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# Synthesis and Bioactivities of 1,3,2-Benzodiazaphosphorin-2-carboxamide 2-Oxides Containing $\alpha$-Aminophosphonate Groups

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ABSTRACT: *In order to search for novel antitumor and antiviral agents with high activity and low toxicity, a series of 1-ethoxycarbonylmethyl-3-ethyl-1,2,3,4-tetrahydro-4-oxo-1,3,2-benzodiazaphosphorin-2-carboxamide 2-oxides containing α-aminophosphonate groups have been designed and synthesized by a convenient one-pot procedure in good yields. The structures of products were confirmed by $^1H$ NMR, $^{31}P$ NMR, IR spectra, and elemental analyses. The bioassay results showed that some of them possess excellent anti–tobacco mosaic virus activities and exhibit higher inhibitory effects compared with that of the contrast drug 2,4-dioxyhexahydro-1,3,5-triazine.*
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## *INTRODUCTION*

Organophosphorus compounds are ubiquitous in nature and have a broad application in many areas of agriculture and medicine. During the past two decades, $\alpha$-ketophosphonates and their derivatives have attracted considerable attention because these compounds are endowed with special physical, chemical, and pharmacological properties due to the proximity of the carbonyl and the phosphoryl groups [1–4]. On the other hand, as phosphorus analogs of naturally occurring amino carboxylic acids, $\alpha$-aminophosphonic acids have been accorded an increasingly wide interest in chemistry, medicine, and agricultural science. Among them, N-substituted $\alpha$-aminophosphonate derivatives represent a class of compounds that tend to exhibit superior biological activities, such as antibacterial, herbicidal, antitumor, and inhibitory activity to enzymes [5–7]. In a study on new pharmaceuticals and agrochemicals, the application of heterocycles is a very important consideration that can improve the biological activity. Benzoannulated and related analogs of cyclophosphamide possess antitumor activity against lymphoid leukemia in mice [8–10]. As part of our ongoing program aimed at searching for novel antitumor and antiviral agents with high activity and low toxicity, a series of 1-ethoxycarbonylmethyl-3-ethyl-1,2,3,4-tetrahydro-4-oxo-1,3,2-benzodiazaphosphorin-2-carboxamide 2-oxides containing $\alpha$-aminophosphonate groups were designed and synthesized by a convenient one-pot procedure in yields of 57.2–78.6%. The bioassay results showed that some of them possess excellent anti–tobacco mosaic virus (TMV) activities and exhibit higher inhibitory effects compared with that of the contrast drug 2,4-dioxyhexahydro-1,3,5-triazine (DHT).

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## *RESULTS AND DISCUSSION*

### *Synthesis of the Products*

Efficient methodologies for forming the bond connecting the carbonyl and the phosphoryl groups are available in the arsenal of the synthetic chemist [1,11]. However, to the best of our knowledge, there is no general method to synthesize the $\alpha$-ketophosphondiamidates under mild conditions. We herein wish to report a simple and direct method utilizing the addition reaction of the phosphorus reagent containing a P–H bond with isocyanates that were prepared in a one-pot procedure from amines by the action of triphosgene [bis(trichloromethyl) carbonate]. Generally, these reactions are carried out under base-catalyzed conditions. Isocyanates are usually prepared by phosgene gas, which requires the hazards of handling of phosgene and drastic conditions. An improved method for the preparation of isocyanates involves the use of triphosgene that is a safe and stable replacement for phosgene [12]. The title compounds were synthesized by a convenient one-pot procedure, as shown in Scheme 1, in which the appearance of isocyanates **2** and the reaction terminal point were monitored by IR spectroscopy.

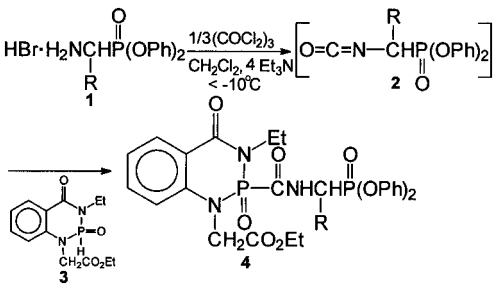
The intermediate **3**, 1-ethoxycarbonylmethyl-3-ethyl-2,3-dihydro-1,3,2-benzodiazaphosphorin-4(1H)-one 2-oxide was prepared according to literature methods [13,14] by the multistep route outlined in Scheme 2. The corresponding data of **3** are given as following: 62% overall yield, m.p. 75–77°C. $^1$H NMR ($CDCl_3$, ppm; *J*, Hz): 1.22–1.36 (m, 6H, $2CH_2CH_3$); 3.79 (m, 2H, $NCH_2CH_3$); 4.09–4.28 (m, 3H, $1/2NCH_2CO_2CH_2CH_3$ + $CO_2CH_2CH_3$); 4.74 (dd, 1H, $1/2PNCH_2CO_2Et$, $^3J_{PH}$ = 8.7, $^2J_{HH}$ = 18.3); 6.60–8.22 (m, 4H, $C_6H_4$); 7.95 (d, 1H, P(O)**H**, $^1J_{PH}$ = 649.8). $^{31}$P NMR ($CDCl_3$, ppm): 5.31 (s). Anal. calcd. for $C_{13}H_{17}N_2O_4P$: C, 52.70; H, 5.78; N, 9.46. Found: C, 52.72; H, 5.84; N, 9.53.

Preparation of the hydrobromides of $\alpha$-aminophosphonates **1** was readily accomplished in a two-step sequence (45–80% overall yield) starting from aldehydes, benzyl carbamate, and triphenyl phosphite as shown in Scheme 3 [15]. Compound **1** reacted smoothly with bis(trichloromethyl) carbonate with the help of four molar equivalents of triethylamine to give isocyanates **2** that then formed the title compounds by the addition with **3**. We could transform **1** into the corresponding diphenyl $\alpha$-aminophosphonates by the reaction of ammonia in dry benzene; nevertheless, our experiment revealed that the yields could not be increased further by the one-pot reaction of diphenyl $\alpha$-aminophosphonates instead of **1** under the same condition.

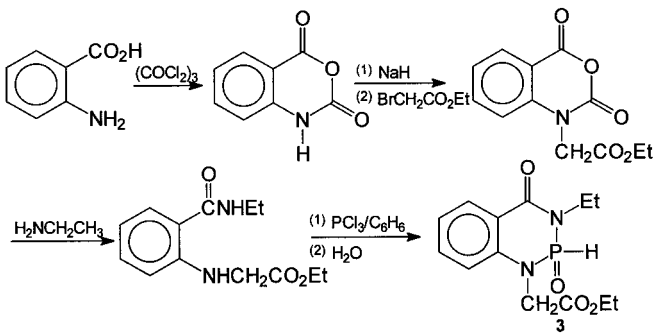
### *The Structures of the Products*

The structures of all of the title compounds were confirmed by $^1$H NMR, $^{31}$P NMR, IR spectra, and elemental analyses. Their physical constants are listed in Table 1, and data of the $^1$H NMR, $^{31}$P NMR, and IR spectra are listed in Table 2 and Table 3.

The title compound **4** may be discussed by recognition of the presence of two chiral centers, for example, CH and P, and consequently existence of two pair of enantiomers (a pair of diastereoisomers) $^{31}$P NMR spectra (Table 3) exhibited doublets of the chiral P in the range of $\delta$ 3.1–4.3 accounting for the pair of diastereoisomers, while **3** gave a single peak at $\delta$ 5.31 due to the presence of only one chiral phosphorus center. The separation of the diastereoisomers was not successful by the column chromatography method. The two diastereoisomers of compound **4a** were separated by partial recrystallization, and their $^{31}$P NMR data were determined as $\delta$ 3.33, 16.83, and $\delta$ 4.25, 16.83 respectively. There is no $^{31}$P–$^{31}$P coupling between the two phosphorus atoms in the title compounds. The ratios of diastereoisomers given in Table 3 were determined by integration of suitable signals in the $^{31}$P NMR spectra of the crude products, which show that, unfortunately, the synthetic reactions are not significantly stereoselective in affording the products. In the $^1$H



**SCHEME 1**



**SCHEME 2**

**TABLE 1** Experimental Data of Products

| *Compound* | *R* | *Yield[a] (%)* | *State m.p. (°C)* | *Molecular Formula* | *Found/Calcd (%)* | | |
|---|---|---|---|---|---|---|---|
| | | | | | *C* | *H* | *N* |
| **4a** | Me | 57.2 | 162–164 | $C_{28}H_{31}N_3O_8P_2$ | 56.00 (56.10) | 5.45 (5.21) | 7.14 (7.01) |
| **4b** | *I*-Pr | 64.5 | syrup | $C_{30}H_{35}N_3O_8P_2$ | 57.15 (57.41) | 5.87 (5.62) | 6.49 (6.70) |
| **4c** | *n*-Bu | 61.2 | syrup | $C_{31}H_{37}N_3O_8P_2$ | 58.12 (58.03) | 6.13 (5.81) | 6.43 (6.55) |
| **4d** | *I*-Bu | 66.5 | syrup | $C_{31}H_{37}N_3O_8P_2$ | 57.89 (58.03) | 6.10 (5.81) | 6.63 (6.55) |
| **4e** | $C_6H_5$ | 78.6 | syrup | $C_{33}H_{33}N_3O_8P_2$ | 59.72 (59.91) | 5.25 (5.03) | 6.13 (6.35) |
| **4f** | *p*-$MeC_6H_4$ | 74.6 | syrup | $C_{34}H_{35}N_3O_8P_2$ | 60.07 (60.45) | 5.49 (5.22) | 5.96 (6.22) |
| **4g** | *p*-$ClC_6H_4$ | 72.5 | syrup | $C_{33}H_{32}ClN_3O_8P_2$ | 56.47 (56.95) | 4.84 (4.63) | 5.77 (6.04) |
| **4h** | 3,4-$OCH_2OC_6H_3$ | 68.4 | syrup | $C_{34}H_{33}N_3O_{10}P_2$ | 57.70 (57.88) | 5.00 (4.71) | 5.81 (5.96) |
| **4i** | 2,4-$Cl_2C_6H_3$ | 67.8 | syrup | $C_{33}H_{31}Cl_2N_3O_8P_2$ | 54.48 (54.26) | 4.55 (4.28) | 5.48 (5.75) |

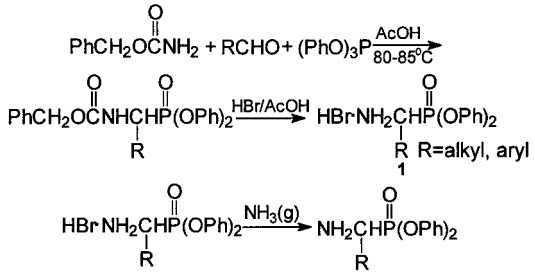
[a]Yield determined by isolation based on **4**.

**TABLE 2** $^1$H NMR Data of Products

| *Compound* | *$^1$H NMR (200 MHz, $CDCl_3$, TMS) $\delta_H$* |
|---|---|
| **4a** | 1.23–1.36 (m, 6H, $2CH_2C\mathbf{H}_3$); 1.45–1.60 (m, 3H, $C\mathbf{H}_3$); 3.69, 3.84 (m, 2H, $NC\mathbf{H}_2CH_3$); 4.05–4.30 (m, 3H, 1/2$NC\mathbf{H}_2CO_2Et$ + $CO_2C\mathbf{H}_2CH_3$); 4.65–4.92 (m, 2H, 1/2$NC\mathbf{H}_2CO_2Et$ + C**H**); 6.58–8.21 (m, 14H, $2C_6\mathbf{H}_5$ + $C_6\mathbf{H}_4$); 8.3, 8.5 (br, 1H, C(O)N**H**) |
| **4b** | 0.82–1.16 (m, 6H, $CH(C\mathbf{H}_3)_2$); 1.21–1.35 (m, 6H, $2CH_2C\mathbf{H}_3$); 2.25 (m, 1H, $C\mathbf{H}(CH_3)_2$); 3.67, 3.83 (m, 2H, $NC\mathbf{H}_2CH_3$); 4.08–4.32 (m, 3H, 1/2$NC\mathbf{H}_2CO_2Et$ + $CO_2C\mathbf{H}_2CH_3$); 4.66–4.93 (m, 2H, 1/2$NC\mathbf{H}_2CO_2Et$ + C**H**); 6.60–8.23 (m, 14H, $2C_6\mathbf{H}_5$ + $C_6\mathbf{H}_4$); 8.3, 8.6 (br, 1H, C(O)N**H**) |
| **4c** | 0.88–1.34 (m, 15H, $2CH_2C\mathbf{H}_3$ + $C\mathbf{H}_2C\mathbf{H}_2C\mathbf{H}_2C\mathbf{H}_3$); 3.67, 3.84 (m, 2H, $NC\mathbf{H}_2CH_3$); 4.07–4.35 (m, 3H, 1/2$NC\mathbf{H}_2CO_2Et$ + $CO_2C\mathbf{H}_2CH_3$); 4.60–4.96 (m, 2H, 1/2$NC\mathbf{H}_2CO_2Et$ + CH); 6.62–8.25 (m, 14H, $2C_6\mathbf{H}_5$ + $C_6\mathbf{H}_4$); 8.4, 8.6 (br, 1H, C(O)N**H**) |
| **4d** | 0.68–0.92 (m, 8H, $C\mathbf{H}_2CH(C\mathbf{H}_3)_2$); 1.22–1.35 (m, 6H, $2CH_2C\mathbf{H}_3$); 1.85 (m, 1H, $CH_2C\mathbf{H}(CH_3)_2$); 3.68, 3.85 (m, 2H, $NC\mathbf{H}_2CH_3$); 4.08–4.36 (m, 3H, 1/2$NC\mathbf{H}_2CO_2Et$ + $CO_2C\mathbf{H}_2CH_3$); 4.61–4.95 (m, 2H, 1/2$NC\mathbf{H}_2CO_2Et$ + C**H**); 6.61–8.26 (m, 14H, $2C_6\mathbf{H}_5$ + $C_6\mathbf{H}_4$); 8.5, 8.7 (br, 1H, C(O)N**H**) |
| **4e** | 1.20–1.32 (m, 6H, $2CH_2C\mathbf{H}_3$); 3.70, 3.91 (m, 2H, $NC\mathbf{H}_2CH_3$); 4.05–4.42 (m, 3H, 1/2$NC\mathbf{H}_2CO_2Et$ + $CO_2C\mathbf{H}_2CH_3$); 4.82 (m, 1H, 1/2$NC\mathbf{H}_2CO_2Et$); 5.80 (m, 1H, C**H**); 6.64–8.25 (m, 19H, $3C_6\mathbf{H}_5$ + $C_6\mathbf{H}_4$); 8.8, 9.2 (br, 1H, C(O)N**H**) |
| **4f** | 1.21–1.34 (m, 6H, $2CH_2C\mathbf{H}_3$); 2.30 (s, 3H, $C_6H_4C\mathbf{H}_3$); 3.69, 3.90 (m, 2H, $NC\mathbf{H}_2CH_3$); 4.03–4.45 (m, 3H, 1/2$NC\mathbf{H}_2CO_2Et$ + $CO_2C\mathbf{H}_2CH_3$); 4.85 (1H, 1/2$NC\mathbf{H}_2CO_2Et$); 5.82 (m, 1H, C**H**); 6.64–8.26 (m, 18H, $2C_6\mathbf{H}_5$ + $2C_6\mathbf{H}_4$); 8.7, 9.1 (br, 1H, C(O)N**H**) |
| **4g** | 1.20–1.33 (m, 6H, $2CH_2C\mathbf{H}_3$); 3.73, 3.92 (m, 2H, $NC\mathbf{H}_2CH_3$); 4.05–4.48 (m, 3H, 1/2$NC\mathbf{H}_2CO_2Et$ + $CO_2C\mathbf{H}_2CH_3$); 4.88 (1H, 1/2$NC\mathbf{H}_2CO_2Et$); 5.85 (m, 1H, C**H**); 6.65–8.24 (m, 18H, $2C_6\mathbf{H}_5$ + $2C_6\mathbf{H}_4$); 8.9, 9.3 (br, 1H, C(O)N**H**) |
| **4h** | 1.19–1.30 (m, 6H, $2CH_2C\mathbf{H}_3$); 3.71, 3.92 (m, 2H, $NC\mathbf{H}_2CH_3$); 4.04–4.45 (m, 3H, 1/2$NC\mathbf{H}_2CO_2Et$ + $CO_2C\mathbf{H}_2CH_3$); 4.85 (1H, 1/2$NC\mathbf{H}_2CO_2Et$); 5.78 (m, 1H, C**H**); 5.89, 5.91 (s, 2H, $OC\mathbf{H}_2O$); 6.59–8.21 (m, 17H, $2C_6\mathbf{H}_5$ + $C_6\mathbf{H}_3$ + $C_6\mathbf{H}_4$); 8.7, 9.2 (br, 1H, C(O)N**H**) |
| **4i** | 1.22–1.34 (m, 6H, $2CH_2C\mathbf{H}_3$); 3.72, 3.94 (m, 2H, $NC\mathbf{H}_2CH_3$); 4.06–4.49 (m, 3H, 1/2$NC\mathbf{H}_2CO_2Et$ + $CO_2C\mathbf{H}_2CH_3$); 4.88 (1H, 1/2$NC\mathbf{H}_2CO_2Et$); 6.05 (m, 1H, C**H**); 6.62–8.25 (m, 17H, $2C_6\mathbf{H}_5$ + $C_6\mathbf{H}_3$ + $C_6\mathbf{H}_4$); 9.0, 9.3 (br, 1H, C(O)N**H**) |

**TABLE 3** $^{31}$P NMR and IR Data of Products

| | $^{31}P$ NMR (80.96 MHz, $CDCl_3$, 85% $H_3PO_4$) | | | IR (In Chloroform, Wavenumbers [$cm^{-1}$]) | | |
|---|---|---|---|---|---|---|
| *Compound* | $\delta_P$ *of Product (Ratio of Diastereoisomers)* | | | *(N–H)* | *(C=O)* | *(P=O)* |
| **4a** | 3.33, 16.83; | 4.25, 16.83 | (0.98:1) | 3458 | 1665, 1740 | 1325, 1240 |
| **4b** | 3.45, 16.00; | 3.83, 16.00 | (0.97:1) | 3450 | 1672, 1742 | 1328, 1242 |
| **4c** | 3.32, 16.33; | 4.00, 16.33 | (0.97:1) | 3452 | 1668, 1738 | 1332, 1248 |
| **4d** | 3.16, 16.78; | 3.98, 16.78 | (0.96:1) | 3460 | 1675, 1735 | 1330, 1245 |
| **4e** | 3.30, 12.63; | 3.80, 12.45 | (0.94:1) | 3468 | 1678, 1745 | 1335, 1250 |
| **4f** | 3.36, 12.87; | 3.87, 12.67 | (0.93:1) | 3470 | 1674, 1745 | 1340, 1252 |
| **4g** | 3.11, 12.05; | 3.69, 11.92 | (0.93:1) | 3465 | 1670, 1742 | 1342, 1250 |
| **4h** | 3.24, 12.56; | 3.83, 12.40 | (0.92:1) | 3475 | 1675, 1746 | 1345, 1254 |
| **4i** | 3.11, 11.34; | 3.62, 11.14 | (0.93:1) | 3472 | 1680, 1745 | 1340, 1255 |




**SCHEME 3**

NMR spectra of **4**, the two methylene protons in the 3-ethyl group of the benzodiazaphosphorine resonated as two multiplets at $\delta$ 3.67–3.73 and $\delta$ 3.83–3.94, respectively. They are magnetically nonequivalent due to their orientation in the six-membered conformation of the benzadiazaphosphorine, which has been described in previous articles [13,14]. With regard to the corresponding methylene group attached to the 1-position of the benzadiazaphosphorine, the signals appeared as two multiplets at $\delta$ 4.0–4.5 and $\delta$ 4.6–5.0 because of the same reason. The chemical shift of the H atom in CH (R = aryl) is in the range of $\delta$ 5.78–6.05. Owing to the deshielding effect of the $\alpha$-(substituted) benzene ring, these chemical shifts are at much larger values of $\delta$ than those of CH (R = alkyl), which are in the range of $\delta$ 4.6–5.0.

The infrared spectra of compounds **4a–i** (taken in chloroform) showed normal stretching absorption bands that indicate the existence of the groups N–H (3450–3475 $cm^{-1}$), amide carbonyl (1665–1680 $cm^{-1}$), ester carbonyl (1735–1746 $cm^{-1}$), P=O (in benzodiazaphosphorine, 1325–1345 $cm^{-1}$), and P=O (in $\alpha$-aminophosphonate, 1240–1255 $cm^{-1}$).

### *Biological Activities*

The preliminary bioassay of the products in acetone with the concentration of $100 \times 10^{-6}$ against the TMV was surveyed, and the results are listed in Table 4. In contrast, the inhibition rate of DHT (2,4-dioxyhexahydro-1,3,5-triazine), one commercially used virucide, is 45%. The compounds **4g**, **4h**, and **4i** possess good anti-TMV activities and exhibit higher inhibitory effects compared with that of the contrast drug DHT.

## *EXPERIMENTAL*

### *Instruments*

Melting points were determined with a model YANACO MP-500 apparatus and are uncorrected. IR spectra were recorded on a SHIMADZU-435 spectrometer, and band positions are reported in wavenumbers ($cm^{-1}$). The $^1$H and $^{31}$P NMR spectra were recorded on a BRUKER AC-P200 instrument. Tetramethylsilane (TMS) was used as an internal standard for $^1$H NMR, and 85% phosphoric acid ($H_3PO_4$) was used as an external standard for $^{31}$P NMR spectroscopy. The nuclei that are deshielded relative to their respective standards are assigned a positive chemical shift. Coupling constants, $J$, are given in Hz. Elemental analyses were carried out on a Yana MT-3 instrument. Column chromatography was performed using silica gel H (10–40 $\mu$m, Haiyang Chemical Factory of Qingdao).

### *General Procedure for the Preparation of N-Substituted 1-Ethoxycarbonylmethyl-3-ethyl-1,2,3,4-tetrahydro-4-oxo-1,3,2-benzodiazaphosphorin-2-carboxamide 2-Oxides (**4a–i**)*

A mixture of 3.0 mmol of the hydrobromides of $\alpha$-aminoalkanephosphonates (**1**) and 12.0 mmol of dry triethylamine in 20 mL of anhydrous dichloromethane was added dropwise over a period of 20 minutes to a solution of 1.0 mmol of bis(trichloromethyl)

**TABLE 4** The Antivirus Activity of Products against TMV

| *Compounds* | **4a** | **4b** | **4c** | **4d** | **4e** | **4f** | **4g** | **4h** | **4i** | *DHT*[b] |
|---|---|---|---|---|---|---|---|---|---|---|
| Concentration (ppm) | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Inhibition rate (%) | 40 | 35 | 25 | 30 | 40 | 45 | 55 | 50 | 65 | 45 |

[b]2,4-dioxyhexahydro-1,3,5-triazine as contrast drug.

carbonate in 10 mL of anhydrous dichloromethane at the temperature of −10°C (ice-salt bath). After completion of the addition, the temperature of the reaction mixture was maintained at 0°C for half an hour, and the appearance of the isocyanate groups, N=C=O (2200–2300 $cm^{-1}$) was monitored by IR spectroscopy. Then, another solution of 3.0 mmol of 1-ethoxycarbonylmethyl-3-ethyl-2,3-dihydro-1,3,2-benzodiazaphosphorin-4(1H)-one 2-oxide (**3**) in 10 mL of anhydrous dichloromethane was added dropwise again. The mixture continued to react at ambient temperature for 2 hours. Reaction terminal points could be detected by the disappearance of the isocyanate group in IR spectroscopy. Triethylamine hydrochloride and hydrobromide was filtered off, and the solvent was evaporated from the filtrate under reduced pressure to produce the crude product, which was purified by column chromatography on silica gel, the eluent solvent being ethyl acetate / light petroleum (b.p. 60–90°C) (v/v, 1:1). The physical and chemical data of compounds **4a–i** are listed in Tables 1–3.

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