SYNTHESIS OF 2-[AMINO ACID ESTER / BIS- (2-CHLOROETHYL)AMINO]-6-METHYL-4*H*-1,3,2-DIOXAPHOSPHORINO(5,4-*b*)PYRIDINE 2-SULFIDES

P. Vasu Govardhana Reddy, Y.B. Kiran and C. Suresh Reddy*
Department of Chemistry, Sri Venkateswara University College of Engineering,
Tirupati - 517 502, India

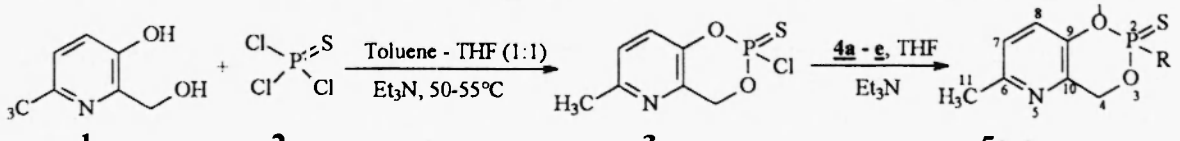
Abstract : Novel 2-[amino acid ester/bis-(2-chloroethyl)amino]-6-methyl-4*H*-1,3,2-dioxaphos-phorino(5,4-b)pyridine 2-sulfides **5a-e** have been synthesized with a two step process starting from 3-hydroxy-6-methyl pyridine methanol **1** and thiophosphoryl chloride **2**. Initially, the intermediate 2-chloro-6-methyl-4*H*-1,3,2-dioxaphosphorino(5,4-*b*)pyridine 2-sulfide **3** was obtained which on subsequent reaction with amino acid ester hydrochlorides **4a-d** and bis-2-(chloroethyl)amine hydrochloride **4e** in dry tetrahydrofuran afforded the title products. The chemical structures were characterized by elemental analyses, IR, ^{1}H, ^{13}C and ^{31}P NMR spectral studies.

Introduction

Phosphoramide derivatives bearing an esterified amino acid group on phosphorus display useful antineoplastic properties (1-4). Recently Mc Guigan reported that phosphorus triester derivatives of AZT bearing amino acid moieties have shown anti HIV activity (5). 1-Ethoxy carbonyl methyl-3-ethyl-1,2,3,4-tetrahydro-4-oxo-1,3,2-bezoxazaphosphorin 2-carboxamide containing α-aminophosphate groups or α-amino acid esters found to possess significant antiviral activity against Tobacco Mosaic Virus (6). Misiura *et al* (7) synthesized [N-3-bis-(2-chloro-1,1-dideuteroethyl) tetrahydro-2*H*-1,3,2-oxazaphosphorin 2-amine 2-oxide with anticipation of anticancer activity for it. In view of the possible potential bioactivity, the title compounds were synthesized.

Results and Discussion

Synthesis of 2-chloro-6-methyl-4*H*-1,3,2-dioxaphosphorino(5,4-*b*)pyridine 2-sulfide **3** was achieved by reacting 3-hydroxy-6-methyl pyridine methanol **1** with thiophosphoryl chloride **2** in the presence of triethylamine in dry toluene-tetrahydrofuran (1:1) mixture at 50-55°C. The intermediate monochloride **3** was reacted with glycine methyl ester hydrochloride **4a**, alanine methyl ester hydrochloride **4b**, anthranilic acid methyl ester hydrochloride **4c**, glycine ethyl ester hydrochloride **4d** and bis-(2-chloroethyl)amine hydrochloride **4e** in dry tetrahydrofuran in the presence of triethylamine at room temperature for **5a-b** and **5d** and at 40-45°C for **5c** and **5e**. Progress of the reaction was monitored by TLC analysis. The title compounds **5a-e** were purified by flash chromatographic method using hexane-ethyl acetate step gradient mixture as eluents (Scheme 1). Reaction yields, elemental analyses, IR and ^{31}P NMR spectral data of **5** are given in Table 1. ^{1}H and ^{13}C NMR spectral data of **5** are presented in Tables 2 and 3 respectively.



1 **2** **3** **5a-e**

4a-d : Amino acid ester hydrochlorides
4e : Bis-(2-chloroethyl)amine hydrochloride

	R
5a	$HN\text{-}CH_2\text{-}COOCH_3$
5b	$HN\text{-}CH\text{-}COOCH_3$
5c	$HN\text{-}C_6H_4\text{-}COOCH_3(2')$
5d	$HN\text{-}CH_2\text{-}COOCH_2CH_3$
5e	$N(CH_2CH_2Cl)_2$

Scheme 1

All members of **5** exhibited characteristic IR absorption bands (Table 1) (8) in the regions for 725-755 cm^{-1} for (P=S), 3168-3260 cm^{-1} for (P-NH) and 1721-1744 cm^{-1} for (C=O).

The methylene (4-CH_2) protons of compounds **5a-e** (Table 2) resonated downfield due to the effect of pyridine ring and appeared as two multiples at δ 4.35-5.28 and 5.01-5.60 respectively. Further, appearance of two multiplets for the C-4 methylene protons indicates that they are magnetically non-equivalent because of their orientation in axial and equatorial positions in the six-membered chair conformation of the dioxaphosphorin ring (Figure 1) and are coupling with phosphorus (9). The two aromatic protons 7-H and 8-H appeared as doublets in the region δ 7.13-7.31 (J = 7.9-8.8 Hz) and 7.07-7.19 (J = 7.4-8.3 Hz) respectively due to their *ortho* coupling..

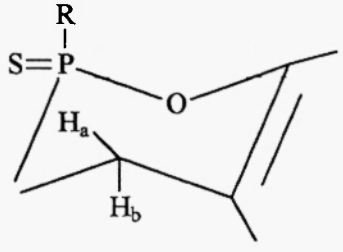


Figure 1

^{13}C NMR chemical shifts of **5a-e** are given in Table 3. A low intense downfield signal at 151.7-153.6 ppm was assigned to C-6 which is deshielded due to its attachment with nitrogen. C-9 of the pyridine ring resonated at 138.6-140.2 ppm. C-10 signal appeared down field at 143.1-144.9 ppm because it is *ortho* to nitrogen. The methylene carbon (C-4) of the heterocycle gave a doublet in the region 50.8-67.9 ppm (J = 5.6-6.0 Hz) because of its coupling with phosphorus (10). The C-11 of the methyl group gave a singlet in the region 22.5-23.6 ppm.

^{31}P NMR spectral data of the compounds **5a-e** are furnished in Table 1. Phosphorus chemical shifts in the amino acid ester groups **5a-d** were observed at 62.08 to 66.58 ppm whereas the nitrogen mustard compound **5e** showed ^{31}P signal at 1.92 ppm. These values are in good agreement with the reported values of similar compounds (11, 12).

Experimental

The melting points were determined on a Mel-Temp apparatus and were uncorrected. Elemental analyses were performed at the Central Drug Research Institute, Lucknow, India. IR spectra were recorded as KBr pellets on a Perkin-Elmer 1000 unit. The ^{1}H, ^{13}C and ^{31}P NMR

spectra were taken on AMX 400 MHz NMR spectrometer operating at 400 MHz for ^{1}H, 100MHz for ^{13}C and 161.89 MHz for ^{31}P NMR NMR data were recorded in $CDCl_3$ or DMSO-d_6 and were referenced to TMS (^{1}H and ^{13}C) and 85% H_3PO_4 (^{31}P).

Amino acid methyl ester hydrochlorides **4a-d** were prepared according to the reported procedure (13), 3-hydroxy-6-methyl pyridine methanol **1** and bis-(2-chloroethyl)amine hydrochloride **4e** were procured from Aldrich Chemical Company, Inc., USA and were used without further purification.

Synthesis of 2-chloro-6-methyl-4*H*-1,3,2-dioxaphosphorino(5,4-*b*)pyridine 2-sulfide 3

A solution of thiophosphoryl chloride (**2**,0.85 g, 0.05 mol) in 30 mL of dry toluene-tetrahydrofuran mixture (1:1) was added to the cooled (0°C) and stirred solution of 6-methyl-3-hydroxy-2-pyridine methanol (**1**, 0.66 g, 0.005 mol) and triethylamine (1.01 g, 0.01 mol) in 20 mL of dry toluene-tetrahydrofuran mixture (1:1). After addition, the reaction mixture was stirred for 2 hours at room temperature and at 50-55°C for another 5 hours. The completion of the reaction is monitored by TLC analysis. The triethylamine hydrochloride was separated from the reaction mixture by filtration and the solvent was evaporated from the filtrate under reduced pressure. The residue was washed with petroleum ether (60-80°C) and used for the next step of the reaction without further purification.

Synthesis of 2-(alanine methyl ester)-6-methyl-4*H*-1,3,2-dioxaphosphirino(5,4-*b*)pyridine 2-sulfide 5b

A mixture of alanine methyl ester hydrochloride (**4b**, 0.28 g, 0.002 mol), 2-chloro-6-methyl-4*H*-1,3,2-dioxaphosphorino (5,4-*b*)pyridine 2-sulfide (**3**, 0.42 g, 0.002 mol) and triethylamine (0.41 g, 0.004 mol) in 40 mL of dry tetrahydrofuran was stirred for 8 hours at room temperature. The progress of the reaction was monitored by TLC analysis. Triethylamine hydrochloride was separated by filtration and the solvent from the filtrate was evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel using hexane-ethyl acetate (7:3) as eluent, yield 0.34 g (54%), mp 130-132°C. Physical and Spectral data of members of **5** are given in Tables 1-3.

Synthesis of other compounds **5a** and **5c-e** were achieved by adopting the above procedure.

Acknowledgements

The authors express their thanks to Prof C. Devendranath Reddy, Department of Chemistry, Sri Venkateswara University, Tirupati and Dr. C. Naga Raju, Assistant Professor, Department of Chemical Engineering, Sri Venkateswara University for helpful discussions. P V G Reddy thanks to CSIR, New Delhi for the award of Senior Research Fellowship. Authors also thank UGC, New Delhi, for providing Financial Assistance.

Table 1 : Physical, IR and ^{31}P NMR data[a] of compounds **5**

Compd.	m.p (°C)	Yield (%)	molecular formula	Elemental Analyses Found (Calcd) %			IR (cm^{-1})		C=O	^{31}P NMR[a]
				C	H	N	P=S	P-NH		
5a	122-124	56	$C_{10}H_{13}N_2PO_4S$	41.52 (41.67	4.43 (4.54)	9.65 (9.72)	736	3182	1734	63.24
5b	130-132	54	$C_{11}H_{15}N_2PO_4S$	43.83 (43.71)	4.89 (5.00)	9.12 (9.27)	751	3260	1737	62.08
5c	149-151	52	$C_{15}H_{15}N_2PO_4S$	51.24 (51.43)	4.16 (4.31)	7.81 (7.99)	755	3242	1721	66.58
5d	78-80	58	$C_{11}H_{15}N_2PO_4S$	43.56 (43.71)	4.87 (5.00)	9.45 (9.27)	725	3168	1744	64.00
5e	184-186	55	$C_{11}H_{15}N_2PO_2Cl_2S$	38.59 (38.72)	4.26 (4 43)	8.10 (8.21)	728	-	-	1.92

[a] ^{31}P NMR chemical shifts were expressed in ppm from 85% H_3PO_4 as external standard

Table 2 : ^{1}H NMR data[a-c] of compounds **5**

Compd	Aromatic-H (7-H, 8-H)	Methylene-H	6-CH_3	Amino acid ester-H/bis-(2-chloroethyl)amine-H
[b]**5a**	7.31 (d, J = 8.2 Hz, 1H) 7.14 (d, J = 7.9 Hz, 1H)	5.34-5.52 (m, H_a) 5.12-5.19 (m, H_b)	2.42 (s, 3H)	4.10 (s, 2H, NH-CH_2) 3.84 (s, 3H, OCH_3)
[b]**5b**	7.20 (d, J = 8.8 Hz, 1H) 7.07 (d, J = 7.4 Hz, 1H)	5.53-5.60 (m, H_a) 5.16-5.28 (m, H_b)	2.49 (s, 3H)	4.16-4.19 (m, 1H, CH) 1.46 (d, 3H, CH3)
[b]**5C**	6.82-7.90 (m, 6H)	5.01-5.29 (m, H_a) 4.35-4.89 (m, H_b)	2.46 (s, 3H)	3.79 (s, 3H, OCH_3)
[b]**5d**	7.13 (d, J = 8.4 Hz,1H) 7.00 (d, J = 8.3 Hz, 1H)	5.47-5.52 (m, Ha) 5.11-5.21 (m, Hb)	2.41 (s, 3H)	4.18 (d, J = 7.1 Hz, 2H, CH_2) 3.76 (q, 2H, OCH_2) 1.20 t, 3H, CH_3)
[c]**5e**	7.28 (d, J = 7.9 Hz, 1H) 7.19 (d, J = 8.0 Hz, 1H)	5.37-5.42 (m, H_a) 4.91-4.93 (m, H_b)	2.40 (s, 3H)	3.65-3.97 [m, 8H, $N(CH_2CH_2Cl)_2$]

[a] Chemical shifts in δ from TMS and J (Hz) given in parentheses
[b] Recorded in $CDCl_3$
[c] Recorded in DMSO-d_6

Table 3 : ^{13}C NMR data of compounds **5**

Compd.	Chemical shifts (in ppm)
5a	56.4 (d, J = 5.6 Hz, 1C, C-4), 151.9 (s, 1C, C-6), 124.9 (s, 1C, C-7), 126.3 (s, 1C, C-8), 139.5 (s, 1C, C-9), 143.7 (s, 1C, C-10), 22.9 (s, 1C, C-11), 40.8 (s, 1C, CH_2), 171.4 (s, 1C, CO), 64.2 (s, 1C, OCH_3)
5b	50.8 (d, J = 5.9 Hz, 1C, C-4), 153.6 (s, 1C, C-6), 123.7 (s, 1C, C-7), 126.9 (s, 1C, C-8), 139.6 (s, 1C, C-9), 144.9 (s, 1C, C-10), 23.5 (s, 1C, C-11), 52.7 (s, 1C, -CH-CH_3), 21.0 (s, 1C, -CH-CH_3), 173.4 (s, 1C, CO), 68.4 (s, 1C, OCH_3)
5c	58.2 (d, J = 5.8 Hz, C-4), 152.8 (s, 1C, C-6), 125.6 (s, 1C, C-7), 125.1 (s, 1C, C-8), 139.9 (s, C-9), 144.2 (s, 1C, C-10), 22.9 (s, 1C, C-11), 150.6 (s, 1C, C-1′), 119.2 (s, 1C, C-2′), 129.6 (s, 1C, C-3′), 122.8 (s, 1C, C-4′), 132.4 (s, 1C, C-5′), 116.7 (s, 1C-C-6′), 172.9 (s, 1C, CO), 59.8 (s, 1C, OCH_3)
5d	67.4 (d, J = 5.9 Hz, C-4), 152.6 (s, 1C, C-6), 127.7 (s, 1C, C-7), 125.9 (d, J = 8.2 Hz, C-8), 138.6 (d, J = 12.1 Hz, C-9), 143.9 (d, J = 5.8 Hz, C-10), 22.5 (s, 1C, C-11), 42.3 (s, 1C, CH_2), 168.7 (s, 1C, CO), 60.8 (s, 1C, OCH_2), 13.1 (s, 1C, CH_3).
5e	67.9 (d, J = 6.0 Hz, C-4), 151.8 (s, 1C, C-6), 125.6 (s, 1C, C-7), 129.8 (s, 1C, C-8), 140.2 (s, 1C, C-9), 143.1 (s, 1C, C-10), 23.6 (s, 1C, C-11), 45.5 [s, 2C, $N(CH_2)_2$], 34.0 [s, 2C, $(CH_2Cl)_2$]

References

(1) P.J. Cox, Biochem. Pharmacol. **28,** 2045 (1979).

(2) K.G. Devine, C. Mc Guigan, T.G. O'Connor, S.R.Nicholis and D. Kinchington, AIDS. **4,** 371 (1990).

(3) C. Mc Guigan and P. Narashiman, Synthesis. 1993, pp. 311.

(4) M. Szekerke, Cancer Treatment Rept. **60**, 347 (1976).

(5) A.Q. Siddiqui, C. Ballatore, C. Mc Guigan, E.D. Clereq and J. Balzarini, J. Med. Chem. **42**, 393 (1999).

(6) J-M. Huang, H. Chen and R-Y.Chen, Synthetic Commun. **32**, 1357 (2002).

(7) K. Misiura, R.W. Kinas and H. Kusnierczyk, Bio. Org. Med. Chem. Lett. **12**, 427 (2002).

(8) L.C. Thomas. The interpretation of the Infrared Spectra of Organophosphorus Compounds, Heydon, London. 1974.

(9) Y. Hari Babu, P. Vasu Govardhana Reddy, C. Suresh Reddy, C. Devendranath Reddy and P. Uma Maheswari Devi, J. Heterocycl. Chem. **39** (1093) 2002.

(10) R.M. Silverstein and F.X. Webster, Spectrometric Identification of Organic Compounds, 6th edition, Wiley, New York, 1998.

(11) Z-W. Miao, H. Fu, B.Han, Y.Chen and Y-F. Zhao, Synthetic Commun. **32,** 1159 (2002).

(12) Z-W. Miao, H. Fu, G-Z Tu and Y-F Zhao, Synthetic Commun. **32**, 3301 (2002).

(13) R.G. Webb, M.W. Haskell and C.H. Stammer, J. Org. Chem. **34**, 576 (1969).

Received on January 2, 2004.