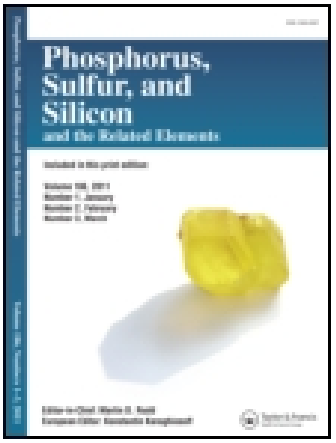

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Synthesis, Characterization, and Activity of Cyclotriphosphazene-Cyclene Conjugates

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SYNTHESIS, CHARACTERIZATION, AND ACTIVITY OF CYCLOTRIPHOSPHAZENE-CYCLENE CONJUGATES

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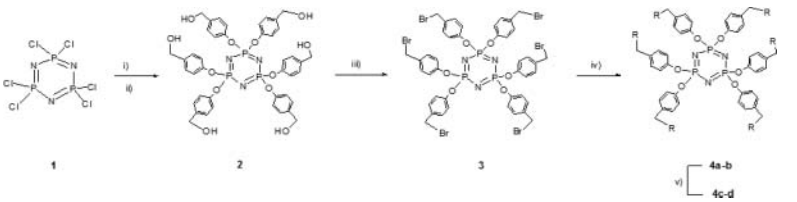
GRAPHICAL ABSTRACT



Abstract *Two novel cyclotriphosphazene derivatives were synthesized from hexachloro cyclotriphosphzene. Their structures were characterized by ^{1}H, ^{31}P, and ^{13}C NMR spectroscopy as well as by IR spectroscopy and electrospray ionization-mass spectrometry (ESI-MS). The Zn complex of **4d** was effective in hydrolytic DNA cleavage reactions.*

Keywords Synthesis; cyclotriphosphazene; DNA; cleavage reactions

INTRODUCTION

DNA phosphodiester bonds are exceptionally resistant to hydrolysis at neutral pH and 25°C: the half-life for the cleavage of dimethyl phosphate is estimated to be 130,000 years.[1] Consequently, much effort has been devoted to the development of efficient catalysts for this reaction.[2–5] Chemical nucleases present some advantages over conventional enzymatic nucleases in that they are smaller in size and thus can reach more sterically hindered regions

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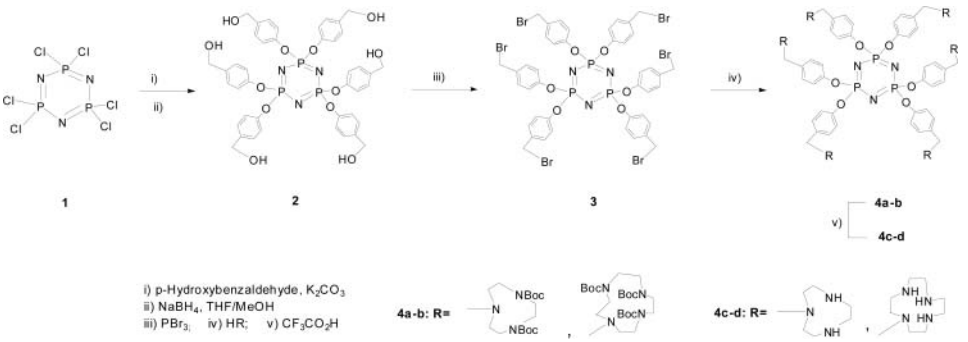
of a macromolecule. Therefore, designing new small molecules suitable for cleaving DNA under hydrolytic conditions is of considerable importance.

Phosphazenes, particularly cyclophosphazenes, have received considerable interest, not only because of their wide spectrum of chemical and physical properties, but also due to their importance in synthetic chemistry.[6–9] Different side-group structures affect the chemical and physical properties of ring systems and high polymers based on a phosphazene skeleton.[10–17] Recently, we reported a series of polydentate cyclotriphosphazene ligands,[18,19] which showed good nuclease activity with hydrolytic cleavage mechanism. In continuation of our efforts to obtain more efficient DNA cleavage reagent, we report here the synthesis of two novel star-branched cyclotriphosphazene derivatives.

RESULTS AND DISCUSSION

The synthetic route to the new cyclotriphosphazene-cyclene conjugates is shown in Scheme 1. Compound **2** was synthesized from the reaction of hexachloro cyclotriphosphazene (**1**) with the potassium salt of 4-hydroxybenzaldehyde, followed by reduction with sodium borohydride in THF/MeOH. Several reagents were available for converting hydroxy groups of **2** to alkyl bromides. Bromination reagents based on hydrobromic acid/sulfuric acid were found unsuitable and the reaction conditions were hard to control. Insoluble intermediates were formed from the reaction with the hydroxy groups of compound **2**. The bromination reaction was achieved by using PBr_3. Compound **3** was characterized by ^{1}H NMR spectroscopy and electrospray ionization-mass spectrometry (ESI-MS). ^{1}H NMR spectroscopy showed that all six hydroxy groups were replaced by bromine atoms. In the spectrum, two doublets for the aromatic ring protons appeared at 6.89 and 7.25 ppm, and a single peak for the protons of the bromomethyl group was observed at 4.48 ppm.



Scheme 1

Compound **3** was treated with Boc-protected cyclene to generate compounds **4a**,**b**. Subsequent deprotection of the Boc group was then carried out by treatment of a CH_2Cl_2 solution of **4a**,**b** with trifluoroacetic acid (TFA) at room temperature. Basification with NaOH then yields the free cyclotriphosphazenes **4c**,**d**.

The new cyclotriphosphazenes **4c**,**d** were characterized by ^{1}H, ^{31}P, and ^{13}C NMR spectroscopy, IR spectroscopy and ESI-MS. ESI mass spectra for all compounds showed the respective $[M+H]^+$ ion. Their structures are similar and **4c** was chosen as an example for the discussion. In its ^{31}P NMR spectrum a single peak is observed at 9 ppm. In the ^{1}H

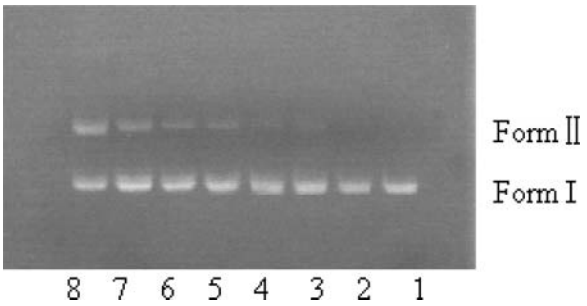


Figure 1 Effect of time on the cleavage activity of pUC19 DNA by the Zn complex of **4d** (10 μM). pUC19 DNA: 0.5 *u*g/*u*L, 37°C. Na_2HPO_4/HCl buffer (0.05 M Na_2HPO_4/HCl, 0.1 M NaCl), pH = 7.5. Lane 1: DNA control, lanes 2–8: 1, 3, 5, 7, 12, 24, and 48 h.

NMR spectrum, two doublets at 6.9 ppm and 7.27 ppm were assigned to the protons of the benzene ring. A singlet for methylene protons was observed at 3.8 ppm. A singlet at 3.52 ppm and two triplets at 3.11 and 2.58 ppm were assigned to the methylene protons of the cyclone moiety.

The absorption band at 3039 cm^{-1} in the IR spectrum of **4c** is assigned to the stretching vibration of C–H. The stretching vibration of the methylene moiety appears at 2357 cm^{-1}. The absorptions of the stretching vibration of the C=C bond appear at 1600 and 1499 cm^{-1}. The absorption of the stretching vibration of C–O bond at 1155 cm^{-1} was also observed.

The progress of the hydrolytic DNA cleavage by the Zn complex of **4d** at pH 7.5 is shown in Figure 1. Obviously the cleavage by the metal complex is time dependent. With increasing reaction time the amount of form I of DNA diminished gradually, whereas the amount of form II increased.

CONCLUSION

In conclusion, we designed and synthesized two novel cyclotriphosphazene derivatives. The reactions are convenient and efficient. The target compounds were characterized by ^{1}H, ^{31}P, and ^{13}C NMR spectroscopy, IR spectroscopy and ESI-MS. The Zn complex of **4d** was effective in the hydrolytic cleavage of DNA.

EXPERIMENTAL

Melting points were measured using a WC-1 microscopic apparatus and are corrected. Infrared spectra were recorded with a Bruker VECTOR22 spectrophotometer in KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded using a Bruker DTX-400 spectrometer. ^{31}P NMR spectra are measured with a Bruker AMX 400 MHz spectrometer with 85% H_3PO_4 as external reference.

Mass spectra were acquired in positive ion mode using a Bruker ESQUIRE 3000 ion trap spectrometer equipped with a gas nebulizer probe, capable of analyzing ions up to *m*/*z* 6000. The high-resolution mass spectral data for compound **4c** was recorded with a Waters Q-Tof microTM spectrometer by ESI method. The sample was analyzed as a solution in methanol. All chemicals and materials were of analytical grade and were obtained from Chenzhuo Company. The solvents used were purified and dried by standard procedures.

Preparation of Compound 3

Into a 500-mL round-bottom flask was placed 3.495 g (4 mmol) of **2** with 200 mL of THF under argon. Then, 3.8 mL (40 mmol) of phosphorus tribromide in 50 mL THF was added dropwise at 0°C and the mixture was stirred overnight. The solvent was removed. The residue was dissolved in $CHCl_3$ and washed with aq. $NaHCO_3$. The organic layer was dried over Na_2SO_4 and then the solvent was evaporated under reduced pressure. After recrystallization from ethyl acetate white solid **4** was obtained (yield 81%). Mp: 144–146 °C; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.89$ (d, $J = 8.2$ Hz, 12H), 7.25 (d, $J = 8.4$ Hz, 12H), 4.48 (s, 12H); ESI-MS: *m/z* 1252 $[M+H]^+$.

Synthesis of Compounds 4a,b

To a solution of compound **3** (0.254 g, 0.2 mmol) in 20 mL of CH_3CN was added 1 g Na_2CO_3. After the mixture was warmed to 80°C, a solution of ^{Boc}Cyclen (0.821 g, 1.7 mmol) in 10 mL of CH_3CN was dropped in under N_2, followed by stirring for 48 h. Then, the reaction mixture was concentrated in vacuo. Column chromatography of the residue (eluant: $CHCl_3$/MeOH 50:1) provided 0.668 g (91.4%) of crude compound **4b**; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.08$ (d, $J = 8$ Hz, 12H, $-C_6H_4$), 6.93 (s, 12H, $-C_6H_4$), 3.74 (s, 12H, $-CH_2$–pH), 3.55 (s, 24H, $-CH_2$–cyclen), 3.39–3.26 (m, 48H, $-CH_2$–cyclen), 2.61 (s, 24H, $-CH_2$–cyclen), 1.44 (s, 162H, $-CH_3$); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 156.0, 155.6, 155.2, 149.9, 132.8, 131.4, 120.5, 79.5, 79.2, 55.1, 54.2, 53.4, 49.9, 47.5, 47.2, 28.7, 28.4; ^{31}P NMR (162 MHz, $CDCl_3$): $\delta = 7.9$; ESI-MS: $[M+H]^+$ 3622.

By a similar method **4a** was obtained in 90% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.88 (d, $J = 8$ Hz, 12H, $-C_6H_4$), 6.73 (s, 12H, $-C_6H_4$), 3.64 (s, 12H, $-CH_2$–aryl), 3.25 (s, 24H, $-CH_2$–cyclen), 3.22–3.20 (m, 24H, $-CH_2$–cyclen), 2.60 (s, 24H, $-CH_2$–cyclen), 1.42 (s, 108H, -CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 156.0, 155.7, 155.4, 150.0, 132.8, 131.6, 121.5, 79.5, 70.9, 55.2, 54.2, 49.9, 47.3, 28.6, 28.4; ^{31}P NMR (162 MHz, $CDCl_3$): $\delta = 8.6$; ESI-MS: $[M+H]^+$ 2742.

Synthesis of Compounds 4c,d

To a solution of **4b** (0.668 g, 1.84 mmol) in dry CH_2Cl_2 (15 mL) at 0°C, CF_3COOH (3 mL) was added dropwise under N_2. The mixture was stirred for 7 h at room temperature. After removal of the solvent, the residue was adjusted to pH 12 by aqueous solution of NaOH and extracted with $CHCl_3$ (3 × 30 mL). The organic phase was collected and dried over Na_2SO_4. After removal of the solvent 0.313 g solid **4d** was obtained. Yield: 93.8%; ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 7.11$ (d, $J = 8.4$ Hz, 12H, $-C_6H_4$), 6.99 (d, $J = 8.4$ Hz, 12H, $-C_6H_4$), 3.59 (s, 12H, $-CH_2$–aryl), 2.78 (d, $J = 4.8$ Hz, 24H, $-CH_2$–cyclen), 2.64 (d, J = 4.8 Hz, 24H, $-CH_2$–cyclen), 2.55 (d, $J = 4$ Hz, 48H, $-CH_2$–cyclen); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 149.7, 134.8, 129.9, 120.8, 58.3, 50.9, 47.0, 46.2, 45.0; ^{31}P NMR (162 MHz, $CDCl_3$): $\delta = 8.3$.

Similarly compound **4c** was obtained in 90% yield, mp: 68–70°C. ^{1}H NMR (400 MHz, D_2O): $\delta = 7.27$ (d, $J = 8.4$ Hz, 12H), 6.90 (d, $J = 8.4$ Hz, 12H), 3.80 (s, 12H), 3.52 (s, 24H), 3.11 (t, $J = 5.6$ Hz, 24H), 2.58 (t, $J = 5.6$ Hz, 24H); ^{13}C NMR (100 MHz, D_2O): δ = 149.3, 133.1, 131.8, 120.8, 57.7, 47.2, 43.6, 42.1; ^{31}P NMR (162 MHz, $CDCl_3$): δ = 9.0; IR (cm^{-1}, KBr): 3039, 2911, 2843, 2357, 1895, 1660, 1599, 1552, 1499, 1450, 1356,

1266, 1198, 1155, 948, 887, 837, 747, 694; ESI-MS: $[M+H]^+$: 1541.7. HR-MS (ESI): m/z Calcd. for $C_{78}H_{120}N_{21}O_6P_3$ $[M+H]^+$: 1540.9012, found 1540.9010.

DNA Cleavage Experiments

The hydrolytic DNA cleavage activities of **4** were evaluated by monitoring the conversion of supercoiled plasmid DNA (pUC19) (Form I) to nicked circular DNA (Form II) or linear DNA (Form III) by agarose gel electrophoresis. In a typical experiment, reaction solutions were prepared by mixing 2 μL of a Zn(II) complex solution (50 μM, pH 7.5) with 2 μL of supercoiled DNA (50 μM), 2 μL of Na_2HPO_4/HCl buffer (100 mM), followed by dilution with sterilized water to a final volume of 20 μL. All reaction mixtures were incubated at 37°C. Hydrolytic cleavage experiment was performed both at pH 7.5 in Na_2HPO_4/HCl buffer. All reactions were quenched by addition of 2 μL of a quench buffer containing EDTA, bromophenol blue, xylene cyanol, and sucrose. Cleavage products were separated by electrophoresis on 1% agarose gels in **1** · TAE buffer for 1.5 h at 60 V and stained with 0.5 μg/mL EB for 45 min. The gel was visualized by UV transillumination.

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