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Dehydroaminophosphonic acids: Synthesis, reactivity and biological applications

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Memoria que, para optar al grado de Doctora en Química, presenta

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CERTIFICAN

que la Memoria "Dehydroaminophosphonic acids: synthesis, reactivity and biological applications" ha sido realizada en el Departamento de Química Orgánica de la Facultad de Ciencias de la Universidad de Zaragoza bajo nuestra inmediata dirección y reúne las condiciones necesarias para su presentación.

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MICHAEL ENDE, *Momo*

Abbreviations

$[\alpha]^{T}_{D}$	Specific rotation
Å	Angstrom
Ac	Acetyl
APT	Attached proton test
aq.	Aqueous
Ar	Aromatic ring
$B^{\oplus}$	Base
BHI	Brain heart infusion
Bn	Benzyl
Boc	*Tert*-butoxycarbonyl
BOP	(Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate
br s	Broad singlet
Bz	Benzoyl
°C	Celsius degrees
c	Concentration
Cbz	Benzyloxycarbonyl
CFU	Colony-forming unit
CLSI	Clinical and Laboratory Standards Institute
cm	Centimetres
corr	Corrected
COSY	Correlation Spectroscopy
CP	Cyclopentadiene
CPCM	Conductor-like polarizable continuum model
d	Doublet
δ	Chemical shift
Dabco	1,4-diazabicyclo[2.2.2]octane
dd	Doublet of doublets
ddd	Doublet of doublet of doublets
ddt	Doublet of doublet of triplets
dec	Decomposition
DFT	Density Functional Theory
Dhp	Dehydrophos
DIPEA	*N*,*N*-Diisopropylethylamine

DMF	*N*,*N*-Dimethylformamide
dq	Doublet of quartets
dt	Doublet of triplets
E	Energy
EDC	*N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride
ent	Enantiomer
eq.	Equivalents
ESI	Electrospray ionization
Et	Ethyl
Fmoc	9-fluorenylmethoxycarbonyl
FPP	Farnesyl pyrophosphate
FTIR	Fourier-transform infrared spectroscopy
g	Grams
G	Free energy
Gly	Glycine
^{1}H	Proton
h	Hours
HOBt	1-Hydroxybenzotriazole
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
HSQC	Heteronuclear Correlation Spectroscopy
H-W-E	Horner-Wadsworth-Emmons
Hz	Hertz
*i*Pr	Isopropyl
IR	Infrared
IRC	Intrinsic reaction coordinate
J	Coupling constant
Leu	Leucine
µg	Microgram
µL	Microlitres
µm	Micrometres
µM	Micromolar
m	Multiplet
M.p.	Melting point
max	Maximum
Me	Methyl

mg	Milligram
MHz	Megahertz
MIC	Minimun inhibitory concentration
mL	Millilitre
mM	millimolar
mmol	Millimol
mol	Mol
MTPA	α-Methoxy-α-(trifluoromethyl)phenylacetyl
N	Normal
ν	Wave number
NCI	Non covalent interactions
nd	Not determined
NMM	*N*-Methylmorpholine
NMR	Nuclear magnetic resonance
NOE	Nuclear Overhauser effect
NOESY	Nuclear Overhauser effect spectroscopy
Nos	Nosyl
NR	Not resolved
OD	Optical density
^{31}P	Phosphorus 31
PepA	Aminopeptidase A
PepB	Aminopeptidase B
PG	Protecting group
Ph	Phenyl
ppm	Parts per million
PyBOP	(Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
q	Quartet
qd	Quartet of doublets
qt	Quartet of triplets
rt	Room temperature
s	Singlet
SPS	Solvent purification system
Su	Succinimide
t	Triplet
T	Temperature

TBDMS	*Tert*-butyldimethylsilyl
td	Triplet of doublets
TEA	Triethylamine
temp.	Temperature
TFA	Trifluoroacetyl
TFAA	Trifluoroacetic acid
THF	Tetrahydrofurane
TLC	Thin-layer chromatography
Ts	Tosyl
TS	Transition state
tt	Triplet of triplets
UV	Ultraviolet
VMD	Visual Molecular Dynamics
WHO	World Health Organization
wt.	Weight
Xaa	Amino acid

Table of contents

General introduction

Aminophosphonic acids are organophosphorus compounds which present an amino group and a phosphonic group in their structure. The biological activity of these compounds was discovered about sixty years ago,[1] and since then, they have been widely studied. Many of these compounds have proven to be potent enzyme inhibitors[2] and therefore find application as: fungicides,[3] agrochemicals,[4] antibiotics[5] or antitumors.[6]

Among these compounds, α-aminophosphonic acids are analogues of α-amino acids in which the carboxyl acid group has been replaced by a phosphonic acid. Some of them have been isolated from natural sources both in free form and as a part of complex molecules. It is presently known that α-aminophosphonic acids are capable of generating physiological and pathological responses in diverse organisms.[7–10]

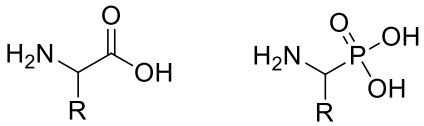

Figure 1. α-Amino acids and their analogues α-aminophosphonic acids.

The N-C-P molecular fragment is usually the main responsible of the biological activity of these compounds. There are many structural possibilities to this fragment, consequently changing the corresponding biological properties (Figure 2).[8]

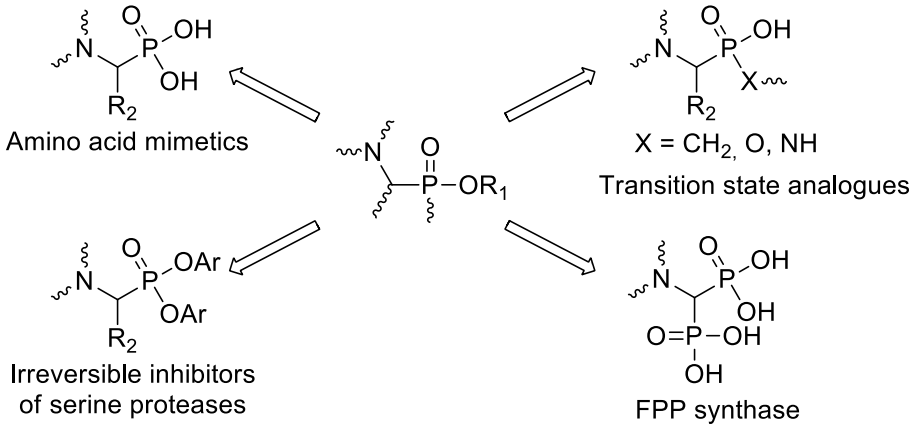

Figure 2. α-Aminophosphonic acid derivatives structural motifs and mechanisms of action.

Phosphonic and carboxylic acids differ in geometry (tetrahedral versus trigonal planar), acidity (phosphonic acid being significantly more acidic) and steric bulk

(phosphorus atom has bigger atomic radius than carbon atom). Despite these differences, they show similar properties and behaviour, as phosphonic acids are usually recognized by enzymes as a false substrate or inhibitor. This is the more frequent activity mode, since the phosphonic acid is believed to bind to the affected enzyme more strongly than the carboxylic acid.[8,11]

Alternatively, the tetrahedral geometry of substituents around the phosphorus atom causes it to resemble the high-energy transition state of ester and amide bond hydrolysis.[8] Some α-aminophosphonic acids are among the most effective inhibitors of metalloproteinases via this mechanism and as a consequence they have been thoroughly studied.[12]

Another mechanism of action involves diaryl α-aminophosphonates acting as serine proteases competitive irreversible inhibitors which, after the formation of an initial enzyme-substrate complex, bind to the active site via a transesterification reaction, thus blocking its catalytic function. [7,8,10]

Finally, aminomethylbisphosphonic acids constitute a distinct class of α-aminophosphonic acid derivatives.[13] These compounds are hydrolytically stable analogues of pyrophosphates, since the P-C-P fragment resembles the P-O-P scaffold of the latter. Consequently, many α-aminomethylbisphosphonic acid derivatives are potent inhibitors of farnesyl pyrophosphate synthases, blocking the biosynthesis of geranyl and farnesyl pyrophosphates, which are crucial for controlling cell survival and signalling pathways.[8] These processes are of great importance not only in bone resorption[15] but also in the proliferation of relevant parasites[16] or myeloma tumors.[17]

Due to the high potential in terms of utility and applications, many efforts have been devoted to the search of synthetic strategies to prepare α-aminophosphonic acids and α-aminophosphonates. Initially they were focused on the synthesis of the analogues of protein

amino acids,[18] but recently the interest have been focused on the phosphorous analogues of non-protein amino acids with chemical or biological interest.[19]

It should be noted that many non-protein amino acids, such as *N*-alkylated amino acids, quaternary amino acids, or proline analogues are introduced into the sequence of native biologically active peptides in order to enhance their bioavailability, while maintaining the bioactivity.[20,21] Furthermore, α,β-dehydroamino acids are also present in the structure of many peptides, where their chemical reactivity or conformational properties are crucial for the bioactivity.[22] Thus, there are many naturally occurring bioactive peptides containing α,β-dehydroamino acid residues in their structure.[23] This is the case of some lantibiotics, including nisin,[24] azinomicines A and B,[25] antitumor compounds like cyclic dehydropeptide kahalalide F[26] or some natural herbicides like tentoxin.[27]

In addition, the synthesis of α,β-dehydroamino acids has been extensively studied, but their reactivity is what have made them compounds of exceptional interest.[28–31] Thus, through the double bond they can undergo many different reactions that provide several α-amino acids derivatives. Hydrogenation is the simplest reaction to obtain an α-amino acid from α,β-dehydroamino acids, and enantioselective hydrogenation is an excellent way of achieving enantioenriched α-amino acids.[32] Hydroboration of the double bond allows to obtain β-boronate-α-amino acids of interest, that could be converted into β-hydroxy-α-amino acids.[33] It is possible to halogenate the double bond and use the halogen-substituted α,β-dehydroamino acid derivatives as substrates in Sonogashira[34] or Suzuki[35] couplings. Ring-closing metathesis has been used to provide heterocycles containing a α,β-dehydroamino acid moiety.[36] Finally, α,β-dehydroamino acid derivatives have been widely used as appropriate substrates in cycloaddition reactions (Figure 3).[37]

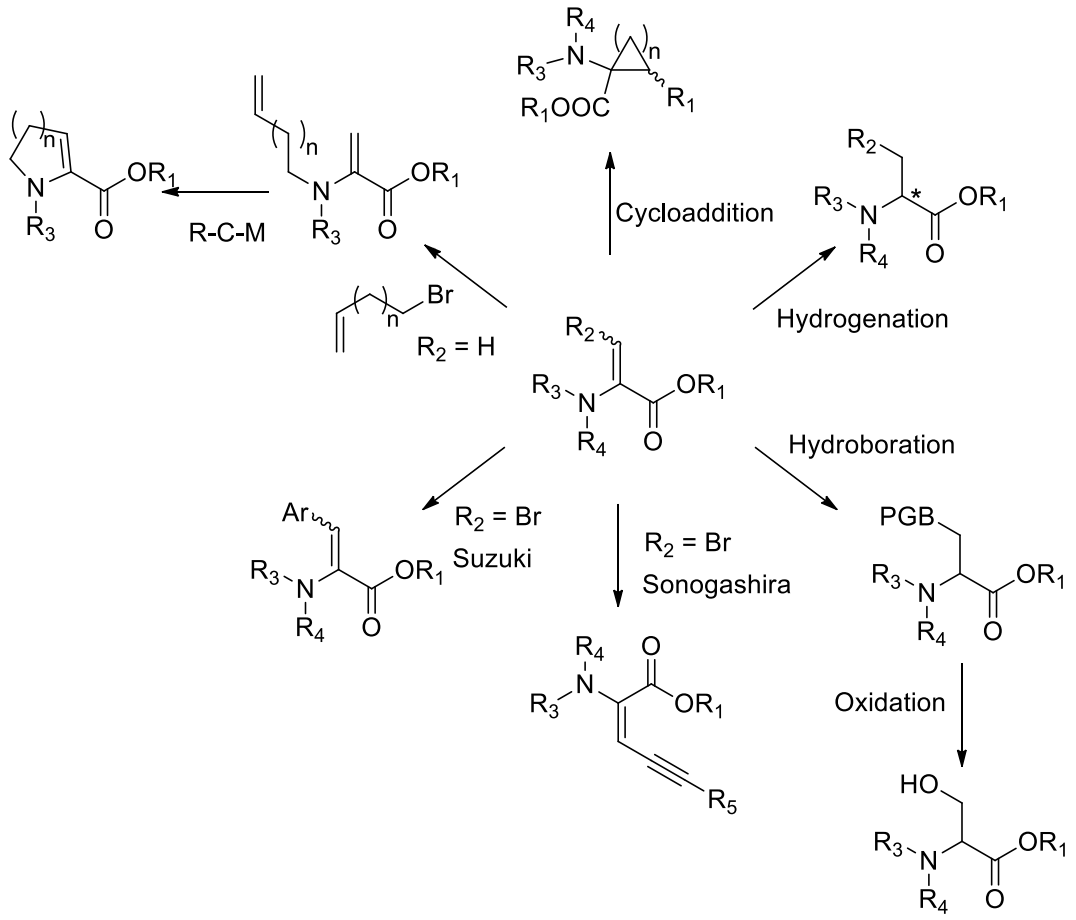

Figure 3. Most common reactions of dehydroamino acids.

In contrast, only a bioactive peptide with a dehydrophosphonate residue in its structure is known so far: the broad-spectrum antibiotic dehydrophos.[38] The mechanism of action of dehydrophos differs significantly from the described for both dehydropeptides and compounds containing an aminophosphonic acid residue in their structure. Thus, it is based on the release into the target microorganism of a toxic phosphopyruvate.[39] Indeed, acylphosphonic acids derivatives are inhibitors of thiamin diphosphate-dependent enzymes and, therefore, many metabolic processes may be targeted by dehydrophos.

On this basis, the first chapter of this doctoral dissertation describes an efficient synthesis for the peptide dehydrophos, which is predicated on the generation of the dehydroaminophosphonates moiety by means of the Horner-Wadsworth-Emmons reaction of formaldehyde with a suitable peptidyl-bisphosphonate (Figure 4).

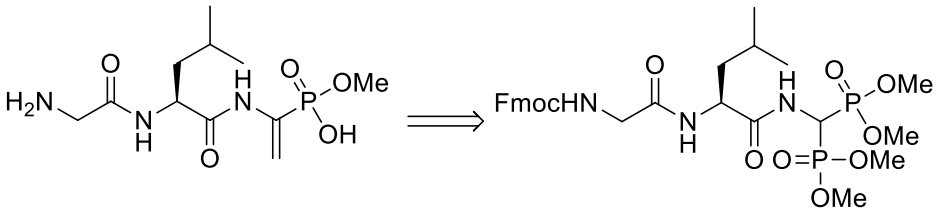

Figure 4. Retrosynthetic scheme for the synthesis of dehydrophos.

According to the above mentioned regarding the bioactivity of the acylphosphonic acid derivatives, dehydrophos analogues bearing different substituents would probably be endowed with interesting biological properties. The synthetic procedure developed in chapter 1 is then exploited for the synthesis of dehydrophos analogues in which different substituents are placed in the phosphonic acid group or in the vinyl residue. Furthermore, the peptidic sequence in dehydrophos may be also changed. Thus, the second chapter of this doctoral dissertation is devoted to the study of the synthesis and biological activities of the dehydrophos derivatives depicted in figure 5.

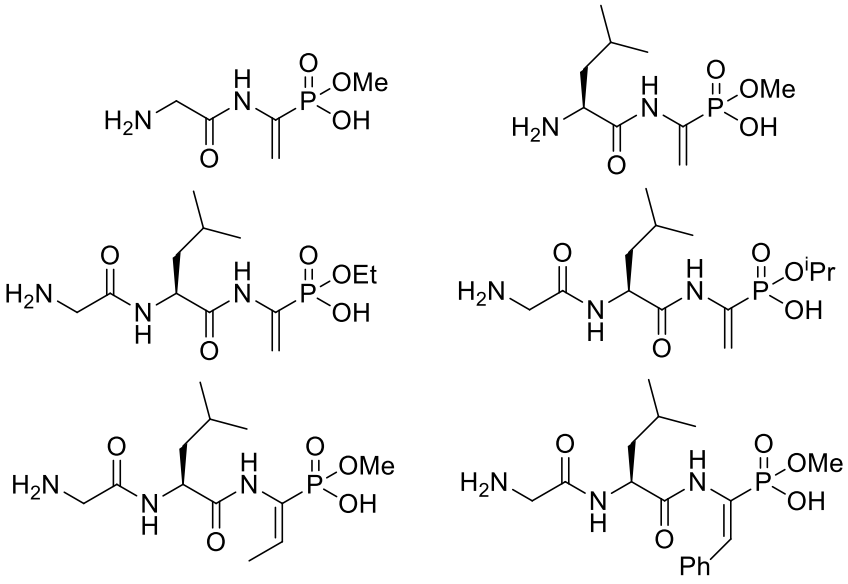

Figure 5. Dehydrophos derivatives synthetized for biological tests.

In addition, the reactivity of α,β-dehydroaminophosphonates is barely studied in comparison with α,β-dehydroamino acids. Thus, the use of the former as synthetic intermediates is limited to the enantioselective hydrogenation of the double bond;[40] the

Rhodium-catalyzed asymmetric 1,4-addition of organotrifluoroborates;[41] the 1,3-cycloaddition of diazoalkanes;[42] and electrocyclization reactions.[43]

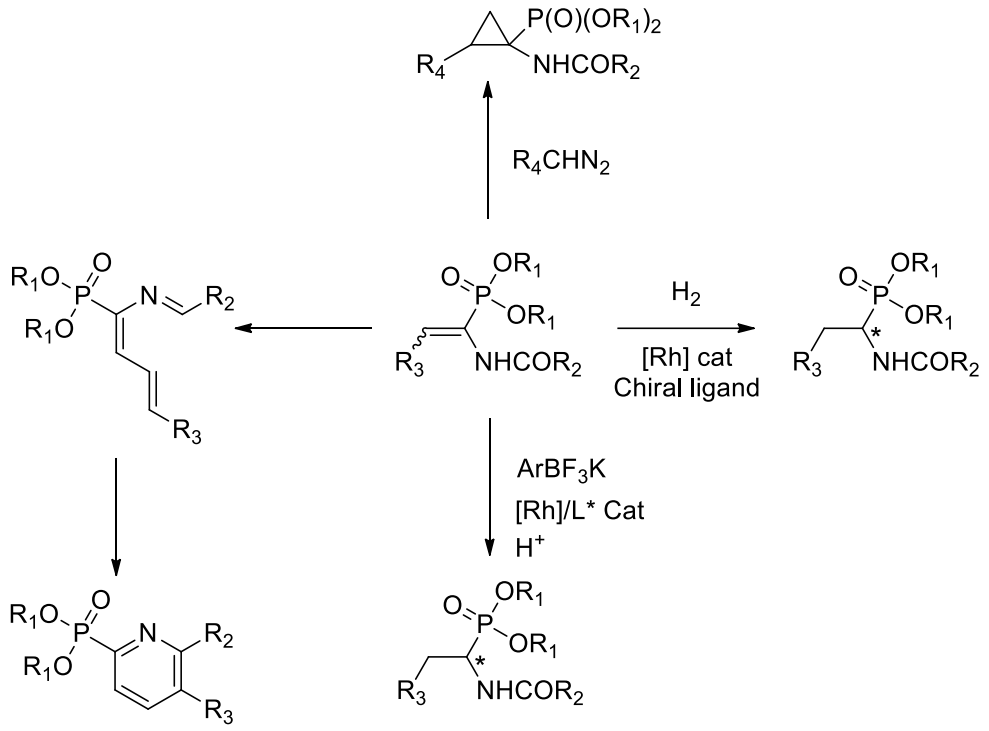

Figure 6. Reactions of α,β-dehydroaminophosphonic acids.

On this basis, the third chapter of this dissertation is focused on the possibilities of α,β-dehydroaminophosphonates as substrates in cycloaddition reactions. Particularly, the synthetic and theoretical study of the Diels-Alder reaction using α,β-dehydroaminophosphonates with different protecting groups in the amine moiety and the phosphonic acid (Figure 7).

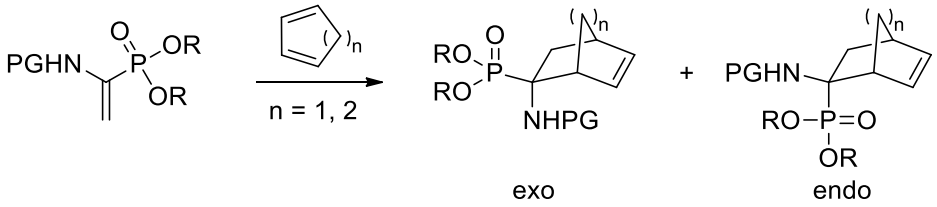

Figure 7. Diels-Alder reaction between α,β-dehydroaminophosphonates and cyclodienes.

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Chapter 1

Improved synthesis of dehydrophos

Introduction

The peptide dehydrophos is a broad-spectrum antibiotic with activity against Gram-positive and Gram-negative bacteria obtained for the first time in 1984 from *Streptomyces Luridus*.[1–3] This naturally occurring phosphonopeptide possesses a glycine-leucine sequence with an *O*-monomethyl ester dehydrophosphoalanine residue at the *C*-terminal position,[4] which is responsible for its biological activity (Figure 1).

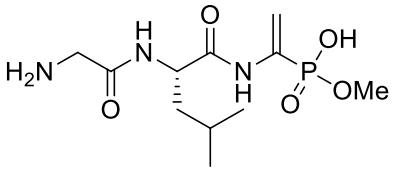


Figure 1. Structure of dehydrophos.

The coupling of phosphonic acids to amino acids is observed in numerous bioactive natural products. Once dehydrophos is inside the cell, cleavage of peptide bonds and hydrolysis release methyl acetylphosphonate, a toxic pyruvate mimic.[5] This mechanism differs from the most common modes of action of α-aminophosphonate derivatives, which usually act as amino acid mimetics, analogues of the transition state of the peptide bond cleavage or irreversible inhibitors of serine proteases.[6] Furthermore, it is also different from the mode of action of dehydropeptides, where the α,β-dehydroamino acid residues influence the bioactivity by means of both their conformational properties and chemical reactivity.[7]

Due to these unusual features of dehydrophos, there have been many efforts to elucidate both its biosynthetic pathway and gene cluster.[8–14] Despite this, the synthetic procedures leading to the indicated antibiotic are very scarce in the literature, limited to obtaining the molecule in order to its biological study. Thus, van der Donk and coworkers[4] first synthesized dehydrophos for a reliable reassignment of its structure. The critical steps in the synthesis of dehydrophos were the introduction of the dehydroaminophosphonate moiety, which was accomplished in low yields through the condensation of a suitable amido

peptide with dimethyl acetylphosphonate, and the deprotection of the thus obtained dehydrophosphonopeptide to obtain dehydrophos, which was carried out under harsh basic conditions and requires HPLC purification of the final compound (Figure 2).

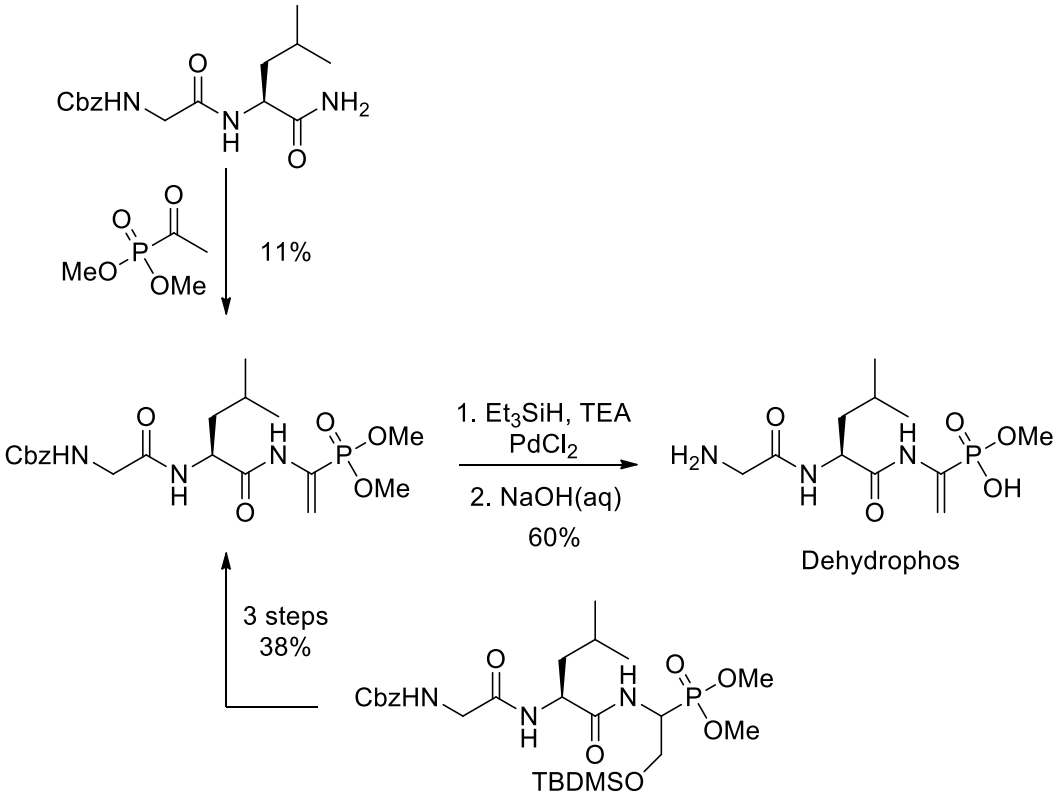


Figure 2. Reported procedures for the synthesis of dehydrophos.

Afterwards, other synthetic route was used in order to enhance yields. Thus, the dehydroaminophosphonate moiety was generated by elimination of a phosphoserine residue.[15] However, the formation of the double bond required a 3-step procedure with a 38% yield, leaving room to improvement. Furthermore, the phosphoserine derivative must be prepared from the commercially available *N*-(benzyloxycarbonyl)serine by previously described protocols.[16–18]

There are different procedures for the preparation of α,β-dehydroaminophosphonic acids.[19,20] The synthesis of α,β-dehydroaminophosphonates by direct formation of the C_α-C_β bound via Horner-Wadsworth-Emmons reaction with bisphosphonates presents many

possibilities as it can be performed with different aldehydes therefore introducing different substituents in the double bond.

Thus, throughout this chapter, we explore the Horner-Wadsworth-Emmons reaction starting from bisphosphonates as a suitable strategy for generating the dehydroaminophosphonate residue. Furthermore, a high-yielding deprotection procedure leading simultaneously to the free amino and the phosphonic acid monomethyl ester has been developed. In both cases, the synthetic procedures described in this work involve the use of smooth bases and conditions, which ensure the stereochemical integrity of the amino acid residue.

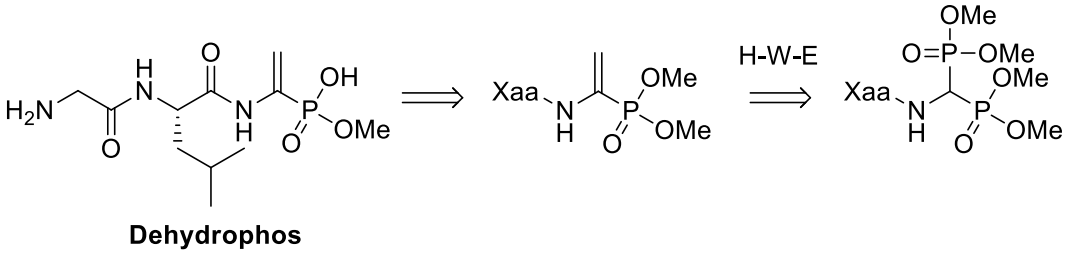
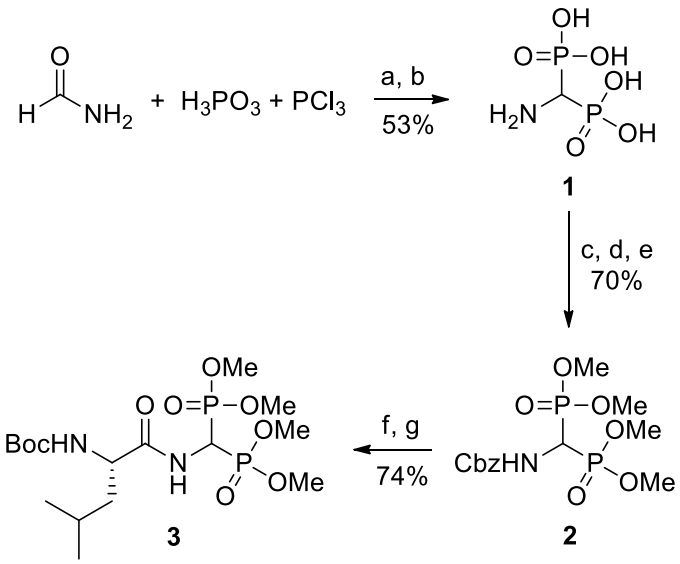


Figure 3. Retrosynthesis of dehydrophos developed in this chapter.

Results and Discussion

In first place, we approached the synthesis of an appropriate aminomethylbisphosphonate derivative and the preparation of a peptide containing it (Scheme 1). Thus, the aminomethylbisphosphonic acid **1** was prepared from formamide using the conditions described by Schöllkopf et al. with slight modifications.[21] The completely deprotected aminomethylbisphosphonic acid is an excellent starting material, as it is a solid obtainable in gram scale, and could be modified by choice.

Benzyloxycarbonylation of compound **1** followed by esterification of the phosphonic acid groups using trimethyl orthoformate as alkylating agent, provided the suitably protected aminomethylbisphosphonate **2** in gram scale without isolation of intermediates. Finally, hydrogenolysis of compound **2** followed by peptide coupling with Boc-Leu-OH yielded the peptidyl-bisphosphonate **3**.

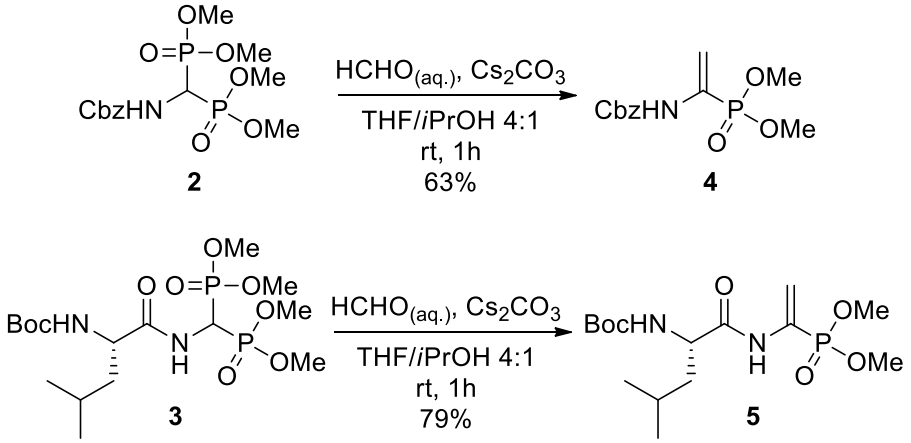


Scheme 1. Reagent and conditions: (a) 70 °C, 8 h., then H_2O, room temp., 12 h.; (b) 6 M HCl, reflux, 72 h.; (c) Cbz-OSu, Et_3N, H_2O/CH_3CN 4:1, room temp., 12 h.; (d) Dowex® 50WX8; (e) $CH(OCH_3)_3$, reflux, 72 h.; (f) H_2, Pd/C, MeOH, room temp., 1 h.; (g) Boc-Leu-OH, NMM, EDC, HOBt, DMF, 0 °C, 18 h.

Bisphosphonate derivatives **2** and **3** were considered as suitable models for exploring the introduction of a methylene group by means of the Horner-Wadsworth-Emmons

reaction, and subsequent cleavage of one methyl ester group to obtain the phosphonic acid monoester.

Firstly, we addressed the Horner-Wadsworth-Emmons reaction of the corresponding bisphosphonate derivative with formaldehyde. The reaction conditions described so far in the literature for the synthesis of α-amino vinylphosphonates through the Horner-Wadsworth-Emmons reaction[19–22] involve the use of strong bases, both ionic[21] and non-ionic.[22] However, while searching for milder conditions, we found that the use of a 37% formaldehyde aqueous solution in the presence of cesium carbonate generated the corresponding dehydrophosphoalanine derivatives **4** and **5** in good yields (Scheme 2).



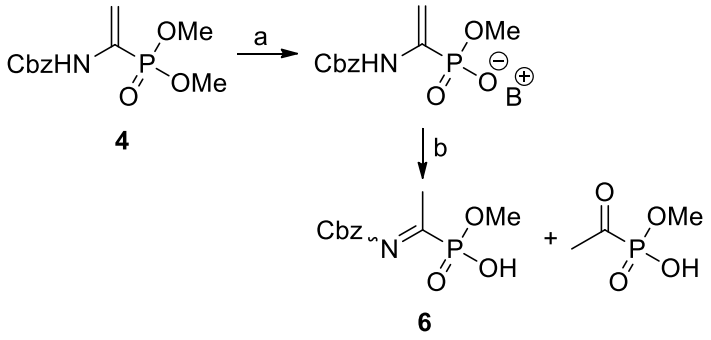
Scheme 2. Horner-Wadsworth-Emmons reaction of aminomethylbisphosphonates **2** and **3**.

These reaction conditions compare favorably with those described before, since the use of a weak base avoids the racemization in compounds possessing an amino acid moiety. It should be noted that the introduction of a methylene group under such smooth conditions is also very rare in the case of the carboxylic acid counterparts. Indeed, the synthetic methodologies described in the literature for the preparation of peptides containing a dehydroalanine moiety comprise the elimination reaction of serine,[23,24] cysteine[25] or selenocysteine[26] residues.

First attempts to deprotect the dimethyl phosphonate group in derivative **4** were carried out using LiOH, which is preferred to NaOH in compounds bearing a chiral amino

acid residue.[27] However, after stirring at room temperature overnight, the ^{31}P NMR spectrum of the crude reaction mixture showed many side products in addition to remaining starting material.

Alternatively, the use of a non-ionic nucleophile base, Dabco®,[28] was tested. Therefore, a solution of compound **4** in an acetone/toluene 1:1 mixture was refluxed in the presence of Dabco® for 5 hours. Both the ^{1}H and ^{31}P NMR spectra of the crude reaction mixture showed the total conversion of compound **4** into the 1-methyl-1,4-diazabicyclo[2.2.2]octan-1-ium salt of the corresponding phosphonic acid monoester, without significant presence of side products (Scheme 3). It should be noted that these reaction conditions compared favorably with the partial hydrolysis of dimethyl α-aminophosphonate derivatives using *tert*-butylamine as the base,[29,30] which usually requires long reaction times and implies stronger conditions.



Scheme 3. Reagent and conditions: (a) Dabco®, acetone/toluene 1:1, reflux, 5 h.; (b) Dowex® 50WX8.

Despite this promising result, treatment of the above mentioned salt with acidic resin Dowex® 50WX8 resulted in a mixture of *Z*/*E* isomers of the iminophosphonic acid **6** instead of the desired α-amino vinylphosphonic acid monoester. Thus, the ^{1}H NMR spectrum of **6** showed two doublets for three protons at 1.50 and 1.47 ppm respectively (Figures 4 and 5). Both signals exhibit a $^{3}J_{P,C}$ of about 12 Hz, which is typical for the methyl groups of compounds possessing an iminoethylphosphonate structure.[31–35] However, compound **6**

decomposed upon standing, and the ^{1}H NMR spectrum showed the presence of the methyl acetylphosphonate[36] formed from the hydrolysis of the imino group.

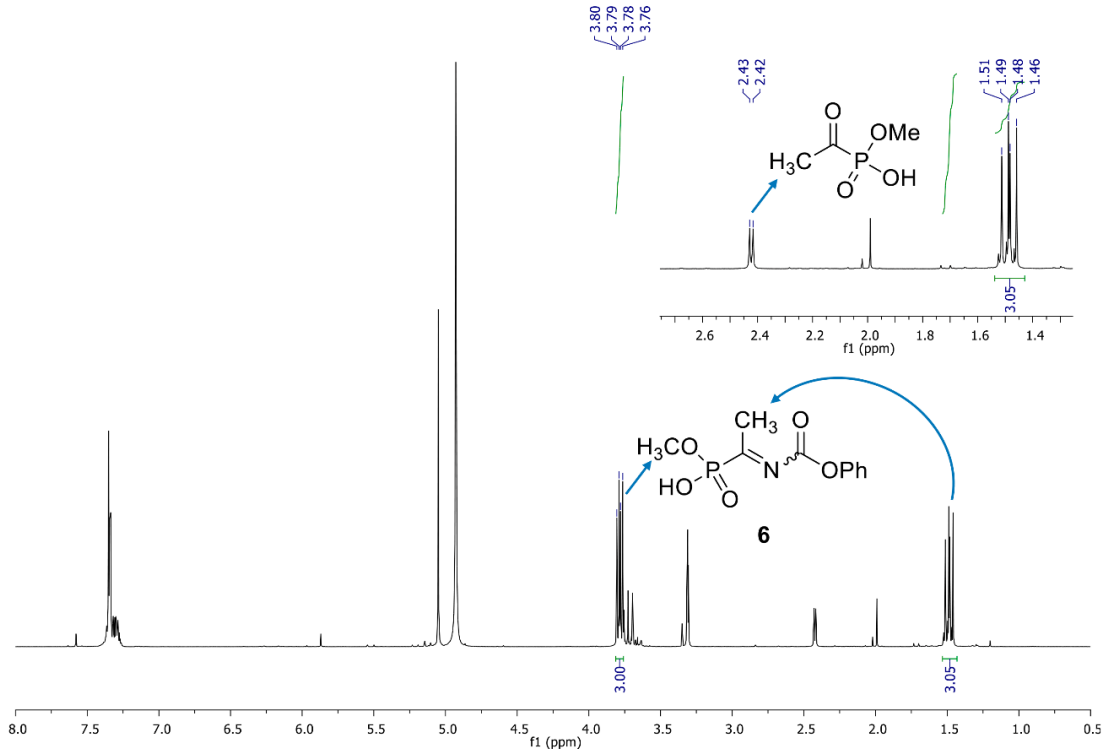


Figure 4: ^{1}H NMR of **6** with signals about 1.50 ppm, showing the presence of methyl acetyl phosphonate (2.42 doublet).

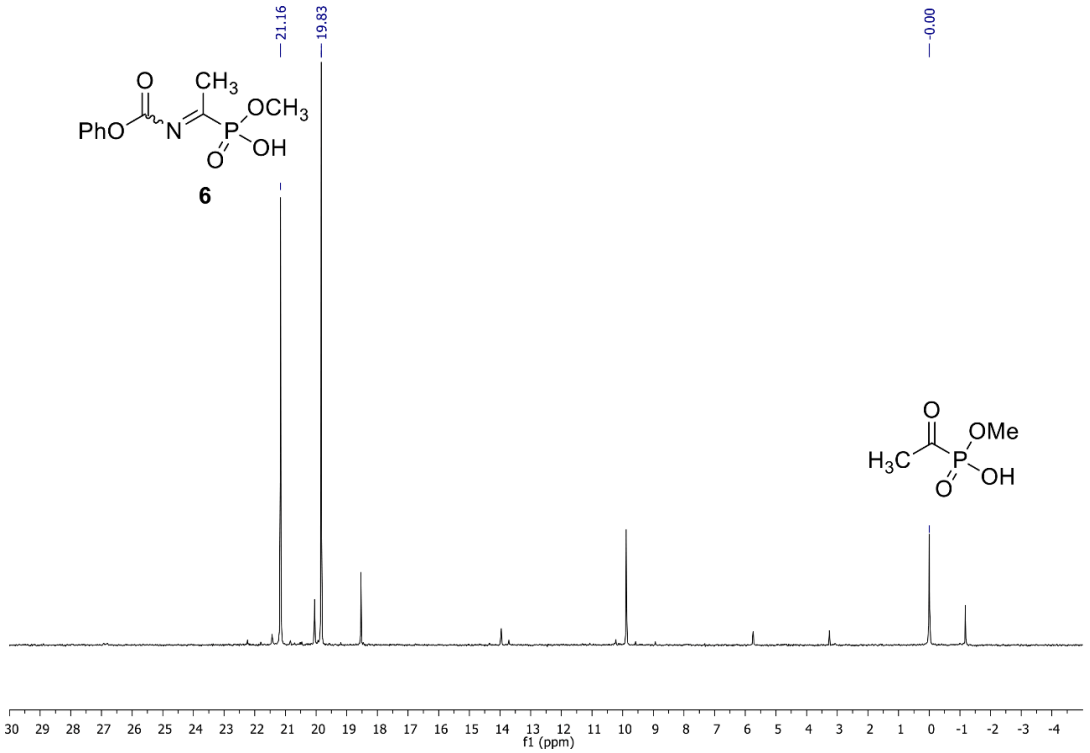
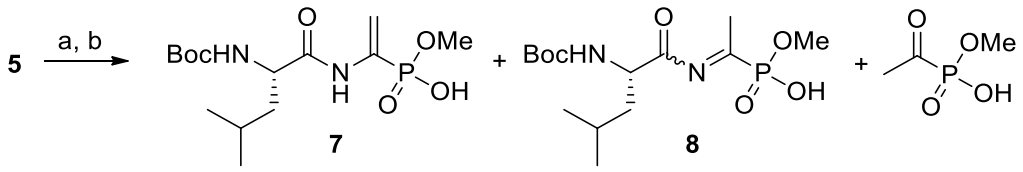


Figure 5: ^{31}P NMR of **6** *Z*/*E* isomers mixture, with the presence of methyl acetyl phosphonate.

Interestingly, when compound **5** was subjected to the procedure described above for **4**, the main compound was the desired α-amino vinylphosphonic acid monoester **7**, although both the ^{1}H and the ^{31}P NMR spectra showed signals compatible with the presence of the iminophosphonate **8** and the methyl acetylphosphonate (Figure 6). However, deprotection of the amine group in compound **7** with a trifluoroacetic acid/dichloromethane mixture resulted in decomposition (Scheme 4).



Scheme 4. Reagent and conditions: (a) Dabco®, acetone/toluene 1:1, reflux, 5 h.; (b) Dowex® 50WX8.

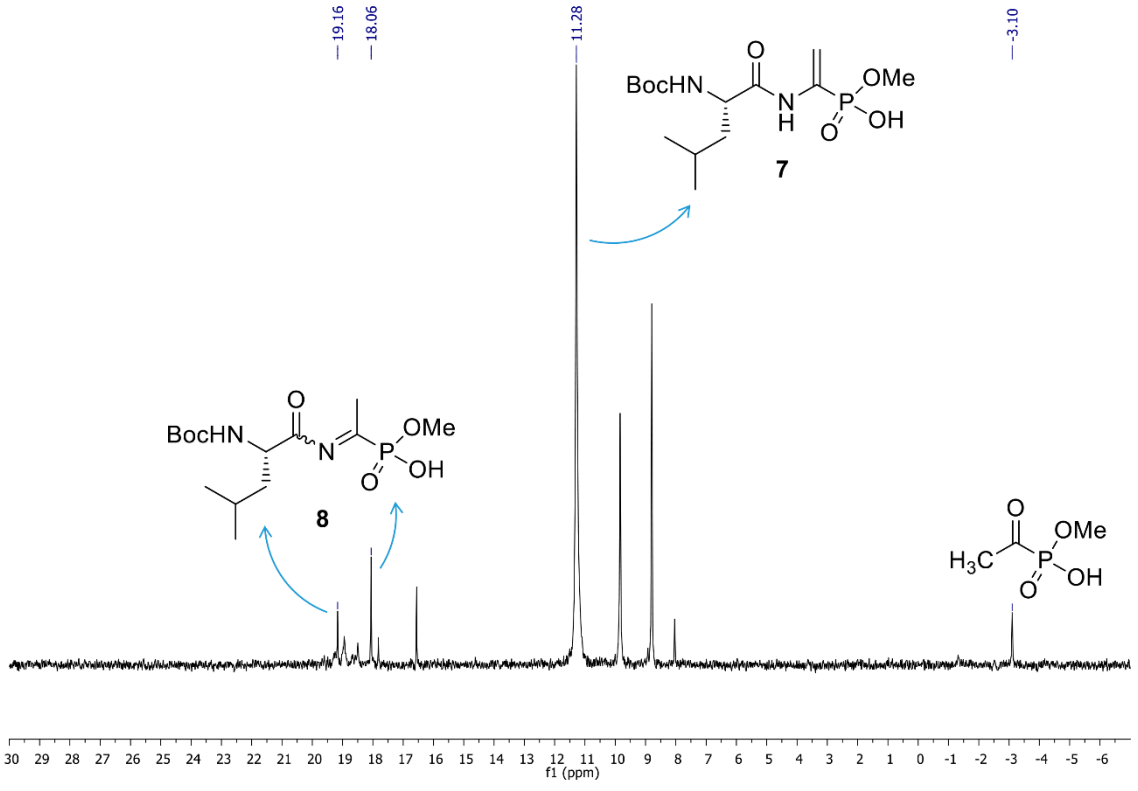
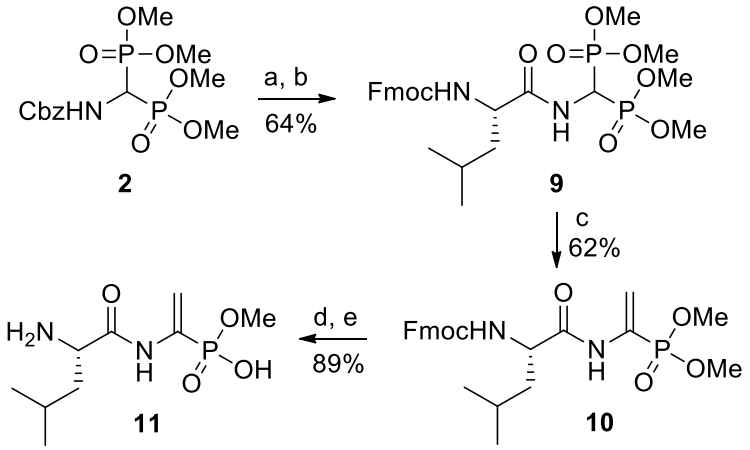


Figure 6. ^{31}P NMR of **7** (11.28 ppm), iminophosphonate **8** *Z*/*E* mixture (19.16 and 18.06 ppm) and methyl acetyl phosphonate (-3.10 ppm) mixture.

These results indicated that the α-amino vinylphosphonic monoesters were sensitive to acidic conditions, including their own phosphonic acid group. Furthermore, we reasoned that the presence of an amine group in the molecule would neutralize the phosphonic acid group and thus would stabilize the final compound. Accordingly, both deprotection of the amino group and partial hydrolysis of the dimethyl phosphonate group should take place at the same time, and procedures for the partial hydrolysis of the phosphonate group based on acidic conditions[37–41] or iodide salts[42–45] were discarded.

With this aim in mind, the Fmoc protected dehydrophosphonopeptide **10** was prepared. Thus, the hydrogenolysis of bisphosphonate **2** followed by the peptide coupling with Fmoc-Leu-OH afforded the peptidyl-bisphosphonate **9**, which was converted into **10** using the procedure described above for the Horner-Wadsworth-Emmons reaction (Scheme 5).

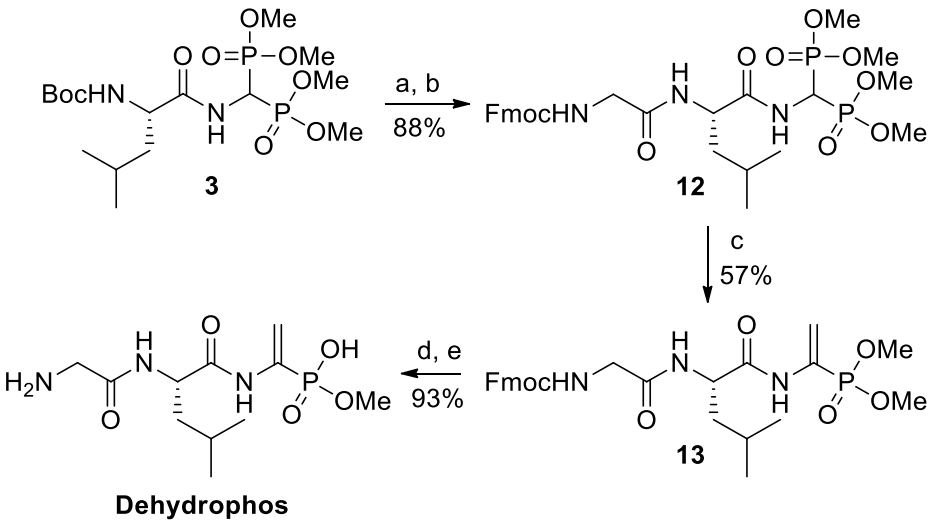


Scheme 5. Reagents and conditions: (a) H_2, Pd/C, MeOH, room temp., 1 h.; (b) Fmoc-Leu-OH, NMM, EDC, HOBt, DMF, 0 °C, 18 h.; (c) 37% HCHO(aq.), Cs_2CO_3, THF/*i*PrOH 4:1, room temp., 1 h.; (d) Dabco®, acetone/toluene 1:1, reflux, 5 h.; (e) Dowex® 50WX8.

Interestingly, when compound **10** was treated with Dabco® partial hydrolysis of the dimethyl phosphonate group and deprotection of the amine occurred simultaneously. Thus, both the ^{31}P and the ^{1}H NMR spectra of the reaction crude showed the total conversion of **10** into the 1-methyl-1,4-diazabicyclo[2.2.2]octan-1-ium salt of the corresponding

deprotected dehydrophosphonopeptide monoester, in addition to the formation of 9-methylenefluorene from the deprotection of the 9-fluorenylmethoxycarbonyl group. To our delight, treatment of the crude reaction product with acidic resin Dowex® 50WX8 yielded the desired free dehydrophosphonopeptide **11** as the only product.

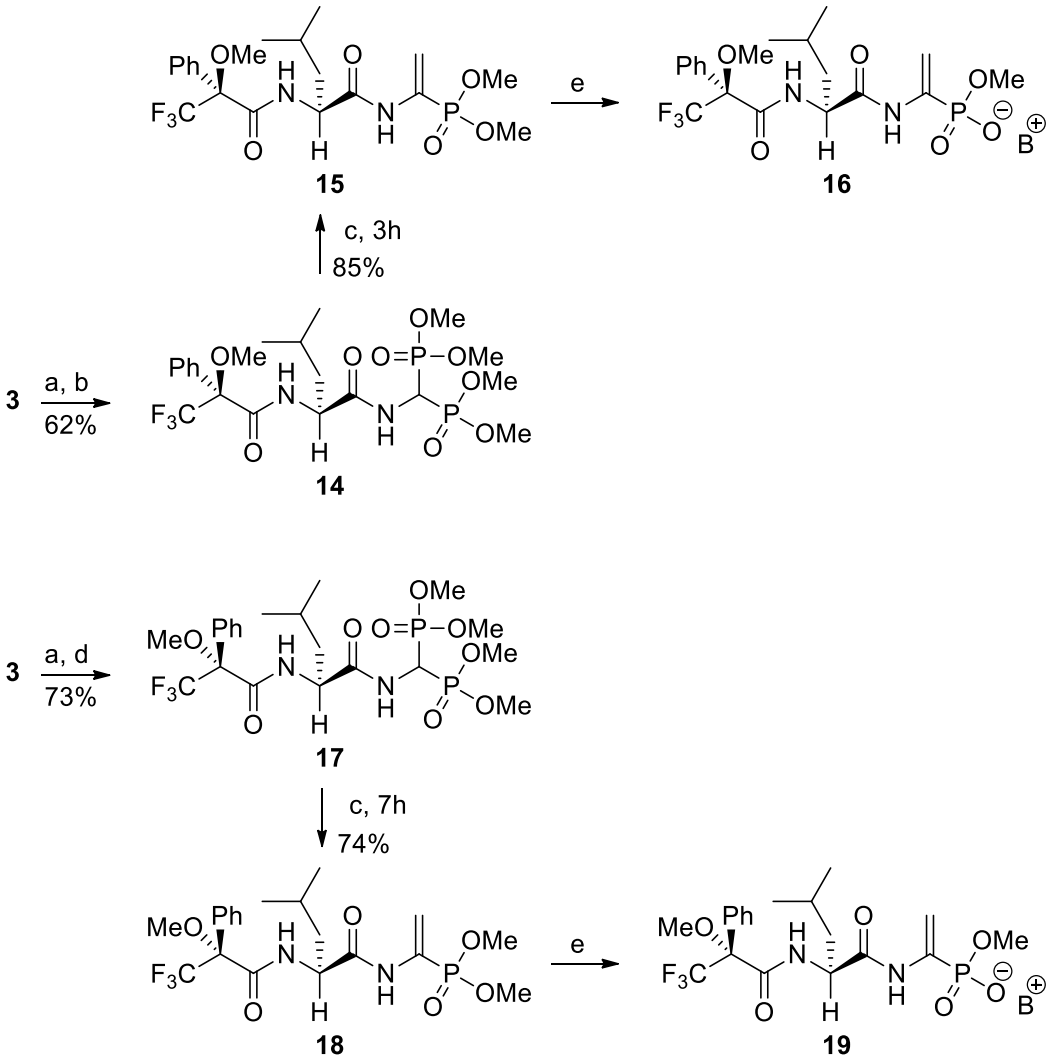
The synthetic procedures developed both for the introduction of a methylene group through the Horner-Wadsworth-Emmons reaction and for the partial hydrolysis of the dimethyl phosphonate group were then applied to the synthesis of dehydrophos (Scheme 6). Therefore, an intermediate peptidyl-bisphosphonate bearing an Fmoc protecting group at the *N*-terminal position was synthesized. Thus, compound **12** was prepared starting from **3** by the peptide coupling of an Fmoc-Gly-OH residue. Treatment of the bisphosphonate derivative **12** with a 37% formaldehyde aqueous solution in the presence of cesium carbonate yielded the dehydrophosphonepeptide **13**, which afforded the antibiotic dehydrophos in a 93% yield by the selective cleavage of a methyl group of the dimethyl phosphonate moiety and simultaneous deprotection of the amine group with Dabco®. It should be mentioned that in this case the reaction crude was treated with the weakly acidic resin Amberlite® CG50 instead of the strongly acidic one Dowex® 50WX8, since the latter leaded to decomposition of the desired compound.



Scheme 6. (a) TFAA, CH_2Cl_2, room temp., overnight; (b) Fmoc-Gly-OH, NMM, EDC, HOBt, DMF, 0 °C, 1 h., then 4 °C, 14 h.; (c) 37% HCHO(aq.), Cs_2CO_3, THF/*i*PrOH 4:1, room temp., 1 h.; (d) Dabco®, acetone/toluene 1:1, reflux, 2 h.; (e) Amberlite® CG50.

It is worth noting that the synthetic procedure developed in this work involves a considerable improvement of the two key transformations for the synthesis of dehydrophos. Thus, the yield of both the generation of a dehydrophosphoalanine residue and the deprotection of the amine and the dimethyl phosphonate groups has increased notably, whereas the number of synthetic steps for both transformations has been reduced. Furthermore, the peptide dehydrophos thus obtained was easily purified using a weakly acidic resin instead of HPLC.

Finally, we wanted to confirm that the chiral integrity of the amino acid residue was not affected throughout the synthetic route. The compounds could not be analyzed with chiral chromatography, so we decided to use the Mosher method based on the use of derivatizing agents instead, in order to detect the possibly presence of enantiomeric substances. Thus, peptides **14** and **17** bearing an (*S*)- and an (*R*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid residue respectively were synthesized for this purpose (Scheme 7).



Scheme 7. Reagents and conditions: (a) TFAA, CH_2Cl_2, room temp., overnight; (b) (*R*)-MTPACl, CH_2Cl_2, DIPEA, room temp., 21 h.; (c) 37% HCHO(aq.), Cs_2CO_3, THF/*i*PrOH 4:1, room temp.; (d) (*S*)-MTPACl, CH_2Cl_2, DIPEA, room temp., 21 h.; (e) Dabco®, acetone/toluene 1:1, reflux, 5 h.

Compounds **14** and **17** were submitted to the synthetic procedure described in this chapter for the synthesis of dehydrophos. Thus, they were firstly treated with a 37% formaldehyde aqueous solution in the presence of cesium carbonate to afford the vinylphosphonates **15** y **18**, which were converted into the 1-methyl-1,4-diazabicyclo[2.2.2]octan-1-ium salts of the corresponding phosphonic acid monoesters **16** and **19** by reaction with Dabco®. It should be noted that **16** and **19** were not treated with an acidic ion-exchange resin to avoid decomposition, and therefore NMR spectra of the reaction crude products were recorded.

The analysis of ^{1}H NMR spectra of compounds described in scheme 6 showed that the stereochemistry of the α-methoxy-α-(trifluoromethyl)phenylacetyl amide did not substantially affect the chemical shift of the α-proton of the leucine residue, appearing about 4.50 ppm. However, signals corresponding to the leucine side chain, around 1.50 ppm and 0.75 ppm respectively, were more shielded for compounds bearing an (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl moiety (Figures 7 and 8). These data are in agreement with the model proposed for Mosher derivatives[46] and with that observed for leucine derivatives.[47] Furthermore, signals corresponding to the methoxy group of the (*S*)-α-methoxy-α-trifluoromethylphenylacetyl amide (compounds **14**, **15** and **16**) were less shielded than those observed for the (*R*)-isomer (compounds **17**, **18** and **19**).

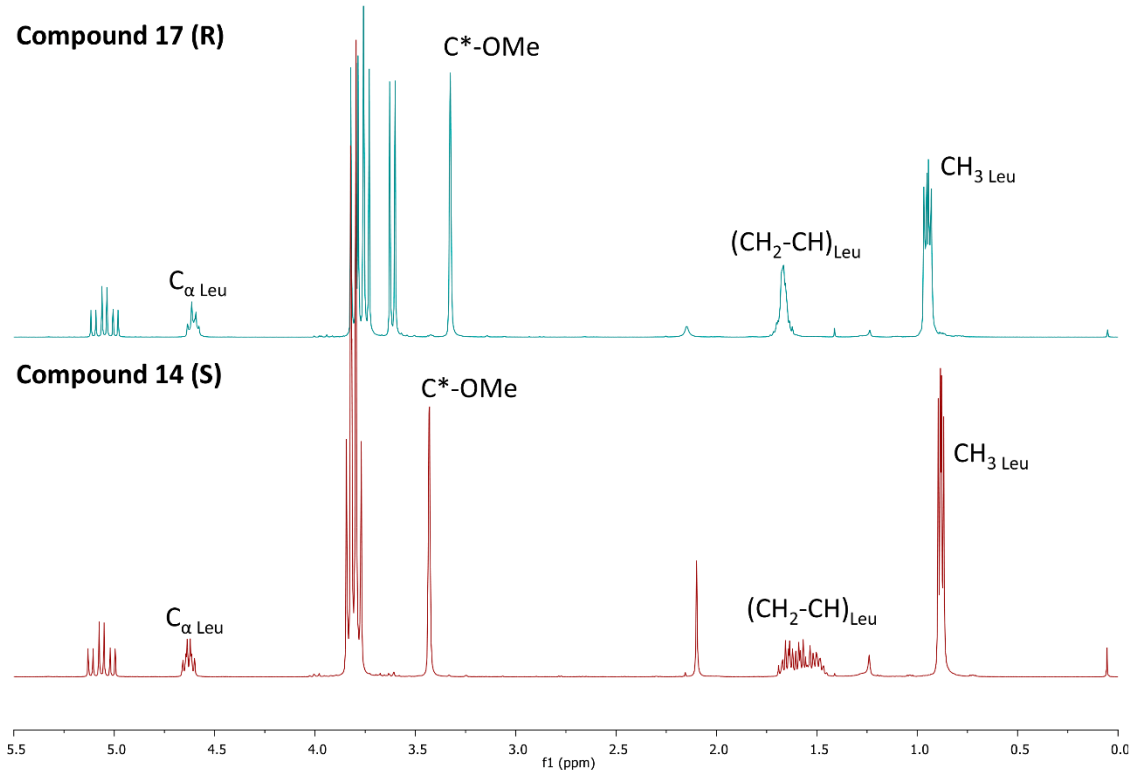


Figure 7: ^{1}H NMR spectra of compounds **14** and **17**. Singlets about 3.50 ppm correspond to the methoxy group, multiplets about 1.50 and 0.75 ppm correspond to leucine side chain.

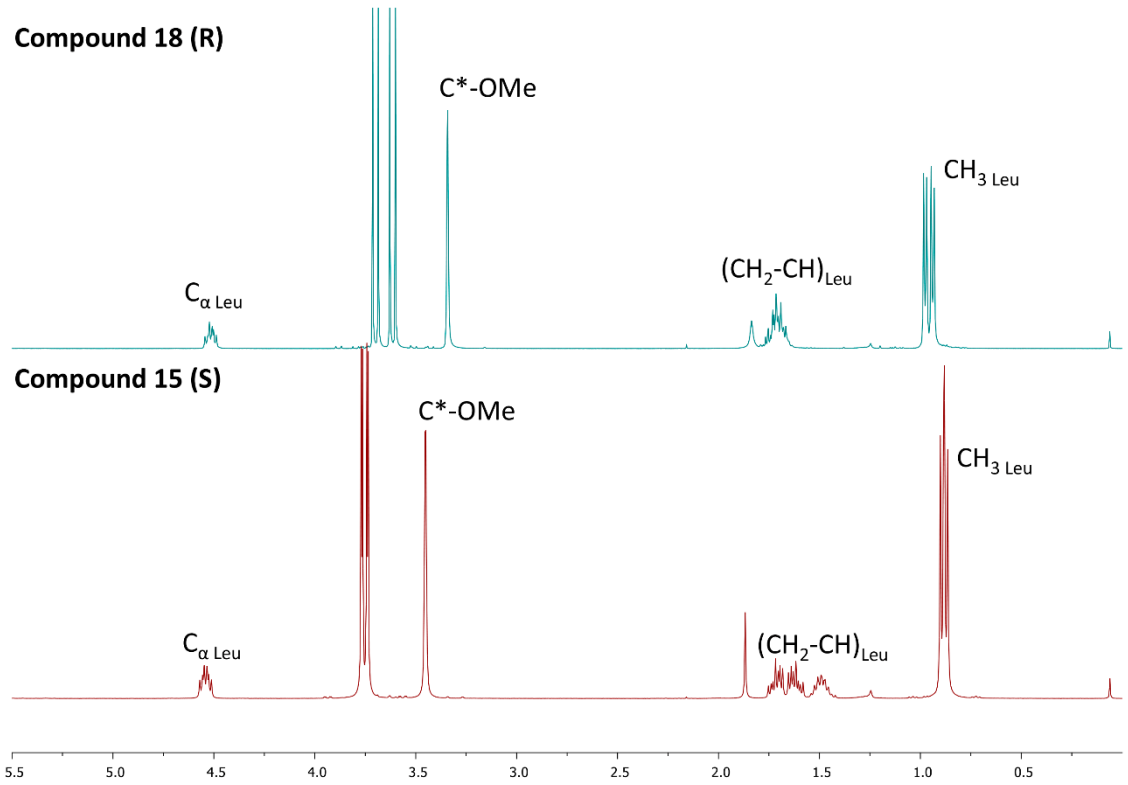


Figure 8: ^{1}H NMR spectra of compounds **15** and **18.** Singlets about 3.50 ppm correspond to the methoxy group, multiplets about 1.50 and 0.75 ppm correspond to leucine side chain.

Considering that epimerization of the leucine residue in (*S*)- or (*R*)-α-methoxy-α-trifluoromethylphenylacetyl amide derivatives would result in the enantiomer of the opposite derivative, it should be easily detected in the ^{1}H NMR spectra of the crude reaction products (Figure 9). The differences between the signals of leucine chain maintain the tendency showed in previous compounds, and signals corresponding to epimerization products were not observed, thus we concluded that our improved protocol for the synthesis of dehydrophos proceeded without racemization.

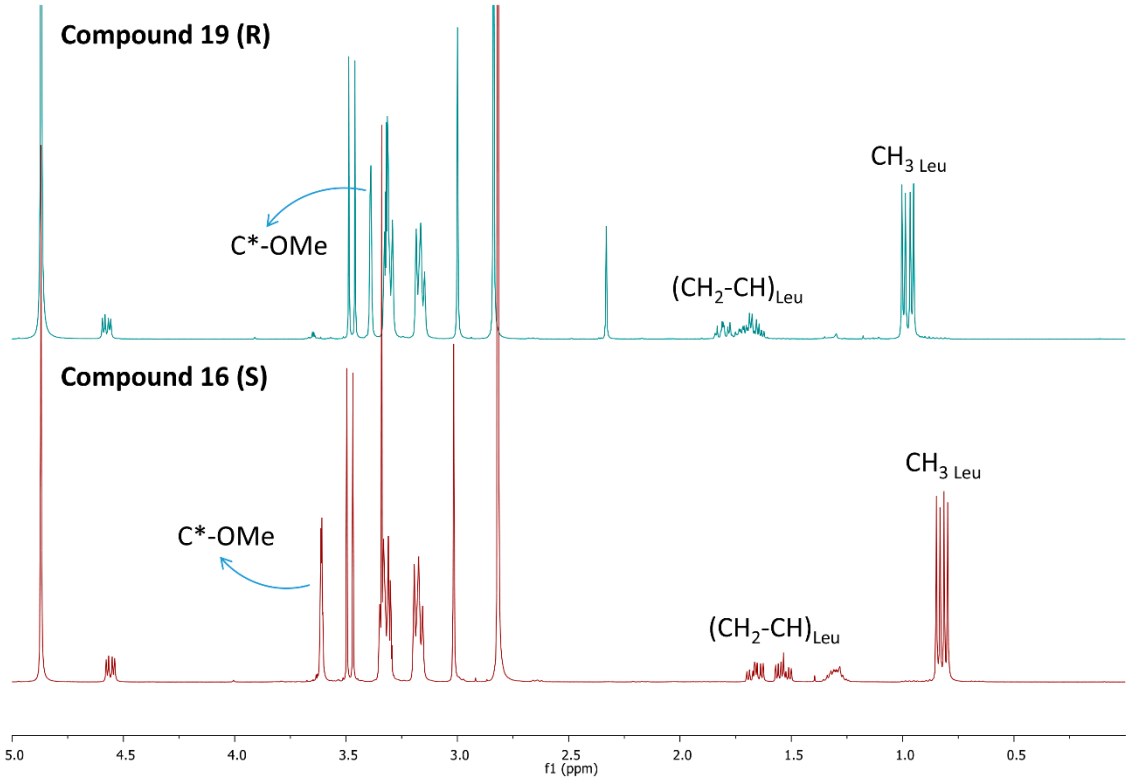
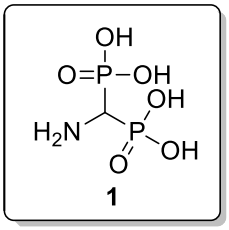


Figure 9: ^{1}H NMR spectra of reaction crudes of compound **16** and **19**. Signals about 3.50 ppm correspond to the methoxy group, multiplets about 1.50 and 0.75 ppm correspond to leucine side chain.

Experimental Section

All reagents were used as received from commercial suppliers without further purification. Anhydrous solvents were dried using a Solvent Purification System (SPS). *N*,*N*-Dimethylformamide were purchased at anhydrous grade from Aldrich. Thin-layer chromatography (TLC) was performed on Macherey–Nagel Polygram® SIL G/UV254 precoated silica gel polyester plates. The products were visualized by exposure to UV light or submersion in ninhydrin, phosphomolybdic acid or permanganate. Column chromatography was performed using 60 Å (0.04–0.063 mm) silica gel from Macherey–Nagel. Melting points were determined on a Gallenkamp apparatus. Optical rotations were measured in a JASCO P-1020 polarimeter. IR spectra were registered on a Nicolet Avatar 360 FTIR spectrophotometer; ν_{max} is given for the main absorption bands. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker AV-400 instrument at room temperature using the residual solvent signal, the solvent signal, or the chemical shift of the lock solvent as the internal standard; chemical shifts (δ) are expressed in parts per million and coupling constants (J) in Hertz. High-resolution mass spectra were recorded on a Bruker Microtof-Q spectrometer.

Synthesis of aminomethylbisphosphonic acid (1)



1

A mixture of phosphorous acid (4.55 g, 55.49 mmol), phosphorus trichloride (20.20 mL, 166.51 mmol) and formamide (8.80 mL, 222.04 mmol) was stirred under argon for 8 hours at 70 °C. It was then cooled to room temperature, water was added dropwise (30 mL) and then stirred 12 hours at room temperature. Solvent was removed and the crude mixture was suspended in a hydrogen chloride 6N aqueous solution and stirred under reflux for 3 days. Solvent was removed under reduced pressure and then a solvent mixture of methanol/diethyl ether 2:1 (70 mL) was added in order to form a precipitate. This precipitate was filtered and washed with a methanol/water 2:1 mixture (25 mL) to give the product as a white solid (11.15 g, 58.37 mmol, 53% yield).

M.p.: 298–300 °C.

IR (KBr): ν 3162, 1200, 1162 cm^{-1}.

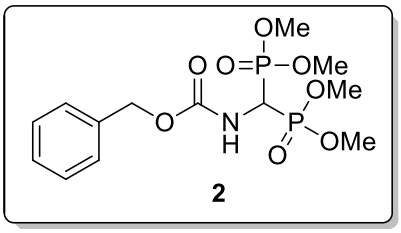
^{1}H NMR (400 MHz, D_2O): δ 3.13 (t, 1H, J = 16.4 Hz, N-CH-P_2) ppm.

^{13}C NMR (100 MHz, D_2O): δ 50.83 (t, J = 119.6 Hz, N-CH-P_2) ppm.

^{31}P NMR (162 MHz, D_2O): δ 9.53 ppm.

HRMS (ESI) $CH_6NO_6P_2$ $[M-H]^-$: 189.9676, found 189.9673.

Synthesis of tetramethyl (benzyloxycarbonylaminomethyl)bisphosphonate (2)



2

To a solution of **1** (969 mg, 5.07 mmol) in water (5 mL) and triethylamine (3.00 mL, 21.50 mmol) was added a solution of *N*-(benzyloxycarbonyloxy)succinimide (1.28 g, 5.14 mmol) in acetonitrile (1.3 mL). The reaction mixture was stirred 12 hours at room temperature, and solvent was removed under reduced pressure. The residue was dissolved in water, washed with diethyl ether, treated with an ion-exchange resin (Dowex® 50WX8, hydrogen form) and evaporated to afford a colourless oil which was used in the next step without further purification. Previous residue was dissolved in trimethyl orthoformate (15.32 mL, 0.14 mol) and stirred at reflux under argon for 72 hours. Subsequently, the orthoformate in excess was removed under reduced pressure. The resulting oil was purified by column chromatography on silica gel (dichloromethane/diethyl ether/methanol 55:40:5) to afford **2** as a yellow oil (1.35 g, 3.54 mmol, 70% yield).

IR (neat): ν 1708, 1270, 1240, 1149, 1053, 1024 cm^{-1}.

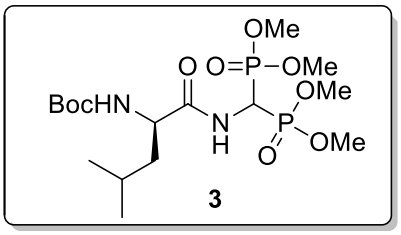
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37–7.29 (m, 5H, CH_{Ar}), 5.41 (d, 1H, J = 10.4 Hz, NH), 5.15 (s, 2H, CH_2-Ph), 4.66 (td, 1H, J = 22.1, 10.4 Hz, N-CH-P_2), 3.85–3.76 (m, 12H, $CH_{3\ OMe}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 155.73 (t, J = 5.0 Hz, CO), 135.97 (C_{Ar}), 128.61 (CH_{Ar}), 128.41 (CH_{Ar}), 128.12 (CH_{Ar}), 67.84 (CH_2-Ph), 54.18–54.04 ($CH_{3\ OMe}$), 45.29 (t, J = 148.5 Hz, N-CH-P_2) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ = 18.85 ppm.

HRMS (ESI) $C_{13}H_{21}NNaO_8P_2$ $[M+Na]^+$: 404.0635, found 404.0628.

Synthesis of tetramethyl [*N*-(*tert*-butoxycarbonyl)-L-leucylamidomethyl]bisphosphonate (3)



3

To a solution of **2** (2.50 g, 6.56 mmol) in methanol (75 mL) 10% wt. palladium on carbon (250 mg) was added. The mixture was stirred under a hydrogen atmosphere at room temperature for one hour. Then, the reaction mixture was filtered (Celite) and evaporated to afford an oil which was used in the next step. A solution of previous oil and Boc-L-leucine (1.79 g, 7.18 mmol) in dry *N*,*N*-dimethylformamide (30 mL) was cooled to 0 °C. *N*-Methylmorpholine (0.93 mL, 8.46 mmol) and 1-hydroxybenzotriazole hydrate (12% H_2O) (1.20 g, 7.82 mmol) were added and the mixture was stirred for 15 minutes at 0 °C. Then *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (1.50 g, 7.82 mmol) was added and the reaction mixture was stirred for 18 hours at 0 °C. Solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate (30 mL) and washed with an aqueous 5% solution of sodium bicarbonate (20 mL). The aqueous phase was extracted with ethyl acetate (3 x 20 mL) until no final product was detected by TLC in the aqueous phase. The combined organic layers were dried over anhydrous magnesium sulphate, filtered and solvent removed under reduced pressure. The resulting oil was purified by column chromatography on silica gel (dichloromethane/2-propanol 9:1) to afford **3** as a colourless oil (2.22 g, 4.82 mmol, 74% yield).

$[\alpha]^{23}_D$: –26.7 (*c* 0.36, $CHCl_3$).

IR (KBr): ν 3280, 2959, 2241, 1716, 1266, 1030 cm^{-1}.

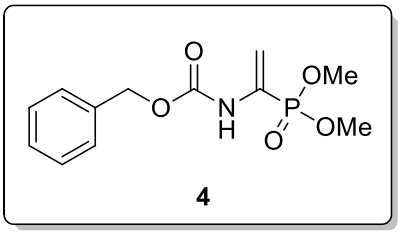
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.18 (br s, 1H, NH), 5.14–4.97 (m, 2H, N-CH-P_2+NH), 4.23–4.12 (m, 1H, $CH_{\alpha\ Leu}$), 3.84–3.75 (m, 12H, $CH_{3\ OMe}$), 1.74–1.41 (m, 3H, $CH_{2\ Leu}$+CH_{Leu}) overlapped with 1.41 (s, 9H, $CH_{3\ Boc}$), 0.95–0.88 (m, 6H, $CH_{3\ Leu}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 172.41 (CO), 155.52 (CO), 80.30 (C_{Boc}), 54.31–54.03 ($CH_{3\ OMe}$), 53.37 ($CH_{\alpha\ Leu}$), 42.68 (t, J = 147.7 Hz, N-CH-P_2), 41.05 ($CH_{2\ Leu}$), 28.34 ($CH_{3\ Boc}$), 24.81 (CH_{Leu}), 22.97 ($CH_{3\ OMe}$), 22.00 ($CH_{3\ OMe}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 18.55 ppm.

HRMS (ESI) $C_{16}H_{34}N_2NaO_9P_2$ $[M+Na]^+$: 483.1632, found 483.1624.

Synthesis of dimethyl [1-(benzyloxycarbonylamino)ethen-1-yl]phosphonate (4)



4

To a solution of **2** (395 mg, 1.04 mmol) in a mixture of tetrahydrofuran/2-propanol 4:1 (8 mL) was added cesium carbonate (422 mg, 1.29 mmol) and 37% aqueous formaldehyde solution (78 μL, 1.04 mmol). After stirring for one hour at room temperature, solvent was removed under reduced pressure, the crude suspended in dichloromethane and the solid removed by filtration. Then, solvent was removed under reduced pressure and the resulting oil was purified by column chromatography on silica gel (ethyl acetate/hexane 3:1) to afford **4** as a yellow oil (188 mg, 0.66 mmol, 63% yield).

IR (neat): ν 3238, 3034, 2955, 1732, 1237, 1030 cm^{-1}.

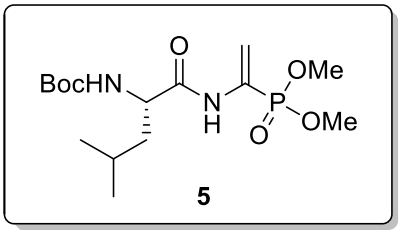
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39–7.31 (m, 5H, CH_{Ar}), 6.62 (d, 1H, J = 6.6 Hz, NH), 6.39 (d, 1H, J = 41.6 Hz, E-CH_2=C), 5.47 (d, 1H, J = 19.1 Hz, Z-CH_2=C), 5.15 (s, 2H, CH_2-Ph), 3.76 (d, 6H, J = 11.2 Hz, $CH_{3\ OMe}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 153.41 (d, J = 16.7 Hz, CO), 135.75 (C_{Ar}), 130.16 (d, J = 202.1 Hz, N-C-P), 128.78 (CH_{Ar}), 128.62 (CH_{Ar}), 128.48 (CH_{Ar}), 111.54 (d, J = 10.0 Hz, $\underline{C}H_2$=C), 67.42 (CH_2-Ph), 53.37 (d, J = 5.5 Hz, $CH_{3\ OMe}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 15.18 ppm.

HRMS (ESI) $C_{12}H_{16}NNaO_5P$ $[M+Na]^+$: 308.0658, found 308.0661.

Synthesis of dimethyl {1-[*N*-(*tert*-butoxycarbonyl)-L-leucylamido]ethen-1-yl}phosphonate (5)



5

To a solution of **3** (518 mg, 1.12 mmol) in a mixture of tetrahydrofuran/2-propanol 4:1 (10 mL) was added cesium carbonate (459 mg, 1.41 mmol) and 37% aqueous formaldehyde solution (84 μL, 1.12 mmol). After stirring two hours at room temperature, solvent was removed under reduced pressure, the crude suspended in dichloromethane and the solid removed by filtration. Then, solvent was removed under reduced pressure and the resulting oil was purified by column chromatography on silica gel (ethyl acetate/hexane 3:1) to afford **5** as a yellow oil (322 mg, 0.88 mmol, 79% yield).

$[\alpha]^{21}_{D}$: –54.83 (*c* 0.50. $CHCl_3$).

IR (neat): ν 3297, 2957, 1700, 1525, 1257, 1029 cm^{-1}.

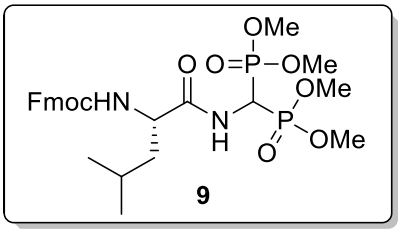
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.92 (br s, 1H, NH), 6.69 (d, 1H, *J* = 42.0 Hz, *E*-CH_2=C), 5.62 (dd, 1H, *J* = 19.3, 1.0 Hz, *Z*-CH_2=C), 4.87 (br s, 1H, NH), 4.19–4.05 (m, 1H, $CH_{\alpha\ Leu}$), 3.76 (d, 3H, *J* = 11.2 Hz, $CH_{3\ OMe}$), 3.75 (d, 3H, *J* = 11.2 Hz, $CH_{3\ OMe}$), 1.77–1.61 (m, 2H, $CH_{2\ Leu}$+CH_{Leu}), 1.53–1.45 (m, 1H, $CH_{2\ Leu}$) overlapped with 1.44 (s, 9H, $CH_{3\ Boc}$), 0.98–0.90 (m, 6H, $CH_{3\ Leu}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 172.06 (d, *J* = 9.9 Hz, CO), 155.90 (CO), 130.09 (d, *J* = 200.5 Hz, N-C-P), 115.29 (d, *J* = 9.8 Hz, $\underline{C}H_2$=C), 80.67 (C_{Boc}), 54.02 ($CH_{\alpha\ Leu}$), 53.33 (d, *J* = 5.5 Hz, $CH_{3\ OMe}$), 53.30 (d, *J* = 5.5 Hz, $CH_{3\ OMe}$), 40.65 ($CH_{2\ Leu}$), 28.36 ($CH_{3\ Boc}$), 24.92 (CH_{Leu}), 23.09 ($CH_{3\ Leu}$), 21.92 ($CH_{3\ Leu}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 15.07 ppm.

HRMS (ESI) $C_{15}H_{29}N_2NaO_6P$ $[M+Na]^+$: 387.1655, found 387.1668.

Synthesis of tetramethyl [*N*-(9-fluorenylmethoxycarbonyl)-L-leucylamidomethyl]bisphosphonate (9)



To a solution of **2** (512 mg, 1.34 mmol) in methanol (30 mL) 10% wt. palladium on carbon (75 mg) was added. The mixture was stirred under a hydrogen atmosphere at room temperature for one hour. Then, the reaction mixture was filtered (Celite) and evaporated to afford an oil which was used in the next step. A solution of previous oil and Fmoc-L-leucine (475 mg, 1.34 mmol) in dry *N*,*N*-dimethylformamide (6.7 mL) was cooled to 0 °C. *N*-Methylmorpholine (190 µL, 1.73 mmol) and 1-hydroxybenzotriazole hydrate (12% H_2O) (248 mg, 1.61 mmol) were added and the mixture was stirred for 15 minutes at 0 °C. Then *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (309 g, 1.61 mmol) was added, and the reaction mixture was stirred for 18 hours at 0 °C. Solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate (30 mL) and washed with an aqueous 5% solution of sodium bicarbonate (2 x 20 mL). The aqueous phase was extracted with ethyl acetate (3 x 15 mL) until no final product was detected by TLC in the aqueous phase. The combined organic layers were dried over anhydrous magnesium sulphate, filtered and solvent removed under reduced pressure. The resulting oil was purified by column chromatography on silica gel (dichloromethane/2-propanol 95:5) to afford **9** as a colourless oil (502 mg, 0.86 mmol, 64% yield).

$[\alpha]^{21}_D$: –19.86 (*c* 0.51, $CHCl_3$).

IR (KBr): ν 3270, 2960, 1720, 1670, 1270, 1050 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 (d, 2H, *J* = 7.6 Hz, $CH_{Ar\ Fmoc}$), 7.56 (d, 2H, *J* = 7.5 Hz, $CH_{Ar\ Fmoc}$), 7.38 (t, 2H, *J* = 7.5Hz, $CH_{Ar\ Fmoc}$), 7.29 (td, 2H, *J* = 7.5, 1.1 Hz, $CH_{Ar\ Fmoc}$), 5.42 (d, 1H,

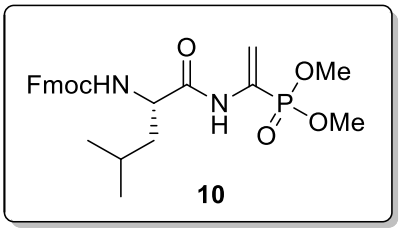
J = 8.3 Hz, NH), 5.08 (td, 1H, *J* = 21.9, 10.0 Hz, N-CH-P_2), 4.46–4.29 (m, 3H, $CH_{2\ Fmoc}$+$CH_{\alpha\ Leu}$), 4.24–4.15 (m, 1H, CH_{Fmoc}), 3.86–3.69 (m, 12H, $CH_{3\ OMe}$), 1.71–1.50 (m, 3H, $CH_{2\ Leu}$+CH_{Leu}), 0.97–0.90 (m, 6H, $CH_{3\ Leu}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 172.22 (CO), 156.12 (CO), 143.91 (C_{Ar}), 143.78 (C_{Ar}), 141.40 (C_{Ar}), 141.39 (C_{Ar}), 127.87 (CH_{Ar}), 127.19 (CH_{Ar}), 125.15 (CH_{Ar}), 125.07 (CH_{Ar}), 120.13 (CH_{Ar}), 120.11 (CH_{Ar}), 67.16 ($CH_{2\ Fmoc}$), 54.37–54.02 ($CH_{3\ OMe}$), 53.62 ($CH_{\alpha\ Leu}$), 47.23 (CH_{Fmoc}), 42.79 (t, *J* = 148.2 Hz, N-CH-P_2), 41.54 ($CH_{2\ Leu}$), 24.77 (CH_{Leu}), 23.02 ($CH_{3\ Leu}$), 22.06 ($CH_{3\ Leu}$) ppm.

^{31}P NMR (162 MHz, CDCl3): δ 18.64, 18.57 (AB spin system, 2P, *J* = 32.0 Hz)

HRMS (ESI) $C_{26}H_{36}N_2NaO_9P_2$ $[M+Na]^+$: 605.1788, found 605.1805.

Synthesis of dimethyl {1-[*N*-(9-fluorenylmethoxycarbonyl)-L-leucylamido]ethen-1-yl}phosphonate (10)



10

To a solution of **9** (582 mg, 1.00 mmol) in a mixture of tetrahydrofuran/2-propanol 4:1 (12 mL) was added cesium carbonate (407 mg, 1.25 mmol) and 37% aqueous formaldehyde solution (75 μL, 1.00 mmol). After stirring for 1 hour at room temperature, solvent was removed under reduced pressure, the crude suspended in dichloromethane and the solid removed by filtration. Then, the solvent was removed under reduced pressure and the resulting oil was purified by column chromatography on silica gel (dichloromethane/2-propanol 97:3) to afford **10** as a white solid (303 mg, 0.62 mmol, 62% yield).

$[\alpha]^{29}_{D}$: –40.7 (*c* 0.41, $CHCl_3$).

M.p.: 150–152 °C.

IR (KBr): ν 3306, 2954, 1713, 1686, 1536, 1257, 1035 cm^{-1}.

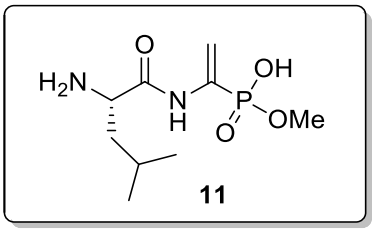
^{1}H NMR (400 MHz, $CDCl_3$): *δ* 7.90 (d, 1H, *J* = 4.8 Hz, NH), 7.75 (d, 2H, *J* = 7.5 Hz, $CH_{Ar\ Fmoc}$), 7.57 (d, 2H, *J* = 7.4 Hz, $CH_{Ar\ Fmoc}$), 7.39 (t, 2H, *J* = 7.5 Hz, $CH_{Ar\ Fmoc}$), 7.30 (t, 2H, *J* = 7.4 Hz, $CH_{Ar\ Fmoc}$), 6.70 (d, 1H, *J* = 41.8 Hz, *E*-CH_2=C), 5.63 (dd, 1H, *J* = 19.2, 0.6 Hz, *Z*-CH_2=C), 5.39 (d, 1H, *J* = 8.1 Hz, NH), 4.47–4.35 (m, 2H, $CH_{2\ Fmoc}$), 4.33–4.18 (m, 2H, CH_{Fmoc}+$CH_{\alpha\ Leu}$), 3.76–3.67 (m, 6H, $CH_{3\ OMe}$), 1.75–1.51 (m, 3H, $CH_{2\ Leu}$+CH_{Leu}), 1.00–0.88 (m, 6H, $CH_{3\ Leu}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): *δ* 171.87 (d, *J* = 10.1 Hz, CO), 156.41 (CO), 143.87 (C_{Ar}), 143.73 (C_{Ar}), 141.40 (C_{Ar}), 130.03 (d, *J* = 200.9 Hz, N-C-P), 127.88 ($CH_{Ar\ Fmoc}$), 127.23 ($CH_{Ar\ Fmoc}$), 125.14 ($CH_{Ar\ Fmoc}$), 125.12 ($CH_{Ar\ Fmoc}$), 120.14 ($CH_{Ar\ Fmoc}$), 120.12 ($CH_{Ar\ Fmoc}$), 115.71 (d, *J* = 9.9 Hz, $\underline{C}H_2$=C), 67.33 ($CH_{2\ Fmoc}$), 54.35 ($CH_{\alpha\ Leu}$), 53.40 (d, *J* = 5.3 Hz, $CH_{3\ OMe}$), 47.23 (CH_{Fmoc}), 41.24 ($CH_{2\ Leu}$), 24.85 (CH_{Leu}), 23.09 ($CH_{3\ Leu}$), 21.98 ($CH_{3\ Leu}$) ppm

^{31}P NMR (162 MHz, $CDCl_3$): δ 14.77 ppm.

HRMS (ESI) $C_{25}H_{31}N_2NaO_6P$ $[M+Na]^+$: 509.1812, found 509.1814.

Synthesis of [1-(L-leucylamido)ethen-1-yl]phosphonic acid monomethyl ester (11)



11

To a solution of **10** (129 mg, 0.27 mmol) in a mixture of acetone/toluene 1:1 (5 mL) 1,4-diazabicyclo[2.2.2]octane (74 mg, 0.55 mmol) was added. The reaction mixture was stirred at reflux for 5 hours. Solvent was removed, and the resulting residue was purified using an acidic ion-exchange resin (Dowex® 50WX8, hydrogen form) to afford **11** as a white solid (61 mg, 0.24 mmol, 89% yield).

$[\alpha]^{24}_{D}$: +33.3 (*c* 0.41, MeOH).

M.p.: 148–150 °C.

IR (KBr): ν 2959, 2360, 1684, 1540, 1207, 1049 cm^{-1}.

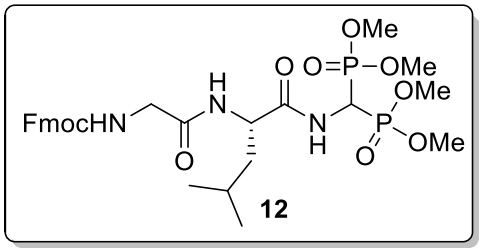
^{1}H NMR (400 MHz, MeOD): δ 6.37 (d, 1H, *J* = 35.2 Hz, *E*-CH_2=C), 5.57 (d, 1H, *J* = 16.4 Hz, *Z*-CH_2=C), 4.04–3.97 (m, 1H, $CH_{\alpha\ Leu}$), 3.50 (d, 3H, *J* = 11.0 Hz, $CH_{3\ OMe}$), 1.83–1.65 (m, 3H, $CH_{2\ Leu}$+CH_{Leu}), 1.04–0.98 (m, 6H, $CH_{3\ Leu}$) ppm.

^{13}C NMR (100 MHz, MeOD): δ 169.71 (d, *J* = 9.7 Hz, CO), 137.80 (d, *J* = 187.1 Hz, N-C-P), 113.49 (d, *J* = 9.5, $\underline{C}H_2$=C), 53.68 ($CH_{\alpha\ Leu}$), 52.34 (d, *J* = 5.3 Hz, $CH_{3\ OMe}$), 41.53 ($CH_{2\ Leu}$), 25.51 (CH_{Leu}), 23.08 ($CH_{3\ Leu}$), 22.30 ($CH_{3\ Leu}$) ppm.

^{31}P NMR (162 MHz, MeOD): δ 7.99 ppm.

HRMS (ESI) $C_9H_{19}N_2NaO_4P$ $[M+Na]^+$: 273.0975, found 273.0976.

Synthesis of tetramethyl [*N*-(9-fluorenylmethoxycarbonyl)glycyl-L-leucylamidomethyl]bisphosphonate (12)



A solution of **3** (1.44 g, 3.13 mmol) in dichloromethane (60 mL) was cooled to 0 °C, trifluoroacetic acid (4.84 mL, 62.68 mmol) was added and then the solution stirred overnight at room temperature. Subsequently, solvent was removed under reduced pressure and the residue lyophilized to afford a colourless oil which was used without further purification. A solution of previous oil and Fmoc-glycine (1.02 g, 3.43 mmol) in dry *N*,*N*-dimethylformamide (16 mL) was cooled to 0 °C. *N*-Methylmorpholine (0.86 mL, 7.82 mmol) and 1-hydroxybenzotriazole hydrate (12% H_2O) (577 mg, 3.76 mmol) were added and the mixture was stirred for 15 minutes at 0 °C. Then *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (720 mg, 3.76 mmol) was added, the reaction mixture was stirred for 1 hour at 0 °C and then for 14 hours at 4 °C. Solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate (30 mL) and washed with an aqueous 5% solution of sodium bicarbonate. The aqueous phase was extracted with ethyl acetate (3 x 15 mL) until no final product was detected by TLC in the aqueous phase. The combined organic layers were dried over anhydrous magnesium sulphate, filtered and solvent removed under reduced pressure. The resulting oil was purified by column chromatography on silica gel (dichloromethane/diethyl ether/methanol 85:10:5) to afford **12** as a colourless oil (1.76 g, 2.75 mmol, 88% yield).

$[\alpha]^{25}_{D}$: –22.7 (*c* 0.41, $CHCl_3$).

IR (KBr): ν 3280, 2957, 1679, 1538, 1264, 1086 cm^{-1}.

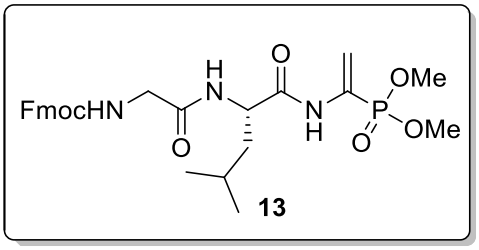
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.75 (d, 2H, J = 7.5 Hz, $CH_{Ar\ Fmoc}$), 7.65–7.56 (m, 3H, $CH_{Ar\ Fmoc}$+NH), 7.39 (t, 2H, J = 7.4 Hz, $CH_{Ar\ Fmoc}$), 7.30 (tt, 2H, J = 7.5, 1.0 Hz, $CH_{Ar\ Fmoc}$), 6.77 (d, 1H, J = 8.0 Hz, NH), 6.09–6.02 (m, 1H, NH), 5.07 (td, 1H, J = 22.0, 10.0 Hz, N-CH-P_2), 4.75–4.65 (m, 1H, $CH_{\alpha\ Leu}$), 4.45–4.35 (m, 2H, $CH_{2\ Fmoc}$), 4.21 (t, 1H, J = 7.0 Hz, CH_{Fmoc}), 4.00–3.84 (m, 2H, $CH_{2\ Gly}$), 3.84–3.72 (m, 12H, $CH_{3\ OMe}$), 1.73–1.50 (m, 3H, $CH_{2\ Leu}$+CH_{Leu}), 0.96–0.83 (m, 6H, $CH_{3\ Leu}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 171.96 (CO), 169.74 (CO), 156.95 (CO), 143.90 (C_{Ar}), 143.86 (C_{Ar}), 141.42 (C_{Ar}), 127.90 ($CH_{Ar\ Fmoc}$), 127.2 ($CH_{Ar\ Fmoc}$), 125.19 ($CH_{Ar\ Fmoc}$), 120.13 ($CH_{Ar\ Fmoc}$), 67.36 ($CH_{2\ Fmoc}$), 54.44–54.08 ($CH_{3\ OMe}$), 51.82 ($CH_{\alpha\ Leu}$), 47.22 (CH_{Fmoc}), 44.74 ($CH_{2\ Gly}$), 42.84 (t, J = 148.3 Hz, N-CH-P_2), 41.18 ($CH_{2\ Leu}$), 24.87 (CH_{Leu}), 22.89 ($CH_{3\ Leu}$), 22.27 ($CH_{3\ Leu}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 18.60 ppm.

HRMS (ESI) $C_{28}H_{39}N_3NaO_{10}P_2$ $[M+Na]^+$: 662.2003, found 662.2016.

Synthesis of dimethyl {1-[*N*-(9-fluorenylmethoxycarbonyl)glycyl-L-leucylamido]ethen-1-yl}phosphonate (13)



13

To a solution of **12** (344 mg, 0.54 mmol) in a mixture of tetrahydrofuran/2-propanol 4:1 (7 mL) was added cesium carbonate (219 mg, 0.67 mmol) and 37% aqueous formaldehyde solution (43 μL, 0.57 mmol). After stirring at room temperature for 1 hour, the solvent was removed under reduced pressure, the crude suspended in dichloromethane and the solid removed by filtration. Solvent was removed under reduced pressure and the resulting oil was purified by column chromatography on silica gel (ethyl acetate/hexane/2-propanol 7:2:1) to afford **13** as a yellow oil (168 mg, 0.31 mmol, 57% yield).

$[\alpha]^{27}_{D}$: –36.2 (*c* 0.40, $CHCl_3$).

M.p.: 63–65 °C.

IR (KBr): ν 3281, 3064, 2955, 1700, 1669, 1539, 1258, 1032 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03 (d, 1H, *J* = 7.2 Hz, NH), 7.76 (d, 2H, *J* = 7.5 Hz, $CH_{Ar\ Fmoc}$), 7.59 (d, 2H, *J* = 7.4 Hz, $CH_{Ar\ Fmoc}$), 7.39 (t, 2H, *J* = 7.5 Hz, $CH_{Ar\ Fmoc}$), 7.30 (tt, 2H, *J* = 7.5, 1.0 Hz, $CH_{Ar\ Fmoc}$), 6.66 (d, 1H, *J* = 7.9 Hz, NH) overlapped with 6.65 (d, 1H, *J* = 41.7 Hz, *E*-CH_2=C), 5.77 (t, 1H, *J* = 5.6 Hz, NH), 5.61 (d, 1H, *J* = 19.0 Hz, *Z*-CH_2=C), 4.62–4.54 (m, 1H, $CH_{\alpha\ Leu}$), 4.41 (d, 2H, *J* = 6.9 Hz, $CH_{2\ Fmoc}$), 4.21 (t, 1H, *J* = 7.0 Hz, CH_{Fmoc}), 3.95–3.90 (m, 2H, $CH_{2\ Gly}$), 3.77–3.70 (m, 6H, $CH_{3\ OMe}$), 1.74–1.51 (m, 3H, $CH_{2\ Leu}$+CH_{Leu}), 0.94–0.87 (m, 6H, $CH_{3\ Leu}$) ppm.

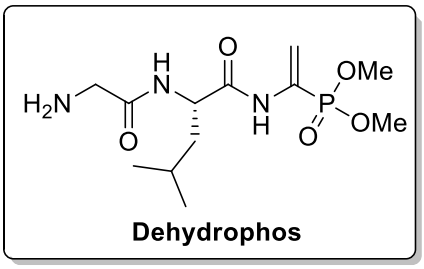
^{13}C NMR (100 MHz, $CDCl_3$): δ 171.84 (d, *J* = 10.2 Hz, CO), 169.77 (CO), 156.88 (CO), 143.83 (C_{Ar}), 141.38 (C_{Ar}), 130.21 (d, *J* = 201.3 Hz, N-C-P), 127.87 ($CH_{Ar\ Fmoc}$), 127.19 ($CH_{Ar\ Fmoc}$), 125.17 ($CH_{Ar\ Fmoc}$), 120.11 ($CH_{Ar\ Fmoc}$), 115.87 (d, *J* = 9.9 Hz, $\underline{C}H_2$=C), 67.39 ($CH_{2\ Fmoc}$),

53.52 (d, *J* = 5.6 Hz, $CH_{3\ OMe}$), 53.49 (d, *J* = 5.6 Hz, $CH_{3\ OMe}$), 52.62 ($CH_{\alpha\ Leu}$), 47.17 (CH_{Fmoc}), 44.46 ($CH_{2\ Gly}$), 40.93 ($CH_{2\ Leu}$), 24.90 (CH_{Leu}), 23.98 ($CH_{3\ Leu}$), 22.03 ($CH_{3\ Leu}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 14.65 ppm.

HRMS (ESI) $C_{27}H_{34}N_3NaO_7P$ $[M+Na]^+$: 566.2027, found 566.2025.

Synthesis of dehydrophos



Dehydrophos

To a solution of **13** (146 mg, 0.27 mmol) in a mixture of acetone/toluene 1:1 (5 mL) 1,4-diazabicyclo[2.2.2]octane (76 mg, 0.68 mmol) was added. The reaction mixture was stirred at reflux for 2 hours. Solvent was removed, the crude dissolved in water (10 mL) and filtered through 0.22 μm HPLC filter. Then, the aqueous solution was washed with dichloromethane (2 x 5 mL) and ethyl acetate (2 x 5 mL) and concentrated in vacuo. The resulting residue was purified using a weakly acidic ion-exchange resin (Amberlite® CG50, hydrogen form) to afford dehydrophos as a pale yellow solid (76 mg, 0.25 mmol, 93% yield).

$[\alpha]^{23}_{D}$: –46.5 (*c* 0.39, H_2O).

M.p.: 137–139 °C (dec).

IR (KBr): ν 3290, 3046, 2949, 1688, 1665, 1531, 1246, 1072 cm^{-1}.

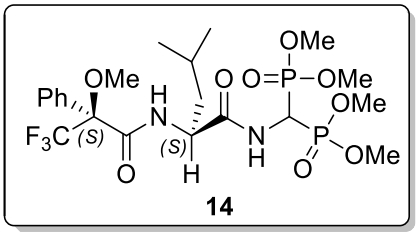
^{1}H NMR (400 MHz, D_2O): *δ* 6.20 (d, 1H, *J* = 36.3 Hz, *E*-CH_2=C), 5.71 (d, 1H, *J* = 15.9 Hz, *Z*-CH_2=C), 4.50–4.42 (m, 1H, $CH_{\alpha\ Leu}$), 3.90 (s, 2H, $CH_{2\ Gly}$), 3.52 (d, 3H, *J* = 11.0 Hz, $CH_{3\ OMe}$), 1.74–1.63 (m, 3H, $CH_{2\ Leu}$+CH_{Leu}), 0.97 (d, 3H, *J* = 6.1 Hz, $CH_{3\ Leu}$), 0.92 (d, 3H, *J* = 6.1 Hz, $CH_{3\ Leu}$) ppm.

^{13}C NMR (100 MHz, D_2O): *δ* 173.57 (d, *J* = 7.6 Hz, CO), 167.11 (CO), 134.53 (d, *J* = 189.9 Hz, N-C-P), 117.07 (d, *J* = 11.7 Hz, $\underline{C}H_2$=C), 53.35 ($CH_{\alpha\ Leu}$), 52.02 (d, *J* = 5.2 Hz, $CH_{3\ OMe}$), 40.28 ($CH_{2\ Gly}$), 39.65 ($CH_{2\ Leu}$), 24.32 (CH_{Leu}), 22.06 ($CH_{3\ Leu}$), 20.67 ($CH_{3\ Leu}$) ppm.

^{31}P NMR (162 MHz, D_2O): *δ* 9.56 ppm.

HRMS (ESI) $C_{11}H_{22}N_3NaO_5P$ $[M+Na]^+$: 330.1189, found 330.1179.

Synthesis of tetramethyl {*N*-[(*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl]-L-leucylamidomethyl}bisphosphonate (14)



14

A solution of **3** (290 mg, 0.63 mmol) in dichloromethane (12 mL) was cooled to 0 °C and trifluoroacetic acid (0.96 mL, 12.44 mmol) was added. The mixture was stirred overnight at room temperature. Then, solvent was removed under reduced pressure and the resulting residue lyophilized. The resulting oil was dissolved in dry dichloromethane (10 mL), *N*,*N*-diisopropylethylamine (0.27 mL, 1.55 mmol) and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (0.14 mL, 0.75 mmol) were added. The mixture was stirred at room temperature under an argon atmosphere for 21 hours. Solvent was removed under reduced pressure and the resulting oil was purified by column chromatography on silica gel (dichloromethane/2-propanol 93:7) to afford **14** as a colourless oil (225 mg, 0.39 mmol, 62% yield).

$[\alpha]^{25}_{D}$: –37.2 (*c* 0.41, $CHCl_3$).

IR (KBr): ν 3245, 2959, 1693, 1678, 1666, 1513, 1263, 1165, 1035 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52–7.46 (m, 2H, CH_{Ar}), 7.42–7.36 (m, 4H, CH_{Ar}+NH), 7.20 (d, 1H, *J* = 8.5 Hz, NH), 5.06 (td, 1H, *J* = 22.0, 9.9 Hz, N-CH-P_2), 4.63 (td, 1H, *J* = 8.7, 6.0 Hz, $CH_{\alpha\ Leu}$), 3.86–3.76 (m, 12H, $CH_{3\ POMe}$), 3.44–3.42 (m, 3H, $CH_{3\ OMe}$), 1.70–1.44 (m, 3H, $CH_{2\ Leu}$+CH_{Leu}), 0.89 (d, 3H, *J* = 6.5 Hz, $CH_{3\ Leu}$) overlapped with 0.88 (d, 3H, *J* = 6.4 Hz, $CH_{3\ Leu}$) ppm.

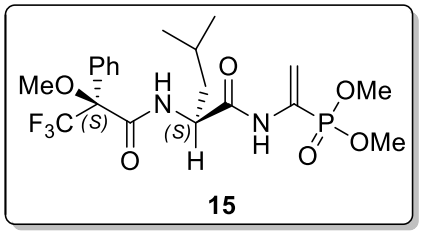
^{13}C NMR (100 MHz, $CDCl_3$): δ 171.24 (t, *J* = 4.1 Hz, CO), 166.49 (CO), 132.51 (C_{Ar}), 129.72 (CH_{Ar}), 128.71 (CH_{Ar}), 127.54 (CH_{Ar}), 123.78 (q, *J* = 290.0 Hz, CF_3), 84.09 (q, *J* = 26.3 Hz, C_S), 55.26–55.14 (m, $CH_{3\ OMe}$), 54.30 (d, *J* = 5.7 Hz, $CH_{3\ POMe}$), 54.17 (d, *J* = 5.5 Hz, $CH_{3\ POMe}$),

54.15 (d, *J* = 6.1 Hz, $CH_{3\ POMe}$), 54.05 (d, *J* = 6.0 Hz, $CH_{3\ POMe}$), 51.63 ($CH_{\alpha\ Leu}$), 42.83 (t, *J* = 148.0 Hz, N-CH-P_2), 41.11 ($CH_{2\ Leu}$), 24.77 (CH_{Leu}), 22.85 ($CH_{3\ Leu}$), 21.98 ($CH_{3\ Leu}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 18.42, 18.18 (AB spin system, 2P, *J* = 30.6 Hz) ppm.

HRMS (ESI) $C_{21}H_{33}F_3N_2NaO_9P_2$ $[M+Na]^+$: 599.1506, found 599.1504.

Synthesis of dimethyl {1-{*N*-[(*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl]-L-leucylamido}ethen-1-yl}phosphonate (15)



15

To a solution of **14** (152 mg, 0.26 mmol) in a mixture of tetrahydrofuran/2-propanol 4:1 (5 mL) cesium carbonate (108 mg, 0.33 mmol) and 37% aqueous formaldehyde solution (20 μL, 0.26 mmol) were added. After stirring for 3 hours at room temperature, solvent was removed under reduced pressure, the crude suspended in dichloromethane and the solid removed by filtration. Then, the solvent was removed under reduced pressure and the resulting oil was purified by column chromatography on silica gel (dichloromethane/2-propanol 95:5) to afford **15** as a white solid (104 mg, 0.22 mmol, 85% yield).

$[\alpha]^{29}_{D}$: –66.5 (*c* 0.42, $CHCl_3$).

IR (KBr): ν 1682, 1537, 1248, 1169, 1045 cm^{-1}.

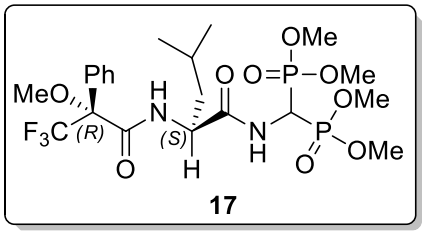
^{1}H NMR (400 MHz, $CDCl_3$): *δ* 7.87 (d, 1H, *J* = 6.7 Hz, NH), 7.54–7.47 (m, 2H, CH_{Ar}), 7.43–7.37 (m, 3H, CH_{Ar}), 7.15 (d, 1H, *J* = 8.2 Hz, NH), 6.67 (d, 1H, *J* = 41.8 Hz, *E*-CH_2=C), 5.68 (d, 1H, *J* = 19.3 Hz, *Z*-CH_2=C), 4.54 (td, 1H, *J* = 9.0, 5.5 Hz, $CH_{\alpha\ Leu}$), 3.80–3.71 (m, 6H, $CH_{3\ POMe}$), 3.47–3.44 (m, 3H, $CH_{3\ OMe}$), 1.76–1.56 (m, 2H, $CH_{2\ Leu}$), 1.55–1.42 (m, 1H, CH_{Leu}), 0.93–0.84 (m, 6H, $CH_{3\ Leu}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): *δ* 170.67 (d, *J* = 10.3 Hz, CO), 167.13 (CO), 132.41 (C_{Ar}), 130.21 (d, *J* = 201.6 Hz, N-C-P), 129.81 (CH_{Ar}), 128.75 (CH_{Ar}), 127.51 (CH_{Ar}), 123.73 (q, *J* = 290.1 Hz, CF_3), 116.04 (d, *J* = 10.1 Hz, $\underline{C}H_2$=C), 84.10 (q, *J* = 26.5 Hz, C_S), 55.38–55.25 ($CH_{3\ OMe}$), 53.35 (d, *J* = 5.6 Hz, $CH_{3\ POMe}$), 53.33 (d, *J* = 5.6 Hz, $CH_{3\ POMe}$), 52.42 (d, *J* = 1.6 Hz, $CH_{\alpha\ Leu}$), 40.36 ($CH_{2\ Leu}$), 24.80 (CH_{Leu}), 23.04 ($CH_{3\ Leu}$), 21.77 ($CH_{3\ Leu}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 14.37 ppm.

HRMS (ESI) $C_{20}H_{28}F_3N_2NaO_6P$ $[M+Na]^+$: 503.1529, found 503.1550.

Synthesis of tetramethyl {*N*-[(*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl]-L-leucylamidomethyl}bisphosphonate (17)



17

A solution of **3** (295 mg, 0.64 mmol) in dichloromethane (13 mL) was cooled to 0 °C and trifluoroacetic acid (0.98 mL, 12.70 mmol) was added. The mixture was stirred overnight at room temperature. Then, solvent was removed under reduced pressure and the resulting residue lyophilized. The resulting oil was dissolved in dry dichloromethane (10 mL), *N*,*N*-diisopropylethylamine (0.28 mL, 1.61 mmol) and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (0.14 mL, 0.75 mmol) were added. The mixture was stirred at room temperature under an argon atmosphere for 22 hours. Solvent was removed under reduced pressure and the resulting oil was purified by column chromatography on silica gel (dichloromethane/2-propanol 9:1) to afford **17** as a colourless oil (272 mg, 0.47 mmol, 73% yield).

$[\alpha]^{25}_{D}$: –23.6 (*c* 0.42, CHCl3).

IR (KBr): ν 3412, 3245, 2959, 2360, 1684, 1507, 1266, 1163, 1037 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50–7.45 (m, 2H, CH_{Ar}), 7.40–7.32 (m, 5H, CH_{Ar} +NH+NH), 5.05 (td, 1H, *J* = 22.1, 9.9 Hz, N-CH-P_2), 4.64–4.57 (m, 1H, $CH_{\alpha\ Leu}$), 3.84–3.72 (m, 9H, $CH_{3\ POMe}$), 3.61 (d, 3H, *J* = 11.0 Hz, $CH_{3\ POMe}$), 3.34–3.31 (m, 3H, $CH_{3\ OMe}$), 1.74–1.61 (m, 3H, $CH_{2\ Leu}$+CH_{Leu}), 1.00–0.89 (m, 6H, $CH_{3\ Leu}$) ppm.

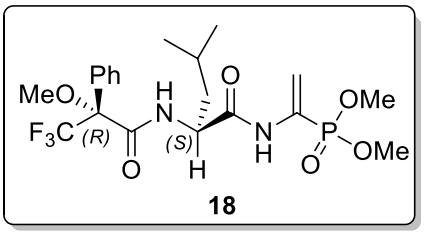
^{13}C NMR (100 MHz, $CDCl_3$): δ 171.49 (t, *J* = 4.1 Hz, CO), 166.41 (CO), 132.05 (C_{Ar}), 129.58 (CH_{Ar}), 128.74 (CH_{Ar}), 128.07 (CH_{Ar}), 123.94 (q, *J* = 290.2 Hz, CF_3), 84.14 (q, *J* = 26.4 Hz, C_R), 55.01–54.90 (m, $CH_{3\ OMe}$), 54.25 (d, *J* = 6.0 Hz, $CH_{3\ POMe}$), 54.21 (d, *J* = 6.5 Hz, $CH_{3\ POMe}$),

54.14 (d, *J* = 6.1 Hz, $CH_{3\ POMe}$), 54.07 (d, *J* = 6.4 Hz, $CH_{3\ POMe}$), 51.82 ($CH_{\alpha\ Leu}$), 42.79 (t, *J* = 147.9 Hz, N-CH-P_2), 41.23 ($CH_{2\ Leu}$), 24.77 (CH_{Leu}), 22.94 ($CH_{3\ Leu}$), 21.93 ($CH_{3\ Leu}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): *δ* 18.48, 18.15 (AB spin system, 2P, *J* = 30.5 Hz) ppm.

HRMS (ESI) $C_{21}H_{33}F_3N_2NaO_9P_2$ $[M+Na]^+$: 599.1506, found 599.1492.

Synthesis of dimethyl {1-{*N*-[(*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl]-L-leucylamido}ethen-1-yl}phosphonate (18)



18

To a solution of **17** (272 mg, 0.47 mmol) in a mixture of tetrahydrofuran/2-propanol 4:1 (6 mL) cesium carbonate (191 mg, 0.59 mmol) and 37% aqueous formaldehyde solution (35 μL, 0.47 mmol) were added. After stirring at room temperature for 7 hours, the solvent was removed under reduced pressure, the crude suspended in dichloromethane and the solid removed by filtration. Solvent was removed under reduced pressure and the resulting oil was purified by column chromatography on silica gel (dichloromethane/2-propanol 95:5) to afford **18** as a white solid (168 mg, 0.35 mmol, 74% yield).

$[\alpha]^{28}_{D}$: –69.82 (*c* 0.33, $CHCl_3$).

IR (neat): ν 2959, 1684, 1507, 1266, 1037 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): *δ* 7.83 (d, 1H, *J* = 6.9 Hz, NH), 7.51–7.44 (m, 2H, CH_{Ar}), 7.42–7.37 (m, 3H, CH_{Ar}), 7.34 (d, 1H, *J* = 8.0 Hz, NH), 6.65 (d, 1H, *J* = 41.8 Hz, *E*-CH_2=C), 5.65 (d, 1H, *J* = 19.3 Hz, *Z*-CH_2=C), 4.52 (td, 1H, *J* = 8.5, 5.5 Hz, $CH_{\alpha\ Leu}$), 3.70 (d, 3H, *J* = 11.2 Hz, $CH_{3\ POMe}$), 3.61 (d, 3H, *J* = 11.1 Hz, $CH_{3\ POMe}$), 3.36–3.32 (m, 3H, $CH_{3\ OMe}$), 1.80–1.62 (m, 3H, $CH_{2\ Leu}$+CH_{Leu}), 0.98 (d, 3H, *J* = 6.1 Hz, $CH_{3\ Leu}$), 0.94 (d, 3H, *J* = 6.2 Hz, $CH_{3\ Leu}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): *δ* 170.67 (d, *J* = 10.4 Hz, CO), 167.14 (CO), 131.85 (CH_{Ar}), 130.28 (d, *J* = 201.5 Hz, N-C-P), 129.72 (CH_{Ar}), 128.87 (CH_{Ar}), 128.06 (d, *J* = 1.4 Hz, CH_{Ar}), 123.91 (q, *J* = 290.2 Hz, CF_3), 115.78 (d, *J* = 10.1 Hz, $\underline{C}H_2$=C), 84.22 (q, *J* = 26.5 Hz, C_R), 55.08–54.96 ($CH_{3\ OMe}$), 53.35 (d, *J* = 5.6 Hz, $CH_{3\ POMe}$), 53.28 (d, *J* = 5.7 Hz, $CH_{3\ POMe}$), 52.66 (d, *J* = 1.6 Hz, $CH_{\alpha\ Leu}$), 40.29 ($CH_{2\ Leu}$), 24.81 (CH_{Leu}), 23.09 ($CH_{3\ Leu}$), 21.81 ($CH_{3\ Leu}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 14.33 ppm.

HRMS (ESI) $C_{20}H_{28}F_3N_2NaO_6P$ $[M+Na]^+$: 503.1529, found 503.1523.

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Chapter 2

Synthesis of dehydrophos derivatives and biological studies

Introduction.

Antibiotic dehydrophos is a tripeptide consisting in a glycine-leucine dipeptide linked to an *O*-methylated aminovinylphosphonate.[1] It is a unique structure as it is unusual for a dehydroaminophosphonic acid to be a part of a natural product and, consequently, thorough studies on its biosynthetic pathway have been reported.[2]

The coupling of α-aminophosphonic acids to amino acids or peptide chains is usually found in bioactive products.[3–5] In some instances, the whole peptide structure is responsible for its biological activity. That is the case of K-26, the first natural peptide which was discovered to contain a phosphorous moiety,[6–8] where the entire peptide binds to the enzyme.[9,10] Contrary, in other phosphonopeptides as alafosfalin, the presence of an amino acid or peptide in the molecule enables the compound to be transported through bacterial cell membrane by means of peptide permeases. Once inside the cell, enzymatic cleavage of the peptide bond releases the phosphorous analogue of alanine, which was discovered to be a powerful inhibitor of alanine racemase. Moreover, phosphoalanine was unable to permeate the cell membrane and resulted non-toxic to bacteria.[11] However, when an amino acid or peptide was added to the α-aminophosphonic acid, a series of compounds with high antibacterial activities were obtained.[12,13] This mechanism is known as "Trojan horse" mechanism.[14-16]

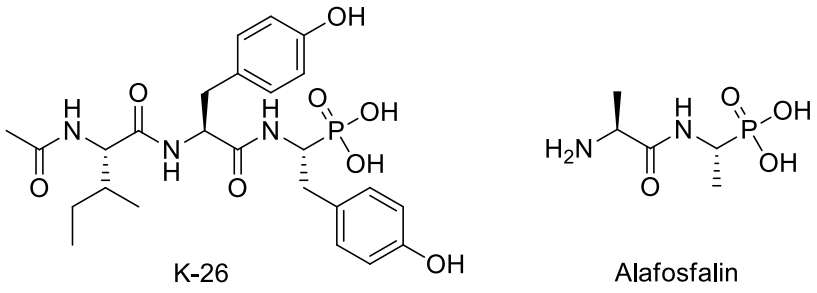


Figure 1. K-26 and alafosfalin structures.

Kuemin and van der Donk provided a first insight into dehydrophos mode of action by means of an structure-activity relationships study involving dehydrophos and synthetic

analogues where the vinyl and/or the phosphonic acid monomethyl ester residues were modified (Figure 2).[17] These studies showed the importance of the *O*-methyl phosphonate and vinyl moieties for the biological activity, as they were critical in preventing bacterial growth, but no conclusions were obtained regarding whether peptide cleavage was required for bioactivity.

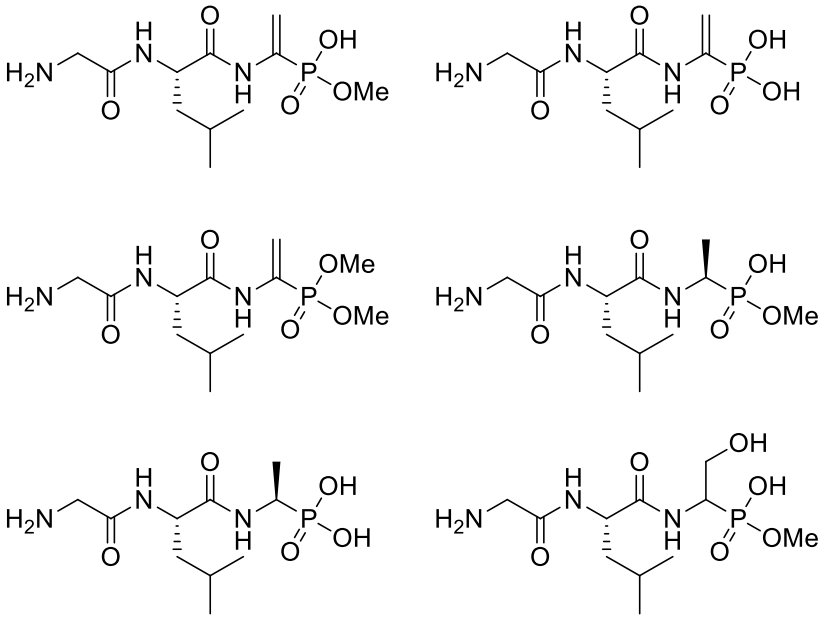


Figure 2. Compounds studied by Kuemin and van der Donk.

Afterwards, Circello et al. proved that dehydrophos mode of action involves a "Trojan horse" mechanism (Figure 3).[18] They demonstrated that the entire molecule is transported into the cell. Then, cleavage of peptide bonds releases 1-aminovinylphosphonate *O*-monomethyl ester, which rearranges to the preferred imine. This imine is hydrolyzed to yield methyl acetylphosphonate, a pyruvate mimic that inhibits pyruvate oxidase, pyruvate dehydrogenase and other thiamine diphosphate-dependent enzymes where pyruvate is involved.[19–23]

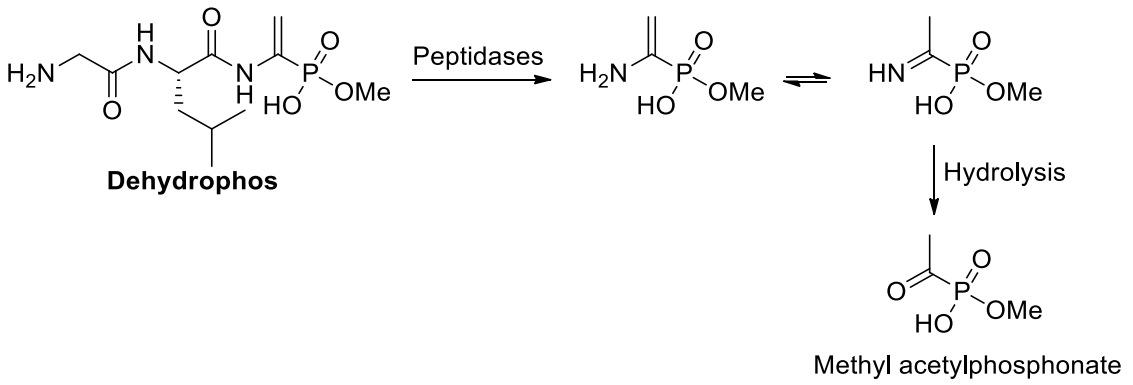


Figure 3. Mode of action of dehydrophos.

It should be noted that pyruvate and other 2-oxo acids are natural substrates of thiamine diphosphate-dependent enzymes, a diverse family of proteins which participate in many metabolic processes, mainly in C-C bond formation/scission reactions. Within this context, acylphosphonic acid derivatives, the phosphorus analogues of the former, act as inhibitors of these enzymes, therefore finding application as herbicides, antibacterial and antifungal compounds (Figure 4).[24] The biological activity of these compounds depends on the substitution in both the acyl moiety and the phosphonic acid group, which causes changes in specificity and reversible or irreversible inhibition.[25,26] In addition, acylphosphonates tend to exhibit more activity in vitro than in vivo, since their polarity impedes an efficient transport through bacterial cell wall.[27]

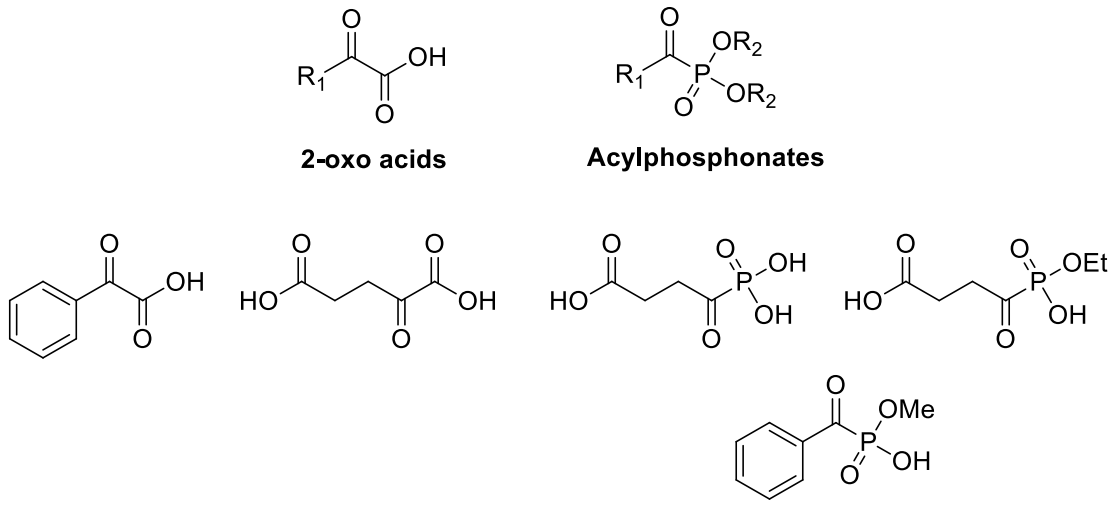


Figure 4. Examples of 2-oxo acids and their bioactive phosphorus analogues.

Within this context, dehydrophos peptide chain is the responsible for making a potential bioactive acylphosphonate pass through the cell wall. Therefore, the introduction of structural variations in the vinyl moiety or the phosphonic acid group of the peptide dehydrophos would lead to the release of different acylphosphonate derivatives into the cell, thus changing the biological activity of the native phosphonopeptide. In addition, changes in the peptide chain would affect both the transport of the molecule through bacterial cell wall and the enzymatic hydrolysis of the peptide bond to release the acylphosphonate derivative and, as a result, the specificity and bioactivity of dehydrophos.

Herein, we exploit the synthetic procedure described in chapter 1 for the synthesis of dehydrophos derivatives. Thus, the introduction of substituents in the vinyl moiety can be achieved by the selection of an appropriate aldehyde in the Horner-Wadsworth-Emmons reaction, whereas the structural variations in the phosphonic acid groups relies in the selection of a suitable aminomethylbisphosphonic acid derivative. Finally, the peptidic sequence can be changed during the peptide coupling (Figure 5). Furthermore, dehydrophos derivatives synthesized in this chapter have been tested against several bacterial strains for testing the effects of structural variations on biological activity.

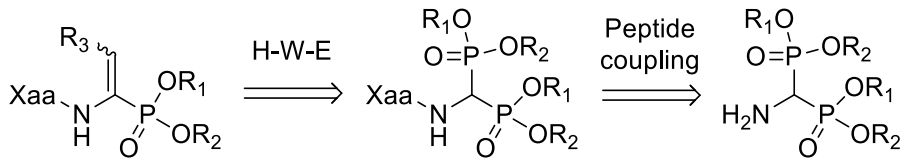


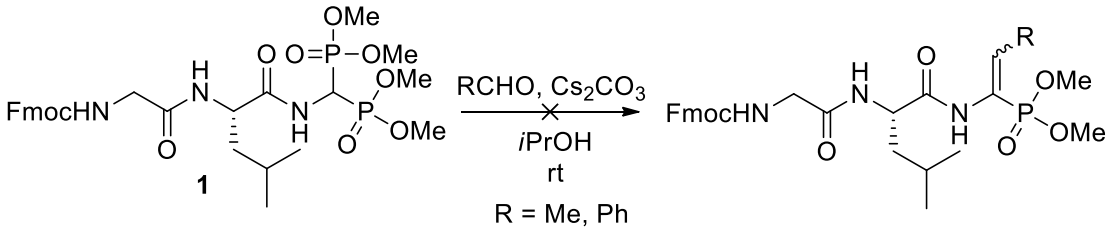
Figure 5. Retrosynthetic analysis for the synthesis of dehydrophos derivatives.

It is worth noting that the study described in this work substantially differs from that described before, mainly focused on the elucidation of dehydrophos mode of action.[17]

Results and discussion.

According to the above exposed we first addressed the introduction of a substituent in the vinyl moiety by means of the Horner-Wadsworth-Emmons olefination. This transformation was carried out under the smooth conditions described in chapter 1, using cesium carbonate as the base and changing the aldehyde.

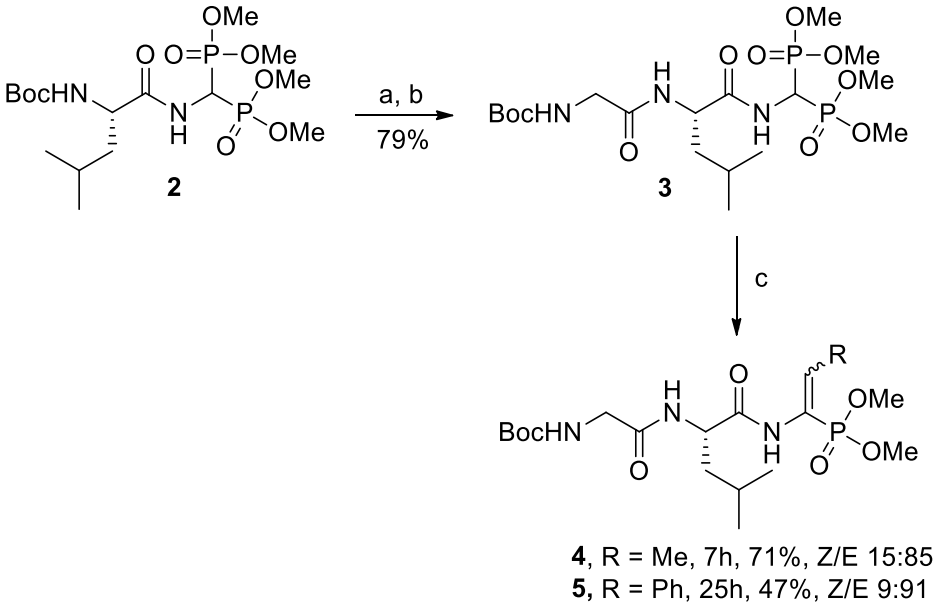
Apart from the generation of the vinyl group under mild conditions, the main point of the synthetic procedure described in chapter 1 is the simultaneous deprotection of the amine and dimethyl phosphonate groups through treatment with Dabco®. Thus, the Fmoc protected peptidyl bisphosphonate **1**, prepared in previous chapter, was used as starting material for the Horner-Wadsworth-Emmons reaction. Two different aldehydes were used this time: acetaldehyde, in order to introduce the simplest alkyl side chain, and benzaldehyde, to introduce an aryl chain. However, the use of more hindered aldehydes in comparison with formaldehyde led to longer reaction times. When the reaction crudes were analysed by ^{1}H and ^{31}P NMR, we found many side compounds and barely the anticipated product (Scheme 1). The Fmoc protecting group is moderately labile in basic media; in addition, bulkier aldehydes increase reaction times. These two facts may lead to the deprotection of the amino group, causing a drop in the yield.



Scheme 1. First attempts of olefination using acetaldehyde and benzaldehyde.

To overcome this problem, we decided to use Boc instead of Fmoc as the nitrogen protecting group, as it is stable under basic conditions and we had experience in its deprotection inside a phosphonopeptide. However, as it will be discussed below, the deprotection sequence of the nitrogen and the dimethyl phosphonate group should be

performed in such a way as to avoid decomposition. Thus, we coupled Boc-Gly-OH to bisphosphonate **2**, which was obtained as previously described in chapter 1, to obtain bisphosphonate **3**. Then, the Horner-Wadsworth-Emmons olefination was performed as described before, using the above mentioned aldehydes (Scheme 2), to afford derivatives **4** and **5** in mild yields.



Scheme 2. Reagents and conditions: (a) TFAA, CH_2Cl_2, room temp., overnight; (b) Boc-Gly-OH, NMM, EDC, HOBt, DMF, 0 °C, 16 h; (c) 37% HCHO(aq.), Cs_2CO_3, *i*PrOH 4:1, room temp.

The olefination with acetaldehyde and benzaldehyde proceeded with the formation of the *Z* and *E* stereoisomers. In both cases, the isomers were isolated by column chromatography on silica gel. Double bond configuration was assigned on the basis of the values of vinyl proton-phosphorous ($^3J_{HP}$) and vicinal carbon-phosphorous ($^3J_{CP}$) coupling constants. Thus, according to literature,[28,29] the value of vicinal coupling constants ($^3J_{HP}$) for the *E*-isomers are in the range of 14-24 Hz, while for the *Z*-isomers the value is larger and vary between 39-44 Hz. When it comes to $^3J_{CP}$ coupling constants, the relationship is inversed and for *Z*-isomers they are smaller, with values between 5-7 Hz, than the corresponding for *E*-isomers, that ranges form 14-22 Hz.[30] As shown in table 1, the experimental data for compounds **4** and **5** are in agreement to the described before.

Additionally, the relationship between the *Z* and *E* isomers of **4** and **5** was determined by direct integration of the corresponding signals in the ^{31}P NMR spectra of the reaction crudes.

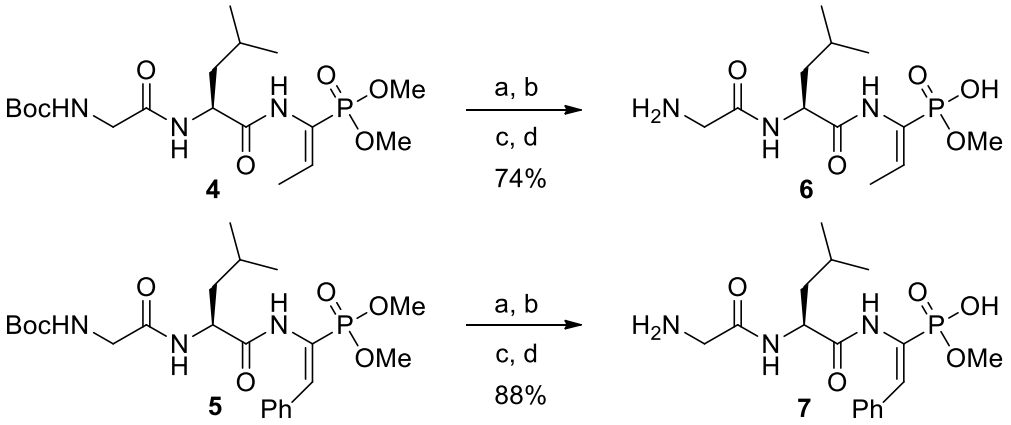
Table 1. Relevant vicinal coupling constants for the stereochemical determination of compounds **4** and **5**.

	$^{3}J_{HP}$ (Hz)	$^{3}J_{CP}$ (Hz)
E-4	13.9	15.4
Z-4	42.3	5.3
E-5	NR	18.6
Z-5	41.0	5.8

Before carrying out the reaction with Dabco® to obtain the corresponding *O*-monomethyl esters **6** and **7**, the Boc protecting group needed to be removed. Otherwise, as discussed in chapter 1, the own acidity of the phosphonic acid monoester or the acidic conditions required for the Boc-deprotection would led to decomposition. Thus, the main isomers *E*-**4** and *E*-**5** were treated with a solution of trifluoroacetic acid in dichloromethane to afford the trifluoroacetate salts of the corresponding aminophosphonates.

Surprisingly, when the trifluoroacetate salts resulting from deprotection of compounds *E*-**4** and *E*-**5** were treated with Dabco®, many side-products were observed in the ^{31}P NMR spectrum of the reaction crude, while the desired phosphonic acid monoesters were hardly identified. As a result, we hypothesized that the trifluoroacetate anion might be responsible for decomposition, and the free aminophosphonates were submitted to deprotection with Dabco® instead of the trifluoroacetate salts. The most usual procedure for obtaining the free amine involves treatment of the salt with an aqueous saturated sodium bicarbonate solution and subsequent extractions with organic solvents. However, due to the high water solubility of the peptidyl-bisphosphonate, this procedure was not appropriate. Instead, we treated the salt with weakly basic anion-exchange resin Dowex® 66 free base to afford the peptide with the free amino group. Afterwards, compounds **4** and **5** were dissolved respectively in an acetone/toluene 1:1 mixture and refluxed in the presence of Dabco® to

finally obtain **6** and **7** in good yields (Scheme 3). It should be noted that these results are applicable to both isomers, even though we only performed the reaction on main *E* compounds, as the *Z* compounds could not be obtained in a suitable scale.



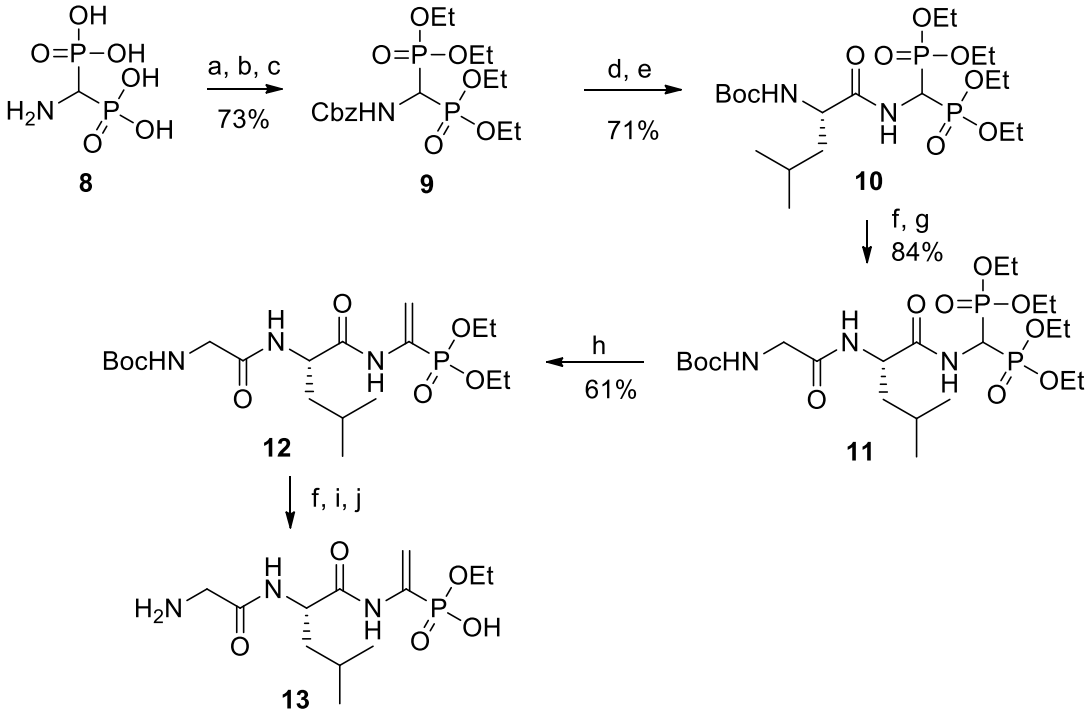
Scheme 3. Reagents and conditions: (a) TFAA, CH_2Cl_2, room temp., overnight; (b) Dowex® 66 free base; (c) Dabco®, acetone/toluene 1:1, reflux, 5 h.; (d) Amberlite® CG50.

Next, the introduction of structural variations in the phosphonic acid group was addressed. On one hand, the ethyl group was selected for testing the effect of a longer linear ester. On the other hand, the isopropyl group was considered representative of branched alkyl phosphonates.

A first approach was attempted selecting a tetraethyl bisphosphonate as a suitable precursor for obtaining a dehydrophos derivative in which the phosphonic acid monomethyl ester has been replaced by an ethyl ester group. The diethyl dehydrophosphonopeptide **12** was synthesized from the aminomethylbisphosphonic acid **8** in a similar way to that described in chapter 1 for the synthesis of dehydrophos (Scheme 4). Thus, *N*-benzyloxycarbonylation followed by alkylation with triethyl orthoformate, instead of trimethyl orthoformate, afforded the tetraethyl bisphosphonate **9**. Next, hydrogenolysis of **9** followed by the coupling with pertinent amino acids provided peptides **10** and **11** respectively. Finally, treatment of peptidyl-bisphosphonate **11** with a 37% formaldehyde solution in the presence of cesium carbonate provided the dehydrophosphonopeptide **12** after stirring at room temperature for 2.5 hours. It is worth

mentioning that the presence of the more hindered ethyl esters led to larger reaction times compared with the dimethyl phosphonate derivatives, so the use of Fmoc as protecting group was discarded.

Moreover, the higher steric hindrance also dramatically affected the outcome of the reaction with Dabco®. The Boc protecting group in compound **12** was deprotected as previously described, and the thus-obtained amino phosphonate was submitted to the partial hydrolysis with Dabco®. However, these reaction conditions proved not to be appropriate to the hydrolysis of an ethyl ester. Indeed, the reaction mixture was refluxed for 24 hours until the starting material disappeared. Furthermore, the ^{1}H and ^{31}P NMR spectra of the crude showed the presence of many side-products along with compound **13**, being impossible the isolation of the desired phosphonic acid monoester.



Scheme 4. Reagents and conditions: (a) Cbz-OSu, Et_3N, H_2O/CH_3CN 4:1, room temp., 12 h.; (b) Dowex® 50WX8; (c) $CH(OEt_3)_3$, reflux, 72 h.; (d) H_2, Pd/C, EtOH, room temp., 1 h.; (e) Boc-Leu-OH, NMM, EDC, HOBt, DMF, 0 °C, 16 h; (f) TFAA, CH_2Cl_2, room temp., overnight; (g) Boc-Gly-OH, NMM, EDC, HOBt, DMF, 0 °C, 18 h; (h) 37% HCHO(aq.), Cs_2CO_3, THF/*i*PrOH 4:1, room temp., 2.5 h.; (i) Dowex® 66 free base; (j) Dabco®, acetone/toluene 1:1, reflux, 29 h.

To overcome this problem, given that the cleavage of one methyl ester can be easily performed under smooth conditions (see chapter 1), a synthetic strategy has been designed based on the use of mixed phosphonate diesters in which the phosphonate group bears a methyl ester and the alkyl group desired to remain in the target compound. Consequently, an aminomethylbisphosphonate derivative in which each phosphonate group possesses the aforementioned feature will be a suitable starting material (Figure 6).

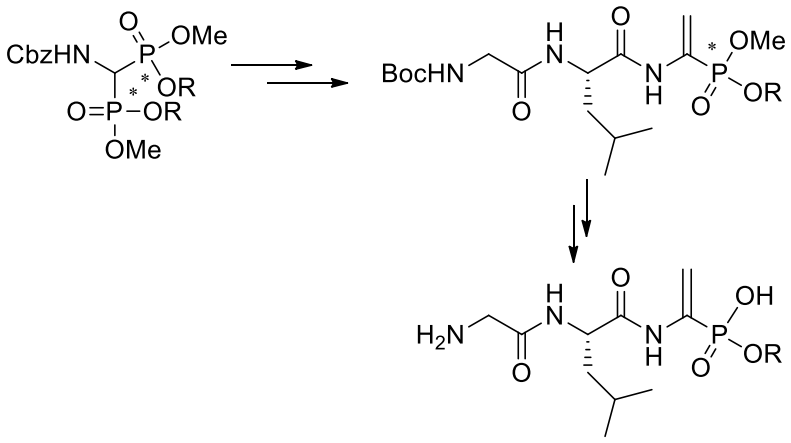
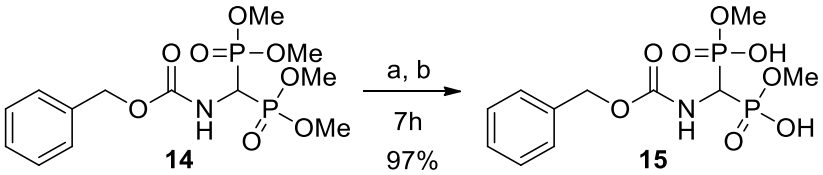


Figure 6. Proposed synthetic strategy for alkyl analogues of dehydrophos.

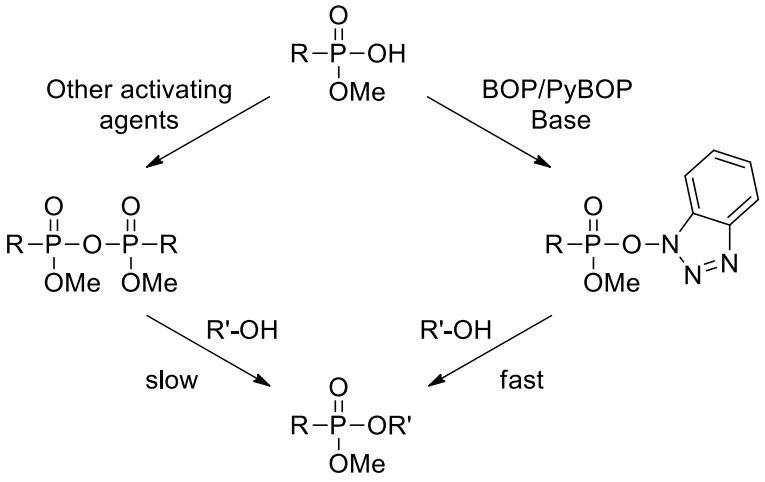
Furthermore, since the phosphorous atom is attached to four different substituents. Each mixed phosphonate diester group is a chiral center. As it will be discussed below, this leads to a mixture of diastereoisomers. However, after deprotection and partial hydrolysis to get the corresponding phosphonic acid monoalkyl ester, a single compound is formed.

Accordingly, the starting mixed aminomethylbisphosphonates were prepared beginning from the tetramethyl bisphosphonate **14**, which has been described in chapter 1. Firstly, compound **14** was treated with Dabco® to afford the dimethyl bis(hydrogen phosphonate) **15** in almost quantitative yield (Scheme 5).



Scheme 5. Reagents and conditions: (a) Dabco®, acetone/toluene 1:1, reflux, 7 h; (b) Dowex® 50WX8.

The esterification of compound **15** with the desired (ethyl and isopropyl) alcohols was then attempted. The synthesis of mixed α-aminophosphonate diesters is usually accomplished by the conversion of an hemiester into the corresponding phosphonochloridate and subsequent reaction with the alcohol.[31,32] However, the direct esterification of the phosphonate hemiester with the alcohol became advantageous when (benzotriazolyloxy)-phosphonium reagents, BOP and PyBOP, were used as activating agents instead of others coupling reagents.[33] Thus, esterifications using BOP or PyBOP usually afforded the mixed phosphonate diesters in higher yields, since the reaction proceeded through a reactive benzotryazolyl phosphonate intermediate, avoiding the formation of the less reactive pyrophosphonate anhydride (Scheme 6).

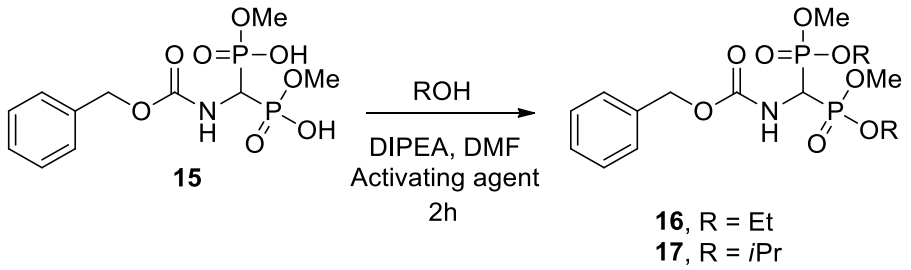


Scheme 6: Proposed reaction mechanism for the synthesis of mixed phosphonates.

Indeed, the esterification of α-aminophosphonic acid monomethyl esters using (benzotriazolyloxy)-phosphonium activating agents usually provided the mixed phosphonate diester in higher yield than treatment of the phosphonochloridate with the alcohol.[34–36] It should be noted that phosphonochloridates are moisture-sensitive, and their hydrolysis leads to the starting phosphonic acid monomethyl ester. In addition, methyl phosphonates are sensitive to acidic conditions, and may be hydrolysed in the presence of hydrogen chloride.[37]

Accordingly, the esterification reaction of compound **15** with ethanol and 2-propanol was performed using BOP and PyBOP as activating agents. As shown in table 1, esterifications in the presence of PyBOP afforded the bisphosphonates **16** and **17** in higher yields than those performed with BOP. The yield improvement is particularly significant for the diisopropyl dimethyl bisphosphonate **17**, which is 20% higher when using PyBOP (Table 2).

Table 2. Esterification of bis(hydrogen phosphonate) **15**.



Final compound	R	BOP	PyBOP
16	Et	86%	91%
17	*i*Pr	42%	62%

It is worth mentioning that the introduction of an ethyl or isopropyl group makes each phosphonate group in **16** and **17** become a chiral center, since the phosphorus atom is attached to four different substituents. Thus, for mixed phosphonates in **16** and **17** both and (*R*) and an (*S*) configuration are possible. This leads to the formation of two *meso* compounds, with symmetric phosphonate groups (phosphonate groups show inverse configuration) and a chiral bridging carbon, and two enantiomeric bisphosphonates, which possess the same configuration for both phosphonate groups (*R*,*R* and *S*,*S* respectively), as shown in figure 7.

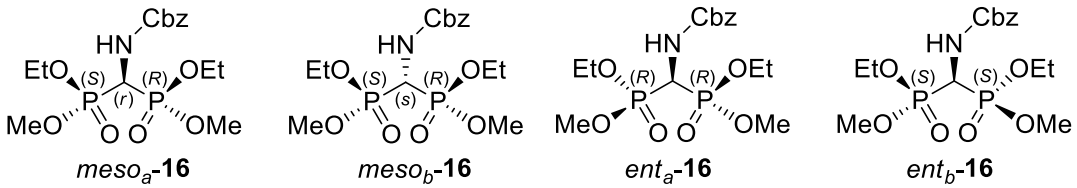


Figure 7. Structures of the diethyl dimethyl (benzyloxycarbonylaminomethyl)bisphosphonate.

The ^{31}P NMR spectra of compounds **16** and **17** show the existence of the stereoisomers. In this manner, the phosphonate groups of enantiomeric phosphonates appeared as an AB system, whereas the singlets prove the presence of both *meso*-compounds (Figure 8). These results are in agreement with those described before for aminomethylbisphosphonic acid derivatives.[38] Accordingly, mixed dialkyl dimethyl bisphosphonates **16** and **17** are actually an inseparable mixture of stereoisomers, and not a single compound.

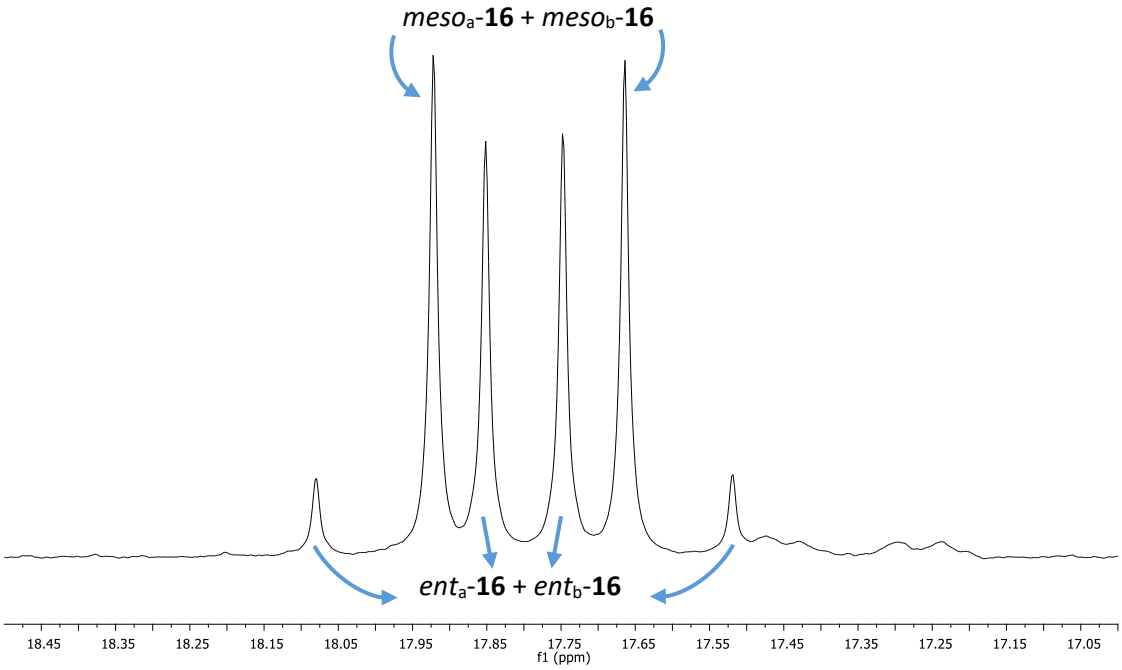
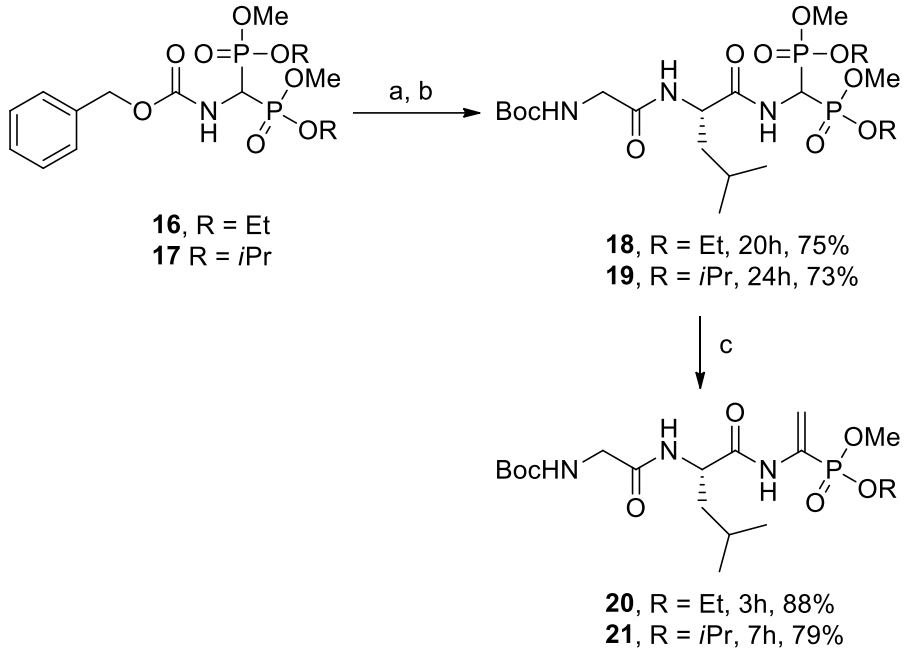


Figure 8. ^{31}P NMR spectra of **16**.

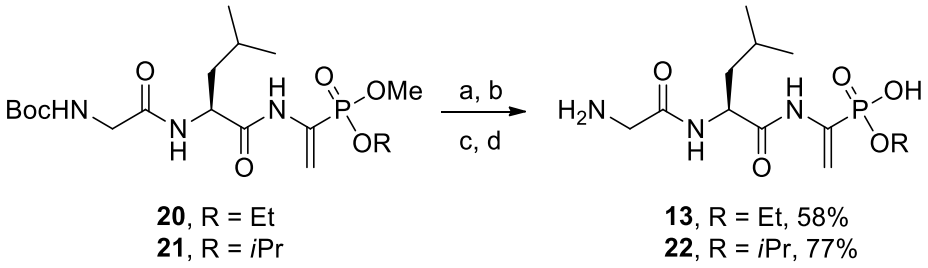
Next, bisphosphonates **16** and **17** were used as intermediates in the synthesis of dehydrophos analogues bearing an ethyl and an isopropyl ester instead of the methyl one of the naturally occurring phosphonopeptide. Firstly, peptidyl bisphosphonates featuring the peptidic sequence of dehydrophos were synthesized (Scheme 7). Nonetheless, in this case, the corresponding bisphosphonates **16** and **17** were coupled to the Boc-Gly-Leu-OH peptide instead of starting the peptide synthesis from the aminomethylbisphosphonate residue. It should be noted that peptiyl-bisphosphonates **18** and **19** were obtained in good yields (75% and 73% respectively) as an inseparable mixture of stereoisomers. Thus, direct

coupling with the whole peptide sequence was preferred to make the synthetic route involving product mixtures as short as possible. Then, peptidyl-bisphosphonates **18** and **19** were submitted to the Horner-Wadsworth-Emmons reaction with formaldehyde to afford the phosphonopeptides **20** and **21** in good yields as a mixture of two diastereoisomers since only one chiral phosphonate group remains.



Scheme 7. Reagents and conditions: (a) H_2, Pd/C, MeOH, room temp., 1 h.; (b) Boc-Gly-Leu-OH, NMM, EDC, HOBt, DMF, 0 °C; (c) 37% HCHO(aq.), Cs_2CO_3, THF/*i*PrOH 4:1, room temp.

Afterwards, compounds **20** and **21** were subjected to the deprotection sequence described above to provide the ethyl and isopropyl phosphonic acid monoesters **13** and **22**, in good yields as single compounds (Scheme 8).



Scheme 8. Reagents and conditions: (a) TFAA, CH_2Cl_2, room temp., overnight; (b) Dowex® 66 free base; (c) Dabco®, acetone/toluene 1:1, reflux, 4.5 h.; (d) Amberlite® CG50.

Finally, dehydrophos analogues featuring modified peptide sequences were prepared. For a first insight into these modifications, dehydrophosphonopeptides **23** and **24** were considered (Figure 9).

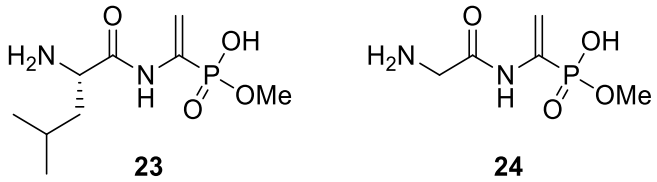
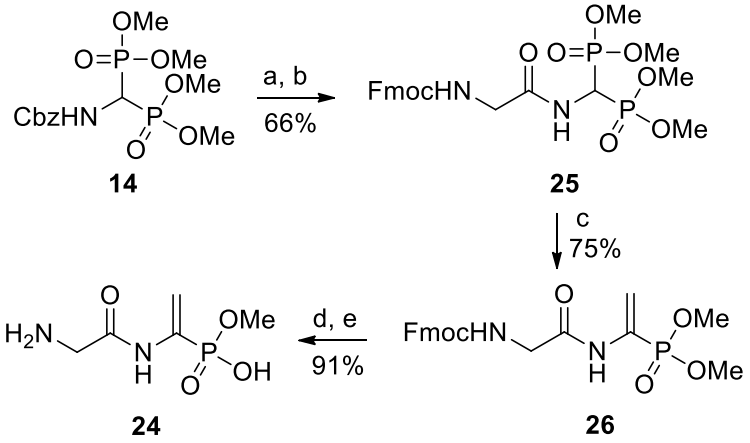


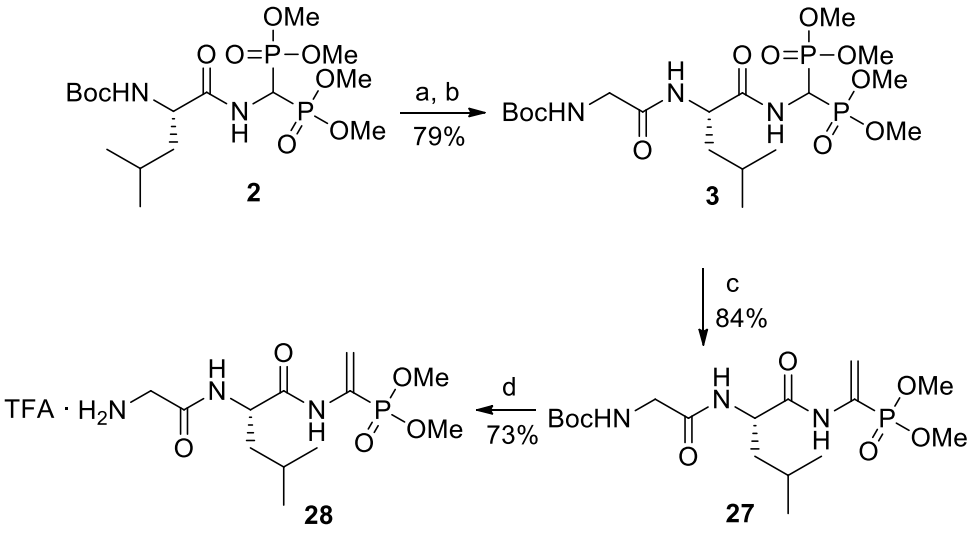
Figure 9. Structure of dehydrophos analogues featuring modified peptidic chains.

On one hand, compound **23** results from the deletion of the *N*-terminal amino acid residue in dehydrophos sequence, and was synthesized as described in chapter 1 (page 23, scheme 5). On the other hand, dehydrophosphonopeptide **24** is the simplest stable compound that is capable of containing a vinylphosphonic acid monomethyl ester in its structure, since, as discussed in chapter 1, an amine group is needed for stabilizing the molecule. Compound **24** was synthesized similarly to that described for **23** in chapter 1, as depicted in scheme 9.



Scheme 9. Reagents and conditions: H_2, Pd/C, MeOH, room temp., 1 h.; (b) Fmoc-Gly-OH, NMM, EDC, HOBt, DMF, 0 °C, 17 h.; (c) 37% HCHO(aq.), Cs_2CO_3, THF/*i*PrOH 4:1, room temp., 1 h.; (d) Dabco®, acetone/toluene 1:1, reflux, 2 h.; (e) Amberlite® CG50.

In addition, the dimethyl phosphonate derivative of dehydrophos, **28**, was synthesized using the above-described procedure excluding partial hydrolysis with Dabco® (Scheme 10). Compound **28** was prepared for studying the effect of a dimethyl phosphonate on biological activity.



Scheme 10. Reagents and conditions: a) TFAA, CH_2Cl_2, room temp., overnight; (b) Boc-Gly-OH, NMM, EDC, HOBt, DMF, 0 °C, 16 h; c) 37% HCHO(aq.), Cs_2CO_3, THF/*i*PrOH 4:1, room temp., 1 h.; d) TFAA, CH_2Cl_2, room temp., overnight.

The antimicrobial activity of compounds **6**, **7**, **13**, **22-24** and **28** was tested against the strains listed in table 3, using the antibiotic dehydrophos for comparison. As shown, *Pseudomonas aeruginosa* (critical), *Staphylococcus aureus* (high) and *Salmonella thyphimurium* (high) are included in the World Health Organization global priority list of antibiotic-resistant bacteria.[39]

Table 3. Bacterial strains used in the biological activity study of compounds **6**, **7**, **13**, **22-24** and **28**.

Strain	Priority by WHO	Gram	Source or reference
E. coli		Gram Negative	Mycobacterial Genetics Group
Bacillus subtilis		Gram Positive	CECT356
Pseudomonas aeruginosa	critical	Gram Negative	ATCC15442
Enterococcus faecalis		Gram Positive	ATCC29212
Staphylococcus aureus	high	Gram Positive	CECT794
Salmonella thyphimurium	high	Gram Negative	Mycobacterial Genetics Group
Mycobacterium smegmatis		Mycobacteria	Mycobacterial Genetics Group

None of these compounds showed bioactivity when tested in a rich MH medium, as previously observed for dehydrophos.[17] However, bioassays performed in M9 medium resulted in growth inhibition zones for compounds **13** and **23** against several of the bacterial strains studied, whereas the rest showed no biological activity (Table 4). Thus, the leucylvinylphosphonic acid monomethyl ester **23** showed a biological activity similar to dehydrophos, since both compounds inhibited the growth of *B. subtilis*, *P. areuginosa*, *E. faecalis* and *S. thyphimurium*. Furthermore, the ethyl analogue of dehydrophos, **13**, also proved biologically active against *B. subtilis*, *P. aeruginosa* and *S. thyphimurium*, although did not impede the growth of *E. faecalis*.

Table 4. Antibacterial activities of compounds **6**, **7**, **13**, **22-24** and **28**.

	Solid agar diffusion[a,b]								MIC (μM)[a]		
Strain	**Dhp**	**6**	**7**	**13**	**22**	**23**	**24**	**28**	**Dhp**	**13**	**23**
E. coli	–	–	–	–	–	–	–	nd	nd	nd	nd
B. subtilis	++	–	–	+	–	++	–	+	3.9–7.8	15.6	15.6
P. aeruginosa	+	–	–	+	–	+	–	nd	7.8	15.6	62.5
E. faecalis	+	–	–	–	–	+	–	nd	15.6	>125	15.6–31.2
S. aureus	–	nd	nd	–	nd	–	nd	nd	nd	nd	nd
S. thyphimurium	++	–	–	++	–	++	–	nd	15.6	125	62.5
M. smegmatis	–	–	–	–	–	–	–	nd	nd	nd	nd

[a] Solid agar diffusion assays and minimal inhibitory concentration determination were performed in M9 minimal medium

[b] + zone of inhibition, – no activity

nd: Not determined

Accordingly, we concluded that the introduction of substituents in the vinyl moiety results in a loss of bioactivity. Similarly, replacement of the phosphonic acid monomethyl ester by a branched alkyl phosphonate (isopropyl group) leads to an inactive dehydrophos derivative, while the corresponding monoethyl ester derivative remains active, especially against *Bacillus subtilis* and *Pseudomonas aeruginosa*. In addition, the *N*-terminal glycyl residue does not seem to be essential for biological activity, since the leucylvinylphosphonic acid derivative **23** exhibits an antimicrobial activity comparable to that of dehydrophos. Contrary, the glycylvinylphosphonic acid monomethyl ester **24** proved inactive.

Indeed, the lack of bioactivity of the abovementioned dehydrophos derivatives may be the result of several factors, such as the inactivity of the released acylphosphonate derivatives, low cell permeability to these compounds, resistance to peptidase activity, and susceptibility to efflux, which has also been described for free acylphosphonate derivatives.[19] Determining the specific cause for any of these compounds requires extensive

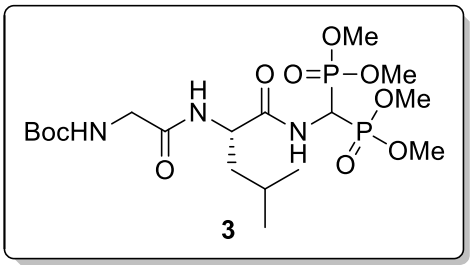
research beyond a structure-activity relationship study. However, based on the structure of these compounds, several hypotheses can be formulated to guide future investigations. Thus, the greater stability of β-substituted enamines might be a cause of the lack of bioactivity of derivatives **6** and **7**, since the hydrolysis of the related imine is essential for the release of an acylphosphonate.

The activity difference between the leucylvinylphosphonate **23** and the glycylvinylphosphonate **24** is particularly interesting. On one hand, it might be caused by an L-specificity of permeases and peptidases involved in the compound uptake and intracellular hydrolysis respectively, in a similar way to that found for alafosfalin derivatives.[12,40] However, both the enantiomer dehydrophos and the naturally occurring peptide have very similar antimicrobial activities[17] and this hypothesis can be discarded. On the other hand, it might indicate that the release of the dehydroaminophosphonic acid residue can only be achieved by an specific enzyme, as previously demonstrated for alafosfalin.[41] In this case, the presence of a leucyl moiety next to the vinylphosphonic acid residue would be essential for biological activity. Indeed, naturally occurring dehydrophos is mainly hydrolysed by leucyl aminopeptidases PepA and PepB.[18] Nonetheless, the efflux of compound **24** can not be ruled out.

Experimental Section

All reagents were used as received from commercial suppliers without further purification. *N*,*N*-Dimethylformamide were purchased at anhydrous grade from Aldrich. Thin-layer chromatography (TLC) was performed on Macherey–Nagel Polygram® SIL G/UV254 precoated silica gel polyester plates. The products were visualized by exposure to UV light or submersion in ninhydrin, phosphomolybdic acid or permanganate. Column chromatography was performed using 60 Å (0.04–0.063 mm) silica gel from Macherey–Nagel. Melting points were determined on a Gallenkamp apparatus. Optical rotations were measured in a JASCO P-1020 polarimeter. IR spectra were registered on a Nicolet Avatar 360 FTIR spectrophotometer; ν_{max} is given for the main absorption bands. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker AV-400 instrument at room temperature using the residual solvent signal, the solvent signal, or the chemical shift of the lock solvent as the internal standard; chemical shifts (δ) are expressed in parts per million and coupling constants (J) in Hertz. High-resolution mass spectra were recorded on a Bruker Microtof-Q spectrometer.

Synthesis of tetramethyl [*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamidomethyl]bisphosphonate (3)



A solution of tetramethyl [*N*-(*tert*-butoxycarbonyl)-L-leucylamidomethyl)] bisphosphonate, compound **2** from chapter 1, (1.07 g, 2.32 mmol) in dichloromethane (40 mL) was cooled at 0 °C, trifluoroacetic acid (3.57 mL, 46.65 mmol) was added and then the solution stirred overnight at room temperature. Subsequently, solvent was removed under reduced pressure and the crude was used without further purification. A solution of previous oil and Boc-glycine (449 mg, 2.56 mmol) in dry *N,N*-dimethylformamide (12 mL) was cooled at 0 °C. *N*-Methylmorpholine (0.64 µL, 5.82 mmol) and 1-hydroxybenzotriazole hydrate (12% H_2O) (430 mg, 2.80 mmol) were added and the mixture was stirred 15 minutes at 0 °C. Then *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide (536 mg, 2.80 mmol) was added, the reaction mixture was stirred 16 hours at 0 °C. Solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate (30 mL) and washed with an aqueous 5% solution of sodium bicarbonate (20 mL). The aqueous phase was extracted with ethyl acetate (3 x 20 mL) until no final product was detected by TLC in the aqueous phase. The combined organic layers were dried over anhydrous magnesium sulphate, filtered and solvent removed under reduced pressure. The resulting oil was purified by column chromatography on silica gel (dichloromethane/methanol 95:5) to afford **3** as a white solid (945 mg, 1.83 mmol, 79% yield).

$[\alpha]^{30}_D$: –27.3 (*c* 0.44, $CHCl_3$).

M.p.: 60–62 °C.

IR (neat): ν 3285, 2959, 1765, 1530, 1269, 1029 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.67 (d, 1H, *J* = 9.9 Hz, NH), 6.88 (br s, 1H, NH), 5.51 (br s, 1H, NH), 5.06 (td, 1H, *J* = 22.1, 10.0 Hz, N-CH-P_2), 4.68–4.59 (m, 1H, $CH_{\alpha\ Leu}$), 3.89–3.73 (m, 14H, $CH_{2\ Gly}$+$CH_{3\ OMe}$), 1.71–1.51 (m, 3H, CH_{Leu}+$CH_{2\ Leu}$), 1.43 (s, 9H, $CH_{3\ Boc}$), 0.92 (d, 3H, *J* = 6.1 Hz, $CH_{3\ Leu}$), 0.91 (d, 3H, *J* = 6.1 Hz, $CH_{3\ Leu}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 172.05 (t, *J* = 4.1 Hz, CO), 170.17 (CO), 156.25 (CO), 80.33 (C_{Boc}), 54.46–54.00 ($CH_{3\ OMe}$), 51.77 ($CH_{\alpha\ Leu}$), 44.45 ($CH_{2\ Gly}$), 42.84 (t, *J* = 148.3 Hz, N-CH-P_2), 41.02 ($CH_{2\ Leu}$), 28.40 ($CH_{3\ Boc}$), 24.79 (CH_{Leu}), 22.98 ($CH_{3\ Leu}$), 22.15 ($CH_{3\ Leu}$) ppm.

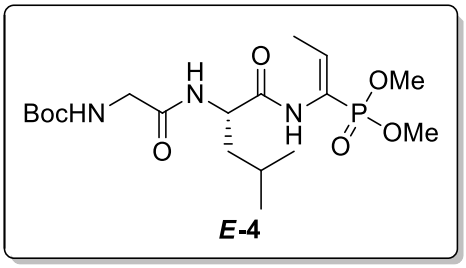
^{31}P NMR (162 MHz, $CDCl_3$): δ 18.64, 18.57 (AB spin system, 2P, *J* = 32.0 Hz) ppm.

HRMS (ESI) $C_{18}H_{37}N_3NaO_{10}P_2$ $[M+Na]^+$: 540.1846, found 540.1861.

Synthesis of dimethyl (*E*)-{1-[*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamido]prop-1-en-1-yl}phosphonate (*E*-4) and dimethyl (*Z*)-{1-[*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamido]prop-1-en-1-yl}phosphonate (*Z*-4)

To a solution of **3** (500 mg, 0.97 mmol) in 2-propanol (10 mL) was added cesium carbonate (394 mg, 1.21 mmol) and acetaldehyde (109 µL, 1.94 mmol). After 7 hours stirring the solvent was removed under reduced pressure, the crude suspended in dichloromethane and the solid removed by filtration. Solvent was removed under reduced pressure and the resulting oil was purified by column chromatography on silica gel (dichloromethane/2-propanol 9:1) to afford *Z*-**4** as a white solid (50 mg, 0.11 mmol, 12% yield) and *E*-**4** as a white solid (247 mg, 0.57 mmol, 59% yield).

Compound *E*-4:



***E*-4**

$[\alpha]^{24}_D$: –44.7 (*c* 0.44, $CHCl_3$).

M.p.: 53–55 °C.

IR (KBr): ν 3423, 2960, 1665, 1524, 1249, 1171, 1029 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68 (s, 1H, NH), 6.94 (d, 1H, *J* = 7.2 Hz, NH), 6.65 (dq, 1H, *J* = 13.9, 6.9 Hz, *Z*-CH=C), 5.42 (br s, 1H, NH), 4.60–4.51 (m, 1H, $CH_{\alpha\ Leu}$), 3.88–3.73 (m, 2H, $CH_{2\ Gly}$), 3.70 (d, 3H, *J* = 11.2 Hz, $CH_{3\ OMe}$), 3.70 (d, 3H, *J* = 11.1 Hz, $CH_{3\ OMe}$), 1.77–1.52 (m, 6H, CH_3+CH_{Leu}+$CH_{2\ Leu}$), 1.43 (s, 9H, $CH_{3\ Boc}$), 0.94 (d, 3H, *J* = 6.3 Hz, $CH_{3\ Leu}$), 0.92 (d, 3H, *J* = 6.2 Hz, $CH_{3\ Leu}$) ppm.

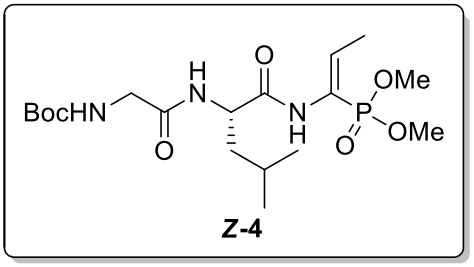
^{13}C NMR (100 MHz, $CDCl_3$): δ 170.33 (d, *J* = 2.6 Hz, CO), 156.33 (CO), 142.51 (d, *J* = 21.1 Hz, CH=C), 123.40 (d, *J* = 213.5 Hz, N-C-P), 80.57 (C_{Boc}), 53.12 (d, *J* = 5.4 Hz, $CH_{3\ OMe}$), 53.08

(d, *J* = 5.4 Hz, $CH_{3\ OMe}$), 52.09 ($CH_{\alpha\ Leu}$), 44.55 ($CH_{2\ Gly}$), 40.69 ($CH_{2\ Leu}$), 28.39 ($CH_{3\ Boc}$), 24.92 (CH_{Leu}), 23.05 ($CH_{3\ Leu}$), 22.05 ($CH_{3\ Leu}$), 14.67 (d, *J* = 15.4 Hz, CH_3) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): *δ* 16.10 ppm.

HRMS (ESI) $C_{18}H_{34}N_3NaO_7P$ [M+Na]$^+$: 458.2027, found 458.2035.

Compound *Z*-4:



***Z*-4**

[α]$^{23}_D$: –35.4 (*c* 0.25, $CHCl_3$).

M.p.: 136–138 °C.

IR (KBr): ν 3275, 2957, 1674, 1546, 1244, 1021 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): *δ* 7.77 (d, 1H, *J* = 5.5 Hz, NH), 7.12 (dq, 1H, *J* = 42.3, 7.6 Hz, *E*-CH=C), 6.75 (d, 1H, *J* = 7.8 Hz, NH), 5.38 (br s, 1H, NH), 4.52–4.49 (m, 1H, $CH_{\alpha\ Leu}$), 3.93–3.71 (m, 2H, $CH_{2\ Gly}$) overlapped with 3.74 (d, 3H, *J* = 11.5 Hz, $CH_{3\ OMe}$), 3.73 (d, 3H, *J* = 11.5 Hz, $CH_{3\ OMe}$), 1.97 (d, 3H, *J* = 7.6, 3.2 Hz, CH_3), 1.76–1.51 (m, 3H, CH_{Leu}+$CH_{2\ Leu}$), 1.44 (s, 9H, $CH_{3\ Boc}$), 0.93 (d, 3H, *J* = 6.7 Hz, $CH_{3\ Leu}$), 0.92 (d, 3H, *J* = 6.6 Hz, $CH_{3\ Leu}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): *δ* 171.09 (d, *J* = 7.2 Hz, CO), 170.09 (CO), 156.25 (CO), 134.57 (d, *J* = 12.8 Hz, C̲H=C), 122.90 (d, *J* = 200.5 Hz, N-C-P), 80.50 (C_{Boc}), 52.92 (d, *J* = 5.1 Hz, $CH_{3\ OMe}$), 52.61 ($CH_{\alpha\ Leu}$), 44.58 ($CH_{2\ Gly}$), 40.92 ($CH_{2\ Leu}$), 28.40 ($CH_{3\ Boc}$), 24.94 (CH_{Leu}), 23.08 ($CH_{3\ Leu}$), 22.00 ($CH_{3\ Leu}$), 14.56 (d, *J* = 5.3 Hz, CH_3) ppm.

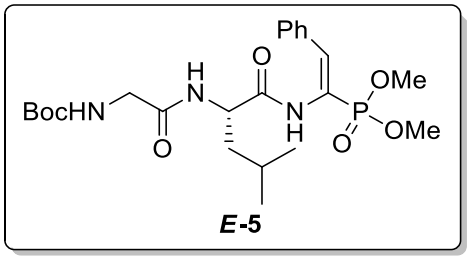
^{31}P NMR (162 MHz, $CDCl_3$): *δ* 16.29 ppm.

HRMS (ESI) $C_{18}H_{34}N_3NaO_7P$ [M+Na]$^+$: 458.2027, found 458.2018.

Synthesis of dimethyl (*E*)-{1-[*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamido]-2-phenylethen-1-yl}phosphonate (*E*-5) and dimethyl (*Z*)-{1-[*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamido]-2-phenylethen-1-yl}phosphonate (*Z*-5)

To a solution of **3** (1.12 g, 2.16 mmol) in 2-propanol (20 mL) was added cesium carbonate (878 mg, 2.69 mmol) and benzaldehyde (0.44 mL, 4.33 mmol). After 25 hours stirring the solvent was removed under reduced pressure, the crude suspended in dichloromethane and the solid removed by filtration. Solvent was removed under reduced pressure and the resulting oil was purified by column chromatography on silica gel (ethyl acetate/hexane/2-propanol 8:1.5:0.5) to afford *Z*-**5** as a white solid (49 mg, 0.10 mmol, 5% yield) and *E*-**5** as a colourless oil (450 mg, 0.90 mmol, 42% yield).

Compound *E*-5:



***E*-5**

[α]$^{22}_{D}$: –70.0 (*c* 0.40, $CHCl_3$).

M.p.: 64–66 °C.

IR (KBr): ν 3278, 2957, 1662, 1449, 1243, 1027 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): *δ* 7.95 (d, 1H, *J* = 2.8 Hz, NH), 7.48–7.43 (m, 2H, CH_{Ar}), 7.35–7.26 (m, 4H, CH_{Ar}+ *Z*-CH=C), 6.91 (d, 1H, *J* = 7.9 Hz, NH), 5.37 (br s, 1H, NH), 4.61–4.51 (m, 1H, $CH_{\alpha\ Leu}$), 3.87–3.68 (m, 2H, $CH_{2\ Gly}$) overlapped with 3.77 (d, 3H, *J* = 11.2 Hz, $CH_{3\ OMe}$), 3.75 (d, 3H, *J* = 11.1 Hz, $CH_{3\ OMe}$), 1.78–1.60 (m, 2H, CH_{Leu}+$CH_{2\ Leu}$), 1.59–1.49 (m, 1H, CH_{Leu}+$CH_{2\ Leu}$), 1.41 (s, 9H, $CH_{3\ Boc}$), 0.93 (d, 3H, *J* = 6.4 Hz, $CH_{3\ Leu}$), 0.90 (d, 3H, *J* = 6.3 Hz, $CH_{3\ Leu}$) ppm.

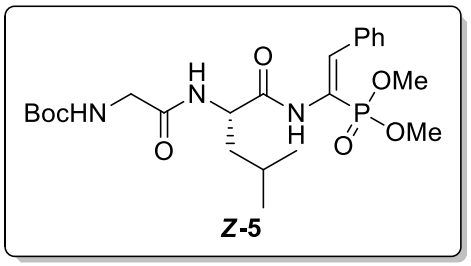
^{13}C NMR (100 MHz, $CDCl_3$): *δ* 170.43 (d, *J* = 12.1 Hz, CO), 156.34 (CO), 141.50 (d, *J* = 22.5 Hz, CH=C), 133.56 (d, *J* = 18.6 Hz, C_{Ar}), 129.85 (CH_{Ar}), 129.76 (CH_{Ar}), 128.64 (CH_{Ar}),

121.87 (d, *J* = 212.3 Hz, N-C-P), 80.62 (C_{Boc}), 53.36 (d, *J* = 5.3 Hz, $CH_{3\ OMe}$), 53.25 (d, *J* = 5.4 Hz, $CH_{3\ OMe}$), 52.06 ($CH_{\alpha\ Leu}$), 44.57 ($CH_{2\ Gly}$), 40.13 ($CH_{2\ Leu}$), 28.38 ($CH_{3\ Boc}$), 24.84 (CH_{Leu}), 22.95 ($CH_{3\ Leu}$), 22.10 ($CH_{3\ Leu}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): *δ* 16.87 ppm.

HRMS (ESI) $C_{23}H_{36}N_3NaO_7P$ $[M+Na]^+$: 520.2183, found 520.2167.

Compound *Z*-5:



***Z*-5**

[α]$^{23}_D$: –45.5 (*c* 0.28, $CHCl_3$).

IR (nujol): ν 3291, 1711, 1682, 1259, 1041 cm^{-1}.

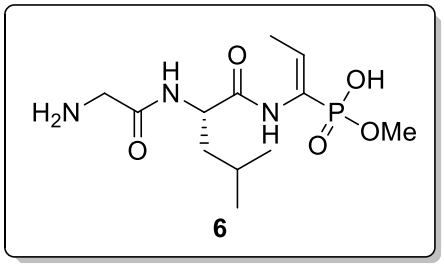
^{1}H NMR (400 MHz, $CDCl_3$): *δ* 8.18 (s, 1H, NH) overlapped with 8.15 (d, 1H, *J* = 41.0 Hz, *E*-CH=C), 7.41–7.36 (m, 2H, CH_{Ar}), 7.34–7.27 (m, 3H, CH_{Ar}), 6.89 (d, 1H, *J* = 6.8 Hz, NH), 5.44 (br s, 1H, NH), 4.58–4.49 (m, 1H, $CH_{\alpha\ Leu}$), 3.97–3.76 (m, 2H, $CH_{2\ Gly}$), 3.57 (d, 3H, *J* = 11.5 Hz, $CH_{3\ OMe}$), 3.55 (d, 3H, *J* = 11.5 Hz, $CH_{3\ OMe}$), 1.82–1.56 (m, 3H, CH_{Leu}+$CH_{2\ Leu}$), 1.44 (s, 9H, $CH_{3\ Boc}$), 0.96 (d, 3H, *J* = 6.7 Hz, $CH_{3\ Leu}$), 0.94 (d, 3H, *J* = 6.8 Hz, $CH_{3\ Leu}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): *δ* 171.06 (d, *J* = 8.1 Hz, CO), 170.21 (CO), 156.29 (CO), 134.52 (d, *J* = 5.8 Hz, C_{Ar}), 133.99 (d, *J* = 9.8 Hz, CH=C), 129.01 (d, *J* = 1.5 Hz, CH_{Ar}), 128.33 (CH_{Ar}), 128.02 (CH_{Ar}), 122.94 (d, *J* = 199.5 Hz, N-C-P), 80.46 (C_{Boc}), 53.13 (d, *J* = 5.4 Hz, $CH_{3\ OMe}$), 53.09 (d, *J* = 5.5 Hz, $CH_{3\ OMe}$), 52.92 ($CH_{\alpha\ Leu}$), 44.53 ($CH_{2\ Gly}$), 40.86 ($CH_{2\ Leu}$), 28.49 ($CH_{3\ Boc}$), 24.96 (CH_{Leu}), 23.13 ($CH_{3\ Leu}$), 21.91 ($CH_{3\ Leu}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): *δ* 15.39 ppm.

HRMS (ESI) $C_{23}H_{36}N_3NaO_7P$ $[M+Na]^+$: 520.2183, found 520.2186.

Synthesis of (*E*)-[1-(glycyl-L-leucylamido)prop-1-en-1-yl]phosphonic acid monomethyl ester (6)



6

A solution of *E*-**4** (135 mg, 0.31 mmol) in dichloromethane (6 mL) was cooled at 0 °C, trifluoroacetic acid (0.47 mL, 6.14 mmol) was added and then the solution stirred overnight at room temperature. Subsequently, solvent was removed under reduced pressure and the resulting residue was treated with weakly basic anion-exchange resin (Dowex® 66 free base) to afford an oil which was used in the next step. Previous oil was dissolved in a mixture of acetone/toluene 1:1 (5 mL) and 1,4-diazabicyclo[2.2.2]octane (65 mg, 0.58 mmol) was added. The reaction mixture was stirred at reflux for 5 hours. Solvent was removed, the crude dissolved in water (10 mL) and filtered through 0.22 μm HPLC filter. Then, the aqueous solution was washed with dichloromethane (2 x 5 mL) and ethyl acetate (2 x 5 mL) and concentrated in vacuo. The resulting residue was purified using a weakly acidic ion-exchange resin (Amberlite® CG50, hydrogen form) to afford **6** as a white solid (75 mg, 0.23 mmol, 74% yield).

$[\alpha]^{23}_{D}$: –60.3 (*c* 0.30, MeOH).

M.p.: 79–81 °C.

IR (neat): ν 3208, 2955, 1663, 1518, 1197, 1070, 1039 cm^{-1}.

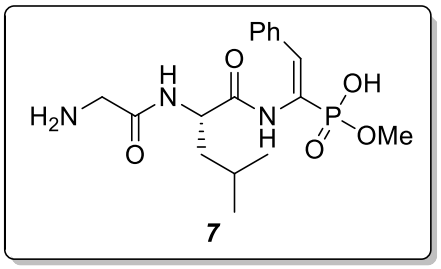
^{1}H NMR (400 MHz, MeOD): δ 6.39 (dq, 1H, *J* = 13.7, 6.9 Hz, *Z*-CH=C), 4.55–4.48 (m, 1H, $CH_{\alpha\ Leu}$), 3.78–3.64 (m, 2H, $CH_{2\ Gly}$), 3.44 (d, 3H, *J* = 11.1 Hz, $CH_{3\ OMe}$), 1.80–1.64 (m, 3H, CH_{Leu}+$CH_{2\ Leu}$) overlapped with 1.62 (dd, 3H, *J* = 6.9, 2.8 Hz, CH_3), 0.99 (d, 3H, *J* = 6.3 Hz, $CH_{3\ Leu}$), 0.95 (d, 3H, *J* = 6.1 Hz, $CH_{3\ Leu}$) ppm.

^{13}C NMR (100 MHz, MeOD): δ 171.83 (d, J = 3.1 Hz, CO), 168.34 (CO), 136.09 (d, J = 18.3, CH=C), 130.23 (d, J = 197.4, N-C-P), 53.87 ($CH_{\alpha\ Leu}$), 52.37 (d, J = 4.9 Hz, $CH_{3\ OMe}$), 41.76 ($CH_{2\ Gly}$), 41.69 ($CH_{2\ Leu}$), 26.00 (CH_{Leu}), 23.43 ($CH_{3\ Leu}$), 21.87 ($CH_{3\ Leu}$), 14.60 (d, J = 13.9, CH_3) ppm.

^{31}P NMR (162 MHz, MeOD): δ 10.04 ppm.

HRMS (ESI) $C_{11}H_{22}N_3NaO_5P$ $[M+Na]^+$: 344.1346, found 344.1327.

Synthesis of (*E*)-[1-(glycyl-L-leucylamido)-2-phenylethen-1-yl]phosphonic acid monomethyl ester (7)



7

A solution of *E*-**5** (130 mg, 0.26 mmol) in dichloromethane (6 mL) was cooled at 0 °C, trifluoroacetic acid (0.49 mL, 5.22 mmol) was added and then the solution stirred overnight at room temperature. Subsequently, solvent was removed under reduced pressure and the resulting residue was treated with weakly basic anion-exchange resin (Dowex® 66 free base) to afford an oil which was used in the next step. Previous oil was dissolved in a mixture of acetone/toluene 1:1 (5 mL) and 1,4-diazabicyclo[2.2.2]octane (73 mg, 0.65 mmol) was added. The reaction mixture was stirred at reflux for 5 hours. Solvent was removed, the crude dissolved in water (10 mL) and filtered through 0.22 μm HPLC filter. Then, the aqueous solution was washed with dichloromethane (2 x 5 mL) and ethyl acetate (2 x 5 mL) and concentrated in vacuo. The resulting residue was purified using a weakly acidic ion-exchange resin (Amberlite® CG50, hydrogen form) to afford **7** as a white solid (90 mg, 0.23 mmol, 88% yield).

$[\alpha]^{24}_{D}$: −71.5 (*c* 0.22, MeOH).

M.p.: 177–179 °C (dec).

IR (KBr): ν 3214, 2957, 1675, 1539, 1195, 1050 cm^{-1}.

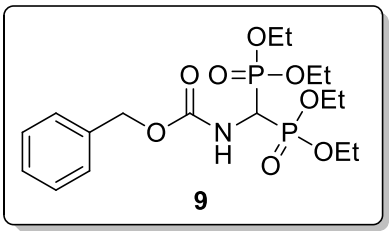
^{1}H NMR (400 MHz, MeOD): δ 7.45 (d, 2H, *J* = 7.3 Hz, CH_{Ar}), 7.32–7.27 (m, 2H, CH_{Ar}), 7.26–7.20 (m, 1H, CH_{Ar}), 7.15 (d, 1H, *J* = 14.6 Hz, *Z*-CH=C), 4.52–4.45 (m, 1H, $CH_{\alpha\ Leu}$), 3.84–3.63 (m, 2H, $CH_{2\ Gly}$), 3.51 (d, 3H, *J* = 11.1 Hz, $CH_{3\ OMe}$), 1.75–1.52 (m, 3H, CH_{Leu}+$CH_{2\ Leu}$), 0.97 (d, 3H, *J* = 6.2 Hz, $CH_{3\ Leu}$), 0.92 (d, 3H, *J* = 5.4 Hz, $CH_{3\ Leu}$) ppm.

^{13}C NMR (100 MHz, MeOD): δ 171.68 (d, J = 2.7 Hz, CO), 168.05 (CO), 136.80 (d, J = 18.9, CH=C), 136.70 (d, J = 16.6 Hz, C_{Ar}), 130.22 (CH_{Ar}), 129.10 (d, J = 194.9 Hz, N-C-P), 129.32 (CH_{Ar}), 129.21 (CH_{Ar}), 53.83 ($CH_{\alpha\ Leu}$), 52.05 (d, J = 5.1 Hz, $CH_{3\ OMe}$), 41.66 ($CH_{2\ Gly}$), 41.08 ($CH_{2\ Leu}$), 25.88 (CH_{Leu}), 23.38 ($CH_{3\ Leu}$), 21.87 ($CH_{3\ Leu}$) ppm.

^{31}P NMR (162 MHz, MeOD): δ 10.46 ppm.

HRMS (ESI) $C_{17}H_{26}N_3NaO_5P$ $[M+Na]^+$: 406.1502, found 406.1492.

Synthesis of tetraethyl (bencyloxycarbonylaminomethyl)bisphosphonate (9)



9

A solution of aminomethylphosphonic acid, compound **1** from chapter 1, (969 mg, 5.07 mmol) in water (5 mL) and triethylamine (2.97 mL, 21.3 mmol) was added a solution of *N*-(benzyloxycarbonyloxy)succinimide (1.28 g, 5.12 mmol) in acetonitrile (1.3 mL). The reaction mixture was stirred 12 hours at room temperature, and solvent was removed under reduced pressure. The residue was dissolved in water, washed with diethyl ether, treated with an ion-exchange resin (Dowex® 50WX8, hydrogen form) and evaporated to afford a colourless oil which was used in the next step without further purification. Previous residue was dissolved in triethyl orthoformate (22.1 mL, 133 mmol) and stirred at reflux under argon for 72 hours. Subsequently, the orthoformate in excess was removed under reduced pressure. The resulting oil was purified by column chromatography on silica gel (dichloromethane/methanol 95:5) to afford **9** as a yellow oil (1.61 g, 3.68 mmol, 73% yield).

IR (neat): ν 3223, 2983, 1717, 1270, 1027 cm^{-1}.

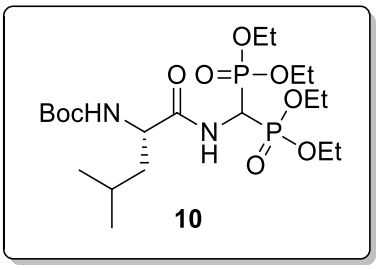
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37–7.26 (m, 5H, CH_{Ar}), 5.32 (d, 1H, J = 10.5 Hz, NH), 5.14 (s, 2H, CH_2-Ph), 4.59 (td, 1H, J = 21.9, 10.5 Hz, N-CH-P_2), 4.24–4.09 (m, 8H, $CH_{2\ OEt}$), 1.32 (t, 6H, J = 7.1 Hz, $CH_{3\ OEt}$), 1.29 (t, 6H, J = 7.1 Hz, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 155.73 (t, J = 4.9 Hz, CO), 136.14 (C_{Ar}), 128.67 (CH_{Ar}), 128.46 (CH_{Ar}), 128.30 (CH_{Ar}), 67.81 (CH_2-Ph), 63.81–63.s68 ($CH_{2\ OEt}$), 46.28 (t, J = 147.7 Hz, N-CH-P_2), 16.58–16.40 ($CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 16.45 ppm.

HRMS (ESI) $C_{17}H_{29}NNaO_8P_2$ $[M+Na]^+$: 460.1261, found 460.1285.

Synthesis of tetraethyl [*N*-(*tert*-butoxycarbonyl)-L-leucylamidomethyl]bisphosphonate (10)



10

To a solution of **9** (1.60 g, 3.66 mmol) in methanol (40 mL) 10% wt. palladium on carbon (160 mg) was added. The mixture was stirred under hydrogen atmosphere at room temperature for one hour and then filtered (Celite) to afford an oil which was used in the next step. A solution of previous oil and Boc-L-leucine (959 mg, 4.15 mmol) in dry *N,N*-dimethylformamide (17 mL) was cooled at 0 °C. *N*-Methylmorpholine (500 µL, 4.55 mmol) and 1-hydroxybenzotriazole hydrate (12% H_2O) (644 mg, 4.19 mmol) were added and the mixture was stirred 15 minutes at 0 °C. Then *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (804 mg, 4.19 mmol) was added and the reaction mixture was stirred for 16 hours at 0 °C. Solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate (30 mL) and washed with an aqueous 5% solution of sodium bicarbonate (20 mL). The aqueous phase was extracted with ethyl acetate (3 x 20 mL) until no final product was detected by TLC in the aqueous phase. The combined organic layers were dried over anhydrous magnesium sulphate, filtered and solvent removed under reduced pressure. The resulting oil was purified by column chromatography on silica gel (ethyl acetate/hexane/2-propanol 50:45:5) to afford **10** as a colourless oil (1.34 g, 2.59 mmol, 71% yield).

$[\alpha]^{27}_D$: –23.5 (*c* 0.41, $CHCl_3$).

IR (neat): ν 3307, 2979, 1691, 1514, 1261, 1023 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.81 (d, 1H, *J* = 9.9 Hz, NH), 4.97 (td, 1H, *J* = 21.7, 10.1 Hz, N-CH-P_2), overlapped with 4.90 (d, 1H, *J* = 7.6 Hz, NH), 4.24–4.11 (m, 9H, $CH_{\alpha\ Leu}$+$CH_{2\ OEt}$),

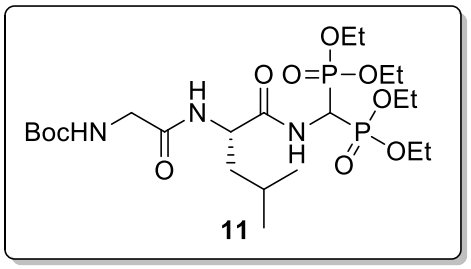
1.74–1.59 (m, 2H, CH_{Leu}+$CH_{2\ Leu}$), 1.42 (s, 10H, $CH_{3\ Boc}$+$CH_{2\ Leu}$), 1.33–1.28 (m, 12H, $CH_{3\ OEt}$), 0.93 (d, 3H, J = 6.4 Hz, $CH_{3\ Leu}$), 0.92 (d, 3H, J = 6.3 Hz, $CH_{3\ Leu}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 172.10 (t, J = 3.9 Hz, CO), 155.51 (CO), 80.29 (C_{Boc}), 63.95–63.62 ($CH_{2\ OEt}$), 53.30 ($CH_{\alpha\ Leu}$), 43.73 (t, J = 146.9 Hz, N-CH-P_2), 41.36 ($CH_{2\ Leu}$), 28.40 ($CH_{3\ Boc}$), 24.82 (CH_{Leu}), 22.99 ($CH_{3\ Leu}$), 21.99 ($CH_{3\ Leu}$), 16.51 (d, J = 5.5 Hz, $CH_{3\ OEt}$), 16.48 (d, J = 5.5 Hz, $CH_{3\ OEt}$), 16.45 (d, J = 5.6 Hz, $CH_{3\ OEt}$), 16.43 (d, J = 6.1 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 16.10 ppm.

HRMS (ESI) $C_{20}H_{42}N_2NaO_9P_2$ $[M+Na]^+$: 539.2258, found 539.2244.

Synthesis of tetraethyl [*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamidomethyl]bisphosphonate (11)



A solution of **10** (1.89 g, 3.66 mmol) in dichloromethane (50 mL) was cooled at 0 °C, trifluoroacetic acid (5.81 mL, 75.87 mmol) was added and then the solution stirred overnight at room temperature. Subsequently, solvent was removed under reduced pressure and the residue was lyophilised to obtain an oil which was used in the next step. A solution of previous oil and Boc-glycine (707 mg, 4.04 mmol) in dry *N*,*N*-dimethylformamide (6.5 mL) was cooled at 0 °C. *N*-Methylmorpholine (1.00 mL, 9.09 mmol) and 1-hydroxybenzotriazole hydrate (12% H_2O) (676 mg, 4.40 mmol) were added and the mixture was stirred 15 minutes at 0 °C. Then, *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (843 mg, 4.40 mmol) was added and the reaction mixture was stirred for 18 hours at 0 °C. Solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate (30 mL) and washed with an aqueous 5% solution of sodium bicarbonate (20 mL). The aqueous phase was extracted with ethyl acetate (3 x 20 mL) until no final product was detected by TLC in the aqueous phase. The combined organic layers were dried over anhydrous magnesium sulphate, filtered and solvent removed under reduced pressure. The resulting oil was purified by column chromatography on silica gel (dichloromethane/2-propanol 95:5) to afford **11** as a colourless oil (1.76 g, 3.07 mmol, 84% yield).

$[\alpha]^{29}_D$: –24.9 (*c* 0.37, $CHCl_3$).

M.p.: 123–125 °C.

IR (neat): ν 3294, 2980, 1716, 1640, 1263, 1031 cm^{-1}.

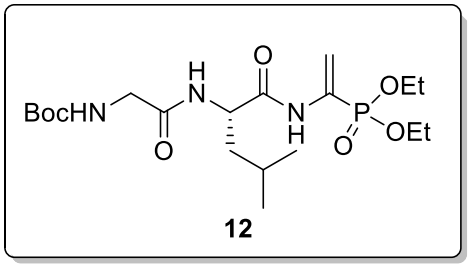
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.12 (d, 1H, J = 8.2 Hz, NH), 6.71 (br s, 1H, NH), 5.36 (br s, 1H, NH), 4.95 (td, 1H, J = 21.8, 10.1 Hz, N-CH-P_2), 4.60–4.52 (m, 1H, $CH_{\alpha\ Leu}$), 4.26–4.09 (m, 8H, $CH_{2\ OEt}$), 3.90–3.70 (m, 2H, $CH_{2\ Gly}$), 1.72–1.50 (m, 3H, CH_{Leu}+$CH_{2\ Leu}$), 1.44 (s, 9H, $CH_{3\ Boc}$), 1.35–1.27 (m, 12H, $CH_{3\ OEt}$), 0.93 (d, 3H, J = 5.4 Hz, $CH_{3\ Leu}$), 0.91 (d, 3H, J = 5.7 Hz, $CH_{3\ Leu}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 171.56 (t, J = 4.0 Hz, CO), 170.01 (CO), 156.14 (CO), 80.39 (C_{Boc}), 63.93–63.65 ($CH_{2\ OEt}$), 51.72 ($CH_{\alpha\ Leu}$), 44.49 ($CH_{2\ Gly}$), 43.87 (t, J = 147.3 Hz, N-CH-P_2), 40.96 ($CH_{2\ Leu}$), 28.41 ($CH_{3\ Boc}$), 24.77 (CH_{Leu}), 22.99 ($CH_{3\ Leu}$), 22.11 ($CH_{3\ Leu}$), 16.52 (d, J = 5.7 Hz, $CH_{3\ OEt}$), 16.49 (d, J = 5.7 Hz, $CH_{3\ OEt}$), 16.46 (d, J = 5.5 Hz, $CH_{3\ OEt}$), 16.43 (d, J = 5.5 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 16.14 ppm.

HRMS (ESI) $C_{22}H_{45}N_3NaO_{10}P_2$ $[M+Na]^+$: 596.2472, found 596.2500.

Synthesis of diethyl {1-[*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamido]ethen-1-yl}phosphonate (12)



12

To a solution of **11** (1.56 g, 2.72 mmol) in a tetrahydrofuran/2-propanol 4:1 solvent mixture (20 mL) was added cesium carbonate (1.11 mg, 3.41 mmol) and 37% aqueous formaldehyde solution (254 μL, 3.38 mmol). After 2.5 hours stirring the solvent was removed under reduced pressure, the crude suspended in dichloromethane and the solid removed by filtration. Solvent was removed under reduced pressure and the resulting oil was purified by column chromatography on silica gel (ethyl acetate/hexane/2-propanol 7.2:1.8:1) to afford **12** as a colourless oil (746 mg, 1.66 mmol, 61% yield).

$[\alpha]^{25}_{D}$: –44.6 (*c* 0.44, $CHCl_3$).

IR (neat): ν 3294, 2980, 1720, 1667, 1537, 1255, 1022 cm^{-1}.

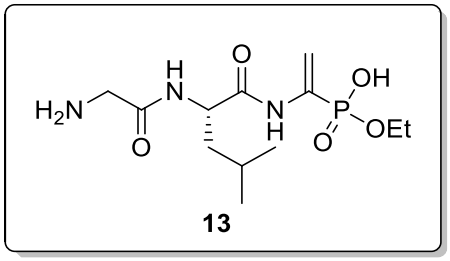
^{1}H NMR (400 MHz, $CDCl_3$): *δ* 8.19 (br s, 1H, NH), 7.16 (br s, 1H, NH), 6.55 (d, 1H, *J* = 41.4 Hz, *E*-CH_2=C), 5.56 (d, 1H, *J* = 18.9 Hz, *Z*-CH_2=C) overlapped with 5.55 (br s, 1H, NH), 4.59–4.49 (m, 1H, $CH_{\alpha\ Leu}$), 4.13–3.97 (m, 4H, $CH_{2\ OEt}$), 3.84–3.71 (m, 2H, $CH_{2\ Gly}$), 1.69–1.49 (m, 3H, CH_{Leu}+$CH_{2\ Leu}$), 1.38 (s, 9H, $CH_{3\ Boc}$), 1.32–1.24 (m, 6H, $CH_{3\ OEt}$), 0.87 (d, 3H, *J* = 9.4 Hz, $CH_{3\ Leu}$), 0.86 (d, 3H, *J* = 9.9 Hz, $CH_{3\ Leu}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): *δ* 171.67 (d, *J* = 10.3 Hz, CO), 170.18 (CO), 156.15 (CO), 131.45 (d, *J* = 200.4 Hz, N-C-P), 115.20 (d, *J* = 9.5 Hz, $\underline{C}H_2$=C), 80.10 (C_{Boc}), 63.12 (d, *J* = 5.5 Hz, $CH_{2\ OEt}$), 52.47 ($CH_{\alpha\ Leu}$), 44.13 ($CH_{2\ Gly}$), 40.61 ($CH_{2\ Leu}$), 28.32 ($CH_{3\ Boc}$), 24.74 (CH_{Leu}), 23.98 ($CH_{3\ Leu}$), 21.92 ($CH_{3\ Leu}$), 16.22 (d, *J* = 6.3 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): *δ* 11.95 ppm.

HRMS (ESI) $C_{19}H_{36}N_3NaO_7P$ $[M+Na]^+$: 472.2183, found 472.2168.

Synthesis of [1-(glycyl-L-leucylamido)ethen-1-yl]phosphonic acid monoethyl ester (13)



13

A solution of **20** (402 mg, 0.92 mmol) in dichloromethane (15 mL) was cooled at 0 °C, trifluoroacetic acid (1.41 mL, 18.41 mmol) was added and then the solution stirred overnight at room temperature. Subsequently, solvent was removed under reduced pressure and the resulting residue was treated with weakly basic anion-exchange resin (Dowex® 66 free base) to afford an oil used in the next step. Previous oil was dissolved in a mixture of acetone/toluene 1:1 (5 mL) and 1,4-diazabicyclo[2.2.2]octane (215 mg, 1.92 mmol) was added. The reaction mixture was stirred at reflux for 4.5 hours. Solvent was removed, the crude dissolved in water (10 mL) and filtered through 0.22 μm HPLC filter. Then, the aqueous solution was washed with dichloromethane (2 x 5 mL) and ethyl acetate (2 x 5 mL) and concentrated in vacuo. The resulting residue was purified using a weakly acidic ion-exchange resin (Amberlite® CG50, hydrogen form) to afford **13** as a white solid (170 mg, 0.53 mmol, 58% yield).

$[\alpha]^{28}_{D}$: –6.0 (*c* 0.27, H_2O).

M.p.: 167–169 °C (dec).

IR (neat): ν 3113, 2961, 1670, 1537, 1206, 1071, 1043 cm^{-1}.

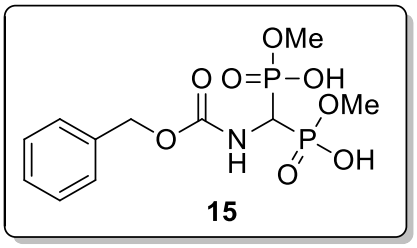
^{1}H NMR (400 MHz, D_2O): δ 6.15 (d, 1H, *J* = 36.3 Hz, *E*-CH_2=C), 5.68 (d, 1H, 16.0 Hz, *Z*-CH_2=C), 4.47–4.40 (m, 1H, $CH_{\alpha\ Leu}$), 3.90–3.80 (m, 4H, $CH_{2\ Gly}$+$CH_{2\ OEt}$), 1.73–1.60 (m, 3H, CH_{Leu}+$CH_{2\ Leu}$), 1.24–1.18 (m, 3H, $CH_{3\ OEt}$), 0.97–0.88 (m, 6H, $CH_{3\ Leu}$) ppm.

^{13}C NMR (100 MHz, D_2O): δ 173.52 (d, J = 7.7 Hz, CO), 167.13 (CO), 135.25 (d, J = 189.8 Hz, N-C-P), 116.49 (d, J = 11.5 Hz, $\underline{C}H_2$=C), 61.63 (d, J = 5.1 Hz, $CH_{2\ OEt}$), 53.36 ($CH_{\alpha\ Leu}$), 40.29 ($CH_{2\ Gly}$), 39.69 ($CH_{2\ Leu}$), 24.33 (CH_{Leu}), 22.07 ($CH_{3\ Leu}$), 20.65 ($CH_{3\ Leu}$), 15.7 (d, J = 6.3 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, D_2O): δ 8.01 ppm.

HRMS (ESI) $C_{12}H_{24}N_3NaO_5P$ $[M+Na]^+$: 344.1346, found 344.1342.

Synthesis of (benzyloxycarbonylaminomethyl)bisphosphonic acid monomethyl ester (15)



15

To a solution of tetramethyl (benzyloxycarbonylaminomethyl)bisphosphonate, compound **2** from chapter 1, (1.85 g, 4.85 mmol) in a mixture of acetone/toluene 1:1 (50 mL) 1,4-diazabicyclo[2.2.2]octane (1.38 g, 12.30 mmol) was added. The reaction mixture was stirred at reflux for 7 hours. Then, solvent was removed, and the resulting residue was purified using an acidic ion-exchange resin (Dowex® 50WX8, hydrogen form) to afford **15** as a white solid (1.66 g, 4.70 mmol, 97% yield).

M.p.: 156–158 °C.

IR (KBr): ν 3385, 2622, 1717, 1518, 1263, 1056 cm^{-1}.

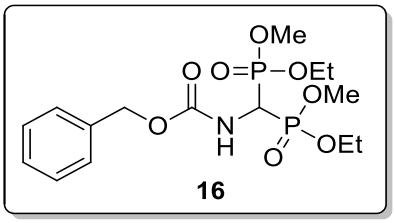
^{1}H NMR (400 MHz, D_2O): δ (duplicated signals are observed for some protons, asterisk indicate those corresponding to the minor rotamer) 7.47–7.36 (m, 5H, CH_{Ar}), 5.20* (s, 2H, CH_2-Ph), 5.16 (s, 2H, CH_2-Ph), 4.41* (t, 1H, J = 21.4 Hz, N-CH-P_2), 4.32 (t, 1H, J = 21.6 Hz, N-CH-P_2), 3.66–3.58 (m, 6H, $CH_{3\ OMe}$) ppm.

^{13}C NMR (100 MHz, D_2O): δ (duplicated signals are observed for some carbons, asterisk indicate those corresponding to the minor rotamer) 157.58 (t, J = 5.0 Hz, CO), 136.27 (C_{Ar}), 128.71 (CH_{Ar}), 128.34 (CH_{Ar}), 127.94* (CH_{Ar}), 127.61 (CH_{Ar}), 67.85* (CH_2-Ph), 67.53 (CH_2-Ph), 52.87 ($CH_{3\ OMe}$), 46.13 (t, J = 141.1 Hz, N-CH-P_2) ppm.

^{31}P NMR (162 MHz, D_2O): δ (duplicated signals are observed, asterisk indicate the corresponding to the minor rotamer) 16.49, 16.19* ppm.

HRMS (ESI) $C_{11}H_{16}NO_8P_2$ [M-H]$^-$:352.0357, found 352.0347.

Synthesis of diethyl dimethyl (benzyloxycarbonylaminomethyl)bisphosphonate (16)



16

To a solution of **15** (151 mg, 0.43 mmol) and (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (668 mg, 1.28 mmol) in dry *N*,*N*-dimethylformamide (2 mL) were added absolute ethanol (75 µL, 1.28 mmol) and *N*,*N*-diisopropylethylamine (0.60 mL, 3.44 mmol). The reaction mixture was stirred under argon for 2 hours. The solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate (20 mL), washed with a saturated solution of sodium bicarbonate (3 x 10 mL) and brine (3 x 10 mL). The combined organic layers were dried over anhydrous magnesium sulphate, filtered and solvent removed under reduced pressure. The resulting oil was purified by column chromatography on silica gel (ethyl acetate/hexane/methanol 6:3:0.5) to afford **16** as a colourless oil (159 mg, 0.39 mmol, 91% yield).

IR (neat): ν 3225, 2984, 1721, 1539, 1282, 1016 cm^{-1}.

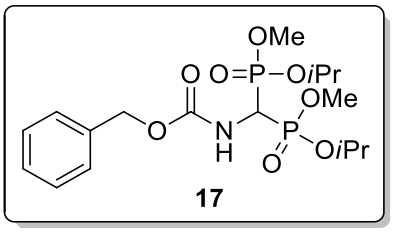
^{1}H NMR (400 MHz, $CDCl_3$): δ (signals appear as a mixture of the four diastereoisomers) 7.38–7.29 (m, 4 x 5H, CH_{Ar}), 5.86–5.75 (m, 4 x 1H, NH), 5.19–5.12 (m, 4 x 2H, CH_2-Ph), 4.73–4.56 (m, 4 x 1H, N-CH-P_2), 4.24–4.08 (m, 4 x 4H, $CH_{2\ OEt}$), 3.83–3.71 (m, 4 x 6H, $CH_{3\ OMe}$), 1.33–1.22 (m, 4 x 6H, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ (signals appear as a mixture of the four diastereoisomers) 155.92–155.72 (4 x CO), 136.00 (4 x C_{Ar}), 128.69 (4 x CH_{Ar}), 128.54 (4 x CH_{Ar}), 128.42–128.25 (4 x CH_{Ar}), 67.92 (4 x CH_2-Ph), 64.32–63.96 (4 x $CH_{2\ OEt}$), 54.25–53.88 (4 x $CH_{3\ OMe}$), 47.27–43.98 (4 x N-CH-P_2), 16.52–16.31 (4 x $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 17.92 (1 x 2P, *meso*-**16**), 17.66 (1 x 2P, *meso*-**16**), and 17.91, 17.68 (AB spin system, 2 x 2P, J = 37.0 Hz, *ent*$_a$-**16**+*ent*$_b$-**16**) ppm.

HRMS (ESI) $C_{15}H_{25}NNaO_8P_2$ $[M+Na]^+$: 432.0948, found 432.0945.

Synthesis of dimethyl diprop-2-yl (benzyloxycarbonylaminomethyl)bisphosphonate (17)



17

To a solution of **15** (252 mg, 0.71 mmol) and (benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate (1.11 g, 2.13 mmol) in dry *N*,*N*-dimethylformamide (2 mL) were added 2-propanol (0.16 mL, 2.09 mmol) and *N*,*N*-diisopropylethylamine (0.99 mL, 5.68 mmol). The reaction mixture was stirred under argon for 2 hours. The solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate (20 mL), washed with a saturated solution of sodium bicarbonate (3 x 10 mL) and brine (3 x 10 mL). The combined organic layers were dried over anhydrous magnesium sulphate, filtered and solvent removed under reduced pressure. The resulting oil was purified by column chromatography on silica gel (ethyl acetate/hexane/methanol 6:3:0.5) to afford **17** as a colourless oil (192 mg, 0.44 mmol, 62% yield).

IR (neat): ν 3229, 2982, 1733, 1541, 1235, 990 cm^{-1}.

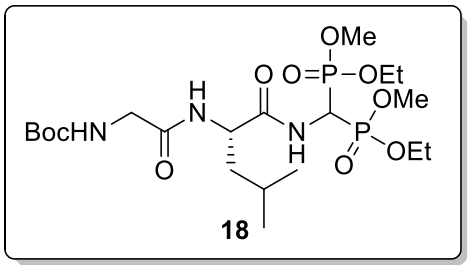
^{1}H NMR (400 MHz, $CDCl_3$): δ (signals appear as a mixture of the four diastereoisomers) 7.37–7.30 (m, 4 x 5H, CH_{Ar}), 5.35–5.29 (m, 4 x 1H, NH), 5.18–5.12 (m, 4 x 2H, CH_2-Ph), 4.83–4.71 (m, 4 x 2H, CH_{OiPr}), 4.66–4.48 (m, 4 x 1H, N-CH-P_2), 3.83–3.74 (m, 4 x 6H, 4 x $CH_{3\ OMe}$), 1.36–1.23 (m, 4 x 12H, $CH_{3\ OiPr}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ (signals appear as a mixture of the four diastereoisomers) 155.77–155.56 (4 x CO), 136.16–136.02 (4 x C_{Ar}), 128.64 (4 x CH_{Ar}), 128.49–128-40 (4 x CH_{Ar}), 128.37–128.25 (4 x CH_{Ar}), 73.22–72.89 (4 x CH_{OiPr}), 67.88–67.71 (4 x CH_2-Ph), 53.89–53.67 (4 x $CH_{3\ OMe}$), 48.12–44.43 (4 x N-CH-P_2), 24.34–23.18 (4 x $CH_{3\ OiPr}$), 23.86–23.64 (4 x $CH_{3\ OiPr}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 17.01 (1 x 2P, *meso*-**17**), 16.40 (1 x 2P, *meso*-**17**), and 17.09, 16.44 (AB spin system, 2 x 2P, J = 36.6 Hz, *ent*$_a$-**17**+*ent*$_b$-**17**) ppm.

HRMS (ESI) $C_{17}H_{29}NNaO_8P_2$ $[M+Na]^+$: 460.1261, found 460.1289.

Synthesis of diethyl dimethyl [*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamidomethyl]bisphosphonate (18)



To a solution of **16** (616 mg, 1.50 mmol) in ethanol (25 mL) 10% wt. palladium on carbon (62 mg) was added. The mixture was stirred under a hydrogen atmosphere at room temperature for one hour. Then, the reaction mixture was filtered (Celite) and evaporated to afford an oil which was used in the next step. A solution of previous oil and Boc-glycine-L-leucine (486 mg, 1.69 mmol) in dry *N,N*-dimethylformamide (7.6 mL) was cooled at 0 °C. *N*-Methylmorpholine (220 µL, 2.00 mmol) and 1-hydroxybenzotriazole hydrate (12% H_2O) (282 mg, 1.84 mmol) were added and the mixture was stirred 15 minutes at 0 °C. Then *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (353 mg, 1.84 mmol) was added, the reaction mixture was stirred 20 hours at 0 °C. Solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate (30 mL) and washed with an aqueous 5% solution of sodium bicarbonate (20 mL). The aqueous phase was extracted with ethyl acetate (3 x 20 mL) until no final product was detected by TLC in the aqueous phase. The combined organic layers were dried over anhydrous magnesium sulphate, filtered and solvent removed under reduced pressure. The resulting oil was purified by column chromatography on silica gel (dichloromethane/2-propanol 9:1) to afford **18** as a colourless oil (616 mg, 1.13 mmol, 75% yield).

IR (KBr): ν 3291, 2958, 1717, 1675, 1558, 1272, 1244, 1030 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ (signals appear as a mixture of the four diastereoisomers) 7.38–7.27 (m, 4 x 1H, NH), 6.81–6.71 (m, 4 x 1H, NH), 5.46–5.31 (m, 4 x 1H, NH), 5.09–4.92 (m, 4 x 1H, N-CH-P_2), 4.64–4.53 (m, 4 x 1H, $CH_{\alpha\,Leu}$), 4.25–4.11 (m, 4 x 4H,

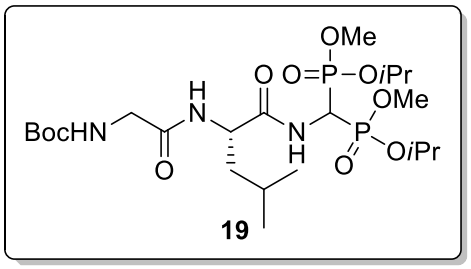
$CH_{2\ OEt}$), 3.89–3.73 (m, 4 x 8H, $CH_{2\ Gly}$+$CH_{3\ OMe}$), 1.72–1.59 (m, 4 x 3H, CH_{Leu}+$CH_{2\ Leu}$), 1.49–1.40 (m, 4 x 9H, $CH_{3\ Boc}$), 1.35–1.28 (m, 4 x 6H, $CH_{3\ OEt}$), 0.97–0.86 (m, 4 x 6H, $CH_{3\ Leu}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ (signals appear as a mixture of the four diastereoisomers) 171.88–171.65 (4 x CO), 170.01 (4 x CO), 156.17 (4 x CO), 80.37 (4 x C_{Boc}), 64.19–63.83 (4 x $CH_{2\ OEt}$), 54.22–53.91 (4 x $CH_{3\ OMe}$), 51.69 (4 x $CH_{\alpha\ Leu}$), 44.98–41.79 (4 x CH-P_2), 44.46 (4 x $CH_{2\ Gly}$), 40.98 (4 x $CH_{2\ Leu}$), 28.41 (4 x $CH_{3\ Boc}$), 24.77 (4 x CH_{Leu}), 22.99 (4 x $CH_{3\ Leu}$), 22.12 (4 x $CH_{3\ Leu}$), 16.58–16.36 (4 x $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 17.74–16.98 (1 x 2P, *meso*-**18**, 1 x 2P, *meso*-**18**, 2 x 2P, *ent*$_a$-**18+** *ent*$_b$-**18**) ppm.

HRMS (ESI) $C_{20}H_{41}N_3NaO_{10}P_2$ $[M+Na]^+$: 568.2159, found 568.2147.

Synthesis of dimethyl diprop-2-yl [*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamidomethyl]bisphosphonate (19)



To a solution of **17** (321 mg, 0.73 mmol) in ethanol (12 mL) 10% wt. palladium on carbon (32 mg) was added. The mixture was stirred under a hydrogen atmosphere at room temperature for one hour. Then, the reaction mixture was filtered (Celite) and evaporated to afford an oil which was used in the next step. A solution of previous oil and Boc-glycine-L-leucine (228 mg, 0.79 mmol) in dry *N,N*-dimethylformamide (4 mL) was cooled at 0 °C. *N*-Methylmorpholine (103 µL, 0.94 mmol) and 1-hydroxybenzotriazole hydrate (12% H_2O) (133 mg, 0.87 mmol) were added and the mixture was stirred 15 minutes at 0 °C. Then *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (166 mg, 0.87 mmol) was added, the reaction mixture was stirred 24 hours at 0 °C. Solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate (30 mL) and washed with an aqueous 5% solution of sodium bicarbonate (20 mL). The aqueous phase was extracted with ethyl acetate (3 x 20 mL) until no final product was detected by TLC in the aqueous phase. The combined organic layers were dried over anhydrous magnesium sulphate, filtered and solvent removed under reduced pressure. The resulting oil was purified by column chromatography on silica gel (ethyl acetate/hexane/2-propanol 6:3:1) to afford **19** as a colourless oil (306 mg, 0.53 mmol, 73% yield).

IR (KBr): ν 3290, 2979, 1717, 1650, 1548, 1269, 1245, 1047, 993 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ (signals appear as a mixture of the four diastereoisomers) 7.17–6.95 (m, 4 x 1H, NH), 6.74–6.61 (m, 4 x 1H, NH), 5.43–5.27 (m, 4 x 1H, NH), 5.03–4.84 (m, 4 x 1H, N-CH-P_2), 4.83–4.69 (m, 4 x 2H, CH_{OiPr}), 4.61–4.52 (m, 4 x 1H,

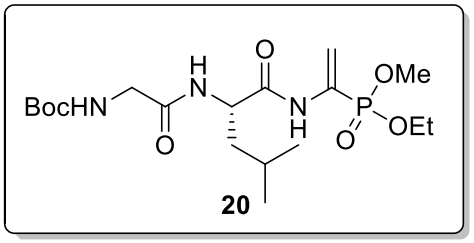
$CH_{\alpha\ Leu}$), 3.90–3.72 (m, 4 x 8H, $CH_{2\ Gly}$+$CH_{3\ OMe}$), 1.73–1.51 (m, 4 x 3H, CH_{Leu}+$CH_{2\ Leu}$), 1.48–1.41 (m, 4 x 9H, $CH_{3\ Boc}$), 1.37–1.29 (m, 4 x 12H, $CH_{3\ O\mathit{i}Pr}$), 0.95–0.89 (m, 4 x 6H, $CH_{3\ Leu}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ (signals appear as a mixture of the four diastereoisomers) 171.55–171.38 (4 x CO), 170.01 (4 x CO), 156.11 (4 x CO), 80.39 (4 x C_{Boc}), 73.29–72.85 (4 x $CH_{O\mathit{i}Pr}$), 54.08–53.77 (4 x $CH_{3\ OMe}$), 51.81–51.53 (4 x $CH_{\alpha\ Leu}$), 45.70–42.27 (4 x CH-P_2), 44.49 (4 x $CH_{2\ Gly}$), 40.94 (4 x $CH_{2\ Leu}$), 28.41 (4 x $CH_{3\ Boc}$), 24.74 (4 x CH_{Leu}), 24.36–24.17 (4 x $CH_{3\ O\mathit{i}Pr}$), 24.00–23.73 (4 x $CH_{3\ O\mathit{i}Pr}$), 23.09–22.96 (4 x $CH_{3\ Leu}$), 22.18–21.99 (4 x $CH_{3\ Leu}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 16.70 (1 x 2P, *meso*-**19**), 16.18 (1 x 2P, *meso*-**19**) and 16.72, 16.21 (AB spin system, 2 x 2P, J = 30.3 Hz, *ent*$_a$-**19**+*ent*$_b$-**19**) ppm.

HRMS (ESI) $C_{22}H_{45}N_3NaO_{10}P_2$ $[M+Na]^+$: 596.2472, found 596.2499.

Synthesis of ethyl methyl {1-[*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamido]ethen-1-yl}phosphonate (20)



20

To a solution of **18** (138 mg, 0.25 mmol) in a mixture of tetrahydrofuran/2-propanol 4:1 (5 mL) was added cesium carbonate (103 mg, 0.32 mmol) and 37% aqueous formaldehyde solution (24 μL, 0.32 mmol). After stirring for 3 hours at room temperature, solvent was removed under reduced pressure, the crude suspended in dichloromethane and the solid removed by filtration. Then, solvent was removed under reduced pressure and the resulting oil was purified by column chromatography on silica gel (ethyl acetate/hexane/2-propanol 6:3:0.5) to afford **20** as a colourless oil (97 mg, 0.22 mmol, 88% yield).

IR (neat): ν 3303, 2959, 1709, 1678, 1537, 1254, 1167, 1026 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ (some signals appear as a mixture of two diastereoisomers) 8.02 (t, 2 x 1H, J = 7.1 Hz, NH), 6.81 (d, 2 x 1H, J = 6.3 Hz, NH), 6.61 (d, 1H, J = 41.6, *E*-CH_2=C), 6.61 (d, 1H, J = 41.6, *E*-CH_2=C), 5.62 (d, 2 x 1H, J = 19.0, *Z*-CH_2=C), 5.61 (d, 2 x 1H, J = 19.0, *Z*-CH_2=C), 5.36 (br s, 2 x 1H, NH), 4.57–4.49 (m, 2 x 1H, $CH_{\alpha\ Leu}$) 4.20–4.05 (m, 2 x 2H, $CH_{2\ OEt}$), 3.89–3.77 (m, 2 x 2H, $CH_{2\ Gly}$), 3.74 (d, 3H, J = 11.2 Hz, $CH_{3\ OMe}$), 3.74 (d, 3H, J = 11.2 Hz, $CH_{3\ OMe}$), 1.73–1.52 (m, 2 x 3H, CH_{Leu}+$CH_{2\ Leu}$), 1.44 (s, 2 x 9H, $CH_{3\ Boc}$), 1.34 (t, 2 x 3H, J = 7.1 Hz, $CH_{3\ OEt}$), 0.92 (d, 2 x 3H, J = 8.9 Hz, $CH_{3\ Leu}$), 0.91 (d, 2 x 3H, J = 8.8 Hz, $CH_{3\ Leu}$) ppm.

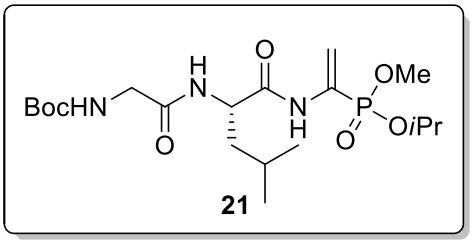
^{13}C NMR (100 MHz, $CDCl_3$): δ (some signals appear as a mixture of two diastereoisomers) 171.37 (d, J = 10.1 Hz, 2 x CO), 170.22 (2 x CO), 156.23 (2 x CO), 130.83 (d, J = 200.8 Hz, 2 x N-C-P), 115.47 (d, J = 7.5 Hz, 2 x $\underline{C}H_2$=C), 80.57 (2 x C_{Boc}), 63.40 (d, J = 5.5

Hz, $CH_{2\ OEt}$), 63.38 (d, *J* = 5.5 Hz, $CH_{2\ OEt}$), 53.30 (d, *J* = 5.5 Hz, $CH_{3\ OMe}$), 53.29 (d, *J* = 5.5 Hz, $CH_{3\ OMe}$), 52.53 (2 x $CH_{\alpha\ Leu}$), 44.49 (2 x $CH_{2\ Gly}$), 40.69 ($CH_{2\ Leu}$), 40.64 ($CH_{2\ Leu}$), 28.39 (2 x $CH_{3\ Boc}$), 24.84 (CH_{Leu}), 23.07 (2 x $CH_{3\ Leu}$), 22.01 ($CH_{3\ Leu}$), 22.00 ($CH_{3\ Leu}$), 16.3 (d, *J* = 6.2 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 13.32 (2 x 1P) ppm.

HRMS (ESI) $C_{18}H_{34}N_3NaO_7P$ $[M+Na]^+$: 458.2027, found 458.2065.

Synthesis of methyl prop-2-yl {1-[*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamido]ethen-1-yl}phosphonate (21)



21

To a solution of **19** (222 mg, 0.39 mmol) in a tetrahydrofuran/2-propanol 4:1 solvent mixture (5 mL) was added cesium carbonate (158 mg, 0.48 mmol) and 37% aqueous formaldehyde solution (36 µL, 0.48 mmol). After stirring for 7 hours, solvent was removed under reduced pressure, the crude suspended in dichloromethane and the solid removed by filtration. Then, solvent was removed under reduced pressure and the resulting oil was purified by column chromatography on silica gel (ethyl acetate/hexane/2-propanol 6:3:0.5) to afford **21** as a yellow oil (141 mg, 0.31 mmol, 79% yield).

IR (KBr): ν 3290, 2979, 1717, 1650, 1550, 1269, 1179, 1047, 993 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ (some signals appear as a mixture of two diastereoisomers) 7.99 (d, 2 x 1H, *J* = 7.1 Hz, NH), 6.86 (d, 2 x 1H, *J* = 7.0 Hz, NH), 6.59 (d, 2 x 1H, *J* = 41.5 Hz, *E*-CH_2=C), 5.60 (d, 2 x 1H, *J* = 19.1 Hz, *Z*-CH_2=C), 5.38 (br s, 2 x 1H, NH), 4.75–4.63 (m, 2 x 1H, $CH_{O\textit{i}Pr}$), 4.56–4.48 (m, 2 x 1H, $CH_{\alpha\ Leu}$), 3.89–3.77 (m, 2 x 2H, $CH_{2\ Gly}$), 3.71 (d, 2 x 3H, *J* = 11.3 Hz, $CH_{3\ OMe}$), 1.74–1.49 (m, 2 x 3H, CH_{Leu}+$CH_{2\ Leu}$), 1.43 (s, 2 x 9H, $CH_{3\ Boc}$), 1.35 (d, 2 x 3H, *J* = 6.2 Hz, $CH_{3\ O\textit{i}Pr}$), 1.30 (d, 2 x 3H, *J* = 6.2 Hz, $CH_{3\ O\textit{i}Pr}$), 0.92 (d, 2 x 3H, *J* = 6.2 Hz, $CH_{3\ Leu}$), 0.90 (d, 2 x 3H, *J* = 6.1 Hz, $CH_{3\ Leu}$) ppm.

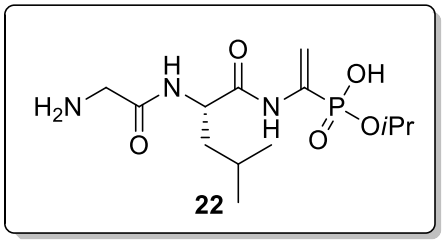
^{13}C NMR (100 MHz, $CDCl_3$): δ (some signals appear as a mixture of two diastereoisomers) 171.31 (d, *J* = 10.2 Hz, 2 x CO), 170.18 (d, *J* = 1.4 Hz, 2 x CO), 156.25 (2 x CO), 131.45 (d, *J* = 201.4 Hz, 2 x N-C-P), 115.14 (d, *J* = 5.7 Hz, 2 x $\underline{C}H_2$=C), 80.55 (2 x C_{Boc}), 72.72 (d, *J* = 5.8 Hz, $CH_{O\textit{i}Pr}$), 72.69 (d, *J* = 5.7 Hz, $CH_{O\textit{i}Pr}$), 53.13 (d, *J* = 5.4 Hz, $CH_{3\ OMe}$), 53.09 (d, *J* = 5.4 Hz, $CH_{3\ OMe}$), 52.57 (2 x $CH_{\alpha\ Leu}$), 44.58 (2 x $CH_{2\ Gly}$), 40.76 ($CH_{2\ Leu}$), 40.68 ($CH_{2\ Leu}$),

28.40 (2 x $CH_{3\ Boc}$), 24.86 (2 x CH_{Leu}), 24.09 (d, *J* = 3.9, $CH_{3\ O\mathit{i}Pr}$), 24.06 (d, *J* = 3.9, $CH_{3\ O\mathit{i}Pr}$), 23.79 (d, *J* = 4.9 Hz, $CH_{3\ O\mathit{i}Pr}$), 23.78 (d, *J* = 4.9 Hz, $CH_{3\ O\mathit{i}Pr}$), 23.06 ($CH_{3\ Leu}$), 23.05 ($CH_{3\ Leu}$), 22.04 ($CH_{3\ Leu}$), 22.01 ($CH_{3\ Leu}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 12.22 (2 x 1P) ppm.

HRMS (ESI) $C_{19}H_{36}N_3NaO_7P$ $[M+Na]^+$: 472.2183, found 472.2204.

Synthesis of [1-(glycyl-L-leucylamido)ethen-1-yl]phosphonic acid monoprop-2-yl ester (22)



A solution of **21** (134 mg, 0.30 mmol) in dichloromethane (6 mL) was cooled at 0 °C, trifluoroacetic acid (0.45 mL, 5.88 mmol) was added and then the solution stirred overnight at room temperature. Subsequently, solvent was removed under reduced pressure and the resulting residue was treated with weakly basic anion-exchange resin (Dowex® 66 free base) to afford an oil which was used in the next step. Previous oil was dissolved in a mixture of acetone/toluene 1:1 (5 mL) and 1,4-diazabicyclo[2.2.2]octane (66 mg, 0.59 mmol) was added. The reaction mixture was stirred at reflux for 4.5 hours. Solvent was removed, the crude dissolved in water (10 mL) and filtered through 0.22 μm HPLC filter. Then, the aqueous solution was washed with dichloromethane (2 x 5 mL) and ethyl acetate (2 x 5 mL) and concentrated in vacuo. The resulting residue was purified using a weakly acidic ion-exchange resin (Amberlite® CG50, hydrogen form) to afford **22** (77 mg, 0.23 mmol, 77% yield).

$[\alpha]^{27}_D$: –5.5 (*c* 0.28, H_2O).

M.p.: 175–177 °C (dec).

IR (neat): ν 3342, 2971, 2684, 1684, 1662, 1529, 1212, 1065 cm^{-1}.

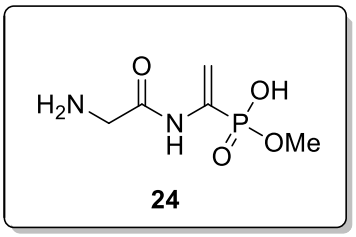
^{1}H NMR (400 MHz, D_2O): δ 6.15 (d, 1H, *J* = 36.3 Hz, *E*-CH_2=C), 5.67 (d, 1H, *J* = 16.1 Hz, *Z*-CH_2=C), 4.47–4.40 (m, 1H, $CH_{\alpha\ Leu}$), 4.39–4.29 (m, 1H, CH_{OiPr}), 3.87 (s, 2H, $CH_{2\ Gly}$), 1.73–1.61 (m, 3H, CH_{Leu}+$CH_{2\ Leu}$), 1.20 (d, 3H, *J* = 6.2 Hz, $CH_{3\ OiPr}$), 1.20 (d, 3H, *J* = 6.2 Hz, $CH_{3\ OiPr}$), 0.97–0.88 (m, 6H, $CH_{3\ Leu}$) ppm.

^{13}C NMR (100 MHz, D_2O): δ 173.43 (d, *J* = 8.1 Hz, CO), 167.14 (CO), 135.91 (d, *J* = 190.0 Hz, N-C-P), 115.45 (d, *J* = 11.1 Hz, $\underline{C}H_2$=C), 70.09 (d, *J* = 5.4 Hz, $CH_{O\textit{i}Pr}$), 53.43 ($CH_{\alpha\ Leu}$), 40.32 ($CH_{2\ Gly}$), 39.72 ($CH_{2\ Leu}$), 24.36 (CH_{Leu}), 23.28 (d, *J* = 3.9 Hz, $CH_{3\ O\textit{i}Pr}$), 23.24 (d, *J* = 3.9 Hz, $CH_{3\ O\textit{i}Pr}$), 22.10 ($CH_{3\ Leu}$), 20.64 ($CH_{3\ Leu}$) ppm.

^{31}P NMR (162 MHz, D_2O): δ 6.60 ppm.

HRMS (ESI) $C_{13}H_{26}N_3NaO_5P$ $[M+Na]^+$: 358.1502, found 358.1499.

Synthesis of [1-(glycylamido)ethen-1-yl]phosphonic acid monomethyl ester (24)



24

To a solution of **26** (199 mg, 0.46 mmol) in a mixture of acetone/toluene 1:1 (7 mL) 1,4-diazabicyclo[2.2.2]octane (131 mg, 1.17 mmol) was added. The reaction mixture was stirred at reflux for 2 hours. The solvent was then removed, water (10 mL) was added and filtered with 0.45-1 μm HPLC filter and with 0.20 μm HPLC filter. Then, the aqueous layer was washed with dichloromethane (2 x 5 mL) and ethyl acetate (2 x 5 mL) and concentrated in vacuo. The resulting residue was purified using a weakly acidic ion-exchange resin (Amberlite® CG50, hydrogen form) to afford **24** as a pale yellow solid (82 mg, 0.42 mmol, 91% yield).

M.p.: 153–155 °C (dec).

IR (KBr): ν 3418, 1694, 1623, 1543, 1202, 1042 cm^{-1}.

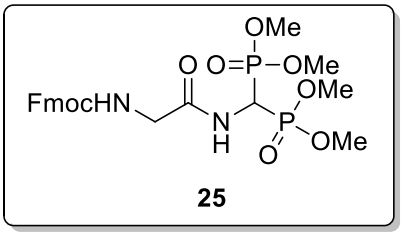
^{1}H NMR (400 MHz, D_2O): δ 6.17 (d, 1H, J = 36.2 Hz, E-CH_2=C), 5.67 (d, 1H, J = 15.9 Hz, Z-CH_2=C), 3.88 (s, 2H, $CH_{2\ Gly}$), 3.49 (d, 3H, J = 11 Hz, $CH_{3\ OMe}$) ppm.

^{13}C NMR (100 MHz, D_2O): δ 166.04 (d, J = 8.6 Hz, CO), 134.41 (d, J = 191.0 Hz, N-C-P), 117.01 (d, J = 11.7 Hz, $\underline{C}H_2$=C), 51.88 (d, J = 5.2 Hz, $CH_{3\ OMe}$), 41.01 ($CH_{2\ Gly}$) ppm.

^{31}P NMR (162 MHz, D_2O): δ 9.48 ppm.

HRMS (ESI) $C_5H_{11}N_2NaO_4P$ $[M+Na]^+$: 217.0349, found 217.0343.

Synthesis of tetramethyl [*N*-(9-fluorenylmethoxycarbonyl)glycylamidomethyl]bisphosphonate (25)



25

To a solution of tetramethyl (benzyloxycarbonylaminomethyl)bisphosphonate, compound **2** from chapter 1, (507 mg, 1.33 mmol) in methanol (15 mL) 10% wt. palladium on carbon (50 mg) was added. The mixture was stirred under a hydrogen atmosphere at room temperature for one hour. Then, the reaction mixture was filtered (Celite) and evaporated to afford an oil which was used in the next step. A solution of previous oil and Fmoc-glycine (434 mg, 1.46 mmol) in dry *N,N*-dimethylformamide (7 mL) was cooled at 0 °C. *N*-Methylmorpholine (190 μL, 1.73 mmol) and 1-hydroxybenzotriazole hydrate (12% H_2O) (245 mg, 1.60 mmol) were added and the mixture was stirred 15 minutes at 0 °C. Then *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (305 mg, 1.59 mmol) was added and the reaction mixture was stirred 17 hours at 0 °C. The solvent was removed under reduced pressure, the residue was dissolved in ethyl acetate (30 mL) and washed with a 5% solution of sodium bicarbonate. The aqueous phase was extracted with ethyl acetate (3 x 15 mL) until no final product was detected by TLC in the aqueous phase. The combined organic layers were dried with magnesium sulphate anhydrous, filtered and the solvent removed under reduced pressure. The resulting oil was purified by column chromatography on silica gel (dichloromethane/2-propanol 9:1) to afford **25** as a white solid (464 mg, 0.88 mmol, 66% yield).

M.p.: 146–148 °C.

IR (KBr): ν 3288, 2958, 1717, 1539, 1261, 1042 cm^{-1}.

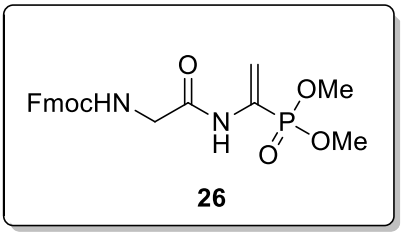
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08 (d, 1H, J = 9.9 Hz, NH), 7.74 (d, 2H, J = 7.5 Hz, $CH_{Ar\ Fmoc}$), 7.58 (d, 2H, J = 7.4 Hz, $CH_{Ar\ Fmoc}$), 7.37 (t, 2H, J = 7.4 Hz, $CH_{Ar\ Fmoc}$), 7.29 (td, 2H, J = 7.5, 1.1 Hz, $CH_{Ar\ Fmoc}$), 5.89 (t, 1H, J = 5.2 Hz, NH), 5.14 (td, 1H, J = 22.1, 10.0 Hz, N-CH-P_2), 4.36 (d, 2H, J = 7.2 Hz, $CH_{2\ Fmoc}$), 4.20 (t, 1H, J = 7.1 Hz, CH_{Fmoc}), 4.05 (d, 2H, J = 5.5 Hz, $CH_{2\ Gly}$), 3.87–3.72 (m, 12 H, $CH_{3\ OMe}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 169.29 (t, J = 4.2 Hz, CO), 156.53 (CO), 143.84 ($C_{Ar\ Fmoc}$), 141.31 ($C_{Ar\ Fmoc}$), 127.79 ($CH_{Ar\ Fmoc}$), 127.11 ($CH_{Ar\ Fmoc}$), 125.15 ($CH_{Ar\ Fmoc}$), 120.05 ($CH_{Ar\ Fmoc}$), 67.22 ($CH_{2\ Fmoc}$), 54.26 (d, J = 3.0 Hz, $CH_{3\ OMe}$), 54.22 (d, J = 3.1 Hz, $CH_{3\ OMe}$), 47.11 (CH_{Fmoc}), 44.09 ($CH_{2\ Gly}$), 42.74 (t, J = 148.8 Hz, N-CH-P) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 18.45 ppm.

HRMS (ESI) $C_{22}H_{28}N_2NaO_9P_2$ $[M+Na]^+$: 549.1162, found 549.1160.

Synthesis of dimethyl {1-[*N*-(9-fluorenylmethoxycarbonyl)glycylamido]ethen-1-yl}phosphonate (26)



26

To a solution of **25** (255 mg, 0.48 mmol) in a tetrahydrofuran/2-propanol 4:1 solvent mixture (5 mL) was added cesium carbonate (198 mg, 0.61 mmol) and 37% aqueous formaldehyde solution (36 μL, 0.48 mmol). After 1 hour stirring the solvent was removed under reduced pressure, the crude suspended in dichloromethane and the solid removed by filtration. Solvent was removed under reduced pressure and the resulting oil was purified by column chromatography on silica gel (ethyl acetate/hexane/2-propanol 7:2:1) to afford **26** as a colourless oil (154 mg, 0.36 mmol, 75% yield).

M.p.: 116–118 °C.

IR (KBr): ν 3337, 3038, 1723, 1683, 1539, 1247, 1044 cm^{-1}.

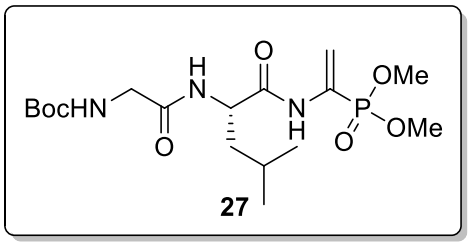
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.95 (br s, 1H, NH), 7.76 (d, 2H, J = 7.5 Hz, $CH_{Ar\ Fmoc}$), 7.59 (d, 2H, J = 7.4 Hz, $CH_{Ar\ Fmoc}$), 7.40 (t, 2H, J = 7.3 Hz, $CH_{Ar\ Fmoc}$), 7.31 (td, 2H, J = 7.5, 1.1 Hz, $CH_{Ar\ Fmoc}$), 6.71 (d, 1H, J = 41.6 Hz, *E*-CH_2=C), 5.61 (d, 1H, J = 19.0 Hz, *Z*-CH_2=C) overlapped with 5.59 (d, 1H, J = 10.6 Hz, NH), 4.42 (d, 2H, J = 7.1 Hz, $CH_{2\ Fmoc}$), 4.23 (t, 1H, J = 7.1 Hz, CH_{Fmoc}), 3.98 (d, 2H, J = 5.3 Hz, $CH_{2\ Gly}$), 3.75 (d, 6H, J = 11.2 Hz, $CH_{3\ OMe}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 168.68 (d, J = 11.3 Hz, CO), 156.73 (CO), 143.83 ($C_{Ar\ Fmoc}$), 141.39 ($C_{Ar\ Fmoc}$), 129.90 (d, J = 201.9 Hz, N-C-P), 127.88 ($CH_{Ar\ Fmoc}$), 127.21 ($CH_{Ar\ Fmoc}$), 125.17 ($CH_{Ar\ Fmoc}$), 120.13 ($CH_{Ar\ Fmoc}$), 115.44 (d, J = 9.8 Hz, $\underline{C}H_2$=C), 67.51 ($CH_{2\ Fmoc}$), 53.57 (d, J = 5.7 Hz, $CH_{3\ OMe}$), 47.17 (CH_{Fmoc}), 45.27 ($CH_{2\ Gly}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 14.58 ppm.

HRMS (ESI) $C_{21}H_{23}N_2NaO_6P$ $[M+Na]^+$: 453.1186, found 453.1201.

Synthesis of dimethyl {1-[*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamido]ethen-1-yl}phosphonate (27)



27

To a solution of **3** (224 mg, 0.43 mmol) in a tetrahydrofuran/2-propanol 4:1 solvent mixture (5 mL) was added cesium carbonate (176 mg, 0.54 mmol) and 37% aqueous formaldehyde solution (34 μL, 0.46 mmol). After one hour stirring the solvent was removed under reduced pressure, the crude suspended in dichloromethane and the solid removed by filtration. Solvent was removed under reduced pressure and the resulting oil was purified by column chromatography on silica gel (ethyl acetate/hexane/2-propanol 7.2:1.8:1) to afford **27** as a yellow oil (152 mg, 0.36 mmol, 84% yield).

$[\alpha]^{26}_{D}$: –49.5 (*c* 0.47, $CHCl_3$).

IR (neat): ν 2985, 1715, 1566, 1186, 1024, 969 cm^{-1}.

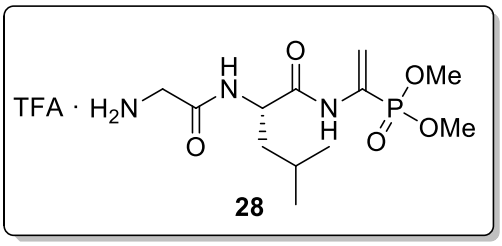
^{1}H NMR (400 MHz, $CDCl_3$): *δ* 8.18 (d, 1H, *J* = 7.1 Hz, NH), 6.96 (d, 1H, *J* = 6.2 Hz, NH), 6.62 (d, 1H, *J* = 41.8 Hz, *E*-CH_2=C), 5.61 (d, 1H, *J* = 19.0 Hz, *Z*-CH_2=C), 5.44 (br s, 1H, NH), 4.61–4.52 (m, 1H, $CH_{\alpha\ Leu}$), 3.89–3.74 (m, 2H, $CH_{2\ Gly}$), overlapped with 3.74 (d, 3H, *J* = 11.2 Hz, $CH_{3\ OMe}$), 3.74 (d, 3H, *J* = 11.2 Hz, $CH_{3\ OMe}$), 1.72–1.52 (m, 3H, CH_{Leu}+$CH_{2\ Leu}$), 1.42 (s, 9H, $CH_{3\ Boc}$), 0.92 (d, 3H, *J* = 6.2 Hz, $CH_{3\ Leu}$), 0.89 (d, 3H, *J* = 6.1 Hz, $CH_{3\ Leu}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): *δ* 171.68 (d, *J* = 10.1 Hz, CO), 170.30 (CO), 156.23 (CO), 130.18 (d, *J* = 201.2 Hz, N-C-P), 116.03 (d, *J* = 9.8 Hz, $\underline{C}H_2$=C), 80.42 (C_{Boc}), 53.46 (d, *J* = 5.6 Hz, $CH_{3\ OMe}$), 52.49 ($CH_{\alpha\ Leu}$), 44.33 ($CH_{2\ Gly}$), 40.63 ($CH_{2\ Leu}$), 28.37 ($CH_{3\ Boc}$), 24.81 (CH_{Leu}), 23.07 ($CH_{3\ Leu}$), 21.95 ($CH_{3\ Leu}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): *δ* 14.67 ppm.

HRMS (ESI) $C_{17}H_{32}N_3NaO_7P$ $[M+Na]^+$: 444.1870, found 444.1862.

Synthesis of dimethyl [1-(glycyl-L-leucylamido)ethen-1-yl]phosphonate trifluoroacetate (28)



28

A solution of **27** (157 mg, 0.37 mmol) in dichloromethane (7 mL) was cooled at 0 °C, trifluoroacetic acid (0.29 mL, 3.73 mmol) was added and then the solution stirred overnight at room temperature. Subsequently, solvent was removed under reduced pressure and the crude was lyophilized to afford **28** as a white solid (119 mg, 0.27 mmol, 73% yield).

$[\alpha]^{24}_{D}$: –44.7 (*c* 0.43, $CHCl_3$).

M.p.: 63–65 °C.

IR (KBr): ν 3267, 2963, 1677, 1544, 1204, 1037 cm^{-1}.

^{1}H NMR (400 MHz, MeOD): δ 6.44 (d, 1H, *J* = 41.6 Hz, *E*-CH_2=C), 5.79 (d, 1H, *J* = 18.0 Hz, *Z*-CH_2=C), 4.60–4.54 (m, 1H, $CH_{\alpha\ Leu}$), 3.78 (m, 2H, $CH_{2\ Gly}$) overlapped with 3.78 (d, 3H, *J* = 11.2 Hz, $CH_{3\ OMe}$), 3.78 (d, 3H, *J* = 11.2 Hz, $CH_{3\ OMe}$), 1.78–1.55 (m, 3H, CH_{Leu}+$CH_{2\ Leu}$), 0.98 (d, 3H, *J* = 6.5 Hz, $CH_{3\ Leu}$), 0.95 (d, 3H, *J* = 6.5 Hz, $CH_{3\ Leu}$) ppm.

^{13}C NMR (100 MHz, MeOD): δ 173.91 (d, *J* = 8.1 Hz, CO), 167.43 (CO), 163.01 (q, *J* = 34.0 Hz, CO_{TFA}), 132.12 (d, *J* = 204.5 Hz, N-C-P), 120.48 (d, *J* = 13.1 Hz, $\underline{C}H_2$=C), 118.22 (q, *J* = 298.9 Hz, $CF_{3\ TFA}$), 54.06 (d, *J* = 5.6 Hz, $CH_{3\ OMe}$), 54.00 (d, *J* = 5.5 Hz, $CH_{3\ OMe}$), 53.66 ($CH_{\alpha\ Leu}$), 41.78 ($CH_{2\ Gly}$), 41.39 ($CH_{2\ Leu}$), 25.99 (CH_{Leu}), 23.40 ($CH_{3\ Leu}$), 21.89 ($CH_{3\ Leu}$) ppm.

^{31}P NMR (162 MHz, MeOD): δ 14.81 ppm.

HRMS (ESI) $C_{12}H_{24}NaN_3O_5P$ $[M+Na]^+$: 344.1346, found 344.1354.

Bacterial strains and growth conditions

The strains utilized in the study are listed in table 5. They are grown in rich medium BHI at 37 °C except for *B. subtilis* grown at 30 °C.

The antimicrobial activity assays were done in MH medium as recommended in the CLSI Standards for Antimicrobial Susceptibility Testing,[42] and also in M9 minimal medium as stated for dehydrophos.[17]

In vitro antimicrobial activity: Solid agar diffusion assays.

The bacterial cultures were grown overnight in BHI media at 37 °C in case of *E. coli*, , *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Enterococcus faecalis*. *Bacillus subtilis* was incubated at 30 °C. The grown culture was spread on the surface of M9 minimal medium agar plates. 5 µl of the compound stock solution (Dhp and derivatives, 20 mM in water) were absorbed on a sterile filter disc and dried. These disks were carefully placed on the plate, and the plates were incubated overnight at 30 °C for *B. subtilis* or 37 °C for the other bacteria.

***In vitro* antimicrobial activity. MIC determination**

The minimal inhibitory concentrations (MICs) were determined by the broth microdilution method according to the CLSI Standards for Antimicrobial Susceptibility Testing,[42] in 96-well flat-bottom microplates. A serial 2-fold dilutions of the compounds were prepared in 100 µL of M9 medium so as the range of final concentrations tested spanned from 0.12 to 125 µM. The bacterial inoculum was prepared by diluting a culture grown overnight to an OD of 0.1, then dilute 1/100 in M9 minimal medium, leading to a bacterial concentration of approx. 10^5 CFU/ml. 100 µL of this bacterial inoculum were added to each well. The plates were subsequently incubated 20h at 30 °C or 37 °C. Bacterial growth inhibition was assessed using alamar blue stain, and results were analysed visually for a clear change in colour. The lowest concentration of compound that prevents a colour

change was recorded as the MIC.[43] The MIC of vancomycin (concentration range 0.06–64 μg/mL) was determined as a control of the test.

Table 5. Bacterial strains used in the biological activity study.

Strain	**Source or reference**
E. coli DH5α	Mycobacterial Genetics Group
Bacillus subtilis	CECT356
Pseudomonas aeruginosa	ATCC15442
Enterococcus faecalis	ATCC29212
Staphylococcus aureus	CECT794
Salmonella thyphimurium	Mycobacterial Genetics Group
Mycobacterium smegmatis mc^2155	Mycobacterial Genetics Group

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Chapter 3

Diels-Alder reaction of

dehydroaminophosphonates

Introduction.

Among organophosphorous compounds, α-aminophosphonic acids have attracted a great interest over the last decades as a result of the outstanding biological properties showed by many of them.[1–9] Initially, the procedures developed for the synthesis of these amino acid analogues were aimed to those which structurally resembled proteinogenic amino acids,[10] but soon an increasing interest was focused on the synthesis of α-aminophosphonic acid derivatives structurally related to non-proteinogenic amino acids of biological or synthetic interest.[11]

Within this context, quaternary α-amino acids constitute a family of compounds which find application in several research fields from the development of new catalytic systems to the synthesis of pharmaceuticals.[11b,12] In particular, carbocyclic amino acid derivatives have attracted a great interest, as they induce conformational restrictions into peptides[12e,h,13] and this may conduct to an improvement in selectivity and efficiency of the interaction with receptors.[14] In addition, peptides containing these amino acids present a special biostability due to their quaternary centre.[15] Therefore, many synthetic procedures leading to these compounds have been described in the literature.[11b,12c–i,k,l]

α,β-Dehydroamino acids have proven to be useful precursors of carbocyclic amino acids, since they allow the synthesis of a wide variety of them by means of cycloaddition reactions (Figure 1).[12b,e,g,k,l,16–20] Thus, these compounds are appropriate substrates for [3+2] cycloaddition reactions, including the cyclopropanation reaction with diazoalkanes,[21] the 1,3-dipolar cycloaddition of azomethine ylides,[22] the Lu's reaction with allenes[16,23] and the palladium-catalysed cycloaddition of vinyl cyclopropane derivatives.[24] Moreover, α,β-dehydroamino acids lead to four-membered or six-membered carbocyclic derivatives by means of [2+2] cycloaddition[25] and Diels-Alder[26] reactions respectively, as well as to bicyclic systems through [2+2+2] cycloaddition reactions.[27]

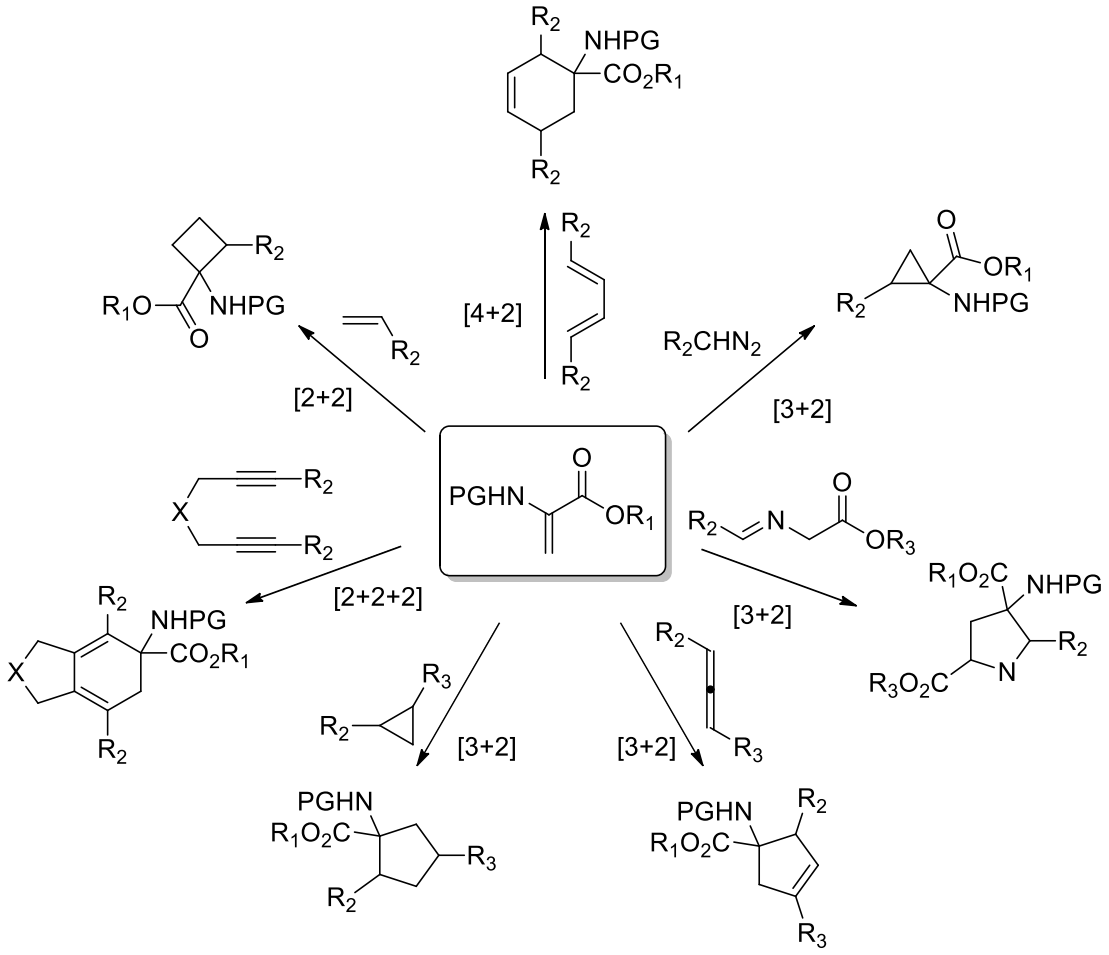


Figure 1. Cycloaddition of α,β-dehydroamino acids.

It is within this context that many efforts have been devoted to the synthesis of α,β-dehydroaminophosphonic acids, the phosphorylated counterparts of α,β-dehydroamino acids derivatives.[28] These compounds are indeed valuable synthetic intermediates in the synthesis of structurally diverse α-aminophosphonic acids.[28a,b,d,g,29] However, α,β-dehydroaminophosphonic acids have been scarcely investigated as substrates for cycloaddition reactions, and the examples described in the literature are limited to the 1,3-dipolar cycloaddition reaction of diazoalkanes, mainly aimed at the synthesis of 1-aminocyclopropylphosphonic acid derivatives (Figure 2).[29a–c,h]

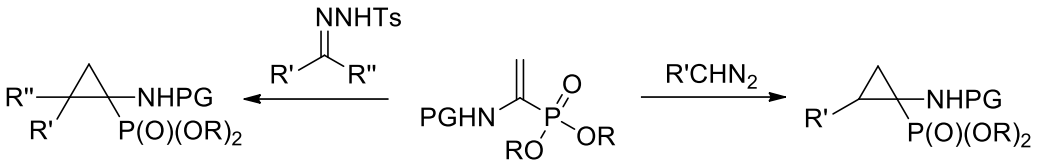


Figure 2. Cyclopropanation of α,β-dehydroaminophosphonates.

This absence of studies on α,β-dehydroaminophosphonates as substrates for cycloaddition reactions made us focus our interest on these transformations. Within this chapter, the first synthetic and theoretical study of the Diels-Alder reaction of α,β-dehydroaminophosphonates is described. Cyclopentadiene was used as a suitable model to assess the reactivity and the *exo*/*endo*-selectivity of a variety of α,β-dehydroaminophosphonic acid derivatives bearing different protecting groups in the phosphonic acid and the amine functions. In addition, the reactivity and selectivity has been rationalized by DFT methods. After completing the study with cyclopentadiene, two other dienes were also tested to expand the study.

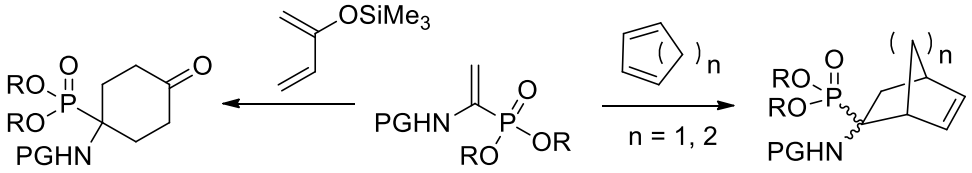


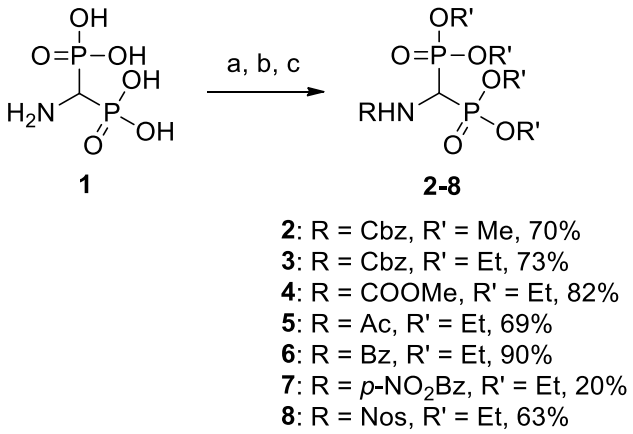
Figure 3. Diels-Alder reaction with dehydroaminophosphonates.

The α-aminophosphonic acid derivatives obtained in this chapter via Diels-Alder reaction are characterized by a norbornane bicyclic system. It is worth mentioning that the corresponding α-amino acid counterparts have attracted a great interest as a result of their biological properties.[30] Furthermore, compounds described in this chapter were obtained with a high *exo*-selectivity. In contrast, previously reported procedures giving access to these α-aminophosphonic acid derivatives were limited to the Kabachnik-Fields reaction with 2-norbornanone, and provided the desired compound as a 3:2 mixture of diastereomers.[31,32]

Results and discussion.

Firstly, the synthesis of a collection of protected α,β-dehydroaminophosphonic acid derivatives was addressed. A variety of carbamates (benzyloxycarbonyl and methoxycarbonyl groups) and amides (benzoyl, acetyl, formyl, trifluoroacetyl and *p*-nitrobenzoyl groups) were selected for this study. Thus, in addition to studying the effects of the protecting groups nature, the influence of steric, inductive and electron-withdrawing effects has also been investigated.

We chose the Horner-Wadsworth-Emmons reaction as a suitable procedure to provide these compounds, since the starting α-aminomethylbisphosphonic acid, **1**, can be obtained in multi-gram scale (see chapter 1). Compound **1** was then acylated and subsequently esterified to afford *N*-protected tetraalkyl aminomethylbisphosphonates **2**-**8** in yields ranging from 20 to 90% (Scheme 1).

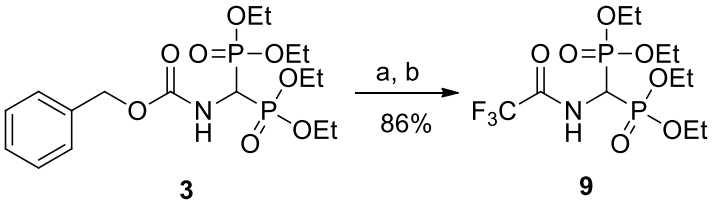


Scheme 1. Reagent and conditions: (a) R-OSu, Et_3N, H_2O/CH_3CN 4:1, room temp., 12 h.; (b) Dowex® 50WX8; (c) $CH(OR')_3$, reflux, 72 h.

On one hand, the selected acylation procedure involves the use of triethylamine as base and treatment of the reaction crude with a strongly-acidic cation exchange resin. This methodology differs from the classical Schotten-Baumann procedure, often involving the use of a basic aqueous solution, acidification of the reaction mixture and extraction of the *N*-protected aminophosphonic acid in an organic solvent.[33–37] However, the high water-

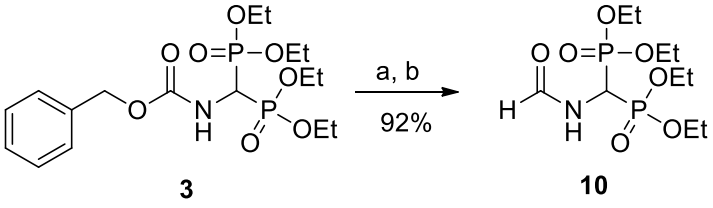
solubility of α-aminomethylbisphosphonic acid derivatives made us discard these reaction conditions. On the other hand, the esterification reaction was carried out using trialkyl orthoformate derivatives as alkylating agents, which usually compare favourably with other procedures described in the literature.[38–45]

N-Trifluoroacetyl bisphosphonate **9** could not be obtained by acylation and subsequent esterification of **1** as described above, since the reaction provided exclusively the starting compound. Thus, as an alternative route, hydrogenolysis of compound **3** followed by treatment with trifluoroacetic anhydride[46] provided the bisphosphonate **9** in high yield (Scheme 2).



Scheme 2. Reagent and conditions: (a) H_2, Pd/C, EtOH, room temp., 1 h.; (b) TFA_2O, CH_2Cl_2, room temp., 2.5 h.

Similarly, *N*-formyl derivative **10** was obtained through hydrogenolysis of **3** and subsequent *N*-formylation, using an equimolecular mixture of acetic anhydride and formic acid, obtaining **10** with high yields (Scheme 3)

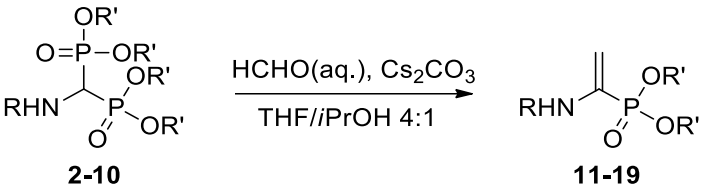


Scheme 3. Reagent and conditions: (a) H_2, Pd/C, EtOH, room temp., 1 h.; (c) Ac_2O, HCO_2H, CH_2Cl_2, room temp., 10 min.

Then, the *N*-protected α,β-dehydroaminophosphonates **11-16, 18** and **19** were obtained by means of the Horner-Wadsworth-Emmons olefination with formaldehyde. Thus, α-aminomethylbisphosphonates **2-10** were treated with a 37% formaldehyde

aqueous solution in the presence of cesium carbonate to afford the corresponding α-amino vinylphosphonates in good to high yields. It should be noted that in the case of the nosyl protected bisphosphonate **8** the corresponding dehydrophosphoalanine could not be obtained as the reaction resulted in decomposition, so compound **17** was discarded for the study.

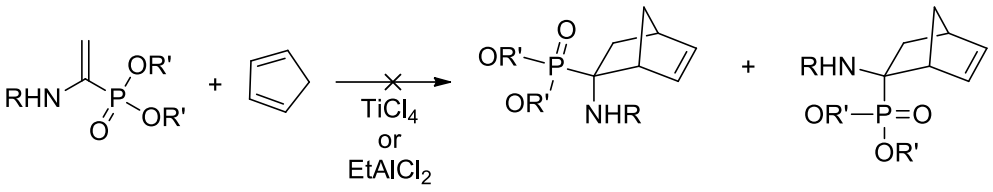
Table 1. Synthesis of *N*-protected α,β-dehydroaminophosphonates.



2-10 **11-19**

Substrate	R	R'	Time (h)	Yield
2	Cbz	Me	1	63
3	Cbz	Et	5	70
4	COOMe	Et	3	51
5	Ac	Et	2	86
6	Bz	Et	7	92
7	*p*-NO_2Bz	Et	1	89
8	Nos	Et	–	–
9	TFA	Et	2	92
10	COH	Et	1	19

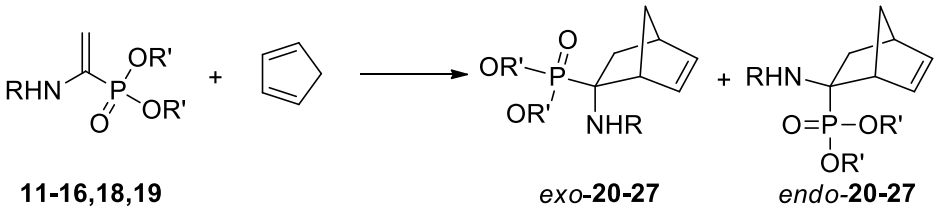
The Diels-Alder reaction of cyclopentadiene with α,β-dehydroaminophosphonates **11-16**, **18** and **19** was then investigated. First attempts were conducted at room temperature under neat conditions or in the presence of Lewis acids ($TiCl_4$ and $EtAlCl_2$) (Scheme 4). The reaction crude ^{31}P NMR spectrum of the experiment performed with titanium tetrachloride showed only a 6% conversion after six days of reaction. Furthermore, the use of aluminium trichloride as Lewis acid led to decomposition, and non-conclusive NMR spectra were recorded. These results discarded the possibility of developing a catalytic process.



Scheme 4. Diels-Alder reaction with Lewis Acids.

After that, reactions were performed under neat conditions, without any additive, and the temperature was increased. Table 2 shows the different temperatures and times used, the corresponding yields and the *exo*/*endo* ratio. On one hand, the *exo*/*endo* ratio was determined by integration of the corresponding signals in the reaction crude ^{31}P NMR spectrum. On the other hand, the *exo*- and *endo*-stereochemistry of the isolated adducts were assigned by means of 2D-NOESY experiments and the analysis of the vicinal $^{3}J_{P\text{-}C}$ coupling constants in the ^{13}C NMR spectra, as it would be explained later.

Table 2. Diels-Alder reaction of α,β-dehydroaminophosphonates **11-16**, **18** and **19** with cyclopentadiene.



11-16,18,19 *exo*-**20-27** *endo*-**20-27**

Entry	Substrate	R	R'	Temperature °C	Time	Yield[a]	*exo*/*endo*[b]
A	**11**	Cbz	Me	100	7 d	26	86:14
B	**11**	Cbz	Me	160	22 h	32	86:14
C	**12**	Cbz	Et	160	36 h	44	86:14
D	**13**	COOMe	Et	160	27 h	54	88:12
E	**14**	Ac	Et	160	18 h	51	82:18
F	**15**	Bz	Et	160	24 h	54	84:16
G	**16**	*p*-NO_2Bz	Et	160	–	–	–
H	**18**	TFA	Et	160	5 h	66	86:14
I	**19**	COH	Et	160	12 h	37	82:18

[a] Isolated yield for both Diels-Alder adducts.

[b] Determined by direct integration of the ^{31}P NMR of the crude reaction mixture.

First attempt was performed stirring the *N*-benzyloxycarbonyl derivative **11** at 100 °C for 7 days (entry A). The ^{31}P NMR spectrum of the reaction crude showed the formation of many side-products along with the signals corresponding to Diels-Alder adducts *exo*- and *endo*-**20**, which were isolated in a 26% yield. Under these reaction conditions, an 86:14 *exo*/*endo* ratio was observed.

Next, the cycloaddition of cyclopentadiene to the dehydroaminophosphonate **11** was carried out at 160 °C (entry B). After stirring at this temperature for 22 hours the reaction was complete, and the Diels-Alder products *exo*- and *endo*-**20** were isolated in 32% yield. Thus, under these conditions reaction time was dramatically reduced with a slight improvement in the yield. Moreover, the *exo*/*endo* ratio remained unchanged and formation of side-products was reduced. Consequently, the Diels-Alder reaction of the vinylphosphonates **12-16, 18** and **19** was performed at 160 °C.

Once the reaction conditions were optimized, the effect of the substitution in the phosphonate group was investigated. Reaction of the diethyl dehydroaminophosphonate **12** with cyclopentadiene was completed in 32 hours (entry C). The ^{31}P NMR spectra of the reaction crude showed less formation of side-products in comparison to that observed for compound **11**, whereas the same *exo*/*endo* ratio was observed. Indeed, adducts *exo*-**21** and *endo*-**21** were obtained in significantly higher yield (44%). This result prompted us to use diethyl phosphonate derivatives instead the corresponding dimethyl phosphonates. The use of more voluminous substituents (*i*Pr or Ph) at the phosphonate group was discarded because of rather longer reaction times would be required to achieve similar results.

After that, the effect of the nitrogen protecting groups was investigated. As showed in entries C-F and I, shorter reaction times were needed for amide derivatives (acetyl, benzoyl and formyl, entries E, F and I) in comparison with the corresponding carbamates (benzyloxycarbonyl and methoxycarbonyl, entries C and D). Moreover, larger substituents led to slightly longer reaction times when benzyloxycarbonyl and methyloxycarbonyl

groups (entries C and D), or formyl, acetyl and benzoyl groups (entries I, E and F) were compared, whereas the *exo*/*endo* ratios remained almost unchanged in all cases. Such small differences indicate that the steric hindrance of the protecting group at the amine function is not decisive in the reaction outcome.

It is worth noting that these results differ from those of the reaction of α,β-dehydroalanine derivatives with cyclopentadiene, where the observed *exo*/*endo* ratios depend on the nature of the *N*-acyl and ester groups. Moreover, they are generally lower than those observed for the corresponding α,β-dehydroaminophosphonates.[47–49]

Since differences in the steric hindrance of the amine protecting groups did not led to a significant improvement of the reaction performance, the influence of inductive and electron-withdrawing effects was addressed (entries G and H). The reaction between cyclopentadiene and dehydrophosphoalanine derivatives is a typical normal demand Diels-Alder reaction; consequently, it should be expected that the presence of electron-withdrawing groups tend to increase the reaction rate. Unfortunately, the reaction of cyclopentadiene with the *p*-nitrobenzoyl derivative **16** resulted in decomposition and no conclusions could be drawn regarding the electron-withdrawing effects. However, reaction with the *N*-trifluoroacetyl dehydroaminophosphonate **18** was completed in only 5 hours, affording the Diels-Alder adducts *exo*-**26** and *endo*-**26** in 66% yield (entry H). Moreover, the *exo*/*endo* ratio observed was very close to those obtained before. Accordingly, this result represents an improvement over the reactions with compounds **12-15** and **19**, the best chemical yield was obtained and the *exo*-selectivity remained unaffected.

Although it is generally accepted that the solvent does not significantly affect the Diels-Alder reaction outcome as a result of a negligible difference in polarity between the initial state and the transition state,[50,51] it is well known that both the reaction time and the stereoselectivity can be dramatically influenced by polar protic or aqueous media.[52] For instance, in the case of dehydroalanine derivatives, for which significant changes in

stereoselectivity are observed when using ethanol[48] or water[53] as solvents. Consequently, the effect of a polar protic medium was investigated for the cycloaddition of cyclopentadiene to compounds **16** and **18** (Table 3). Thus, α,β-dehydroaminophosphonates **16** and **18** and cyclopentadiene were stirred at 75 °C in an ethanol/water mixture (higher temperatures led to decomposition). Under these conditions, the *p*-nitrobenzoyl derivative **16** did not lead to decomposition, presumably due to the lower temperature used, and adducts *exo*-**25** and *endo*-**25** were obtained in 45% yield after 17 days of reaction (entry B). An 82:18 *exo/endo* ratio was determined. The reaction with the *N*-trifluoroacetyl derivative **18** (entry A) was completed after 7 days affording adducts *exo*-**26** and *endo*-**26** in a 65% yield and 84:16 *exo/endo* ratio. These results confirmed the limited effect exerted by including a polar solvent which, on the other side, favours decomposition at high temperatures, forcing the use of lower temperatures that increase the reaction time in a large extent.

Table 3. Diels-Alder reaction of compounds **16** and **18** in EtOH/H_2O.

Entry	Substrate	R	Time (d)	Yield (%)	*exo*/*endo*
A	**18**	TFA	7	65	84:16
B	**16**	*p*-NO_2Bz	17	45	82:18

As mentioned above, the stereochemistry of the Diels-Alder adducts *exo*-**20-27** and *endo*-**20-27** was determined by the analysis of both 2D-NOESY experiments and the values of characteristic $^3J_{P\text{-}C}$ coupling constants in the ^{13}C NMR spectra.

Firstly, the 2D-NOESY spectra of compounds *endo*-**20**-**27** showed a spatial relationship between NH and H_{7a} protons (Figure 4), confirming the *endo* stereochemistry for these compounds. In addition, the 2D NOESY experiments of compounds *exo*-**20**-**23** showed a relationship between NH and H_6 protons, indicative of and exo configuration, whereas for compounds *exo*-**24**-**27** could not be observed because NH and H_6 signals were too close.

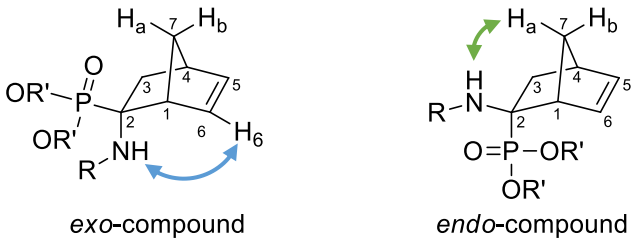


Figure 4. NOE interactions for *exo* and *endo* adducts

Moreover, the configuration assignment of the Diels-Alder adducts was supported by the analysis of the vicinal $^{3}J_{P\text{-}C}$ coupling constants for indicative signals. Since the angular dependence of P-C coupling constants was observed, the application of such data to stereochemical determination and conformational analysis was developed.[54] Within this context, vicinal $^{3}J_{P\text{-}C}$ constants drew a special attention, since a Karplus-like dihedral dependence was found.[55] This feature proved to be very useful both in conformational studies and the configuration assignment of organophosphorous compounds, and prompted the development of several Karplus-type equations.[56] These equations could not be generalized as a result of their dependence on many factors, including the substituents on the coupling route. However, the analysis of vicinal $^{3}J_{P\text{-}C}$ coupling constants has been successfully applied to the stereochemistry determination of several organophosphorous compounds, especially of those based on constrained cyclic systems.[57]

As shown in table 5, compounds *exo*-**20-27** showed a $^{3}J_{P\text{-}C6}$ coupling constant ranging from 11.3 to 11.6 Hz, while no coupling was observed between the phosphorus atom and the C_7 carbon. In contrast, compounds *endo*-**20-27** showed much smaller values for the $^{3}J_{P\text{-}C6}$ coupling constants (2.0-2.8 Hz) and a $^{3}J_{P\text{-}C7}$ coupling constant close to 10 Hz. These observations are in agreement with the data found in the literature for organophosphorous compounds characterised by a norbornane structure.[56b,57b] These bicyclic systems showed greater $^{3}J_{P\text{-}C6}$ coupling constants for *exo* compounds than for *endo* ones, whereas the opposite is true for the $^{3}J_{P\text{-}C7}$ coupling constants. In addition, characteristic $^{3}J_{P\text{-}C}$ coupling constants for *exo*-**20-27** are very close to those observed for α-hydroxyl- ($^{3}J_{P\text{-}C4}$ = 4.7 Hz, $^{3}J_{P\text{-}}$

$_{C6}$ = 9.1 Hz and $^{3}J_{P\text{-}C7}$ < 0.6 Hz) and α-phenylaminophosphonates ($^{3}J_{P\text{-}C4}$ = 4.7 Hz, $^{3}J_{P\text{-}C6}$ = 9.1 Hz and $^{3}J_{P\text{-}C7}$ = NR) derived from 2-norbornanone and showing an *exo*-stereochemistry.[31]

Table 4. Selected ^{13}C NMR data of Diels-Alder.

Compound		C_4		C_6		C_7	
		δ (ppm)	J (Hz)	δ (ppm)	J (Hz)	δ (ppm)	J (Hz)
20	***exo***	42.11	3.5	134.31	11.3	47.55	0
	endo	42.57	7.7	132.24	2.8	47.62	10.1
21	***exo***	42.03	3.5	134.47	11.4	47.76	0
	endo	42.61	7.6	132.35	2.4	47.65	10.2
22	***exo***	42.22	3.4	134.58	11.3	47.65	0
	endo	42.60	7.7	132.49	2.0	47.63	10.1
23	***exo***	42.04	3.4	134.84	11.5	47.44	0
	endo	42.45	7.5	132.48	2.7	47.69	10.2
24	***exo***	42.09	3.4	134.83	11.6	47.61	0
	endo	42.58	7.4	132.43	2.8	47.83	10.1
25	***exo***	42.20	3.4	134.59	11.4	47.68	0
	endo	42.50	7.3	132.40	2.7	47.90	10.2
26	***exo***	42.21	3.2	133.93	11.4	47.76	0
	endo	42.28	7.0	131.88	2.6	47.63	10.1
27	***exo***	42.36	3.5	133.82	10.4	47.60	0
	endo	42.87	7.7	132.30	2.7	47.51	10.2

All experiments were performed in CDCl3. NMR frequency and instrument are detailed in the experimental section.

The reversibility of the Diels-Alder reaction of cyclopentadiene with dehydrophosphoalanine derivatives was also investigated. To this end, a 2:1 mixture of *exo*-**26** and *endo*-**26** was stirred in cyclopentadiene at 160°C. After 5 h, the ^{1}H NMR spectra of the reaction mixture did not showed any changes, concluding that the reaction was not reversible and that thermodynamic control can be discarded for the observed selectivity.

Moreover, the reaction was also investigated computationally by DFT methods in collaboration with the Institute for Biocomputation and Physics of Complex Systems to provide a rationale of the experimental results. The *exo* and *endo* transition structures were located for the reaction of cyclopentadiene with alkenes **AKa-g** equivalent to the corresponding alkenes **11-16**, **18** and **19**, replacing Et by Me (i.e: R^2 = Me) as the only approximation (Figure 5). In addition, alkenes **AKh,i** were also studied for the purpose of comparison. The energy barriers for the reaction were calculated in gas phase and in ethanol as a solvent. Results are collected in figure 5. The observed values for energy barriers are in a good agreement with the experimental observations. The lower energy barrier corresponds to **AKg** (13.2 kcal/mol) corroborating the best result obtained with **18**. Similarly, **AKf** showed also a low barrier (13.6 kcal/mol), while the more electron-rich alkene **AKi** exhibited a higher barrier (14.2 kcal/mol), as expected. Little influence was observed by moving from gas phase to ethanol a solvent, the former presenting lower barriers, in agreement with an apolar concerted reaction. In all cases, the *exo* adduct was predicted to be the preferred one although the differences with *endo* adducts predicted that the latter might be obtained as minor isomers. Finally, similar results were obtained for **AKe** (14.8 kcal/mol) and **AKh** (14.8 kcal/mol) confirming the absence of steric effects of the amino protecting group.

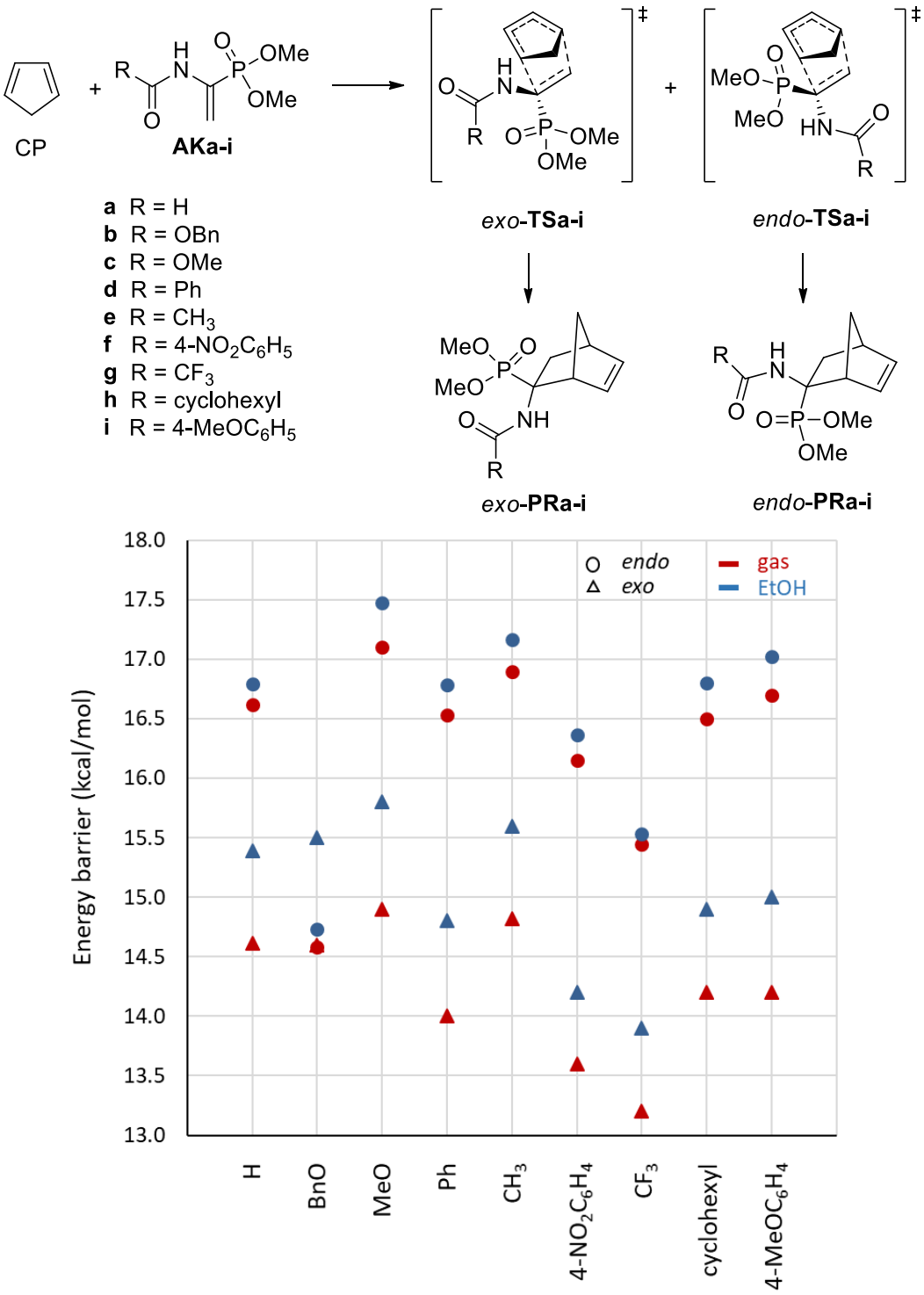


Figure 5: Energy barriers (b3lyp-d3bj/def2-tzvp) for the reactions between cyclopentadiene and alkenes **AKa-i**

The geometry of the transition structures evidenced differences between *endo* and *exo* approaches (see experimental section). Whereas the less favoured *endo* transition states showed to be clearly concerted, the preferred *exo* transition structures showed a marked

asynchronicity, based on the different lengths of forming bonds. Figure 6 illustrates the preferred *exo*-**TSg** (the most favoured reaction) in which the asynchronicity is exemplified with distances of 1.97 and 2.65 Å for the C-C forming bonds. A topological analysis of non-covalent interactions (NCI) confirmed H-F interactions that might also be responsible of the enhanced stability of *exo*-**TSg**.

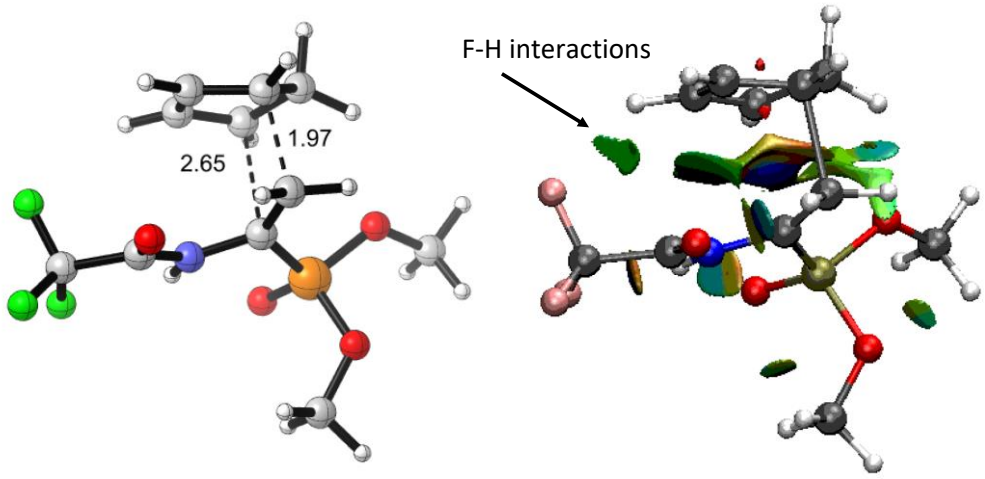
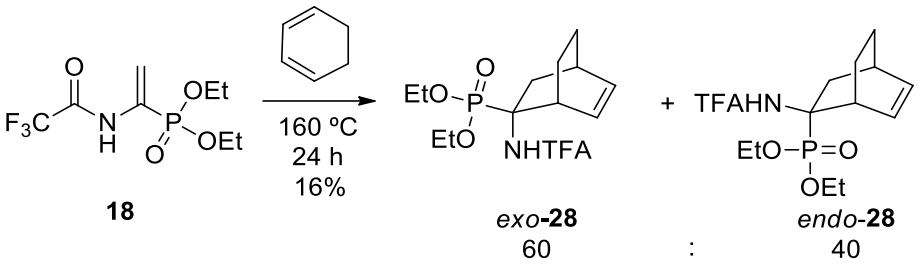


Figure 6: Optimized (b3lyp-d3bj/def2-svp) geometry for *exo*-**TSg** (right) and NCI topological analysis (right).

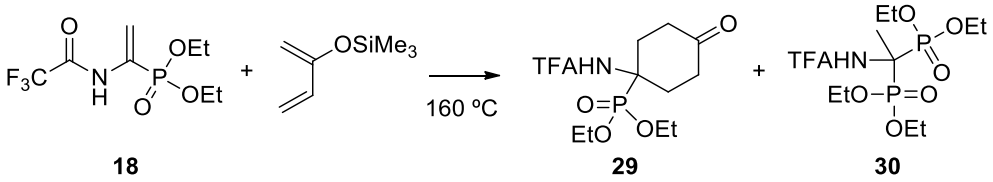
To conclude and expand the Diels-Alder studies, other dienes were tested on compound **18**, the *N*-trifluoroacetyl-protected dehydrophosphoalaninate, as it provided the best results in the cycloaddition with cyclopentadiene. First, cyclohexadiene was tested. The reaction was followed by ^{31}P NMR and finished after 24 hours due to disappearance of the signal corresponding to the starting compound (10.33 ppm) and the presence of decomposition compounds in the spectrum. Two main signals about 25 and 24 ppm appeared, and a 60:40 ratio was obtained by direct integration of the corresponding signals. These chemical sifts were similar to the *exo* and *endo* compounds obtained previously with cyclopentadiene, so the two compounds were isolated by column chromatography on silica gel, obtaining a global yield of 16%. They were analysed separately by means of the ^{1}H and ^{31}P NMR with COSY and HSQC experiments, and we concluded that they were the expected bicyclo[2.2.2]octenes. The stereochemistry was assigned by means of 2D-NOESY

experiments, where the minor compound exhibited a NOE interaction between the NH and H_{7a} proton; and the $^{3}J_{P\text{-}C6}$ coupling constant, as the main compound had a value of 13.5 Hz whereas the minor one had a $^{3}J_{P\text{-}C6}$ of 0 Hz. These data are in agreement with the corresponding found in literature for constrained systems with a greater $^{3}J_{P\text{-}C6}$ coupling constants for *exo* compounds than for *endo* ones[57] and therefore the *endo* stereochemistry was assigned to the minor compound (Scheme 5).



Scheme 5. Reaction with cyclohexadiene.

Lastly, the reaction with 2-(trimethylsiloxy)-1,3-butadiene, an equivalent of Danishefsky's diene, was tested. The reaction was heated in a sealed tube at 160 °C and was followed by ^{31}P NMR. The reaction was stopped after 5 hours, due to the emerging of decomposition compounds and the minimal presence of the starting compound. However, when analysing the reaction crude, and trying to purify it via column chromatography, the main compound was identified as **30**, and the expected compound **29** was present in a barely 5% conversion in the ^{31}P NMR of the reaction crude.



Scheme 6. Reaction with 2-(trimethylsiloxy)-1,3-butadiene.

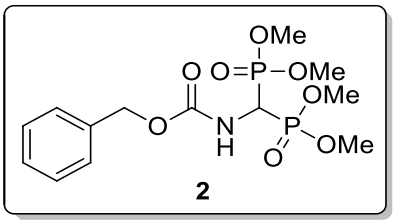
Experimental Section

All reagents were used as received from commercial suppliers without further purification. Anhydrous solvents were dried using a Solvent Purification System (SPS). Thin-layer chromatography (TLC) was performed on Macherey–Nagel Polygram® SIL G/UV254 precoated silica gel polyester plates. The products were visualized by exposure to UV light or submersion in ninhydrin, phosphomolybdic acid or permanganate. Column chromatography was performed using 60 Å (0.04–0.063 mm) silica gel from Macherey–Nagel. Melting points were determined on a Gallenkamp apparatus. IR spectra were registered on a Nicolet Avatar 360 FTIR spectrophotometer; ν_{max} is given for the main absorption bands. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker AV-400 instrument at room temperature using the residual solvent signal, the solvent signal, or the chemical shift of the lock solvent as the internal standard; chemical shifts (δ) are expressed in parts per million and coupling constants (J) in Hertz. High-resolution mass spectra were recorded on a Bruker Microtof-Q spectrometer.

Synthesis of aminomethylbisphosphonates. General Procedure.

To a solution of aminomethylbisphosphonic acid, compound **1** from chapter 1, (1 equivalent) in water (1 mL per equivalent) and triethylamine (6 equivalent) was added a solution of the corresponding succinimidyl carbonate, anhydride, acyl chloride or sulfonyl chloride (2.5 equivalent) in acetonitrile (0.5 mL for equivalent). The reaction mixture was stirred 12 hours at room temperature, and solvent was removed under reduced pressure. The residue was dissolved in water, washed with diethyl ether, treated with an acidic resin Dowex® 50WX8 and evaporated to afford an residue which was used in the next step without further purification. Previous residue was suspended in the correspondent orthoformate and stirred at reflux under argon for 72 hours. Subsequently, the orthoformate in excess was removed under reduced pressure. The resulting oil was purified by column chromatography on silica gel to afford the bisphosphonate.

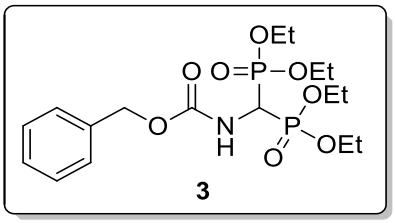
Tetramethyl (benzyloxycarbonylaminomethyl)bisphosphonate (2)



2

Compound **2** was prepared according to the procedure in chapter 1, see page 32.

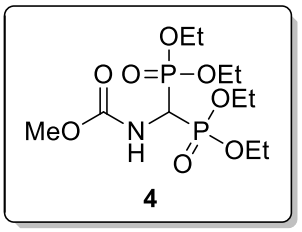
Tetraethyl (benzyloxycarbonylaminomethyl)bisphosphonate (3)



3

Compound **3** was prepared according to the procedure in chapter 2, see page 91.

Tetraethyl (methoxycarbonylaminomethyl)bisphosphonate (4)



4

Compound **4** was prepared according to the general procedure described above starting from aminomethylbisphosphonic acid (1.03 g, 5.39 mmol), using *N*-(methoxycarbonyloxy)succinimide (1.98 g, 11.44 mmol) and triethyl orthoformate (50 mL, 0.30 mol) to afford **4** (column eluent: dichloromethane/methanol 95:5) as a colourless oil (1.60 g, 4.43 mmol, 82% yield).

IR (neat): ν 3223, 2981, 1710, 1535, 1261, 1010, 949 cm^{-1}.

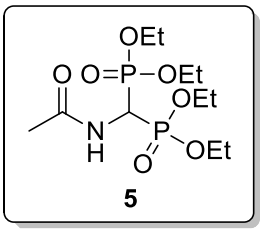
^{1}H NMR (400 MHz, $CDCl_3$): δ 5.24 (d, 1H, J = 10.3 Hz, NH), 4.56 (td, 1H, J = 21.9, 10.5 Hz, N-CH-P_2), 4.25–4.13 (m, 8H, $CH_{2\ OEt}$), 3.71 (s, 3H, $CH_{3\ OMe}$), 1.33 (t, 6H, J = 7.1 Hz, $CH_{3\ OEt}$), 1.32 (t, 6H, J = 7.1 Hz, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 156.36 (t, J = 4.9 Hz, CO), 63.87–63.54 ($CH_{2\ OEt}$), 53.11 ($CH_{3\ OMe}$), 46.23 (t, J = 147.8 Hz, N-CH-P_2), 16.50 (d, J = 3.2 Hz, $CH_{3\ OEt}$), 16.43 (d, J = 3.2 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 16.57 ppm.

HRMS (ESI) $C_{11}H_{25}NNaO_8P_2$ $[M+Na]^+$: 384.0948, found 384.0926.

Tetraethyl (acetylaminomethyl)bisphosphonate (5)



5

Compound **5** was prepared according to the general procedure described above starting from aminomethylbisphosphonic acid (2.13 g, 11.15 mmol), using acetic anhydride (2.20 mL, 23.27 mmol) and triethyl orthoformate (65 mL, 390.79 mmol) to afford **5** (column eluent: dichloromethane/2-propanol 95:5) as a white solid (2.64 g, 7.65 mmol, 69% yield).

M.p.: 56–58 °C.

IR (neat): ν 3260, 1677, 1530, 1235, 1013, 944 cm^{-1}.

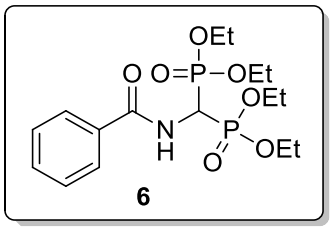
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.58 (d, 1H, J = 10.0 Hz, NH), 5.02 (td, 1H, J = 21.8, 10.1 Hz, N-CH-P_2), 4.23–4.09 (m, 8H, $CH_{2\ OEt}$), 2.05–2.03 (m, 3H, CH_3), 1.31 (t, 6H, J = 7.0 Hz, $CH_{3\ OEt}$), 1.29 (t, 6H, J = 7.0 Hz, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 169.28 (t, J = 4.1 Hz, CO), 63.74 (d, J = 3.1 Hz, $CH_{2\ OEt}$), 63.71 (d, J = 3.1 Hz, $CH_{2\ OEt}$), 63.62 (d, J = 3.2 Hz, $CH_{2\ OEt}$), 63.59 (d, J = 3.2 Hz, $CH_{2\ OEt}$), 43.67 (t, J = 147.4 Hz, N-CH-P_2), 22.87 (CH_3), 16.48 (d, J = 2.9 Hz, $CH_{3\ OEt}$), 16.45 (d, J = 3.1 Hz, $CH_{3\ OEt}$), 16.41 (d, J = 3.2 Hz, $CH_{3\ OEt}$), 16.38 (d, J = 3.1 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 16.55 ppm.

HRMS (ESI) $C_{11}H_{25}NNaO_7P_2$ $[M+Na]^+$: 368.0998, found 368.0997.

Tetraethyl (*N*-benzoylaminomethyl)bisphosphonate (6)



Compound **6** was prepared according to the general procedure described above starting from aminomethylbisphosphonic acid (441 mg, 2.31 mmol), using benzoyl chloride (0.67 mL, 5.75 mmol) and triethyl orthoformate (12 mL, 72 mmol) to afford **6** (column eluent: dichloromethane/methanol 95:5) as a white solid (842 mg, 2.07 mmol, 90% yield).

M.p.: 144–146 °C.

IR (KBr): ν 3215, 1662, 1538, 1311, 1260, 1041 cm^{-1}.

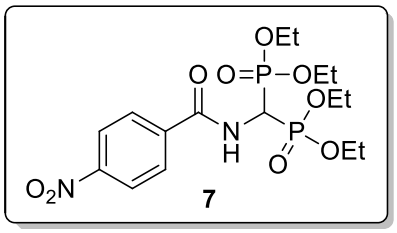
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82–7.77 (m, 2H, CH_{Ar}), 7.56–7.50 (m, 1H, CH_{Ar}), 7.49–7.41 (m, 2H, CH_{Ar}), 6.72 (d, 1H, J = 10.1 Hz, NH), 5.25 (td, 1H, J = 21.6, 10.1 Hz, N-CH-P_2), 4.32–4.12 (m, 8H, $CH_{2\ OEt}$), 1.33 (t, 6H, J = 7.1 Hz, $CH_{3\ OEt}$), 1.30 (t, 6H, J = 7.1 Hz, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 166.57 (t, J = 4.1 Hz, CO), 133.41 (C_{Ar}), 132.25 (CH_{Ar}), 128.88 (CH_{Ar}), 127.27 (CH_{Ar}), 63.90 (d, J = 3.1 Hz, $CH_{2\ OEt}$), 63.87 (d, J = 3.1 Hz, $CH_{2\ OEt}$), 63.74 (d, J = 3.2 Hz, $CH_{2\ OEt}$), 63.71 (d, J = 3.2 Hz, $CH_{2\ OEt}$), 44.19 (t, J = 146.8 Hz, N-CH-P_2), 16.54 (d, J = 2.9 Hz, $CH_{3\ OEt}$), 16.51 (d, J = 2.9 Hz, $CH_{3\ OEt}$), 16.47 (d, J = 3.1 Hz, $CH_{3\ OEt}$), 16.44 (d, J = 3.1 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 16.48 ppm.

HRMS (ESI) $C_{16}H_{27}NNaO_7P_2$ $[M+Na]^+$: 430.1155, found 430.1170.

Tetraethyl (*p*-nitrobenzoylaminomethyl)bisphosphonate (7)



7

Compound **7** was prepared according to the general procedure described above starting from aminomethylbisphosphonic acid (1.00 g, 5.23 mmol), using *p*-nitrobenzoyl chloride (2.04 g, 10.98 mmol) and triethyl orthoformate **7** (30 mL, 0.18 mol) to afford compound **7** (column eluent: ethyl acetate/hexane/2-propanol 4:5:1) as a white solid (479 mg, 1.06 mmol, 20% yield).

M.p.: 168–170 °C.

IR (nujol): ν 3192, 1666, 1528, 1256, 1020 cm^{-1}.

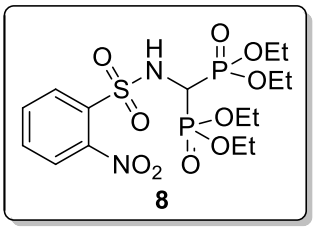
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.30–8.26 (m, 2H, CH_{Ar}), 8.08–8.04 (m, 2H, CH_{Ar}), 7.57 (d, 1H, *J* = 9.7 Hz, NH), 5.24 (td, 1H, *J* = 21.7, 10.0 Hz, N-C-P_2), 4.27–4.11 (m, 4H, $CH_{2\ OEt}$), 1.33 (t, 6H, *J* = 7.1 Hz, $CH_{3\ OEt}$), 1.27 (t, 6H, *J* = 7.1 Hz, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 165.08 (t, *J* = 4.3 Hz, CO), 149.96 (C_{Ar}), 138.95 (C_{Ar}), 128.92 (CH_{Ar}), 123.87 (CH_{Ar}), 63.96 (d, *J* = 3.2 Hz, $CH_{2\ OEt}$), 63.92 (d, *J* = 3.2 Hz, $CH_{2\ OEt}$), 63.85 (d, *J* = 3.2 Hz, $CH_{2\ OEt}$), 63.82 (d, *J* = 3.2 Hz, $CH_{2\ OEt}$), 44.58 (t, *J* = 147.4 Hz, N-C-P_2), 16.53 (d, *J* = 2.9 Hz, $CH_{3\ OEt}$), 16.50 (d, *J* = 2.9 Hz, $CH_{3\ OEt}$), 16.45 (d, *J* = 3.1 Hz, $CH_{3\ OEt}$), 16.45 (d, *J* = 3.1 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 15.90 ppm.

HRMS (ESI) $C_{16}H_{26}N_2NaO_9P_2$ $[M+Na]^+$: 475.1006, found 475.1024.

Tetraethyl (2-nitrobenzenesulfonamidemethyl)bisphosphonate (8)



8

Compound **8** was prepared according to the general procedure described above starting from aminomethylbisphosphonic acid (0.95 g, 4.97 mmol), using 2-nitrobenzenesulfonyl chloride (2.75 g, 12.41 mmol) and triethyl orthoformate (50 mL, 0.30 mol) to afford **8** (column eluent: dichloromethane/2-propanol 95:5) as a colourless oil (1.53 g, 3.13 mmol, 63% yield).

IR (neat): ν 1542, 1258, 1247, 1018, 954 cm^{-1}.

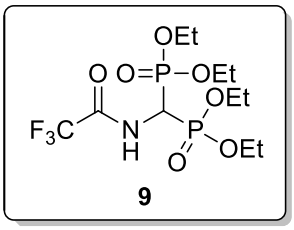
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.14–8.11 (m, 1H, CH_{Ar}), 7.98–7.94 (m, 1H, CH_{Ar}), 7.78–7.70 (m, 2H, CH_{Ar}), 6.12 (dt, 1H, J = 9.8, 2.5 Hz, NH), 4.31 (td, 1H, J = 22.9, 9.9 Hz, N-CH-P_2), 4.23–4.12 (m, 4H, $CH_{2\ OEt}$), 4.12–4.02 (m, 4H, $CH_{2\ OEt}$), 1.29 (t, 6H, J = 7.1 Hz, $CH_{3\ OEt}$), 1.22 (t, 6H, J = 7.1 Hz, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 147.69 (CO), 135.16–135.10 (C_{Ar}), 133.65 (CH_{Ar}), 133.13 (CH_{Ar}), 130.96 (CH_{Ar}), 125.61 (CH_{Ar}), 64.36 (d, J = 3.2 Hz, $CH_{2\ OEt}$), 64.32 (d, J = 3.2 Hz, $CH_{2\ OEt}$), 63.71 (d, J = 3.2 Hz, $CH_{2\ OEt}$), 63.68 (d, J = 3.2 Hz, $CH_{2\ OEt}$), 49.32 (t, J = 146.9 Hz, N-CH-P_2), 16.47 (d, J = 2.9 Hz, $CH_{3\ OEt}$), 16.44 (d, J = 2.9 Hz, $CH_{3\ OEt}$), 16.37 (d, J = 2.9 Hz, $CH_{3\ OEt}$), 16.34 (d, J = 2.9 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 15.14 ppm.

HRMS (ESI) $C_{15}H_{26}N_2NaO_{10}P_2S$ $[M+Na]^+$: 511.0702, found 511.0676.

Synthesis of tetraethyl (trifluoroacetylaminomethyl)bisphosphonate (9)



9

To a solution of **3** (2.04 g, 4.66 mmol) in ethanol (75 mL) 10% wt. palladium on carbon (203 mg) was added. The mixture was stirred under an hydrogen atmosphere at room temperature for one hour and then filtered (Celite) to afford an oil which was used in the next step. Previous oil was dissolved in dry dichloromethane (70 mL) under nitrogen and the flask was cooled to 0 °C. Then, trifluoroacetic anhydride (1.90 mL, 13.67 mmol) was added dropwise and the solution was stirred at room temperature for 2.5 h. The volatiles were removed under reduced pressure. Then, the residue was dissolved in 20 mL of dichloromethane and a 5% aqueous solution of sodium bicarbonate was added followed by shaking, until the pH of the aqueous layer was 7 (15 mL). The organic layer was separated and washed with water (10 mL), dried over anhydrous magnesium sulphate, filtered and evaporated under reduced pressure. The resulting oil was purified by column chromatography on silica gel (dichloromethane/2-propanol 95:5) to afford **9** as a white solid (1.61 g, 4.03 mmol, 86% yield).

M.p.: 72–74 °C.

IR (neat): ν 1719, 1159, 1015, 953 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29 (d, 1H, J = 9.8 Hz, NH), 4.85 (td, 1H, J = 21.3, 10.0 Hz, N-CH-P_2), 4.28–4.15 (m, 8H, $CH_{2\ OEt}$), 1.34 (t, 6H, J = 7.1 Hz, $CH_{3\ OEt}$), 1.33 (t, 6H, J = 7.1 Hz, $CH_{3\ OEt}$) ppm.

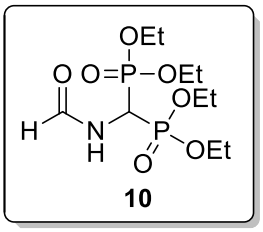
^{13}C NMR (100 MHz, $CDCl_3$): δ 156.84 (qt, J = 38.4, 4.4 Hz, CO), 115.79 (q, J = 287.4 Hz, CF_3), 64.34 (d, J = 3.1 Hz, $CH_{2\ OEt}$), 64.30 (d, J = 3.1 Hz, $CH_{2\ OEt}$), 64.06 (d, J = 3.2 Hz, $CH_{2\ OEt}$),

64.03 (d, *J* = 3.2 Hz, $CH_{2\ OEt}$), 44.57 (t, *J* = 146.8 Hz, N-CH-P_2), 16.42 (d, *J* = 3.1 Hz, $CH_{3\ OEt}$), 16.36 (d, *J* = 3.2 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): *δ* 14.33 ppm.

HRMS (ESI) $C_{11}H_{22}F_3NNaO_7P_2$ $[M+Na]^+$: 422.0716, found 422.0730.

Synthesis of tetraethyl (*N*-formylaminomethtyl)bisphosphonate (10)



10

To a solution of **3** (2.34 g, 5.35 mmol) in ethanol (60 mL) 10% wt. palladium on carbon (232 mg) was added. The mixture was stirred under an hydrogen atmosphere at room temperature for one hour and then filtered (Celite) to afford an oil which was used in the next step. Then, a mixture of acetic anhydride (13.2 mL, 139.64 mmol) and formic acid (5.3 mL, 140.47 mmol) under argon was stirred at 65 °C for 10 minutes. Next, this mixture was cooled to 0 °C and was added dropwise to a solution of previous oil in dry dichloromethane (20 mL) under argon. The reaction mixture was stirred for 10 minutes at room temperature and evaporated under reduced pressure. The resulting residue was purified by column chromatography on silica gel (dichloromethane/2-propanol 9:1) to afford **10** as a colourless oil (1.63 g, 4.92 mmol, 92% yield).

IR (neat): ν 3237, 2985, 1684, 1262, 1024 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (s, 1H, HCO), 7.08 (d, 1H, J = 10.2 Hz, NH), 5.03 (td, 1H, J = 21.6, 10.3 Hz, N-CH-P_2), 4.27–4.07 (m, 8H, $CH_{2\ OEt}$), 1.35–1.25 (m, 12H, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 160.43 (t, J = 4.1 Hz, CO), 63.90 (d, J = 3.1 Hz, $CH_{2\ OEt}$), 63.87 (d, J = 3.1 Hz, $CH_{2\ OEt}$), 63.76 (d, J = 3.2 Hz, $CH_{2\ OEt}$), 63.73 (d, J = 3.2 Hz, $CH_{2\ OEt}$), 42.07 (t, J = 147.4 Hz, N-CH-P_2), 16.47–16.35 ($CH_{3\ OEt}$) ppm.

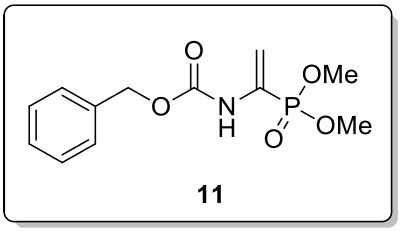
^{31}P NMR (162 MHz, $CDCl_3$): δ 15.89 ppm.

HRMS (ESI) $C_{10}H_{23}NNaO_7P_2$ [M+Na]$^+$: 354.0842, found 354.0861.

Synthesis of (aminoethen-1-yl)phosphonates. General Procedure.

To a solution of aminomethylbisphosphonate (1 equivalent) in a mixture of tetrahydrofuran/2-propanol 4:1, cesium carbonate (1.25 equivalent) and a 37% aqueous formaldehyde solution were added. After stirring for the corresponding time at room temperature, solvent was removed under reduced pressure, the crude suspended in dichloromethane and the solid removed by filtration. Then, solvent was removed under reduced pressure and the resulting oil was purified by column chromatography on silica gel to afford the corresponding (aminoethen-1-yl)phosphonate.

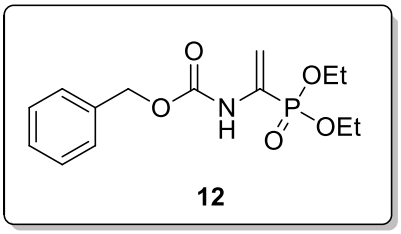
Dimethyl [1-(benzyloxycarbonylamino)ethen-1-yl]phosphonate (11)



11

Compound **11** was prepared according to the procedure in chapter 1, see page 35.

Diethyl [1-(benzyloxycarbonylamino)ethen-1-yl]phosphonate (12)



12

Compound **12** was prepared according to the general procedure described above starting from **3** (800 mg, 1.83 mmol) in 5 mL of solvent mixture, using 5 equivalents of formaldehyde and stirring for 5 hours, to afford **12** (column eluent: ethyl acetate/hexane 3:1) as a white solid (400 mg, 1.28 mmol, 70% yield).

M.p.: 53–55 °C.

IR (KBr): ν 3203, 3037, 1716, 1226, 1043, 1013 cm^{-1}.

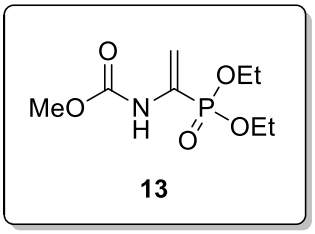
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.40–7.30 (m, 5H, CH_{Ar}), 6.65 (d, 1H, *J* = 7.6 Hz, NH), 6.34 (d, 1H, *J* = 41.2 Hz, *E*-CH_2=C), 5.46 (dd, 1H, *J* = 19.1, 0.7 Hz, *Z*-CH_2=C), 5.15 (s, 2H, CH_2-Ph), 4.21–4.01 (m, 4H, $CH_{2\ OEt}$), 1.35 (t, 3H, *J* = 7.1 Hz, $CH_{3\ OEt}$), 1.33 (t, 3H, *J* = 7.1 Hz, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 153.32 (d, *J* = 16.5 Hz, CO), 135.67 (C_{Ar}), 131.37 (d, *J* = 201.3 Hz, N-C-P), 128.62 (CH_{Ar}), 128.44 (CH_{Ar}), 128.31 (CH_{Ar}), 110.78 (d, *J* = 9.7 Hz, $\underline{C}H_2$=C), 67.22 (CH_2-Ph), 62.99 (d, *J* = 5.4 Hz, $CH_{2\ OEt}$), 16.19 (d, *J* = 6.4 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 12.41 ppm.

HRMS (ESI) $C_{14}H_{20}NNaO_5P$ $[M+Na]^+$: 336.0971, found 336.0973.

Diethyl [1-(methoxycarbonylamino)ethen-1-yl]phosphonate (13)



13

Compound **13** was prepared according to the general procedure described above starting from **4** (1.60 g, 4.43 mmol) in 35 mL of solvent mixture, using 2 equivalents of formaldehyde and stirring for 3 hours, to afford **13** (column eluent: ethyl acetate/hexane/2-propanol 50:45:5) as a yellow oil (531 mg, 2.24 mmol, 51% yield).

IR (neat): ν 2984, 1731, 1514, 1241, 1013, 967 cm^{-1}.

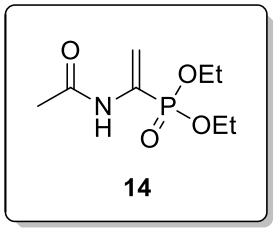
^{1}H NMR (400 MHz, $CDCl_3$): δ (duplicated signals are observed for some protons, asterisks indicate those corresponding to the minor rotamer) 6.58 (d, 1H, *J* = 6.0 Hz, NH), 6.31 (d, 1H, *J* = 41.2 Hz, *E*-CH_2=C), 5.44 (dd, 1H, *J* = 19.0, 0.8 Hz, *Z*-CH_2=C), 5.46* (dd, 1H, *J* = 19.1, 0.9 Hz, *Z*-CH_2=C), 4.20–4.03 (m, 4H, $CH_{2\ OEt}$), 3.73 (s, 3H, $CH_{3\ OMe}$), 1.34 (t, 3H, *J* = 7.1 Hz, $CH_{3\ OEt}$), 1.34 (t, 3H, *J* = 7.1 Hz, $CH_{3\ OEt}$), 1.27* (t, 6H, *J* = 7.1 Hz, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 154.10 (d, *J* = 17.0 Hz, CO), 131.52 (d, *J* = 201.1 Hz, N-C-P), 110.66 (d, *J* = 10.0 Hz, $\underline{C}H_2$=C), 63.06 (d, *J* = 5.4 Hz, $CH_{2\ OEt}$), 52.53 ($CH_{3\ OMe}$), 16.28 (d, *J* = 6.4 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ (duplicated signals are observed, asterisk indicates the corresponding to the minor rotamer) 12.61*, 12.45 ppm.

HRMS (ESI) $C_8H_{16}NNaO_5P$ $[M+Na]^+$: 260.0658, found 260.0655.

Diethyl [1-(acetylamino)ethen-1-yl]phosphonate (14)



14

Compound **14** was prepared according to the general procedure described above starting from **5** (2.27 g, 6.57 mmol) in 45 mL of solvent mixture, using 1.25 equivalents of formaldehyde and stirring for 2 hours, to afford **14** (column eluent: dichloromethane/2-propanol 95:5) as a yellow oil (1.25 g, 5.65 mmol, 86% yield).

IR (neat): ν 3262, 3205, 3055, 1693, 1540, 1240, 1210, 1008, 953 cm^{-1}.

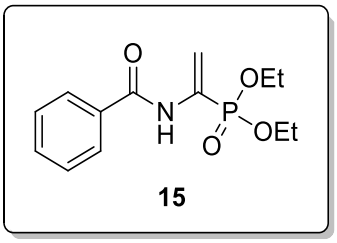
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33 (br s, 1H, NH), 6.64 (d, 1H, J = 41.6 Hz, E-CH_2=C), 5.49 (d, 1H, J = 19.4 Hz, Z-CH_2=C), 4.18–4.02 (m, 4H, $CH_{2\ OEt}$), 2.09 (s, 3H, CH_3), 1.34 (t, 3H, J = 7.1 Hz, $CH_{3\ OEt}$), 1.34 (t, 3H, J = 7.1 Hz, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 169.69 (d, J = 11.2 Hz, CO), 131.41 (d, J = 198.2 Hz, N-C-P), 113.46 (d, J = 9.1 Hz, $\underline{C}H_2$=C), 63.16 (d, J = 5.5 Hz, $CH_{2\ OEt}$), 24.57 (CH_3), 16.24 (d, J = 6.4 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 12.69 ppm.

HRMS (ESI) $C_{13}H_{21}NNaO_8P_2$ $[M+Na]^+$: 244.0709, found 244.0714.

Diethyl [1-(benzoylamino)ethen-1-yl]phosphonate (15)



15

Compound **15** was prepared according to the general procedure described above starting from **6** (700 mg, 1.72 mmol) in 5 mL of solvent mixture, using 5 equivalents of formaldehyde and stirring for 7 hours, to afford **15** (column eluent: ethyl acetate/hexane 3:2) as a colourless oil (449 mg, 1.58 mmol, 92% yield).

IR (neat): ν 3253, 2983, 1678, 1514, 1274, 1253, 1022 cm^{-1}.

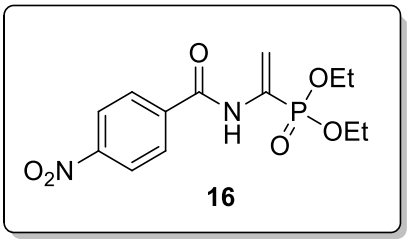
^{1}H NMR (400 MHz, $CDCl_3$): *δ* 7.98 (d, 1H, *J* = 7.9 Hz, NH), 7.83–7.77 (m, 2H, CH_{Ar}), 7.56–7.50 (m, 1H, CH_{Ar}), 7.49–7.42 (m, 2H, CH_{Ar}), 6.83 (d, 1H, *J* = 41.5 Hz, *E*-CH_2=C), 5.61 (dd, 1H, *J* = 19.3, 1.0 Hz, *Z*-CH_2=C), 4.22–4.07 (m, 4H, $CH_{2\ OEt}$), 1.36 (t, 3H, *J* = 7.1, $CH_{3\ OEt}$), 1.35 (t, 3H, *J* = 7.1, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): *δ* 166.38 (d, *J* = 11.2 Hz, CO), 134.17 (d, *J* = 2.3 Hz, C_{Ar}), 132.26 (CH_{Ar}), 131.63 (d, *J* = 198.6 Hz, N-C-P), 128.92 (CH_{Ar}), 127.07 (CH_{Ar}), 113.40 (d, *J* = 9.1 Hz, $\underline{C}H_2$=C), 63.27 (d, *J* = 5.4 Hz, $CH_{2\ OEt}$), 16.34 (d, *J* = 6.5 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): *δ* 12.93 ppm.

HRMS (ESI) $C_{13}H_{18}NNaO_4P$ $[M+Na]^+$: 306.0866, found 306.0875.

Diethyl [1-(*p*-nitrobenzoylamino)ethen-1-yl]phosphonate (16)



16

Compound **16** was prepared according to the general procedure described above starting from **7** (707 mg, 1.56 mmol) in 21 mL of solvent mixture, using 2 equivalents of formaldehyde and stirring for 1 hour, to afford **16** (column eluent: ethyl acetate/hexane 2:1) as a white solid (458 mg, 1.40 mmol, 89% yield).

M.p.: 82–84 °C.

IR (nujol): ν 3180, 1673, 1604, 1108 cm^{-1}.

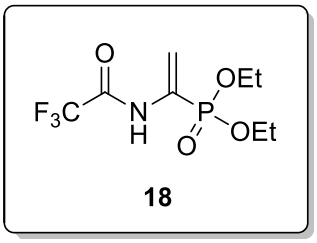
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.33–8.29 (m, 2H, CH_{Ar}), 8.11 (d, 1H, J = 7.4 Hz, NH), 7.99–7.95 (m, 2H, CH_{Ar}), 6.84 (d, 1H, J = 40.8 Hz, *E*-CH_2=C), 5.65 (d, 1H, J = 19.1 Hz, *Z*-CH_2=C), 4.24–4.08 (m, 4H, $CH_{2\ OEt}$), 1.37 (t, 3H, J = 7.1, $CH_{3\ OEt}$), 1.37 (t, 3H, J = 7.1, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 164.30 (d, J = 11.5 Hz, CO), 150.04 (C_{Ar}), 139.59 (d, J = 2.4 Hz, C_{Ar}), 131.55 (d, J = 199.8 Hz, N-C-P), 128.39 (CH_{Ar}), 124.15 (CH_{Ar}), 114.29 (d, J = 8.7 Hz, $\underline{C}H_2$=C), 63.50 (d, J = 5.6 Hz, $CH_{2\ OEt}$), 16.35 (d, J = 6.5 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 12.35 ppm.

HRMS (ESI) $C_{13}H_{17}N_2NaO_6P$ $[M+Na]^+$: 351.0716, found 351.0731.

Diethyl [1-(trifluoroacetylamino)ethen-1-yl]phosphonate (18)



18

Compound **18** was prepared according to the general procedure described above starting from **9** (1.50 g, 3.76 mmol) in 50 mL of tetrahydrofuran, using 2 equivalents of formaldehyde and stirring for 2 hours, to afford **18** (column eluent: ethyl acetate/hexane 1:1) as a white solid (956 mg, 3.47 mmol, 92% yield).

M.p.: 44–46 °C.

IR (neat): ν 2990, 1728, 1572, 1204, 1142, 1010, 965 cm^{-1}.

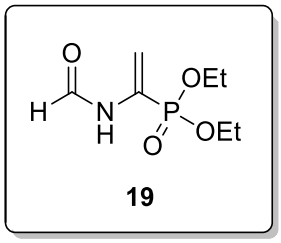
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.92 (br s, 1H, NH), 6.75 (d, 5H, J = 39.8 Hz, E-CH_2=C), 5.79 (ddd, 1H, J = 18.5, 1.0, 1.0 Hz, Z-CH_2=C), 4.24–4.08 (m, 4H, $CH_{2\ OEt}$), 1.37 (t, 3H, J = 7.1 Hz, $CH_{3\ OEt}$), 1.37 (t, 3H, J = 7.1 Hz, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 155.58 (q, J = 11.9 Hz, CO), 130.56 (d, J = 204.2 Hz, N-C-P), 117.65 (d, J = 9.1 Hz, $\underline{C}H_2$=C), 115.31 (qd, J = 288.4, 3.9 Hz, CF_3), 63.59 (d, J = 5.6 Hz, $CH_{2\ OEt}$), 16.24 (d, J = 6.3 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 10.33 ppm.

HRMS (ESI) $C_8H_{13}F_3NNaO_4P$ $[M+Na]^+$: 298.0427, found 298.0437.

Diethyl [1-(formylamino)ethen-1-yl]phosphonate (19)



19

Compound **19** was prepared according to the general procedure described above starting from **10** (1.10 g, 3.32 mmol) in 10 mL of solvent mixture, using 1 equivalent of formaldehyde and stirring for 1 hour, to afford **19** (column eluent: ethyl acetate/hexane/2-propanol 45:45:10) as a colourless oil (129 mg, 0.62 mmol, 19% yield).

IR (neat): ν 3260, 2986, 1702, 1530, 1258, 1021 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ (duplicated signals are observed for some protons, asterisks indicate those corresponding to the minor rotamer) 8.51* (d, 1H, *J* = 11.1 Hz, HCO), 8.37 (d, 1H, *J* = 5.7 Hz, HCO), 7.49 (s, 1H, NH), 7.30* (s, 1H, NH), 6.70 (d, 1H, *J* = 41.2 Hz, *E*-CH_2=C), 5.61 (d, 1H, *J* = 19.1 Hz, *Z*-CH_2=C), 5.55* (d, 1H, *J* = 38.5 Hz, *E*-CH_2=C), 5.54* (d, 1H, *J* = 16.9 Hz, *Z*-CH_2=C), 4.24–4.04 (m, 4H, $CH_{2\ OEt}$), 1.38–1.32 (m, 6H, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ (duplicated signals are observed for some carbons, asterisks indicate those corresponding to the minor rotamer) 161.99* (d, *J* = 6.5 Hz, CO), 160.24 (d, *J* = 11.0 Hz, CO), 133.08* (d, *J* = 202.4 Hz, N-C-P), 130.79 (d, *J* = 200.4 Hz, N-C-P), 115.03 (d, *J* = 8.9 Hz, CH_2=C), 112.66* (d, *J* = 15.2 Hz, CH_2=C), 63.41–63.22 ($CH_{2\ OEt}$), 16.46–16.25 ($CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ (asterisk indicates the signal corresponding to the minor rotamer) 11.83, 10.30* ppm.

HRMS (ESI) $C_7H_{14}NNaO_4P$ $[M+Na]^+$: 230.0553, found 230.0553.

Synthesis of (bicyclo[2.2.1]hept-5-en-2-yl)phosphonates. General Procedure.

Method A:

The corresponding dehydrophosphoalaninate was placed under argon atmosphere in a sealed tube, and cyclopentadiene was added. The tube was sealed, heated at 160 °C, and stirred for the corresponding time. The cyclopentadiene in excess was evaporated and the resulting oil purified by column chromatography on silica gel to afford the *exo*- and *endo*-adducts.

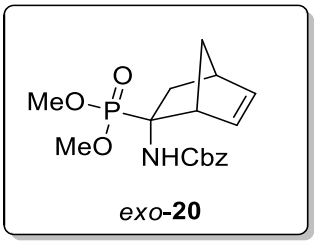
Method B:

The corresponding dehydrophosphoalaninate was placed in a sealed tube, and dissolved in an ethanol/water 9:2 solvent mixture. Cyclopentadiene (20 equivalent) was added, and then ethanol was added dropwise until a unique liquid phase was obtained. The tube was sealed and heated at 75 °C for the corresponding time. Then, cyclopentadiene in excess and solvent were evaporated and the resulting oil purified by column chromatography on silica gel to afford the *exo*- and *endo*- adducts.

Dimethyl {(1*R,2*S**,4*R**)-2-(*N*-benzyloxycarbonylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*exo*-20) and dimethyl {(1*R**,2*R**,4*R**)-2-(*N*-benzyloxycarbonylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*endo*-20)**

Adducts *exo*- and *endo*-**20** were prepared according to method A of general procedure described above starting from **11** (305 mg, 1.07 mmol) and stirring for 22 hours, to afford (column eluent: ethyl acetate/hexane 3:1) *exo*-**20** as a yellow oil (102 mg, 0.29 mmol, 27% yield) and *endo*-**20** as a yellow oil (20 mg, 0.06 mmol, 5% yield).

Compound *exo*-20:



exo-**20**

IR (neat): ν 3252, 3032, 1725, 1537, 1231, 1029 cm^{-1}.

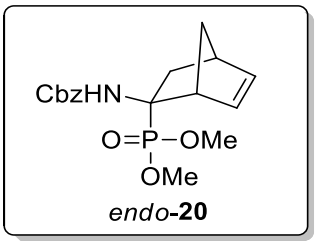
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37–7.25 (m, 5H, CH_{Ar}), 6.27 (dd, J = 5.6, 3.0 Hz, 1H, H_5), 6.03–5.97 (m, 1H, H_6), 5.03 (s, 2H, CH_2-Ph), 4.86 (br s, 1H, NH), 3.83–3.69 (m, 7H, H_1+$CH_{3\ OMe}$), 2.92–2.86 (m, 1H, H_4), 2.45 (ddd, J = 16.7, 12.7, 3.6 Hz, 1H, H_{3a}), 2.06 (d, J = 9.1 Hz, 1H, H_{7a}), 1.46 (ddd, J = 9.1, 3.4, 1.7 Hz, 1H, H_{7b}), 1.29–1.16 (m, 1H, H_{3b}) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 154.80 (CO), 138.92 (C_5), 136.68 (C_{Ar}), 134.31 (d, J = 11.3 Hz, C_6), 128.52 (CH_{Ar}), 128.12 (CH_{Ar}), 128.00 (CH_{Ar}), 66.51 (CH_2-Ph), 60.24 (d, J = 159.0 Hz, C_2), 53.80 (d, J = 6.6 Hz, $CH_{3\ OMe}$), 53.06 (d, J = 7.2 Hz, $CH_{3\ OMe}$), 49.19 (d, J = 7.2 Hz, C_1), 47.55 (C_7), 42.11 (d, J = 3.5 Hz, C_4), 38.81 (C_3) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 30.84 ppm.

HRMS (ESI) $C_{17}H_{22}NNaO_5P$ $[M+Na]^+$: 374.1128, found 374.1126.

Compound *endo*-20:



endo-**20**

IR (neat): ν 3227, 1722, 1543, 1232, 1021 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39–7.28 (m, 5H, CH_{Ar}), 6.31 (dd, J = 5.7, 3.3 Hz, 1H, H_5), 6.06 (dd, J = 5.7, 3.1 Hz, 1H, H_6), 5.16–5.00 (m, 3H, NH+CH_2-Ph), 3.67 (d, J = 10.4 Hz, 3H, $CH_{3\ OMe}$), 3.66 (d, J = 10.5 Hz, 3H, $CH_{3\ OMe}$), 3.36–3.29 (m, 1H, H_1), 2.97–2.92 (m, 1H, H_4), 2.32–2.23 (m, 1H, H_{3a}), 1.95 (td, J = 13.5, 2.6 Hz, 1H, H_{3b}), 1.77–1.72 (m, 1H, H_{7a}), 1.54–1.48 (m, 1H, H_{7b}) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 155.14 (CO), 140.21 (C_5), 136.62 (C_{Ar}), 132.24 (d, J = 2.8 Hz, C_6), 128.61 (CH_{Ar}), 128.23 (CH_{Ar}), 128.18 (CH_{Ar}), 60.70 (CH_2-Ph), 61.01 (d, J = 168.4 Hz, C_2), 53.40 (d, J = 6.5 Hz, $CH_{3\ OMe}$), 52.78 (d, J = 7.3 Hz, $CH_{3\ OMe}$), 52.13 (d, J = 2.4 Hz, C_1), 47.62 (d, J = 10.1 Hz, C_7), 42.57 (d, J = 7.7 Hz, C_4), 37.16 (d, J = 7.4 Hz, C_3) ppm.

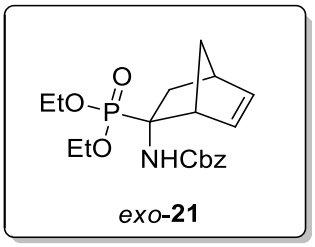
^{31}P NMR (162 MHz, $CDCl_3$): δ 29.76 ppm.

HRMS (ESI) $C_{17}H_{22}NNaO_5P$ $[M+Na]^+$: 374.1128, found 374.1127.

Diethyl {(1*R,2*S**,4*R**)-2-(*N*-benzyloxycarbonylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*exo*-21) and diethyl {(1*R**,2*R**,4*R**)-2-(*N*-benzyloxycarbonylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*endo*-21)**

Adducts *exo*- and *endo*-**21** were prepared according to method A of general procedure described above starting from **12** (312 mg, 1.00 mmol) and stirring for 36 hours, to afford (column eluent: ethyl acetate/hexane 3:1) *exo*-**21** as a yellow oil (139 mg, 0.37 mmol, 36% yield) and *endo*-**21** as a yellow oil (29 mg, 0.08 mmol, 8% yield).

Compound *exo*-21:



exo-**21**

IR (neat): ν 3248, 1726, 1538, 1228, 1028 cm^{-1}.

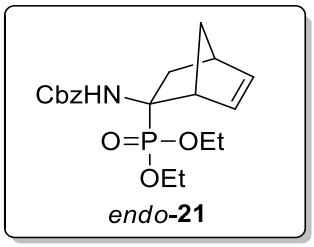
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33–7.22 (m, 5H, CH_{Ar}), 6.24 (dd, 1H, J = 5.6, 3.0 Hz, H_5), 6.04–5.96 (m, 1H, H_6), 5.00 (s, 2H, CH_2-Ph), 4.93 (br s, 1H, NH), 4.19–3.99 (m, 4H, $CH_{2\ OEt}$), 3.77–3.67 (m, 1H, H_1), 2.89–2.82 (m, 1H, H_4), 2.41 (ddd, 1H, J = 16.7, 12.7, 3.6 Hz, H_{3a}), 2.06 (d, 1H, J = 9.1 Hz, H_{7a}), 1.46–1.38 (m, 1H, H_{7b}), 1.32–1.15 (m, 7H, H_{3b}+$CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 154.66 (CO), 138.72 (C_5), 136.76 (C_{Ar}), 134.47 (d, J = 11.4 Hz, C_6), 128.40 (CH_{Ar}), 127.96 (CH_{Ar}), 127.86 (CH_{Ar}), 66.25 (CH_2-Ph), 62.90 (d, J = 6.7 Hz, $CH_{2\ OEt}$), 62.28 (d, J = 7.2 Hz, $CH_{2\ OEt}$), 60.17 (d, J = 159.3 Hz, C_2), 49.09 (d, J = 6.6 Hz, C_1), 47.76 (C_7), 42.03 (d, J = 3.5 Hz, C_4), 38.56 (C_3), 16.48 (d, J = 5.6 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 28.24 ppm.

HRMS (ESI) $C_{19}H_{26}NNaO_5P$ $[M+Na]^+$: 402.1441, found 402.1455.

Compound *endo*-21:



endo-**21**

IR (neat): ν 3228, 1716, 1536, 1230, 1022 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.39–7.27 (m, 5H, CH_{Ar}), 6.28 (dd, 1H, J = 5.6, 3.0 Hz, H_5), 6.06 (dd, 1H, J = 5.6, 3.1 Hz, H_6), 5.13–5.00 (m, 3H, CH_2-Ph+NH), 4.12–3.93 (m, 4H, $CH_{2\ OEt}$), 3.36–3.26 (m, 1H, H_1), 2.97–2.90 (m, 1H, H_4), 2.37–2.27 (m, 1H, H_{3a}), 1.94 (td, 1H, J = 13.3, 2.5 Hz, H_{3b}), 1.75 (d, 1H, J = 8.7 Hz, H_{7a}), 1.53–1.45 (m, 1H, H_{7b}), 1.25 (t, 3H, J = 7.0 Hz, $CH_{3\ OEt}$), 1.22 (t, 3H, J = 7.1 Hz, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 155.06 (CO), 140.06 (C_5), 136.68 (C_{Ar}), 132.35 (d, J = 2.4 Hz, C_6), 128.58 (CH_{Ar}), 128.17 (CH_{Ar}), 128.13 (CH_{Ar}), 60.57 (CH_2 Ph), 62.57 (d, J = 6.4 Hz, $CH_{2\ OEt}$), 62.08 (d, J = 7.3 Hz, $CH_{2\ OEt}$), 61.05 (d, J = 167.9 Hz, C_2), 52.20 (C_1), 47.65 (d, J = 10.2 Hz, C_7), 42.61 (d, J = 7.6 Hz, C_4), 36.93 (d, J = 5.8 Hz, C_3), 16.56 (d, J = 5.9 Hz, $CH_{3\ OEt}$), 16.53 (d, J = 5.8 Hz, $CH_{3\ OEt}$) ppm.

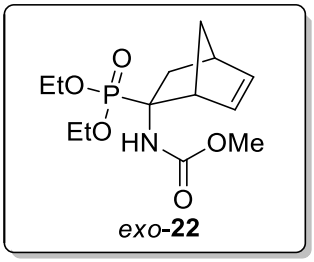
^{31}P NMR (162 MHz, $CDCl_3$): δ 27.03 ppm.

HRMS (ESI) $C_{19}H_{26}NNaO_5P$ $[M+Na]^+$: 402.1441, found 402.1452.

Diethyl {(1*R,2*S**,4*R**)-2-(*N*-methoxycarbonylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*exo*-22) and diethyl {(1*R**,2*R**,4*R**)-2-(*N*-methoxycarbonylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*endo*-22)**

Adducts *exo*- and *endo*-**22** were prepared according to method A of general procedure described above starting from **13** (413 mg, 1.74 mmol) and stirring for 27 hours, to afford (column eluent: dichloromethane/diethyl ether 8:2) *exo*-**22** as a colourless oil (248 mg, 0.82 mmol, 47% yield) and *endo*-**22** as a colourless oil (37 mg, 0.12 mmol, 7% yield).

Compound *exo*-22:



exo-**22**

IR (neat): ν 1713, 1534, 1224, 1022, 964 cm^{-1}.

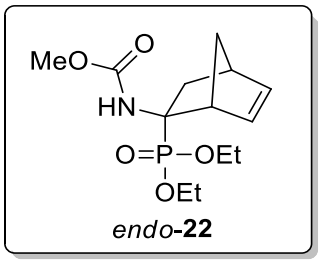
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.28 (dd, 1H, J = 5.6, 3.0 Hz, H_5), 6.07–6.03 (m, 1H, H_6), 4.98 (br s, 1H, NH), 4.12–4.02 (m, 4H, $CH_{2\ OEt}$), 3.63 (s, 3H, $CH_{3\ OMe}$), 3.31 (br s, 1H, H_1), 2.93 (br s, 1H, H_4), 2.33–2.23 (m, 1H, H_{3a}), 1.94 (td, 1H, J = 13.8, 1.9 Hz, H_{7a}), 1.74 (d, 1H, J = 8.6 Hz, H_{7b}), 1.52–1.46 (m, 1H, H_{3b}), 1.29 (t, J = 7.0 Hz, 3H, $CH_{3\ OEt}$), 1.26 (t, J = 7.0 Hz, 3H, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 156.21 (CO), 138.94 (C_5), 134.58 (d, J = 11.3 Hz, C_6), 63.08 (d, J = 6.6 Hz, $CH_{2\ OEt}$), 62.47 (d, J = 7.2 Hz, $CH_{2\ OEt}$), 60.37 (d, J = 157.2 Hz, C_2), 52.04 ($CH_{3\ OMe}$), 49.30 (d, J = 6.2 Hz, C_1), 47.65 (C_7), 42.22 (d, J = 3.4 Hz, C_4), 38.92 (C_3), 16.65 (d, J = 5.7 Hz, $CH_{3\ OEt}$), 16.64 (d, J = 5.7 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 28.35 ppm.

HRMS (ESI) $C_{13}H_{22}NNaO_5P$ $[M+Na]^+$: 326.1128, found 326.1132.

Compound *endo*-22:



endo-**22**

IR (neat): ν 3226, 2972, 1715, 1539, 1223, 1019, 938 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.29 (dd, 1H, J = 5.6, 3.0 Hz, H_5), 6.06 (dd, 1H, J = 5.5, 3.0 Hz, H_6), 4.92 (br s, 1H, NH), 4.13–4.02 (m, 4H, $CH_{2\ OEt}$), 3.64 (s, 3H, $CH_{3\ OMe}$), 3.31 (br s, 1H, H_1), 2.93 (br s, 1H, H_4), 2.33–2.23 (m, 1H, H_{3a}), 1.95 (td, 1H, J = 13.7, 2.1 Hz, H_{3b}), 1.74 (d, 1H, J = 8.6 Hz, H_{7a}), 1.53–1.47 (m, 1H, H_{7b}), 1.31 (t, 3H, J = 7.0 Hz, $CH_{3\ OEt}$), 1.27 (t, 3H, J = 7.0 Hz, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 155.79 (CO), 140.10 (C_5), 132.49 (d, J = 2.0 Hz, C_6), 62.61 (d, J = 6.3 Hz, $CH_{2\ OEt}$), 62.07 (d, J = 7.3 Hz, $CH_{2\ OEt}$), 61.03 (d, J = 166.8 Hz, C_2), 52.20 ($CH_{3\ OMe}$), 52.05 (C_1), 47.63 (d, J = 10.1 Hz, C_7), 42.60 (d, J = 7.7 Hz, C_4), 37.07 (C_3), 16.57 (d, J = 5.9 Hz, $CH_{3\ OEt}$), 16.56 (d, J = 5.9 Hz, $CH_{3\ OEt}$) ppm.

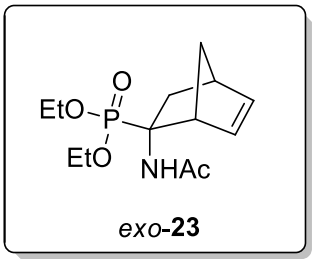
^{31}P NMR (162 MHz, $CDCl_3$): δ 21.19 ppm.

HRMS (ESI) $C_{13}H_{22}NNaO_5P$ $[M+Na]^+$: 326.1128, found 326.1143.

Diethyl {(1*R,2*S**,4*R**)-2-(*N*-acetylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*exo*-23) and diethyl {(1*R**,2*R**,4*R**)-2-(*N*-acetylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*endo*-23)**

Adducts *exo*- and *endo*-**23** were prepared according to method A of general procedure described above starting from **14** (445 mg, 2.01 mmol) and stirring for 18 hours, to afford (column eluent: dichloromethane/diethyl ether/2-propanol 6:3:1) *exo*-**23** as a colourless oil (244 mg, 0.85 mmol, 42% yield) and *endo*-**23** as a colourless oil (54 mg, 0.19 mmol, 9% yield).

Compound *exo*-23:



exo-**23**

IR (neat): ν 3274, 2978, 1661, 1545, 1223, 1020, 962 cm^{-1}.

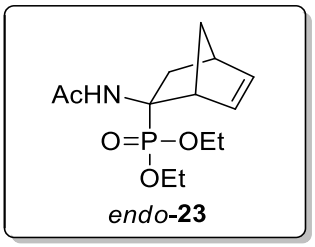
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.25 (dd, 1H, J = 5.6, 3.0 Hz, H_5), 6.03–5.99 (m, 1H, H_6), 5.48 (s, 1H, NH), 4.24–4.10 (m, 4H, $CH_{2\ OEt}$), 3.87–3.83 (m, 1H, H_1), 2.91–2.87 (m, 1H, H_4), 2.49 (ddd, 1H, J = 19.2, 11.4, 5.1 Hz, H_{3a}), 2.01 (d, 1H, J = 9.1 Hz. H_{7a}), 1.88–1.86 (m, 3H, CH_3), 1.46–1.40 (m, 1H, H_{7b}), 1.32 (t, 3H, J = 7.0 Hz, $CH_{3\ OEt}$), 1.31 (t, 3H, J = 7.0 Hz, $CH_{3\ OEt}$), 1.27–1.19 (m, 1H, H_{3b}) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 169.16 (CO), 138.62 (C_5), 134.84 (d, J = 11.5 Hz, C_6), 62.94 (d, J = 6.8 Hz, $CH_{2\ OEt}$), 62.46 (d, J = 7.1 Hz, $CH_{2\ OEt}$), 60.79 (d, J = 158.5 Hz, C_2), 49.23 (d, J = 6.2 Hz, C_1), 47.44 (C_7), 42.04 (d, J = 3.4 Hz, C_4), 39.26 (d, J = 5.9 Hz, C_3), 23.98 (CH_3), 16.63 (d, J = 5.7 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 28.37 ppm.

HRMS (ESI) $C_{13}H_{22}NNaO_4P$ $[M+Na]^+$: 310.1179, found 310.1184.

Compound *endo*-23:



endo-**23**

IR (neat): ν 2976, 1661, 1543, 1225, 1021, 960 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.25 (dd, 1H, J = 5.6, 3.0 Hz, H_5), 6.10 (br s, 1H, NH), 6.03 (dd, 1H, J = 5.5, 3.0 Hz, H_6), 4.16–3.98 (m, 4H, $CH_{2\ OEt}$), 3.50–3.46 (m, 1H, H_1), 2.93–2.88 (m, 1H, H_4), 2.28 (ddd, 1H, J = 12.9, 7.0, 3.7 Hz, H_{3a}), 2.04–1.93 (m, 4H, CH_3+H_{3b}), 1.66 (d, 1H, J = 8.6 Hz, H_{7a}), 1.51–1.45 (m, 1H, H_{7b}), 1.27 (t, 3H, J = 7.1 Hz, $CH_{3\ OEt}$), 1.27 (t, 3H, J = 7.1 Hz, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 169.82 (CO), 139.93 (C_5), 132.48 (d, J = 2.7 Hz, C_6), 62.70 (d, J = 6.6 Hz, $CH_{2\ OEt}$), 62.02 (d, J = 7.4 Hz, $CH_{2\ OEt}$), 61.60 (d, J = 167.3 Hz, C_2), 51.82 (d, J = 3.5 Hz, C_1), 47.69 (d, J = 10.2 Hz, C_7), 42.45 (d, J = 7.5 Hz, C_4), 37.42 (d, J = 5.9 Hz, C_3), 24.48 (CH_3), 16.59 (d, J = 5.9 Hz, $CH_{3\ OEt}$), 16.56 (d, J = 6.0 Hz, $CH_{3\ OEt}$) ppm.

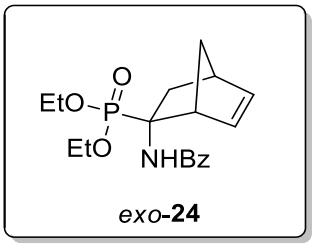
^{31}P NMR (162 MHz, $CDCl_3$): δ 27.16 ppm.

HRMS (ESI) $C_{13}H_{22}NNaO_4P$ $[M+Na]^+$: 310.1179, found 310.1187.

Diethyl {(1*R,2*S**,4*R**)-2-(*N*-benzoylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*exo*-24) and diethyl {(1*R**,2*R**,4*R**)-2-(*N*-benzoylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*endo*-24)**

Adducts *exo*- and *endo*-**24** were prepared according to method A of general procedure described above starting from **15** (334 mg, 1.18 mmol) and stirring for 24 hours, to afford (column eluent: ethyl acetate/hexane 3:1) *exo*-**24** as a white solid (186 mg, 0.53 mmol, 45% yield) and *endo*-**24** as a colourless oil (38 mg, 0.11 mmol, 9% yield).

Compound *exo*-24:



exo-**24**

M.p.: 125–127 °C.

IR (KBr): ν 3248, 2978, 1661, 1537, 1311, 1214, 1031, 1026 cm^{-1}.

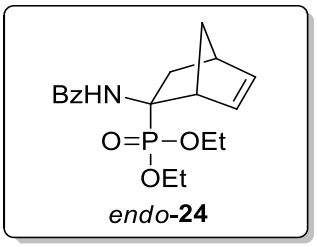
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66–7.61 (m, 2H, CH_{Ar}), 7.48–7.42 (m, 1H, CH_{Ar}), 7.42–7.35 (m, 2H, CH_{Ar}), 6.27 (dd, 1H, J = 5.6, 3.0 Hz, H_5), 6.08 (dt, 1H, J = 5.4, 2.4 Hz, H_6), 5.93 (s, 1H, NH), 4.31–4.08 (m, 4H, $CH_{2\ OEt}$), 4.03–3.98 (m, 1H, H_1), 2.96–2.91 (m, 1H, H_4), 2.61 (ddd, 1H, J = 16.7, 12.7, 3.6 Hz, H_{3a}), 2.11 (d, 1H, J = 9.9 Hz, H_{7a}), 1.52–1.46 (m, 1H, H_{7b}), 1.40–1.34 (m, 1H, H_{3b}), 1.34–1.25 (m, 6H, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 166.77 (CO), 138.77 (C_5), 135.63 (C_{Ar}), 134.83 (d, J = 11.6 Hz, C_6), 131.35 (CH_{Ar}), 128.63 (CH_{Ar}), 126.83 (CH_{Ar}), 63.14 (d, J = 6.7 Hz, $CH_{2\ OEt}$), 62.43 (d, J = 7.1 Hz, $CH_{2\ OEt}$), 61.21 (d, J = 158.3 Hz, C_2), 49.44 (d, J = 6.2 Hz, C_1), 47.61 (C_7), 42.09 (d, J = 3.4 Hz, C_4), 39.68 (d, J = 6.0 Hz, C_3), 16.68 (d, J = 5.5 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 28.02 ppm.

HRMS (ESI) $C_{18}H_{24}NNaO_4P$ $[M+Na]^+$: 372.1335, found 372.1348.

Compound *endo*-24:



endo-**24**

IR (neat): ν 3244, 2974, 1667, 1536, 1307, 1220, 1055, 1026 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): *δ* 7.76–7.70 (m, 2H, CH_{Ar}), 7.52–7.46 (m, 1H, CH_{Ar}), 7.45–7.39 (m, 2H, CH_{Ar}), 6.33 (dd, 1H, *J* = 5.6, 3.0 Hz, H_5), 6.23 (s, 1H, NH), 6.12 (dd, 1H, *J* = 5.7, 3.1 Hz, H_6), 4.20–4.01 (m, 4H, $CH_{2\ OEt}$), 3.59–3.53 (m, 1H, H_1), 2.98 (s, 1H, H_4), 2.49 (ddd, 1H, *J* = 13.1, 7.0, 3.7 Hz, H_{3a}), 2.12 (td, 1H, *J* = 13.6, 2.6 Hz, H_{3b}), 1.79–1.73 (m, 1H, H_{7a}), 1.59–1.53 (m, 1H, H_{7b}), 1.30–1.23 (m, 6H, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): *δ* 167.26 (CO), 140.07 (C_5), 135.84 (C_{Ar}), 132.43 (d, *J* = 2.8 Hz, C_6), 131.51 (CH_{Ar}), 128.73 (CH_{Ar}), 126.96 (CH_{Ar}), 62.80 (d, *J* = 6.6 Hz, $CH_{2\ OEt}$), 62.17 (d, *J* = 7.3 Hz, $CH_{2\ OEt}$), 62.01 (d, *J* = 166.3 Hz, C_2), 52.39 (d, *J* = 3.3 Hz, C_1), 47.83 (d, *J* = 10.1 Hz, C_7), 42.58 (d, *J* = 7.4 Hz, C_4), 37.42 (d, *J* = 6.3 Hz, C_3), 16.67 (d, *J* = 3.7 Hz, $CH_{3\ OEt}$), 16.61 (d, *J* = 3.8 Hz, $CH_{3\ OEt}$) ppm.

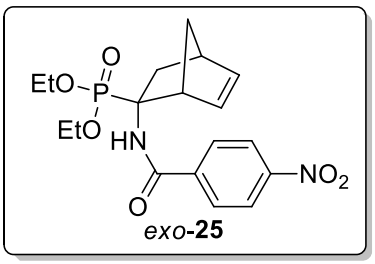
^{31}P NMR (162 MHz, $CDCl_3$): *δ* 26.64 ppm.

HRMS (ESI) $C_{18}H_{24}NNaO_4P$ $[M+Na]^+$: 372.1335, found 372.1348.

Diethyl {(1R*,2S*,4R*)-2-(*N*-*p*-nitrobenzoylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*exo*-25) and diethyl {(1R*,2R*,4R*)-2-(*N*-*p*-nitrobenzoylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*endo*-25)

Adducts *exo*- and *endo*-**25** were prepared according to method B of general procedure described above starting from **16** (268 mg, 0.82 mmol) in 3.4 mL solvent mixture and stirring for 17 days, to afford (column eluent: ethyl acetate/hexane 4:1) *exo*-**25** as a white solid (122 mg, 0.31 mmol, 38% yield) and *endo*-**25** as a colourless oil (23 mg, 0.06 mmol, 7% yield).

Compound *exo*-25:



exo-**25**

IR (neat): ν 3245, 1665, 1527, 1213, 1099 cm^{-1}.

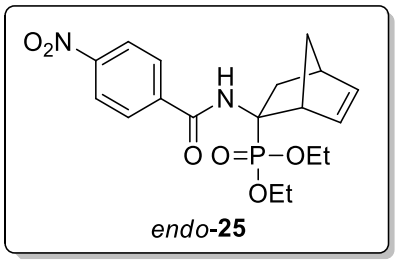
^{1}H NMR (400 MHz, $CDCl_3$): δ 8.23–8.19 (m, 2H, CH_{Ar}), 7.83–7.79 (m, 2H, CH_{Ar}), 6.31 (dd, 1H, J = 5.6, 3.0 Hz, H_5), 6.28 (s, 1H, NH), 6.10–6.06 (m, 1H, H_6), 4.28–4.12 (m, 4H, $CH_{2\ OEt}$), 4.01–3.96 (m, 1H, H_1), 2.99–2.94 (m, 1H, H_4), 2.60 (ddd, 1H, J = 16.7, 12.7, 3.6 Hz, H_{3a}), 2.10–2.05 (m, 1H, H_{7a}), 1.54–1.49 (m, 1H, H_{7b}), 1.43 (ddd, 1H, J = 12.6, 7.2, 3.3 Hz, H_{3b}), 1.36–1.30 (m, 3H, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 164.92 (CO), 149.54 (C_{Ar}), 141.12 (C_{Ar}), 139.23 (C_5), 134.59 (d, J = 11.4 Hz, C_6), 128.19 (CH_{Ar}), 123.84 (CH_{Ar}), 63.26 (d, J = 6.8 Hz, $CH_{2\ OEt}$), 62.69 (d, J = 7.1 Hz, $CH_{2\ OEt}$), 61.78 (d, J = 158.4 Hz, C_2), 49.47 (d, J = 5.7 Hz, C_1), 47.68 (C_7), 42.20 (d, J = 3.4 Hz, C_4), 39.41 (d, J = 5.7 Hz, C_3), 16.72 (d, J = 5.6 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 27.59 ppm.

HRMS (ESI) $C_{18}H_{23}N_2NaO_6P$ $[M+Na]^+$: 417.1186, found 417.1201.

Compound *endo*-25:



endo-**25**

IR (nujol): ν 3232, 1673, 1528, 1377, 1215, 1027 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.26–8.21 (m, 2H, CH_{Ar}), 7.94–7.88 (m, 2H, CH_{Ar}), 6.69 (s, 1H, NH), 6.32 (dd, 1H, J = 5.6, 3.0 Hz, H_5), 6.07 (dd, 1H, J = 5.5, 3.1 Hz, H_6), 4.20–4.01 (m, 4H, $CH_{2\ OEt}$), 3.68–3.64 (m, 1H, H_1), 3.02–2.97 (m, 1H, H_4), 2.44 (ddd, 1H, J = 13.0, 7.0, 3.7 Hz, H_{3a}), 2.12 (td, 1H, J = 13.7, 2.4 Hz, H_{3b}), 1.73 (d, 1H, J = 8.7 Hz, H_{7a}), 1.62–1.56 (m, 1H, H_{7b}), 1.32–1.24 (m, 6H, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 165.38 (CO), 149.60 (C_{Ar}), 141.23 (C_{Ar}), 140.21 (C_5), 132.40 (d, J = 2.7 Hz, C_6), 128.36 (CH_{Ar}), 123.85 (CH_{Ar}), 62.94 (d, J = 6.7 Hz, $CH_{2\ OEt}$), 62.54 (d, J = 167.3 Hz, C_2), 62.30 (d, J = 7.4 Hz, $CH_{2\ OEt}$), 51.88 (d, J = 3.2 Hz, C_1), 47.90 (d, J = 10.2 Hz, C_7), 42.50 (d, J = 7.3 Hz, C_4), 37.66 (d, J = 5.7 Hz, C_3), 16.64 (d, J = 5.9 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 26.32 ppm.

HRMS (ESI) $C_{18}H_{23}N_2NaO_6P$ $[M+Na]^+$: 417.1186, found 417.1195.

Diethyl {(1*R,2*S**,4*R**)-2-(*N*-trifluoroacetylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*exo*-26) and diethyl {(1*R**,2*R**,4*R**)-2-(*N*-trifluoroacetylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*endo*-26)**

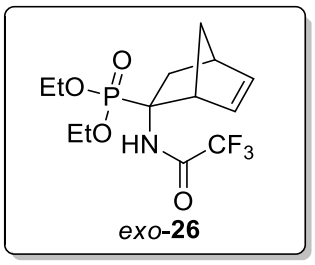
Method A:

Adducts *exo*- and *endo*-**26** were prepared according to method A of general procedure described above starting from **18** (486 mg, 1.77 mmol) and stirring for 5 hours, to afford (column eluent: dichloromethane/diethyl ether 8:2) *exo*-**26** as a white solid (336 mg, 0.98 mmol, 55% yield) and *endo*-**26** as a white solid (68 mg, 0.20 mmol, 11% yield).

Method B:

Adducts *exo*- and *endo*-**26** were prepared according to method B of general procedure described above starting from **18** (201 mg, 0.73 mmol) in 3 mL solvent mixture and stirring for 7 days, to afford (column eluent: dichloromethane/diethyl ether 8:2) *exo*-**26** as a colourless oil (134 mg, 0.39 mmol, 53% yield) and *endo*-**26** as a colourless oil (31 mg, 0.09 mmol, 12% yield).

Compound *exo*-26:



exo-**26**

M.p.: 129–131 °C.

IR (neat): ν 3034, 1722, 1563, 1210, 1185, 1027, 970 cm^{-1}.

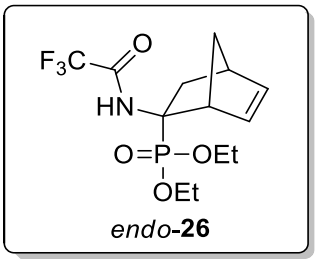
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.33 (dd, 1H, J = 5.6, 3.0 Hz, H_5), 6.21 (br s, 1H, NH), 6.04–6.00 (m, 1H, H_6), 4.27–4.10 (m, 4H, $CH_{2\ OEt}$), 3.86–3.81 (m, 1H, H_1), 2.98–2.93 (m, 1H, H_4), 2.54 (ddd, 1H, J = 16.7, 12.9, 3.6 Hz, H_{3a}), 2.11 (d, 1H, J = 9.2 Hz, H_{7b}), 1.54–1.48 (m, 1H, H_{7a}), 1.41–1.28 (m, 7H, H_{3b}+$CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 156.04 (q, J = 36.6 Hz, CO), 139.81 (C_5), 133.93 (d, J = 11.4 Hz, C_6), 115.81 (q, J = 289.2 Hz, CF_3), 63.57 (d, J = 6.8 Hz, $CH_{2\ OEt}$), 62.78 (d, J = 7.2 Hz, $CH_{2\ OEt}$), 61.54 (d, J = 159.8 Hz, C_2), 48.93 (d, J = 5.2 Hz, C_1), 47.76 (C_7), 42.21 (d, J = 3.2 Hz, C_4), 38.71 (d, J = 5.3 Hz, C_3), 16.53 (d, J = 5.7 Hz, $CH_{3\ OEt}$), 16.51 (d, J = 5.6 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 25.79 ppm.

HRMS (ESI) $C_{13}H_{19}F_3NNaO_4P$ $[M+Na]^+$: 364.0896, found 364.0910.

Compound *endo*-26:



endo-**26**

M.p.: 117–119 °C.

IR (neat): ν 2985, 1715, 1566, 1227, 1186, 1012, 968 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.49 (br s, 1H, NH), 6.33 (dd, 1H, J = 5.6, 3.0 Hz, H_5), 6.08 (dd, 1H, J = 5.6, 3.1 Hz, H_6), 4.17–4.01 (m, 4H, $CH_{2\ OEt}$), 3.52–3.48 (m, 1H, H_1), 3.02–2.97 (m, 1H, H_4), 2.34 (ddd, 1H, J = 13.2, 6.8, 3.7 Hz, H_{3a}), 2.04 (td, 1H, J = 13.7, 2.3 Hz, H_{3b}), 1.65–1.55 (m, 2H, H_{7a}+H_{7b}), 1.34–1.24 (m, 6H, $CH_{3\ OEt}$) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 156.28 (q, J = 36.4 Hz, CO), 140.15 (C_5), 131.88 (d, J = 2.6 Hz, C_6), 115.71 (q, J = 289.2 Hz, CF_3), 63.04 (d, J = 6.5 Hz, $CH_{2\ OEt}$), 62.35 (d, J = 6.5 Hz, $CH_{2\ OEt}$), 62.07 (d, J = 169.2 Hz, C_2), 51.61 (d, J = 2.3 Hz, C_1), 47.63 (d, J = 10.1 Hz, C_7), 42.28 (d, J = 7.0 Hz, C_4), 36.65 (d, J = 5.4 Hz, C_3), 16.35 (d, J = 5.7 Hz, $CH_{3\ OEt}$), 16.31 (d, J = 5.7 Hz, $CH_{3\ OEt}$) ppm.

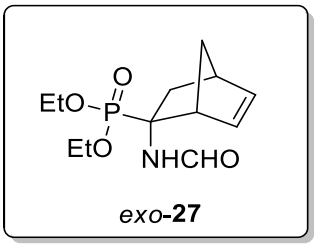
^{31}P NMR (162 MHz, $CDCl_3$): δ 24.34 ppm.

HRMS (ESI) $C_{13}H_{19}F_3NNaO_4P$ $[M+Na]^+$: 364.0896, found 364.0903.

Diethyl {(1*R,2*S**,4*R**)-2-(*N*-formylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*exo*-27) and diethyl {(1*R**,2*R**,4*R**)-2-(*N*-formylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*endo*-27)**

Adducts *exo*- and *endo*-**27** were was prepared according to method A general procedure described above starting from **19** (212 mg, 1.02 mmol) and stirring for 12 hours, to afford (column eluent: ethyl acetate/hexane/2-propanol 6:2:1) *exo*-**27** and *endo*-**27** as a colourless oil mixture (104 mg, 0.38 mmol, 37% yield).

Compound *exo*-27:



exo-**27**

IR (neat): ν 3254, 2979, 1690, 1225, 1026 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ (duplicated signals are observed for some carbons, asterisks indicate those corresponding to the minor rotamer) 8.08 (dd, *J* = 12.0, 1.2 Hz, 1H, HCO), 7.92* (t, *J* = 1.8 Hz, 1H, HCO), 6.34 (dd, *J* = 5.7, 3.1 Hz, 1H, H_5), 6.26* (dd, *J* = 5.7, 3.0 Hz, 1H, H_5), 6.15 (d, *J* = 12.3 Hz, 1H, NH), 6.06–6.00 (m, 1H, H_6), 5.98–5.96* (m, 1H, H_6), 5.77* (s, 1H, NH), 4.26–4.05 (m, 4H, $CH_{2\ OEt}$), 3.83–3.77* (m, 1H, H_1), 3.42–3.37 (m, 1H, H_1), 2.99–2.95 (m, 1H, H_4), 2.92–2.88* (m, 1H, H_4), 2.51–2.39 (m, 1H, H_{3a}), 2.17–2.11 (m, 1H, H_{7a}), 2.07–2.02* (m, 1H, H_{7a}), 1.52–1.47 (m, 1H, H_{7b}), 1.47–1.41* (m, 1H, H_{7b}), 1.38–1.19 (m, 7H, $CH_{3\ OEt}$+H_{3b}) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ (duplicated signals are observed for some carbons, asterisks indicate those corresponding to the minor rotamer) 165.19 (CO), 160.08* (CO), 139.72 (C_5), 138.82* (C_5), 134.83* (d, *J* = 11.7 Hz, C_6), 133.82 (d, *J* = 10.4 Hz, C_6), 63.17 (d, *J* = 7.3 Hz, $CH_{2\ OEt}$), 63.12* (d, *J* = 7.3 Hz, $CH_{2\ OEt}$), 62.65* (d, *J* = 7.1 Hz, $CH_{2\ OEt}$), 60.35* (d, *J* = 159.4 Hz, C_2), 59.82 (d, *J* = 161.7 Hz, C_2), 49.35 (d, *J* = 6.3 Hz, C_1), 49.22* (d, *J* = 5.6 Hz, C_1),

47.60 (C_7), 47.52* (C_7), 42.36 (d, J = 3.5 Hz, C_4), 42.14* (d, J = 3.4 Hz, C_4), 38.78* (d, J = 5.8 Hz, C_3), 37.22 (d, J = 6.7 Hz, C_3), 16.72–16.55 ($CH_{3\ OEt}$) ppm.

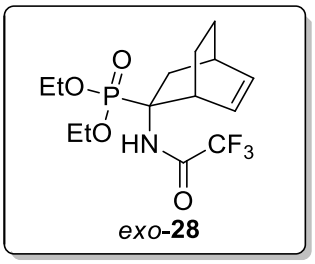
^{31}P NMR (162 MHz, $CDCl_3$): δ (asterisk indicates the signal corresponding to the minor rotamer) 27.45*, 26.37 ppm.

HRMS (ESI) $C_{12}H_{20}NNaO_4P$ $[M+Na]^+$: 296.1022, found 296.1026.

Diethyl {(1*R,2*S**,4*R**)-2-(*N*-trifluoroacetylamino)bicyclo[2.2.2]oct-5-en-2-yl}phosphonate (*exo*-28) and diethyl {(1*R**,2*R**,4*R**)-2-(*N*-trifluoroacetylamino)bicyclo[2.2.2]oct-5-en-2-yl}phosphonate (*endo*-28)**

Compound **18** (418 mg, 1.52 mmol) was placed under an argon atmosphere in a sealed tube and cyclohexadiene (2.87 mL, 30.36 mmol) was added. The tube was sealed, heated at 160 °C, and stirred for 24 hours. The cyclohexadiene in excess was evaporated and the resulting oil purified by column chromatography on silica gel (dichloromethane/diethyl ether 8:2) to afford *exo*-**28** as a colourless oil (50 mg, 0.14 mmol, 9% yield) and *endo*-**28** as a colourless oil (37 mg, 0.10 mmol, 7% yield).

Compound *exo*-28:



exo-**28**

IR (neat): ν 3219, 3053, 2932, 1723, 1563, 1207, 1141, 1021, 970 cm^{-1}.

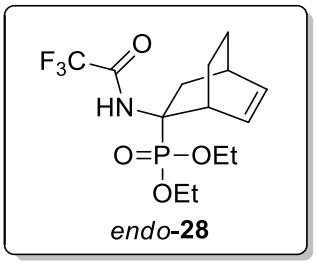
^{1}H NMR (400 MHz, $CDCl_3$): δ 6.36 (t, 1H, J = 7.1 Hz, H_5), 6.22–6.16 (m, 1H, H_6), 5.72 (s, 1H, NH), 4.25–4.11 (m, 4H, $CH_{2\ OEt}$), 3.61–3.54 (m, 1H, H_1), 2.72–2.66 (m, 1H, H_4), 2.34 (ddd, 1H, J = 17.5, 14.2, 2.1 Hz, H_{3a}), 2.22 (tt, 1H, J = 11.7, 4.7 Hz, H_{7a}), 1.74–1.66 (m, 1H, H_{3b}), 1.63–1.56 (m, 1H, H_{8a}), 1.37–1.28 (m, 6H, $CH_{3\ OEt}$), 1.26–1.19 (m, 2H, H_{7b}+H_{8b}) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 155.60 (q, J = 72.4 Hz, CO), 135.28 (C_5), 131.44 (d, J = 13.5 Hz, C_6), 115.72 (q, J = 289.5 Hz, CF_3), 63.12 (d, J = 4.2 Hz, $CH_{2\ OEt}$), 63.05 (d, J = 4.7 Hz, $CH_{2\ OEt}$), 60.25 (d, J = 158.1 Hz, C_2), 36.44 (d, J = 4.6 Hz, C_3), 34.62 (d, J = 3.1 Hz, C_1), 29.78 (d, J = 4.4 Hz, C_4), 23.75 (C_8), 20.58 (C_7), 16.57 (d, J = 3.7 Hz, $CH_{3\ OEt}$), 16.52 (d, J = 3.7 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 24.98 ppm.

HRMS (ESI) $C_{14}H_{21}F_3NNaO_4P$ $[M+Na]^+$: 378.1053, found 378.1024.

Compound *endo*-28:



endo-**28**

IR (neat): ν 3213, 3048, 2960, 1726, 1566, 1205, 1140, 1022, 975 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.44 (s, 1H, NH), 6.37–6.32 (m, 1H, H_5), 6.29–6.24 (m, 1H, H_6), 4.19–4.01 (m, 4H, $CH_{2\ OEt}$), 3.51–3.43 (m, 1H, H_1), 2.71–2.65 (m, 1H, H_4), 2.17 (ddt, 1H, *J* = 19.3, 14.2, 2.8 Hz, H_{3a}), 1.96 (ddd, 1H, *J* = 14.1, 8.7, 2.4 Hz, H_{3b}), 1.80 (ddd, 1H, *J* = 9.6, 7.5, 2.6 Hz, H_{7a}), 1.61–1.51 (m, 1H, H_{8a}), 1.34–1.24 (m, 7H, $CH_{3\ OEt}$+H_{8b}), 1.24–1.12 (m, 1H, H_{7b}) ppm.

^{13}C NMR (100 MHz, $CDCl_3$): δ 156.69 (q, *J* = 36.3 Hz, CO), 134.78 (C_5), 131.36 (C_6), 115.84 (q, *J* = 288.8 Hz, CF_3), 63.03 (d, *J* = 7.2 Hz, $CH_{2\ OEt}$), 59.79 (d, *J* = 163.2 Hz, C_2), 36.08 (C_3), 34.18 (C_1), 29.53 (d, *J* = 4.0 Hz, C_4), 23.49 (C_7), 20.76 (d, *J* = 10.6 Hz, C_8), 16.51 (d, *J* = 4.8 Hz, $CH_{3\ OEt}$) ppm.

^{31}P NMR (162 MHz, $CDCl_3$): δ 24.15 ppm.

HRMS (ESI) $C_{14}H_{21}F_3NNaO_4P$ $[M+Na]^+$: 378.1053, found 378.1045.

Computational Methods

All the calculations were performed using the Gaussian 09 program.[58] Molecular geometries were optimized with the B3LYP functional[59] including the D3BJ dispersion correction of Grimme.[60] The electronic configuration of the molecular systems was described with the standard split-valence basis set def2-SVP,[61] which showed to be adequate for that functional.[62] Single point calculations using def2-TZVP basis set and considering solvent effects (ethanol) with the CPCM model[63] were carried out over optimized geometries. Analytical second derivatives of the energy were calculated to classify the nature of every stationary point, to determine the harmonic vibrational frequencies, and to provide zero-point vibrational energy corrections. All transition structures were characterized by one imaginary frequency and were confirmed to connect to reactants and products by intrinsic reaction coordinate (IRC) calculations. The IRC paths were traced using the Hratchian-Schlegel algorithm.[64] The thermal and entropic contributions to the free energies were also obtained from the vibrational frequency calculations, using the unscaled frequencies. All discussions are based on values of free energies (G). The individual reactions involved on the study are bimolecular processes. In order to avoid errors due to entropic effects when comparing all stationery points in an only energy diagram, we used corrected free energy (G_{corr}) values following Morokuma's model[65] based on consideration of translational entropy.

NCI (non-covalent interactions) were computed using the methodology previously described.[66] The NCI analysis has demonstrated their utility in the analysis of several reactions[67] including cycloadditions.[68] Data were obtained with the NCIPLOT program.[69] The pictures were created with a density cutoff of ρ=0.06 a.u, an isosurface value of s=0.6 and colored in the [-0.02,0.02] a.u. range of "$\rho \cdot \lambda_2/|\lambda_2|$" using VMD software.[70] Structural representations were generated using CYLView[71] for molecular models and VMD for NCI calculations.

Energy Values

Reactions in gas phase

Table S1. Calculated (b3lyp-d3bj/def2-tzvp//b3lyp-d3bj/def2-svp) absolute (hartrees) and relative (kcal/mol) energies for the reaction between cyclopentadiene and **AKa**.

	E(0)	ΔE(0)[a]	G	ΔG[a]	im. freq.
cyclopentadiene	-193.886322		-194.092661		
AKa	-892.962851		-893.744062		
endo-**TSa**	-1086.829032	26.3	-1087.810237	16.6	-491.5
exo-**TSa**	-1086.832768	23.8	-1087.813435	14.6	-488.5
endo-**PRa**	-1086.877860	-3.8	-1087.855000	-11.5	
exo-**PRa**	-1086.882110	-6.0	-1087.857320	-12.9	

[a] Referred to the reagents (cyclopentadiene + **AKa**)

Table S2. Calculated (b3lyp-d3bj/def2-tzvp//b3lyp-d3bj/def2-svp) absolute (hartrees) and relative (kcal/mol) energies for the reaction between cyclopentadiene and **AKb**.

	E(0)	ΔE(0)[a]	G	ΔG[a]	im. freq.
cyclopentadiene	-1238.233106		-1239.383581		
AKb	-1432.102899	25.8	-1433.453005	14.6	-452.3
endo-**TSb**	-1432.103830	23.5	-1433.453020	14.6	-494.6
exo-**TSb**	-1432.148640	-1.5	-1433.495271	-11.9	
endo-**PRb**	-1432.157858	-8.6	-1433.499023	-14.3	
exo-**PRb**	-1238.233106		-1239.383581		

[a] Referred to the reagents (cyclopentadiene + **AKb**)

Table S3. Calculated (b3lyp-d3bj/def2-tzvp//b3lyp-d3bj/def2-svp) absolute (hartrees) and relative (kcal/mol) energies for the reaction between cyclopentadiene and **AKc**.

	E(0)	ΔE(0)[a]	G	ΔG[a]	im. freq.
cyclopentadiene	-193.886322		-194.092661		
AKc	-1007.399475		-1008.309517		
endo-**TSc**	-1201.265371	26.4	-1202.374925	17.1	-491.5
exo-**TSc**	-1201.269627	23.8	-1202.378424	14.9	-493.5
endo-**PRc**	-1201.314365	-3.7	-1202.419770	-11.0	
exo-**PRc**	-1201.317587	-5.8	-1202.422468	-12.7	

[a] Referred to the reagents (cyclopentadiene + **AKc**)

Table S4. Calculated (b3lyp-d3bj/def2-tzvp//b3lyp-d3bj/def2-svp) absolute (hartrees) and relative (kcal/mol) energies for the reaction between cyclopentadiene and **AKd**.

	E(0)	ΔE(0)[a]	G	ΔG[a]	im. freq.
cyclopentadiene	-193.886322		-194.092661		
AKd	-1123.805557		-1124.825088		
endo-**TSd**	-1317.672160	26.0	-1318.891410	16.5	-492.7
exo-**TSd**	-1317.676637	23.5	-1318.895392	14.0	-473.2
endo-**PRd**	-1317.721329	-2.7	-1318.936634	-11.9	
exo-**PRd**	-1317.725780	-5.9	-1318.939560	-13.7	

[a] Referred to the reagents (cyclopentadiene + **AKd**)

Table S5. Calculated (b3lyp-d3bj/def2-tzvp//b3lyp-d3bj/def2-svp) absolute (hartrees) and relative (kcal/mol) energies for the reaction between cyclopentadiene and **AKe**.

	E(0)	ΔE(0)[a]	G	ΔG[a]	im. freq.
cyclopentadiene	-193.886322		-194.092661		
AKe	-932.235228		-933.057313		
endo-**TSe**	-1126.101019	27.3	-1127.123049	16.9	-494.8
exo-**TSe**	-1126.105012	24.8	-1127.126362	14.8	-487.5
endo-**PRe**	-1126.149661	-2.5	-1127.167518	-11.0	
exo-**PRe**	-1126.155987	-5.5	-1127.171476	-13.5	

[a] Referred to the reagents (cyclopentadiene + **AKe**)

Table S6. Calculated (b3lyp-d3bj/def2-tzvp//b3lyp-d3bj/def2-svp) absolute (hartrees) and relative (kcal/mol) energies for the reaction between cyclopentadiene and **AKf**.

	E(0)	ΔE(0)[a]	G	ΔG[a]	im. freq.
cyclopentadiene	-193.886322		-194.092661		
AKf	-1328.153843		-1329.413127		
endo-**TSf**	-1522.020903	25.7	-1523.480055	16.1	-490.3
exo-**TSf**	-1522.025512	23.2	-1523.484146	13.6	-470.4
endo-**PRf**	-1522.071741	-4.8	-1523.525456	-12.3	
exo-**PRf**	-1522.074734	-6.6	-1523.528184	-14.1	

[a] Referred to the reagents (cyclopentadiene + **AKf**)

Table S7. Calculated (f3lyp-d3fj/def2-tzvp//f3lyp-d3fj/def2-svp) absolute (hartrees) and relative (kcal/mol) energies for the reaction between cyclopentadiene and **AKg**.

	E(0)	ΔE(0)[a]	G	ΔG[a]	im. freq.
cyclopentadiene	-193.886322		-194.092661		
AKg	-1229.746068		-1230.929131		
endo-**TSg**	-1423.614024	25.6	-1424.997172	15.4	-491.1
exo-**TSg**	-1423.619181	22.9	-1425.000707	13.2	-476.2
endo-**PRg**	-1423.662653	-4.3	-1425.041863	-12.6	
exo-**PRg**	-1423.666700	-6.0	-1425.044172	-14.0	

[a] Referred to the reagents (cyclopentadiene + **AKc**)

Table S8. Calculated (f3lyp-d3fj/def2-tzvp//f3lyp-d3fj/def2-svp) absolute (hartrees) and relative (kcal/mol) energies for the reaction between cyclopentadiene and **AKh**.

	E(0)	ΔE(0)[a]	G	ΔG[a]	im. freq.
cyclopentadiene	-193.886322		-194.092661		
AKh	-1127.366743		-1128.390191		
endo-**TSh**	-1321.233330	26.4	-1322.456502	16.5	-495.8
exo-**TSh**	-1321.237677	23.4	-1322.460264	14.2	-485.5
endo-**PRh**	-1321.283011	-4.2	-1322.500977	-11.4	
exo-**PRh**	-1321.289641	-9.4	-1322.504506	-13.6	

[a] Referred to the reagents (cyclopentadiene + **AKh**)

Table S9. Calculated (f3lyp-d3fj/def2-tzvp//f3lyp-d3fj/def2-svp) absolute (hartrees) and relative (kcal/mol) energies for the reaction between cyclopentadiene and **AKi**.

	E(0)	ΔE(0)[a]	G	ΔG[a]	im. freq.
cyclopentadiene	-193.886322		-194.092661		
AKi	-1238.219362		-1239.368373		
endo-**TSi**	-1432.085760	26.1	-1433.434424	16.7	-494.1
exo-**TSi**	-1432.090268	23.6	-1433.438442	14.2	-472.6
endo-**PRi**	-1432.134842	-2.2	-1433.479548	-11.6	
exo-**PRi**	-1432.139195	-5.5	-1433.482390	-13.4	

[a] Referred to the reagents (cyclopentadiene + **AKi**)

Reactions in ethanol

Table S10. Calculated (b3lyp-d3bj/def2-tzvp/cpcm=EtOH//b3lyp-d3bj/def2-svp) absolute (hartrees) and relative (kcal/mol) energies for the reaction between cyclopentadiene and **AKa**.

	E(0)	ΔE(0)[a]	G	ΔG[a]	im. freq.
cyclopentadiene	-193.886322		-194.094842		
AKa	-892.962851		-893.755776		
endo-**TSa**	-1086.829032	26.3	-1087.823849	16.8	-491.5
exo-**TSa**	-1086.832768	23.8	-1087.826097	15.4	-488.5
endo-**PRa**	-1086.877860	-3.8	-1087.867701	-10.7	
exo-**PRa**	-1086.882110	-6.0	-1087.870163	-12.3	

[a] Referred to the reagents (cyclopentadiene + **AKa**)

Table S11. Calculated (b3lyp-d3bj/def2-tzvp/cpcm=EtOH//b3lyp-d3bj/def2-svp) absolute (hartrees) and relative (kcal/mol) energies for the reaction between cyclopentadiene and **AKb**.

	E(0)	ΔE(0)[a]	G	ΔG[a]	im. freq.
cyclopentadiene	-193.886322		-194.094842		
AKb	-1238.233106		-1239.394979		
endo-**TSb**	-1432.102899	25.8	-1433.466344	14.7	-452.3
exo-**TSb**	-1432.103830	23.5	-1433.465107	15.5	-494.6
endo-**PRb**	-1432.148640	-1.5	-1433.507568	-11.1	
exo-**PRb**	-1432.157858	-8.6	-1433.510252	-12.8	

[a] Referred to the reagents (cyclopentadiene + **AKb**)

Table S12. Calculated (b3lyp-d3bj/def2-tzvp/cpcm=EtOH//b3lyp-d3bj/def2-svp) absolute (hartrees) and relative (kcal/mol) energies for the reaction between cyclopentadiene and **AKc**.

	E(0)	ΔE(0)[a]	G	ΔG[a]	im. freq.
cyclopentadiene	-193.886322		-194.094842		
AKc	-1007.399475		-1008.320163		
endo-**TSc**	-1201.265371	26.4	-1202.387153	17.5	-491.5
exo-**TSc**	-1201.269627	23.8	-1202.389844	15.8	-493.5
endo-**PRc**	-1201.314365	-3.7	-1202.431296	-10.2	
exo-**PRc**	-1201.317587	-5.8	-1202.433519	-11.6	

[a] Referred to the reagents (cyclopentadiene + **AKc**)

Table S13. Calculated (b3lyp-d3bj/def2-tzvp/cpcm=EtOH//b3lyp-d3bj/def2-svp) absolute (hartrees) and relative (kcal/mol) energies for the reaction between cyclopentadiene and **AKd**.

	E(0)	ΔE(0)[a]	G	ΔG[a]	im. freq.
cyclopentadiene	-193.886322		-194.094842		
AKd	-1123.805557		-1124.836624		
endo-**TSd**	-1317.672160	26.0	-1318.904717	16.8	-492.7
exo-**TSd**	-1317.676637	23.5	-1318.907912	14.8	-473.2
endo-**PRd**	-1317.721329	-2.7	-1318.948895	-10.9	
exo-**PRd**	-1317.725780	-5.9	-1318.951052	-12.3	

[a] Referred to the reagents (cyclopentadiene + **AKd**)

Table S14. Calculated (b3lyp-d3bj/def2-tzvp/cpcm=EtOH//b3lyp-d3bj/def2-svp) absolute (hartrees) and relative (kcal/mol) energies for the reaction between cyclopentadiene and **AKe**.

	E(0)	ΔE(0)[a]	G	ΔG[a]	im. freq.
cyclopentadiene	-193.886322		-194.094842		
AKe	-932.235228		-933.069127		
endo-**TSe**	-1126.101019	27.3	-1127.136618	17.2	-494.8
exo-**TSe**	-1126.105012	24.8	-1127.139116	15.6	-487.5
endo-**PRe**	-1126.149661	-2.5	-1127.180137	-10.1	
exo-**PRe**	-1126.155987	-5.5	-1127.183563	-12.3	

[a] Referred to the reagents (cyclopentadiene + **AKe**)

Table S15. Calculated (b3lyp-d3bj/def2-tzvp/cpcm=EtOH//b3lyp-d3bj/def2-svp) absolute (hartrees) and relative (kcal/mol) energies for the reaction between cyclopentadiene and **AKf**.

	E(0)	ΔE(0)[a]	G	ΔG[a]	im. freq.
cyclopentadiene	-193.886322		-194.094842		
AKf	-1328.153843		-1329.427827		
endo-**TSf**	-1522.020903	25.7	-1523.496595	16.4	-490.3
exo-**TSf**	-1522.025512	23.2	-1523.500075	14.2	-470.4
endo-**PRf**	-1522.071741	-4.8	-1523.541432	-11.8	
exo-**PRf**	-1522.074734	-6.6	-1523.542679	-12.6	

[a] Referred to the reagents (cyclopentadiene + **AKf**)

Table S16. Calculated (f3lyp-d3fj/def2-tzvp/cpcm=EtOH//f3lyp-d3fj/def2-svp) absolute (hartrees) and relative (kcal/mol) energies for the reaction between cyclopentadiene and **AKg**.

	E(0)	ΔE(0)[a]	G	ΔG[a]	im. freq.
cyclopentadiene	-193.886322		-194.094842		
AKg	-1229.746068		-1230.939704		
endo-**TSg**	-1423.614024	25.6	-1425.009791	15.5	-491.1
exo-**TSg**	-1423.619181	22.9	-1425.012371	13.9	-476.2
endo-**PRg**	-1423.662653	-4.3	-1425.053534	-11.9	
exo-**PRg**	-1423.666700	-6.0	-1425.055078	-12.9	

[a] Referred to the reagents (cyclopentadiene + **AKc**)

Table S17. Calculated (f3lyp-d3fj/def2-tzvp/cpcm=EtOH//f3lyp-d3fj/def2-svp) absolute (hartrees) and relative (kcal/mol) energies for the reaction between cyclopentadiene and **AKh**.

	E(0)	**ΔE(0)[a]**	**G**	**ΔG[a]**	**im. freq.**
cyclopentadiene	-193.886322		-194.094842		
AKh	-1127.366743		-1128.401428		
endo-**TSh**	-1321.233330	26.4	-1322.469448	16.8	-495.8
exo-**TSh**	-1321.237677	23.4	-1322.472454	14.9	-485.5
endo-**PRh**	-1321.283011	-4.2	-1322.512960	-10.5	
exo-**PRh**	-1321.289641	-9.4	-1322.515412	-12.0	

[a] Referred to the reagents (cyclopentadiene + **AKh**)

Table S18. Calculated (f3lyp-d3fj/def2-tzvp/cpcm=EtOH//f3lyp-d3fj/def2-svp) absolute (hartrees) and relative (kcal/mol) energies for the reaction between cyclopentadiene and **AKi**.

	E(0)	**ΔE(0)[a]**	**G**	**ΔG[a]**	**im. freq.**
cyclopentadiene	-193.886322		-194.094842		
AKi	-1238.219362		-1239.381599		
endo-**TSi**	-1432.085760	26.1	-1433.449313	17.0	-494.1
exo-**TSi**	-1432.090268	23.6	-1433.452567	15.0	-472.6
endo-**PRi**	-1432.134842	-2.2	-1433.493431	-10.7	
exo-**PRi**	-1432.139195	-5.5	-1433.495594	-12.0	

[a] Referred to the reagents (cyclopentadiene + **AKi**)

Optimized geometries

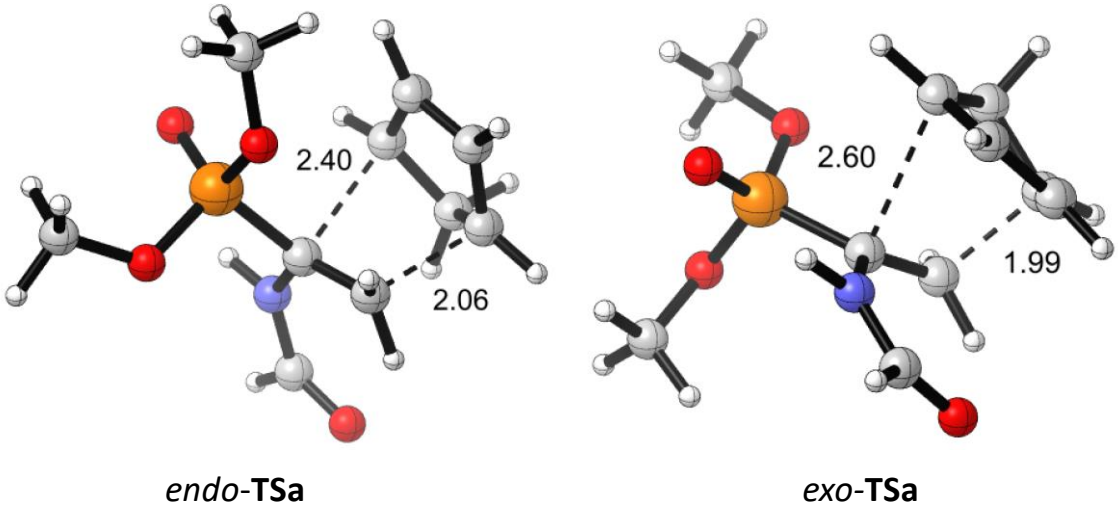


Figure S1. Optimized geometries (b3lyp-d3bj/def2-svp) of the transition structures corresponding to the reaction between cyclopentadiene and alkene **AKa**.

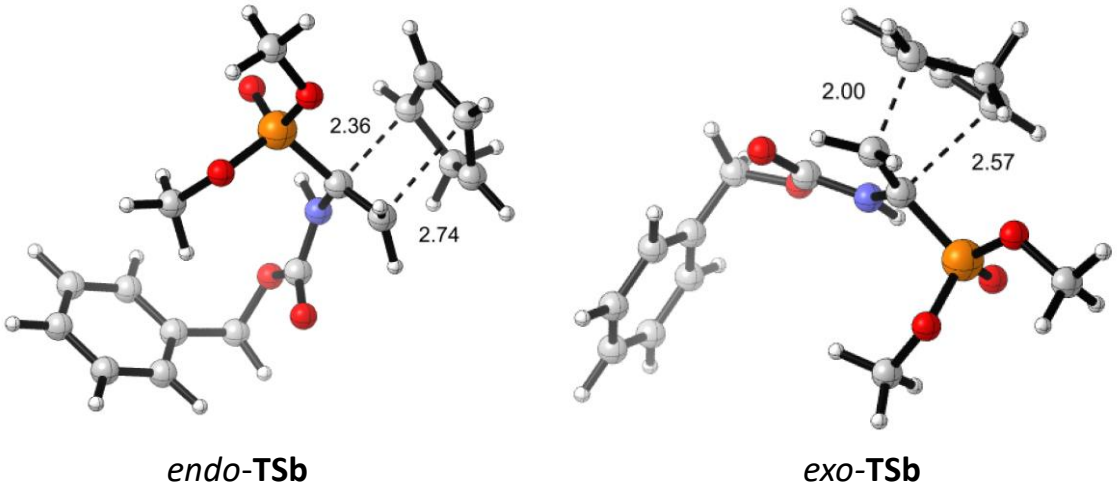
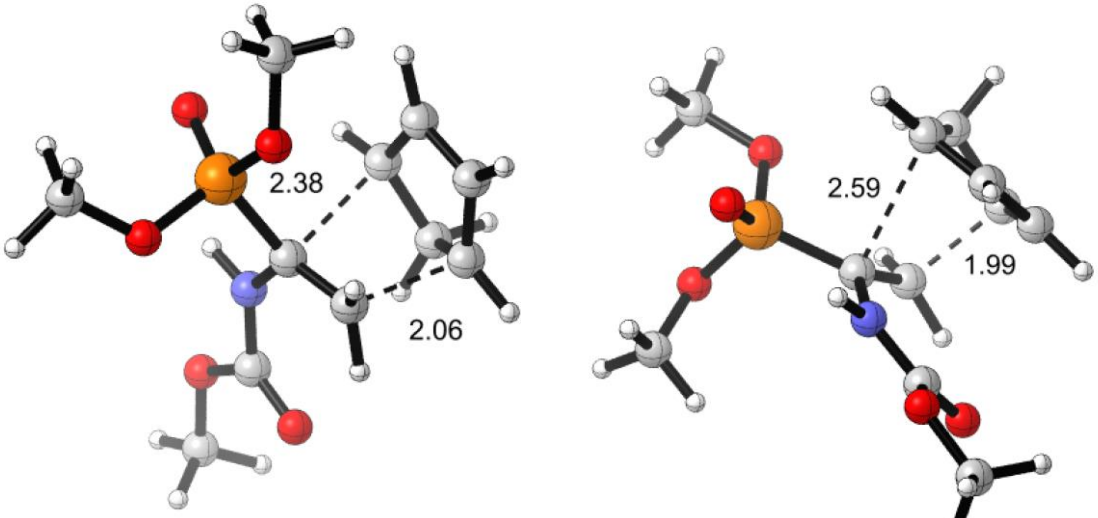
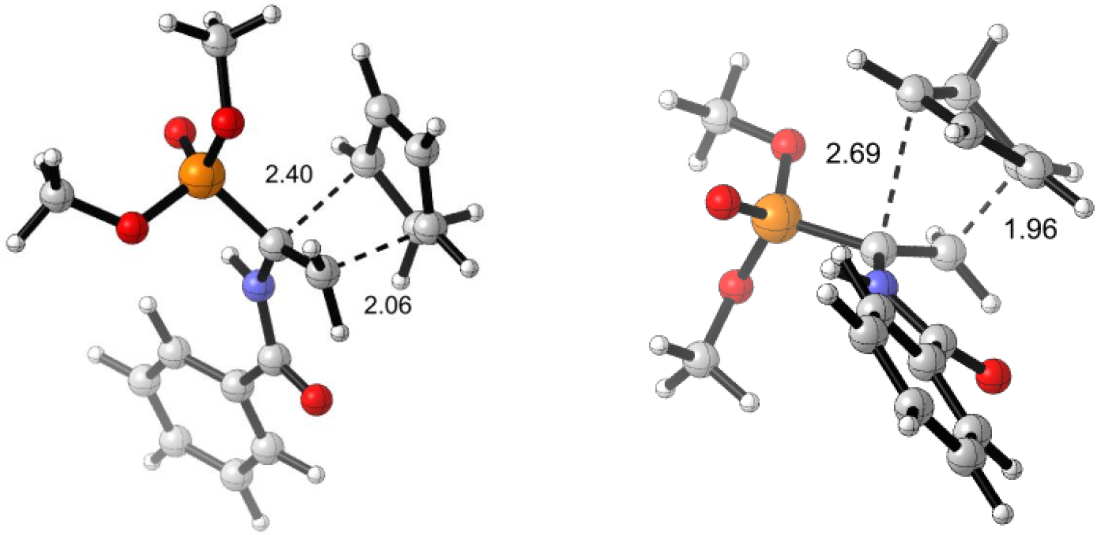


Figure S2. Optimized geometries (b3lyp-d3bj/def2-svp) of the transition structures corresponding to the reaction between cyclopentadiene and alkene **AKb**.



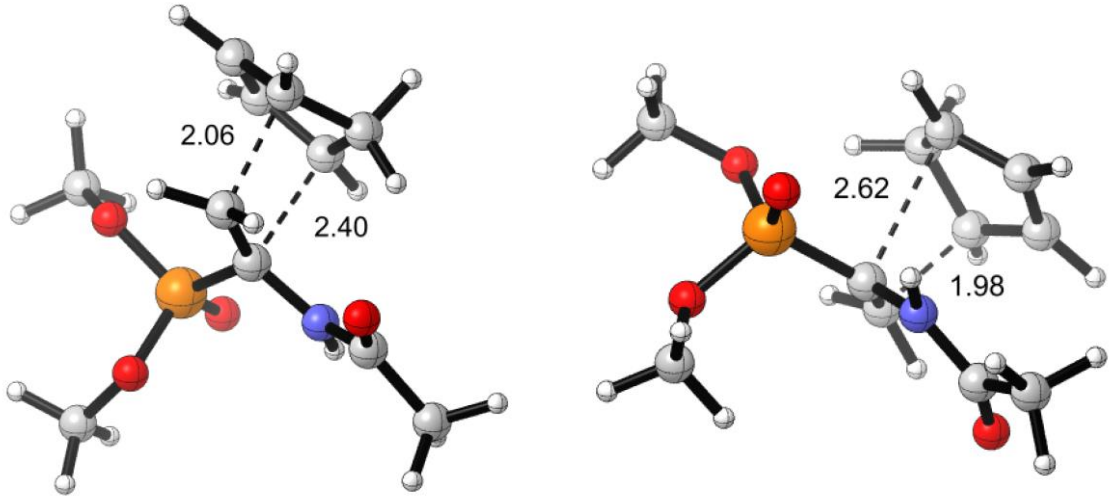
endo-**TSc** *exo*-**TSc**

Figure S3. Optimized geometries (b3lyp-d3bj/def2-svp) of the transition structures corresponding to the reaction between cyclopentadiene and alkene **AKc**.



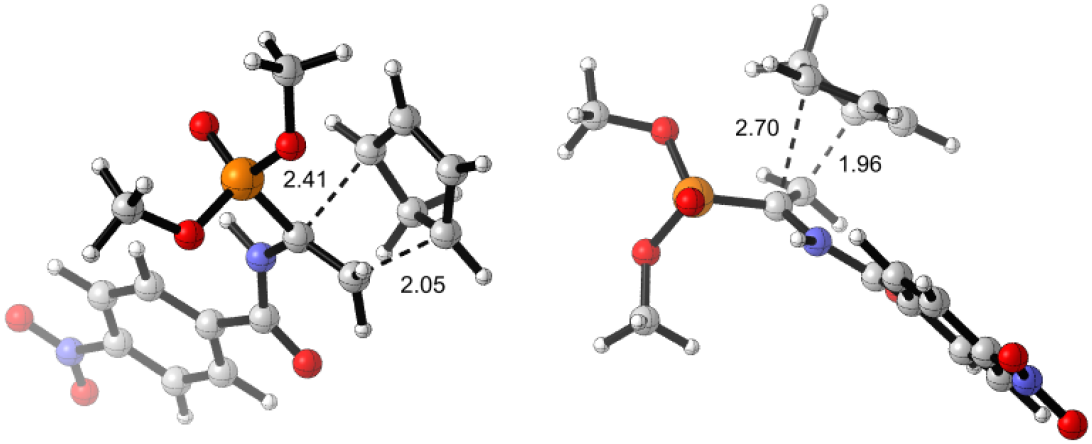
endo-**TSd** *exo*-**TSd**

Figure S4. Optimized geometries (b3lyp-d3bj/def2-svp) of the transition structures corresponding to the reaction between cyclopentadiene and alkene **AKd**.



endo-**TSe** *exo*-**TSe**

Figure S5. Optimized geometries (b3lyp-d3bj/def2-svp) of the transition structures corresponding to the reaction between cyclopentadiene and alkene **AKe**.



endo-**TSf** *exo*-**TSf**

Figure S6. Optimized geometries (b3lyp-d3bj/def2-svp) of the transition structures corresponding to the reaction between cyclopentadiene and alkene **AKf**.

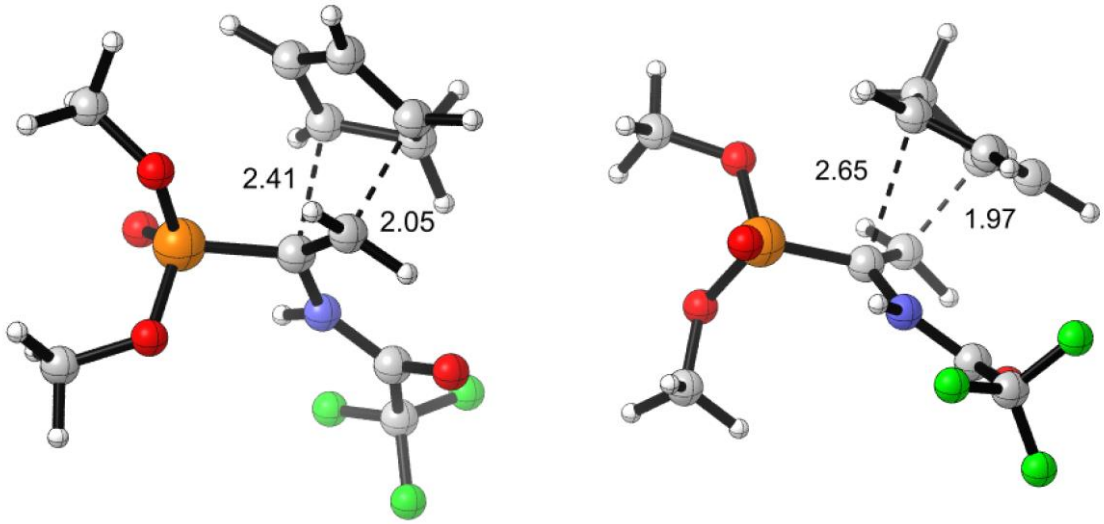


Figure S7. Optimized geometries (b3lyp-d3bj/def2-svp) of the transition structures corresponding to the reaction between cyclopentadiene and alkene **AKg**.

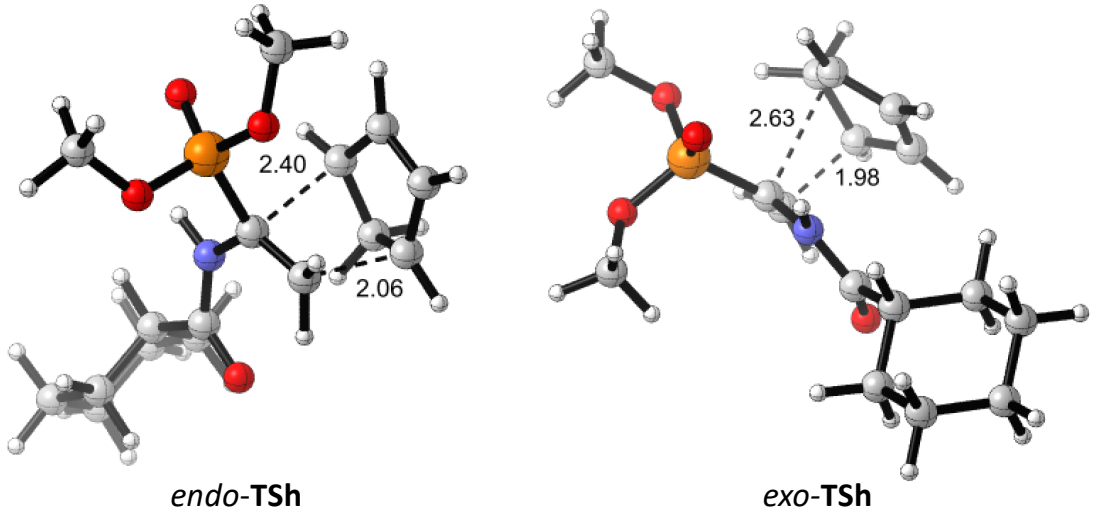


Figure S8. Optimized geometries (b3lyp-d3bj/def2-svp) of the transition structures corresponding to the reaction between cyclopentadiene and alkene **AKh**.

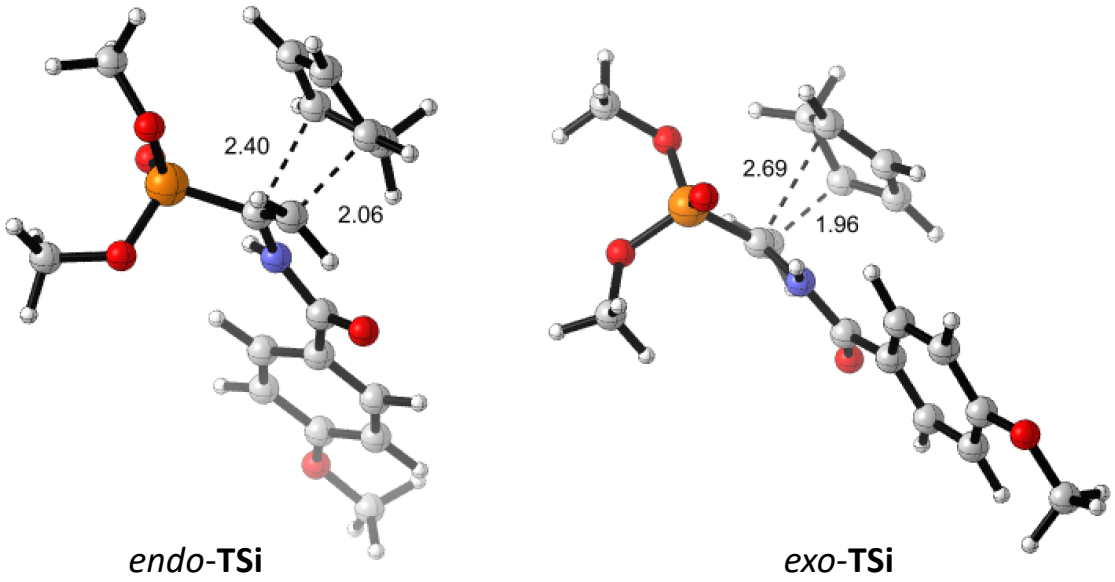


Figure S9. Optimized geometries (b3lyp-d3bj/def2-svp) of the transition structures corresponding to the reaction between cyclopentadiene and alkene **AKi**.

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Conclusions

1. An efficient procedure for the synthesis of dehydrophos with good yields has been provided. The procedure is based on the Horner-Wadsworth-Emmons reaction of an adequate bisphosphonate to generate the dehydroaminophosphonate residue at the *C*-terminal position and the simultaneous cleavage of the methyl ester of the phosphonate moiety and the protecting group of the *N*-terminal amino acid using Dabco®. In addition, it has been confirmed that the chiral integrity of the leucine residue was not affected throughout these synthetic transformations.
2. The versatility of this synthetic procedure provided variations of the natural peptide in which different substituents are placed in the phosphonic acid group or in the vinyl residue. Furthermore, the peptidic sequence in dehydrophos may be also changed. The antimicrobial activity of dehydrophos derivatives was tested against several bacterial strains of medical interest. The introduction of substituents in the vinyl moiety results in a loss of bioactivity. On the other hand, the presence of a leucyl moiety next to the vinylphosphonic acid residue appears to be essential for biological activity.
3. It has been demonstrated that α,β-dehydroamino phosphonates can be used as suitable substrates in Diels-Alder reactions leading to quaternary α-amino phosphonates. The reaction has some limitations including the lack of reactivity or decomposition when conducted in the presence of Lewis acids. Also, while the substituent at the amino group has no steric influence in the stereochemical course of the reaction, the presence of bulky substituents at the phosphonate function hinders the reaction. These results prevent the use of chiral catalysts or auxiliaries to induce asymmetry, a subject that will require further investigations. On the other hand, the reaction takes place with good chemical yields in shorter reaction times by placing electron-withdrawing groups at the amino functionality. In particular, the trifluoroacetylamido derivative provided the best results. Moreover, the trifluoroacetyl group is a convenient protective group that can be easily eliminated.

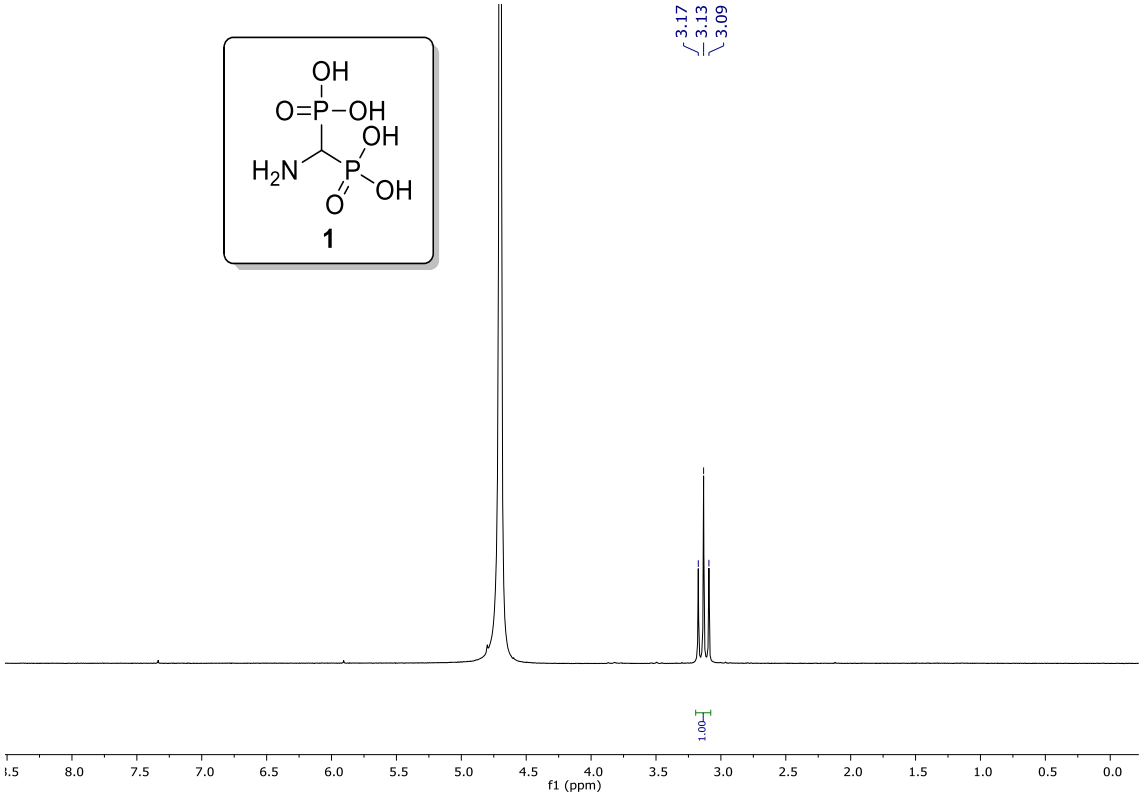
Dehydroaminophosphonic acids: synthesis, reactivity and biological applications

Appendix

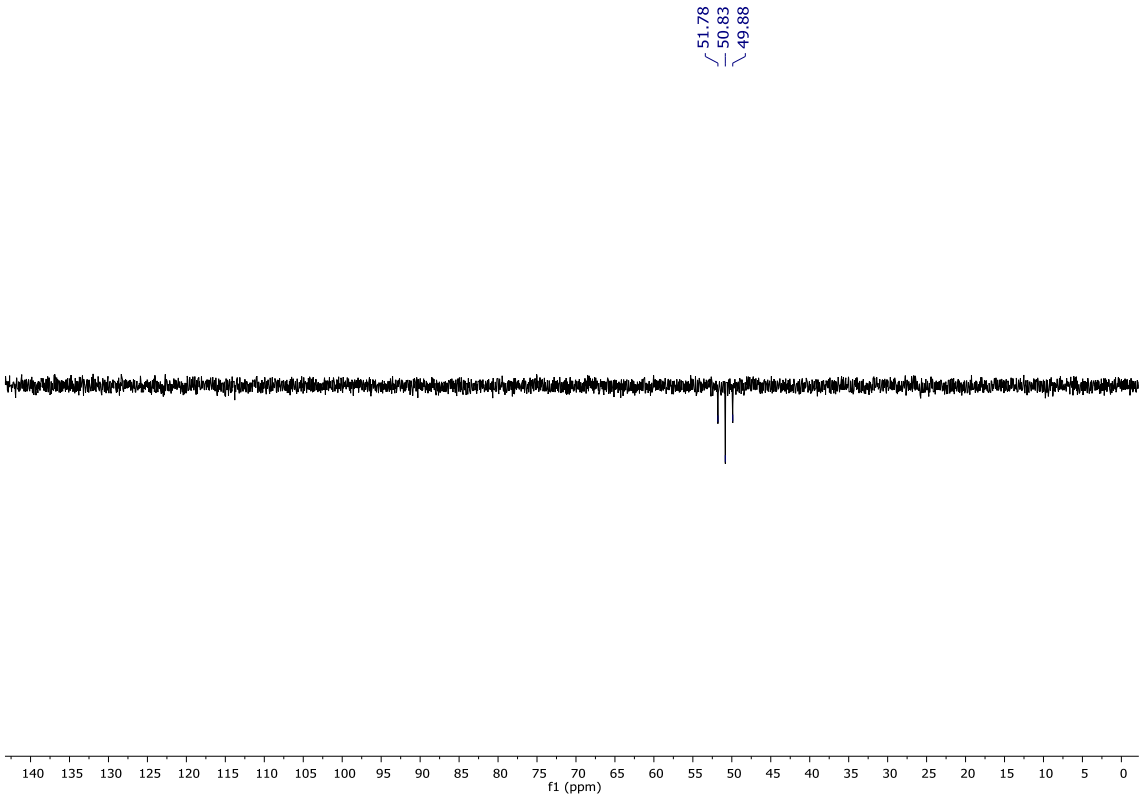
Appendix

NMR Spectra of Chapter 1

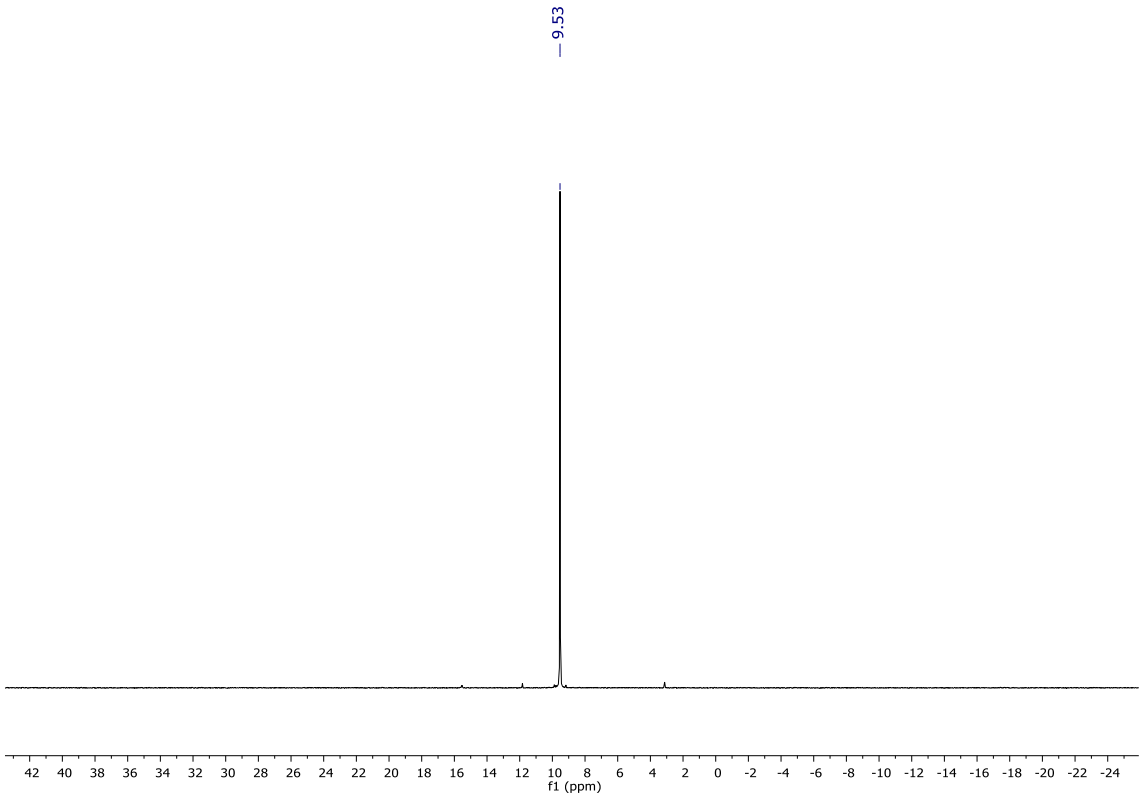
Aminomethylbisphosphonic acid (1)



^{1}H NMR (400 MHz, D_2O):

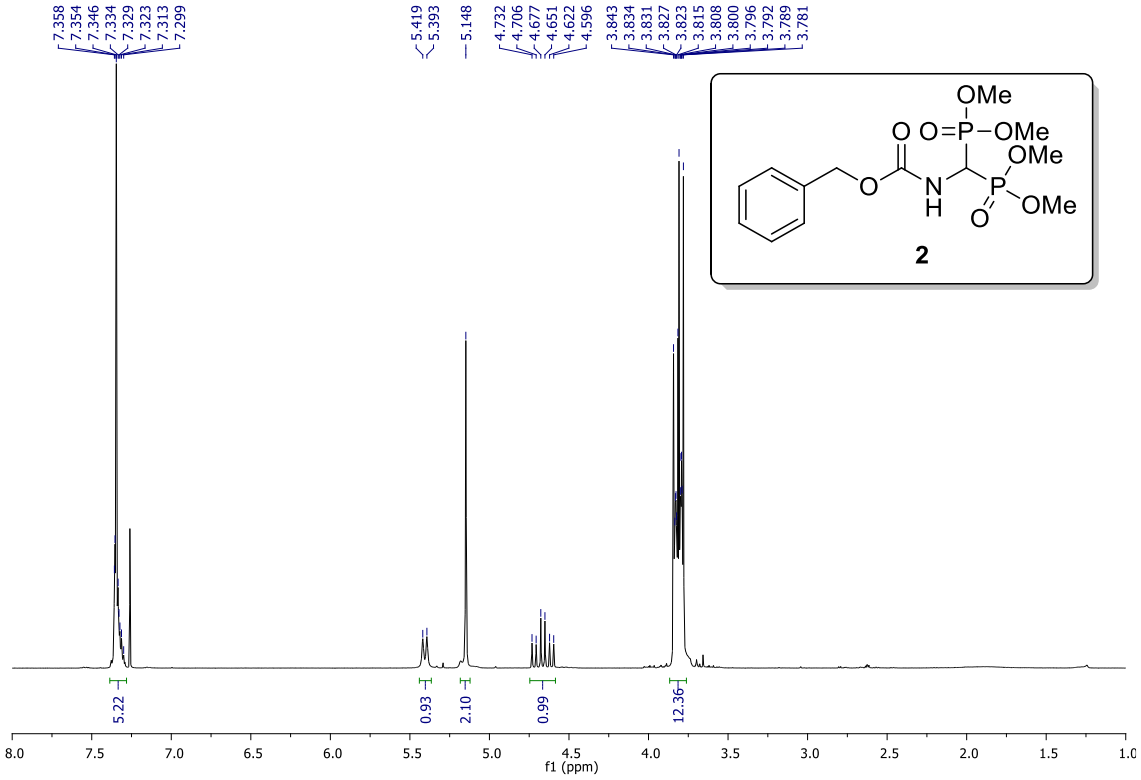


APT NMR (100 MHz, D_2O):

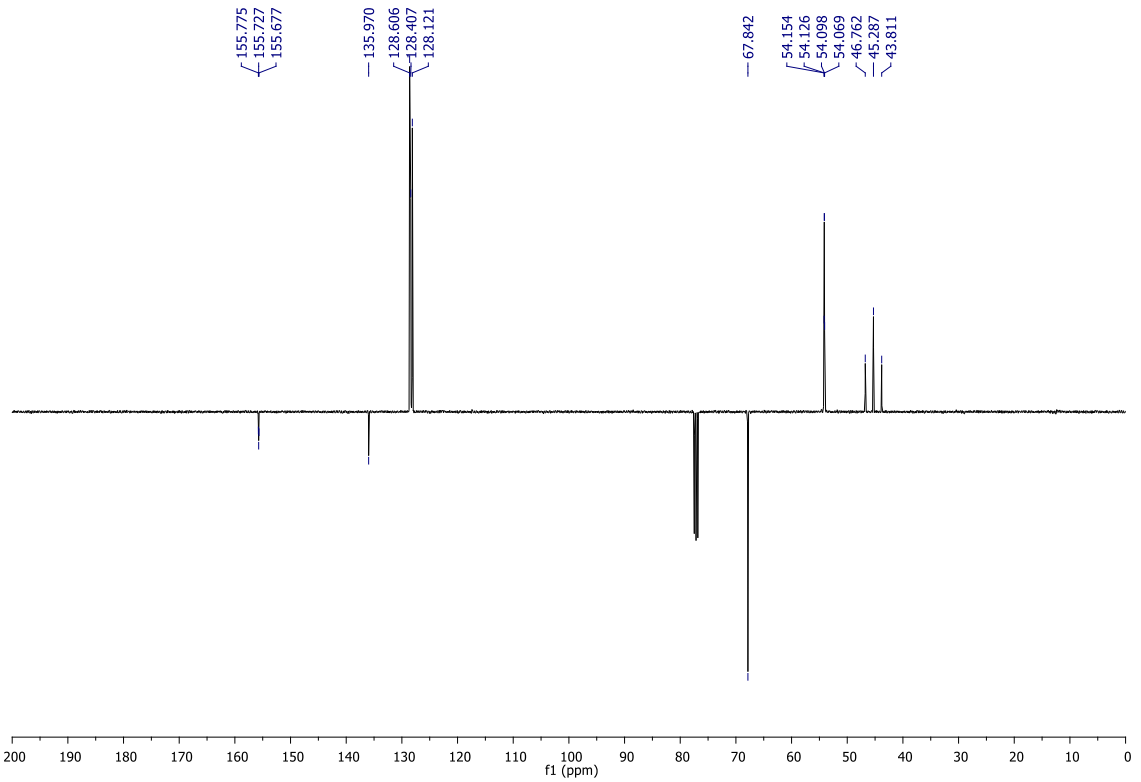


^{31}P NMR (162 MHz, D_2O):

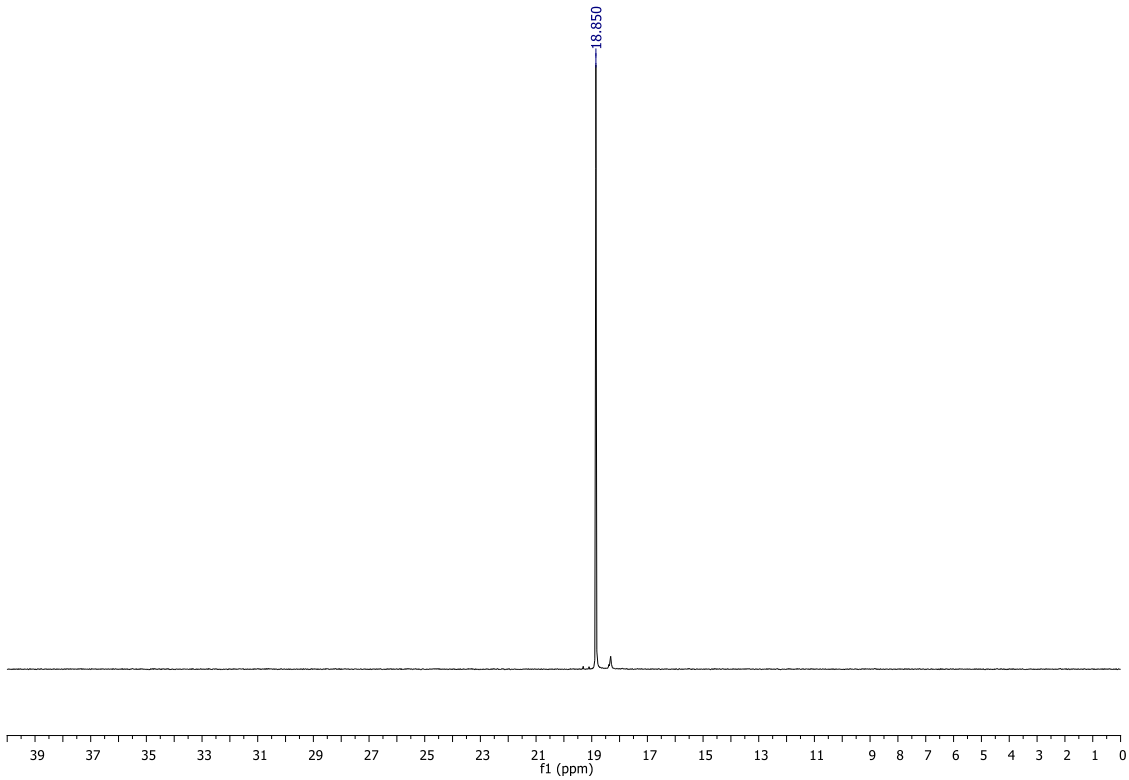
Tetramethyl (benzyloxycarbonylaminomethyl)bisphosphonate (2)



^{1}H NMR (400 MHz, $CDCl_3$)

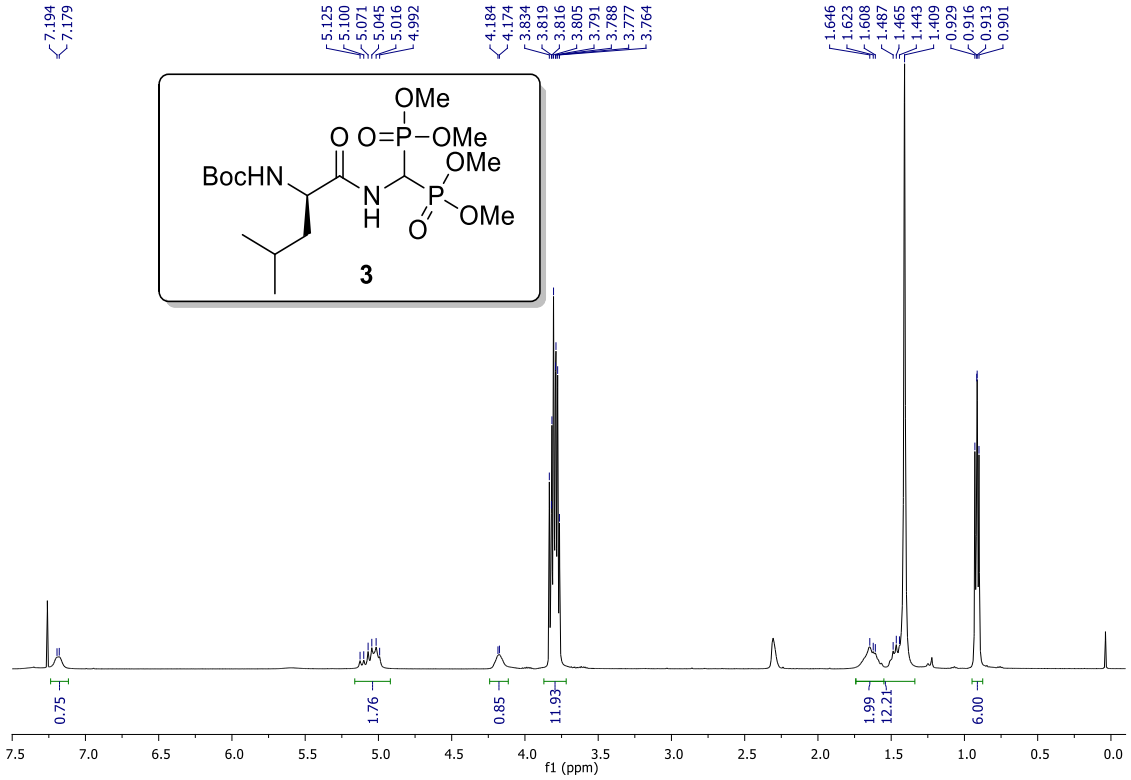


APT NMR (100 MHz, $CDCl_3$)

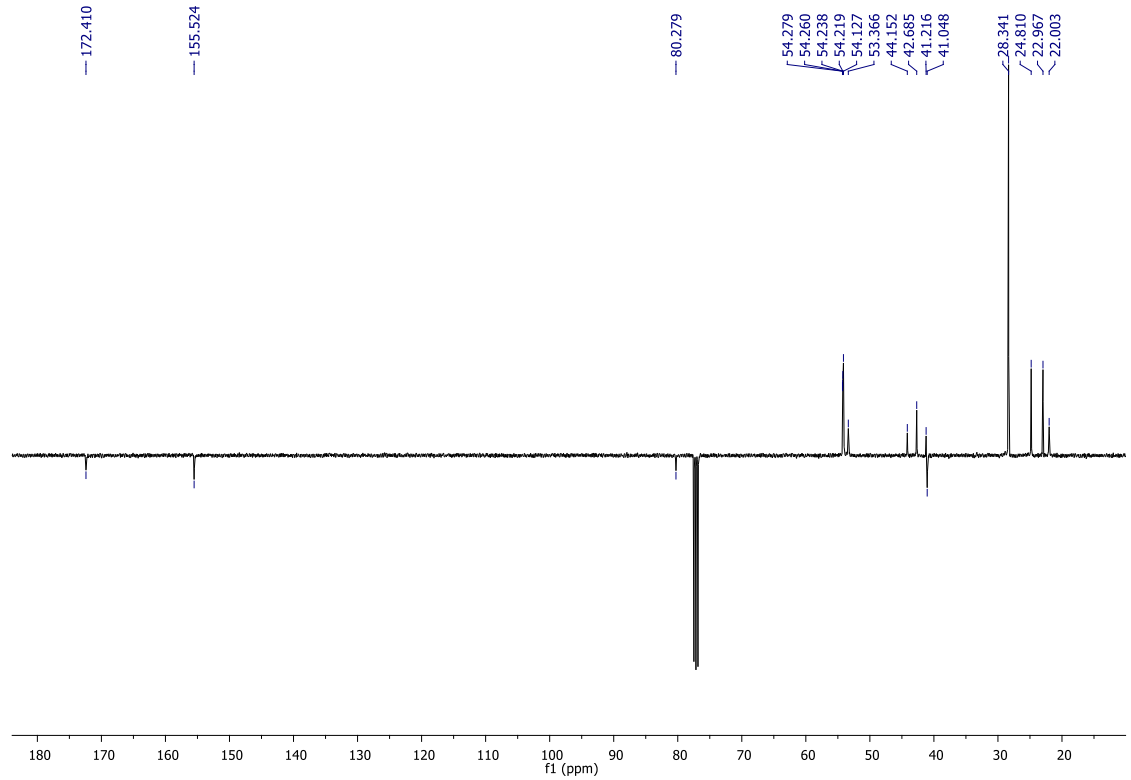


^{31}P NMR (162 MHz, $CDCl_3$)

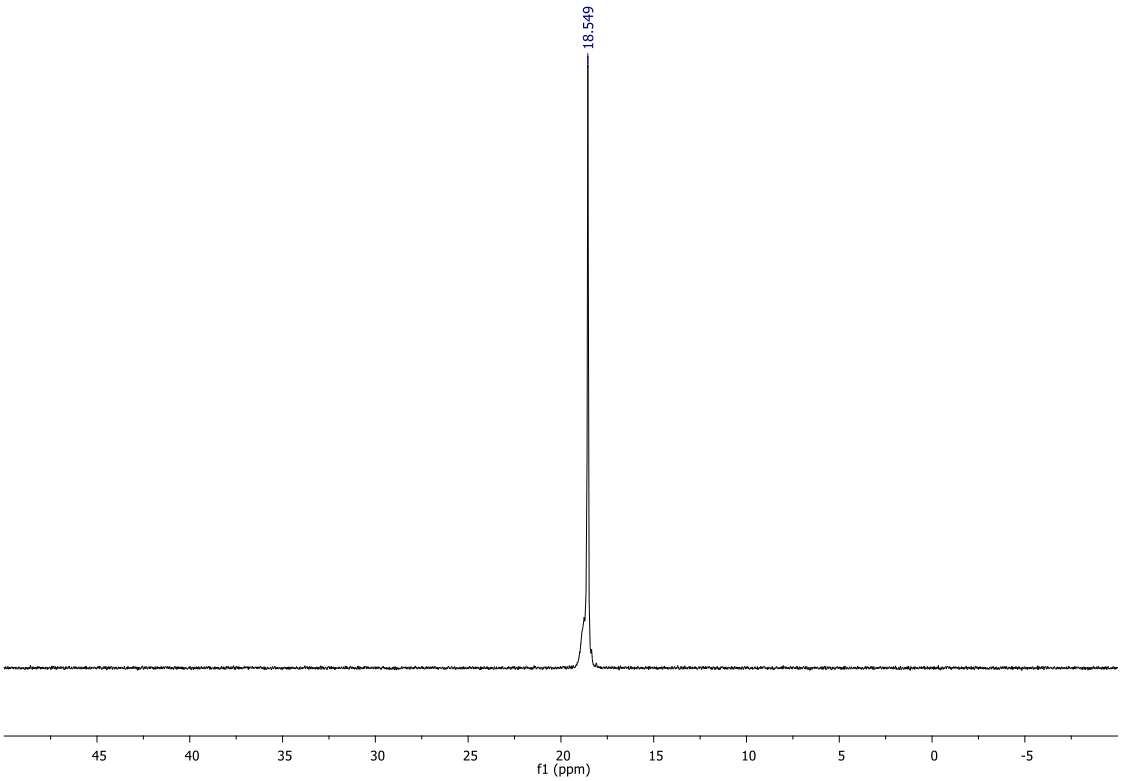
Tetramethyl [*N*-(*tert*-butoxycarbonyl)-L-leucylamidomethyl]bisphosphonate (3)



^{1}H NMR (400 MHz, $CDCl_3$)

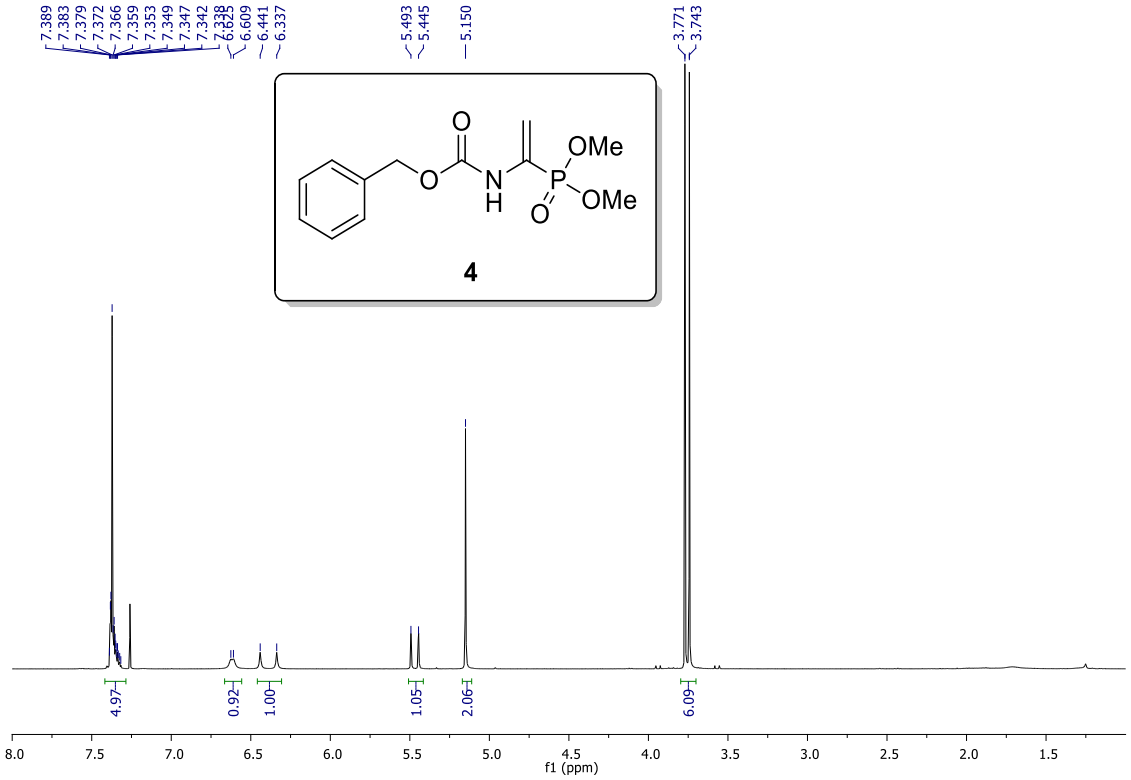


APT NMR (100 MHz, $CDCl_3$)

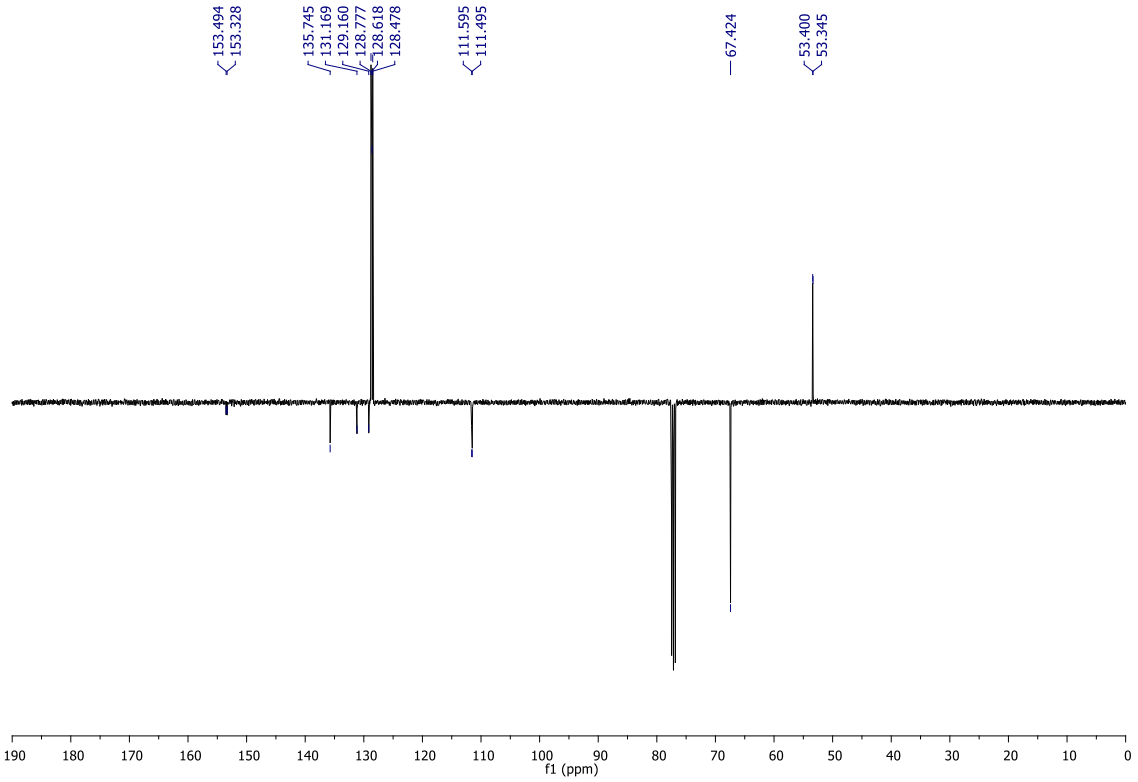


^{31}P NMR (162 MHz, $CDCl_3$)

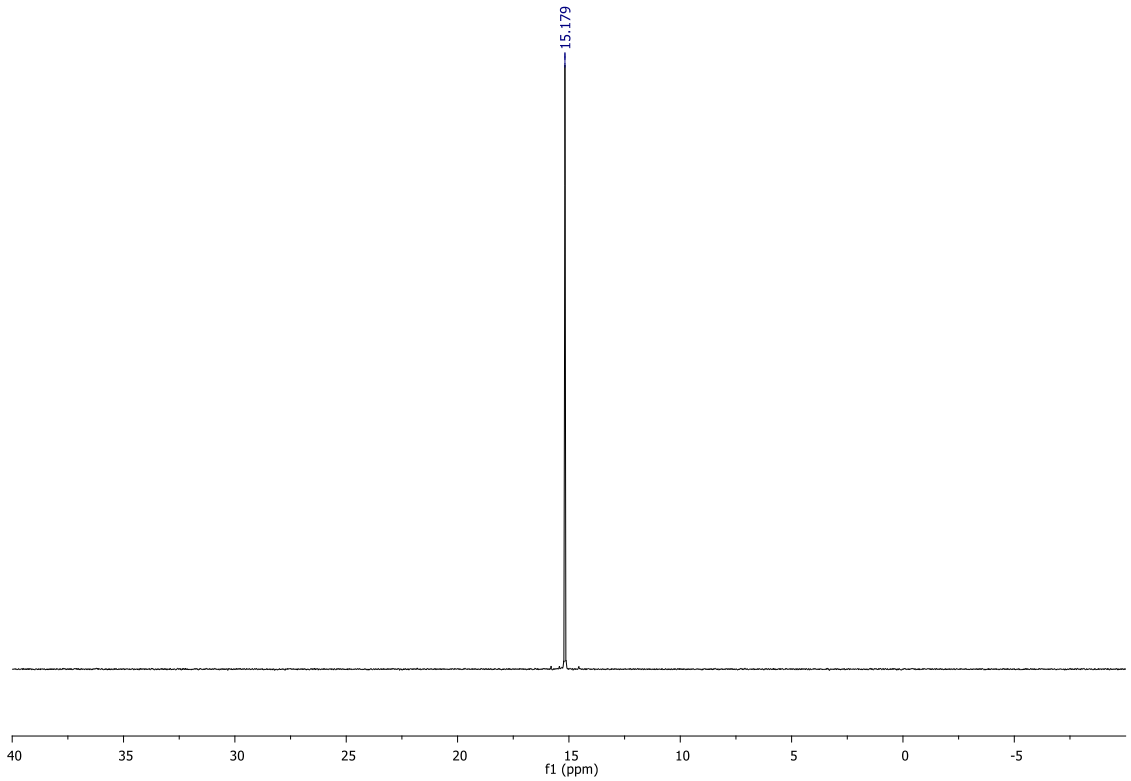
Dimethyl [1-(benzyloxycarbonylamino)ethen-1-yl]phosphonate (4)



^{1}H NMR (400 MHz, $CDCl_3$)

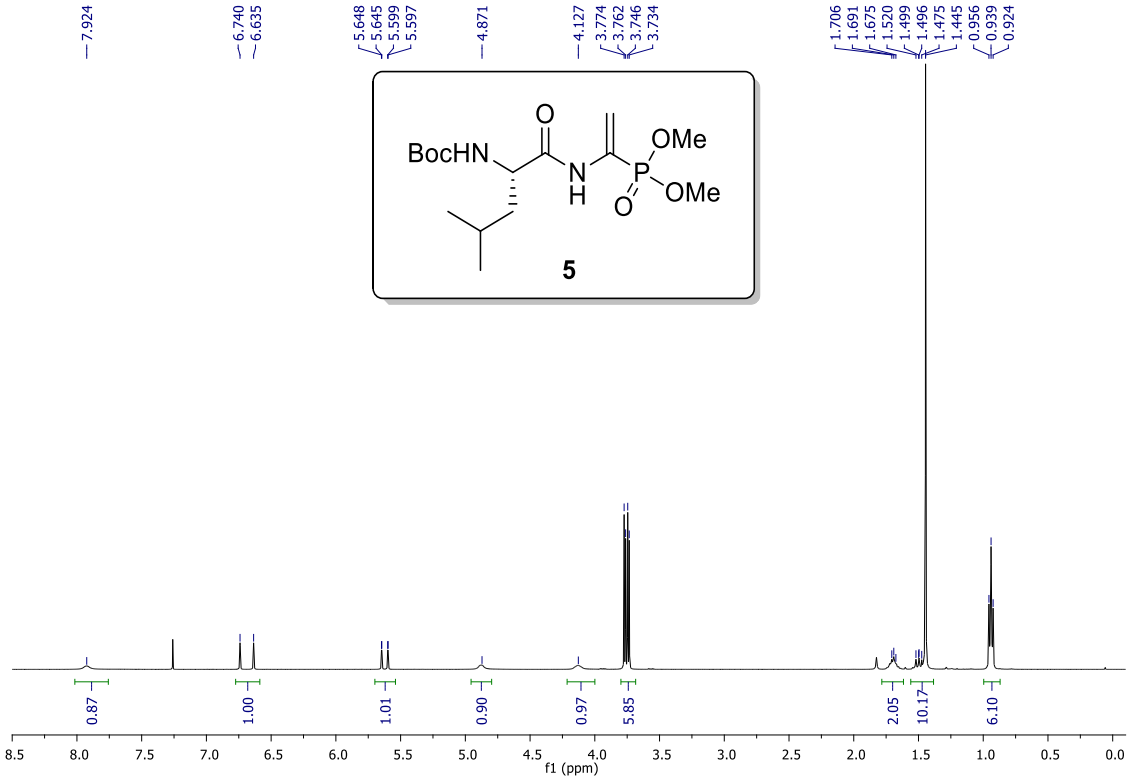


APT NMR (100 MHz, $CDCl_3$)

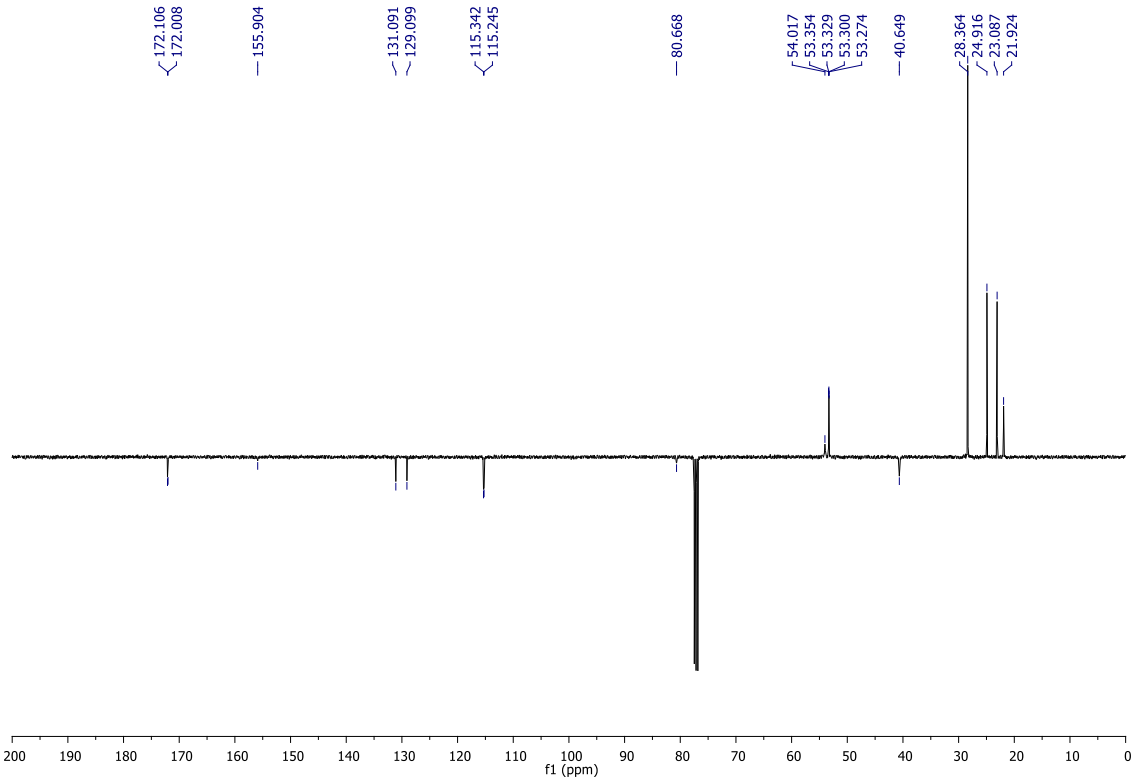


^{31}P NMR (162 MHz, $CDCl_3$)

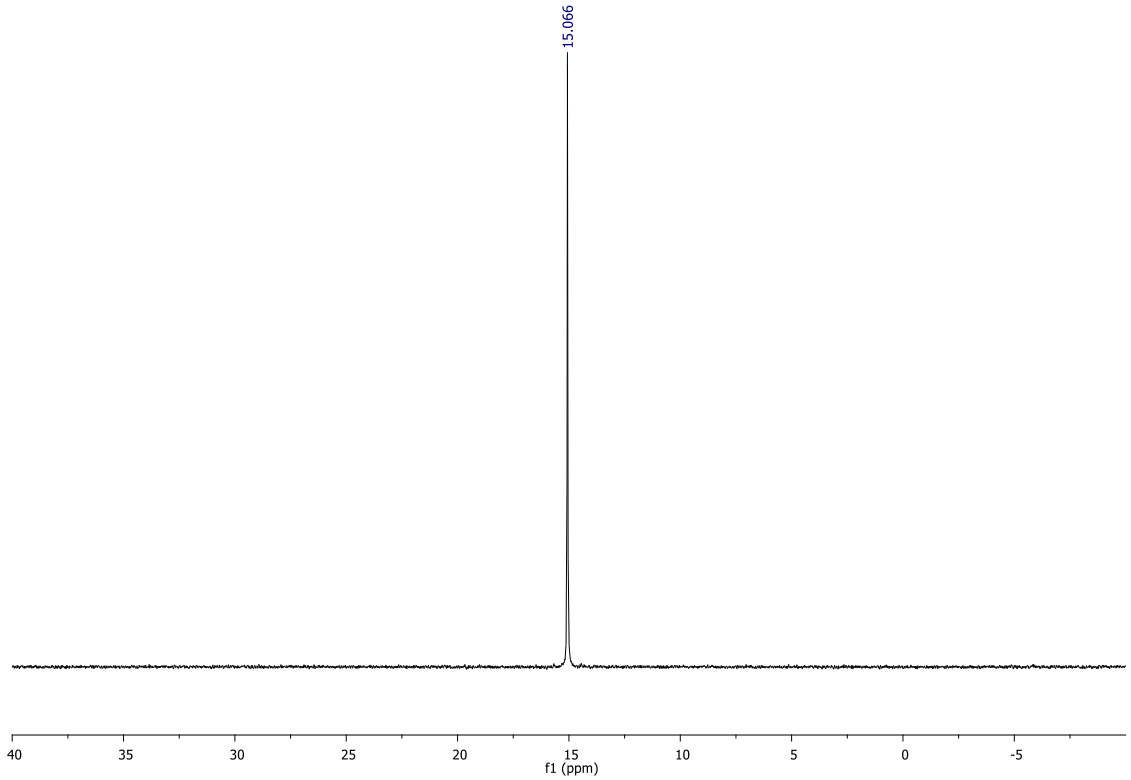
Dimethyl {1-[*N*-(*tert*-butoxycarbonyl)-L-leucylamido]ethen-1-yl}phosphonate (5)



^{1}H NMR (400 MHz, $CDCl_3$)

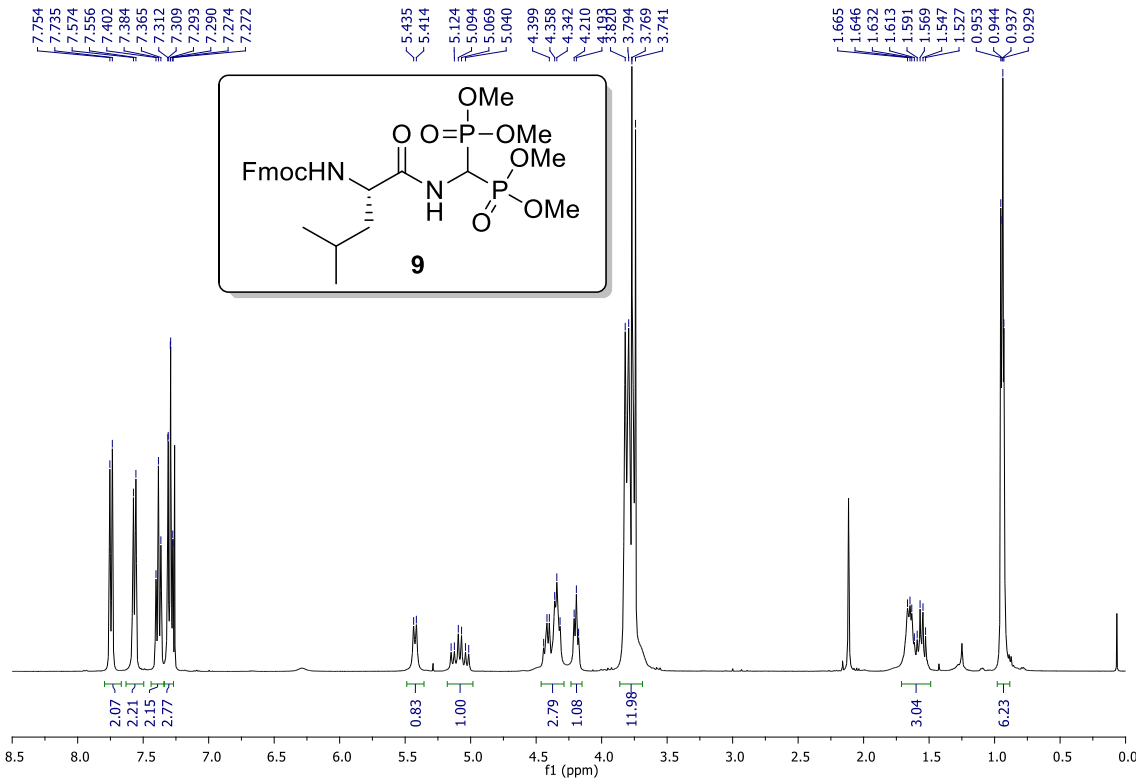


APT NMR (100 MHz, $CDCl_3$)

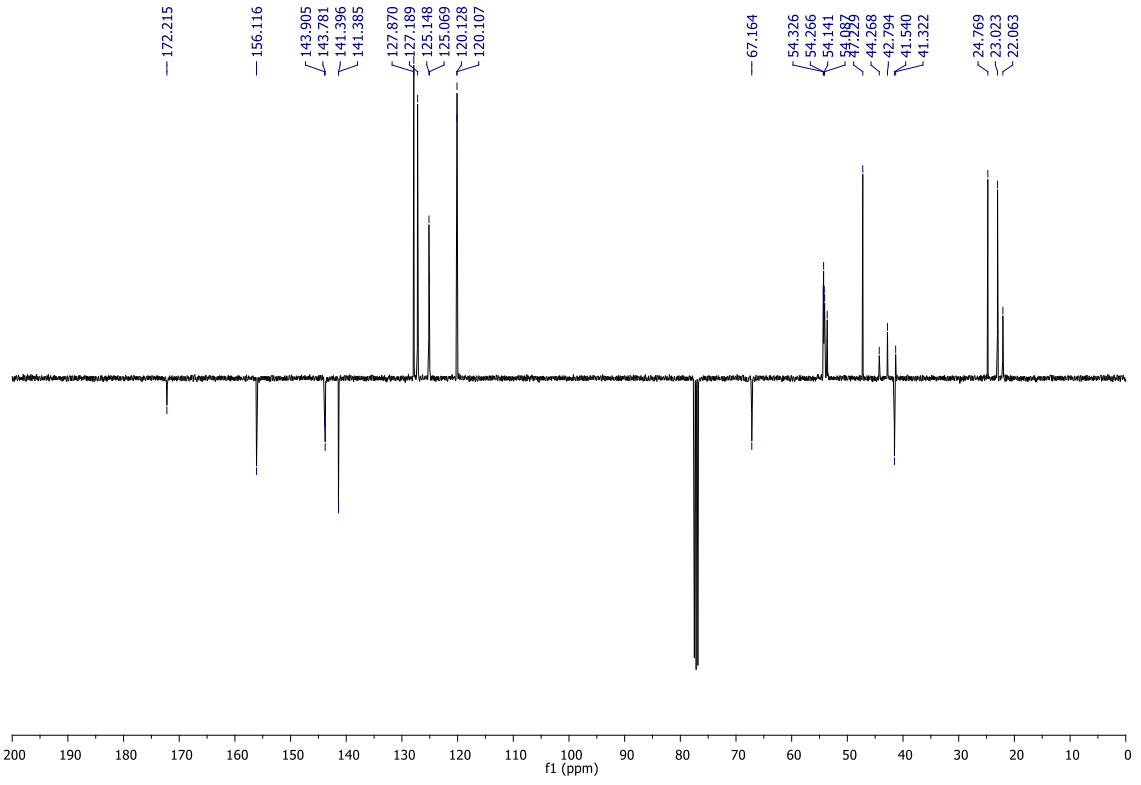


^{31}P NMR (162 MHz, $CDCl_3$)

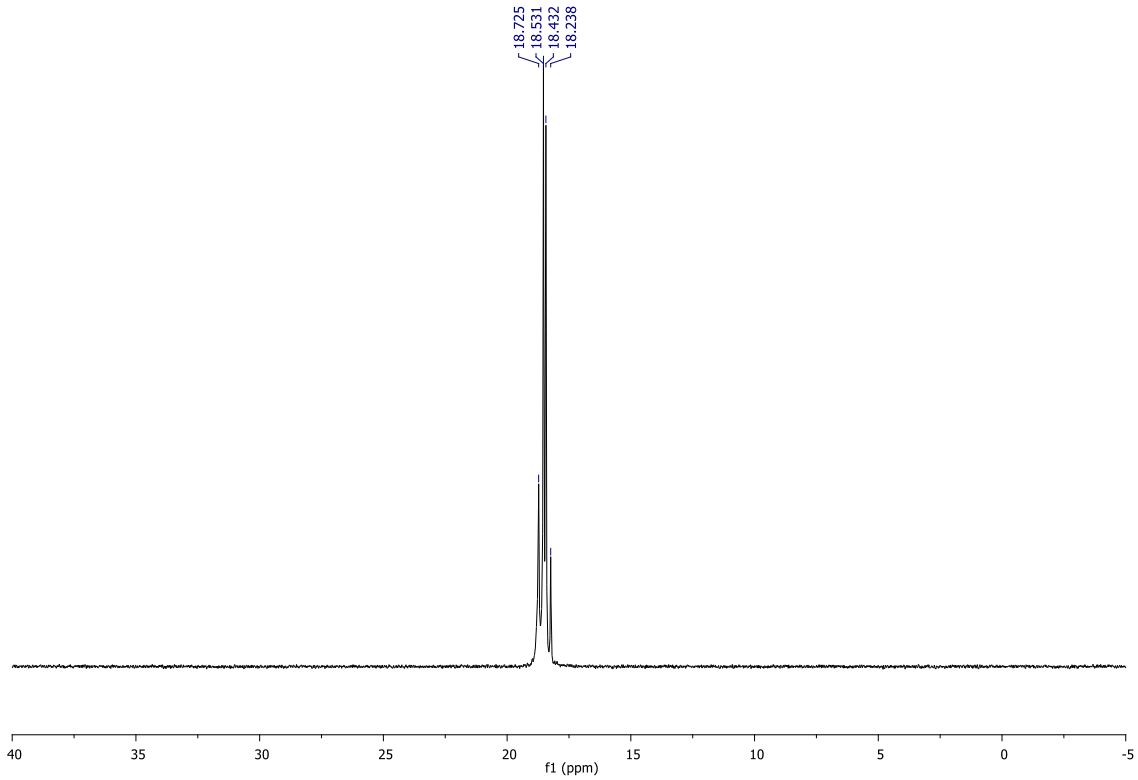
Tetramethyl [*N*-(9-fluorenylmethoxycarbonyl)-L-leucylamidomethyl]bisphosphonate (9)



^{1}H NMR (400 MHz, $CDCl_3$)

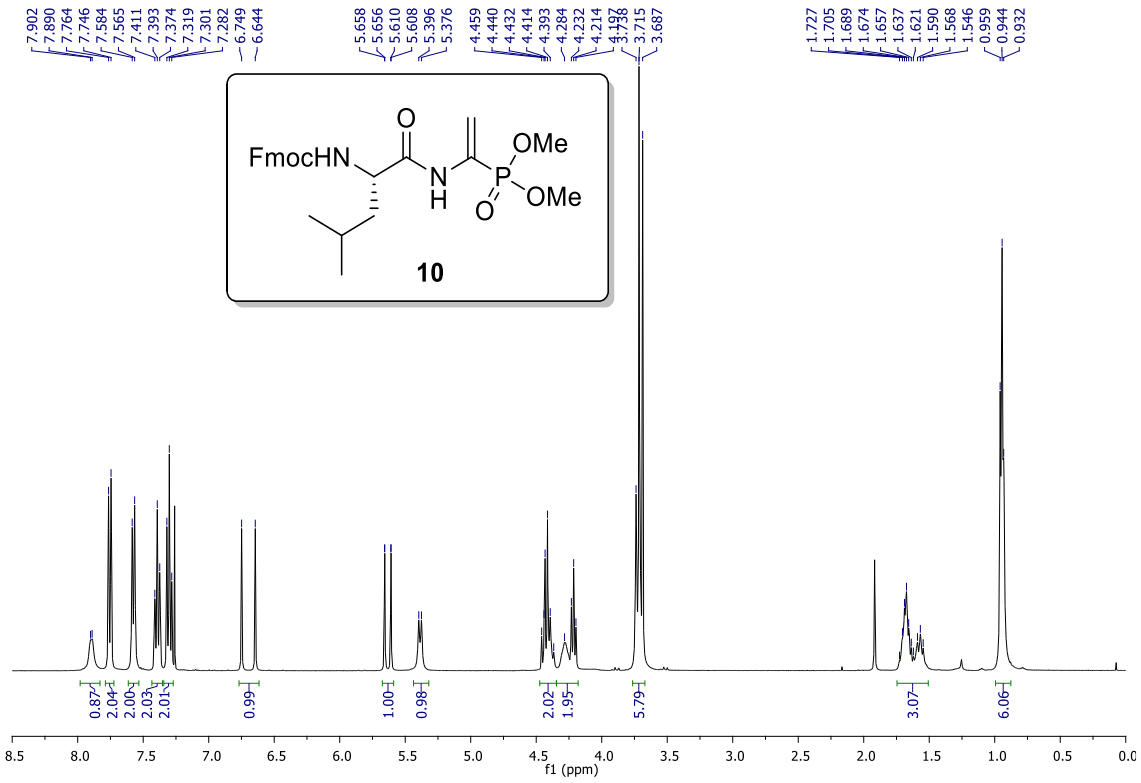


APT NMR (100 MHz, $CDCl_3$)

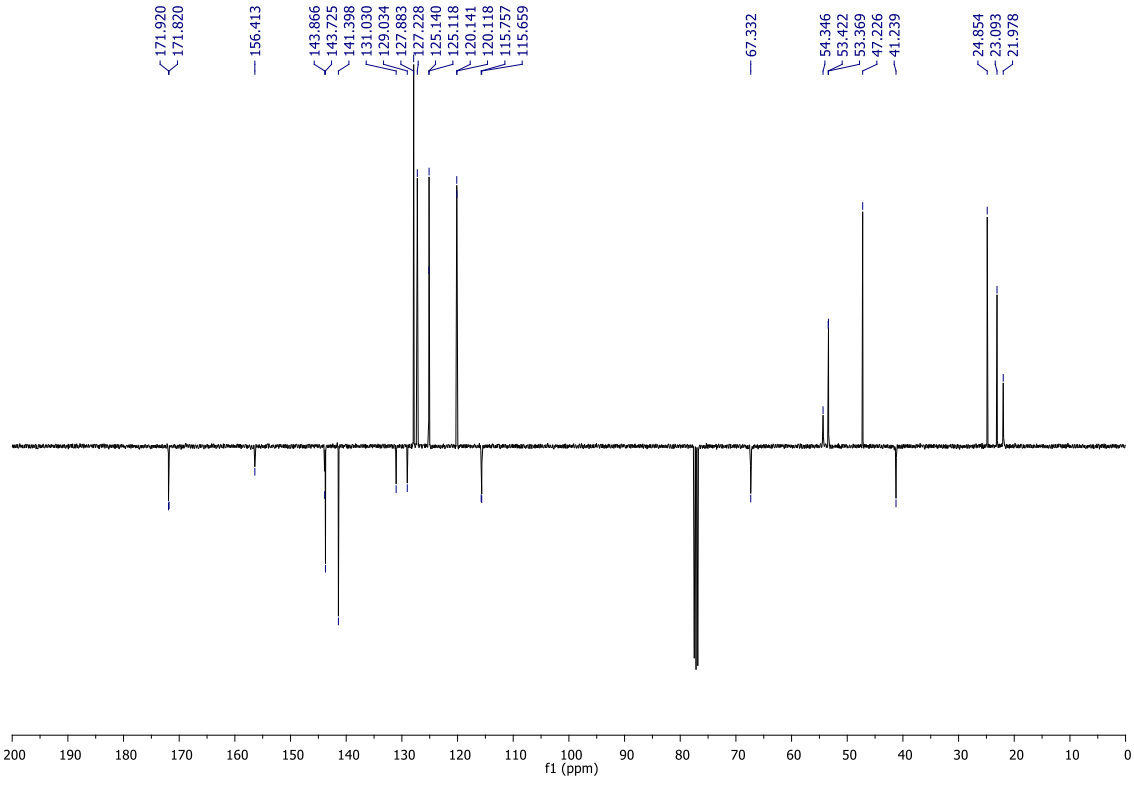


^{31}P NMR (162 MHz, $CDCl_3$)

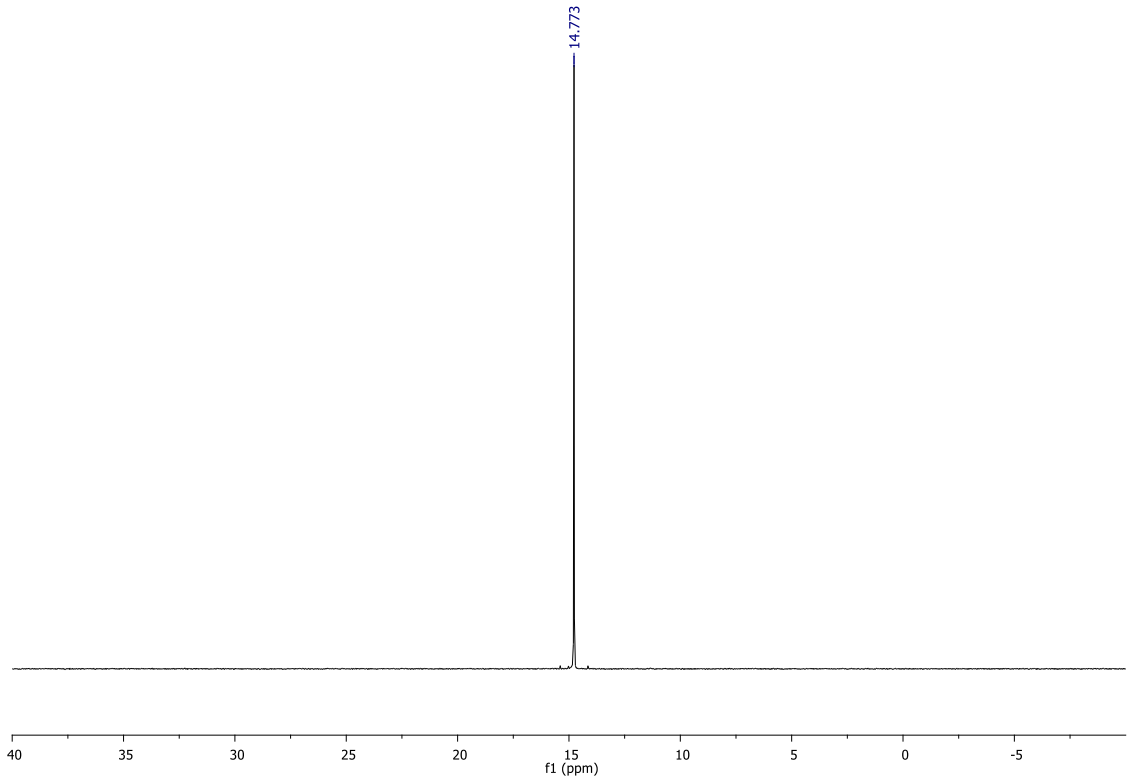
Dimethyl {1-[*N*-(9-fluorenylmethoxycarbonyl)-L-leucylamido]ethen-1-yl}phosphonate (10)



^{1}H NMR (400 MHz, $CDCl_3$)

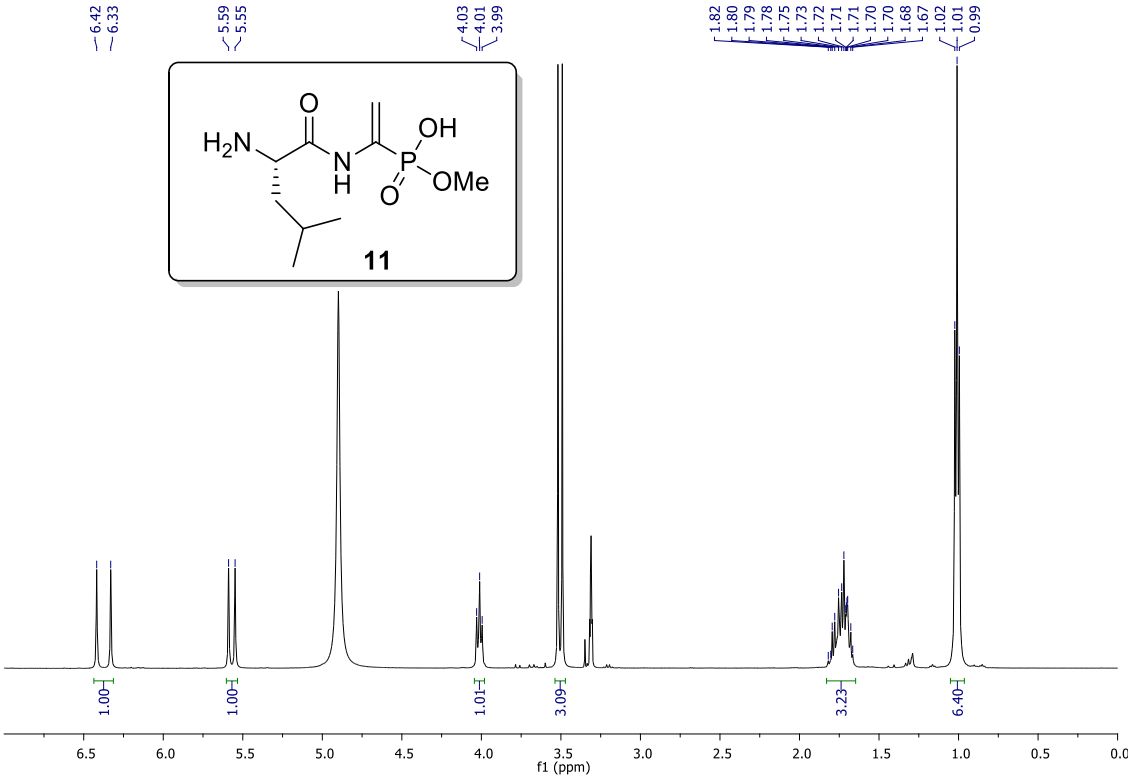


APT NMR (100 MHz, $CDCl_3$)

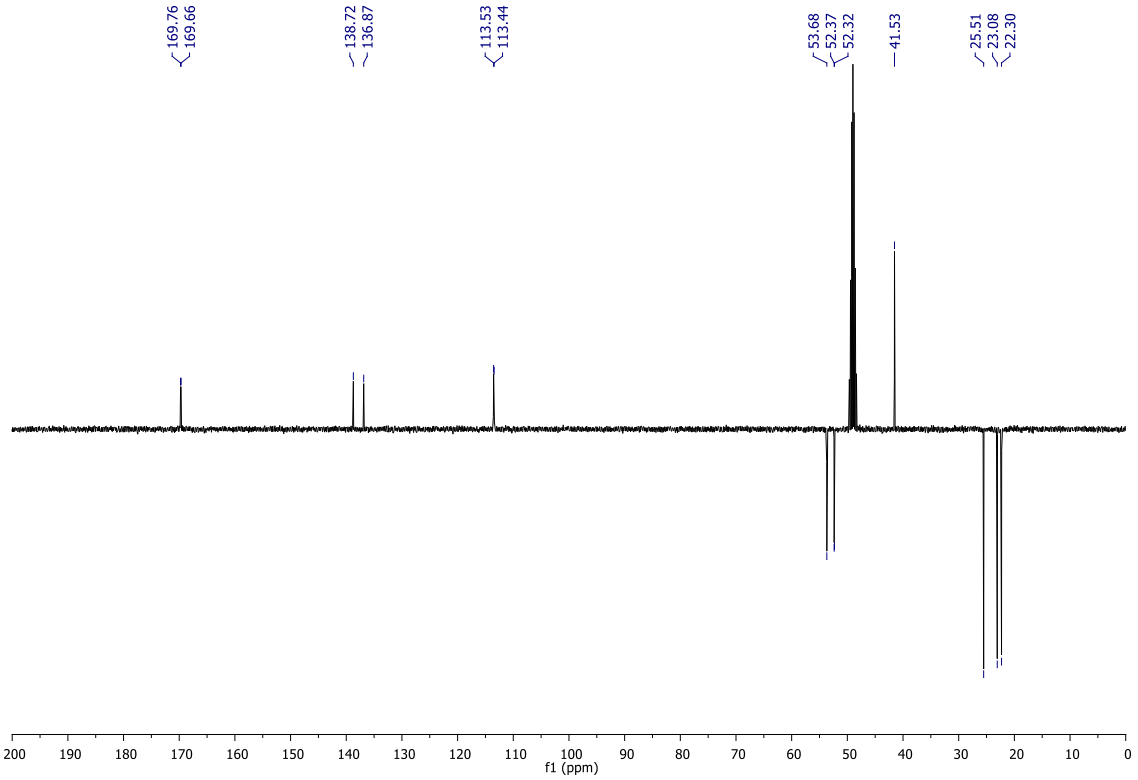


^{31}P NMR (162 MHz, $CDCl_3$)

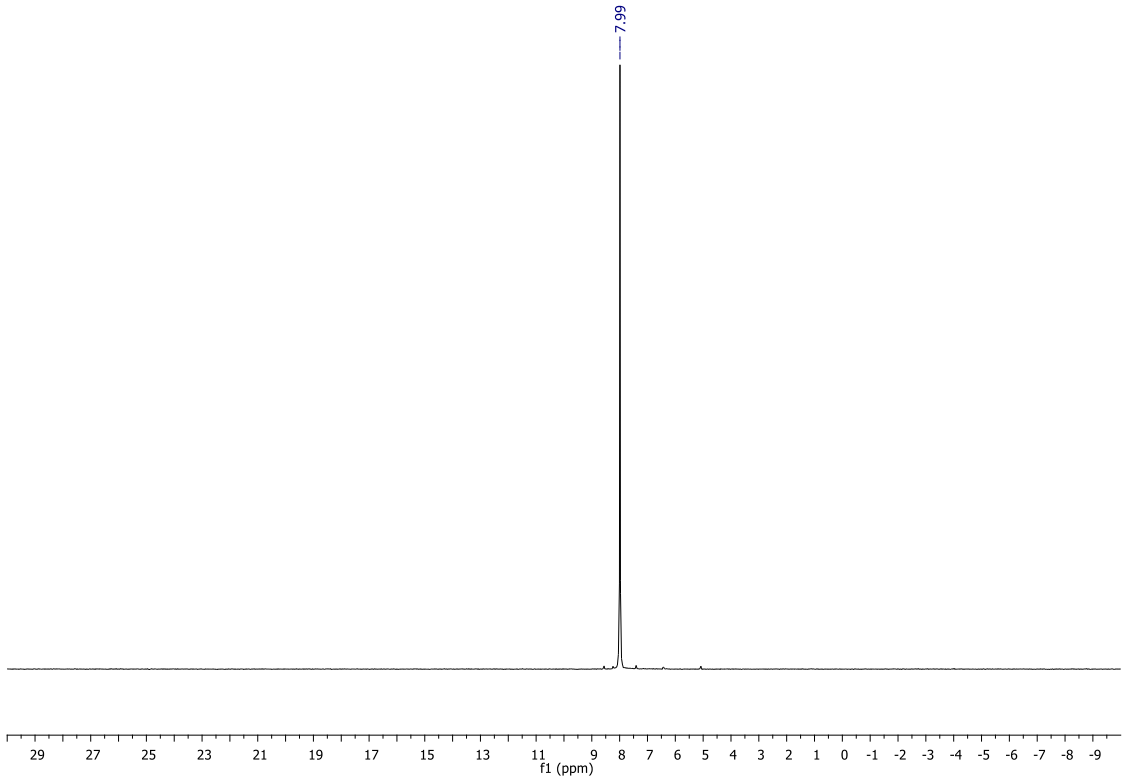
[1-(L-leucylamido)ethen-1-yl]phosphonic acid monomethyl ester (11)



^{1}H NMR (400 MHz, MeOD)

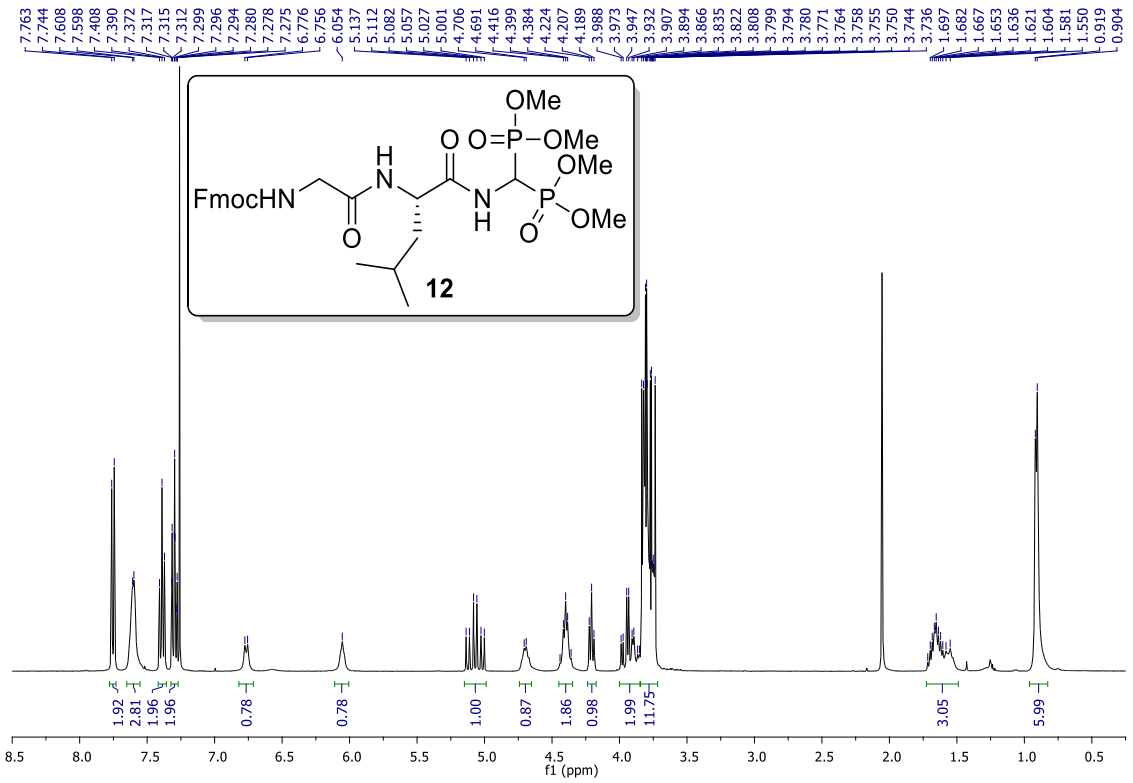


APT NMR (100 MHz, MeOD)

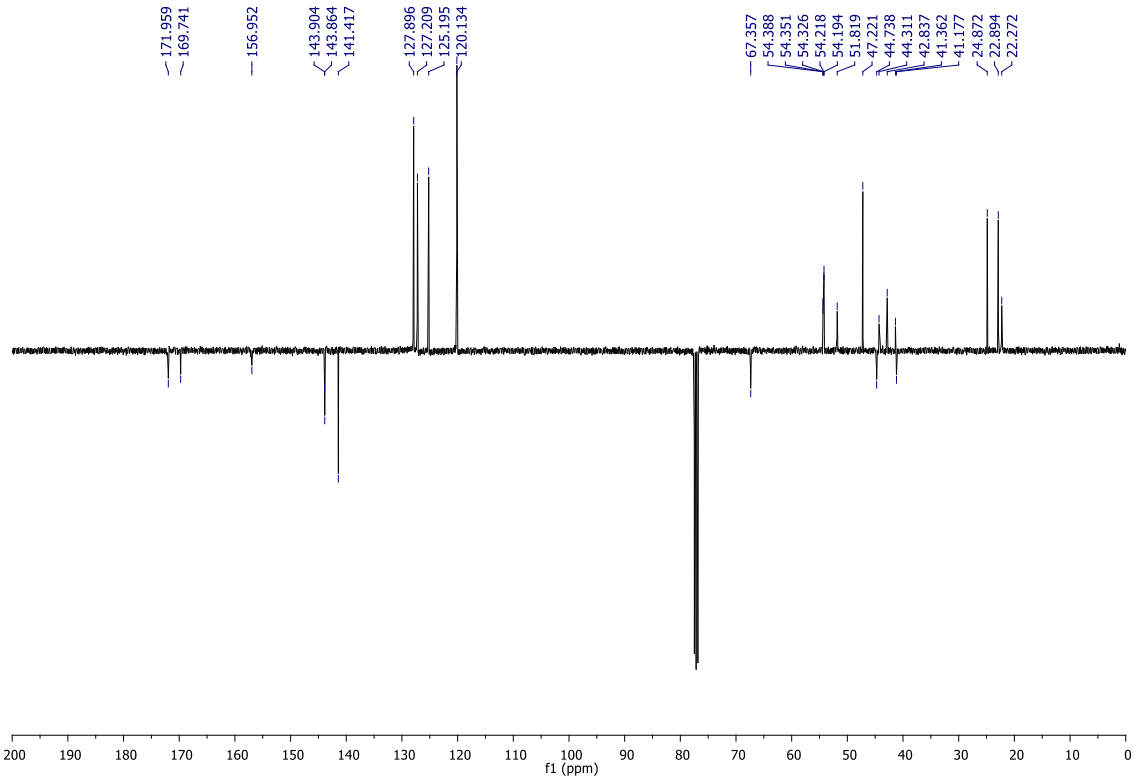


^{31}P NMR (162 MHz, $CDCl_3$)

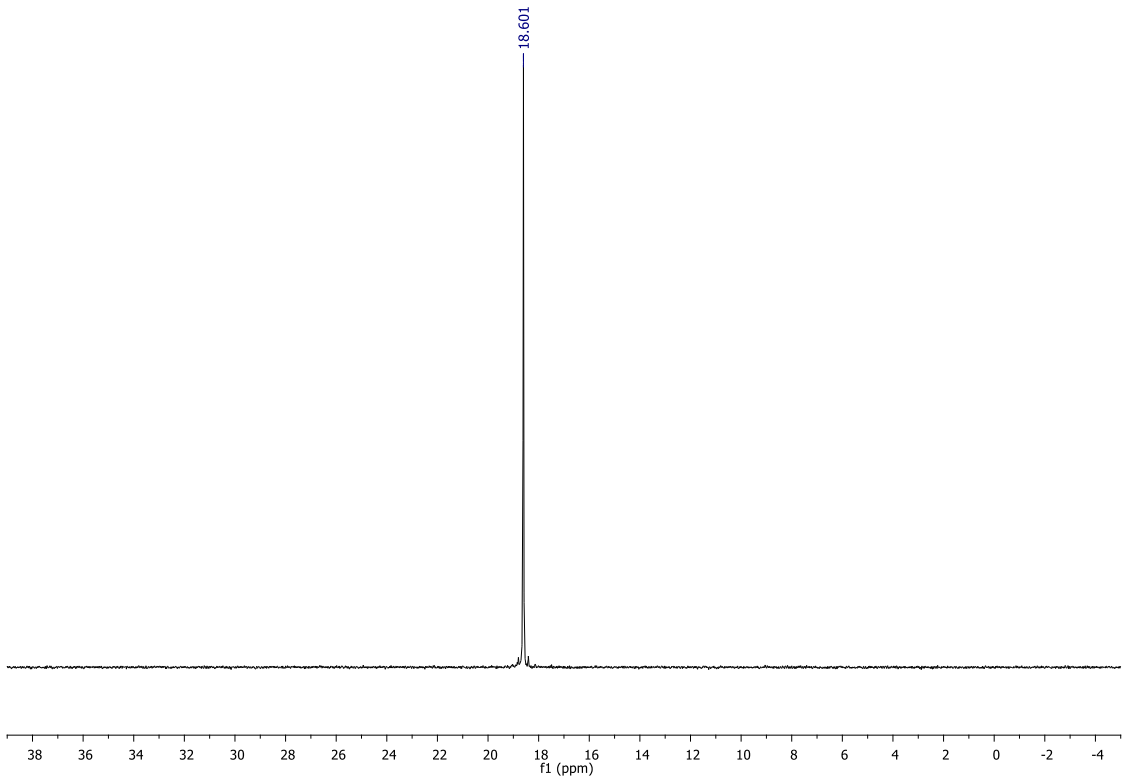
Tetramethyl [*N*-(9-fluorenylmethoxycarbonyl)glycyl-L-leucylamidomethyl]bisphosphonate (12)



^{1}H NMR (400 MHz, $CDCl_3$)

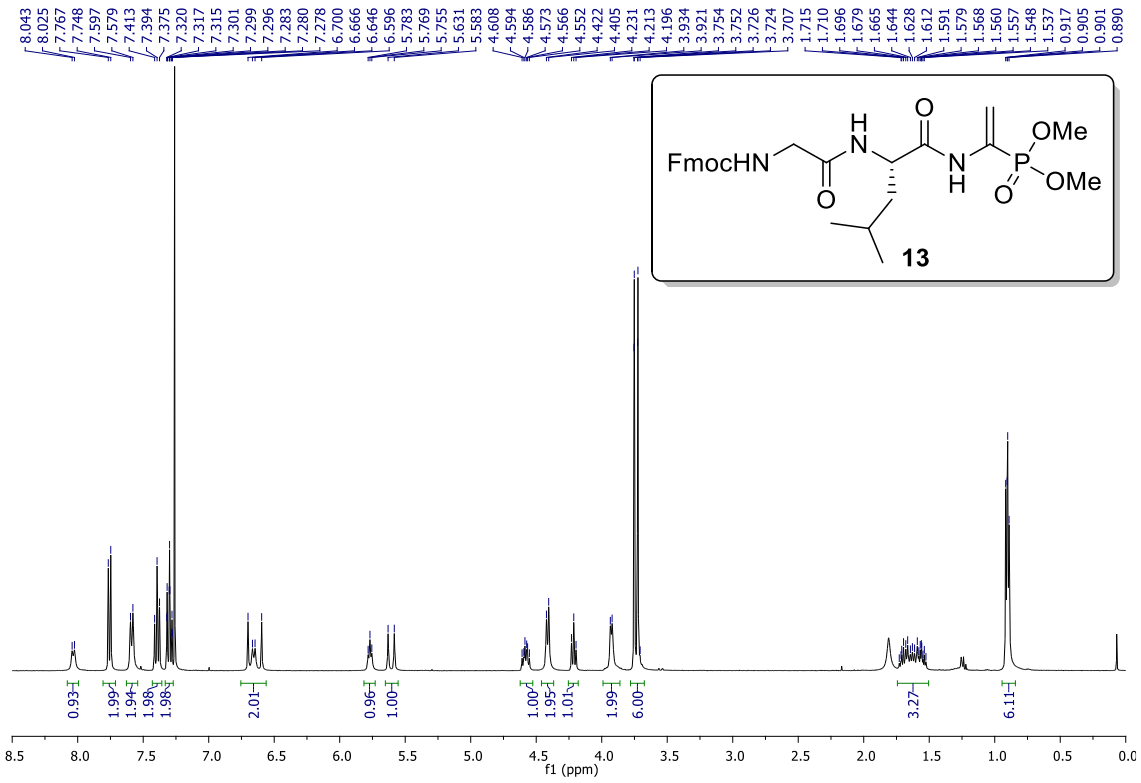


APT NMR (100 MHz, $CDCl_3$)

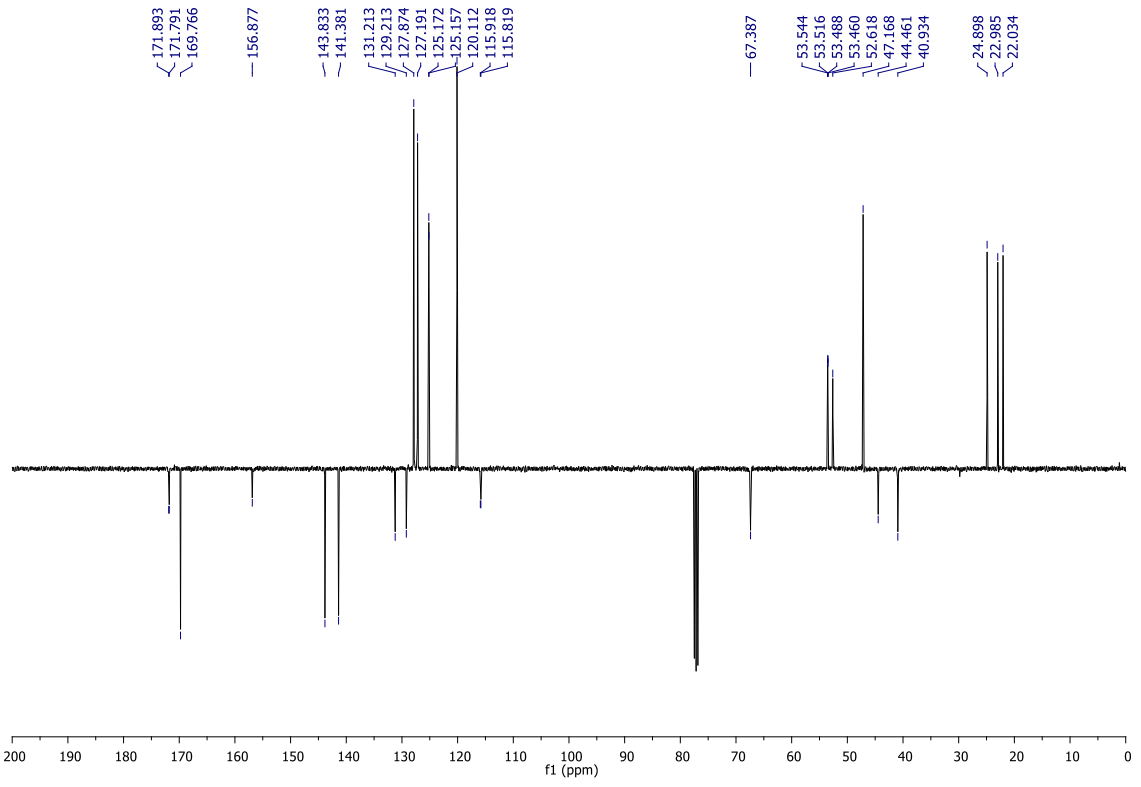


^{31}P NMR (162 MHz, $CDCl_3$)

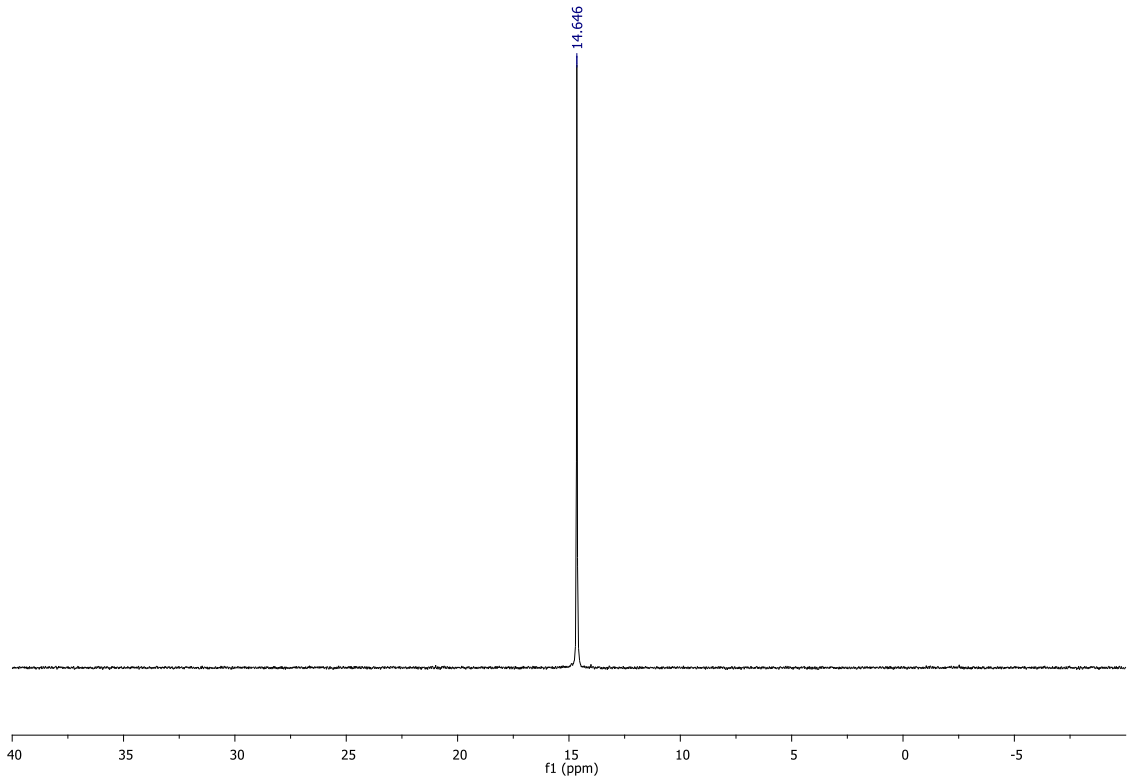
Dimethyl {1-[*N*-(9-fluorenylmethoxycarbonyl)glycyl-L-leucylamido]ethen-1-yl}phosphonate (13)



^{1}H NMR (400 MHz, $CDCl_3$)

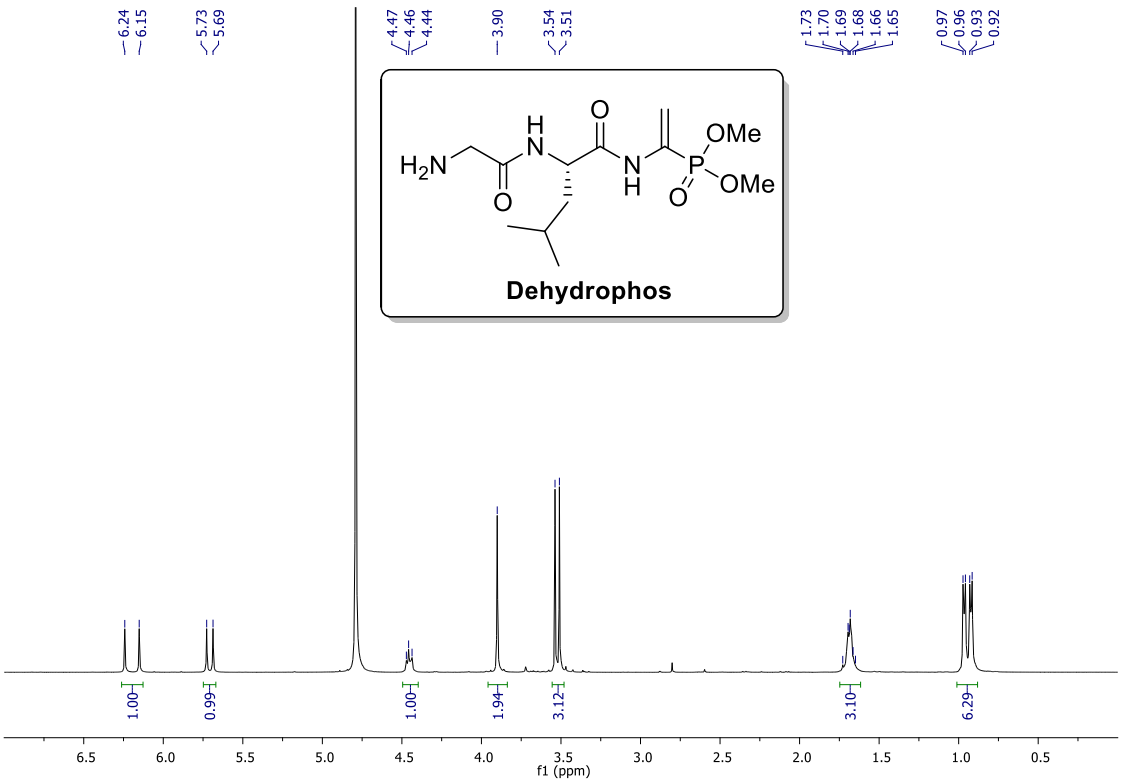


APT NMR (100 MHz, $CDCl_3$)

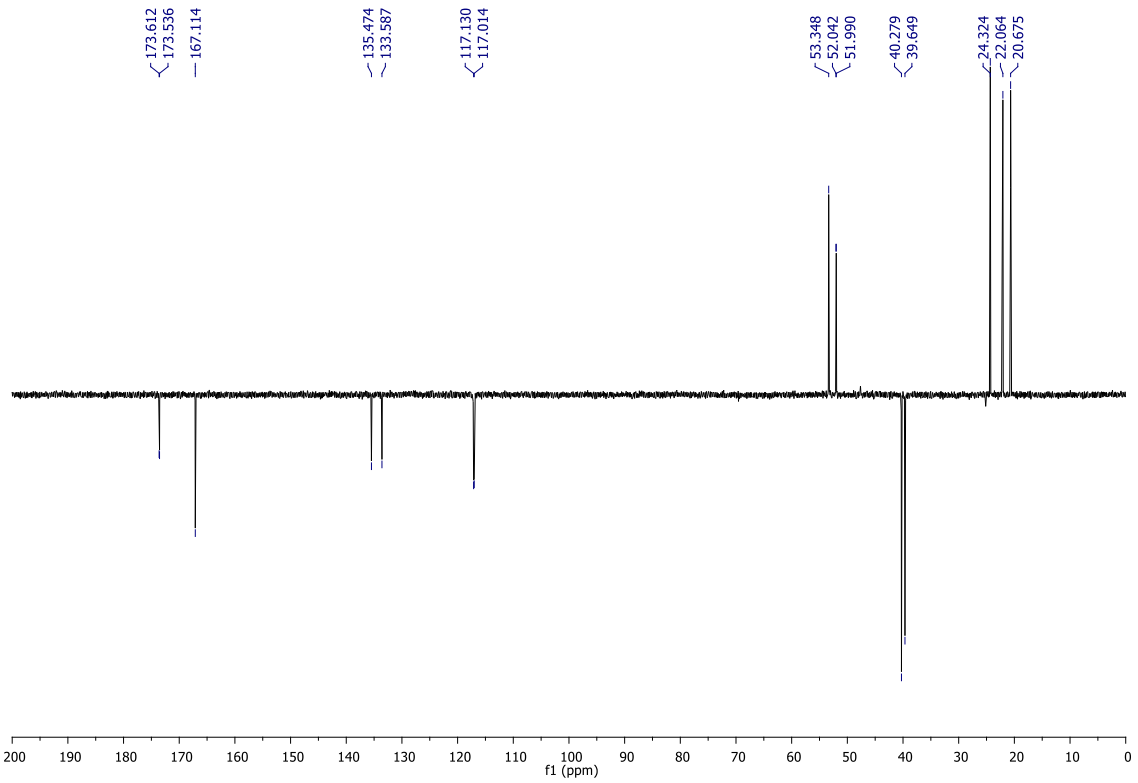


^{31}P NMR (162 MHz, $CDCl_3$)

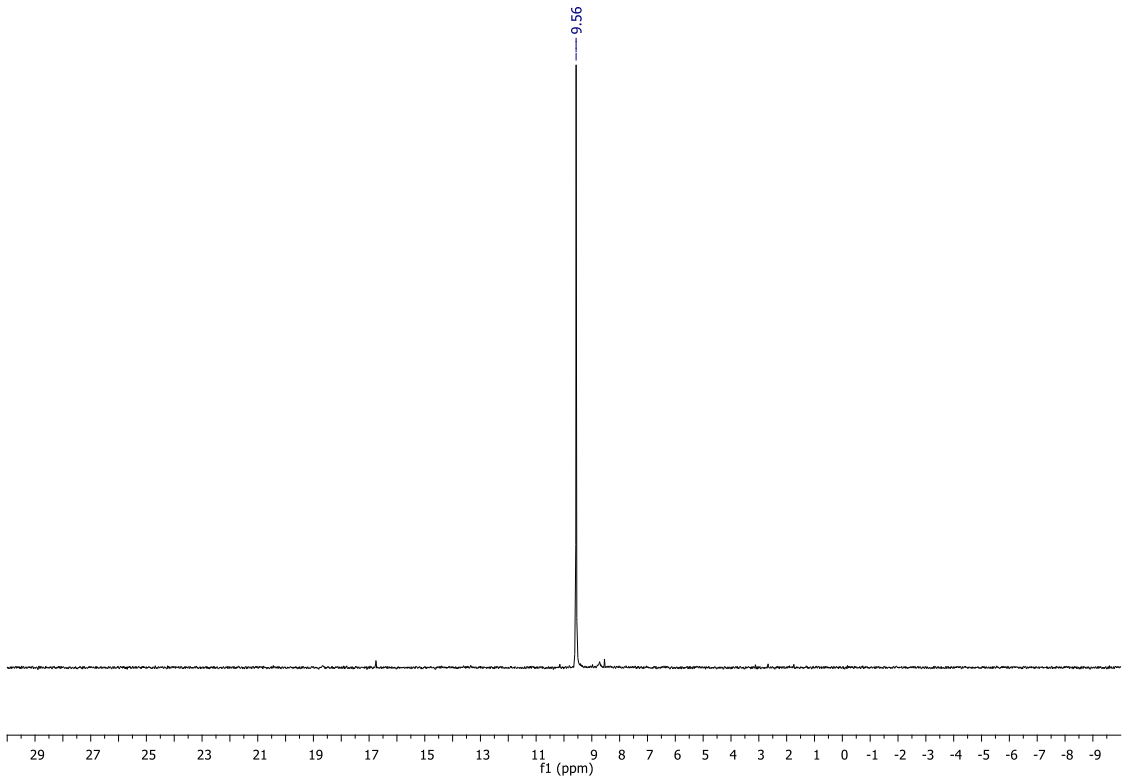
Dehydrophos



^{1}H NMR (400 MHz, D_2O)

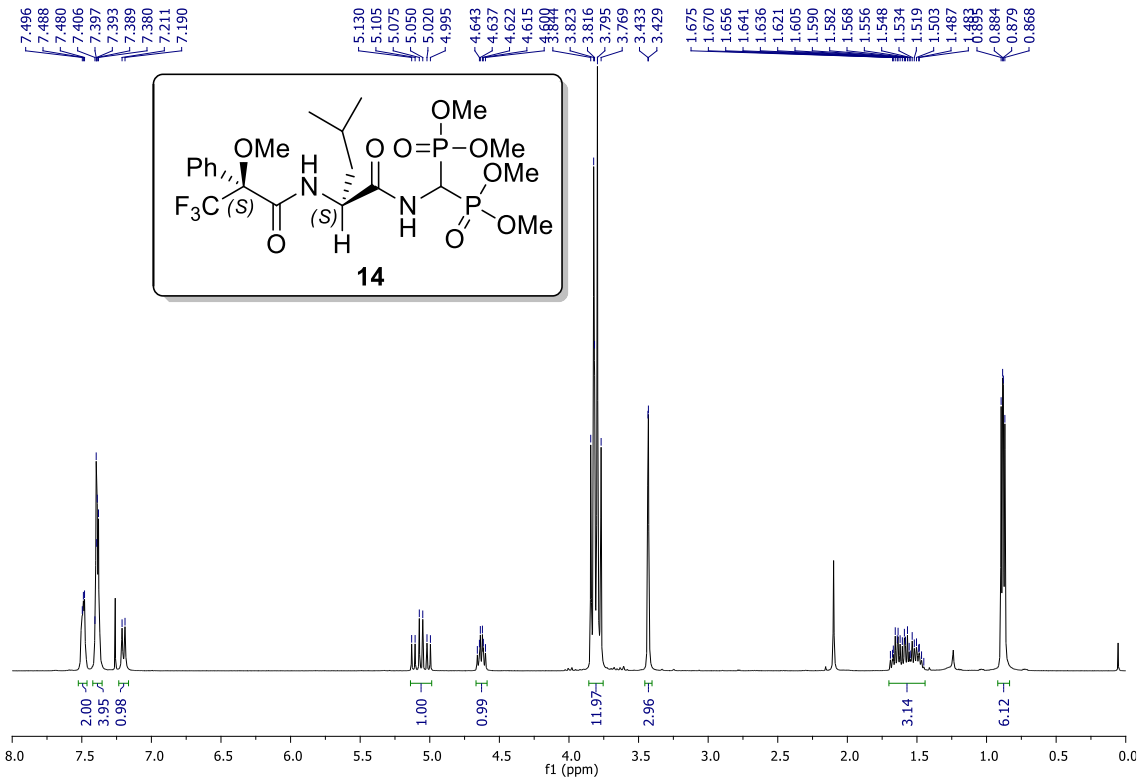


APT NMR (100 MHz, D_2O)

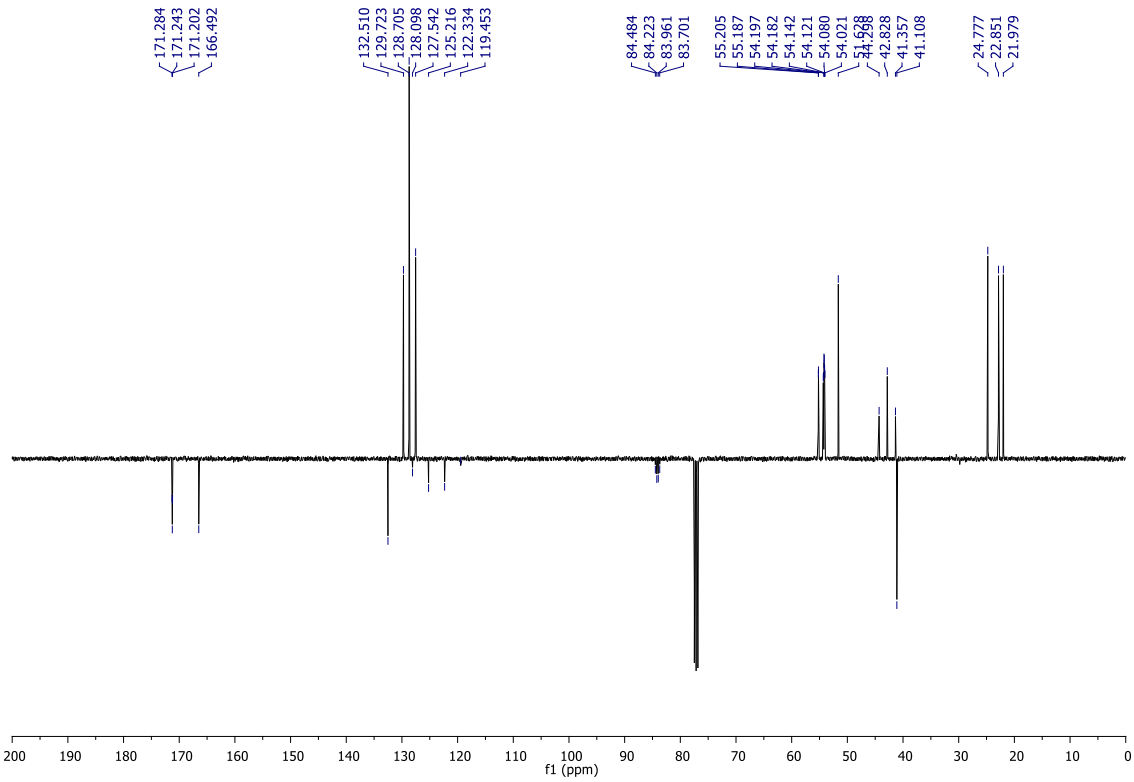


^{31}P NMR (162 MHz, D_2O)

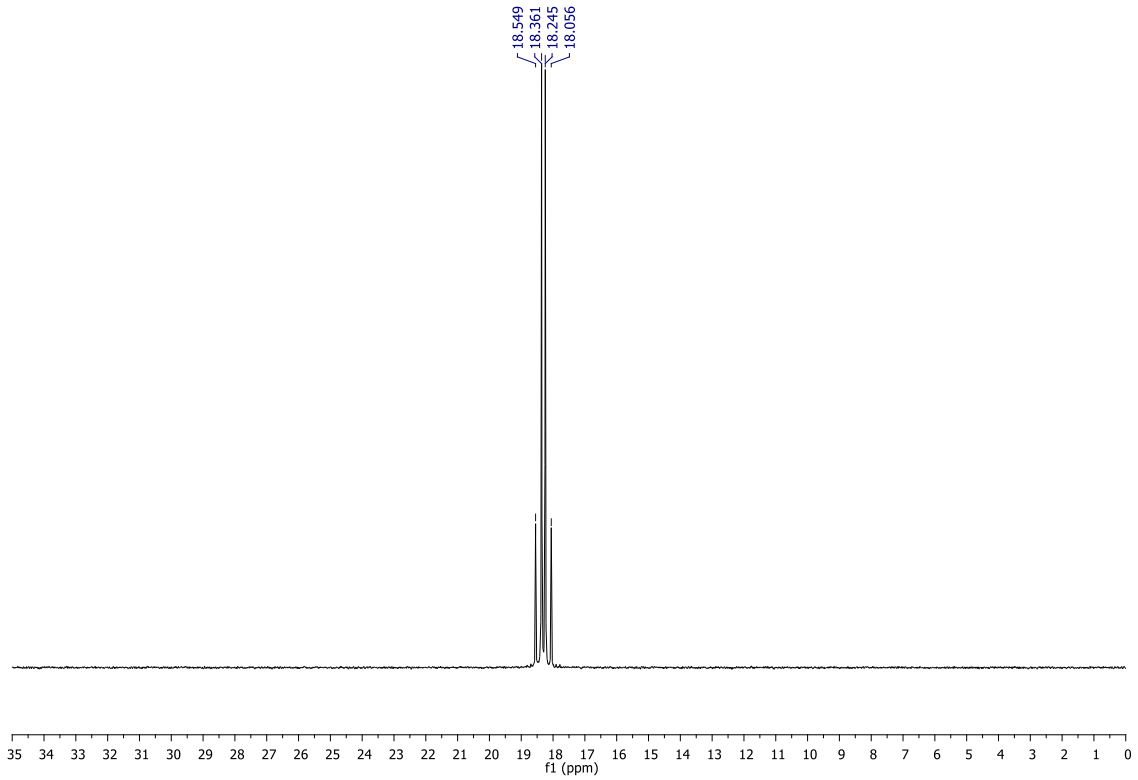
Tetramethyl {*N*-[(*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl]-L-leucylamidomethyl}bisphosphonate (14)



^{1}H NMR (400 MHz, $CDCl_3$)

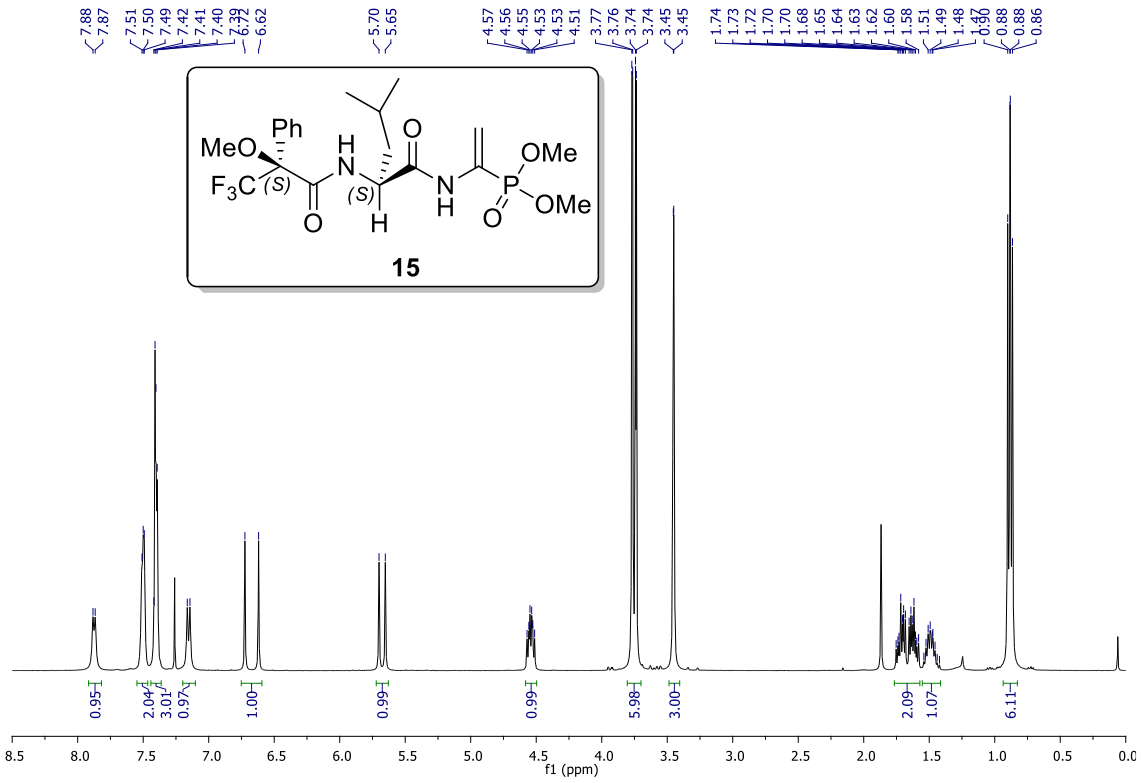


APT NMR (100 MHz, $CDCl_3$)



^{31}P NMR (162 MHz, $CDCl_3$)

Dimethyl {1-{*N*-[(*S*)-α-methoxy-α-(trifluoromethyl)phenylacetyl]-L-leucylamido}ethen-1-yl}phosphonate (15)



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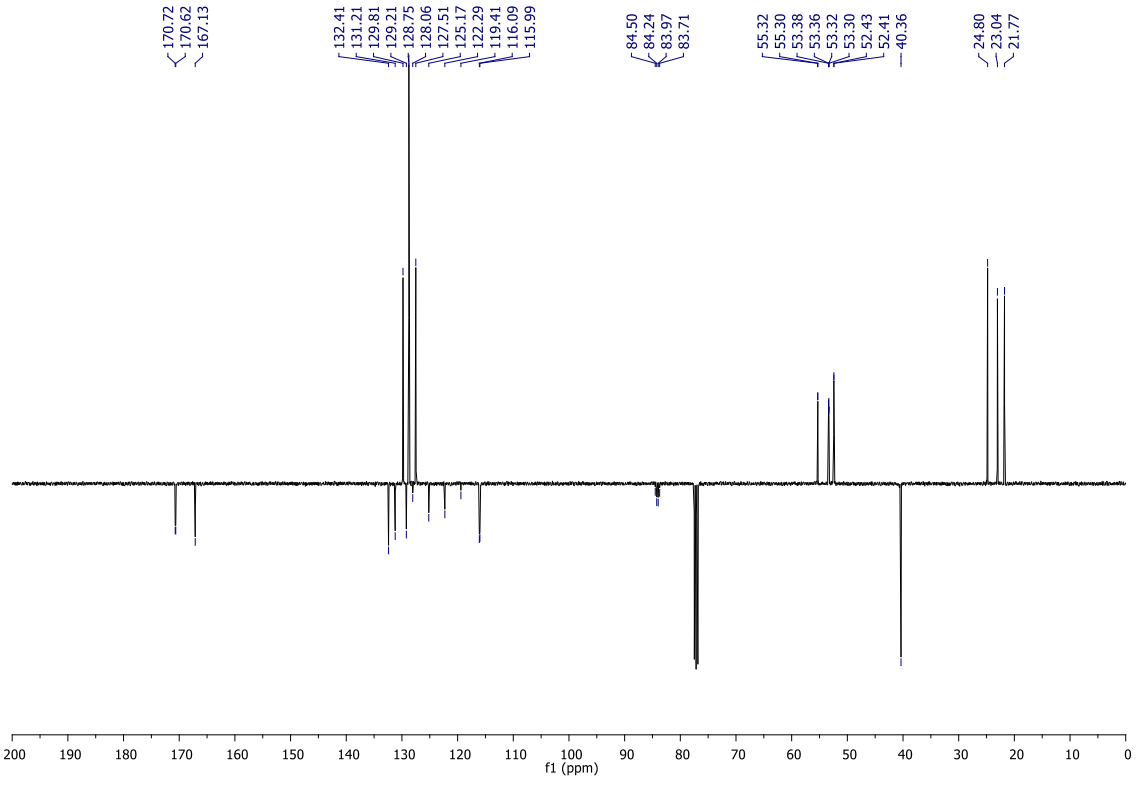
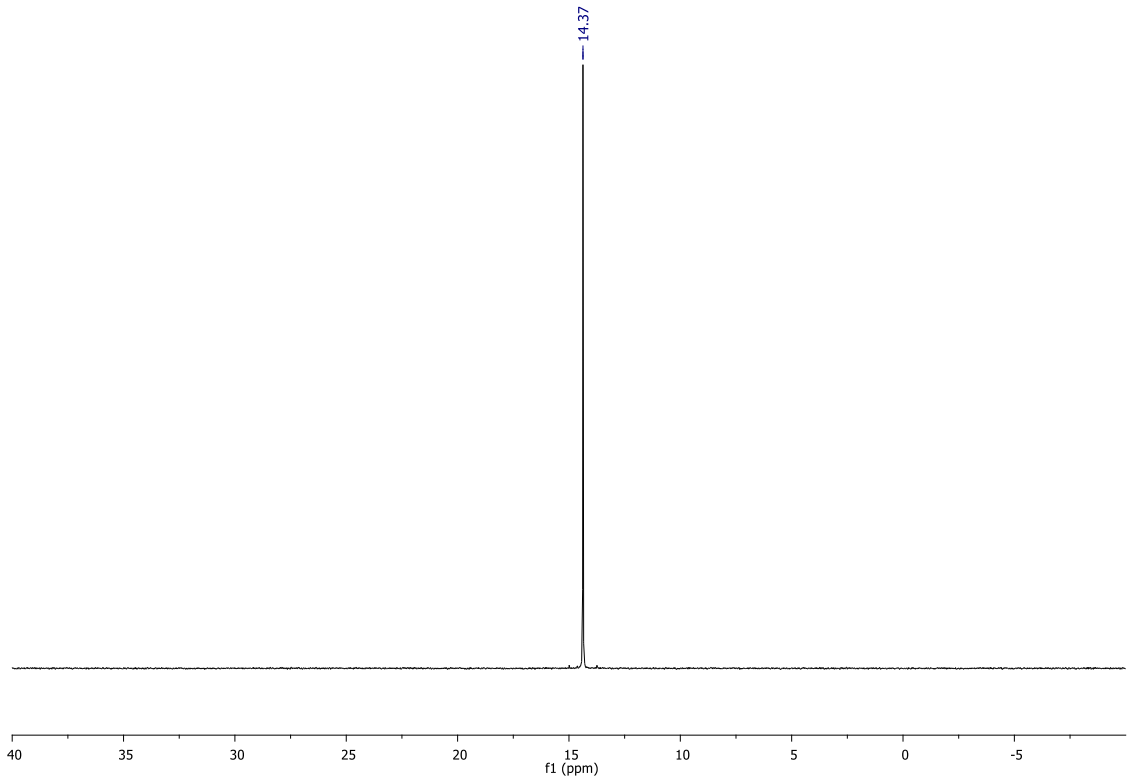
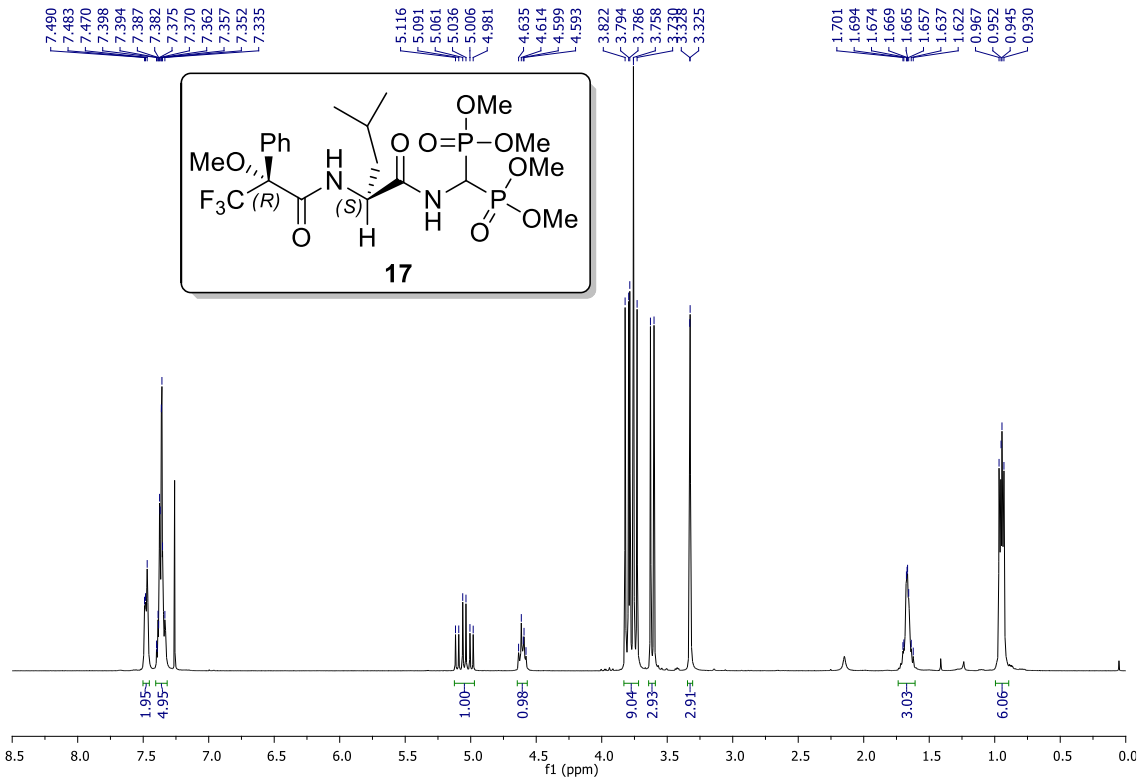


APT NMR (100 MHz, $CDCl_3$)

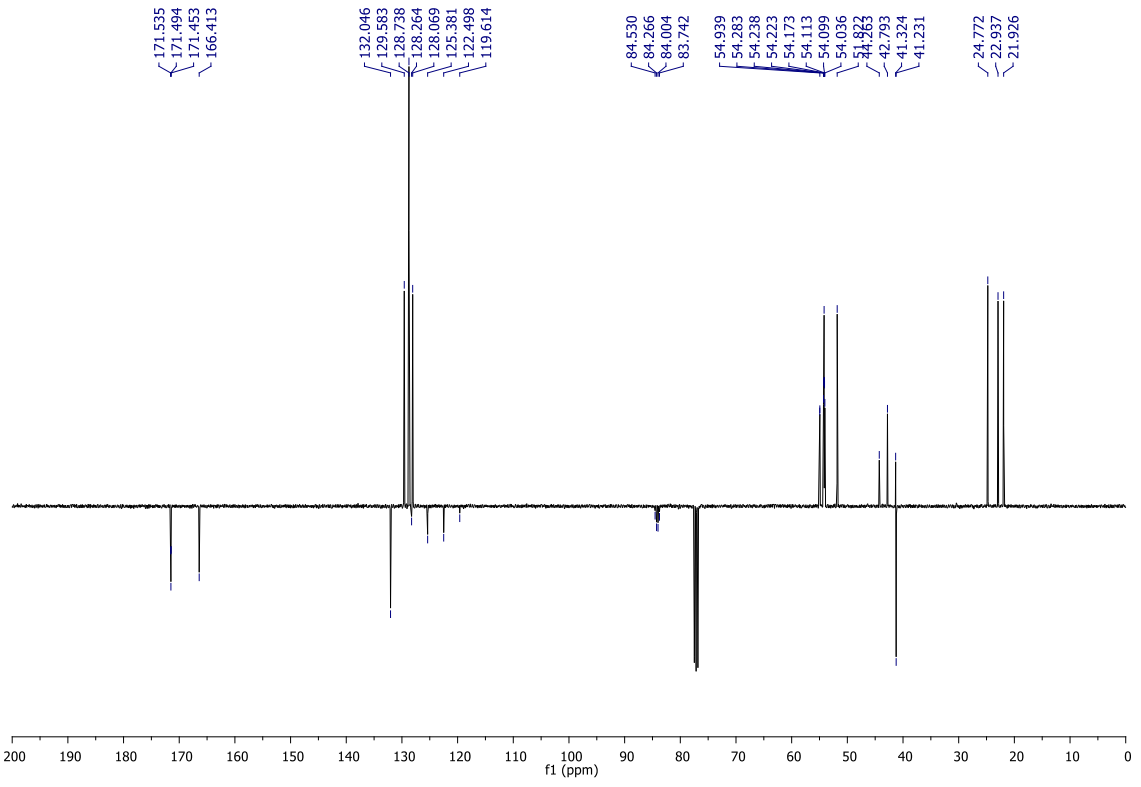


^{31}P NMR (162 MHz, $CDCl_3$)

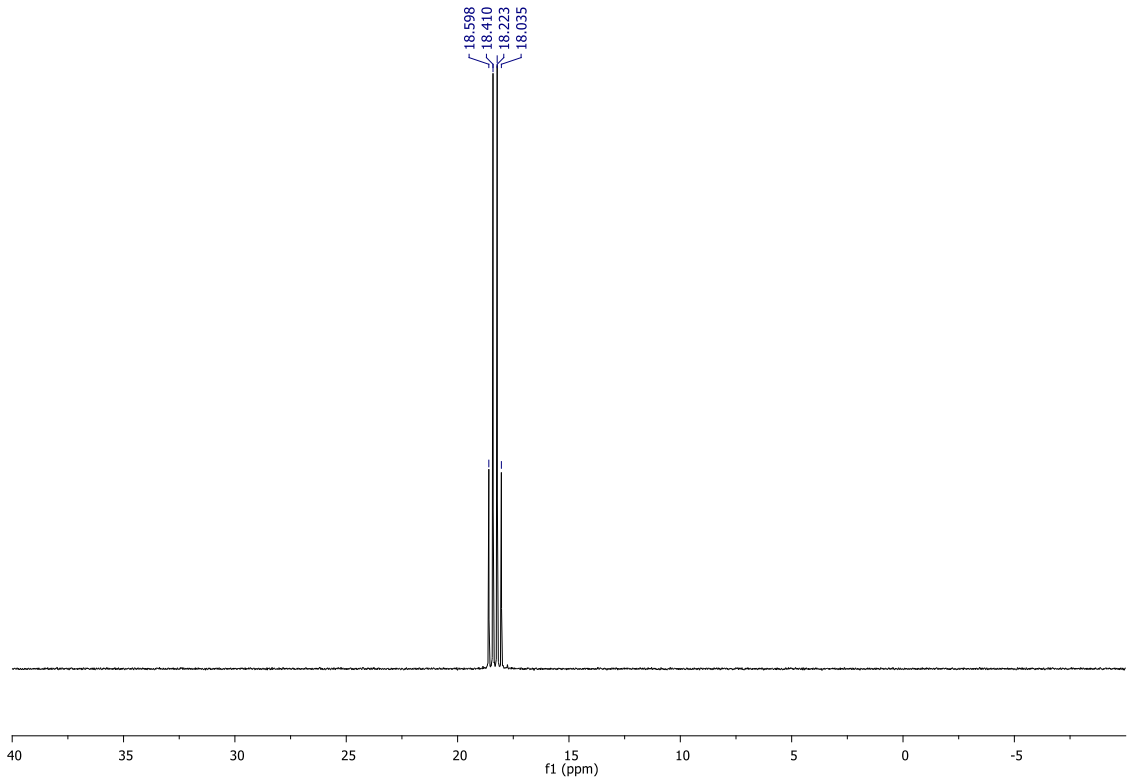
Tetramethyl {*N*-[(*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl]-L-leucylamidomethyl}bisphosphonate (17)



^{1}H NMR (400 MHz, $CDCl_3$)

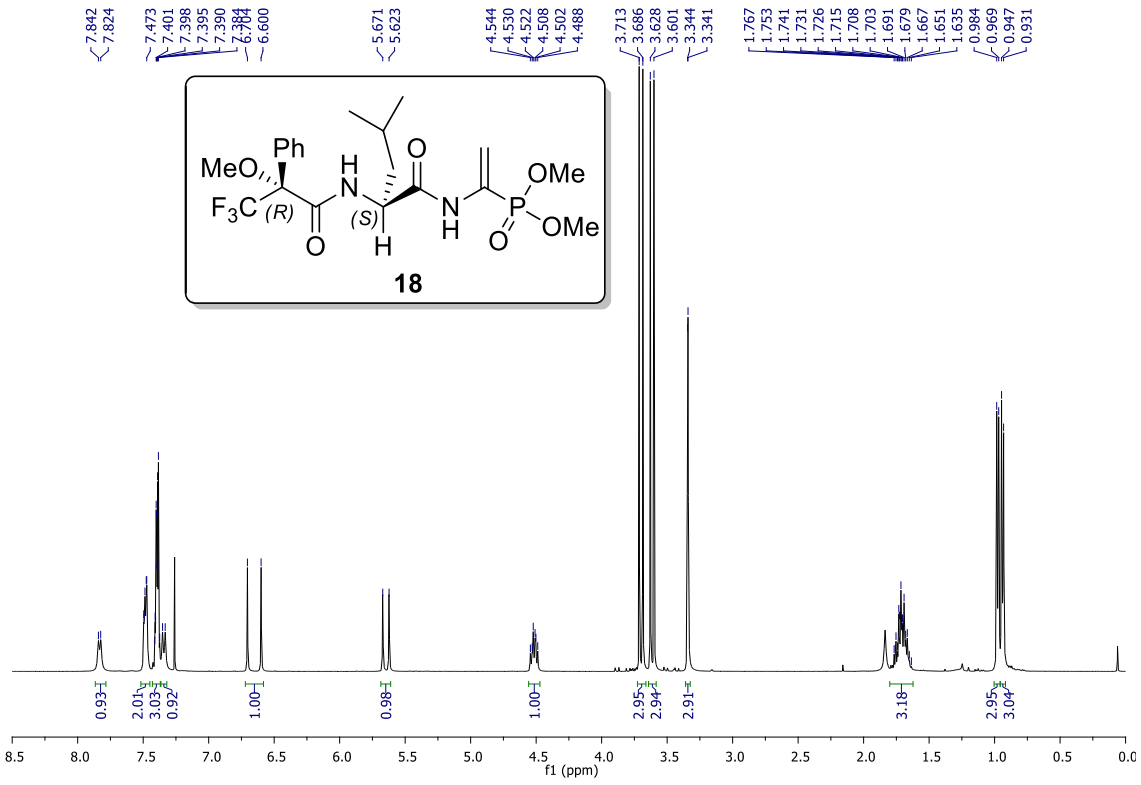


APT NMR (100 MHz, $CDCl_3$)

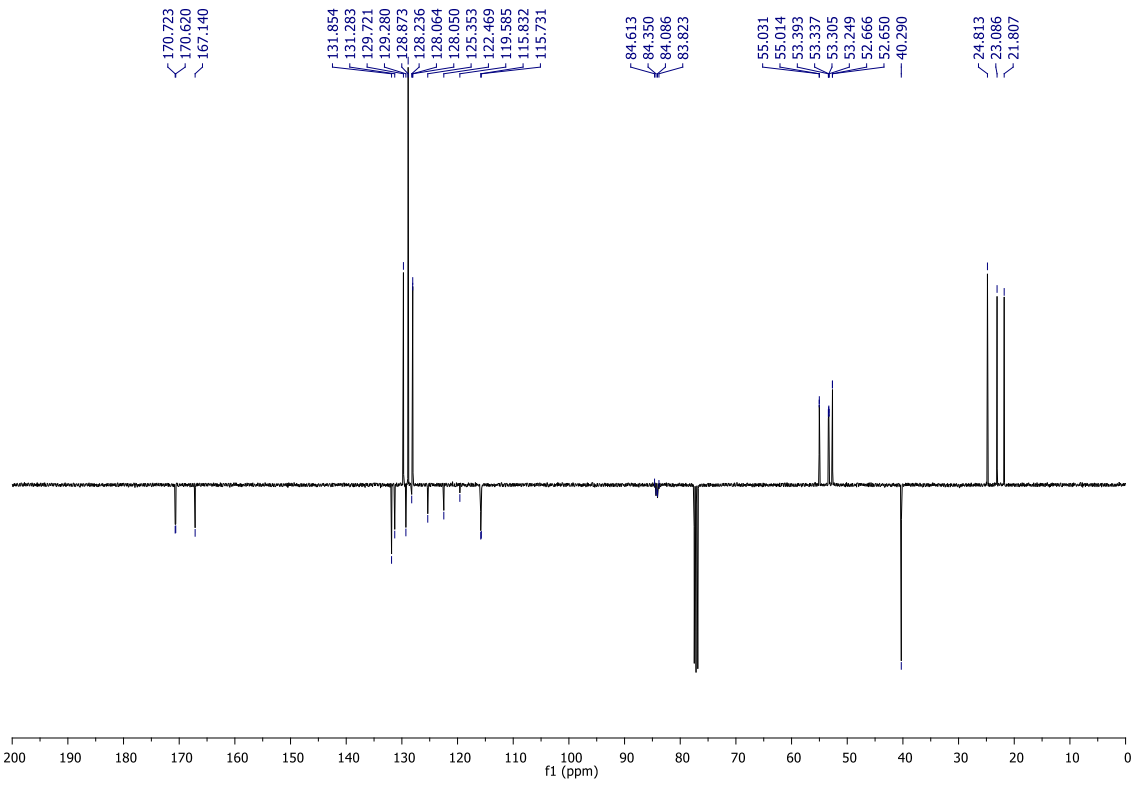


^{31}P NMR (162 MHz, $CDCl_3$)

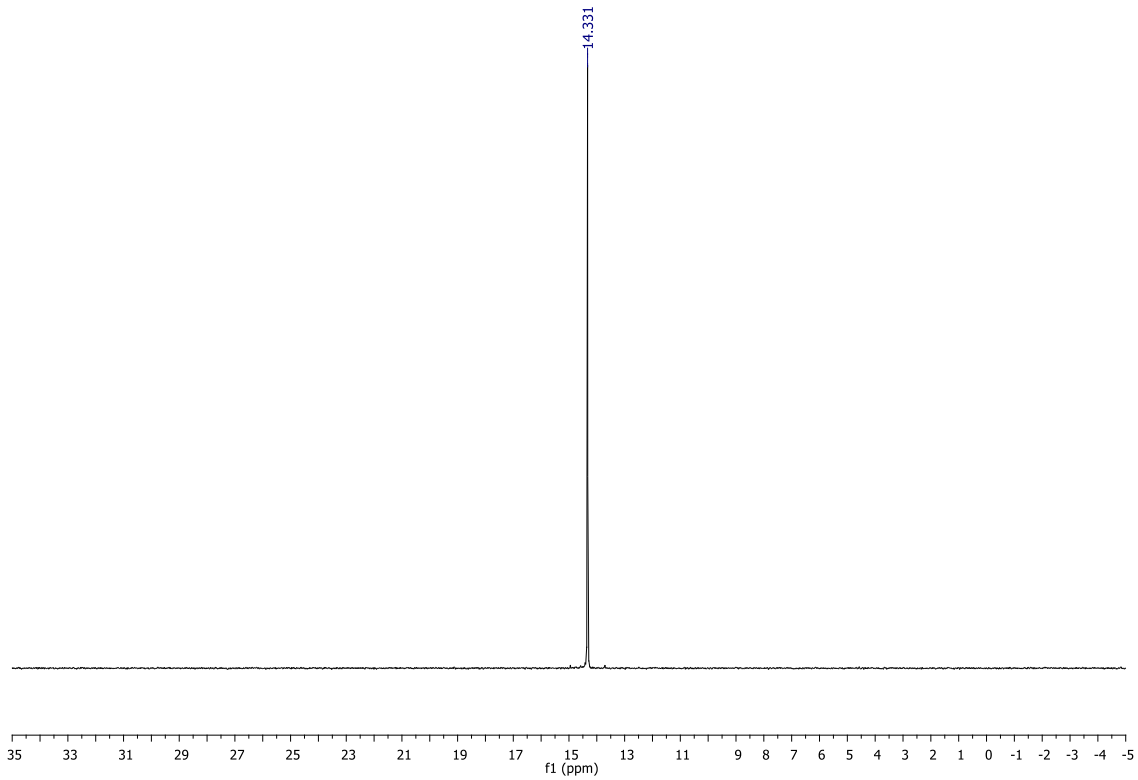
Dimethyl {1-{*N*-[(*R*)-α-methoxy-α-(trifluoromethyl)phenylacetyl]-L-leucylamido}ethen-1-yl}phosphonate (18)



^{1}H NMR (400 MHz, $CDCl_3$)



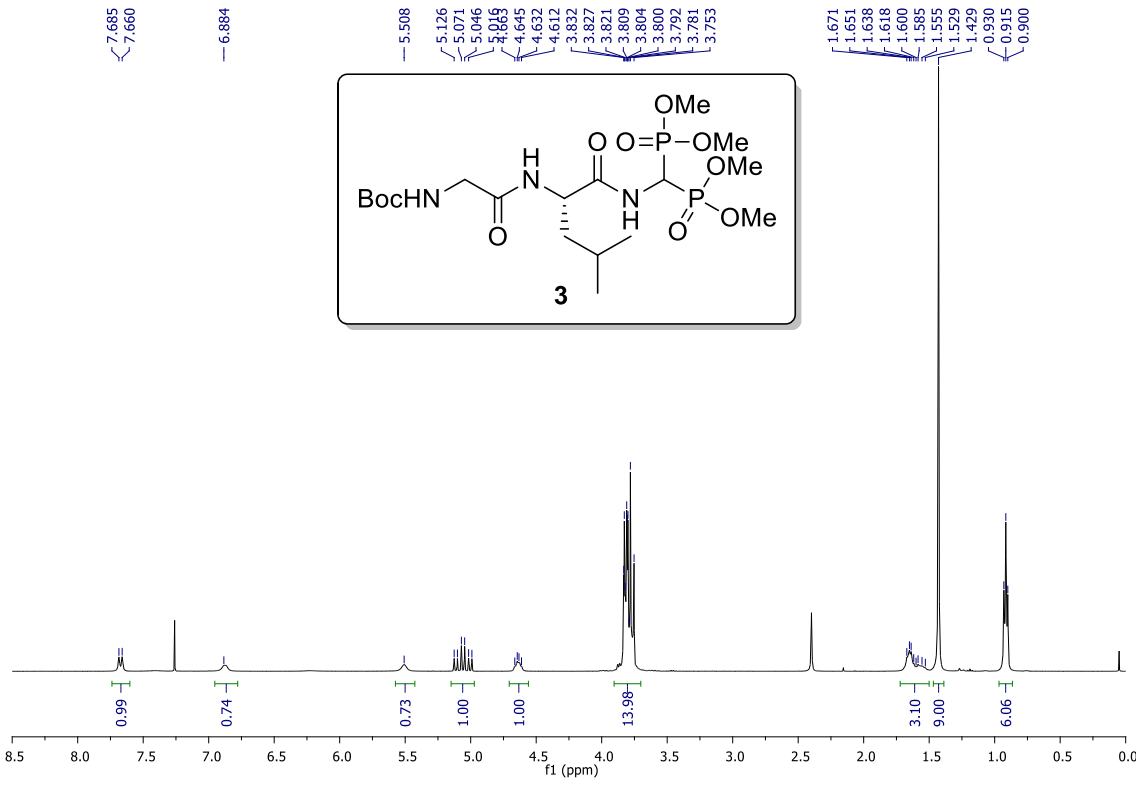
APT NMR (100 MHz, $CDCl_3$)



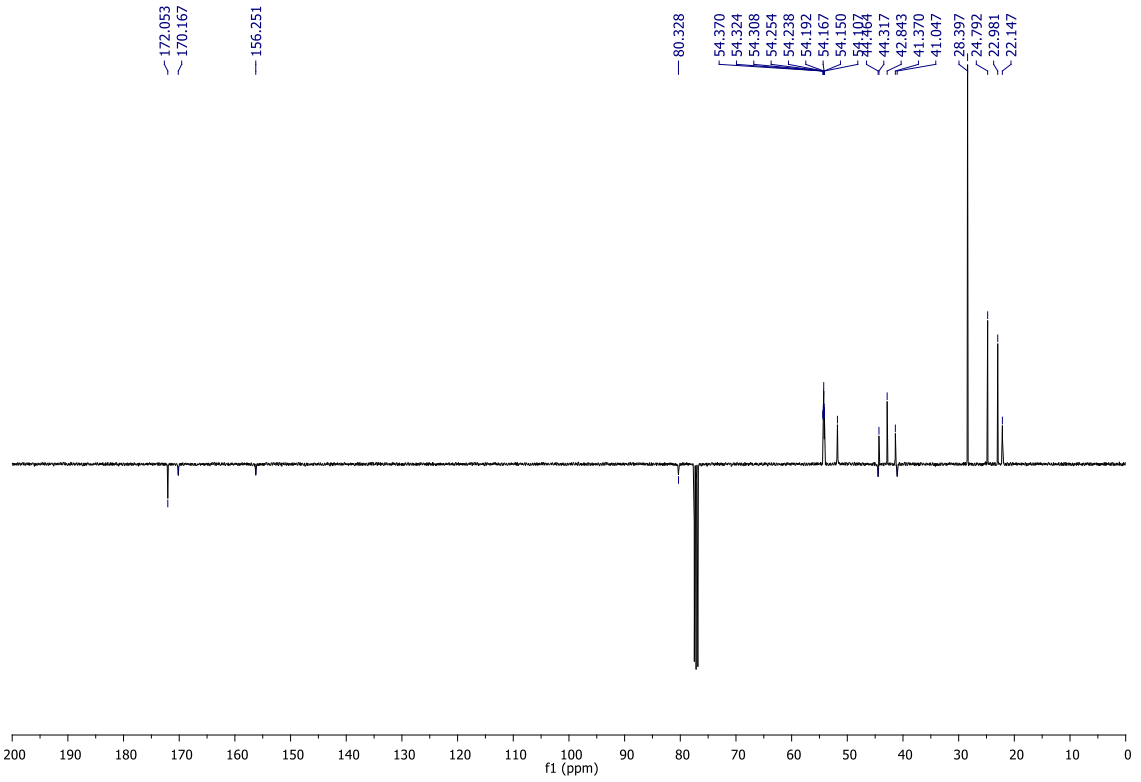
^{31}P NMR (162 MHz, $CDCl_3$)

NMR Spectra of Chapter 2

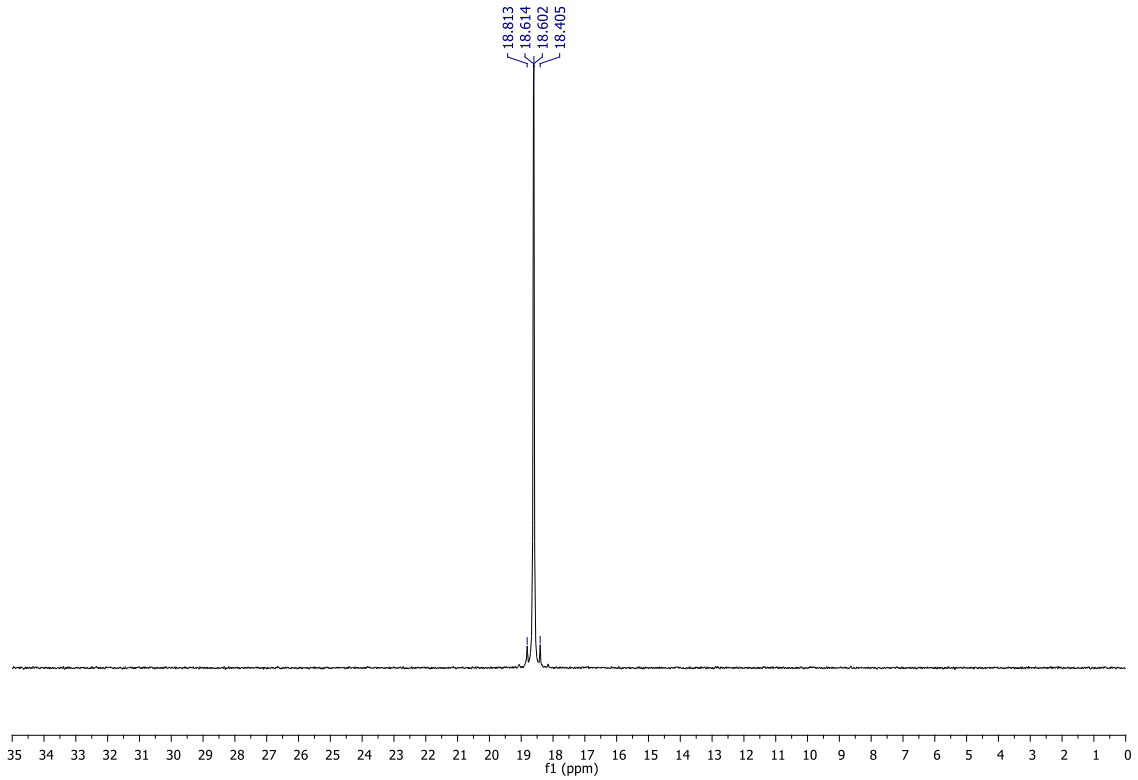
Tetramethyl [*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamidomethyl]bisphosphonate (3)



^{1}H NMR (400 MHz, $CDCl_3$)

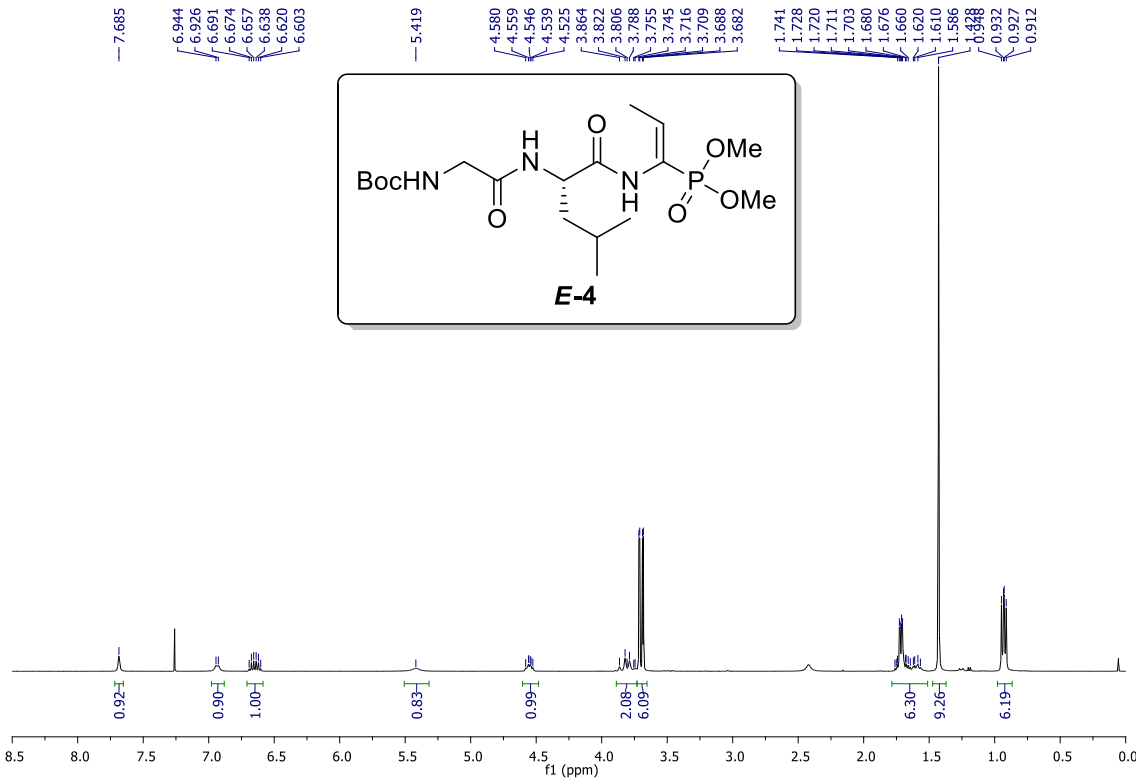


APT NMR (100 MHz, $CDCl_3$)

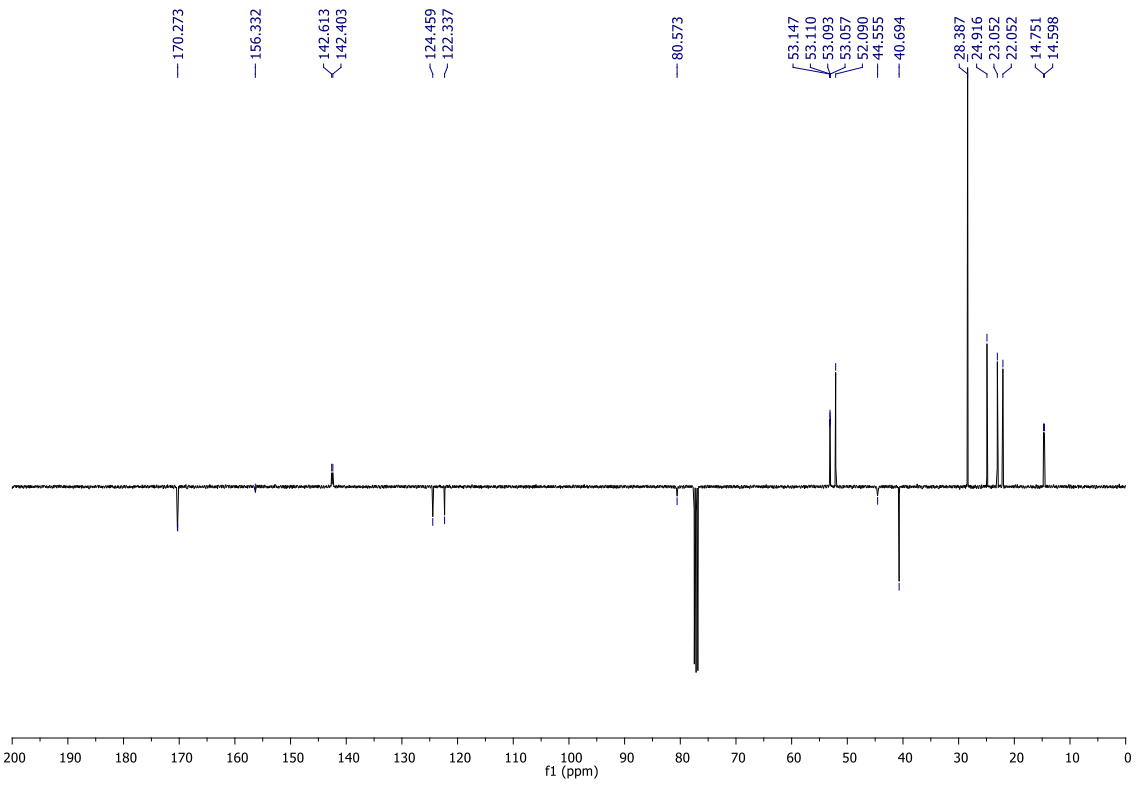


^{31}P NMR (162 MHz, $CDCl_3$)

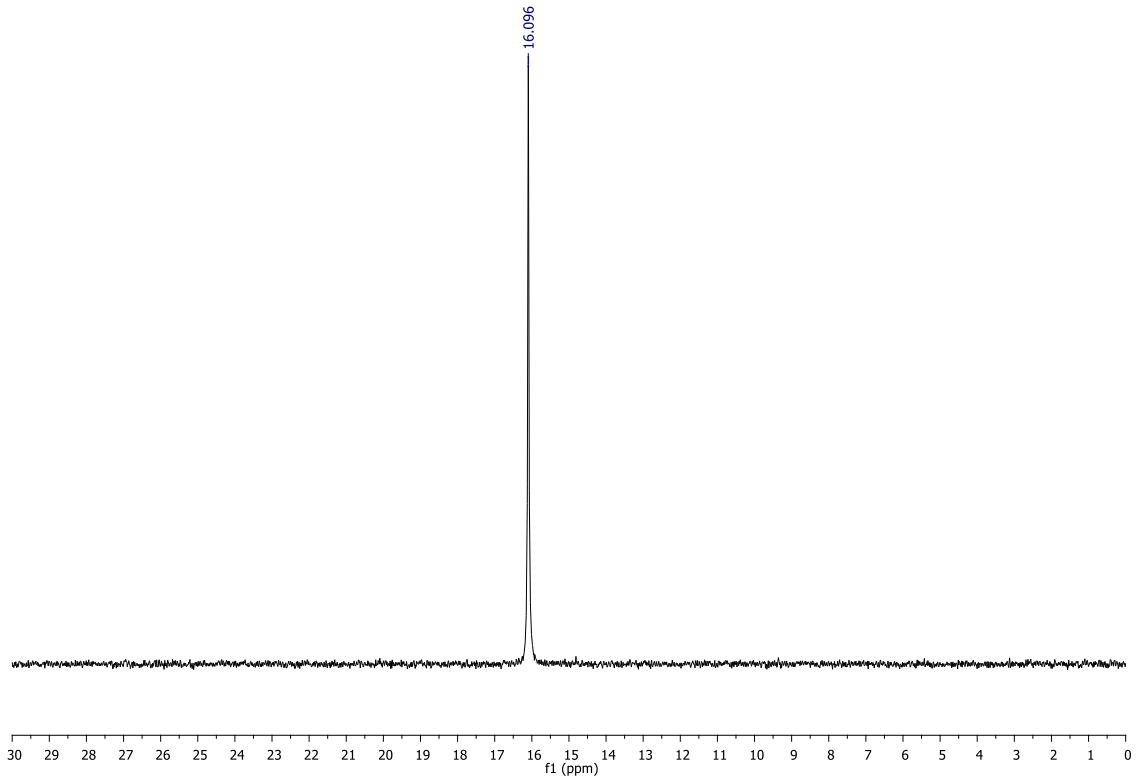
Dimethyl (*E*)-{1-[*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamido]prop-1-en-1-yl}phosphonate (*E*-4)



^{1}H NMR (400 MHz, $CDCl_3$)

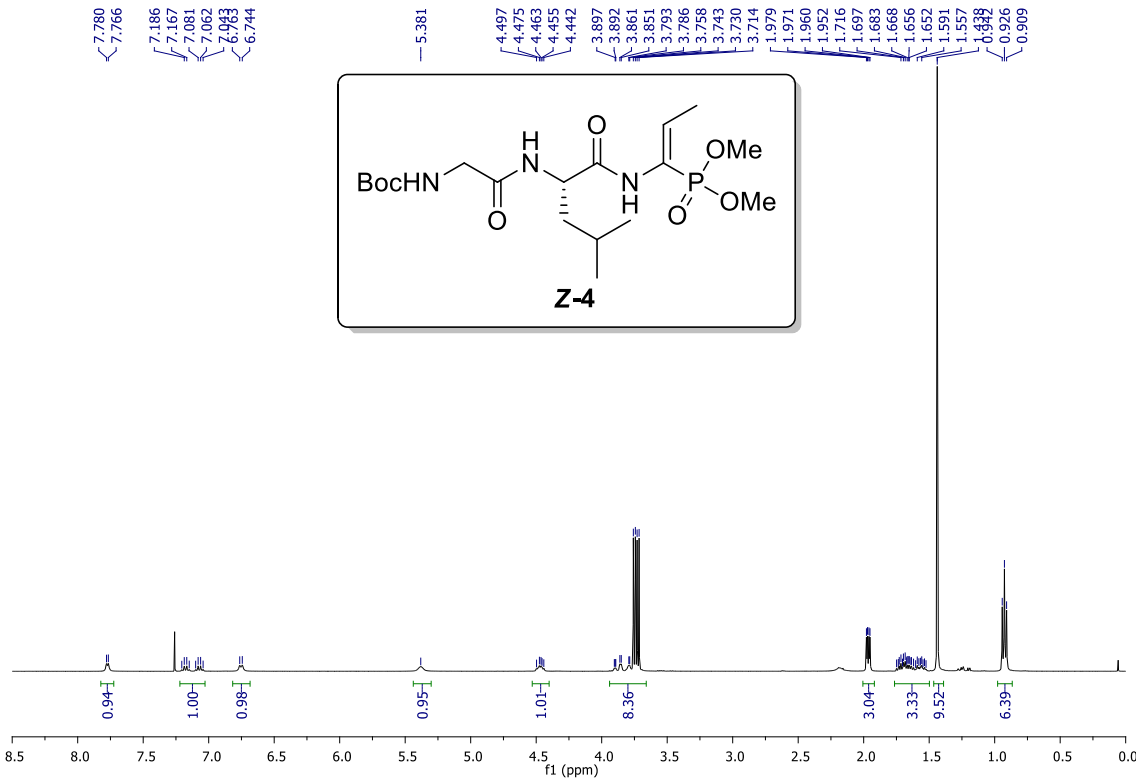


APT NMR (100 MHz, $CDCl_3$)

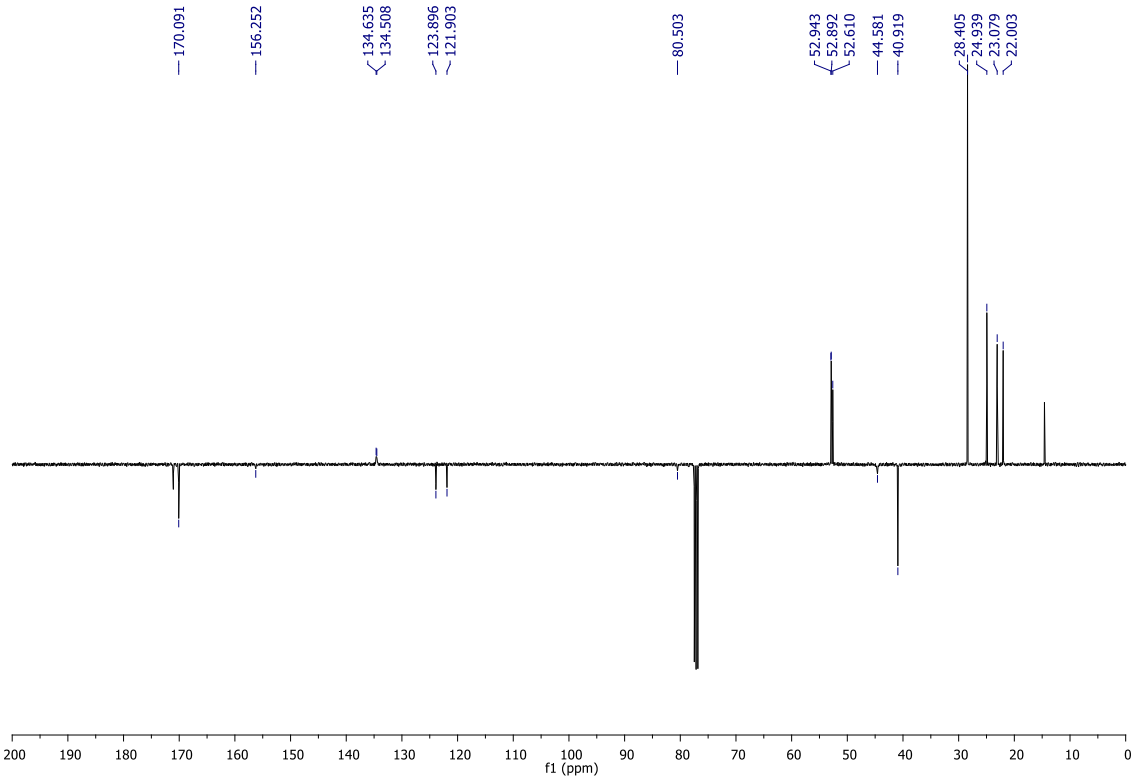


^{31}P NMR (162 MHz, $CDCl_3$)

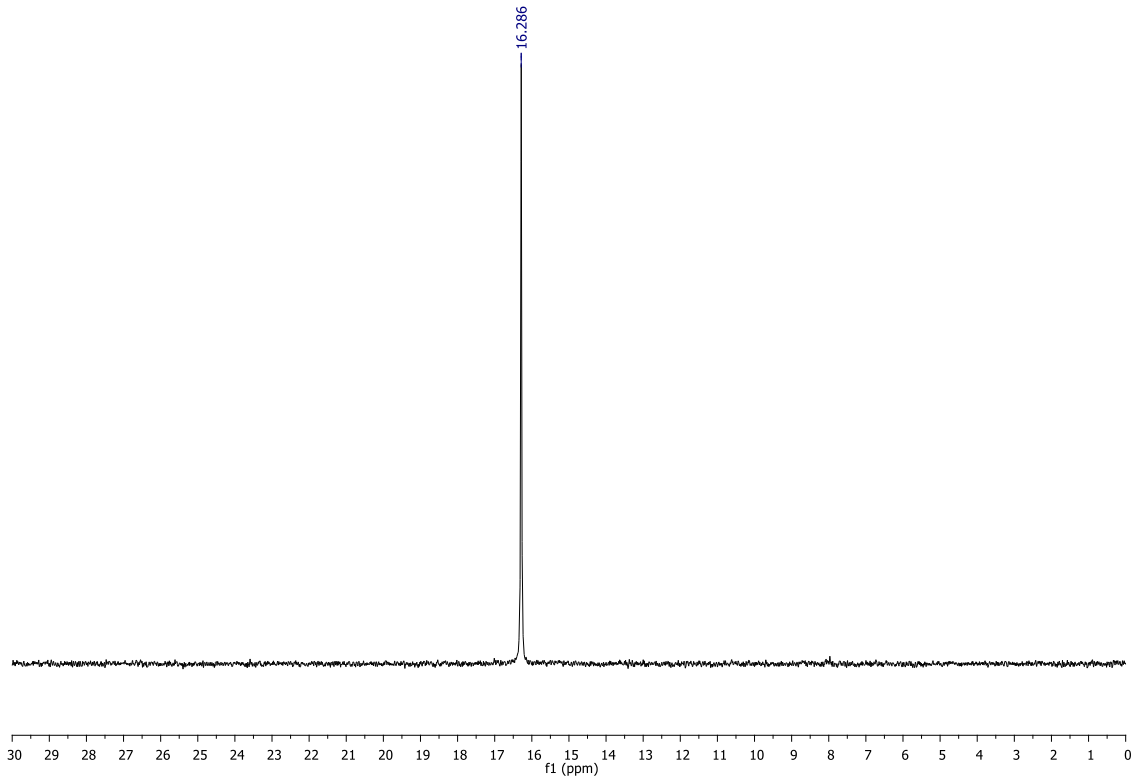
Dimethyl (*Z*)-{1-[*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamido]prop-1-en-1-yl}phosphonate (*Z*-4)



^{1}H NMR (400 MHz, $CDCl_3$)

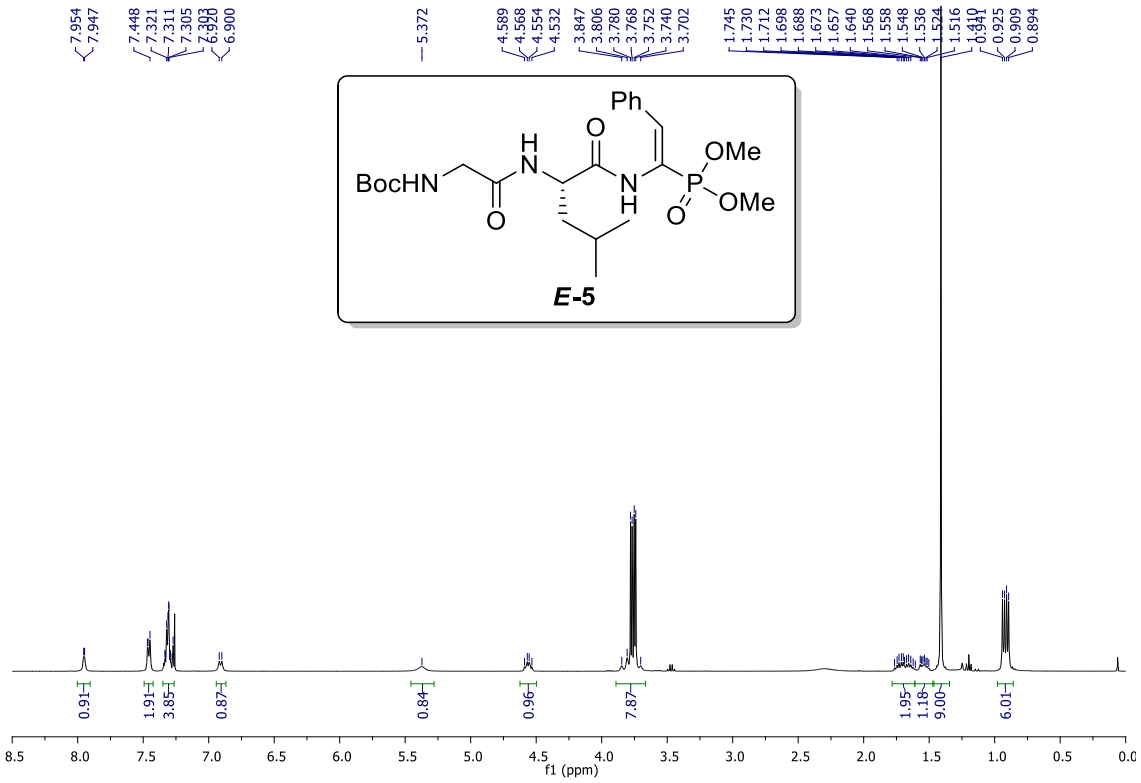


APT NMR (100 MHz, $CDCl_3$)

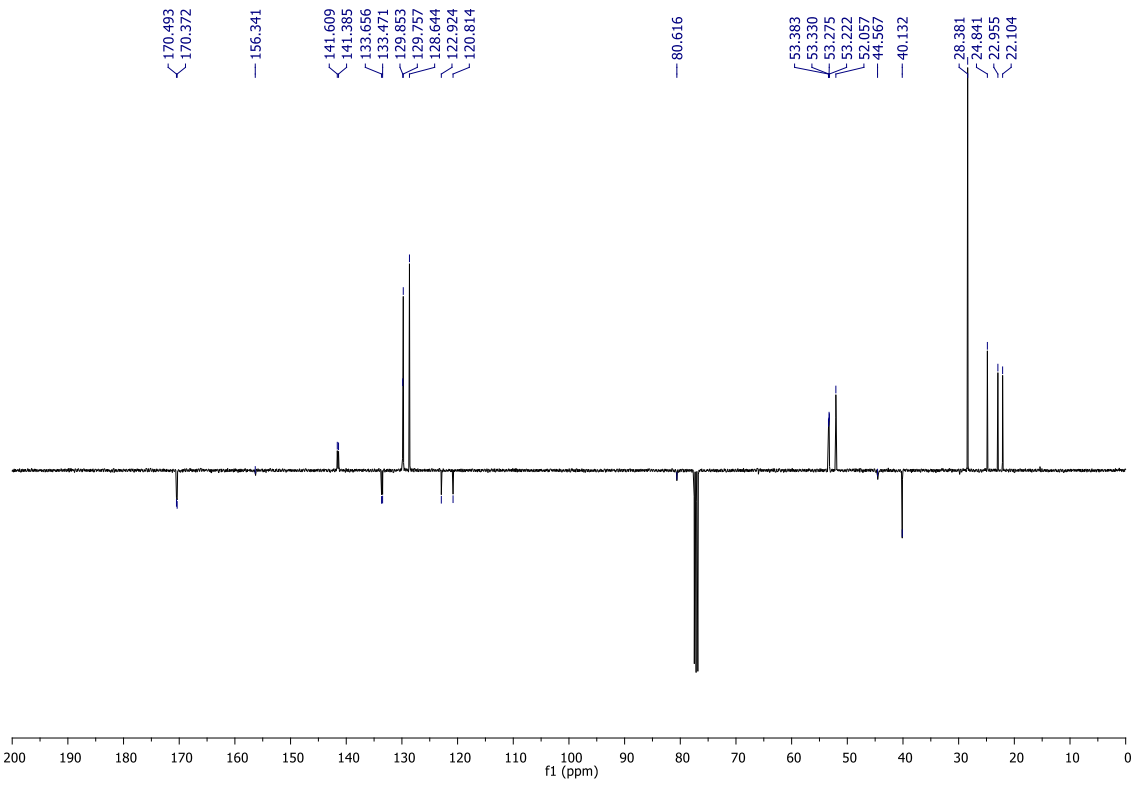


^{31}P NMR (162 MHz, $CDCl_3$)

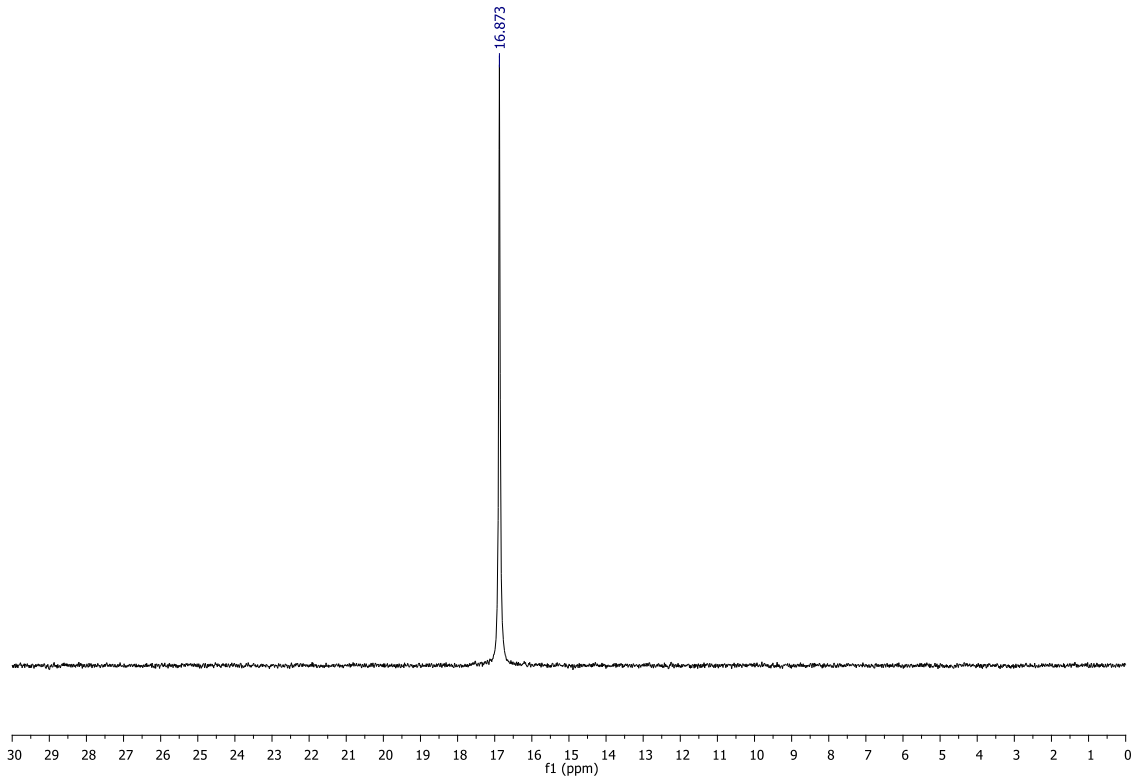
Dimethyl (*E*)-{1-[*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamido]-2-phenylethen-1-yl}phosphonate (*E*-5)



^{1}H NMR (400 MHz, $CDCl_3$)

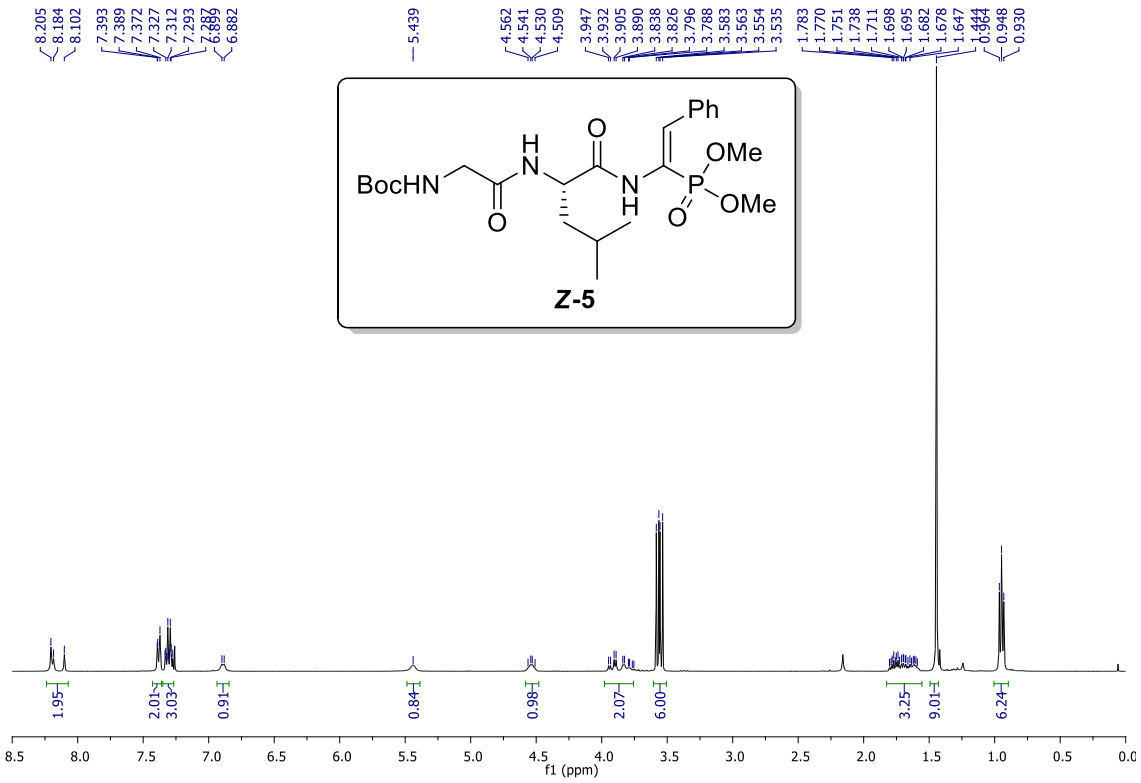


APT NMR (100 MHz, $CDCl_3$)

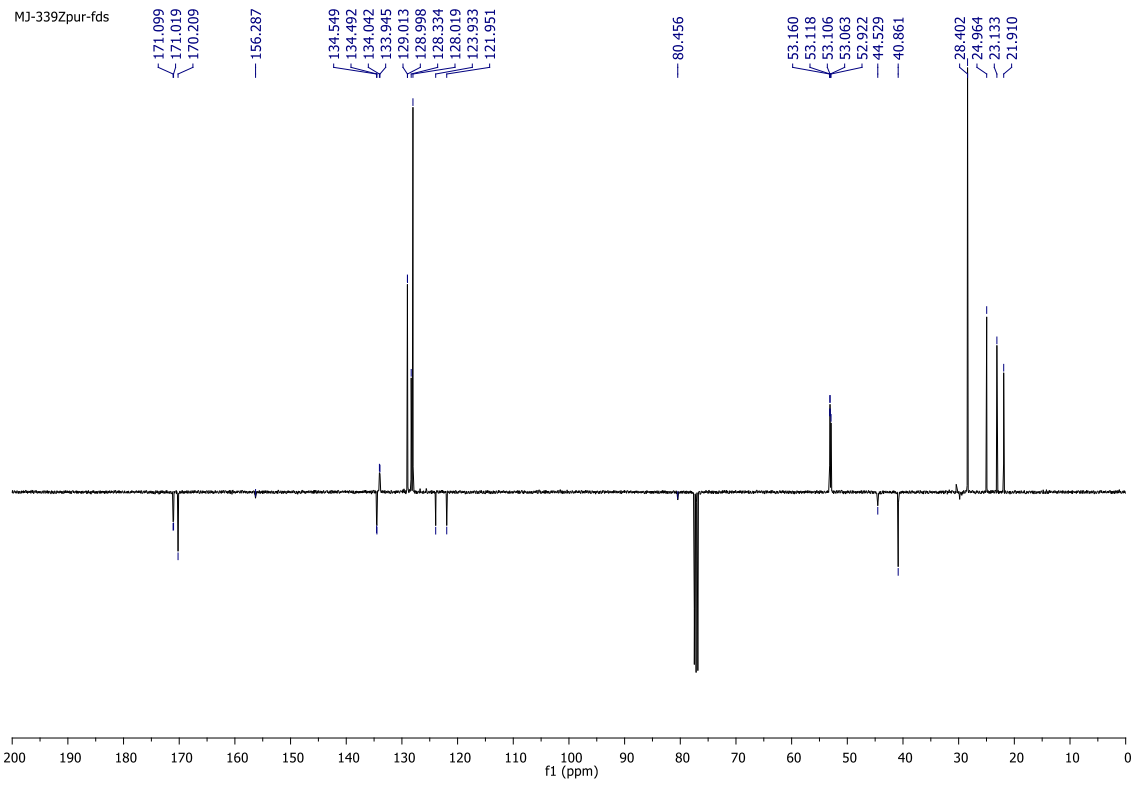


^{31}P NMR (162 MHz, $CDCl_3$)

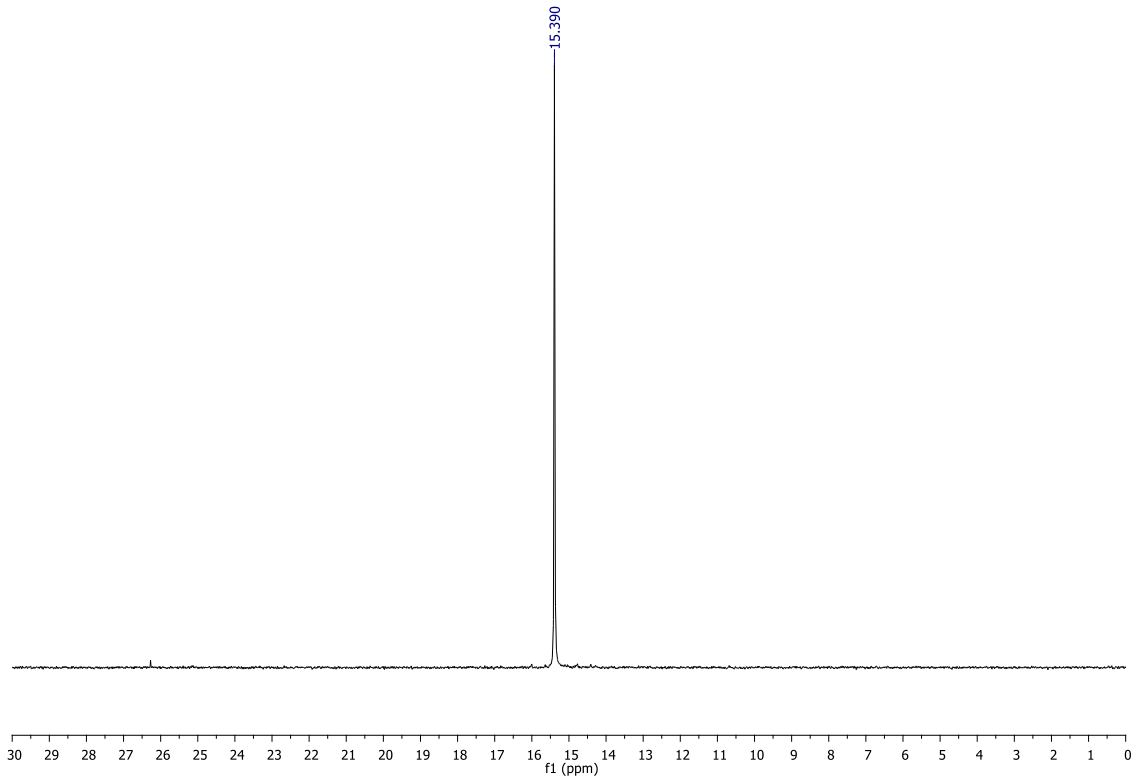
Dimethyl (*Z*)-{1-[*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamido]-2-phenylethen-1-yl}phosphonate (*Z*-5)



^{1}H NMR (400 MHz, $CDCl_3$)

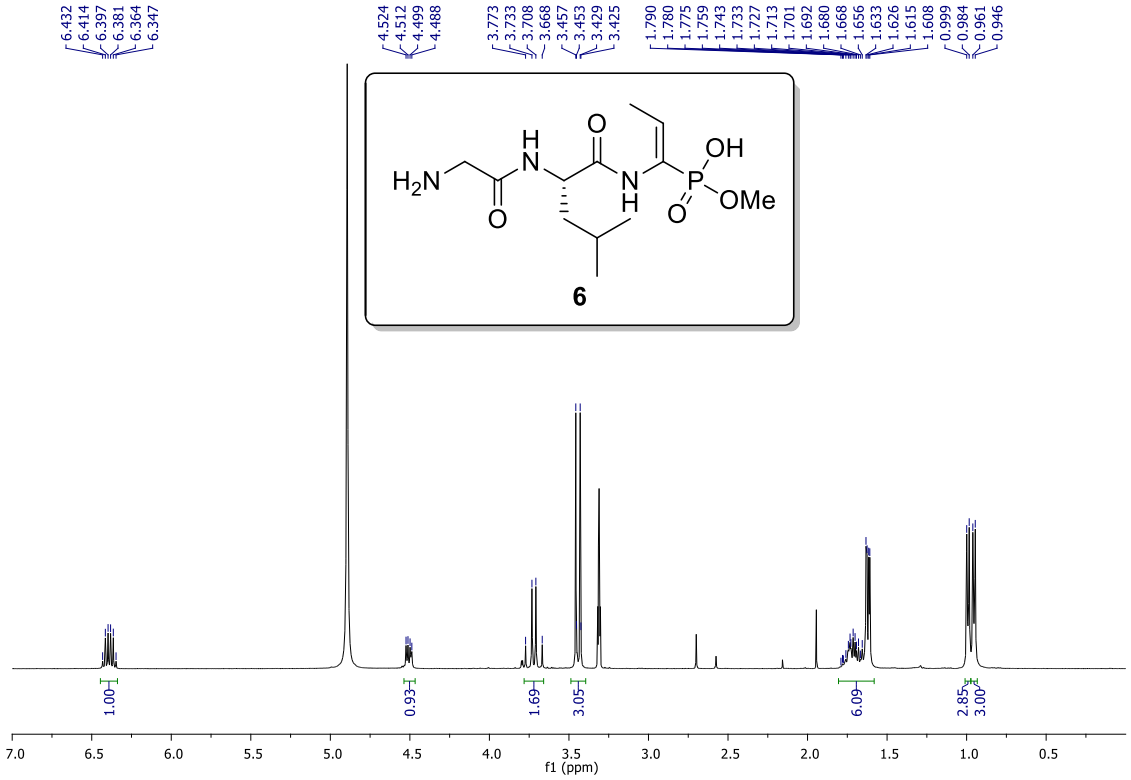


APT NMR (100 MHz, $CDCl_3$)

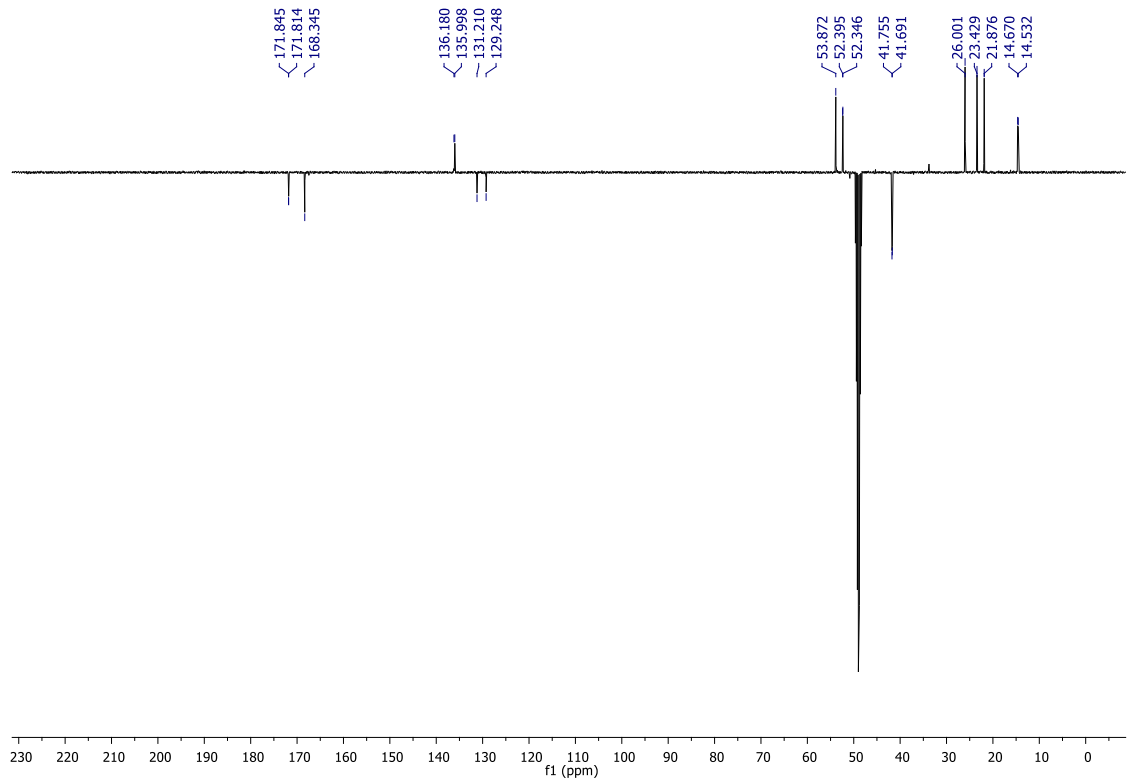


^{31}P NMR (162 MHz, $CDCl_3$)

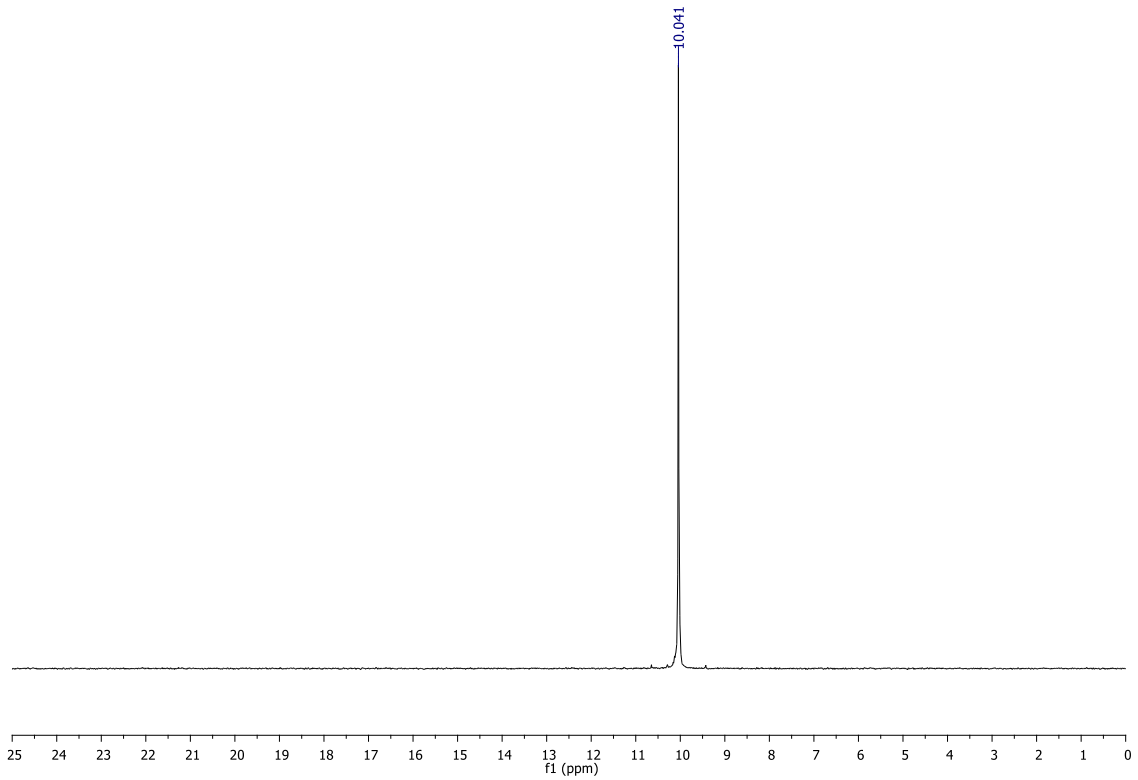
(*E*)-[1-(glycyl-L-leucylamido)prop-1-en-1-yl]phosphonic acid monomethyl ester (6)



^{1}H NMR (400 MHz, MeOD)

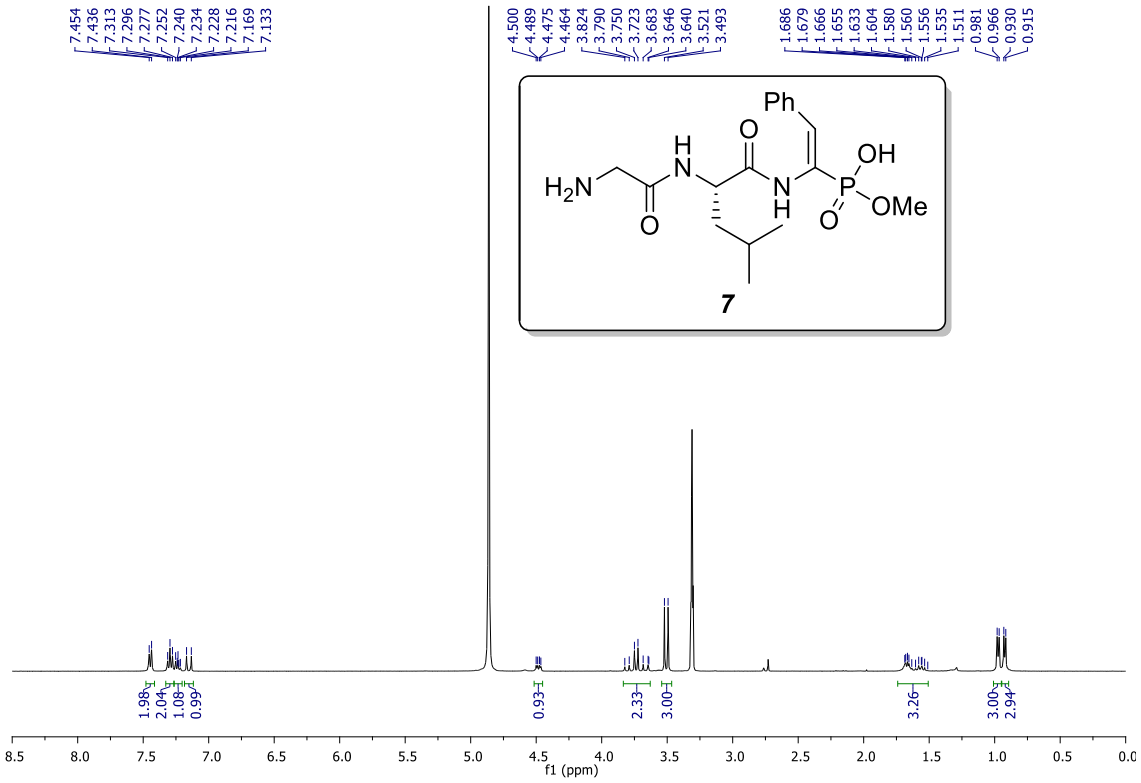


APT NMR (100 MHz, MeOD)

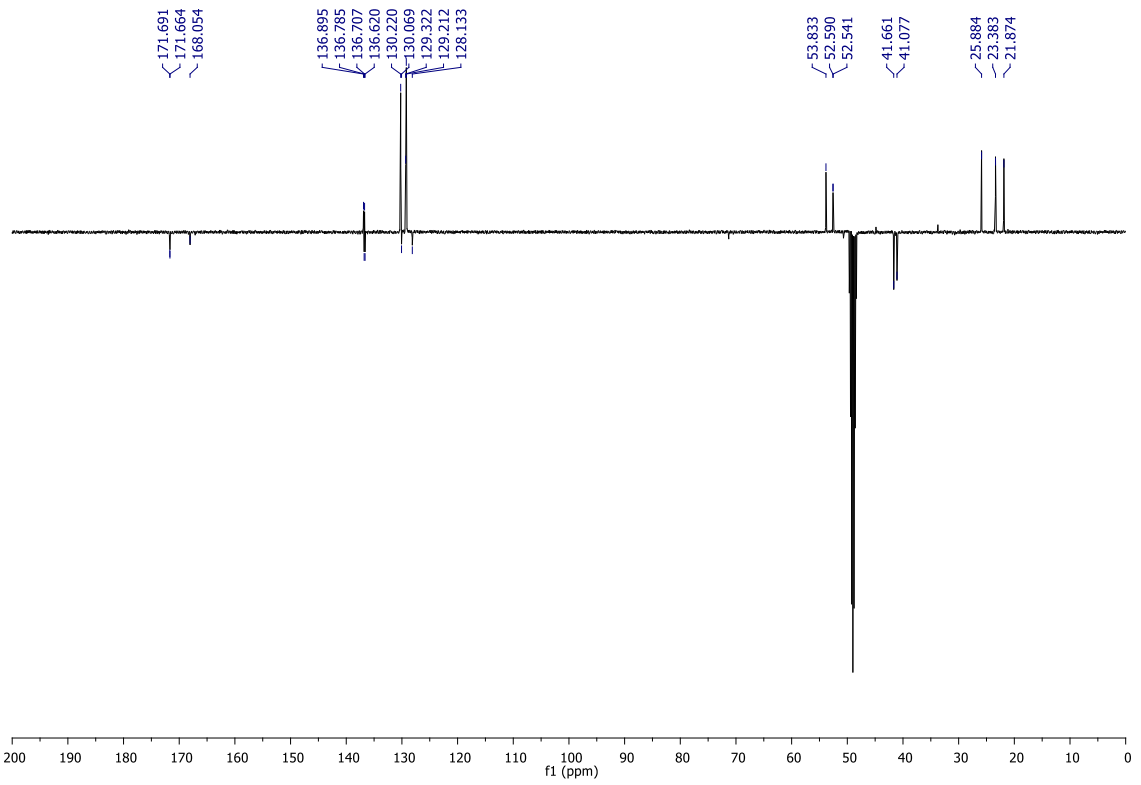


^{31}P NMR (162 MHz, MeOD)

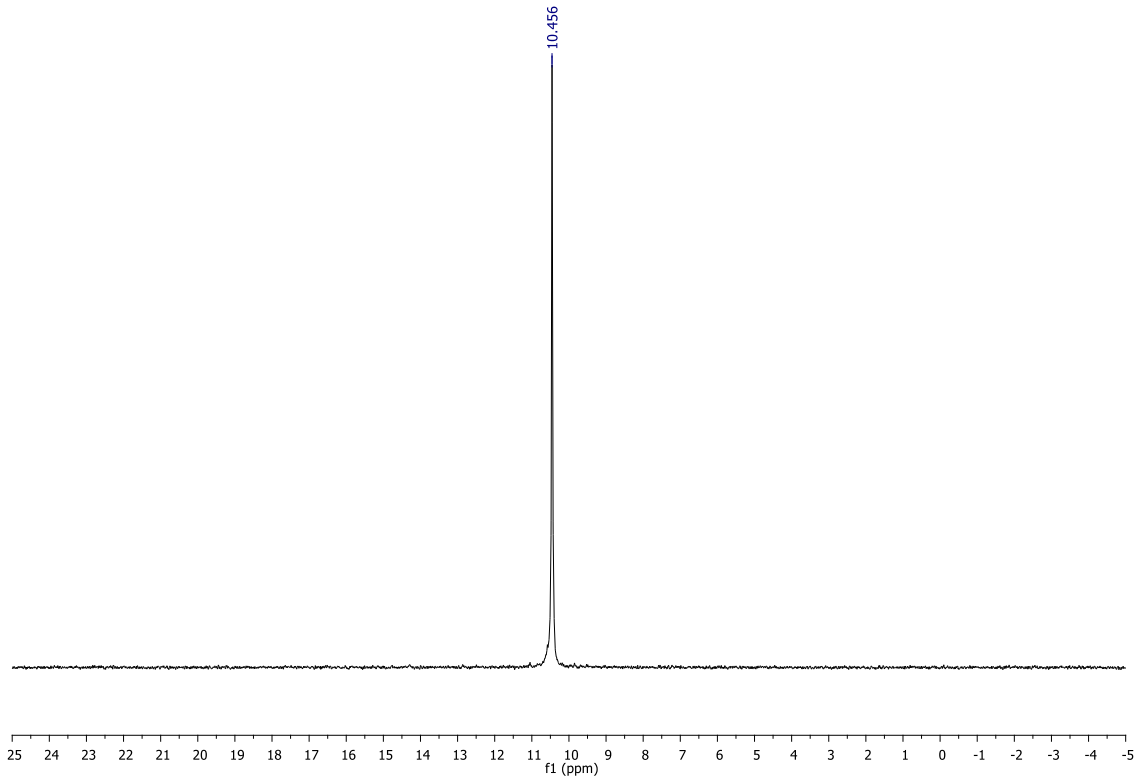
(*E*)-[1-(glycyl-L-leucylamido)-2-phenylethen-1-yl]phosphonic acid monomethyl ester (7)



^{1}H NMR (400 MHz, MeOD)

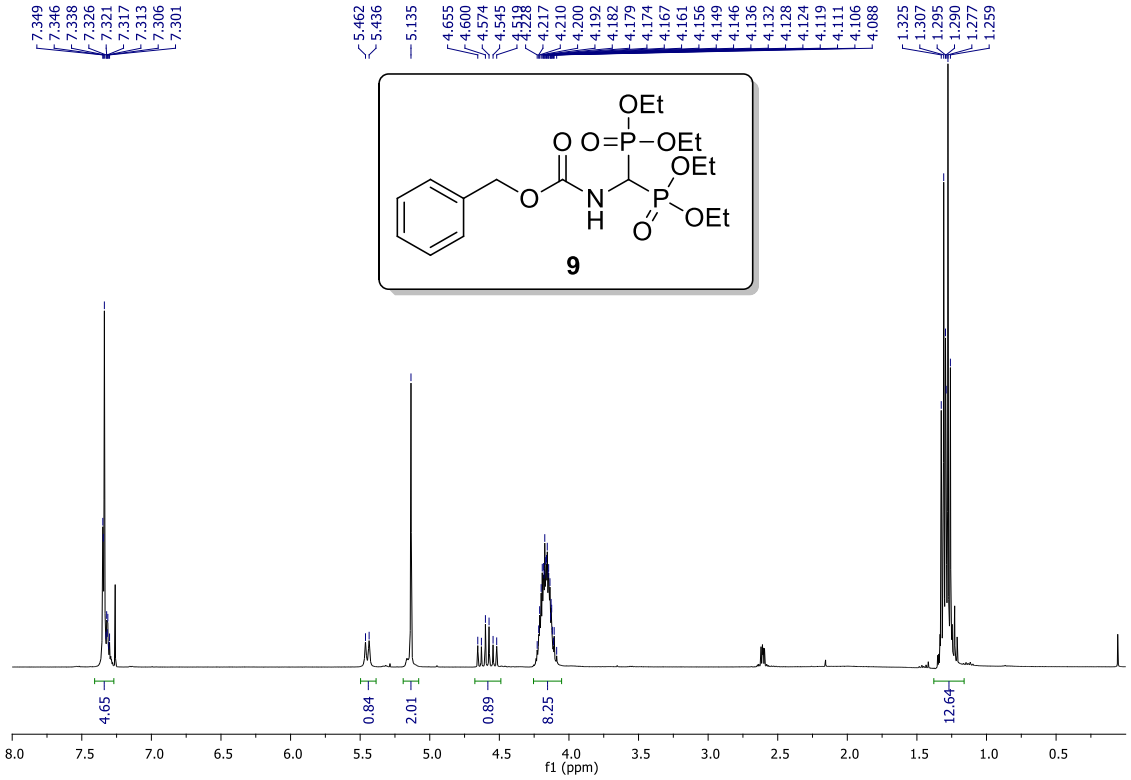


APT NMR (100 MHz, MeOD)

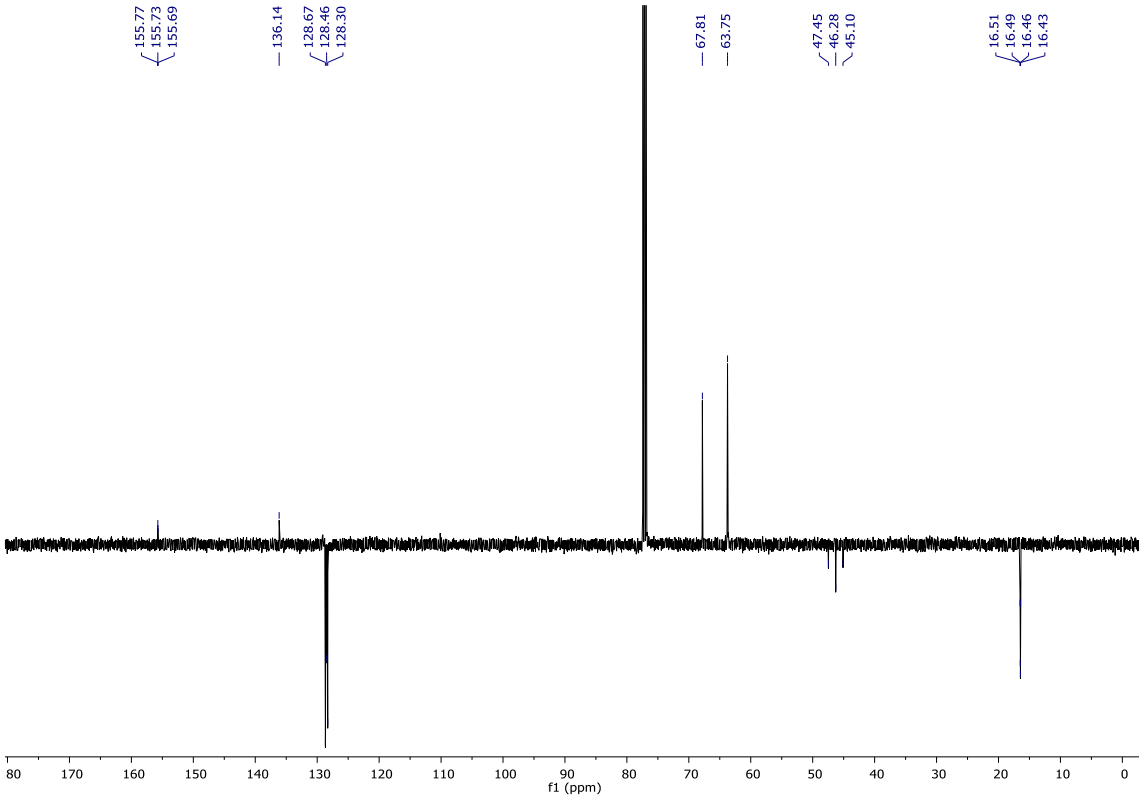


^{31}P NMR (162 MHz, MeOD)

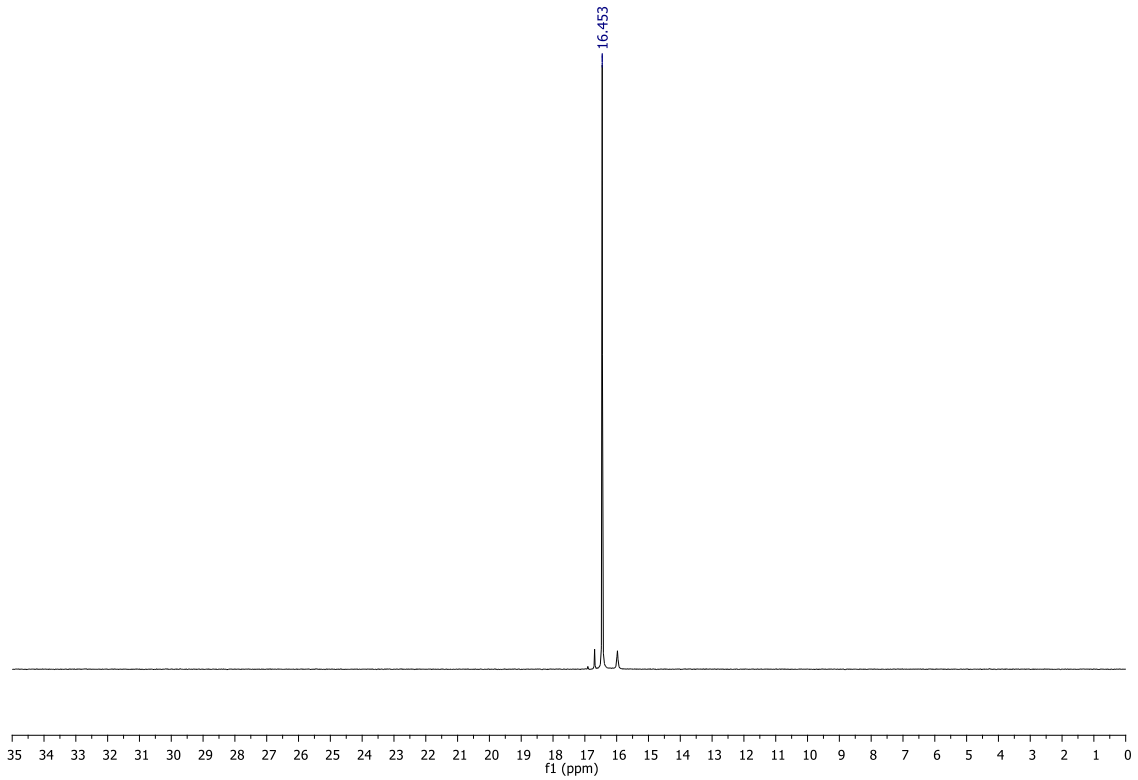
Tetraethyl (bencyloxycarbonylaminomethyl)bisphosphonate (9)



^{1}H NMR (400 MHz, $CDCl_3$)

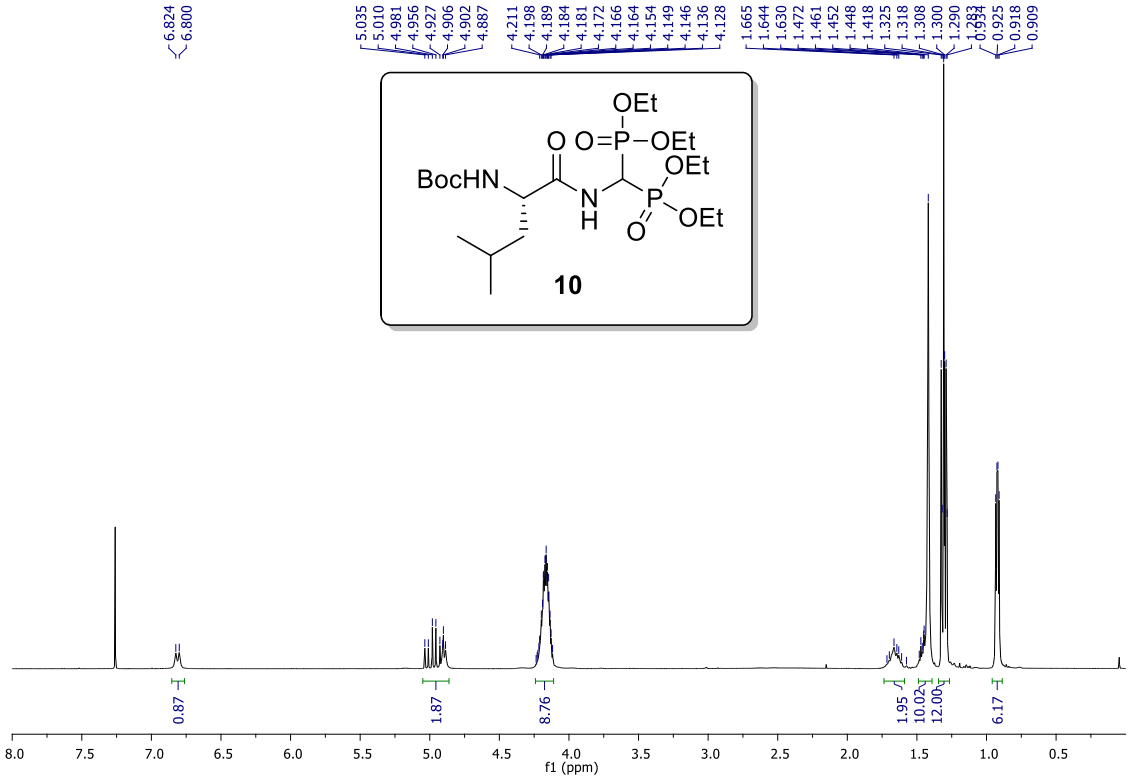


APT NMR (100 MHz, $CDCl_3$)

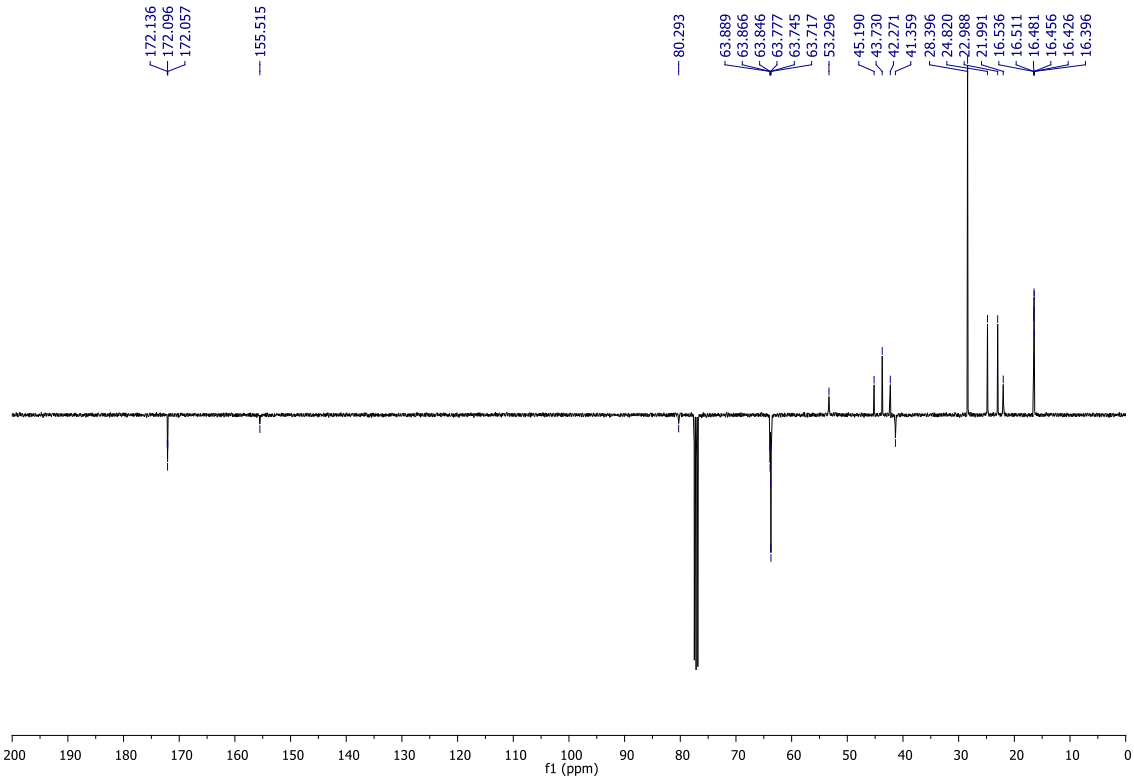


^{31}P NMR (162 MHz, $CDCl_3$)

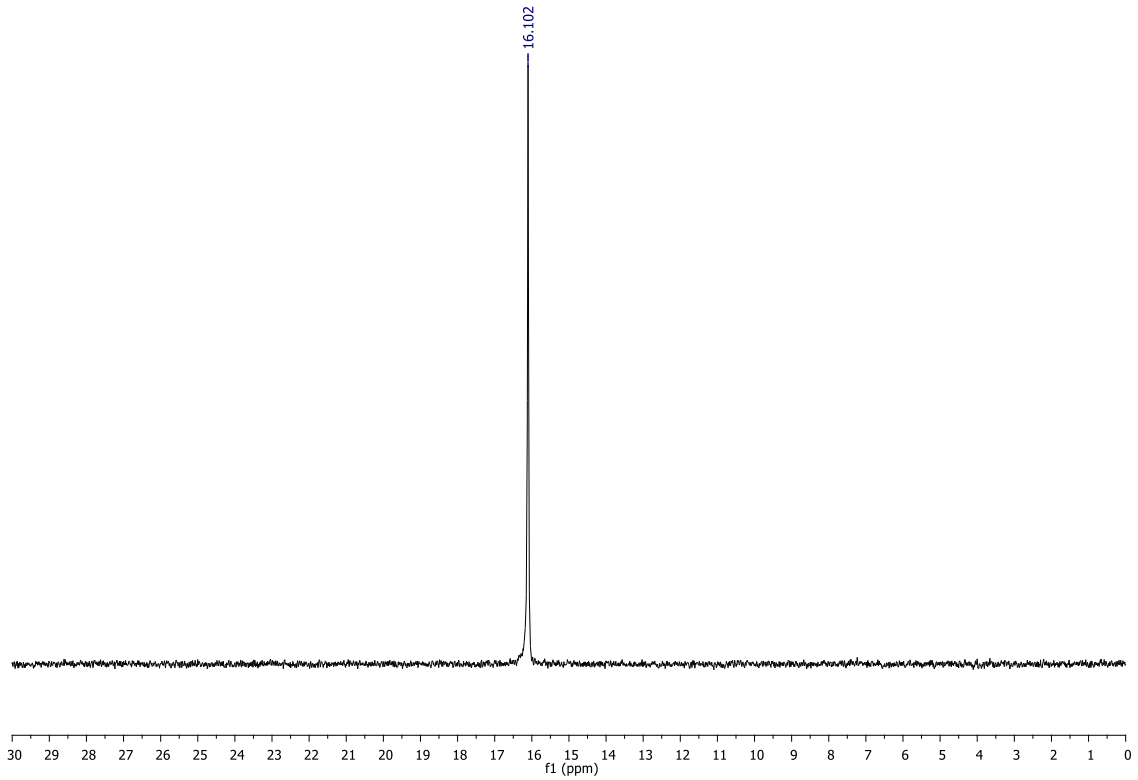
Tetraethyl [*N*-(*tert*-butoxycarbonyl)-L-leucylamidomethyl]bisphosphonate (10)



^{1}H NMR (400 MHz, $CDCl_3$)

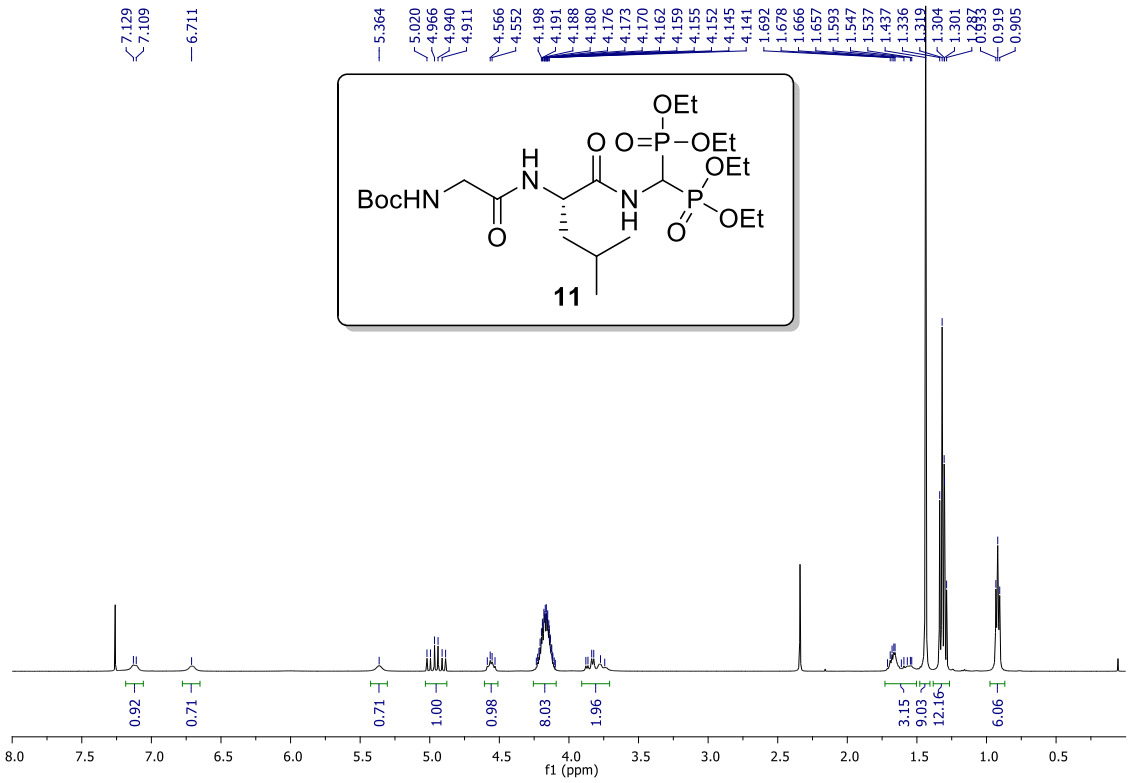


APT NMR (100 MHz, $CDCl_3$)

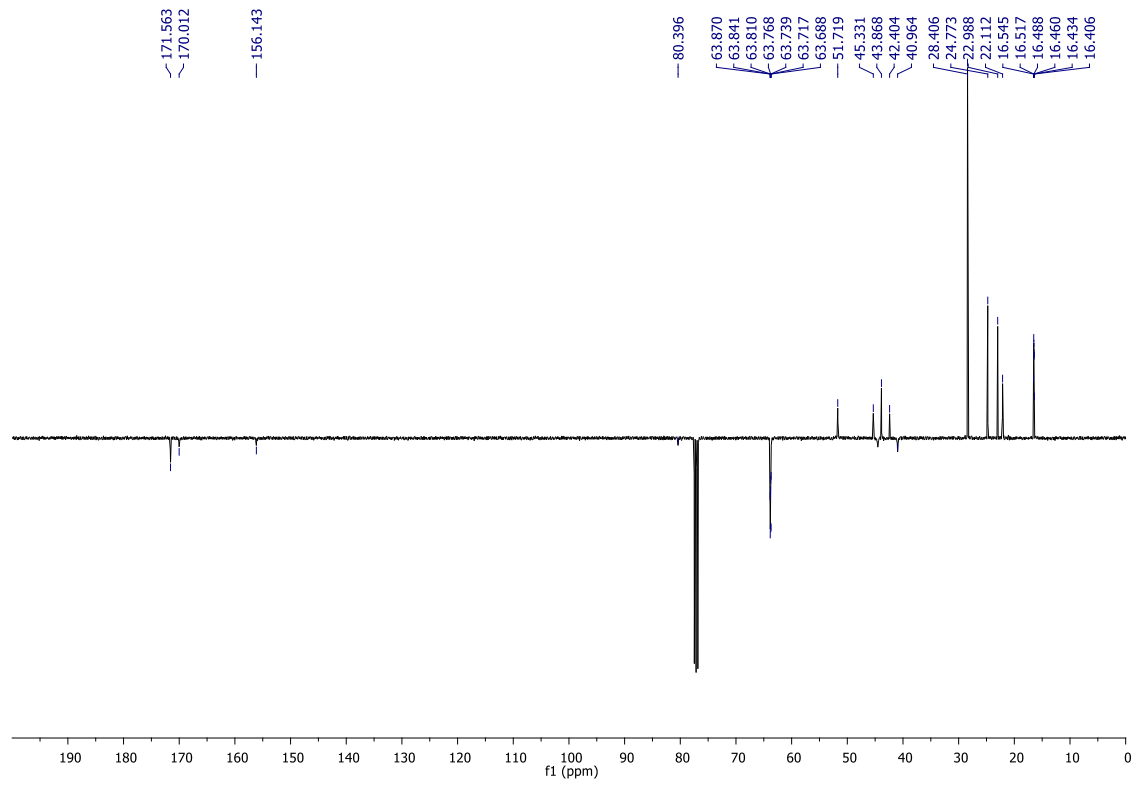


^{31}P NMR (162 MHz, $CDCl_3$)

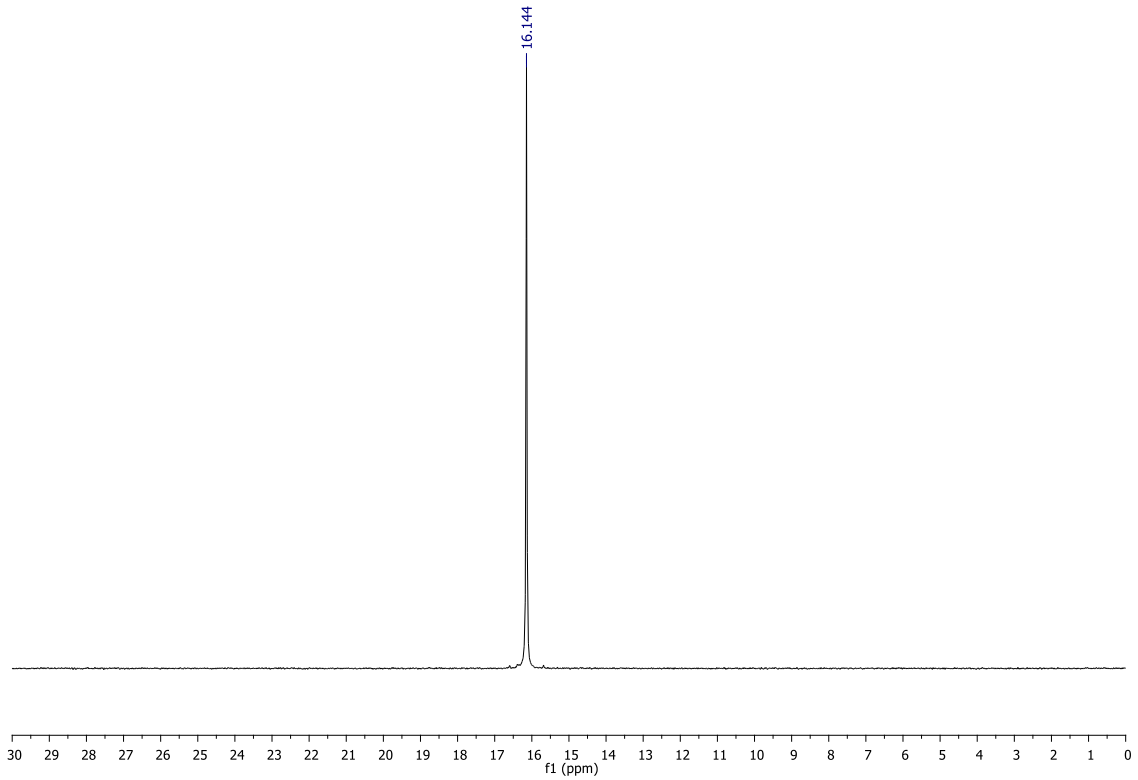
Tetraethyl [*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamidomethyl]bisphosphonate (11)



^{1}H NMR (400 MHz, $CDCl_3$)

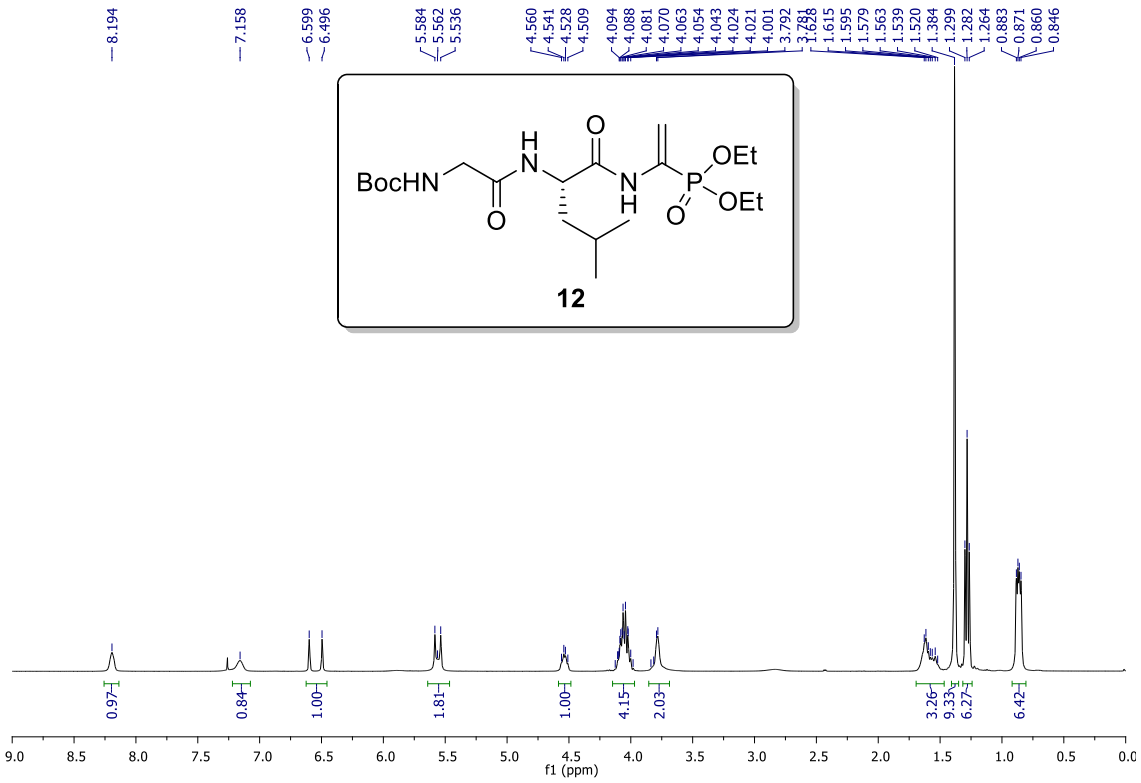


APT NMR (100 MHz, $CDCl_3$)

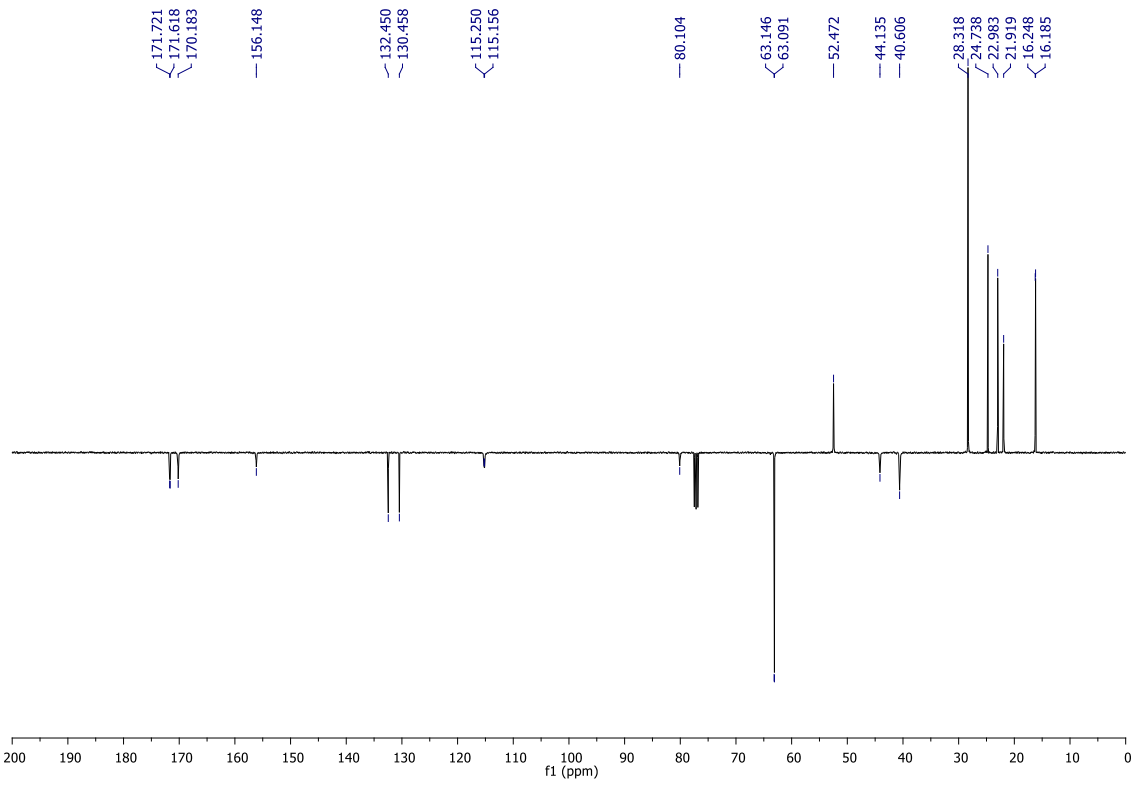


^{31}P NMR (162 MHz, $CDCl_3$)

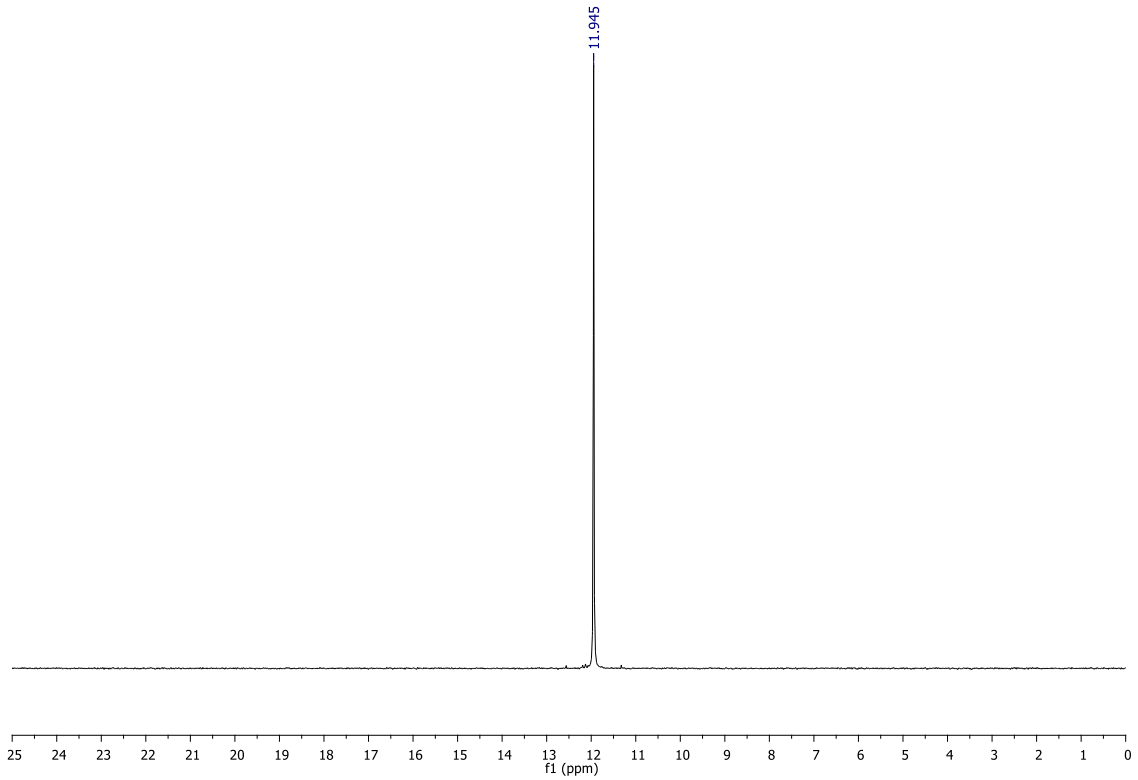
Diethyl {1-[*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamido]ethen-1-yl}phosphonate (12)



^{1}H NMR (400 MHz, $CDCl_3$)

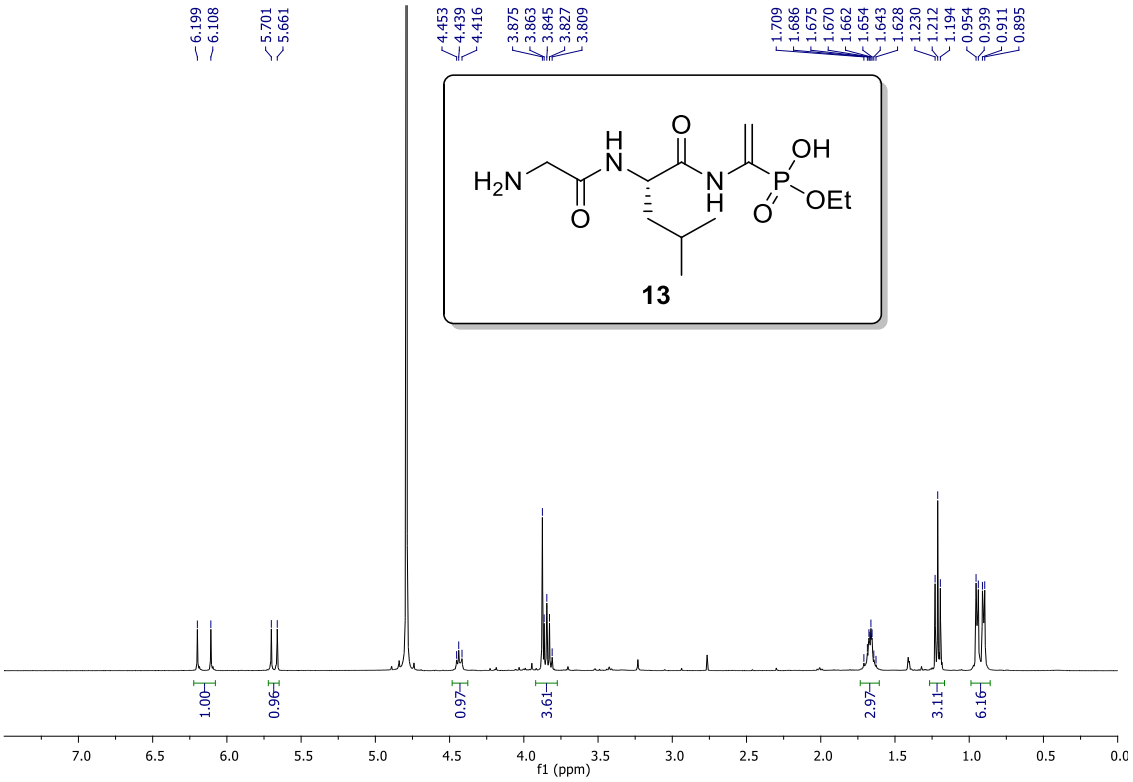


APT NMR (100 MHz, $CDCl_3$)

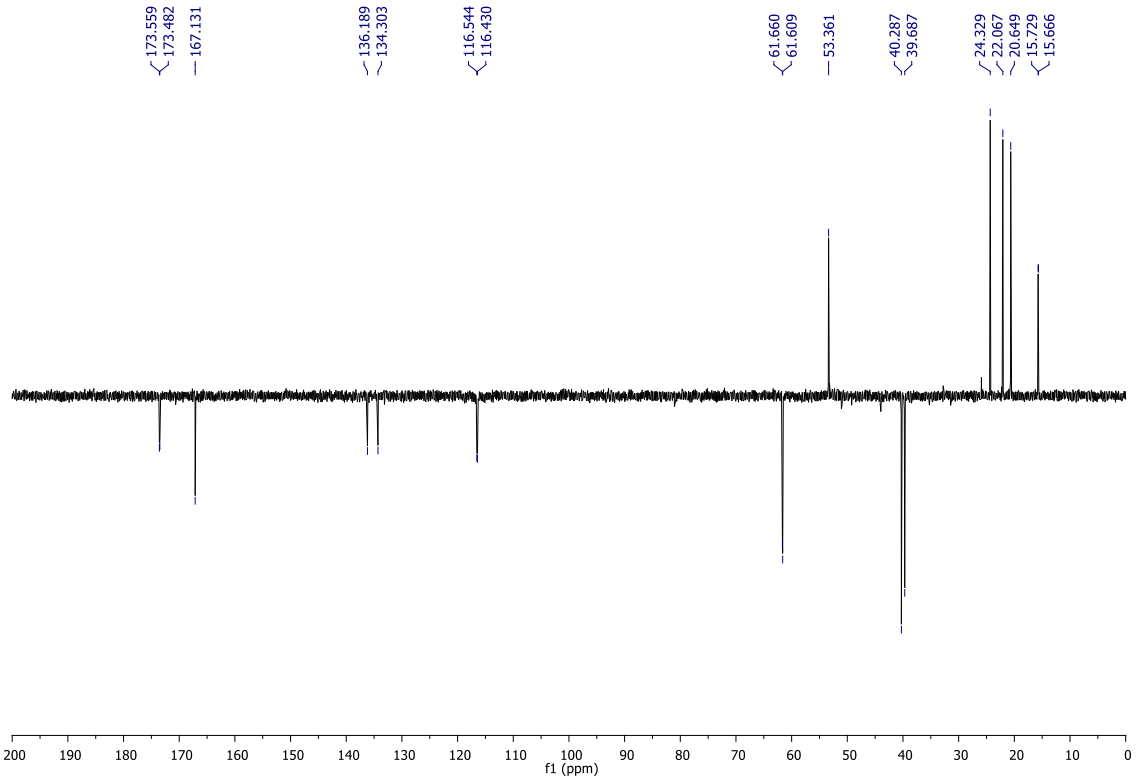


^{31}P NMR (162 MHz, $CDCl_3$)

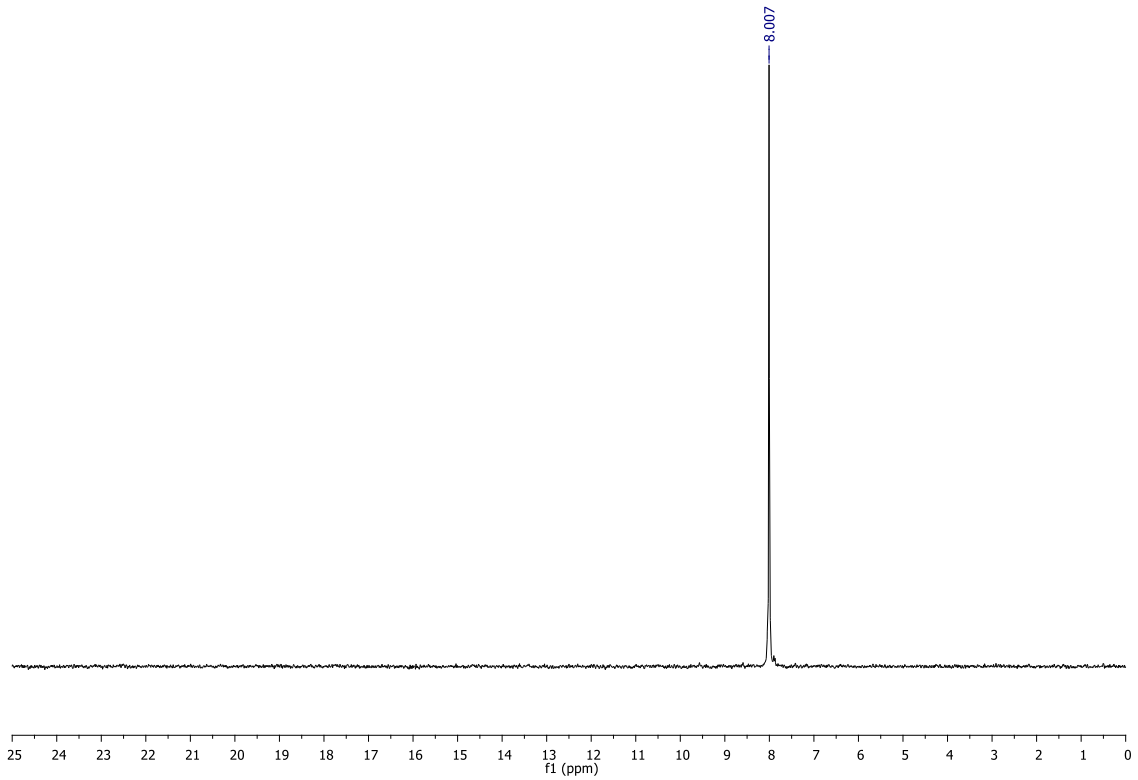
[1-(glycyl-L-leucylamido)ethen-1-yl]phosphonic acid monoethyl ester (13)



^{1}H NMR (400 MHz, D_2O)

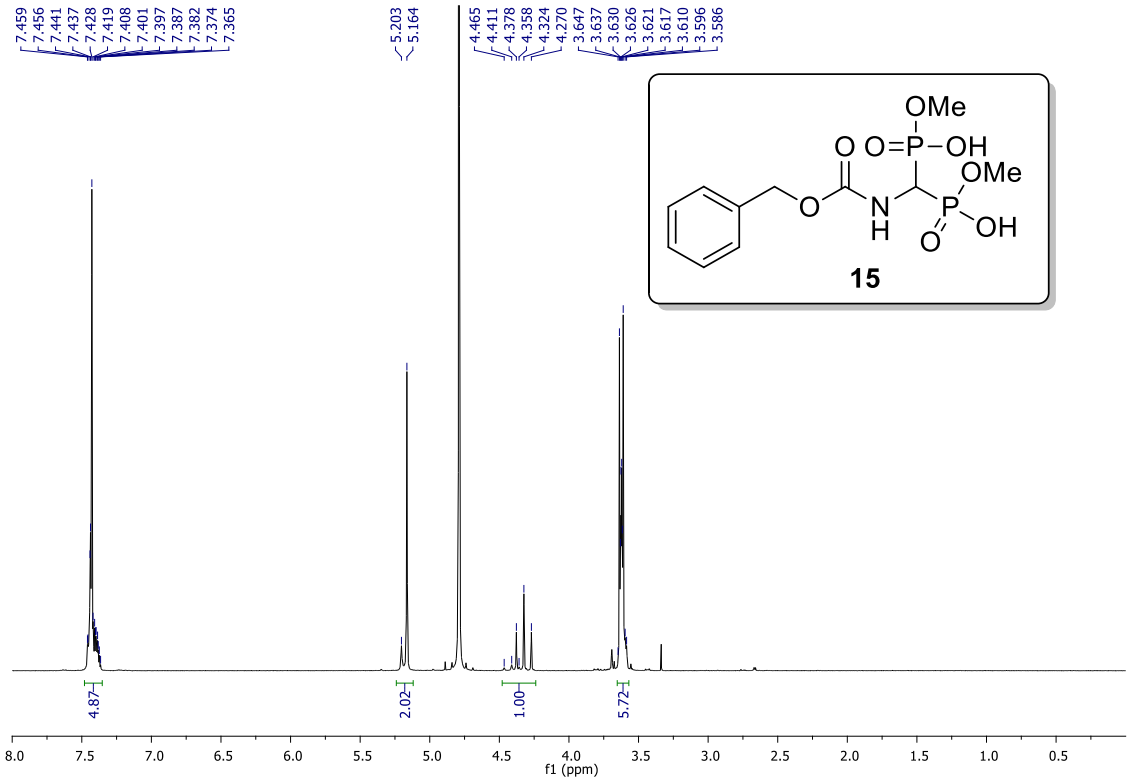


APT NMR (100 MHz, D_2O)

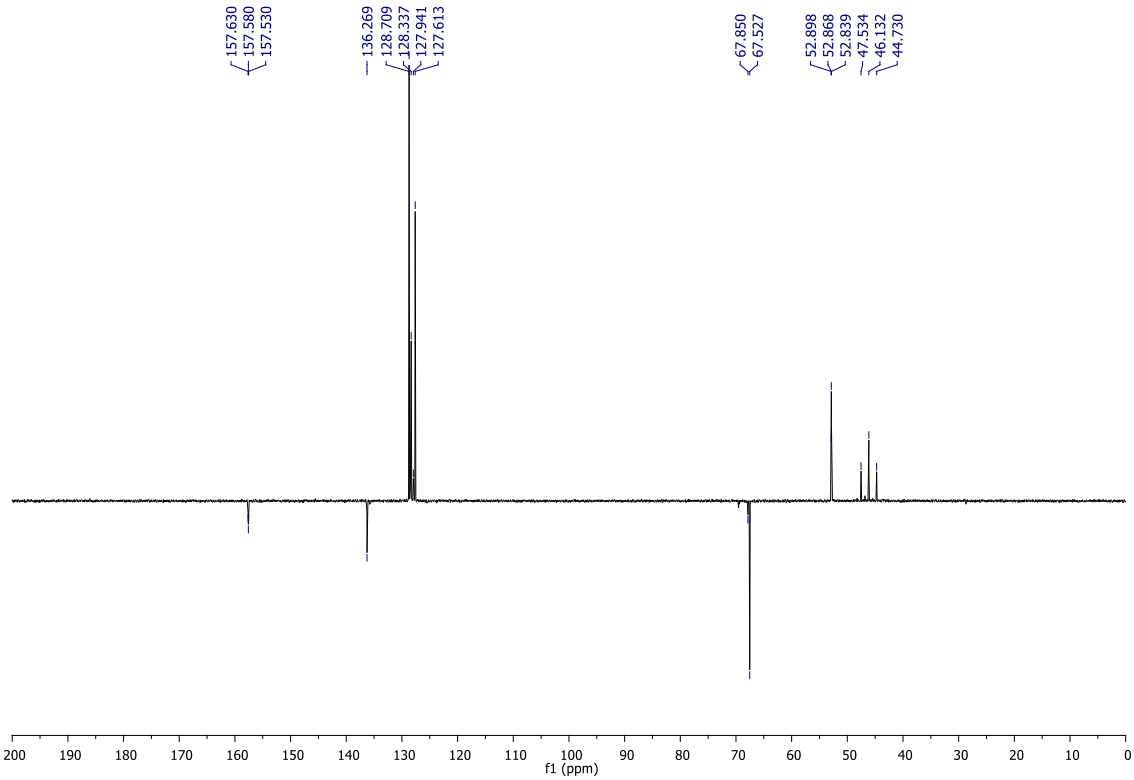


^{31}P NMR (162 MHz, D_2O)

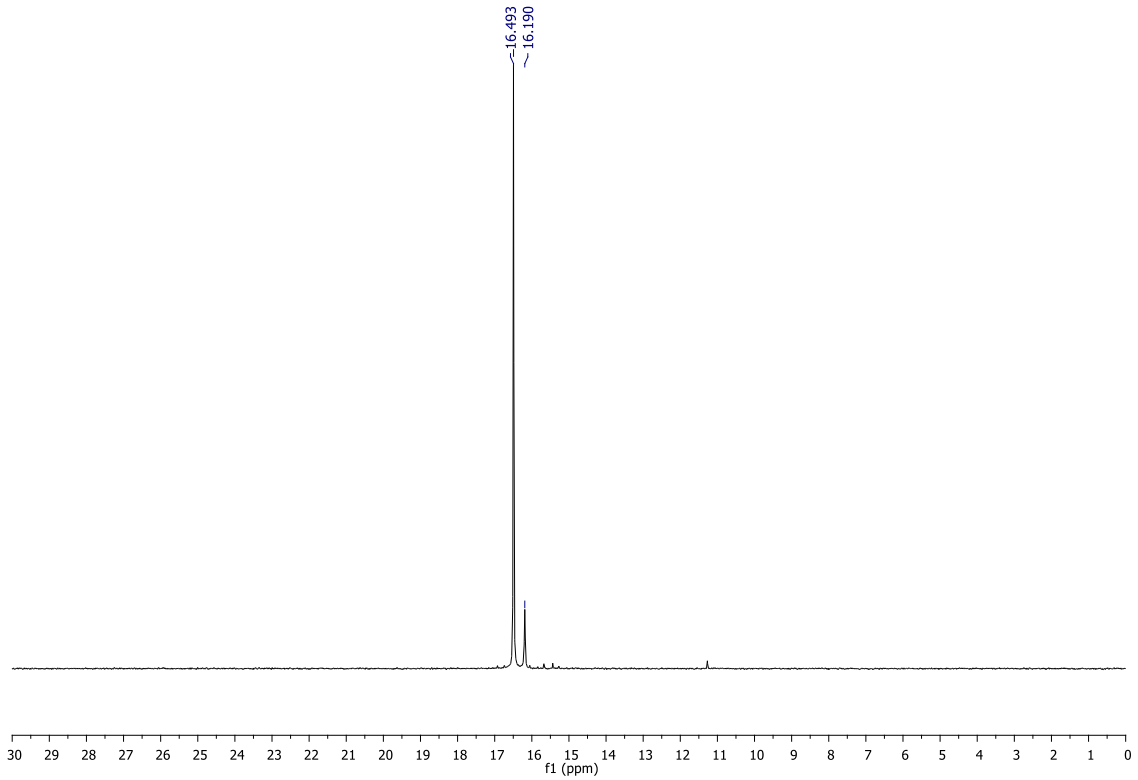
(Benzyloxycarbonylaminomethyl)bisphosphonic acid monomethyl ester (15)



^{1}H NMR (400 MHz, D_2O)

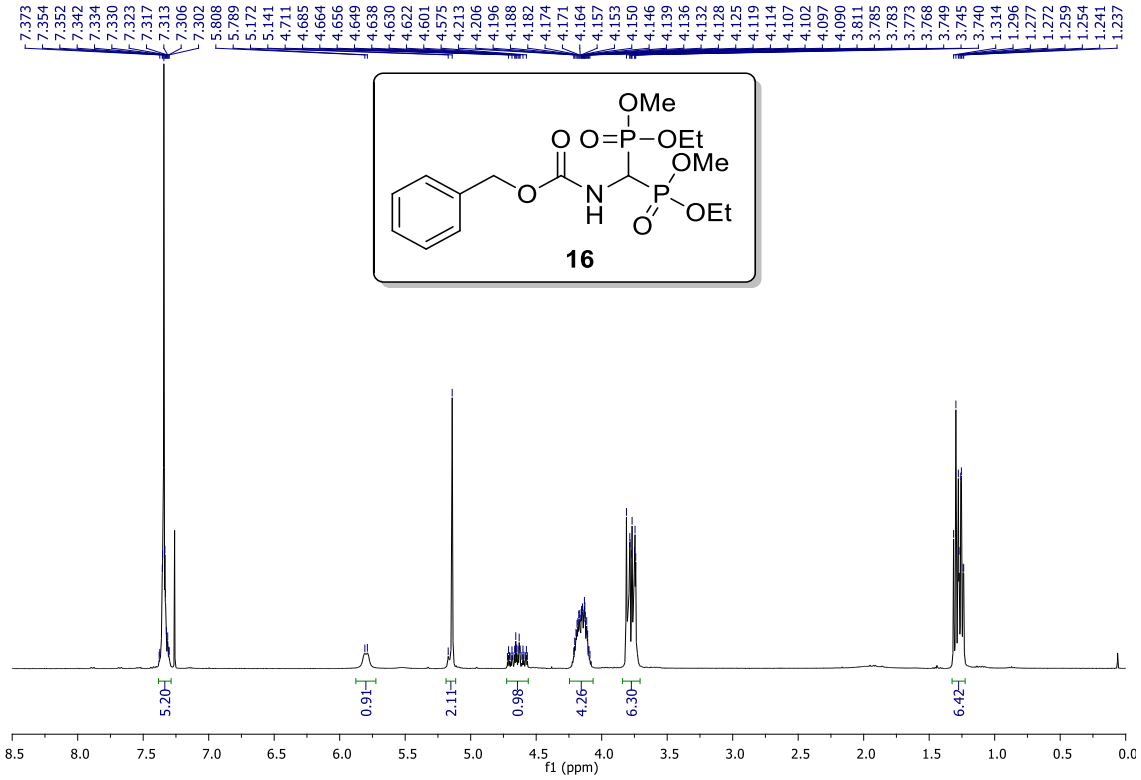


APT NMR (100 MHz, D_2O)

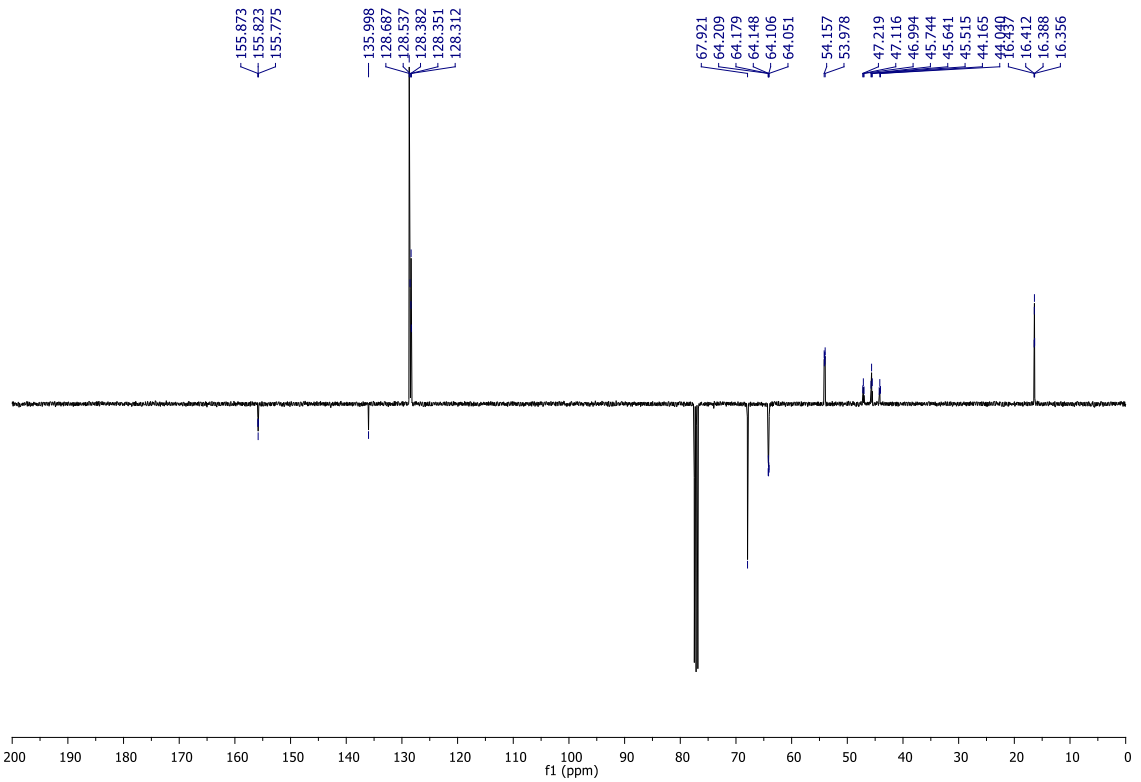


^{31}P NMR (162 MHz, D_2O)

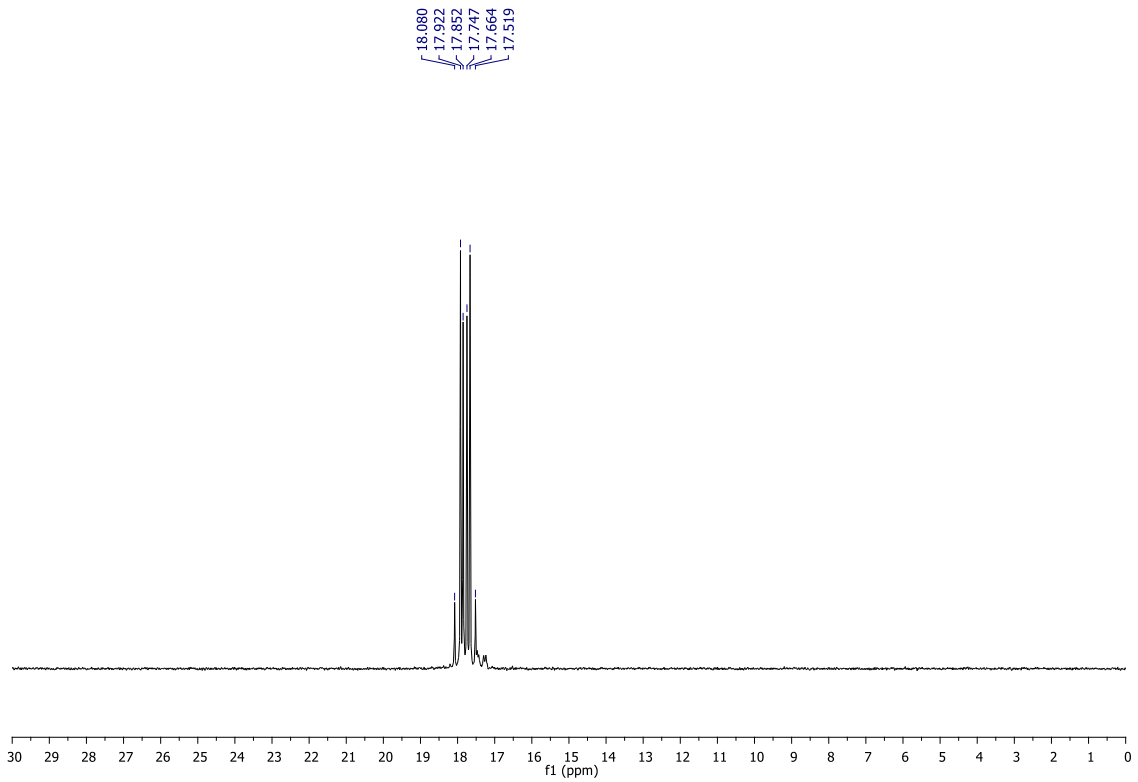
Diethyl dimethyl (benzyloxycarbonylaminomethyl)bisphosphonate (16)



^{1}H NMR (400 MHz, $CDCl_3$)

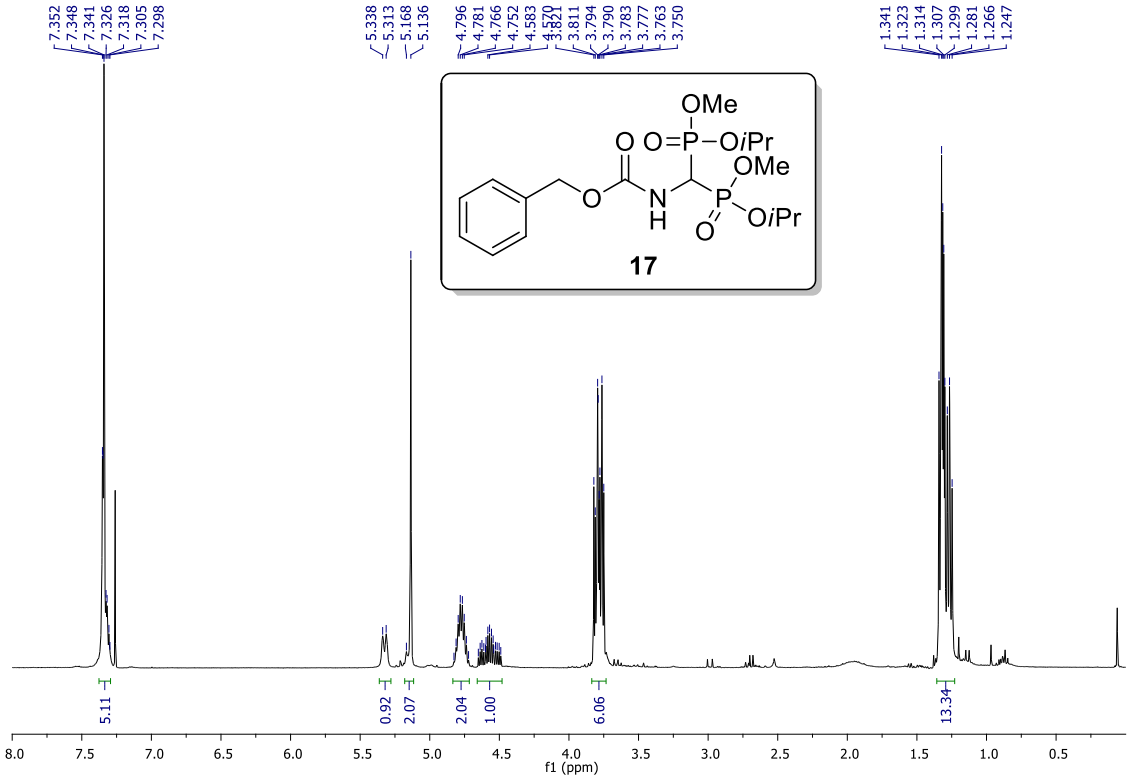


APT NMR (100 MHz, $CDCl_3$)

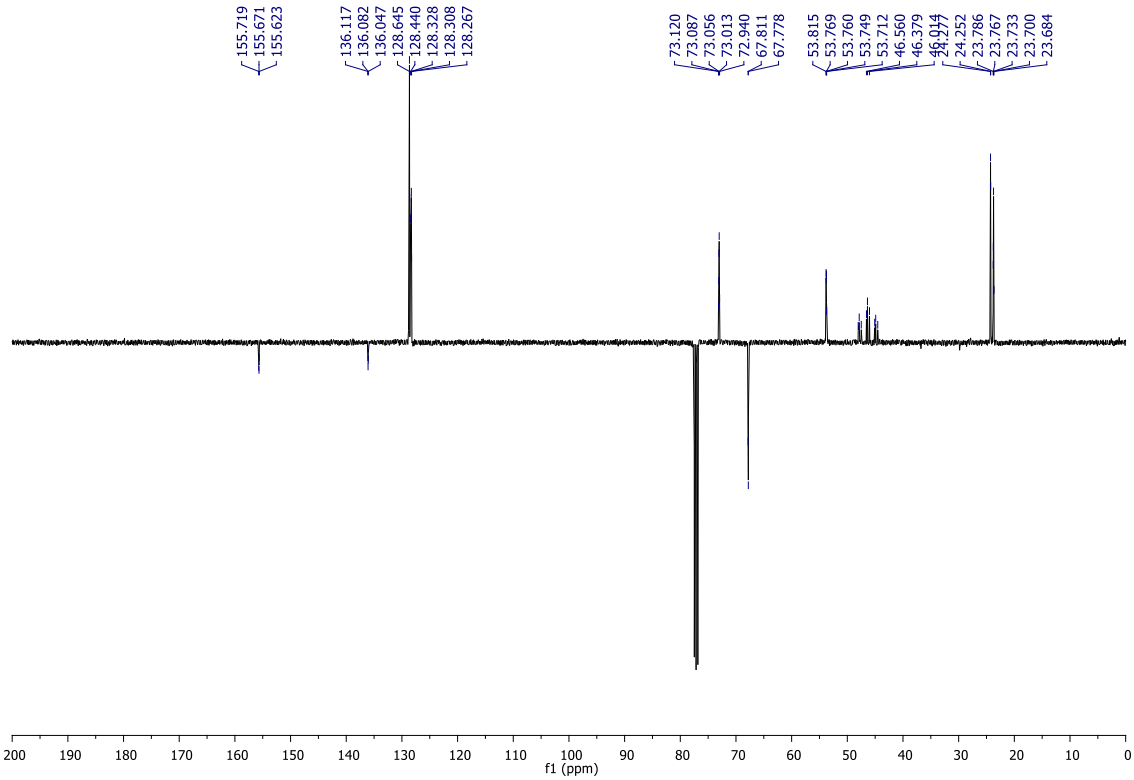


^{31}P NMR (162 MHz, $CDCl_3$)

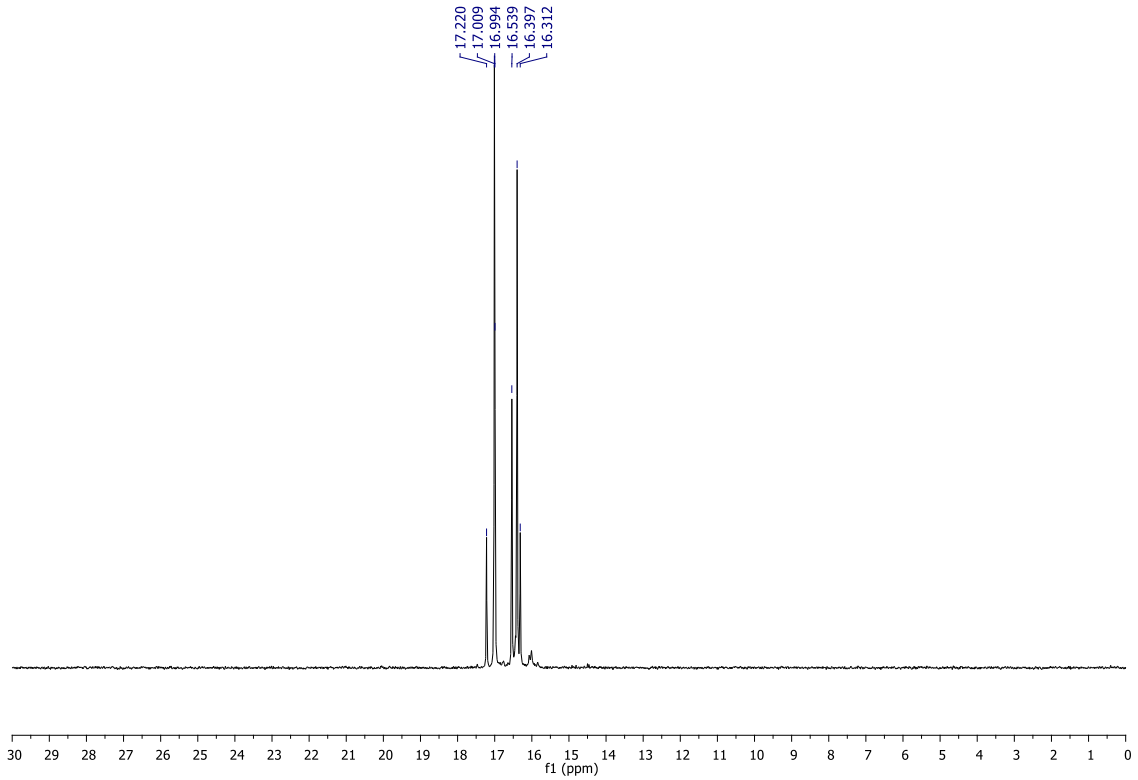
Dimethyl diprop-2-yl (benzyloxycarbonylaminomethyl)bisphosphonate (17)



^{1}H NMR (400 MHz, $CDCl_3$)

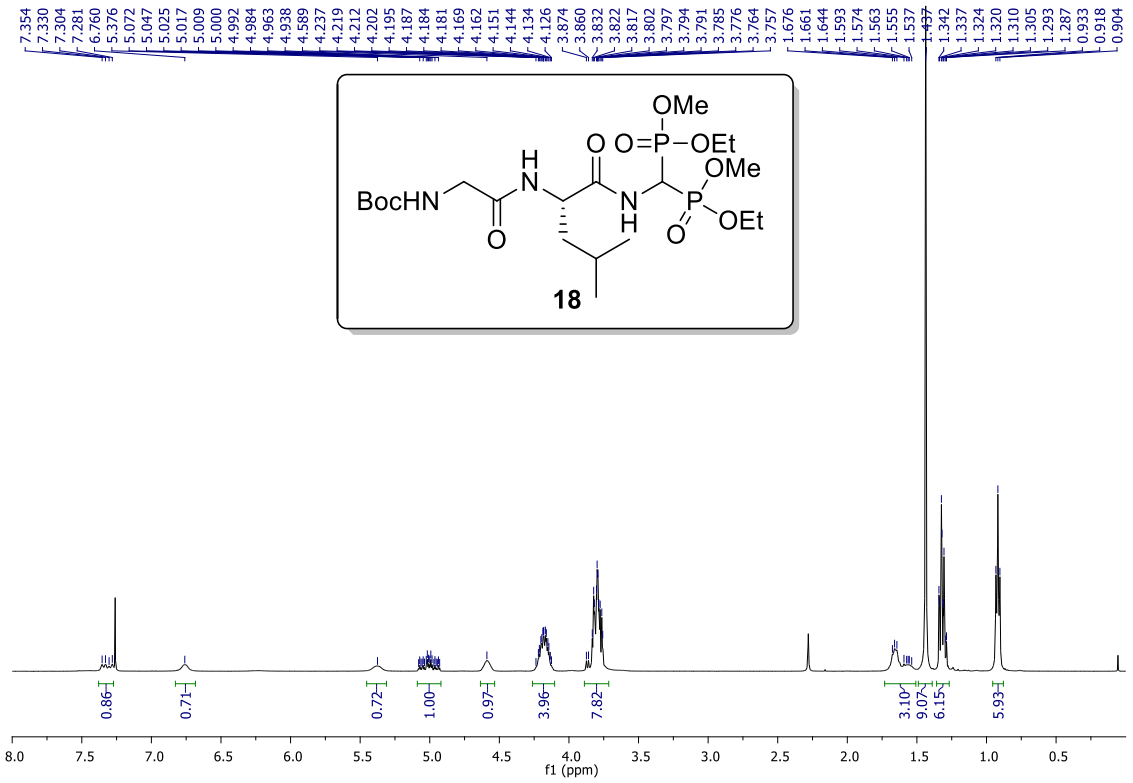


APT NMR (100 MHz, $CDCl_3$)

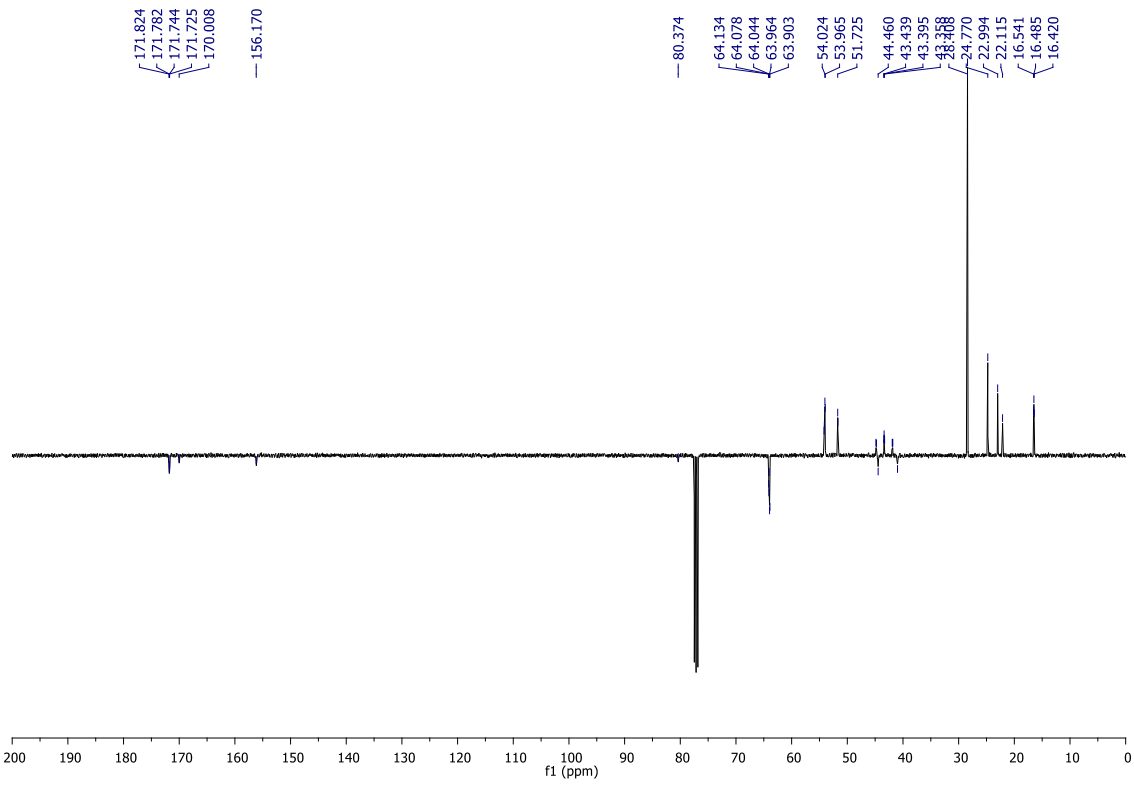


^{31}P NMR (162 MHz, $CDCl_3$)

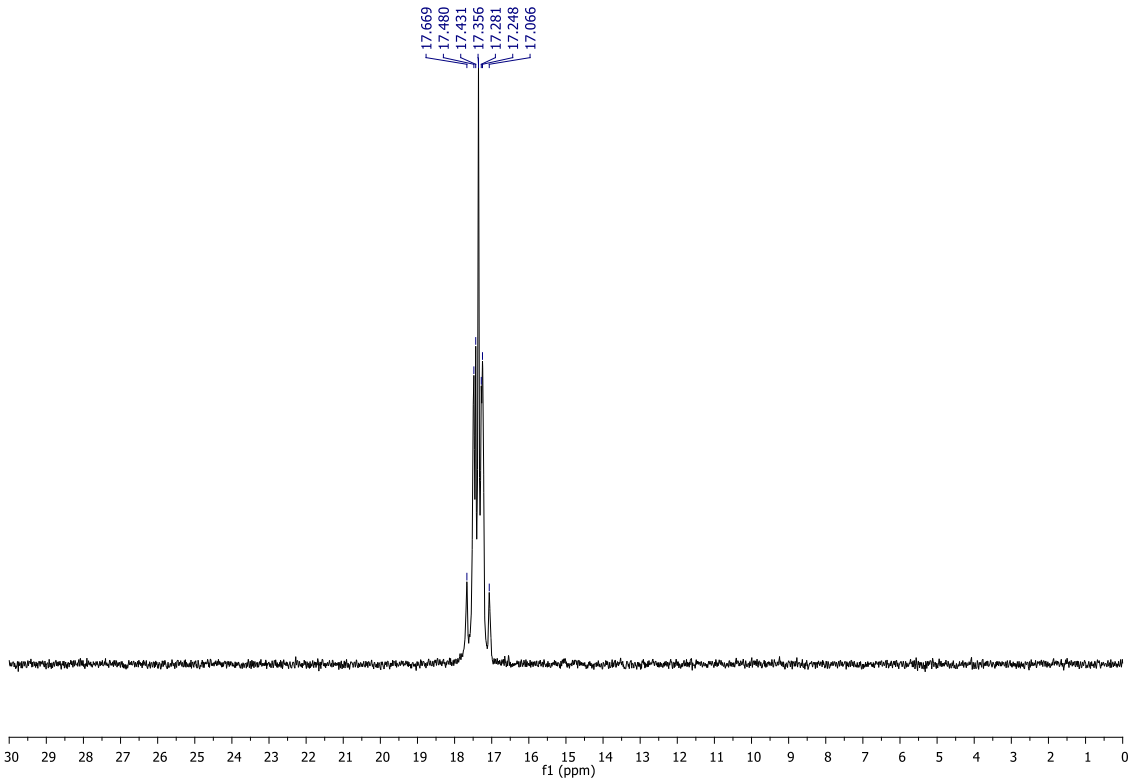
Diethyl dimethyl [*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamidomethyl]bisphosphonate (18)



^{1}H NMR (400 MHz, $CDCl_3$)

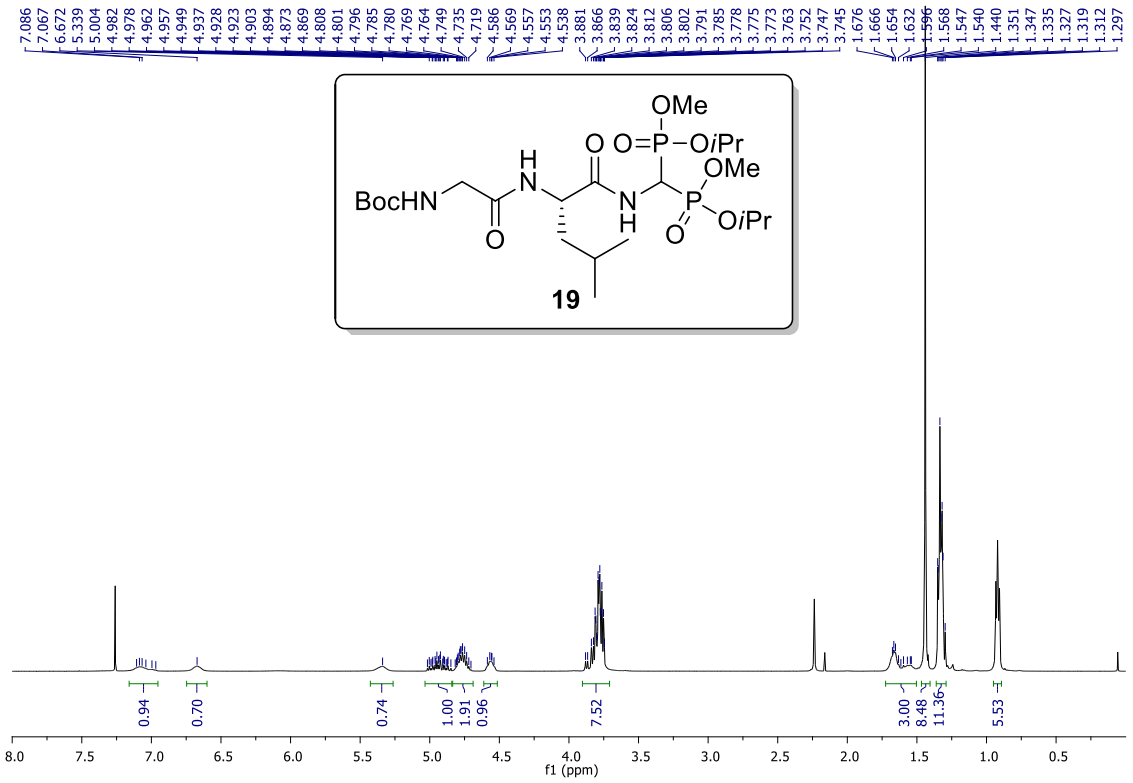


APT NMR (100 MHz, $CDCl_3$)

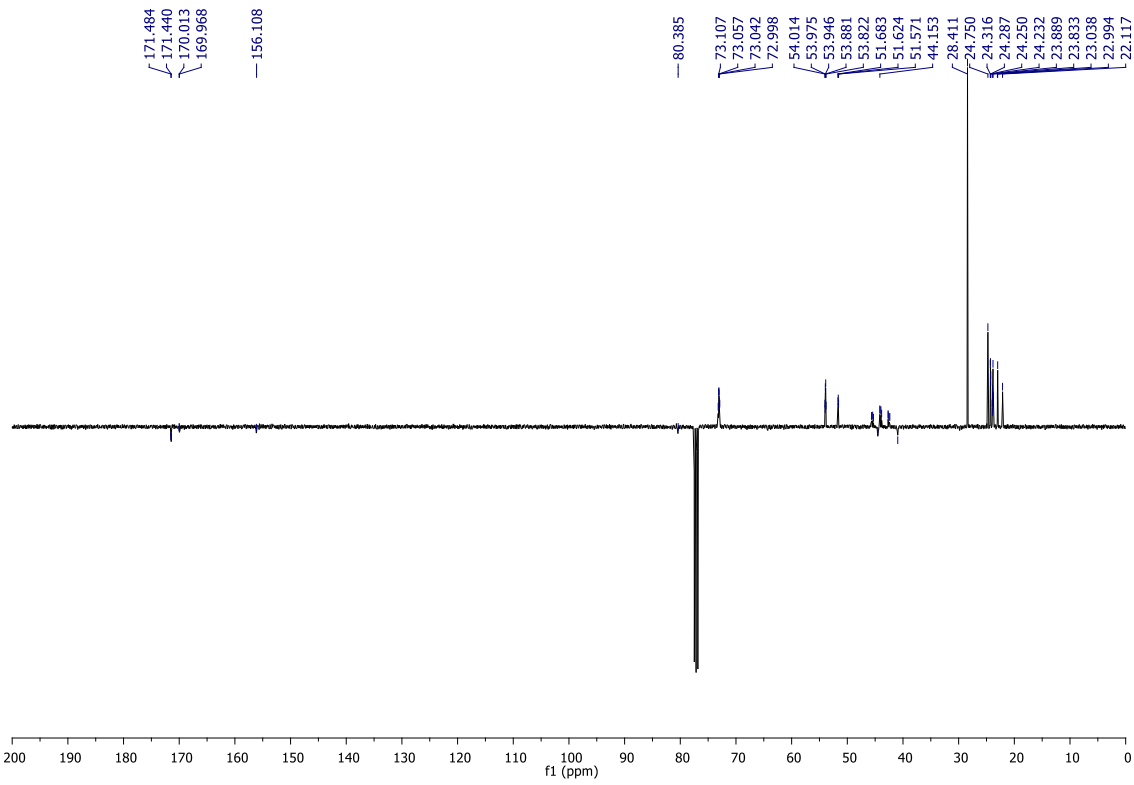


^{31}P NMR (162 MHz, $CDCl_3$)

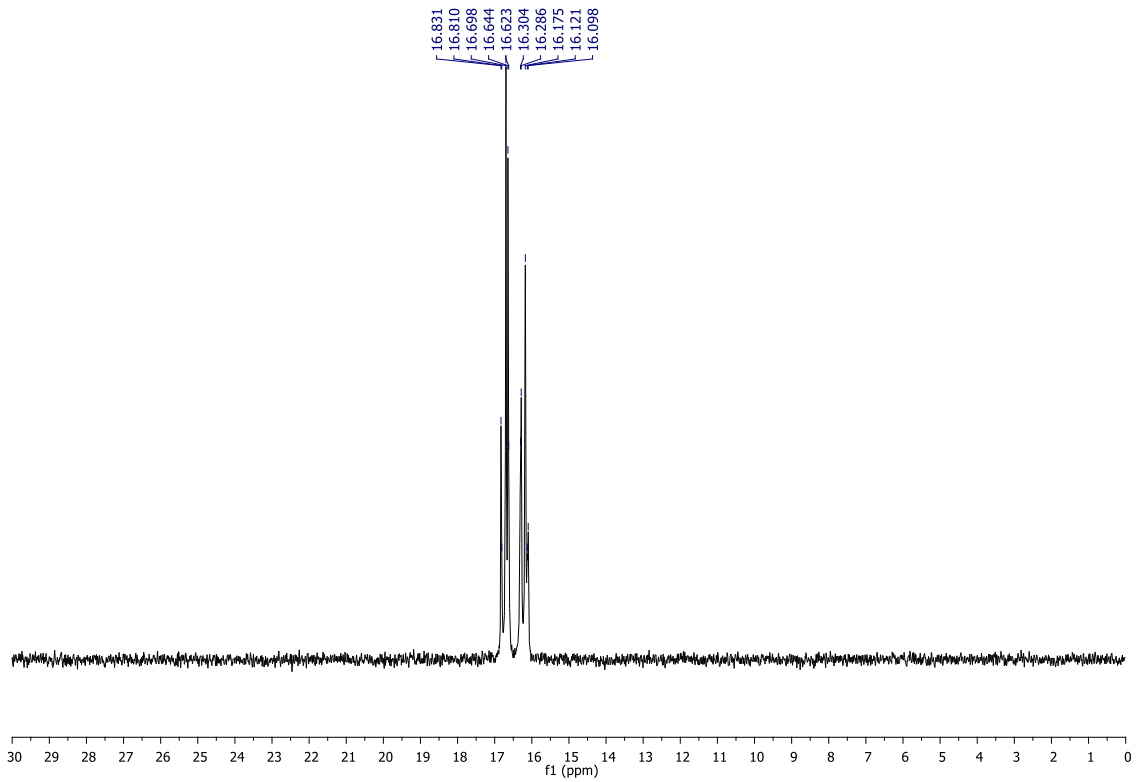
Dimethyl diprop-2-yl [*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamidomethyl]bisphosphonate (19)



^{1}H NMR (400 MHz, $CDCl_3$)

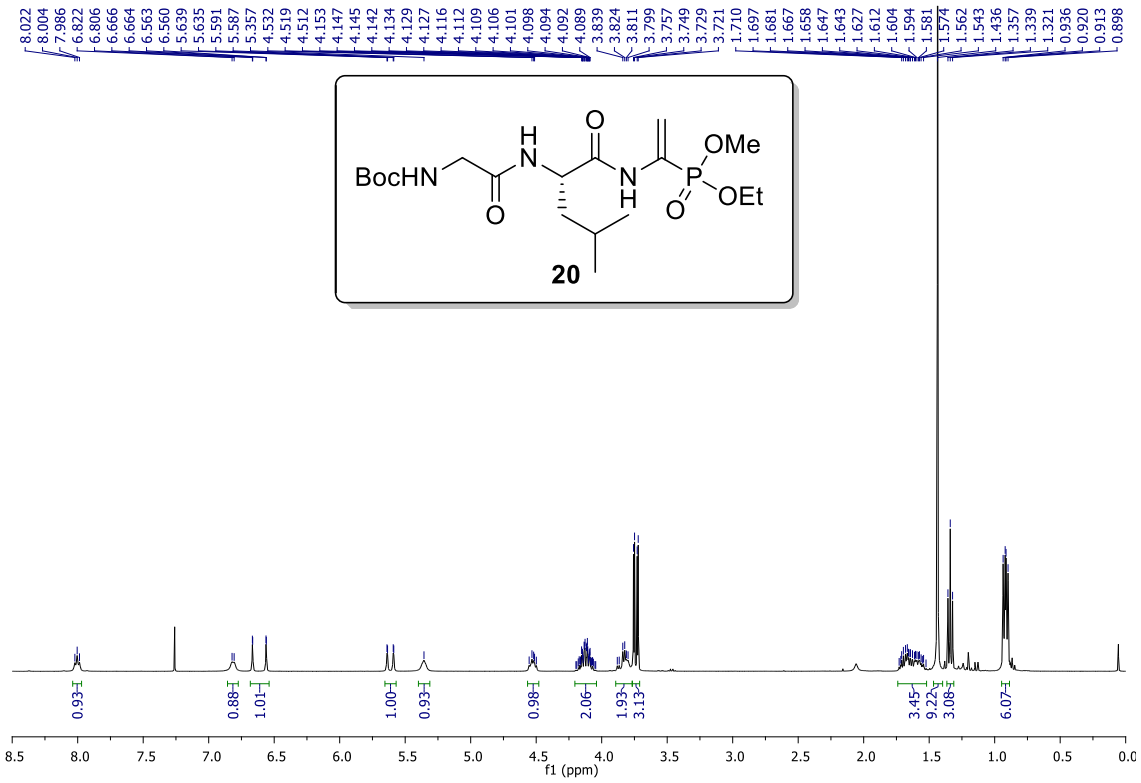


APT NMR (100 MHz, $CDCl_3$)

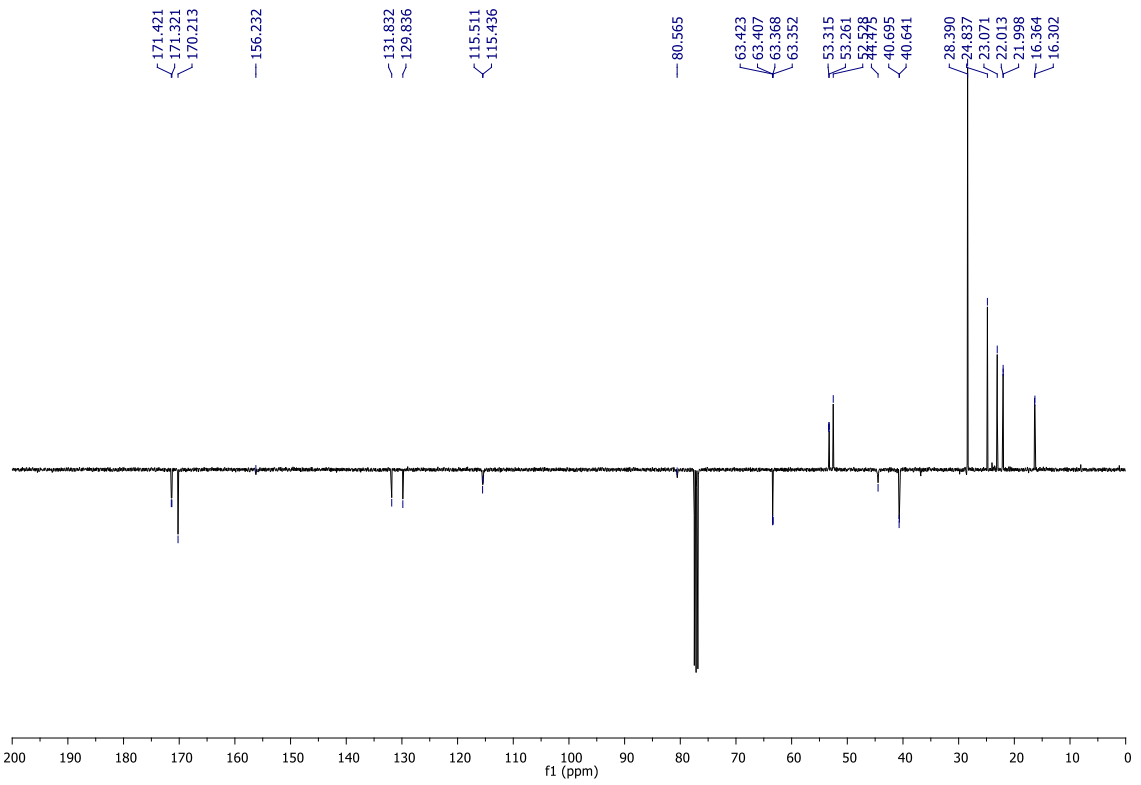


^{31}P NMR (162 MHz, $CDCl_3$)

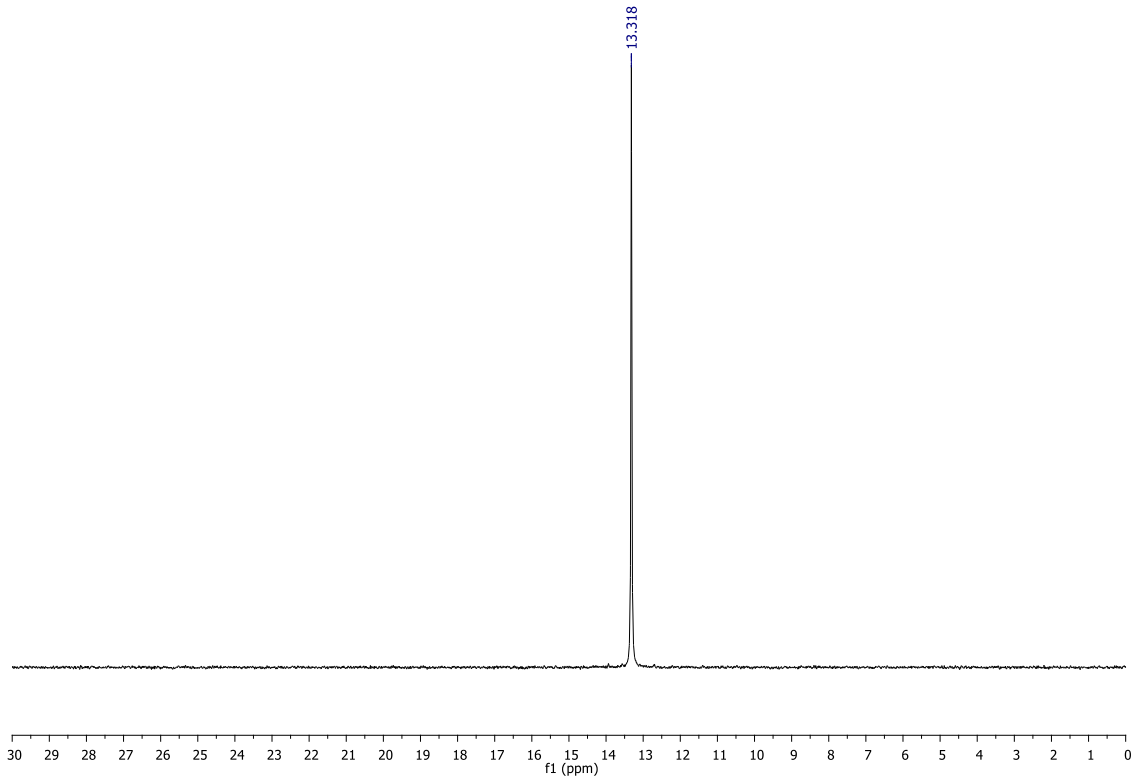
Ethyl methyl {1-[*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamido]ethen-1-yl}phosphonate (20)



^{1}H NMR (400 MHz, $CDCl_3$)

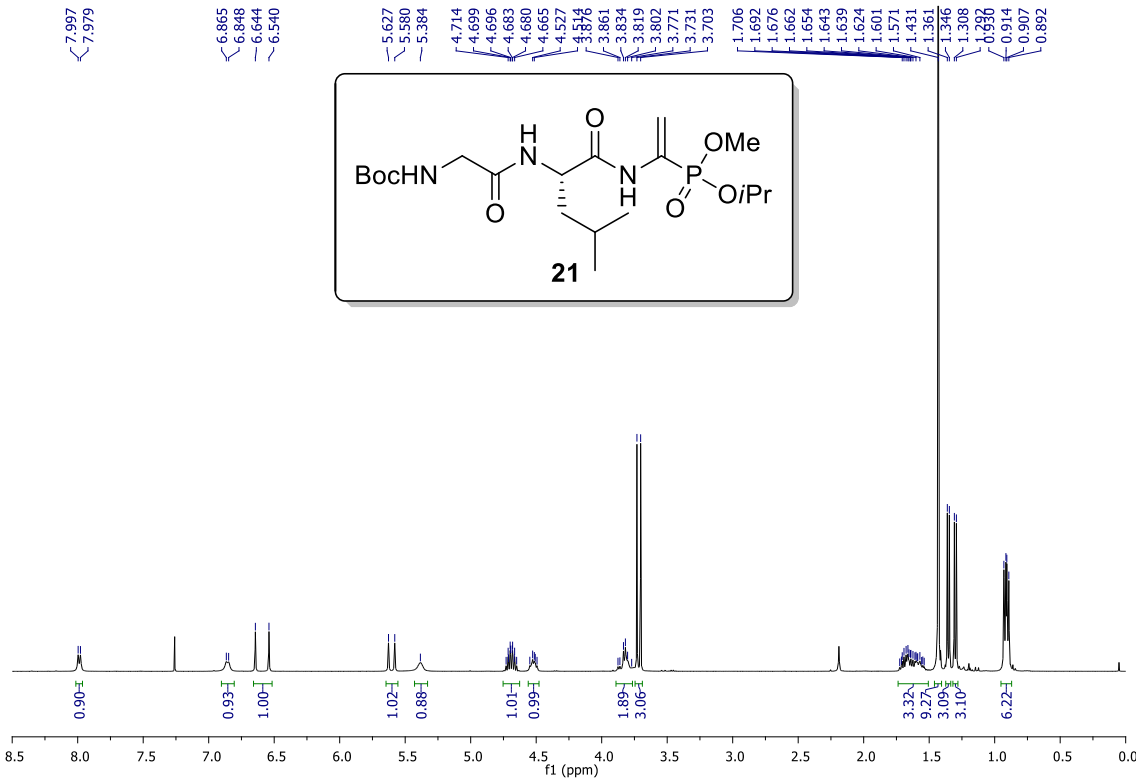


APT NMR (100 MHz, $CDCl_3$)

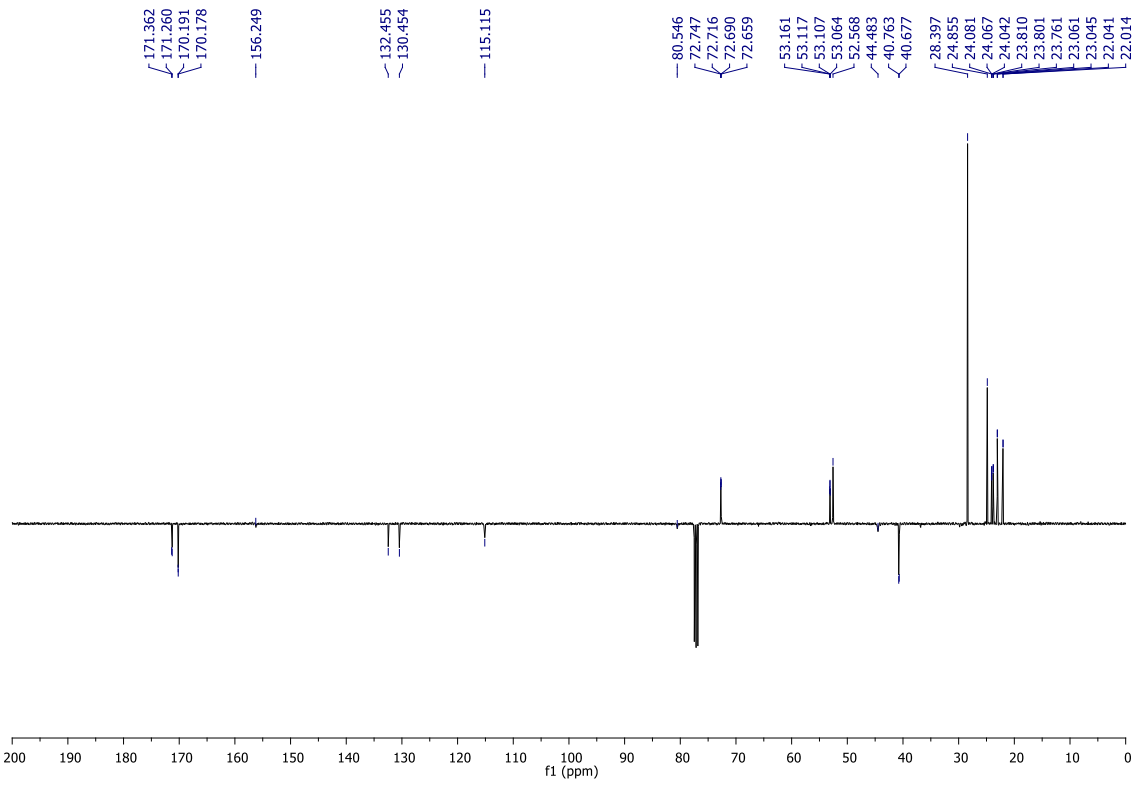


^{31}P NMR (162 MHz, $CDCl_3$)

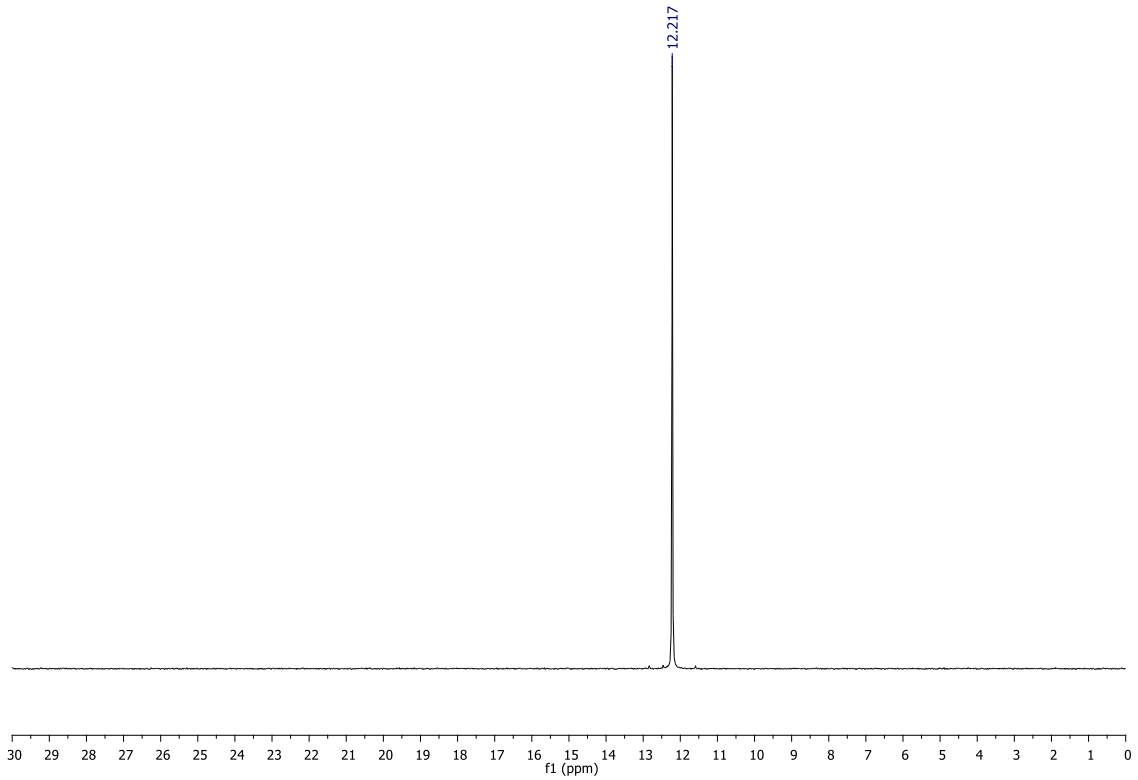
Methyl prop-2-yl {1-[*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamido]ethen-1-yl}phosphonate (21)



^{1}H NMR (400 MHz, $CDCl_3$)

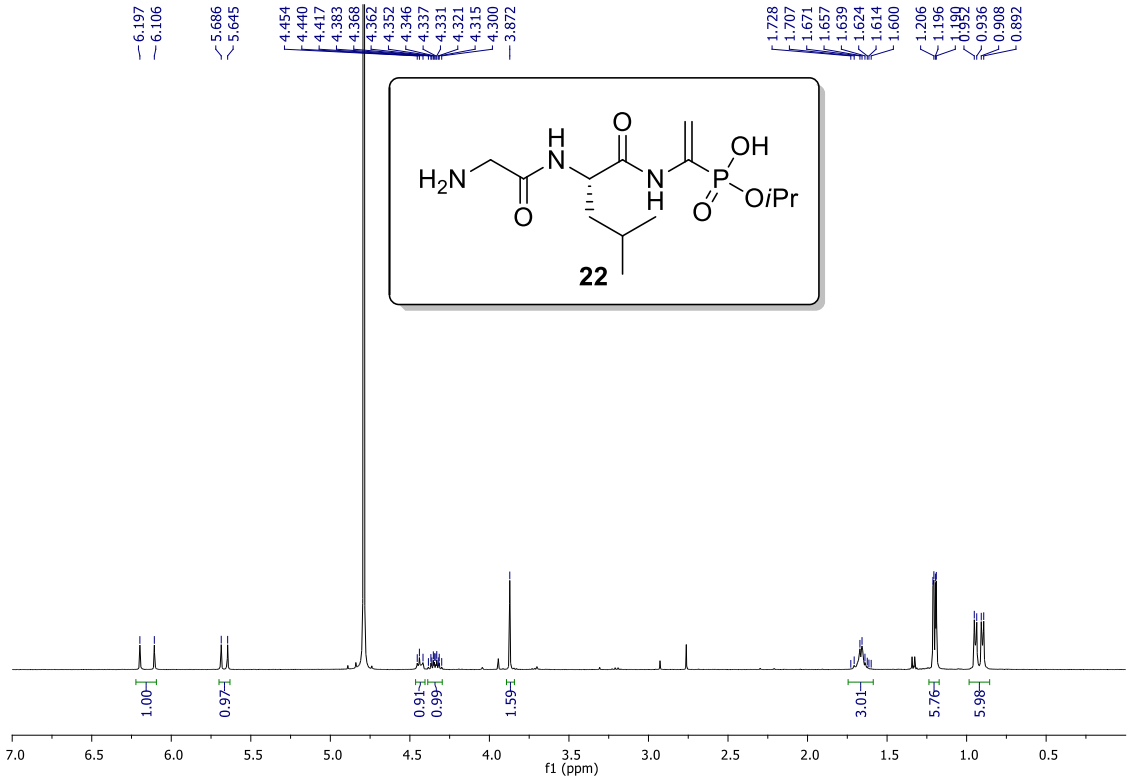


APT NMR (100 MHz, $CDCl_3$)

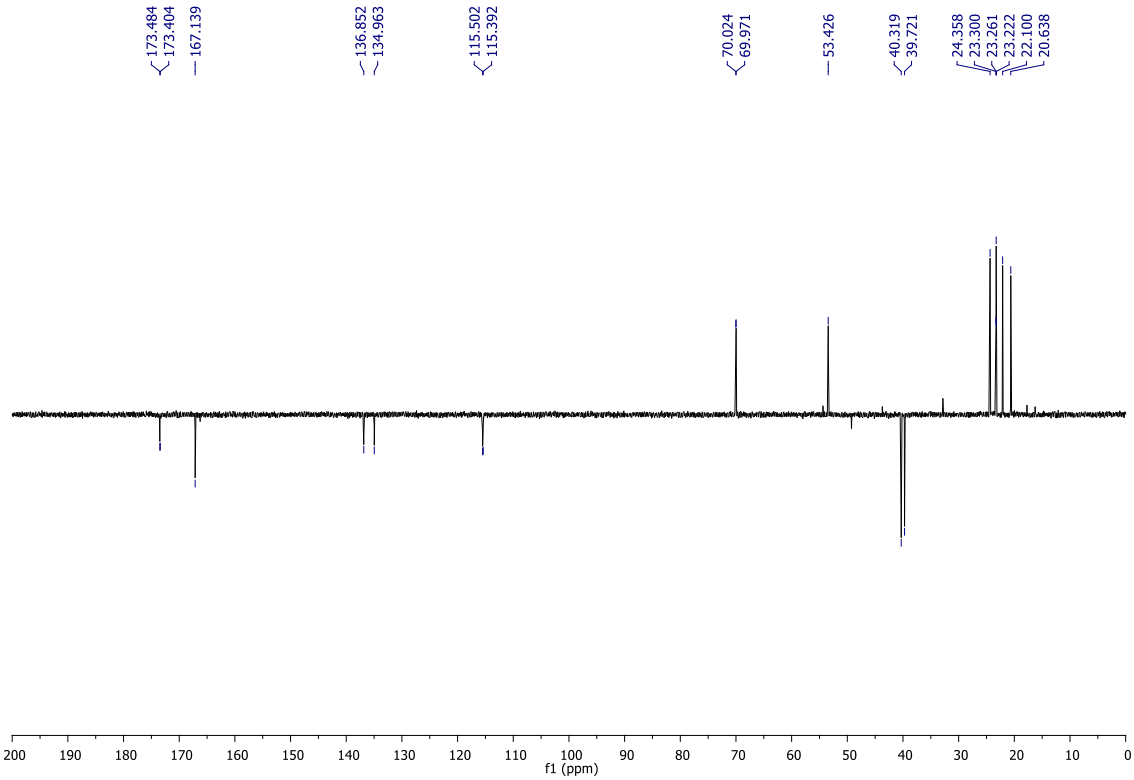


^{31}P NMR (162 MHz, $CDCl_3$)

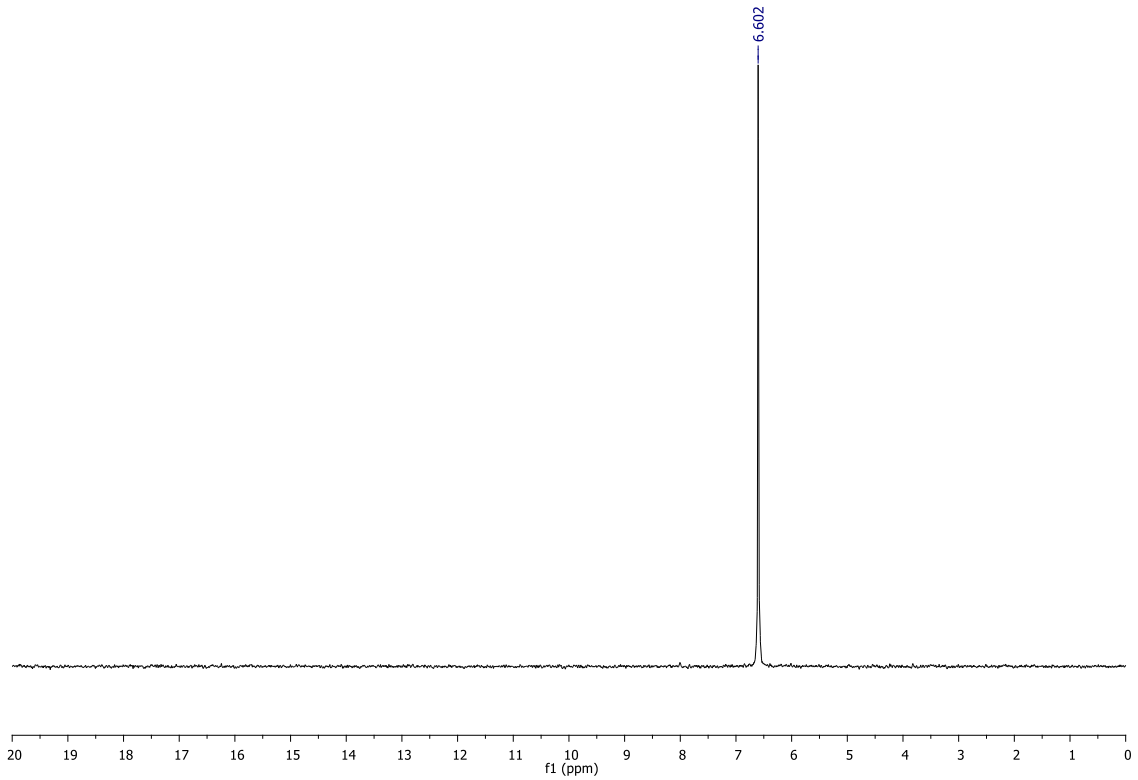
[1-(glycyl-L-leucylamido)ethen-1-yl]phosphonic acid monoprop-2-yl ester (22)



^{1}H NMR (400 MHz, D_2O)

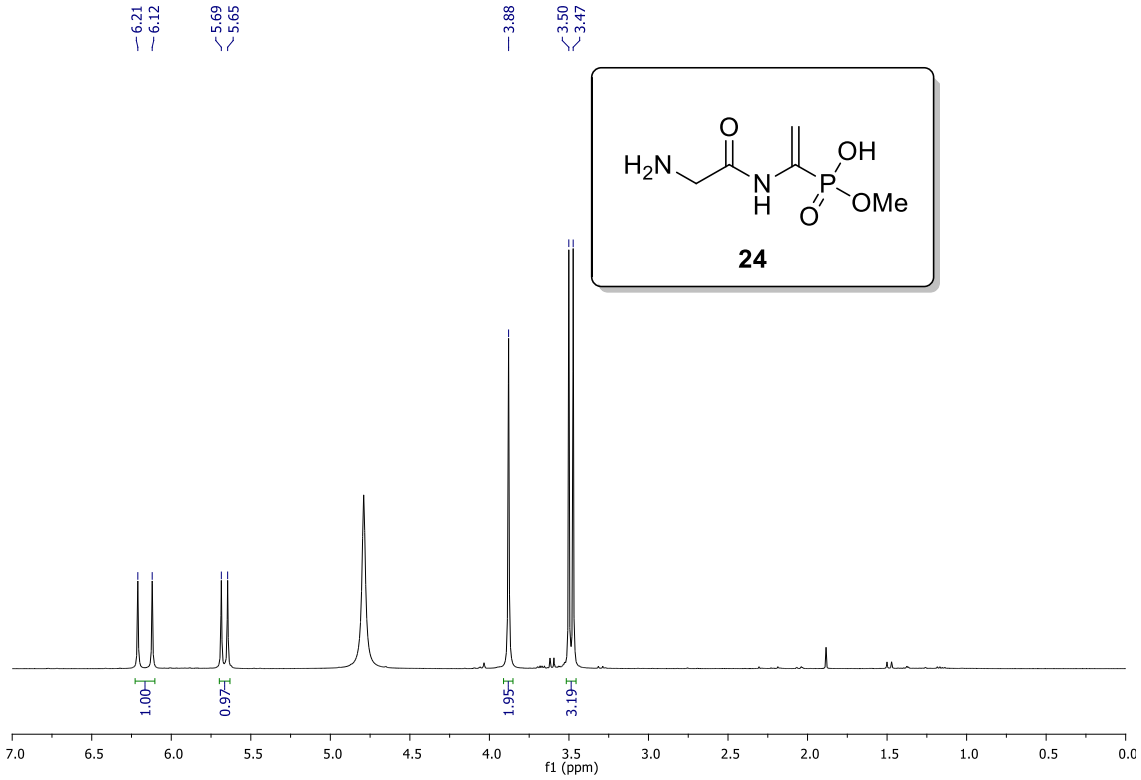


APT NMR (100 MHz, D_2O)

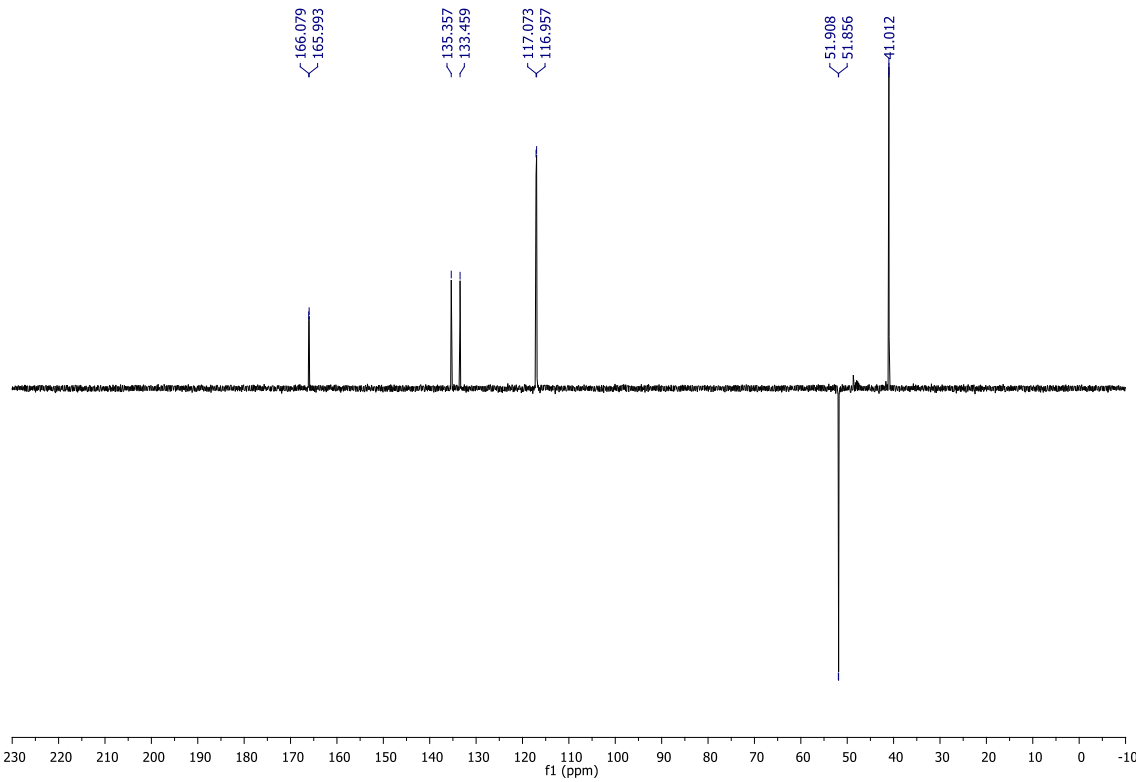


^{31}P NMR (162 MHz, D_2O)

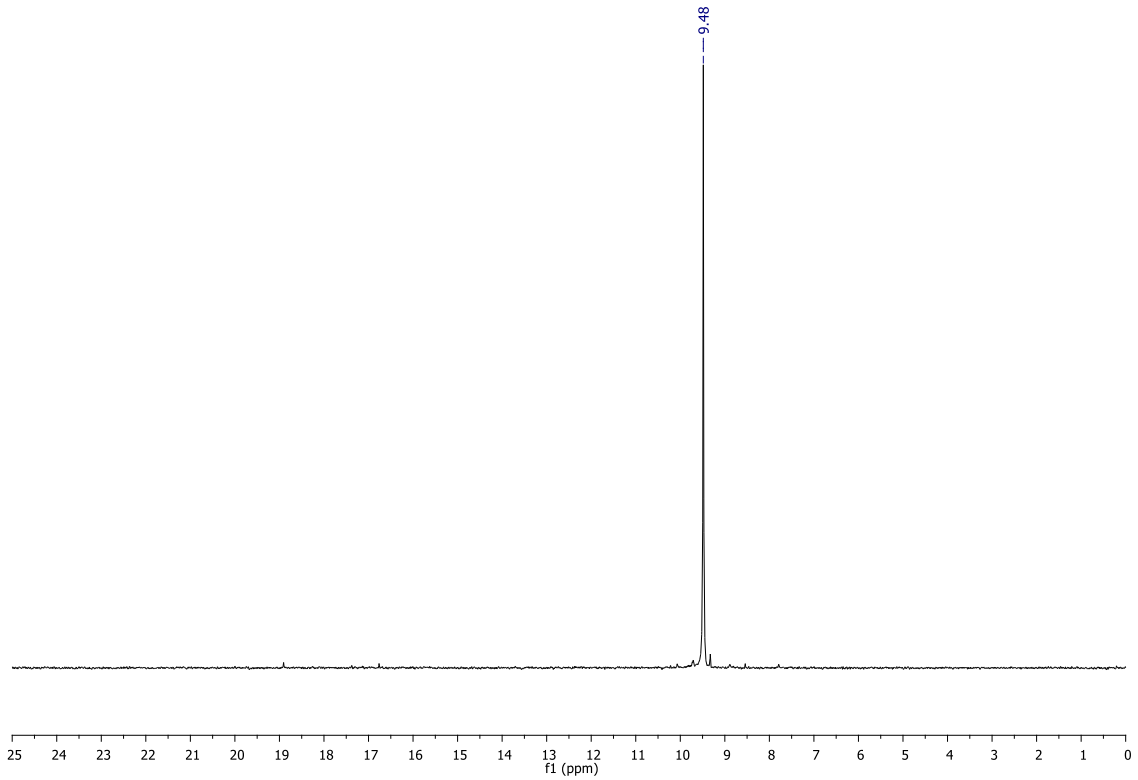
[1-(glycylamido)ethen-1-yl]phosphonic acid monomethyl ester (24)



^{1}H NMR (400 MHz, D_2O)

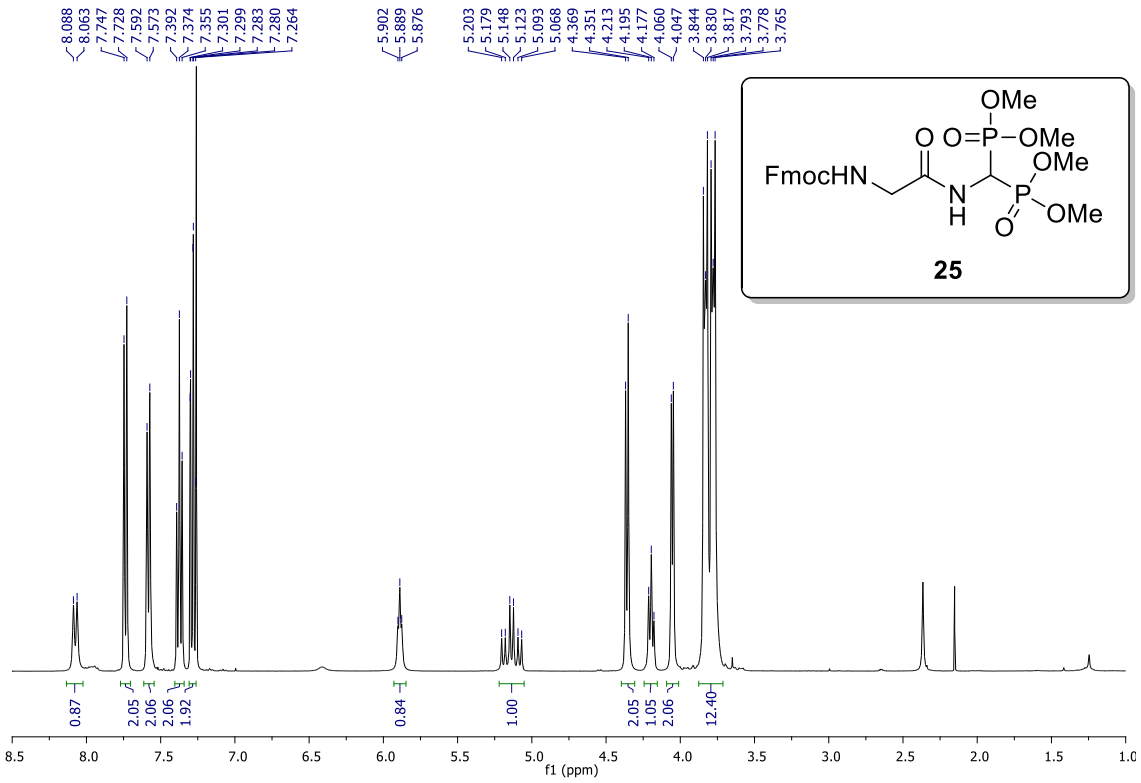


APT NMR (100 MHz, D_2O)

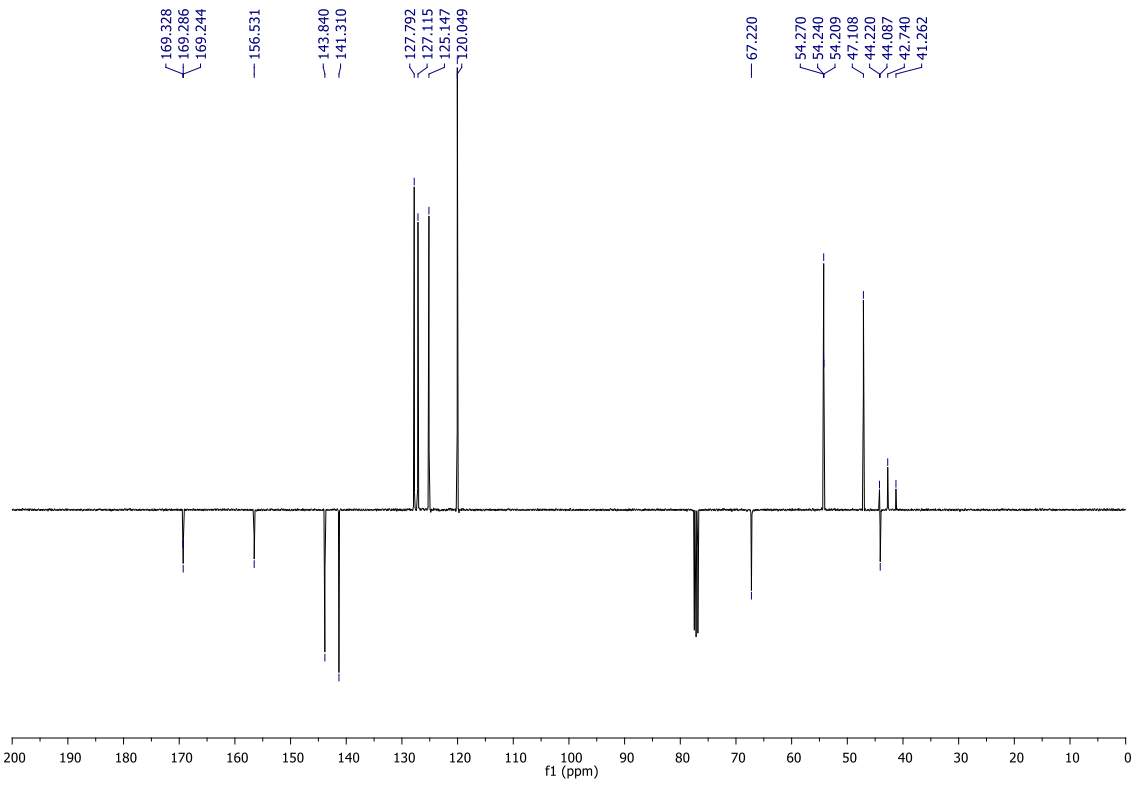


^{31}P NMR (162 MHz, D_2O)

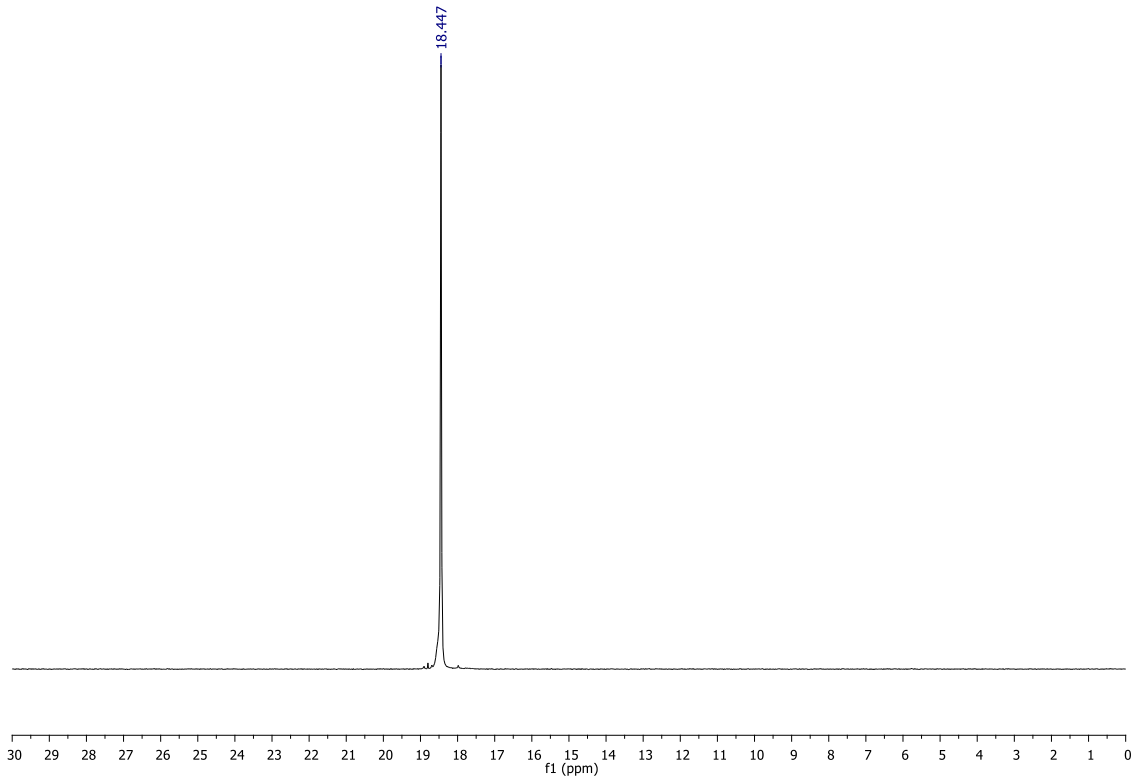
Tetramethyl [*N*-(9-fluorenylmethoxycarbonyl)glycylamidomethyl]bisphosphonate (25)



^{1}H NMR (400 MHz, $CDCl_3$)

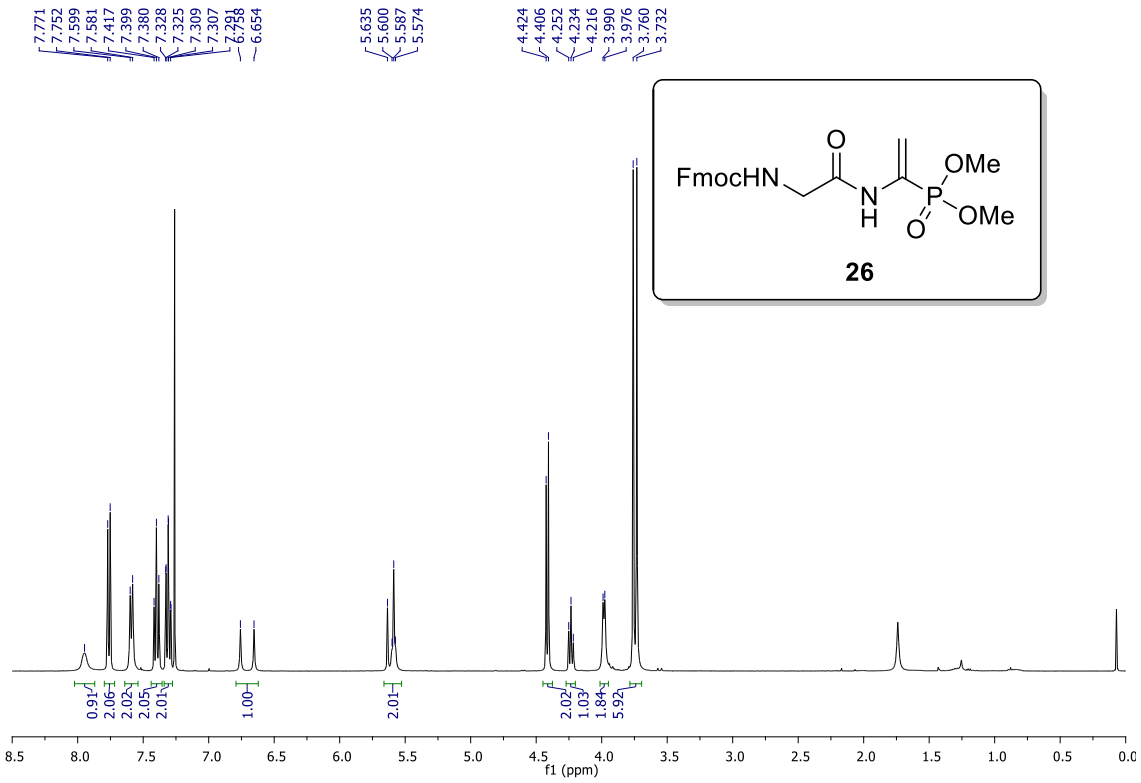


APT NMR (100 MHz, $CDCl_3$)

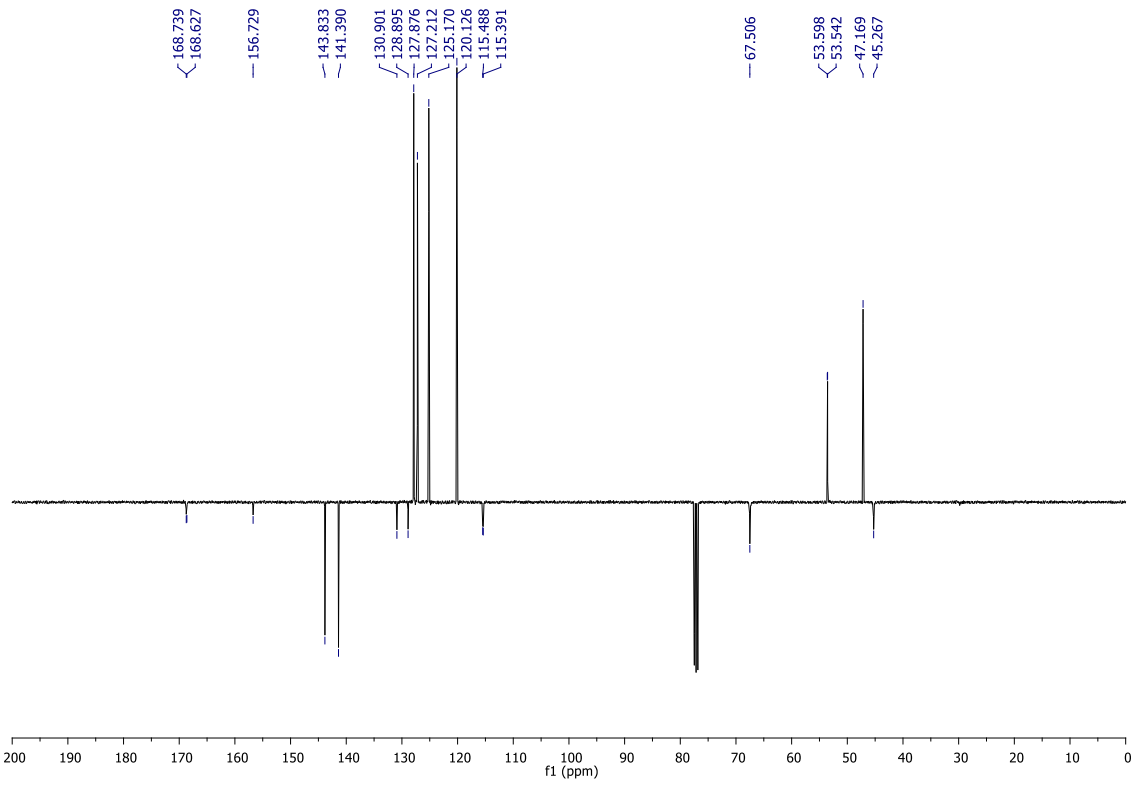


^{31}P NMR (162 MHz, $CDCl_3$)

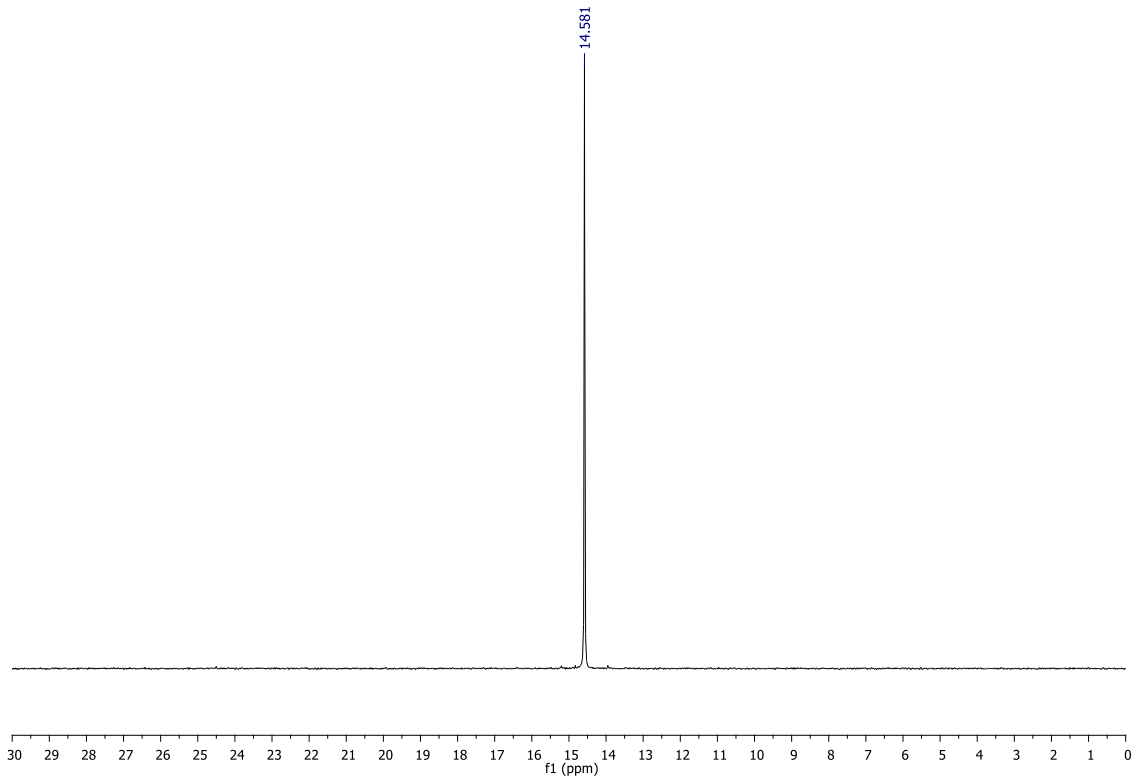
Dimethyl {1-[*N*-(9-fluorenylmethoxycarbonyl)glycylamido]ethen-1-yl}phosphonate (26)



^{1}H NMR (400 MHz, $CDCl_3$)

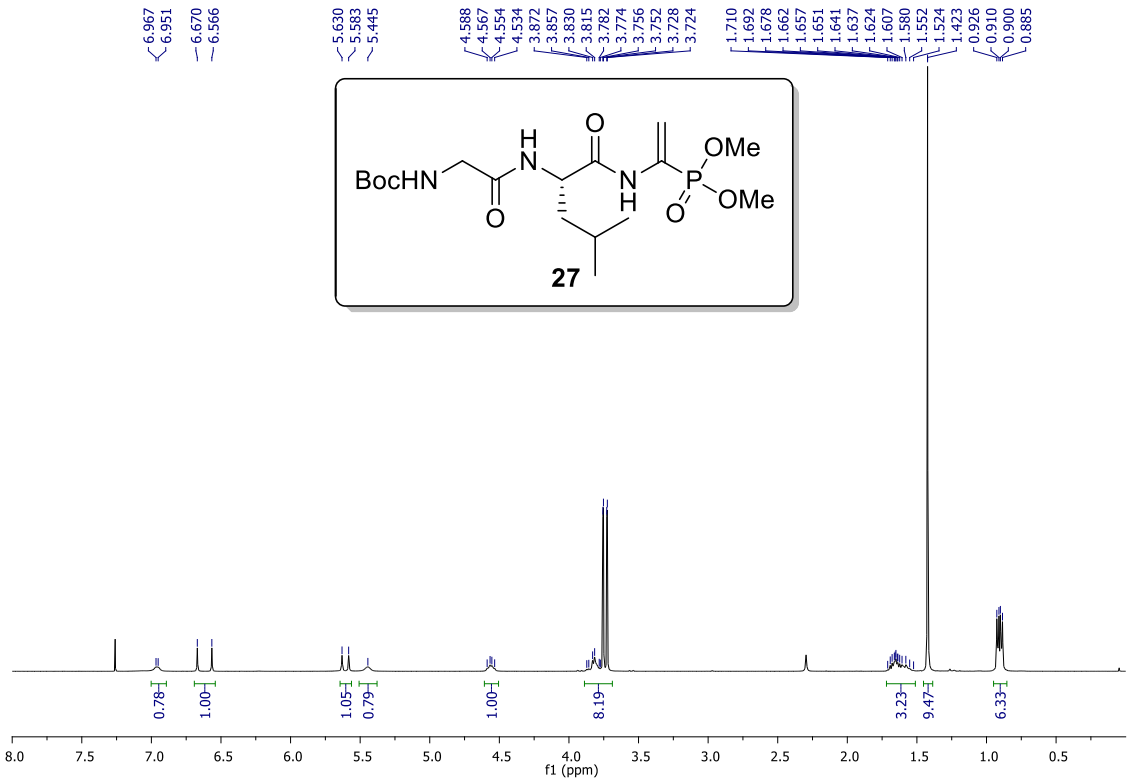


APT NMR (100 MHz, $CDCl_3$)

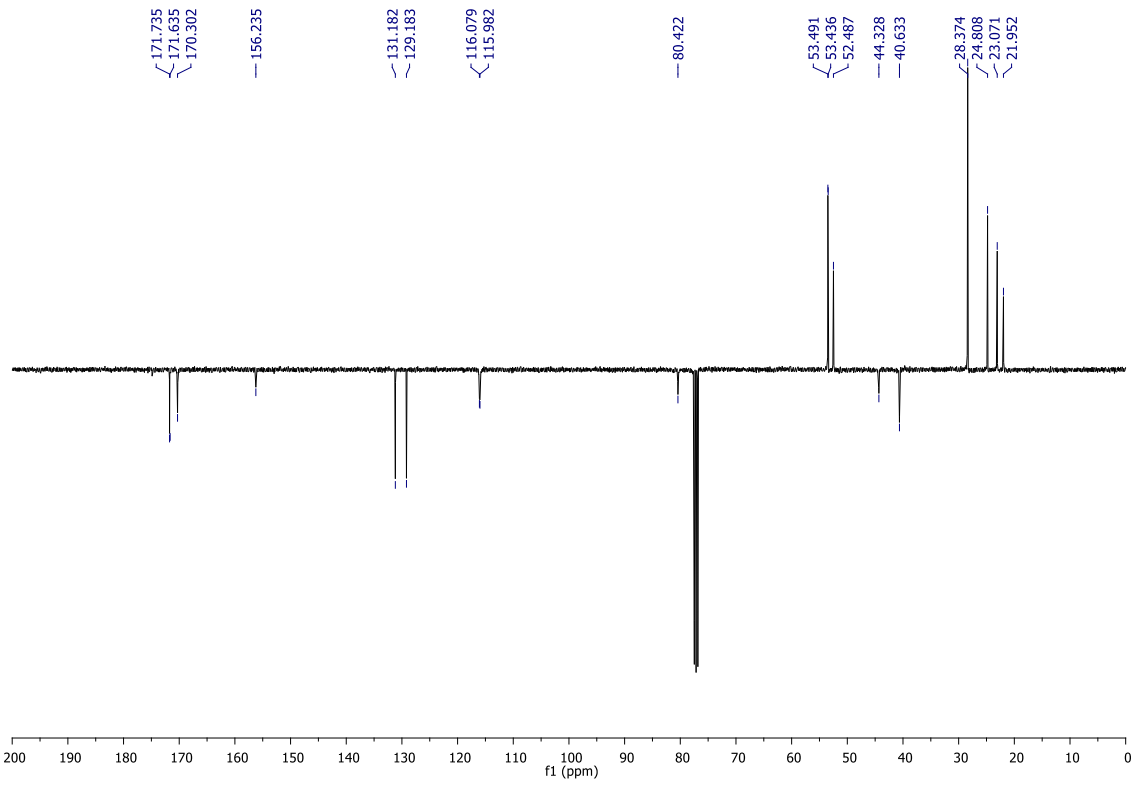


^{31}P NMR (162 MHz, $CDCl_3$)

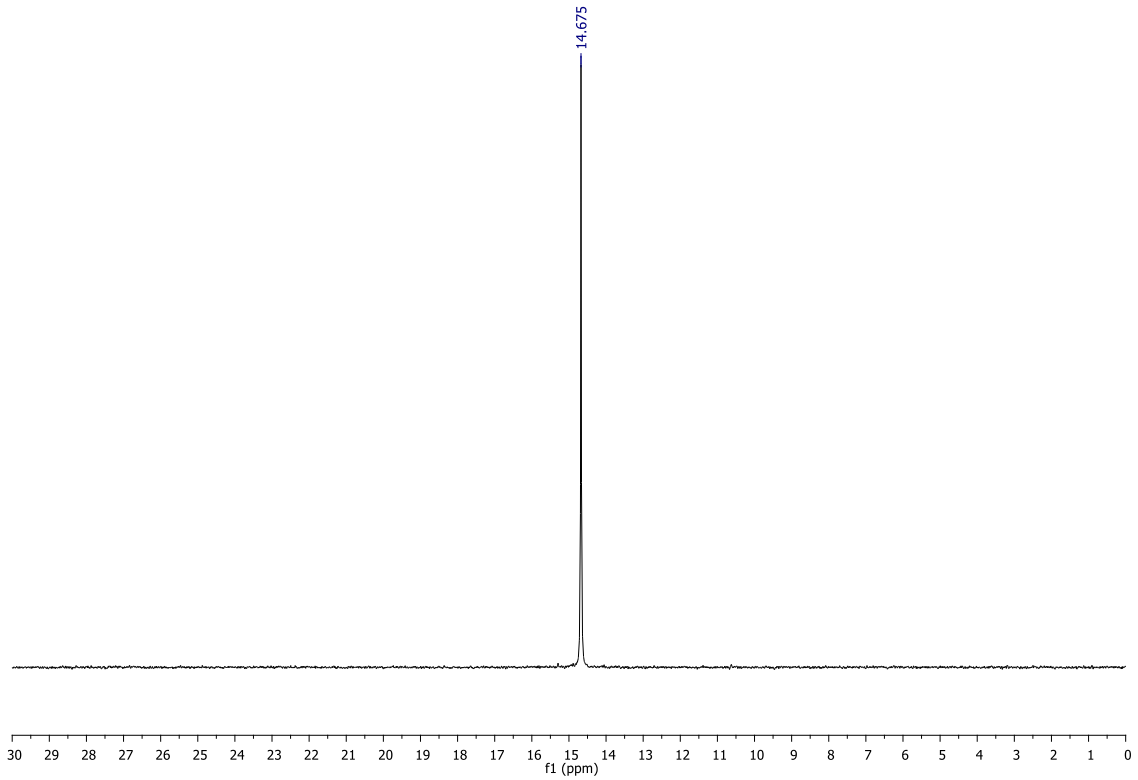
Dimethyl {1-[*N*-(*tert*-butoxycarbonyl)glycyl-L-leucylamido]ethen-1-yl}phosphonate (27)



^{1}H NMR (400 MHz, $CDCl_3$)

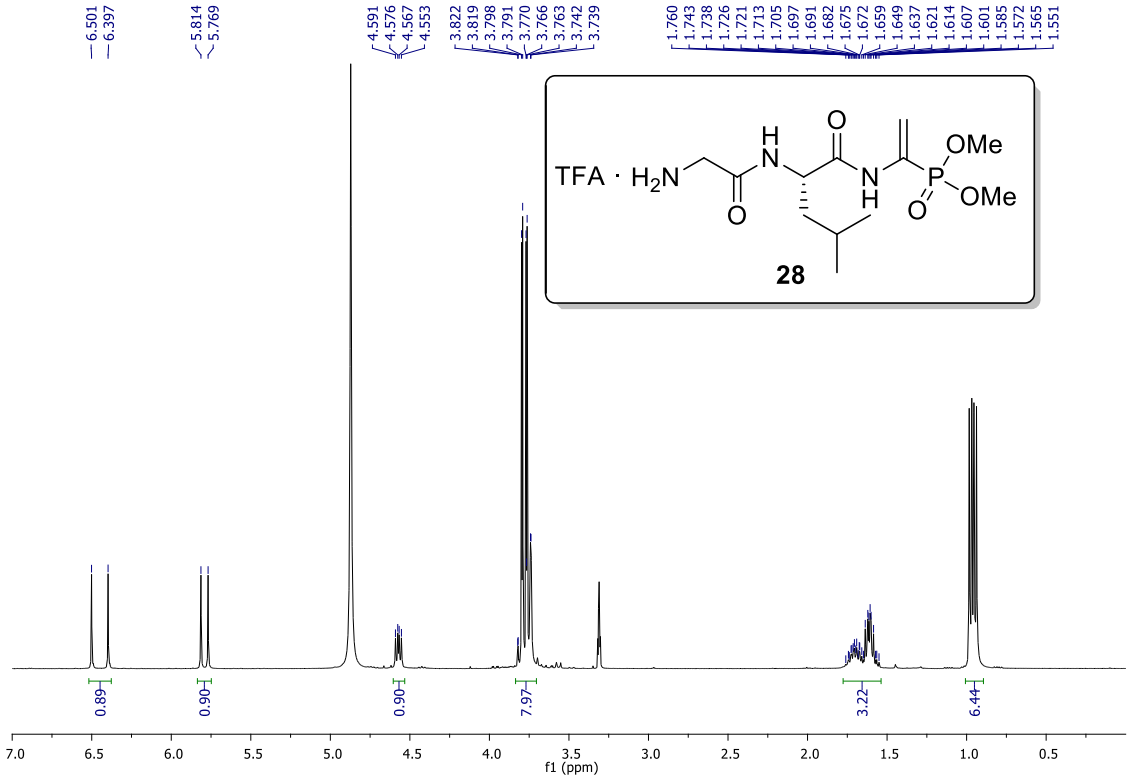


APT NMR (100 MHz, $CDCl_3$)

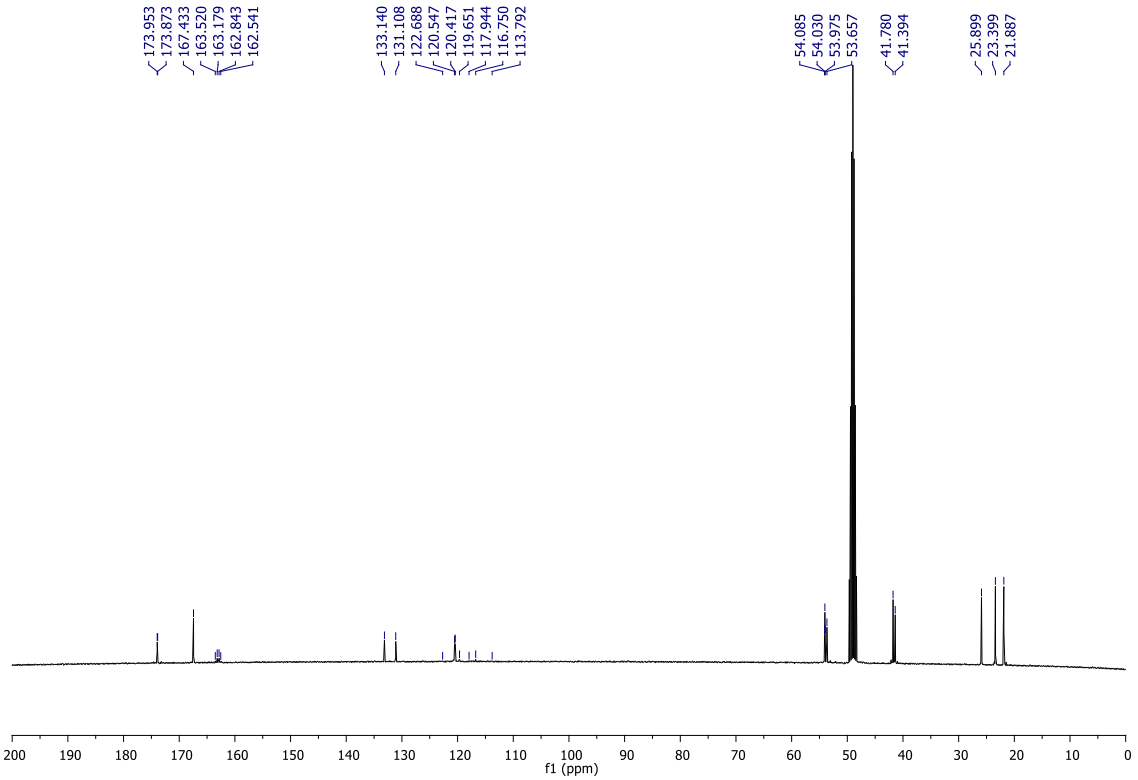


^{31}P NMR (162 MHz, $CDCl_3$)

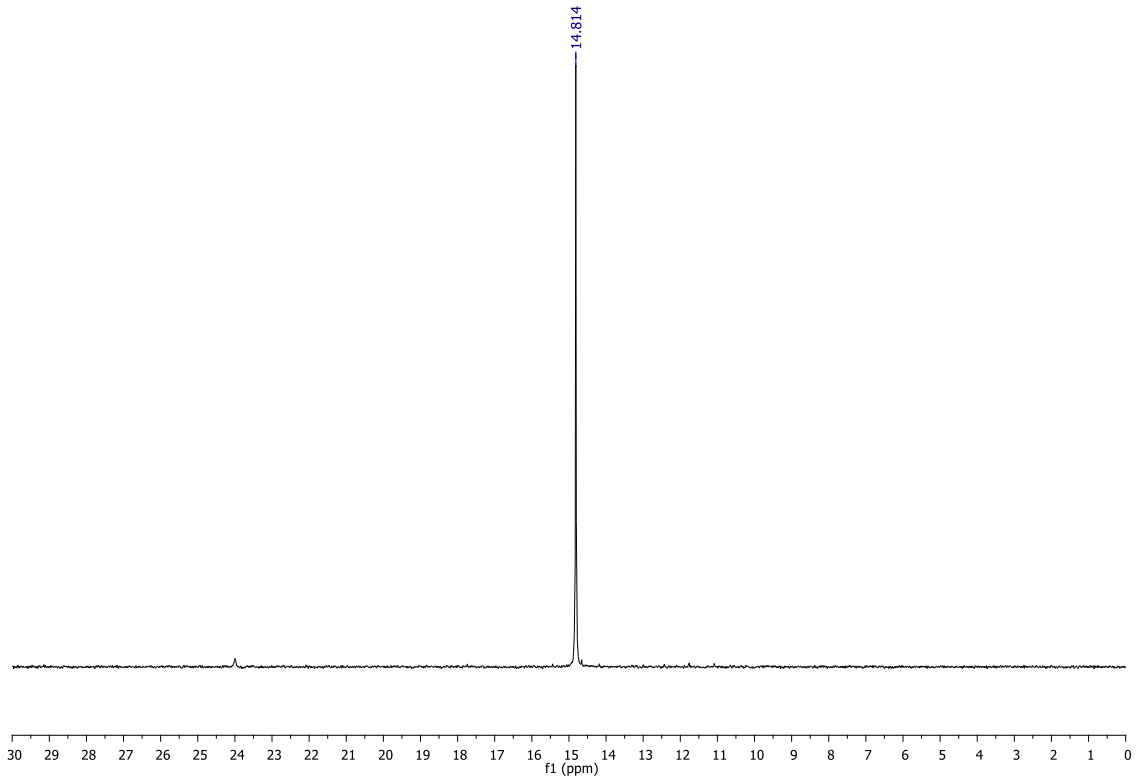
Dimethyl [1-(glycyl-L-leucylamido)ethen-1-yl]phosphonate trifluoroacetate (28)



^{1}H NMR (400 MHz, MeOD)



C NMR (100 MHz, MeOD)



^{31}P NMR (162 MHz, MeOD)

NMR Spectra of Chapter 3

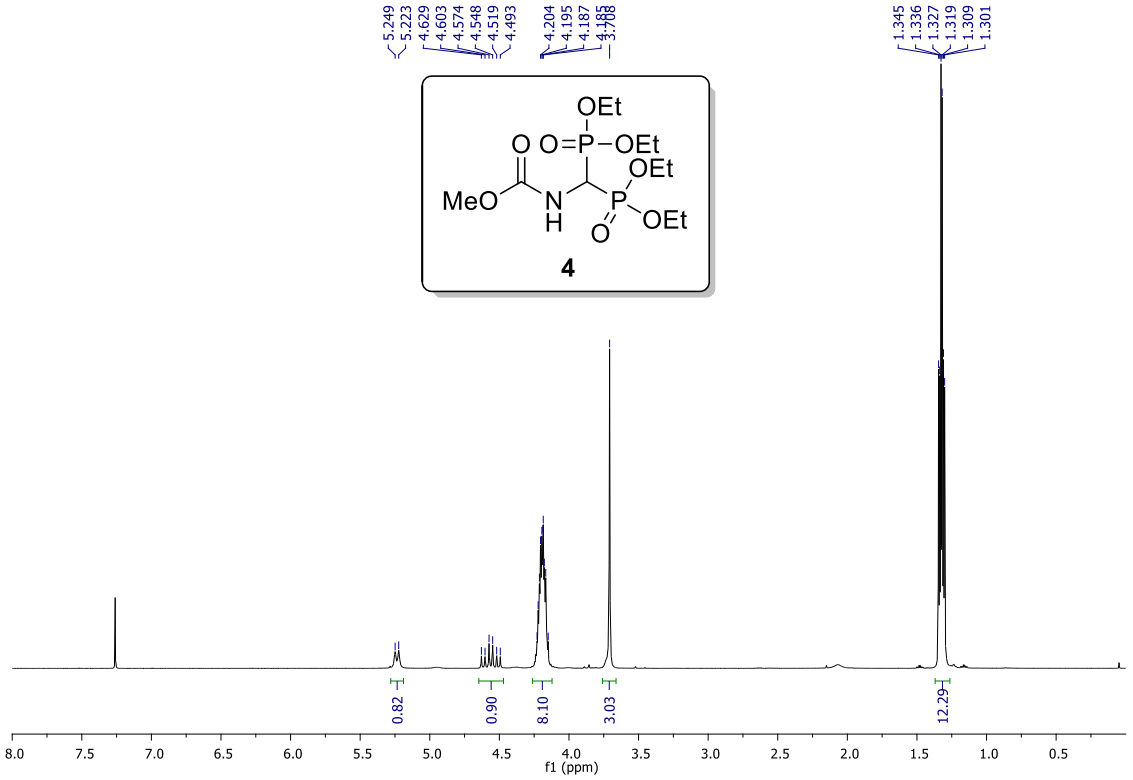
Tetramethyl (benzyloxycarbonylaminomethyl)bisphosphonate (2)

See Chapter I, compound **2**, page 8.

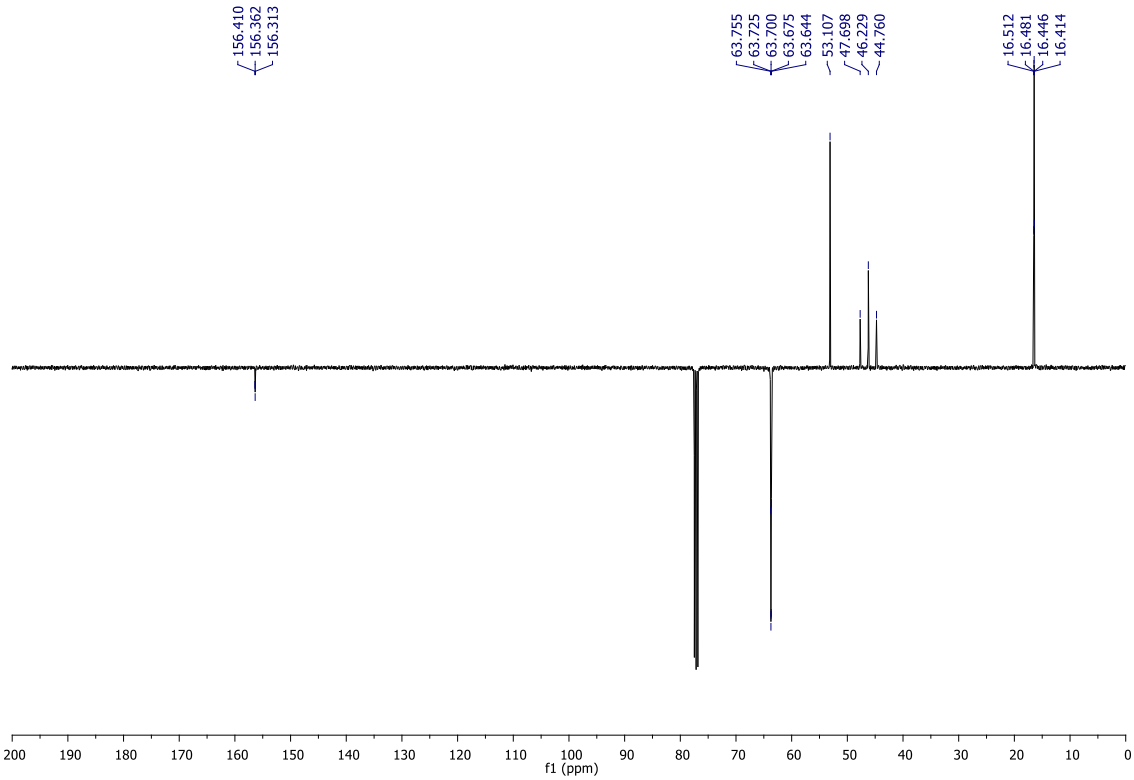
Tetraethyl (bencyloxycarbonylaminomethyl)bisphosphonate (3)

See Chapter 2, compound **9**, page 52.

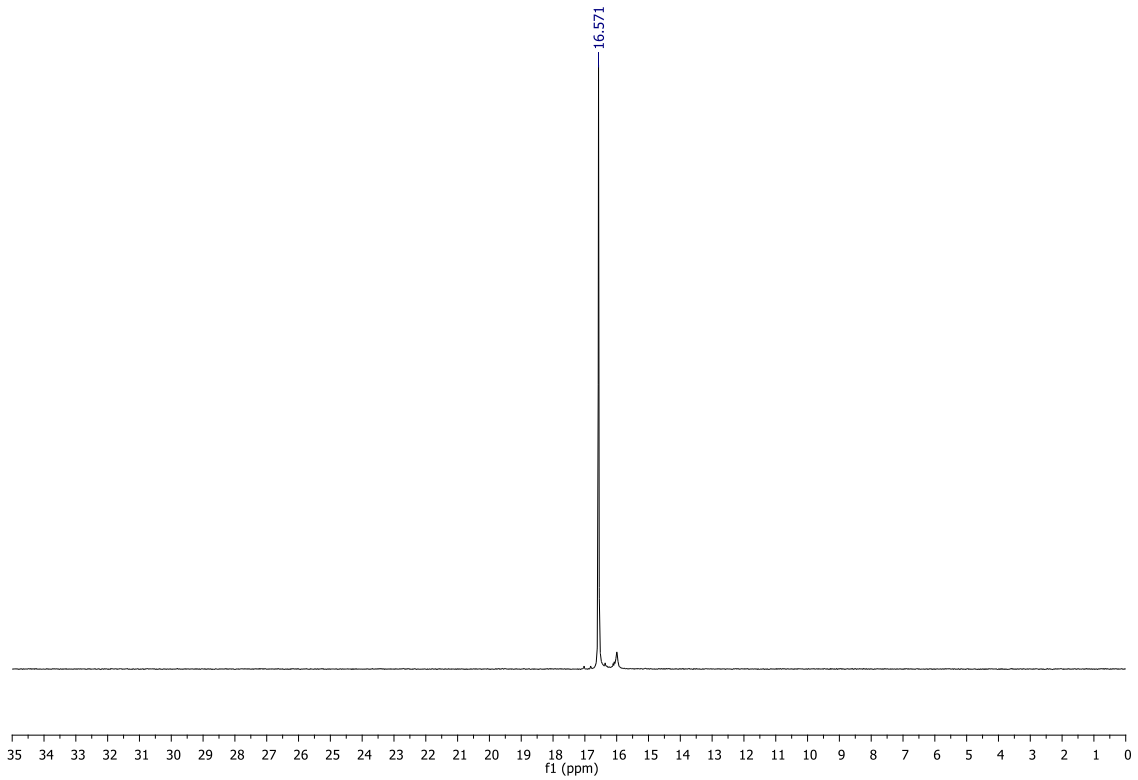
Tetraethyl (methoxycarbonylaminomethyl)bisphosphonate (4)



^{1}H NMR (400 MHz, $CDCl_3$)

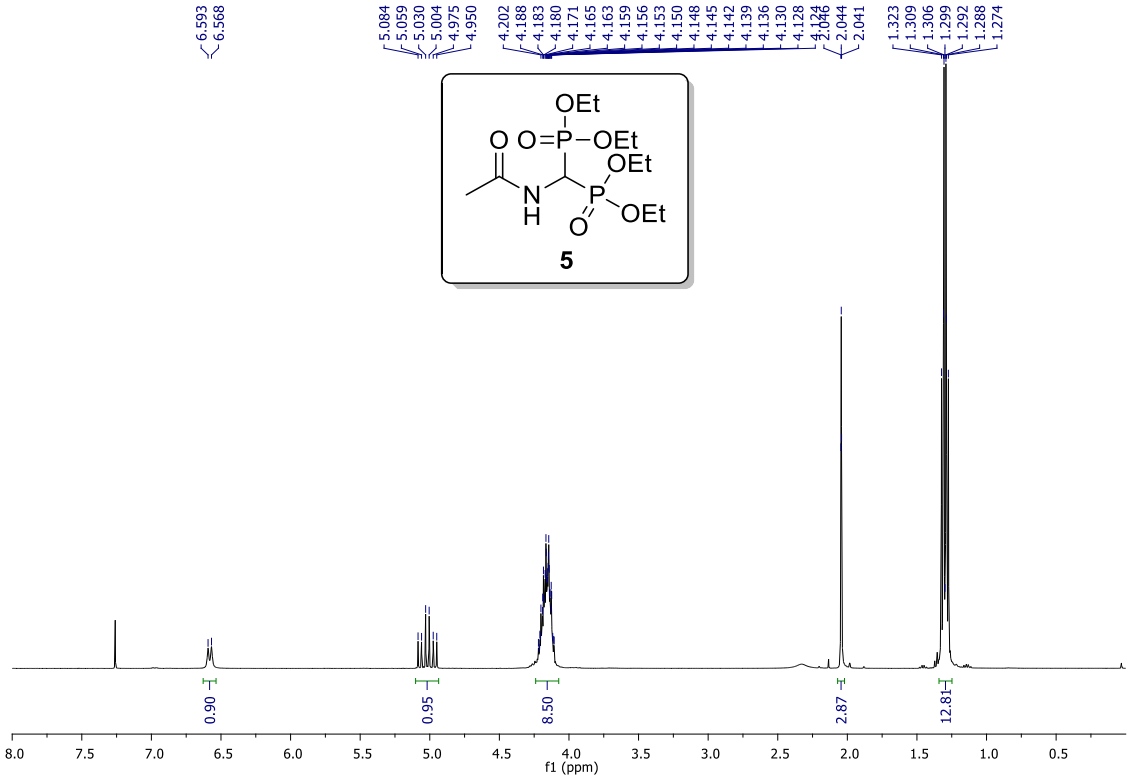


APT NMR (100 MHz, $CDCl_3$)

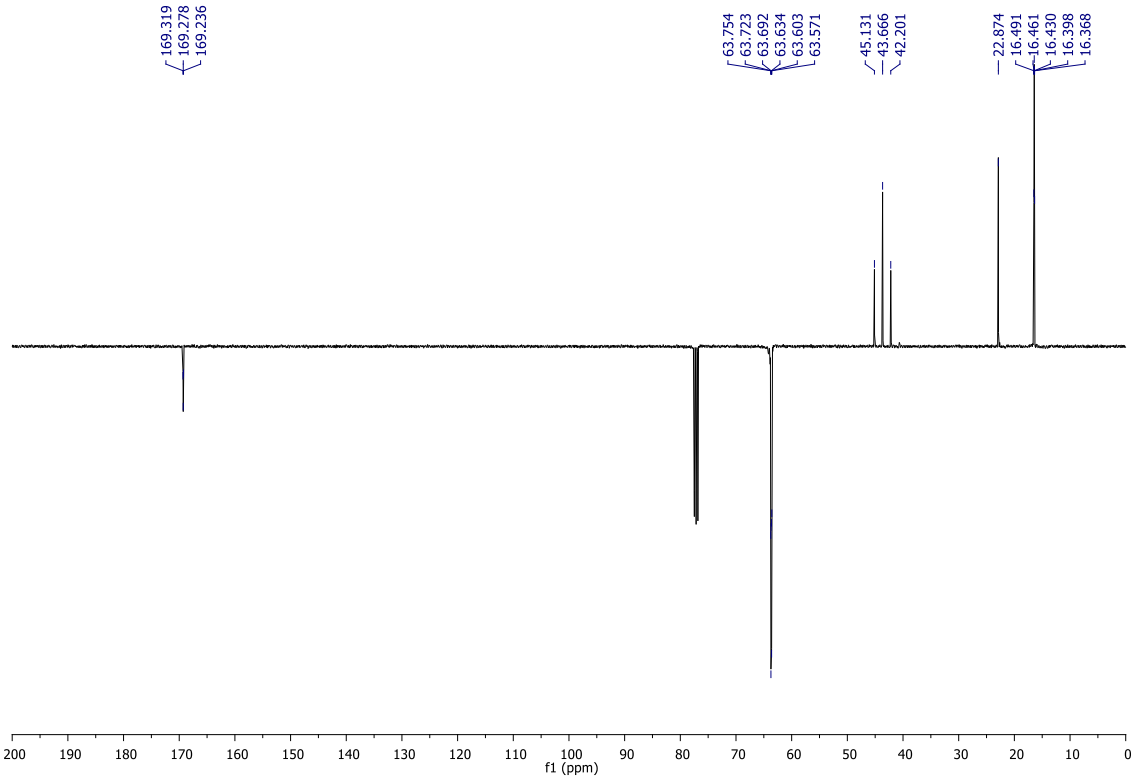


^{31}P NMR (162 MHz, $CDCl_3$)

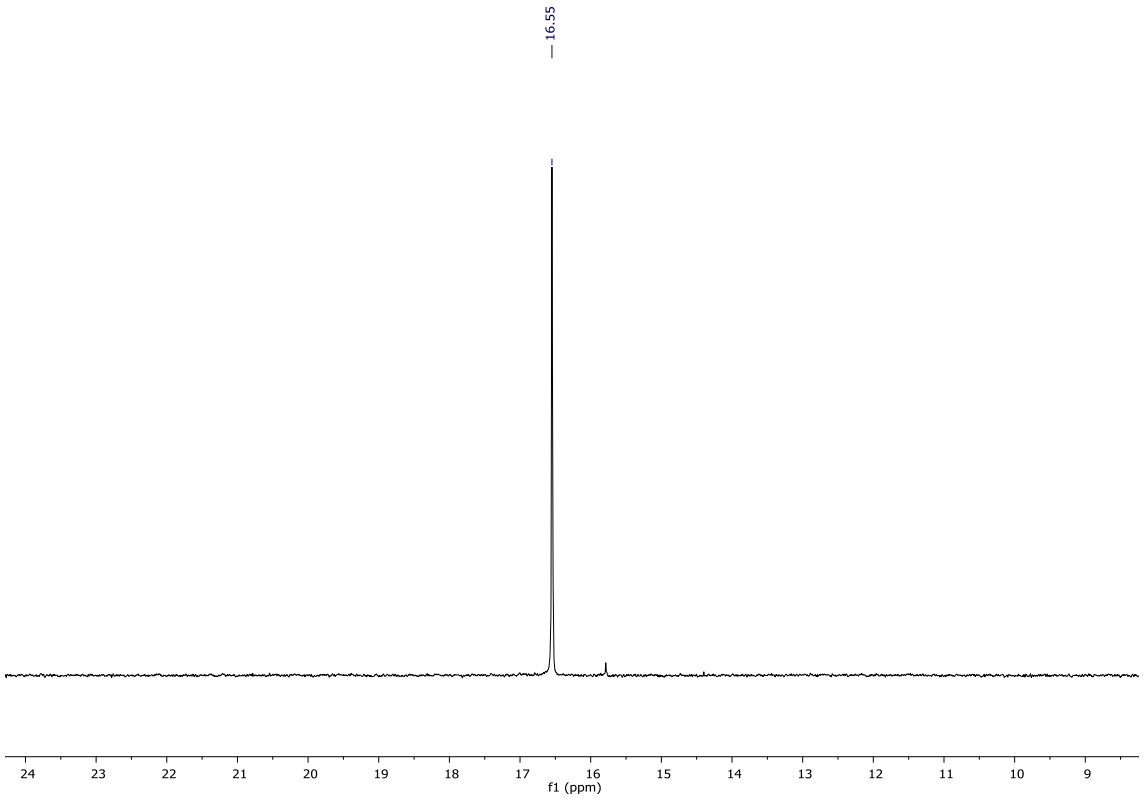
Tetraethyl (acetylaminomethyl)bisphosphonate (5)



^{1}H NMR (400 MHz, $CDCl_3$)

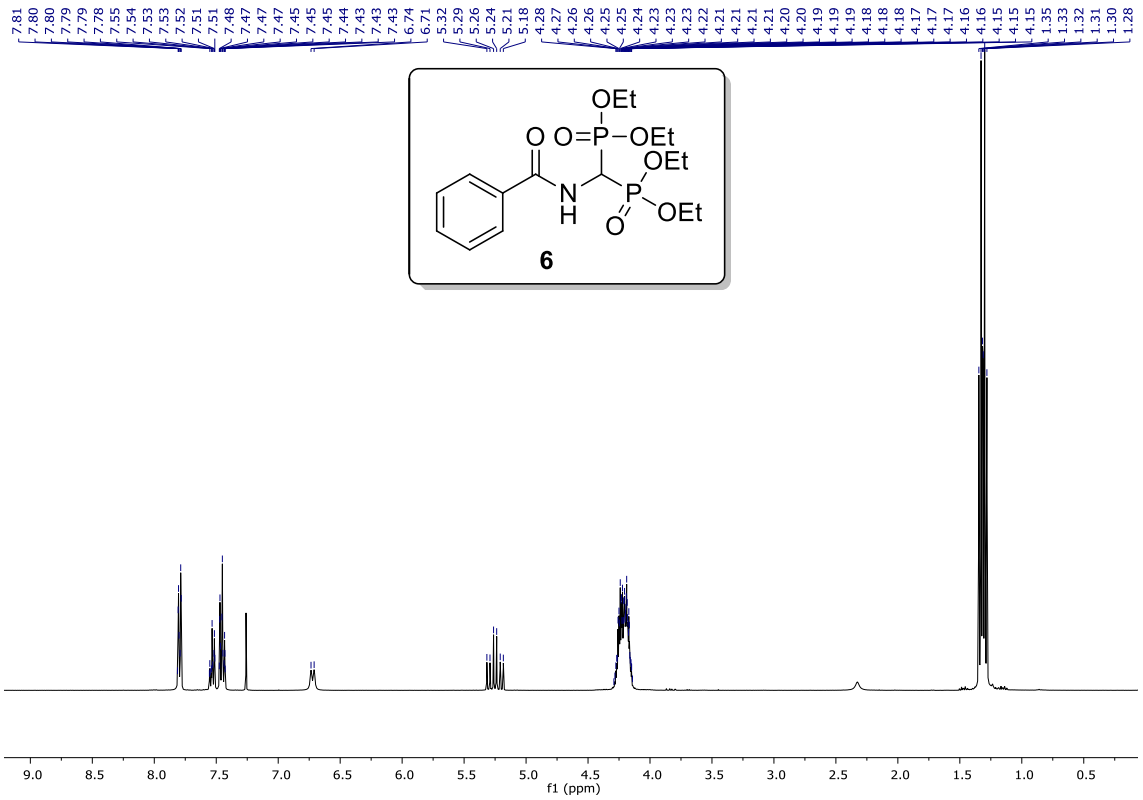


APT NMR (100 MHz, $CDCl_3$)

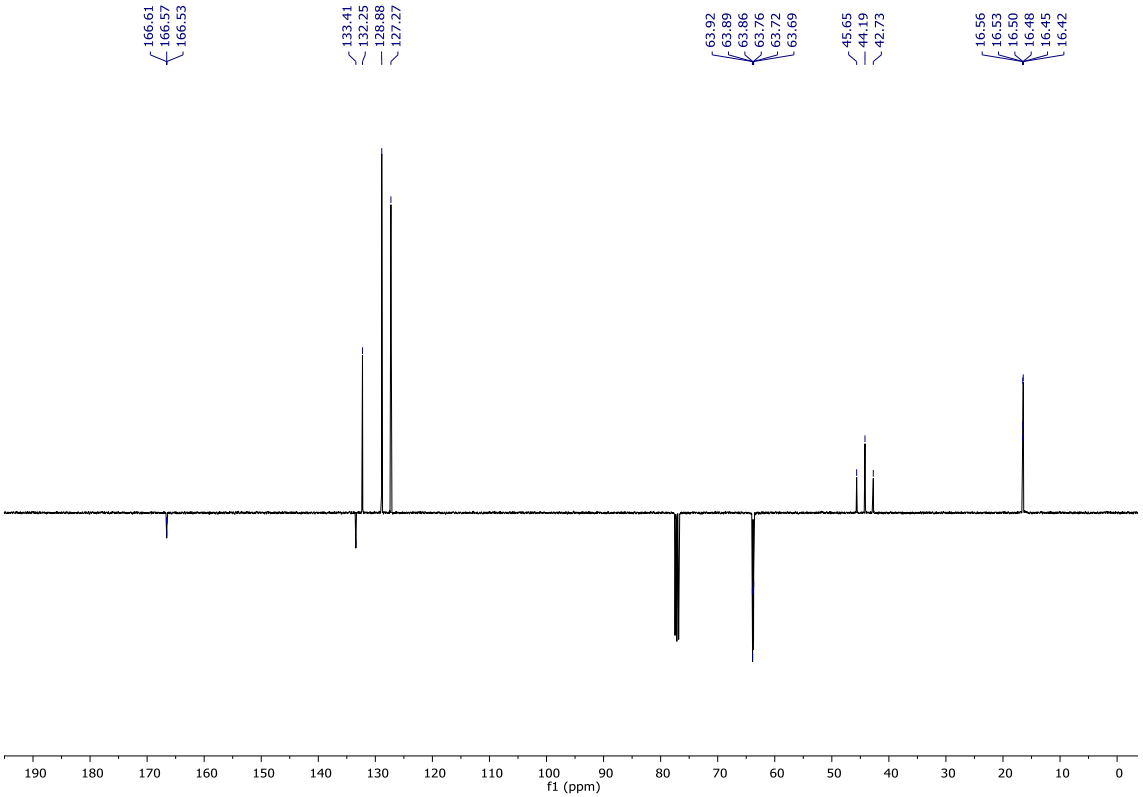


^{31}P NMR (162 MHz, $CDCl_3$)

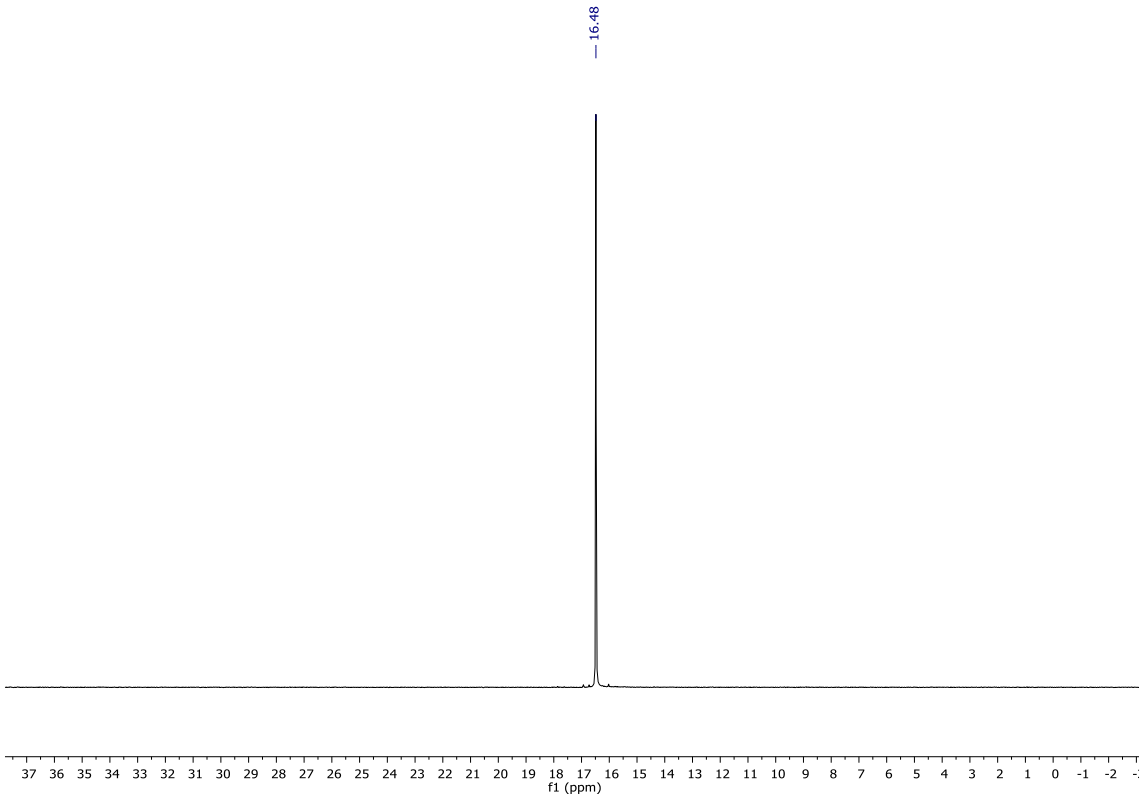
Tetraethyl (*N*-benzoylaminomethyl)bisphosphonate (6)



^{1}H NMR (400 MHz, $CDCl_3$)

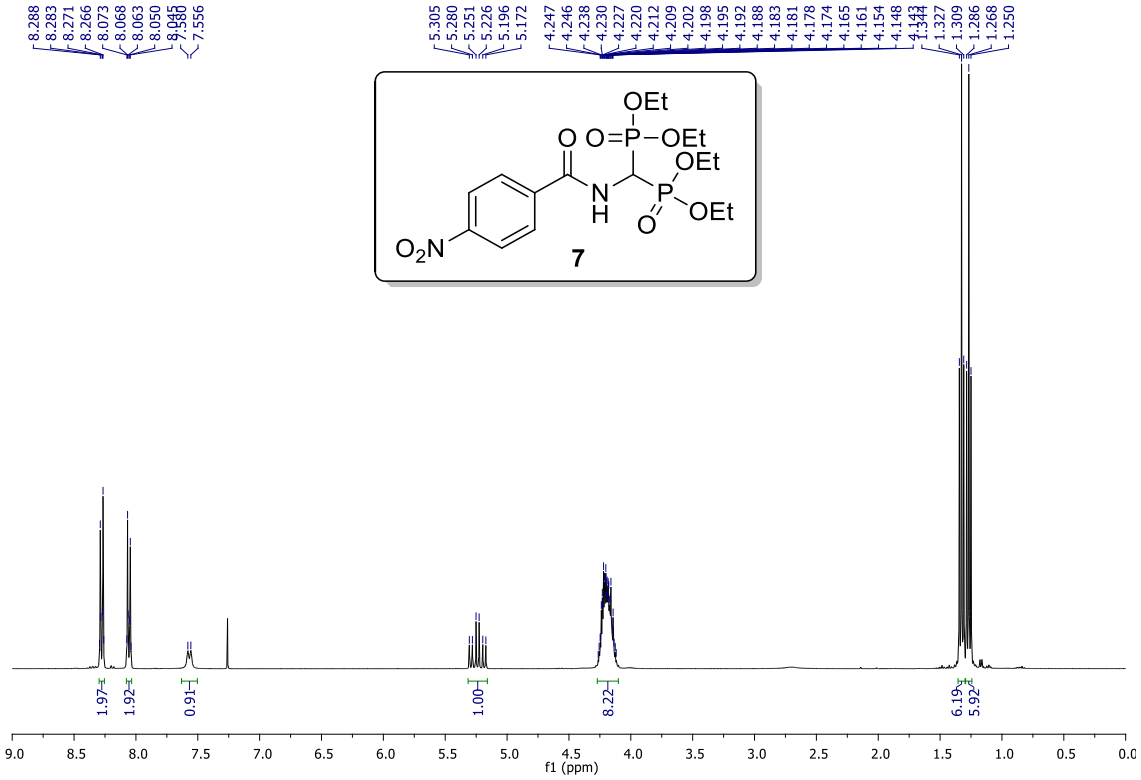


APT NMR (100 MHz, $CDCl_3$)

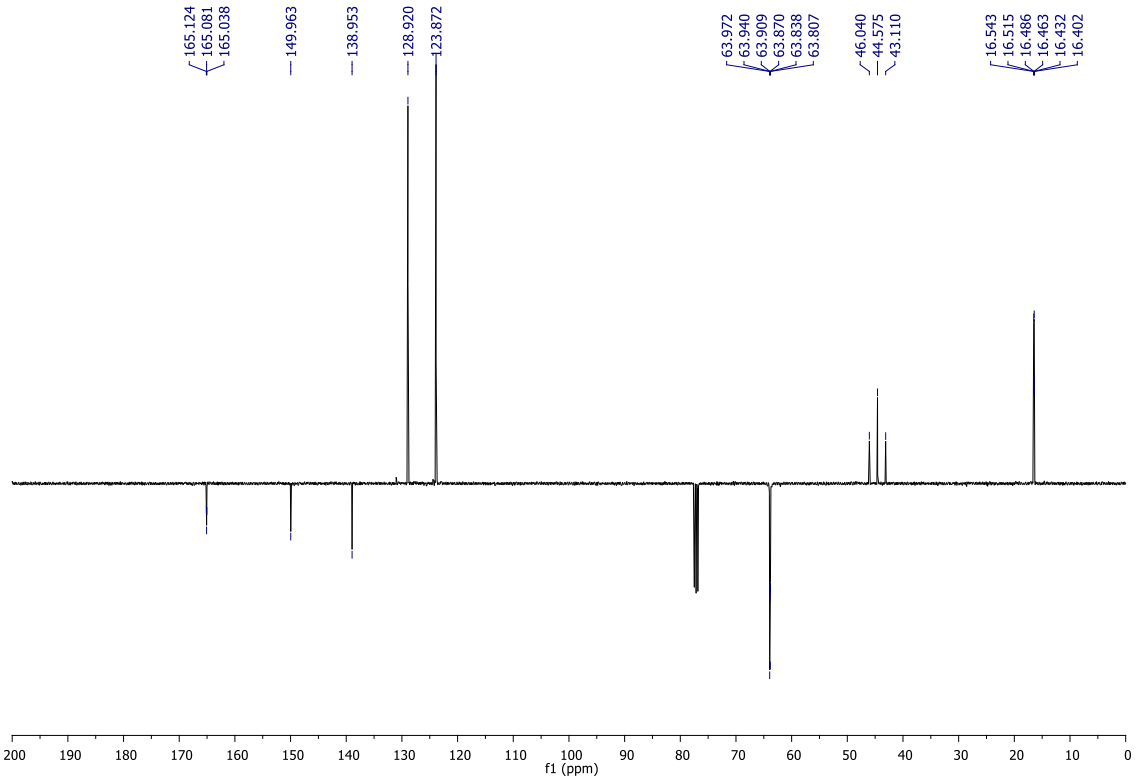


^{31}P NMR (162 MHz, $CDCl_3$)

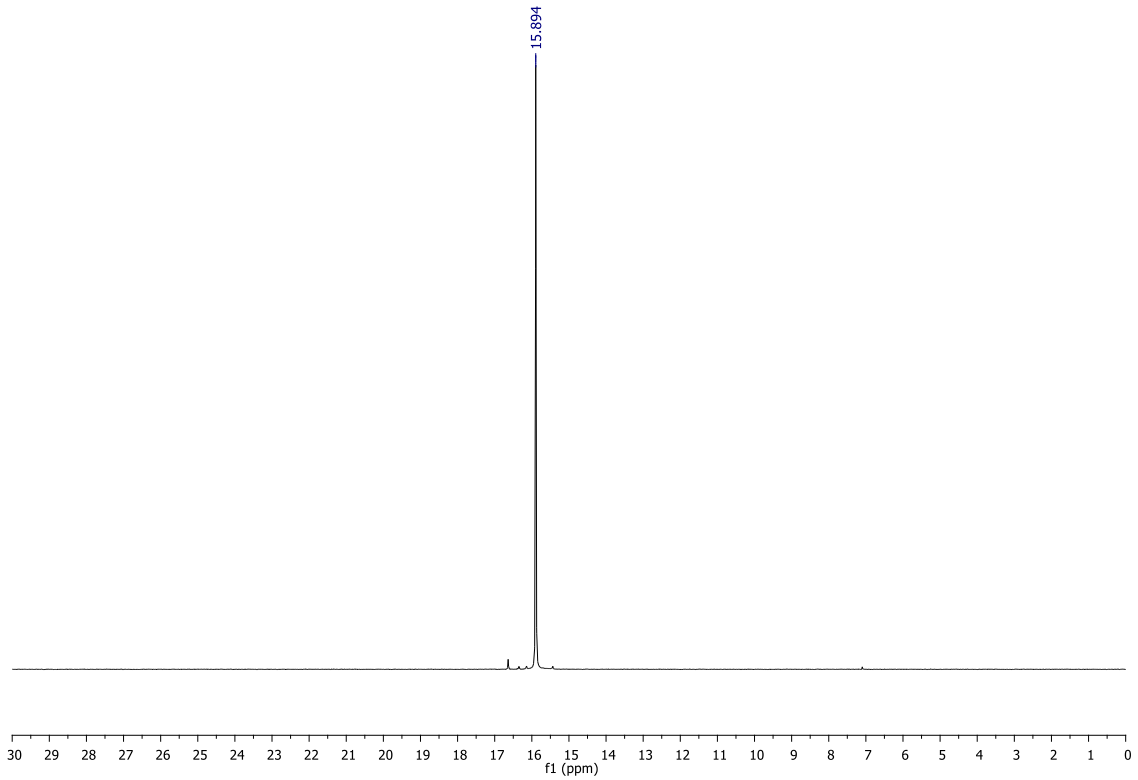
Tetraethyl (*p*-nitrobenzoylaminomethyl)bisphosphonate (7)



^{1}H NMR (400 MHz, $CDCl_3$)

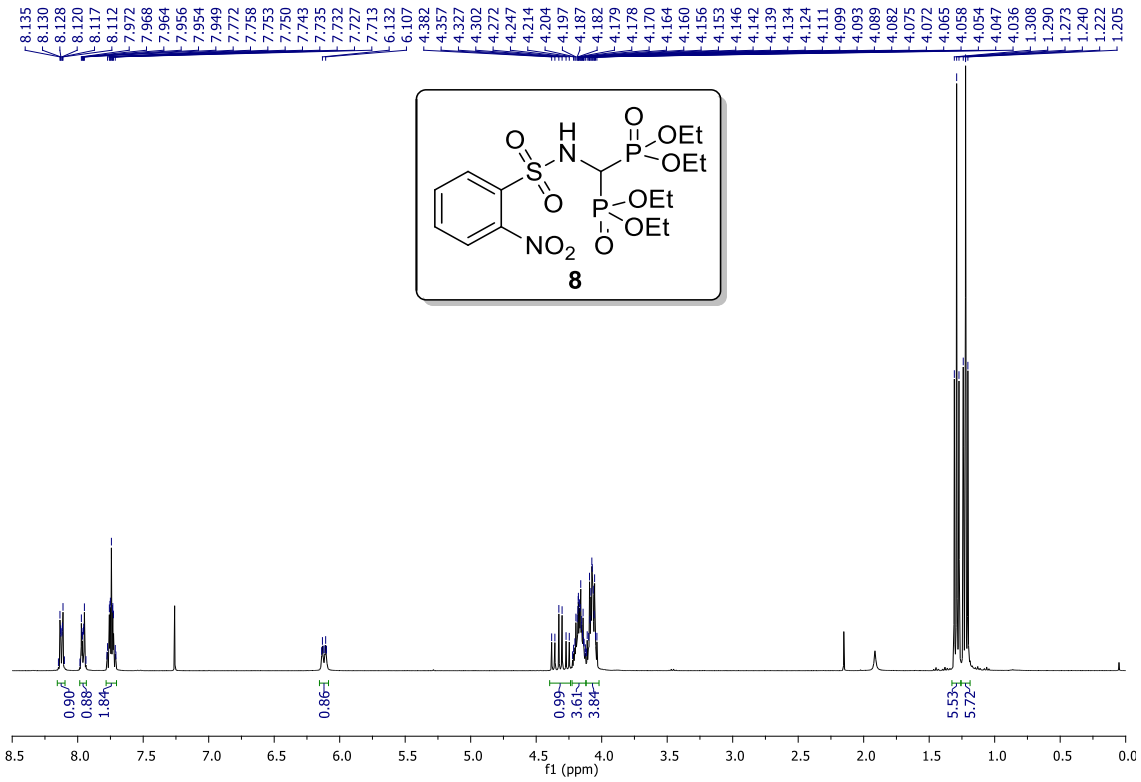


APT NMR (100 MHz, $CDCl_3$)

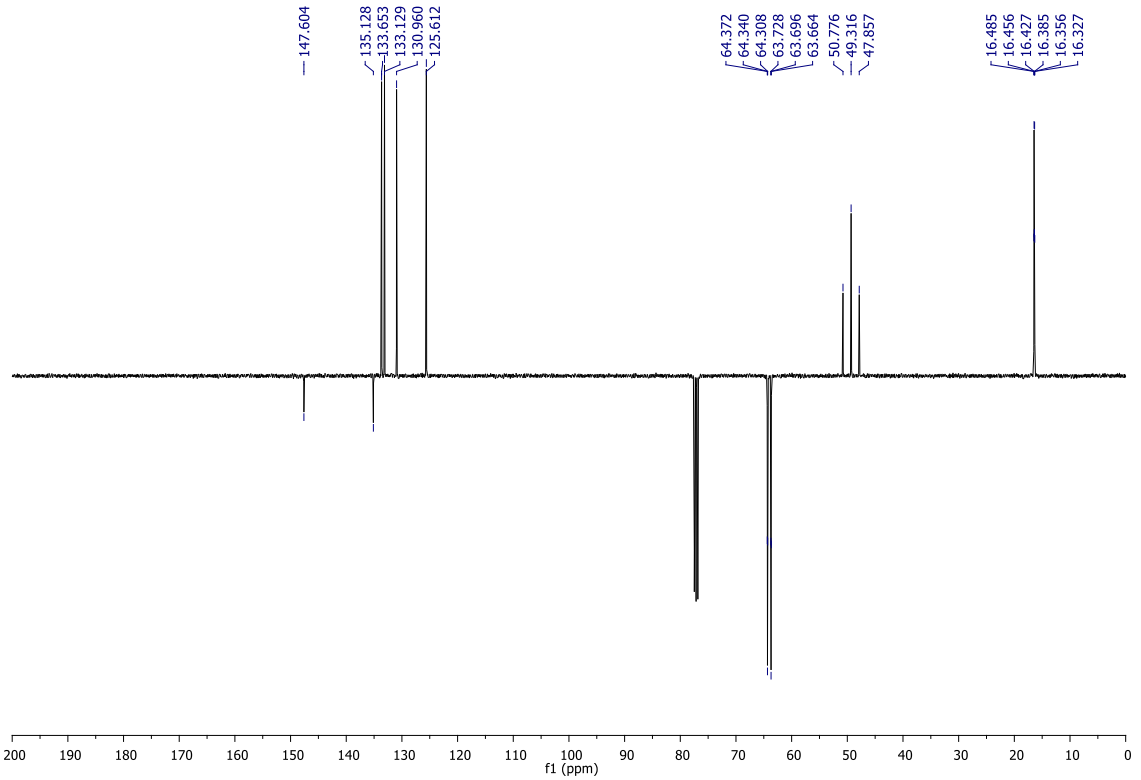


^{31}P NMR (162 MHz, $CDCl_3$)

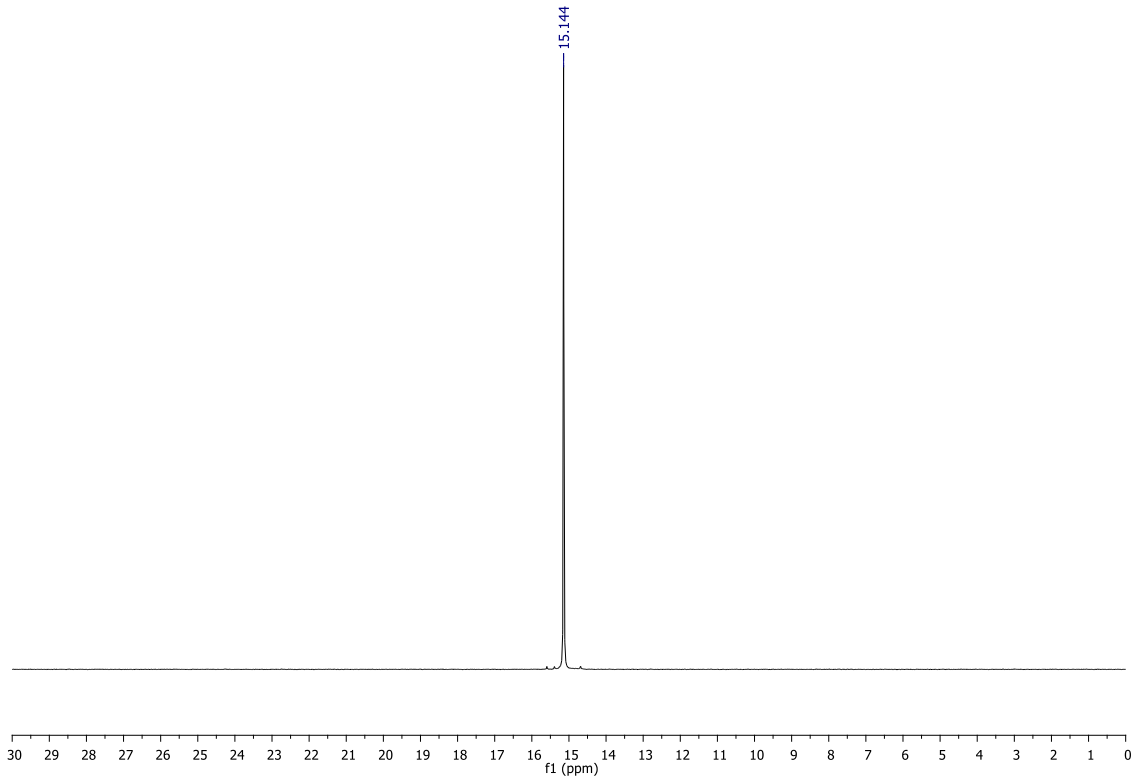
Tetraethyl (2-nitrobenzenesulfonamidemethyl)bisphosphonate (8)



^{1}H NMR (400 MHz, $CDCl_3$)

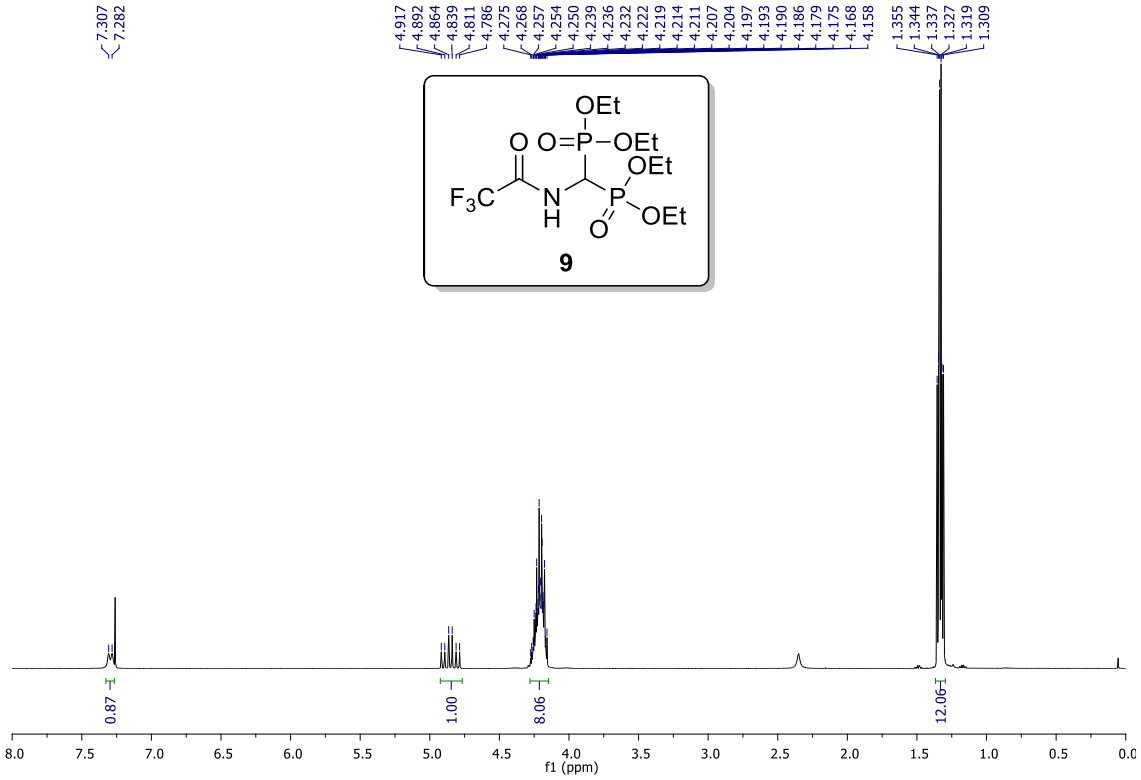


APT NMR (100 MHz, $CDCl_3$)

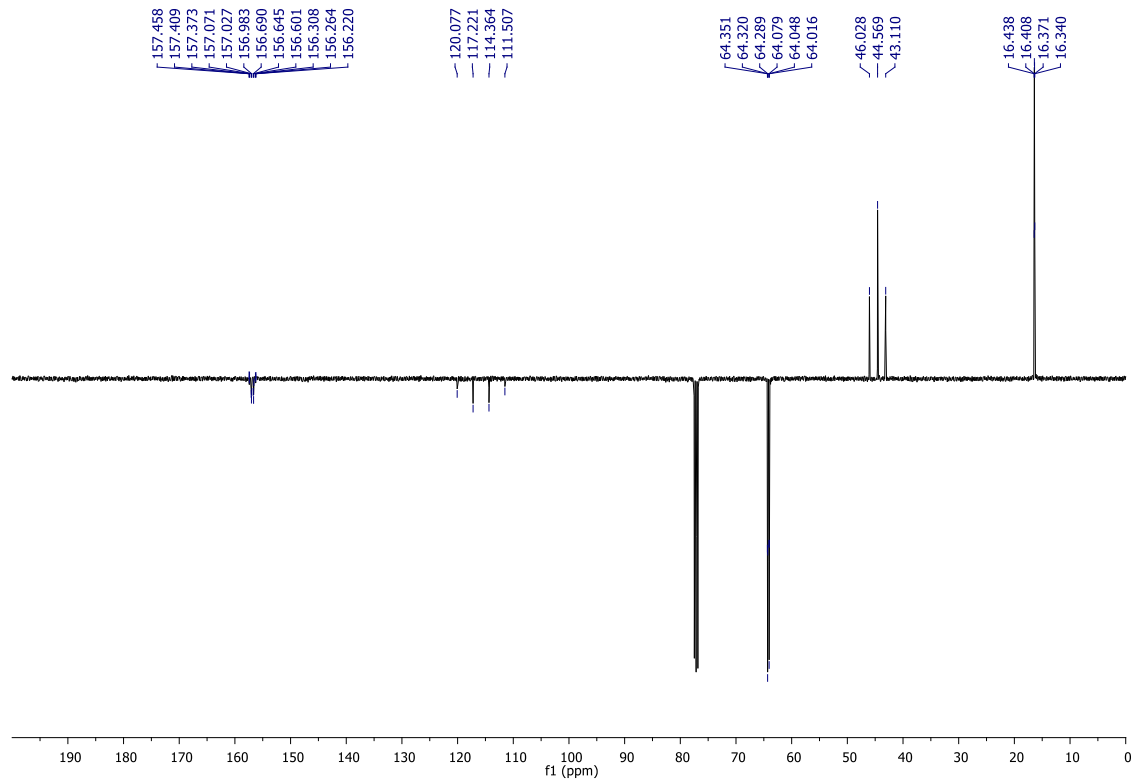


^{31}P NMR (162 MHz, $CDCl_3$)

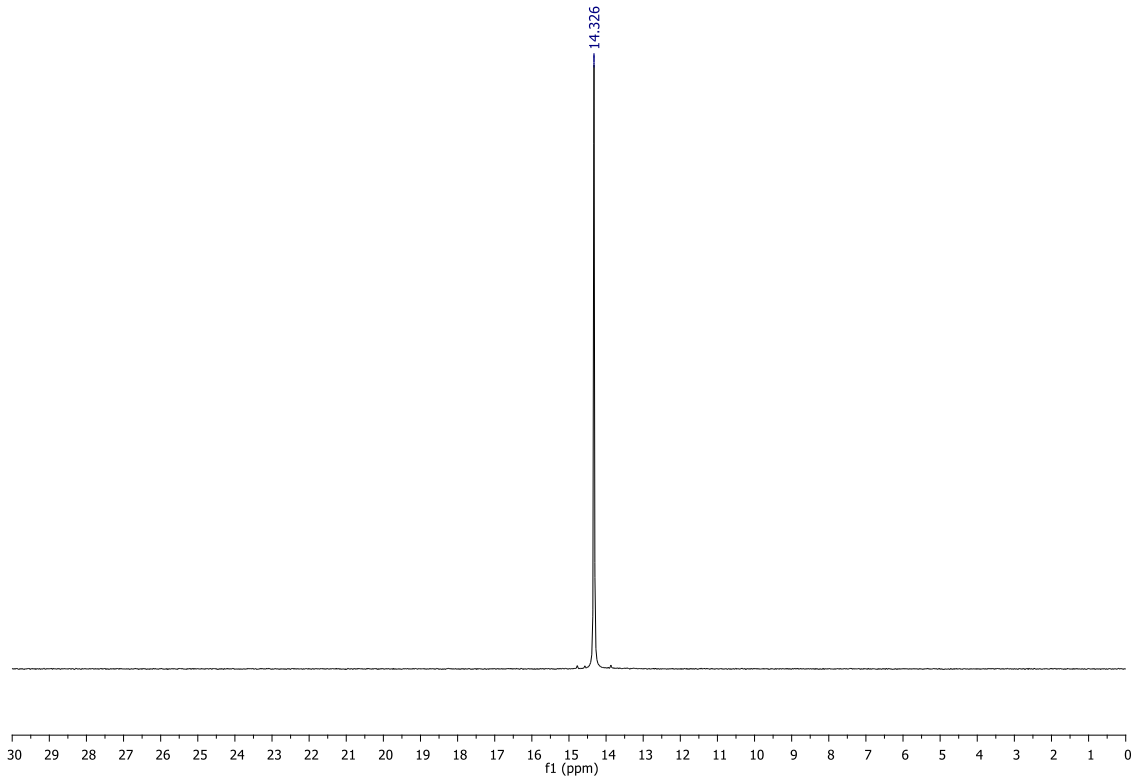
Tetraethyl (trifluoroacetylaminomethyl)bisphosphonate (9)



^{1}H NMR (400 MHz, $CDCl_3$)

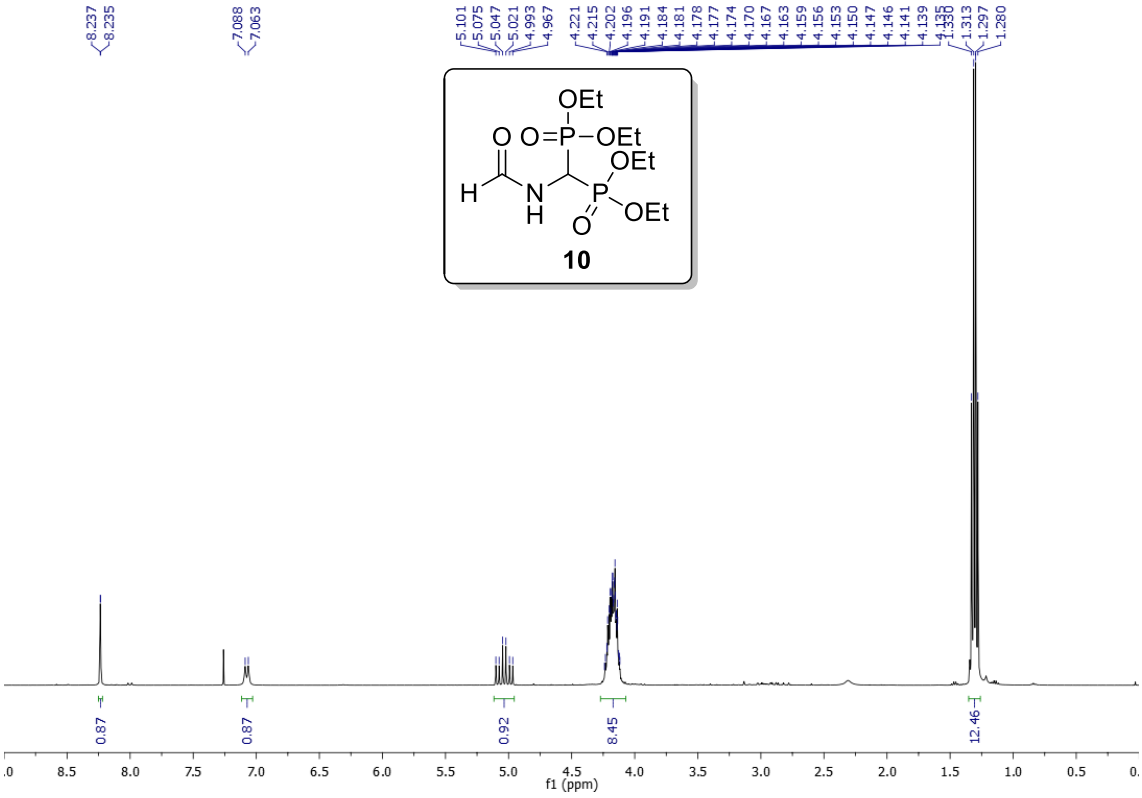


APT NMR (100 MHz, $CDCl_3$)

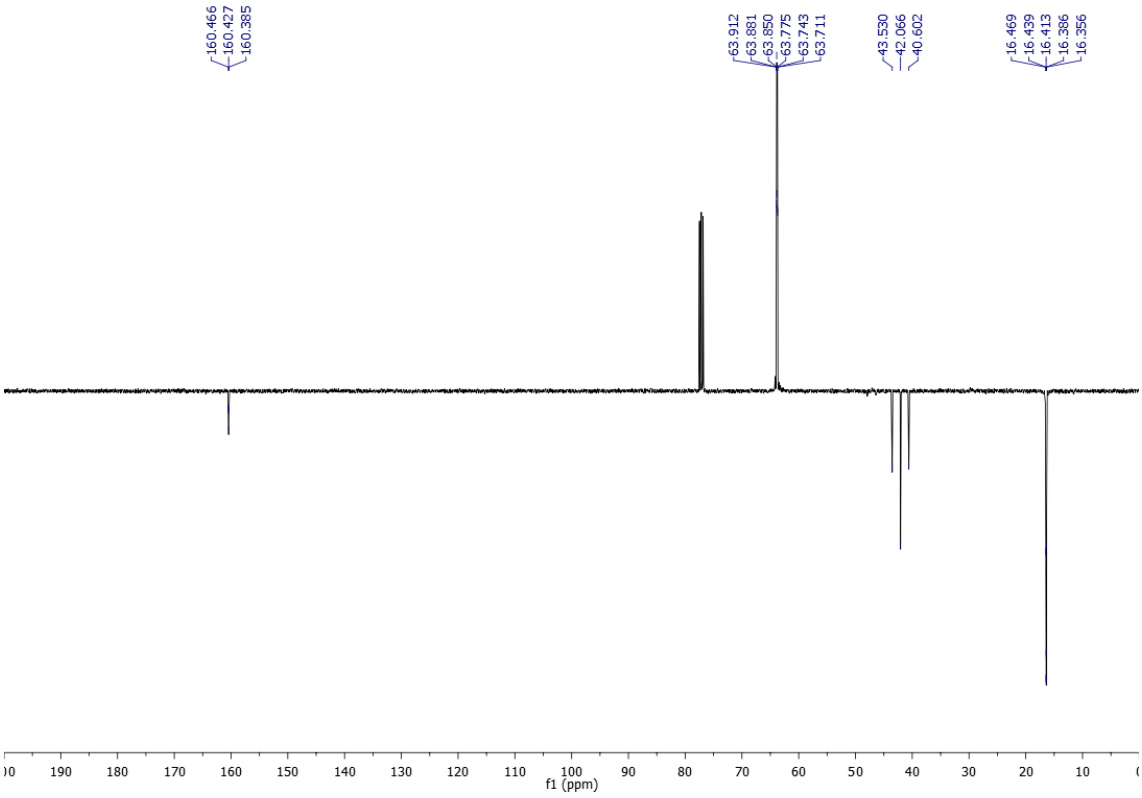


^{31}P NMR (162 MHz, $CDCl_3$)

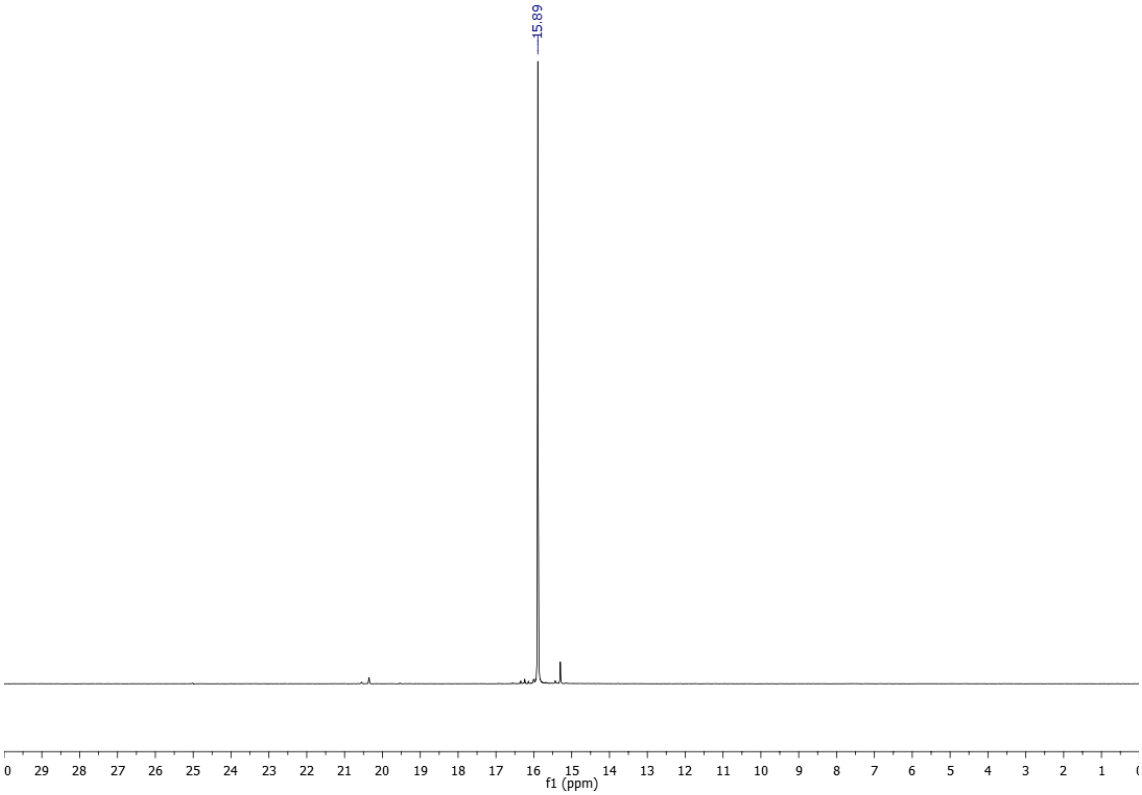
Tetraethyl (*N*-formylaminomethtyl)bisphosphonate (10)



^{1}H NMR (400 MHz, $CDCl_3$)



APT NMR (100 MHz, $CDCl_3$)

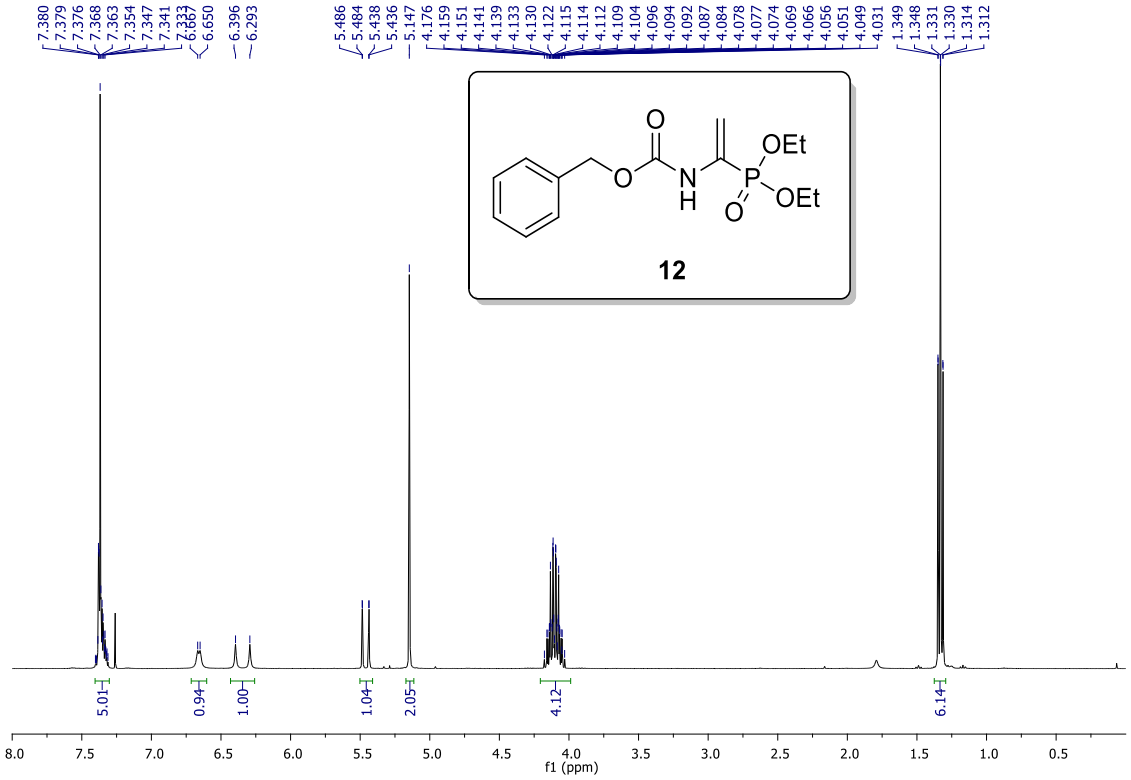


^{31}P NMR (162 MHz, $CDCl_3$)

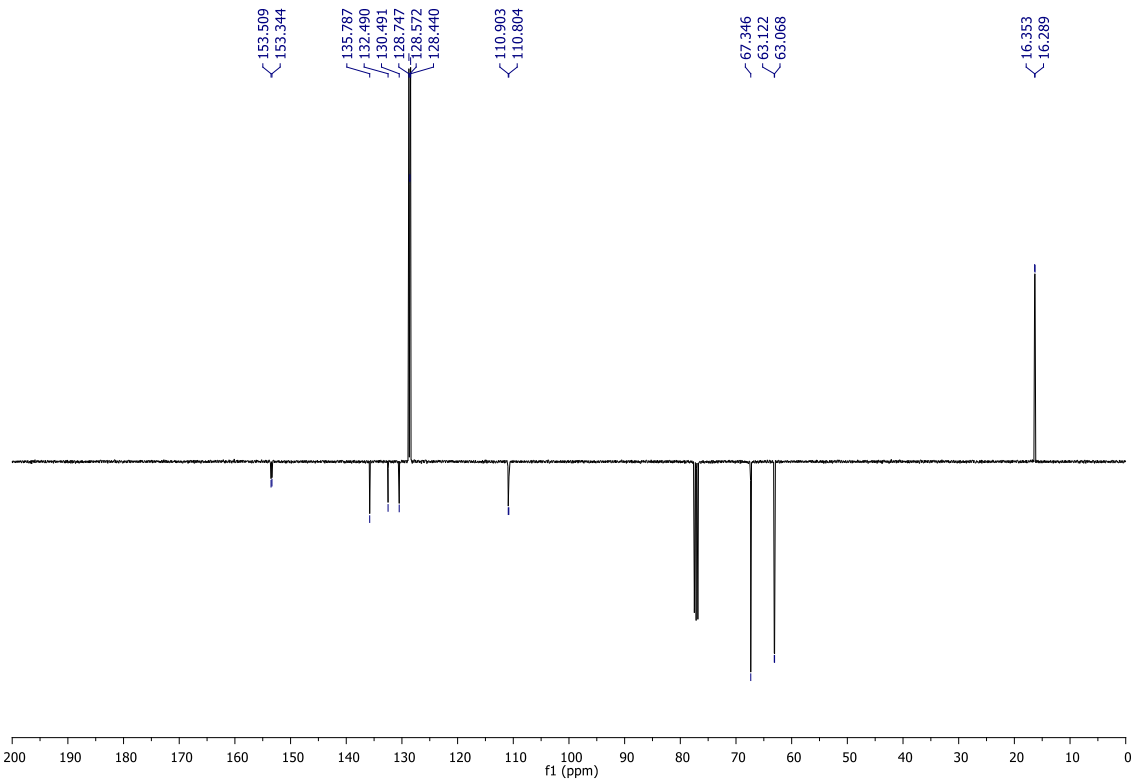
Dimethyl [1-(benzyloxycarbonylamino)ethen-1-yl]phosphonate (11)

See Chapter I, compound **4**, page 12.

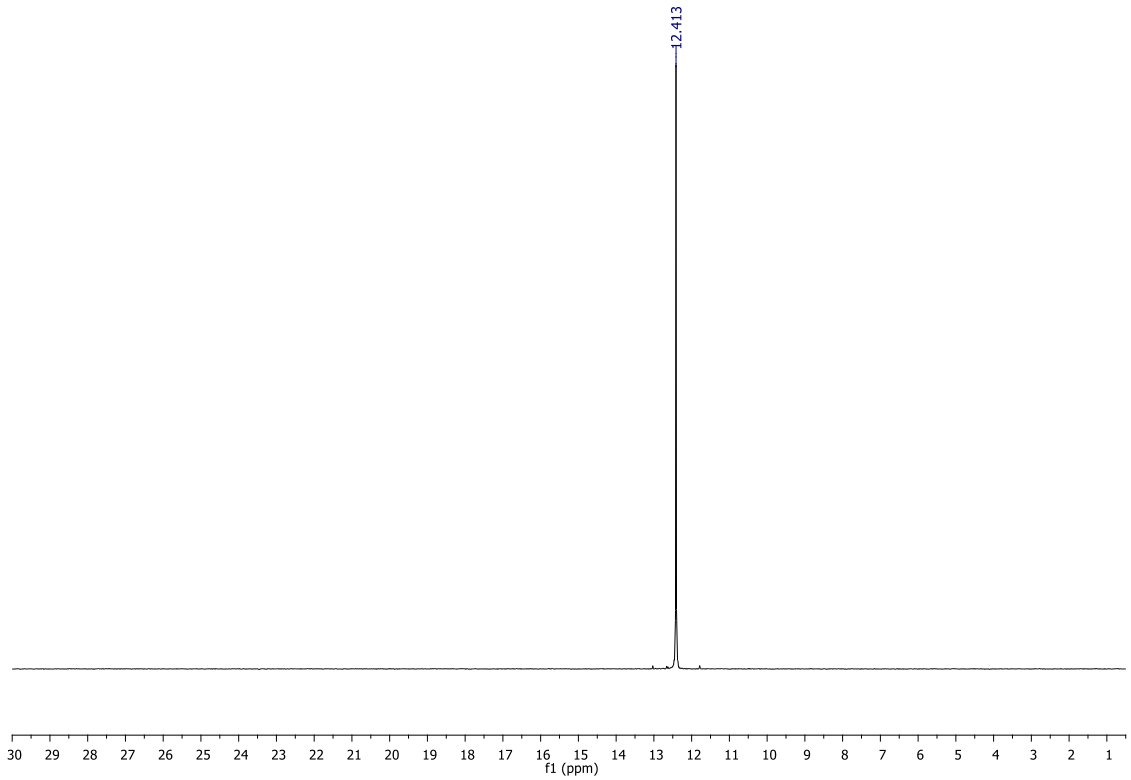
Diethyl [1-(benzyloxycarbonylamino)ethen-1-yl]phosphonate (12)



^{1}H NMR (400 MHz, $CDCl_3$)

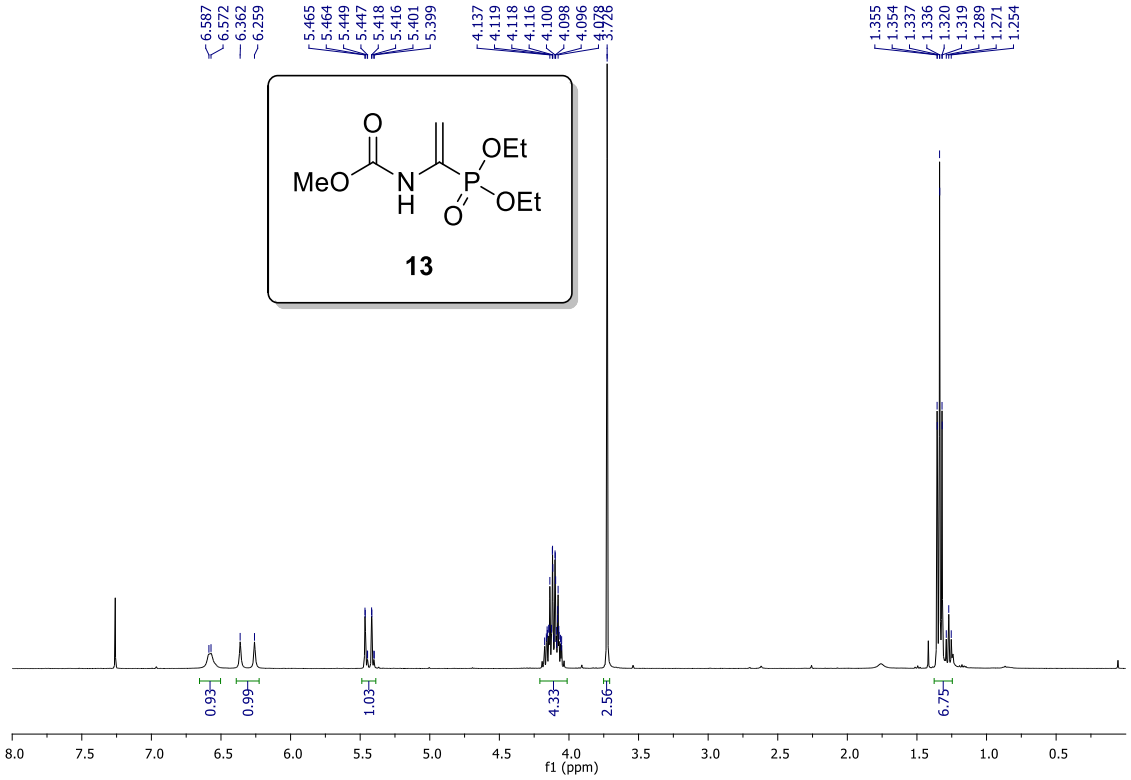


APT NMR (100 MHz, $CDCl_3$)

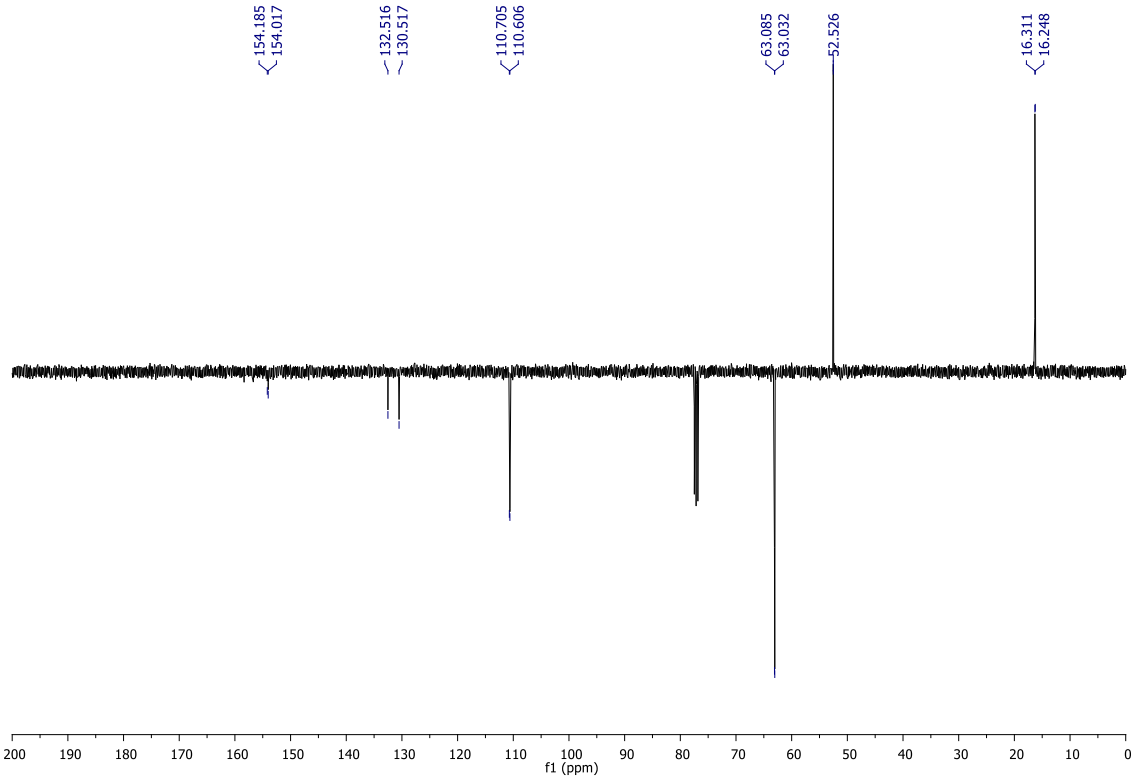


^{31}P NMR (162 MHz, $CDCl_3$)

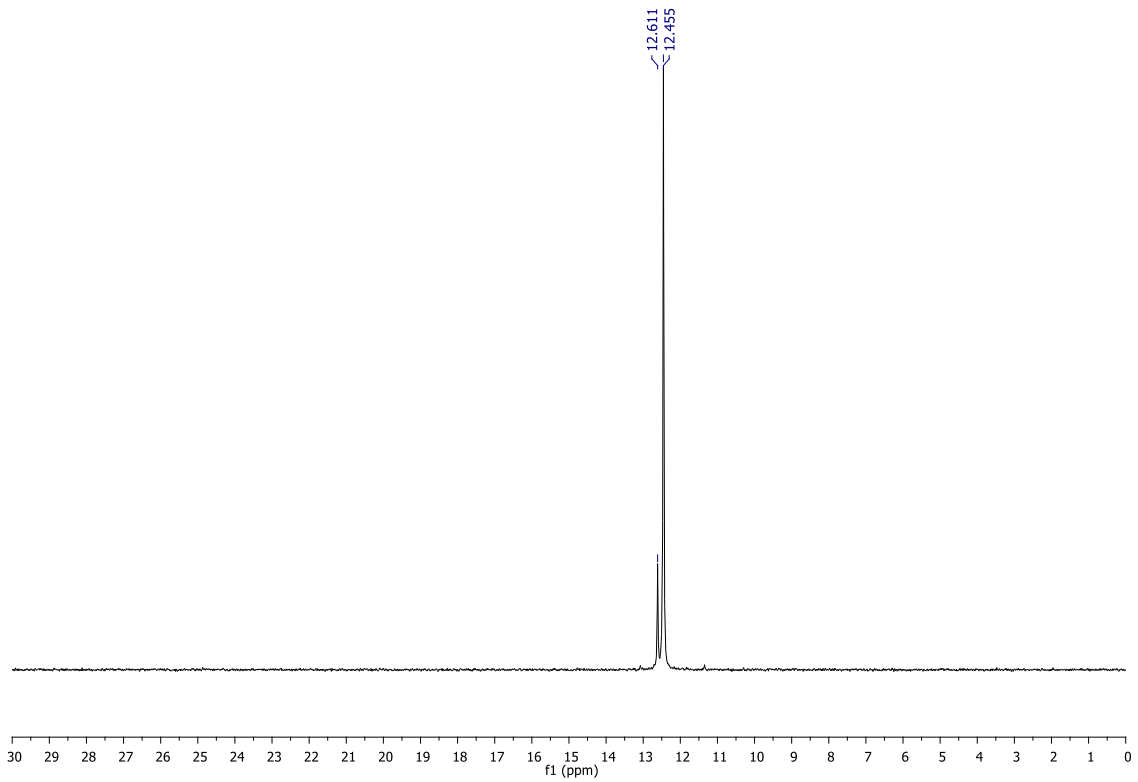
Diethyl [1-(methoxycarbonylamino)ethen-1-yl]phosphonate (13)



^{1}H NMR (400 MHz, $CDCl_3$)

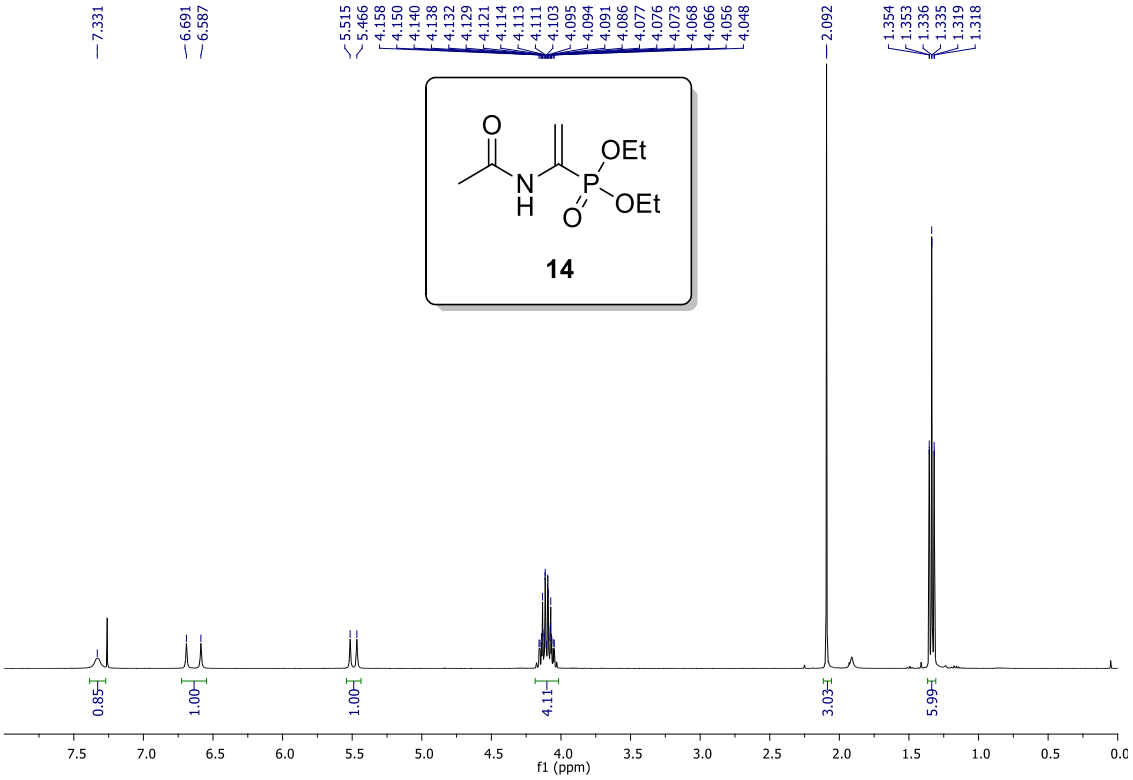


APT NMR (100 MHz, $CDCl_3$)

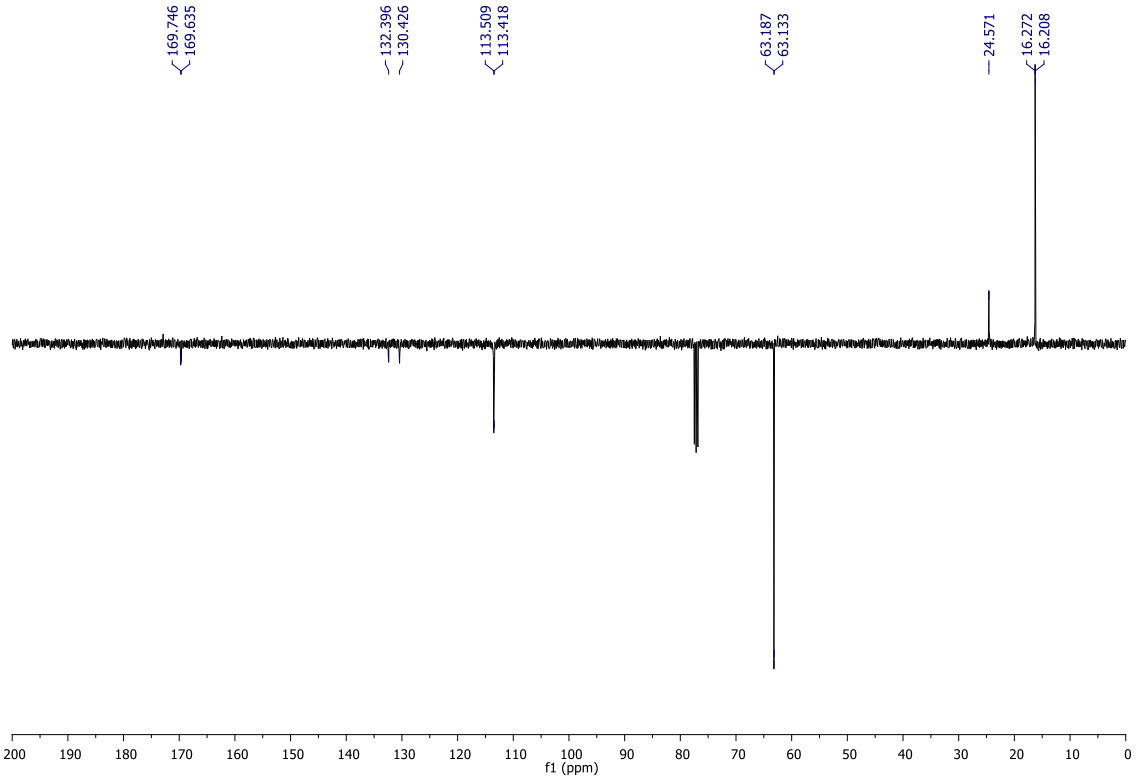


^{31}P NMR (162 MHz, $CDCl_3$)

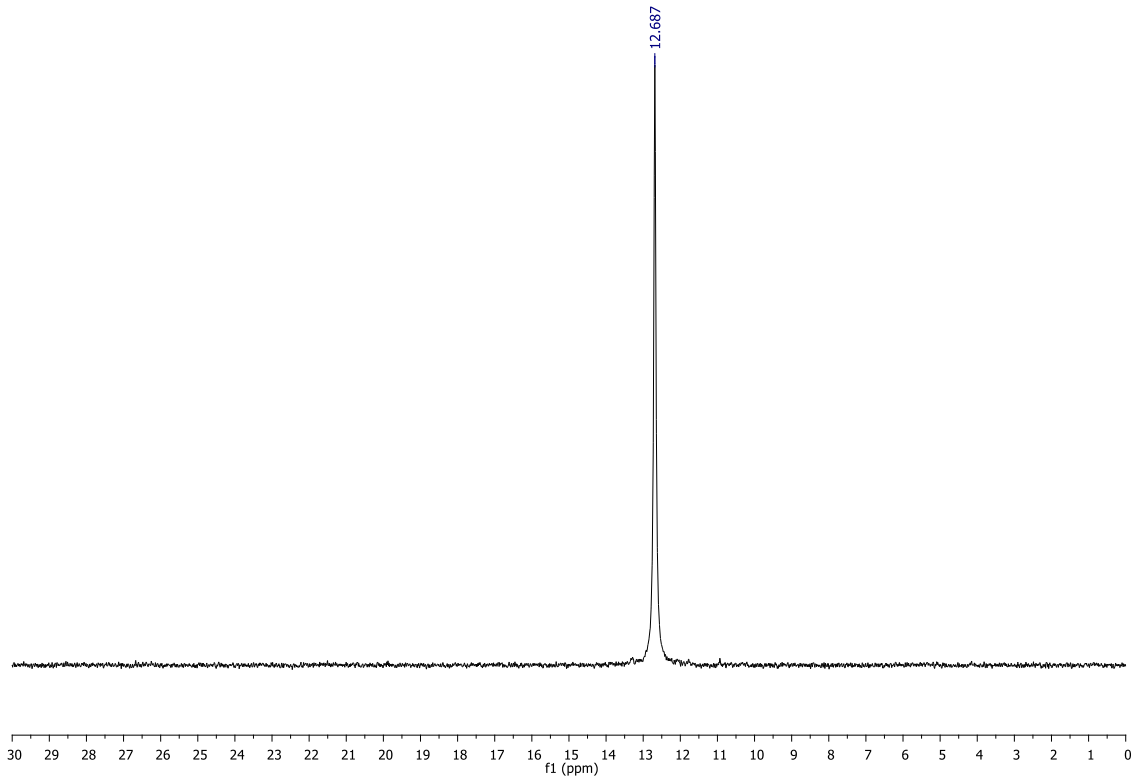
Diethyl [1-(acetylamino)ethen-1-yl]phosphonate (14)



^{1}H NMR (400 MHz, $CDCl_3$)

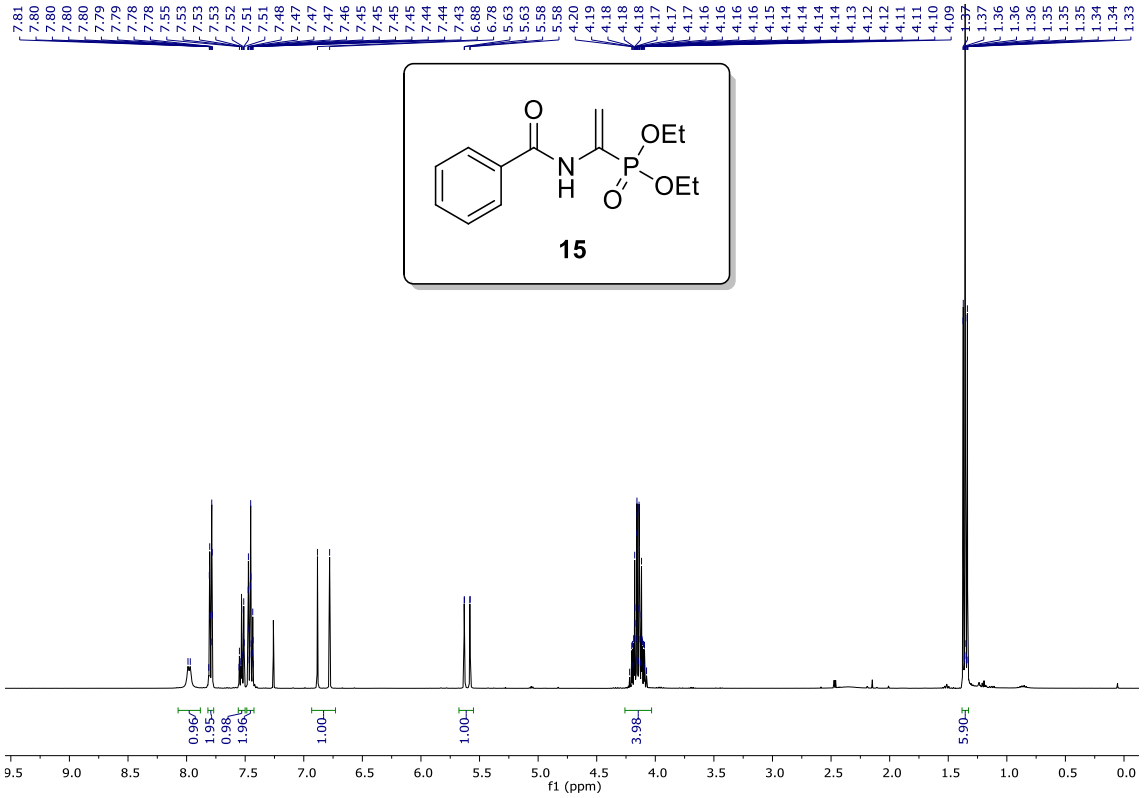


APT NMR (100 MHz, $CDCl_3$)

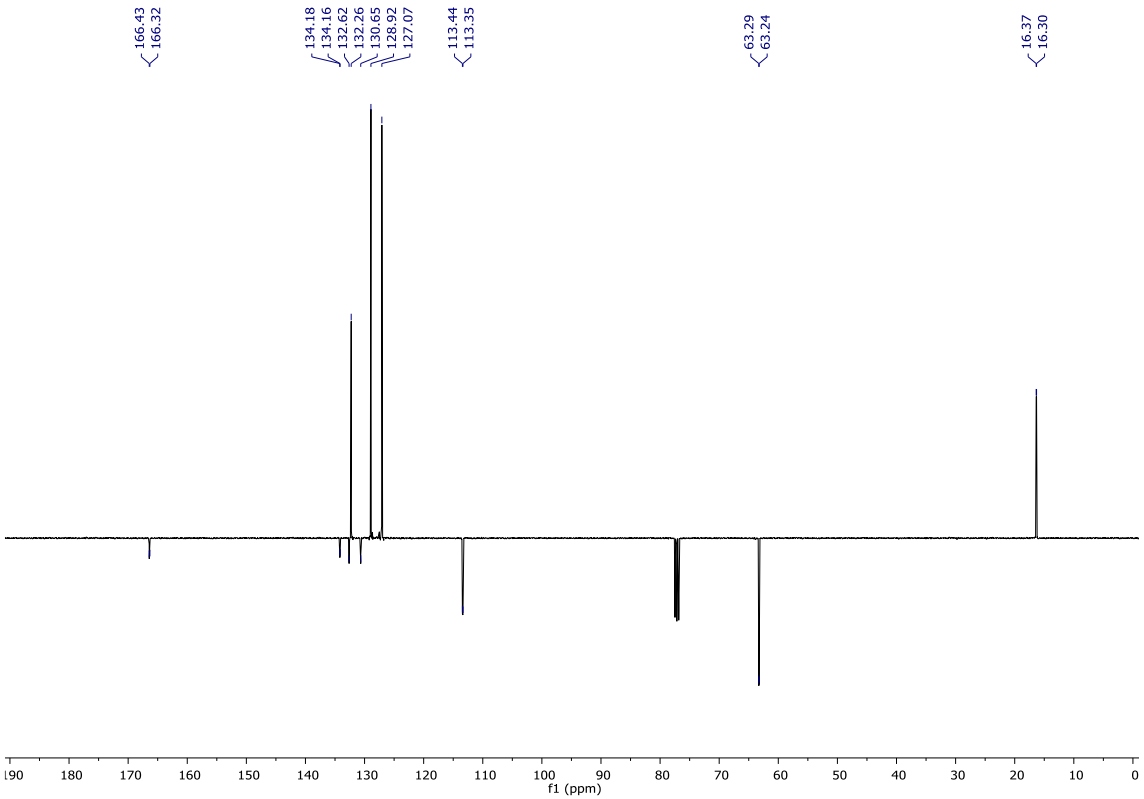


^{31}P NMR (162 MHz, $CDCl_3$)

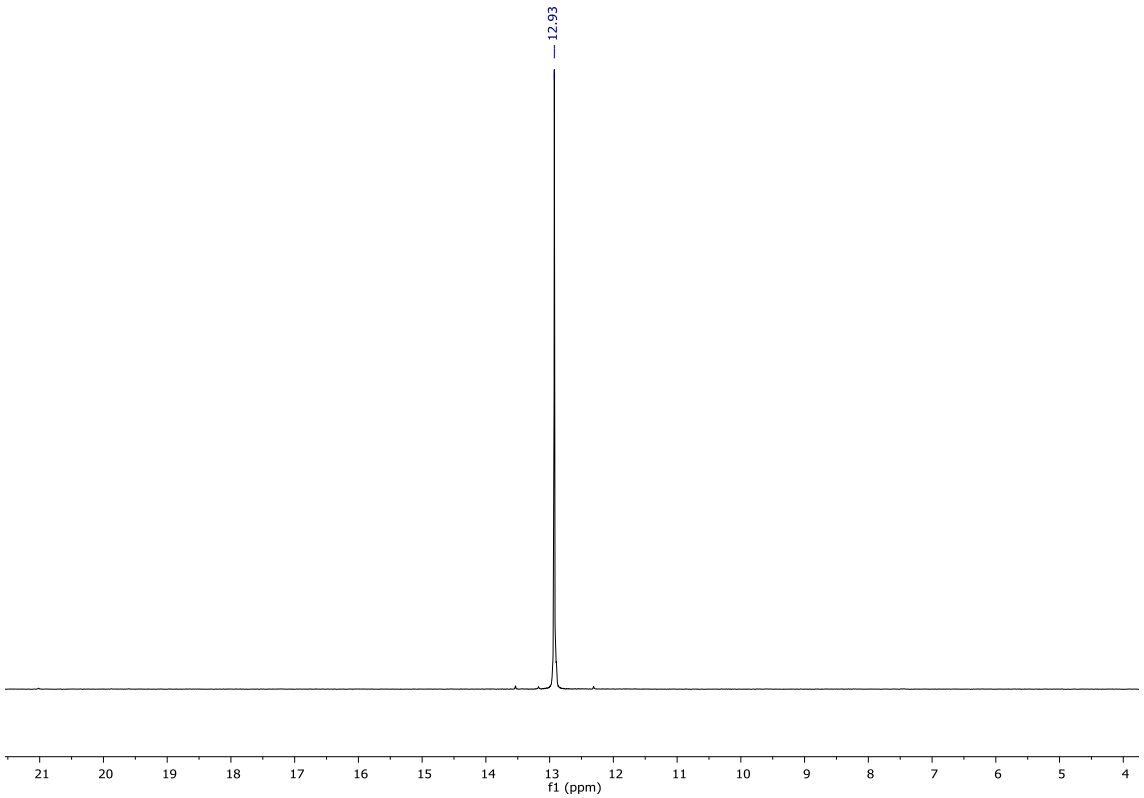
Diethyl [1-(benzoylamino)ethen-1-yl]phosphonate (15)



^{1}H NMR (400 MHz, $CDCl_3$)

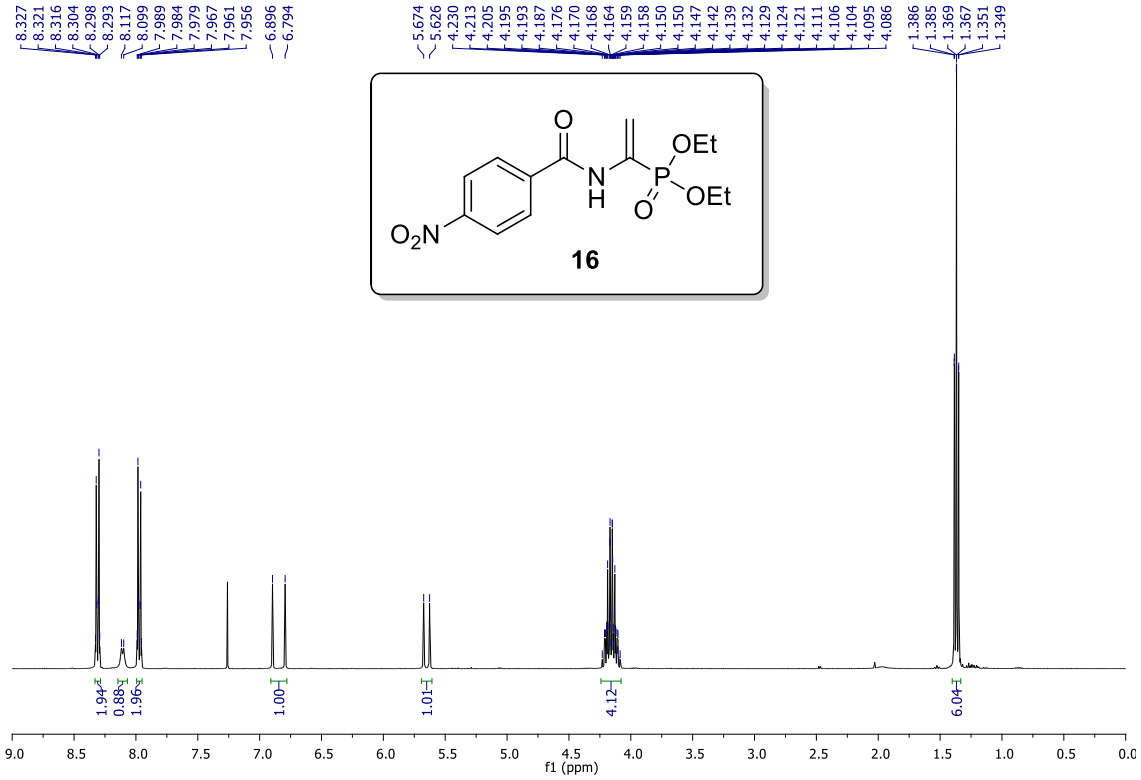


APT NMR (100 MHz, $CDCl_3$)

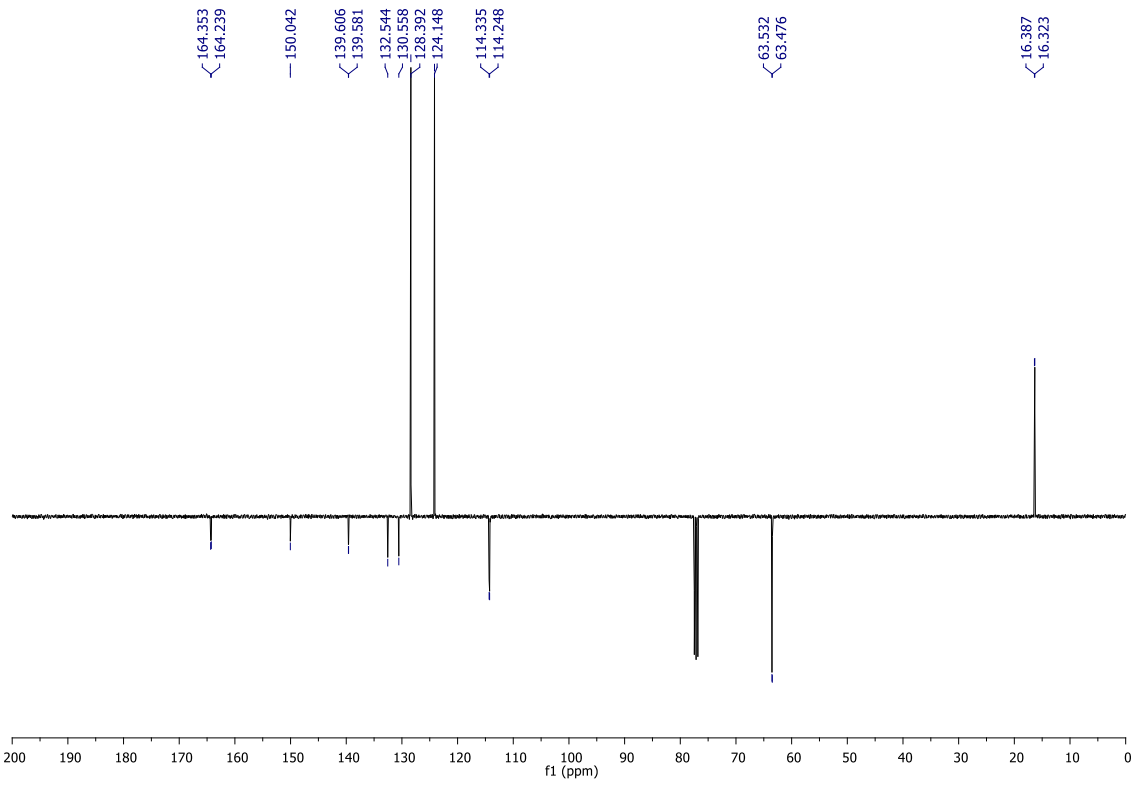


^{31}P NMR (162 MHz, $CDCl_3$)

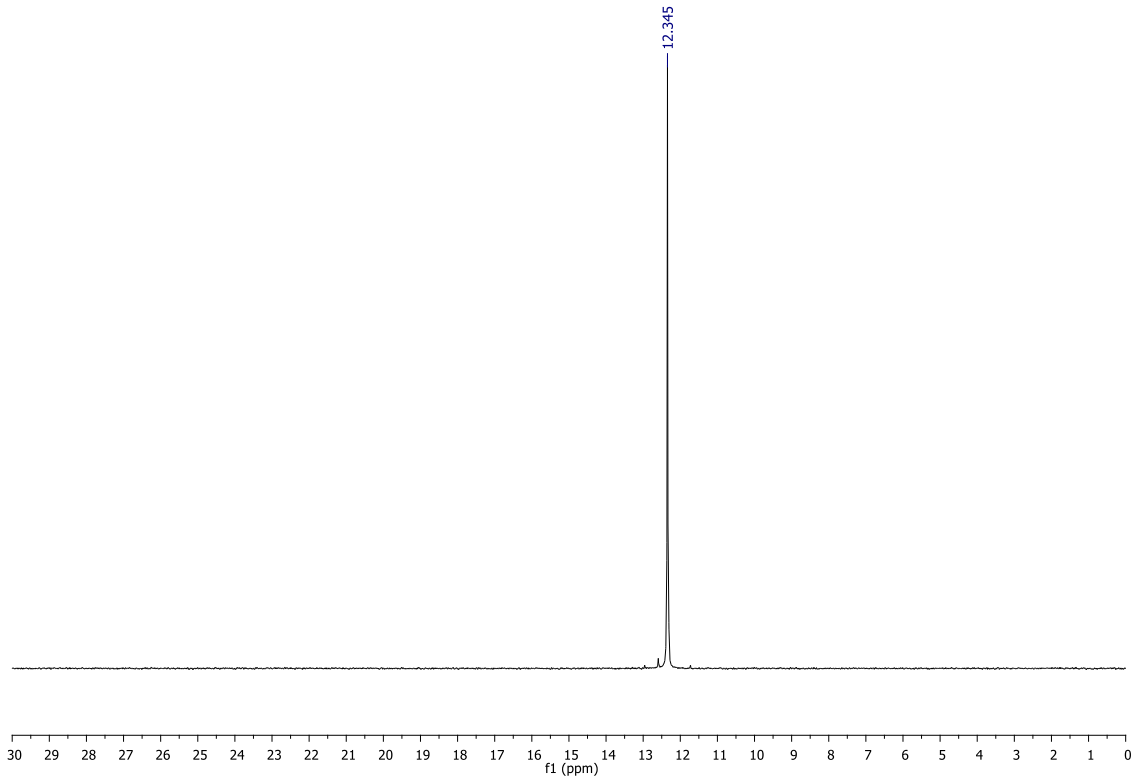
Diethyl [1-(*p*-nitrobenzoylamino)ethen-1-yl]phosphonate (16)



^{1}H NMR (400 MHz, $CDCl_3$)

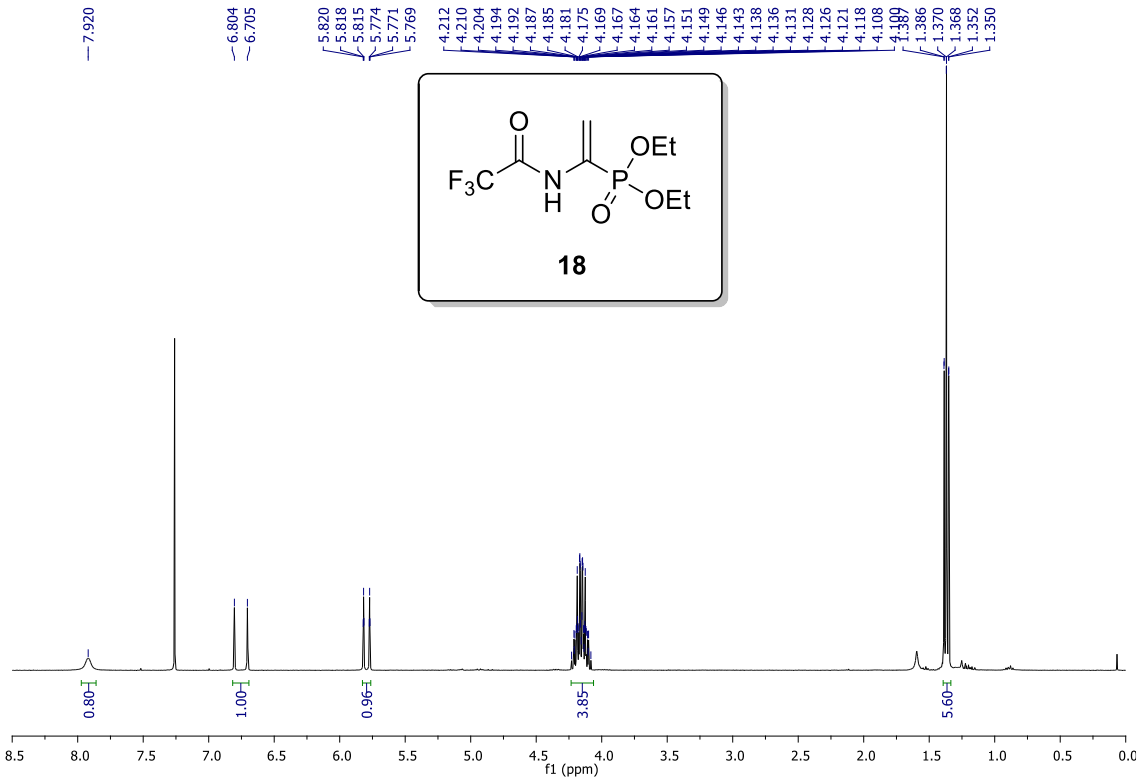


APT NMR (100 MHz, $CDCl_3$)

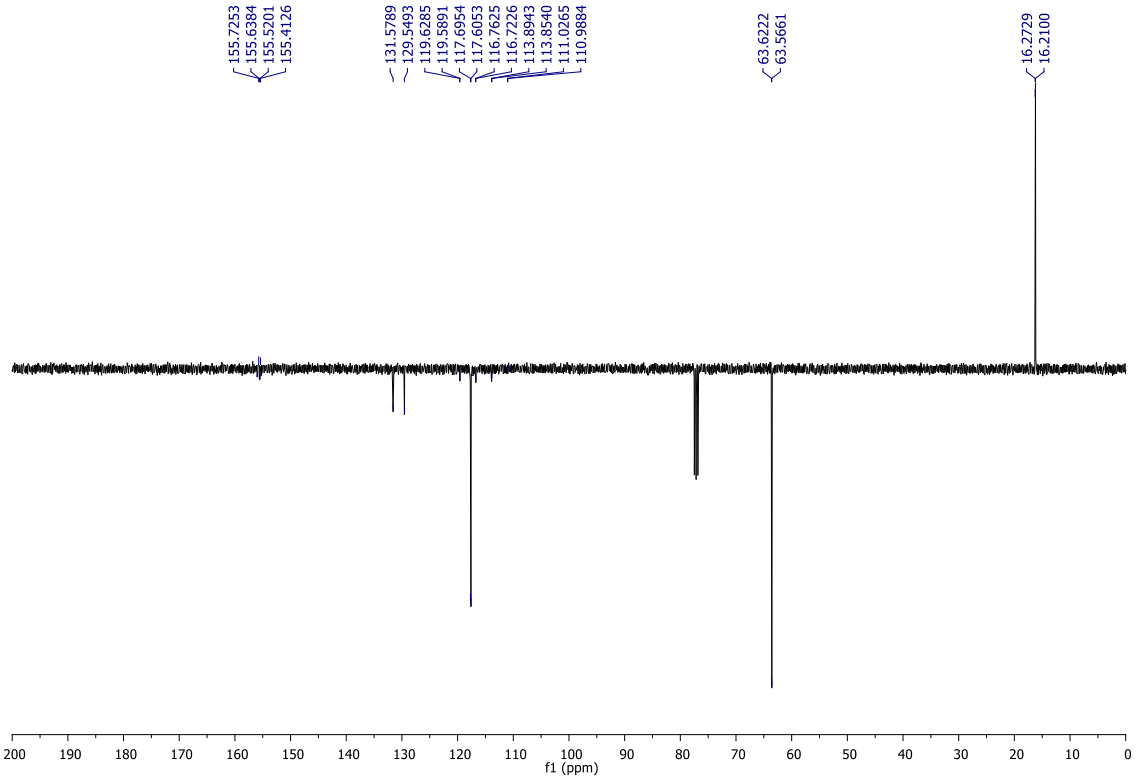


^{31}P NMR (162 MHz, $CDCl_3$)

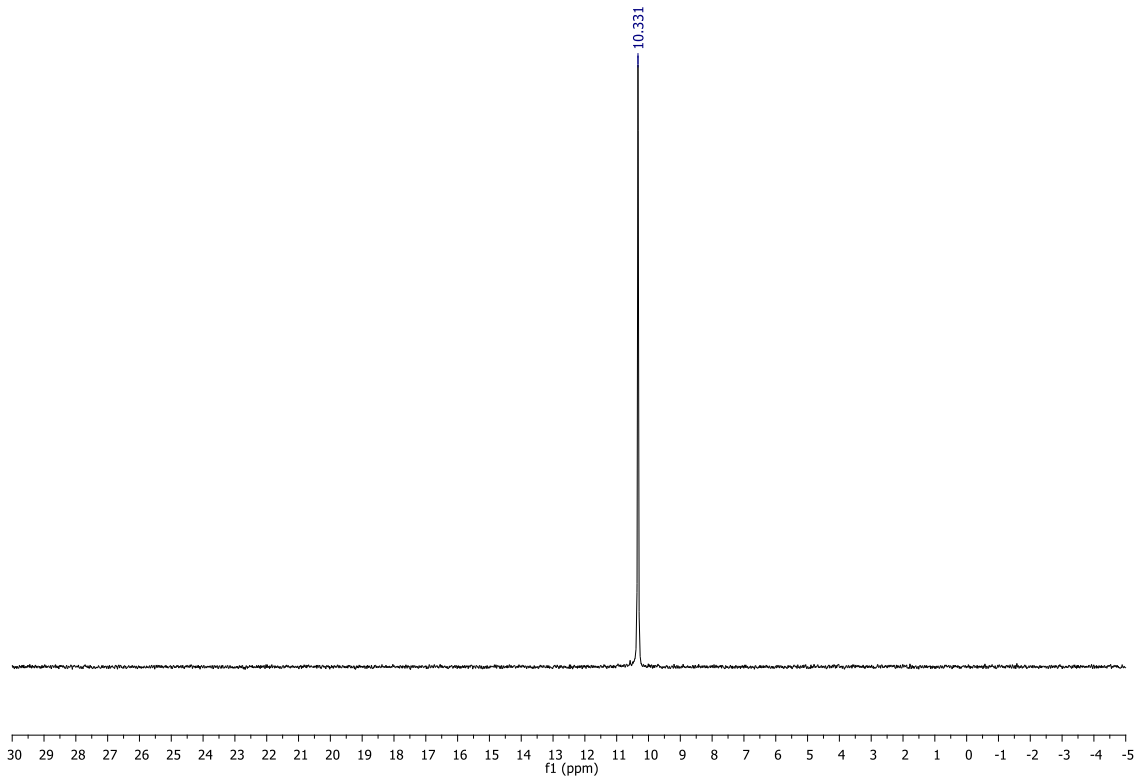
Diethyl [1-(trifluoroacetylamino)ethen-1-yl]phosphonate (17)



^{1}H NMR (400 MHz, $CDCl_3$)

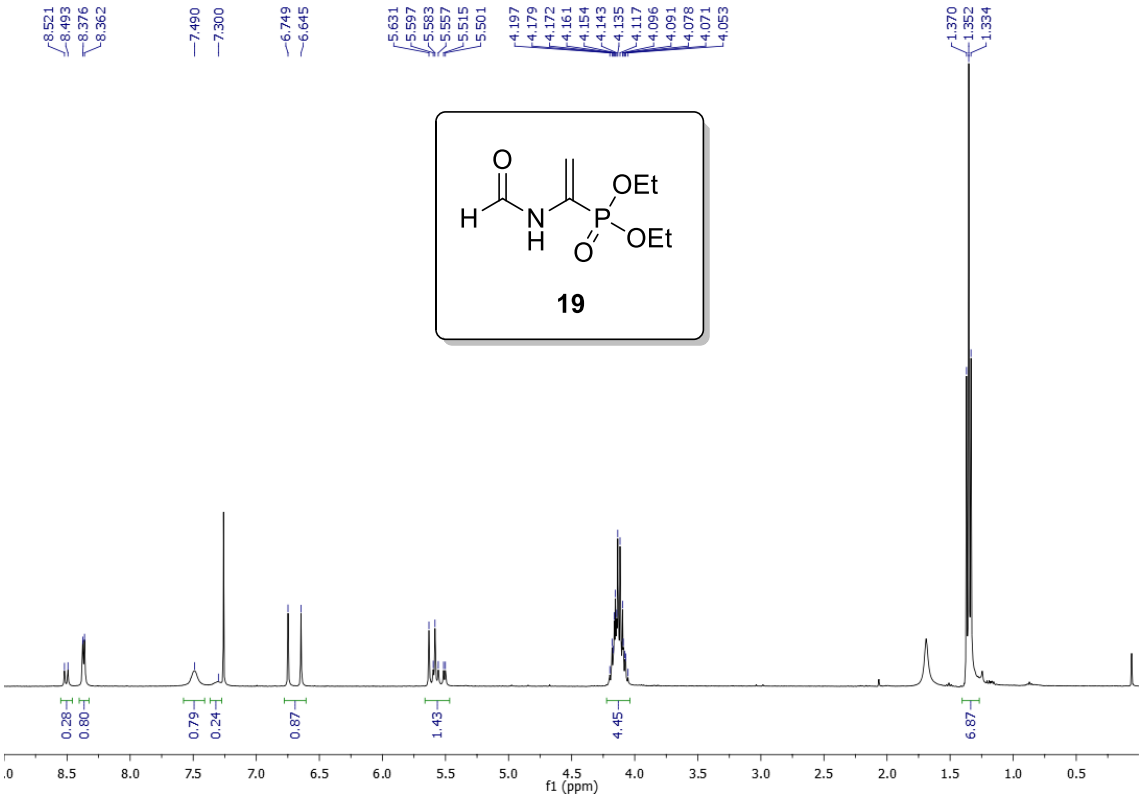


APT NMR (100 MHz, $CDCl_3$)

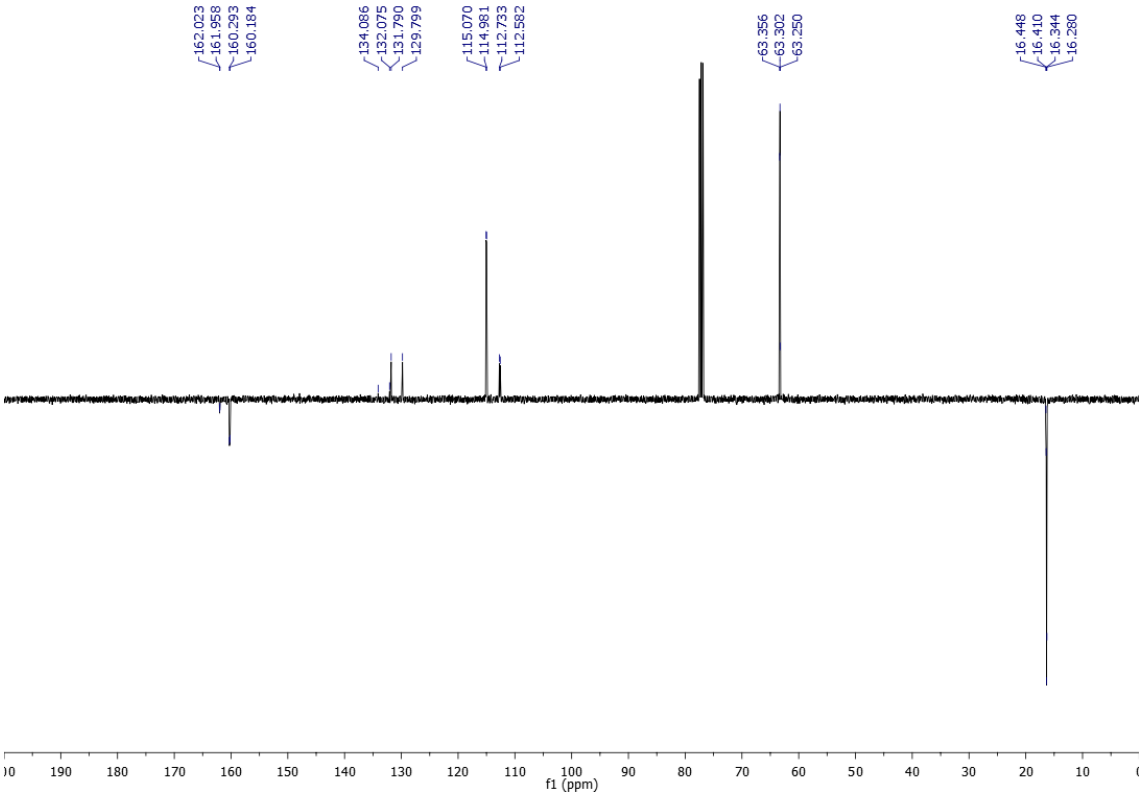


^{31}P NMR (162 MHz, $CDCl_3$)

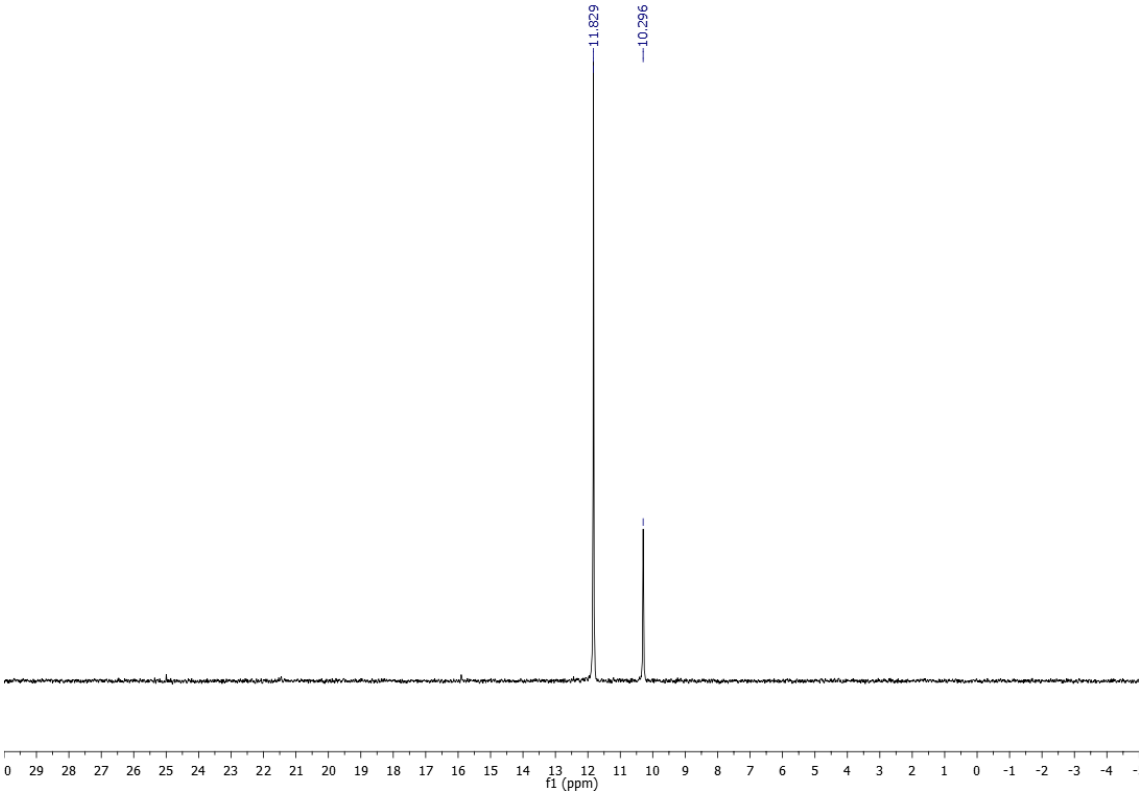
Diethyl [1-(formylamino)ethen-1-yl]phosphonate (19)



^{1}H NMR (400 MHz, $CDCl_3$)

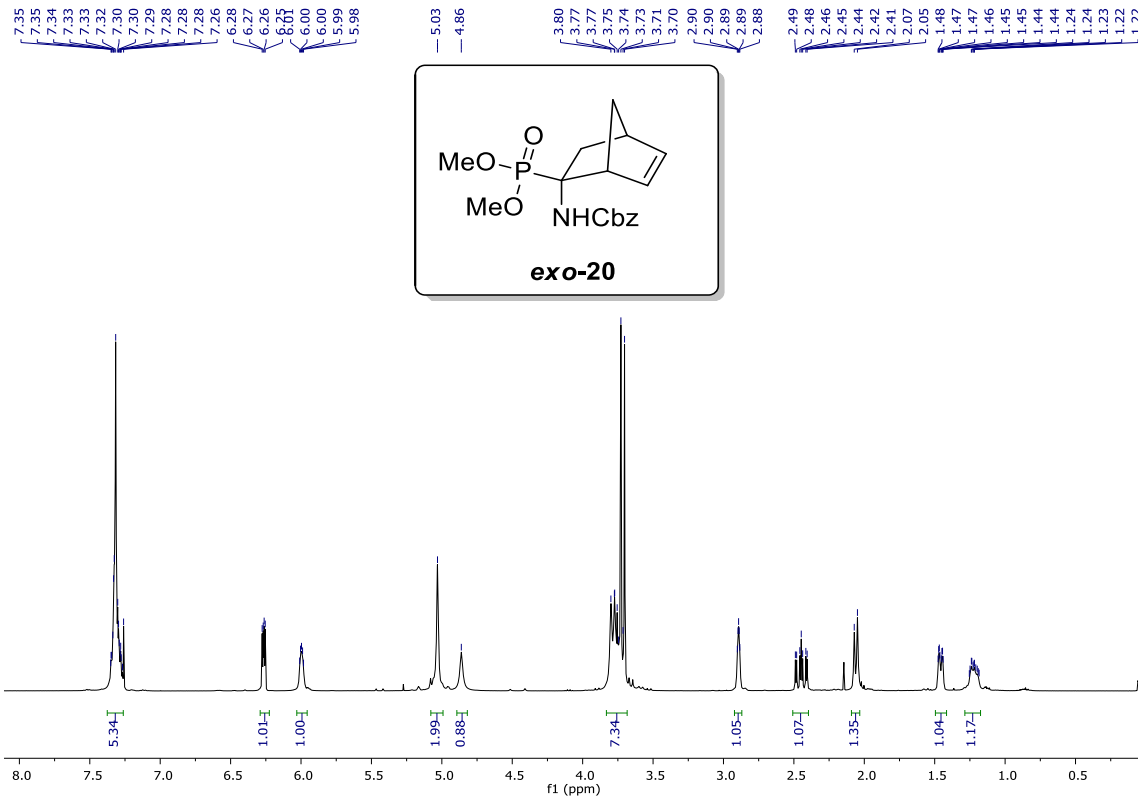


APT NMR (100 MHz, $CDCl_3$)

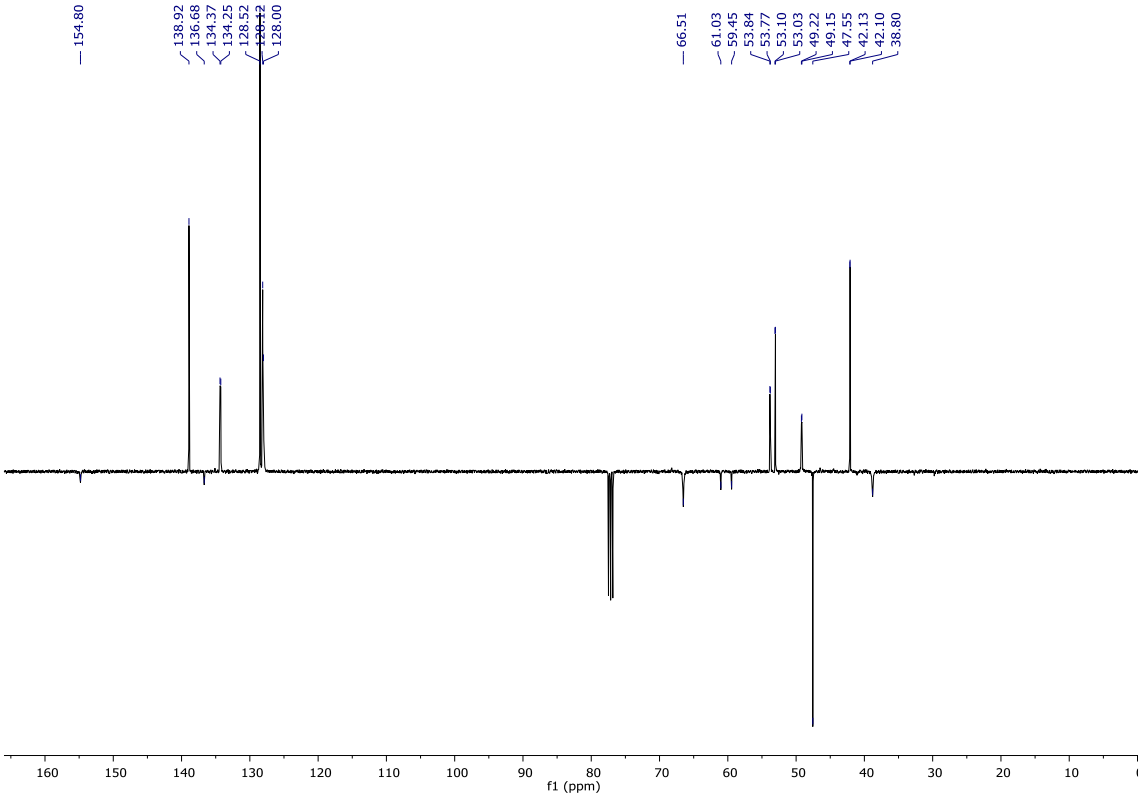


^{31}P NMR (162 MHz, $CDCl_3$)

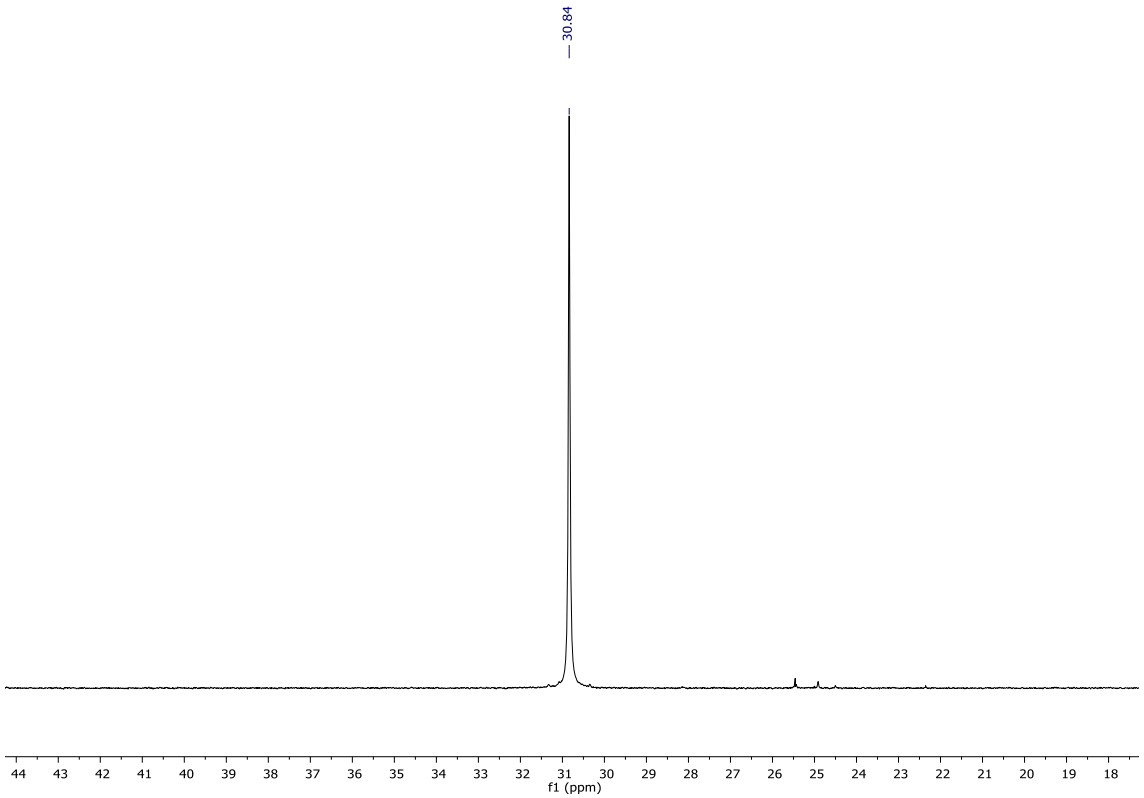
Dimethyl {(1*R,2*S**,4*R**)-2-(*N*-bencyloxycarbonylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*exo*-20)**



^{1}H NMR (400 MHz, $CDCl_3$)

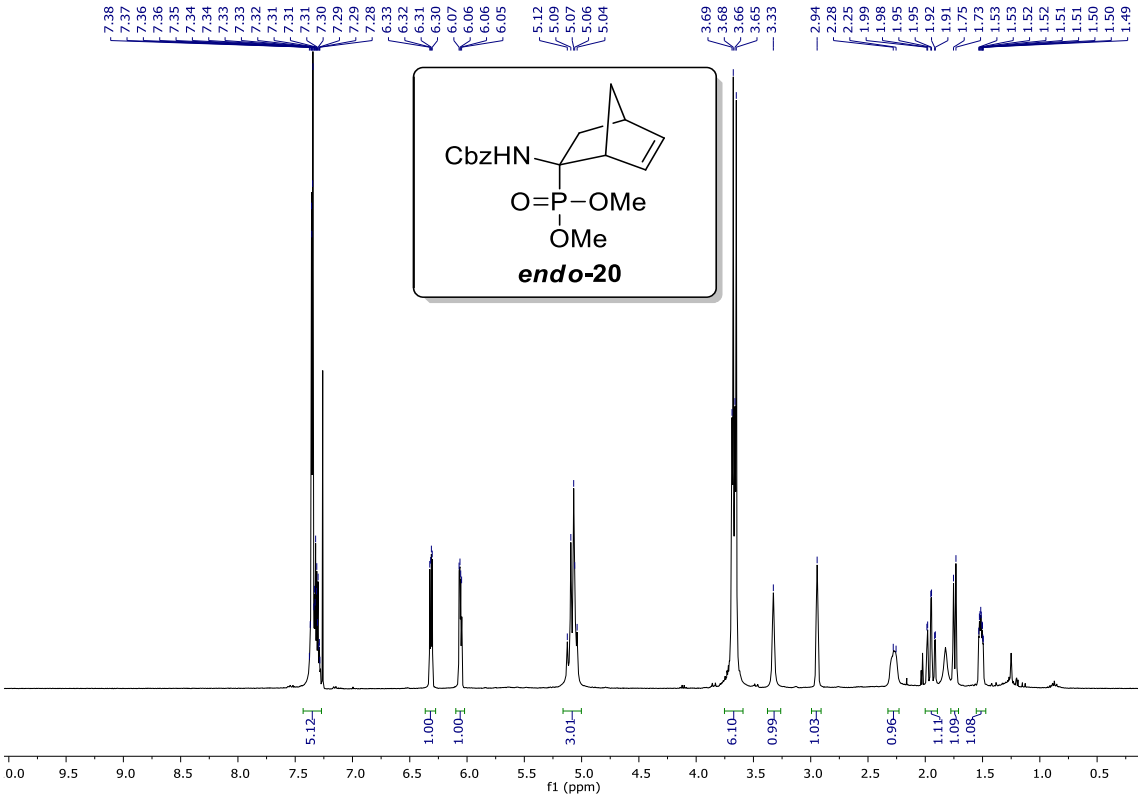


APT NMR (100 MHz, $CDCl_3$)

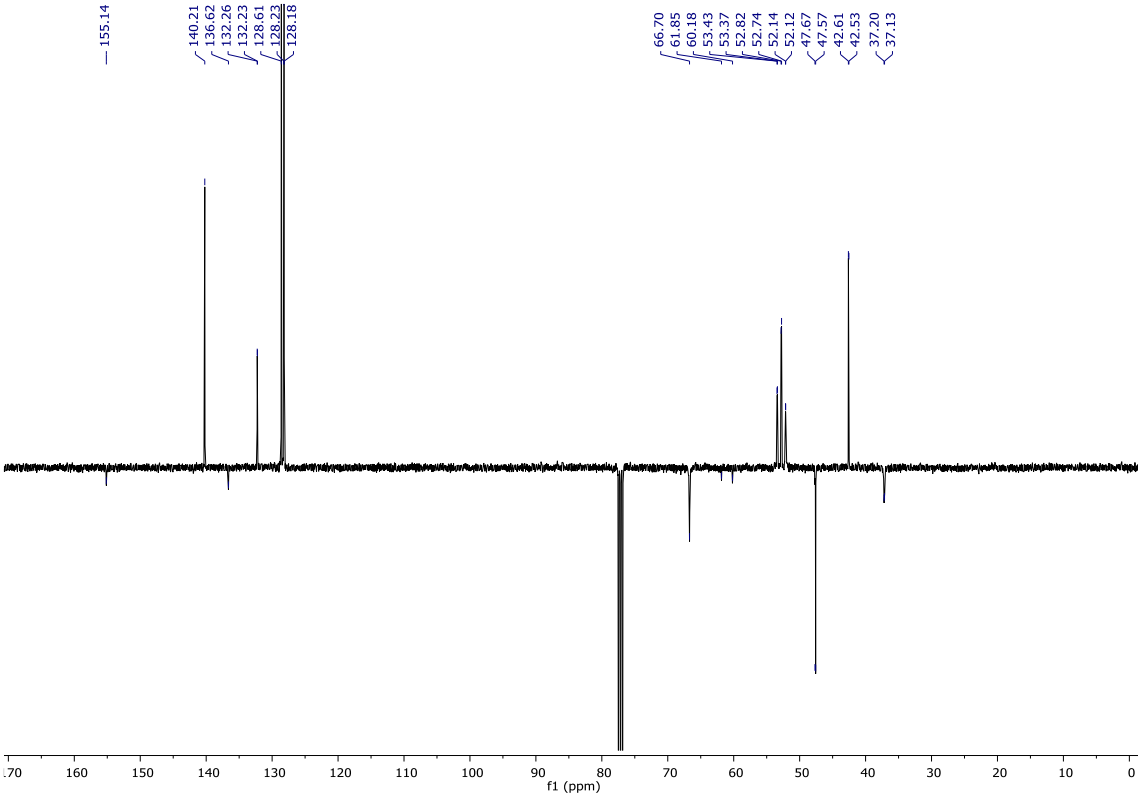


^{31}P NMR (162 MHz, $CDCl_3$)

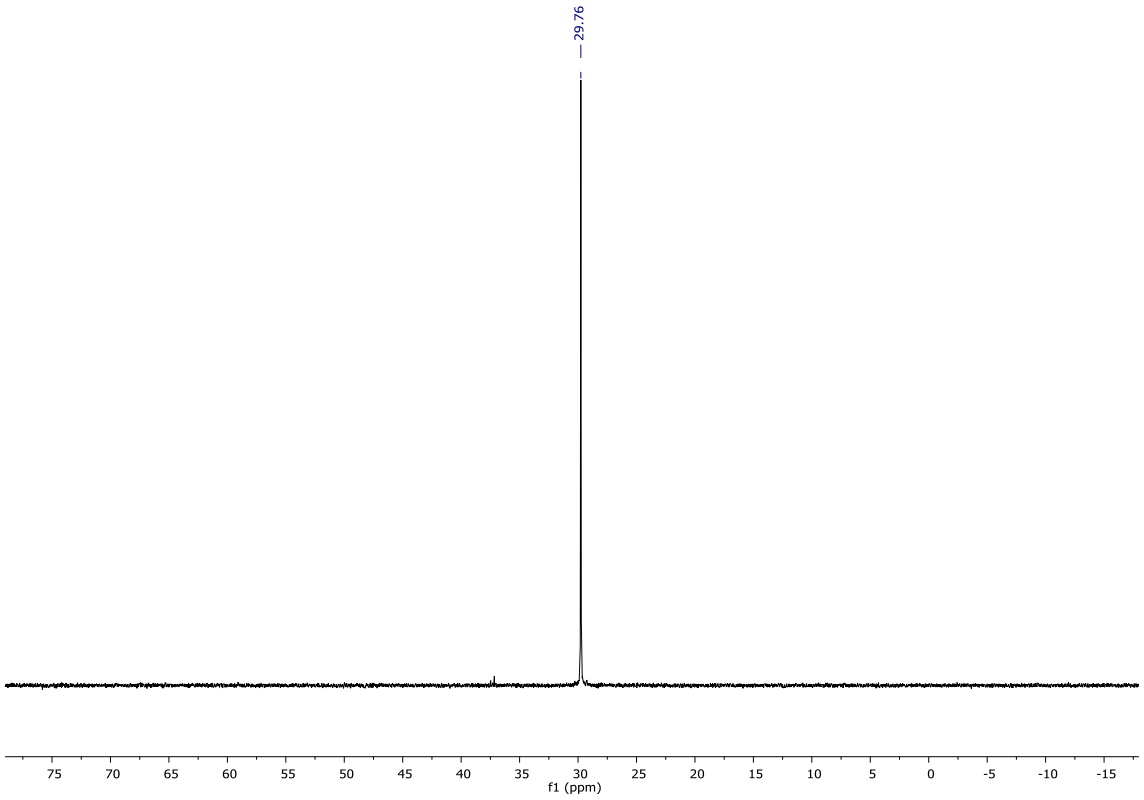
Dimethyl {(1*R,2*R**,4*R**)-2-(*N*-bencyloxycarbonylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*endo*-20)**



^{1}H NMR (400 MHz, $CDCl_3$)

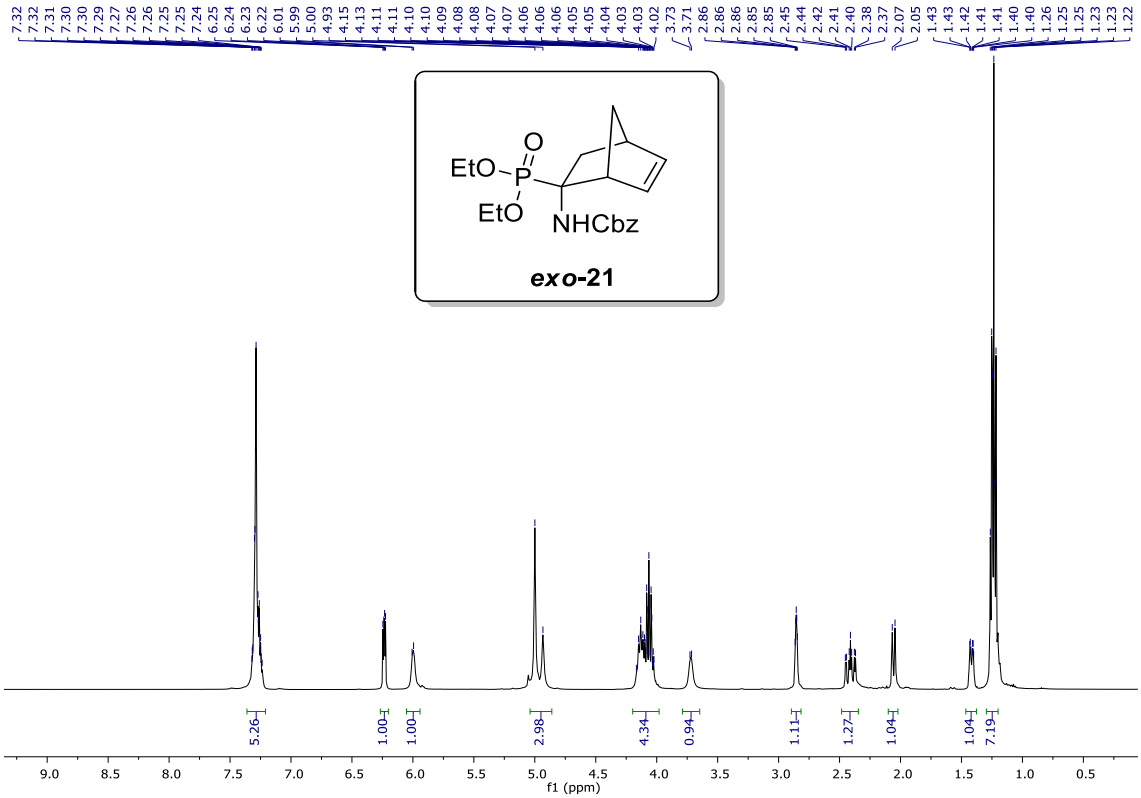


APT NMR (100 MHz, $CDCl_3$)

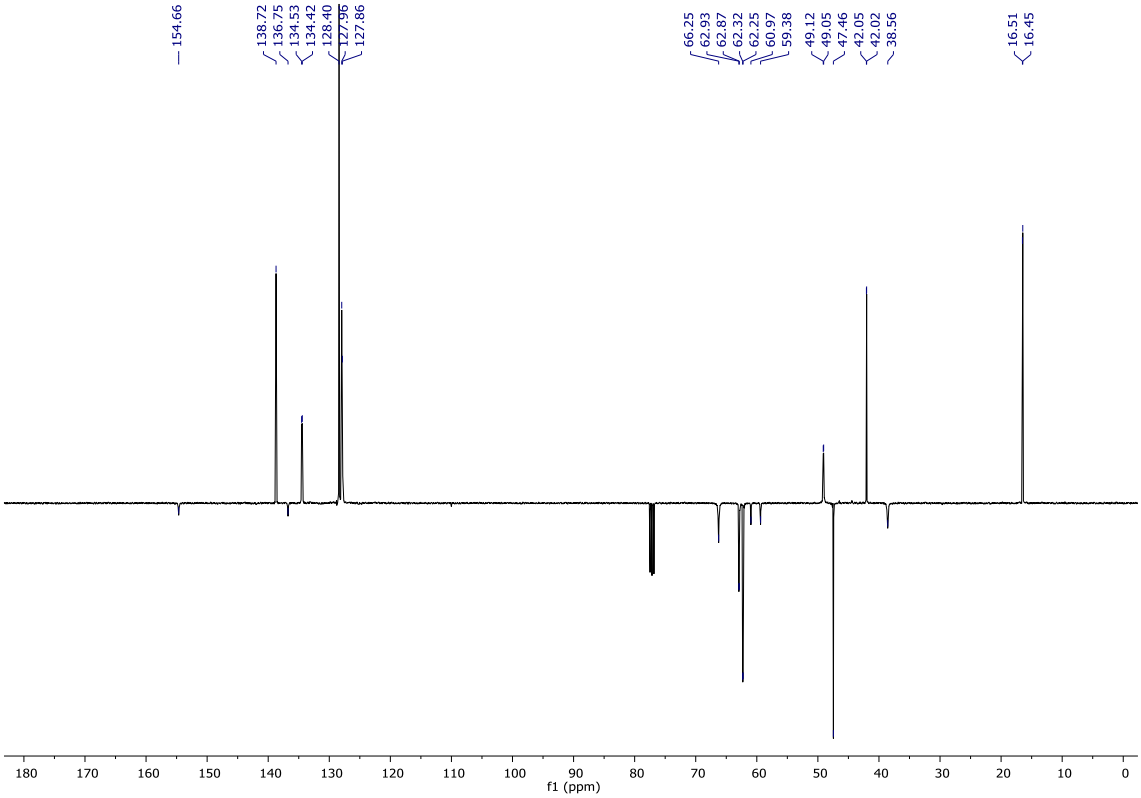


^{31}P NMR (162 MHz, $CDCl_3$)

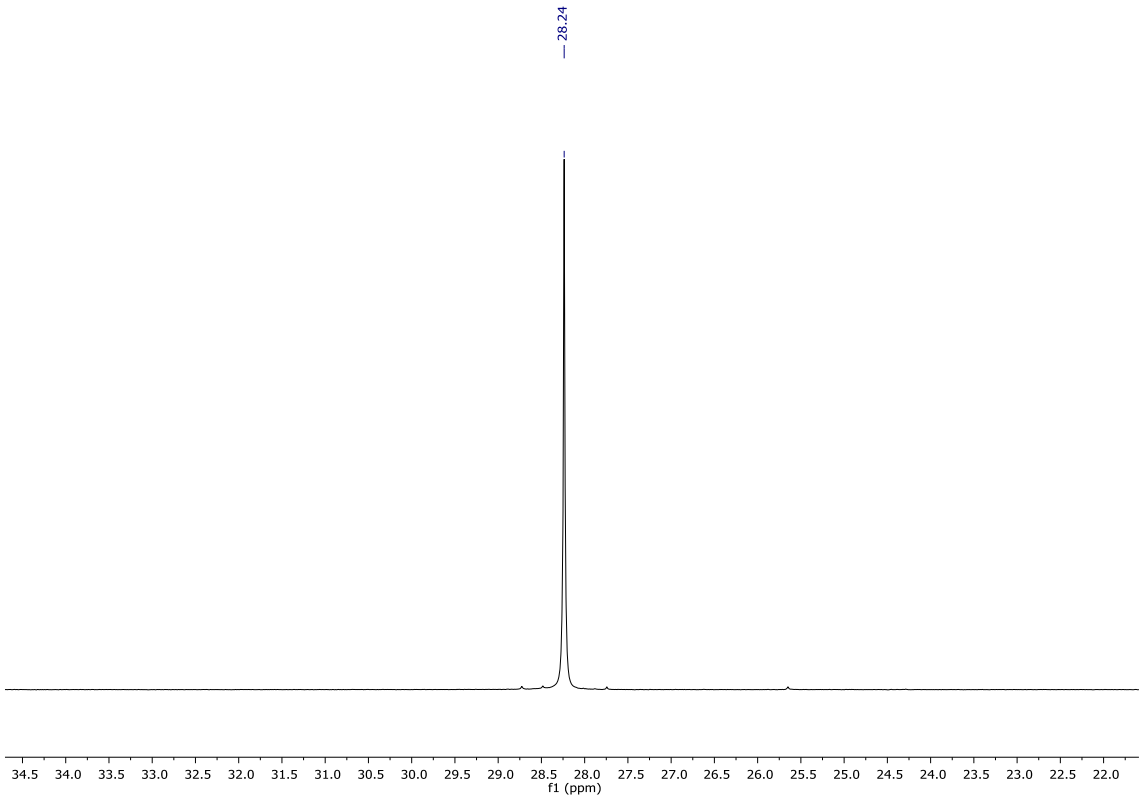
Diethyl {(1*R,2*S**,4*R**)-2-(*N*-bencyloxycarbonylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*exo*-21)**



^{1}H NMR (400 MHz, $CDCl_3$)

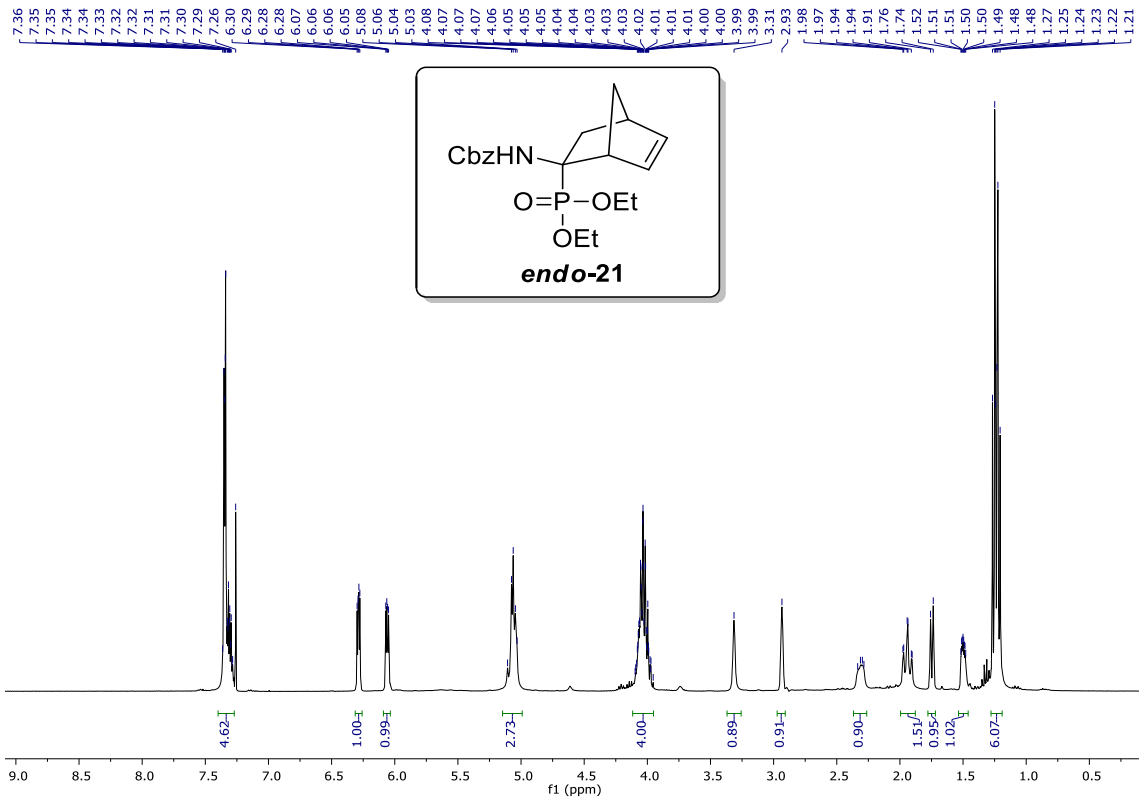


APT NMR (100 MHz, $CDCl_3$)

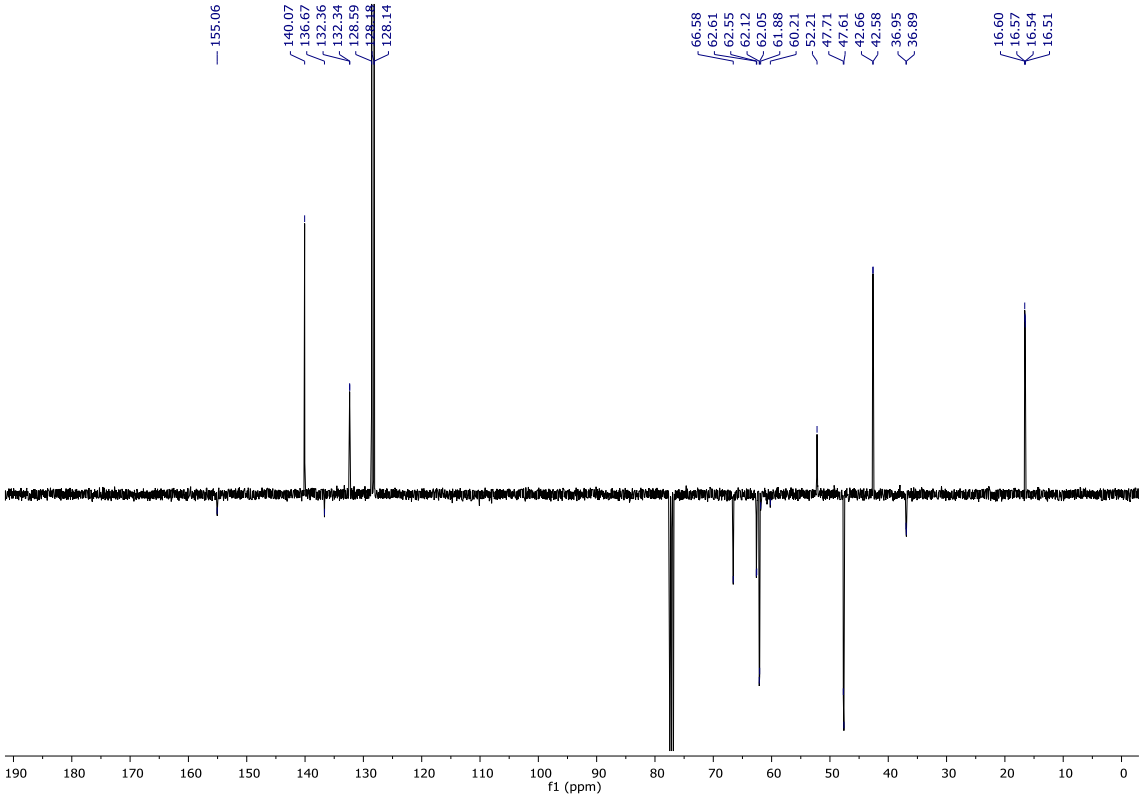


^{31}P NMR (162 MHz, $CDCl_3$)

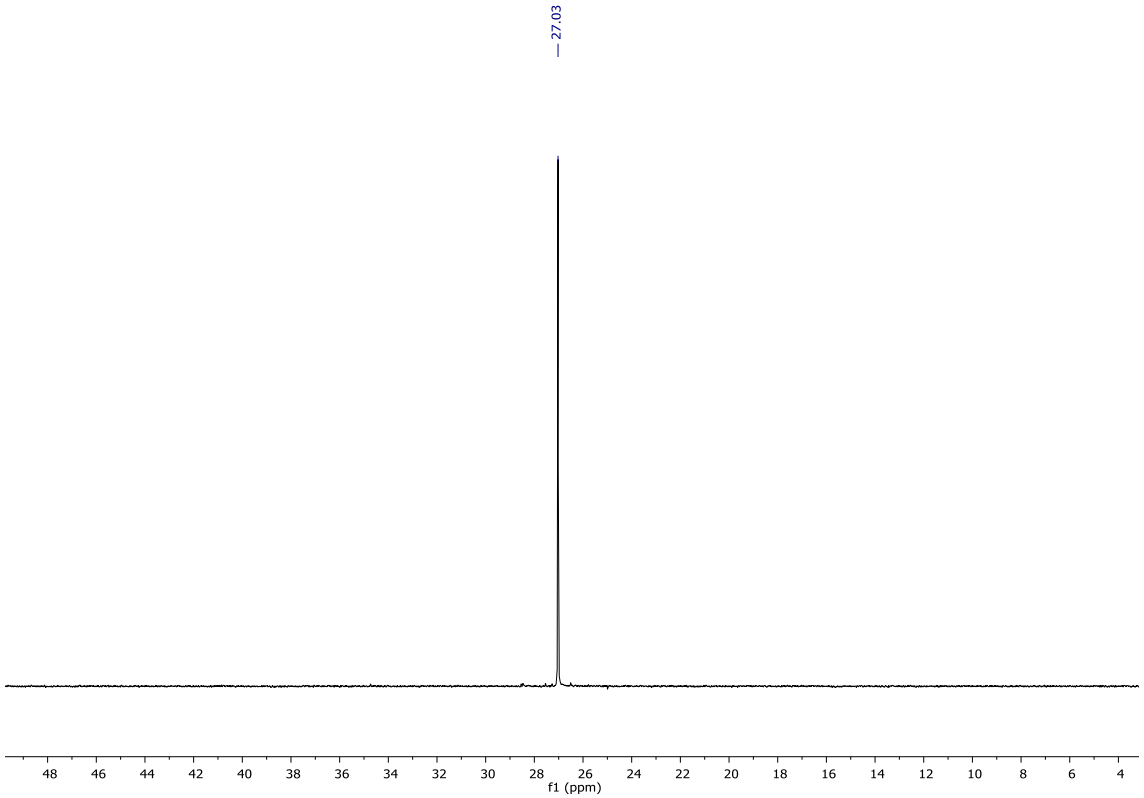
Diethyl {(1*R,2*R**,4*R**)-2-(*N*-bencyloxycarbonylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*endo*-21)**



^{1}H NMR (400 MHz, $CDCl_3$)

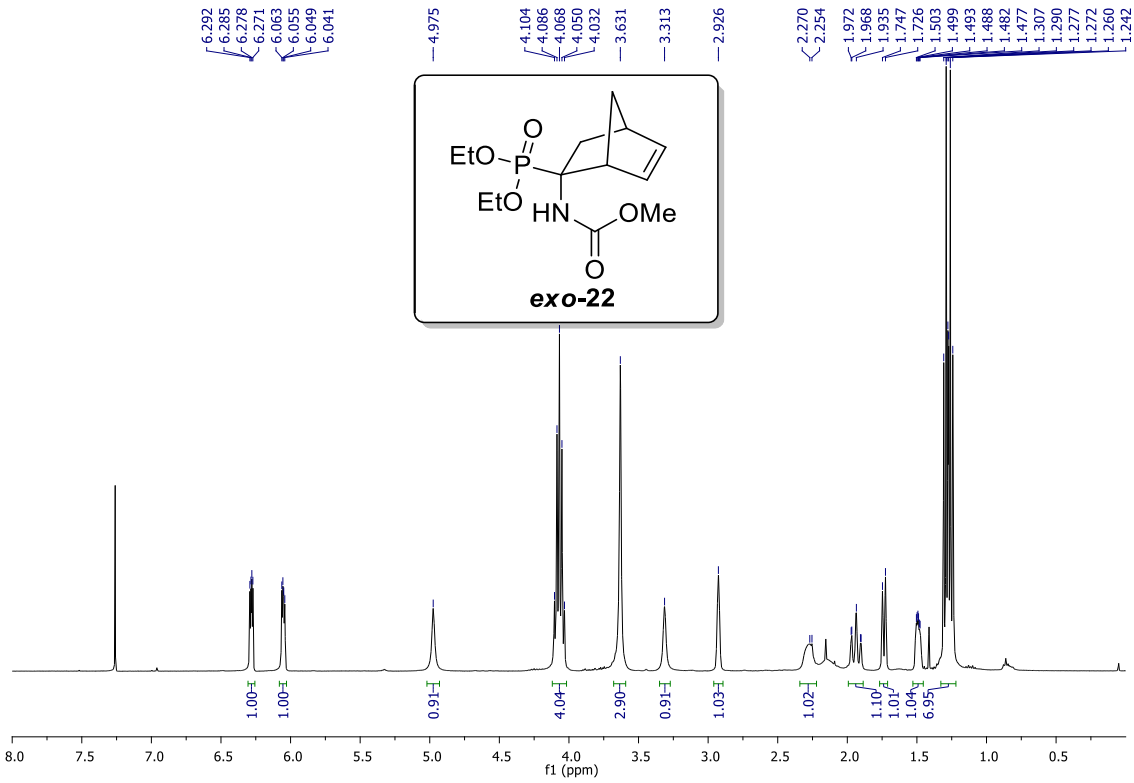


APT NMR (100 MHz, $CDCl_3$)

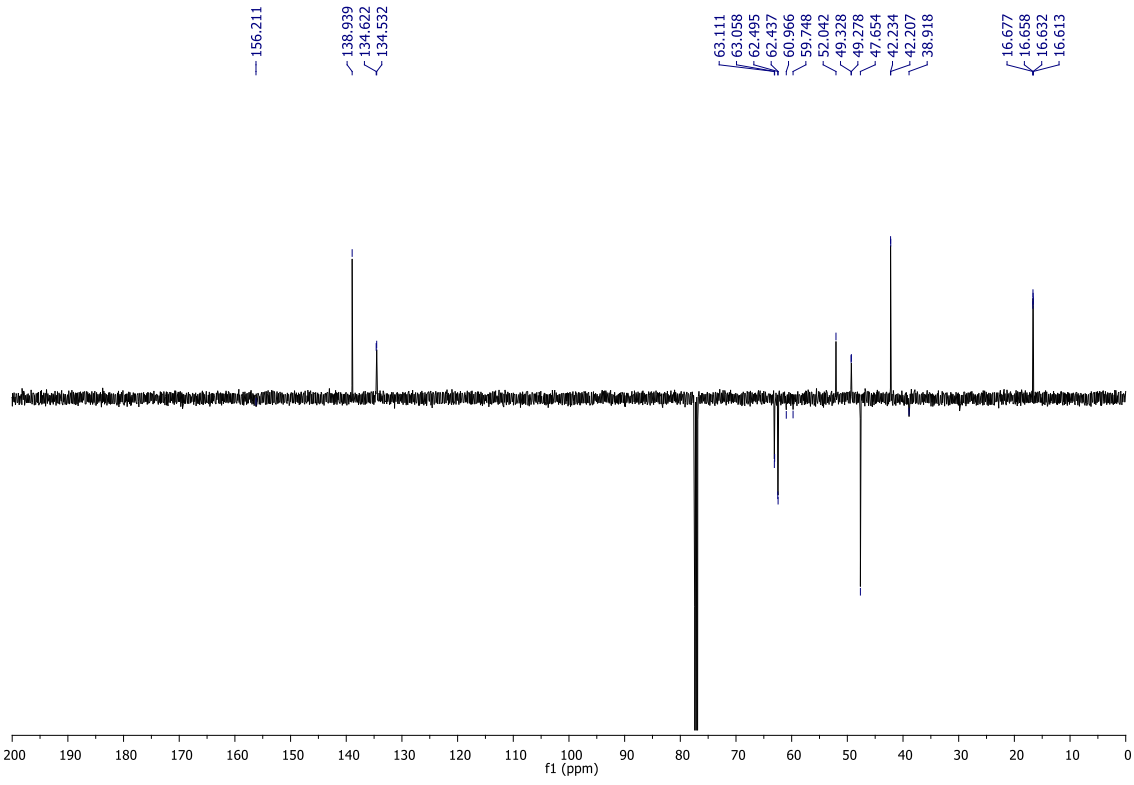


^{31}P NMR (162 MHz, $CDCl_3$)

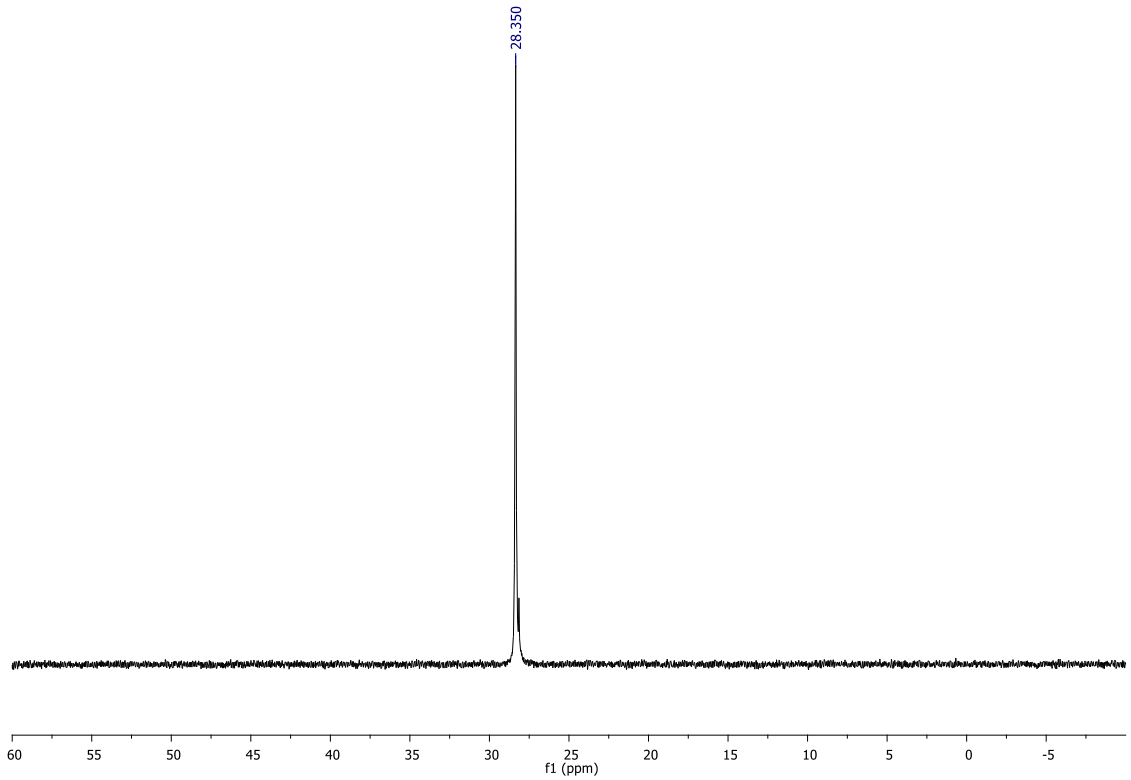
Diethyl {(1*R,2*S**,4*R**)-2-(*N*-methoxycarbonylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*exo*-22)**



^{1}H NMR (400 MHz, $CDCl_3$)

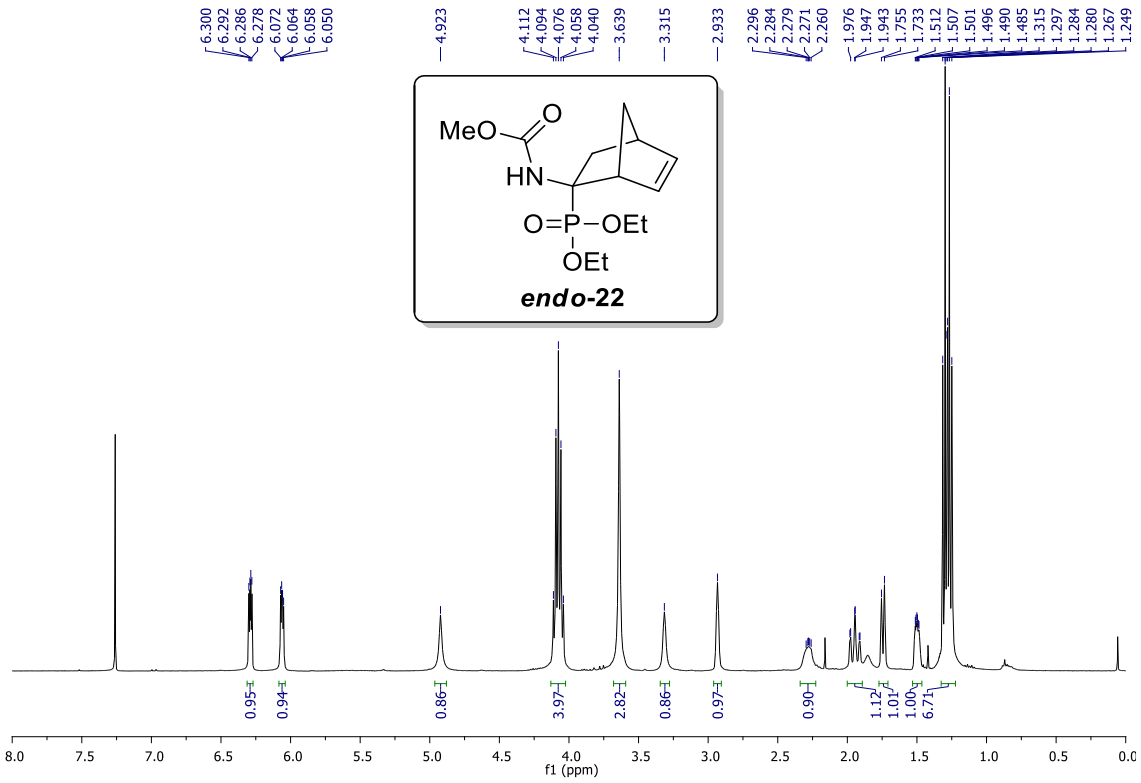


APT NMR (100 MHz, $CDCl_3$)

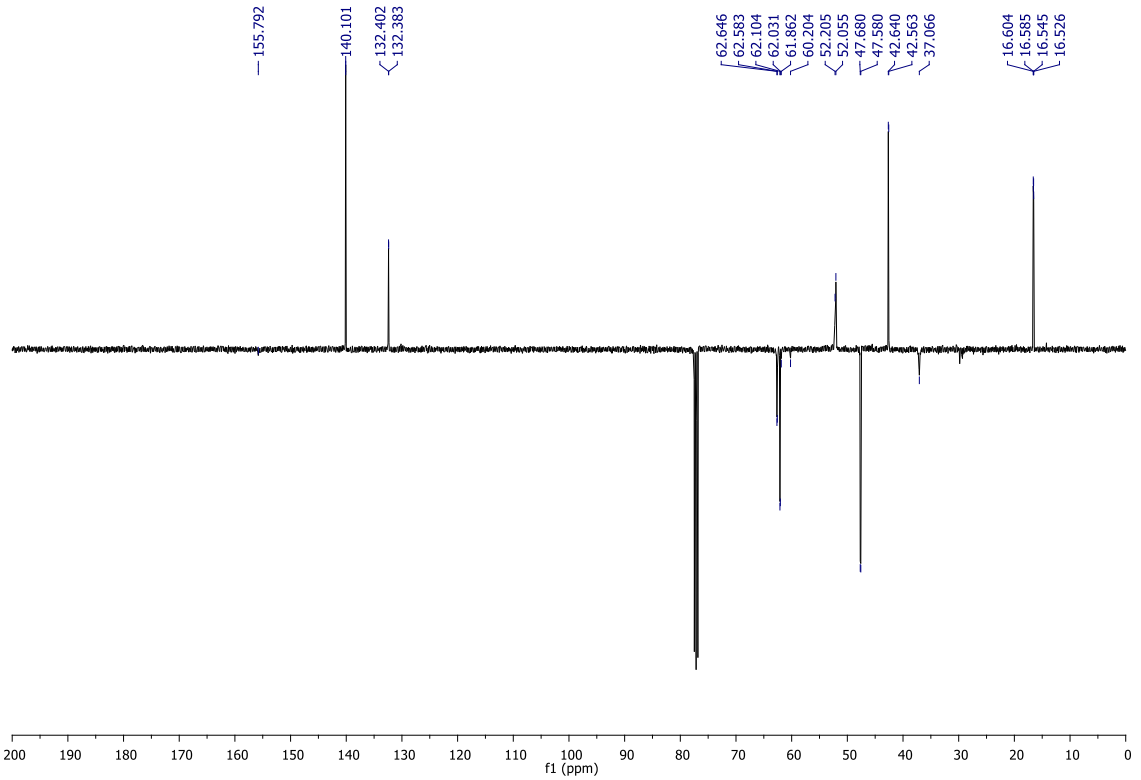


^{31}P NMR (162 MHz, $CDCl_3$)

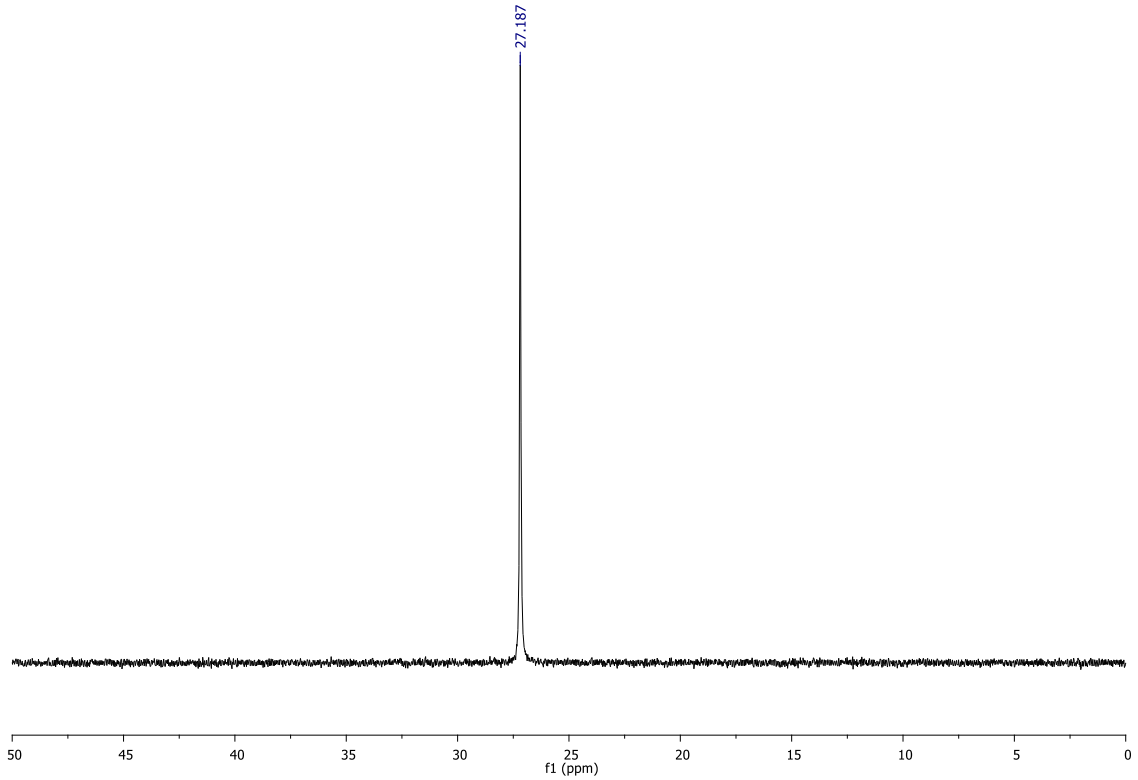
Diethyl {(1*R,2*R**,4*R**)-2-(*N*-methoxycarbonylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*endo*-22)**



^{1}H NMR (400 MHz, $CDCl_3$)

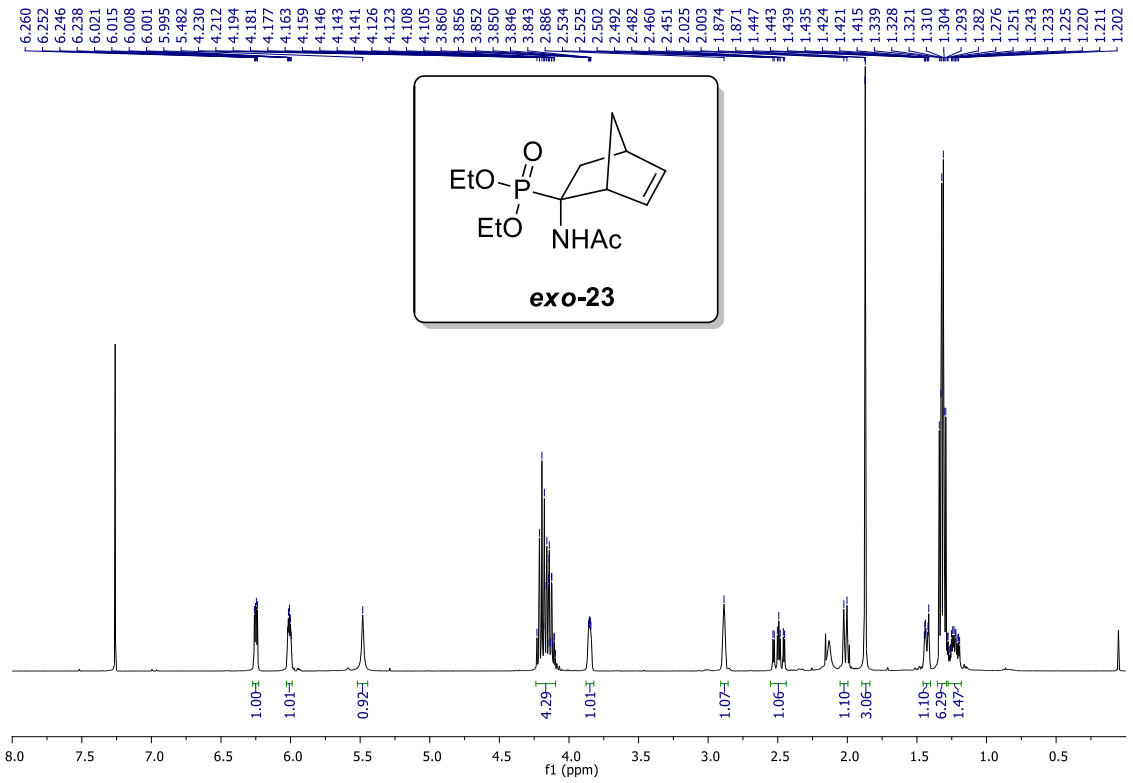


APT NMR (100 MHz, $CDCl_3$)

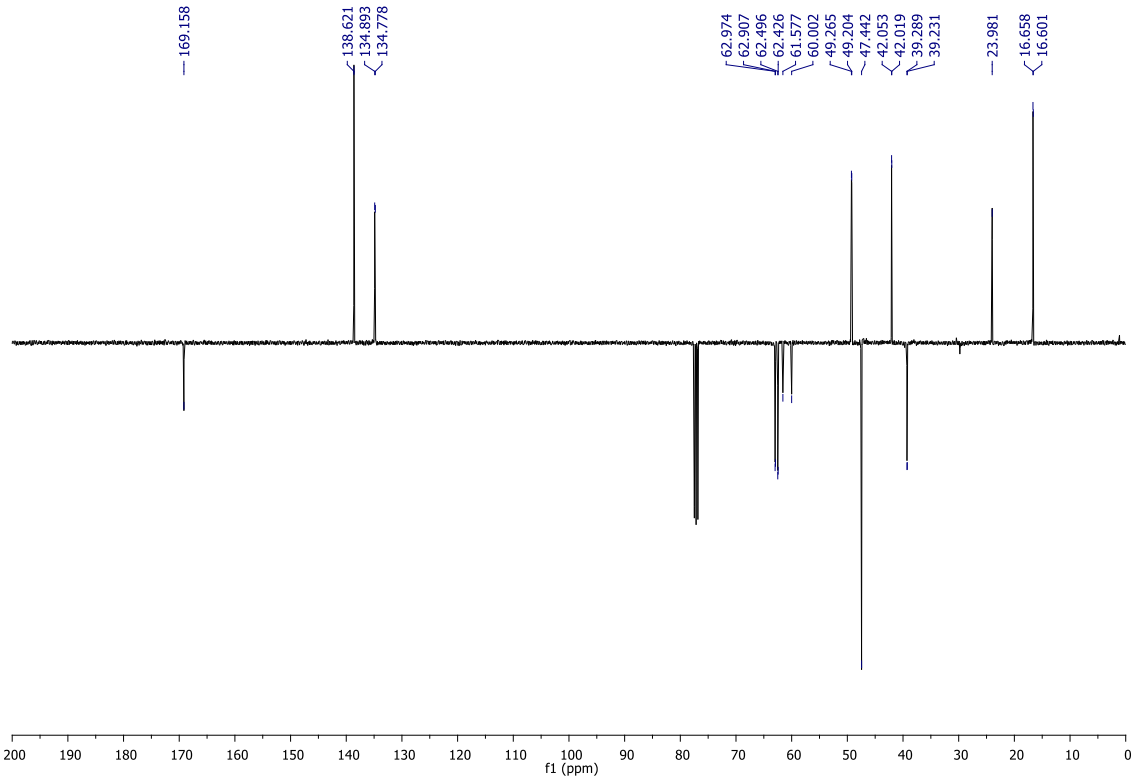


^{31}P NMR (162 MHz, $CDCl_3$)

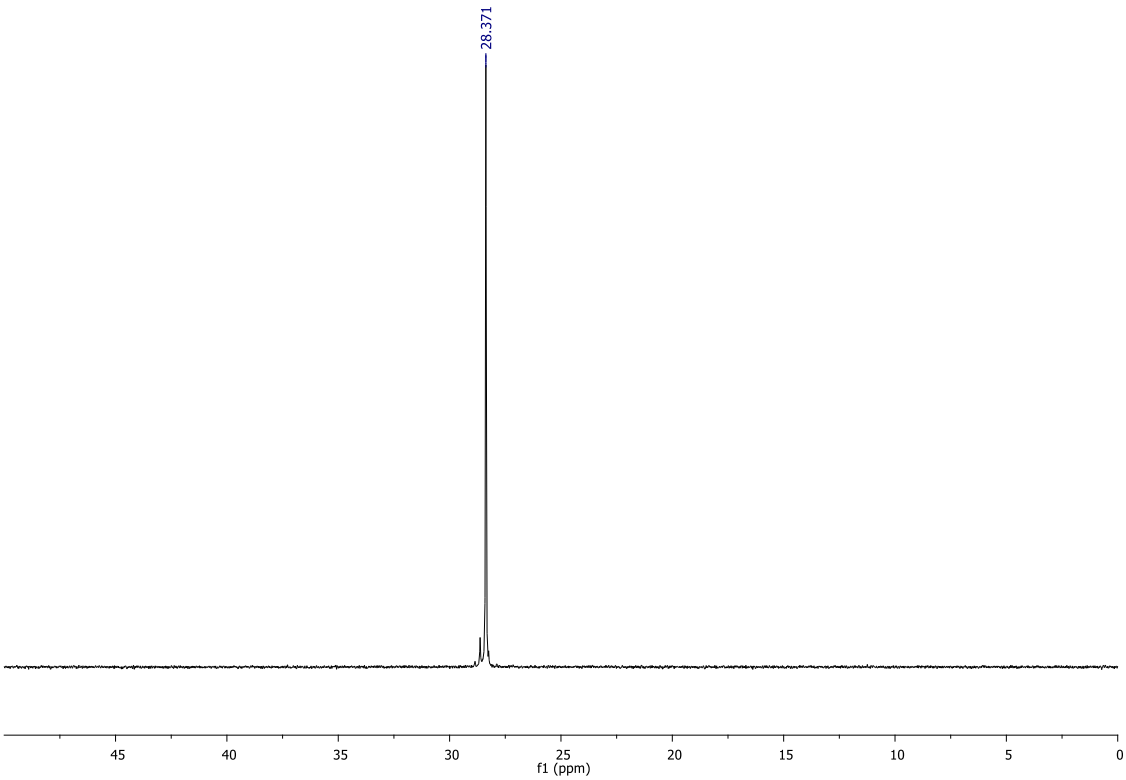
Diethyl {(1*R,2*S**,4*R**)-2-(*N*-acetylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*exo*-23)**



^{1}H NMR (400 MHz, $CDCl_3$)

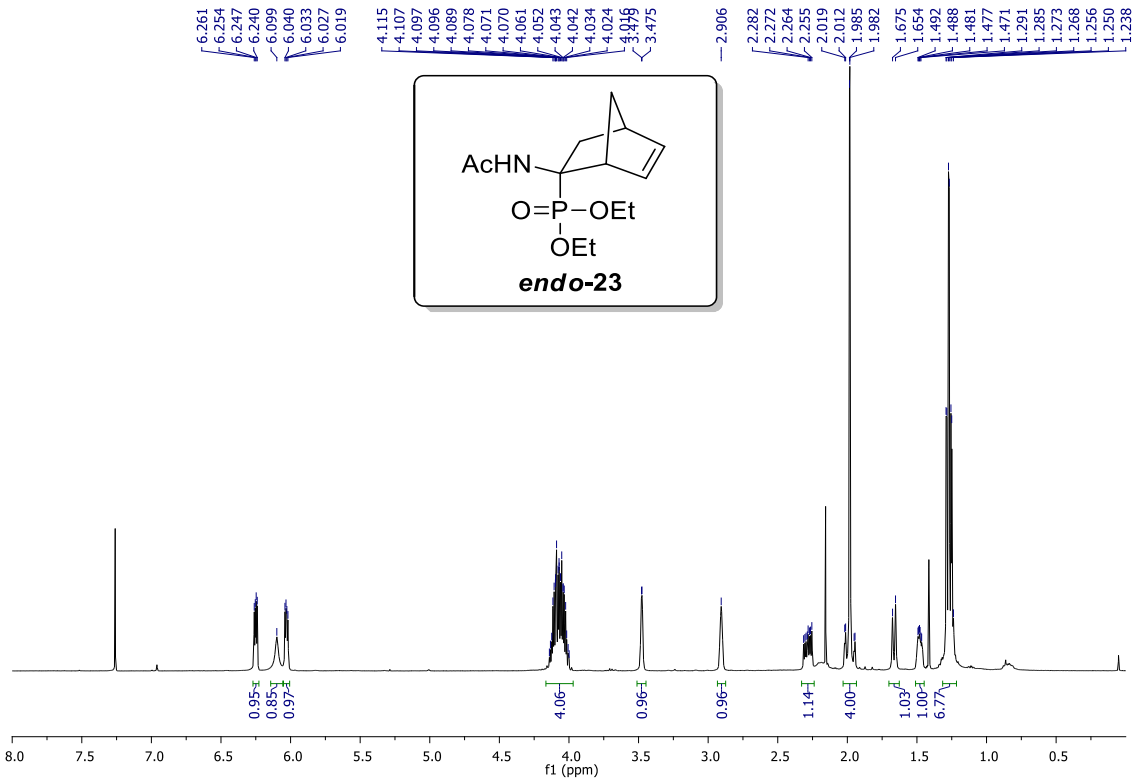


APT NMR (100 MHz, $CDCl_3$)

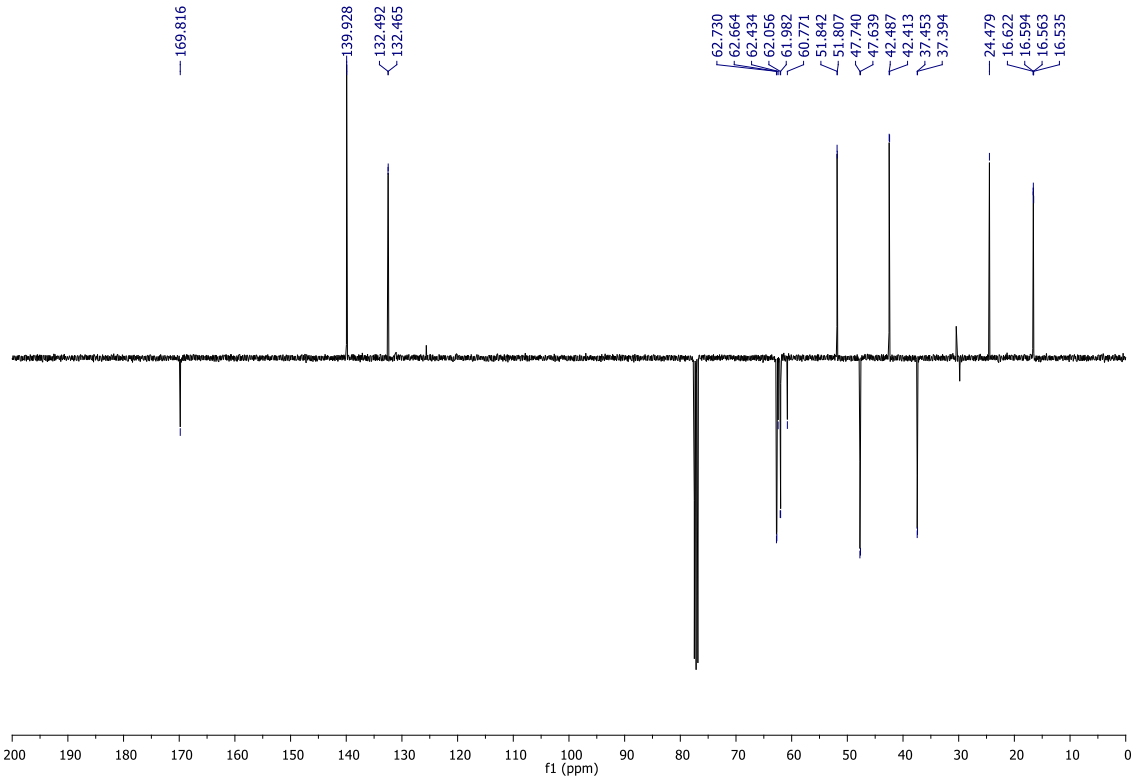


^{31}P NMR (162 MHz, $CDCl_3$)

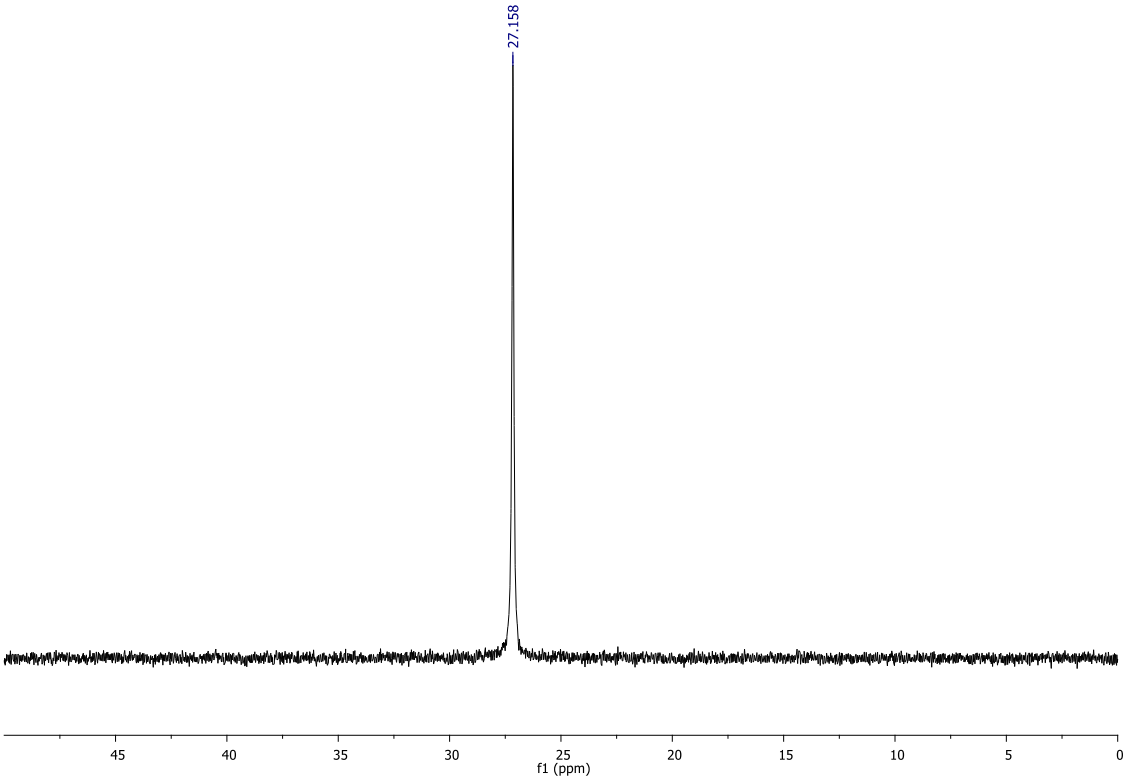
Diethyl {(1*R,2*R**,4*R**)-2-(*N*-acetylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*endo*-23)**



^{1}H NMR (400 MHz, $CDCl_3$)

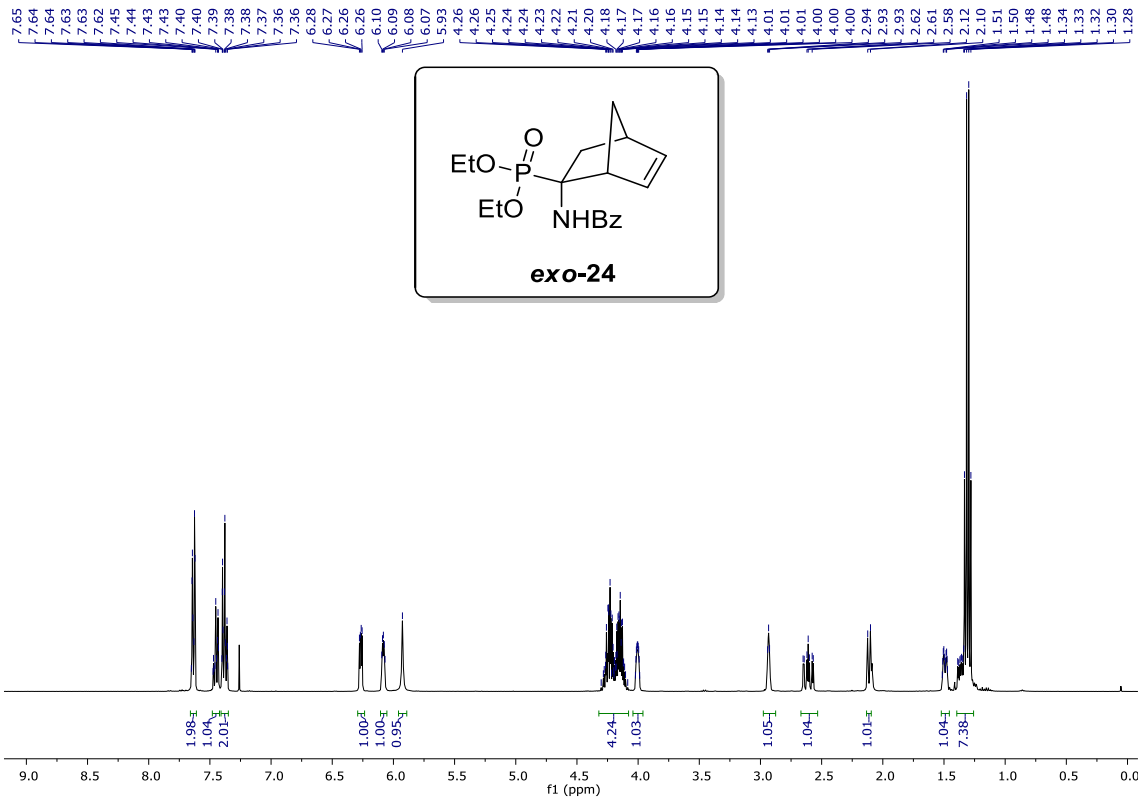


APT NMR (100 MHz, $CDCl_3$)

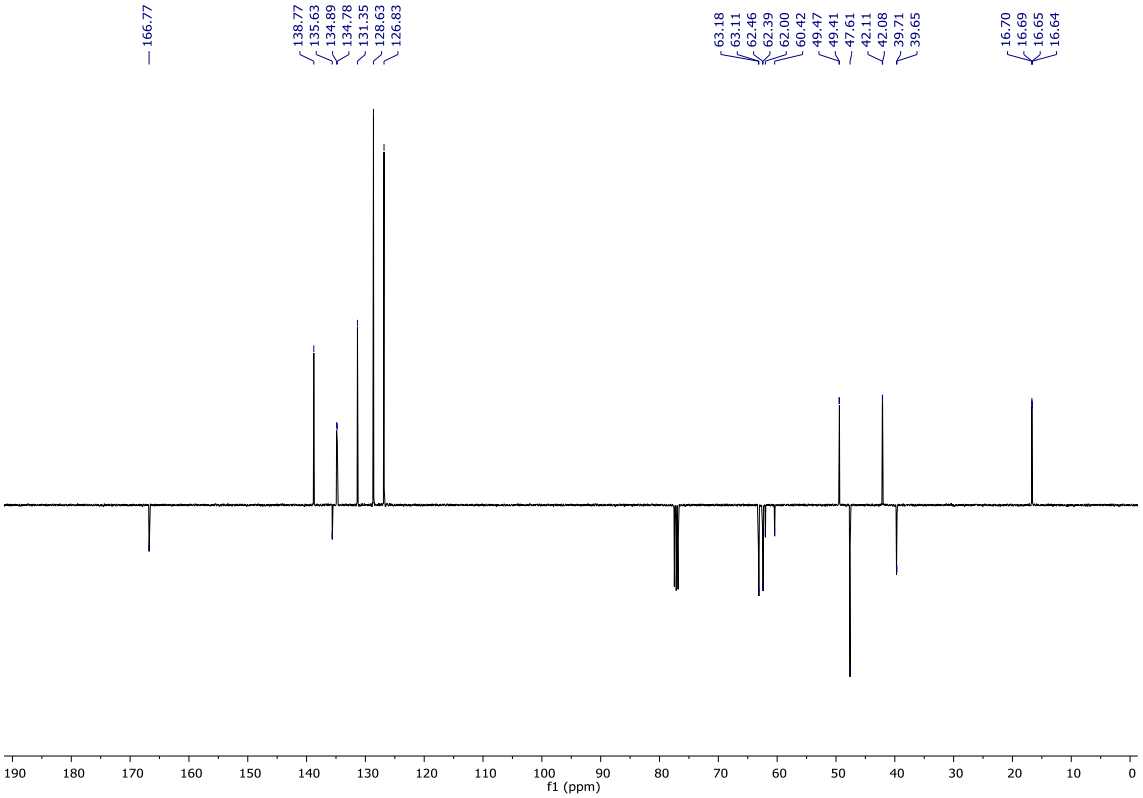


^{31}P NMR (162 MHz, $CDCl_3$)

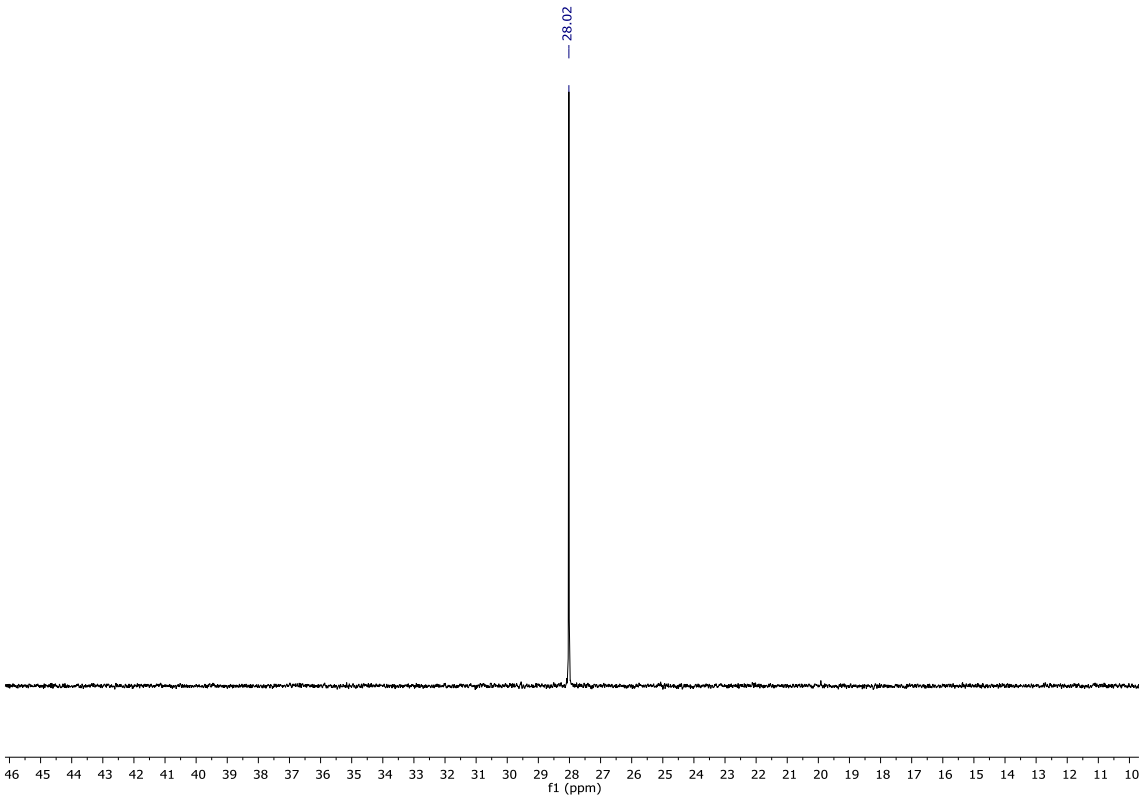
Diethyl {(1*R,2*S**,4*R**)-2-(*N*-benzoylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*exo*-24)**



^{1}H NMR (400 MHz, $CDCl_3$)

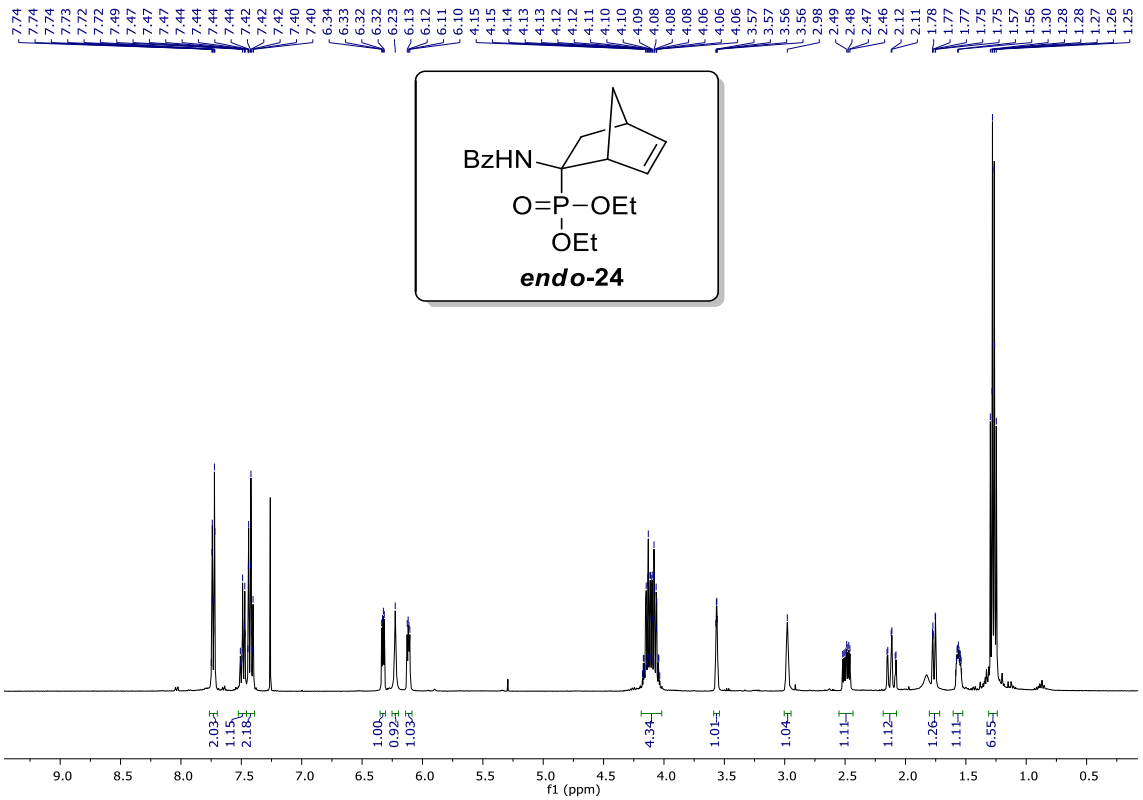


APT NMR (100 MHz, $CDCl_3$)

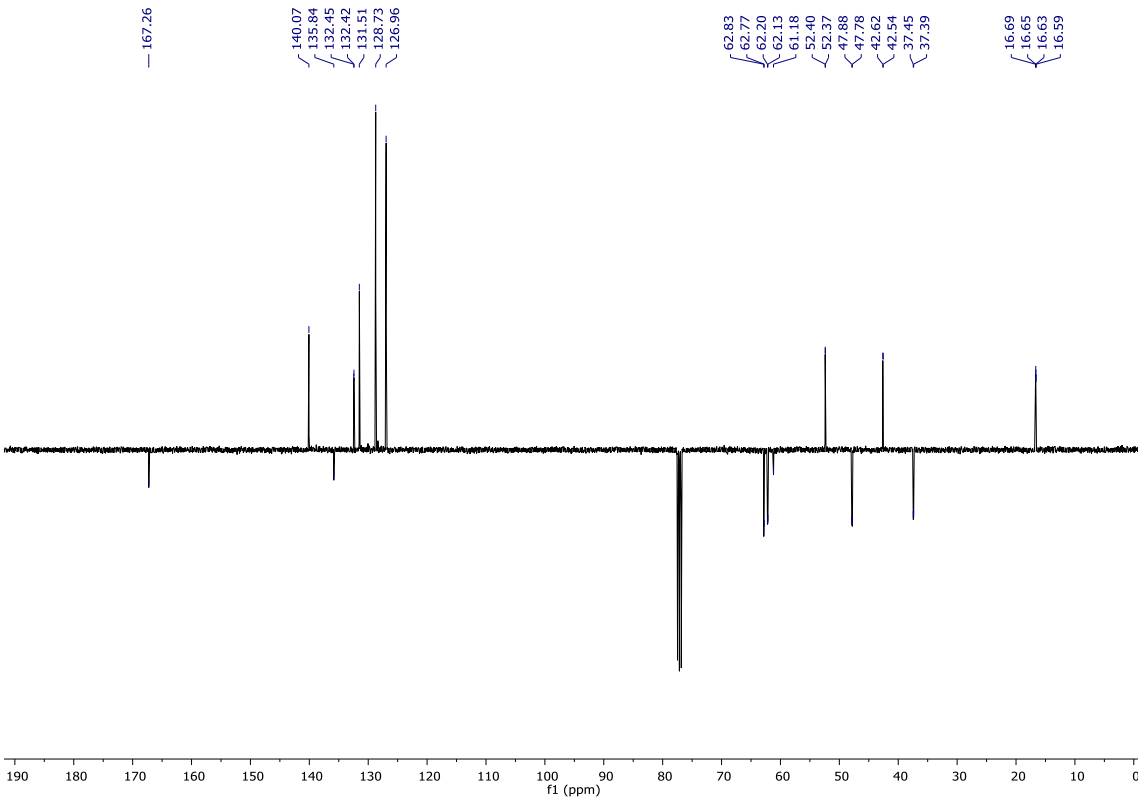


^{31}P NMR (162 MHz, $CDCl_3$)

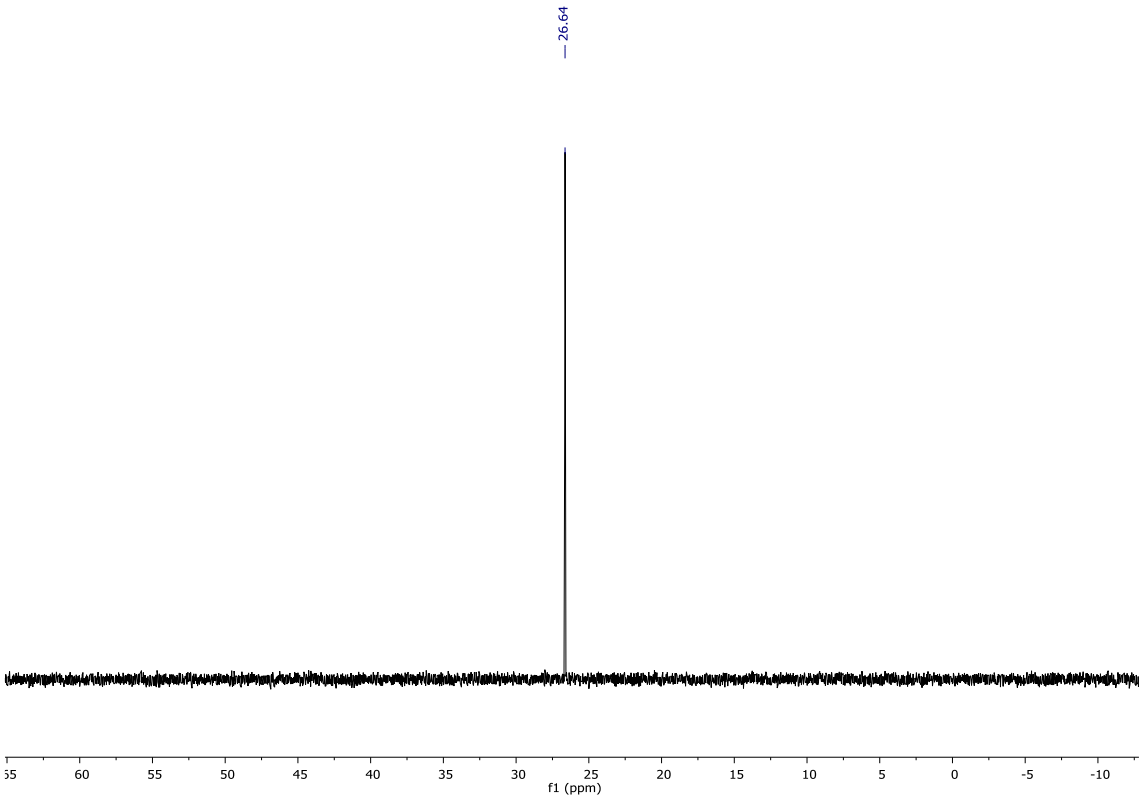
Diethyl {(1*R,2*R**,4*R**)-2-(*N*-benzoylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*endo*-24)**



^{1}H NMR (400 MHz, $CDCl_3$)

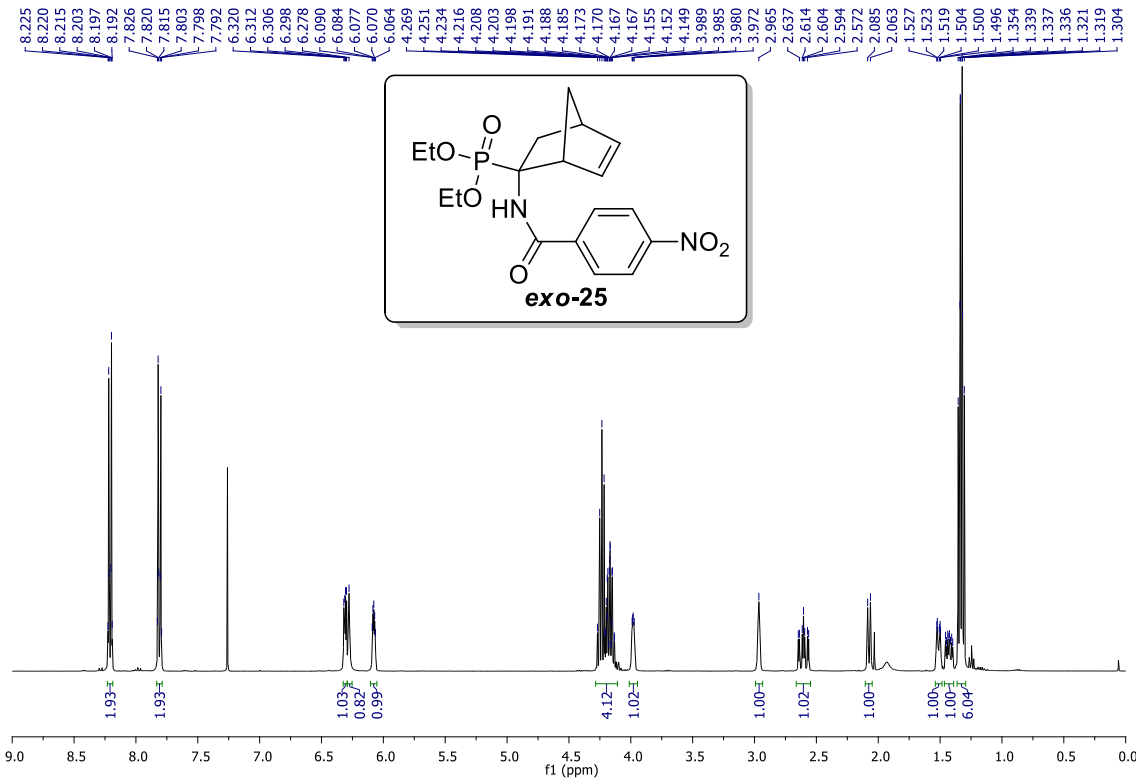


APT NMR (100 MHz, $CDCl_3$)

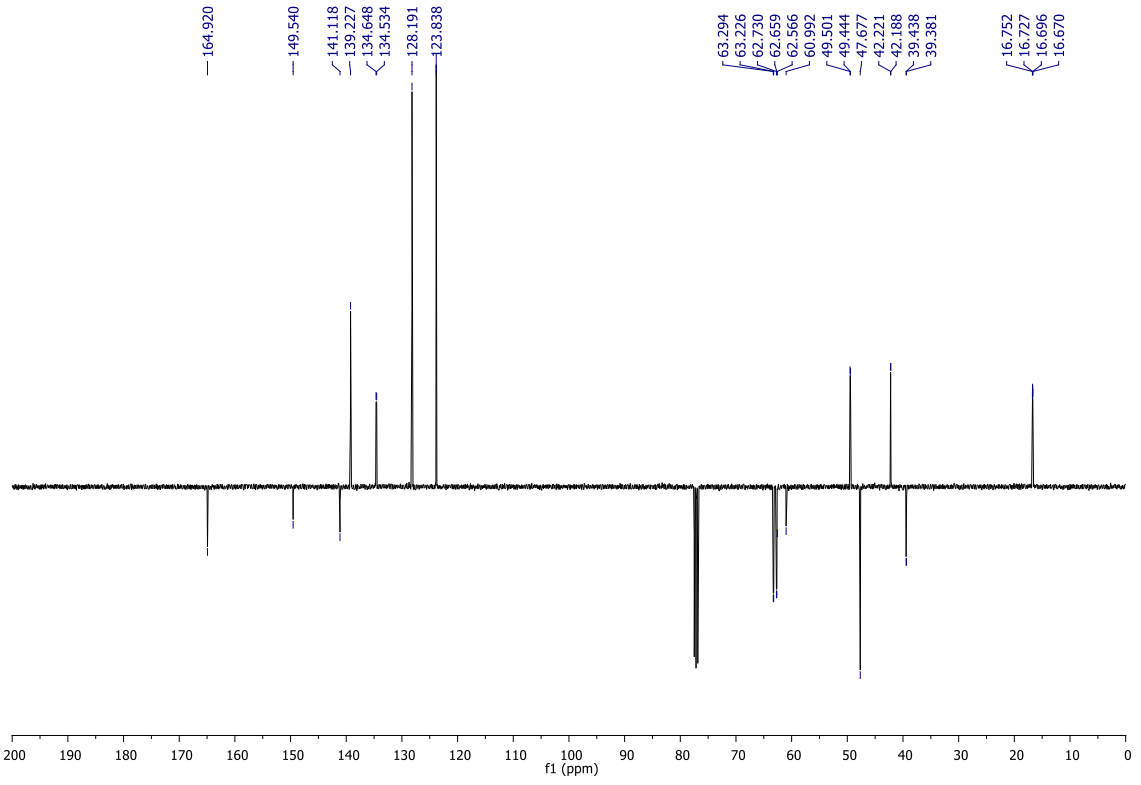


^{31}P NMR (162 MHz, $CDCl_3$)

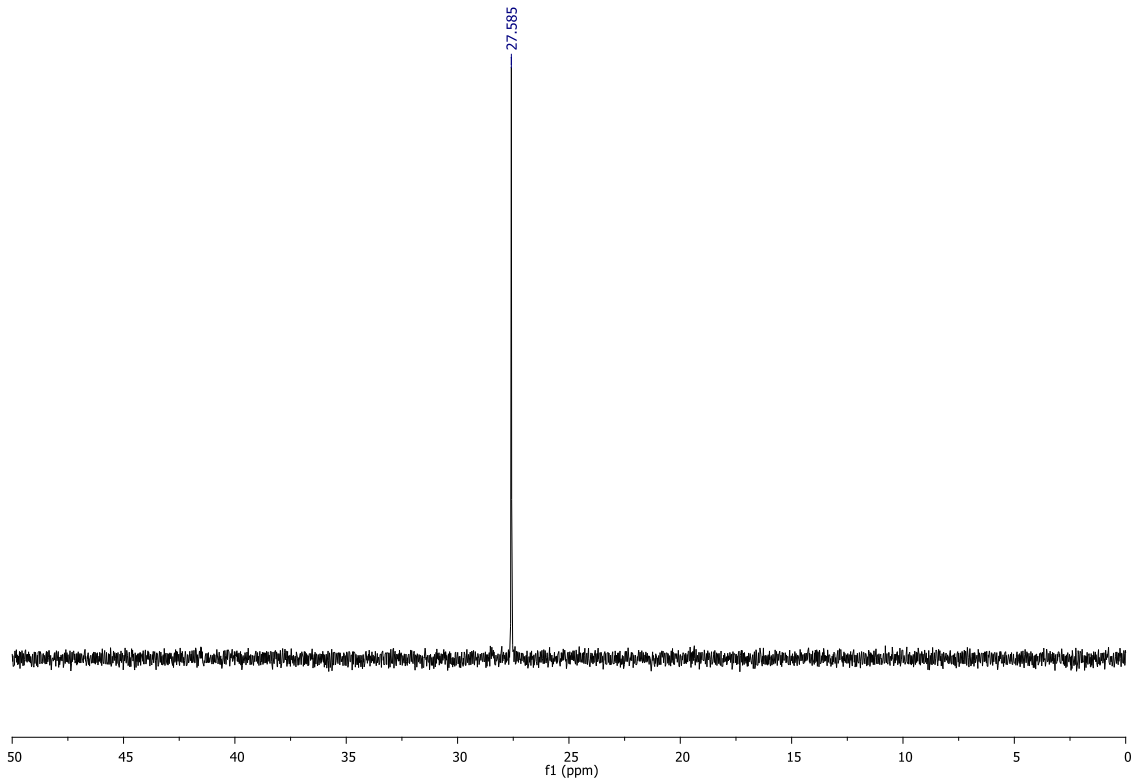
Diethyl {(1*R,2*S**,4*R**)-2-(*N*-*p*-nitrobenzoylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*exo*-25)**



^{1}H NMR (400 MHz, $CDCl_3$)

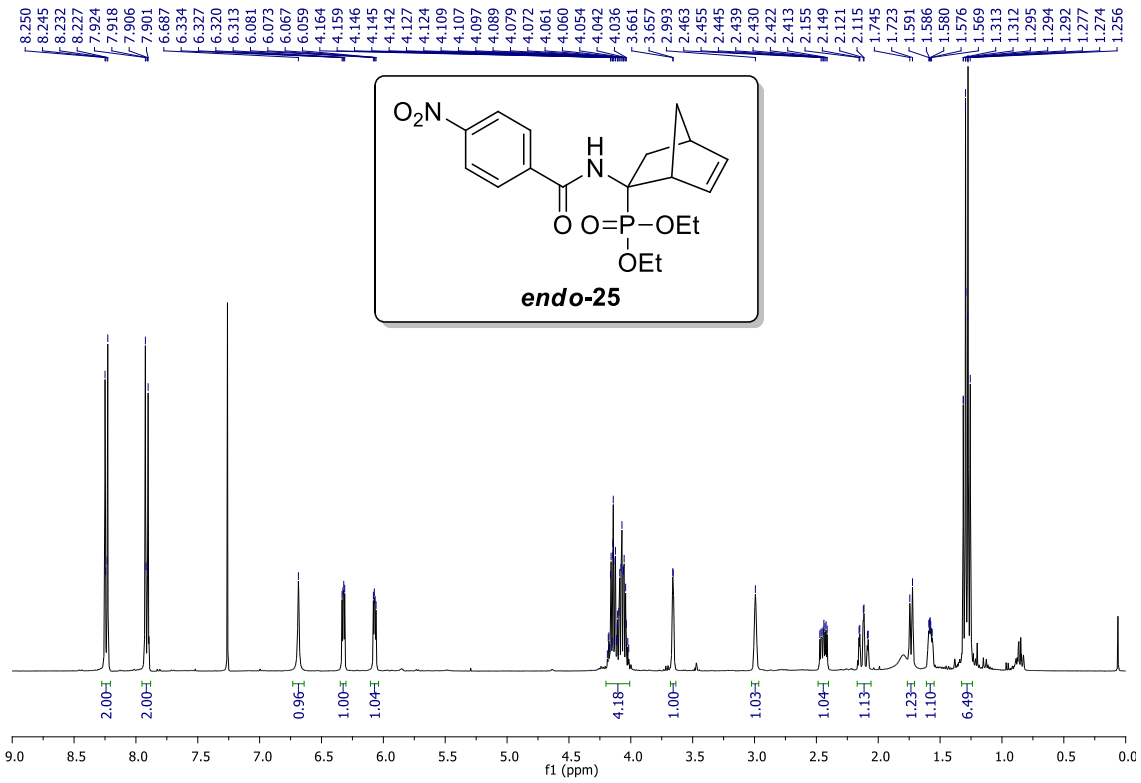


APT NMR (100 MHz, $CDCl_3$)

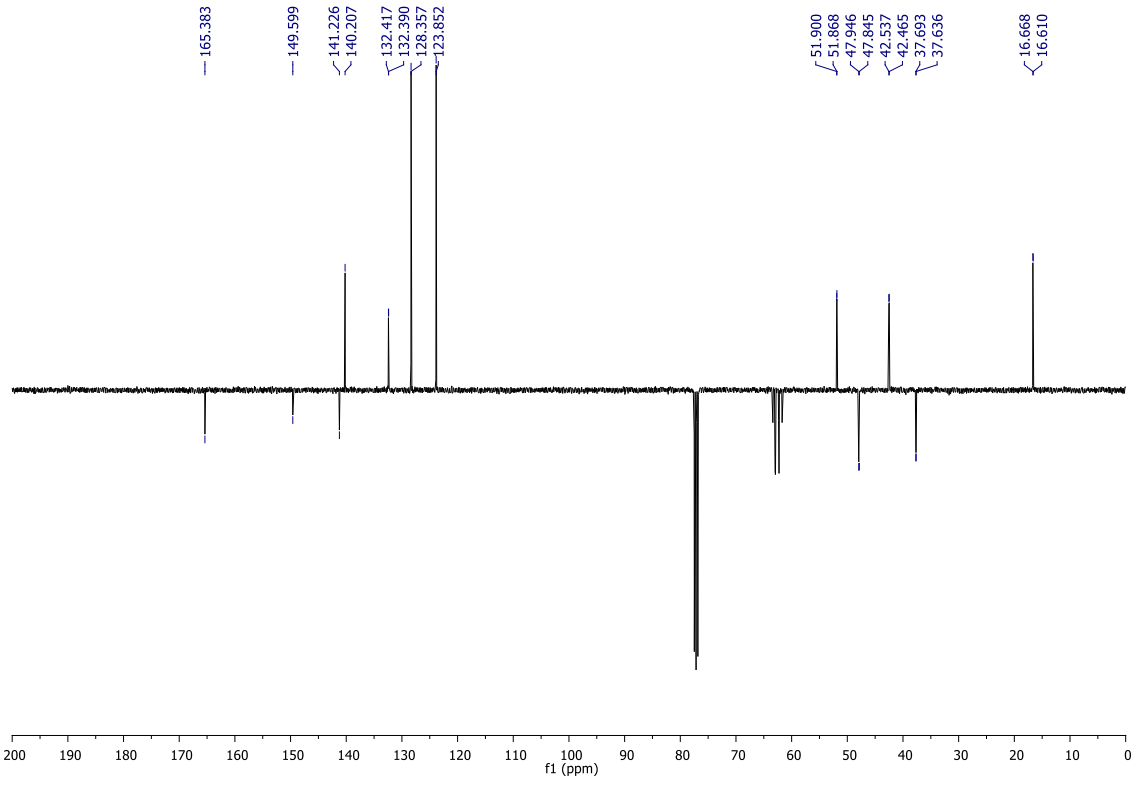


^{31}P NMR (162 MHz, $CDCl_3$)

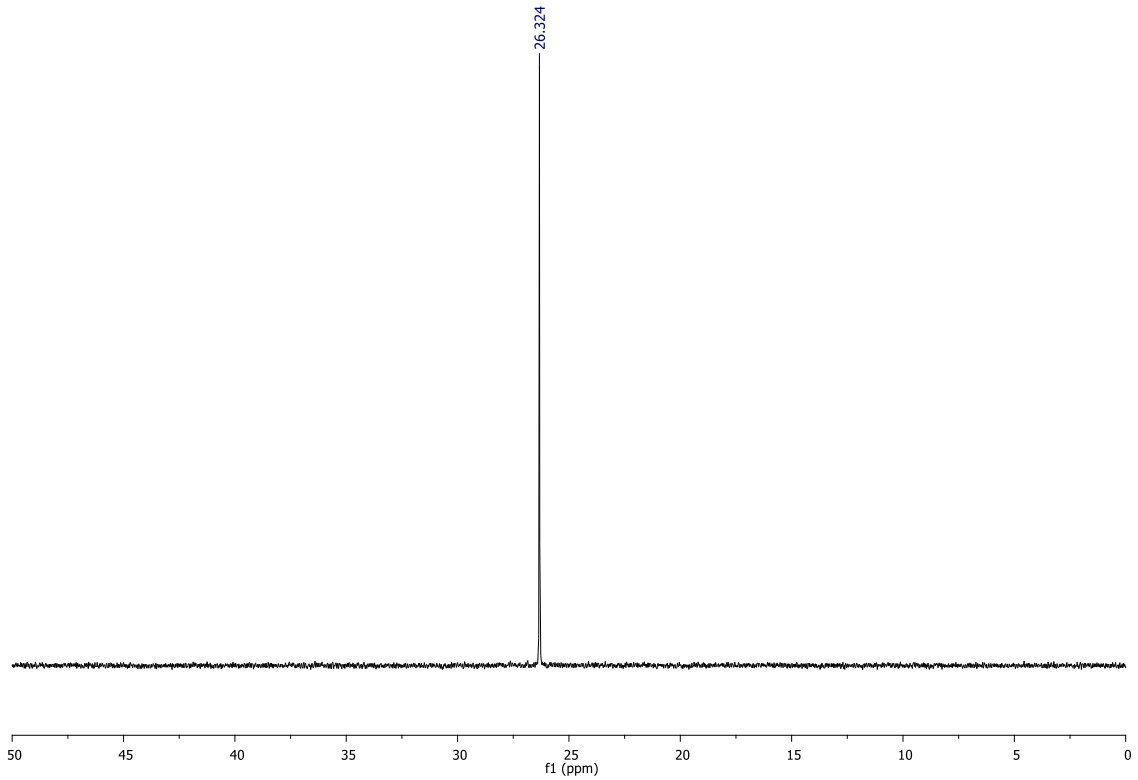
Diethyl {(1*R,2*R**,4*R**)-2-(*N*-*p*-nitrobenzoylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*endo*-25)**



^{1}H NMR (400 MHz, $CDCl_3$)

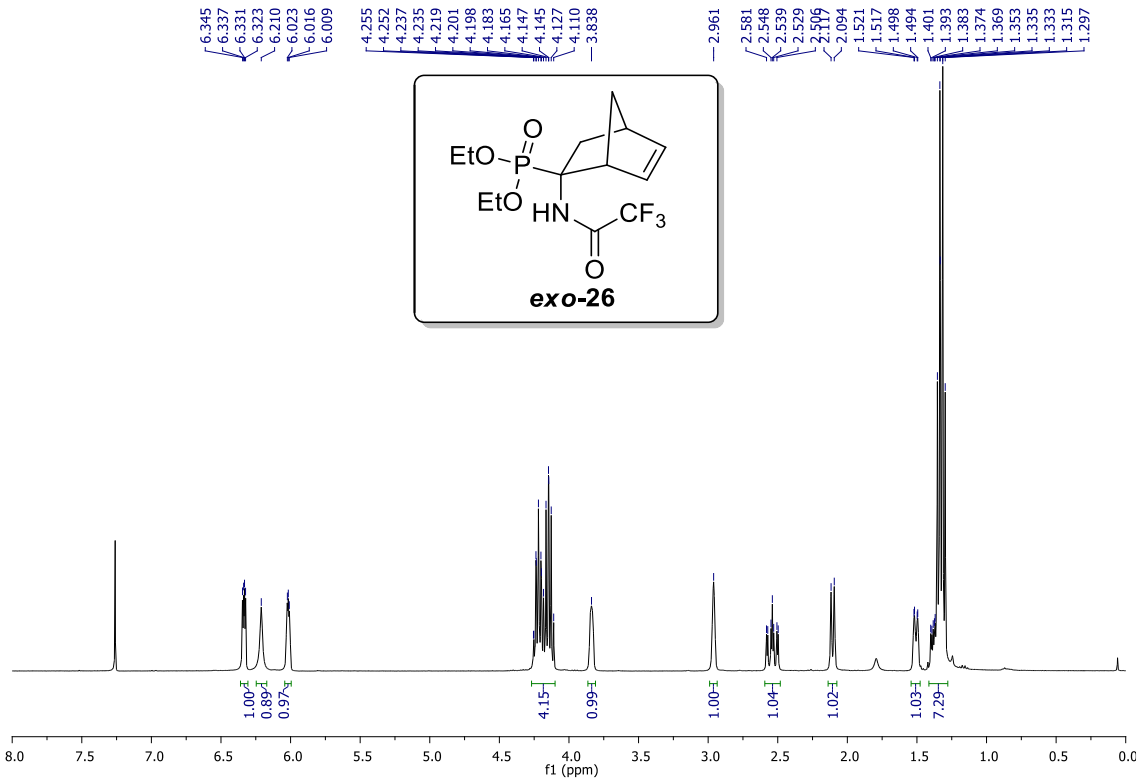


APT NMR (100 MHz, $CDCl_3$)

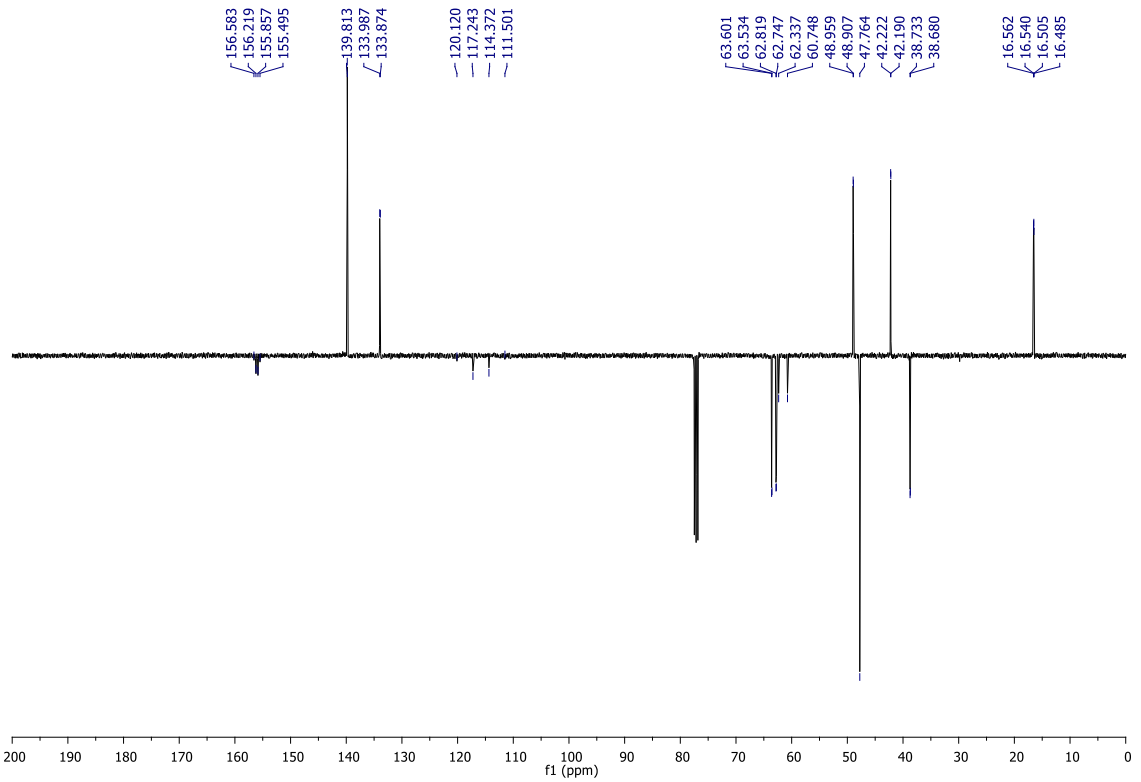


^{31}P NMR (162 MHz, $CDCl_3$)

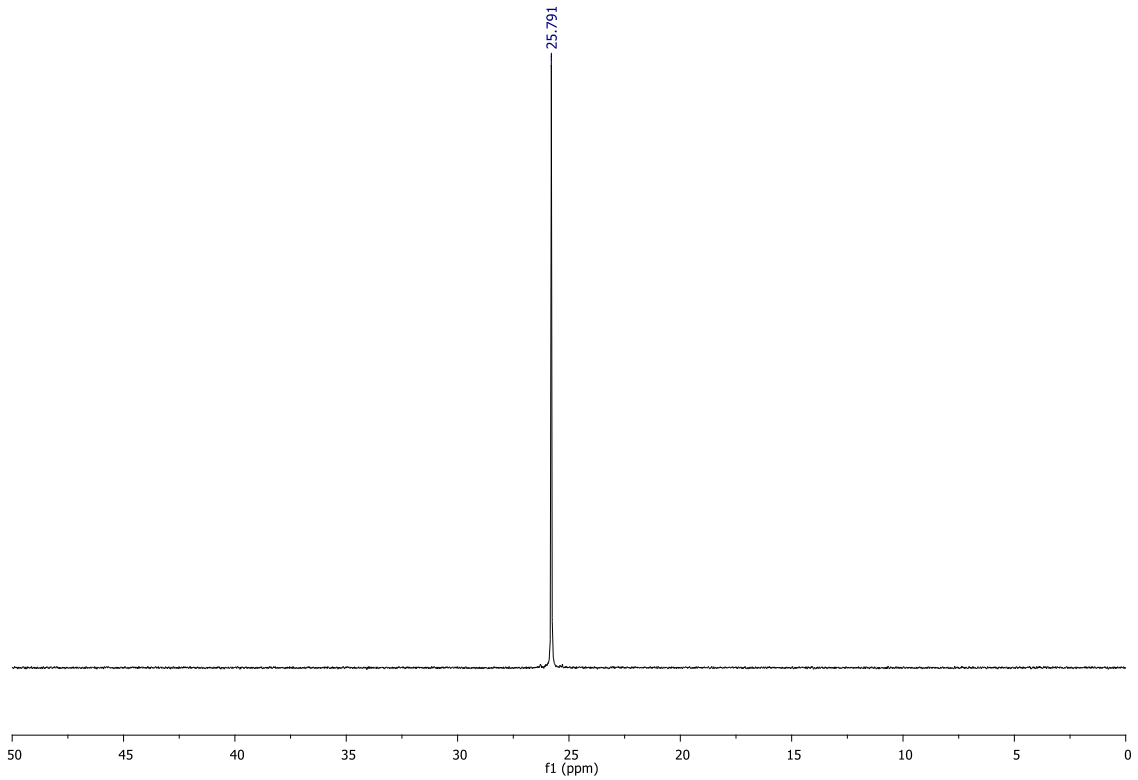
Diethyl {(1*R,2*S**,4*R**)-2-(*N*-trifluoroacetylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*exo*-26)**



^{1}H NMR (400 MHz, $CDCl_3$)

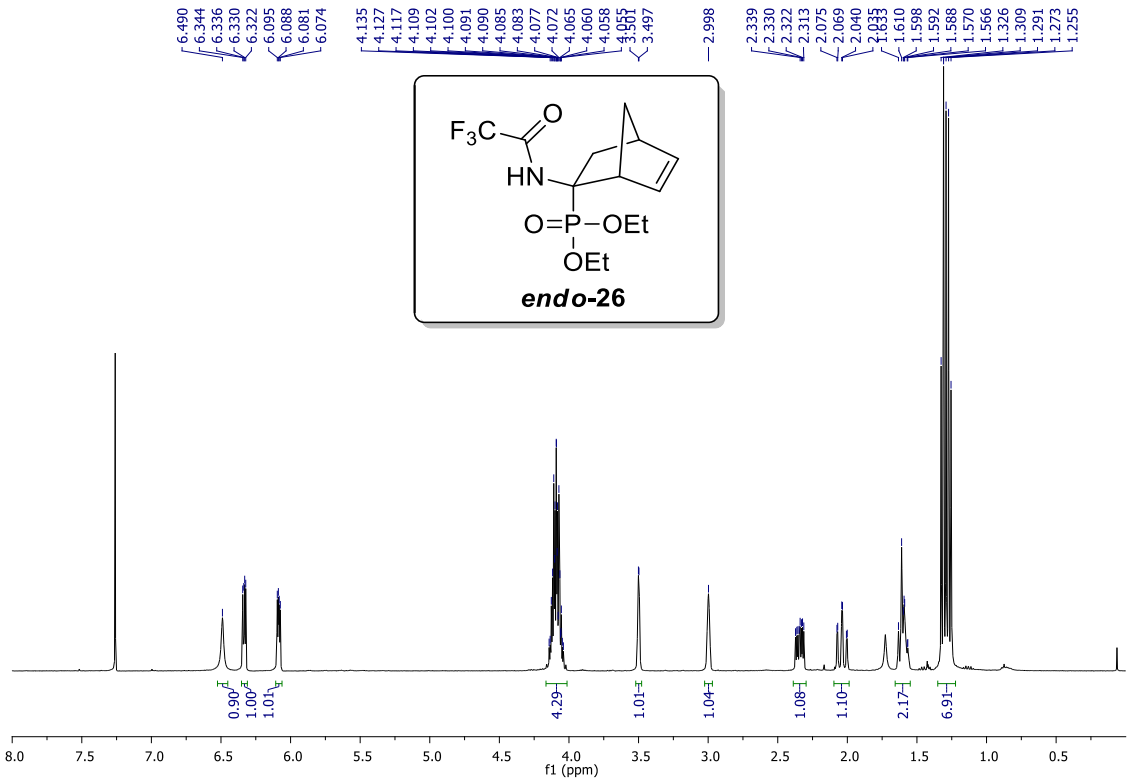


APT NMR (100 MHz, $CDCl_3$)

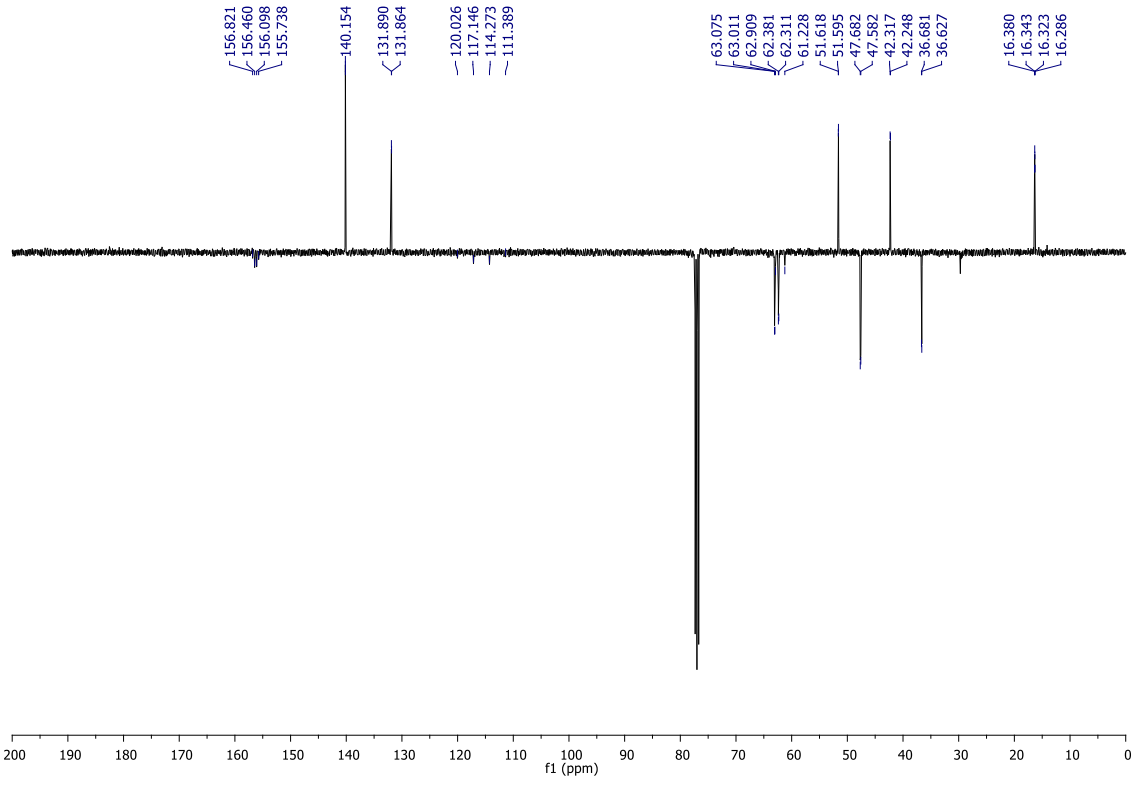


^{31}P NMR (162 MHz, $CDCl_3$)

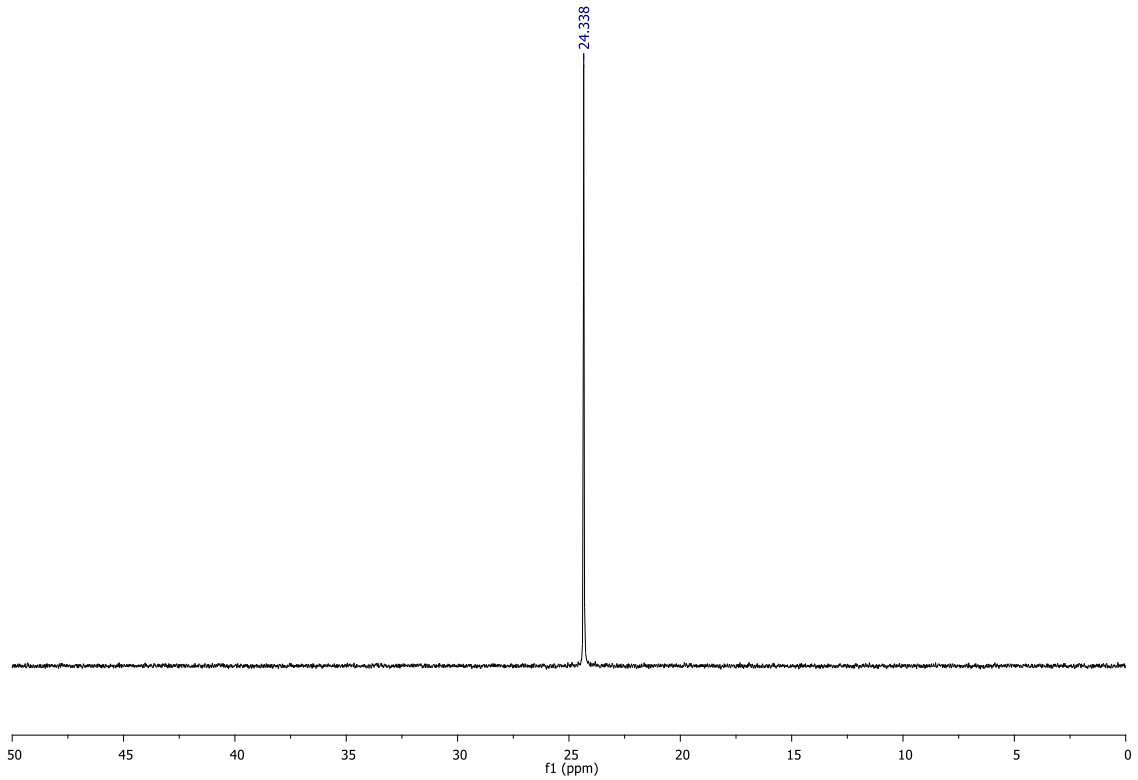
Diethyl {(1*R,2*R**,4*R**)-2-(*N*-trifluoroacetylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*endo*-26)**



^{1}H NMR (400 MHz, $CDCl_3$)

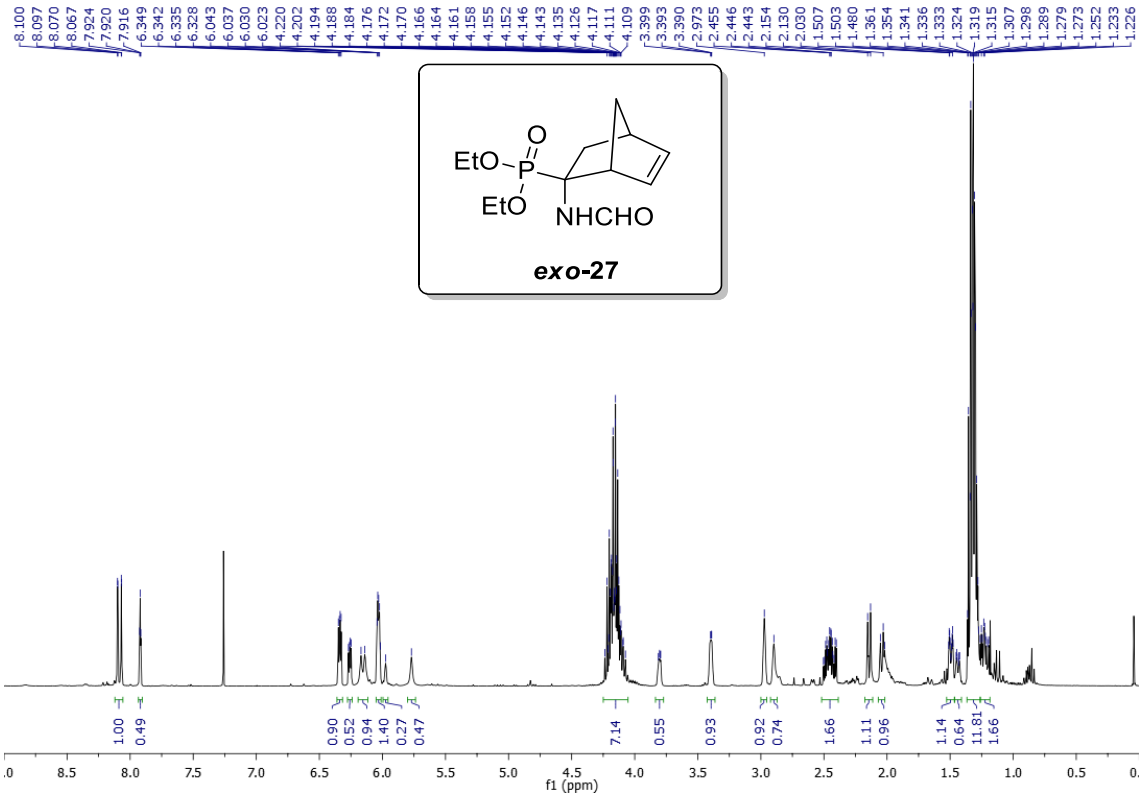


APT NMR (100 MHz, $CDCl_3$)

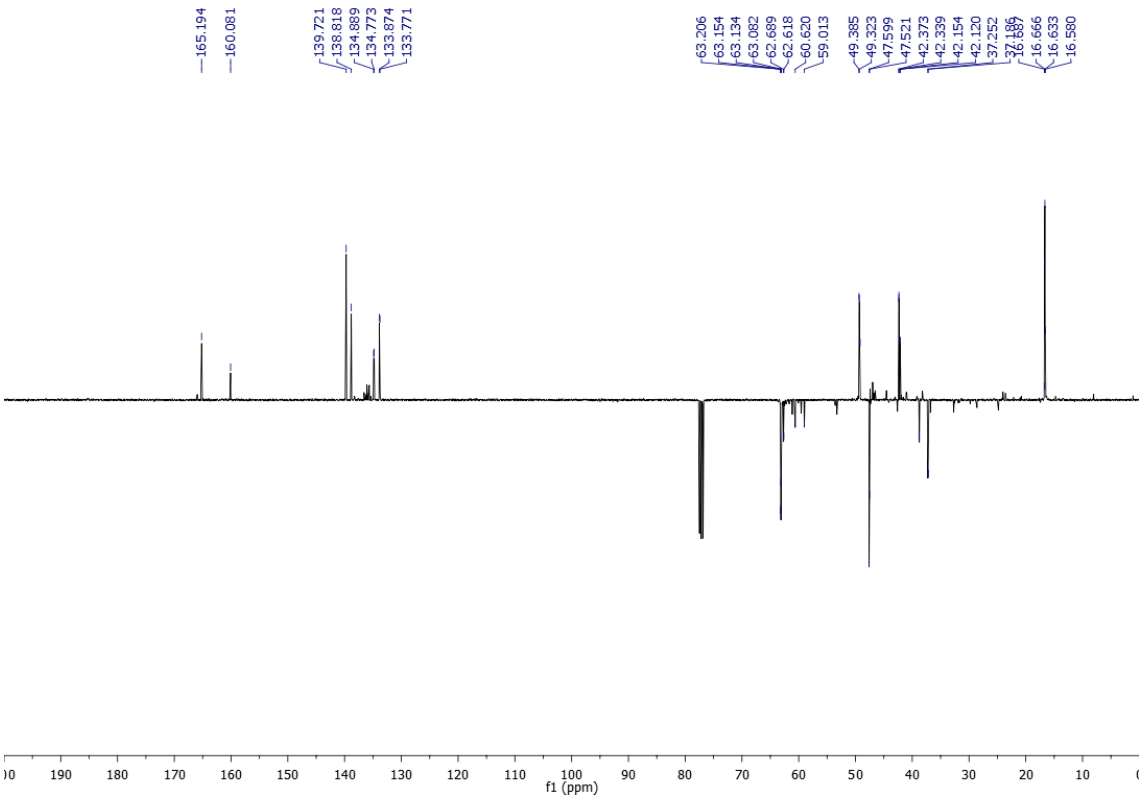


^{31}P NMR (162 MHz, $CDCl_3$)

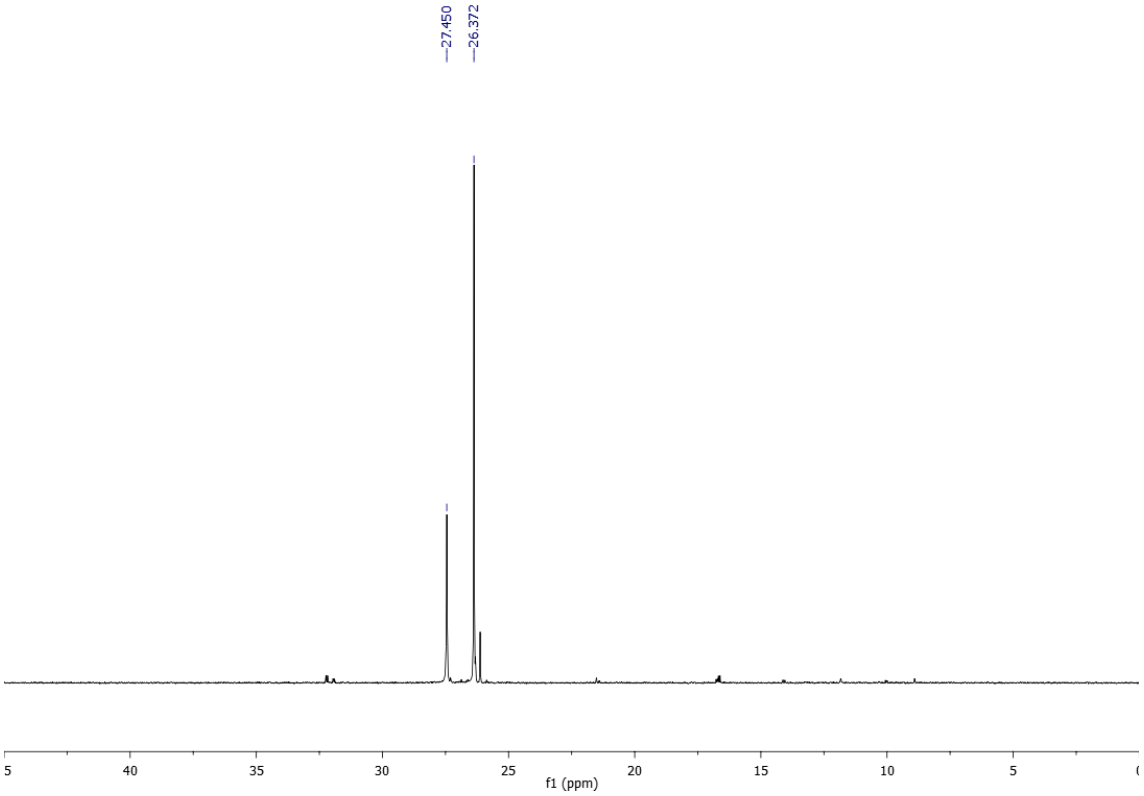
Diethyl {(1*R,2*S**,4*R**)-2-(*N*-formylamino)bicyclo[2.2.1]hept-5-en-2-yl}phosphonate (*exo*-27)**



^{1}H NMR (400 MHz, $CDCl_3$)

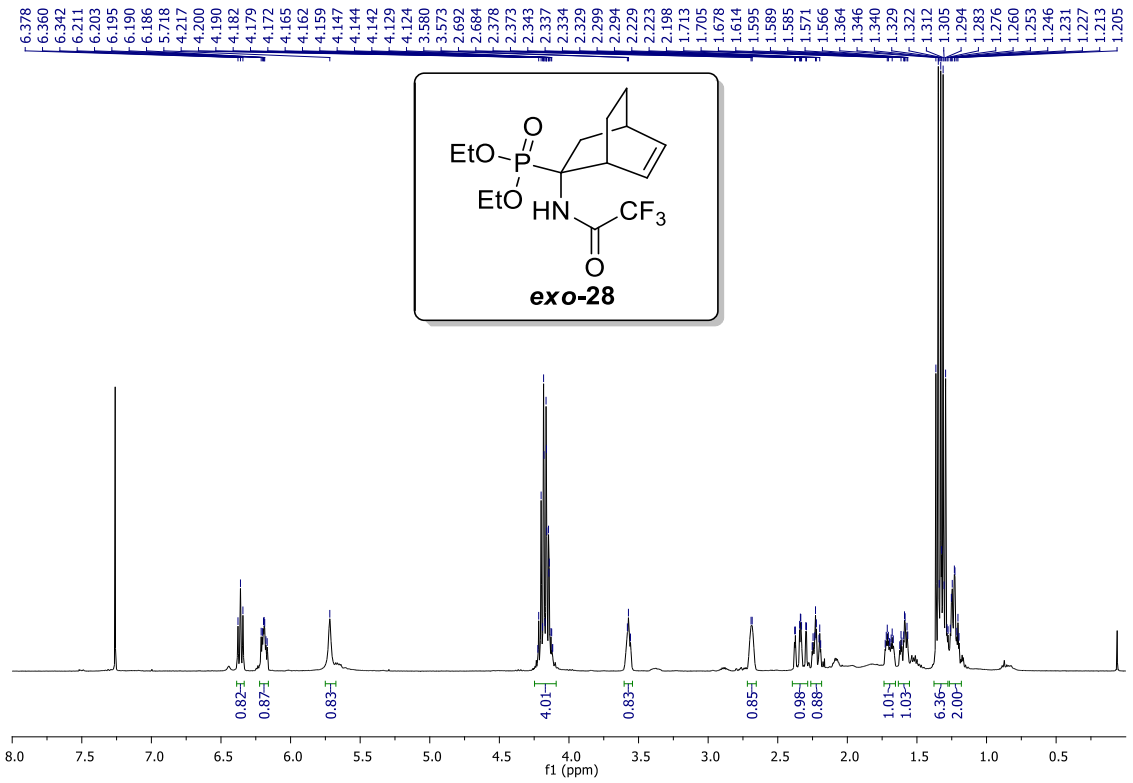


APT NMR (100 MHz, $CDCl_3$)

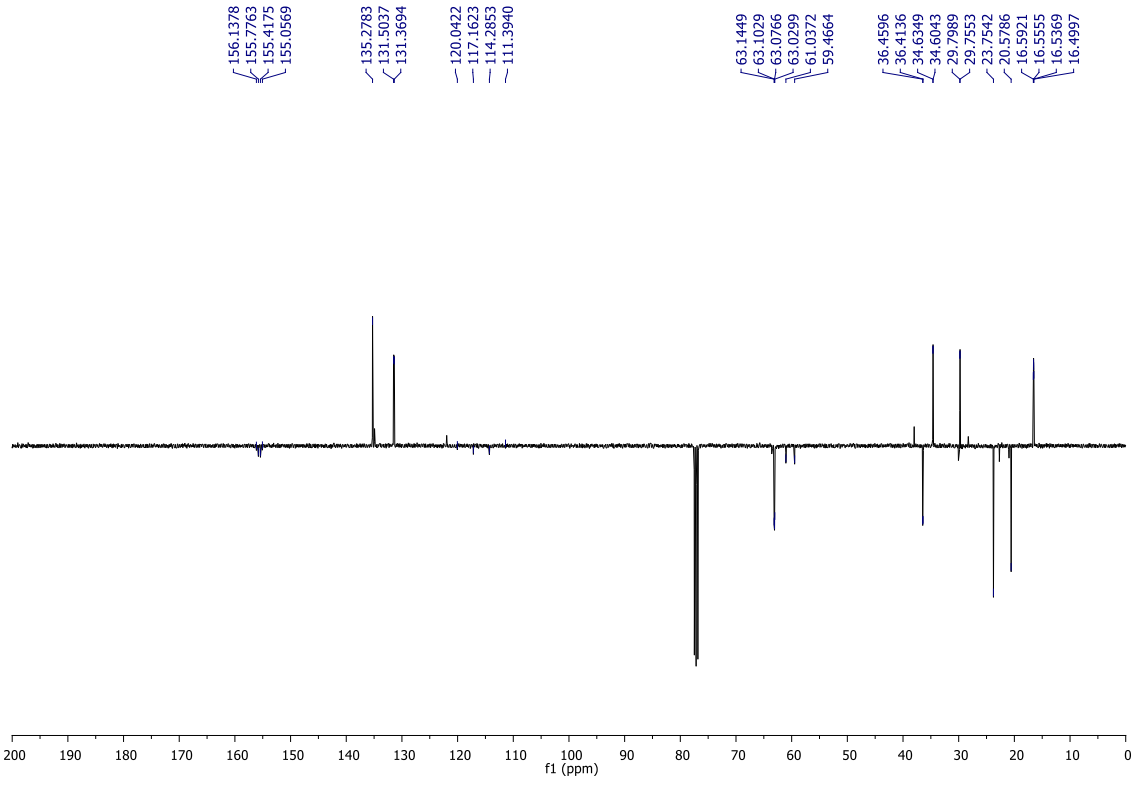


^{31}P NMR (162 MHz, $CDCl_3$)

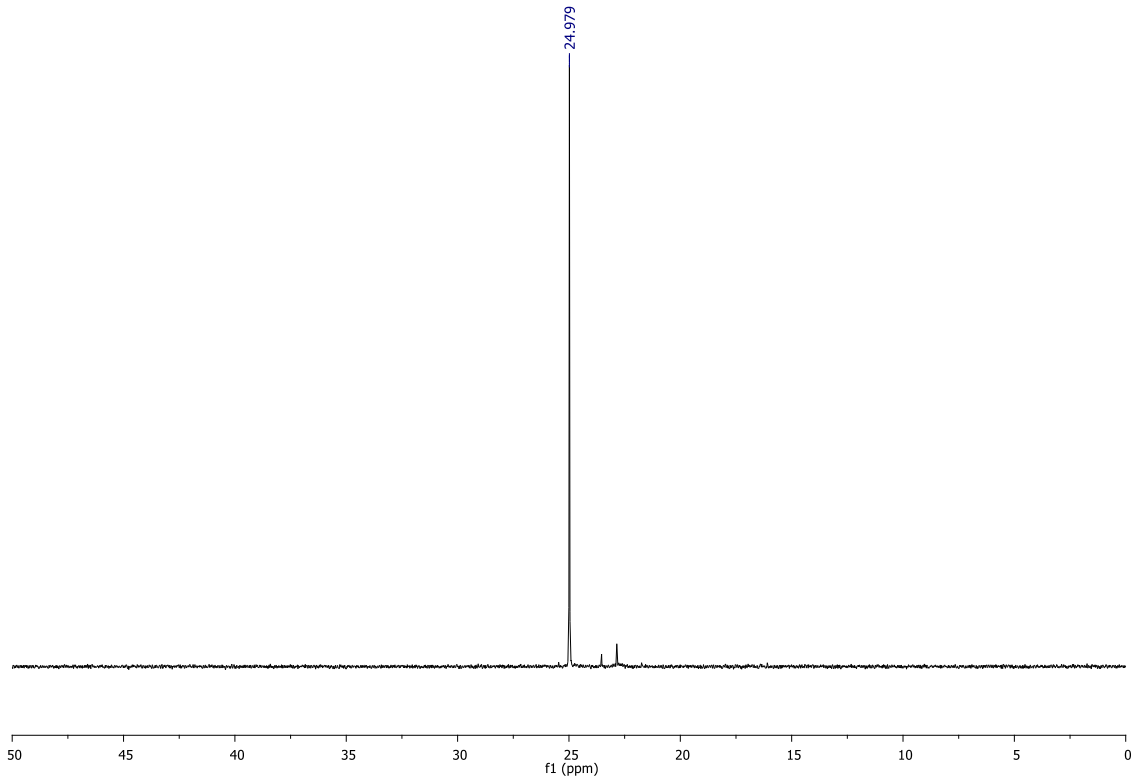
Diethyl {(1*R,2*S**,4*R**)-2-(*N*-trifluoroacetylamino)bicyclo[2.2.2]oct-5-en-2-yl}phosphonate (*exo*-28)**



^{1}H NMR (400 MHz, $CDCl_3$)

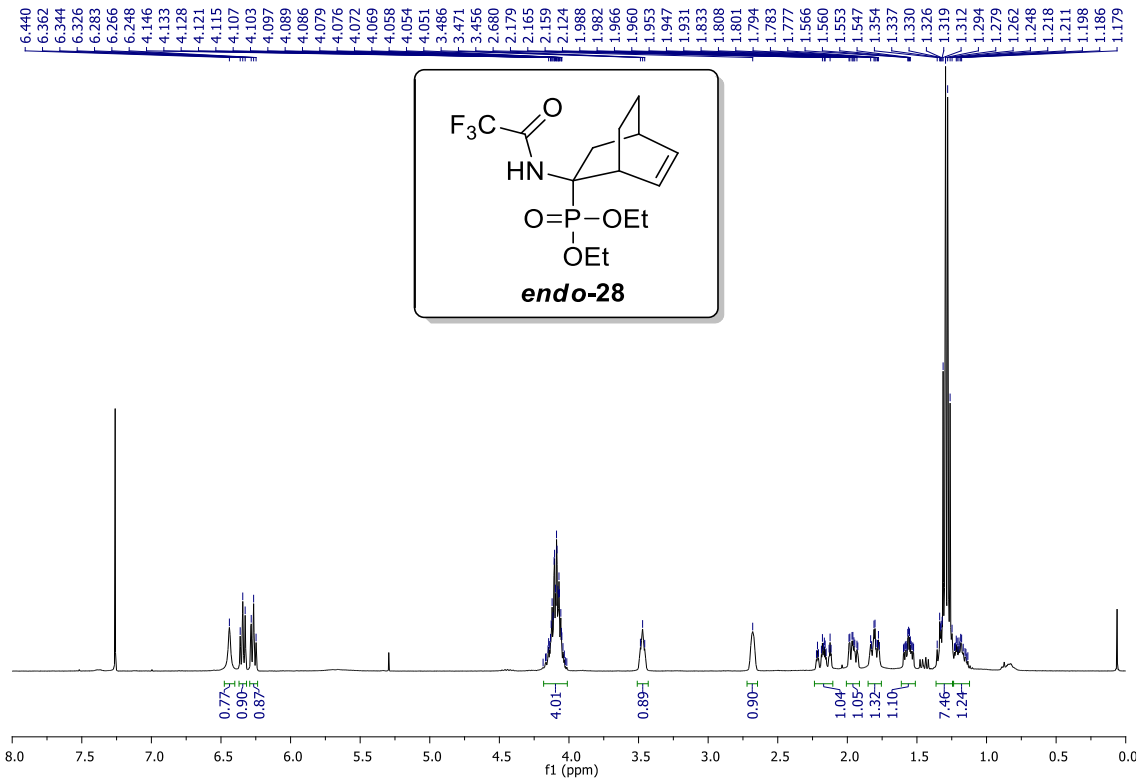


APT NMR (100 MHz, $CDCl_3$)

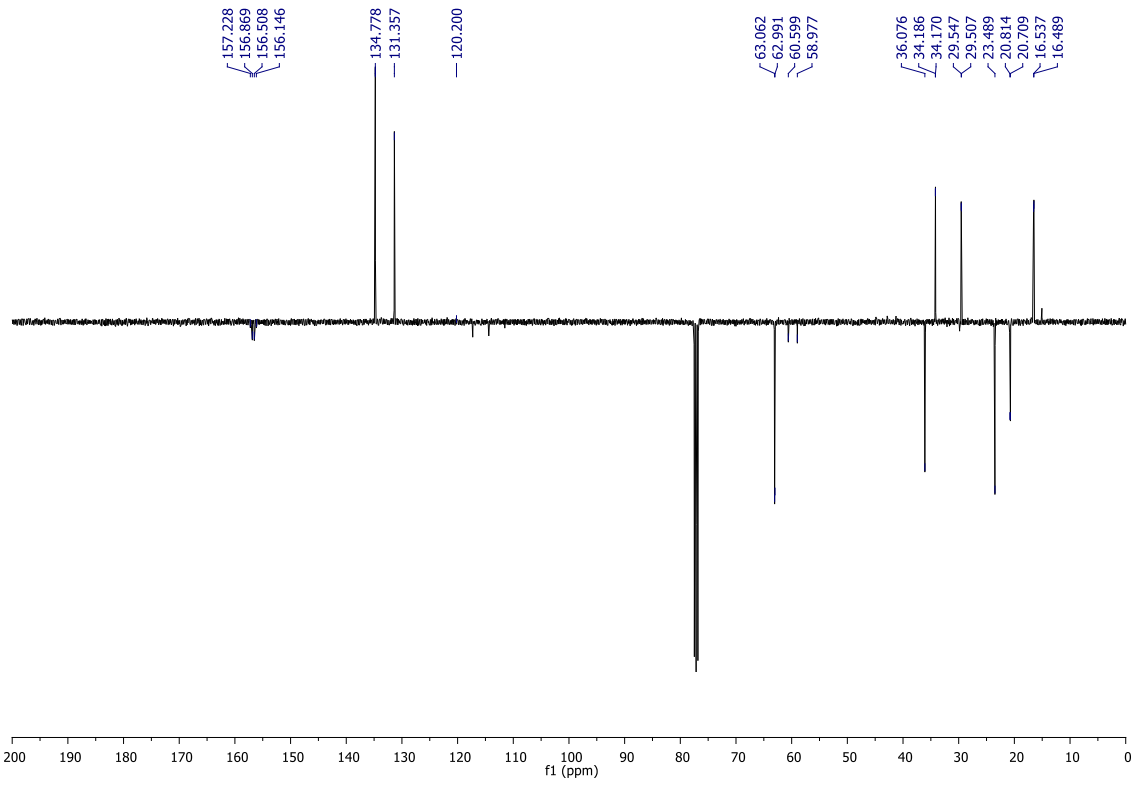


^{31}P NMR (162 MHz, $CDCl_3$)

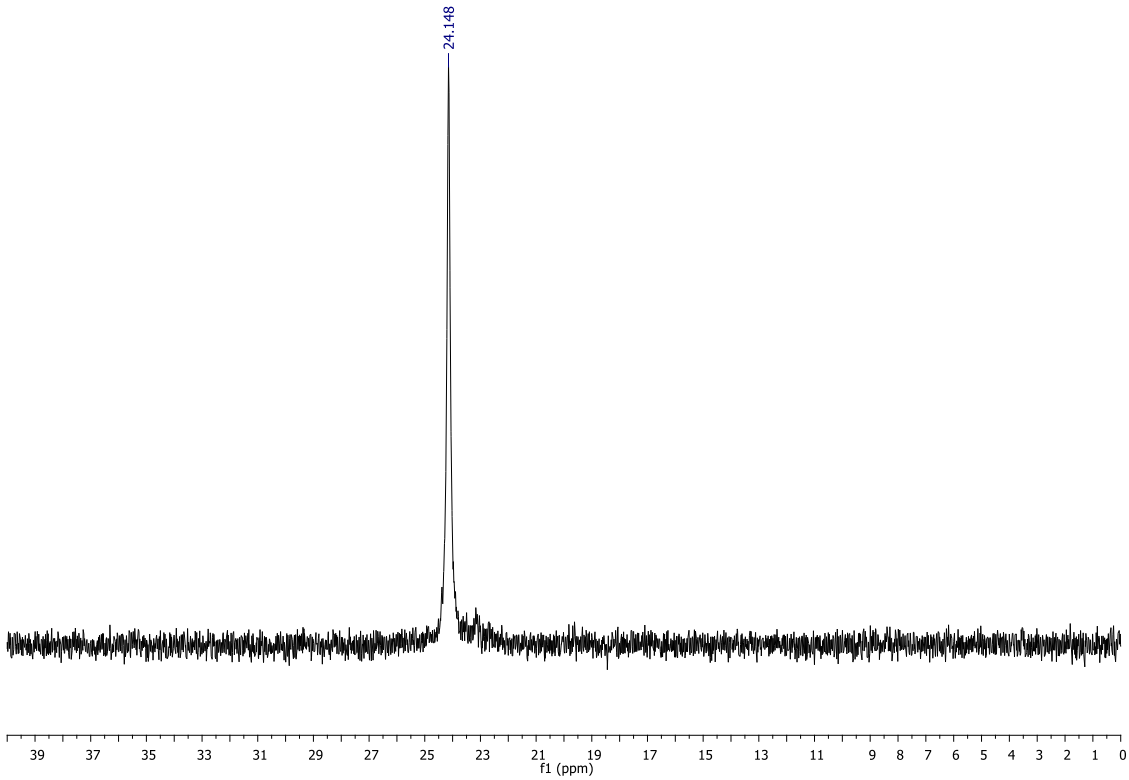
Diethyl {(1*R,2*R**,4*R**)-2-(*N*-trifluoroacetylamino)bicyclo[2.2.2]oct-5-en-2-yl}phosphonate (*endo*-28)**



^{1}H NMR (400 MHz, $CDCl_3$)



APT NMR (100 MHz, $CDCl_3$)



^{31}P NMR (162 MHz, $CDCl_3$)

Cartesian Coordinates of Chapter 3

TSa-*endo*

0 1

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C	0.1857124808	3.0628491994	-0.4572171800
C	-1.1005025624	2.5787860295	-0.7989904495
C	-1.7303672170	2.1265166323	0.5007842649
C	-0.4981716784	1.7419144913	1.2716593420
H	1.5320538251	2.6178252351	1.2515442254
H	0.8458738193	3.6188199031	-1.1247452337
H	-1.6933108382	2.9911108552	-1.6187479366
H	-2.5148690722	1.3672696097	0.4112341168
H	-0.4991816557	1.2290282132	2.2337648874
H	-2.1805922780	3.0132200448	0.9905422497
C	-0.6772413953	0.6709743122	-1.4468091865
C	-0.4319538031	-0.0834431179	-0.2819760771
H	0.1830520279	0.8768131543	-2.0819256112
H	-1.6242098085	0.4994145698	-1.9572363747
N	-1.4103356711	-0.9025521283	0.3409078506
H	-1.1229159311	-1.3123547535	1.2261640041
C	-2.6603329316	-1.2299380568	-0.0976957657
O	-3.2144464771	-0.8100407791	-1.0937976266
P	1.2013295896	-0.6666681208	0.1531883902
O	1.4447621221	-0.9100680997	1.6081066597
O	1.3217661357	-2.0138305040	-0.7502034962
O	2.2274815458	0.3702822282	-0.5549596273
C	2.4044417418	-2.9125479755	-0.5246362937
H	2.1390087329	-3.8721434516	-0.9890339899
H	3.3297229406	-2.5336394777	-0.9920423970
H	2.5755343027	-3.0567490219	0.5538496637
C	3.3984023290	0.8355118352	0.1115398140
H	3.5047653309	1.9099987405	-0.0948829009
H	3.3234279257	0.6663244288	1.1960390634
H	4.2825485599	0.3062232791	-0.2784239517
H	-3.1489485708	-1.9510200486	0.6022845728

TSa-*exo*

0 1

C	-1.9262762834	-1.6071876812	1.1665064206
C	-2.5175738662	-1.7594223563	-0.1004687077
C	-1.5205594470	-2.1782129132	-1.0193271475
C	-0.3948336844	-2.7495156671	-0.1761178302
C	-0.5900091371	-1.9862414830	1.0991146920
H	-2.4148671952	-1.1635970819	2.0354187076
H	-3.5297260907	-1.4558293596	-0.3688522441

H	-1.7755943301	-2.5964827555	-1.9969006958
H	0.6104661952	-2.6910900056	-0.6049429002
H	0.1198320680	-1.9705901092	1.9258564787
H	-0.6248858932	-3.8179092119	0.0139949934
C	-0.5758821763	-0.5037578559	-1.5397889036
C	-0.1815910572	0.1297295882	-0.3480811727
H	-1.3860148228	-0.0404397291	-2.1008395524
H	0.2298163455	-0.9341482382	-2.1381719474
P	1.4851821380	0.2960394747	0.2607351757
O	1.5145191874	0.7785442311	1.6750834950
O	2.2093639615	-1.1002620855	-0.0981935356
O	2.3237064360	1.2706405125	-0.7496723540
C	3.6193114156	-1.2300504835	0.0950670689
H	3.8623314285	-2.2937383152	-0.0269627414
H	4.1667467682	-0.6329233652	-0.6497670083
H	3.9039722717	-0.9027579936	1.1074253890
C	2.2184009093	2.6829671438	-0.6066297323
H	3.0468554265	3.1340136391	-1.1701921926
H	1.2637364532	3.0490607058	-1.0220479894
H	2.2883207632	2.9749814472	0.4528145418
H	-0.7314131261	1.2617924941	1.2666736351
N	-1.0341877724	1.0299876963	0.3220122361
C	-2.1914033498	1.6111152101	-0.1169195220
O	-2.7067063199	1.4909918503	-1.2104533233
H	-2.6296488460	2.2580416372	0.6819865169

TSb-*endo*

0 1

C	-4.2128113887	-0.6573597450	0.7547027450
C	-4.5295117458	-1.0672448163	-0.5478188439
C	-3.6017892686	-2.0517011315	-0.9554231016
C	-2.9845282521	-2.5963127622	0.3148283755
C	-3.1118837371	-1.3926005231	1.2065327742
H	-4.6611721772	0.1865319784	1.2777372788
H	-5.2640374169	-0.5889278517	-1.1975760478
H	-3.7282967402	-2.6594493737	-1.8544889363
H	-1.9737526215	-3.0125202666	0.2235145368
H	-2.6777202166	-1.3084695734	2.2036409382
H	-3.6457571432	-3.3945281544	0.7069546918
C	-1.9344937185	-0.8612173592	-1.4094560894
C	-1.4131667716	-0.4923569023	-0.1599566004
H	-2.4133396183	-0.0824663933	-2.0017814385
H	-1.3972675882	-1.6178080522	-1.9818978102
N	-0.3167001541	-1.1972433456	0.4222180412
H	-0.2213194442	-1.1083148246	1.4302502840
C	0.8392524614	-1.5246940995	-0.2385786579
O	1.0230714903	-1.4948805280	-1.4346225847

P	-1.4365035872	1.1821464416	0.4786492492
O	-1.2057920119	1.2493261424	1.9529704170
O	-0.3570617131	2.0578794303	-0.3725026044
O	-2.8055624418	1.8022384621	-0.1100106194
C	1.0301752870	2.0379136541	-0.0374331820
H	1.5532354264	1.2680124923	-0.6245411205
H	1.4526039123	3.0206478378	-0.2892002087
H	1.1740462131	1.8488849923	1.0368968661
C	-3.1393394671	3.1572217363	0.1838451210
H	-4.1895082552	3.3025288325	-0.1028883822
H	-3.0190073367	3.3637677699	1.2592463495
H	-2.4997178891	3.8445765590	-0.3918320004
O	1.7736180500	-1.9093463976	0.6684362296
C	3.1137467927	-2.0263222921	0.1630204492
H	3.1083414813	-2.6114487137	-0.7667805144
H	3.6504547667	-2.5835798591	0.9428610192
C	3.7242127098	-0.6645492553	-0.0578852378
C	3.9621604098	-0.1826884146	-1.3509714740
C	3.9860461738	0.1682613361	1.0400638316
C	4.4647180769	1.1068151290	-1.5445151643
H	3.7233627826	-0.8148412351	-2.2083521696
C	4.4802311027	1.4594416841	0.8491266422
H	3.7879370352	-0.1966406525	2.0514161137
C	4.7218038204	1.9308664249	-0.4457923961
H	4.6435169082	1.4737328735	-2.5579558903
H	4.6780225605	2.1007017707	1.7114165255
H	5.1085692440	2.9415869754	-0.5970584043

TSb-*exo*

0 1

C	-1.4731463000	-2.5111648280	0.8920755323
C	-2.2500798905	-2.4616014494	-0.2789810269
C	-1.3869325041	-2.2570561500	-1.3868211864
C	-0.0042994039	-2.6717912138	-0.9169093914
C	-0.1265291205	-2.4137454959	0.5553199507
H	-1.8732274169	-2.5121170886	1.9072798947
H	-3.3390302549	-2.4247486820	-0.3174604024
H	-1.7172218802	-2.4210953033	-2.4162890391
H	0.8414279391	-2.1791185848	-1.4075262080
H	0.7069357058	-2.4206265124	1.2573460627
H	0.0940751453	-3.7648373683	-1.0790140309
C	-1.0306979444	-0.2917167553	-1.3866044922
C	-0.5872224354	0.0343249468	-0.0941372484
H	-2.0272531688	0.0413909066	-1.6716438045
H	-0.2736980447	-0.2431089865	-2.1715899127
P	1.0655795999	0.5263148665	0.3555752400
O	1.3013354571	0.4576006699	1.8291092903

O	2.0238553062	-0.3243514931	-0.6253495000
O	1.3464769680	2.0194502589	-0.2491621227
C	3.4263716467	-0.0531576590	-0.6389869896
H	3.9032544131	-0.8581987998	-1.2133600788
H	3.6261789878	0.9172691962	-1.1183623141
H	3.8302005641	-0.0446906126	0.3856957316
C	0.9141616970	3.1609443904	0.4849977743
H	1.4281981264	4.0365526765	0.0648167752
H	-0.1754495923	3.3032711156	0.3839566768
H	1.1668310897	3.0578003530	1.5516430237
H	-1.0860006740	0.3637019668	1.8758986122
N	-1.4814753899	0.3728440076	0.9381647489
C	-2.8212565157	0.6193400405	0.8534845992
O	-3.4886714981	0.7092277372	-0.1584320929
O	-3.3145346517	0.7688502113	2.1078675895
C	-4.6800434122	1.2112538405	2.2134506976
H	-5.2613648586	0.7616995104	1.3970889660
H	-5.0289051447	0.8143249056	3.1758512138
C	-4.7783481663	2.7159360207	2.1867431425
C	-4.8226007988	3.4073574433	0.9656160469
C	-4.7882532535	3.4463400816	3.3829428562
C	-4.8762118925	4.8025759926	0.9470081367
H	-4.7995112768	2.8387990437	0.0341336378
C	-4.8441545904	4.8420313738	3.3652591506
H	-4.7526894077	2.9148751320	4.3380338045
C	-4.8876596230	5.5228235572	2.1455910468
H	-4.9119961873	5.3312501983	-0.0087575695
H	-4.8546094128	5.3992708861	4.3051698020
H	-4.9327064355	6.6145803731	2.1289995169

TSc-*endo*

0 1

C	0.6342940198	2.5831375078	0.7801963732
C	0.2644518821	3.1303514034	-0.4560258253
C	-1.0443501614	2.6911909379	-0.7717854185
C	-1.6652497454	2.2642666781	0.5406428578
C	-0.4326598582	1.8338559643	1.2869818150
H	1.6271873504	2.6365074981	1.2261358043
H	0.9316359558	3.6594595891	-1.1382877314
H	-1.6373768760	3.1194548876	-1.5832701198
H	-2.4754518764	1.5302100881	0.4665816925
H	-0.4344652504	1.3258761145	2.2516518248
H	-2.0758742015	3.1653497517	1.0387371477
C	-0.7017318218	0.7575144501	-1.4099996303
C	-0.4693125117	0.0059573059	-0.2412926242
H	0.1580810648	0.9258107943	-2.0560614311
H	-1.6590151217	0.6167912990	-1.9106757015

N	-1.4627081231	-0.7705238172	0.4067812084
H	-1.1981189593	-1.1804761331	1.2978066475
C	-2.7122788081	-1.0868179138	-0.0432548970
O	-3.2552206418	-0.6727869896	-1.0459660395
P	1.1503701471	-0.6332594364	0.1687723160
O	1.4351988875	-0.8466937690	1.6205143283
O	1.1886897132	-2.0096456234	-0.6978121846
O	2.1908679542	0.3469645141	-0.5983111772
C	2.2043306754	-2.9748105352	-0.4391233704
H	1.8675106997	-3.9304148007	-0.8640877126
H	3.1523492354	-2.6833781866	-0.9235936705
H	2.3704989299	-3.0874604838	0.6437188245
C	3.4016084715	0.7781297833	0.0171560883
H	3.5368075678	1.8463343469	-0.2049858028
H	3.3625612466	0.6228620506	1.1056341680
H	4.2514005907	0.2145492262	-0.4003512739
O	-3.2951098796	-1.9433040388	0.8312985584
C	-4.6233217707	-2.3387093868	0.5095632939
H	-4.6551312239	-2.8676731542	-0.4551616478
H	-4.9458955045	-3.0069179672	1.3177471967
H	-5.2920370761	-1.4663266650	0.4480628132

TSc-*exo*

0 1

C	-1.9127416539	-1.6428802608	1.1657921871
C	-2.4991998005	-1.8375086695	-0.0975482116
C	-1.4921899304	-2.2624254170	-1.0036281389
C	-0.3617946827	-2.7984131822	-0.1434068607
C	-0.5699474648	-2.0024441347	1.1104208908
H	-2.4089218511	-1.1785461727	2.0196319185
H	-3.5150396536	-1.5571812138	-0.3767167648
H	-1.7371435974	-2.7080154913	-1.9718033314
H	0.6434055627	-2.7369455391	-0.5726853392
H	0.1365336877	-1.9539946337	1.9386496753
H	-0.5781974600	-3.8648925294	0.0724116146
C	-0.5704913670	-0.5861276279	-1.5574039918
C	-0.2056418947	0.0833590246	-0.3770138574
H	-1.3811331600	-0.1554949368	-2.1433372650
H	0.2540706176	-1.0108670834	-2.1330446571
P	1.4511318324	0.2922178186	0.2445202636
O	1.4742386037	0.7305976481	1.6725735474
O	2.2148576648	-1.0694661889	-0.1641365157
O	2.2526860558	1.3296499705	-0.7341268760
C	3.6251943870	-1.1678686507	0.0398946358
H	3.8993894573	-2.2192539992	-0.1177668200
H	4.1636008830	-0.5293116507	-0.6768647326
H	3.8909156913	-0.8697111049	1.0663750323

C	2.1369947313	2.7309862966	-0.5126903006
H	2.9547613979	3.2200742991	-1.0601938149
H	1.1740336717	3.1102770671	-0.8957748572
H	2.2156219330	2.9648010152	0.5603453562
H	-0.8203270602	1.2114036710	1.2291249853
N	-1.0785811951	0.9731295220	0.2740271751
C	-2.2856905376	1.4454369632	-0.1618487530
O	-2.7819315131	1.3080296662	-1.2601700546
O	-2.8701808408	2.1408153004	0.8458476738
C	-4.1288554019	2.7264892671	0.5368140692
H	-4.4534707859	3.2475805836	1.4461144269
H	-4.0404533771	3.4387056930	-0.2978483366
H	-4.8646842796	1.9567538505	0.2574979475

TSd-*endo*

0 1

C	0.5121323245	2.5995416387	0.6760052998
C	0.1561591761	3.0625916044	-0.5987460887
C	-1.1210590758	2.5465431528	-0.9274912170
C	-1.7619843947	2.1570411044	0.3869839724
C	-0.5343345524	1.8303046363	1.1922227608
H	1.4862887254	2.7278171289	1.1470258238
H	0.8184580560	3.5889455784	-1.2878322400
H	-1.7089373129	2.9082467826	-1.7742819622
H	-2.5346216297	1.3830397469	0.3246981743
H	-0.5433860318	1.3713946108	2.1812844228
H	-2.2291185572	3.0628121494	0.8232874749
C	-0.6770165446	0.6132611293	-1.4682144627
C	-0.4405975323	-0.0762572985	-0.2628410077
H	0.1894436212	0.7869266709	-2.1050906461
H	-1.6198874955	0.4123680033	-1.9753110195
N	-1.4038217221	-0.8694414242	0.4069158282
H	-1.0965100770	-1.2087428643	1.3128862426
C	-2.6551945867	-1.2420904563	-0.0055887913
O	-3.2146916778	-0.7742159365	-0.9892554226
P	1.1824333818	-0.6481403197	0.2182922701
O	1.3704266220	-0.8794269422	1.6845001145
O	1.3307827847	-2.0048039837	-0.6676458270
O	2.2415891615	0.3715041721	-0.4641251730
C	2.4269113142	-2.8809850711	-0.4217355279
H	2.1881616969	-3.8469405066	-0.8873942980
H	3.3523707568	-2.4851714148	-0.8745911853
H	2.5829182836	-3.0197907142	0.6599350387
C	3.3767287131	0.8583107558	0.2473544702
H	3.5023216542	1.9223223436	0.0017268416
H	3.2405852847	0.7354429176	1.3320907133
H	4.2777257306	0.3086912225	-0.0691767288

C	-3.3235139924	-2.2654197594	0.8704867920
C	-4.7173064397	-2.3867426274	0.7709246137
C	-2.6207468230	-3.1151185432	1.7399716886
C	-5.4014502922	-3.3212723538	1.5471202385
H	-5.2411907061	-1.7334595837	0.0712853398
C	-3.3064221763	-4.0540816767	2.5130322292
H	-1.5303250070	-3.0743920513	1.7997817116
C	-4.6975589923	-4.1551373239	2.4223912222
H	-6.4881546053	-3.4033165531	1.4688713013
H	-2.7504170678	-4.7149669362	3.1819801491
H	-5.2327251347	-4.8896522077	3.0290630138

TSd-*exo*

0 1

C	-1.8331163990	-1.5027770627	1.2901641951
C	-2.4865571247	-1.6227642108	0.0481598562
C	-1.5506645341	-2.0880528657	-0.9150309689
C	-0.4171044576	-2.7140085765	-0.1181245207
C	-0.5279760040	-1.9576570960	1.1706319278
H	-2.2642604682	-1.0351474232	2.1768980754
H	-3.4949571620	-1.2742666006	-0.1746311997
H	-1.8790208530	-2.5154853301	-1.8667338996
H	0.5725312768	-2.6902580477	-0.5859481695
H	0.2217696995	-1.9666737248	1.9611125077
H	-0.6816193344	-3.7754089022	0.0671892247
C	-0.5856996852	-0.4966311512	-1.5363718610
C	-0.1432744078	0.1946872219	-0.3950375093
H	-1.3967701042	-0.0356888137	-2.0986193727
H	0.1972863737	-0.9668611372	-2.1350874141
P	1.5278169534	0.3238076716	0.2026540541
O	1.5718987757	0.8264898658	1.6107878724
O	2.2223037900	-1.0903358406	-0.1440862975
O	2.3839724565	1.2689858362	-0.8209769148
C	3.6338889449	-1.2412289479	0.0184188225
H	3.8559113459	-2.3105931083	-0.0939798510
H	4.1740887400	-0.6638058500	-0.7468544967
H	3.9479765319	-0.9053920396	1.0192855237
C	2.2776414175	2.6843280399	-0.7097562519
H	3.0911229228	3.1230258381	-1.3039062332
H	1.3119759549	3.0382508684	-1.1101429682
H	2.3716044362	3.0021066385	0.3403566160
H	-0.6144879203	1.3264709219	1.2196303416
N	-0.9753827892	1.0766687071	0.3024850669
C	-2.1913027696	1.5955857413	-0.0716250183
O	-2.6650557673	1.4929861001	-1.1961510345
C	-2.9079522274	2.3495296747	1.0154005982
C	-3.9918064302	3.1507068717	0.6252109850

C	-2.5719512106	2.2713915542	2.3770972645
C	-4.7138445433	3.8752523318	1.5722358561
H	-4.2467770511	3.1832368674	-0.4351096818
C	-3.2979372979	2.9948650417	3.3254824685
H	-1.7558570236	1.6319234311	2.7215845921
C	-4.3674654124	3.8006259506	2.9254517750
H	-5.5523253170	4.5003794201	1.2560036207
H	-3.0295422145	2.9232502321	4.3819740666
H	-4.9339874409	4.3662242626	3.6691385328

TSe-*endo*

0 1

C	-0.5292150588	2.5331609853	-0.7858334452
C	-0.1576826135	3.0624746139	0.4580634169
C	1.1228788800	2.5622273200	0.7987542988
C	1.7476585782	2.1057743604	-0.5019743233
C	0.5106177331	1.7358282914	-1.2728798375
H	-1.5091902202	2.6356750306	-1.2510300868
H	-0.8118050801	3.6239373163	1.1269645790
H	1.7211273597	2.9668520215	1.6184172734
H	2.5223400078	1.3367404970	-0.4111540348
H	0.5061876571	1.2244889259	-2.2358198754
H	2.2080359252	2.9881705897	-0.9902617342
C	0.6818991258	0.6602660774	1.4428102943
C	0.4354021972	-0.0924177711	0.2770976493
H	-0.1801700680	0.8706448448	2.0742169384
H	1.6265426249	0.4805507794	1.9542224294
N	1.4006070960	-0.9171742953	-0.3559153129
H	1.0884048535	-1.3211610556	-1.2336219905
C	2.6585728830	-1.2547768788	0.0736423011
O	3.1865752972	-0.7949340798	1.0733277466
C	3.3687417489	-2.2683795532	-0.8083517076
H	2.8281532186	-2.5109913098	-1.7345632056
H	4.3675313103	-1.8814020300	-1.0572713327
H	3.5138712615	-3.1923214932	-0.2273468861
P	-1.2003364275	-0.6680401373	-0.1567039141
O	-1.4351832045	-0.9291644722	-1.6105016541
O	-1.3364765637	-2.0035312551	0.7632803641
O	-2.2302541266	0.3785667971	0.5317646022
C	-2.4361909148	-2.8843451676	0.5531389126
H	-2.1919666606	-3.8392871285	1.0384432202
H	-3.3553401972	-2.4773576323	1.0090763331
H	-2.6072736123	-3.0481422212	-0.5226728920
C	-3.3844991955	0.8539372110	-0.1557045372
H	-3.4879446321	1.9282590157	0.0529351165
H	-3.2897838923	0.6882896318	-1.2391871901
H	-4.2798332904	0.3291031721	0.2144914845

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C	1.9141714655	1.5286983825	1.2360968183
C	2.5176920241	1.7090382586	-0.0217755599
C	1.5334045808	2.1714388459	-0.9345828545
C	0.4118927356	2.7370731749	-0.0809471293
C	0.5860124396	1.9341854584	1.1728112994
H	2.3888307025	1.0508496127	2.0948244116
H	3.5259964682	1.3963621232	-0.2938480556
H	1.8048617814	2.6205185844	-1.8939994061
H	-0.5914459503	2.7062177500	-0.5175389685
H	-0.1306014482	1.9033643249	1.9931259970
H	0.6572457644	3.7963432727	0.1394919608
C	0.5732645415	0.5406839893	-1.5254377641
C	0.1752078947	-0.1431501447	-0.3635937037
H	1.3785343882	0.0928831292	-2.1055445098
H	-0.2314110269	1.0006496628	-2.1029265294
P	-1.4906718727	-0.3143979547	0.2429850479
O	-1.5239041863	-0.8382849826	1.6429868465
O	-2.2083203438	1.0954707702	-0.0750235381
O	-2.3376302128	-1.2553198289	-0.7933225447
C	-3.6175949526	1.2258858691	0.1199139422
H	-3.8558430911	2.2938009289	0.0288812461
H	-4.1668649819	0.6533944545	-0.6427608384
H	-3.9056907456	0.8703718793	1.1218026575
C	-2.2297809033	-2.6708909290	-0.6958473338
H	-3.0563752778	-3.1053856745	-1.2750437610
H	-1.2735989327	-3.0219273369	-1.1209139962
H	-2.3008883954	-2.9971580122	0.3535621426
H	0.7083798472	-1.3032071362	1.2225312419
N	1.0223238939	-1.0582215439	0.2863231976
C	2.2027908863	-1.6107359238	-0.1538333064
O	2.6727353981	-1.4456084721	-1.2674568194
C	2.9068299673	-2.4532526358	0.8973723251
H	2.2261924580	-2.8493266062	1.6655408353
H	3.4246123495	-3.2809110449	0.3949694491
H	3.6703017344	-1.8346012452	1.3969742002

TSf-*endo*

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C	0.5010032140	2.6018184034	0.6465035414
C	0.1447094128	3.0515739962	-0.6329491206
C	-1.1311029434	2.5305123620	-0.9581142521
C	-1.7710530123	2.1492745311	0.3592477785

C	-0.5434618236	1.8350582347	1.1690511801
H	1.4734703291	2.7405713716	1.1175000700
H	0.8049925917	3.5763793203	-1.3250260470
H	-1.7193101323	2.8852066147	-1.8075551831
H	-2.5421982840	1.3733701504	0.3036364645
H	-0.5524191091	1.3848809308	2.1622312157
H	-2.2415666185	3.0571675963	0.7870812703
C	-0.6797840704	0.5971071039	-1.4881927546
C	-0.4386400298	-0.0849140979	-0.2803363119
H	0.1845661936	0.7682299688	-2.1289833383
H	-1.6222987857	0.3926354365	-1.9946469915
N	-1.3998506274	-0.8739565480	0.3999832684
H	-1.0783449486	-1.2055548211	1.3046592776
C	-2.6494190850	-1.2485897561	-0.0004259639
O	-3.2195316974	-0.8038127392	-0.9881365485
P	1.1782573008	-0.6699144071	0.2074705900
O	1.3126197274	-0.9581708204	1.6699675037
O	1.3515805252	-1.9855579510	-0.7310329113
O	2.2580885478	0.3721753262	-0.4001606796
C	2.4665997655	-2.8490253803	-0.5210877688
H	2.2529227633	-3.7942157602	-1.0381755116
H	3.3853635699	-2.4087872477	-0.9449582816
H	2.6169821664	-3.0410863795	0.5532319287
C	3.3674885633	0.8351461841	0.3679122608
H	3.5215174777	1.8977568512	0.1337756764
H	3.1787394156	0.7084363616	1.4441776640
H	4.2729725787	0.2721590566	0.0907758755
C	-3.3180380271	-2.2582852265	0.8977310951
C	-4.7108733903	-2.3877942314	0.7853557561
C	-2.6152846979	-3.0851798610	1.7903302520
C	-5.4030379312	-3.3060898527	1.5692084368
H	-5.2305373734	-1.7527449580	0.0667098686
C	-3.2948490776	-4.0122883714	2.5779346274
H	-1.5265342503	-3.0362231269	1.8625810773
C	-4.6808789445	-4.1033942023	2.4576777173
H	-6.4844492182	-3.4205555328	1.5077712038
H	-2.7742934042	-4.6681124377	3.2742953051
N	-5.4064742915	-5.0827248327	3.2937616175
O	-4.7466413032	-5.7710919793	4.0549468149
O	-6.6186433657	-5.1392716394	3.1697053271

TSf-*exo*

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C	-1.8312272198	-1.4981030532	1.3008727538
C	-2.4878197323	-1.6184859961	0.0603502527
C	-1.5551673181	-2.0835495973	-0.9060280071
C	-0.4174147181	-2.7060317149	-0.1125782935

C	-0.5268136859	-1.9530335713	1.1774459393
H	-2.2607156093	-1.0349607975	2.1907837812
H	-3.4988878107	-1.2754015316	-0.1589788280
H	-1.8879153419	-2.5138534615	-1.8548150549
H	0.5711399044	-2.6802680931	-0.5823054456
H	0.2265791856	-1.9595742394	1.9644740805
H	-0.6788438977	-3.7679960079	0.0731132994
C	-0.5994006925	-0.4929133659	-1.5372134664
C	-0.1442572266	0.2022999494	-0.4032134244
H	-1.4160234086	-0.0352364413	-2.0941463917
H	0.1773277295	-0.9646611418	-2.1428566927
P	1.5296195746	0.3350191647	0.1895910742
O	1.5616165100	0.8686098192	1.5870431068
O	2.2163936008	-1.0893122687	-0.1219431835
O	2.3915055486	1.2525031462	-0.8507430997
C	3.6297436650	-1.2444829177	0.0321870416
H	3.8443552942	-2.3169110517	-0.0623571037
H	4.1658962750	-0.6840817863	-0.7481235110
H	3.9529239578	-0.8922847962	1.0243266961
C	2.3030005700	2.6717319563	-0.7675476522
H	3.1237096946	3.0874291903	-1.3678898084
H	1.3432415906	3.0293650875	-1.1785215152
H	2.3984804135	3.0085753350	0.2763060042
H	-0.5887606070	1.3421200497	1.2096776914
N	-0.9707645747	1.0843370199	0.3024842394
C	-2.1889062095	1.5968520015	-0.0571359050
O	-2.6878500563	1.4906843136	-1.1702604334
C	-2.8913093581	2.3592025701	1.0376178189
C	-3.9842865517	3.1520332948	0.6526347213
C	-2.5338210024	2.2910000058	2.3952975365
C	-4.7001868014	3.8825120719	1.5958805661
H	-4.2536072616	3.1734804160	-0.4040117331
C	-3.2436365957	3.0147384097	3.3513730044
H	-1.7101936035	1.6588441644	2.7330556939
C	-4.3155408084	3.8027811694	2.9347182643
H	-5.5476004994	4.5090706131	1.3207453273
H	-2.9878649902	2.9762392147	4.4092384391
N	-5.0708334440	4.5719340536	3.9451389467
O	-4.7158786134	4.4736518418	5.1084460701
O	-6.0007680956	5.2563781149	3.5514818398

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C	0.5211271443	2.5130504046	0.7996630599
C	0.1537092247	3.0528665918	-0.4406674675
C	-1.1242828138	2.5546054065	-0.7936319594
C	-1.7534508699	2.0829793434	0.5000566821

C	-0.5206249324	1.7118590945	1.2750073117
H	1.4988011510	2.6117439785	1.2703006534
H	0.8081490052	3.6257579235	-1.0994019231
H	-1.7188351215	2.9695890589	-1.6107369801
H	-2.5294589822	1.3156042462	0.4058165111
H	-0.5179251504	1.1865097909	2.2303413359
H	-2.2181838543	2.9598120626	0.9934561946
C	-0.6649848309	0.6700591506	-1.4641308255
C	-0.4098644768	-0.0959430430	-0.3098434953
H	0.1948110164	0.8962517722	-2.0928427172
H	-1.6049386882	0.4921432325	-1.9847218928
N	-1.3801568222	-0.9277408627	0.3105652218
H	-1.0895842184	-1.3398354976	1.1943489200
C	-2.6198653377	-1.2422211182	-0.1339177790
O	-3.1719221565	-0.8474796640	-1.1423708920
C	-3.3979378118	-2.1794431335	0.8224127903
P	1.2258564611	-0.6619843690	0.1381373262
O	1.4532415942	-0.9051052727	1.5951603654
O	1.3660840713	-2.0042893837	-0.7662591391
O	2.2351154047	0.3975288759	-0.5572881406
C	2.4199897594	-2.9284029553	-0.5018270383
H	2.1453505864	-3.8829873883	-0.9707594820
H	3.3679206844	-2.5735744199	-0.9413495955
H	2.5520206834	-3.0705393031	0.5820919646
C	3.4076969869	0.8612707293	0.1092526601
H	3.5067817076	1.9383844128	-0.0863403074
H	3.3379171231	0.6803204222	1.1921802756
H	4.2925109386	0.3410317030	-0.2907062953
F	-2.6398279947	-2.6163826975	1.8519758550
F	-3.8464037359	-3.2449586561	0.1622470053
F	-4.4452378552	-1.5289316952	1.3398988273

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C	-1.9197128299	-1.4962336463	1.1977910730
C	-2.5181069618	-1.6370895905	-0.0682285297
C	-1.5406359313	-2.1043638090	-0.9874119917
C	-0.4331329398	-2.7065633816	-0.1387087933
C	-0.6032844148	-1.9335742323	1.1325858428
H	-2.3921425140	-1.0208650881	2.0583645444
H	-3.5204699138	-1.2997660854	-0.3327075488
H	-1.8235808583	-2.5385229631	-1.9504161181
H	0.5743411922	-2.6811951845	-0.5661075390
H	0.1149727448	-1.9217332570	1.9519391498
H	-0.6960079670	-3.7666552333	0.0542856416
C	-0.5594159211	-0.4959171163	-1.5662432134
C	-0.1464164668	0.1782938299	-0.4036820113

H	-1.3541187981	-0.0356210309	-2.1518651800
H	0.2382408760	-0.9631730285	-2.1473236399
P	1.5096775830	0.3228666057	0.2332052557
O	1.5093736187	0.8174323329	1.6432856554
O	2.2081368510	-1.0910202469	-0.1039791270
O	2.3845565559	1.2731950182	-0.7657164927
C	3.6162100885	-1.2479909553	0.0893997201
H	3.8373090170	-2.3167084826	-0.0293111774
H	4.1746232237	-0.6641851183	-0.6575529558
H	3.9075347365	-0.9237462226	1.1007634865
C	2.3188136058	2.6892186990	-0.6225180814
H	3.1679832708	3.1148659131	-1.1744861628
H	1.3812417030	3.0828038426	-1.0512560712
H	2.3818582170	2.9777794660	0.4379963140
H	-0.7076369798	1.2976898711	1.2156586389
N	-1.0076205448	1.0649204305	0.2688509754
C	-2.2008819345	1.5539127628	-0.1561238174
O	-2.6899565726	1.4835281834	-1.2670713045
C	-3.0062597493	2.2279893313	0.9814582514
F	-3.9003496048	1.3532411210	1.4746504467
F	-3.6641400296	3.2900967953	0.5325998893
F	-2.2207574921	2.6190863895	2.0066961705

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C	2.7813660000	-2.0731000000	-0.9092020000
C	2.9206010000	-2.6749750000	0.3492660000
C	1.6360330000	-2.8114660000	0.9306310000
C	0.6530780000	-2.7366140000	-0.2178860000
C	1.4238610000	-1.8615120000	-1.1674490000
H	3.5993070000	-1.7250920000	-1.5394270000
H	3.8659870000	-2.8493910000	0.8653780000
H	1.4457880000	-3.4207370000	1.8172430000
H	-0.3593950000	-2.4116450000	0.0448170000
H	1.0226980000	-1.4349370000	-2.0871380000
H	0.5727690000	-3.7467470000	-0.6674440000
C	1.2616140000	-0.9018100000	1.5918830000
C	0.9256700000	-0.1568710000	0.4435000000
H	2.2201490000	-0.6715710000	2.0547300000
H	0.4461590000	-1.1609350000	2.2657600000
N	-0.4047510000	0.1047680000	0.0288970000
H	-0.4787170000	0.5742020000	-0.8686910000
C	-1.5810500000	-0.1916560000	0.6634800000
O	-1.6548870000	-0.8015050000	1.7210410000
P	2.0093110000	1.0926840000	-0.2339100000
O	1.8461190000	1.3798330000	-1.6925560000
O	1.6699820000	2.3732440000	0.7109590000

O	3.5050810000	0.6616640000	0.2213840000
C	2.1670480000	3.6585450000	0.3498830000
H	1.5864080000	4.4059960000	0.9078310000
H	3.2317330000	3.7567350000	0.6240510000
H	2.0538310000	3.8327010000	-0.7318680000
C	4.6135740000	0.7539150000	-0.6693530000
H	5.2341570000	-0.1436330000	-0.5361370000
H	4.2718200000	0.8234670000	-1.7128020000
H	5.2142480000	1.6445900000	-0.4243500000
C	-2.8298970000	0.2560840000	-0.0885650000
C	-3.5487190000	-0.9808810000	-0.6628730000
C	-3.7625170000	1.0437730000	0.8450410000
H	-2.5390850000	0.9106170000	-0.9304210000
C	-4.8624180000	-0.6055660000	-1.3533210000
H	-3.7459500000	-1.6742420000	0.1722750000
H	-2.8783750000	-1.5082420000	-1.3626620000
C	-5.0775200000	1.4136240000	0.1534860000
H	-3.9590510000	0.4168750000	1.7303380000
H	-3.2471480000	1.9483280000	1.2080800000
C	-5.7836820000	0.1773020000	-0.4122820000
H	-5.3671090000	-1.5145860000	-1.7201960000
H	-4.6434410000	0.0111040000	-2.2444770000
H	-5.7371330000	1.9432310000	0.8604470000
H	-4.8717970000	2.1220260000	-0.6702660000
H	-6.7091090000	0.4687210000	-0.9359840000
H	-6.0877730000	-0.4791060000	0.4232330000

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0 1

C	-0.7207830275	2.3689173117	-0.9300795915
C	-1.0549284631	2.7192648840	0.3910639807
C	0.1361423782	2.7642199360	1.1632911418
C	1.2711649331	2.9056666258	0.1634016286
C	0.6598847141	2.2603959149	-1.0434233394
H	-1.4406066784	2.1232836345	-1.7126230551
H	-2.0659742370	2.7899237740	0.7925239842
H	0.1690245963	3.2568010673	2.1390770872
H	2.2394415445	2.4965414767	0.4692444586
H	1.2069613970	1.9919802611	-1.9467189283
H	1.4061187451	3.9872898399	-0.0430935818
C	0.5038525385	0.8854976555	1.6664192925
C	0.4664144284	0.1315692977	0.4807171028
H	-0.3254380739	0.7489154370	2.3587683289
H	1.4895750446	1.0041965490	2.1211739626
P	1.8533589424	-0.6217488731	-0.3426837342
O	1.5101109242	-1.0659105787	-1.7282929794
O	3.0798750017	0.4068960421	-0.1380493902

O	2.4106302095	-1.8507855550	0.5827243990
C	4.3979748193	0.0132505455	-0.5235704628
H	5.0274421611	0.9115529938	-0.4753617781
H	4.7873613379	-0.7539578579	0.1626776912
H	4.4004498743	-0.3810978908	-1.5520003422
C	1.7599420200	-3.1148550548	0.5223682383
H	2.4226021382	-3.8501835023	0.9994955698
H	0.8020257660	-3.0900412620	1.0703096053
H	1.5715000807	-3.4083427503	-0.5222171941
H	-0.6751644795	-0.6898512572	-0.9958806896
N	-0.7388616364	-0.3810576106	-0.0288099487
C	-1.9632559971	-0.4853113381	0.5838280035
O	-2.1814189478	-0.2005886028	1.7525334771
C	-3.0705441374	-1.0104207104	-0.3239639933
C	-3.6580356900	-2.3086697642	0.2568096299
C	-4.1654617925	0.0618868864	-0.4748254347
H	-2.6563313313	-1.2285197372	-1.3248983686
C	-4.8450428840	-2.8141981609	-0.5675142016
H	-3.9743458413	-2.1007413697	1.2925170451
H	-2.8709013042	-3.0792209355	0.3146436813
C	-5.3511444018	-0.4473002779	-1.2983676690
H	-4.4998669703	0.3442716662	0.5375483102
H	-3.7365816245	0.9695449764	-0.9312330787
C	-5.9273594055	-1.7394158949	-0.7105647543
H	-5.2636956191	-3.7231127373	-0.1047942154
H	-4.4925962313	-3.1103310030	-1.5727822347
H	-6.1300827188	0.3308331056	-1.3563827836
H	-5.0211209438	-0.6360065085	-2.3366169964
H	-6.7564769795	-2.1096821563	-1.3359002257
H	-6.3556296795	-1.5234103518	0.2850990627

TSi-*endo*

0 1

C	0.6209002164	2.6421247588	0.5475541055
C	0.2367861547	3.0633813765	-0.7335473076
C	-1.0629849905	2.5686778287	-1.0014460898
C	-1.6719127918	2.2499070817	0.3468722264
C	-0.4280154999	1.9226218938	1.1267394178
H	1.6127171173	2.7624733800	0.9825598487
H	0.8906053949	3.5426076273	-1.4639058438
H	-1.6681588206	2.9114293457	-1.8439621544
H	-2.4652750903	1.4946262491	0.3391376149
H	-0.4176334375	1.5044669712	2.1336977950
H	-2.1019026288	3.1846807605	0.7597105241
C	-0.6865891262	0.6039288299	-1.4750452895
C	-0.4336309550	-0.0436382464	-0.2497512550
H	0.1650259230	0.7275179499	-2.1429122761

H	-1.6493611393	0.4087307846	-1.9457207765
N	-1.3967567471	-0.7813657688	0.4793610057
H	-1.0706257071	-1.0929178740	1.3884134644
C	-2.6718160460	-1.1348602566	0.1187988011
O	-3.2460842246	-0.6807014597	-0.8641235736
P	1.1867057399	-0.6415095588	0.2075168050
O	1.4115514757	-0.8220503110	1.6758091246
O	1.2740918084	-2.0352104643	-0.6281538777
O	2.2538295003	0.3209010428	-0.5430336602
C	2.3466976580	-2.9362174784	-0.3700395856
H	2.0669419306	-3.9117534037	-0.7908491068
H	3.2741974037	-2.5896164894	-0.8578994383
H	2.5225081322	-3.0366977982	0.7128285205
C	3.4239925592	0.7973603180	0.1162114576
H	3.5708191313	1.8493066161	-0.1670160566
H	3.3197696945	0.7123037750	1.2081055689
H	4.2990618825	0.2129159585	-0.2107324282
C	-3.3367593097	-2.1075335560	1.0437452139
C	-4.7283972637	-2.2341418337	0.9600450060
C	-2.6435640988	-2.9227288416	1.9588590102
C	-5.4278131854	-3.1225783082	1.7759073075
H	-5.2557305360	-1.6152300799	0.2321261043
C	-3.3257479765	-3.8146966940	2.7750881668
H	-1.5529961795	-2.8909623298	2.0220477046
C	-4.7261410685	-3.9195733314	2.6954290436
H	-6.5117289213	-3.1890420682	1.6879051146
H	-2.7975611179	-4.4563616699	3.4822107706
O	-5.3045834273	-4.8118604329	3.5308478307
C	-6.7046753851	-4.9786516446	3.4962593308
H	-7.0508506299	-5.3240153671	2.5058856014
H	-6.9470620376	-5.7428209897	4.2462285365
H	-7.2342433306	-4.0435824421	3.7516256181

TSi-*exo*

0 1

C	-1.7157963021	-1.6564966680	1.2691657115
C	-2.3959334649	-1.7541830479	0.0394744299
C	-1.4680860083	-2.1476705124	-0.9629082930
C	-0.2929635472	-2.7609349750	-0.2177832493
C	-0.3986377942	-2.0580194239	1.1015194908
H	-2.1406618703	-1.2373726225	2.1828259544
H	-3.4210619537	-1.4338758127	-0.1458527207
H	-1.8045289520	-2.5516950628	-1.9220343016
H	0.6829543848	-2.6823596918	-0.7080718846
H	0.3703929059	-2.0689734291	1.8731945917
H	-0.5137302978	-3.8378676476	-0.0678218279
C	-0.5781850895	-0.5004142685	-1.5469468513

C	-0.1393734136	0.1677058177	-0.3905454378
H	-1.4185360430	-0.0520384419	-2.0753819872
H	0.2099747292	-0.9171938187	-2.1776536826
P	1.5375795547	0.3394254002	0.1780353093
O	1.5959414767	0.7851865282	1.6047301770
O	2.2806745419	-1.0294111218	-0.2432950153
O	2.3325498166	1.3607296751	-0.8226656426
C	3.6997279175	-1.1293200211	-0.1126104308
H	3.9624779162	-2.1827953917	-0.2760496193
H	4.2016123719	-0.4975590839	-0.8609481809
H	4.0196732954	-0.8246724136	0.8963973808
C	2.1708127974	2.7642278460	-0.6491381270
H	2.9491382720	3.2613259271	-1.2445030328
H	1.1808451600	3.0932561948	-1.0095084952
H	2.2797461269	3.0423224743	0.4107885120
H	-0.6209470907	1.2146064108	1.2758495209
N	-0.9904833351	0.9872101186	0.3566856708
C	-2.2374019672	1.4684331515	0.0284997647
O	-2.7259882165	1.3886071851	-1.0924415071
C	-2.9611065011	2.1418406532	1.1549013809
C	-4.1110456460	2.8726922284	0.8312975447
C	-2.5783441879	2.0658292836	2.5082059628
C	-4.8574575692	3.5284680935	1.8091854475
H	-4.4095209084	2.9132152162	-0.2174830893
C	-3.3129447949	2.7113898822	3.4937980961
H	-1.7078961866	1.4828527541	2.8184819978
C	-4.4587083045	3.4526901617	3.1538317928
H	-5.7434474453	4.0913671909	1.5171662467
H	-3.0258665716	2.6540842072	4.5449810579
O	-5.1089986472	4.0478913557	4.1791915096
C	-6.2698815650	4.8027913873	3.9112497700
H	-6.6231672611	5.1864306066	4.8772499131
H	-6.0576913676	5.6551947628	3.2416544804
H	-7.0643722348	4.1838615422	3.4577260619