Faculty of Chemistry UAM

INDIVIDUAL RESEARCH PROJECT IN CHEMISTRY

Synthesis and spectroscopic characterization of phosphonates analogues of phenylglycine

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The work was carried out in the Department of Synthesis and Structure of Organic Compounds under the supervision of prof. UAM dr. hab, Donata Pluskota- Karwatka.

ABSTRACT:

There are a lot of studies focused on α-aminophosphonates due to their big potential for biological activity. In the frame of this project, new α-aminophosphonates were synthesized based on Kabachnik-Fields reaction. Four different amines: p-chloroaniline, p-bromoanilline, p-anisidine and benzylamine were subjected to reactions with 2,3,4,5,6-pentafluorobenzaldehyde and diethyl phosphite result in obtaining α-aminophosphonates. The structures of all synthesized compounds were confirmed by ^{1}H, ^{31}P and ^{19}F NMR spectral data and spectrometric studies.

Keywords: α-aminophosphonates; Kabachnik-Fields reaction; biological activity; corrosion inhibitors; fluorine.

1. INTRODUCTION

Cancer is the second leading cause of mortality worldwide. In the last decades this disease has been the main cause of death. Aminophosphonates, known as phosphorus analogues of amino acids, have received much more attention due to their applications in medicine. [1]

Recently, aminophosphonates are proved to have potent biological activities such as: selective inhibitory of tyrosine kinase and cytotoxicity to cancer cells. They also exhibit antifungal, antibacterial, antitumor, and antiviral activities. Moreover the compounds are known to act as enzyme inhibitors and plant growth regulators [2].

α-Aminophosphonates are important because they are used as key intermediates in organic synthesis focused on obtaining biologically active compounds [3]. Biological activity of α-aminophosphonates can be explained by their structural similarity to natural α-amino acids. In the structure of α-

aminophosphonates the planar carboxylic group is replaced by tetrahedral phosphorus group [4]. Combination of fluorine atoms with α-aminophosphonates offers the opportunity to modify physicochemical and biological properties [5].

Kabachnik-Fields reaction and hydrophosphonylation of imine, the intermediate, will be the way to obtain such compounds just as this project will show on the next pages. Microwave irradiation, catalyst and solvent-free conditions could also be applied to obtaining α-aminophosphonates [4].

2. LITERATURE PART

2.1. α-Aminophosphonates.

α-Aminophosphonates are a group of compounds with attractive properties. Their biological and pharmacological properties can be modified by introducing fluorine into aminophosphonates molecules.

Fluorinated analogues of phenylglycine are shown to play a remarkable role within the protein fold. Amino acids interactions within protein fold can be tracked with ^{19}F NMR spectroscopy also in living systems [4,6].

2.2. α-aminophosphonic acids and phosphonates.

α-Aminophosphonic acids are the most important analogues of α-amino acids due to the isosteric substitution of planar carboxylic acid (CO_2H) by tetrahedral phosphonic acid (PO_3H_2). Isosteric substitution creates a new molecule called bioisostere with similar properties to the parent one [7].

In this context, thanks to the several applications of α-aminophosphonates, phosphonates constitutes an interesting group of compounds which can be obtained by Michaelis-Arbuzov and Michaelis-Becker reactions. The addition of ethyl phosphite ($HP(O)(OEt)_2$) to imine is a part of Pudovnik reaction [8]. The formation of C-P bond can be achieved by addition of a weak nucleophilic reagent to the reactive C=N bond under Ar atmosphere and using toluene as solvent [9].

2.3. Properties of fluorine.

Due to its high electronegativity and enhanced lipophilic activity fluorine provides useful tool for probing and modifying the functions of biological systems [10].

Fluorine is the most electronegativity element, thus it introduces a potent electronic effect. The acidity of an adjacent carboxylic moiety is increased.

Thanks to similar size of fluorine and hydrogen atoms, fluorine is considered as steric isoster of hydrogen. It is assumed due to the difference in C-H and C-F bonds lengths (106 and 134 pm, respectively) which does not affect the size of fluorinated molecules, especially those containing more than one fluorine atom. The replacement of hydrogen with fluorine increases the lipophilicity, a remarkable property for medicine agents.

Fluorine in organic molecules can cause changes in the preferred conformation [11].

2.4. α-Aminophosphonates as corrosion inhibitors.

Nowadays there are several areas where α-aminophosphonates are being used to develop new studies. Recently, they are proved to improve the corrosion studies.

Investigators claim that the protecting activity of an inhibitor relies on electronic and physicochemical properties. Therefore, some α-aminophosphonates are proved on molecular dynamic simulations to understand how they fix on metal surface. It permits determining the adsorption energy value. Thanks to such studies it is known that these compounds are effective inhibitors and their adsorption on the carbon steel surface obeys Langmuir adsorption isotherm [12].

2.5. α- Aminophosphonates as anti-cancer agents.

In 2009, Kraicheva reported the synthesis of a series of furan-derived aminophosphonates and their cytostatic action against human leukemia cell lines. A new study of dymetil(2-methoxyphenyl)amino(2-furyl)-methylphosphonate showed a strong cytotoxic impact on KYSE 30, 150 and 270 squamous esophageal cancer cell lines and moreover it was found to induce apoptosis of these cells [13,14]. Apotosis is genetically designed process to active cell destruction, being a vital process of a normal cell. However, inappropriate apoptosis produces neurogenerative diseases, autoimmune disorders and many types of cancer. In this way, activation of apoptosis pathways is the key mechanism of action of cytotoxic drugs based on α-aminophosphonates [15].

2.6. Synthesis of α-aminophosphonates.

There are different synthetic ways for obtaining α-aminophosphonates. Most of methods are based on Kabachnik – Fields reaction (**Fig 1.**).

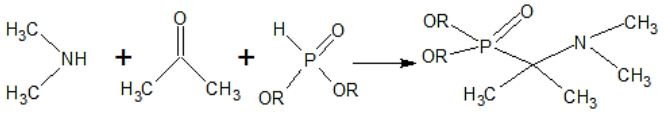

Fig 1. Scheme of Kabachnik-Fields reaction.

Studies of α-aminophosphonates showed that using catalysts such as 10%mol of BF_3:Et_2O α-aminophosphonates could be obtained in high yields (70,6 – 83,5%) after shorter reaction time (30 min). In absence of catalyst, the yields were lower but it increased from 37,5 to 65,6% in increasing the temperature. Therefore, temperature was a parameter which was optimized [16].

Chmarthi and co-workers found that Kabachnik-Fields reaction proceeds much faster and with higher yields under ultrasonification or microwave irradiation and under solvent-free conditions compared to convencional conditions [9].

On the other hand, Kabachnik- Fields reaction was studied in detail by Cherkasov who suggested two routes: one was called *imine* route. Due to the fact that the imine was formed as intermediate through the reaction of carbonyl compound and primary amine. α-Aminophosphonate was formed by de addition of diekyl phosphite on the C=N unit of the imine. The second route called *hydroxyphosphonate route* was suggested in order to follow the reaction with more nucleophilic cyclohexyl-amine, benzaldehyde and diakyl phosphite. Later, Zefirov and Matveeva proved that it follows the imine route [17].

Table 1. Name of synthesized compounds.

Symbol	Product
1a	*Diethyl(((4-chlorophenyl)amino)(perfluorophenyl)methyl) phosphonate*
1b	*Diethyl(((4-bromophenyl)amino)(perfluorophenyl)methyl) phosphonate*
1c	*Diethyl(((4-methoxyhenyl)amino)(perfluorophenyl)methyl) phosphonate*
1d	*Diethyl(((4-methoxyhenyl)amino)(perfluorophenyl)methyl) phosphonate*

3. AIM OF THIS WORK

The aim of this project consisted of the synthesis of fluorophosphonates analogues of phenylglycine in order to obtain compounds with new properties. Apart the synthesis, structural studies of the obtained compounds performed with the use of spectrometric and spectroscopic tecniques were also planned.

4. RESULTS AND DISCUSSION

Synthesis of phosphonates analogues of phenylglycine was carried out following the method based on Kabachnik- Fields reaction. In spite of conventional Kabachnik-Fields reaction that occurs in one pot mixing three component, this synthesis was performed in two steps: firstly, perfluorinated benzaldehyde was reacted with amine compound. In this way, imine was formed. On the second step, diethyl phosphite was added to reaction mixture. The reaction was monitored by TLC.

In the frame of this project, reactions with the following amines: p- chloroaniline, p-bromoaniline, p-anisidine and benzylamine were carried out.

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

2,3,4,5,6 –pentafluorobenzaldehyde was subjected to reaction with p-chloroaniline and diethyl phosphite. The obtained product was analyzed by the use of GC-MS technique. The resulted mass spectrum revealed the presence of the expected compound (**1a**).

In the spectrum (recorded in positive mode) signal located at m/z= 443 corresponded with the protonated molecular ion of the α-aminophosphonate formed. Two additional peaks, at m/z= 306 and m/z=111, present in the spectrum attracted attention therefore were studied. The first one was attributed to the protonated imine ion (**Fig 2.**), the second resulted from breaking the bond between the nitrogen atom of imine and carbon atom from p-chlorobenzene (**Fig 3.**).

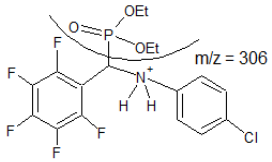

Fig 2. Fragment which corresponds to the signal at m/z= 306.

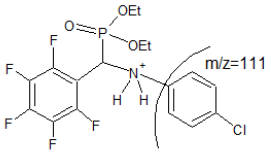

Fig 3. Fragment which corresponds to the signal m/z= 111.

The mechanism proposed for the product formation was based on two step reaction which consisted of nucleophile attacks. Firstly, the C-N bond was formed due to the nucleophile attack of p-chloroaniline to pentafluorobenzaldehyde thanks to the free pair electrons of nitrogen atom. Then, due to all these reactions are reversible, the water formed was removed from the reaction mixture. The second nucleophile attack took place between diethyl phosphite and imine compound through the double bond C=N. These reactions lead to obtaining α-aminophosphonate.

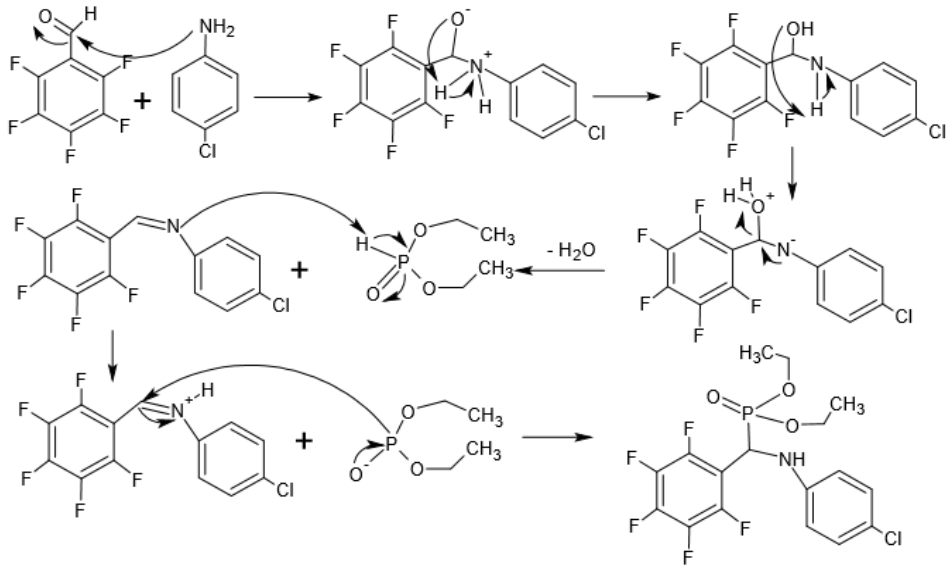

Fig 4. Mechanism proposed for the reaction of 2,3,4,5,6 -pentafluorobenzaldehyde with p-chloroaniline.

The mechanism was detailed for p-chloroaniline responsible for formation α-aminophosphonates with other amines as well. (**Fig 4**).

4.2. Reaction of 2,3,4,5,6 – pentafluorobenzaldehyde with p-bromoaniline.

The α-aminophosphonate **1b** resulted from the reaction between 2,3,4,5,6- pentafluorobenzaldehyde, p-bromoaninline and diethyl phosphite was analyzed by the use of GC-MS technique. The obtained mass spectrum confirmed the presence of the expected compound.

In the spectrum, the peak located at m/z=487.1 corresponded with the protonated molecular ion of the α-aminophosphonate formed. One additional peak attracted attention at m/z=351.1. It was attributed to the protonated imine ion (**Fig 5.**).

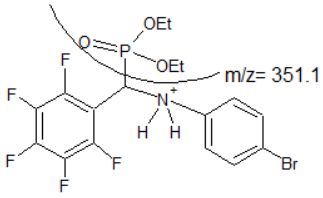

Fig 5. Fragment which corresponds to the signal at m/z= 351.1

4.3. Reaction of 2,3,4,5,6 – pentafluorobenzaldehyde with p-anisidine.

From the reaction between 2,3,4,5,6 – pentafluorobenzaldehyde, p-anisidine and diethyl phosphite, the α-aminophosphonate **1c** was formed. GC-MS technique was required to analyze the product of the reaction. GC spectrum showed two relevant signals, at t_r= 15.306min and t_r=17.912min, therefore MS studies for each were required.

The peak located at m/z= 439.1 in the second mass spectrum revealed the presence of the expected product. Two additional peaks, at m/z= 302.1 and m/z= 287.5, attracted attention thus were studied. The first one was attributed to the protonated imine ion (**Fig 6.**) and the second was resulted from breaking C-O bond of p-anisidine (**Fig 7.**).

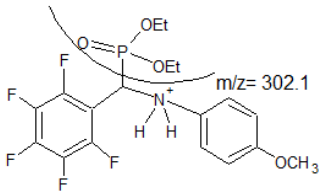

Fig 6. Fragment which corresponds to the signal at m/z= 302.1

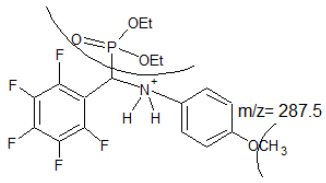

Fig 7. Fragment which corresponds to the signal at m/z= 287.5

5. CONCLUSIONS

This study has drowned some remarkable conclusions. Firstly, α-aminophosphonates have been prepared and characterized by spectroscopic techniques. Through the determination of the structures obtained on the basis of ^{1}H, ^{19}F and ^{31}P NMR spectra, the structure of α-aminophosphonates were proved. Reaction with benzylamine resulted in significant lower yield of the obtained product.. Therefore it can be concluded that the synthetic protocol applied to these studies is appropriate rather for aromatic amines than aliphatic ones.

6. EXPERIMENTAL PART

6.1 General procedure for α-aminophosphonates (1a-d) synthesis.

2,3,4,5,6 –pentafluorobenzaldehyde was subjected to reaction with an appropriate amine in the ratio 1:1. The reaction was performed in boiling anhydrous toluene and was monitored by TLC. When the imine formation was completed, diethyl phosphite was added to the reaction mixture. The mixture was kept at 110.6 ºC for 16 hours. Then, the reaction mixture was evaporated to dryness and the residue was subjected to purification by flash chromatography (silica gel, hexane/AcOEt, 1:1, v/v).

Structures of obtained compounds were determined on the basis of ^{1}H, ^{19}F and ^{31}P NMR spectra. The signals observed were compared to the chemical shifts values reported in the literature for the similar α-aminophosphonates [4].

6.1.1*Diethyl(((4-chlorophenyl)amino)(perfluorophenyl)methyl) phosphonate (**1a**).*

From 2,3,4,5,6- pentafluorobenzaldehyde (120 mg, 0.5mmol), p-chloroaniline (77mg, 0.5 mmol) and diethyl phosphite (0.013ml, 0,1 mmol) a white solid was obtained. ^{1}H NMR (403 MHz, $CDCl_3$) δ=7.12 (dm, 2H, Ph), δ= 6.57 (dm, 2H, Ph), δ=5.19 (dd, 1H, -CH-), δ=4.65 (m, 1H, -NH-), δ=4.28- 4.19 (m, 2H, -OCH_2), δ=4.16- 4.04 (m, 2H, -OCH_2), δ=1.34 (td,3H, -OCH_2CH_3), δ=1.26 (td, 3H, -OCH_2CH_3) ppm. ^{31}P NMR (121MHz, $CDCl_3$) δ=18.31 (m, 1P) ppm. ^{19}F NMR (282 MHz, $CDCl_3$) δ= -142.6 to -143.1 (m, 2F), δ= -153.4 to -153.9 (m, 1F), δ= -161.3 to -161.8 (m, 2F) ppm.

6.1.2*Diethyl(((4-bromophenyl)amino)(perfluorophenyl)methyl) phosphonate (**1b**).*

From 2,3,4,5,6- pentafluorobenzaldehyde (120 mg, 0.5mmol), p-bromoaniline (86mg, 0.5 mmol) and diethyl phosphite (0.013ml, 0,1 mmol) a brown liquid was obtained. ^{1}H NMR (403 MHz, $CDCl_3$) δ=7.25 (m, 2H, Ph), δ= 6.54 (m, 2H, Ph), δ=5.19 (dd, 1H,J= 14.9, 9.2 Hz, -CH-), δ=4.73 (dd, 1H,J= 4.5, 6.7 Hz, -NH-), δ=4.05- 4.32 (m, 4H, 2 x -OCH_2), δ=1.35 (td, 3H, J= 0.5, 7 Hz, -OCH_2CH_3), δ=1.26 (td, 3H, J= 0.5, 7.1 Hz, -OCH_2CH_3) ppm. ^{31}P NMR (121MHz, $CDCl_3$) δ=19 (m, 1P) ppm. . ^{19}F NMR (282 MHz, $CDCl_3$) δ= -142.7 to -143.1 (m, 2F), δ= -153.5 to -153.8 (m, 1F), δ= -161.3 to -161.7 (m, 2F) ppm.

*6.1.3Diethyl(((4-methoxyhenyl)amino)(perfluorophenyl)methyl) phosphonate (**1c**).*

From 2,3,4,5,6- pentafluorobenzaldehyde (120 mg, 0.5mmol), p-anisidine (61.5mg, 0.5 mmol) and diethyl phosphite (0.013ml, 0,1 mmol) a yellow solid was obtained. ^{1}H NMR (403 MHz, $CDCl_3$) δ=6.75 (m, 2H, Ph), δ= 6.63 (m, 2H, Ph), δ=5.18 (dd, 1H, -CH-), δ=4.42 (s, 1H, -NH-), δ=4.05- 4.32 (m, 4H, 2 x -OCH_2), δ=3.71 (s, 3H, -OCH_3), δ=1.36 (td, 3H, J= 0.6, 7.1 Hz, -OCH_2CH_3), δ=1.26 (td, 3H, J= 0.5, 7.1 Hz, -OCH_2CH_3) ppm. ^{31}P NMR (121MHz, $CDCl_3$) δ=19 (m, 1P) ppm. ^{19}F NMR (282 MHz, $CDCl_3$) δ= -142.6 to -143.3 (m, 2F), δ= -154.2 to -154.6 (m, 1F), δ= -161.4 to -162.3 (m, 2F) ppm.

*6.1.4Diethyl(((4-methoxyhenyl)amino)(perfluorophenyl)methyl) phosphonate (**1d**).*

From 2,3,4,5,6- pentafluorobenzaldehyde (120 mg, 0.5mmol), benzylamine (0.0546 ml, 0.5 mmol) and diethyl phosphite (0.013ml, 0,1 mmol) a colorless oil was obtained. The concentration of obtained product was not enough to get ^{1}H, ^{19}F and ^{31}P NMR spectra.

7. REFERENCES

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8. SUPPLEMENTARY INFORMATION

Mass and NMR spectra of obtained α-aminophosphonates: ^{1}H, ^{19}F and ^{31}P NMR spectra.

7.1 GC-MS spectra.

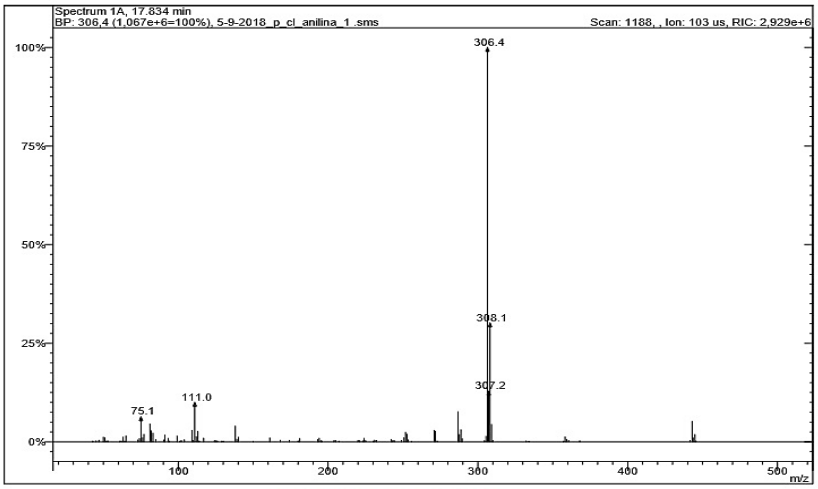

Fig 7. MS spectrum of **1a**.

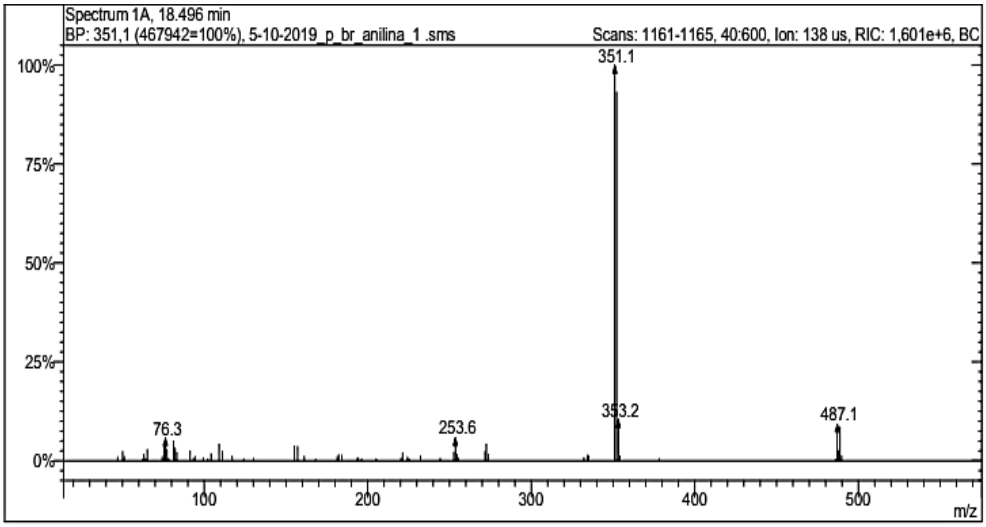

Fig 8. MS spectrum of **1b**.

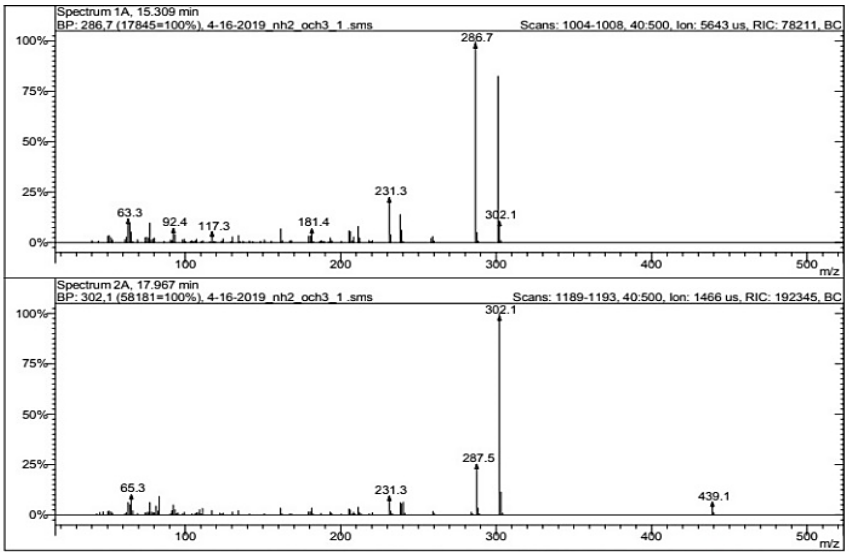

Fig 9. MS spectrum of **1c**.

7.2 ^{1}H NMR spectra.

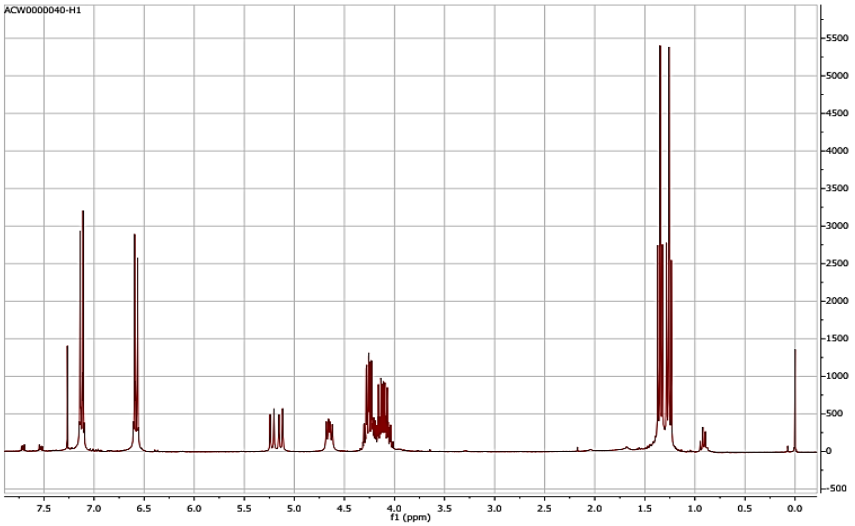

Fig 10. ^{1}H NMR spectrum **1a**.

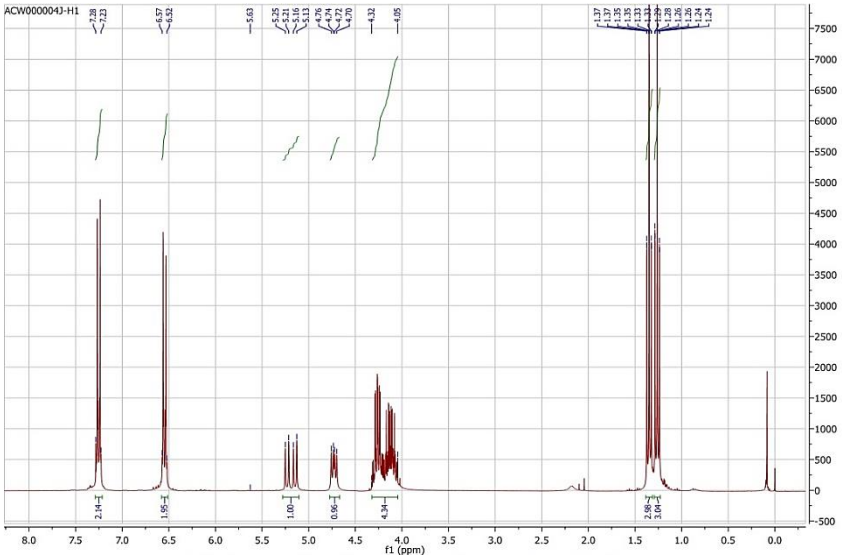

Fig 11. ^{1}H NMR spectrum **1b**.

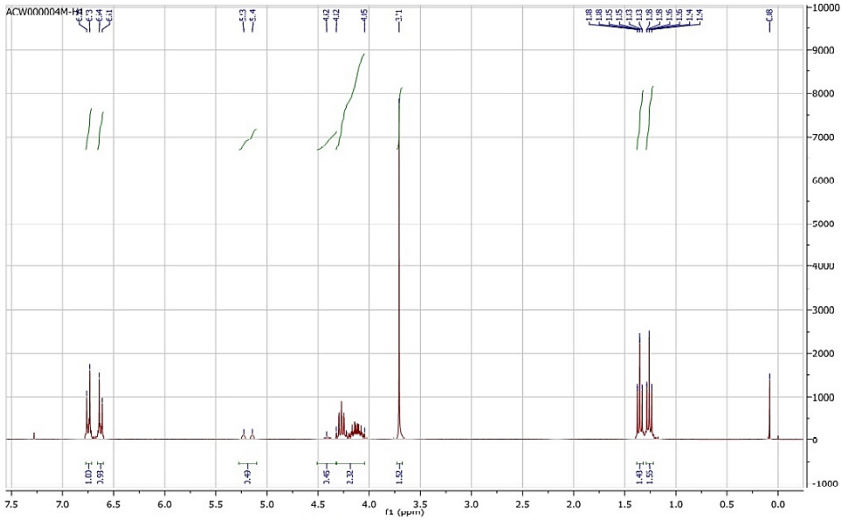

Fig 12. ^{1}H NMR spectrum **1c**.

7.3 ^{31}P NMR spectra.

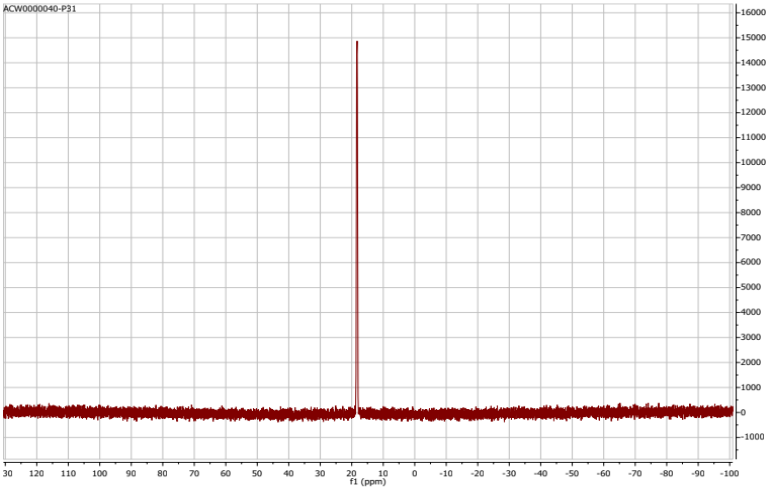

Fig 13. ^{31}P NMR spectrum **1a**.

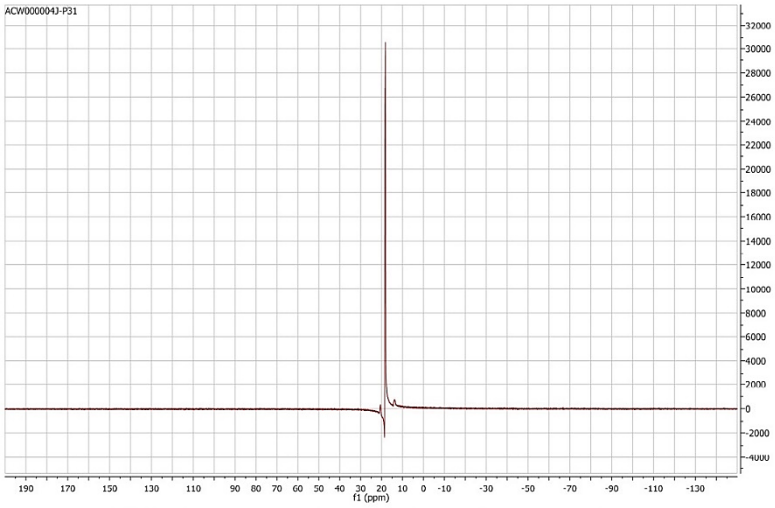

Fig 14. ^{31}P NMR spectrum **1b**.

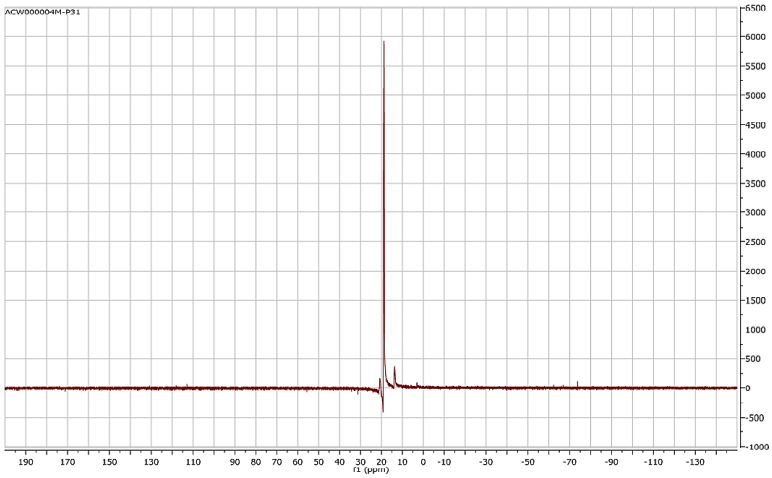

Fig 15. ^{31}P NMR spectrum **1c**.

7.4 ^{19}F NMR spectra.

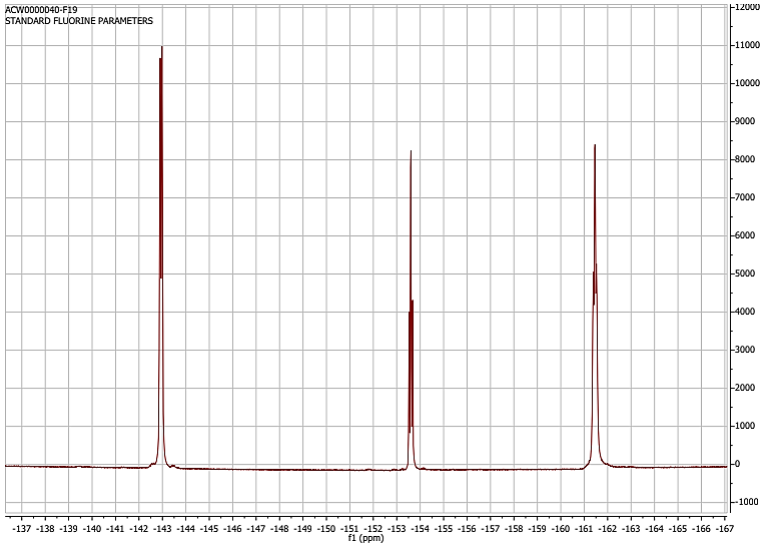

Fig 16. ^{19}F NMR spectrum **1a**.

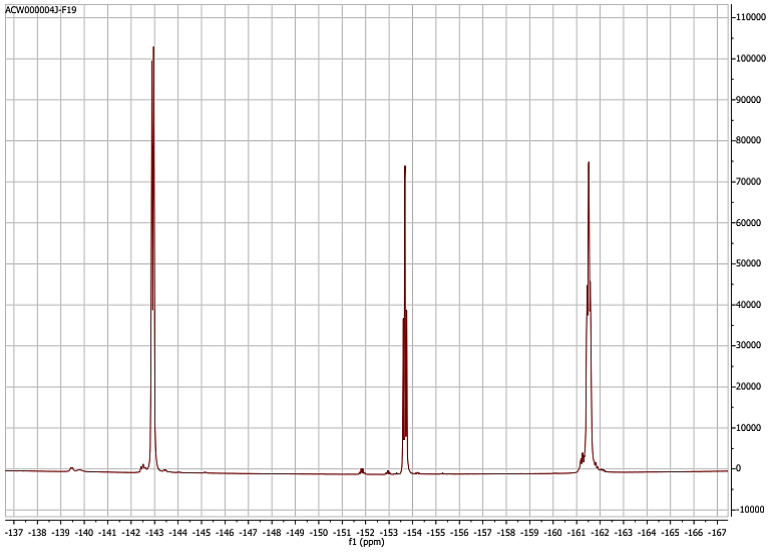

Fig 17. ^{19}F NMR spectrum **1b**.

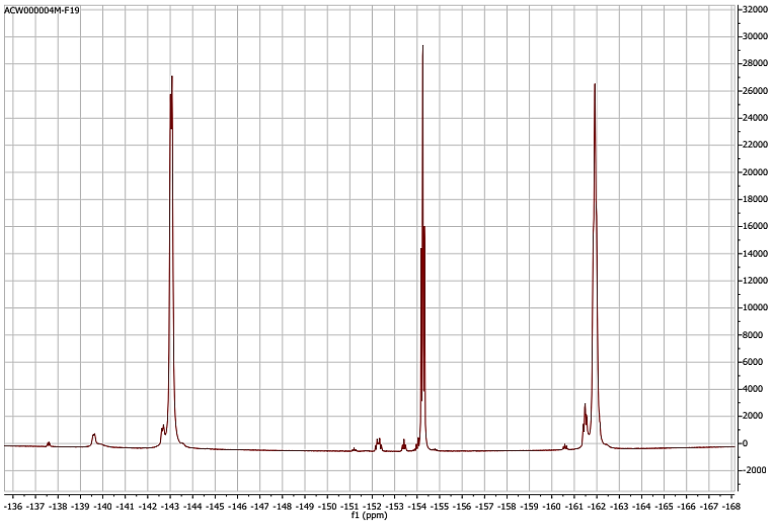

Fig 18. ^{19}F NMR spectrum **1c**.