Synthesis and Antimicrobial Activity of New 2,10-Dichloro-6-phenylaminobenzyl-dibenzo[*d,g*][1,3,6,2]dioxathiaphosphocin 6-Oxides

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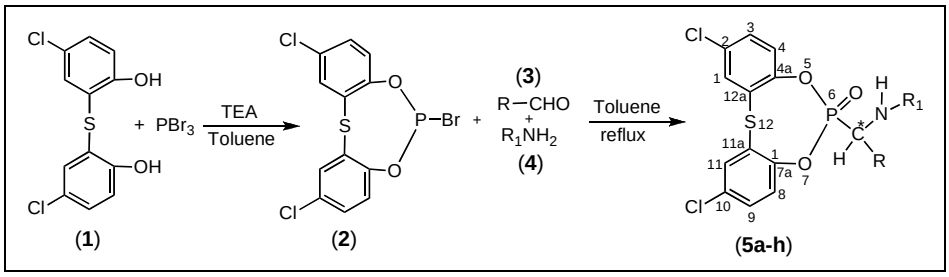
January 3, 2007



A series of novel 2,10-dichloro-6-phenylaminobenzyldibenzo[*d,g*][1,3,6,2]dioxa-thiaphosphocin 6-oxides were synthesized in two steps. In the first step, 5,5′-dichloro-2,2′-dihydroxydiphenyl sulphide **1** was reacted with phosphorus tribromide in the presence of triethylamine in dry toluene at room temperature to yield an intermediate, phosphorobromodite **2**. In the second step, **2** was treated *in situ* with aromatic aldehydes **3** and aromatic amines **4** in toluene at refluxing temperature yielded the title compounds **5a-h**. Their structures were established by analytical, IR, NMR (^{1}H, ^{13}C and ^{31}P) and mass spectra. Compounds **5a-h** have been screened for their antimicrobial activity. They exhibited significant antibacterial and antifungal activity.

J. Heterocyclic Chem., **45**, 103 (2008).

INTRODUCTION

In recent years, considerable interest has been focussed on the synthesis of phosphoric acids. They are very important carbon phosphorus bond-forming reagents in the synthesis of organophosphorus compounds with P-C bonds. The versatility and potential to create both functional and structural diversity using these reagents have long been stimulating the creativity of organophosphorus chemists.

In recent years, dioxathiaphosphocins have been used as antioxidants [1] and superior ligands [2]. The o-alkyl α-substituted aminophosphonates are very important class of compounds since they find applications as antibiotics, antiviral agents and enzyme inhibitors. The corresponding α-functionalised phosphoric acids, α-amino phosphonic acid derivatives are currently gaining importance in medicinal chemistry as they are analogues of naturally occurring α-amino acids in biological systems. Extensive investigations over the last twenty years have shown that they are of great importance in biological and medicinal research; in this connection the use of α-aminophosphonates can serve as peptidomimetics. Hapten design in antibodies generation and also in enzyme inhibitor activity [3] may be cited as examples. Recently it has been reported that they are also useful as antibiotics, neurotransmitters and herbicides [4]. Some aminodiphosphonate derivatives have been successfully used in the treatment of disorders of calcium metabolism. In view of the wide range spectrum of their physiological activity, efforts have been made to develop efficient strategies for the synthesis of amino phsophonate derivatives with diverse structural pattern.

In view of this, 2,10-dichloro-6-phenylaminobenzyl dibenzo[*d,g*][1,3,6,2] dioxathiaphosphocin 6-oxides were synthesized expecting them to possess broad spectrum of biological activity.

RESULTS AND DISCUSSION

The first step in the synthesis of 6-substituted phenyl-amino benzyldibenzo[*d,g*][1,3,6,2]dioxathiaphosphocin 6-oxides, involves the cyclisation of 5,5′-dichloro-2,2′-dihydroxy diphenylsulfide **1** with phosphorus tribromide in the presence of TEA in dry toluene to obtain the intermediate phosphorobromodite **2**.

The second step involves reaction of the phosphoro-bromodite **2** with an aldehyde **3** and phenylamine **4** to form 6-substituted phenylamino benzyldibenzo[*d,g*][1,3, 6,2]dioxathiaphosphocin 6-oxides [5]. The reaction went smoothly in toluene at reflux temperature with stirring for 30-45 minutes. Compounds **5a-h** were obtained in high yields (86-96%). Toluene is found to be an ideal solvent to effect this reaction. Isolation of the products involves simple work-up.

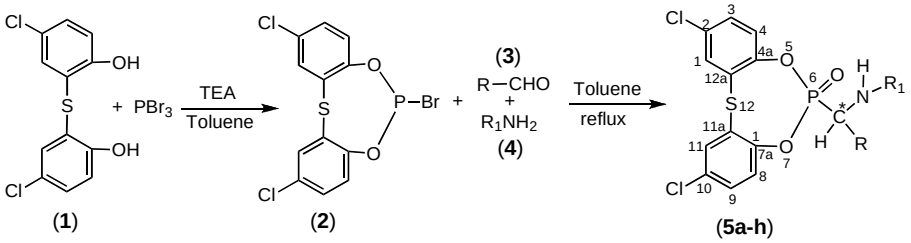
After completion of the reaction which was judged by TLC, the solvent was evaporated under reduced pressure,

crude products obtained were further purified by column chromatography on 60-120 mesh silica gel using elthyl acetate: hexane (1:2) as an eluent.

The merit of the reaction is that sterically hindered and functionalized substrates also reacted under similar conditions and afforded the compounds in excellent yields. Their structures were established by elemental analysis IR, ^{1}H, ^{13}C, ^{31}P and mass spectral data.

The oxygen bearing C(4a) and C(7a) resonated in the downfield region [10] at δ 146.4 – 151.3 ($^{2}J_{poc}$ (4a, 7a) = 7.9 – 14.9 Hz) (Table-III). The bridged C(11a) and C(12a) resonated as low intensity doublets at δ 126.2 – 126.8 [$^{3}J_{poc}$ = 3.1 – 3.2 Hz]. Another doublet at δ 125.0 – 126.4 ($^{3}J_{poc}$ = 4.1 – 4.8 Hz) is assigned to C(4) and C(8). The chlorine bearing C(2) and C(10) resonated as singlets at δ 131.2–131.5, chemical shifts at δ 130.00–130.40 and δ 134.10–

Scheme I



P=O and P-NH stretching frequencies were observed in the expected regions 1298-1253 and 3396-3301 cm^{-1}, respectively [6,7] (Table-1). The P-C (aliphatic) stretching frequencies [8] appeared in the region 758-715 cm^{-1}.

The aromatic protons of the four benzene rings present in these compounds **5a-h** showed complex multiplet [9] at δ 6.71 – 7.94 (Table-II). The α-hydrogen of the side chain only experienced coupling with phosphorus and gave signal as a doublet at δ 4.29 – 5.05 (d, J = 23.0 – 24.3 Hz). Methyl, methoxy, methylene and ethylene protons in **5a-h** were observed in the expected regions.

134.40 are assigned to C(3) and C (9), C (1) and C (11) respectively.

The signals of the carbons of 6-phenylamino benzyl moieties in compounds **5a-h** were observed downfield when compared with the signals of the free phenyl benzyl amine. The chiral α-carbon gave doublet at δ 53.8 – 54.8 (J_{pc} =130.0 - 146.8 Hz) [11].

All the phenylaminobenzyldibenzo[*d,g*][1,3,6,2]dioxathiaphosphocin 6-oxides gave two ^{31}P NMR signals except **5a** and **5h** in the region 33.10 – 23.30 ppm. Appearance of two distinct signals reveals that

Scheme II

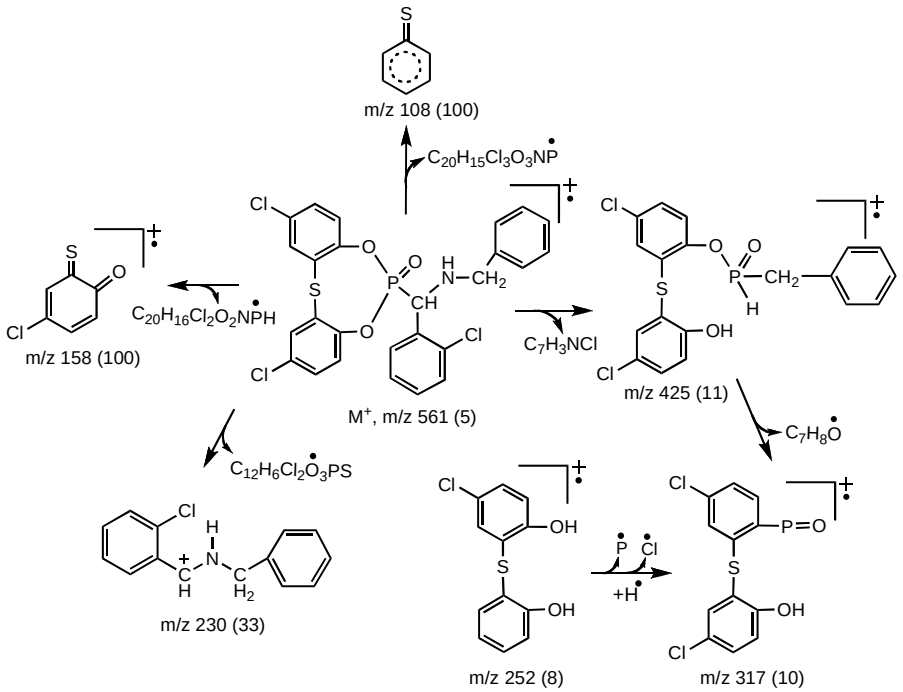


Table 1

Physical and IR Spectral Data (cm^{-1}) of Compounds (**5a-h**)

Compound	R	R′	Yield %	Mp. (°C)	Molecular Formula (Mol. Wt)	Analysis % Found / Calcd. C	H	-NH	P=O	P-$C_{(ali)}$
5a	C_6H_5	C_6H_5	96	210-211	$C_{25}H_{18}Cl_2NO_3PS$ (514.3)	58.42 58.37	3.43 3.52	3327	1274	715
5b	C_6H_5	C_6H_4-Cl(4″)	93	192-193	$C_{25}H_{17}Cl_3NO_3PS$ (548.7)	54.80 54.71	3.16 3.12	3335	1298 6	742
5c	C_6H_5	C_6H_4-NO_2(4″)	86	220-221	$C_{25}H_{17}Cl_2N_2O_5PS$ (559.3)	53.76 53.68	3.13 3.06	3320	1298	735
5d	C_6H_5	C_6H_3-$(OCH_3)_2$ (3″ & 4″)	94	156-157	$C_{27}H_{21}Cl_2NO_5PS$ (573.3)	56.54 56.46	3.73 3.68	3380	1280	758
5e	C_6H_4-F(4′)	C_6H_4-CH_3 (4″)	90	192-193	$C_{26}H_{19}Cl_2FNO_3PS$ (546.3)	57.30 57.15	3.55 3.50	3308	1260	740
5f	C_6H_4-Cl (2′)	$CH_2C_6H_5$	92	208-209	$C_{26}H_{19}Cl_{13}NO_3PS$ (562.7)	55.62 55.48	3.45 3.40	3315	1253	736
5g	C_6H_4-OCH_3 (4′)	C_6H_4-I (4″)	93	216-217	$C_{26}H_{19}Cl_2NO_4PS$ (543.3)	57.32 57.43	2.91 2.85	3301	1260	733
5h	CH=CHC_6H_5	C_6H_5	89	150-151	$C_{27}H_{20}Cl_2NO_3PS$ (540.3)	60.13 60.00	3.78 3.72	3396	1254	750

Table 2

^{1}H and ^{31}P NMR Data of Compounds (**5a-h**)

Compound	Ar-H [a,b]	N-H [a]	R-H [b]	^{31}P NMR [c]
5a	6.95-7.50 (m, 16H)	6.17 (d, *J*=7.9 Hz, 1H, NH)	6.01 (d, *J*=24.3 Hz, 1H, 1′-H)	23.3
5b	6.78-7.89 (m, 15H)	6.20 (br, s, 1H, NH)	4.80 (d, *J*=23.1 Hz, 1H, 1′-H)	24.8, 26.2
5c	6.84-7.92 (m, 15H)	6.38 (br, s, 1H, NH)	4.62 (d, *J*=24.1 Hz, 1H, 1′-H)	31.1, 31.8
5d	6.71-7.90 (m, 14H)	6.25 (d, *J*=5.8 Hz, 1H, NH)	4.73 (d, *J*=23.1 Hz, 1H, 1′-H) 3.56, 3.60 (s, 6H, 3″ & 4″ - $2OCH_3$)	23.8, 24.2
5e	6.78-7.93 (m, 14H)	4.70 (br, s, 1H, NH)	4.28 (m, 1H, 1′-H), 2.28 (s, 3H, 4″-CH_3)	29.2, 30.4
5f	6.81-7.90 (m, 14H)	4.66 (br, s, 1H, NH)	4.29 (d, *J*=23.0 Hz, 1H, 1′-H), 4.1 (m, 2H, 1″-CH_2)	28.4, 30.0
5g	6.96-7.93 (m, 14H)	5.80 (d, *J*=5.8 Hz, 1H, NH)	4.61 (d, *J*=23.4 Hz, 1H, 1′-H), 3.52 (s, 3H, 4′-OCH_3)	30.3, 31.0
5h	6.82-9.94 (m, 16H)	---	6.12-6.30 (m, 2H), 4.4 (m, 1H, 1′-H)	33.1

[a] Chemical shifts in ppm from TMS and coupling constants J (Hz) given in parenthesis. [b] Recorded in deuteriochloroform. [c] Chemical shifts in ppm from 85% H_3PO_4.

phosphorus resonances are very sensitive in its stereo electronic environment suggesting the presence of two conformers [10,12,13] in the solution state.

The electron impact mass spectrum of **5f** showed M^+ ion and characteristic daughter ions (Scheme-II).

Antibacterial Activity. The title compounds **5a-h** were screened against the growth of *Staphylococcus aureus* (Gram +ve) and *Klebsiella pneumoniae* (Gram –ve). Vincent and Vincent technique [14] was followed for evaluation of antibacterial activity at 200 and 400 ppm. Majority of the compounds were highly active against the growth of both *Staphylococcus aureus* and *Klebsiella pneumoniae*. Penicillin was tested as standard reference compound to compare the activity of the title compounds. It is observed that the presence of dimethoxy substituent exhibited the highest antibacterial activity (Table-IV).

Compounds **5a**, **5d**, **5e**, **5f** and **5g** exhibited higher activity against Klebsiella pneumoniae when compared to that of the standard penicillin.

Antifungal Activity. All the compounds **5a-h** were tested for their antifungal activity against the *Pellicularia solmanicolor* (pink disease) and *Macrophomina*

Table 3

Carbon-13 NMR Spectral Data [a,b] of Compounds **5a-h**

Compound	C(1/11)	C(2/10)	C(3/9)	C(4/8)	C(4a/7a)	C(11a/12a)	C(1′)	C(2′)	C(3′)	C(4′)	C(5′)	C(6′)	C*
5a	134.2	131.5	130.0	126.4	146.4 (d,14.9)	126.5	134.0	126.8	128.1	128.0	128.1	126.8	53.8 (146.6)
5b	134.2	131.2	130.2	125.0 (d,4.1)	150.4 (d, 8.2)	126.7 (d, 3.2)	132.0	126.7	128.2	128.0	128.2	126.7	54.2 (138.0)
5c	134.3	131.4	130.3	125.2	150.6 (d, 8.0)	126.4	132.1	126.9	127.9	128.2	127.9	126.9	54.4 (138.0)
5d	134.1	131.2	130.1	125.1	151.0	126.7	132.0	126.8	127.9	128.4	127.9	126.8	54.8 (130.0)
5e	134.3	131.4	130.4	125.2 (d, 4.8)	150.8 (d, 7.9)	126.7 (d, 3.1)	132.2	126.8	127.8	-	127.8	126.8	53.9 (139.0)
5f	134.4	131.4	130.3	125.3	150.7 (d, 8.2)	126.8	133.2	133.2	128.3	128.6	128.3	126.9	54.2 (142.0)
5g [c]	134.2	131.2	130.4	125.3	151.2 (d, 7.9)	126.2 (d, 3.2)	133.2	126.9	128.6	-	128.6	126.9	54.4 (130.0)
5h [d]	134.3	131.3	130.2	125.6	151.3 (d, 8.2)	126.2	133.0	126.8	128.6	-	128.6	126.9	54.6 (133.0)

[a] Chemical shifts in ppm from TMS and coupling constants J (Hz) given in parenthesis. [b] Recorded in deuteriochloroform. [c] C-4′, O-CH_3, δ 55.4. [d] δ 126.12, 154.40 (-CH=CH-C_6H_5).

Table 3 (Continued)

^{13}C NMR data of R (amino moieties) of **5a-h**

Compound.	C(1′′)	C(2′′)	C(3′′)	C(4′′)	C(5′′)	C(6′′)	C2′′
5a	153.8	116.8	130.1	119.0	130.1	116.8	-
5b	153.4	117.3	130.2	133.0	130.2	117.3	-
5c	152.8	117.4	130.4	145.6	130.4	117.4	-
5d	153.4	117.9	156.0	-	130.4	117.5	C3′′-55.4
5e	153.8	117.9	131.0	137.2	131.0	117.9	C4′′-20.9
5f	-	119.2	130.3	120.2	130.3	119.2	C1′′-51.1
5g	152.6	116.0	129.0	102.0	129.0	116.0	-
5h	153.6	117.0	129.8	119.4	129.8	117.0	-

[a] Chemical shifts in ppm from TMS and coupling constants J (Hz) given in parenthesis. [b] Recorded in deuteriochloroform

Table 4

Antibacterial and Antifungal Activity Data of Compounds with Statistical Evaluation (**5a-h**)

	Zone of inhibition of spore germination											
	Bacteria (μg/mL) [b]						Fungi (μg/mL) [b]					
Compound [a]	*Staphyloccus aureus*			*Klebsiella pneumonia*			*Pellicularia solmanicolor*			*Macrophomina phaseolina*		
	200		400	200		400	200		400	200		400
	Mean [c]	S.D.		Mean	S.D.		Mean	S.D.		Mean	S.D.	
5a	38	1.41	58	25	3.39	48	30	2.92	52	27	3.24	55
5b	36	2.00	60	19	3.67	40	33	2.12	54	21	3.46	38
5c	31	2.83	56	18	3.74	42	36	2.00	58	31	2.83	50
5d	38	1.41	60	26	3.32	52	37	1.58	59	37	1.58	55
5e	32	2.65	54	29	3.00	55	36	2.00	55	40	1.22	56
5f	28	3.16	47	34	2.10	56	43	1.00	62	34	2.10	51
5g	36	2.00	56	33	2.12	55	17	3.81	35	20	3.61	35
5h	28	3.16	46	25	3.39	45	30	2.92	39	20	3.61	32
Pencillin	24	-	-	24	-	-	-	-	-	-	-	-
Grieseofulvin	-	-	-	-	-	-	22	-	-	23	-	-

S.D. - Standard Deviation [a] Recrystallized from ethanol; [b] Concentration in ppm; [c] Average Value of five experimental values.

phaseolina (dry root rot sunflower and citrus), Benson technique [15] was followed for testing the compounds at two different concentrations (200 and 400 ppm). Most of the compounds showed significant antifungal activity against the growth of both fungi. Griseofulvin is used as reference compound to compare the activity of these compounds.

It is gratifying to note that the compound **5c**, **5d** and **5f** exhibited higher activity than that of the standard griseofulvin.

EXPERIMENTAL

Melting points were taken on a Mel-temp apparatus and are uncorrected. Microanalyses were performed at RSIC, Central Drug Research Institute, Lucknow. IR spectra (ν_{max} in cm^{-1}) were recorded in KBr on a Perkin-Elmer 283 double beam spectrophotometer. ^{1}H, ^{13}C and ^{31}P NMR were recorded on a Varian XLAA-400 spectrometer operating at 400 MHz for ^{1}H, 100 MHz for ^{13}C and 161.89 Mz for ^{31}P. All NMR spectra were taken in DMSO-d_6 (or) CD_3OD and were referenced to TMS (^{1}H and ^{13}C) or 85% H_3PO_4 for ^{31}P, chemical shifts in (δ) and mass spectra were recorded on a Auto Spec Q instrument either by EI (or) CI methods.

General Procedure for Preparation of (5a). A solution of phosphorus tribromide (1.35 g, 0.005 mole) in 25 ml of dry toluene was added dropwise over a period of 20 minutes to a cold (0°C) and stirred solution of 5,5′-dichloro-2,2′-dihydroxydiphenyl sulfide (**1**) (1.43 g, 0.005 mole) and triethylamine (1.01 g, 0.01 mole) in dry toluene (20 ml). After addition, the temperature was slowly raised to room temperature and stirring was continued for an additional one hour. Progress of the reaction was monitored by TLC analysis. Triethylamine hydrobromide was then filtered off and the intermediate (**2**), 6-bromo-dibenzodioxathiaphosphocin was used for the second step without further purification.

To the intermediate (**2**), benzaldehyde (0.53 g, 0.005 mole) and aniline (0.46 g, 0.005 mole) were added and the temperature was raised to reflux and stirring was continued for an additional 30 minutes. Progress of the reaction was monitored by TLC analysis. The solvent was removed under reduced pressure. The residue was purified by column chromatography on 60-120 mesh silica gel using ethyl acetate: hexane (1:2) as an eluent to yield 2.46 g (96%) of **5a**, mp 210-211°C.

Acknowledgement. Authors express thanks to Prof. C.D. Reddy, S.V. University, Tirupati, for useful discussions.

REFERENCES

[1] Spivack, J.D.; Brit. Pat, **1982**, 1, 087, 399, *Chem. Abstr.* **1982**, 1, 97, 98374.

[2] Billig, E.; Abvatjoglou, A.G.; Bryant, D.R.; Murray, R.E.; and Mather, J.M.; US Pat **1986**, 4, 599, 206, *Chem. Abstr.* **1986**, 105, 81142.

[3] Allen, M.C.; Fuhrer, W.; Tuck, B.; Wade, R.; and Wood, J.M.; *J. Med. Chem.* **1989**, *32*, 1652.

[4] Hirschmann, R.; Smith, A.B. III.; Taylor, C.M.; Benkovic, P.A.; Taylor, S.D.; Yager, K.M.; Spengler, P.A.; Venkovic, S. J. *Science* **1994**, *265*, 234.

[5] Qing dai, *Ruyuchen, Chunxiangzhang, ZhunLiu, *Synthesis*, **1998**, *4*, 405.

[6] Thomas, L.C.; and Chittenden, R.A.; *Spectrochimi. Acta.,* **1964**, *20*, 489.

[7] Thomas, L.C.; *The Interpretation of the Infrared Spectra of Organophosphorus Compounds*, Heydon, London, **1974**.

[8] Nyquist, R.A.; *Spectrochim. Acta,* **1963**, *19*, 713.

[9] Kumar, K.A.; Reddy, C.S.; Rao, L.N.; and Raju, C.N.; *Heterocycl. Commun.* **2001**, *7(3)*, 253,.

[10] Harnath, P.; Anasuyamma, U.; Syam Prasad, G.; Naga Raju, C.; and Suresh Reddy, C .*; Heterocyclic. Commun.* **2004**, *10(6)*, 457.

[11] Hanessian, S.; Bennani, Y.L.; and Delorme, D.; *Tetrahedron. Lett.* **1990**, *31*, 6461.

[12] Gorenstein, D.G.; Rowell, R.; and Findlay, J.; *J. Am. Chem. Soc.* **1980**, *102*, 5077.

[13] Vasu Govardhan Reddy, P.; Suresh Reddy, C.; Nagaraju, C.; *Chem. Pharm. Bull.* **2003**, *51(7)*, 860.

[14] Vincent, J.C.; and Vincent, H.W.; *Proc. Soc. Expt. Biol. Med.* **1944**, *55*, 162.

[15] Benson, H.J.; *Microbial Application*, WC Brown Publications, USA, **1990**, 134.