Faculty of Chemistry UAM

Individual Research Project

Phosphonate analogues of fluorophenylglycine: Synthesis and structural studies

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ABSTRACT

α-Aminophosphonates are analogues of amino acids with a big potential for biological activity. In addition, fluorination of these molecules can provide them interesting changes in their properties.
This paper reviews previously published data and presents new results to support the synthesis of fluorinated α-aminophosphonates using a variant of Kabachnik-Fields reaction based on a one-pot two step synthesis.

Keywords: α-aminophosphonates, Kabachnik Fields reaction, fluorinated aminophosphonates

1. Introduction

α-Aminophosphonates are structural and isoelectronic [1] analogues of natural amino acids. Due to their biological activities, they are very interesting for synthetic organic chemistry, and they have received special attention because of their wide range of different applications. Among them, α-aminophosphonates have been used as powerful corrosion inhibitors [2,3], anticancer agents, enzyme inhibitors [4,5], pharmacological agents [6], and in agriculture as herbicides and insecticides [7,8].
Because of this, lots of methods have been designed for their synthesis. One of them is the three-component Kabachnik-Fields reaction, which can be carried out using microwave irradiation, under solvent-free conditions or applying different kinds of catalysts. However, a limited attention has been paid to the addition of fluorine to organic molecules such as the fluorinated α-aminophosphonates. Presence of fluorine in molecules can provide interesting changes in their physical, chemical and biological properties. Therefore, fluorinated α-aminophosphonates can be used to synthesize compounds with potential biological activity [9-11].

2. Literature part

2.1 Aminophosphonic acids

Aminophosphonic acids are broadly defined as analogues of amino acids, in which the less bulky carboxylic acid (CO_2H) group has been replaced by a sterically more hindered tetrahedral phosphonic acid (PO_3H_2) group. They can be considered as important metabolites due to their negligible mammalian toxicity and their efficient mimicry of amino acids.

Because of their structural similarities, carboxylic and phosphonic acids compete for the active sites of enzymes and other cell receptors. However, they differ in shape (planar versus tetrahedral geometry), acidity (phosphonic acid is significantly more acidic), and steric bulk (phosphorus atom has bigger radius than carbon atom), what defies the intuitive understanding of their structural analogy [9,12].

2.2 α-Aminophosphonates

α-Aminophosphonates are esters of aminophosphonic acids, and very significant bioisosteres of amino acids, thus displaying a broad spectrum of biological applications [7,8].

2.2.1 Biological activity of α-aminophosphonates

Recently, due to the bacterial resistance increase, the currently available for medical use antibiotics and chemotherapeutics are not sufficient and it has been necessary to search for new antimicrobial substances [7]. α-Aminophosphonates seem to be an interesting solution for this problem due to their wide variety of promising physiological activities, enabling to apply these compounds in different medicinal fields [12].

2.2.3 α-Aminophosphonates as inhibitors

One of the most interesting applications of α-aminophosphonates is their use as enzyme inhibitors. This activity is attributed to the tetrahedral geometry of the substituents around the phosphorous moiety that mimic the tetrahedral high-energy transition state of the peptide bond hydrolysis, what benefits the inhibition of a wide range of proteases and ligases.
Chirality of the molecules plays an important role in this kind of enzyme inhibition. Nowadays, it is well-known that the biological activity of the α-aminophosphonates depends on the absolute configuration of the stereogenic α-carbon bonded to the phosphorus atom. For example, (R)-phospholeucine is a more potent inhibitor of leucine aminopeptidase than (S)-phospholeucine.
Stereochemistry may be induced in the α-aminophosphonates through a source of phosphorus, an amine, a carboxylic group, or using a chiral catalyst [4,5].

2.3 Fluorine in organic compounds

Fluorine is a curious element with unique properties such as high electronegativity, electron density, blocking and lipophilic effects [13]. In consequence, the presence of this atom in organic molecules can influence their solubility, chemical reactivity and biological activity [11,14,15]. An example of how fluorine can influence different organic molecules is the combination of a -CF_3 moiety with α-aminophosphonates, which can be an option for modulating the physicochemical and biological properties**,** and improve the metabolic stability of the molecules [16]. Another example is the fluorination of amines for increasing their metabolic stability, that consequently leads to a decrease in their pKa values, and an increase in their lipophilicity.

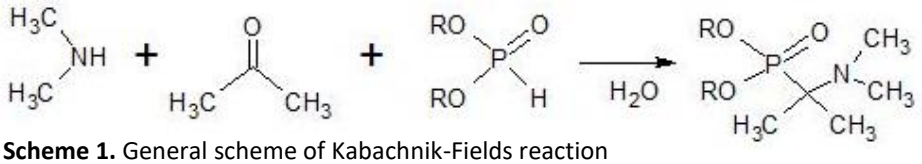
Because of the influence of fluorine in organic molecules, the process of fluorination can be very useful, and the development of new enantioselective methodologies that can lead to enantiopure fluorine compounds is getting a lot of interest within chemical industry [17]. Nucleophilic fluorination is a way of introducing fluorine into organic compounds [18].

2.3.1 Fluorinated amino acids and their analogues

Fluorinated amino acids and their analogues are of special interest for the design of new fluorine-containing peptides with unusual folding patterns [19]. The amino acids fluorination has also effects on protein stability and protein-protein interactions since fluorine atoms have unique stereoelectronic properties. Some previous approaches to assessing those properties have been focused on helical systems, in which fluoro-amino acids have shown lower intrinsic helix activity than their hydrocarbon analogues. Within globular proteins, fluorination has been carried out in specific β-sheet positions with a consequent stabilizing effect. This suggests that fluorinated amino acids may be suitable for modulating non-helical structures [20].
In protein biochemistry, recognition of different mechanisms can be accomplished by different fluorinated molecules. The interactions of amino acids within the proteins can be studied replacing natural amino acids with their fluorinated analogues and monitoring with ^{19}F NMR spectroscopy [16], since ^{19}F NMR spectroscopy can be used in the investigation of the interactions between fluorinated compounds and biological systems [18].

2.4 Kabachnik-Fields Reaction

Two important methods for synthesize α-aminophosphonates are Kabachnik-Fields reaction (Scheme 1) and aza-Pudovik reaction. The three-component Kabachnik-Fields reaction involves the reaction of primary or secondary amines, oxo compounds such as aldehydes or ketones and >P(O)H species, specially dialkyl phosphites. On the other hand, in aza-Pudovik reaction a >P(O)H reagent is added to the C=N double bond of an imine [21,22].
The three-component Kabachnik-Fields reaction can be carried out through different ways including synthesis under microwave irradiation, under solvent-free conditions, and applying different catalysts such as Lewis and Bronsted acids. Nevertheless, the use of organic solvents can cause a serious impact on the environment, and due to the increasing demand of environmentally friendly synthesis, the solvent-free method is taking importance.
Kabachnik-Fields reaction may be carried out as one-pot procedure where the three components are mixed together [23-26].
Cherkasov et al. studied carefully the mechanism of Kabachnik-Fields reaction concluding that two different possibilities could occur. In the first one, an imine is formed in the reaction between a carbonyl compound and an amine, then the dialkyl phosphite is added on the C=N unit of the intermediate. The second possibility assumes the formation of an α-hydroxyphosphonate by the addition of the dialkylphosphite to



Scheme 1. General scheme of Kabachnik-Fields reaction

the carbonyl group of the oxo compound. Then the hydroxyphosphonate suffers a substitution by the amine to achieve the desired α-aminophosphonate [10,27]. However, some years later Zefirov and Matveeva proved that there's no real experimental evidence for the hydroxyphosphonate route [28].

3. Aim of the work

The aim of this work is to synthesize different phosphonate analogues of fluorophenylglycine following a variant of the Kabachnik-Fields Reaction with the later characterization of the final products by using different spectroscopic methods.

4. Results and discussion

Typical Kabachnik-Field's reactions are carried out in just one step, but the synthesis of the different fluorinated α-aminophosphonates (Table 1) was based on the results obtained earlier [1], and was performed in two steps. The first one was the mixing of pentafluorobenzaldehyde with an aniline, using anhydride toluene as solvent, and the second one was the addition of diethyl phosphite to the reaction mixture.

Table 1. Name of synthesized compounds.

Symbol	Product
1a	Diethyl(((4-fluorophenyl)amino)(perfluorophenyl)methyl) phosphonate
1b	Diethyl(((4-chlorophenyl)amino)(perfluorophenyl)methyl) phosphonate
1c	Diethyl(((4-nitrophenyl)amino)(perfluorophenyl)methyl) phosphonate
1d	Diethyl((trans-1,2-diaminciclohexane)(perfluorophenyl) methyl) phosphonate

4.1 Reaction of 2,3,4,5,6-pentafluorobenzaldehyde with p-chloroaniline

Preparation of perfluorinated α-aminophosphonate **1a** was carried out mixing the commercially available 2,3,4,5,6-pentafluorobenzaldehyde with p-chloroaniline and diethyl phosphite.
Once synthesized, the final product was analysed by GC-MS showing different signals in the mass spectra. The most relevant signals located at m/z = 306.4 and m/z = 444, corresponded to the protonated imine ion and the protonated molecular ion respectively. The analysed mass spectra also showed some fragment ion signals of less importance as the signal located at m/z = 111, which corresponded to the chlorobenzene fragment **(Fig. 1)**.

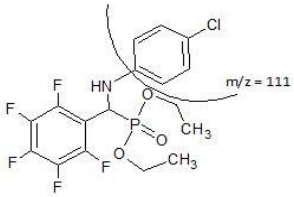


Fig. 1 Possible fragmentation of α-aminophosphonate **1a**

4.2 Reaction of 2,3,4,5,6-pentafluorobenzaldehyde with p-nitroaniline

The synthesis of the α-aminophosphonate **1b** was done mixing 2,3,4,5,6-pentafluorobenzaldehyde, p-nitroaniline and diethyl phosphite.
The final product was analysed by GC-MS. The mass spectra showed signals located at m/z = 455 and 333.4. The first one corresponded to the protonated molecular ion, and the second one to the protonated imine ion. The mass spectra also showed fragment ion signals corresponded to the loss of

4.3 Reaction of 2,3,4,5,6-pentafluorobenzaldehyde with p-fluoroaniline

The α-aminophosphonate **1c** resulted from the reaction between 2,3,4,5,6-pentafluorobenzaldehyde, p-fluoroaniline and diethyl phosphate, was analysed by GC-MS. The mass spectra showed two relevant signals. The first one placed at m/z = 290.4 corresponded to the protonated imine ion, and the second one located at m/z= 428, corresponded to the protonated molecular ion. There were also some fragment ions signals corresponded to the fluorobenzene fragment at m/z = 95, and to the loss of both $HP(O)(OEt)_2$ and perfluorophenyl from the molecular ion at m/z = 123 **(Fig. 2)**.

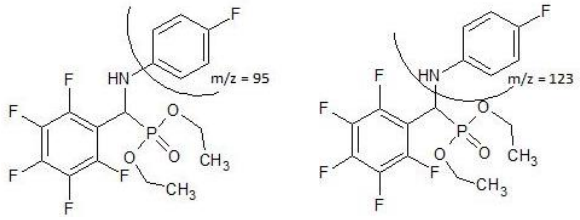


Fig. 2 Possible fragmentations of α-aminophosphonate **1c**

4.4 Reaction of 2,3,4,5,6-pentafluorobenzaldehyde with trans-1,2-diaminecyclohexane

2,3,4,5,6-pentafluorobenzaldehyde was subjected to reaction with 1,2-diaminecyclohexane and diethyl phosphite. The obtained product was analysed by GC-MS. The resulted mass spectra revealed the presence of the expected compound **1d**.
In the spectra, the signal located at m/z = 431 corresponded with the protonated molecular ion of the α-aminophosphonate formed and the signal located at m/z = 293.4 corresponded with the protonated imine ion.

5. Conclusion

Four perfluorophenyl phosphonate analogues of phenylglycine were synthesized using a variant of Kabachnick-Fields reaction. In this variant the reaction was carried out in two step reactions. Four products were obtained, from which three were characterised by the use of spectroscopic and spectrometric methods. These three α-aminophosphonates were synthesized using aromatic amines. The one was synthesized using an aliphatic amine. The obtained amount of product was not relevant.

It can be concluded that this method is more useful for synthesize α-aminophosphonates which include aromatic amine unit.

6. Experimental part

To the mixture of pentafluorobenzaldehyde in dry toluene (10 mL), aniline or amine was added under an Ar atmosphere. The reaction mixture was heated under reflux for 4h, then $HP(O)(OEt)_2$ was added, and the mixture was heated under reflux for 16h. After cooling to room temperature, the solvent was removed under reduced pressure, and the crude product was purified by chromatography (silica gel, cyclohexane/ethyl acetate 1:1, v/v).

5.1. Diethyl (((4-fluorophenyl)amino) (perfluorophenyl)methyl) phosphonate (**1a**)

From 2,3,4,5,6-pentafluorobenzaldehyde (0.10 g, 0.51 mmol), p-fluoroaniline (0.0489 ml, 0.51 mmol), $HP(O)(OEt)_2$ (13 µl, 0.10 mmol), the final product was obtained. ^{1}H NMR (403 MHz, $CDCl_3$) δ = 6.88 (m, 2H, Ph), 6.61 (m, 2H, Ph), 5.18 (dd, 1H, J = 15, 11 Hz, -CH-), 4.58 (m, 1H, -NH-), 4.32-4.05 (m, 4H, -OC<u>H</u>$_2$-), 1.35 (td, 3H, J = 7, 0.6 Hz, -OCH$_2$C<u>H</u>$_3$), 1.26 (td, 3H, J = 7.1, 0.5 Hz, -OCH$_2$C<u>H</u>$_3$) ppm. ^{19}F NMR (282 MHz, $CDCl_3$) δ = -125.5 to -125.7 (m, 1F), -143.2 to -143.4 (m, 2F), -153.9 to -154.3 (m, 1F), -161.8 to -162.1 (m, 2F) ppm. ^{31}P NMR (121 MHz, $CDCl_3$) δ = 19.80-19.50 ppm.

5.2. Diethyl (((4-chlorophenyl)amino) (perfluorophenyl)methyl) phosphonate (**1b**)

From 2,3,4,5,6-pentafluorobenzaldehyde (0.12 g, 0.61 mmol), p-chloroaniline (0.077 g, 0.60 mmol), $HP(O)(OEt)_2$ (13 µl, 0.10 mmol), a white product was obtained. ^{1}H NMR (403 MHz, $CDCl_3$) δ = 7.12 (dm, 2H, Ph), 6.57 (m, 2H, Ph), 5.19 (dd, 1H, -CH-), 4.65 (m, 1H, -NH-), 4.28-4.19 (m, 2H, -OC<u>H</u>$_2$-), 1.34 (td, 3H, -OCH$_2$C<u>H</u>$_3$), 1.26 (td, 3H, -OCH$_2$CH$_3$) ppm. ^{19}F NMR (282 MHz, $CDCl_3$) δ = -142.30 to -142.70 (m, 2F), -152.4 to -152.6 (m, 1F), -160.50 to -160.80 (m, 2F) ppm. ^{31}P NMR (121 MHz, $CDCl_3$) δ = 18.50-18.00 (m, 1P) ppm.

5.3. Diethyl (((4-nitrophenyl)amino) (perfluorophenyl)methyl) phosphonate (**1c**)

From 2,3,4,5,6-pentafluorobenzaldehyde (0.031 ml, 0.51 mmol), p-nitroaniline (0.030 ml, 0.25 mmol), $HP(O)(OEt)_2$ (13 µl, 0.10 mmol), a yellow crystal was obtained. ^{1}H NMR (403 MHz, $CDCl_3$) δ = 8.11 (m, 2H, Ph), 6.65 (m, 2H, Ph), 5.38 (m, 1H, -NH-), 5.26 (dd, 1H, J = 15, 10 Hz, -NH-), 4.30-4.05 (m, 4H, -OC<u>H</u>$_2$-), 1.35 (td, 3H, J = 7, 0.5 Hz, -OCH$_2$C<u>H</u>$_3$), 1.27 (td, 3H, J = 7, 0.5 Hz, -OCH$_2$C<u>H</u>$_3$) ppm. ^{19}F NMR (282 MHz, $CDCl_3$) δ = -142.40 to -142.60 (m, 2F), -152.40 to -152.60 (m, 1F), -160.50 to -160.80 (m, 2F) ppm. ^{31}P NMR (121 MHz, $CDCl_3$) δ = 17.50–17.00 (m, 1P) ppm.

5.4. Diethyl ((trans-1,2-diaminciclohexane) (perfluorophenyl)methyl) phosphonate (**1d**)

From 2,3,4,5,6-pentafluorobenzaldehyde (0.100 g, 0.51 mmol), trans-1,2-diaminecyclohexane (0.030 ml, 0.25 mmol), $HP(O)(OEt)_2$ (13 µl, 0.10 mmol) the final product was obtained but it wasn't sufficient for doing the different NMR spectra.

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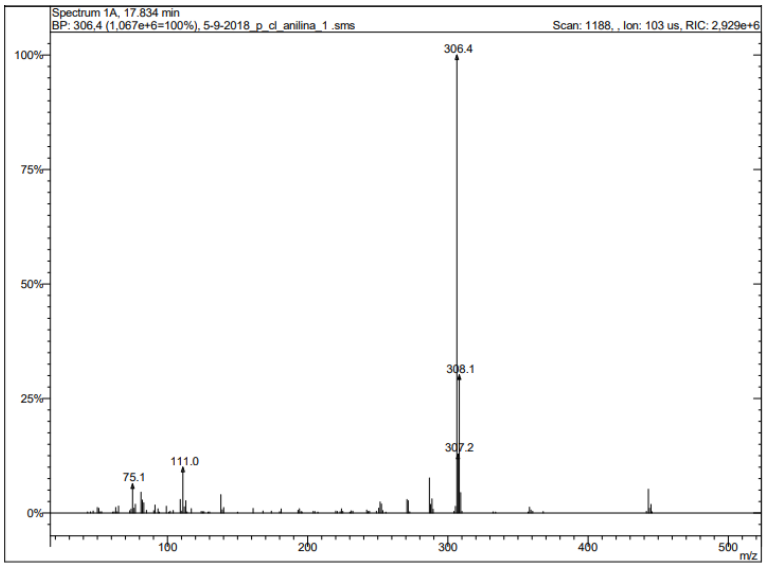
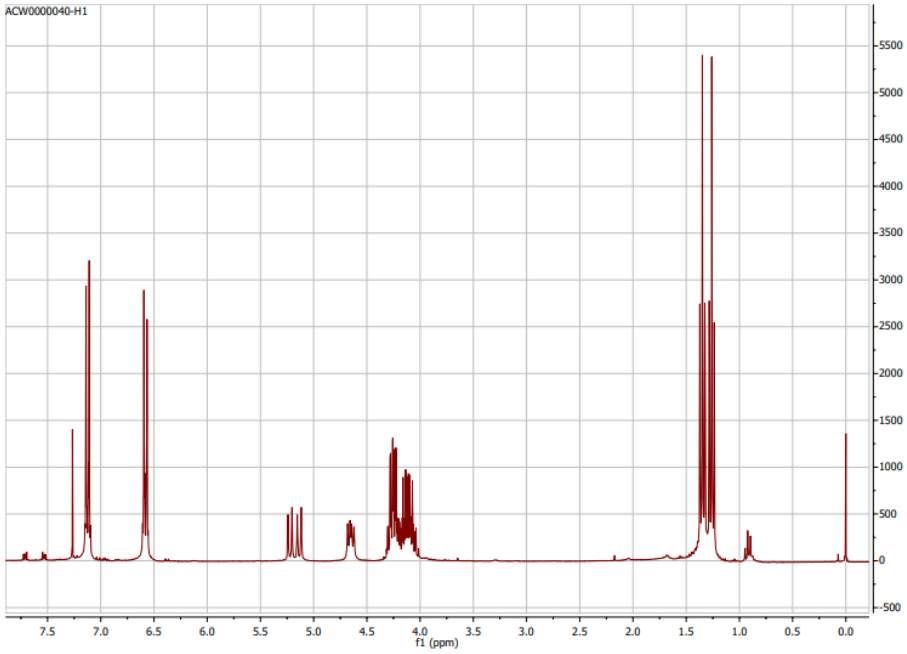
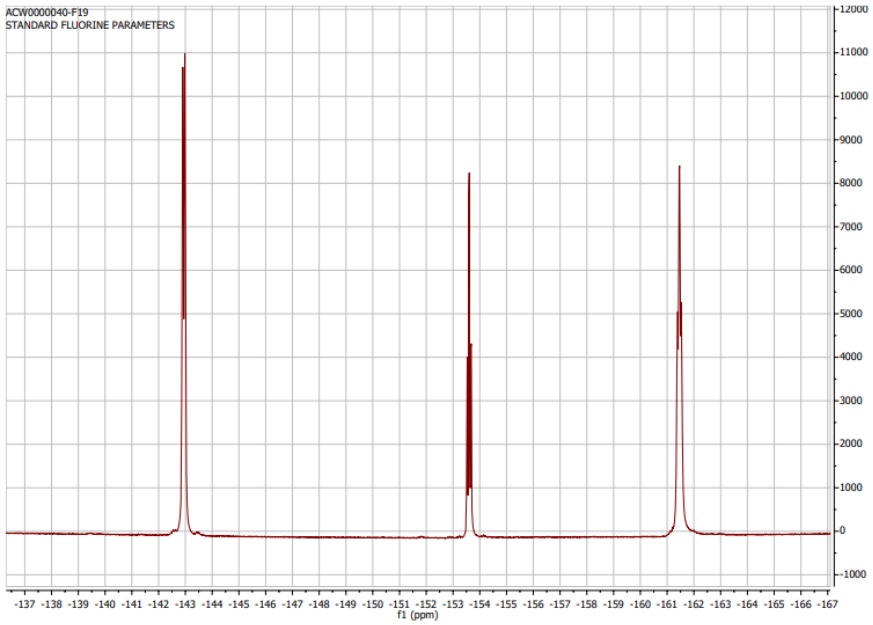
8. Supplementary information



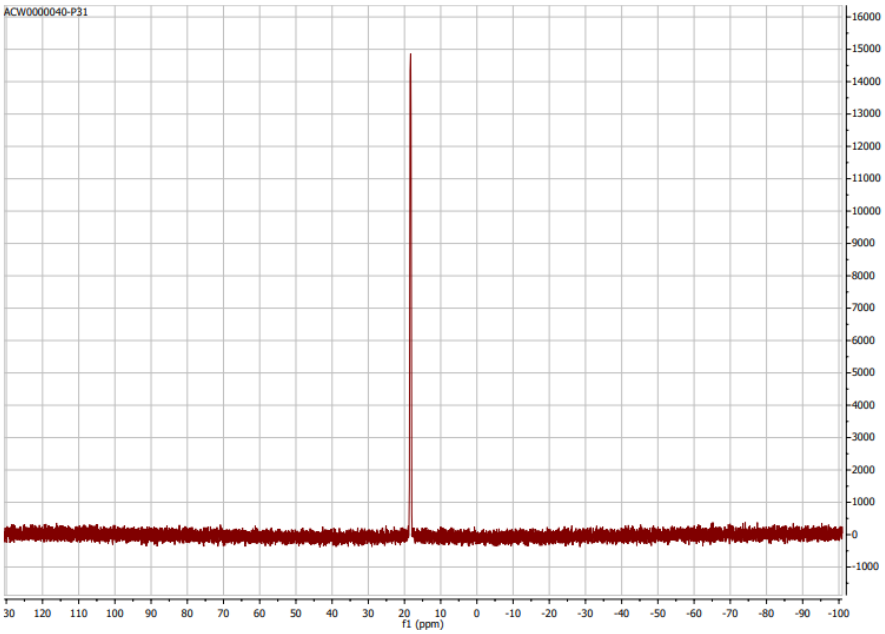
*Mass spectra of α-aminophosphonate **1a***



*^{1}H NMR of α-aminophosphonate **1a***



*^{19}F NMR of α-aminophosphonate **1a***



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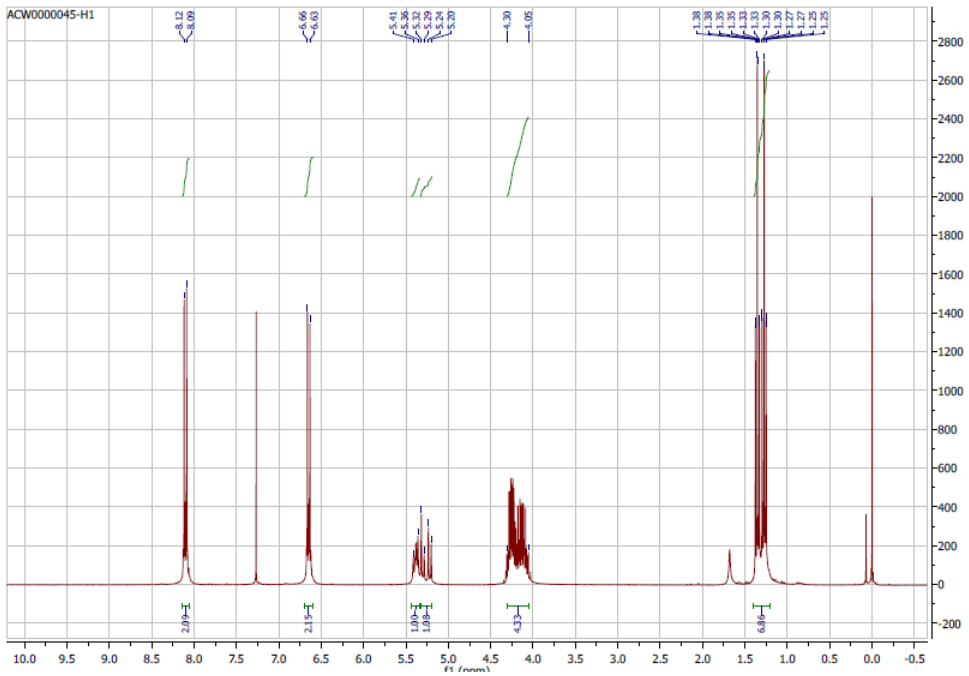

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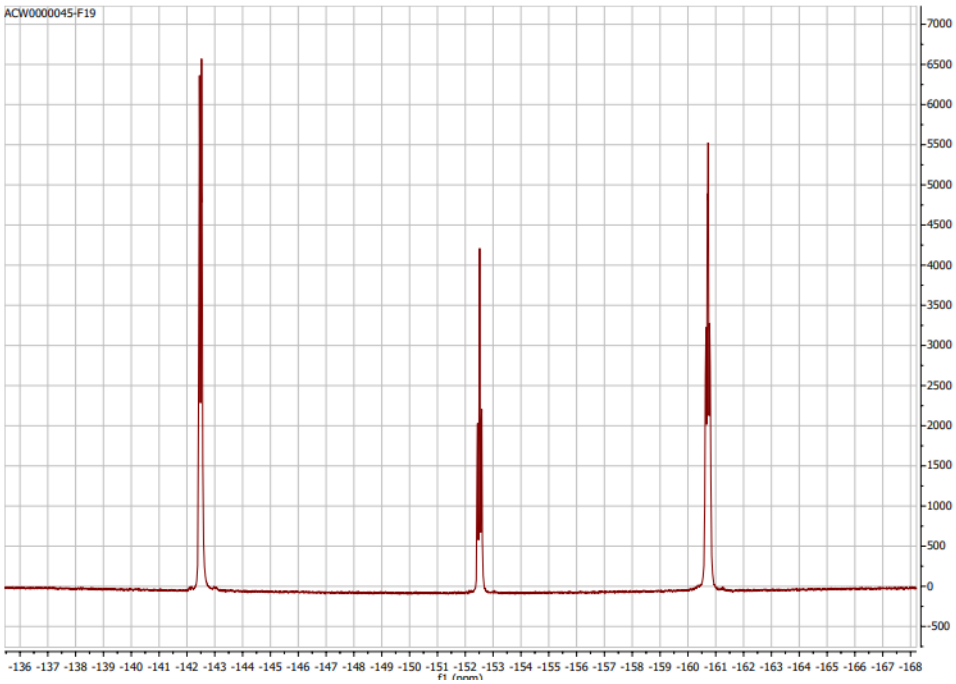

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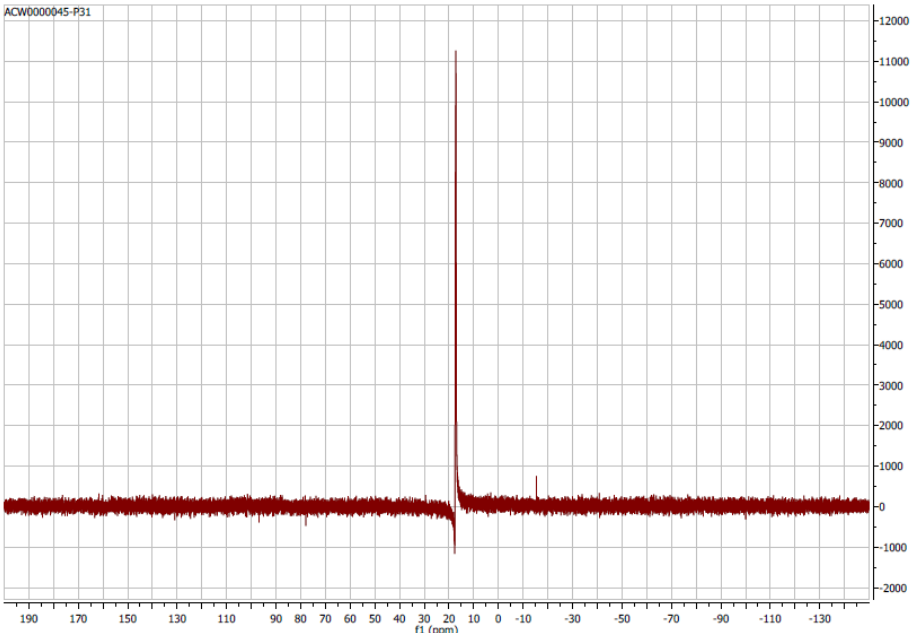

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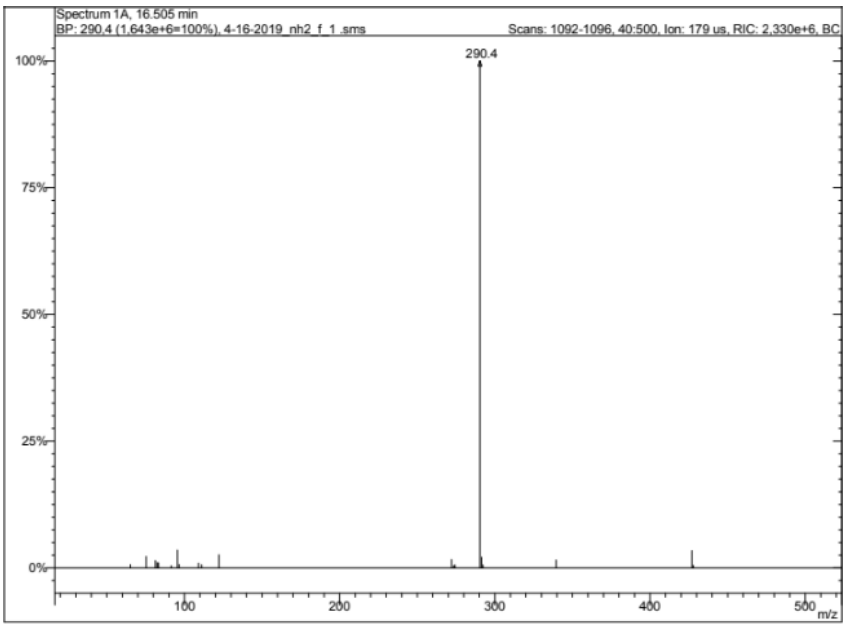
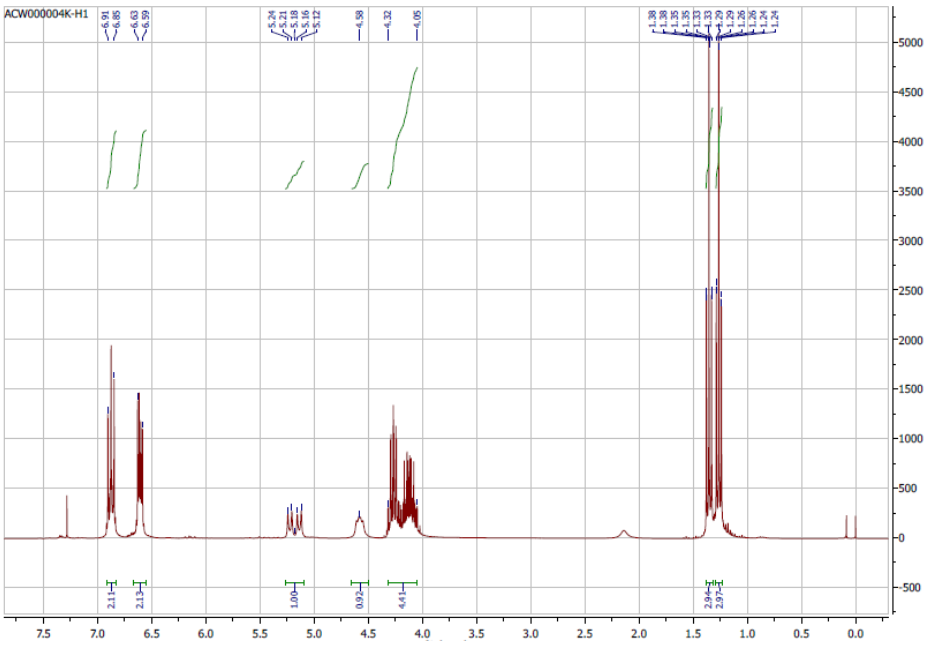
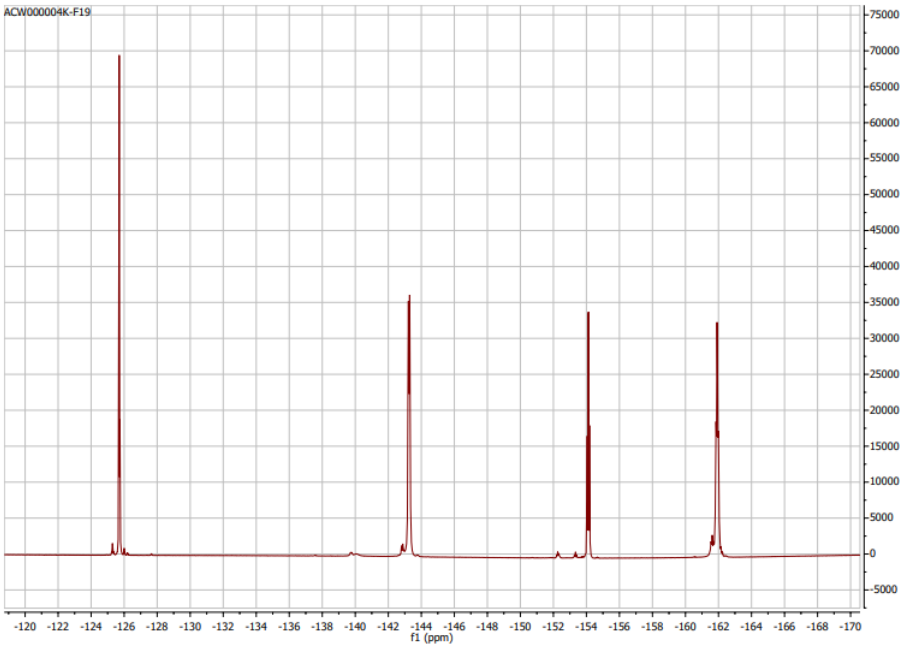


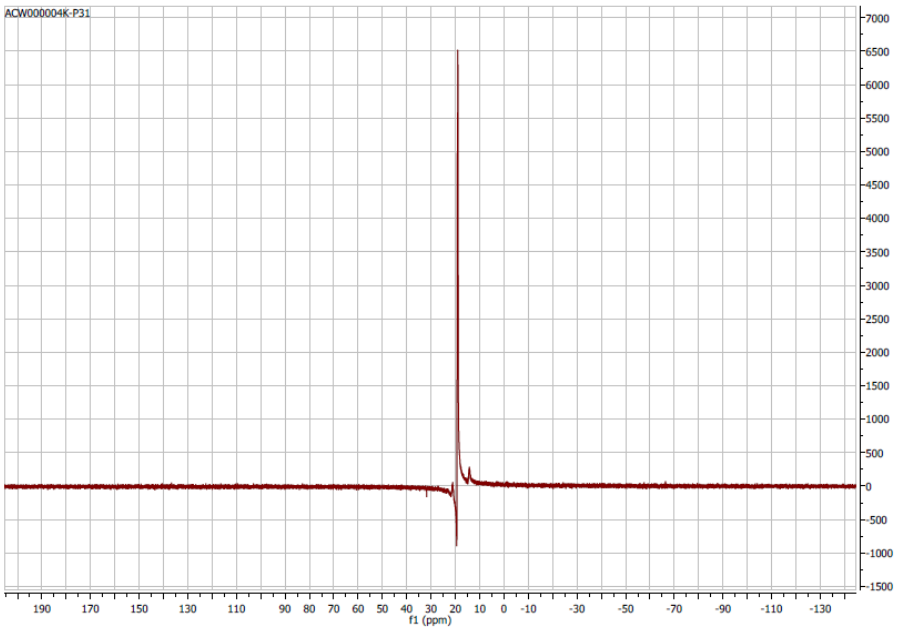
*Mass spectra of α-aminophosphonate **1c***



*^{1}H NMR of α-aminophosphonate **1c***



*^{19}F NMR of α-aminophosphonate **1c***



*^{31}P NMR of α-aminophosphonate **1c***