KJE-3900

Master thesis in organic chemistry

Synthesis of Potential Metallo-β-Lactamase Inhibitors

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June, 2012

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Abbreviations

Ac – acetyl

Bn – benzyl

BnX – benzyl halide

DMF – dimethylformamide

DMSO- dimethylsulfoxide

EWG - electron-withdrawing groups

IMP, VIM, GIM, SPM, SIM, KHM, AIM, DIM, TMB, and NDM – different types of MBLs

IR – infrared spectroscopy

MBL – metallo β-lactamse

HR-MS – High resolution mass spectroscopy

Nitrocefin - (6R,7R)-3-[(2,4-dinitro)styryl]-8-oxo-7-[(2-thienylacetyl) amino]-5-thia-1-azabicyclo[4.2.0]oct-2-ene-2-carboxylic acid

NMR –nuclear magnetic resonance

Ph - phenyl

PG – protecting group

TEA- triethylamine

THF – tetrahydrofuran

TLC – thin layer chromatography

TMS – trimethylsilyl

TMSBr – trimethylsilylbromide

TMSCl – trimethylsilylchloride

VIM – Verona integron-borne metallo-β-lactamase

Acknowledgments

The work presented in this thesis has been performed at the Department of Chemistry, University of Tromsø, in the period from January 2011 to June 2012. During this time, many have contributed to this work.

First I would like to thank my supervisor, Annette Bayer, for presenting me a motivating and very interesting project, helpful insights and proofreading my thesis. I am beyond grateful.

My gratitude goes to my co-supervisor, Magnus Engquist, being a reliable source of helpful suggestions and advice during this work.

Moreover, I would like to thank the engineering crew, Jostein Johansen, Truls Ingebrigtsen, and Arnfinn Kvarsnes, for kindly helping me with MS analysis and operation of the various analytical instruments.

To my lab and office partners; thank you for the inspirational and good atmosphere.

Last, but not least, I wish to thank my family and friends for inspiration and support. To my sister, Britt Paulsen, for showing outmost patience and whom without I would have starved. To Hanne-Kristin Paulsen for proofreading. To mom, making me want to save the world. And to dad, being who you are.

Tromsø, June 2012

Marianne Hagensen Paulsen

1 Introduction

Bacterial resistance against antibiotics has in the last decades become an increasing problem worldwide. One cause of antibiotic resistance is the bacterial production of enzymes that hydrolyze the β-lactam ring found in many antibiotics. These enzymes, called β-lactamases, are classified into four main groups, one of them being metallo-β-lactamases (MBLs). The structural complexity and heterogeneity of MBLs have posed challenges finding a common inhibitor to treat infections caused by MBL-producing bacteria. To date, the MBLs subgroup has no clinically developed inhibitors, and the search for inhibitors is therefore highly important.

The aim of this project is to synthesize new potential MBL inhibitors, and test for their activities on two specifically selected Verona integron-borne metallo-β-lactamase (VIM) enzymes, VIM-2 and VIM-7. VIM-2 is one of the most clinically relevant MBLs since it is most widespread. One of the characteristics of the VIM enzymes is the presence of two Zn(II) ions in the catalytic site. These Zn(II) ions are important for both enzymatic and inhibitory activity.

Only a few MBL inhibitors have been reported so far, and even fewer have been described for the VIM class. No VIM-7 inhibitors have been found. For VIM-2, some inhibitors have been described in literature, among them, 2-substituted-3-sulfanylpropanoic acids. The inhibitor shown in Figure 1 is the only inhibitor for which an X-ray structure of the VIM-2 inhibitor complex is available. So far, MBL inhibitors containing both thiol and carboxylate functional groups are the most potent broad spectrum MBL inhibitors.[1] A study based on the structure-activity relationship (SAR) of inhibitors has shown that the proximity of the thiol and carboxylate group is important in enabling successful inhibition.[2]

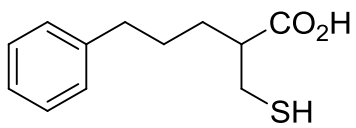
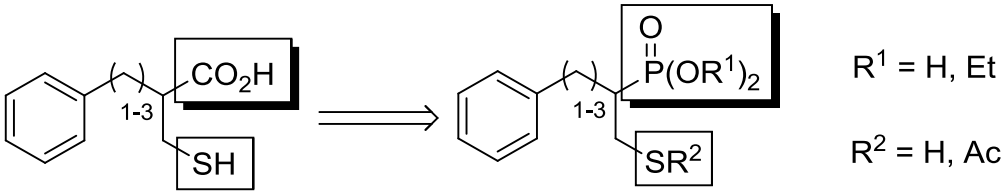


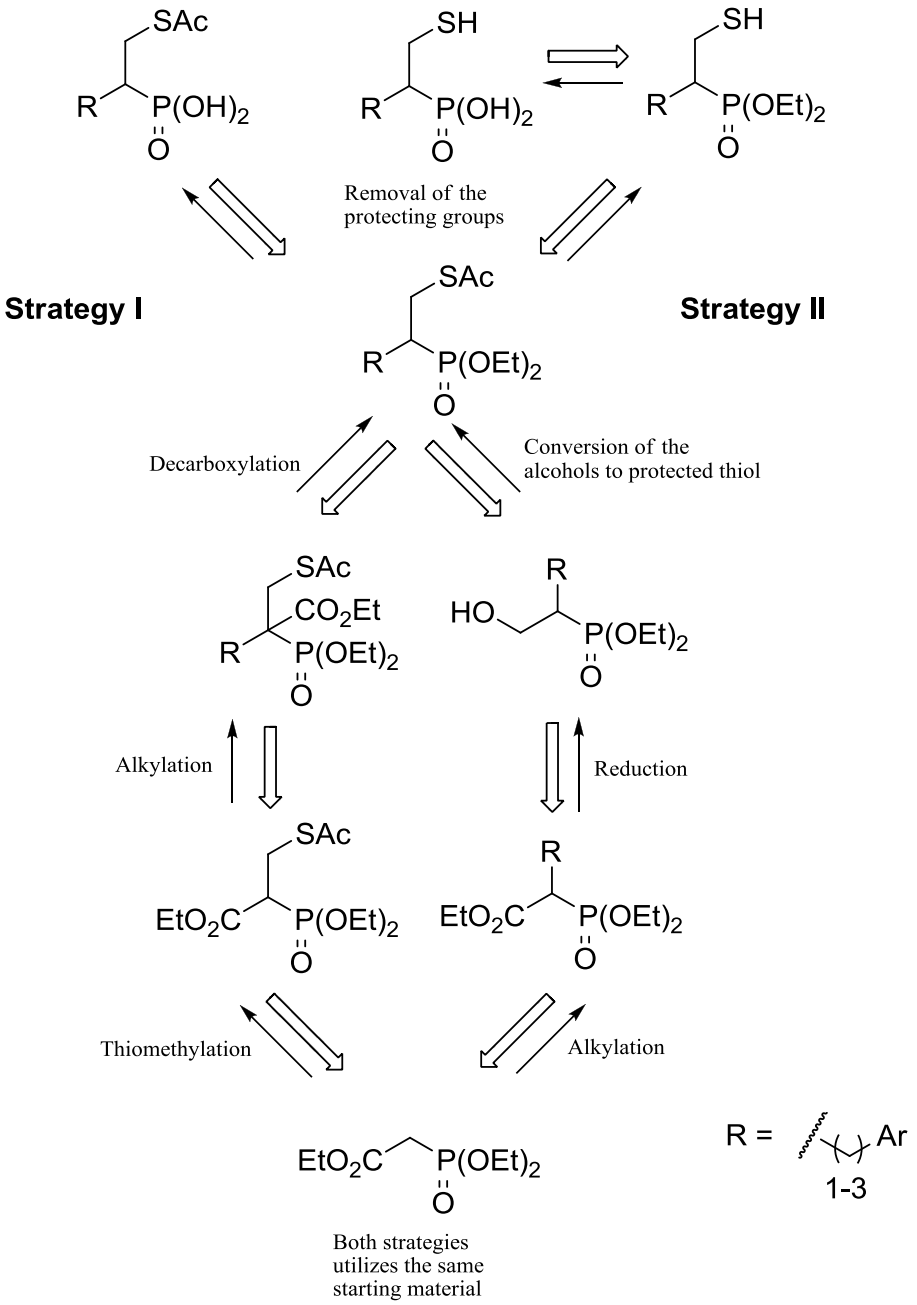
Figure 1 Structure of rac-2-ω-phenylpropyl-3-sulfanylpropanoic acid, the only inhibitor co-crystallized with the VIM-2 enzyme.

Within this project a new group of inhibitors combining structural features of the 2-substituted-3-sulfanylpropanoic acids and phosphonate chemistry is envisioned (Scheme 1). The plan is to conserve the thiol functionality as it is an important group for binding to the Zn(II) ions in the catalytic site, while the carboxylate group will be replaced with a phosphonic acid or a diethyl phosphonate. Phosphonates and phosphonic acids are effective chelating agents, and are therefore potential candidates for MBL inhibitors. One article on VIM-4 inhibitors combining a sulfanyl and a phosphonate group has been found in literature.[1] In addition, the thioacetate protected derivates of the envisioned inhibitor were of interest to see whether the MBLs are capable to hydrolyse the acetate, generating the free thiol in situ.



Scheme 1 The 2-substituted-3-sulfanylpropanoic acid inhibitor to the left will be modified as shown to the right. The sidechain will be fixed, whereas the sulfanyl functionality will be both a free thiol and a protected thioacetate. The carboxylic acid functional group is exchanged with a phosphonic acid and a phosphonates ester.

Retrosynthetic analysis of the target molecules revealed two possible strategies (Scheme 2). Strategy I is the most step efficient synthesis with respect to derivation of the substituent in 2-position as the thiomethyl group common for all compounds is introduced earlier in the synthesis by a thiomethylation reaction. The thiomethylation of the phosphonoacetate is followed by alkylation, decarboxylation, and removal of the protecting groups. Thiomethylation of phosphonoacetates has not been reported to date, and strategy II was considered as a backup plan. Strategy II is based on a sequence of alkylation, reduction, replacement of the alcohol with a protected sulfanyl group, and removal of the protecting groups. As the synthetic intermediates also contain structural elements accounting for potential inhibitor activity, they will also be converted to their phosphonic acid derivate. The testing will be performed in collaboration with the group of Hanna-Kirsti S. Leiros and Ørjan Samuelsen at The Norwegian Structural Biology Centry (NorStruct) and UNN, respectively. All compounds will be tested for their inhibitor activity towards VIM-2.



Scheme 2 Strategy I and II.

2 Background

2.1 Metallo-β-Lactamases

The bacterial resistance to β-lactam antibiotics poses a major threat to society. The β-lactam antibiotics hold a beta-lactam ring as its key feature (Figure 2) and works by inhibition of bacterial peptidyltransferase by forming covalent bonds with the enzymes that have transpeptidase activity. Peptidyltransferase is critical for the peptidoglycan biosynthesis of bacterial cell wall.

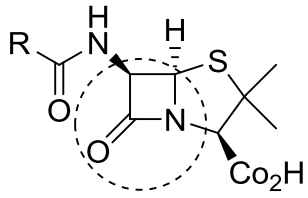


Figure 2 Penicillin core structure, with the β-lactam ring.

One cause of bacterial resistance to β-lactam antibiotics is bacterial development of enzymes able to hydrolyze the β-lactam ring of the antibiotic. These enzymes are called β-lactamases. On the basis of Amblers initial proposal, β-lactamases can be divided into four classes; A, B, C, and D. Class A, C, and D hydrolyze the beta-lactam antibiotics by a nucleophilic attack from a serine residue in the active site (serine beta-lactamases, SBLs), while class B are zinc-dependent hydrolases (also called metallo-β-lactamases, MBLs).

So far, ten types of MBLs have been identified: IMP, VIM, GIM, SPM, SIM, KHM, AIM, DIM, TMB, and NDM. Based on the known sequences, three lineages of MBLs can be characterized (B1, B2, and B3). All three subclasses have a binuclear active site, requiring one or two zinc ions for full activity. The active site containing the zinc ions is located at the edge of two β-sheets. These β-sheets are a part of the characteristic αβ-βα-fold, called the metallo β-lactamase fold. [3, 4]

The enzyme relevant for this thesis, VIM-2, belongs to the VIM class, categorized in subgroup B1. VIM enzymes are the globally most widespread MBLs.[5, 6] This subgroup bind two Zn(II) ions in a binuclear center formed by the conserved amino acids His116, His118, and His 196.[7] The hydrolyzing mechanism for VIM enzymes is assumed to be similar to the mechanism Wang and coworkers proposed for nitrocefin hydrolysis of CcrA (an enzyme also belonging to the B1 subgroup).[8] According to this mechanism, the water/hydroxide molecule

coordinated by the Zn(II) ions performs a nucleophilic attack on the carbonyl carbon in the β-lactam ring of the antibiotic, resulting in a cleavage of the amide bond, and deactivation of the antibiotic activity.

VIM-2 was first isolated from the bacteria *Psaudomonas aeruginosa*, and is considered to be one of the more clinically relevant MBLs [9, 10] VIM-2 has been found to be the source of several outbreaks, and has been isolated in Europe, Asia and America.[10, 11]

In parallel to the search for new antibiotics resisting β-lactamases, a second strategy is to co-administer the antibiotic with a β-lactamse inhibitor/inactivator. To date, no such inhibitors of therapeutic use are available for MBLs.[1] In the search for such inhibitors, some positive results have been reported. Minond and coworkers published in 2009 two potent sulfonyltriazole inhibitors (Figure 3, K_i = 0.41 μM (left) and 1.41μM (right)).[12]

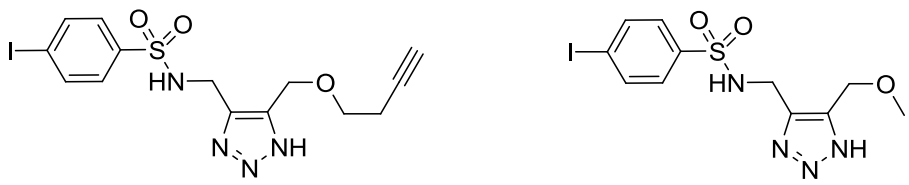


Figure 3 Sulfonyltriazole inhibitors reported by Minond and coworkers (2009).

Weide and coworkers used these sulfonyltriazole inhibitors as starting point developing second generation sulfonyltriazole inhibitors (Figure 4, left).[13] Overall, they discovered that the most potent inhibitors contained a dichlorobenzene group, and a hydrogen acceptor of the C4 methyl of the triazole, with nitrogen being superior to oxygen. The most potent inhibitor was the sulfonyltriazole with chlorine in the 2 and 5 position on the aromatic ring, and adamantyl attached to the nitrogen (Figure 4, right, K_1 = 0.01μM). This is in accordance with the predicted mode of binding for these molecules. The arylsulfonamide is exposed to the solvent, while the alkoxy group is buried into a hydrophobic sub-pocket in the active site.

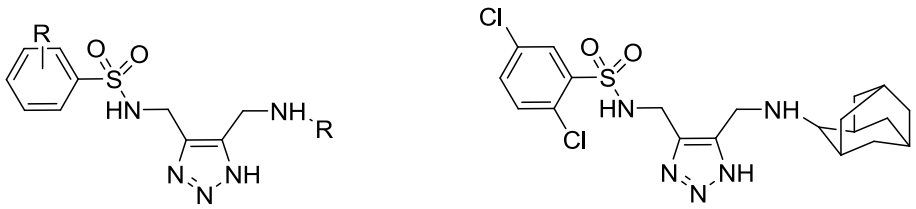


Figure 4 Second generation sulfonyltriazole showing a general structure (left) and the most potent inhibitor (right).

Jin and coworkers tested a series of 2-substituted-3-sulfanylpropanoic acid (Figure 5, left) and N-substituted 2-mercaptoacetamide derivatives (Figure 5, right) on VIM-2, varying the length of the carbon chain. When n = 4, the inhibitory effect was greatest (IC_{50} = 1.1 μM and 2.4 μM, respectively).[14]

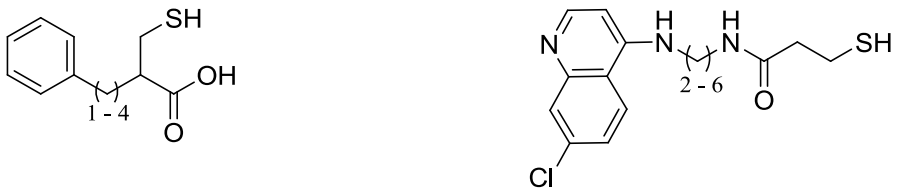


Figure 5 Chemical structure of 2-substituted-3-sulfanylpropanoic acid and N-substituted 2-mercaptoacetamide derivatives, potent inhibitors for VIM-2.

By structural analysis of the co-crystallized enzyme-inhibitor complex (Figure 6), it was discovered that the thiol group of 2-(sulfanylmethyl)-5-phenylpentanoic acid replaced the water molecule between the two Zn(II) ions in the active site, due to the high affinity sulfur holds for Zn(II).[15] Furthermore, the phenyl group interacted with Tyr67 on loop 1 close to the active site by π-π-stacking interactions. The methylene group interacted with Phe61 located at the bottom of loop 1 through CH-π interactions. The carboxyl group was involved in hydrogen bonding and electrostatic interactions with preserved residues, such as Lys. Arg228 and Asn233 on loop 2 were moved compared with the native structure. These results suggests that the above-mentioned four amino acid residues play important roles in the binding and recognition of inhibitors or substrates, and in stabilizing a loop in the VIM-2 enzyme.

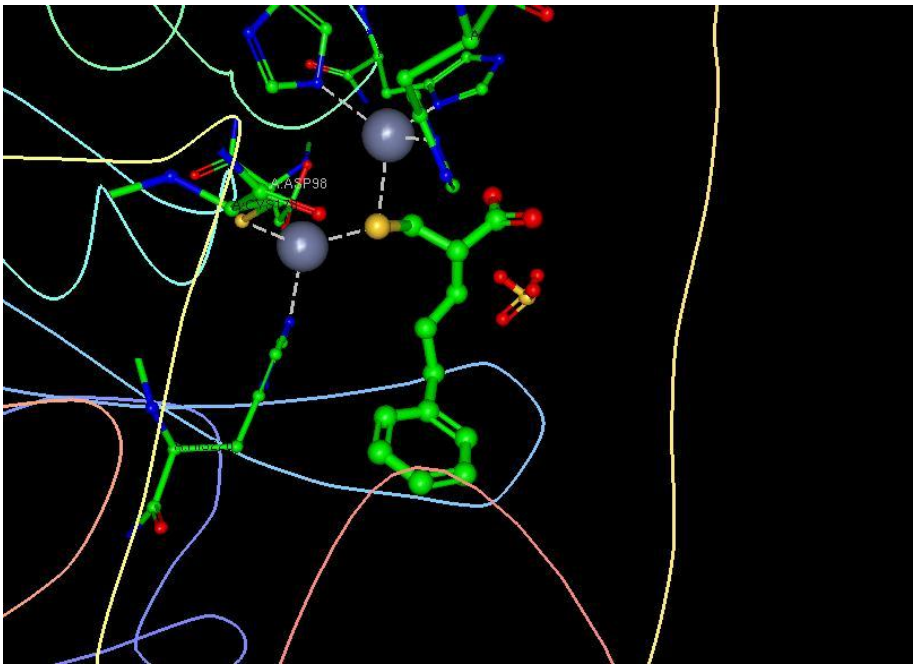


Figure 6 A suggested coordination pattern for the sulfur-Zn(II) atoms.

Phosphorous analogs have also shown good results in the search for potent broad spectrum inhibitors, as phosphonates and phosphonic acids are known as effective chelating agents. Lassaux and coworkers (2010) replaced the carboxylate functionality of the previously reported potent broad spectrum inhibitor thiomandelic acid (Figure 7, left, $K_i < 0.80$ μM) with a phosphorous analog with good results (Figure 7, middle, $K_i = 3$ μM) for VIM-4.[1] Of the fourteen sulfanylphosphonate derivates tested, the analog with two chlorine atoms in *para* and *ortho* position on the phenyl ring (Figure 7, right) showed an increased inhibition for all three representative enzymes (VIM-4 (B1), CphA (B2), and L1(B3)) compared to the thiomandelic analog. The K_i values were 1, 5, and 0,4 μM, respectively.

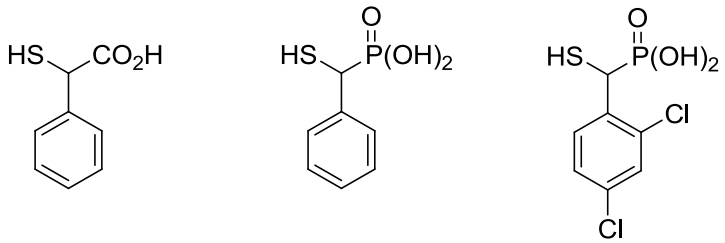
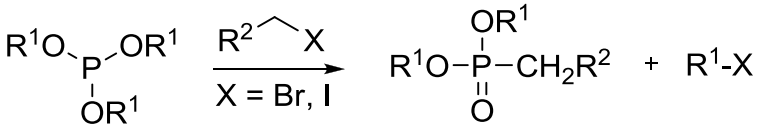


Figure 7 The potent broad spectrum inhibitor thiomandelic acid (left), its phosphorous analog (middle) and the inhibitor showing most promising results of the analog tested by Lassaux and coworkers (2010) (right).

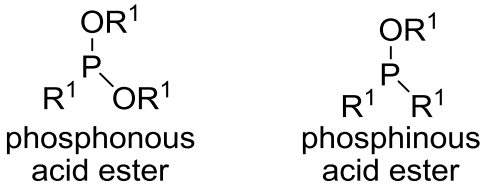
2.2 Relevant Reactions Used in This Thesis

2.2.1 The Michaelis-Arbuzov Reaction

The starting material for both strategy I and II is a diethyl phosphonoacetate and is typically made by a Michaelis-Arbuzov reaction, which is one of the most versatile ways to form carbon-phosphorus bonds. The best known variant of the Arbuzov reaction employs trialkyl phosphites ($P(OR)_3$) and alkyl halides to form the phosphonate (Scheme 3). Alternative variants utilize phosphonous acid esters or phosphinous acid esters as substrates (Scheme 4) instead of trialkyl phosphite.



Scheme 3 Arbuzov reaction with trialkyl phosphites.

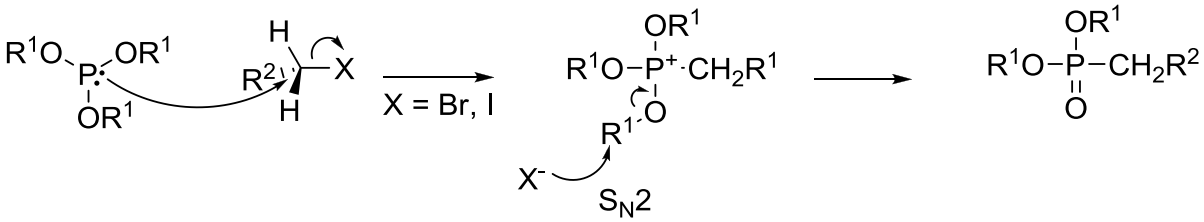


Scheme 4 Phosphonous acid ester and phosphinous acid ester that can be used in the Arbuzov reaction.

The Arbuzov reaction can be done with a variety of alkyl halides. The key feature to a successful Arbuzov reaction is the possibility for the alkyl halide to undergo a S_N2 reaction. Primary alkyl halides with bromide or iodide usually proceed well, but most secondary and tertiary alkyl halides do not react due to the competing elimination reaction. Other organic halides that are good substrates for the reaction are benzyl halides, halogenated esters, acyl halides, and chloroformic acid esters.[16]

The reaction is one of the most thoroughly investigated among organophosphorus reactions, and the reaction mechanism has been extensively debated. One of the debated mechanisms, originally suggested by Arbuzov is referred to as the "textbook" mechanism (Scheme 5).[17, 18] The lone pair on the phosphorus of the phospite attacks the alkyl halide in a S_N2 fashion to form a phosphonium salt intermediate. The phosphonium salt intermediate is unstable at

elevated temperatures. The halide ion attacks one of the alkyl groups in a S_N2 manner, and the phosphonium salt undergoes a C-O bond cleavage to afford the phosphonate. The transformation is driven by the formation of the highly stable P=O bond (128-139 kcal/mol, 178kcal/mol for a C=O bond).[19]



Scheme 5 Arbuzov reaction mechanism

2.2.2 Alkylation of Malonate Ester Derivatives

In the following section, two different alkylating reactions will be described. The thiomethylation reaction is relevant for strategy I, while the alkylation with alkyl halides is relevant for strategy II. Due to the 1,3 relationship between the electron-withdrawing groups, malonate ester and its derivates are able to form stable resonance forms, making them good nucleophiles.

2.2.2.1 Thiomethylation - Strategy I

Very little research has been carried out on thiomethylation (Scheme 6). Smissmann and coworkers published in 1970 an article on thiomethylation of the methylene group of 1,3 dicarbonyl compounds utilizing piperidinomethyl thiobenzoate hydrochloride (Figure 8, right) and piperidinomethyl thioacetate hydrochloride (Figure 8, middle) as alkylating agent.[20] They reported successful alkylation of β diketones (dimedone, 89% yield) and β ketoesters in refluxing dioxane, but failed to thiomethylate malonic esters due to decomposition of the starting material at the temperatures and reaction time required. Yamauchi and coworkers published in 1982 an article concerning thiomethylation with methylthiomethylenepiperidine hydrochloride (Figure 8, left).[15] The same reaction conditions as Smissmann employed resulted in successful thiomethylation of selected acyclic and cyclic keto esters and acyclic diketones yielding 74-95%. Smissmann thiomethylated dimedone in refluxing dioxane, ethanol, chloroform, and DMF. When using piperidinomethyl thiobenzoate hydrochloride they suggested dioxane as the optimum solvent due to the insolubility of the piperidine hydrochloride in this solvent. As dimedone was thiomethylated, the piperidine hydrochloride precipitated. Mikolajczyk and coworkers published in 1984 an article concerning thiomethylation of β-keto phosphonates with methylthiomethylenepiperidine hydrochloride based on Yamauchi's article from 1982.[21] They used methylthiomethylenepiperidine hydrochloride in dioxane under gentle reflux which resulted in a 60-80% yield. Thiomethylation of other substrates has also been reported. Bugaev and coworkers published in 2010 a different approach to alkylate phenols, reacting the diethylaminomethylsulfide and phenol in boiling glacial acetic acid resulting in 95% yields.[22]

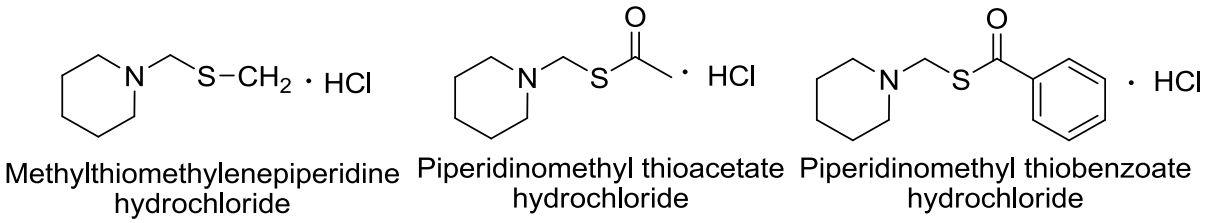
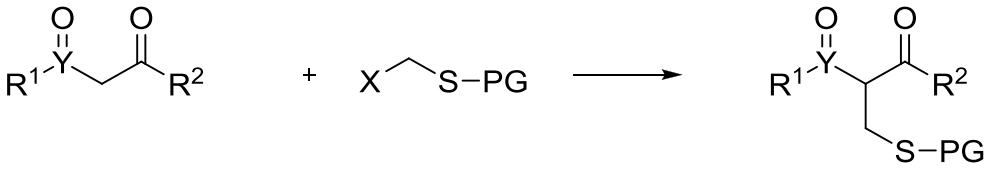
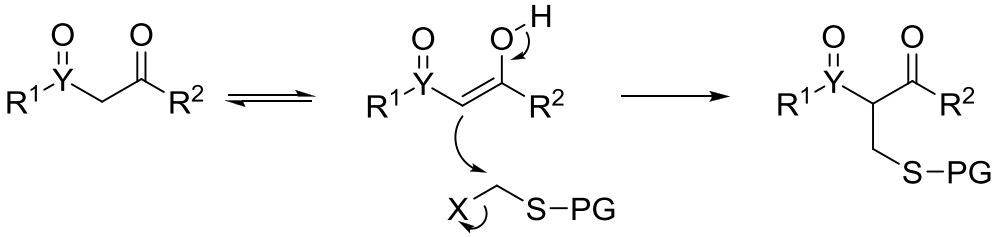


Figure 8 Different alkylating agents used to thiomethylate active methylene compounds.



Scheme 6 Thiomethylation of malonate derivates.

The reaction mechanism for thiomethylation with malonate ester derivates is not described in literature, but is assumed to follow the standard mechanism for acid-catalyzed alkylation of enoles (Scheme 7). The methylene compound exists in equilibrium between the keto and enol form (due to the mentioned 1,3 relationship of the electron withdrawing groups). The enol acts as the nucleophile and attacks the electrophilic carbon, and the leaving group departs.

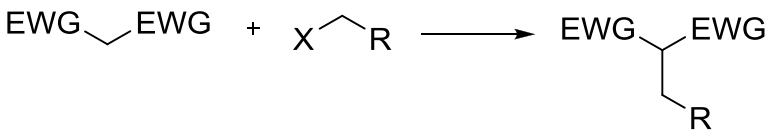


Scheme 7 Assumed reaction mechanism of thiomethylation of active methylene compounds.

Under the reaction conditions tested, it was not possible to synthesize the thiomethylated phosphonoacetate. The following sections in the background chapter will therefore only present reactions relevant for strategy II.

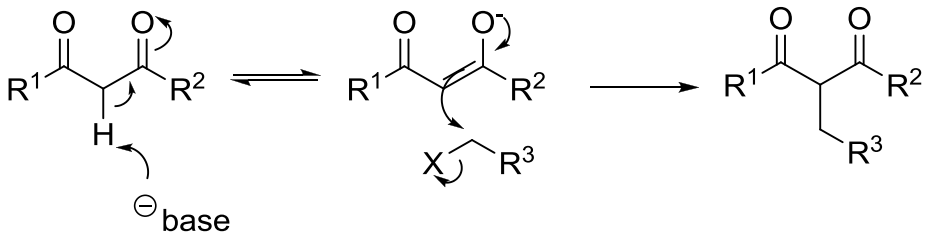
2.2.2.2 Alkylation with alkyl halides - Strategy II

Malonate esters and their derivatives can be alkylated with various primary and secondary alkyl halides (Scheme 8). Different electron-withdrawing groups (EWG) can be used in almost any combination with good results.[23]



Scheme 8 Alkylation of malonate ester derivates.

As mentioned in the previous section, the malonate ester and its derivatives are able to form stable resonance forms, making them good nucleophiles (due to the 1,3 relationship between the electron-withdrawing groups). The presence of the electron-withdrawing groups makes the protons acidic, which then can be removed by a suitable base. The reaction mechanism for alkylation of the malonate ester with alkyl halides is shown in Scheme 9. The base enolizes the malonate ester, which attacks the alkyl halide, and the halide anion departs as the leaving group.



Scheme 9 Reaction mechanism for alkylation of phosphonoacetates.

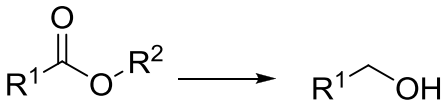
Depending on the anion stabilizing ability to the electron-withdrawing groups, different bases are required to enolize the malonate ester derivates. The bases used to deprotonate the malonate derivates are selected to fit the pK_a value of the acidic protons. As an example, the malonate ester has a pK_a of 16,4 in DMSO and can be deprotonated by alkoxides (pK_a 16), while phosphonoacetates have a pK_a of 18,6 in DMSO and can be deprotonated with potassium *tert*-butoxide (*tert*-BuOK) (pK_a 19). Sodium hydride (NaH) (pK_a 35) and more

rarely K_2CO_3 are also used to deprotonate phosphonoacetates.[24-26] NaH and *tert*-BuOK are both strong bases, and will readily generate the carbanion. K_2CO_3 is a weaker base, and will reach equilibrium at a lower concentration compared to NaH and t-BuOK.

Both the alkylation agent and the solvent are important in determining the outcome of the reaction. Primary halides, especially allylic and benzylic, are the most reactive alkylation agents. Some primary halides, including benzyl bromide, are so reactive that mild conditions are necessary to avoid dialkylation.[27] In literature, the occurrence of di-alkylating products with benzyl bromide and allyllic compounds are reported.[27] The solvent affects the rate of alkylation. Aprotic polar solvents as DMSO and DMF are good options, solvating enolates and other carbanions from ion pairs.[28]

2.2.3 Reduction of Esters to Alcohols – Strategy II

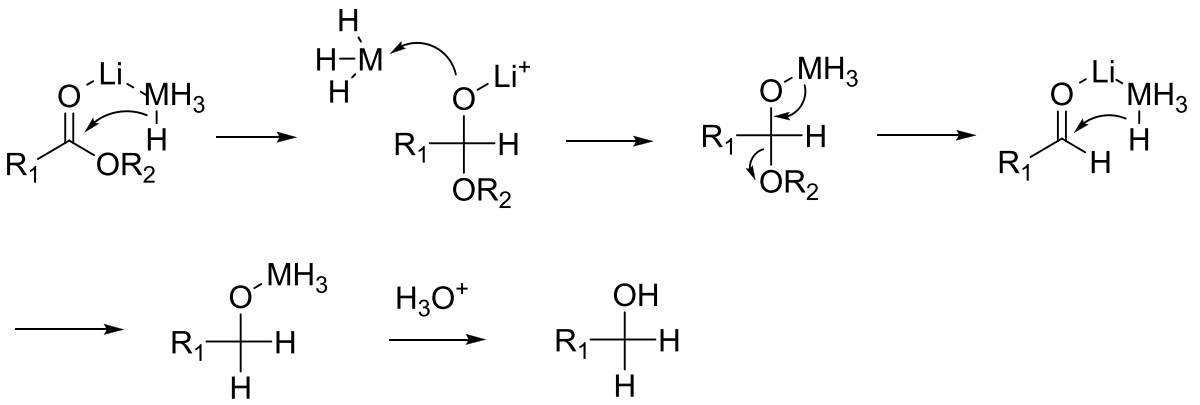
Esters are relatively resistant to reduction compared to ketones and aldehydes. There are several reducing agents that can reduce a carboxylic ester to an alcohol, the most common being; aluminiumhydrides, borohydrides, and boranes, which all have at least one hydrogen attached to a less electronegative atom. A reduction of an ester to an alcohol is shown in Scheme 10.



Scheme 10 Reduction of an ester to an alcohol.

2.2.3.1 Aluminiumhydrides and Borohydrides

Aluminiumhydrides and borohydrides work by a nucleophilic approach, donating a hydride to the electrophilic atom which then is reduced.[29] The reducing agent donates a second hydride, and the alcohol is formed (Scheme 11).



Scheme 11 Reaction mechanism of aluminiumhydrides and borohydrides.

The reactivity is determined by the difference in electronegativity, making the aluminiumhydrides more powerful reducing agent and borohydrides milder reducing agents. Among the aluminiumhydrides, lithiumaluminum hydride ($LiAlH_4$) is the strongest and best known reducing agent, reducing a variety of functional groups like esters, carboxylic acids, acyl chlorides, aldehydes, and ketones to the corresponding alcohol, and phosphonates, which

is of special interest in this project, into phosphonic acids.[29, 30] $LiAlH_4$ are not particularly selective, but the selectivity can be altered by replacing the hydride groups by alkoxy groups.

There are several variants of LiAlH4 are available, all having different selectivity; Sodium bis(2-methoxyethoxy)aluminium hydrid (SMEAH/Red-Al), Lithium tris(tert-butoxy)aluminium hydride (LTBA), lithium tris(methoxy)aluminium hydride (LTMA), and Calcium alkoxyaluminium hydrides (CALH).[31, 32] Red-Al has a reducing selectivity similar to $LiAlH_4$, but is stable in air in contrast to $LiAlH_4$. Aprotic solvents such as THF, ether, and diglyme are suitable since protic solvents cause decomposition of aluminiumhydrides.

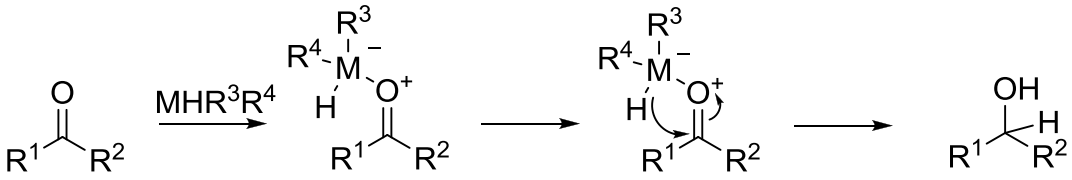
When more selective reducing agents are needed, borohydrides can be used. There are several borohydrid reducing agents available, including; sodium borohydride ($NaBH_4$), lithium borohydride ($LiBH_4$), sodium cyanoborohydride ($NaBH_3CN$), potassium borohydride (KBH_4) and lithium triethylborohydride ($LiBH(C_2H_5)_3$, also known as Super Hydride).[33] The strength of the borohydride reagents is regulated by the substituents on the hydridic anion, BH_4^-, its counter ion, and the solvents utilized.[34] By exchanging the hydride atoms with other functional groups, the selectivity is altered e.g. $NaBH_3CN$ and $LiBH(C_2H_5)_3$. The cyanide group is electron-withdrawing, and makes $NaBH_3CN$ a milder reducing agent compared to $NaBH_4$. $NaBH_3CN$ is well known for its selectivity for reductive amination. By exhanging the hydride atom with an electron donating group, the reducing ability is increased dramatically, as in $LiBH(C_2H_5)_3$.[35] By exchanging the counter ion to the hydridic anion, the electrophility of the carbonyl group is altered, and as shown in the reaction mechanism (Scheme 11), affecting the nucleophilic attack from the hydride. The lithium cation has a higher Lewis acidity than e.g. the sodium cation, and will there be a stronger reducing agent and causing an increased reducing rate.

The reductions can be executed in both aprotic and protic solvents, depending on which borohydride is used. Because of the different solubility properties the borohydrides have, there several common solvents. $NaBH_4$ is by example restricted to diglyme and ethanol, due to very low solubility in other solvents such as 2-propanol, THF and diethylether. $LiBH_4$ on the other hand is very soluble in THF, which makes this a good choice. In protic solvents, decomposition of the borohydrides is observed to different extent, making aprotic solvents a better option when possible.[34] Higher solubility of the reducing agent leads to an increased availability for the lithium counter ion to coordinate to the ester/substrate, promoting reaction. The solubility is not the only factor determining the rate of reaction. Brown and coworkers[34] investigated solubility and reaction speed for $NaBH_4$, $Ca(BH_4)_2$, and $LiBH_4$, and established

that the polarity of the solvents affect the reaction speed. For $LiBH_4$, the reaction speed is in the following order from fastest to slower: diethylether > THF > diglyme. For $NaBH_4$, the solubility in the different solvents was so low that there was not possible to establish a trend.

2.2.3.2 Diisobutylaluminium Hydride (DIBAL-H) and Borane (BH_3)

Diisobutylaluminium hydride (DIBAL-H) and borane (BH_3) work by a different mechanism than aluminiumhydrides and borohydrides (Scheme 12). They are not charged species, and must bind to the carbonyl before it is "activated" and can donate a hydride to the carbonyl carbon.



Scheme 12 Reaction mechanism for reduction with borane and DIBAL-H.

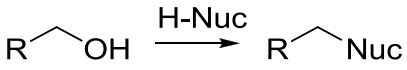
Borane is a good Lewis acid, and coordinates to atoms with high electron density, giving it its selectivity. It will reduce carboxylic acids in the presence of halides, esters, nitriles, and ketones. DIBAL-H is on the other hand a strong reducing reagent, reducing most functional groups. Its synthetic utility lies in three areas: reduction of esters, lactones and nitriles. A major advantage of DIBAL-H is the ability to stop the reduction after one step, reducing the ester to an aldehyde. DIBAL-H reduction can be executed in all aliphatic and aromatic hydrocarbons, which is often used because it solvates many substrates to be reduced. Other solvents utilized are ethers, chlorobenzene, and dichloromethane. The latter two is found to be compatible with refluxing DIBAL-H over several hours. Ethers such as diethylether and THF complexes with DIBAL-H making it a milder reducing agent.

2.2.4 Conversion of Alcohols to Protected Thiols

In the following section, two reactions for converting alcohols to protected thiols will be described, the Mitsunobu reaction and S_N2 nucleophilic substitution of mesylated alcohols with thio nucleophiles.

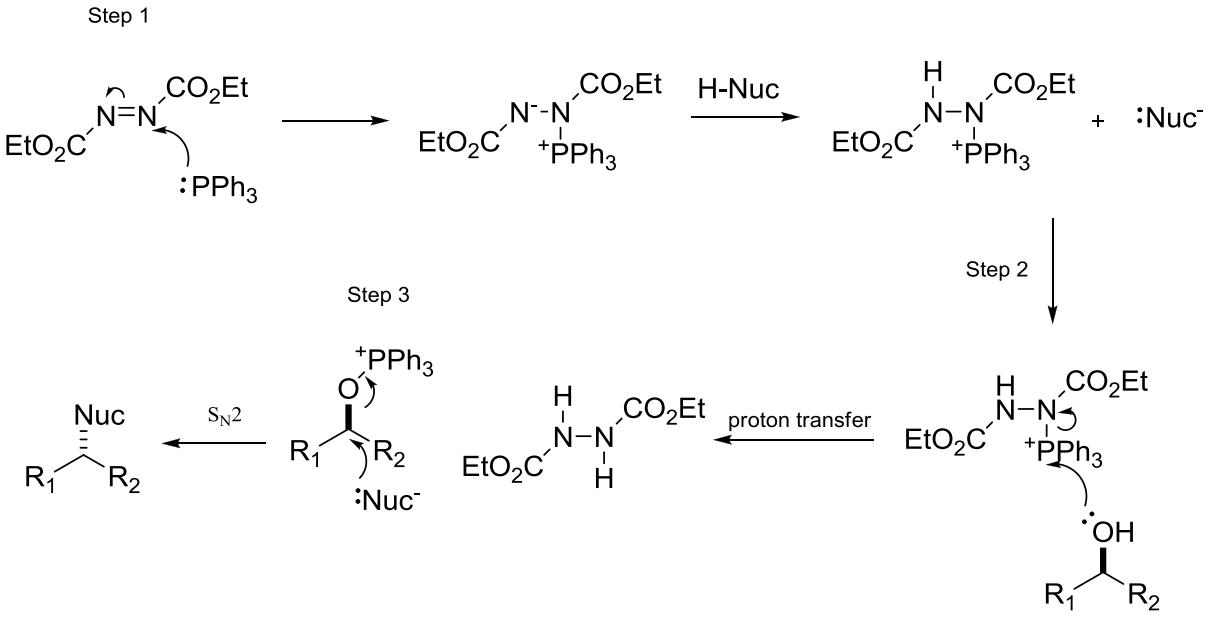
2.2.4.1 Mitsunobu Reaction

In 1967 Mitsunobu discovered that primary and secondary alcohols reacted with carboxylic acids in the presence of diethyl azodicarboxylate (DEAD) and triphenylphosphine (TPP) to give the corresponding esters in high yield with retention of stereochemistry (Scheme 13).[36] Today, the Mitsunobu reaction is known for its high reliability and extensive versatility and constitutes one of the most important organic reactions.



Scheme 13 Mitsunobu reaction.

The Mitsunobu reaction can be done with a variety of nucleophiles containing an acidic O-H, N-H or S-H proton.[37, 38] Some common nucleophiles are: carboxylic acids, phenols, imides, purine/purimidine bases, thiocarboxylic acids, and thiols, which are of special interest in this project. If the less crowded trimethylphosphine is employed, even C-H based nucleophiles such as malonate esters, diketones, and ketoesters can be used with reasonable yield replacing the OH with the expected retention of stereochemistry.
The procedures containing the Mitsunobu reaction generally follow the same pattern; a solution of trialkylphosphine in the chosen solvent, usually THF, toluene, CH_2Cl_2, DMF, or acetonitrile is slowly added dialkyl azodicarboxylate at 0ºC, stirred for 30min. A solution of the diluted alcohol and nucleophile is added at 0ºC, allowed to reach RT and stirred for 1-24h. This is in accordance to the proposed reaction mechanism (Scheme 14). [37]



Scheme 14 Mitsunobu reaction mechanism.

The mechanism proceeds in three steps; the adduct formation, alcohol activation, and S_N2 reaction. The first step generates the zwitterionic adduct, which gets protonated by the acidic nucleophile. In the second step, the alcohol is activated through a highly discussed oxyphosphonium ion intermediate.[39-42] This happens by a proton transfer from the alcohol, via the nucleophile, to the dialkyl azodicarboxylate molecule, followed by a nucleophilic attack on the phosphorous ion, reducing the dialkyl azodicarboxylate to dialkyl hydrazinedicarboxylate. Since the alcohol oxygen is now activated as a leaving group, step three, in the form of a S_N2 reaction, takes place with inversion of the stereochemistry of the alcohol, and the trialkyl phosphine is oxidized to trialkyl phosphine oxide. As mentioned in the Arbuzov chapter, the formation of the P=O bond is a driving force in the reaction due to its stability.

The original reagents employed in the Mitsunobu reaction have a few drawbacks. In 2009, Swamy and coworkers published a review article giving an overall picture of the development concerning the Mitsunobu reaction and its related reactions, considering these drawbacks.[43] All modifications described below are described in this article.

One important issue is the separation of product from a considerable amount of byproducts, mostly being reduced dialkyl azodicarboxylate and oxidized trialkylphosphine. Several alternatives have been developed to aid this issue. For example, when the original triphenylphosphine is replaced with either tributylphosphine or trimethylphosphine, the oxide

is water soluble and can be removed by a simple aquatic work up. Diphenyl(2-pyridyl)phosphine (Figure 9, left) (4-dimethylaminophenyl)diphenylphosphine (Figure 9, middle) and tris-(4-dimethylaminophenyl)phosphine (Figure 9, right) utilizes the amino functionality, and the corresponding oxide can be removed by washing the reaction mixture with dilute hydrochloric acid.

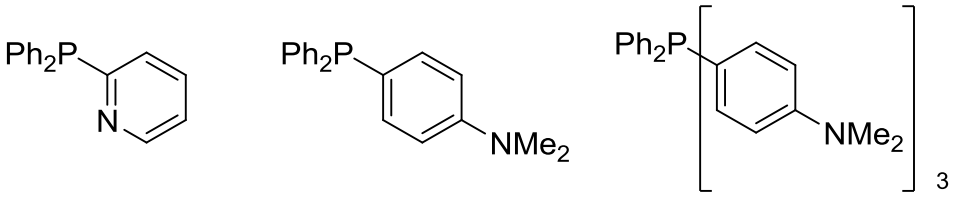


Figure 9 Substituted phosphines utilizing the amino functionality, making the work up easier in the Mitsunobu reaction.

Another strategy is anchoring the triphenylphosphine to a polystyrene resin. At the end of the reaction, the immobilised phosphine and the resulting oxide are still linked to the polystyrene resin, and can easily be removed by filtration.[44]

Optional reagents to diethyl azodicarboxylate (DEAD) have also been explored. In a similar way to triphenylphosphine, DEAD has been immobilized by linking it to a polystyrene unit, and can be removed by filtration.[45] In addition to be difficult to separate from the product, DEAD is highly toxic and potentially explosive. To avoid the toxicity and separation issue other dialkyl azodicarboxylates have been developed, e.g. diisopropyl azodicarboxylate (DIAD), bis(4-chlorobenzyl)azodicarboxylate (DCAD), and di-tert-butyl azodicarboxylate (DTBAD) (Figure 10). The reduced DCAD is by example insoluble in CH_2Cl_2, making it easy to filtrate off after the reaction is complete, and DTBAD decomposes to gaseous byproducts by acidic work up.

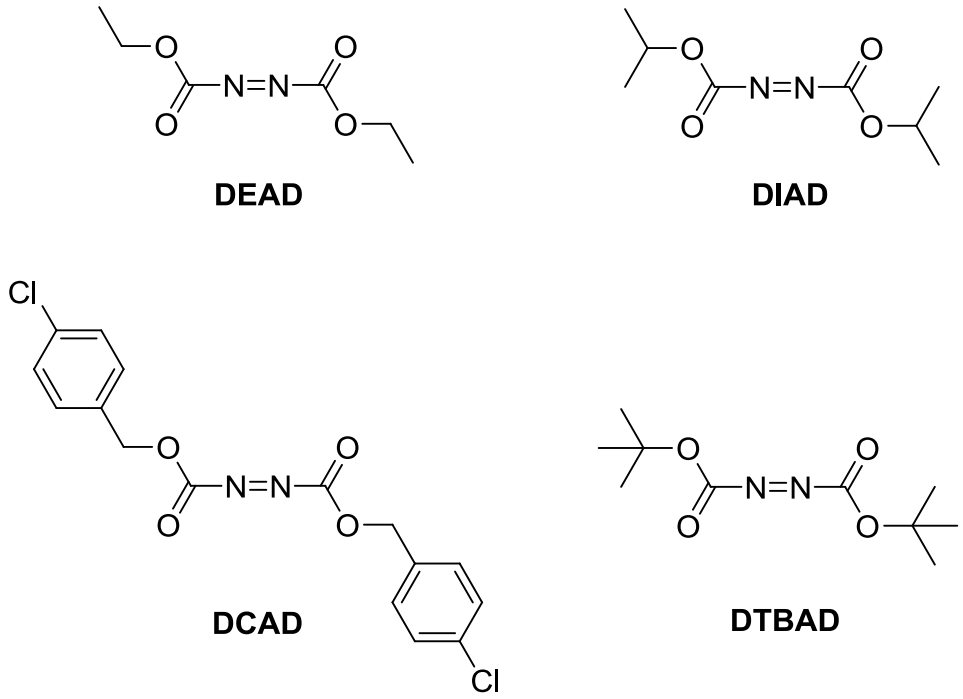


Figure 10 Some of the dialkyl azodicarboxylate reagents available.

The Mitsunobu reaction is restricted by the acidity of the nucleophile. If the pKa is above 15, the reaction will not go with the original reagents. To overcome this limitation, Tsunoda and coworkers developed an equivalent to the n-Bu_3P-DEAD system, cyanomethylene-tri-n-butylphosphorane (CMBP) (Figure 11). This system has been utilized for C-C bond formation where the methylene compound has a pKa > 20.

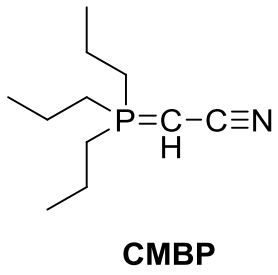
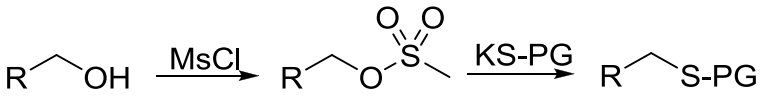


Figure 11 Cyanomethylene-tri-n-butylphosphorane (CMBP) can be used in the Mitsunobu reaction when the pKa of the substrate exceeds 15.

2.2.4.2 SN2 Nucleophilic Substitution of Mesylated Alcohols with Thio Nucleophiles

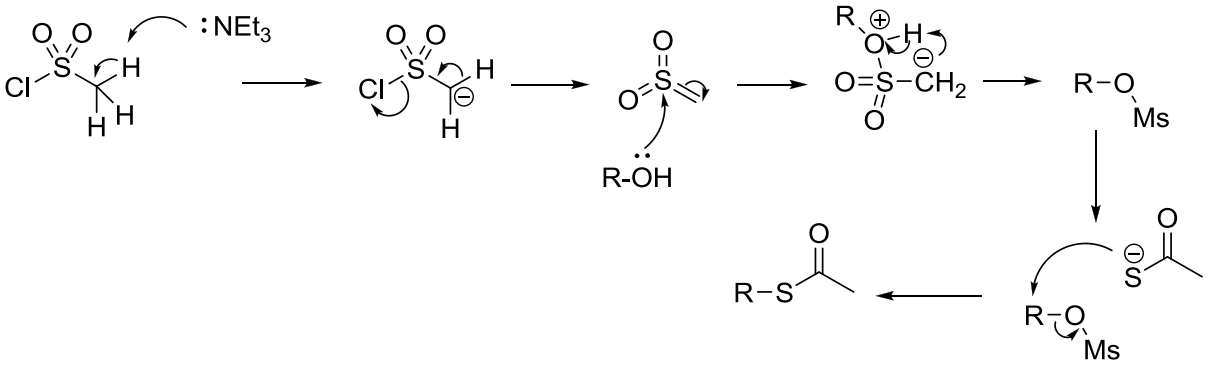
A second explored synthetic approach to replace alcohols with protected thiols is the nucleophilic replacement of the mesylate with the corresponding thio nucleophile (Scheme 15).



Scheme 15 S_N2 Nucleophilic substitution of mesylated alcohols with thio nucleophiles.

Potassium thioactetate is often used as the nucleophile, and is of special interest in this project.[46] If thioacetic acid is used, it is necessary to generate the anion before addition of the mesylate. Potassium thiocyanate can also be employed as the nucleophile. The substitution can also be done with halides as leaving group with good yields.[46]

The reaction mechanism is different from the reaction mechanism for tosylates (Scheme 16).[23] The first step is an elimination of HCl from the sulfonyl chloride to give a sulfene. The sulfene is highly electrophilic and will react with any alcohol to yield the methanesulfonate ester. After the methanesulfonate ester is formed, the thioacetate anion attacks the electrophilic carbon next to the mesylate in a S_N2 manner to yield the thioacetate ester.



Scheme 16 Reaction mechanism of methanesulfonation and the formation of thioacetate ester.

2.2.5 Thiol Protecting Groups and Their Removal

Thiols are easily oxidized to give the corresponding disulfide bond. In order to work with thiols, protection is often necessary. There are about 70 protecting groups available, being mostly thioethers, thioesters and thiocyanate.

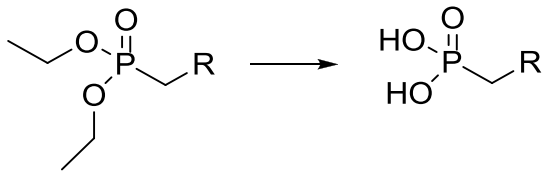
Thioethers are among the most used protecting groups, due to the stability to hydrolysis. The most common thioethers are tert-butyl, - benzyl,.- substituted benzyl, trityl, and 2-cyanoethyl thioethers. The tert-butyl group is for example less influenced by acidolysis with TFA and HCl than the corresponding oxygen tert-butyl protecting group derivates (Boc), allowing selective cleavage of Boc groups. Trityl (triphenylmethyl) thiolether is another used protecting group, and can be removed by either HCl, aq ACOH, 90 degrees, 1,5h or trifluoroacetic acid.

Thioesters are very vulnerable towards hydrolysis. This is due to the poor overlap between the orbitals between the sulfur and the carbonyl, lowering the resonance stabilization and thereby raising the ground state energy. The carbonyl of the thioester is more electrophilic than the corresponding carboxylic ester, and hence more reactive. The thioacetate protecting group can be removed by reduction with LiAlH4, hydrolysis or sodium thiomethoxide. Using LiAlH4, little selectivity is achieved as mentioned in page 15 in the section Aluminiumhydrides and Borohydrides. Both acidic and basic methanolysis is reported in literature.

Removing the thioacetate protecting group with sodium thiomethoxide is a very mild and selective deprotection procedure. It can be done in room temperature, and is often complete within minutes.[47] This procedure is preferable for water soluble and volatile thiols, as the thiol is isolated as its sodium salt. Another procedure for isolating the thiol as its sodium salt is by using 0.1 M NaOMe/MeOH under H_2 atmosphere for 5-10 minutes.[48]

2.2.6 Dealkylation of Phosphonate Esters

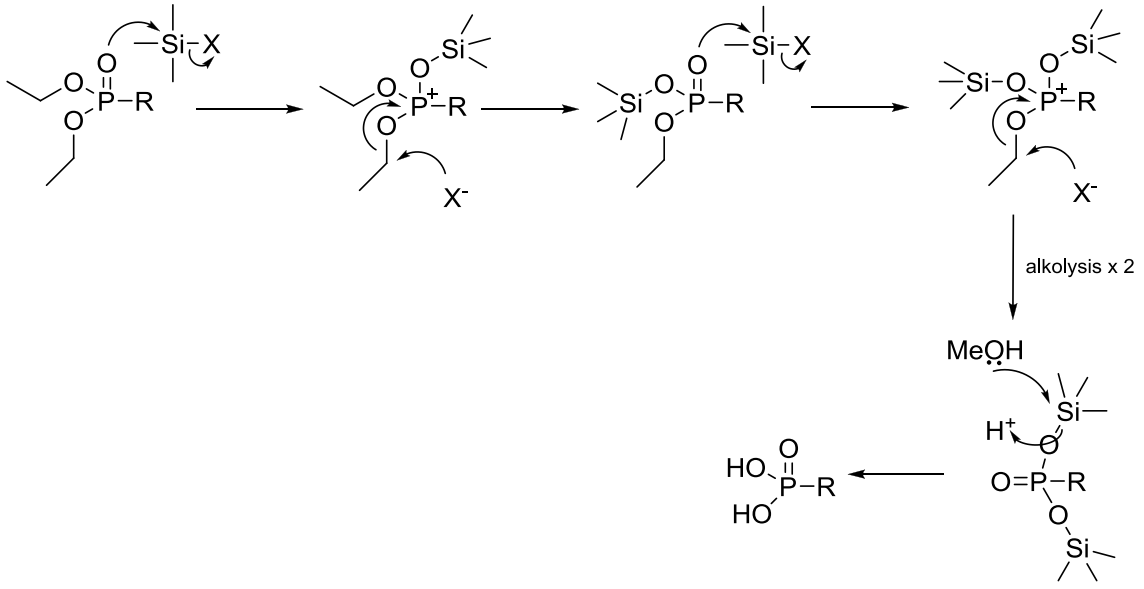
The most common reagents to dealkylate phosphonates to the corresponding phosphonic acid are BBr_3, trimethylsilyl bromide (TMSBr) or trimethylsilyl chloride (TMSCl).[49-52] All these reagents are mild and will dealkylate most phosphonates to phosphonic acids. A overview of the reaction is shown in Scheme 17.



Scheme 17 Dealkylation of phosphonates to phosphonic acids.

BBr_3 is a versatile reagent which in addition to dealkylate phosphonates can cleave ethers, amines, and thiols.[53] It can also reduce phosphonates containing an allylic R-group in contrast to the trimethylsilyl halides, which cannot. The reactions with BBr_3 are usually done at -78ºC-0ºC. The reaction conditions for trimethylsilylhalides are dependent on the halide. Trimethylsilylbromide needs only a few hours stirring in room temperature to give high conversion rates. Trimethylsilylchloride need higher reaction temperature and longer reaction times than the corresponding bromide due to the lower reactivity. This is a drawback associated with the use of trimethylsilylchloride, even though the costs are lower and it is easier to handle. In 2001, Gutierrez and coworkers published a procedure, using NaI as catalyst.[49] With conversion rates close to a 100%, and reaction times down to mostly below 12h, there is no doubt that this procedure can be a valid option to trimethylsilylbromide dealkylation. The solvents used are normally chlorobenzene, DMF, dichloroethane, or acetonitrile.[49]

The reaction mechanism was not found to be described in literature. A suggestion is shown in Scheme 18.



Scheme 18 Suggested mechanism for dealkylation of phosphonates.

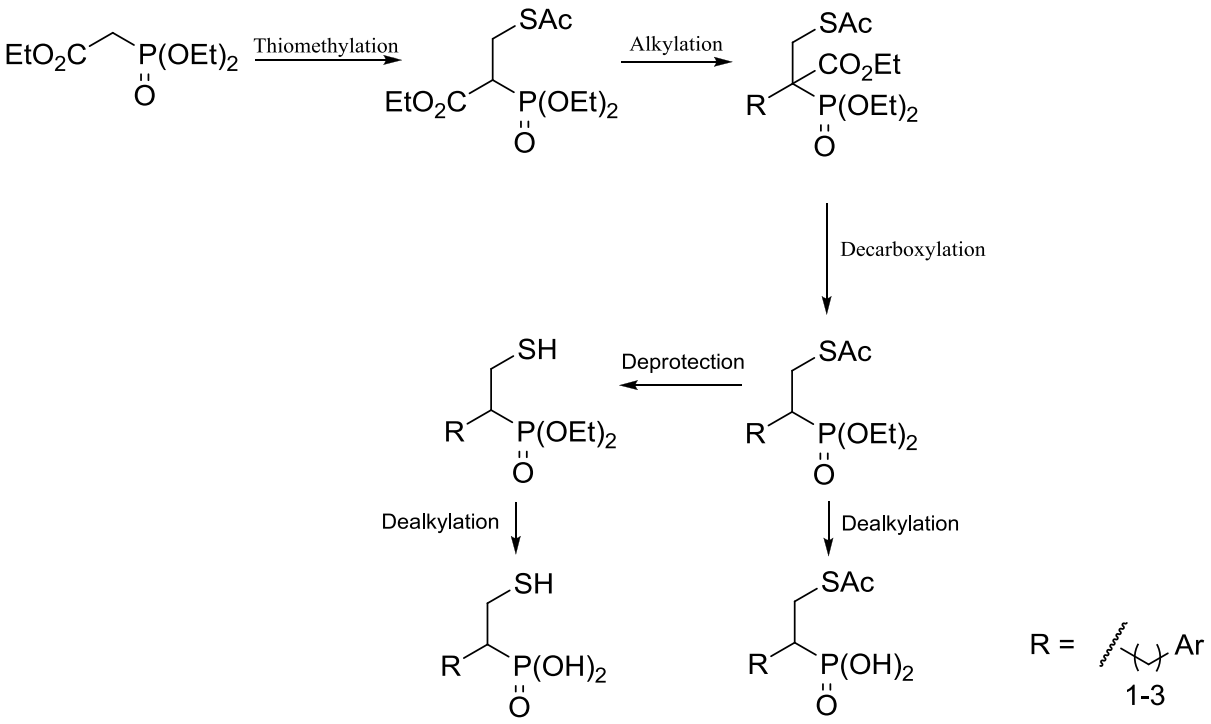
3 Result and Discussion

All the compounds produced are synthesized as racemic mixtures. No attempts towards separation have been executed.

3.1 Strategy I

As described in the introduction, strategy I is the most step efficient synthesis. The first step has not been reported to date, and is the Achilles heel in strategy I. The following sequence is alkylation and decarboxylation (Scheme 19). The results for the reactions executed in strategy I are presented in the following section.

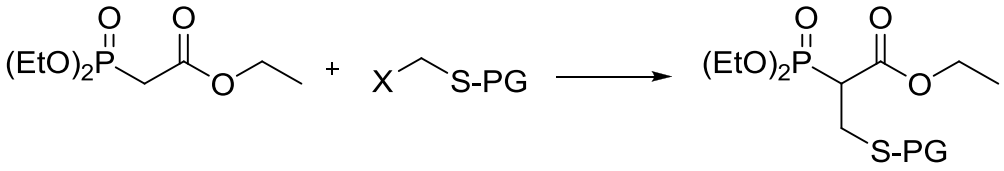
Strategy I



Scheme 19 Strategy I.

3.1.1 Thiomethylation

The investigation was started exploring the thiomethylation of phosphonaoacetate (Scheme 20), which has only been described for the related β ketophosphonate, ketoesters, and diketones.[21] Since all the target molecules have a thiol functionality, the thioalkylated phosphonoacetate could be synthesized first and used as the starting material for the alkylation, varying only the length of the carbon chain on the alkyl halide. In the attempts to thiomethylate the phosponoacetate factors as solvents, leaving groups, bases, thiol protecting groups and temperature were varied (
Table **1**). As mentioned in the background chapter, according to literature, the solvent utilized did not appear to be critical. The hydrochloride salt is very hygroscopic and will decompose easily. If a base is added in the presence of piperidinomethyl thioacetate hydrochloride, the compound will rearrange spontaneously to the piperidinoamide.[54]



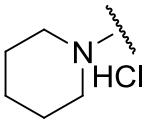
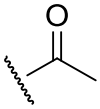
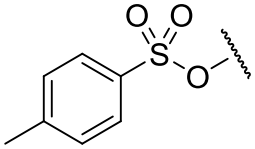
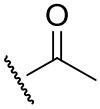
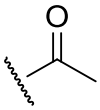
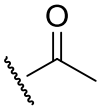
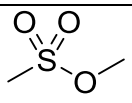
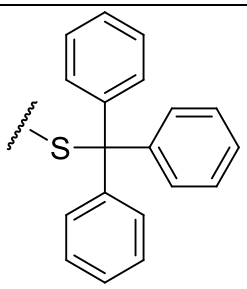
Scheme 20 Reaction scheme of thiomethylation of phosphonoactetate.

According to Smissman (1970), the piperidine hydrochloride precipitated after few minutes when the reactions were executed in dioxane. In the reaction described in Entry 1 (Table 1), the described precipitate was observed but no product was seen when analyzing the crude by ^{1}H NMR. Varying the solvent and increasing the reaction time (entry 2-6) did not yield the product.

Piperidine hydrochloride is a good leaving group but sensitive to bases. To avoid the sensitivity issue, piperidine hydrochloride was replaced with four different leaving groups (tosylate, mesylate, bromide, and iodine, entry 7-15). By exchanging the base sensitive piperidine hydrochloride by other more stable (and less nucleophilic) leaving groups, bases could be introduced. The attempts to generate the phosphonoacetate anion, leading to a Sn2 reaction did not succeed (entry 7, 10-15). Thiomethylation under neutral conditions did not succeed either (entry 8-9). The piperidinomethyl thioacetate hydrochloride and its derivates all have two nucleophilic centers; the piperidinomethyl carbon and the thioacetate carbonyl

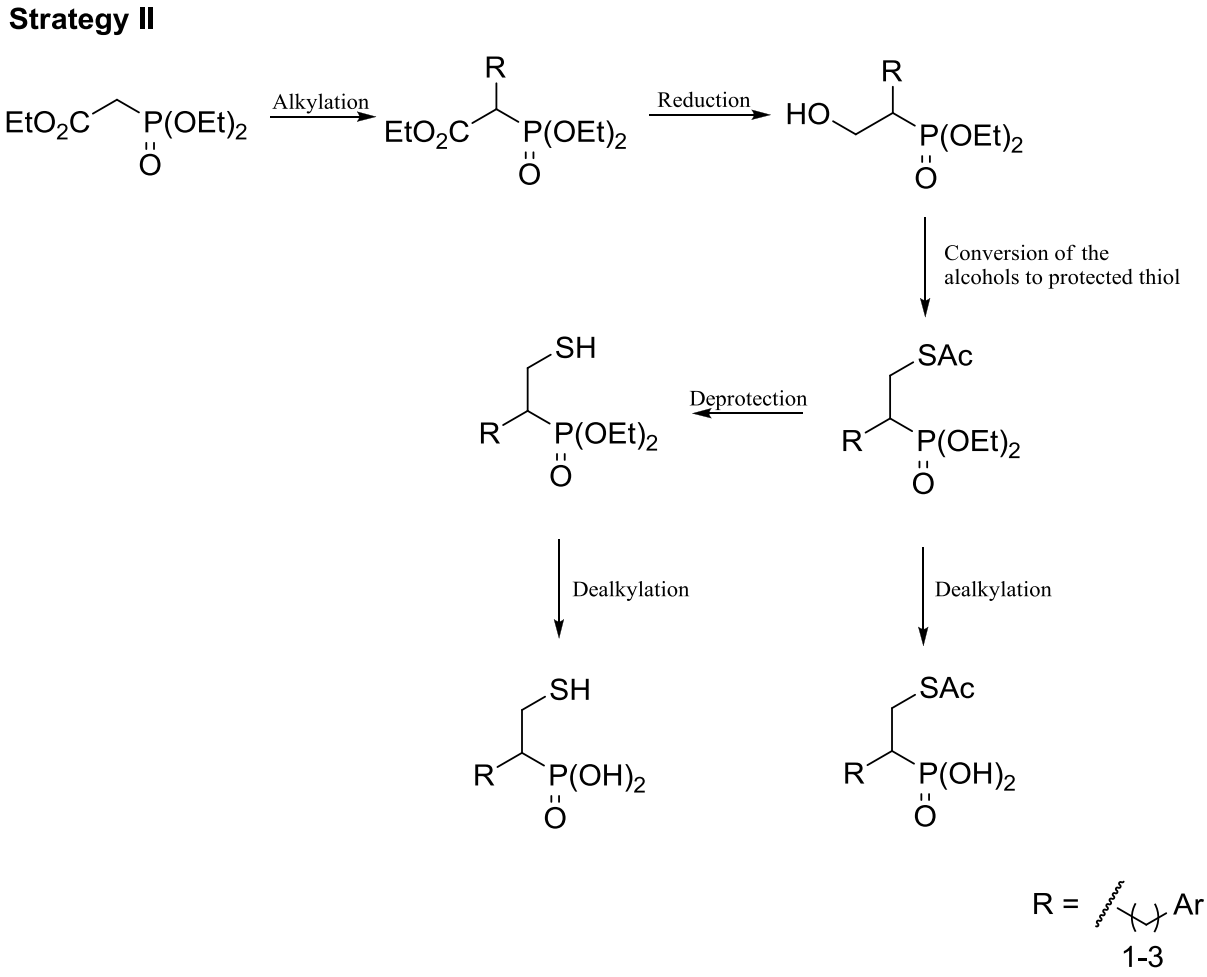
carbon. To reduce the possibility of a possible unwanted side reaction on the thioacetate carbonyl carbon, the acetate protecting group was exchanged with the trityl protecting group. The leaving group was the mesylate (entry 16). This did not yield the product. Due to insufficient analysis of the reagents in entry 7-16, it cannot be concluded with 100% certainty that the lack of success is due to the reaction, and not the reagents.

Table 1 An overview of different attempts to thiomethylate phosphonoacetate.

Leaving group, X	Protecting group	Entry	Reaction conditions	Solvent
		1	85°C, 2 h	Dioxane
		2	85°C, 23 h	Ethanol
		3	85°C, 23 h	Dioxane/Ethanol
		4	85°C, 48 h	Dichloroethane
		5	85°C, 5 d	Ethanol
		6	80°C, 1 h, MW	Ethanol
		7	NaH, RT, 4 h	THF
		8	90°C, 16 h	Dioxane
		9	90°C, 24 h	Dioxane
		10	NaH, RT, 1 h	THF
		11	TEA, LiBr, 0°C, 2 h, RT ON	Acetonitrile
		12	LDA, -78°C, 1 h, 67°C, 2 h	THF
		13	*tert*-BuOK, RT, ON	DMF
		14	*Tert*-BuOK, RT ON	DMF
		15	*Tert*-BuOK, RT ON	DMF
		16	*Tert*-BuOK, RT ON	DMF

3.2 Strategy II

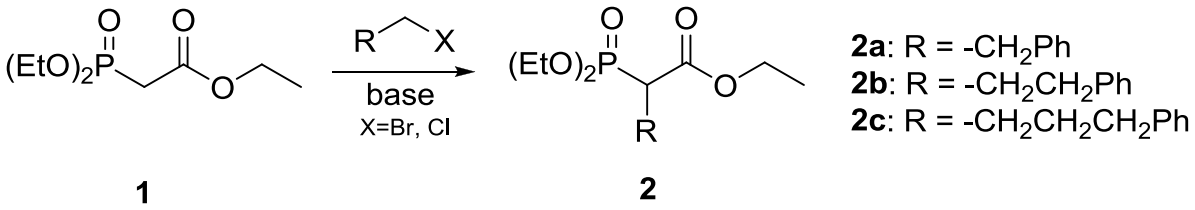
When the thiomethylating step failed to give the thiomethylated product, strategy II was followed (Scheme 21). The results for the reactions executed in strategy II are presented in the following sections.



Scheme 21 Strategy II.

3.2.1 Alkylation

The first step in strategy II is alkylation of diethyl phosphonoacetate with alkyl halides (Scheme 22). The synthesis of compound **2a** by this reaction has been described in literature. The synthesis of compound **2b** and **2c** has not been described before.



Scheme 22 Alkylation of diethyl phosphonoacetates with alkyl halides.

Regarding the synthesis of **2a**, three procedures have been published. Kirschleger and coworkersreported an alkylation with benzylchloride as substrate, K_2CO_3 as base, and NaI as catalyst.[27] The reaction mixture was stirred at 60ºC and 4 days yielding 78% of **2a**. The article did not report any solvent. Hackelöer and coworkers[55] described a similar approach, but without NaI as catalyst and DMF as a solvent at 70ºC and 5 days yielding 68% of **2a**. [55] Rodriguez and coworkers reported the synthesis of **2a**, using benzylbromide and EtONa in ethanol providing 55% yield. [25] In the same publication an improved procedure employing NaH in THF was described, resulting in 92% yield after purification. Rodriguez did not report reaction temperature or reaction time.

An overview of a number of benzylation reactions performed within this work is given in Table 2. Alkylation with benzylchloride according to the procedure by Kirschleger et al.[27] with DMF as solvent gave 7% of **2a** after 7 days reaction time (Table 2, entry 1). A similar reaction with benzylbromide resulted in a 20% yield of **2a** (entry 2). An attempt to decrease the reaction time by utilizing stronger bases was made (entry 3,4,5,6, and 7). Analyzing the reactions raised awareness of a side reaction resulting in dialkylated phosphonoacetate. None of the three articles mentioned dialkylation as a side reaction, but Kirschleger mentioned dialkylation as a common side reaction when using reactive alkylating reagents such as allylic and propargylic halides.[27] To confirm the suspicion of the formation of the dialkylated phosphonoacetate, a reaction with excess *tert*-BuOK and benzyl bromide was executed. ^{1}H NMR analyzes confirmed that the additional peaks in the spectrum of the monoalkylated phosphonoacetate corresponded to the dialkylated phosphonoacetate. The dialkylated and monoalkylated phosphonoacetates were difficult to separate by flash chromatography, so it became very to avoid this challenge.

It was believed that two things could lower the rate of di-alkylation; making the alkyl halide less electrophilic (benzylbromide vs benzylchloride) or make the reaction conditions less favorable for alkylation. Both strategies were to some extent tested. Since DMSO is the most

favorable solvent for alkylation it was exchanged for less favorable, but still adequate solvents as DMF and THF. Decreasing the reaction time and adjusting the equivalent ratio adding less base and alkyl halide (entry 8 and 9) did not avoid dialkylation. Exhanging benzylbromide with benzylchloride (entry 10) gave the lowest rate of dialkylation, but had only 20% converted starting material. To summarize, all attempts utilizing NaH or *tert*-BuOK resulted in dialkylation. Therefore, the reaction described in entry 2 was chosen to synthesize **2a**. This reaction has potential to increase the yield by prolong reaction time.

Table 2 Overview over attempts to lower the reaction time and avoid dialkylation of the diethyl phosphonoacetate.

Entry	Solvent	Base	Alkylating reagent	Rate of dialkylation	Reaction conditions	Equivalents Stm:BzX:Base	Converted amount of starting material*
1	DMF	K2CO3	BnCl	0 %	60°C, 7d	1:1.2:2	7 %
2	DMF	K2CO3	BnBr	0 %	RT O.N. 60°C O.N.	1:0.9:1	20 %
3	DMSO	*tert*-BuOK	BnBr	21 %	60 C O.N.	1:1.1:1.1	90 %
4	DMSO	*tert*-BuOK	BnBr	23 %	60 C 3h, 13% NaI	1:1:1.2	94 %
5	DMSO	NaH	BnBr	13 %	RT 1h, 40 C O.N.	1:1:1	82 %
6	THF	NaH	BnBr	20 %	RT O.N. / 4h 60C	1:1:1.1	93 %
7	THF	NaH	BnBr	20 %	RT 48h	1:1.1:1.2	93 %
8	DMF	*tert*-BuOK	BnBr	15 %	RT 1h	1:1:0.9	83 %
9	DMF	*tert*-BuOK	BnBr	11 %	RT 1h	1:0.5:0.7	95 %
10	DMF	*tert*-BuOK	BnCl	7 %	RT 1h	1:1:0.95	20 %

*by integration of NMR peaks

2b and **2c** were synthesized from the phoshphonoacetate and the corresponding alkyl bromides (1.2 eqv) with *tert*-BuOK as base (1 eqv). Because of the lower reactivity of the alkyl halide, *tert*-BuOK was employed as base, but the reaction time was set to overnight. The phosphonoacetate and the base were stirred in DMF to generate the anion before the alkyl halide was added. Isolated yields of **2b** and **2b** were 58% and 62%, respectively. No dialkylated byproduct was observed in these reactions, probably due to the lower reactivity of the alkyl halide.

In all the reactions, unreacted phosphonoacetate was one of the impurities in the crude product and separation by flash chromatography lead to a significant loss of product.

Motivated by these difficulties, a new work up procedure was developed, which greatly simplified the separation of the starting material and the product. The new process exploits the water solubility of the starting material, diethyl phosphonoacetate. The reaction mixture was portioned between water and pentane. The starting material dissolves in the water phase, while the product stays in the organic phase. This was especially useful for the purification of **2a**, where a large amount of unreacted starting material was present.

The alkylated phosphonoacetates **2a - c** were characterized by ^{1}H,- and ^{13}C NMR, HR-MS and IR analysis. The ^{1}H and ^{13}C NMR spectra of **2a** were in accordance to the previously reported data.[55] The spectrum of **2b** is chosen to represent the typical spectra for the alkylated phosphonoacetates (Figure 12).

The aromatic protons gave rise to two unsymmetrical multiplets at 7.29-7.18 ppm, and integrated to 2 and 3. The multiplet at 4.25-4.19 ppm belongs to the methylene protons on C5. The multiplet at 4.15-4.07 ppm belongs to the methylene protons at C7 and C9. The extensive splitting of the multiplets is due to coupling with the NMR active isotope of phosphorous (^{31}P spin quantum number ½), which is able to split signals as far apart as three bonds from phosphorous. The double double dublet (ddd) at 2.95 ppm, belonging to the proton at the kiral carbon (C2), is also split by phosphorous. The coupling constants to the ddd are 22 Hz, 10,9 Hz and 3,6 Hz. The largest coupling constant is most likely ^{2}J P-H coupling, and is in accordance to the reported value for dimethyl ethylphosphonate and dimethyl methylphosphonate (17-18 Hz).[56] The smaller couplings are originating from the interaction with the vicinal protons. Vicinal couplings constants range from 0 to 18 Hz, but are typically found to be 7.5 Hz. The multiplets at 2.74 ppm and at 2.67 ppm belong to the protons in the benzylic position. The diastereotopic protons on C3 give two separate multiplets at 2.31 and 2.15 ppm. The appearing quartet at 1.31 ppm corresponds to the methyl protons in the ethoxy group of the phosphonate and ester C6, C8, and C10). The signals overlap, resulting in an asymmetrical quartet.

The ^{13}C NMR spectrum of **2b** demonstrates a common coupling pattern between carbon and phosphorous. The coupling constants for phosphonates are normally 143 Hz for ^{1}J (P-C), 7 Hz

for ^{2}J (P-C), and 6 Hz for ^{3}J (P-C). The carbonyl carbon at 169.0 ppm is split to a doublet with 5.0 Hz coupling constant. The aromatic protons were observed in the expected area at 140.4, 128.5, and 126.2 ppm. The appearing triplet at 62.8 ppm are two overlapping doublets, corresponding to C7 and C9 (J = 7.5 Hz). The singlet at 61.4 ppm belongs to C5. The chiral carbon (C2) gave, as expected, rise to a doublet with a large splitting (130.9 Hz) at 45.1 ppm. The doublet at 34.3 ppm corresponds to the benzylic carbon (C4). A coupling constant J = 15.4 Hz is seen, and is in accordance with literature.[56] The doublet at 28.6 ppm (J = 4.4 Hz) belongs to C3. The remaining peaks at 16.3 (multiplet) and 14.2 belong to C8, C10, and C6, respectively.

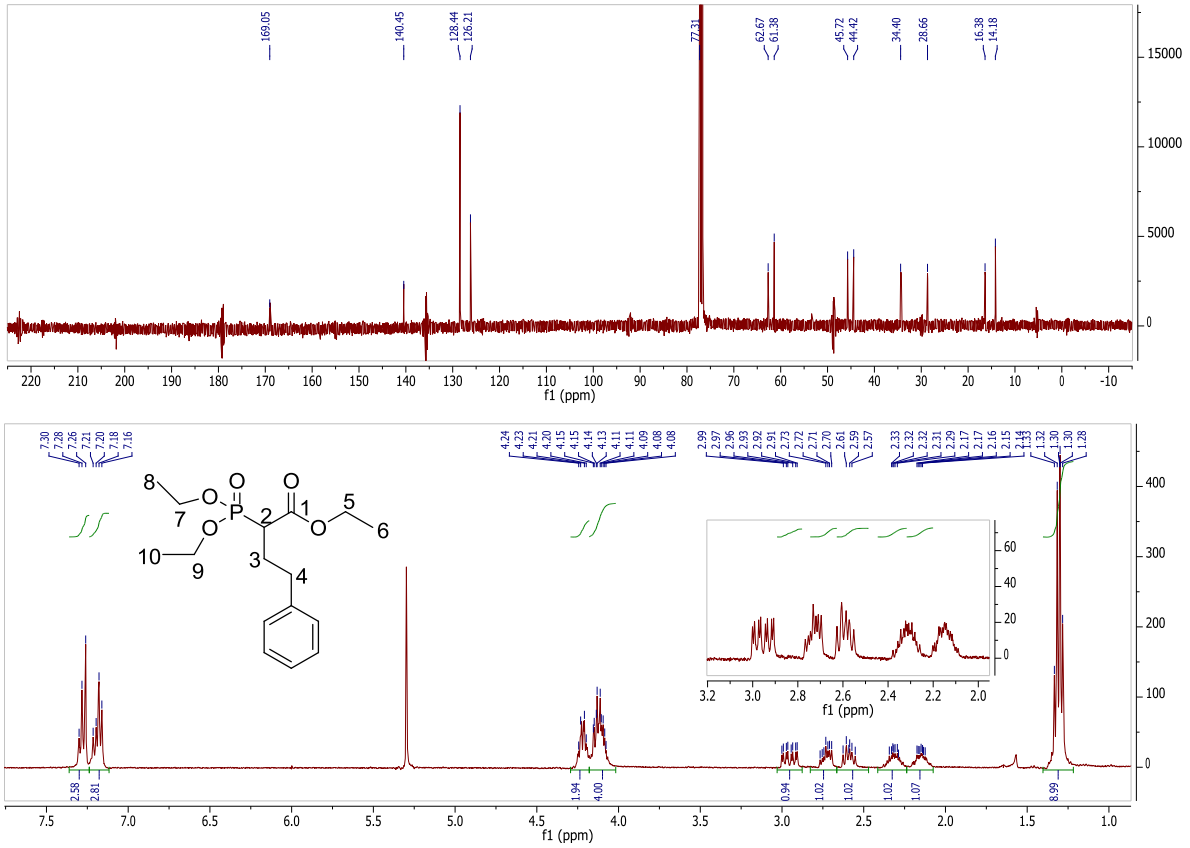
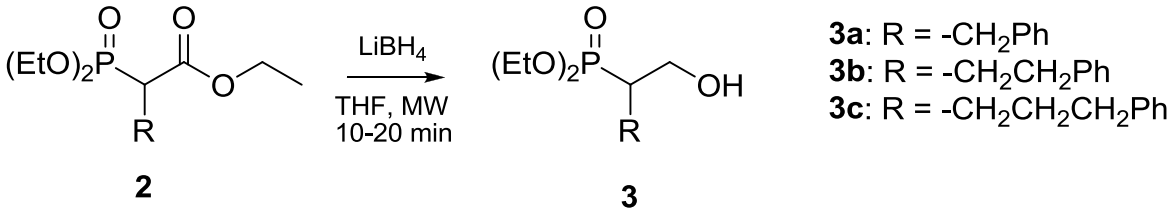


Figure 12 ^{1}H and ^{13}C NMR of 2b.

3.2.2 Ester Reduction

The next step in Strategy II is the reduction of the ester functionality in **2a**-**c** to the corresponding alcohol (Scheme 23).



2 **3**

Scheme 23 Reduction of the carboxylate ester to the corresponding alcohol.

The phosphonate functionality of **2a**-**c** must be taken into consideration when reducing phosphonoacetates. The most common reagent for ester reduction, $LiAlH_4$, was not suitable because $LiAlH_4$ has been reported to reduce phosphonates to the corresponding phosphonic acids.[30] A few reports on the reduction of esters in the presence of a phosphonates have however, been found in the literature. In 2002, Zhang and coworkers published a procedure using BH_3·THF for 2 days, reporting 82 to 87% yields.[57] Moreover, Biraboneye and coworkers described the use of $LiBH_4$, reporting a 22% yield.[58] The reaction was done at room temperature overnight.

During this work, both procedures were tested for the reduction of **2a**. Reduction with BH_3·THF (3.8eqv) was attempted for 48 hours at room temperature. NMR analysis of the crude showed only starting material. As BH_3·THF is not a good reducing agent for esters, harsher conditions were then tested. After several attempts varying equivalents and higher temperature without any success, we turned our attention to reduction with $LiBH_4$. However, the procedure of Biraboneye and coworkers (2009) was modified taking the MW assisted reduction described by Feng and coworkers into account.[59]

In an initial reaction, the ester **2a** was added to a suspension of $LiBH_4$ (2,5 eqv) in THF at 0ºC and irradiated in the microwave at 80ºC for 17 minutes. The reaction mixture was quenched with water and extracted with diethyl ether. After final purification with flash chromatography the yield of **3a** was 22%. It was suspected that under the very basic work up condition, the product alcohol **3a** was to some extent water soluble as the alkoxy anion was formed. The reaction was repeated and acidic work up resulted in a 56% yield of **3a**. An attempt to raise the yield by increasing the relative amount of $LiBH_4$ and reaction time did not

succeed. As an example, **3a** gave 52% yield from a reaction employing 6 equivalents of $LiBH_4$ and increased reaction time.

The alcohols **3b** and **3c** were prepared in a 60% and 95% yield, respectively. In an attempt to raise the yields for **3a** and **3b** varying the reaction time in the microwave from 8 to 40 minutes did not seem to influence the yield in any way.

The hydroxyphosphonates **3a - c** have not been reported before. They were characterized by ^{1}H and ^{13}C NMR, HR-MS and IR. The spectrum of **3b** is chosen to represent the typical appearance of the spectra for the hydroxyphosphonates (Figure 14).

Figure 13 shows a representative ^{1}H NMR and ^{13}C NMR spectrum for the hydroxyphosphonates, **3b**. The spectrum was run in $CDCl_3$. The aromatic and phosophonate ethyl groups are in the previously described area, 7.27-7.17, 4.08 and 1.29 ppm, respectively. The diastereotopic protons at C1 gave rise to two multiplets at 3.85 and 3.79 ppm. These protons are split by a geminal coupling to the other diastereotopic proton, a vicinal coupling to the neighbouring proton at C2, and coupling to the phosphorous atom. Geminal coupling constants are normally from -8 to -18 Hz, depending on electronegative substituents. Vicinal couplings constants range from 0 to 18 Hz, but are typically found to be 7. The phosphorous coupling constant is typically 19.5 Hz. Due to the unsymmetrical splitting pattern, it is not possible to calculate coupling constants, or deduce the coupling to each of the protons. The benzylic protons (C4) gave rise to two multiplets integrated to 1 each at 2.79 ppm and 2.68 ppm. The proton at the chiral center (C2) and the other diastereotopic protons at C3 gave multiplets between 2.03 and 1.78 ppm.

^{13}C NMR analysis is as expected. There is no carbonyl peak, or peaks corresponding to the ethoxy group of the ester. The new signal at 60.6 ppm corresponding to C1, appeared as an expected doublet with a coupling J = 5.2 Hz.

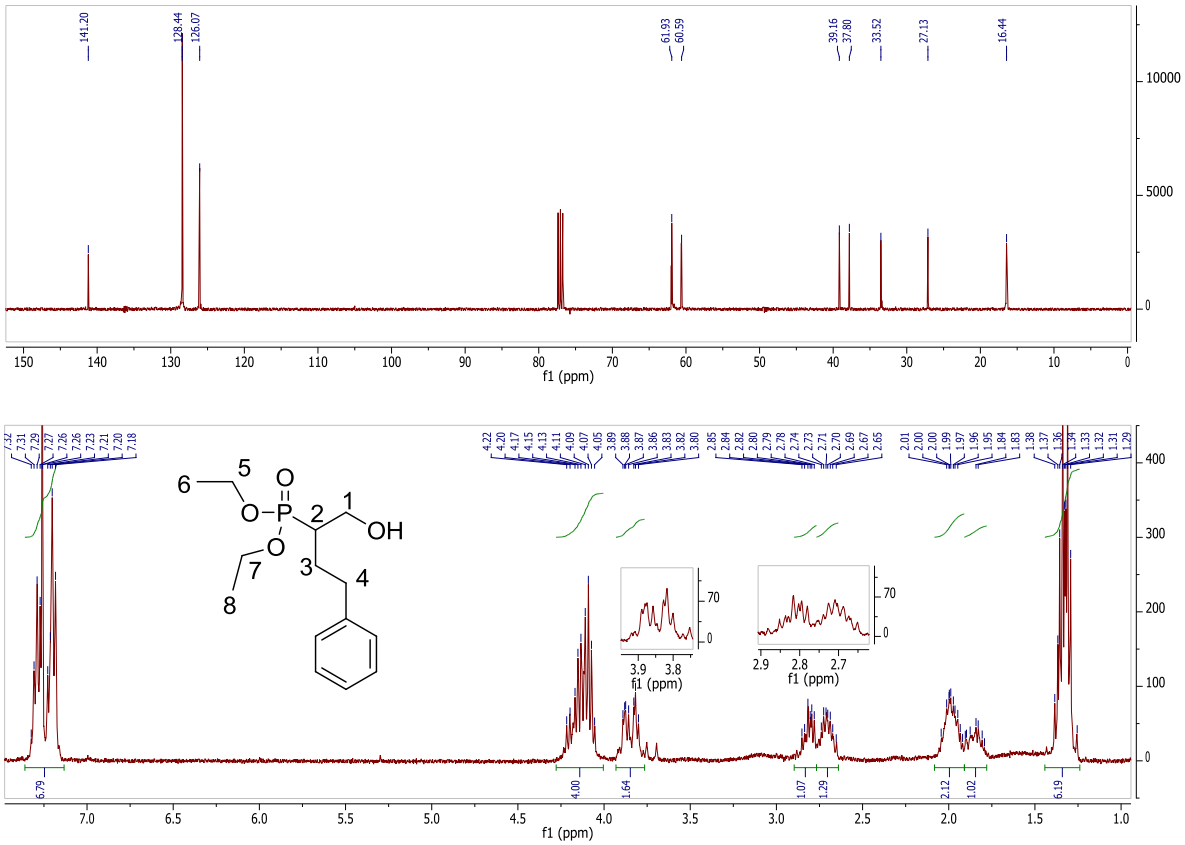
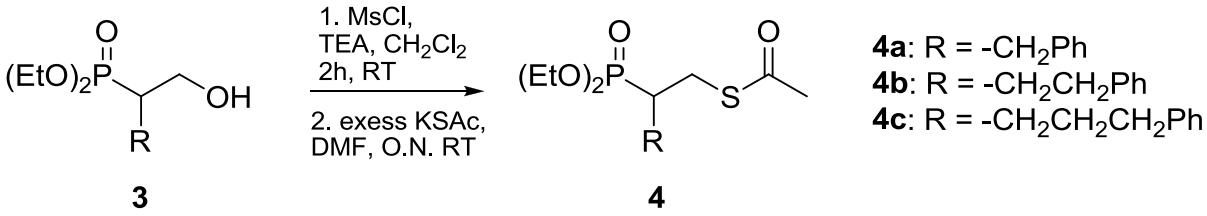


Figure 13 ^{1}H NMR and ^{13}C NMR spectra of 3b.

3.2.3 Conversion of Alcohols to Protected Thiols

Two synthetic routes were executed to yield the protected thiols (Scheme 24). The first reaction was the Mitsunobu reaction, and the second was nucleophilic substitution of the mesylated intermediate. Results from both reactions are presented in the following section.



Scheme 24 Conversion of the hydroxyphosphonates to the protected thiol.

All three componds **4a-c** were synthesized by the Mitsunobu reaction. The initial test on synthesizing **4a** with 1.5 eqv of PPh_3, DEAD and thioacetic acid in THF did succeed, but was not possible to purify. **4c** was synthesized by the same procedure, but with DIAD instead of DEAD. NMR analysis after purification revealed significant contamination with DIAD and PPh_3. DCAD was employed in hope to ease the work up procedure, due to its insolubility in CH_2Cl_2, making it possible to filtrate off after the reaction was complete. The reaction was done with the alcohol to **4b** (**3b**). After flash chromatography a considerable amount of PPh_3 and PPh_3 oxide was observed on the NMR analysis and a small amount of unreacted DCAD. PPh_3 was then exchanged with tributylphosphine (TBP), bearing in mind that the oxide is water soluble. The reaction was first tested in CH_2Cl_2. DCAD precipitated shortly after addition of the thioacetic acid and the alcohol. However, after flash chromatography, only starting material could be isolated. The same reaction was therefore executed in THF. NMR analysis after purification showed remaining TBP. Attempts to oxidize the remaining TBP with H_2O_2 did not succeed. [60]

An obvious approach to solve the described purification problems is to apply immobilized DEAD and PPh_3, which was not attempted within this project. Instead, we turned our attention to a classic substitution of alcohol with nucleophile via a mesylate intermediate. First, the alcohol was transformed into a mesylate, making the hydroxyl group a good leaving group. The alcohol (1 eqv) and triethylamine (1.05 eqv) in dry CH_2Cl_2 were stirred for 5 minutes, and added methanesulfonyl chloride (1.05 eqv). The reaction was complete within two hours as confirmed by ^{1}H NMR of the crude. The crude was subject to a nucleophilic

substitution with potassium thioacetate. An excess of potassium thioactetate (7 eqv) was dissolved in DMF, and added the mesylate in a drop wise manner. The reaction was stirred at room temperature overnight. All three compounds **4a-c** were synthesized by this procedure. The thioacetate **4b** and **4c** were isolated with small contaminations of unreacted mesylate in 54% and 34% yield. **4a** was not isolated in its pure form.

None of the thioacetates **4a-c** have been reported previously. **4b** and **4c** were characterized by ^{1}H and ^{13}C NMR, HR-MS, and IR analysis.

Figure 14 shows a representative ^{1}H NMR spectrum of **4b**. The spectra were run in $CDCl_3$. The aromatic and phosophonate ethyl protons are, as described earlier, found at 7.27-7.17, 4.13 and 1.34 ppm, respectively. The two diastereotopic protons at C1 gave rise to two separate double triplets at 3.39 ppm and 3.02 ppm. The splitting is due to the coupling to the geminal and vicinal protons, and to the phosphorous atom as described for the hydroxyphosphonates. It is not possible to deduce the coupling constants found in the spectrum for **4b** to be either geminal, vicinal or phosphorous couplings with certainty. The benzylic protons (C4) gave rise to a triplet at 2.80 ppm with a coupling constant 7.2 Hz. The singlet at 2.33 ppm corresponds to the methyl group of the thioacetate (C6). The protons corresponding to the remaining positions C2 and C3, was observed as multiplets between 2.12 ppm and 1.82 ppm. The appearing quartet at 1.34 ppm integrated to 6 (as mentioned previously) belong to the methylene protons at C8 and C10. The diviation from the expected triplet can be explained by the two ethyl groups being diastereotopic and resonate at slightly different frequency resulting in a partial signal overlap.

^{13}C NMR analysis of **4b** is as expected. The carbonyl carbon (C5) corresponds to the peak at 195.1 ppm, while the methyl carbon (C6) is observed at 30.43 ppm.

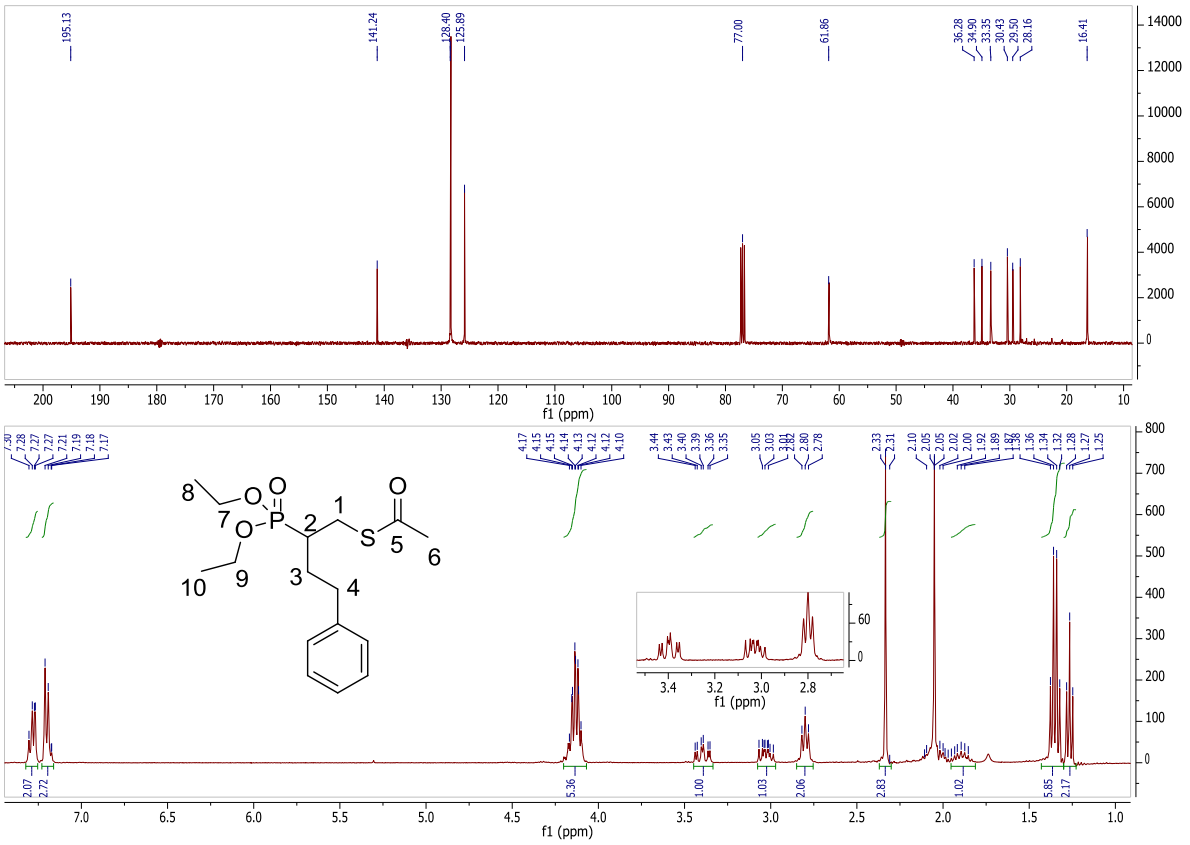
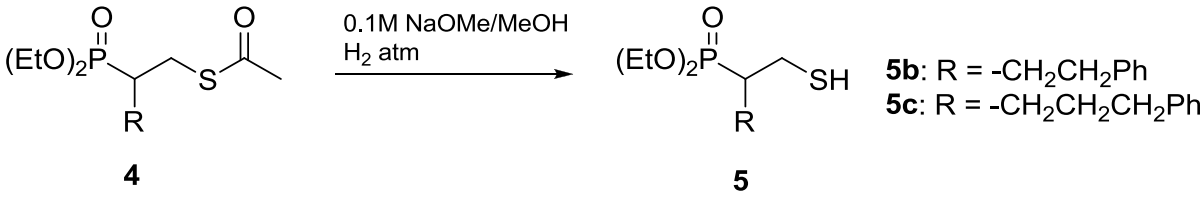


Figure 14 ^{1}H and ^{13}C NMR of 4b.

3.2.4 Removal of Sulfur Protecting Groups

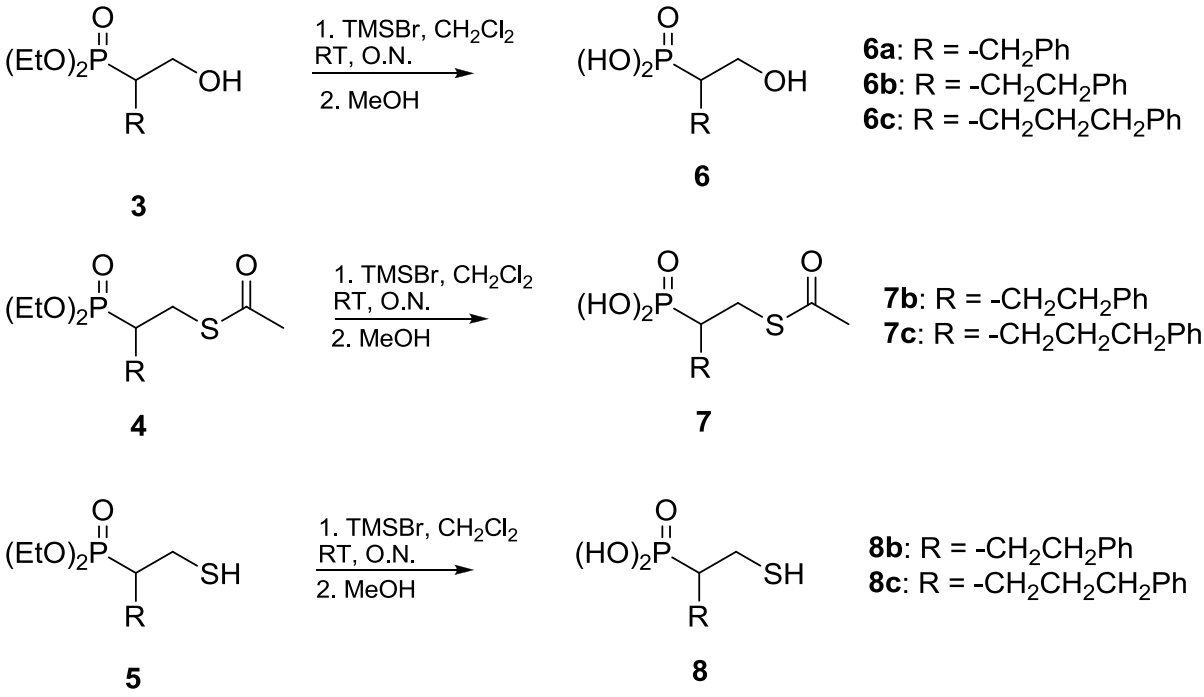
Removal of the thioacetate protecting group has been reported extensively in literature.[47, 61-64] Two methods (acidic + basic hydrolysis) were applied in this work. The phosphonate functionality is not affected by acidic or basic hydrolysis, so no precautions were necessary.[19] Initially, deprotection by acid-catalyzed hydrolysis was attempted by refluxing the protected thiol in concentrated HCl in methanol overnight.[64] This method was not successful. Base-catalyzed hydrolysis employing 0.1 M of MeONa/MeOH under H_2 atmosphere at room temperature for 30 min gave the free thiol (Scheme 25).[48] Compounds **5b** and **5c** were hydrolyzed to yield 30% and 16%, respectively. The low yields are to some extent explained by unreacted starting material, loss of product during flash chromatography and byproduct formation observed by analyzing the different fractions by ^{1}H NMR.



Scheme 25 Removal of the thioactetate protecting group.

3.2.5 Dealkylation of Phosphonate Esters

We envisioned that dealkylation of several of the phosphonates prepared during this project (**3**-**5**) may provide phosphonic acids with potential inhibitor activity (Scheme 26, **6**-**8**).



Scheme 26 Dealkylation of the hydroxyphosphonates, phosphonate thioacetates, and phosphonate thiols.

The reported procedure by Gutierrez and coworkers[49] was used as a starting point for the dealkylation, since trimethylsilyl chloride (TMSCl) was available at the time and not trimethylsilyl bromide (TMSBr). Gutierrez reported reaction times varying from 8-36h, refluxing the phosphonates at 130°C -140°C with NaI as catalyst.

Dealkylation was first tested with TMSCl and NaI at reflux or microwave. Extraction work up after quenching with water gave a crude, which contained the phosphonic acid as the ^{1}H NMR spectrum revealed no multiplet at 4.12ppm. However, NMR analysis also revealed that the

crude was contaminated with TMS residues, probably TMS-OH. The phosphonic acids were not possible to purify on a regular silica flash chromatography.

The work-up procedure was modified hydrolyzing unreacted trimethylsilyl chloride with MeOH instead of water. After stirring with MeOH overnight, the reaction mixture was concentrated to yield the phosphonic acid as a dark brown oil. In addition, the yield corresponded to 120%, due to contamination with NaI. TMSBr is a more reactive reagent and neither heating nor addition of NaI is needed in the dealkylation of phosphonates. We therefore hoped that by applying TMSBr, the purification of the resulting phosphonic acids could be simplified.

The phosphonates were stirred with TMSBr (3 eqv) overnight at room temperature, and hydrolyzed with MeOH. The reduction of the phosphonates with the thioacetate (**7b** and **7c**) was of special interest, because of the suspected possibility to hydrolyse the thioacetate under the same conditions as the phosphonates. HR-MS of **7c** showed a peak corresponding to the calculated mass for the thiol **8c** with a 15% intensity. It was not done any further attempt to identify the peak. For **7b**, no such peak was observed.

The solvent in the samples was difficult to remove, and resulted in yields above 100% (except for compound **7b**), even though they were dried under vacuum for up to five days. The high yields in sample **6b**, **6c**, **7b**, **7c**, and **8c**, could in addition be caused by unidentified byproducts.

None of the phosphonic acids **6**-**8** have been reported previously. All compounds were characterized by ^{1}H and ^{13}C NMR, HR-MS, and IR.

As expected, ^{1}H and ^{13}C NMR of the phosphonic acids were as the phosphonates, only missing the two multiplets corresponding to the phosphonates ethyl groups. Figure 15 shows a ^{1}H NMR and ^{13}C NMR spectrum typical for the hydroxyphosphonic acids (**6b**). The splitting of the proton in the benzylic position has shifted from being two multiplets integrated to 1 each, to one multiplet integrated to 2. This decreased splitting pattern is also observed for the protons at position C2 and C3.

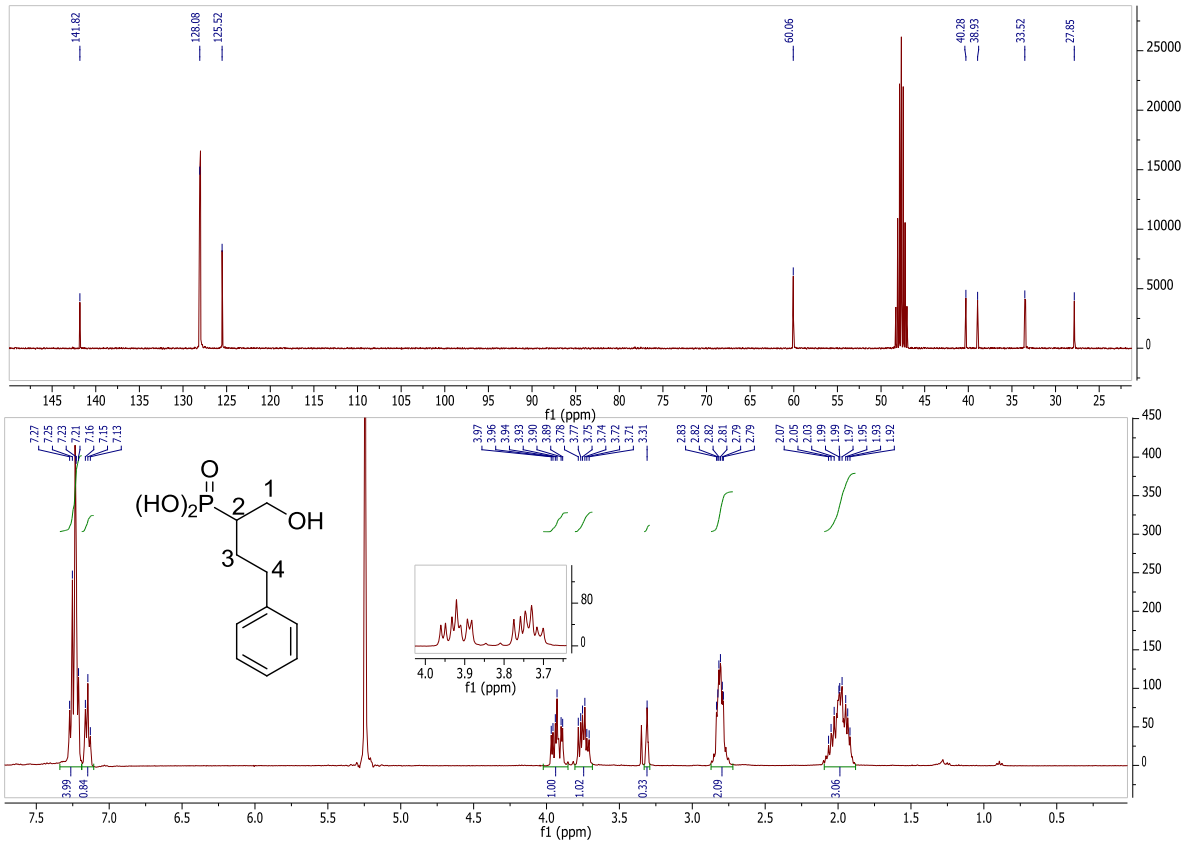
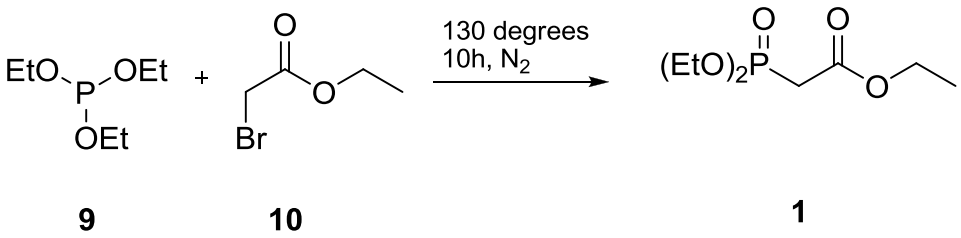


Figure 15 ^{1}H NMR and ^{13}C NMR of 6b.

3.3 Preparation of Starting Materials

3.3.1 Diethyl phosphonacetate

The starting material diethyl phosphonoacetate (**1**) was synthesized according to a procedure published by Sharma and coworkers (Scheme 27).[65] Triethylphospite (**9**) was mixed with ethyl-2-bromoacetate (**10**) and heated to 130°C for 10 hours under nitrogen to yield 98%. **1** was used without further purification.



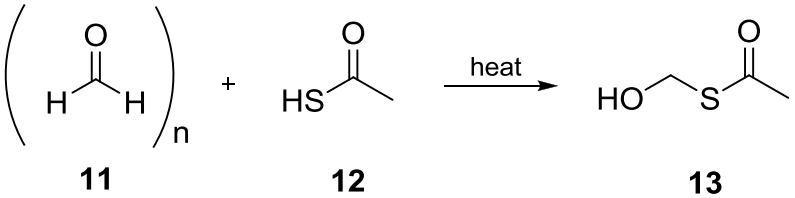
9 **10** **1**

Scheme 27 The starting material diethyl phosphonoacetate (1) was made with triethylphosphite (9) and ethyl-2-bromoacetate (10).

3.3.2 S-hydroxymethyl ethanethioate

Hydroxymethyl-acetyl-sulfide (**13**) was synthesized according to Böhme and coworkers procedure[66] from 1959. Thioacetic acid (**12**) was slowly added *n*-paraformaldehyde (**11**) under N_2 and heated to 100°C to the paraformaldehyde was dissolved. The reaction mixture was allowed to cool down to room temperature and used without further purification necessary resulting in 85% yield.

The procedure was originally executed with freshly distilled thioacetic acid. In our hands, this "purification step" gave more contamination compared with the reaction done with non-distilled commercial thioacetic acid, so the commercial thioacetic acid was used without further purification.

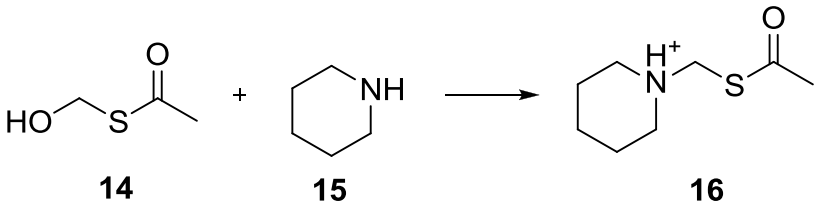


11 **12** **13**

Scheme 28 Hydroxymethyl-acetyl-sulfide (13) was made with thioacetic acid (12) and *n*-paraformaldehyde (11).

3.3.3 Piperidinomethyl thioacetate hydrochloride

Piperidinomethyl thioacetate hydrochloride (**16**) was synthesized according to Smissman and coworkers procedure.[20] Piperidine (**15**) was dissolved in ether and treated with a large excess of anhydrous magnesium sulfate. The mixture was cooled in an ice bath and added an ethereal solution of hydroxymethyl-acetyl-sulfide (**14**). The mixture was stirred for 1h, and filtered into diethylether saturated with HCl to precipitate in 82% yield. The hydrochloride salt is hygroscopic and must be stored in the absence of moisture.



14 **15** **16**

Scheme 29 Piperidinomethyl thiobenzoate hydrochloride (16) was synthesized with piperidine (15) and hydroxymethyl-acetyl-sulfide (14).

3.4 Future Outlook

3.4.1 Thiomethylation

In future attempts to thiomethylate phosphonoacetate, the combination of leaving groups and sulfanyl protecting group could be varied. An interesting combination would be trityl ($(HSC(C_6H_5)_3$) or methanethiol as protecting groups with mesylate as leaving group.

3.4.2 Conversion of Alcohols to Protected Thiols

To achieve higher yields of the nucleophilic replacement of the mesylate with thioacetate, the synthesis could be optimized by altering the reaction temperature and/or reaction time.

3.4.3 Removal of Sulfur Protecting Group

Due to the low yields, a more thorough testing of the procedures removing the thioacetate protecting group could be profitable.

3.4.4 Suggestions for Additional Potential Metallo-β-Lactamase Inhibitors

Based on the article by Jin and coworkers (2004), it would be very interesting to add one or two carbons in the alkyl chain. It is also envisioned to use alkyl halides with halide substituted phenyl rings, or to replace the aromatic ring with heterocyclic groups.

Purification, removal of the thioacetate protecting group and dealkylation should also be executed on **4a**.

If the inhibitors show good results, it would be desirable to co-crystallize the inhibitors with the VIM-2 enzyme for X-ray analysis.

4 Conclusion

16 new racemic compounds have been synthesized and characterized by ^{1}H and ^{13}C NMR, HR-MS, and IR. 14 of these will be tested for inhibitor activity against VIM-2.

Attempts to thiomethylate phosphonoacetate failed. Factors as solvents, reaction time, leaving groups, and sulfur protecting groups were varied.

The alkylation of the phosphonoacetate yielded 20% for **2a** and 62% for **2b** and **2c**. The synthesis of **2a** demanded mild reaction conditions and long reaction time to avoid the formation of dialkylated phosphonoacetate. A new convenient work up procedure was developed using pentane and water for extraction.

The reduction of **2a-c** provided the hydroxyphosphonates **3a-c** with moderate to high yields, 52, 60 and 95%, respectively. The reaction time did not appear to affect the reduction.

The Mitsunobu reaction synthesizing thioacetates **4a-c** gave the desired product, which was not possible to purify. The alternative reaction with nucleophilic substitution via the mesylate was preferred. **4b** and **4c** was synthesized in 54% and 34% yields, respectively. Attempts to purify **4a** did not succeed.

Removal of the thioacetate protecting group resulted in low yields, 30% (**5b**) 16% (**5c**).

Dealkylation with TMSBr was preferred over TMSCl. Dealkylation of compound **6a-c**, **7b-c**, and **8b-c** with TMSBr resulted in high conversion rates.

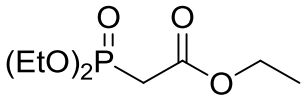
14 inhibitors have been delivered for testing against the VIM-2 enzyme. To date, the results are not available. If the results are positive and preparation of analogs is desirable, the developed synthetic protocol can be followed.

5 Experimental Section

5.1 Experimental

The solvents and reagents were purchased from commercial suppliers and used without further purification (except from *para*-toluene sulfonyl chloride, which was recrystallized). The reactions monitored by TLC was run on 60 F_{254} silica gel plates and visualized with UV light and stains. Microwave irradiation was carried out in a Biotage initiator microwave synthesizer. IR spectra were obtained on a Varian 7000e FT-IR spectrometer. Mass spectra were recorded on a LTQ Orbitrap XL in a positive and negative Electron Spray Ionization mode. ^{1}H NMR, ^{13}C NMR and ^{31}P NMR were recorded on a Varian Mercury 400 MHz at room temperature. ^{1}H NMR data are reported in the following order: multiplicity (s, singlet; d, dublet; dd, double dublet; t, triplet; dt, duble triplet; m, multiplet), coupling constant and number of protons.

5.1.1 The Arbuzov Reaction



Ethyl 2-(diethoxyphosphoryl)acetate (**1**)[65]

A mixture of ethylbromoacetate **10** (60g, 0.358 mol, 1 eq) and triethylphosphite **9** (59.65g, 0.359mol, 1 eq) were heated in a flame-dried flask to 130ºC overnight under N_2. The mixture was allowed to cool to RT to give **1** (79g, 0.35mol, 98%) as a colorless oil. The compound was used without further purification. Analysis data were in accordance with literature.[65]

^{1}H NMR (400 MHz, Chloroform-d) δ 4.18 (h, *J* = 7.2 Hz, 1H), 2.96 (d, *J* = 21.6 Hz, 0H), 1.34 (t, *J* = 7.1 Hz, 1H), 1.28 (t, *J* = 7.1 Hz, 1H).
^{13}C NMR (101 MHz, Chloroform-d) δ 165.7, 62.6 (d, *J* = 6.3 Hz), 34.3 (d, *J* = 134.3 Hz), 16.3, 14.0.
^{31}P NMR (162 MHz, Benzene-d6) δ 19.58.

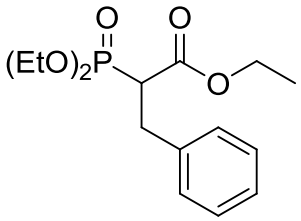
High resolution mass spectroscopy (ESI): $(M+Na)^+$: calculated for $C_8H_{17}O_5NaP$: 247.0711; found 247.0707 (100%). $(M+H)^+$ calculated for $C_8H_{18}O_5P$: 225.0886; found 225.0890 (5%).

IR: 2983, 1735, 1258

5.1.2 Alkylation Reaction

5.1.2.1 General procedure:

The base was added to a solution of *ethyl 2-(diethoxyphosphoryl)acetate* **1** in DMF at 0ºC, and stirred until the base had dissolved (approx. 10 min). The alkyl halide was added drop wise and heated to 60ºC for 12h-7d. The mixture was acidified with concentrated HCl or 10% citric acid and extracted with pentane. The combined organic phases were washed with pure water (3x), brine (1x), dried with $NaSO_4$, and evaporated. The residue was purified with flash chromatography (pentane/ether 1:1).



Ethyl 2-(diethoxyphosphoryl)-3-phenylpropanoate (**2a**):

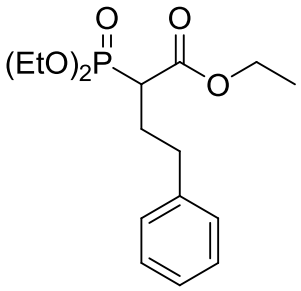
Potassium carbonate (2 eq, 0.11 mol, 15.4g), ethyl 2-(diethoxyphosphoryl)acetate **1** (1 eq, 0.056 mol, 12.49g) and benzyl bromide (1.2 eq, 0.067 mol, 11.46g) gave **2a** (0,01 mol, 3.44g, 20%) as a pale yellow oil.

^{1}H NMR (400 MHz, DMSO-d6) δ 7.32 – 7.09 (m, 5H), 4.24 – 3.81 (m, 6H), 3.39 (ddd, *J* = 22.2, 10.8, 4.6 Hz, 1H), 3.09 – 2.98 (m, 2H), 1.23 (td, *J* = 7.0, 2.5 Hz, 6H), 1.03 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (101 MHz, DMSO-d6) δ 168.4 (d, *J* = 4.8 Hz), 138.8 (d, *J* = 16.0 Hz), 128.9 (d, *J* = 18.8 Hz), 127.0, 61.2, 46.8 (d, *J* = 127.1 Hz), 32.7 (d, *J* = 4.3 Hz), 16.7 (d, *J* = 5.8 Hz), 14.3.

High resolution mass spectroscopy (ESI): $(M+Na)^+$: calculated for $C_{15}H_{23}O_5NaP$: 337.1181; found 337.1178 (100%). $(M+H)^+$ calculated for $C_{15}H_{24}O_5P$: 315.1356; found 315.1363 (20%).

IR: 2982, 1732, 1253, 1018



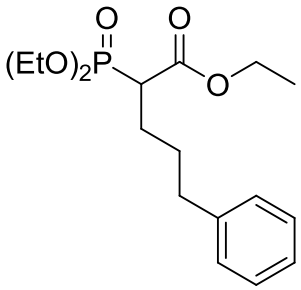
Ethyl 2-(diethoxyphosphoryl)-4-phenylbutanoate (**2b**):

Potassium *tert*-butoxide (1 eq, 0.028mol, 3.15g), ethyl 2-(diethoxyphosphoryl)acetate (1 eq, 0.028 mol, 6.3g) and (2-bromoethyl)benzene (1 eq, 0.028 mol, 5.18g) gave **2b** (0.018mol, 5.76g, 62%) as a pale yellow oil.

^{1}H NMR (400 MHz, Chloroform-d) δ 7.31 – 7.24 (m, 2H), 7.19 (m, 3H), 4.22 (m, 2H), 4.12 (m, 4H), 2.95 (ddd, *J* = 22.9, 10.9, 3.7 Hz, 1H), 2.73 (m, 1H), 2.59 (m, 1H), 2.31 (m, H), 2.22 – 2.07 (m, 1H), 1.31 (m, 9H).
^{13}C NMR (101 MHz, Chloroform-d) δ 169.0 (d, *J* = 5.0 Hz), 140.5, 128.49 (d, *J* = 10.7 Hz), 126.2, 62.7, 61.4, 45.1 (d, *J* = 130.9 Hz), 34.3 (d, *J* = 15.4 Hz), 28.6 (d, *J* = 4.4 Hz), 16.3 (dd, *J* = 6.1, 2.3 Hz), 14.2.

High resolution mass spectroscopy (ESI): $(M+Na)^+$: calculated for $C_{16}H_{25}O_5NaP$: 351.1332; found 351.1333 (100%). $(M+H)^+$ calculated for $C_{15}H_{26}O_5P$: 329.1512; found 329.1519 (15%).

IR: 2982, 1731, 1251, 1019



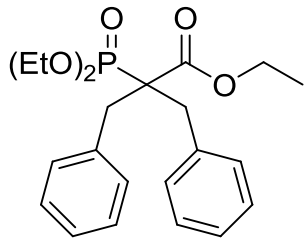
Ethyl 2-(diethoxyphosphoryl)-5-phenylpentanoate (**2c**):

Potassium *tert*-butoxide (0.7 eq, 0.014mol, 1.549g), ethyl 2-(diethoxyphosphoryl)acetate (1 eq, 0.019 mol, 4.25g) and (3-bromopropyl)benzene (0.5 eq, 0.01mol, 2.01g) gave **2c** (5.9 mmol, 2.01g, 62%) as a pale yellow oil.

^{1}H NMR (400 MHz, Chloroform-d) δ 7.27 (m, 2H), 7.17 (m, 3H), 4.15 (m, 6H), 2.95 (ddd, *J* = 22.7, 11.5, 3.8 Hz, 1H), 2.63 (dt, *J* = 7.8, 1.8 Hz, 2H), 2.10-2.00 (m, 1H), 1.94-1.84 (m, 1H), 1.75 – 1.56 (m, 2H), 1.38 – 1.24 (m, 6H).
^{13}C NMR (101 MHz, Chloroform-d) δ 169.2 (d, *J* = 4.7 Hz), 141.6, 128.3 (d, *J* = 1.8 Hz), 125.9, 65.4 – 61.8 (m), 61.3, 45.7 (d, *J* = 131.2 Hz), 35.4, 30.2 (d, *J* = 14.9 Hz), 26.7 (d, *J* = 5.0 Hz), 16.4 (dd, *J* = 6.0, 3.2 Hz), 14.1.

High resolution mass spectroscopy (ESI): $(M+Na)^+$: calculated for $C_{17}H_{27}O_5NaP$: 365.1488; found 365.1484 (100%).

IR: 2982, 1732, 1252, 1019



Ethyl 2-benzyl-2-(diethoxyphosphoryl)-3-phenylpropanoate (**2d**):

^{1}H NMR (400 MHz, Chloroform-d) δ 7.36 – 7.12 (m, 10H), 4.21 (q, *J* = 7.2 Hz, 2H), 3.99 (dp, *J* = 10.1, 7.0 Hz, 2H), 3.86 (ddq, *J* = 10.0, 8.5, 7.1 Hz, 2H), 1.24 (t, *J* = 7.2 Hz, 3H), 1.12 (t, *J* = 7.0 Hz, 6H).
^{13}C NMR (101 MHz, Chloroform-d) δ 170.7 (d, *J* = 2.2 Hz), 136.6 (d, *J* = 8.1 Hz), 130.9, 127.7, 126.7, 64.9 – 58.9 (m), 55.4 (d, J = 140.4 Hz), 40.0 (d, J = 3.2 Hz), 16.2 (d, J = 6.3 Hz), 13.9.

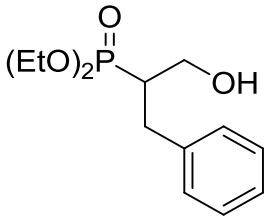
High resolution mass spectroscopy (ESI): $(M+H)^+$: calculated for $C_{22}H_{30}O_5P$: 405.1825; found 405.1830 (100%). $(M+Na)^+$ calculated for $C_{22}H_{29}O_5NaP$:427.1645; found 427.1647 (35%).

IR: 3023, 2929, 1119, 930, 696

5.1.3 Ester Reduction

5.1.3.1 General procedure:

$LiBH_4$ was dissolved in THF and added the alkylated phosphonates (**2**) drop wise at 0ºC. The suspension was stirred at room temperature for 30 minutes under argon, and irradiated at 80ºC for 10-20 minutes. The mixture was allowed to cool to RT, quenched with MeOH and stirred until the evolution of H_2 gas stopped. The solution was acidified with 10% citric acid, saturated with NaCl, extracted with ether, dried with $NaSO_4$ or $MgSO_4$, and evaporated to give the crude product. The residue was purified with flash chromatography (Pentane/MeOH or CH_2Cl_2/MeOH).



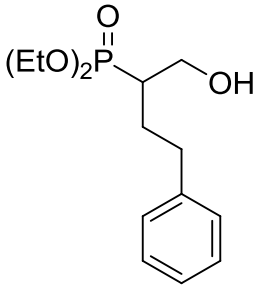
Diethyl 1-hydroxy-3-phenylpropan-2-ylphosphonate (**3a**):

Ethyl 2-(diethoxyphosphoryl)-4-phenylbutanoate **2a** (1 eq, 2.6 mmol, 0.81g) and $LiBH_4$ (6 eq, 0.015mol, 0.34g) gave **3a** (2.5mmol, 0.37g, 52%) as a colorless oil, after flash chromatography (ether/pentane).

^{1}H NMR (400 MHz, Chloroform-d) δ 7.26 – 7.02 (m, 5H), 4.11 – 3.90 (m, 4H), 3.70-3.54 (m, 2H), 2.92 (ddd, *J* = 14.0, 11.3, 4.9 Hz, 1H), 2.79-2.71 (m, 1H), 2.13 (ddq, *J* = 19.7, 9.9, 5.0 Hz, 1H), 1.19 (dt, *J* = 15.3, 7.1 Hz, 6H).
^{13}C NMR (101 MHz, Chloroform-d) δ 138.7 (d, *J* = 14.7 Hz), 129.0, 128.5, 126.5, 62.3 (d, *J* = 6.9 Hz), 62.1 (d, *J* = 6.7 Hz), 59.6 (d, *J* = 5.6 Hz), 41.1 (d, *J* = 136.4 Hz), 31.2 (d, *J* = 2.6 Hz), 16.4 (t, *J* = 6.4 Hz).

High resolution mass spectroscopy (ESI): $(M+Na)^+$: calculated for $C_{13}H_{21}O_4NaP$: 295.1070; found 295.1071 (100%). $(M+H)^+$ calculated for $C_{13}H_{22}O_4P$: 273.1250; found 273.1255 (10%).

IR: 3365, 2981, 1220, 1021



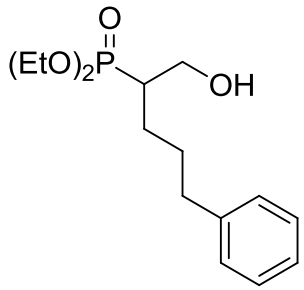
Diethyl 1-hydroxy-4-phenylbutan-2-ylphosphonate (**3b**):

Ethyl 2-(diethoxyphosphoryl)-4-phenylbutanoate **2b** (1 eq, 1.6 mmol, 0.53g) and $LiBH_4$ (3 eq, 4.8 mmol, 0.12g) gave **3b** (1.0 mmol, 0.28g, 60%) as a colorless oil, after flash chromatography (CH_2Cl_2/MeOH).

^{1}H NMR (400 MHz, Chloroform-d) δ 7.33 – 7.25 (m, 2H), 7.23-7.18 (m, 3H), 4.22-4.05 (m, 4H), 3.95 – 3.78 (m, 2H), 2.89 – 2.76 (m, 1H), 2.76 – 2.63 (m, 1H), 2.10 – 1.91 (m, 2H), 1.91 – 1.76 (m, 1H), 1.41 – 1.27 (m, 6H).
^{13}C NMR (101 MHz, Chloroform-d) δ 141.2, 128.4, 128.4, 126.1, 61.9 (t, *J* = 6.6 Hz), 60.6 (d, *J* = 5.2 Hz), 38.5 (d, *J* = 136.7 Hz), 33.5 (d, *J* = 11.0 Hz), 27.1 (d, *J* = 3.4 Hz), 16.4 (t, *J* = 5 Hz).

High resolution mass spectroscopy (ESI): $(M+H)^+$: calculated for $C_{14}H_{24}O_4P$: 287.1407; found 287.1408 (100%). $(M+Na)^+$ calculated for $C_{14}H_{24}O_4NaP$: 309.1226; found 209.1227 (35%).

IR: 3365, 2981, 1217, 1021



Diethyl 1-hydroxy-5-phenylpentane-2-ylphosphonate (**3c**):

Ethyl 2-(diethoxyphosphoryl)-5-phenylpentanoate **2c** (1 eq, 4.7 mmol, 1.60g) and $LiBH_4$ (2.5 eq, 0.012 mol, 0.25g) gave **3c** (4.5 mmol, 1.342g, 95%) as a colorless oil, after flash chromatography (CH_2Cl_2/MeOH).

^{1}H NMR (400 MHz, Chloroform-d) δ 7.31 – 7.23 (m, 2H), 7.22 – 7.13 (m, 3H), 4.16-4.07 (m, 4H), 3.90 – 3.69 (m, 2H), 3.08 (t, *J* = 6.3 Hz, 1H), 2.63 (t, *J* = 7.4 Hz, 2H), 2.02-1.95 (m, 1H), 1.90 – 1.76 (m, 1H), 1.76-1.65 (m, 2H), 1.62 – 1.49 (m, 1H), 1.32 (td, *J* = 7.1, 3.9 Hz, 2H). ^{13}C NMR (101 MHz, Chloroform-d) δ 141.8, 128.3 (d, *J* = 1.7 Hz), 125.8, 62.0 (d, *J* = 6.7 Hz), 61.9 (d, *J* = 6.6 Hz), 60.8 (d, *J* = 5.7 Hz), 39.2 (d, *J* = 136.6 Hz), 35.7, 29.3 (d, *J* = 11.0 Hz), 25.0 (d, *J* = 3.6 Hz), 16.5 (d, *J* = 5.8 Hz).

High resolution mass spectroscopy (ESI): $(M+H)^+$: calculated for $C_{15}H_{26}O_4P$: 301.1563; found 301.1566 (100%). $(M+Na)^+$ calculated for $C_{15}H_{25}O_4NaP$: 323.1383; found 301.1566 (20%).

IR: 3365, 2931, 1217, 1021

5.1.4 Conversion of Alcohols to Protected Thiols

5.1.4.1 The Mitsunobu Reaction

5.1.4.1.1 General procedure for reactions with DEAD or DIAD:

Trialkyl phosphine (1.55 eq) was dissolved in THF and slowly added dialkyl azodicarboxylate (1.55 eq) at 0ºC. After 30 min, a solution of the hydroxyphosphonate (1 eq) and thioacetic acid (1.5) in THF was added at 0ºC, allowed to warm to RT and stirred overnight. The reaction mixture was quenched with 10% citric acid and extracted with diethyl ether. The combined organic phases were washed with brine, attempted purified with flash chromatography, dried over Na_2SO_4, filtered, and evaporated.

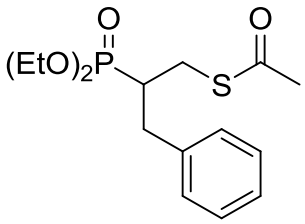
5.1.4.1.2 Variation for reactions with DCAD:

Trialkyl phosphine (1.55 eq) was dissolved in THF and slowly added DCAD (1.55 eq) at 0ºC. After 30 min, a solution of the hydroxyphosphonate (1 eq) and thioacetic acid (1.5) in THF was added at 0ºC, allowed to warm to RT and stirred overnight. The reaction mixture was filtrated, quenched with NH_4Cl and extracted with CH_2Cl_2. The organic phase was concentrated, added CH_2Cl_2, filtrated, attempted purified with flash cromatography (CH_2Cl_2/MeOH), dried over Na_2SO_4, filtered, and evaporated.

5.1.4.2 S_N2 Nucleophilic Substitution of Mesylated Alcohols with Thio Nucleophiles

5.1.4.2.1 General procedure:

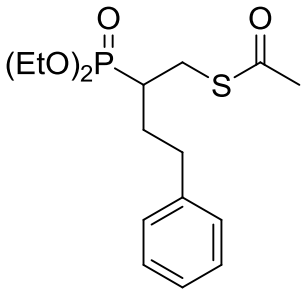
Hydroxyphosphonate (**3**) was dissolved in CH_2Cl_2 and added triethylamine (1.05 eqv) and a catalytic amount of 4-dimethylaminopyridine (DMAP) and stirred for 5 minutes. The mesylate was added in a drop wise manner, and the reaction mixture was stirred at room temperature for 2 hours. The solvent was evaporated, and the remaining crude was quenched with NH_4Cl and extracted with ether and dried with Na_2SO_4, filtered, and evaporated. Without further purification, the crude was added a solution of excess potassium thioacetate (7-10 eqv) in DMF and stirred overnight at room temperature. The reaction mixture went from being a yellow solution to a black porridge. The resulting porridge was quenched with NH_4Cl, extracted with ether, dried over Na_2SO_4, filtered and purified with flash chromatography.



S-2-(diethoxyphosphoryl)-3-phenylpropyl ethanethioate (**4a**):

Diethyl 1-hydroxy-3-phenylpropan-2-ylphosphonate **3a** (1 eq, 0.55 mmol, 0.15g), triethylamine (1.05 eq, 0.58 mmol, 0.06 g), methanesulfonyl chloride (1.05 eq, 0.58 mmol, 0.13 g), DMAP (cat.amount.), and potassium thioacetate (7 eq, 3.8 mmol, 0.44 g) gave **4a** as an orange oil. Attempts to purify **4a** did not succeed.

^{1}H NMR (400 MHz, Chloroform-d) δ 7.37 – 7.08 (m, 5H), 4.19 – 3.93 (m, 4H), 3.33 – 3.16 (m, 1H), 3.05 – 2.96 (m, 1H), 2.89 – 2.77 (m, 2H), 2.28 (s, 3H), 1.36 – 1.17 (m, 6H).



S-2-(diethoxyphosphoryl)-4-phenylbutyl ethanethioate (**4b**):

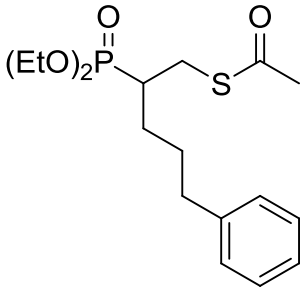
Diethyl 1-hydroxy-4-phenylbutan-2-ylphosphonate **3b** (1 eq, 2.8 mmol, 0.82g), triethylamine (1.05 eq, 2.9 mmol, 0.3 g), methanesulfonyl chloride (1.05 eq, 2.9 mmol, 0.35 g), DMAP (cat.amount.), and potassium thioacetate (7 eq, 20.3 mmol, 2.31 g) gave **4b** (1.5 mmol, 0.52g, 54%) as an orange oil, after purification on flash chromatography (CH_2Cl_2/MeOH).

^{1}H NMR (400 MHz, Chloroform-d) δ 7.36 – 7.24 (m, 2H), 7.20 (m, 3H), 4.25 – 4.03 (m, 4H), 3.40 (td, *J* = 15.6, 14.8, 4.7 Hz, 1H), 3.03 (m, 1H), 2.80 (t, *J* = 7.6 Hz, 2H), 2.33 (s, 3H), 2.13 – 1.96 (m, 2H), 1.90 (m, 1H), 1.35 (q, *J* = 7.2 Hz, 6H).

^{13}C NMR (101 MHz, Chloroform-d) δ 195.1, 141.2, 128.4, 128.3, 126.0, 61.9 (d, *J* = 2.1 Hz), 61.8 (d, *J* = 2.5 Hz), 35.6 (d, *J* = 138.1 Hz), 33.3 (d, *J* = 7.4 Hz), 30.4, 29.5 (d, *J* = 3.3 Hz), 28.2 (d, *J* = 1.8 Hz), 16.4 (d, *J* = 5.9 Hz).

High resolution mass spectroscopy (ESI): $(M+Na)^+$: calculated for $C_{16}H_{25}O_4NaP$: 367.1103; found 367.1109 (100%).

IR: 2981, 1691, 1241, 1021,



S-2-(diethoxyphosphoryl)-5-phenylpentyl ethanethioate (**4c**):

Diethyl 1-hydroxy-5-phenylpentane-2-ylphosphonate **3c** (1 eq, 0.93 mmol, 0.28g), triethylamine (1.05 eq, 0.98 mmol, 0.1 g), methanesulfonyl chloride (1.05 eq, 0.98 mmol, 0.11 g), DMAP (cat.amount.), and potassium thioacetate (10 eq, 9.3 mmol, 1.06 g) gave **4c**

(0.32 mmol, 0.11g, 34%) as an orange oil, after purification twice on flash chromatography (Ether/pentane, ether)

^{1}H NMR (400 MHz, Chloroform-d) δ 7.29 – 7.23 (m, 2H), 7.19 – 7.14 (m, 3H), 4.14-4.06 (m, 4H), 3.32 (ddd, *J* = 15.2, 13.9, 5.1 Hz, 1H), 2.97 (ddd, *J* = 13.8, 11.9, 8.2 Hz, 1H), 2.61 (t, *J* = 7.3 Hz, 2H), 2.32 (s, 3H), 2.05 – 1.91 (m, 1H), 1.86 – 1.69 (m, 2H), 1.67 – 1.53 (m, 1H), 1.31 (td, *J* = 7.0, 2.5 Hz, 6H).
^{13}C NMR (101 MHz, Chloroform-d) δ 195.3, 141.9, 128.3 (d, *J* = 16.0 Hz), 125.7 , 61.9 (t, *J* = 6.8 Hz), 36.4 (d, *J* = 139.0 Hz), 30.5, 29.0 (d, *J* = 7.4 Hz), 28.3 (d, *J* = 1.8 Hz), 27.5 (d, *J* = 3.5 Hz).

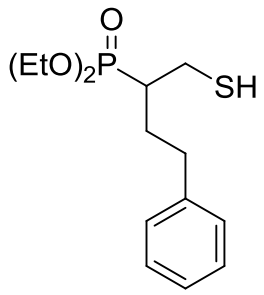
High resolution mass spectroscopy (ESI): $(M+Na)^+$: calculated for $C_{17}H_{27}O_4NaPS$: 381.1260; found 381.1263 (100%). $(M+H)^+$ calculated for $C_{17}H_{28}O_4PS$: 359.1440; found 359.1449 (5%).

IR: 2981, 1691, 1241, 1020

5.1.5 Removal of the Sulfur Protecting Group

5.1.5.1 General procedure

To the thioacetate, **4**, was added a solution of NaOMe in MeOH (0.1M) and stirred under H_2 gas at room temperature for 30 minutes. The solvent was removed under reduced pressure and the resulting salt was quenched with aq. NH_4Cl, acidified with HCl, extracted with ether and purified with flash cromatograpy (ether/EtOAc).



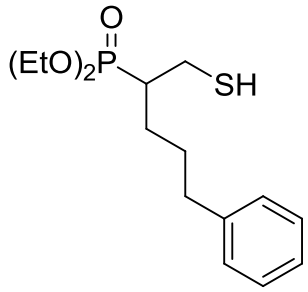
Diethyl 1-sulfanyl-4-phenylbutan-2-ylphosphonate (**5b**):

S-2-(diethoxyphosphoryl)-4-phenylbutyl ethanethioate **4b** (1.5 mmol, 0.52g) and 0.1 M NaOMe/MeOH gave **5b** (0.46 mmol, 0.139 g, 30%) as a slightly orange oil, after purification twice with flash chromatography (CH_2Cl_2/MeOH and EtOAc/Ether).

^{1}H NMR (400 MHz, Chloroform-d) δ 7.33 – 7.16 (m, 1H), 4.22 – 4.01 (m, 1H), 2.95 (dddd, *J* = 18.5, 13.9, 8.3, 4.6 Hz, 0H), 2.86 – 2.62 (m, 1H), 2.17 – 1.89 (m, 1H), 1.32 (td, *J* = 7.1, 4.6 Hz, 1H).
^{13}C NMR (101 MHz, Chloroform-d) δ 141.3, 128.5, 128.4, 126.1, 61.8 (dd, *J* = 6.8, 4.2 Hz), 38.9 (d, *J* = 137.5 Hz), 33.3 (d, *J* = 8.1 Hz), 28.7 (d, *J* = 3.2 Hz), 23.5 (d, *J* = 2.5 Hz), 16.5 (d, *J* = 6.1 Hz).

High resolution mass spectroscopy (ESI): $(M+H)^+$: calculated for $C_{16}H_{28}O_3PS$: 331.1491; found 331.1494 (100%). $(M+Na)^+$ calculated for $C_{16}H_{27}O_3NaPS$: 353.1311; found 353.1310 (10%).

IR: 2981, 1691, 1241, 1021



Diethyl 1-sulfanyl-5-phenylpentan-2-ylphosphonate (**5c**):

S-2-(diethoxyphosphoryl)-5-phenylpentyl ethanethioate **4c** (0.46 mmol, 0.17g) and 0.1 M NaOMe/MeOH gave **5c** (0.08 mmol, 0.024 g, 16%) as a slightly orange oil, after purification with flash chromatography (CH_2Cl_2/MeOH).

^{1}H NMR (400 MHz, Methanol-d4) δ 7.31 – 7.05 (m, 1H), 4.21 – 3.94 (m, 1H), 2.89 (ddd, *J* = 18.6, 13.9, 4.6 Hz, 0H), 2.73 – 2.54 (m, 1H), 2.12 – 1.92 (m, 0H), 1.88 – 1.66 (m, 1H), 1.38 – 1.22 (m, 2H).
^{13}C NMR (101 MHz, Methanol-d4) δ 141.8, 128.1 (d, *J* = 0.7 Hz), 127.9, 125.4, 63.4 – 60.8 (m), 38.8 (d, *J* = 137.2 Hz), 35.3, 28.6 (d, *J* = 7.6 Hz), 26.0 (d, *J* = 3.4 Hz), 22.4 (d, *J* = 2.3 Hz), 15.3 (d, *J* = 5.9 Hz).

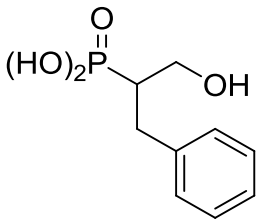
High resolution mass spectroscopy (ESI): $(M+H)^+$: calculated for $C_{15}H_{26}O_3PS$: 317.1335; found 317.1337 (100%). $(M+Na)^+$ calculated for $C_{15}H_{23}O_3NaPS$: 339.1154; found 339.1154 (35%).

IR: 3025, 2980, 2931, 1239, 1023, 699

5.1.6 Dealkylation of the Phosphonate Ester

5.1.6.1 General procedure:

TMSBr was added dropwise to the phosphonate in dry CH_2Cl_2 under argon and stirred at room temperature overnight. The solvent and excess TMSBr were removed under reduced pressure, and added MeOH to stir overnight. The reaction mixture was evaporated to dryness providing0 the phosphonic acid without further purification necessary.



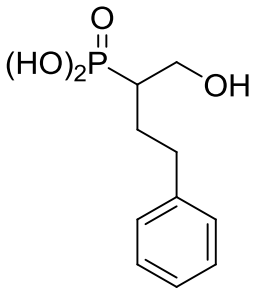
1-hydroxy-3-phenylpropan-2-ylphosphonic acid (**6a**):

Diethyl 1-hydroxy-3-phenylpropan-2-ylphosphonate **3a** (1 eq, 0.20 mmol, 0.07g) and TMSBr (3 eq, 0.61 mmol, 0.09g) gave **6a** (0.58 mmol, 0.13 g, 162%) as a white solid.

^{1}H NMR (400 MHz, Methanol-d4) δ 7.32–7.23 (m, 3H), 7.21–7.14 (m, 2H), 3.70 (dd, *J* = 18.3, 5.3 Hz, 2H), 3.08 dt, *J* = 12.0, 4.0 Hz, 1H), 2.88-2.79 (m, 1H), 2.21-2.10 (m, 1H).
^{13}C NMR (101 MHz, Methanol-d4) δ 139.7 (d, *J* = 13.4 Hz), 130.9, 128.7, 128.0, 127.4, 125.9, 59.1 (d, *J* = 3.3 Hz), 42.1 (d, *J* = 135.2 Hz), 31.3 (d, *J* = 2.1 Hz).

High resolution mass spectroscopy (ESI): $(M+H)^+$: calculated for $C_9H_{14}O_4P$: 217.0624; found 217.0621 (100%). $(M+Na)^+$ calculated for $C_9H_{13}O_4NaP$: 239.0444; found 239.0443 (5%).

IR: 3025, 2952, 1013, 696



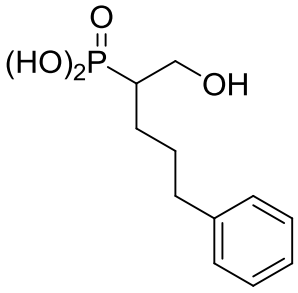
1-hydroxy-4-phenylbutan-2-ylphosphonic acid (**6b**)

Diethyl 1-hydroxy-4-phenylbutyl-2-ylphosphonate **3b** (1 eq, 0.31 mmol, 0.09g) and TMSBr (3 eq, 0.92 mmol, 0.14g) gave **6b** (0.49 mmol, 0.11 g, 160%) as a colorless oil.

^{1}H NMR (400 MHz, Methanol-d4) δ 7.29 – 7.20 (m, 3H), 7.15 (t, *J* = 6.8 Hz, 2H), 4.00 – 3.87 (m, 1H), 3.78-3.71 (m, 1H), 2.81 (td, *J* = 8.1, 7.3, 3.3 Hz, 2H), 2.12 – 1.86 (m, 3H).
^{13}C NMR (101 MHz, Methanol-d4) δ 141.8, 128.1, 128.0, 125.5, 60.1, 39.6 (d, *J* = 134.9 Hz), 33.5 (d, *J* = 8.8 Hz), 27.8 (d, *J* = 3.1 Hz).

High resolution mass spectroscopy (ESI): $(M+H)^+$: calculated for $C_{10}H_{16}O_4P$: 231.0781; found 231.0779 (100%). $(M+Na)^+$ calculated for $C_{10}H_{15}O_4NaP$: 253.0600; found 253.0601 (10%).

IR: 3255, 3027, 2930, 929, 697



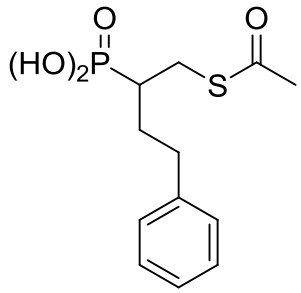
1-hydroxy-5-phenylpentane-2-ylphosphonic acid (**6c**):

Diethyl 1-hydroxy-5-phenylpentan-2-ylphosphonate **3c** (1 eq, 0.18 mmol, 0.05g) and TMSBr (3 eq, 0.53 mmol, 0.08g) gave **6c** (0.40 mmol, 0.098 g, 220%) as a colorless oil.

^{1}H NMR (400 MHz, Methanol-d4) δ 7.35 – 7.05 (m, 5H), 3.97 – 3.81 (m, 1H), 3.71-3.63 (m, 1H), 2.63 (t, *J* = 7.4 Hz, 2H), 1.97-1.64 (m, 5H).
^{13}C NMR (101 MHz, Methanol-d4) δ 142.1, 128.0, 127.9, 125.3, 60.3, 40.1 (d, *J* = 134.7 Hz), 35.7, 29.6 (d, *J* = 8.4 Hz), 25.7 (d, *J* = 3.4 Hz).

High resolution mass spectroscopy (ESI): $(M+H)^+$: calculated for $C_{11}H_{18}O_4P$: 245.0937; found 245.0936 (100%). $(M+Na)^+$ calculated for $C_{11}H_{17}O_4NaP$: 267.0757; found 267.0758 (40%).

IR: 3257, 3025, 2934, 993, 697



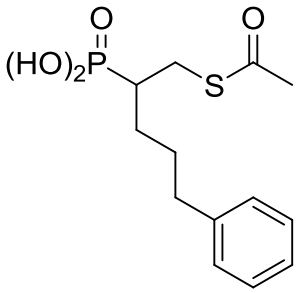
1-(acetylthio)-4-phenylbutane-2-ylphosphonic acid (**7b**):

S-2-(diethoxyphosphoryl)-4-phenylbutyl ethanethioate **4b** (1 eq, 0.2 mmol, 0.07g) and TMSBr (3 eq, 0.61 mmol, 0.09g) gave **7b** (1.5 mmol, 0.044 g, 72%) as an orange oil.

^{1}H NMR (400 MHz, Chloroform-d) δ 10.34 (s), 7.09 – 6.64 (m, 5H), 3.18 – 2.96 (m, 1H), 2.83 – 2.55 (m, 1H), 2.43 (q, *J* = 10.6, 9.1 Hz, 2H), 1.97 (s, 3H), 1.87 – 1.65 (m, 2H), 1.63-1.52 (m, 1H).
^{13}C NMR (101 MHz, Chloroform-d) δ 195.6, 140.9 (d, *J* = 4.1 Hz), 128.5, 128.5, 128.4, 126.1, 38.1 (d, *J* = 141.0 Hz), 35.2 (d, *J* = 142.3 Hz), 33.2 (t, *J* = 7.8 Hz), 30.5 , 29.2 (d, *J* = 2.6 Hz), 28.3, 27.7, 23.2.

High resolution mass spectroscopy (ESI): $(M+H)^+$: calculated for $C_{12}H_{18}O_4PS$:289.0658; found 289.0660 (100%). $(M+Na)^+$ calculated for $C_{12}H_{17}O_4NaPS$: 311.0477; found 311.0481 (25%).

IR: 2919, 1691, 1128, 931, 697



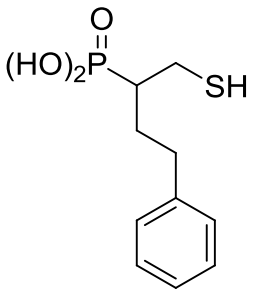
1-(acetylthio)-5-phenylpentan-2-ylphosphonic acid (**7c**):

S-2-(diethoxyphosphoryl)-5-phenylpentan ethanethioate **4c** (1 eq, 0.09 mmol, 0.033g) and TMSBr (3 eq, 0.28 mmol, 0.042g) gave **7c** (0.13 mmol, 0.041g, 147%) as an orange oil.

^{1}H NMR (400 MHz, Methanol-d4) δ 7.32 – 7.07 (m, 5H), 3.41 – 3.25 (m, 1H), 3.07 – 2.90 (m, 1H), 2.59 (t, *J* = 7.4 Hz, 2H), 2.29 (s, 3H), 2.01 – 1.47 (m, 5H).
^{13}C NMR (101 MHz, Methanol-d4) δ 195.5, 141.9, 128.1, 127.9 (d, *J* = 1.5 Hz), 125.3, 37.1 (d, *J* = 136.7 Hz), 35.4, 29.1, 29.0 (d, *J* = 3.8 Hz), 28.0, 27.4 (d, *J* = 3.0 Hz).

High resolution mass spectroscopy (ESI): $(M+H)^+$: calculated for $C_{13}H_{20}O_4PS$: 303.0814; found 303.0818 (100%). $(M+Na)^+$ calculated for $C_{13}H_{19}O_4NaPS$: 325.0634; found 325.0636 (40%).

IR: 3025, 2931, 1691, 946, 698



1-sulfanyl-4-phenylbutan-2-ylphosphonic acid (**8b**):

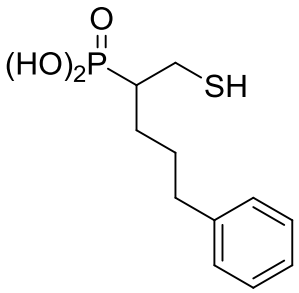
Diethyl 1-sulfanyl-4-phenylbutan-2-ylphosphonate **5b** (1 eq, 0.14 mmol, 0.043g) and TMSBr (3 eq, 0.43 mmol, 0.065g) gave **8b** (0.13 mmol, 0.033 g, 96%) as an orange oil.

^{1}H NMR (400 MHz, Methanol-d4) δ 7.34 – 7.07 (m, 5H), 2.97 (ddd, *J* = 17.8, 13.8, 4.5 Hz, 1H), 2.79 (tt, *J* = 7.4, 3.6 Hz, 2H), 2.68 (ddd, *J* = 13.9, 9.9, 7.8 Hz, 1H), 2.13 – 1.98 (m, 2H), 1.97 – 1.81 (m, 1H).

^{13}C NMR (101 MHz, Methanol-d4) δ 141.7, 128.1, 128.0, 125.5, 33.1 (d, *J* = 7.6 Hz), 28.9 (d, *J* = 2.4 Hz), 22.9.

High resolution mass spectroscopy (ESI): $(M+H)^+$: calculated for $C_{10}H_{16}O_3PS$: 247.0552; found 247.0552 (100%).

IR: 3025, 2929, 2863, 1453, 1132, 932, 697



1-sulfanyl-5-phenylpentan-2-ylphosphonic acid (**8c**):

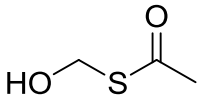
Diethyl 1-sulfanyl-5-phenylpentan-2-ylphosphonate **5c** (1 eq, 0.05 mmol, 0.015g) and TMSBr (3 eq, 0.14 mmol, 0.022g) gave **8b** (0.04 mmol, 0.011 g, 90%) as an orange oil.

^{1}H NMR (400 MHz, Methanol-d4) δ 7.29 – 7.17 (m, 5H), 2.93 (ddd, *J* = 17.8, 13.6, 3.9 Hz, 1H), 2.70 – 2.57 (m, 3H), 1.97 – 1.69 (m, 5H).
^{13}C NMR (101 MHz, Methanol-d4) δ 142.1, 128.0, 127.9, 125.3, 71.8, 40.3 (d, *J* = 135.5 Hz), 35.5, 29.0 (d, *J* = 7.5 Hz), 26.6 (d, *J* = 3.0 Hz), 23.0 (d, *J* = 1.4 Hz).

High resolution mass spectroscopy (ESI): $(M+Na)^+$: calculated for $C_{11}H_{17}O_3NaPS$: 283.0528; found 283.0532 (100%). $(M+H)^+$: calculated for $C_{11}H_{18}O_3PS$: 261.0709; found 261.0712 (20%)

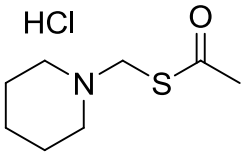
IR: 3023, 2927, 1119, 930, 696

5.1.7 Preparation of Starting Materials



S-hydroxymethyl ethanethioate (**13**):

Thioacetic acid **12 (**1 eq, 0.17 mol, 5 g) and *n*-paraformaldehyde **11** (1 eq, 0.17 mol, 12.7 g) gave **13** (0.14 mol, 14.68 g, 84%) as a colorless oil. The spectra was in accordance with literature.[66]



Piperidinomethyl thioacetate hydrochloride (**16**):

Piperidine **15 (**1.2 eq, 0.8 mmol, 1.56 g**)** and hydroxymethyl-acetyl-sulfide **14** (1 eq, 0.55 mmol, 1.95 g) gave **16** (0.15 mmol, 3.19 g, 82%) as a white powder. The spectra was in accordance with literature.[20]

6 References

[1] Lassaux, P.; Hamel, M.; Gulea, M.; Delbrück, H.; Mercuri, P. S.; Horsfall, L.; Dehareng, D.; Kupper, M.; Frère, J.-M.; Hoffmann, K.; Galleni, M.; Bebrone, C., *Journal of Medicinal Chemistry*, (2010) **53**, 4862.

[2] Mollard, C.; Moali, C.; Papamicael, C.; Damblon, C.; Vessilier, S.; Amicosante, G.; Schofield, C. J.; Galleni, M.; Frère, J.-M.; Roberts, G. C. K., *Journal of Biological Chemistry*, (2001) **276**, 45015.

[3] Carfi, A.; Pares, S.; Duee, E.; Galleni, M.; Duez, C.; Frere, J. M.; Dideberg, O., *E M B O. J.*, (1995) **14**, 4914.

[4] Ambler, R. P., *Phil. Trans. R. Soc. Lond.*, (1980), 321.

[5] Borra, P. S.; Leiros, H.-K. S.; Ahmad, R.; Spencer, J.; Leiros, I.; Walsh, T. R.; Sundsfjord, A.; Samuelsen, Ø., *Journal of Molecular Biology*, (2011) **411**, 174.

[6] Garau, G.; Garcia-Saez, I.; Bebrone, C.; Anne, C.; Mercuri, P.; Galleni, M.; Frere, J.-M.; Dideberg, O., *Antimicrob. Agents. Chemother*, (2004) **48**, 2347.

[7] Oelschlaeger, P.; Ai, N., DuPrez, K. T.; ; Welsh, W. J.; Toney, J. H. J., *Med. Chem*, (2010) **53**, 3013.

[8] Wang, Z.; Fast, W.; Benkovic, S. J., *Biochemistry*, (1999) **38**, 100013.

[9] Lauretti, L.; Riccio, M. L.; Mazzariol, A.; Cornaglia, G.; Amicosante, G.; Fontana, R.; Rossolini, G. M., *Chemother*, (1999) **43**, 1584.

[10] T. R. Walsh; M. A. Toleman; L. Poirel; Nordmann, P., *Clinical Microbiology Reviews*, (2005), 306.

[11] Lagatolla, C.; Tonin, C.; Monti-Bragadin E. A.; Dolzani, L.; Gombac, F.; Bearzi, C.; Edalucci, E.; Gionechetti, E.; Rossolini, G. M., *Emerg. Infect. Dis.*, (2004) **10**, 535.

[12] Minond, D.; Saldanha, S. A.; Subramaniam, P.; Spaargaren, M.; Spicer, T.; Fotsing, J. R.; Weide, T.; Fokin, V. V.; Sharpless, K. B.; Galleni, M.; Bebrone, C.; Lassaux, P.; Hodder, P., *Bioorganic & Medicinal Chemistry*, (2009) **17**, 5027.

[13] Weide, T.; Saldanha, S. A.; Minond, D.; Spicer, T. P.; Fotsing, J. R.; Spaargaren, M.; Frère, J.-M.; Bebrone, C.; Sharpless, K. B.; Hodder, P. S.; Fokin, V. V., *ACS Medicinal Chemistry Letters*, (2010) **1**, 150.

[14] Jin, W.; Arakawa, Y.; Yasuzawa, H.; Taki, T.; Hashiguchi, R.; Mitsutani, K.; Shoga, A.; Yamaguchi, Y.; Kurosaki, H.; Shibata, N.; Ohta, M.; Goto, M., *Biol. Pharm. Bull.*, (2004) **27**, 851.

[15] Yamauchi, M.; Katayama, S.; Watanabe, T., *Synthesis, Communications*, (1982), 935.

[16] Kürti, L.; Czako, B., *Strategic applications of named reactions in organic synthesis*, Hayhurst, J. Ed. Elsevier Academic Press: USA, (2005); p 758.

[17] Lewis, E. S.; Hamp, D., *The Journal of Organic Chemistry*, (1983) **48**, 2025.

[18] Bhattacharya, A. K.; Thyagarajan, G., *Chemical Reviews*, (1981) **81**, 415.

[19] Quin, L. D., *A guide to Organophosphorus Chemistry*, John Wiley & Sons: (2000).

[20] Smissman, E. E.; Sorenson, J. R. J.; Albrecht, W. A.; Creese, M. W., *The Journal of Organic Chemistry*, (1970) **35**,

[21] Mikolajczyk, M.; Balczewski, P., *Synthesis*, (1984), 691.

[22] Bugaev, I. M.; Prosenko, A. E., *Russian Chemical Bulletin, International Edition*, (2010) **59**, 861.

[23] Clayden, J.; Greeves, N.; Warren, S.; Wothers, P., Organic Chemistry, In Oxford University Press Inc: New York USA, (2001).

[24] Clark, R. D.; Kozar, L. G.; Heathcock, C. H., *Synthesis*, (1975) **10**,

[25] Rodriguez, M.; Heitz, A.; Martinez, J., *Tetrahedron Letters*, (1990) **31**, 7319.

[26] Stritzke, K.; Schulz, S.; Nishida, R., *European Journal of Organic Chemistry*, (2002) **2002**, 3884.

[27] Kirschleger, B.; Queignec, R., *Synthesis Communications*, (1986), 926.

[28] Parker, A. J., *Chemical Reviews*, (1969) **69**, 1.

[29] Smith, M. B., *Organic Synthesis*, (1994).

[30] Hurtado, M.; Yánez, M.; Herrero, R.; Guerrero, A.; Dávalos, J. Z.; Abboud, J.-L. M.; Khater, B.; Guillemin, J.-C., *Chemistry – A European Journal*, (2009) **15**, 4622.

[31] Itsuno, S., *Organic Reactions*, (1998) **52**, 395.

[32] Brown, H. C.; Yoon, N. M., *Journal of the American Chemical Society*, (1966) **88**, 1464.

[33] Gemal, A. L.; Luche, J. L., *Journal of the American Chemical Society*, (1981) **103**, 5454.

[34] Brown, H. C.; Narasimhan, S.; Choi, Y. M., *The Journal of Organic Chemistry*, (1982) **47**, 4702.

[35] Brown, H. C.; Krishnamurthy, S., *Aldrichimica Acta*, (1979) **12**, 3.

[36] Mitsunobu, O.; Yamada, M., *Bulletin of the Chemical Society of Japan*, (1967) **40**, 2380.

[37] Hughes, D. L., *Organic Reactions*, (1992) **42**, 335.

[38] Mitsunobu, O., *Synthesis*, (1981) **1**, 1.

[39] Grochowski, E.; Hilton, B. D.; Kupper, R. J.; Michejda, C. J., *Journal of the American Chemical Society*, (1982) **104**, 6876.

[40] Hughes, D. L.; Reamer, R. A.; Bergan, J. J.; Grabowski, E. J. J., *Journal of the American Chemical Society*, (1988) **110**, 6487.

[41] Camp, D.; Jenkins, I. D., *The Journal of Organic Chemistry*, (1989) **54**, 3045.

[42] Camp, D.; Jenkins, I. D., *The Journal of Organic Chemistry*, (1989) **54**, 3049.

[43] K. C. Kumara Swamy; Kumar, N. N. B.; Balaraman, E.; Kumar, K. V. P. P., *Chemical Reviews*, (2009) **109**, 2551.

[44] Amos, R. A.; Emblidge, R. W.; Havens, N., *The Journal of Organic Chemistry*, (1983) **48**, 3598.

[45] Arnold, L. D.; Assil, H. I.; Vederas, J. C., *Journal of the American Chemical Society*, (1989) **111**, 3973.

[46] Zheng, T.-C.; Burkart, M.; Richardson, D. E., *Tetrahedron Letters*, (1999) **40**, 603.

[47] Wallace, O. B.; Springer, D. M., *Tetrahedron Letters*, (1998) **39**, 2693.

[48] Rvasi, L.; Elis, P. H. T. N.; Photaki, I.; Ghelis, N., (1962) **85**, 1337.

[49] Gutierrez, A. J.; Prisbe, E. J.; Rohloff, J. C., *Nucleosides, Nucleotides and Nucleic Acids*, (2001) **20**, 1299.

[50] Hocková, D.; Holý, A.; Masojídková, M.; Keough, D. T.; Jersey, J.; Guddat, L. W., *Bioorganic & Medicinal Chemistry*, (2009) **17**, 6218.

[51] Gauvry, N.; Mortier, J., *Synthesis*, (2001) **4**,

[52] McKenna, C. E.; Schmidhuser, J., *Journal of the Chemical Society, Chemical Communications*, (1979), 739.

[53] Doyagüez, E. G., *Synlett*, (2005) **10**, 1636.

[54] Smissman, E. E.; Sorenson, J. R. J., *The Journal of Organic Chemistry*, (1965) **30**, 300.

[55] Hackelöer, K.; Schnakenburg, G.; Waldvogel, S. R., *European Journal of Organic Chemistry*, (2011) **2011**, 6314.

[56] Pretsch, E.; Bühlmann, P.; Affolter, C., *Structure Determination of Organic Compounds*, springer-verlag berlin heidelberg 2000: (2000).

[57] Zhang, N.; Casida, J. E., *Bioorganic & Medicinal Chemistry*, (2002) **10**, 1281.

[58] Biraboneye, A. C.; Madonna, S.; Laras, Y.; Krantic, S.; Maher, P.; Kraus, J.-L., *Journal of Medicinal Chemistry*, (2009) **52**, 4358.

[59] Feng, J.-C.; Liu, B.; Dai, L.; Yang, X.-L.; Tu, S.-J., *Synthetic Communications*, (2001) **31**, 1875.

[60] Bendle, M.; Huynh, K.; Haddow, M. F.; Manners, I., *Inorganic Chemistry*, (2011) **50**, 10292.

[61] Dia, R.-M.; Belaqziz, R.; Romane, A.; Antoniotti, S.; Duñach, E., *Tetrahedron Letters*, (2010) **51**, 2164.

[62] Zervas, L.; Photaki, I.; Ghelis, N., *Journal of the American Chemical Society*, (1963) **85**, 1337.

[63] Pachamuthu, K.; Zhu, X.; Schmidt, R. R., *The Journal of Organic Chemistry*, (2005) **70**, 3720.

[64] Braverman, S.; Cherkinsky, M.; Meridor, D.; Sprecher, M., *Tetrahedron*, (2010) **66**, 1925.

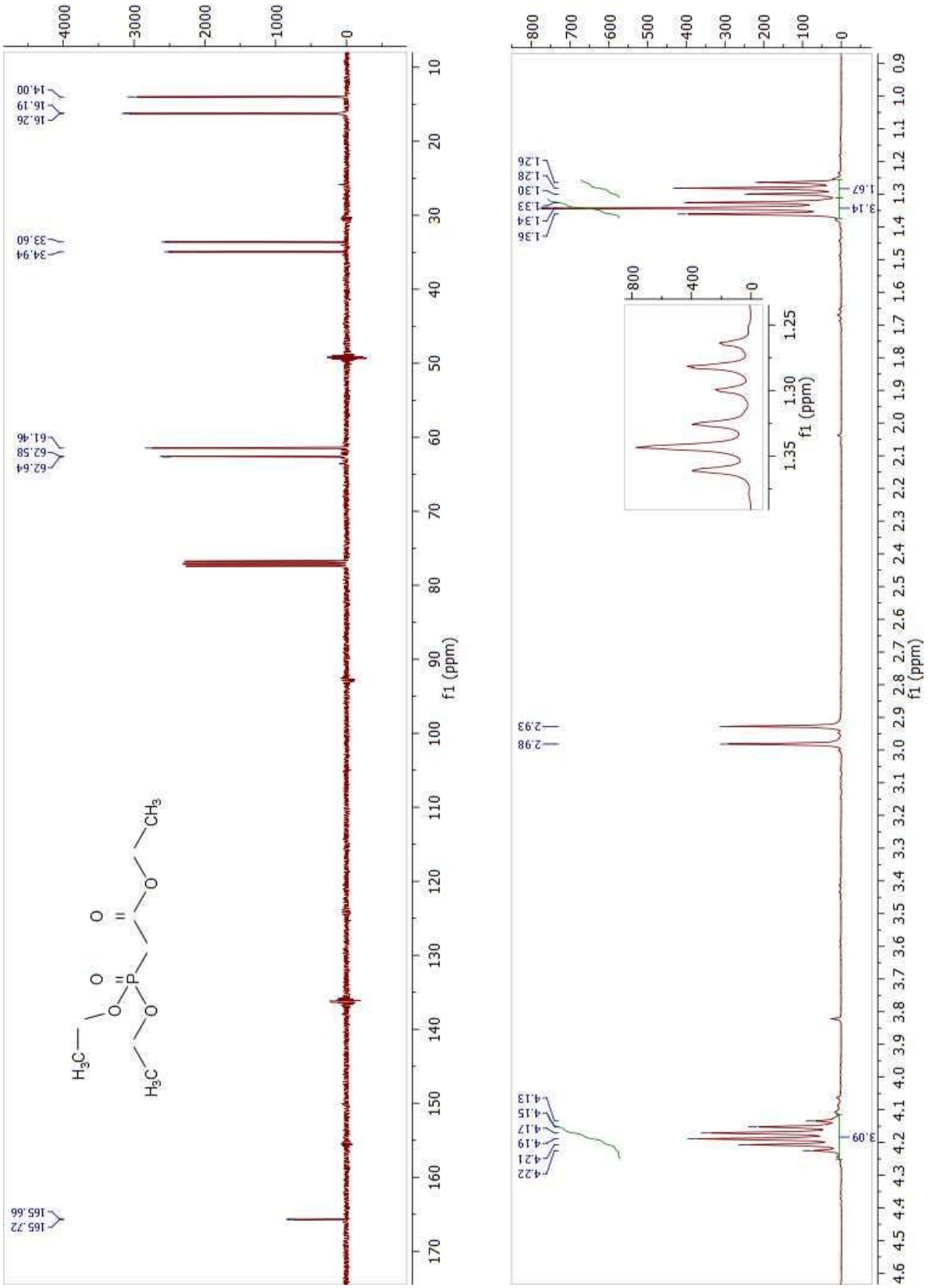
[65] Sharma, V.; Kelly, G. T.; Watanabe; H., C. M., *Organic Letters*, (2008) **10**, 4815.

[66] Böhme, H., *Liebigs Ann. Chem.*, (1959),

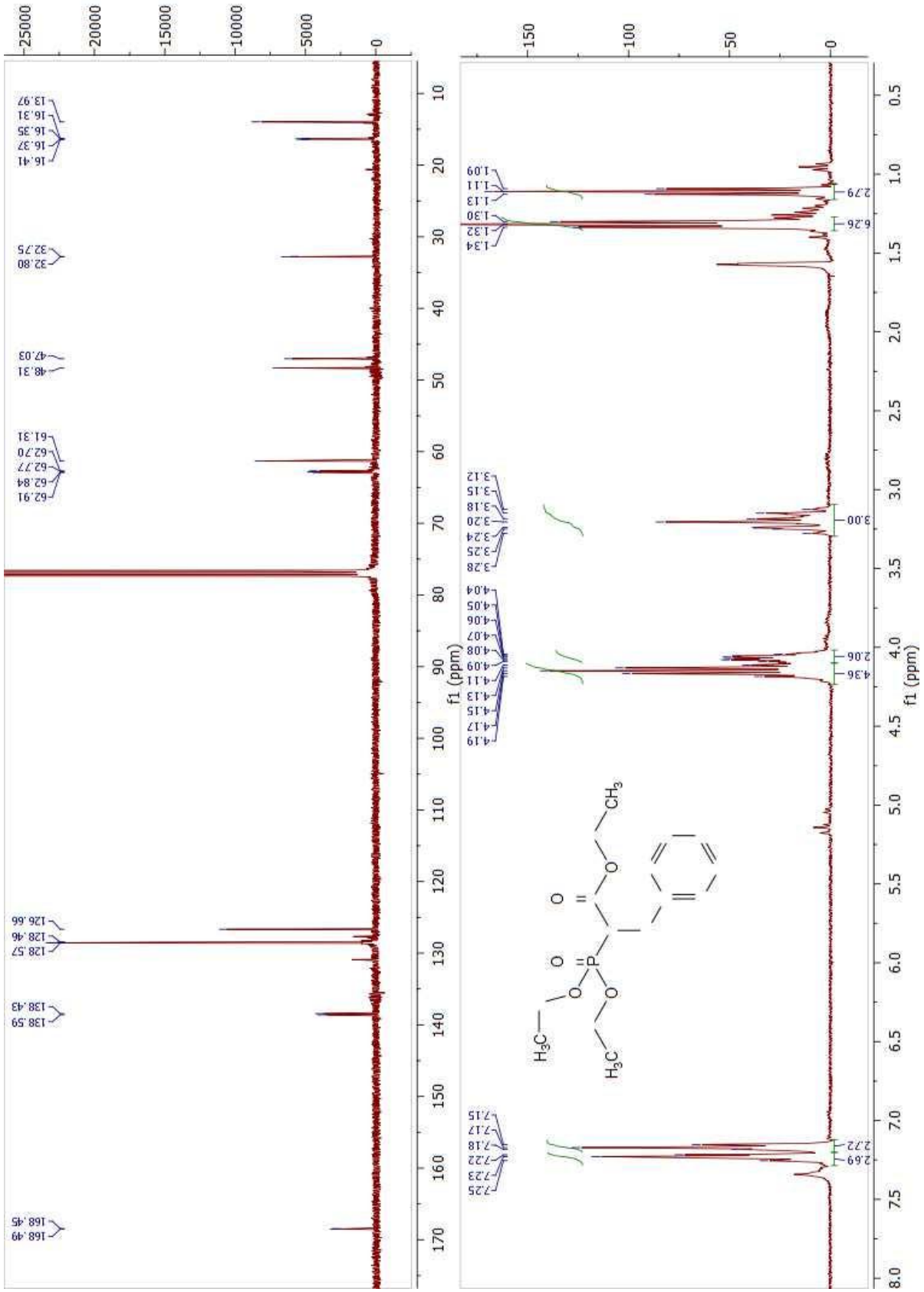
7 Appendices

7.1 NMR Spectra

1, Ethyl 2-(diethoxyphosphoryl)acetate



2a, Ethyl 2-(diethoxyphosphoryl)-3-phenylpropanoate



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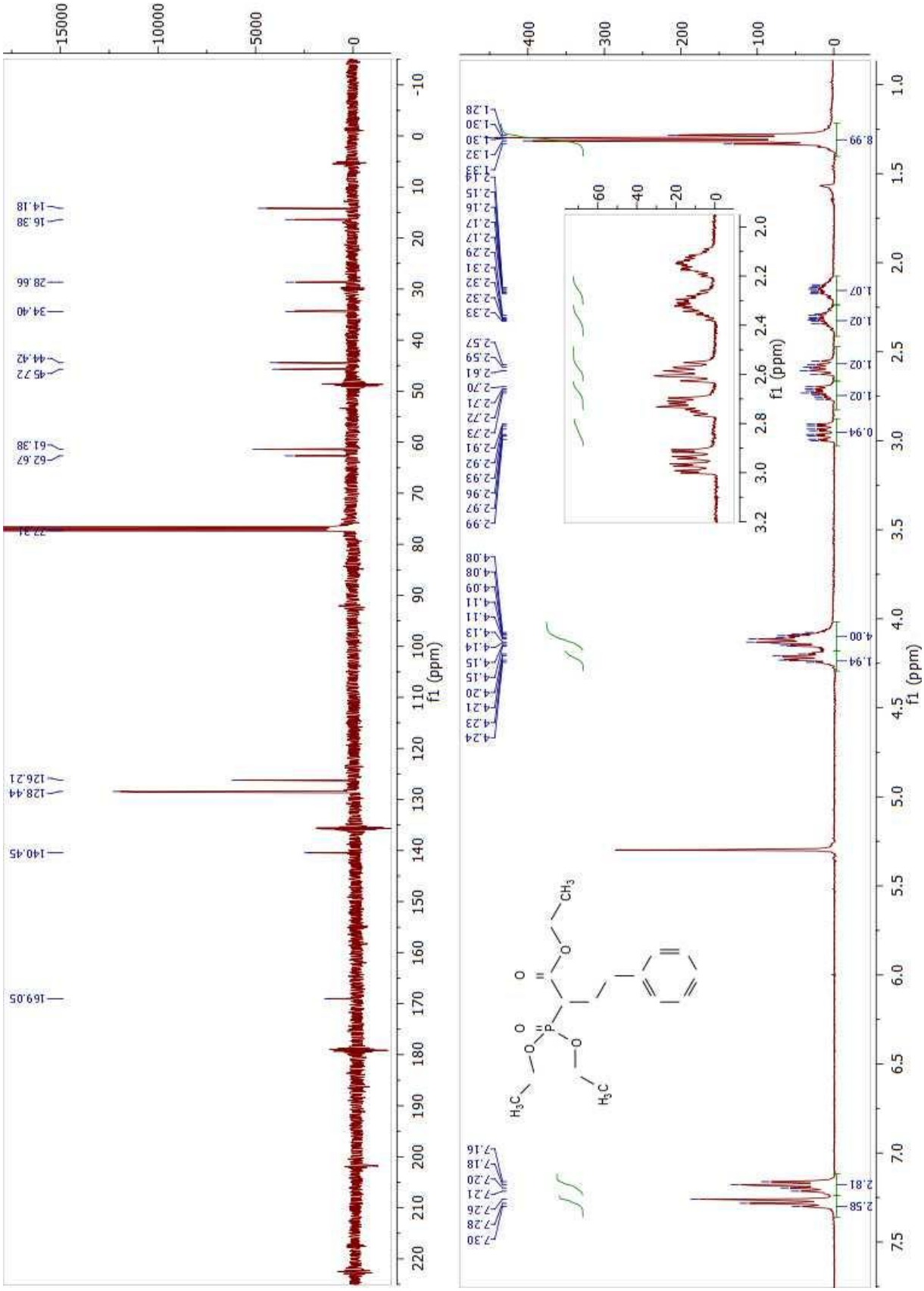

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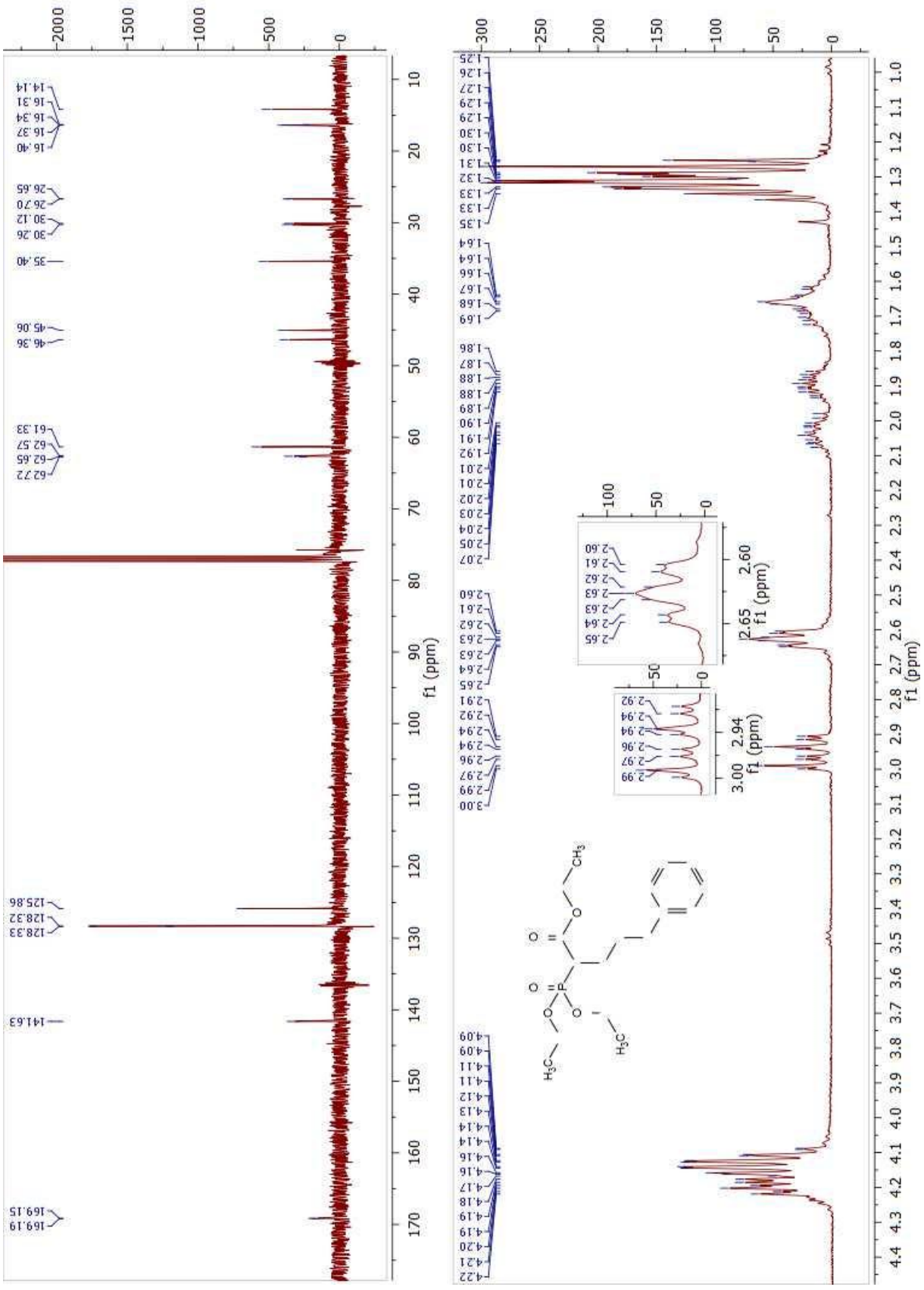

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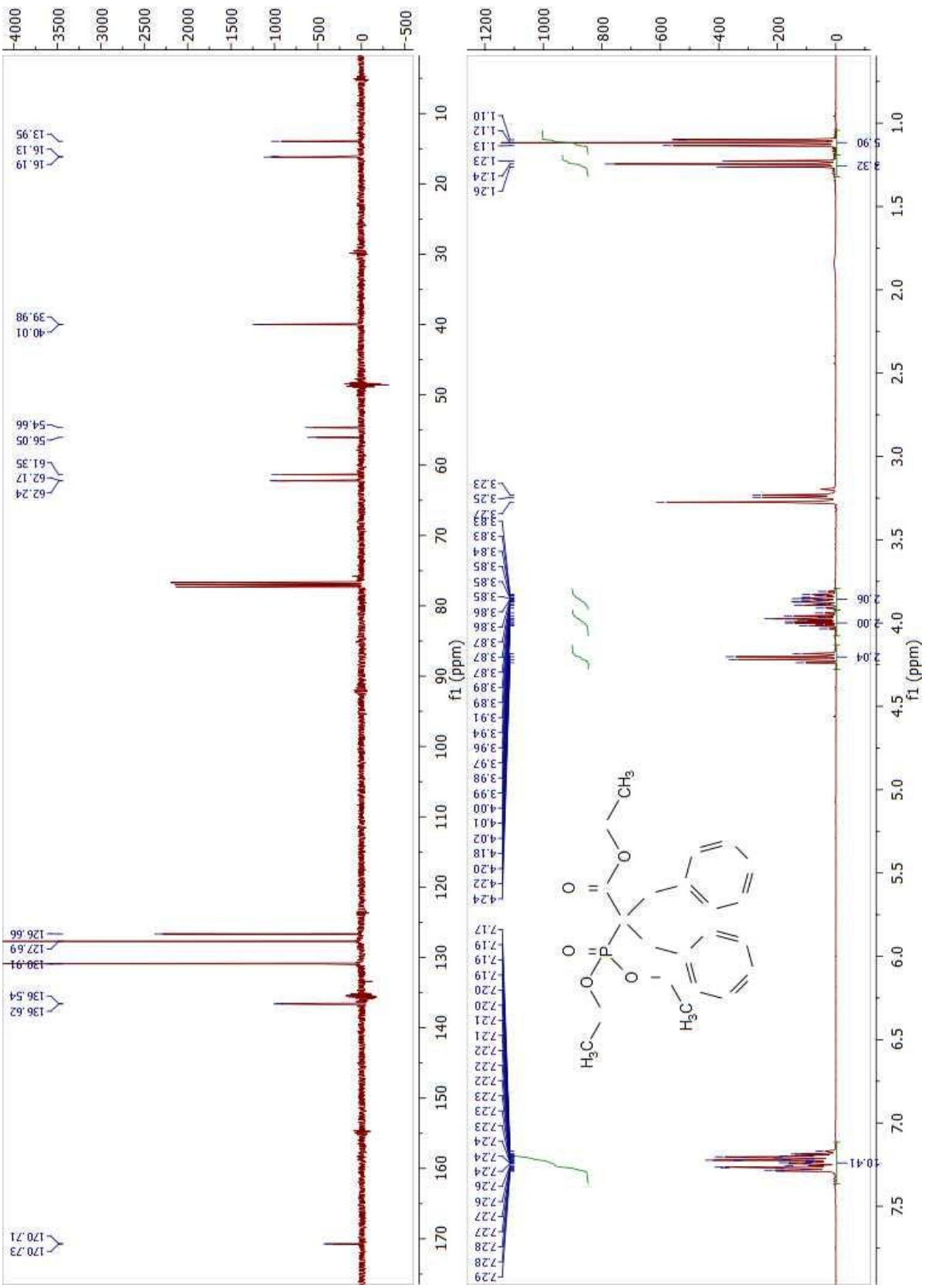
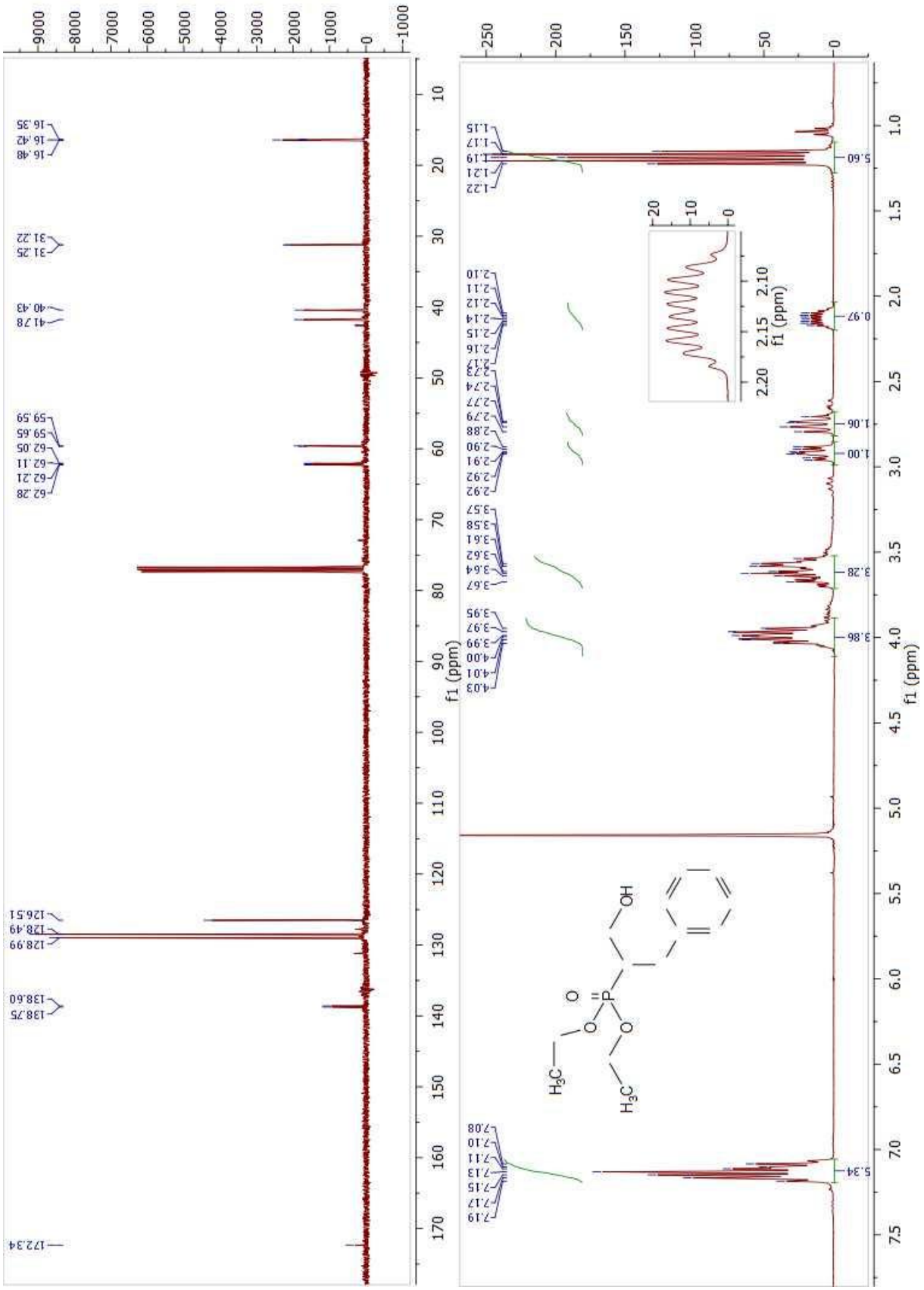


3a, Diethyl 1-hydroxy-3-phenylpropan-2-ylphosphonate



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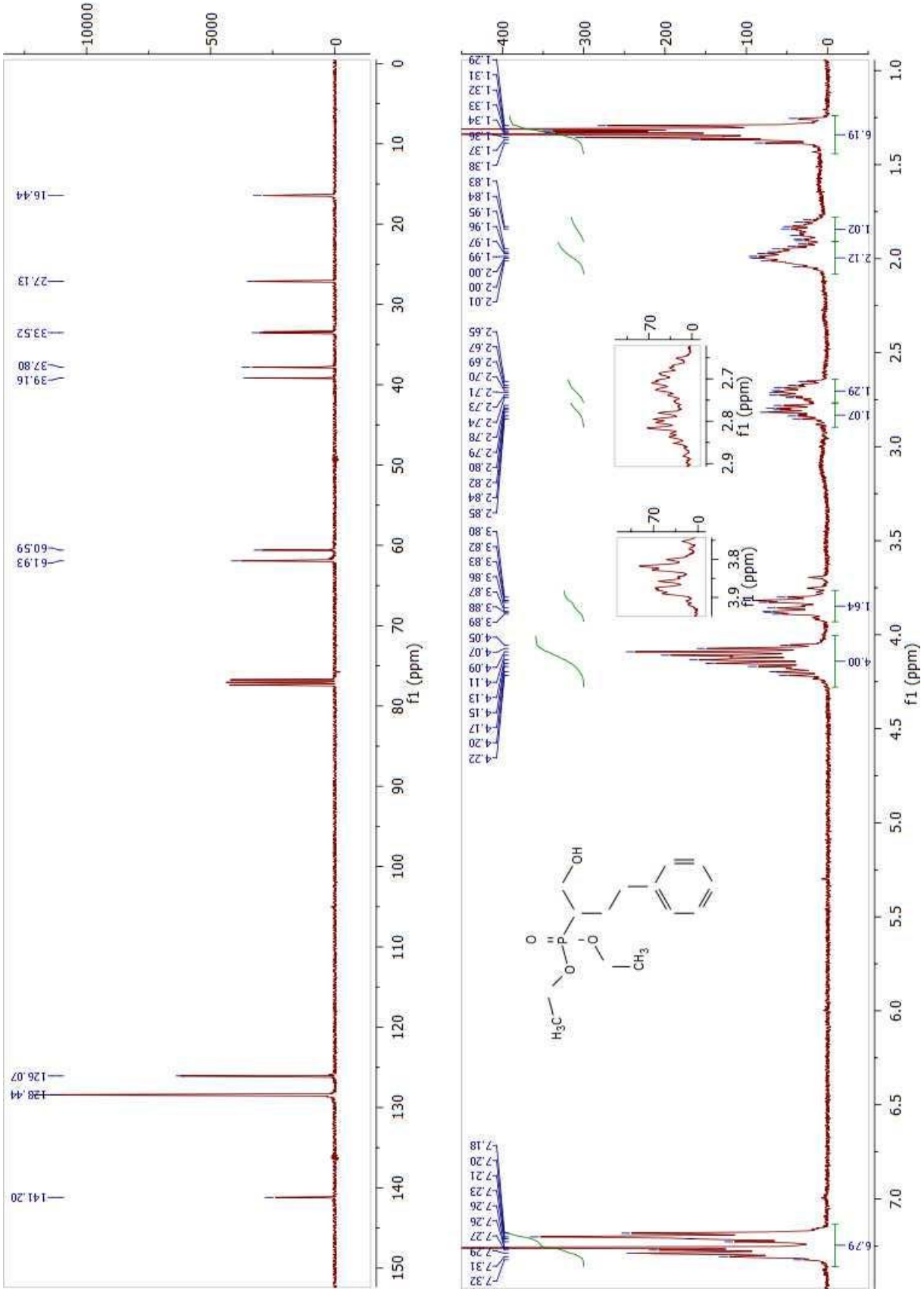

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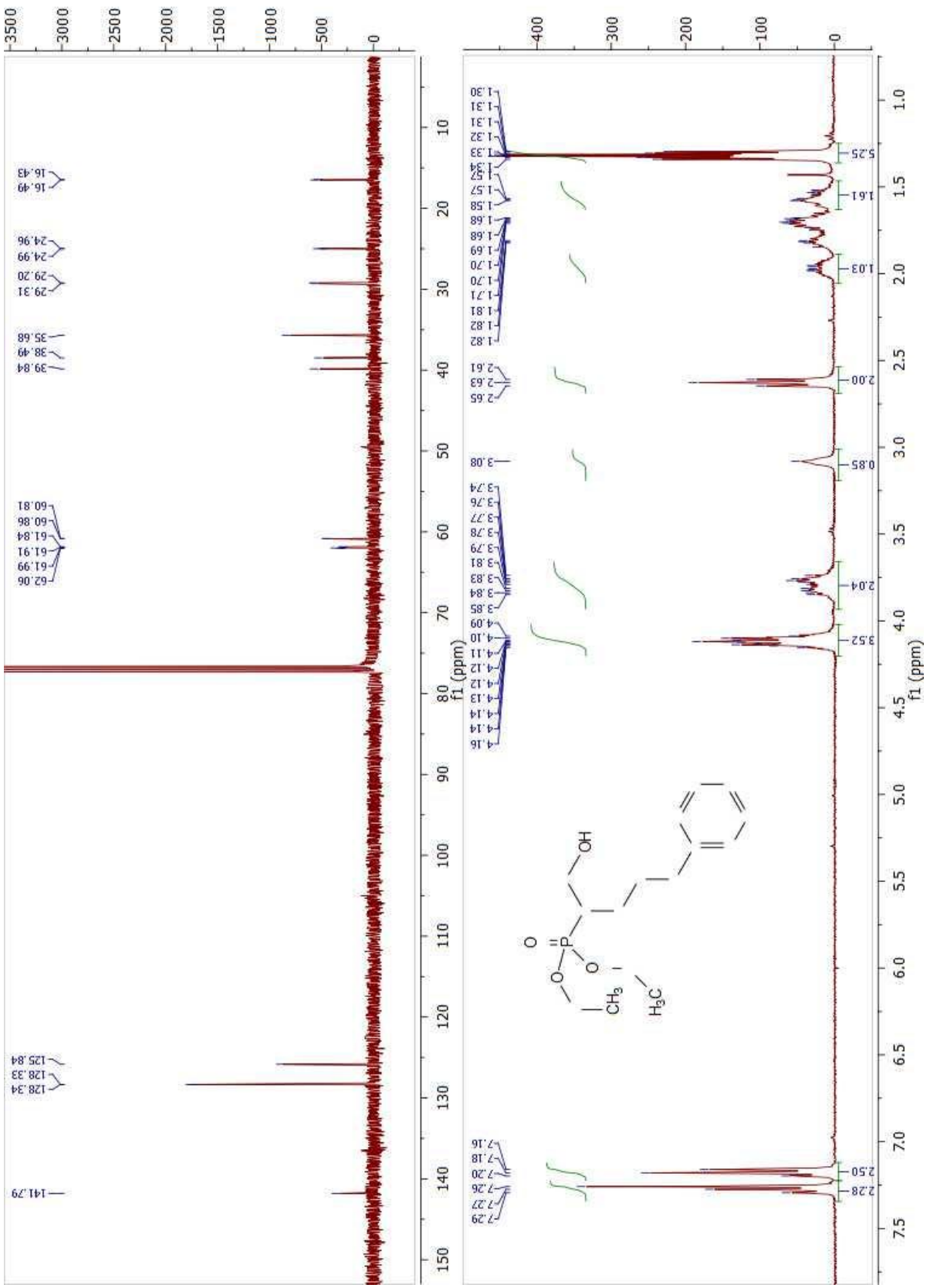

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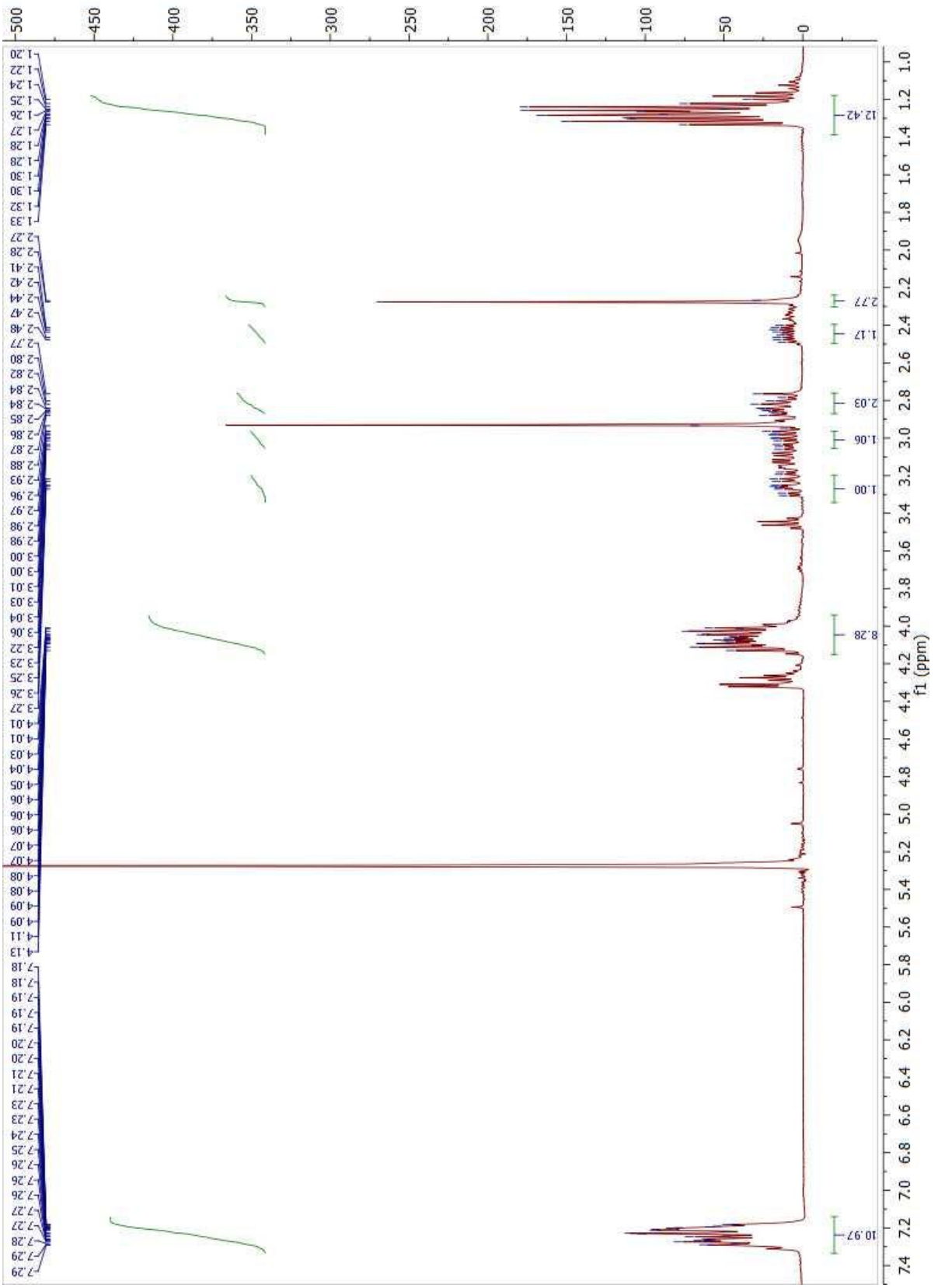

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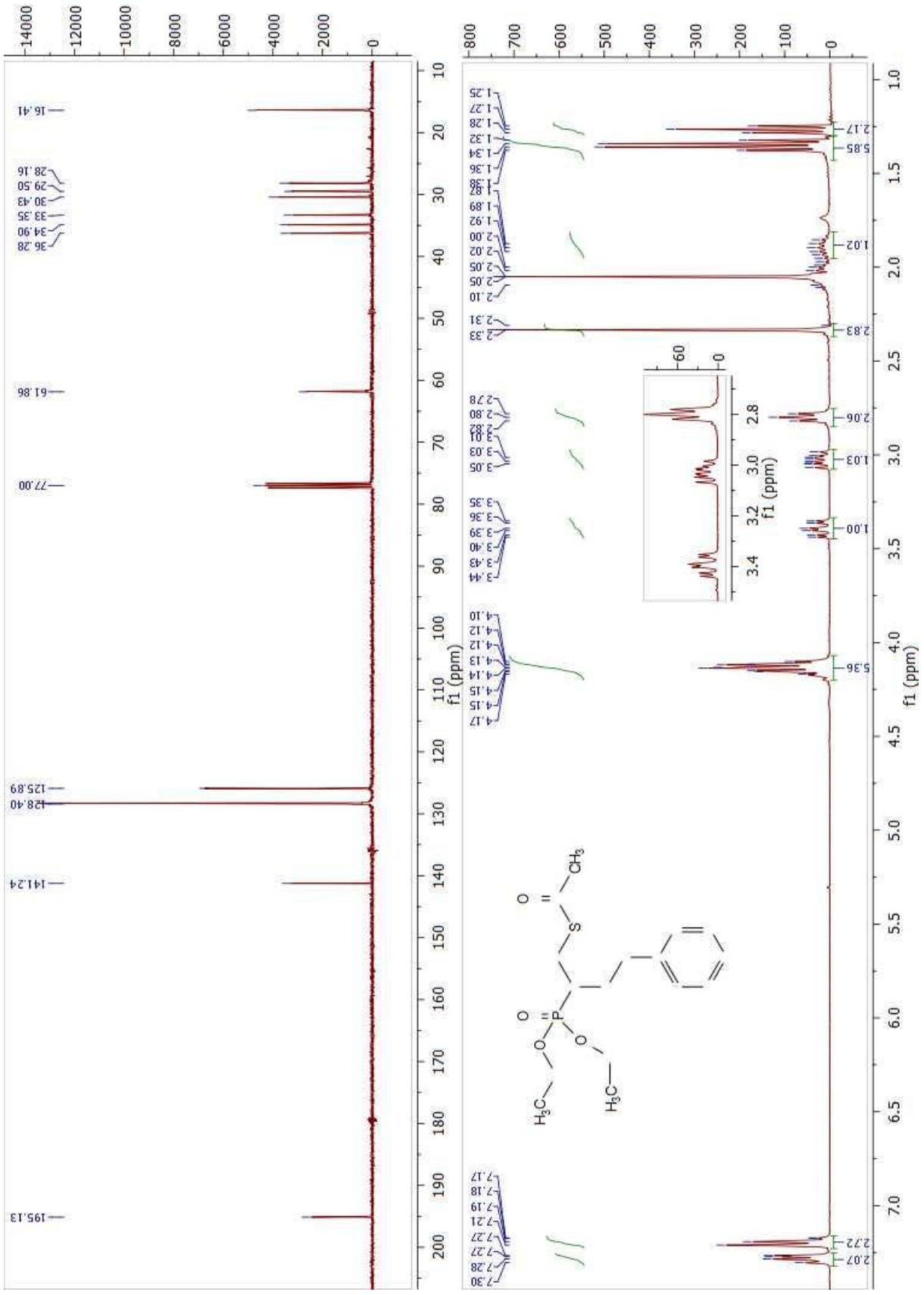

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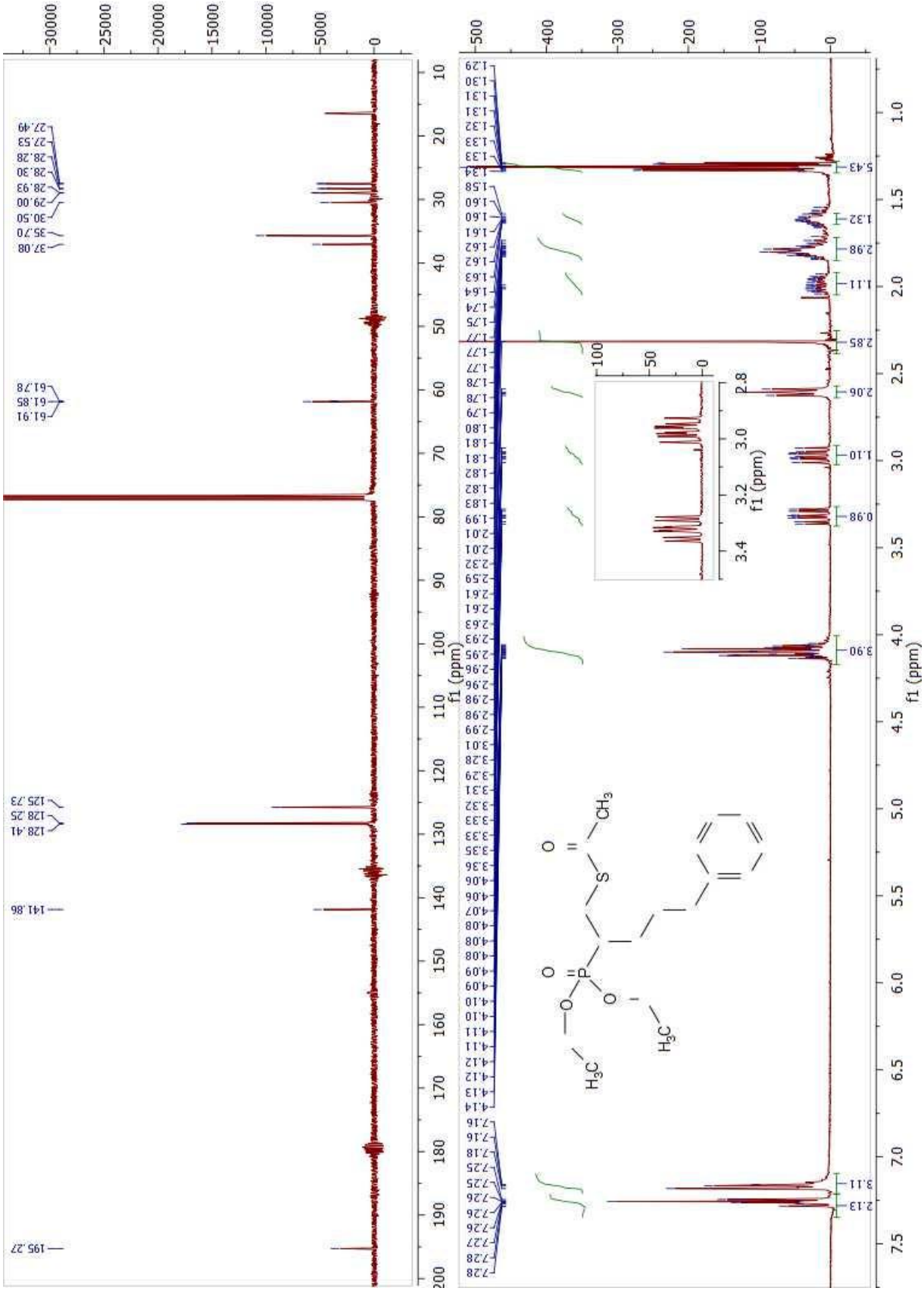

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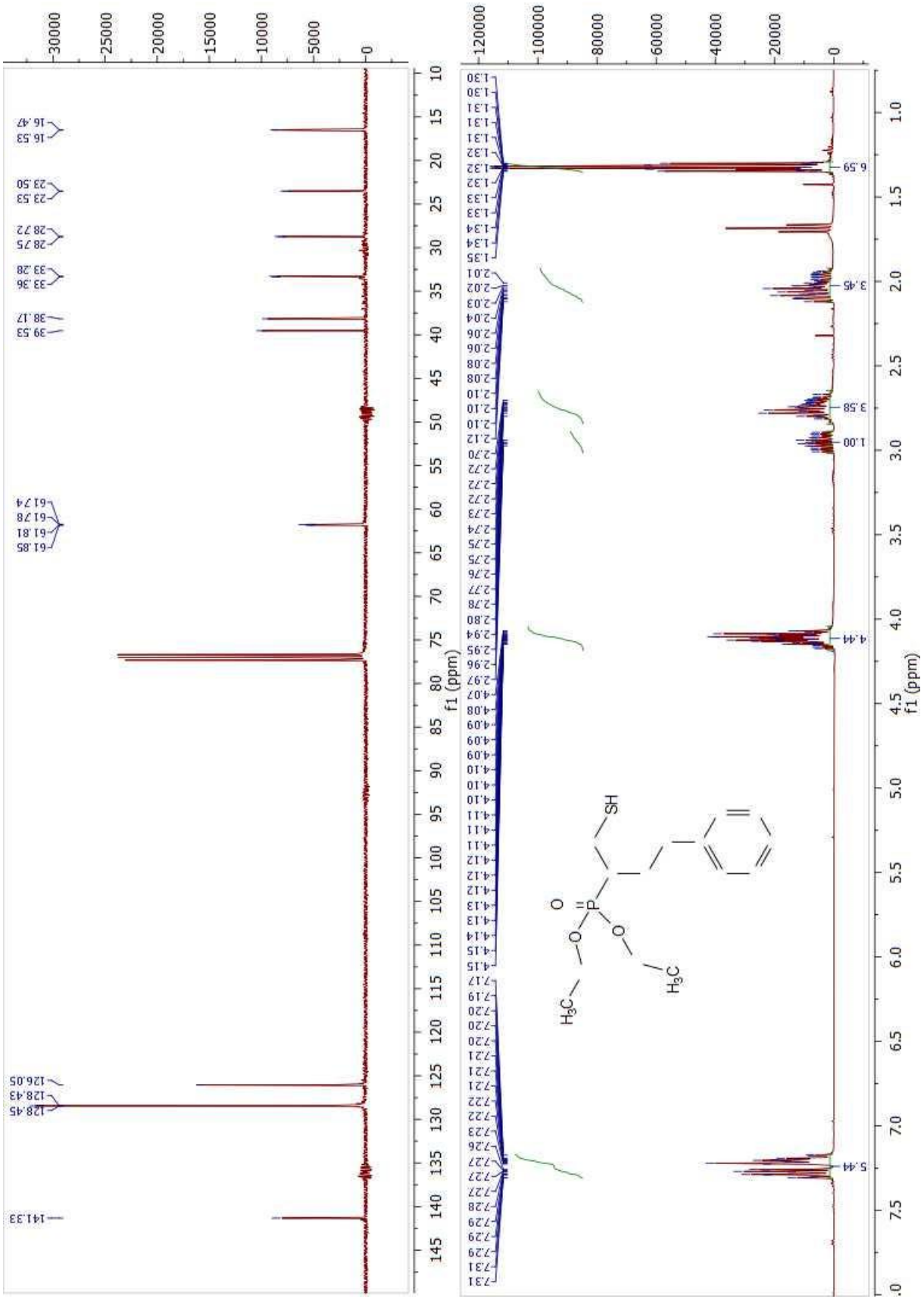

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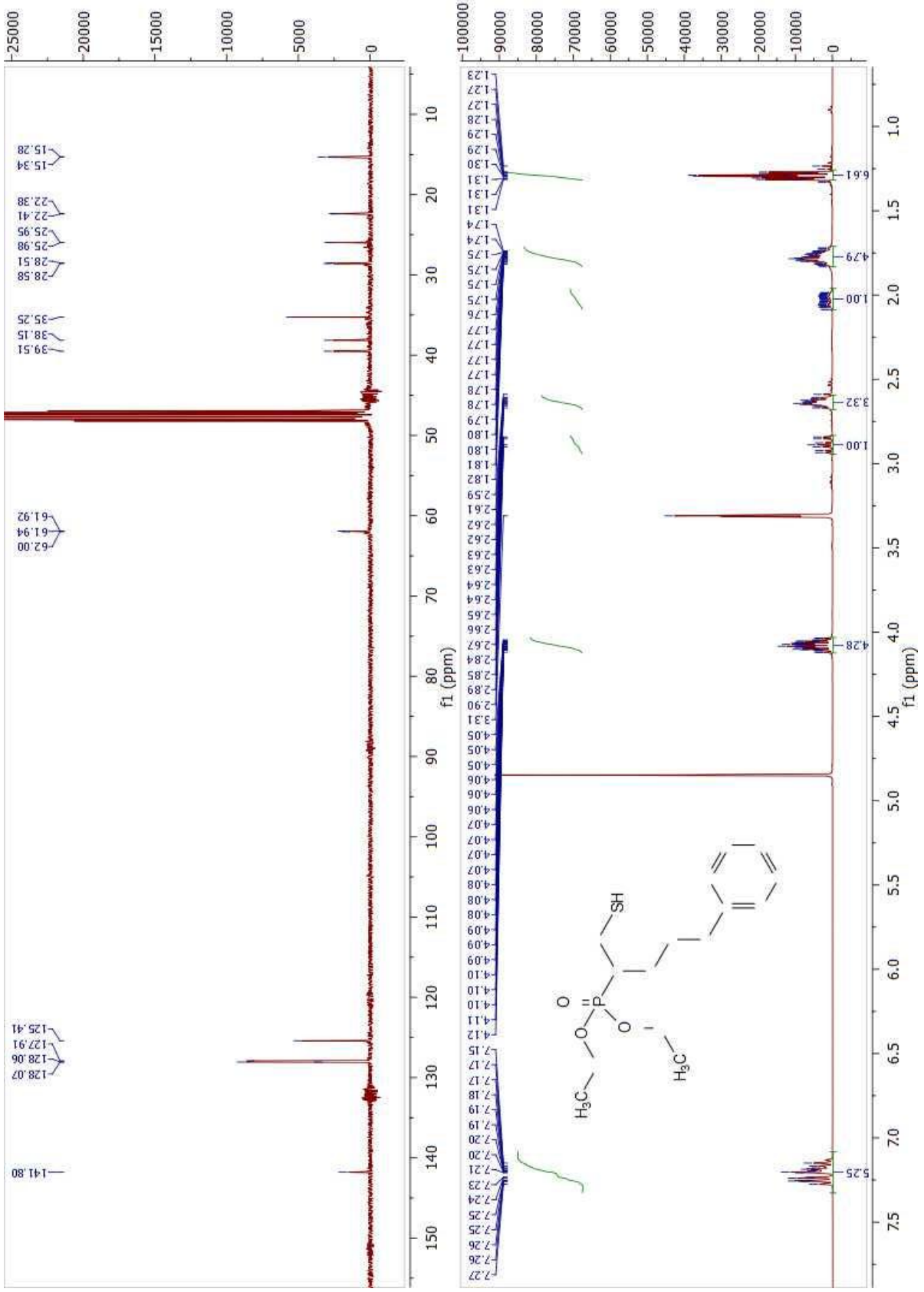
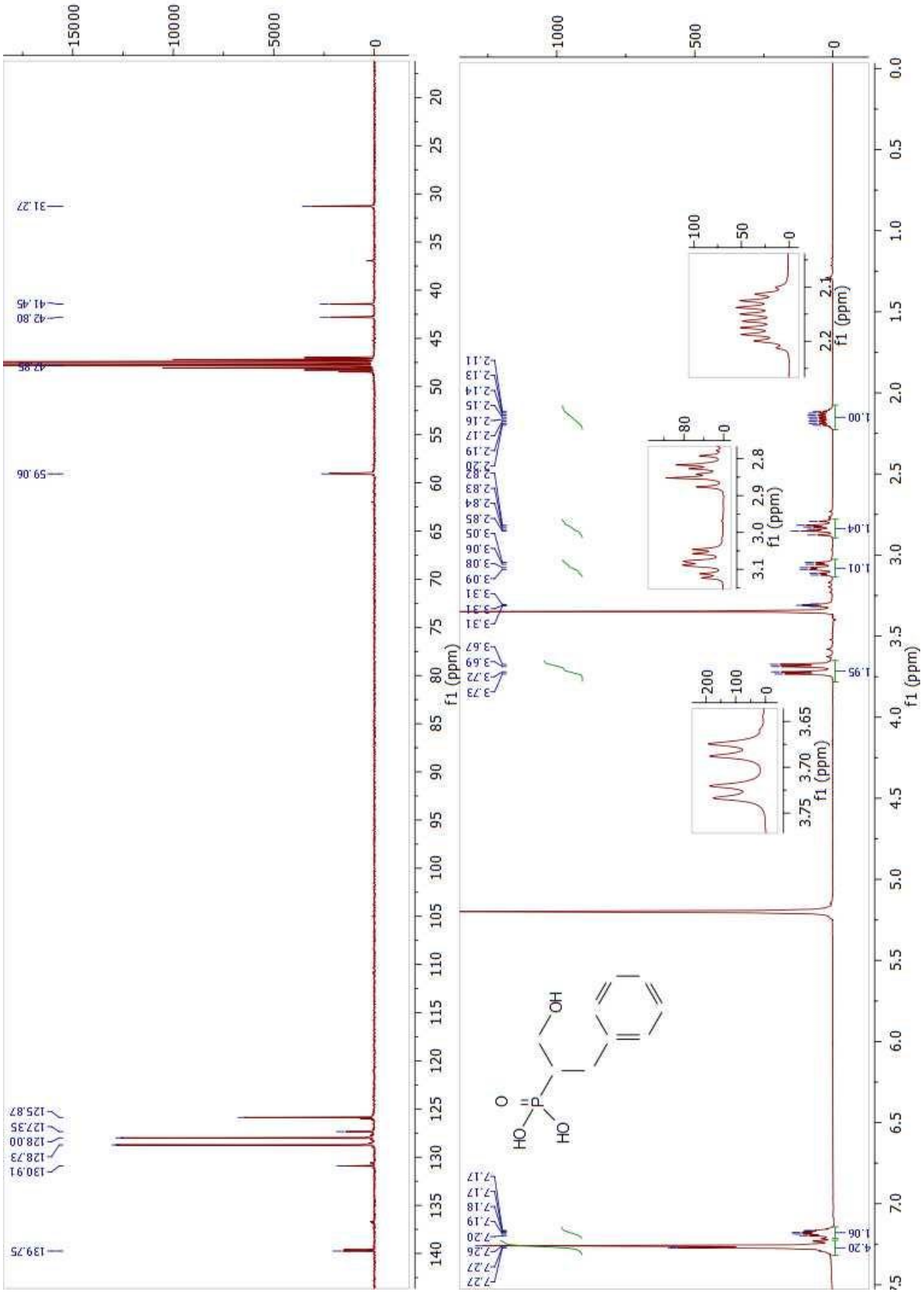


6a, 1-hydroxy-3-phenylpropan-2-ylphosphonic acid



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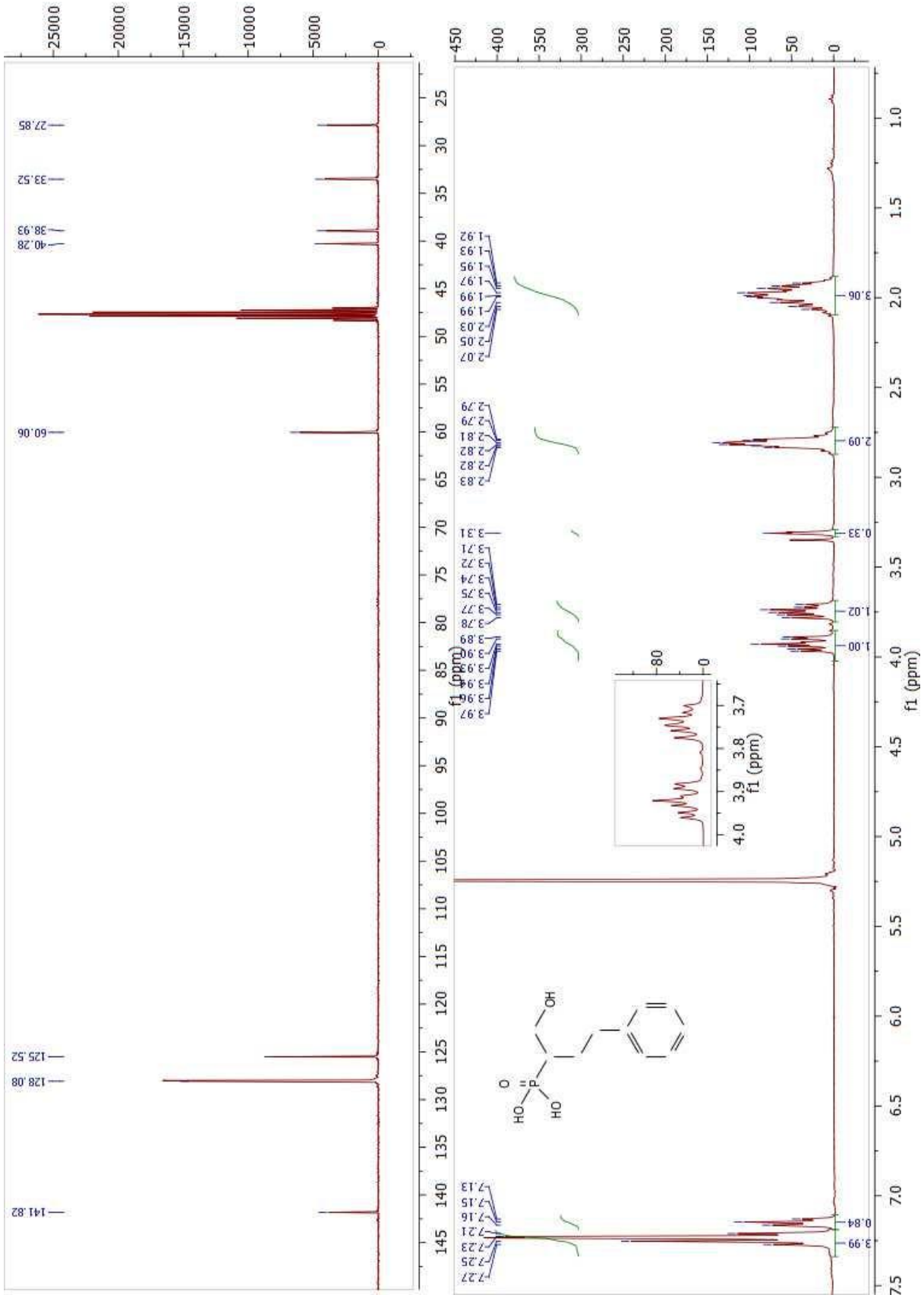

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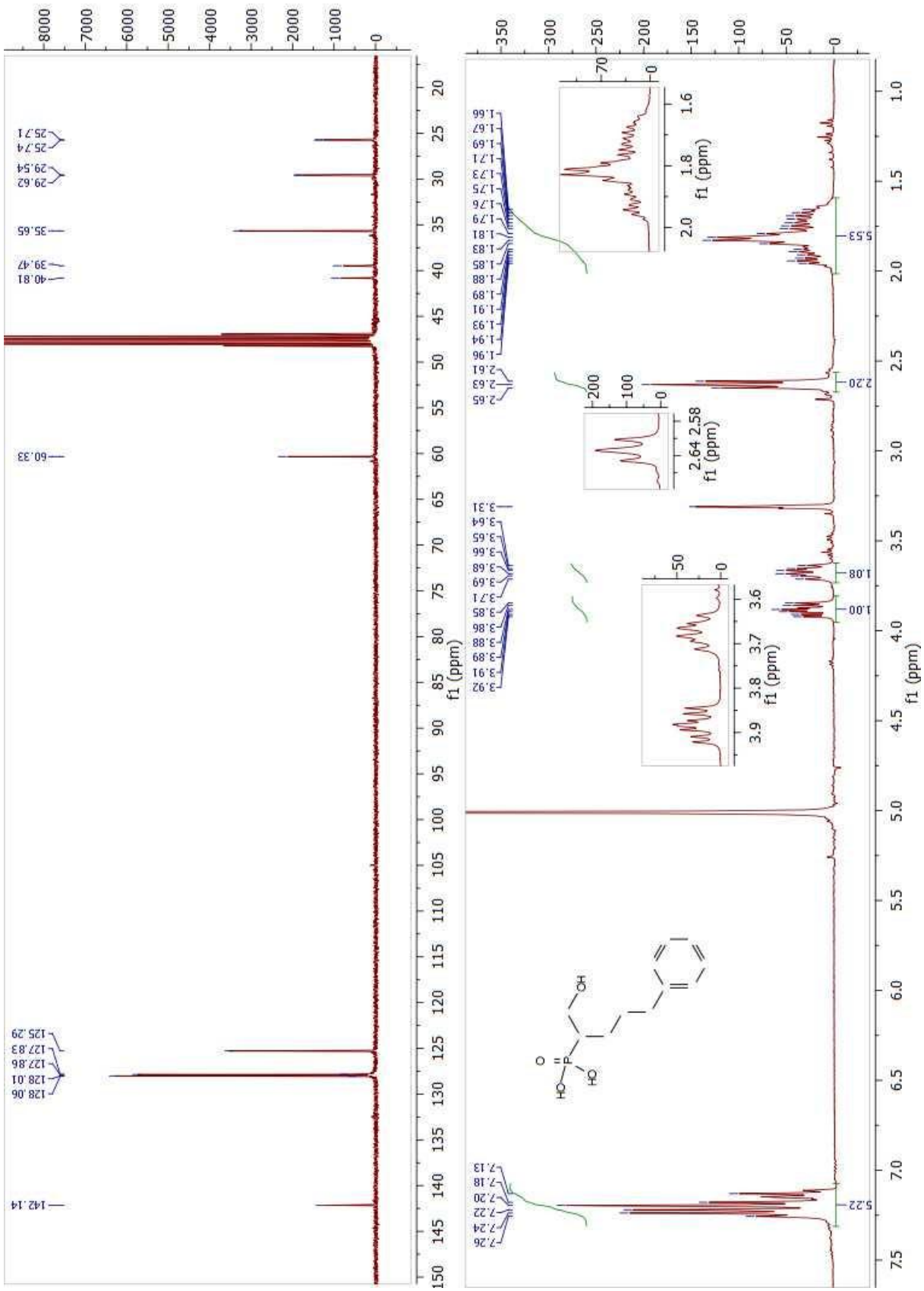
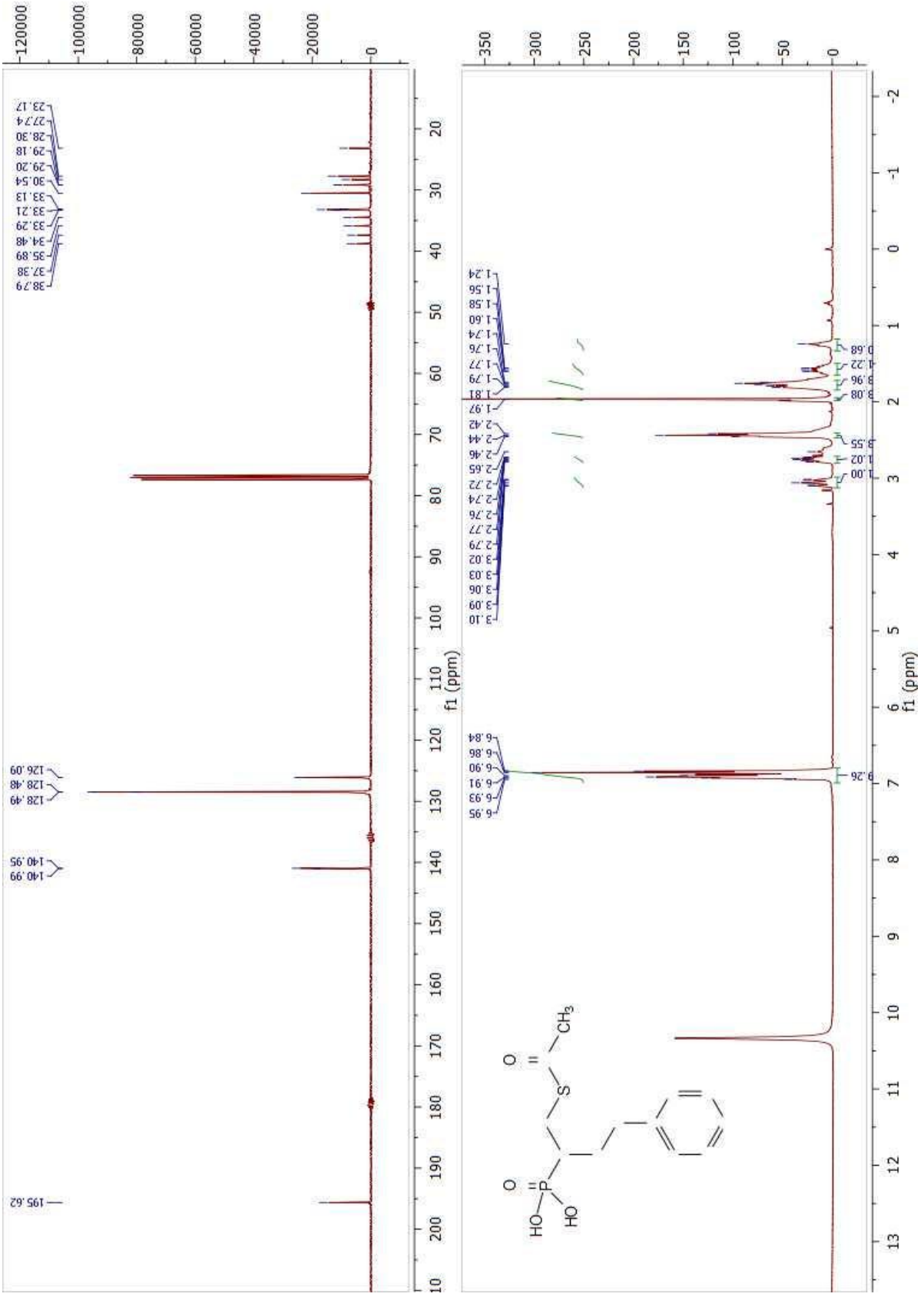


7b, 1-(acetylthio)-4-phenylbutane-2-ylphosphonic acid



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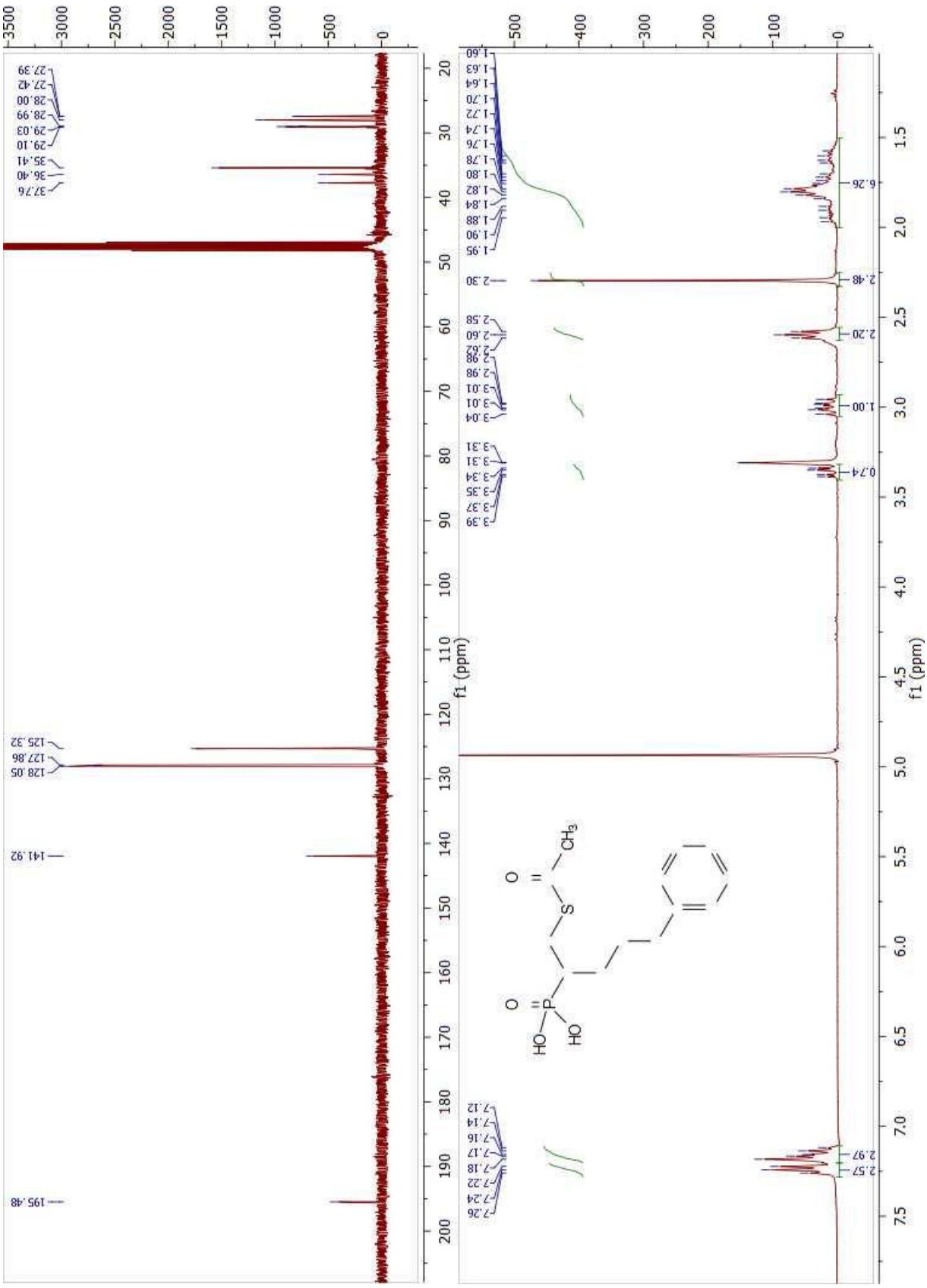

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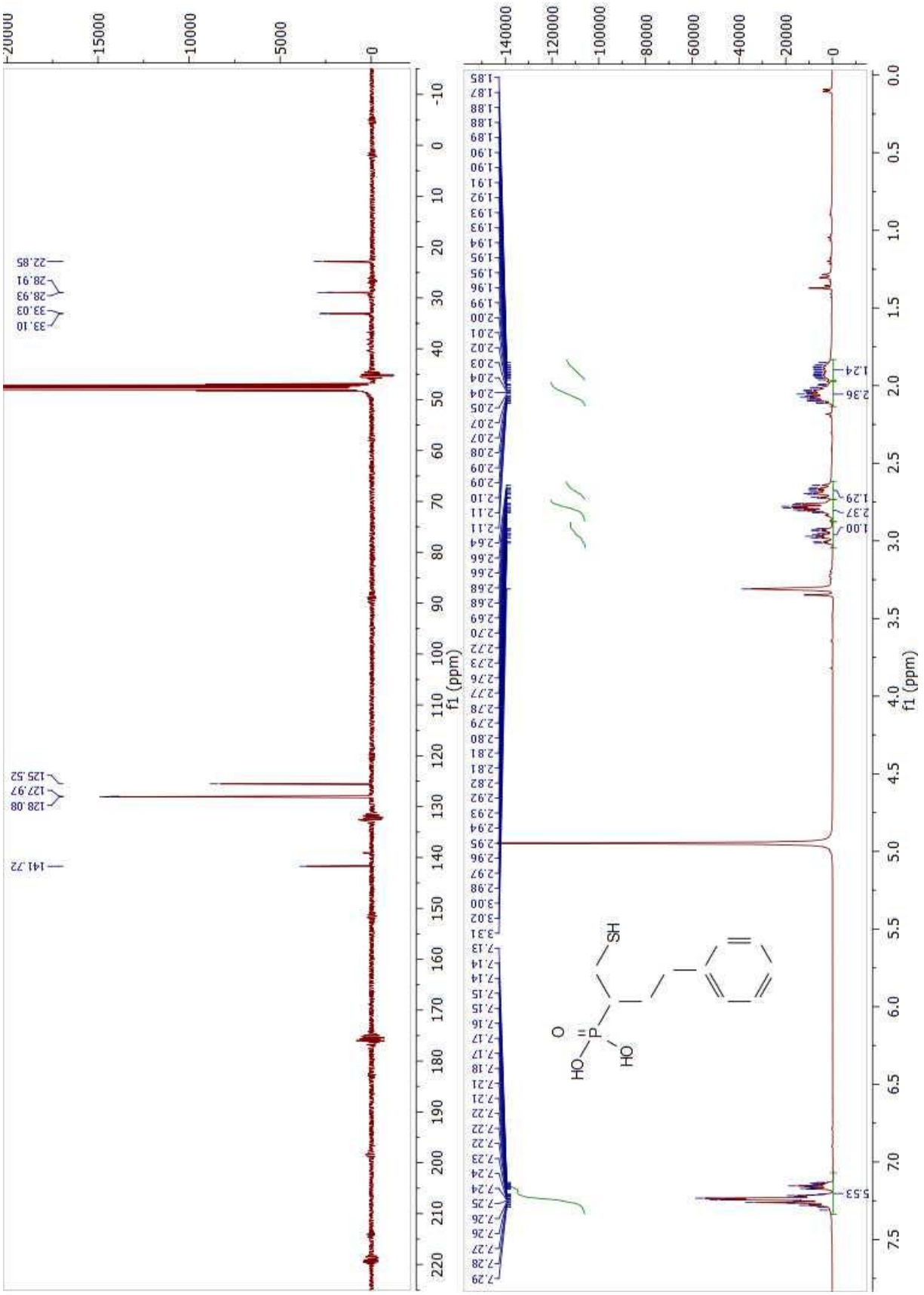

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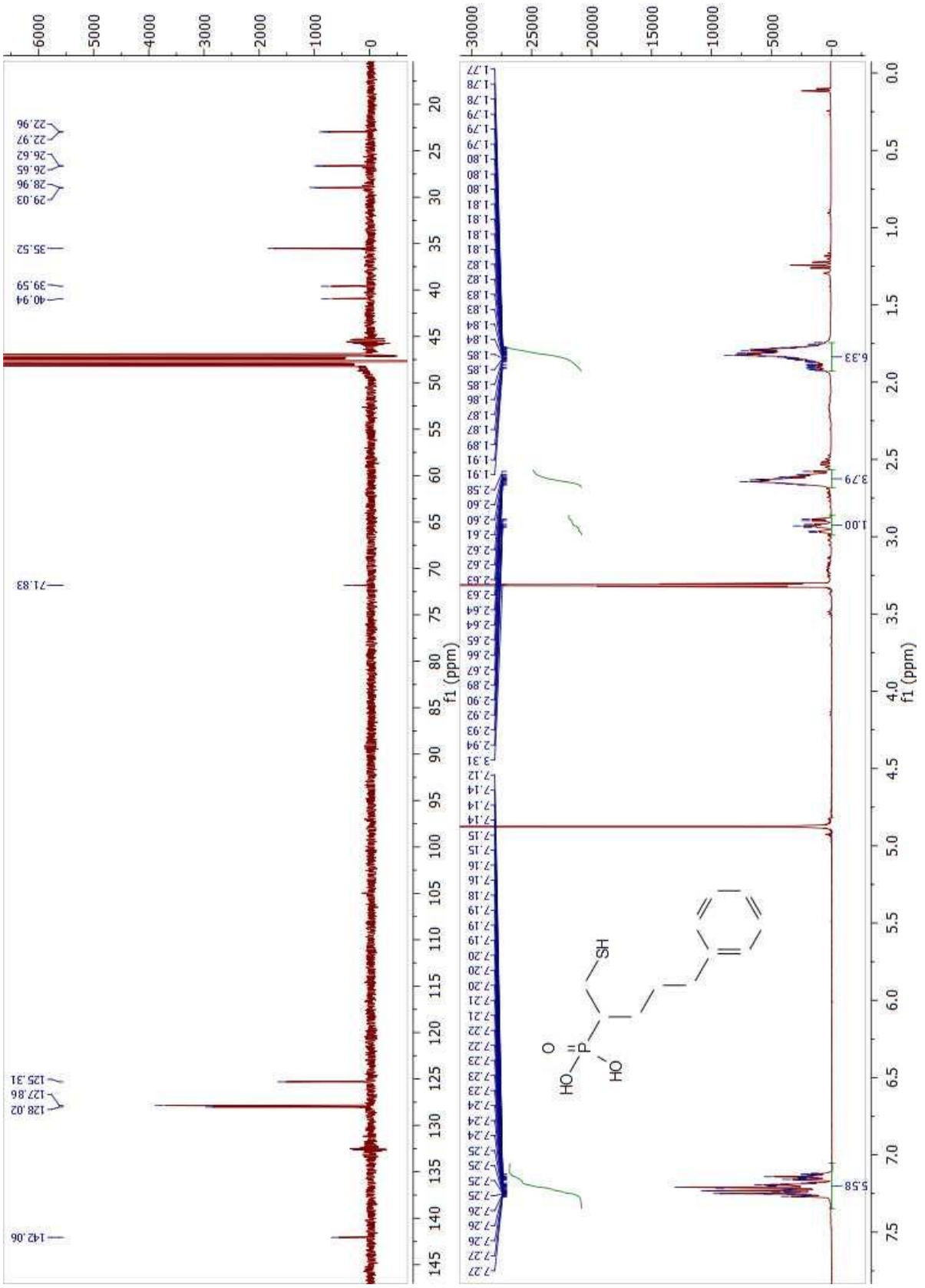

7.2 Mass Spectra, HR-MS

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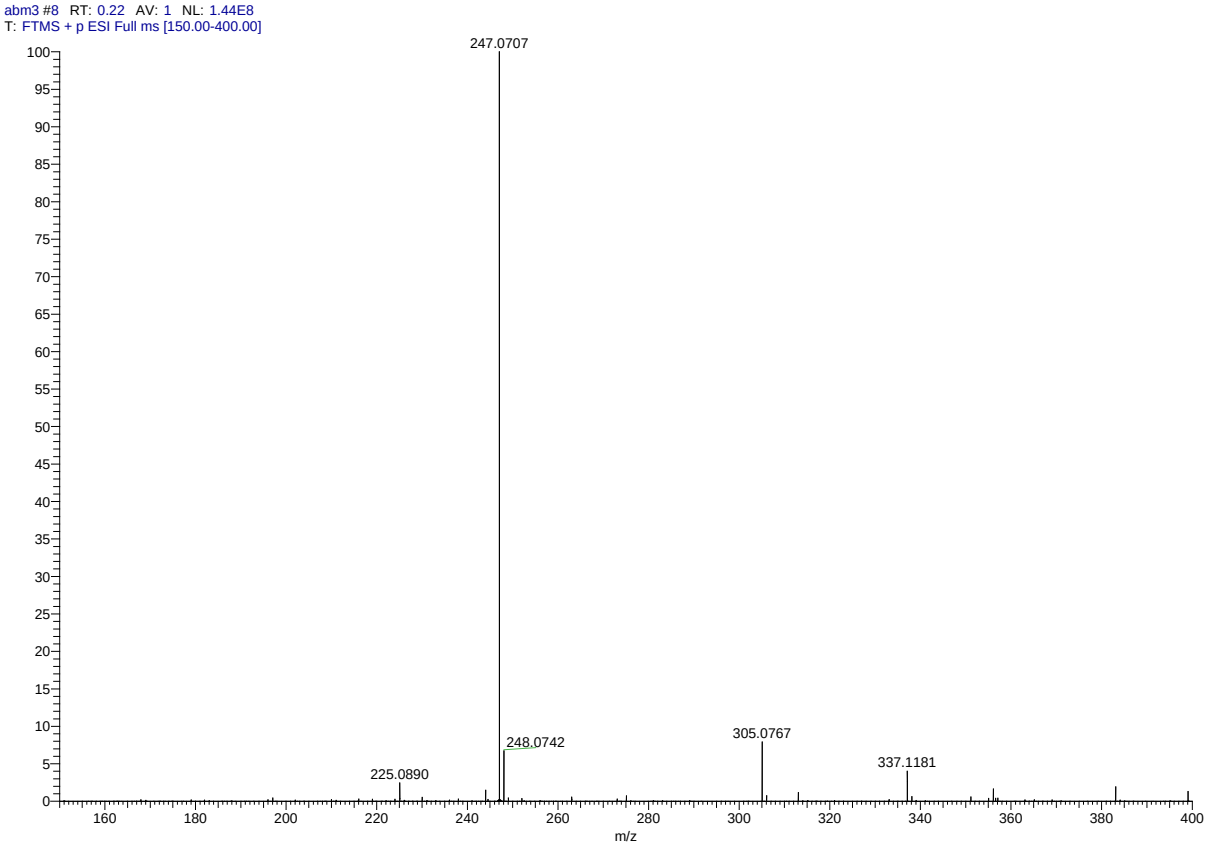
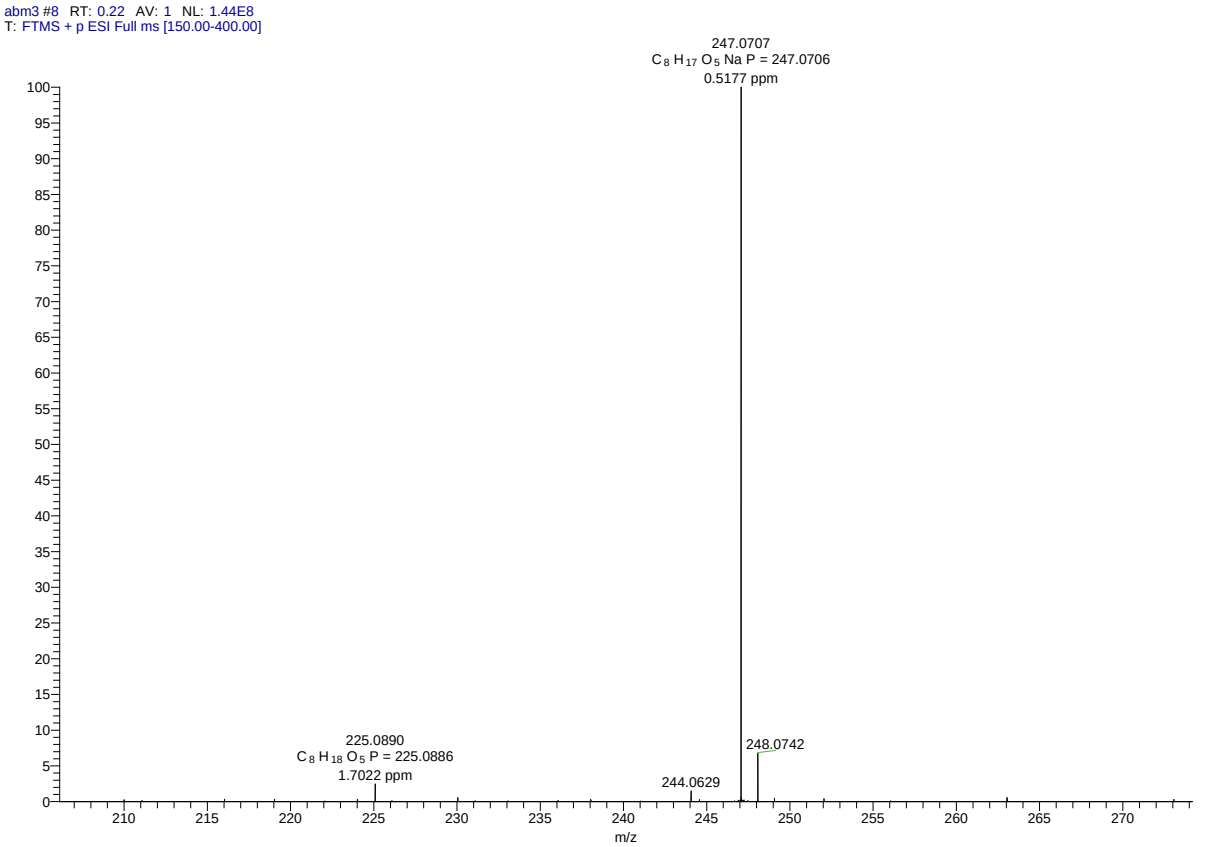
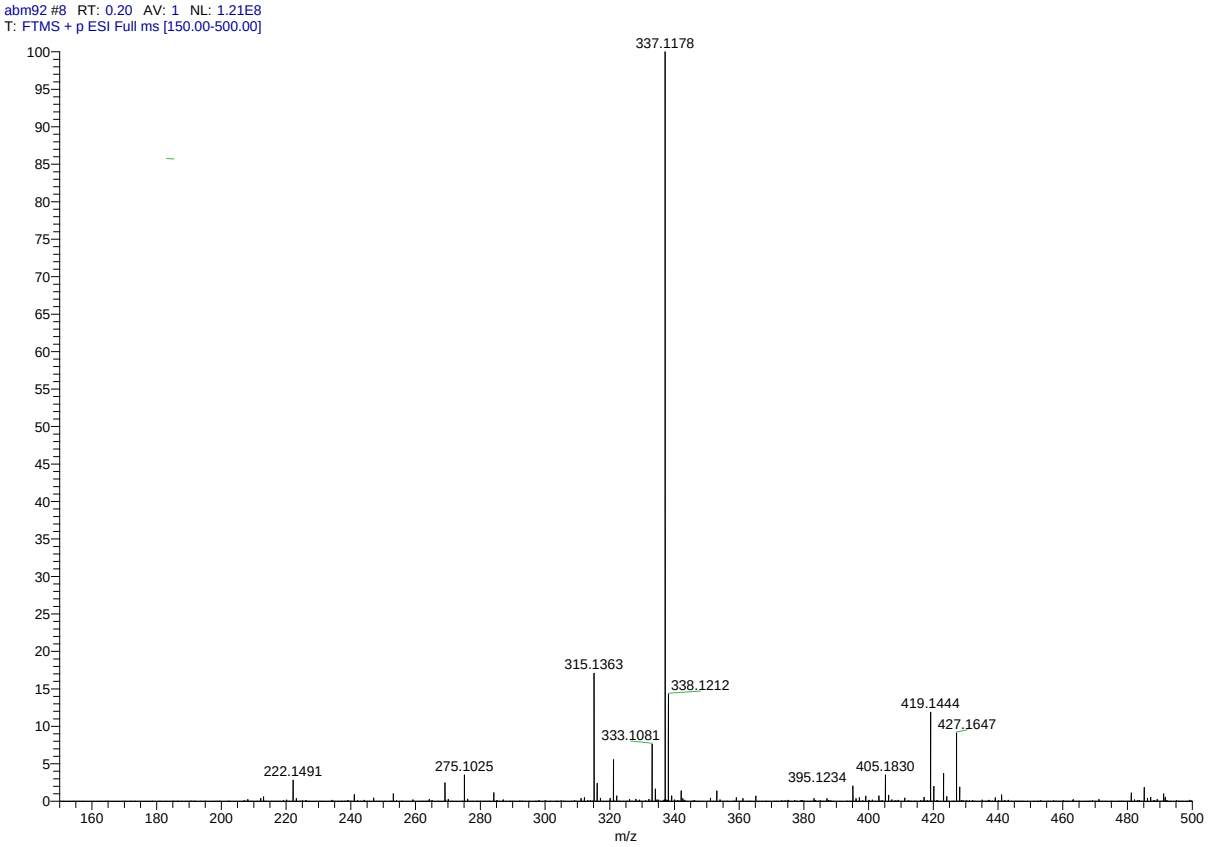
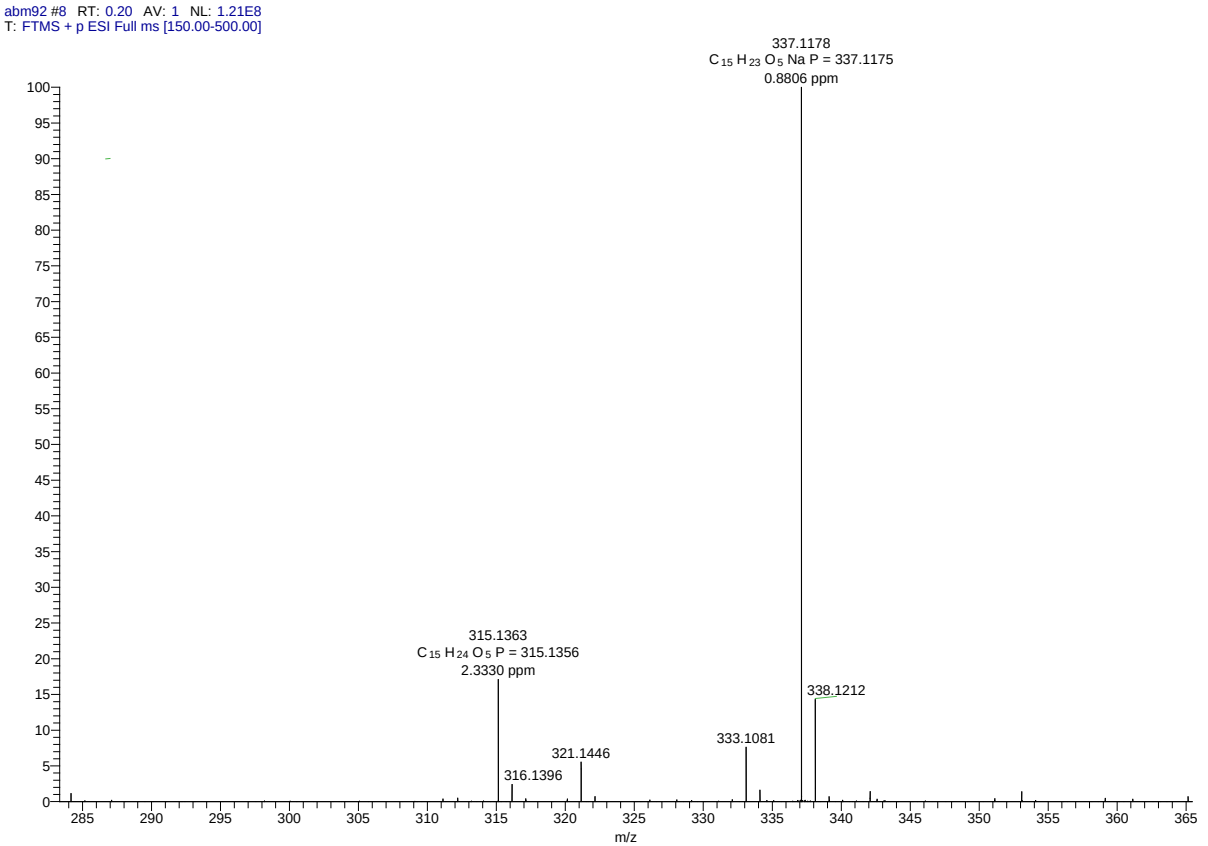




2a, Ethyl 2-(diethoxyphosphoryl)-3-phenylpropanoate





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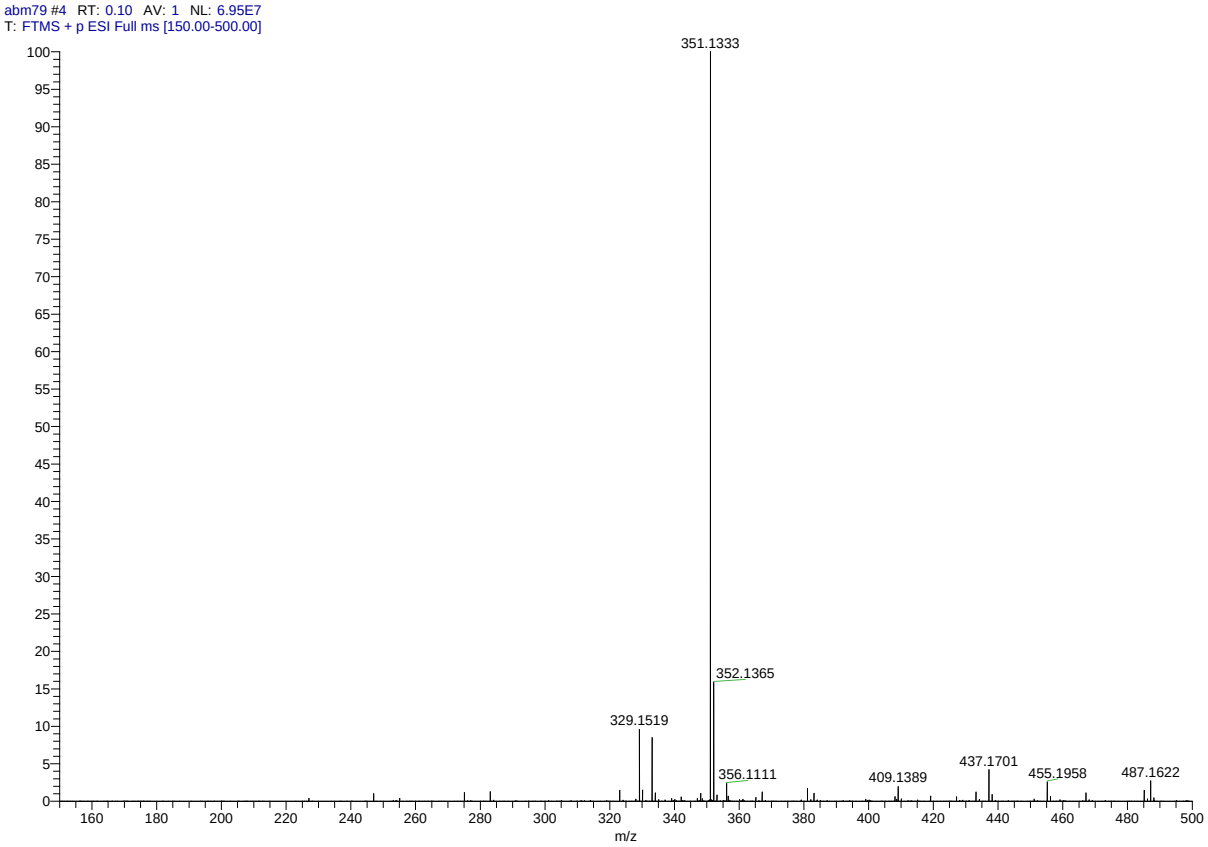

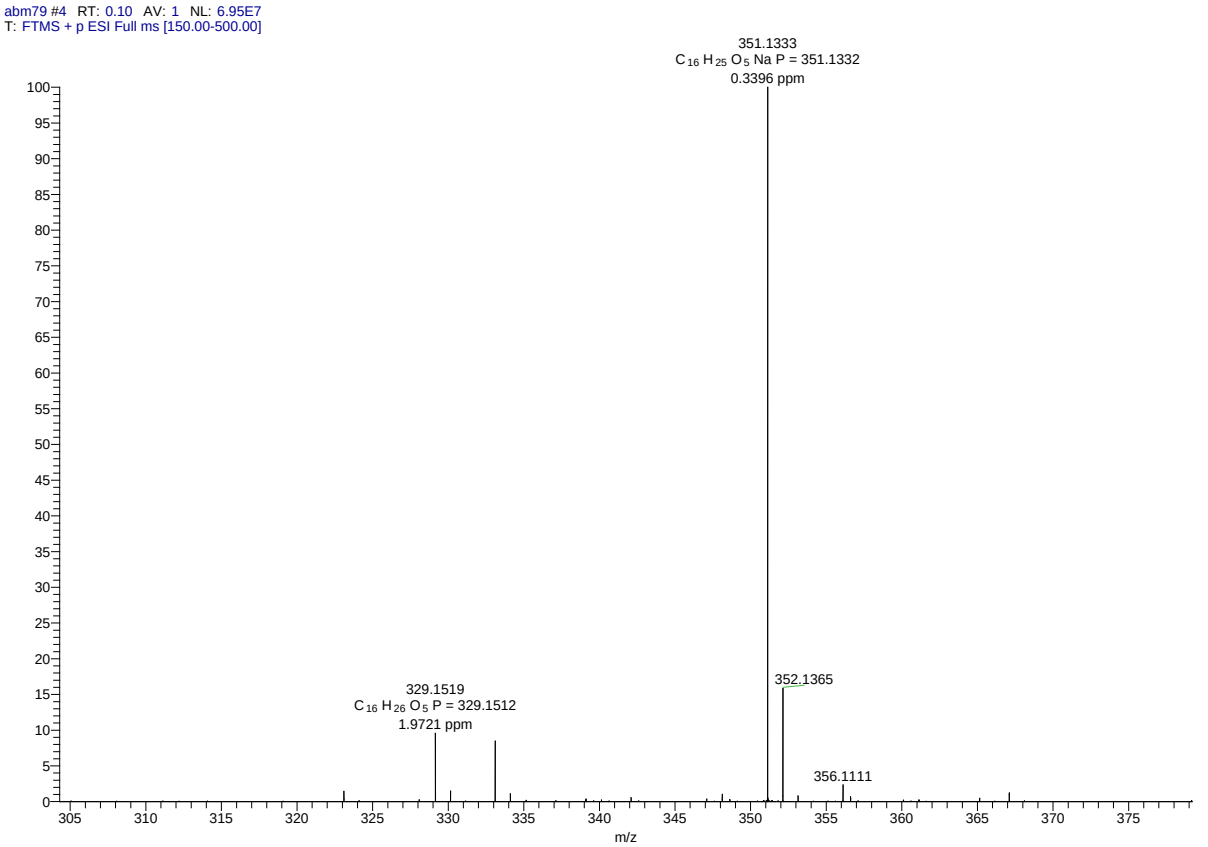

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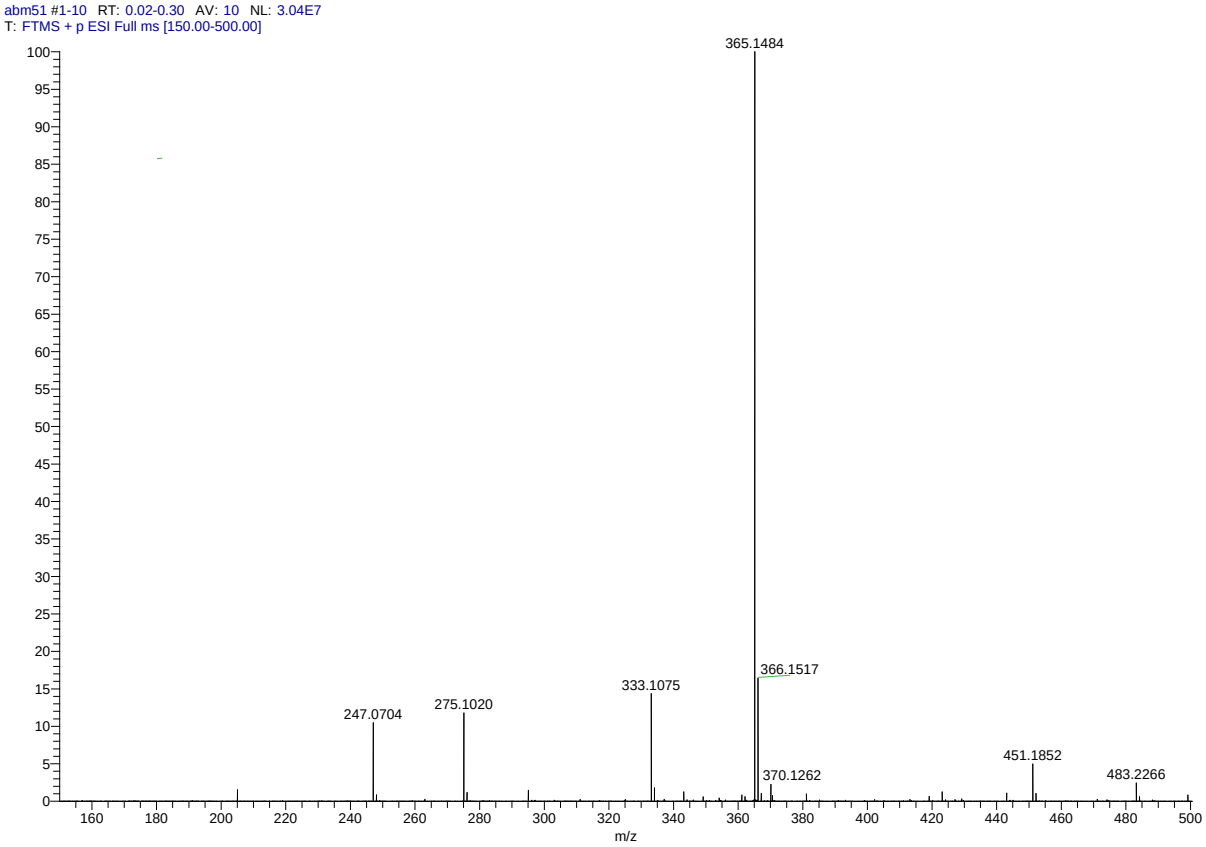
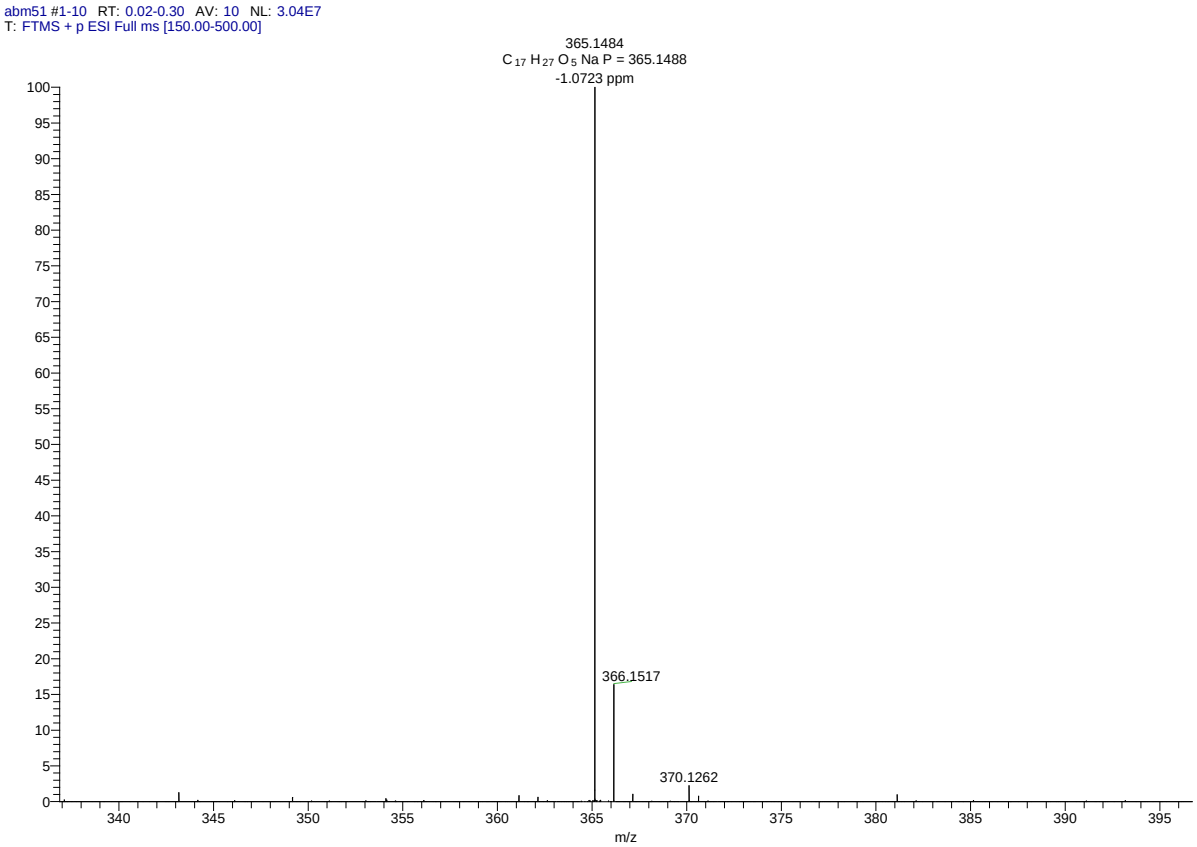
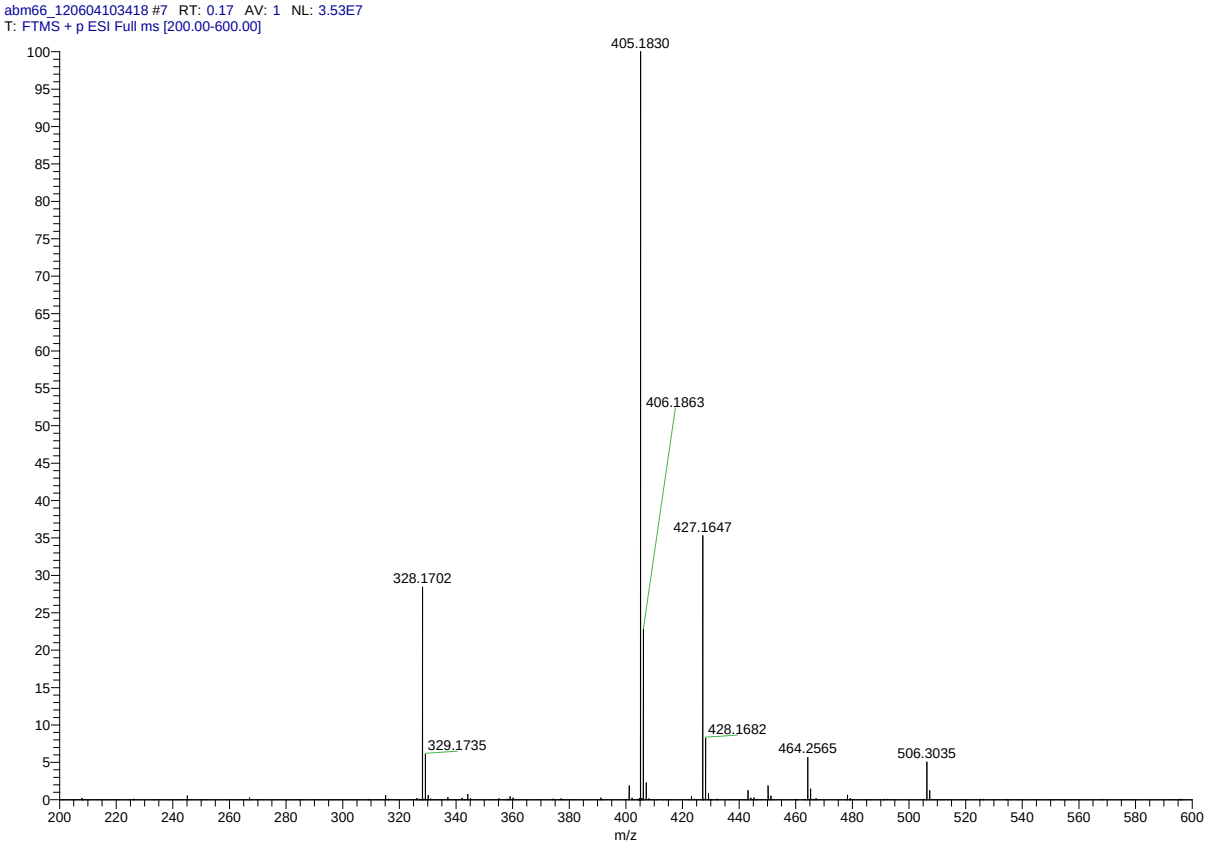
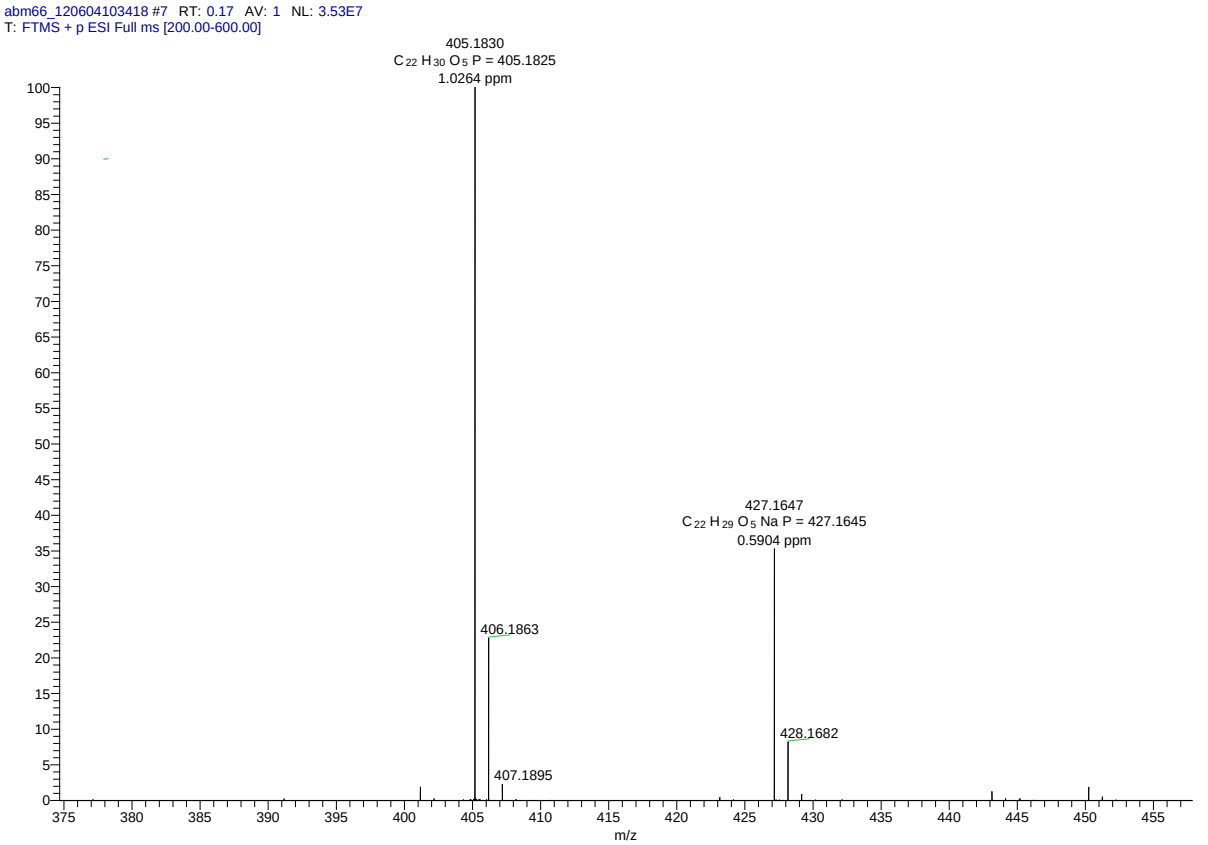




2d, Ethyl 2-benzyl-2-(diethoxyphosphoryl)-3-phenylpropanoate





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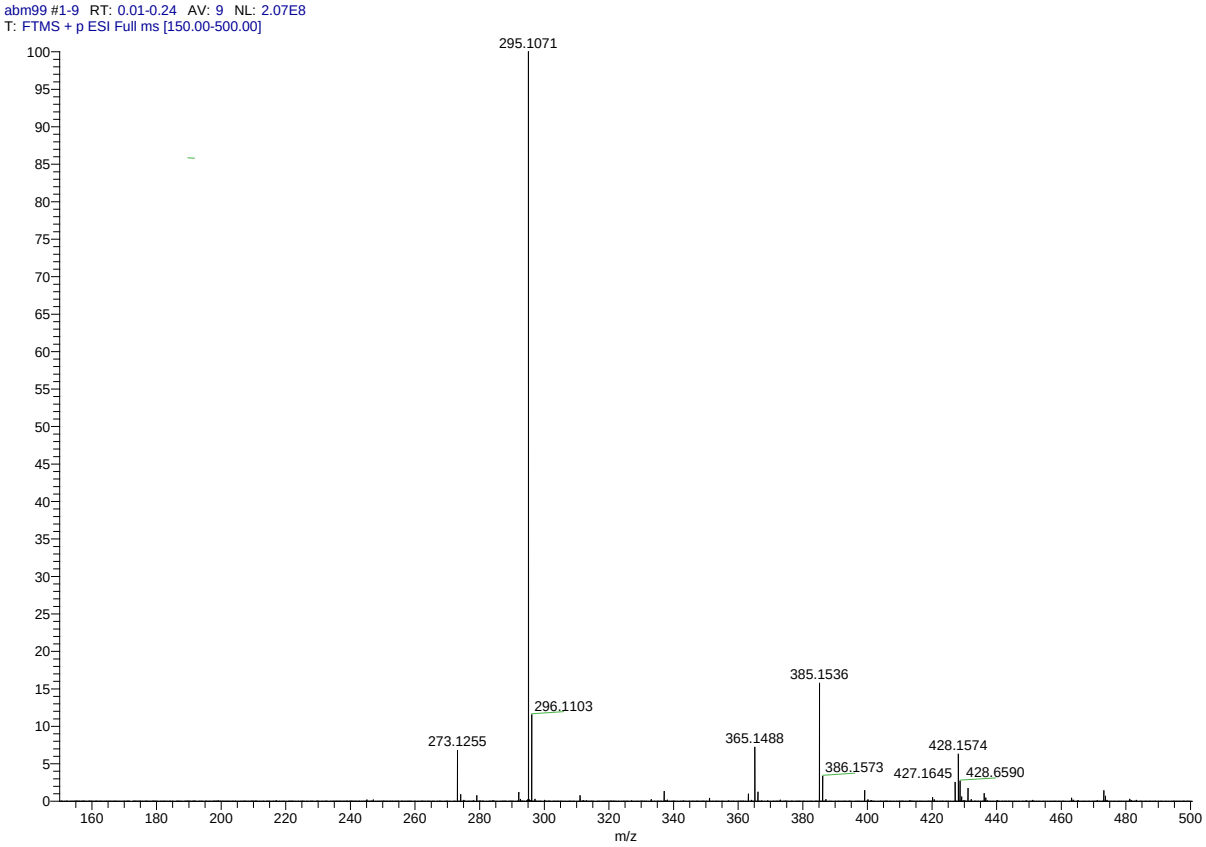

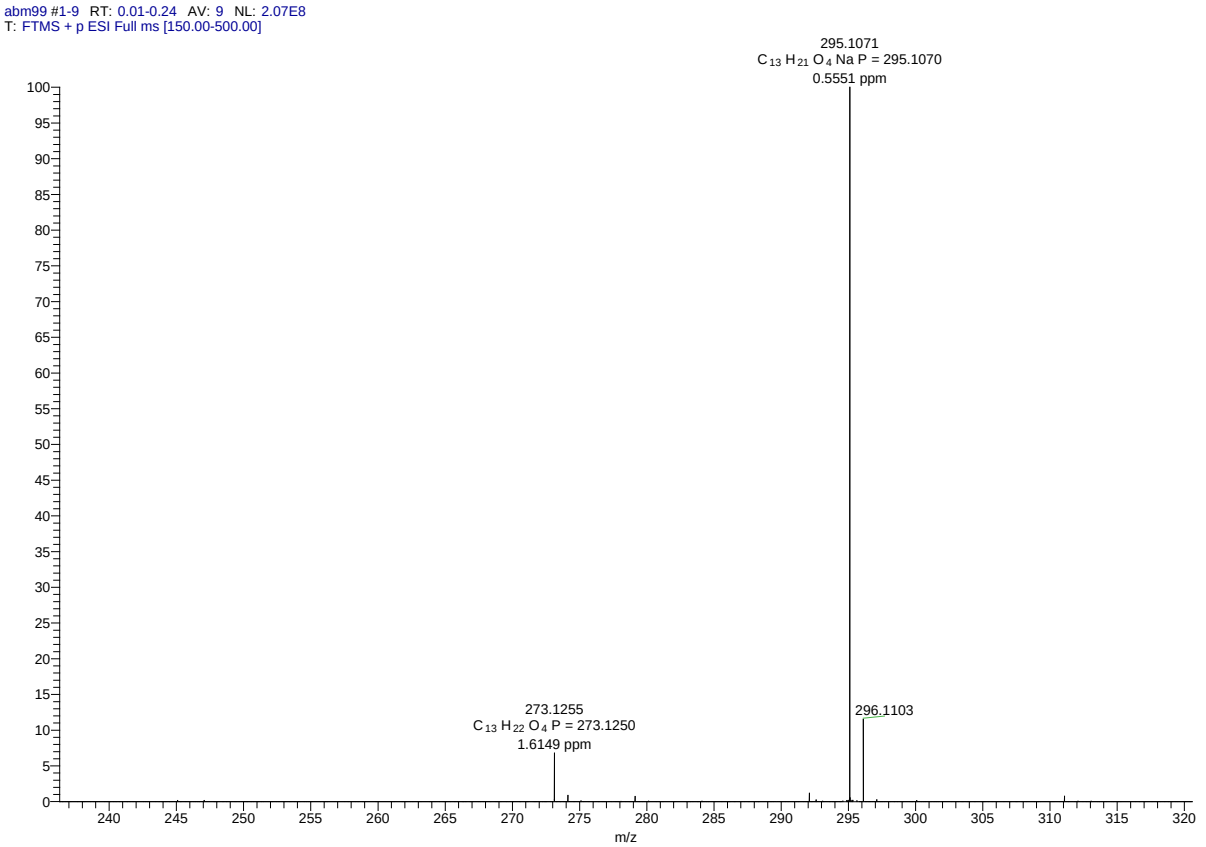

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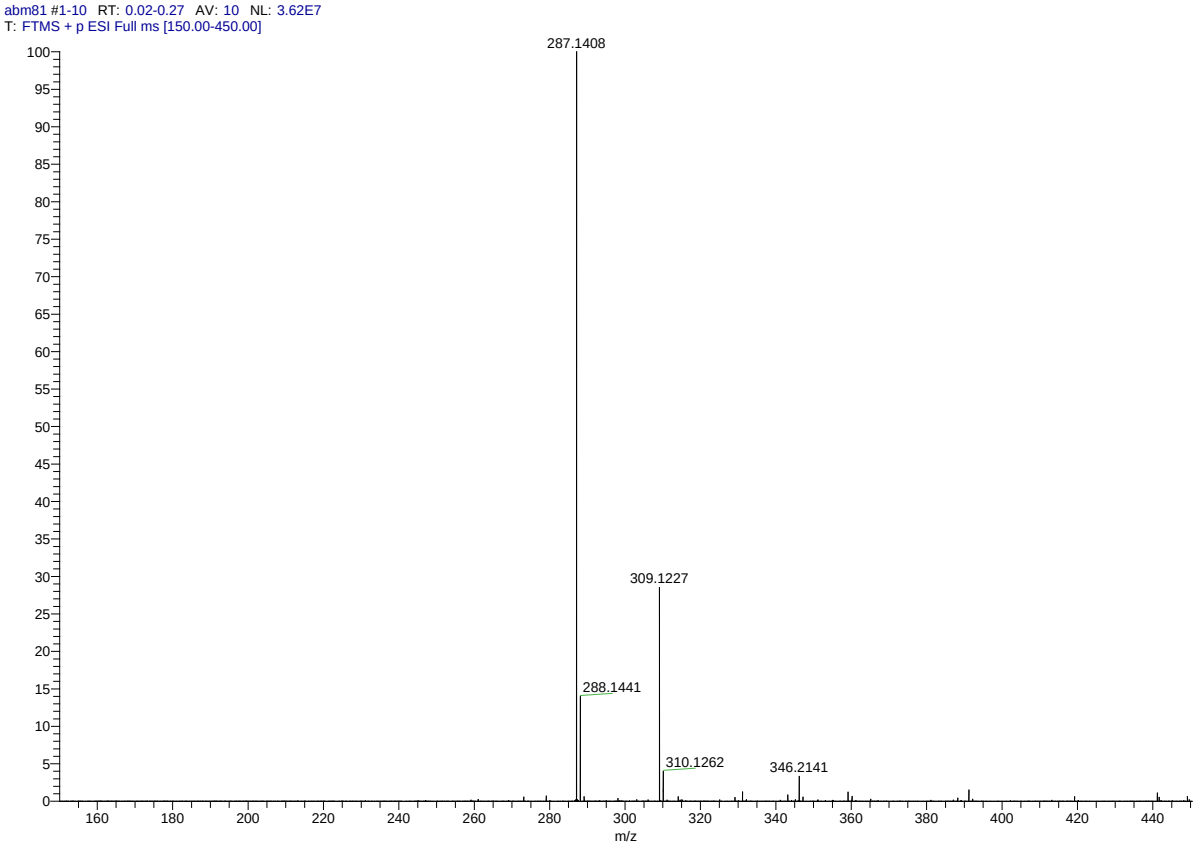
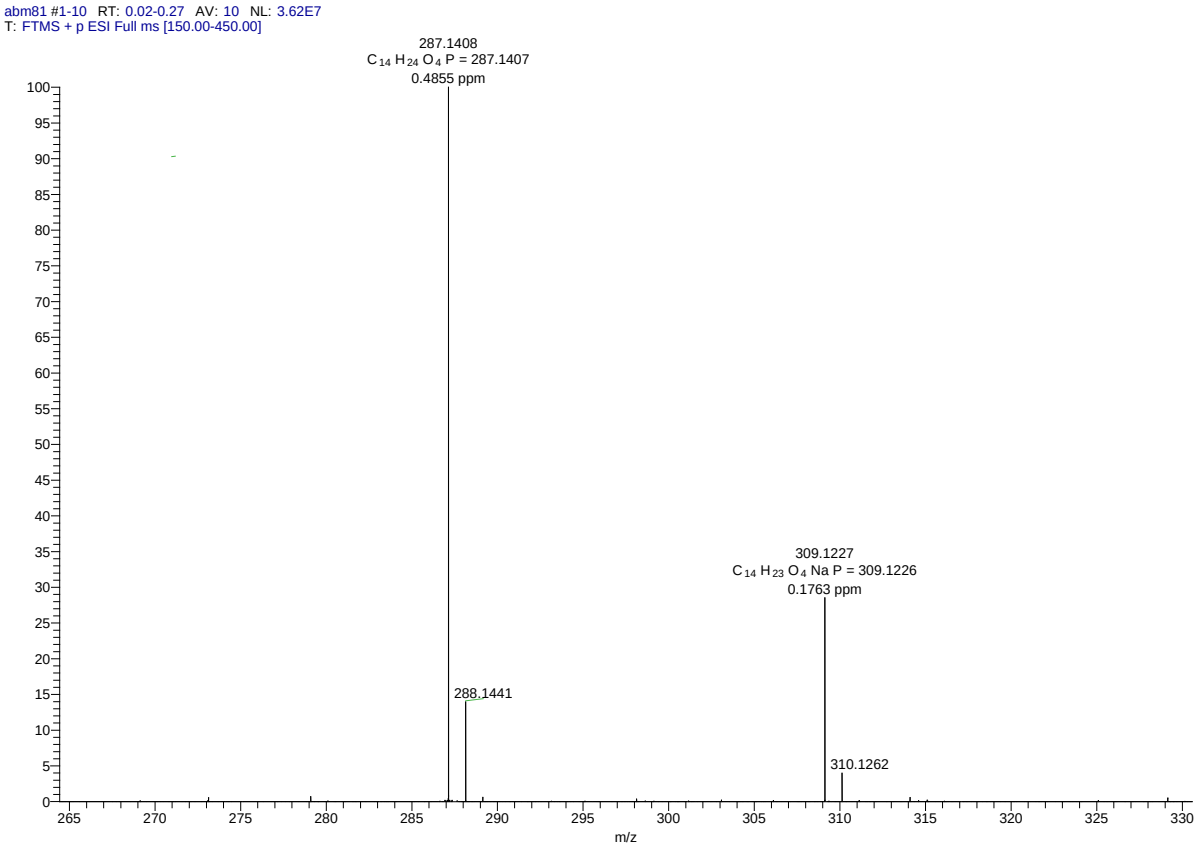
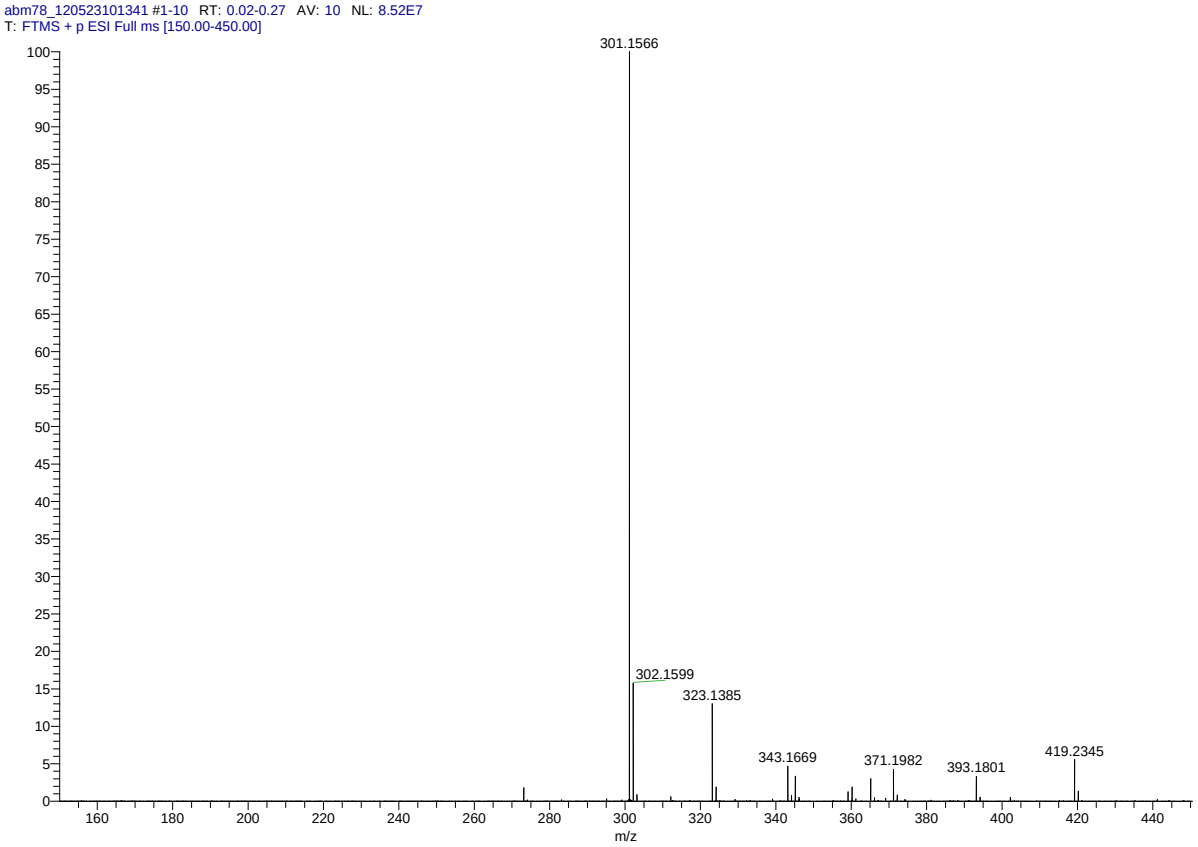
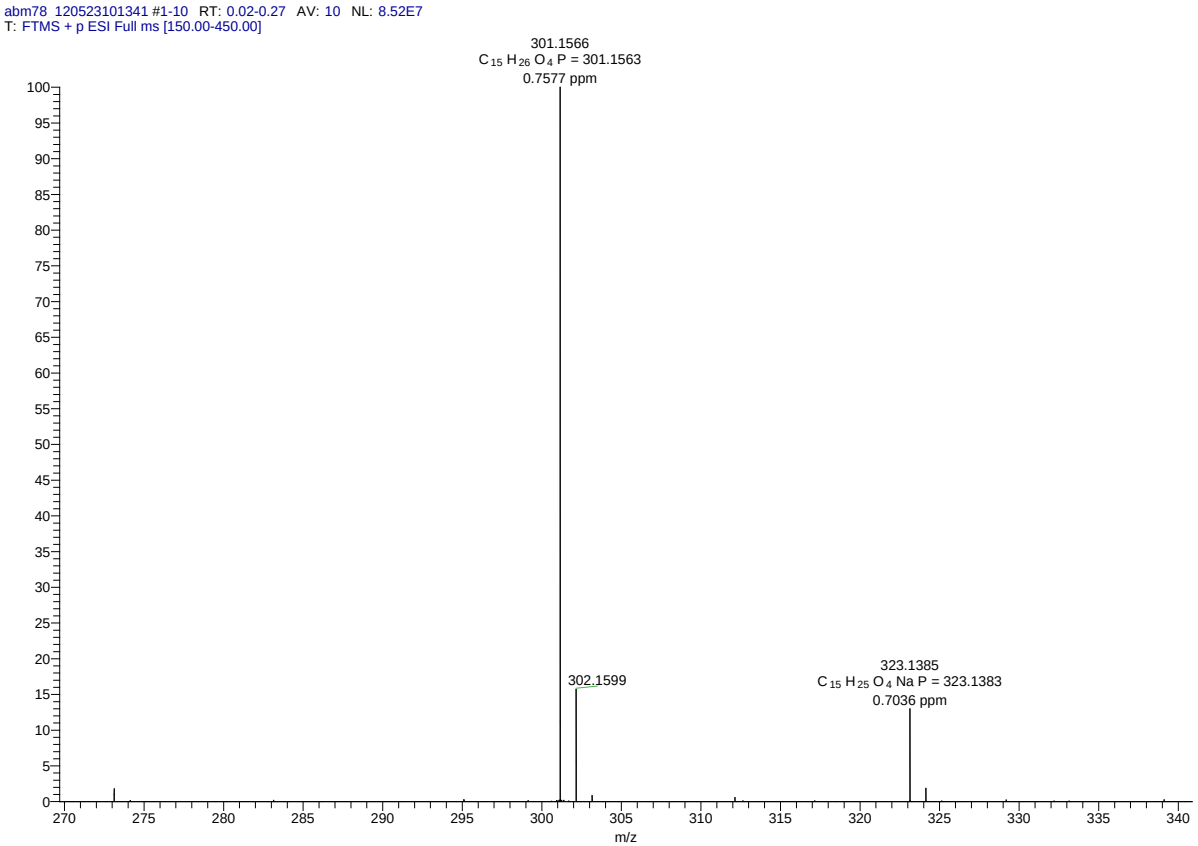


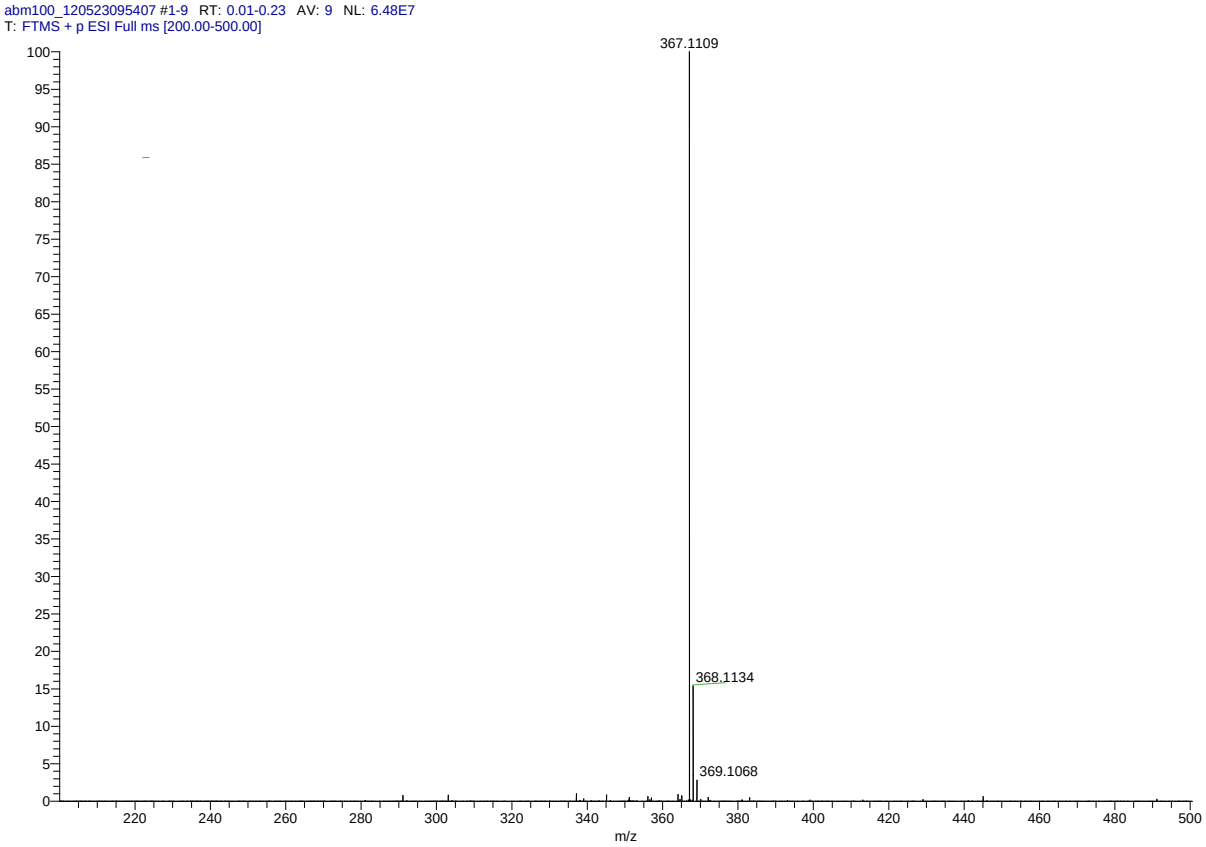


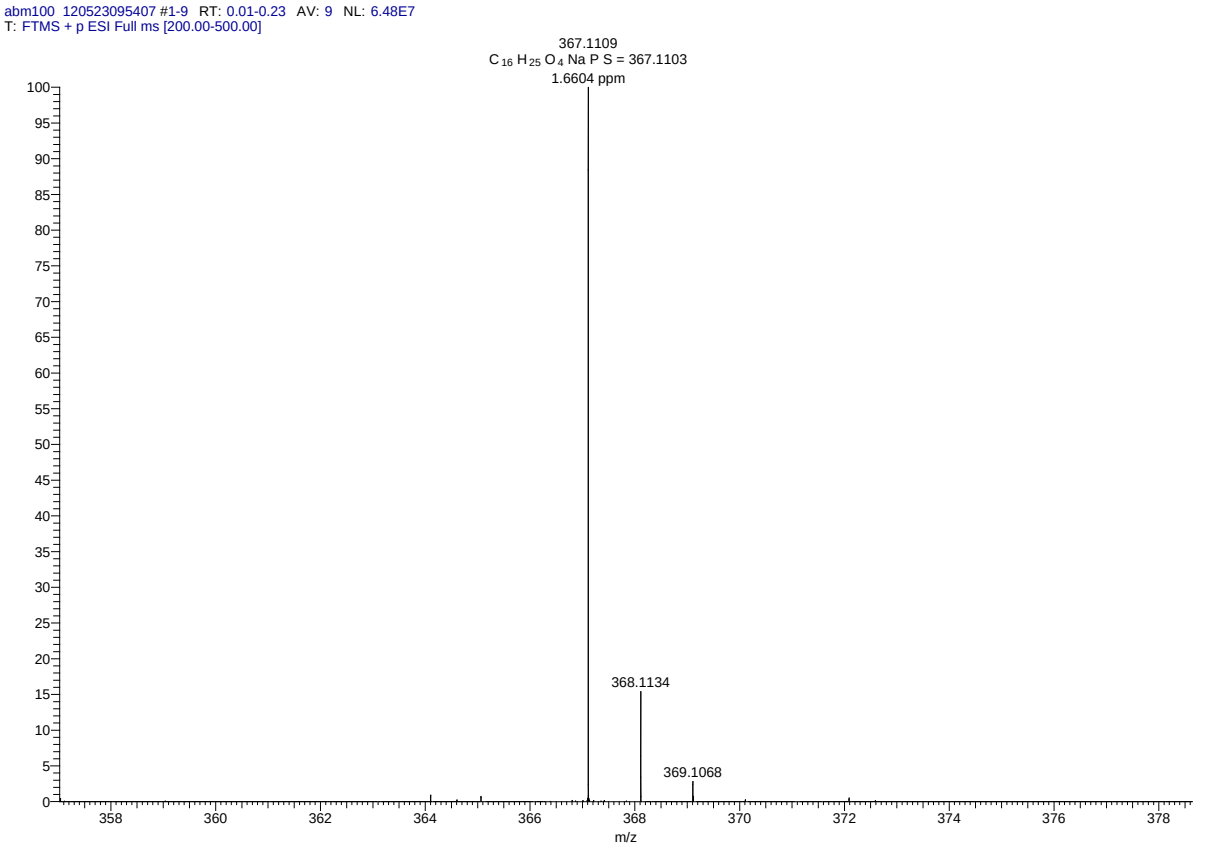
3c, Diethyl 1-hydroxy-5-phenylpentane-2-ylphosphonate





4b, S-2-(diethoxyphosphoryl)-4-phenylbutyl ethanethioate





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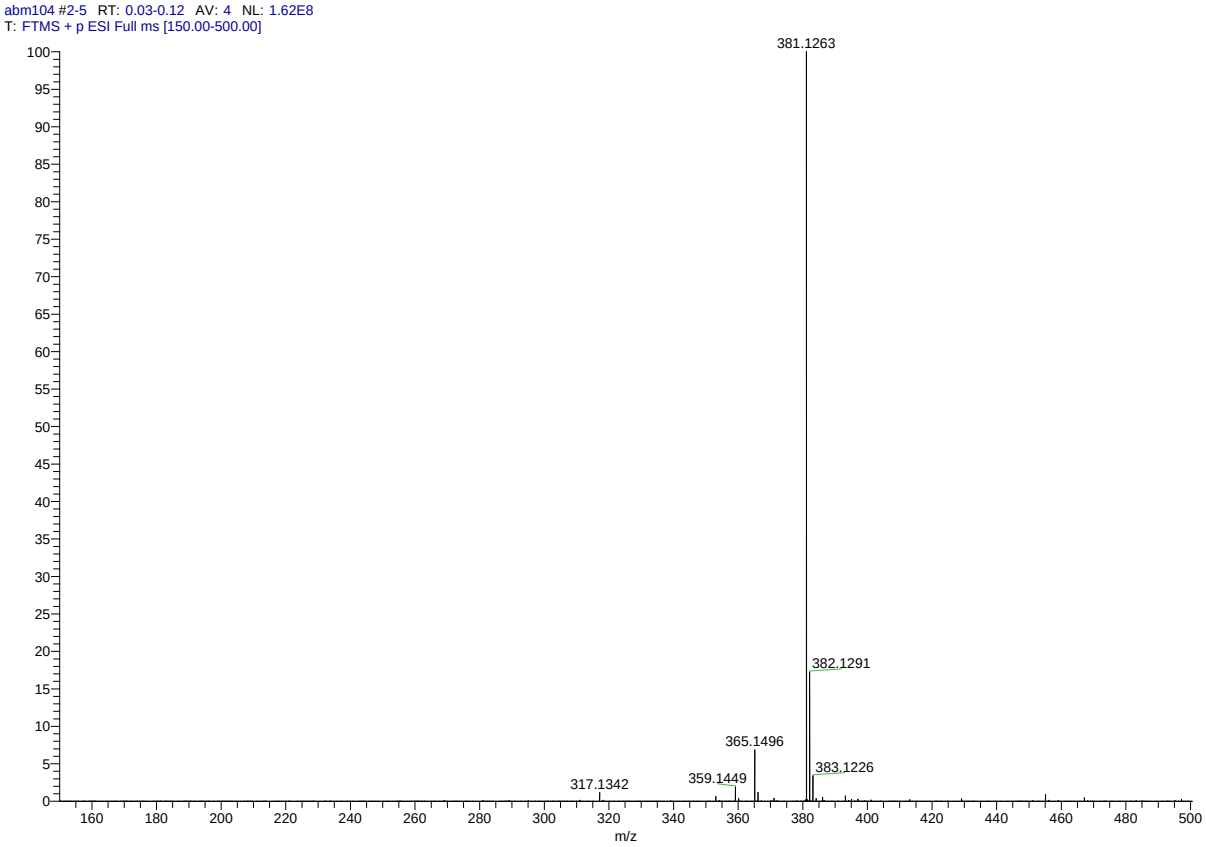
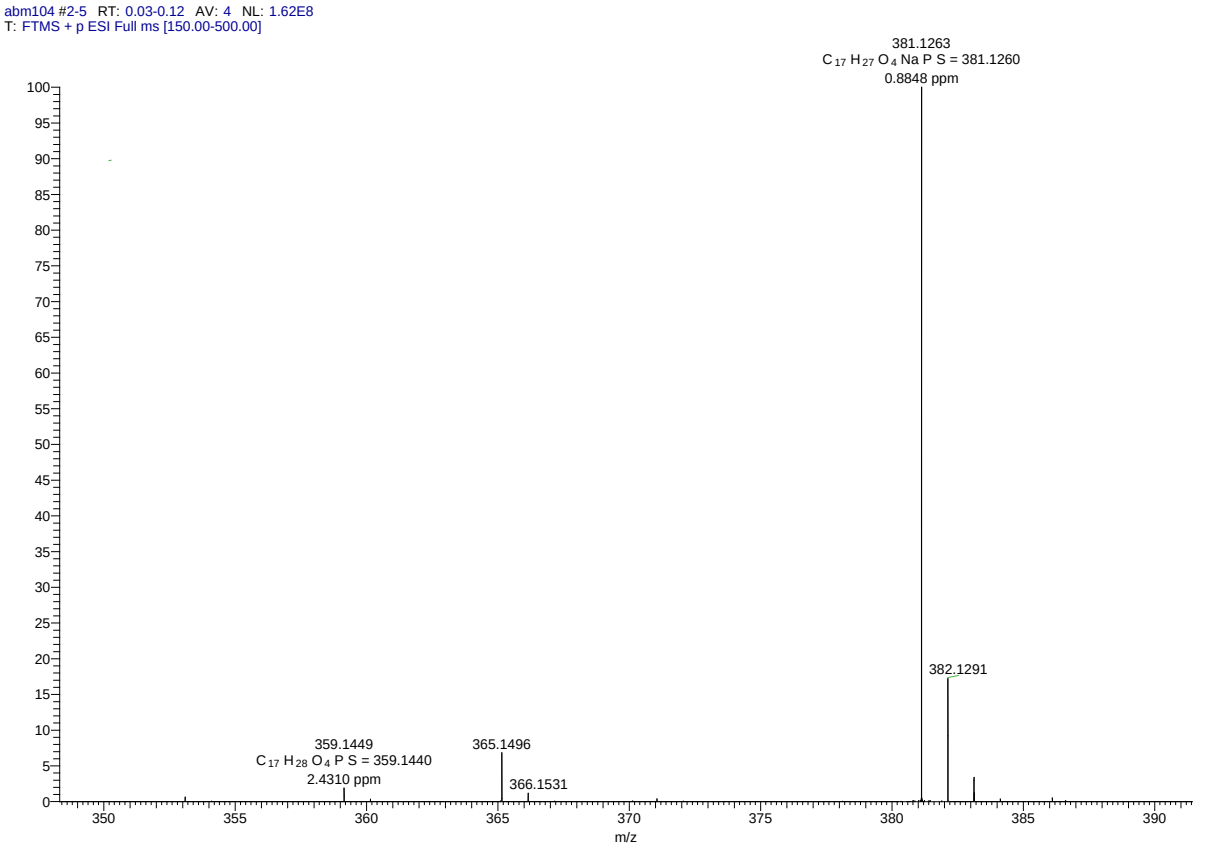
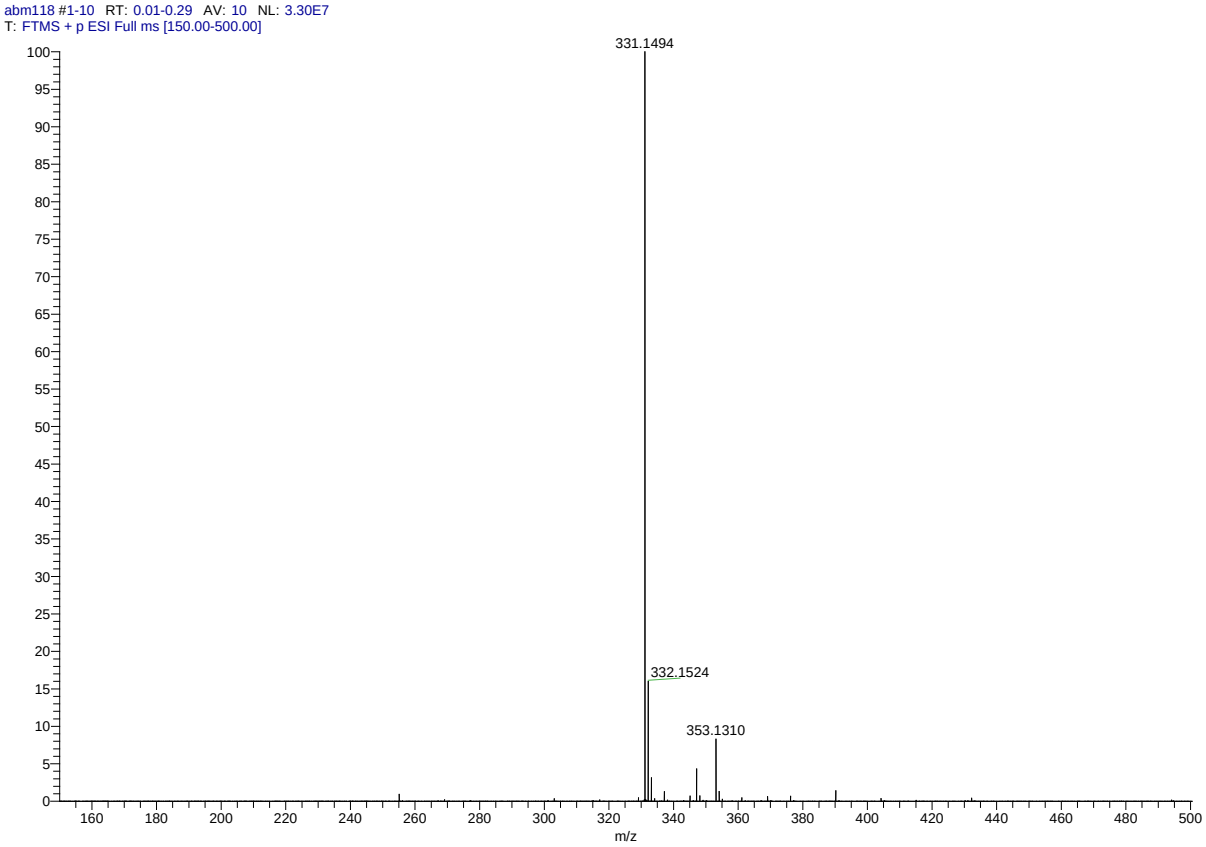
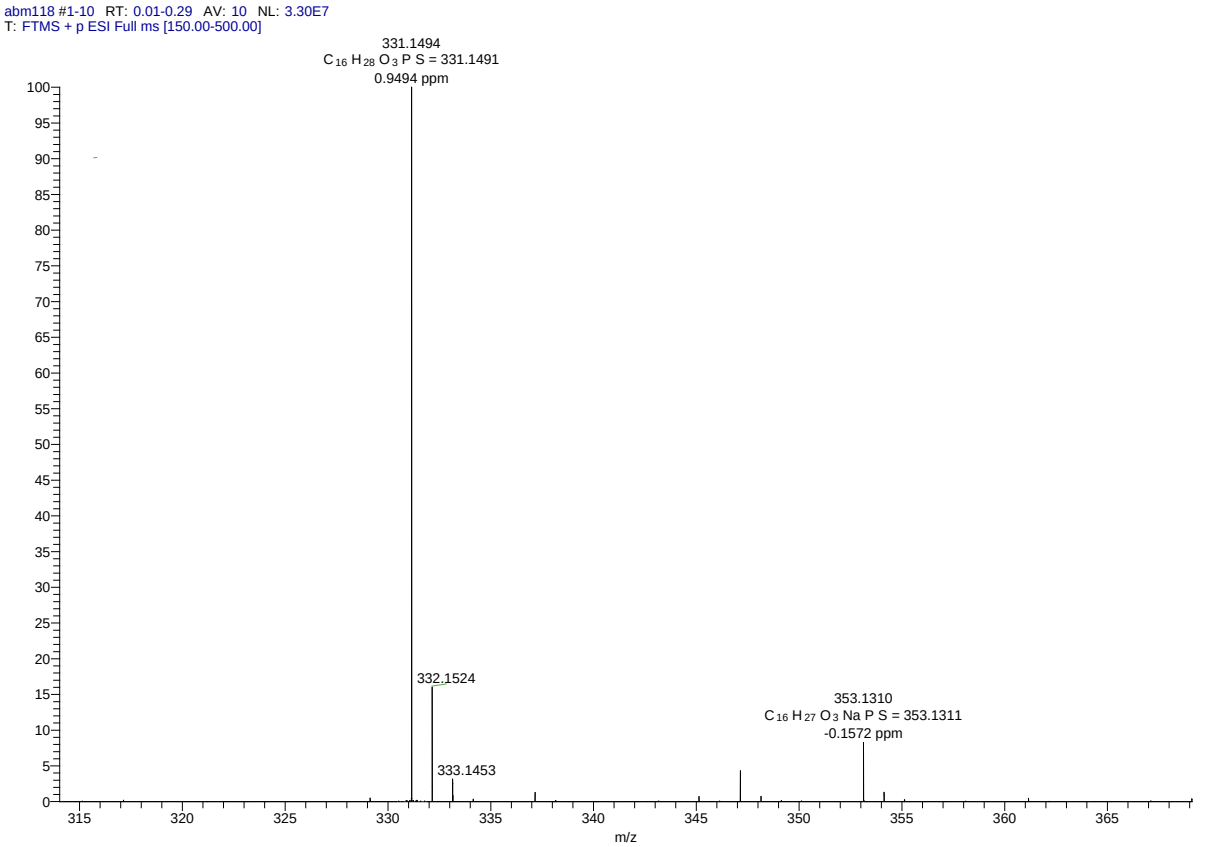




5b, Diethyl 1-mercapto-4-phenylbutan-2-ylphosphonate





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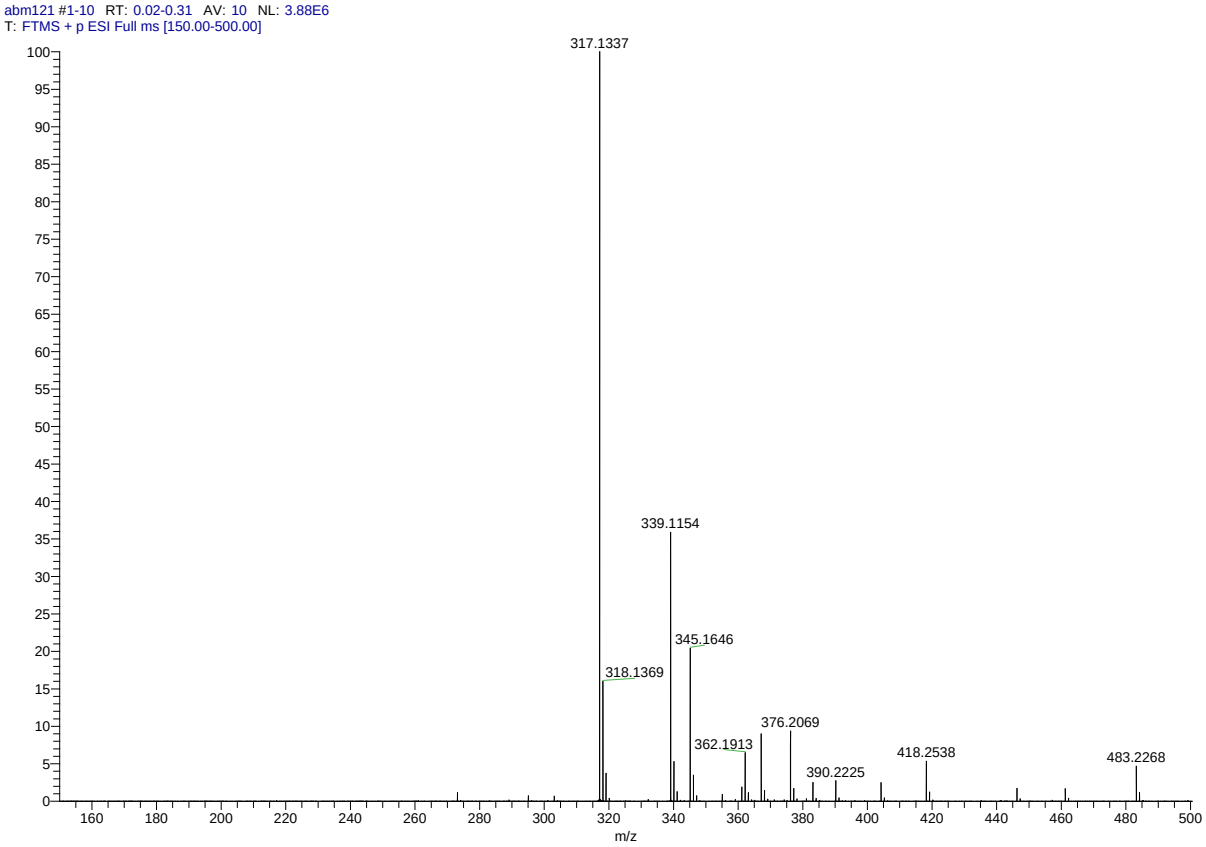
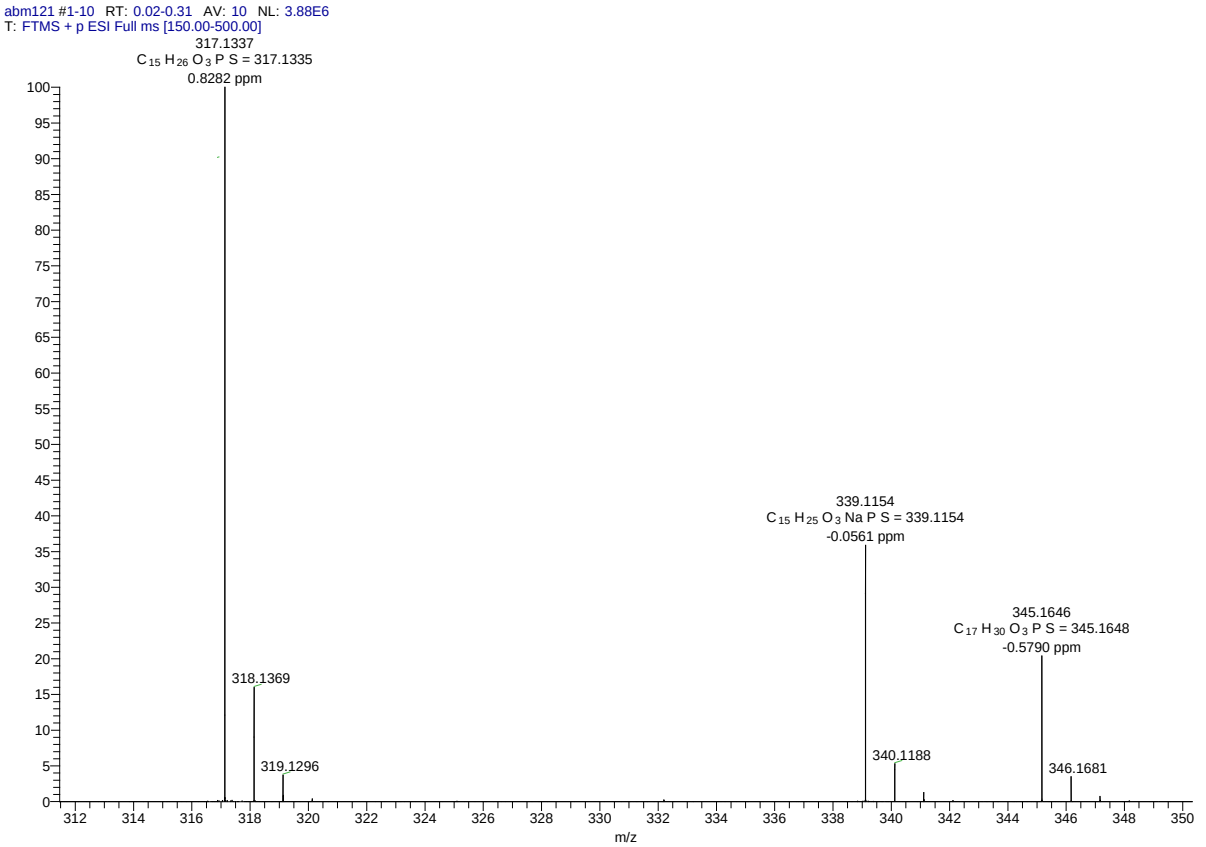
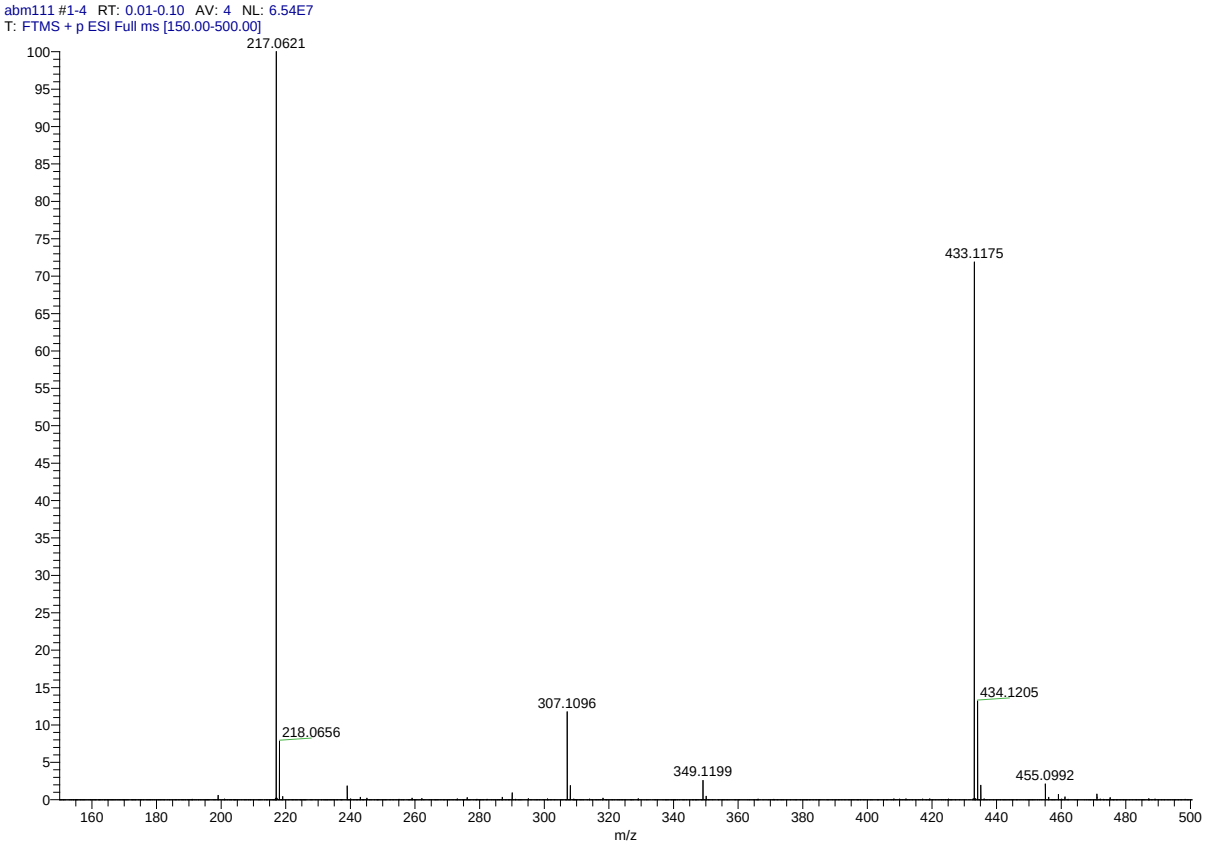
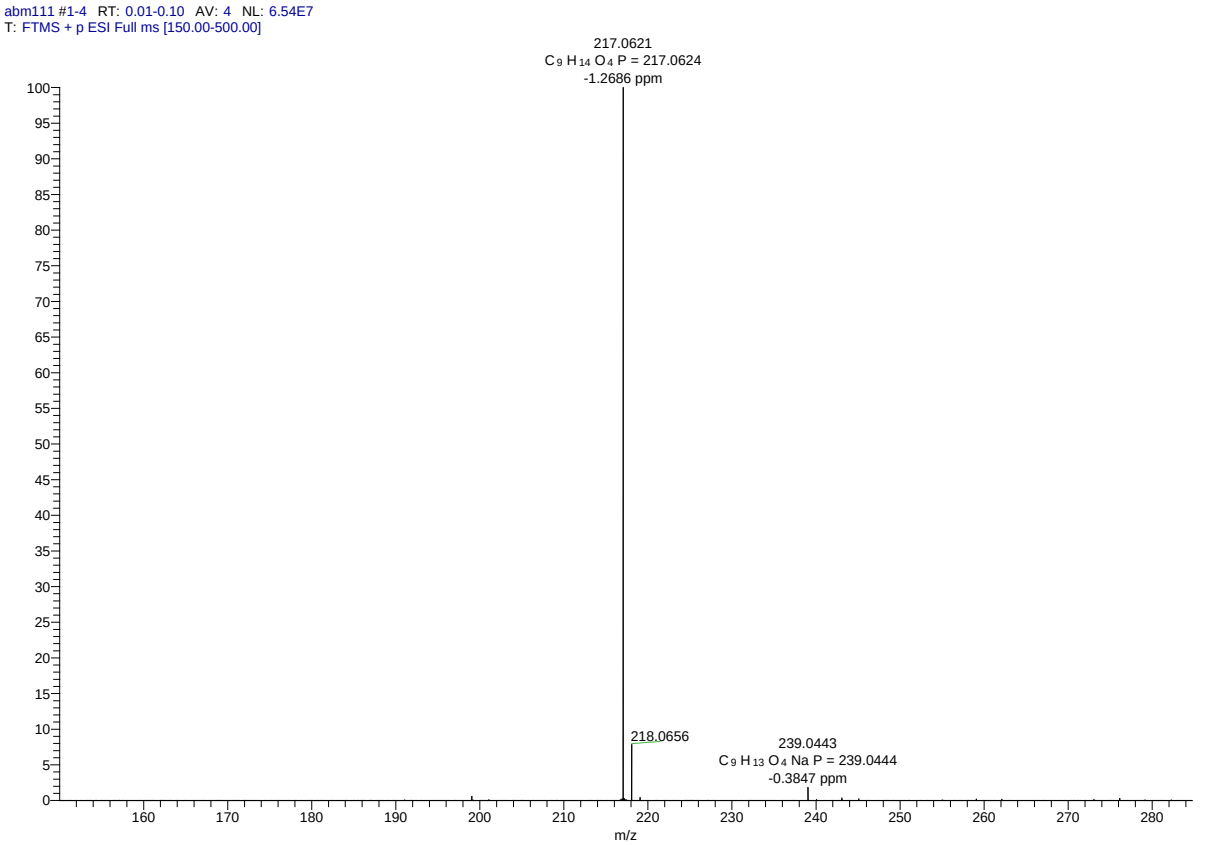


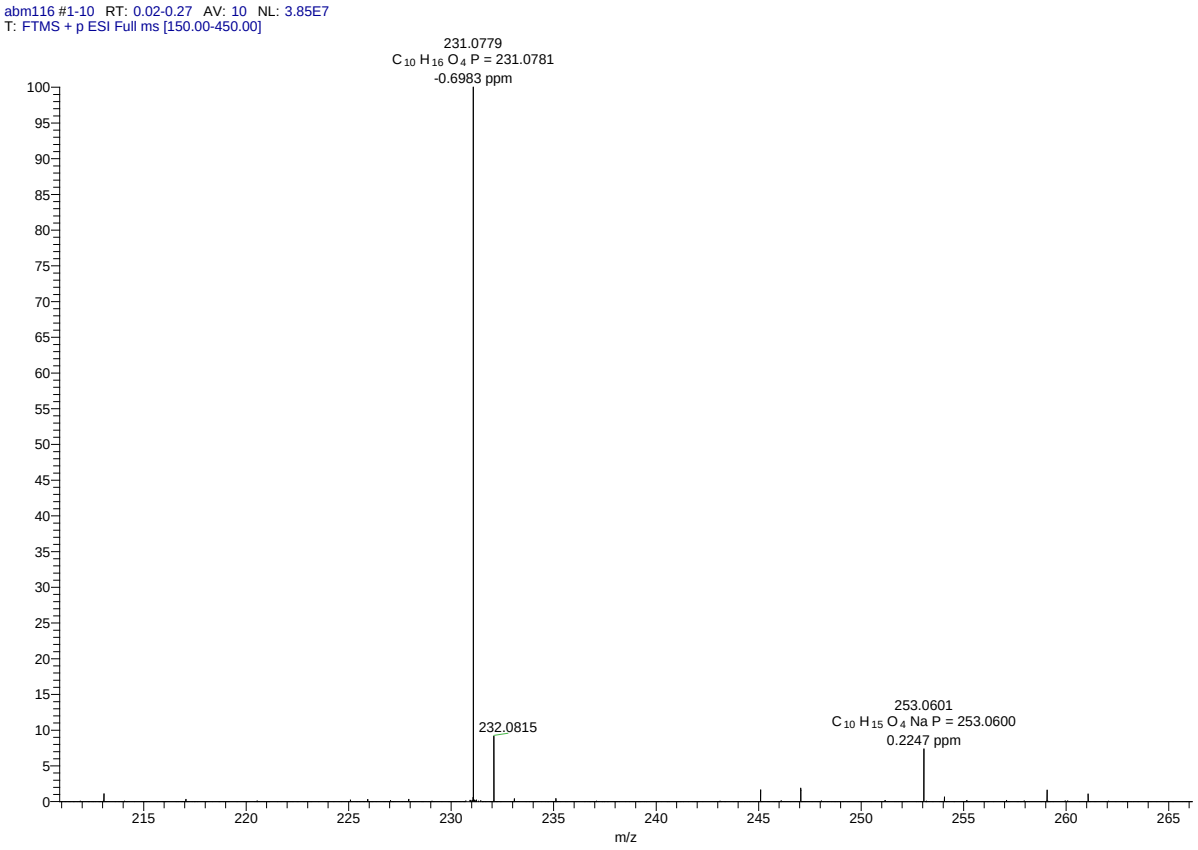


6a, 1-hydroxy-3-phenylpropan-2-ylphosphonic acid

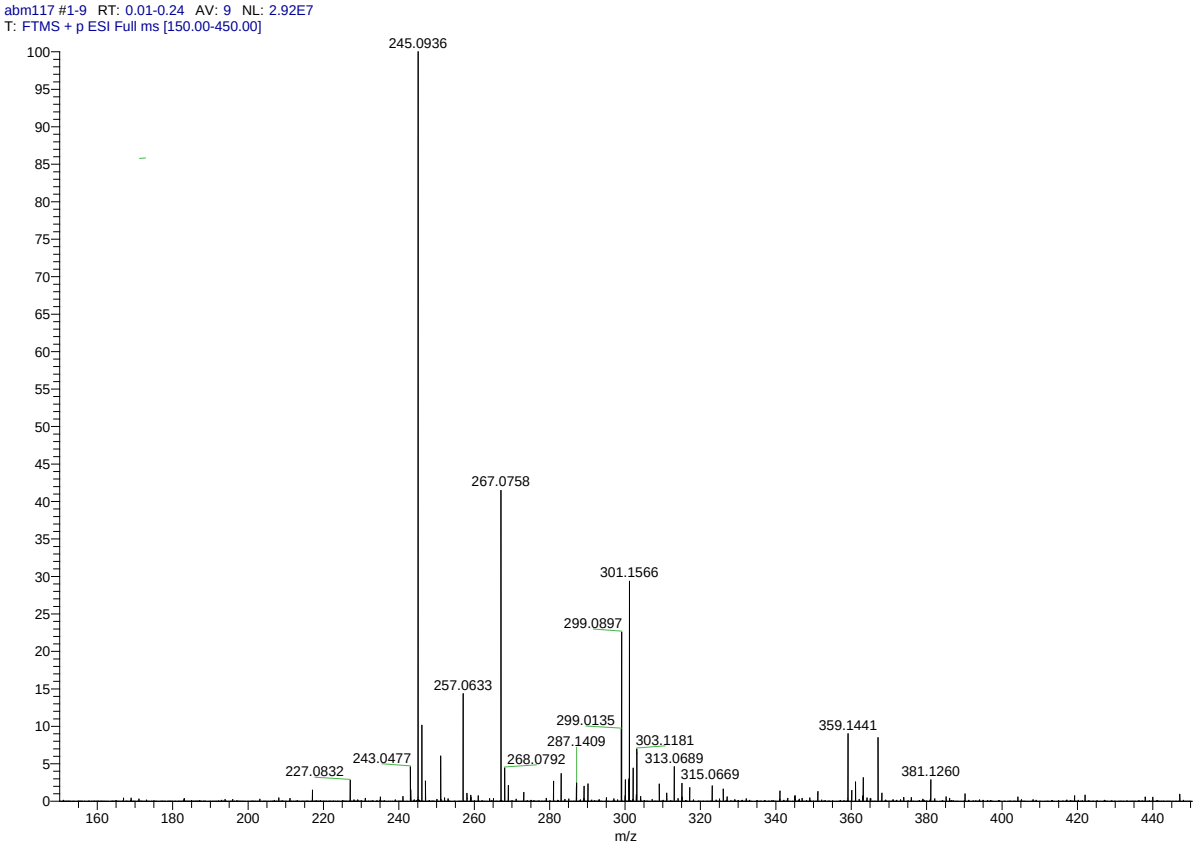


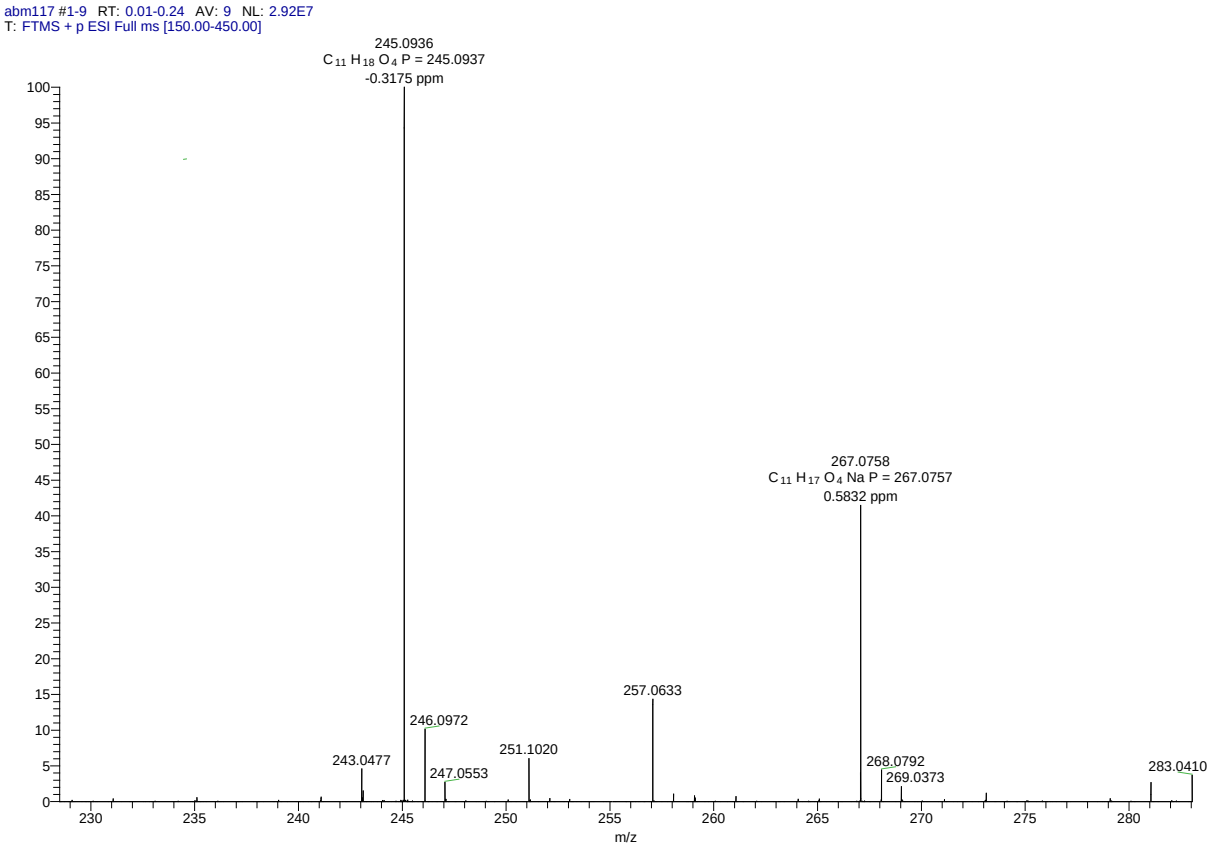


6b, 1-hydroxy-4-phenylbutan-2-ylphosphonic acid

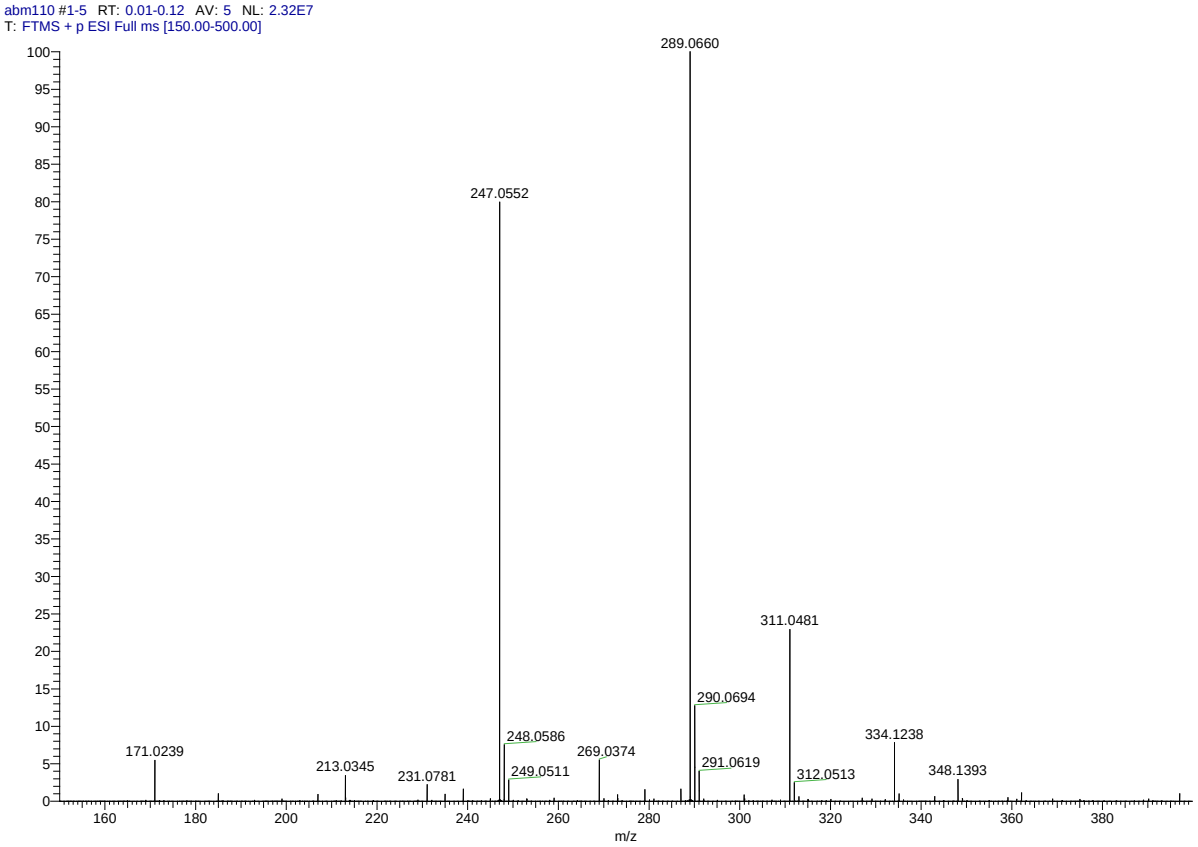


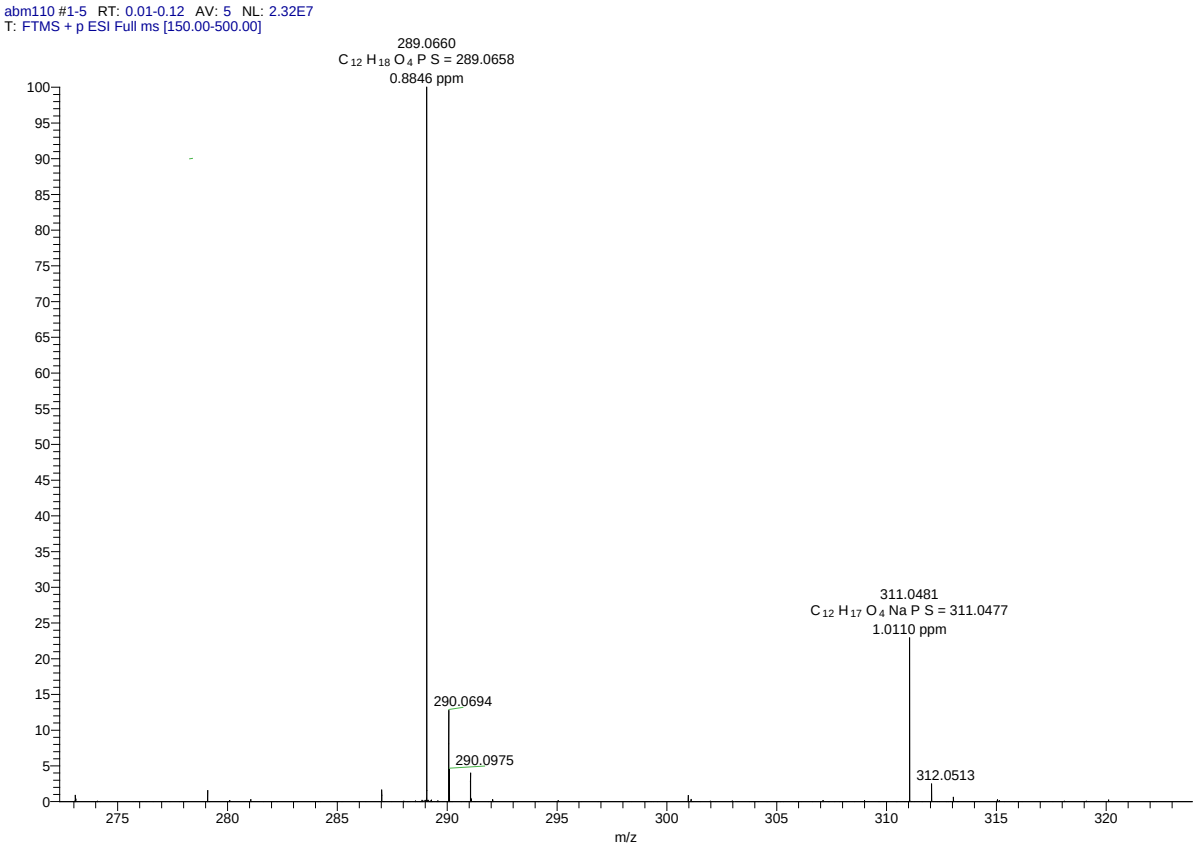
6c, 1-hydroxy-5-phenylpentane-2-ylphosphonic acid



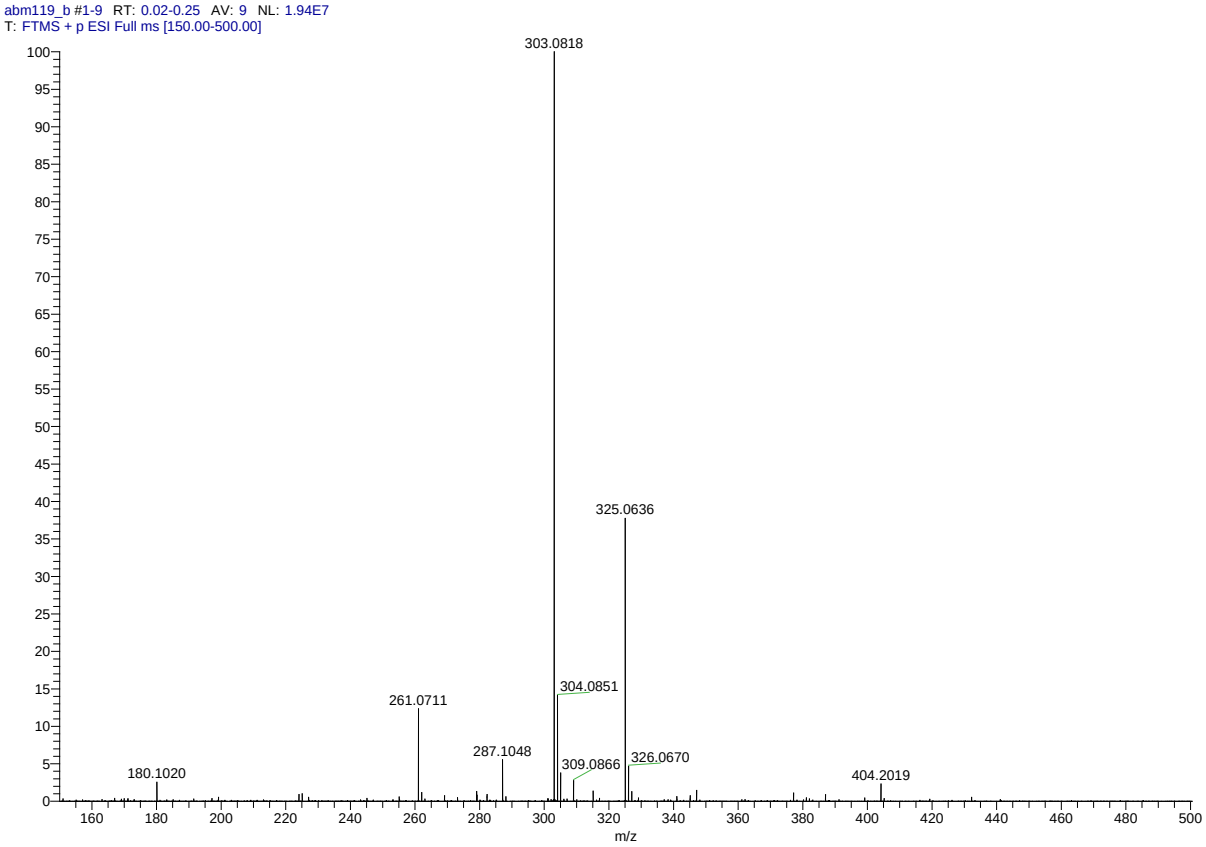


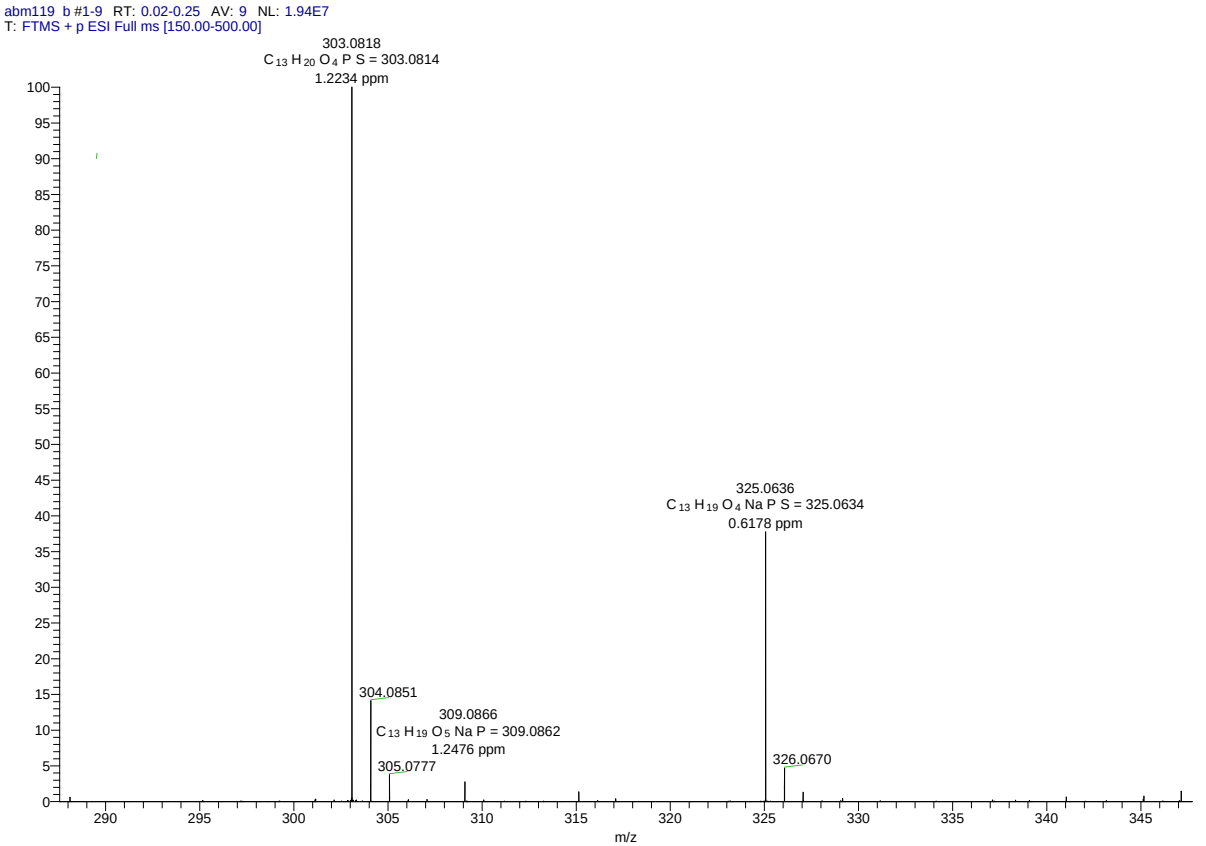
7b, 1-(acetylthio)-4-phenylbutane-2-ylphosphonic acid



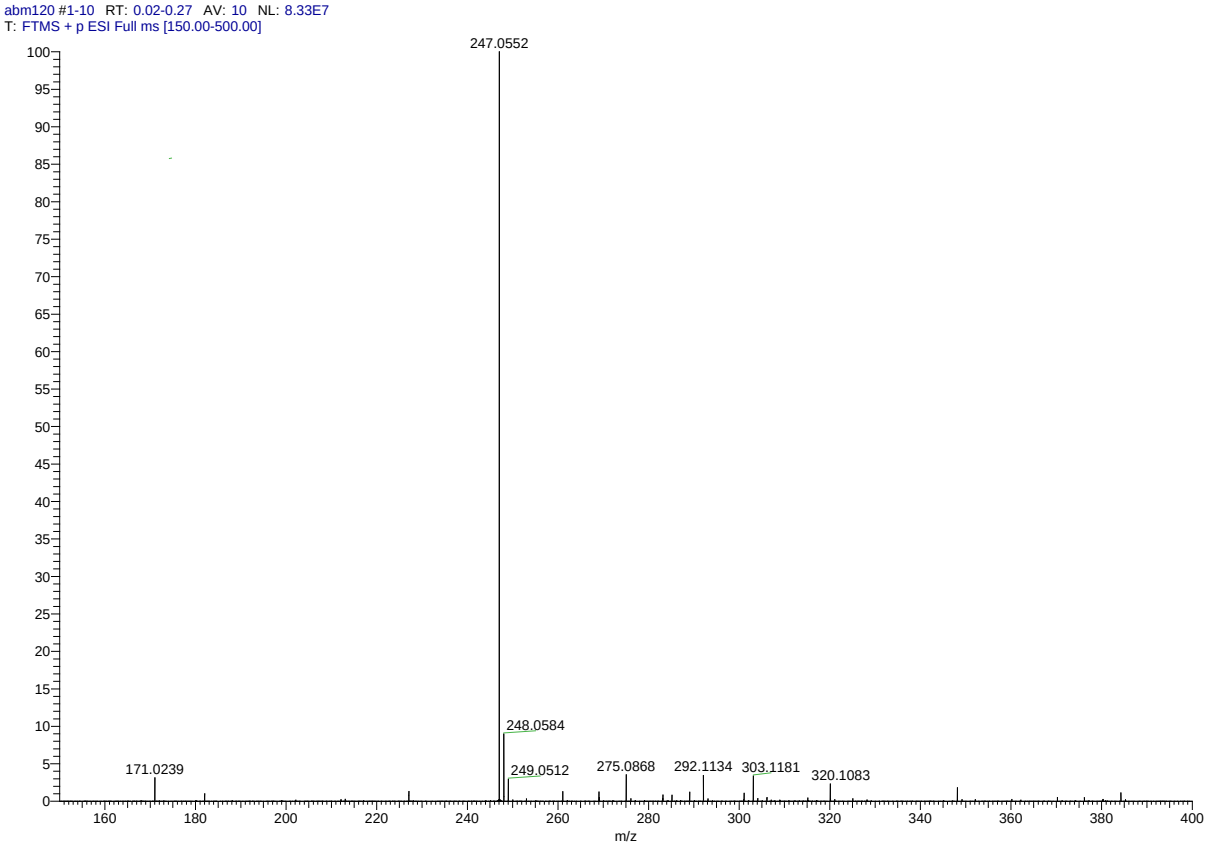


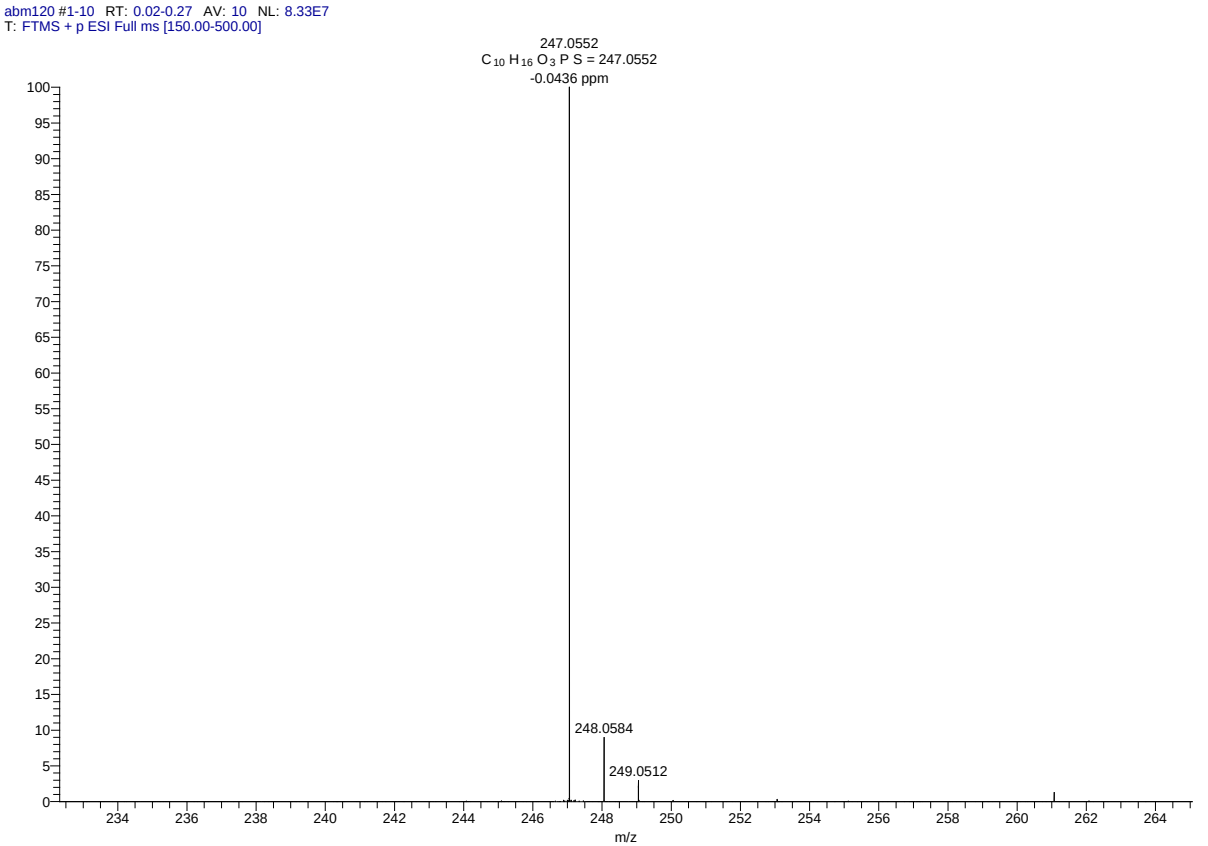
7c, 1-(acetylthio)-5-phenylpentan-2-ylphosphonic acid



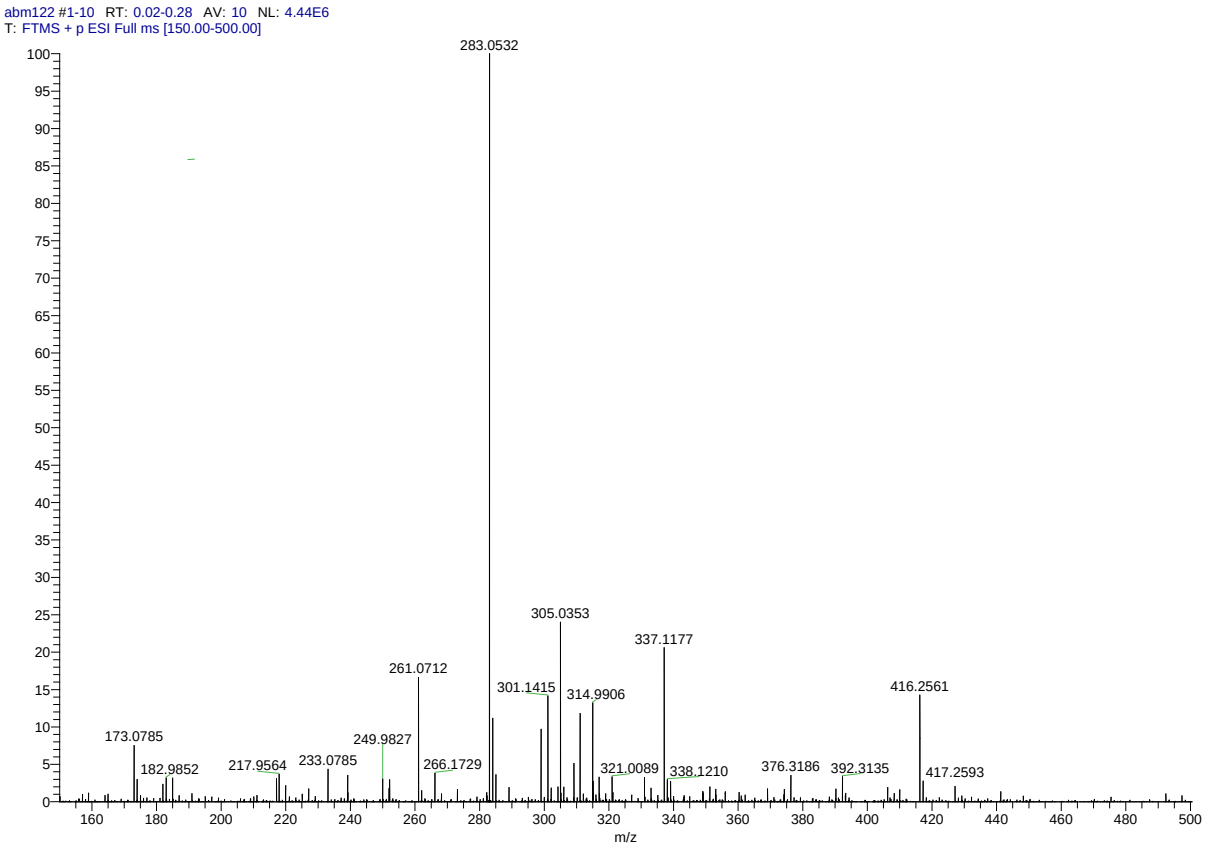


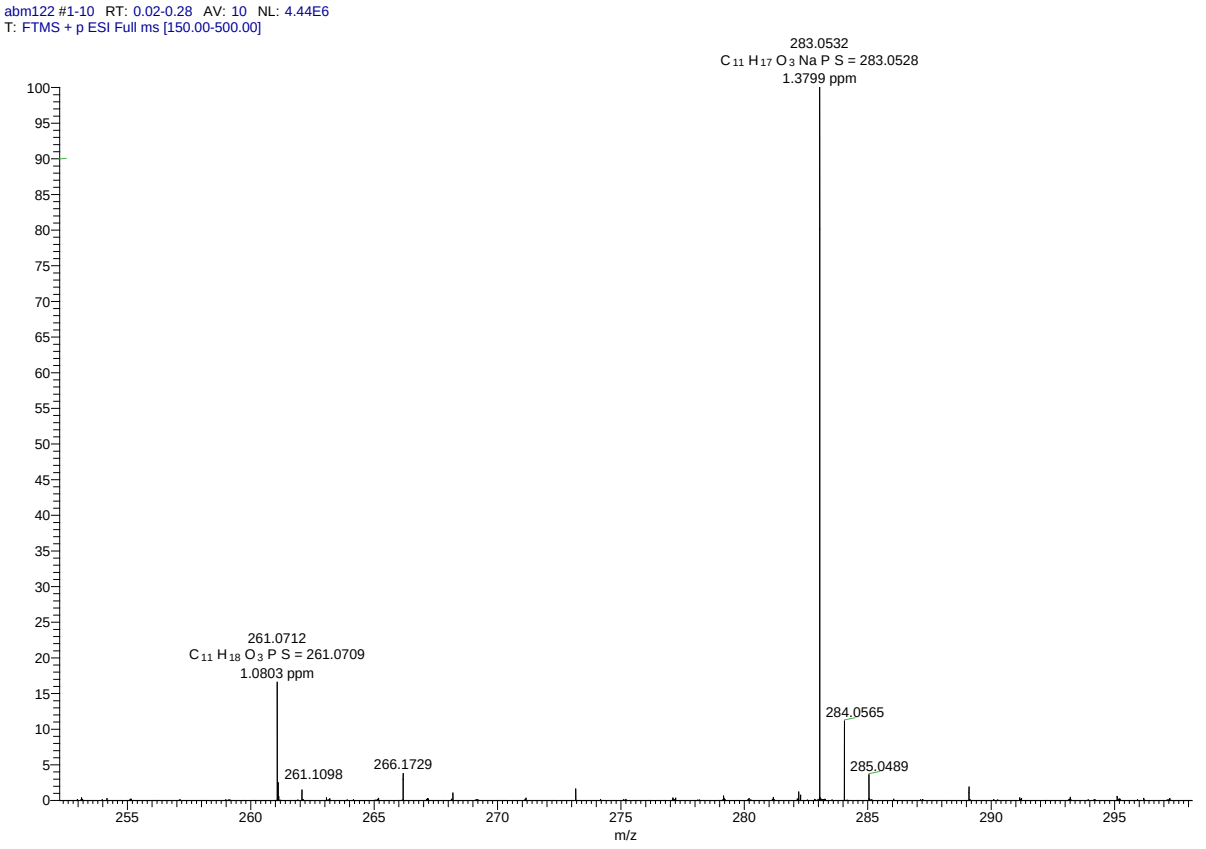
8b, 1-mercapto-4-phenylbutan-2-ylphosphonic acid





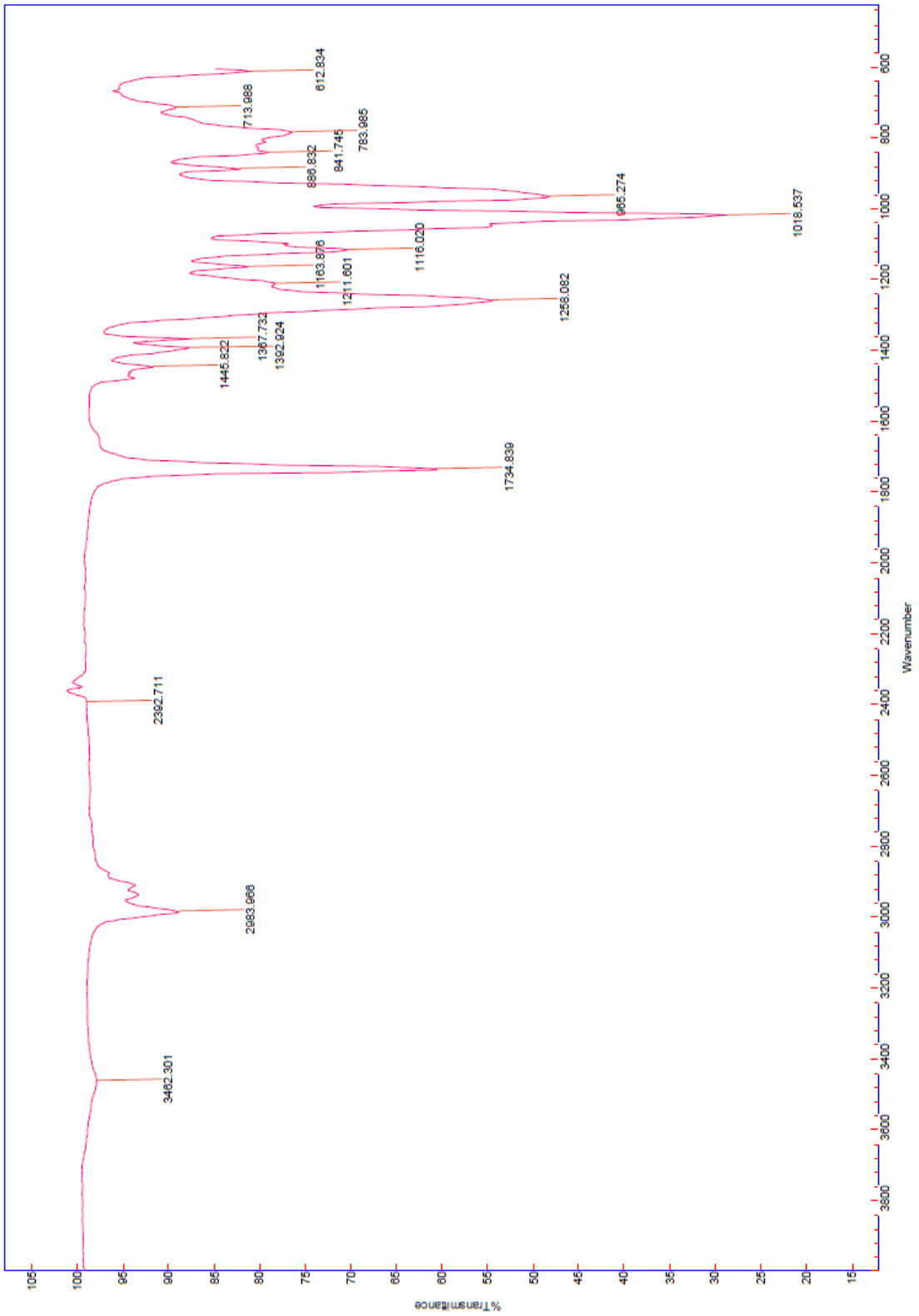
8c, 1-mercapto-5-phenylpentan-2-ylphosphonic acid



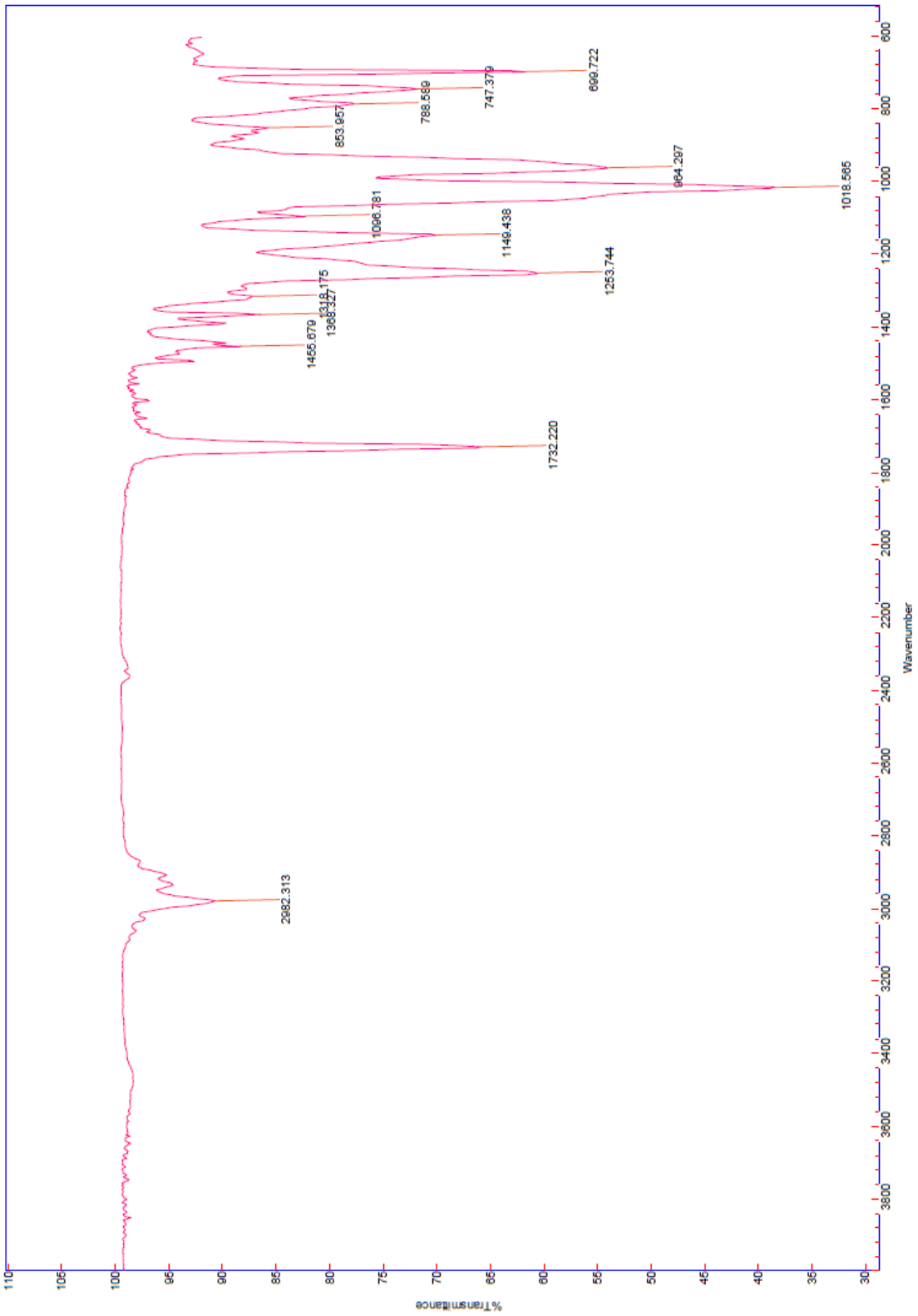


7.3 IR Spectra

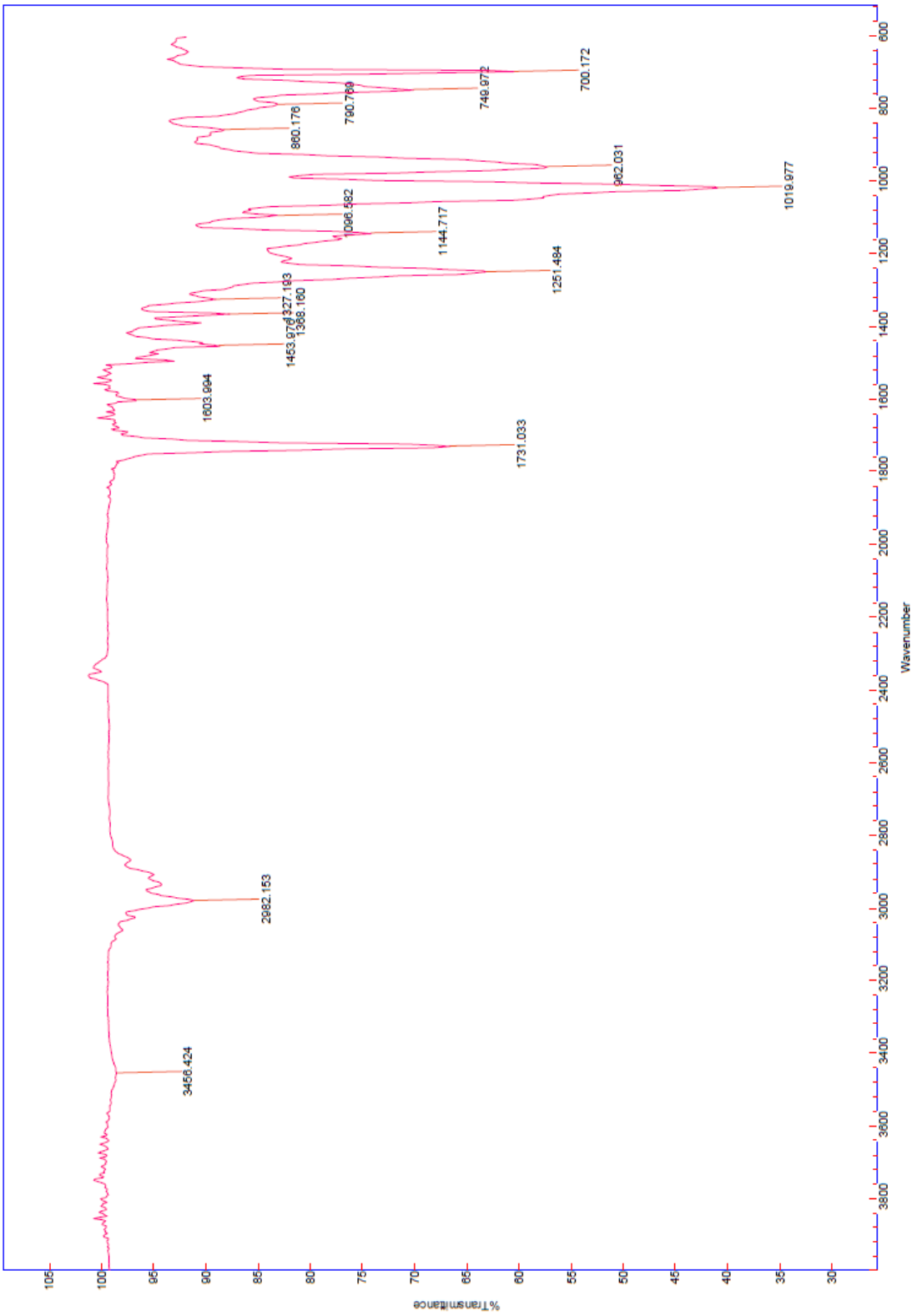
1, Ethyl 2-(diethoxyphosphoryl)acetate



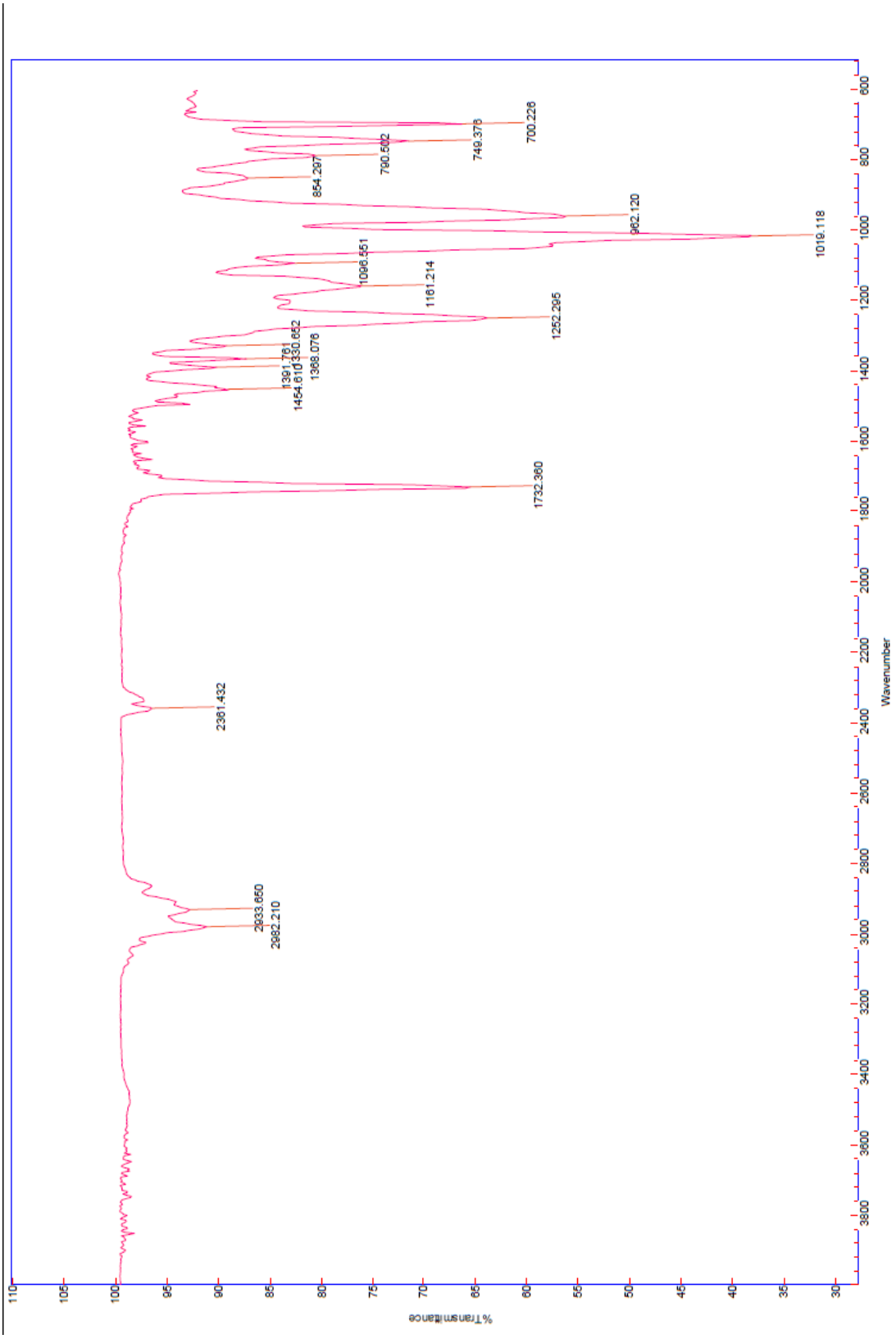
2a, Ethyl 2-(diethoxyphosphoryl)-3-phenylpropanoate



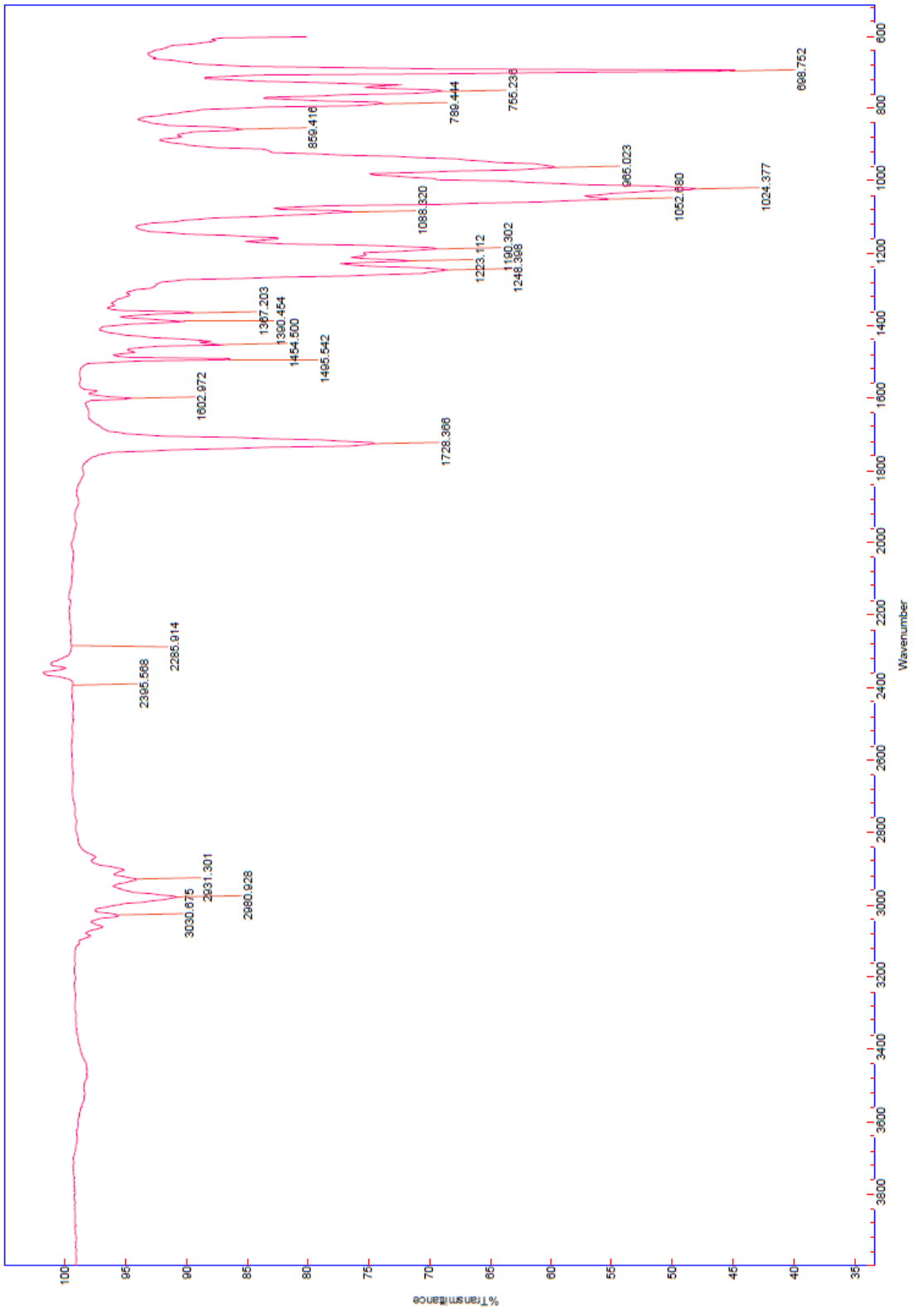
2b, Ethyl 2-(diethoxyphosphoryl)-4-phenylbutanoate



2c Ethyl 2-(diethoxyphosphoryl)-5-phenylpentanoate

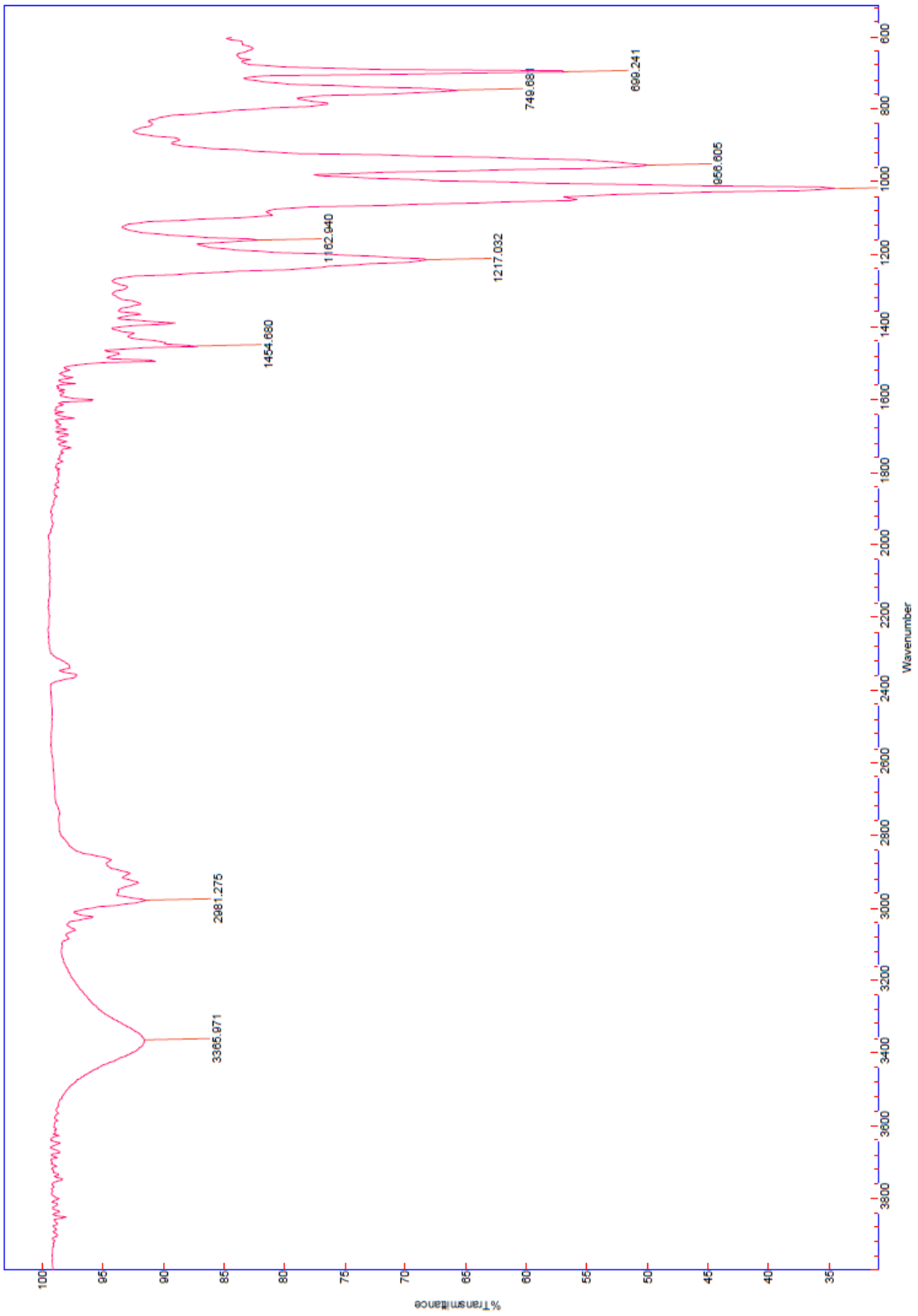


2d, Ethyl 2-benzyl-2-(diethoxyphosphoryl)-3-phenylpropanoate

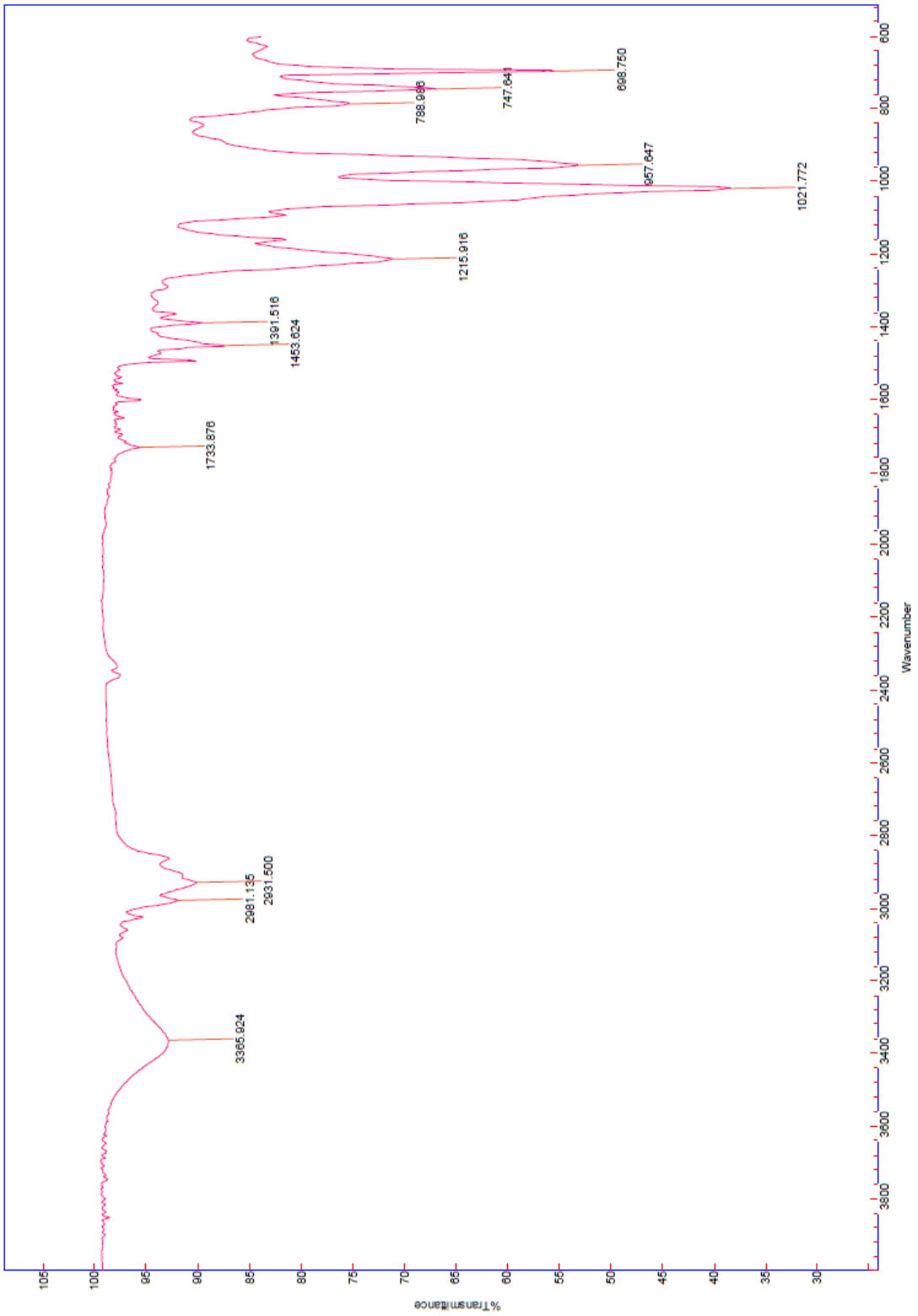


3a, Diethyl 1-hydroxy-3-phenylpropan-2-ylphosphonate

3b, Diethyl 1-hydroxy-4-phenylbutan-2-ylphosphonate

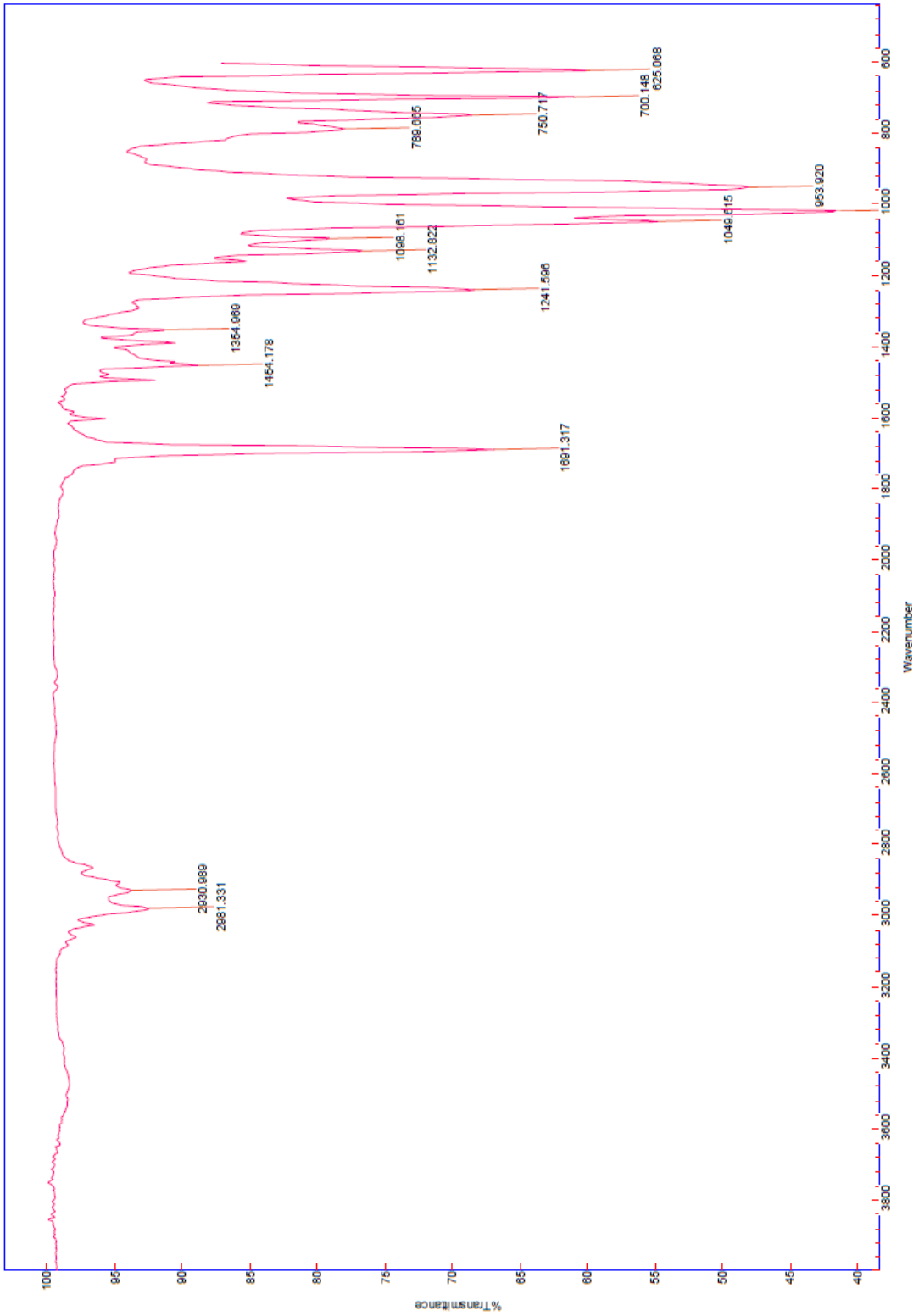


3c, Diethyl 1-hydroxy-5-phenylpentane-2-ylphosphonate

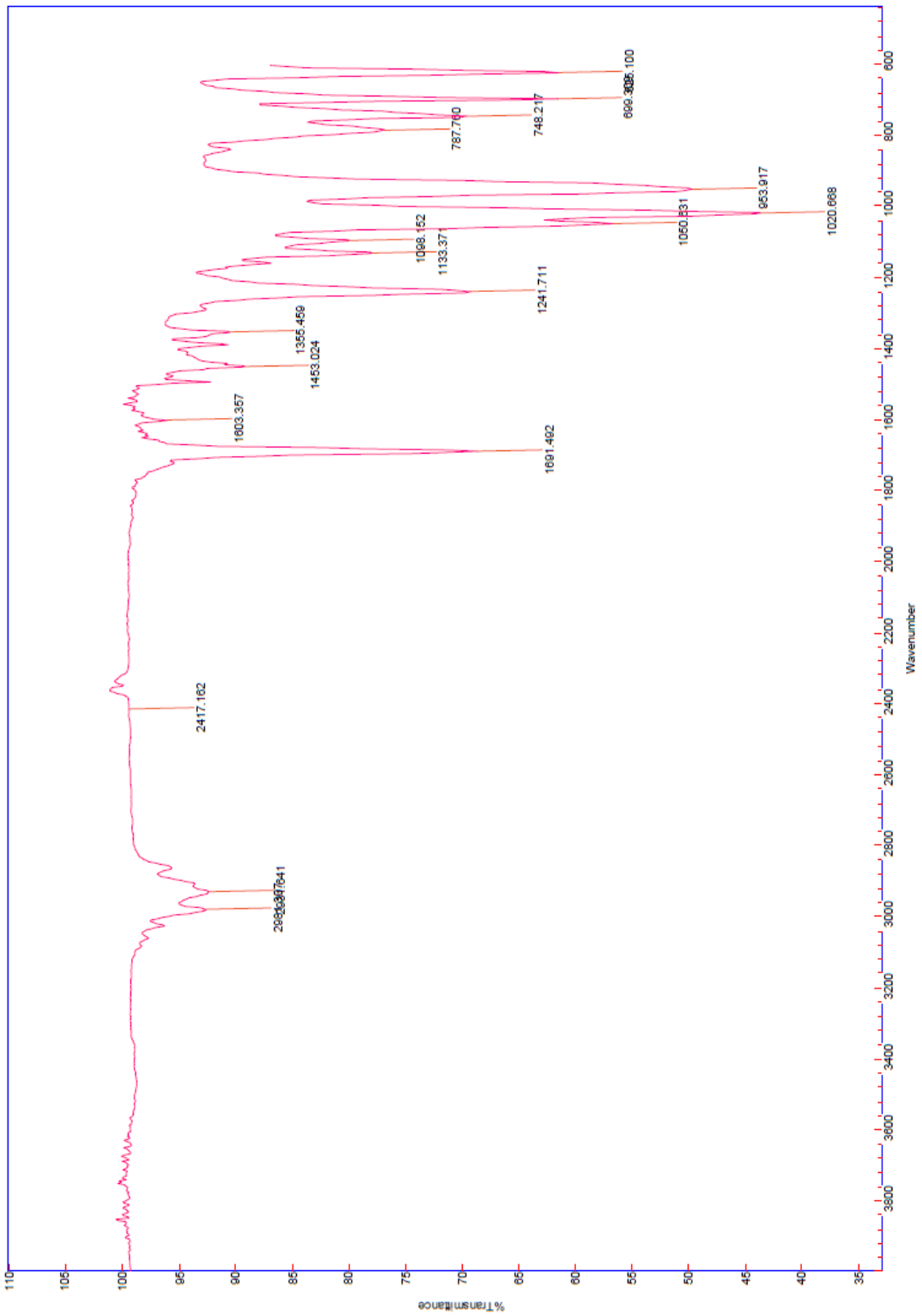


4a, S-2-(diethoxyphosphoryl)-3-phenylpropyl ethanethioate

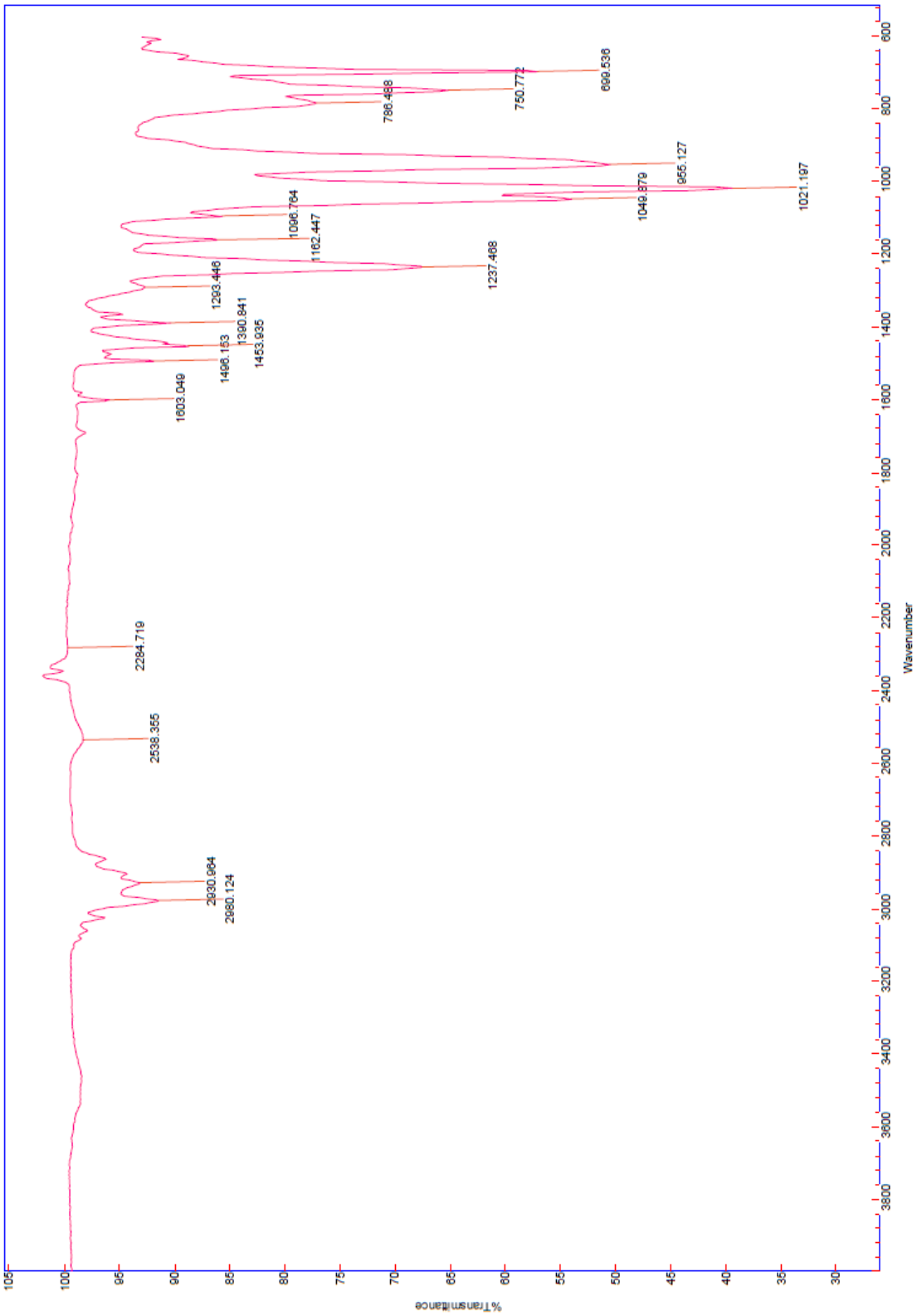
4b, S-2-(diethoxyphosphoryl)-4-phenylbutyl ethanethioate



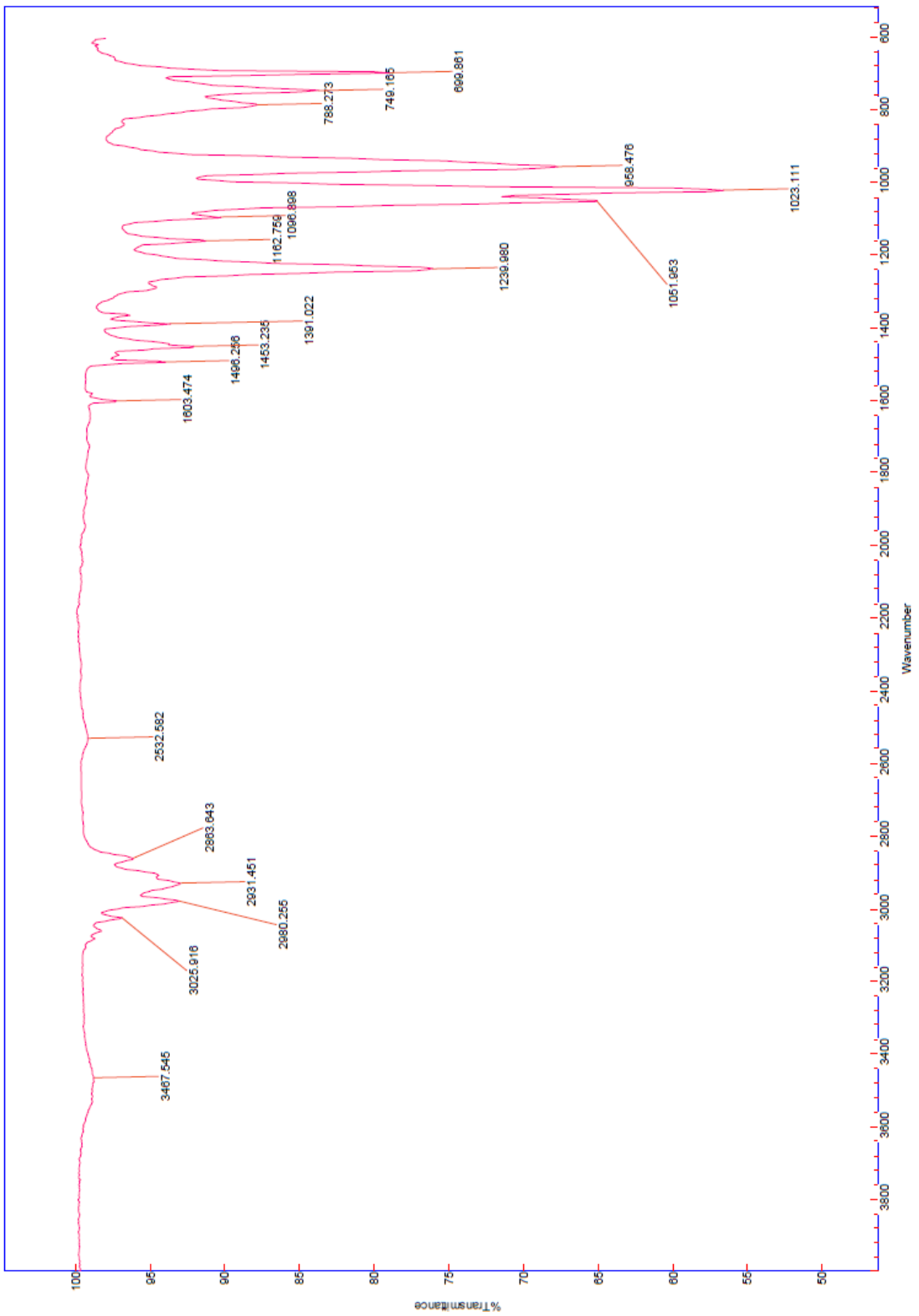
4c, S-2-(diethoxyphosphoryl)-5-phenylpentyl ethanethioate



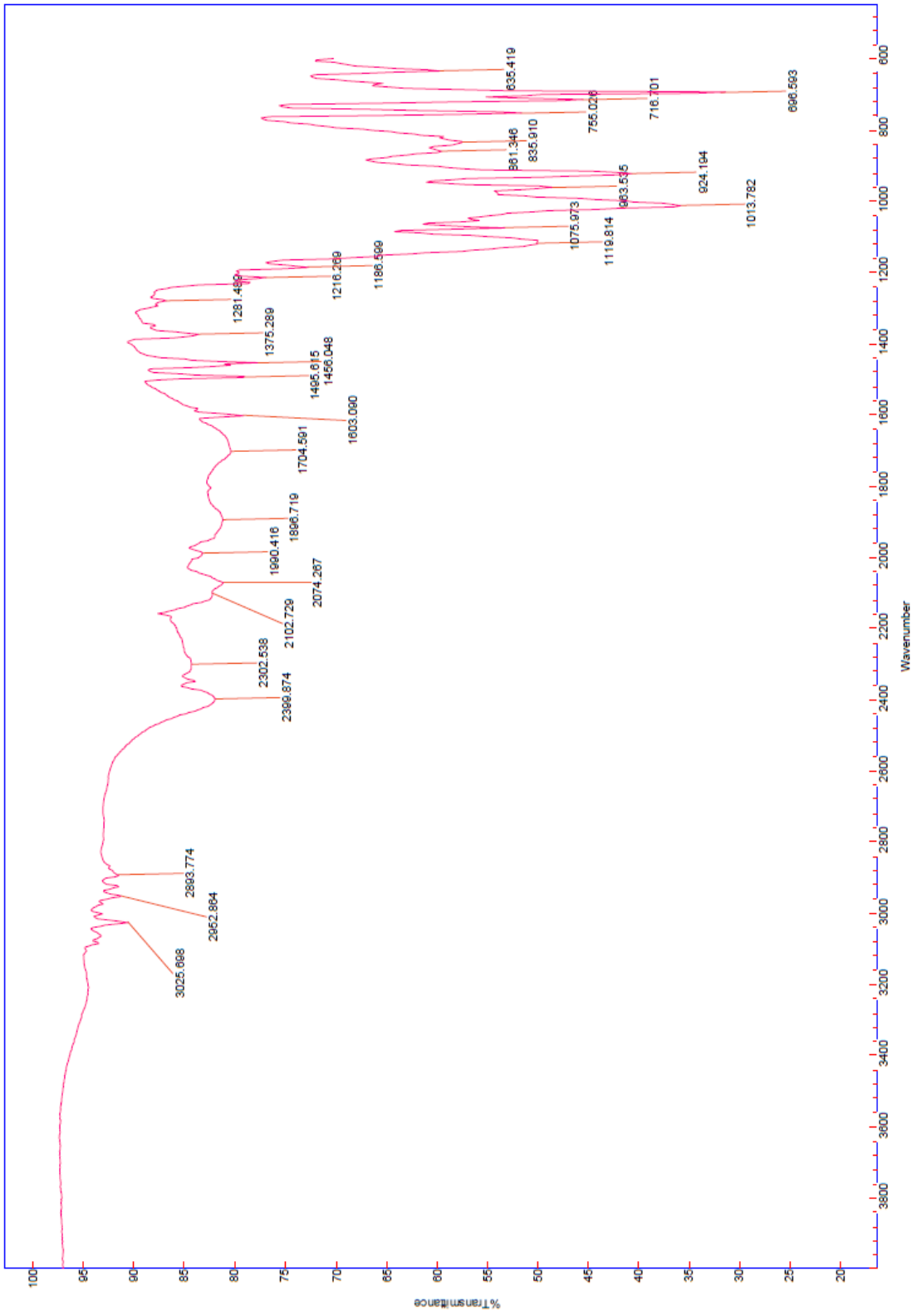
5b, Diethyl 1-mercapto-4-phenylbutan-2-ylphosphonate



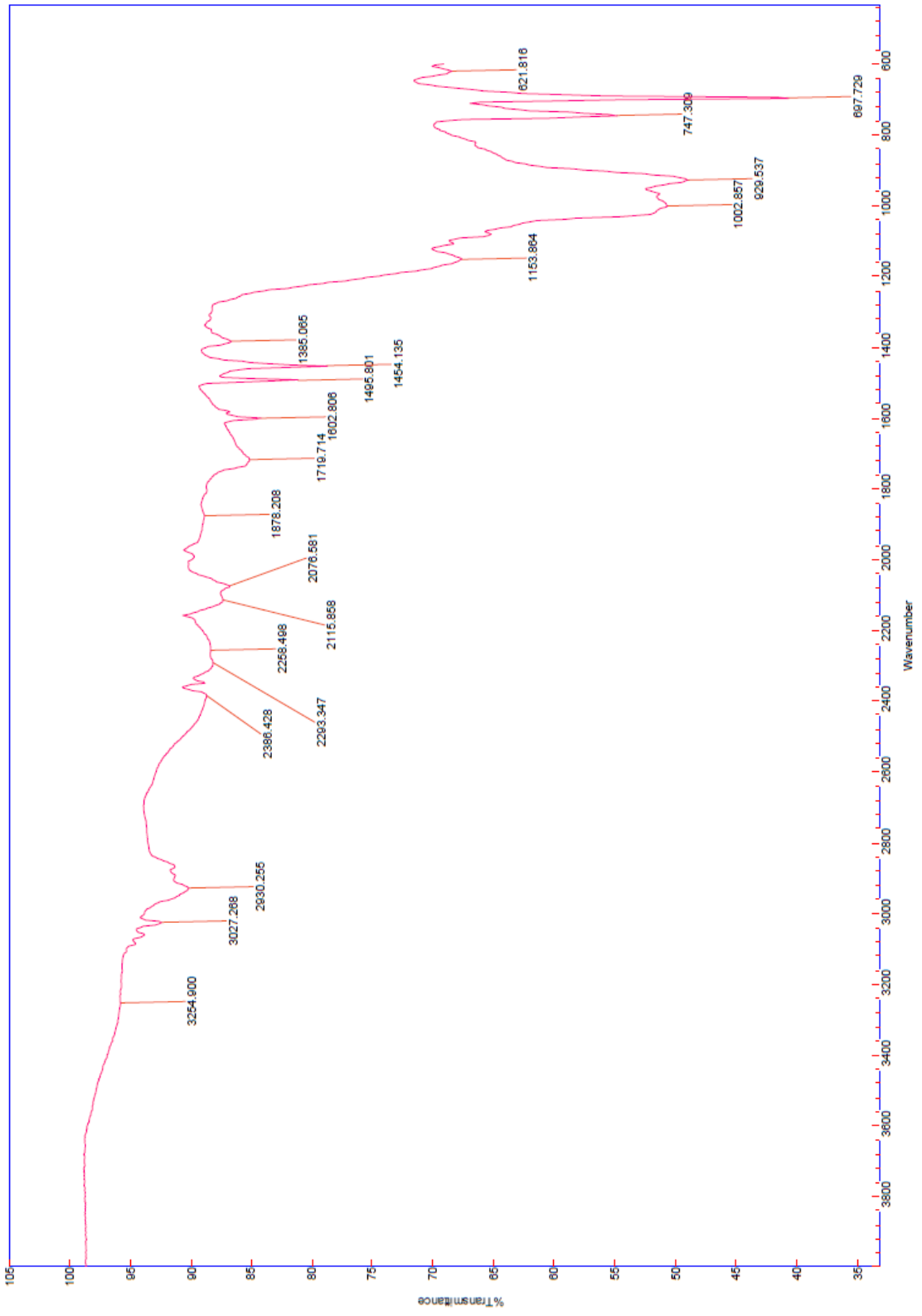
5c, Diethyl 1-mercapto-5-phenylpentan-2-ylphosphonate



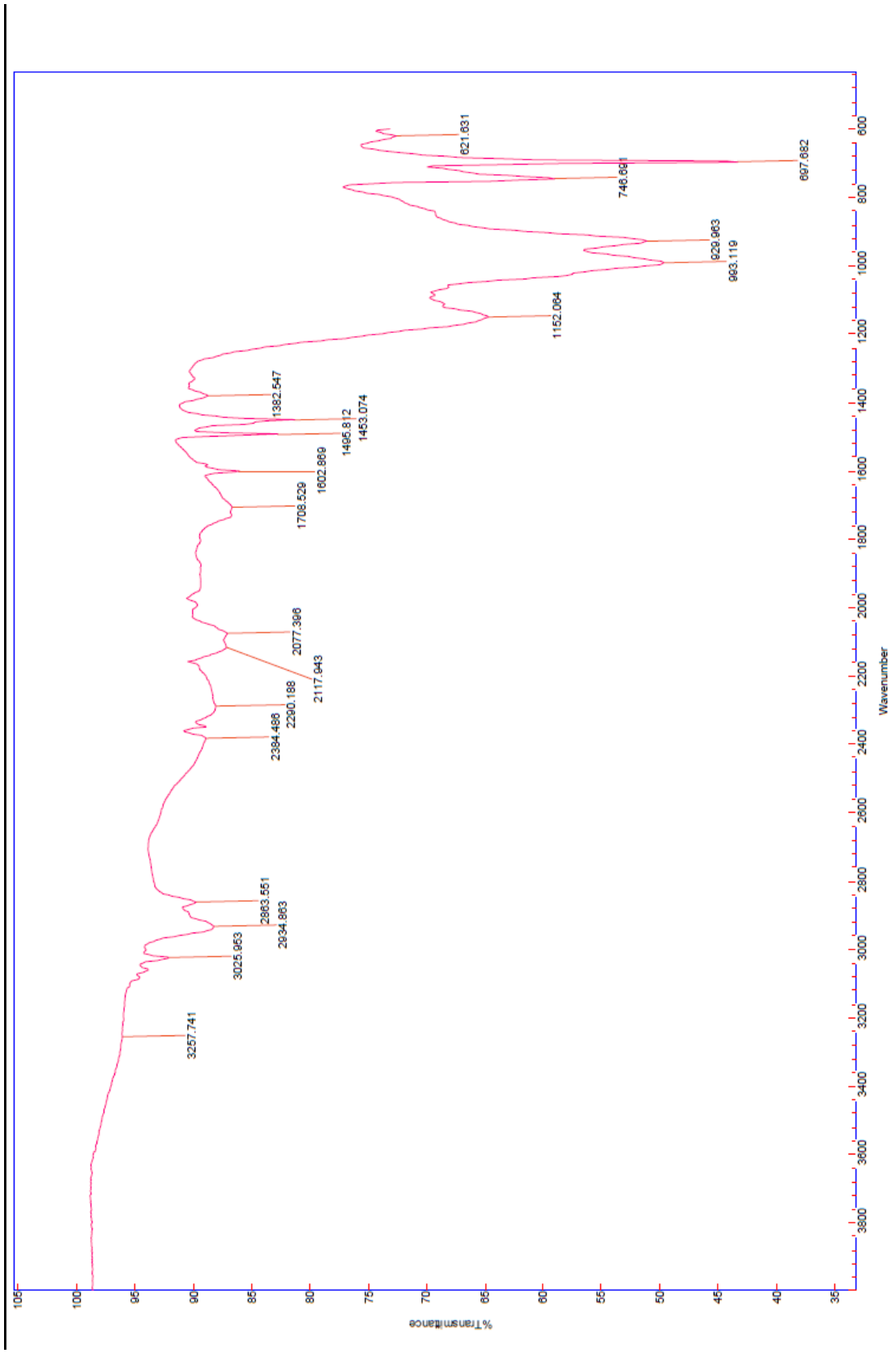
6a, 1-hydroxy-3-phenylpropan-2-ylphosphonic acid



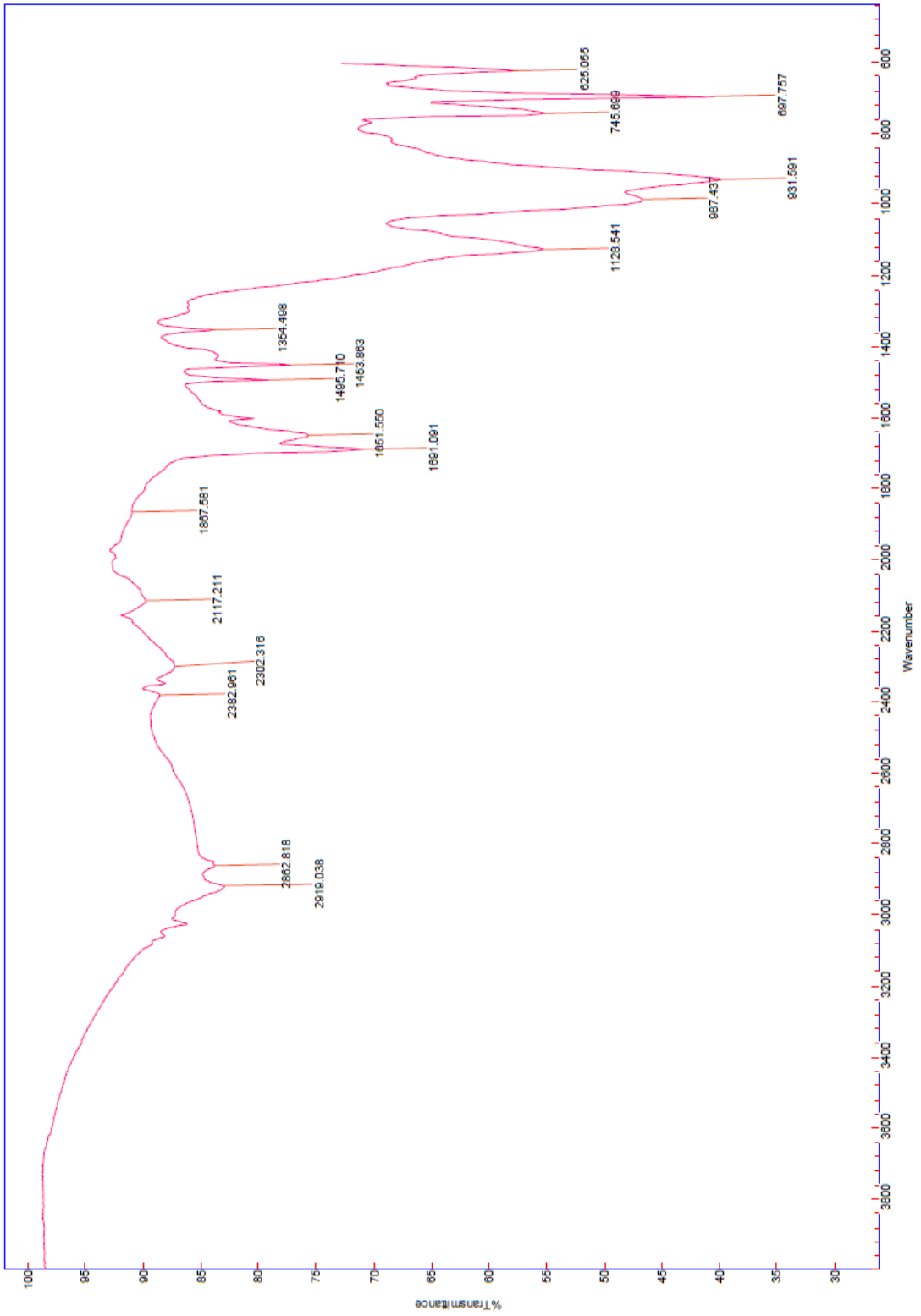
6b, 1-hydroxy-4-phenylbutan-2-ylphosphonic acid



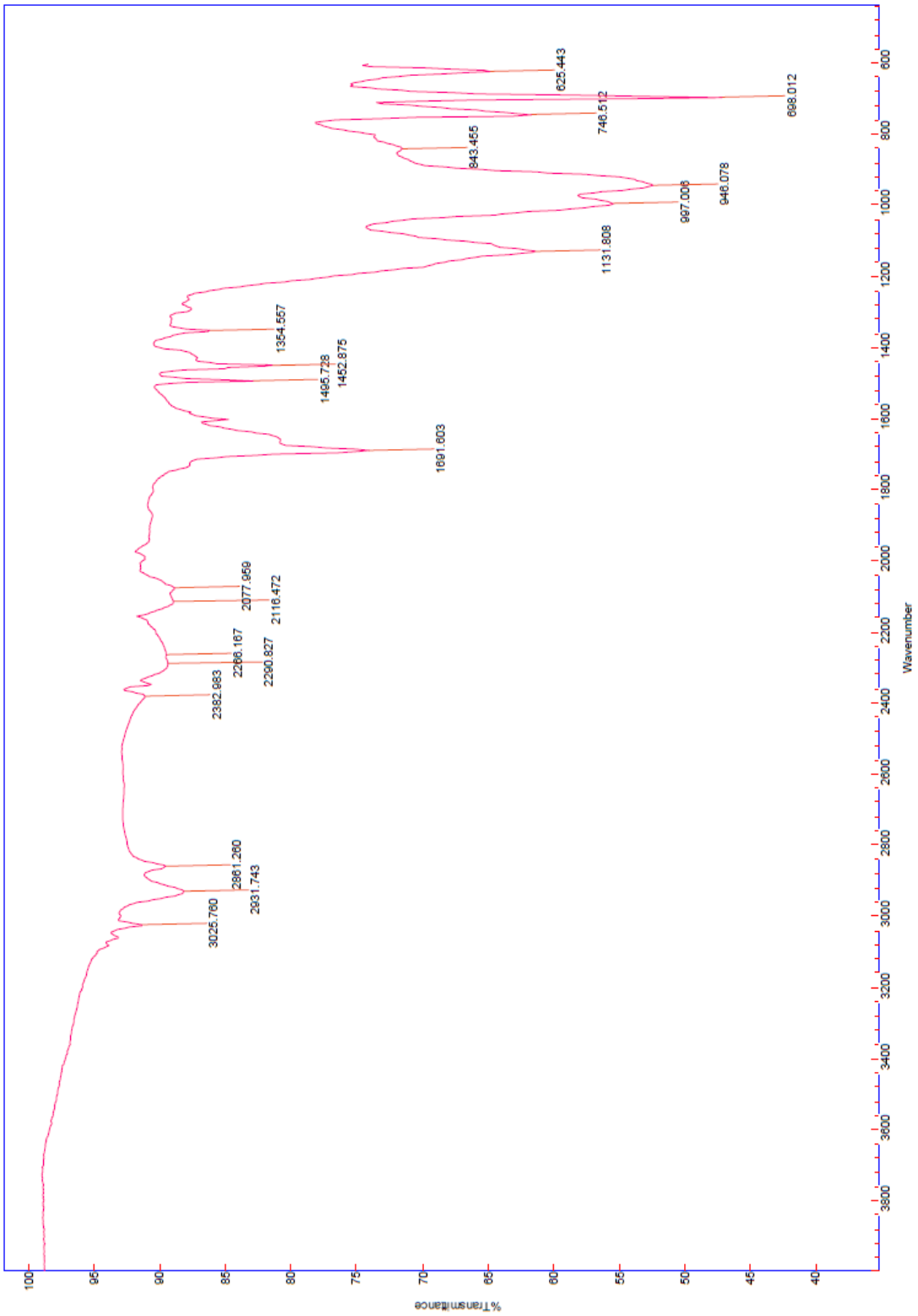
6c, 1-hydroxy-5-phenylpentane-2-ylphosphonic acid



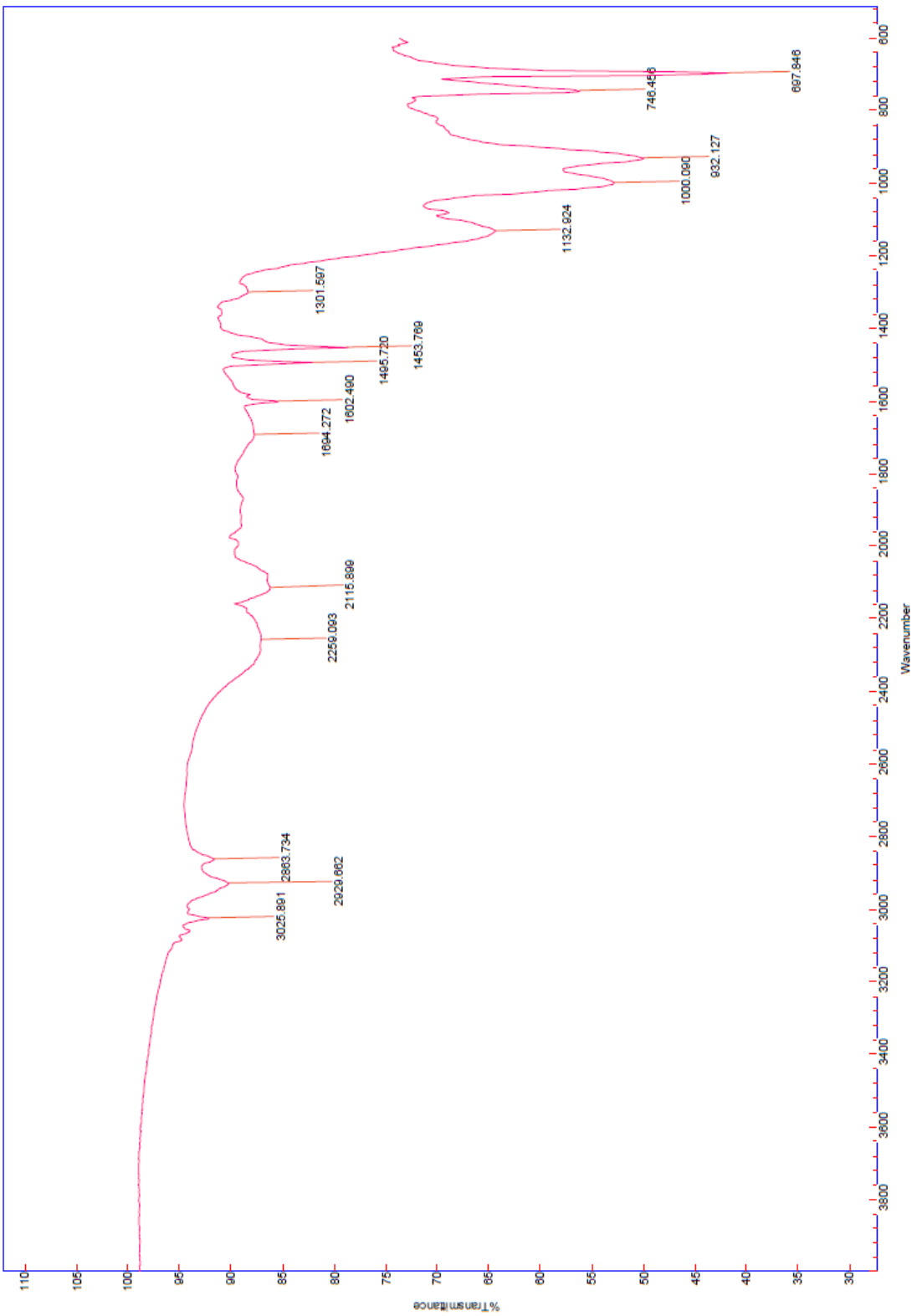
7b, 1-(acetylthio)-4-phenylbutane-2-ylphosphonic acid



7c, 1-(acetylthio)-5-phenylpentan-2-ylphosphonic acid



8b, 1-mercapto-4-phenylbutan-2-ylphosphonic acid



8c, 1-mercapto-5-phenylpentan-2-ylphosphonic acid