

^1H -NMR, IR spectra and elemental analyses. The antitumor activity of some of these polymers were tested in mice. The polyphosphates containing both 5-fluorouracil and nitrogen mustard showed inhibition ratio of 58.6%—66.0%. Their antitumor activity are comparable to that of 5-fluorouracil or nitrogen mustard, but with much lower toxicity.

EXPERIMENTAL

Monomer synthesis

N,N-bis(2-chloroethyl)phosphoramidate dichloride (I) was synthesized according to the literature^[2]. m.p. 52—53°C (lit. 53—55°C).

1,3-dihydroxyethyluracil (II_c) was synthesized according to the literature^[4]. m.p. 151—152°C (lit. 152.5—153°C). 1,3-dihydroxyethyl-5-fluorouracil (II_a)^[2], 1,3-dihydroxypropyl-5-fluorouracil (II_b)^[2], 1,3-dihydroxypropyluracil (II_d), 1,3-dihydroxyethylthymine (II_e), 1,3-dihydroxypropylthymine (II_f) were prepared in a similar way. 1,3-dihydroxy-methyl-5-fluorouracil (II_g) was prepared from 5-fluorouracil with formaldehyde^[2]. Monomers II_a , II_b , II_d , II_f , II_g were purified by chromatography on silica gel. The experimental data of monomers (II_{a-g}) are given in Table 1.

Table 1 Yield and analytical data of monomer (II_{a-g})

Compd.	Yield (%)	M.P. (°C)	Eluant	Elemental analysis					
				Found (%)			Calcd. (%)		
				C	H	N	C	H	N
II_a	61.9	gel. ^[2]	CH_3COCH_3	43.76	5.09	12.55	44.04	5.05	12.84
II_b	56.9	gel. ^[2]	$\text{C}_6\text{H}_6 + \text{EtOH}$	48.24	5.62	11.86	48.78	6.10	11.38
II_c	55.0	152 (lit. ^[4] 152)		47.84	5.99	13.81	48.00	6.00	14.00
II_d	70.1	gel.	CH_3COCH_3	51.72	6.84	12.20	52.63	7.02	12.28
II_e	49.1	99—102		49.94	6.73	13.15	50.47	6.54	13.08
II_f	53.7	gel.	CH_3COCH_3	54.30	7.04	11.31	54.55	7.44	11.57
II_g	63.0	gel. ^[2]	CH_3COCH_3	37.84	4.27	14.67	37.89	3.68	14.74

General procedure for the polycondensation of monomers (II) with N,N-bis(2-chloroethyl)phosphoramidate dichloride (I).

To a solution of monomer (II) (0.01 mole) in DMF (10 ml) was added sodium bicarbonate (0.02 mole). The mixture was stirred for 30 min at room temperature. N,N-bis(2-chloroethyl)phosphoramidate dichloride (I) (2.58g, 0.01 mole) in 10 ml DMF was then added dropwise and the resulting mixture was stirred for 30 h at 25—30°C. The sodium salt was filtered off and the filtrate was concentrated under reduced pressure. The residue was dissolved in ethanol and the precipitate was filtered off. The clear solution was poured into ether, the precipitate, thus formed, was dried at 80—100°C under reduced pressure.

The yield, intrinsic viscosity, molecular weight and elemental analyses data of polymers (III_{a-g}) are given in Table 2.

Table 2 Analytical data, intrinsic viscosities and molecular weights of polymer (III_{a-g})

Compd.	Yield (%)	[η]* (ml/g)	$\bar{M}_n^* \times 10^{-3}$	Elemental analysis							
				Found (%)				Calcd. (%)			
				C	H	N	Cl	C	H	N	Cl
III _a	86.6	8.5	3.3	34.62	5.76	9.58	16.69	34.12	4.50	9.90	16.82
III _b	81.0	8.6	3.7	36.09	5.53	9.07	15.22	37.33	5.11	9.33	15.77
III _c	83.3	9.7	3.6	34.94	5.25	9.65	17.05	35.64	4.95	10.40	17.57
III _d	81.8	9.3	4.7	38.48	5.57	9.24	16.66	38.88	5.56	9.72	16.43
III _e	87.5	8.9	4.3	38.61	6.17	9.22	16.97	37.32	5.26	10.05	16.99
III _f	88.8	7.1	3.4	38.89	6.55	8.60	14.84	40.36	5.83	9.42	15.92
III _g	87.0	6.7	2.8	30.76	4.11	10.28	17.78	30.46	3.81	10.66	18.02

* $\bar{M}_n$ were determined by dynamic osmometry (DMF, 30°C). Intrinsic viscosities were measured in H₂O at 30°C using Ubbelohde viscometer.

RESULTS AND DISCUSSION

Structures of monomers (II)

The structures of monomers (II) are explained by taking monomer (II_c) as an example. The ¹H-NMR spectrum of 1,3-dihydroxyuracil (II_c) (Figure 1a) showed two doublets at 7.56 ppm (*J*=8 Hz) and 5.62 ppm (*J*=8 Hz) indicating the presence of C-6 and C-5 protons at uracil ring; The broad singlet at 4.56 ppm disappeared when D₂O was added, this fact

Table 3 ¹H-NMR and UV data of monomer (II_{a-g})

Compd.	Solvent	¹ H-NMR* δ(ppm)						UV* λ _{max} (nm)
		C ₆ H	C ₅ H	OH	NCH ₂ CH ₂ O	C-CH ₂ -C	CH ₃	
II _a	CD ₃ CN	7.60(d,1H)		3.70 (m, 10H)				272
II _b	CD ₃ CN	7.60(d,1H)		3.70 (m, 10H)		1.80(m,4H)		272
II _c	DMSO-d ₆	7.50(d,1H)	5.55(d,1H)	4.3(b,2H)	3.65(m,8H)			267
II _d	CD ₃ CN	7.45(d,1H)	5.50(d,1H)	3.70 (m, 10H)		1.83(m,4H)		267
II _e	DMSO-d ₆	7.45(s,1H)		4.2(b,2H)	3.65(m,8H)		1.80(s,3H)	270
II _f	CF ₃ COOH	7.45(s,1H)			4.3(m,8H)	2.10(m,4H)	2.00(s,3H)	270
II _g	DMSO-d ₆	8.1(d,1H)	6.5 (b, 2H, OH),	5.0 (m,4H, NCH ₂ O)				268

* ¹H-NMR spectra were recorded with EM-360L instrument, TMS as an internal reference. UV spectra were determined in H₂O (pH=7) with UV-240 spectrophotometer.

can be attributed to hydrogen exchange due to OH group; The multiplets at 3.65 ppm can be assigned to the $\text{OCH}_2\text{CH}_2\text{N}$ protons. On the other hand, by comparison with the IR spectrum of uracil (Fig. 2a), the presence of hydroxy group attached to carbon is shown by the characteristic absorptions at 3346 cm^{-1} (O—H stretching), 1374 cm^{-1} (O—H bending), and 1061 cm^{-1} (C—O stretching). The 1675 cm^{-1} absorption demonstrates the presence of carbonyl groups of uracil (Fig. 2b). Both the ^1H -NMR and IR spectra showed no proton signals (Fig. 1a) or absorption bands (Fig. 2b) corresponding to N-1 and N-3 hydrogen of uracil. All these results are consistent with the monomer structure (II_c).

The ^1H -NMR data of the other six monomers (II) (Table 3) showed that they have similar structures. The UV (Table 3) and elemental analysis data (Table 1) have further confirmed the structure of these monomers (II_{a-g}).

Synthesis of polyphosphates (III)

In our earlier paper^[5], we reported the synthesis of polyphosphates containing 5-fluorouracil by the condensation of 1,3-dihydroxyalkyl-5-fluorouracil with ethyldichlorophosphate or phenyldichlorophosphate. The polycondensation reaction was carried out at 50°C . The remaining hydrogen chloride in the reaction mixture was removed by the treatment with an aqueous potassium carbonate. In the present investigation, N,N-bis(2-chloroethyl)-phosphoramidate dichloride was used instead of ethyldichlorophosphate. Since the pendant nitrogen mustard group on the polyphosphate is labile to hydrolysis in alkaline medium, especially at elevated temperature. In order to protect this group from hydrolysis, an excess of potassium carbonate should be avoided, but it is not easy to adjust during the neutralizing treatment. Nevertheless, we successfully synthesized the polyphosphates (III) by varying the reaction conditions described in the experimental section.

Structure of polymer (III)

The structures of polymers (III_{a-g}) are explained by taking polymer (III_c) as an example. The ^1H -NMR spectrum of polymer (III_c) (Fig. 1b) also showed the chemical shifts of C-6

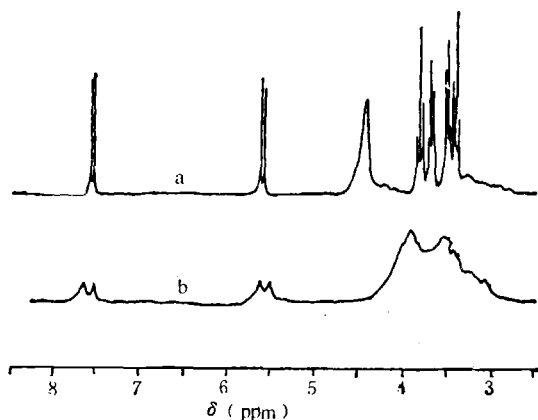


Fig. 1 a. ^1H -NMR spectrum of monomer (II_c) (200MHz) (using a Varian XL-200 instrument).
b. ^1H -NMR spectrum of polymer (III_c) (60MHz) (using a Varian EM-360L instrument).

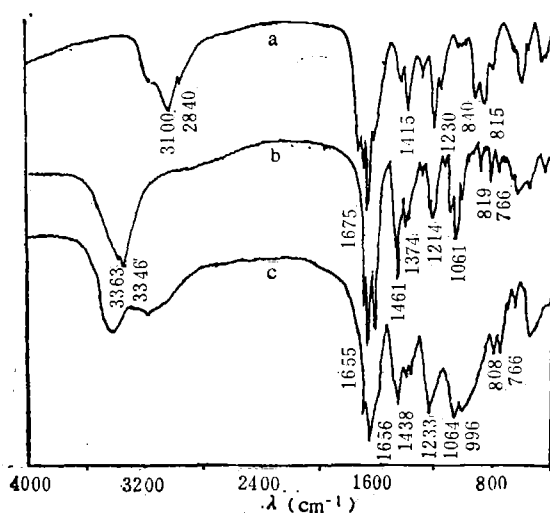


Fig. 2 a. IR spectrum of uracil. b. IR spectrum of monomer (II_c). c. IR spectrum of polymer (III_c).

IR spectra were determined on a Nicolet 170SX instrument.

and C-5 protons, the multiplet from 3 ppm to 4 ppm are due to the protons of CH₂ linking to chlorine, oxygen and nitrogen atoms in the polymer. On the other hand, in comparison with monomer (II_c), the IR spectrum of polymer (III_c) (Fig. 2c) exhibited the characteristic absorption of phosphate group: $\nu_{\text{P=O}}$ (1233 cm⁻¹ strong) and $\nu_{\text{P-O-O}}$ (996–1064 cm⁻¹ strong), indicating that the phosphate bond were formed in the polycondensation. In combination with elemental analysis data, these results confirmed that the polymers had the expected structures (III_{a-g}).

Solubility of the polymer

All the polyphosphates obtained are very soluble in water, dimethyl formamide and benzyl alcohol; soluble in ethanol, glycerin and chloroform; insoluble in acetone, ethyl acetate and ether. Water soluble polyphosphate is good for biological transport and systematic administration.

Antitumor activity of the polymer

The antitumor activity of some of these polymers were determined in mice against Ehrlich Ascites. By comparing the polymers (V_a) (V_b) in the previous paper, the data in Table 4 indicated that the polymers (III_a) (III_b), which contain both 5-fluorouracil and nitrogen mustard, are more active than that of the polymers (V_a) (V_b). This may be attributed to the different component and linking of the drug units in the polymer molecule. As a main component, monomer 1,3-dihydroxyalkyl-5-fluorouracil has higher antitumor activity than that of N,N'-dihydroxyalkyl-5-fluorouracil-1,3-diacetamide monomer.

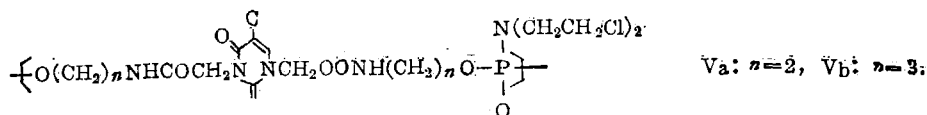
The contrastive test between polymers (III_a) (III_b) and 5-fluorouracil or nitrogen mustard hydrochloride against Ehrlich Ascites in mice showed that the antitumor activity of polymers

(III_a) (III_b) are comparable to that of 5-fluorouracil or nitrogen mustard. However the toxicity of polymers (III_a) (III_b) are much lower than that of 5-fluorouracil or nitrogen mustard.

Table 4 Antitumor activity of polymers (V_{a,b}) (III_{a,b}) and 5-fluorouracil (5-Fu), nitrogen mustard hydrochloride (MHCl) tested against Ehrlich Ascite in mice

Compd.	Dose (mg/kg)	Number of mice in treatment		Average weight of tumor (g)		Inhibition ratio (%)	P value
		start	end	control	treated		
V _a	i.p. 6 × 180	9	9	1.16	0.68	41.3	<0.001
V _b	i.p. 6 × 180	11	11	1.16	0.63	45.7	<0.001
III _a	i.p. 6 × 180	9	9	1.16	0.39	66.0	<0.001
III _b	i.p. 6 × 130	9	9	1.16	0.48	58.6	<0.001
5-Fu	i.p. 4 × 30	12	5			58.9	
MHCl	i.p. 1 × 120	9	6	1.16	0.45	61.2	<0.001

Structures of polymers (V_{a,b}):



Acknowledgment The author is indebted to Mr. Zheng Fanbo of Wuhan Medical College for carrying out the animal test.

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