Phosphorus–nitrogen compounds. Part 41. Ferrocenyl pendant-armed spirocyclopiperidinocyclotriphosphazatrienes: Langmuir–Blodgett thin films and biological activity studies

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Abstract. The Cl replacement reactions of N/N or N/O spirocyclic monoferrocenylcyclotriphosphazatrienes (**1–5**) with the piperidine resulted in the geminal- (**6–10**) and tetra-piperidinophosphazenes with monoferrocenyl pendant arm (**11–15**). The structures of all the new compounds were determined using spectroscopic techniques. The ultrathin Langmuir–Blodgett (LB) films of two compounds (**3** and **12**) were prepared. The characterization of the LB films using p-polarized grazing angle (GAIR) and horizontal attenuated total reflectance (HATR) techniques was carried out. The molecular and crystal structure of the compound **6** was examined using X-ray crystallography. In addition, the interactions between six compounds (**6**, **7**, **11**, **12**, **14** and **15**) and pBR322 plasmid DNA were investigated by agarose gel electrophoresis.

Keywords. Pendant-armed ferrocenyl phosphazenes; spectroscopy; thin films; DNA cleavage.

1. Introduction

The chemistry of hexachlorocyclotriphosphazenes ($N_3P_3Cl_6$; trimer) and octachlorocyclotetraphosphazenes ($N_4P_4Cl_8$; tetramer) has been widely studied since 1960.¹ Trimer and tetramer are used as scaffolds for the construction of numerous substituted cyclotriphosphazenes.² The sequential Cl replacement reactions of trimer and tetramer with mono-functional reagents produce the partly and fully-substituted phosphazenes.³ However, the reactions carried out with di-, tri- and multi-functional reagents give different geometrical and optical isomers, e.g., spiro-, ansa-, dispiro-, trispiro, ansa-spiro and bino-architectures.^{2–4} In addition, trimer and tetramer are also used as building blocks for dendrimers⁵ and phosphazene polymers.⁶ In recent years, the coordination complexes of the aminocyclophosphazene have attracted interest as an active research area.⁷ In the last two decades, the stereogenic properties of cyclophosphazenes have been widely investigated,⁸

and recently a nice review was published as well.⁹ Although the stereochemical terms such as prochirality, enantio-, diastereo- and homotopic atoms or substituents and pseudo-asymmetric centers were known for a long time in organic chemistry, they were incorporated into phosphazene derivatives only in two papers.¹⁰ On the other hand, phosphazene derivatives and polymeric phosphazenes are used as inflammable textile fibers and elastomers,¹¹ antibacterial¹² and anticancer¹³ reagents, lubricants,¹⁴ membranes,¹⁵ synthetic bones,¹⁶ ion-transferring agents for rechargeable lithium batteries¹⁷ and photophysics.¹⁸ Ferrocene (Fc) derivatives are used in organometallic chemistry,¹⁹ photochemistry,²⁰ redox-active probe materials²¹ and electron transfer mediators for nonlinear optical devices²² as well.

In the meantime, the ultrathin films of inorganic and organic materials were obtained with different methods such as thermal evaporation, adsorption from solution, electrodeposition, sputtering, self-assembly, molecular beam epitaxy and Langmuir–Blodgett (LB)

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techniques.²³ The LB films are very useful materials in industry e.g., as detectors, sensors and electronic circuit components.²⁴ Our group published two papers about the LB thin films of trimeric phosphazene derivatives.²⁵

The literature survey shows that there are very limited number of reports about the pendant armed spirocyclo-triphosphazenes, their reactions, and spectral and structural properties.²⁶ Actually, several papers have been reported by our group.^{3,4a, 27}

This paper deals with the condensation reactions of the tetrachloro monoferrocenyl pendant-armed spiro-phosphazenes (**1–5**) with piperidine for determining their biological activities and DNA interactions, and also for preparing the LB thin films of the partly and fully-piperidino-substituted compounds. Thus, the bis- (**6–10**) and tetrakis-piperidino-substituted (**11–15**) N/N and N/O spirocyclo-triphosphazenes with monoferrocenyl pendant arm were obtained from the substitution reactions. The structures of all the new phosphazenes (**6–15**) were analyzed using mass spectrometry, elemental analyses, Fourier transform (FTIR) and one-dimensional (1D) ¹H, ¹³C{¹H}, and ³¹P{¹H} NMR techniques. The molecular and crystal structures of **6** were established by X-ray crystallography. The purpose of combining the trimer with the ferrocenyl group is to create compounds with new properties from these two groups. According to our previous studies,^{3a, 8c, 25b} it has been observed that geminal products have occurred from the reactions of the ferrocenylphosphazenes with the secondary amines. So, ferrocenyl pendant-armed spiro precursors have also been chosen for the formation of geminal products instead of non-geminal products with piperidine. In addition, piperidine substituents increase the basic character of the phosphazene ring as well. As understood in this study, these novel compounds may be used for different purposes in the future in addition to the above-mentioned uses, taking into account their different properties. To confirm this view, due to the difficulties in preparing LB films of all compounds, only two phosphazenes **3** and **12** were selected as examples and LB films were prepared. The LB films of other analog phosphazenes can also be prepared.

2. Experimental

2.1 Reagents

Ferrocenecarboxaldehyde (Aldrich), hexachlorocyclo-triphosphazatriene (Aldrich) and aliphatic amines (Fluka) were purchased and used without further purification. THF was dried over 3 Å molecular sieves. All the reactions were monitored with thin-layer chromatography (TLC) on Merck DC Alufolien Kiesegel 60 B₂₅₄ sheets using toluene as

solvent. The column chromatography was performed on Merck Kiesegel 60 (230–400 mesh ATSM) silica gel purchased and used without further purification.

2.2 Instruments

The melting points were measured with a Gallenkamp apparatus using a capillary tube. The elemental analyses were performed by Leco CHNS-932. The ¹H, ¹³C and ³¹P NMR spectra were recorded on a Varian Mercury FT-NMR spectrometer (SiMe₄ as an internal standard and 85% H₃PO₄ as an external standard), operating at 400.00, 100.59 and 161.92 MHz. The spectrometer was equipped with a 5 mm PABBO BB inverse-gradient probe. Standard Bruker pulse programs were used.²⁸ APIES mass spectrometric analyses were performed on a Waters 2695 Alliance Micromass ZQ spectrometer. IR spectra with KBr pellet were recorded on a Perkin-Elmer Fourier Transform Infrared (FTIR) Spectrometer equipped with transmission accessories. A commercially available computer-controlled round alternate trough manufactured by NIMA (Model TKB 2410A) was used for the preparation of the LB films. In order to elucidate the structures of the LB films, FTIR, p-polarized GAIR and HATR spectra were recorded. Transmittance spectra at normal incidence and p-polarized GAIR spectra at an incidence angle of 82° were measured on a Perkin Elmer Spectrum One FTIR Spectrometer equipped with a Deuterated Triglycine Sulfate (DTGS) detector. A ZnSe polarizer was employed for the polarization measurements of the LB films. Both GAIR and HATR spectra were collected for 1000 interferograms with a resolution of 4 cm⁻¹ using the same sample. A Perkin Elmer horizontal attenuated total reflectance attachment was used for HATR measurements. The angle of incidence was 45° with ZnSe crystal. In this technique, LB film on aluminum covered glass substrate was pressed on ZnSe crystal using a very sensitive computer controlled pressure arm to obtain a good contact between the sample and the infrared element (ZnSe crystal) at the interface that was essential requisite for the technique.²⁹

Antimicrobial susceptibility testing was performed by the BACTEC MGIT 960 (Becton Dickinson, Sparks, MD) system using the agar-well diffusion method (Section S1, Supplementary Information). The DNA binding abilities were examined using agarose gel electrophoresis (Section S2, Supplementary Information).

2.3 LB film deposition

The floating Langmuir monolayers of **3** and **12** were obtained in pure chloroform (3.5 and 3.6 mg/mL). The investigations of the surface pressure-area (π -A) isotherms, the Langmuir monolayers at the air-water interface and the stabilities of the monolayer of **3** and **12** were performed. To determine these parameters, the solutions of the compounds in chloroform were added dropwise (62 μ L) using a Hamilton microsyringe on to the subphase surface. After that, for the evaporation of the solvent, the solution waited 2–3 min. The π -A isotherms were obtained with a compression rate of 50 cm² min⁻¹ until

Scheme 1. The Cl replacement reaction pathway of monoferrocenyl-cyclotriphosphazenes.

the given surface pressure value was reached. The Wilhelmy Method was used for the measure. The estimated standard deviations were found to be $\pm 0.1 \text{ mN m}^{-1}$ for pressure and $\pm 0.1 \text{ cm}^2$ for the area. While the targeted pressure was reached, after approximately 17 min, the stable monolayer occurred. The monolayer was stable for 1.5 h. This stability or the surface equilibrium at the interface is very important because of the ordered film deposition. Otherwise, a little change of this parameter affects the whole deposition process. The LB films of **3** and **12** on the substrates were obtained with the thicknesses of four monolayers. The substrates were glass slide coated with a 50 nm film of thermally evaporated aluminium. The dipping rate was adjusted to 10 mm min^{-1} when the deposition was performed. The LB films were deposited by only upstroke at ambient temperature. Thus, all the transferred monolayers were Z-type.

2.4 X-ray crystallography

Suitable crystals of compounds (**3**, **6** and **12**) were obtained from acetonitrile at room temperature. Crystallographic data were recorded on a Bruker Kappa APEXII CCD area-detector diffractometer using Mo K α radiation ($\lambda = 0.71073 \text{ \AA}$) at $T = 173(2) \text{ K}$ (for **6**) and $T = 296(2) \text{ K}$ (for **3** and **12**). Absorption corrections by multi-scan³⁰ were applied. Structures were solved by direct methods and refined by full-matrix least squares against F^2 using all data.^{31,32} All non-H atoms were refined anisotropically. Aromatic, methylene and methyl H atoms were positioned geometrically at distances of 0.93 (aromatic CH), 0.97 (CH₂) and 0.96 (CH₃) from the parent C atoms; a riding model was used during the refinement process and the Uiso(H) values were constrained to be 1.2 Ueq (for aromatic and methylene carrier atoms) and 1.5 Ueq (for methyl carrier atoms). Crystallographic data of **6** are; Empirical formula : C₂₄H₃₈Cl₂FeN₇P₃, formula weight: 644.27, crystal system : monoclinic, space group : C2/c, a (Å) : 27.5472(5), b (Å) : 11.0280(3), c (Å) : 20.4061(4), α (°) : 90.00, β (°) : 90.00, γ (°) : 90.00, V (Å³) : 5965.4(2), Z : 8, μ

(cm⁻¹) : 0.873 (Mo K α), ρ (Calc.) (g cm⁻³) : 1.435, number of reflections total : 27883, number of reflections unique: 5278, R_{int} : 0.0253, $2\theta_{\text{max}}$ (°) : 50.06, $T_{\text{min}}/T_{\text{max}}$: 0.8765/0.9543, number of parameters : 335, R [$F^2 > 2\sigma(F^2)$] : 0.0519, wR : 0.1381.

2.5 Preparation of the compounds

The tetrachloro NN and NO spirocyclic monoferrocenyl pendant-armed spirocyclotriphosphazenes (**1–5**) were obtained from the reactions of monoferrocenylamines with trimer according to the methods reported in the literature.^{3c, 33} See Scheme 1 for structures.

2.5a Synthesis of 6: 0.31 mL of piperidine (3.10 mmol) in THF (25 mL) was added to **1** (0.850 g, 1.55 mmol) and triethylamine (0.87 mL) in dry THF (100 mL) with stirring and refluxing for 10 h. The reaction was followed on TLC silica gel plates using toluene-THF (7:1). After the solvent was evaporated, the product was purified by column chromatography with toluene-THF (7:1). Yield: 0.82 g (82%), M.p. 162 °C. *Anal.* Calc. for C₂₄H₃₈N₇FeP₃Cl₂: C, 44.74; H, 5.94; N, 15.22. Found: C, 45.01; H, 5.86; N, 15.34. APIES-MS (fragments were based on ³⁵Cl and ⁵⁶Fe, Ir %): m/z 644 ([MH]⁺, 100.0). FTIR (KBr, cm⁻¹): 3079 (C-H arom.), 2960, 2854 (C-H aliph.), 1187 (P=N), 553 (asymm.), 511 (symm.) (PCl). ¹H NMR (CDCl₃, ppm, numberings of protons are given in Scheme 1): δ 1.56 [m, 4H, NCH₂CH₂CH₂ (pip)], 1.62 [m, 8H, NCH₂CH₂ (pip)], 2.53 (d, 3H, ³J_{PH} = 12.0 Hz, CH₃), 2.98–3.18 (m, 2H, NCH₂), 2.98–3.18 [m, 8H, NCH₂ (pip)], 2.98–3.18 (m, 2H, CH₃NCH₂), 3.85 (dd, 1H, ³J_{PH} = 7.2 Hz, ²J_{HH} = 13.8 Hz, H5), 3.91 (dd, 1H, ³J_{PH} = 7.0 Hz, ²J_{HH} = 13.8 Hz, H5), 4.12 (5H, H4), 4.09 (2H, H3), 4.13 (2H, H2). ¹³C NMR (CDCl₃, ppm, numberings of carbons are given in Scheme 1): δ 24.81 [NCH₂CH₂CH₂ (pip)], 24.96 [NCH₂CH₂CH₂ (pip)], 26.13 [d, ³J_{PC} = 6.8 Hz, NCH₂CH₂ (pip)], 26.29 [d, ³J_{PC} = 6.1 Hz, NCH₂CH₂ (pip)], 31.43 (d, ²J_{PC} = 3.9 Hz, CH₃), 43.48 (d, ²J_{PC} = 13.0 Hz,

CH₃NCH₂), 44.31 (d, ²J_{PC} = 6.1 Hz, C5), 45.12 [NCH₂ (pip)], 45.24 [NCH₂ (pip)], 47.06 (d, ²J_{PC} = 12.2 Hz, NCH₂), 68.41 (C4), 68.25 (C3), 69.89 (C2), 83.72 (d, ³J_{PC} = 11.8 Hz, C1).

2.5b Synthesis of 7: For the synthesis, the procedure used for **6** was followed; using **2** (0.77 g, 1.37 mmol), piperidine (0.27 mL, 2.74 mmol) and triethylamine (0.77 mL). Yield: 0.76 g (84%), M.p. 154 °C. *Anal.* Calc. for C₂₅H₄₀N₇FeP₃Cl₂: C, 45.61; H, 6.12; N, 14.89. Found: C, 45.36; H, 6.43; N, 14.62. APIES-MS (fragments were based on ³⁵Cl and ⁵⁶Fe, Ir %): *m/z* 658 ([MH]⁺, 100.0). FTIR (KBr, cm⁻¹): 3078 (C-H arom.), 2933, 2850 (C-H aliph.), 1170 (P=N), 578 (asymm.) 518 (symm.) (PCl). ¹H NMR (CDCl₃, ppm, numberings of protons are given in Scheme 1): δ 1.15 (t, 3H, ³J_{HH} = 7.2 Hz, CH₃), 1.55 [m, 4H, NCH₂CH₂CH₂ (pip)], 1.63 [m, 8H, NCH₂CH₂ (pip)], 2.86–3.06 (m, 2H, NCH₂CH₃), 2.86–3.06 (m, 2H, NCH₂), 3.09 [m, 4H, NCH₂ (pip)], 3.18 [m, 4H, NCH₂ (pip)], 2.86–3.06 (m, 2H, ³J_{HH} = 7.2 Hz, C₂H₅NCH₂), 3.71 (dd, 1H, ³J_{PH} = 6.8 Hz, ²J_{HH} = 14.0 Hz, H5), 3.86 (dd, 1H, ³J_{PH} = 6.8 Hz, ²J_{HH} = 14.0 Hz, H5), 4.10 (5H, H4), 4.09 (1H, H3), 4.12 (1H, H3), 4.34 (2H, H2). ¹³C NMR (CDCl₃, ppm, numberings of carbons are given in Scheme 1): δ 13.81 (d, ³J_{PC} = 5.3 Hz, CH₃), 24.83 [NCH₂CH₂CH₂ (pip)], 24.98 [NCH₂CH₂CH₂ (pip)], 26.16 [d, ³J_{PC} = 6.8 Hz, NCH₂CH₂ (pip)], 26.32 [d, ³J_{PC} = 6.9 Hz, NCH₂CH₂ (pip)], 39.23 (d, ²J_{PC} = 4.6 Hz, NCH₂CH₃), 43.52 (d, ²J_{PC} = 13.0 Hz, NCH₂), 43.65 (d, ²J_{PC} = 12.9 Hz, C₂H₅NCH₂), 44.28 (d, ²J_{PC} = 6.1 Hz, C5), 45.28 [d, ²J_{PC} = 2.3 Hz, NCH₂ (pip)], 45.95 [d, ²J_{PC} = 2.5 Hz, NCH₂ (pip)], 68.41 (C4), 68.00 (C3), 68.38 (C3), 69.34 (C2), 69.94 (C2), 83.50 (d, ³J_{PC} = 9.2 Hz, C1).

2.5c Synthesis of 8: The work-up procedure was similar to that of compound **6**, using **3** (0.72 g, 1.28 mmol), piperidine (0.25 mL, 1.56 mmol) and triethylamine (0.44 mL). Yield: 0.66 g (78%), M.p. 101 °C. *Anal.* Calc. for C₂₅H₄₀N₇FeP₃Cl₂: C, 45.61; H, 6.12; N, 14.89. Found: C, 45.28; H, 6.33; N, 15.03. APIES-MS (fragments were based on ³⁵Cl and ⁵⁶Fe, Ir %): *m/z* 658 ([MH]⁺, 100.0). FTIR (KBr, cm⁻¹): 3089 (C-H arom.), 2956, 2879 (C-H aliph.), 1197 (P=N), 561 (asymm.) 512 (symm.) (PCl). ¹H NMR (CDCl₃, ppm, numberings of protons are given in Scheme 1): δ 1.53 [m, 4H, ³J_{HH} = 5.0 Hz, NCH₂CH₂CH₂ (pip)], 1.58 [m, 8H, ³J_{HH} = 5.7 Hz, NCH₂CH₂ (pip)], 1.66 [m, 2H, NCH₂CH₂(spiro)], 2.52 (d, 3H, ³J_{PH} = 12.4 Hz, CH₃), 2.89 (m, 2H, CH₃NCH₂), 2.92 (m, 2H, NCH₂), 3.12 [m, 4H, NCH₂ (pip)], 3.16 [m, 4H, NCH₂ (pip)], 3.74 (dd, 1H, ³J_{PH} = 5.8 Hz, ²J_{HH} = 13.6 Hz, H5), 3.88 (dd, 1H, ³J_{PH} = 6.0 Hz, ²J_{HH} = 13.6 Hz, H5), 4.13 (5H, H4), 4.10 (1H, H3), 4.22 (2H, H2). ¹³C NMR (CDCl₃, ppm, numberings of carbons are given in Scheme 1): δ 24.46 (NCH₂CH₂), 24.94 [NCH₂CH₂CH₂ (pip)], 25.06 [NCH₂CH₂CH₂ (pip)], 26.82 [d, ³J_{PC} = 9.1 Hz, NCH₂CH₂ (pip)], 26.94 [d, ³J_{PC} = 9.0 Hz, NCH₂CH₂ (pip)], 36.37 (CH₃), 44.95 (CH₃NCH₂), 45.42 [NCH₂ (pip)], 45.67 [NCH₂ (pip)], 46.09 (C5), 49.95 (NCH₂), 68.52 (C4), 68.39 (C3), 68.45 (C3), 69.88 (C2), 70.02 (C2), 84.93 (d, ³J_{PC} = 11.6 Hz, C1).

2.5d Synthesis of 9: The procedure used for **6** was followed; using **4** (0.85 g, 1.59 mmol), piperidine (0.31 mL, 3.18 mmol) and triethylamine (0.90 mL). Yield: 0.83 g (83%), M.p. 108 °C. *Anal.* Calc. for C₂₃H₃₅N₆OFeP₃Cl₂: C, 43.76; H, 5.59; N, 13.31. Found: C, 43.95; H, 5.67; N, 13.24. APIES-MS (fragments were based on ³⁵Cl and ⁵⁶Fe, Ir %): *m/z* 31 ([MH]⁺, 100.0). FTIR (KBr, cm⁻¹): 3079 (C-H arom.), 2982, 2853 (C-H aliph.), 1192 (P=N), 572 (asymm.), 528 (symm.) (PCl). ¹H NMR (CDCl₃, ppm, numberings of protons are given in Scheme 1): δ 1.54 [m, 4H, NCH₂CH₂CH₂ (pip)], 1.62 [m, 8H, NCH₂CH₂ (pip)], 2.86–3.06 (m, 2H, ³J_{HH} = 4.8 Hz, NCH₂), 3.04 [m, 4H, NCH₂ (pip)], 3.15 [m, 4H, NCH₂ (pip)], 3.75 (dd, 1H, ³J_{PH} = 7.2 Hz, ²J_{HH} = 13.4 Hz, H5), 3.88 (dd, 1H, ³J_{PH} = 6.8 Hz, ²J_{HH} = 13.4 Hz, H5), 4.11 (5H, H4), 4.13 (2H, H3), 4.20 (m, 2H, ³J_{PH} = 6.8 Hz, ³J_{HH} = 4.8 Hz, OCH₂), 4.33 (2H, H2). ¹³C NMR (CDCl₃, ppm, numberings of carbons are given in Scheme 1): δ 24.78 [NCH₂CH₂CH₂ (pip)], 24.93 [NCH₂CH₂CH₂ (pip)], 25.99 [d, ³J_{PC} = 6.5 Hz, NCH₂CH₂ (pip)], 26.25 [d, ³J_{PC} = 6.4 Hz, NCH₂CH₂ (pip)], 44.06 (d, ²J_{PC} = 5.7 Hz, C5), 45.51 [NCH₂ (pip)], 45.71 [NCH₂ (pip)], 45.56 (d, ²J_{PC} = 14.8 Hz, NCH₂), 64.29 (OCH₂), 68.67 (C4), 68.37 (C3), 68.56 (C3), 69.46 (C2), 69.93 (C2), 83.20 (d, ³J_{PC} = 10.3 Hz, C1).

2.5e Synthesis of 10: For the synthesis, the procedure used for **6** was followed; using **5** (0.65 g, 1.19 mmol), piperidine (0.23 mL, 2.38 mmol) and triethylamine (0.67 mL). The first product eluted was the mono-DASD-substituted derivative (**5a**). Yield: 0.61 g (79%), M.p. 162 °C. *Anal.* Calc. for C₂₄H₃₇N₆OFeP₃Cl₂: C, 44.67; H, 5.78; N, 13.02. Found: C, 44.89; H, 5.77; N, 12.85. APIES-MS (fragments were based on ³⁵Cl and ⁵⁶Fe, Ir %): *m/z* 644 ([M]⁺, 100.0). FTIR (KBr, cm⁻¹): 3092 (C-H arom.), 2952, 2844 (C-H aliph.), 1186 (P=N), 548 (asymm.), 521 (symm.) (PCl). ¹H NMR (CDCl₃, ppm, numberings of protons are given in Scheme 1): 1.54 [m, 4H, NCH₂CH₂CH₂ (pip)], 1.62 [m, 8H, NCH₂CH₂ (pip)], 1.93 [m, 2H, ³J_{HH} = 6.0 Hz, NCH₂CH₂ (spiro)], 2.92–3.22 (m, 2H, NCH₂), 2.92–3.22 [m, 8H, NCH₂ (pip)], 3.68 (dd, 1H, ³J_{PH} = 6.0 Hz, ²J_{HH} = 13.2 Hz, H5), 3.93 (dd, 1H, ³J_{PH} = 5.6 Hz, ²J_{HH} = 13.2 Hz, H5), 4.09 (5H, H4), 4.11 (1H, H3), 4.12 (1H, H3), 4.21 (1H, H2), 4.25 (1H, H2), 4.31 (m, 2H, ³J_{PH} = 6.8 Hz, ³J_{HH} = 6.0 Hz, OCH₂). ¹³C NMR (CDCl₃, ppm, numberings of carbons are given in Scheme 1): δ 24.83 [NCH₂CH₂CH₂ (pip)], 24.96 [NCH₂CH₂CH₂ (pip)], 26.01 [d, ³J_{PC} = 6.4 Hz, NCH₂CH₂ (pip)], 26.29 [d, ³J_{PC} = 7.1 Hz, NCH₂CH₂ (pip)], 26.09 (d, ³J_{PC} = 3.8 Hz, NCH₂CH₂), 45.04 [NCH₂ (pip)], 45.18 [NCH₂ (pip)], 45.06 (NCH₂), 47.24 (C5), 67.11 (d, ²J_{PC} = 7.1 Hz, OCH₂), 68.44 (C4), 68.12 (C3), 68.36 (C3), 69.82 (C2), 70.60 (C2), 82.59 (d, ³J_{PC} = 11.5 Hz, C1).

2.5f Synthesis of 11: A solution of piperidine (1.45 mL, 14.63 mmol) in dry THF (25 mL) was added to a solution of **1** (1.00 g, 1.83 mmol) and triethylamine (0.72 mL) in dry THF (100 mL) with stirring and refluxing for 18 h. After the solvent was evaporated, the product was purified by column

chromatography with toluene-THF (2:1). The first product eluted was the geminal-piperidino-substituted derivative (**6**). Yield: 0.25 g (21%). The second product was the tetrakis-piperidino-substituted derivative (**11**). Yield: 0.96 g (71%), M.p. 160 °C. *Anal.* Calc. for $C_{34}H_{58}N_9FeP_3$: C, 55.06; H, 7.88; N, 17.00. Found: C, 54.75; H, 7.51; N, 17.36. APIES-MS (fragments were based on ^{56}Fe , Ir %): m/z 742 ($[MH]^+$, 100.0). FTIR (KBr, cm^{-1}): 3072 (C-H arom.), 2929, 2848 (C-H aliph.), 1195 (P=N). 1H NMR ($CDCl_3$, ppm, numberings of protons are given in Scheme 1): δ 1.51 [m, 8H, $NCH_2CH_2CH_2$ (pip)], 1.58 [m, 16H, NCH_2CH_2 (pip)], 2.51 (d, 3H, $^3J_{PH} = 11.2$ Hz, CH_3), 2.93 (m, 2H, $^3J_{PH} = 10.4$ Hz, $^3J_{HH} = 6.4$ Hz, CH_3NCH_2), 3.09 (m, 2H, $^3J_{PH} = 10.4$ Hz, $^3J_{HH} = 6.4$ Hz, NCH_2), 3.07 [m, 8H, NCH_2 (pip)], 3.16 [m, 8H, NCH_2 (pip)], 3.72 (d, 2H, $^3J_{PH} = 5.2$ Hz, H5), 4.09 (m, 5H, $^4J_{HH} = 1.8$ Hz, $^4J_{HH} = 1.8$ Hz, H4), 4.06 (m, 2H, $^3J_{HH} = 3.6$ Hz, $^4J_{HH} = 1.8$ Hz, H3), 4.15 (m, 2H, $^3J_{HH} = 3.6$ Hz, $^4J_{HH} = 1.8$ Hz, H2). ^{13}C NMR ($CDCl_3$, ppm, numberings of carbons are given in Scheme 1): δ 25.33 [$NCH_2CH_2CH_2$ (pip)], 25.47 [$NCH_2CH_2CH_2$ (pip)], 26.69 [d, $^3J_{PC} = 8.4$ Hz, NCH_2CH_2 (pip)], 26.77 [d, $^3J_{PC} = 6.7$ Hz, NCH_2CH_2 (pip)], 32.38 (d, $^2J_{PC} = 3.8$ Hz, CH_3), 44.35 (d, $^2J_{PC} = 10.7$ Hz, CH_3NCH_2), 45.62 [NCH_2 (pip)], 45.74 [NCH_2 (pip)], 45.95 (d, $^2J_{PC} = 4.5$ Hz, C5), 47.29 (d, $^2J_{PC} = 12.0$ Hz, NCH_2), 68.52 (C4), 68.10 (C3), 69.84 (C2), 85.03 (d, $^3J_{PC} = 12.2$ Hz, C1).

2.5g Synthesis of 12: For the preparation of **12** the procedure used for **11** was followed; using **2** (0.70 g, 1.25 mmol), piperidine (0.99 mL, 10.00 mmol) and triethylamine (1.41 mL). The first product eluted was the geminal-piperidino-substituted derivative (**7**). Yield: 0.15 g (18%). The second product was the tetrakis-piperidino-substituted derivative (**12**). Yield: 0.71 g (75%), M.p. 186 °C. *Anal.* Calc. for $C_{35}H_{60}N_9FeP_3$: C, 55.63; H, 8.00; N, 16.68. Found: C, 55.63; H, 8.30; N, 16.45. APIES-MS (fragments were based on ^{56}Fe , Ir %): m/z 756 ($[M]^+$, 100.0). FTIR (KBr, cm^{-1}): 3087 (C-H arom.), 2954, 2840 (C-H aliph.), 1193 (P=N). 1H NMR ($CDCl_3$, ppm, numberings of protons are given in Scheme 1): δ 1.10 (t, 3H, $^3J_{HH} = 7.1$ Hz, CH_3), 1.51 [m, 8H, $NCH_2CH_2CH_2$ (pip)], 1.58 [m, 16H, NCH_2CH_2 (pip)], 2.86 (m, 2H, $^3J_{HH} = 7.1$ Hz, NCH_2CH_3), 2.93 (m, 2H, $^3J_{HH} = 6.4$ Hz, $^3J_{PH} = 10.0$ Hz, $C_2H_5NCH_2$), 3.00 (m, 2H, $^3J_{HH} = 6.42$ Hz, NCH_2), 3.06 [m, 8H, NCH_2 (pip)], 3.15 [m, 8H, NCH_2 (pip)], 3.73 (d, 2H, $^3J_{PH} = 4.8$ Hz, H5), 4.09 (5H, H4), 4.06 (1H, H3), 4.15 (2H, H2). ^{13}C NMR ($CDCl_3$, ppm, numberings of carbons are given in Scheme 1): δ 14.39 (d, $^3J_{PC} = 7.6$ Hz, CH_3), 25.32 [$NCH_2CH_2CH_2$ (pip)], 25.46 [$NCH_2CH_2CH_2$ (pip)], 26.67 [d, $^3J_{PC} = 6.6$ Hz, NCH_2CH_2 (pip)], 26.77 [d, $^3J_{PC} = 6.7$ Hz, NCH_2CH_2 (pip)], 39.88 (d, $^2J_{PC} = 5.0$ Hz, NCH_2CH_3), 43.70 (d, $^2J_{PC} = 12.2$ Hz, NCH_2), 44.38 (d, $^2J_{PC} = 11.5$ Hz, $C_2H_5NCH_2$), 45.92 (d, $^2J_{PC} = 5.8$ Hz, C5), 45.60 [NCH_2 (pip)], 45.77 [NCH_2 (pip)], 68.52 (C4), 68.09 (C3), 69.91 (C2), 84.99 (d, $^3J_{PC} = 12.9$ Hz, C1).

2.5h Synthesis of 13: The procedure used for **11** was followed; using **3** (0.70 g, 1.25 mmol), piperidine (0.99 mL, 10.00 mmol) and triethylamine (1.41 mL). The first product

eluted was the geminal-piperidino-substituted derivative (**8**). Yield: 0.16 g (16%). The second product was the tetrakis-piperidino-substituted derivative (**13**). Yield: 0.69 g (73%), M.p. 191 °C. *Anal.* Calc. for $C_{35}H_{60}N_9FeP_3$: C, 55.63; H, 8.00; N, 16.68. Found: C, 55.79; H, 8.09; N, 16.35. APIES-MS (fragments were based on ^{56}Fe , Ir %): m/z 756 ($[MH]^+$, 100.0). FTIR (KBr, cm^{-1}): 3087 (C-H arom.), 2929, 2819 (C-H aliph.), 1193 (P=N). 1H NMR ($CDCl_3$, ppm, numberings of protons are given in Scheme 1): δ 1.50 [m, 8H, $^3J_{HH} = 5.6$ Hz, $NCH_2CH_2CH_2$ (pip)], 1.55 [m, 16H, $^3J_{HH} = 5.6$ Hz, NCH_2CH_2 (pip)], 1.65 [m, 2H, NCH_2CH_2 (spiro)], 2.50 (d, 3H, $^3J_{PH} = 12.8$ Hz, CH_3), 2.93 (m, 2H, CH_3NCH_2), 2.97 (m, 2H, NCH_2), 3.10 [m, 8H, NCH_2 (pip)], 3.13 [m, 8H, NCH_2 (pip)], 3.77 (d, 2H, $^3J_{PH} = 5.6$ Hz, H5), 4.09 (m, 5H, $^4J_{HH} = 1.6$ Hz, $^4J_{HH} = 1.2$ Hz, H4), 4.10 (m, 1H, $^3J_{HH} = 2.0$ Hz, $^4J_{HH} = 1.6$ Hz, H3), 4.22 (m, 2H, $^3J_{HH} = 2.0$ Hz, $^4J_{HH} = 1.2$ Hz, H2). ^{13}C NMR ($CDCl_3$, ppm, numberings of carbons are given in Scheme 1): δ 24.33 (NCH_2CH_2), 25.14 [$NCH_2CH_2CH_2$ (pip)], 25.23 [$NCH_2CH_2CH_2$ (pip)], 26.48 [d, $^3J_{PC} = 9.2$ Hz, NCH_2CH_2 (pip)], 26.51 [d, $^3J_{PC} = 9.2$ Hz, NCH_2CH_2 (pip)], 36.46 (CH_3), 44.96 (CH_3NCH_2), 45.56 [NCH_2 (pip)], 45.59 [NCH_2 (pip)], 46.17 (C5), 50.88 (NCH_2), 68.29 (C4), 68.39 (C3), 70.14 (C2), 85.07 (d, $^3J_{PC} = 11.9$ Hz, C1).

2.5i Synthesis of 14: The work-up procedure was similar to that of compound **11**, using **4** (0.90 g, 1.69 mmol), piperidine (1.34 mL, 13.52 mmol) and triethylamine (1.90 mL). The first product eluted was the geminal-piperidino-substituted derivative (**9**). Yield: 0.17 g (16%). The second product was the tetrakis-piperidino-substituted derivative (**14**). Yield: 0.69 g (73%), M.p. 204 °C. *Anal.* Calc. for $C_{33}H_{55}N_8OFeP_3$: C, 54.40; H, 7.61; N, 15.38. Found: C, 54.59; H, 7.40; N, 15.07. APIES-MS (fragments were based on ^{56}Fe , Ir %): m/z 729 ($[MH]^+$, 100.0). FTIR (KBr, cm^{-1}): 3070 (C-H arom.), 2929, 2848 (C-H aliph.), 1191 (P=N). 1H NMR ($CDCl_3$, ppm, numberings of protons are given in Scheme 1): δ 1.51 [m, 8H, $NCH_2CH_2CH_2$ (pip)], 1.60 [m, 16H, NCH_2CH_2 (pip)], 3.02–3.12 (m, 2H, $^3J_{HH} = 6.4$ Hz, NCH_2), 3.18 [m, 16H, NCH_2 (pip)], 3.80 (d, 2H, $^3J_{PH} = 5.2$ Hz, H5), 4.09 (5H, H4), 4.08 (2H, H3), 4.15 (m, 2H, $^3J_{PH} = 9.6$ Hz, $^3J_{HH} = 6.4$ Hz, OCH_2), 4.20 (2H, H2). ^{13}C NMR ($CDCl_3$, ppm, numberings of carbons are given in Scheme 1): δ 25.09 [$NCH_2CH_2CH_2$ (pip)], 25.22 [$NCH_2CH_2CH_2$ (pip)], 26.30 [d, $^3J_{PC} = 6.8$ Hz, NCH_2CH_2 (pip)], 26.56 [d, $^3J_{PC} = 7.6$ Hz, NCH_2CH_2 (pip)], 45.23 (C5), 45.17 [NCH_2 (pip)], 45.49 [NCH_2 (pip)], 46.16 (d, $^2J_{PC} = 13.7$ Hz, NCH_2), 63.23 (OCH_2), 68.34 (C4), 68.05 (C3), 69.65 (C2), 84.36 (d, $^3J_{PC} = 11.5$ Hz, C1).

2.5j Synthesis of 15: The procedure used for **11** was followed; using **5** (0.75 g, 1.37 mmol), piperidine (1.08 mL, 10.95 mmol) and triethylamine (1.54 mL). The first product eluted was the geminal-piperidino-substituted derivative (**10**). Yield: 0.19 g (22%). The second product was the tetrakis-piperidino-substituted derivative (**15**). Yield: 0.71 g (70%), M.p. 218 °C. *Anal.* Calc. for $C_{34}H_{57}N_8OFeP_3$: C, 54.99; H, 7.74; N, 15.09. Found: C, 55.26; H, 7.59; N, 14.81. APIES-MS (fragments were based on ^{56}Fe , Ir %): m/z 743 ($[MH]^+$,

100.0). FTIR (KBr, cm^{-1}): 3089 (C-H arom.), 2954, 2844 (C-H aliph.), 1193 (P=N). ^1H NMR (CDCl_3 , ppm, numberings of protons are given in Scheme 1): 1.59 [m, 8H, $\text{NCH}_2\text{CH}_2\text{CH}_2$ (pip)], 1.58 [m, 16H, NCH_2CH_2 (pip)], 1.73 [m, 2H, $^3J_{\text{HH}} = 5.6$ Hz, $^3J_{\text{HH}} = 5.6$ Hz, NCH_2CH_2 (spiro)], 2.89–3.04 (m, 2H, $^3J_{\text{HH}} = 5.6$ Hz, NCH_2), 2.89–3.04 [m, 16H, NCH_2 (pip)], 3.73 (d, 2H, $^3J_{\text{PH}} = 6.4$ Hz, H5), 4.08 (5H, H4), 4.07 (1H, H3), 4.17 (m, 2H, $^3J_{\text{PH}} = 10.8$ Hz, $^3J_{\text{HH}} = 5.4$ Hz, OCH_2), 4.21 (1H, H2). ^{13}C NMR (CDCl_3 , ppm, numberings of carbons are given in Scheme 1): δ 25.16 [$\text{NCH}_2\text{CH}_2\text{CH}_2$ (pip)], 25.24 [$\text{NCH}_2\text{CH}_2\text{CH}_2$ (pip)], 26.33 [d, $^3J_{\text{PC}} = 7.7$ Hz, NCH_2CH_2 (pip)], 26.55 [d, $^3J_{\text{PC}} = 7.1$ Hz, NCH_2CH_2 (pip)], 26.25 (NCH_2CH_2), 45.23 [NCH_2 (pip)], 45.57 [NCH_2 (pip)], 45.39 (NCH_2), 47.78 (C5), 65.69 (d, $^2J_{\text{PC}} = 7.0$ Hz, OCH_2), 68.27 (C4), 68.91 (C3), 70.46 (C2), 83.96 (d, $^3J_{\text{PC}} = 14.1$ Hz, C1).

3. Results and Discussion

3.1 Syntheses

The tetrachloro N/N (**1–3**) and N/O (**4** and **5**) spirocycloctriphosphazenes are the fertile starting compounds for the preparation of partly and fully-substituted phosphazenes. They have four reactive Cl atoms and they can give the substitution reactions with different mono, bidentate and multidentate ligands. Thus, the reactions of one equimolar amount of the compounds **1–5** with two equimolar amounts of piperidine afford the geminal-piperidino-substituted cycloctriphosphazenes (**6–10**), regioselectively. The thin-layer chromatography (TLC) exhibits no other compounds in the

Figure 1. (a) ^1H -decoupled and (b) coupled ^{31}P NMR spectra of **9** and **14**.

Table 1. ^{31}P -NMR (decoupled) spectral data of the phosphazenes. [Chemical shifts (δ) reported in Hz]^a.

Compound	Spin System	δ (ppm)			$^2J_{\text{PP}}$ (Hz)
		PCl_2	$\text{P}(\text{NR})_2$ (spiro)	$\text{P}(\text{NR})_2$	
6	AMX	25.40(dd)	22.06(dd)	18.57(dd)	41.3; 43.7; 51.0
7	AMX	25.26(dd)	21.26(dd)	18.60(dd)	40.1; 43.7; 51.0
8	AMX	26.62(dd)	18.26(dd)	16.18(dd)	43.7; 48.6; 51.9
9	ABX	26.03(dd)	25.16(dd)	19.41(dd)	49.0; 50.4; 51.6
10	AMX	23.58(dd)	13.90(dd)	19.13(dd)	39.2; 45.4; 52.9
11	AX_2	—	27.08(t)	23.04(d)	40.1
12	AX_2	—	27.02(t)	23.04(d)	41.7
13	AX_2	—	22.97(t)	21.19(d)	36.4
14	AX_2	—	31.62(t)	23.26(d)	46.2
15	AX_2	—	20.12(t)	22.40(d)	43.8

^a ^{31}P NMR measurements in CDCl_3 solutions at 293 K.

reaction mixture. The reaction yields of the compounds **6–10** are found to be in the range of 78–84%. On the other hand, the reactions of one equimolar amount of the compounds **1–5** with excess piperidine give the geminal (**6–10**) and fully-piperidino-substituted cyclotriphosphazenes (**11–15**) (Scheme 1). The fully-substituted products (**11–15**) occur predominantly, and the reaction yields are calculated as 71, 75, 73, 73 and 70%, respectively. In these reactions, triethylamine, Et_3N , was used as an HCl acceptor. As an example, the coupled and decoupled ^{31}P NMR spectra of the reaction mixture of **4** with excess piperidine are depicted in Figure 1, showing that the geminal (**9**) and fully-piperidino-substituted ferrocenylphosphazenes (**14**) are present in the mixture.

Besides, each of the geminal products (**6–10**) contains one stereogenic P-centers and they ought to behave as the enantiomeric mixtures (R and S). Luckily, the structure of **6** is exactly solved by X-ray crystallography (see section 3.4), and its absolute configuration is found to be as S.

The microanalysis, FTIR, APIES-MS and NMR data are coherent with the proposed structures of the compounds. The mass spectra of **6, 7, 8, 9, 11, 13, 14** and **15** disclose the protonated molecular ion ($[\text{MH}]^+$) peaks, whereas the mass spectra of **10** and **12** exhibit the molecular ion peaks (M^+).

3.2 NMR and IR spectroscopy

The ^1H -decoupled ^{31}P NMR data of the new phosphazenes (**6–15**) are given in Table 1. The ^{31}P NMR spectra of **6, 7, 11, 12** and **14** are presented in Figure S1 (Supplementary Information). The spin systems of all the geminal (except **9**) and tetrakis-piperidino-substituted ferrocenylphosphazenes were designated as AMX and AX_2 , respectively. Besides, the compound **9** has an ABX spin system. The 12-line resonance pattern in the ^{31}P NMR spectrum of the geminal-substituted compounds (**6–10**) arise from the doublet of doublets for each phosphorus atom. As expected, the fully-substituted phosphazenes (**11–15**) have a triplet for one P(spiro) atom and a doublet for two other P atoms. The ^1H -coupled ^{31}P NMR spectra were also recorded in order to determine the geminal structures of the compounds **6–10**. The $\delta\text{P}(\text{spiro})$ -shifts of the geminal- and tetrakis-piperidino-substituted phosphazenes, bearing the six-membered spiro-rings, are smaller than those of the five-membered ones. Moreover, the average $^2J_{\text{PP}}$ coupling constant of **11–15** is 41.6 Hz.

Taking into account the coupling constants, chemical shifts and multiplicities; the ^{13}C and ^1H NMR signals of the new phosphazenes are definitely assigned, and the ^{13}C and ^1H NMR spectra of the two phosphazenes

Table 2. LB Film Parameters.

Compound	Spreading Solution Volume and Concentration	Target Pressure (mN/m)	Area per Molecule from Isotherm ($\text{\AA}^2$ / molecule)	Deposition Type and Deposition Cycles
3	62 μL ; 3.5 mg/mL	2.0	12.5	Z; 4
12	62 μL ; 3.6 mg/mL	19.0	13.5	Z; 6

were given in Figures S2 and S3 (Supplementary Information). The two piperidino-groups bonded to the same phosphorus atom show two groups of $\text{NCH}_2\text{CH}_2\text{CH}_2$, NCH_2CH_2 and NCH_2 (pip) signals with small separations in the ^{13}C NMR spectra. The methyl carbons, NCH_3 , of **6**, **8**, **11** and **13** were assigned between 31.43 and 36.46 Hz. The ferrocenyl carbon peaks, C1, C2, C3 and C4, were also determined in the range of 68.00–70.60 ppm. Additionally, the average $^2J_{\text{PC}}$ couplings between the NCH_2 spiro-carbon and the P atoms of the phosphazenes containing the five-membered spiro rings (**6**, **7**, **9**, **11**, **12** and **14**) is 13.0 Hz. The $^3J_{\text{PC}}$ values between the ipso-C (C1) and P atoms are observed for all the phosphazene derivatives, and the average $^3J_{\text{PC}}$ values of the geminal and tetrakis-compounds are calculated as 10.9 Hz and 12.5 Hz, respectively.

The ^1H NMR spectra of **6–10** are highly complex because of the diastereotopic protons. The chemical shifts of NCH_3 protons are assigned between 2.50 ppm and 2.53 ppm, and the average $^3J_{\text{PH}}$ value of all the ferrocenylphosphazenes is calculated as 11.6 Hz. The H5 protons of the fully-substituted- phosphazenes (**11–15**) are determined at *ca.* 3.75 ppm as doublets. The average $^3J_{\text{PH5}}$ value of all the phosphazenes is 6.2 Hz.

3.3 The Interpretations of FTIR, GAIR and HATR Spectra of LB films of **3** and **12**

The determination of the LB film deposition parameters given in Table 2 is very crucial. The area per molecule on the subphase may reveal information on the conformation adopted by the molecules. The values of the area of **3** and **12** were determined, extrapolating the most steeply rising part of the π –A isotherms to the zero pressure.

The π –A isotherm of **12** is depicted in Figure 2, as an example. The best value of the deposition pressure of **12** is found to be 16 mN/m. Figure 3 illustrates the reduced area of the monolayer during the deposition of the LB films of **12** in the ranges of one and four layers during the four monolayers transferring. It was understood from the surface pressure–time (π –t) and area–time (A–t) curves of **12** (Figure 4) that the monolayers of the molecules were considerably stable.

Figure 2. The surface pressure – area (π – A) isotherm of **12**. **Figure 3.** The reduced area of the monolayers during the deposition of the LB films of **12** between one and four layers, while four monolayers were transferring on to the substrate.

The transfer ratio (TR) is the best measure of transferring a Langmuir–Blodgett film to a substrate. This ratio is given by the following formula:

$$\text{TR} = (\text{Area of monolayer removed from subphase at constant pressure}) / (\text{Area of substrate immersed in water})$$

The TR ought to be measured for each substrate pass through the air–water interface. Nevertheless, it must be kept in mind that the measured transfer rate is an

Figure 4. The π - t and A - t curves of monolayers of **12**.

aggregate value over the entire immersed surface and ought to be interpreted most of the time. For instance, if there is 100% deposition on one side of the substrate and the film 100% is stripped from the other side, it is possible to measure a TR of 0%.

The monolayers area of **12** removed from subphase at constant pressure (same for 4 layer) ≈ 40 – 42 cm²

The area of substrate [superforest (Cole-Parmer) 75 x 25 x 1 mm³] immersed in water = 7.5 cm x 2.5 cm x 2 (two side immersed) = 37.5 cm²

For good deposition, TR should be 0.95–1.05. In compound **12**, TR is calculated as 1.06 and greater than 1.00 because molecular stability changes when substrate immersed the subphase. This is nearly the same for all deposition cycle.

On the other hand, the deposition type (Z) and deposition cycle values of **3** and **12** given in Table 2 are comparable to those of mono- and gem-DASD-substituted phosphazene derivatives.^{25b} The deposition type of four phosphazene derivatives is Z, whereas the deposition cycles of the partly-substituted phosphazenes and the fully-substituted one (**12**) are found to be as 4 and 6, respectively.

The FTIR spectra of the piperidinocyclotriphosphazenes were recorded on KBr pellet. The GATR and HATR spectra of the LB ultrathin films of **3** and **12** were obtained. Figures 5, 6 and 7, respectively, illustrate the FTIR, p-polarized GATR and HATR spectra of **12**, as examples. In Table 3, the characteristic bands of **12** were given, and the characteristic p-polarized GATR and HATR spectral data of **3** and **12** were also listed in Tables 4 and 5.

The asymmetric methyl [$\nu(\text{CH}_3)$] and methylene [$\nu(\text{CH}_2)$] stretching bands of **3** and **12** emerge at *ca.* 2963 and 2928 cm⁻¹ in the FTIR spectra, respectively. In-plane bending frequencies of $\delta(\text{CH}_2)$ and $\delta(\text{CH}_3)$ are observed in the ranges of 1469–1329 cm⁻¹ for **3**

Figure 5. The infrared spectrum of **12** in a KBr pellet by transmission.

Figure 6. p-Polarized GATR spectra of LB film of **12**.

Figure 7. HATR spectra of LB film of **12**.

and 1448–1377 cm⁻¹ for **12**. The characteristic $\nu(\text{P}=\text{N})$ strong bands of the phosphazene rings are assigned at 1232 cm⁻¹, 1170 cm⁻¹ and 860 cm⁻¹ (for **3**) and

Table 3. The characteristic vibrational wavenumbers (cm^{-1}) of FTIR, GAIR and HATR spectra of **12**.

KBr Pellet (Solid Sample) (cm^{-1})	GAIR of LB Films (cm^{-1})	HATR of LB Films (cm^{-1})	Assignments
2963s	2964m	2963m	$\nu_a(\text{CH}_3)$
2928s	2930m	2927m	$\nu_a(\text{CH}_2)$
2846s	2849m	2849m	$\nu_s(\text{CH}_2)$
1448s	1450w	1448m	$\delta(\text{CH}_3), \delta(\text{CH}_2)$
1377s	1377w	1377m	$\delta(\text{CH}_3), \delta(\text{CH}_2)$
1200s,b	1201s	1187s	$\nu(\text{P}=\text{N}), (\text{CH})_{\text{ring}}, (\text{CC})_{\text{ring}}$
1156–1027s	1159–1028m	1154–1022s	$(\text{CH})_{\text{ring}}, (\text{CC})_{\text{ring}}$
861s	864w	861m	$\nu(\text{P}=\text{N})$

ν , stretching; δ , in-plane bending; b, broad; w, weak; m, medium; s, strong; Subscript: a, asymmetric; s, symmetric.

Table 4. p-Polarized GAIR Bands for LB Films of **3** and **12**.

Compound	IR Bands Belongs Rings (cm^{-1})	Methyl and Methylene Groups (cm^{-1})						IR Bands for Phosphazene Ring (cm^{-1})	
		(CH) ve (CC)	$\nu_a(\text{CH}_3)$	$\nu_a(\text{CH}_2)$	$\nu_s(\text{CH}_3)$	$\nu_s(\text{CH}_2)$	$\delta(\text{CH}_2)$ ve $\delta(\text{CH}_3)$	$\nu(\text{P}=\text{N})$	$\nu_a(\text{P-Cl})$
3	1180–1003m	2963m	2923w	–	2855w	1406, 1351w	1236, 1180s, 858w	–	–
12	1201–1028m	2964m	2930m	–	2849m	1450, 1377w	1201s, 864w	–	–

ν , stretching; δ , in-plane bending; b, broad; w, weak; m, medium; s, strong; Subscript: a, asymmetric; s, symmetric.

Table 5. HATR Bands for LB Films of **3** and **12**.

Compound	IR Bands Belongs Rings (cm^{-1})	Methyl and Methylene Groups (cm^{-1})						IR Bands for Phosphazene Ring (cm^{-1})	
		(CH) ve (CC)	$\nu_a(\text{CH}_3)$	$\nu_a(\text{CH}_2)$	$\nu_s(\text{CH}_3)$	$\nu_s(\text{CH}_2)$	$\delta(\text{CH}_2)$ ve $\delta(\text{CH}_3)$	$\nu(\text{P}=\text{N})$	$\nu_a(\text{P-Cl})$
3	1179–1003s	2958w	2921w	–	2850sh	–	1234, 1179, 857m	657m	–
12	1187–1022s	2963m	2927m	–	2849m	1448, 1377m	1187s, 861m	–	–

ν , stretching; δ , in-plane bending; b, broad; w, weak; m, medium; s, strong; Subscript: a, asymmetric; s, symmetric.

1200 cm^{-1} and 861 cm^{-1} (for **12**). The $(\text{CC})_{\text{ring}}$ and $(\text{CH})_{\text{ring}}$ vibrations are found to be in the ranges of 1150–1062 cm^{-1} and 1156–1027 for **3** and **12**, respectively. The bands at ca. 660 cm^{-1} and 430 cm^{-1} in accordance with the published literature values,^{34,35} and they may be attributed to characteristic asymmetric and symmetric stretching frequencies of $\nu(\text{P-Cl})$, respectively.

The GAIR and HATR spectra are excellent data for investigating the orientation of ultrathin films at the molecular level.³⁶ The comparative p-polarized GAIR and HATR spectral data of **3** and **12** were listed in Tables 4 and 5, indicating the clear ‘coupling modes’ of $\nu(\text{P}=\text{N})$ stretching bands at both spectra. As expected, the asymmetric $\nu(\text{P-Cl})$ stretching band of **3** is observed at 657 cm^{-1} in the HATR spectrum, implying that the PCl_2 groups of LB films of **3** parallel oriented to the substrate surface. The $\nu(\text{P}=\text{N})$ bands disclose at 1236,

1234, 1180 and 1179 cm^{-1} ; 1201 and 1187 cm^{-1} for **3** and **12**, respectively. As seen in Tables 4 and 5, methyl [$\nu(\text{CH}_3)$] and methylene [$\nu(\text{CH}_2)$] modes are interpreted in the ranges of 2963–2850 cm^{-1} (for **3**) and 2964–2849 cm^{-1} (for **12**). Compared to GAIR and HATR intensities of $\nu(\text{CH}_3)$ and $\nu(\text{CH}_2)$ bands, both of the band modes are likely to be nearly parallel to the substrate surface for **12**. But, in **3**, these bands appear dominant on p-polarized GAIR spectrum, and they ought to be nearly parallel to the normal of the surface. The $\delta(\text{CH}_2)$ and $\delta(\text{CH}_3)$ modes were found to be at 1406 and 1351 cm^{-1} (for **3**), and 1450 and 1377 cm^{-1} (for **12**). Eventually, the spectral data of **3** and **12** indicate that both of the phosphazenes were deposited with the monolayers on the aluminium covered glass substrates as the Z deposited ultrathin LB films.

Figure 8. ORTEP-3⁴¹ drawing of **6** with the atom numbering scheme. Displacement ellipsoids are drawn at the 30% probability level.

3.4 X-Ray structure of the compound **6**

The molecular and crystal structure of **6** was established using X-ray crystallography. The ORTEP diagram of **6** is depicted in Figure 8 with the atom-numbering. The P1/N1/P2/N2/P3/N3 [Figure S4a; $\varphi_2 = 173.34(3.10)^\circ$, $\theta_2 = 44.66(2)^\circ$] phosphazene ring of **6** is nearly planar with total puckering amplitude Q_T of 0.068(0.002) Å.³⁷ The five-membered spiro-ring (P3/N4/C3/C2/N5) is also almost planar (Figure S4b, Supplementary Information). Compound **6** has one chiral P-center, and the chirality at P3 is S. The space group is C2/c. It is among the Sohncke space groups, implying that only one enantiomer of **6** is crystallized from the racemic mixture. The chiral and non-chiral compounds crystallized in the well-known Sohncke space groups are discussed in the literature.³⁸

The endocyclic N2–P3–N3 [113.18(15)°] and N1–P1–N3 [115.46(16)°] bond angles of **6** are narrow, whereas P1–N3–P3 [126.94(19)°] bond angle expands with respect to the corresponding values in the standard compound, N₃P₃Cl₆.³⁹ On the other hand, there are regular variations with the distances from P2: P2–N2 $\approx$ P2–N1 < P3–N3 $\approx$ P1–N3 < P3–N2 = P1–N1. The average endocyclic P–N bond length [1.588(3) Å] in the phosphazene ring is considerably shorter than the average exocyclic P–N bond length [1.636(3) Å]. Consequently, the bond lengths and bond angles values are in good agreement with the reported data in the literature.^{2,40}

3.5 Antimicrobial activity

In vitro antimicrobial activities of **6**, **7**, **9**, **10**, **11**, **12**, **14** and **15** were studied against eight different bacteria

and three fungi (Table S2, Supplementary Information). Ampicillin and chloramphenicol were used as standard antibacterial agents, Ketoconazole was used as an anti-fungal control. All the compounds have generally weak activities against the tested strain. However, the compound **11** has activity against all of the yeast strains. In addition, the compounds **9**, **11** and **15** exhibit activity as Ketoconazole against *C. albicans* ATCC 10231 at high concentration. Consequently, according to the results given in Table S2 (Supplementary Information), most of these phosphazenes show weak to mild inhibition against tested bacterial and fungal strain.

The findings obtained in this paper are comparable with the literature data. It was found that the pyrrolidino and 1,4-dioxo-8-azaspiro[4,5]decane(DASD)-substituted cyclotriphosphazenes bearing ferrocenyl spirocyclic pendant arms were found to be active against some bacteria and fungi.^{3a, 25b, 33,42} While, tetra-morpholinoferrocenylphosphazenes exhibited no effect against these bacteria.^{27b} Consequently, the antibacterial and anti-fungal activities seem to be considerably depending on the formation of hydrogen bonds with the substituents (pyrrolidino, morpholino, DASD and piperidino groups) bonded to the P-atoms and the active centers of bacterial cell components.

3.6 Interactions of DNA with the compounds **6**, **7**, **9**, **10**, **12**, **14** and **15**

The interactions of the phosphazenes **6**, **7**, **9**, **10**, **12**, **14** and **15** with supercoiled DNA were investigated using the agarose gel electrophoresis. Figure 9 shows the electrophoretic mobility of the closed circular form I and open circular form II bands for the incubated mixtures of plasmid DNA at various concentrations of the compounds. In the electrophoretograms Lane P applies to untreated plasmid DNA as a control and Lanes 1–4 apply to plasmid DNA incubated with the compounds ranging from 2500 μM to 312 μM. The presence of the smaller bands from form I and bigger bands from form II was observed in **9**, **10**, **12**, **14** and **15** in the concentration range tested. The decreasing intensities of form I were also observed in compound **12**, **14**, and **15**. Compounds **6** and **7** are found to be more damaging to plasmid DNA, causing DNA cleavage (existence of form III linear DNA in Lanes 1 and 2) and increased the mobilities and intensities of form II of the DNA. In addition, there is no form I DNA at the highest concentrations of **6** and **7**. As shown in Figure 9, at a concentration above 625 μM, the compound **6** shows the DNA nuclease activity which converts supercoiled form I DNA into the linear form III DNA. The nuclease activity does not occur when DNA incubated with either 625 μM or 312 μM of **6**. It

Figure 9. Gel electrophoretic mobility of plasmid DNA when incubated with various concentrations of **6**, **7**, **9**, **10**, **12**, **14** and **15** (P: untreated plasmid DNA, Lanes 1–4: DNA treated by compounds at the concentration as follows: 2500, 1250, 625 and 312 μ M). The top and the bottom bands correspond to Form II (open circular) and Form I (covalently closed circular) and form III (linear), respectively.

Figure 10. The electrophoretograms for *Bam*HI (P/B) and *Hind*III (P/H) digested mixtures of DNA after treatment with the compounds. Lane P applies to untreated plasmid DNA and the other lanes apply to *Bam*HI and *Hind*III digestion of untreated DNA.

is apparent that the compound **6** has begun to damage the DNA, causing it to lose its compact form and move slower through the gel and all of the plasmid DNA has been converted to the nicked form (Lane 4). Subsequent lanes begin to show a smear on the gel indicative of DNA double-strand cleavage. In the case of compound **7**, as shown in Figure 9, at all concentration tested, compound shows DNA nuclease activity which converts supercoiled form I DNA into the linear form III DNA. It is apparent that the compound **7** has strong DNA damage, causing it to lose its compact form and slow mobility through the gel and all of the plasmid DNA has been converted to the nicked form (Lanes 3 and 4). Subsequent lanes also exhibit a smear on the gel indicative of DNA double-strand cleavage (Lanes 1 and 2). Similar results were found to be for the analogous DASD-substituted ferrocenylphosphazene derivatives.^{3a} As known, the mobility and conformational changes may exhibit the binding activity of the compounds with hydrogen bonding and/or intercalation through the active sites of the DNA.

3.7 Restriction digest analysis

In order to assess the compounds **6**, **7**, **9**, **10**, **12**, **14** and **15** showing affinity toward guanine-guanine (GG)

and/or adenine-adenine (AA) regions, the restriction endonuclease analyses of the DNA-compound incubated mixtures by *Hind*III and *Bam*HI enzymes for 1 h at the 37 °C are carried out. When plasmid DNA was digested with *Bam*HI or *Hind*III only one band corresponding to linear (form III) band was formed, whereas in the undigested plasmid DNA generally two bands corresponding to supercoiled and partially nicked form I and form II were observed. When plasmid DNA interacted with the compounds **6**, **7**, **9**, **10**, **12**, **14** and **15** followed by *Bam*HI digestion, two bands corresponding to forms I and II were observed at all the compounds.

*Bam*HI and *Hind*III enzymes bind the specific 5'-A/AGCTT-3', 5'-G/GATCC-3' sequences and cleave the adenine and guanine sites, respectively, as a result, Form I and Form II converts to a linear form of DNA (Form III) (Figure 10, PB). Eventually, the results reveal that the enzymes do not cleave the DNA for all the compounds indicating these phosphazenes binding to G/G and A/A nucleotides

4. Conclusions

In this paper, the partly (**6–10**) and fully-substituted (**11–15**) ferrocenyl pendant-armed

spirocyclotriposphazenes were prepared by the reactions of the unsymmetrical N/N (**1–3**) and N/O (**4** and **5**) spirocyclotriposphazenes with piperidine. It is an important result that the formation of geminal-products occurs only when the partly-substituted phosphazene compounds containing bulky ferrocenyl-pendant-armed group are reacted with the two equimolar amounts of piperidine. Despite all the efforts made in this study, the theoretical expected non-geminal products (cis and/or trans) could not be obtained. In addition, the fully-substituted phosphazenes (**11–15**) obtained in this paper ought to be strong phosphazene-bases as well. In the future studies, the phosphazene-bases can give the protic molten salts (PMOS) with inorganic and organic acids, and they can be used as phosphazanium base catalysts and ionic liquids. The preparations and the characterizations using GAIR and HATR techniques of ultrathin and highly ordered Langmuir–Blodgett films of **3** and **12** are the most important part of this paper. As known, the phosphazene thin films are likely to be useful components in electronic and optical devices. In addition, DNA and the phosphazenes were analyzed by agarose gel electrophoresis which illustrated the ability of phosphazenes to break double-stranded DNA.

Supplementary Information (SI)

Crystallographic data for the structure reported herein have been deposited with the Cambridge Crystallographic Data Centre as Supporting Information, CCDC No. 1813168 (for **6**). Copies of the data can be obtained through application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK. (fax: +44 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk or at <http://www.ccdc.cam.ac.uk>). Listings of the ^{31}P , ^{13}C and ^1H NMR spectra of the some phosphazenes (Figures S1, S2 and S3), the ring conformations and packing diagram of **6** (Figure S4), selected the bond lengths (Å) and the angles (deg) for **6** (Table S1), the antimicrobial activities of **6,7,9,10, 11,12,14** and **15** (Table S2), antimicrobial activities (Section S1) and DNA-compound interactions (Section S2). Supplementary Information is available at www.ias.ac.in/chemsci.

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Compliance with ethical standards

Conflicts of interest

There are no conflicts of interest to declare.

References

- (a) Stewart F F 2015 *Organophosphorus Chemistry* Vol. 44 (Cambridge, UK: Royal Society of Chemistry) Ch. 9 p. 397; (b) Gleria M and De Jaeger R 2004 *Applicative Aspect of Cyclophosphazenes* (New York: Nova Science Publishers); (c) Allen C W 1991 Regio- and stereochemical control in substitution reactions of cyclophosphazenes *Chem. Rev.* **91** 119
- (a) Bilge Koçak S, Koçoğlu S, Okumuş A, Kılıç Z, Öztürk A, Hökelek T, Öner Y and Açıık L 2013 Syntheses, spectroscopic properties, crystal structures, biological activities, and DNA interactions of heterocyclic amine substituted spiro-ansa-spiro- and spiro-bino-spiro-phosphazenes *Inorg. Chim. Acta* **406** 160; (b) Okumuş A, Bilge S, Kılıç Z, Öztürk A, Hökelek T and Yılmaz F 2010 Phosphorus-nitrogen compounds. Part 20: Fully substituted spiro-cyclotriposphazenic lariat (PNP-pivot) ether derivatives *Spectrochim. Acta A* **76** 401; (c) Satish Kumar N and Kumara Swamy K C 2004 Synthesis and structures of unsymmetrical bis- and tris-cyclotriposphazenes *Polyhedron* **23** 979; (d) Kumaraswamy S, Vijjulatha M, Muthiah C, Kumara Swamy K C and Engelhardt U 1999 Synthesis, reactivity and structures of spirocyclic products derived from octachlorocyclo-tetraphosphazene: comparison with spirocyclic cyclotriposphazenes and linear phosphazenes *J. Chem. Soc., Dalton Trans.* 891; (e) Işıklıan M, Sayın L, Sonkaya Ö, Hökelek T, Türk M and Oğuztüzün S 2016 Synthesis, structural characterization, and cytotoxic activity of new spirocyclic octachlorocyclo-tetraphosphazenes *Phosphorus, Sulfur Silicon Relat. Elem.* **191** 1216
- (a) Asmafiliz N, Kılıç Z, Hökelek T, Açıık L, Koç L Y, Süzen Y and Öner Y 2013 Phosphorus–nitrogen compounds: Part 26. Syntheses, spectroscopic and structural investigations, biological and cytotoxic activities, and DNA interactions of mono and bisferrocenylspirocyclo-triposphazenes *Inorg. Chim. Acta* **400** 250; (b) Tümer Y, Koç L Y, Asmafiliz N, Kılıç Z, Hökelek T, Soltanzade H, Açıık L, Yola M L and Solak A O 2015 Phosphorus-nitrogen compounds: part 30. Syntheses and structural investigations, antimicrobial and cytotoxic activities and DNA interactions of vanillinato-substituted NN or NO spirocyclic monoferrocenyl cyclotriposphazenes *J. Biol. Inorg. Chem.* **20** 165; (c) Egemen G, Hayvalı M, Kılıç Z, Solak A O and Üstündağ Z 2010 Phosphorus-nitrogen compounds Part 17: The synthesis, spectral and electrochemical investigations of porphyrino-phosphazenes *J. Porphyrins Phthalocyanines* **14** 227; (d) Bešli S, Mutlu C, İbişoğlu H, Yüksel F and Allen C W 2015 Synthesis of a New Class of Fused Cyclo-tetraphosphazene Ring Systems *Inorg. Chem.* **54** 334; (e) Kılıç Z, Contractor S R and Shaw R A 1987 Phosphorus–nitrogen compounds. Part 50. Further studies on the formation of bicyclic cyclo-tetraphosphaz-atetraene derivatives 2023
- (a) Bešli S, Mutlu Balcı C, Uslu A and Allen C 2017 Newsynthetic approach to molecular rods using cyclophosphazene-based oligospiranes *Inorg. Chem.* **56** 9413; (b) Bilge S, Demiriz Ş, Okumuş A, Kılıç Z, Tercan B, Hökelek T and Büyükgüngör O 2006

- Phosphorus-nitrogen Compounds. Part 13. Syntheses, crystal structures, spectroscopic, stereogenic, and anisochronic properties of novel spiro-ansa-spiro-, spiro-bino-spiro-, and spiro-cryptaphosphazene derivatives *Inorg. Chem.* **45** 8755; (c) Muralidharan K and Elias A 2003 Preparation of the first examples of ansa-spiro substituted fluorophosphazenes and their structural studies: Analysis of C-H...F-P weak interactions in substituted fluorophosphazenes *J. Inorg. Chem.* **42** 7535; (c) Asmafiliz N, İltar E E, Kılıç Z, Hökelek T and Şahin E 2008 Phosphorus-nitrogen compounds: Part 15. Synthesis, anisochronism and the relationship between crystallographic and spectral data of monotopic spiro-cryptaphosphazenes *J. Chem. Sci.* **120** 363; (d) İbişoğlu H, Yenilmez Çiftçi G, Kılıç A, Tanrıverdi E, Ün İ, Dal H and Hökelek T 2009 Formation of novel spiro, spiroansa and dispiroansa derivatives of cyclotetraphosphazene from the reactions of polyfunctional amines with octachlorocyclotetraphosphazetetraene *J. Chem. Sci.* **121** 125
5. Çosut B, Hacivelioglu F, Durmuş M, Kılıç A and Yeşilot S 2009 The synthesis, thermal and photophysical properties of phenoxycyclotriphosphazene-substituted cyclic and polymeric phosphazenes *Polyhedron* **28** 2510
 6. (a) Allcock H R 2006 Recent developments in polyphosphazene materials science *Curr. Opin. Solid State Mater. Sci.* **10** 231; (b) Mark J E, Allcock H R and West R 2005 *Inorg. Polym.* (USA: Oxford University Press)
 7. (a) Steiner A, Zacchini S and Richards P I 2002 From neutral iminophosphoranes to multianionic phosphazenes. The coordination chemistry of imino-aza-P(V) ligands *Coord. Chem. Rev.* **227** 193; (b) Chandrasekhar V and Nagendran S 2001 Phosphazenes as scaffolds for the construction of multi-site coordination ligands *Chem. Soc. Rev.* **30** 193
 8. (a) Bhuvan Kumar N N and Kumara Swamy K C 2008 Single diastereomers of unsymmetrical tris-spirocyclic cyclotriphosphazenes based on 1, 1'-bi-2-naphthol—Synthesis and structures *Chirality* **20** 781; (b) Şahin Ün Ş 2014 Cyclotriphosphazenes having stereogenic phosphorus atoms: synthesis, characterization and their stereogenic properties *Polyhedron* **70** 148; (c) Asmafiliz N 2014 Syntheses of chiral phosphazenes with stereogenic centers: NMR behavior in the presence of a chiral solvating agent *Heteroatom Chem.* **25** 83; (d) Porwollik-Czomperlik I, Brandt K, Clayton T A, Davies D B, Eaton R J and Shaw R A 2002 Diastereoisomeric singly bridged cyclophosphazene-macrocyclic compounds *Inorg. Chem.* **41** 4944; (e) Elmas G, Okumuş A, Kılıç Z, Çam M, Açık L and Hökelek T 2018 Phosphorus-Nitrogen Compounds. Part 40. The syntheses of (4-fluorobenzyl) pendant armed cyclotetraphosphazene derivatives: spectroscopic, crystallographic and stereogenic properties, DNA interactions and antimicrobial activities *Inorg. Chim. Acta* **476** 110; (f) Elmas G, Okumuş A, Koç L Y, Soltanzade H, Kılıç Z, Hökelek T, Dal H, Açık L, Üstündağ Z, Dünder D and Yavuz M 2014 Phosphorus-nitrogen compounds. Part 29. Syntheses, crystal structures, spectroscopic and stereogenic properties, electrochemical investigations, antituberculosis, antimicrobial and cytotoxic activities and DNA interactions of ansa-spiro-ansa cyclotetraphosphazenes *Eur. J. Med. Chem.* **87** 662
 9. Uslu A and Yeşilot S 2015 Chiral configurations in cyclophosphazene chemistry *Coord. Chem. Rev.* **291** 28
 10. Beşli S, Allen C W, Mutlu Balcı C, Kara G, Yeşilot S and Uslu A 2017 Stereochemical aspects of cyclotriphosphazenes: Prochiral and pseudo-asymmetric phosphorus atoms *Polyhedron* **135** 49
 11. Allcock H R, Napierala M E, Cameron C G and O'Connor S J M 1996 Synthesis and characterization of ionically conducting alkoxy ether/alkoxy mixed-substituent poly(organophosphazenes) and their use as solid solvents for ionic conduction *Macromolecules* **29** 1951
 12. (a) Çil E, Tanyıldızı M A, Özen F, Boybay M, Arslan M and Görgülü O 2012 Synthesis, characterization, and biological-pharmacological evaluation of new phosphazenes bearing dioxybiphenyl and schiff base groups *Arch. Pharm. Chem. Life Sci.* **345** 476; (b) Tümer Y, Asmafiliz N, Zeyrek C T, Kılıç Z, Açık L, Çelik S P, Türk M, Çağdaş Tunalı B, Ünver H and Hökelek T 2018 Syntheses, spectroscopic and crystallographic characterizations of cis- and trans-dispirocyclic ferrocenylphosphazenes: molecular dockings, cytotoxic and antimicrobial activities *New J. Chem.* **42** 1740
 13. (a) Brandt K, Bartczak T J, Kruszynski R and Porwollik-Czomperlik I 2001 AIDS-related lymphoma screen results and molecular structure determination of a new crown ether bearing aziridinylcyclophosphazene, potentially capable of ion-regulated DNA cleavage action *Inorg. Chim. Acta* **322** 138; (b) Berberoğlu İ, Asmafiliz N, Kılıç Z, Hökelek T, Koç L Y, Açık L, Türk M, Soltanzade H and Dal H 2016 Phosphorus nitrogen compounds: Part 34. Syntheses, structural investigations, cytotoxic and biological activities of spiro-ansa-spiro and spiro-bino-spiro tetrameric phosphazene derivatives *Inorg. Chim. Acta* **446** 75; (c) Elmas G, Okumuş A, Sevinç P, Kılıç Z, Açık L, Atalan M, Türk M, Deniz G and Hökelek T 2017 Phosphorus-nitrogen compounds. Part 37. Syntheses and structural characterizations, biological activities of mono and bis(4-fluorobenzyl)spirocyclotetraphosphazenes *New J. Chem.* **41** 5818; (d) Elmas G, Okumuş A, Cemaloğlu R, Kılıç Z, Çelik S P, Açık L, Çağdaş Tunalı B, Türk M, Aytuna Çerçi N, Güzel R and Hökelek T 2017 Phosphorus-nitrogen compounds. part 38. Syntheses, characterizations, cytotoxic, antituberculosis and antimicrobial activities and DNA interactions of spirocyclotetraphosphazenes with bis-ferrocenyl pendant arms *J. Organomet. Chem.* **853** 93; (e) Koran K, Tekin Ç, Bıryan F, Tekin S, Sandal S and Görgülü A O 2017 Synthesis, structural and thermal characterizations, dielectric properties and in vitro cytotoxic activities of new 2,2,4,4-tetra (4'-oxy-substituted-chalcone)-6, 6-diphenylcyclotriphosphazene derivatives *Med. Chem. Res.* **26** 962
 14. Zhu J, Liu W, Chu R and Meng X 2007 Tribological properties of linear phosphazene oligomers as lubricants *Tribology Int.* **40** 10
 15. Singh A, Krogman N R, Sethurman S, Nair L S, Sturgeon J L, Brown P W, Laurencin C T and Allcock H R 2006 Effect of side group chemistry on

- the properties of biodegradable L-alanine cosubstituted polyphosphazenes *Biomacromolecules* **7** 914
16. Greish Y E, Bender J D, Lakshmi S, Brown P W, Allcock H R and Laurencin C T 2005 Low temperature formation of hydroxyapatite-poly(alkyl oxybenzoate)phosphazene composites for biomedical applications *Biomaterials* **26** 1
 17. Klein R, Welna D T, Weikel A, Allcock H R and Runt J 2007 Counterion effects on ion mobility and mobile ion concentration of doped polyphosphazene and polyphosphazene ionomers *Macromolecules* **40** 3990
 18. Yeşilot S, Çoşut B, Ardiç Alidağı H, Hacıvelioğlu F, Özpınar G A and Kılıç A 2014 Intramolecular excimer formation in hexakis(pyrenyloxy)cyclotriphosphazene: photophysical properties, crystal structure, and theoretical investigation *Dalton Trans.* **43** 3428
 19. Cao R and Diaz-Garcia A 2009 Coordination compounds built on metal surfaces. *Coord. Chem. Rev.* **253** 1262
 20. (a) Chandrasekhar V, Andavan G T S, Nagendran S, Krishnan V, Azhakar R and Butcher R J 2003 Cyclophosphazene hydrazides as scaffolds for multi-ferrocenyl assemblies: synthesis, structure, and electrochemistry *Organometallics* **22** 976; (b) Şenkuytu E and Tanrıverdi Eçik E 2017 Octa-BODIPY derivative dendrimeric cyclotetraphosphazenes; photophysical properties and fluorescent chemosensor for Co^{2+} ions *Spectrochim. Acta Part A* **173** 863
 21. Barlow S and Marder S R 2000 Electronic and optical properties of conjugated group 8 metallocene derivatives *Chem. Commun.* 155
 22. Barlow S, Bunting H E, Ringham C, Green J C, Bublit G U, Boxer S G, Perry J W and Marder S R 1999 Studies of the electronic structure of metallocene-based second-order nonlinear optical dyes *J. Am. Chem. Soc.* **121** 3715
 23. (a) Gill R, Batool S, Qureshi R and Khalid S S 2015 Nanofabrication of Block Copolymers of PDMS/Polyamide having Trichlorogermyl Pendant Using LbL Technique *J. Chem. Soc. Pak.* **37** 468; (b) Petty M C 1992 Possible applications for Langmuir-Blodgett films *Thin Solid Films* **210–211** 417
 24. (a) Swalen J D, Allara D L, Andrade J D, Chandross E A, Garoff S, Israelachvili J, McCarthy T J, Murray R, Pease R F, Rabolt J F, Wynne K J and Yu H 1987 Molecular monolayers and films. A panel report for the Materials Sciences Division of the Department of Energy *Langmuir* **3** 932; (b) Roberts G 1990 *Langmuir-Blodgett Films* (New York: Plenum Press)
 25. (a) Asmafiliz N, Kılıç Z, Civan M, Avcı O, Gönder L Y, Açıık L, Aydın B, Türk M and Hökelek T 2016 Phosphorus–nitrogen compounds. Part 36. Syntheses, Langmuir–Blodgett thin films and biological activities of spiro-bino-spiro trimeric phosphazenes *New J. Chem.* **40** 9609; (b) Asmafiliz N, Civan M, Özben A, Kılıç Z, Ramazanoğlu N, Açıık L and Hökelek T 2018 *Appl. Organomet. Chem.* **32** e4223
 26. (a) Muralidharan K, Reddy N D and Elias A J 2000 Syntheses of Novel Exo and Endo Isomers of Ansa-Substituted Fluorophosphazenes and Their Facile Transformations into Spiro Isomers in the Presence of Fluoride Ions *Inorg. Chem.* **39** 3988; (b) Myer C M and Allen C W 2002 N-(Ferrocenylmethyl)-N-methylaminocyclotriphosphazenes *Inorg. Chem.* **41** 60; (c) Sengupta S 2003 A hexaferrocenyl cluster based on a cyclotriphosphazene core: synthesis and electrochemistry *Polyhedron* **22** 1237; (d) Sengupta S 2003 A ferrocene dendrimer based on a cyclotriphosphazene core *Tetrahedron Lett.* **44** 7281
 27. (a) Okumuş A, Akbaş H, Kılıç Z, Koç L Y, Açıık L, Aydın B, Türk M, Hökelek T and Dal H 2016 Phosphorus–nitrogen compounds: part 33: in vitro cytotoxic and antimicrobial activities, DNA interactions, syntheses and structural investigations of new mono(nitrobenzyl)cyclotriphosphazenes *Res. Chem. Intermed.* **42** 4221; (b) Asmafiliz N, Kılıç Z, Öztürk A, Süzen Y, Hökelek T, Açıık L, Çelik Z B, Koç L Y, Yola M L and Üstündağ Z 2013 Phosphorus-Nitrogen Compounds: Part 25. Syntheses, spectroscopic, structural and electrochemical investigations, antimicrobial activities, and DNA interactions of ferrocenyldiaminocyclotriphosphazenes *Phosphorus Sulfur* **188** 1723; (c) Okumuş A, Elmas G, Kılıç Z, Ramazanoğlu N, Açıık L, Türk M and Akça G 2017 The reactions of $\text{N}_3\text{P}_3\text{Cl}_6$ with monodentate and bidentate ligands: the syntheses and structural characterizations, in vitro antimicrobial activities, and DNA interactions of 4-fluorobenzyl(N/O)spirocyclotriphosphazenes *Turk. J. Chem.* **41** 525; (d) Elmas G, Okumuş A, Kılıç Z, Çelik S P and Açıık L 2017 The Spectroscopic and Thermal Properties, Antimicrobial Activities and DNA Interactions of 4-(Fluorobenzyl)Spiro(N/O) Cyclotriphosphazanium Salts *J. Turk. Chem. Soc. Sect. A: Chem.* **4** 993
 28. Bruker program 1D WIN-NMR (release 6.0) and 2D WIN-NMR (release 6.1).
 29. (a) Blaudez D, Buffeteau T, Desbat B, Orrit M and Turlet J M 1992 Characterization of Langmuir-Blodgett monolayers using polarization modulated FTIR spectroscopy *Thin Solid Films* **210–211** 648; (b) Civan M, Topaçlı C, Topaçlı A, Gürol I and Ahsen V 2007 An infrared study on Langmuir–Blodgett films of 12,13-bis(hydroxyimino)-11,14-diazatetracosane *J. Mol. Struct.* **834–836** 482
 30. Bruker 2005 SADABS Bruker AXS Inc. Madison Wisconsin USA
 31. Sheldrick G M 1997 SHELXS-97 SHELXL-97 University of Göttingen: Göttingen Germany
 32. Sheldrick G M 2008 A short history of SHELX *Acta Crystallogr. Sect. A* **64** 112
 33. İlter E E, Asmafiliz N, Kılıç Z, Açıık L, Yavuz M, Bali E B, Solak A O, Büyükkaya F, Dal H and Hökelek T 2010 Phosphorus–nitrogen compounds: Part 19. Syntheses, structural and electrochemical investigations, biological activities, and DNA interactions of new spirocyclic monoferrocenylcyclotriphosphazenes *Polyhedron* **29** 2933
 34. Carriedo G A, Alonso F G, Gonzalez P A and Menendez J R 1998 Infrared and Raman spectra of the phosphazene high polymer $[\text{NP}(\text{O}_2\text{C}_{12}\text{H}_8)]_n$ *J. Raman Spectrosc.* **29** 327
 35. Workman Jr J 2000 *Handbook of Organic Compounds Methods and Interpretation* (Location: Academic Press) p.1
 36. (a) Roeges N P G 1994 *A Guide To The Complete Interpretation of Infrared Spectra of Organic Structure* (Chichester UK: John Wiley); (b) Bellamy L J 1975

- The Infrared Spectra of Complex Molecules* (New York: Chapman and Hall); (c) Mielczarski J A and Mielczarski E 1995 Determination of Molecular Orientation and Thickness of Self-Assembled Monolayers of Oleate on Apatite by FTIR Reflection Spectroscopy *J. Phys. Chem.* **99** 3206
37. Cremer D and Pople J A 1975 General definition of ring puckering coordinates *J. Am. Chem. Soc.* **97** 1354
38. Pidcock E 2005 Achiral molecules in non-centrosymmetric space groups *Chem. Commun.* **27** 3457
39. Bullen G J 1971 An improved determination of the crystal structure of hexachlorocyclotriphosphazene (phosphonitrilic chloride) *J. Chem. Soc. A* 1450
40. Tümer Y, Asmafiliz N, Kılıç Z, Hökelek T, Koç L Y, Açık L, Yola M L, Solak A O, Öner Y, Dünder D and Yavuz M 2013 Phosphorus–nitrogen compounds: Part 28. Syntheses, structural characterizations, antimicrobial and cytotoxic activities, and DNA interactions of new phosphazenes bearing vanillinato and pendant ferrocenyl groups *J. Mol. Struct.* **1049** 112
41. Farrugia L J 1997 ORTEP-3 for Windows – a version of ORTEP-III with a Graphical User Interface (GUI) *J. Appl. Crystallogr.* **30** 565
42. Asmafiliz N, Kılıç Z, Öztürk A, Hökelek T, Koç L Y, Açık L, Kısa Ö, Albay A, Üstündağ Z and Solak A O 2009 Phosphorus-nitrogen compounds. 18. Syntheses, stereogenic properties, structural and electrochemical investigations, biological activities, and DNA interactions of new spirocyclic mono- and bis-ferrocenylphosphazene derivatives *Inorg. Chem.* **48** 10102