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Novel Synthetic Route to α-Aminophosphonates Containing Benzothiazole Moiety

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A novel and efficient synthetic route to α-aminophosphonates containing benzothiazole moiety via a cascade three-component reaction from conveniently available starting materials has been developed. The target compounds **3a**—**3g**, **7** and **8a**, **8b** were evaluated for their anticancer activities against the cancer cell line HL-60 *in vitro* by the MTT method. Compound **3g** showed good cancer inhibitory activity against the tested cell line. Further study is necessary to find out the potential antitumor activities.

Keywords cascade three-component reaction, α-aminophosphonates, antitumor activity

Introduction

In past decades, the synthesis of heterocycles has continued to attract the interest of synthetic chemists.[1] Among these heterocycles, benzothiazoles with a nitrogen substituent in the 2-position in natural products play pharmacologically important roles because of the broad spectrum of their biological activities.[2]

On the other hand, α-aminophosphonic acids and their corresponding α-aminophosphonates have received much interest in organic and medicinal chemistry because they are analogues to both natural and unnatural amino acids.[3] Some of them have been used as potent enzyme inhibitors, antimicrobial, antitumor and antiviral agents.[4-6] Among these compounds, studies are mainly concentrated on heterocycle moieties such as thiophene, furan, benzothiazole. α-Aminophosphonates containing benzothiazole moiety have been suggested as antitumor agent.[7]

In view of this research and our desire to develop new anticancer agents of high potency, we fused biological benzothiazole and aminophosphonate structures to obtain compounds possessing better biological activities. Among numerous synthetic methodologies of α-aminophosphonate, the most noteworthy and remarkable one is Kabachnik-Fields reaction, generally using amines, dialkyl phosphites and carbonyl compounds as the reactants.[8] In connection with our ongoing project on the Cu-catalyzed reaction,[9] we herein describe a new strategy to construct α-aminophosphonates containing benzothiazole moiety.

Experimental

Synthesis

Materials and equipment Reagents were obtained commercially and used as received. Solvents were purified and dried by standard methods. Primary 1-aminophosphonates were synthesized according the literature methods.[10] The melting points were determined on an XT-4 micro melting point apparatus and uncorrected. IR spectra were recorded on an EQUINOX-55 spectrometer on a KBr matrix. NMR spectra were recorded on an INOVA-400 NMR instrument at room temperature using TMS as internal standard. Coupling constants (*J*) were measured in Hz. Elemental analyses were performed on a Vario EL III CHNS analyzer. Electrospray mass spectra were obtained with an MALDI-TOF mass spectrometer. 200—300 mesh silica gel was used for column chromatography.

Typical procedure for the synthesis of target compounds A reaction mixture of 1-bromo-2-nitrobenzene **4** (1 mmol), CS_2 (1.5 mmol), primary 1-aminophosphonate **5** (1.4 mmol), K_2CO_3 (3 mmol), $CuCl_2$•$2H_2O$ (1 mmol) and $SnCl_2$•$2H_2O$ (4 mmol) in DMF (3.5 mL) was stirred at 110 ℃ for 7 h. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was washed with water and brine, and dried over anhydrous $MgSO_4$ and concentrated under vacuum to yield the crude product. The crude product was purified by flash chromatography (ethyl acetate ∶ petroleum ether, 1 ∶ 2, volum ratio).

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***O*,*O′*-Diethyl-*α*-(benzothiazole-2-yl) amino-(phenylmethyl) phosphonate (3a)** White powder, yield 84%, m.p. 112—114 ℃ (lit.[11] m.p. 112 ℃); ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 7.54 (dd, *J*=7.2, 7.2 Hz, 4H), 7.06—7.39 (m, 5H), 5.54 (d, J_{H-P}=22.4 Hz, 1H), 4.84 (bs, 1H), 3.98—4.22 (m, 4H), 1.30 (t, *J*=7.2 Hz, 3H), 1.15 (t, *J*=7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) *δ*: 151.9, 135.0, 131.0, 128.5, 128.2, 128.1, 125.8, 121.8, 120.7, 119.3, 63.6 (d, J_{C-P}=10.7 Hz), 56.7, 55.1, 16.4, 16.2; ^{31}P NMR ($CDCl_3$, 200 MHz) *δ*: 22.7; IR (KBr) *v*: 3210, 2907, 1256 and 1031 cm^{-1}; MALDI-TOF MS *m/z*: 399 $[M+Na]^+$. Anal. calcd for $C_{18}H_{21}N_2O_3PS$: C 57.44, H 5.62, N 7.44, S 8.52; found C 57.56, H 5.69, N 7.41, S 8.49.

***O*,*O′*-Diethyl-*α*-(benzothiazole-2-yl) amino-(4-fluoro-phenylmethyl) phosphonate (3b)** White powder, yield 83%, m.p. 167—169 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 7.06—7.61 (m, 8 H), 5.57 (d, J_{H-P}=22.0 Hz, 1H), 4.81 (bs, 1H), 3.81—4.24 (m, 4H), 1.32 (t, *J*=7.0 Hz, 3H), 1.15 (t, *J*=7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) *δ*: 165.3 (d, *J*=9.3 Hz), 150.9, 131.0, 129.9 (t, J_{C-F}=6.0 Hz), 129.8, 125.8, 122.0, 120.7, 119.4, 115.6, 115.4, 63.5 (d, J_{C-P}=4.8 Hz), 55.8, 54.3, 16.4 (d, J_{C-P}=4.2 Hz), 16.2 (d, J_{C-P}=4.2 Hz); ^{31}P NMR ($CDCl_3$, 200 MHz) *δ*: 21.3; IR (KBr) *v*: 3216, 2903, 2828, 1233, 1021 cm^{-1}; MALDI-TOF MS *m/z*: 417 $[M+Na]^+$. Anal. calcd for $C_{18}H_{20}FN_2O_3PS$: C 54.82, H 5.11, N 7.10, S 8.13; found C 54.91, H 5.20, N 7.11, S 8.09.

***O*,*O′*-Diethyl-*α*-(benzothiazole-2-yl) amino-(4-chlorophenylmethyl) phosphonate (3c)** Pale yellow solid, yield 85%, m.p. 194—195 ℃ (lit.[11] m.p. 195 ℃); ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 7.07—7.56 (m, 8 H), 5.59 (d, J_{H-P}=22.8 Hz, 1 H), 4.81 (bs, 1H), 3.85—4.25 (m, 4H), 1.29 (t, *J*=7.2 Hz, 3H), 1.20 (t, *J*=7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) *δ*: 165.6 (d, *J*=9.1 Hz), 151.8, 134.1, 133.8, 131.0, 129.5, 128.7, 125.8, 122.0, 120.7, 119.4, 63.7 (d, J_{C-P}=3.6 Hz), 55.9, 54.3, 16.4 (d, J_{C-P}=4.2 Hz), 16.2 (d, J_{C-P}=4.2 Hz); ^{31}P NMR ($CDCl_3$, 200 MHz) *δ*: 19.7; IR (KBr) *v*: 3239, 3021, 2831, 1225, 1024 cm^{-1}; MALDI-TOF MS *m/z*: 433 $[M+Na]^+$. Anal. calcd for $C_{18}H_{20}ClN_2O_3PS$: C 52.62, H 4.91, N 6.82, S 7.80; found C 52.71, H 4.83, N 6.88, S 7.86.

***O*,*O′*-Diethyl-*α*-(benzothiazole-2-yl) amino-(4-dimethylaminophenylmethyl) phosphonate (3d)** White solid, yield 85%, m.p. 181—182 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 7.07—7.56 (m, 7 H), 6.70 (d, *J*=8.8 Hz, 1H), 5.23 (d, J_{H-P}=22.0 Hz, 1H), 4.48 (bs, 1H), 3.75—4.20 (m, 4H), 2.95 (d, *J*=19.6 Hz, 6H), 1.28 (t, *J*=7.2 Hz, 3H), 1.15 (t, *J*=7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) *δ*: 166.2 (d, *J*=9.9 Hz), 152.0, 131.3, 131.0, 129.0, 128.9, 125.7, 121.7, 120.7, 119.3, 112.3, 63.3 (d, J_{C-P}=5.4 Hz), 56.5, 54.9, 40.5, 40.4, 16.4 (d, J_{C-P}=4.2 Hz), 16.3 (d, J_{C-P}=4.2 Hz); ^{31}P NMR ($CDCl_3$, 200 MHz) *δ*: 21.3; IR (KBr) *v*: 3223, 3029, 2891, 1236, 1018 cm^{-1}; MALDI-TOF MS *m/z*: 442 $[M+Na]^+$. Anal. calcd for $C_{20}H_{26}N_3O_3PS$: C 57.27, H 6.25, N 10.02, S 7.64; found C 57.31, H 6.19, N 10.11, S 7.66.

***O*,*O′*-Diethyl-*α*-(benzothiazole-2-yl) amino-(4-methoxyphenylmethyl) phosphonate (3e)** Pale yellow solid, yield 84%, m.p. 175—177 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 7.08—7.56 (m, 6H), 6.88 (d, *J*=8.4 Hz, 2H), 5.49 (d, J_{H-P}=22.0 Hz, 1H), 4.81 (bs, 1H), 3.74—4.24 (m, 7H), 1.32 (t, *J*=7.2 Hz, 3H), 1.22 (t, *J*=7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) *δ*: 165.9 (d, *J*=9.3 Hz), 152.0, 131.1, 129.4, 127.0, 125.7, 121.8, 120.6, 119.3, 114.0, 63.4 (d, J_{C-P}=5.3 Hz), 56.0, 55.2, 54.5, 16.4 (d, J_{C-P}=4.2 Hz), 16.2 (d, J_{C-P}=4.2 Hz); ^{31}P NMR ($CDCl_3$, 200 MHz) *δ*: 20.6; IR (KBr) *v*: 3228, 3011, 2989, 1232, 1025 cm^{-1}; MALDI-TOF MS *m/z*: 429 $[M+Na]^+$. Anal. calcd for $C_{19}H_{23}N_2O_4PS$: C 56.15, H 5.70, N 6.89, S 7.89; found C 56.20, H 5.66, N 6.71, S 7.81.

***O*,*O′*-Dimethyl-*α*-(benzothiazole-2-yl) amino-(4-methoxyphenylmethyl) phosphonate (3f)** Brownish yellow solid, yield 81%, m.p. 169—171 ℃ (lit.[12] m.p. 170 ℃); ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 7.04—7.54 (m, 6H), 6.89 (d, *J*=8.8 Hz, 2H), 5.56 (d, J_{H-P}=21.6 Hz, 1H), 4.50 (bs, 1H), 3.82 (d, *J*=10.8 Hz, 3H), 3.76 (s, 3H), 3.54 (d, *J*=10.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) *δ*: 165.7 (d, *J*=9.1 Hz), 151.9, 131.1, 129.4, 129.3, 126.7, 125.7, 121.8, 120.7, 119.3, 114.1, 55.5, 55.2, 54.0 (d, J_{C-P}=5.3 Hz), 53.9 (d, J_{C-P}=2.7 Hz); ^{31}P NMR ($CDCl_3$, 200 MHz) *δ*: 21.3; IR (KBr) *v*: 3221, 3031, 2941, 1239, 1018 cm^{-1}; MALDI-TOF MS *m/z*: 401 $[M+Na]^+$. Anal. calcd for $C_{17}H_{19}N_2O_4PS$: C 53.96, H 5.06, N 7.40, S 8.47; found C 54.01, H 5.17, N 7.47, S 8.56.

***O*,*O′*-Diethyl-*α*-(benzothiazole-2-yl) amino-(4-nitrophenylmethyl) phosphonate (3g)** Yellow solid, yield 86%, m.p. 162—164 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 8.49 (d, *J*=2.0 Hz, 1H), 8.15 (d, *J*=7.6 Hz, 1H), 7.96 (d, *J*=7.2 Hz, 1H), 7.10—7.56 (m, 5H), 5.83 (d, J_{H-P}=22.8 Hz, 1H), 4.69 (bs, 1H), 3.96—4.34 (m, 4H), 1.36 (t, *J*=7.2 Hz, 3H), 1.26 (t, *J*=7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) *δ*: 165.2 (d, *J*=9.1 Hz), 151.7, 137.9, 134.2, 134.1, 131.1, 129.4, 125.8, 123.1, 123.0, 122.1, 120.7, 119.5, 64.0 (d, J_{C-P}=10.4 Hz), 55.7, 54.1, 16.5 (d, J_{C-P}=4.2 Hz), 16.2 (d, J_{C-P}=4.2 Hz); ^{31}P NMR ($CDCl_3$, 200 MHz) *δ*: 19.6; IR (KBr) *v*: 3229, 3011, 2972, 1502, 1334, 1225, 1022 cm^{-1}; MALDI-TOF MS *m/z*: 444 $[M+Na]^+$. Anal. calcd for $C_{18}H_{20}N_3O_5PS$: C 51.30, H 4.78, N 9.97, S 7.61; found C 51.39, H 4.85, N 9.90, S 7.72.

***O*,*O′*-Diethyl-*α*-(benzothiazole-2-yl) amino-(ferrocenylmethyl) phosphonate (7)** Yellow solid, yield 76%, m.p. 153—155 ℃; ^{1}H NMR ($CDCl_3$, 400 MHz) *δ*: 7.11—7.64 (m, 5H), 5.44 (d, J_{H-P}=19.2 Hz, 1H), 4.68 (bs, 1H), 3.92—4.42 (m, 13H), 1.24 (t, *J*=4.4 Hz, 3H), 1.20 (t, *J*=4.4 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) *δ*: 165.7 (d, *J*=4.2 Hz), 152.1, 130.9, 125.9, 121.8, 120.8, 119.2, 84.0, 83.9, 68.9, 68.2, 67.9, 66.2, 63.3 (d, J_{C-P}=5.2 Hz), 52.3, 50.8, 16.4, 16.3; ^{31}P NMR ($CDCl_3$, 200 MHz) *δ*: 21.1; IR (KBr) *v*: 3233, 3089, 2931, 1443,

1272, 1220, 1039, 751 cm^{-1}; MALDI-TOF MS *m/z*: 507 $[M+Na]^+$. Anal. calcd for $C_{22}H_{25}FeN_2O_3PS$: C 54.56, H 5.20, N 5.78, S 6.62; found C 54.67, H 5.23, N 5.80, S 6.69.

***O,O′*-Diethyl-α-(5-methylbenzothiazole-2-yl) amino-(phenylmethyl) phosphorrnate (8a)** White powder, yield 85%, m.p. 133—134 ℃; ^1H NMR ($CDCl_3$, 400 MHz) *δ*: 7.18—7.89 (m, 8H), 5.58 (d, J_{H-P}=22.4 Hz, 1H), 4.54 (bs, 1H), 3.76—4.24 (m, 4H), 2.22 (s, 3H), 1.32 (t, *J*=6.0 Hz, 3H), 1.15 (t, *J*=6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) *δ*: 164.2 (d, *J*=8.9 Hz), 150.7, 136.9, 133.2, 133.1, 131.2, 129.3, 125.8, 123.0, 122.1, 120.1, 118.5, 63.8 (d, J_{C-P}=22.5 Hz), 55.7, 54.2, 22.4, 16.4 (d, J_{C-P}=4.2 Hz), 16.2 (d, J_{C-P}=4.2 Hz); ^{31}P NMR ($CDCl_3$, 200 MHz) *δ*: 19.9; IR (KBr) *v*: 3194, 3030, 2903, 1233, 1027 cm^{-1}; MALDI-TOF MS *m/z*: 413 $[M+Na]^+$. Anal. calcd for $C_{19}H_{23}N_2O_3PS$: C 58.45, H 5.94, N 7.17, S 8.21; found C 58.51, H 5.88, N 7.23, S 8.29.

***O,O′*-Diethyl-α-(5-methoxybenzothiazole-2-yl) amino-(phenylmethyl) phosphonate (8b)** Pale yellow solid, yield 81%, m.p. 142—144 ℃; ^1H NMR ($CDCl_3$, 400 MHz) *δ*: 7.08—7.55 (m, 7 H), 6.78 (d, *J*=9.2 Hz, 1H), 5.53 (d, J_{H-P}=22.0 Hz, 1H), 4.53 (bs, 1H), 3.79—4.28 (m, 7H), 1.30 (t, *J*=7.2 Hz, 3H), 1.19 (t, *J*=7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) *δ*: 165.4 (d, *J*=9.1 Hz), 151.2, 134.2, 131.4, 128.0, 126.7, 122.8, 121.2, 118.4, 116.3, 64.1 (d, J_{C-P}=5.1 Hz), 55.1, 54.2, 53.7, 16.5, 16.3; ^{31}P NMR ($CDCl_3$, 200 MHz) *δ*: 20.2; IR (KBr) *v*: 3239, 3023, 2991, 1241, 1015 cm^{-1}; MALDI-TOF MS *m/z*: 429 $[M+Na]^+$. Anal. calcd for $C_{19}H_{23}N_2O_4PS$: C 56.15, H 5.70, N 6.89, S 7.89; found C 56.08, H 5.75, N 6.80, S 7.92.

***O,O′*-Diethyl-α-(5-chlorobenzothiazole-2-yl) amino-(phenylmethyl) phosphonate (8c)** Pale yellow solid, yield 80%, m.p. 117—119 ℃; ^1H NMR ($CDCl_3$, 400 MHz) *δ*: 7.61 (d, *J*=6.0 Hz, 1H), 7.26—7.44 (m, 6H), 7.10 (t, *J*=7.6 Hz, 1H), 5.64 (d, J_{H-P}=22.4 Hz, 1H), 4.72 (bs, 1H), 3.86—4.23 (m, 4H), 1.32 (t, *J*=7.2 Hz, 3H), 1.11 (t, *J*=7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) *δ*: 163.8 (d, *J*=9.1 Hz), 149.1, 135.0, 132.4, 131.3, 128.5, 128.1, 121.2, 120.7, 119.7, 62.7 (d, J_{C-P}=10.4 Hz), 55.1, 54.2, 16.5 (d, J_{C-P}=4.1 Hz), 16.3 (d, J_{C-P}=4.1 Hz); ^{31}P NMR ($CDCl_3$, 200 MHz) *δ*: 22.8; IR (KBr) *v*: 3293, 3017, 2911, 1226, 1021 cm^{-1}; MALDI-TOF MS *m/z*: 433 $[M+Na]^+$. Anal. calcd for $C_{18}H_{20}ClN_2O_3PS$: C 52.62, H 4.91, N 6.82, S 7.80; found C 52.71, H 4.85, N 6.92, S 7.88.

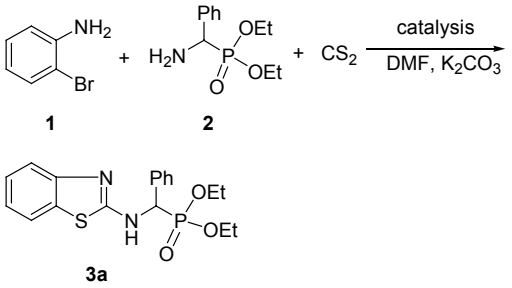
Antitumor activities assay *in vitro*

The antitumor activities of the target compounds (**3a**—**3g**, **7** and **8a**, **8b**) were evaluated with HL-60 (human acute promyelocytic leukemia). HL-60 was obtained from the American Type Culture Collection (Manassas, VA). Cells were cultured in RPMI 1640 and maintained in a Thermo incubator (Waltham, MA) with a humidified air containing 5% CO_2, 95% air. All culture media contained 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution (10000 units of penicillin and 10 mg of streptomycin in 1 mL 0.9% NaCl). The cancer cell line was cultured in minimum essential medium (MEM). Four-thousand cells (per well) suspended in MEM, were plated onto each well of a 96-well plate and incubated for 24 h. The tested compounds at the indicated final concentrations were added to the culture medium and the cell cultures were continued for 72 h. Fresh MTT was added to each well at the terminal concentration of 5 μg/mL and incubated with cells at 37 ℃ for 4 h. The formazan crystals were dissolved in 100 μL DMSO each well, and the absorbency at 492 nm (for absorbance of MTT formazan) and 630 nm (for the reference wavelength) was measured using the ELISA reader. All of the compounds were tested in triplicate in the cell line. The results expressed as IC_{50} (inhibitory concentration 50%) were the averages of three measurements and calculated by using the Bacus Laboratories Incorporated Slide Scanner (Bliss) software.

Results and Discussion

Ma and his group have developed a copper-catalyzed three-component synthesis of 2-*N*-substituted benzothiazoles in 2011.[2] According to the structure of *O,O′*-diethyl-α-(benzothiazole-2-yl) amino-(phenylmethyl) phosphonate, we envisioned that 2-bromoaniline **1** may be used to react with primary 1-aminophosphonate **2** under the catalysis of $CuCl_2•2H_2O$ in presence of carbon disulfide, K_2CO_3 and *N*,*N*-dimethylformamide (DMF) to synthesize such products. Performing the reaction using the conditions similar to those reported by Ma and coworkers, the desired product was obtained in 84% yield (Scheme 1).

Scheme 1 Synthesis of α-aminophosphonate derivative **3a**

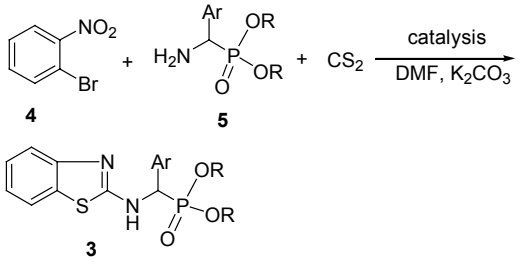


Encouraged by the result, we replaced 2-bromoaniline **1** with 1-bromo-2-nitrobenzene **4** and carried out the reaction using the following reaction conditions: 1-bromo-2-nitrobenzene (1 equiv.), CS_2 (1.5 equiv.), primary 1-aminophosphonate (1.4 equiv.), K_2CO_3 (4 equiv.), $CuCl_2•2H_2O$ (1 equiv.) and $SnCl_2•2H_2O$ (3 equiv.) in DMF. To our delight, the desired product was isolated in 76% yield after reacting for 7 h at 110 ℃. Then, we briefly examined the effect of different temperatures and ratio of **4**/$SnCl_2•2H_2O$. The results showed that at 110 ℃, the reaction preceded smoothly in high

yield. To further evaluate the influence of the ratio of **4**/$SnCl_2$•$2H_2O$, the reaction was carried out in DMF at 110 ℃ using a 1∶1 to 1∶5 ratio of **4**/$SnCl_2$•$2H_2O$, leading to **3a** in 29%, 43%, 74%, 84%, and 79% yields, respectively. We concluded the best ratio of **4**/$SnCl_2$•$2H_2O$ was 1∶4.

With optimized conditions in hand, a series of substituted primary 1-aminophosphonates **5** were investigated, as shown in Table 1. Both electron-donating and -withdrawing primary 1-aminophosphonates could be successfully converted to the corresponding α-aminophosphonates in high yields. In addition, a high level of functional group tolerance was observed, and the efficiency of the reaction was not affected in the presence of halides, ether, and nitro group.

Table 1 Substrate scope of primary 1-aminophosphonates **5**



Entry	Ar	R	Product	Yield[a]/%
1	Ph	Et	**3a**	84
2	*p*-F-Ph	Et	**3b**	83
3	*p*-Cl-Ph	Et	**3c**	85
4	*p*-N(Me)$_2$-Ph	Et	**3d**	85
5	*p*-MeO-Ph	Et	**3e**	84
6	*p*-MeO-Ph	Me	**3f**	81
7[b]	*m*-NO_2-Ph	Et	**3g**	86

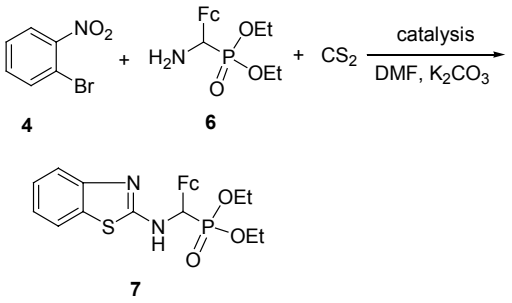
[a] Isolated yields. [b] Reaction conditions: 2-bromoaniline (1 equiv.), CS_2 (1.5 equiv.), primary 1-aminophosphonate (1.4 equiv.), K_2CO_3 (4 equiv.), $CuCl_2$•$2H_2O$ (1 equiv.) in DMF.

To highlight the utility of this transformation, primary 1-aminophosphonate **6** containing ferrocenyl group is subjected to the standard reaction conditions under a nitrogen atmosphere (Scheme 2). The desired product was isolated in good yield (76%). In the case, the purification of the final product has been achieved by column chromatography on cellulose powder.

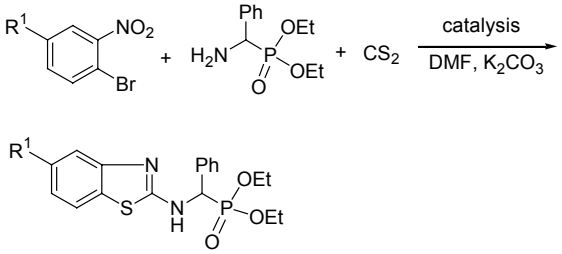
To further explore the potential of our methodology, 4-substituted 1-bromo-2-nitrobenzenes **9** were investigated as shown in Scheme 3. The substrates **9** also provided the expected α-aminophosphonates **8a**—**8c** in high yields under the standard reaction condition (Scheme 3).

A possible mechanism for the formation of the target compounds was proposed and shown in Scheme 4. 1-Bromo-2-nitrobenzene **4** was reduced by tin(II) chloride to 2-bromoaniline **1**, and the dithiocarbamate salt **A** was generated via the reaction of primary 1-aminophosphonate **2** with carbon disulfide in the presence of K_2CO_3. Next, the reaction of dithiocarbamate salts **A** with 2-bromoaniline **1** afforded dithiocarbamates **B**. The amine group of **B** could attack the C-S double bond to generate intermediate **C**, which would deliver the desired products **3** via elimination of hydrogen sulfide with the aid of $CuCl_2$•$2H_2O$.

Scheme 2 Synthesis of α-aminophosphonate derivative **7**

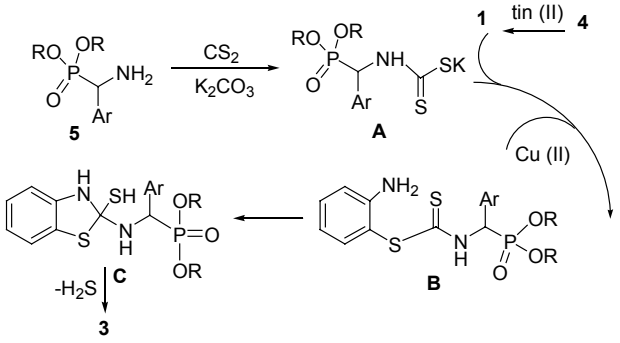


Scheme 3 Synthesis of α-aminophosphonate derivatives **8**



8a: R^1 = Me, **8b**: R^1 = OMe,
8c: R^1 = Cl

Scheme 4 Proposed reaction mechanism



The structure of α-aminophosphonate derivatives **3a**—**3g**, **7** and **8a**—**8c** was thoroughly characterized with spectral and elemental analysis. The infrared spectrum of compound **7** displayed bands at 3233, 1220 and 1039 cm^{-1} due to N—H, P=O, and P—O stretching frequencies, respectively. The characteristic bands of the ferrocenyl group in the infrared spectrum of the compound appear at 3089, 1443 and 751 cm^{-1}.[13]

The ^1H NMR spectrum of compound **7** exhibited a multiplet at δ 7.11—7.64, which accounts for the aromatic protons of the benzothiazole ring; a doublet appeared at δ 5.44, which accounts for CH proton; a mul-

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tiplet appeared at δ 3.92—4.42, which accounts for the CH_2 and ferrocenyl protons. The two ethoxys of the phosphonates are magnetically nonequivalent, which is caused by the different shielding effects of α-ferrocenyl on the two ethoxy groups. The mass spectrum displayed the molecular ion $[M+Na]^+$ peak at *m*/*z* 507. All of the nonequivalent carbon atoms were identified in ^{13}C NMR and the total number of protons calculated from the integration curve accorded (in ^1H NMR) with the assigned structures.

Biological activities

The antitumor activities were determined by the standard MTT assay against the cancer cell line HL-60.[14] The screening results expressed as IC_{50} are summarized in Table 2. The IC_{50} values were the average of three independent experiments. The antitumor screening results revealed that some target compounds exhibited moderate to good antitumor activity against HL-60. Compound **3b** bearing a fluorine atom at 4-position of phenyl ring showed moderate cancer inhibitory activities against HL-60 with IC_{50} of 22.4 μmol/L, removal of the fluorine atom of **3b** to give **3a** resulted in loss of potency, and replacement of the fluorine with chlorine atom in the 4-position of phenyl ring rendered lower activity (**3b** vs. **3c**, Table 2). Compounds **3d** and **3e**, which have 4-dimethylamino and 4-methoxyl substituted phenyl rings, respectively, were inactive against HL-60. The antitumor activity of **3f** to HL-60 that was substituted by Me of phosphonate was inactive. Compound **7** bearing a ferrocenyl moiety showed moderate cancer inhibitory activity against HL-60 with IC_{50} of 13.1 μmol/L. Interestingly, compound **3g** bearing a nitro group at 3-position of phenyl ring showed good cancer inhibitory activity against the tested cell line.

From the above results, some interesting structure-activity relationships can be disclosed: the substitution of electron-donating groups at 4-position of phenyl ring is not important for potent activity, and incorporation of an electrowithdrawing group in the phenyl ring leads to significant improvement of potency.

Conclusions

In conclusion, we have developed a novel and efficient synthetic route to α-aminophosphonates containing benzothiazole moiety via a cascade three-component reaction from conveniently available starting materials. The approaches described here obviously have significant advantages in terms of experimental simplicity and easy work-up. The antitumor activities of the target compounds were evaluated against HL-60. Compound **3g** showed good cancer inhibitory activity against the tested cell line. Influence of subtle structural modification and steric parameters on structure activity relationships for identifying lead bioactive compound would be taken up in our future course of investigation.

Table 2 The antitumor activities of the target compounds

Entry	Compound	IC_{50}/(μmol•L^{-1})
1	**3a**	＞100
2	**3b**	22.4±2.0
3	**3c**	35.7±2.5
4	**3d**	＞100
5	**3e**	＞100
6	**3f**	＞100
7	**3g**	8.2±0.3
8	**7**	13.1±1.1
9	**8a**	＞100
10	**8b**	＞100

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