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Synthesis of Ethyl and Phenyl Amido(Ethoxyphenylalaninyl)Phosphate Compounds

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The synthetic route for preparation of three phosphoramides containing amino acid and their precursors in good yield and mild conditions is reported. This involved the synthesis of the ethyl and phenyl phosphorodichloridate and its reaction with *bis*(β-chloroethyl) amine or diethylamine, followed by the reaction of the products with phenyl alanine ethyl ester hydrochloride. In all the three compounds, because of the presence of chiral centre, the final products were obtained as a mixture of two diastereoisomers, which can be determined using ^{31}P NMR and TLC methods. The structures of all the prepared compounds were confirmed by ^{1}H NMR, ^{31}P NMR, ^{13}C NMR spectroscopy.

Key Words: Amino acid, Phosphoramide, *Bis*(β-chloroethyl)amine.

INTRODUCTION

Phosphoramides shows the anticancer activity containing amino acid moiety have advantage to cyclophosphamide because of releasing the non toxic compounds after hydrolysis in body. The phosphoramides containing the *bis*-(2-chloroethyl)-amino group, are known to be highly effective alkylating agents, which were widely used over years in antitumor chemotherapy[1,2]. Among them the clinically useful anticancer agents such as cyclophosphamide A, phosphamide B and trophosphamide C, (Fig. 1) has been investigated and is well documented[2-4]. These compounds metabolize through similar pathways in body to give phosphoramide mustard D a therapeutic agent and the acrolein toxic compound[5].

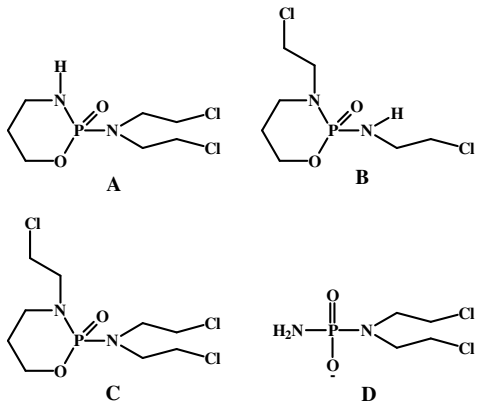

Fig. 1. Cyclophosphamide A, ifosphamide B, trofosphamide C and their metabolize D

McGuigan *et al.*[6] synthesized some phosphoramide derivatives containing an esterified amino acid group from reaction of ethyl phosphorodichloridate and some esters of amino acids to test and decrease byproduct toxicity and also to increase chemotherapeutic properties. The properties of novel amino acid linked phosphoryl nitrogen mustard derivatives containing phosphoramidates of BVdU and thymectacin also investigated[7,8].

This work reports the synthesis of phenyl and ethyl *N,N-bis*(β-chloroethyl)amido(methoxyphenylalaninyl)phosphate as two diasteromeric mixture. ^{31}P NMR spectroscopy was used to determine the ratio of the diastereomers.

EXPERIMENTAL

^{31}P NMR spectra were recorded on a varian DPX300 spectrometer operating at 101.249 MHz and shifts are reported in units of δ relative to 85 % phosphoric acid as external standard, positive shifts are downfield. ^{13}C NMR spectra were recorded on a varian DPX300 spectrometer operating at 62.902 MHz and shifts are reported in units of δ relative to TMS. Both ^{13}C and ^{31}P NMR spectra were proton noise decoupled and all signals were singlets unless otherwise stated. ^{1}H NMR spectra were recorded on a varian DPX300 spectrometer operating at 250.132 MHz and are reported in units of δ relative to TMS. All NMR spectra were recorded in $CDCl_3$ and TMS as internal standard or 85 % H_3PO_4 as external standard unless otherwise stated and all coupling constants are reported in Hz. All experiments involving water sensitive reagents were carried out under scrupulously dry conditions. Where needed, anhydrous solvents

and reagents were obtained in the following ways: Et_3N, Et_2O, hexane and CH_2Cl_2, were refluxed over CaH_2 for several hours and distilled. All but Et_3N were further dried over activated 4 Å molecular sieves. All alcohols were distillated onto activated 4Å molecular sieves. The starting materials were prepared as described in the literature[1]. The reactions were monitored by TLC and visualized with UV light followed by development using *n*-hexane and ethyl acetate 7:3 (v/v) as an eluent.

Ethyl phosphorodichloridate (1a): Dry Et_3N (7.48 mL, 53.6 mmol) and EtOH (3.15 mL, 53.6 mmol) in Et_2O (100 mL) were added dropwise with vigorous stirring to phosphoryl chloride (5.0 mL, 53.6 mmol) in Et_2O (100 mL) at -78 ºC under an atmosphere of nitrogen. The mixture was allowed to warm to ambient temperature, stirred for 20 h, then filtered and the solvent removed under reduced pressure to give the crude product. Yield: 85 %; b.p.: 166-168 ºC; ^{31}P NMR: δ = 6.25.

Phenyl phosphorodichloridate (1b): It was prepared as described for (**1a**) above, using C_6H_5OH (5.04 g, 53.6 mmol). The solvent removed under reduced pressure to give the crude product. Yield: 87 %; b.p.: 241-243 ºC; ^{31}P NMR: δ = 4.33.

Ethyl *N,N-bis*(ß-chloroethyl)amidophosphorochloridate (2a): Dry Et_3N (3.42 mL, 24.6 mmol) in Et_2O (50 mL) was added dropwise with vigorous stirring to mixture of ethyl phosphorodichloridate (2.0 g, 12.3 mmol) and $(ClCH_2CH_2)_2NH$. HCl (2.19 g, 12.3 mmol) in Et_2O (50 mL) at -78 ºC under an atmosphere of nitrogen. The mixture was allowed to warm to ambient temperature, stirred for 42 h, then filtered and the solvent removed under reduced pressure. Hexane (150 mL) was added to the residue and the mixture was filtered and solvent removed under reduced pressure and the crude product was purified by column chromatography on silica-gel 60 using a mixture of *n*-hexane and ethyl acetate 7:3 (v/v) as an eluent. Yield: 53 %; b.p.: 117-122 ºC; ^{31}P NMR: δ = 15.59; ^{1}H NMR: δ = 4.3 (2H, q, CH_2OP), 3.6 (4H, m, CH_2N), 3.5 (4H, m, CH_2Cl), 1.4 (3H, t, Me); ^{13}C NMR: δ = 64.48 - 64.58 (d, CH_2OP, *J* = 6.30 Hz), 49.35 - 49.41 (d, CH_2N, *J* = 3.78 Hz), 40.99 - 41.03 (d, CH_2Cl, *J* = 2.52 Hz), 15.19 - 15.32 (d, Me, *J* = 8.19 Hz).

Phenyl *N,N-bis*(ß-chloroethyl)amidophosphorochloridate (2b): Dry Et_3N (3.42 mL, 24.6 mmol) in CH_2Cl_2 (50 mL) was added dropwise with vigorous stirring to mixture of phenyl phosphorodichloridate (2.59 g, 12.3 mmol) and $(ClCH_2CH_2)_2NH$. HCl (2.19 g, 12.3 mmol) in CH_2Cl_2 (50 mL) at -78 ºC under an atmosphere of nitrogen. The mixture was allowed to warm to ambient temperature, stirred for 42 h, then filtered and the solvent removed under reduced pressure. Hexane (150 mL) was added to the residue and the mixture was filtered and solvent removed under reduced pressure, to give the crude product. Yield: 90 %; b.p.: 232-237 ºC; ^{31}P NMR: δ = 11.06, ^{1}H NMR: δ = 7.2-7.4 (5H, m, Ph), 3.5-3.7 (8H, m, CH_2N, CH_2Cl). ^{13}C NMR: δ = 119.90-149.30 (Ph), 49.20-49.30 (d, CH_2N, *J* = 6.30 Hz), 40.90 (CH_2Cl).

One pot synthesis of Phenyl *N,N*-diethylamido phosphorochloridate (2c): Mixture of phenol (9.45 g, 0.1 mol) and pyridine (9.25 mL, 0.11 mmol) in dry benzene (30 mL) was added dropwise with vigorous stirring to mixture of phosphoryl chloride (0.1 mol) in benzene (15 mL) at -20 ºC. To the mixture diethylamine (921.55 mL, 0.2 mmol) was added dropwise and allowed to stirred at room temperature, for 1 h, then filtered. The solvent removed under reduced pressure. And product was purified by column chromatography on silica-gel 60 using a mixture of *n*-hexane and ethyl acetate 7:3 (v/v) as an eluent. Yield: 65 %; b.p.: 212-217 ºC; ^{31}P NMR ($CDCl_3$): δ = 12.42, ^{13}C NMR ($CDCl_3$, 63 MHz): δ = 120-149 (Ph), 39.5 (d, CH_2N, *J* = 6.3 Hz), 12.95 (d, CH_3CH_2N, *J* = 6.3 Hz). ^{1}H NMR ($CDCl_3$, 250 MHz): δ = 7.1-7.2 (5H, m, Ph), 3.1-3.3 (4H, m, CH_2N), 1.0-1.1 (6H, m, CH_3CH_2N).

Ethyl *N,N-bis*(β-chloroethyl)amido(ethoxyphenylalaninyl)phosphate (3a): To a stirred solution of ethyl *N,N-bis*(β-chloroethyl)amidophosphorochloridate (**2a**) (2 g, 0.74 mmol) and phenylalanine ethyl ester hydrochloride (0.16 g, 0.74 mmol) in dry dichloromethane (20 mL) at -78 ºC (dry-ice/aceton) a solution of dry triethylamine (1.5 mmol) in dichloromethane (10 mL) was added dropwise under atmosphere of nitrogen and the mixture was allowed to reach at room temperature for 42 h. The obtained salt was filtered and the solvent was removed under reduced pressure to give the crude product after work up and purification by column chromatography on silica-gelusing a mixture of chloroform as an eluent, then pooling and evaporation of appropriate fractions gave the product as a white solid. Yield: 64 %; m.p.: 187-189 ºC; ^{31}P NMR: δ = 13.87, 13.99; R_f = 0.23, 0.26; ^{1}H NMR: δ = 7.1-7.3 (5H, m, Ph), 4.1-4.2 (4H, m, $COOCH_2CH_3$, $POCH_2CH_3$), 3.9 (1H, q, CHNH), 3.7 (4H, m, CH_2N), 3.4-3.5 (4H, m, CH_2Cl), 2.8-3.1 (2H, 2xd, $PhCH_2$), 2.7 (1H, s, NH), 1.1-1.2 (6H, m, $COOCH_2CH_3$, $POCH_2CH_3$). ^{13}C NMR: δ = 174.60 (C=O), 137 -126 (Ph), 61.30-61.40 (d, CH_2OP, *J* = 6.30 Hz), 55.60 (NHCH), 49.24-49.30 (d, CH_2N, *J* = 3.78 Hz), 42.50 (CH_2Cl), 40.70-40.80 (d, Ph-CH_2, *J* = 6.30 Hz), 16.23-16.36 (d, Me, *J* = 8.19 Hz), 14.20 ($COOCH_2CH_3$).

Phenyl *N,N-bis*(β-chloroethyl)amido(ethoxyphenylalaninyl)phosphate (3b): This was prepared as described for (**3a**) above, using $(ClCH_2CH_2)_2NP(O)(OC_6H_5)Cl$ (0.23 g, 0.74 mmol). Except that two consecutive chromatographic columns were required, with elution by 2.5 % MeOH in $CHCl_3$, then pooling and evaporation of appropriate fractions gave the product as a colourless oil. Yield: 67 %; b.p.: 220-225 ºC; ^{31}P NMR: δ = 10.15, 10.22; R_f = 0.15, 0.17; ^{1}H NMR: δ = 7.2-7.3 (10H, m, Phe, Ph), 4.1-4.3 (2H, m, $COOCH_2CH_3$), 3.3-3.6 (9H, m, CH_2N, CH_2Cl, CHNH), 3.0-3.1 (3H, m, $PhCH_2$, NH), 1.1-1.3 (3H, m, $COOCH_2CH_3$). ^{13}C NMR: δ = 172.81-173.03 (d, C=O, *J* = 13.86 Hz), 120.50-151.10 (Phe, Ph), 61.82-61.96 (d, $COOCH_2CH_3$, *J* = 8.82 Hz), 55.50 (NHCH), 49.42-49.54 (d, CH_2N, *J* = 7.56 Hz), 42.60 (CH_2Cl), 41.11-41.17 (d, Phe-CH_2, *J* = 3.78 Hz), 14.47-14.53 (d, $COOCH_2CH_3$, *J* = 3.78 Hz).

Phenyl *N,N*-diethylamido(ethoxyphenylalaninyl)phosphate (3c): This compound was prepared as described for (**3a**) and (**3b**) above. R_f = 0.43, 0.44; yield: 76 %; b.p.: 184-189 ºC; ^{31}P NMR ($CDCl_3$): δ = 11.29, 11.14; ^{13}C NMR ($CDCl_3$, 63 MHz): δ = 172.516 (C=O), 119.9-150.9 (Phe, Ph), 54.372, 55.031 (NHCH), 39.2 (NCH_2CH_3), 13.730 (NCH_2CH_3), 40.8 (Phe-CH_2), 13.730 ($COOCH_2CH_3$), 60, 60.8 ($COOCH_2CH_3$). ^{1}H NMR ($CDCl_3$, 250 MHz): δ = 7.1-7.3 (10H, m, Phe, Ph), 4.1-4.2 (2H, m, $COOCH_2CH_3$), 3.3-3.6 (5H, m, CH_2N, CHNH), 3.0-3.1 (3H, m, $PhCH_2$, NH), 1.1-1.2 (9H, tt, $COOCH_2CH_3$, CH_3CH_2N).

TABLE-1
^{3}C NMR DATA FOR **2a**, **2b**, **3a** AND **3b** RECORDED AT 62.902 MHz IN $CDCl_3$: PHOSPHORUS–CARBON COUPLING CONSTANT (Hz) ARE GIVEN IN PARENTHESES. MANY PEAKS ARE SPLIT DUE TO DIASTEREOISOMERS; IN THESE CASES THE MEAN δ AND COUPLING CONSTANT ARE GIVEN

Signal	2a	2b	3a	3b
$N\underline{C}H_2CH_2Cl$	49.35-49.41 (3.78)	49.2-49.3 (6.3)	49.24-49.30 (3.78)	49.42-49.54 (7.56)
$NCH_2\underline{C}H_2Cl$	40.99-41.03 (2.52)	40.9	42.5	42.6
NHCH	-	-	55.6	55.5
C=O	-	-	174.6	172.8-173.0
$O\underline{C}H_2CH_3$	-	-	61.0	61.82-61.96 (8.82)
$OCH_2\underline{C}H_3$	-	-	14.2	14.47-14.53 (3.78)
Phe	-	-	136-127	128.9-136.33
Phe-$\underline{C}H_2$	-	-	40.7-40.8	41.11-41.17 (3.78)
P-O-C	64.48-64.58 (6.3)	-	61.3-41.4 (6.3)	-
P-O-C$\underline{C}$	15.19-15.32 (8.19)	-	16.23-16.36 (8.19)	-
P-O-Phe	-	119.9-149.3	-	120.5-151.1

RESULTS AND DISCUSSION

The reaction of ethanol and phenol with phosphoryl chloride in diethyl ether in the presence of triethylamine at -78 ºC gave ethyl phosphorodichloridate (**1a**) and phenyl phosphorodichloridate (**1b**), as a crude product with 70 % and 75 % yields, respectively. Ethyl phosphorodichloridate (**1a**) were allowed to react with *bis*(β-chloroethyl)amine hydrochloride, under similar condition to the first stage, to give the phosphorochloridate (**2a**) with 53 % yield. The phenyl phosphorodichloridate (**1b**) react with *bis*(β-chloroethyl)amine hydrochloride or diethylamine in presence of base (triethylamine or pyridine) at -78 ºC to affored (**2b**) or (**2c**) with 33 % and 35 % yields, respectively. ^{31}P NMR of products changes from 6.25 and 4.33 to upfield and appears at 15.59, 11.06 and 12.42 for (**2a**), (**2b**) and (**2c**) respectively.

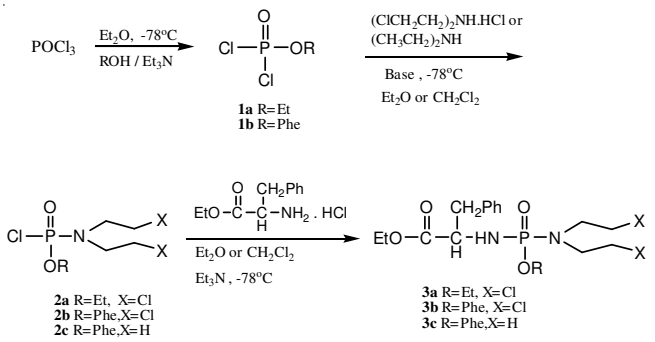

Fig. 2. Reaction pathway to prepare target compounds **3a**, **3b** and **3c**

The ^{31}P, ^{13}C and ^{1}H NMR data were entirely consistent with the proposed structure. Compounds (**2a**), (**2b**) and (**2c**) were allowed to react with chiral (*S*)-phenylalanine ethyl ester hydrochloride in dichloromethane in the presence of triethylamine to give ethyl *N,N-bis*(β-chloroethyl)amido(ethoxyphenylalaninyl)phosphate (**3a**), phenyl *N,N-bis*(β-chloroethyl)-amido(ethoxyphenylalaninyl)phosphate (**3b**) and phenyl *N,N*-diethylamido(ethoxyphenylalaninyl)phosphate (**3c**). It is worth mentioning that in every case the target compounds (**3a**), (**3b**) and (**3c**) were isolated as mixtures of two diastereoisomers corresponding to roughly 60:40, 50:50 and 60:40 mixed stereochemistry, respectively.

The structural assignment of the products was based on their ^{1}H NMR, ^{13}C NMR, ^{31}P NMR spectroscopic data and comparison with literature data[6]. Attempt to separate the mixtures by preparative TLC or by column chromatography was not successful due to the poor resolution of the two diastereoisomers[9]. They are readily distinguished by ^{31}P NMR [δp = 13.87, 13.99], [δp = 10.15, 10.22] and [δp = 11.14, 11.29] respectively. Full ^{13}C NMR data for the compounds (**2**) and (**3**) are given in Table-1. In the ^{13}C NMR of (**2a**) and (**2b**), all resonances displayed phosphorus coupling for ethoxy and chloroethylene groups. The ^{13}C NMR of (**3a**) and (**3b**) were more complicated due to a combination of diastereomeric splitting and phosphorus coupling.

The existence of signals at 13.87, 13.99 ppm and the disappears of signal at 15.5 ppm in the ^{31}P-{^{1}H} NMR spectrum of crude product show the presence of (**3a**) and consumption of phosphate starting material (**2a**). For (**3b**) appearing the signals at 10.15, 10.22 and disappearing the signal at 11.22 indicate consumption of phosphate starting material (**2b**). For (**3c**) presence the signals at 11.14 and 11.29 and absence the signal at 12.42 show the completing the reaction of (**2c**) with amino acid ester. The ^{1}H NMR data were completely consistent with the proposed structures.

Conclusion

The reaction of ethyl and phenyl phosphorodichloridates with *bis*(β-chloroethyl)amine or diehtylamine gives the corresponding phosphorochloridates in a good yield and these three react with phenylalanine ethyl ester hydrochloride to afford ethyl *N,N-bis*(β-chloroethyl)amido(ethoxyphenylalaninyl)phosphate (**3a**), Phenyl *N,N-bis*(β-chloroethyl) amido(ethoxyphenylalaninyl)phosphate (**3b**) and phenyl *N,N*-diethylamido(ethoxyphenylalaninyl)phosphate (**3c**) as a diastereomeric mixtures, in good yields.

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