University of Hamburg
Faculty of Chemistry

Structure-Activity-Studies on the Natural Antibacterial Compound SF-2312

Dissertation submitted in partial satisfaction of the requirements for the degree Doctor of Philosophy in pharmaceutical chemistry

by
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Hamburg 2007

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Acknowlegdements

There are lots of people I would like to thank for a huge variety of reasons. It is difficult to overstate my gratitude to my Ph.D. advisor, **Prof. Dr. Detlef Geffken** for the opportunity to work in his group and also for help with various applications for funding of the project. His inspiration and his many efforts to explain things clearly and simply are highly appreciated. Thank you also for managing to read the whole thesis so thoroughly and for helpful comments on the text.

I would also like to gratefully acknowledge the enthusiastic supervision of **Dr. Thomas Kurz** during this work. Thanks for the many insightful conversations during the development of the ideas in this thesis. Without your knowlegde, perceptiveness, cracking-of-the-whip and encouragement I would never have finished.

I wish to acknowledge the other members of the editorial and disputation panels **Prof. Dr. H.-J. Duchstein** and **JProf. Dr. D. Dartsch.**

Colleagues and faculty members are thanked for numerous stimulating discussions, help with experimental setup and general advice; among others I would like to acknowledge the support of **Dr. Tobias Mauz**, **Dr. Tanja Pies**, **Ms. Katharina Wehner**,and **Dr. Andrea Nicola Lubbe**. Help in translating the summary to a correct german version by **Ms. Miriam Pein**, **Ms. Steffi Jaeger**, **Mr. Guido Schmeink** and **Mr. Hassan Karimzadeh** is gratefully acknowleged.

Sincere thanks are also due to **Prof. Dr. P. Heisig** and **Ms. Tatjana Claussen** for providing all the required facilities to carry out the tests for antibacterial activity and big “thank you” to **Ms. Yvonne Krueper** at the chemistry library for help with speedily securing scientific articles from other libraries.

For financial support, I thank the state of Hamburg for the “Hamburg state youth scholarship award”

I am especially grateful to our mentors **Astrid** and **Jochen Winand** for making the transition into german culture possible and fun.

Finally, I am forever indebted to my family; my husband **Peter** for his understanding, endless patience and encouragement when it was most required, my siblings **Jerry**, **Francis**, **Miriam** and **Loise** for long-distance cheerleading and most of all my **Mum** and **Dad** for their courage to send me off into the world to pursue my dreams.

Standard Abbreviations and Acronyms

Ar	Aromatic
Bn	Benzyl
B.p.	boiling point
°C	degrees Celsius
calcd.	Calculated
Ch.	chapter (abbreviation used with period)
cm^{-1}	wavenumber(s)
Δ	Reflux
δ	chemical shift in parts per million downfield from tetramethylsilane
d	doublet (spectral)
CDI	1,1′-carbonyldiimidazole
COESY	Correlated spectroscopy
Conc.	concentrated (abbreviation used with period)
DMF	dimethylformamide
DMSO-d_6	dimethylsulfoxide-deuterated
DOXP	1-Desoxy-D-xylulose-5-phosphate
e.g	for example (latin: exempli gratia)
equiv.	Equivalent (abbreviation used with period)
Et	Ethyl
et al.	et alii (and others)
Et_2O	diethyl ether
EtOAc	ethyl acetate
EtOH	Ethanol
FAB	fast atom bombardment
g	gram(s)
GP	general procedure

h	hour(s)
Hz	Hertz
ICU	intensive care unit
ie	that is (latin: id est)
IR	Infrared
J	coupling constant (in NMR spectrometry)
L	liter(s)
lit.	literature (abbreviation used with period)
m	Meta
m	multiplet (spectral)
MDR	Multidrug resistant
Me	Methyl
MeOH	Methanol
MEP	2-C-methyl-D-erythritol-4-phosphate
min	minute(s)
mL	Milliliter
MMP	matrix metalloproteinase
mmol	Millimole(s)
M.p.	melting point
MS	mass spectrometry
MW	Molecular weight
n-BuLi	*n*-Butyllithium
NMR	nuclear magnetic resonance
NOE	nuclear overhauser effect
p	Para
Pd/C	palladium on carbon
Ph	Phenyl
POM-Cl	chloromethyl pivalate
ppm	part(s) per million

psi	pounds per square inch - unit of measure of pressure
Pt/C	platinum on carbon
q	quartet (spectral)
quart.	Quartiary
RT	room temperature
s	singlet (spectral); second(s)
sp.	species (abbreviation used with period)
t	triplet (spectral)
TEA	triethylamine
TEP	triethylphosphite
tert.	Tertiary
THF	tetrahydrofuran
TLC	thin-layer chromatography
TMSBr	trimethylsilyl bromide
W	Watt

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Preface

The discovery and development of new anti-infectives is a permanent challenge due to the increasingly frightening build up of resistant pathogens against established drugs. The emergence of drug-resistant pathogenic bacteria continues to be a serious threat to human health[1]. In developed countries antibiotic resistance appears to be a new kind of epidemic due to over use of broad range antibiotics and possibly overuse of antimicrobials in food animals[2]. 15% of all antibiotics manufactured in Europe are used on animals[3] and although the rest are banned, related antibiotics are often used as growth promoters and have been associated with the development of resistant strains. An European Prevalence of Infection (EPIC) study revealed that as many as 45% of all ICU patients had one or more nosocomial infection on the continent on the day of the study. 21% were ICU acquired while 10% and 14% were hospital and community acquired respectively[4]. The rapid spread of methicillin resistant *staphylococcus aureus* (MRSA) and vancomycin resistant *enterococci* (VRE) and their multi-drug resistant (MDR) strains not only as nosocomial but as community acquired infections as well causes the most concern in our time. MRSA infections are now no longer confined to the hospital setting, but also appear in healthy community-dwelling individuals[5].

On a different but equally grave front, any little progress made against Malaria –a disease whose mortality exceeds 1 million per year, most of them children in poor countries[6] - is threatened by growing resistance against known anti-malarials.

There is therefore an urgent need to replenish our arsenal of anti-microbials and the new anti-infectives should not only be based on one of the conventional fundamentally different chemical structures, but also based on new modes of action, in order to confront the danger of rapid formation of resistance. This thesis is a step in that direction. Analogues of natural antibiotic SF-2312 have been synthesized and studied. The mode of action of SF-2312 is not yet fully understood. However, it is known to hinder microbial growth in a mode different from the common bacterial cell wall synthesis inhibition.

1 Introduction

1.1 Antibiotic resistance

1.1.1 Defining Antibiotic resistance

Antibiotic resistance is the ability of a microorganism to prevail against the effects of an antimicrobial agent. Micro-evolutionary natural selection and mutation have led to microorganisms developing different mechanisms to render the antibiotic inactive through either physical removal from the cell via efflux pumps (Chloramphenicol) or through modification of its target site to reduce uptake into the cell (Tetracycline) or chemical modification of the antibiotic. (Fig 1.1) Chemical modification is mostly effected by enzymes that either breakdown the antibiotic for example hydrolysis of β-lactams or sequestering of the antibiotic by protein binding.

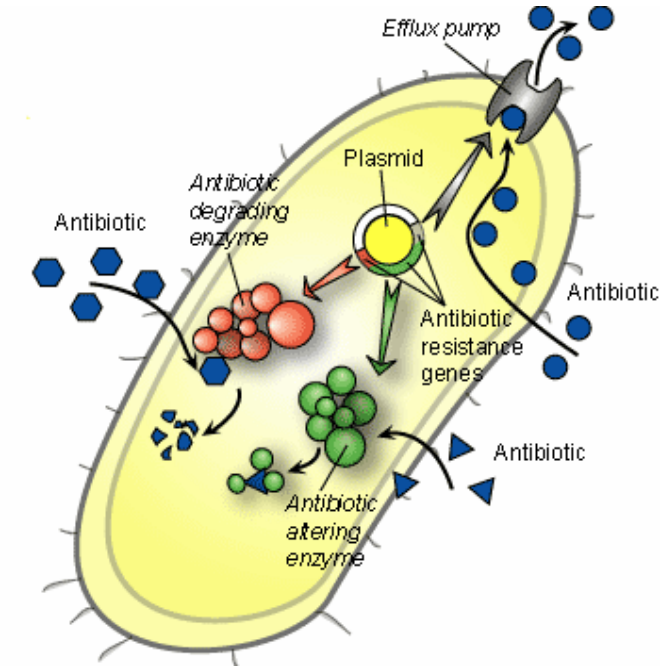


Fig. 1.1: *Mechanisms of Antibiotic Resistance*[7]

1.1.2 State of the art in Combating Antibiotic Resistance

From Fleming's discovery of Penicillin in 1928 and German Gerhard Domagk's discovery of synthetic sulfonamide antimicrobials in 1932, antibiotics have been essential in the fight against many diseases and infections[7]. Penicillin was mass produced from 1943 and by 1946 was available for oral use to the public without prescription. Although Fleming, as early as 1945, warned that misuse of penicillin could lead to resistant bacteria[8], penicillin resistant *Staphylococcus aureus* strains had already been observed in 1944[9]. 30 yrs later this bug beat medicine again when Methicillin resistant strains were observed.

The prevailing group-think that infection and resistance problems had been conquered, the antibiotic saturated market and poor return of investments[10] led pharmaceutical companies to abandon antibacterial development in the late 1980s.

Then, came the rapid spread of Methicillin resistant *Staphylococcus aureus* (MRSA) and Vancomycin resistant *enterococci* (VRE) strains in hospitals affecting patients in intensive care units and organ transplant stations. This resulted in renewed efforts and investments for identifying compounds active against Gram-positive pathogens yielding, in the past 5 years[11], four newly licensed antimicrobials - Linezolid, Daptomycin, Quinupristin-dalfopristin and Tigecycline[*].

The Gram-negative bacteria were ignored in the renewed interest on antibiotics. Enterobacteriaceae such as *Pseudomonas sp.* and *Klebsiella pneumoniae* and other gram-negative bacilli have become resistant to most frontline antibiotics including third generation cephalosporins, monobactams, aminoglycosides and quinolones[4].

Recent structurally novel classes of antimicrobials such as oxazolidinones and peptide deformylase (PDF) inhibitors are poor inhibitors of Gram-negative bacteria due to poor permeability[12].

There is urgent need to pay attention to MDR Gram negative bacteria.

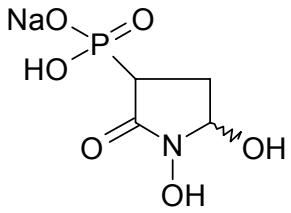
Commensals, bacteria that colonize individuals without causing disease, like *Escherichia coli* now exist in MDR strains that could cause urinary tract infections (UTI) and septicemias[13].

[*] Of the four only Linezolid is available for oral use and only Tigecycline is also active against Gram-negative bacteria though its spectrum does not include *Pseudomonas aeruginosa* which is emerging as multi-resistant Gram-negative bacteria.

In what has become a vicious cycle, Community-acquired MRSA (CA-MRSA) are now reported in Hospitals as well[14].
Antibiotic resistance is far from combated, it still leads to chronic illnesses, more doctor visits or extended hospital stays. Rice reported of a US study[11] estimating that nosocomial *Staphylococcus aureus* infections alone resulted in 2.7 million extra days in the hospital, 9.5 billion dollars in extra hospital charges and almost 12,000 impatient deaths per year.

1.2 Natural antibiotic SF-2312

SF-2312 (**I**) is a structurally novel broad spectrum antibiotic which was isolated in 1986 from *Micromonospora sp.* by Watabe et al. The antibiotic, extracted in small quantities (0.5µg/mL of microorganism culture), was obtained via a complex method that involved successive application of culture broth fermentation*, extraction and both ion-exchange and silica gel chromatography. **I** is characterized by a cyclic N-hydroxyimide and a phosphonic acid functionality.



SF-2312

Fig. 1.2*: SF-2312*

Antibacterial activity tests against eight genera of both Gram-positive and Gram-negative organisms revealed that SF-2312 was more active under anaerobic conditions than in aerobic conditions. To list a few; *Staphylococcus epidermis, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae* were included.

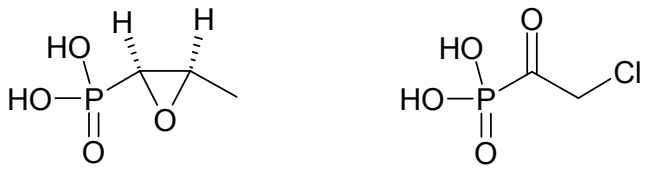
Initial studies on SF-2312 indicate that it does not exhibit its anti-microbial activity through bacterial cell wall inhibition like Fosfomycin[15-16]. Its availability in no more than micro-liter quantities represents an important task of synthesis and is a step toward renewing humanity's antibiotic arsenal.

* SF-2312 was extracted from a mixed culture of *Micromonospora sp.* and *Coryneform* bacterium (strain BN-258) where the bacterium does not produce the antibiotic but enhances its production by the actinomycete *Micromonospora sp.*

1.3 Phosphono-carbohydroxamic acids as bioactive compounds

1.3.1 Phosphono-functionalised compounds

Phosphonic acid containing antimicrobials have attracted considerable interest in medicinal chemistry. Examples are Fosfomycin and Fosfonochlorin, which are reported to have low toxicity, are effective and more importantly are compatible with other antimicrobials[17]. Fosfomycin[18] is a cell wall synthesis inhibiting bactericidal antibiotic. It inactivates the enzyme enolpyruvyl transferase, thereby irreversibly blocking the condensation of uridine diphosphate-N-acetylglucosamine with *p*-enolpyruvate, one of the first steps in bacterial cell wall synthesis.
Fosfomycin is prescribed as a tromethamine monosalt for oral use and is indicated for the treatment of uncomplicated urinary tract infections due to susceptible strains of *Escherichia coli* and *Enterococcus faecalis*. It is also prescribed as a free acid for parenteral use.
Fosfonochlorin was isolated from *Fusarium sp.* and is moderately active against some species of Gram-negative bacteria[19].



Fosfomycin **Fosfonochlorin**

Fig. 1.3: *Phosphonic acid containing antimicrobials*

1.3.2 Hydroxamic acid functionalised compounds

The first hydroxamic acids was synthesised in 1869 by Lossen W. Although discrepancies arose, it has been shown that hydroxamic acids are stable in both solid and solution state in their carbonyl-form **II**. Research continues inorder to determine whether the hydrogen atom of the amino group NH in **II** dissociates predominately to form the mesomeric monoanion **B** making **II** an N-acid or whether it is the hydrogen atom of the OH group in **II** that dissociates predominately to form **A**, hence an O-acid. Some authors suggest N-acid behavior for hydroxamic acids in gas phase and in DMSO solution but O-acid character in aqueous solution while others argue that hydroxamic acids are essentially N-acids except when the H of the amino group is substituted with an alkyl group[20-24].

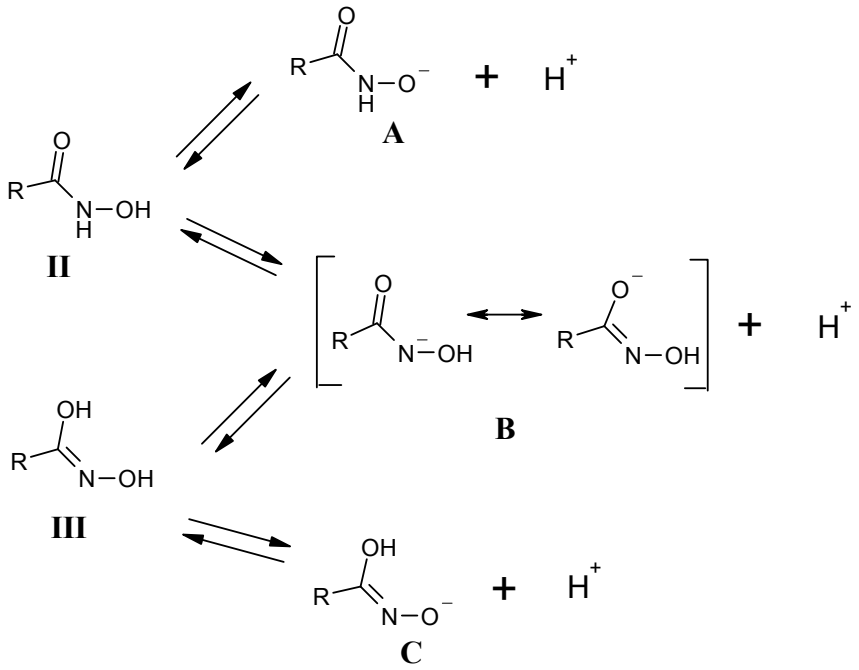
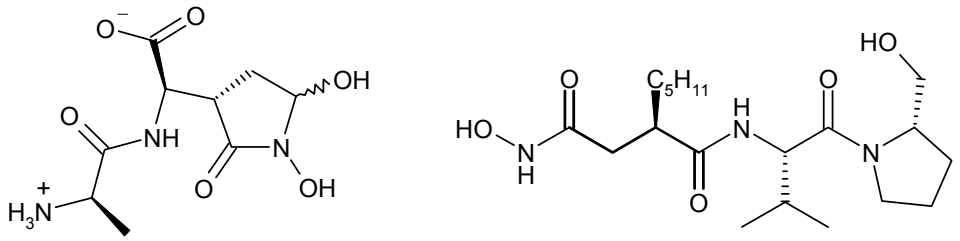


Fig. 1.4 *Diprotic nature of hydroxamic acids*[25]

Though now well studied for 137 yrs, hydroxamic acids continue to be intriguing and important pharmacophores in medicinal chemistry. While considered derivatives of carboxylic acids and hydroxylamines, they have significantly stronger metal chelating abilities than corresponding carboxylic acids[26]. The detection and isolation of hydroxamates

especially of naturally occurring hydroxamic acids is facilitated by a deep red colour produced upon addition of ferric chloride to the culture supernatant fluid.
Due to their metal chelating properties, hydroxamic acids for example Desferrioxamine[27] are used to deal with transfusional iron overload in thalassaemic and sickle cell patients and also iron intoxication in children[28]. However, the acid's biomedical applications are not only associated with uptake or removal of iron from the body. Their role as potent and selective inhibitors of a range of enzymes has increasing interest. These include zinc, nickel and iron metalloproteases such as MMPs, angiotensin converting enzyme (ACE), ureases, cyclooygenases and peptide deformylases (PDF) which have been implicated in a number of diseases such as arthritis, allergies, urinary track infections (UTI), antibiotic and anti-tumour drug agents[29]. Alahopcin, Actinonin are well known antibiotic agents functionalized by hydroxamic acids.



Alahopcin **Actinonin**

Fig. 1.5: *Hydroxamic acid containing Antibiotics*

Alahopcin

Alahopcin and the natural antibiotic SF-2312 are both functionalized by the 1,5-dihydroxypyrrolidin-2-one system.
Alahopcin (Fig. 1.5) was first isolated from a culture of *Streptomyces albulus* by Higashide et al. It was shown[30] to be active against both Gram-positive and Gram-negative bacteria with especially strong activity against a previously antibiotic resistant type of *Staphylococcus aureus*. It is reported to be identical to the natural antibiotic Nourseimycin[31] that had earlier been isolated from *Streptomyces noursei*[32] through physical and spectroscopic data.
In 1991, Baldwin et al. reported the first stereospecific synthesis of Alahopcin, starting from (L)-aspartic acid[33].

Actinonin

Like SF-2312, Actinonin[34] (Fig. 1.5) is a naturally occurring antibacterial agent that was isolated from *Actinomyces sp.* and *Streptomyces sp.* in 1962. It is active against both gram positive and gram negative bacteria potentially as a PDF inhibitor[35]. Although a supposed human mitochondrial PDF has been identified[36] and demonstrated to have a low level of catalytic activity, known inhibitors of the bacterial enzymes do not demonstrate toxicity toward human cell growth except at extremely high concentrations[37]. In addition to this new approach to antimicrobial chemotherapy, PDF has also been identified as a novel potential anticancer drug target[36].
Like the 1-hydroxypyrrolidin-2,5-dione analogues of SF-2312 studied in this work, Actinonin is also a succinyl derivative whose C_4 chain is optimal for its antibiotic activity in comparison to malonyl or glutaryl derivatives[38]. Actinonin, other MMP inhibitors such as Barimastat, Marimastat and Kelatorphan - a potent LTA_4 hydrolase (Leukotriene A_4) inhibitor - are all functionalized by a succinyl moiety which might imply its importance in drug synthesis.

Others

Other hydroxamic acid containing antibiotics include the natural products Hadacidin (*Penicillium purpurrescens*)[39], Mycelianamide (*Penicillium griseofulvum*)[40] and Aspergillic acid[41] (*Aspergillus flavus*). (Fig. 1.6)

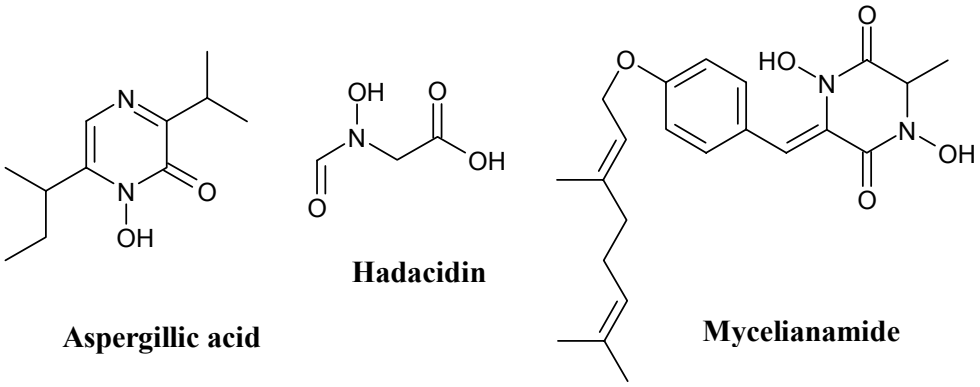


Fig. 1.6: *Natural antibiotics containing a hydroxamic acid group*

Marmion et al. have suggested that hydroxamic acids may act as nitric oxide donors[42]. This heightens the importance of the hydroxamic acid pharmacophore as nitric oxide is a key inter- and intracellular molecule involved in the maintenance of host response to infection, vascular tone and neuronal signaling*.

1.3.3 Phosphono-carbohydroxamic acid functionalized compounds

Compounds containing both the hydroxamic acid and phosphonic acid functions have been receiving growing interest in current medicinal chemistry because of their broad antimicrobial spectra[44]. One acclaimed example is Fosmidomycin (Fig. 1.7), an antibiotic active against many Gram-negative and some Gram-positive bacteria[45]. SF-2312 bears not only similarity in isolation and activity spectra but also an obvious structural relationship with open chained antimicrobial Fosmidomycin and might be regarded as a rigid analogue.

Fosmidomycin not only revealed a new structure but was of great interest in medicinal chemistry because of its novel mode of action of inhibiting the non-mevalonate synthesis of isoprenoids.
This non-mevalonate pathway to isoprenoids has been found in most eubacteria including pathogens for humans, in green algae and in chloroplasts of phototrophic organisms such as algae, liverworts, higher plants and unicellular eukaryotes such as *Plasmodium sp.*
DOXP (1-deoxyxylulose-5-phosphate) and MEP (2-C-methyl-D-erythritol-4-phosphate) are the first intermediates, formed by DOXP synthase (DXS) and DOXP reductoisomerase (DXR).
Of these, DXR is the most promising target for the development of drugs and even herbicides[46].

* Nitric oxide or nitric oxide donors are used as therapeutic agents to counterbalance the nitric oxide deficit. These donors are rapidly and often, non-selectively transformed to nitric oxide in mammals and have a short half-life in vivo[43], there is need for more stable nitric oxide donors that would be selectively oxidised to nitric oxide in situ with formation of nitric oxide in a given tissue.

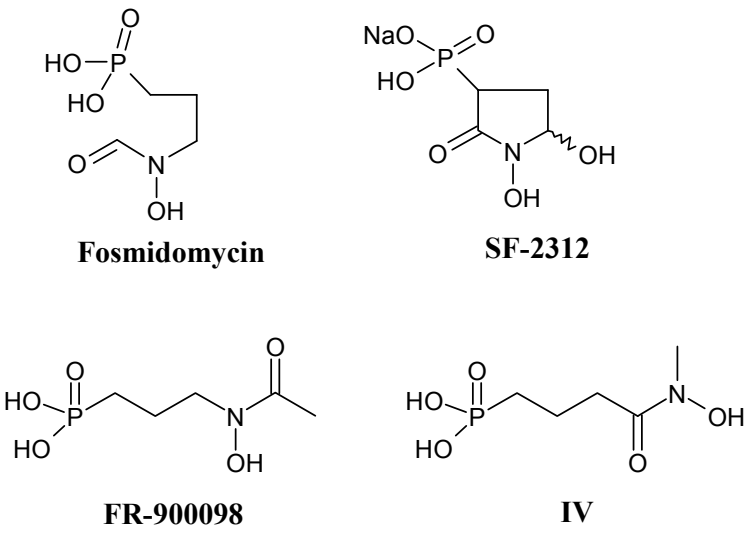


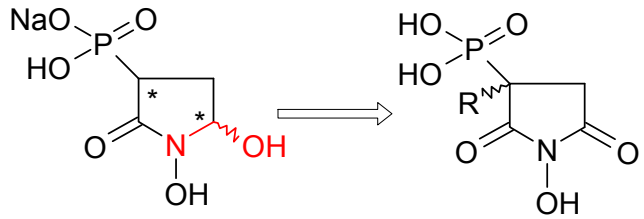
Fig. 1.7: *Antimicrobial agents functionalized by both phosphonic and hydroxamic acids*

Fosmidomycin specifically inhibits DXR and was reported to inhibit this enzyme in *Escherichia coli*. Fosmidomycin and its acetyl analogue FR-900098* (Fig. 1.7) had been known as the most efficient inhibitors of DXR[48] until the report of their more active α-phenyl analogues as pivaloyloxymethyl ester pro-drugs by Kurz et al.[49].
Interestingly, some strains of bacteria are reported to have shown cross resistance to Fosmidomycin and Fosfomycin while the N-methylated derivative **IV** (Fig. 1.7) inhibits a Fosmidomycin/ Fosfomycin-resistant strain of *E. coli*[50-56].
No synthesis of SF-2312 has been reported to date.

* Both Fosmidomycin and FR-900098 are also effective against Malaria where they inhibit the DXR of *Plasmodium falciparum*. Fosmidomycin has been used to treat Malaria in humans but due to high recrudescence rates is proposed for use in combination with Clindamycin[47].

1.4 Aim of Thesis

The aim of this thesis is to synthesize and study stable analogues of the naturally occurring antibiotic SF-2312. This is an important study for medicinal chemistry due to the reported activity of SF-2312 against both Gram-negative and Gram-positive bacteria, its similarity to known antibiotics like Alahopcin, Actinonin, and Fosfomycin and also to anti-malarials like Fosmidomycin.



SF-2312 **V**

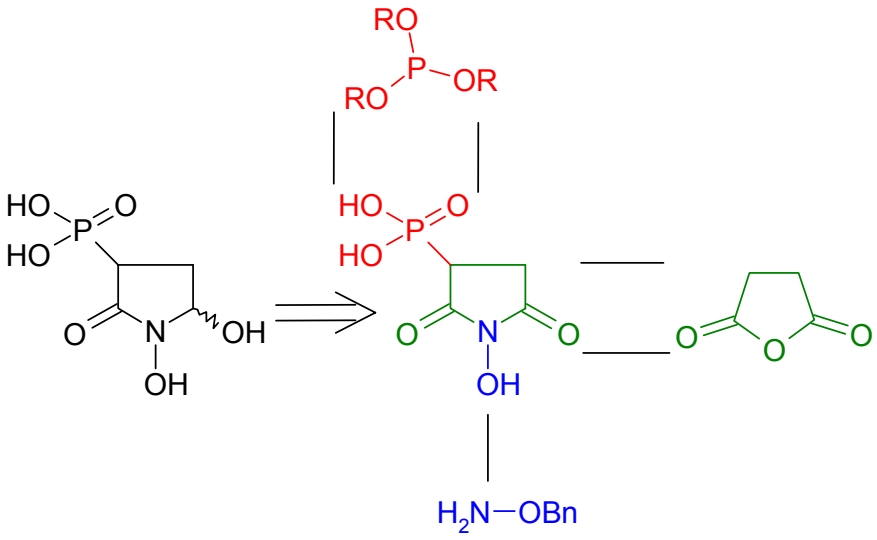
Fig. 1.8: *N,O-Acetal and chiral centers of SF-2312*

The corner stone of the strategy towards the synthesis of SF-2312 was the preparation of 1-hydroxypyrrolidin-2,5-dione (**V**) (Fig. 1.8).
This is due to N,O-Acetal instability and also to the two chiral centers of SF-2312 whose isomers would present purification problems during multi-step synthesis. It was therefore planned to synthesise compounds **V** with only one chiral center, as the initial target compounds.

1.5 Synthesis Plan

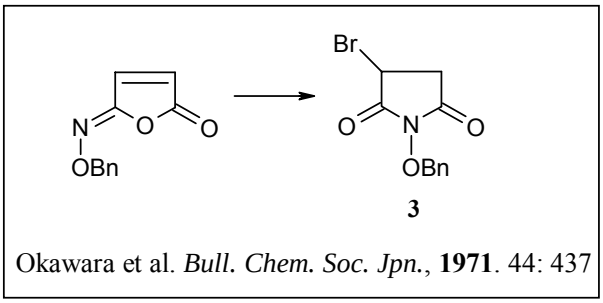
Retrosynthetic analysis shows that natural compound SF-2312 (**I**) consists of three identifiable functional groups; phosphonyl, succinyl and hydroxylamine moieties. (Scheme 1.1)

The pyrrolidin-dione nucleus would be elaborated via coupling of maleic anhydride with a protected hydroxylamine, subsequent cyclisation and reacting with trialkyl phosphite in a Michaelis-Arbusov reaction would then produce the target compounds **V** (Fig. 1.8).



Scheme 1.1: *Retro-synthetic analysis of* ***V***

The plan was to approach the synthesis from the known and easily synthesised 1-benzyloxy-3-bromopyrrolidin-2,5-dione (**3**). Okawara et al.[57] successfully brominated N-benzyloxyisomaleimide to yield the rearranged compound **3**. (Fig.1.9)



Okawara et al. *Bull. Chem. Soc. Jpn.*, **1971**. 44: 437

Fig. 1.9: *Synthesis of 1-benzyloxy-3-bromopyrrolidin-2,5-dione (**3**)*

Synthesis of open chained analogues of SF-2312 **VI** and **VII** (Fig. 1.10) was envisioned via reactions of γ-butyrolacton with hydrazine hydrate or coupling 2-(diethylphosphono)-butanoic acid with benzyloxyamine and 1,1′-carbonyldiimidazole (CDI).

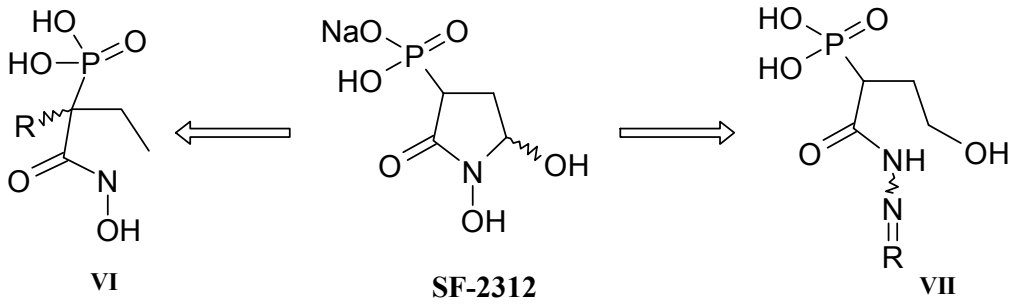


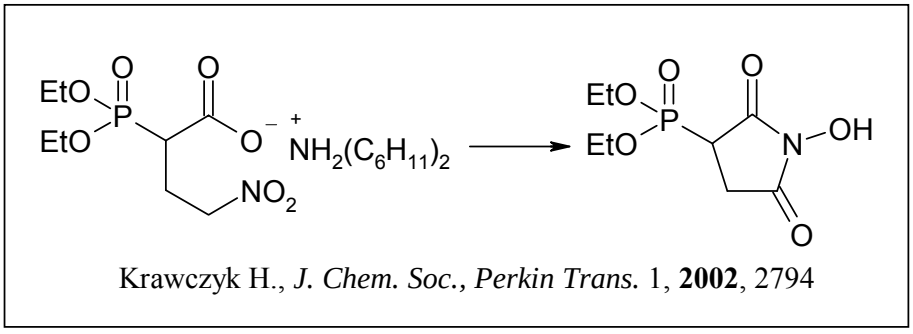
Fig. 1.10: *Open chained analogues of SF-2312*

The synthesised analogues of SF-2312 would then be modified by reacting them with various electrophiles and via a Mannich reaction to yield 3 (or α)-substituted derivatives.

2 Synthesis of 3-dialkoxyphosphoryl-1-benzyloxysuccinimides

2.1 Literature review

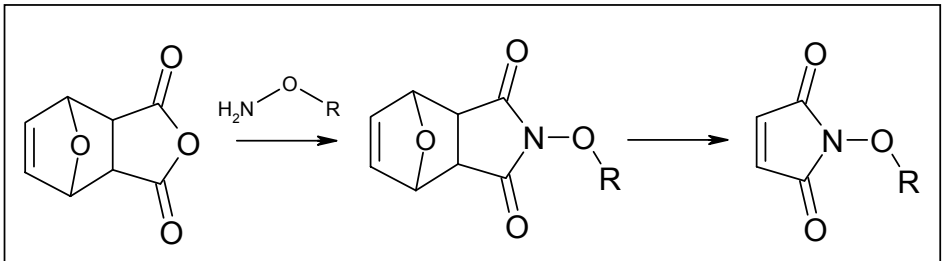
Krawczyk et al. have reported the synthesis of 3-diethoxyphosphoryl-1-hydroxysuccinimide and also a monoethyl phosphonate N-cyclohexanamine salt derivative of the same via self-catalytic Michael reaction using nitroalkanes[58].



Krawczyk H., *J. Chem. Soc., Perkin Trans.* 1, **2002**, 2794

Fig. 2.1: *Synthesis of 3-diethoxyphosphoryl-1-hydroxysuccinimide*

Diels Alder reactions[59] are also reported to facilitate the synthesis of N-alkyloxymaleimides.



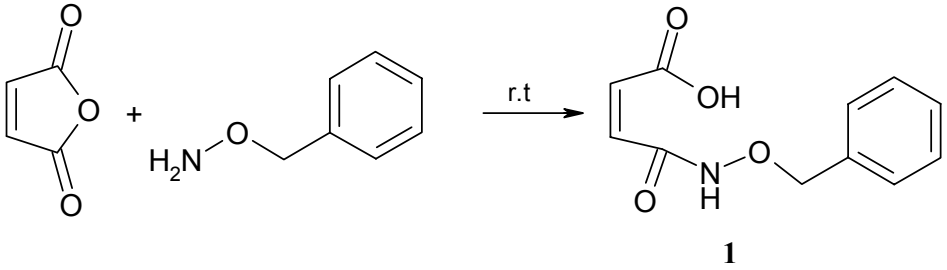
Narita M., *J.Bull. Chem. Soc. Jpn.,* 44, **1971**, 1084

Fig. 2.2: *Diels Alder Synthesis of N-Benzyloxymaleimide*

2.2 Educt Synthesis

2.2.1 Synthesis of N-Benzyloxyisomaleimide

N-Benzyloxyisomaleimide (**1**) was prepared in two steps from maleic anhydride. First the anhydride was reacted with benzyloxyamine in an exothermic ring opening reaction according to Ames et al. to afford *N*-benzyloxymaleimic acid[60]. This crystalline compound was obtained in 60% yield and its structure was confirmed by IR spectra and NMR spectra. The IR spectrum showed strong carbonyl bands at 1709 cm^{-1} (C=O), and at 1639 cm^{-1} (CONH).



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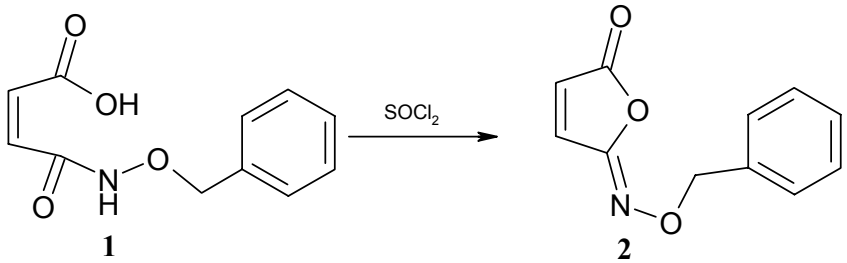
Scheme 2.1: *Synthesis of N-benzyloxymaleimic acid*

Next, *N*-benzyloxymaleimic acid was reacted with thionyl chloride to close the ring yielding 74% *N*-benzyloxyisomaleimide. Sharp 1793 cm^{-1} (C=O) and 1639 cm^{-1} (C=N) bands were observed in IR spectra.

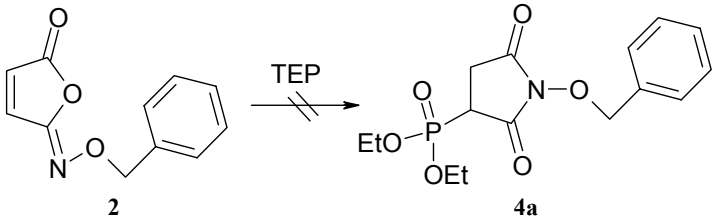


1 **2**

Scheme 2.2: *Synthesis of N-benzyloxyisomaleimide*

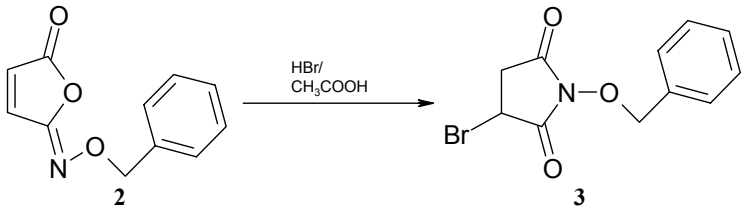
2.2.2 Synthesis of 3-bromopyrrolidin-2,5-dione

N-Benzyloxyisomaleimide was refluxed in triethyl phosphite in an attempted phosphonation but did not yield the desired product **4a** (Scheme 2.3).
In a different approach, the brominated product was first obtained for successive Michaelis-Arbusov reactions. It was isolated in 93% yield when *N*-benzyloxyisomaleimide was stirred at room temperature for 5 h in a solution of 32% hydrogen bromide in glacial acetic acid.



Scheme 2.3: *Synthesis of 3-diethoxyphosphoryl-1-benzyloxysuccinimide*

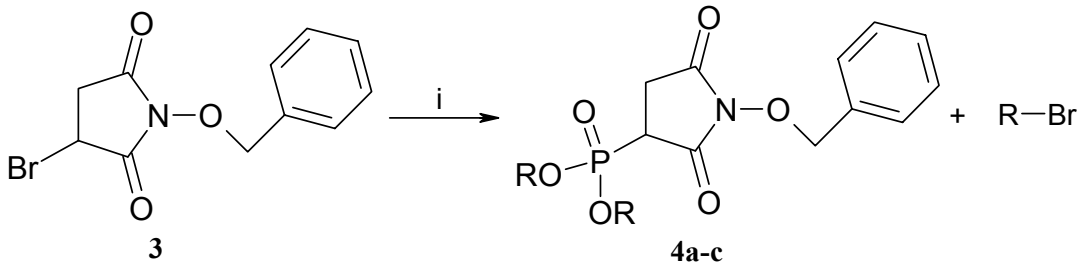
Internal rearrangement of the N-benzyloxy group from isoimide to imide was observed as reported[57]. The IR spectra confirmed the carbonyl groups at 1797 cm^{-1} and 1732 cm^{-1} respectively (Scheme 2.4).



Scheme 2.4: *Synthesis of 3-diethoxyphosphoryl-1-benzyloxysuccinimide via bromination*

2.3 Michaelis-Arbusov reactions

3-Bromo-1-benzyloxysuccinimide was refluxed in the appropriate trialkyl phosphite in Michaelis-Arbusov reactions to yield the phosphonic esters **4a-c** in good yields of 63-77% (Scheme 2.5)[61-63].



i: Δ, triethyl phosphite or trimethyl phosphite or triisopropyl phosphite

Scheme 2.5: *Michaelis-Arbusov reactions*

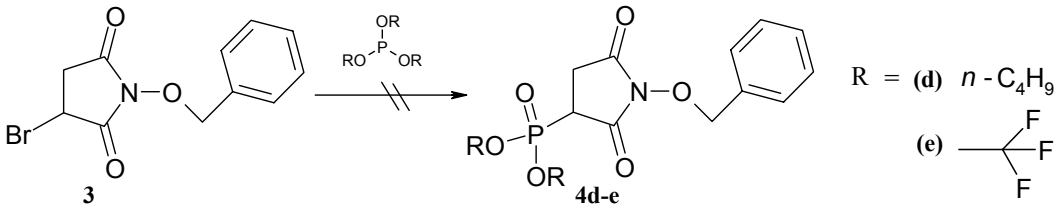
Table 2.1: Products **4a-c**

4	R	Yield %
a	Ethyl	77
b	Methyl	63
c	Isopropyl	64

Treatment of 3-bromo-1-benzyloxysuccinimide with trifluoromethyl phosphite under similar conditions (Scheme 2.5) to get **4e** left the educt **3** unreacted.

Although mass spectra and thin layer chromatography confirmed that dibutyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4d**) was the major product in the reaction using tributyl phosphite, its realization in analytically pure quantities proved to be a challenge. Fractional distillation and column chromatography methods did not fully remove impurities.

Even use of other solvents such as toluene and xylol and use of $ZnCl_2$ as a catalyst as reported in literature for similar reactions was to no avail for these two compounds (Scheme 2.6)[64].



Scheme 2.6: *Michaelis-Arbusov reactions using n-butyl- and trifluoromethyl phosphites*

The IR spectra of **4a-c** show two carbonyl bands at 1787 – 1799 cm^{-1} and 1724 – 1739 cm^{-1} while the (P=O) bands appear in the range 1251 – 1261 cm^{-1} (Fig. 2.3)

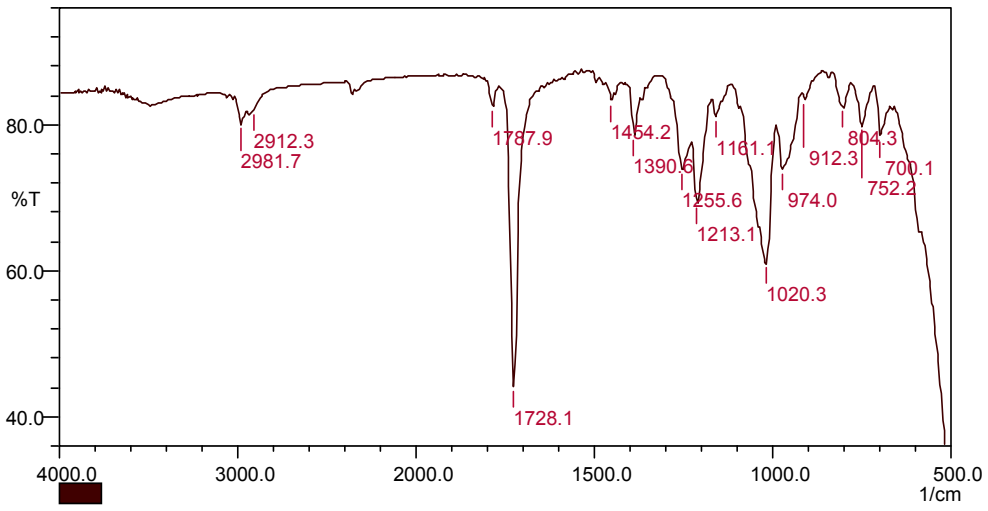


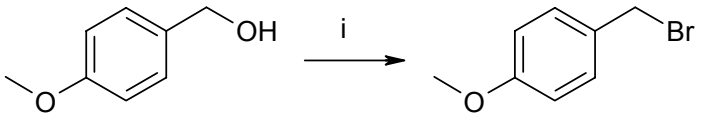
Fig. 2.3: *IR-spectrum of 3-diethoxyphosphoryl-1-benzyloxysuccinimide* ***4a***

2.4 Synthesis of 3-(aryl, alkyl)-3-dialkoxyphosphoryl-1-benzyloxysuccinimides

The acidity of the alpha C-H in position 3 due to the phosphonate group in the 3-alkoxyphosphoryl-1-benzyloxysuccinimides provides access to fuctionalization of the 2,5-pyrrolidindiones **4a-c** using electrophiles. Alkylation of 3-alkoxyphosphoryl-1-benzyloxysuccinimide compounds with alkyl (benzyl) halides in the presence of sodium hydride (Scheme 2.8) afforded 3-substituted 1-benzyloxypyrrolidin-2,5-diones **5a-t** in good to high yields (see Table 2.2).

2.4.1 Educt synthesis – electrophiles

Some electrophiles such as *p*-methoxy-benzylbromide and 3-furylmethylbromide are not commercially available due to instability and were thus prepared by reacting the commercially available alcohols for 15 - 20 minutes in 62% hydrobromic acid (Scheme 2.7). These electrophiles were used immediately after isolation in the alkylation reactions for products **5e,f,m,p** (Table 2.2).

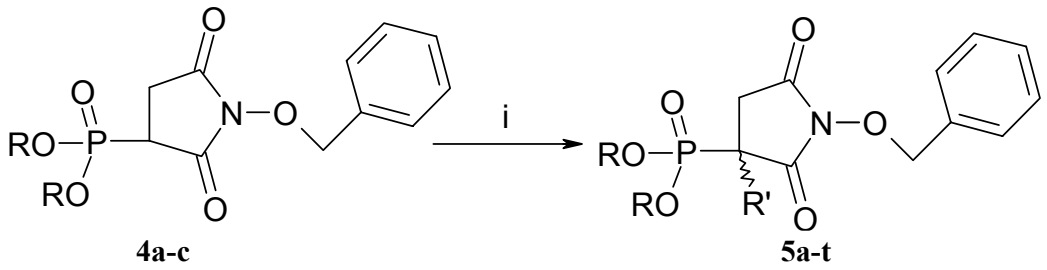


i: 62% HBr

Scheme 2.7: *Synthesis of 4-substituted benzyl bromide.*

2.4.2 Alkylation of 1-Benzyloxysuccinimides

The 1-benzyloxysuccinimides **4a-c** were dissolved in dry THF under nitrogen atmosphere and reacted with 60% sodium hydride. This mixture was left to stir for 10 min. The appropriate electrophile was then added either as a solution in dry THF, as a liquid or as a solution of toluene as in the case of methyl iodide. (Scheme 2.8)



i: NaH, R'Br or R'I, THF

Scheme 2.8: *Synthesis of arylalkyl, alkyl-substituted compounds* ***5a-t***

Table 2.2: Alkylation products **5**

5	R	R'	Yield%
a	ethyl	allyl	71
b	ethyl	4-methylbenzyl	78
c	ethyl	benzyl	80
d	ethyl	4-fluorobenzyl	64
e	ethyl	4-methoxybenzyl	51
f	ethyl	3-phenoxybenzyl	93
g	ethyl	methyl	60
h	ethyl	2-ethoxy-2-oxoethyl	47
i	ethyl	4-(trifluoromethyl)benzyl	81
j	ethyl	3-chlorobenzyl	80
k	methyl	benzyl	91
L	ethyl	2-chlorobenzyl	90
M	ethyl	3,5-dimethylbenzyl	89
N	ethyl	3,5-dichlorobenzyl	84
O	methyl	4-(trifluoromethyl)benzyl	89
P	ethyl	3-furylmethyl	69
Q	ethyl	3,5-difluorobenzyl	67
R	methyl	3,4-difluorobenzyl	53
S	Isopropyl	benzyl	70
T	Isopropyl	4-fluorobenzyl	65

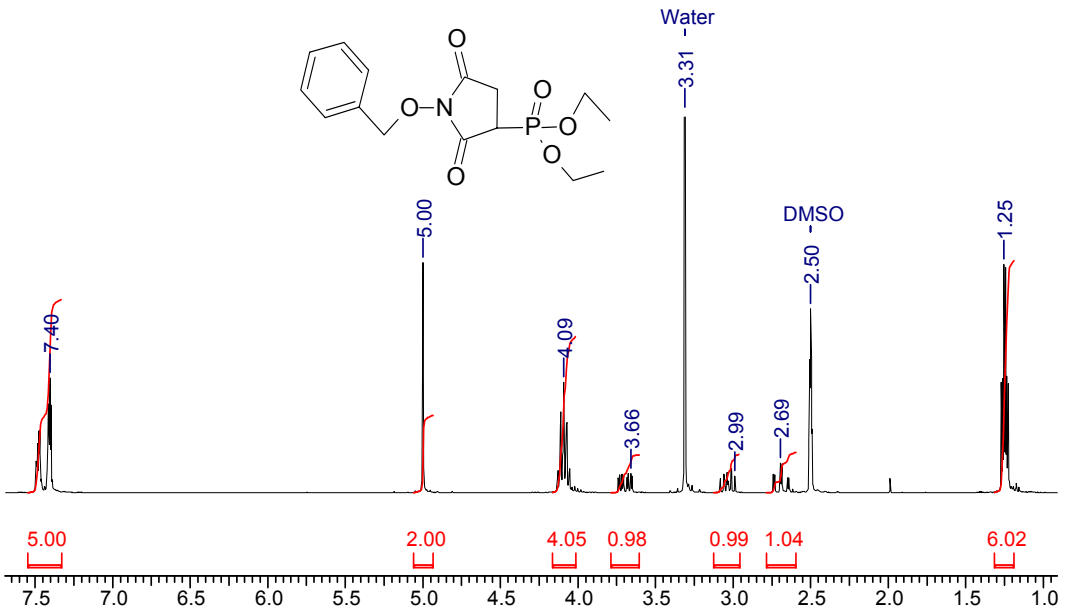


Fig. 2.4:
^{1}H-NMR spectrum of 3-diethoxyphosphoryl-1-benzyloxysuccinimide ***4a***

Whereas 3-diethoxyphosphoryl-1-benzyloxysuccinimide **4a** has the ^{1}H-NMR spectrum (Fig. 2.4) showing the two methylene and one methine protons as three distinct multiplets (2.69, 2.99 and 3.66 ppm), it was remarkably different for the alkylated products such as the *p*-fluorobenzyl derivative **5d** (Fig.2.5)

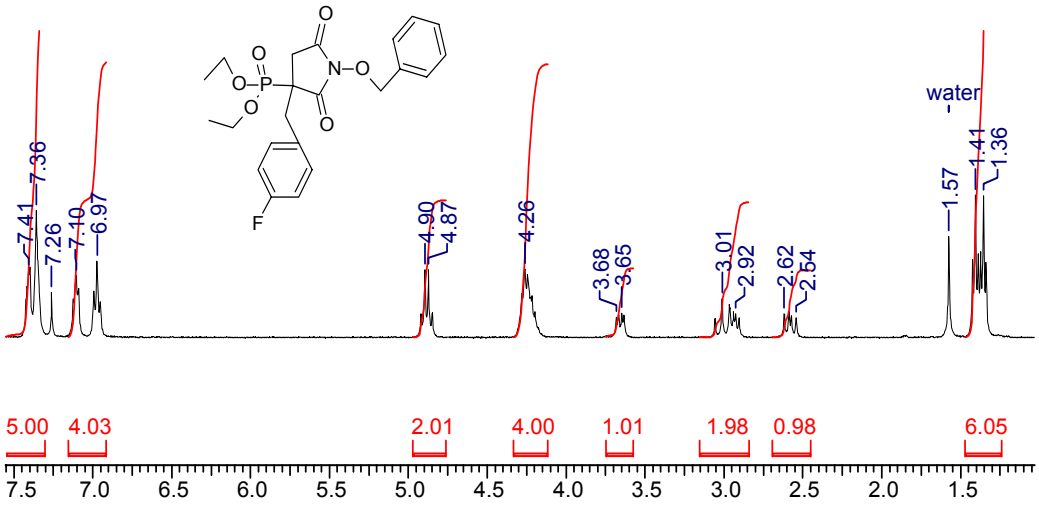


Fig.2.5: *^{1}H-NMR spectrum of the 3-(p-fluorobenzyl) derivative* ***5d***

Here it was observed that each of the two protons of each methylene carbon split and were found further apart as quartets and triplets. This phenomenon was confirmed by CH-Coesy spectra and was observed in other benzyl alkylated derivatives as well.

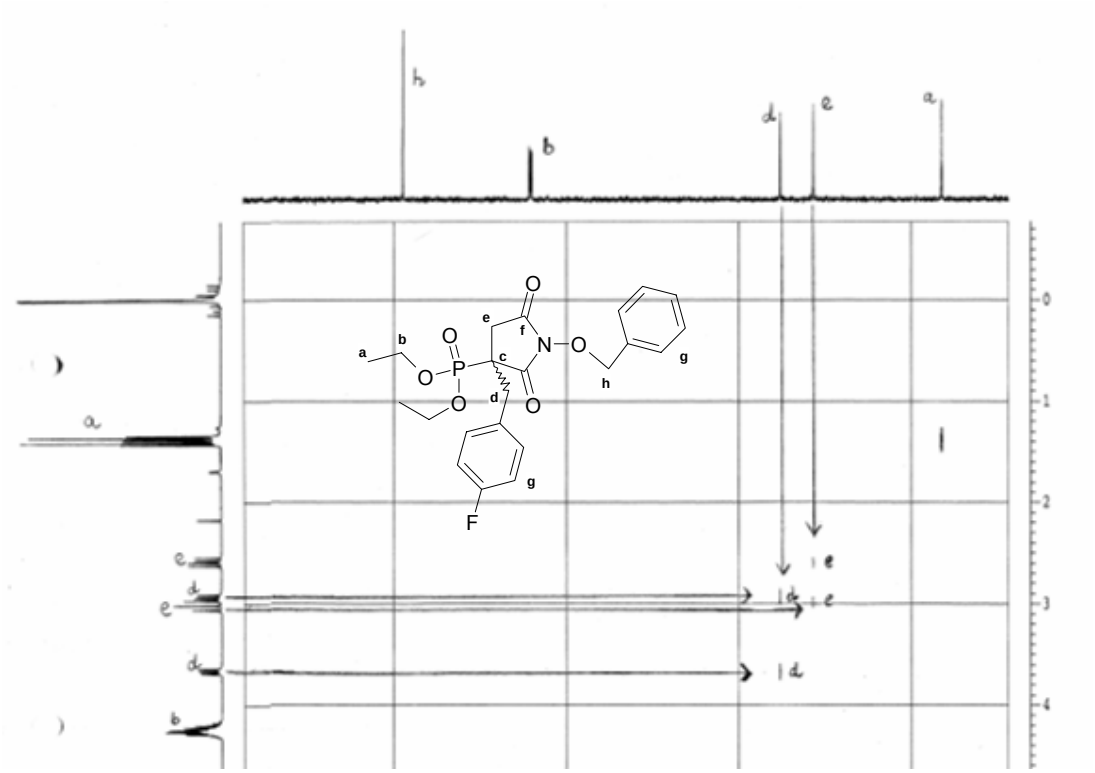
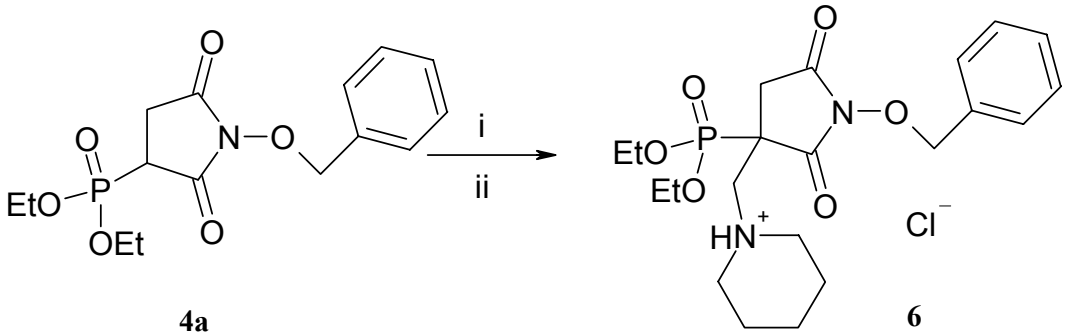


Fig.2.6: CH-Coesy spectrum of the 3-(p-fluorobenzyl) derivative **5d** showing seperated methylene protons

2.4.3 Mannich reaction

Functionalisation of the pyrrolidin-2,5-dione nucleus was also achieved through Mannich reaction of **4a** with formalin and piperidine in acetic acid[65]. The Mannich base was isolated as crystalline hydrogen chloride salt **6** in 20% yield after passing dry hydrogen chloride gas through an ethyl acetate solution of the base. (Scheme 2.9)
The structure of compound **6** has also been characterized by X-ray crystallography. (Fig. 2.7)

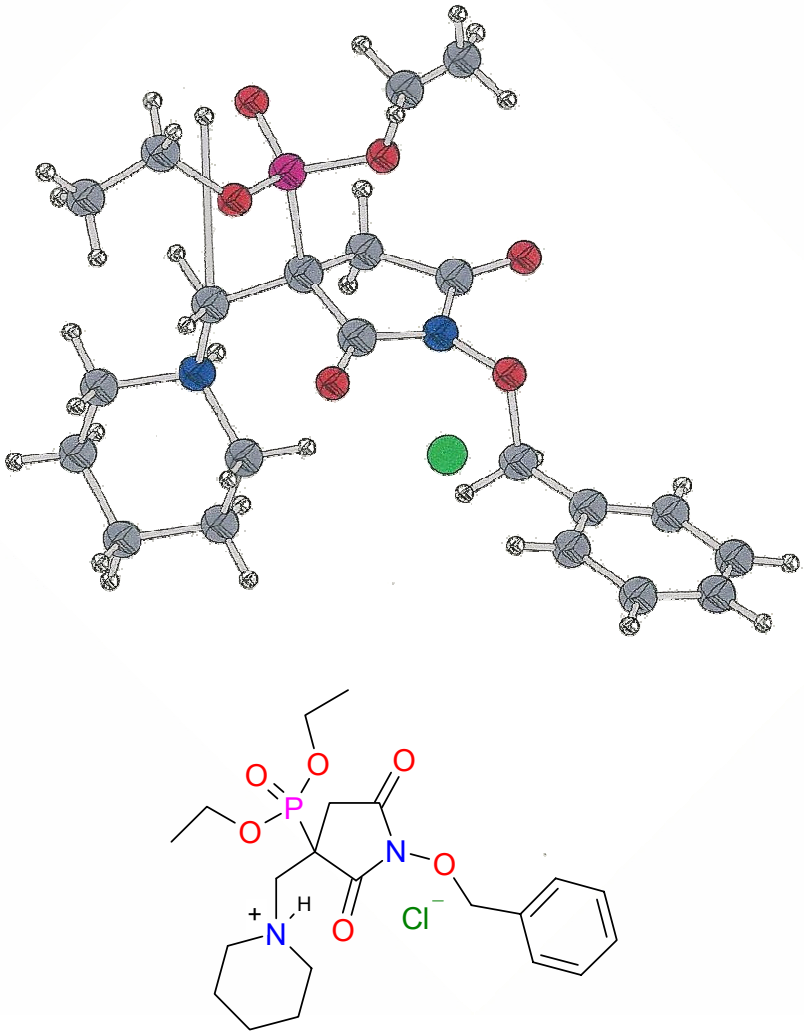


4a **6**

i: CH_3COOH, HCHO, Piperidine ii: HCl(g), EtOAc

Scheme 2.9: *Mannich reaction*

Fig.2.7: *X-ray crystallograph of compound* ***6*** *(Diamond-Visual Crystal Structure)*



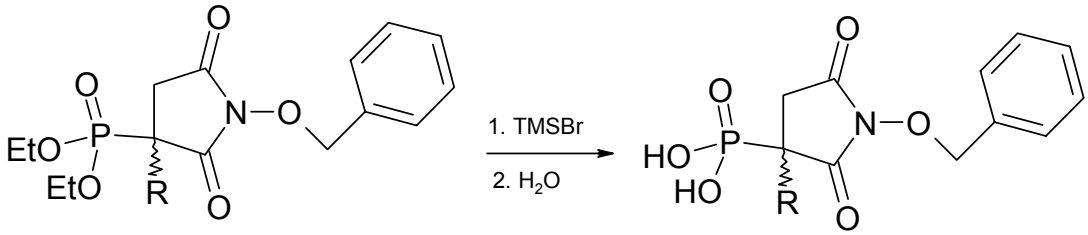
3 Synthesis of Phosphonic acids, protecting esters and salts

3.1 Literature review

The phosphonic acid group is present in various pharmaceuticals and pesticides and represents an important pharmacophore/ toxophore. The phosphonic acid functionality offers access to water soluble derivatives[66] and also serves as a chelating agent for various metal cations[67].

3.2 Synthesis of Phosphonic acids

Cleavage of the phosphonic esters **4a** and **5a-e** with trimethylsilyl bromide cleanly led to the phosphonic acids **7a-f**.



4a, **5a-e** **7a-f**

Scheme 3.1: *Cleavage of ethyl esters **4a** and **5a-e***

Conclusive evidence that the triplets of methyl (1.5 - 2.0 ppm) and the multiplets of methylene (4.0 - 4.4 ppm) protons were completely removed was provided by ^{1}H-NMR spectra (Fig. 3.1).

Table 3.1: *Cleavage of ethyl esters **4a** and **5a-e***

7	R
a	H
b	Alkyl
c	Aryl
d	substituted aryl

However these were highly hygroscopic compounds and did not furnish satisfactory elemental analysis data. It was therefore envisioned that protected phosphonic acids would not be as hygroscopic and thus more stable.

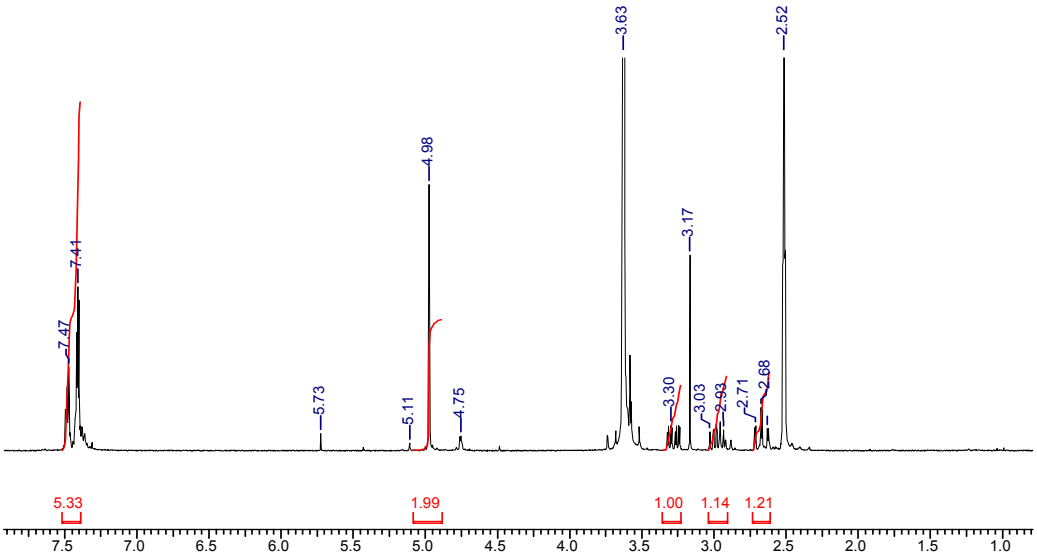
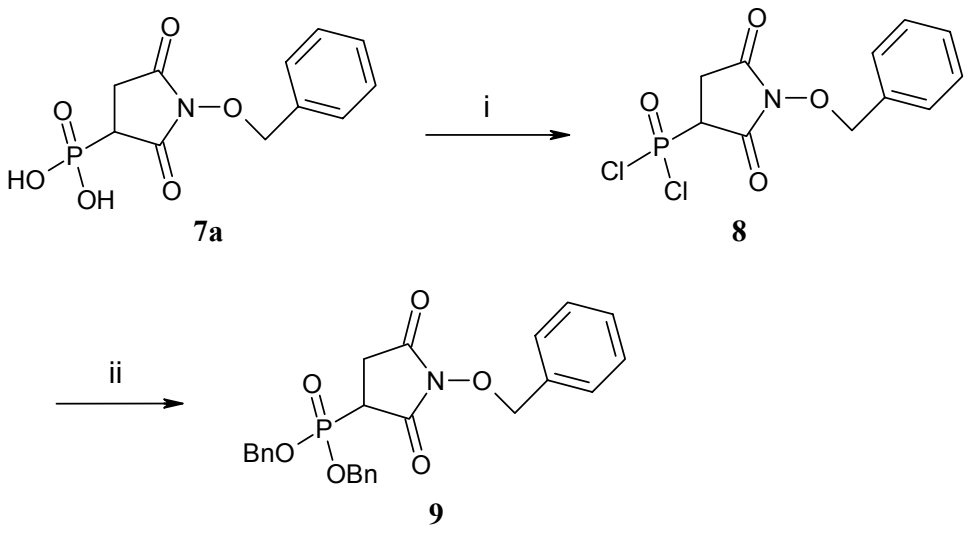


Fig.3.1: *^{1}H-NMR spectrum of **7a** showing cleanly cleaved triplet methyl (~1.5 ppm) and multiplet methylene (~4.0 ppm) protons.*

3.3 Benzyl protected phosphonates

The benzyl group has found considerable use in organic chemistry as a protecting group as it can be removed under neutral conditions by hydrogenolysis. In this case, it would be possible not only to easily purify the product by column chromatography but more importantly to remove the three benzyl groups protecting both the hydroxamic acid and the phosphonic acid at the same time via catalytic hydrogenation. According to a procedure by Reichenberg et al.[54], the phosphonic acid was chlorinated using phosphorous pentachloride in dry chloroform. The reagents and solvents were carefully removed and the benzylation conducted in dry tetrahydrofuran using pyridine as the base and benzyl alcohol to yield dibenzyl [1-(benzyloxy)-2,5-dioxopyrrolidin-3-yl]phosphonate (**9**).



i: PCl_5 or oxalyl chloride ii: pyridine, BnOH

Scheme 3.2: *Synthesis of Dibenzyl [1-(benzyloxy)-2,5-dioxopyrrolidin-3-yl]phosphonate* ***9***

This reaction proved to be very moisture sensitive yielding only 10% of the product as pink needle-like crystals due to the instability of the chloride **8** that in the slightest presence of atmospheric moisture reverted to the acid just before the second benzylation step.

Despite carrying out the reaction under nitrogen it was inevitable that the mixture be slightly exposed while evaporating out the excess PCl_5. A different approach of chlorination via oxalylchloride resulted in similar non-remarkable yields (Scheme 3.2).

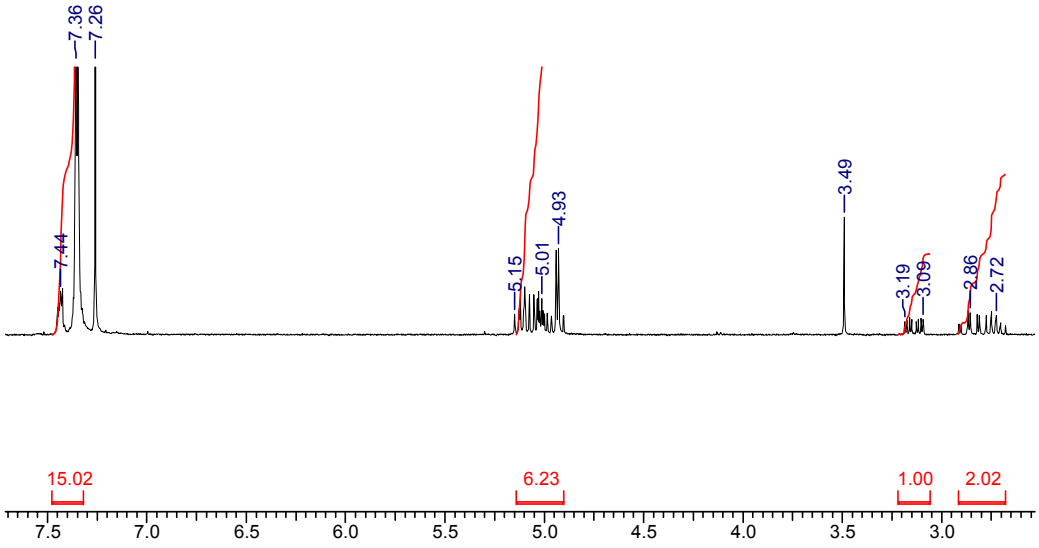


Fig. 3.2: *^{1}H-NMR spectrum of tribenzyl derivative **9** in $CDCl_3$*

IR-Spectra of **9** showcased both carbonyls at 1784 cm^{-1} and 1724 cm^{-1} while the ^{1}H-NMR spectra is clean with 15 aromatic protons at 7.36 ppm. (Fig. 3.2)

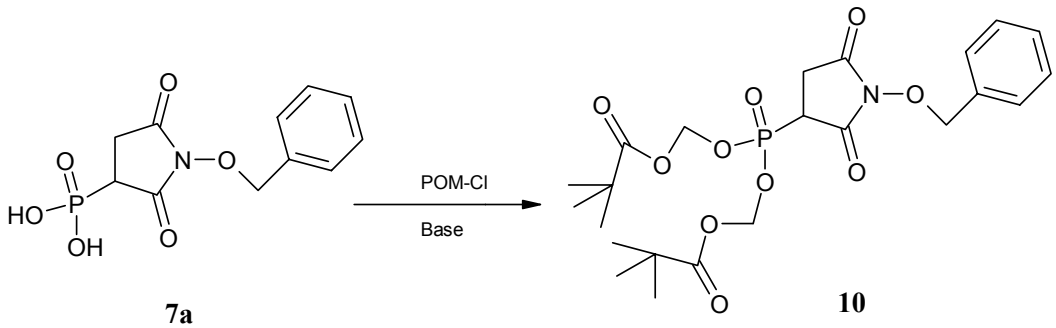
3.4 Prodrug synthesis

3.4.1 Phosphono prodrugs

Because phosphonate groups impart an ionic charge at nearly all physiological pH values making them very polar, there arise many deficiencies in the efficacy of drug delivery. Highly ionized species do not readily undergo passive diffusion across biological membranes. Due to this, these agents often exhibit low volume of distribution and therefore tend to be subject to efficient clearance from the body.

Prodrugs are thus used to overcome these drug delivery obstacles. To combat these shortcomings, the ionizable phosphonate groups have been neutralized via chemical derivatization which generally involves derivation of the phosphorus-coupled oxygen to form neutral esters[68]. These are supposed to be non-polar and membrane permeable derivates that could be hydrolysed by intracellular enzymes to release the native ionic phosphate. Acyloxymethyl esters such as the pivaloyloxymethyl esters used here, have been prepared and shown to be readily hydrolysed in the presence of esterases[69].

3.4.2 Synthesis of pivaloyloxymethyl pro-drug products



7a **10**

Scheme 3.3: S*ynthesis of **10***

The phosphonic acid **7a** was dissolved in DMF and reacted with chloromethyl pivalate in the presence of triethylamine yielding only traces of the product after work up and purification by column chromatography (Scheme 3.3). However, further attempts to improve

yield using different combinations of solvents (DMF, THF, toluene, DMA), bases and even catalysts (triethylamine, Hunig's base, silver carbonate, sodium iodide) as reported, proved futile[69-74].

The product's (**10**) IR spectrum shows carbonyl bands at 1786, 1755 and 1726 cm^{-1} (Fig. 3.3)

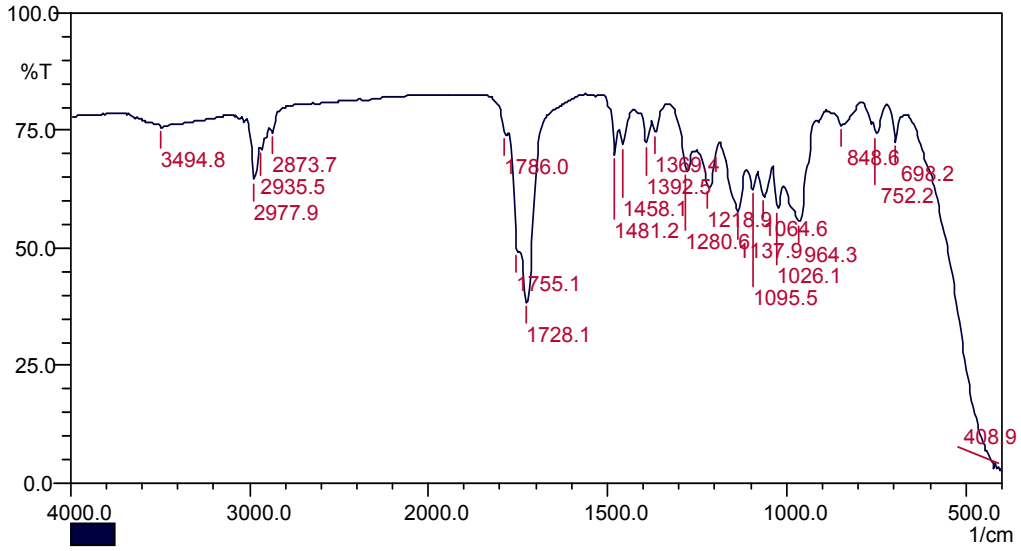
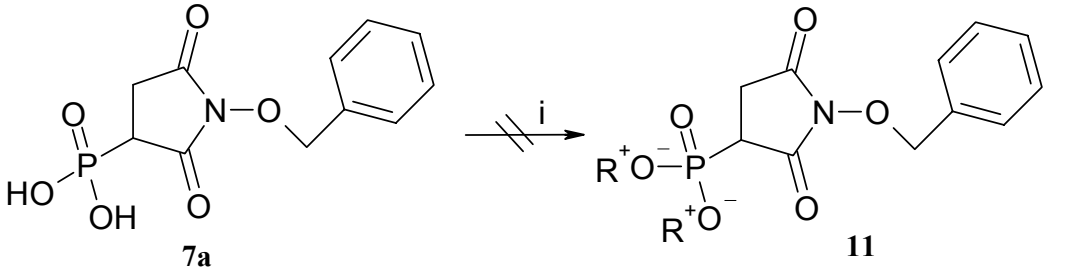


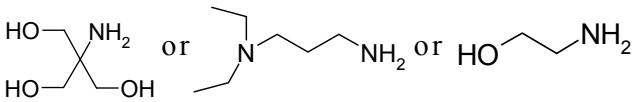
Fig. 3.3: *IR-spectrum of pivaloyloxymethyl ester derivative **10***

3.5 Synthesis of Ethanolamine monosalts

In a different but successful approach, the phosphonic acids were converted into stable non-hygroscopic salts. A compound's solid state properties and also its properties in solution can be modified by salt formation. Nearly half of all drug molecules in medicinal therapy are administered as salts. While most organic acids and bases are only poorly soluble in water, salification has been used to improve their solubility properties. This has in turn eased drug candidate screening, facilitating in-vitro studies as well as studies on isolated organs[75]. For example, SF-2312[15] was isolated as a sodium monosalt of its phosphonic acid moiety. Salt formation has also been used to render medicinal compounds in stable solid-state forms as attested to by the synthesis of solid ethanolamine monosalts in this section.

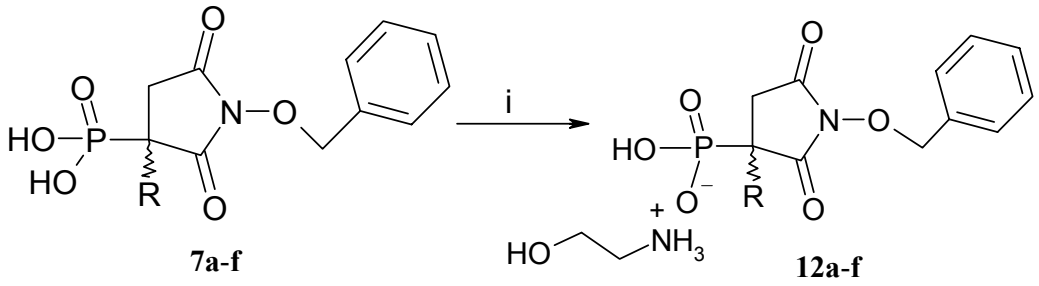
The phosphonic acids were treated with ethanolamine, trometamol, N,N-diethylpropyldiamine in a molar ratio of 1:2 in dry methanol to synthesize disalts but did not yield the desired products **11** (Scheme 3.4).



i: MeOH, Base (2 equiv.): R-  or or

Scheme 3.4: *Synthesis of disalts **11***

Reaction of the phosphonic acids **7a-f** and ethanolamine in a molar ratio of 1:0.9 respectively and subsequent cooling at −18°C for two days[76] afforded the monosalts **12a-f** as analytically pure compounds in yields of 53 – 68% (Scheme 3.5, Table 3.2). The IR spectra show carbonyl bands between 1782 - 1786 cm^{-1} and 1706 - 1724 cm^{-1}.



7a-f **12a-f**

i: ethanolamine

Scheme 3.5: *Salification of **7a-f***

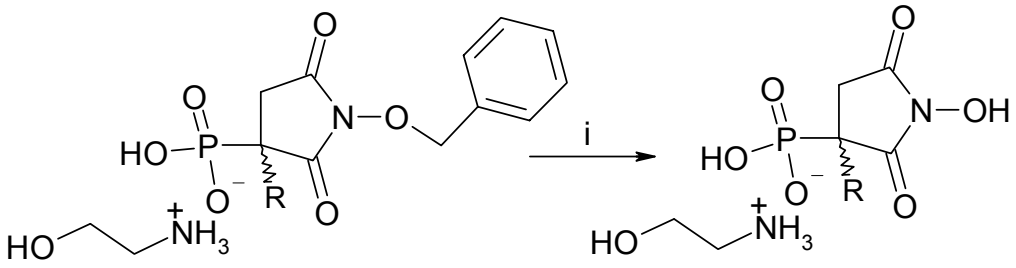
Table 3.2: *Salification products **12***

12	R	Solvent (mL)	Yield%
a	H	5mL THF	53
b	allyl	5mL THF	64
c	4-methylbenzyl	5mL THF	65
d	benzyl	3mL THF: 1.5mL MeOH	57
e	4-fluorobenzyl	7mL THF	68
f	4-methoxybenzyl	5mL THF	66

4 Synthesis of 1-Hydroxysuccinimides

4.1 O-Benzyl deprotection of 1-benzyloxy-succinimides 12a-f

Deprotection of the cyclic hydroxamate moiety by cleavage of the benzyl group of the monosalts **12a-f** via catalytic hydrogenation on Pd/C in methanol provided the monosalts **13a-f** in excellent yields of 90-96%. (Scheme 4.1, Table 4.1)



12a-f **13a-f**

i: Pd/C, $H_{2(g)}$, MeOH, 90min

Scheme 4.1: *Hydrogenation of the benzyl protected monosalts **12a-f***

Table 4.1: *Debenzylated monosalst **13a-f***

13	R	Yield%
a	H	95
b*	propyl	92
c	4-methylbenzyl	90
d	benzyl	96
e	4-fluorobenzyl	90
f	4-methoxybenzyl	96

* Hydrogenation of **12b** led to simultaneous debenzylation and hydrogenation of the allyl double bond yielding 3-propyl substituted **13b**

The IR spectra of compounds **12a-f** show two carbonyl bands at 1706 – 1724 and 1782 – 1786 cm^{-1} whereas the 1-hydroxypyrrolidin-2,5-diones **13a-f** exhibit the carbonyl bands bathochromically shifted to 1697 – 1718 and 1774 – 1782 cm^{-1} (Fig 4.1).

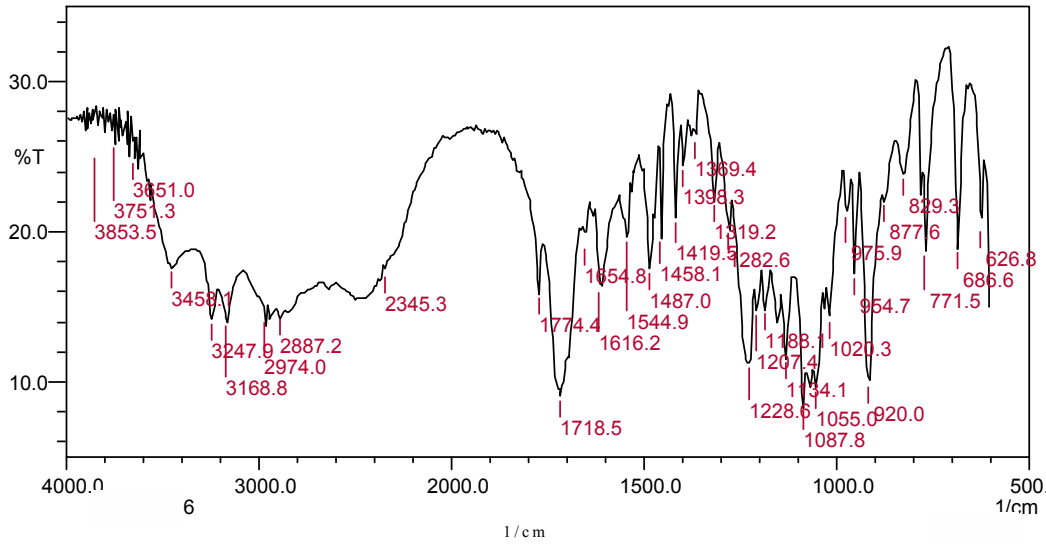
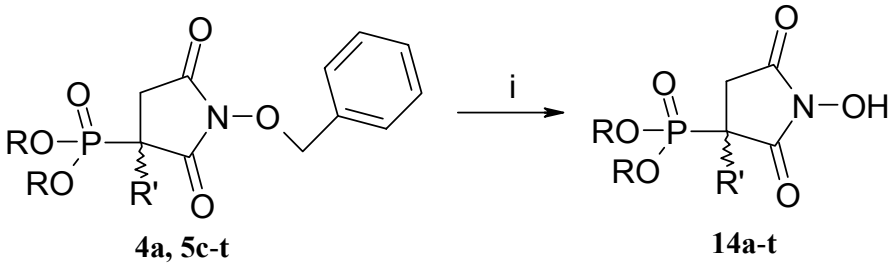


Fig. 4.1: *Characteristic IR-spectrum of the ethanolamine salt* ***13a***

4.2 Synthesis of Diethyl phosphonate 1-Hydroxy-succinimides

Catalytic hydrogenation of **4a** and **5c-t** (Table 2.2) on Pd/C in methanol provided the novel cyclic hydroxamic acids **14a-t** in good yields of 50 - 85% (Table 4.2, Scheme 4.3). Chemoselective hydrogenation of halogen-substituted compounds **5j,l,n** proved to be a challenging task as it resulted in the reductive removal of their chlorine atoms.

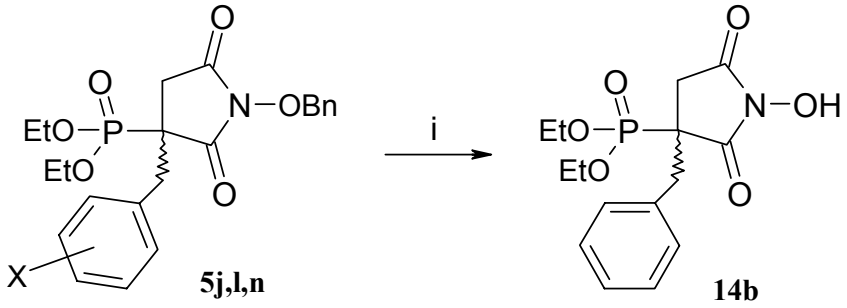


4a, 5c-t **14a-t**

i: MeOH, Pd/C, $H_{2(g)}$, 90 min.

Scheme 4.3: *hydrogenation of **5** in ethyl acetate or methanol on Pd/C for 90 min under 2bar of pressure*

Deprotection of **5j,l,n** conducted in methanol on Pd/C for 90 min as applied for compounds **5c-i,k,m,o-t** afforded only **14b**. Using ethyl acetate as a less polar solvent, lower pressure and the same time of 90 min did yield the desired products **14j,l,n** but only in trace amounts as exhibited by mass spectra (Scheme 4.4, Table 4.2)[77].



5j,l,n **14b**

i: MeOH, Pd/C, $H_{2(g)}$, 90 min

Scheme 4.4: *hydrogenation of **5j,l,n** in ethyl acetate or methanol on Pd/C for 40-90 min*

Tabel 4.2: *Hydrogenation products **14a-t***

14	R	R'	Yield%
a	ethyl	H	76
b	ethyl	Benzyl	69
c	ethyl	4-fluorobenzyl	85
d	ethyl	4-methoxybenzyl	49
e	ethyl	3,5-dimethylbenzyl	79
f	ethyl	3-phenoxybenzyl	52
g	ethyl	Methyl	72
h	ethyl	2-ethoxy-2-oxoethyl	68
i	ethyl	4-(trifluoromethyl)benzyl	66
j	ethyl	3-chlorobenzyl	traces
k	methyl	Benzyl	82
l	ethyl	2-chlorobenzyl	traces
m	ethyl	3-furylmethyl	62
n	ethyl	3,5-dichlorobenzyl	traces
o	methyl	4-(trifluoromethyl)benzyl	66
p	ethyl	3,5-difluorobenzyl	82
q	n-butyl	benzyl	9*
r	ethyl	3,4-difluorobenzyl	77
s	Isopropyl	benzyl	85
t	Isopropyl	4-fluorobenzyl	85

The 3-chlorobenzyl derivative **14j** and 2-chlorobenzylated **14l** could be obtained in modest quantities of 25% and 28% respectively after purification via multiple recrystallization to separate **14j,l** from **14b**.
In these cases, ethyl acetate was used as the solvent while less pressure of 1 bar and shorter time of 40 min conditions were applied. The shorter reaction time also left some un-reacted educts **5j,l** that were similarly removed by crystallization.

Diethyl (3,5-chlorobenzyl-1-hydroxy-2,5-dioxopyrrolidin-3-yl) phosphonate **14n** was however realized in a good yield of 84% when hydrogenated under a lower pressure of 1 bar in ethyl acetate for 35 minutes (Table 4.3).

* **14q** was realized in yield of 9% after benzylation of crude educt **4d** and subsequent catalytic hydrogenation of the resulting crude benzylated product.

Table 4.3: *Deprotection of halogenated products **5j,l,n** under varied conditions*

14	R'	Solvent	Time	Catalyst	Pressure	Yield%
j	3-chlorobenzyl	MeOH	90'	Pd/C	2bar	--
		EtOAc	90'	Pd/C	1bar	traces
		EtOAc	40'	Pd/C	1bar	25
		EtOAc	9h	Pd/C	1bar	56
			20'	$ZnBr_2$		
l	2-chlorobenzyl	MeOH	90'	Pd/C	2bar	--
		EtOAc	70'	Pd/C	1bar	traces
		EtOAc	40'	Pd/C	1bar	28
n	3,5-dichlorobenzyl	MeOH	90'	Pd/C	2bar	--
		MeOH	30'	Pd/C	1bar	--
		EtOAc	35'	Pd/C	1bar	84

4.3 Discussion

A literature search revealed that different groups of researchers have used varied methods to prevent similar undesired hydrodehalogenations. Sajiki et al. used protein-supported metal catalysts for chemoselective hydrogenations. They reported using a silk fibrion-supported Pd(0) catalyst * to hydrogenate aromatic halide containing compounds in methanol with H_2 gas to get desired products in high yield[78].

Similar chemoselective hydrogenation of diverse functional groups in compounds containing aromatic halides has been realised using autoclaves where catalysts include Pt/C with formamidine acetate and Raney Ni in ethanol. Other selective hydrogenation reactions utilize Pt/C in presence of CO_2 gas, tungsten carbide (WC) in methanol and Pd/C with conc. HCl [79-82].

* The catalyst was prepared by impregnating silk fibrion with $Pd(OAc)_2$ in methanol. Silk fibrion is one of the two components of silk fiber from the silk worm *bombyx mori*.

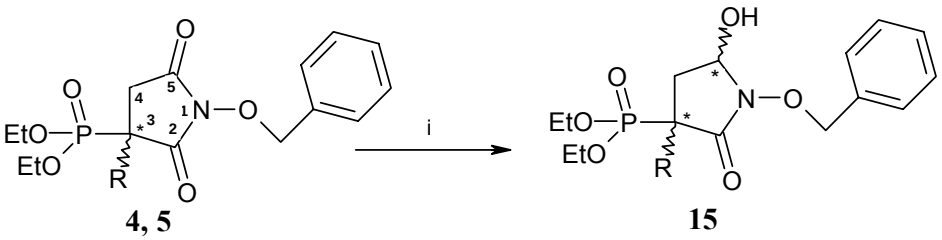
Some of these methods use expensive catalysts such as platinum or toxic nickel-based ones, have additives with low selectivity, low reaction rates or yield undesired by-products.
Wu et al. reported of using $ZnBr_2$-modulated Pd/C as a catalyst in selective hydrogenation reactions of chloro-substituted compounds. There is indication that all the de-chlorination active sites on the palladium surface are completely deactivated by $ZnBr_2$ while the hydrogenation reactivity toward the targeted groups is retained[83].

The yield of **14j** was increased by more than two fold from 25% to 56% when $ZnBr_2$ was added to the catalytic hydrogenation reaction (Table 4.3).

5 Reactivity of Succinimides

5.1 Carbonyl reduction

Synthesis of stereochemically pure compounds **15** proved to be a challenge. The reduction of the sp^2 carbonyl group to an sp^3 secondary alcohol created a new chiral center. This led to four possible isomeric products due to the additional chiral center.
In addition, there are two carbonyls in **4**,**5** that are both possible targets of reduction or even complete removal. The carbonyl at C_5 is more likely to be regioselectively reduced as it is less hindered by bulky groups unlike the one at C_2.

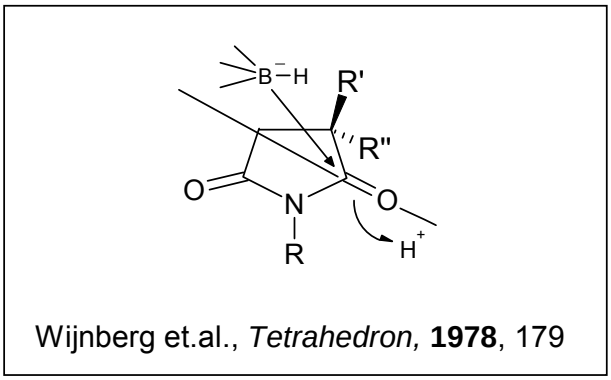


i: $NaBH_4$ or $LiAlH_4$

Scheme 5.1: R*eduction of the carbonyl moiety to hydroxyl*

Even then, Wijnberg et al. observed the opposite phenomena where the C_2 carbonyls were reduced in higher ratios compared to the evidently less hindered C_5 carbonyl groups.

They indicate that in these reactions, the hydride ion possibly approaches via the less hindered C_5 carbonyl and adds to the C_2 atom virtually along a straight line through the C=O bond (Fig. 5.1)[84].



Wijnberg et.al., *Tetrahedron,* **1978**, 179

Fig. 5.1: *Reduction via less hindered carbonyl group of succinimides*

Ring cleavage has been observed when succinimides were reduced in acidic conditions and also subsequent alkoxy formation when they were worked-up under these conditions in the presence of alcohols (Fig.5.2)[85]. Thus reduction of **4a** and **5c** was performed in basic media. The reactions were conducted in ethanol, methanol or a combination of either of them with tetrahydrofuran or water using various reducing agents such as natrium borohydride or lithium aluminium borohydride or lithium triethylborohydride[86-87].
The reaction temperatures were varied from 0ºC to -78ºC depending on which reductant was used. **15** could not be obtained in analytically pure form even though during the reaction the IR spectrum showed the replacement of imide C=O bond bands 1786 cm^{-1} and 1732 cm^{-1} by 1693 cm^{-1} which could represent an amide carbonyl.

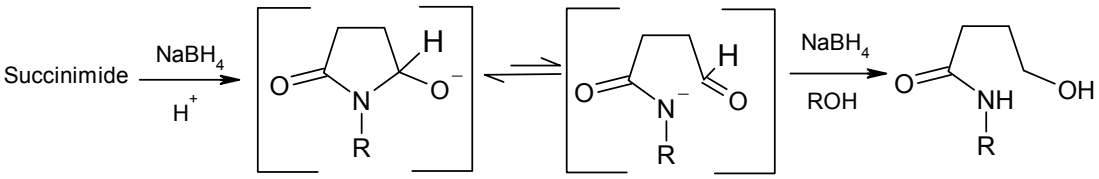
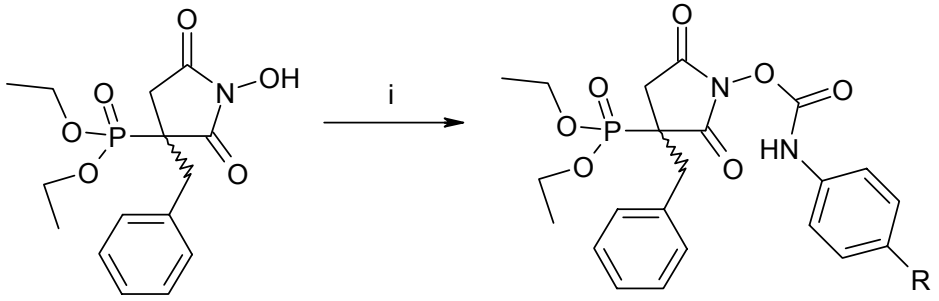


Fig. 5.2: *Ring cleavage of initially formed oxy-anion A to the amide aldehyde B and further reduction to amide alcohol C as observed by Hubert et al.*[85]

5.2 Reaction of 1-Hydroxysuccinimide 14b with isocyanates

Diethyl (3-benzyl-1-hydroxy-2,5-dioxopyrrolidin-3-yl)phosphonate (**14b**) was reacted with *p*-substituted-phenylisocyanates realizing compounds **16a-b** as analytically pure solids (Scheme 5.2, Table 5.3). The IR spectrum of **14b**[*] depicts the carbonyl bands as one very short band at 1786 cm^{-1} and one strong band at 1724 cm^{-1} whereas IR spectra of **16a-b** show two strong carbonyl bands in the ranges 1779 - 1785 cm^{-1} and 1733 -1739 cm^{-1}.



14b **16a-b**

i: TEA, 4-methoxy-phenylisocyanate or 4-trifluoromethyl-phenyl isocyanate

Scheme 5.2: *Synthesis of **16a-b***

Table 5.3: *Synthesis of **16a-b***

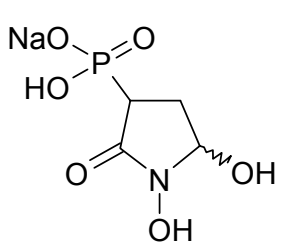
16	R
a	trifluoromethyl
b	methoxy

[*] The IR spectrum of **4a** which is very similar to that of **14b** can be seen at Fig. 2.3 in Chapter 2, Section 2.3.2.

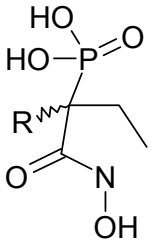
6 Opened chained analogues of SF-2312

6.1 Synthesis of N-(benzyloxy)-butanamides

The synthesis of *N*-(hydroxy)-butanamides **17a-c** as open chained and 3-substituted analogues of SF-2312 was envisioned (Fig. 6.1).



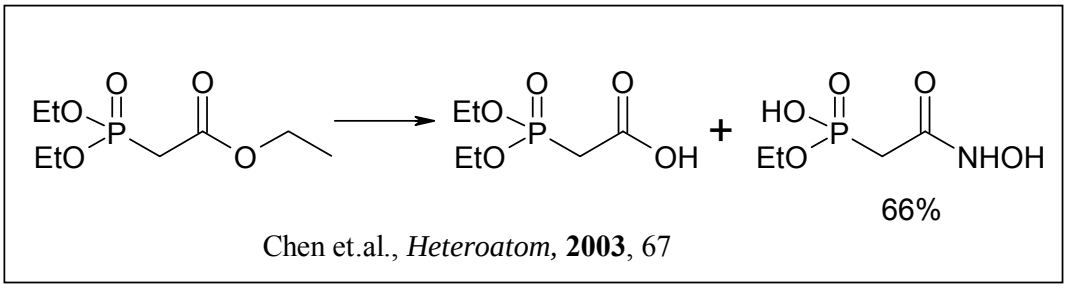
SF-2312



17a R = H
17b R = alkyl
17c R = aryl

Fig. 6.1: *Open chained analogues of SF-2312*

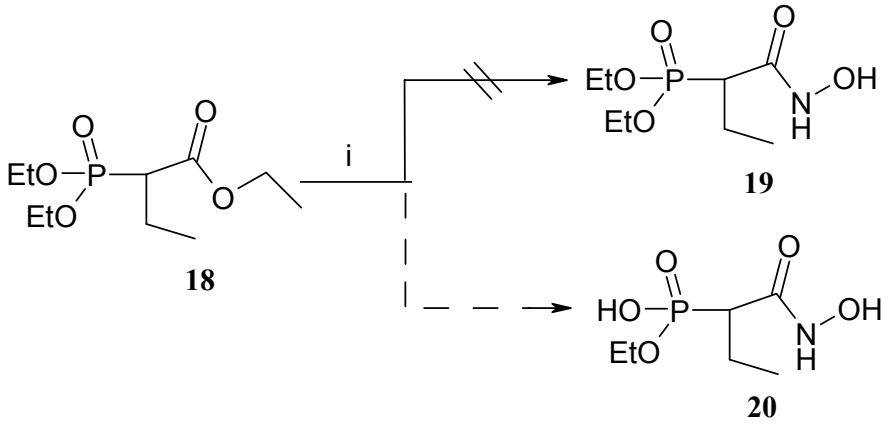
The strategy of synthesis of open chained analogues of SF-2312 (**17a-c**) was to start with the commercially available triethyl 2-phosphonobutyrate (**18**) and transform the carboxylic ester into a hydroxamic acid.
The faster route of using hydroxylamine in direct transformation of the carboxylate ester **18** to hydroxamic acid **19** was deemed unattractive due to reported intramolecular monodealkylation[88].



Chen et.al., *Heteroatom,* **2003**, 67

Fig.6.2: *Intramolecular monodealkylation by the formed hydroxamic acid.*

Chen et al. suggest that an intramolecular nucleophilic catalysis mechanism, in which an anion of the hydroxamic OH group attacks the phosphorous intramoleculary leads to monodealkylated phosphonates (Fig. 6.2).
Such a reaction would then afford **20** among other byproducts and not the desired **19** (Fig. 6.3).



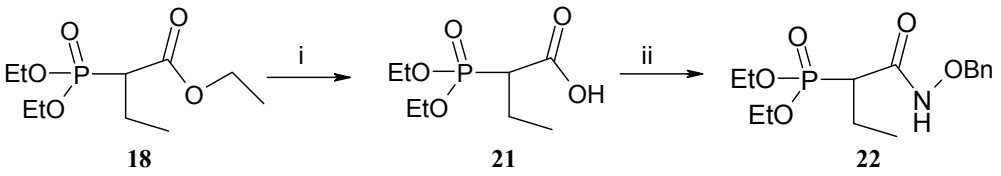
i: $NH_2OH.HCl$, KOH

Fig. 6.3: *Synthesis of open chained hydroxamic acids*

Compounds **17a-c** (Fig. 6.1) would thus be elaborated via hydrolysis of esters to form carboxylic acids and subsequent hydroxamic acid formation to avoid phosphonate ester dealkylation (Scheme 6.1).

6.1.1 Ethyl esters as phosphonate protecting groups

In a two step procedure, triethyl 2-phosphonobutyrate **18** was reacted with potassium hydroxide base in a 7:3 solution of ethanol and water. After work up and acidification the pure acid **21** was obtained as oil in good yield of 80%. This oil was dried in vacuo for 1 h and then reacted with 1,1′-carbonyldiimidazole (CDI) and benzyloxyamine in dry dichloromethane to afford 46% of the targeted benzyl 2-(diethoxyphosphoryl)butanoate (**22**). (Scheme 6.1)



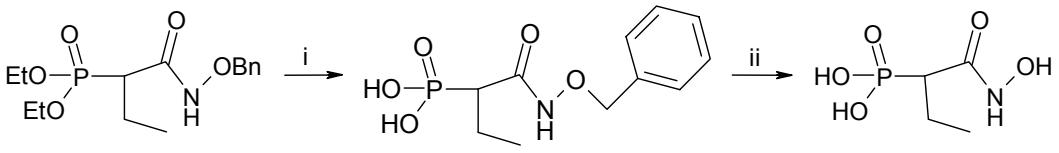
18 **21** **22**

i: KOH, EtOH/ H_2O, 24h ii: CDI, NH_2OBn, CH_2Cl_2

Scheme 6.1: *Synthesis of benzyl-protected hydroxamic acid **22***

The reaction was monitored by IR spectroscopy until complete replacement of the carboxylic acid's carbonyl band 1732 cm^{-1} with a carbonyl band of the benzyl protected hydroxamic acid **22** at 1685 cm^{-1}.

Trimethylsilyl bromide was used to cleave the phosphonate ethyl groups to afford the phosphonic acid **23** in good yield of 82% (Scheme 6.2). Unlike the very hygroscopic phosphonic acids **7** (Chapter 3), this phosphonic acid was obtained as a stable white powder. The IR spectrum of **23** exhibits a shift from **22**'s carbonyl band at 1685 cm^{-1} to 1628 cm^{-1} (CONH).



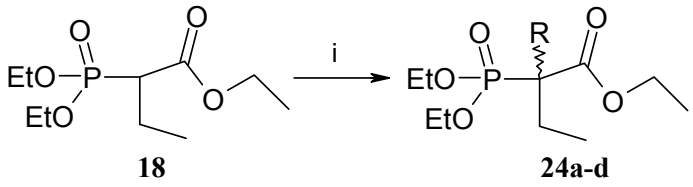
22 **23** **17a**

i: 1. TMSBr 2. H_2O; ii: Pd/ C, $H_{2(g)}$

Scheme 6.2: *Deprotection of phosphonic and hydroxamic acid functionalities*

Catalytic hydrogenation of **23** yielded a rather hygroscopic hydroxamic acid **17a** whose structure was confirmed by ^{1}H-NMR spectra. It's IR spectrum shows the carbonyl band at 1654 cm^{-1} (Scheme 6.2).

The next step was to vary the open chained compound **17a** by substitution at C-α. Here triethyl 2-butylphosphonate **17** was first alkylated in dry THF using NaH to afford **24a-d** in good yields of 70% - 85% (Scheme 6.3, Table 6.1).



i: NaH, RBr

Scheme 6.3: *Synthesis of 3-substituted compounds **24a-d***

Table 6.1: *3-Substituted open chained compounds **24a-d***

24	R	Yield%
a	benzyl	85
b	4-fluorobenzyl	70
c	methyl	85
d	allyl	85

The yields of the methyl and allyl substituted derivatives **24c** and **24d** were calculated from the ^{1}H-NMR spectra where the amount of remaining methine protons from the educt indicated that 85% had already reacted and been substituted with an alkyl group.

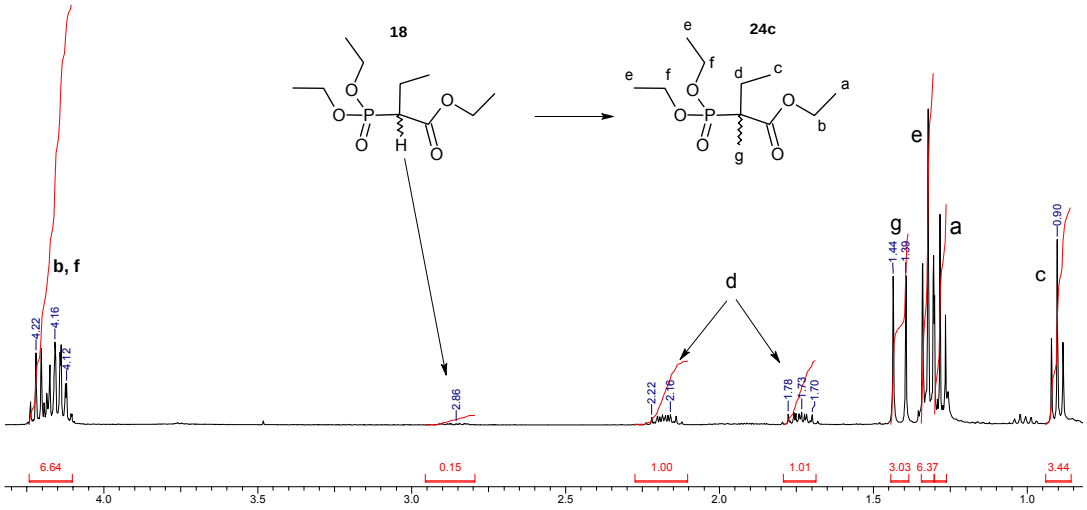
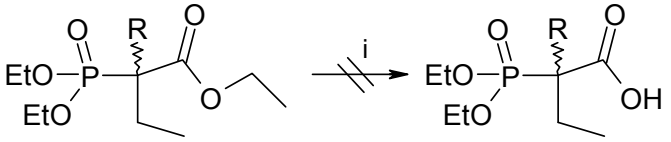


Fig. 6.4: *^{1}H-NMR spectrum of **24c** in $CDCl_3$ showing 0.15 protons* at 2.86 ppm left from the methine proton of the educt triethyl 2-butylphosphonate (**18**)*

* 0.15 proton at 2.86 ppm was intergrated in relation to 1 proton of the methylene proton of one of the methylene protons at 2.16 ppm.

Subsequent cleavage of the carbonate ethyl ester for eventual hydroxamic acid formation via 1,1′-carbonyldiimidazole (CDI) like in the synthesis of **17a** (Scheme 6.2) was envisioned.

Compounds **24a**,**b** were dissolved in a mixture of ethanol and water or just methanol and treated with equimolar amounts of potassium hydroxide or barium hydroxide. Thin layer chromatography for these hydrolysis reactions showed a lot of non-reacted educt despite use of higher temperatures and longer time (Scheme 6.3) unlike compound **18** (Scheme 6.2) which was hydrolyzed in exactly 24 hours at room temperature.

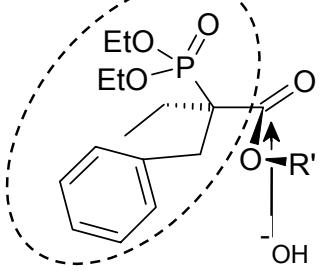


24a-b **25a-b**

i: KOH or $Ba(OH)_2$ or NaOH, EtOH /H_2O

Scheme 6.4: *Futile hydrolysis of **24a-b***

Roberston J.(**Fig. 6.5**) suggests that OH group attack of the carbonyl carbon is hindered by increased steric bulk in its proximity, in this case by the bulky benzyl groups.

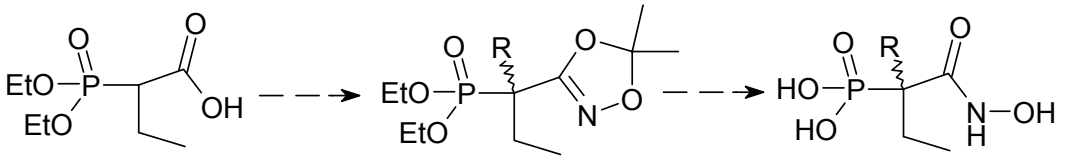


*Robertson J., **Protecting Group Chemistry**, Oxford University Press-USA. **2000**, Ch. 3*

Fig. 6.5: *Nucleophilic attack of carbonyl carbon in **24a***

To resolve this, the hydroxamic acid would be introduced at an earlier stage* in the synthesis sequence in a different approach where it would be masked in a dioxazole ring according to a procedure by Geffken et al.[90].

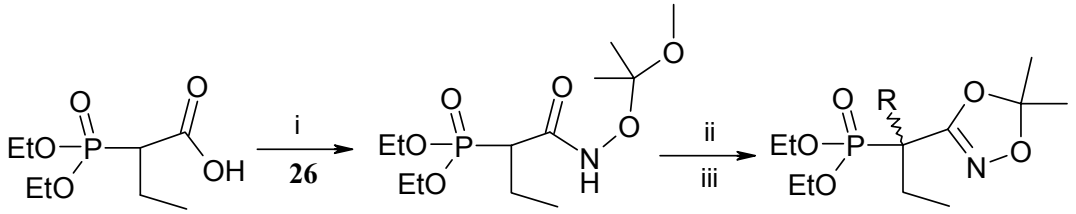
The dioxazole derivative (**28a**) would subsequently be substituted via alkylation then phosphonate ester deprotection before ion exchange deprotection to free the hydroxamic acid. (Fig. 6.6)



21 **28a-b** **17a-c**

Fig. 6.6: *Proposed synthesis of open chained analogues **17a-c***

O-(1-methoxy-1-methylethyl) hydroxylamine (**26**) was prepared in a slightly modified procedure of Froböse[91] in 56% yield. **21** was reacted with CDI and hydroxylamine **26** in dry dichloromethane affording **27** in high yield of 95% colourless oil. The IR spectrum of **27** shows the carbonyl band at 1673 cm^{-1} (Scheme 6.5).



21 **27** **28a-b**

i: CDI, NH_2OR (**26**) ii: cyclohexane, reflux iii: NaH, benzylbromide

Scheme 6.5: *Synthesis of dioxazole derivatives **28a-b***

* The hydroxamate is generally added in its protected form at the end of synthesis sequences because of its labile and diprotic nature[89](Fig. 1.4, Ch. 1).

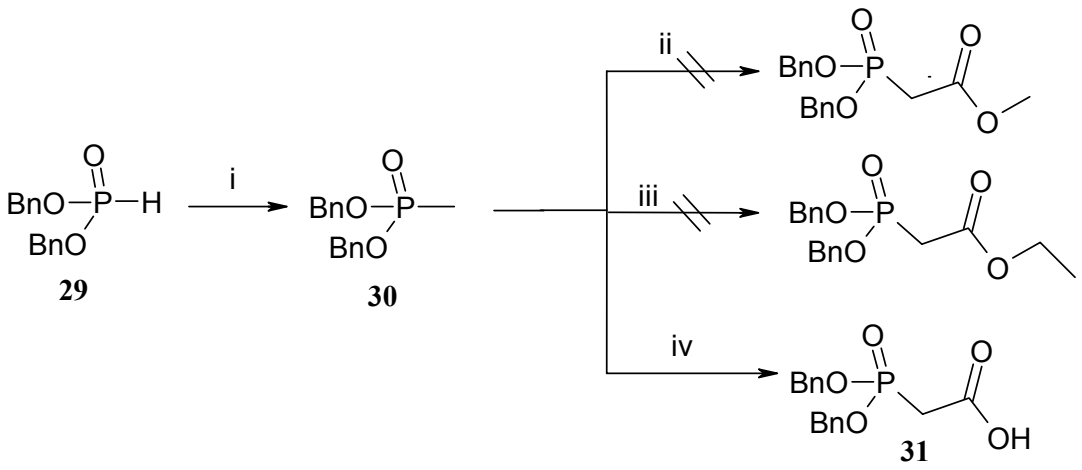
Table 6.2: *Synthesis of dioxazole derivatives* ***28a-b***

28	**R**
a	H
b	benzyl

The 5,5-dimethyl-1,4,2-dioxazole derivative **28a** was realized by reflux of **27** in cyclohexane in 48% yield. **28a** was alkylated using benzylbromide to yield benzylated **28b**. Only 15% was however achieved in analytically pure quantity as column chromatography resulted in an additional compound with ester like band at 1736 cm^{-1} (Scheme 6.5).

6.1.2 Benzyl esters as phosphonate protecting groups

The same reactions and procedures used for ethyl phosphonate protected compounds as reported in section 6.1.1 were tested on benzyl phosphonate derivatives. This was in attempt to improve yields in the reactions that did not work or that led to non remarkable results while using ethyl esters as protecting groups

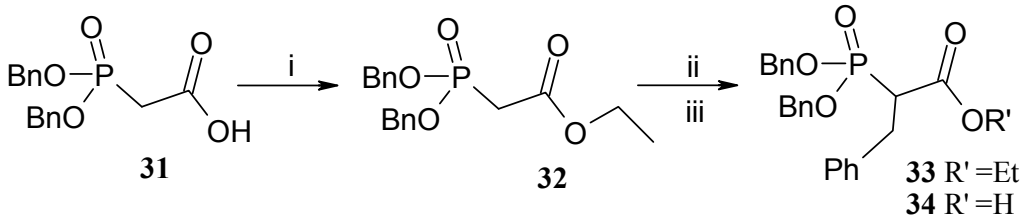


i: n-BuLi, MeI; ii: n-BuLi, dimethyl carbonate; iii: n-BuLi, ethyl choroformiate; iv: n-BuLi, dry ice

Scheme 6.6: *Synthesis of [bis(benzyloxy)phosphoryl] acetic acid **31***

Dibenzyl methylphosphonate (**30**) was prepared via methylation of dibenzyl phosphite (**29**) with methyl iodide. Reaction of **30** with dimethylcarbonate or ethylchloroformiate to form methyl and ethyl carboxylic esters yielded many undesired by-products.

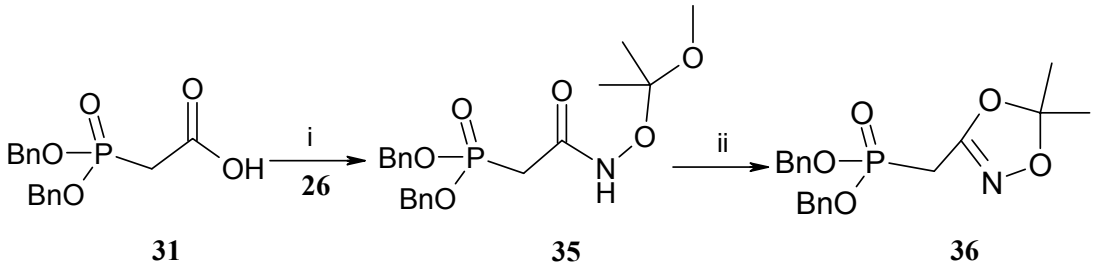
Therefore, 2-[bis(benzyloxy)phosphoryl] acetic acid (**31**) was first prepared for subsequent esterification by carboxylation of **30** with dry ice (CO_2) in good yield of 65% (Scheme 6.6).



i: EtOH, H_2SO_4, 4days ii: NaH, BnBr iii: KOH

Scheme 6.7: *Esterification and alkylation reactions*

Compound **32** was readily synthesised as yellow oil in 87% yield by stirring **31** in ethanol with 3-5 drops of concentrated sulphuric acid. This product was then alkylated to yield benzyl derivative **33** in 54% yield (Scheme 6.7)
Unlike the di-substituted ethyl phosphonates **24a-b** (Scheme 6.4), **33** could successfully be hydrolyzed to yield the carboxylic acid **34**.



Scheme 6.8: *Dioxazole ring masked hydroxamic acids*

(Dibenzylphosphono) acetic acid **31** was also reacted with CDI and *O*-(1-methoxy-1-methylethyl) hydroxylamine (**26**) to yield the benzyl phosphonate analogue of **27** (Scheme 6.5) as stable and solid compound **35** in 69% yield (Scheme 6.8).
Cyclization of **35** to get dibenzyl [1-(5,5-dimethyl-1,4,2-dioxazol-3-yl)propyl]phosphonate (**36**) was realized under microwave-assisted synthesis.

Microwave irradiation enables rapid and uniform heating of a reaction mixture. This occurs when molecules present in the reaction meduim absorb microwave energy and convert it to heat. This direct and efficient internal (incore) heating leads to reactions with less byproducts, dramatically reduced reaction times and higher yields[92].

Having observed the formation of an unknown and insoluble sticky brownish oil in the cyclization of ethyl phosphonate **27*** in toluene and cyclohexane using conventional synthesis (Scheme 6.5), the aim for using microwave irradiation in this case was then not confined to just reducing reaction times but also aimed at avoiding of this sticky and oily byproduct.
Thus after establishing a method (12psi, 290W, 100°C)** in the microwave-assisted synthesis where most of the intermediate **35** was cyclized into its dioxazole derivative **36** in an impressive 5 - 20 min, the solvents were varied in attempt to avoid the oily byproduct.

In a microwave enhanced organic reaction, boiling points of solvents are less of an important factor as they are in conventional chemistry. This is because microwave processing will reach and bypass the boiling point of most solvents in a matter of seconds, especially in sealed-vessel systems[93].
In this cyclization reaction, the temperature needed as reported[90] is 80°C and the solvent used was cyclohexane (B.p = 81°C). This rules out the use of solvents such as ethyl acetate (B.p. = 77°C) or dichloromethane (B.p. = 40°C) in conventional chemistry even though they might and did indeed give higher yields and purer products in the cyclization of benzyl phosphonate derivative **35** when microwaves were applied.

As shown in table 6.3; THF, dichloromethane and ethyl acetate gave clear and near complete reactions according to IR spectra where the 1684 cm^{-1} (C=O) was replaced with 1630 cm^{-1}.

* See conventional synthesis of **28a** in the experimental part at Section 8.6.1 where the solution had to be decanted to separate the brownish oily sediment from the decantant (solution got from decantation) that contained the cyclized product before further purification by column chromatography.
** Input values

Table 6.3: *Variation of irradiation times and solvents during cyclization of* ***35***

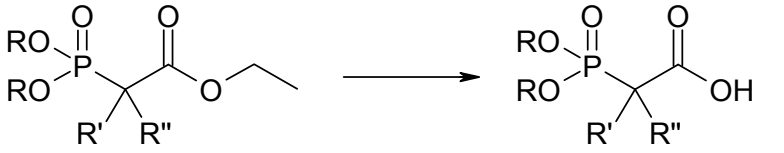
Solvent	Time (min)	Solution
THF	15	clear
Cyclohexane	10	oily sediment
EtOAc	10	clear
None	5	-
CH_2Cl_2	20	clear

However, after THF and dichloromethane were removed by evaporation and oil dissolved in less polar diethyl ether for cystallisation purposes, the oily by-product was formed at the bottom of the flask. This was not observed in the ethyl acetate reaction.
Though the standard time was set at 10 min with IR spectra checked every 5 min, the THF and dichloromethane reactions needed extra time to be completed while in the case where no solvent was used, the reaction was complete in 5 min. Isolation of analytically pure product **35** from these reactions via cystallisation in dichloromethane/ hexane or ethyl acetate/hexane was futile. More irradiation time in these reactions led to decomposition

The reaction in ethyl acetate was most successful where **36** was prepared in good yield of 83% as analytically pure solid which would allow for further development of this synthesis sequence to achieve open-chained analogues of SF-2312 (**17a-c**).

6.1.3 Conclusion

Unlike compounds **24a-b** that are di-substituted at the α-position, both ethyl and benzyl ester protected phosphonates **18** and **33** with only one alkyl or aryl substitution at α-position were readily hydrolyzed into their respective carboxylic acids **21** and **34** using potassium hydroxide in ethanol (Scheme 6.9).

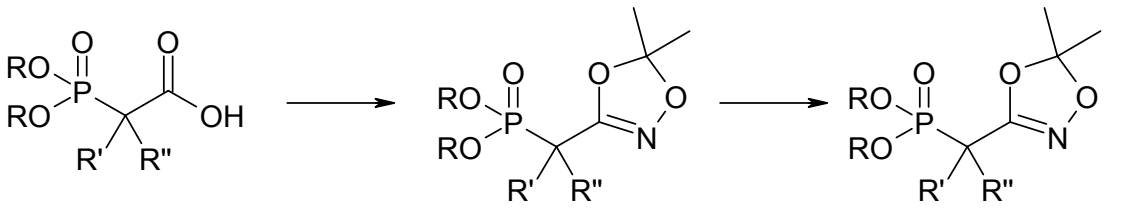


18 R,R' = Et; R" = H
24a-b R,R' = Et; R" = Aryl
33 R = Bn; R',R" = H

21 R,R' = Et; R" = H
34 R = Bn; R',R" = H

Scheme 6.9

2-(Diethoxyphosphoryl) butanoic acid (**19**) was successfully transformed into dioxazole functionalized **28a** in 48% yield and also subsequently alkylated into **28b** albeit in low yield of 15%. (Dibenzylphosphono) acetic acid (**31**) was also successfully made into dioxazole functionalized **36** in good yield of 83% (Scheme 6.10).



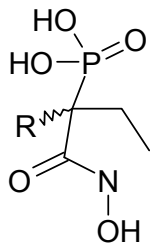
19 R,R' = Et; R" = H
31 R = Bn; R',R" = H

28a R,R' = Et; R" = H
36 R = Bn; R',R" = H

28b R,R' = Et; R" = Bn

Scheme 6.10

Although they have different substitution at C-α, the benzyl ester protected phosphonate compounds generally gave higher yields than ethyl ester protected ones.



17a R =H
17b R=alkyl
17c R=aryl

Fig. 6.7

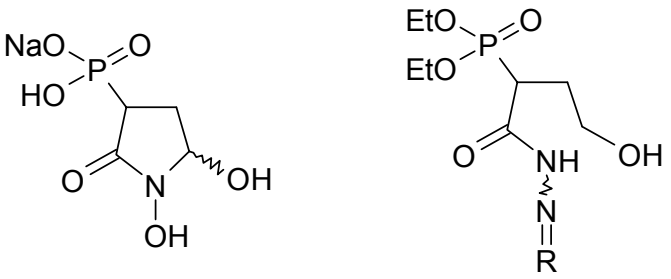
The targeted open chained analogues of SF-2312 (**17a-c**) may be prepared in the following steps: alkylation of the ethyl carbonate derivative **32** (Scheme 6.7) → hydrolysis to carboxylic acids → benzyl protected hydroxamic acid formation → deprotection of all benzyl protecting groups by catalytic hydrogenation. Alternatively, alkylation of dioxazole masked derivative **36** (Scheme 6.10) and subsequent deprotection of the hydroxamic acid and phosphonic acid moieties could be used.

With regard to hygroscopic derivative **17a**, it is pertinent to note that deprotection of both phosphonic and hydroxamic acid moieties could lead to similar hygroscopic products and thus phosphonate pro-drug synthesis or salt formation is recommended.

6.2 Synthesis of acylhydrazone analogues of SF-2312

Hydrazides and hydrazones are well used pharmacophores in medicinal chemistry of antimicrobial drugs. Isonicotinic acid hydrazide (Isoniazid) has been an important agent in tuberculosis therapy since 1952, when its action against *Mycobacterium tuberculosis* was first discovered**.** Hydrazone derivatives of isoniazid demonstrate comparable antimicrobial activity[94].

With this knowledge, open chained analogues of SF-2312 where the hydroxamic acid is replaced by either hydrazide (**39**) or acylhydrazone (**40a-c**) moieties were synthesised (Fig.6.8). Due to reported and hitherto experienced hygroscopicity of phosphonic acid derivatives (Chapter 3) compounds **39** and **40a-c** were obtained as ethyl phosphonates.



SF-2312 **39, 40a-c**

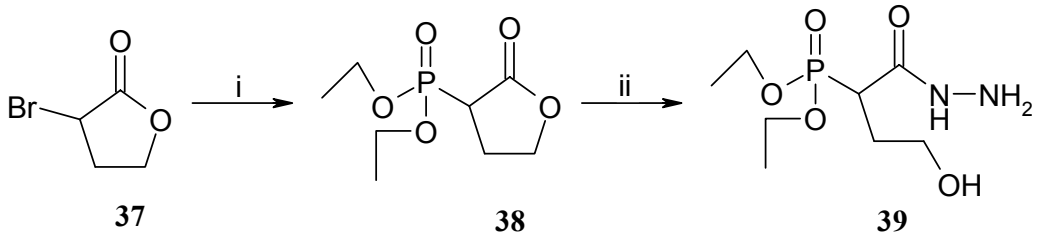
Fig. 6.8: *Acylhydrazide (**39**) and acylhydrazone (**40a-c**) open chained analogues of **SF-2312***

The commercially available α-bromo-γ-butyrolacton (**37**) was reacted in a Michaelis-Arbusov reaction using triethyl phosphite to form diethyl (2-oxotetrahydrofuran-3-yl)phosphonate (**38**) in good yield of 89%.
The IR spectrum shows the carbonyl band at 1770 cm^{-1} (Scheme 6.9).

The γ-butyrolacton ring of **38** was opened using hydrazine hydrate* in methanol affording diethyl [1-(hydrazinocarbonyl)-3-hydroxypropyl]

* The very toxic and carcinogenic hydrazine has for long been thought to be a man-made compound until recent discovery of unusual bacteria such as Brocadia anammoxidans that consume ammonia, producing hydrazine that they safely store in specialized organelles[95]

phosphonate (**39**) in high yield of 90%. This reaction was monitored by IR spectroscopy showing a shift of the carbonyl band from 1770 cm^{-1} of **38** to 1670 cm^{-1} (**39**).

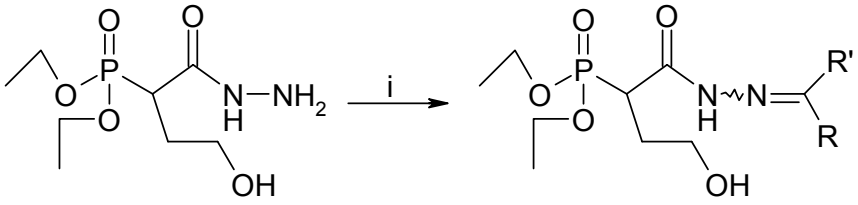


37 **38** **39**

i: TEP, 130°C ii $N_2H_4.H_2O$

Scheme 6.9: *Synthesis of acylhydrazide **39***

Acylhydrazones **40a-c** were obtained when **39** was condensed with respective ketones and aldehydes (Scheme 6.10, Table 6.4). The solid compounds were afforded in yields of 32 – 97%.



39 **40a-c**

i: 2,6-dichlorobenzaldehyde or 1-(4-phenoxyphenyl)propan-1-one or 1-(4-chlorophenyl) ethanone, MeOH

Scheme 6.10: *Synthesis of acylhydrazones **40a-c***

Table 6.4: *Acylhydrazone derivatives* ***40a-c***

40	R	R′	Yield%
a	4-chlorophenyl	methyl	42
b	4-phenoxyphenyl	ethyl	32
c	2,6-dichloro	H	97

The carbonyl bands on the IR spectra were observed at 1664 cm^{-1} – 1670 cm^{-1} (CONH) and a strong NH band that had not been observed in IR spectra of **39** was present at 3297 – 3393 cm^{-1} (Fig. 6.9).

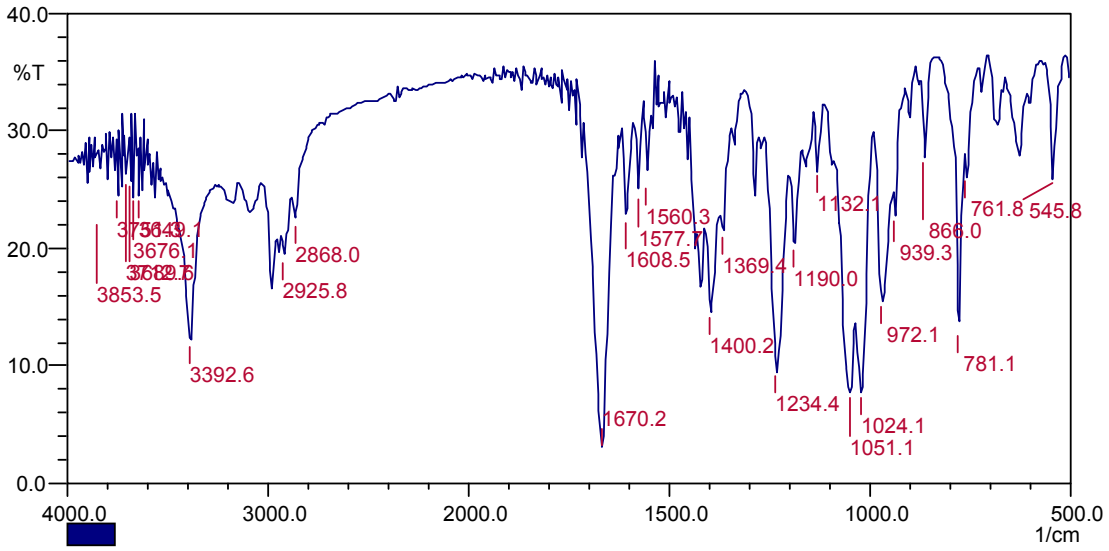


Fig. 6.9: *IR-spectrum (E/Z)-**40c** showing a sharp NH band at 3392cm^{-1}*

^{1}H-NMR spectra of compounds **40a-c** revealed split signals for the NH proton for the mixture of *Z* and *E* isomers (Fig. 6.11).
The isomeric mixture's signals were also observed in ^{13}C-NMR spectra as shown in the ^{13}C-NMR spectrum for **40c** (Fig. 6.10).

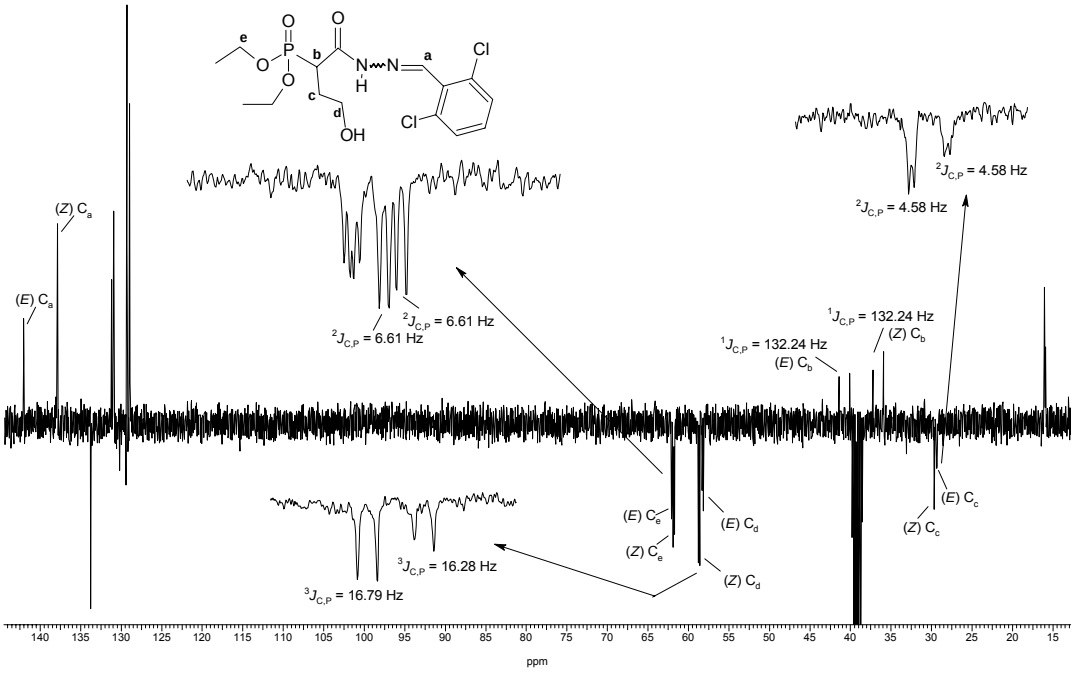


Fig. 6.10: *^{13}C-NMR spectrum of (E/Z)-**40c** showing the $J_{C,P}$ coupling constants.*

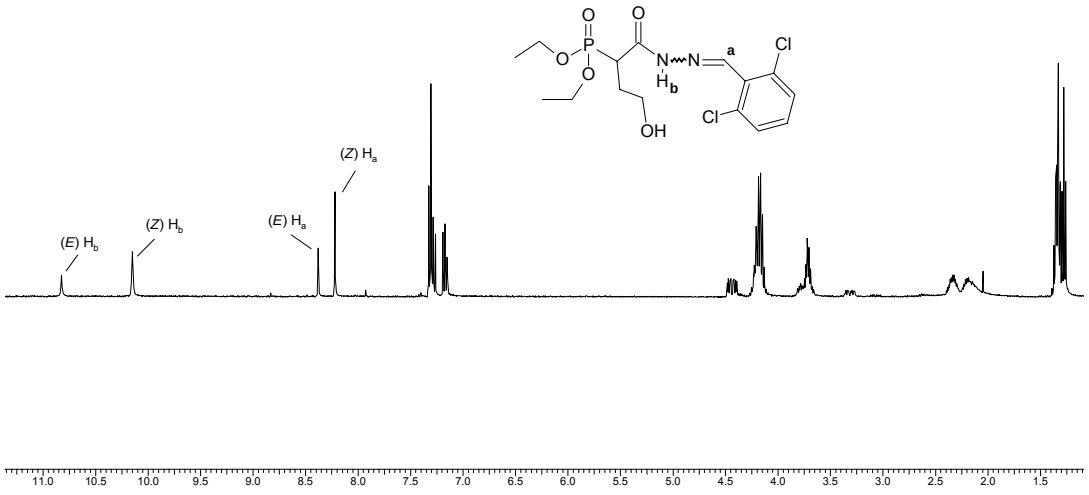
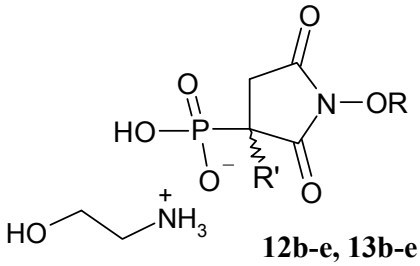


Fig. 6.11: *^{1}H-NMR spectrum of (E/Z)-**40c***

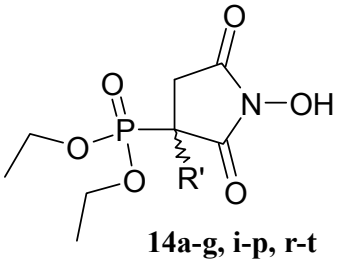
7 Biological studies

7.1 Biological studies on ethanolamine monosalts 12 and 13

12b-e and **13b-e** did not inhibit the growth of *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecalis* and *Pseudomonas aeruginosa* when screened under in vitro conditions. It is however pertinent to note that there exists an 'in vitro in vivo paradox' in antibiotic therapy whereby successful treatment with antibiotics has been achieved against bacteria that have shown resistance to the same antibiotics in vitro. Scortti et al. have reported that the Sodium salt of Fosfomycin is indeed effective against *Listeria monocytogenes* a bacteria that causes septicemia, abortion and meningoencephalitis although it shows resistance to this drug under in vitro conditions [96].



12b-e, 13b-e



14a-g, i-p, r-t

Fig. 7.1: *Compounds tested for biological activity**

7.2 Biological studies on dialkyl [3-(aryl)-1-hydroxy-2,5-dioxopyrrolidin-3-yl] phosphonates (14)

In vitro fungicidal activity by 1-hydroxy derivatives **14a-g,i-p,r-t** was observed in tests run by E.I Dupont de Nemours, Nework-Wilmington /USA. These results were unfortunately not confirmed in the greenhouse. These and other selected compounds are still under investigation for other antimicrobial activity in conjunction with Bayer AG and the University of Antwerp/ Belgium.

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* See Table 3.2, Ch. 3 for R definition in compounds **12b-e**, Table 4.1, Ch.4 for R definition in compounds **13b-e** and Table 4.2 , Ch. 4 for R definition in compounds **14a-g,i-p,r-t**

8 Experimental

8.1 General information

Melting points

Mettler FP62

IR spectra

Shimadzu FT-IR 8300
Measured as KBr -pressling or as film on NaCl plate

^{1}H-NMR - spectra

Bruker AMX 400 (400 MHz)
Chemical shift δ measured in ppm
Internal standard: Trimethylsilane (TMS)
Number of protons determined by integration
Abbreviation of signal multiplicity;
s = singlet, d = duplet, t = triplet, q = quartet, m = multiplet
Coupling constant *J* measured in Hz

^{13}C-NMR - spectra

Bruker AMX 400 (100,6 MHz)
Chemical shift δ measured in ppm
Internal standard: Trimethylsilane (TMS)

^{31}P-NMR - spectra

Bruker DXR 500 (202,5 MHz)
Chemical shift δ measured in ppm

Mass Spectra

HRFAB-Mass spectra: Mass spectrometer VG 70-250S
ESI- Mass spectra: Varian MS 1200L

Elemental analysis

Heraeus CHN-O-Rapid

Thin layer chromatography

DC-Mikrokarten Polygram SIL G/UV_{254}, from the Macherey-Nagel Firm, Duren
Thickness: 0.25 mm

Column chromatography

Kieselgel ICN Silica 100-200, aktiv 60A
Kieselgel 60 (particle size 0.015-0.040mm), Merck

Drying agent for organic phases

Dry Magnesiumsulfat

8.2 General procedures

General Michaelis-Arbusov procedure **GP-1**

5 mmol of 1-benzyloxy-3-bromopyrrolidin-2,5-dione (**3**) was refluxed in a solution of the appropriate trialkyl phosphite (30 mmol) for 2 h. Excess trialkyl phosphite was removed in vacuo and the oily residue was purified by column chromatography (4:1 ethyl acetate: *n*-hexane, 100 mL). The resulting pale yellow oils were dried under vacuum to yield solid compounds **4a-c**.

General alkylation procedure using sodium hydride **GP-2**

To a stirred solution of the electrophiles (2 mmol) in dry THF (5 mL), under nitrogen atmosphere was added sodium hydride (2 mmol) portion wise over a period of 10 min. After stirring for 10 min, a solution of the appropriate alkyl (aryl) halide (2 mmol) in dry THF (2 mL) was added drop wise. The reaction mixture was stirred for 90 min, poured into ice cold 1M HCl solution and extracted with ethyl acetate (3 x 10 mL). The combined organic layers were washed with brine (1 x 10 mL), dried over $MgSO_4$ and evaporated. The oily residues were purified by column chromatography (3:2, ethyl acetate/ *n*-hexane elution) to give oily or solid compounds crystallised from EtOAc/ *n*-hexane.

General ethanolamine monosalt synthesis procedure **GP-3**

To a solution of compounds **4a, 5a-e** (3 mmol) in dry dichloromethane (5 mL) was added trimethylsilyl bromide (6 mmol) and the reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated and the remaining oil was dissolved in MeOH (3 mL), treated with water (0.1 mL), and stirred for 10 min. The solvents were removed under reduced pressure and the residue dried in vacuo for 12 h. The resulting phosphonic acids **7** were dissolved in dry THF (5 mL), and ethanolamine (2.7 mmol) dissolved in 2 mL dry THF was added drop wise. The solution was stirred for 1 h at RT and then kept at −18°C for 2 days. The solids formed were filtered and recrystallized from methanol/ diethyl ether to give **12a–f** as white solids.

General catalytic hydrogenation procedure **GP-4**

Compounds **12a–f, 5a-t** and **22** (5 mmol) were dissolved in MeOH (100 mL), Pd/C 10% (300 mg) was added and the resulting mixture was hydrogenated for 90 min at 2 bar. The suspension was then filtered through an SPE tube RP-18. The solvent was evaporated and the respective solid products crystallized from ethyl acetate/ *n*-hexane
Compounds **5j,l,n** (5 mmol) were hydrogenated using this procedure but in ethyl acetate (100 mL) under 1 bar of pressure. 0.2 equivalent $ZnBr_2$ was added as catalyst during the a second yield improving reaction for **5j**.

General synthesis procedure for **16a** and **16b**: **GP-5**

To a solution of diethyl (3-benzyl-1-hydroxy-2,5-dioxopyrrolidin-3-yl)-phosphonate (**14b**) in dry dichloromethane (3 mL) was added 1 molar equivalent 4-trifluoromethyl-phenylisocyanate for (**16a**) or for **16b** 4-methoxy-phenylisocyanate. After stirring for 48 h, the solvent was evaporated and the crude product crystallized from diethyl ether/ *n*-hexane to yield solid compounds **16a** and **16b**.

Hydrolysis of ethyl esters to synthesize carboxylic acids **GP-6**

2 mmol of the appropriate ethyl ester was dissolved in a 7:3 solution (2 mL) of ethanol and water. Potassium hydroxide (2 mmol, 0.112 g) dissolved in similar ethanol/ water solution (3 mL) was added and the mixture stirred for 24 h at room temperature. Solvents were evaporated at reduced pressure; the oily residue was washed with diethyl ether (3 x 10 mL) and water (5 mL) added to the residue followed by acidification to pH 1 using 6M HCl. NaCl was added to saturation to this aqueous phase which was the extracted with dichloromethane (3 x 10 mL). The organic layer was dried ($MgSO_4$) and evaporated to yield the desired carboxylic acids

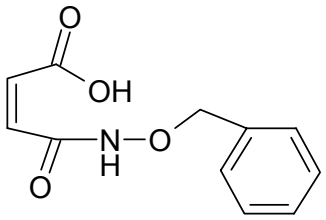
8.3 Procedures and Analytical Data for Chapter 2

8.3.1 Educt synthesis (Section 2.2.1)

Synthesis procedure for **1**

According to Lit.[60], benzyloxyamine (50 mmol) was added drop wise to a stirred suspension of maleic anhydride (4.90g, 50 mmol) in dichloromethane (50 mL). The exothermic reaction was complete after 20 min. The solvent was removed in vacuo and the compound crystallized from 2-butanone.
1 was used immediately after structure confirmation by melting point and IR spectra for the synthesis of intermediate **2**.

N-Benzyloxymaleimic acid **1**



Yield: 98%, colourless rods

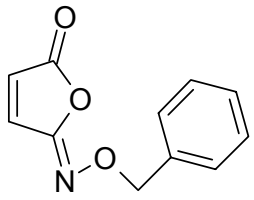
M.p.: 122 °C (Lit.[60])

IR (KBr): 1705 cm^{-1}, 1633 cm^{-1}, 1567 cm^{-1}, 1537 cm^{-1}

Synthesis procedure for **2**

Following a slightly modified literature method[97], 50 mmol (11.1 g) of *N*-benzyloxymaleimic acid (**1**) was refluxed for 2.5 h with thionyl chloride (22 mL). The solution was cooled to 5°C and pyridine (22 mL) was added drop wise. After 30 min stirring, the mixture was slowly poured onto 200 mL ice water while stirring. The separated aqueous layer was extracted with dichloromethane (2 x 15 mL) and the combined organic layers washed with 0.1M HCl (2 x 10 mL), then $NaHCO_3$ (2 x 10 mL), dried over $MgSO_4$ and filtered . The solvent was reduced under pressure and the compound crystallized from dichloromethane and *n*-hexane.
As **2** is fully characterized in Lit., it was used immediately for further synthesis of intermediate compound **3** after structure confirmation by IR spectra and melting point.

N-benzyloxyisomaleimide **2**



Yield: 88%, colourless needles

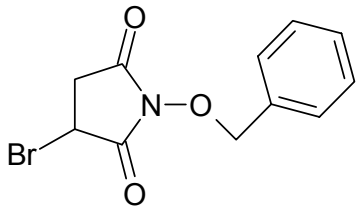
M.p.: 79 °C (Lit.[97])

IR (KBr): 1793 cm^{-1} (C=O), 1693 cm^{-1} (CONH)

Synthesis procedure for **3**

According to Lit.[57] procedure, a solution of *N*-benzyloxyisomaleimide (**2**) (20 mmol, 4.06g) and 33% HBr in acetic acid was stirred at room temperature for 5h. The mixture was poured into 100 mL water stirred and then filtered. The solid was washed with water (2 x 10 mL) and then recrystallized from ethanol.
3 was used immediately for further synthesis of fully characterized compounds **4a-c** after structure confirmation by IR spectra and melting point.

1-benzyloxy-3-bromopyrrolidin-2,5-dione **3**



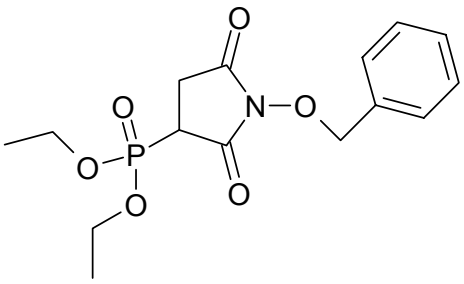
Yield: 93%, colourless needle-like crystals

M.p.: 98 °C (Lit.[57])

IR (KBr): 1797 cm^{-1}, 1733 cm^{-1} (CO)

8.3.2 Michaelis-Arbusov synthesis of phosphonates 4a-c (Section 2.3)

Diethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4a**)



From 14.2 g 1-benzyloxy-3-bromopyrrolidin-2,5-dione (**3**) according to **GP-1**

Yield: 77%, light green powder

M.p.: 88 °C

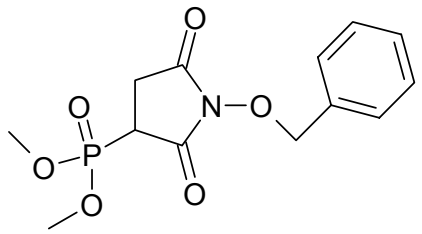
IR (KBr): 1787 cm^{-1}, 1728 cm^{-1}, (C=O), 1255 cm^{-1} (P=O), 1020 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.49 - 7.40 (m, 5H, ArH), 5.00 (s, 2H, CH_2Ph), 4.13 - 4.05 (m, 4H, CH_2), 3.74 - 3.65 (m, 1H, PCH), 3.08 – 2.99 (m, 1H, CH_2), 2.69 - 2.66 (m, 1H, CH_2), 1.23 - 1.27 (m, 6H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 169.1, 166.6 (C=O), 133.2 ($ArC_{quart.}$), 128.6, 129.5, 129.9 ($ArC_{tert.}$), 78.9 (CH_2Ph), 63.4 (d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 63.9 (d, $^{2}J_{C,P}$ = 7.12 Hz , POC), 36.9 (d, $^{1}J_{C,P}$ = 142.9 Hz, PCH), 27.9 (CH_2), 16.4 (t, $^{3}J_{C,P}$ = 5.10 Hz, CH_3)

$C_{15}H_{20}NO_6P$
Requires [%]: C 52.79, H 5.91, N 4.10
Found [%]: C 52.97, H 5.99, N 4.09

Dimethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4b**)



From 14.2 g 1-benzyloxy-3-bromopyrrolidin-2,5-dione (**3**) according to **GP-1**

Yield: 64%, amorphous powder

M.p.: 85 °C

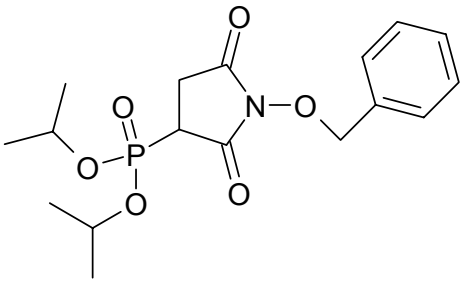
IR (KBr): 1787 cm^{-1}, 1728 cm^{-1}(C=O), 1257 cm^{-1} (P=O), 1031 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, DMSO-d_6) = δ (ppm) 7.49 - 7.40 (m, 5H, ArH), 5.01 (s, 2H, CH_2Ph), 3.70 - 3.62 (m, 4H, $POCH_2$), 3.08 - 2.98 (m, 1H, PCH), 2.76 - 2.74 (m, 1H, CH_2), 3.81 - 3.76 (m, 1H, CH_2)

^{13}C-NMR: (101 MHz, DMSO-d_6) = δ (ppm) 169.9, 167.3 (C=O), 134.1 ($ArC_{quart.}$), 129.9, 129.5, 128.8 ($ArC_{tert.}$), 78.5 (CH_2Ph), 54.0 (d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 53.7 (d, $^{2}J_{C,P}$ = 6.62 Hz, POC), 27.9 (CH_2), δ 35.9 (d, $^{1}J_{C,P}$ =140.39 Hz, PC)

$C_{13}H_{16}NO_6P$
Requires [%]: C 49.85, H 5.15, N 4.47
Found [%]: C 49.51, H 5.20, N 4.48

Diisopropyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4c**)



From 14.2 g 1-benzyloxy-3-bromopyrrolidin-2,5-dione (**3**) according to **GP-1**

Yield: 63%, yellow oil

IR (Film): 1799 cm^{-1}, 1739 cm^{-1} (C=O), 1261 cm^{-1} (P=O), 999 cm^{-1} (POC)

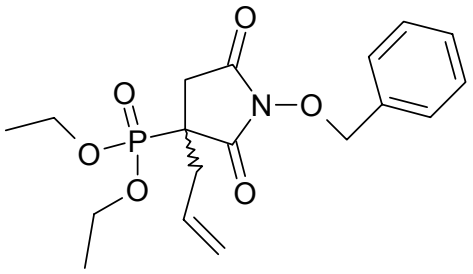
^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.52 - 7.37 (m, 5H, ArH), 5.08 (s, 2H, CH_2Ph), 4.82 - 4.74 (m, 2H, POCH), 3.21 - 3.12 (m, 1H, CH), 2.99 - 2.81 (m, 2H, CH_2), 1.38 - 1.33 (m, 12H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 169.7, 167.2 (C=O), 133.6 ($ArC_{quart.}$), 130.7, 130.3, 129.8, 128.9 ($ArC_{tert.}$), 79.3 (CH_2Ph), 73.2 (d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 72.8 (d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 37.7 (d, $^{1}J_{C,P}$ = 142.42 Hz, PC), 28.3 (CH_2), 24.5 (d, $^{3}J_{C,P}$ = 3.07 Hz, CH_3), 24.4 (d, $^{3}J_{C,P}$ = 4.07 Hz, CH_3), 24.2 (t, $^{3}J_{C,P}$ = 5.83 Hz, CH_3)

$C_{17}H_{24}NO_6P$
Requires [%]: C 55.28, H 6.55, N 3.79
Found [%]: C 55.26, H 6.57, N 3.50

8.3.3 Alkylation of phosphonates 4a-c (Section 2.4.2)

Diethyl [3-allyl-1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate **5a**



From 0.68 g diethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4a**) according to **GP-2**

Yield: 71%, colourless oil

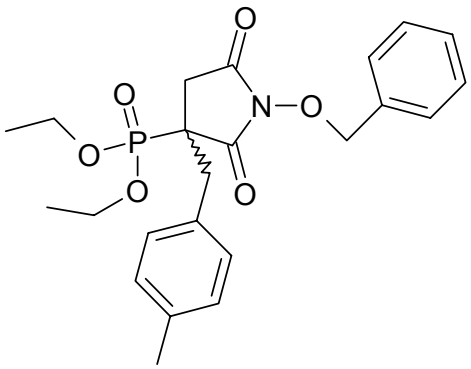
IR (Film): 1789 cm^{-1}, 1728 cm^{-1} (C=O), 1249 cm^{-1} (P=O), 1018 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.51 - 7.37 (m, 5H, ArH), 5.38 - 5.28 (m, 1H, CH_2CH), 5.19 - 5.15 (m, 2H, CH_2CH), 5.10 (ABs, J = 12.86 Hz, 2H, CH_2Ph), 4.22 - 4.16 (m, 4H, $POCH_2$), 3.04 – 2.87(m, 2H, CH_2), 2.65 - 2.58 (m, 1H, CH_2), 2.46 - 2.39 (m, 1H, CH_2), 1.38 - 1.31 (m, 6H, $(CH_3)_2$)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 169.9, 169.2 (C=O), 133.3 ($ArC_{quart.}$), 129.7, 129.4, 128.5 ($ArC_{tert.}$), 122.1 (CH_2), 78.7 (OCH_2Ph), 63.7 (d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 64.0 (d, $^{2}J_{C,P}$ = 7.12 Hz, POC), 45.6 (d, $^{1}J_{C,P}$ = 139.96 Hz, PC), 35.4 (CH_2), 31.9 (CH_2), 16.4 (d, $^{3}J_{C,P}$ = 5.08 Hz CH_3), 16.5 (d, $^{3}J_{C,P}$ = 6.11 Hz, CH_3)

$C_{18}H_{24}NO_6P$
Requires [%]: C 56.69, H 6.50, N 3.67
Found [%]: C 56.76, H 6.54, N 3.72

Diethyl [1-benzyloxy-3-(4-methylbenzyl)-2,5-dioxopyrrolidin-3-yl]phosphonate (**5b**)



From 0.68 g diethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4a**) according to **GP-2**

Yield: 78%, amorphous powder

M.p.: 54 °C

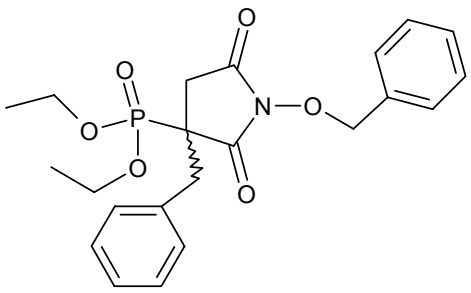
IR (KBr): 1782 cm^{-1}, 1720 cm^{-1} (C=O), 1257 cm^{-1} (P=O), 1020 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.40 - 7.01 (m, 9H, ArH), 4.87 (ABs, *J* = 27.15 Hz, 2H, CH_2Ph), 4.28 - 4.17 (m, 4H, $POCH_2$), 3.69 - 2.64 (m, 1H, CH_2), 3.03 - 2.88 (m, 2H, CH_2), 2.67 - 2.60 (m, 1H, CH_2), 2.29 (s, 3H, CH_3), 1.42 - 1 .34 (m, 6H, $(CH_3)_2$)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.2, 168.9 (C=O), 137.7, 133.4 ($ArC_{quart.}$), 130.1, 129.7, 129.3, 128.5 ($ArC_{tert.}$), 78.9 (CH_2Ph), 64.1 (d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 63.9 (d, $^{2}J_{C,P}$ = 7.12 Hz, POC), 47.6 (d, $^{1}J_{C,P}$ = 139.37 Hz PC), 35.7 (CH_2), 31.4 (CH_2), 21.0 (CH_3), 16.4 (t, $^{3}J_{C,P}$ = 5.85 Hz, CH_3)

$C_{23}H_{28}NO_6P$
Requires [%]: C 62.02, H 6.34, N 3.14
Found [%]: C 61.85, H 6.35, N 3.20

Diethyl[3-benzyl-1-benzyloxy-2,5-dioxopyrrolidin-3-yl] phosphonate **5c**



From 0.68 g diethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4a**) according to **GP-2**

Yield: 80%, yellow oil

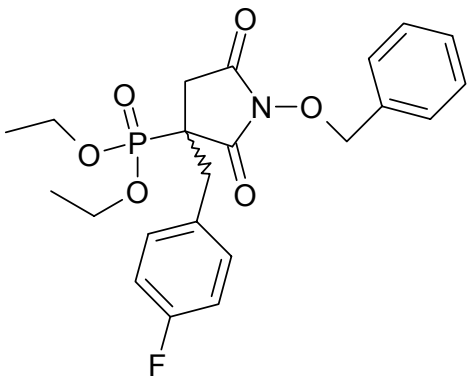
IR (Film): 1787cm^{-1}, 1726cm^{-1} (C=O), 1249cm^{-1} (P=O), 1018cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.38 - 7.23 (m, 10H, ArH), 4.64 (ABs, *J* = 46.36 Hz, 2H, CH_2Ph), 4.20 - 4.12 (m, 4H, $POCH_2$), 3.49 - 3.44 (m, 1H, CH_2), 3.01 – 2.77 (m, 3H, CH_2), 1.28 (dt, *J* = 1.78 Hz, *J* =7.09 Hz, 6H $(CH_3)_2$)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.0, 168.8 (C=O), 134.2, 133.8 ($ArC_{quart.}$), 130.7, 129.9, 129.5, 129.1, 128.8, 128.0 ($ArC_{tert.}$), 78.6 (CH_2Ph), 64.0 (d, $^{2}J_{C,P}$ = 7.12 Hz, POC), 63.8 (d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 47.6 (d, $^{1}J_{C,P}$ = 139.88 Hz PC), 36.1 (CH_2), 31.7 (CH_2),16.6 (d, $^{3}J_{C,P}$ = 5.08 Hz, CH_3)

$C_{22}H_{26}NO_6P$
Requires [%]: C 61.25, H 6.07, N 3.25
Found [%]: C 61.06, H 6.22, N 3.08

Diethyl [1-benzyloxy-3-(4-fluorobenzyl)-2,5-dioxopyrrolidin-3-yl]phosphonate (**5d**)



From 0.68 g diethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4a**) according to **GP-2**

Yield: 64%, amorphous powder

M.p.: 66 °C.

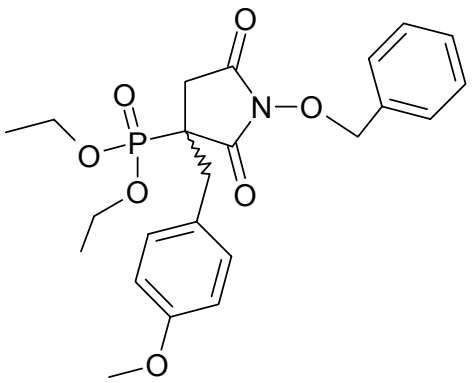
IR (KBr): 1786 cm^{-1}, 1726 cm^{-1} (C=O), 1251 cm^{-1} (P=O), 1018 cm^{-1} (POC).

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.40 - 6.97 (m, 9H, ArH), 4.92 - 4.85 (m, 2H, CH_2Ph), 4.26 - 4.24 (m, 4H, $POCH_2$), 3.68 - 3.63 (m, 1H, CH_2), 3.06 - 2.93 (m, 2H, CH_2), 2.62 - 2.54 (m, 1H,CH_2), 1.36 (t, J = 6.94 Hz, 6H, $(CH_3)_2$)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.0, 168.6 (C=O), 162.2 (d, $J_{C,F}$ = 247.60 Hz, CF), 133.2, 129.5 ($ArC_{quart.}$), 131.9, 129.7, 128.5, 116.1 ($ArC_{tert.}$), 78.93 (CH_2Ph), 64.3 (d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 64.0 (d, $^{2}J_{C,P}$ = 7.63 Hz, POC), 47.8 (d, $^{1}J_{C,P}$ = 141.47 Hz, PC), 35.2 (CH_2), 31.4 (CH_2), 16.5 (t, $^{3}J_{C,P}$ = 5.85 Hz, CH_3)

$C_{22}H_{25}FNO_6P$
Requires [%]: C 58.80, H 5.61, N 3.12
Found [%]: C 58.90, H 5.61, N 3.19

Diethyl [1-benzyloxy-3-(4-methoxybenzyl)-2,5-dioxopyrrolidin-3-yl]phosphonate (**5e**)



From 0.68 g diethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4a**) according to **GP-2**

Yield: 51%, yellow oil

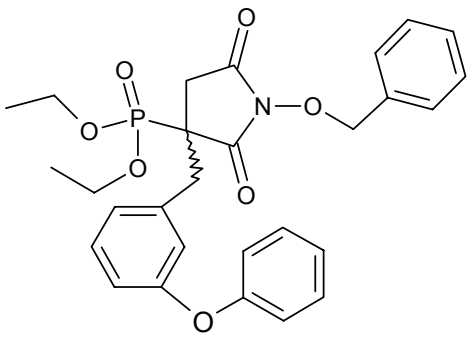
IR (Film): 1791 cm^{-1}, 1726 cm^{-1}, (C=O), 1251 cm^{-1} (P=O), 1018 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.41 - 6.79 (m, 9H, ArH), 4.85 (ABs, *J* = 23.46 Hz, 2H, CH_2Ph), 4.28- 4.17 (m, 4H, $POCH_2$), 3.75 (s, 3H, OCH_3), 3.67 - 3.62 (m, 1H, CH_2) , 3.03- 2.86 (m, 2H, CH_2), 2.67 - 2.60 (m, 1H, CH_2), 1.45 - 1 .34 (m, 6H, $(CH_3)_2$).

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.4, 168.9 (C=O), 159.3, 133.4, 125.5 ($ArC_{quart.}$), 131.3, 129.8, 129.3, 128.5, 114.4 ($ArC_{tert.}$), 78.9 (CH_2Ph), 63.9 (d, $^{2}J_{C,P}$ = 7.12 Hz, POC), 64.1 (d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 55.3 (OCH_3), 35.7 (CH_2), 31.4 (CH_2), 16.5 (t, $^{3}J_{C,P}$ = 5.85 Hz, CH_3)

$C_{23}H_{28}NO_6P$
Requires [%]: C 59.87, H 6.12, N 3.04
Found [%]: C 59.52, H 6.34, N 2.95

Diethyl [1-benzyloxy-3-(3-phenoxybenzyl)-2,5-dioxopyrrolidin-3-yl]phosphonate (**5f**)



From 0.68 g diethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4a**) according to **GP-2**

Yield: 68%, colourless oil

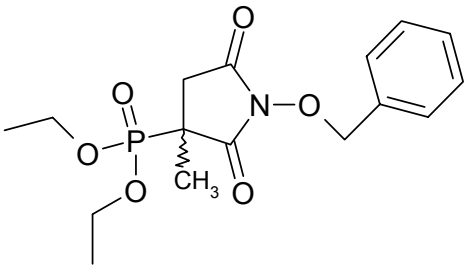
IR (Film): 1789 cm^{-1}, 1724 cm^{-1} (C=O), 1252 cm^{-1} (P=O), 1018 (POC)

^{1}H-NMR: (400 MHz, DMSO-d_6) = δ (ppm) 7.37 - 6.93 (m, 14H, ArH), 4.75 (ABs, J = 4.83 Hz, CH_2Ph), 4.17 - 4.10 (m, 4H, $POCH_2$), 3.45 - 3.39 (m, 1H, CH_2), 3.03 - 2.80 (m, 3H, CH_2), 1.25 (t, J = 7.42 Hz, 6H, CH_3)

^{13}C-NMR: (101 MHz, DMSO-d_6) = δ (ppm) 170.0, 168.9 (C=O), 156.9, 136.4, 133.8 ($ArC_{quart.}$), 130.7, 130.4, 129.9, 128.8, 125.9, 123.3, 120.9, 118.9 ($ArC_{tert.}$), 78.7 (CH_2Ph), 63.8 (d, $^{2}J_{C,P}$ = 7.12 Hz, POC), 63.5 (d, $^{2}J_{C,P}$ = 7.12 Hz, POC), 47.5 (d, $^{1}J_{C,P}$ = 139.90 Hz PC), 35.9 (CH_2), 31.9 (CH_2CH_2), 16.6 (d, $^{3}J_{C,P}$ = 5.30 Hz, CH_3)

$C_{28}H_{30}NO_7P$
Requires [%]: C 64.24, H 5.78 N 2.68
Found [%]: C 64.35, H 6.13 N 2.44

Diethyl [1-benzyloxy-3-methyl-2,5-dioxopyrrolidin-3-yl]phosphonate(**5g**)



From 0.68 g diethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4a**) according to **GP-2**

Yield: 60%, amorphous powder

M.p.: 54 °C.

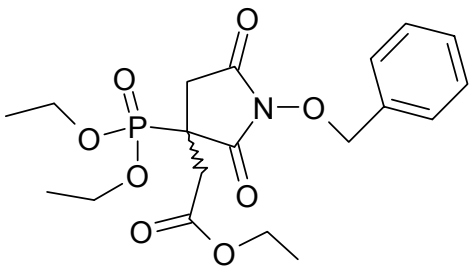
IR (KBr): 1784 cm^{-1}, 1732 cm^{-1} (C=O), 1247 cm^{-1} (P=O), 1020 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.42 - 7.38 (m, 5H, ArH), 5.01 (ABs, J = 6.36 Hz, 2H, CH_2Ph), 4.04 (m, 4H, CH_2), 2.76 - 2.71 (m, 2H, CH_2), 1.44 (d, J = 16.53 Hz, 3H, CH_3), 1.25 - 1 .23 (m, 6H, (CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.6, 168.9 (C=O), 133.7 ($ArC_{quart.}$), 129.4, 129.0, 128.4 ($ArC_{tert.}$), 78.0 (OCH_2Ph), 63.5 (d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 63.3 (d, $^{2}J_{C,P}$ = 7.12 Hz, POC), 41.7 (d, $^{1}J_{C,P}$ = 139.90 Hz PC), 35.5 (CH_2), 17.6 (d, $^{2}J_{C,P}$ = 4.07 Hz, CH_3), 16.3 (d, $^{3}J_{C,P}$ =5.09 Hz, CH_3)

$C_{16}H_{22}NO_6P$
Requires [%]: C 54.08, H 6.24, N 3.94
Found [%]: C 53.76, H 6.30, N 3.90

Ethyl [1-benzyloxy-3-(diethoxyphosphoryl)-2,5-dioxopyrrolidin-3-yl]acetate (**5h**)



From 0.68 g diethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4a**) according to **GP-2**

Yield: 47%, yellow oil

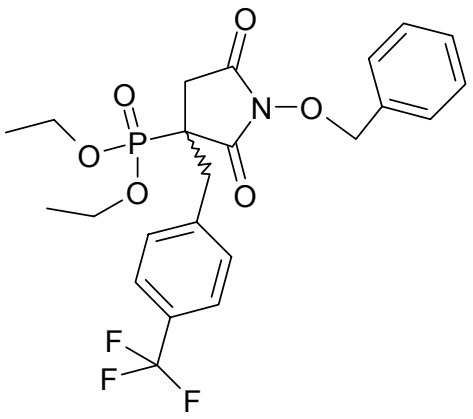
IR (Film): 1791 cm^{-1}, 1729 cm^{-1} (C=O), 1254 cm^{-1} (P=O), 1019 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.56 – 7.37 (m, 5H, ArH), 5.11 (s, 2H, CH_2Ph), 4.23 – 4.11 (m, 6H, OCH_2), 3.36 – 2.72 (m, 4H, CH_2), 1.36 – 1.33 (m, 6H, CH_3), 1.24 (t, J = 7.33 Hz, 3H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 169.7, 169.5, 169.0 (C=O), 133.7 ($ArC_{quart.}$), 129.8, 129.1, 128.5 ($ArC_{tert.}$), 78.9 (CH_2Ph), 64.6 (d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 63.9 (d, $^{2}J_{C,P}$ = 7.12 Hz, POC), 61.7 (OCH_2), 44.4 (PC), 35.4 (CH_2), 33.9 (CH_2), 16.4 (d, $^{3}J_{C,P}$ = 5.60 Hz, CH_3), 14.0 (CH_2)

$C_{19}H_{26}NO_8P$
Requires [%]: C 53.40, H 6.13, N 3.28
Found [%]: C 53.79, H 6.30, N 3.01

Diethyl {3-[4-(trifluoromethyl)benzyl]-1-benzyloxy-2,5-dioxopyrrolidin-3-yl}phosphonate (**5i**)



From 0.68 g diethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4a**) according to **GP-2**

Yield: 81%, amorphous powder

M.p.: 88 °C

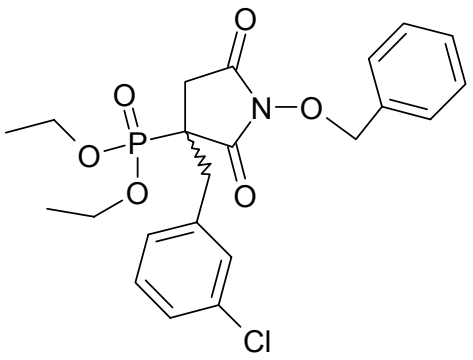
IR (KBr): 1786 cm^{-1}, 1724 cm^{-1} (C=O), 1247 cm^{-1} (P=O), 1017 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.55 -7.24 (m, 9H, ArH), 4.90 (ABs, *J* = 10.04 Hz, 2H, CH_2Ph), 4.28 – 4.22 (m, 4H, CH_2), 3.75 -3.70 (m, 1H, CH_2), 3.07 - 2.98 (m, 2H, CH_2), 2.57 – 2.49 (m, 1H, CH_2), 1.43 -1.34 (m, 6H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 168.9, 168.8 (C=O), 133.6 $ArC_{quart.}$), 131.1, 130.1, 129.8, 128.9, 126.4 ($ArC_{tert.}$), 79.3 (CH_2Ph), 64.8 (d, $^{2}J_{C,P}$ = 7.12 Hz, CH_2), 64.5 (d, $^{2}J_{C,P}$ = 7.63 Hz, CH_2), 48.0 (d, $^{1}J_{C,P}$ = 138.86 Hz, PC), 35.9 (CH_2), 31.8 (CH_2), 16.9 (t, $^{3}J_{C,P}$ = 4.83 Hz, CH_3)

$C_{23}H_{25}F_3NO_6P$
Requires [%]: C 55.31 H 5.05, N 2.80
Found [%]: C 55.35 H 5.07, N 2.64

Diethyl [3-(3-chlorobenzyl)-1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**5j**)



From 0.68 g diethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4a**) according to **GP-2**

Yield: 80%, yellow oil

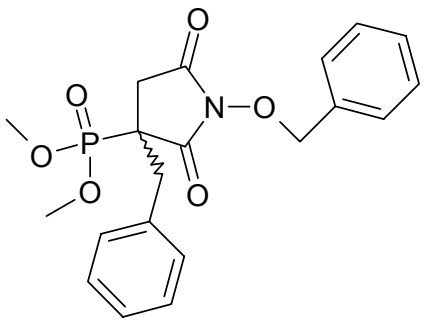
IR (Film): 1789 cm^{-1}, 1724 cm^{-1} (C=O), 1252 cm^{-1} (P=O), 1018 (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.42 - 7.03 (m, 9H, ArH), 4.90 (ABs, *J* = 18.56 Hz, CH_2Ph), 4.28 - 4.20 (m, 4H, CH_2), 3.69 – 3.64 (m, 1H, CH_2), 3.06 – 2.91 (m, 2H, CH_2), 2.62 – 2.54 (m, 1H, CH_2), 1.42 – 1.34 (m, 6H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 169.0 (C=O), 136.1, 135.3, 133.6 $ArC_{quart.}$), 130.7, 130.2, 129.8, 128.9, 128.8, 128.7 ($ArC_{tert.}$), 79.5 (CH_2Ph), 64.8 (d, $^{2}J_{C,P}$ = 6.61 Hz, CH_2), 64.5 (d, $^{2}J_{C,P}$ = 7.12 Hz, CH_2), 48.0 (d, $^{1}J_{C,P}$ = 139.87 Hz, PC), 36.1 (CH_2), 31.8 (CH_2), 16.9 (t, $^{3}J_{C,P}$ = 5.10 Hz, CH_3)

$C_{22}H_{25}ClNO_6P$
Requires [%]: C 56.72, H 5.41, N 3.01
Found [%]: C 57.20, H 5.54, N 2.98

Dimethyl [3-benzyl-1-benzyloxy-2,5-dioxopyrrolidin-3-yl] phosphonate (**5k**)



From 0.63 g dimethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4b**) according to **GP-2**

Yield: 91%, amorphous powder

M.p.: 79 °C.

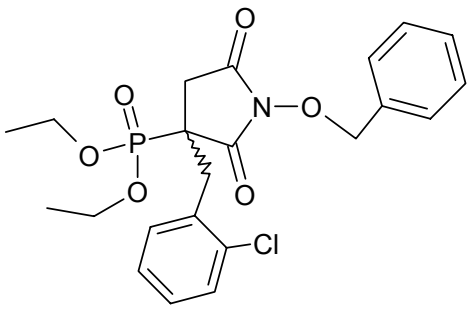
IR (KBr): 1789 cm^{-1}, 1724 cm^{-1} (C=O), 1258 cm^{-1} (P=O), 1014 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.41 - 7.13 (m, 10H, ArH), 4.83 (ABs, *J* = 14.00 Hz, CH_2Ph) 3.89 (t, *J* = 10.93 Hz, 6H, CH_3), 3.69 - 3.66 (m, 1H, CH_2), 3.05- 2.93 (m, 2H, CH_2), 2.65 - 2.63 (m, 1H, CH_2)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.4, 168.9 (C=O), 133.9, 133.7 ($ArC_{quart.}$), 130.7, 130.2, 129.7, 129.5, 128.9, 128.5 ($ArC_{tert.}$), 79.4 (CH_2Ph), 55.6 (d, $^{2}J_{C,P}$ = 7.12 Hz, POC), 55.7 (d, $^{2}J_{C,P}$ = 7.12 Hz, POC), 48.1 (d, $^{1}J_{C,P}$ = 140.89 Hz PC), 36.6 (CH_2), 31.8 (CH_2).

$C_{20}H_{22}NO_6P$
Requires [%]: C 59.55, H 5.50, N 3.47
Found [%]: C 59.53, H 5.52, N 3.30

Diethyl [3-(2-chlorobenzyl)-1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**5l**)



From 0.68 g diethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4a**) according to **GP-2**

Yield: 90%, yellow oil

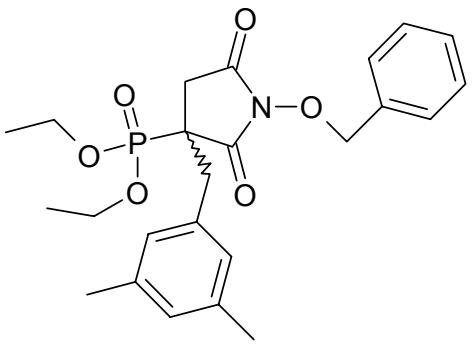
IR (film): 1789 cm^{-1}, 1724 cm^{-1} (C=O), 1252 cm^{-1} (P=O), 1018 (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.44 – 7.16 (m, 9H, ArH), 4.91 (ABs, *J* =9.42 Hz, 2H, CH_2Ph), 4.30 - 4.23 (m, 4H, CH_2) 3.61 -3.53 (m, 2H, CH_2), 3.02 (t, *J* = 17.77 Hz, 1H, CH_2), 2.58 (q, *J* = 9.96 Hz, 1H, CH_2), 1.42 -1.33 (m, 6H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 169.1 (C=O), 136.1, 135.3, 133.6 $ArC_{quart.}$), 130.7, 130.2, 129.8, 128.9, 128.8, 128.7 ($ArC_{tert.}$), 79.5 (CH_2Ph), 64.8 (d, $^{2}J_{C,P}$ = 6.61 Hz, CH_2), 64.5 (d, $^{2}J_{C,P}$ = 7.12 Hz, CH_2), 48.0 (d, $^{1}J_{C,P}$ = 139.87 Hz, PC), 36.1 (CH_2), 31.8 (CH_2), 16.9 (t, $^{3}J_{C,P}$ = 5.10 Hz, CH_3)

$C_{22}H_{25}ClNO_6P$
Requires [%]: C 56.72, H 5.41, N 3.01
Found [%]: C 56.49, H 5.69, N 2.94

Diethyl [3-(3,5-dimethylbenzyl)-1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**5m**)



From 0.68 g diethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4a**) according to **GP-2**

Yield: 89%, yellow oil

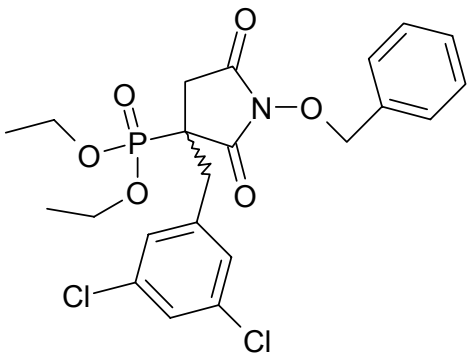
IR (Film): 1789 cm^{-1}, 1729 cm^{-1} (C=O), 1250 cm^{-1} (P=O), 1018 (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.40 – 7.35 (m, 5H, ArH), 6.89 (s, 1H, ArH), 6.77 (s, 2H, ArH), 4.80 (ABs, *J* = 19.48 Hz, 2H, CH_2Ph), 4.30 – 4.18 (m, 4H, CH_2), 3.66 – 3.61 (m, 1H, CH_2), 3.03 - 2.85 (m, 2H, CH_2), 2.72 - 2.62 (m, 1H, CH_2), 2.25 (s, 3H, CH_3), 1.41 (t, *J* = 7.29 Hz, ,3H, CH_3), 1.36 (t, *J* = 7.67 Hz, ,3H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 169.4, 169.3 (C=O), 139.0, 133.7 ($ArC_{quart.}$), 130.1, 129.9, 129.7, 128.9, 128.5 ($ArC_{tert.}$), 79.4 (CH_2Ph), 64.5 (d, $^{2}J_{C,P}$ = 7.12 Hz, CH_2), 64.2 (d, $^{2}J_{C,P}$ = 7.12 Hz, CH_2), 47.9 (d, $^{1}J_{C,P}$ = 139.88 Hz, PC), 36.4 (CH_2), 31.9 (CH_2), 21.7 (CH_2), 16.9 (CH_3)

$C_{23}H_3NO_6P$
Requires [%]: C 62.75, H 6.58, N 3.05
Found [%]: C 62.80, H 6.86, N 2.78

Diethyl [3-(3,5-dichlorobenzyl)-1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**5n**)



From 0.68 g diethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4a**) according to **GP-2**

Yield: 84%, amorphous powder

M.p.: 77 °C

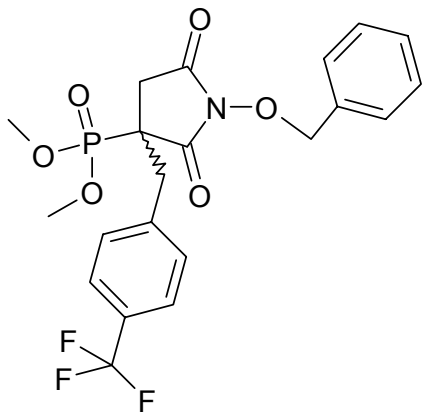
IR (KBr): 1785 cm^{-1}, 1724 cm^{-1} (C=O), 1255 cm^{-1} (P=O), 1014 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.42 -7.09 (m, 8H, ArH), 4.90 (ABs, *J* = 19.28 Hz, 2H, CH_2Ph), 4.26 – 4.20 (m, 4H, CH_2), 3.63 (q, *J* = 7.12 Hz, 1H, CH_2), 3.08 – 2.90 (m, 2H, CH_2), 2.53 (q, *J* = 9.13 Hz, 1H, CH_2), 1.41 – 1.34 (m, 6H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.2, 168.7 (C=O), 136.0, 133.5 ($ArC_{quart.}$), 130.1, 129.8, 129.1, 128.9, 128.8 (ArC), 79.6 (CH_2Ph), 64.9 (d, $^{2}J_{C,P}$ = 7.12 Hz, CH_2), 64.4 (d, $^{2}J_{C,P}$ = 7.63 Hz, CH_2), 35.9 (CH_2), 31.8 (CH_2), 16.8 (CH_3)

$C_{22}H_{24}Cl_2NO_6P \cdot H_2O$
Requires [%]: C 50.98, H 5.06, N 2.70
Found [%]: C 50.95, H 5.19, N 2.65

Dimethyl {3-[4-(trifluoromethyl)benzyl]-1-benzyloxy-2,5-dioxopyrrolidin-3-yl}phosphonate (**5o**)



From 0.63 g dimethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4b**) according to **GP-2**

Yield: 89%, amorphous powder

M.p.: 103 °C

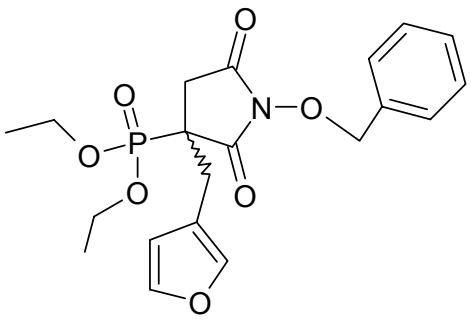
IR (KBr): 1793 cm^{-1}, 1725 cm^{-1} (C=O), 1258 cm^{-1} (P=O), 1017 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.56 – 7.24 (m, 9H, ArH), 4.94 - 4.87 (m, 2H, CH_2Ph), 3.89 (t, J = 10.94 Hz, 6H, CH_3), 3.74 – 3.69 (m, 1H, CH_2), 3.07 - 2.98 (m, 2H, CH_2), 2.58 - 2.50 (m, 1H, CH_2)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.1, 168.7 (C=O), 138.1, 137.9, 135.6 $ArC_{quart.}$), 131.1, 130.1, 128.9, 126.8, 126.4 ($ArC_{tert.}$), 79.3 (CH_2Ph), 55.2 (d, $^{2}J_{C,P}$ = 6.87 Hz, POC), 54.7 (d, $^{2}J_{C,P}$ = 6.86 Hz, CH_2), 48.0 (d, $^{1}J_{C,P}$ = 140.39 Hz, PC), 36.1 (CH_2), 31.1 (CH_2)

$C_{21}H_{21}F_3NO_6P$
Requires [%]: C 53.51 H 4.49, N 2.97
Found [%]: C 53.44 H 4.76, N 2.93

Diethyl [3-(3-furylmethyl)-1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**5p**)



From 0.68 g diethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4a**) according to **GP-2**

Yield: 69%, yellow oil

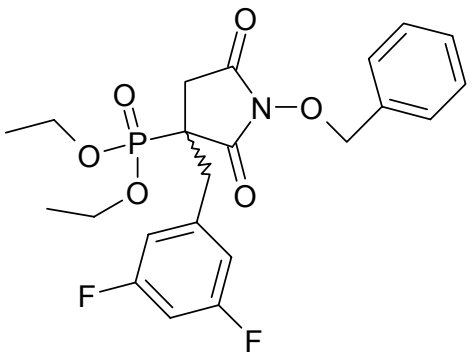
IR (Film): 1790 cm^{-1}, 1726 cm^{-1} (C=O), 1248 cm^{-1} (P=O), 1020 (POC)

^{1}H-NMR: (400 MHz, DMSO-d_6) = δ (ppm) 7.61 - 7.60 (m, 2H, OCH), 7.39 (s, 5H, ArH), 6.29 (s, 1H, CH), 4.83 (ABs, J = 14.05 Hz, CH_2Ph), 4.15 - 4.11 (m, 4H, OCH_2), 3.25 – 3.19 (m, 1H, CH_2), 2.97 – 2.78 (m, 3H, CH_2), 1.27 (dt, J = 6.91 Hz, 6H, CH_3)

^{13}C-NMR: (101 MHz, DMSO-d_6) = δ (ppm) 173. 1, 169.1 (C=O), 144.3, 142.5 (OCH), 133.8 ($ArC_{quart.}$), 129.9, 129.6, 128.8 ($ArC_{tert.}$), 111.9 (CCH), 78.6 (CH_2Ph), 64.0 (d, $^{2}J_{C,P}$ = 6.61 Hz, CH_2), 63.7 (d, $^{2}J_{C,P}$ = 7.13 Hz, CH_2), 46.9 (d, $^{1}J_{C,P}$ = 139.88 Hz, PC), 32.2 (CH_2), 26.3 (CH_2), 16.6 (t, $^{3}J_{C,P}$ = 5.60 Hz, CH_3)

$C_{20}H_{24}NO_7P$
Requires [%]: C 57.01, H 5.74, N 3.32
Found [%]: C 57.95, H 6.17, N 3.05

Diethyl [3-(3,5-difluorobenzyl)-1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**5q**)



From 0.68g diethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4a**) according to **GP-2**

Yield: 67%, pinkish powder

M.p.: 71 °C

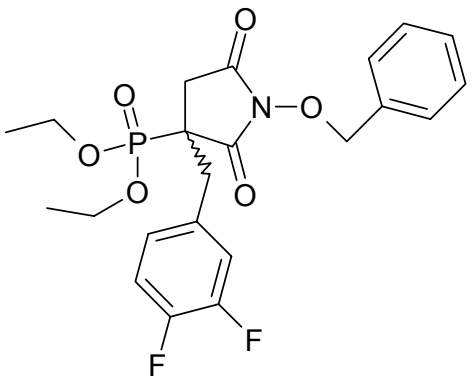
IR (KBr): 1791 cm^{-1}, 1724 cm^{-1} (C=O), 1250 cm^{-1} (P=O), 1009 (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.44 - 7.35 (m, 5H, ArH), 6.74 - 6.70 (m, 3H, ArH), 4.93 (ABs, *J* = 11.23 Hz, 2H, CH_2Ph), 4.27 – 4.19 (m, 4H, CH_2), 3.67 – 3.62 (m, 1H, CH_2), 3.07 - 2.91 (m, 2H, CH_2), 2.59 – 2.51 (m, 1H, CH_2), 1.42 - 1.34 (m, 6H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 169.8, 168.4 (C=O), 133.2, 103.7 ($ArC_{quart.}$), 129.8, 129.4, 128.4, 113.4, 113.2 ($ArC_{tert.}$), 79.1 (CH_2Ph), 64.5 (CH_2), 64.1 (CH_2), 47.5 (d, $^{1}J_{C,P}$ = 138.35 Hz, PC), 35.6 (CH_2), 31.4 (CH_2), 16.4 (CH_3)

$C_{22}H_{24}F_2NO_6P$
Requires [%]: C 56.53, H 5.18, N 3.00
Found [%]: C 56.53, H 5.16, N 3.01

Diethyl [3-(3,4-dichlorobenzyl)-1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**5r**)



From 0.68 g diethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4a**) according to **GP-2**

Yield: 53%, yellow rod-like crystals

M.p.: 91 °C

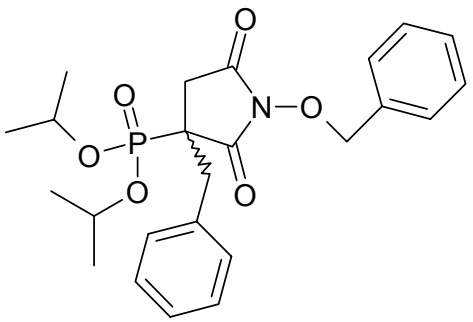
IR (KBr): 1786 cm^{-1}, 1724 cm^{-1} (C=O), 1243 cm^{-1} (P=O), 1020 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, DMSO-d_6) = δ (ppm) 7.41 - 7.09 (m, 8H, ArH), 4.81 (ABs, J = 11.79 Hz, 2H, CH_2Ph), 4.16 - 4.11 (m, 4H, CH_2), 3.43 - 3.37 (m, 1H, CH_2), 3.07 - 2.98 (m, 3H, CH_2), 1.25 (t, J = 7.54 Hz, 3H, CH_3)

^{13}C-NMR: (101 MHz, DMSO-d_6) = δ (ppm) 172.1, 168.9 (C=O), 150.6, 148.0, 133.8 ($ArC_{quart.}$), 129.9, 128.8, 127.7, 119.9, 118.1 ($ArC_{tert.}$), 78.6 (CH_2Ph), 64.1 (d, $^{2}J_{C,P}$ = 7.12 Hz, CH_2), 63.4 (d, $^{2}J_{C,P}$ = 7.63 Hz, CH_2), 47.1 (d, $^{1}J_{C,P}$ = 139.37 Hz, PC), 35.0 (CH_2), 31.9 (CH_2), 16.5 (t, $^{3}J_{C,P}$ = 5.60 Hz, CH_3)

$C_{22}H_{24}F_2NO_6P$
Requires [%]: C 56.53, H 5.18, N 3.00
Found [%]: C 56.49, H 5.44, N 2.95

Diisopropyl [3-benzyl-1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**5s**)



From 0.74 g diisopropyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4c**) according to **GP-2**

Yield: 70%, colourless oil

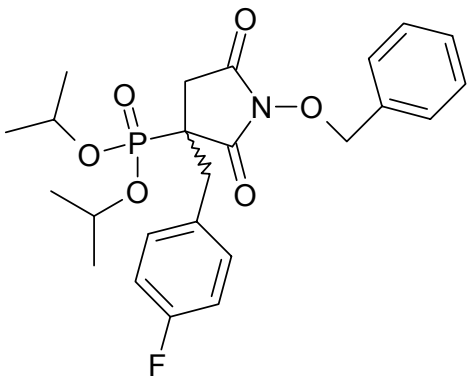
IR (Film): 1789 cm^{-1}, 1728 cm^{-1} (C=O), 1261 cm^{-1} (P=O), 989 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.40 - 7.13 (m, 10H, ArH), 4.87 - 4.77 (m, 2H, POCH), 3.70 - 3.69 (m, 1H, CH_2), 3.02 - 2.89 (m, 2H, CH_2), 2.62 - 2.60 (m, 1H, CH_2), 1.42 - 1.35 (m, 12H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.6, 169.5 (C=O), 134.3, 133.8 ($ArC_{quart.}$), 130.7, 130.1, 129.7, 129.5, 128.9, 128.8 ($ArC_{tert.}$), 79.4 (CH_2Ph), 73.5 (d, $^{2}J_{C,P}$ = 7.12 Hz, POC), 73.1 (d, $^{2}J_{C,P}$ = 7.63 Hz, POC), 48.2 (d, $^{1}J_{C,P}$= 139.88 Hz PC), 36.5 (CH_2), 31.9 (CH_2), 24.6 (d, $^{3}J_{C,P}$ = 3.05 Hz, CH_3), 24.5 (d, $^{3}J_{C,P}$ = 4.07 Hz, CH_3), 24.2 (d, $^{3}J_{C,P}$ = 5.59 Hz, CH_3), 24.1 (d, $^{3}J_{C,P}$ = 6.11 Hz, CH_3)

$C_{24}H_{30}NO_6P$
Requires [%]: C 62.74, H 6.58, N 3.05
Found [%]: C 62.63, H 6.78, N 2.97

Diisopropyl [3-(4-fluorobenzyl)-1 -benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**5t**)



From 0.74 g diisopropyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4c**) according to **GP-2**

Yield: 78%, rod-like crystals

M.p.: 84 °C

IR (KBr): 1787 cm^{-1}, 1731 cm^{-1} (C=O), 1249 cm^{-1} (P=O), 975 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.42 - 6.95 (m, 9H, ArH), 4.92 - 4.86 (m, 2H, CH_2Ph), 4.85 - 4.77 (m, 2H, POCH), 3.65 - 3.63 (m, 1H, CH_2), 3.03 - 2.87 (m, 2H, CH_2), 2.56 - 2.64 (m, 1H, CH_2), 1.41 - 1 .35 (m, 12H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.4, 169.3 (C=O), 162.7 (d, $J_{C,F}$ = 247.19 Hz, CF), 133.6 ($ArC_{quart.}$), 132.3, 130.1, 129.70, 128.9, 116.5 ($ArC_{tert.}$), 79.3 (CH_2Ph), 73.6 (d, $^{2}J_{C,P}$ = 7.12 Hz, POC), 73.2 (d, $^{2}J_{C,P}$ = 7.63 Hz, POC), 48.2 (d, $^{1}J_{C,P}$= 139.87 Hz PC), 35.6 (CH_2), 31.9 (CH_2), 24.6 (d, $^{3}J_{C,P}$ = 3.05 Hz, CH_3), 24.5 (d, $^{3}J_{C,P}$ = 4.07 Hz, CH_3), 24.3 (d, $^{3}J_{C,P}$ = 5.09 Hz, CH_3), 24.1 (d, $^{3}J_{C,P}$ = 6.10 Hz, CH_3)

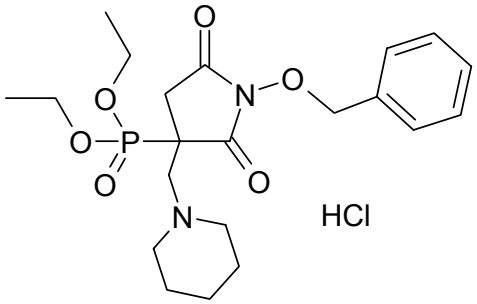
$C_{24}H_{29}NO_6P$
Found [%]: C 60.33, H 6.26, N 2.91
Requires [%]: C 60.37, H 6.12, N 2.93

8.3.4 Mannich reaction of 4a with piperidine and formaldehyde (Section 2.4.3)

Synthesis procedure for **6**

To a stirred solution of **4a** (2 mmol, 0.68 g) in glacial acetic acid (4.6 mL) was added piperidine (12 mmol) drop wise. The mixture was cooled to 30°C and 35% formaldehyde (0.5g, 16 mmol) was added. The solution was stirred slowly for 1hr and then poured onto 12 g crushed ice. After the ice had melted, the resulting mixture was made alkaline by slow addition of 30% NaOH (5 mL) and then extracted with Et_2O (3 × 10 mL). The organic solvent was evaporated and resulting residue acidified to pH 2 using 1M HCl (3 mL). This mixture was washed with ethyl acetate (2 × 5 mL). 30% NaOH (2 mL) was added to the aqueous solution to reach pH 9. The crude base was extracted with ethyl acetate (3 × 5 mL) and the organic solution dried ($MgSO_4$). Dry HCl gas was passed through this ethyl acetate solution which was then cooled for 24 h at 4°C, affording crystalline **6**

1-{[1-Benzyloxy-3-(diethoxyphosphoryl)-2,5-dioxopyrrolidin-3-yl]methyl} piperidinum chloride **6**



Yield: 21%, rod-like crystals

M.p.: 177 °C

IR (KBr): 1793 cm^{-1}, 1720 cm^{-1} (C= O), 1245 cm^{-1} (P=O), 1010 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, DMSO-d_6) = δ (ppm) 10.49 (s, 1H, NH), 7.50 – 7.41 (m, 5H, ArH), 5.08 (s, 2H, CH_2Ph), 4.16 – 4.12 (m, 4H, $POCH_2$), 3.60 – 1.63 (m, 14H, CH_2), 1.29 – 1.23 (m, 6H, CH_3)

^{13}C-NMR: (101 MHz, DMSO-d_6) = δ (ppm) 170.0, 168.6 (C=O), 134.9 ($ArC_{quart.}$), 129.9, 129.5, 128.8 ($ArC_{tert.}$), 78.7 (CH_2Ph), 55.9 (d, $^{2}J_{C,P}$ = 4.07 Hz, POC), 48.1 (CH_2), 35.2 (CH_2), 28.1 (CH_2), 22.4 (CH_2), 21.9 (CH_2), 16.5 (q, $^{3}J_{C,P}$ = 5.59 Hz, CH_3)

$C_{21}H_{31}N_2O_6P \cdot HCl$
Requires [%]: C 53.11, H 6.79, N 5.90
Found [%]: C 53.00, H 6.84, N 5.92

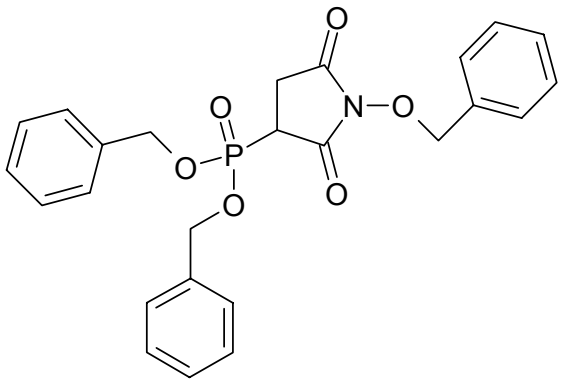
8.4 Procedures and Analytical Data for Chapter 3

8.4.1 Synthesis of dibenzyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (Section 3.3)

Synthesis procedure for **9**

Trimethylsilyl bromide (3.8 mL) was added to a solution of diethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4a**) (4 mmol, 6.82g) in dry dichloromethane (5 mL) and the reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated and the remaining oil was dissolved in MeOH (5 mL), treated with water (0.1 mL), and stirred for 10 min. The solvents were removed under reduced pressure and the residue dried in vacuo for 12 h.
The resulting phosphonic acid was dissolved in dry dichloromethane (10 mL) under nitrogen atmosphere, PCl_5 (8 mmol, 1.63g) was added portion wise over 5 min then the mixture was stirred for another 30 min before being refluxed for 2 h. The contents were evaporated under pressure at 60°C and the resulting brown oil dissolved in dry dichloromethane (10 mL) under nitrogen atmosphere.
After cooling to 0°C, a mixture of absolute pyridine (7.2 mmol, 0.57g) and benzyl alcohol (7.2 mmol, 0.78g) was added drop wise over 10 min. The mixture was stirred for 15 min at 0°C and then for 14 h at room temperature. The solution was then washed with ice cold 1N NaOH (5 mL), ice cold 1N HCl (5 mL), saturated $NaHCO_3$ solution (5 mL), water (5 mL), saturated NaCl solution (5 mL) and then dried with $MgSO_4$. Evaporation in vacuo resulted in yellow oil that was purified via column chromatography (diethyl ether) to yield colourless oil that became pink needle-like crystals.

Dibenzyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**9**) (Section 3.3)



Yield: 10%, pink needle-like crystals

M.p.: 74 °C

IR (KBr): 1784 cm^{-1}, 1724 cm^{-1} (C=O), 1247 cm^{-1} (P=O), 995 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.44 - 7.36 (m, 15H, Ar-H), 5.15 - 4.96 (m, 4H, POCH2), 4.93 (ABs, 2H, CH2Ph), 3.19 - 3.09 (m, 1H, PCH), 2.98 - 2.68 (m, 2H, CH2)

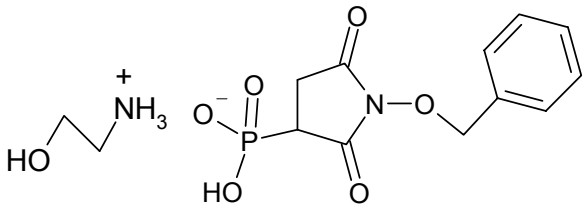
^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 168.9 (C=O), 133.1 ($ArC_{quart.}$) 128.3, 128.4, 128.5, 128.8, 128.9, 129.4 ($ArC_{tert.}$), 78.9 (CH_2Ph), 69.3 (d, $^{2}J_{C,P}$ = 6.61 Hz , POC), 68.8 (d, $^{2}J_{C,P}$ = 6.61 Hz , POC), 36.9 (d, $^{1}J_{C,P}$ = 142.9 Hz, PCH), 27.7 (CH_2)

^{31}P-NMR: (203 MHz, $CDCl_3$) = δ (ppm) 21.25 (POBn)

$C_{25}H_{24}NO_6P$
Requires [%]: C 64.51, H 5.20, N 3.01
Found [%]: C 64.46, H 5.30, N 2.87

8.4.2 Preparation of Ethanolamine Monosalts 12a-f (Section 3.4)

2-Hydroxyethanaminium hydrogen (1-benzyloxy-2,5-dioxopyrrolidin-3-yl)phosphonate **12a**



From 1.02 g diethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4a**) according to **GP-3**

Yield: 53%, amorphous powder

M.p.: 178 °C

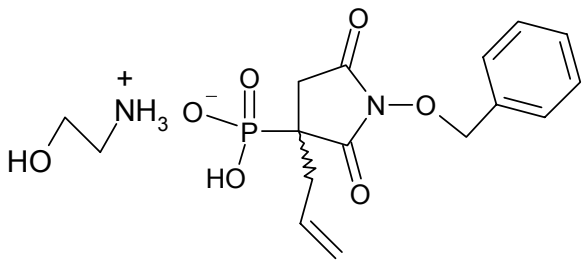
IR (KBr): 1724cm^{-1}, 1782cm^{-1} (C=O)

^{1}H-NMR: (400 MHz, D_2O) = δ (ppm) 7.44 - 7.35 (m, 5H, Ar-H), 5.13 (ABs, *J* = 16.87 Hz, 2H, CH_2Ph), 3.70 (t, *J* = 6.33 Hz, 2H, CH_2NH_3), 3.19 - 3.10 (m, 1H, PCH), 3.02 (t, *J* = 5.97 Hz, 2H, CH_2OH), 2.94 - 2.84 (m, 1H, CH_2), 2.76 - 2.66 (m, 1H, CH_2)

^{13}C-NMR (101 MHz, D_2O) = δ (ppm) 174.3, 172.6 (C=O), 133.5 ($ArC_{quart.}$), 130.8, 130.2, 129.2(ArC_{tert}), 79.6 (CH_2Ph), 57.9 (CH_2OH), 41.6 (CH_2NH_3), 38.9 (d, $^{1}J_{C,P}$ = 125.12 Hz, PCH), 28.9 (CH_2CO)

$C_{13}H_{19}N_2O_7P$
Requires [%]: C 45.09, H 5.53, N 8.09
Found [%]: C 44.95, H 5.62, N 7.94

2-Hydroxyethanaminium hydrogen (3-allyl-1-benzyloxy-2,5-dioxopyrrolidin-3-yl)phosphonate **12b**



From 1.14 g diethyl [3-allyl-1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**5a**) according to **GP-3**

Yield: 64%, amorphous powder

M.p.: 169 °C

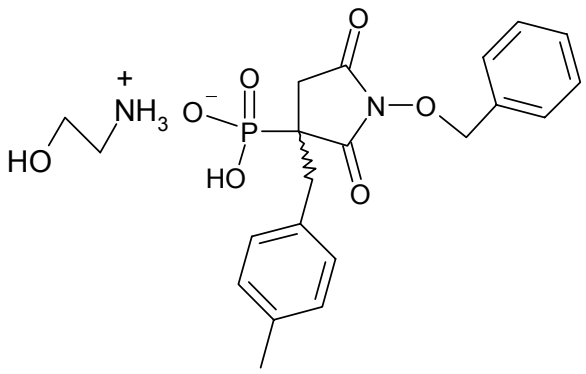
IR (KBr): 1786 cm^{-1}, 1706 cm^{-1} (C=O)

^{1}H-NMR: (400 MHz, D_2O) = δ (ppm) 7.47 – 7.45 (m, 5H, ArH), 5.38–5.28 (m, 1H, $CHCH_2$), 5.17 – 5.13 (m, 2H, $CHCH_2$), 5.10 (s, 2H, CH_2Ph), 3.78 (t, J = 5.12, 2H, CH_2NH_3), 3.10 (t, J = 5.12, 2H, CH_2OH), 2.92–2.67 (m, 3H, CH_2), 2.40 – 2.33 (m, 1H, CH_2)

^{13}C-NMR: (101 MHz, D_2O) = δ (ppm) 174.1, 174.0 (C=O), 133.4 ($ArC_{quart.}$), 131.4, 130.3, 129.2 ($ArC_{tert.}$), 121.4 (CH_2), 79.5 (CH_2Ph), 57.9 (CH_2OH), 47.7 (d, $^{1}J_{C,P}$ = 125.12, PC), 41.6 (CH_2NH_3), 35.7 (CH_2CH), 32.7 (CH_2)

$C_{16}H_{23}N_2O_7P$
Requires [%]: C 49.74, H 6.00, N 7.25
Found [%]: C 49.73, H 6.13, N 7.15

2-Hydroxyethanaminium hydrogen [1-benzyloxy-3-(4-methylbenzyl)-2,5-dioxopyrrolidin-3-yl]phosphonate **12c**



From 1.34 g diethyl [1-benzyloxy-3-(4-methylbenzyl)-2,5-dioxopyrrolidin-3-yl]phosphonate (**5b**) according to **GP-3**

Yield: 65%, amorphous powder

M.p.: 187 °C

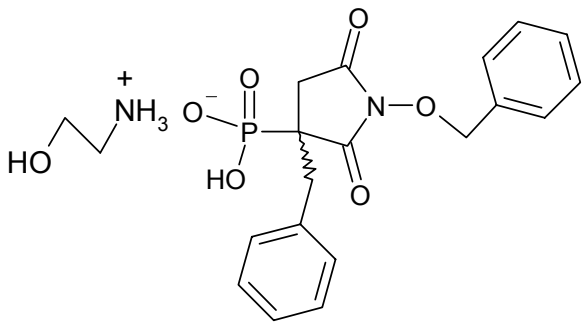
IR (KBr): 1786cm^{-1}, 1712cm^{-1} (C=O)

^{1}H-NMR: (400 MHz, D_2O) = δ (ppm) 7.91 – 6.93 (m, 9H, ArH), 4.81 – 4.58 (m, 2H, CH_2Ph), 3.72 (t, *J* = 6.09 Hz, 2H, CH_2NH_3), 3.54 – 3.50 (m, 1H, CH_2), 3.04 (t, *J* = 6.47 Hz, 2H, CH_2OH), 2.87 – 2.79 (m, 2H, CH_2), 2.65 – 2.58 (m, 1H, CH_2), 2.21 (s, 3H, CH_3)

^{13}C-NMR: (101 MHz, D_2O) = δ (ppm) 173.7, 173.6 (C=O), 138.3, 130.7 ($ArC_{quart.}$), 130.6, 130.1, 130.0, 129.1 (ArC_{tert}), 79.7 (CH_2Ph), 57.9 (CH_2OH), 49.6 (d, $^{1}J_{C,P}$ = 123.60 Hz, PC), 41.6 (CH_2NH_3), 36.4 (CH_2), 32.3 (CH_2), 20.5 (CH_3)

$C_{21}H_{22}N_2O_7P$
Requires [%]: C 56.00, H 6.04, N 6.22
Found [%]: C 55.68, H 6.05, N 6.19

2-Hydroxyethanaminium hydrogen (3-benzyl-1-benzyloxy-2,5-dioxopyrrolidin-3-yl)phosphonate **12d**



From 1.29 g diethyl [3-benzyl-1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**5c**) according to **GP-3**

Yield: 57%, amorphous powder

M.p.: 175 °C

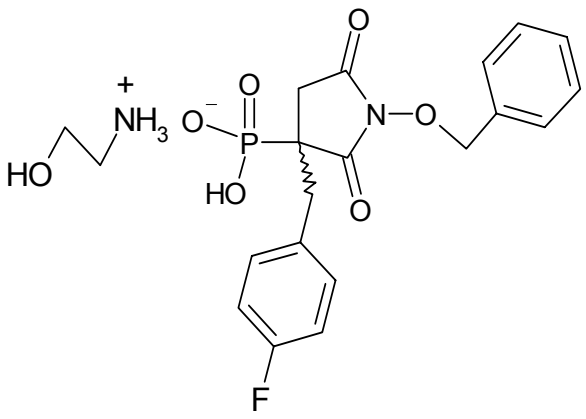
IR (KBr): 1784 cm^{-1}, 1710 cm^{-1} (C=O)

^{1}H-NMR: (400 MHz, D_2O) = δ (ppm) 7.27–6.95 (m, 10H, ArH), 4.74 – 4.55 (m, 2H, CH_2Ph), 3.66 (t, *J* = 6.49, 2H, CH_2NH_3), 3.53 (CH_2), 2.98 (t, *J* 6.48, 2H, CH_2OH), 2.86 – 2.76 (m, 2H, CH_2), 2.60 (q, 1H, CH_2)

^{13}C-NMR: (101 MHz, D_2O) = δ (ppm) 175.2, 173.6 (C=O), 135.7, 133.4 ($ArC_{quart.}$), 130.7, 129.4, 129.1, 128.1 (ArC_{tert}), 79.7 (CH_2Ph), 57.9 (CH_2OH), 41.6 (CH_2NH_3), 48.2 (d, $^{1}J_{C,P}$ = 142.92, PC), 36.9 (CH_2), 32.4 (CH_2)

$C_{20}H_{25}N_2O_7P$
Requires [%]: C 55.05, H 5.77, N 6.42
Found [%]: C 54.51, H 6.30, N 5.79

2-Hydroxyethanaminium hydrogen [1-benzyloxy-3-(4-fluorobenzyl)-2,5-dioxopyrrolidin-3-yl]phosphonate **12e**



From 1.35 g diethyl [1-benzyloxy-3-(4-fluorobenzyl)-2,5-dioxopyrrolidin-3-yl]phosphonate (**5d**)according to **GP-3**

Yield: 68%, amorphous powder

M.p.: 172 °C

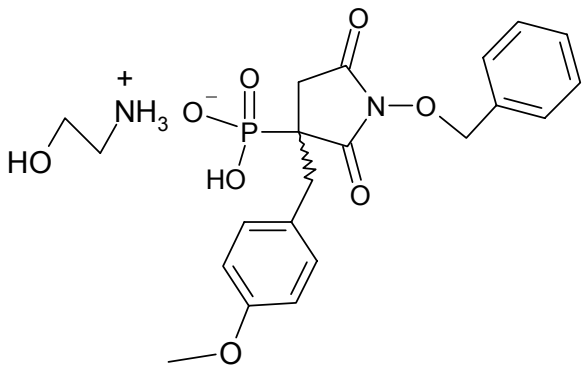
IR (KBr): 1786 cm^{-1}, 1712 cm^{-1} (C=O)

^{1}H-NMR: (400 MHz, D_2O) = δ (ppm) 7.39 – 7.18 (m, 9H, ArH), 4.94 – 4.70 (m, 2H, CH_2Ph), 3.83 (t, J = 6.35 Hz, 2H, CH_2NH_3), 3.75 – 3.60 (m, 1H, CH_2), 3.17 (t, J = 6.38, 2H, CH_2OH), 3.05 – 2.90 (m, 2H, CH_2), 2.70 – 2.60 (m, 1H, CH_2)

^{13}C-NMR: (101 MHz, D_2O) = δ (ppm) 175.1, 173.5 (C=O), 162.5 (d, $J_{C,F}$ = 243.13 Hz, CF), 133.4 ($ArC_{quart.}$), 132.4, 130.6, 129.1, 116.2, 115.9 (ArC_{tert}), 79.7 (CH_2Ph), 57.9 (CH_2OH), 49.6 (d, $^{1}J_{C,P}$ = 122.58 Hz, PC), 41.6 (CH_2NH_3), 35.9 (CH_2), 32.3 (CH_2)

$C_{20}H_{24}FN_2O_7P$
Requires [%]: C 52.87, H 5.32, N 6.17
Found [%]: C 52.52, H 6.01, N 5.29

2-Hydroxyethanaminium hydrogen [1-benzyloxy-3-(4-methoxybenzyl)-2,5-dioxopyrrolidin-3-yl]phosphonate **12f**



From 1.38 g diethyl [1-benzyloxy-3-(4-methoxybenzyl)-2,5-dioxopyrrolidin-3-yl]phosphonate (**5e**) according to **GP-3**

Yield: 66%, amorphous powder

M.p.: 205 °C

IR (KBr): 1784cm^{-1}, 1712cm^{-1} (C=O)

^{1}H-NMR: (400 MHz, D_2O) = δ (ppm) 7.52 – 7.11 (m, 9H, ArH), 5.01 – 4.79 (m, 2H, CH_2Ph), 3.91 (s, 3H, OCH_3), 3.93 – 3.90 (m, 2H, CH_2NH_3), 3.72 – 3.68 (m, 1H, CH_2), 3.23 (t, *J* = 5.30 Hz, 2H, CH_2OH), 3.07 – 2.98 (m, 2H, CH_2), 2.84 – 2.76 (m, 1H, CH_2)

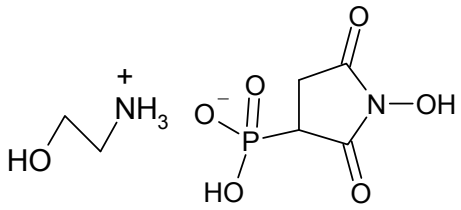
^{13}C-NMR: (101 MHz, D_2O) = δ (ppm) 174.5, 173.7 (C=O), 158.7, 133.5 ($ArC_{quart.}$), 132.1, 130.6, 130.1, 129.0, 114.8 ($ArC_{tert.}$), 79.7 (CH_2Ph), 57.9 (CH_2OH), 55.8 (OCH_3), 41.6 (CH_2NH_3), 36.0 (CH_2), 32.3 (CH_2)

$C_{21}H_{27}N_2O_8P \cdot ½H_2O$
Found [%]: C 53.27, H 5.91, N 5.77
Requires [%]: C 53.04, H 5.93, N 5.89

8.5 Procedures and Analytical Data for Chapter 4

8.5.1 Catalytic hydrogenation of 12a-f monosalts (Section 4.1)

2-Hydroxyethanaminium hydrogen (1-hydroxy-2,5-dioxopyrrolidin-3-yl)phosphonate **13a**



From 1.73 g 2-hydroxyethanaminium hydrogen (1-benzyloxy-2,5-dioxopyrrolidin-3-yl)phosphonate (**12a**) according to **GP-4**

Yield: 95%, amorphous powder

M.p.: 190 °C

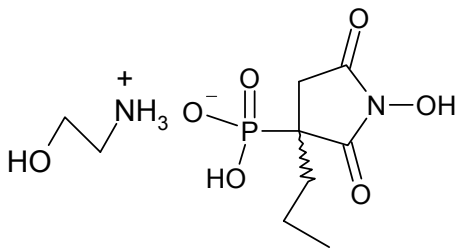
IR (KBr): 1774 cm^{-1}, 1718 cm^{-1} (C=O)

^{1}H-NMR: (400 MHz, D_2O) = δ (ppm) 3.82 (t, *J* = 4.83 Hz, 2H, CH_2NH_3), 3.36 - 3.26 (m, 1H, PCH), 3.15 (t, *J* = 7.78 Hz, 2H, CH_2OH), 3.12 - 3.03 (m, 1H, CH_2), 2.93 - 2.83 (m, 1H, CH_2)

^{13}C-NMR: (101 MHz, D_2O) = δ (ppm) 175.2, 173.4 (C=O), 57.9 (CH_2OH), 41.6 (CH_2NH_3), 38.7 (d, $^{1}J_{C,P}$ = 126.21 Hz, PC), 28.8 (CH_2)

$C_6H_{13}N_2O_7P$
Requires [%]: C 28.13, H 5.12, N 10.94
Found [%]: C 28.11, H 5.28, N 10.78

2-Hydroxyethanaminium hydrogen (1-hydroxy-3-propyl-2,5-dioxopyrrolidin-3-yl)phosphonate **13b**



From 1.93 g 2-hydroxyethanaminium hydrogen (3-allyl-1-benzyloxy-2,5-dioxopyrrolidin-3-yl)phosphonate (**12b**) according to **GP-4**

Yield: 92%, amorphous powder

M.p.: 168 °C

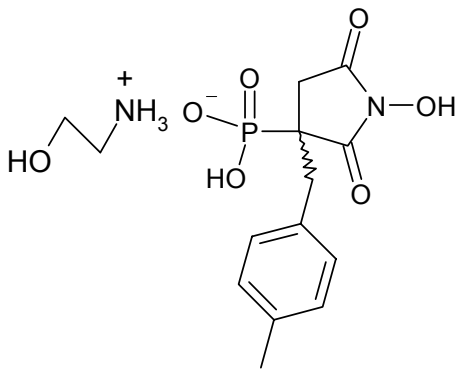
IR (KBr): 1780 cm^{-1}, 1701 cm^{-1} (C=O)

^{1}H-NMR: (400 MHz, D_2O) = δ (ppm) 3.82 (t, *J* = 5.06 Hz, 2H, CH_2NH_3), 3.14 (t, *J* = 5.29 Hz, 2H, CH_2OH), 3.07 – 2.81 (m, 2H, CH_2), 2.15 – 2.05 (m, 1H, CH_2), 1.83 – 1.74 (m, 1H, CH_2), 1.41 – 1.33 (m, 1H, CH_2), 1.19 – 1.06 (m, 1H, CH_2), 0.92 (t, *J* = 7.40 Hz, 3H, CH_3)

^{13}C-NMR: (101 MHz, D_2O) = δ (ppm) 176.9, 175.8 (C=O), 57.9 (CH_2OH), 48.0 (d, $^{1}J_{C,P}$ = 127.16, PC), 41.6 (CH_2NH_3), 33.8 (d, $^{2}J_{C,P}$ = 2.55, CCH_2), 33.3 (CH_2), 17.4 (d, $^{3}J_{C,P}$ = 10.69 Hz, CH_2CH_3), 13.8 (CH_3)

$C_9H_{19}N_2O_7P$
Requires [%]: C 36.25, H 9.39, N 6.42
Found [%]: C 35.94, H 9.28, N 6.45

2-Hydroxyethanaminium hydrogen [1-hydroxy-3-(4-methylbenzyl)-2,5-dioxopyrrolidin-3-yl]phosphonate **13c**



From 2.25 g 2-hydroxyethanaminium hydrogen [1-benzyloxy-3-(4-methylbenzyl)-2,5-dioxopyrrolidin-3-yl]phosphonate (**12c**) according to **GP-4**

Yield: 90%, amorphous powder

M.p.: 214 °C

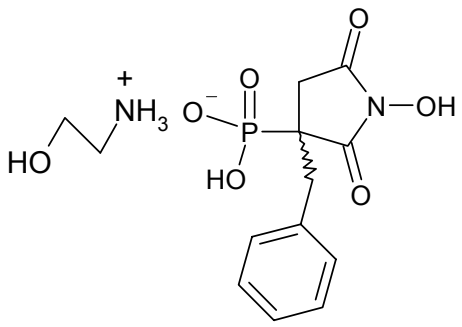
IR (KBr): 1780cm^{-1}, 1701cm^{-1} (C=O)

^{1}H-NMR: (400 MHz, D_2O) = δ (ppm) 6.95 – 6.83 (m, 4H, ArH), 3.56 (m, *J* = 6.52 Hz, 2H, CH_2NH_3), 3.36 – 3.32 (m, 1H, CH_2), 2.88 (t, *J* = 8.14 Hz, 2H, CH_2OH), 2.78 – 2.67 (m, 2H, CH_2), 2.48 – 2.41 (m, 1H, CH_2), 2.03 (s, 3H, CH_3)

^{13}C-NMR: (101 MHz, D_2O) = δ (ppm) 174.5, 174.3 (C=O), 138.1, 132.3 ($ArC_{quart.}$), 130.2, 129.8 ($ArC_{tert.}$), 57.9 (CH_2OH), 48.0 (d, $^{1}J_{C,P}$ = 127.05 Hz, PC), 41.6 (CH_2NH_3), 36.8 (CH_2), 32.3 (CH_2), 20.4 (CH_3)

$C_{14}H_{21}N_2O_7P \cdot ½H_2O$
Found [%]: C 45.83, H 5.87, N 7.60
Requires [%]: C 45.51, H 6.01, N 7.59

2-Hydroxyethanaminium hydrogen (3-benzyl-1-hydroxy-2,5-dioxopyrrolidin-3-yl)phosphonate **13d**



From 2.18 g 2-hydroxyethanaminium hydrogen (3-benzyl-1-benzyloxy-2,5-dioxopyrrolidin-3-yl)phosphonate (**12d**) according to **GP-4**

Yield: 96%, amorphous powder

M.p.: 232 °C

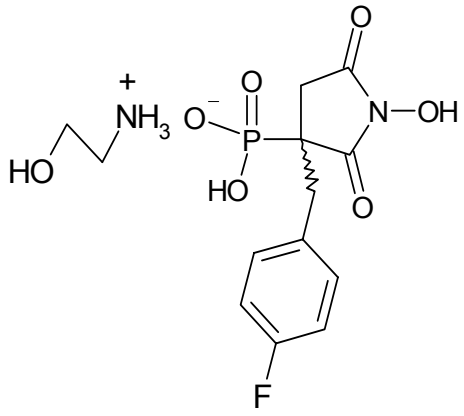
IR (KBr): 1780 cm^{-1}, 1697 cm^{-1} (C=O)

^{1}H-NMR: (400 MHz, D_2O) = δ (ppm) 7.38 – 7.21 (m, 5H, ArH), 3.82 (t, *J* = 5.92 Hz, 2H, CH_2NH_3), 3.67 – 3.62 (m, 1H, CH_2), 3.14 (t, *J* = 6.30 Hz, 2H, CH_2OH), 3.05 – 2.97 (m, 2H, CH_2), 2.96 – 2.75 (m, 1H, CH_2)

^{13}C-NMR: (101 MHz, D_2O) = δ (ppm) 176.1, 174.3 (C=O), 135.5 ($ArC_{quart.}$), 130.0, 129.2, 127.9 ($ArC_{tert.}$), 57.9 (CH_2OH), 49.0 (d, $^{1}J_{C,P}$ = 126.65 Hz, PC), 41.6 (CH_2NH_3), 37.2 (CH_2), 32.3 (CH_2)

$C_{13}H_{19}N_2O_7P$
Requires [%]: C 45.09, H 5.53, N 8.09
Found [%]: C 44.83, H 5.36, N 7.92

2-Hydroxyethanaminium hydrogen [3-(4-fluorobenzyl)-1-hydroxy-2,5-dioxopyrrolidin-3-yl]phosphonate **13e**



From 2.27 g 2-hydroxyethanaminium hydrogen [1-benzyloxy-3-(4-fluorobenzyl)-2,5-dioxopyrrolidin-3-yl]phosphonate (**12e**) according to **GP-4**

Yield: 90%, amorphous powder

M.p.: 160 °C

IR (KBr): 1782cm^{-1}, 1697cm^{-1} (C=O)

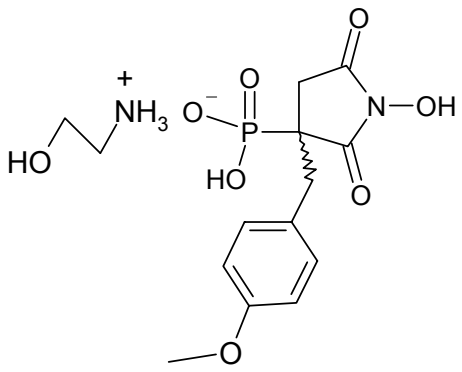
^{1}H-NMR: (400 MHz, D_2O) = δ (ppm) 7.09 – 7.06 (m, 4H, ArH), 3.82 (t, J = 7.75 Hz, 2H, CH_2NH_3), 3.64 – 3.59 (m, 1H, CH_2), 3.14 (t, J = 7.74 Hz, 2H, CH_2OH), 3.06 – 2.90 (m, 2H, CH_2), 2.74 – 2.67 (m, 1H, CH_2)

^{13}C-NMR: (101 MHz, D_2O) = δ (ppm) 176.0, 174.2 (C=O), 162.4 (d, $J_{C,F}$ = 243.13 Hz, CF), 131.8, 115.7 ($ArC_{tert.}$), 57.9 (CH_2OH), 48.9 (d, $^{1}J_{C,P}$ = 126.65 Hz, PC), 41.6 (CH_2NH_3), 36.4 (CH_2), 32.2 (CH_2)

.

$C_{13}H_{18}FN_2O_7P \cdot ½H_2O$
Requires [%]: C 41.81, H 5.13, N 7.51
Found [%]: C 41.91, H 5.04, N 7.39

2-Hydroxyethanaminium hydrogen [1-hydroxy-3-(4-methoxybenzyl)-2,5-dioxopyrrolidin-3-yl]phosphonate **13f**



From 2.33 g 2-hydroxyethanaminium hydrogen [1-benzyloxy-3-(4-methoxybenzyl)-2,5-dioxopyrrolidin-3-yl]phosphonate (**12f**) according to **GP-4**

Yield: 96%, amorphous powder

M.p.: 214 °C

IR (KBr): 1780cm^{-1}, 1716cm^{-1} (C=O)

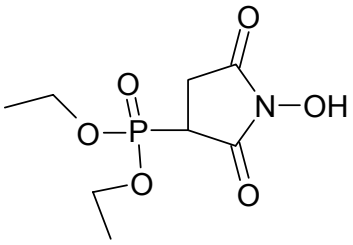
^{1}H-NMR: (400 MHz, D_2O) = δ (ppm) 7.17 – 6.95 (m, 4H, ArH), 3.84 – 3.82 (m, 2H, CH_2NH_3), 3.81 (s, 3H, OCH_3), 3.61 – 3.56 (m, 1H, CH_2), 3.15 (t, J = 5.16 Hz, 2H, CH_2OH), 3.05 – 2.92 (m, 2H, CH_2), 2.74 – 2.68 (m, 1H, CH_2)

^{13}C-NMR: (101 MHz, D_2O) = δ (ppm) 176.2, 174.4 (C=O), 158.5, 127.9 ($ArC_{quart.}$), 132.5, 114.6 ($ArC_{tert.}$), 49.1 (d, $^{1}J_{C,P}$ = 124.62 Hz, PC), 57.9 (CH_2OH), 55.7 (OCH_3), 41.6 (CH_2NH_3), 36.5 (CH_2), 32.3 (CH_2)

$C_{14}H_{21}N_2O_8P$
Requires [%]: C 44.69, H 5.62, N 7.44
Found [%]: C 44.49, H 5.67, N 7.37

8.5.2 Catalytic hydrogenation of diethyl phosphonate 1-benzyloxysuccinimides (5) (Section 4.2)

Diethyl (1-hydroxy-2,5-dioxopyrrolidin-3-yl)phosphonate (**14a**)



From 0.34 g diethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4a**) according to **GP-4**

Yield: 76%, amorphous powder

M.p.: 95 °C

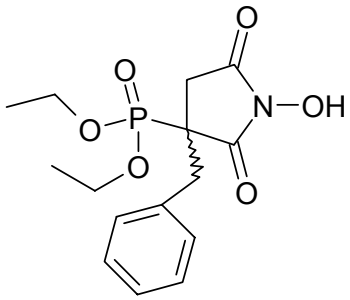
IR (KBr): 1791 cm^{-1}, 1724 cm^{-1} (C=O), 1230 cm^{-1} (P=O), 1026 cm^{-1} (POC) cm^{-1}

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 4.19 (m, 4H, CH_2), 3.32 (m, 1H, PCH), 2.92 (m, 2H, CH_2), 1.35 (m, 6H, (CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.2, 167.4 (C=O), 64.8, (CH_2), 63.8 (CH_2), 27.6 (CH_2), 36.6 (d, $^{1}J_{C.P}$ = 147.00 Hz, PCH), 16.3 (CH_3)

$C_8H_{14}NO_6P$
Requires [%]: C 38.26, H 5.62, N 5.58
Found [%]: C 38.25, H 5.51, N 5.60

Diethyl (3-benzyl-1-hydroxy-2,5-dioxopyrrolidin-3-yl)phosphonate **14b**



From 0.43 g diethyl [3-benzyl-1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**5c**) according to **GP-4**

Yield: 69%, amorphous powder

M.p.: 162 °C

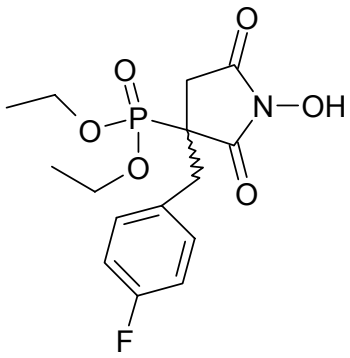
IR (KBr): 1786cm^{-1}, 1724cm^{-1} (C=O), 1242 (P=O), 1026 (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.11 – 7.04 (m, 5H, ArH), 4.22 – 4.19 (m, 4H, CH_2), 3.64 – 3.61 (m, 2H, CH_2), 2.96 – 2.86 (m, 2H, CH_2), 2.58 – 2.56 (m, 2H, CH_2), 1.38 (m, 6H, (CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.9, 169.8 (C=O), 133.9 ($ArC_{quart.}$), 130.5, 129.4, 128.3 ($ArC_{tert.}$), 66.1 (d, $^{2}J_{C,P}$ = 7.12 Hz, POC), 64.1 (d, $^{2}J_{C,P}$ = 7.63 Hz, POC), 47.8 (d, $^{1}J_{C.P}$ = 143.4 Hz, PC), 37.1 (CH_2), 31.3 (CH_2), 16.8 (q, $^{3}J_{C,P}$ = 6.60 Hz, CH_3)

$C_{15}H_{20}NO_6P$
Requires [%]: C 52.79, H 5.91, N 4.10
Found [%]: C 52.37, H 5.97, N 3.91

Diethyl [3-(4-fluorobenzyl)-1-hydroxy-2,5-dioxopyrrolidin-3-yl]phosphonate **14c**



From 0.45 g diethyl [1-benzyloxy-3-(4-fluorobenzyl)-2,5-dioxopyrrolidin-3-yl]phosphonate (**5d**) according to **GP-4**

Yield: 85%, amorphous powder

M.p.: 155 °C

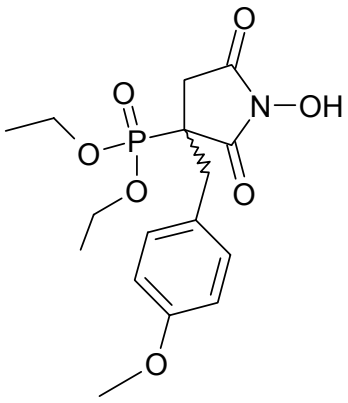
IR (KBr): 1782cm^{-1}, 1720cm^{-1} (C=O), 1245cm^{-1} (P=O), 1010cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.06- 7.04 (m, 4H, ArH), 4.32 - 4.30 (m, 4H, CH_2), 3.60 - 2.85 (m, 2H, CH_2Ph), 2.98 - 2.54 (m, 2H, CH_2), 1.37 -1.35 (m, 6H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.8, 169.7 (C=O), 162.8 (d, $J_{C.F}$ = 247.7 Hz, CF), 132.2, 116.5 ($ArC_{tert.}$), 129.6 ($ArC_{quart.}$), 66.2 (CH_2), 64.2 (CH_2), 47.8 (d, $^{1}J_{C.P}$ = 143.43 Hz, PC), 36.3 (CH_2), 31.3 (CH_2), 16.7 (CH_3)

$C_{15}H_{19}FNO_6P$
Requires [%]: C 50.14, H 5.33, N 3.90
Found [%]: C 50.05, H 5.44, N 3.75

Diethyl (1-hydroxy-3-(4-methoxybenzyl)-2,5-dioxopyrrolidin-3-yl)phosphonate **14d**



From 0.46 g diethyl [1-benzyloxy-3-(4-methoxybenzyl)-2,5-dioxopyrrolidin-3-yl]phosphonate (**5e**) according to **GP-4**

Yield: 49%, amorphous powder

M.p.: 156 °C

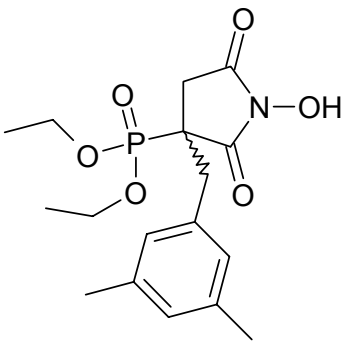
IR (KBr): 1783cm^{-1}, 1721 cm^{-1} (C=O), 1245 cm^{-1} (P=O), 1018 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.02 – 6.78 (m, 4H, ArH), 4.37 - 4.20 (m, 4H, CH_2), 3.76 (s, 3H, CH_3), 3.62 – 3.57 (m, 1H, CH_2), 3.01 - 2.78 (m, 2H, CH_2), 2.61 – 2.54 (m, 1H, CH_2), 1.43 - 1.36 (m, 6H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 167.1 (C=O), 131.6, 114.8 ($ArC_{tert.}$), 63.9 (d, $^{2}J_{C,P}$ = 7.12 Hz, POC), 64.1 (d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 55.6 (OCH_3), 36.1 (CH_2), 31.4 (CH_2), 16.5 (CH_3)

$C_{16}H_{22}NO_7P \cdot ½H_2O$
Requires [%]: C 50.53, H 6.10, N 3.68
Found [%]: C 50.18, H 6.00, N 3.86

Diethyl [3-(3,5-dimethylbenzyl)-1-hydroxy-2,5-dioxopyrrolidin-3-yl]phosphonate **14e**



From 0.46 g diethyl [3-(3,5-dimethylbenzyl)-1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**5m**) according to **GP-4**

Yield: 79%, amorphous powder

M.p.: 149 °C

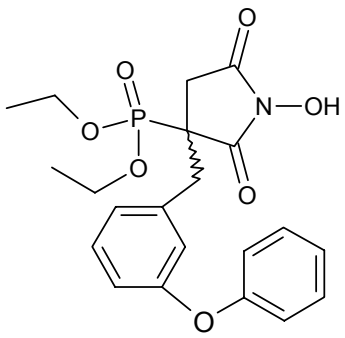
IR (KBr): 1785 cm^{-1}, 1723 cm^{-1} (C=O), 1237 cm^{-1} (P=O), 1020 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 9.61 (s, 1H, OH), 6.96 (s, 1H, ArH), 6.86 (s, 2H, ArH), 4.36 – 4.22 (m, 4H, CH_2), 3.57 (t, *J* = 6.63 Hz, 1H, CH_2), 2.93 (t, *J* = 17.99 Hz, 1H, CH_2), 2.77 (t, *J* = 6.94 Hz, 1H CH_2), 2.59 (t, *J* = 9.15 Hz, 1H, CH_2), 2.24 (s, 6H, CH_3), 1.42 - 1.38 (m, 6H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.9, 169.9 (C=O), 138.9, 133.6 ($ArC_{quart.}$), 129.9, 128.2 ($ArC_{tert.}$), 66.0 (d, $^{2}J_{C,P}$ = 6.11 Hz, CH_2), 64.0 (d, $^{2}J_{C,P}$ = 7.12 Hz, CH_2), 36.4 (CH_2), 36.7 (CH_2), 31.2 (CH_2), 21.6(CH_3) 16.8 (CH_3)

$C_{17}H_{24}NO_6P$
Requires [%]: C 55.28, H 6.55, N 3.79
Found [%]: C 54.47, H 6.60, N 3.98

Diethyl [1-hydroxy-3-(3-phenoxybenzyl)-2,5-dioxopyrrolidin-3-yl]phosphonate **14f**



From 0.52 g diethyl [1-benzyloxy-3-(3-phenoxybenzyl)-2,5-dioxopyrrolidin-3-yl]phosphonate (**5f**)according to **GP-4**

Yield: 52%, amorphous powder

M.p.: 174 °C

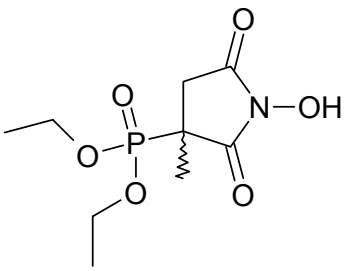
IR (KBr): 1787 cm^{-1}, 1723 cm^{-1} (C=O), 1254 cm^{-1} (P=O), 971 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 9.37 (s, 1H, OH), 7.35 – 6.76 (m, 9H, ArH), 4.36 – 4.19 (m, 4H, CH_2), 3.63 – 3.60 (m, 1H, CH_2), 2.99 – 2.93 (m, 1H, CH_2), 2.82 – 2.79 (m, 1H, CH_2), 2.58 – 2.55 (m, 1H, CH_2), 1.39 – 1.36 (m, 6H, $(CH_3)_2$)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 171.6, 169.6 (C=O), 158.1, 152.3 ($ArC_{quart.}$), 125.1, 123.9, 120.8, 119.4, 118.6 ($ArC_{tert.}$), 65.9 (d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 64.0 (d, $^{2}J_{C,P}$ = 7.63 Hz, POC), 47.8 (d, $^{1}J_{C.P}$ = 142.42 Hz, PC), 36.8 (CH_2), 31.3 (CH_2), 16.7 (q, $^{3}J_{C,P}$ = 5.76 Hz, CH_3)

$C_{21}H_{24}NO_7P$
Requires [%]: C 58.20, H 5.58, N 3.23
Found [%]: C 57.85, H 5.76, N 3.17

Diethyl (1-hydroxy-3-methyl-2,5-dioxopyrrolidin-3-yl)phosphonate **14g**



From 0.36 g diethyl [1-benzyloxy-3-methyl-2,5-dioxopyrrolidin-3-yl]phosphonate (**5g**) according to **GP-4**

Yield: 76%, amorphous powder

M.p.: 118 °C

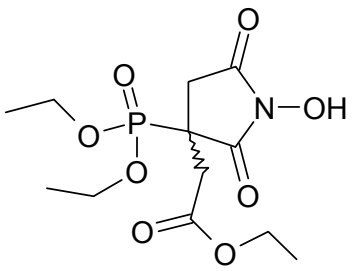
IR (KBr): 1782 cm^{-1}, 1720 cm^{-1} (C=O), 1230 cm^{-1} (P=O), 1014 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 4.31 – 4.17 (m, 4H, CH_2), 3.20 (t, *J* = 17.14 Hz, 1H, CH_2), 2.45 (q, *J* = 9.40 Hz, 1H, CH_2), 1.55 (d, *J* = 16.85 Hz, 3H, CH_3), 1.40 – 1.34 (m, 6H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 171.4, 169.7 (C=O), 65.4 (d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 63.6 (d, $^{2}J_{C,P}$ = 7.63 Hz, POC), 41.9 (d, $^{1}J_{C.P}$ = 147.50 Hz, PC), 35.9 (CH_2), 31.9 (CH_2), 19.7 (d, $^{3}J_{C,P}$ = 4.98 Hz, CH_3), 16.3 (d, $^{3}J_{C,P}$ = 5.93 Hz, CH_3)

$C_9H_{16}NO_6P$
Requires [%]: C 40.76, H 6.08, N 5.28
Found [%]: C 40.76, H 6.08, N 5.25

Ethyl [3-(diethoxyphosphoryl)-1-hydroxy-2,5-dioxopyrrolidin-3-yl]acetate **14h**



From 0.43 g ethyl [1-benzyloxy-3-(diethoxyphosphoryl)-2,5-dioxopyrrolidin-3-yl]acetate (**5h**) according to **GP-4**

Yield: 68%, amorphous powder

M.p.: 109 °C

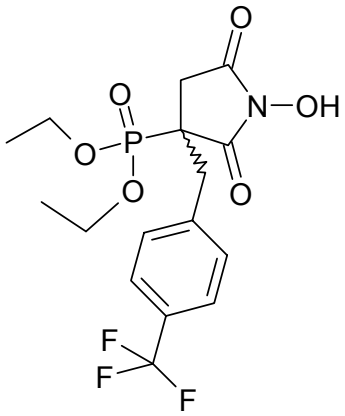
IR (KBr): 1792 cm^{-1}, 1720 cm^{-1} (C=O), 1225 cm^{-1} (P=O), 1020 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 4.28 – 4.11 (m, 4H, CH_2), 3.36 -3.30 (m, 1H, CH_2), 3.13 (q, J = 18.18 Hz, 1H, CH_2), 2.83 – 2.67 (m, 2H, CH_2), 1.39 – 1.35 (m, 6H, CH_3) 1.55 (d, J = 7.31 Hz, 3H, CH_3),

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 169.5, 169.2, 169.0 (C=O), 65.9 (d, $^{2}J_{C,P}$ = 6.10 Hz, POC), 63.8 (d, $^{2}J_{C,P}$ = 7.12 Hz, POC),61.8 (OCH_2), 43.5 (d, $^{1}J_{C.P}$ = 144.42 Hz, PC), 35.6 (CH_2), 33.4 (CH_2), 16.4 (CH_3), 13.9 (CH_3)

$C_{12}H_{20}NO_8P$
Requires [%]: C 42.74, H 5.98, N 4.15
Found [%]: C 42.86, H 5.94, N 4.03

Diethyl {1-hydroxy-3-[4-(trifluoromethyl)benzyl]-2,5-dioxopyrrolidin-3-yl}phosphonate **14i**



From 0.50 g diethyl {3-[4-(trifluoromethyl)benzyl]-1-benzyloxy-2,5-dioxopyrrolidin-3-yl}phosphonate (**5i**) according to **GP-4**

Yield: 66%, amorphous powder

M.p.: 149 °C

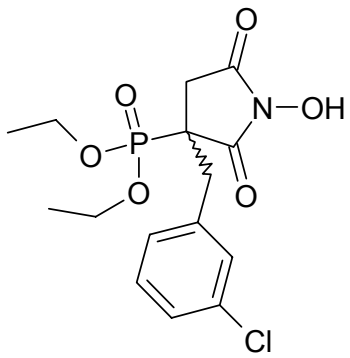
IR (KBr): 1786 cm^{-1}, 1731 cm^{-1} (C=O), 1247 cm^{-1} (P=O), 1020 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 9.65 (s, 1H, OH), 7.55 – 7.23 (m, 4H, ArH), 4.38 – 4.20 (m, 4H, CH_2), 3.71 – 3.66 (m, 1H, CH_2), 3.06 – 2.91 (m, 2H, CH_2), 2.54 – 2.46 (m, 1H, CH_2), 1.44 - 1.37 (m, 6H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.5, 169.3 (C=O), 138.1 (d, $J_{C,F}$ = 15.77 Hz, CF), 130.9, 126.3 ($ArC_{tert.}$), 66.3 (d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 64.3 (d, $^{2}J_{C,P}$ = 7.63 Hz, POC), 47.6 (d, $^{1}J_{C,P}$ = 143.94 Hz, PC), 36.8 (CH_2), 31.3 (CH_2), 16.8 (d, $^{3}J_{C,P}$ = 5.60 Hz, CH_3), 16.3 (d, $^{3}J_{C,P}$ = 5.59 Hz, CH_3)

$C_{16}H_{19}F_3NO_6P$
Requires [%]: C 46.95, H 4.68, N 3.42
Found [%]: C 46.75, H 4.68, N 3.42

Diethyl [3-(3-chlorobenzyl)-1-hydroxy-2,5-dioxopyrrolidin-3-yl]phosphonate **14j**



From 0.47 g diethyl [3-(3-chlorobenzyl)-1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**5j**) according to **GP-4**

Yield: 56%, amorphous powder

M.p.: 139 °C

IR (KBr): 1787 cm^{-1}, 1731 cm^{-1} (C=O), 1247 cm^{-1} (P=O), 1023 cm^{-1} (POC)

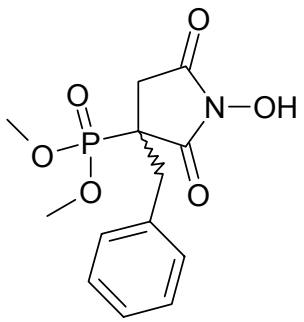
^{1}H-NMR: (400 MHz, DMSO-d_6) = δ (ppm) 7.34 - 7.17 (m, 4H, ArH), 4.15 - 4.11 (m, 4H, CH_2), 3.44 - 3.42 (m, 1H, CH_2), 3.02 - 2.75 (m, 3H, CH_2), 1.29 - 1.25 (m, 6H, CH_3)

^{13}C-NMR: (101 MHz, DMSO-d_6) = δ (ppm) 169.8 (C=O), 137.2, 136.8 $ArC_{quart.}$), 130.7, 130.5, 129.3, 129.0, 127.9 ($ArC_{tert.}$), 64.0 (CH_2), 63.7 (CH_2), 47.2 (d, $^{1}J_{C,P}$ = 138.86 Hz, PC), 35.6 (CH_2), 31.6 (CH_2), 16.5 (CH_3)

$C_{15}H_{19}ClNO_6P \cdot ½H_2O$

Requires [%]: C 46.83, H 5.24, N 3.64
Found [%]: C 46.96, H 5.11, N 3.62

Dimethyl (3-benzyl-1-hydroxy-2,5-dioxopyrrolidin-3-yl)phosphonate **14k**



From 0.40 g dimethyl [3-benzyl-1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**5k**) according to **GP-4**

Yield: 82%, amorphous powder

M.p.: 198 °C

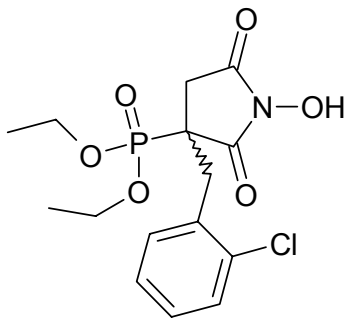
IR (KBr): 1783 cm^{-1}, 1724 cm^{-1} (C=O), 1227 cm^{-1} (P=O), 1039 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 9.58 (s, 1H, OH), 7.28 – 7.08 (m, 5H, ArH), 3.99 (d, *J* = 10.93 Hz, OCH_3), 3.85 (d, *J* = 10.93 Hz, 3H, OCH_3), 3.65 – 3.62 (m, 1H, CH_2), 2.98 – 2.96 (m, 1H, CH_2), 2.89 – 2.85 (m, 1H, CH_2), 2.59 – 2.54 (m, 1H, CH_2)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 169.5 (C=O), 133.5 ($ArC_{quart.}$), 130.4, 129.5, 128.4 ($ArC_{tert.}$), 56.3 (d, $^{2}J_{C,P}$ = 6.62 Hz, POC), 54.1 (d, $^{2}J_{C,P}$ = 7.63 Hz, POC), 48.7 (PC), 31.3 (CH_2), 30.1 (CH_2)

$C_{13}H_{16}NO_6P$
Requires [%]: C 49.85, H 5.15, N 4.47
Found [%]: C 49.55, H 5.13, N 4.46

Diethyl [3-(2-chlorobenzyl)-1-hydroxy-2,5-dioxopyrrolidin-3-yl]phosphonate **14l**



From 0.47 g diethyl [3-(2-chlorobenzyl)-1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**5l**) according to **GP-4**

Yield: 28%, amorphous powder

M.p.: 138 °C

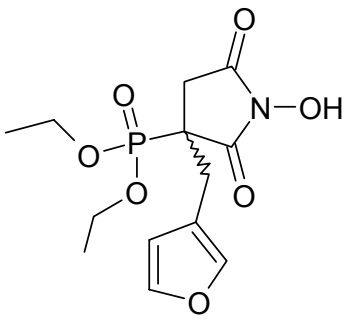
IR (KBr): 1787 cm^{-1}, 1732 cm^{-1} (C=O), 1247 cm^{-1} (P=O), 1023 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, DMSO-d_6) = δ (ppm) 7.49 - 7.16 (m, 4H, ArH), 4.18 - 4.15 (m, 4H, CH_2), 3.45 – 3.40 (m, 1H, CH_2), 2.89 (t, J = 17.55 Hz, 1H, CH_2), 2.64 – 2.33 (m, 2H, CH_2), 1.30 – 1.26 (m, 6H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.7, 169.6 (C=O), 134.9, ($ArC_{quart.}$), 132.1, 131.9, 129.6, ($ArC_{tert.}$), 65.7 (d, $^{2}J_{C,P}$ = 7.23 Hz, POC), 63.9 (d, $^{2}J_{C,P}$ = 8.03 Hz, POC), 47.0 (d, $^{1}J_{C,P}$ = 141.35 Hz, PC), 32.3 (CH_2), 30.6 (CH_2), 16.6 (CH_3)

$C_{15}H_{19}ClNO_6P$
Requires [%]: C 47.95, H 5.10, N 3.73
Found [%]: C 47.77, H 5.11, N 3.59

Diethyl [3-(3-furylmethyl)-1-hydroxy-2,5-dioxopyrrolidin-3-yl]phosphonate **14m**



From 0.42 g diethyl [3-(3-furylmethyl)-1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**5p**) according to **GP-4**

Yield: 62%, pinkish powder

M.p.: 109 °C

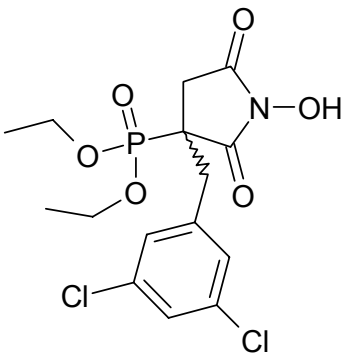
IR (KBr): 1793 cm^{-1}, 1732 cm^{-1} (C=O), 1242 cm^{-1} (P=O), 1020 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 9.83 (s, 1H, OH), 7.32 - 7.27 (m, 2H, OCH), 6.17 (s, 1H, CCH), 4.36 - 4.21 (m, 4H, CH_2), 3.43 -3.39 (m, 1H, CH_2), 3.00(t, *J* = 17.96 Hz, 1H, CH_3), 2.79 (m, 1H, CH_2), 2.57 – 2.51 (m, 1H, CH_2) 1.42 - 1.37 (m, 6H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 171.9, 169.9 (C=O), 144.4, 141.9 ($ArC_{tert.}$), 117.3 ($ArC_{quart.}$), 111.4 (CCH), 66.1 (d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 64.2 (d, $^{2}J_{C,P}$ = 7.12 Hz, CH_2), 47.0 (d, $^{1}J_{C,P}$ = 143.94 Hz, PC), 31.9 (CH_2), 27.2 (CH_2), 16.7 (CH_3)

$C_{13}H_{18}NO_7P$
Requires [%]: C 47.14, H 5.48, N 4.23
Found [%]: C 46.59, H 5.90, N 4.15

Diethyl [3-(3,5-dichlorobenzyl)-1-hydroxy-2,5-dioxopyrrolidin-3-yl]phosphonate **14n**



From 0.50 g diethyl [3-(3,5-dichlorobenzyl)-1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**5n**) according to **GP-4**

Yield: 84%, amorphous powder

M.p.: 169 °C

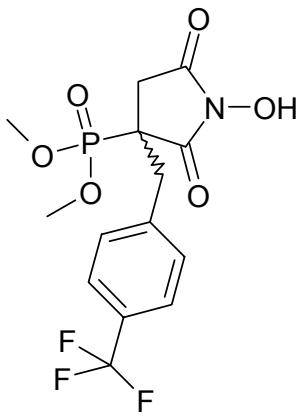
IR (KBr): 1792 cm^{-1}, 1724 cm^{-1} (C=O), 1240 cm^{-1} (P=O), 1010 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 11.47 (s, 1H, OH), 7.54 - 7.32 (m, 3H, ArH), 4.13 – 4.09 (m, 4H, CH_2), 3.38 – 3.30 (m, 1H, CH_2), 3.09 – 2.82 (m, 2H, CH_2), 1.36 – 1.22 (m, 6H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.5, 169.5 (C=O), 138.9, 134.3 ($ArC_{quart.}$), 129.5, 127.5 ($ArC_{tert.}$), 64.0 (CH_2), 63.7 (CH_2), 47.0 (d, $^{1}J_{C,P}$ = 149.03 Hz, PC), 39.2 (CH_2), 31.8 (CH_2), 16.5 (CH_3)

$C_{15}H_{18}Cl_2NO_6P$
Requires [%]: C 43.92, H 4.42, N 3.41
Found [%]: C 44.36, H 4.84, N 3.36

Dimethyl {1-hydroxy-3-[4-(trifluoromethyl)benzyl]-2,5-dioxopyrrolidin-3-yl}phosphonate **14o**



From 0.47 g dimethyl {3-[4-(trifluoromethyl)benzyl]-1-benzyloxy-2,5-dioxopyrrolidin-3-yl}phosphonate (**5o**) according to **GP-4**

Yield: 66%, amorphous powder

M.p.: 200 °C

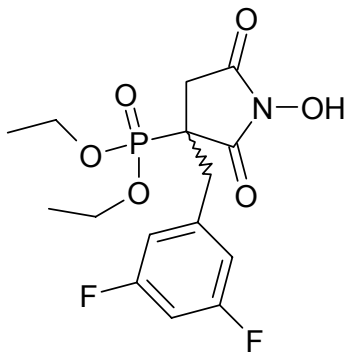
IR (KBr): 1786 cm^{-1}, 1731 cm^{-1} (C=O), 1249 cm^{-1} (P=O), 1019 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.56 – 7.23 (m, 4H, ArH), 3.99 (d, *J* = 10.72 Hz, 3H, CH_3), 3.87 (d, *J* = 11.04 Hz, 3H, CH_3), 3.68 (q, *J* = 6.91 Hz, 1H, CH_2), 3.05 - 2.93 (m, 2H, CH_2), 2.53 (q, *J* = 9.38 Hz, 1H, CH_2)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.1, 169.2 (C=O), 138.9 $ArC_{quart.}$), 131.1, 128.2 ($ArC_{tert.}$), 54.3 (d, $^{2}J_{C,P}$ = 6.42 Hz, CH_2), 53.9 (d, $^{2}J_{C,P}$ = 7.23 Hz, CH_2), 46.7 (d, $^{1}J_{C,P}$ = 141.35 Hz, PC), 35.3 (CH_2), 31.0 (CH_2)

$C_{14}H_{15}F_3NO_6P$
Requires [%]: C 44.11, H 3.97, N 3.67
Found [%]: C 44.05, H 4.04, N 3.68

Diethyl [3-(3,5-difluorobenzyl)-1-hydroxy-2,5-dioxopyrrolidin-3-yl]phosphonate **14p**



From 0.47 g diethyl [3-(3,5-difluorobenzyl)-1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**5q**) according to **GP-4**

Yield: 82%, amorphous powder

M.p.: 166 °C

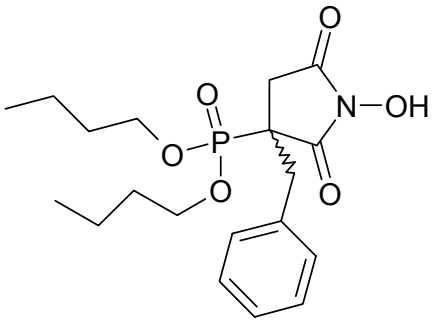
IR (KBr): 1790 cm^{-1}, 1724 cm^{-1} (C=O), 1249 cm^{-1} (P=O), 990 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 9.88 (s, 1H, OH), 6.66 – 6.73 (m, 3H, ArH), 4.36 - 4.20 (m, 4H, CH_2), 3.60 (t, *J* = 6.90 Hz, 1H, CH_3), 3.01 (t, *J* = 17.01 Hz, 1H, CH_2), 2.86 (q, *J* = 9.66 Hz, 1H, CH_2), 2.52 (q, *J* = 14.71 Hz, 1H, CH_2), 1.42 (t, *J* = 7.36 Hz, 3H, CH_3), 1.38 (t, *J* = 7.36 Hz, 3H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.4, 169.4 (C=O), 137.6, ($ArC_{quart.}$), 113.6, 104.1 ($ArC_{tert.}$), 66.3 (d, $^{2}J_{C,P}$ = 6.61 Hz, CH_2), 64.4 (d, $^{2}J_{C,P}$ = 7.63 Hz, CH_2), 47.5 (d, $^{1}J_{C,P}$ = 143.44 Hz, PC), 36.5 (CH_2), 31.3 (CH_2), 16.7 (CH_3)

$C_{15}H_{18}F_2NO_6P$
Requires [%]: C 47.75, H 4.81, N 3.71
Found [%]: C 47.74, H 4.81, N 3.73

Dibutyl [3-benzyl-1-hydroxy-2,5-dioxopyrrolidin-3-yl]phosphonate **14q**



From 1.97 g dibutyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**4d**) according to **GP-2** and **GP-4**

Yield: 9%, amorphous powder

M.p.: 147 °C

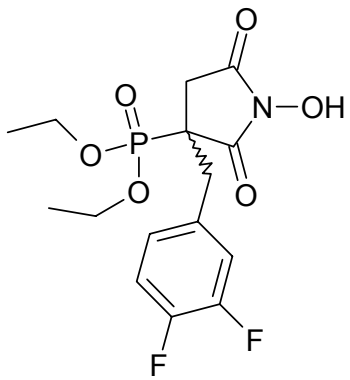
IR (KBr): 1783 cm^{-1}, 1731 cm^{-1} (C=O), 1245 cm^{-1} (P=O), 1024 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 9.57 (OH), 7.29 -7.09 (m, 5H, ArH), 4.32 - 4.13 (m, 4H, CH_2), 3.65 (q, *J* = 7.41 Hz, 1H, CH_2), 2.96 – 2.83 (m, 2H, CH_2), 2.55 (q, *J* = 9.15 Hz, CH_2), 1,75 – 1,65 (m, 4H, CH_2), 1,48 – 1,39 (m, 4H, CH_2), 1.00 - 0.94 (m, 6H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.9, 169.8 (C=O), 133.8 ($ArC_{quart.}$), 130.4, 129.4, 128.3 ($ArC_{tert.}$), 69.7 (d, $^{2}J_{C,P}$ = 6.86 Hz, POC), 67.8 (d, $^{2}J_{C,P}$ = 8.40 Hz, POC), 47.9 (d, $^{1}J_{C.P}$ = 143.44 Hz, PC), 37.2 (CH_2), 32.9 (d, $^{3}J_{C,P}$ = 5.35 Hz, CH_2), 32.7 (d, $^{3}J_{C,P}$ = 6.10 Hz, CH_2), 31.3 (CH_2), 19.1 (CH_2), 13.9 (CH_3)

$C_{19}H_{28}NO_6P$
Requires [%]: C 57.19, H 7.10, N 3.50
Found [%]: C 57.42, H 7.31, N 3.52

Diethyl [3-(3,4-difluorobenzyl)-1-hydroxy-2,5-dioxopyrrolidin-3-yl]phosphonate **14r**



From 0.47 g diethyl [3-(3,4-difluorobenzyl)-1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**5r**) according to **GP-4**

Yield: 77%, amorphous powder

M.p.: 151 °C

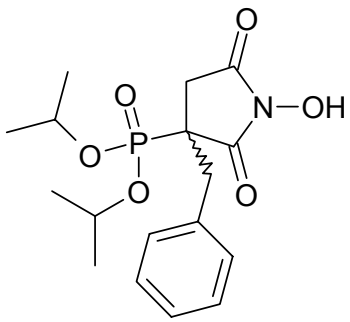
IR (KBr): 1790 cm^{-1}, 1724 cm^{-1} (C=O), 1247 cm^{-1} (P=O), 1004 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.41 - 7.09 (m, 8H, ArH), 4.81 (ABs, *J* = 11.79 Hz, 2H, CH_2Ph), 4.16 - 4.11 (m, 4H, CH_2), 3.43 - 3.37 (m, 1H, CH_2), 3.07 - 2.98 (m, 3H, CH_2), 1.25 (t, *J* = 7.54 Hz, 3H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 169.4 (C=O), 126.7, 119.6, 118.3 ($ArC_{tert.}$), 66.3 (d, $^{2}J_{C,P}$ = 6.61 Hz, CH_2), 64.3 (d, $^{2}J_{C,P}$ = 7.63 Hz, CH_2), 36.2 (CH_2), 31.3 (CH_2), 16.7 (CH_3)

$C_{15}H_{18}F_2NO_6P$
Requires [%]: C 47.75, H 4.81, N 3.71
Found [%]: C 47.84, H 5.10, N 3.64

Diisopropyl (3-benzyl-1-hydroxy-2,5-dioxopyrrolidin-3-yl)phosphonate **14s**



From 0.46 g diisopropyl [3-benzyl-1 -benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**5s**) according to **GP-4**

Yield: 85%, amorphous powder

M.p.: 156 °C

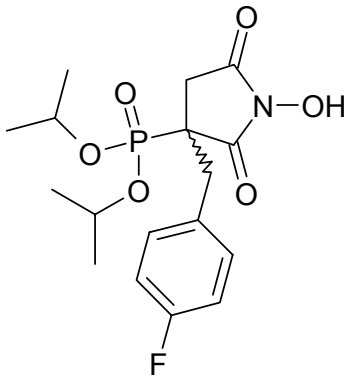
IR (KBr): 1785 cm^{-1}, 1724cm^{-1} (C=O), 1243cm^{-1} (P=O), 1001cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 9.69 (s, 1H, OH), 7.28 – 7.08 (m, 5H, ArH), 4.91 – 4.76 (m, 2H, POCH), 3.85 – 3.82 (m, 1H, CH_2), 2.96 – 2.91 (m, 1H, CH_2), 2.82 – 2.79 (m, 1H, CH_2), 2.54 – 2.51 (m, 1H, CH_2), 1.50 – 1.36 (m, 12H, $(CH_3)_4$)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.9, 169.5 (C=O), 134.2 ($ArC_{quart.}$), 130.5, 129.3, 128.2 ($ArC_{tert.}$), 75.3 (d, $^{2}J_{C,P}$ = 7.12 Hz, POC), 73.3 (d, $^{2}J_{C,P}$ = 7.63 Hz, POC), 47.9 (d, $^{1}J_{C.P}$ = 144.45 Hz, PC), 37.3 (CH_2), 31.4 (CH_2), 24.9 (d, $^{3}J_{C,P}$ = 1.52 Hz, CH_3), 24.3 (d, $^{3}J_{C,P}$ = 3.56 Hz, CH_3), 24.2 (d, $^{3}J_{C,P}$ = 5.08 Hz, CH_3), 23.8 (d, $^{3}J_{C,P}$ = 7.12 Hz, CH_3)

$C_{17}H_{24}NO_6P$
Requires [%]: C 55.28, H 6.55, N 3.79
Found [%]: C 54.90, H 6.55, N 3.82

Diisopropyl [3-(4-fluorobenzyl)-1-hydroxy-2,5-dioxopyrrolidin-3-yl]phosphonate **14t**



From 0.48 g diisopropyl [3-(4-fluorobenzyl)-1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate (**5t**) according to **GP-4**

Yield: 85%, amorphous powder

M.p.: 180 °C

IR (KBr): 1790 cm^{-1}, 1724 cm^{-1} (C=O), 1245 cm^{-1} (P=O), 990 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 9.91 (s, 1H, OH), 7.08 – 6.94 (m, 4H, ArH), 4.90 – 4.76 (m, 2H, POCH), 3.59 – 3.57 (m, 1H, CH_2CH_2), 2.98 – 2.95 (m, 1H, CH_2), 2.81 – 2.78 (m, 1H, CH_2), 3.50 – 3.47 (m, 1H, CH_2), 1.49 -1.38 (m, 12H, $(CH_3)_4$)

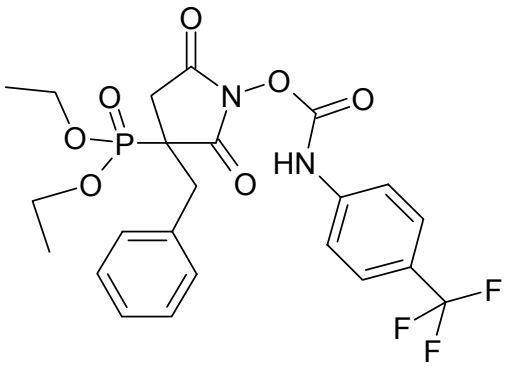
^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.8, 169.7 (C=O), 162.7 (d, $J_{C,F}$ = 247.70 Hz, CF), 132.1, 116.4, 116.2 ($ArC_{tert.}$), 75.4 (d, $^{2}J_{C,P}$ = 7.12 Hz, POC), 73.3 (d, $^{2}J_{C,P}$ = 7.63 Hz, POC), 47.9 (d, $^{1}J_{C.P}$ = 149.9 Hz, PC), 36.5 (CH_2), 31.3 (CH_2), 24.9 (d, $^{3}J_{C,P}$ = 1.53 Hz, CH_3), 24.3 (d, $^{3}J_{C,P}$ = 3.56 Hz, CH_3), 24.1 (d, $^{3}J_{C,P}$ = 5.09 Hz, CH_3), 23.7 (d, $^{3}J_{C,P}$ = 6.61 Hz, CH_3)

$C_{17}H_{23}FNO_6P \cdot ½H_2O$
Requires [%]: C 51.52, H 6.10, N 3.53
Found [%]: C 51.82, H 5.92, N 3.58

8.6 Procedures and Analytical Data for Chapter 5

8.6.1 Reaction of 1-hydroxysuccinimide 14b with isocyanates (section 5.2)

Diethyl [3-benzyl-1-({[(4-trifluoromethylphenyl)amino]carbonyl}oxy)-2,5-dioxopyrrolidin-3-yl]phosphonate **16a**



From 0.05g diethyl (3-benzyl-1-hydroxy-2,5-dioxopyrrolidin-3-yl)phosphonate (**14b**) according to **GP-5**

Yield: 34%, amorphous powder

M.p.: 151.3 °C

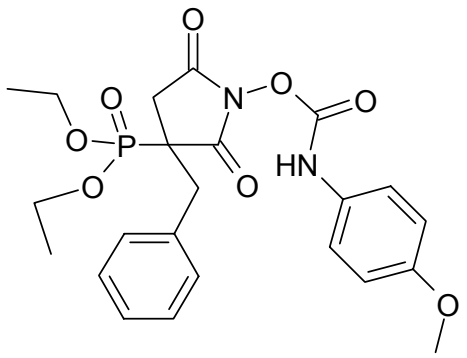
IR (KBr): 1785 cm^{-1}, 1733 cm^{-1} (C=O), 1226 cm^{-1} (P=O), 1025 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.70 – 7.16 (m, 9H, ArH), 4.30 – 4.24 (m, 4H, OCH_2), 3.78 -3.73 (m, 1H, CH_2), 3.18 – 2.74 (m, 3H, CH_2), 1.44 – 1.37 (m, 3H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 169.4, 167.1 (C=O), 130.2, 129.8, 129.1, 128.0 ($ArC_{tert.}$), 64.6 (POC), 64.4 (POC), 35.6 (CH_2), 31.4 (CH_2), 16.3 (CH_3)

$C_{23}H_{24}F_3N_2O_7P \cdot H_2O$
Requires [%]: C 50.36, H 4.80, N 5.13
Found [%]: C 49.86, H 4.46, N 5.22

Diethyl [3-benzyl-1-({[(4-methoxyphenyl)amino]carbonyl}oxy)-2,5-dioxopyrrolidin-3-yl]phosphonate **17b**



From 0.11g diethyl (3-benzyl-1-hydroxy-2,5-dioxopyrrolidin-3-yl)phosphonate (**14b**) according to **GP-5**

Yield: 84%, short white needles

M.p.: 154 °C

IR (KBr): 1779 cm^{-1}, 1739 cm^{-1} (C=O), 1248 cm^{-1} (P=O), 1017 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.32 – 7.16 (m, 7H, ArH), 7.00 (s, 1H, NH), 6.87 – 6.85 (m, 2H, ArH), 4.31 – 4.25 (m, 4H, OCH_2),3.79 (m, 1H, OCH_3), 3.78–3.73 (m, 1H, CH_2), 3.17 – 3.02 (m, 1H, CH_2), 2.79 – 2.72 (m, 1H, CH_2), 1.41 (t, J = 7.01 Hz, 3H, CH_3), 1.37 (t, J = 3.76 Hz, 3H, CH_3)

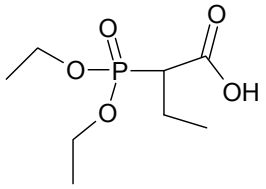
^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 169.5, 167.9, 148.7 (C=O), 133.9, 133.8 ($ArC_{quart.}$), 130.6, 129.4, 128.4, 121.6, 114.8 ($ArC_{tert.}$), 64.8 (d, $^{2}J_{C,P}$ = 7.12 Hz, POC), 64.6 (d, $^{2}J_{C,P}$ =7.12 Hz, POC), 55.9 (OCH_3), 48.4 (d, $^{1}J_{C,P}$ = 140.40 Hz, PC), 36.7 (CH_2), 31.9 (CH_2), 16.8 (q, $^{3}J_{C,P}$ = 5.43 Hz, CH_3).

$C_{23}H_{27}N_2O_8P$
Requires [%]: C 56.33, H 5.55, N 5.71
Found [%]: C 56.32, H 5.47, N 5.77

8.7 Procedures and Analytical Data for Chapter 6

8.7.1 Synthesis of ethyl protected phosphonates (Section 6.1.1)

2-(Diethoxyphosphoryl)butanoic acid **21**



From 0.5g triethyl 2-phosphonobutyrate according to **GP-6**

Yield: 96%, colourless oil

IR (film): 1736 cm^{-1} (C=O) 1231 cm^{-1} (P=O), 1022 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, DMSO-d_6) = δ (ppm) 4.06 - 3.99 (m, 4H, CH_2), 2.87 - 2.77 (m, 1H, CH_2) 1.85 - 1.67 (m, 2H, CH_2), 1.25 - 1.21 (m, 6H, CH_3), 0.91 (t, J = 7.43 Hz, 3H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.0 (C=O), 63.6(d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 62 7 (d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 47.4 (d, $^{1}J_{C,P}$ = 130.72 Hz, PC), 20.7 (d, $^{2}J_{C,P}$ = 5.60 Hz, CH_2), 16.3 (t, $^{3}J_{C,P}$ = 6.10 Hz, 6H, CH_3), 12.9 (CH_3)

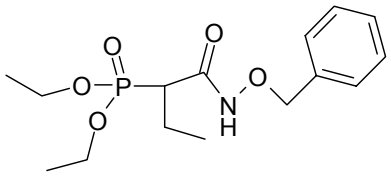
Compound **21** was used with this determined purity for further steps.

Synthesis procedure for **22**

2-(Diethoxyphosphoryl)butanoic acid (**21**) (9.9 mmol, 2.2g) was dissolved in dry dichloromethane (20 mL) and 1,1′-carbonyldiimidazole (11 mmol, 1.77g) was added. The solution was stirred for another 40 min then benzyloxyamine (11 mmol, 1.15g) was added. After 1 h, reaction completion was determined by complete removal of the 1720 cm^{-1} (CO) band on IR spectra. The solution was washed with 1M HCl

(2 x 5mL), then brine (1 x 5mL) and dried with $MgSO_4$. Solvent was evaporated leaving oil from which the solid was crystallised using dichloromethane and *n*-hexane.

Diethyl (1-{[(benzyloxy)amino]carbonyl}propyl)phosphonate **22**



Yield: 46%, colourless needle-like crystals

M.p.: 58 °C

IR (KBr): 1687 cm^{-1} (CONH), 1240 cm^{-1} (P=O), 1025 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 9.10 (s, 1H, NH), 7.43 -7.34 (m, 5H, ArH), 4.93 (s, 2H, CH_2Ph), 4.12 – 4.08 (m, 4H, CH_2), 2.57 – 2.51 (m, 2H, CH_2), 1.31 (t, *J* = 7.12 Hz, 6H, CH_3), 0.98 (t, *J* = 7.41 Hz, 3H, CH_3)

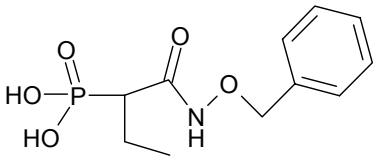
^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 167.5 (C=O), 129.2, 128.7, 128.6 ($ArC_{tert.}$), 78.3 (CH_2Ph), 63.1(CH_2), 62.7 (CH_2), 20.4 (CH_2), 16.4 (CH_3), 12.8 (CH_3)

$C_{15}H_{24}NO_5P$
Requires [%]: C 54.71, H 7.35, N 4.29
Found [%]: C 54.94, H 7.36, N 4.29

Synthesis procedure for **23**

To a solution of diethyl (1-{[(benzyloxy)amino]carbonyl}propyl) phosphonate (**22**) (3 mmol, 0.99g) in dry dichloromethane (5 mL) was added trimethylsilyl bromide (2.9 mL) and the reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated and the remaining oil was dissolved in methanol (3 mL), and stirred for 10 min. The solvents were removed under reduced pressure, the residue dissolved in dichloromethane and dried ($MgSO_4$). Solid compound **23** was crystallised from the oil remaining after solvent evaporation using ethyl acetate and *n*-hexane

Diethyl (1-{[(benzyloxy)amino]carbonyl}propyl)phosphonate **23**



Yield: 82%, amorphous powder

M.p.: 121 °C

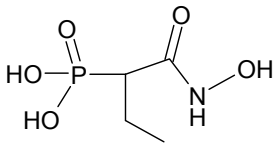
IR (KBr): 1628 cm^{-1} (CONH)

^{1}H-NMR: (400 MHz, DMSO-d_6) = δ (ppm) 7.43 – 7.34 (m, 5H, ArH), 4.78 (ABs, J = 7.36 Hz, 2H, CH_2Ph), 2.34 – 2.26 (m, 1H, CH_2), 1.82 -1.65 (m, 2H, CH_2) 0.91 (t, J = 7.39 Hz, 3H, CH_3)

^{13}C-NMR: (101 MHz, DMSO-d_6) = δ (ppm) 166.3 (C=O), 136.4 ($ArC_{quart.}$), 129.2, 128.6 ($ArC_{tert.}$), 77.3 (CH_2Ph), 45.9 (d, $^1J_{C,P}$ = 130.72 Hz, PC), 20.4 (d, $^2J_{C,P}$ = 4.07 Hz, CH_2), 13.0 (d, $^3J_{C,P}$ = 16.28 Hz, CH_3)

$C_{11}H_{16}NO_5P \cdot H_2O$
Requires [%]: C 45.37, H 6.23, N 4.81
Found [%]: C 45.10, H 6.20, N 4.64

Diethyl {1-[(hydroxyamino)carbonyl]propyl}phosphonate **17a**



From 0.1g diethyl (1-{[(benzyloxy)amino]carbonyl}propyl)phosphonate (**23**) according to **GP-4**

Yield: 95%, pinkish foam-like solid

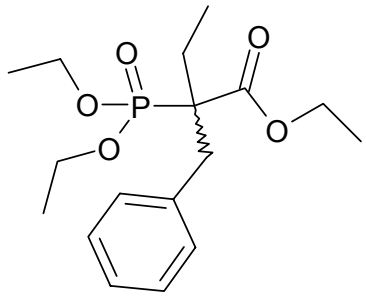
IR (KBr): 1654 cm^{-1} (CONH)

^{1}H-NMR: (400 MHz, D_2O) = δ (ppm) 2.34 – 2.25 (m, 1H, CH_2), 1.68 -1.61 (m, 2H, CH_2) 0.91 (t, *J* = 7.37 Hz, 3H, CH_3)

^{13}C-NMR: (101 MHz, D_2O) = δ (ppm) 165.0 (C=O), 20.6 (CH_2) 12.3 (CH_3)

Correct elemental analysis could not be obtained due to high hygroscopic properties

Ethyl 2-benzyl-2-(diethoxyphosphoryl)butanoate **24a**



From 2.52g triethyl 2-butylphosphonate (**18**) according to **GP-2**

Yield: 65%, amorphous powder

M.p.: 109 °C

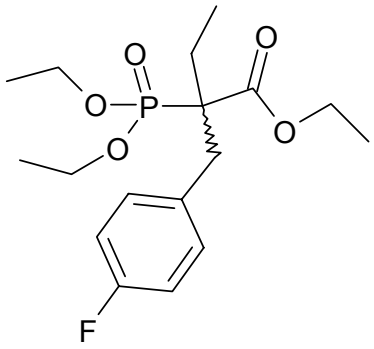
IR (Film): 1731 cm^{-1} (C=O), 1249 cm^{-1} (P=O), 1026 (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.24 -7.17 (m, 5H, ArH), 4.22 - 4.13 (m, 6H, CH_2), 3.45 – 3.05 (m, 2H, CH_2), 1.94 – 1.76 (m, 2H, CH_2), 1.31 – 1.25 (m, 9H, CH_3), 1.07 (t, *J* = 7.38 Hz, 3H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 170.9 (C=O), 136.4 ($ArC_{quart.}$), 130.5, 127.9, 126.8 ($ArC_{tert.}$), 62.6 (d, $^{2}J_{C,P}$ = 7.12 Hz, POC), 62.3 (d, $^{2}J_{C,P}$ = 7.13 Hz, POC), 61.2 (CH_2), 54.1 (d, $^{1}J_{C,P}$ = 134.79 Hz, PC), 39.5 (CH_2), 25.6 (CH_2), 16.4 (CH_3), 14.1(CH_3), 10.2 (CH_3)

$C_{17}H_{27}O_5P$
Requires [%]: C 59.64, H 7.95
Found [%]: C 59.21, H 7.86

Ethyl 2-(diethoxyphosphoryl)-2-(4-fluorobenzyl)butanoate **24b**



From 1.26g triethyl 2-butylphosphonate (**18**) according to **GP-2**

Yield: 70%, amorphous powder

M.p.: 108 °C

IR (KBr): 1732 cm^{-1} (C=O), 1261 cm^{-1} (P=O), 1026 (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.16 -7.14 (m, 2H, ArH), 6.95 -6.91 (m, 2H, ArH), 4.22 - 4.11 (m, 6H, CH_2), 3.35 – 3.06 (m, 2H, CH_2), 1.89 – 1.70 (m, 2H, CH_2), 1.32 – 1.26 (m, 9H, CH_3), 1.06 (t, J = 7.38 Hz, 3H, CH_3)

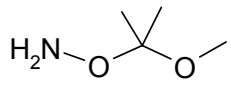
^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 163.1 (C=O), 132.1 ($ArC_{quart.}$), 132.0, 114.8, 114.6 ($ArC_{tert.}$), 62.6 (d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 62.4 (d, $^{2}J_{C,P}$ = 7.63 Hz, POC), 61.3 (CH_2), 38.5 (CH_2), 25.7 (CH_2), 16.4 (CH_3), 14.1(CH_3), 10.1 (CH_3)

$C_{17}H_{26}FO_5P$
Requires [%]: C 56.66, H 7.27
Found [%]: C 56.36, H 7.19

Synthesis procedure for **26**

In a 2-step modified Froböse procedure[91], N-hydroxyphthalimide (16.3g, 100 mmol) was suspended in dichloromethane (100 mL). 2-methoxypropene (10.8g, 150 mmol) was added drop wise and then 3 - 6 drops of phosphorylchloride. The mixture was stirred at room temperature for 3 h. Some more drops of $POCl_3$ were added and the mixture was left to stir overnight. 1 mL of triethylamine was added and after 15 min the solvent was reduced by half in vacuo. The remaining solution was washed with a saturated $NaHCO_3$ solution (2 x 20) and dried ($MgSO_4$). Evaporation gave an oily residue from which 2-(1-methoxy-1-methyl-ethoxy)-isoindole-1,3-dione as a yellowish powder was crystallised using dichloromethane and *n*-hexane (1793 cm^{-1}, 1736 cm^{-1} (C=O)). To 10 g of 2-(1-methoxy-1-methyl-ethoxy)-isoindole-1,3-dione (4.3 mmol) was added ethanolamine (17.4 mL) and the mixture stirred at 50°C for 2 h. Then it was poured into water (50 mL) and extracted with dichloromethane, dried ($MgSO_4$) and evaporated. The resulting oil was purified by fractional distillation (33 – 35°C, 14 - 16 x 10^{-1} mbar) to give **26** as colourless oil.

O-(1-methoxy-1-methylethyl) hydroxylamine (**26**)



Yield: 56%, colourless oil

IR (Film): 3321 cm^{-1} (NH), 1593 cm^{-1}

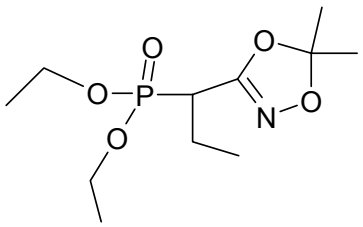
^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 4.94 (s, 2H, NH_2), 3.25 (s, 3H, CH_3), 1.36 (s, 6H, CH_3)

Compound **26** was used with this determined purity for further steps.

Synthesis procedure for **28a**

1,1′-Carbonyldiimidazole (11 mmol, 1.77g) was added to a solution of 2-(diethoxyphosphoryl)butanoic acid (**21**) (9.9 mmol, 2.2g) in dry dichloromethane (20 mL). After stirring for 20 min, *O*-(1-methoxy-1-methylethyl) hydroxylamine (11 mmol, 1.15g) was added. Reaction completion was determined by disappearance of the 1720 cm^{-1} (CO) band on IR spectra after 1 h. The solution was washed with 1M HCl (2 x 5mL), then brine (1 x 5mL) and dried with $MgSO_4$. Solvent was evaporated to yield **27** as colourless oil (1667 cm^{-1} (C=O)) which was dried and used immediately for further synthesis. This oily diethyl (1-{[(1-methoxy-1-methylethoxy)amino]carbonyl}propyl)phosphonate (**27**) (3 mmol, 0.98g) was refluxed in 10 mL cyclohexane for 1 h[90]. The solution was decanted leaving a brownish oily substance at the bottom. The decantant (solution got from decanting) was evaporated and resulting oil purified by fast column chromatography. Elution with ethyl acetate/ dichloromethane (4:6) yielded an oily product.

Diethyl [1-(5,5-dimethyl-1,4,2-dioxazol-3-yl)propyl]phosphonate **28a**



Yield: 48%, colourless oil

IR (Film): 1630cm^{-1} (C=N), 1236 cm^{-1} (P=O), 1020 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 4.21 - 4.13 (m, 4H, $POCH_2$), 2.91 – 2.82 (m, 1H, CH), 2.00 - 2.90 (m, 2H, CH_2), 1.60 (s, 6H, CH_3), 1.36 – 1.33 (m, 6H, CH_3), 1.04 (t, J = 7.38 Hz, 3H, CH_3)

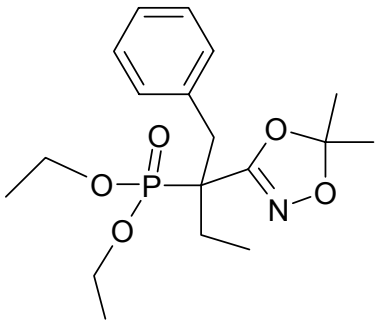
^{13}C-NMR: (101 MHz,) = δ (ppm) 156.8 (C=N), 115.3 ($C(O)_2$), 62.7 (POC), 62.6 (POC), 35.5 (d, $^{1}J_{C,P}$ = 137.58 Hz, PC), 24.5 (CH_3), 19.9 (d, $^{3}J_{C,P}$ = 4.07 Hz, CH_3) 16.6 (d, $^{3}J_{C,P}$ = 2.55 Hz, CH_3), 16.5 (d, $^{3}J_{C,P}$ = 2.54 Hz, CH_3) 12.3 (d, $^{3}J_{C,P}$ = 14.75 Hz, CH_3)

$C_{11}H_{22}NO_5P \cdot ½H_2O$
Requires [%]: C 47.31, H 7.94, N 5.02
Found [%]: C 46.74, H 8.00, N 4.99

Synthesis procedure for **(28b)**

Under nitrogen atmosphere, diethyl [1-(5,5-dimethyl-1,4,2-dioxazol-3-yl)propyl]phosphonate (**28a**) (1.6 mmol, 0.45g) was dissolved in dry THF (5 mL) at -78°C. *n*-Butyllithium (1.6M in *n*-hexane, 1.9 mmol, 0.8 mL) was added and the solution stirred at -78°C for 1 h. Benzyl bromide (1.6 mmol, 0.44g) was added drop wise and solution stirred for 30 min at -78°C before being allowed to warm up and stir at room temperature overnight. The mixture was quenched with 10% NH_4Cl and extracted with diethyl ether. The diethyl ether solution was dried ($MgSO_4$) and evaporated to yield the crude product that was purified by column chromatography. Elution with diethyl ether/ *n*-hexane (1:1) led to oily product **28b**

Diethyl [1-benzyl-1-(5,5-dimethyl-1,4,2-dioxazol-3-yl)propyl]phosphonate **28b**



Yield: 15%, colourless oil

IR (Film): 1621 cm^{-1} (C=N), 1247 cm^{-1} (P=O), 1023 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, DMSO-d_6) = δ (ppm) 7.30 – 7.18 (m, 5H, ArH), 4.01 - 4.10 (m, 4H, $POCH_2$), 3.24 – 2.98 (m, 2H, CH_2), 1.73 – 1.68 (m, 2H, CH_2), 1.65 (s, 3H, CH_3), 1.51 (s, 3H, CH_3), 1.31 – 1.25 (m, 6H, CH_3), 1.05 (t, J = 7.62 Hz, 3H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 159.6 (C=N), 115.3 ($C(O)_2$), 135.8 ($ArC_{quart.}$), 62.7 (d, $^{2}J_{C,P}$ = 6.87 Hz, POC), 45.0 (d, $^{1}J_{C,P}$ = 138.86 Hz, PC), 37.8 (CH_2), 25.3 (CH_3), 24.8 (CH_2), 16.9 (CH_3), 10.0 (CH_3)

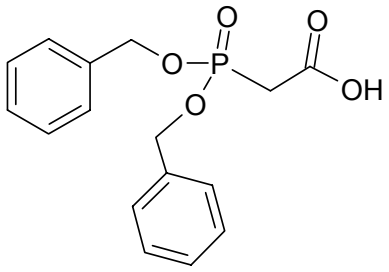
$C_{18}H_{28}NO_5P \cdot ½H_2O$
Requires [%]: C 57.13, H 7.72, N 3.70
Found [%]: C 57.25, H 7.84, N 3.72

8.7.2 Synthesis of benzyl protected phosphonates (Section 6.1.2)

Synthesis procedure for **31**

To 5 mL cooled (-65°C) tetrahydrofuran was added *n*-butyllithium (1.6M in *n*-hexane, 5.2 mmol, 3.3 mL)[98]. Dibenzyl methylphosphonate[99] (**30**) (5 mmol, 1.38g) in 2mL tetrahydrofuran was added and the solution was stirred at -65°C for 30 min. then poured with stirring into a Dewar containing a saturated dry ice/ diethyl ether solution (Et_2O, 10 mL). After 5 min., the mixture was poured into a beaker and warmed to room temperature with stirring for 2 h. Water (5 mL) was added and the organic layer washed with 10% Na_2CO_3 (2 x 5 mL). The combined aqueous layers were washed with diethyl ether (2 x 10 mL), acidified to pH 1 with 2M H_2SO_4, saturated with NaCl and extracted with dichloromethane (3 x 15 mL). The organic layer was dried with $MgSO_4$ and solvent evaporated. A white solid was crystallized from the resulting oil using ethyl acetate/ *n*-hexane.

(Dibenzylphosphono)acetic acid (**31**)



Yield: 65%, amorphous powder

M.p.: 58 °C

IR (KBr): 1730 (C=O), 1253 cm^{-1} (P=O), 968 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.33 (m, 10H, ArH), 6.59 (s, 1H, OH), 5.11 – 5.07 (m, 4H, CH_2), 3.01 (d, J = 21.62 Hz, 2H, CH_2)

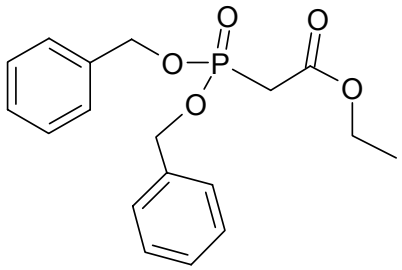
^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 167.6 (C=O), 135.7 ($ArC_{quart.}$), 128.6, 128.1 ($ArC_{tert.}$), 68.6 (d, $^{2}J_{C,P}$ = 6.10 Hz, POC), 34.5 (d, $^{1}J_{C,P}$ = 135.30 Hz, PC)

$C_{16}H_{17}O_5P$
Requires [%]: C 60.00, H 5.35
Found [%]: C 59.91, H 5.53

Synthesis procedure for **32**

To a solution of (dibenzylphosphono)acetic acid (**31**) (2 mmol 0.64g) in ethanol (4 mL) was added conc. H_2SO_4 (0.03 mL). The mixture was stirred at room temperature for 5 days, solvent evaporated under reduced pressure and then 3 mL $NaHCO_3$ solution was added. The mixture was extracted with ethyl acetate which was dried ($MgSO_4$) and evaporated leaving a crude product. Purification by column chromatography with diethyl ether/ *n*-hexane (7:3) elution led to oily product **32**.

Ethyl [bis(benzyloxy)phosphoryl]acetate **32**



Yield: 87%, yellow oil

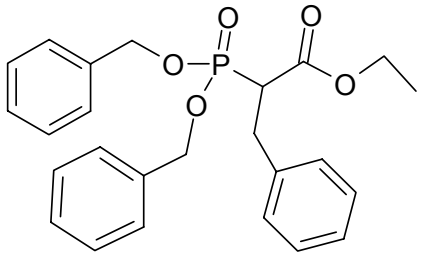
IR (Film): 1732 cm^{-1} (C=O), 1267 cm^{-1} (P=O), 995 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.39 -7.36 (m, 5H, ArH), 5.07 – 5.04 (m, 4H, CH_2), 4.07 (q, J = 7.12 Hz, 2H, CH_2), 1.12 (t, J = 7.12 Hz, 3H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 128.6, 128.5, 127.9 ($ArC_{tert.}$), 68.1 (POC), 61.7 (CH_2), 34.7 (d, $^1J_{C,P}$ = 135.80 Hz, PC), 14.0 (CH_3)

$C_{18}H_{21}O_5P \cdot ½H_2O$
Requires [%]: C 60.50, H 6.21
Found [%]: C 60.69, H 6.48

Ethyl 2-[bis(benzyloxy)phosphoryl]-3-phenylpropanoate **33**



From 0.74 g ethyl [bis(benzyloxy)phosphoryl]acetate (**32**) according to **GP-2**

Yield: 54%, colourless oil

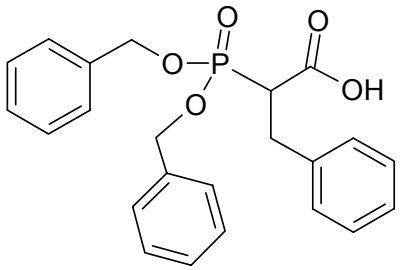
IR (Film): 1731 cm^{-1} (C=O), 1257 cm^{-1} (P=O), 995 (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.34 -7.09 (m, 15H, ArH), 5.10 – 4.66 (m, 4H, CH_2), 4.18 – 4.02 (m, 2H, CH_2), 3.32 – 3.31 (m, 1H, CH), 3.30 - 3.23 (m, 2H, CH_3), 1.20 – 1.03 (m, 3H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 167.9 (C=O), 135.9 ($ArC_{quart.}$), 130.6, 128.4, 128.2, 127.9, 127.6, 127.2, 126.6 ($ArC_{tert.}$), 67.3 (POC), 60.7 (CH_2), 32.3 (CH_2), 13.6 (CH_3)

$C_{25}H_{27}O_5P$
Requires [%]: C 68.48, H 6.21
Found [%]: C 68.83, H 6.34

2-[Bis(benzyloxy)phosphoryl]-3-phenylpropanoic acid **34**



From 0.66g ethyl 2-[bis(benzyloxy)phosphoryl]-3-phenylpropanoate (**33**) according to **GP-6**

Yield: 42%, amorphous powder

M.p.: 94 °C

IR (KBr): 1726 cm^{-1} (C=O), 1245 cm^{-1} (P=O), 992 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.39 – 7.18 (m, 15H, ArH), 5.10 – 5.07 (m, 4H, CH_2), 3.47 – 3.37 (m, 1H, CH), 3.11 – 3.01 (m, 2H, CH_2)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 169.5 (C=O), 139.0, 136.8 ($ArC_{quart.}$), 128.8, 128.1, 126.9 ($ArC_{tert.}$), 67.8 (q, $^{2}J_{C,P}$ = 3.05 Hz, POC), 46.9 (d, $^{1}J_{C,P}$ = 125.89 Hz, PC) 32.6 (CH_2)

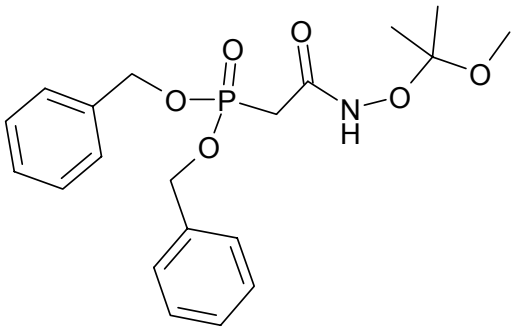
$C_{23}H_{23}O_5P$
Requires [%]: C 67.31, H 5.65
Found [%]: C 66.60, H 5.71

Synthesis procedure for **35**

The dry (dibenzylphosphono)acetic acid (**31**) (6 mmol, 1.92g) was dissolved in dry dichloromethane (12 mL). 1,1′-carbonyldiimidazole (6.6 mmol, 1.06g) was added, the solution stirred for 20 min then *O*-(1-methoxy-1-methylethyl) hydroxylamine (6.6 mmol, 0.69g) was added.

Reaction completion was determined by completed replacement of the 1720 cm^{-1} (C=O) band, with 1678 cm^{-1} (CONH). The solution was washed with 1M HCl (2 x 5 mL), then brine (1 x 5 mL) and dried with $MgSO_4$. Solvent was evaporated and the white powdered product was crystallized from the resulting colourless oil using diethyl ether.

Dibenzyl (1-{[(1-methoxy-1-methylethoxy)amino]carbonyl}propyl) phosphonate **(35)**



Yield: 69%, amorphous powder

M.p.: 55 °C

IR (KBr): 1684 cm^{-1} (C=O), 1250 cm^{-1} (P=O), 1018 cm^{-1} (POC)

^{1}H-NMR: (400 MHz, DMSO-d_6) = δ (ppm) 7.38 – 7.35 (m, 10H, ArH), 5.04 – 5.03 (m, 4H, $POCH_2$), 3.21 (s, 3H, CH_3), 3.00 (d, J = 21.36 Hz, 2H, CH_2), 1.29 (s, 6H, CH_3)

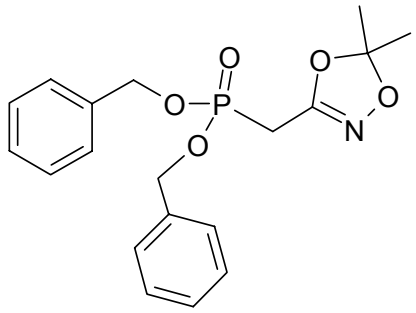
^{13}C-NMR: (101 MHz, DMSO-d_6) = δ (ppm) 136.7 ($ArC_{quart.}$), 128.7, 128.5, 128.1 ($ArC_{tert.}$), 105.8 ($C(O)_2$), 67.3 (d, $^{2}J_{C,P}$ = 6.10 Hz, POC), 49.5 (CH_3), 32.0 (d, $^{1}J_{C,P}$ = 134.79 Hz, PC) 22.9 (CH_3)

$C_{20}H_{26}NO_6P$
Requires [%]: C 58.96, H 6.43, N 3.44
Found [%]: C 58.85, H 6.37, N 3.32

Synthesis procedure for **36**

Dibenzyl (1-{[(1-methoxy-1-methylethoxy)amino]carbonyl}propyl) phosphonate **(35)** (0.5 mmol, 0.2g) was dissolved in ethyl acetate (2 mL) and heated under microwave conditions (12 psi, 100°C, 290 watts) for 10 min. Solvent was evaporated under reduced pressure. The resulting oil was dissolved in *n*-hexane and drops of diethyl ether and kept in fridge (4 °C) where a white solid formed.

Dibenzyl [1-(5,5-dimethyl-1,4,2-dioxazol-3-yl)propyl]phosphonate **36**



Yield: 83%, amorphous powder

M.p.: 37 °C

IR (KBr): 1638 cm^{-1} (C=N), 1260 cm^{-1} (P=O), 1007 cm^{-1} (POC)

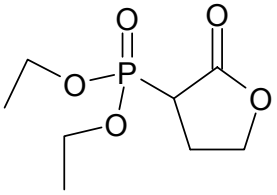
^{1}H-NMR: (400 MHz, $CDCl_3$) = δ (ppm) 7.35 (s, 10H, ArH), 5.09 – 5.04 (m, 4H, $POCH_2$), 2.93 (d, J = 21.44 Hz, 2H, CH_2), 1.53 (s, 6H, CH_3)

^{13}C-NMR: (101 MHz, $CDCl_3$) = δ (ppm) 154.1 (C=O), 136.2 ($ArC_{quart.}$), 129.1, 128.8 ($ArC_{tert.}$), 116.5 ($C(O)_2$), 68.7 (d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 49.5 (CH_3), 25.0 (CH_3), 23.8 (d, $^{1}J_{C,P}$ = 142.92 Hz, PC)

$C_{19}H_{22}NO_5P$
Requires [%]: C 60.80, H 5.91, N 3.73
Found [%]: C 60.62, H 5.97, N 3.78

8.7.3 Synthesis of Acylhydrazones 40a-c (Section 6.2)

Diethyl (2-oxotetrahydrofuran-3-yl)phosphonate **38**



From α-bromo-γ-butyrolacton (50 mmol) according to **GP-1** and purified by fractional distillation (122 - 125 °C, 7 x 10^{-1} mbar)

Yield: 89%, colourless oil

IR (film): 1772 cm^{-1} (C=O), 1249 cm^{-1} (P=O), 1026 cm^{-1} (POC)

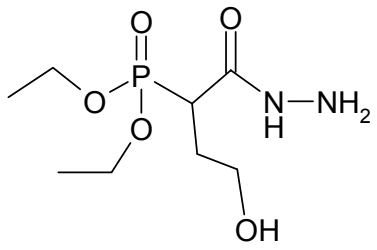
^{1}H-NMR: (400 MHz, DMSO-d_6) = δ (ppm) 4.32 - 4.26 (m, 2H, OCH_2), 4.10 - 4.05 (m, 4H, CH_2), 3.53 – 3.44 (m, 1H, CH), 2.55 – 2.33 (m, 2H, CH_2) 1.27 - 1.23 (m, 3H, CH_3)

^{13}C-NMR: (101 MHz, DMSO-d_6) = δ (ppm) 172.4 (C=O), 67.3 (d, $^{3}J_{C,P}$ = 7.12 Hz, OCH_2),62.6 (d, $^{2}J_{C,P}$ = 6.62 Hz, POC), 62.3 (d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 37.6 (d, $^{1}J_{C,P}$ = 142.42 Hz, PC), 24.0 (CH_2), 16.1 (CH_3)

Synthesis procedure for **39**

To a solution of diethyl (2-oxotetrahydrofuran-3-yl)phosphonate (**38**) (5.5 mmol) in 5 mL methanol was added hydrazine hydrate (5.5 mmol) dissolved in 5 mL methanol. The mixture was stirred at room temperature for 18 h. The solvent was evaporated and the crude product dissolved in dichloromethane and dried ($MgSO_4$). The resulting colourless oil became a white solid after 2 weeks at -18 °C which was recrystallised from ethyl acetate and *n*-hexane.

Diethyl [1-(hydrazinocarbonyl)-3-hydroxypropyl]phosphonate **39**



Yield: 90%, amorphous powder

M.p.: 47 °C

IR (KBr): 1670 cm^{-1} (C=O)

^{1}H-NMR: (400 MHz, DMSO-d_6) = δ (ppm) 9.08 (s, 1H, NH), 4.52 – 4.50 (m, 1H, OH), 4.26 (m, 2H, NH_2), 4.04 – 3.96 (m, 4H, CH_2), 3.38 – 3.20 (m, 2H, CH_2), 3.98 – 2.86 (m, 1H, CH), 1.97 – 1.74 (m, 2H, CH_2) 1.24 - 1.19 (m, 6H, CH_3)

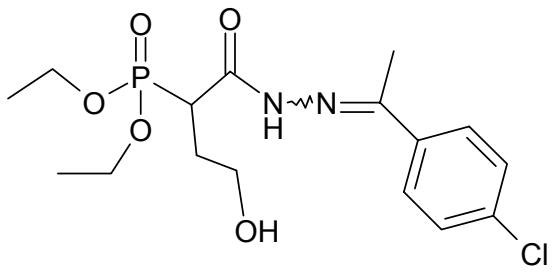
^{13}C-NMR: (101 MHz, DMSO-d_6) = δ (ppm) 166.6 (C=O), 61.8 (d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 61.6 (d, $^{2}J_{C,P}$ = 6.10 Hz, POC), 58.5 (d, $^{3}J_{C,P}$ = 16.78 Hz, OCH_2), 40.5 (PC), 29.7 (CH_2), 16.2 (d, $^{3}J_{C,P}$ = 6.10 Hz, CH_3)

$C_8H_{19}N_2O_5P$
Requires [%]: C 37.08, H 7.53, N 11.02
Found [%]: C 37.14, H 7.60, N 11.10

Synthesis procedure for **40a**

Diethyl [1-(hydrazinocarbonyl)-3-hydroxypropyl]phosphonate (**39**) (2 mmol, 0.51g) was dissolved in 5 mL methanol and then 2 mmol of 4-chloroacetophenone were added. The mixture was stirred overnight at room temperature. The solvent was evaporated and a white powder recrystallised from the resulting crude product using ethyl acetate and methanol.

(E/Z) Diethyl [1-({2-[1-(4-chlorophenyl)ethylidene]hydrazino}carbonyl)-3-hydroxypropyl]phosphonate **40a**



Yield: 42%, amorphous powder

M.p.: 132 °C

Ratio(E:Z): 49:51

IR (KBr): 1676 cm^{-1} (C=O)

^{1}H-NMR: (400 MHz, DMSO-d_6) = δ (ppm):

E-Isomer 10.76 (s, 1H, NH), 7.81 – 7.47 (m, 4H, ArH), 4.60 (t, *J* = 4.96 Hz, 1H, OH), 4.44 - 4.35 and 3.55 -3.52 (m, 1H, CH), 4.06 – 3.95 (m, 4H, CH_2), 3.46 – 3.28 (m, 2H, CH_2), 2.25 (d, J = 10.42 Hz, 3H, CH_3), 2.13 – 1.84 (m, 2H, CH_2), 1.25 - 1.17 (m, 6H, CH_3), 1.08 – 0.88 (m, 3H, CH_3)

Z-Isomer 10.49 (s, 1H, NH), 7.81 – 7.47 (m, 4H, ArH), 4.55 (t, *J* = 4.95 Hz, 1H, OH), 4.44 - 4.35 and 3.55 -3.52 (m, 1H, CH), 4.06 – 3.95 (m, 4H, CH_2), 3.46 – 3.28 (m, 2H, CH_2), 2.25 (d, J = 10.42 Hz, 3H, CH_3), 2.13 – 1.84 (m, 2H, CH_2), 1.25 - 1.17 (m, 6H, CH_3), 1.08 – 0.88 (m, 3H, CH_3)

^{13}C-NMR: (101 MHz, DMSO-d_6) = δ (ppm):

E-Isomer 169.7 (C=O), 127.6, 127.4 ($ArC_{tert.}$), 61.4 (POC), 61.2 (POC), 57.9 (OCH_2), 28.9 (CH_2), 15.5 (CH_3), 13.2 (CH_3)

Z-Isomer 169.7 (C=O), 127.6, 127.4 ($ArC_{tert.}$), 61.4 (POC), 61.2 (POC), 57.9 (OCH_2), 28.9 (CH_2), 15.5 (CH_3), 13.2 (CH_3)

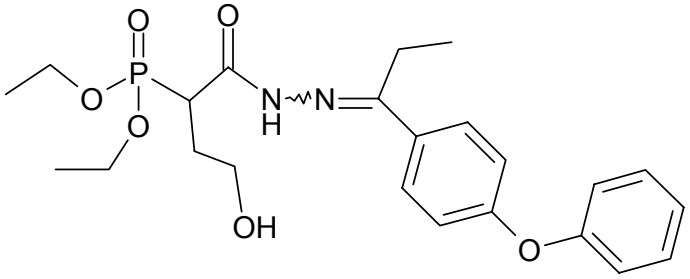
$C_{16}H_{24}ClN_2O_5P \cdot ½H_2O$
Requires [%]: C 48.07, H 6.30, N 7.01

Found [%]: C 48.21, H 6.35, N 7.14

Synthesis procedure for **40b**

Diethyl [1-(hydrazinocarbonyl)-3-hydroxypropyl]phosphonate (**39**) (4 mmol, 1.01g) and *p*-phenoxyphenylethyl ketone (4 mmol) were dissolved in 10 mL methanol and stirred for 2 days at room temperature. The solvent was evaporated and a white powder recrystallised from the resulting crude product using petrol ether.

(E/Z) Diethyl [1-({2-[1-(4-phenoxyphenyl)propylidene]hydrazino} carbonyl)-3-hydroxypropyl]phosphonate **40b**



Yield: 33%, amorphous powder

M.p.: 122 °C

Ratio (E:Z): 54: 46

IR (KBr): 1670 cm^{-1} (C=O), 3279 cm^{-1} (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) = δ (ppm):

E-Isomer 10.79 (s, 1H, NH), 7.81 – 7.01 (m, 9H, ArH), 4.60 (t, *J* = 5.09 Hz, 1H, OH), 4.47 - 4.38 and 3.69 -3.50 (m, 1H, CH), 4.06 – 3.97 (m, 4H, CH_2), 3.45 – 3.26 (m, 2H, CH_2), 2.81 – 3.77 (m, 2H, CH_2), 2.08 – 1.86 (m, 2H, CH_2), 1.24 - 1.14 (m, 6H, CH_3), 1.08 – 0.88 (m, 3H, CH_3)

Z-Isomer 10.43 (s, 1H, NH), 7.81 – 7.01 (m, 9H, ArH), 4.55 (t, *J* = 4.95 Hz, 1H, OH), 4.47 - 4.38 and 3.69 -3.50 (m, 1H, CH), 4.06 – 3.97 (m, 4H, CH_2), 3.45 – 3.26 (m, 2H, CH_2), 2.81 – 3.77 (m, 2H, CH_2), 2.08 – 1.86 (m, 2H, CH_2), 1.24 - 1.14 (m, 6H, CH_3), 1.08 – 0.88 (m, 3H, CH_3)

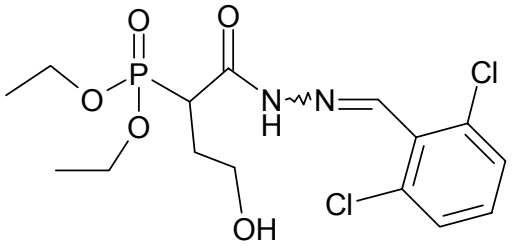
^{13}C-NMR: (101 MHz, DMSO-d_6) = δ (ppm):
E-Isomer 165.0 (C=O), 131.7, 130.1, 128.2, 123.8, 119.0, 118.0 ($ArC_{tert.}$), 61.8 (POC), 61.5 (POC), 58.6 (OCH_2), 29.8 (CH_2), 19.4 (CH_2), 16.2 (CH_3), 10.7 (CH_3)
Z-Isomer 165.0 (C=O), 131.7, 130.1, 128.2, 123.8, 119.0, 118.0 ($ArC_{tert.}$), 61.8 (POC), 61.5 (POC), 58.6 (OCH_2), 29.8 (CH_2), 19.4 (CH_2), 16.2 (CH_3), 10.7 (CH_3)

$C_{23}H_{31}N_2O_6P \cdot ½H_2O$
Requires [%]: C 57.49, H 6.92, N 5.83
Found [%]: C 57.97, H 6.81, N 6.15

Synthesis procedure for **40c**

Diethyl [1-(hydrazinocarbonyl)-3-hydroxypropyl]phosphonate (**39**) (4 mmol, 1.01g) was dissolved in 10 mL methanol and 2,6-dichlorobenzylaldehyde (4 mmol) was added, then the mixture was stirred for 2 days at room temperature. The solvent was evaporated and a white powder recrystallised from the resulting crude product using ethyl acetate and petrol ether.

(E/Z) Diethyl (1-{[2-(2,6-dichlorobenzylidene)hydrazino]carbonyl}-3-hydroxypropyl)phosphonate **40c**



Yield: 97%, amorphous powder

M.p.: 138 °C

Ratio(E:Z): 33: 67

IR (KBr): 1670 cm^{-1} (C=O), 3392 cm^{-1} (NH)

^{1}H-NMR: (400 MHz, DMSO-d_6) = δ (ppm):

E-Isomer 10.83 (s, 1H, NH), 8.38 (s, 1H, NCH), 7.33 – 7.15 (m, 3H, ArH), 4.49 - 4.39 and 3.34 -3.27 (m, 1H, CH), 4.21 – 4.15 (m, 4H, CH_2), 3.79 – 3.69 (m, 2H, CH_2), 2.34 – 2.18 (m, 2H, CH_2), 1.35 – 1.26 (m, 6H, CH_3)

Z-Isomer 10.15 (s, 1H, NH), 8.22 (s, 1H, NCH), 7.33 – 7.15 (m, 3H, ArH), 4.49 - 4.39 and 3.34 -3.27 (m, 1H, CH), 4.21 – 4.15 (m, 4H, CH_2), 3.79 – 3.69 (m, 2H, CH_2), 2.34 – 2.18 (m, 2H, CH_2), 1.35 – 1.26 (m, 6H, CH_3)

^{13}C-NMR: (101 MHz, DMSO-d_6) = δ (ppm):

E-Isomer 163.9 (C=O), 142 (C=N), 133.7 ($ArC_{quart.}$), 130.2, 129.1, 128.9 ($ArC_{tert.}$), 62.2, 62.1 (POC), 58.3 ($^{3}J_{C,P}$ = 16.28 Hz, OCH_2), 40.8 (d, $^{1}J_{C,P}$ = 134.24 Hz, PC), 29.4 (d, $^{2}J_{C,P}$ = 4.58 Hz, POC), 16.1 (CH_3)

Z-Isomer 169.9 (C=O), 137.9 (C=N), 133.7 ($ArC_{quart.}$), 130.2, 129.1, 128.9 ($ArC_{tert.}$), 61.9 (d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 61.8 (d, $^{2}J_{C,P}$ = 6.61 Hz, POC), 58.8 ($^{3}J_{C,P}$ = 16.79 Hz, OCH_2), 36.6 (d, $^{1}J_{C,P}$ = 134.24 Hz, PC), 29.7 (d, $^{2}J_{C,P}$ = 4.58 Hz, POC), 16.1 (CH_3)

$C_{15}H_{21}Cl_2N_2O_5P$
Requires [%]: C 43.81, H 5.15, N 6.81
Found [%]: C 43.75, H 5.17, N 6.94

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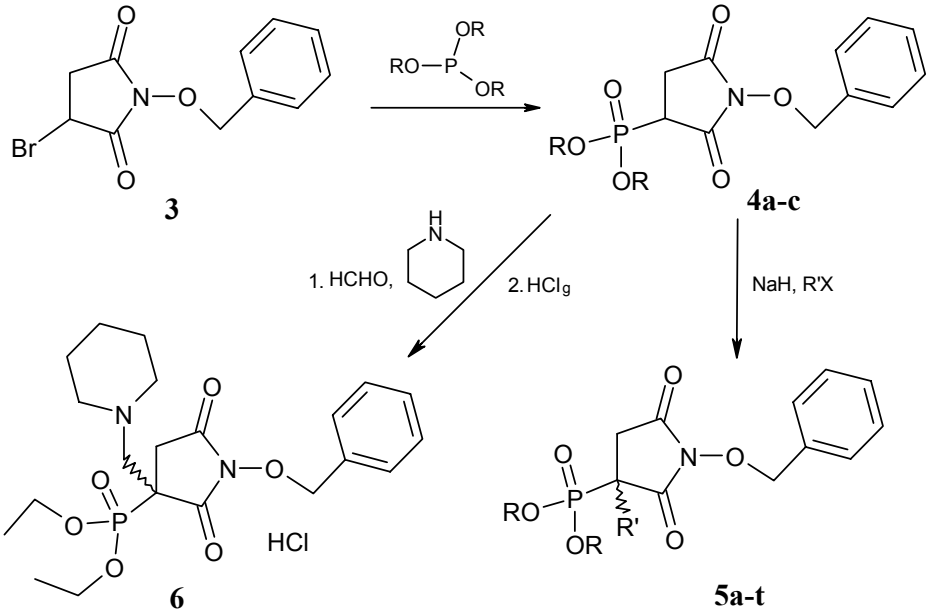
10 Summary

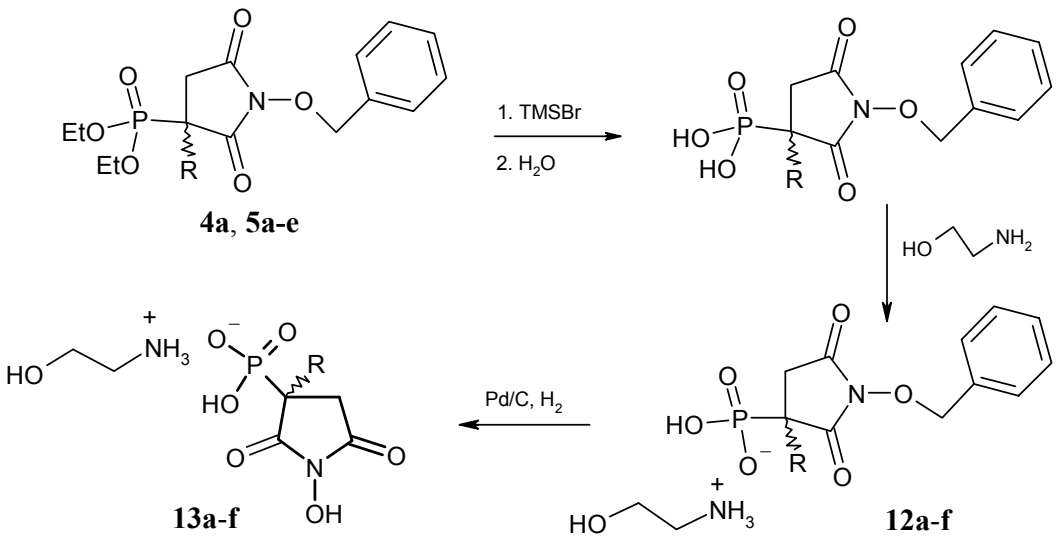
This work deals with the synthesis, analysis and biological activity studies on analogues of natural antibiotic SF-2312. The objective of the first part was to synthesise 1-hydroxypyrrolidin-2,5-diones **5a-t**,**6** that do not have either multiple chiral centers or the unstable N,O-Acetal present in SF-2312. 1-Benzyloxypyrrolidin-2,5-diones (**4a-c**) were synthesised via Michaelis-Arbusov reactions on 1-benzyloxy-3-bromopyrrolidin-2,5-dione (**3**). (Scheme 1)



Scheme 1.

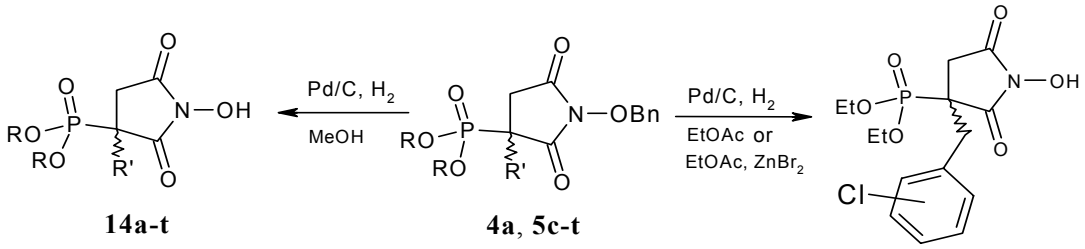
The acidity of the alpha C-H in ring position 3 due to the phosphonate in the 3-alkoxyphosphoryl-1-benzyloxysuccinimides **4a-c** provided access to their fuctionalization. First, by alkylation using electrophiles to get **5a-t** and second via a Mannich reaction yielding **6** as a hydrochloride salt (Scheme 1).

Cleavage of phosphonic acid esters was realized with TMSBr and water. Due to hygroscopicity of the free phosphonic acids, the target compounds were synthesised as ethanolamine monosalts. Salification of the phosphonic acids with 0.9 equivalent of ethanolamine in methanol or THF afforded the salts **12a-f** (Scheme 2).



Scheme 2.

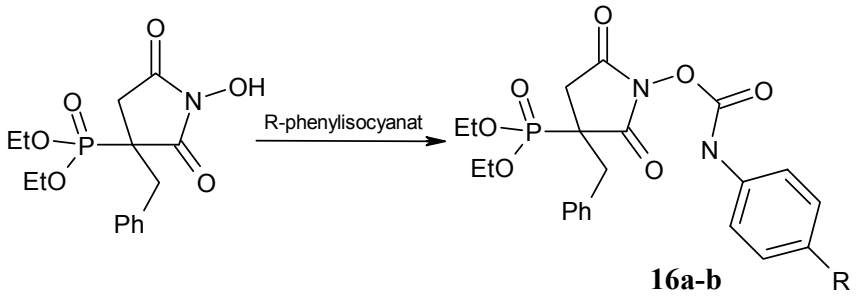
Catalytic hydrogenation of the benzyl-protected cyclic hydroxamic acids of **12a-f** finally led to the targeted ethanolamine monosalt 1-hydroxy-3-(alkyl/ aryl)-2,5-dioxopyrrolidine analogues of SF-2312 (**13a-f**) (Scheme 2).
Similar deprotection of **4a**, **5c-t** in methanol led to the 1-hydroxysuccinimides **14a-t** whose phosphonic acid moieties were masked as various alkyl phosphonates in order to overcome hygroscopicity of phosphonic acids (Scheme 3).
Undesired dehalogenations during the catalytic hydrogenation of halogenated compounds were overcome by using less polar solvents, lower pressure and their yield improved by adding catalysts such as $ZnBr_2$ (Scheme 3).



Scheme 3.

All attempts towards reduction of the carbonyl in ring position 5 of diethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonate using $NaBH_4$ or $LiAlH_4$ were futile.

Diethyl (3-benzyl-1-hydroxy-2,5-dioxopyrrolidin-3-yl)phosphonate could however be reacted with 4-trifluoromethyl-phenylisocyanate or 4-methoxy-phenylisocyanate to successfully afford compounds **16a-b** (Scheme 4).

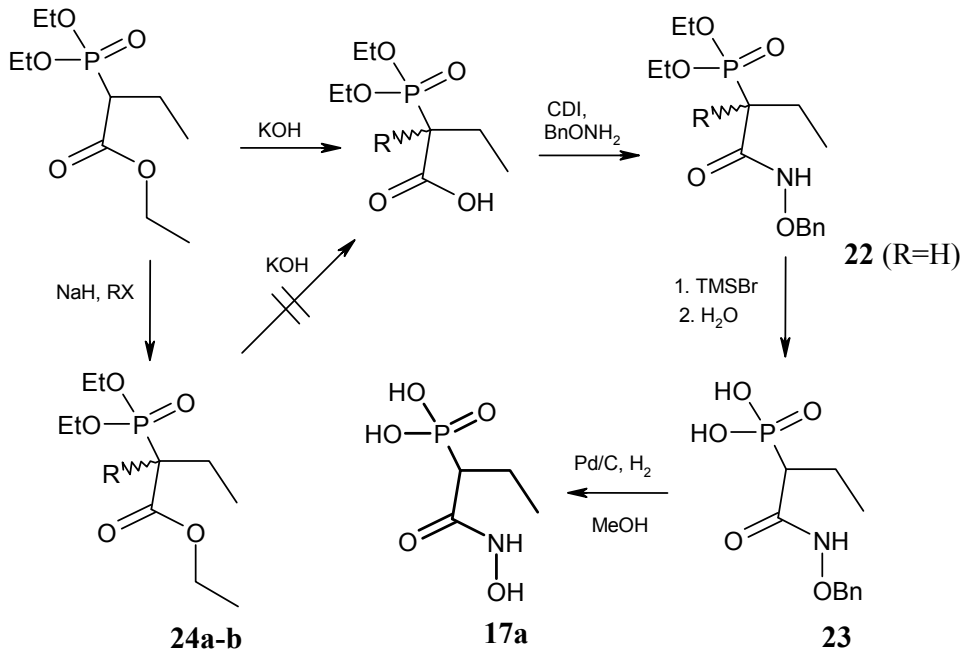


Scheme 4.

Another compound synthesised within the scope of this thesis as an open-chained analogue of SF-2312 (**17a**). Hydrolysis of triethyl 2-phosphonobutyrate provided 2-(diethoxyphosphoryl)butanoic acid that was then treated with 1,1′-carbonyldiimidazole (CDI) and benzyloxyamine to afford O-benzyl protected hydroxamic acid **22**.

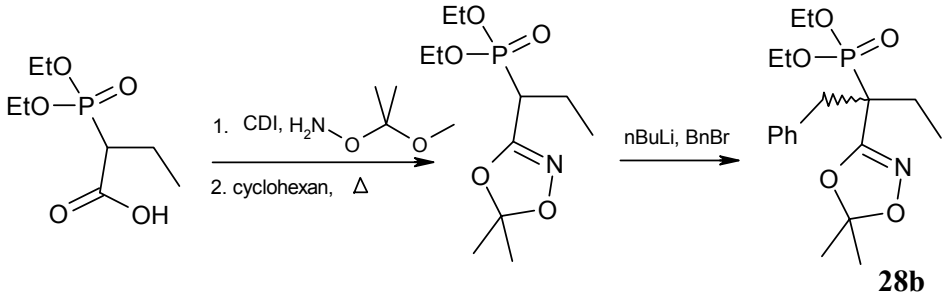
Dealkylation of the phosphonic ester gave the stable and non-hygroscopic phosphonic acid **23** whereas debenzylation via catalytic hydrogen led to hygroscopic **17a** (Scheme 5).

Functionalization of the α-position in triethyl 2-phosphonobutyrate successfully afforded 3-substituted compounds **24a-b** but hydrolysis using KOH and $Ba(OH)_2$ to give carboxylic acids was unsuccessful (Scheme 5).



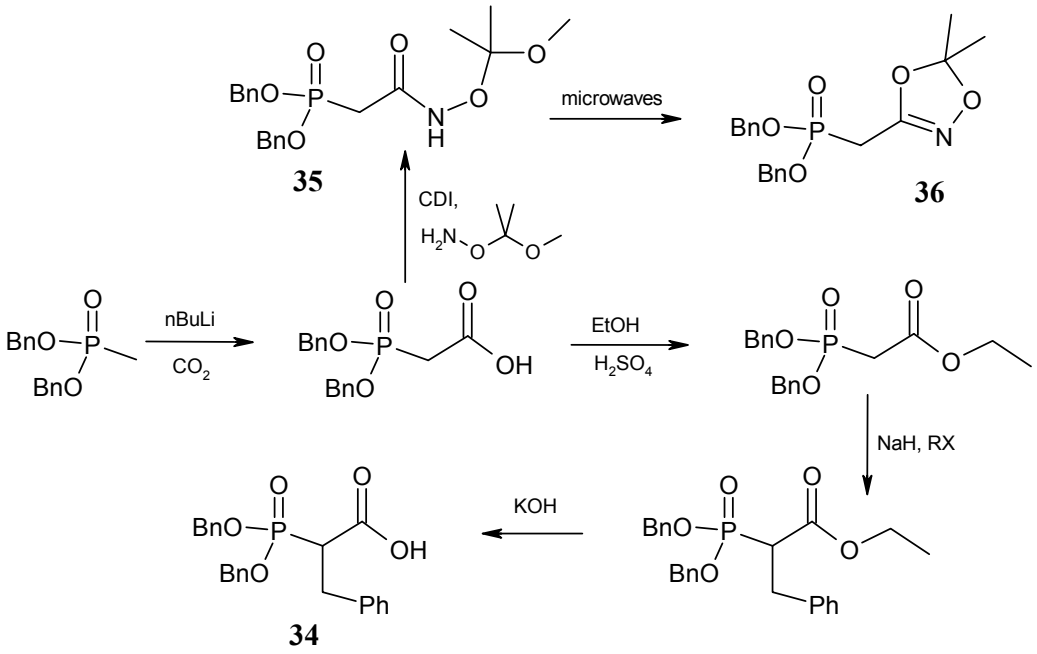
Scheme 5.

The hydroxamic acid was masked in dioxazole ring when 2-(diethoxyphosphoryl)butanoic acid was treated with CDI and O-(1-methoxy-1-methylethyl) hydroxylamine and the resulting hydroxamic acid cyclized into diethyl [1-(5,5-dimethyl-1,4,2-dioxazol-3-yl)propyl] phosphonate. Alkylation using benzyl bromide gave benzylated derivative **28b** (Scheme 6).



Scheme 6.

The ethyl phosphonates were replaced by benzyl phosphonates in similar reactions to improve yields of this synthesis sequence aiming at open-chained analogues. Dibenzyl methylphosphonate was activated with *n*-BuLi and then reacted with dry ice (CO_2) to afford (dibenzylphosphono) acetic acid in good yield.



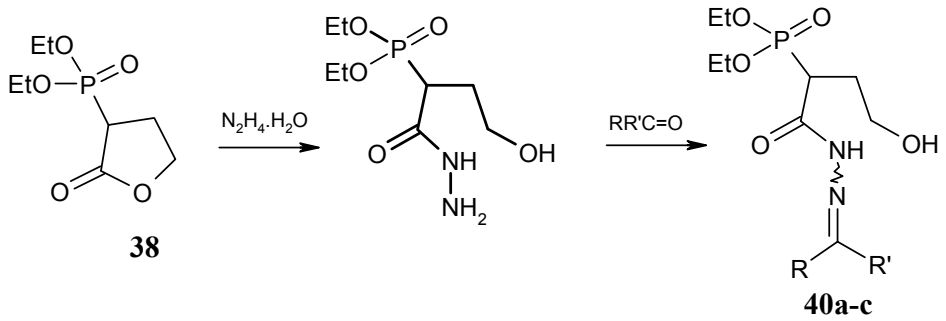
Scheme 7.

Ethyl [bis(benzyloxy)phosphoryl] acetate was realised after the acid was esterified. It was subsequently benzylated at the α-position to yield ethyl 2-[bis(benzyloxy)phosphoryl]-3-phenylpropanoate (Scheme 7).

Hydrolysis readily resulted in good yields of stable 2-[bis(benzyloxy)phosphoryl]-3-phenylpropanoic acid (**34**) that may be transformed in hydroxamic acid using CDI and benzyloxyamine then subsequently deprotected in another scope of work as was done here for ethyl phosphonate derivatives (Scheme 7). It would be imperative to consider pro-drug or salt formation while reckoning the observed herein hygroscopicity of **17a** that contains both free phosphonic and hydroxamic acids.

Benzyl phosphonate-dioxazole derivatives were also successfully synthesised when (dibenzylphosphono) acetic acid was treated with 1,1′-carbonyldiimidazole and then O-(1-methoxy-1-methylethyl) hydroxylamine and the resulting hydroxamic acid subsequently cyclized under microwave irradiation (Scheme 7). This section revealed that benzyl phosphonates afforded higher yielding and stable intermediates than ethyl phosphonates for the synthesis sequence towards open-chained analogues.

This study finally describes the synthesis of hydrazide and hydrazone open-chained analogues of SF-2312. **38** was treated with hydrazine hydrate to afford diethyl [1-(hydrazinocarbonyl)-3-hydroxypropyl]phosphonate in good yield. This hydrazide was then reacted with various aldehydes and ketones to yield their corresponding hydrazones as *E*/*Z*-isomeric mixtures (Scheme 8).

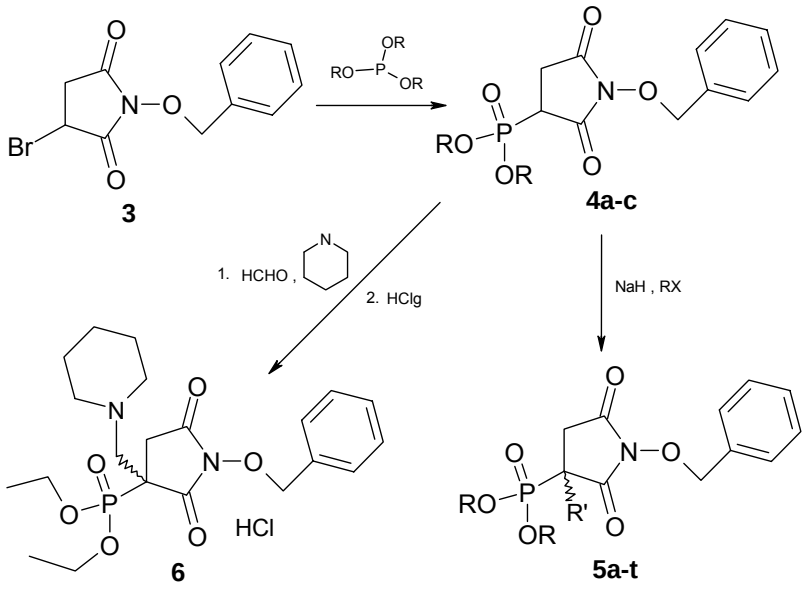


Scheme 8.

Compounds **12** and **13** were tested for antibacterial activity, but did not inhibit growth of various bacterial strains.
Selected compounds of **14** exhibited in-vitro fungicidal activity when tested in collaboration with Dupont de Nemours, Newark-Welmington/ USA but these results were not confirmed in greenhouse tests.
These and other selected compounds are still under investigation for other antimicrobial activity in conjunction with Bayer/ Germany and the University of Antwerp/ Belgium.

11 Zusammenfassung

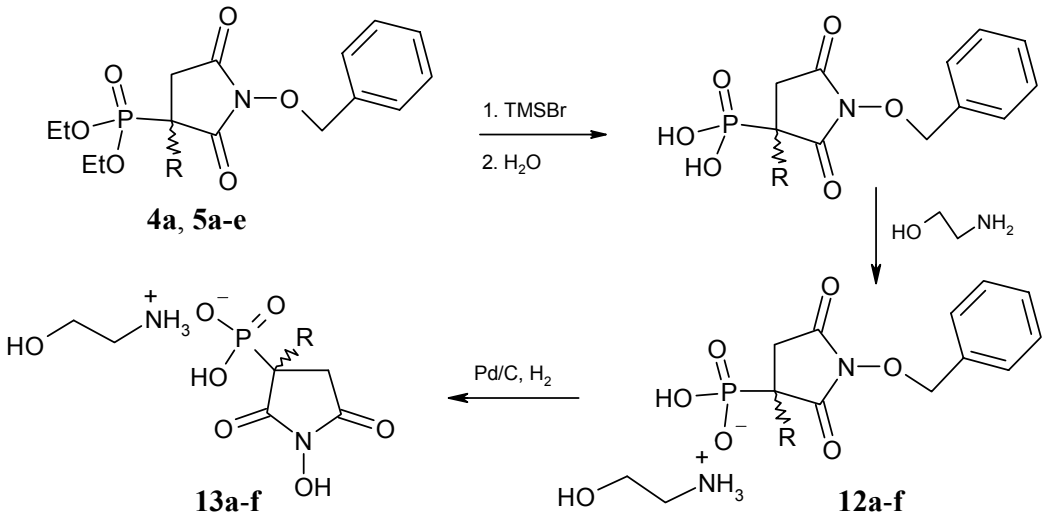
Die vorliegende Arbeit beschäftigt sich mit der Synthese, der Analytik und den biologischen Eigenschaften von Strukturanaloga des antibiotisch wirksamen Naturstoffs SF-2312. Im ersten Teil der Arbeit werden 1-Hydroxypyrrolidin-2,5-dione **5a-t**, **6** als Derivate dieses Antibiotikums beschrieben.
1-Benzyloxypyrrolidin-2,5-dione (**4a-c**) wurden mittels Michaelis-Arbusov-Reaktion aus 1-Benzyloxy-3-brompyrrolidin-2,5-dion (**3**) gewonnen (Schema 1).



Schema 1.

Die C,H-Acidität der Verbindungen **4a-c** erlaubte ihre Funktionalisierung an Ringposition 3 - einerseits durch den Einsatz von Alkylhalogeniden (**5a-t**) und andererseits über eine Mannich-Reaktion, die das Hydrochlorid **6** hervorbrachte (Schema 1).

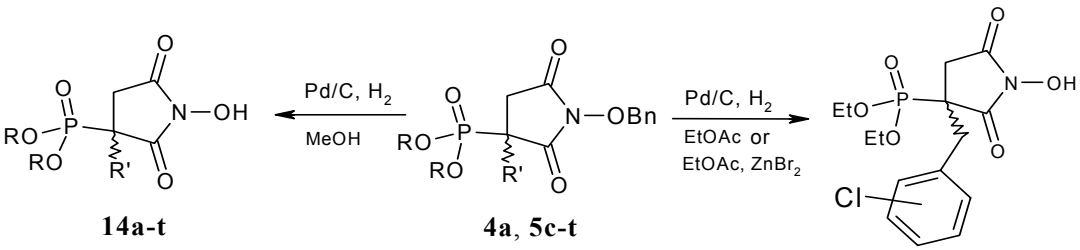
Die Spaltung des Phosphonsäureesters erfolgte mit TMSBr und Wasser. Da die resultierenden Phosphonsäuren eine starke Hygroskopizität aufwiesen, wurden sie als Ethanolaminmonosalze dargestellt. Um das Salz zu erhalten, wurden die Phosphonsäuren mit 0,9 Äquivalenten des Ethanolamins in MeOH oder THF versetzt (**12a-f**) (Schema 2).



Schema 2.

Die katalytische Hydrierung der O-benzyl-geschützten cyclischen N-Hydroxysuccinimide **12a-f** führte schließlich zu den gewünschten Ethanolaminmonosalzen der 1-Hydroxy-3-(alkyl/ aryl)-2,5-dioxopyrrolidin-Analoga von SF-2312 (**13a-f**) (Schema 2).

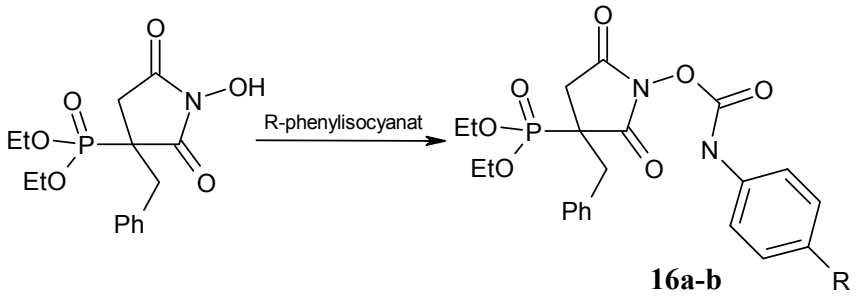
Bei den Verbindungen **4a**, **5c-t** wurde besonderes Augenmerk auf die Darstellung verschiedener Phosphonsäuredialkylester gelegt, um die Hygroskopizität zu vermindern. In Methanol gelöst wurden sie entsprechend obiger Methode in die debenzylierten N-Hydroxysuccinimide überführt (Schema 3).



Schema 3.

Durch die Verwendung schwach polarer Lösungsmittel wie Ethylacetat und unter geringerem Druck konnte die unter Standardbedingungen auftretende, unerwünschte Dehalogenierung des Benzylrests unterbunden werden. Gleichzeitig wurde die Ausbeute durch Zugabe von Katalysatoren wie $ZnBr_2$ verbessert (Schema 3). Die Versuche, die

Carbonylgruppe in Ringposition 5 des Diethyl [1-benzyloxy-2,5-dioxopyrrolidin-3-yl]phosphonats mit $NaBH_4$ oder $LiAlH_4$ zu reduzieren, schlugen fehl.
Diethyl (3-benzyl-1-hydroxy-2,5-dioxopyrrolidin-3-yl) phosphonat reagierte mit 4-Trifluormethyl-phenylisocyanat und 4-Methoxy-phenylisocyanat dennoch erfolgreich zu den Verbindungen **16a-b**.

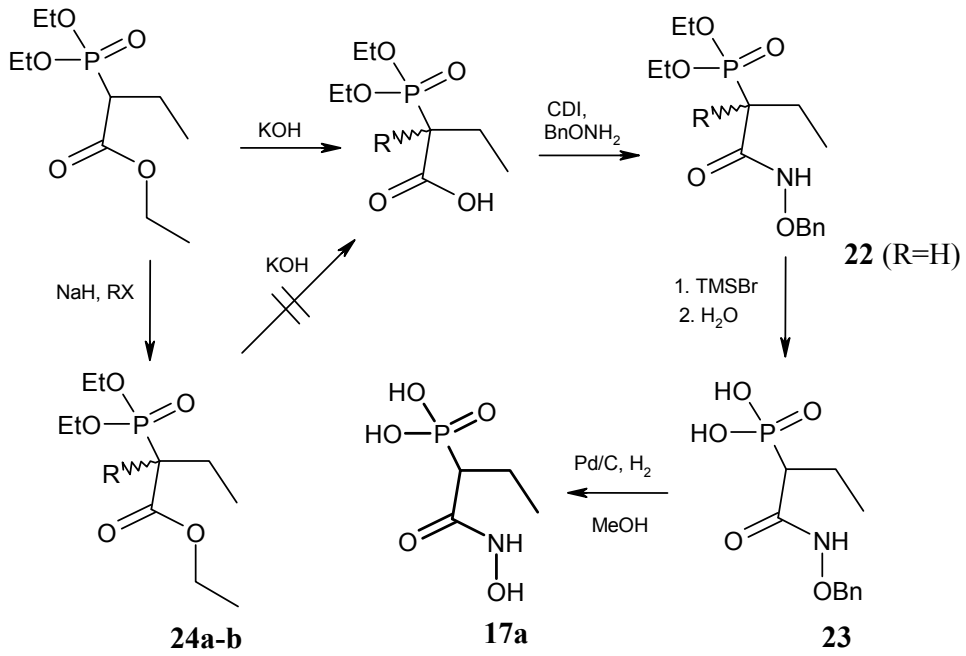


Schema 4.

Im Rahmen dieser Arbeit wurde ein weiteres offenkettiges Analogon von SF-2312 (**17a**) synthetisiert.

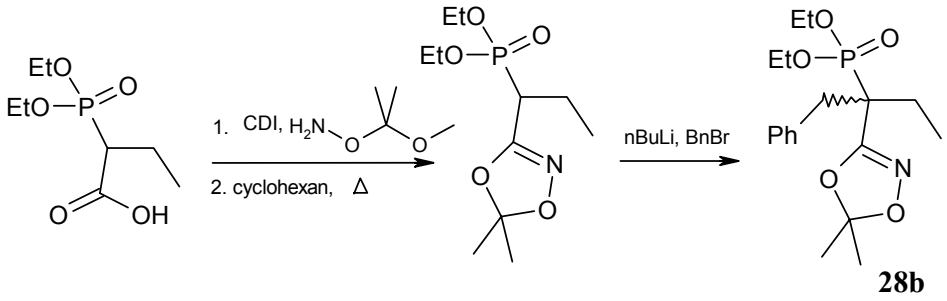
Die Hydrolyse des 2-Phosphonobutyrats lieferte 2-(Diethoxyphosphoryl) buttersäure, die durch die Behandlung mit 1,1′-Carbonyldiimidazol (CDI) und O-Benzyloxyamin die O-benzylisch geschützte Hydroxamsäure **22** ausbildete (Schema 5).

Die Desalkylierung des Phosphonsäureesters ergab die stabile und nicht hygroskopische Phosphonsäure **23**, während die Debenzylierung durch katalytischen Wasserstoff zu **17a** führte (Schema 5).



Schema 5.

Die erfolgreiche Funktionalisierung der α-Position des Triethyl 2-phosphonobutyrats brachte die Verbindungen **24a-b** hervor. Der Versuch, diese Ester mit KOH oder $Ba(OH)_2$ zur freien Carbonsäure zu verseifen, um im Anschluß daran die Hydroxamsäure darstellen zu können, scheiterte (Schema 5).

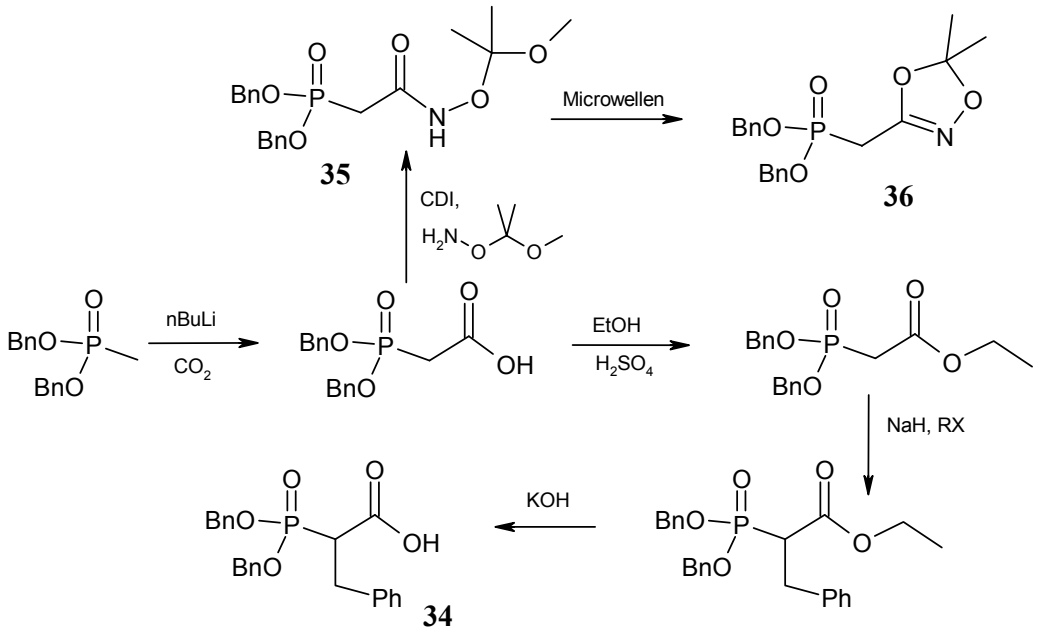


Schema 6.

Die Hydroxamsäure wurde in einem Dioxazolring maskiert. Dieser Ring wurde durch die Behandlung der 2-(Diethoxyphosphoryl)buttersäure mit CDI und O-(1-Methoxy-1-methylethyl) hydroxylamin dadurch erhalten, indem die zunächst resultierende Hydroxamsäure unter Ringschluß zu Diethyl[1-(5,5-dimethyl-1,4,2-dioxazol-3-yl)propyl]phosphonat reagierte.

Eine Alkylierung unter Verwendung von Benzylbromid ergab die benzylierte Verbindung **28b** (Schema 6).

Um die Ausbeute im Hinblick auf die offenkettigen Produkte zu steigern, wurden die zunächst verwendeten Ethylphosphonate durch Benzylphosphonate ersetzt.
Dibenzylmethylphosphonat wurde mit n-BuLi aktiviert und anschließend mit Trockeneis (CO_2) zur Reaktion gebracht, um Dibenzylphosphonoessigsäure in guten Ausbeuten zu erhalten (Schema 7).

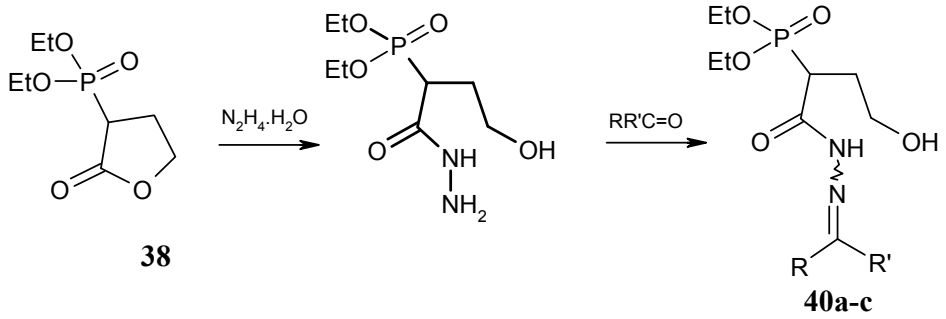


Schema 7.

Die freie Säure wurde anschließend zum Ethyl[bis(benzyloxy)-phosphoryl]acetat verestert. Anschließend erfolgte eine Benzylierung an der α-Position, um Ethyl-2-[bis(benzyloxy)phosphoryl]-3-phenylpropanoat darzustellen.
Die Hydrolyse ergab problemlos die stabile 2-[Bis(benzyloxy)phosphoryl]-3-phenylpropansäure in guten Ausbeuten. In einem weiteren Arbeitsschritt konnte diese Verbindung mit CDI und Benzyloxyamin umgesetzt werden, um die O-Benzyl-geschützte Hydroxamsäure zu erhalten. Die drei benzylischen Schutzgruppen konnten gleichzeitig durch katalytische Hydrierung abgespalten werden (Schema 7). Für die so erhaltenen Verbindungen sollte eine Salzbildung oder die Darstellung von prodrugs in Erwägung gezogen werden, um

die beobachtete Hygroskopizität von Verbindung **17a,** basierend auf den freien Phosphon- und Hydroxamsäuren, zu vermindern.
Benzylphosphonatdioxazol-Derivate wurden ebenfalls erfolgreich synthetisiert, indem (Dibenzylphosphono) essigsäure zunächst mit CDI und im Anschluß mit O-(1-Methoxy-1-methylethyl) hydroxylamin umgesetzt wurde. Die resultierende Hydroxamsäure cyclisierte in einem letzten Schritt unter Mikrowellenbestrahlung (Schema 7).
Aus diesem Abschnitt der Arbeit ging hervor, dass man durch die Verwendung von Benzylphosphonaten - im Gegensatz zur Synthese mit Ethylphosphonaten - höhere Ausbeuten und stabile Zwischenprodukte im Hinblick auf die Darstellung offenkettiger Derivate erzielen kann.

Der letzte Teil dieser Arbeit beschäftigt sich mit der Synthese von offenkettigen Hydrazid- und Hydrazonanaloga von SF-2312. Durch die Umsetzung von **38** mit Hydrazinhydrat konnte man Diethyl[1-(hydrazinocarbonyl)-3-hydroxypropyl]phosphonat in guten Ausbeuten erzielen.
Diese Hydrazide wurden dann mit verschiedenen Aldehyden und Ketonen zur Reaktion gebracht, um die entsprechenden E/Z-Isomerengemische der Acylhydrazone zu erhalten (Schema 8).



Schema 8.

Verbindungen **12** und **14** wurden auf ihre antibakterielle Aktivität getestet. Es wurde keine Wachstumshemmung bei unterschiedlichen Bakterienstämmen beobachtet. In einer Zusammenarbeit mit Dupont de Nemours, Newark-Welmington/ USA wiesen Verbindungen von **14** in vitro auf fungizide Eigenschaften hin, allerdings wurde dieses Ergebnis nicht durch Tests im Gewächshaus bestätigt. Diese und andere ausgesuchte Verbindungen werden weiterhin bei Bayer AG und Antwerp Universitaet getestet.

Hazardous substances

No information about the toxicological characteristics of the compounds synthesized within the scope of this thesis is available. Hence, hazardous properties cannot be excluded.
These chemicals should therefore be regarded as hazardous substances and treated with the appropriate caution.
Toxicological properties of the solvents and the chemicals employed within the course of this project are summarized in the tables below.

Solvents	Category of danger*	Safety phrases
Acetone	F, Xi	S 9-16-26
Acetic acid	C	S 2-23-26
2-butanone	F,Xi	S 9-16
Chloroform	Xn	S 36/37
Dichloromethane	Xn	S 23.2-24/25-36/37
Diethyl ether	F+, Xn	S 9-16-29-33
Ethanol	F	S 7-16
Ethyl acetate	F, Xn	S 16-26-33
N,*N*-Dimethylformamide	T	S 53.1-45
n-Hexane	F, Xn, N	S 9-16-29-33-36/37-61-62
Methanol	F, T	S 7-16-36/37-45
Tetrahydrofuran	F, Xn	S 16-29-33
Toluene	Xn, F	S 16-25-29-33

* C – Corrosive, F – Highly flammable, F+ – Extremely flammable, N – Environmentally dangerous, O – Oxidative, T – toxic, T+ – Very toxic, Xi – Irritant, Xn – Harmful

Solvents	Category of danger	Safety phrases
Acetyl chloride	Xn, F+	S 16-33-36/37
Ammonium chloride	Xn	S 22-36/22
α-bromo-γ-butyrolacton	Xn	S 22
Benzylbromide	Xi	S 39
n-Butyllithium-solution	F, C, N	S 16-26-36/37/39-43.11-45
1,1′-Carbonyldiimidazole	Xn	S 22-36/37/38
4-chloroacetophenone	Xn	S 26-37/39
Chloromethyl pivalate	Xn	S 16-26-36
2,6-dichlorobenzylaldehyde	Xi	S 26-37/39
Hydrazine hydrate	T, N	S 53-45-60-61
Hydrochloric acid	C	S 26-36/37/39-45
Hydrogen	F+	S 9-16-33
Hydrogen chloride	C	S 26-36/37/39-45
Hydrobomic acid	C	S 7/9-26-45
Maleic anhydride	C, Xn	S 22-26-36/37/39-45
4-Methoxy-phenylisocyanate	Xi	S 26-37/39
Phosphorous pentachloride	C	S 7/8-26-36/37/39-45
Potassium hydroxide	C	S 22-26-37/39
Pyridine	F, Xn	S 26-28.1
Sodium carbonate	Xi	S 22-26
Sodium hydride	F, Xi	S 24/25-26-43.11-7/8
Sodium hydroxide	C	S 26-37/39-45
Thionyl chloride	C	S 26-36/37/39-45
Triethylamine	C, F	S 3-16-26-29-36/37-45
Triethyl 2-butylphosphonate	Xi	S 26-37/39
Triethylphosphite	Xn	S 16-26-36
4-Trifluoromethyl-phenylisocyanate	Xn	S 26-36/37/39
Trimethyl phosphite	T	S 53-26-36/37/39-43
Trimethylsilyl bromide	C	S 16-26-36/37/39-45

Curriculum vitae

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Ph.D.Topic: „Structure-Activity-Studies on the Natural Antibacterial Compound SF-2312"

Publications:
Owotoki, Wamuyu; Geffken, Detlef; Kurz, Thomas.
"Synthesis of 1-Hydroxypyrrolidin-2,5-dione Derivatives of the Phosphonic-Hydroxamic Acid Antibiotic SF-2312" *Australian Journal of Chemistry* (**2006**), 59(4), 283-288