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The Gem-tetraziridinocyclotriphosphazene Diazide, $N_3P_3Az_4(N_3)_2$, a Synthon to Monophosphazenylmonoazido Derivatives of Biological Interest

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Abstract

This contribution reports on the synthesis of the gem-tetraziridinodiazidocyclotriphosphazene which is a suitable synthon for the synthesis of monophosphazenylmonoazidocyclotriphosphazenes of biological interest.

Introduction

The gem-tetraziridinocyclotriphosphazenes, $N_3P_3Az_4XY$, are both anticancer agents [1] and immunomodulators [2] whatever X and Y are. However, pharmacokinetics reveal that their selectivity for malignant cells is commonly poor, a large amount of the injected dose 'missing the target' and being actually spread out over the body without effective therapeutic benefits. Also, penalizing side-effects happen which dramatically limit clinical treatments.

Efforts have been made during the last few years to enhance the selectivity of these drugs through covalent binding to biogenic polyamines (mainly 1,3-diaminopropane, putrescine, cadaverine, spermidine and spermine) [3], polyamines playing the role of tumor finders. The benefits from such a targeting for the gem-$N_3P_3Az_4Cl_2$ are now well documented, mainly about therapeutic index and proper toxicity [4, 5].

Another way to attempt the production of more selective drugs is the covalent binding of 'nude' drugs to monoclonal antibodies [6]. The chemical principle of such a linkage requires the presence of a specific hook, either an NH_2 or an N_3 group, in the molecular structure of the nude drug, for hitching on carbonyl groups of immunoglobulins.

This contribution reports on the synthesis of the gem-tetraziridinocyclotriphosphazene diazide **I**, gem-$N_3P_3Az_4(N_3)_2$, as a synthon to monophosphazenylmonoazido nude drugs which could be convenient for covalent linkage to antibodies.

Experimental

Synthesis and Identification of I

Synthesis of **I** was carried out by reaction of sodium azide, NaN_3, on the gem-tetraziridinodichlorocyclotriphosphazene, gem-$N_3P_3Az_4Cl_2$, in refluxing acetonitrile:

$$N_3P_3Az_4Cl_2 + 2NaN_3 \xrightarrow{MeCN} N_3P_3Az_4(N_3)_2 + 2NaCl \quad (1)$$

Such a reaction proceeds under heterogeneous conditions, since NaN_3 is poorly soluble in acetonitrile. However, the final diazidocyclotriphosphazene **I** is obtained quite easily, in contrast to the reaction using Me_3SiN_3 as azido reactant, where the persubstitution of Cl atoms by N_3 groups cannot be achieved. Reaction (1) took a few hours and persubstitution was checked by ^{31}P NMR. NaCl was then filtered off and the solvent removed *in vacuo* at ambient temperature. Compound **I** is a colourless oil, its oily state being probably due to an acetonitrile clathration. The gem-diazido structure was assigned by IR spectroscopy ($\nu(N_3) = 2120$ cm^{-1}, KBr cell, Perkin-Elmer 683) and by ^{31}P NMR. The NMR spectrum of **I**, as recorded at 32.4 MHz (Bruker AC 80 FT), is an A_2B system: the doublet and the triplet centered on 36 and 14 ppm (in C_6D_6 with H_3PO_4 85% as the standard) correspond to PAz_2 and $P(N_3)_2$ moieties, respectively. The coupling constant $^2J_{AB}$ is equal to 38 Hz.

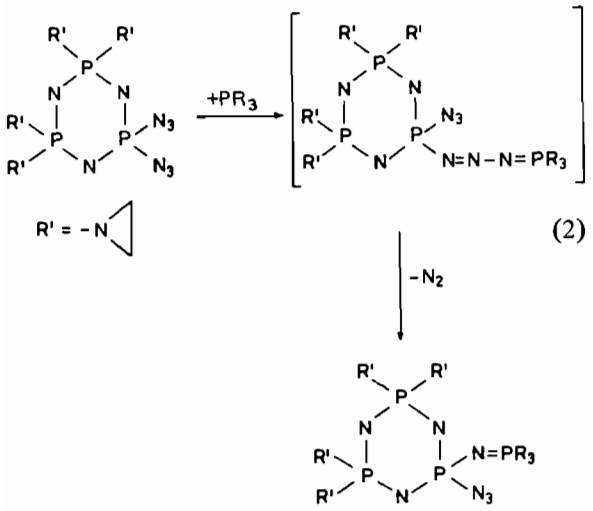
0020-1693/87/$3.50

Incidentally, the pentaziridinomonoazidocyclotriphosphazene, $N_3P_3Az_5(N_3)$, can be prepared in the same way from $N_3P_3Az_5Cl$ (δPAz_2 = 36.5 ppm, $\delta PAzN_3$ = 27.0 ppm, $^2J_{PP}$ = 34 Hz in C_6D_6).

Thus, reaction of NaN_3 on suitable chlorinated aziridinocyclophosphazenes leads to two new species whith the field of azido derivatives of $N_3P_3Cl_6$, which is up-to-now rather poorly documented: only three such compounds have been reported in literature, namely $N_3P_3(N_3)_6$ [7], $N_3P_3(NH_2)_2(N_3)_4$ [8] and $N_3P_3(Ph)_5(N_3)$ [9].

Synthesis and Identification of Phosphazenyl Derivatives of I

The Staudinger and Meyer reaction [10] of suitable R_3P derivatives on **I** leads to monophosphazenylmonoazidotetraziridinocyclotriphosphazenes **II** to **V** of Table I, according to the following scheme:

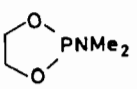


(2)

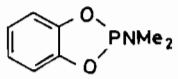
Such a reaction, when proceeding under 1:1 stoichiometric conditions with acetonitrile (or benzene) as the solvent, leads to the expected monophosphazenyl derivatives (νN_3 = 2120 cm^{-1}, KBr disk, Perkin-Elmer 683) as white microcrystalline powders. ^{31}P NMR data are reported in Table I.

It is noteworthy that the intermediate adduct of reaction (2) could be observed in two cases, *i.e.* with Ph_3P and $(Me_2N)_3P$, when the reaction is conducted with the first step at room temperature. ^{31}P NMR data of these two adducts, **II'** and **III'**, are reported in Table II. Such an observation of the Staudinger intermediate is common with $(Me_2N)_3P$ [11] but quite scarce for Ph_3P.

Thus, the four R_3P starting materials reported here do not behave in the same way when they are added in a large excess to **I**: under such conditions, Ph_3P leads to the monophosphazenyl species **II**, whereas $(Me_2N)_3P$ and the dioxaphospholane

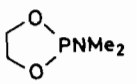


yield mixtures of mono- *and* diphosphazenyl compounds, as demonstrated by ^{31}P NMR. Incidentally, the fourth R_3P starting material, namely the orthodiphenylenedioxaphospholane



gives complex mixtures of monomeric and probably dimeric final products when reacting in large excess with **I**.

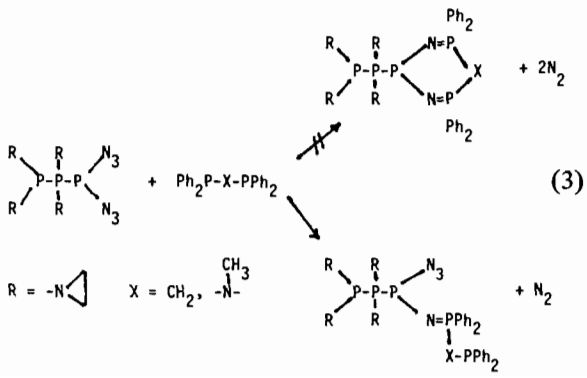
In other words, $(Me_2N)_3P$ is more nucleophilic than Ph_3P *versus* the remaining azido function of compounds **II** to **V**. This conclusion is supported by the fact that $(Me_2N)_3P$ and



react with **II**, in contrast with Ph_3P, to give the fused diphosphazenyl derivatives **VI** and **VII** (Table III).

Anyhow, the graft of one and/or two phosphazenyl groups (–N=PR_3) on the gem-$N_3P_3Az_4Cl_2$ through its azido derivatives as intermediates is facile, actually much easier than when following the synthetic route recently reported by Krishnamurthy [12] for $N_3P_3Az_5(N=PPh_3)$. The work reported here constitutes a new way for the synthesis of phosphazenylcyclotriphosphazenes amongst many other pathways [13–17].

Because of the poor nucleophilic character of Ph_3P and the reactivity of the second azido group within the series of phosphines studied here, it was of interest to investigate the reaction of diphosphinic reagents $Ph_2P–X–PPh_2$ (X = CH_2 and NMe) on compound **I**, in an attempt to prepare new spiro derivatives of aziridinocyclotriphosphazenes [3]. Actually, reaction occurs through an open linkage of the diphosphine, as demonstrated by NMR spectroscopy (compounds **VIII** and **IX** of Table III).



(3)

TABLE I. ^{31}P NMR Data of Compounds II to V.

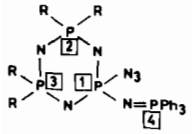
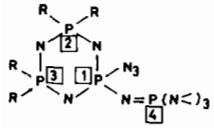
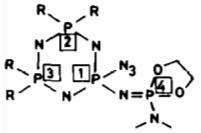
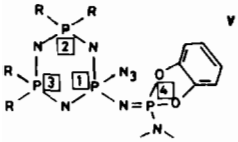
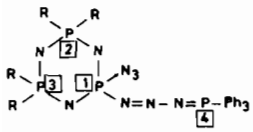
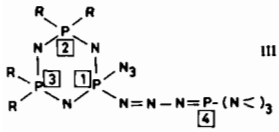
		δ_1	$\delta_2\delta_3$	δ_4	$J_{12}J_{13}$	J_{14}	$J_{42}J_{43}$
	II	*2t* 9.7 9.4	*2d* 38.5 38.6	*2t* 11.6 11.3	36	27	2.5
	III	*2t* 2.7 0.9	*2d* 36.5 36.3	*2t* 20.6 18.8	39	59	2.9
	IV	*2t* 5.4 6.9	*2d* 36.7 36.8	*2t* 25.5 27.0	38	50	3.8
	V	*2t* 6.0 6.5	*2d* 38.5 38.6	*2t* 18.5 17.1	37	52	3.6

TABLE II. ^{31}P NMR Data of Intermediates II' and III'

		δ_1	$\delta_2 = \delta_3$	δ_4	$J_{12} = J_{13}$
	II'	*1t* 9	*1d* 37	*1s* 25	35
	III'	*1t* 19	*1d* 37	*1s* 41	34

Thus, the poor nucleophilic character of $(Ph_2)P$ groups is once more revealed.

Mass Spectrometry

Spectra were recorded on an R1010 Ribermag Quadrupole Mass Spectrometer using a direct inlet system. The source temperature was 150 °C. Spectra were analysed by means of a DEC PDP 8/M computer and stored on disk. A small sample (~1 μg) was introduced into the probe, the temperature of which was then gradually increased from ambient to 100 °C, taking care that neither the electron multiplier nor the amplifier is in a saturated condition at any time. The areas under the curves corresponding to the current carried by the various ions were calculated by computer.

Mass spectra of compounds **II** ($M = 621.5$) and **III** ($M = 521.3$) are reported in Figs. 1 and 2. They are very simple and we shall detail the spectrum of **II** as an example.

TABLE III. ^{31}P NMR Data of Compounds VI to IX

	δ_1	$\delta_2\delta_3$	δ_4	δ_5	J_{12}	J_{14}	J_{45}	J_{15}
VI	*4t* 4–10	*1d* 36	*1d* 18.7	*1d* 0.3	40	33		12
VII	*4t* 2.2 2.4 2.4 3.0	*1d* 37.5	*1d* 21	*1d* 1.0	40	32		16
VIII	*2t* 9.0 10.0	*1d* 35.9	*2d* 11.7 12.4	*1d* −29.6	34	21	63	
IX	*2t* 2.2 3.4	*1d* 36.1	*2d* 18.3 21.3	*1d* 51.9	36	36	97	

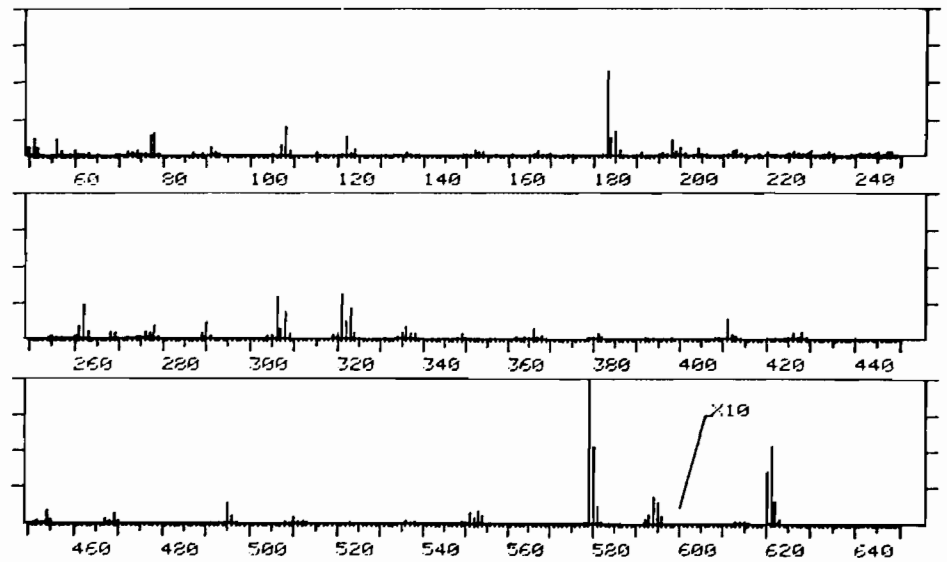


Fig. 1. Mass spectrum of compound II.

The molecular ion M^+ is observed at *m/z* 621 (5.3%) with two satellites at *m/z* 622 (1.5%) and 620 (3%).

The main fragmentation route involves the successive loss of one N_2 molecule as a whole (*m/z* 594, 17.9%), one N_3 ligand (*m/z* 579, 100%, base peak), and one and two pairs of Az groups giving maximal peaks at *m/z* 495 (13.4%) and 411 (14.0%). Afterwards, the three phenyl groups leave as a whole, leading to the $N_3P_3NPH_3$ fragment (*m/z* 183, 57.5%)

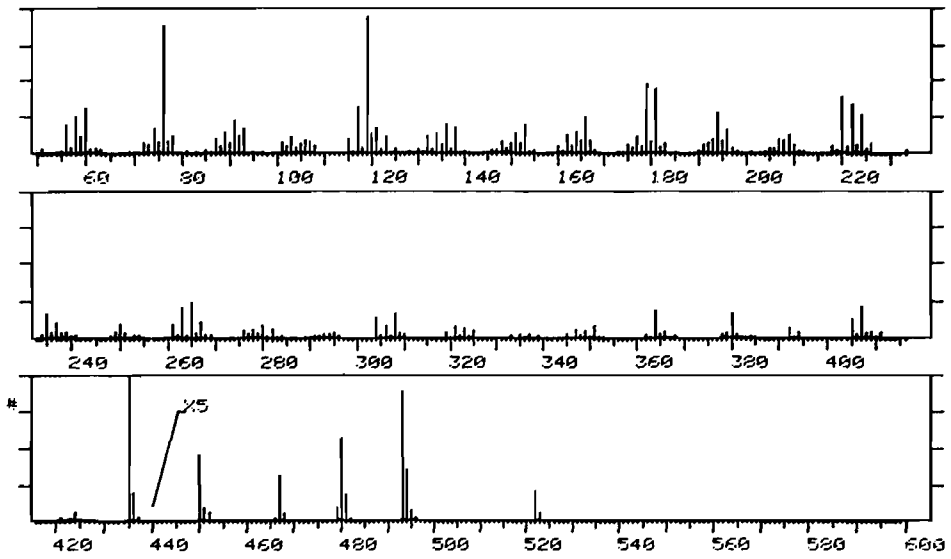


Fig. 2. Mass spectrum of compound **III**.

which looses one PH_3 molecule (*m/z* 149, 1.2%) and then its N atom (*m/z* 135, 2.2%). The intensity of all other peaks is less than 0.5%.

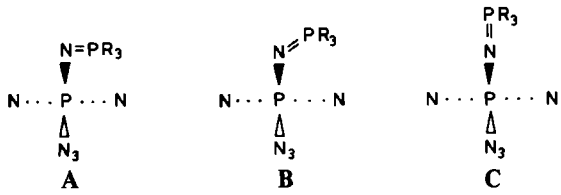
EI mass spectra of **IV** and **V** could not be recorded owing to their extremely weak solubility in any organic solvent. Further attempts were however made with these insoluble compounds by using ^{252}Cf-Plasma Desorption Mass Spectrometry [18], but they were unsuccessful, except for compound **II** in which traces of the gem-diphosphazenyl derivative $N_3P_3Az_4(-N{=}PPh_3)_2$ were detected at *m/z* 855 (1.2%). Such detection of an impurity weighing more than 700 is really an advantage of this new technique, neither the EI nor the DCI mass spectrometry being able to reveal in routine such impurities with large molecular weights.

NMR Spectroscopy and Molecular Conformation

^{31}P NMR spectroscopy at 32.4 MHz was able to assign unambiguously the structure of **I**. The situation is less favourable when an exocyclic P atom exists in the molecular structure, as is the case for phosphazenyl derivatives of **I**.

Then, ^{31}P NMR spectra were recorded at 101.27 MHz (Bruker WM 250) and some interesting features were observed, mainly about the value of $^4J_{PP}$ in **II** to **V**, which varies from 2.5 to 3.6 Hz. Figure 3 displays four spectra (as examples) which can be analysed as first-order patterns.

According to Shaw [19], three different conformations, coded as **A**, **B** and **C**, may exist for a phosphazenyl group linked to a N_3P_3 ring, depending on the value of the $N{-}P_1{-}N{=}P_4$ dihedral angle: ±90° for **A**, 45° or 135° for **B**, and 0° or 180° for **C**.



The $^4J_{PP}$ value reveals the conformation adopted by the phosphazenyl ligand: it varies from 3.3 to 5.7 Hz for **A** to 0 Hz for **C**. Experimental values for compounds **II** to **V**, being within the 2.5–3.6 Hz range, would suggest that the phosphazenyl group in these derivatives could be in the conformation **B** which would be stabilized by some dipole-dipole intramolecular interactions.

In contrast, $^4J_{PP}$ is equal to zero in compound **VI**, suggesting a conformation **C** for the two phosphazenyl groups, probably for steric reasons.

Conclusion

We report on the synthesis of the gem-tetraziridinodiazidocyclotriphosphazene which is a suitable synthon for the synthesis of monophosphazenyl-monoazidocyclotriphosphazenes of biological interest. These molecules contain in their molecular structure a labile N_3 group which is convenient for covalent binding to monoclonal antibodies. The reactivity of the remaining azido group in these compounds depends on the nature of the phosphine involved in the Staudinger process. Such azido derivatives may open new synthetic routes which are not accessible from their chlorinated parents.

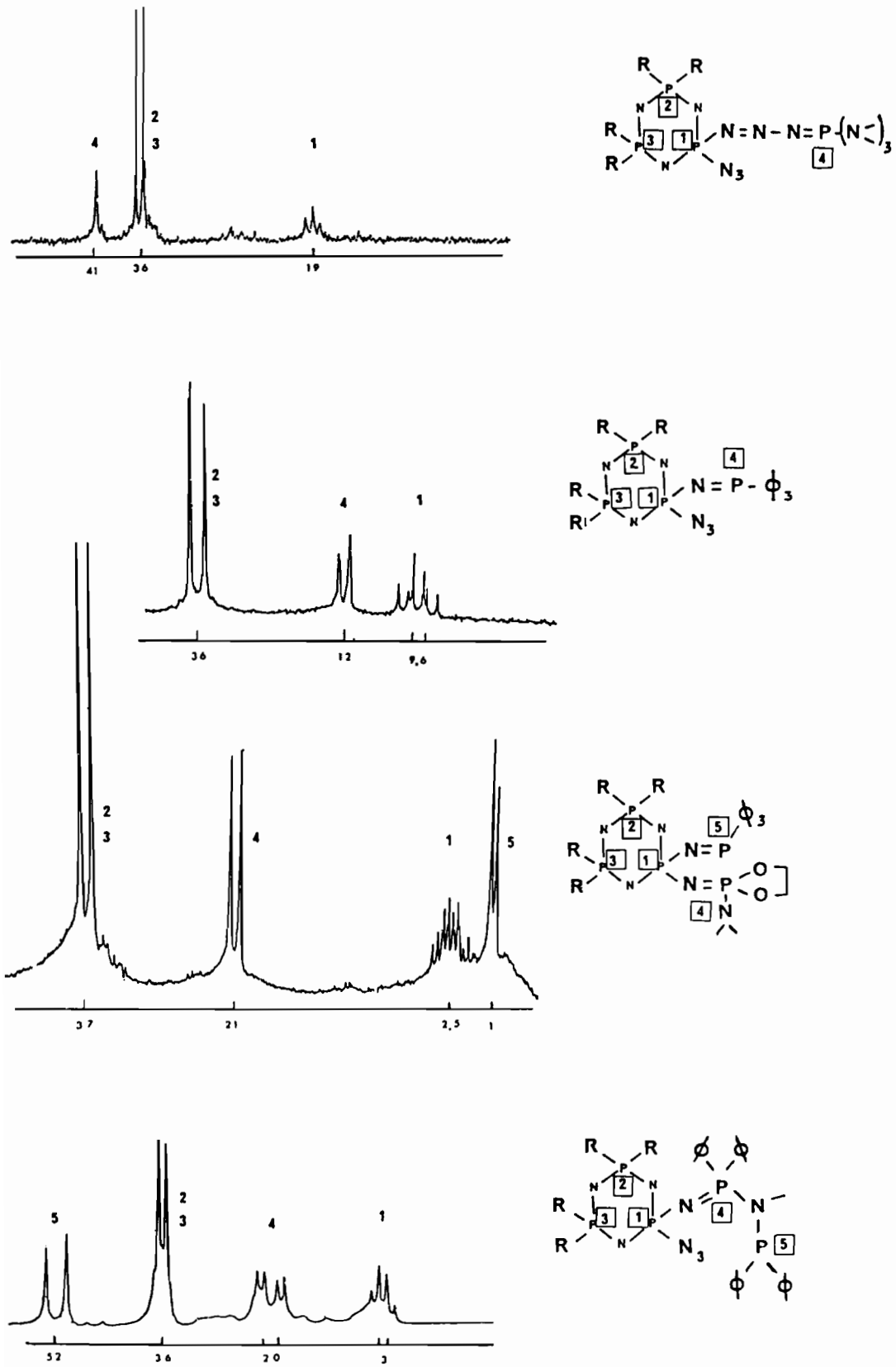


Fig. 3. ^{31}P NMR data (101.27 MHz) of compounds III, II, VII and IX.

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