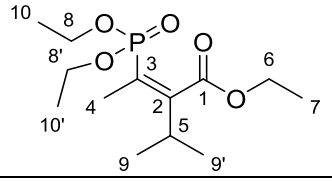
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.74 – 7.66 (m, 4H, **CH_{Ar}**), 7.56 – 7.50 (m, 2H, **CH_{Ar}**), 7.49 – 7.42 (m, 4H, **CH_{Ar}**), 3.92 (q, $^{3}J_{HH}$ = 7.2 Hz, 2H, **$^{6}CH_2$**), 3.03 (heptd, $^{3}J_{HH}$ = 6.7 Hz, $^{4}J_{PH}$ = 1.9 Hz, 1H, **^{5}CH**), 1.78 (d, $^{2}J_{PH}$ = 13.2 Hz, 3H, **$^{4}CH_3$**), 1.16 (d, $^{3}J_{HH}$ = 6.9 Hz, 6H, **$^{9}CH_3$**, **$^{9'}CH_3$**), 1.12 (t, $^{3}J_{HH}$ = 7.2 Hz, 3H, **$^{7}CH_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 30.21 (s).

^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 167.97 (d, $^{3}J_{PC,cis}$ = 9.1 Hz, **^{1}C**), 154.40 (d, $^{2}J_{PC}$ = 4.1 Hz, **^{2}C**), 132.28 (d, $^{2}J_{PC}$ = 9.9 Hz, **^{10}CH**), 131.95 (d, $^{1}J_{PC}$ = 102.8 Hz, **^{8}C**), 131.77 (d, $^{4}J_{PC}$ = 2.7 Hz, **^{12}CH**), 128.33 (d, $^{3}J_{PC}$ = 12.0 Hz, **^{11}CH**), 125.17 (d, $^{1}J_{PC}$ = 93.7 Hz, **^{3}C**), 60.74 (s, **$^{6}CH_2$**), 30.46 (d, $^{2}J_{PC}$ = 11.7 Hz, **$^{4}CH_3$**), 20.34 (d, $^{4}J_{PC}$ = 1.1 Hz, **$^{9}CH_3$**, **$^{9'}CH_3$**), 15.28 (d, $^{3}J_{PC,trans}$ = 12.1 Hz, **^{5}CH**), 13.84 (s, **$^{7}CH_3$**).

Diethyl (Ethyl 2-isopropylbut-2-enoat-3-yl)phosphonate 132:

The synthesis of diethyl (ethyl 2-isopropylbut-2-enoat-3-yl)phosphonate was performed according to the general cross coupling procedure, starting from 920 mg of ethyl 2-isopropyl-3-[(trifluoromethylsulfonyl)oxo]but-2-enoate and 467 µL of diethyl phosphite. The product was purified with a flash column chromatography with ethyl acetate/ethanol (90/10).

	$C_{13}H_{25}O_5P$ M = 292.31 g.mol^{-1} 43% (380 mg) Yellow oil

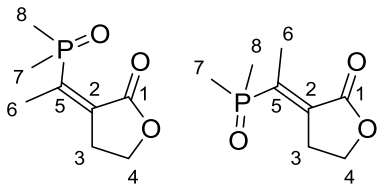
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.24 (q, $^3J_{HH}$ = 7.2 Hz, 2H, **$^6CH_2$**), 4.14 – 3.95 (m, 4H, **$^8CH_2$**, **$^{8'}CH_2$**), 2.91 (heptd, $^3J_{HH}$ = 6.9 Hz, $^4J_{PH}$ = 2.6 Hz, 1H, **^{5}CH**), 1.85 (d, $^3J_{PH}$ = 13.6 Hz, 3H, **$^4CH_3$**), 1.32 – 1.26 (m, 9H, **$^7CH_3$**, **$^{10}CH_3$**, **$^{10'}CH_3$**), 1.08 (d, $^3J_{HH}$ = 6.9 Hz, 6H, **$^9CH_3$**, **$^{9'}CH_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 17.65 (s).

(1-(Dihydrofuran-2(3H)-on-3-yl)ethyliden-1-yl)dimethylphosphine oxide 133:

The synthesis of (1-(dihydrofuran-2(3H)-on-3-yl)ethyliden-1-yl)dimethylphosphine oxide was performed according to the general cross coupling procedure, starting from 1.0 g of 1-(2-oxodihydrofuran-3(2H)-ylidene)ethyl trifluoromethanesulfonate and 360 mg of dimethylphosphine oxide. The product wasn't in the organic phase but in the aqueous one, which was evaporated and purified with a flash column chromatography with ethyl acetate/ethanol (80/20).

A mixture of 60/40 of E/Z isomers was obtained.

 (*Z*) (*E*)	$C_8H_{13}O_3P$ M = 188.16 g.mol^{-1} 56% (403 mg) Colourless oil

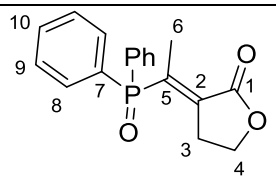
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.43 (t, $^3J_{HH}$ = 7.3 Hz, 2H, **$^4CH_2$**, *Z*), 4.29 (t, $^3J_{HH}$ = 7.3 Hz, 2H, **$^4CH_2$**, *E*), 3.68 – 3.55 (m, 2H, **$^3CH_2$**, *Z*), 3.12 – 2.94 (m, 2H, **$^3CH_2$**, *E*), 2.24 (dt, $^3J_{PH}$ = 14.7 Hz, $^5J_{HH}$ = 2.5 Hz, 3H, **$^6CH_3$** *E*), 2.16 (dt, $^3J_{PH}$ = 10.8 Hz, $^5J_{HH}$ = 2.1 Hz, 3H, **$^6CH_3$**, *Z*), 1.78 (d, $^2J_{PH}$ = 14.1 Hz, 6H, **$^7CH_3$**, *Z*), 1.59 (d, $^2J_{PH}$ = 12.9 Hz, 6H, **$^8CH_3$**, *E*).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 38.84 (s, *E*), 37.82 (s, *Z*).

((*E*)-1-(dihydrofuran-2(3H)-on-3yl)ethyliden-1-yl)diphenylphosphine oxide 134:

The synthesis of ((*E*)-1-(dihydrofuran-2(3H)-on-3yl)ethyliden-1-yl)diphenylphosphine oxide was performed according to the general cross coupling procedure, starting from 1.0 g of 1-(2-oxodihydrofuran-3(2H)-ylidene)ethyl trifluoromethanesulfonate and 932 mg of diphenylphosphine oxide. The product was purified by trituration in toluene and filtration of the brown solid.

The stereochemistry of the C-C double bond was determined by ^{13}C NMR thanks to the comparison of $^3J_{PC,cis}$ and $^3J_{PC,trans}$ coupling constants of **^{1}C** and **$^3CH_2$**.

	$C_{18}H_{17}O_3P$ M = 312.30 g.mol^{-1} 36% (436 mg) Brown solid

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.81 – 7.66 (m, 5H, **CH_{Ar}**), 7.65 – 7.58 (m, 2H, **CH_{Ar}**), 7.58 – 7.47 (m, 3H, **CH_{Ar}**), 4.33 (t, $^{3}J_{HH}$ = 7.1 Hz, 2H, **$^{4}CH_2$**), 3.52 – 3.22 (m, 2H, **$^{3}CH_2$**), 2.22 (d, $^{3}J_{PH}$ = 15.0 Hz, 3H, **$^{6}CH_3$**).

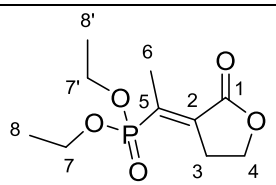
^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 31.63 (s).

^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 169.63 (d, $^{3}J_{PC,trans}$ = 21.2 Hz, **^{1}C**), 141.31 (d, $^{1}J_{PC}$ = 86.2 Hz, **^{5}C**), 136.81 (d, $^{2}J_{PC}$ = 7.7 Hz, **^{2}C**), 132.49 (d, $^{4}J_{PC}$ = 2.7 Hz, **^{10}CH**), 131.67 (d, $^{2}J_{PC}$ = 9.8 Hz, **^{8}CH**), 130.78 (d, $^{1}J_{PC}$ = 103.1 Hz, **7C**), 128.91 (d, $^{3}J_{PC}$ = 12.1 Hz, **^{9}CH**), 65.61 (s, **$^{4}CH_2$**), 29.00 (d, $^{3}J_{PC,cis}$ = 5.1 Hz, **$^{3}CH_2$**), 17.40 (d, $^{2}J_{PC}$ = 9.1 Hz, **$^{6}CH_3$**).

Diethyl ((*E*)-1-(dihydrofuran-2(3H)-on-3-yl)ethyliden-1-yl)phosphonate 135:

The synthesis of diethyl ((*E*)-1-(dihydrofuran-2(3H)-on-3yl)ethyliden-1-yl)phosphonate was performed according to the general cross coupling procedure, starting from 1.0 g of 1-(2-oxodihydrofuran-3(2H)-ylidene)ethyl trifluoromethanesulfonate and 594 µL of diethyl phosphite. The product was purified with a flash column chromatography with ethyl acetate/ethanol (80/20).

The stereochemistry of the C-C double bond was determined by ^{13}C NMR thanks to the comparison of $^{3}J_{PC,cis}$ and $^{3}J_{PC,trans}$ coupling constants of **^{1}C** and **$^{3}CH_2$**.

	$C_{10}H_{17}O_5P$ M = 248.21 g.mol^{-1} 38% (361 mg) Yellow oil

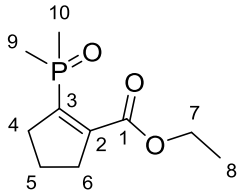
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.29 (t, *J* = 7.3 Hz, 2H, **$^{4}CH_2$**), 4.15 – 4.00 (m, 4H, **$^{7}CH_2$**, **$^{7'}CH_2$**), 3.35 – 3.26 (m, 2H, **$^{3}CH_2$**), 2.31 (dt, $^{3}J_{PH}$ = 15.3 Hz, $^{5}J_{HH}$ = 2.6 Hz, 3H, **$^{6}CH_3$**), 1.30 (t, $^{3}J_{HH}$ = 7.1 Hz, 6H, **$^{8}CH_3$**, **$^{8'}CH_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 16.32 (s).

^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 169.44 (d, $^{3}J_{PC,trans}$ = 30.6 Hz, **^{1}C**), 137.51 (d, $^{1}J_{PC}$ = 171.9 Hz, **^{5}C**), 135.14 (d, $^{2}J_{PC}$ = 13.0 Hz, **^{2}C**), 65.27 (s, **$^{4}CH_2$**), 62.24 (d, $^{2}J_{PC}$ = 5.8 Hz, **$^{7}CH_2$**, **$^{7'}CH_2$**), 29.59 (d, $^{3}J_{PC,cis}$ = 4.9 Hz, **$^{3}CH_2$**), 16.32 (d, $^{3}J_{PC}$ = 6.1 Hz, **$^{8}CH_3$**, **$^{8'}CH_3$**), 14.96 (d, $^{2}J_{PC}$ = 6.0 Hz, **$^{6}CH_3$**).

[1-(ethyl carboxylate)cyclopent-1-en-2-yl]dimethylphosphine oxide 136:

The synthesis of ethyl [1-(ethyl carboxylate)cyclopent-1-en-2-yl]dimethylphosphine oxide was performed according to the general cross coupling procedure, starting from 1.0 g of ethyl 2-[(trifluoromethyl)sulfonyloxy]cyclopent-1-ene-1-carboxylate and 325 mg of dimethylphosphine oxide. The product wasn't in the organic phase but in the aqueous one, which was evaporated and purified with a flash column chromatography with ethyl acetate/ethanol (80/20).

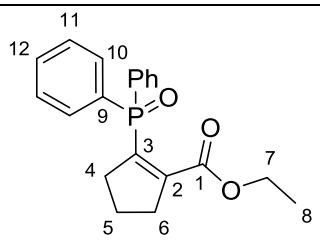
	$C_{10}H_{17}O_3P$ M = 216.22 g.mol^{-1} 40% (304 mg) Colourless oil

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.15 (q, $^{3}J_{HH}$ = 7.2 Hz, 2H, **$^{7}CH_2$**), 2.93 – 2.66 (m, 4H, 5CH2, **$^{6}CH_2$**), 1.89 (dd, $^{3}J_{PH}$ = 12.6 Hz, $^{3}J_{HH}$ = 5.5 Hz, 2H, **$^{4}CH_2$**), 1.70 (d, $^{2}J_{PH}$ = 5.8 Hz, 3H, **$^{9}CH_3$**), 1.67 (d, $^{2}J_{PH}$ = 5.7 Hz, 3H, **$^{10}CH_3$**), 1.24 (t, $^{3}J_{HH}$ = 7.2 Hz, 3H, **$^{8}CH_3$**).

^{31}P NMR (162 MHz, $CDCl_3$) δ (ppm): 35.55 (s).

[1-(ethyl carboxylate)cyclopent-1-en-2-yl]diphenylphosphine oxide 137:

The synthesis of [1-(ethyl carboxylate)cyclopent-1-en-2-yl]diphenylphosphine oxide was performed according to the general cross coupling procedure, starting from 1.0 g of ethyl 2-[(trifluoromethyl)sulfonyloxy]cyclopent-1-ene-1-carboxylate and 842 mg of diphenylphosphine oxide. The product was purified by a flash column chromatography with ethyl acetate/heptane (75/25).

	$C_{20}H_{21}O_3P$ M = 340.36 g.mol^{-1} 80% (946 mg) Orange oil

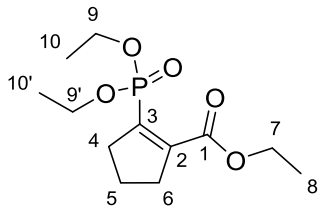
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.73 – 7.64 (m, 4H, **CH_{Ar}**), 7.51 – 7.45 (m, 2H, **CH_{Ar}**), 7.45 – 7.37 (m, 4H, **CH_{Ar}**), 3.67 (q, $^{3}J_{HH}$ = 7.1 Hz, 2H, **$^{7}CH_2$**), 2.90 – 2.76 (m, 2H, **$^{6}CH_2$**), 2.64 – 2.52 (m, 2H, **$^{5}CH_2$**), 1.96 – 1.82 (m, 2H, **$^{4}CH_2$**), 0.95 (t, $^{3}J_{HH}$ = 7.1 Hz, 3H, **$^{8}CH_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 22.85 (s).

^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 165.26 (d, $^{3}J_{PC}$ = 3.6 Hz, **^{1}C**), 148.44 (d, $^{2}J_{PC}$ = 7.5 Hz **^{2}C**), 141.23 (d, $^{1}J_{PC}$ = 96.0 Hz, **^{3}C**), 132.44 (d, $^{1}J_{PC}$ = 106.9 Hz, **^{9}C**), 131.73 (d, $^{4}J_{PC}$ = 2.7 Hz, **^{12}CH**), 131.38 (d, $^{2}J_{PC}$ = 10.2 Hz, **^{10}CH**), 128.35 (d, $^{3}J_{PC}$ = 12.4 Hz, **^{11}CH**), 60.91 (s, **$^{7}CH_2$**), 38.03 (d, $^{4}J_{PC}$ = 10.9 Hz, **$^{6}CH_2$**), 37.20 (d, $^{3}J_{PC}$ = 12.9 Hz, **$^{5}CH_2$**), 22.66 (d, $^{2}J_{PC}$ = 9.3 Hz, **$^{4}CH_2$**), 13.57 (s, **$^{8}CH_3$**).

Diethyl [1-(ethyl carboxylate)cyclopent-1-en-2-yl]phosphonate 138:

The synthesis of triethyl [1-(ethyl carboxylate)cyclopent-1-en-2-yl] phosphonate was performed according to the general cross coupling procedure, starting from 1.35 g of ethyl 2-[(trifluoromethyl)sulfonyloxy]cyclopent-1-ene-1-carboxylate and 727 µL of diethyl phosphite. The product was purified with a flash column chromatography with ethyl acetate/heptane (75/25).

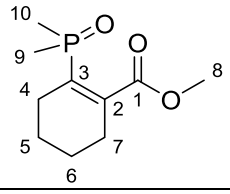
	$C_{12}H_{21}O_5P$ M = 276.27 g.mol^{-1} 93% (1.21 g) Yellow oil

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.26 (q, $^3J_{HH}$ = 7.1 Hz, 2H, **$^7CH_2$**), 4.18 – 4.05 (m, 4H, **$^9CH_2$**, **$^{9'}CH_2$**), 2.84 – 2.69 (m, 4H, **$^6CH_2$**, **$^4CH_2$**), 1.96 (p, $^3J_{HH}$ = 7.6 Hz, 2H, **$^5CH_2$**), 1.32 (t, $^3J_{HH}$ = 7.1 Hz, 9H, **$^8CH_3$**, **$^{10}CH_3$**, **$^{10'}CH_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 12.87 (s).

[1-(methyl carboxylate)cyclohex-1-en-2-yl]dimethylphosphine oxide 139:

The synthesis of [1-(methyl carboxylate)cyclohex-1-en-2-yl]dimethylphosphine oxide was performed according to the general cross coupling procedure, starting from 1.0 g of methyl 2-[(trifluoromethyl)sulfonyloxy]cyclohex-1-ene-1-carboxylate and 329 mg of dimethylphosphine oxide. The product was purified with a flash column chromatography with ethyl acetate/ethanol (80/20).

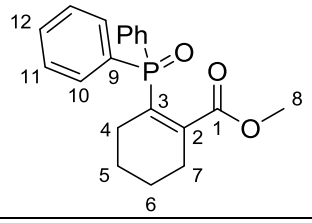
	$C_{10}H_{17}O_3P$ M = 216.22 g.mol^{-1} 62% (466 g) Colourless oil

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 3.87 (s, 3H, **$^8CH_3$**), 2.68 – 2.56 (m, 2H, **$^7CH_2$**), 2.56 – 2.42 (m, 2H, **$^6CH_2$**), 2.19 (d, $^2J_{PH}$ = 14.5 Hz, 6H, **$^9CH_3$**, **$^{10}CH_3$**), 1.74 (m, 4H, **$^4CH_2$**, **$^5CH_2$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 70.72 (s).

[1-(methyl carboxylate)cyclohex-1-en-2-yl]diphenylphosphine oxide 140:

The synthesis of [1-(methyl carboxylate)cyclohex-1-en-2-yl]diphenylphosphine oxide was performed according to the general cross coupling procedure, starting from 1.0 g of methyl 2-[(trifluoromethyl)sulfonyloxy]cyclohex-1-ene-1-carboxylate and 842 mg of diphenylphosphine oxide. The product was purified with a flash column chromatography with pure ethyl acetate.

	$C_{20}H_{21}O_3P$ M = 340.36 g.mol^{-1} 60% (711 mg) Brown solid

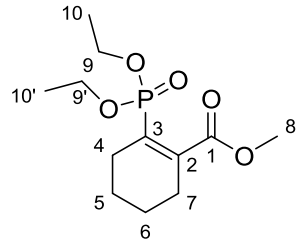
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.77 – 7.66 (m, 4H, **CH_{Ar}**), 7.57 – 7.50 (m, 2H, **CH_{Ar}**), 7.49 – 7.41 (m, 4H, **CH_{Ar}**), 3.40 (s, 3H, **$^8CH_3$**), 2.53 – 2.45 (m, 2H, **$^7CH_2$**), 2.12 – 2.05 (m, 2H, **$^6CH_2$**), 1.74 – 1.58 (m, 4H, **$^4CH_2$**, **$^5CH_2$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 28.30 (s).

^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 169.83 (d, $^3J_{PC}$ = 8.7 Hz, **^1C**), 145.58 (d, $^2J_{PC}$ = 3.9 Hz, **^2C**), 131.96 (d, $^2J_{PC}$ = 9.9 Hz, **^{10}CH**), 131.89 (d, $^1J_{PC}$ = 103.7 Hz, **^3C**), 131.76 (d, $^4J_{PC}$ = 2.7 Hz, **^{12}CH**), 131.72 (d, $^1J_{PC}$ = 92.9 Hz, **^9C**), 128.37 (d, $^3J_{PC}$ = 12.1 Hz, **^{11}CH**), 51.93 (s, **$^8CH_3$**), 28.98 (d, $^2J_{PC}$ = 11.0 Hz, **$^4CH_2$**), 27.19 (d, $^3J_{PC}$ = 9.2 Hz, **$^7CH_2$**), 21.74 (d, $^3J_{PC}$ = 8.1 Hz, **$^5CH_2$**), 21.12 (s, **$^6CH_2$**).

Diethyl [1-(methyl carboxylate)cyclohex-1-en-2-yl]phosphonate 141:

The synthesis of diethyl [1-(methyl carboxylate)cyclohex-1-en-2-yl]phosphonate was performed according to the general cross coupling procedure, starting from 1.0 g of methyl 2-[(trifluoromethyl)sulfonyloxy]cyclohex-1-ene-1-carboxylate and 536 µL of diethyl phosphite. The product was purified with a flash column chromatography with ethyl acetate/heptane (75/25).

	$C_{12}H_{21}O_3P$ M = 276.27 g.mol^{-1} 63% (600 mg) Yellow oil

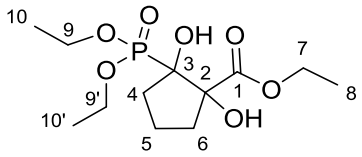
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.17 – 3.93 (m, 4H, **$^9CH_2$**, **$^{9'}CH_2$**), 3.75 (s, 3H, **$^8CH_3$**), 2.37 – 2.29 (m, 2H, **$^7CH_2$**), 2.27 – 2.17 (m, 2H, **$^6CH_2$**), 1.70 – 1.60 (m, 4H, **$^4CH_2$**, **$^5CH_2$**), 1.29 (t, $^3J_{HH}$ = 7.1 Hz, 6H, **$^{10}CH_3$**, **$^{10'}CH_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 16.18 (s).

^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 170.46 (d, $^{3}J_{PC}$ = 10.9 Hz, **^{1}C**), 145.81 (d, $^{2}J_{PC}$ = 7.9 Hz, **^{2}C**), 126.40 (d, $^{1}J_{PC}$ = 178.2 Hz, **^{3}C**), 61.79 (d, $^{2}J_{PC}$ = 5.4 Hz, **$^{9}CH_2$**, **$^{9'}CH_2$**), 52.24 (s, **$^{8}CH_3$**), 28.23 (d, $^{2}J_{PC}$ = 15.7 Hz, **$^{4}CH_2$**), 25.31 (d, $^{2}J_{PC}$ = 7.4 Hz, **$^{7}CH_2$**), 21.31 (d, $^{3}J_{PC}$ = 9.0 Hz, **$^{5}CH_2$**), 21.01 (d, $^{4}J_{PC}$ = 1.2 Hz, **$^{6}CH_2$**), 16.23 (d, $^{3}J_{PC}$ = 6.2 Hz, **$^{10}CH_3$**, **$^{10'}CH_3$**).

Diethyl (1-ethylcarboxylate-1,2-dihydroxycyclopentan-2-yl)phosphonate 143:

In a 10 mL flask, to a solution of ethyl diethyl [1-(ethyl carboxylate)cyclopent-1-en-2-yl]phosphonate (100 mg, 0.362 mmol, 1 eq) in acetone (3.2 mL) was added, in the following order, piperidine (50 µL, 0.507 mmol, 1.4 eq) and potassium permanganate (74 mg, 0.471 mmol, 1.3 eq). After 1h of stirring at room temperature acetic acid (21 µL, 0.362 mmol, 1 eq) was added to the medium as a solution in acetone (260 µL). The completion of the rection was monitored by TLC (ethyl acetate/ethanol 80/20). The reaction was quenched by a solution of sodium hydrogensulfite (1 M, 1 mL), followed by the addition of a hydrogen chloride solution (2 M, 1.7 mL). After 15 min of stirring, the acetone was evaporated under vacuum and the residue was extracted with dichloromethane (twice with 2.6 mL). The organic phase was dried with $MgSO_4$ and evaporated under vacuum. The crude orange solid obtained was purified by a flash column chromatography with ethyl acetate/ethanol (80/20) to lead to 32 mg of product as a white solid with 29% yield.

	$C_{12}H_{23}O_7P$ M = 310.28 g.mol^{-1} 29% (32 mg) White solid

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.32 – 3.96 (m, 6H, **$^{7}CH_2$**, **$^{9}CH_2$**, **$^{9'}CH_2$**), 2.34 – 2.22 (m, 1H from **$^{6}CH_2$**), 2.16 – 2.06 (m, 1H from **$^{4}CH_2$**), 2.05 – 1.92 (m, 3H, 1 from **$^{4}CH_2$**, 1 from **$^{5}CH_2$**, 1 from **$^{6}CH_2$**), 1.89 – 1.77 (m, 1H from **$^{6}CH_2$**), 1.32 – 1.20 (m, 9H, **$^{8}CH_3$**, **$^{10}CH_3$**, **$^{10'}CH_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 21.48 (s).

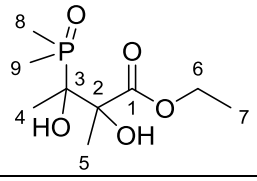
^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 173.88 (s, **^{1}C**), 84.59 (d, $^{2}J_{PC}$ = 9.5 Hz, **^{2}C**), 81.15 (d, $^{1}J_{PC}$ = 168.5 Hz, **^{3}C**), 63.09 (d, $^{2}J_{PC}$ = 6.8 Hz, **$^{9}CH_2$**), 62.70 (d, $^{2}J_{PC}$ = 7.4 Hz, **$^{9'}CH_2$**), 62.52 (s, **$^{7}CH_2$**), 34.04 (d, $^{3}J_{PC}$ = 11.5 Hz, **$^{6}CH_2$**), 32.23 (d, $^{4}J_{PC}$ = 8.4 Hz, **$^{5}CH_2$**), 20.18 (d, $^{3}J_{PC}$ = 14.4 Hz, **$^{4}CH_2$**), 16.49 (d, $^{3}J_{PC}$ = 4.4 Hz, **$^{10}CH_3$**), 16.44 (d, $^{3}J_{PC}$ = 5.2 Hz, **$^{10'}CH_3$**), 13.87 (s, **$^{8}CH_3$**).

MS (ES+) *m/z* = 311.13 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_{12}H_{24}O_7P$ 311.1260, found 311.1259.

(Ethyl 2,3-dihydroxy-2-methylbutanoat-3-yl)dimethylphosphine oxide 148:

The synthesis of (ethyl 2,3-dihydroxy-2-methylbutanoat-3-yl)dimethylphosphine oxide was performed according to the previous dihydroxylation procedure, starting from 82 mg of ((*E*)-ethyl 2-methylbut-2-enoat-3-yl)dimethylpshophine oxide.

	$C_9H_{19}O_5P$ M = 238.22 g.mol-1 9% (9 mg) Brown solid

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 4.14 (q, $^3J_{HH}$ = 7.1 Hz, 2H, **$^6CH_3$**), 1.56 (d, $^2J_{PH}$ = 12.9 Hz, 3H, **$^8CH_3$**), 1.44 (s, 3H, **$^5CH_3$**) 1.43 (d, $^2J_{PH}$ = 12.5 Hz, 3H, **$^9CH_3$**), 1.41 (d, $^3J_{PH}$ = 2.1 Hz, 3H, **$^4CH_3$**), 1.22 (t, $^3J_{HH}$ = 7.1 Hz, 3H, **$^7CH_3$**).

^{31}P CPD NMR (162 MHz, CD_3OD) δ (ppm): 61.15 (s).

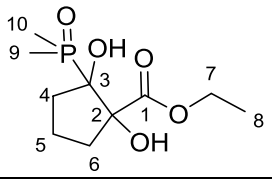
^{13}C APT NMR (101 MHz, CD_3OD) δ (ppm): 174.75 (d, $^3J_{PC}$ = 8.0 Hz, **^1C**), 78.99 (d, $^2J_{PC}$ = 3.6 Hz, **^2C**), 76.22 (d, $^1J_{PC}$ = 86.5 Hz, **^3C**), 61.56 (s, **$^6CH_2$**), 21.09 (d, $^3J_{PC}$ = 1.7 Hz, **$^5CH_3$**), 17.44 (d, $^2J_{PC}$ = 6.2 Hz, **$^4CH_3$**), 13.16 (d, $^1J_{PC}$ = 67.3 Hz, **$^8CH_3$**), 13.09 (d, $^1J_{PC}$ = 65.2 Hz, **$^9CH_3$**), 12.97 (s, **$^7CH_3$**).

MS (ES+) *m/z* = 239.10 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_9H_{20}O_5P$ 239.1048, found 239.1050.

(1-(ethyl carboxylate)-1,2-dihydroxycyclopentan-2-yl)dimethylphosphine oxide 152:

The synthesis of (1-(ethyl carboxylate)-1,2-dihydroxycyclopentan-2-yl)dimethylphosphine oxide was performed according to the previous dihydroxylation procedure, starting from 86 mg of [1-(ethyl carboxylate)cyclopent-1-en-2-yl]dimethylphosphine oxide.

	$C_{10}H_{19}O_5P$ M = 250.23 g.mol-1 7% (7 mg) Yellow oil

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 4.19 – 4.01 (m, 2H, **$^7CH_2$**), 2.46 – 2.33 (m, 1H from **$^6CH_2$**), 2.10 (m, 1H from **$^6CH_2$**), 2.01 – 1.73 (m, 4H, **$^4CH_2$**, **$^5CH_2$**), 1.51 (d, $^2J_{PH}$ = 13.5 Hz, 3H, **$^9CH_3$**), 1.38 (d, $^2J_{PH}$ = 13.0 Hz, 3H, **$^{10}CH_3$**), 1.20 (t, $^3J_{HH}$ = 7.2 Hz, 3H, **$^8CH_3$**).

^{31}P CPD NMR (162 MHz, CD_3OD) δ (ppm): 52.86 (s).

^{13}C APT NMR (101 MHz, CD_3OD) δ (ppm): 173.85 (d, $^3J_{PC}$ = 1.3 Hz, **^1C**), 84.18 (d, $^2J_{PC}$ = 7.2 Hz, **^2C**), 80.42 (d, $^1J_{PC}$ = 89.0 Hz, **^3C**), 61.59 (s, **$^7CH_2$**), 36.11 (d, $^3J_{PC}$ = 7.8 Hz, **$^6CH_2$**), 30.85 (d, $^3J_{PC}$ = 8.7 Hz, **$^5CH_2$**), 19.97 (d, $^2J_{PC}$ = 11.4 Hz, **$^4CH_2$**), 12.76 (s, **$^8CH_3$**), 11.94 (d, $^1J_{PC}$ = 67.7 Hz, **$^9CH_3$**), 10.37 (d, $^1J_{PC}$ = 65.1 Hz, **$^{10}CH_3$**).

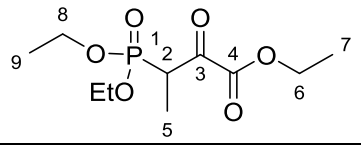
MS (ES+) *m/z* = 251.11 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_{10}H_{20}OP$ 251.1048, found 251.1050.

Synthesis of HPPD-like potential inhibitors

Ethyl 3-(diethoxyphosphoryl)-2-oxobutanoate 163b:

In a 10 mL flask dried and flushed with N_2, to a solution of diethyl ethylphosphonate (500 mg, 3 mmol, 1 eq) in THF (950 µL) at -78 °C was added dropwise a solution of butyllithium (1.6 M in THF, 1.9 mL, 3 mmol, 1 eq). The medium was stirred for 20 min at -78 °C. Then copper iodide (628 mg, 3.3 mmol, 1.1 eq) was introduced in one portion at -30 °C. The medium was stired for 30 min at -30 °C. A solution of ethyl chlorooxoacetate (370 µL, 3.3 mmol, 1.1 eq) in THF (630 µL) was added dropwise. The medium was stirred 40 min at -30 °C. A GC-MS analysis indicated the completion of the reaction. The reaction was quenched by 3 mL of distilled water and extracted by diethyl ether. The aqueous phase was acidified by HCl (4 N) until pH = 1 and it was extracted by diethyl ether. The organic phases were reunited, dried over MgSO4 and reduced under pressure. The oily crude was purified by a flash column chromatography with ethyl acetate/ethanol (100/0 to 90/10) leading to 545 mg of yellow oil pure in product with 68%.

	$C_{10}H_{19}O_6P$ M = 266.23 g.mol^{-1} 68%

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.37 – 4.29 (m, 2H, **^{6}CH$_2$**), 4.28 – 4.19 (m, 1H, **^{2}CH**), 4.19 – 4.08 (m, 4H, **^{8}CH$_2$**), 1.43 – 1.25 (m, 12H, **^{5}CH$_3$**, **^{7}CH$_3$**, **^{9}CH$_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 21.33 (s).

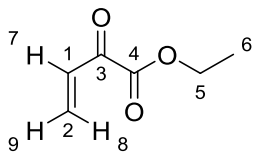
^{13}C APT NMR (101 MHz, CDCl3) δ (ppm): 190.26 (s, **^{4}C**), 161.08 (s, **^{3}C**), 63.33 (d, $^2J_{PC}$ = 6.8 Hz, **^{8}CH$_2$**), 63.31 (d, $^2J_{PC}$ = 7.1 Hz, **^{8}CH$_2$**) 62.82 (s, **^{6}CH$_2$**), 41.91 (d, $^1J_{PC}$ = 127.4 Hz, **^{2}CH**), 16.22 (d, $^3J_{PC}$ = 6.5 Hz, **^{9}CH$_3$**), 16.15 (d, $^3J_{PC}$ = 6.8 Hz, **^{9}CH$_3$**) 13.91 (s, **^{7}CH$_3$**), 9.69 (d, $^2J_{PC}$ = 6.0 Hz, **^{5}CH$_3$**).

MS (ES+) *m/z* = 267.10 (M+H$^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_{10}H_{20}O_6P$ 267.0997, found 267.0999.

Ethyl 2-oxobutenoate 174:

In a 100 mL flask dried and flushed with N_2, to a solution of diethyl oxalate (929 µL, 6.8 mmol, 1 eq) in a mixture of THF (6.8 mL) and diethyl ether (6.8 mL) at -78 °C was added dropwise a solution of vinylmagnesium bromide (1 M in THF, 8.2 mL, 8.2 mmol, 1.2 eq). The medium was stirred for 3h from -78 °C to room temperature. The completion was controlled by GC-MS analysis. The reaction was quenched by a solution of H_2SO_4 (2 N, 10 mL) and extracted by diethyl ether. The organic phase was dried over $MgSO_4$ and reduced under pressure to give 657 mg of yellow oil in 75 % yield.

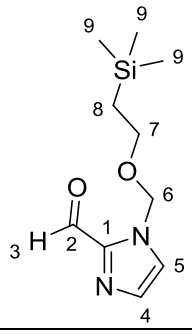
	$C_6H_8O_3$ M = 128.13 g.mol^{-1} 75%

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 6.92 (dd, $^{3}J_{HH,\ trans}$ = 17.6 Hz, $^{3}J_{HH,\ cis}$ = 10.7 Hz, 1H, **^{7}H**), 6.57 (dd, $^{3}J_{HH,\ trans}$ = 17.6 Hz, $^{2}J_{HH}$ = 0.9 Hz, 1H, **^{8}H**), 6.13 (dd, $^{3}J_{HH,\ cis}$ = 10.7 Hz, $^{2}J_{HH}$ = 1.0 Hz, 1H, **^{9}H**), 4.37 (q, $^{3}J_{HH}$ = 7.1 Hz, 2H, **$^{5}CH_2$**), 1.40 (t, $^{3}J_{HH}$ = 7.2 Hz, 3H, **$^{6}CH_3$**).

Bioisosteres and mimics of carboxylic acids

***N*-[(trimethylsilyl)ethoxy]methyl-2-carboxaldehydeimidazole 181f:**

In a dried two-necked flask was introduced NaH (60% dispersion in mineral oil, 149 mg, 5.7 mmol, 1.1 eq) under nitrogen, dry DMF (8 mL) and 2-imidazolecarboxaldehyde (500 mg, 5.2 mmol, 1 eq). After stirring at room temperature for 1.5h, the reaction mixture was treated dropwise with 2-(trimethylsilyl)ethoxymethyl chloride (SEMCl) (1 mL, 5.7 mmol, 1.1 eq). The reaction mixture became slightly warm upon addition of SEMCl. After stirring for 4h at room temperature, the reaction mixture was quenched with distilled water (40 mL) and extracted with ethyl acetate (3 x 10 mL). The organic extract was dried with $MgSO_4$ and concentrated to give a yellow oil corresponding to the product in 88% yield.

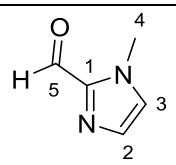
	$C_{10}H_{18}N_2O_2Si$ M = 226.35 g.mol^{-1} 88% yellow oil

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.84 (s, 1H, **^{3}CH**), 7.38 – 7.32 (m, 2H, **^{4}CH**, **^{5}CH**), 5.78 (s, 2H, **^{6}CH$_2$**) 3.56 (dd, $^3J_{HH}$ = 8.8 Hz, 7.7 Hz, 2H, **^{7}CH$_2$**), 0.92 (dd, $^3J_{HH}$ = 8.8, 7.7 Hz, 2H, **^{8}CH$_2$**), -0.02 (s, 9H, **^{9}CH$_3$**).

^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 183.70 (s, **^{2}CH**), 144.97, (s, **^{1}C**), 133.41 (s, **^{4}CH**), 126.95 (s, **^{5}CH**), 77.23 (s, **^{6}CH$_2$**), 68.42 (s, **^{7}CH$_2$**), 19.26 (s, **^{8}CH$_2$**), -0.00 (s, **^{9}CH$_3$**).

N-methyl-2-carboxaldehydeimidazole 181g:

In a dried and N_2 flushed 25 mL flask was introduced imidazole 2-carbaldehyde (500 mg, 5.2 mmol, 1 eq), K_2CO_3 (1.45 g, 10.5 mmol, 2 eq) and DMF (5 mL). To the reaction mixture was then added iodomethane (395 µL, 6.3 mmol, 1.2 eq) and heated to 100 °C overnight. The reaction was then quenched with water (20 mL) and extracted with ethyl acetate (3x5 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under vacuum to give the product as an oil in 41% yield.

	C_6H_7NO M = 109.13 g.mol^{-1} 41%

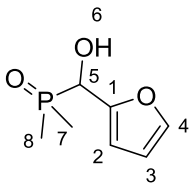
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.80 (s, 1H, **^{5}CH**), 7.25 (s, 1H, **^{3}CH**), 7.09 (s, 1H, **^{2}CH**), 4.00 (s, 3H, **^{4}CH$_3$**)

General procedure for the base free Pudovik reactions:

In a test tube equipped with a cap pierced with a syringe needle, a mixture of heterocycle 2-carboxaldehyde (1 eq), and diorganylphosphine oxide (1 eq) in dioxane (1.5 M) was stirred at room temperature for 1h or 1h30. The solvent was removed under vacuum and the residue was washed with ether and dried.

(Furan-2-yl(hydroxy)methyl)dimethylphosphine oxide 180h:

The synthesis of (furan-2-yl(hydroxy)methyl)dimethylphosphine oxide was performed according to the base free Pudovik procedure, starting from dimethylphosphine oxide (100 mg, 1.28 mmol) and furan 2-carboxaldehyde (106 µL, 1.28 mmol).

	$C_7H_{11}O_3P$ M = 174.14 g.mol^{-1} 58% (129 mg) Pale yellow solid

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 7.57 (s, 1H, **^{4}CH**), 6.52 (t, *J* = 2.8 Hz, 1H, **^{2}CH**), 6.49 – 6.46 (m, 1H, **^{3}CH**), 5.01 (d, $^2J_{PH}$ = 9.6 Hz, 1H, **^{5}CH**), 1.61 (d, $^2J_{PH}$ = 13.2 Hz, 3H, **^{7}CH$_3$**), 1.56 (d, $^2J_{PH}$ = 13.3 Hz, 3H, **^{8}CH$_3$**).

^{31}P CPD NMR (162 MHz, CD_3OD) δ (ppm): 51.26 (s).

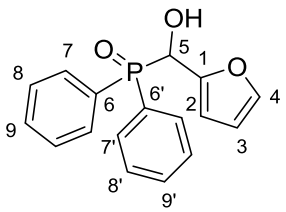
^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 150.63 (d, $^2J_{PC}$ = 2.3 Hz **^{1}C**), 142.70 (s, **^{4}CH**), 110.77 (d, $^3J_{PC}$ = 1.5 Hz **^{2}CH**), 108.96 (s, **^{3}CH**), 67.60 (d, $^1J_{PC}$ = 84.1 Hz, **^{5}CH**), 16.72 (d, $^1J_{PC}$ = 95.8 Hz, **^{7}CH$_3$**), 13.69 (d, $^1J_{PC}$ = 95.9 Hz, **^{8}CH$_3$**).

MS (ES+) *m/z* = 175.05 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_7H_{12}O_3P$ 175.0524, found 175.0525.

(Furan-2-yl(hydroxy)methyl)diphenylphosphine oxide 180j:

The synthesis of (furan-2-yl(hydroxy)methyl)diphenylphosphine oxide was performed according to the base free Pudovik procedure, starting from diphenylphosphine oxide (100 mg, 0.49 mmol) and furan 2-carboxaldehyde (40 µL, 0.49 mmol).

	$C_{17}H_{15}O_3P$ M = 298.28 g.mol^{-1} 70% (103 mg) Beige solid

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 7.95 – 7.88 (m, 2H, **CH_{Ar}**), 7.85 – 7.78 (m, 2H, **CH_{Ar}**), 7.68 – 7.48 (m, 6H, **CH_{Ar}**), 7.42 – 7.40 (m, 1H, **^{4}CH**), 6.34 (m, 1H, **^{2}CH**), 6.28 (m, 1H, **^{3}CH**), 5.65 (d, $^{2}J_{PH}$ = 7.7 Hz, 1H, **^{5}CH**).

^{31}P CPD NMR (162 MHz, CD_3OD) δ (ppm): 32.00 (s).

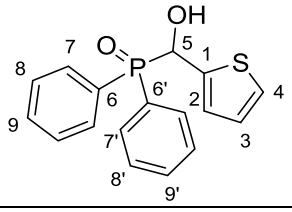
^{13}C APT NMR (101 MHz, CD_3OD) δ (ppm): 151.78 (s, **^{4}CH**), 144.16 (d, $^{2}J_{PC}$ = 2.3 Hz **^{1}C**), 133.63 (d, $^{4}J_{PC}$ = 2.9 Hz, **^{9}CH**), 133.53 (d, $^{4}J_{PC}$ = 2.9 Hz, **$^{9'}$CH**), 133.42 (d, $^{2}J_{PC}$ = 9.1 Hz, **^{7}CH**), 132.69 (d, $^{2}J_{PC}$ = 9.2 Hz, **$^{7'}$CH**) 131.35 (d, $^{1}J_{PC}$ = 63.8 Hz, **^{6}C**), 129.75 (d, $^{1}J_{PC}$ = 63.8 Hz, **$^{6'}$C**), 129.72 (d, $^{3}J_{PC}$ = 11.7 Hz, **^{8}CH**), 129.55 (d, $^{3}J_{PC}$ = 11.8 Hz, **$^{8'}$CH**), 111.66 (s, **^{3}CH**), 111.06 (d, $^{3}J_{PC}$ = 5.4 Hz, **^{2}CH**), 68.60 (d, $^{1}J_{PC}$ = 91.9 Hz, **^{5}CH**).

MS (ES+) *m/z* = 299.08 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_{17}H_{16}O_3P$ 299.0837, found 299.0840.

(Hydroxy(thiophen-2-yl)methyl)diphenylphosphine oxide 180k:

The synthesis of (hydroxy(thiophen-2-yl)methyl)diphenylphosphine oxide was performed according to the base free Pudovik procedure, starting from diphenylphosphine oxide (100 mg, 0.49 mmol) and thiophene 2-carboxaldehyde (46 µL, 0.49 mmol).

	$C_{17}H_{15}O_2PS$ M = 314.34 $g.mol^{-1}$ 83% (129 mg) White solid

^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ (ppm): 7.95 – 7.79 (m, 4H, **CH_{Ar}**), 7.65 – 7.57 (m, 2H, **CH_{Ar}**), 7.57 – 7.47 (m, 4H, **CH_{Ar}**), 7.30 (dt, $^{3}J_{HH}$ = 5.1, $^{4}J_{HH}$ = 1.4 Hz, 1H, **^{4}CH**), 6.99 (m, 1H, **^{3}CH**), 6.92 (m, 1H, **^{2}CH**), 5.88 (dd, $^{2}J_{PH}$ = 7.2, $^{4}J_{HH}$ = 0.8 Hz, 1H, **^{5}CH**).

^{31}P CPD NMR (162 MHz, $(CD_3)_2SO$) δ (ppm): 26.84 (s).

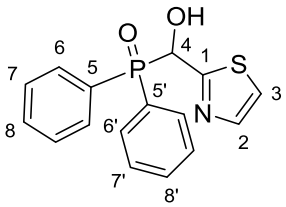
^{13}C NMR (101 MHz, $(CD_3)_2SO$) δ (ppm): 141.39 (d, $^{2}J_{PC}$ = 6.8 Hz, **^{1}C**), 132.48 (d, $^{1}J_{PC}$ = 94.0 Hz, **^{6}CH**), 131.82 (d, $^{2}J_{PC}$ = 8.5 Hz, **^{7}CH**), 131.69 (d, $^{4}J_{PC}$ = 2.6 Hz, **^{9}CH**), 131.62 (d, $^{4}J_{PC}$ = 2.5 Hz, **$^{9'}$CH**), 131.27 (d, $^{2}J_{PC}$ = 8.5 Hz, **$^{7'}$CH**), 130.87 (d, $^{1}J_{PC}$ = 94.4 Hz, **$^{6'}$CH**) 128.33 (d, $^{3}J_{PC}$ = 11.1 Hz, **^{8}CH**), 128.17 (d, $^{3}J_{PC}$ = 11.2 Hz, **$^{8'}$CH**), 126.33 (s, **^{4}CH**), 125.83 (d, $^{3}J_{PC}$ = 2.4 Hz, **^{2}CH**), 125.78 (s, **^{3}CH**), 68.63 (d, $^{1}J_{PC}$ = 90.1 Hz, **^{5}CH**).

MS (ES+) *m/z* = 315.05 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_{17}H_{16}O_2PS$ 315.0609, found 315.0606.

(Hydroxy(thiazol-2-yl)methyl)diphenylphosphine oxide 180l:

The synthesis of (hydroxy(thiazol-2-yl)methyl)diphenylphosphine oxide was performed according to the base free Pudovik procedure, starting from diphenylphosphine oxide (100 mg, 0.49 mmol) and thiazole 2-carboxaldehyde (43 µL, 0.49 mmol).

	$C_{16}H_{14}NO_2PS$ M = 315.33 g.mol^{-1} 73% (114 mg) White solid

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 7.96 – 7.80 (m, 4H, **CH_{Ar}**), 7.69 (d, $^{3}J_{HH}$ = 3.3 Hz, 1H, **^{2}CH**), 7.67 – 7.61 (m, 2H, **CH_{Ar}**), 7.57 – 7.51 (m, 4H, **CH_{Ar}**), 7.52 (d, $^{3}J_{HH}$ = 3.3 Hz, 1H, **^{3}CH**), 5.99 (d, $^{2}J_{PH}$ = 8.0 Hz, 1H, **^{4}CH**).

^{31}P CPD NMR (162 MHz, CD_3OD) δ (ppm): 31.58 (s).

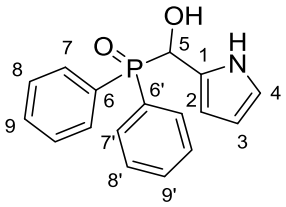
^{13}C APT NMR (101 MHz, CD_3OD) δ (ppm): 169.96 (s, **^{1}C**), 143.18 (s, **^{2}CH**), 133.81 (d, $^{4}J_{PC}$ = 2.9 Hz, **^{8}CH**), 133.70 (d, $^{4}J_{PC}$ = 2.9 Hz, **$^{8'}$C**), 133.57 (d, $^{2}J_{PC}$ = 8.9 Hz, **^{6}CH**), 132.84 (d, $^{2}J_{PC}$ = 9.2 Hz, **$^{6'}$CH**), 131.55 (d, $^{1}J_{PC}$ = 99.1 Hz, **^{5}C**), 129.77 (d, $^{3}J_{PC}$ = 11.9 Hz, **^{7}CH**), 129.58 (d, $^{3}J_{PC}$ = 11.9 Hz, **$^{7'}$CH**), 129.53 (d, $^{1}J_{PC}$ = 98.9 Hz, **$^{5'}$C**), 121.93 (s, **^{3}CH**), 72.71 (d, $^{1}J_{PC}$ = 88.1 Hz, **^{4}CH**).

MS (ES+) *m/z* = 316.06 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_{16}H_{15}NO_2PS$ 316.0561, found 316.0562.

(Hydroxy(1H-pyrrol-2-yl)methyl)diphenylphosphine oxide 180m:

The synthesis of (hydroxy(1H-pyrrol-2-yl)methyl)diphenylphosphine oxide was performed according to the base free Pudovik procedure, starting from diphenylphosphine oxide (100 mg, 0.49 mmol) and pyrrole 2-carboxaldehyde (46 mg, 0.49 mmol).

	$C_{18}H_{16}NO_2P$ M = 297.29 g.mol^{-1} 74% (109 mg) White solid

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 10.06 (s, 1H, **NH**), 7.90 – 7.82 (m, 2H, **CH_{Ar}**), 7.73 – 7.61 (m, 3H, **CH_{Ar}**), 7.60 – 7.50 (m, 3H, **CH_{Ar}**), 7.51 – 7.42 (m, 2H, **CH_{Ar}**), 6.70 (m, **^{4}CH**), 5.99 (m, 1H, **^{2}CH**), 5.91 (m, 1H, **^{3}CH**), 5.60 (d, $^{2}J_{PH}$ = 4.3 Hz, 1H, **^{5}CH**).

^{31}P CPD NMR (162 MHz, CD_3OD) δ (ppm): 32.93 (s).

^{13}C APT NMR (101 MHz, CD_3OD) δ (ppm): 144.60 (s, **^{1}C**), 133.43 (d, $^{4}J_{PC}$ = 2.8 Hz, **^{9}CH**), 133.32 (d, $^{2}J_{PC}$ = 8.9 Hz, **^{7}CH**), 133.28 (d, $^{1}J_{PC}$ = 42.3 Hz, **^{6}C**), 133.27 (d, $^{4}J_{PC}$ = 2.7 Hz, **$^{9'}$CH**), 132.72 (d, $^{2}J_{PC}$ = 9.0 Hz,

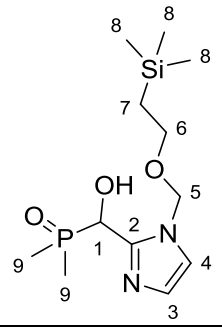
$^{7'}$CH), 129.53 (d, $^{3}J_{PC}$ = 11.6 Hz, **^{8}CH**), 129.51 (d, $^{3}J_{PC}$ = 11.5 Hz, **$^{8'}$CH**), 119.56 (s, **^{4}CH**), 109.48 (s, **^{2}CH**), 109.00 (s, **^{3}CH**), 68.74 (d, $^{1}J_{PC}$ = 92.8 Hz, **^{5}CH**).

MS (ES+) *m/z* = 320.08 ($M+Na^+$)

HRMS (ES+): *m/z*: [M+Na] calculated for $C_{17}H_{16}NO_2PNa$ 320.0816, found 320.0817.

(Hydroxy(1-((2-(trimethylsilyl)ethoxy)methyl)-1H-imidazol-2-yl)methyl)dimethylphosphine oxide 180n:

The synthesis of (hydroxy(1-((2-(trimethylsilyl)ethoxy)methyl)-1H-imidazol-2-yl)methyl)dimethyl phosphine oxide was performed according to the base free Pudovik procedure, starting from diphenylphosphine oxide (100 mg, 0.49 mmol) and *N*-SEM imidazole 2-carboxaldehyde (111 mg, 0.49 mmol).

	$C_{12}H_{25}N_2O_3PSi$ M = 304.40 $g.mol^{-1}$ 96% (375 mg) Yellow oil

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.03 (d, $^{3}J_{HH}$ = 17.0 Hz, 2H, **^{3}CH**, **^{4}CH**), 5.71 (d, $^{3}J_{HH}$ = 10.9 Hz, 1H, **^{5}CH$_2$**), 5.33 (d, $^{3}J_{HH}$ = 10.9 Hz, 1H, **^{5}CH$_2$**), 5.19 (d, $^{2}J_{PH}$ = 6.5 Hz, 1H, **^{1}CH**), 3.53 (t, $^{3}J_{HH}$ = 7.9 Hz, 2H, **^{6}CH$_2$**), 1.70 (d, $^{2}J_{PH}$ = 13.1 Hz, 3H, **^{9}CH$_3$**), 1.46 (d, $^{2}J_{PH}$ = 12.9 Hz, 3H, **^{9}CH$_3$**), 0.96 – 0.89 (t, $^{3}J_{HH}$ = 7.9 Hz, 2H, **^{7}CH$_2$**), 0.00 (s, 9H, **^{8}CH$_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 49.68 (s).

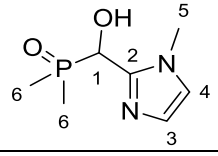
^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 145.39 (s, **^{2}C**), 128.75 (s, **^{4}CH**), 123.38 (s, **^{3}CH**), 77.32 (s, **^{5}CH$_2$**), 68.37 (d, $^{1}J_{PC}$ = 82.5 Hz, **^{1}CH**), 67.99 (s, **^{6}CH$_2$**), 19.20 (s, **^{7}CH$_2$**), 15.35 (d, $^{1}J_{PC}$ = 67.0 Hz, **^{8}CH$_3$**), 13.11 (d, $^{1}J_{PC}$ = 67.5 Hz, **^{9}CH$_3$**), -0.00 (s, **^{8}CH$_3$**).

MS (ES+) *m/z* = 305.15 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_{12}H_{26}N_2O_3PSi$ 305.1450, found 305.1452.

(Hydroxy(1-methyl-1H-imidazol-2-yl)methyl)dimethylphosphine oxide 180o:

The synthesis of (hydroxy(1-methyl-1H-imidazol-2-yl)methyl)dimethylphosphine oxide was performed according to the base free Pudovik procedure, starting from dimethylphosphine oxide (38 mg, 0.49 mmol) and *N*-methylimidazole 2-carboxaldehyde (53 mg, 0.49 mmol).

	$C_7H_{13}N_2O_2P$ M = 188.17 g.mol^{-1} 31% (27 mg) White solid

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 7.12 (s, 1H, **^{4}CH**), 6.99 (s, 1H, **^{3}CH**), 5.20 (d, $^{2}J_{PH}$ = 7.8 Hz, 1H, **^{1}CH**), 3.83 (s, 3H, **^{5}CH$_3$**), 1.63 (d, $^{2}J_{PH}$ = 4.8 Hz, 3H, **^{6}CH$_3$**), 1.60 (d, $^{2}J_{PH}$ = 4.6 Hz, 3H, **^{6}CH$_3$**).

^{31}P CPD NMR (162 MHz, CD_3OD) δ (ppm): 52.74 (s).

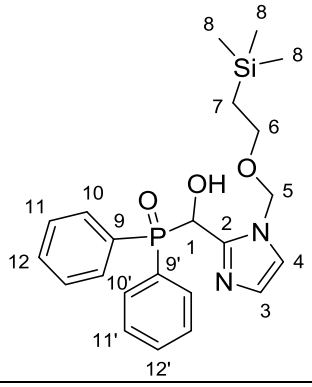
^{13}C APT NMR (101 MHz, CD_3OD) δ (ppm): 144.96 (s, **^{2}C**), 127.90 (s), 124.36 (s), 67.64 (d, $^{1}J_{PC}$ = 87.8 Hz, **^{1}CH**), 34.11 (s), 13.64 (d, $^{1}J_{PC}$ = 67.9 Hz, **^{6}CH$_3$**), 12.03 (d, $^{1}J_{PC}$ = 67.7 Hz, **^{6}CH$_3$**).

MS (ES+) *m/z* = 211.06 ($M+Na^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_7H_{14}N_2O_2P$ 189.0793, found 189.0792.

(Hydroxy(1-((2-(trimethylsilyl)ethoxy)methyl)-1H-imidazol-2-yl)methyl)diphenylphosphine oxide 180m:

The synthesis of (hydroxy(1-((2-(trimethylsilyl)ethoxy)methyl)-1H-imidazol-2-yl)methyl)diphenylphosphine oxide was performed according to the base free Pudovik procedure, starting from diphenylphosphine oxide (100 mg, 0.49 mmol) and *N*-SEM imidazole 2-carboxaldehyde (111 mg, 0.49 mmol).

	$C_{22}H_{29}N_2O_3PSi$ M = 428.54 g.mol^{-1} 80% (168 mg) White solid

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 7.94 – 7.87 (m, 2H, **CH$_{Ar}$**), 7.77 – 7.46 (m, 8H, **CH$_{Ar}$**), 7.23 (s, 1H, **^{4}CH**), 6.85 (s, 1H, **^{3}CH**), 5.89 (d, $^{2}J_{PH}$ = 6.2 Hz, 1H, **^{1}CH**), 5.62 (d, $^{2}J_{HH}$ = 10.7 Hz, 1H, **^{5}CH**), 5.53 (d, $^{2}J_{HH}$ = 10.7 Hz, 1H, **^{5}CH**), 3.62 – 3.51 (m, 2H, **^{6}CH$_2$**), 0.99 – 0.83 (m, 2H, **^{7}CH$_2$**), -0.00 (s, 9H, **^{8}CH$_3$**).

^{31}P CPD NMR (162 MHz, CD_3OD) δ (ppm): δ 32.84 (s).

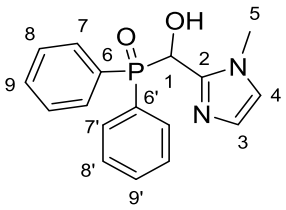
^{13}C APT NMR (101 MHz, CD_3OD) δ (ppm): 146.10 (d, $^{2}J_{PC}$ = 1.2 Hz, **^{2}C**), 135.08 (d, $^{3}J_{PC}$ = 10.6 Hz, **^{11}CH**), 135.05 (d, $^{3}J_{PC}$ = 10.6 Hz, **$^{11'}$CH**), 134.69 (d, $^{2}J_{PC}$ = 9.1 Hz, **^{10}CH**), 133.95 (d, $^{2}J_{PC}$ = 9.1 Hz, **$^{10'}$CH**), 134.00 (d, $^{1}J_{PC}$ = 96.8 Hz, **^{9}C**), 131.73 (d, $^{1}J_{PC}$ = 98.0 Hz, **$^{9'}$C**), 131.15 (d, $^{4}J_{PC}$ = 1.1 Hz, **^{12}CH**), 131.04 (d, $^{4}J_{PC}$ = 0.9 Hz, **$^{12'}$CH**) 129.56 (s, **^{3}CH**), 124.26 (s, **^{4}CH**), 78.55 (s, **^{5}CH$_2$**), 70.56 (d, J = 89.3 Hz, **^{1}CH**), 68.69 (s, **^{6}CH$_2$**), 20.10 (s, **^{7}CH$_2$**), -0.00 (s, **^{8}CH$_3$**).

MS (ES+) *m/z* = 429.18 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_{22}H_{30}N_2O_3PSi$ 429.1767, found 429.1766.

(Hydroxy(1-methyl-1H-imidazol-2-yl)methyl)diphenylphosphine oxide 180q:

The synthesis of (hydroxy(1-methyl-1H-imidazol-2-yl)methyl)diphenylphosphine oxide was performed according to the base free Pudovik procedure, starting from diphenylphosphine oxide (100 mg, 0.49 mmol) and *N*-methylimidazole 2-carboxaldehyde (53 mg, 0.49 mmol).

	$C_{17}H_{18}N_2O_2P$ M = 312.31 $g.mol^{-1}$ 71% (110 mg) White solid

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 7.98 – 7.85 (m, 2H, **CH_{Ar}**), 7.76 – 7.47 (m, 8H, **CH_{Ar}**), 7.06 (s, 1H, ^{4}**CH**), 6.82 (s, 1H, ^{3}**CH**), 5.86 (d, $^2J_{PH}$ = 6.1 Hz, 1H, ^{1}**CH**), 3.81 (s, 3H, ^{5}**CH_3**).

^{1}P CPD NMR (162 MHz, CD_3OD) δ (ppm): 32.97 (s).

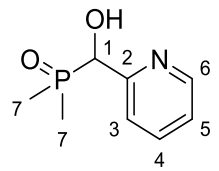
^{13}C APT NMR (101 MHz, CD_3OD) δ (ppm): 144.40 (d, $^2J_{PC}$ = 1.8 Hz, ^{2}**C**), 133.77 (d, $^4J_{PC}$ = 2.7 Hz, ^{9}**CH**), 133.65 (d, $^4J_{PC}$ = 2.6 Hz, $^{9'}$**CH**), 133.30 (d, $^2J_{PC}$ = 9.1 Hz, ^{7}**CH**), 132.58 (d, $^2J_{PC}$ = 9.2 Hz, $^{7'}$**CH**), 129.76 (d, $^3J_{PC}$ = 11.9 Hz, ^{8}**CH**), 129.72 (d, $^3J_{PC}$ = 11.9 Hz, $^{8'}$**CH**) 127.35 (s, ^{4}**CH**), 124.76 (s, ^{3}**CH**), 69.27 (d, $^1J_{PC}$ = 90.0 Hz, ^{1}**CH**), 34.59 (s, ^{5}**CH_3**).

MS (ES+) *m/z* = 313.1 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_{17}H_{18}N_2O_2P$ 313.1106, found 313.1104.

(Hydroxy(pyridin-2-yl)methyl)dimethylphosphine oxide 183a:

The synthesis of (hydroxy(pyridin-2-yl)methyl)dimethylphosphine oxide was performed according to the base free Pudovik procedure, starting from dimethylphosphine oxide (100 mg, 1.28 mmol) and pyridine 2-carboxaldehyde (122 µL, 1.28 mmol).

	$C_8H_{12}NO_2P$ M = 185.16 $g.mol^{-1}$ 32% (77 mg) Yellow solid

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 8.58 (d, $^3J_{HH}$ = 4.9 Hz, 1H, ^{6}**CH**), 7.88 (td, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 1.6 Hz, 1H, ^{4}**CH**), 7.63 (dd, $^3J_{HH}$ = 7.8 Hz, $^4J_{HH}$ = 0.8 Hz, 1H, ^{3}**CH**), 7.42 – 7.33 (m, 1H, ^{5}**CH**), 5.09 (d, $^2J_{PH}$ = 7.9 Hz, 1H, ^{1}**CH**), 1.60 (d, $^2J_{PH}$ = 13.2 Hz, 3H, ^{7}**CH_3**), 1.48 (d, $^2J_{PH}$ = 13.1 Hz, 3H, ^{7}**CH_3**).

^{31}P CPD NMR (162 MHz, CD_3OD) δ (ppm): 51.94 (s).

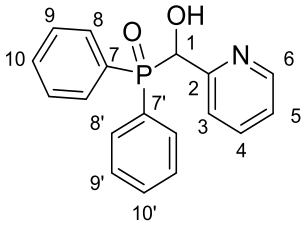
^{13}C APT NMR (101 MHz CD_3OD) δ (ppm): 158.09 (s, **^{2}C**), 149.76 (s, **^{6}CH**), 138.49 (s, **^{4}CH**), 124.48 (s, **^{3}CH**), 123.66 (s, **^{5}CH**), 74.56 (d, $^{1}J_{PC}$ = 81.8 Hz, **^{1}CH**), 13.35 (d, $^{1}J_{PC}$ = 67.7 Hz, **$^{7}CH_3$**), 11.86 (d, $^{1}J_{PC}$ = 67.5 Hz, **$^{7}CH_3$**).

MS (ES+) *m/z* = 186.07 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_8H_{13}NO_2P$ 186.0684, found 186.0684.

(Hydroxy(pyridin-2-yl)methyl)diphenylphosphine oxide 183b:

The synthesis of (hydroxy(pyridin-2-yl)methyl)diphenylphosphine oxide was performed according to the base free Pudovik procedure, starting from diphenylphosphine oxide (100 mg, 0.49 mmol) and pyridine 2-carboxaldehyde (47 µL, 0.49 mmol).

	$C_8H_{12}NO_2P$ M = 309.30 $g.mol^{-1}$ 72% (111 mg) Slightly yellow solid

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 8.38 (d, $^{3}J_{HH}$ = 4.9 Hz, 1H, **^{6}CH**), 7.94 – 7.71 (m, 5H, **CH_{Ar}**), 7.65 – 7.60 (m, 2H, **CH_{Ar}**), 7.56 – 7.39 (m, 5H, **CH_{Ar}**), 7.29 (dd, *J* = 6.8, 5.7 Hz, 1H, **CH_{Ar}**), 5.76 (d, $^{2}J_{PH}$ $^{2}J_{PH}$ = 7.0 Hz, 1H, **^{1}CH**).

^{31}P CPD NMR (162 MHz, CD_3OD) δ (ppm): 32.97 (s).

^{13}C APT NMR (101 MHz, CD_3OD) δ (ppm): 157.18 (d, $^{2}J_{PC}$ = 1.0 HZ, **^{2}C**), 149.03 (s, **^{6}CH**), 138.19 (s, **^{4}CH**), 133.61 (d, $^{4}J_{PC}$ = 2.8 Hz, **^{10}CH**), 133.57 (d, $^{4}J_{PC}$ = 2.7 Hz, **$^{10'}$CH**), 133.49 (d, $^{2}J_{PC}$ = 8.8 Hz, **^{8}CH**), 132.83 (d, $^{2}J_{PC}$ = 9.2 Hz, **$^{8'}$CH**), 132.07 (d, $^{1}J_{PC}$ = 97.0 Hz, **^{7}C**), 129.79 (d, $^{1}J_{PC}$ = 97.8 Hz, **$^{7'}$C**), 129.74 (d, $^{3}J_{PC}$ = 11.7 Hz, **^{9}CH**), 129.46 (d, $^{3}J_{PC}$ = 11.8 Hz, **$^{9'}CH_{Ar}$**), 124.41 (s, **^{3}CH**), 124.31 (s, **^{5}CH**)74.52 (d, $^{1}J_{PC}$ = 84.3 Hz, **^{1}CH**).

MS (ES+) *m/z* = 310.10 ($M+H^+$)

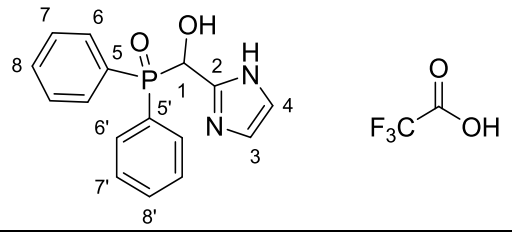
HRMS (ES+): *m/z*: [M+H] calculated for $C_{18}H_{17}NO_2P$ 310.0997, found 310.1000.

General procedure for the SEM deprotection:

In a 5 mL V-shaped Wheaton tube was introduced the SEM-protected Pudovik adduct (1 eq) in a mixture of ethanol/dichloromethane (3% v/v of ethanol in dichloromethane, 0.28 M). Trifluoroacetic acid (15 eq) was added in one portion, the medium was stirred overnight at room temperature. Then, it was concentrated under vacuum and purified by flash column chromatography with a mixture of ethyl acetate and ethanol as eluent (80/20) which afforded the pure deprotected product as a trifluoroacetate salt.

(Hydroxy(1H-imidazol-2-yl)methyl)diphenylphosphine oxide trifluoroacetate 180r:

The synthesis of (hydroxy(1H-imidazol-2-yl)methyl)diphenylphosphine oxide trifluoroacetate was performed according to the SEM deprotection procedure, starting from 100 mg of (hydroxy(1-((2-(trimethylsilyl)ethoxy)methyl)-1H-imidazol-2-yl)methyl)diphenylphosphine oxide.

	$C_{18}H_{16}F_3N_2O_4P$ M = 412.30 g.mol^{-1} Quantitative (96 mg) Beige solid

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 8.00 – 7.52 (m, 10H, **CH_{Ar}**), 7.43 (s, 2H, **^{3}CH**, **^{4}CH**), 6.05 (d, $^2J_{PH}$ = 11.6 Hz, 1H, **^{1}CH**).

^{31}P CPD NMR (162 MHz, CD_3OD) δ (ppm): 30.57 (s).

^{13}C APT NMR (101 MHz, CD_3OD) δ (ppm): 145.47 (s, **^{2}C**), 134.55 (d, $^4J_{PC}$ = 1.8 Hz, **^{8}CH**, **$^{8'}$CH**), 133.36 (d, $^2J_{PC}$ = 9.1 Hz, **^{6}CH**), 132.80 (d, $^2J_{PC}$ = 9.5 Hz, **$^{6'}$CH**), 130.28 (d, $^3J_{PC}$ = 12.3 Hz, **$^7CH_{Ar}$**), 130.22 (d, $^1J_{PC}$ = 102.1 Hz, **^{5}C**), 129.96 (d, $^3J_{PC}$ = 12.0 Hz, **$^{7'}$CH**), 127.83 (d, $^1J_{PC}$ = 100.3 Hz, **$^{5'}$C**), 120.95 (s, **^{3}CH**, **^{4}CH**), 67.89 (d, $^1J_{PC}$ = 85.4 Hz, **^{1}CH**).

^{19}F CPD NMR (376 MHz, CD_3OD) δ (ppm): -77.30 (s).

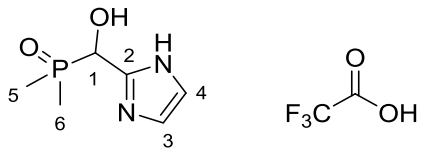
MS (ES+) *m/z* = 299.09 ($M+H^+$) imidazolium

MS(ES-) *m/z* = 113.0 ($M-H^+$) trifluoroacetate

HRMS (ES+): *m/z*: [M+H] calculated for $C_{16}H_{16}N_2O_2P$ 299.0949, found 299.0946.

(Hydroxy(1H-imidazol-2-yl)methyl)dimethylphosphine oxide trifluoroacetate 180a:

The synthesis of (hydroxy(1H-imidazol-2-yl)methyl)dimethylphosphine oxide trifluoroacetate was performed according to the SEM deprotection procedure procedure, starting from 150 mg of (hydroxy(1-((2-(trimethylsilyl)ethoxy)methyl)-1H-imidazol-2-yl)methyl)dimethylphosphine oxide.

	$C_8H_{12}F_3N_2O_4P$ M = 288.16 g.mol^{-1} 61% (52 mg) Yellow oil

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 7.55 (s, 2H, **^{3}CH**, **^{4}CH**), 5.50 (d, $^2J_{PH}$ = 10.8 Hz, 1H, **^{1}CH**), 1.73 (d, $^2J_{PH}$ = 13.5 Hz, 3H, **$^5CH_3$**), 1.49 (d, $^2J_{PH}$ = 13.3 Hz, 3H, **$^6CH_3$**).

^{31}P CPD NMR (162 MHz, CD_3OD) δ (ppm): 51.97 (s).

^{19}F CPD NMR (376 MHz, CD_3OD) δ (ppm): -77.27 (s).

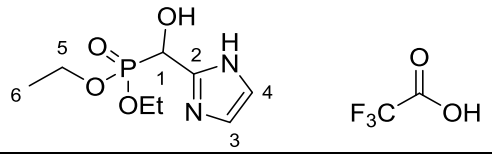
^{13}C APT NMR (101 MHz, CD_3OD) δ (ppm): 145.64 (s, **^{2}C**), 121.09 (s, **^{3}CH**, **^{4}CH**), 66.78 (d, $^{1}J_{PC}$ = 81.3 Hz, **^{1}CH**), 13.47 (d, $^{1}J_{PC}$ = 69.3 Hz, **$^{5}CH_3$**), 11.15 (d, $^{1}J_{PC}$ = 68.4 Hz, **$^{6}CH_3$**).

MS (ES+) *m/z* = 175.06 ($M+H^+$ without trifluoroacetic acid)

HRMS (ES+): *m/z*: [M+H] calculated for $C_6H_{12}N_2O_2P$ 175.0636, found 175.0637.

Diethyl (hydroxy(1H-imidazol-2-yl)methyl)phosphonate trifluoroacetate 180t:

The synthesis of diethyl (hydroxy(1H-imidazol-2-yl)methyl)phosphonate trifluoroacetate was performed according to the SEM deprotection procedure, starting from 200 mg of diethyl (hydroxy(1-((2-(trimethylsilyl)ethoxy)methyl)-1H-imidazol-2-yl)methyl)phosphonate.

	$C_{10}H_{16}F_3N_2O_6P$ M = 348.21 g.mol^{-1} 59% (112 mg) Yellow solid

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 7.54 (s, 2H, **^{3}CH**, **^{4}CH**), 5.48 (d, $^{2}J_{PH}$ = 15.7 Hz, 1H, **^{1}CH**), 4.32 – 4.11 (m, 4H, **$^{5}CH_2$**), 1.32 (t, $^{3}J_{HH}$ = 7.1 Hz, 6H, **$^{6}CH_3$**).

^{31}P CPD NMR (162 MHz, CD_3OD) δ (ppm): 14.91 (s).

^{19}F CPD NMR (377 MHz, CD_3OD) δ (ppm): -76.97 (s).

^{13}C APT NMR (101 MHz, CD_3OD) δ (ppm): 145.59 (s, **^{2}C**), 120.97 (s, **^{3}CH**, **^{4}CH**), 65.84 (d, $^{2}J_{PC}$ = 13.2 Hz, **$^{5}CH_2$**), 65.76 (d, $^{2}J_{PC}$ = 12.9 Hz, **$^{5}CH_2$**) 64.46 (d, $^{1}J_{PC}$ = 171.4 Hz, **^{1}CH**), 16.75 (s, **$^{6}CH_3$**), 16.70 (s, **$^{6}CH_3$**).

MS (ES+) *m/z* = 235.08 ($M+H^+$ without trifluoroacetic acid)

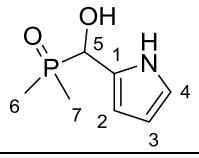
HRMS (ES+): *m/z*: [M+H] calculated for $C_8H_{16}N_2O_4P$ 235.0848, found 235.0847.

General conditions for Pudovik reactions with CsF-Al_2O_3:

In a 5 mL V-shaped Wheaton tube was introduced the P-H compound (1 eq), 1,4-dioxane (1.5 M), the heterocycle 2-carboxaldehyde (1 eq), then caesium fluoride (1.6 eq) and alumina (2.5 eq). The heterogenous mixture was stirred at room temperature for 2h to 3 days. The medium was filtered through celite. The solid was rinsed with dichloromethane. The filtrate was evaporated under vacuum leading to the Pudovik product.

(Hydroxy(1H-pyrrol-2-yl)methyl)dimethylphosphine oxide 180b:

The synthesis of (hydroxy(1H-pyrrol-2-yl)methyl)dimethylphosphine oxide was performed according to Texier-Boullet's Pudovik conditions, starting from dimethylphosphine oxide (100 mg, 1.28 mmol) and pyrrole2-carboxaldehyde (122 mg, 1.28 mmol).

	$C_7H_{12}NO_2P$ M = 173.15 g.mol^{-1} 94% (219 mg)

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 6.78 (m, 1H, **^{4}CH**), 6.17 (m, 1H, **^{2}CH**), 6.11 (m, 1H, **^{3}CH**), 4.96 (d, $^2J_{PH}$ = 6.3 Hz, 1H, **^{5}CH**), 1.50 (d, $^2J_{PH}$ = 13.0 Hz, 1H, **^{6}CH$_3$**), 1.46 (d, $^2J_{PH}$ = 13.1 Hz, 1H, **^{7}CH$_3$**).

^{31}P CPD NMR (162 MHz, CD_3OD) δ (ppm): 51.32 (s).

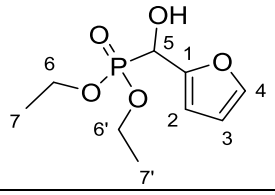
^{13}C APT NMR (101 MHz, CD_3OD) δ (ppm): 127.71 (d, $^2J_{PC}$ = 1.5 Hz, **^{1}C**), 119.50 (s, **^{4}CH**), 109.06 (d, $^4J_{PC}$ = 1.8 Hz, **^{3}CH**), 108.36 (d, $^3J_{PC}$ = 5.5 Hz, **^{2}CH**), 68.82 (d, $^1J_{PC}$ = 90.5 Hz, **^{5}CH**), 12.63 (d, $^1J_{PC}$ = 67.1 Hz, **^{6}CH$_3$**), 11.83 (d, $^1J_{PC}$ = 67.2 Hz, **^{7}CH$_3$**).

MS (ES+) *m/z* = 174.07 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_7H_{13}NO_2P$ 174.0684, found 174.0690.

Diethyl (furan-2-yl(hydroxy)methyl)phosphonate 180c:

The synthesis of diethyl (furan-2-yl(hydroxy)methyl)phosphonate was performed according to Texier-Boullet's Pudovik conditions, starting from diethyl phosphite (463 µL, 3.6 mmol) and furan 2-carboxaldehyde (298 µL, 3.6 mmol).

	$C_9H_{15}O_5P$ M = 234.19 g.mol^{-1} 85% (1.0 g) Colourless oil

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.41 – 7.38 (m, 1H, **^{5}CH**), 6.50 (m, 1H, **^{3}CH**), 6.35 (s, 1H, **^{4}CH**), 5.00 (d, $^2J_{PH}$ = 13.2 Hz, 1H, **^{1}CH**), 4.20 – 3.99 (m, 4H, **^{6}CH$_2$**, **$^{6'}$CH$_2$**), 1.29 (t, $^3J_{HH}$ = 7.0 Hz, 3H, **^{7}CH$_3$**), 1.23 (t, $^3J_{HH}$ = 7.1 Hz, 3H, **$^{7'}$CH$_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 19.50 (s).

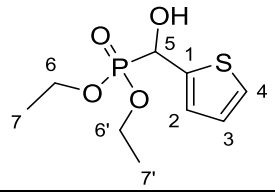
^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 150.26 (s, **^{2}C**), 142.65 (s, **^{5}CH**), 110.66 (s, **^{6}CH**), 109.19 (s, **^{4}CH**), 64.67 (d, $^1J_{PC}$ = 167.1 Hz, **^{1}CH**), 63.46 (d, $^2J_{PC}$ = 6.9 Hz, **^{6}CH$_2$**), 63.23 (d, *J* = 7.0 Hz, **$^{6'}$CH$_2$**), 16.38 (d, $^3J_{PC}$ = 5.8 Hz, **^{7}CH$_3$**), 16.31 (d, $^3J_{PC}$ = 5.7 Hz, **$^{7'}$CH$_3$**).

MS (ES+) *m/z* = 235.07 ($M+H^+$) and 257.06 ($M+Na^+$)

HRMS (ES+): *m/z*: [M+Na] calculated for $C_9H_{15}O_5PNa$ 255.0555, found 257.0555.

Diethyl (hydroxy(thiophen-2-yl)methyl)phosphonate 180d:

The synthesis of diethyl (hydroxy(thiophen-2-yl)methyl)phosphonate was performed according to Texier-Boullet's Pudovik conditions, starting from diethyl phosphite (185 µL, 1.44 mmol) and thiophene 2-carboxaldehyde (135 µL, 1.44 mmol).

	$C_9H_{15}O_4PS$ M = 250.25 g.mol^{-1} 78% (225 mg) Brown oil

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.29 (m, 1H, **^{5}CH**), 7.20 – 7.16 (m, 1H, **^{3}CH**), 7.02 – 6.96 (m, 1H, **^{4}CH**), 5.23 (d, $^2J_{PH}$ = 11.0 Hz, 1H, **^{1}CH**), 4.18 – 4.03 (m, 4H, **^{6}CH$_2$**, **$^{6'}$CH$_2$**), 1.30 (t, $^3J_{HH}$ = 7.1 Hz, 3H, **^{7}CH$_3$**), 1.25 (t, $^3J_{HH}$ = 7.1 Hz, 3H, **$^{7'}$CH$_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 19.80 (s).

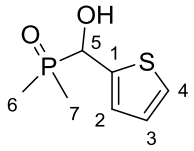
^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 139.60 (s, **^{2}C**), 126.79 (s, **^{5}CH**), 126.11 (d, $^3J_{PC}$ = 7.5 Hz, **^{3}CH**), 125.68 (s, **^{4}CH**), 67.00 (d, $^1J_{PC}$ = 167.0 Hz, **^{1}CH**), 63.65 (d, $^2J_{PC}$ = 7.0 Hz, **^{6}CH$_2$**), 63.32 (d, $^2J_{PC}$ = 7.3 Hz, **$^{6'}$CH$_2$**), 16.39 (d, $^3J_{PC}$ = 5.6 Hz, **^{7}CH$_3$**), 16.36 (d, $^3J_{PC}$ = 5.5 Hz, **$^{7'}$CH$_3$**).

MS (ES+) *m/z* = 251.05 ($M+H^+$) and 273.03 ($M+Na^+$)

HRMS (ES+): *m/z*: [M+Na] calculated for $C_9H_{15}O_4NaPS$ 273.0326, found 273.0327.

(Hydroxy(thiophen-2-yl)methyl)dimethylphosphine oxide 180g:

The synthesis of (hydroxy(thiophen-2-yl)methyl)dimethylphosphine oxide was performed according to Texier-Boullet's Pudovik conditions, starting from dimethylphosphine oxide (100 mg, 1.28 mmol) and thiophene 2-carboxaldehyde (120 µL, 1.28 mmol).

	$C_7H_{10}O_2PS$ M = 190.20 g.mol^{-1} Quantitative (243 mg) Orange solid

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.49 (m, 1H, **^{5}CH**), 7.27, (m, 1H, **^{3}CH**) 7.21 (m, 1H, **^{4}CH**), 5.37 (d, $^2J_{PH}$ = 8.1 Hz, 1H, **^{1}CH**), 1.68 (d, $^2J_{PH}$ = 12.8 Hz, 3H, **^{6}CH$_3$**), 1.56 (d, $^2J_{PH}$ = 12.7 Hz, 3H, **^{7}CH$_3$**).

^{31}P NMR (162 MHz, $CDCl_3$) δ (ppm): 48.88 (s).

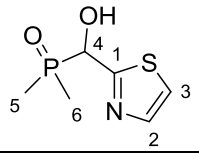
^{13}C NMR (101 MHz, $CDCl_3$) δ (ppm): 140.66 (s, **^{2}C**), 127.14 (s, **^{5}CH**), 125.15 (s, **^{3}CH**), 125.09 (s, **^{4}CH**), 69.69 (d, $^1J_{PC}$ = 86.0 Hz, **^{1}CH**), 12.41 (d, $^1J_{PC}$ = 67.2 Hz, **^{6}CH$_3$**), 11.65 (d, $^1J_{PC}$ = 66.8 Hz, **^{7}CH$_3$**).

MS (ES+) *m/z* = 213.01 ($M+Na^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_7H_{12}O_2PS$ 191.0296, found 191.0297.

(Hydroxy(thiazol-2-yl)methyl)dimethylphosphine oxide 180i:

The synthesis of (hydroxy(thiazol-2-yl)methyl)dimethylphosphine oxide was performed according to Texier-Boullet's Pudovik conditions, starting from dimethylphosphine oxide (100 mg, 1.28 mmol) and thiazole 2-carboxaldehyde (112 µL, 1.28 mmol).

	$C_6H_9NO_2PS$ M = 191.18 $g.mol^{-1}$ 51% (126 mg) Orange solid

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 7.72 (d, $^3J_{HH}$ = 3.3 Hz, 1H, **^{3}CH**), 7.51 (dd, $^3J_{HH}$ = 3.3, 1H, **^{4}CH**), 5.23 (d, $^2J_{PH}$ = 7.5 Hz, 1H, **^{1}CH**), 1.50 (d, $^2J_{PH}$ = 4.3 Hz, 3H, **^{5}CH$_3$**), 1.47 (d, $^2J_{PH}$ = 4.3 Hz, 3H, **^{6}CH$_3$**).

^{31}P CPD NMR (162 MHz, CD_3OD) δ (ppm): 50.48 (s).

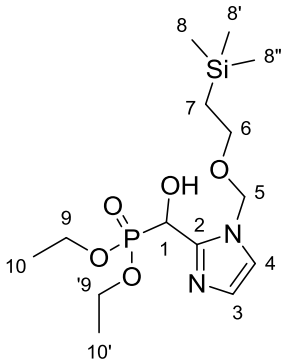
^{13}C APT NMR (101 MHz, CD_3OD) δ (ppm): 173.40 (s, **^{2}C**), 146.39 (s, **^{3}CH**), 123.97 (s, **^{4}CH**), 74.84 (d, $^1J_{PC}$ = 83.5 Hz, **^{1}CH**), 15.47 (d, $^1J_{PC}$ = 59.3 Hz, **^{5}CH$_3$**), 14.79 (d, $^1J_{PC}$ = 59.0 Hz, **^{6}CH$_3$**).

MS (ES+) *m/z* = 192.02 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_6H_{11}NO_2PS$ 192.0248, found 192.0246.

Diethyl (Hydroxy(1-((2-(trimethylsilyl)ethoxy)methyl)-1H-imidazol-2-yl)methyl)phosphonate 180s:

The synthesis of 2-[(diethylphosphono)hydroxymethyl]-*N*-[(trimethylsilyl)ethoxy]methylimidazole was performed according to Texier-Boullet's Pudovik conditions, starting from diethyl phosphite (627 µL, 4.9 mmol) and N-SEM imidazole 2-carboxaldehyde (1.10 g, 4.9 mmol).

	$C_{14}H_{29}N_2O_5PSi$ M = 364.45 $g.mol^{-1}$ 43% (704 mg) Colourless oil

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.04 (m, 2H, **^{3}CH**, **^{4}CH**), 5.52 (q, *J* = 10.9 Hz, 2H, **^{5}CH$_2$**), 5.23 (d, $^{2}J_{PH}$ = 11.7 Hz, 1H, **^{1}CH**), 4.22 – 4.01 (m, 4H, **^{9}CH$_2$**, **$^{9'}$CH$_2$**), 3.57 – 3.45 (m, 2H, **^{6}CH$_2$**), 1.36 – 1.22 (m, 6H, **^{10}CH$_3$**, **$^{10'}$CH$_3$**), 0.94 - 0.88 (m, 2H, **^{7}CH$_2$**), -0.01 (s, 9H, **^{8}CH$_3$**, **$^{8'}$CH$_3$**, **$^{8''}$CH$_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 18.28 (s).

^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 145.22 (d, $^{2}J_{PC}$ = 0.7 Hz, **^{2}C**), 128.88 (s, **^{3}CH**), 122.24 (s, **^{4}CH**), 77.44 (s, **^{5}CH$_2$**), 67.78 (s, **^{6}CH$_2$**), 66.42 (d, $^{1}J_{PC}$ = 167.6 Hz, **^{1}CH**), 64.85 (d, $^{2}J_{PC}$ = 6.9 Hz, **^{9}CH$_2$**), 64.53 (d, $^{2}J_{PC}$ = 7.0 Hz, **$^{9'}$CH$_2$**), 19.26 (s, **^{7}CH$_2$**), 17.84 (d, $^{3}J_{PC}$ = 5.6 Hz, **^{10}CH$_2$**), 17.77 (d, $^{3}J_{PC}$ = 5.5 Hz, **$^{10'}$CH$_2$**), -0.00 (s, **^{8}CH$_3$**, **$^{8'}$CH$_3$**, **$^{8''}$CH$_3$**).

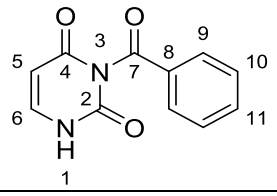
MS (ES+) *m/z* = 387.15 ($M+Na^+$).

HRMS (ES+): *m/z*: [M+Na] calculated for $C_{14}H_{29}N_2O_5PNaSi$ 387.1481, found 387.1476.

N-hydroxyimido derivatives and hydroxamate derivatives as complexing units

N^3-benzoyluracil 195a:

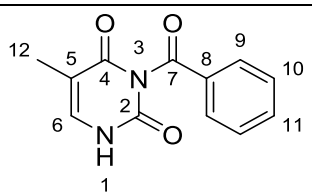
In a 100 mL round-bottom flask were introduced uracil (3.00 g, 26.7 mmol, 1 eq), benzoyl chloride (6.8 mL, 58.5 mmol, 2.2 eq) and pyridine (10.5 mL, 130 mmol, 4.9 eq) in acetonitrile (27.5 mL). The mixture was stirred at room temperature during 24 hours. After evaporation under reduced pressure, an extraction was performed using dichloromethane (3 × 50 mL) and water (30 mL). The organic layer was dried over magnesium sulfate and concentrated under vacuum. The crude was dissolved in dioxane (50 mL) and a saturated aqueous solution of potassium carbonate was introduced (25 mL). The mixture was stirred at room temperature during 30 min. Acetic acid was added slowly until reached pH = 5 and the mixture was concentrated under vacuum. A saturated aqueous solution of sodium hydrogencarbonate (130 mL) was added and the mixture was stirred at room temperature during 1 hour. The white solid was filtered off and washed with water and acetone leading to 5.6 g of white solid N^3-benzoyluracil.

	$C_{11}H_8N_2O_3$ M = 216.20 g.mol^{-1} 97% (5.6 g) White solid

^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ (ppm): 7.94 – 7.89 (m, 2H, **CH_{Ar}**), 7.79 – 7.73 (m, 1H, **CH_{Ar}**), 7.60 (m, 2H, **CH_{Ar}**), 7.58 (d, $^3J_{HH}$ = 7.6 Hz, 1H, **^{6}CH**), 5.71 (d, $^3J_{HH}$ = 7.6 Hz, 1H, **^{5}CH**).

N^3-benzoylthymine 195b:

The synthesis of N^3-benzoylthymine was performed according to N^3-benzoyluracil starting from 4 g of thymine.

	$C_{12}H_{10}N_2O_3$ M = 230.22 g.mol^{-1} 93% (6.8 g) White solid

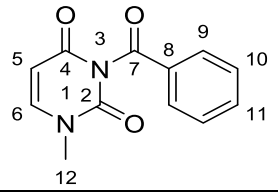
^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 7.98 – 7.92 (m, 2H, **CH_{Ar}**), 7.75 – 7.69 (m, 1H, **CH_{Ar}**), 7.60 – 7.53 (m, 2H, **CH_{Ar}**), 7.40 (q, $^4J_{HH}$ = 1.2 Hz, 1H, **^{6}CH**), 1.91 (d, $^4J_{HH}$ = 1.2 Hz, 3H, **$^{12}CH_3$**).

General procedure for the alkylation of N^1 of N^3-benzoyluracil and N^3-benzoylthymine

A mixture of N^3-benzoylthymine or N^3-benzoyluracil (1 eq) and anhydrous K_2CO_3 (1 eq) in DMF (0.1 M) was stirred for 1 h at room temperature, then alkyl halide (1.1 eq) was added. After stirring overnight at room temperature, insoluble material was removed by filtration and the filtrate was concentrated to dryness. The crude residue was dissolved in CH_2Cl_2 and washed with water, the organic layer was dried and concentrated under vacuum. In general, the N^1-alkylated N^3-benzoyluracil or N^3-benzoylthymine were obtained without further purifications. If not, a flash column chromatography was done to afford the pure products.

N^1-methyl-N^3-benzoyluracil 196a:

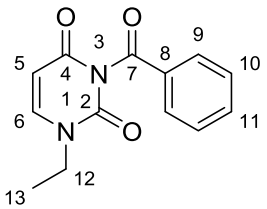
The synthesis of N^1-methyl-N^3-benzoyluracil was performed according to the general alkylation procedure, starting from 2 g of N^3-benzoyluracil.

	$C_{12}H_{10}N_2O_3$ M = 230.22 g.mol^{-1} 99% (2.12 g) White solid

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.98 – 7.88 (m, 2H, **CH_{Ar}**), 7.70 – 7.60 (m, 1H, **CH_{Ar}**), 7.54 – 7.46 (m, 2H, **CH_{Ar}**), 7.26 (d, $^3J_{HH}$ = 7.9 Hz, 1H, **^{6}CH**), 5.79 (d, $^3J_{HH}$ = 7.9 Hz, 1H, **^{5}CH**), 3.40 (s, 3H, **$^{12}CH_3$**).

N^1-ethyl-N^3-benzoyluracil 196b:

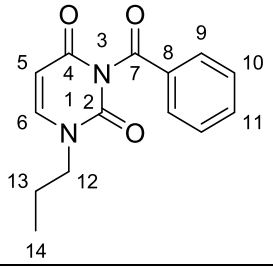
The synthesis of N^1-ethyl-N^3-benzoyluracil was performed according to the general alkylation procedure, starting from 625 mg of N^3-benzoyluracil.

	$C_{13}H_{12}N_2O_3$ M = 244.08 g.mol^{-1} 97% (683 mg) Yellow solid

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.98 – 7.78 (m, 2H, **CH_{Ar}**), 7.63 – 7.47 (m, 1H, **CH_{Ar}**), 7.47 – 7.35 (m, 2H, **CH_{Ar}**), 7.21 (d, $^3J_{HH}$ = 7.9 Hz, 1H, **^{6}CH**), 5.72 (d, $^3J_{HH}$ = 7.9 Hz, 1H, **^{5}CH**), 3.74 (q, $^3J_{HH}$ = 7.2 Hz, 2H, **$^{12}CH_2$**), 1.26 (t, $^3J_{HH}$ = 7.2 Hz, 3H, **$^{13}CH_3$**).

N^1-propyl-N^3-benzoyluracil 196c:

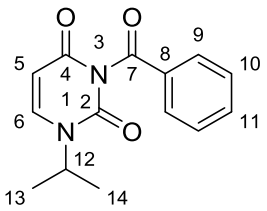
The synthesis of N^1-propyl-N^3-benzoyluracil was performed according to the general alkylation procedure, starting from 1.0 g of N^3-benzoyluracil.

	$C_{14}H_{14}N_2O_3$ M = 258.28 g.mol^{-1} 89% (1.23 g) Pale yellow solid

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.97 – 7.90 (m, 2H, **CH_{Ar}**), 7.69 – 7.62 (m, 1H, **CH_{Ar}**), 7.55 – 7.47 (m, 2H, **CH_{Ar}**), 7.26 (d, $^3J_{HH}$ = 8.0 Hz, 1H, **^{6}CH**), 5.82 (d, $^3J_{HH}$ = 8.0 Hz, 1H, **^{5}CH**), 3.76 – 3.71 (m, 2H, **$^{12}CH_2$**), 1.77 (h, $^3J_{HH}$ = 7.4 Hz, 2H, **$^{13}CH_2$**), 0.99 (t, $^3J_{HH}$ = 7.4 Hz, 3H, **$^{14}CH_3$**).

N^1-isopropyl-N^3-benzoyluracil 196d:

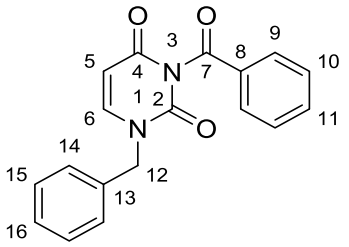
The synthesis of N^1-isopropyl-N^3-benzoyluracil was performed according to the general alkylation procedure, starting from 1.0 g of N^3-benzoyluracil. The product was purified by flash column chromatography with dichloromethane/methanol (80/20).

	$C_{14}H_{14}N_2O_3$ M = 258.28 g.mol^{-1} 29% (334 mg) Pale yellow solid

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.98 – 7.87 (m, 2H, **CH_{Ar}**), 7.72 – 7.59 (m, 1H, **CH_{Ar}**), 7.55 – 7.43 (m, 2H, **CH_{Ar}**), 7.34 (d, $^3J_{HH}$ = 8.2 Hz, 1H, **^{6}CH**), 5.87 (d, $^3J_{HH}$ = 8.1 Hz, 1H, **^{5}CH**), 4.86 (hept, $^3J_{HH}$ = 6.9 Hz, 1H, **^{12}CH**), 1.39 (d, $^3J_{HH}$ = 6.9 Hz, 6H, **$^{13}CH_3$**, **$^{14}CH_3$**).

N^1-benzyl-N^3-benzoyluracil 196e:

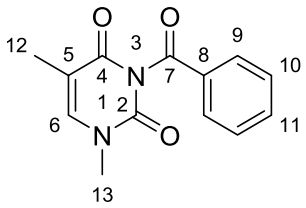
The synthesis of N^1-benzyl-N^3-benzoyluracil was performed according to the general alkylation procedure, starting from 1.0 g of N^3-benzoyluracil. The product was purified by a flash column chromatography with ethyl acetate/dichloromethane (0/100 to 30/70).

	$C_{18}H_{14}N_2O_3$ M = 306.32 g.mol^{-1} 98 % (1.39 g) Yellow oil

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (m, 2H, **CH$_{Ar}$**), 7.67 (m, 1H, **CH$_{Ar}$**), 7.51 (m, 2H, **CH$_{Ar}$**), 7.44 – 7.30 (m, 5H, **CH$_{Ar}$**), 7.27 (d, $^3J_{HH}$ = 8.0 Hz, 1H, **^{6}CH**), 5.79 (d, $^3J_{HH}$ = 8.0 Hz, 1H, **^{5}CH**), 4.93 (s, $^3J_{HH}$ = 9.2 Hz, 2H, **^{12}CH**).

***N*^{1}-methyl-*N*^{3}-benzoylthymine 196f:**

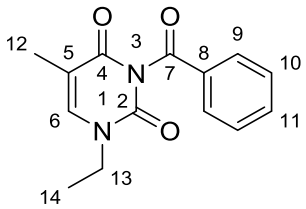
The synthesis of *N*^{1}-methyl-*N*^{3}-benzoylthymine was performed according to the general alkylation procedure, starting from 1.0 g of *N*^{3}-benzoylthymine.

	$C_{13}H_{12}N_2O_3$ M = 244.25 g.mol^{-1} 86% (777 mg) White solid

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.97 – 7.88 (m, 2H, **CH$_{Ar}$**), 7.68 – 7.60 (m, 1H, **CH$_{Ar}$**), 7.52 – 7.47 (m, 2H, **CH$_{Ar}$**), 7.11 (q, $^4J_{HH}$ = 1.2 Hz, 1H, **^{6}CH**), 3.38 (s, 3H, **^{13}CH$_3$**), 1.96 (d, $^4J_{HH}$ = 1.2 Hz, 3H, **^{12}CH$_3$**).

***N*^{1}-ethyl-*N*^{3}-benzoylthymine 196g:**

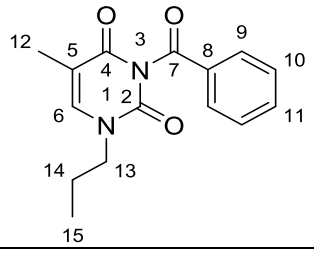
The synthesis of *N*^{1}-ethyl-*N*^{3}-benzoylthymine was performed according to the general alkylation procedure, starting from 1.0 g of *N*^{3}-benzoylthymine.

	$C_{14}H_{15}N_2O_3$ M = 258.28 g.mol^{-1} 75% (891 mg) Pale yellow solid

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.98 – 7.85 (m, 2H, **CH$_{Ar}$**), 7.71 – 7.57 (m, 1H, **CH$_{Ar}$**), 7.54 – 7.45 (m, 2H, **CH$_{Ar}$**), 7.11 (q, $^4J_{HH}$ = 1.0 Hz, 1H, **^{6}CH**), 3.81 (q, $^3J_{HH}$ = 7.2 Hz, 2H, **^{13}CH$_2$**), 1.97 (d, $^4J_{HH}$ = 1.0 Hz, 3H, **^{12}CH$_3$**), 1.34 (t, $^3J_{HH}$ = 7.2 Hz, 3H, **^{14}CH$_3$**).

N^1-propyl-N^3-benzoylthymine 196h:

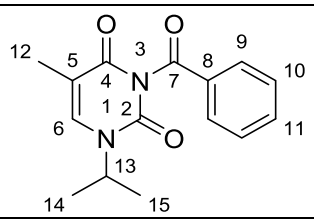
The synthesis of N^1-propyl-N^3-benzoylthymine was performed according to the general alkylation procedure, starting from 1.0 g of N^3-benzoylthymine.

	$C_{15}H_{16}N_2O_3$ M = 272.30 g.mol^{-1} 89% (1.04 g) Pale yellow solid

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.96 – 7.88 (m, 2H, **CH_{Ar}**), 7.69 – 7.60 (m, 1H, **CH_{Ar}**), 7.53 – 7.45 (m, 2H, **CH_{Ar}**), 7.10 (q, $^4J_{HH}$ = 1.2 Hz, 1H, **^6CH**), 3.70 (t, $^3J_{HH}$ = 7.3 Hz, 2H, **$^{13}CH_2$**), 1.96 (d, $^4J_{HH}$ = 1.2 Hz, 3H, **$^{12}CH_3$**), 1.74 (h, $^3J_{HH}$ = 7.4 Hz, 2H, **$^{14}CH_2$**), 0.97 (t, $^3J_{HH}$ = 7.4 Hz, 3H, **$^{15}CH_3$**).

N^1-isopropyl-N^3-benzoylthymine 196i:

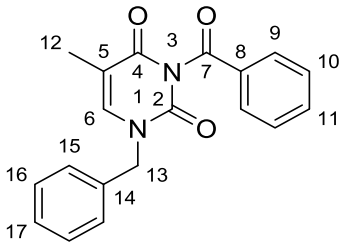
The synthesis of N^1-isopropyl-N^3-benzoylthymine was performed according to the general alkylation procedure, starting from 2.0 g of N^3-benzoylthymine. The product was purified by a flash column chromatography with ethyl acetate/heptane (75/25 to 100/0).

	$C_{15}H_{16}N_2O_3$ M = 272.30 g.mol^{-1} 40% (585 mg) Yellow oil solidifying

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.96 – 7.88 (m, 2H, **CH_{Ar}**), 7.69 – 7.61 (m, 1H, **CH_{Ar}**), 7.55 – 7.45 (m, 2H, **CH_{Ar}**), 7.15 (d, $^4J_{HH}$ = 1.2 Hz, 1H, **^6CH**), 4.86 (hept, $^3J_{HH}$ = 6.8 Hz, 1H, **^{13}CH**), 1.99 (d, $^4J_{HH}$ = 1.2 Hz, 3H, **$^{12}CH_3$**), 1.37 (d, $^4J_{HH}$ = 6.8 Hz, 6H, **$^{14}CH_3$**, **$^{15}CH_3$**).

N^1-benzyl-N^3-benzoylthymine 196j:

The synthesis of N^1-benzyl-N^3-benzoylthymine was performed according to the general alkylation procedure, starting from 3.0 g of N^3-benzoylthymine. The product was purified by a flash column chromatography with dichloromethane/ethyl acetate (100/0 to 70/30).

	$C_{19}H_{16}N_2O_3$ M = 320.35 g.mol^{-1} 97% (4.04 g) White solid

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.97 – 7.92 (m, 2H, **CH_{Ar}**), 7.68 – 7.63 (m, 1H, **CH_{Ar}**), 7.54 – 7.47 (m, 2H, **CH_{Ar}**), 7.41 – 7.30 (m, 5H, **CH_{Ar}**), 7.10 (d, $^4J_{HH}$ = 1.2 Hz, 1H, **^{6}CH**), 4.92 (s, 2H, **$^{13}CH_2$**), 1.93 (d, $^4J_{HH}$ = 1.2 Hz, 3H, **$^{12}CH_3$**).

Deprotection of *N*^{1}-alkyl-*N*^{3}-benzoyluracil and *N*^{1}-alkyl-*N*^{3}-benzoylthymine:

Procedure A: ammonia in methanol

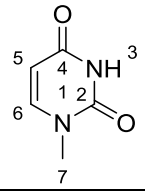
N^1-alkyl-*N*^{3}-benzoyluracil or *N*^{1}-alkyl-*N*^{3}-benzoylthymine was stirred as a solution in methanol (0.031 mol.L^{-1}) with NH_4OH (25 % w/w in water, 10 eq) for 4 h to 6 h at room temperature. Ammonium benzoate precipitated and was filtered and washed with dichloromethane. The filtrate was introduced into a separatory funnel in order to recover the organic phase. The aqueous phase was washed with dichloromethane. The organic layers were gathered together and were dried on $MgSO_4$. The solvent evaporated under vacuum. The crude was then submitted to flash column chromatography in order to obtain the pure product.

Procedure B: diethylamine in methanol

N^1-alkyl-*N*^{3}-benzoyluracil or *N*^{1}-alkyl-*N*^{3}-benzoylthymine was stirred as a solution in methanol (0.37 mol.L^{-1}) at room temperature. A solution of diethylamine (2 M in THF, 2 eq) was added. The medium was stirred for 4-6 h. The precipitate of *N*^{1}-alkyluracil or *N*^{1}-alkylthymine which appeared was filtered off the solution and eventually purified by flash column chromatography.

N^1-methyluracil 197a:

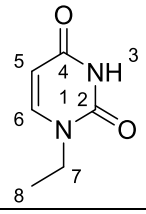
The synthesis of *N*^{1}-methyluracil was performed according to procedure A, starting from 500 mg of *N*^{1}-methyl-*N*^{3}-benzoyluracil. The product was purified by a flash column chromatography with ethyl acetate/heptane (20/80 to 100/0).

	$C_5H_6N_2O_2$ M = 126.12 g.mol^{-1} 78% (260 mg) White solid

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 7.54 (d, $^3J_{HH}$ = 7.8 Hz, 1H, **^{6}CH**), 5.62 (d, $^3J_{HH}$ = 7.8 Hz, 1H, **^{5}CH**), 3.33 (s, 3H, **$^7CH_3$**).

N^1-ethyluracil 197b:

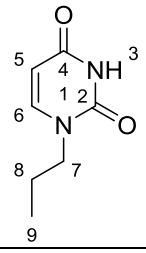
The synthesis of N^1-ethyluracil was performed according to procedure A, starting from 500 mg of N^1-ethyl-N^3-benzoyluracil. The product was purified by a flash column chromatography with pure ethyl acetate.

	$C_6H_8N_2O_2$ M = 140.14 g.mol^{-1} 25% (72 mg) White solid

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.18 (d, $^3J_{HH}$ = 7.9 Hz, 1H, **^{6}CH**), 5.72 (d, $^3J_{HH}$ = 7.9 Hz, 1H, **^{5}CH**), 3.80 (q, $^3J_{HH}$ = 7.2 Hz, 2H, **^{7}CH$_2$**), 1.32 (t, $^3J_{HH}$ = 7.2 Hz, 3H, **^{8}CH$_3$**).

N^1-propyluracil 197c:

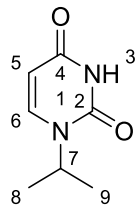
The synthesis of N^1-propyluracil was performed according to procedure A, starting from 475 mg of N^1-propyl-N^3-benzoyluracil. The product was purified by a flash column chromatography ethyl acetate/heptane (60/40 to 100/0).

	$C_7H_{10}N_2O_2$ M = 154.17 g.mol^{-1} 77% (219 mg) White solid

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 7.59 (d, $^3J_{HH}$ = 7.8 Hz, 1H, **^{6}CH**), 5.64 (d, $^3J_{HH}$ = 7.8 Hz, 1H, **^{5}CH**), 3.74 – 3.66 (m, 2H, **^{7}CH$_2$**), 1.70 (h, $^3J_{HH}$ = 7.3 Hz, 2H, **^{8}CH$_2$**), 0.94 (t, $^3J_{HH}$ = 7.4 Hz, 3H, **^{9}CH$_3$**).

N^1-isopropyluracil 197d:

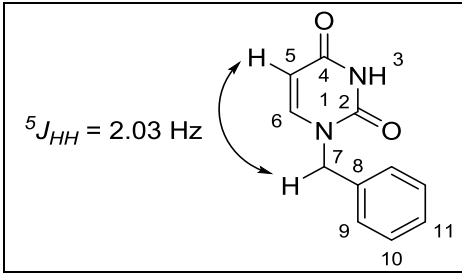
The synthesis of N^1-isopropyluracil was performed according to procedure B, starting from 681 mg of N^1-isopropyl-N^3-benzoyluracil. The product was purified by a flash column chromatography ethyl acetate/heptane (50/50 to 100/0).

	$C_7H_{10}N_2O_2$ M = 154.17 g.mol^{-1} 93% (377 mg) White solid

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.24 (d, $^{3}J_{HH}$ = 8.1 Hz, 1H, **^{6}CH**), 5.75 (dd, $^{3}J_{HH}$ = 8.0, $^{5}J_{HH}$ = 2.4 Hz, 1H, **^{5}CH**), 4.87 (hept, $^{3}J_{HH}$ = 6.8 Hz, 1H, **^{7}CH**), 1.34 (d, $^{3}J_{HH}$ = 6.9 Hz, 6H, **$^{8}CH_3$**, **$^{9}CH_3$**).

***N*^{1}-benzyluracil 197e:**

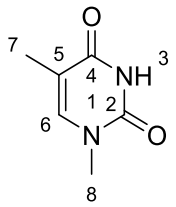
The synthesis of *N*^{1}-benzyluracil was performed according to procedure B, starting from 2.69 g of *N*^{1}-benzyl-*N*^{3}-benzoyluracil.

	$C_{11}H_{10}N_2O_2$ M = 202.21 g.mol^{-1} 67% (1.19 g) White solid

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.42 – 7.28 (m, 5H, **CH_{Ar}**), 7.17 (d, $^{3}J_{HH}$ = 7.9 Hz, 1H, **^{6}CH**), 5.70 (dd, $^{3}J_{HH}$ = 7.9 Hz, $^{5}J_{HH}$ = 2.0 Hz, 1H, **^{5}CH**), 4.93 (s, 2H, **$^{7}CH_2$**).

***N*^{1}-methylthymine 197f:**

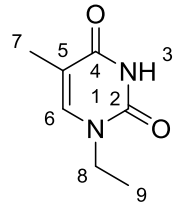
The synthesis of *N*^{1}-methylthymine was performed according to procedure A, starting from 2.75 g of *N*^{1}-methyl-*N*^{3}-benzoylthymine. The product was purified by a flash column chromatography ethyl acetate/heptane (80/20 to 100/0).

	$C_6H_8N_2O_2$ M = 140.14 g.mol^{-1} 84% (1.33 g) White solid

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.00 (q, $^{4}J_{HH}$ = 1.1 Hz, 1H, **^{6}CH**), 3.36 (s, 3H, **$^{8}CH_3$**), 1.93 (d, $^{4}J_{HH}$ = 1.2 Hz, 3H, **$^{7}CH_3$**).

***N*^{1}-ethylthymine 197g:**

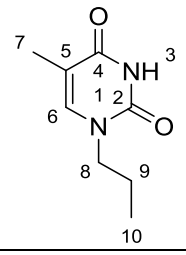
The synthesis of *N*^{1}-ethylthymine was performed according to procedure A, starting from 805 mg of *N*^{1}-ethyl-*N*^{3}-benzoylthymine. The product was purified by a flash column chromatography ethyl acetate/heptane (80/20 to 100/0).

	$C_7H_{10}N_2O_2$ M = 154.17 g.mol^{-1} 75% (357 mg) White solid

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 7.45 (q, $^4J_{HH}$ = 1.1 Hz, 1H, **^{6}CH**), 3.76 (q, $^3J_{HH}$ = 7.2 Hz, 2H, **^{8}CH$_2$**), 1.87 (d, $^4J_{HH}$ = 1.2 Hz, 3H, **^{7}CH$_3$**), 1.26 (t, $^3J_{HH}$ = 7.2 Hz, 3H, **^{9}CH$_3$**).

***N^1*-propylthymine 197h:**

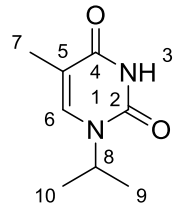
The synthesis of *N^1*-propylthymine was performed according to procedure A, starting from 1 g of *N^1*-propyl-*N^3*-benzoylthymine. The product was purified by a flash column chromatography ethyl acetate/heptane (60/40 to 100/0).

	$C_8H_{12}N_2O_2$ M = 168.20 g.mol^{-1} 17% (106 mg) White solid

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 7.45 (q, $^4J_{HH}$ = 1.1 Hz, 1H, **^{6}CH**), 3.71 – 3.67 (m, 2H, **^{8}CH$_2$**), 1.89 (d, $^4J_{HH}$ = 1.2 Hz, 3H, **^{7}CH$_3$**), 1.71 (h, $^3J_{HH}$ = 7.4 Hz, 2H, **^{9}CH$_2$**), 0.96 (t, $^3J_{HH}$ = 7.4 Hz, 3H, **^{10}CH$_3$**).

***N^1*-isopropylthymine 197i:**

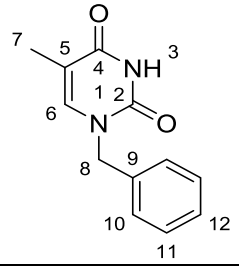
The synthesis of *N^1*-isopropylthymine was performed according to procedure B, starting from 292 mg of *N^1*-isopropyl-*N^3*-benzoylthymine.

	$C_8H_{12}N_2O_2$ M = 168.20 g.mol^{-1} 69% (124 mg) White solid

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.04 (q, $^4J_{HH}$ = 1.0 Hz, 1H, **^{6}CH**), 4.85 (hept, $^3J_{HH}$ = 6.8 Hz, 1H, **^{8}CH**), 1.95 (d, $^4J_{HH}$ = 1.2 Hz, 3H, **^{7}CH$_3$**), 1.33 (d, $^3J_{HH}$ = 6.9 Hz, 6H, **^{9}CH$_3$**, **^{10}CH$_3$**).

N^1-benzylthymine 197j:

The synthesis of N^1-benzylthymine was performed according to procedure B, starting from 2.05 g of N^1-benzyl-N^3-benzoylthymine.

	$C_{12}H_{12}N_2O_2$ M = 216.24 g.mol^{-1} 43% (597 mg) White solid

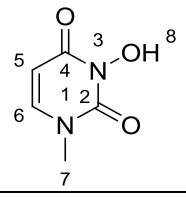
^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.43 – 7.28 (m, 5H, **CH_{Ar}**), 6.99 (q, $^4J_{HH}$ = 1.1 Hz, 1H, **^{6}CH**), 4.90 (s, 2H, **$^8CH_2$**), 1.89 (d, $^4J_{HH}$ = 1.2 Hz, 3H, **$^7CH_3$**).

General procedure for the oxidation of N^3

To a solution of N^1-alkyluracil or N^1-alkylthymine (1 eq) in anhydrous THF (0.091 M) was added NaH (10 eq, 60 % dispersion in mineral oil) at 0 °C. This reaction mixture was allowed to warm to room temperature over 1 h, and then cooled to 0 °C followed by the addition of solid *m*-CPBA (5 eq). The reaction mixture was stirred overnight at room temperature. Then the reaction was quenched by adding distilled water, the aqueous phase was acidified with 1 N HCl to pH = 7, and then extracted 3 times with ethyl acetate. The combined organic extracts were dried over $MgSO_4$ and concentrated under reduced pressure. The solid obtained was grinded in methanol and filtered. The filtrate was evaporated and the process was done one or two more times to get rid of the sodium chloride. No yields could be calculated.

N^3-hydroxy-N^1-methyluracil 192a:

The synthesis of N^3-hydroxy-N^1-methyluracil was performed according to the general procedure for N^3 oxidation, starting from 119 mg of N^1-methyluracil.

	$C_5H_6N_2O_3$ M = 142.11 g.mol^{-1} 138 mg White solid

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 7.60 (d, $^3J_{HH}$ = 7.7 Hz, 1H, **^{6}CH**), 5.79 (d, $^3J_{HH}$ = 7.7 Hz, 1H, **^{5}CH**), 3.45 (s, 3H, **$^7CH_3$**).

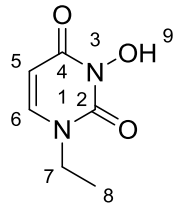
^{13}C APT NMR (101 MHz, CD_3OD) δ (ppm): 162.87 (s, **^{4}C**), 152.17 (s, **^{2}C**), 145.12 (s, **^{6}CH**), 100.86 (s, **^{5}CH**), 37.32 (s, **^{7}CH$_3$**).

MS (ES+) *m/z* = 143.1 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_5H_7N_2O_3$ 143.0457, found 143.0488.

***N^3*-hydroxy-*N^1*-ethyluracil 192b:**

The synthesis of *N^3*-hydroxy-*N^1*-ethyluracil was performed according to the general procedure for *N^3* oxidation, starting from 72 mg of *N^1*-ethyluracil.

	$C_6H_8N_2O_3$ M = 156.14 g.mol^{-1} 94 mg Yellow oil

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 7.63 (d, $^3J_{HH}$ = 8.0 Hz, 1H, **^{6}CH**), 5.81 (d, $^3J_{HH}$ = 8.0 Hz, 1H, **^{5}CH**), 3.90 (q, $^3J_{HH}$ = 7.2 Hz, 2H, **^{7}CH$_2$**), 1.32 (t, $^3J_{HH}$ = 7.2 Hz, 3H, **^{8}CH$_3$**).

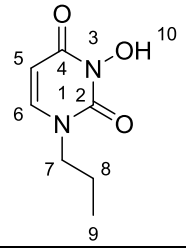
^{13}C APT NMR (101 MHz, CD_3OD) δ (ppm): 162.78 (s, **^{4}C**), 151.69 (s, **^{2}C**), 144.20 (s, **^{6}CH**), 101.32 (s, **^{5}CH**), 46.26 (s, **^{7}CH$_2$**), 14.54 (s, **^{8}CH$_3$**).

MS (ES+) *m/ z*= 157.1 ($M+H^+$), MS (ES^-) m/z = 155.1 ($M-H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_6H_9N_2O_3$ 157.0613, found 157.0630

***N^3*-hydroxy-*N^1*-propyluracil 192c:**

The synthesis of *N^3*-hydroxy-*N^1*-propyluracil was performed according to the general procedure for *N^3* oxidation, starting from 219 mg of *N^1*-propyluracil.

	$C_7H_{10}N_2O_3$ M = 170.17 g.mol^{-1} 163 mg Pale yellow solid

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 7.53 (d, $^3J_{HH}$ = 7.9 Hz, 1H, **^{6}CH**), 5.70 (d, $^3J_{HH}$ = 7.9 Hz, 1H, **^{5}CH**), 3.71 (t, $^3J_{HH}$ = 7.2 Hz, 2H, **^{7}CH$_2$**), 1.64 (h, $^3J_{HH}$ = 7.1 Hz, 2H, **^{8}CH$_2$**), 0.85 (t, $^3J_{HH}$ = 7.4 Hz, 3H, **^{9}CH$_3$**).

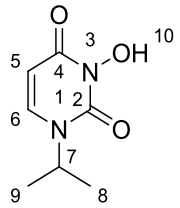
^{13}C CPD NMR (101 MHz, CD_3OD) δ (ppm): 162.70 (s, **^{4}C**), 151.87 (s, **^{2}C**), 144.46 (s, **^{6}CH**), 101.02 (s, **^{5}CH**), 52.41 (s, **$^{7}CH_2$**), 23.20 (s, **$^{8}CH_2$**), 11.09 (s, **$^{9}CH_3$**).

MS (ES+) *m/z* = 171.2 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_7H_{11}N_2O_3$ 171.0770, found 171.0763.

***N^3*-hydroxy-*N^1*-isopropyluracil 192d:**

The synthesis of *N^3*-hydroxy-*N^1*-isopropyluracil was performed according to the general procedure for *N^3* oxidation, starting from 200 mg of *N^1*-isopropyluracil.

	$C_7H_{10}N_2O_3$ M = 170.17 g.mol^{-1} 124 mg White solid

^{1}H NMR (400 MHz, methanol-*d4*) δ (ppm): 7.56 (d, $^{3}J_{HH}$ = 8.1 Hz, 1H, **^{6}CH**), 5.72 (d, $^{3}J_{HH}$ = 8.1 Hz, 1H, **^{5}CH**), 4.71 (hept, $^{3}J_{HH}$ = 6.8 Hz, 1H, **^{7}CH**), 1.27 (d, $^{3}J_{HH}$ = 6.8 Hz, 6H, **$^{8}CH_3$, $^{9}CH_3$**).

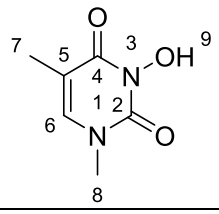
^{13}C APT NMR (101 MHz, D_2O) δ (ppm): 162.61 (s, **^{4}C**), 151.37 (s, **^{2}C**), 140.20 (s, **^{6}CH**), 100.55 (s, **^{5}CH**), 50.11 (s, **^{7}CH**), 20.20 (s, **$^{8}CH_3$**, **$^{9}CH_3$**).

MS (ES+) *m/z* = 171.08 ($M+H^+$) and 193.06 ($M+Na^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_7H_{11}N_2O_3$ 171.0770, found 171.0768.

***N^3*-hydroxy-*N^1*-methylthymine 193a:**

The synthesis of *N^3*-hydroxy-*N^1*-methylthymine was performed according to the general procedure for *N^3* oxidation, starting from 500 mg of *N^1*-methylthymine.

	$C_6H_8N_2O_3$ M = 156.14 g.mol^{-1} 726 mg White solid

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 7.45 (q, $^{4}J_{HH}$ = 1.2 Hz, 1H, **^{6}CH**), 3.42 (s, 3H, **$^{8}CH_3$**), 1.95 (d, $^{4}J_{HH}$ = 1.2 Hz, 3H, **$^{7}CH_3$**).

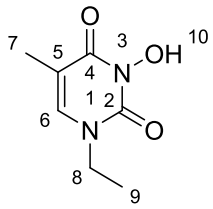
^{13}C APT NMR (101 MHz, CD_3OD) δ (ppm): 163.30 (s, **^{4}C**), 151.97 (s, **^{2}C**), 141.11 (s, **^{5}C**), 109.90 (s, **^{6}CH**), 36.95 (s, **$^{8}CH_3$**), 12.71 (s, **$^{7}CH_3$**).

MS (ES+): m/z = 157.1 ($M+H^+$), 179.1 ($M+Na^+$)

HRMS (ES+): m/z: [M+H] calculated for $C_6H_9N_2O_3$ 157.0613, found 157.0686.

N^3-hydroxy-N^1-ethylthymine 193b:

The synthesis of N^3-hydroxy-N^1-ethylthymine was performed according to the general procedure for N^3 oxidation, starting from 157 mg of N^1-ethylthymine.

	$C_7H_{10}N_2O_3$ M = 170.17 g.mol^{-1} 100 mg White solid

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 7.48 (d, $^4J_{HH}$ = 1.2 Hz, 1H, **^{6}CH**), 3.86 (q, $^3J_{HH}$ = 7.2 Hz, 2H, **$^8CH_2$**), 1.96 (d, $^4J_{HH}$ = 1.2 Hz, 3H, **$^7CH_3$**), 1.31 (t, $^3J_{HH}$ = 7.2 Hz, 3H, **$^9CH_3$**).

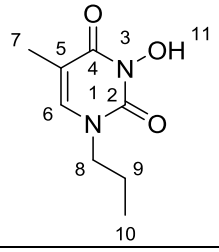
^{13}C APT NMR (101 MHz, CD_3OD) δ (ppm): 163.19 (s, **^{4}C**), 151.45 (s, **^{2}C**), 140.09 (s, **^{6}CH**), 110.31 (s, **^{5}C**), 45.89 (s, **$^8CH_2$**), 14.55 (s, **$^9CH_3$**), 12.80 (s, **$^7CH_3$**).

MS (ES+) *m/z* = 171.2 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_7H_{11}N_2O_3$ 171.0770, found 171.0755.

N^3-hydroxy-N^1-propylthymine 193c:

The synthesis of N^3-hydroxy-N^1-propylthymine was performed according to the general procedure for N^3 oxidation, starting from 106 mg of N^1-propylthymine.

	$C_8H_{12}N_2O_3$ M = 184.20 g.mol^{-1} 151 mg White solid

^{1}H NMR (400 MHz, CD_3OD) δ (ppm): 7.51 (d, $^4J_{HH}$ = 1.2 Hz, 1H, **^{6}CH**), 3.79 (t, $^3J_{HH}$ = 7.2 Hz, 2H, **$^8CH_2$**), 1.95 (d, $^4J_{HH}$ = 1.2 Hz, 3H, **$^7CH_3$**), 1.74 (h, $^3J_{HH}$ = 7.4 Hz, 2H, **$^9CH_2$**), 0.95 (t, $^3J_{HH}$ = 7.4 Hz, 3H, **$^{10}CH_3$**).

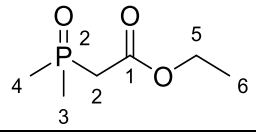
^{13}C APT NMR (101 MHz, CD_3OD) δ (ppm): 163.15 (s, **^{4}C**), 151.68 (s, **^{2}C**), 140.46 (s, **^{6}CH**), 110.06 (s, **^{5}C**), 52.10 (s, **$^8CH_2$**), 23.25 (s, **$^9CH_2$**), 12.77 (s, **$^{10}CH_3$**), 11.12 (s, **$^7CH_3$**).

MS (ES+) *m/z* = 185.2 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_8H_{13}N_2O_3$ 185.0926, found 185.0913.

Ethyl 2-dimethylphosphorylacetate 210:

In a two necked 25 mL round bottom flask equipped with a condenser dimethylphosphine oxide (400 mg, 4.76 mmol, 1 eq) was dissolved under N_2 in DMF (10 mL). Then caesium carbonate (1.86 g, 5.72 mmol, 1.2 eq) was added in one portion and ethyl bromoacetate (620 µL, 5.72 mmol, 1.2 eq) was introduced portionwise with a syringe. The medium was stirred 1 day at 80 °C and the conversion controlled by ^{31}P CPD NMR. After completion, the crude was filtered through a celite pad and the cake was washed with ethyl acetate. The filtrate was evaporated under vacuum. The crude oil was fractionally distilled with a Kugelrohr apparatus at ambient pressure. 555 mg (71% yield) of the product was obtained as a colorless oil between 145 °C and 149 °C.

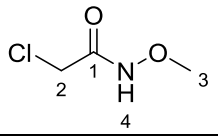
	$C_6H_{16}O_3P$ M = 164.14 g.mol^{-1} 71%

^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.54 (d, $^2J_{PH}$ = 11.6 Hz, 2H, **$^2CH_2$**), 4.20 (q, $^3J_{HH}$ = 7.0 Hz, 2H, **$^5CH_2$**), 1.56 (d, $^2J_{PH}$ = 14.1 Hz, 6H, **$^3CH_3$, $^4CH_3$**), 1.26 (t, $^3J_{HH}$ = 6.8 Hz, 3H, **$^6CH_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 57.27 (s).

2-Chloro-*O*-methylacetohydroxamate 216:

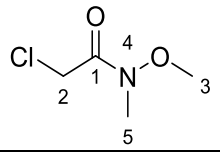
In a two necked 250 mL flask equipped with a condenser and a 100 mL addition funnel, chloroacetyl chloride (3.6 mL, 45.3 mmol, 1 eq) was dissolved in THF (30 mL) at 0 °C. A solution of *O*-methylhydroxylamine hydrochloride (3.75 g, 45 mmol, 1 eq) and sodium hydrogenocarbonate (7.5 g, 90 mmol, 2 eq) in water (60 mL) was added portionwise with the addition funnel to the medium at 0 °C. The reaction was let to stir and heat up to room temperature overnight. The completion was checked by TLC with pure ethyl acetate as eluent. The medium was extracted three times with ethyl acetate. The organic phase was dried with $MgSO_4$ and evaporated under vacuum leading to 2.21 g of a colorless and unpure oil. The oil was purified by Kugelrohr distillation (7 mbar, 100 °C), giving 2.04 g of pure product, in 36% yield.

	$C_3H_6ClNO_2$ M = 123.54 g.mol^{-1} 36%

^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.12 (s, 2H, **$^2CH_2$**), 3.81 (s, 3H, **$^3CH_3$**).

2-Chloro-*N*,*O*-dimethylacetohydroxamate 222:

In a two necked 250 mL flask equipped with a condenser and a 100 mL addition funnel, chloroacetyl chloride (3.6 mL, 45.3 mmol, 1 eq) was dissolved in THF (30 mL) at 0 °C. A solution of *N*,*O*-dimethylhydroxylamine hydrochloride (4.41 g, 45 mmol, 1 eq) and sodium hydrogenocarbonate (7.5 g, 90 mmol, 2 eq) in water (60 mL) was added portionwise with the addition funnel to the medium at 0 °C. The reaction was let to stir and heat up to room temperature overnight. The completion was checked by TLC with pure ethyl acetate as eluent. The medium was extracted using dichloromethane. The organic phase was dried with $MgSO_4$ and evaporated under vacuum. No additional purification was needed, leading to 4.92 g of pure product, in 79% yield, as a slightly yellow oil.

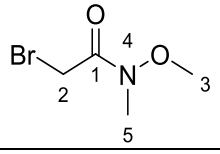
	$C_4H_8ClNO_2$ M = 137.56 g.mol^{-1} 79%

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.24 (s, 2H, **$^2CH_2$**), 3.73 (s, 3H, **$^3CH_3$**), 3.21 (s, 3H, **$^5CH_3$**).

2-Bromo-*N*,*O*-dimethylacetohydroxamate 223:

In a two necked 50 mL flask equipped with a condenser, and previously dried with a heat gun and inerted with N_2, *N,O*-dimethylhydroxylamine hydrochloride (2 g, 20.4 mmol, 1 eq) and potassium carbonate (6.24 g, 45.1 mmol, 2.2 eq) were introduced with acetonitrile (50 mL). Then bromoacetyl bromide (2.1 mL, 24.6 mmol, 1.2 eq) was added portionwise in the medium which after the addition was stirred overnight at room temperature. The completion of the reaction was controlled by GC-MS analysis. After evaporation of the solvent under vacuum, the crude was dissolved in 45 mL of distilled water followed by three extractions, with 45 mL of dichloromethane each. The organic phase was dried with $MgSO_4$ and evaporated under vacuum. The oil obtained was purified by a Kugelrohr distillation at 30 °C and 6 mbar leading to 1.63 g (44% yield) of the pure product as a yellow oil.

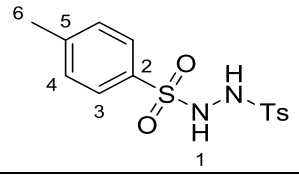
2-chloro-*N,O*-dimethylacetohydroxamate can be observed during the reaction because of a Finkelstein reaction with the side product KCl.

	$C_4H_8BrNO_2$ M = 182.02 g.mol^{-1} 44%

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.03 (br s, 2H, **$^2CH_2$**), 3.80 (s, 3H, **$^3CH_3$**), 3.25 (s, 3H, **$^5CH_3$**).

***N,N'*-ditosylhydrazine 230:**

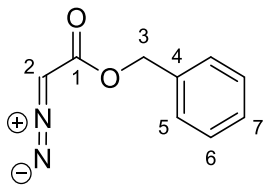
A dried 500 mL flask flushed with N_2 was charged with *p*-toluenesulfonyl hydrazide (5.0 g, 26.8 mmol, 1 eq) and *p*-toluenesulfonyl chloride (7.7 g, 40.2 mmol, 1.5 eq) in dichloromethane (27 mL). The suspension was stirred at room temperature while pyridine (3.2 mL, 40.2 mmol, 1.5 eq) was added dropwise over 1 min. During the addition, the reaction mixture became homogeneous and turned yellow. A white precipitate was observed and the reaction mixture was stirred for 1.5 h. Diethyl ether (107 mL) and distilled water (54 mL) were added and stirred at 0 °C for 15 min. The white solid which precipitated was collected by a Büchner filtration and washed with diethyl ether. The solid thus obtained was dissolved in boiling MeOH (215 mL). After cooling to the room temperature, a precipitate appeared. About 100mL of MeOH was removed by evaporation under vacuum and cooled to 0 °C. The precipitate was collected by filtration and washed with cold MeOH to give 6.02 g (66% yield) of *N,N*'-ditosylhydrazine as a beige solid.

	$C_{14}H_{16}N_2O_4S_2$ M = 340.41 g.mol^{-1} 66%

^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ (ppm): 9.58 (s, 2H, **NH**), 7.64 (d, $^3J_{HH}$ = 8.2 Hz, 4H, $^{\mathbf{3}}$**CH**), 7.38 (d, $^3J_{HH}$ = 8.0 Hz, 4H, $^{\mathbf{4}}$**CH**), 2.39 (s, 6H, $^{\mathbf{6}}$**CH$_3$**).

Benzyl 2-diazoacetate 232:

A dried and N_2 flushed 50 mL three-necked flask was charged with benzyl bromoacetate (1.04 mL, 6.6 mmol, 1.00 eq) and 23 mL of THF. *N,N'*-ditosyl hydrazine (3.37 g, 9.9 mmol, 1.50 eq) was added. The suspension was stirred in an ice bath while DBU (3.9 mL, 26.4 mmol, 4.00 eq) was added dropwise from a syringe over 5 min. During the addition, the reaction mixture became homogenous and turned yellow. The reaction mixture was stirred for 30 min and TLC analysis (Heptane/ ethyl acetate 70/30) showed no starting material. Saturated aqueous sodium hydrogen carbonate solution (10 mL) was added and the reaction mixture was poured into diethyl ether (20 mL) and distilled water (10mL) in a separatory funnel. The layers were separated and the organic solutions were combined, washed with brine, and dried over $MgSO_4$. The solvents are removed by evaporation under vacuum. The crude oil was purified by silica gel column chromatography (Heptane/ ethyl acetate 70/30) giving 732 mg (63 % yield) of benzyl diazoacetate as a yellow oil.

	$C_9H_8N_2O_2$ M = 176.18 g.mol^{-1} 63%

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.42 – 7.32 (m, 5H, **CH$_{Ar}$**), 5.21 (s, 2H, $^{\mathbf{3}}$**CH$_2$**), 4.81 (br s, 1H, $^{\mathbf{2}}$**CH**).

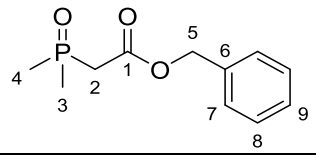
Benzyl 2-dimethylphosphorylacetate 229:

Protocol A:

In a two necked 100 mL round bottom flask equipped with a condenser dimethylphosphine oxide (1 g, 12.8 mmol, 1 eq) was dissolved under N_2 in DMF (25 mL). Then caesium carbonate (6.3 g, 19.2 mmol, 1.5 eq) was added in one portion along with benzyl bromoacetate (2.1 mL, 19.2 mmol, 1.5 eq). The medium was stirred 5 days at 80 °C and the conversion controlled by ^{31}P CPD NMR. After completion, the crude was filtered through a celite pad and the cake washed with ethyl acetate. The filtrate was evaporated under vacuum. The crude oil was purified by column chromatography ethyl acetate/ethanol 90/10 giving 753 mg (26% yield) of the product as a yellow oil.

Protocol B:

In a dried 100 mL round bottom flask, flushed with N_2, was introduced benzyl 2-diazoacetate (1.59 g, 9,0 mmol, 1 eq), dimethylphosphine oxide (772 mg, 9.90 mmol, 1.1 eq) 1,2-dichloroethane (36 mL) and copper bromide (64 mg, 0.45 mmol, 5 mol%).The suspension was stirred overnight at room temperature. After completion of the reaction, checked by ^{31}P CPD NMR, the medium was evaporated under vacuum and directly purified by silicagel column chromatography (Ethyl acetate/ethanol: 90/10 to 80/20) giving 2.03 g of oily product in quantitative yield.

	$C_{11}H_{15}O_3P$ M = 226.21 g.mol^{-1} 26% (protocol A) or quantitative (protocol B)

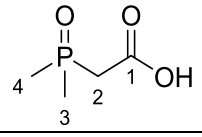
^1H NMR (400 MHz, $CDCl_3$) δ (ppm): 7.40-7.35 (m, 5H, **CH_{Ar}**), 5.19 (s, 2H, **$^5CH_2$**), 3.06 (d, $^2J_{PH}$ = 16.1 Hz, 2H, **$^2CH_2$**), 1.58 (d, $^2J_{PH}$ = 13.1 Hz, 6H, **$^3CH_3$**, **$^4CH_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 39.69 (s).

^{13}C APT NMR (101 MHz, $CDCl_3$) δ (ppm): 166.65 (d, $^2J_{PC}$ = 3.4 Hz, **^1C**), 135.04 (s, **^6C**), 128.70 (s, **^8CH**), 128.69 (s, **^9CH**), 128.66 (s, **^7CH**), 67.45 (s, **$^5CH_2$**), 39.42 (d, $^1J_{PC}$ = 57.8 Hz, **$^2CH_2$**), 16.66 (d, $^1J_{PC}$ = 70.9 Hz, **$^3CH_3$**, **$^4CH_3$**).

2-dimethylphosphorylacetic acid 211:

In a 25 mL flask, benzyl 2-dimethylphosphorylacetate (423 mg, 1.87 mmol, 1 eq) was dissolved in ethanol (5 mL). Palladium on charcoal (10% Pd/C, 199 mg, 0.19 mmol, 0.1 eq) was introduced and the flask was flushed with H_2. The medium was stirred 4h at room temperature. After checking the completion of the reaction by ^{31}P CPD NMR spectroscopy, the suspension was filtered through celite. The solution was evaporated under vacuum leading to 254 mg of white solid whish were the pure 2-dimethylphosphorylacetic acid in quantitative yield.

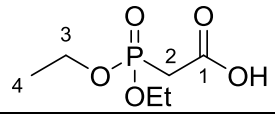
	$C_{11}H_{15}O_3P$ M = 136.09 g.mol^{-1} Quantitative

^{1}H NMR (400 MHz, D_2O) δ (ppm): 3.01 (d, $^3J_{PH}$ = 15.1 Hz, 2H, **^{2}CH$_2$**), 1.59 (d, $^3J_{PH}$ = 13.6 Hz, 6H, **^{3}CH$_3$**, **^{4}CH$_3$**).

^{31}P CPD NMR (162 MHz, D_2O) δ (ppm): 50.00 (s).

2-Diethylphosphonacetic acid 237:

In a 10 mL flask was introduced ethyl 2-diethylphosphonoacetate (500 mg, 2.2 mmol, 1 eq) and a sodium hydroxide solution at 1 M (2.4 mL, 2.4 mmol, 1.1 eq). The medium was stirred at room temperature for 4 h. Then it was acidified to pH = 1 by HCl 4 N. The aqueous solution was extracted by ethyl acetate three times. The organic phase was dried over $MgSO_4$ and evaporated under vacuum leading to 319 mg of colourless oil in 89% yield.

	$C_6H_{13}O_5P$ M = 186.14 g.mol^{-1} 89%

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.62 (s, 1H, **OH**), 4.24 – 4.14 (m, 4H, **^{3}CH$_2$**), 3.00 (d, $^2J_{PH}$ = 21.7 Hz, 2H, **^{2}CH$_2$**), 1.34 (t, $^3J_{HH}$ = 7.1 Hz, 6H, **^{4}CH$_3$**).

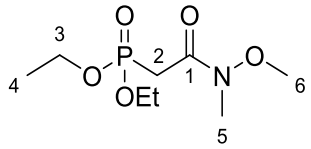
^{31}P CPD NMR (162 MHz $CDCl_3$) δ (ppm): 21.22 (s).

Peptidic coupling conditions with PyBOP:

Triethylamine (1 eq) was introduced dropwise to hydroxylammonium chloride derivative (1 eq) in suspension in CH_2Cl_2 (0.23 M). The neutrality of the pH was verified. A solution of diethyl phosphonoacetic acid (1 eq) in CH_2Cl_2 (0.15 M) and then PyBOP (1 eq) in solution in CH_2Cl_2 (0.46 M) were added to the reaction. Finally, triethylamine (3 eq) diluted in CH_2Cl_2 (1.4 M) were added in order to have pH ≈ 9. After stirring overnight at room temperature, the reaction was diluted with CH_2Cl_2 and washed with an aqueous solution of H_2SO_4 (3 times, 2 M) and a saturated solution of $NaHCO_3$ (3 times, same amount as for H_2SO_4). The organic layer was dried with $MgSO_4$ and concentrated under vacuum. The crude product was purified by silicagel chromatography (AcOEt/EtOH – 80/20).

***N,O*-dimethyl-2-diethylphosphonoacetohydroxamate 240c:**

The synthesis of *N,O*-dimethyl-2-diethylphosphonoacetohydroxamate was performed according to the general procedure for peptidic coupling, starting from 200 mg of 2-diethylphosphonoacetic acid.

	$C_8H_{18}O_5NP$ M = 239.21 g.mol^{-1} 50% (121 mg) Colourless oil

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 4.23 – 4.23 (m, 4H, **^{3}CH$_2$**), 3.78 (s, 3H, **^{6}CH$_3$**), 3.22 (s, 3H, **^{5}CH$_3$**), 3.17 (d, $^2J_{PH}$ = 22.1 Hz, 2H, **^{2}CH$_2$**), 1.35 (t, $^3J_{HH}$ = 7.1 Hz, 6H, **^{4}CH$_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ 21.31 (s).

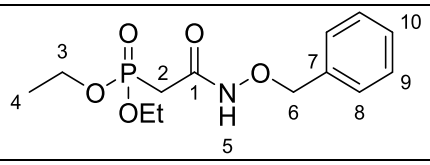
^{13}C APT NMR (101 MHz, $CDCl_3$) δ 166.05 (s, **^{1}C**), 62.03 (d, $^2J_{PC}$ = 6.4 Hz, **^{3}CH$_2$**), 61.49 (s, **^{6}CH$_3$**), 32.16 (s, **^{5}CH$_3$**), 31.41 (d, $^1J_{PC}$ = 136.4 Hz, **^{2}CH$_2$**), 16.32 (d, $^3J_{PC}$ = 6.4 Hz, **^{4}CH$_3$**).

MS (ES+) *m/z* = 240.10 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_8H_{19}NO_5P$ 240.1001, found 240.1003.

***O*-benzyl-2-diethylphosphonoacetohydroxamate 240a:**

The synthesis of *O*-benzyl-2-diethylphosphonoacetohydroxamate was performed according to the general procedure for peptidic coupling, starting from 200 mg of 2-diethylphosphonoacetic acid. This product was purified by a flash column chromatography with ethyl acetate/ethanol (90/10).

	$C_{13}H_{20}O_5NP$ M = 301.28 g.mol^{-1} 65% (162 mg) Colourless solid

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 9.50 (br s, **NH**), 7.49 – 7.32 (m, 5H, **CH_{Ar}**), 4.94 (s, 2H, **^{6}CH$_2$**), 4.25 – 4.02 (m, 4H, **^{3}CH$_2$**), 2.81 (d, $^2J_{PH}$ = 20.8 Hz, 2H, **^{2}CH$_2$**), 1.32 (t, $^3J_{HH}$ = 7.1 Hz, 6H, **^{4}CH$_3$**).

^{31}P CPD NMR (162 MHz, $CDCl_3$) δ (ppm): 21.83 (s).

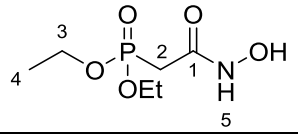
^{13}C APT NMR (101 MHz, CD_3OD) δ (ppm): 164.01 (s, ^{1}**C**), 136.92 (s, **^{7}C**), 130.34 (s, **^{8}CH**), 129.70 (s, **^{10}CH**), 129.54 (s, **^{9}CH**), 79.14 (s, **^{6}CH$_2$**), 64.24 (d, $^2J_{PC}$ = 6.5 Hz, **^{3}CH$_2$**), 32.79 (d, $^1J_{PC}$ = 136.8 Hz, **^{2}CH$_2$**), 16.67 (d, $^3J_{PC}$ = 6.2 Hz, **^{4}CH$_3$**).

MS (ES+) *m/z* = 302.12 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_{13}H_{21}NO_5P$ 302.1157, found 302.1158.

Benzyl cleavage, 2-(diethylphosphono)acetohydroxamic acid synthesis 240d:

In a 10 mL flask was introduced *O*-benzyl-2-(diethylphosphono)acetohydroxamate (100 mg, 0.33 mmol, 1 eq) and Pd 10% on charcoal (3.5 mg, 0.033 mmol, 10 mol%) in ethyl acetate (3 mL). The flask was flushed with H_2 and the reaction was stirred for 4h at room temperature. The suspension was filtered through a celite pad and the filtrate evaporated under vacuum leading to 66 mg of the deprotected product in 87% yield as a white solid.

	$C_6H_{14}O_5NP$ M = 211.15 g.mol^{-1} 87%

^{1}H NMR (400 MHz, CD_3CN) δ (ppm): 8.42 (s, 1H, **NH**), 4.21 – 3.98 (m, 4H, **^{3}CH$_2$**), 2.75 (d, $^2J_{PH}$ = 21.0 Hz, 2H, **^{2}CH$_2$**), 1.29 (t, $^3J_{HH}$ = 7.0 Hz, 6H, **^{4}CH$_3$**).

The first carbon NMR was made in methanol-*d4* but due to a proton-deuterium exchange, the methylene group bridging the phosphorus atom and the carbonyl wasn't visible. Another analysis was done with fresh product in CD_3CN, the methylene group gave a signal at 31.5 ppm (d, J = 134 Hz). However the carbonyl carbon signal was in the baseline in the second spectrum due to insufficient accumulation time.

^{13}C NMR APT (101 MHz, CD_3OD) δ (ppm): 164.09 (s, **^{1}C**), 64.22 (d, $^2J_{PC}$ = 6.5 Hz, **^{3}CH$_2$**), 16.64 (d, $^3J_{PC}$ = 6.2 Hz, **^{4}CH$_3$**).

^{13}C NMR APT (101 MHz, CD_3CN) δ (ppm): 62.20 (d, $^2J_{PC}$ = 6.4 Hz, **^{3}CH$_2$**), 31.51 (d, $^1J_{PC}$ = 134.4 Hz, **^{2}CH$_2$**), 15.32 (d, $^3J_{PC}$ = 6.2 Hz, **^{4}CH$_3$**).

^{31}P CPD NMR (162 MHz, CD_3CN) δ (ppm): 21.52 (s).

MS (ES+) *m/z* = 212.07 ($M+H^+$)

HRMS (ES+): *m/z*: [M+H] calculated for $C_6H_{15}NO_5P$ 212.0688, found 212.0687.

Synthesis of chelating phosphorus compounds for agrochemistry

Abstract:

The branched-chain amino acids metabolic pathway is present in many living beings such as plants, bacteria and fungi but not in mammals. This is why it has been interesting to target this enzymatic pathway with specific inhibitors in order to develop non-selective herbicides. The main non-selective herbicides commercialised inhibit the first enzyme of this metabolic route. However more and more cases of resistant weed appeared and spread. Thus it raises the interest and importance of designing new compounds targeting another enzyme from the biological pathway in order to circumvent the resistance issue.

The Ketolacid Reductoisomerase (KARI) protein also intervenes in the aforementioned enzymatic pathway. Although two inhibitors, IpOHA and Hoe 704, were synthesised during the 80s they have not proven active enough in field treatment. Nonetheless both inhibitors remain as references for *in vivo* biological activity.

The X-ray diffraction representations of KARI including each inhibitor or its natural substrate show that the functional groups borne by the latter chelate two metal cations within the active site. Moreover the comparison between inhibitor structures and the substrate transition states reveals a general pattern in order to design and develop new potential biologically active compounds. For that purpose three major substructure units have to be considered: a double chelating pincer, a lipophilic group and a hydrogen bond accepting moiety.

In this context different functional groups such as diorganylphosphine oxides, dihydropyrimidinediones, carboxylic acid or hydroxamic acid derivatives could be modified and used as chelating motifs. The functional groups listed have already proven their efficacy as part of other metalloenzyme inhibitors.

Keywords: Herbicide, Metalloenzyme, Ketolacid Reductoisomerase (KARI), IpOHA, Hoe 704, phosphorus

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Synthèse de composés phosphorés chélatants à visée herbicide

Résumé :

La voie de synthèse des acides aminés ramifiés est présente chez les plantes, les bactéries et les champignons mais est absente chez les mammifères. C'est pourquoi il est intéressant de cibler cette voie métabolique par des inhibiteurs spécifiques des enzymes qui la compose afin de développer des herbicides non sélectifs. Une famille d'herbicide déjà commercialisée agit sur la première enzyme de cette chaine enzymatique. Néanmoins des cas de mauvaises herbes résistantes à ces composés sont apparus et leur incidence continue d'augmenter. Il apparait donc important de cibler une autre enzyme de la voie de synthèse de ces acides aminés ramifiés afin de contourner ce problème de résistance.

L'enzyme cétoacide réductoisomérase (KARI) intervient également dans la voie métabolique citée précédemment. Deux inhibiteurs IpOHA et Hoe 704 ont été développés dans les années 1980, mais malheureusement n'ont pas montré d'activité lors de traitements en plein champ. Cependant ils restent tous les deux, les références en tant qu'inhibiteurs *in vitro*.

L'analyse de la diffraction par rayons X de KARI cristallisée avec un des inhibiteurs ou son substrat naturel montre que les groupements fonctionnels portés par ces derniers viennent complexer deux cations métalliques au sein du site actif. De plus la comparaison des structures des inhibiteurs ou des états de transition du substrat permet de dégager une structure générale pour le développement de nouvelles molécules potentiellement actives. Ainsi trois sous-structures doivent être présentes avec une double pince complexante, un groupement lipophile et un groupement accepteur de liaison hydrogène.

Dans ce contexte, différents groupements comme des oxydes de diorganylphosphines, des dihydropyrimidinediones, des dérivés d'acides carboxyliques ou hydroxamiques peuvent être modifiés et utilisés comme motifs complexants. Les fonctions et groupements fonctionnels cités ont déjà prouvé leur efficacité lors du développement d'autres inhibiteurs de métalloenzymes.

Mots clés : Herbicide, Métalloenzyme, cétoacide réductoisomérase (KARI), IpOHA, Hoe 704, phosphore.