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Synthesis, Spectral Studies, Antimicrobial Activity and Crystal Structures of Phosphaza-Lariat Ethers

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Phosphaza-lariat ethers, which are known as 2,2-[4,4'-(2,2'-oxy*bis*(ethane-2,1-diyl)*bis*(oxy))dianilino]-4,4,6,6-tetrachlorocyclo-2λ^5,4λ^5,6λ^5-triphosphazatriene(spiro) (**1**), 2,2-[4,4'-(2,2'-(ethane-1,2-diyl*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy)dianilino]-4,4,6,6-tetrachlorocyclo-2λ^5,4λ^5,6λ^5-triphosphazatriene(spiro) (**2**) and 2,2-[4,4'-(2,2'-(ethane-1,2-diyl*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy)dianilino]-4,4-*bis*(*tert*-butylamino)-6,6-dichlorocyclo-2λ^5,4λ^5,6λ^5-triphosphazatriene (**3**) have been synthesized. The structures of the compounds (**1**, **2** and **3**) are characterized by elemental analysis, IR, ^{1}H, ^{13}C, ^{31}P NMR and mass spectroscopic techniques. The antimicrobial activities of the compounds have been screened *in vitro* against the organisms. The structures of the compounds **1**, **2** and **3** have also been examined by means of cyrstallographically. The compounds **1**, **2** and **3** crystallize in the orthorhombic space group Pbca, hexagonal space group $P4_32_12$ and tetragonal space group $P4_2/n$, respectively. They have unit cell parameters: a = 15.607(1), 10.6723(2) and 26.0308(9), b = 15.667(2), 10.6723(2) and 26.0308(9), c = 19.481(3), 23.3633(6) and 10.6033(4) Å, V = 4763.4(1), 2661.03(1) and 7184.8(4) Å^3, D_x = 1.570, 3.031 and 1.258 g cm^{-3} and Z = 8, 8 and 8, respectively.

Key Words: Phosphazene, Spectroscopic studies, Antimicrobial activities, Crystal structure, Spiro.

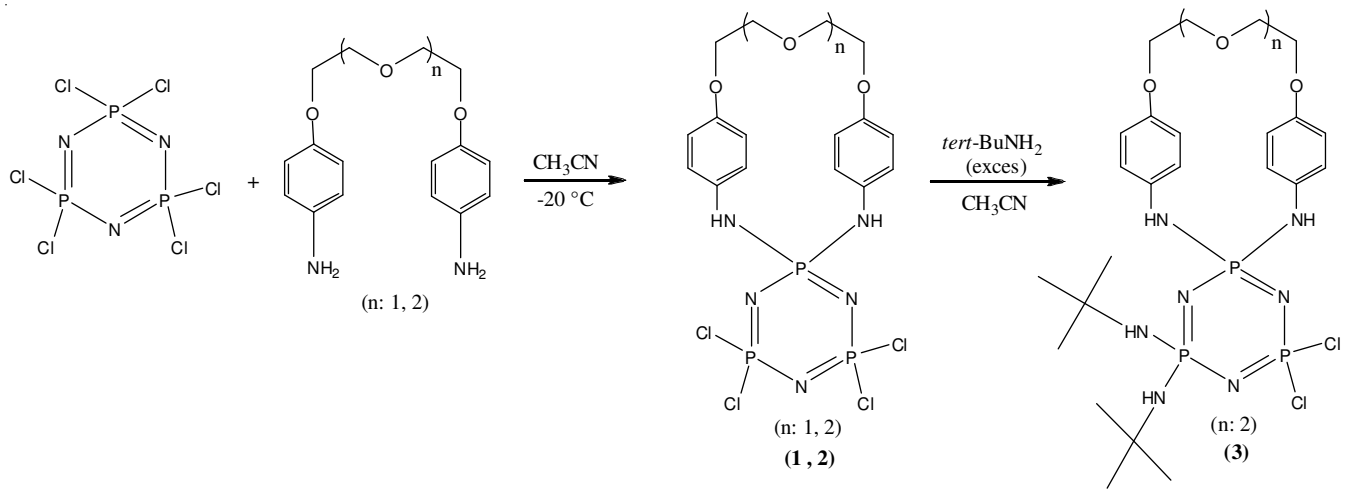
INTRODUCTION

A large variety of application areas of phosphazene compounds have been found in the last three decades by examining their features. There have been considerable work in the literature concerning the reactions of phosphazenes with amine and alcohol by mono- and di-functions[1-13]. Recently, phosphaza-lariat ethers, which are new types of compounds, have been obtained by reacting phosphazenes with amino-podand, cryptand and oligoethyleneglycol[14-23]. The design and synthesis of phosphaza-lariat ethers are significant; as ligating agents for alkali-, alkaline-earth and transition metal cations[24-26]. Despite the early studies on lariat ethers only a few phosphaza-lariat ethers have been reported[14-18]. There have not also been satisfactory studies on their antimicrobial activities on bacterial and yeast cells.

In this study, the reactions of aminopodand with hexachlorocyclotriphosphazene and partly gem-*bis*amino substituted phosphazene derivative obtained from the reaction of spiro phosphaza-lariat ether (**2**) with an excess of *tert*-butylamine are reported (**Scheme-I**). The structures of the compounds are characterized by elemental analysis, IR, ^{1}H, ^{13}C, ^{31}P NMR, mass spectroscopies and X-ray cyrstallographically and then subjected to in *in vitro* assays of antimicrobial activity.

EXPERIMENTAL

The ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded on a Bruker DPX FT-NMR spectrometer operating at 400, 101.6 and 161.99 MHz. Infrared absorption spectra were obtained from a Perkin-Elmer BX II spectrometer in KBr discs. Carbon, nitrogen and hydrogen analyses were performed on a LECO CHNS-932 analyzer. Melting points were determined on an Electro Thermal IA 9100 apparatus using a capillary tube. LC mass spectra were obtained on an AGILENT 1100 MSD spectrometer with an ion source temperature at 240 ºC. Hexachlorocyclotriphosphazene was purchased from Aldrich. It was recrystallized from hexane and purified by fractional vacuum sublimation at 55 ºC before use. CH_3CN was purchased from Merck, distilled over sodium hydride and stored over molecular sieves. $CHCl_3$ (Merck), CH_2Cl_2 (Merck), *n*-hexane (Merck), THF (Merck), petroleum ether (50:70) (Merck), *tert*-butylamine (Merck), 4-nitrophenol (Merck), triethyleneglycoldichloride (Merck), diethyleneglycoldichloride (Merck), sodium hydride (Merck), Pd-C (10 %) (Merck), hydrazine monohydrate (Merck), DMF (Merck), Na_2CO_3 (Merck), silica gel (Aldrich, 70-230 mesh, 60 Å) were used as received and all reactions were monitored by using Kieselgel 60 F_{254} (silica gel) precoated TLC plates. All reactions and manipulations were carried out under an atmosphere of dry argon.



Scheme-I: Synthesis route for the compounds **1-3**

Synthesis of 2,2-[4,4'-(2,2'-oxy*bis*(ethane-2,1-diyl)*bis*-(oxy))dianilino]-4,4,6,6-tetrachlorocyclo-2λ^5, 4λ^5,6λ^5-triphosphazatriene(spiro) (1): 4,4'-(2,2'-Oxy*bis*(ethane-2,1-diyl)*bis*(oxy))dianiline[16,27-28] (1.50 g; 5.20 × 10^{-3} mol) in CH_3CN (50 mL) was added drop wise to a stirred solution of hexachlorocyclotriphosphazene (1.81 g; 5.20 × 10^{-3} mol) and triethylamine (1.05 g; 10.40 × 10^{-3} mol) in CH_3CN (150 mL) at -20 ºC for over 1 h, with argon being passed over the reaction mixture. After the mixture had been allowed to come to ambient temperature, it was boiled under reflux (12 h) using a condenser fitted with a $CaCl_2$ drying tube. The precipitated salt was filtered off and the solvent removed by rotary evaporation. The crude product was dried *in vacuo* and chromatographed (silica gel, 100 g, eluent; $CHCl_3$/THF, 5:1) to give the compound **1**. Then, it was recrystallized from CH_2Cl_2/petroleum ether (50:70) by the slow diffusion method yielding a white solid, m.p. 218 ºC, 0.23 g (12 %) yields. Found (%): C, 34.01; H, 3.19; N, 12.43; calcd. (%) for $C_{16}H_{18}N_5O_3P_3Cl_4$: C, 34.13; H, 3.19; N, 12.44. IR (KBr, ν_{max}, cm^{-1}) (N-H) 3397-3301 m, (Ar-H) 3024 w, (C-H, aliphatic) 2936-2866 s, (C=C) 1509 s, (P=N) 1197 s, (C-O-C) 1247-1059 s, (P-Cl) 587-518 s. ^{31}P NMR ($CDCl_3$); δ ppm, 21.98 (d, 2P, PCl_2, $^2J_{PNP}$: 44.55 Hz), 1.45 (t, 1P, P ((NH-Ar-$OCH_2CH_2)_2O$), $^2J_{PNP}$: 44.55 Hz). MS (highest peak in multiplet, based on Cl^{35}): m/z; 563 (M^+, 100 %), 289 (M-(NH-Ar-$OCH_2CH_2OCH_2CH_2OAr$) 60 %), 136 (M-((NH-Ar-$OCH_2CH_2)_2O$ + 4Cl) 75 %).

Synthesis of 2,2-[4,4'-(2,2'-(ethane-1,2-diyl*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy)dianilino]-4,4,6,6-tetrachlorocyclo-2λ^5, 4λ^5,6λ^5-triphosphazatriene(spiro) (2): 4,4'-(2,2'-(Ethane-1,2-diyl*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*-(oxy)dianiline[16,27,28] (1.50 g; 4.51 × 10^{-3} mol) in CH_3CN (50 mL) was added drop wise to a stirred solution of hexachlorocyclotriphosphazene (1.57 g; 4.51 × 10^{-3} mol) and triethylamine (0.913 g; 9.02 × 10^{-3} mol) in CH_3CN (150 mL) at -20 ºC for over 1 h, with argon being passed over the reaction mixture. The compound **2** was isolated as the compound **1**. Compound **2**, R_f = 0.76, m.p. 189-190 ºC, 0.47 g (17 %) yields. Found (%): C, 35.60; H, 3.65; N, 11.54; calcd. (%) for $C_{18}H_{22}N_5O_4P_3Cl_4$: C, 35.61; H, 3.65; N, 11.54. IR (KBr, ν_{max}, cm^{-1}): (N-H) 3274 m, (Ar-H) 3047 w, (C-H, aliphatic) 2927-2873 s, (C=C) 1511 s, (P=N) 1200 s, (C-O-C) 1246-1107-1058 s, (P-Cl) 583-517 s. ^{31}P NMR ($CDCl_3$); δ ppm, 21.98 (d, 2P, PCl_2, $^2J_{PNP}$: 45.99 Hz), 0.83 (t, 1P, P(NH-Ar-$OCH_2CH_2OCH_2)_2$, $^2J_{PNP}$: 45.99 Hz). MS (highest peak in multiplet, based on Cl^{35}): m/z; 607(M^+, 100 %), 252(M-(NH-Ar-$OCH_2CH_2OCH_2)_2$ + Cl, 10 %).

Synthesis of 2,2-[4,4'-(2,2'-(ethane-1,2-diyl*bis*(oxy))*bis*(ethane-2,1-diyl))*bis*(oxy)dianilino]-4,4-*bis*(*tert*-butylamino)-6,6-dichlorocyclo-2λ^5,4λ^5,6λ^5-triphosphazatriene (3): *Tert*-butylamine (0.49 g; 67.10 × 10^{-4} mol), in CH_3CN (50 mL) was added drop wise to a stirred solution of compound **2** (0.5 g; 8.23 × 10^{-4} mol), in CH_3CN (150 cm^3) at -20 ºC for over 1 h, with argon being passed over the reaction mixture. After the mixture had been allowed to come to ambient temperature, it was boiled under reflux (24 h) using a condenser fitted with a $CaCl_2$ drying tube. The precipitated amine hydrochloride was filtered off and the solvent removed by rotary evaporation. The crude product was dried *in vacuo* and chromatographed (silica gel, 60 g, eluent; $CHCl_3$/THF, 3:1) to give the compound **3**. Then, it was recrystallized from CH_2Cl_2/petroleum ether (50:70) by the slow diffusion method yielding a white solid, m.p. 158-159 ºC, 0.196 g (27 %) yields. Found (%): C, 45.86; H, 6.17; N, 14.41; calcd. (%) For $C_{26}H_{42}N_7O_4P_3Cl_2$: C, 45.89; H, 6.22; N, 14.41. IR (KBr, ν_{max}, cm^{-1}): (N-H) 3401-3339 s, (Ar-H) 3050 w, (C-H, aliphatic) 2971-2914-2870 s, (C=C) 1512 s, (P=N) 1190 s, (C-O-C) 1254-1169-1043 s, (P-Cl) 569-544-514 m. ^{31}P NMR ($CDCl_3$); δ ppm, 22.22 (t, 1P, PCl_2), 5.06 (t, 1P, P(HN-Bu-*tert*)$_2$), 2.90 (dd, 1P, (NH-Ar-$OCH_2CH_2OCH_2)_2$). MS (highest peak in multiplet, based on Cl^{35}): m/z; 680 (M^+, 100 %), 607 (M-(NH-Bu-*tert*), 8 %), 551 (M-(NH-Bu-*tert*), 7 %).

Antimicrobial test: The compounds were dissolved in DMSO to a final concentration of 100 µg/mL. Empty sterilized antibiotic discs having a diameter of 6 mm (Schleicher & Schull No. 2668, Germany) were each impregnated with 20 µL of solution. All the bacteria mentioned above were incubated at 30 ± 0.1 ºC for 24 h by inoculution into Nutrient Broth (Difco) and the yeasts studied were incubated in Malt Extract Broth (Difco) for 48 h. An inoculum containing 10^6 bacterial cells or 10^8 yeast cells/mL was spread on Mueller-Minton Agar (Oxoid) plates (1 mL inoculum/plate). The discs injected with solutions were placed on the inoculated agar by pressing slightly and incubated at 35 ºC (24 h) for bacteria and at 25 ºC (72 h) for yeast. On each plate an appropriate reference

antibiotic disc was applied depending on the test microorganisms[29,30].

X-Ray crystallographic studies: The data collection for both compounds was performed on a STOE IPDS-2 diffractometer employing graphite-monochromatized MoK_α radiation (λ = 0.71073 Å). Data collection, reduction and corrections for absorption and crystal decomposition for compounds **1**, **2** and for compound **3** were achieved using X-AREA, X-RED software[31]. The structure was solved by SHELXS-97 and refined with SHELXL-97[32,33]. The positions of the H atoms bonded to C atoms were calculated (C-H distance 0.96 Å) and refined using a riding model. The H atom displacement parameters were restricted to be $1.2U_{eq}$ of the parent atom. The details of the X-ray data collection, structure solution and structure refinements are given in Table-1. The molecular structures with the atom-numbering schemes are shown in Fig. 1[34] for the compounds **1**, **2** and **3**, respectively. Crystallographic data (excluding structure factors) for the structure reported in this work have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no CCDC 689709, 729434 and 729435[35].

RESULTS AND DISCUSSION

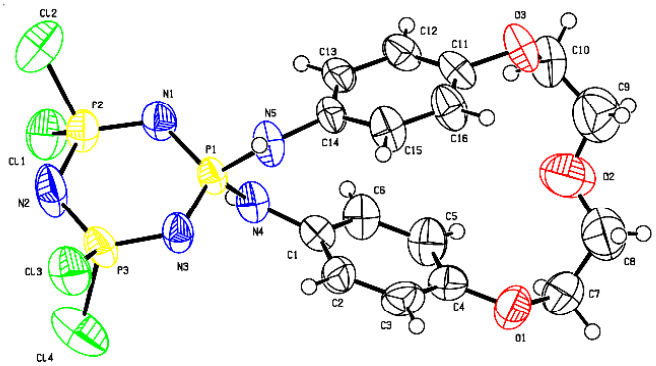
Spectroscopic studies: The IR spectra of the compounds are given in synthetic procedures (Fig. 2). The characteristic N-H, P=N, C-O-C and P-Cl bands with the wave numbers of 3397-3301 s, 3274 m, 3401-3339 s ν(N-H), 1197 s, 1200 s, 1190 s ν(P=N), 1247-1059 s, 1246-1107-1058 s, 1254-1169-1043 s ν(C-O-C) and 587-518 s, 583-517 s, 569-544-514 m ν(P-Cl) were observed for compounds **1**, **2** and **3**. The P=N vibration bands of **3** observed in a lower frequency such as 9 and 13 cm^{-1} than the same band of **1** and **2**. The distribution of electron density of phosphazene bond depends strongly from substituents of phosphorus atom and fairly from P=N bond environment, thus strength of P=N oscillators shows similar dependence (these values in **1** and **2** are close and in **3** are distinctly shifted).

In the ^{1}H NMR spectra, the N-H protons were observed δ = 4.86 ppm doublet ($^2J_{\underline{P}N\underline{H}}$: 8.85 Hz) (**1**), 4.92 ppm (doublet, $^2J_{\underline{P}N\underline{H}}$ = 10.52) (**2**) and δ = 4.90, 2.55 ppm broad-singlet (**3**). The phenyl protons were observed 6.65-6.58 ppm, 6.68, 6.65 ppm and 6.71, 6.60 ppm doublets-doublets for compounds **1**, **2** and **3**. The three bond proton couplings $^3J_{\underline{H}CC\underline{H}}$ = 8.65, 9.07 and 8.77 Hz, were observed respectively, for compounds **1**, **2** and **3**. The protons of the etheric group at $ArOC\underline{H}_2$ and $ArOCH_2C\underline{H}_2$ in also gave a triplet at δ = 4.19, 4.15, 4.13 ppm and δ = 3.71, 3.74, 3.72 ppm ($^3J_{\underline{H}CC\underline{H}}$ = 4.19, 4.59 and 4.68 Hz), respectively, for **1**, **2** and **3**. All of the $OC\underline{H}_2$ protons were singlets at δ = 3.70 ppm and 3.68 ppm in **2** and **3**. The *tert*-Bu protons $C(C\underline{H}_3)_3$ in **3** also gave a singlet at δ = 1.37 ppm.

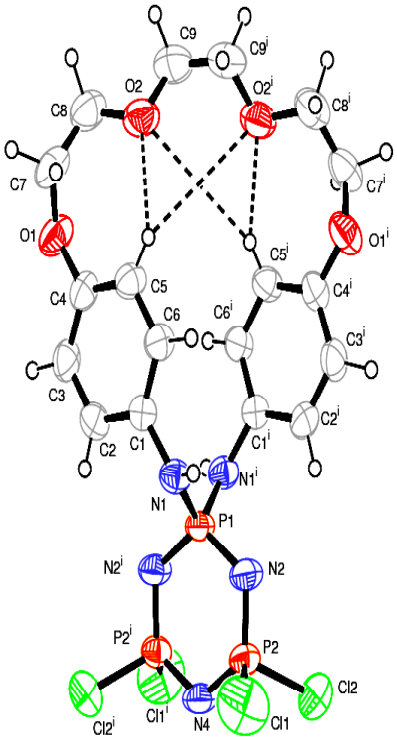
According to the proton de-coupled ^{13}C NMR spectra compounds **1**, **2** and **3** have 6, 7 and 9 signals. The compounds (**1**, **2** and **3**) seem to have symmetric molecular structures in solution. The chemical shifts δ ppm; 155.43 (s, 2C), 131.52 (s, 2C), 122.11 (d, 4C, $^3J_{\underline{P}NC\underline{C}}$: 5.60 Hz), 116.52 (s, 4C), 71.68 (s, 2C), 69.20 (s, 2C), in **1**, 155.33 (s, 2C), 131.36 (s, 2C), 122.13 (d, 4C, $^3J_{\underline{P}NC\underline{C}}$: 5.81 Hz), 115.86 (s, 4C), 71.39 (s, 2C), 69.81(s, 2C), 69.46 (s, 2C) in **2** and 154.19 (s, 2C), 133.23 (s, 2C), 120.97 (d, 4C, $^3J_{\underline{P}NC\underline{C}}$: 5.50 Hz), 115.77 (s, 4C), 71.32 (s, 2C), 69.59 (s, 2C), 69.40 (s, 2C), 51.57 (s, 2C), 31.77 (d, 6C, $^3J_{\underline{P}NC\underline{C}}$: 4.96 Hz) in **3**.

TABLE-1
CRYSTAL AND EXPERIMENTAL DATA

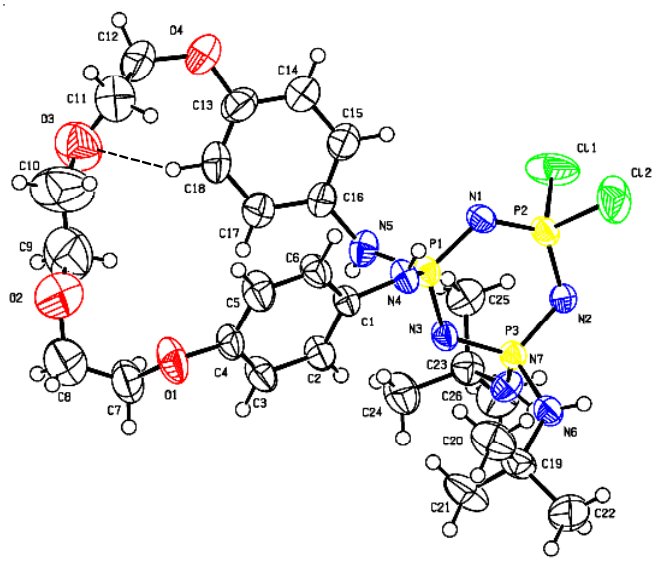
Compound	**1**	**2**	**3**
Formula	$C_{16}H_{18}N_5O_3P_3Cl_4$	$C_{18}H_{22}N_5O_2P_3Cl_4$	$C_{26}H_{42}N_7O_4P_3Cl_2$
Colour	White	White	White
Formula weight	563.06	607.12	680.48
Crystal system	Orthorhombic	Tetragonal	Tetragonal
Space group	P b c a	$P\,4_32_12$	$P\,4_2/n$
Crystal dimension	0.55 mm × 0.10 mm × 0.10 mm	0.20 mm × 0.39 mm × 0.70 mm	0.48 mm × 0.44 mm × 0.24 mm
Unit cell parameters	a = 15.607(1) Å	a = 10.6723(2) Å	a = 26.0308(9) Å
	b = 15.667(2) Å	b = 10.6723(2) Å	b = 26.0308(9) Å
	c = 19.481(3) Å	c = 23.3633(6) Å	c = 10.6033(4) Å
V	4763.4(1) $Å^3$	2661.03(1) $Å^3$	7184.8(4) $Å^3$
Z	8	8	8
D_c (g cm^{-3})	1.570 g cm^{-3}	3.031 g cm^{-3}	1.258 g cm^{-3}
μ (MoK_α)	6.689 mm^{-1}	1.320 mm^{-1}	0.354 mm^{-1}
F(000)	2288	2480	2864
$2\theta_{max}$	39.66°	51.36 °	52.74°
h, k, l range	-18 ≤ h ≤ 0	-12 ≤ h ≤ 12	-32 ≤ h ≤ 28
	-18 ≤ k ≤ 0	-12 ≤ k ≤ 12	-32 ≤ k ≤ 27
	-23 ≤ l ≤ 1	-28 ≤ l ≤ 28	-13 ≤ l ≤ 6
No. of measured reflections	4254	39898	30055
No. of independent reflections	4244	1523	7315
No. of observed reflections	2613	1500	4602
Goodness-of-fit on F^2	1.080	1.103	1.086
Measurement	STOE IPDS 2	STOE IPDS 2	STOE IPDS 2
Program system	STOE X-AREA	STOE X-AREA	STOE X-AREA
Structure determination	SHELXS-97	SHELXS-97	SHELXS-97
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
R, R_w ($I > 2\sigma(I)$)	0.0812, 0.2458	0.0321, 0.0868	0.0748, 0.2153
(Δρ)max, (Δρ)min	0.959, -0.702 e $Å^{-3}$	0.319, -0.222 e $Å^{-3}$	1.757, -0.652 e $Å^{-3}$



(**1**)



(**2**)



(**3**)

Fig. 1. Molecular structure of the compounds **1-3**, displacement ellipsoids is plotted at the 50 % probability level[34]

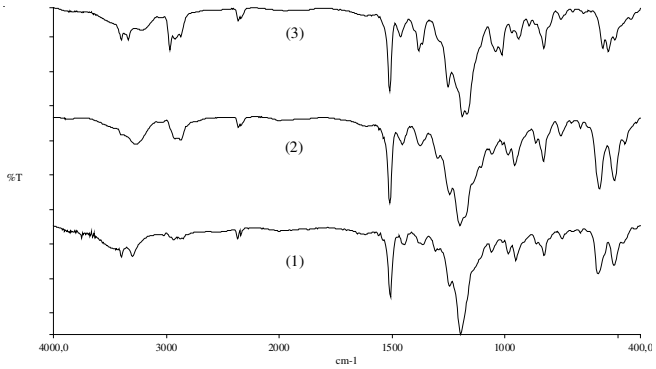
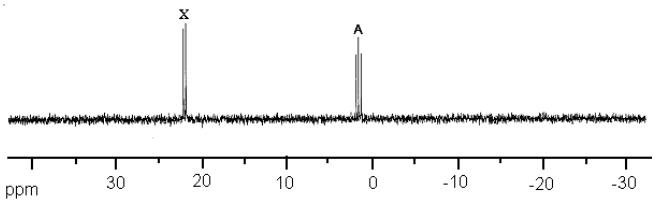
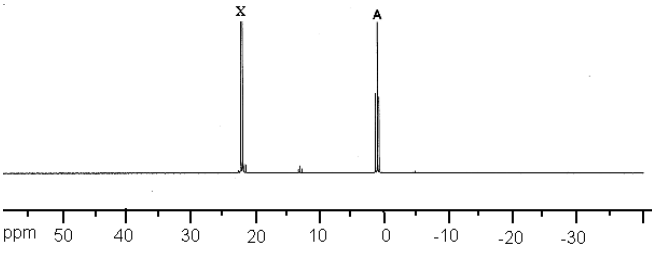


Fig. 2. FT-IR spectra of the compounds **1-3**

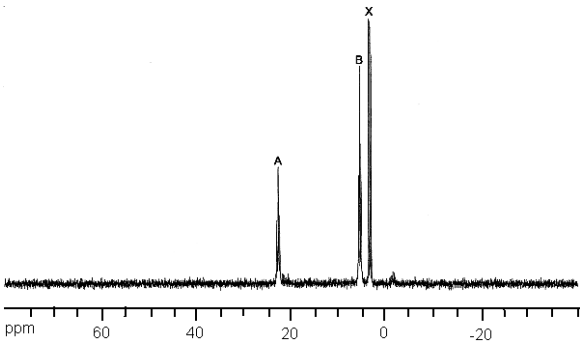
The proton de-coupled ^{31}P NMR spectra of compounds were interpreted as a result of a simple AX_2, AX_2 and ABX spin system for **1**, **2** and **3** (Fig. 3). According to the pattern of proton coupled ^{31}P NMR spectra of compounds (**1**, **2** and **3**), it was concluded that the only spiro (**1**, **2**) and gem-*bis*amino (**3**) architectures were possible. ^{31}P NMR data of the compounds are listed in Table-2.



(**1**)



(**2**)

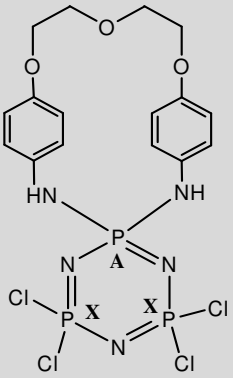
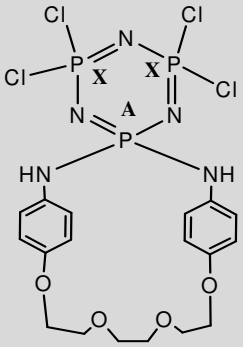
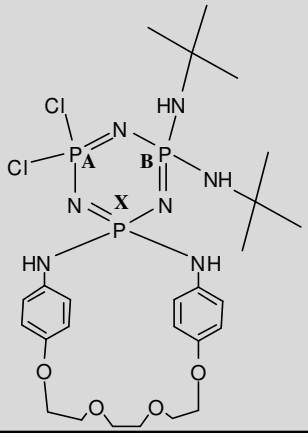


(**3**)

Fig. 3. ^{31}P NMR spectra of the compounds **1-3**

The MS spectra of compounds (**1**, **2** and **3**) showed a well-defined parent ion at m/z 563, 607 and 680 (100 %) with the expected isotope pattern. The peaks, at m/z values of 289 and 136 in **1**, 252 in **2** and 607 and 551 in **3** correspond to the loss of (M-(NH-Ar-$OCH_2CH_2OCH_2CH_2OAr$), 60 %) and (M-((NH-Ar-$OCH_2CH_2)_2O$ + 4Cl), 75 %), (M-(NH-Ar-

TABLE-2
^{31}P NMR SPECTRAL DATA IN $CDCl_3$. CHEMICAL SHIFTS (δ) ARE REPORTED IN ppm

Formula	Compound	Spin system	δP_A	δP_B	δP_X	$^2J_{PANPB}$ (Hz)	$^2J_{PANPX}$ (Hz)	$^2J_{PBNPX}$ (Hz)
	1	AX_2	1.45	–	21.98	44.55	–	–
	2	AX_2	0.83	–	21.98	45.99	–	–
	3	ABX	22.22	5.06	2.90	48.50	52.95	53.16

$OCH_2CH_2OCH_2)_2$ + Cl, 10 %) and (M-(NH-Bu-*tert*)), 8 %) and (M-(NH-Bu-*tert*)), 7 %) groups. N_3P_3 ring system in **2** and **3** was not stable (dominant ion was not observed: m/z 134) during the fragmentation that indicates the first loss of aminopodand and chloride fragments.

Antimicrobial activities: The bacteria *Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 11230, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus epidermidis* ATCC NRRL 3284, *Enterobacter aerogenes* ATCC 13048, *Micrococcus luteus* LA 2971, *Proteus vulgaris* ATCC 8427, *Salmonella typhi* ATCC 19430, *Salmonella typhimurium* CCM 5445, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas fluorescens* ATCC 17400, *Listeria monocytogenes* ATCC 19117 and the yeast cultures *Rhodotorula rubra* DSM 70403, *Debaryomyces hansenii* DSM 70238, *Hanseniaspora guilliermondii* DSM 3432, *Kluyveromyces fragilis* NRRL 2415, *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 90018, *Candida tropicalis* ATCC 13803 were used in this study as the test microorganisms. The data reported in Table-3 are the average data of three experiments.

Table-3 shows antimicrobial activities of the compounds and standard antibacterial and antifungal antibiotic discs. As can clearly be seen from Table-3, the compounds showed antibacterial activity against both gram-positive and gram-negative bacteria and the yeast cultures in this study. In classifying the antimicrobial activity, it would be generally expected that a much greater number would be active against the yeast cultures than the bacterial cultures. However, in this study, the compounds are active against both gram-positive and gram-negative bacteria as well as active against yeasts, which may indicate broad-spectrum properties. The compound **2** has

TABLE-3
ANTIMICROBIAL ACTIVITIES OF COMPOUNDS (**1-3**) AND SOME STANDARD ANTIBIOTICS

Microorganisms	Inhibition zone (mm)									
	Compounds			Antibiotics						
	1	2	3	AK30[a]	SAM20[b]	CTX30[c]	VA30[d]	NY100[e]	KETO20[f]	CLT10[g]
B. subtilis	10	9	10	20	14	16	20	–	–	–
B. cereus	11	12	13	16	12	14	18	–	–	–
E. coli	10	–	–	17	12	10	22	–	–	–
S. aureus	–	11	10	24	16	12	13	–	–	–
S. epidermidis	10	12	12	23	18	15	15	–	–	–
E. aerogenes	11	11	10	18	15	14	18	–	–	–
S. typhimurium	9	11	10	20	20	18	16	–	–	–
S. typhi	12	14	15	19	18	18	18	–	–	–
L. monocytogenes	10	10	12	20	12	16	26	–	–	–
M. luteus	8	8	7	24	32	32	34	–	–	–
P. vulgaris	10	12	9	18	16	18	20	–	–	–
P. aeruginosa	12	14	15	19	10	54	10	–	–	–
P. fluorescens	12	16	18	18	16	36	16	–	–	–
H. guilliermondii	12	15	17	–	–	–	–	21	24	22
K. fragilis	11	13	15	–	–	–	–	18	16	18
C. albicans	14	15	17	–	–	–	–	20	21	15
C. parapsilosis	13	15	17	–	–	–	–	22	20	16
C. tropicalis	11	13	15	–	–	–	–	18	18	16
R. rubra	10	10	12	–	–	–	–	18	22	16
D. hansenii	11	11	11	–	–	–	–	16	14	18

[a]Amikacin 30 µg, [b]Ampicillin 10 µg, [c]Cefotaxime 30 µg, [d]Vancomycin 30 µg, [e]Nystatin 100 µg, [f]Ketaconazole 20 µg, [g]Clotrimazole 10 µg.

among the most active against the test microorganism. The compound **1** and **2** have a moderate activity against all microorganisms used in this study. The compound **3** against *Bacillus cereus* and *Pseudomonas aeruginosa* has stronger antibacterial effect than those of some standard antibacterial antibiotic SAM20. Notably, *Pseudomonas flurosescens* is equivalent or susceptible to the compound **3**, as compared to standards SAM20, VA30 and AK30, respectively. Similarly, the same compound has higher antifungal activity against species of *Candida* species than those of the standard antifungal antibiotic CLT10. Fungi used in this study were chosen primarily on the basis of their importance as opportunistic pathogens of humans. According to findings from the National Nosocomial Infection Surveillance System (NNIS), 61 % of reported nosocomial fungal infections were due to *Candida albicans*, followed by other *Candida* spp.[36,37]. *Candida albicans*, while naturally occurring in the intestinal flora, can cause oral thrush and systemic infections.

The results of our study indicate that the compounds especially the compound **3** have the potential to generate novel metabolites. Their strong effect on many tested organisms, particularly their lethal anticandidal activity could result in the discovery of novel anticandidal agents, demonstrating broad-spectrum characteristic. These compounds could be selected for further pharmacological tests to be evaluated as potential drugs against many infectious diseases.

Crystallographic study: The compounds **1**, **2** and **3**, $C_{16}H_{18}N_5O_3P_3Cl_4$, $C_{18}H_{22}N_5O_2P_3Cl_4$ and $C_{26}H_{42}N_7O_4P_3Cl_2$, are phosphazene derivatives with a bulky substituted spirocyclic ring. The C_4PN_1 spirocyclic ring has a sofa conformation, while the phosphazene ring is a perfect planar. The spirocyclic ring, with eighteen- and twenty one-members, has a total puckering amplitude of 1.006(3) Å[38]. The structures of **1** and **3** are mirror symmetric and **2** centro symmetric (Fig. 1). The dihedral angles between the phosphazene ring and phenyl ring planes are 82.7 (2), 82.7 (2) and 82.7 (2) for **1**, **2** and **3**, respectively. The endocyclic P2-N2-P3, P2-N1-P1, P3-N3-P1 and P2-N1-P1, P2-N2-P3, P1-N3-P3 bond angles [118.4(4), 123.0(4), 122.7(4)º and 120.1(2), 121.2(2), 125.7(2)º, respectively], P2-N2-P1, P2-N4-P2 and N2-P2-N4, N2-P1-N2 bond angles [122.36(17), 120.1(2) and 119.61(15), 115.45(18), respectively] and N3-P1-N1, N1-P2-N2, N3-P3-N2 and N3-P1-N1, N1-P2-N2, N3-P3-N2 bond angles [113.4(3), 120.9(4), 118.7(4)º and 115.74(2), 121.9(2), 113.88(2)º, respectively] for the compounds **1**, **2** and **3** are larger than the standard compound, $N_3P_3Cl_6$ and spirocyclic phenoxyphosphazene[13]. In the standard compound, $N_3P_3Cl_6$ (trimer), the endocyclic P-N-P and exocyclic Cl-P-Cl angles are 118.3(2)º and 101.2(1)º[39]. In compounds **1**, **2** and **3**, the endocyclic angles of substituents, bonded to phosphorus atoms, 113.4(3), 115.45(1) and 115.74(2), 113.88(2) are smaller than the other endocyclic angles. The exocyclic N4-P1-N5 [106.5(3)º] (**1**), N1-P1-N1' [109.60(1)º] (**2**) and N5-P1-N4 [104.8(2)º] (**3**) angles, however, are larger than the other exocyclic angles due to the replacement of the bulky aminopodand group by Cl atoms. The P-N bond lengths have been correlated with the orbital electronegativities of groups of atoms in phosphazenes[40]. The lengths of the P-N bonds depend on the electronegativities of the substituents. In compounds **1**, **2** and **3**, the aminopodand group attached to P1 seems to have a strong electron withdrawing character whereas *tert*-butylamino group attached to P3 seems to have a strong electron release character. Thus, the lengths of P-N(exocyclic) and P-N(endocyclic) bonds are changed considerably. The P-N bonds lengths in $N_3P_3R_6$ are generally equal provided that all the substituents R are the same. If R is difunctional bulky substituent[41] or contains different substituents, then the P-N bonds may show significant variations[42,43]. The P-N bond lengths in the structure of **1**, **2**

TABLE-4
GEOMETRIC DETAILS OF INTRA- AND INTERMOLECULAR HYDROGEN BONDING FOR THE COMPOUNDS **1-3**

Compound	D^a-H···A^b (Å)	D^a-H	H···A^b (Å)	D^a···A^b (Å)	∠D^a - H···A^b (º)
1	N(4)-H(4N)···O(3)[i]	0.86	2.11	2.922(8)	156
	N(5)-H(5N)···N(5)[ii]	0.86	2.59	3.233(9)	133
2	N1···O1[iii]	0.78(3)	2.43(3)	3.188(3)	164(3)
3	C(18)-H(18)···O(3)	0.93	2.55	3.292(9)	137
	N(4)-H(4N)···N(2)[iv]	0.84(5)	2.22(5)	3.058(5)	175(5)
	N(5)-H(5N)···O(2)[v]	0.82(5)	2.45(5)	3.086(9)	135(4)

[a]Donor, [b]Acceptor, [i]1/2-x, -1/2+y, z; [ii]1-x, 1-y, 1-z; [iii]1/2-y, -1/2 + x, -1/4+z; [iv]1/2-y, x, 3/2-z; [v]1-y, -1/2+x, 1/2+z.

and **3** vary from 1.534(7), 1.556(7), 1.558(6), 1.598(6), 1.603(8)-1.619(7) Å (**1**), 1.544(3), 1.572(2)-1.601(3) Å (**2**) and from 1.548(4), 1.561(4), 1.585(4), 1.595(4), 1.561(4)-1.626(4) Å (**3**) because of the influence of difunctional bulky and *tert*-butylamino substituent. Bond lengths of the P1-N1 in **1** and **2** and P3-N2 in **3** are 1.619(7) Å and 1.642(3) and 1.641(3) 5 Å 1.626(4) Å, which are longer than other P-N bonds in the ring. The P-Cl bond lengths, on the other hand, are in good agreement with the expected values[13,16,39,44-46].

The crystal structures are stabilized by intramolecular and intermolecular hydrogen bonding and their geometrical details are listed in Table-4. There are intermolecular hydrogen bonds between N4···O3 [2.922(8) Å] and N5···N5 [3.233(9) Å], for the molecule **1**, N1···O1 [3.188(3) Å], for the molecule **2** and N4···N2 [3.058(5) Å] and N5···O2 [3.086(9) Å] atoms of neighbouring molecules for the compound **3**. In the compounds **2** and **3**, there are an intramolecular hydrogen bonds C5-H5···O2 atoms [3.098(5) Å] and C18-H18···O3 atoms [3.292(9) Å. The bond distances for C5-H5 and H5···O2 are 0.92(4) and 2.34(4)Å, respectively and the C5-H5···O2 angle is 139(3)º in the compound **2**. The sum of the Van der Waals radius of the O and N atoms (3.07 Å) is significantly longer than the intramolecular O···N hydrogen bond length[47].

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