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Short communication

SPERMIDINE- AND SPERMINE-LINKED CYCLOPHOSPHAZENES: NEW FORCED (*BI-DANGLING* AND *SPIRO-BIDANGLING*) MOLECULAR CONFIGURATIONS

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INTRODUCTION

The reactions of hexachlorocyclotriphosphazene, $N_3P_3Cl_6$, with trifunctional or higher functional reagents, which have been studied, are very few. Thus, the reaction of $N_3P_3Cl_6$ with trifunctional triamine spermidine, $H_2N-(CH_2)_3-NH-(CH_2)_4-NH_2$, was reported [1] to give a unique product (Fig. 1) in which the spermidino moiety adopts the so-called *spiro-bino* configuration with a six-membered *spiro* loop. The reaction of $N_3P_3Cl_6$ with spermine, $H_2N-(CH_2)_3-NH-(CH_2)_4-NH-(CH_2)_3-NH_2$, a tetrafunctional tetraamine, was also reported [2] to give exclusively one product (Fig. 1) amongst the seventeen structures which could have been expected considering possible *spiro*, *bridging* or *bino* [3, 4] and *dangling* derivatives. More recently, Shaw's

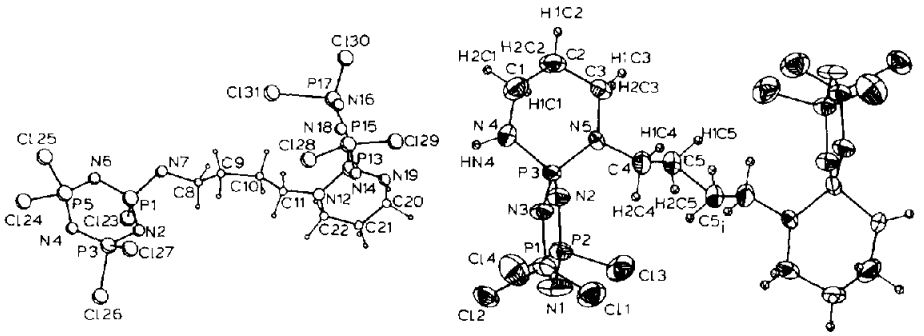

Fig. 1. X-Ray structures of the spermidine and spermine innate derivatives of $N_3P_3Cl_6$.

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group in London reported on products of the reaction of $N_3P_3Cl_6$ with glycerol [5] and they isolated two derivatives in which the triol adopts either a *spiro-dangling* or a *spiro-bridging* configuration, the latter looking quite similar to the *spiro-bino* one we obtained with spermidine.

Thus, polyamines yield unique products whilst polyols lead to at least two compounds; this is due to the fact that amino groups are more nucleophilic versus $N_3P_3Cl_6$ than hydroxy groups.

Polyamine-linked cyclophosphazenes were developed in our group with the aim of designing selective anticancer immuno-modulating drugs, natural (biogenic) polyamines playing the role of tumor finders for malignant cells [6]. The reactions of $N_3P_3Cl_6$ with 1,3-diaminopropane (DAP) [7, 8], putrescine [9], cadaverine [3], spermidine [1] and spermine [2] in suitable stoichiometric conditions lead unequivocally to neat final products, according to the rules of the BASIC system [10]. Indeed, the covalent binding of the polyamines leads to *spiro* (for DAP and putrescine), *bino* (for cadaverine), *spiro-bino* (for spermidine) and *dispiro-bino* (for spermine) innate configurations which induce a dramatic variation of the water solubility of the drugs depending on the configuration: for example, *spiro*, *spiro-bino* and *dispiro-bino* drugs are soluble in physiological serum at 2 kg l^{-1} when the solubility of *bino* drugs is less than 2 g l^{-1}. In other words, if a very high water solubility is an advantage for drugs which have to be injected through intravenous routes, the concomitant poor lipophilicity may be a disadvantage, mainly when bioreceptors of drugs are located on the phospholipids of the cell membrane.

Thus, we were urged to investigate new configurations of natural polyamines linked to cyclophosphazenes which would induce more balanced water solubility and lipophilicity.

The present contribution reports on two examples of new configurations in spermidine- and spermine-linked cyclophosphazenes where the polyamines were forced to react along unusual pathways.

EXAMPLE 1: A *BI-DANGLING* SPERMIDINE-LINKED CYCLOPHOSPHAZENE

Bergeron et al. [11, 12] recently described the synthesis of N4-substituted spermidines (homospermidines) of biological interest by transposing classical reactions of organic chemistry to polyamines syntheses.

For linking spermidine to a cyclophosphazene at the N4 position, we had to make the primary amino functions unreactive by protecting them with phthalic anhydride (Gabriel's reaction).

In our case, the synthesis of the di-protected spermidine on its primary amino functions proceeds as follows. Phthalic anhydride is dissolved in refluxing chloroform and spermidine is then added dropwise in suitable (2:1) stoichiometric conditions. The medium is stirred for 4 days, maintaining the $CHCl_3$ reflux. The final limpid organic solution is dried over $MgSO_4$ and solvent is removed in vacuo to give a sticky white oil which is stirred for a few hours with

n-heptane, in order to remove $CHCl_3$ from the clathrated moiety. Di-protected spermidine is obtained as a white powder in 70% yield after removal of the solvent.

The ^1H NMR spectrum at 60 MHz and analytical data are very consistent with the expected structure.

In a second step, the di-protected spermidine is dissolved in toluene and reacted with $N_3P_3Cl_6$ in the same solvent and in the presence of a saturated aqueous sodium carbonate solution, which picks up HCl from the reaction. This heterogeneous medium is stirred for 1 day. The organic phase is poured off, dried with $MgSO_4$, and toluene is then removed in vacuo to give a white powder in 70% yield. Incidentally, such a pathway in heterogeneous conditions keeps HCl in the water phase, leading to hydrochloride-free final samples.

The final product (**I**) is either washed with n-heptane or refined by SiO_2 column chromatography with toluene:acetonitrile (7:3) as the eluant for removing the non-reactive $N_3P_3Cl_6$. $R_f=0.57$ in t.l.c. with the same eluant.

The structure of this product was assigned by ^{31}P NMR at 32.4 MHz and DCI mass spectrometry.

The ^{31}P NMR spectrum is of the expected A_2B type, the doublet PCl_2 being at 16.64 and 17.81 ppm and the triplet $PClNRR'$ being centered on 2.58 ppm. The coupling constant $^2J_{PP}$ is equal to 37.2 Hz. It must be pointed out that both the PCl_2 and the $PClNRR'$ signals are noticeably shielded with respect to normal PCl_2 and $PClNH$ signals which are both generally observed within the 19–21 ppm range.

The DCI mass spectrum is reported in Fig. 2. The molecular ion is observed

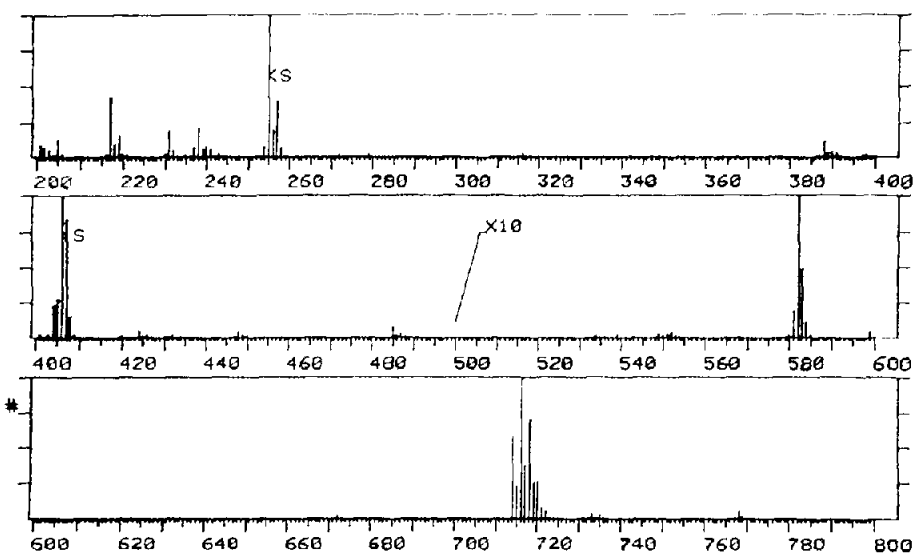

Fig. 2. DCI mass spectrum of (**I**).

at m/z 714 (mol.wt.=714 with ^{35}Cl) for the MH^+–1H entity. The isotopic $^{37}Cl/^{35}Cl$ distribution is consistent with 5 chlorine atoms in the molecule. Incidentally, the M, 1 NH_4^+ (m/z 731) and the M, 3 NH_4^+ (m/z 768) entities are also detected.

The IR spectrum (KBr discs) reveals the linked phthalimide functions at 1760 (vs) and 1700 cm^{-1} (s), a very significant splitting of the breathing frequency of the N_3P_3 ring at 1230 and 1160 cm^{-1} (vs with a shoulder at 1140 cm^{-1}), and an unusual pattern for the P–Cl vibrations at 570, 525 and 510 cm^{-1}.

Thus, the di-protected spermidine links the cyclophosphazenic ring through its N4 position as a genuine secondary monoamine, keeping two *dangling* primary amino functions free (when unblocked) for further reactions. In other

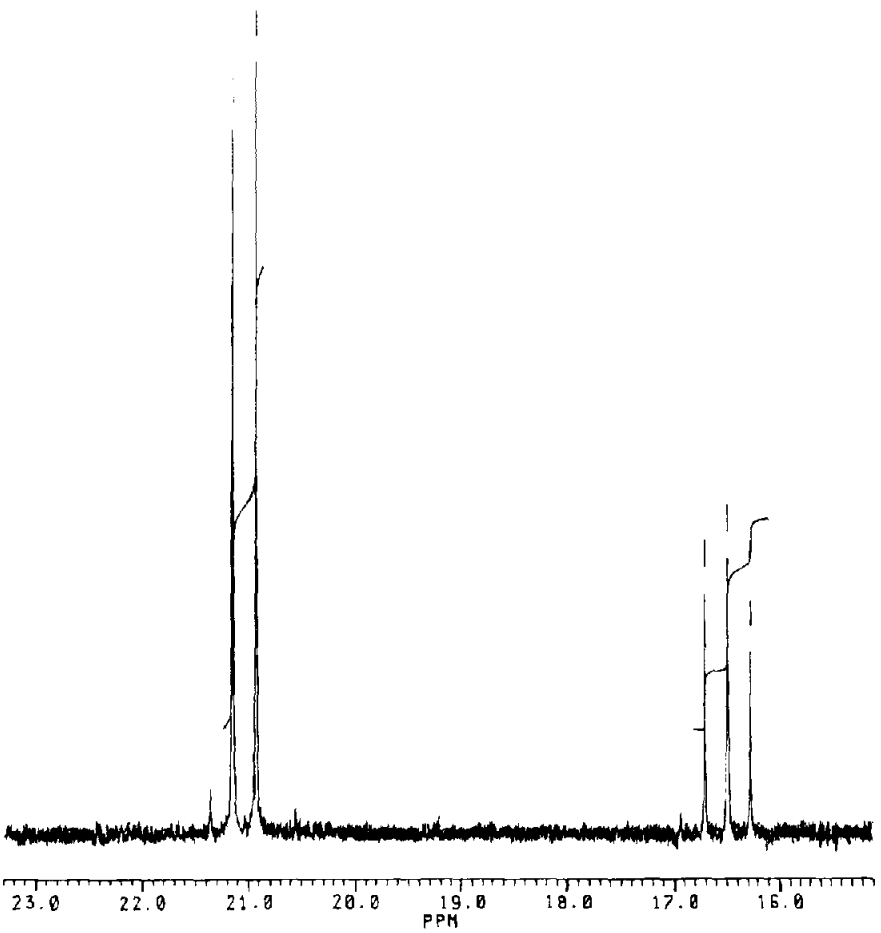

Fig. 3. 202 MHz ^{31}P NMR spectrum of (**II**).

words, we forced spermidine to react as a secondary monoamine and so prepared the first *bi-dangling* spermidine-linked cyclophosphazene.

EXAMPLE 2: A *SPIRO-BIDANGLING* SPERMINE-LINKED CYCLOPHOSPHAZENE

In the same manner, we protected by phthalic anhydride the two primary amino functions of spermine (same experimental conditions as above) in order to leave the two secondary amino functions to react with $N_3P_3Cl_6$. This reaction was carried out in (1:1) conditions (heterogeneous medium, again with reactants in toluene, versus a saturated aqueous sodium carbonate solution). The medium is stirred for 2 days and the final product is extracted as previously described, submitted to SiO_2 column chromatography with a toluene: acetonitrile (7:3) mixture as the eluant. The final product (**II**) is a white powder, obtained in 70% yield; its R_f in t.l.c. = 0.62 with the same eluant.

According to the BASIC rules, we could normally expect that the di-protected spermine would link $N_3P_3Cl_6$ in a *spiro* configuration, its two NH functions being separated by only 4 CH_2 groups as in putrescine, $H_2N-(CH_2)_4-NH_2$. However, we could not rule out the possibility of a linkage in a *bino* configuration, owing to the bulky character of the di-protected spermine and to the common behaviour of secondary amines which leads normally to *non-geminal* disubstituted derivatives of $N_3P_3Cl_6$ [13].

The 202 MHz ^{31}P NMR [14] spectrum of (**II**) in $CDCl_3$ with 85% H_3PO_4 as standard shows a doublet at 21.14 and 20.92 ppm and a triplet centered on 16.49 ppm, $^2J_{PP} = 44.3$ Hz (Fig. 3). According to the classical A_2B spectra of

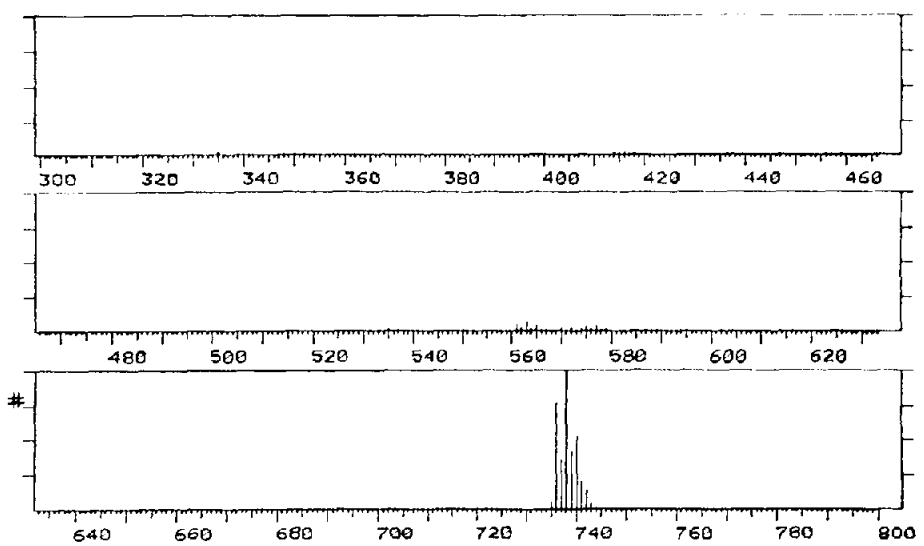

Fig. 4. DCI mass spectrum of (**II**).

both *bino* [3] and *spiro* [7, 9] species, we were inclined to conclude (**II**) had a *spiro* configuration. Indeed, the gap between the doublet and the triplet in the latter is at least 5–10 ppm while it is less than 2 ppm in *bino* patterns.

The DCI mass spectrum of (**II**) (Fig. 4) was recorded on a RIBERMAG R1010 quadrupole mass spectrometer. The molecular ion MH^+ is observed at m/z 736 (mol. wt. 735 with ^{35}Cl) with a satellite distribution showing 4 chlorine atoms in the molecule. Thus, mass spectrometry gave the answer: (**II**) is a *spiro* species and not a *bino* (which would be expected to have a molecular weight of 1080 with ^{35}Cl and 10 chlorine atoms in the molecule).

Then, the di-protected spermine links the cyclophosphazenic ring through its N4 and N8 positions leading to a spermino moiety in a *spiro* configuration, the two primary amino functions of the spermino entity each being in a *dangling* situation. In other words, the di-protected spermine gives a *spiro-bidangling* derivative.

CONCLUSION

Skillful handling of both spermidine and spermine when protected at their primary amino functions leads to unique forced configurations (Fig. 5) upon reaction with hexachlorocyclotriphosphazene, $N_3P_3Cl_6$. The former reacts as a secondary mono-amine, yielding a *bi-dangling* structure. The two secondary

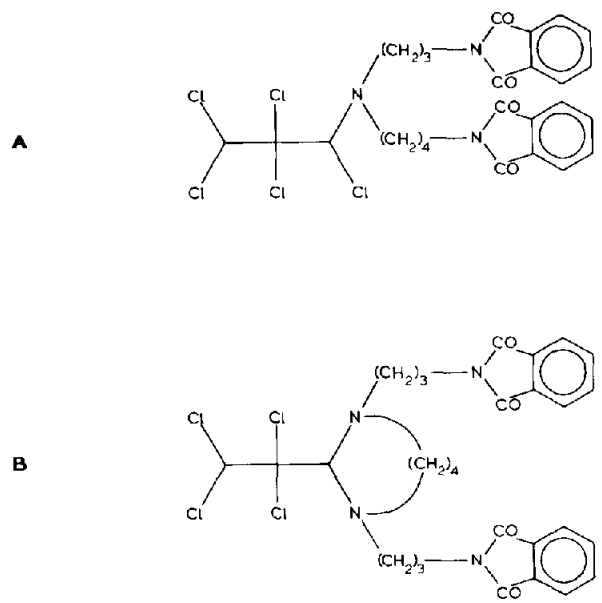

Fig. 5. The two *bi-dangling* (A) and *spiro-bidangling* (B) forced configurations from spermidine and spermine.

amino functions of the latter link $N_3P_3Cl_6$ in a *spiro* configuration, the two protected primary amino groups each being in a *dangling* situation. Thus, polyamines may now be linked on demand to cyclophosphazenes either in innate or in forced configurations.

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