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Synthesis and Properties of Novel Amino Acid Linked Phosphoryl Nitrogen Mustard Derivatives

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ABSTRACT

In order to look for novel antitumor drugs and virucides with high activity and low toxicity, a series of amino acid linked phosphoryl nitrogen mustard derivatives have been synthesized. The structures of all compounds prepared were confirmed by ^1H NMR, ^{31}P NMR, IR, MS spectroscopy, and elemental analyses. In the case of dl-α-amino acid derivatives, the presence of multiple chiral centers in the target products leads to the generation of diastereoisomers, which can be detected by spectroscopic and analytical (TLC) methods. Preliminary bioassays indicate that some of compounds **5** *have high inhibitory activities against some tumor cells and tobacco mosaic virus (TMV). In addition, some of them display certain herbicidal activities and significant fungicidal activities against wheat leaf rust.*

INTRODUCTION

Endoxan, one of the most effective antitumor agents prepared by Arnold and Bourseaux [1], has become very widely used in cancer chemotherapy [2]. However, acrolein, metabolized from it by the action of hepatic mixed function oxidases in the liver, is toxic to the urinary system [3]. We wondered whether phosphoramidate derivatives bearing an (esterified) amino acid group would improve the antineoplastic properties. The attachment of an amino acid to a drug is an effective vehicle for enhanced cellular uptake [4]. On the other hand, it may lead to certain limitations of the toxicity [5] and an enhancement of chemotherapeutic properties [6].

For the purpose of searching for new, effective antitumor drugs and antivirals, we designed and synthesized a number of novel phosphoramidates **5**. Preliminary biological tests show that some of these compounds have good antitumor activity and potent activity against tobacco mosaic virus (TMV).

RESULTS AND DISCUSSION

Synthesis of N',N' -Bis (2-chloroethyl) O-Aryl N^3-Ethoxycarbonyl Substituted Methylene Thioureido Phosphoramidates

The title compounds **5** were synthesized by a multistep route outlined in Scheme 1.

The reactions of isothiocyanato phosphoramidates **3** with α-amino acid esters **4** were carried out under mild conditions to give yields of products of 46–61%. It was found that two main factors affected the conversion of **3** to **5**. One is the apparent steric hindrance of the system, especially attributable to the nitrogen mustard group and the bulky side chain of the amino acid. The other one, which is predominant, is the electronic effect of the α-substituents of the amino acids. Thus, the nucleophilic addition reaction of a carboxyl-protected amino acid bearing an electron-donating side chain, such as dl-isoleucine ester, with the intermediates **3** proceeded more smoothly. All the products **5** were purified by recrystallization except for **5j**, which was purified by flash column chromatography on silica gel.

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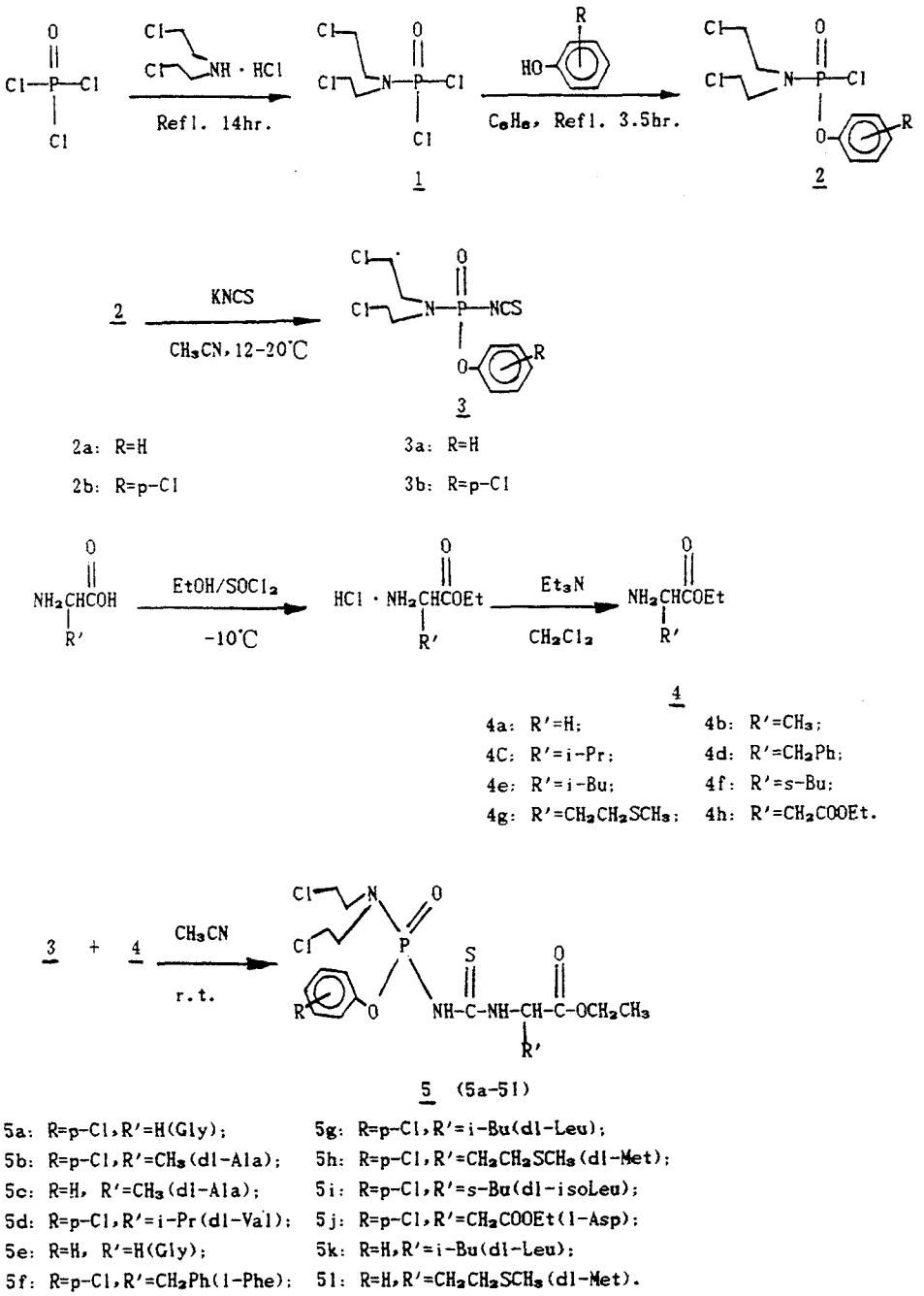


1

2

3

2a: R=H
2b: R=p-Cl

3a: R=H
3b: R=p-Cl

4

4a: R'=H; 4b: R'=CH3;
4C: R'=i-Pr; 4d: R'=CH2Ph;
4e: R'=i-Bu; 4f: R'=s-Bu;
4g: R'=CH2CH2SCH3; 4h: R'=CH2COOEt.

5 (5a-5l)

5a: R=p-Cl, R'=H(Gly);
5b: R=p-Cl, R'=CH3(dl-Ala);
5c: R=H, R'=CH3(dl-Ala);
5d: R=p-Cl, R'=i-Pr(dl-Val);
5e: R=H, R'=H(Gly);
5f: R=p-Cl, R'=CH2Ph(l-Phe);
5g: R=p-Cl, R'=i-Bu(dl-Leu);
5h: R=p-Cl, R'=CH2CH2SCH3(dl-Met);
5i: R=p-Cl, R'=s-Bu(dl-isoLeu);
5j: R=p-Cl, R'=CH2COOEt(l-Asp);
5k: R=H, R'=i-Bu(dl-Leu);
5l: R=H, R'=CH2CH2SCH3(dl-Met).

SCHEME 1

Structures of the Products

The molecular structures of the products **5a–l** were confirmed by ^{1}H NMR, IR, MS, and ^{31}P NMR spectroscopy and by elemental analyses. The experimental data for **5a–l** are listed in Table 1.

In the ^{1}H NMR spectra of compounds **5**, the methylene protons of the nitrogen mustard group appear as a set of characteristic complex absorption peaks in the range of δ 3.32–3.78. Noteworthy, both the splitting and phosphorus coupling of many protons in the corresponding products of l- and of dl-α-amino acids are much different due to the diastereoisomerism of the latter. Thus, the methyl and methylene protons of the ethoxy group, the NH proton in P–N–H, and the NH proton in C–N–H of **5a**, **5e**, **5f**, and **5j** appear as a triplet (δ = 1.18–1.32), a quartet (δ = ~4.17), a doublet (δ = 7.1–7.9, $^2J_{PNH}$ = 7.4–8.6 Hz), and another doublet (δ = 8.98–9.3, $^3J_{HNCH}$ = 7.2–7.4 Hz), respectively. In

TABLE 1 Experimental Data of Compounds **5**[a]

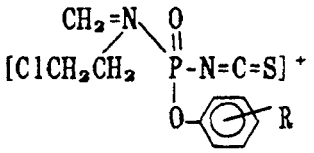
Number	*Yield (%)*	*State*	*(mp °C)*	*^{1}H NMR (TMS, $CDCl_3$, δ, ppm)*[b]	*^{31}P NMR*[c] *(δ, ppm)*
5a	52.6	solid	(131–132)	1.32 (t, 3H, CH_3), 3.36–3.76 (m, 8H, $2ClCH_2CH_2$), 4.12–4.36 (m, 4H, $2CH_2$), 7.08–7.4 (m, 4H, 4Ph–H), 7.9 (d, 1H, NH, J = 7.4 Hz), 8.92–9.12 (w, 1H, NH)	1.785
5b	54.8	solid	(143–144)	1.0–1.62 (m, 6H, $2CH_3$), 3.32–3.76 (m, 8H, $2ClCH_2CH_2$), 4.22 (q, 2H, CH_2), 4.86 (m, 1H, CH), 7.12–7.42 (m, 4H, 4Ph–H), 8.18 (d, 1H, NH, J = 7.6 Hz), 9.0–9.2 (w, 1H, NH)	1.030 and 0.725 (3.76:1)
5c	53.9	solid	(126–127)	1.20 (t, 3H, CH_3), 1.44 (d, 3H, CH_3), 3.32–3.72 (m, 8H, $2ClCH_2CH_2$), 4.16 (q, 2H, CH_2), 4.82 (m, 1H, CH), 7.14–7.42 (m, 5H, 5Ph–H), 7.81 (d, 1H, NH, J = 7.6 Hz), 9.10 (d, 1H, NH, J = 7.2 Hz)	—[d]
5d	61.9	solid	(125)	0.82–0.99 (m, 6H, $2CH_3$), 1.2 (m, 3H, CH_3), 2.4 (m, 1H, CH), 3.4–3.68 (m, 8H, $2ClCH_2CH_2$), 4.18 (m, 2H, CH_2), 4.82 (m, 1H, CH), 7.10–7.36 (m, 4H, 4Ph–H), 7.8 (w, 1H, NH), 9.2 (q, 1H, NH, J = 7.11 Hz)	1.091 and 0.916 (0.91:1)
5e	49.6	solid	(116)	1.30 (t, 3H, CH_3), 3.42–3.78 (m, 8H, $2ClCH_2CH_2$) 4.1–4.4 (m, 4H, CH_2O, $CH_2C(O)$), 7.2–7.4 (m, 5H, 5Ph–H), 7.7 (d, 1H, NH, J = 8.6 Hz), 9.0–9.2 (w, 1H, NH)	—
5f	51.4	solid	(132–133)	1.23 (t, 3H, CH_3), 3.16 (t, 2H, CH_2), 3.48–3.66 (m, 8H, $2ClCH_2CH_2$), 4.17 (q, 2H, CH_2), 5.12 (q, 1H, CH), 7.07–7.31 (m, 5H, 4Ph–H, NH), 8.98 (d, 1H, NH, J = 7.40 Hz)	1.837
5g	58.7	solid	(118–119)	0.85–0.95 (m, 6H, $2CH_3$), 1.22–1.32 (m, 3H, CH_3), 1.32–1.98 (m, 3H, CH_2, CH), 3.50–3.65 (m, 8H, $2ClCH_2CH_2$), 4.16–4.24 (m, 2H, CH_2), 4.8–4.9 (m, 1H, CH), 7.19–7.34 (m, 4H, 4Ph–H), 7.98 (d, 1H, NH, J = 7.6 Hz), 9.1 (d, 1H, NH, J = 7.40 Hz)	—
5h	54.7	solid	(139)	1.24–1.29 (m, 3H, CH_3), 2.05–2.10 (ds, 3H, CH_3), 1.8–2.6 (m, 4H, $2CH_2$), 3.36–3.72 (m, 8H, $2ClCH_2CH_2$), 4.19–4.25 (m, 2H, CH_2), 4.90 (q, 1H, CH), 7.21–7.32 (m, 4H, 4Ph–H), 7.6 (w, 1H, NH), 9.3 (q, 1H, NH, J = 7.2 Hz)	0.952 and 0.901 (1.04: 1)
5i	60.2	solid	(134)	0.7–0.96 (m, 6H, $2CH_3$), 1.2–1.31 (m, 3H, CH_3), 1.0–1.6 (m, 2H, CH_2), 1.9–2.1 (m, 1H, CH), 3.52–3.63 (m, 8H, $2ClCH_2CH_2$), 4.17–4.22 (m, 2H, CH_2), 4.85 (m, 1H, CH), 7.22–7.34 (m, 4H, 4Ph–H), 7.76–7.9 (w, 1H, NH), 9.2 (q, 1H, NH, J = 7.64 Hz)	—
5j	45.8	syrup		1.18–1.32 (m, 6H, $2CH_3$), 3.0–3.2 (m, 2H, $CH_2C(O)$), 3.4–3.66 (m, 8H, $2ClCH_2CH_2$), 4.1–4.3 (m, 4H, $2CH_2O$), 5.3 (m, 1H, CH), 7.14–7.36 (m, 5H, 4Ph–H, NH), 9.3 (d, 1H, NH, J = 7.2 Hz)	−0.262
5k	59.8	solid	(131–132)	0.83–0.96 (m, 6H, $2CH_3$), 1.22–1.32 (m, 3H, CH_3), 1.32–1.98 (m, 3H, CH_2, CH), 3.36–3.67 (m, 8H, $2ClCH_2CH_2$), 4.18 (m, 2H, CH_2), 4.8 (m, 1H, CH), 7.20–7.36 (m, 5H, 5Ph–H), 7.5–7.7 (w, 1H, NH), 9.0 (q, 1H, NH, J = 7.40 Hz)	—
5l	56.2	solid	(122–123)	1.29 (t, 3H, CH_3), 2.03 (s, 3H, CH_3), 1.98–2.40 (m, 4H, CH_2CH_2), 3.4–3.72 (m, 8H, $2ClCH_2CH_2$), 4.22 (q, 2H, CH_2), 5.06 (q, 1H, CH), 7.26–7.4 (m, 6H, 5Ph–H, NH), 9.2 (d, 1H, NH, J = 7.4 Hz)	—

[a]Satisfactory microanalyses obtained: C, ±0.32; H, ±0.29; N, ±0.27.
[b]^{1}H NMR spectra of **5a**, **5b**, **5c**, and **5e** were recorded with a JEOL-FX-90Q spectrometer, others with a BRUKER AC-P200 spectrometer.
[c]The ratio of the diastereomers is given in parentheses from the relative intensities of ^{31}P NMR signals, which were recorded with a BRUKER AC-P200 spectrometer.
[d]Data were not recorded.

contrast, the protons in the counterpart mentioned previously of the products from dl-amino acids displayed as multiplets, multiplets, a broad peak, or multiplets and a quartet ($^3J_{HNCH}$ = 7.11–7.64 Hz), respectively. To our great interest, the ^{31}P NMR spectrum revealed a single peak for the compounds containing a glycine ester or l-amino acid ester but double peaks for the corresponding products of a dl-amino acid. In the case of dl-amino acid derivatives, the presence of double chiral centers in the target products leads to the generation of diastereomers, which can be observed by TLC and ^{31}P NMR. A separation was not possible; however, from ^{31}P NMR spectra, the ratio of the diastereomers could be determined.

The IR spectra of compounds **5** showed normal stretching absorption bands, indicating the existence of the groups NH (3239–3399 cm^{-1}), $\diagdown C{=}O$ (~1732 cm^{-1}), $\diagdown C{=}S$ (~1328 cm^{-1}), $-P{=}O$ (1196–1228 cm^{-1}), Ar C=C (~3092, 1482–1546 cm^{-1}), and P–N–C (912–1192 cm^{-1}).

The EI mass spectra of compounds **5** demonstrate the existence of the weak molecular ion peaks (M^+) and (M^+ + 2) peaks. The main fragmentation products of **5** are $[R'-CH=NH_2]^+$ (R': the side chain of α-amino acid), $[NH_2=CH-COOEt]^+$, $[NH_2=CH-COOH]^+$,



and $[O=P\equiv O]^+$, which are obtained via a four-membered H-rearrangement and its further cleavage. All fragmentation ions are consistent with their structures and can be clearly assigned.

Biological Activities

The preliminary biological activities were determined for some of compounds **5**. The antitumor activities given in Table 2 indicate that some of them have high inhibitory effects against human gastric carcinoma (MGC) cells and human leukemia$_{60}$ (HL_{60}) cells. Furthermore, it was found that some of compounds **5** display good inhibitory activities against TMV; the results are listed in Table 3. In addition, compounds **5** possess certain herbicidal activities (**5c**: the inhibition rate against rape for 1.5 kg/ha is 53.4%) and significant activities against wheat leaf rust (**5h**: IR for 500 ppm is 80%).

EXPERIMENTAL

Instruments

Elemental analysis was performed with a CHNCORDERD MT-3 elementary analyzer. Mass spectra were recorded with a VG-7070E spectrometer using the GAB method. NMR spectra were recorded with a JEOL-FX-90Q spectrometer and BRUKER AC-P200. TMS was used as an internal standard for ^1H NMR, and 85% H_3PO_4 was used as an external standard for ^{31}P NMR. IR spectra were measured by using a SHIMA DZU-435 instrument. Melting points were determined with a model YANACO MP-500 apparatus and are uncorrected. Column chromatography was performed on silica gel H (10–40 μ, Hai Yang Chemical Factory of Qingdao).

α-Amino acids were available commercially and used without purification. All the reaction solvents were anhydrous and were dried according to conventional methods before use.

Bis(2-chloroethyl)aminophosphoryl Dichloride (**1**)

A mixture of dry bis(2-chloroethyl)amine hydrochloride (30.0 g, 0.17 mol) [7] and phosphoryl chloride (78 mL, 0.81 mol) was heated under reflux at 120–140°C for 14 hours. Excess phosphoryl chloride was evaporated under reduced pressure to yield the crude product that was crystallized from acetone-petroleum ether to give colorless crystals (34.0 g, 78.2%), mp 53–54°C (Ref. [8]: yield 80%, mp 53–56°C). ^{31}P NMR ($CDCl_3$): 15.8. ^1H NMR ($CDCl_3$): 3.42–3.84 (m, 8H, $2ClCH_2CH_2$). ^{13}C NMR: 49.2 (d, CH_2N, J = 4.1), 40.6 (d, CH_2Cl, J = 2.7).

O-Phenyl N,N-Bis(2-chloroethyl)aminophosphoryl Chloride (**2a**)

A solution of 1.24 g (12.3 mmol) of dry triethylamine in 10 mL of anhydrous benzene was added dropwise to a solution of 3.0 g (11.6 mmol) of *N,N*-bis(2-chloroethyl)aminophosphoryl dichloride and 1.06 g (11.6 mmol) of phenol in 20 mL of anhydrous benzene under reflux over 40 minutes. Then, reflux (80–100°C) was continued for 3.5 hours, and triethylamine hydrochloride was filtered off. The filtrate was concentrated under reduced pressure, and the residue was purified by flash chromatography on a silica gel column using a mixture of ethyl ether and petroleum ether (1:4.5 v/v) as the eluent. The product was obtained as a light yellowish syrup, 2.85 g, yield 77.7%. ^1H NMR ($CDCl_3$): 3.40–3.88 (m, 8H, $2ClCH_2CH_2$), 7.12–7.60 (m, 5H, 5Ph–H).

Similarly, O-*p*-chlorophenyl *N,N*-bis(2-chloroethyl)aminophosphoryl chloride (**2b**) was prepared in a yield of 74.8%. ^1H NMR ($CDCl_3$): 3.40–3.79 (m, 8H, $2ClCH_2CH_2$), 7.20–7.64 (m, 4H, 4Ph–H).

α-Amino Acid Esters (**4a–h**)

General procedure. Each α-amino acid ester hydrochloride was prepared from the appropriate

TABLE 2 Antitumor Activities of Some of Compounds **5**

Compounds	**5a**	**5b**	**5c**	**5d**	**5f**	**5g**	**5h**	**5i**	**5d**	**5g**	**5h**	**5i**
Cell line	MGC	MGC	MGC	MGC	MGC	MGC	MGC	MGC	HL_{60}	HL_{60}	HL_{60}	HL_{60}
Time (hours)	72	72	72	72	72	72	72	72	72	72	72	72
IC_{50} ($\times 10^{-5}$ M)	9.0	6.0	8.0	7.0	8.0	8.0	8.0	8.0	0.95	6.14	2.24	2.71

TABLE 3 Antivirus Activity of Some of **5** against TMV

Compounds	**5b**	**5c**	**5d**	**5f**	**5g**	**5h**	**5k**	**5l**
Concentration (ppm)	100	100	100	100	100	100	100	100
Inhibition rate (%)	40	36	64	60	35	62	62	70

α-amino acid by the method given in the literature [9]. Then, the hydrochloride was neutralized. To a solution of each α-amino acid ester hydrochloride in 20 mL of CH_2Cl_2 was added dropwise an equimolecular amount of triethylamine at room temperature, the mixture stirred for 3–4 hours, then filtered. After the removal of the solvent, the residue was extracted with anhydrous benzene, and the extract was rotarily evaporated under reduced pressure to give each intermediate **4a–h**.

N',N'-Bis(2-chloroethyl) O-Aryl N^3-Ethoxycarbonyl Substituted Methylene Thioureido Phosphoramidates (**5**)

General Procedure. To a solution of 4.0 mmol of *O*-aryl *N,N*-bis (2-chloroethyl)aminophosphoryl chloride (**2**) in 30 mL of anhydrous acetonitrile was added 8.2 mmol of potassium thiocyanate at 12–20°C. The reaction mixture was kept at this temperature for 8–12 hours until the phosphoryl chloride (**2**) disappeared, this being monitored by TLC. After vacuum evaporation, the residue was extracted with anhydrous benzene and the extract was rotarily evaporated under reduced pressure, and then 15 mL of acetonitrile was added. The mixture was reacted with an equimolecular amount of α-amino acid ester (**4**) at room temperature for 6–15 hours. After the removal of the solvent, the residue was purified by crystallization or by column chromatography.

Solvents for recrystallization of **5a**, **5d–e**, **5g–i**: chloroform-ethyl ether; for **5b–c**, **5f**: ethyl ether-petroleum ether; and for **5k–l**: acetone-ethyl ether.

5j was purified by column chromatography on silica gel (ethyl acetate:petroleum ether 1:3 as the eluent).

The physical and chemical data of compounds **5a–l** are given in Table 1.

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