

Full Paper

One Pot Synthesis of α -Aminophosphonates Containing Bromo and 3,4,5-Trimethoxybenzyl Groups under Solvent-free Conditions

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Abstract: New α -aminophosphonates were synthesized by the Kabachnik-Fields reaction of 3,4,5-trimethoxybenzaldehyde (TMB) with *p*- or *m*-bromoaniline and a dialkyl phosphite under solvent-free conditions. TMB was prepared from gallic acid via a four step synthetic sequence involving etherification, esterification, hydrazidation and potassium ferricyanide oxidation. The structures of all synthesized compounds were confirmed by elemental analysis, IR, ^1H -, ^{13}C - and ^{31}P -NMR spectral data. Compound **7g** was also characterized by X-ray crystallography. A half-leaf method was used to determine the *in vivo* curative efficacy of the eight title products against tobacco mosaic virus (TMV). It was found that compounds **7g** and **7h** possess good *in vivo* curative effects against TMV.

Keywords: α -Aminophosphonates; bromo group; 3,4,5-trimethoxybenzyl group; Kabachnik-Fields reaction; solvent-free conditions; crystallography; biological activity.

Introduction

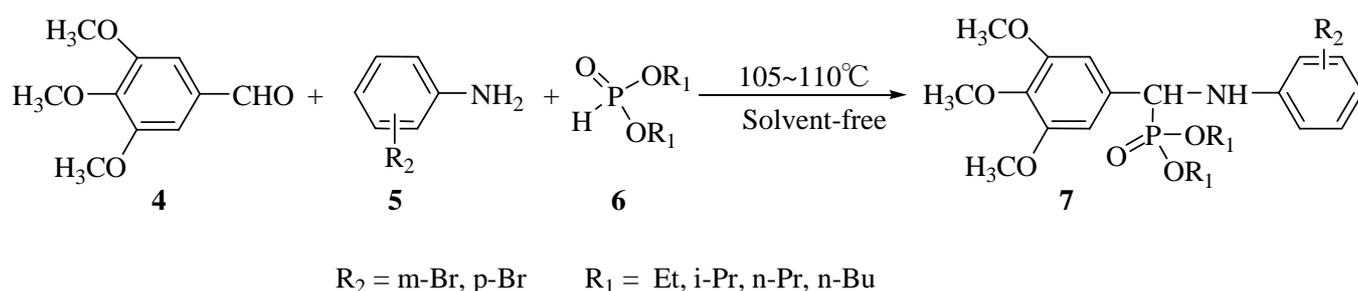
α -Aminophosphonates, structural analogues of natural amino acids, have received wide attention in medicinal, bioorganic and organic chemistry. The applications of α -aminophosphonates have ranged from agriculture to medical uses as anti-cancer agents [1], enzyme inhibitors [2], peptide mimetics [3], antibiotics and pharmacological agents [4]. To the best of our knowledge, only a few α -aminophosphonates containing bromo and 3,4,5-trimethoxybenzyl groups have been reported. As typical of a halogen, bromine has high electronegativity and is also known for its steric and lipophilic effects. As an active group, bromine is often introduced in the design of bioactive compounds. Many pesticides containing bromine are widely used commercially, and have a broad spectrum of activity, high efficiency and low toxicity, associated with their easy decomposition and minimal residues [5].

Gallic acid derivatives are compounds of significant biological and pharmaceutical interest [6-8]. Among them, 3,4,5-trimethoxybenzaldehyde (TMB) is an important pharmaceutical intermediate, which is employed, among other examples, in the synthesis of trimethoprim, tetroquinol and podophyllotoxin [9].

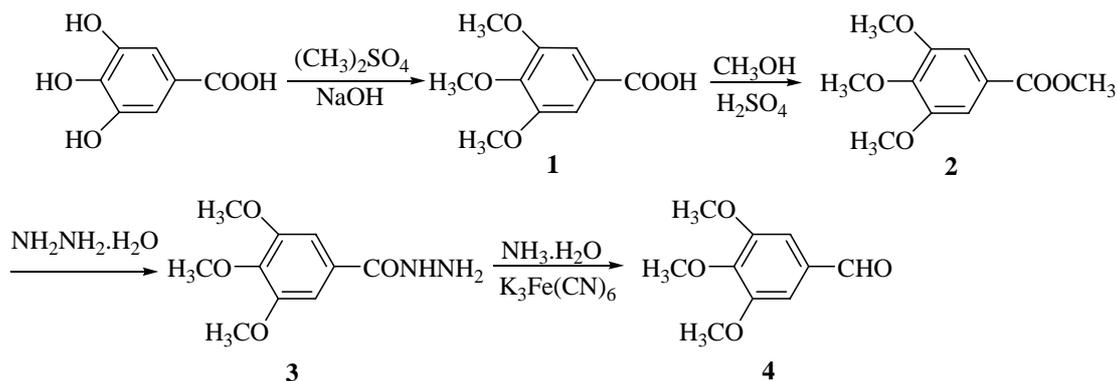
In view of all this information, a series of α -aminophosphonates containing bromo and 3,4,5-trimethoxybenzyl groups were designed and synthesized in our laboratory for the purpose of exploring them as potential bioactive compounds. Substituted α -aminophosphonates are often synthesized in an organic solvent via a traditional Kabachnik-Fields reaction [10], but the use of organic solvents has a serious impact on the environment, whereas in contrast, solvent free reactions are benign and do not call for any drastic work-ups.

Herein we report an easy one pot synthesis of new α -aminophosphonates **7a-7g** by Kabachnik-Fields reaction of equimolar mixtures of 3,4,5-trimethoxybenzaldehyde (TMB) and *p*- or *m*-bromoaniline with dialkyl phosphite under solvent-free conditions (Scheme 1).

Scheme 1. Synthesis of compounds **7a-7h** by the Kabachnik-Fields reaction..



The TMB starting material **4** was prepared in turn in high yield from gallic acid (abundant in plant gallnut) in four steps: etherification, esterification, hydrazidation and potassium ferricyanide oxidation (Scheme 2).

Scheme 2. Synthesis of the starting material **4** from gallic acid.

Results and Discussion

A systematic study of the effect of reaction parameters on the process, including reaction temperature, the molar ratios of reagents and the amount of catalyst, was undertaken for optimization of the reaction. For this purpose, compound **7g** was synthesized under different conditions.

First, the effect of reaction temperature was investigated. When the reaction temperature was increased from 85~90 °C to 95~100 °C and then to 105~110°C, the yields obtained were 37.5 %, 61.2 % and 65.6 %, respectively (Table 1, entries 1-3). When the temperature was further increased to 115~120°C, no improvement was noticed (56.3 %, Table 1, entry 4), compared to the results obtained at 105~110°C (entry 3). Next, the effect of molar ratio of the reagents was investigated at the 105~110°C temperature. As the molar ratio of the reagents (dialkyl phosphate/amine/aldehyde) was varied from 1:1:1, 1.2:1:1 to 2:1:1, no significant changes were observed in the yields (65.6%, 61.8 %, 60.3%, respectively Table 1, entries 3, 5 and 6).

The yield of the product was found to be significantly lower under otherwise similar conditions when no catalyst was used (Table 2, entry 2). The yield was improved as the amount of catalyst was increased from 5 mol% to 10 mol% (Table 2, entries 3, 4), but no noteworthy change was observed when the amount of catalyst used was increased to 15 mol% (Table 2, entry 5). Based on these results, the optimal conditions for the synthesis was established as the use of equivalent molar concentrations of the reactants and 10 mol% $\text{BF}_3 \cdot \text{Et}_2\text{O}$ with a reaction temperature of 105~110 °C and a reaction time of 30 min.

Table 1. The influence of the molar ratio of reagents and the reaction temperature on the synthesis of **7a**.

Entry	Dialkyl phosphate: amine: aldehyde (molar ratio)	Reaction temperature (°C)	Reaction time (h)	Yield (%)
1	1:1:1	85~90	2	37.5
2	1:1:1	95~100	2	61.2
3	1:1:1	105~110	2	65.6
4	1:1:1	115~120	2	56.3
5	1.2:1:1	105~110	2	61.8
6	2:1:1	105~110	2	60.3

Table 2. The influence of the amount of catalyst $\text{BF}_3 \cdot \text{Et}_2\text{O}$ on the synthesis of **7a**.

Entry	Dialkyl phosphate: amine: aldehyde (molar ratio)	Catalyst	Amount of catalyst (mol %)	Reaction time (min.)	Yield (%)
1	1:1:1	-	-	120	65.6
2	1:1:1	-	-	30	41.0
3	1:1:1	$\text{BF}_3 \cdot \text{Et}_2\text{O}$	5	30	66.6
4	1:1:1	$\text{BF}_3 \cdot \text{Et}_2\text{O}$	10	30	76.0
5	1:1:1	$\text{BF}_3 \cdot \text{Et}_2\text{O}$	15	30	71.7

As may be seen from Table 3, using optimal conditions and with 10 mol% $\text{BF}_3 \cdot \text{Et}_2\text{O}$ catalyst, the compounds **7a-7h** could be obtained in high yields (70.6-83.5%) after a much shorter reaction time (30 min). The compounds **7a-7h** were obtained in 61.3-74.2% yield when the reaction was conducted for longer periods of time (2 h), but without the use of a catalyst.

Table 3. Yields of **7a-7h**.

Compounds	Substitutents		With Catalyst		Without Catalyst	
	R ₁	R ₂	Reaction time (min.)	Yield (%)	Reaction time (h)	Yield (%)
7a	Et	<i>p</i> -Br	30	76.0	2	65.6
7b	Et	<i>m</i> -Br	30	75.9	2	61.5
7c	<i>i</i> -Pr	<i>p</i> -Br	30	82.0	2	67.8
7d	<i>i</i> -Pr	<i>m</i> -Br	30	81.0	2	71.0
7e	<i>n</i> -Bu	<i>p</i> -Br	30	76.1	2	62.5
7f	<i>n</i> -Bu	<i>m</i> -Br	30	70.6	2	61.4
7g	<i>n</i> -Pr	<i>p</i> -Br	30	83.5	2	74.2
7h	<i>n</i> -Pr	<i>m</i> -Br	30	75.8	2	61.3

The structures of compounds **7a-7h** were identified by elemental analysis, IR, ^1H -, ^{13}C - and ^{31}P -NMR spectral data. Taking compound **7g** as an example, the absorption band at 3400 cm^{-1} (s) in its IR spectrum corresponds to an N-H stretch, the absorption bands at 1240 cm^{-1} (s) and 1001 cm^{-1} (s) correspond to P=O and P-O-C stretching, respectively. The ^1H -NMR spectra of **7g** showed well-resolved doublets at δ 4.64 ($J_{\text{P-H}} = 24.05\text{ Hz}$) for the P(O)CH proton, while a singlet at δ 3.04 was due to the presence of the NH proton. In the ^{31}P -NMR spectra the phosphonate group resonance appeared at $\delta_{\text{p}} 22.69$. The structure of compound **7g** was definitively confirmed by X-ray diffraction analysis.

Crystal Structure Analysis of **7g**.

The crystal belongs to the tetragonal system with space group P 2(1)/c, $a = 12.7429(14)\text{ nm}$, $b = 14.1740(16)\text{ nm}$, $c = 4.2643(15)\text{ nm}$, $\alpha = 90.00^\circ$, $\beta = 107.360(6)^\circ$, $\gamma = 90.00^\circ$, $V = 2459.0(5)\text{ nm}^3$, $Z =$

4, $D_c = 1.395 \text{ mg/m}^3$, $\mu = 1.77 \text{ mm}^{-1}$, $F(000) = 1072.0$. The molecular structure and cell packing of the compound are presented in Figures 1 and 2, respectively. It can be seen from Figure 1 that the two phenyl rings are planar, with a C(4)-C(5)-C(7)-N(1) torsion angle of about 56.58° . As shown in Figure 2, there is one N-H \cdots O hydrogen bond intermolecular interaction. The N(1)-O(4) bond distance is 2.960 nm and N(1)-H(1) \cdots O(4) bond angle is 165.94° (Table 4). In the solid state, the hydrogen bonds form a two-dimensional network to stabilize the crystal structure.

Figure 1. Molecular structure of compound **7g**.

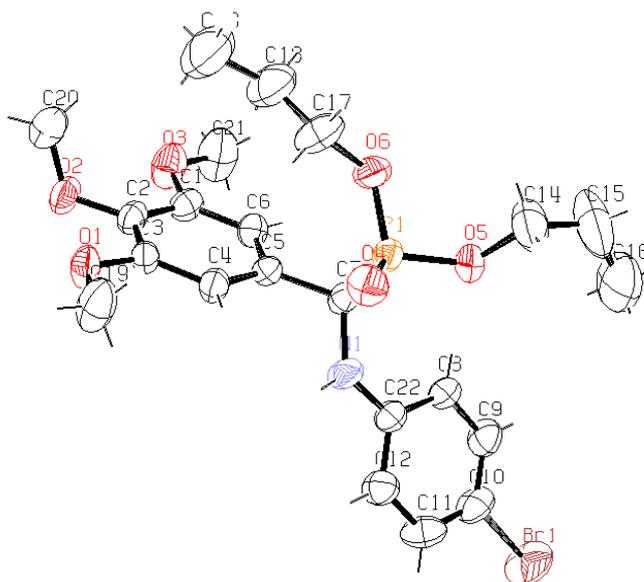


Figure 2. Cell packing of the compound **7g**.

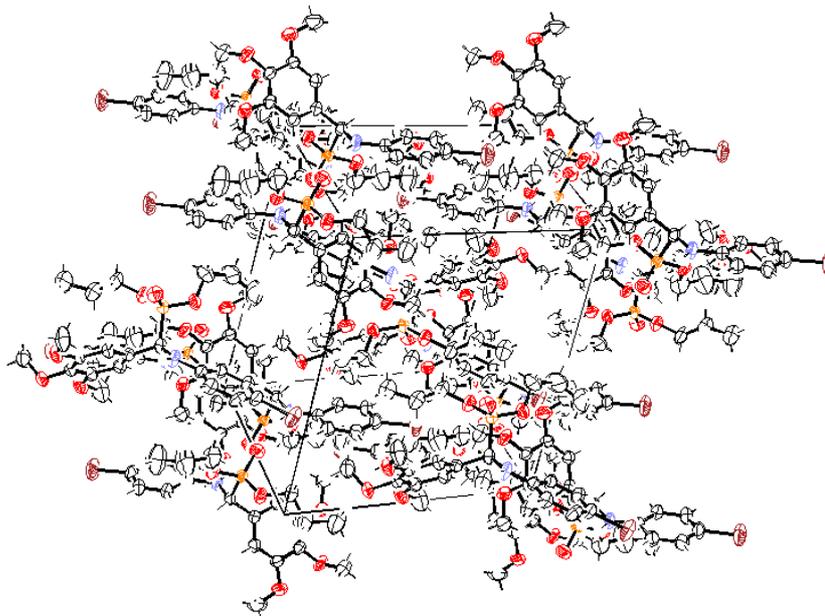


Table 4. Hydrogen bonds for compound **7g**.

D-H \cdots A	$d(\text{D-H})/\text{nm}$	$d(\text{H-A})/\text{nm}$	$d(\text{D-A})/\text{nm}$	$\angle\text{DHA}(\text{^\circ})$
N(1)-H(1) \cdots O(4)	0.860	2.118	2.960	165.94

*Symmetry transformations used to generate equivalent atoms: #1: $-x+1, -y, -z+2$

Antiviral activity bioassays

The results of the *in vivo* activity against TMV bioassays are given in Table 5. Ningnanmycin was used as reference antiviral agent. The data indicate that a change in the substituent might also affect the curative activity of title compounds **7a-7h**. Compound **7h** ($R_1 = n\text{-Pr}$, $R_2 = 3\text{-Br}$) and compound **7g** ($R_1 = n\text{-Pr}$, $R_2 = 4\text{-Br}$) could cure TMV up to 54.5% and 44.3% at 500 $\mu\text{g/mL}$. The other compounds all have relatively lower curative activity than **7h** and **7g**.

Table 5. The curative effect of title compounds **7a-h** at 500 $\mu\text{g/mL}$ against TMV.

Compd.	7a	7b	7c	7d	7e	7f	7g	7h	Ningnamycin
Inhibition rate (%)	35.0	23.8	28.0	26.2	22.5	11.7	44.3	54.5	57.5

Conclusions

A series of α -aminophosphonates **7a-h** containing bromo and 3,4,5-trimethoxybenzyl groups were synthesized by Kabachnik-Fields reaction under solvent-free condition. The procedure offers a great alternative to existing methodologies due to its ease of work up, faster reaction rates and high yields. The method is clean, free of any significant byproducts, environmental friendly and does not employ any solvent. A half-leaf method was used to determine the curative efficacy *in vivo* of the eight title products against tobacco mosaic virus (TMV). It was found that compound **7h** had a good curative effect *in vivo* against TMV, with an inhibition rate of 54.5%.

Acknowledgements

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Experimental

General

Melting points (uncorrected) were measured on a XT-4 binocular microscope (Beijing Tech Instrument Co., China). The IR spectra (KBr disks) were recorded on a Bruker VECTOR 22 spectrometer. ^1H - and ^{13}C -NMR spectra were determined at room temperature in the indicated solvents on a JEOL-ECX 500 NMR spectrometer (500 MHz for ^1H and 125 MHz for ^{13}C), with TMS as internal standard. ^{31}P -NMR spectra were measured using as external reference an 85% H_3PO_4 sample prepared by sealing a capillary containing 85% H_3PO_4 in a 5 mm NMR tube containing a suitable amount of CDCl_3 for field locking. Elemental analysis was performed on an Elementar Vario-III CHN analyzer. The reagents were all of analytical reagent grade or chemically pure. All solvents were dried, deoxygenated and redistilled before use. TMB was prepared from gallic acid according to literature

methods [11-13]. *O,O*-Dialkyl phosphites were prepared as described in the literature [14].

General procedure for the preparation of products 7a-h

A mixture of 3,4,5-trimethoxybenzaldehyde (3 mmol), *p*- (or *m*-) bromoaniline (3 mmol) and dialkyl phosphite (3 mmol) was stirred in silicone oil bath at 108 °C for 2 h. The reaction was followed and monitored by TLC (petroleum ether-ethyl acetate=1:2 v/v). The resultant viscous liquid was dissolved in ether and then washed, first with saturated aqueous NaHCO₃ and next with distilled water. The organic layer was separated, followed by extraction of the aqueous layer with ethyl acetate (3×10 mL). The combined organic layers were dried over MgSO₄ and concentrated under reduced pressure. The residue was purified by recrystallization from petroleum ether to give compounds **7a-7h** as white crystals. At the same time, compounds **7a-7h** were also synthesized using BF₃·Et₂O as catalyst by a similar procedure.

Yields and physicochemical properties:

O,O'-diethyl- α -(4-bromophenylamino)-3,4,5-trimethoxybenzylphosphonate (**7a**). M.p. 123-124 °C; IR: 3398.6, 1591.3, 1242.2, 1055.1, 1029.9 cm⁻¹; ¹H-NMR (CD₃OCD₃) δ : 6.82-7.18 (m, 6H, Ar-H), 4.65 (d, *J*= 24.0 Hz, 1H, CH-P), 3.95-4.15 (m, 4H, 2OCH₂), 3.69-3.79 (m, 9H, 3OCH₃), 3.06 (br s, 1H, NH), 1.12-1.27 (m, 6H, 2CH₃); ¹³C-NMR (CD₃OCD₃) δ : 16.75, 55.30, 56.42, 56.52, 60.49, 63.59, 106.71, 109.51, 116.53, 132.34, 132.75, 138.63, 147.60, 154.23; ³¹P-NMR δ : 22.78; Anal. Calcd. for C₂₀H₂₇BrNO₆P(488.31): C, 49.19; H, 5.57; N, 2.87. Found C, 49.28; H, 5.54; N, 3.09.

O,O'-diethyl- α -(3-bromophenylamino)-3,4,5-trimethoxybenzylphosphonate (**7b**). M.p. 113-114 °C; IR: 3305.9, 1591.3, 1228.7, 1047.4, 1022.3 cm⁻¹; ¹H-NMR (CD₃OCD₃) δ : 6.96-7.08 (m, 6H, Ar-H), 4.66 (d, *J*=24.6Hz, 1H, CH-P), 3.93-4.16 (m, 4H, m, 2OCH₂), 3.69-3.79 (m, 9H, 3OCH₃), 3.07 (br s, 1H, NH), 1.13-1.27 (m, 6H, 2CH₃); ¹³C-NMR (CD₃OCD₃) δ : 16.77, 55.04, 56.26, 56.44, 60.52, 63.65, 106.77, 113.30, 117.28, 120.91, 123.34, 131.36, 132.74, 138.71, 149.88, 154.23; ³¹P-NMR δ : 22.65; Anal. Calcd. for C₂₀H₂₇BrNO₆P(488.31): C, 49.19; H, 5.57; N, 2.87. Found C, 49.24; H, 5.40; N, 3.00.

O,O'-di-*i*-propyl- α -(4-bromophenylamino)-3,4,5-trimethoxybenzylphosphonate (**7c**). M.p. 134-135 °C; IR: 3284.8, 1591.3, 1228.7, 1001.1 cm⁻¹; ¹H-NMR (CDCl₃) δ : 6.47-7.21 (m, 6H, Ar-H), 4.68-4.50 (m, 3H, CH-P+2OCH), 3.82-3.83 (m, 9H, m, 3OCH₃), 3.08 (br s, 1H, NH), 1.00-1.23 (m, 12H, 4CH₃); ¹³C-NMR (CDCl₃) δ : 23.47, 24.35, 56.24, 56.39, 57.60, 60.95, 72.31, 104.94, 110.29, 115.50, 131.46, 132.00, 137.74, 145.83, 153.36; ³¹P-NMR δ : 20.99; Anal. Calcd. for C₂₂H₃₁BrNO₆P (516.36): C, 51.17; H, 6.05; N, 2.71. Found C, 51.38; H, 6.14; N, 2.95.

O,O'-di-*i*-propyl- α -(3-bromophenylamino)-3,4,5-trimethoxybenzylphosphonate (**7d**). M.p. 123-124°C; IR: 3278.9, 1591.3, 1228.7, 1008.8 cm⁻¹; ¹H-NMR (CDCl₃) δ : 6.52-6.98 (m, 6H, Ar-H), 4.48-4.58 (m, 3H, CH-P+2OCH), 3.82-3.84 (m, 9H, m, 3OCH₃), 3.08 (br s, 1H, NH), 0.98-1.22 (m, 12H, 4CH₃); ¹³C-NMR (CDCl₃) δ : 23.95, 24.35, 56.10, 56.25, 57.31, 60.96, 72.33, 106.77, 112.44, 116.65, 121.32, 123.16, 130.56, 131.40, 137.76, 148.01, 154.23; ³¹P-NMR δ : 20.88; Anal. Calcd. for C₂₂H₃₁BrNO₆P (516.36): C, 51.17; H, 6.05; N, 2.71. Found C, 51.31; H, 5.98; N, 2.81.

O,O'-di-*n*-butyl- α -(4-bromophenylamino)-3,4,5-trimethoxybenzylphosphonate (**7e**). M.p. 87-88°C; IR: 3278.9, 1591.3, 1226.7, 1018.4 cm⁻¹; ¹H-NMR (CDCl₃) δ : 6.48-7.21 (m, 6H, Ar-H), 4.62 (d, J = 23.50 Hz, 1H, CH-P), 3.94-4.05 (m, 4H, 2OCH₂), 3.84-3.90 (m, 9H, 3OCH₃), 3.08 (br s, 1H, NH), 1.24-1.61 (m, 8H, 4CH₂), 0.84-0.88 (m, 6H, 2CH₃); ¹³C-NMR (CDCl₃) δ : 13.63, 18.74, 32.60, 55.85, 56.21, 57.06, 60.91, 67.07, 104.71, 110.38, 115.52, 131.21, 132.02, 137.76, 145.61, 153.50; ³¹P-NMR δ : 22.75; Anal. Calcd. for C₂₄H₃₅BrNO₆P (544.42): C, 52.95; H, 6.48; N, 2.57. Found C, 53.06; H, 6.42; N, 2.73.

O,O'-di-*n*-butyl- α -(3-bromophenylamino)-3,4,5-trimethoxybenzylphosphonate (**7f**). M.p. 80-81°C; IR: 3304.1, 1591.3, 1238.3, 1026.1 cm⁻¹; ¹H-NMR (CDCl₃) δ : 6.50-7.21 (m, 6H, Ar-H), 4.63 (d, J = 23.50 Hz, 1H, CH-P), 3.95-4.05 (m, 4H, 2OCH₂), 3.84-3.91 (m, 9H, 3OCH₃), 3.04 (br s, 1H, NH), 1.25-1.61 (m, 8H, 4CH₂), 0.84-0.90 (m, 6H, 2CH₃); ¹³C-NMR (CDCl₃) δ : 13.56, 18.68, 32.40, 55.52, 56.16, 56.73, 60.84, 66.99, 104.75, 112.39, 116.56, 121.34, 123.09, 130.49, 131.11, 137.73, 147.84, 53.44; ³¹P-NMR δ : 22.65; Anal. Calcd. for C₂₄H₃₅BrNO₆P (544.42): C, 52.95; H, 6.48; N, 2.57. Found C, 52.97; H, 6.44; N, 2.67.

O,O'-di-*n*-propyl- α -(4-bromophenylamino)-3,4,5-trimethoxybenzylphosphonate (**7g**). M.p. 111-112°C; IR: 3400.5, 1591.3, 1240.2, 1001.1 cm⁻¹; ¹H-NMR (CDCl₃) δ : 6.50-7.21 (m, 6H, Ar-H), 4.64 (d, J = 24.05 Hz, 1H, CH-P), 3.93-4.01 (m, 4H, 2OCH₂), 3.81-3.83 (m, 9H, 3OCH₃), 3.04 (br s, 1H, NH), 1.51-1.67 (m, 4H, 2CH₂), 0.82-0.92 (m, 6H, 2CH₃); ¹³C-NMR (CDCl₃) δ : 9.97, 23.96, 55.85, 56.23, 57.06, 60.91, 68.82, 104.73, 110.38, 115.52, 131.20, 132.01, 137.77, 145.61, 153.49; ³¹P-NMR δ : 22.69; Anal. Calcd. for C₂₂H₃₁BrNO₆P (516.36): C, 51.17; H, 6.05; N, 2.71. Found C, 51.25; H, 5.79; N, 2.76%.

O,O'-di-*n*-propyl- α -(3-bromophenylamino)-3,4,5-trimethoxybenzylphosphonate (**7h**). M.p. 85-86°C; IR: 3305.9, 1591.3, 1234.4, 1002.9 cm⁻¹; ¹H-NMR (CDCl₃) δ : 6.51-6.97 (m, 6H, Ar-H), 4.66 (d, J = 23.45 Hz, 1H, CH-P), 3.92-4.02 (m, 4H, 2OCH₂), 3.84-3.86 (m, 9H, 3OCH₃), 3.07 (br s, 1H, NH), 1.50-1.67 (m, 4H, 2CH₂), 0.84-0.92 (m, 6H, 2CH₃); ¹³C-NMR (CDCl₃) δ : 9.98, 23.97, 55.58, 56.24, 56.79, 60.91, 68.81, 104.86, 112.43, 116.67, 121.37, 123.15, 130.56, 131.81, 147.96, 153.49; ³¹P-NMR δ : 22.59; Anal. Calcd. for C₂₂H₃₁BrNO₆P (516.36): C, 51.17; H, 6.05; N, 2.71. Found C, 51.26; H, 5.95; N, 2.66.

X-Ray diffraction experiment

The X-ray diffraction data of compound **7g** were collected at 293(2)K on a Rigaku Raxis-IV diffractometer with graphite-monochromated MoK α (λ = 0.71073 Å) radiation using an ω scan mode in the 1.67° ≤ θ ≤ 24.99° range. A total of 8805 reflections were collected and 3737 were independent (R_{int} = 0.0288), of which 2577 with $I > 2\sigma(I)$ were observed. The crystal structure was solved by the direct method and refined by full-matrix least squares with the SHELXS-97 program [15]. The non-hydrogen atoms were refined on F^2 anisotropically, and the hydrogen atoms were determined with theoretical calculation. The least-square cycle gave $R = 0.0461$, $wR = 0.1317$ ($w = 1 / [\sigma^2(F_o^2) + (0.0884 P)^2 + 1.2039 P]$, where $P = (F_o^2 + 2 F_c^2) / 3$), $S = 0.970$, $(\Delta/\sigma)_{\text{max}} = 0.005$, $(\Delta\rho)_{\text{max}} = 0.83$ and $(\Delta\rho)_{\text{min}} = -0.59 \text{ e}/\text{Å}^3$. Atomic scattering factors and anomalous dispersion corrections were taken from

International Table for X-ray Crystallography [16]. Crystallographic data (excluding structure factors) for the structure have been deposited with the Cambridge Crystallographic Data Center as supplementary publication No. CCDC-634425. These data can be obtained free of charge from the CCDC via www.ccdc.cam.ac.uk/data_request/cif.

Antiviral Bioassay

Nicotiana tabacum. L leaves of the same age were selected. Whole leaves were dipped in a solution containing tobacco mosaic virus (6×10^{-3} mg/mL), then the leaves were washed with water and dried. The test compound solution was smeared on the left side and the solvent was smeared on the right side for control. The local lesion numbers were then recorded 3-4 days after inoculation [17]. For each compound, three repetitions were carried out to ensure the reliability of the results. Inhibition rates were calculated using the expression:

$$\text{Inhibition rate (\%)} = \frac{\text{av local lesion numbers of control (not treated with compound)} - \text{av local lesion numbers smeared with drugs}}{\text{av local lesion numbers without drugs}}$$

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Sample Availability: Samples of the compounds are available from authors.

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