A Through Route for the Synthesis of Pure 2,2,4,4-Tetrakis(aziridinyl)-6,-amino-6,-methoxycyclotriphosphaza-1,3,5-triene,gem-$N_3P_3AZ_4(NH_2)(OCH_3)$

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Introduction

Interest in inorganic ring systems as anticancer drugs has recently been enhanced by the finding that the aziridinocyclophosphazenes $N_3P_3Az_6$ and $N_4P_4Az_8$ (Az = Aziridinyl) [1–4] and the aziridinocyclodiphosphathiazene $N_3P_2SOAz_5$ and its relatives [4–7] were active on a large series of experimental neoplasms.

In subsequent studies conducted with the E.O.R.T.C. Screening and Pharmacology Group, and employing a range of rodent neoplasms including leukemias and solid tumors of different histological nature, growth rate and chemotherapeutic sensitivity, $N_3P_3Az_6$ (code name MYKO 63) and especially $N_3P_2SOAz_5$ (code name SOAz) were found to be the most effective through the series.

In view of the fact that SOAz was non-mutagenic for various bacterial systems and in preliminary tests in dogs and monkeys showed no significant nephro-, hepato- or cardiotoxicity and little hematotoxicity, it was of interest (i) to explore the biological activity of additional molecules, and (ii) to design suitable derivatives of MYKO 63 and SOAz which could be grafted on some selective antibodies of various tumors with the aim of improving the selectivity of anticancer cyclophosphazenes *versus* malignant cells.

Concerning (i), we have recently reported [8] on the antineoplastic activity of 2,2,4,4-tetrakis-(Aziridinyl)6,6,-dichlorocyclotriphosphaza-1,3,5-triene, gem-$N_3P_3Az_4Cl_2$ (code name MYCLAz), which appeared as a promising novel anticancer agent and which constitutes a suitable starting material for the synthesis of synergic combinations with other antitumor drugs. Incidentally, the antitumor activity is not dramatically altered when passing from MYKO 63 and SOAz to MYCLAz, despite the fact that the number of aziridinyl rings decreases from six to four within the series. In other words, the presence of four gem-linked Az ligands is enough for antitumour activity.

Concerning (ii), a possible way was to prepare some *monomethoxy* derivatives of our drugs. It is well known [9] that OCH_3 groups of the drug can be easily removed upon interaction with several antibodies, the linkage between the drug and the antibody occurring through the OCH_3-bearing phosphorus atom.

Chemically speaking, there is no possibility for preparing methoxy derivatives of MYKO 63 and SOAz, at least in large quantities and in a pure state. On the other hand, MYCLAz contains two labile chlorine atoms which can be substituted by methoxy groups in a very facile way. However, the presence of *two* OCH_3 groups on the same phosphorus induces intricate side-reactions with antibodies which do not occur with *mono*methoxy cyclophosphazenes.

This contribution deals with an unforeseen through route for the synthesis of such a monomethoxy derivative of MYCLAz and with some of its physico-chemical properties.

Synthesis and Purity

A solution of 500 mg of MYCLAz in anhydrous methanol (15 ml) is saturated with gaseous dry NH_3 during 15 min and kept at 80 °C overnight in a sealed tube The reaction mixture was then allowed to stand at room temperature for 1 hr. The solvent was removed *in vacuo* at room temperature. The residue was taken up in CH_2Cl_2 and filtered in order to remove NH_4Cl. The filtrate contains two products as indicated by thin-layer chromatography (Rf = 0.32 and 0.40 with 90% CH_2Cl_2/10% CH_3OH as eluent and vapour of iodine as revealing), which are separated by column chromatography (silica gel). The major product (Rf = 0.40) was identified as the title derivative (see below), MP = 107–108 °C. The minor product, $N_3P_3Az_4(NH_2)_2$, is firmly retained by the column and needs pure methanol to be extracted, with a poor yield.

Incidentally, MYCLAz does not react with NH_3 in CH_2Cl_2 at room temperature. In other words, methanol is necessary for the reaction. Furthermore, the yield of the reaction increases sharply from 20 °C to 80 °C but then decreases sharply with formation of sticky resins which harden and become insoluble on exposure to atmospheric moisture or on storing for 2–3 days.

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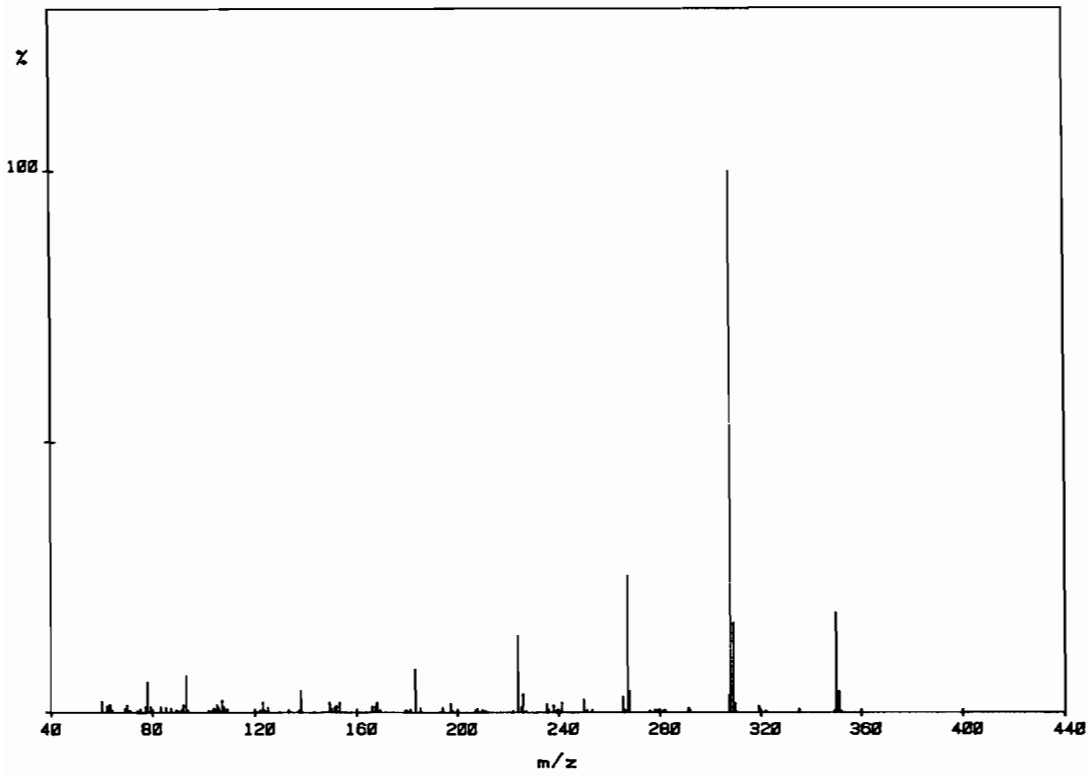

Fig. 1. 70 eV electron impact mass spectrum of the title compound.

Mass Spectrometry

The spectra were recorded on a R1010 Ribermag quadrupole mass spectrometer, using a direct inlet system. The source temperature was 150 °C, electron energy 70 eV. The spectra were recorded by means of a DEC PDP 8M computer and stored on disk. About 1 μg of sample was introduced into the probe. The probe temperature was then slowly and continously increased from ambient temperature to 100 °C, taking care that neither the electron multiplier nor the amplifier were in a saturated condition at any time. The area of the curves corresponding to the current carried by the selected ions were calculated by the computer.

The 70 eV electron impact mass spectrum is presented in Fig. 1.

The molecular ion is observed at m/z 350. One main fragmentation route is detected: an aziridino radical (42 mass units) is expelled giving the base peak at m/z 308. Further consecutive losses of the aziridino substituents give peaks at m/z 267 (loss of 41 mass units *i.e.* Az minus H), 224 and 183, each of them being associated with H-transfers. Minor peaks of very low intensity (less than 2%) are also detected which correspond to the loss of either NH_2 or OCH_3 moieties from the main fragments mentioned above

From the simplicity of this spectrum, it was quite easy to check the purity of the sample by looking at eventual superimposition of peaks due to possible contaminants. In this way, we did not observe any peak related either to MYCLAz (m/z 373) or to di-substituted $N_3P_3Az_4(NH_2)_2$ (m/z 335) and $N_3P_3Az_4(OCH_3)_2$ (m/z 365) terms.

Thus, mass spectrometry appeared to be a very adequate tool for controlling the purity of the title compound in an unambiguous way, as was demonstrated previously in the case of other cyclophosphazenes for biological uses [8, 10].

NMR Spectroscopy

The ^{31}P NMR spectrum of the title compound was recorded on a Brucker WH 90 instrument. The doublet at 38.66 and 37.45 ppm corresponds to the PAz_2 entity and the triplet at 24.70, 23.57 and 22.44 ppm at the $P(NH_2)(OCH_3)$ moiety (intensity ratio 2:1). No trace of MYCLAz was ever detected (doublet at 35.89 and 35.21 ppm and triplet around 25.1 ppm) [8]. Data reported here are consistent with very accurate data recently published [6] for other aziridinocyclotriphosphazenes.

Infrared Spectroscopy

The infrared spectrum (KBr disks) was recorded at room temperature on a microprocessor-assisted

TABLE I. IR Vibrational Frequencies of the Title Compound (Solid State).

Frequency	Tentative Assignment
495 m	$\rho_{\omega}(NC_2)$ + PN_{exo} (IP deformation)
635 s	$\nu_{as}(PAz_2)$ + $\rho_{\omega,s}(PAz_2)$
698 s	Ring deformation IP, δ_s(NPN)
795 m	Ring breathing, ν_s(PN)
810 w, 820 m, 840 m	Ring deformation IP, $\delta_s(NC_2)$ / $\rho_{T,s}(CH_2)$ + $\rho_{r,as}(CH_2)$
870 s	ν_s(PN)
930 s, 945 s, 960 m	$\rho_{\omega,s}(CH_2)$
1037 s	ν_{as}(PN)
1080 m	$\rho_{T,s}(CH_2)$
1150 m	$\rho_{\omega,as}(CH_2)$ + ν_{as}(PN)
1190 vs, 1210 vs	ν_{as}(PN) + $\rho_{T,as}(CH_2)$
1255 s	$\nu_{as}(NC_2)$
1440 w	$\delta_{as}(CH_2)$
1460 vw	$\delta_s(CH_2)$
2985 m	ν_{as}(CH)
3060 w	ν_s(CH)
3260 m	$\nu_s(NH_2)$
3370 m	$\nu_{as}(NH_2)$

Perkin-Elmer 683 spectrometer (Range 4000–200 cm^{-1} calibration with polystyrene lines). This spectrum was assigned (Table I) by comparison with previous data from our own works [11–15].

Conclusion

Reaction of gem-$N_3P_3Az_4Cl_2$ with NH_3 in methanol provides a through route for the synthesis of pure gem-$N_3P_3Az_4(NH_2)(OCH_3)$ with a high yield. Antitumoral tests *in vivo* on rodent neoplasms of this chemical are now in progress, as well as attempts for linking it to specific antibodies of human tumors.

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