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The reactions of hexachlorocyclotriphosphazene with piperidine, *N*-(1-naphthyl)ethylenediamine and 2-(2-hydroxyethylamino)ethanol. Antimicrobial activities of iminodiethoxy-substituted cyclotriphosphazene derivatives

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ABSTRACT

A series of cyclotriphosphazene derivatives (**5**–**16**) were synthesized from the reactions of hexachlorocyclotriphosphazene (**1**, $N_3P_3Cl_6$) with piperidine (**2**), *N*-(1-naphthyl)ethylenediamine (**3**) and 2-(2-hydroxyethylamino)ethanol (**4**). Of the synthesized compounds, 2-piperidino-2,4,4,6,6-pentachlorocyclotri phosphazatriene, $N_3P_3Cl_5[N\text{-}(C_5H_{10})]$ (**5**); 2,4-piperidino-2,4,6,6-tetrachlorocyclo triphosphazatriene, $N_3P_3Cl_4[N\text{-}(C_5H_{10})]_2$ (**6**); 2,4,6-piperidino-2,4,6-trichlorocyclo triphosphazatriene, $N_3P_3Cl_3[N\text{-}(C_5H_{10})]_3$ (**7**); 2,2,4,6-piperidino-4,6-dichlorocyclotri phosphazatriene, $N_3P_3Cl_2[N\text{-}(C_5H_{10})]_4$ (**8**); the 2,4,6,6-tetrachloro-2,4-*non-gem*-*N*-(1-naphthyl)ethylendiamino)-cyclotri phosphazatriene, $N_3P_3Cl_4[NH\text{–}(CH_2)_2\text{–}NH\text{–}(C_{10}H_7)]_2$ (**9**); and the 2,2,4,4,6,6-trispiro-2,2′-iminodiethoxy-cyclotriphosphazatriene, $N_3P_3[O\text{–}(CH_2)_2\text{–}NH\text{–}(CH_2)_2O]_3$ (**14**) derivatives are reported for the first time, others (**10**–**13**, **15** and **16**) were previously reported. The derived compounds (**5**–**16**) were structurally elucidated by elemental analysis and spectral data of ^1H and ^{31}P NMR and TLC-MS. Water-soluble hexachlorocyclotriphosphazene derivatives (**10–16**) were screened for their antimicrobial activities against three human pathogens; *Escherichia coli* W3110, *Staphylococcus aureus* ATCC 25923, and *Candida albicans* ATCC 10231 and compounds **10**, **12**, and **16** found to exhibit significant antimicrobial activities against the indicated microorganism.

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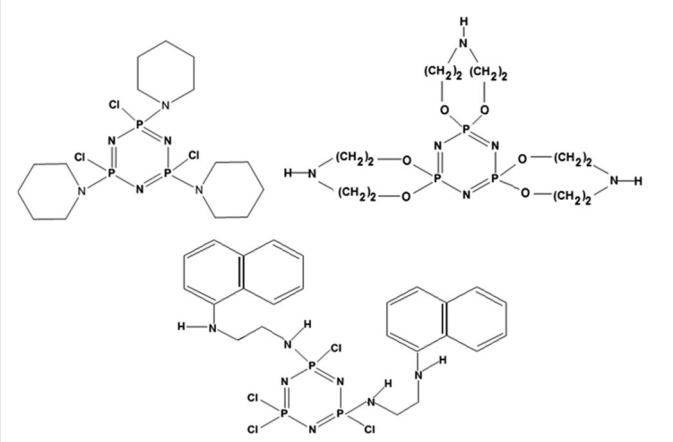
GRAPHICAL ABSTRACT



Introduction

Cyclophosphazenes are an important family of inorganic cyclic systems due to their wide range of applications in science and technology.[1] Since cyclophosphazenes have active chlorine atoms on the phosphorus atoms of the cyclic rings, they can form different types of cyclophosphazene derivatives by undergoing nucleophilic substitution reactions with wide variety of nucleophilic reagents and the compounds formed depending, on the anorganic, organic or organometallic groups, have also different properties.[1–36] Therefore, cyclophosphazenes have received increasing attention

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because of their numerous potential applications. For example, the biological activities of cyclophosphazene derivatives have been investigated by many research groups for the treatment of various diseases especially against cancer, microbial and various bacterial and fungal species.[5–19,21–24]

The antimicrobial and antifungal activities of different hexachlorocyclotriphosphazene derivatives on Gram (−), Gram (+) and eukaryotic organisms were extensively investigated. Where compounds bearing N/O–, 4-fluoro-benzyl, fluorobenzylspiro (N/O) and ferrocenyl groups were found to be effective on Gram (−), Gram (+) and eukaryotic organisms.[5,14,15,25,26]

In one study, on the antimicrobial activities of 4-fluorobenzyl spirocyclotriphosphazene derivatives with salicylic acid have been found that these compounds are more effective against eukaryotic organisms than Gram (−) and Gram (+) organisms.[23]

In another study, spermine containing cylotriphosphazene (**1**) complexes had no effect at all against these microorganisms.[21] Similarly, studies with ferrocenyldiaminocyclotriphosphazene complexes showed that not all compounds had antimicrobial activity against the mentioned bacteria and fungal strains.[16]

Moreover, the chemistry of amino- group containing cyclophosphazene derivatives such as pyrrolidine, aziridine, spermine, and spermidine side-groups have gained popularity due to their potential anti-carcinogenic properties.[2,13,21] They show cytotoxicity against HT-29, Hep2, and Vero cells and stimulated apoptosis.[13] As known, octapyrrolidinocyclotetraphosphazene and the Cu (II) complexes of fully phenoxy-substituted star-branched cyclotetraphosphazene derivatives[36] exhibit significant anticancer activities as well. In the present study, we wished to expand our research with the reactions of cyclotriphosphazene (**1**) with different nucleophilic reagents (**2–4**) and accordingly, we studied the structural characterization of the newly synthesized cyclotriphosphazene derivatives (**5**-**9** and **14**) and evaluated the antimicrobial activities of the previously reported 2,2′-iminodiethoxy-substituted cyclotriphosphazene derivatives (**10–16**, Scheme 1).[29] The effect of these compounds were tested against the three bacteria; (*E. coli* W3110 and *S. aureus* ATCC 25923) and a yeast strain (*Candida albicans* ATCC 10231) and all of the compounds have antimicrobial effect on bacterial and yeast strain within the range of 0.009–0.333 mg/mL of MIC values. It is found that compound **16** is the most effective agent against yeast strains (with a MIC value of 0.009 mg/mL). We can conclude that from the evaluated compounds; **10**, **12**, and **16** seem to be good candidates for being antimicrobial agents.

Results and discussion

Synthesis and structural characterization of the compounds

The reactions of hexachlorocyclotriphosphazene, $N_3P_3Cl_6$ (**1**), with piperidine (**2**), *N*-(1-naphthyl)ethylenediamine (**3**), and 2-(2-hydroxyethylamino)ethanol(**4**) resulted in the formation of the following novel (**5–9** and **14**) cyclotriphosphazene derivatives: 2-piperidino-2,4,4,6,6-pentacholorocyclotriphosphazatriene, $N_3P_3Cl_5[N\text{-}(C_5H_{10})]$ (**5**); *non-gem*-2,4-piperidino-2,4,6,6-tetrachlorocyclotriphosphazatriene, $N_3P_3Cl_4[N\text{-}(C_5H_{10})]_2$ (**6**); *non-gem*-2,4,6-piperidino-2,4,6-trichloro-cyclotriphosphazatriene, $N_3P_3Cl_3[N\text{-}(C_5H_{10})]_3$ (**7**); and the 2,2,4,6-piperidino-4,6-dichlorocyclotriphosphazatriene, $N_3P_3Cl_2[N\text{-}(C_5H_{10})]_4$ (**8**); 2,4,6,6-tetrachloro-2,4-*non-gem*-*N*-(1-naphthyl)ethylendiamino)-cyclotriphosphazatriene, $N_3P_3Cl_4[NH\text{–}(CH_2)_2\text{–}NH\text{–}(C_{10}H_7)]_2$ (**9**); and tri-spiro, 2,2,4,4,6,6-trispiro-2,2′-iminodiethoxy-cyclotriphosphazatriene, $N_3P_3[O\text{–}(CH_2)_2\text{–}NH\text{–}(CH_2)_2O]_3$ (**14**). Other compounds (**10–13**, **15**, and **16**) that we have synthesized have been previously reported by our research group.[29] Molecular structures of the synthesized compounds (**5–16**) were characterized by elemental analysis, TLC-MS, ^{1}H, ^{31}P-{^{1}H} and ^{31}P{^{1}H} NMR spectral data.

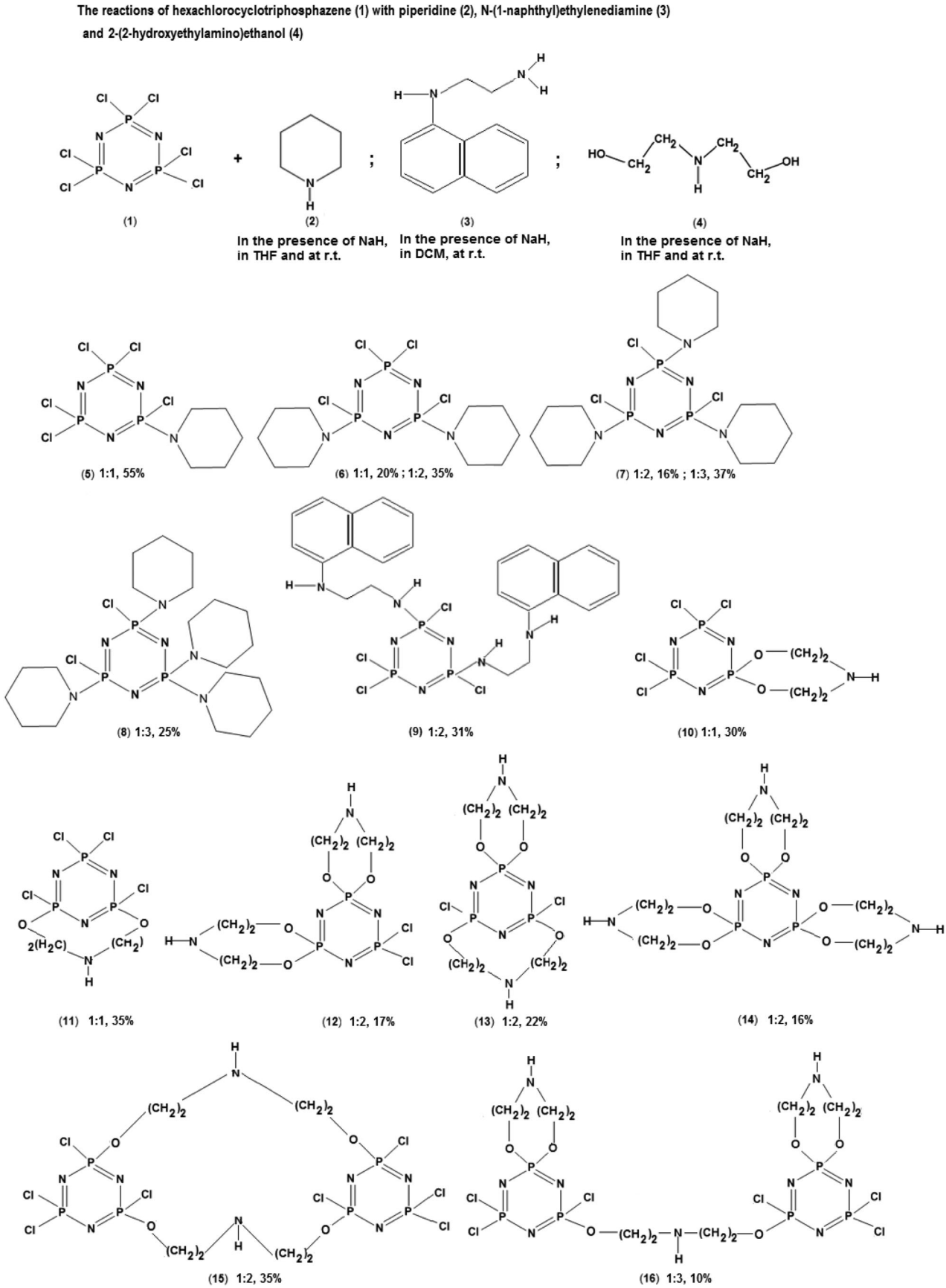
Compounds (**5–8**) are stable at room temperature. Although their solubility is high in common organic solvents such as chloroform, dichloromethane and THF, they have low solubility in water. The other compounds (**10–16**) have high solubility in water, but their stability in the laboratory environment (at r.t.) is limited to a few days.

Therefore, compounds **10–16** were selected to be screened for their antimicrobial activities against three human pathogens; *Escherichia coli* W3110, *Staphylococcus aureus* ATCC 25923, and *Candida albicans* ATCC 10231. The minimum inhibitory concentration (MIC) and the minimum cidal concentration (MCC) values of the above mentioned compounds were determined by using broth-agar microdilution technique. Compounds **10**, **12**, and **16** exhibit significant antimicrobial activities against the indicated microorganisms (Supplemental Materials Table S1). Structures of the derived compounds (**5–16**) are shown in Scheme 1. ^{1}H and ^{31}P NMR data are provided as part of the analytical data in the experimental section on synthesis.

In general, in the reaction of cyclotriphosphazene (**1**) with a shorter chain length reagent and at low temperature, nucleophilic substitution takes place on different phosphorus atoms and leads to open chain products. The substitution can be via the *geminal* or *non-geminal* route. If there is a *geminal* and *non-geminal* isomer distribution, one of them is dominant. If the nucleophilic power of the first bonding amine is high, the electron density on the phosphorus atom in the (PClR) group increases, resulting in a partial negative charge on the phosphorus and it becomes difficult for the second amine to bind to the same phosphorus atom. In this case, the *non-geminal* product is dominant or completely formed. Steric hindrance in bulky amines makes it difficult to bind to the same phosphorus atom and there is *non-geminal* substitution.

Although these features are a general trend, there are exceptions. For example, a bulky amine such as tertiary butylamine is expected to give *non-geminal* substitution while it gives *geminal* substitution[37a–c]. In addition, the formation of the hydrogen bond of the second amine with the amino group bound to the phosphazene makes geminal substitution more effective.

Cyclotriphosphazene (**1**) shows quite different behavior in reactions with ammonia and primary amines. While there



Scheme 1. Structures of the hexachlorocyclotriphosphazene derivatives (**5**–**16**). Compounds **5**–**9** and **14** are the newly synthesized derivatives in this system. Other compounds (**10**–**13**, **15**, and **16**) have recently been reported by our research group.[29]

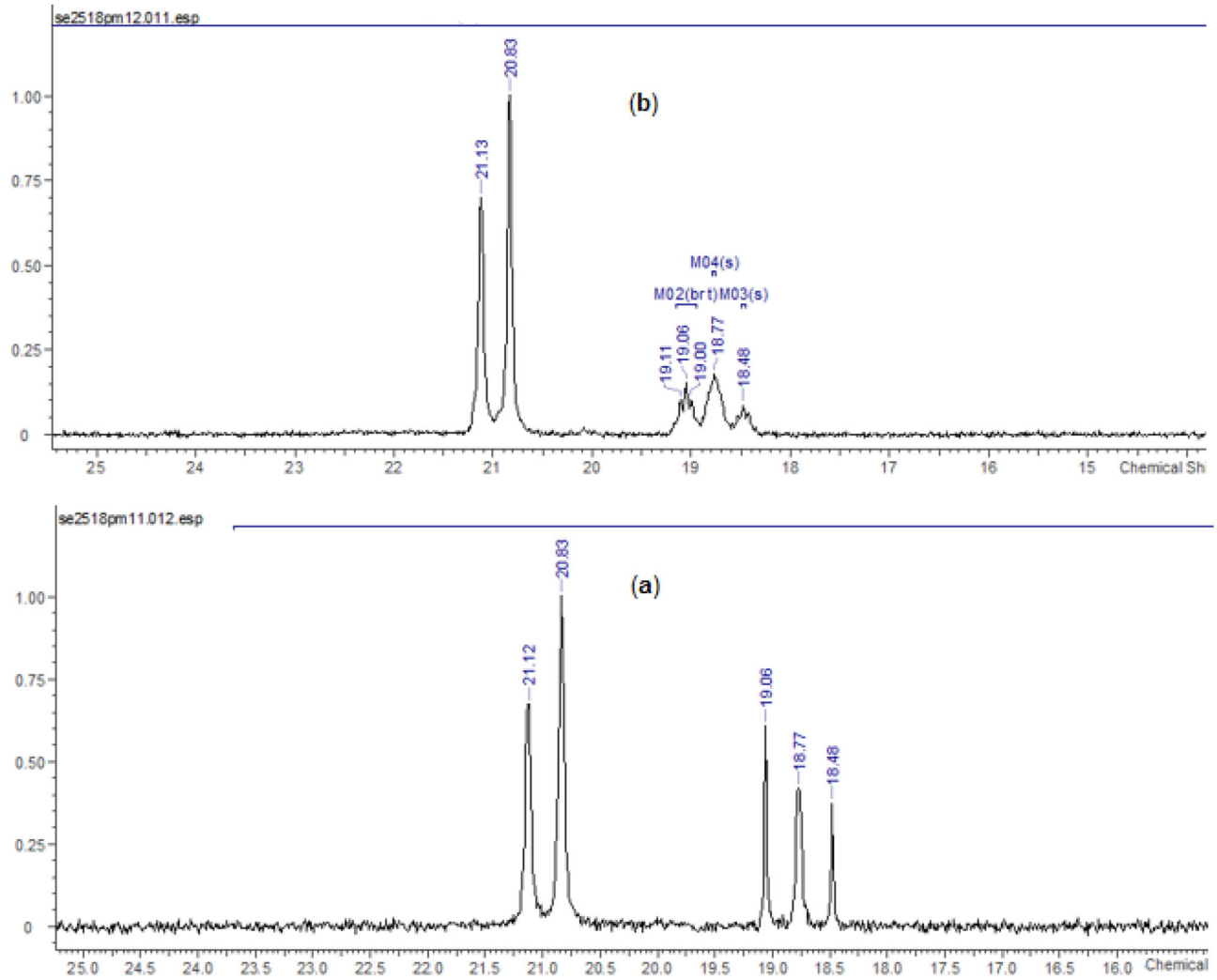


Figure 1. (a) $^{31}P\{-^{1}H\}$ decoupled and (b) $^{31}P\{^{1}H\}$ coupled NMR spectra of compound **5**, in $CDCl_3$ at 162.00 MHz, (room temperature), referenced to external 85% H_3PO_4.

is substitution in the *geminal* way in ammonia and primary amines, such as methylamine and ethylamine have a *non-geminal* substitution through the S_N2 mechanism and a shift from *non-geminal* to *geminal* substitution is observed as steric hindrance increases. In addition to steric effects, it has also been found that *geminal* isomers are formed in the reactions of (*β*-haloethyl)amines due to electronic effects[37a–c]. The reactions of secondary amines are much more harmonious than primary amines.

Piperidine (**2**) many secondary amines, such as dimethylamine, diethylamine, pyrrolidine, and piperidine give rise to *non-geminal* substitution. The substitution pathways in the reactions of cyclotriphosphazene (**1**) with and *N*-(1-naphthyl)ethylenediamine (**3**) are stereo and regio selective and therefore, we expect to have compound **9** in transformation.

^{31}p NMR spectra

Initially, the $^{31}P-\{^{1}H\}$ NMR spectra of the reaction mixtures were measured to determine which types of products are formed and their relative proportions. The assertions for the number of compounds supported at first by the ^{31}P NMR proton decoupled $^{31}P-\{^{1}H\}$ and coupled $^{31}P\{^{1}H\}$ NMR spectra. $^{31}P-\{^{1}H\}$ NMR spectra of hexachlorocyclotriphosphazene (**1**) derivatives provide examples of different types of a three-spin system as A_3, AB_2, AX_2 (or A_2B), AMX (ABC or AXX). As in compounds **5**, **6**, **8**, and **9**, most hexachlorocyclotriphosphazene (**1**) derivatives contain only two distinctly substituted phosphorus centers, and subsequently give rise to A_2B, AX_2, or AB_2 type spectra. However, examples of cylotriphosphazene derivatives exhibiting ABC, ABX, or AMX type spin system rare (as in the formation of compound **16**). The form of the spectra in conjunction with the chemical shifts, give extra proof for structural information, particularly for those with the ^{1}H NMR analysis are uninformative. As indicated above, the ^{31}P NMR spectra of compounds (**5**, **6**, **8**, and **9**) exhibit A_2B (or A_2X) and AB_2 spin systems, while that of the *non-gem* tri-open chain derivative (**7**) and *gem*-spiro derivative (**14**) are of A_3 types. $^{31}P\{^{1}H\}$ NMR coupling affects the B part (≡P(NR)Cl) of compound **5** (A_2B), A_2 parts of compound **6** (A_2X), and X_2 parts of compound **9** (AX_2), and both A and B_2 parts of compound **8** (AB_2), where each group split into further lines. Proton decoupled and coupled ^{31}P NMR spectra of compounds (**5, 8**, and **9**) are shown in Figures 1–3, respectively.

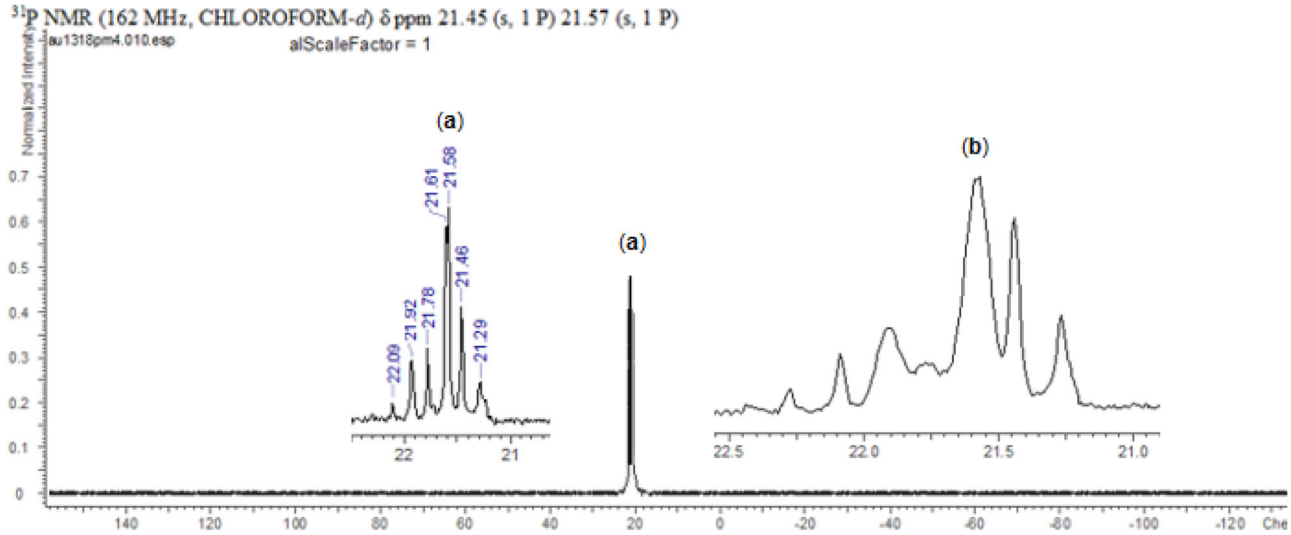


Figure 2. (a) ^{31}P $\{-^{1}H\}$ decoupled and (b) coupled NMR spectra of compound **8**, in $CDCl_3$ at 162.00 MHz, (room temperature), referenced to external 85% H_3PO_4.

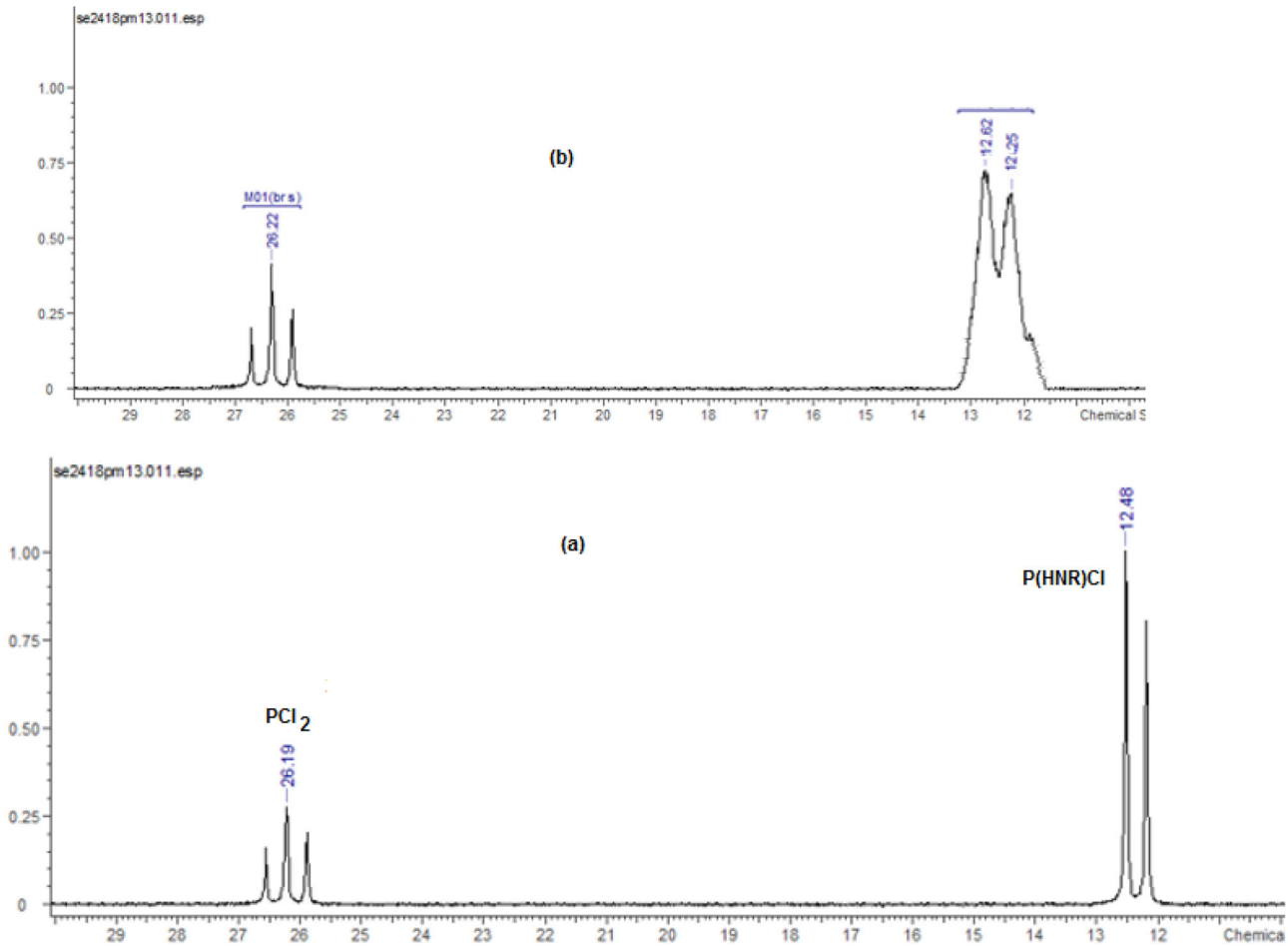


Figure 3. (a) $^{31}P\{-^{1}H\}$ decoupled and (b) $^{31}P\{^{1}H\}$ coupled NMR spectra of compound **9**, in $CDCl_3$ at 162.00 MHz, (room temperature), referenced to external 85% H_3PO_4.

Comprehensive ^{31}P NMR spectra are also presented for compounds **5** and **8** in Supplemental Materials in Figures **S1** and **S3** as well. Reaction mixture of piperidino-substituted cyclotriphosphazene derivatives may also be found in Supplemental Materials in Figure **S5**.

On the other hand, in compounds (**7** and **14**), the three phosphorus atoms are substituted with the ≡P(NR)Cl and the ≡$P(OR)_2$ moieties, respectively, and therefore all the phosphorus atoms are chemically and magnetically in the same environment and tri-open chain and tri-spiro moieties are symmetrically located in the molecule. Therefore, the ^{31}P NMR spectrum of this compound gave rise to a single line (A_3 type spin system). The $^{31}P\{-^{1}H\}$ NMR decoupled spectra of compounds **7** and **14** are presented in Supplemental

Table 1. Selected ^{31}P NMR parameters for compounds **5–16**.

Comp.	δPCl_2	[b]$\delta P(OR)_2$[b]	$\delta P(OR)Cl$[b]	$\delta P(HNR)Cl$[b]	$\delta P(NR)Cl$[b]	$\delta P(NR)_2$[b]	$^2JPCl_2$–P(NR)Cl[c]	$^2JP(NR)_2$–P(NR)Cl[c]
5	20.98				18.77		59.30	
6	22.16				17.98		55.76	
7					12.59			
8					21.37	21.73		48.57
(9)	26.32			12.26			50.45[d]	

Comp.	δPCl_2[b]	$\delta P(OR)_2$[b]	$\delta P(OR)Cl$[b]	$^2JPCl_2$-P(OR)$_2$[c]	$^2JPCl_2$-P(OR)Cl[c]	
(**10**)	22.40	14.60		68.70		[29]
(**11**)	25.80		16.50		76.30	[29]
(**12**)	28.10		13.20		62.70	[29]
(**13**)		15.40	28.20		83.40[e]	[29]
(**14**)		18.47				
(**15**)	27.50		20.30		64.30	[29]
(**16**)	29.40	48.80	1.60	[f]	[f]	[29]

[a]In $CDCl_3$ (with respect to 85% phosphoric acid external reference) at 162 and 202.38 MHz.
[b]In ppm.
[c]In Hz.
[d]$^2JPCl_2$–P(OR)$_2$.
[e]$^2JP(OR)_2$–P(OR)Cl.
[f]Unresolved spectrum.

Materials in Figures **S2** and **S4**, respectively. Selected ^{31}P NMR data for the newly synthesized compounds (**5–9** and **14**) are shown in Table 1 and the ^{31}P NMR data of the reported compounds (**10–13** and **16**) may be found in our previously reported study.[29]

^1H NMR data

The ^1H NMR cylotriphosphazene derivatives reveal valuable information regarding the positional and geometric disposition of the substituents. The *mono- non-gem di- and tri-, and tetra* piperidino-substituted cyclotriphosphazene derivatives (**5–8**) show very similar ^1H NMR spectra with close chemical shift values for the $\delta PNCH_2$ (in between 2.97 and 3.33 ppm) and the $\delta PNCCH_2$ (in between 1.40 and 155 ppm) groups. The ^1H NMR spectrum of compound **8** is shown in Figure 4.

The ^1H NMR spectrum of compound, 2,4,6,6-tetrachloro-2,4-*non-gem-N*-(1-naphthyl) ethylendiamino)cyclotriphosphazatriene (**9**) is very complex and the $\equiv PNCH_2$ and the $\equiv PNCCH_2$ signals are unresolved. However, the $\equiv PNCH_2$ and Ar–N–CH_2 protons of the open-chain moieties are obviously distinguishable from the $\equiv PN$–H and benzylic H–N–Ar protons. We observed a complex multiplet for the NCH_2 protons resonate at 3.32 ppm, while the $\equiv PN$–H protons gave rise to a doublet structure at 3.55 ppm. The two bond coupling constants ($^2J_{PH}$) for the $\equiv PNH$ protons observed at 9.5 Hz. Whilst the three bond coupling for NCH_2 protons are observed at 7.86 Hz., which is larger than the previously reported spiro compounds.[35]

The tri-spiro derivative (2,2,4,4,6,6-trispiro-2,2′-iminodiethoxy-cyclotriphosphazatriene), $N_3P_3[O–(CH_2)_2–NH–(CH_2)_2O]_3$ (**14**) gave rise to similar in appearance to those described for the reported mono-spiro derivative (**10**).[29] The methylene protons in each group are equivalent, and split by coupling with the adjacent methylene protons and with the phosphorus nucleus. The six lines of the $POCH_2$ protons and triplet lines of the $POCCH_2$ protons are well resolved, further splitting by the phosphorus nucleus and the N–H proton is observed as well. Selected ^1H NMR parameters for the newly synthesized compounds are summarized in Table 2. ^1H NMR spectra of compounds **5** and **6** are presented in Supplemental Materials in Figures S6 and S7, respectively. The ^1H NMR spectral data for the reported compounds (**10–13**, **15**, and **16**) may be found in our earlier reported studies.[29]

Antimicrobial screening of the synthesized compounds (10–16)

In this study, antimicrobial and antifungal activities of novel synthesized *gem* and *non-gem* hexachlorocyclotriphosphazene (**1**) derivatives (**10–16**) were investigated. All compounds have different antimicrobial effects on prokaryotic and eukaryotic organisms. Considering the MIC values of the derived compounds (**10–16**), the most effective compound is the single-bridged with spiro-substituted units (**16**), which has an effect on *C. albicans* (0.009 mg/mL), on *E. coli* (0.0014 mg/mL), and on *S. aureus* (0.0018 mg/mL). Studies on the antimicrobial activities of the cyclotriphosphazene derivatives showed that starting material ($N_3P_3Cl_6$, **1**) on different organisms has a MIC value of 0.013–0.054 mg/mL.[9] This result shows that all of the synthesized compounds, except for compound **16**, have lower MIC values against *E. coli*, *S. aureus*, and *C. albicans* which are less effective than the starting material $N_3P_3Cl_6$ (**1**). On the other hand, compound **16** have MIC values similar to the starting material $N_3P_3Cl_6$ (**1**). In addition, compounds **10** and **12** have similar MIC values on *S. aureus*, a Gr (+) organism and *C. albicans*, an eukaryotic organism, while on *E. coli*, a Gr (-) organism, have different MIC and MBC values. As seen in Table S1, the MIC value of the mono-spiro derivative (**10**) is about 0.208 mg/mL, while the MIC value of the mono-ansa compound (**12**) is 65 mg/mL. All of the screened compounds (**10–16**) were found to be more effective to eukaryotic organisms than prokaryotes. This result is also confirmed by some of the studies in the literature as well.[7–9,18] Both the MIC and MCC values of the

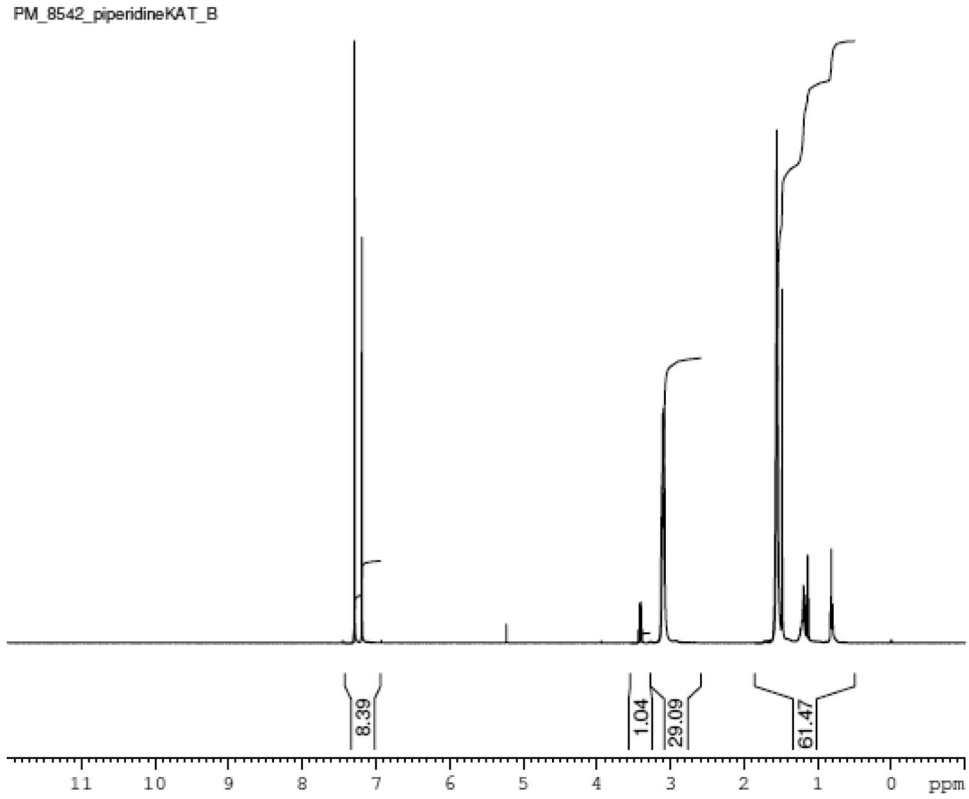


Figure 4. ^{1}H NMR spectrum of compound (**8**), at room temperature, in $CDCl_3$ (TMS as internal reference) and at 399.95 MHz.

Table 2. Selected ^{1}H NMR parameters for compounds **5**–**9**, and **14**.[a]

Comp.	δNCH_2[b]	$\delta NCCH_2$[b]				$^{3}J_{(PH)}$[c]	
(**5**)	3.30 (t)	1.55 (q)				10.10	
(**6**)	3.10 (t)	1.52 (q)				9.82	
(**7**)	2.98 (t)	1.46 (q)				9.40	
(**8**)	3.12 (t)	1.39 (overlapped struct.)				9.10	
(**9**)	δPNH[b]	$\delta ArNH$[b]	$\delta PNHCH_2$[b]	$\delta PNHCCH_2$[b]	$\delta Ar\text{-}H$[b]	$^{2}J_{(PH)}$[c]	$^{3}J_{(PH)}$[c]
	3.52 (d)	8.34	3.35	3.22/3.25	8.26	9.5	7.95
	$\delta POCH_2$[b]		$\delta PCCH_2$[b]		δNH[b]		
(**14**)	4.40		1.66		7.28		

^{1}H NMR data for compounds (**10–13, 15**, and **16**) may be found in our previously reported manuscript.[29]
[a]In $CDCl_3$ (referenced to internal TMS internal) at 399.95 MHz. (room temperature).
[b]In ppm.
[c]In Hz.

compounds (**10–16**) are presented in Supplemental Materials Table S1.

Experimental

Materials

All the chemicals and reagent grade solvents were used throughout the work, benzene, light petroleum (b.p. 40–60 °C), petroleum ether, anhydrous diethyl ether, acetonitrile, butanol, *n*-hexane (>96%), dichloromethane (>99.0%), chloroform, acetone, THF, and acetone were purchased through local suppliers (Sigma Aldrich).Solvents were purified by standard techniques and were freshly distilled prior to use. THF was distilled over a sodium-potassium alloy under an argon atmosphere. $CDCl_3$, deuteriated solvent for NMR spectroscopy (Sigma Aldrich), Silica gel (60, 0.063–0.200 mm Merck) was used for column chromatography, Kieselgel 60° 254 (silica gel) precoated TLC plates (Merck). Nutrient Broth and Nutrient Agar (from Merck); Kanamycin and Cycloheximide (from Sigma Aldrich); and *E. coli* (W3110), *S. aureus* (ATCC 25923), and *C. albicans* (ATCC 10231), micro plate (Nest) and incubator k (Nüve ES120) were obtained from the American Type Culture Collection. The *N*-(1-naphthyl)ethylenediamine, piperidine and 2-(2-hydroxyethylamino)ethanol were obtained from Southampton University Chemistry Laboratories with a stated purity of greater than 98% and used as such without further purification. The following materials were also obtained from Sigma Aldrich Chemicals: hexachlorocyclotriphosphazene (**1**) (purified by fractional crystallization from hexane), piperidine, ninhydrin (0.5%w/v), NaH (60% dispersion in mineral oil, which was removed by washing with dry *n*-heptane followed by decantation). The Supplemental Materials presents sample ^{1}H and ^{31}P NMR spectra of the products (Supplemental Materials Figures S1–S7).

Methods

All reactions were monitored using Kieselgel 600 254 (silica gel) precoated TLC plates and sprayed with ninhydrine (0.5% w/v) in butanol solution, and developed at approximately 130 °C. UV for TLC measurements. Required separations of mixtures were carried out by crystallization techniques, TLC and by column chromatography using Kieselgel 60. (Merck 60, 0.063–0.200 mm; for 2 g crude mixture, 100 g silica gel was used in a column of 2.5 cm in diameter and 90 cm in length). Melting points were determined on a Hot Stage Microscopy and hot stage connected

to a FP 800 central processor both fitted with a polarizing microscope at Southampton University.

The antimicrobial properties of the derived compounds were determined by using broth microdilution technique for the measurement of the MIC and MCC values. For incubation, micro plates (Nest) and Nüve ES120 incubator were used. Thermo Scientific GENESYS 150 Vis/UV-Spectrophotometry was used for obtaining antibacterial data. FT-IR spectra were recorded in Perkin Elmer BX II FT model spectrometer with a number of scans at 4 cm^{-1} resolution in the range 4000–350 cm^{-1}. Elemental analyses were performed using a ThermoFinnigan Flash 1112 Instrument.^{1}H NMR spectra were recorded using a Bruker AVII and AVIIIHD 400 MHz spectrometer at the indicated frequencies (as operating at 399.5 MHz; Southampton University). Samples were dissolved in $CDCl_3$ and placed in 5 mm NMR tubes. Measurements were carried out using a $CDCl_3$ lock, TMS as internal reference, and sample concentrations of 15–20 mg/cm^3. ^{31}P NMR spectra were recorded using a Bruker AVII and AVIIIHD 400 MHz spectrometer (operating at 161.97 MHz., Southampton University); in $CDCl_3$ and 85% H_3PO_4 was used as an external reference. Mass spectra were recorded at room temperature using a TLC/MS (obtained by a Bruker MicroTOF LC/MS spectrometer using electro spray ionization (ESI) method). ^{31}P NMR and ^{1}H NMR data are provided in Tables 1 and 2, respectively and the MIC and MCC values of hexachlorocyclotriphosphazene derivatives (**10**–**16**) (mg/mL) may be found in Supplemental Materials Table S3.

Synthesis

(**a**) The reactions of hexachlorocyclotriphosphazene (**1**) with piperidine (**2**):
Synthesis of the novel 2-piperidino-2,4,4,6,6-pentacholorocyclotriphosphazatriene (**5**); 2,4-piperidino-2,4,6,6-tetrachlorocyclotriphosphazatriene (**6**); 2,4,6-piperidino-2,4,6-trichlorocyclotriphosphazatriene (**7**); and the 2,2,4,6-piperidino-4,6-dichlorocyclotriphosphazatriene (**8**) derivatives.

*One equivalent of piperidine (**2**), in the presence of NaH, in THF and at room temperature:*

Hexachlorocycloriphosphazene (**1**, 1 g, 0.0028 mol) and two equivalents of NaH (60% oil suspension, 0.14 g, 0.0057 mol) were dissolved in dry THF (100 mL) in a 250 mL three-necked round-bottom flask. This mixture was stirred approximately for 30 min at room temperature then one equivalent of piperidine (**2**, 0.24 g, 0.0028 mol) in THF (30 mL) was added dropwise to this stirred solution under an argon atmosphere. The mixture was stirred (36 h) at room temperature until TLC indicated the completion of the reaction. The reaction mixture was filtered to remove sodium chloride and any other insoluble materials. Then the reaction mixture was followed on TLC silica gel plates using dichloromethane–diethylether (4:1) as the mobile phase. The solvent was removed under reduced pressure and the resulting colorless solid was subjected to column chromatography using dichloromethane–diethylether (4:1) as the eluent. Two main fractions were synthesized and products were recrystallized from benzene: hexane (1;5) containing a few drops of light petroleum (b.p. 40–60 °C).(**i**) The first product was identified as the2-piperidino-2,4,4,6,6-pentachlorocyclotriphosphazatriene, $N_3P_3Cl_5[N\text{-}(C_5H_{10})]$ derivative (**5**). White crystal; yield: 0.42 g; 55%; m.p: 137–139 °C; Anal. Calc. for M.F. $N_4P_3Cl_5C_5H_{10}$: C, 15.15; H, 2.54; N, 14.13, M: 396.42. Found: C, 14.99; H, 2.61; N, 14.19, M^+, 397.29. ^{1}H NMR ($CDCl_3$, at 399.95 MHz.); δPNC$\underline{H}_2$:3.3 (t, 4H, CH_2–N–CH_2); δPNCCH_2: 1.55 (q, 6H, –C$\underline{H}_2$–CH_2–CH_2–); $^{3}J_{(PH)}$: 13.24 Hz.^{31}PNMR ($CDCl_3$, at 162.00 MHz.); δPCl_2: 20.98, δP(NR)Cl: 18.77, $^{2}J(PCl_2$–P(NR)Cl: 59.30 Hz.

(**ii**) The second product was identified as the non-gem-2,4-piperidino-2,4,6,6-tetrachlorocyclotriphosphazatriene, $N_3P_3Cl_4[N\text{–}(C_5H_{10})]_2$ derivative (**6**). White crystal; yield: 0.22 g, 20%; m.p: 169–172 °C. Anal. Calc. for M.F. $N_5P_3Cl_4C_{10}H_{20}$: C, 26.98; H, 4.52; N, 15.73, M, 445.12. Found: C, 27.05; H, 4.57; N, 15.70, M^+, 446.07.^{1}H NMR ($CDCl_3$, at 399.95 MHz.); δPNC$\underline{H}_2$:3.1 (t, 4H, CH_2–N–CH_2); δPNCCH_2: 1.52 (q, 6H, –CH_2–C$\underline{H}_2$–CH_2–); $^{3}J_{(PH)}$: 11.89 Hz. ^{31}PNMR ($CDCl_3$, at 162.00 MHz.); δPCl_2: 22.16, δP(NR)Cl: 17.98, $^{2}J(PCl_2$-P(NR)Cl: 55.76 Hz.

*Two equivalents of piperidine (**2**), in the presence of NaH, in THF and at room temperature (47 h):* The reaction procedure as for (**a**). Starting materials used as hexachlorocyclotriphosphazene (**1**, 2 g, 0.0058 mol); NaH (0.55 g, 0.023 mol); Piperidine (**2**, 0.98 g, 0.012 mol). Two compounds were synthesized and identified as the (**i**) non-gem-2,4-piperidino-2,4,6,6-tetrachlorocyclotriphosphazatriene, $N_3P_3Cl_4[N\text{-}(C_5H_{10})]_2$ derivative (**6**, 35%); and (**ii**) the non-gem-2,4,6-piperidino-2,4,6-trichlorocyclotriphosphazatriene, $N_3P_3Cl_3[C_5H_{10}N]_3$ (**7**) derivative. off-white; yield: 0.20 g, 16%; m.p: 156–159 °C. Anal. Calc. for M.F. $N_6P_3Cl_3C_{15}H_{30}$: C, 36.48; H, 6.12; N, 17.01, M, 493.80. Found: C, 36.55; H, 6.26; N, 16.99, M^+, 494.67. ^{1}H NMR ($CDCl_3$, at 399.95 MHz.); δPNC$\underline{H}_2$:2.98 (t, 4H, CH_2–N–CH_2); δPNCCH_2: 1.46 (6H, –CH_2–CH_2–CH_2–); $^{3}J_{(PH)}$: 11.89 Hz. ^{31}PNMR ($CDCl_3$, at 162.00 MHz.); δP(NR)Cl:12.59.

*Three equivalents of piperidine (**2**), in the presence of NaH, in THF and at room temperature (82 h):* The reaction procedure as for (**a**). Starting material used as hexachlorocyclotriphosphazene (**1**, 2 g, 0.006 mol); Piperidine (**2**, 1.47 g, 0,018 mol); and NaH (0.83 g, 0.035 mol). Two products were synthesized and identified as (**i**) the non-gem-2,4,6-piperidino-2,4,6-trichlorocyclotriphosphazatriene, $N_3P_3Cl_3[C_5H_{10}N]_3$(**7**, 37%); and (**ii**) the2,2,4,6-piperidino-4,6-dichlorocyclotriphosphazatriene, $N_3P_3Cl_2[C_5H_{10}N]_4$ (**8**) derivative. White amorphous solid; yield: 0.28 g, 25%; m.p: 146–148 °C. Anal. Calc. for M.F. $N_7P_3Cl_2C_{20}H_{40}$: C, 44.28; H, 7.43; N, 18.1, M, 542.52. Found: C, 44.36; H, 7.63; N, 18.23, M^+, 543.37.^{1}H NMR ($CDCl_3$, at 399.95 MHz.); δPNC$\underline{H}_2$:2.97 (t, 4H, CH_2–N–CH_2); δPNCCH_2: 1.39 (unclear, 6H, –CH_2–CH_2–CH_2–); $^{3}J_{(PH)}$: 11.89 Hz. ^{31}PNMR ($CDCl_3$, at 162.00 MHz.); $\delta P(NR)_2$: 21.37, δP(NR)Cl: 21.73, $^{2}J(PNR)_2$–P(NR)Cl: 48.57 Hz.

(**b**) The reactions of hexachlorocyclotriphosphazene (**1**) with *N*-(1-naphthyl)ethylenediamine (**3**). Synthesis of the novel 2,4,6,6-tetrachloro-2,4-*non-gem-N*-(1-naphthyl)ethylendiamino)-cyclotriphosphazatriene,

$N_3P_3Cl_4[NH–(CH_2)_2–NH–C_{10}H_7)]_2$ (**9**). *Two equivalents of compound **3**, in the presence of NaH, in dichloromethane solution and at room temperature (48 h):*

The reaction procedure as for (**a**). Starting materials were used as: cyclotriphosphazene (**1**, 2 g, 0.0057 mol); NaH (0.54 g, 0.023 mol) and *N*-(1-naphthyl)ethylenediamine (**2**, 2.95 g, 0.0114 mol). The novel *non-gem*, $N_3P_3Cl_4[NH–(CH_2)_2–NH–(C_{10}H_7)]_2$ derivative (**9**) was separated by column chromatography using dichloromethane and hexane (3:1) as the eluent. Light brown solid; yield: 0.36 g, 31%; m.p: 186–188 °C. Anal. Calc. for M.F. $N_7P_3Cl_4C_{24}H_{26}$: C, 44.53; H, 4.05; N, 15.15, M, 647.36. Found: C, 44.57; H, 4.11; N, 15.18, M^+, 648.17. ^{1}H NMR ($CDCl_3$, at 399.95 MHz); δPNH:3.52(d, 1H); δPNCH$_2$: 3.35 (t, 2H, N-CH$_2$-C-); δPNCCH$_2$: 3.22/3.25 (t, 2 H, N–C–CH$_2$); δAr-H: 8.26 (s, 1H); δArNH:8.34; $^3J_{(PH)}$: 7.95 Hz. ^{31}PNMR ($CDCl_3$, at 162.00 MHz.); δPCl$_2$: 26.32, δP(HNR)Cl: 12.26, ^2J(PCl$_2$–P(HNR)Cl: 50.45 Hz. Previously reported derivatives of this reaction system may be found in Ref.[34]

(**c**) The reactions of hexachlorocyclotriphosphazene (1) with 2-(2-hydroxyethylamino)-ethanol. Synthesis of the novel 2,2,4,4,6,6-trispiro-2,2′-iminodiethoxy-cyclotriphosphazatriene, $N_3P_3[O–(CH_2)_2–NH–(CH_2)_2O]_3$ (**14**): *Two equivalents of compound **3**, in the presence of NaH, in THF and at room temperature (38 h):*The reaction procedure as for (**a**). Starting materials were used as: one equivalent of hexachlorocyclotriphosphazene (**1**, 2 g, 0.006 mol), two equivalents of 2-(2-hydroxyethylamino)ethanol (**2**, 1.26 g, 0.012 mol), and four equivalents of NaH (0.58 g, 0.024 mol). The novel trispiro derivative, $N_3P_3[O–(CH_2)_2–NH–(CH_2)_2O]_3$ (**14**) was separated using dichloromethane-hexane (3:1) as the eluent and recrystallized from benzene: hexane (1:3) containing a few drops of light petroleum (b.p. 40–60 °C). Off-white solid; yield: 0.28 g, 16%; m.p: 143–145 °C. Anal. Calc. for M.F. $N_6P_3C_{12}H_{27}O_6$: C, 32.43; H, 6.12; N, 18.91, M, 444.39. Found: C, 32.37; H, 6.19; N, 18.88, M^+, 445.23.^{1}H NMR ($CDCl_3$, at 399.95 MHz.); δPOCH$_2$:4.40 (t, 2H, O–CH$_2$); δPOCCH$_2$: 1.66 (overlapped quintet of quintet, 2H, O–C–CH$_2$–NH); δ-C–NH–C: 7.28. ^{31}PNMR ($CDCl_3$, at 162.00 MHz.); δP(OR)$_2$: 18.47.

Other cyclotriphosphazene derivatives (**10–13**, **15**, and **16**) from this reaction system may be found in our previously reported paper).[29]

Determination of the antimicrobial activity of the derived compounds (10–16)

The antimicrobial properties of the derived compounds (**10–16**) were determined by using microdilution techniques for the MIC and MCC values.[27,28]

Due to the amount of synthesized compounds were low, the antimicrobial activities of these compounds could only be tested against targeted *E. coli* W3110, *S. aureus* ATCC 25923, and *C. albicans* ATCC 10231 microorganism. MIC and MCC were determined using ½ serial dilution in microplates (Nest) using two different concentrations of each substance.

The substances were prepared by dissolving in different concentrations of water and DMSO and used for MIC tests.

Growth method

To prepare pre-cultures, each microorganism was incubated into 5 mL nutrient broth medium and incubated at 37 °C for 18 h at 160 rpm with shaking. After incubation, the density of the microorganisms was adjusted to 0.1 absorbance at OD_{600} nm on the Thermo Scientific GENESYS 150 Vis/UV-Spectrophotometer and then the required culture was prepared in 60 mL sterile nutrient broth with 400 µL of microorganism (0.4 mL in 59.6 mL broth).

Broth microdilution

From this culture, 180 µL of culture was added to the first well of the 96-well plate and 100 µL of culture to the other wells. To complete the total volume of 200 µL, 20 µL of chemical compound was added to the first wells containing 180 µL of bacterial culture and ½ serial dilution was performed in 12 wells. Micro plates were incubated at 37 °C for 18 h and then the MIC values were determined the next day. In addition, to obtain more accurate results, the experiments were repeated by reducing the intervals at close concentrations of the determined MIC values. (20 µL in 180 µL bacterial suspension and 30 µL in 170 µL bacterial suspension were added from the synthesized compounds.)

Moreover, in order to determine the cidal effects of the compounds, 10 µL of cultures were taken from the wells where no growth was observed and dropped into the nutrient agar containing medium. Then, the plates were incubated at 37 °C for 18 h and MCC values were determined the next day. Experiments were repeated at least three times as required.[28]

Conclusions

In this study, from the nucleophilic substitution reactions of hexachlorocyclotriphosphazene (**1**) with piperidine (**2**), *N*-(1-naphtyl)ethylenediamine (**3**) and 2-(2-hydroxyethylamino)ethanol (**4**) *geminal* and *non-geminal* substituted derivatives were synthesized. Novel cyclotriphosphazenecompounds (**6–9** and **14**) were structurally characterized by spectral data of ^{1}H-NMR and ^{31}P-NMR, TLC-MS, and elemental analysis. Selected cyclotriphosphazene derivatives (**10–16**) were screened for their antimicrobial activities against three human pathogens; *E. coli* W3110, *S. aureus* ATCC 25923, and *C. albicans* ATCC 10231. Investigations of the MIC and MBC values of compound **16** indicated that they are more effective than antibiotics against eukaryotic microorganisms, but as effective as antibiotics against prokaryotic microorganisms. It is concluded that among the evaluated compounds; **10**, **12**, and **16** seem to be good candidates for being antimicrobial agents.

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