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Synthesis and characterization of new cyclotriphosphazene compounds

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ABSTRACT

In the present study, spiro (**1a**), dispiro (**1b**, **2**, **3**), per-substituted spermine-bridged (**6**–**9**) and dispiroansa spermine (**10**) derivatives of cyclotriphosphazene have been synthesized. The structures of the novel compounds (**1b**, **6**–**10**) have been characterized by elemental analysis, FTIR, mass spectrometry, ^{1}H and ^{31}P NMR spectroscopy. The molecular structures of **1b**, **2**, **8**, and **10** were determined by single crystal X-ray crystallography. In order to investigate the anti-tumour properties of the newly synthesized cyclotriphosphazene derivatives, in vitro cytotoxic activity test (MTT assay) has been performed using HT-29 (human colon adenocarcinoma) and Hep2 (human epidermoid larynx carcinoma) cell lines. The result of the MTT assay showed that while compound **1a** has cytotoxic effect on both Hep2 and HT-29 cell lines, compound **3** has only cytotoxic effect towards the Hep 2 cells.

1. Introduction

Many compounds with different structures have been tested in an effort to find new anticancer drugs, as cancer is the leading cause of death worldwide. Although a number of compounds exhibit therapeutical properties and are currently used for treatment, the search for new drugs with improved efficiency and minimum side effects still continues. One of the compounds that has attracted attention as a potential anti-cancer agent is hexachlorocyclotriphosphazene; $N_3P_3Cl_6$, this compound has a robust heterocyclic ring with six pendant chlorine atoms that can be substituted with special groups.[1] The cyclophosphazene core can exhibit different physical and chemical characteristics depending on the side groups replacing the chlorine atoms.[2] This important feature enables the design of new molecules containing cyclotriphosphazenyl groups for diverse applications; such as anti-cancer agents,[3–6] antibacterial reagents,[7,8] biomedical materials,[9] liquid crystals,[10,11] lubricants,[12] electrical conductors,[13] and re-chargeable batteries.[14] In recent years $N_3P_3Cl_6$ and its derivatives have raised considerable interest as anticancer agents, although the mechanism has remained largely unknown. The reactions of $N_3P_3Cl_6$ with diols can give rise to two types of cyclic derivatives: spiro (in which the two P—O bonds are formed to the same P-atom) and ansa (in which the two P—O bonds are formed to different P-atoms) when the linking $-(CH_2)_n-$ group of the reagent consisted of n=2,3,4 moieties.[15–18] Di-amines with $N_3P_3Cl_6$ predominantly give spiro derivatives.[4,19,20] It is known that on increasing the chain length of the diol or di-amine, there is a decrease in the relative proportion of intra-molecular reactions giving cyclic derivatives and an increase in the amount of bridged cyclotriphosphazene molecules via intermolecular reactions.[21–24] Most bridged cyclotriphosphazene compounds are based on the linkage of two cyclotriphosphazene rings with linear diols, di-amines and polyamines such as spermidine or spermine.[22,24] The structure of the molecule can be changed by modifying the side groups of the cyclic moiety, this important characteristic enables the synthesis of novel compounds with unique properties including biologic activity.[3–9] For example in recent studies, it has been shown that polyamine derivatives of cyclotriphosphazene compounds such as spermine exhibit cytotoxic activity against human cancer cell lines.[6,25] In a study performed by Isiklan et al., it has been showed that the pyrrolidine derivative of cyclotriphosphazene has antimicrobial properties against *Bacillus subtilis*, *Bacillus cereus* and *Staphylococcus aureus*.[8] In another study, Siwy et al. tested the cytotoxic effects of 1,3-(oxytetraethylenoxy) cyclotriphosphazene derivatives against four cell lines and they observed antiproliferative activity of the compounds in the range of the international criterion.[5] In addition, the DNA binding abilities of cyclotriphosphazenes have been shown by a number of research groups.[7,8,26]

We report here (i) the synthesis of spiro (**1a**),[27] dispiro (**1b**, **2**,[20] **3**[28]) derivatives of cyclotriphosphazene, (ii) the preparation of per-substituted single spermine-bridged (**6**–**9**) and dispiroansa

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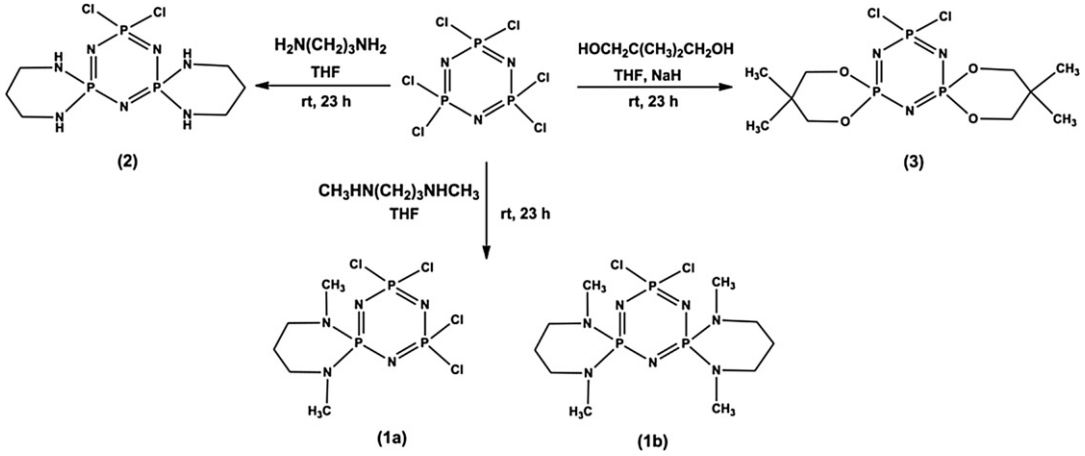
spermine (**10**) derivatives of cyclotriphosphazene, (iii) the determination of the structures of novel compounds (**1b**, **6**–**10**) by elemental analysis, IR, mass spectrometry, ^{1}H and ^{31}P NMR spectroscopy, (iv) the solid-state structures of **1b**, **2**, **8** and **10** established by X-ray diffraction techniques, (v) the cytotoxic properties of the synthesized compounds against cancer cell lines.

2. Results and discussion

2.1. Formation of the cyclotriphosphazene compounds

The reactions of $N_3P_3Cl_6$ with difunctional alcohols and amines lead to the formation of regioisomers; spiro-, ansa-, and bridged-structures.[18,20] In this study, spiro-substituted *N*,*N*′-dimethyl-1,3-propanediamino (**1a**),[27] dispiro-substituted *N*,*N*′-dimethyl-1,3-propanediamino (**1b**), 1,3-propanediamino (**2**)[20] and 2,2-dimethyl-1,3-propanedioxy (**3**)[28] derivatives of cyclotriphosphazene were first synthesized. The reaction of **1b**, **2**, and **3** with spermine (**4**) in dichloromethane solution and the presence of triethylamine lead to formation of per-substituted single spermine-bridged (**5**–**7**) derivatives. In order to obtain compound **9**, the reaction of single spermine-bridged compound (**8**) with 2,2,3,3-tetrafluorobutane-1,4-diol was preferred over the reaction between $N_3P_3Cl_6$ and 2,2,3,3-tetrafluorobutane-1,4-diol that gives both spiro and ansa derivatives.[17] In addition, a new per-substituted dispiroansa spermine derivative of cyclotriphosphazene (**10**) was synthesized from the reaction of spiro-substituted *N*,*N*′-dimethyl-1,3-propanediamino (**1a**) with spermine in a 1:2 molar ratio in chloroform.

The syntheses of these compounds (**1**–**10**) are described in the synthesis section and a graphical presentation is given in Schemes 1 and 2. Each of the new compounds (**1b**, **6**–**10**) was characterized by FTIR, MS, elemental analysis, ^{1}H and ^{31}P NMR spectroscopy and X-ray crystallography (**1b**, **8**, **10**). The synthesis of compound **2** was reported previously,[20] but X-ray crystallography analysis has been completed in this study.



Scheme 1. Preparation of spiro (**1a**), dispiro (**1b**, **2**, **3**) derivatives of cyclotriphosphazene.

2.2. NMR characterization of compounds 1b, 6–10

The proton decoupled ^{31}P NMR spectra of compound **1b**, **6**–**10** showed as AX_2 spin systems due to the different environments for the two different phosphorus nuclei of the cyclotriphosphazene ring. The PN(bridge or ansa) group has chemical shifts between 10.3 and 19.7 ppm and the PX_2 groups occur in different regions of the spectra between 8.0 and 27.6 ppm depending on the substituent X. For example, chemical shifts of the PX_2 (X=$[OCH_2C(CH_3)_2CH_2O]_{0.5}$ or $[OCH_2(CF_2)_2CH_2O]_{0.5}$) groups move to high frequency (downfield) by about 8.0–14.4 ppm. An example is given in Fig. 1 for the single-bridged compound **8**, which clearly shows the triplet signal ca. 19.4 ppm and doublet signal ca. 13.9 ppm, corresponding to the PN(bridge) and PX_2 (X=$[OCH_2C(CH_3)_2CH_2O]_{0.5}$), respectively. The proton decoupled ^{31}P NMR spectrum of the sample taken from the reaction mixture of compound **10** shows the predominant formation of dispiroansa spermine derivative of cyclotriphosphazene (**10**) (Fig. 2a), and trace amounts of other products. Compound **10** gave the ^{31}P NMR spectrum shown in Fig. 2b, consisting of one triplet for PX_2 (X=$[CH_3N(CH_2)_3NCH_3]_{0.5}$) and one doublet for >PN(ansa) due to two different phosphorus environments within the molecule. The chemical shifts and coupling constants of all compounds are summarized in Table 1.

2.3. X-ray crystallography of compounds 1b, 2, 8, 10

The molecular structures of compounds **1b**, **2**, **8** and **10** were determined by X-ray crystallography. The data collection and refinement parameters are presented in Table 2 and the molecular structures are shown in Figs. 3–6 respectively. The asymmetric units of compounds **2** and **10** contain two crystallographically independent molecules, which have been denoted as A and B but only molecules **2-A** and **10-A** are shown in Figs. 4 and 6, respectively.

The crystal structures of compounds **1b** and **2** shown in Figs. 3 and 4, each have one six-membered cyclotriphosphazene (P_3N_3) ring, which is dispiro-substituted with two 1,3-propanediamino- and *N*,*N*′-dimethyl-1,3-propanediamino-moieties, respectively. The bond lengths and bond angles of the N_3P_3 rings, and also the substituted moieties of both structures, are within the normal range found for many cyclotriphosphazene derivatives, which are spiro-substituted with same or similar groups.[29–33] The cyclotriphosphazene rings are not planar in both structures; in a twisted-boat conformation in compound **1** with the maximum deviation from the plane of the cyclotriphosphazene ring being 0.1145(16) Å (N2) whilst, in a flattened boat conformation in **2** with the maximum deviations being 0.1696(7) Å (P1) for **2-A** and 0.1749 (7)Å (P4) for **2-B**. In all structures, the six-membered spiro rings (C_3N_2P) are in the chair conformation as expected, except one spiro ring of **2-B**, which is in the twisted conformation as rarely observed in the literature.[29,33,34]

The molecular structure of compound **8** shown in Fig. 5 contains a spermine moiety bridging two cyclotriphosphazene (P_3N_3) rings, which are dispiro-substituted with 2,2′-dimethylpropanedioxy-groups. The molecule sits on an inversion centre and the asymmetric unit contains one CH_2Cl_2 solvent per molecule. The cyclotriphosphazene ring is approximately planar with the maximum deviation from the plane being 0.0826(8) Å (P1). All the