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The Syntheses and Structural Characterizations, Antimicrobial Activity, and *in vitro* DNA Binding of 4-Fluorobenzylspiro(N/O)Cyclotriphosphazenes and their Phosphazenium Salts

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Abstract: In the present study, the condensation reaction of $N_3P_3Cl_6$ (**1**) with sodium 3-(4-fluorobenzylamino)-1-propanoxide gave partly substituted 4-fluorobenzylspirocyclotriphosphazene (**2**). The Cl replacement reactions of **2** with excess benzylamine, n-hexylamine, n-butylamine and n-propylamine led to the formation of the corresponding 4-fluorobenzylspiro(N/O)tetrabenzylamino (**3a**), tetrahexylamino (**3b**), tetrabutylamino (**3c**) and tetrapropylamino (**3d**) cyclotriphosphazenes. With the protic ionic liquids (PILs), phosphazenium salts (**4a-4d**), were obtained from the reactions of the corresponding phosphazene bases (**3a**-**3d**) with gentisic acid in dry THF. The structures of all the isolated cyclotriphosphazene derivatives were determined by elemental analyses, FTIR and ^{1}H, $^{13}C\{^{1}H\}$, $^{31}P\{^{1}H\}$ NMR techniques. The crystal structure of **4d** was verified by X-ray diffraction analysis. All the compounds were screened for antibacterial and antifungal activities against bacteria and yeast strains. The interactions of the compounds with supercoiled pUC18 plasmid DNA were investigated.

Keywords: Spirocyclotriphosphazenes, crystallography, spectroscopy, antimicrobial activity, DNA binding.

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INTRODUCTION

Hexachlorocyclotriphosphazene, $N_3P_3Cl_6$, is one of the best known and studied one as the starting compound in the family of inorganic heterocyclic ring systems [1]. Various cyclotriphosphazene derivatives have been prepared from the Cl replacement reactions of $N_3P_3Cl_6$ with the different nucleophiles [2]. The condensation reactions of $N_3P_3Cl_6$ with excess monodentate and bidentate ligands resulted in the formation of fully substituted cyclotriphosphazenes [3]. For instance, there are several studies in the literature on the reactions of cyclotriphosphazenes with NO donor type difunctional reagents [4-6]. Some of the cyclotriphosphazene derivatives are used as liquid crystals [7,8], rechargeable lithium-ion batteries [9,10], OLEDs [11,12], and lubricants [13,14]. It is found that aminocyclophosphazene derivatives have antimicrobial activity against bacteria and fungi [15-17]. The interactions between DNA and the phosphazene derivatives have also been investigated in the two last decades [18,19].

On the other hand, the oldest protic ionic liquids (PILs), *e.g.* ethanolammonium nitrate and ethylammonium nitrate were reported in 1888 and 1914 by Gabriel and Walden, respectively [20]. The amine-based PILs were also reported by Bicak [21] and by Karadag [22]. Nevertheless, there is only one paper about the PILs based on cyclotriphosphazene with salicylic acid in the literature [23].

The present study is focused on the preparation of the partly substituted 4-fluorobenzylspirocyclotriphosphazene (**2**), and the fully substituted phosphazene ligands (**3a**-**3d**) for the goal of the preparation of the PILs (**4a-4d**) of the phosphazene ligands with gentisic acid. In addition, this paper also describes features of spectroscopic crystallographic properties, the evaluation of antimicrobial activity, and DNA interactions of all the compounds.

EXPERIMENTAL SECTION

Materials and Methods: $N_3P_3Cl_6$ (Aldrich), 4-fluorobenzaldehyde, 3-amino-1-propanol, benzylamine, n-hexylamine, n-butylamine, n-propylamine and 2,5-dihydroxybenzoic acid (Merck) were purchased. The solvents were distilled by standard methods before use. All the Cl replacement reactions were carried out under argon atmosphere and the reactions were monitored using thin-layer chromatography (TLC) on Merck DC Alufolien Kiesegel 60 B254 sheets in different solvents. Column chromatography was performed on Merck Kiesegel 60 (230-400 mesh ATSM) silica gel. The melting points were determined on a Gallenkamp apparatus using a capillary tube and are uncorrected. The FTIR spectra of all the phosphazenes were recorded on a Jasco FT/IR-430 spectrometer in KBr discs and reported in cm^{-1} units. The mass spectra (ESI-MS) of the phosphazenes were recorded on the Waters 2695 Alliance Micromass ZQ spectrometer. Elemental analyses were carried out by using a Leco CHNS-932 instrument (microanalytical service of Ankara University). ^1H and $^{13}C\{^1H\}$ NMR spectra were recorded on a Varian Mercury FT-NMR (400 MHz) spectrometer ($SiMe_4$ as an internal standard), operating at 400.13 and 100.62 MHz, respectively. The spectrometer was equipped with a 5 mm PABBO BB inverse-gradient probe, and standard Bruker pulse programs [24] were used. The $^{31}P\{^1H\}$ NMR spectra of the cyclotriphosphazenes were obtained on a Bruker Ascend™ 600 ULH spectrometer (85% H_3PO_4 as an external standard), operating at 242.93 MHz.

Synthesis of 3-(4-fluorobenzylamino)-1-propanol: A solution of 3-amino-1-propanol (1.20 g, 16.0 mmol) in ethanol (25 mL) was added into the solution of 4-fluorobenzaldehyde (2.00 g, 16.0 mmol) in ethanol (25 mL) with stirring at -5°C. The mixture was stirred for three days at room temperature. The solvent was evaporated at reduced pressure and the Schiff base (oily product) was obtained. $NaBH_4$ (2.92 g, 76.50 mmol) in a small portion was added into the solution of the Schiff base (2.80 g, 15.30 mmol) in ethanol (150 mL). The mixture was stirred for 24 h, and then the solvent was evaporated at reduced pressure. The crude product was extracted with $CHCl_3$ (3 x 100 mL), and dried over Na_2SO_4. The colorless oily product was dried overnight *in vacuo*. Yield: 2.60 g (89%). FTIR (KBr, cm^{-1}): v3402 (N-H), 3065 (asymm.), 3028 (symm.) (C-H arom.),1030 (C-F). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): *δ* 7.25(dd, 2H, $^3J_{FH}$=5.4 Hz, $^3J_{HH}$=8.6 Hz, $H_3$and H_5), 6.98(dd, 2H, $^3J_{FH}$=8.8 Hz, $^3J_{HH}$=8.6 Hz, $H_2$and H_6), 3.71 (s, 2H, Ar-CH_2-N), 3.70 (t, 2H, $^3J_{HH}$=6.4 Hz, O-CH_2), 3.61 (b, 2H, N*H* and O*H*),2.80 (t, 2H, $^3J_{HH}$=6.0 Hz, N-CH_2), 1.70 (m, 2H, $^3J_{HH}$=6.4 Hz, $^3J_{HH}$=6.0 Hz, N-CH_2-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 161.94 (d, $^1J_{FC}$=244.5 Hz, C_1), 135.12(d, $^4J_{FC}$=2.5 Hz, C_4), 129.77 (d, $^3J_{FC}$=7.8 Hz, C_3 and C_5), 115.19 (d, $^2J_{FC}$=21.2 Hz, C_2 and C_6), 62.81 (s, O-*C*H_2), 52.98 (s, Ar-*C*H_2-N), 48.17 (s, N-*C*H_2), 31.04 (s, N-CH_2-*C*H_2).

Synthesis of compound 2: A total of 4.42 g of $N_3P_3Cl_6$ (**1**) (12.70 mmol) in dry THF (150 mL) was added into the solution of sodium (3-amino-1-propanoxide) (3.13 g, 15.0 mmol) and triethylamine (7.10 mL, 50.8 mmol) at -10 °C. The mixture was stirred for three days at room temperature. The precipitated triethylammonium hydrochloride and sodium chloride were filtered off and the solvent was evaporated completely. The product was eluted by column chromatography using toluene, and it was crystallized from toluene. Yield: 3.96 g (68%). mp: 71 °C. Anal. Calcd. for $C_{10}H_{12}FCl_4N_4OP_3$: C, 26.23; H, 2.64; N, 12.23. Found: C, 26.18; H, 3.05; N, 11.73. ESI-MS (fragments are based on ^{35}Cl, Ir %, Ir designates the fragment abundance percentage): *m/z* 459 ([M+H]$^+$, 100). FTIR (KBr, cm^{-1}): v 3067 (asymm.), 3025 (symm.) (C-H arom.), 2927, 2855(C-H aliph.), 1243 (asymm.), 1198 (symm.) (P=N), 1046 (C-F), 575 (asymm.), 531 (symm.) (PCl). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): *δ* 7.35 (dd, 2H, $^3J_{FH}$=5.6 Hz, $^3J_{HH}$=8.4 Hz, H_3 and H_5), 7.03 (dd, 2H, $^3J_{FH}$=8.8 Hz, $^3J_{HH}$=8.4 Hz, H_2 and H_6), 4.41 (m, 2H, $^3J_{PH}$=13.6 Hz, $^3J_{HH}$=5.6 Hz, O-CH_2), 3.94 (d, 2H, $^3J_{PH}$=9.6 Hz, Ar-CH_2-N), 3.04 (m, 2H, $^3J_{PH}$=14.0 Hz, $^3J_{HH}$=6.3 Hz, N-CH_2), 1.92 (m, 2H, $^3J_{HH}$=6.3 Hz, $^3J_{HH}$=5.6 Hz, N-CH_2-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 162.43 (d, $^1J_{FC}$=245.8 Hz, C_1), 131.92 (dd, $^3J_{PC}$=9.6 Hz, $^4J_{FC}$=3.2 Hz, C_4), 130.13 (d, $^3J_{FC}$=8.4 Hz, C_3 and C_5), 115.48 (d, $^2J_{FC}$=21.2 Hz, C_2 and C_6), 62.18 (d, $^2J_{PC}$=7.1 Hz, O-*C*H_2), 50.34 (d, $^2J_{PC}$=3.2 Hz, Ar-*C*H_2-N), 45.43 (s, N-*C*H_2), 25.85 (d, $^3J_{PC}$=4.5 Hz, N-CH_2-*C*H_2).

Synthesis of compound 3a: A solution of benzylamine (2.30 mL, 21.0 mmol) in dry THF (50 mL) was slowly added into a stirred solution of triethylamine (0.97 mL, 7.00 mmol) and **2** (0.80 g, 1.80 mmol) in dry THF (100 mL) at room temperature. The mixture was refluxed for over 72 h. The precipitated triethylammonium hydrochloride was filtered off and the solvent was evaporated. The product was purified by column chromatography using toluene-THF (3:2), and the light yellow powder was crystallized from toluene. Yield: 0.80 g (62%). mp: 91 °C. Anal. Calcd. for $C_{38}H_{44}FN_8OP_3.H_2O$: C, 60.19; H, 6.11; N, 14.78. Found: C, 60.31; H, 5.73; N, 14.68. ESI-MS (Ir %, Ir designates the fragment abundance percentage): *m/z* 741 ([M+H]$^+$, 100). FTIR (KBr, cm^{-1}): v3374, 3200 (b, N-H), 3063 (asymm.), 3028 (symm.) (C-H arom.), 2954, 2850 (C-H aliph.), 1198 (b, P=N), 1052 (C-F).^{1}H NMR (400 MHz, $CDCl_3$, ppm): *δ* 7.35 (dd, 2H, $^3J_{FH}$=5.2 Hz, $^3J_{HH}$=8.8 Hz, H_3 and H_5), 7.31 (m, 4H, H_{10} and $H_{10'}$), 7.25 (m, 8H, H_8 and $H_{8'}$), 7.19 (m, 8H, H_9 and $H_{9'}$), 6.96(dd, 2H, $^3J_{FH}$=9.2 Hz, $^3J_{HH}$=8.4 Hz, H_2 and H_6), 4.34 (m, 2H, $^3J_{PH}$=13.2 Hz, $^3J_{HH}$=5.6 Hz, O-CH_2), 4.15 (m, 8H, NH-CH_2), 3.82 (d, 2H, $^3J_{PH}$=7.6 Hz, Ar-CH_2-N), 2.97 (m, 2H, $^3J_{PH}$=13.2 Hz, $^3J_{HH}$=5.2 Hz, N-CH_2), 2.56 (b, 4H, N*H*), 1.85 (m, 2H, $^3J_{HH}$=5.6 Hz, $^3J_{HH}$=5.2 Hz, N-CH_2-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ

162.02 (d, $^{1}J_{FC}$=244.6 Hz, C_1), 140.87 (t, $^{3}J_{PC}$=7.8 Hz, C_7), 140.71 (t, $^{3}J_{PC}$=7.7 Hz, $C_{7'}$), 134.17 (dd, $^{3}J_{PC}$=7.7 Hz, $^{4}J_{FC}$=2.9 Hz, C_4), 129.73 (d, $^{3}J_{FC}$=7.7 Hz, C_3 and C_5), 128.31 (s, C_8 and $C_{8'}$), 127.44 and 127.31 (s, C_9 and $C_{9'}$), 126.85 and 126.82 (s, C_{10} and $C_{10'}$), 115.00 (d, $^{2}J_{FC}$=20.7 Hz, C_2 and C_6), 66.36 (d, $^{2}J_{PC}$=6.9 Hz, O-CH_2), 50.83 (d, $^{2}J_{PC}$=2.1 Hz, Ar-CH_2-N), 45.95 (s, N-CH_2), 45.09 and 45.00 (s, Ar-CH_2-NH), 26.62 (d, $^{3}J_{PC}$=3.8 Hz, N-CH_2-CH_2).

Synthesis of compound 3b: The work-up procedure was similar to that of compound **3a**, using **2** (0.80 g, 1.80 mmol), n-hexylamine (2.78 mL, 21.0 mmol) and triethylamine (0.97 mL, 7.00 mmol). The product was purified by column chromatography using toluene-THF (3:2), and the yellow oily product was crystallized from toluene. Yield: 0.65 g (52%). Anal. Calcd. for $C_{34}H_{68}FN_8OP_3$: C, 57.00; H, 7.94; N, 14.01. Found: C, 56.23; H, 7.36; N, 13.42. ESI-MS (Ir %): m/z 717 ([M+H]$^+$, 100). FTIR (KBr, cm^{-1}): v 3377, 3244(b, N-H), 3068 (asymm.), 3037 (symm.) (C-H arom.), 2956, 2856(C-H aliph.), 1198 (b, P=N), 1052 (C-F). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.39(dd, 2H, $^{3}J_{FH}$=5.6 Hz, $^{3}J_{HH}$=8.4 Hz, H_3 and H_5), 6.98(dd, 2H, $^{3}J_{FH}$=8.8 Hz, $^{3}J_{HH}$=8.8 Hz, H_2 and H_6), 4.30 (m, 2H, $^{3}J_{PH}$=12.4 Hz, $^{3}J_{HH}$=5.2 Hz, O-CH_2), 3.93 (d, 2H, $^{3}J_{PH}$=7.6 Hz, Ar-CH_2-N), 2.98 (m, 2H, $^{3}J_{PH}$=13.2 Hz, $^{3}J_{HH}$=5.2 Hz, N-CH_2), 2.90 (b, 8H, NH-CH_2), 2.24 (b, 4H, NH), 1.82 (m, 2H, $^{3}J_{HH}$=5.2 Hz, $^{3}J_{HH}$=4.8 Hz, N-CH_2-CH_2), 1.48 (m, 8H, NH-CH_2-CH_2), 1.27 (m, 24H, NH-CH_2-CH_2-(CH_2)$_3$), 0.88 (t, 6H, $^{3}J_{HH}$=7.2 Hz, CH_3), 0.84 (t, 6H, $^{3}J_{HH}$=7.2 Hz, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 161.91 (d, $^{1}J_{FC}$=244.5 Hz, C_1), 134.25 (dd, $^{3}J_{PC}$=10.4 Hz, $^{4}J_{FC}$=2.6 Hz, C_4), 129.68 (d, $^{3}J_{FC}$=7.7 Hz, C_3 and C_5), 114.82 (d, $^{2}J_{FC}$=21.5 Hz, C_2 and C_6), 65.51 (d, $^{2}J_{PC}$=6.9 Hz, O-CH_2), 50.78 (s, Ar-CH_2-N), 45.74 (s, N-CH_2), 40.97 and 40.85 (s, NH-CH_2), 31.92 (d, $^{3}J_{PC}$=7.7 Hz,NH-CH_2-CH_2), 31.79 (d, $^{3}J_{PC}$=7.7 Hz,NH-CH_2-CH_2), 26.57 (s,N-CH_2-CH_2), 22.51 and 22.47 (s, NH-CH_2-CH_2-(CH_2)$_3$), 13.90 and 13.85 (s, CH_3).

Synthesis of compound 3c: The work-up procedure was similar to that of compound **3a**, using **2** (0.80 g, 1.80 mmol), n-butylamine (2.10 mL, 21.0 mmol) and triethylamine (0.97 mL, 7.00 mmol). The product was eluted by column chromatography using toluene-THF (3:2), and the light yellow powder was crystallized from toluene. Yield: 0.75 g (71%). mp: 53 °C. Anal. Calcd. for $C_{26}H_{52}FN_8OP_3.H_2O$: C, 50.18; H, 8.75; N, 18.00. Found: C, 50.74; H, 8.26; N, 17.96. ESI-MS (Ir %): m/z 607 ([M+H]$^+$, 100). FTIR (KBr, cm^{-1}): v 3386, 3243(b, N-H), 3068 (asymm.), 3040 (symm.) (C-H arom.), 2958, 2869(C-H aliph.), 1202(b, P=N), 1053(C-F). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 7.40(dd, 2H, $^{3}J_{FH}$=5.2 Hz, $^{3}J_{HH}$=8.4 Hz, H_3 and H_5), 6.99(dd, 2H, $^{3}J_{FH}$=8.8 Hz, $^{3}J_{HH}$=8.4 Hz, H_2 and H_6), 4.30 (m, 2H, $^{3}J_{PH}$=12.4 Hz, $^{3}J_{HH}$=5.6 Hz, O-CH_2), 3.92 (d, 2H, $^{3}J_{PH}$=7.2 Hz, Ar-CH_2-N), 2.96 (m, 2H, $^{3}J_{PH}$=13.6 Hz, $^{3}J_{HH}$=5.6 Hz, N-CH_2), 2.89 (b, 8H, NH-CH_2), 2.13 (b, 4H, NH), 1.80 (m, 2H, $^{3}J_{HH}$=5.6 Hz, $^{3}J_{HH}$=5.2 Hz, N-CH_2-CH_2), 1.47 (m, 8H, NH-CH_2-CH_2), 1.33 (m, 8H, NH-CH_2-CH_2-CH_2), 0.90 (t, 6H, $^{3}J_{HH}$=7.6 Hz, CH_3), 0.83 (t, 6H, $^{3}J_{HH}$=7.6 Hz, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 161.97 (d, $^{1}J_{FC}$=244.6 Hz, C_1), 134.38 (dd, $^{3}J_{PC}$=10.4 Hz, $^{4}J_{FC}$=2.7 Hz, C_4), 129.78 (d, $^{3}J_{FC}$=7.8 Hz, C_3 and C_5), 114.90 (d, $^{2}J_{FC}$=20.6 Hz, C_2 and C_6), 66.09 (d, $^{2}J_{PC}$=6.9 Hz, O-CH_2), 50.88 (d, $^{2}J_{PC}$=2.3 Hz,Ar-CH_2-N), 45.85 (s, N-CH_2), 40.70 and 40.59 (s, NH-CH_2), 34.09 (d, $^{3}J_{PC}$=8.5 Hz,NH-CH_2-CH_2), 34.00 (d, $^{3}J_{PC}$=8.5 Hz,NH-CH_2-CH_2), 26.57 (d,$^{3}J_{PC}$=3.0 Hz,N-CH_2-CH_2), 20.07 and 20.04 (s, NH-CH_2-CH_2-CH_2), 13.82 and 13.74 (s, CH_3).

Synthesis of compound 3d: The work-up procedure was similar to that of compound **3a**, using **2** (0.80 g, 1.80 mmol), n-propylamine (1.73 mL, 21.0 mmol) and triethylamine (0.97 mL, 7.00 mmol). The product was purified by column chromatography using toluene-THF (3:2), and and the light yellow powder was crystallized from toluene. Yield: 0.73 g (76%). mp: 77 °C. Anal. Calcd. for $C_{22}H_{44}FN_8OP_3$: C, 57.00; H, 8.01; N, 13.99.

Found: C, 56.23; H, 7.36; N, 13.42. ESI-MS (Ir %): m/z 549 ([M+H]$^+$, 100). FTIR (KBr, cm^{-1}): v 3371, 3237(b, N-H), 3068 (asymm.), 3033 (symm.) (C-H arom.), 2957, 2853(C-H aliph.), 1204(b, P=N), 1051(C-F). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): *δ* 7.37(dd, 2H, $^3J_{FH}$=5.6 Hz, $^3J_{HH}$=8.8 Hz, H_3 and H_5), 6.96(dd, 2H, $^3J_{FH}$=8.8 Hz, $^3J_{HH}$=8.8 Hz, H_2 and H_6), 4.28 (m, 2H, $^3J_{PH}$=12.4 Hz, $^3J_{HH}$=5.6 Hz, O-CH_2), 3.89 (d, 2H, $^3J_{PH}$=7.6 Hz, Ar-CH_2-N), 2.92 (m, 2H, $^3J_{PH}$=13.2 Hz, $^3J_{HH}$=5.6 Hz, N-CH_2), 2.84 (b, 8H, NH-CH_2), 2.14 (b, 4H, N*H*), 1.78 (m, 2H, $^3J_{HH}$=5.6 Hz, $^3J_{HH}$=5.2 Hz, N-CH$_2$-CH_2), 1.46 (m, 8H, NH-CH$_2$-CH_2), 0.88 (t, 6H, $^3J_{HH}$=7.2 Hz, CH_3), 0.81 (t, 6H, $^3J_{HH}$=7.6 Hz, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 161.99 (d, $^1J_{FC}$=244.6 Hz, C_1), 134.41 (dd, $^3J_{PC}$=10.0 Hz, $^4J_{FC}$=2.8 Hz, C_4), 129.82 (d, $^3J_{FC}$=7.7 Hz, C_3 and C_5), 114.92 (d, $^2J_{FC}$=21.5 Hz, C_2 and C_6), 66.11 (d, $^2J_{PC}$=6.9 Hz, O-*C*H$_2$), 50.89 (d, $^2J_{PC}$=3.0 Hz,Ar-*C*H$_2$-N), 45.87 (s, N-*C*H$_2$), 42.81 and 42.73 (s, NH-*C*H$_2$), 26.58 (d, $^3J_{PC}$=3.0 Hz,N-CH$_2$-*C*H$_2$), 25.07 (d, $^3J_{PC}$=6.9 Hz,NH-CH$_2$-*C*H$_2$), 24.99 (d, $^3J_{PC}$=8.4 Hz,NH-CH$_2$-*C*H$_2$), 11.44 and 11.35 (s, *C*H$_3$).

Synthesis of compound 4a: A solution of **3a** (0.50 g, 0.67 mmol) in dry THF (30 mL) was slowly added by the dropwise addition of gentisic acid (0.10 g, 0.67 mmol) in dry THF (10 mL) at room temperature. The reaction mixtures were refluxed for over 30 h. Afterwards, the solvent was evaporated under vacuum, and the light yellow oily crude product was crystallized from toluene. Yield: 0.45 g (75%). mp: 62 °C. Anal. Calcd. for $C_{45}H_{50}FN_8O_5P_3.H_2O$: C, 59.27; H, 5.75; N, 12.28. Found: C, 58.81; H, 5.05; N, 11.82. FTIR (KBr, cm^{-1}): v 3277 (b, N-H), 3062 (asymm.), 3029 (symm.) (C-H arom.), 2923, 2858 (C-H aliph.), 2668 (N$^+$-H), 1571 (asymm.), 1377 (symm.) (COO$^-$), 1260 (b, P=N), 1050 (C-F). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): *δ* 7.70 (b, 1H, H_b), 7.26-7.03 (m, 24H, H_3, H_5, H_8, $H_{8'}$, H_9, $H_{9'}$, H_{10}, $H_{10'}$, H_d and H_e*),* 6.93(dd, 2H, $^3J_{FH}$=8.8 Hz, $^3J_{HH}$=8.4 Hz, H_2 and H_6), 4.24 (m, 2H, $^3J_{PH}$=12.4 Hz, $^3J_{HH}$=6.0 Hz, O-CH_2), 3.98 (m, 8H, NH-CH_2), 3.80 (b, 4H, N*H*), 3.61 (d, 2H, $^3J_{PH}$=7.6 Hz, Ar-CH_2-N), 2.89 (m, 2H, $^3J_{PH}$=12.8 Hz, $^3J_{HH}$=6.0 Hz, N-CH_2), 2.34 (s, 1H, N*H*), 1.80 (m, 2H, $^3J_{HH}$=5.2 Hz, $^3J_{HH}$=4.4 Hz, N-CH$_2$-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 175.05 (s, COO$^-$), 162.17 (d, $^1J_{FC}$=245.3 Hz, C_1), 155.26 (s, C_f), 148.09 (s, C_c), 139.44 (t, $^3J_{PC}$=6.0 Hz, C_7), 139.31 (t, $^3J_{PC}$=6.0 Hz, $C_{7'}$), 132.92 (dd, $^3J_{PC}$=7.6 Hz, $^4J_{FC}$=2.8 Hz, C_4), 129.64 (d, $^3J_{FC}$=7.7 Hz, C_3 and C_5), 128.46 and 128.42 (s, C_8 and $C_{8'}$), 127.44 and 127.23 (s, C_9 and $C_{9'}$), 127.16 and 127.10 (s, C_{10} and $C_{10'}$), 121.70 (s, C_a), 117.55 (s, C_d), 117.32 (s, C_e), 116.32 (s, C_b), 115.22 (d, $^2J_{FC}$=21.5 Hz, C_2 and C_6), 67.49 (d, $^2J_{PC}$=6.1 Hz, O-*C*H$_2$), 50.17 (s, Ar-*C*H$_2$-N), 45.64 (s, N-*C*H$_2$), 44.77 and 44.76 (s, Ar-*C*H$_2$-NH), 26.18 (s, N-CH$_2$-*C*H$_2$).

Synthesis of compound 4b: The work-up procedure was similar to that of compound **4a**, using **3b** (0.50 g, 0.70 mmol) and gentisic acid (0.11 g, 0.70 mmol). The reaction mixture was refluxed for over 30 h. Afterwards, the solvent was evaporated under reduced pressure, and the light yellow oily crude product was crystallized from toluene. Yield: 0.49 g (80%). mp: 90 °C. Anal. Calcd. for $C_{41}H_{74}FN_8O_5P_3.H_2O$: C, 54.32; H, 8.67; N, 12.36. Found: C, 54.21; H, 7.84; N, 11.95. FTIR (KBr, cm^{-1}): v 3263(b, N-H), 3065 (asymm.), 3040 (symm.) (C-H arom.), 2956, 2857(C-H aliph.), 2667 (N$^+$-H), 1579(asymm.), 1381 (symm.) (COO$^-$), 1257(asymm.), 1193 (symm.) (P=N), 1055(C-F). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): *δ* 7.59 (b, 1H, $^4J_{HH}$=3.1 Hz, H_b), 7.33 (dd, 2H, $^3J_{FH}$=5.2 Hz, $^3J_{HH}$=8.4 Hz, H_3 and H_5), 7.00 (dd, 2H, $^3J_{FH}$=8.4 Hz, $^3J_{HH}$=8.4 Hz, H_2 and H_6), 6.93 (dd, 1H, $^3J_{HH}$=8.5 Hz, $^4J_{HH}$=3.1 Hz, H_d), 6.77 (d, 1H, $^3J_{HH}$=8.5 Hz, H_e), 4.36 (m, 2H, $^3J_{PH}$=11.6 Hz, $^3J_{HH}$=5.2 Hz, O-CH_2), 3.94 (d, 2H, $^3J_{PH}$=6.0 Hz, Ar-CH_2-N), 3.02 (m, 2H, N-CH_2), 2.86 (b, 8H, NH-CH_2), 2.84 (b, 4H, N*H*), 2.28 (s, 1H, N*H*), 1.73 (m, 2H, $^3J_{HH}$=5.2 Hz, N-CH$_2$-CH_2), 1.40 (m, 8H, NH-CH$_2$-CH_2), 1.18 (m, 24H, NH-CH$_2$-CH$_2$-(CH_2)$_3$), 0.85 (t, 6H, $^3J_{HH}$=6.8 Hz, CH_3), 0.78 (t, 6H, $^3J_{HH}$=7.6 Hz, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): δ 174.03 (s, COO$^-$), 162.23 (d, $^1J_{FC}$=245.4 Hz, C_1), 155.45 (s, C_f), 148.22 (s, C_c), 132.93 (dd, $^3J_{PC}$=10.0 Hz, $^4J_{FC}$=2.6 Hz, C_4), 129.65 (d, $^3J_{FC}$=7.8 Hz, C_3 and C_5), 122.63 (s, C_a), 117.52 (s, C_d), 115.94 (s, C_b), 115.43 (s, C_e), 115.27 (d, $^2J_{FC}$=21.4 Hz, C_2 and C_6), 67.40 (d, $^2J_{PC}$=4.6 Hz, O-*C*H$_2$), 50.40 (s, Ar-*C*H$_2$-N), 45.73 (s, N-*C*H$_2$), 41.21 and 41.08 (s, NH-

*C*H$_2$), 31.64 (d, $^3J_{PC}$=7.0 Hz,NH-CH$_2$-*C*H$_2$), 31.53 (d, $^3J_{PC}$=7.8 Hz,NH-CH$_2$-*C*H$_2$), 26.45 (s,N-CH$_2$-*C*H$_2$), 22.56 and 22.50 (s, NH-CH$_2$-*C*H$_2$-(*C*H$_2$)$_3$), 14.00 and 13.94 (s, *C*H$_3$).

Synthesis of compound 4c: The work-up procedure was similar to that of compound **4a**, using **3c** (0.50 g, 0.83 mmol) and gentisic acid (0.13 g, 0.83 mmol). The reaction mixtures were refluxed for over 30 h. Afterwards, the solvent was evaporated under vacuum, and the light yellow oily crude product was crystallized from toluene. Yield: 0.53 g (84%). mp: 87 °C. Anal. Calcd. for $C_{33}H_{58}FN_8O_5P_3$: C, 52.27; H, 6.46; N, 14.78. Found: C, 52.40; H, 6.54; N, 14.50.FTIR (KBr, cm^{-1}): v 3361, 3314, 3209 (b, N-H), 3072 (asymm.), 3040 (symm.) (C-H arom.), 2959, 2873 (C-H aliph.), 2667 (N$^+$-H), 1579 (asymm.), 1382 (symm.) (COO$^-$), 1255 (asymm.), 1189 (symm.) (P=N), 1057 (C-F). ^{1}H NMR (400 MHz, CDCl$_3$, ppm): *δ* 7.69 (b, 1H, $^4J_{HH}$=2.9 Hz, *H*$_b$), 7.34 (dd, 2H, $^3J_{FH}$=5.2 Hz, $^3J_{HH}$=8.4 Hz, *H*$_3$ and *H*$_5$), 6.99 (dd, 2H, $^3J_{FH}$=8.8 Hz, $^3J_{HH}$=8.8 Hz, *H*$_2$ and *H*$_6$), 6.83 (dd, 1H, $^3J_{HH}$=8.7 Hz, $^4J_{HH}$=2.9 Hz, *H*$_d$), 6.73 (d, 1H, $^3J_{HH}$=8.7 Hz, *H*$_e$), 4.34 (m, 2H, $^3J_{PH}$=12.0 Hz, $^3J_{HH}$=6.4 Hz, O-C*H*$_2$), 3.94 (d, 2H, $^3J_{PH}$=6.0 Hz, Ar-C*H*$_2$-N), 3.01 (m, 2H, N-C*H*$_2$), 2.86 (b, 8H, NH-C*H*$_2$), 2.85 (b, 4H, N*H*), 2.35 (s, 1H, N*H*), 1.86 (m, 2H, $^3J_{HH}$=6.4 Hz, N-CH$_2$-C*H*$_2$), 1.46 (m, 4H, NH-CH$_2$-C*H*$_2$), 1.38 (m, 4H, NH-CH$_2$-C*H*$_2$), 1.32 (m, 4H, NH-CH$_2$-CH$_2$-C*H*$_2$), 1.18 (m, 4H, NH-CH$_2$-CH$_2$-C*H*$_2$), 0.85 (t, 6H, $^3J_{HH}$=7.4 Hz, C*H*$_3$), 0.73 (t, 6H, $^3J_{HH}$=7.4 Hz, C*H*$_3$). ^{13}C NMR (100 MHz, CDCl$_3$, ppm): δ 174.91 (s, COO$^-$), 162.20 (d, $^1J_{FC}$=244.6 Hz, *C*$_1$), 155.20 (s, *C*$_f$), 148.09 (s, *C*$_c$), 133.03 (dd, $^3J_{PC}$=6.7 Hz, $^4J_{FC}$=2.7 Hz, *C*$_4$), 129.67 (d, $^3J_{FC}$=8.4 Hz, *C*$_3$ and *C*$_5$), 121.43 (s, *C*$_a$), 117.53 (s, *C*$_d$), 117.10 (s, *C*$_e$), 116.36 (s, *C*$_b$), 115.24 (d, $^2J_{FC}$=21.5 Hz, *C*$_2$ and *C*$_6$), 67.21 (d, $^2J_{PC}$=4.6 Hz, O-*C*H$_2$), 50.43 (s, Ar-*C*H$_2$-N), 45.75 (s, N-*C*H$_2$), 40.81 and 40.73 (s, NH-*C*H$_2$), 33.62 (d, $^3J_{PC}$=6.1 Hz,NH-CH$_2$-*C*H$_2$), 33.58 (d, $^3J_{PC}$=6.2 Hz,NH-CH$_2$-*C*H$_2$), 26.21 (d, $^3J_{PC}$=3.2 Hz,N-CH$_2$-*C*H$_2$), 19.87 and 19.83 (s, NH-CH$_2$-*C*H$_2$-*C*H$_2$), 13.70 and 13.57 (s, *C*H$_3$).

Synthesis of compound 4d: The work-up procedure was similar to that of compound **4a**, using **3d** (0.50 g, 0.90 mmol) and gentisic acid (0.14 g, 0.90 mmol). The reaction mixtures were refluxed for over 30 h. Afterwards, the solvent was evaporated under vacuum, and the light yellow oily crude product was crystallized from toluene. Yield: 0.55 g (86%). mp: 114 °C. Anal. Calcd. for $C_{29}H_{50}FN_8O_5P_3.H_2O$: C, 48.36; H, 7.28; N, 15.56. Found: C, 48.88; H, 6.81; N, 15.37. FTIR (KBr, cm^{-1}): v3373, 3289(b, N-H), 3062 (asymm.), 3033 (symm.) (C-H arom.), 2962, 2875 (C-H aliph.), 2669 (N$^+$-H), 1580(asymm.), 1372 (symm.) (COO$^-$), 1256 (asymm.), 1218 (symm.) (P=N), 1046(C-F). ^{1}H NMR (400 MHz, CDCl$_3$, ppm): *δ* 7.70 (b, 1H, $^4J_{HH}$=3.1 Hz, *H*$_b$), 7.33(dd, 2H, $^3J_{FH}$=5.6 Hz, $^3J_{HH}$=8.8 Hz, *H*$_3$ and *H*$_5$), 6.99(dd, 2H, $^3J_{FH}$=8.8 Hz, $^3J_{HH}$=8.8 Hz, *H*$_2$ and *H*$_6$), 6.82 (dd, 1H, $^3J_{HH}$=8.9 Hz, $^4J_{HH}$=3.1 Hz, *H*$_d$), 6.73 (d, 1H, $^3J_{HH}$=8.9 Hz, *H*$_e$), 4.33 (m, 2H, $^3J_{PH}$=11.6 Hz, $^3J_{HH}$=6.4 Hz, O-C*H*$_2$), 3.93 (d, 2H, $^3J_{PH}$=6.0 Hz, Ar-C*H*$_2$-N), 2.99 (m, 2H, N-C*H*$_2$), 2.83 (b, 8H, NH-C*H*$_2$), 2.80 (b, 4H, N*H*), 2.35 (s, 1H, N*H*), 1.86 (m, 2H, $^3J_{HH}$=6.4 Hz, N-CH$_2$-C*H*$_2$), 1.48 (m, 4H, NH-CH$_2$-C*H*$_2$), 1.41 (m, 4H, NH-CH$_2$-C*H*$_2$), 0.86 (t, 6H, $^3J_{HH}$=7.4 Hz, C*H*$_3$), 0.74 (t, 6H, $^3J_{HH}$=7.5 Hz, C*H*$_3$).^{13}C NMR (100 MHz, CDCl$_3$, ppm): δ 175.02 (s, COO$^-$), 162.18 (d, $^1J_{FC}$=245.3 Hz, *C*$_1$), 155.12 (s, *C*$_f$), 148.10 (s, *C*$_c$), 133.07 (dd, $^3J_{PC}$=7.3 Hz, $^4J_{FC}$=2.8 Hz, *C*$_4$), 129.70 (d, $^3J_{FC}$=8.5 Hz, *C*$_3$ and *C*$_5$), 121.23 (s, *C*$_a$), 117.93 (s, *C*$_d$), 117.02 (s, *C*$_e$), 116.42 (s, *C*$_b$), 115.21 (d, $^2J_{FC}$=21.4 Hz, *C*$_2$ and *C*$_6$), 67.18 (d, $^2J_{PC}$=4.5 Hz, O-*C*H$_2$), 50.46 (s, Ar-*C*H$_2$-N), 45.79 (s, N-*C*H$_2$), 42.82 and 42.76 (s, NH-*C*H$_2$), 26.20 (d, $^3J_{PC}$=3.0 Hz,N-CH$_2$-*C*H$_2$), 24.71 (d, $^3J_{PC}$=6.7 Hz,NH-CH$_2$-*C*H$_2$), 24.68 (d, $^3J_{PC}$=6.7 Hz,NH-CH$_2$-*C*H$_2$), 11.23 and 11.14 (s, *C*H$_3$).

X-Ray Crystal Structure Determinations: The light yellow crystals **4d** were obtained from toluene at ambient temperature. The crystallographic data (Table 1) and the selected bond lengths and angles (Table 2) were given. Crystallographic data were recorded on a Bruker Kappa APEXII CCD area-detector diffractometer using MoK_α

radiation (λ=0.71073 Å) at T=173(2) K. Absorption correction by multi-scan was applied [25]. The structure was solved by direct methods and refined by full-matrix least squares against F^2 using all data [26,27]. All non-H atoms were refined anisotropically. Atoms H2A (for N2), H51 (for N5), H61 (for N6), H71 (for N7), H81 (for N8) and H5A (for O5) were located in a difference Fourier map and refined isotropically. The remaining H atoms were positioned geometrically at distances of 0.82 Å (OH), 0.93 Å (CH), 0.97 Å (CH_2) and 0.96 Å (CH_3) from the parent C and O atoms; a riding model was used during the refinement process and the U_{iso}(H) values were constrained to be $1.2U_{eq}$ (for methine and methylene carrier atoms) and $1.5U_{eq}$ (for hydroxyl and methyl carrier atoms).

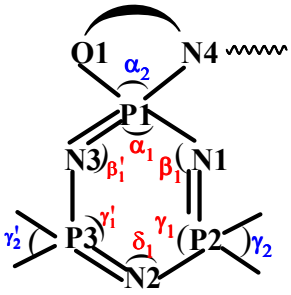
Determination of antimicrobial activity: The antimicrobial susceptibility testing was performed by the BACTEC MGIT 960 (Becton Dickinson, Sparks, MD) system. The antimicrobial efficacy of the compounds (**3a-3d** and **4a-4d**) was examined using the standard broth dilution method [28]. The microorganisms used in antimicrobial screening included three bacteria {*Escherichia coli* ATCC 25922 (G-), *Klebsiella pneumoniae* ATCC 13883 (G) and *Enterobacter faecalis* ATCC 29212 (G+)} and a fungus (*Candida albicans* ATCC 10231). The MIC values were determined in benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH) broth using serial dilution of the compounds ranging from 3000-15,63 µM with adjusted bacterial and fungal concentration ($1x10^6$ CFU/mL, 0,5 McFarland's standard). Bacterial strains were grown in nutrient agar medium and incubated at 37 ºC for 24 h. The yeast cells were cultured on Sabouraud dextrose agar (SDA) medium and incubated at 30 ºC for 48 h. Ampicillin (Amp, 10 µg/mL) and Chloramphenicol (C, 30 µg/mL) (antibacterial), and Ketoconazole (K, 50 µg/mL) (antifungal) were used as controls. The solutions (4000 µM) of the compounds were obtained in DMF. All the experiments were repeated three times, and the mean values were used. The MIC is the lowest concentration of compounds that inhibits 90% growth. The MBC and MFC are determined by inoculating previous culture which showed no growth in agar plates. The MBC and MFC are the lowest concentration of the compound that kills 99,9% of the initial microorganism concentration.

Table 1: Crystallographic data for **4d**.

Empirical Formula	$C_{29}H_{50}O_5N_8P_3F$	Z	4
Fw	702.68	μ(MoKα) (cm^{-1})	2.172
Crystal System	monoclinic	ρ(calcd) (g cm^{-1})	1.289
Space Group	P 2_1/n	Number of Reflections Total	22308
a (Å)	11.9537(3)	Number of Reflections Unique	6402
b (Å)	24.0902(5)	R_{int}	0.094
c (Å)	12.7785(3)	$2\theta_{max}$ (°)	50.1
α (°)	90.00	T_{min} / T_{max}	0.80/0.90
β (°)	100.331(3)	Number of Parameters	443
γ (°)	90.00	R [$F^2 > 2\sigma(F^2)$]	0.079
V ($Å^3$)	3620.1(2)	wR	0.199

Table 2: The selected bond lengths and angles for **4d**.

Bond Lengths (Å)		Bond Angles (°)	
P1-N1	1.589(4)	α_1	115.3(2)
P1-N3	1.585(4)	α_2	102.3(2)
P2-N1	1.579(4)	β_1	123.5(2)
P2-N2	1.655(4)	β_1'	128.5(3)
P3-N2	1.666(4)	γ_1	111.6(2)
P3-N3	1.575(4)	γ_1'	108.5(2)
P1-N4	1.649(4)	γ_2	105.9(3)
P1-O1	1.581(4)	γ_2'	104.0(2)
P2-N7	1.626 (5)	δ_1	126.7(3)
P2-N8	1.598(5)		
P3-N5	1.610(4)		
P3-N6	1.610(5)		



Determination of the pUC18 plasmid DNA interaction with the compounds: The interactions of the cyclotriphosphazenes and the PILs with the pUC18 plasmid DNA were evaluated using agarose gel electrophoresis [29]. The compounds were incubated with pUC18 plasmid DNA in an incubator at 37 ºC for 24 h in the dark. The compound/DNA mixtures were loaded onto the 1% agarose gel with a loading buffer (0.1% bromophenol blue, 0.1% xylene cyanol). Electrophoresis was made in 0.05 M Tris base, 0.05 M glacial acetic acid, and 1 mM EDTA (TAE buffer, pH = 8.0) for 3 h at 60V [30]. Subsequently, the gel was stained with ethidium bromide (0.5 µg/mL), visualized under UV light using a transilluminator (BioDoc Analyzer, Biometra), photographed with a video camera, and saved as a TIFF file. The experiments were repeated three times.

Determination of *BamH*I and *Hind*III restriction enzyme digestion of the compounds-pUC18 plasmid DNA: In order to assess whether the compounds **3a-3d** and **4a-4d** show affinity towards adenine-adenine (AA) and/or guanine-guanine (GG) regions of DNA, the restriction analyses of the compound-pUC18 plasmid DNA adducts by *BamH*Iand *Hind*III enzymes are performed. The compound/pUC18 plasmid DNA mixtures were incubated for 24 h,and then restricted with *BamH*I or *Hind*III enzymes at 37 ºC for 2 h. The restricted DNA was run in 1% agarose gel electrophoresis for 1 h at 70 V in TAE buffer. Consequently, the gel was stained with ethidium bromide (0.5 µg/mL), and it was viewed using a transilluminator, and the image was photographed using a video-camera, and saved as a TIFF file[20].

RESULTS and DISCUSSION

Syntheses and Characterizations. The intermediate Schiff base, "N-[(E)-(4-fluorophenyl)methylidene]-3-(hydroxy)propan-1-amine", was prepared from the condensation reaction of 4-fluorobenzaldehyde with 3-amino-1-propanol. Subsequently, this compound was reduced with $NaBH_4$ in methanol to give the starting compound 3-(4-fluorobenzylamino)-1-propanol. The total reaction yield was 89%. In the literature, the latter compound was also prepared from the reaction of 4-fluorobenzyl chloride with 3-amino-1-propanol in moderate yield 64%, and patented [31].

The condensation reaction of $N_3P_3Cl_6$ (**1**) with an equimolar amount of sodium 3-(4-fluorobenzylamino)-1-propanoxide gave 4-fluorobenzylspiro(N/O)cyclotriphosphazene (**2**) in a high yield (68%). The reactions of partly substituted **2** with excess benzylamine, n-hexylamine, n-butylamine and n-propylamine led to the formation of the corresponding 4-fluorobenzylspiro(N/O)tetrabenzylamino (**3a**), tetrahexylamino (**3b**), tetrabutylamino (**3c**) and tetrapropylamino (**3d**) cyclotriphosphazenes. The calculated yields of these compounds were found to be 62, 52, 71 and 76%, respectively. The PILs (**4a-4d**) of phosphazene bases (**3a-3d**) with gentisic acid were obtained in a high yield in dry THF (Figure 1). The crystal structure of **4d** was verified by X-ray diffraction analysis. The results indicate that compound **4d** was monoprotonated with the nitrogen of phosphazene ring non-adjacent to the NO spiro ring. However, in the literature the protonation was observed from the nitrogen atom of phosphazene ring adjacent to the NN spiro ring [23]. The NMR results exhibit that all the compounds are likely to be protonated in the same fashion. All the PILs were highly soluble in the common organic polar and apolar solvents. However, they are dissolved slightly in water. The solubilities of these PILs increased with the increasing temperature.

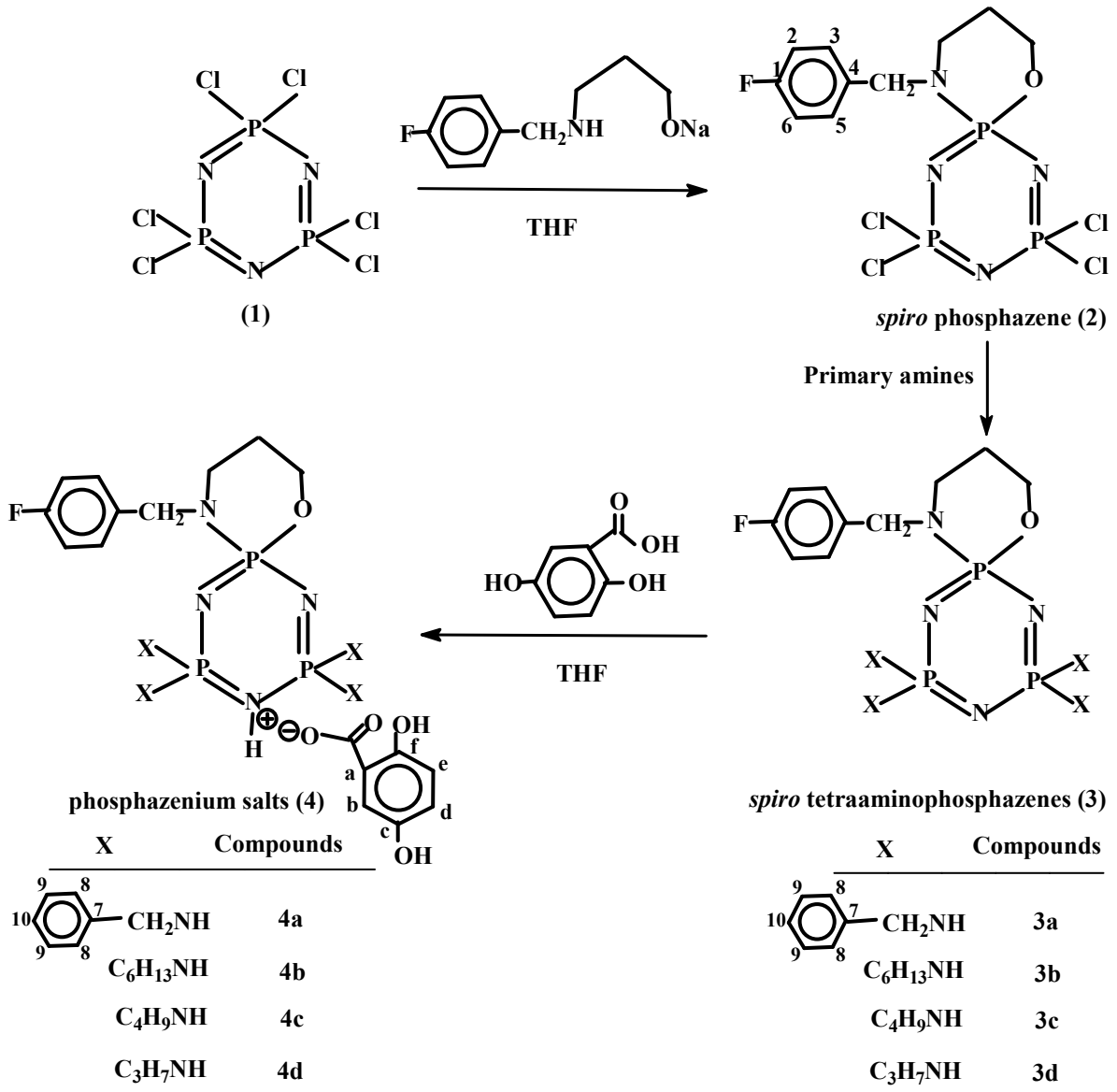


Figure 1. The cyclotriphosphazene derivatives obtained from the reactions of 4-fluorobenzylspiro(N/O)cyclotriphosphazene with primary amines and their phosphazenium salts.

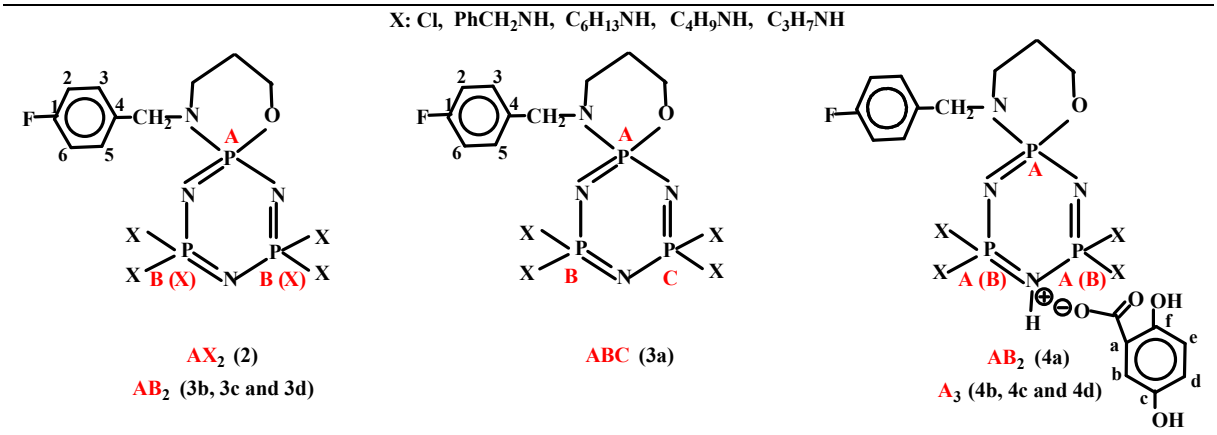
The structures of all the isolated cyclotriphosphazene derivatives were determined by elemental analyses, FTIR and ^{1}H, $^{13}C\{^{1}H\}$, $^{31}P\{^{1}H\}$ NMR techniques. All the compounds were screened for antibacterial and antifungal activities against bacteria and yeast strains. The interactions of the compounds with supercoiled pUC18 plasmid DNA were investigated.

The microanalyses, FTIR and^{1}H, $^{13}C\{^{1}H\}$, $^{31}P\{^{1}H\}$, and NMR results were confirmed the suggested structure of the compounds with one mole of water for **3a**, **3c, 4a, 4b** and **4d**. The protonated molecular $[MH]^+$ ion peaks were observed in the mass spectra of the free phosphazene bases (**3a-3d**). The analytical data and NMR results are given in the "Experimental Section".

The spin systems and the ^{31}P-NMR spectral data of the cyclotriphosphazenes are given in Table 3. The starting compound **2** has AX_2 spin system. It gives rise to one triplet (P_{spiro}, P_A) and one doublet (P_X). Compound **3a** has an ABC spin system and displays a total of twelve signals for the expected ABC spin system. The fully substituted phosphazenes; **3b**, **3c** and **3d** have AB_2 spin systems and exhibit a total of eight signals. The $^{2}J_{PP}/\Delta v$

values of these compounds are calculated and listed in Table 3. The $\delta P_{(spiro)}$-shifts of the phosphazene bases (**3a-3d**) are considerably larger than that of the starting compound (**2**). However, when the Cl atoms exchanged with amine groups, the δP-shifts of the free bases were decreasing. The spin systems of the PILs are found to be as AB_2 for **4a** and A_3 for **4b**, **4c** and **4d**. The chemical shifts of these salts were smaller than those of the corresponding free bases, depending on the salt formation with bulky organic acid.

Table 3: ^{31}P NMR parameters of compounds.[a]



	Spin System	$\delta PNO_{(spiro)}$	δPCl_2	δPN_2	$^2J_{PP}$ (Hz)	$^2J_{PP}/\Delta v$
2	AX_2	P_A 9.06	P_X 23.32	-	$^2J_{AX}$ 50.2	-
3a	ABC	P_A 21.20		P_B 20.22 P_C 18.83	$^2J_{AB}$ 53.4 $^2J_{BC}$ 53.2 $^2J_{AC}$ 51.0	-
3b	AB_2	P_A 19.90		P_B 18.75	$^2J_{AB}$ 48.6	0.17
3c	AB_2	P_A 20.04	-	P_B 18.88	$^2J_{AB}$ 46.2	0.15
3d	AB_2	P_A 20.04	-	P_B 18.80	$^2J_{AB}$ 48.6	0.16
4a	AB_2	P_A 14.84	-	P_B 14.35	$^2J_{AB}$ 56.7	0.48
4b	A_3	P_A 14.70	-	P_A 14.70	-	Broad singlet
4c	A_3	P_A 14.75	-	P_A 14.75	-	Broad singlet
4d	A_3	P_A 14.74	-	P_A 14.74	-	Broad singlet

[a]242.93 MHz ^{31}P NMR measurements in $CDCl_3$ solutions at 298 K. Chemical shifts referenced to external H_3PO_4.

The assignments of the chemical shifts, multiplicities, and coupling constants were elucidated from the ^{13}C and ^1H-NMR spectra of all the cyclotriphoshazenes (**2** and **3a-3d**) and their salts (**4a-4d**), and given in "Experimental Section". In the ^{13}C NMR spectra of the cyclotriphosphazene bases (**3a-3d**) and the PILs (**4a-4d**), the geminal substituents display two small separated peaks for $NHCH_2$, $NHCH_2CH_2$, CH_3, and $ArCH_2NH$ and *ipso*-C_7 carbons (for **3a** and **4a**), implying that the two geminal groups are not equivalent. The average coupling constants of the free bases (**3a-3d**), $^2J_{POC}$=6.9 Hz, is very higher than that of the PILs ($^2J_{POC}$=5.0 Hz). The coupling constants, $^3J_{PNCC}$, of the free bases (**3a-3d**) and the PILs (**4a-4d**) emerge to triplets of the $NHCH_2CH_2$ and $NHCH_2C_7$ carbons depending on the second-order effects (Figure 2). The $^3J_{PNCC}$ values are calculated using the external transitions of the triplets as it is estimated in the literature [32]. In addition, the coupling constants of $^1J_{FC}$, $^2J_{FC}$, $^3J_{FC}$ and $^4J_{FC}$ are also assigned for the cyclotriphosphazene and the PILs.

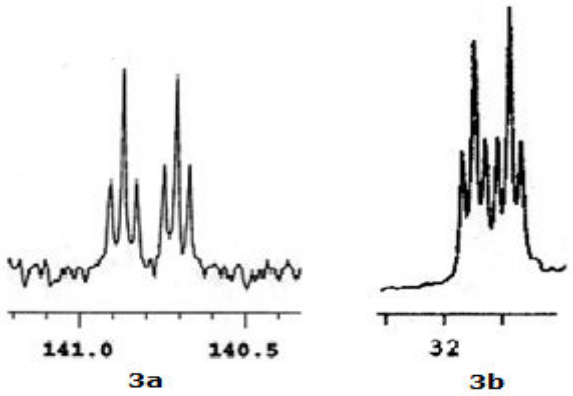


Figure 2. The second order effects in ^{13}C NMR spectra of **3a** and **3b.**

The interpretations of the free bases (**3a-3d**) and the PILs (**4a-4d**) were made using the coupling constants of $^{3}J_{FH}$ and $^{4}J_{FH}$, and the multiplicities. The results were presented in "Experimental Section". The average values of $^{3}J_{FH}$ and $^{4}J_{FH}$ were found to be at 8.8 Hz and 5.4 Hz, respectively. The protons of ArCH_2N were observed asa doublet. The average values of $^{3}J_{PH}$ of ArCH_2N protons in the free bases and the PILs were found to be at 7.5 and 6.4 Hz, respectively. The δ_H-shifts of OCH_2 spiro protons of the cyclotriphosphazenes and the PILs were observed in the range of 4.24-4.34 ppm, and the average $^{3}J_{PH}$ value, 12.3 Hz, was very large.

The characteristic stretching band (ν_{N-H}, 3402 cm^{-1}, broad) was observed for 3-(4-fluorobenzylamino)-1-propanol disappeared in the FTIR spectra of the compound **2**. Whereas, in the FTIR spectra of the free bases and the PILs exhibit the broad ν_{N-H} peaks. They are observed in the ranges of 3200-3289 cm^{-1} and 3361-3389 cm^{-1}. All the salts display ν_{+N-H} (ca. 2668 cm^{-1}), ν_{coo-}(asymm.) (*ca*. 1578 cm^{-1}) and ν_{coo-} (symm.) (*ca*. 1377 cm^{-1}) absorption frequencies clearly indicating the salt formation. The cyclotriphosphazenes and the PILs show intense stretching vibrations between 1202-1257 cm^{-1} and 1193-1218 cm^{-1}, attributed to the $\nu_{P=N}$ bonds of the phosphazene skeletons [33,34].

The crystal structure of 4d: The molecular and the solid state structure of **4d** were verified by X-ray crystallography. Figure 3 depicts the molecular structure of **4d** along with the atom-numbering scheme. The phosphazene ring of **4d** is in twisted boat conformation [Figure 3b: φ_2 =-84.6(6)°, θ_2 =100.0(6)°(P1/N1/P2/N2/P3/N3)] with a total puckering amplitude, Q_T of 0.270(4)Å] [35]. The six-membered *spiro* ring (P1/N4/C8/C9/C10/O1) is in the chair conformation [Figure 3c: φ_2 =34.7(4)°, θ_2 =88.1(5)°] with a total puckering amplitude, Q_T of 0.673(5)Å]. Moreover, the torsion angles of N_3P_3 ring of **4d** exhibit that the cyclotriphosphazene ring does not have any pseudo-mirror plane (Figure 3d).

In PILs **4d**, the endocyclic PN bond lengths of the phosphazene ring were found to be in the range of 1.575(4)–1.666(4) Å (average value is 1.608(2) Å), compared with the corresponding values [1.588(1)–1.599(1) Å, average value is 1.595(1) Å] of the analogues phosphazene free bases [23]. In addition, the exocyclic PN bond lengths of **4d** are in the range of 1.581(4)–1.649(4) Å (average value is 1.612(5) Å). The corresponding values of analogues phosphazene free bases and its salts are in the ranges of 1.652(1)–1.674(1) Å (average value is 1.663(1) Å) and 1.625(2)–1.647(2) Å (average value is 1.639(2) Å) [23] (Table 2). In **4d**, the P2N2 (1.655(4) Å) and P3N2 (1.666(4) Å) bond lengths are considerably larger than those of other PN bond lengths, depending on the protonation of N2 atom.

The considerable narrowings of the endocylic α_1 [115.3(2)º] and γ_1 [111.6(2)º and 108.5(2)º] angles and the considerable expanding of the endocylic β_1 [123.5(2)º], β_1' [128.5(3)º] and δ_1 [126.7(3)º] angles may indicate the electron delocalization in the phosphazene ring (Table 2). These findings may be compared with the corresponding values of analogues phosphazene free bases and its salts reported in the literature [23]. In addition, it was observed that the great changes are observed at around the N2 atom indicating HN^+ bond formation.

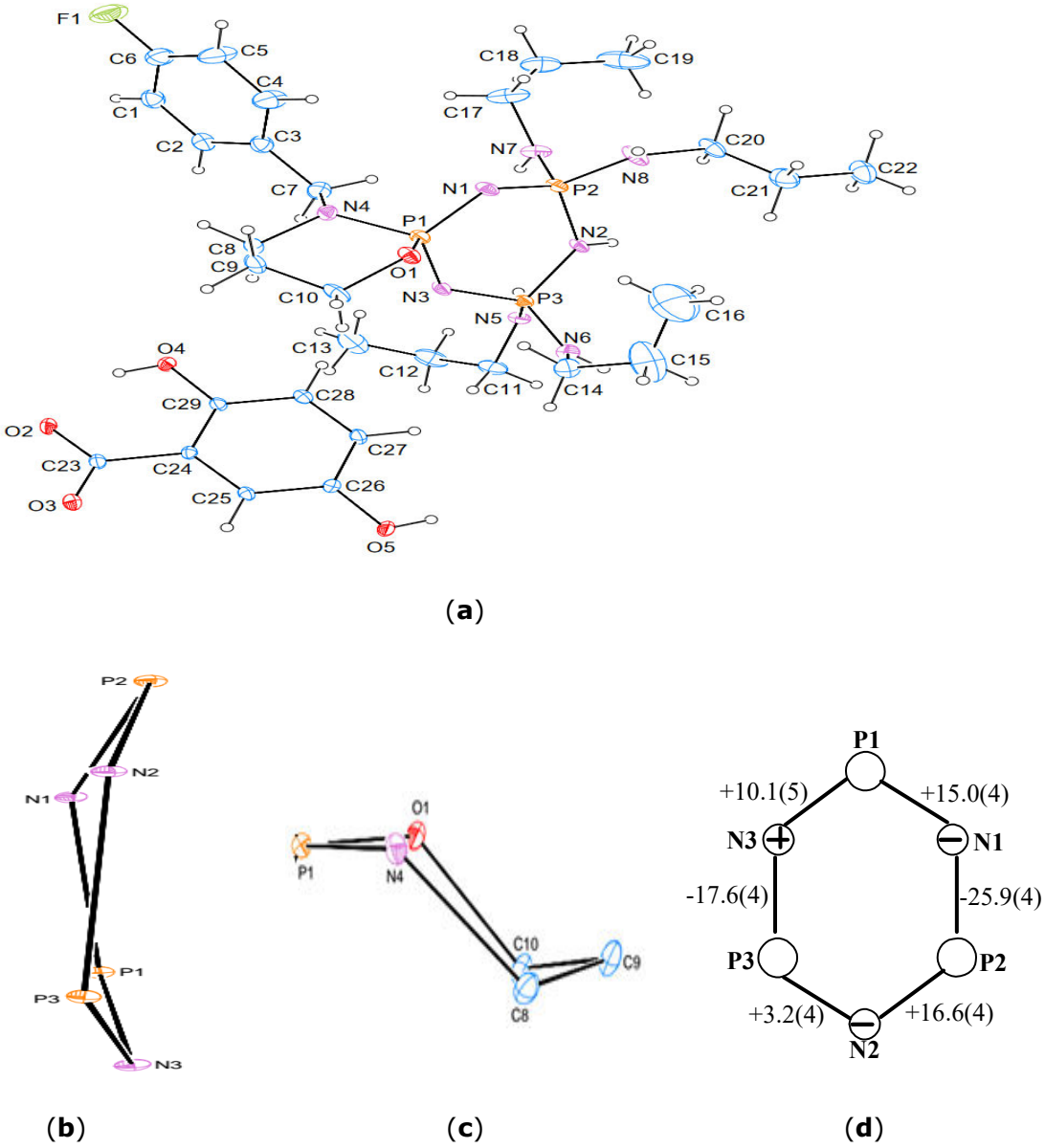
Hydrogen-bond geometries of **4d** were listed in Table 4. It was observed that the strong intramolecular hydrogen bonds (O–H … O) were present in the gentisic acid anion. Furthermore, the strong intramolecular N–H … O hydrogen bond links the gentisic acid anion to the phosphazene molecule [36].

Crystallographic data for the structural analysis have been deposited with the Cambridge Crystallographic Data Centre, CCDC1487569 Copies of this information may be obtained free of charge from The Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: 44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

Table 4: Hydrogen-bond geometries (Å, º) for **4d.**

D-H …A	D-H	H …A	D …A	D-H …A
N2-H2A ... O3^{iv}	0.84(5)	2.83(1)	2.01(5)	166.9(4.0)
C8-H8A ... O4	0.97(1)	3.41(1)	2.59(1)	142.3(3.0)
N5-H51 ... O5^{iii}	0.86(5)	2.95(1)	2.09(5)	172.7(4.0)
N8-H81 ... N1^{ii}	0.86(5)	3.12(1)	2.30(4)	156.8(4.0)
O4-H4A ... O2	0.82(1)	2.52(1)	1.81(1)	146.1(3.0)
N6-H61 ... O4^{i}	0.85(1)	2.99(1)	2.31(1)	137.4(4.0)
O5-H5A ... O2^{i}	0.82(1)	2.56(1)	1.74(1)	170.3(3.0)
N7-H71 ... O5^{iii}	0.87(1)	3.09(1)	2.27(1)	157.3(2.0)

Symmetry codes (i) x + ½, -y + ½, z + ½, (ii) –x, -y, -z +1, (iii) x – ½, -y + ½, z + ½, (iv) x, y, z + 1.



(**a**) (**b**) (**c**) (**d**)

Figure 3. (**a**) An ORTEP-3 [37] drawing of **4d** with the atom-numbering scheme. Displacement ellipsoids are drawn at the 30% probability level. The conformations of (**b**) the trimer ring and (**c**) the six-membered spiro-ring of **4d,** and (**d**) the shape of the phosphazene ring in **4d** with torsion angles (deg) given.

Antimicrobial activity evaluation: In this paper, the antimicrobial activity of the cyclotriphosphazene bases and the PILs have been investigated for discovering the new antimicrobial agents against some (G−) and (G+) bacteria and fungi. Table 5 illustrates *in vitro* antimicrobial activity of cyclotriphosphazene bases (**3a-3d**) and the PILs (**4a-4d**) against three types of bacteria and one type of fungus. The MIC, MBC and MFC values of the compounds have ranges of 15.63-1000 µM, 31.25-2500 µM and 15.63-250µM, respectively. Compounds **3c** and **4c** are strongly active against *E. faecalis* (G+). As known, *E. faecalis*, is a facultative anaerobic Gram-positive coccus and causes root canal failure and persistent apical periodontitis [38]. In addition, the free base **3d**, and the PILs, **4a**, **4c** and **4d,** are very active against *C. albicans.* On the other hand, compared to the salts with the corresponding free bases, the salts (**4a** and **4c**) and (**4b** and **4d**) are found to be more effective than corresponding free bases (**3a** and **3c**) and (**3b** and **3d**), respectively, against *C. albicans* and *K. pneumoniae,* indicating that the salt formation considerably increases antimicrobial activity. This situation may attribute to the strong

hydrogen bond formation of the anion and/or cation of the saltswith the DNA of microorganisms.

Interactions of pUC18 plasmid DNA with the compounds: The interactions of pUC18 plasmid DNA with the free bases (**3a-3d**) and the PILs (**4a-4d**) were studied using agarose gel electrophoresis (Figure 4). In the electrophoretograms, in all the cases run with untreated pUC18 plasmid DNA was included as a control, while lanes 1 to 4 contained plasmid DNA interacted with decreasing concentrations of the compounds (from 4000 µM to 500 µM). It appears that all of the compounds, exhibit similar effects against plasmid DNA except **3d**. As the concentrations of the compounds decrease, the mobility of Form I DNA increases slightly. In the case of **3d**, two bands co-migrate for the concentration of the compound at the highest concentration (4000 µM). In the lower concentrations, the seperated two bands are observed,indicating that compound **3d** is binding to DNA.

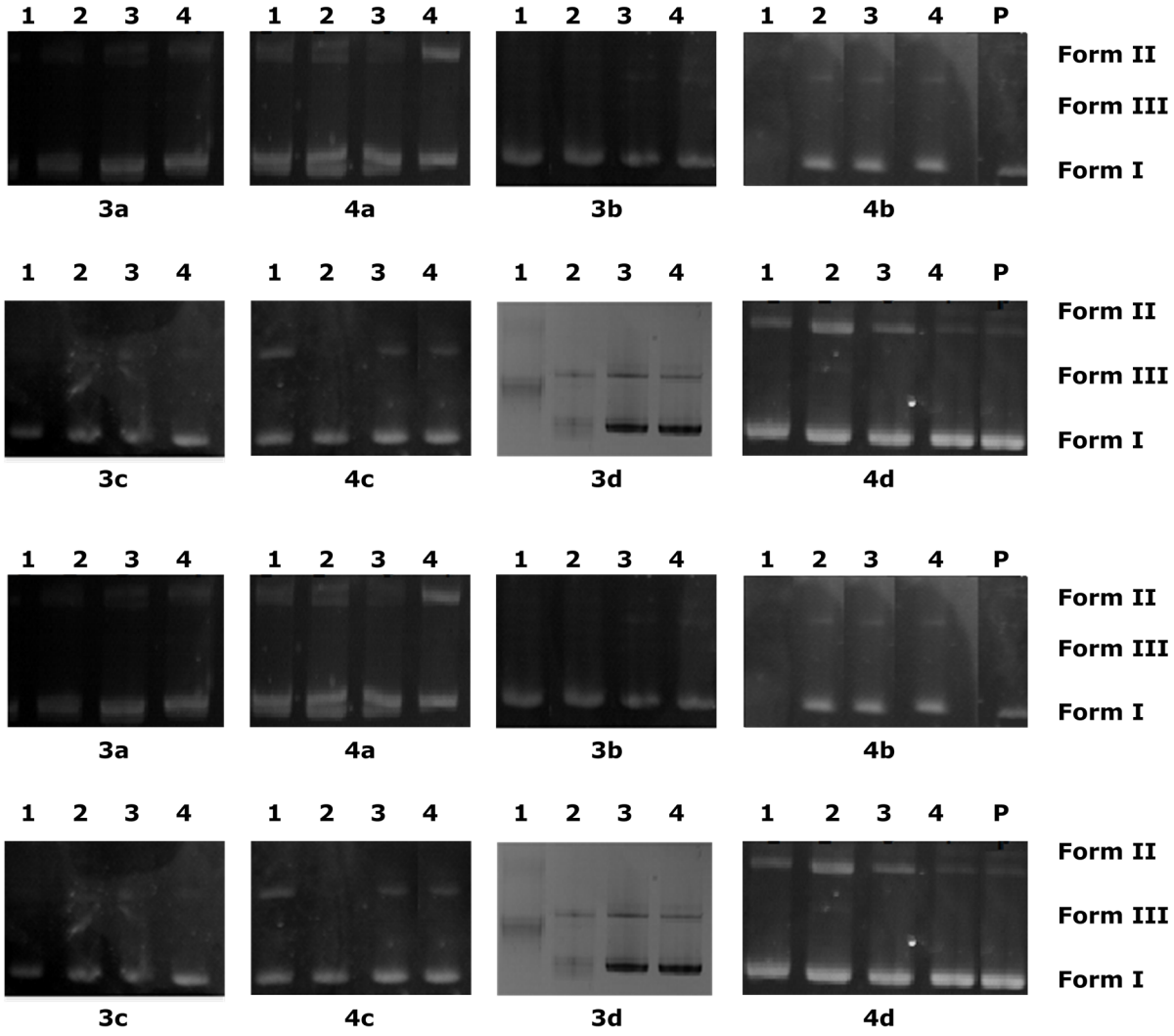


Figure 4. Interaction of pUC18 plasmid DNA with decreasing concentrations of the compounds (lanes 1–4; 1: 4000 µM; 2: 2000 µM; 3: 1000 µM; 4: 500 µM, and P: untreated pUC18 plasmid DNA as a control).

Table 5: The *in vitro* antimicrobial activities of compounds **3a-3d**and **4a-4d**against test strains (MIC: Minimum Inhibitory Concentration, MBC: Minimum Bactericidal Concentration, and MFC: Minimum Fungicidal Concentration. MIC, MBC and MFC values are reported in µM).

	E. coli ATCC 25922		***K. pneumoniae ATCC 13883***		***E. faecalis ATCC 29212***		***C. albicansATCC 10231***	
Compounds	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MFC**
3a	250	500	125	125	250	500	125	125
4a	250	500	250	250	500	1000	<15.63	<15.63
3b	250	1000	250	500	250	250	125	125
4b	125	125	125	125	250	250	125	250
3c	125	125	62.5	62.5	<15.63	<15.63	62.5	125
4c	125	125	62.5	62.5	<15.63	<15.63	31.25	62.5
3d	250	500	250	250	500	1000	31.25	31.25
4d	250	500	125	125	500	1000	62.50	62.50
Amp	<19.5	NS	1250	NS	312.5	NS	NS	NS
C	1250	NS	2500	NS	625	NS	NS	NS
Keto	NS	NS	NS	NS	NS	NS	1250	NS

Amp: Ampicillin, C: Chloramphenicol, Keto: Ketoconazole (NS: Not studied).

***BamH*I and *Hind*III digestion of compounds-pUC18 plasmid DNA:** DNA–compound mixture was digested with *BamH*I and *Hind*III restriction enzymes in order to understand DNA-compound binding. Figure 5 displays the electrophoretograms for the incubated mixtures of pUC18 plasmid DNA with the compounds followed by *BamH*I and *Hind*III digestion. In the absence of the compounds, when untreated plasmid DNA is digested with the enzymes, only the linear Form III band is observed, indicating that two strands of pUC18 plasmid DNA arecut by *BamH*I at the specific GG site and *Hind*III enzymes at the specific AA site. For *BamH*I digestion, a mixture of Form I and Form II bands are observed for all the compounds, **3a-3d** and **4a-4d**. Form III band is also detected for *BamH*I digestion for **3d**, **4a** and **4c**. On the other hand, for *Hind*III digestion, only one band is determined for **3a**, **3c**, **3d**, **4c** and **4d**. Whereas two bands, Form I and Form II, were observed for **3b**, **4a** and **4b**. Eventually, the prevention of *BamH*I digestion by **3b**, **4a** and **4b**, disclosed binding with GG nucleotides in DNA.

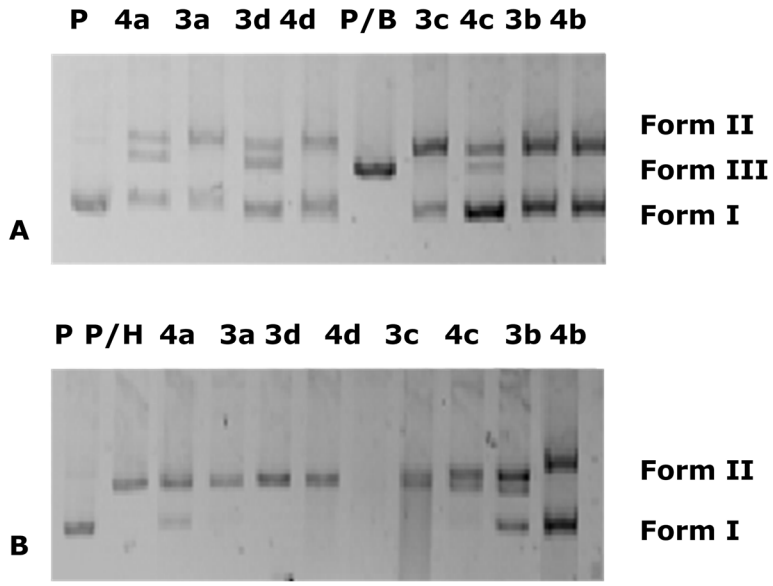


Figure 5. Electrophoretogram for the incubated mixtures of pUC18 plasmid DNA followed by digestion with (**A**) *BamH*I and (**B**) *Hind*III. Lane P applies to untreated DNA, P/B and P/H, untreated, but digested pUC18 plasmid DNA.

CONCLUSIONS

The structures of the cyclotriphosphazenes (**3a-3d**) and the PILs (**4a-4d**) were elucidated using NMR data in $CDCl_3$ solution. The crystallographic results of **4d** unambiguously indicate that the nitrogen of the phosphazene ring is monoprotonated. The fully substituted cyclotriphosphazenes (**3a-3d**) appear to be the ligating agents for transition metal cations. The antimicrobial activities of all the compounds were scrutinized against bacteria and fungi. Compounds **3c** and **4c** are the most active compounds against *E. faecalis* (G+). In addition, the phosphazene base, **3d**, and the PILs, **4a**, **4c** and **4d,** are found to be considerably active against *C. albicans.* The gel electrophoretic data demonstrate that compound **3d** is binding to pUC18 plasmid DNA.

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Türkçe Öz ve Anahtar Kelimeler

4-FLOROBENZİLSPİRO(N/O) TRİMERİK HALKALI FOSFAZENLERİN VE TUZLARININ SENTEZİ, YAPILARININ KARAKTERİZASYONU, ANTİMİKROBİYAL AKTİVİTELERİ VE DNA İLE ETKİLEŞİMLERİ

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Özet: Bu makalede, $N_3P_3Cl_6$ (**1**) bileşiğinin sodyum 3-(4-florobenzilamino)-1-propanoksit ile kondenzasyon tepkimesinden kısmen sübstitüe 4-florobenzilspiro(N/O) trimerik halkalı fosfazen (**2**) bileşiği elde edildi. Bu bileşiğin klorlarının aşırı miktarda benzilamin, n-hekzilamin, n-bütilamin ve n-propilamin ile yer değiştirme tepkimelerinden 4-florobenzilspiro(N/O)tetrabenzilamino (**3a**), tetrahekzilamino (**3b**), tetrabütilamino (**3c**) and tetrapropilamino (**3d**) halkalı trifosfazen bileşikleri oluştu. Oluşan bu bazların (**3a**-**3d**), kuru THF'de gentisik asit ile etkileştirilmesinden fosfazenyum tuzları (**4a-4d**), protik iyonik sıvılar (protic ionic liquids; PILs), sentezlendi. Elde edilen tüm bileşiklerin yapıları; element analizleri, FTIR ve ^{1}H, $^{13}C\{^{1}H\}$, $^{31}P\{^{1}H\}$ NMR verileri kullanılarak belirlendi. Bileşik **4d'**nin kristal yapısı X-ışını kırınımmetre yöntemi ile aydınlatıldı. Tüm bileşiklerin bakteri ve mayalara karşı antibakteriyal ve antifungal aktiviteleri incelendi. Ayrıca, bu bileşiklerin süper sarmal plasmid pUC18 DNA ile etkileşimleri araştırıldı.

Anahtar Kelimeler: Spirosiklotrifosfazenler, kristallografi, spektroskopi, antimikrobiyal aktivite, DNA'ya bağlanma.

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