A High Dilution Route for the Synthesis of Pure 2,2,4,4-Tetrakis(aziridinyl)-6,6-diaminocyclotriphosphazene, GEM-$N_3P_3Az_4(NH_2)_2$

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Received February 16, 1984

Introduction

Interest in inorganic ring systems as anticancer drugs has been enhanced by finding that the aziridinocyclophosphazenes $N_3P_3Az_6$ and $N_4P_4Az_8$ (Az = Aziridinyl) [1–3] 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 of Mario Negri Institute (Milan, Italy) and employing a wide 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 $N_3P_2SOAz_5$ (code name SOAz) were found to be most effective.

In view of the fact that SOAz was non-mutagenic for various bacterial systems [8] and in preliminary tests in dogs, monkeys and humans [9] showed no significant nephro-, hepato- or cardiotoxicity and controllable leucopenia, it was of interest to explore the biological activity of suitable derivatives of MYKO 63 and SOAz when grafted on some tumor finders (natural polyamines) and/or selective antibodies with the aim of improving the selectivity of these anticancer cyclophosphazenes *versus* malignant cells and, consequently, of decreasing their unique

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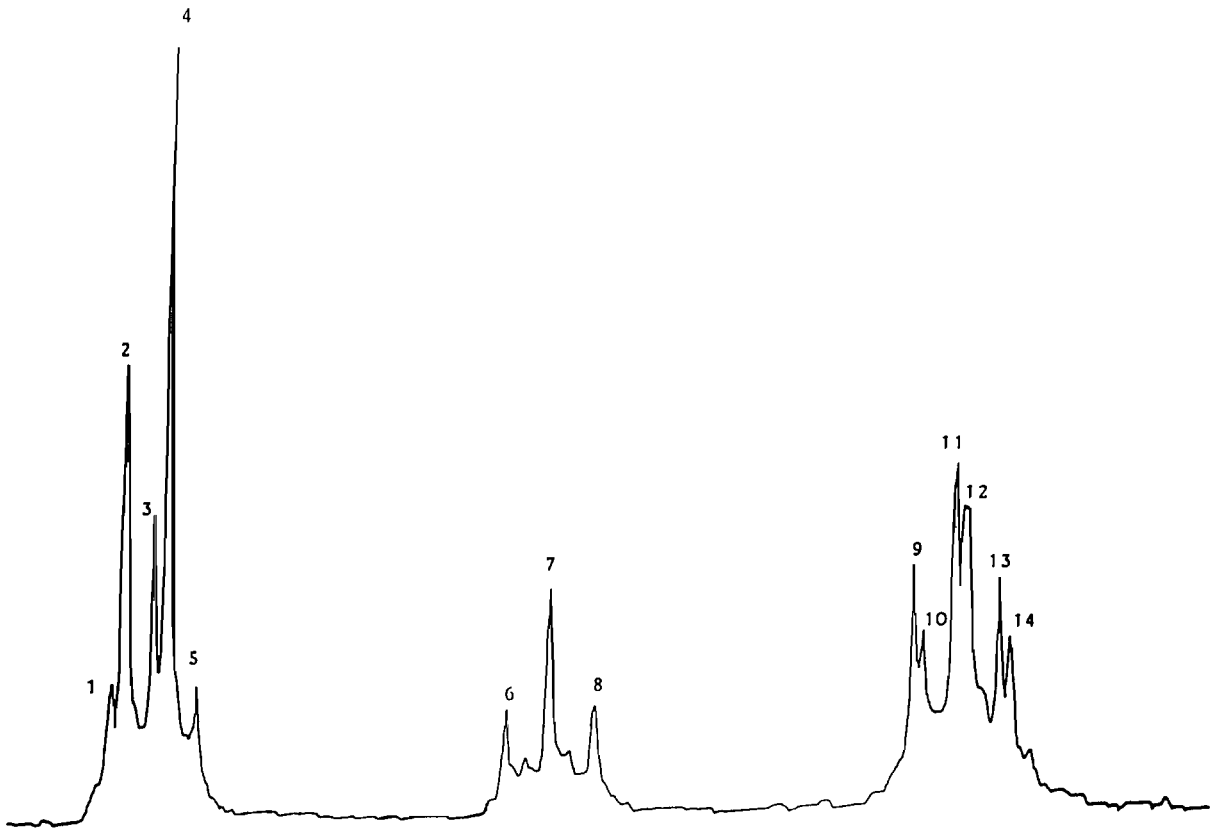

Fig. 1. ^{31}P nmr spectrum (BRUCKER WH 90) of the A + B mixture.

1	1402.70	38.49	380.37	6	1061.49	29.13	255.58	11	670.28	18.39	921.02
2	1393.88	38.25	1221.54	7	1023.26	28.08	631.64	12	664.40	18.23	1022.83
3	1367.41	37.52	681.98	8	985.02	27.03	264.04	13	634.98	17.42	394.16
4	1355.64	37.20	1553.13	9	708.52	19.44	663.22	14	626.16	17.18	383.93
5	1332.11	36.55	226.83	10	699.70	19.20	446.93				

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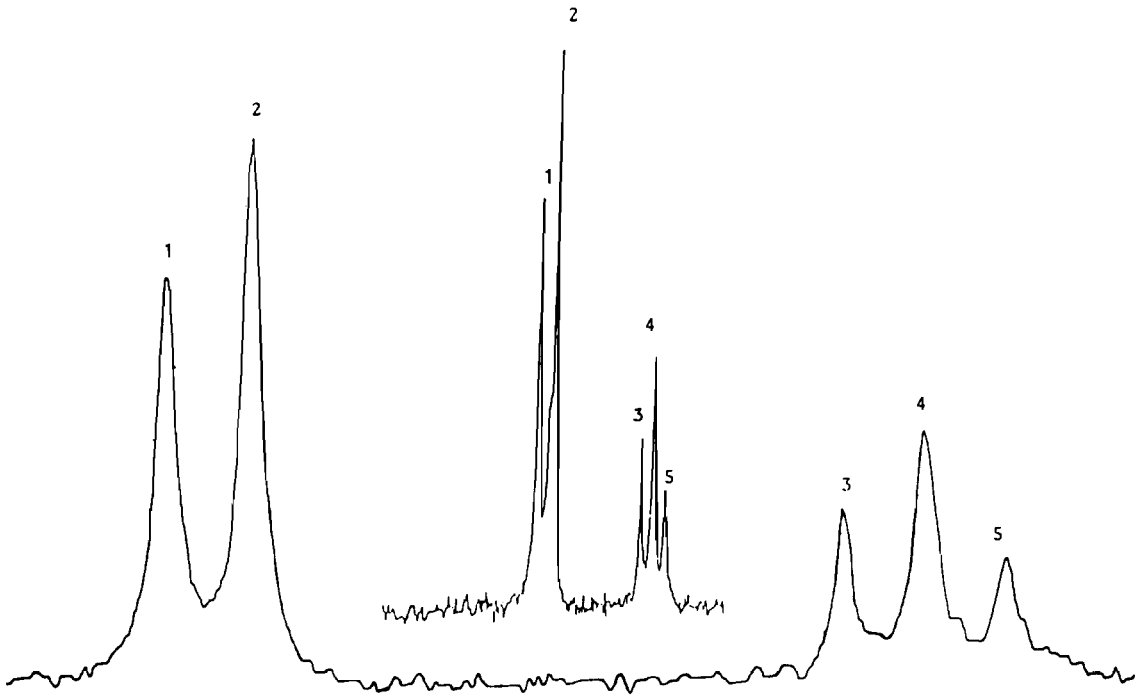

Fig. 2. ^{31}P nmr spectrum (BRUCKER WH 90) of pure A from high dilution technique.

1	1399.47	38.40	356.07
2	1361.23	37.35	335.21
3	717.06	19.67	87.49
4	678.82	18.62	202.61
5	643.52	17.65	66.77

dose-dependent side-effect, *i.e.* thrombocytopenia.

Benefits from such a targeting through polyamines were recently demonstrated about the antineoplastic activity of 2,2,4,4-Tetrakis(aziridinyl)6,6-dichlorocyclotriphosphazene, gem-$N_3P_3Az_4Cl_2$ (code name MYCLAz) which had appeared as a promising MYKO 63-like novel anticancer agent [10] and which constituted a suitable starting material for linkage to natural polyamines [11–15]. We demonstrated on this opportunity that polyamines represent a useful means for targeting to neoplastic tissues compounds which possess cell inhibitory capacity [16].

Concerning the targeting through selective monoclonal antibodies [17] we had to prepare some mono- and/or diamino derivatives of our drugs. It is well-known [18] that NH_2 groups of the drug can be easily removed upon interaction with carbonyl groups of immunoglobulins, the linkage between the drug and the antibody occuring through (C=N) bonds.

We reported recently [19] on the synthesis of pure 2,2,4,4-Tetrakis(aziridinyl)-6,-amino-6,-methoxy-cyclotriphosphazene, gem-$N_3P_3Az_4(NH_2)(OCH_3)$ which can be linked to antibodies through its amino group. The presence of the methoxy group seems however to induce some intricate side reactions which do not occur with pure aminocyclotriphosphazenes.

This contribution deals with a route for the synthesis of pure aminocyclotriphosphazene, *i.e.* gem-$N_3P_3Az_4(NH_2)_2$, by using a high dilution technique.

Synthesis and Purity: Low Dilution *versus* High Dilution Results

The synthesis of the diaminotetrachloro starting material, gem$N_3P_3Cl_4(NH_2)_2$, is trivial [20]: ammonia gas is bubbled (ambient, 4 hours) through a solution of $N_3P_3Cl_6$ in ether or dioxane.

Aziridinolysis of this chlorinated starting material (P) was originally performed as following. 155.3 mmole of aziridine in 50 ml of CH_2Cl_2 is added dropwise in two hours to a mixture of 38.8 mmole of (P) and 186 mmole of anhydrous Et_3N in 400 ml of CH_2Cl_2. The medium is stirred under argon pressure in an ice-bath during 2 days and the reaction is considered completed when the nmr PCl_2 doublet of (P) (21.61 and 20.16 ppm, BRUCKER WH 250) has disappeared. Hydrochlorides are then filtered off, solvent is removed *in vacuo* to give a sticky light yellow powder. T.l.c. of this crude product reveals 2 spots: $Rf_A = 0.75$ and $Rf_B = 0.70$ with a 4:1 mixture of ether and methanol as eluant and iodine vapor as developer.

The ^{31}P nmr spectrum of this A + B mixture at 36.44 MHz is shown in Fig. 1 (BRUCKER WH 90). The triplet at 29.13, 28.08 and 27.03 ppm and the doublet at 38.25 and 37.20 ppm reveal the existence in the mixture of a large amount of 2,2,4,4,6-pentakis(aziridinyl)-6,-aminocyclotriphosphazene, $N_3P_3Az_5(NH_2)$ [21]. The expected gem-$N_3P_3Az_4(NH_2)_2$ compound is responsible for the 'split' triplet centered on 18.23 ppm and the false triplet (*i.e.* 2 doublets with overlap of one line of each) centered on 37.52 ppm. The splitting of the foreseeable A_2B-type structure for gem-$N_3P_3Az_4(NH_2)_2$ is presumably due to the existence of 2 conformers (50:50 ratio): coalescence indeed occurs in VTP nmr at 233 K.

Aziridinolysis of gem-$N_3P_3Cl_4(NH_2)_2$ in the experimental conditions described above yields a mixture of gem-$N_3P_3Az_4(NH_2)_2$ (2 conformers) and of a large amount of $N_3P_3Az_5(NH_2)$ as undesirable by-product. Aziridinolysis was repeated several times modifying the temperature of the reaction and/or the degree of dilution (*i.e.* the volume of CH_2Cl_2 in the medium). The yield in crude final product (and the A and B contents of it) is not dramatically modified within the −10 °C to +25 °C range. On the other hand, A, *i.e.* gem-$N_3P_3Az_4(NH_2)_2$, becomes the unique final product (t.l.c.: 0.75) as soon as the volume of CH_2Cl_2 in the medium is equal or larger to 1600 ml, *i.e.* four times larger than previously.

In other words, high dilution clarifies the matter: the ^{31}P nmr spectrum at 36.44 MHz in such conditions is shown in Fig. 2 where a first order A_2B pattern (one conformer only here) is revealed with a doublet at 38.40 and 37.35 ppm and a triplet at 19.67, 18.62 and 17.65 ppm.

Conclusions

Thus, aziridinolysis of gem-$N_3P_3Az_4(NH_2)_2$ takes advantage of high dilution for providing the gem-$N_3P_3Az_4(NH_2)_2$ expected derivative in a pure state.

Several classical aminolysis reactions in cyclophosphazenes are now currently being re-investigated in our laboratory within high dilution conditions, with the aim of preparing pure amino derivatives.

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