ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

Synthesis of Mesylated and Tosylated α-Hydroxy-Benzylphosphonates; Their Reactivity and Cytostatic Activity

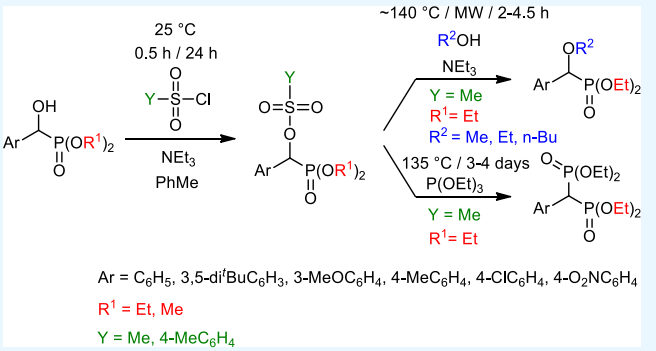
Zsuzsanna Szalai, Márton Debrei, Péter Ábrányi-Balogh, Szilvia Bősze, Rita Oláhné Szabó,* Konstantin Karaghiosoff, László Drahos, and György Keglevich*

Cite This: *ACS Omega* 2024, 9, 31043−31055

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: α-Hydroxyphosphonates and their acylated and phosphorylated derivatives may be of significant biological activity including cytotoxic effects. To extend the pool of the potentially bioactive species, new methane- and arenesulfonyloxyphosphonates were synthesized by the sulfonylation of differently substituted α-hydroxy-benzylphosphonates using methanesulfonyl chloride or *p*-toluenesulfonyl chloride at 25 °C in the presence of triethylamine in toluene. The new sulfonyl derivatives were obtained in 54−80% yields. In the case of the 4- or 2-methoxy substituent in the aromatic ring, surprisingly the corresponding α-chlorophosphonates were the exclusive products, whose formation was explained assuming a quinoid intermediate and supported by theoretical calculations. With a 3-methoxyphenyl substituent, the expected mesylation of the hydroxy group took place. Attempted alcoholyses of the diethyl α-methanesulfonyloxy-benzylphosphonates with different substituents in the benzyl ring at ∼140 °C in the presence of triethylamine under microwave irradiation left the P-function intact under the conditions applied, instead, the mesyloxy group was substituted by an alkoxy unit in a selective new reaction. The α-alkoxy-benzylphosphonates were isolated in 60−77% yields. While α-chloro- or α-bromo-benzylphosphonates proved to be rather inefficient in the Michaelis−Arbuzov reaction with triethyl phosphite, according to a new possibility, the α-methansulfonyloxy-benzylphosphonates underwent an efficient Arbuzov fission using the phosphite in excess at 135 °C. The arylmethylenebisphosphonates were obtained in yields of 76−81%. Bioactivity studies with the members of the phosphonate library revealed pronounced in vitro cytostatic effect of the α-hydroxy- and α-mesyloxy-3,5-di-*tert*-butylbenzylphosphonates on human breast carcinoma cell culture with IC_{50} values of 16.4 and 28.0 μM, respectively. The mesyloxy species was also cytostatic on melanoma cells (IC_{50} = 34.9).



1. INTRODUCTION

The field of α-hydroxyphosphonates represents an evergreen topic, as their chemistry hides a lot of possibilities regarding synthesis and reactions. The principal method for the synthesis of α-hydroxyphosphonates is the Pudovik-reaction, according to which a dialkyl phosphite is added to the carbonyl group of aldehydes or ketones.[1] It is also possible that the oxo compound is reacted with a trialkyl phosphite to afford eventually the corresponding hydroxyphosphonate.[2] This addition may be catalyzed by bases or acids.[3−7] Solvent-free methods on the surface of solid catalysts were also described,[8−10] however, in most of the cases, a great amount of solvent had to be used during the workup. An indeed green protocol was when the hydroxyphosphonate crystallized out from acetone solution on the addition of pentane.[11] The reversibility of the Pudovik reaction was also observed in certain cases.[12] The α-hydroxyphosphonates may be involved in a series of reactions. A simple, but relatively less studied modification is alkylation.[1] A much better-studied reaction is their acylation.[13−29] This change in the functionality was especially useful in making available biologically active species.[13−16] The phosphorylation of hydroxyphosphonates was elaborated by us.[30,31] At first sight, it may be seen as surprising that the α-hydroxy-benzylphosphonates underwent substitution with primary amines on microwave (MW) irradiation.[32,33] The reaction was promoted by an adjacent group effect. Additional transformations of α-hydroxyphosphonates include rearrangement,[34,35] dealkylation,[1] and oxidation and reduction.[1] It was a further development to obtain hydroxyphosphonates in high enantiomeric excess following sequential asymmetric reactions.[36]

Received: May 8, 2024
Revised: June 18, 2024
Accepted: June 20, 2024
Published: July 2, 2024

The α-hydroxyphosphonates and their derivatives are also of importance due to their many-sided biological activity. The antibacterial and antiviral activity of α-hydroxyphosphonates is in connection with their enzyme−inhibitory properties.[37−41] A part of the acylated hydroxyphosphonates obtained on reaction with carboxylic acid chlorides or anhydrides included aryloxy-butyryloxy- or- valeroxy[13] and heterocyclic derivatives[14,15] that were described as herbicidal agents. Dialkyl α-hydroxy-benzylphosphonates and their acylated derivatives showed cytotoxic effects on certain cell lines.[16] Phosphorylated species were active against the sarcoma cell line.[30]

It was a challenge for us to synthesize sulfonylated hydroxyphosphonates as newer derivatives and to explore their reactivity and cytotoxic activity.

2. RESULTS AND DISCUSSION

2.1. Synthesis of Sulfonylated α-Hydroxy-benzyl-phosphonates. In the series of studying the reactivity of α-hydroxy-benzylphosphonates, the next task was to synthesize and investigate the properties of their sulfonylated derivatives. The starting α-hydroxyphosphonates (**2a,d−g** and **3a,c−h**) were prepared by the method described by us earlier.[11] The two 3,5-di-*tert*-butylphenyl species (**2b** and **3b**) synthesized by our earlier procedure[11] were new compounds and were fully characterized. The dimethyl and diethyl α-hydroxy-benzyl-phosphonates (**2a,b,d−f** and **3a−f**, respectively) were reacted with 1.5 equiv of methanesulfonyl chloride at 25 °C in the presence of 1.5 equiv of triethylamine in toluene for 0.5 h. The reaction involves a nucleophilic substitution on the SO_2 moiety of the sulfonyl chloride by the hydroxyphosphonates (**2** and **3**). The workup included evaporation of the solvent and column chromatography of the residue so obtained. The expected mesyloxy-phosphonates **4a,b,d−f** and **5a−f** were isolated in 54−80% yields (Table 1, entries 1−11), and they were characterized by ^{31}P, ^{13}C, and ^{1}H NMR spectral data, as well as HRMS. α-Hydroxy-benzylphosphonates **3a,d,e** were also reacted with *p*-toluenesulfonyl chloride under similar conditions to afford products **6a,d,e**, respectively, in 65−71% yields after the workup (Table 1, entries 12−14). As these products (**6a,d,e**) were described earlier,[42] they were identified by ^{31}P NMR spectral data, as well as HRMS (See Experimental Section).

Mesyloxy-phosphonate **5e** was also subjected to single-crystal X-ray diffraction studies. The compound crystallizes in the monoclinic space group $P21/n$ with four formula units in the unit cell. The molecular structure of **5e** is shown in Figure 1, and views of the molecular packing in the crystal are displayed in SI Figures S1−S3. There are, to the best of our knowledge, only three molecules having a *P*-bonded phosphonate ($P(O)(OR)_2$) unit and an *O*-bonded sulfonate (RSO_3) unit, attached to the same tetrahedrally coordinated carbon atom, of which the molecular structures in the solid state have been determined.[43,44,29] The first two cases[43,44] refer to special molecules, structurally quite different from **5e**, and only compound **6a**[29] is available for a direct structural comparison. The structures of **6a** and **5e** are very similar in terms of bond lengths, angles, and molecular geometry. In both cases, phosphorus displays a slightly distorted tetrahedral surrounding and the P,C bond length (1.817(2) Å for **5e** and 1.822(3) Å for **6a**) are typical for a P,C single bond. The most interesting feature of both structures is the orientation of the *C*-bonded phenyl ring (torsion angle P−C−C-C 82.2(2)° for **5e** and 82.5(3)° for **6a**) tending to minimize steric repulsion by phosphonate and sulfonate substituents.

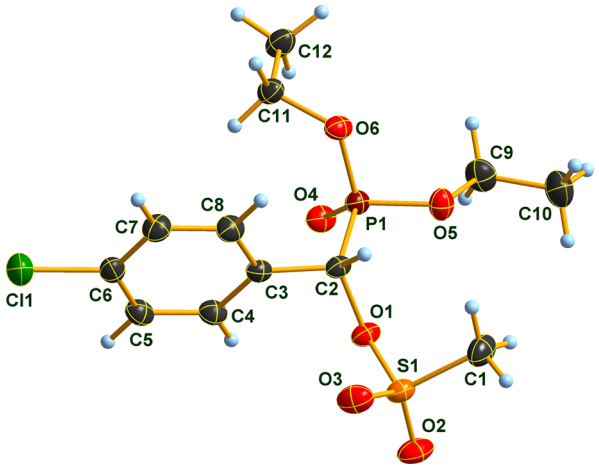
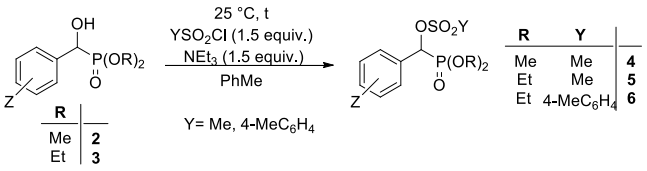


Figure 1. Molecular structure of compound **5e** in the crystal. DIAMOND[45] representation thermal ellipsoids are drawn at 50% probability level.

Table 1. Synthesis of α-Methane- and *p*-Toluenesulfonyloxyphosphonates 4−6



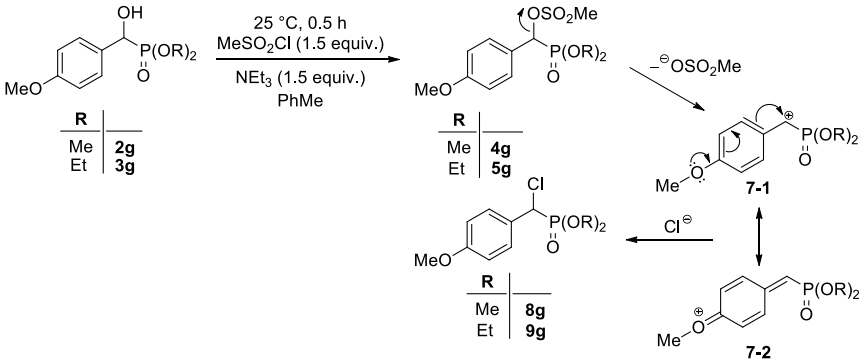
Z = 4-H (**a**), 3,5-di^{t}Bu (**b**), 3-MeO (**c**), 4-Me (**d**), 4-Cl (**e**), 4-O_2N (**f**)

	starting material					product	
entry		Z	R	Y of the sulfonyl chloride	t (h)		yield (%)
1	**2a**	4-H	Me	Me	0.5	**4a**	67
2	**2b**	3,5-di^{t}Bu	Me	Me	0.5	**4b**	60
3	**2d**	4-Me	Me	Me	0.5	**4d**	61
4	**2e**	4-Cl	Me	Me	0.5	**4e**	66
5	**2f**	4-O_2N	Me	Me	0.5	**4f**	76
6	**3a**	4-H	Et	Me	0.5	**5a**	78
7	**3b**	3,5-di^{t}Bu	Et	Me	0.5	**5b**	54
8	**3c**	3-MeO	Et	Me	0.5	**5c**	80
9	**3d**	4-Me	Et	Me	0.5	**5d**	76
10	**3e**	4-Cl	Et	Me	0.5	**5e**	79
11	**3f**	4-O_2N	Et	Me	0.5	**5f**	76
12	**3a**	4-H	Et	4-MeC_6H_4	24	**6a**	65
13	**3d**	4-Me	Et	4-MeC_6H_4	24	**6d**	71
14	**3e**	4-Cl	Et	4-MeC_6H_4	24	**6e**	70

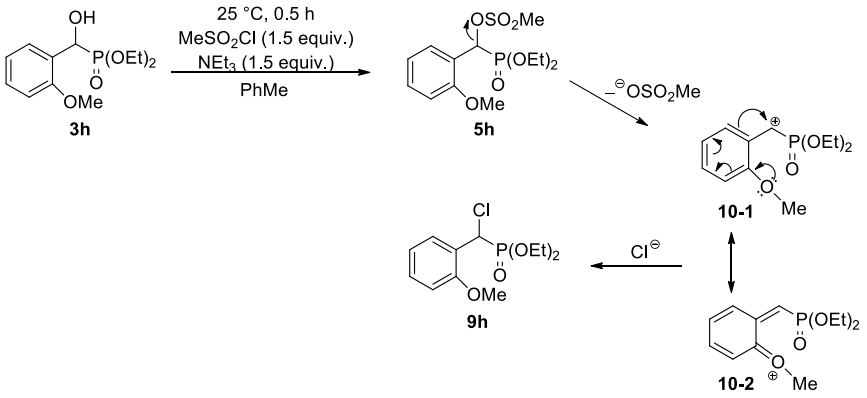
It was surprising to observe that the interaction of α-hydroxy-α-(4-methoxyphenyl)-methanephosphonates **2g** and **3g** led selectively to the corresponding **α-chloro-benzyl-phosphonates 8g** and **9g**, respectively (Scheme 1). It is assumed that, in these cases, the expected mesyl derivatives (**4g** and **5g**) are only intermediates that give the chlorophosphonates (**8g** and **9g**) in reaction with the chloride anion deriving from the hydrochloric acid liberated. Departure of the $MeSO_3^-$ anion may lead to a cationic intermediate that may exist under two resonant forms (**7−1** and **7−2**), from among **7−2** is of a quinoid structure meaning a stabilization.

https://doi.org/10.1021/acsomega.4c04382
ACS Omega 2024, 9, 31043−31055

Scheme 1. Reaction of Dialkyl α-Hydroxy-4-methoxybenzylphosphonates (2g and 3g) with Methanesulfonyl Chloride



Scheme 2. Reaction of Diethyl α-Hydroxy-2-methoxybenzylphosphonate 3h with Methanesulfonyl Chloride

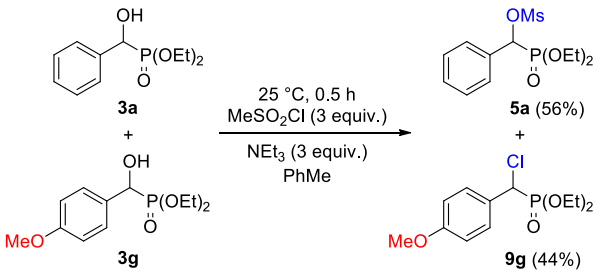


The reaction of α-hydroxy-α-(2-methoxyphenyl)-methanephosphonate **3h** with methanesulfonyl chloride also gave the corresponding α-chloro-benzylphosphonate (**9h**) (Scheme 2). It can be said that the α-chlorophosphonates (**8g**, **9g**, and **9h**) are formed via an S_N1 substitution.

To confirm this unexpected reactivity, an equimolar mixture of α-hydroxy-benzylphosphonate (**3a**) and 4-methoxy derivative (**3g**) was reacted with 3 equiv of the methanesulfonyl chloride at room temperature in toluene under the same conditions. ^{31}P NMR spectrum of the crude mixture confirmed the presence of the mesyloxyphosphonate (**5a**) and **chlorophosphonates** (**9g**) in comparative quantities (Scheme 3).

2.2. Quantum Chemical Calculations. Notably, in the case of compounds **5a** and **5c**, no substitution of the mesyloxy group to chloro atom was observed, and the mesylated derivatives (**5a** and **5c**) were proved to be stable, contrary to mesylates **5g** and **5h** that were only intermediates and transformed in situ to chlorophosphonate **9g** and **9h**, respectively. These reactions were assumed to take place via an S_N1 mechanism involving the triethylammonium-assisted cleavage of the C-OMs bond, where the driving force is surely the stability of the quinoidal carbocation **7−2** and **10−2**, respectively. The energies for the formation of intermediates **7** or **10** formed from diethyl α-mesyloxy-benzylphosphonate (**5a**), diethyl α-mesyloxy-3-methoxybenzylphosphonate (**5c**), diethyl α-mesyloxy-4-methoxybenzylphosphonate (**5g**), and diethyl α-mesyloxy-2-methoxybenzylphosphonate (**5h**) were computed. It was found that the transformation of phosphonates **5g** and **5h** to the corresponding carbocations (**7** and **10**, respectively) is an exothermic procedure, as suggested by the formation of Gibbs free energies of −9.0 and −14.1 kJ mol^{-1}, respectively. On the contrary, and in accord with the experimental observations, the Gibbs free energies for the formation of the carbocations from **5a** and **5c** were found to be highly unfavorable, as marked by a Gibbs free energy of 49.1 and 53.2 kJ mol^{-1}, respectively. This endothermic process is not supposed to occur at room temperature. The full details of the computations and the data set can be found in the Experimental Section and in the Supporting Information.

Scheme 3. Competitive Reaction of Diethyl α-Hydroxybenzylphosphonate (3a) and 4-Methoxybenzylphosphonate (3g) with Methanesulfonyl Chloride



3. REACTIONS OF THE α-MESYLOXY-BENZYLPHOSPHONATES

3.1. Unexpected Substitution of Methanesulfonyloxy-benzylphosphonates. In the series of transesterification reactions investigated by us,[46,47] dimethyl α-hydroxybenzylphosphonate (**2a**) was rather reluctant to be involved in alcoholysis with butyl alcohol. Even under MW conditions at 150 °C using 20% of [bmim][BF_4] as the catalyst, the conversion remained incomplete (72%), and a complex mixture comprising the butoxy-metoxy ester (**11**, δ_P ($CDCl_3$)

https://doi.org/10.1021/acsomega.4c04382
ACS Omega 2024, 9, 31043−31055

Scheme 4. Alcoholysis of Dimethyl α-Hydroxy-benzylphosphonate 2a with Butyl Alcohol

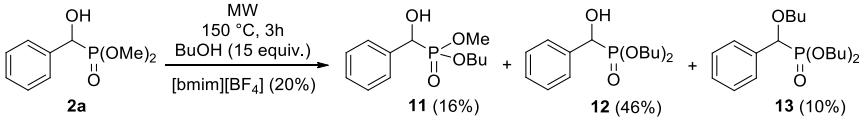
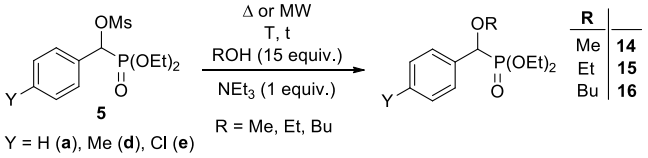


Table 2. Conversion of Methanesulfonyloxy-benzylphosphonates (5a,d,e) to the Alkoxy Derivatives



entry	starting material	*Y*	*R* of the alcohol	heating source	*T* (°C)	*t* (h)	conversion (%)[a]	product	yield (%)
1	**5a**	H	Me	MW	135	2	100	**14a**	63
2	**5d**	Me	Me	MW	135	2	100	**14d**	77
3	**5e**	Cl	Me	MW	135	3	95	**14e**	60
4	**5a**	H	Et	Δ	135	2.5	33	**15a**	
5	**5a**	H	Et	Δ	135	10	97	**15a**	
6	**5a**	H	Et	MW	135	2.5	96	**15a**	75
7	**5a**	H	Et	MW	135	2.5	96[b,c]	**15a**	
8	**5d**	Me	Et	MW	135	2.5	100	**15d**	66
9	**5e**	Cl	Et	MW	135	2.5	55	**15e**	
10	**5e**	Cl	Et	MW	145	3	97	**15e**	74
11	**5a**	H	Bu	MW	135	2.5	28	**16a**	
12	**5a**	H	Bu	MW	135	4	70	**16a**	
13	**5a**	H	Bu	MW	150	4	100[d]	**16a**	
14	**5a**	H	Bu	MW	145	4.5	98	**16a**	62
15	**5d**	Me	Bu	MW	145	4.5	100	**16d**	70
16	**5e**	Cl	Bu	MW	145	4.5	100[d]	**16e**	61

[a]On the basis of relative ^{31}P NMR intensities. [b]Using pyridine instead of triethylamine. [c]Unknown byproduct appeared at δ_P 16.8. [d]Some decomposition occurred.

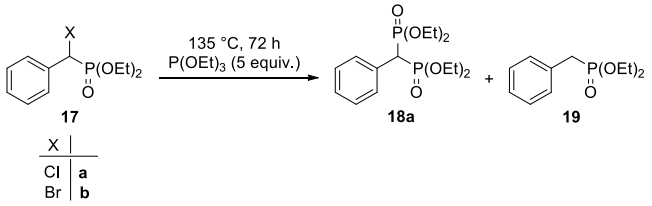
22.5/22.6, $[M + H]^+$ = 259), the dibutoxy ester (**12**, δ_P ($CDCl_3$) 21.4, $[M + H]^+$ = 301), and, surprisingly, the tributoxy product (**13**, δ_P ($CDCl_3$) 19.1, $[M + H]^+$ = 357) coming from O-alkylation of the fully transesterified product (**12**) was obtained (Scheme 4).

In the hope of a more selective α-substitution, we reacted mesyloxyphosphonates (**5a**, **5d**, and **5e**) with 15 equiv of alcohol in the presence of 1 equiv of triethylamine. The interaction of mesyloxyphosphonate **5a** with methyl alcohol at 135 °C for 2 h under MW irradiation led to the substitution of the α-mesyloxy group by a methoxy unit in a selective manner. No replacement of the ethoxy group(s) on the P atom to methoxy unit(s), i.e., no transesterification occurred. The α-methoxyphosphonate (**14a**) was formed in an S_N2 nucleophilic substitution reaction in quantitative conversion (Table 2, entry 1). Then, this new and entirely selective reaction was extended also to other mesyloxyphosphonates. The 4-methylphenyl- and the 4-chlorophenyl model compounds (**5d** and **5e**, respectively) reacted in a similar way (Table 2, entries 2 and 3). To see the role of MW irradiation, the reaction of diethyl α-mesyloxy-benzylphosphonate (**5a**) with ethyl alcohol was performed first at 135 °C in a bomb. After heating for 2.5 h, the conversion was only 33%, however, a reaction time of 10 h led to an almost complete transformation (Table 2, entries 4 and 5). An exposure of 2.5 h on MW irradiation, led to a 96% conversion (Table 2, entry 6). Using pyridine instead of triethylamine under similar conditions was not advantageous, as a minor byproduct was also formed (Table 2, entry 7). The reaction of diethyl α-mesyloxy-α-(4-methylphenyl-)-methylphosphonate (**5d**) took place in a similar way (Table 2, entry 8), however, the conversion of the 4-chlorophenyl derivative (**5e**) was reluctant (Table 2, entry 9), and there was need for 145 °C/3 h to reach an almost quantitative transformation (Table 2, entry 10). Changing for butyl alcohol, an irradiation of 2.5 h, or even 4 h at 135 °C was not enough, there was need for 4 h and 150 °C to reach a complete conversion (Table 2, entries 11−13). To avoid the minor decomposition, an irradiation at 145 °C for 4.5 h was the best option (Table 2, entry 14). The situation was similar for the reaction of the 4-methylphenyl derivative (**5d**) (Table 2, entry 15). The MsO → BuO substitution with the 4-chlorophenyl species (**5e**) was also complete after an irradiation time of 4.5 h at 145 °C (Table 2, entry 16). The α-alkoxy-benzylphosphonates (**14-16**) were obtained in 60−77% yields after purification by column chromatography from the best experiments. The α-alkoxyphosphonates (**14-16**) were characterized by ^{31}P, ^{13}C, and ^{1}H NMR spectral data, as well as HRMS. Products **16a,d,e** are new compounds.

3.2. Novel Michaelis−Arbuzov Reaction of Methanesulfonyloxy-benzylphosphonates. It was a disappointing experience that the Michaelis−Arbuzov reaction of α-chloro- and even α-bromo-benzylphosphonates (**17a** and **17b**) with triethyl phosphite led to the desired bisphosphonic derivative (**18a**) with a low efficiency. Besides the unreacted triethyl phosphite, diethyl phosphite and diethyl ethylphosphonate were also present in the mixture. Using the **α-chlorophosphonate** (**17a**), the conversion was as low as 6% (Table 3, entry 1). The application of the α-bromophosphonate was more

https://doi.org/10.1021/acsomega.4c04382
ACS Omega 2024, 9, 31043−31055

Table 3. Attempted Michaelis–Arbuzov Reaction of α-Halogeno-benzylphosphonates (17a and 17b) with Triethyl Phosphite



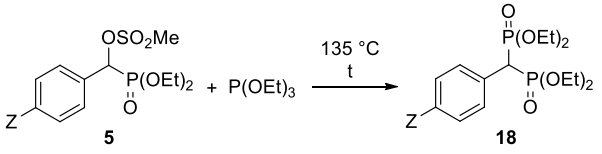
entry	X	starting material	conversion (%)[a]	composition of the mixture (%)[a] 18a	19	others
1	Cl	**17a**	~6	5	<1	$P(OEt)_3$, **$(EtO)_2P(O)H$**, $(EtO)_2P(O)Et$
2	Br	**17b**	98	45	53	$(EtO)_2P(O)H$, $(EtO)_2P(O)Et$

[a]On the basis ^{31}P NMR relative intensities.

encouraging, however, the debromination of the starting substrate to benzylphosphonate **19** predominated (Table 3, entry 2). There are examples when triethylphosphite acted as a reducing agent.[48]

To the best of our knowledge, there is no mention in the literature about Michaelis–Arbuzov reactions applying an alkylmethanesulfonate ester instead of the halogeno reagents. Moreover, in one study, the mesyloxy group was replaced by an iodo atom before performing the Michaelis–Arbuzov reaction.[49] However, a far analogy involving the reaction of thiosulfonates and phosphoramidites was described.[50] We wished to try out the α-mesyloxy-benzylphosphonates (**5**) in Arbuzov-type fission. It was nice to find that the diethyl α-mesyloxy-benzylphosphonates (**5a,d,e**) could be involved in an efficient Michaelis–Arbuzov reaction using the triethyl phosphite in an excess at 135 °C, as shown in Table 4.

Table 4. Michaelis–Arbuzov Reaction of α-Mesyloxy-benzylphosphonates (5a,d,e) with Triethyl Phosphite



entry	starting material	Z	$P(OEt)_3$ (equiv)	t (day)	conversion (%)[a]	yield (%)
1	**5a**	H	5	3	83	
2	**5a**	H	9	4	92	80 (**18a**)
3	**5d**	Me	5	3	100	76 (**18d**)
4	**5e**	Cl	5	3	80	
5	**5e**	Cl	9	4	90	81 (**18e**)

[a]On the basis ^{31}P NMR relative intensities.

Bisphosphonates **18a,d,e** were isolated in 76–81% yields after purification by column chromatography. Their structure was supported by ^{31}P, ^{13}C, and ^{1}H NMR, as well as HRMS. Arylmethylenebisphosphonates **18a** and **18e** were described earlier,[51,52] while compound **18d** is a new species.

4. IN VITRO CYTOSTATIC ACTIVITY OF THE COMPOUNDS PREPARED

For the evaluation of the in vitro antiproliferative activity of the compounds, the cell viability was determined by resazurin (Alamar Blue) assay on MDA-MB 231 breast carcinoma and A2058 melanoma cell culture. The control wells were treated only with the serum-free medium. The IC_{50} values (the concentration which decreases the viability of the cells to 50% from the maximal viability) were determined from the dose–response curves and presented as micromolar (μM) units (data are summarized in Table 5). Compounds **3b** and **5b** induce

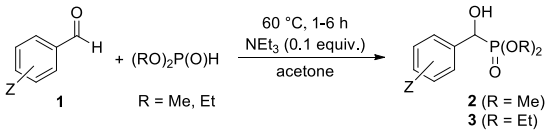
Table 5. In Vitro Cytostatic Effect of the Compounds, IC_{50} Values Presented as μM Units

	IC_{50} (μM)	
cell culture		
compound	MDA-MB 231	A2058
2b	95.4	>250
3b	16.4	110.5
4a	>250	>250
4b	92.1	106.0
4d	>250	>250
4e	>250	>250
4f	130.8	119.9
5a	>250	>250
5b	28.0	34.9
5c	>250	>250
5d	>250	>250
5e	>250	>250
5f	>250	>250
7a	>250	>250
7e	>250	>250
14a	>250	>250
14d	>250	>250
14e	>250	>250
15a	>250	>250
15d	>250	>250
15e	>250	>250
16a	>250	109.8
16d	>250	>250
16e	91.6	114.5
18a	>250	>250
18d	>250	>250
18e	>250	>250
daunomycin	0.7	0.9
tamoxifen	3.4	1.0

cytostasis on MDA-MB231 cells with IC_{50} of 16.4 and 28.0 μM, respectively. The other phosphonates have no or only slight in vitro cytostatic effect on these cells. Compounds **2b**, **4b**, and **4f** revealed IC_{50} values of 95.4, 92.1, and 130.8 μM, respectively, on MDA-MB 231 cell culture. On the melanoma culture, phosphonate **5b** showed a fair antiproliferative effect (IC_{50} = 34.9 μM). Other compounds had no or limited inhibiting activity. Species **3b**, **4b**, and **4f** revealed IC_{50} values of 110.5, 106.0, and 119.9 μM, respectively, on A2058 cells. It is worthy to mention that diethyl α-hydroxy-di-*tert*-butylbenzylphosphonate **3b** showed a selective activity on one of the two cell cultures investigated. One may also see that the effect is somehow connected with the *tert*-butyl substituent in the phenyl ring. However, one can see that our best compounds

https://doi.org/10.1021/acsomega.4c04382
ACS Omega 2024, 9, 31043–31055

Table 6. Preparation of the Starting α-Hydroxy-benzylphosphonates 2 and 3



Z = 4-H (**a**), 3,5-di^{t}Bu (**b**), 3-MeO (**c**), 4-Me (**d**), 4-Cl (**e**), 4-NO_2 (**f**), 4-MeO (**g**), 2-MeO (**h**)

Z	R	t (h)	product	yield (%)	δ ^{31}P ($CDCl_3$)	δ ^{31}P ($CDCl_3$) lit.	$[M + H]^+$	mp (°C)	mp (°C) lit.
H[11]	Me	1	**2a**	77	23.6	23.8	217	100−101	101−102[53]
3,5-di^{t}Bu	Me	6	**2b**	64	24.0		329	130−131	
4-Me[11]	Me	1.5	**2d**	88	24.1	24.1	231	99−100	98[54]
4-Cl[11]	Me	1	**2e**	80	22.9	23.2	251	101−102	104−105[55]
4-NO_2[11]	Me	1.5	**2f**	77	21.9	22.3	262	129−130	129−131[56]
4-MeO[57]	Me	2	**2g**	85	23.4	23.4	247	73−74	72[54]
H[11]	Et	1	**3a**	87	21.4	21.7	245	83−84	83−84[58]
3,5-di^{t}Bu	Et	6	**3b**	78	21.8		357	84−85	
3-MeO[59]	Et	2	**3c**	87	21.4	25.2	275		
4-Me[57]	Et	3	**3d**	71	21.6	21.7	259	96−97	94−95[60]
4-Cl[11]	Et	2	**3e**	74	20.9	21.0	279	74−75	73−74[61]
4-NO_2[11]	Et	1.5	**3f**	75	19.8	20.0	290	89−90	90−91[62]
4-MeO[57]	Et	2	**3g**	76	21.7	21.5	275	121−122	120−121.5[60]
2-MeO[59]	Et	3	**3h**	77	22.3	26.3	275		

(**3b** and **5b**) are less efficient than the reference compounds Daunomycin and Tamoxifen.

5. CONCLUSIONS

A new family of compounds, methane- and arenesulfonyloxy-benzylphosphonates were prepared by the reaction of α-hydroxy-benzylphosphonates and the corresponding sulfonyl chloride. When the starting hydroxyphosphonate bore a methoxy substituent in position 4 or 2 in the phenyl ring, the respective chlorophosphonate was the only product, whose formation could be explained by the intermediacy of a stabilized quinoid species, as confirmed by quantum chemical calculations. The reactivity of the methanesulfonyloxy-benzylphosphonates was explored in reaction with alcohols and in the Michaelis−Arbuzov reaction. In the first case, on MW irradiation at ∼140 °C, in the presence of triethylamine, not alcoholysis, but a selective nucleophilic substitution on the α-carbon atom to provide the α-alkoxy derivatives took place. In the second case, an efficient Michaelis−Arbuzov fission occurred, which is a novel and valuable experience, as the similar reaction of the related α-halogeno phosphonates is insufficient. Among the phosphonate derivatives, the α-hydroxy- and α-mesyloxy-3,5-di-*tert*-butylbenzylphosphonates showed significant cytostatic effect on the MDA-MB 231 human breast carcinoma cell line, and the mesyloxy derivative also on A2058 melanoma cell culture.

6. EXPERIMENTAL SECTION

6.1. General Information. The MW reactions were carried out in a CEM Discover (300 W) focused MW reactor (CEM Microwave Technology Ltd., Buckingham, U.K.) equipped with a stirrer and a pressure controller using 80−100 W irradiation under isothermal conditions. The reaction mixtures were irradiated in sealed glass vessels (with a volume of 10 mL) available from the supplier of CEM. The reaction temperature was monitored by an external IR sensor.

The ^{31}P, ^{13}C, ^{1}H NMR spectra were taken on a Bruker DRX-500 or Bruker Avance-300 spectrometer operating at 202, 126, and 500 MHz or 122, 75, and 300 MHz, respectively. The couplings were given in Hertz. LC−MS measurements were performed with an Agilent 1200 liquid chromatography system, coupled with a 6130 quadrupole mass spectrometer equipped with an ESI ion source (Agilent Technologies, Palo Alto, CA, USA). High-resolution mass spectrometric measurements were performed using a Thermo Velos Pro Orbitrap Elite hybrid mass spectrometer in positive electrospray mode.

6.2. General Procedure for the Synthesis of Dialkyl α-Hydroxy-benzylphosphonates. 11.0 mmol of aromatic aldehyde (benzaldehyde 1.2 g, *p*-chlorobenzaldehyde 1.5 g, *p*-nitrobenzaldehyde 1.7 g, *p*-methylbenzaldehyde 1.3 g, *p*-methoxybenzaldehyde 1.5 g, *m*-methoxybenzaldehyde 1.5 g, *o*-methoxybenzaldehyde 1.5 g, 3,5-di-*tert*-butyl-benzaldehyde 2.4 g) and 11.0 mmol of dialkyl phosphite (dimethyl phosphite 1.1 mL, diethyl phosphite 1.4 mL) and 1.1 mmol (0.15 mL) of triethylamine were stirred in 1 mL acetone at reflux. After 1−6 h, 6 mL of *n*-pentane was added to the reaction mixture, and it was cooled to 5 °C. Compounds **2a**,**b**,**d**−**g** and **3a**,**b**,**d**−**g** crystallized from the reaction mixture. The crystals were filtered off and washed with *n*-pentane. In two cases, the products (**3c**,**h**) were purified by column chromatography (using DCM−MeOH 97:3 as the eluent on silica gel). Products **2a**,**b**,**d**,**e**,**g** and **3a**,**b**,**d**,**e**,**g** are white, hydroxyphosphonates **2f** and **3f** are yellow crystalline compounds, **3c** and **3h** are colorless oils. The exact conditions are shown in Table 6.

*6.2.1. Dimethyl α-Hydroxy-3,5-di-tert-butylbenzylphosphonate (**2b**).* Yield: 2.3 g (64%); white solid; m.p.: 130−131 °C; ^{31}P {^{1}H} NMR (202 MHz, $CDCl_3$) δ 24.0; ^{13}C {^{1}H} NMR (126 MHz, $CDCl_3$) δ 31.5 (s, 6 CCH_3), 34.9 (s, 2 CCH_3), 53.6 and 53.9 (d, J = 7.3 Hz, 2 OCH_3), 71.2 (d, J = 158.5 Hz, CH), 121.5 (d, J = 5.9 Hz, C_β), 122.0 (bs, C_δ), 135.4 (s, C_α), 150.7 (s, C_γ); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.35 (s, 18H, CCH_3), 3.64 and 3.73 (d, J = 10.5 Hz, 6H, OCH_3), 5.06 (dd, J_1 = 10.6 Hz, J_2 = 5.0 Hz, 1H, CH), 7.34−7.35 and 7.40−7.41 (m, 3H, ArH); $[M + H]^+$ = 329; HRMS *m*/*z*: $[M + Na]^+$ calculated for $C_{17}H_{29}O_4PNa$ 351.1701; found 351.1697.

*6.2.2. Diethyl α-Hydroxy-3,5-di-tert-butylbenzylphosphonate (**3b**).* Yield: 3.1 g (78%); white solid; m.p.: 84−85 °C; ^{31}P {^{1}H} NMR (202 MHz, $CDCl_3$) δ 21.8; ^{13}C {^{1}H} NMR (126

https://doi.org/10.1021/acsomega.4c04382
ACS Omega 2024, 9, 31043−31055

MHz, $CDCl_3$) δ 16.4 (t, J = 6.2 Hz, 2 CH_2CH_3), 31.5 (s, 6 CCH_3), 34.9 (2 CCH_3), 62.8 and 63.2 (d, J = 7.2 Hz, 2 OCH_2), 71.4 (d, J = 158.7 Hz, CH), 121.5 (d, J = 5.9 Hz, C_β), 121.9 (bs, C_δ), 135.6 (s, C_α), 150.5 (s, C_γ); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.21 and 1.29 (t, J = 6.9 Hz, 6H, CH_2CH_3), 1.35 (s, 18H, CCH_3), 3.93−4.10 (m, 4H, OCH_2), 5.03 (d, J = 1.3 Hz, 1H, CH), 7.34−7.39 (m, 3H, ArH); $[M + H]^+$ = 357; HRMS m/z: $[M + Na]^+$ calculated for $C_{19}H_{33}O_4PNa$ 379.2014; found 379.2007.

6.3. General Procedure for the Synthesis of Dialkyl α-Methanesulfonyloxy-arylphosphonates. 1.0 mmol of dialkyl α-hydroxy-benzylphosphonate (**2a**: 0.22 g; **3a**: 0.24 g; **2b**: 0.33 g; **3b**: 0.36 g; **3c**: 0.27 g; **2d**: 0.23 g; **3d**: 0.26 g; **2e**: 0.25 g, **3e**: 0.28 g, **2f**: 0.26 g, **3f**: 0.29 g), 1.5 mmol (0.12 mL) of methanesulfonyl chloride and 1.5 mmol (0.21 mL) of triethylamine in 5 mL of toluene were mixed at room temperature for half an hour. The precipitated triethylamine hydrochloride salt was filtered off, the filtrate was evaporated under vacuum, and the crude product was purified by column chromatography (using DCM−MeOH 95:5 as the eluent on silica gel).

*6.3.1. Dimethyl α-Methanesulfonyloxy-benzylphosphonate (**4a**).* Yield: 0.20 g (67%); white solid; m.p.: 105−106 °C; ^{31}P {^{1}H} NMR (202 MHz, $CDCl_3$) δ 16.9; ^{13}C {^{1}H} NMR (126 MHz, $CDCl_3$) δ 39.5 (s, SCH_3), 54.1 and 54.5 (d, J = 7.1 Hz, 2 OCH_3), 77.0 (d, J = 171.4 Hz, CH), 128.1 (d, J = 5.9 Hz, C_β), 129.0 (d, J = 2.0 Hz, C_γ), 129.8 (d, J = 2.7 Hz, C_δ), 131.7 (bs, C_α); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.87 (s, 3H, SCH_3), 3.70 and 3.84 (d, J = 10.7 Hz, 6H, OCH_3), 5.79 (d, J = 15.1 Hz, 1H, CH), 7.43−7.57 (m, 5H, ArH), $[M + H]^+$ = 295; HRMS m/z: $[M + Na]^+$ calculated for $C_{10}H_{15}O_6PSNa$ 317.0225; found 317.0222.

*6.3.2. Dimethyl α-Methanesulfonyloxy-3,5-di-tert-butylbenzylphosphonate (**4b**).* Yield: 0.24 g (60%); colorless oil; ^{31}P {^{1}H} NMR (202 MHz, $CDCl_3$) δ 17.3; ^{13}C {^{1}H} NMR (126 MHz, $CDCl_3$) δ 31.4 (s, 6 CCH_3), 35.0 (s, 2 CCH_3), 39.7 (s, SCH_3), 54.1 and 54.4 (d, J = 6.9 Hz, 2 OCH_3), 77.8 (d, J = 170.3 Hz, CH), 122.7 (d, J = 6.0 Hz, C_β), 123.7 (d, J = 2.6 Hz, C_δ), 130.6 (s, C_α), 151.6 (d, J = 2.0 Hz, C_γ); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.33 (s, 18H, CCH_3), 2.79 (s, 3H, SCH_3), 3.65 and 3.80 (d, J = 10.7 Hz, 6H, OCH_3), 5.77 (d, J = 14.7 Hz, 1H, CH), 7.36−7.37 and 7.44−7.46 (m, 3H, ArH); $[M + H]^+$ = 407; HRMS m/z $[M + Na]^+$ calculated for $C_{18}H_{31}O_6PSNa$ 429.1477; found 429.1477.

*6.3.3. Dimethyl α-Methanesulfonyloxy-4-methylbenzylphosphonate (**4d**).* Yield: 0.20 g (61%); white solid; m.p.: 85−86 °C; ^{31}P {^{1}H} NMR (202 MHz, $CDCl_3$) δ 17.1; ^{13}C {^{1}H} NMR (126 MHz, $CDCl_3$) δ 21.3 (s, $ArCH_3$), 39.7 (s, SCH_3), 54.1 and 54.5 (d, J = 6.8 Hz, 2 OCH_3), 77.2 (d, J = 172.9 Hz, CH), 128.3 (d, J = 6.1 Hz, C_β), 128.6 (d, J = 1.7 Hz, C_α), 129.7 (d, J = 2.0 Hz, C_γ), 140.1 (d, J = 2.7 Hz, C_δ); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.39 (s, 3H, $ArCH_3$), 2.84 (s, 3H, SCH_3), 3.70 and 3.86 (d, J = 10.7 Hz, 6H, OCH_3), 5.76 (d, J = 14.9 Hz, 1H, CH), 7.25−7.26 and 7.45−7.46 (m, 4H, ArH); $[M + H]^+$ = 309; HRMS m/z: $[M + Na]^+$ calculated for $C_{11}H_{17}O_6PSNa$ 331.0381; found 331.0376.

*6.3.4. Dimethyl α-Methanesulfonyloxy-4-chlorobenzylphosphonate (**4e**).* Yield: 0.22 g (66%); white solid; m.p.: 120−121 °C; ^{31}P {^{1}H} NMR (202 MHz, $CDCl_3$) δ 16.6; ^{13}C {^{1}H} NMR (126 MHz, $CDCl_3$) δ 39.6 (s, SCH_3), 54.2 and 54.6 (d, J = 6.7 Hz, 2 OCH_3), 76.1 (d, J = 172.6 Hz, CH), 129.3 (d, J = 1.9 Hz, C_γ), 129.5 (d, J = 6.1 Hz, C_β), 130.4 (d, J = 1.8 Hz, C_α), 135.9 (d, J = 3.2 Hz, C_δ); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.94 (s, 3H, SCH_3), 3.74 and 3.85 (d, J = 10.7 Hz, 6H, OCH_3), 5.77 (d, J = 15.1 Hz, 1H, CH), 7.43−7.52 (m, 4H, ArH), $[M + H]^+$ = 329; HRMS m/z: $[M + Na]^+$ calculated for $C_{10}H_{14}ClO_6PSNa$ 350.9835; found 350.9831.

*6.3.5. Dimethyl α-Methanesulfonyloxy-4-nitrobenzylphosphonate (**4f**).* Yield: 0.26 g (76%); pale yellow solid; m.p.: 163−164 °C; ^{31}P {^{1}H} NMR (202 MHz, $CDCl_3$) δ 15.8; ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$) δ 39.4 (s, SCH_3), 54.4 and 54.7 (d, J = 6.8 Hz, 2 OCH_3), 75.3 (d, J = 169.1 Hz, CH), 124.0 (d, J = 2.3 Hz, C_γ), 128.6 (d, J = 5.3 Hz, C_β), 139.2 (d, J = 1.9 Hz, C_α), 148.5 (bs, C_δ); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.09 (s, 3H, SCH_3), 3.81 and 3.85 (d, J = 10.8 Hz, 6H, OCH_3), 5.90 (d, J = 15.9 Hz, 1H, CH), 7.72−7.74 and 8.30−8.32 (m, 4H, ArH); $[M + H]^+$ = 340; HRMS m/z: $[M + Na]^+$ calculated for $C_{10}H_{14}NO_8PSNa$ 362.0075; found 362.0072.

*6.3.6. Diethyl α-Methanesulfonyloxy-benzylphosphonate (**5a**).* Yield: 0.25 g (78%); white solid; m.p.: 72−73 °C; ^{31}P {^{1}H} NMR (202 MHz, $CDCl_3$) δ 14.5; ^{13}C {^{1}H} NMR (126 MHz, $CDCl_3$) δ 16.2 and 16.4 (d, J = 5.7 Hz, 2 CH_2CH_3), 39.6 (s, SCH_3), 63.8 and 64.1 (d, J = 6.9 Hz, 2 OCH_2), 77.5 (d, J = 171.2 Hz, CH), 128.2 (d, J = 5.9 Hz, C_β), 128.8 (d, J = 1.8 Hz, C_γ), 129.7 (d, J = 2.6 Hz, C_δ), 131.9 (d, J = 1.6 Hz, C_α); ^{1}H NMR (500 MHz, CDC1$_3$) δ 1.24 and 1.35 (t, J = 7.1 Hz, 6H, CH_2CH_3), 2.86 (s, 3H, SCH_3), 3.95−4.23 (m, 4H, OCH_2), 5.76 (d, J = 15.1 Hz, 1H, CH), 7.41−7.58 (m, 5H, ArH), δ lit.[63] 1.20 and 1.30 (t, 6H, J = 7.0 Hz, CH_2CH_3), 2.82 (s, 3H, SCH_3), 3.70−4.40 (m, 4H, OCH_2), 5.70 (d, 1H, J = 15.0 Hz, CH), 7.20−7.60 (m, 5H, ArH); $[M + H]^+$ = 323; HRMS m/z: $[M + Na]^+$ calculated for $C_{12}H_{19}O_6PSNa$ 345.0538; found 345.0536.

*6.3.7. Diethyl α-Methanesulfonyloxy-3,5-di-tert-butylbenzylphosphonate (**5b**).* Yield: 0.24 g (54%); colorless oil; ^{31}P {^{1}H} NMR (202 MHz, $CDCl_3$) δ 14.9; ^{13}C {^{1}H} NMR (126 MHz, $CDCl_3$) δ 16.2 and 16.4 (d, J = 5.9 Hz, 2 CH_2CH_3), 31.4 (s, 6 CCH_3), 34.9 (s, 2 CCH_3), 39.7 (s, SCH_3), 63.7 and 63.9 (d, J = 6.5 Hz, 2 OCH_2), 78.5 (d, J = 171.1 Hz, CH), 122.7 (d, J = 6.0 Hz, C_β), 123.5 (d, J = 2.6 Hz, C_δ), 130.8 (s, C_α), 151.4 (d, J = 1.9 Hz, C_γ); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.21 and 1.35 (t, J = 7.1 Hz, 6H, CH_2CH_3), 1.35 (s, 18H, CCH_3), 2.81 (s, 3H, SCH_3), 3.93−4.21 (m, 8H, OCH_2), 5.76 (d, J = 14.7 Hz, 1H, CH), 7.37−7.46 (m, 3H, ArH); $[M + H]^+$ = 437; HRMS m/z: $[M + Na]^+$ calculated for $C_{20}H_{35}O_6PSNa$ 457.1790; found 457.1776.

*6.3.8. Diethyl α-Methanesulfonyloxy-3-methyoxybenzylphosphonate (**5c**).* Yield: 0.28 g (80%), pale yellow oil; ^{31}P {^{1}H} NMR (202 MHz, $CDCl_3$) δ 14.5; ^{13}C {^{1}H} NMR (126 MHz, $CDCl_3$) δ 16.3 and 16.4 (d, J = 5.8 Hz, 2 CH_2CH_3), 39.6 (s, SCH_3), 55.4 (s, OCH_3), 63.8 and 64.2 (d, J = 6.9 Hz, 2 OCH_2), 77.5 (d, J = 171.2 Hz, CH), 113.5 (d, J = 5.7 Hz, C_6*), 115.6 (d, J = 2.6 Hz, C_5*) 120.5 (d, J = 6.0 Hz, C_2*), 129.9 (d, J = 1.9 Hz, C_4), 133.3 (d, J = 1.7 Hz, C_1) 159.8 (s, C_3(OMe)), *tentative; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.17 and 1.26 (t, J = 7.1 Hz, 6H, CH_2CH_3), 2.79 (s, 3H, SCH_3), 3.76 (s, 3H, OCH_3), 3.87−4.14 (m, 4H, OCH_2), 5.63 (d, J = 15.1 Hz, 1H, CH), 6.86−6.87, 7.01−7.04 and 7.23−7.27 (m, 4H, ArH); $[M + H]^+$ = 353; HRMS m/z: $[M + Na]^+$ calculated for $C_{13}H_{21}O_7PSNa$ 375.0643; found 375.0642.

*6.3.9. Diethyl α-Methanesulfonyloxy-4-methylbenzylphosphonate (**5d**).* Yield: 0.26 g (76%), colorless oil; ^{31}P {^{1}H} NMR (202 MHz, $CDCl_3$) δ 14.7; ^{13}C {^{1}H} NMR (126 MHz, $CDCl_3$) δ 16.3 and 16.4 (d, J = 5.7 Hz, 2 CH_2CH_3), 21.3 (s, $ArCH_3$), 39.7 (s, SCH_3), 63.7 and 64.1 (d, J = 6.7 Hz, 2 OCH_2), 77.6 (d, J = 172.4 Hz, CH), 128.3 (d, J = 5.9 Hz, C_β),

https://doi.org/10.1021/acsomega.4c04382
ACS Omega 2024, 9, 31043−31055

128.8 (d, J = 1.6 Hz, C_α), 129.6 (d, J = 1.9 Hz, C_γ), 139.8 (d, J = 2.8 Hz, C_δ); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.24 and 1.36 (t, J = 7.1 Hz, 6H, CH_2CH_3), 2.39 (s, 3H, $ArCH_3$) 2.83 (s, 3H, SCH_3), 3.94−4.23 (m, 4H, OCH_2), 5.72 (d, J = 14.9 Hz, 1H, CH), 7.23−7.25 and 7.45−7.46 (m, 4H, ArH); $[M + H]^+$ = 337; HRMS m/z $[M + Na]^+$ calculated for $C_{13}H_{21}O_6PSNa$ 359.0694; found 359.0694.

*6.3.10. Diethyl α-Methanesulfonyloxy-4-chlorobenzylphosphonate (**5e**).* Yield: 0.28 g (79%); white crystals; m.p.: 76−77 °C; ^{31}P {^{1}H} NMR (202 MHz, $CDCl_3$) δ 14.2; ^{13}C {^{1}H} NMR (126 MHz, $CDCl_3$) δ 16.2 and 16.3 (d, J = 5.7 Hz, 2 CH_2CH_3), 39.5 (s, SCH_3), 63.9 and 64.2 (d, J = 6.8 Hz, 2 OCH_2), 76.5 (d, J = 171.6 Hz, CH), 129.0 (d, J = 2.0 Hz, C_γ), 129.5 (d, J = 5.7 Hz, C_β), 130.7 (bs, C_α), 135.6 (bs, C_δ); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.27 and 1.35 (t, J = 7.0 Hz, 6H, CH_2CH_3), 2.93 (s, 3H, SCH_3), 3.99−4.23 (m, 4H, OCH_2), 5.73 (d, J = 15.1 Hz, 1H, CH), 7.41−7.51 (m, 4H, ArH); $[M + H]^+$ = 357; HRMS m/z $[M + Na]^+$ calculated for $C_{12}H_{18}ClO_6PSNa$ 379.0148; found 379.0143.

*6.3.11. Diethyl α-Methanesulfonyloxy-4-nitrobenzylphosphonate (**5f**).* Yield: 0.28 g (76%); yellow oil; ^{31}P {^{1}H} NMR (202 MHz, $CDCl_3$) δ 13.3; ^{13}C {^{1}H} NMR (75 MHz, $CDCl_3$) δ 16.3 and 16.4 (d, J = 5.7 Hz, 2 CH_2CH_3), 39.4 (s, SCH_3), 64.2 and 64.5 (d, J = 6.8 Hz, 2 OCH_2), 75.8 (d, J = 168.4 Hz, CH), 123.8 (d, J = 2.2 Hz, C_γ), 128.6 (d, J = 5.2 Hz, C_β), 139.6 (d, J = 1.9 Hz, C_α), 148.3 (d, J = 3.1 Hz, C_δ); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.31 and 1.34 (t, J = 7.1 Hz, 6H, CH_2CH_3), 3.09 (s, 3H, SCH_3), 4.10−4.21 (m, 4H, OCH_2), 5.87 (d, J = 15.9 Hz, 1H, CH), 7.71−7.73 and 8.29−8.30 (m, 4H, ArH); $[M + H]^+$ = 368; HRMS m/z $[M + Na]^+$ calculated for $C_{12}H_{18}NO_8PSNa$ 390.0388; found 390.0390.

6.4. General Procedure for the Synthesis of Dialkyl α-Toluenesulfonyloxy-arylphosphonates. 1.0 mmol of dialkyl α-hydroxy-benzylphosphonate (**3a**: 0.24 g; **3d**: 0.26 g; **3e**: 0.28 g), 1.5 mmol (0.29 g) of *p*-toluenesulfonyl chloride and 1.5 mmol (0.21 mL) of triethylamine in 5 mL of toluene were mixed at room temperature for 24 h. The precipitated salt was filtered off, the filtrate evaporated under vacuum, and the crude product purified by column chromatography (using DCM−MeOH 97:3 as the eluent on silica gel).

*6.4.1. Diethyl α-4-Methylbenzenesulfonyloxy-benzylphosphonate (**6a**).* Yield: 0.26 g (65%); white solid; m.p.: 63−64 °C, mp lit.:[29] 63−65 °C; ^{31}P {^{1}H} NMR (202 MHz, $CDCl_3$) δ 14.6, δ_P lit.[42] 14.6; $[M + H]^+$ = 399; HRMS m/z: $[M + Na]^+$ calculated for $C_{18}H_{23}O_6PSNa$ 421.0851; found 421.0857.

*6.4.2. Diethyl α-4-Methylbenzenesulfonyloxy-4-methylbenzylphosphonate (**6d**).* Yield: 0.29 g (71%); colorless oil; ^{31}P {^{1}H} NMR (202 MHz, $CDCl_3$) δ 14.7, δ_P lit.[42] 14.8; $[M + H]^+$ = 413; HRMS m/z: $[M + Na]^+$ calculated for $C_{19}H_{25}O_6PSNa$ 435.1007; found 435.1006.

*6.4.3. Diethyl α-4-Methylbenzenesulfonyloxy-4-chlorobenzylphosphonate (**6e**).* Yield: 0.30 g (70%); colorless oil; ^{31}P {^{1}H} NMR (202 MHz, $CDCl_3$) δ 14.1, δ_P lit.[42] 14.1; $[M + H]^+$ = 433; HRMS m/z: $[M + Na]^+$ calculated for $C_{18}H_{22}ClO_6PSNa$ 455.0461; found 455.0460.

6.5. General Procedure for the Synthesis of Diethyl α-Chloro-arylphosphonates. 1.0 mmol of dialkyl **α-hydroxy-benzylphosphonate** (**2g**: 0.25 g; **3g**: 0.27 g; **3h**: 0.27 g), 1.5 mmol (0.12 mL) of methanesulfonyl chloride and 1.5 mmol (0.21 mL) of triethylamine in 5 mL of toluene were mixed at room temperature for half an hour. The precipitated salt was filtered off, the filtrate evaporated under vacuum, and the crude product purified by column chromatography (using DCM−MeOH 95:5 as the eluent on silica gel).

*6.5.1. Dimethyl α-Chloro-4-methyoxybenzylphosphonate (**8g**).* Yield: 0.18 g (68%); pale yellow oil; ^{31}P {^{1}H} NMR (202 MHz, $CDCl_3$) δ 19.7; ^{13}C {^{1}H} NMR (126 MHz, $CDCl_3$) δ 53.0 (d, J = 163.1 Hz, CH), 54.3 and 54.6 (d, J = 6.9 Hz, 2 OCH_3), 55.3 (s, OCH_3), 114.1 (d, J = 1.7 Hz, C_γ), 125.8 (d, J = 3.6 Hz, C_α), 130.3 (d, J = 6.5 Hz, C_β), 160.2 (d, J = 2.3 Hz, C_δ); ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.63 and 3.85 (d, J = 10.7 Hz, 6H, OCH_3), 3.82 (s, 3H, OCH_3), 4.90 (d, J = 13.8 Hz, 1H, CH), 6.91 (d, J = 8.7 Hz, 2H, ArH), 7.47 (dd, J_1 = 8.8 Hz, J_2 = 1.9 Hz, 2H, ArH), δ lit.[64] 3.60 and 3.81 (d, J = 10.0 Hz, 6H, OCH_3), 3.79 (s, 3H, OCH_3), 4.90 (d, J = 14.0 Hz, 1H, CH), 6.90 (d, J = 8 Hz, 2H, ArH), 7.50 (dd, J_1 = 8 Hz, J_2 = 2 Hz, 2H, ArH); $[M + H]^+$ = 265; HRMS m/z: $[M + Na]^+$ calculated for $C_{10}H_{14}ClO_4PNa$ 287.0216; found 287.0210.

*6.5.2. Diethyl α-Chloro-4-methyoxybenzylphosphonate (**9g**).* Yield: 0.20 g (70%); pale yellow oil; ^{31}P {^{1}H} NMR (202 MHz, $CDCl_3$) δ 17.4, δ_P lit.[65] 17.6; $[M + H]^+$ = 293; HRMS m/z $[M + Na]^+$ calculated for $C_{12}H_{18}ClO_4PNa$ 315.0529; found 315.0528.

*6.5.3. Diethyl α-Chloro-2-methyoxybenzyl]phosphonate (**9h**).* Yield: 0.18 g (65%); pale yellow oil; ^{31}P {^{1}H} NMR (202 MHz, $CDCl_3$) δ 18.1; ^{13}C {^{1}H} NMR (126 MHz, $CDCl_3$) δ 16.2 and 16.4 (d, J = 5.9 Hz, 2 CH_2CH_3), 45.6 (d, J = 163.6 Hz, CH), 55.7 (s, OCH_3), 63.7 and 63.8 (d, J = 7.0 Hz, 2 OCH_2), 110.5 (d, J = 1.4 Hz, C_5), 121.0 (d, J = 2.3 Hz, C_4*), 122.8 (d, J = 2.7 Hz, C_1), 130.2 (d, J = 2.3 Hz, C_3*), 130.6 (d, J = 4.0 Hz, C_6), 156.4 (d, J = 7.7 Hz, C_2 (OCH_3)), *may be reversed; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.18 and 1.34 (t, J = 7.1 Hz, 6H, CH_2CH_3), 3.87 (s, 3H, OCH_3), 3.89−3.97, 4.03−4.10 and 4.19−4.27 (m, 4H, OCH_2), 5.64 (d, J = 14.0 Hz, 1H, CH), 6.88 (d, J = 8.3 Hz, 1H, ArH), 7.02 (t, J = 7.6 Hz, 1H, ArH), 7.29−7.33 and 7.77−7.79 (m, 2H, ArH); $[M + H]^+$ = 293; HRMS m/z: $[M + Na]^+$ calculated for $C_{12}H_{18}ClO_4PNa$ 315.0529; found 315.0526.

6.6. General Procedure for the Synthesis of Diethyl α-Alkoxy-arylphosphonates. 0.50 mmol of diethyl α-methanesulfonyloxy-arylphosphonate (**5a**: 0.16 g; **5d**: 0.17 g; **5e**: 0.18 g), 7.5 mmol of primary alcohol (methyl alcoholl: 0.30 mL, ethyl alcohol: 0.44 mL, butyl alcohol: 0.69 mL), and 0.50 mmol (0.07 mL) of triethylamine were mixed at 135−145 °C for 2−4.5 h under MW irradiation (for the details see Table 2). The reaction mixture was evaporated under vacuum, and the crude product was purified by column chromatography (using DCM−MeOH 97:3 as the eluent on silica gel).

*6.6.1. Diethyl α-Methoxybenzylphosphonate (**14a**).* Yield: 0.08 g (63%); colorless oil; ^{31}P {^{1}H} NMR (202 MHz, $CDCl_3$) δ 19.1, δ_P lit.[66] 19.1; $[M + H]^+$ = 259; HRMS m/z: $[M + Na]^+$ calculated for $C_{12}H_{19}O_4PNa$ 281.0919; found 281.0913.

*6.6.2. Diethyl α-Methoxy-4-methylbenzylphosphonate (**14d**).* Yield: 0.11 g (77%); colorless oil; ^{31}P {^{1}H} NMR (122 MHz, $CDCl_3$) δ 19.3, δ_P lit.[66] 19.3; $[M + H]^+$ = 273; HRMS m/z: $[M + Na]^+$ calculated for $C_{13}H_{21}O_4PNa$ 295.1075; found 295.1068.

*6.6.3. Diethyl α-Methoxy-4-chlorobenzylphosphonate (**14e**).* Yield: 0.09 g (60%); colorless oil; ^{31}P {^{1}H} NMR (202 MHz, $CDCl_3$) δ 18.4, δ_P lit.[66] 18.4; $[M + H]^+$ = 293; HRMS m/z: $[M + Na]^+$ calculated for $C_{12}H_{18}ClO_4PNa$ 315.0529; found 315.0523.

*6.6.4. Diethyl α-Ethoxy-benzylphosphonate (**15a**).* Yield: 0.10 g (75%); colorless oil; ^{31}P {^{1}H} NMR (202 MHz,

https://doi.org/10.1021/acsomega.4c04382
ACS Omega 2024, 9, 31043−31055

$CDCl_3$) δ 19.2, δ_P lit.[67] 19.6; ^{13}C {^1H} NMR (75 MHz, $CDCl_3$) δ 15.2 (s, CH_2CH_3), 16.4 (t, J = 5.7 Hz, 2 CH_2CH_3), 62.9 and 63.1 (d, J = 7.0 Hz, 2 OCH_2), 66.5 (d, J = 13.8 Hz, OCH_2), 78.6 (d, J = 168.1 Hz, CH), 127.9 (d, J = 5.8 Hz, C_β), 128.2 (d, J = 4.2 Hz, C_δ), 128.3 (d, J = 2.5 Hz, C_γ), 135.1 (d, J = 1.8 Hz, C_α); ^1H NMR ($CDCl_3$) δ lit.[68] 1.25 (t, J = 7.0 Hz, 9H, CH_2CH_3), 3.62 (q, J = 7.0 Hz, 2H, OCH_2), 4.20 (qq, J = 7.0 Hz, 4H, OCH_2), 4.80 and 5.00 (2d, J = 15.0 Hz, 1H, CH), 7.45 (s, 5H, ArH); $[M + H]^+$ = 273; HRMS m/z: $[M + H]^+$ calculated for $C_{13}H_{22}O_4P$ 273.1256; found 273.1260; $[M + Na]^+$ calculated for $C_{13}H_{21}O_4PNa$ 295.1075; found 295.1083.

*6.6.5. Diethyl α-Ethoxy-4-methylbenzylphosphonate (**15d**).* Yield: 0.09 g (66%); colorless oil; ^{31}P {^1H} NMR (122 MHz, $CDCl_3$) δ 19.5, δ_P lit.[67] 19.9; ^{13}C {^1H} NMR ($CDCl_3$) δ lit.[69] 14.9 (s, CH_2CH_3), 16.1 and 16.2 (d, J = 6.0 Hz, 2 CH_2CH_3), 20.9 (s, $ArCH_3$), 62.6 and 62.8 (d, J = 7.0 Hz, 2 OCH_2), 66.0 (d, J = 14.2 Hz, OCH_2), 78.2 (d, J = 168.0 Hz, CH), 127.7 (d, J = 6.0 Hz, C_β), 128.8 (d, J = 2.4 Hz, C_γ), 131.8 (d, J = 1.9 Hz, C_α), 137.7 (d, J = 3.4 Hz, C_δ); ^1H NMR ($CDCl_3$) δ lit.[69] 1.15−1.32 (m, 9H, CH_2CH_3), 2.34 (s, 3H, ArCH3), 3.40−3.64 (m, 2H, OCH_2), 4.15−3.93 (m, 4H, OCH_2), 4.59 (d, J = 15.8 Hz, 1H, CH), 7.16 (d, J = 7.4 Hz, 2H, ArH), 7.34 (d, J = 6.8 Hz, 2H, ArH); $[M + H]^+$ = 287; HRMS m/z: $[M + Na]^+$ calculated for $C_{14}H_{23}O_4PNa$ 309.1232; found 309.1229.

*6.6.6. Diethyl α-Ethoxy-4-chlorobenzylphosphonate (**15e**).* Yield: 0.10 g (74%); colorless oil; ^{31}P {^1H} NMR (202 MHz, $CDCl_3$) δ 18.6; δ_P lit.[67] 18.7; ^{13}C {^1H} NMR (126 MHz, $CDCl_3$) δ 15.1 (s, CH_2CH_3), 16.3 and 16.4 (d, J = 5.7 Hz, 2 CH_2CH_3), 63.0 and 63.3 (d, J = 7.0 Hz, 2 OCH_2), 66.7 (d, J = 13.6 Hz, OCH_2), 78.0 (d, J = 168.5 Hz, CH), 128.5 (d, J = 2.6 Hz, C_γ), 129.2 (d, J = 5.9 Hz, C_β), 133.9 (d, J = 1.8 Hz, C_α), 134.1 (d, J = 3.8 Hz, C_δ); ^1H NMR ($CDCl_3$) δ lit.[68] 1.22 and 1.27 (t, J = 7.0 Hz, 9H, CH_2CH_3), 3.60 (q, J = 7.0 Hz, 2H, OCH_2), 4.06 (qq, 4H, OCH_2), 4.66 (d, J = 16.0 Hz, 1H, CH), 7.44 (s, 4H, ArH); $[M + H]^+$ = 307; HRMS m/z: $[M + Na]^+$ calculated for $C_{13}H_{20}ClO_4PNa$ 329.0685; found 329.0608.

*6.6.7. Diethyl α-Butoxy-benzylphosphonate (**16a**).* Yield: 0.09 g (62%); colorless oil; ^{31}P {^1H} NMR (202 MHz, $CDCl_3$) δ 19.2; ^{13}C {^1H} NMR (75 MHz, $CDCl_3$) δ 13.8 (s, CH_2CH_3), 16.3 and 16.4 (d, J = 5.8 Hz, 2 CH_2CH_3), 19.2 (s, CH_2CH_3), 31.7 (s, CH_2CH_2), 62.9 and 63.1 (d, J = 6.9 Hz, 2 OCH_2), 70.9 (d, J = 13.5 Hz, OCH_2), 78.8 (d, J = 168.2 Hz, CH), 127.9 (d, J = 5.8 Hz, C_β), 128.2 (d, J = 3.2 Hz, C_δ), 128.3 (d, J = 2.5 Hz, C_γ), 135.2 (d, J = 1.8 Hz, C_α); ^1H NMR (500 MHz, $CDCl_3$) δ 0.91 (t, J = 7.4 Hz, 3H, CH_2CH_3), 1.25 and 1.27 (t, J = 7.1 Hz, 6H, CH_2CH_3) 1.34−1.46 (m, 2H, CH_2CH_3), 1.56−1.64 (m, 2H, CH_2CH_2), 3.44−3.55 (m, 2H, OCH_2), 3.95−4.13 (m, 4H, OCH_2), 4.63 (d, J = 16.1 Hz, 1H, CH), 7.31−7.47 (m, 5H, ArH); $[M + H]^+$ = 301; HRMS m/z: $[M + Na]^+$ calculated for $C_{15}H_{25}O_4PNa$ 323.1388; found 323.1385.

*6.6.8. Diethyl α-Butoxy-4-methylbenzylphosphonate (**16d**).* Yield: 0.11 g (70%); colorless oil; ^{31}P {^1H} NMR (122 MHz, $CDCl_3$) δ 19.5; ^{13}C {^1H} NMR (75 MHz, $CDCl_3$) δ 13.8 (s, CH_2CH_3), 16.3 and 16.4 (d, J = 6.0 Hz, 2 CH_2CH_3), 19.2 (s, CH_2CH_3), 21.2 (s, $ArCH_3$), 31.7 (s, CH_2CH_2), 62.8 and 63.1 (d, J = 6.9 Hz, 2 OCH_2), 70.7 (d, J = 13.7 Hz, OCH_2), 78.6 (d, J = 169.0 Hz, CH), 127.9 (d, J = 5.9 Hz, C_β), 129.0 (d, J = 2.5 Hz, C_γ), 132.1 (d, J = 1.9 Hz, C_α), 138.0 (d, J = 3.3 Hz, C_δ); ^1H NMR (500 MHz, $CDCl_3$) δ 0.91 (t, J = 7.4 Hz, 3H, CH_2CH_3), 1.25 and 1.28 (t, J = 6.8 Hz, 6H, CH_2CH_3) 1.35−1.43 (m, 2H, CH_2CH_3), 1.59−1.62 (m, 2H, CH_2CH_2), 2.37 (s, $ArCH_3$), 3.41−3.54 (m, 2H, OCH_2), 3.95−4.13 (m, 4H, OCH_2); 4.59 (d, J = 15.7 Hz, 1H, CH), 7.18−7.20 and 7.34−7.36 (m, 4H, ArH); $[M + H]^+$ = 315; HRMS m/z: $[M + Na]^+$ calculated for $C_{16}H_{27}O_4PNa$ 337.1545; found 337.1533.

*6.6.9. Diethyl α-Butoxy-4-chlorobenzylphosphonate (**16e**).* Yield: 0.11 g (61%); colorless oil; ^{31}P {^1H} NMR (202 MHz, $CDCl_3$) δ 18.6; ^{13}C {^1H} NMR (126 MHz, $CDCl_3$) δ 13.8 (s, CH_2CH_3), 16.3 and 16.4 (d, J = 5.7 Hz, 2 CH_2CH_3), 19.2 (s, CH_2CH_3), 31.7 (s, CH_2CH_2), 63.0 and 63.2 (d, J = 7.0 Hz, 2 OCH_2), 71.1 (d, J = 13.2 Hz, OCH_2), 78.2 (d, J = 168.9 Hz, CH), 128.5 (d, J = 2.7 Hz, C_γ), 129.2 (d, J = 5.8 Hz, C_β), 133.9 (d, J = 1.8 Hz, C_α), 134.1 (d, J = 3.8 Hz, C_δ); ^1H NMR (500 MHz, $CDCl_3$) δ 0.91 (t, J = 7.4 Hz, 3H, CH_2CH_3), 1.26 and 1.28 (t, J = 7.1 Hz, 6H, CH_2CH_3) 1.34−1.43 (m, 2H, CH_2CH_3), 1.60−1.63 (m, 2H, CH_2CH_2), 3.44−3.53 (m, 2H, OCH_2), 3.99−4.14 (m, 4H, OCH_2); 4.59 (d, J = 16.1 Hz, 1H, CH), 7.35−7.43 (m, 4H, ArH); $[M + H]^+$ = 335; HRMS m/z: $[M + Na]^+$ calculated for $C_{15}H_{24}ClO_4PNa$ 357.0998; found 357.1006.

6.7. General Procedure for the Synthesis of Tetraethyl 4-Substituted-(phenylmethylene)-bisphosphonates. 0.50 mmol of diethyl ***α*-methanesulfonyloxy-arylphosphonate** (**5a**: 0.16 g; **5d**: 0.17 g; **5e**: 0.18 g), 2.5 or 4.5 mmol of triethyl phosphite (0.43 or 0.77 mL, respectively) were mixed at 135 °C for 3 or 4 days in a sealed tube (for the details see Table 4). The crude product was purified by column chromatography (using DCM−MeOH 97:3 as the eluent on silica gel).

*6.7.1. Tetraethyl (Phenylmethylene)bisphosphonates (**18a**).* Yield: 0.15 g (80%); colorless oil; ^{31}P {^1H} NMR (202 MHz, $CDCl_3$) δ 18.6, δ_P lit.[51] 19.1 $[M + H]^+$ = 365; HRMS m/z: $[M + Na]^+$ calculated for $C_{15}H_{26}O_6P_2Na$ 387.1102; found 387.1099.

*6.7.2. Tetraethyl (4-Methylphenyl)-methylenebisphosphonates (**18d**).* Yield: 0.14 g (76%); colorless oil; ^{31}P {^1H} NMR (202 MHz, $CDCl_3$) δ 18.8; ^{13}C {^1H} NMR (75 MHz, $CDCl_3$) δ 16.1−16.3 (m, 4 CH_2CH_3), 21.1 (s, $ArCH_3$), 45.2 (t, J = 133.0 Hz, CH), 62.8−62.8 and 63.3−63.4 (dm, 4 OCH_2), 126.8 (t, J = 7.8 Hz, C_α), 129.2 (t, J = 2.1 Hz, C_γ), 130.2 (t, J = 6.4 Hz, C_β), 137.3 (t, J = 2.7 Hz, C_δ); ^1H NMR (500 MHz, $CDCl_3$) δ 1.18 and 1.30 (t, J = 7.1 Hz, 12H, CH_2CH_3), 2.35 (s, 3H, $ArCH_3$), 3.72 (t, J = 25.1 Hz, 1H, CH), 3.93−4.18 (m, 8H, OCH_2), 7.15 (d, J = 7.8 Hz, 2H, ArH), 7.36−7.38 (m, 2H, ArH); $[M + H]^+$ = 379; HRMS m/z: $[M + Na]^+$ calculated for $C_{16}H_{28}O_6P_2Na$ 401.1259; found 401.1260.

*6.7.3. Tetraethyl (4-Chlorophenyl)-methylenebisphosphonates (**18e**).* Yield: 0.16 g (81%); colorless oil; ^{31}P {^1H} NMR (202 MHz, $CDCl_3$) δ 18.1, δ_P lit.[52] 15.3; ^{13}C {^1H} NMR (126 MHz, $CDCl_3$) δ 15.2 and 15.3 (d, J = 6.2 Hz, 4 CH_2CH_3), 44.1 (t, J = 133.2 Hz, CH), 62.1 and 62.5 (d, J = 6.5 Hz, 4 OCH_2), 127.7 (t, J = 2.0 Hz, C_γ), 127.9 (t, J = 7.8 Hz, C_α), 130.7 (t, J = 6.4 Hz, C_β), 132.7 (t, J = 3.0 Hz, C_δ); ^1H NMR (500 MHz, $CDCl_3$) δ 1.21 and 1.31 (t, J = 7.1 Hz, 6H, CH_2CH_3), 3.72 (t, J = 25.0 Hz, 1H, CH), 3.98−4.19 (m, 4H, OCH_2), 7.33−7.49 (m, 4H ArH); $[M + H]^+$ = 399; HRMS m/z: $[M + Na]^+$ calculated for $C_{15}H_{25}ClO_6P_2Na$ 421.0713; found 421.0707.

6.8. Experimental for Computations. DFT computations at the M062*X*/6-31+G (d,p) level of theory were performed considering the solvent effect of toluene using the SMD (universal solvation model based on solute electron density) solvent model with the Gaussian 09 program

https://doi.org/10.1021/acsomega.4c04382
ACS Omega 2024, 9, 31043−31055

package.[70−72] The geometries of the molecules were optimized in all cases, and frequency calculations were also performed to ensure that the structures were at a local minimum. The solution-phase enthalpies and Gibbs free energies were obtained by frequency calculations as well. The H and G values obtained were given under standard conditions; the standard state correction and the corrected total energies of the molecules were considered. Entropic and thermal corrections were evaluated for isolated molecules using standard rigid rotor harmonic oscillator approximations, that is, the Gibbs free energy was taken as the "sum of electronic and thermal free energies" printed in a Gaussian 09 vibrational frequency calculation. The standard state correction was taken into account. For details of the calculations, see the Supporting Information.

6.9. Single X-ray Experimental. Single crystals of compound **5e**, suitable for X-ray diffraction, were obtained by slow evaporation of acetone solution. The crystals were introduced into perfluorinated oil and a suitable single crystal was carefully mounted on the top of a thin glass wire. Data collection was performed with an Oxford Xcalibur 3 diffractometer equipped with a Spellman generator (50 kV, 40 mA) and a Kappa CCD detector, operating with Mo−K_α radiation (λ = 0.71071 Å).

Data collection and data reduction were performed with the CrysAlisPro software.[73] Absorption correction using the multiscan method[74] was applied. The structures were solved with SHELXS-97,[75] refined with SHELXL-97[75] and finally checked using PLATON.[76] Details for data collection and structure refinement are summarized in Table 7.

CCDC-2351759 contains supplementary crystallographic data for this compound. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Table 7. Details for X-ray Data Collection and Structure Refinement for Compound 5e

	5e
empirical formula	$C_{12}H_{18}ClO_6PS$
formula mass	356.74
T[K]	123(2)
crystal size [mm]	0.25 × 0.15 × 0.10
crystal description	colorless block
crystal system	monoclinic
space group	$P21/n$
a [Å]	5.30830(10)
b [Å]	17.8886(4)
c [Å]	17.1694(4)
α [°]	90.0
β [°]	97.308(2)
γ [°]	90.0
V [Å^3]	1617.13(6)
Z	4
$\rho_{calcd.}$ [g cm^{-3}]	1.465
μ [mm^{-1}]	0.486
F(000)	744
Θ range [°]	2.39−25.24
index ranges	$-7 \le h \le 7$
	$-25 \le k \le 25$
	$-24 \le l \le 24$
reflns. collected	32826
reflns. obsd.	3935
reflns. unique	4954 (R_{int} = 0.0408)
R_1, wR_2 (2σ data)	0.0356, 0.0789
R_1, wR_2 (all data)	0.0520, 0.0863
GOOF on F^2	1.041
peak/hole [e Å^{-3}]	0.424/−0.360

7. BIOACTIVITY EXPERIMENTAL

7.1. Cell Culturing and Evaluation of In Vitro Cytostasis on Tumor Cell Lines. Cytostatic effect of the compounds was studied on tumor cell cultures in vitro. MDA-MB-231 human breast adenocarcinoma[77] cells were cultured in DMEM medium supplemented with 10% FBS, 2 mM L-glutamine, penicillin-streptomycin antibiotics mixture (50 IU/mL and 50 μg/mL, respectively), 1 mM sodium pyruvate and 1% nonessential amino acid mixture. A2058[78] human melanoma cells were cultured in RPMI medium supplemented with 10% FBS, 2 mM L-glutamine, and penicillin-streptomycin antibiotics mixture (50 IU/mL and 50 μg/mL, respectively). The cultures were maintained at 37 °C in a humidified atmosphere with 5% CO_2. The cells were grown to confluence, and then they were divided into 96-well tissue culture plates with the initial cell number of 5.0 × 10^3 cells/well. After 24 h incubation at 37 °C, the cells were treated with the compounds in 200 μL final volume containing 1.0 v/v% DMSO at 10−250 μM (daunomycin: 0.016−10 μM, tamoxifen: 0.16−100 μM) concentration overnight. Control cells were treated with serum-free medium only or with DMSO (c = 1.0 v/v %) at the same conditions. After this incubation period, cells were washed twice with serum-free medium and following that, they were cultured for another 72 h in 10% serum-containing medium at 37 °C. After that, cell viability was determined by Alamar Blue assay. Alamar Blue is a nontoxic, resazurin-based dye that is reduced by living cells to a fluorescent molecule, resorufin.[79] Resazurin sodium salt (Merck, Darmstadt, Germany) was dissolved in phosphate-buffered saline at c = 0.15 mg/mL, pH 7.4. 32.5 μL of the dye was added to each well and incubated at 37 °C for 3 h until the pink color of the reduced dye appeared. Fluorescence intensity in each well was measured using a Synergy H4 multimode microplate reader (BioTek, Winooski, VT); at λ_{ex} = 530/30 and λ_{em} = 610/10 nm. Cytostatic effect was calculated with the following equation:

$$\text{Cytostatic effect (\%)} = [1 - (\text{Fluorescence intensity}_{\text{treated}} / \text{Fluorescence intensity}_{\text{control}})] \times 100$$

Cytostasis values were expressed in the percentage of untreated control. 50% inhibitory concentration (IC_{50}) was determined by fitting a sigmoid curve on the data points using Microcal Origin2021 software and the calculating X values at Y = 50 and expressed in micromolar units.

■ ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article and its Supporting Information.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.4c04382.

^{31}P, ^{13}C, and ^{1}H NMR spectra, the geometrical data for compound **5e**, as well as data of the theoretical calculations (PDF)

https://doi.org/10.1021/acsomega.4c04382
ACS Omega 2024, 9, 31043−31055

AUTHOR INFORMATION

Corresponding Authors

Rita Oláhné Szabó − *Hungarian Research Network (HUN-REN), HUN-REN-ELTE Research Group of Peptide Chemistry, Eötvös Loránd University, 1117 Budapest, Hungary; Department of Genetics, Cell- and Immunobiology, Semmelweis University, 1089 Budapest, Hungary;* Email: rita.szabo@ttk.elte.hu

György Keglevich − *Department of Organic Chemistry and Technology, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, 1111 Budapest, Hungary;* orcid.org/0000-0002-5366-472X; Email: keglevich.gyorgy@vbk.bme.hu

Authors

Zsuzsanna Szalai − *Department of Organic Chemistry and Technology, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, 1111 Budapest, Hungary*

Márton Debrei − *Department of Organic Chemistry and Technology, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, 1111 Budapest, Hungary*

Péter Ábrányi-Balogh − *Department of Organic Chemistry and Technology, Faculty of Chemical Technology and Biotechnology, Budapest University of Technology and Economics, 1111 Budapest, Hungary; Medicinal Chemistry Research Group and National Drug Research and Development Laboratory, HUN-REN Research Centre for Natural Sciences, 1117 Budapest, Hungary;* orcid.org/0000-0002-9284-5160

Szilvia Bősze − *Hungarian Research Network (HUN-REN), HUN-REN-ELTE Research Group of Peptide Chemistry, Eötvös Loránd University, 1117 Budapest, Hungary*

Konstantin Karaghiosoff − *Department Chemie, Ludwig-Maximilians-Universität München, D-81377 München, Germany;* orcid.org/0000-0002-8855-730X

László Drahos − *MS Proteomics Research Group, Research Centre for Natural Sciences, 1117 Budapest, Hungary;* orcid.org/0000-0001-9589-6652

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.4c04382

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This project was supported by the National Research, Development and Innovation Office (K134318) and the Doctoral Excellence Fellowship Programme (DCEP) is funded by the National Research Development and Innovation Fund of the Ministry of Culture and Innovation and the Budapest University of Technology and Economics, under a grant agreement with the National Research, Development and Innovation Office. The publication, as well as the scientific results presented in its context, were made with the support of the Gedeon Richter Talentum Foundation established by Gedeon Richter Plc. (1103 Budapest, Gyömrői út 19-21.) with the support of the Gedeon Richter Excellence PhD Scholarship. The work of P.Á.-B. was supported by the János Bolyai Research Scholarship of the Hungarian Academy of Sciences and the ÚNKP-22-5 and ÚNKP-23-5 New National Excellence Program of the Ministry for Innovation and Technology.

REFERENCES

(1) Rádai, Z.; Keglevich, G. Synthesis and reactions of α-hydroxyphosphonates. *Molecules* **2018**, *23*, 1493.

(2) Azizi, N.; Saidi, M. R. Lithium perchlorate diethyl ether solution: A highly efficient media for the Abramov reaction. *Phosphorus Sulfur Silicon Relat. Elem.* **2003**, *178*, 1255−1259.

(3) Wang, C.; Zhou, J.; Lv, X.; Wen, J.; He, H. Solvent-free synthesis of tertiary α-hydroxyphosphates by the triethylamine-catalyzed hydrophosphonylation of ketones. *Phosphorus Sulfur Silicon Relat. Elem.* **2013**, *188*, 1334−1339.

(4) Tajbakhsh, M.; Khaksar, S.; Tafazoli, Z.; Bekhradnia, A. $MgCl_2$/Et_3N Base system as a new catalyst for the synthesis of α-hydroxyphosphonate. *Chin. J. Chem.* **2012**, *30*, 827−829.

(5) Nandre, K. P.; Nandre, J. P.; Patil, V. S.; Bhosale, S. V. Barium hydroxide catalyzed greener protocol for the highly efficient and rapid synthesis of α-hydroxyphosphonates under solvent free conditions. *Chem. Biol. Interface* **2012**, *2*, 314−321.

(6) Pandi, M.; Chanani, P. K.; Govindasamy, S. An efficient synthesis of α-hydroxy phosphonates and 2-nitroalkanols using $Ba(OH)_2$ as catalyst. *Appl. Catal., A* **2012**, *441*−*442*, 119−123.

(7) Kulkarni, M. A.; Lad, U. P.; Desai, U. V.; Mitragotri, S. D.; Wadgaonkar, P. P. Mechanistic approach for expeditious and solvent-free synthesis of α-hydroxy phosphonates using potassium phosphate as catalyst. *C. R. Chim.* **2013**, *16*, 148−152.

(8) Kaboudin, B.; Nazari, R. The synthesis of α-hydroxyphosphonates mediated by microwave irradiation under solvent-free conditions. *J. Chem. Res.* **2002**, *6*, 291−292.

(9) Kong, D.-L.; Liu, R.-D.; Li, G.-Z.; Zhang, P.-W.; Wu, M.-S. A rapid, convenient, solventless green approach for the synthesis of α-hydroxyphosphonates by grinding. *Asian J. Chem.* **2014**, *26*, 1246−1248.

(10) Ramananarivo, H. R.; Solhy, A.; Sebti, J.; Smahi, A.; Zahouily, M.; Clark, J.; Sebti, S. An eco-friendly paradigm for the synthesis of α-hydroxyphosphonates using sodium-modified fluorapatite under solventless conditions. *ACS Sustain. Chem. Eng.* **2013**, *1*, 403−409.

(11) Keglevich, G.; Rádai, Z.; Kiss, N. Z. To date the greenest method for the preparation of α-hydroxyphosphonates from substituted benzaldehydes and dialkyl phosphites. *Green Process. Synth.* **2017**, *6*, 197−201.

(12) Gancarz, R.; Gancarz, I.; Walkowiak, U. On the reversibility of hydroxyphosphonate formation in the Kabachnik-Fields reaction. *Phosphorus Sulfur Silicon Relat. Elem.* **1995**, *104*, 45−52.

(13) Wang, W.; Zhou, Y.; Peng, H.; He, H. W.; Lu, X. T. Synthesis and herbicidal activity of α-[(substituted phenoxybutyryloxy or valeryoxy)]alkylphosphonates and 2-(substituted phenoxybutyryloxy)alkyl-5,5-dimethyl-1,3,2-dioxaphosphinan-2-one containing fluorine. *J. Fluorine Chem.* **2017**, *193*, 8−16.

(14) Chen, X. B.; Shi, D. Q. Synthesis and biological activity of novel phosphonate derivatives containing of pyridyl and 1,2,3-triazole rings. *Phosphorus Sulfur Silicon Relat. Elem.* **2008**, *183*, 1134−1144.

(15) Wang, W.; Wang, L. P.; Ning, B. K.; Mao, M. Z.; Xue, C.; Wang, H. Y. Synthesis and insecticidal activities of *O*,*O*-dialkyl-2-[3-bromo-1-(3-chloropyridin-2-yl)-1*H*-pyrazole-5-carbonyloxy] (aryl) methylphosphonates. *Phosphorus Sulfur Silicon Relat. Elem.* **2016**, *191*, 1362−1367.

(16) Varga, P. R.; Belovics, A.; Bagi, P.; Tóth, S.; Szakács, G.; Bősze, S.; Szabó, R.; Drahos, L.; Keglevich, G. Efficient synthesis of acylated, dialkyl α-hydroxy-benzylphosphonates and their anticancer activity. *Molecules* **2022**, *27*, 2067.

(17) Wang, T.; He, H. W. An efficient synthesis of α-(2,4-dichlorophenoxyacetoxy)aryl methyl phosphonate monosodium salts. *Synth. Commun.* **2004**, *34*, 1415−1423.

(18) He, H. W.; Yuan, J. L.; Peng, H.; Chen, T.; Shen, P.; Wan, S. Q.; Li, Y.; Tan, H. L.; He, Y. H.; He, J. B.; Li, Y. Studies of *O*,*O*-dimethyl α-(2,4-dichlorophenoxyacetoxy)-ethylphosphonate (HW02) as a new herbicide. 1. Synthesis and herbicidal activity of HW02 and

https://doi.org/10.1021/acsomega.4c04382
ACS Omega 2024, 9, 31043−31055

analogues as novel inhibitors of pyruvate dehydrogenase complex. *J. Agric. Food Chem.* **2011**, *59*, 4801−4813.
(19) Wang, W.; He, H. W.; Zuo, N.; He, H. F.; Peng, H.; Tan, X. S. Synthesis and herbicidal activity of 2-(substituted phenoxyacetoxy)-alkyl-5,5-dimethyl-1,3,2-dioxaphosphinan-2-one. *J. Agric. Food Chem.* **2012**, *60*, 7581−7587.
(20) Long, Q.; Deng, X.; Gao, Y.; Xie, H.; Peng, H.; He, H. *Phosphorus Sulfur Silicon Relat. Elem.* **2013**, *188*, 819−825.
(21) Sharghi, H.; Jokar, M.; Doroodmand, M. M. Iron-doped single-walled carbon nanotubes as new heterogeneous and highly efficient catalyst for acylation of alcohols, phenols, carboxylic acids and amines under solvent-free conditions. *Adv. Synth. Catal.* **2011**, *353*, 426−442.
(22) Firouzabadi, H.; Iranpoor, N.; Sobhani, S.; Amoozgar, Z. Copper triflate as a useful catalyst for the high-yielding preparation of α-acetyloxyphosphonates under solvent-free conditions. *Synthesis* **2004**, *2004*, 295−297.
(23) Firouzabadi, H.; Iranpoor, N.; Farahi, S.; Farahi, S. Solid trichlorotitanium(IV) trifluoromethanesulfonate $TiCl_3(OTf)$ catalyzed efficient acylation of − OH and − SH: Direct esterification of alcohols with carboxylic acids and transesterification of alcohols with esters under neat conditions. *J. Mol. Catal. A: Chem.* **2008**, *289*, 61−68.
(24) Firouzabadi, H.; Iranpoor, N.; Sobhani, S.; Amoozgar, Z. Facile and high-yielding preparation of α-hydroxyphosphonates assisted by microwave irradiation. *Synthesis* **2004**, *2004*, 1771−1774.
(25) Rostami, A.; Atashkar, B.; Moradi, D. Synthesis, characterization and catalytic properties of magnetic nanoparticle supported guanidine in base catalyzed synthesis of α-hydroxyphosphonates and α-acetoxyphosphonates. *Appl. Catal., A* **2013**, *467*, 7−16.
(26) Yang, J.; Ma, J.; Che, W.; Li, M.; Li, G.; Song, B. Microwave-assisted synthesis and antitumor activity of salicyl acyloxy phosphonate derivatives. *Chin. J. Org. Chem.* **2014**, *34*, 2566−2571.
(27) Jin, C.; He, H. Synthesis and herbicidal activity of novel dialkoxyphosphoryl aryl methyl 2-(4,6-dimethoxypyrimidin-2-yloxy) benzoate derivatives. *Phosphorus Sulfur Silicon Relat. Elem.* **2011**, *186*, 1397−1403.
(28) Iranpoor, N.; Firouzabadi, H.; Khalili, D. The first Mitsunobu protocol for efficient synthesis of α-acyloxyphosphonates using 4,4′-azopyridine. *Phosphorus Sulfur Silicon Relat. Elem.* **2011**, *186*, 2166−2171.
(29) Kong, D. L.; Li, G. Z.; Liu, R. D. Synthesis and crystal structure of diethyl tosyloxybenzylphosphonate. *Asian J. Chem.* **2014**, *26*, 2138−2140.
(30) Rádai, Z.; Windt, T.; Nagy, V.; Füredi, A.; Kiss, N. Z.; Randelovic, I.; Tóvári, J.; Keglevich, G.; Szakács, G.; Tóth, S. Synthesis and anticancer cytotoxicity with structural context of an α-hydroxyphosphonate based compound library derived from substituted benzaldehydes. *New J. Chem.* **2019**, *43*, 14028−14035.
(31) Rádai, Z.; Hodula, V.; Kiss, N. Z.; Kóti, J.; Keglevich, G. Phosphorylation of (1-aryl-1-hydroxymethyl)phosphonates. *Mendeleev Commun.* **2019**, *29*, 153−154.
(32) Kiss, N. Z.; Kaszás, A.; Drahos, L.; Mucsi, Z.; Keglevich, G. A neighbouring group effect leading to enhanced nucleophilic substitution of amines at the hindered α-carbon atom of an α-hydroxyphosphonate. *Tetrahedron Lett.* **2013**, *57*, 207−209.
(33) Kiss, N. Z.; Rádai, Z.; Mucsi, Z.; Keglevich, G. Synthesis of α-aminophosphonates from α-hydroxyphosphonates; A theoretical study. *Heteroatom Chem.* **2016**, *27*, 260−268.
(34) Rádai, Z.; Szabó, R.; Szigetvári, Á.; Kiss, N. Z.; Mucsi, Z.; Keglevich, G. A study on the rearrangement of dialkyl 1-aryl-1-hydroxymethylphosphonates to benzyl phosphates. *Curr. Org. Chem.* **2020**, *24*, 465−471.
(35) Kaim, L. E.; Gaultier, L.; Grimaud, L.; Dos Santos, A. Formation of new phosphates from aldehydes by a DBU-catalysed Phospha-Brook rearrangement in a polar solvent. *Synlett* **2005**, *15*, 2335−2336.
(36) Rowe, B. J.; Spilling, C. D. The synthesis of 1-hydroxy phosphonates of high enantiomeric excess using sequential asymmetric reactions: titanium alkoxide-catalyzed P−C bond formation and kinetic resulotion. *Tetrahedron: Asymmetry* **2001**, *12*, 1701−1708.
(37) Fleisch, H. Bisphosphonates: Mechanisms of Action. *Endocr. Rev.* **1998**, *19*, 80−100.
(38) Zheng, X.; Nair, V. Synthesis of isomeric nucleoside phosphonates: Cyclic analogs of the anti-HIV active compound. *PMEA. Tetrahedron* **1999**, *55*, 11803−11818.
(39) Lee, M. V.; Fong, E. M.; Singer, F. R.; Guenette, R. S. Bisphosphonate treatment inhibits the growth of prostate cancer cells. *Cancer Res.* **2001**, *61*, 2602−2608.
(40) Kafarski, P.; Lejczak, B. Application of bacteria and fungi as biocatalysts for the preparation of optically active hydroxyphosphonates. *J. Mol. Catal. B: Enzym.* **2004**, *29*, 99−104.
(41) Fields, S. C. Synthesis of natural products containing a C−P bond. *Tetrahedron* **1999**, *55*, 12237−12273.
(42) Wang, X.; Cai, Y.; Chen, J.; Verpoort, F. A simple protocol for the synthesis of α-substituted phosphonates. *Phosphorus Sulfur Silicon Relat. Elem.* **2016**, *191*, 1268−1273.
(43) Thiem, J.; Günther, M.; Paulsen, H.; Kopf, J. Ringerweiterung von Furanose-Ringen zu 1,2λ5-Oxaphosphorinanen. *Chem. Ber.* **1977**, *110*, 3190−3200.
(44) Thiery, E.; Reniers, J.; Wouters, J.; Vincent, S. P. Stereoselective synthesis of boat-locked glycosides designed as glycosyl hydrolase conformational probes. *Eur. J. Org. Chem.* **2015**, *2015*, 1472−1484.
(45) *DIAMOND, version 3.2i; Crystal Impact GbR*: Bonn, Germany, 2014.
(46) Harsági, N.; Kiss, N. Z.; Keglevich, G. Synthesis of cyclic phosphinates by microwave-assisted ionic-liquid-promoted alcoholysis. *Synthesis* **2022**, *54*, 3899−3905.
(47) Harsági, N.; Szőllősi, B.; Kiss, N. Z.; Keglevich, G. MW irradiation and ionic liquids as green tools in hydrolyses and alcoholyses. *Green Proc. Synth.* **2021**, *10*, 1−10.
(48) Gangireddy, P.; Patro, V.; Lam, L.; Morimoto, M.; Liebeskind, L. S. Mechanism of acylative oxidation−reduction−condensation reactions using benzoisothiazolones as oxidant and triethylphsophite as stoichiometric reductant. *J. Org. Chem.* **2017**, *82*, 3513−3529.
(49) Hasník, Z.; Pohl, R.; Hocek, M. Synthesis of (purin-6-yl)methylphosphonate bases and nucleosides. *Tetrahedron Lett.* **2010**, *51*, 2464−2466.
(50) Rather, S. A.; Bhat, M. Y.; Hussain, F.; Ahmed, Q. N. Sulfonyl-promoted Michaelis−Arbuzov-type reaction: An approach to S/Se−P bonds. *J. Org. Chem.* **2021**, *86*, 13644−13663.
(51) Plabst, M.; Stock, N.; Bein, T. Hydrogen-bonded networks of ammonium bis- and tetrakisphosphonates—synthesis, high-throughput screening and structural trends. *Cryst. Growth Des.* **2009**, *9*, 5049−5060.
(52) Teulade, M.-P.; Savignac, P.; Aboujaoude, E. E.; Liétge, S.; Collignon, N. Alkylidènediphosphonates et vinylphosphonates: Une démarche synthétiques sélective par voie carbanionique. *J. Organomet. Chem.* **1986**, *304*, 283−300.
(53) Abramov, V. S. Reaction of dialkyl phosphites with aldehydes and ketones. A new method of preparation of esters of hydroxyalkanephosphonic acids. *Zh. Obshch. Khim.* **1952**, *22*, 647−652.
(54) Hudson, H. R.; Yusuf, R. O.; Matthews, R. W. The preparation of dimethyl α-hydroxyphosphonates and the chemical shift non-equivalence of their diastereotopic methyl ester groups. *Phosphorus Sulfur Silicon Relat. Elem.* **2008**, *183*, 1527−1540.
(55) Seven, O.; Polat-Cakir, S.; Hossain, M. S.; Emrullahoglu, M.; Demir, A. S. Reactions of acyl phosphonates with organoaluminum reagents: A new method for the synthesis of secondary and tertiary α-hydroxy phosphonates. *Tetrahedron Lett.* **2011**, *67*, 3464−3469.
(56) Fang, H.; Chen, W.; Hong, B.; Zhao, Y.; Fang, M. Synthesis, characterizations, and crystal structures of α-hydroxyphosphonic acid esters. *Phosphorus Sulfur Silicon Relat. Elem.* **2010**, *185*, 2182−2193.
(57) Keglevich, G.; Tóth, V. R.; Drahos, L. Microwave-assisted synthesis of α-hydroxy-benzylphosphonates and -benzylphosphine oxides. *Heteroatom Chem.* **2011**, *22*, 15−17.

https://doi.org/10.1021/acsomega.4c04382
ACS Omega 2024, 9, 31043−31055

(58) Kabachnik, M. I.; Medved, T. Y. New synthesis of aminophosphonic acids. *Dokl. Akad. Nauk SSSR* **1952**, *83*, 689−692.
(59) Angelini, T.; Bonollo, S.; Lanari, D.; Pizzo, F.; Vaccaro, L. E-Factor minimized hydrophosphonylation of aldehydes catalyzed by polystyryl-BEMP under solvent-free conditions. *Org. Biomol. Chem.* **2013**, *11*, 5042−5046.
(60) Sardarian, A. R.; Kaboudin, B. Surface-mediated solid phase reactions: Preparation of diethyl 1-hydroxyarylmethylphosphonates on the surface of magnesia. *Synth. Commun.* **1997**, *27*, 543−551.
(61) Rajeshwaran, G. G.; Nandakumar, M.; Sureshbabu, R.; Mohanakrishnan, A. K. Lewis acid-mediated Michaelis−Arbuzov reaction at room temperature: A facile preparation of arylmethyl/heteroarylmethyl phosphonates. *Org. Lett.* **2011**, *13*, 1270−1273.
(62) Miller, C. D.; Miller, R. C.; Rogers, W., Jr. Phosphine oxides. V. Intra- and intermolecular association. *J. Am. Chem. Soc.* **1958**, *80*, 1562−1565.
(63) Creary, X.; Geiger, C. C.; Hilton, K. Mesylate derivatives of α-hydroxy phosphonates. Formation of carbocations adjacent to the diethyl phosphonate group. *J. Am. Chem. Soc.* **1983**, *105*, 2851−2858.
(64) Crenshaw, M. D.; Zimmer, H. Synthesis with α-heterosubstituted phosphonates. Part 13. Synthesis of trisubstituted vinyl chlorides. *J. Org. Chem.* **1983**, *48*, 2782−2784.
(65) Iorga, B.; Eymery, F.; Savignac, P. Controlled monohalogenation of phosphonates: A new route to pure α-monohalogenated diethyl benzylphosphonates. *Tetrahedron Lett.* **1999**, *55*, 2671−2686.
(66) Li, X.; Chen, T.; Saga, Y.; Han, L.-B. Iron-catalyzed cross coupling of P−H/C−O bonds: efficient synthesis of α-alkoxyphosphorus compounds. *Dalton Trans.* **2016**, *45*, 1877−1880.
(67) Kim, D. Y.; Oh, D. Y. A facile synthesis of diethyl 1-alkoxy-1-arylmethanephosphonates. *Synth. Commun.* **1986**, *16*, 859−863.
(68) Burkhouse, D.; Zimmer, H. Novel synthesis of 1-alkoxy-1-arylmethanephosphonic acid esters. *Synthesis* **1984**, *4*, 330−332.
(69) Prasad, S. S.; Singh, D. K.; Kim, I. One-pot, three-component approach to diarylmethylphosphonates: A direct entry to polycyclic aromatic systems. *J. Org. Chem.* **2019**, *84*, 6323−6336.
(70) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, G. A. et al. *Gaussian 09, Revision D.01*; Gaussian, Inc.: Wallingford, CT, USA, 2009.
(71) Zhao, Y.; Truhlar, D. G. The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: Two new functionals and systematic testing of four M06-class functionals and 12 other functionals. *Theor. Chem. Acc.* **2008**, *120*, 215−241.
(72) Petersson, G. A.; Bennett, A.; Tensfeldt, T. G.; Al-Laham, M. A.; Shirley, W. A. A complete basis set model chemistry. I. The total energies of closed-shell atoms and hydrides of the first-row elements. *J. Chem. Phys.* **1988**, *89*, 2193−2218.
(73) *CrysAlisPro 1.171.40.82a; Program package*; Rigaku OD: Tokyo, Japan, 2020.
(74) Sheldrick, G. M. *SHELXS-97: Program for Crystal Structure Solution*; University of Göttingen: Germany, 1997.
(75) Sheldrick, G. M. *SHELXL-97: Program for the Refinement of Crystal Structures*; University of Göttingen: Germany, 1997.
(76) Spek, A. L. *PLATON: A Multipurpose Crystallographic Tool*; Utrecht University: Utrecht, The Netherlands, 1999.
(77) Cailleau, R.; Olivé, M.; Cruciger, Q. V. Long-term human breast carcinoma cell lines of metastatic origin: preliminary characterization. *In Vitro.* **1978**, *14*, 911−915.
(78) Fabricant, R. N.; De Larco, J. E.; Todaro, G. J. Nerve growth factor receptors on melanoma cells in culture. *Proc. Natl. Acad. Sci. U. S. A.* **1977**, *74*, 565−569.
(79) O'Brien, J.; Wilson, I.; Orton, T.; Pognan, F. Investigation of the Alamar Blue (resazurin) fluorescent dye for the assessment of mammalian cell cytotoxicity. *Eur. J. Biochem.* **2000**, *267*, 5421−5426.

https://doi.org/10.1021/acsomega.4c04382
ACS Omega 2024, 9, 31043−31055