Inorganic Ring Systems of Physiological Importance. Part III[+]: Structure and Cytostatic Activity *in vitro* of Aziridino Cyclophospha(thia)zenes

A. A. VAN DER HUIZEN, J. C. VAN DE GRAMPEL[++]

Laboratorium voor Anorganische Chemie, Rijksuniversiteit Groningen, Nijenborgh 16, 9747 AG Groningen, The Netherlands

W. AKKERMAN, P. LELIEVELD

Radiobiologisch Instituut TNO, Lange Kleiweg 151, 2288 GJ Rijswijk, The Netherlands

A. VAN DER MEER-KALVERKAMP and H. B. LAMBERTS

Laboratorium voor Radiopathologie, Rijksuniversiteit Groningen, Bloemsingel 1, 9713 BZ Groningen, The Netherlands

Received October 28, 1982

The testing for cytostatic activity of a series of 36 aziridino derivatives of $(NPCl_2)_3$ and $(NPCl_2)_2NSOCl$ gives similar results in two different in vitro *screening systems.*

Evaluation of the dose causing 50 per cent inhibition of colony formation (ID50) in mouse leukaemia L1210 cells and the lowest active dose (LAD) established for the radiosensitive mouse lymphoma L5178Y leads to an insight into activating and deactivating structural requirements. The presence of aziridino $(N(CH_2)_2)$ groups with their alkylating ability and electron releasing substituents is essential for effective tumour growth inhibition. Hydrolytic instability results in loss of activity.

Introduction

The replacement of chlorine atoms in the inorganic ring systems $(NPCl_2)_3$ (I) and $(NPCl_2)_2NSOCl$ (II) by aziridino groups offers a possibility to prepare compounds of biological interest. Several derivatives have been prepared [1–12]. Some of them were investigated with respect to their toxicological and cytostatic properties.

The biological effects of the hexakisaziridino derivative of I, the so-called Apholate or Myko 63 (Fig. 1, XXXVII) have been the subject of numerous studies [13–17]. Its therapeutic potency as an antitumour agent has been proven on leukaemias P388 and L1210, and on B16 melanoma, all in mice [18].

Another derivative, investigated for its cytostatic and mutagenic behaviour, is the compound named Fotrin [4, 17, 19–21]. Compared with XXXVII in this compound one aziridino ligand is replaced by a morpholino residue (Fig. 1, XXXII). The agent has been used clinically in the USSR in the therapy of chronic lympholeukaemia and haemodermia. However, these data are rather poorly documented [4, 17, 22].

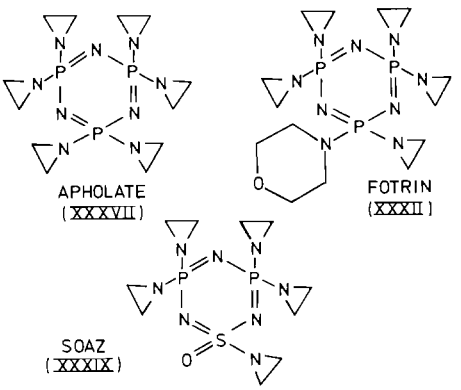

Fig. 1. Examples of cytostatic cyclophospha(thia)zenes.

Recently we reported the antitumour activity of some aziridino derivatives of II in mice [23]. The pentakisaziridino derivative, codename SOAz (Fig. 1, XXXIX), is in Phase I clinical trials [24].

In spite of the number of investigated compounds little is known about the mode of action in biological systems. Some structure activity relationships were proposed by Chernov *et al.* [13] and Safonova [17], assuming the compounds to be alkylating agents. The presence of at least four aziridino ligands would be necessary to get highly active compounds, whereas molecules with a *trans*-arrangement of ligands relative to the plane of the ring are considered more active than their *cis*-isomers. Amino-ester derivatives have been reported to show lower activity than the corresponding compounds with secondary amino residues [13, 17]. Although far from being complete the present paper intends to indicate and clarify the

[+]Part II: Ref. [1].

[++]Author to whom correspondence should be addressed.

0020-1693/83/$03.00

features that determine the cytostatic activity of this class of compounds. Because of their expected high activity mainly tetra- and pentakisaziridino derivatives are investigated. The geminally substituted compounds $N_3P_3Az_4R_2$ in particular offer the possibility of observing electronic substituent effects. To study the cytostatic activity of the derivatives prepared two different *in vitro* screening systems are used: the determination of 50 per cent growth inhibition doses (ID50) in a leukaemia L1210 clonogenic assay and of 'lowest active doses' (LAD) in mouse lymphoma L5178Y cells. The biological behaviour will be discussed in the context of substituent effects, which are expressed in terms of the substituent constants (a_R and γ_R) determined in basicity measurements of a large number of cyclophospha(thia)zenes [12, 25–27]. These constants represent the electron-releasing properties of side groups towards these ring systems. Relative hydrolytic stability as well as isomerism of the compounds investigated will also be considered.

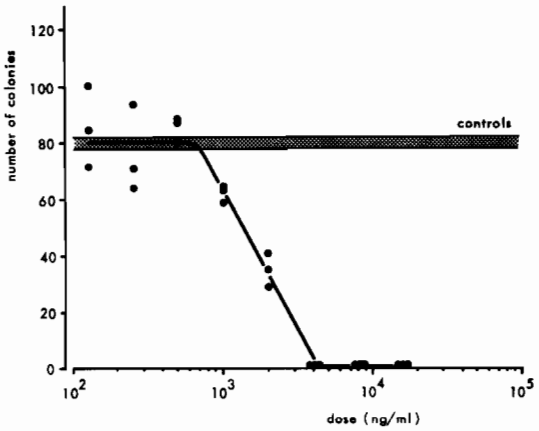

Fig. 2. Effect of gem-$N_3P_3Az_4Pip_2$ (XIX) against leukaemia L1210 in a clonogenic assay. Appropriate drug concentrations were added to 100 L1210 cells in culture dishes containing soft agar medium (3 dishes per drug concentration; 9 dishes for untreated controls). After the incubation period of 8 days colonies were counted.

Screening Methods

Determination of ID50 Values for Leukaemia L1210 Cells

Drug activity on leukaemia L1210 cells was determined using an *in vitro* clonogenic assay. From a suspension culture, 100 L1210 cells were plated into 35 mm culture dishes (Falcon) containing 1 ml of soft agar growth medium and the test compound in appropriate concentrations. The soft agar growth medium consisted of Dulbecco's medium supplemented with 20% horse serum, 4.75 mg l^{-1} 2-mercaptoethanol, 20 mg l^{-1} L-asparagine, 75 mg l^{-1} DEAE dextran (molecular weight 2×10^6) and 0.3% bacto-agar (Difco). The culture dishes were incubated at 37 °C in an atmosphere of 10% CO_2 in humidified air for 8 days. After this period of continuous drug exposure colonies were counted and dose effect curves were made (Fig. 2). From these curves the drug dose causing 50% inhibition of colony formation (ID50) relative to untreated control cells was calculated (Table I).

Determination of LAD Values for Lymphoma L-5178Y

The cytostatic activity of the agents on lymphoma L5178Y cells was determined using a recently described method by Lamberts *et al.* [28]. The cells were grown in a suspension culture, the medium consisting of RPMI 1640 enriched with 10% fetal calf serum. Penicillin and streptomycin were added as antibiotics. After filtration through a Millipore filter (ϕ 0.22 μ) 100 μl of solution of a compound in physiological saline or, as was sometimes required by the low solubility in water, in 96% ethanol, was serially diluted by a factor of 2 over the wells of a microtiter test plate (Cooke Microtiter System, type U). Addition of an equal volume of cell suspension containing 2×10^3 cells gave the final concentration range. A row of wells, only containing culture medium and cell suspension was used as control. The cells were propagated for four days at 37 °C in an incubator, adjusted to standard CO_2 percentage and humidity. After this incubation the lowest growth inhibiting dose was determined by comparing the diameter of each of the precipitation spots with that of the control (growth inhibition causes a decrease in diameter (See Fig. 3)). This gives the lowest active dose (LAD) values within an accuracy factor of 2, as listed in Table I.

Results and Discussion

The tumour cell growth inhibition by the 38 compounds tested, expressed as ID50 and LAD values

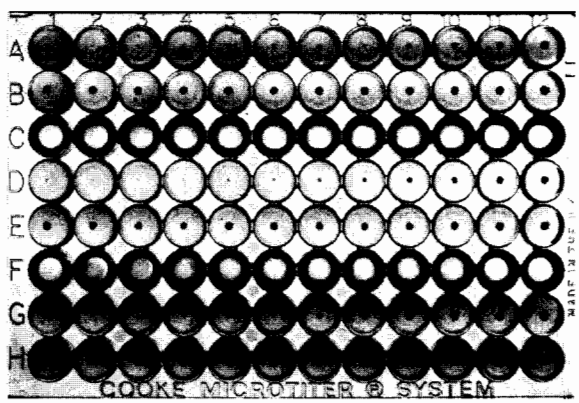

Fig. 3. Determination of LAD's on a test plate.

for the L1210 and L5178Y cells, respectively, ranges from $1.1–31.5 \times 10^{-6}$ mol l^{-1} for the former and from $0.25–250 \times 10^{-6}$ mol l^{-1} for the latter (Table I). Compound III does not possess any activity, whereas compound IV appears cytotoxic only towards L1210 cells. Direct comparison of data, obtained by such different methods, is hardly feasible. However, within both series of values the same trends can be observed.

The cytostatic activities of all compounds are listed together with the substituent constants a_R and γ_R ($R \neq Az$). Obviously, there is a relationship between $a_R(\gamma_R)$ and the ID50 or LAD values. Particularly, in the series with formula gem-$N_3P_3Az_4R_2$ (XI–XXII) and $N_3P_3Az_5R$ (XXIX–XXXVI) higher $a_R(\gamma_R)$ values (*i.e.* stronger electron-releasing capacity) lead to a higher activity for both the leukaemia and the lymphoma cells. It should be mentioned that in a series of bisaziridino-sym-triazines ($N_3C_3Az_2R$) Ihvin *et al.* [31] also found electron-donating side groups R to enhance the antitumour activity (*in vivo* experiments on sarcoma 45 and Walker sarcoma). Compounds XIV, XVI, and XXX do not fit this rule. This can be explained by their relatively high

TABLE I. Cytostatic Activity *in vitro* of Aziridino Cyclophospha(thia)zenes.

Formula[a]	Compound number	a_R[b] $R \neq Az$	γ_R[b]	Leukaemia L1210 ID50 (10^{-6} mol l^{-1})	Lymphoma L5178Y LAD (10^{-6} mol l^{-1})	Ref.[c]
$N_3P_3(GlyE)_6$	III	5.0[d]	2.5[d]	not active	not active	[29]
$N_3P_3Pyr_6$	IV	5.9	2.9	18.0	not active	[30]
gem-$N_3P_3Az_2(GlyE)_4$	V	5.0[d]	2.5[d]	31.5	250	[11]
$N_3P_3Az_2Pyr_2Cl_2$[e]	VI	5.9/0	2.9/0	13.6	250	[1]
trans-$N_3P_3Az_3Pyr_3$	VII	5.9	2.9	4.5	2	
cis-$N_3P_3Az_3Pyr_3$	VIII	5.9	2.9	2.1	2	
gem-$N_3P_3Az_4MorphCl$	IX	5.0/0	2.5/0	25.4	16	[1]
gem-$N_3P_3Az_4PipCl$	X	5.6/0	2.8/0	16.6	16	[1]
gem-$N_3P_3Az_4(OCH_2CF_3)_2$	XI	1.0	0.3	9.1	16	
gem-$N_3P_3Az_4Cl_2$	XII	0	0	8.9	8	[8, 9]
gem-$N_3P_3Az_4(OPh)_2$	XIII	3.1	1.3	7.6	8	
gem-$N_3P_3Az_4(GlyE)_2$	XIV	5.0[d]	2.5[d]	7.2	16	[11]
gem-$N_3P_3Az_4Morph_2$	XV	5.0	2.5	5.8	4	
gem-$N_3P_3Az_4(NH_2)_2$	XVI	6.0	2.7	3.9	4	
gem-$N_3P_3Az_4(NHBu)_2$	XVII	5.8	3.6	3.9	2	
gem-$N_3P_3Az_4Ph_2$	XVIII	4.2	2.2	3.7	2	
gem-$N_3P_3Az_4Pip_2$	XIX	5.6	2.8	3.6	2	
gem-$N_3P_3Az_4Pyr_2$	XX	5.9	2.9	2.7	1.0	
gem-$N_3P_3Az_4(NHMe)_2$	XXI	5.8	3.1	2.5	2	
gem-$N_3P_3Az_4(NHPh)_2$	XXII	4.4	1.0	1.1	1.0	
trans-$N_3P_3Az_4Morph_2$	XXIII	5.0	2.5	4.7	2	
trans-$N_3P_3Az_4Pyr_2$	XXIV	5.9	2.9	2.2	2	[1]
trans-$N_3P_3Az_4Pip_2$	XXV	5.6	2.8	2.1	2	
trans-$N_3P_3Az_4Cl_2$	XXVI	0	0	1.9	2	
cis-$N_3P_3Az_4Pyr_2$	XXVII	5.9	2.9	1.8	1.0	
cis-$N_3P_3Az_4Pip_2$	XXVIII	5.6	2.8	1.6	2	
$N_3P_3Az_5Im$	XXIX	[f]	[f]	8.2	4	
$N_3P_3Az_5(GlyE)$	XXX	5.0[d]	2.5[d]	7.7	4	[11]
$N_3P_3Az_5OPh$	XXXI	3.1	1.3	4.4	2	
$N_3P_3Az_5Morph$	XXXII	5.0	2.5	3.5	2	[1]
$N_3P_3Az_5Cl$	XXXIII	0	0	3.5	2	[9]
$N_3P_3Az_5Pyr$	XXXIV	5.9	2.9	1.6	1.0	[1]
$N_3P_3Az_5Pip$	XXXV	5.6	2.8	1.5	1.0	[1]
$N_3P_3Az_5(NHMe)$	XXXVI	5.8	3.1	1.2	0.25	[7]
$N_3P_3Az_6$	XXXVII	–	–	2.0	1.0	[8]
$N_3P_2Az_4SOPh$	XXXVIII	–	–	31.0	16	[2]
$N_3P_2Az_4SOAz$	XXXIX	–	–	10.6	6	[2]
$N_3P_2Az_4SOF$	XL	–	–	2.9	1.0	[2]

[a]The following abbreviations have been used: Az (Aziridino), GlyE (Ethylglycinato), Im (Imidazolyl), Morph (Morpholino), Pip (Piperidino), Pyr (Pyrrolidino). [b]Substituent constants for N_3P_3 derivatives. Values taken from [12, 25–27]. [c]Preparation and characterization data described therein. [d]Estimated value: $a(\gamma)_{GlyE} \approx a(\gamma)_{Morph}$. [e]1,1-bisaziridino-*cis*-3,5-dichloro-3,5-bispyrrolidino derivative. [f]Value unknown.

sensitivity towards hydrolysis. In an extended study on compounds of type $N_3P_3R_6$ Allcock *et al.* [32] obtained the following order in hydrolysis rates for R:

Im > GlyE > NH_2 > NHMe > NHPh >Pyr,Pip,Morph

Under physiological conditions aziridino derivatives will also react with water. Whether the first hydrolysis step will be the opening of one of the three membered rings or nucleophilic attack on other sites of the molecule [32] will depend strongly on the nature of the other substituents. For compounds XVI and especially XIV, XXIX and XXX hydrolysis can be expected to overrule the biological alkylating action. Apart from the imidazolyl-, amino-, and ethylglycinato derivatives, compounds with chlorine ligands (*i.e.* VI, IX, X, XII, XXVI and XXXIII) are also easily hydrolyzed. As shown in our experiments, solutions of these compounds in neutral water lose a considerable amount of cytostatic activity within 24 hours on standing at room temperature.

Apart from hydrolysis effects the activity spectrum might be explained from electronic effects. Substituents with high $a_R(\gamma_R)$ values will increase the electron density on the P–N(–S) system as well as on the three-membered rings. The resulting higher basicity of endo- and exocyclic nitrogen atoms leads to easier electrophilic attack, which might be rate-determining in the opening of the aziridino rings [14] and promote the alkylating ability of the compounds, *i.e.* the trifluoroethoxy derivative XI should possess a rather low basicity compared with the corresponding amino derivatives. This might explain the relatively low cytostatic activity of the former compound. To some extent this also applies to the three cyclophospha*thiaz*enes XXXVIII–XL in which the 'S=O' fragment acts as an electron sink. It is noteworthy that the *in vitro* measurements with these agents are in good agreement with previous *in vivo* results in mice [23]. The high activity of XL cannot be understood with regard to electronic effects. This also applies to the ID50 and LAD values of the 'phenyl' derivatives XVIII and XXII, which are rather low compared with other compounds. Whether the relative hydrophilicity of the compounds concerned plays a role in determining the ultimate activity is not clear.

Another question remains, namely, whether or not the activity depends on the number of aziridino groups present in a molecule. The series of ethylglycinato derivatives (III, V, XIV and XXX) would suggest some dependence, but the activity range can also be correlated with sensitivity towards hydrolysis. Besides, the pyrrolidino derivatives, with the exception of IV and VI, all show about the same activity. Thus, a quantification of biological activity in terms of number of aziridino groups seems to be oversimplified. A relationship between biological activity of phosphorus bonded aziridino derivatives and the number of alkylating centres (*i.e.*, P-atoms) in the molecule has been suggested by Yakovenko *et al.* [33]. Slight, but consistent differences between the ID50 values of geminal and nongeminal pyrrolidino, piperidino and morpholino derivatives might point in this direction (see Table I). Changing the number of alkylating centres from two to one might cause a more profound effect as observed for the extensively studied mono- and bifunctional nitrogen mustards [34, 35].

Probably, by comparing isomeric bisaziridino derivatives a most active form can be assigned. The substitution pattern of aziridine towards $(NPCl_2)_3$ being only partly geminal [10] offers the opportunity to prepare these compounds. Preliminary screening results, obtained with the three isomers of $N_3P_3Az_2(NHMe)_4$, show the non-geminal isomers to possess the highest activity. A detailed investigation including the isolation of the various isomeric derivatives will be published [36].

Additional *in vivo* experiments with a selection of the compounds described here are now underway to investigate their therapeutic potencies.

Experimental Section

Only the syntheses of compounds without a reference in Table I are described. All experiments were carried out under dry nitrogen. The amines and the trifluoroethanol were purified before use by distillation over KOH pellets. Imidazole was purified by sublimation. *P.a.* $(NPCl_2)_3$, kindly provided by Otsuka Ltd., Osaka, Japan, was used as received. Solvents were purified and dried by conventional methods.

Elemental analyses (Table II) were carried out under the supervision of Mr. A. F. Hamminga. ^{31}P NMR (Table II) and ^{13}C NMR spectra were taken with a Nicolet 283A FT spectrometer, equipped with a NTCFT-1180 data system in 5 mm tubes at 25 °C at 80.9 MHz. The deuterium resonance of the solvent ($CDCl_3$) was used for field-frequency lock. Purification by HPLC was carried out using a Spectra-Physics 740 B pump and a LDC refractometer (model 1107). Separations were performed on Lichrosorb Si 60/10 columns (Mr. H. P. Velvis, Department of Inorganic Chemistry, University of Groningen). As a routine purity check mass spectra were recorded on an AEI M.S. 9 mass spectrometer operating at 70 eV, using an accelerating voltage of 8 kV. Samples were introduced directly by a conventional inlet system at about 100 °C (Mr. A. Kiewiet, Department of Organic Chemistry, University of Groningen).

TABLE II. ^{31}P NMR[a] and Elemental Analysis[b] Data.

Compound	$\delta(PAz_2)$	$\delta(PR_2)$	$\delta(PAzR)$	$^2J_{PP}$ (Hz)	C(%)	H(%)	N(%)
VII			27.1/27.7	34.9	45.75(45.86)	7.66(7.70)	26.93(26.74)
VIII			26.7	–	45.77(45.86)	7.57(7.70)	26.90(26.74)
XI	38.4	18.0		52.7	28.89(28.75)	3.99(4.02)	19.73(19.56)
XIII	38.2	9.5		52.4	49.11(49.08)	5.39(5.36)	19.93(20.03)
XV	36.7	20.2		37.6	39.88(40.42)	6.79(6.78)	26.44(26.52)
XVI	37.4	18.6		36.6	28.45(28.66)	6.08(6.01)	36.65(37.60)[c]
XVII	37.6	18.5		36.2	42.64(42.95)	8.09(8.11)	27.87(28.17)
XVIII	36.2	18.9		15.6	52.32(52.52)	5.72(5.73)	21.05(21.44)
XIX	36.8	20.7		36.5	46.00(45.86)	7.77(7.70)	26.70(26.74)
XX	36.9	16.5		35.8	43.36(43.34)	7.44(7.27)	28.24(28.43)
XXI	37.6	21.6		36.1	32.87(33.06)	6.68(6.66)	33.81(34.70)[c]
XXII·THF	38.1	7.0		38.5	51.43(51.52)	6.55(6.49)	22.50(22.53)
XXIII	37.1		28.5	33.0	40.36(40.42)	6.85(6.78)	26.50(26.52)
XXIV	36.8		27.4	33.8	43.36(43.34)	7.24(7.27)	28.13(28.43)
XXV	37.4		28.7	33.3	45.76(45.86)	7.70(7.70)	26.53(26.74)
XXVI	37.6		40.0	29.7	25.69(25.69)	4.28(4.31)	26.31(26.21)
XXVII	36.6		26.8	33.3	43.13(43.34)	7.23(7.27)	28.25(28.43)
XXVIII	37.1		27.4	32.6	45.87(45.86)	7.74(7.70)	26.73(26.74)
XXIX	37.1		20.8	35.4	38.03(37.87)	5.76(5.62)	33.07(33.97)[c]
XXXI	37.4		25.1	39.8	43.52(43.84)	5.74(5.75)	25.57(25.56)

[a]Chemical shifts (ppm) are relative to 85% H_3PO_4 and positive in downfield direction; solvent $CDCl_3$. [b]Calculated values in parentheses. [c]Deviation as a result of systematic errors.

Preparation of Compounds $N_3P_3Az_4R_2$

Geminally Substituted Derivatives

Via *gem-$N_3P_3Cl_4R_2$*. (i) Preparation of XVI and XVIII (R = NH_2, Ph). Precursors $N_3P_3Cl_4R_2$ with R = NH_2 and R = Ph were prepared according to literature methods [37, 38]. Thirty-two mmol of aziridine in 30 ml of diethyl ether was added slowly to a stirred solution of 2 mmol of precursor in 30 ml of diethyl ether, cooled at −70 °C. The reaction mixture was allowed to warm up slowly to room temperature and stirred for an additional period of 18 h. The precipitate was removed by filtration and washed with diethyl ether. The combined solutions were evaporated to dryness and the resulting crude products were crystallized from hot benzene (XVI) and diethyl ether (XVIII), respectively, yielding 10% of XVI, m.p. 250 °C (decomp.) and 60% of XVIII, m.p. 155–156 °C.

(ii) Preparation of XXII (R = NHPh). Gem-$N_3P_3Cl_4(NHPh)_2$ was prepared as described by Lederle *et al.* [39]. To a solution of 2.0 mmol of this starting material in 30 ml of benzene, cooled at 5 °C, was added dropwise a solution of 32.0 mmol of aziridine in 30 ml of benzene. After warming up to room temperature and an additional 18 h of stirring the temperature was raised to about 50 °C for 3 h. Removal of the precipitate by filtration and evaporation of the filtrate to dryness gave the crude (solid) product. Recrystallization from THF yielded 75% of XXII as a 1:1 THF adduct, m.p. 177–178 °C.

Via *gem-$N_3P_3Az_4Cl_2$ (XII)*. XII was prepared as described by Kobayashi *et al.* [9]. (i) Preparation of XI (R = OCH_2CF_3). A solution of 3.0 mmol of sodium trifluoroethoxide in 10 ml of THF was added slowly to a solution of 2.0 mmol of XII in 5 ml of THF, cooled at 10 °C. The reaction mixture was allowed to warm up slowly to room temperature and left stirring for an additional 48 h. Filtration of the formed salt and evaporation of the filtrate to dryness yielded XI as a crude solid product. Recrystallization from diethyl ether gave XI in 38% yield, m.p. 132–133 °C.

(ii) Preparation of XIII (R = OPh). A reaction of XII with sodium phenoxide carried out in a similar way to that described for the preparation of XI gave a crude oil, consisting of a 1:1 mixture of gem-$N_3P_3Az_4(OPh)Cl$ and XIII, according to ^{31}P NMR. Separation of both products by HPLC using an acetone/n-hexane mixture as eluent yielded XIII (20%), m.p. 95 °C.

(iii) Preparation of XV, XVII, XIX, XX and XXI (R = Morph, NHBu, Pip, Pyr, NHMe). To a solution of 2.0 mmol of XII in 25 ml of benzene, cooled at 5 °C, was added dropwise a solution of 12.0 mmol of the appropriate amine in 20 ml of benzene. After warming up to room temperature the reaction mixture was stirred for 18 h. Then, for an additional period of 24 h, it was maintained at 55 °C. Filtration of the precipitated amine hydrochloride and evaporation of the filtrate to dryness *in vacuo* yielded the crude products. Recrystallization from diethyl ether/n-pentane mixtures gave analytically pure com-

pounds: XV, yield 75%, m.p. 138–139.5 °C; XVII, yield 60%, m.p. 92.5–94 °C; XIX, yield 75%, m.p. 118.5–119 °C; XX, yield 55%, m.p. 88–90 °C; XXI, yield 60%, m.p. 119–121 °C.

Non-geminally substituted derivatives

Preparation of cis-/trans-$N_3P_3Az_4R_2$ *(XXIII–XXV, XXVII and XXVIII)* via cis-/trans-$N_3P_3Cl_4R_2$ *(R = Pyr, Pip, Morph).* The starting compounds were prepared as described in [1]. To a solution of 2.0 mmol of $N_3P_3Cl_4R_2$ in 25 ml of benzene, cooled at 5 °C, was added dropwise a solution of 60 mmol of aziridine in 25 ml of benzene. The reaction mixture was warmed up slowly to 50–55 °C. Depending on the reactivity of the starting material [1] this temperature was retained for 24–96 h. Filtration and subsequent evaporation of the filtrate to dryness *in vacuo* gave the crude product, which was recrystallized several times from diethyl ether/n-pentane mixtures. XXIII, yield 80%, m.p. 112.5–114 °C; XXIV, yield 40%, m.p. 99–100 °C; XXV, yield 65%, m.p. 99.5–100.5 °C; XXVII, yield 50%, m.p. 71.5–73 °C; XXVIII, yield 40%, m.p. 80–82 °C.

Preparation of trans-$N_3P_3Az_4Cl_2$ *(XXVI).* A (1:8) reaction of I with aziridine, as described by Kobayashi *et al.* [9], gives XXVI as one of the products [36]. It was isolated in 10% yield by preparative HPLC, using diethyl ether with traces of methanol as eluent, and subsequent recrystallizations from diethyl ether. M.p. 102.5–104 °C. The *trans*-structure was assigned due to the simplicity of the right part of the ^{13}C NMR spectrum (Fig. 4, doublet at 23.9 ppm). Moreover, a reaction with excess of pyrrolidine gave XXIV as the ultimate product.

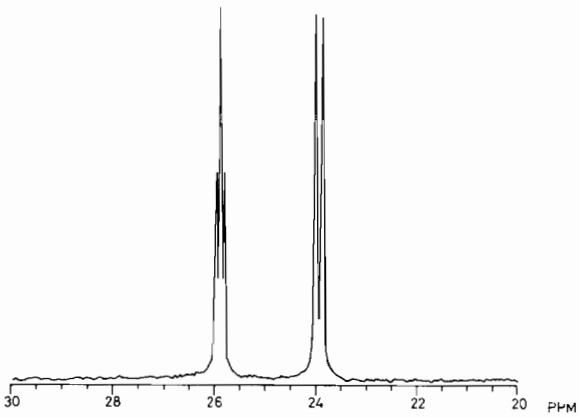

Fig. 4. ^{13}C NMR spectrum of *trans*-$N_3P_3Az_4Cl_2$ (XXVI). $\delta^{13}C(PAz_2) = 23.9$ ppm, $^2J_{PC}{}^* = 6.9$ Hz. $\delta^{13}C(PAzCl) = 25.9$ ppm, $^2J_{PC}{}^* = 8.3$ Hz. Chemical shifts are relative to TMS; $^2J_{PC}{}^* = {}^2J_{PC} + \Sigma^4J_{PC}$.

Preparation of Compounds $N_3P_3Az_5R$ via *XXXIII*

XXXIII was prepared as described in [9].

Preparation of XXIX (R = Im)

To a stirred solution of 2.0 mmol of XXXIII in 30 ml of THF at a temperature of 10 °C, was added slowly a solution of 5.0 mmol of imidazole in 50 ml of THF. The resulting mixture was warmed up to room temperature and stirring was continued for 18 h at that temperature. Afterwards the mixture was kept at *ca.* 45 °C for 3 h. The resulting clear solution was evaporated to dryness *in vacuo.* XXIX was isolated from the remaining hygroscopic oil by fractional crystallization from THF under dry nitrogen. Yield: 30%. Recrystallization from diethyl ether gave the pure product, m.p. 148–149 °C.

Preparation of XXXI (R = OPh)

At room temperature a solution of 4.0 mmol of sodium phenoxide in 15 ml of THF was added dropwise to a stirred solution of 2.0 mmol of XXXIII in 15 ml of THF. The reaction mixture was refluxed for 18 hours. After cooling the precipitated NaCl was removed by filtration. Evaporation of the solvent gave an oil, from which XXXI was isolated by preparative HPLC, using acetone as an eluent, and subsequent recrystallization from n-pentane. Yield: 50%, m.p. 90–92 °C.

Preparation of VII and VIII

The starting compounds *trans*- and *cis*-$(NPPyrCl)_3$ were prepared simultaneously as follows: to a stirred solution of 2.0 mmol of I in 25 ml of acetonitrile, cooled at −14 °C, was added slowly a solution of 12.0 mmol of pyrrolidine in 15 ml of acetonitrile. The reaction mixture was warmed up to room temperature and stirring was continued overnight. Evaporation of the solvent *in vacuo* yielded a yellowish oil, which was washed three times with 25 ml portions of n-hexane. The combined n-hexane solutions were evaporated to 1/10 of the original volume. *Trans*- and *cis*-$(NPPyrCl)_3$ were separated by HPLC, using n-hexane as an eluent and subsequent recrystallization from n-hexane. Yields: *trans*-$(NPPyrCl)_3$ 40%, m.p. 161–163 °C; *cis*-$(NPPyrCl)_3$ 10%, m.p. 73–75 °C. Both compounds were converted to their corresponding aziridino derivatives, VII and VIII. For this purpose, the procedure described above for non-geminally substituted derivatives was used with a reaction time of two days for the *trans*- and 4 days for the *cis*-isomer. Both yields were 30%, m.p. 105 °C (VII) and 89–91 °C (VIII).

Acknowledgements

The authors are much indebted to Ms. E. Bosma and Mr. T. Wilting for their valuable cooperation, and to Ms. H. Jense for typing the manuscript.

References

1 A. A. van der Huizen, A. P. Jekel, J. K. Bolhuis, D. Keekstra, W. H. Ousema and J. C. van de Grampel, *Inorg. Chim. Acta, 66*, 85 (1982).
2 J. C. van de Grampel, A. A. van der Huizen, A. P. Jekel, D. Wiedijk, J.-F. Labarre and F. Sournies, *Inorg. Chim. Acta, 53*, L169 (1981).
3 A. A. Kropacheva and L. E. Mukhina, *J. Gen. Chem. USSR, 31*, 2274 (1961).
4 V. A. Chernov, T. S. Safonova, L. E. Mukhina, A. A. Kropacheva, *Ger. Offen.* 720302, Patent no: 2043128 (1970).
5 A. A. Kropacheva and N. M. Kashnikova, *J. Gen. Chem. USSR, 35*, 2219 (1965).
6 A. A. Kropacheva and N. M. Kashnikova, *J. Gen. Chem. USSR, 38*, 135 (1968).
7 G. F. Ottmann, H. Agahigian, H. Hooks, G. D. Vickers, E. H. Kober and R. F. W. Rätz, *Inorg. Chem. 3*, 753 (1964).
8 R. F. W. Rätz, E. H. Kober, C. Grundmann and G. F. Ottmann, *Inorg. Chem., 3*, 757 (1964).
9 Y. Kobayashi, L. A. Chasin and L. B. Clapp, *Inorg. Chem., 2*, 212 (1963).
10 A. A. van der Huizen, A. P. Jekel, J. Rusch and J. C. van de Grampel, *Recl. Trav. Chim. Pays-Bas, 100*, 343 (1981).
11 A. A. Smaazdÿk, B. de Ruiter, A. A. van der Huizen and J. C. van de Grampel, *Recl. Trav. Chim. Pays-Bas, 101*, 270 (1982).
12 J. C. van de Grampel, *Rev. Inorg. Chem., 3*, 1 (1981).
13 V. A. Chernov, V. B. Lytkina, S. I. Sergievskaya, A. A. Kropacheva, V. A. Parshina and L. E. Sventsitskaya, *Farmakol. Toksikol. (Moscow), 22*, 365 (1959).
14 O. C. Dermer and G. E. Ham, 'Ethyleneimine and Other Aziridines', Ac. Press, New York and London, 1969, p. 394.
15 G. Obe, *Mut. Res., 6*, 467 (1968).
16 T. H. Chang and W. Klassen, *Chromosoma, 24*, 314 (1968).
17 T. S. Safonova, *Zh. Vses. Khim. Ob-va im. Mend., 18*, 6, 657 (1973).
18 J.-F. Labarre, J.-P. Faucher, G. Levy, F. Sournies, S. Cros and G. François, *Eur. J. Cancer, 15*, 637 (1979).
19 V. A. Ovchinnikova, P. P. Filatov, V. A. Chernov, A. S. Singin, T. S. Safonova and G. V. Bornovalova, *Pharm. Chem. J., 11*, 1601 (1977).
20 A. N. Dedenkov and R. B. Susanjan, *Med. Radiol., 20*, 1, 84 (1975).
21 L. M. Fonshtein, S. K. Abilev, L. A. Akin'shina and A. M. Zekhnov, *Pharm. Chem. J., 12*, 35 (1978).
22 M. N. Preobrazhenskaya in 'Development of Anticancer Drugs', ed. J. F. Saunders and S. K. Carter, NCI Monograph 45, 196 (1977).
23 J.-F. Labarre, F. Sournies, S. Cros, G. François, J. C. van de Grampel and A. A. van der Huizen, *Cancer Letters, 12*, 245 (1981).
24 S. Rodenhuis, N. H. Mulder, H. Schraffordt-Koops, P. Coninx and J. C. van de Grampel, personal communication.
25 D. Feakins, S. N. Nabi, R. A. Shaw and P. Watson, *J. Chem. Soc. (A)*, 10 (1968).
26 D. Feakins, W. A. Last, S. N. Nabi, R. A. Shaw and P. Watson, *J. Chem. Soc. (A)*, 196 (1969).
27 D. Feakins, S. N. Nabi, R. A. Shaw and P. Watson, *J. Chem. Soc. (A)*, 2468 (1969).
28 H. B. Lamberts, A. van der Meer-Kalverkamp, J. C. van de Grampel, A. A. van der Huizen, A. P. Jekel and N. H. Mulder, to be published.
29 A. A. Smaardijk, unpublished results.
30 A. A. Kropacheva and N. H. Kashnikova, *J. Gen. Chem. USSR, 35*, 1978 (1965).
31 B. A. Ihvin, V. A. Filov, B. O. Kraiz, V. V. Belogorodsky, L. L. Maljugina, R. I. Pol'kina and Yu. N. Kozlovsky, *Vopr. Oncol., 25*, 7, 71 (1979).
32 H. R. Allcock, T. J. Fuller and K. Matsumura, *Inorg. Chem., 21*, 515 (1982).
33 K. N. Yakovenko, S. A. Azhaev and N. P. Bochkov, *Genetika, 10*, 10, 135 (1974).
34 W. C. J. Ross, 'Biological Alkylating Agents', ed. R. W. Raven, 1962, London, Butterworths.
35 W. C. J. Ross, in 'Antineoplastic and Immunosuppressive Agents', Springer-Verlag, 1974, p. 33.
36 J. W. Rusch, A. P. Jekel, A. A. van der Huizen and J. C. van de Grampel, to be published.
37 G. R. Feistel and T. Moeller, *J. Inorg. Nucl. Chem., 29*, 2731 (1967).
38 M. Biddlestone and R. A. Shaw, *Chem. Commun.*, 407 (1968).
39 H. Lederle, G. Ottmann and E. H. Kober, *Inorg. Chem., 5*, 1818 (1966).