

# New promising generation of phosphates $\alpha$ - aminophosphonates: Design, synthesis, *in-vitro* biological evaluation and computational study

Rim Aissa <sup>a</sup>, Samia Guezane-Lakoud <sup>a, \*</sup>, Lynda Gali <sup>b</sup>, Martial Toffano <sup>c</sup>, Anna Ignaczak <sup>d</sup>, Marta Adamiak <sup>d</sup>, Mounia Merabet-Khelassi <sup>a</sup>, Regis Guillot <sup>c</sup>, Louisa Aribi-Zouioueche <sup>a</sup>

<sup>a</sup> Eco-compatible Asymmetric Catalysis Laboratory (LCAE) Badji Mokhtar Annaba-University, B.P. 12, 230 00 Annaba, Algeria <sup>b</sup> Research Center in Biotechnology, Ali Mendjli Nouvelle Ville UV 03, BP E73 Constantine, Algeria <sup>c</sup> Equipe de Catalyse Moléculaire-ICMMO Bât 420. Université Paris- Saclay <sup>d</sup> Theoretical and Structural Chemistry Group, Department of Physical Chemistry, Faculty of Chemistry, University of Lodz, Pomorska 163/165, 90-236 Lodz, Poland

**Abstract** A novel category of phosphate-phosphonate compounds is synthesized by a sequence multicomponent reactions strategy of *Kabachnik-Fields* and *Atherton-Todd reactions*. All the designed products are obtained with good chemical yields in short reaction times, and confirmed by spectroscopy analyses. The diethyl [(4-diethylphosphate phenyl) (4-trifluoromethyl phenylamino) methyl] phosphonate **6g** is characterized by single-crystal X-ray analysis. These compounds exhibited a strong antifungal effect, a low inhibition of AChE, and no inhibitory activity on BChE, with interesting antioxidant capacity. Results make them promising candidates as original organophosphorus pesticides. The DFT calculations concerning relative stability of various conformers, besides the comparison of the measured and calculated NMR spectra of selected compounds; suggest that conformational interconversion can quite easily occur in this type of molecules, so that many structures of the same compound can coexist in solution.

## 1.

### Introduction

Organophosphates are distinguished for their diverse range of application as therapeutic agents [1], or additional moieties on prodrugs for their stability and ability to aqueous solubility (Fludarabine an antiviral, and Inflamase an anti-inflammatory and anti-allergic) [2]. In industry they are used as additives lubricants [3], flame retardants and plasticizers in textiles, polyurethane foams, thermoplastics, resins, polyvinyl chlorides, electronics and rubber [4]. However, the most known are those employed in agriculture as major pesticides since 1960's representing 80% of phytosanitary products used all over the world as insecticides, herbicides and plant growth regulators [5]. They are appreciated for their relatively easy manufacturing, high toxicity for insects [6], and their biodegradation that ensures low persistence in the environment. Moreover, organophosphorus compounds have been considered as alternatives to toxic organochlorines [7]. However, exposure to organophosphate pesticides can lead to their penetration in body through dermal absorption, inhalation or ingestion [8] and can cause irreversible inhibition of acetylcholinesterase (AChE) in red cells, plasma, cholinergic synapses in both central and peripheral nervous systems and neuromuscular junction [9]. AChE inhibition causes an over stimulation of acetylcholine receptors due to the accumulation of acetylcholine leading to acute neurotoxicity; organophosphates pesticides are also thought to be implied in disturbing the cell redox and lead to oxidative stress caused by the excessive generation of reactive oxygen species (ROS) mainly by mitochondria. ROS could also be produced during organophosphates metabolism [10]. The Oxidative Stress is associated with the onset of numerous illnesses as cardiovascular diseases, cancer, diabetes, Alzheimer's, and Parkinson's diseases [11]. The synthesis of compounds with improved and less toxic properties is an interesting approach to reduce the impact on the environment and on health linked to the excessive use of organophosphates. The  $\alpha$ -aminophosphonates are extensively studied as interesting organophosphorus derivatives known as bioisosteres of amino acids [12]. The  $\alpha$ -aminophosphonates possess promising biological and pharmacological potentials as enzymes inhibitors (compound I) [13], antioxidants (compound II) [14], antibiotics, (compound III) [15] and antifungal agents (compound IV) [16] (Fig. 1).

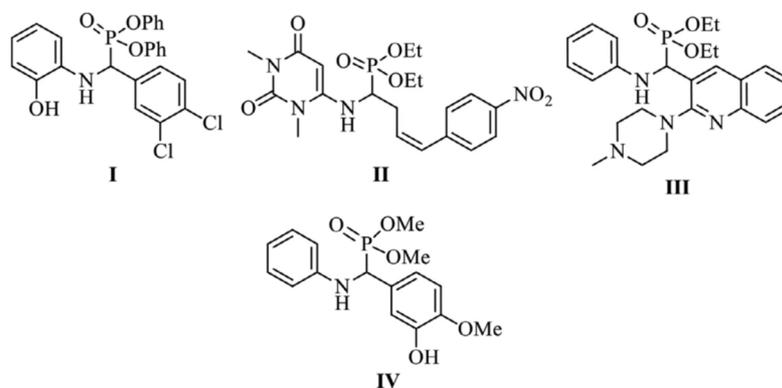
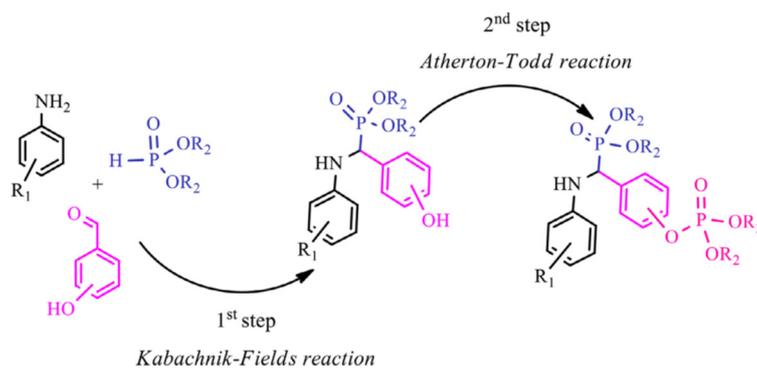


Fig. 1. Structures of  $\alpha$ -aminophosphonates having biological activities.



**Scheme 1.** Sequence strategy of the synthesis of diethylphosphates-  $\alpha$ -aminophosphonates.

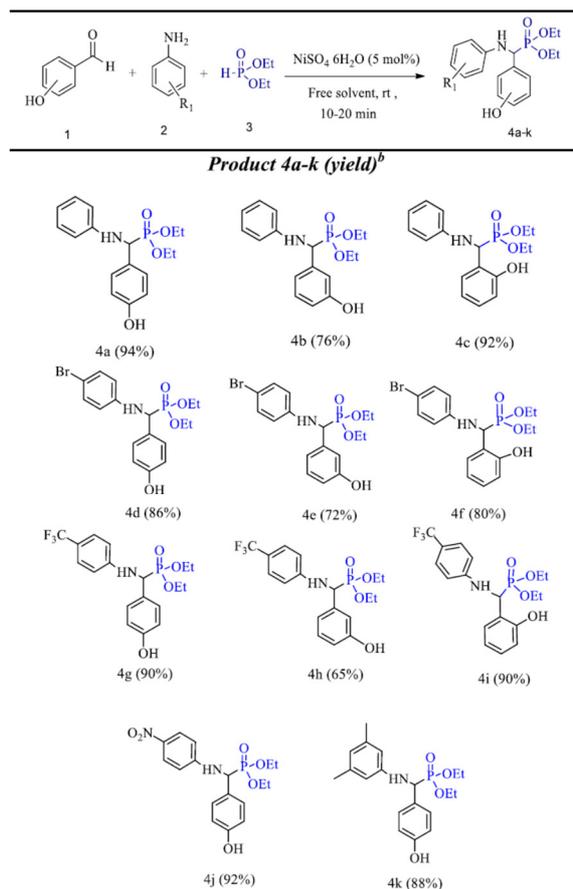
Compared to phosphates compounds, phosphonates derivatives are more stable chemically and metabolically because of the presence of C-P bond instead of P-O bond [17]. The synthesis of  $\alpha$ -aminophosphonates is called often the *Kabachnik-Fields* reaction [18], in environmentally friendly media [19]. This method is based on the direct *in situ* attack of imine formed through condensation of an aldehyde and a primary amine by diethylphosphite in their tautomers form. The  $\alpha$ -aminophosphonates can be used as excellent intermediates in chemistry synthesis [20]. *Atherton-Todd* reaction [21] is a powerful classical synthetic method employed for the preparation of phosphates and efficient protocol to directly relate phosphorus derivatives with heteroatom, to generate a large number of interesting compounds in chemical and pharmacological domains. In order to extend our previous studies on preparation of novel synthetic derivatives of organophosphorus compounds [22], we investigate the synthesis of original phosphate  $\alpha$ -aminophosphonate molecules combining of phosphate and phosphonate moieties. The synthetic strategy will involve as intermediate the diethyl hydroxyphenyl  $\alpha$ -aminophosphonates prepared in the first step by *Kabachnik-Fields* reaction. In the second step, the diethylphosphate moiety will be introduced *via* the *Atherton-Todd* reaction to form desired derivatives (Scheme 1). Moreover, *in vitro* biological evaluation has been carried out to investigate the antifungal, antioxidant, and anti-cholinesterase effects. To our knowledge, these compounds have not been synthesized yet; therefore this article may constitute the first description of their synthesis which further describes the investigation of their biological effects. **2.**

## Results and discussion

### 2.1. Chemistry

**2.1.1. Synthesis of diethyl hydroxyphenyl  $\alpha$ -aminophosphonates** The synthesis of diethyl hydroxy phenyl  $\alpha$ -aminophosphonates compounds as intermediates is realized *via* multicomponents *Kabachnik-Fields* reaction, by the condensation in one pot of hydroxybenzaldehyde (115 mg, 1 mmol), substituted aniline (99 mg, 1 mmol) and diethylphosphite (136 mg, 1.2 mmol), using NiSO<sub>4</sub> · 6H<sub>2</sub>O (5 mol%) as catalyst as described in our previous work [23]. The reaction proceeds without solvent at room temperature within short reaction time (20 min). The results in the Table 1 show that the position of hydroxyl group in *ortho* or *para* of benzaldehyde offers better yields compared with *meta* position in the presence of diethylphosphite and aniline substituted with electron withdrawing groups (CF<sub>3</sub> and NO<sub>2</sub>) and electron-donor groups (Br and 3,5-dimethyl-). In these cases, the diethyl hydroxyphenyl  $\alpha$ -aminophosphonates 4a, 4c, 4d, 4f, 4g, 4j, 4k and 4i are obtained with excellent yields (80 to > 94% yield). The reaction with *meta*-hydroxybenzaldehyde in the presence of various substituted aniline shows a partial decrease in yields under the same conditions, leading to 4b, 4e and 4h compounds with (76, 72 and 65%) yields respectively. The molecular structures of all the diethyl hydroxyphenyl  $\alpha$ -aminophosphonates obtained 4a-k were confirmed by NMR <sup>1</sup>H, <sup>13</sup>C and <sup>31</sup>P spectroscopy and HRMS analyses.

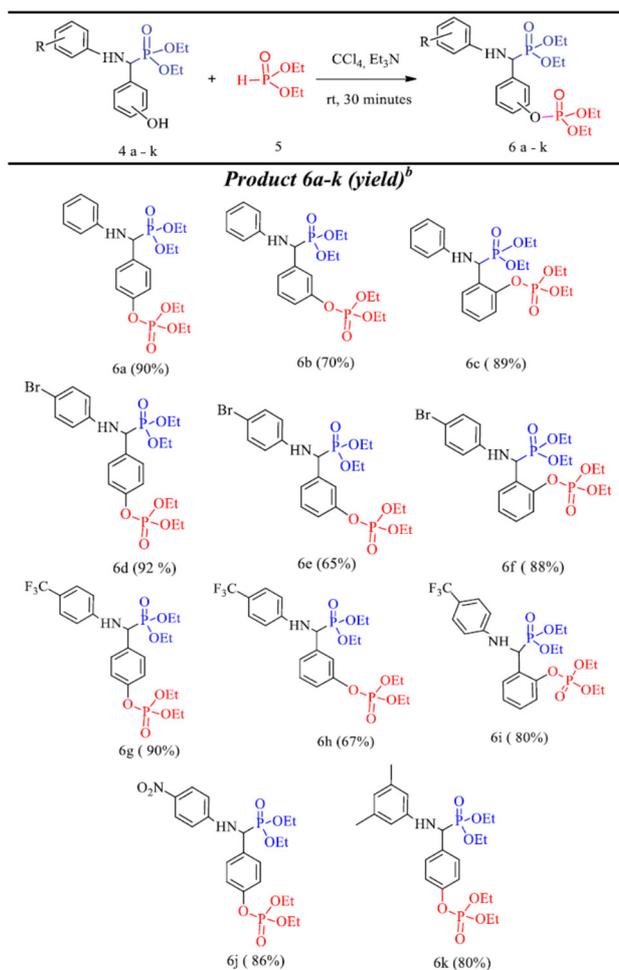
**Table 1** Nickel-catalysed synthesis of diethyl hydroxyphenyl  $\alpha$ -aminophosphonates 4a-k a.



<sup>a</sup> Reaction conditions: hydroxybenzaldehyde (1 mmol), aniline (1 mmol) and diethylphosphite (1.2 mmol), room temperature, 10-20 min. <sup>b</sup>Isolated yield after purification, purified by column chromatography or by crystallization from hexane.

**2.1.2. Phosphorylation of diethyl hydroxyphenyl  $\alpha$ -aminophosphonates** The novel series of phosphates  $\alpha$ -aminophosphonates derivatives were carried out by phosphorylation of diethyl hydroxyphenyl  $\alpha$ -aminophosphonates according to the *Atherton-Todd* reaction. This step is realized by one pot condensation of 1 eq of substituted diethyl hydroxyphenyl  $\alpha$ -aminophosphonate, 1 eq of diethylphosphite, 2.5 eq of Et<sub>3</sub>N and 1 ml of CCl<sub>4</sub>, at room temperature for 30 min. The reactions were monitored by TLC analysis. All the products were obtained with good chemical yields after crystallization from hexane or by chromatography in ethyl acetate and hexane (70/30) (Table 2). The Table 2 shows that the *Atherton-Todd* reaction is efficient for the synthesis of novel phosphates  $\alpha$ -aminophosphonates compounds with chemical yields up to 92%. These yields depend on the electronic effects of diethyl hydroxyphenyl  $\alpha$ -aminophosphonate compounds. The phosphorylation of *ortho*- or *para*-hydroxyphenyl  $\alpha$ -aminophosphonates substituted driving to the corresponding diethylphosphates diethyl  $\alpha$ -aminophosphonates **6a**, **6c**, **6d**, **6f**, **6g**, **6i**, **6j** and **6k** with excellent chemical yields (>80%). Decrease of the chemical yields was observed with *meta*-hydroxyphenyl  $\alpha$ -aminophosphonates which are unfavorable of the phosphorylation, giving the products **6b**, **6e** and **6h** with (70, 65 and 67%) yields respectively. All the products of diethylphosphates diethyl  $\alpha$ -aminophosphonates **6a-k** were confirmed by NMR <sup>1</sup>H, <sup>13</sup>C and <sup>31</sup>P spectroscopy and HRMS analyses. From <sup>1</sup>H NMR, the compounds are characterized by the appearance of a doublet around 4.7 ppm corresponding to the stereogenic carbon with a coupling constant towards *J*<sub>P-H</sub> = 23 Hz. From <sup>31</sup>P NMR, two peaks appear at 22.1 ppm and -6.5 ppm correspond to the phosphonate and phosphate groups respectively. A possible mechanism for the formation of diethylphosphate diethyl  $\alpha$ -aminophosphonate derivatives via multi-components *Atherton-Todd* reaction is shown in Scheme 2. Based on the examination of the results of our experiments and the mechanisms proposed for the *Atherton-Todd* reaction from the literature, we propose a plausible mechanism for the phosphorylation of diethyl hydroxyphenyl  $\alpha$ -aminophosphonate with diethylphosphite. We assume that the first step of the reaction is the formation of a salt [Et<sub>3</sub>N Cl] + CCl<sub>3</sub> between carbon tetrachloride [CCl<sub>4</sub>]

**Table 2** Phosphorylation of diethyl hydroxyphenyl  $\alpha$ -aminophosphonates by *Atherton-Todd* reaction.



<sup>a</sup> Reaction conditions: diethyl hydroxyphenyl  $\alpha$ -aminophosphonates (1 mmol), diethylphosphite (1,2 mmol),  $\text{CCl}_4$  (2mL),  $\text{Et}_3\text{N}$ , room temperature, 30 min. <sup>b</sup>Isolated yield after purified by column chromatography or by crystallization from hexane.

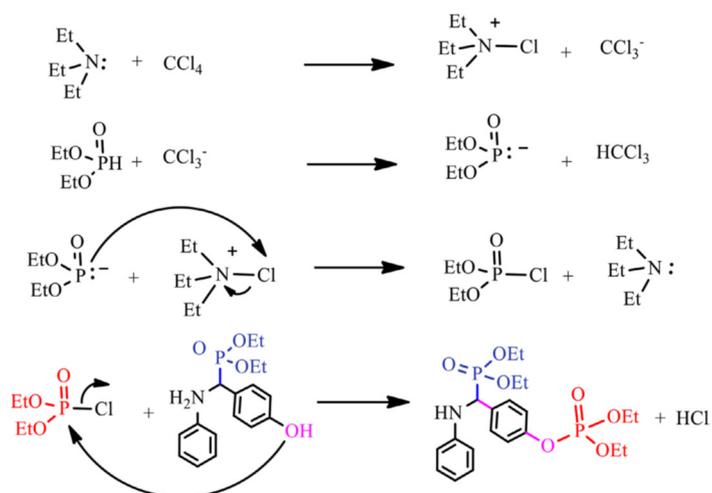
and triethylamine [ $\text{Et}_3\text{N}$ ], of which this step was confirmed by  $^1\text{H NMR}$  by Mitova, *V and al* [24]. Then the [ $\text{CCl}_3^-$ ] anion react as base with the diethylphosphite to give the dialkylphosphonate ions [ $(\text{EtO})_2\text{P}(\text{O})^-$ ]—more nucleophile, and the chloroform [ $\text{CHCl}_3$ ]. After, the diethylphosphonate ion [ $(\text{EtO})_2\text{P}(\text{O})^-$ ]—reacts with [ $\text{Et}_3\text{N Cl}$ ] + resulting in diethyl chlorophosphate, which reacts with diethyl hydroxyphenyl  $\alpha$ -aminophosphonate as nucleophile to form the desired product.

### 2.2. Cristal structure analyses

The single-crystal structure of diethyl [(4-diethylphosphate phenyl) (4-trifluoromethyl phenylamino) methyl] phosphonate **6g** was determined by X-ray crystallography. The crystal data collection and refinement parameters are given in Table 3. The ORTEP drawing of **6a** is shown in Fig. 2.

### 2.3. Biological activity

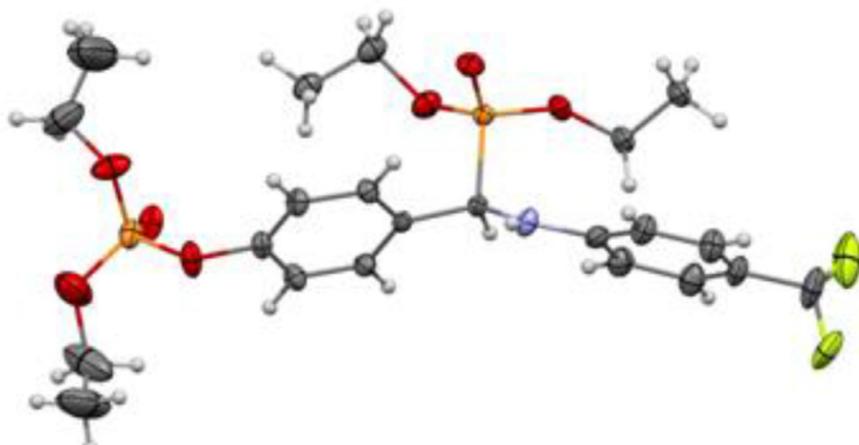
*In vitro* assays were explored to compare the biological effect between the diethyl hydroxyphenyl  $\alpha$ -aminophosphonate compounds (**4a-k**) and diethylphosphate diethyl  $\alpha$ -aminophosphate derivatives (**6a-k**). The evaluation of the antifungal activity against *Fusarium oxysporum* and *Botrytis cinerea* showed a strong growth inhibitory effect for all tested diethyl hydroxyphenyl  $\alpha$ -aminophosphonates and diethylphosphates diethyl  $\alpha$ -aminophosphonates, showing interesting inhibition rates (%) at the concentration of 0.14 mg/m, with low  $\text{IC}_{50}$  values presented in Histogram 1. (Results are detailed in Tables 1–5–8 in the Electronic Supplementary Information (ESI)). We observed that, compound **6d** exhibited the highest inhibition rate of mycelium growth with a value of 87% for *Fusarium oxysporum* and 92% for *Botrytis cinerea* and an  $\text{IC}_{50}$  value under 0.02 mg/ml. Besides, compounds **4a**, **6g**, **6a**, **4d**, **6f**, **4j** and **6j** have inhibited 79 to 80 % of both fungus growth presenting  $\text{IC}_{50}$  values less than 0.02 mg/ml. Similarly, **6g** presented an inhibition of 78% against *Botrytis cinerea* only with  $\text{IC}_{50}$  values below 0.02 mg/ml. An excellent antifungal activity is observed for the rest of compounds having 52 to 78% of inhibition against *Fusarium oxysporum* and  $\text{IC}_{50}$  values from 0.132 to 0.031 mg/ml and while inhibitions varying from 57 to 72% were obtained against *Botrytis cinerea* with  $\text{IC}_{50}$  values from 0.03 to 0.12 mg/ml.



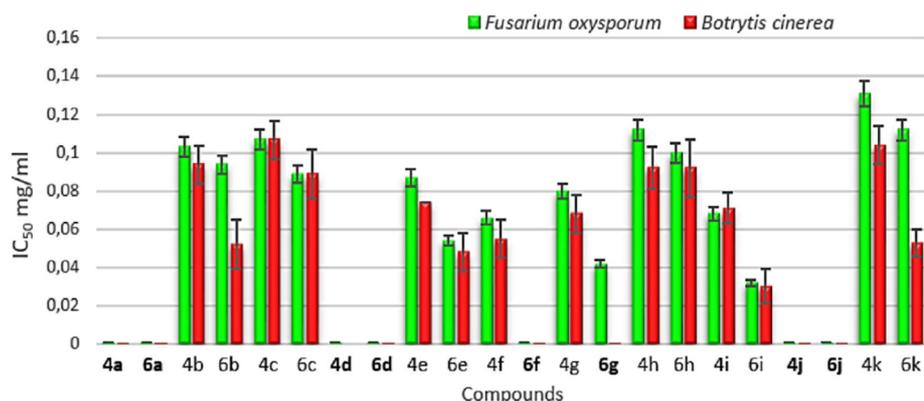
**Scheme 2.** Plausible mechanism for the synthesis of diethylphosphate diethyl  $\alpha$ -aminophosphonate by Atherton-Todd reaction.

**Table 3**  
Crystallographic data and structure refinement details for **6g**.

CCDC	2079186	T, K	200 (1)
Empirical Formula	C <sub>22</sub> H <sub>30</sub> F <sub>3</sub> NO <sub>7</sub> P <sub>2</sub>	Radiation type; wavelength	Moka; 0,717073
Mr	539,41	F <sub>000</sub>	564
Crystal size, mm <sup>3</sup>	0,27 × 0,12 × 0,05	$\mu$ , mm <sup>-1</sup>	0,230
Crystal system	Triclinic	range, °	1,732-30,603
Space group	<i>P</i> -1	Reflection collected	34575
a, Å	7,9750 (4)	Reflections unique	7791
b, Å	12,1873 (6)	R <sub>int</sub>	0,0324
c, Å	14,2085 (8)	GOF	1,087
$\alpha$ , °	103,664 (2)	Refl. obs. ( <i>I</i> >2( <i>I</i> ))	5 730
$\beta$ , °	103,332 (2)	Parameter	411
$\gamma$ , °	93,388 (2)	wR <sub>2</sub> (all data)	0,2798
Cell volume, Å <sup>3</sup>	1296,27 (12)	R value ( <i>I</i> >2( <i>I</i> ))	0,0833
Z; Z'	2 ; 1	Largest diff. peak and hole (e <sup>-</sup> ·Å <sup>-3</sup> )	1,930 ; -0,991



**Fig. 2.** ORTEP drawing of compound **6g**. Thermal ellipsoids are shown at the 30% level (only one of the disordered arrangements show



**Histogram 1.** IC<sub>50</sub> values of antifungal activity of diethyl hydroxyphenyl  $\alpha$ -aminophosphonates and diethyl phosphates diethyl  $\alpha$ -aminophosphonates.

**Table 4**  
Antioxidant capacity results.

Entry	ABTS IC <sub>50</sub> ( $\mu$ g/mL)	DPPH IC <sub>50</sub> ( $\mu$ g/mL)	CUPRAC assay A <sub>0.50</sub> ( $\mu$ g/mL)	GOR assay IC <sub>50</sub> ( $\mu$ g/mL)	Phen assay A <sub>0.5</sub> ( $\mu$ g/mL)
4a	75.37 $\pm$ 1.36 <sup>g</sup>	>800	262.42 $\pm$ 1.04 <sup>j</sup>	n.a	>50
6a	13.4 $\pm$ 2.03 <sup>b</sup>	n.a	119.24 $\pm$ 1.25 <sup>h</sup>	n.a	>50
4b	>800	>800	>800	n.a	>50
6b	>800	727.88 $\pm$ 0.82 <sup>k</sup>	>800	>800	>50
4c	>800	>800	>800	>800	>50
6c	415.00 $\pm$ 0.16 <sup>j</sup>	>800	>800	>800	24.10 $\pm$ 0.45 <sup>f</sup>
4d	>800	31.82 $\pm$ 0.92 <sup>d</sup>	>800	110.59 $\pm$ 0.86 <sup>g</sup>	3.58 $\pm$ 0.55 <sup>d</sup>
6d	57.26 $\pm$ 1.87 <sup>f</sup>	12.5 $\pm$ 0.25 <sup>b</sup>	>800	21.49 $\pm$ 0.33 <sup>d</sup>	0.9 $\pm$ 0.038 <sup>a</sup>
4e	17.79 $\pm$ 1.94 <sup>b,c</sup>	190.10 $\pm$ 3.30 <sup>f</sup>	63.40 $\pm$ 2.12 <sup>e</sup>	151.01 $\pm$ 0.57 <sup>h</sup>	2.75 $\pm$ 0.36 <sup>c</sup>
6e	9.01 $\pm$ 0.20 <sup>b</sup>	18.66 $\pm$ 0.69 <sup>c</sup>	60.85 $\pm$ 1.76 <sup>d,e</sup>	86.55 $\pm$ 2.29 <sup>f</sup>	1.98 $\pm$ 0.05 <sup>b</sup>
4f	49.46 $\pm$ 1.77 <sup>e</sup>	>800	39.68 $\pm$ 0.15 <sup>b</sup>	>800	1.87 $\pm$ 0.03 <sup>a,b</sup>
6f	45.06 $\pm$ 0.37 <sup>e</sup>	214.40 $\pm$ 2.22 <sup>g</sup>	37.26 $\pm$ 1.36 <sup>b</sup>	156.53 $\pm$ 1.27 <sup>i</sup>	1.48 $\pm$ 0.06 <sup>a,b</sup>
4g	37.33 $\pm$ 0.97 <sup>d</sup>	147.72 $\pm$ 2.23 <sup>e</sup>	58.15 $\pm$ 1.85 <sup>c,d</sup>	9.46 $\pm$ 0.18 <sup>b</sup>	1.44 $\pm$ 0.20 <sup>a,b</sup>
6g	21.90 $\pm$ 1.17 <sup>c</sup>	310.67 $\pm$ 1.57 <sup>i</sup>	57.66 $\pm$ 2.29 <sup>c,d</sup>	8.83 $\pm$ 0.23 <sup>b</sup>	0.66 $\pm$ 0.02 <sup>a</sup>
4h	>800	>800	472.64 $\pm$ 1.16 <sup>l</sup>	29.60 $\pm$ 0.62 <sup>e</sup>	>50
6h	>800	>800	353.60 $\pm$ 0.16 <sup>k</sup>	24.64 $\pm$ 2.24 <sup>d</sup>	>50
4i	363.80 $\pm$ 2.26 <sup>i</sup>	592.27 $\pm$ 1.30 <sup>j</sup>	89.95 $\pm$ 1.13 <sup>g</sup>	16.90 $\pm$ 0.65 <sup>c</sup>	5.18 $\pm$ 0.04 <sup>e</sup>
6i	56.64 $\pm$ 2.40 <sup>f</sup>	286.61 $\pm$ 2.14 <sup>h</sup>	78.24 $\pm$ 0.17 <sup>f</sup>	16.85 $\pm$ 1.55 <sup>c</sup>	2.86 $\pm$ 0.05 <sup>c</sup>
4j	358.98 $\pm$ 2.55 <sup>h</sup>	>800	>800	n.a	>50
6j	14.55 $\pm$ 1.52 <sup>b</sup>	>800	130.77 $\pm$ 0.55 <sup>i</sup>	n.a	>50
4k	>800	n.a	>800	>800	>50
6k	>800	n.a	>800	>800	>50
Trolox <sup>a</sup>	3.21 $\pm$ 0.06 <sup>a</sup>	5.12 $\pm$ 0.21 <sup>a</sup>	8.69 $\pm$ 0.14 <sup>a</sup>	4.31 $\pm$ 0.05 <sup>a</sup>	5.21 $\pm$ 0.27 <sup>e</sup>
Ascorbic acid <sup>a</sup>	3.04 $\pm$ 0.05 <sup>a</sup>	4.39 $\pm$ 0.01 <sup>a</sup>	8.310.15 <sup>a</sup>	5.02 $\pm$ 0.02 <sup>a</sup>	3.08 $\pm$ 0.02 <sup>c,d</sup>

Values are expressed as means  $\pm$  S.D of three parallel measurements. Values with different letters are significantly different (Tukey multiple comparison test,  $p < 0.05$ ). <sup>a</sup>Reference compounds.

**Table 5**  
AChE inhibition.

Entry	AChE IC <sub>50</sub> ( $\mu$ g/mL)	Entry	AChE IC <sub>50</sub> ( $\mu$ g/mL)
4a	>800	4g	53.36 $\pm$ 2.56 <sup>b</sup>
6a	>800	6g	>800
4b	>800	4h	>800
6b	>800	6h	n.a
4c	78.00 $\pm$ 2.02 <sup>c</sup>	4i	>800
6c	89.57 $\pm$ 2.86 <sup>d</sup>	6i	n.a
4d	83.54 $\pm$ 2.89 <sup>d</sup>	4j	395.40 $\pm$ 1.30 <sup>e</sup>
6d	n.a	6j	n.a
4e	n.a	4k	>800
6e	n.a	6k	n.a
4f	101.17 $\pm$ 3.59 <sup>e</sup>	Galantamine <sup>a</sup>	6.27 $\pm$ 1.15 <sup>a</sup>
6f	110.33 $\pm$ 2.38 <sup>f</sup>		

Values are reported as means  $\pm$  S.D of three parallel measurements. Values with different letters are significantly different ( $p < 0.05$ ). <sup>a</sup>Reference drug. n.a: no absorbance.

The *in-vitro* antioxidant activities we studied by five spectrophotometric complementary methods; Azinobis-3-ethylbenthiiazoline-6-sulphonic acid, radical scavenging ( **ABTS assay** ), 1, 1-diphenyl, 2-picrylhydrazyl free radical scavenging ( **DPPH assay** ), and Galvinoxyl radical scavenging ( **GOR assay** ), cupric reducing antioxidant capacity ( **CUPRAC** ) assay, and iron reduction by the phenanthroline assay ( **Phen assay** ). Results are determined in terms of the IC<sub>50</sub> or A<sub>0.50</sub> in **Table 4** . Ascorbic acid and Trolox were used as positive standards. The investigation of the antioxidant effect allowed distinguishing essentially three groups including weak, intermediate, and strong compounds based on their IC<sub>50</sub> values that varied according to the test used. The compounds were found to be very potent as reducing agents of iron ions as assessed by phenanthroline assay. Among these compounds, **6d**, **4g**, **6g**, **6f**, **4f**, **6e**, **4e** and **6i** compounds were more powerful than the two standard compounds displaying A<sub>0.50</sub> values between 0.66 and 2.86  $\mu$ g/ml, while the difference isn't significant for **4d** (A<sub>0.50</sub> = 3.58  $\pm$  0.55) compared to ascorbic acid (A<sub>0.50</sub> = 3.08  $\pm$  0.02  $\mu$ g/ml) or for **4i** (A<sub>0.50</sub> = 5.18  $\pm$  0.04  $\mu$ g/ml) and Trolox (A<sub>0.50</sub> = 5.21  $\pm$  0.27  $\mu$ g/ml). Nevertheless, the cupric ions reduction by the compounds is

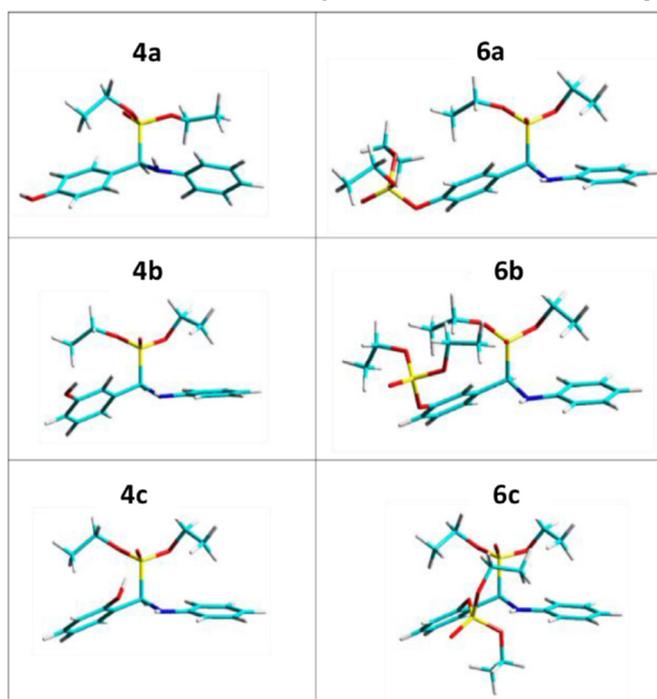
less efficient where **4f** and **6f** having  $A_{0.50}$  values of  $37.26 \pm 1.36$  and  $39.68 \pm 0.15$   $\mu\text{g/ml}$ , respectively, displayed the most powerful effect followed by **6g**, **4g**, **6e** and **4e** ( $A_{0.50}$  from 57.66 to 63.40  $\mu\text{g/ml}$ ). The free radical scavenging activity of the synthesized products was assessed by using three different radicals including DPPH, ABTS, and galvinoxyl. The results showed that the scavenging capacity depends on the free radical nature due to steric bulk caused by the free radical molecule size as well as to the antioxidant process mechanism. The compounds **6d**, **6e** and **4d** were found the most effective in scavenging DPPH with  $IC_{50}$  values of  $12.5 \pm 0.2$ ,  $18.66 \pm 0.69$ , and  $31.82 \pm 0.92$   $\mu\text{g/ml}$  respectively. Whereas, **6e**, **6a**, **6j** and **4e** exhibited the strongest trapping effect against the radical ABTS with  $IC_{50}$  values between 9.01 and 17.79  $\mu\text{g/ml}$ . Galvinoxyl radical was the most sensitive to tested compounds, especially **6g**, **4g**, **6i**, **4i**, **6d**, **6h**, and **4h** with  $IC_{50}$  values of  $8.83 \pm 0.23$  and  $29.60 \pm 0.62$   $\mu\text{g/ml}$ , noting that the activity increases in the descent order of the free radical sizes. On the other hand, the structure of compounds is another major influencing factor as with **6e** that exerted an excellent scavenging capacity against ABTS with  $IC_{50}$  value of  $9.01 \pm 0.20$   $\mu\text{g/ml}$ , a good activity for DPPH with  $IC_{50}$  value of  $18.66 \pm 0.69$   $\mu\text{g/ml}$  and a moderate activity against Galvinoxyl radical ( $IC_{50} = 86.55 \pm 2.29$   $\mu\text{g/ml}$ ). Furthermore, containing electron-withdrawing groups such as Br presents better results than electron-donating groups as CF<sub>3</sub>. For AChE inhibition, (Table 5) we observed that compounds **6d**, **4e**, **6e**, **6h**, **6i**, **6j** and **6k** did not exhibit any inhibitory effect. Also, the  $IC_{50}$  values of **4a**, **6a**, **6g**, **4b**, **6b**, **4h** and **4k** were superior to 800  $\mu\text{g/ml}$ . A moderate inhibition was measured for **4g** with  $IC_{50}$  value of  $53.36 \pm 2.56$   $\mu\text{g/ml}$ , and a weak potential for **4c**, **6c**, **4d**, **4f**, **6f** and **4j** with  $IC_{50}$  values that varied from 53.36 to 395.33  $\mu\text{g/ml}$  compared to the reference drug Galantamine ( $IC_{50} = 6.27 \pm 1.15$   $\mu\text{g/ml}$ ). It is clear that the addition of diethylphosphate moiety to corresponding diethyl hydroxyphenyl  $\alpha$ -aminophosphonate reduces or eliminates the inhibition activity as in the case of **4c** and **6c**, **4d** and **6d**, **4g** and **6g**, **4j** and **6j**. Additionally, no inhibitory effect was exerted on the BChE by the tested diethyl hydroxyphenyl  $\alpha$ -aminophosphonates or diethylphosphates diethyl  $\alpha$ -aminophosphonates. As a conclusion to the evaluation of the biological effects, diethyl phosphates diethyl  $\alpha$ -aminophosphonates have higher antifungal activity than their correspondent diethyl hydroxyphenyl  $\alpha$ -aminophosphonates as observed with **6b**, **6c**, **6e**, **6f**, **6g**, **6h**, **6i** and **6k** compounds, while **6a**, **6c**, **6d**, **6e**, **6f**, **6h** and **6i** compounds exhibited the strongest antioxidant capacity. Nevertheless, the diethylphosphate diethyl  $\alpha$ -aminophosphonate compounds have weakest AChE inhibitory effect, such as **6g**, **6h**, **6c**, **6i**, **6d**, **6j** and **6k** than the correspondent diethyl hydroxyphenyl  $\alpha$ -aminophosphonate compounds and have no inhibitory effect on BChE. These conclusions may indicate the importance of diethylphosphate moiety inserted to diminish the toxic impact of these compounds destined to be used as pesticides.

#### 2.4. Computational study

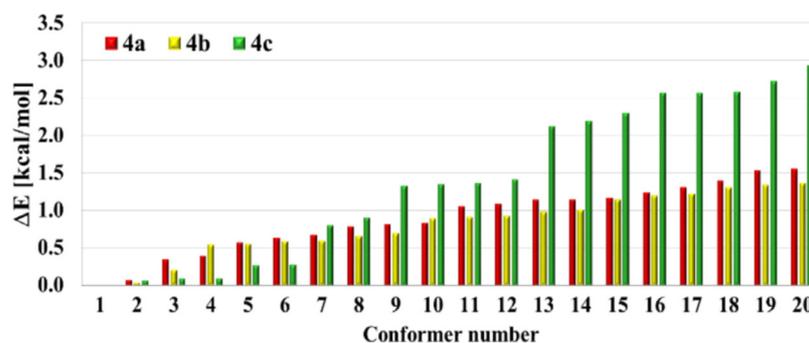
The formulas in Tables 1 and 2 and the measured NMR spectra give only a general idea of the structure of these molecules. In order to deepen knowledge about properties of such compounds and compare an effect of the hydroxyl or phosphate group positions, we performed the quantum chemical calculations for the three diethyl hydroxyphenyl  $\alpha$ -aminophosphonate molecules **4a-c** and the corresponding diethylphosphate diethyl  $\alpha$ -aminophosphonate compounds **6a-c**. For each molecule, at the density functional theory (DFT) level, we analyzed its low energy conformers in vacuum, identified the most stable structure, computed the corresponding <sup>1</sup>H and <sup>13</sup>C NMR chemical shifts and compared them to the measured values.

The lowest energy structures obtained from the DFT calculations are presented in Fig. 3. As can be seen, all structures have a similar arrangement of ethyl arms belonging to the PO(OC<sub>2</sub>H<sub>5</sub>)<sub>2</sub> group. Except for **4a**, the orientation of this group is always with the P = O bond pointing in the opposite direction than the (P)C-H bond. The corresponding methyl groups are always located above the aromatic rings. The spatial arrangement of the OPO(OC<sub>2</sub>H<sub>5</sub>)<sub>2</sub> substituent present in the molecules **6a-c** is similar, thus the two ethyl groups are also stretched away, but their orientation with respect to the aromatic ring depends on whether the diethylphosphate group occupies the *para*-, *meta*- and *ortho*- positions. In **6a** and **6b** compounds, the P = O bond points away from the aromatic ring and the -O-C<sub>2</sub>H<sub>5</sub> groups are arranged approximately parallel to its plane. In **6c** the whole group is rotated by ca. 90°, which enables formation of the hydrogen bond between oxygen and the hydrogen atom (NH). In the corresponding **4c** molecule, the hydroxyl group is also reoriented with respect to the aromatic ring in order to form the energetically profitable hydrogen bond with the oxygen P = O. Due to much more distant positions of *meta* and *para*, no such hydrogen bonds can be created in the molecules **4a** and **4b**. However, it should be mentioned that the energy surface of these molecules is very flat, which is demonstrated in the both Histograms 2 and 3 for 20 lowest energy conformers of each molecule. The energy differences calculated with respect to the most stable conformer 1 for all six structures are very small; in each case  $\Delta E$  for the 20<sup>th</sup> conformer is below 3.5 kcal/mol. Thus, all these structures, and probably also some other conformers having somewhat higher energy, should be thermodynamically accessible, unless the rotation around a single bond encounters a high energy barrier. The exact energy values for all conformers are given in Tables 9 and 10 in the ESI, where additionally dipole moments for each structure are included. The DFT results indicate that generally diethylphosphates diethyl  $\alpha$ -aminophosphonates have significantly higher dipole moments than the corresponding diethyl hydroxyphenyl  $\alpha$ -aminophosphonates (with a few exceptions). The dipole moments for the most stable conformers 1 increase as follows: **4a** < **4b** < **4c** < **6c** < **6a** < **6b**. The same trend appears in the Mulliken charges (Table 11 in ESI) on atoms **N11**, **P24** and **O21** (for the atom numbers see Fig. 2 in ESI) in diethyl hydroxyphenyl  $\alpha$ -aminophosphonates, while it is different in diethylphosphates diethyl  $\alpha$ -aminophosphonates. Among the latter, the *ortho* isomer **6c** has the lowest dipole moment, but at the same time the highest charges on the atoms **N11**, **P24** and **O21**. Of course, the dipole moment results from the partial charges on all atoms and their spatial arrangement, which is more complex in the molecules **6a-c** due to the presence of the additional diethylphosphate moiety. It should be noted that in all diethylphosphates diethyl  $\alpha$ -aminophosphonates, the atom P34 of this group has the highest positive charge. The

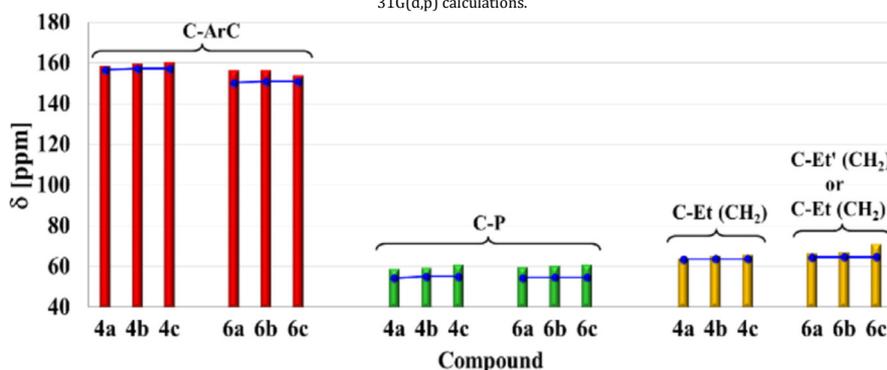
possible coexistence of multiple conformers in a real solution may be responsible for some differences between the computed and measured NMR spectra. All  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shifts  $\delta$  obtained from the DFT calculations for the most stable structures of the six molecules are presented in Tables 12-15 in the ESI, while in the both **Histograms 4** and **5** the computed  $\delta$  for selected atoms are compared to the corresponding measured values. Considering the difference in the environment (vacuum in the theoretical approach, solution in the measurement), the agreement between the calculated and experimental  $\delta$  is quite good. For example, both experimental and theoretical results indicate that the replacement of the hydroxyl group by the diethylphosphate group cause the downfield shift of  $\delta$  (larger values) for most protons. However, when different isomers *para*, *meta* and *ortho* are compared, the theoretical  $\delta$  vary much more than the measured ones, exhibiting clear trends in chemical shifts for particular protons.



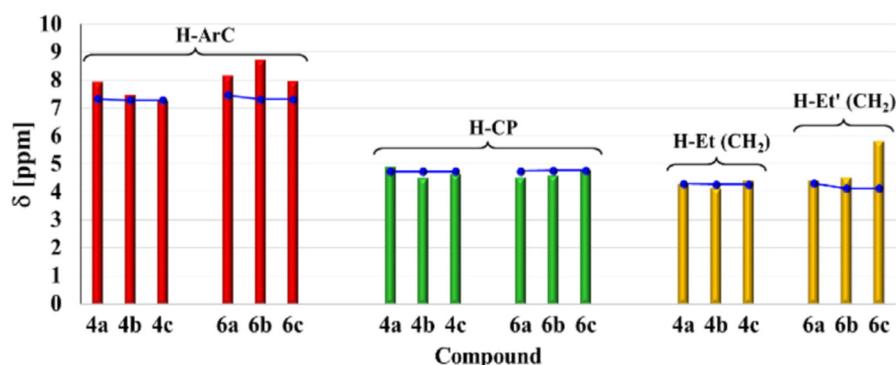
**Fig. 3.** The most stable structures of studied diethyl hydroxyphenyl  $\alpha$ -aminophosphonates and diethylphosphates diethyl  $\alpha$ -aminophosphonates obtained from the B3LYP-GD2/6-31G(d,p) calculations.



**Histogram 2.** The relative energy differences calculated with respect to the most stable structures (conformer 1) for the molecules 4a-c obtained from the B3LYP-GD2/6-31G(d,p) calculations.



**Histogram 4.** Comparison of selected  $^1\text{H}$  NMR chemical shifts for the molecules 4a-c and 6a-c obtained from DF calculations (vertical columns) with the experimental values (blue dots with lines).



**Histogram 5.** Comparison of selected  $^{13}\text{C}$  NMR chemical shifts for the molecules 4a-c and 6a-c obtained from DF calculations (vertical columns) with the experimental values (blue dots with lines).

pared, the theoretical  $\delta$  vary much more than the measured ones, exhibiting clear trends in chemical shifts for particular protons. The possible coexistence of multiple conformers in a real solution may be responsible for some differences between the computed and measured NMR spectra. All  $^1\text{H}$  and  $^{13}\text{C}$  NMR chemical shifts  $\delta$  obtained from the DFT calculations for the most stable structures of the six molecules are presented in Tables 12–15 in the ESI, while in the [Histograms 4](#) and [5](#) the computed  $\delta$  for selected atoms are compared to the corresponding measured values. Considering the difference in the environment (vacuum in the theoretical approach, solution in the measurement), the agreement between the calculated and experimental  $\delta$  is quite good. For example, both experimental and theoretical results indicate that the replacement of the hydroxyl group by the phosphate group cause the downfield shift of  $\delta$  (larger values) for most protons. However, when different isomers *para*, *meta* and *ortho* are compared, the theoretical  $\delta$  vary much more than the measured ones, exhibiting clear trends in chemical shifts for particular protons. For example, the experimental  $\delta$  for the proton HCP in the three isomers **4** (**a**, **b** and **c**) are exactly the same, while the calculated values follow the trend:  $\delta(4a) > \delta(4c) > \delta(4b)$ . For the same proton in diethylphosphate diethyl  $\alpha$ -aminophosphonates the measured value of 4.77 ppm for **6b** and **6c** is only slightly higher than 4.75 ppm for **6a**, while the computed chemical shifts are in the following order:  $\delta(6c) > \delta(6b) > \delta(6a)$  (4.51, 4.56 and 4.74 ppm, respectively). Such differences and sometimes even the reverse ordering of the experimental and theoretical NMR chemical shifts appear also for other protons and for some carbons. One may also note that the quantum calculations predict for some protons much larger values  $\delta$  than these obtained from the spectroscopic analysis. As already mentioned, the discrepancies may be due to the fact that the calculated NMR spectra are obtained in a vacuum for a single structure with the lowest energy, while under experimental conditions they are measured in solution where they are affected by the presence of the solvent and other, less stable conformers. **3**.

## Conclusion

In this work, we have designed the diethylphosphate diethyl  $\alpha$ -aminophosphonate derivatives (**6a-k**) as novel category in organophosphorus compounds. These products were synthesized by two steps using two famous multicomponents reactions; the first one is the *Kabachnik-Fieds* reaction which used for the synthesis of diethyl hydroxyphenyl  $\alpha$ -aminophosphonates in the presence of  $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ . The second step involves the phosphorylation of diethyl hydroxyphenyl  $\alpha$ -aminophosphonates by *Atherton-Todd* reaction to give the diethyl phosphates diethyl  $\alpha$ -aminophosphonates with an excellent chemical yield at room temperature within 30 min. The structure of diethyl [(4-phosphate phenyl) (4-trifluoromethyl phenylamino) methyl] phosphonate **6g** was proved by X-ray diffraction of pure crystal. The most of synthesized phosphates  $\alpha$ -aminophosphonates have exhibited an excellent antifungal activity and a high antioxidant potential. A weak AChE inhibition and no effect on BChE is detected. In addition, we observed that phosphate  $\alpha$ -aminophosphonates have an antifungal effect and antioxidant capacity higher than the corresponding diethyl hydroxyphenyl  $\alpha$ -aminophosphonates and the weakest AChE inhibitory effect with the absence of interaction with BChE. These conclusions may indicate the importance of diethylphosphate inserted moiety to the diethyl hydroxyphenyl  $\alpha$ -aminophosphonates and the possibility of the use of diethylphosphates diethyl  $\alpha$ -aminophosphonates as potential safer pesticides that needs to be confirmed by further biological evaluations. The computational study allowed to identifying the most stable conformers of diethyl hydroxyphenyl  $\alpha$ -aminophosphonates and diethylphosphates diethyl  $\alpha$ -aminophosphonates and analyzed in detail their spatial structure. It is shown that in these molecules the ethyl groups belonging to the  $\alpha$ -aminophosphonate moiety are preferentially placed above the aromatic rings. However, the energy differences between various low energy geometries of the same compound are rather small, so conformational interconversion and the coexistence of multiple structures in a real system is very likely. The calculated NMR spectra were found to be fairly well in agreement with the measured ones and allowed a more accurate assignment of the chemical shifts.

## 4.

### Experimental section

#### 4.1. Chemistry

All reagents were purchased from Sigma-Aldrich or Acros Company used without further purification. Reactions were monitored by thin layer chromatography (TLC) carried out on 0.25-mm Merck silica gel plates (60F-254) using ultraviolet light (254 nm) as the visualizing agent and  $\text{KMnO}_4$  solution as developing agents. NMR spectra were recorded with Bruker spectrometers operating at (360 MHz, 300 MHz and 250 MHz for  $^1\text{H}$ , 90 MHz, 75 MHz or 63 MHz for  $^{13}\text{C}$  and 101 MHz or 121 MHz for  $^{31}\text{P}$ ). Chemical shift of

Solvent reference peaks used were  $\text{CDCl}_3$  ( $\delta = 7.26$  ppm) for  $^1\text{H}$  and ( $\delta = 77$  ppm) for  $^{13}\text{C}$  NMR spectra, while  $\text{H}_3\text{PO}_4$  was used as external standard for chemical shift references for  $^{31}\text{P}$  NMR. Couplings constants ( $J$ ) are given in Hz, with the following abbreviations multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad signal. Mass spectra were taken by a MicrOTOF-Q Bruker spectrometer using electrospray ionization (ESI) analysis. Melting points were measured using Buchi Melting Point B-545.

**4.1.1. General procedure for the synthesis of Diethyl hydroxyphenyl  $\alpha$ -aminophosphonates 4a-k** To the reaction mixture containing hydroxybenzaldehyde (122.12 mg, 1 mmol), aniline (93 mg, 1 mmol), and diethylphosphite (165 mg, 1.2 mmol) a  $\text{NiSO}_4 \cdot \text{H}_2\text{O}$  (5 mol%) was added, it was stirred within 30 min at room temperature. The reaction progress was monitored by TLC. The catalyst was removed by filtration. The filtrate was washed by water (10 mL) and extracted with dichloromethane (10 mL  $\times$  2). The organic phases were combined and evaporated in vacuum. The crude product was purified by recrystallization in hexane or by column chromatography on silica gel (ethyl acetate/hexane: 30/70) to afford majority product 4a-l. Complete experimental data have been provided (NMR spectra and HRMS).

**4.1.1.1. Diethyl [(4-hydroxyphenyl) (phenylamino) methyl] phosphonate (4a).** White solid, 94% yield, mp: 110 °C.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.33–7.17 (m, 2H, H-Ar), 7.22–7.03 (m, 2H, H-Ar), 6.72 (t,  $J = 7.3$  Hz, 3H, H-Ar), 6.63 (d,  $J = 7.7$  Hz, 2H, H-Ar), 4.74 (d,  $J = 23.7$  Hz, 1H, HCP), 4.30–4.04 (m, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>), 4.08–3.91 (m, 3H, -O-CH<sub>2</sub>-CH<sub>3</sub>), 3.91–3.63 (m, 1H, -O-CH<sub>2</sub>-CH<sub>3</sub>), 1.30 (t,  $J = 7.1$  Hz, 3H, -O-CH<sub>2</sub>-CH<sub>3</sub>), 1.18 (t,  $J = 7.1$  Hz, 3H, -O-CH<sub>2</sub>-CH<sub>3</sub>);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  156.54 (d,  $J = 3.0$  Hz), 146.23 (d,  $J = 14.7$  Hz), 129.16, 128.90 (d,  $J = 5.6$  Hz), 125.97 (d,  $J = 2.4$  Hz), 118.49, 116.00 (d,  $J = 2.4$  Hz), 113.97, 63.56 (dd,  $J_{\text{C-P}} = 13.2, 7.2$  Hz), 56.32, 54.28, 16.33 (dd,  $J_{\text{3C-P}} = 12.7, 5.7$  Hz);  $^{31}\text{P}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  22.78 ppm; HRMS (ESI)  $m/z$  calcd for  $\text{C}_{17}\text{H}_{22}\text{NO}_4\text{NaP}$  [ $M + \text{Na}^+$ ]: 358.1178; Found 358.1171.

**4.1.1.2. Diethyl [(3-hydroxyphenyl) (phenylamino) methyl] phosphonate (4b).** White solid, 76% yield, mp: 108 °C.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.28 (s, 1H, H-Ar), 7.21 (t,  $J = 7.6$  Hz, 1H, H-Ar), 7.18–7.06 (m, 2H, H-Ar), 6.95 (d,  $J = 5.2$  Hz, 1H, H-Ar), 6.81 (d,  $J = 8.0$  Hz, 1H), 6.70 (t,  $J = 7.4$  Hz, 1H, H-Ar), 6.61 (d,  $J = 7.6$  Hz, 2H, H-Ar), 4.74 (d,  $J = 24.3$  Hz, 1H, HCP), 4.28–4.05 (m, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.93 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.73–3.55 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.29 (t,  $J = 7.1$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.09 (t,  $J = 7.1$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>).  $^{13}\text{C}$  NMR (91 MHz,  $\text{CDCl}_3$ )  $\delta$  157.14, 146.29 (d,  $J = 14.6$  Hz), 137.05, 129.71, 129.15, 119.99 (d,  $J = 6.3$  Hz), 118.45, 115.64, 114.33 (d,  $J = 4.2$  Hz), 113.90, 63.82 (dd,  $J_{\text{C-P}} = 19.2, 7.1$  Hz), 56.72, 55.04, 16.25 (dd,  $J_{\text{3C-P}} = 22.3, 5.8$  Hz).  $^{31}\text{P}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  22.83 ppm. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{17}\text{H}_{22}\text{NO}_4\text{NaP}$  [ $M + \text{Na}^+$ ]: 358.1178; Found 358.1162.

**4.1.1.3. Diethyl [(3-hydroxyphenyl) (phenylamino) methyl] phosphonate (4c).** White solid, 92% yield, mp: 112 °C.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.28 (s, 1H, H-Ar), 7.21 (t,  $J = 7.6$  Hz, 1H, H-Ar), 7.18–7.06 (m, 2H, H-Ar), 6.95 (d,  $J = 5.2$  Hz, 1H, H-Ar), 6.81 (d,  $J = 8.0$  Hz, 1H), 6.70 (t,  $J = 7.4$  Hz, 1H, H-Ar), 6.61 (d,  $J = 7.6$  Hz, 2H, H-Ar), 4.74 (d,  $J = 24.3$  Hz, 1H, HCP), 4.28–4.05 (m, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.93 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.73–3.55 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.29 (t,  $J = 7.1$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.09 (t,  $J = 7.1$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>).  $^{13}\text{C}$  NMR (91 MHz,  $\text{CDCl}_3$ )  $\delta$  157.14, 146.29 (d,  $J = 14.6$  Hz), 137.05, 129.71, 129.15, 119.99 (d,  $J = 6.3$  Hz), 118.45, 115.64, 114.33 (d,  $J = 4.2$  Hz), 113.90, 63.82 (dd,  $J_{\text{C-P}} = 19.2, 7.1$  Hz), 56.72, 55.04, 16.25 (dd,  $J_{\text{3C-P}} = 22.3, 5.8$  Hz).  $^{31}\text{P}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  22.83 ppm. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{17}\text{H}_{22}\text{NO}_4\text{NaP}$  [ $M + \text{Na}^+$ ]: 358.1178; Found 358.1171.

**4.1.1.4. Diethyl [(4-hydroxyphenyl) (4-bromophenylamino) methyl] phosphonate (4d).** Green solid, 86% yield, mp: 126 °C.  $^1\text{H}$  NMR (250 MHz,  $\text{CDCl}_3$ )  $\delta$  7.24–7.09 (m, 5H, H-Ar), 6.70 (d,  $J = 8.4$  Hz, 1H, H-Ar), 6.47 (d,  $J = 8.8$  Hz, 2H, H-Ar), 4.65 (d,  $J = 23.8$  Hz, 1H, HCP), 4.21–4.02 (m, 2H, -O-CH<sub>2</sub>-CH<sub>3</sub>), 4.02–3.87 (m, 1H, -O-CH<sub>2</sub>-CH<sub>3</sub>), 3.85–3.62 (m, 1H, -O-CH<sub>2</sub>-CH<sub>3</sub>), 1.27 (t,  $J = 7.1$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.14 (t,  $J = 7.1$  Hz, 3H, -O-CH<sub>2</sub>-CH<sub>3</sub>).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  156.79, 145.23 (d,  $J = 14.9$  Hz), 131.89, 128.90 (d,  $J = 5.0$  Hz), 125.35, 115.82 (d,  $J = 34.8$  Hz), 110.28, 63.71 (d,  $J_{\text{C-P}} = 5.5$  Hz), 56.27, 54.23, 16.34 (dd,  $J_{\text{3C-P}} = 13.2, 5.4$  Hz).  $^{31}\text{P}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  22.43 ppm. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{17}\text{H}_{21}\text{BrNO}_4\text{NaP}$  [ $M + \text{Na}^+$ ]: 436.0283; Found 436.0274.

**4.1.1.5. Diethyl [(3-hydroxyphenyl) (4-bromophenylamino) methyl] phosphonate (4e).** Green solid, 72% yield, mp: 126 °C.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  7.35–7.11 (m, 4H, H-Ar), 6.74 (d,  $J = 8.4$  Hz, 2H, H-Ar), 6.50 (d,  $J = 8.9$  Hz, 2H, H-Ar), 4.68 (d,  $J = 23.9$  Hz, 1H, HCP), 4.30–4.03 (m, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>), 4.07–3.89 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.84–3.67 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.30 (t,  $J = 7.1$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.17 (t,  $J = 7.1$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>).  $^{31}\text{P}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  22.47.  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  162.63, 156.52 (d,  $J = 2.9$  Hz), 145.12 (d,  $J = 14.8$  Hz), 132.39, 131.85, 128.88 (d,  $J = 5.5$  Hz), 125.48 (d,  $J = 2.0$  Hz), 117.65–114.55 (m), 110.30, 63.72 (d,  $J_{\text{C-P}} = 7.4$  Hz), 56.21, 54.17, 16.27 (dd,  $J_{\text{3C-P}} = 13.2, 5.6$  Hz).  $^{31}\text{P}$  NMR (101 MHz, Chloroform-*d*)  $\delta$  22.50. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{17}\text{H}_{21}\text{BrNO}_4\text{NaP}$  [ $M + \text{Na}^+$ ]: 436.0283; Found 436.0268.

**4.1.1.6. Diethyl [(2-hydroxyphenyl) (4-bromophenylamino) methyl] phosphonate (4f).** Green solid, 80% yield, mp: 128 °C.  $^1\text{H}$  NMR (250 MHz,  $\text{CDCl}_3$ )  $\delta$  7.33–7.04 (m, 4H, H-Ar), 6.98–6.77 (m, 3H, H-Ar), 6.53 (d,  $J = 6.8$  Hz, 1H, H-Ar), 5.07 (d,  $J = 23.6$  Hz, 1H, HCP), 4.20–4.00 (m, 4H, O-CH<sub>2</sub>-CH<sub>3</sub> + OH), 3.97–3.84 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.28 (t,  $J = 7.1$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.18 (t,  $J = 7.1$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  155.55 (d,  $J = 4.4$  Hz), 145.33 (d,  $J = 13.4$  Hz), 131.92, 129.49, 128.79 (d,  $J = 5.5$  Hz), 121.20,

120.42, 117.58, 115.77, 110.64, 78.17 -76.04(m), 63.85 (dd,  $J_{C-P} = 26.2, 6.9$  Hz), 53.52, 51.47, 16.35 (dd,  $J_{3 C-P} = 15.4, 5.2$  Hz).  $^{31}P$  NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$ 23.57. HRMS (ESI)  $m/z$  calcd for C<sub>17</sub>H<sub>22</sub>BrNO<sub>4</sub>NaP [M + Na + ]: 436.0283; Found 436.0265. 4.1.1.7. *Diethyl [(4-hydroxyphenyl) (4-trifluoromethyl phenylamino) methyl] phosphonate (4g)*. Brawn solid, 90 % yield, mp: 136 °C.  $^1H$  NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ 7.34 (d,  $J = 8.5$  Hz, 2H, H-Ar), 7.21 (dd,  $J = 8.6, 2.2$  Hz, 3H, H-Ar + OH), 6.70 (d,  $J = 8.3$  Hz, 2H, H-Ar), 6.62 (d,  $J = 8.5$  Hz, 2H, H-Ar), 4.72 (d,  $J = 23.5$  Hz, 1H, HCP), 4.22 -4.04 (m, 2H-O-CH<sub>2</sub>-CH<sub>3</sub>), 4.03 -3.89 (m, 1H-O-CH<sub>2</sub>-CH<sub>3</sub>), 3.83 -3.66 (m, 1H-O-CH<sub>2</sub>-CH<sub>3</sub>), 1.28 (t,  $J = 7.1$  Hz, 3H, -O-CH<sub>2</sub>-CH<sub>3</sub>), 1.16 (t,  $J = 7.1$  Hz, 3H-O-CH<sub>2</sub>-CH<sub>3</sub>).  $^{13}C$  NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$ 156.71, 148.82 (d,  $J = 14.2$  Hz), 128.87 (d,  $J = 4.7$  Hz), 126.55, 125.32, 120.34, 119.91, 116.11, 113.13, 63.72 (d,  $J_{C-P} = 5.3$  Hz), 55.88, 53.83, 16.32 (dd,  $J_{3 C-P} = 12.8, 5.3$  Hz).  $^{31}P$  NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$ 22.20 ppm. HRMS (ESI)  $m/z$  calcd for C<sub>20</sub>H<sub>22</sub>BrNO<sub>4</sub>NaP [M + Na + ]: 426.1052; Found 426.1032.

4.1.1.8. *Diethyl [(4-hydroxyphenyl) (3-trifluoromethyl phenylamino) methyl] phosphonate (4h)*. Brawn solid, 65 % yield, mp: 142 °C.  $^1H$  NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ 7.32 (d,  $J = 8.6$  Hz, 2H, H-Ar), 7.28 -7.13 (m, 2H, H-Ar), 6.94 (d,  $J = 7.5$  Hz, 1H, H-Ar), 6.85 (d,  $J = 8.0$  Hz, 1H, H-Ar), 6.59 (d,  $J = 8.6$  Hz, 2H, H-Ar), 4.75 (d,  $J = 24.1$  Hz, 1H, HCP), 4.26 -4.03 (m, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.92 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.69 -3.45 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.30 (t,  $J = 7.1$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.07 (t,  $J = 7.1$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>).  $^{13}C$  NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$ 157.43, 148.81 (d,  $J = 14.2$  Hz), 136.23, 129.96, 126.50, 120.29, 119.88 (d,  $J = 6.0$  Hz), 116.04, 114.12 (d,  $J = 4.3$  Hz), 113.01, 64.01 (dd,  $J_{C-P} = 40.1, 7.1$  Hz), 56.49, 54.46, 16.20 (dd,  $J_{3 C-P} = 20.2, 5.6$  Hz).  $^{31}P$  NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$ 22.32. HRMS (ESI)  $m/z$  calcd for C<sub>20</sub>H<sub>22</sub>F<sub>3</sub>NO<sub>4</sub>NaP [M + Na + ]: 426.1052; Found 426.1032. 4.1.1.9. *Diethyl [(2-hydroxyphenyl) (2-trifluoromethyl phenylamino) methyl] phosphonate (4i)*. Brawn solid, 90% yield, mp: 156 °C.  $^1H$  NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ 7.36 (d,  $J = 8.5$  Hz, 2H, H-Ar), 7.27 (dt,  $J = 7.8, 1.8$  Hz, 1H, H-Ar), 7.19 -7.11 (m, 1H, H-Ar), 6.95 (d,  $J = 8.0$  Hz, 1H, H-Ar), 6.87 (t,  $J = 7.9$  Hz, 1H, H-Ar), 6.70 (d,  $J = 8.5$  Hz, 2H, H-Ar), 5.19 (d,  $J = 23.6$  Hz, 1H, HCP), 4.25 -4.07 (m, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>), 4.03 -3.90 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.31 (t,  $J = 7.1$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.23 (t,  $J = 7.1$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>).  $^{13}C$  NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$ 155.47 (d,  $J = 4.7$  Hz), 148.91 (d,  $J = 12.3$  Hz), 129.63, 128.76 (d,  $J = 6.1$  Hz), 126.85 -125.53 (m), 121.20, 120.45 (d,  $J = 8.2$  Hz), 119.96, 117.73, 113.13, 63.91 (dd,  $J_{C-P} = 26.7, 7.1$  Hz), 52.93, 50.88, 16.31 (dd,  $J_{3 C-P} = 15.4, 5.5$  Hz).  $^{31}P$  NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$ 23.31. HRMS (ESI)  $m/z$  calcd for C<sub>20</sub>H<sub>22</sub>F<sub>3</sub>NO<sub>4</sub>NaP [M + Na + ]: 426.1052; Found 426.1034. 4.1.1.10. *Diethyl [(4-hydroxyphenyl) (4-nitro phenylamino) methyl] phosphonate (4j)*. Orange solid, 92 % yield, mp: 96 °C.  $^1H$  NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ 8.01 (d,  $J = 9.1$  Hz, 2H, H-Ar), 7.22 (dd,  $J = 8.6, 2.2$  Hz, 2H, H-Ar), 6.72 (d,  $J = 8.5$  Hz, 2H, H-Ar), 6.59 (d,  $J = 9.2$  Hz, 2H, H-Ar), 5.64 (t,  $J = 8.5$  Hz, 1H, NH), 4.78 (dd,  $J = 23.4, 7.9$  Hz, 1H, HCP), 4.28 -4.05 (m, 2H, O-CH<sub>2</sub>-CH<sub>3</sub>), 4.05 -3.86 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.76 (dd,  $J = 17.2, 8.4$  Hz, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.28 (t,  $J = 7.1$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.16 (t,  $J = 7.1$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>).  $^{13}C$  NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$ 157.37 -156.43 (m), 151.72 (d,  $J = 13.2$  Hz), 139.04, 128.91 (d,  $J = 5.1$  Hz), 126.08, 116.18, 112.50, 63.91 (dd,  $J_{C-P} = 19.7, 7.3$  Hz), 55.74, 53.69, 16.33 (dd,  $J_{3 C-P} = 12.9, 5.4$  Hz).  $^{31}P$  NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$ 21.31 ppm. HRMS (ESI)  $m/z$  calcd for C<sub>17</sub>H<sub>21</sub>N<sub>2</sub>O<sub>6</sub>NaP [M + Na + ]: 436.0283; Found 436.0256. 4.1.1.11. *Diethyl [(4-hydroxyphenyl) (3,5-methyl phenylamino) methyl] phosphonate (4k)*. Yalow solid, 88% yield, mp: 145 °C.  $^1H$  NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ 7.32 -7.11 (m, 2H, H-Ar), 6.68 (d,  $J = 8.4$  Hz, 2H, H-Ar), 6.35 (s, 1H, H-Ar), 6.25 (s, 2H, H-Ar), 4.70 (d,  $J = 23.7$  Hz, 1H, HCP), 4.21 -3.96 (m, 2H, -O-CH<sub>2</sub>-CH<sub>3</sub>), 3.96 (m, 1H, -O-CH<sub>2</sub>-CH<sub>3</sub>), 3.83 -3.61 (m, 1H, -O-CH<sub>2</sub>-CH<sub>3</sub>), 2.16 (s, 1H, -O-CH<sub>2</sub>-CH<sub>3</sub>), 1.26 (t,  $J = 7.1$  Hz, 3H, -O-CH<sub>2</sub>-CH<sub>3</sub>), 1.14 (t,  $J = 7.1$  Hz, 3H, -O-CH<sub>2</sub>-CH<sub>3</sub>).  $^{13}C$  NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$ 159.98 -152.79 (m), 146.29 (d,  $J = 14.3$  Hz), 138.82, 128.85 (d,  $J = 5.4$  Hz), 126.04, 120.48, 115.98, 111.85, 63.57 (dd,  $J_{C-P} = 16.9, 7.2$  Hz), 56.23, 54.19, 21.46, 16.34 (dd,  $J_{3 C-P} = 13.5, 5.7$  Hz).  $^{31}P$  NMR (101 MHz, CDCl<sub>3</sub>)  $\delta$ 22.96 ppm. HRMS (ESI)  $m/z$  calcd for C<sub>23</sub>H<sub>36</sub>NO<sub>4</sub>NaP [M + Na + ]: 426.1052; Found 426.1032. 4.1.2. *Synthesis of diethylphosphates diethyl  $\alpha$ -aminophosphonates 6 a-k* Tetrachloromethane (2 mL) and triethylamine (0.34 mL, 2.5 mmol) were added to diethyl hydroxyphenyl  $\alpha$ -aminophosphonates (1 mmol) under stirring. After cooling the reaction mixture to 0 °C, a corresponding diethylphosphite (1 mmol) was added at vigorous stirring during 30 min. After the reaction mixture was stirred at room temperature, and the reaction was completed. The reaction progress was monitored by TLC. The mixture was extracted with dichloromethane (10 mL  $\times$  2). The organic phases were combined and evaporated in vacuum. The crude product was purified by column chromatography on silica gel (ethyl acetate/hexane: 40/60) or by crystallization from hexane to afford majority product 6a-l. Complete experimental data have been provided (NMR spectra and HRMS). 4.1.2.1. *Diethyl [(4-diethylphosphate phenyl) (phenylamino) methyl] phosphonate (6a)*. White solid, 90% yield, mp: 180 °C.  $^1H$  NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$ 7.46 (dd,  $J = 8.7, 2.3$  Hz, 2H, H-Ar), 7.21 (d,  $J = 8.7$  Hz, 2H, H-Ar), 7.12 (dd,  $J = 8.5, 7.4$  Hz, 2H, H-Ar), 6.78 -6.65 (m, 1H, H-Ar), 6.58 (dd,  $J = 8.6, 1.0$  Hz, 2H, H-Ar), 4.75 (d,  $J = 24.2$  Hz, 1H, HCP), 4.31 -4.07 (m, 6H, -O-CH<sub>2</sub>-CH<sub>3</sub>), 4.05 -3.88 (m, 1H, -O-CH<sub>2</sub>-CH<sub>3</sub>), 3.85 -3.66 (m, 1H, -O-CH<sub>2</sub>-CH<sub>3</sub>), 1.38 -1.26 (m, 9H, -O-CH<sub>2</sub>-CH<sub>3</sub>), 1.16 (t,  $J = 7.1$  Hz, 3H, -O-CH<sub>2</sub>-CH<sub>3</sub>).  $^{13}C$  NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$ 150.35, 146.10 (d,  $J = 14.3$  Hz), 132.61, 129.17, 129.08, 120.21 -119.99 (m), 118.55, 113.85, 64.58 (d,  $J_{C-P} = 6.1$  Hz), 63.30 (t,  $J = 6.4$  Hz), 56.47, 54.47, 16.42 (d,  $J_{3 C-P} = 5.7$  Hz), 16.13 (dd,  $J_{3 C-P} =$

12.8, 6.3 Hz).  $^{31}\text{P}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$ 22.14, -6.47 ppm. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{21}\text{H}_{31}\text{NO}_7\text{NaP}_2$  [ $M + \text{Na} +$ ]: 494.1467; Found 494.1466. 4.1.2.2. Diethyl [(3-diethylphosphate phenyl) (phenylamino) methyl] phosphonate (6b). Yellow solid, 70% yield, mp: 186 °C.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ 7.31 (m, 4H, H-Ar), 7.10 (t,  $J = 7.9$  Hz, 2H, H-Ar), 6.70 (t,  $J = 7.3$  Hz, 1H, H-Ar), 6.58 (d,  $J = 8.6$  Hz, 2H, H-Ar), 4.77 (d,  $J = 24.5$  Hz, 1H, HCP), 4.13 (m, 6H, O-CH<sub>2</sub>-CH<sub>3</sub>), 4.03–3.85 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.86–3.66 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.42–1.19 (m, 9H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.15 (t,  $J = 7.1$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$ 150.93 (d,  $J = 6.4$  Hz), 146.02 (d,  $J = 14.8$  Hz), 138.24, 129.84, 129.16, 124.52 (d,  $J = 5.0$  Hz), 119.42 (dd,  $J = 14.9, 4.1$  Hz), 118.55, 113.82, 64.66 (d,  $J_{C-P} = 6.1$  Hz), 63.80–63.12 (m), 56.68, 54.68, 16.41 (d,  $J = 5.6$  Hz), 16.12 (dd,  $J_{3C-P} = 13.9, 6.1$  Hz).  $^{31}\text{P}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$ 21.98, -6.59. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{21}\text{H}_{31}\text{NO}_7\text{NaP}_2$  [ $M + \text{Na} +$ ]: 494.1467; Found: 494.1466. 4.1.2.3. Diethyl [(2-diethyl phosphate phenyl) (phenylamino) methyl] phosphonate (6c). Yellow solid, 89% yield, mp: 186 °C.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ 7.31 (m, 4H, H-Ar), 7.10 (t,  $J = 7.9$  Hz, 2H, H-Ar), 6.70 (t,  $J = 7.3$  Hz, 1H, H-Ar), 6.58 (d,  $J = 8.6$  Hz, 2H, H-Ar), 4.77 (d,  $J = 24.5$  Hz, 1H, HCP), 4.13 (m, 6H, O-CH<sub>2</sub>-CH<sub>3</sub>), 4.03–3.85 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.86–3.66 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.42–1.19 (m, 9H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.15 (t,  $J = 7.1$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$ 150.93 (d,  $J = 6.4$  Hz), 146.02 (d,  $J = 14.8$  Hz), 138.24, 129.84, 129.16, 124.52 (d,  $J = 5.0$  Hz), 119.42 (dd,  $J = 14.9, 4.1$  Hz), 118.55, 113.82, 64.66 (d,  $J_{C-P} = 6.1$  Hz), 63.80–63.12 (m), 56.68, 54.68, 16.41 (d,  $J = 5.6$  Hz), 16.12 (dd,  $J_{3C-P} = 13.9, 6.1$  Hz).  $^{31}\text{P}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$ 21.98, -6.59. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{21}\text{H}_{31}\text{NO}_7\text{NaP}_2$  [ $M + \text{Na} +$ ]: 494.1467; Found: 494.1466. 4.1.2.4. Diethyl [(4-diethylphosphate phenyl) (4-bromophenylamino) methyl] phosphonate (6d). Green solid, 92% yield, mp: 220 °C.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ 7.41 (d,  $J = 8.5$  Hz, 2H, H-Ar), 7.19 (t,  $J = 7.9$  Hz, 4H, H-Ar), 6.45 (d,  $J = 8.5$  Hz, 2H, H-Ar), 4.68 (d,  $J = 24.0$  Hz, 1H, HCP), 4.16 (m, 6H, O-CH<sub>2</sub>-CH<sub>3</sub>), 4.03–3.87 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.82–3.62 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.30 (m, 9H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.14 (t,  $J = 7.0$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$ 150.52, 145.14 (d,  $J = 14.8$  Hz), 131.97 (d,  $J = 11.4$  Hz), 129.07 (d,  $J = 5.0$  Hz), 120.19, 115.46, 110.30, 64.63 (d,  $J_{C-P} = 5.9$  Hz), 63.69–63.02 (m), 56.42, 54.42, 16.43 (d,  $J = 5.5$  Hz), 16.14 (dd,  $J_{3C-P} = 12.1, 6.1$  Hz).  $^{31}\text{P}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$ 21.85, -6.53 ppm. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{21}\text{H}_{30}\text{O}_7\text{BrNP}_2$  [ $M + \text{Na} +$ ]: 574.0573; Found 574.0533. 4.1.2.5. Diethyl [(3-diethylphosphate phenyl) (4-bromophenylamino) methyl] phosphonate (6e). Green solid, 65% yield, mp: 192 °C.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ 7.34–7.26 (m, 4H, H-Ar), 7.20–7.12 (m, 2H, H-Ar), 6.46 (d,  $J = 8.9$  Hz, 2H, H-Ar), 4.99–4.84 (m, 1H, HCP), 4.69 (dd,  $J = 24.3, 7.6$  Hz, 1H), 4.25–4.03 (m, 6H, O-CH<sub>2</sub>-CH<sub>3</sub>), 4.03–3.87 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.83–3.67 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.29 (m, 9H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.14 (t,  $J = 6.9$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$ 157.30, 150.96, 145.07 (d,  $J = 14.8$  Hz), 137.68, 131.82, 129.83 (d,  $J = 17.8$  Hz), 124.43 (d,  $J = 4.9$  Hz), 119.71, 119.28 (t,  $J = 5.1$  Hz), 115.48, 110.36, 64.69 (d,  $J_{C-P} = 6.0$  Hz), 63.85–63.06 (m), 56.72, 54.72, 16.41 (d,  $J = 5.5$  Hz), 16.12 (dd,  $J_{3C-P} = 13.4, 6.0$  Hz).  $^{31}\text{P}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$ 22.41, 21.58. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{21}\text{H}_{30}\text{O}_7\text{BrNP}_2$  [ $M + \text{Na} +$ ]: 574.0573; Found 574.0533. 4.1.2.6. Diethyl [(2-diethylphosphate phenyl) (4-bromophenylamino) methyl] phosphonate (6f). Green solid, 88% yield, mp: 232 °C.  $^1\text{H}$  NMR (250 MHz,  $\text{CDCl}_3$ )  $\delta$ 7.55–7.38 (m, 2H, H-Ar), 7.32–7.21 (m, 1H, H-Ar), 7.15 (t,  $J = 8.1$  Hz, 3H, H-Ar), 6.54 (d,  $J = 8.9$  Hz, 2H, H-Ar), 5.21 (d,  $J = 24.5$  Hz, 1H, HCP), 4.36–4.04 (m, 6H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.95–3.73 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.72–3.51 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.41–1.25 (m, 9H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.05 (t,  $J = 7.1$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>).  $^{13}\text{C}$  NMR (91 MHz,  $\text{CDCl}_3$ )  $\delta$ 149.15, 144.96 (d,  $J = 14.3$  Hz), 131.87, 129.27 (d,  $J = 3.2$  Hz), 128.43 (d,  $J = 4.2$  Hz), 126.48 (d,  $J = 7.5$  Hz), 125.19, 119.08, 115.31, 110.17, 64.85 (t,  $J = 6.5$  Hz), 63.64 (d,  $J_{C-P} = 7.2$  Hz), 63.16 (d,  $J_{C-P} = 6.8$  Hz), 49.75, 48.05, 16.66–15.94 (m).  $^{31}\text{P}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$ 22.37, -6.54 ppm. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{21}\text{H}_{30}\text{O}_7\text{BrNP}_2$  [ $M + \text{Na} +$ ]: 574.0573; Found 574.0533. 4.1.2.7. Diethyl [(4-diethylphosphate phenyl) (4-trifluoromethyl phenylamino) methyl] phosphonate (6g). Yellow solid, 90% yield, mp: 186 °C.  $^1\text{H}$  NMR (250 MHz,  $\text{CDCl}_3$ )  $\delta$ 7.44 (dd,  $J = 8.7, 2.2$  Hz, 2H, H-Ar), 7.35 (d,  $J = 8.5$  Hz, 2H, H-Ar), 7.23 (d,  $J = 8.6$  Hz, 2H, H-Ar), 6.61 (d,  $J = 8.5$  Hz, 2H, H-Ar), 4.77 (d,  $J = 24.2$  Hz, 1H, HCP), 4.29–4.05 (m, 7H, O-CH<sub>2</sub>-CH<sub>3</sub> + NH), 4.03–3.85 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.82–3.65 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.34 (t,  $J = 6.5$  Hz, 6H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.15 (t,  $J = 7.1$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>).  $^{13}\text{C}$  NMR (63 MHz,  $\text{CDCl}_3$ )  $\delta$ 148.92, 148.63, 131.81 (d,  $J = 3.1$  Hz), 129.04 (d,  $J = 5.4$  Hz), 126.53 (q,  $J = 3.8$  Hz), 120.28 (dd,  $J = 5.2, 2.7$  Hz), 113.09, 64.62 (d,  $J = 6.2$  Hz), 63.48 (dd,  $J_{C-P} = 8.8, 7.1$  Hz), 56.29, 53.87, 16.53–15.86 (m).  $^{31}\text{P}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$ 21.59, -6.50. HRMS (ESI)  $m/z$  calcd for  $\text{C}_{22}\text{H}_{30}\text{F}_3\text{NNaO}_7\text{P}$  [ $M + \text{Na} +$ ]: 562.1341; Found 562.1314. 4.1.2.8. Diethyl [(4-diethylphosphate phenyl) (3-trifluoromethyl phenylamino) methyl] phosphonate (6h). Yellow solid, 67% yield, mp: 181 °C.  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$ 7.36–7.32 (m, 5H, H-Ar), 7.18 (d,  $J = 11.0$  Hz, 1H, H-Ar), 6.63 (d,  $J = 8.5$  Hz, 2H, H-Ar), 4.79 (d,  $J = 24.5$  Hz, 1H, HCP), 4.29–4.05 (m, 6H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.98 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.83–3.68 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.29 (m, 9H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.16 (t,  $J = 7.1$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>).  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$ 151.01, 148.81, 137.43, 130.05, 126.51, 124.39, 119.57 (d,  $J = 45.4$  Hz), 113.09, 64.70 (d,  $J = 5.9$  Hz), 63.66 (dd,  $J_{C-P} = 12.1, 7.2$  Hz), 56.29, 54.29, 16.93–15.18 (m).  $^{31}\text{P}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$ 21.26, -6.59.

HRMS (ESI)  $m/z$  calcd for  $C_{22}H_{30}F_3NNaO_7P$  [M + Na + ]; 562.1341; Found 562.1323. 4.1.2.9. *Diethyl [(2-diethylphosphate phenyl) (2-trifluoromethyl phenyl-amino)] methylphosphonate (6i)*. Yellow solid, 80 % yield, mp: 186 °C.  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$ 7.54 (d,  $J = 7.4$  Hz, 1H, H-Ar), 7.44 (d,  $J = 8.0$  Hz, 1H, H-Ar), 7.30 (dd,  $J = 15.4, 5.4$  Hz, 3H, H-Ar), 7.12 (t,  $J = 7.5$  Hz, 1H, H-Ar), 6.71 (d,  $J = 8.5$  Hz, 2H, H-Ar), 5.51–5.11 (m, 2H, NH + HCP), 4.40–4.06 (m, 4H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.91 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.73–3.51 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.53–1.25 (m, 9H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.07 (t,  $J = 7.1$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>).  $^{13}C$  NMR (91 MHz,  $CDCl_3$ )  $\delta$ 149.13 (t,  $J = 6.4$  Hz), 148.69 (d,  $J = 13.8$  Hz), 129.39 (d,  $J = 2.2$  Hz), 128.45 (d,  $J = 4.1$  Hz), 126.62–126.17 (m), 125.21, 120.06, 119.70, 119.19, 112.90, 64.88 (t,  $J_{C-P} = 6.0$  Hz), 63.75 (d,  $J_{C-P} = 7.0$  Hz), 63.13 (d,  $J_{C-P} = 6.9$  Hz), 49.43, 47.73, 16.28 (dd,  $J_{C-P} = 24.6, 5.6$  Hz).  $^{31}P$  NMR (101 MHz,  $CDCl_3$ )  $\delta$ 22.08, -6.46 ppm. HRMS (ESI)  $m/z$  calcd for  $C_{22}H_{30}F_3NNaO_7P$  [M + Na + ]; 562.1341; Found 562.1316. 4.1.2.9. *Diethyl [(4-diethylphosphate phenyl) (4-nitro phenylamino) methyl] phosphonate (6j)*. Orange solid, 86% yield, mp: 194 °C.  $^1H$  NMR (300 MHz,  $CDCl_3$ )  $\delta$ 8.02 (d,  $J = 9.1$  Hz, 2H, H-Ar), 7.43 (dd,  $J = 8.7, 2.2$  Hz, 3H, H-Ar), 7.31–7.10 (m, 2H, H-Ar), 6.57 (d,  $J = 9.2$  Hz, 2H, H-Ar), 5.87–5.65 (m, 1H, NH), 4.79 (dd,  $J = 23.9, 7.4$  Hz, 1H, HCP), 4.29–4.05 (m, 6H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.96 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.77–3.62 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.32 (m, 9H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.14 (t,  $J = 7.1$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>).  $^{13}C$  NMR (75 MHz,  $CDCl_3$ )  $\delta$ 151.57 (d,  $J = 13.2$  Hz), 150.80, 139.18, 131.13, 129.03 (d,  $J = 4.7$  Hz), 126.07, 120.45, 112.45, 64.70 (d,  $J = 5.6$  Hz), 63.67 (dd,  $J_{C-P} = 29.7, 7.1$  Hz), 55.91, 53.90, 16.77–15.70 (m).  $^{31}P$  NMR (101 MHz,  $CDCl_3$ )  $\delta$ 20.85, -6.54 ppm. HRMS (ESI)  $m/z$  calcd for  $C_{21}H_{28}N_2NaO_9P$  [M + Na + ] 562,1341 Found 562,1316. 4.1.2.10. *Diethyl [(4-diethylphosphate phenyl) (3,5-methyl phenyl-amino) methyl] phosphonate (6k)*. White solid, 80% yield, mp: 196 °C.  $^1H$  NMR (250 MHz,  $CDCl_3$ )  $\delta$ 7.44 (dd,  $J = 8.7, 2.4$  Hz, 2H, H-Ar), 7.19 (d,  $J = 8.7$  Hz, 3H), 6.36 (s, 1H, H-Ar), 6.20 (s, 2H, H-Ar), 4.73 (d,  $J = 24.3$  Hz, 1H, HCP), 4.31–4.02 (m, 6H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.96 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 3.74 (m, 1H, O-CH<sub>2</sub>-CH<sub>3</sub>), 2.16 (s, 6H, CH<sub>3</sub>-Ar), 1.46–1.22 (m, 9H, O-CH<sub>2</sub>-CH<sub>3</sub>), 1.14 (t,  $J = 7.1$  Hz, 3H, O-CH<sub>2</sub>-CH<sub>3</sub>).  $^{13}C$  NMR (75 MHz,  $CDCl_3$ )  $\delta$ 146.15 (d,  $J = 14.1$  Hz), 138.86, 132.84, 129.08 (d,  $J = 5.4$  Hz), 120.55, 120.46–119.40 (m), 111.70, 64.59 (d,  $J = 6.1$  Hz), 63.28 (dd,  $J_{C-P} = 12.3, 7.0$  Hz), 56.35, 54.34, 16.42 (d,  $J = 5.7$  Hz), 16.13 (dd,  $J_{C-P} = 13.3, 6.2$  Hz).  $^{31}P$  NMR (101 MHz,  $CDCl_3$ )  $\delta$ 22.37, -6.50 ppm. HRMS (ESI)  $m/z$  calcd for  $C_{23}H_{35}NNaO_7P_2$  [M + Na + ] 522.1780; Found 522.1760.

#### 4.2. Crystallographic data

X-ray diffraction data for compound **6g** were collected by using a Kappa X8 APPEX II Bruker diffractometer with graphite-monochromated  $M\alpha$  radiation ( $\lambda = 0.71073 \text{ \AA}$ ). Crystals were mounted on a CryoLoop (Hampton Research) with Paratone-N (Hampton Research) as cryoprotectant and then flashfrozen in a nitrogen-gas stream at 200 K. For compound, the temperature of the crystal was maintained at the selected value by means of a 700 series Cryostream cooling device to within an accuracy of  $\pm 1$ K. The data were corrected for Lorentz polarization, and absorption effects. The structure was solved by direct methods using SHELXS-97 [25] and refined against  $F^2$  by full-matrix least-squares techniques using SHELXL-2018 [26] with anisotropic displacement parameters for all non-hydrogen atoms. Hydrogen atoms were located on a difference Fourier map and introduced into the calculations as a riding model with isotropic thermal parameters. All calculations were performed by using the Crystal Structure crystallographic software package WINGX [27]. CCDC 2079186 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via [www.ccdc.cam.ac.uk/data\\_request/cif](http://www.ccdc.cam.ac.uk/data_request/cif).

#### 4.3. In-vitro biological effect evaluation

Antioxidant and enzymatic inhibition activities were performed using a 96-well microplate reader, PerkinElmer Multimode Plate Reader (Perkin Elmer Enspire, Singapore) at the national research center in biotechnology, Constantine, Algeria. The chemical products and reagents used were: 1,1-diphenyl-2-picrylhydrazyl (DPPH), Trolox, Ascorbic acid, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), Neocuproine, Acetylcholinesterase (AChE, EC 3.1.1.7) type VI-S, from electric eel < 10 000 U/mg solid, Butyrylcholinesterase (BChE, EC 3.1.1.8) from equine serum 100 U/mg solid, Butyrylthiocholine iodide, 5,5'-Dithiobis (2-nitrobenzoic acid) (DTNB), Galantamine, Potassium persulfate were obtained from Sigma Aldrich while iron (III) chloride ( $FeCl_3$ ), Copper (II) chloride, Acetylthiocholine iodide was purchased from BioChemica. Potato-dextrose agar (PDA) for antifungal investigation is purchased from Sigma Aldrich. 4.3.1. *Antifungal activity* All synthesized products were tested for their antifungal activity by evaluation the mycelial growth inhibition of two phytopathogenic fungi *Fusarium oxysporum* f. sp. *lycopersici* (FOL) strain 4287, and *Botrytis cinerea*, according to Song method [28]. Growth inhibition capacity was calculated through radial growth of the fungal colonies as described in Dennis and Webster work [29]. The tests were repeated for divergent concentration from 0.14 to 0.02 mg/ml in order to identify the IC<sub>50</sub> values of tested compounds by a linear regression. (Inhibition rates (%) and statistics are joined in supplement information). The following antioxidant activities and enzymatic inhibition assays are spectrophotometric methods adapted to multimode plate reader. 4.3.2. *Antioxidant activity essays* Five of effective different complementary spectrophotometric methods were implemented for testing the synthesized compounds basing on the complex structures and nature of interactions involved in the antioxidant effect. 4.3.2.1. *ABTS radical scavenging activity*. ABTS radical was generated by the oxidation of 2mM of ABTS by 2.45mM potassium persulfate ( $K_2S_2O_8$ ). The resulted solution was mixed with samples and the absorbance was monitored at 734 nm [30]. 4.3.2.2. *DPPH scavenging activity*. DPPH scavenging activity was assessed following the method described by Blois [31]. Briefly, 1mM DPPH solution was added to samples at different concentrations and the absorbance was measured at 517nm. 4.3.2.3. *Phenanthroline assay*. Iron ions reduction was assessed by the phenanthroline assay as reported in the literature [32]. The reaction mixture contains sample, 0.2%  $FeCl_3$ , and 0.5% phenanthroline. The absorbance was

read at 510nm. **4.3.2.4. Cupric Reducing Antioxidant Capacity (CUPRAC).** The reduction of copper ions was investigated by the reduction of the colourless copper (II) neocuproine (2,9-dimethyl-1,10-phenanthroline) complex to the coloured copper(I)-neocuproine complex [33]. Briefly, 10 mM CuCl<sub>2</sub>, 7.5 mM neocuproine, and 1M CH<sub>3</sub>COONH<sub>4</sub> were mixed with samples in a microplate and the absorbance was read at 450nm. **4.3.2.5. Galvinoxyl radical scavenging activity.** The reduction of Galvinoxyl radical was determined as previously reported [34]. 0.1 mM of Galvinoxyl radical solution was added to the samples and the absorbance was recorded at 428 nm. **4.3.3. Cholinesterase inhibition essays** The anticholinesterase activity of compounds **4a**, **4b**, **4e**, **4h**, **4k** and **4l** was investigated according to the colorimetric method of Ellman [35]. AChE (5.32 × 10<sup>-3</sup> U) and BChE (6.85 × 10<sup>-3</sup> U) in phosphate buffer (0.1 M, pH8) were incubated in a 96-well microplate with different concentrations of the compounds. Afterwards, 0.5 mM of DTNB and 0.71 mM acetylthiocholine iodide or 0.2 mM butyrylthiocholine chloride were added and the absorbance was monitored at 412 nm using a multimode plate reader (Perkin Elmer, EnSpire®, and Singapore).

**4.3.4. Statistical analysis** Results are reported as mean value ±SD of three measurements; the IC<sub>50</sub> and A<sub>0.50</sub> values were calculated by linear regression analysis, and one-way analysis of variance ANOVA followed by Tukey's multiple comparison test to detect significant differences (p < 0.05) using GraphPad prism software (GraphPad Software Inc, San Diego, CA, USA).

#### 4.4. Computational Methods

In order to find the most stable structures of the molecules **4a**, **6a**, **4b**, **6b**, **4c** and **6c** the computational methods were used. For each compound the configurational space was explored in three steps using the "hierarchical" approach, in which each subsequent step was performed with a more advanced theoretical method. The first part of calculations (Stage I) was conducted using the HyperChem program [36], that was also used to create the initial models of particles. For each molecule, using the "Conformational search" module available in this program, a large number of structures was generated by a random variation of 9 (diethyl hydroxyphenyl α-aminophosphonates) or 14 (diethylphosphates diethyl α-aminophosphonates) torsion angles selected in each molecule (Fig. 3 (ESI)). The number of angles simultaneously changed in a single step was varied from 1 to 9 for diethyl hydroxyphenyl α-aminophosphonates and from 1 to 14 for diethylphosphates diethyl α-aminophosphonates and they were modified in the range of 0- 180°. As a result, after automatic removal of duplicates, for each diethyl hydroxyphenyl α-aminophosphonates were created from seven to thirteen thousand, and for each diethylphosphate diethyl α-aminophosphonates up to thirty thousand of different conformers, which were next optimized in the molecular mechanics force field AMBER99. In Stage II all structures obtained in Stage I were re-optimized in vacuum using the semi-empirical method PM7 in the program MOPAC 2016 [37]. Next, from a set of conformers obtained for each molecule, were selected three hundred of the lowest energy structures (again duplicates were removed), which were used in the next stage. Finally, for the six sets of structures selected in Stage II were performed the DFT calculations using the Gaussian 16 program [38]. Each structure was fully optimized (without any constraints) in vacuum using the method B3LYP-GD2/6-31G (d,p), which is the hybrid density functional B3LYP [39] with the Grimme GD2 empirical corrections [40], combined with the People's 6-31G(d,p) basis set. For the six most stable structures selected in Stage III the <sup>1</sup>H and <sup>13</sup>C NMR isotropic shielding tensors were obtained using the B3LYP/6-31++ G(d,p) method and the Gauge-Independent Atomic Orbital (GIAO) approach [41]. The chemical shifts δ (relative to TMS) were obtained as in our earlier works [42], thus, they were corrected using the procedure proposed by Tantillo [43]. The parameters used are given in the article.

#### Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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#### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi: [10.1016/j.molstruc.2021.131336](https://doi.org/10.1016/j.molstruc.2021.131336).

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