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Alkynyl and β-ketophosphonates: Selective and potent butyrylcholinesterase inhibitors

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ABSTRACT

A series of thirty-three alkynyl and β-ketophosphonates were evaluated for their in vitro acetyl- and butyryl-cholinesterase (AChE and BChE) inhibitory activities using Ellman's spectrophotometric method. None of the examined compounds inhibited AChE activity at tested concentrations while twenty-nine of them showed significant and selective inhibition of BChE with IC_{50} values between 38.60 μM and 0.04 μM. In addition, structure-activity relationships were discussed. The most effective inhibitors were the dibutyl *o*-methoxyphenyl alkynylphosphonate **3dc** and dibutyl *o*-methoxyphenyl β-ketophosphonate **4dc**. Activities of most potent compounds were also compared with a commercial organophosphorus compound. These results could inspire the design of new inhibitors with stronger activity against BChE.

1. Introduction

Cholinesterases (ChEs) belong to the serine hydrolase family and therefore possess an αβ-hydrolase fold structure [1]. Their main function is to hydrolyze the neurotransmitter acetylcholine (ACh) to choline and acetic acid, terminating impulse transmission at cholinergic synapses, which is an essential process for the restoration of cholinergic neurons [2]. There are two major forms of ChEs in vertebrates: acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BChE, EC 3.1.1.8), also called pseudo-cholinesterase or nonspecific cholinesterase [3]. These two enzymes share about 54% amino acid sequence identity, but differ in their specificity towards various substrates and inhibitors [4]. Their crystal structures have revealed similar architecture, with one catalytic triad located at the bottom of a deep gorge [5,6]. Compounds with the ability of reversibly or irreversibly inhibit ChEs, restrict the enzymes from breaking down ACh, increasing the amount of neurotransmitter available for neuronal and neuromuscular transmission. ChEs inhibitors have been successfully used in the treatment of various diseases, such as myasthenia gravis, Alzheimer's disease (AD) and some other dementias [7–9], parasitic infections, glaucoma, obstipation or to antagonize muscle relaxation [6,10]. Other important applications include their use as insecticides, herbicides, antifungal agents and chemical warfare nerve agents [11–13].

Organophosphorus compounds (OPs) are some of the most powerful and well-studied ChEs irreversible inhibitors [6,14,15]. The chemical warfare agents VX and Sarin, the insecticide malathion and the potential drug for AD metrifonate are examples of this group of inhibitors. The electrophilic phosphorous atom of OPs can react with the hydroxyl group of the serine residue in the active site through nucleophilic attack, which leads to the enzymes' phosphorylation and deactivation. Even though most of these compounds are highly toxic, investigations on the ChEs inhibition of OPs is widely appreciated [16–18]. Less toxic forms of these agents have the potential to serve as therapeutic alternatives. In addition, insect and fungus chemical resistance requires new insecticides for agricultural uses. Therefore, the search for new and potent ChEs inhibitors with phosphorus moiety in its structure is an ongoing quest mobilizing the scientific community around the world.

Phosphonates are well known as non-hydrolyzable analogs of biological phosphates and are of widespread interest in synthetic organic chemistry [19]. In a previous work some of us reported a new simple and mild protocol for the direct synthesis of alkynylphosphonates and β-ketophosphonates from terminal alkynes catalyzed by Cu_2O or copper nanoparticles supported on zinc oxide (CuNPs/ZnO), respectively [20,21]. In this study, we present our results on the evaluation of their AChE and BChE inhibitory activities. In addition, structure-activity relationships are discussed. Since differences in the inhibition potency of organophosphorus agents are a manifestation of differing molecular properties of the inhibitors involved in the interaction with the active site of the enzyme, we were interested in studying the inhibition potency

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of a wide variety of new phosphonates, whose synthesis and biological activity is reported here for the first time. Moreover, activities shown by the most potent of these phosphonates were compared with that of a commercial organophosphorus compound.

2. Results and discussion

A library of twenty previously reported alkynyl- and β-ketophosphonates were tested for their ChEs inhibition using Ellman's method [22]. The effectiveness of the inhibitors is expressed as IC_{50}, representing the concentration of an inhibitor required for 50% inhibition of the enzyme. For this study, compounds with IC_{50} values over 50 μM were considered to be inactive. Tacrine, a well-known ChEs inhibitor and FDA approved drug for AD treatment, was used as reference inhibitor [23].

We also prepared and tested a series of thirteen new phosphonates (entries 5, 10, 15–19, Table 1, and entries 4, 8–10, Table 2), which allowed us to study the influence of the substitution pattern at the aromatic moiety on enzyme inhibition. Alkynylphosphonates were prepared in good yields (64–97%) by Cu_2O-catalyzed cross-coupling of a dialkylphosphite and the appropriate terminal alkyne, using acetonitrile as solvent at 70 °C (Scheme 1). On the other hand, β-ketophosphonates were obtained also in good yields (60–97%) by direct reaction of a dialkylphosphite and terminal alkynes catalyzed by CuNPs/ZnO using acetonitrile as reaction media at 70 °C (Scheme 2).

All compounds, except **3jb** and **3mb**, displayed potent inhibitory activity against BChE at micromolar and sub-micromolar range (IC_{50} = 38.60–0.04 μM). The inhibition was found to be highly selective since none of the phosphonates were active against AChE at tested concentrations. BChE inhibitory activity results are summarized in Tables 1 and 2.

In order to explain the selectivity of our phosphonates towards BChE main differences in the ligand binding sites in both enzymes should be considered, i.e., active site and peripheral anionic site (PAS). It is known that the active site size in BChE is larger than in AChE [5,24,25]. Therefore, BChE can accommodate ligands with larger molecular structures. In addition, in the PAS and along the gorge structure, there are many hydrophobic amino acids, being predominantly aromatic in AChE and mainly aliphatic in BChE [26,27]. Since tested compounds could be considered of medium size when compared with other inhibitors, the larger active site in BChE does not provide an explanation for the observed selectivity. On the contrary, it could be hypothesized that the interactions between our compounds and the aromatic residues in AChE's PAS would be reducing favorable interactions with the active site residues, consequently avoiding AChE inhibition.

It is important to note that, unlike results obtained in this work, several authors have reported that even though OPs behave more selectively towards BChE, most of them elicit inhibition of AChE [28–31].

2.1. Evaluation of alkynylphosphonates as BChE inhibitors

Based on the IC_{50} values obtained and the nature of R^1 and R^2 substituents in the alkynylphosphonates, some trends in terms of structure-activity relationships could be envisaged (Table 1). A clear difference in activity was observed between compounds **3aa** and **3ab** (R^2 = Me and Et, respectively) when compared with compound **3ac** (R^2 = Bu), the latter being six/ten times more potent than its congeners. This variation on activity could be attributed to the increase in the hydrophobicity of the R^2 chain. Comparable results were obtained by Nakayama and co-workers for dialkylphosphates where, increasing the length of the *n*-alkyl moiety of substituents attached to the oxygen produced an increase in their affinity with ChEs [32].

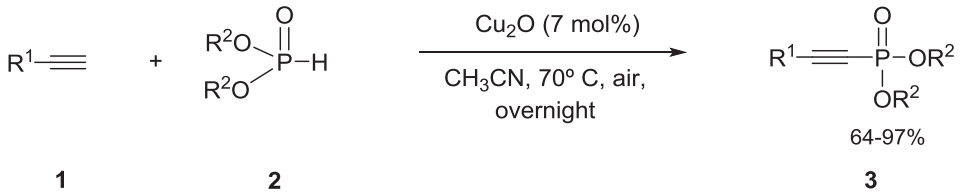
According to the obtained IC_{50} values for compounds **3ab** and **3ib** it could be assumed that BChE active site can interact positively with both alkyl chains and phenyl groups attached to the carbon-carbon triple bond. These results led us to think whether these interactions would depend on the alkyl chain length. Therefore, we synthetized compound **3jb** with an alkyl chain four carbon atoms larger, but resulted to be inactive against BChE. Then, it could be hypothesized that compound **3jb** with a larger R^1 substituent could not fit well in the enzyme active site, consequently diminishing its activity.

With regard to electronic effects, electron-withdrawing groups in the phenyl ring showed a detrimental effect on inhibition as it is illustrated with the IC_{50} values of compounds **3gb** and **3hb** (27.60 μM and 14.50 μM, respectively) when compared with the unsubstituted phosphonate **3ab**. Moreover, a chlorine atom

Table 1
BChE inhibitory activity of alkynylphosphonates expressed as IC_{50} (μM).

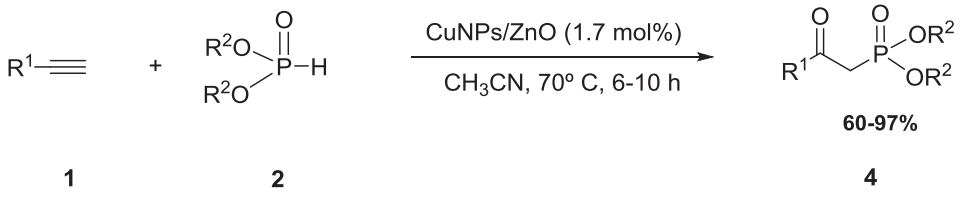
Entry	R^1	R^2	Compound[a]	IC_{50} (μM)[b]
1	Ph (**1a**)	Me (**2a**)	**3aa**	6.60 ± 1.22
2	Ph	Et (**2b**)	**3ab**	10.10 ± 1.48
3	*m*-CH_3-Ph (**1b**)	Et	**3bb**	3.80 ± 0.77
4	*p*-CH_3-Ph (**1c**)	Et	**3cb**	4.70 ± 1.15
5	*o*-OCH_3-Ph (**1d**)	Et	**3db**	2.17 ± 0.47
6	*p*-OCH_3-Ph (**1e**)	Et	**3eb**	10.50 ± 2.14
7	*p*-$N(CH_3)_2$-Ph (**1f**)	Et	**3fb**	1.90 ± 0.11
8	*m*-Cl-Ph (**1g**)	Et	**3gb**	27.60 ± 4.98
9	*m*-CF_3-Ph (**1h**)	Et	**3hb**	14.50 ± 5.54
10	CH_3-$(CH_2)_5$- (**1i**)	Et	**3ib**	9.50 ± 0.81
11	CH_3-$(CH_2)_9$- (**1j**)	Et	**3jb**	>50.0
12	HO-CH_2- (**1k**)	Et	**3 kb**	20.40 ± 6.57
13	HO-$(CH_2)_2$- (**1l**)	Et	**3 lb**	4.50 ± 0.44
14	Cl-$(CH_2)_3$- (**1m**)	Et	**3mb**	>50.0
15	Ph	Bu (**2c**)	**3ac**	1.60 ± 0.30
16	*m*-CH_3-Ph	Bu	**3bc**	0.31 ± 0.04
17	*p*-CH_3-Ph	Bu	**3 cc**	4.50 ± 1.06
18	*o*-CH_3-Ph (**1n**)	Bu	**3nc**	1.52 ± 0.06
19	*o*-OCH_3-Ph	Bu	**3dc**	0.08 ± 0.01
20	3,4-OCH_3-Ph (**1o**)	Bu	**3oc**	0.21 ± 0.06
21	Tacrine	–	–	0.004 ± 0.001

[a] Reaction conditions: alkyne (0.5 mmol) added to a suspension of dialkyl phosphite (0.7 mmol) and Cu_2O (14 mol%) in MeCN (2 mL), stirred overnight at 70 °C under air.
[b] BChE inhibitory activity was measured in vitro by the spectrophotometric method developed by Ellman with slight modifications [20].



1 2 3

Scheme 1. Direct synthesis of alkynylphosphonates from terminal alkynes and dialkylphosphites catalyzed by Cu_2O.



1 2 4

Scheme 2. Synthesis of β-ketophosphonates from terminal alkynes and dialkyl phosphite catalyzed by copper nanoparticles supported on ZnO.

Table 2
BChE inhibitory activity of β-ketophosphonates expressed as IC_{50} (μM).

Entry	R^1	R^2	Compound[a]	IC_{50} (μM)[b]
1	Ph (**1a**)	Me (**2a**)	**4aa**	38.60 ± 3.57
2	Ph	Et (**2b**)	**4ab**	34.80 ± 5.90
3	*m*-CH_3-Ph (**1b**)	Et	**4bb**	0.79 ± 0.05
4	*o*-OCH_3-Ph (**1d**)	Et	**4db**	1.85 ± 0.05
5	*p*-OCH_3-Ph (**1e**)	Et	**4eb**	4.60 ± 0.62
6	*p*-$N(CH_3)_2$-Ph (**1f**)	Et	**4fb**	3.40 ± 0.84
7	*p*-NH_2-Ph (**1p**)	Et	**4pb**	18.70 ± 1.34
8	Ph	Bu (**2c**)	**4ac**	0.43 ± 0.04
9	*p*-$N(CH_3)_2$-Ph	Bu	**4fc**	0.16 ± 0.05
10	*o*-OCH_3-Ph	Bu	**4dc**	0.04 ± 0.01
11	3,4-OCH_3-Ph (**1o**)	Bu	**4oc**	0.09 ± 0.03
12	Tacrine	–	**–**	0.004 ± 0.001

[a] Reaction conditions: alkyne (0.5 mmol), dialkyl phosphite (1 mmol), catalyst (20 mg, 1.7 mol% Cu), in acetonitrile (2 mL) at 70 °C under air atmosphere, 6–10 h. Isolated yield after chromatographic purification (hexane/AcOEt).
[b] BChE inhibitory activity was measured in vitro by the spectrophotometric method developed by Ellman with slight modifications [20].

attached to an alkyl chain (**3mb**) also increase the IC_{50} value. In the case of structurally related compounds **3kb** and **3lb**, it seems that electron withdrawing effect of hydroxyl group might be balanced by its capacity to electrostatically interact through hydrogen bonds with the enzyme residues. Again, structure length appears to play an important role in inhibitory activity since compound **3lb**, with an extra carbon atom in the R^1 chain, resulted four times more potent than **3kb**. Therefore, it could be inferred that alkynylphosphonates with small molecular size cannot interact well with the enzyme either.

As shown in Table 1, substitution with methyl (**3bb**/**3cb**) and amine (**3fb**) electron donating groups, produced a 2/5-fold improvement in BChE inhibitory activity. Furthermore, methyl group seem to exert almost the same effect regardless its location. Substitution in meta (**3bb**) and para (**3cb**) positions did not show significant differences in the IC_{50} values (3.80 μM and 4.70 μM for para and meta derivatives, respectively). On the other hand, the introduction of a methoxy group in different aromatic ring positions appeared to have a considerable impact on activity. The *ortho* substituted compound **3db** turned out to be a 5-fold more potent inhibitor in comparison with the unsubstituted congener, while substitution in para position (**3eb**) seemed not to influence inhibitory activity. Hence, it can be concluded that in order to obtain an alkynylphosphonate with potent inhibitory activity, both the electronic nature of the substituent and its position at the aromatic ring are crucial.

With the aim of obtaining a more powerful inhibitor, we decided to make modifications based on the structures of those phosphonates which showed lower IC_{50}, i.e., those bearing a butyl group as R^2 and electron-donor groups in the aryl ring.

Thus, we investigated the inhibitory activity which have a methyl group as substitution of the aromatic ring at *o*, *m* and *p* position respectively (compounds **3bc**, **3cc** and **3nc**). As can be seen from the IC_{50} values, there is a marked difference in activity, with the meta-substituted phosphonate being the most active.

The introduction of a methoxy substituent instead of a methyl group in the phenyl ring led to a 19-fold more potent derivative. Compound **3dc**, synthesized from the 1-ethynyl-2-methoxybenzene (**1d**), proved to be the most active of the entire series with an IC_{50} of 0.08 μM.

Building on the observation that methoxy substitution leads to a better inhibitor compared to the unsubstituted aryl ring, we next tested an analog with two methoxy groups at the 3,4-positions. Thus, compound **3oc** produced an eight-fold more potent inhibitor when compared with the unsubstituted phosphonate.

2.2. Evaluation of β-ketophosphonates as BChE inhibitors

We next examined β-ketophosphonates analogs as BChE inhibitors, the results are presented in Table 2.

As it was observed for alkynylphosphonates **3**, the inhibitory activity increased with the increment in hydrophobicity of R^2 alkyl chain (Me < Et < Bu) showing IC_{50} values of 34.80 μM for R^2 = Et, 38.60 μM for R^2 = Me and 0.43 μM for R^2 = Bu. Dimethyl and diethyl β-ketophosphonates derivatives elicited threefold lower activity than the corresponding alkynyl analogs, while the opposite

was observed for R^2 = Bu, being the β-ketophosphonate **4ac** more active than the alkynylphosphonate **3ac**.

The presence of an electron donating group in R^1 aromatic ring (Table 2, entries 3–7) rendered phosphonates with increased activity. Dimethylated amine group (**4fb**) resulted in a five times better inhibitor than **4pb**. β-ketophosphonates with electron-withdrawing groups in the phenyl ring were not evaluated due to the detrimental effect in inhibition observed for alkynylphosphonates.

Similar to the trend observed for alkynylphosphonates, the inhibitory activity when a methoxy group is present in R^1 aromatic ring, seemed to be dependent on its location (Table 2, compare entries 4 and 5).

Three new dibutyl β-ketophosphonates were synthetized with the aim to obtain more potent inhibitors (Scheme 2). Compounds **4fc**, **4dc** and **4oc** which have electron donor groups at R^1 aromatic ring shown to be more active than the unsubstituted analog. The most active compound was the dibutyl *ortho*-methoxyphenyl derivative **4dc**, which shown the lowest IC_{50} value (0.04 μM). It could be also concluded that the introduction of an extra methoxy group decreases the ability to inhibit BChE (**4oc**), when compared with that of the mono methoxylated analog (**4dc**).

2.3. Chemical transformations on the phosphonate structure and derivatives BChE inhibition

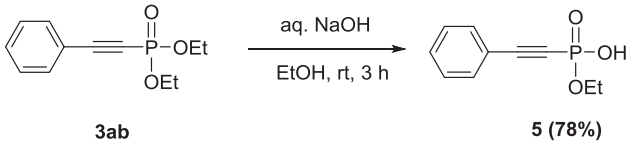
In order to study the influence of R^2 substituent on the phosphonate-enzyme interaction, an alkynyl phosphonic acid monoethyl ester was synthesized and tested against BChE (Scheme 3). Racemic mixture **5** was obtained by reaction of the diethyl alkynylphosphonate **3ab**, with aqueous NaOH and ethanol at room temperature [33]. As expected, enantiomers mixture **5** resulted inactive against BChE, in line with the loss of hydrophobicity in its structure.

The presence of the carbonyl group in β-ketophosphonates and its susceptibility to be easily reduced to a hydroxyl group, encouraged us to test the impact of this transformation in the anticholinesterase activity. Reduction of the carbonyl group in the β-ketophosphonate **4ab** was carried out by reaction with sodium borohydride at 0 °C affording racemic **6** in very good yield (Scheme 4) [34].

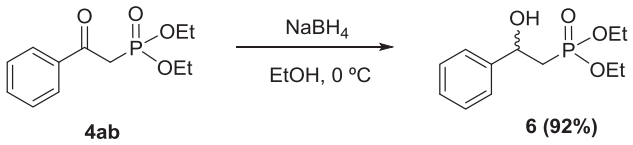
Enantiomers mixture **6** proved to be inactive towards BChE since its determined IC_{50} value was superior to 50 μM. Despite this fact, careful examination of **4ab** and **6** chemical structures suggested that ketone function in **4ab** could produce significant influence on compound potency, making this group necessary for the β-ketophosphonate interaction with residues at binding site in BChE.

2.4. Comparison of most active phosphonates with a commercial compound

In Table 3 the inhibitory activity of the best of our alkynyl- and beta-keto phosphonates is compared with a structurally related commercial compound (2-chloroethyl)-phosphonic acid (Ethephon). This is a major agrochemical used as a plant growth regulator. It penetrates the plant tissue and decomposes to ethylene

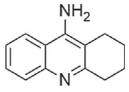
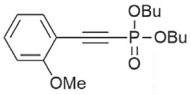
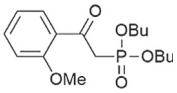
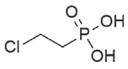


Scheme 3. Synthesis of phenyl alkynyl phosphonic acid monoethyl ester.



Scheme 4. Chemical reduction of carbonyl group.

Table 3
Comparison of BChE inhibitory activities of different compounds.

Entry	Structure	IC_{50} (μM)
1	 **Tacrine**	0.004
2	 **3dc**	0.08
3	 **4dc**	0.04
4	 **Ethephon**	166[a]

[a] Haux et al. [33].

which affects growth, flowering, fruit maturation, and other vital processes. This compound has also been proven to inhibit the activity of plasma BChE in humans, dogs, rats, and mice.[35] As it can be observed from Table 3, the activity of compounds **3dc** and **4dc** resulted more than 2000- and 4000-fold higher compared with Ethephon. Tacrine IC_{50} is also included in Table 3 as reference inhibitor.

3. Conclusions

A series of a wide variety of alkynyl and β-ketophosphonates, eleven of them synthesized and reported for the first time, were evaluated against AChE and BChE. Most compounds showed significant and selective inhibition of BChE with IC_{50} values in the micromolar and submicromolar range. In general, aromatic dibutyl phosphonates with electron-donor groups in the aryl ring presented the best activities. Also, the importance of electron donor group position in the aromatic moiety was determined. Most active derivatives were much stronger than commercial agrochemical Ethephon. According to the results obtained in this study, it seems that dibutyl *ortho*-methoxyphenyl derivatives, **3dc** and **4dc**, are good candidates for further optimization studies.

4. Experimental section

4.1. General experimental details

All starting materials were of the best available grade (Aldrich, Merck) and were used without further purification. AChE from electric eel (type VI-S), 5,50-dithiobis(2-nitrobenzoic acid) (DTNB), acetylthiocholine iodide (ATCI), butyrylthiocholine iodide (BTCI)

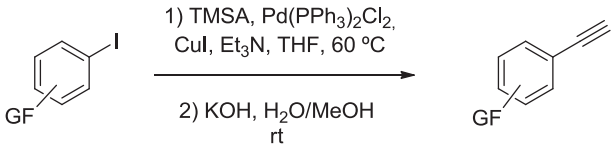
and tacrine were purchased from Sigma Aldrich. BChE (horse serum) was obtained from MP Biomedicals. Column chromatography was performed with Merck silica gel 60 (0.040–0.063 μm, 240–400 mesh). Reactions were monitored by thin-layer chromatography on silica gel plates (60F-254) visualized under UV light and/or using 5% $KMnO_4$ in water.

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker ARX-300 (300 MHz for ^1H NMR; 75 MHz for ^{13}C NMR; 121 MHz for ^{31}P MNR) spectrometer using $CDCl_3$ as solvent and tetramethylsilane (TMS) as internal reference. Chemical shifts (δ) are reported in parts per million (ppm) from tetramethylsilane (TMS) using the residual solvent resonance ($CDCl_3$: 7.26 ppm for ^1H NMR, 77.16 ppm for ^{13}C NMR). Multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, brs = broad signal. Coupling constants (J) were reported in Hz.

Mass spectra (EI) were obtained at 70 eV on a Hewlett Packard HP-5890 GC/MS instrument equipped with a HP-5972 selective mass detector. Infrared (FT-IR) spectra were obtained on a Nicolet-Nexus spectrophotometer. The purity of volatile compounds and the chromatographic analyses (GC) were determined with a Shimadzu GC-14B instrument equipped with a flame-ionization detector and a 30 m column (HP-5MS, 0.25 mm, 0.25 μm), using nitrogen as carrier gas. High resolution mass spectra were recorded on Thermo Fisher LTQ Orbitrap XL, (for EI) and a Finnigen MAT 95 (for ESI).

All known compounds were characterized by comparison of their physical and spectroscopic data with those described in literature. For new compounds, copies of ^1H, ^{13}C, and ^{31}P NMR graphical spectra are also provided (see the Supporting Information).

4.2. Synthesis of 1-ethynyl-2-methoxybenzene (1d) [36] and 1-ethynyl-2-methylbenzene (**1n**) [37]

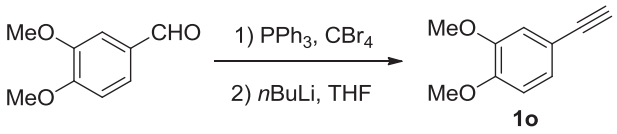


To a solution of the corresponding iodoarene (2 mmol) and trimethylsilylacetylene (2.4 mmol, 1.2 equiv) in Et_3N (2 mL) and THF (2 mL) were added $PdCl_2(PPh_3)_2$ (28.1 mg, 2 mol%) and CuI (3.82 mg, 1 mol%). The resulting mixture was then heated under an N_2 atmosphere at 60 °C. The reaction was complete in 2 h. The mixture was allowed to cool to room temperature, and the ammonium salt was removed by filtration. The solvent was removed under reduced pressure and the residue was desylilated. A solution of KOH (56.0 mg, 1 mmol) in 2 mL of water was added dropwise to the residue in 4 mL of CH_3OH at room temperature for 1 h. The crude was added to 10 mL of brine solution, and the mixture was extracted with EtOAc (3 × 10 mL), dried over $MgSO_4$, filtered, and the solvent removed under vacuum. The residue was purified by column chromatography (hexane/EtOAc, 9:1) affording the desired product.

1-Ethynyl-2-methoxybenzene (1d). Yellow liquid. (224.5 mg, 85%). ^1H NMR (300 MHz, $CDCl_3$) δ 7.39 (dd, J = 7.5, 1.7 Hz, 1H), 7.29–7.20 (m, 1H), 6.87–6.77 (m, 2H), 3.82 (s, 3H), 3.23 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 160.7, 134.3, 130.4, 120.6, 111.3, 110.7, 81.2, 80.2, 77.6, 55.9.

1-Ethynyl-2-methylbenzene (1n). Yellow liquid. (150.8 mg, 65%). ^1H NMR (300 MHz, $CDCl_3$) δ 7.39 (d, J = 7.6 Hz, 1H), 7.20–7.02 (m, 3H), 3.20 (s, 1H), 2.39 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.9, 132.7, 129.6, 128.9, 125.7, 122.1, 82.7, 81.1, 20.7.

4.3. Synthesis of 4-ethynyl-1,2-dimethoxybenzene (**1o**) [38]



1o

3,4-Dimethoxybenzaldehyde (249.8 mg, 1.5 mmol) was added to a solution of CBr_4 (995.0 mg, 3.0 mmol) and PPh_3 (1.57 g, 6.0 mmol) in anhydrous dichloromethane (15 mL) at room temperature. After stirring for 60 min, the reaction was quenched with water (25 mL) and extracted with dichloromethane (3 × 15 mL). The combined organic layers were washed with water, brine, and dried over $MgSO_4$. The solvent was removed under reduced pressure. The crude residue was used without further purification. Then, *n*-BuLi (1.58 mmol, 0.63 mL, 2.5 M solution in hexane) was added dropwise to a stirred solution of crude residue in anhydrous THF (5.0 mL) under argon at −78 °C. The solution was stirred at −78 °C for 1 h and at rt for 2 h. The reaction mixture was quenched with saturated aqueous NH_4Cl solution. The reaction mixture was extracted with EtOAc (3 × 10 mL) and washed with H_2O and brine. The organic solution was dried over $MgSO_4$ and the solvent was removed under reduced pressure. The obtained residue was purified by column chromatography (hexane/EtOAc, 8:2) affording the desired product **1o** (194.5 mg, 80%) as white solid, ^1H NMR (300 MHz, $CDCl_3$) δ 7.05 (dd, J = 7.8, 1.8 Hz, 1H), 6.98 (t, J = 7.8 Hz, 1H), 6.91 (dd, J = 7.8, 1.8 Hz, 1H), 3.94 (s, 3H), 3.85 (s, 3H), 3.26 (1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 152.8, 151.2, 125.7, 123.9, 116.9, 113.6, 81.3, 79.8, 61.1, 56.1.

4.4. General procedure of the synthesis of alkynylphosphonates

The H-phosphonate (0.7 mmol) was added to a suspension of the Cu_2O (5 mg, 14 mol%) in MeCN (2 mL). Then the alkyne (0.5 mmol) was added and the reaction mixture was warmed to 70 °C overnight under air atmosphere. The solvent was evaporated under vacuum and the product was purified by flash column chromatography (hexane-EtOAc) to give the corresponding isolated alkynylphosphonate.

Compounds **3aa** [39], **3ab, 3cb**, **3eb**, **3fb** [40], **3bb** [41], **3gb**, **3hb, 3lb**, **3mb** [21], **3ib** [42], **3kb** [43] and **3ac** [44] were already reported in literature, while spectral data for remaining new compounds are provided.

4.4.1. Compound characterization data of alkynylphosphonates

Dimethyl (phenylethynyl)phosphonate (3aa). Colorless oil. (73.5 mg, 70%). ^1H NMR (300 MHz, $CDCl_3$) δ 7.55–7.48 (m, 2H), 7.44–7.28 (m, 3H), 3.79 (d, J = 12.3 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 132.8 (d, J = 2.5 Hz), 131.0, 128.7, 119.4 (d, J = 5.7 Hz), 100.2 (d, J = 53.4 Hz), 76.9 (d, J = 303.3 Hz), 53.6 (d, J = 5.5 Hz). ^{31}P NMR (121 MHz, $CDCl_3$) δ −2.7 ppm.

Diethyl (phenylethynyl)phosphonate (3ab): Yellow oil. (102.4 mg, 86%). ^1H NMR (300 MHz, $CDCl_3$) δ 7.61–7.53 (m, 2H), 7.49–7.33 (m, 3H), 4.31–4.17 (m, 4H), 1.41 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 132.7 (d, J = 2.5 Hz), 130.8, 128.7, 119.7 (d, J = 5.7 Hz), 99.2 (d, J = 53.0 Hz), 78.5 (d, J = 300.0 Hz), 63.4 (d, J = 5.5 Hz), 16.2 (d, J = 7.0 Hz). ^{31}P NMR (121 MHz, $CDCl_3$) δ −5.9 ppm.

Diethyl (*m*-tolylethynyl)phosphonate (3bb): Yellow oil. (88.2 mg, 70%). ^1H NMR (300 MHz, $CDCl_3$) δ 7.41–7.33 (m, 2H), 7.30–7.22 (m, 2H), 4.30–4.17 (m, 4H), 2.35 (s, 3H), 1.41 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.5, 133.2 (d, J = 2.5 Hz), 131.7, 129.9

(d, J = 2.5 Hz), 128.6, 119.4 (d, J = 5.6 Hz), 99.6 (d, J = 53.1 Hz), 78.0 (d, J = 300.7 Hz), 63.4 (d, J = 5.5 Hz), 21.2, 16.2 (d, J = 7.0 Hz). ^{31}P NMR (121 MHz, $CDCl_3$) δ −5.9 ppm.

Diethyl (*p*-methylphenyl)ethynyl phosphonate (3cb): Pale yellow oil. (112.2 mg, 89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, J = 8.0 Hz, 2H), 7.16 (d, J = 7.9 Hz, 2H), 4.26–4.16 (m, 4H), 2.36 (s, 3H), 1.39 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.3, 132.5 (d, J = 2.5 Hz), 129.3, 116.4 (d, J = 5.6 Hz), 99.6 (d, J = 53.3 H z), 77.7 (d, J = 301.1 Hz), 63.2 (d, J = 5.5 Hz), 21.6, 16.1 (d, J = 7.0 Hz). ^{31}P NMR (121 MHz, $CDCl_3$) δ -5,7 ppm.

Diethyl ((*o*-methoxyphenyl)ethynyl)phosphonate (3db). Yellow oil. (123.3 mg, 92%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.50 (dd, J = 7.6, 1.6 Hz, 1H), 7.46–7.28 (m, 1H), 6.98–6.87 (m, 3H), 4.33–4.17 (m, 4H), 3.33 (s, 3H), 1.41 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.7 (d, J = 2.0 Hz), 134.5 (d, J = 2.6 Hz), 132.5, 120.5, 110.9, 108.8 (d, J = 5.7 Hz), 96.9 (d, J = 54.4 Hz), 81.8 (d, J = 302.8 Hz), 63.5 (d, J = 5.5 Hz), 55.8, 16.1 (d, J = 7.0 Hz). ^{31}P NMR (121 MHz, $CDCl_3$) δ -5.62 ppm. IR (neat): 2960, 2940, 2866, 2188, 1489, 1267, 1032, 966, 810, 762 cm^{-1}. MS (EI) m/z (%): 268 (M^+, 4), 212 (21), 159 (21), 158 (11), 132 (48), 131 (100), 102 (10), 91 (11), 89 (13), 77 (10); HRMS calcd. for $C_{13}H_{17}O_4P$ (M): m/z 268.0864, found: 268.0870.

Diethyl (*p*-methoxyphenyl)ethynylphosphonate (3eb): Yellow oil. (118.0 mg, 88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (d, J = 8.8 Hz, 2H), 6.82 (d, J = 8.9 Hz, 2H), 4.22–4.09 (m, 4H), 3.77 (s, 3H), 1.34 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.5, 134.4 (d, J = 2.5 Hz), 114.3, 111.3 (d, J = 5.7 Hz), 99.8 (d, J = 53.9 H z), 77.1 (d, J = 302.3 Hz), 63.1 (d, J = 5.5 Hz), 55.4, 16.1 (d, J = 7.1 Hz). ^{31}P NMR (121 MHz, $CDCl_3$) δ −5.5 ppm.

Diethyl (*p*-dimethylaminophenyl)ethynylphosphonate (3fb): maroon oil. (126.5 mg, 90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (d, J = 8.9 Hz, 2H), 6.53 (d, J = 8.9 Hz, 2H), 4.20–4.07 (m, 4H), 2.94 (s, 6H), 1.32 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.6, 134.2 (d, J = 2.5 Hz), 111.5, 105.3 (d, J = 5.9 Hz), 102.3 (d, J = 54.7 Hz), 76.4 (d, J = 305.1 Hz), 63.0 (d, J = 5.4 Hz), 40.1, 16.3 (d, J = 7.1 Hz). ^{31}P NMR (121 MHz, $CDCl_3$) δ −4.5 ppm.

Diethyl (*m*-chlorophenyl)ethynylphosphonate (3gb): Pale yellow oil. (127.9 mg, 94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (s, 1H), 7.47–7.37 (m, 2H), 7.35–7.26 (m, 1H), 4.26–4.16 (m, 4H), 1.39 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 134.6, 132.4 (d, J = 2.6 Hz), 131.1, 130.8 (d, J = 2.4 Hz), 130.0, 121.4 (d, J = 5.7 H z), 97.1 (d, J = 52.5 Hz), 79.7 (d, J = 298.0 Hz), 63.5 (d, J = 5.6 Hz), 16.2 (d, J = 6.9 Hz). ^{31}P NMR (121 MHz, $CDCl_3$) δ −6.7 ppm.

Diethyl (*m*-trifluoromethylphenyl)ethynylphosphonate (3hb): Pale yellow oil. (128.5 mg, 84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.74–7.64 (m, 2H), 7.58–7.49 (m, 2H), 4.29–4.13 (m, 4H), 1.37 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.2 (d, J = 2.7 Hz), 132.7 (qd, J = 31.1, 1.7 Hz), 131.8, 130.6, 126.2 (q, J = 5.0 Hz), 123.1 (q, J = 273.6 Hz), 117.9–117.7 (m), 94.1 (d, J = 51.8 Hz), 83.8 (d, J = 293.0 Hz), 63.6 (d, J = 5.7 Hz), 16.1 (d, J = 7.1 Hz). ^{31}P NMR (121 MHz, $CDCl_3$) δ −7.3 ppm.

Diethyl oct-1-yn-1-ylphosphonate (3ib): Yellow oil. (113.2 mg, 92%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.21–4.08 (m, 4H), 2.34 (td, J = 7.1, 4.4 Hz, 2H), 1.65–1.52 (m, 2H), 1.43–1.25 (m, 6H), 1.37 (t, J = 7.1 Hz, 6H), 0.89 (t, J = 6.8 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 103.4 (d, J = 53.2 Hz), 70.5 (d, J = 303.9 Hz), 63.0 (d, J = 5.5 Hz), 31.2, 28.5, 27.5 (d, J = 2.2 Hz), 22.5, 19.3 (d, J = 4.5 Hz), 16.1 (d, J = 7.1 Hz), 14.0. ^{31}P NMR (121 MHz, $CDCl_3$) δ −6.1 ppm.

Diethyl dodec-1-yn-1-ylphosphonate (3jb). Yellow oil. (124.0 mg, 81%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.22–4.08 (m, 4H), 2.39–2.28 (m, 2H), 1.64–1.51 (m, 2H), 1.44–1.21 (m, 14H), 1.37 (t, J = 7.1 Hz, 6H), 0.88 (t, J = 6.7 Hz, 3H).^{13}C NMR (75 MHz, $CDCl_3$) δ 103.3 (d, J = 53.0 Hz), 70.5 (d, J = 303.2 Hz), 63.0 (d, J = 5.5 Hz), 32.0, 29.6, 29.5, 29.4, 29.0, 28.9, 27.5 (d, J = 2.2 Hz), 19.3 (d, J = 4.5 Hz), 22.7, 16.2 (d, J = 7.1 Hz), 14.2. ^{31}P NMR (121 MHz, $CDCl_3$) δ −6.11 ppm. IR (neat): 2925, 2855, 2202, 1474, 1266, 1033, 967, 808, 763 cm^{-1}. MS (EI) m/z (%): 302 (M^+, 8), 273 (15), 259 (17), 247 (14), 246 (10), 245 (24), 233 (3), 232 (100), 231 (35), 219 (11), 218 (79), 217 (36), 204 (26), 203 (27), 191 (15), 190 (31), 189 (82), 178 (28), 177 (17), 176 (87), 175 (28), 163 (18), 162 (41), 161 (66), 152 (17), 150 (25), 149 (21), 148 (70), 147 (38), 138 (10), 135 (24), 134 (10), 133 (41), 125 (12), 124 (11), 123 (13), 122 (23), 121 (32), 120 (51), 115 (10), 111 (2), 109 (22), 108 (17), 107 (33), 105 (11), 102 (12), 95 (16), 94 (20), 93 (52), 91 (27), 83 (10), 82 (30), 80 (16), 79 (55), 77 (19), 67 (17), 65 (21), 55 (22); HRMS calcd. for $C_{16}H_{31}O_3P$ (M): m/z 302.2011, found: 302.2015.

Diethyl (3-hydroxyprop-1-yn-1-yl)phosphonate (3kb): Pale yellow oil. (61.5 mg, 64%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.84 (s, 1H), 4.34 (d, J = 3.8 Hz, 2H), 4.20–4.10 (m, 4H), 1.35 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 100.4 (d, J = 50.6 Hz), 74.1 (d, J = 298.7 Hz), 63.6 (d, J = 5.6 Hz), 50.6 (d, J = 4.4 Hz), 16.1 (d, J = 7.1 H z). ^{31}P NMR (121 MHz, $CDCl_3$) δ −6.8 ppm.

Diethyl (4-hydroxybut-1-yn-1-yl)phosphonate (3lb): Yellow oil. (74.2 mg, 72%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.85 (s, 1H), 4.22–4.07 (m, 4H), 3.79 (t, J = 6.5 Hz, 2H), 2.61 (td, J = 6.4, 4.6 Hz, 2H), 1.37 (t, J = 7.2 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 101.1 (d, J = 53.2 Hz), 71.4 (d, J = 302.8 Hz), 63.3 (d, J = 5.5 Hz), 59.8 (d, J = 2 .5 Hz), 23.6 (d, J = 4.5 Hz), 16.1 (d, J = 7.1 Hz).^{31}P NMR (121 MHz, $CDCl_3$) δ −6.3 ppm.

Diethyl (5-chloropent-1-yn-1-yl)phosphonate (3mb): Colorless oil. (115.5 mg, 97%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.24–4.07 (m, 4H), 3.65 (t, J = 6.2 Hz, 2H), 2.57 (td, J = 6.9, 4.4 Hz, 2H), 2.10–2.02 (m, 2H), 1.37 (t, J = 7.1 Hz, 6H).^{13}C NMR (75 MHz, $CDCl_3$) δ 100.9 (d, J = 53.0 Hz), 71.6 (d, J = 302.4 Hz), 63.2 (d, J = 5.5 Hz), 43.2, 30.2 (d, J = 2.3 Hz), 16.7 (d, J = 4.6 Hz), 16.1 (d, J = 7.0 Hz). ^{31}P NMR (121 MHz, $CDCl_3$) δ −6.7 ppm.

Dibutyl (phenylethynyl)phosphonate (3ac): Colorless oil. (132.4 mg, 90%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.60–7.53 (m, 2H), 7.50–7.42 (m, 1H), 7.40–7.34 (m, 2H), 4.21–4.12 (m, 4H), 1.79–1.67 (m, 4H), 1.53–1.39 (m, 4H), 0.95 (d, J = 7.4 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 132.8 (d, J = 2.5 Hz), 130.8, 128.7, 119.8 (d, J = 5.6 Hz), 99.2 (d, J = 52.7 Hz), 78.5 (d, J = 299.5 Hz), 67.1 (d, J = 5.9 Hz), 32.4 (d, J = 7.1 Hz), 18.9, 13.7. ^{31}P NMR (121 MHz, $CDCl_3$) δ −5.56 ppm.

Dibutyl (*m*-tolylethynyl)phosphonate (3bc). Yellow oil. (124.0. mg, 81%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40–7.34 (m, 2H), 7.28–7.25 (m, 2H), 4.15 (dd, J = 14.3, 6.5 Hz, 4H), 2.35 (s, 3H), 1.80–1.66 (m, 4H), 1.54–1.39 (m, 4H), 0.95 (t, J = 7.4 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.5, 133.2 (d, J = 2.2 Hz), 131.7, 129.9 (d, J = 2.2 Hz), 128.6, 119.5 (d, J = 5.3 Hz), 99.5 (d, J = 52.7 Hz), 78.1 (d, J = 300.1 Hz), 66.9 (d, J = 5.8 Hz), 32.3 (d, J = 7.1 Hz), 21.3, 18.8, 13.7. ^{31}P NMR (121 MHz, $CDCl_3$) δ −5.39 ppm. IR (neat): 2994, 2925, 2173, 1723, 1486, 1274, 1029, 984, 792, 690, 649 cm^{-1}.MS (EI) m/z (%): 308 (M^+, 4), 253 (24), 252 (19), 251 (16), 198 (14), 197 (100), 196 (12), 179 (32), 171 (23), 170 (25), 155 (11), 143 (11), 129 (10), 116 (27), 115 (40); HRMS calcd. for $C_{17}H_{25}O_3P$ (M): m/z 308.1541, found: 308.1547.

Dibutyl (*p*-tolylethynyl)phosphonate (3cc). Yellow oil. (134.0 mg, 87%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (d, J = 7.5 Hz, 2H), 7.11 (d, J = 7.5 Hz, 2H), 4.08 (dd, J = 13.4, 6.5 Hz, 4H), 2.31 (s, 3H), 1.72–1.55 (m, 4H), 1.45–1.31 (m, 4H), 0.88 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.4, 132.7 (d, J = 2.3 Hz), 129.4, 116.6 (d, J = 5.6 Hz), 99.7 (d, J = 53.2 Hz), 77.9 (d, J = 301.1 Hz), 66.9 (d, J = 5.9 Hz), 32.3 (d, J = 7.1 Hz), 21.8, 18.8, 13.7. ^{31}P NMR (121 MHz, $CDCl_3$) δ −5.15 ppm. IR (neat): 2994, 2925, 2185, 1511, 1274, 1025, 976, 861, 812, 780 cm^{-1}. MS (EI) m/z (%): 308 (M^+, 3), 253 (21), 252 (19), 251 (17), 210 (10), 198 (15), 197 (100), 196 (20), 179 (36), 171 (33), 170 (31), 155 (13), 143 (10), 129 (12), 117 (12, 116 (61), 115 (52); HRMS calcd. for $C_{17}H_{25}O_3P$ (M): m/z 308.1541, found: 308.1550.

Dibutyl (*o*-tolylethynyl)phosphonate (3nc). Yellow oil. (116.0 mg, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, J = 7.7 Hz, 1H), 7.27

(td, J = 7.7, 1.2 Hz, 1H), 7.19–7.07 (m, 2H), 4.14–4.04 (m 4H), 2.40 (s, 3H), 1,72–1.60 (m, 4H), 1.46–1.31 (m, 4H), 0.88 (t, J = 7.4 Hz, 6H).^{13}C NMR (75 MHz, $CDCl_3$) δ 141.9 (d, J = 2.1 Hz), 133.1 (d, J = 2.5 Hz), 130.7, 129.8, 125.9, 119.6 (d, J = 5.5 Hz), 98.3 (d, J = 52.6 Hz), 82.1 (d, J = 299.1 Hz), 66.9 (d, J = 5.9 Hz), 32.3 (d, J = 7.1 Hz), 20.6, 18.8, 13.6. ^{31}P NMR (121 MHz, $CDCl_3$) δ −5.51 ppm. IR (neat): 2958, 2937, 2859, 2185, 1466, 1274, 1029, 988, 878, 792, 763 cm^{-1}. MS (EI) m/z (%): 308 (M^+, 6), 231 (19), 230 (100), 229 (48), 228 (75), 227 (22), 226 (35), 215 (25), 202 (18), 115 (59), 114 (11), 113 (12), 101 (13); HRMS calcd. for $C_{17}H_{25}O_3P$ (M): m/z 308.1541, found: 308.1544.

Dibutyl ((*o*-methoxyphenyl)ethynyl)phosphonate (3dc). Yellow oil. (123.1 mg, 76%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.49 (dd, J = 7.6, 1.4 Hz, 1H), 7.45–7.36 (m, 1H), 6.97–6.86 (m, 2H), 4.17 (dd, J = 14.4, 6.6 Hz, 4H), 3.87 (s, 3H), 1.81–1.66 (m, 4H), 1.54–1.39 (m, 4H), 0.95 (t, J = 7.4 Hz, 6H).^{13}C NMR (75 MHz, $CDCl_3$) δ 161.6 (d, J = 1.9 Hz), 134.4 (d, J = 2.6 Hz), 132.3, 120.5, 110.9, 109.0 (d, J = 5.7 Hz), 96.5 (d, J = 53.5 Hz), 82.0 (d, J = 299.8 Hz), 66.9 (d, J = 5.9 Hz), 55.8, 32.2 (d, J = 7.1 Hz), 18.8, 13.6. ^{31}P NMR (121 MHz, $CDCl_3$) δ −5.26 ppm. IR (neat): 2962, 2945, 2872, 2185, 1597, 1486, 1258, 1033, 996, 878, 792, 755 cm^{-1}. MS (EI) m/z (%): 324 (M^+, 3), 253 (17), 237 (10), 213 (27), 212 (40), 186 (10), 171 (10), 132 (45), 131 (100), 103 (10), 102 (10); HRMS calcd. for $C_{17}H_{25}O_4P$ (M): m/z 324.1490, found: 324.1494.

Dibutyl ((3,4-dimethoxyphenyl)ethynyl)phosphonate (3oc). Yellow oil. (116.8 mg, 66%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.11–7.06 (m, 1H), 7.05–6.98 (m, 2H), 4.17 (dd, J = 14.3, 6.6 Hz, 1H), 3.94 (s, 3H), 3.87 (s, 3H), 1.80–1.66 (m, 4H), 1.54–1.39 (m, 4H), 0.95 (t, J = 7.4 Hz, 1H).^{13}C NMR (75 MHz, $CDCl_3$) δ 152.7, 151.9 (d, J = 2.2 Hz), 125.6 (d, J = 2.5 Hz), 124.2, 115.2, 114.5 (d, J = 5.5 Hz), 95.9 (d, J = 53.0 Hz), 82.0 (d, J = 298.1 Hz), 67.1 (d, J = 5.9 Hz), 61.4, 56.1, 32.3 (d, J = 7.2 Hz), 18.8, 13.7.^{31}P NMR (121 MHz, $CDCl_3$) δ −5.72 ppm. IR (neat): 2970, 2941, 2876, 2177, 1576, 1478, 1425, 1274, 1086, 1029, 792, 742 cm^{-1}.MS (EI) m/z (%): 354 (M^+, 9), 243 (24), 242 (100), 224 (10), 162 (30), 161 (67), 151 (10), 147 (11); HRMS calcd. for $C_{18}H_{27}O_5P$ (M): m/z 354.1596, found: 354.1599.

4.5. General procedure for the synthesis of β-ketophosphonates

The alkyne (0.5 mmol) and dialkylphosphite (0.65 mmol) were added to a suspension of the CuNPs/ZnO catalyst (20 mg, 1.7 mol% Cu) in MeCN (2 mL) under air atmosphere. The reaction mixture was warmed to 70 °C and monitored by TLC and/or GLC until total conversion of the starting material. Water (10 mL) was added to the reaction mixture followed by extraction with EtOAc (3 × 10 mL). The collected organic phases were dried over $MgSO_4$ and the solvent was evaporated under vacuum to give the corresponding β-ketophosphonate, which was purified by flash column chromatography (hexane-EtOAc).

Compounds **4aa, 4ac** [45], **4ab**, **4bb**, **4cb** [44], **4db** [46], **4eb** [20] were already reported in literature, while spectral data for remaining new compounds are provided.

4.5.1. Compound characterization data of β-ketophosphonates

Dimethyl 2-oxo-2-phenylethylphosphonate (4aa): Yellow oil. (88.9 mg, 78%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.93 (d, J = 7.5 Hz, 2H), 7.59–7.48 (m, 1H), 7.47–7.36 (m, 2H), 3.71 (d, J = 11.2 Hz, 6H), 3.58 (d, J = 22.6 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.9 (d, J = 6.6 Hz), 136.5 (d, J = 2.6 Hz), 133.9, 129.1, 128.8, 53.3 (d, J = 6.5 Hz), 37.6 (d, J = 131.5 Hz). ^{31}P NMR (121 MHz, $CDCl_3$) δ 22.9.

Diethyl 2-oxo-2-phenylethylphosphonate (4ab): Yellow oil. (110.1 mg, 86%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 (d, J = 7.8 Hz, 2H), 7.55 (t, J = 7.3 Hz, 1H), 7.44 (d, J = 7.6 Hz, 2H), 4.15–4.05 (m, 4H), 3.60 (d, J = 22.7 Hz, 2H), 1.24 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 191.9 (d, J = 6.6 Hz), 136.6 (d, J = 2.1 Hz), 133.7, 129.1, 128.6, 62.8 (d, J = 6.5 Hz), 38.5 (d, J = 130.2 Hz), 16.3 (d, J = 6.4 Hz). ^{31}P NMR (121 MHz, $CDCl_3$) δ 20.0.

Diethyl (2-oxo-2-(*m*-tolyl)ethyl)phosphonate (4bb): Yellow oil. (126.9 mg, 94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80–7.78 (m, 2H), 7.40–7.32 (m, 2H), 4.17–4.08 (m, 4H), 3.61 (d, J = 22.7 Hz, 2H), 2.40 (s, 3H), 1.27 (t, J = 7.0 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.1 (d, J = 6.7 Hz), 138.4, 136.6 (d, J = 1.9 Hz), 134.4, 129.4, 128.4, 126.3, 62.6 (d, J = 6.5 Hz), 38.4 (d, J = 130.2 Hz), 21.3, 16.2 (d, J = 6.4 Hz). ^{31}P NMR (121 MHz, $CDCl_3$) δ 20.0.

Diethyl (2-(*o*-methoxyphenyl)-2-oxoethyl)phosphonate (4db): Yellow oil. (125.8 mg, 88%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (dd, J = 7.7, 1.8 Hz, 1H), 7.47–7.37 (m, 1H), 6.99–6.36 (m, 2H), 4.10–3.98 (m, 4H), 3.86 (s, 3H), 3.77 (d, J = 21.9 Hz, 2H), 1.18 (t, J = 7.0 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 193.4 (d, J = 7.3 H z), 162.3, 158.8, 134.4, 131.1, 120.9, 111.7, 62.6 (d, J = 6.4 Hz), 55.7, 42.5 (d, J = 131.0 Hz), 16.3 (d, J = 6.5 Hz). ^{31}P NMR (121 MHz, $CDCl_3$) δ 21.6 ppm. IR (neat): 3458, 2960, 2945, 2882, 1668, 1610, 1481, 1288, 1232, 1019, 989, 751 cm^{-1}. MS (EI) m/z (%): 286 (M^+, 3), 268 (29), 158 (12), 135 (100), 131 (19), 77 (19); HRMS calcd. for $C_{13}H_{19}O_5P$ (M): m/z 286.0970, found: 286.0975.

Diethyl (2-(*p*-methoxyphenyl)-2-oxoethyl)phosphonate (4eb): Orange oil. (120.1 mg, 84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.92 (d, J = 8.8 Hz, 2H), 6.87 (d, J = 8.9 Hz, 2H), 4.16–3.98 (m, 4H), 3.79 (s, 3H), 3.52 (d, J = 22.8 Hz, 2H), 1.21 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 190.3 (d, J = 6.5 Hz), 164.1, 131.6, 129.7 (d, J = 2.0 Hz), 113.9, 62.8 (d, J = 6.5 Hz), 55.6, 38.1 (d, J = 130.1 H z), 16.3 (d, J = 6.3 Hz). ^{31}P NMR (121 MHz, $CDCl_3$) δ 20.6.

Diethyl (2-(*p*-(dimethylamino)phenyl)-2-oxoethyl)phosphonate (4fb): Orange oil. (89.7 mg, 60%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (d, J = 9.0 Hz, 2H), 6.64 (t, J = 9.0 Hz, 2H), 4.17–4.07 (m, 4H), 3.53 (d, J = 22.6 Hz, 2H), 3.06 (s, 6H), 1.28 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 189.3 (d, J = 6.4 Hz), 153.7, 131.4, 124.5 (d, J = 2.2 Hz), 110.5, 62.5 (d, J = 6.5 Hz), 39.9, 37.8 (d, J = 129.8 Hz), 16.3 (d, J = 6.2 Hz). ^{31}P NMR (121 MHz, $CDCl_3$) δ 21.4.

Diethyl (2-(*p*-aminophenyl)-2-oxoethyl)phosphonate (4pb): Orange solid. (108.4 mg, 80%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.81 (d, J = 8.6 Hz, 2H), 6.60 (d, J = 8.7 Hz, 2H), 4.35 (brs, 2H), 4.19–4.01 (m, 4H), 3.50 (d, J = 22.6 Hz, 2H), 1.26 (t, J = 7.1 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 189.5 (d, J = 6.2 Hz), 152.0, 131.7, 126.8 (d, J = 2.0 Hz), 113.6, 62.6 (d, J = 6.5 Hz), 37.8 (d, J = 130.0 Hz), 16.3 (d, J = 6.3 Hz). ^{31}P NMR (121 MHz, $CDCl_3$) δ 21.1.

Dibutyl (2-oxo-2-phenylethyl)phosphonate (4ac): Yellow oil. (138.8 mg, 89%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.97–7.91 (m, 2H), 7.56–7.48 (m, 1H), 7.44–7.36 (m, 2H), 4.05–3.93 (m, 4H), 3.56 (d, J = 22.8 Hz, 2H), 1.59–1.46 (m, 4H), 1.33–1.16 (m, 4H), 0.81 (t, J = 7.4 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 192.0 (d, J = 6.8 Hz), 136.7 (d, J = 1.8 Hz), 133.7, 129.3, 128.7, 66.4 (d, J = 6.7 Hz), 38.4 (d, J = 129.5 Hz), 32.5 (d, J = 6.4 Hz), 18.7, 13.6. ^{31}P NMR (121 MHz, $CDCl_3$) δ 19.9 ppm.

Dibutyl (2-(*p*-(dimethylamino)phenyl)-2-oxoethyl)phosphonate (4fc). Brown oil. (150.9. mg, 85%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, J = 9.1 Hz, 2H), 6.58 (d, J = 9.1 Hz, 2H), 4.06–3.92 (m, 4H), 3.47 (d, J = 22.6 Hz, 2H), 3.00 (s, 6H), 1.61–1.46 (m, 4H), 1.35–1.16 (m, 4H), 0.82 (t, J = 7.4 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 189.5 (d, J = 6.4 Hz), 153.9, 131.6, 124.8, 110.7, 66.3 (d, J = 6.7 Hz), 40.1, 37.9 (d, J = 129.4 Hz), 32.6 (d, J = 6.4 Hz), 18.8, 13.7. ^{31}P NMR (121 MHz, $CDCl_3$) δ 21.4 ppm. IR (neat): 3456, 2966, 2933, 2872, 1658, 1539, 1376, 1294, 1188, 1029, 808 cm^{-1}. MS (EI) m/z (%): 355 (M^+, 16), 163 (16), 149 (11), 148 (100), 134 (11); HRMS calcd. for $C_{18}H_{30}NO_4P$ (M): m/z 355.1912, found: 355.1916.

Dibutyl (2-(*o*-methoxyphenyl)-2-oxoethyl)phosphonate (4dc). Yellow oil. (122.0 mg, 77%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (dd, J = 7.7, 1.8 Hz, 1H), 7.41 (ddd, J = 8.4, 7.4, 1.8 Hz, 1H), 6.98–6.93 (m, 2H), 3.94 (dd, J = 13.7, 6.7 Hz, 4H), 3.85 (s, 3H), 3.76 (d, J = 22.1 Hz, 2H), 1.55–1.40 (m, 4H), 1.30–1.15 (m, 4H), 0.81 (t, J = 7.4 Hz, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 193.5 (d, J =

7.3 Hz), 158.7, 134.2, 131.0, 127.9, 120.9, 111.6, 66.0 (d, J = 6.6 Hz), 55.7, 42.5 (d, J = 129.6 Hz), 32.51 (d, J = 6.4 Hz), 18.7, 13.7.^{31}P NMR (162 MHz, $CDCl_3$) δ 21.2 ppm. IR (neat): 3460, 2966, 2941, 2872, 1670, 1601, 1486, 1294, 1249, 1021, 984, 755 cm^{-1}. MS (EI) m/z (%): 342 (M^+, 3), 324 (15), 268 (11), 229 (11), 213 (11), 212 (23), 235 (100), 131 (19), 123 (22), 97 (14), 77 (23); HRMS calcd. for $C_{17}H_{27}O_5P$ (M): m/z 342.1596, found: 342.1599.

Dibutyl (2-(3,4-dimethoxyphenyl)-2-oxoethyl)phosphonate (4oc). Yellow oil. (126.0 mg, 68%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.19 (dd, J = 6.8, 2.7 Hz, 1H), 7.13–7.03 (m, 2H), 4.03 (q, J = 6.7 H z, 4H), 3.92 (s, 3H), 3.89 (s, 3H), 3.79 (d, J = 22.1 Hz, 2H), 1.63–1.50 (m, 4H), 1.37–1.23 (m, 4H), 0.89 (t, J = 7.4 Hz, 6H).^{13}C NMR (75 MHz, $CDCl_3$) δ 194.4 (d, J = 7.2 Hz), 152.9, 148.6, 133.5, 124.2, 121.6, 116.4, 66.2 (d, J = 6.6 Hz), 61.8, 56.2, 42.2 (d, J = 129.3 Hz), 32.5 (d, J = 6.4 Hz), 18.8, 13.7. ^{31}P NMR (162 MHz, $CDCl_3$) δ 20.7 ppm. IR (neat): 3488, 2966, 2937, 2868, 1683, 1576, 1478, 1421, 1266, 1074, 1017, 792, 739 cm^{-1}.MS (EI) m/z (%): 372 (M^+, 4), 354 (26), 311 (21), 298 (31), 255 (13), 243 (18), 242 (39), 229 (23), 179 (14), 178 (20), 166 (10), 165 (100), 162 (20), 161 (96), 150 (11), 148 (23), 139 (10), 135 (15), 123 (41), 122 (26), 121 (12), 107 (15), 105 (12), 97 (32), 92 (10), 91 (11), 77 (28), 57 (12); HRMS calcd. for $C_{18}H_{29}O_6P$ (M): m/z 372.1702, found: 372.1708.

4.6. Preparation of racemic ethyl hydrogen (phenylethynyl) phosphonate (**5**) [33]

A solution of **3ab** (83.3 mg, 0.35 mmol), NaOH (82.5 mg, 3.5 mmol) and ethanol (3.0 mL) were stirred at room temperature for 3 h. The solvent was evaporated under vacuum and the residue was diluted with water (5 mL). The solution was neutralized with cooled concentrated hydrochloric acid and extracted with EtOAc (3 × 5 mL). The extracts were evaporated under reduced pressure to give the desired product.

Ethyl hydrogen (phenylethynyl)phosphonate (5). Brown oil. (57.3 mg, 78%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 11.3 (brs, 1H), 7.55 (d, J = 7.3 Hz, 2H), 7.47–7.39 (m, 1H), 7.38–7.29 (m, 2H), 4.29–4.17 (m, 2H), 1.40 (t, J = 7.0 Hz, 3H).^{13}C NMR (75 MHz, $CDCl_3$) δ 132.8 (d, J = 2.2 Hz), 130.7, 128.6, 119.7 (d, J = 5.8 Hz), 98.8 (d, J = 56.4 Hz), 79.1 (d, J = 312.9 Hz), 63.4 (d, J = 5.4 Hz), 16.1 (d, J = 7.2 Hz).^{31}P NMR (121 MHz, $CDCl_3$) δ −4.64 ppm.

4.7. Synthesis of racemic 2-hydroxyalkanephosphonate [34]

A solution of **4ab** (64.0 mg, 0.25 mmol) in ethanol (2.5 mL) was treated with sodium borohydride (37.8 mg, 1.0 mmol) at 0 °C. The unreacted $NaBH_4$ was decomposed with acetone (2.5 mL) and the crude product was purified by column chromatography (hexane-EtOAc) to afford the desired product.

Diethyl (2-hydroxy-2-phenylethyl)phosphonate (6). Yellow oil. (63.2 mg, 92%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36–7.16 (m, 5H), 5.10–4.98 (m, 1H), 4.13–3.95 (m, 4H), 2.83 (s broad, 1H), 2.23–2.04 (m, 2H), 1.27 (t, J = 7.2 Hz), 1.23 (t, J = 7.2 Hz). ^{13}C NMR (75 MHz, $CDCl_3$) δ 143.6 (d, J = 16.1 Hz), 128.7, 127.9, 125.7, 68.9 (d, J = 4.6 Hz), 62.2 (d, J = 6.3 Hz), 62.1 (d, J = 6.7 Hz), 36.1 (d, J = 136.1 Hz), 16.6 (d, J = 23.7 Hz), 16.5 (d, J = 23.7 Hz).^{31}P NMR (121 MHz, $CDCl_3$) δ 29.1 ppm.

4.8. Preparation of copper nanoparticles (CuNPs) supported on ZnO

Anhydrous copper (II) chloride (135.0 mg, 1.0 mmol) was added to a suspension of lithium (14.0 mg, 2.0 mmol) and 4,4′-di-*tert*-butylbiphenyl (DTBB, 27.0 mg, 0.10 mmol) in THF (2 mL) at room temperature under a nitrogen atmosphere. The reaction mixture, which was initially dark blue, rapidly changed to black, indicating that the suspension of copper nanoparticles was formed. This suspension was diluted with THF (18 mL) followed by the addition of the zinc oxide (800 mg). The resulting mixture was stirred for 1 h at room temperature, filtered, and the solid successively washed with THF (20 mL) and diethyl ether (20 mL), and then dried under vacuum. The full characterization is reported in Gutierrez et.al., 2015 [47].

4.9. Cholinesterase inhibition assay

Electric eel AChE and horse serum BChE were used as sources of both cholinesterases. AChE and BChE inhibitory activities were measured in vitro by the spectrophotometric method developed by Ellman with slight modifications. The lyophilized enzyme, 500 U AChE (300 U BChE), was dissolved in buffer phosphate A (8 mM K_2HPO_4, 2.3 mM NaH_2PO_4) to obtain 5 (3) U/mL stock solution. Further enzyme dilution was carried out with buffer phosphate B (8 mM K_2HPO_4, 2.3 mM NaH_2PO_4, 0.15 M NaCl, 0.05% Tween 20, pH 7.6) to produce 0.126 (0.06) U/mL enzyme solution. Samples were dissolved in buffer phosphate B with 2.5% of MeOH as cosolvent. 300 μL of enzyme solution and 300 μL of sample solution were mixed in a test tube and incubated for 60/120 min at room temperature. The reaction was started by adding 600 μL of the substrate solution (0.5 mM DTNB, 0.6 mM ATCI/BTCI, 0.1 M Na_2HPO_4, pH 7.5). The absorbance was read at 405 nm for 120 s at 25 °C. Enzyme activity was calculated by comparing reaction rates for the samples to the blank. All reactions were performed in triplicate. IC_{50} values were determined with GraphPad Prism 5. Tacrine (99%) was used as the reference AChE and BChE inhibitor.

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Appendix A. Supplementary material

Copies of ^{1}H, ^{13}C and ^{31}P NMR spectra of all phosphonates are available as supplementary information. Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bioorg.2018.01.030.

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