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The Spectroscopic and Thermal Properties, Antibacterial and Antifungal Activity and DNA Interactions of 4-(Fluorobenzyl)Spiro(N/O) Cyclotriphosphazenium Salts

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Abstract: The protic ionic liquids (PILs) (**3a-3d**) were synthesized from the reactions of tetraamino-4-(fluorobenzyl)spiro(N/O)cyclotriphosphazenes (**2a-2d**) with gentisic acid in dry THF. The structures of the PILs were verified by the elemental analyses, Fourier transform infrared (FTIR), ^{1}H, ^{13}C $\{^{1}H\}$ and ^{31}P $\{^{1}H\}$ NMR techniques. The thermal properties of the PILs were evaluated using TG/DTG/DTA instrument. The antifungal and antibacterial activity of the PILs were examined against some fungi and bacteria, and these obtained results were compared with the evaluations of the corresponding fully substituted cyclotriphosphazene bases. The interactions between the PILs and supercoiled PBR322 DNA were scrutinized, and it was determined that they exhibited significant conformational changes.

Keywords: PILs, Thermal analysis, Spectroscopy, Antimicrobial activity, DNA binding.

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INTRODUCTION

Chlorocyclophosphazenes [$(N=PCl_2)_n$] (n=3-40) are a well-known family of inorganic heterocyclic ring systems in linear, cyclic and polymeric forms composed of a backbone that contains the repeating unit with trivalent nitrogen and pentavalent phosphorus (1). Hexachlorocyclotriphosphazene, $N_3P_3Cl_6$, is one of the most popular starting compound used in the syntheses of the phosphazene derivatives, and its nucleophilic substitution reactions have been examined thoroughly in the area of phosphazene chemistry (2). There are diverse reports on the Cl substitution reactions of $N_3P_3Cl_6$ with different mono and difunctional reagents in the literature (3,4). For instance, the condensation reactions of $N_3P_3Cl_6$ with N/O donor typed bidentate ligands cause to the formation of the partly substituted (N/O)spirocyclotriphosphazenes (Figure 1) (5-8). The fully substituted products were prepared for the reactions of these (N/O)spirocyclotriphosphazenes with the excess of monoamines as well. It has been reported that some of the pyrrolidinocycloptrihosphazenes exhibited substantial antibacterial activity (6). In addition, they are designated to be efficient compounds in modifying the mobility of the DNA. A wide range of cyclotriphosphazene-based derivatives is used as ionic liquids (9), liquid crystals (10), Li-ion batteries (11), OLEDs (12), organic light emitting diodes (13) and fluorescence sensors (14,15).

On the other hand, it is also known that the fully substituted cyclotriphosphazenes have high thermal stabilities and strong basic characters (16). In recent years, the cyclophosphazene-based salts were obtained with bulky acids; eg. salicylic, gentisic, decanoic, and boric acid (17-19). Such salts ought to be named as protic ionic liquids (PILs), and their pharmacological activity are likely to be very attractive.

This study reveals that the preparation of the fully secondary amine substituted (4-fluorobenzyl)spiro(N/O)cyclotriphosphazenium bases (**2a-2d**) with the gentisic acid. The aim of these syntheses of the salts (**3a-3d**) is to determine the spectral and thermal properties, antibacterial and antifungal activity and DNA interactions of the salts, and to compare these obtained results with those of the phosphazene free bases.

EXPERIMENTAL PART

Materials and Methods

$N_3P_3Cl_6$ (Aldrich), 3-amino-1-propanol, 4-fluorobenzaldehyde, pyrrolidine, piperidine, morpholine, DASD, and 2,5-dihydroxybenzoic acid (Merck) were purchased. All the used solvents were firstly distilled and then dried using common methods. $N_3P_3Cl_6$ were recrystallized from *n*-hexane before use. The reactions were performed under Ar and monitored by TLC on Merck DC Alufolien Kiesegel 60 B254 sheets in various solvents. The melting points of the salts were

designated on a Gallenkamp apparatus by a capillary tube. The FTIR spectra of the PILs were obtained a Jasco FT/IR-430 spectrometer in KBr discs and reported in cm^{-1} units. The microanalyses were made by a Leco CHNS-932 instrument. ^{1}H and ^{13}C $\{^{1}H\}$ 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 (at Ankara University). The spectrometer was fitted with a 5 mm PABBO BB inverse-gradient probe, and standard Bruker pulse programs (20) were used. The ^{31}P $\{^{1}H\}$ NMR spectra of the salts were done on a Bruker Ascend™ 600 ULH spectrometer, 85% H_3PO_4 as an external standard, operating at 242.93 MHz (at Inönü University). The thermogravimetric (TG) and differential thermal analysis (DTA) curves were performed by a PYRIS Diamond TG/DTA apparatus in a dynamic nitrogen atmosphere (heating rate: 10 °C/min, platinum crucibles, mass ~10 mg and temperature range 35–1000 °C).

Preparation of the amino alcohol and cyclotriphosphazene bases

The amino alcohol, 3-(4-fluorobenzylamino)-1-propanol, was prepared from the reaction of 4-fluorobenzaldehyde with 3-amino-1-propanol in methanol according to the published procedure (21). The partly (**1**) and fully substituted cyclotriphosphazene derivatives (**2a-2d**) were also synthesized with respect to the literature (18, 22).

Preparation of the PILs.

Synthesis of **3a:** A solution of **2a** (0.50 g, 0.84 mmol) in dry THF (30 mL) was slowly added dropwise to gentisic acid (0.13 g, 0.84 mmol) in dry THF (10 mL) at ambient temperature. The reaction mixture was then refluxed for over 40 h. Thereafter, the solvent was evaporated under vacuum, and the light yellow oily crude product was recrystallized from toluene. Yield: 0.54 g (86%). mp: 149 °C. Anal. Calcd. for $C_{33}H_{50}O_5N_8FP_3$: C, 52.79; H, 6.73; N, 14.93. Found: C 53.06; H, 6.64; N, 14.49. FTIR (KBr, cm^{-1}): *v* 3227 (O-H), 2948, 2869 (C-H aliph.), 2640 (N^+-H), 1577 (asym.), 1377 (sym.) (COO^-), 1247 (asym.), 1201 (sym.) (P=N), 1045 (C-F). ^{1}H NMR (400 MHz, $CDCl_3$, ppm, in Figure 1 the numberings of protons were given): δ 9.01 (b, 2H, Ar-O*H*), 7.66 (d, 1H, $^{3}J_{HH}$=2.8 Hz, H_b), 7.34 (dd, 2H, $^{4}J_{FH}$=5.6 Hz, $^{3}J_{HH}$=8.0 Hz, H_3 and H_5), 6.97 (dd, 2H, $^{3}J_{FH}$=8.8 Hz, $^{3}J_{HH}$=8.4 Hz, H_2 and H_6), 6.89 (dd, 1H, $^{3}J_{HH}$=8.8 Hz, $^{3}J_{HH}$=2.8 Hz, H_d), 6.70 (d, 1H, $^{3}J_{HH}$=8.4 Hz, H_e), 4.39 (m, 2H, $^{3}J_{PH}$=10.8 Hz, $^{3}J_{HH}$=5.2 Hz, O-CH_2), 4.02 (d, 2H, $^{3}J_{PH}$=6.4 Hz, Ar-CH_2-N), 3.19 [m, 8H, N-CH_2(pyrr)], 3.13 [m, 8H, N-CH_2(pyrr)], 3.05 (m, 2H, $^{3}J_{PH}$ 12.0 Hz, $^{3}J_{HH}$ 6.0 Hz, N-CH_2), 2.34 (s, 1H, N*H*), 1.80 (m, 2H, $^{3}J_{HH}$=6.0 Hz, N-CH_2-CH_2), 1.78 [m, 8H, N-CH_2-CH_2(pyrr)], 1.72 [m, 8H, N-CH_2-CH_2(pyrr)]. ^{13}C NMR (100 MHz, $CDCl_3$, ppm, in Figure 1 the numberings of protons were given): δ 173.58 (s, COO^-), 162.18 (d, $^{1}J_{FC}$=245.4 Hz, C_1), 154.67 (s, C_f), 148.70 (s, C_c), 132.92 (dd, $^{3}J_{PC}$=10.6 Hz, $^{4}J_{FC}$=2.7 Hz, C_4), 129.97 (d, $^{3}J_{FC}$=7.5 Hz, C_3 and C_5), 121.00 (s, C_a), 118.41 (s, C_d), 116.65 (s, C_b), 116.52 (s, C_e), 115.15 (d, $^{2}J_{FC}$=21.4 Hz, C_2 and C_6), 67.58 (d, $^{2}J_{PC}$=6.4 Hz, O-*C*H_2), 50.44 (s, Ar-*C*H_2-N), 46.49 and 46.33 [s, N-*C*H_2 (pyrr)], 45.63 (s, N-*C*H_2), 26.36 and 26.32 [(d, $^{3}J_{PC}$=6.4 Hz and $^{3}J_{PC}$=6.9 Hz, N-CH_2-*C*H_2 (pyrr)], 26.25 (d, $^{3}J_{PC}$=4.6 Hz, N-CH_2-*C*H_2).

Synthesis of **3b:** The work-up procedure was similar to that of compound **3a**, using **2b** (0.50 g, 0.77 mmol) and gentisic acid (0.12 g, 0.77 mmol). The reaction mixture was then refluxed for over 40 h. Thereafter, the solvent was evaporated under vacuum, and the light yellow oily crude product was recrystallized from toluene. Yield: 0.51 g (82%). mp: 130 °C. Anal. Calcd. for $C_{37}H_{58}O_5N_8FP_3$. 0.5 C_7H_8: C, 57.07; H, 7.33; N, 13.15. Found: C, 56.90; H, 7.43; N, 13.60. FTIR (KBr, cm^{-1}): *v* 3283 (O-H), 2934, 2844 (C-H aliph.), 2528 (N^+-H), 1577 (asym.), 1362 (sym.) (COO^-), 1249 (asym.), 1194 (sym.) (P=N), 1052 (C-F). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): *δ* 9.65 (b, 2H, Ar-O*H*), 7.56 (d, 1H, $^3J_{HH}$=2.4 Hz, H_b), 7.32 (dd, 2H, $^4J_{FH}$=5.6 Hz, $^3J_{HH}$=8.4 Hz, H_3 and H_5), 6.95 (dd, 2H, $^3J_{FH}$=8.8 Hz, $^3J_{HH}$=8.0 Hz, H_2 and H_6), 6.92 (dd, 1H, $^3J_{HH}$=8.0 Hz, $^3J_{HH}$=2.8 Hz, H_d), 6.73 (d, 1H, $^3J_{HH}$=8.4 Hz, H_e), 4.44 (m, 2H, $^3J_{PH}$=13.2 Hz, $^3J_{HH}$=5.2 Hz, O-CH_2), 4.05 (d, 2H, $^3J_{PH}$=8.8 Hz, Ar-CH_2-N), 3.04 [m, 16H, N-CH_2(pip)], 3.04 (m, 2H, N-CH_2), 2.33 (s, 1H, N*H*), 1.81 (m, 2H, N-CH_2-CH_2), 1.50 [m, 8H, N-CH_2-CH_2(pip)], 1.41 [m, 8H, N-CH_2-CH_2(pip)], 1.40 [m, 8H, N-CH_2-CH_2-CH_2(pip)]. ^{13}C NMR (100 MHz, $CDCl_3$, ppm): *δ* 173.26 (s, COO^-), 162.22 (d, $^1J_{FC}$=245.3 Hz, C_1), 154.91 (s, C_f), 148.51 (s, C_c), 132.56 (dd, $^3J_{PC}$=8.5 Hz, $^4J_{FC}$=3.1 Hz, C_4), 129.87 (d, $^3J_{FC}$=7.6 Hz, C_3 and C_5), 122.16 (s, C_a), 117.06 (s, C_d), 116.59 (s, C_b), 116.40 (s, C_e), 115.22 (d, $^2J_{FC}$=21.5 Hz, C_2 and C_6), 68.08 (d, $^2J_{PC}$=7.8 Hz, O-CH_2), 50.13 (s, Ar-CH_2-N), 45.58 (s, N-CH_2), 45.45 and 45.36 [s, N-CH_2 (pip)], 25. 94 and 25.92 [(s, N-CH_2-CH_2 (pip)], 25.72 (d, $^3J_{PC}$=4.6 Hz, N-CH_2-CH_2), 24.49 and 24.30 [(s, N-CH_2-CH_2-CH_2 (pip)].

Synthesis of **3c:** The work-up procedure was similar to that of compound **3a**, using **2c** (0.50 g, 0.76 mmol) and gentisic acid (0.11 g, 0.76 mmol). The reaction mixture was then refluxed for over 40 h. Thereafter, the solvent was evaporated under vacuum, and the light yellow oily crude product was recrystallized from toluene. Yield: 0.49 g (79%). mp: 90 °C. Anal. Calcd. for $C_{33}H_{50}O_9N_8FP_3$. H_2O: C, 47.63; H, 6.31; N, 13.47. Found: C, 47.72; H, 6.75; N, 12.98. FTIR (KBr, cm^{-1}): *v* 3270 (O-H), 2965, 2850 (C-H aliph.), 2686 (N^+-H), 1588 (asym.), 1373 (sym.) (COO^-), 1239 (asym.), 1200 (sym.) (P=N), 1049 (C-F). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): *δ* 7.38 (d, 1H, $^3J_{HH}$=2.8 Hz, H_b), 7.32 (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, $^3J_{HH}$=8.4 Hz, H_2 and H_6), 6.95 (dd, 1H, $^3J_{HH}$=7.6 Hz, $^3J_{HH}$=2.8 Hz, H_d), 6.78 (d, 1H, $^3J_{HH}$=8.4 Hz, H_e), 4.39 (m, 2H, $^3J_{PH}$=13.6 Hz, $^3J_{HH}$=5.2 Hz, O-CH_2), 3.99 (d, 2H, $^3J_{PH}$=8.0 Hz, Ar-CH_2-N), 3.67 [t, 8H, $^3J_{HH}$ 8.8 Hz,O-CH_2 (m)], 3.59 [t, 8H, $^3J_{HH}$ 8.4 Hz,O-CH_2(m)], 3.12 [m, 16H, N-CH_2(m)], 3.01 (m, 2H, $^3J_{PH}$ 11.2 Hz, $^3J_{HH}$ 5.6 Hz, N-CH_2), 2.35 (s, 1H, N*H*), 1.84 (m, 2H, $^3J_{HH}$=5.2 Hz, N-CH_2-CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, ppm): *δ* 172.73 (s, COO^-), 162.21 (d, $^1J_{FC}$=246.1 Hz, C_1), 155.23 (s, C_f), 148.51 (s, C_c), 132.77 (dd, $^3J_{PC}$=9.2 Hz, $^4J_{FC}$=3.1 Hz, C_4), 129.76 (d, $^3J_{FC}$=8.5 Hz, C_3 and C_5), 123.05 (s, C_a), 117.67 (s, C_d), 115.59 (s, C_b), 115.32 (d, $^2J_{FC}$=21.5 Hz, C_2 and C_6), 114.78 (s, C_e), 67.37 (d, $^2J_{PC}$=6.9 Hz, O-CH_2), 66.99 and 66.95 [(d, O-CH_2 (morp)], 50.63 (s, Ar-CH_2-N), 45.66 (s, N-CH_2), 44.66 and 44.57 [s, N-CH_2 (morp)], 26.07 (d, $^3J_{PC}$=3.8 Hz, N-CH_2-CH_2).

Synthesis of **3d:** The work-up procedure was similar to that of compound **3a**, using **2d** (0.50 g, 0.57 mmol) and gentisic acid (0.08 g, 0.57 mmol). The reaction mixture was then refluxed for over 40 h. Thereafter, the solvent was evaporated under vacuum, and the light yellow oily crude product was recrystallized from toluene. Yield: 0.48 g (81%). mp: 96 °C. Anal. Calcd. for $C_{45}H_{66}O_{13}N_8FP_3$. 0.5 C_7H_8: C, 53.68; H, 6.52; N, 10.33. Found: C, 54.02; H, 7.03; N, 10.11. FTIR (KBr, cm^{-1}): *v* 3201 (O-H), 2967, 2870 (C-H aliph.), 2642 (N^+-H), 1571 (asym.), 1370 (sym.) (COO^-), 1236 (asym.), 1199 (sym.) (P=N), 1050 (C-F). ^{1}H NMR (400 MHz, $CDCl_3$, ppm): *δ* 7.45 (d, 1H, $^3J_{HH}$=3.2 Hz, H_b), 7.32 (dd, 2H, $^3J_{FH}$=5.6 Hz, $^3J_{HH}$=8.4 Hz, H_3 and H_5), 6.96 (dd, 2H, $^3J_{FH}$=8.4 Hz, $^3J_{HH}$=8.8 Hz, H_2 and H_6), 6.90 (dd, 1H, $^3J_{HH}$=8.8 Hz, $^3J_{HH}$=3.2 Hz, H_d), 6.76 (d, 1H, $^3J_{HH}$=8.8 Hz, H_e), 4.43 (m, 2H, $^3J_{PH}$=13.2 Hz, $^3J_{HH}$=5.2 Hz, O-CH_2), 4.00 (d, 2H, $^3J_{PH}$=8.0 Hz, Ar-CH_2-N), 3.92 [s, 8H, O-CH_2(d)], 3.87 [s, 8H, O-CH_2(d)], 3.19 [m, 16H, N-CH_2(d)], 3.09 (m, 2H, ^{3}J$_{PH}$ 12.8 Hz, ^{3}J$_{HH}$=5.6 Hz, N-CH_2), 2.33 (s, 1H, N*H*), 1.82 (m, 2H, $^3J_{HH}$=5.6 Hz, N-CH_2-CH_2), 1.67 [t, 8H, ^{3}J$_{PH}$=10.8 Hz, ^{3}J$_{HH}$=5.6 Hz, N-CH_2-CH_2(d)], 1.59 [t, 8H, ^{3}J$_{PH}$=10.4 Hz, ^{3}J$_{HH}$=5.2 Hz, N-CH_2-CH_2(d)]. ^{13}C NMR (100 MHz, $CDCl_3$, ppm): *δ* 173.06 (s, COO^-), 162.18 (d, $^1J_{FC}$=244.6 Hz, C_1), 155.20 (s, C_f), 148.25 (s, C_c), 132.83 (dd, $^3J_{PC}$=9.3 Hz, $^4J_{FC}$=2.4 Hz, C_4), 129.88 (d, $^3J_{FC}$=7.7 Hz, C_3 and C_5), 121.78 (s, C_a), 117.25 (s, C_d), 116.77 (s, C_b), 115.94 (s, C_e), 115.19 (d, $^2J_{FC}$=21.4 Hz, C_2 and C_6), 107.25 and 107.03 (s, O-*C*-O), 67.63 (d, $^2J_{PC}$=7.7 Hz, O-*C*H_2), 64.25 and 64.22 [s, O-*C*H_2 (DASD)], 50.38 (s, Ar-*C*H_2-N), 45.72 (s, N-*C*H_2), 42.80 and 42.72 [s, N-*C*H_2 (DASD)], 35.38 [d, $^3J_{PC}$=6.1 Hz, N-CH_2-*C*H_2(DASD)], 35.31 [d, $^3J_{PC}$=6.1 Hz, N-CH_2-*C*H_2(DASD)], 25.95 (d, $^3J_{PC}$=4.6 Hz, N-CH_2-*C*H_2).

Determination of the antimicrobial activity

The antibacterial and antifungal activity of the PILs (**3a-3d**) was examined using the BACTEC MGIT 960 (Becton Dickinson, Sparks, MD) system by the standard broth dilution method (23). The antimicrobial efficacy of the salts was assessed against five G (+) {(*Bacillus cereus* NRRL B-3711, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Enterococcus hirae* ATCC 10541)} and five G (-) {(*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 35218, *Escherichia coli* ATCC 25922, *P. Vulgaris* RSKK 96029, *Klebsiella pneumaniae* ATCC 13883, *Salmonella typhimurium* ATCC 14028)} bacteria and three fungi {(*Candida albicans* ATCC 10231, *Candida krusei* ATCC 6258, *Candida tropicalis* Y-12968)}. The microorganisms were provided from the collections of G.U. Culture Collection, Turkey. Ampicillin (Amp, 10 μg/mL) and chloramphenicol (C, 30 μg/mL) (antibacterial), and ketoconazole (K, 50 μg/mL) (antifungal) were chosen as the standard antimicrobial agents. The bacterial cells were incubated in McFarland Mueller Hinton agar (MHA) at 37 °C for 24 h, and the yeast strains were incubated on Sabouraud dextrose agar (SDA) medium at 30 °C for 48 h. The MIC values, which are the lowest concentration of the salts that inhibits 90% growth, found to be in benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH) broth using serial dilution of the salts ranging from 2500-78.13 μM with adjusted bacterial and fungous concentrations (1x10^6 CFU/mL, 0.5 McFarland's standard). The solutions (50 μL) of the 4000 μM

PILs were obtained in DMSO. All the experiments were repeated three times, and the mean values were estimated. The MBC and MFC values, which are the lowest concentration of the PILs that kills 99.9 % of the initial microorganism concentration, determined by inoculating previous culture which showed no growth in agar plates.

Determination of the pBR322 plasmid DNA interaction with the PILs

The interactions of the salts with the pBR322 plasmid DNA were investigated using agarose gel electrophoresis (24). The stock solutions of the salts were prepared in DMSO. The aliquots of decreasing concentrations of the PILs ranging from 5000 to 625 μM were incubated with plasmid DNA in an incubator and at 37 °C for 24 h in the dark. The aliquots of salt/DNA mixtures were loaded onto the 1 % agarose gel with a loading buffer (0.1% bromophenol blue, 0.1% xylene cyanol). The electrophoresis was performed 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 70 V. After that, the gel was stained with ethidium bromide (0.5 μg/mL), visualized under UV light using a transilluminator (BioDoc Analyzer, Biometra). The image was photographed with a video camera, and recorded as a TIFF file. The experiments were conducted three times, and the mean values were used.

Determination of *BamH*I and *Hind*III restriction enzyme digestion of the PILs-pBR322 plasmid DNA

Evaluation of whether the salts (**3a-3d**) exhibit affinity towards adenine-adenine (AA) and/or guanine-guanine (GG) regions of the DNA, the restriction analyses of the PIL-pBR322 plasmid DNA adducts are made by *BamH*I and *Hind*III enzymes. The PIL/pBR322 plasmid DNA mixtures were incubated for 24 h, and then restricted with *BamH*I or *Hind*III enzymes at 37 °C in order for the salt to bind to DNA. The restricted DNA was run in 1 % agarose gel electrophoresis for 1 h at 70 V in TAE buffer. Eventually, the gel was stained with ethidium bromide (0.5 μg/mL), and then it was viewed with a transilluminator. The electrophoretograms were viewed using a video-camera, and saved as a TIFF file.

RESULTS AND DISCUSSION

Syntheses

The reactions of the tetraamino phosphazene bases (**2a-2d**) with gentisic acid in dry THF afforded the corresponding phosphazenium salts (**3a-3d**) with high yields (79-86 %) (Figure 1). The suitable crystals of the salts were not able to acquired. However, the solid state structure of 4-fluorobenzylspiro(N/O) tetrapropylaminocyclotriphosphazenium salt was solely identified using X-ray diffraction analysis in the literature (18). The crystallographic data displayed that this compound was monoprotonated with the nitrogen (N2) of cyclotriphosphazene ring non-adjacent to the N/O spiro ring (Figure 1). Taking into account of the NMR data, it was determined that all the PILs (**3a-3d**) protonated in the same fashion. These type of PILs are the first types

of the phosphazenium salts synthesized from the reaction of the fully secondary amine substituted cyclotriphosphazene bases and bulky organic acid. Because, in (4-fluorobenzyl)spiro(N/N)cyclotriphosphazene derivatives, the protonations were formed with the nitrogen atom (N1 and/or N3) of the cyclotriphosohazene ring adjacent to the NN spiro ring in the literature (17).

The structures of the prepared cyclotriphosphazenium salts (**3a**, one mole of water for **3c** and half mole of toluene for **3b** and **3d**) were elucidated by the elemental analysis, FTIR and ^{1}H, ^{13}C $\{^{1}H\}$, ^{31}P $\{^{1}H\}$ NMR techniques. The NMR and analytical data are presented in the Experimental Section. The thermal properties of the PILs were evaluated by TG/DTG/DTA instrument, and it was found that all of the salts had different thermal decomposition patterns. All the PILs were soluble in the common organic polar and apolar solvents such as $CHCl_3$, CH_2Cl_2, CH_3CN, DMSO, toluene, and hexane nevertheless they are dissolved slightly in water. The solubility of the obtained PILs (**3a-3d**) did not increase in water even under the increased temperature. Therefore, the PILs were scrutinized for the antibacterial and antifungal activity against bacteria and fungi using DMSO. The interactions of the PILs with supercoiled pBR322 plasmid DNA and their *Bam*HI and *Hind*III restriction enzyme digestion were examined.

The ^{31}P $\{^{1}H\}$ NMR spectral data and the spin systems of the tetrachloro-4-fluorobenzyl(N/O)spirocyclotriphosphazene (**1**) and its tetraamino derivatives (**2a-2d**) [22] for comparison and their salts (**3a-3d**) were tabulated in Table 1. The ^{31}P $\{^{1}H\}$ NMR spectra of the 4-fluorobenzyl(N/O)spirocyclotriphosphazenes have the AX_2 (for **1**) and AB_2 (for **2a-2d**) spin systems due to two different phosphorus environments within the structures. As expected, the ^{31}P spectra of the PILs have the AB_2 (for **3a** and **3c**) and AX_2 (for **3b** and **3d**) spin systems depending on two different phosphorus environments. The δ P_{ON} and δ P_{NN} shifts of the tetraaminocyclotriphosphazene bases (**2a, 2b** and **2d**) are highly larger than those of the PILs (**3a, 3b** and **3d**), but the same shift values of **2c** phosphazene base and their **3c** salt are very close to each other. In addition, it is found that two bond-coupling constants ($^{2}J_{PP}$) of the phosphazene bases (**2a, 2b** and **2d**) are larger than those of the corresponding salts (**3a, 3b** and **3d**) due to the salt formation with the bulky organic acid. Furthermore, the $^{2}J_{PP}/\Delta v$ values of these compounds/salts were estimated and listed in Table 1.

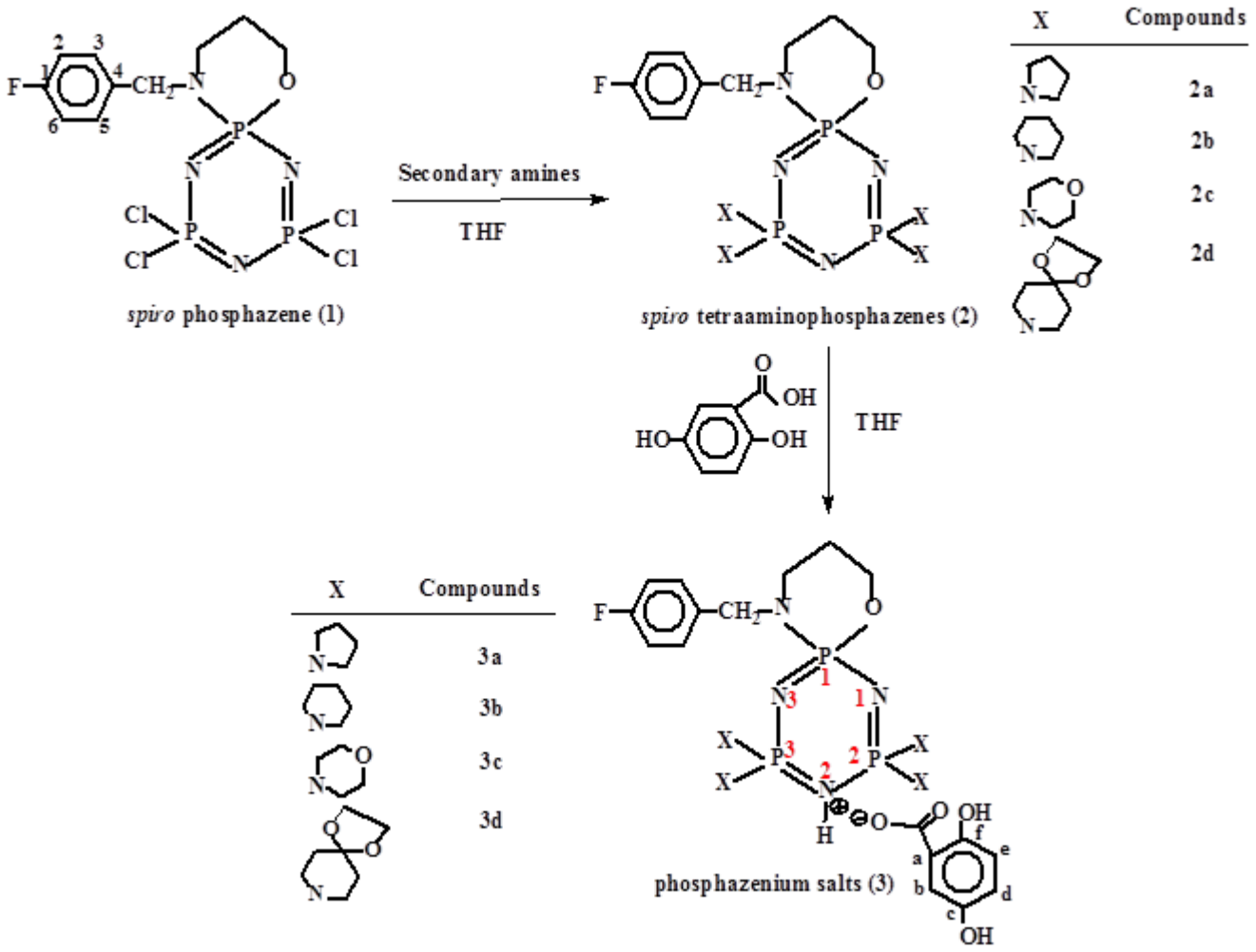
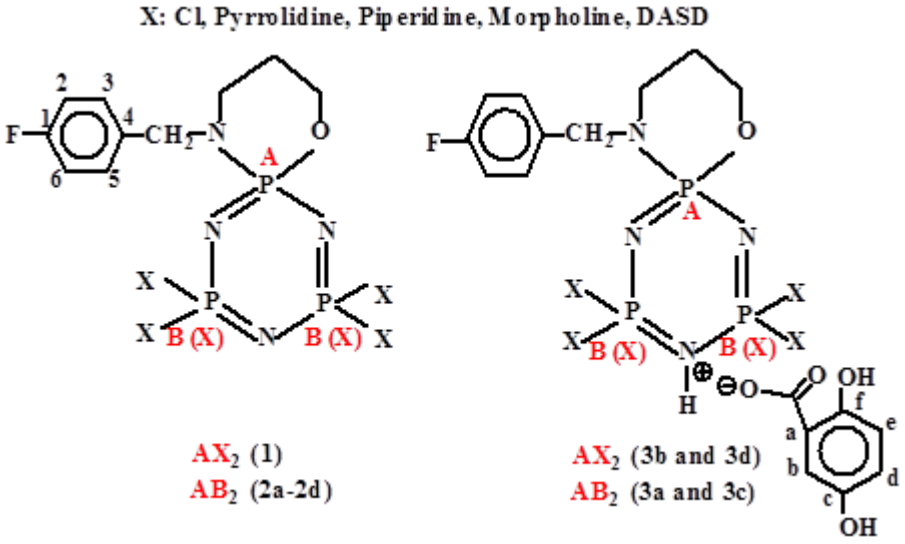


Figure 1: Cyclotriphosphazene architectures prepared from the reactions of (4-fluorobenzyl)spiro(N/O)cyclotriphosphazene with secondary amines and their phosphazenium salts.

Table 1: ^{31}P $\{^{1}H\}$ NMR parameters of (4-fluorobenzyl)spiro(N/O)cyclotriphosphazenes and their PILs.[a]



Compound	Spin Systems	P_A (P_{ON})	P_B/P_X (P_{NN})	P_X (P_{Cl2})	$^2J_{PP}$ (Hz)	$^2J_{PP}/\Delta v$
*1	AX_2	9.06	-	23.32	$^2J_{AX}$ 50.2	-
*2a	AB_2	21.17	18.98	-	$^2J_{AB}$ 44.5	0.08
*2b	AB_2	20.20	22.18	-	$^2J_{AB}$ 46.2	0.10
*2c	AB_2	19.95	21.45	-	$^2J_{AB}$ 47.8	0.13
*2d	AB_2	19.66	21.18	-	$^2J_{AB}$ 50.2	0.14
3a	AB_2	13.36	13.85	-	$^2J_{AB}$ 41.3	0.35
3b	AX_2	11.38	16.84	-	$^2J_{AX}$ 38.9	-
3c	AB_2	19.05	21.04	-	$^2J_{AB}$ 46.2	0.10
3d	AX_2	14.45	18.24	-	$^2J_{AB}$ 41.3	-

[a]242.93 MHz ^{31}P $\{^{1}H\}$ NMR measurements in $CDCl_3$ solutions at 298 K, and the chemical shifts referenced to external H_3PO_4.

*^{31}P $\{^{1}H\}$ NMR values of these compunds were taken from the lit (22).

As an example, ^{31}P $\{^{1}H\}$ NMR spectra of phosphazene base **2d** and its PIL **3d** is illustrated in Figure 2. In the spectra of **2d** and **3d**, the δ P_{ON} of spiro (P_A) and δ P_{NN} (P_B or P_X) were 19.66 and 21.18 ppm, and 14.45 and 18.24 ppm, respectively. The $^2J_{PP}$ values were calculated as 50.2 Hz (for **2d**) and 41.3 Hz (for **3d**). The δ $_P$ shifts and $^2J_{PP}$ values of the other PILs were elucidated as in salt **3d**.

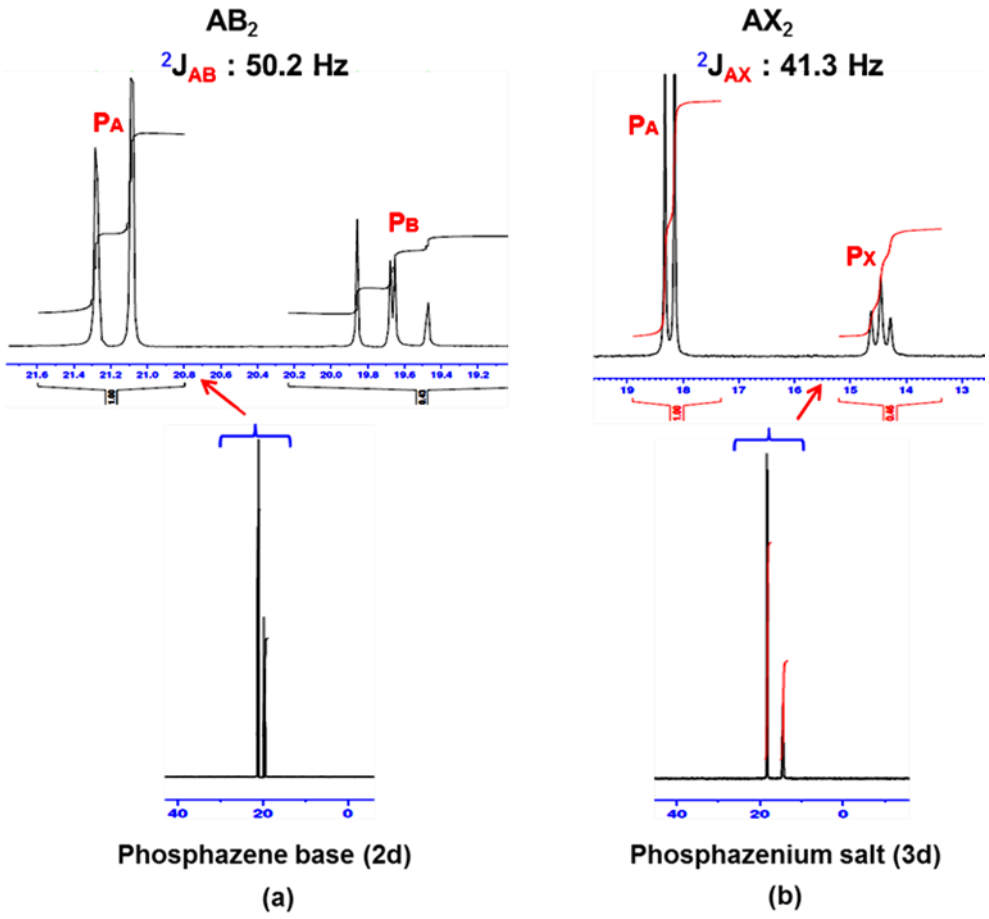


Figure 2: ^{31}P {^{1}H} NMR spectra of the pure (**a**) phosphazene base **2d** and (**b**) PIL **3d**.

The interpretations of the δ-shifts, multiplicities, and coupling constants were assigned from the ^{13}C {^{1}H} and ^{1}H NMR spectra of the PILs (**3a-3d**), and given in Experimental Section. The signals of all the carbon atoms of the PILs were elucidated in the ^{13}C NMR spectra. The *geminal* amine substituents exhibited two small separated peaks for NCH_2, NCH_2CH_2, $NCH_2CH_2CH_2$, OCH_2 and OCO groups in the ^{13}C {^{1}H} NMR spectra of the PILs, since two *geminal* groups were not equivalent. The coupling constants of $^{1}J_{FC}$, $^{2}J_{FC}$, $^{3}J_{FC}$ and $^{4}J_{FC}$ were interpreted for the phosphazenium salts. The average $^{1}J_{FC}$, $^{2}J_{FC}$, $^{3}J_{FC}$ and $^{4}J_{FC}$ values of the salts were 245.4 Hz, 21.5 Hz, 7.8 Hz and 2.8 Hz respectively, and the obtained results were in consistent with the literature findings of (4-fluorobenzyl) substituted cyclotriphosphazene derivatives and their salts (18,22,25). As expected, in DASD substituted compound, **3d**, δ_{OCO} shifts observed at 107.25 ppm and 107.03 ppm as a singlet. Moreover, COO^- and C_a-C_f carbon peaks of gentisic acid are also designated in all salts (Figure 1).

The assignments of the 4-fluorobenzyl protons of the PILs were anticipated using the coupling constants of $^{3}J_{FH}$ and $^{4}J_{FH}$, and the multiplicities of the hydrogen atoms. The $^{3}J_{FH}$ and $^{4}J_{FH}$ values of the rings were found to be in accordance with the literature data (25), and the average $^{3}J_{FH}$ and $^{4}J_{FH}$ values of the salts (**3a-3d**) are 8.7 Hz and 5.6 Hz,

respectively. The average values of NCH_2CH_2, NCH_2 and OCH_2 spiro protons of the PILs were found to be as 1.82 ppm, 3.05 ppm and 4.41 ppm, respectively, in comparison to the values (1.86 ppm, 3.01 ppm and 4.30 ppm) of the free phosphazene bases (**2a-2d**). The average $^3J_{PH}$ value of OCH_2 spiro protons of the PILs, 12.7 Hz, was very large. Furthermore, the $ArCH_2N$ protons of the salts appeared in the range of 3.99-4.05 ppm as doublets, and the average $^3J_{PH}$ value of them was 7.8 Hz. The $^3J_{PH}$ values of the salts were larger than those of the phosphazene bases.

All the PILs exhibit in the FTIR spectra ν_{O-H} (ca. 3245 cm^{-1}), ν_{N^+-H} (ca. 2600 cm^{-1}), ν_{COO^-} (asymm.) (ca. 1578 cm^{-1}) and ν_{COO^-} (symm.) (ca. 1369 cm^{-1}) absorption frequencies conforming formation of the salt. In addition, the salts display intense asymmetric and symmetric stretching vibrations between 1236-1249 cm^{-1} and 1193-1201 cm^{-1}, refer to the $\nu_{P=N}$ bonds of the cyclotriphosphazene skeletons (26). The PILs also show ν_{C-F} absorption frequencies (ca. 1050 cm^{-1}), and they are in agreement with the literature data of the mono(4-fluorobenzyl)spirophosphazenes and their salts (18,25).

Thermal studies of the PILs

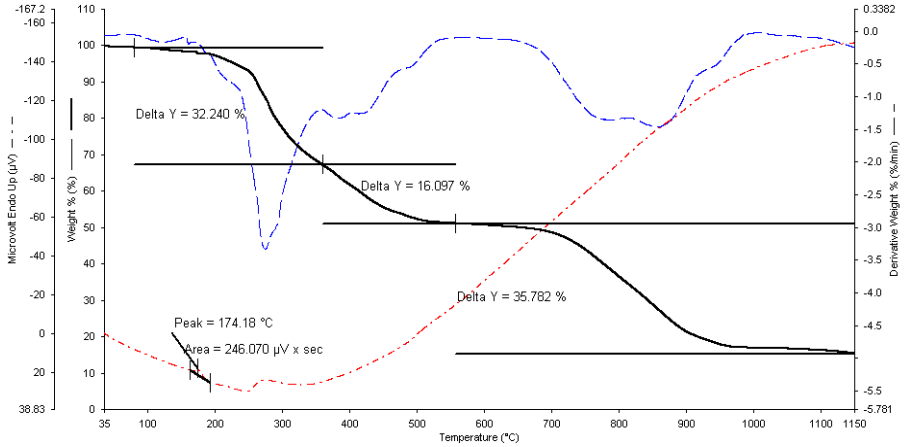
Recently, the thermal properties of some phosphazenium salts of the bulky organic acids were examined using TG and differential scanning calorimetry (DSC) techniques (17,19). The TG and DTA techniques are quite helpful for the materials science to determine the stabilities of inorganic, organic and polymeric compounds. Figure 3 depicted the TG thermograms of the PILs (**3a-3d**), and they were examined under similar experimental conditions. The thermoanalytical data for the salts are also summarized in Table 2. TG analyses of the PILs were carried out in the temperature range from 35 °C to 1150 °C under a flowing atmosphere of N_2.

According to the TG curves, there are some differences in the thermal stabilities of the PILs. The amount of the total mass loss in the PILs slightly varies, and the non-volatile residue was consisting approximately 11-20 wt%. All of the salts had almost similar thermal degradation routes of the thermal analysis, and were comprised of three (**3a, 3c** and **3d**) and five (**3b**) steps (Figure 3). In **3a**, firstly the genticylate anion and a part of 3-(4-fluorobenzylamino)-1-propanol, secondly two equimolar amounts of pyrrolidine, and finally the remaining pyrrolidines and a part of phosphazene cation leave the phosphazenium salt. In PIL **3b**, in the first two steps the toluene solvent is removed from the molecule. Then genticylate anion and a portion of 3-(4-fluorobenzylamino)-1-propanol, the remaining part of amino alcohol, and ca. two equimolar amounts of piperidine were separated in the other three stages, respectively. The phosphazenium salt **3c** consists of three steps, and as a first step, one mole of water in its structure is removed. The second decomposition step corresponds about a mass loss of the genticylate anion, 3-(4-

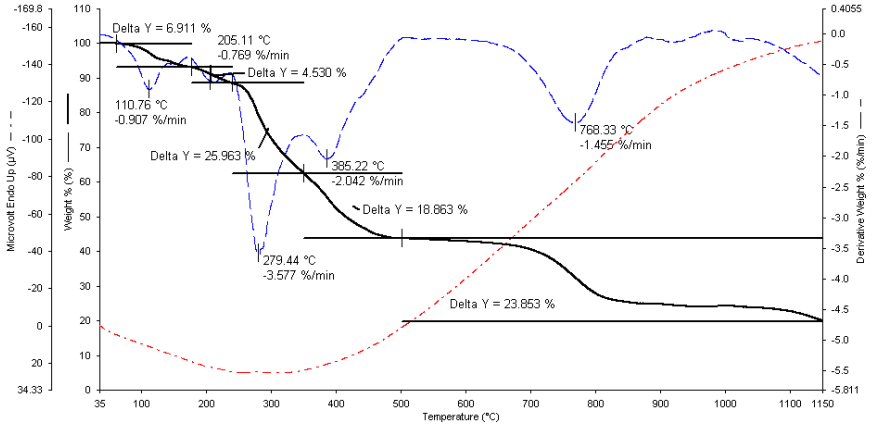
fluorobenzylamino)-1-propanol and one mole morpholine. In the third step, approximately to the mass loss of three equimolar amounts of morpholines and a portion of phosphazene cation is removed from the molecule. In **3d**, primarily the half mole of toluene and genticylate anion in structure leave the molecule, later a mass loss of about 3-(4-fluorobenzylamino)-1-propanol and two moles DASD occur. The rest of DASD is separated in the final decomposition step. It appeared that the genticylate anion is principally left in all the salts.

Table 2: Thermoanalytical data of **3a-3d.**

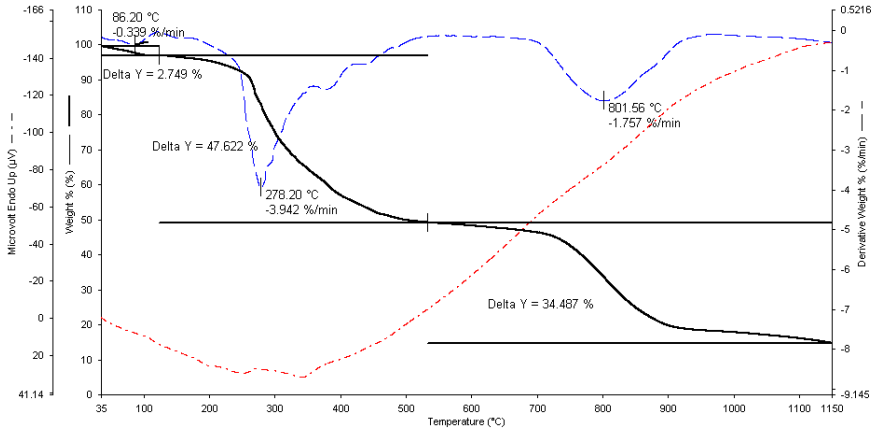
Compounds	**Stage**	**Temperature range (°C)**	**$DTG_{(max)}$ (°C)**	**Mass loss (Obs.) Δm %**	**Total Mass loss (Obs.) Δm %**
3a	1	79-359	275	32.24	84.12
$C_{33}H_{50}O_5N_8FP_3$	2	359-557	384	16.10	
750.31 g mol^{-1}	3	557-1150	857	35.78	
3b	1	61-177	111	6.91	80.11
$C_{37}H_{58}O_5N_8FP_3.1/2\ C_7H_8$	2	177-239	205	4.53	
806.37 g mol^{-1}	3	239-349	279	25.96	
	4	34-501	385	18.86	
	5	501-1150	768	23.85	
3c	1	35-123	86	2.75	84.86
$C_{33}H_{50}O_9N_8FP_3.H_2O$	2	123-533	278	47.62	
814.29 g mol^{-1}	3	533-1150	802	34.49	
3d	1	108-294	278	19.47	89.04
$C_{45}H_{66}O_{13}N_8FP_3.1/2\ C_7H_8$	2	294-575	331	41.89	
1038.40 g mol^{-1}	3	575-1150	941	27.68	



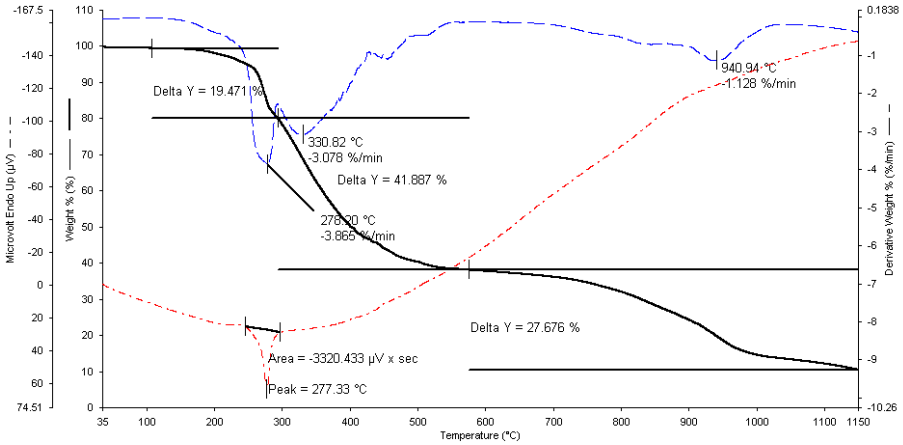
3a



3b



3c



3d

Figure 3: TG curves of the phosphazenium salts (**3a-3d**).

Antimicrobial activity evaluations: The antimicrobial activity of the PILs (**3a-3d**) was screened for comparing with the amino alcohol, cyclotriphosphazene bases (**1** and **2a-2d**) and exploring the compounds against both (G−) and (G+) bacterial and fungal species. The *in vitro* antimicrobial activity of the PILs against eleven types of bacteria and three type of fungi were illustrated in Table 3. The MIC, and MBC/MFC values of the salts were listed in Table 4 and 5, respectively.

The PIL **3c** is the more active against *E. coli* ATCC 35218 than the chloramphenicol. It is well known that *E. coli* is a (G−) anaerobic coccus and ordinarily lives in the intentines of healthy people and animals. The most types of it are harmless or cause brief diarrhea in some degree. The PILs **3a** and **3d** are strongly active against *E. faecalis* ATCC 29212, and they are particularly more efficient than the control antibacterial agent chloramphenicol. It is known that *E. faecalis* is a facultative anaerobic (G+) bacteria. It induces root canal failure and persistent apical periodontitis (27). The salt **3a** appears to be efficient against *B. subtilis* ATCC 6633 as much as the ampicillin and chloramphenicol. Only one (**3b**) of the phosphazenenium salts exhibit anticandidal activity. PIL **3b** is especially more active than the control antifungal agent ketoconazole for *C. albicans* ATCC 10231. As it is known, Candida species lead to fungal infections. On the other hand, all the salts do not display any antibacterial activity against *E. hirae* ATCC 9790 and antifungal activity against *C. krusei* ATCC 6258. In addition, compared to the salts (**3a-3d**) with the corresponding free bases (**2a-2d**), the salts (**3c**) and (**3a** and **3d**) are found to be more efficient or active than corresponding free bases (**2c**) and (**2a** and **2d**), respectively, against *E. coli* ATCC 35218 and *E. faecalis* ATCC 29212*,* implying that the salt formation usually increases antibacterial activity. However, the free bases (**2a-2d**) presented more antifungal activity than corresponding PILs (except **3b**) against test fungi, showing that the salt formation decreases antifungal activity. MIC values changed from 78.13 μM to >2500 μM. The most effective compound was **3b** against *C. tropicalis*. MBC and MFC values were between 78.13 μM to >2500 μM.

Table 3: The antimicrobial activity results of the amino alcohol, 4-(fluorobenzyl)(N/O)spirocyclotriphosphazenes (**1** and **2a-2d**) and their salts (**3a-3d**).

	Compounds									Positive control		
Test microorganism	**Amino alcohol**	**1**	**2a**	**2b**	**2c**	**2d**	**3a**	**3b**	**3c**	**3d**	**Amp**	**C**	**Keto**
***E. coli* ATCC 35218 G(-)**	9 ± 0	-	-	-	-	-	-	-	16 ± 1	-	-	8 ± 0	NS
***E. coli* ATCC 25922 G(-)**	-	9 ± 1	-	9 ± 1	11 ± 2	12 ± 1	-	-	-	-	18 ± 0	25 ± 0	NS
***B. cereus* NRRLB-3711 G(+)**	-	-	-	-	9 ± 0	9 ± 1	19 ± 0	-	-	17 ± 1	-	-	NS
***B. subtilis* ATCC 6633 G(+)**	8 ± 0	-	-	-	13 ± 1	-	20 ± 1	14 ± 1	-	17 ± 1	23 ± 1	21 ± 0	NS
***S. aureus* ATCC 25923 G(+)**	-	-	-	11 ± 1	-	-	18 ± 1	-	-	21 ± 1	44 ± 1	24 ± 1	NS
***E. faecalis* ATCC 29212 G(+)**	10 ± 0	-	9 ± 0	-	-	10 ± 0	22 ± 2	-	16 ± 1	23 ± 2	27 ± 0	20 ± 0	NS
***P. aeruginosa* ATCC 27853 G(-)**	9 ± 1	-	10 ± 1	11 ± 1	12 ± 0	12 ± 1	15 ± 1	12 ± 1	12 ± 1	16 ± 1	60 ± 0	34 ± 0	NS
***K. pneumaniae* ATCC 13883 G(-)**	-	-	-	10 ± 0	11 ± 1	11 ± 1	16 ± 0	17 ± 0	15 ± 1	20 ± 1	-	31 ± 1	NS
***S. typhimurium* ATCC 14028 G(-)**	-	-	-	12 ± 1	8 ± 1	-	11 ± 1	-	14 ± 1	-	19 ± 1	38 ± 1	NS
***E. hirae* ATCC 9790 G(+)**	-	-	-	-	-	-	-	-	-	-	9 ± 1	22 ± 1	NS
***P. vulgaris RSKK 96029* G(-)**	-	-	-	-	-	-	15 ± 1	15 ± 1	12 ± 1	16 ± 1	-	32 ± 1	NS
***C. albicans* ATCC 10231**	-	-	10 ± 1	13 ± 1	19 ± 1	18 ± 1	-	14 ± 1	-	-	NS	NS	11 ± 1
***C. krusei* ATCC 6258**	-	-	-	12 ± 0	14 ± 1	13 ± 0	-	-	-	-	NS	NS	18 ± 1
***C. tropicalis* Y-12968**	-	-	-	-	11 ± 1	14 ± 1	-	17 ± 1	-	-	NS	NS	34 ± 2

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

Table 4. The *in vitro* antimicrobial activity of the 4-(fluorobenzyl)(N/O)spirocyclotriphosphazenium salts (**3a-3d**) against test strains (MIC: Minimum Inhibitory Concentration. MIC values are reported in μM).

M**icroorganism / Compound**	**3a**	**3b**	**3c**	**3d**
K. pneumaniae **ATCC 13883 G(-)**	625 μM	312.5 μM	625 μM	312.5 μM
S. aureus **ATCC 25923 G(+)**	312.5 μM	-	-	625 μM
E. faecalis **ATCC 29212 G(+)**	1250 μM	-	-	1250 μM
P. aeruginosa **ATCC 27853 G(-)**	-	-	-	312.5 μM
B. subtilis **ATCC 6633 G(+)**	>2500 μM	-	-	>2500 μM
E. coli **ATCC 35218 G(-)**	-	-	625 μM	-
S. typhimurium **ATCC 14028 G(-)**	-	-	1250 μM	-
P. vulgaris ***RSKK 96029*** **G(-)**	1250 μM	1250 μM	-	1250 μM
E. hirae **ATCC 9790 G(+)**	-	-	-	2500 μM
B. cereus **NRRLB-3711 G(+)**	>2500 μM	-	-	2500 μM
C. tropicalis **Y-12968**	-	78.13 μM	-	-

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

Table 5. The *in vitro* antimicrobial activity of the 4-(fluorobenzyl)(N/O)spirocyclotriphosphazenium salts (**3a-3d**) against test strains (MBC: Minimum Bactericidal Concentration, and MFC: Minimum Fungicidal Concentration. MBC and MFC values are reported in μM).

M**icroorganism / Compound**	**3a**	**3b**	**3c**	**3d**
K. pneumaniae **ATCC 13883 G(-)**	625 μM	625 μM	1250 μM	625 μM
S. aureus **ATCC 25923 G(+)**	625 μM	-	-	625 μM
E. faecalis **ATCC 29212 G(+)**	2500 μM	-	2500 μM	2500 μM
P. aeruginosa **ATCC 27853 G(-)**	-	-	-	312.5 μM
B. subtilis **ATCC 6633 G(+)**	>2500 μM	-	-	>2500 μM
E. coli **ATCC 35218 G(-)**	-	-	1250 μM	-
S. typhimurium **ATCC 14028 G(-)**	-	-	1250 μM	-
P. vulgaris ***RSKK 96029*** **G(-)**	2500 μM	2500 μM	-	2500 μM
E. hirae **ATCC 9790 G(+)**	-	-	-	2500 μM
B. cereus **NRRLB-3711 G(+)**	>2500 μM	-	-	>2500 μM
C. tropicalis **Y-12968**	-	78.13 μM	-	-

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

Interactions of pBR322 plasmid DNA with the PILs

The plasmid DNA was treated with salts (**3a-3d**) in the dark at 37 °C for 24 h, and then the mobilities of the forms of DNA were investigated with agarose gel electrophoresis (28,29). Figure 4 exhibits the electrophoretograms applied to the incubated mixtures of DNA at various concentrations (5000 - 625 μM) of the PILs. In addition, the interactions of plasmid DNA with the PILs (**3a-3d**) were compared with those of amino alcohol and cyclotriphosphazene bases (**1** and **2a-2d**) in Figure 4. Lane P applies to the untreated plasmid DNA (control DNA), indicating the major supercoiled circular (Form I) and minor singly nicked relaxed circular (Form II) forms of the plasmid DNA. Lanes 1-4 apply to plasmid DNA incubated with compounds ranging from 5000 μM to 625 μM.

When plasmid DNA was incubated with decreasing concentrations of the phosphazenium salts (**3a-3d**), the mobility of Form I DNA decrease at two high concentrations, and afterwards increase slightly. In case of intensity Form I and II, were decreased at the first high concentration, then Form I increased with the decreasing concentration of compounds.

On the other hand, Lanes 1-4 demonstrate that the plasmid DNA is interacted with increasing concentrations of **amino alcohol**, **1**, **2a**, **2b** and **2c**. These compounds caused a slight decrease in the mobility of Form I. The compounds **3a** and **3b** caused depletion of two bands at two higher concentrations indicating cleavage of DNA. In case of **3b**, **3c** and **3d,** linear Form III bands were observed, indicating double strand breaks in DNA.

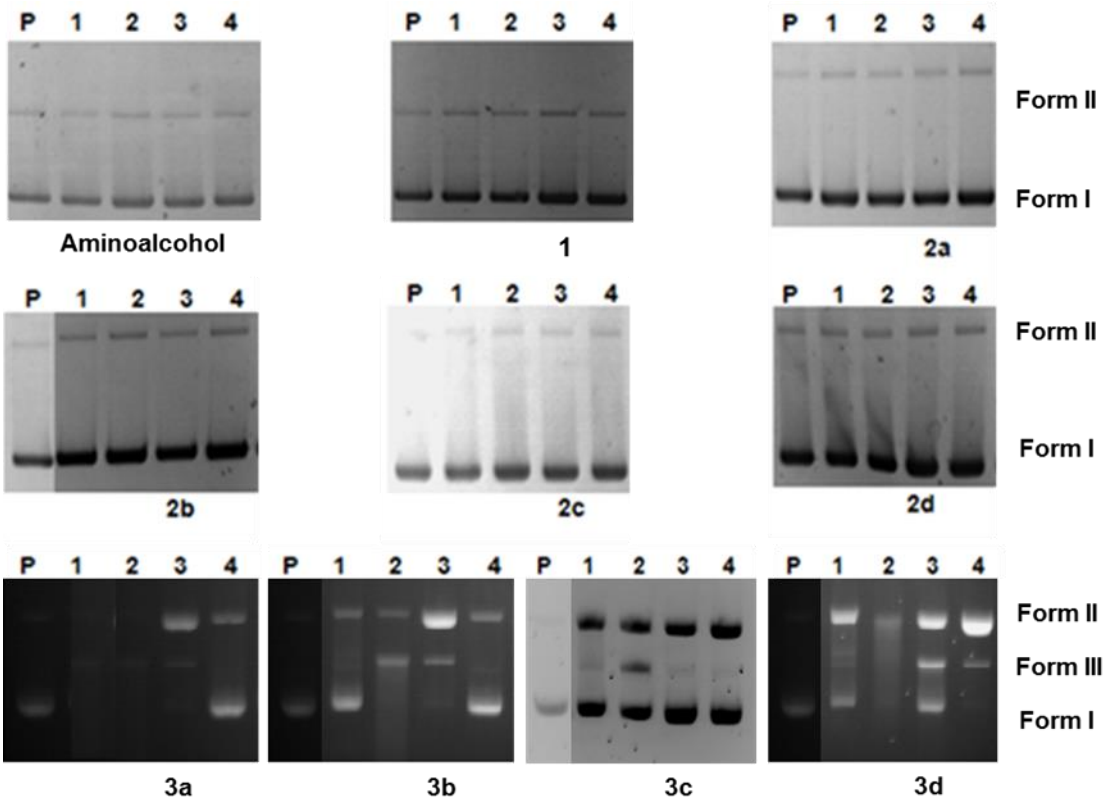


Figure 4. The gel electrophoretic mobilities of the pBR322 DNA after incubated at concentrations ranging from 5000 to 625 μM incubation at 37 °C, Lane 1: 5000, Lane 2: 2500, Lane 3: 1250, Lane 4: 625 (μM), P: untreated plasmid.

Determination of nucleotides interacted with the nucleotides

DNA–PIL mixtures were digested with *BamH*I and *Hind*III restriction enzymes for the purpose of determine DNA-PIL binding. Figure 5 presents the electrophoretograms for the *Bam*HI and *Hind*III digested mixtures of the plasmid DNA after the treatment with the salts. Figure 5 also includes the results of the amino alcohol and cyclotriphosphazene bases (**1** and **2a-2d**). Untreated plasmid DNA was applied to Lane P, Lane PB and PH were applied plasmit DNA digested with *BamH*I and *Hind*III enzymes, respectively. The other lanes were applied to the plasmid DNA interacting with the PILs (**3a-3d**) followed by their digestions with *Bam*HI and *Hind*III. Compounds **3a**, **3b** and **3c** are not cleaved by both enzymes indicating compound binding to A/A and G/G nucleotides, however, all the others compounds, DNA mixture is restricted indicating compounds are not binding to DNA. Therefore, *Bam*HI and *Hind*III restriction digestion revealed that the salts

bind to A/A and G/G nucleotides. On the other hand, when the **amino alcohol, 1**, **2a**, **2b**, **2c** and **2d** were digested with *Bam*HI, only Form III bands were also observed. The findings suggest that the PILs (**3a-3d**) can cause a greater conformational change and cleavage to the DNA than the corresponding phosphazene bases.

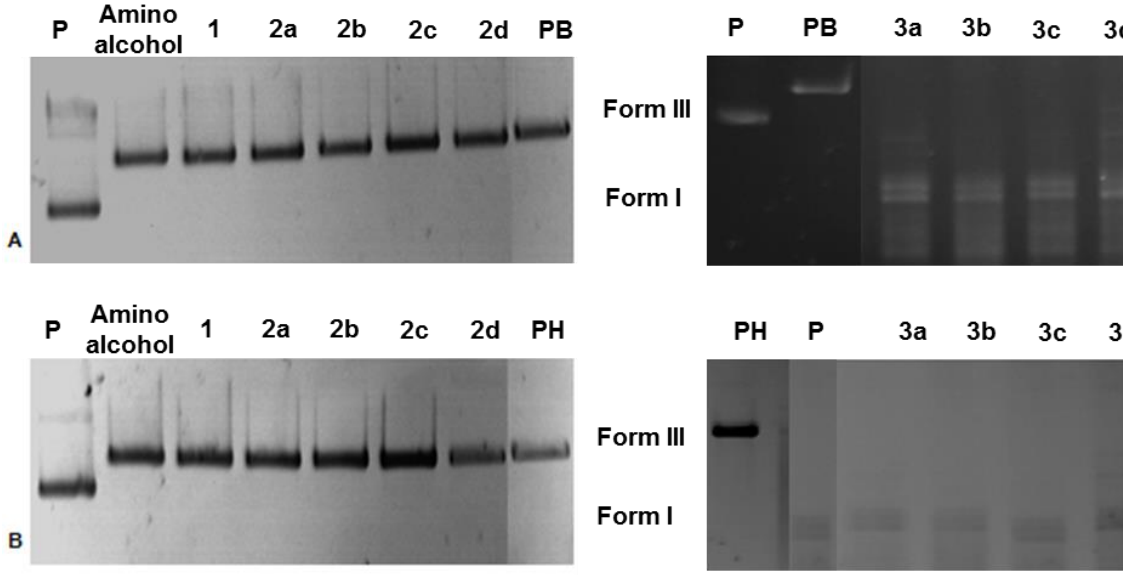


Figure 5. The electrophoretograms for A) *Bam*HI and B) *Hind*III digested mixtures of the pBR322 DNA after treatment with the amino alcohol and cyclotriphosphazene bases (**1** and **2a-2d**), and PILs (**3a-3d**). The numbers above the lanes show the compounds digested with the enzymes.

CONCLUSIONS

The PILs (**3a-3d**) were prepared from the reactions of tetraamino substituted 4-(fluorobenzyl)spiro(N/O)cyclotriphosphazenes (**2a-2d**) with the gentisic acid in dry THF. The structures of PILs were determined in comparison to free phosphazene bases (**2a-2d**) using NMR results obtained in $CDCl_3$ solution. The spectroscopic data of the salts showed that the nitrogen of the phosphazene ring non-adjacent to the N/O spiro ring was mono protonated, indicating that this N atom was more basic than the other two phosphazene ring nitrogens. All the PILs had nearly the same thermal degradation patterns. The antimicrobial activity of all the salts (**3a-3d**) were evaluated against bacteria and fungi, and compared with the corresponding free phosphazene bases (**2a-2d**). Consequently, the tested (**3c**) and (**3a** and **3d*)*** salts were the most promising antibacterial derivatives against *E. coli* ATCC 35218 and *E. faecalis* ATCC 29212, respectively. Similarly, the PIL **3b** was also the most active antifungal agent for *C. albicans* ATCC 10231 among the tested salts. On the other hand, when the salts (**3a-3d**) with the corresponding free bases (**2a-2d**) are

compared, the salts (**3a, 3c** and **3d**) display better antibacterial activity than corresponding free bases (**2a, 2c** and **2d**), while the free bases (**2a-2d**) exhibit better antifungal activity than corresponding PILs (except **3b**). The gel electrophoresis data demonstrate that cause the conformational changes and cleavage to the DNA, **3a**, **3b** and **3c** bind to A/A and G/G nucleotides.

ACKNOWLEDGMENTS

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