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Synthesis and Antiviral Activities of Chiral Thiourea Derivatives Containing an α-Aminophosphonate Moiety

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Starting from benzaldehyde **1**, the title compounds **8** were synthesized in six steps. Benzaldehyde**1** was reacted with ammonium hydroxide, and the resulting imine was then treated with dialkyl phosphite **3** to give dialkyl *N*-(arylmethylene)-1-amino-1-aryl methylphosphonates **4**. Phosphonates **4** were then easily hydrolyzed to give dialkyl 1-amino-1-aryl-methylphosphonates **6**, which on treatment with triethylamine, carbon disulfide, and phosphorus oxychloride provided **7**. Target compounds **8** were then prepared by the reaction of **7** with substituted chiral amine. The structures were clearly verified by spectroscopic data (IR, ^{1}H, ^{13}C, and ^{31}P NMR, and elemental analysis). The bioassay of these compounds revealed them as antivirally active. It was found that title compounds **8g**, **8e**, **8k**, and **8m** had the same curative effects of TMV (inhibitory rate = 54.8, 50.5, 50.4, and 50.4%, respectively) as the commercial product Ningnanmycin (56.2%). This would appear to be the first report of the synthesis and antiviral activity of chiral thiourea derivatives containing an α-aminophosphonate moiety.

KEYWORDS: Chiral thiourea; α-aminophosphonate moiety; antiviral activity; synthesis

INTRODUCTION

Chiral thioureas and their derivatives are known for their wide range of functional and biological activities. Some chiral thiourea derivatives can serve not only as chiral catalysts for the synthesis of optically active compounds (*1*) but also as medicines such as anticancer and anti-HIV agents (*2, 3*). In this context, Venkatachalam and his group (*4*) held absolute configuration of the enantiomer as the key factor responsible for the antileukemic potency of halopyridyl and thiazolyl thiourea compounds. Preliminary screening indicated that the (*S*)-enantiomers were more effective in comparison with (*R*)-enantiomers in inhibiting tubulin polymerization and activating caspase-3. The role of one particular enantiomer of thiourea derivative on the anti-HIV activity was further established and confirmed by this group (*5*). Venkatachalam and his group (*6*) synthesized chiral naphthyl thiourea (CNT) compounds as non-nucleoside inhibitors (NNI) of the reverse transcriptase (RT) enzyme of HIV-1. The (*R*)-enantiomers of all 11 compounds inhibited the recombinant RT in vitro with lower IC_{50} values than their (*S*)-enantiomers. In addition, chiral thioureas, in recent years, have started to gain enormous importance due to their wide bioactivity and ability to serve as potential bifunctional organocatalysts in typical organic transformations (*7*−*9*). However, to date, most of the studies have been focused on anticancer and anti-HIV activity in medicinal formulation, whereas no publication concerning the antiviral activity of these compounds in pesticide formulation is encountered.

Some phosphonates in general (*10*) and some α-aminophosphonic acids and their esters in particular (*11*−*16*) have been found to exhibit a wide range of biological activities and are widely employed as fungicides, plant virucides, herbicides, and plant growth regulators. A large number of papers in the literature on their synthesis and biological activities have been reported during the past 10 years (*17*−*20*).

The plant disease caused by tobacco mosaic virus (TMV) is found worldwide. TMV is known to infect members of 9 plant families, and at least 125 individual species, including tobacco, tomato, pepper, cucumbers, and a number of ornamental flowers. The amount of loss can vary from 5 to 90% depending on the strain of TMV, the total time of infection by TMV, the temperature during disease development and the presence of other diseases. It is found that in certain fields 90−100% of the plants show mosaic or leaf necrosis by harvesting time. Studies have shown that TMV can change plant phenotypes by destruction of mitochondria followed by damage of plant quality. Ningnanmycin, a commercial antiviral agent, isolated from *Strepcomces noursei* var. *xichangensisn* by theChengdu Institute of Biology, Chinese Academy of Sciences, is a kind of microbial pesticide known to impart its action by destruction of the coat protein of TMV, thereby inducing plant host resistance. It is more effective in the treatment of plants against TMV than the

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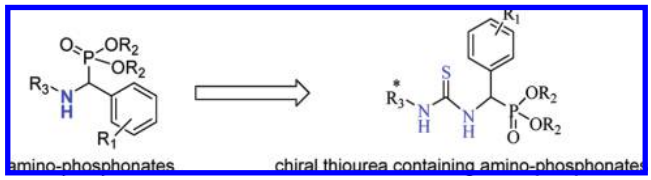


Figure 1. Design of the target compounds.

other existing commercial agents in the Chinese pharmaceutical market. However, the use of this agent for field trial is largely limited by its photosensitivity and water stickiness. Therefore, further research needs to be conducted in this area for the development of a highly efficient, novel, environmentally benign antiviral agent.

In our preliminary work, many substituted diaryl aminophosphonate derivatives containing amide or cyanoacrylate moieties were synthesized and were found to have good antiviral activities (*21*, *22*). To extend our research work to chiral thioureas as antiviral agent against TMV, we designed and synthesized some novel chiral thiourea derivatives **8** containing an α-aminophosphonate moiety (**Figure 1**). The synthetic route is shown in **Scheme 1**. Preliminary bioassay tests showed that some compounds possess a certain degree of antiviral activity against TMV at 500 mg/L in vivo as shown in **Tables 2** and **3**, however, with a degree of variation. The bioassay results showed that title compounds **8g**, **8e**, **8k**, and **8m** had similar curative effects of TMV (inhibitory rate = 54.8, 50.5, 50.4, and 50.4%, respectively) as commercial product Ningnanmycin (56.2%), and the EC_{50} values ranged from 227.0 to 413.9 μg/mL. To the best of our knowledge, this is the first report on the synthesis and antiviral activity of chiral thioureas containing an α-aminophosphonate moiety.

MATERIALS AND METHODS

Instruments. The melting points of the products were determined on an XT-4 binocular microscope (Beijing Tech Instrument Co., China) and were not corrected. The IR spectra were recorded on a Bruker VECTOR 22 spectrometer in a KBr disk. ^{1}H, ^{13}C, and ^{31}P NMR (solvent $CDCl_3$, D_3CCOCD_3, or DMSO-d_6) spectral analyses were performed on a JEOL-ECX 500 NMR spectrometer at room temperature using TMS as an internal standard. Elemental analysis was performed on an Elementar Vario-III CHN analyzer. Analytical TLC was performed on silica gel GF_{254}. Column chromatographic purification was carried out using silica gel. All reagents were of analytical reagent grade or chemically pure. All solvents were dried, deoxygenated, and redistilled before use.

Synthetic Procedures. Dialkyl phosphites **3** were prepared according to the literature method as described (*23*). Intermediates **2**, **4**, and **5** were prepared following standard synthetic protocols (*21*, *22*). Intermediates **6** were prepared according to the reported method (*24*). The synthetic methods and characterization data of intermediates **2**−**6** are provided in the Supporting Information.

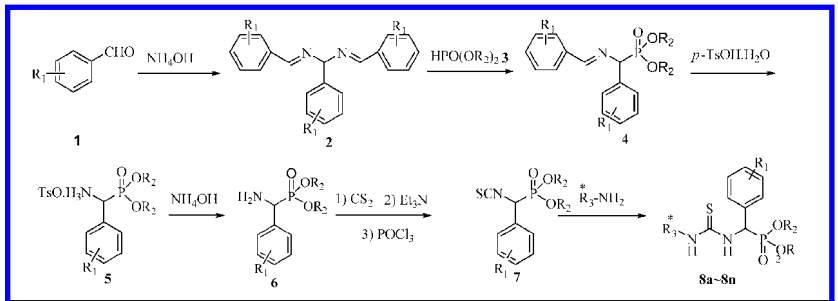
General Procedure for the Preparation of the Intermediates *O,O′*-Dialkyl Isothiocyanato(phenyl)methylphosphonate 7. To a solution of α-aminophosphonates **6** (6 mmol) in ether (15 mL) was added under stirring triethylamine (18 mmol) at room temperature and cooled to 0 °C. Then, carbon disulfide (6 mmol) was added dropwise and stirred for 2 h at 0 °C, the temperature was raised to 25 °C, and stirring was continued for an additional 2 h. Phosphorus oxychloride (6 mmol) dissolved in ether (10 mL) was then added dropwise into the reaction mixture and stirred for 4 h at 25 °C. The solid was filtered off, and the liquid was extracted with ether, treated with saturated sodium bicarbonate, and dried on anhydrous sodium sulfate. Removal of the solvent followed by chromatography of the crude product on silica using a mixture of petroleum ether and ethyl acetate (2:1) as the eluent gave the intermediates **7** in 54.2−61.5% yields (*25*).

*Data for O,O′-diethyl isothiocyanato(phenyl)methylphosphonate (***7a***):* light yellow liquid; n_D^{25} = 1.6365; yield, 60.3%; IR (KBr, cm^{-1}) ν 2981.4, 2063.8, 1494.3, 1456.3, 1392.6, 1259.5, 1022.3 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.25−1.27 (6H, m, $2CH_3$), 4.00−4.03 (4H, m, $2OCH_2$), 5.02 (1H, d, J = 18.02 Hz, CH), 7.37−7.43 (5 H, m, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.14, 131.78, 128.95, 128.80, 127.48, 64.32, 58.58, 16.51, 16.28; ^{31}P NMR (200 MHz, $CDCl_3$) δ 15.99.

*O,O′-Di-n-propyl isothiocyanato(phenyl)methylphosphonate (***7b***):* light yellow liquid; n_D^{25} = 1.6372; yield, 61.5%; IR (KBr, cm^{-1}) ν 2968.4, 2061.9, 1494.3, 1454.3, 1392.6, 1262.5, 1006.8 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.90−0.93 (6H, m, $2CH_3$), 1.61−1.64 (4H, m, $2CH_2$), 3.90−3.95 (4H, m, $2OCH_2$), 5.02 (1H, d, J = 18.02 Hz, CH), 7.37−7.43 (5 H, m, ArH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 137.12, 131.95, 128.95, 128.81, 127.49, 69.62, 58.58, 57.38, 23.95, 10.02; ^{31}P NMR (200 MHz, $CDCl_3$) δ 15.96.

*O,O′-Diisopropyl isothiocyanato (phenyl) methylphosphonate (***7c***):* light yellow liquid; n_D^{25} = 1.6424; yield, 54.2%; IR (KBr, cm^{-1}) ν 2980.0, 2063.8, 1496.3, 1456.3, 1386.6, 1257.5, 995.3 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.13 (12H, d, J = 16.70 Hz, $4CH_3$), 4.54−4.59 (2H, m, 2OCH), 4.88 (1H, d, J = 18.02 Hz, CH), 7.30−7.37 (5H, m, ArH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 137.24, 131.97, 128.95, 128.83, 127.49, 67.93, 67.58, 57.81, 57.40, 18.67, 13.61; ^{31}P NMR (200 MHz, $CDCl_3$) δ 14.04.

Scheme 1. Synthetic Route to Chiral Thiourea Analogues **8** Containing an α-Aminophosphonate Moiety



Compd.	R_2	R_3	Compd.	R_2	R_3
8a	Et	(S)-1-phenylethyl	**8b**	Et	(R)-1-phenylethyl
8c	Et	(S)-1-cyclohexylethyl	**8d**	Et	(R)-1-cyclohexylethyl
8e	*n*-Pr	(S)-1-phenylethyl	**8f**	*n*-Pr	(R)-1-phenylethyl
8g	*n*-Pr	(S)-1-cyclohexylethyl	**8h**	*n*-Pr	(R)-1-cyclohexylethyl
8i	*i*-Pr	(S)-1-phenylethyl	**8j**	*i*-Pr	(R)-1-phenylethyl
8k	*i*-Pr	(S)-1-cyclohexylethyl	**8l**	*i*-Pr	(R)-1-cyclohexylethyl
8m	*n*-Bu	(S)-1-cyclohexylethyl	**8n**	*n*-Bu	(R)-1-cyclohexylethyl

*O,O′-Di-n-butyl isothiocyanato (phenyl) methylphosphonate (***7d***):* light yellow liquid; n_D^{25} = 1.6362; yield, 57.8%; IR (KBr, cm^{-1}) ν 2958.0, 2061.9, 1494.3, 1454.3, 1385.6, 1259.5, 1022.3 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.89−0.92 (6H, m, $2CH_3$), 1.34−1.40 (4H, m, $2CH_2$), 1.54−1.63 (4H, m, $2CH_2$), 3.94−3.99 (4H, m, $2OCH_2$), 4.00 (1H, d, J = 18.02 Hz, CH), 7.37−7.43 (5H, m, ArH); ^{13}C NMR (125 MHz,$CDCl_3$) δ 137.24, 131.97, 128.95, 128.83, 127.49, 67.93, 67.58, 57.81, 57.40, 18.67, 13.61; ^{31}P NMR (200 MHz, $CDCl_3$) δ 16.02.

General Procedure for the Preparation of Title Compounds 8a−8n. A solution of *O,O′*-dialkyl isothiocyanato(phenyl)methylphosphonate **7** (1 mmol) in tetrahydrofuran (10 mL) was stirred, followed by dropwise addition of chiral amine (1.2 mmol). The reaction mixture was stirred for 0.5 h at 25 °C, the solvent was removed by evaporation, and the crude product was purified by chromatography on silica using a mixture of petroleum ether and ethyl acetate (2:1) as the eluent to give the title compounds **8a**−**8n** in 58−70% yields.

*Data for O,O′-diethyl phenyl[3-((S)-1-phenylethyl) thioureido] methylphosphonate (***8a***):* white crystal, mp 97−98 °C; yield, 70%; $[\alpha]_D^{20}$ = +16.8 (*c* 1.7, acetone); IR (KBr, cm^{-1}) ν 3300, 3118, 3057, 2983, 1533, 1492, 1452, 1336, 1207, 1018, 763, 742, 698, 565, 542; ^{1}H NMR (D_3CCOCD_3, 500 MHz) δ 8.31 (1H, br s, NH), 8.19 (1H, br d, J = 7.4 Hz, NH), 7.31−7.25(10H, m, ArH), 6.10 (1H, d, J = 20.0 Hz, N−CH−P), 5.37(1H, br s, N−CH−Ar), 3.97−3.83 (4H, m, $2OCH_2$), 1.38−0.98 (9H, m, $3CH_3$); ^{13}C NMR (125 MHz, DMSO-d_6) δ 182.7, 144.5, 136.6, 128.9, 128.7, 128.4, 128.2, 127.4, 126.5, 63.2, 53.9, 53.6, 21.5, 16.0; ^{31}P NMR (200 MHz, D_3CCOCD_3) δ 22.3, 22.1. Anal. Calcd for $C_{20}H_{27}N_2O_3PS$: C, 59.10; H, 6.70; N, 6.89. Found: C, 59.01; H, 6.39; N, 6.58.

*O,O′-Diethylphenyl [3-((R)-1-phenylethyl) thioureido] methylphosphonate (***8b***):* white crystal; mp 80−82 °C; yield, 70%; $[\alpha]_D^{20}$ = −16.5 (*c* 1.6, acetone); IR (KBr, cm^{-1}) ν 3300, 3116, 3062, 2974, 1537, 1494, 1452, 1336, 1207, 1051, 1018, 763., 742, 698, 565, 542; ^{1}H NMR (D_3CCOCD_3, 500 MHz) δ 8.53 (1H, br s, NH), 7.88 (1H, br d, J = 7.4 Hz, NH), 7.47−7.14 (10H, m, ArH), 6.50 (1H, d, J = 18.0 Hz, N−CH−P), 5.5 (1H, br s, N−CH−Ar), 4.30−3.39 (4H, m, $2OCH_2$), 1.42−0.93 (9H, m, $3CH_3$); ^{13}C NMR ($D_3CCO\ CD_3$, 125 MHz) δ 182.9, 143.7, 135.5, 128.9, 128.7, 128.2, 127.4, 126.7, 126.4, 64.0, 54.9, 53.6, 21.5, 16.3; ^{31}P NMR (200 MHz, D_3CCOCD_3) δ 22.5, 22.4. Anal. Calcd for $C_{20}H_{27}N_2O_3PS$: C, 59.10; H, 6.70; N, 6.89. Found: C, 59.35; H, 6.62; N, 6.51.

*O,O′-Diethylphenyl [3-((S)-1-cyclohexylethyl)thioureido]methylphosphonate (***8c***):* white crystal; mp 134−135 °C; yield, 60%; $[\alpha]_D^{20}$ = +15.3 (*c* 1.3, acetone); IR (KBr, cm^{-1}) ν 3319, 3124, 3078, 2978, 1541, 1492, 1454, 1354, 1207, 1031, 763., 742, 696, 565, 543; ^{1}H NMR (D_3CCOCD_3, 500 MHz) δ 8.19 (1H, br s, NH), 7.64 (1H, bs d, J = 7.4 Hz, NH), 7.34−7.25 (5H, m, ArH), 6.16 (1H, d, J = 13.0 Hz, N−CH−P), 4.11(1H, br s, N−CH−cyclohexyl), 4.00−3.69 (4H, m, $2OCH_2$), 1.16−1.00 (11H, m, cyclohexyl−H), 1.38−0.98 (9H, m, $3CH_3$); ^{13}C NMR (D_3CCOCD_3, 125 MHz) δ 182.7, 136.8, 128.7, 128.4, 128.2, 63.1, 62.8, 54.5, 54.3, 28.8, 26.3, 26.2, 16.7, 16.5; ^{31}P NMR (200 MHz, D_3CCOCD_3) δ 22.6, 22.5. Anal. Calcd for $C_{20}H_{33}N_2O_3PS$: C, 58.23; H, 8.06; N, 6.79. Found: C, 58.11; H, 8.05; N, 6.56.

*O,O′-Diethyl phenyl [3-((R)-1-cyclohexylethyl) thioureido] methylphosphonate (***8d***):* white crystal; mp 136−137 °C; yield, 65%; $[\alpha]_D^{20}$ = −15.6 (*c* 1.4, acetone); IR (KBr, cm^{-1}) ν 3321, 3124, 3078, 2976, 1541, 1492, 1454, 1354, 1207, 1031, 763, 742, 698, 565, 545; ^{1}H NMR (D_3CCOCD_3, 500 MHz) δ 8.24 (1H, br s, NH), 7.64 (1H, br d, J = 7.4 Hz, NH), 7.37−7.31 (5H, m, ArH), 6.21 (1H, d, J = 21.0 Hz, N−CH−P), 4.11 (1H, br s, N−CH−cyclohexyl), 4.03−3.75 (4H, m, $2OCH_2$), 1.20−1.03 (11H, m, cyclohexyl−H), 1.69−0.96 (9H, m, $3CH_3$); ^{13}C NMR (D_3CCOCD_3, 125 MHz) δ 182.7, 136.8, 128.8, 128.4, 128.0, 62.8, 54.5, 54.3, 42.9, 29.2, 26.3, 26.2, 16.8, 16.7; ^{31}P NMR (200 MHz, D_3CCOCD_3) δ 22.5, 22.7. Anal. Calcd for $C_{20}H_{33}N_2O_3PS$: C, 58.23; H, 8.06; N, 6.79. Found: C, 58.34; H, 8.18; N, 6.60.

*O,O′-Di-n-propyl phenyl [3-((S)-1-phenylethyl) thioureido] methylphosphonate (***8e***):* white crystal; mp 76−79 °C; yield, 58%; $[\alpha]_D^{20}$ = +15.1 (*c* 1.8, acetone); IR (KBr, cm^{-1}) ν 3298, 3133, 3064, 2968, 1541, 1494, 1452, 1352, 1213, 1012, 756, 698, 565, 549; ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.36 (1H, s, NH), 8.17 (1H, d, J = 7.4, NH), 7.33−7.30 (10H, m, ArH), 6.17 (1H, s, N−CH−P), 5.41 (1H, br s, N−CH−Ar), 3.84−3.74 (4H, m, $2OCH_2$), 1.53 (3H, d, J = 18.0 Hz, CH_3), 1.42−1.37 (4H, m, $2CH_2$), 0.89(6H, t, J = 5.1 Hz, $2CH_3$); ^{13}C NMR (D_3CCOCD_3, 125 MHz) δ 182.7, 143.2, 135.5, 128.9, 128.5, 128.1, 128.0, 126.9, 126.2, 68.6, 54.8, 53.4, 23.5, 21.8, 9.8; ^{31}P NMR (200 MHz, D_3CCOCD_3) δ 22.4, 22.3. Anal. Calcd for $C_{22}H_{31}N_2O_3PS$: C, 60.81; H, 7.19; N, 6.45. Found: C, 60.67; H, 7.33; N, 6.18.

*O,O′-Di-n-propylphenyl [3-((R)-1-phenylethyl) thioureido] methylphosphonate (***8f***):* white crystal; mp 69−70 °C; yield, 67%; $[\alpha]_D^{20}$ = −15.1 (*c* 1.7, acetone); IR (KBr, cm^{-1}) ν 3305 3130, 3062, 2966, 1512, 1494, 1452, 1352, 1215, 1012, 756, 698, 565, 549; ^{1}H NMR (D_3CCOCD_3, 500 MHz) δ 8.49 (1H, br s, J = 7.4 Hz, NH), 7.87 (1H, br s, NH), 7.49−7.14 (10H, m, ArH), 6.46 (1H, s, N−CH−P), 5.50 (1H, br s, N−CH−Ar), 4.26−3.49 (4H, m, $2OCH_2$), 1.61(3H, d, J = 20.0 Hz, CH_3), 1.43−1.39 (4H, m, $2CH_2$), 1.04 (6H, t, J = 3.5 Hz, $2CH_3$); ^{13}C NMR (D_3CCOCD_3, 125 MHz) δ 183.2, 143.2, 136.2, 128.9, 128.8 128.5, 128.0, 127.3, 126.5, 68.6, 54.8, 53.6, 23.3, 21.8, 9.3; ^{31}P NMR (200 MHz, D_3CCOCD_3) δ 22.8, 22.6. Anal. Calcd for $C_{22}H_{31}N_2O_3PS$: C, 60.81; H, 7.19; N, 6.45. Found: C, 60.57; H, 7.31; N, 6.28.

*O,O′-Di-n-propylphenyl [3-((S)-1-cyclohexylethyl) thioureido] methylphosphonate (***8g***):* white crystal; mp 145−147 °C; yield, 62%; $[\alpha]_D^{20}$ = +14.2 (*c* 1.5, acetone); IR (KBr, cm^{-1}) ν 3302, 3142, 3084, 2966, 1541, 1450, 1359, 1213, 1011, 752, 696, 563, 549; ^{1}H NMR (D_3CCOCD_3, 500 MHz) δ 8.33 (1H, s, NH), 8.75 (1H, d, J = 7.4 Hz, NH), 7.35−7.34 (5H, m, ArH), 6.22 (1H, s, N−CH−P), 5.41 (1H, br s, N−CH−cyclohexyl), 3.84−3.74 (4H, m, $2OCH_2$), 1.66−1.51 (11H, cyclohexyl−H), 1.42−1.27 (4H, m, $2CH_2$), 1.02 (3H, d, J = 7.2 Hz, CH_3), 0.85 (6H, t, J = 6.4 Hz, $2CH_3$); ^{13}C NMR (D_3CCOCD_3, 125 MHz) δ 182.6, 136.8, 128.7, 128.4, 126.7¬68.4, 68.4, 54.9, 53.7, 32.1, 28.8, 26.6, 26.2, 17.6, 10.2; ^{31}P NMR (200 MHz, D_3CCOCD_3) δ 22.5, 22.4. Anal. Calcd for $C_{22}H_{37}N_2\ O_3PS$: C, 59.97; H, 8.46; N, 6.36. Found: C, 60.10; H, 8.39; N, 6.45.

*O,O′-Di-n-propyl phenyl [3-((R)-1-cyclohexylethyl) thioureido] methylphospho-nate (***8h***):* white crystal; mp 143−145 °C; yield, 55%; $[\alpha]_D^{20}$ = −14.4 (*c* 1.6, acetone); IR (KBr, cm^{-1}) ν 3302, 3138, 3084, 2966, 1541, 1450, 1359, 1213, 1010, 732, 696, 563, 549; ^{1}H NMR (D_3CCOCD_3, 500 MHz) δ 8.33 (1H, s, NH), 8.75 (1H, d, J = 7.4, NH), 7.35−7.34 (5H, m, ArH), 6.22 (1H, s, N−CH−P), 4.15 (1H, br s, N−CH−cyclohexyl), 3.94−3.62 (4H, m, $2OCH_2$), 1.69−1.40 (11H, cyclohexyl−H), 1.19−1.07 (4H, m, $2CH_2$), 1.04 (3H, d, J = 22.0 Hz, CH_3), 0.86 (6H, t, J = 3.5 Hz, $2CH_3$); ^{13}C NMR (125 MHz, D_3CCOCD_3) δ 182.7, 136.8, 128.7, 128.4, 126.7¬68.4, 68.4, 54.9, 53.7, 32.1, 28.8, 26.6, 26.2, 17.6, 10.3; ^{31}P NMR (200 MHz, D_3CCOCD_3) δ 22.8, 22.6. Anal. Calcd for $C_{22}H_{37}N_2O_3PS$: C, 59.97; H, 8.46; N, 6.36. Found: C, 59.97; H, 8.46; N, 6.36.

*O,O′-Diisopropyl phenyl [3-((S)-1-phenylethyl) thioureido] methylphosphonate (***8i***):* white crystal; mp 112−114 °C; yield, 65%; $[\alpha]_D^{20}$ = +14.1 (*c* 2.0, acetone); IR (KBr, cm^{-1}) ν 3305, 3116, 3062, 2980, 1541, 1454, 1375, 1338, 1205, 1012, 767, 700, 572; ^{1}H NMR (D_3CCOCD_3, 500 MHz) δ 8.37 (1H, br s, NH), 7.87 (1H, br d, J = 7.4 Hz, NH), 7.52−7.26 (10H, m, ArH), 6.45 (1H, s, N−CH−P), 5.66 (1H, br s, N−CH−Ar), 4.68−4.67 (2H, m, 2OCH), 1.25 (3H, d, J = 6.0 Hz, CH_3), 1.15 (12H, d, J = 6.3, $4CH_3$); ^{13}C NMR (125 MHz, DMSO-d_6) δ 183.4, 144.1, 136.5, 128.7, 128.4, 128.3, 127.8, 126.9, 126.2, 72.7, 55.4, 53.0, 23.8, 22.7; ^{31}P NMR (200 MHz, D_3CCOCD_3) δ 21.0, 20.8. Anal. Calcd for $C_{22}H_{31}N_2O_3PS$: C, 60.81; H, 7.19; N, 6.45. Found: C, 60.89; H, 7.20; N, 6.77.

*O,O′-Diisopropyl phenyl [3-((R)-1-phenylethyl) thioureido] methylphosphonate (***8j***):* white crystal; mp 113−114 °C; yield, 67%; $[\alpha]_D^{20}$ = −14.1 (*c* 1.8, acetone); IR (KBr, cm^{-1}) ν 3305, 3116, 3062, 2980, 1541, 1494, 1452, 1338, 1205, 1014, 767, 700, 572; ^{1}H NMR (D_3CCOCD_3, 500 MHz) δ 8.44 (1H, br s, NH), 7.92 (1H, br d, J = 7.4 Hz, NH), 7.35−7.26 (10H, m, ArH), 6.43 (1H, s, N−CH−P), 5.66 (1H, br s, N−CH−Ar), 2.86−2.81 (2H, m, 2OCH), 1.25 (3H, d, J = 5.0 Hz, CH_3), 1.15 (12H, d, J = 5.3, $4CH_3$); ^{13}C NMR (125 MHz, DMSO-d_6) δ 183.1, 143.7, 136.5, 128.7, 128.4, 128.3, 127.8, 126.9, 126.1, 72.8, 55.8, 53.1, 23.7, 22.6; ^{31}P NMR (200 MHz, D_3CCOCD_3) δ 21.0, 20.8. Anal. Calcd for $C_{22}H_{31}N_2O_3PS$: C, 60.81; H, 7.19; N, 6.45. Found: C, 60.86; H, 7.39; N, 6.75.

*O,O′-Diisopropyl phenyl[3-((S)-1-cyclohexylethyl)thioureido]methylphosphonate (***8k***):* white crystal; mp 123−124 °C; yield, 63%; $[\alpha]_D^{20}$ = +10.5 (*c* 0.18, acetone); IR (KBr, cm^{-1}) ν 3313, 3122, 3064, 2978,

1533, 1494, 1455, 1338, 1207, 1006, 765, 761, 696, 569; ^{1}H NMR (500 MHz, D_3CCOCD_3) δ 8.30 (1H, br s, NH), 7.55 (1H, br d, J = 7.4 Hz, NH), 7.37−7.35 (5H, m, ArH), 6.53 (1H, s, N−CH−P), 4.85 (1H, br s, N−CH−cyclohexyl), 4.54−4.50 (2H, m, 2OCH), 1.71−1.36 (11H, m, cyclohexyl−H), 1.43 (3H, d, J = 5.4 Hz, CH_3), 1.28 (12H, d, J = 6.1, $4CH_3$); ^{13}C NMR (125 MHz, D_3CCOCD_3) δ 183.5, 136.6, 128.7, 128.3, 127.8, 72.8, 72.1, 55.1, 43.0, 26.3, 26.2, 23.9, 22.8, 16.6; ^{31}P NMR (200 MHz, D_3CCOCD_3) δ 21.1, 21.0. Anal. Calcd for $C_{22}H_{37}N_2O_3PS$: C, 59.97; H, 8.46; N, 6.36. Found: C, 59.80; H, 8.49; N, 6.47.

O,O′-Diisopropyl phenyl [3-((S)-1-cyclohexylethyl) thioureido] methylphosphon-ate (**8l**)*:* white crystal; mp 124−125 °C; yield, 58%; $[\alpha]_D^{20}$ = −10.1 (*c* 0.017 acetone); ^{1}H NMR (D_3CCOCD_3, 500 MHz) δ 3313, 3122, 3064, 2978, 1533, 1494, 1455, 1338, 1207, 1006, 765, 761, 696, 569; ^{1}H NMR (D_3CCOCD_3, 500 MHz) δ 8.12 (1H, br s, NH), 7.53 (1H, br d, J = 7.4 Hz, NH), 7.38−7.33 (5H, m, ArH), 6.45 (1H, s, N−CH−P), 4.85 (1H, br s, N−CH−cyclohexyl), 4.54−4.50 (2H, m, 2OCH), 1.71−1.36 (11H, m, cyclohexyl−H), 1.45 (3H, d, J = 7.5 Hz, CH_3), 1.27 (12H, d, J = 6.2, $4CH_3$); ^{13}C NMR (125 MHz, D_3CCOCD_3) δ 183.4, 136.6, 128.7, 128.3, 127.8, 72.8, 72.1, 55.1, 43.0, 26.3, 26.2, 23.9, 22.8, 17.1; ^{31}P NMR (200 MHz, D_3CCOCD_3) δ 21.0, 21.09. Anal. Calcd for $C_{22}H_{37}N_2O_3PS$: C, 59.97; H, 8.46; N, 6.36. Found: C, 59.47; H, 8.73; N, 6.49.

O,O′-Di-n-butylphenyl [3-((S)-1-cyclohexylethyl) thioureido] methylphosphonate (**8m**)*:* colorless viscous liquid; yield, 65%; $[\alpha]_D^{20}$ = +13.6 (*c* 1.3, acetone); IR (KBr, cm^{-1}) ν 3313, 3126, 3064, 2960, 1541, 1494, 1456, 1355, 1209, 1028, 767, 698, 569; ^{1}H NMR (500 MHz, D_3CCOCD_3) δ 8.39 (1H, br s, NH), 7.68 (1H, br d, J = 7.4 Hz, NH), 7.51−7.34 (5H, m, ArH), 6.62 (1H, s, N−CH−P), 4.26−3.76 (4H, m, $2OCH_2$), 2.85 (1H, br s, N−CH−cyclohexyl), 1.71−1.43 (11H, m, cyclohexyl−H), 1.03−0.94 (8H, m, $4CH_2$), 1.00 (3H, d, J = 5.6 Hz, CH_3), 0.94 (6H, t, J = 5.3 Hz, $2CH_3$); ^{13}C NMR (125 MHz, D_3CCOCD_3) δ 183.3, 136.3, 128.8, 128.5, 127.9, 67.7, 66.8, 54.4, 43.1, 32.5, 32.1, 26.3, 26.1, 18.4, 17.0, 13.2; ^{31}P NMR (200 MHz, D_3CCOCD_3) δ 22.8, 22.6. Anal. Calcd for $C_{24}H_{41}N_2O_3PS$: C, 61.51; H, 8.82; N, 5.98. Found: C, 61.11; H, 8.59; N, 5.41.

O,O′-Di-n-butyl phenyl [3-((R)-1-cyclohexylethyl) thioureido] methylphosphonate (**8n**)*:* colorless viscous liquid; yield, 60%; $[\alpha]_D^{20}$ = −13.5 (*c* 1.4, acetone); IR (KBr, cm^{-1}) ν 3313, 3128, 3062, 2958, 1541, 1494, 1450, 1355, 1207, 1028, 767, 798, 567; ^{1}H NMR (500 MHz, D_3CCOCD_3) δ 8.37 (1H, br s, NH), 7.82 (1H, br d, J = 7.4 Hz, NH), 7.53−7.36 (5H, m, ArH), 6.63 (1H, s, N−CH−P), 4.28−3.75 (4H, m, OCH_2), 2.87 (1H, br s, N−CH−cyclohexyl), 1.78−1.42 (11H, m, cyclohexyl−H), 1.12 (3H, d, J = 4.9 Hz, CH_3), 1.03−0.94 (8H, m, $4CH_2$), 0.98 (6H, t, J = 5.8 Hz, $2CH_3$); ^{13}C NMR (125 MHz, D_3CCOCD_3) δ 183.3, 136.3, 128.8, 128.4, 127.9, 67.5, 66.8, 54.4, 43.1, 32.6, 32.1, 26.3, 26.1, 18.6, 18.4, 13.0; ^{31}P NMR (200 MHz, D_3CCOCD_3) δ 22.9, 22.8. Anal. Calcd for $C_{24}H_{41}N_2O_3PS$: C, 61.51; H, 8.82; N, 5.98. Found: C, 61.06; H, 8.35; N, 5.34.

Antiviral Biological Assay. *Purification of TMV.* Using Gooding's method (*26*), the upper leaves of *Nicotiana tabacum* L. inoculated with TMV were selected and ground in phosphate buffer and then filtered through double-layer pledget. The filtrate was centrifuged at 10000*g*, treated twice with PEG, and centrifuged again. The whole experiment was carried out at 4 °C. Absorbance values were estimated at 260 nm using an ultraviolet spectrophotometer.

$$\text{virus concn} = (A_{260} \times \text{dilution ratio})/E_{1\text{cm}}^{0.1\%,260\text{nm}}$$

Protective Effects of Compounds against TMV in Vivo. The compound solution was smeared on the left side, whereas the solvent served as the control on the right side of growing *N. tabacum* L. leaves of the same ages. The leaves were then inoculated with the virus after 12 h. A brush was dipped in tobacco mosaic virus of 6×10^{-3} mg/mL to inoculate the leaves, which were previously scattered with silicon carbide. The leaves were then washed with water and rubbed softly along the nervature once or twice. The local lesion numbers appearing 3−4 days after inoculation were counted (*27*). Three repetitions were conducted for each compound.

Curative Effect of Compounds against TMV in Vivo. Growing leaves of *N. tabacum* L. of the same ages were selected. TMV (concentration of 6×10^{-3} mg/mL) was dipped and inoculated on the whole leaves. 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 counted and recorded 3−4 days after inoculation (*27*). For each compound, three repetitions were measured. The inhibition rate of the compound was then calculated according to the following formula:

$$\text{inhibition rate (\%)} = [(\text{av local lesion no. of control (not treated with compd)} - \text{av local lesion no. smeared with drugs})/\text{av local lesion no. of control (not treated with compd)}] \times 100\%$$

Table 1. Effect of Different Solvents for Synthesis of **8b**

no.	solvent	vol (mL)	reaction time (h)	temperature (°C)	yield (%)
1	THF	10	0.5	room	70.0
2	CH_3CN	10	0.5	room	58.3
3	DMF	10	0.5	room	62.4
4	toluene	10	0.5	room	51.5

RESULTS AND DISCUSSION

Synthesis. To obtain intermediates**7** with high purity, the method reported by Kaboudin (*24*) was modified by employing ether as the extraction solvent and petroleum ether and ethyl acetate as the eluent for column chromatography. To restrict the formation of side products, the reaction was conducted at lower temperature.

To optimize the reaction conditions for the preparation of compound **8b**, the synthesis was carried out in different solvents, such as tetrahydrofuran (THF), acetonitrile, *N*,*N*-dimethylformamide (DMF), and toluene. A maximum yield of up to 70.0% was achieved when the reaction mixture was stirred for 0.5 h in THF. The effect of solvent system is summarized in Table **1**.

The main characteristic of the ^{1}H NMR spectra of **8a**−**8n** is the presence of high-frequency downfield broad singlet δ_H 8.53−8.12, presumably arising due to the deshielded N−H proton of thiourea linked to the phosphonate moiety and benzene ring through an intervening carbon atom. The broad doublets at δ_H 8.19−7.53 are assigned to the N−H proton of thiourea directly attached to the asymmetric center R_3. The doublet at δ_H 6.63−6.16 is assigned to the C−H proton of N−C−P, and the broad singlet at δ_H 5.54−4.11 is assigned to the C−H of N−C−C. Dialkyl phosphites **3**, due to their ability to exist in phosphonate−phosphite equilibrium, showed two singlets in the regions δ_H 6.06−6.13 and 7.45−7.51 for H−P═O and P−OH, respectively. The typical carbon resonance at δ_c 183.6−182.7 in the ^{13}C NMR spectra of **8a**−**8n** also confirms the presence of a carbon−sulfur double bond. The typical phosphorus resonance at δ_P 22.6−21.1 in the ^{31}P NMR spectra of **8a**−**8n** reveals the presence of a phosphorus center coupled to an adjacent CH.

Antiviral Activity and Structure−Activity Relationship. The antiviral activities of compounds **8a**−**8n** against TMV are assayed according to the reported method (*27*).

The results of bioassay in vivo against TMV are given in **Table 2**. Ningnanmycin was used as reference antiviral agent (*28*). The data provided in **Table 2** indicate that the title chiral compounds **8a**−**8n** showed protection rates of 30.9−46.3%. Compounds **8b**, **8e**,**8f**, and **8j** have moderate protection activities (42.6, 42.7, 45.9, and 46.3%, respectively), lower than that of the commercial reference (60.2%). From the data in **Table 2**, it may be observed that the title chiral compounds **8a**−**8n** possess

Table 2. Protection Effect and Curative Effect of the New Compounds against TMV in Vivo[a]

agent	concn (μg/mL)	protection effect (%)	curative effect (%)
8a	500	37.6*	41.8*
8b	500	42.6*	45.2*
8c	500	36.4*	36.3*
8d	500	33.1*	40.1*
8e	500	42.7*	50.5*
8f	500	45.9*	41.9*
8g	500	30.9*	54.8**
8h	500	37.9*	40.7*
8i	500	36.9*	36.8*
8j	500	46.3*	34.5*
8k	500	36.3*	50.4**
8l	500	33.7*	34.6*
8m	500	36.3*	50.4**
8n	500	33.7*	34.6*
Ningnamycin	500	60.6**	56.2**

[a] $n = 3$ for all groups; *, $P < 0.05$; **, $P < 0.01$.

moderate to good curative bioactivities, with values of 41.8, 45.2, 36.3, 40.1, 50.5, 41.9, 54.8, 40.7, 36.8, 34.5, 50.4, 34.6, 50.4, and 34.6% at 500 μg/mL, respectively. The data also indicate that the curative activity of the title chiral compounds **8a**−**8n** is strongly dependent upon the absolute configuration of the enantiomer and the nature of substituents. The enantiomer **8g** [R_2 is *n*-Pr, R_3 is (*S*)-1-cyclohexylethyl] could curate TMV up to 54.3% at the concentration of 500 μg/mL. The other chiral thioureas with different substituents and configuration have a relatively lower curative activity than that of **8g**. At a concentration of 500 μg/mL, compound **8g** [(*S*)-configuration] showed significant enhancement in anti-TMV activities compared to its enantiomer **8h** [(*R*)-configuration] [R_2 is *n*-Pr, R_3 is (*R*)-1-cyclohexylethyl] from the curative effect and inactivation effect. The same trend in terms of improved activity was noted with other (*S*)-enantiomers (**8e**, **8k**, and **8m**) when compared with the activities shown by their corresponding (*R*)-enantiomers (**8f**, **8l**, and **8n**) to TMV. The enantiomers **8a**, **8b**, **8c**, **8d**, **8i**, and**8j** present little variation in the anti-TMV activities and display moderate protection and curative effects with values not exceeding 46%. Although our studies indicate the existence of a definite relationship between anti-TMV activity and the configuration of the enantiomer, structure−activity relationships in view of steric and hydrophobic effects could not be successfully ascertained due to lack of structural diversity.

Beccause the enantiomers **8e**, **8g**, **8k**, and**8m** were found to display good antiviral activities, they were selected for further bioassay with commercial plant antiviral agent Ningnanmycin serving as a control for making a judgment on the antiviral potency of these compounds. As shown in **Table 3**, the curative effects against TMV of the enantiomer **8g** and Ningnanmycin were remarkable. The EC_{50} values on TMV were 239.8 and 227.0 μg/mL, respectively. Compounds **8e**, **8k**, and **8m** were fairly effective in curing TMV, and the EC_{50} values were 399.9, 413.4, and 378.9 μg/mL, respectively.

In summary, a series of new chiral thiourea derivatives containing an α-aminophosphonate moiety, **8a**−**8n**, were designed and synthesized by the addition reaction of *O*,*O*′-dialkyl isothiocyanato(phenyl)methylphosphonate **7** with chiral amine in THF. The in vivo tests indicated that compounds **8g**, **8e**, **8k**, and **8m** exhibited a curative activity level very similar to that of Ningnanmycin against TMV. Therefore, the present work demonstrates that the antiviral activity of chiral thiourea derivatives was significantly improved via the introduction of the appropriate enantiomer with (*S*)-configuration. The (*S*)-enantiomers (**8g**, **8e**, **8k**, and **8m**) display improved activity in comparison with their corresponding (*R*)-enantiomers (**8h**, **8f**, **8l**, and **8n**) to TMV. Effects of steric parameters on structure−activity relationships and studies on structural modification for identifying lead bioactive compounds are currently underway.

Table 3. Antiviral Activities in Vivo of Compounds **8b**, **8e**, **8g**, **8k**, and **8m**

	TMV curative effect (%)			
agent	500 μg/mL	250 μg/mL	125 μg/mL	EC_{50} (μg/mL)
8e	50.5	37.7	30.9	399.9
8 g	54.8	49.0	39.1	239.8
8k	50.4	36.7	29.0	413.4
8m	50.4	37.2	32.9	378.9
Ningnamycin	56.2	50.7	41.9	227.0

Supporting Information Available: Synthetic procedure and characterization data of intermediates **2**−**6**. This material is available free of charge via the Internet at http://pubs.acs.org.

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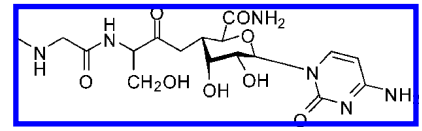
(28) Ningnanmycin is produced by *Strepcomces noursei* var. *xichangensisn*. Ningnanmycin is now commercially available in China as a registered plant virucide. However, its action target is not clear yet. Its structure is as follows:



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