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Full Paper

Synthesis and Antiviral Bioactivities of *α*-Aminophosphonates Containing Alkoxyethyl Moieties

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Abstract: A simple, efficient, and general method has been developed for the synthesis of various *α*-aminophosphonate derivatives **4a-4l** by treatment of substituted benzaldehydes and aniline with bis(2-methoxyethyl)- or bis(2-ethoxyethyl) phosphite under microwave irradiation without solvents and catalysts. The products were characterized by elemental analysis, IR, ^{1}H-NMR, ^{13}C- and ^{31}P-NMR spectra. The X-ray crystallographic data of the representative compound **4l** was determined. The new *α*-aminophosphonate derivatives were found to possess moderate to good antiviral activity.

Keywords: Aminophosphonates, microwave irradiation, synthesis, antiviral activity

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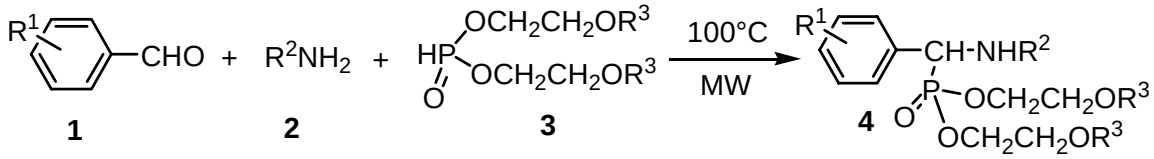
Introduction

α-Aminophosphonate derivatives have attracted much attention in medicinal and pesticide chemistry over the past two decades due to their biological activities. Some of them have been used as potent enzyme inhibitors [1], antimicrobial [2], antitumor [3] and antiviral agents [4]. In our search for new potential plant antiviral agents, we have reported the design and synthesis of a series of *α*-aminophosphonate compounds, among which some were found to possess moderate to good bioactivity [5,6]. Recently, attention has been focused on their potential as antiviral agents after the discovery of the activity of compounds containing the methoxyethyl moiety [7]. We now report the design and synthesis of a series of novel *α*-aminophosphonate compounds containing alkoxyethyl moieties and the investigation of their bioactivity. The synthetic route is shown in Scheme 1. The structures of compounds **4a-4l** were firmly established by IR, ^{1}H-, ^{13}C- and ^{31}P-NMR and elemental analysis. The X-ray crystallographic data of compound **4l** is also provided. Preliminary bioassay tests showed that some of these compounds displayed *in vivo* antiviral activity against Tobacco Mosaic Virus (TMV) at 500 μg/mL.

Scheme 1 Synthetic route to the title compounds.



1 **2** **3** **4**

R^1 = 3,4,5-trimethoxy, H, 2-F, 4-F, 2-CF_3, 3-CF_3;
R^2=4-$CF_3C_6H_4$-, 6-CH_3O-benzothiazol-2-yl-, 3-$NO_2C_6H_4$- ;
R^3 = Me, Et

Results and Discussion

In order to optimize the reaction conditions, the synthesis of **4a** was carried out under a variety of conditions. First, it was determined that microwave irradiation should be used, as the reactions without microwave activation were much slower and, for example, the product was obtained in a yield of only 64.9 %, after 4h of conventional heating as compared with the yield of 83.5% obtained after 10 min. under microwave irradiation (Table 1, entries 2, 11). In addition, we also examined the effects of reaction time on the reactions. When the reaction times were increased from 5 min. to 10 min., 15 min. and 20 min., **4a** was obtained in 78.9, 83.5, 82.7 and 81.8 % yield, respectively (Table 1, entries 1-4). When the reaction time was prolonged further to 25 min. (entry 5), no additional improvement was noted (82.8 % yield) as compared to that obtained after 10 min. (83.5 % yield, entry 2).The effect of reaction temperature was also studied. It could be seen that the yields were relatively lower when the reactions were carried out at 60 or 80 °C (Table 1, entries 6, 7) than at 100 °C (entry 2). A lower yield was also observed when the reaction system was heated to 120 °C, and no yield was observed when the reaction system was heated to 140 °C (Table 1, entries 9, 10). Consequently, it is better for the reaction to be carried out at 100 °C than at lower or higher temperatures.

Table 1. Different reaction conditions used for the microwave irradiation synthesis of **4a**.

Entry	Reaction time / min	Power (W)	Reaction temperature / °C	Yield [c] / %
1 [a]	5	50	100	78.9
2 [a]	10	50	100	83.5
3 [a]	15	50	100	82.7
4 [a]	20	50	100	81.8
5 [a]	25	50	100	82.8
6 [a]	10	50	60	5.0
7 [a]	10	50	80	51.2
8 [b]	10	-	100	0
9 [a]	10	50	120	65.7
10 [a]	10	50	140	0
11 [b]	240	-	100	64.9

[a] Reaction conditions: stirred under microwave irradiation in a Discovery™ LabMate instrument at 50W; [b] stirred with no microwave irradiation; [c] Yields of isolated products.

Using the optimized conditions, the best results were obtained when bis(2-methoxyethyl) phosphite or bis(2-ethoxyethyl) phosphite were treated with 1 equiv. of substituted benzaldehyde and 1 equiv. of substituted aniline under microwave conditions without solvent or catalyst at 100 °C for 10 min. Under these conditions, the Mannich reactions proceeded smoothly, and the results are summarized in Table 2.

Table 2. Yields of the title compounds **4** [a].

Compd.	**R^1-**	**R^2-**	**R^3-**	**Yields /% [b]**
4a	2-F	4-$CF_3C_6H_5$	CH_3	83.5
4b	H	4-$CF_3C_6H_5$	CH_3	81.3
4c	2-F	*6-methoxybenzothiazole-2-yl*	CH_3	76.5
4d	2-F	3-$NO_2C_6H_5$	CH_3	79.8
4e	3,4,5-trimethoxy	4-$CF_3C_6H_5$	CH_3	70.6
4f	4-F	4-$CF_3C_6H_5$	CH_3	73.6
4g	2-CF_3	4-$CF_3C_6H_5$	CH_3	56.8
4h	3-CF_3	4-$CF_3C_6H_5$	CH_3	79.2
4i	3,4,5-trimethoxy	4-$CF_3C_6H_5$	C_2H_5	78.6
4j	2-F	4-$CF_3C_6H_5$	C_2H_5	81.6
4k	H	4-$CF_3C_6H_5$	C_2H_5	82.8
4l	2-F	3-$NO_2C_6H_5$	C_2H_5	80.5

[a] Reaction conditions: stirred under microwave irradiation in a Discovery™ LabMate instrument at 50W; [b] Yields of isolated products.

The structures of title compounds **4a-4l** were established on the basis of their spectroscopic data. They showed IR absorption bands at 3200-3500 (NH) and 1520-1616 cm^{-1}(C=C) (aromatic ring skeletal vibration), while the absorption at 1220~1250 cm^{-1} was assigned to the P=O stretching absorption bands and that at 990~1100 cm^{-1} to the C-O stretching absorption bands in the P-O-C group. In the ^{1}H-NMR all phenyl protons showed multiplets at 6.19-7.78 ppm. The chemical shifts of the ester PCH were about 4.85-6.17 ppm, respectively.

Antifungal activity bioassay

The antifungal bioassay results are given in Table 3. It can be seen that the newly synthesized alkoxyethyl *α*-aminophosphonate derivatives **4a-4l** exhibit weak antifungal activities at 500 μg/mL towards *Fusarium oxysporum*, *Valsa mali* and *Gibberella zeae*, which were obviously lower than that of a hymexazol standard.

Antiviral activity bioassay

The results of the *in vivo* bioassay against Tobacco Mosaic Virus (TMV) are given in Table 4. Ningnanmycin was used as the reference antiviral agent. The results indicated that the antiviral activity depended on the substituents present. When R^1 is H, R^2 is a 4-trifluoromethyl group and R^3 is CH_3, (**4b**) the compound showed a 56.5 % curative rate against TMV at 500μg/mL, slightly higher than that of reference (53.8 %). The inhibitory rates of compounds **4i**, **4d**, **4e**, **4c** and **4g** at the same 500μg/mL concentration were 53.1 %, 51.6 %, 51.3 %, 49.2 % and 46.0%, respectively. These compounds all have an antiviral activity slightly lower than that of the reference compound. The other compounds **4a**, **4k**, **4f**, **4h**, **4j** and **4l** exhibited a weak anti-TMV bioactivity, with inhibitory rates of 38.8%, 34.3 %, 34.2%, 25.0 %, 16.2 % and 13.8 %, respectively, at 500μg/mL.

Table 3. Inhibitory effects on phytopathogenic fungi.

Compd (500 *μ*g/mL)	***Fusarium oxysporum***	***Gibberella zeae***	***Valsa mali***
4a	51.2	42.8	50.3
4b	36.7	30.2	33.5
4c	8.4	11.8	8.9
4d	12.4	17.9	15.5
4e	6.1	10.7	5.7
4f	2.1	6.9	3.2
4g	0	0	0
4h	12.1	23.1	11.0
4i	10.0	0	2.0
4j	23.1	9.0	30.9
4k	33.0	2.9	40.1
4l	20.0	1.0	43.4
Hymexazol	100	90.0	82.3

Table 4. The curative effects of the new compounds **4a-4l** against TMV *in vivo**.

Compound	**Ningnanmycin**	**4a**	**4b**	**4c**	**4d**	**4e**	**4f**
Inhibition rate (%)	53.8	38.8	56.5	49.2	51.6	51.3	34.2
Compound	**4g**	**4h**	**4i**	**4j**	**4k**	**4l**	
Inhibition rate (%)	46.0	25.0	53.1	16.2	34.3	13.8	

* Concentration: 500 mg/L.

Crystal Structure Analysis

As it can be seen from the X-ray single crystal analysis of **4l** (Figure 1), the dihedral angle between the C(8)-C(7)-N(2)-C(5) plane is -72.3(2)°. The N-H···O type of intermolecular interactions plays a major role in stabilizing the molecules in the unit cell. As shown in the packing diagram of this compound (Figure 2), there is a N(2)-H(2)···O(3), intermolecular hydrogen bond interaction in which N(2)-H(2) = 0.860 Å, H(2)..O(2) = 2.1866, N(2))···O(3) = 2.892(3) Å, N(2)-H(2)···O(3) = 139.81°, -x, -y+1/2, -z+1/2). In the solid state, the above hydrogen bonds connecting the molecules form hydrogen bond networks which stabilize the crystal structure.

Conclusions

A new method is presented for the formation under microwave irradiation conditions of *α*-aminophosphonates containing alkoxyethyl moieties. The method offers several advantages: an easy, rapid, one-pot reaction, environmental-friendliness and good yields. It was also found that the title compounds **4b, 4i, 4d** and **4e** displayed good antiviral activity.

Figure 1. The molecular structure of compound **4l**.

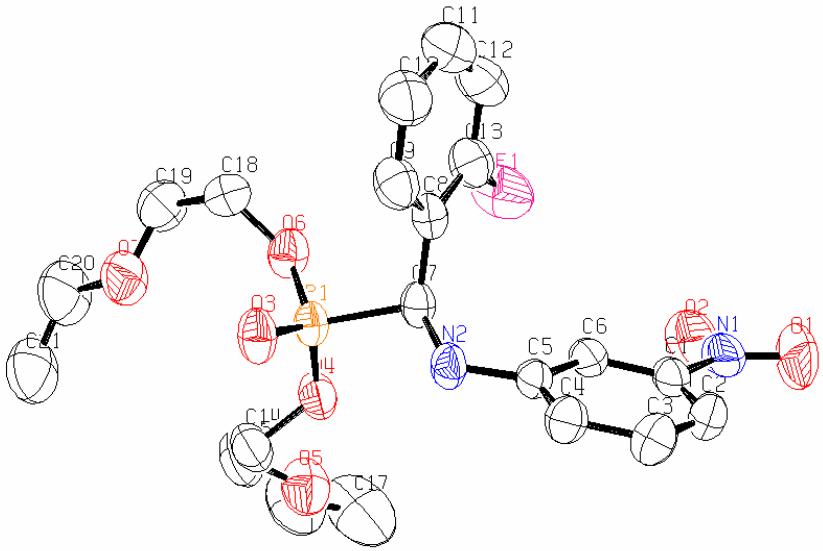
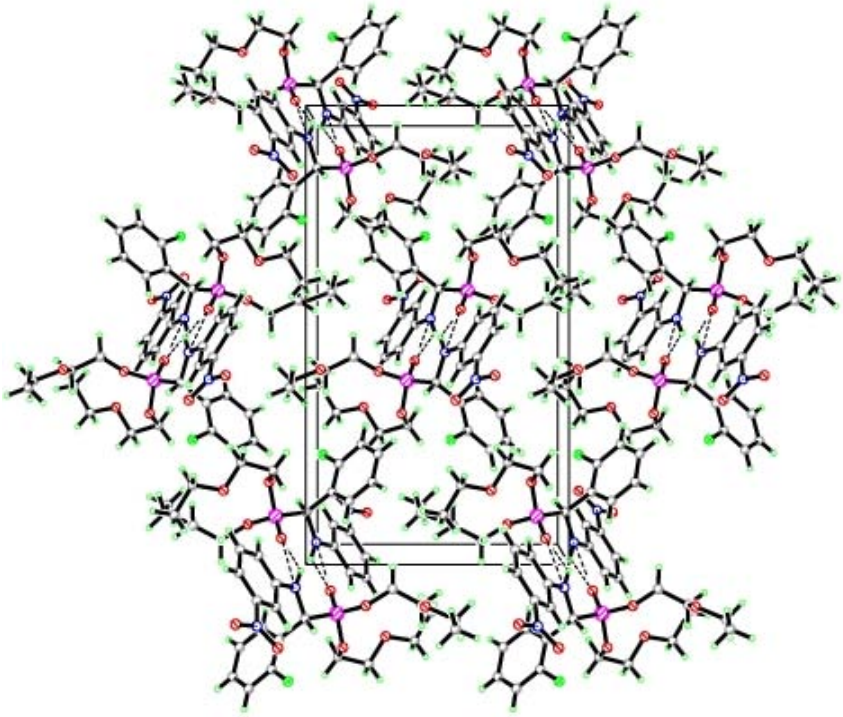


Figure 2. Packing diagram of the unit cell of compound **4l**.



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Experimental

General

The melting points of the products were determined on a XT-4 binocular microscope (Beijing Tech Instrument Co., P.R. China) and are uncorrected. IR spectra (KBr disks) were recorded on a Bruker Vector 22 spectrometer. ^{1}H- and ^{13}C-NMR spectra (solvent CD_3COCD_3) were recorded at room temperature on a JEOL-ECX 500 NMR spectrometer operating at 500 and 125 MHz, respectively, using TMS as internal standard. ^{31}P-NMR spectra were measured with 85% H_3PO_4 as external reference. The reference sample was prepared by sealing a capillary containing 85% H_3PO_4 in a 5 mm NMR tube inside which a suitable amount of $CDCl_3$ was added for field locking. Elemental analysis was performed on an Elementar Vario-III CHN analyzer. Microwave reactions were performed on a Discovery^{TM} LabMate Focused Microwave Synthesizer (50 W power). Analytical TLC was performed on silica gel GF254. All reagents were analytical reagent grade or chemically pure. Solvents were dried, deoxygenated and redistilled before use. Dialkyoxyethyl phosphites were synthesized according to the literature method [8].

Preparation of Alkyloxyethyl-containing α-Aminophosphonates **4a-4l**

Substituted benzaldehyde (5 mmol) and aniline (5 mmol) and di(2-methoxyethyl) phosphite or di(2-ethoxyethyl) phosphite were placed in a microwave tube, which was the sealed and placed in the Discovery ^{TM} synthesizer and irradiated at 100 °C and 50 W for 10 min. Completion of the reaction was checked by TLC. The reaction mixture was cooled and the crude product was recrystallized from 95% ethanol to give title compounds **4a**-**4l**.

N-(4-trifluoromethylphenyl)-α-amino-α-(2-fluorophenyl)-O,O-di(2-methoxyethyl)phosphonate (**4a**). White crystals; yield 83.5%; m.p. 67~68 °C; IR: 3273.2, 1537.3, 1238.3, 1064.7 cm^{-1}; ^{1}H-NMR *δ*: 3.07 (s,1H, N-H), 3.28 (s, 6H, $2CH_3O$), 3.44~4.27(m, 8H, $4CH_2$), 5.37 (d, *J*= 25.2 Hz, 1H, CH), 6.89~7.68(m, 8H, Ar-H); ^{13}C-NMR *δ*: 47.74, 49.01, 58.69, 58.72, 66.67, 66.73, 72.06, 75.15, 113.68, 115.90, 116.06, 125.41, 127.16, 127.20, 130.24, 150.74, 150.86, 160.73, 162.73; ^{31}P-NMR *δ*: 22.19, 22.21; *Anal*. Calcd for $C_{20}H_{24}F_4NO_5P$: C, 51.62; H, 5.20; N, 3.01. Found: C, 51.92; H, 5.17; N, 3.15.
N-(4-trifluoromethylphenyl)-α-amino-α-phenyl-O,O-di(2-methoxyethyl)phosphonate (**4b**). White crystals; yield 81.3%; m.p.71~73 °C; IR: 3309.9, 1539.2, 1230.6, 983.7 cm^{-1}; ^{1}H-NMR *δ*: 3.09 (s, 1H, N-H), 3.27 (s, 6H, $2CH_3O$), 3.43~4.21 (m, 8H, $4CH_2$), 5.14 (d, *J*= 25.2Hz, 1H, CH), 6.91~7.62 (m, 9H, Ar-H); ^{13}C-NMR *δ*: 54.87, 56.09, 58.69, 66.50, 66.55, 72.13, 72.17, 113.90, 118.89, 126.98, 127.01, 128.58, 129.16, 129.23, 137.09, 151.17, 151.27; ^{31}P NMR *δ*: 23.01; *Anal*. Calcd for $C_{20}H_{25}F_3NO_5P$: C, 53.69; H, 5.63; N, 3.13. Found: C, 53.40; H, 5.39; N, 3.30.

N-(6-methoxybenzothiazole-2-yl)-α-amino-α-(2-fluorophenyl)-O,O-di(2-methoxyethyl)phosphonate (**4c**). White crystals; yield 76.5%; m.p. 74~75 °C; IR: 3286.7, 1521.8, 1234.4, 1041.6 cm^{-1}; ^{1}H-NMR *δ*: 3.08 (s,1H, N-H), 3.26 (s, 6H, $2CH_3O$), 3.47~3.56 (m, 4H, $2CH_2$), 3.77 (s, 3H, CH_3O), 4.04~4.29 (m, 4H, $2CH_2$), 6.12 (d, *J*= 22.4 Hz, 1H, CH), 6.85~7.75 (m, 7H, Ar-H); ^{13}C-NMR *δ*: 48.46, 49.70, 56.00, 58.75, 66.77, 66.83, 72.04, 72.09, 105.98, 114.12, 115.93, 116.12, 120.28, 125.24, 130.34 , 130.71, 133.14, 147.03, 156.35, 160.34, 162.25, 164.40, 164.48; ^{31}P-NMR *δ*: 20.67, 20.69; *Anal*. Calcd for $C_{21}H_{26}FN_2O_6PS$: C, 52.06; H, 5.41; N, 5.78. Found: C, 52.09; H, 5.29; N, 5.99.

N-(3-nitrophenyl)-α-amino-α-(2-fluorophenyl)-O,O-di(2-methoxyethyl)phosphonate (**4d**). Yellow crystals; yield 79.8%; m.p. 108~109 °C; IR: 3211.5, 1544.1, 1222.9, 1055.0 cm^{-1}; ^{1}H-NMR *δ*: 3.07(s, 1H, N-H), 3.27 (s, 6H, $2CH_3O$), 3.45~4.31 (m, 8H, $4CH_2$), 5.42 (d, *J* = 25.2 Hz, 1H, CH), 7.17~7.73 (m, 8H, Ar-H); ^{13}C-NMR *δ*: 47.89, 49.17, 58.74, 58.77, 66.85, 66.88, 72.12, 72.18, 108.18, 112.95, 115.97, 116.15, 120.08, 125.50, 130.32, 130.83, 130.90, 148.91, 149.02, 150.16, 160.83, 162.78, 162.84; ^{31}P-NMR *δ*: 21.90, 21.92; *Anal*. Calcd for $C_{19}H_{24}FN_2O_7P$: C, 51.40; H, 5.81; N, 2.61. Found: C, 51.50; H, 5.54; N, 2.85.

N-(4-trifluorophenyl)-α-amino-α-(3, 4, 5-trimethoxyphenyl)-O,O-di(2-methoxyethyl)phosphonate (**4e**). White crystals; yield 70.6 %; m.p. 104~106 °C; IR: 3311.8, 1537.3, 1221.7, 1043.4 cm^{-1}; ^{1}H-NMR *δ*: 3.08 (s,1H, N-H), 3.28 (s, 6H, $2CH_3O$), 3.46~3.56 (m, 4H, $2CH_2$), 3.70 (s, 9H, $3CH_3O$), 3.96~4.22 (m, 4H, $2CH_2$), 5.09 (d, *J* = 24.6 Hz, 1H, CH), 6.95~7.39 (m, 6H, Ar-H); ^{13}C-NMR *δ*: 56.40, 58.72, 60.49, 66.53, 66.59, 72.22, 72.27, 106.61, 106.65, 113.98, 118.97, 119.22, 125.06, 127.01, 127.21, 132.46, 138.65, 151.31, 151.42, 154.26; ^{31}P-NMR *δ*: 22.89; *Anal*. Calcd for $C_{23}H_{31}F_3NO_8P$: C, 51.50; H, 5.54;

N, 2.61. Found: C, 51.50; H, 5.54; N, 2.85.

N-(4-trifluoromethylphenyl)-α-amino-α-(4-fluorophenyl)-O,O-di(2-methoxyethyl)phosphonate (**4f**). Colorless crystals*;* yield 73.6%; m.p. 45~47 °C. IR: 3298.3, 1606.7, 1238.3, 1064.7 cm^{-1}; ^{1}H NMR δ: 3.00 (s, 1H, N-H), 3.33 (s, 6H, $2CH_3O$), 3.48~3.56（m, 4H, $2CH_2$), 4.02~4.19（m, 4H, $2CH_2$), 4.87 (d, J = 32.0Hz, 1H, CH), 6.57~7.46 (m, 8H, Ar-H); ^{13}C-NMR δ: 54.41, 55.62, 58.78, 58.83, 66.01, 66.04, 71.39, 71.43, 112.97, 115.65, 115.81, 126.51, 126.54, 129.43, 129.47, 129.54, 148.94, 149.05, 161.55, 163.54; ^{31}P-NMR δ: 22.60, 22.63; *Anal*. Calcd for $C_{20}H_{24}F_4NO_5P$: C, 51.62; H, 5.20; N, 3.01. Found: C, 51.65; H, 5.26; N, 3.13.

N-(4-trifluoromethylphenyl)-α-amino-α-(2-trifluoromethylphenyl)-O,O-di (2-methoxyethyl)phosphonate (**4g**). White crystals; yield 56.8%; m.p. 47~48 °C; IR: 3319.5, 1616.3, 1323.2, 1060.9 cm^{-1}; ^{1}H-NMR δ: 3.06 (s, 1H, N-H), 3.27 (s, 6H, $2CH_3O$), 3.53~3.55 (m, 8H, $4CH_2$), 5.37 (d, J = 24.05 Hz, 1H, CH), 6.68~7.78 (m, 8H, Ar-H); ^{13}C-NMR δ: 50.72, 51.93, 58.83, 58.94, 66.23, 66.26, 113.00, 126.51, 126.56, 126.64, 126.67, 128.34, 128.98, 129.00, 132.51, 148.49, 148.60; ^{31}P-NMR δ: 22.06; *Anal*. Calcd for $C_{21}H_{24}F_6NO_5P$: C, 48.94; H, 4.69; N, 2.72. Found: C, 48.95; H, 4.80; N, 2.71.

N-(4-trifluoromethylphenyl)-α-amino-α-(3-trifluoromethylphenyl)-O,O-di(2-methoxyethyl) phosphonate (**4h**). White crystals; yield 79.2%; m.p. 46~48 °C. IR: 3296.4, 1614.4, 1327.0, 1064.7 cm^{-1}; ^{1}H-NMR δ: 3.09 (s, 1H, N-H), 3.34 (s, 6H, $2CH_3O$), 3.47~4.20 (m, 8H, $4CH_2$), 4.96 (d, J = 25.2Hz, 1H, CH), 6.58~7.75 (m, 8H, Ar-H); ^{13}C-NMR δ: 54.99, 56.21, 58.83, 58.89, 66.16, 66.21, 66.27, 71.42, 71.46, 112.99, 124.71, 125.06, 126.67, 126.70, 129.26, 129.28, 131.17, 131.21, 148.89, 14901; ^{31}P-NMR δ: 21.66; *Anal*. Calcd for $C_{21}H_{24}F_6NO_5P$: C, 48.94; H, 4.69; N, 2.72. Found: C, 48.96; H, 4.65; N, 2.82.

N-(4-trifluorophenyl)-α-amino-α-(3, 4, 5-trimethoxyphenyl)-O,O-di(2-ethoxyethyl)phosphonate (**4i**). White crystals; yield 78.6%; m.p.103~104 °C; IR: 3309.8, 1614.4, 1332.8, 1060.85 cm^{-1}; ^{1}H-NMR δ: 1.18~1.20 (m, 6H, $2CH_3$), 3.04 (s, 1H, NH), 3.46~3.52 (m, 8H, $4CH_2$), 3.82~3.52 (s, 9H, $3CH_3O$), 3.97~4.21 (4H, $2CH_2$), 4.78 (d, J = 31.4Hz, 1H, CH), 6.62~7.37 (m, 6H, Ar-H); ^{13}C-NMR δ: 56.23, 60.91, 66.63, 66.65, 69.48, 69.53, 104.76, 104.80, 113.06, 119.94, 120.20, 123.76, 125.90, 126.59, 126.62, 130.85, 137.79, 149.23, 149.35, 153.52; ^{31}P- NMR δ: 22.78; *Anal*. Calcd for $C_{25}H_{35}NO_8P$: C, 53.55; H, 5.27; N, 2.84. Found: C, 53.29; H, 6.11; N, 2.55.

N-(4-trifluoromethylphenyl)-α-amino-α-(2-fluorophenyl)-O,O-di(2-ethoxyethyl)phosphonate (**4j**). White crystals; yield 81.6%; m.p. 74~75 °C; IR: 3296.4, 1541.1, 1228.7, 1043.5 cm^{-1}; ^{1}H-NMR δ: 1.16~1.19 (m, 6H, $2CH_3O$), 3.07 (s, 1H, N-H), 3.47~4.20 (m, 12H, $6CH_2O$), 5.29 (d, 1H, J=25.2 Hz, CH), 6.63~7.35(m, 8H, Ar-H); ^{13}C-NMR δ: 47.46, 48.71 66.28, 66.57, 66.60, 77.04, 77.30, 112.75, 115.33, 115.50, 124.64, 126.57, 126.60, 128.81, 148.65, 148.76, 159.74,159.80, 161.71; ^{31}P-NMR δ: 22.06; *Anal*. Calcd for $C_{22}H_{28}F_4NO_5P$: C, 53.10; H, 6.24; N, 2.48. Found: C, 53.38; H, 6.12; N, 2.43.

N-(4-trifluoromethylphenyl)-α-amino-α-(phenyl)-O,O-di(2-ethoxyethyl)phosphonate (**4k**). White crystals; yield 82.8%; m.p. 59~60 °C; IR: 3298.3 , 1537.3, 1226.7, 1035.8 cm^{-1}; ^{1}H-NMR δ: 1.18~1.21 (m, 6H, $2CH_3O$), 3.07 (s, 1H, N-H), 3.46~4.18 (m, 12H, $6CH_2O$), 4.88 (d, 1H, J=25.2 Hz, CH),

6.60~7.34 (m, 8H, Ar-H); ^{13}C-NMR δ: 55.19, 56.39, 66.18, 66.59, 66.64, 76.84, 77.10, 77.36, 113.03, 126.55, 126.58, 127.87, 127.92, 128.22, 128.24, 128.78, 135.31, 149.13, 149.25; ^{31}P-NMR δ: 22.86; *Anal*. Calcd for $C_{22}H_{29}F_3NO_5P$: C, 55.58; H, 6.15; N, 2.95. Found: C, 55.71; H,5.96; N, 3.09.

N-(3-nitrophenyl)-α-amino-α-(2-fluorophenyl)-O,O-di(2-eethoxyethyl)phosphonate (**4l**). Yellow crystals; yield 80.5 %; m.p. 75~76 °C; IR: 3284.8, 1543.1, 1251.8, 1022.3 cm^{-1}; ^{1}H-NMR δ: 1.16~1.20 (m, 6H, 2CH_3O), 3.07 (s, 1H, N-H), 3.49~4.28 (m, 12H, 6CH_2O), 5.32 (d, 1H, *J*=25.2 Hz, CH), 7.10~7.51 (m, 8H, Ar-H); ^{13}C-NMR δ: 47.83, 49.06, 66.41, 66.53, 66.68, 76.87, 77.38, 108.10, 113.02, 115.49, 115.67, 118.88, 124.72, 128.90, 129.91, 129.96, 147.18, 147.29, 149.30, 159.87, 159.93, 161.90; ^{31}P-NMR δ: 21.73; *Anal*. Calcd for $C_{21}H_{28}FN_2O_7P$: C, 53.62; H, 6.00; N, 5.95. Found: C, 53.89; H, 5.86; N, 6.00.

Bioassays: Antifungal Bioassays

The antifungal activity of all synthesized compounds **4a-l** was tested against three pathogenic fungi, namely *Fusarium oxysporum*, *Gibberella zeae*, and *Valsa mali*, by the poison plate technique [9]. Test compounds were dissolved in acetone (10 mL) before mixing with Potato Dextrose Agar (PDA, 90 mL). The final concentration of compounds **4a-l** in the medium was fixed at 500 *μg*/mL. Three kinds of fungi were incubated in PDA at 25±1 °C for 5 days to get new mycelium for antifungal assay, then a mycelia disk of approximately 0.45 cm diameter cut from the culture medium was picked up with a sterilized inoculation needle and inoculated in the center of PDA plate. The inoculated plates were incubated at 25±1°C for 5 days. Acetone in sterilized distilled water served as control, while hymexazole was used as positive control For each treatment, three replicates were carried out. The radial growth of the fungal colonies was measured on the sixth day and the data were statistically analyzed. The *in vitro* inhibiting effects of the test compounds on the fungi were calculated by the formula CV = $\frac{A-B}{A}$, where A represents the diameter of fungi growth on untreated PDA, B represents the diameter of fungi on treated PDA, and CV represents the rate of inhibition.

Antiviral Bioassays

Growing leaves on *Nicotiana tabacum. L* of the same ages were selected. The tobacco mosaic virus (6×10^{-3} mg/mL) and inoculated on the whole leaves by dipping, then the leaves were washed with water and dried. The 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 [10]. For each compound, three repetitions were conducted to ensure the reliability of the results.

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

Crystal structure determination.

For the structure determination of a single crystal of **4l** X-ray intensity data were recorded on a Rigaku Raxis-IV diffraction meter using graphite monochromated MoKα radiation (λ = 0.71073 Å). In the range of 2.14 º ≤ θ ≤ 25.01º, 3641 independent reflections were obtained. Intensities were corrected for Lorentz and polarization effects and empirical absorption, and all data were corrected using SADABS [11] program. The structure was solved by direct methods SHELXS-97 program [12]. All the non-hydrogen atoms were refined on *F*2 anisotropically by full-matrix least squares method. The hydrogen atoms were located from the difference Fourier map, but their position were not refined. The contributions of these hydrogen atoms were included in structure-factor calculations. The final least-square cycle gave *wR*=0.1254, *R*=0.0511 for 5779 reflection with $I>2\sigma(I)$; the weighting scheme, $w = 1 / [\sigma^2(F_o^2) + (0.0603P)^2 + 0.7083P]$, where $P = [(F_o^2) + 2F_c^2] / 3$. The max and min difference peaks and holes are 0.337 and -0.348 e.A^{-3}, respectively. *s*=1.038. Atomic scattering factors and anomalous dispersion corrections were taken from International Table for X-ray Crystallography [13]. Crystallographic data (excluding structure factors) for the structure have been deposited with the Cambridge Crystallographic Data Center as supplementary publication No. CCDC-616465. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via the website www.ccdc.cam.ac.uk/data_request/cif.

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